3rd European BioSensor Symposium
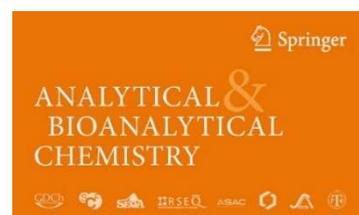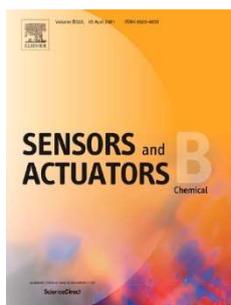
EBS ONLINE 2021

9th – 12th March, 2021

Wildau, Germany

Welcome

Dear friends and colleagues from the area of biosensor research!

We would like to welcome you to the European Biosensor Symposium 2021 Online! This is a conference in the tradition of ten German Biosensor Symposia (DBS) since 1999 and of two European Biosensor Symposia since 2017.

We had 2017 in Potsdam the 1st EBS and 2019 in Florence the 2nd EBS. As you may know, we have planed the next EBS 2021 in Aachen, but because of the Covid 19 pandemic this event had to be postponed.

In order to fill the gap, the scientific committee has decided to go for an online event in 2021. Thus, actual researchers will get the chance to present and interact with their colleagues. This might be not the same as in reality, but it is a good way to keep the international exchange and to allow young scientists to present. So we will organise the 3rd EBS in Wildau and intend to create opportunities for interaction among the participants – particularly in the format of poster discussions.

A peculiarity of the EBS conference series is the strong focus on young scientists. This is also valid for the Online conference. Thus, I would like to encourage students after the master, phd students or young post-docs to submit an abstract. We also would like to stimulate the participation by prizes for good oral presentations and poster prizes.

The conference will cover a broad range of topics which includes all components of biosensors (recognition elements, immobilisation strategies, transducers, micro-fluidics, data analysis) and biosensor application, but also related fields such as bio-nanotechnology, bioenergetics, photobioelectrochemistry and model development.

Thus, we hope to provide a broad view and give stimulus to the own research and future development. Welcome to the virtual meeting in the capital region of Germany!

Fred Lisdat

Chair of EBS 2021 Online

Campus Technical University of Applied Sciences Wildau

Scientific Committee

- Prof. Dr. Till Bachmann (University of Edinburgh)
- Prof. Dr. Antje Baeumner (University of Regensburg)
- Prof. Dr. Francesco Baldini (Institute of Applied Physics of the National Research Council of Italy)
- Prof. Dr. Frank Bier (Potsdam University)
- Prof. Dr. Dieter Beckmann (IBA Heiligenstadt)
- Prof. Dr. Maria Cruz Moreno Bondi (Universidad Complutense de Madrid)
- Prof. Dr. Dario Compagnone (University of Teramo)
- Prof. Dr. Serge Cosnier (Grenoble University)
- Prof. Dr. Jenny Emneus (Technical University of Denmark)
- Prof. Dr. Guenther Gauglitz (Tübingen University)
- Prof. Dr. Hubert Girault (EPFL)
- Prof. Dr. Róbert E. Gyurcsányi (Budapest University of Technology and Economics)
- Prof. Dr. Christine Kranz (Ulm University)
- Prof. Dr. Ilya Kurochkin (Lomonosov Moscow State University)
- Prof. Dr. Laura Lechuga (Catalan Institute of Nanoscience and Nanotechnology)
- Prof. Dr. Fred Lisdat (Technical University Wildau)
- Prof. Dr. med. Peter Luppa (Technical University of Munich)
- Prof. Dr. Pilar Marco (National Research Council of Spain)
- Prof. Dr. Maria Minunni (University of Florence)
- PD Dr. Michael Seidel (Technical University of Munich)
- Prof. Dr. Nicolas Plumere (Ruhr University Bochum)
- Prof. Dr. Juergen Popp (Friedrich Schiller University Jena)
- Dr. Ute Resch-Genger (Bundesanstalt für Materialforschung und -prüfung)
- Prof. Dr. Frieder Scheller (Potsdam University)
- Prof. Dr. Michael J. Schöning (FH Aachen)
- Prof. Dr. Giuseppe Spoto (University of Catania)
- Prof. Dr. Petr Skladal (Masaryk University)
- Dr. Michael Steinwand (Innovendia Consulting Services)
- Prof. Dr. Gerald Urban (University of Freiburg)
- Prof. Dr. Patrick Wagner (KU Leuven)
- Prof. Dr. Joachim Wegener (University of Regensburg)
- Prof. Dr. Ulla Wollenberger (Potsdam University)

Organizing Committee

(Technical University Wildau, Germany)

- Prof. Dr. Fred Lisdat
- Dipl. Ing. Daniel Schäfer
- Dr. Carsten Hille
- Dipl. Ing. Stefanie Radig

Contact Informations

Biosystems Technology
Institute of Life Sciences and Biomedical Technologies
Technical University of Applied Sciences Wildau
Hochschulring 1
15745 Wildau

E-Mail: ebs2021@th-wildau.de
Tel +49 (0)3375 508134/799
Fax +49 (0)3375 508578
www.th-wildau.de/lisdat

Keynote Lectures

- **Hatice Altug**
  Ecole Polytechnique Federale de Lausanne
  Frontiers in Nanophotonic Biosensors

- **Francesco Baldini**
  Institute of Applied Physics-CNR
  Chemical and biochemical optical fibre sensing for invasive and intracellular application: past, present and future

- **Can Dincer**
  University of Freiburg
  CRISPR-powered electrochemical multiplexed nucleic acid testing

- **Steffi Krause**
  Queen Mary University of London
  Photoelectrochemical imaging for live cell monitoring and sensor applications

- **Francesco Ricci**
  University of Rome Tor Vergata
  Electrochemical DNA-based nanodevices for IgG antibodies detection

- **Janos Vörös**
  ETH Zurich
  The role of non-specific binding in determining the limit of detection of diagnostically relevant biosensors

Prizes

The EBS Online intends to give prizes for young researchers (phd students, post docs) who have given excellent oral presentations or have presented a very good poster. This way we want to stimulate the participants further and give gratitude to those who try to transfer their research ideas and results to others in a convincing and engaged way.

This intention of the Scientific Committee is also supported by scientific organisations such as the Bioelectrochemical Society (BES), Publishers of scientific journals such as Elsevier (Sensors & Actuators B: Chemical) and Springer (Analytical and Bioanalytical Chemistry) or companies (Sciospec Scientific Instruments).

In order to have a broad basis for the selection following scientists have agreed to work in the **prize selection committees**:

- Prof. F. Baldini
- Prof. F. Bier
- Dr. P. Bollela
- Prof. M. Bondi
- Dr. M. Bossi
- Prof. D. Compagnone
- Dr. F. Conzuelo
- Dr. V. Diculescu
- Prof. G. Gauglitz
- Prof. Ch. Kranz
- Prof. I. Kurochkin
- Prof. L. Lechuga
- Prof. M.P. Marco
- Prof. M. Minunni
- Prof. N. Plumere
- Dr. G. Proll
- Dr. M. Seidel
- Prof. U. Wollenberger

Prize Winners

Oral Awards

Nako Nakatsuka / ETH Zurich, Switzerland

Towards Simultaneous Neurochemical Sensing and Electrophysiology via Aptamer-Based Bioelectronics

Sponsored by the publisher Elsevier (Sensors & Actuators B: Chemical)

Julia Klüpfel / TU Munich, Germany

Automated, flow based chemiluminescence microarray immunoassay for the rapid multiplex detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (CoVRapid CL-MIA)

Sponsored by the publisher Elsevier (Sensors & Actuators B: Chemical)

Soraya Höfs / TH Wildau, Germany

Enzyme activity determination of human monoamine oxidase B (Mao B) by amperometric hydrogen peroxide detection
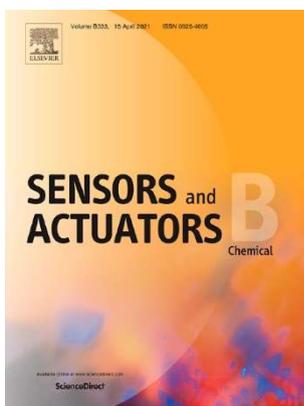
Poster Awards

Alejandro Valverde / Complutense University of Madrid, Spain

Electrochemical bioplatform to unravel neurodegeneration and Alzheimer's disease through the determination of neurofilament light chain protein

Sponsored by the Bioelectrochemical Society

Aysu Yarman / University of Potsdam, Germany

Strep-Tag II-Imprinted Polymer for the Recognition of Recombinant Proteins

Sponsored by the Bioelectrochemical Society

Muqsit Pirzada / Technical University Berlin, Germany

Cancer biomarker detection in human serum samples using nanoparticle decorated epitope-mediated hybrid MIP

Sponsored by the Publisher Springer (*Analytical and Bioanalytical Chemistry*)

Simon Streif / University of Regensburg, Germany

Developing a liposome-based lateral flow assay for the detection of SARS-CoV-2 neutralizing antibodies

Sponsored by the Publisher Springer (Analytical and Bioanalytical Chemistry)

**Federica Mariani** / University of Bologna, Italy

A material-based approach for the development of wearable pH sensors

Sponsored by the company Sciospec Scientific Instruments

Rebeca M. Torrente Rodriguez / Complutense University of Madrid, Spain

A Multiplexed Graphene-Based Telemedicine Platform for Rapid, Remote and Low-Cost COVID-19 Control and Monitoring

Sponsored by the company Sciospec Scientific Instruments
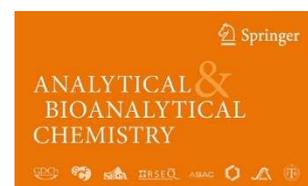
Programme

Tuesday 09.03.2021

08:45 Opening of the meeting room

09:00 Welcome

09:10 <u>Keynote lecture 1</u>   <u>Chair</u>: **Fred Lisdat** (Wildau, Germany)

Hatice. Altug (Lausanne, Switzerland)

Frontiers in Nanophotonic Biosensors

09:35 **Cosimo Trono**, T.K. Dey, S. Tombelli, P. Biswas, A. Giannetti, N. Basumallick,

O1   F. Baldini,

S. Bandyopadhyay (Kolkata, India; Florence, Italy)

Etched long period fiber gratings near turn around point for high sensitive

label-free biosensing

09:50 **Alessandra M. Bossi**, D. Maniglio, N. Cennamo (Verona, Italy)

O2   Flexible molecularly imprinted nanoparticles in plasmonic optical sensing

10:05 Discussion

10:20 **Annalisa Scroccarello**, F.D. Pelle, D. Compagnone, (Teramo, Italy)

O3   Plasmonic-active nanostructured thin-films for sensing and biosensing.

Towards lab-on-a-strip smart devices

10:35 **Francesco Chiavaioli**, F. Esposito, L. Sansone, A. Srivastava, St. Campopiano,

O4   M. Giordano, A. Giannetti, A. Iadicicco, F. Baldini (Florence, Italy)

Long period fiber grating-assisted optical biosensing for the real-time

detection of C-reactive protein in serum

10:50 **Stefanie Michaelis** , S. Azzam, M. Klein, Z. v. Guttenberg, L. Tomasova, J.

O5   Wegener (Regensburg, Germany)

A novel high-precision optical wound healing assay to analyze 2D cell

migration

11:05 Discussion

11:20 Coffee break

11:40 [Keynote lecture 2] Chair: **Till Bachmann** (Edinburgh, UK)

Francesco Baldini (Florence, Italy)

Chemical and biochemical optical fibre sensing for invasive and intracellular application: past, present and future

12:05 **Nako Nakatsuka**, A. Faillétaz, K. Vadodaria, U. Ilgin, F.H. Gage, D. Momo-

O6 tenko, J. Vörös (Zurich, Switzerland)

Towards Simultaneous Neurochemical Sensing and Electrophysiology via Aptamer-Based Bioelectronics

12:20 **Eszter Supala**, L. Tamás, J. Erdössy, R.E. Gyurcsányi (Budapest, Hungary)

O7 Multiplexed redox gating measurements with a microelectrospotter. Towards electrochemical readout of molecularly imprinted polymer microarrays

12:35 Discussion

12:40 Lunch break

13:50 **Milad Eyvazi Hesar**, N. Seyedsadrkhani, D. Khan, M. Piekarski, S. Ingebrandt

O8 (Aachen, Germany)

Disposable paper-based biosensors for continuous remote sensing

14:05 **Veronica Caratelli**, M. Moccia, C. Avitabile, M. Saviano, A.L. Imbriani, D.

O9 Moscone, F. Arduini (Rome, Italy)

Smartphone assisted PNA-based printed electrochemical biosensor for non-invasive coeliac disease diagnosis

14:20 **Farnoosh Vahidpour**, T. Wagner, M.J. Schöning (Aachen, Germany)

O10 Simultaneous online monitoring and sterility assurance in aseptic food processing based on a combined chemical/biosensor

14:35 **Victor Diculescu**, M. M. Barsan, T.A. Enache, E. Matei, M. Enculescu, N.

O11 Apostol, I. Enculescu (Romania)

Magnetic materials for electrodes and nanoparticles and their applications in biosensing

14:50 Discussion

15:10 **Poster discussion** – Session 1

17:00 End of the 1st day

Wednesday 10.03.2021

08:45 Opening of the meeting room

09:00 <u>Keynote lecture 3</u>   <u>Chair</u>: **Nicolas Plumere** (Munich, Germany)

Steffi Krause (London, UK)

Photoelectrochemical imaging for live cell monitoring and sensor applications

09:25 M. Riedel, A. Ruff, W. Schuhmann, F. Lisdat, **Felipe Conzuelo**

O12 (Wildau, Germany; Bochum, Germany)

Light-triggered biocatalytic reactions for multiplexed sensing

09:40 **Jana Becker**, T. Bobrowski, F. Conzuelo, A. Ruff, W. Schuhmann (Bochum,

O13 Germany)

A self-powered glucose sensor with optical readout

09:55 Discussion

10:10 **Alexey K. Orlov**, A.A. Galushin, A.A. Karykin, M.A. Komkova, (Moscow,

O14 Russia)

Bioelectrocatalysis by PQQ-Glucose Dehydrogenase Enhanced by Electro-polymerized Azines for Advanced Biosensors

10:25 **Paolo Bollella**, A. Melman, Zh. Guo, S. Edwardraja, W. Johnston, K.A.

O15 Alexandrov, E. Katz (Potsdam, USA)

PQQ-Glucose Dehydrogenase-Calmodulin Chimera Enzyme: Different

Triggered Activation for Multipurpose Biosensors

10:40 **Soraya Höfs**, A. Talke, F. Lisdat (Wildau, Germany)

O16 Enzyme activity determination of human monoamine oxidase B (Mao B) by amperometric hydrogen peroxide detection

10:55 Discussion

11:10 Coffee break

Chair: **Frank Bier** (Potsdam, Germany)

11:30 **Till Schlotter**, T. Kloter, S. Weaver, D. Momotenko, M. Aramesh, J. Vörös, T.

O17   Zambelli, N. Nakatsuka (Zurich, Switzerland)

Cell Secretion Measurements using Force-Controlled Interface Nanopores

11:45 **Jiaxin Lian**, W. Tang, Y. Yang, S.G. Parker, V.R. Goncales, R.D. Tilley, J.J.

O18   Gooding (Sydney, Australia)

A transparent platform for cell capture and single cell isolation

12:00 **Sorour Darvishi**, H. Pick, E. Oveisi, A. Lesch, H.H. Girault (Lausanne,

O19   Switzerland)

Electrochemical imaging of E.coli biofilms using Soft-Probe-Scanning

Electrochemical Microscopy

12:15 Discussion

12:30 Lunch Break

Chair: **Ulla Wollenberger** (Potsdam, Germany)

13:40 **Florian Gerstl**, U. Pongkitdachoti, A.J. Bäumner (Regensburg, Germany)

O20   Low-cost microfludic device based on laser-induced graphene electrodes for

highly sensitive electrochemiluminescent detection of pathogens

13:55 **Dua Özsoylu**, T. Isik, M.M. Demir, M.J. Schöning, T. Wagner (Jülich,

O21   Germany; Ismir, Turkey)

Elastic electrospun polymer enabling cryo-preservation of a cell-based

biosensor chip for ready-to-use on-site applications

14:10 **Atefeh Shafaat**, F. Neilands, S. Björklund, J. Sotres, T. Ruzgas (Malmö,

O22   Sweden

Wireless sensing of biofilm formation in medically relevant bacteria based on

Ag/AgCl redox conversion

14:25 **Simon Guette-Marquet**, Ch. Roques, A. Bergel (Toulouse, France)

O23   Catalysis of oxygen electro-reduction by animal and human cells: a new

platform for cell-based biosensors

14:40 Discussion

15:00 **Poster discussion** – session 2

16:50 End of the 2nd day

Thursday 11.03.2021

08:45 Opening of the meeting room

09:00 Keynote lecture 4   Chair: **Petr Skladal** (Brno, Czech Republic)

Janos Vörös (Zurich, Switzerland)

The role of non-specific binding in determining the limit of detection of diagnostically relevant biosensors

09:25 **Mihaela Puiu**, L-G. Zamfir, V. Mirceski, C. Bala (Bucharest, Romania)

O24   Bifunctional Peptide Supports Designed for Electrochemical Signalling-off Detection of High-Molecular Weight Targets

09:40 **Zeynep Altintas**, R. Tchinda, A. Tutsch, J. Drzazgowska, B. Schmid, R.

O25   Sussmuth, (Berlin, Germany)

Computationally determined epitopes and high-affinity synthetic protein binders for biosensor applications

09:55 Discussion

10:10 **Rocio Arreguin-Campos**, K. Eersels, H. Dilien, B. v. Grinsven, T.J. Cleij

O26   (Maastricht, Netherlands)

Combined thermal and electrochemical sensor platform employing a novel surface-imprinted polymer as receptor for the real time detection of Escherichia Coli.

10:25 **Viknasvarri Ayerdurai**, A. Garcia-Cruz, M. Cieplak, P.S. Sharma, F. D`Souza,

O27   W. Kutner, (Warsaw, Poland)

Nucleobase-Functionalized Molecularly Imprinted Polymer (MIP) Electro-chemical Sensor for Determination of Heterocyclic Aromatic Amines

10:40 **Francesca Torrini**, P. Palladino, S. Scarano, M. Minunni, (Florence, Italy)

O28 A polynorepinephrine-based molecular imprinting assay targeting a small peptide hormone in doping control analysis

10:55 Discussion

11:10 Coffee Break

11:30 Keynote lecture 5   Chair: **Giuseppe Spoto** (Catania, Italy)

Can Dincer (Freiburg, Germany)

CRISPR-powered electrochemical multiplexed nucleic acid testing

11:55 **Noemi Bellassai**, M. Selvaraj, R. D'Agata, P. Greco, G. Spoto, (Catania, Italy)

O29 Quantification of circulating microRNAs biomarkers using a novel isothermal amplification method in microfluidic devices

12:10 **Christian Warmt**, C.K. Fenzel, C. Yaslanmaz, S. Zwirner, L.-M. Broweleit, J.

O30 Henkel, F.F. Bier (Potsdam, Germany)

Isothermal amplification methods as a versatile option for standard PCR in the field of lab automation and PoC testing

12:25 Discussion

12:40 Lunch Break

13:50 **Alessandro Porchetta**, M. Rossetti, F. Ricci (Rome, Italy)

O31 Programming DNA-based Sensing Platforms through Effective Molarity Enforced by Biomolecular Confinement

14:05 **Andrea Miti**, S. Thamm, P. Müller, A. Csáki, W. Fritzsche, G. Zuccheri

O32 (Bologna, Italy; Jena, Germany)

LSPR based detection based on Hybridization Chain Reaction for miRNA detection

14:20 **Anna Brunauer**, A-S. Kittel, R.D. Verboket, F. v. Stetten, S.M. Früh, (Freiburg,

O33   Germany)

       Multianalyte-Assays: Simultaneous detection of protein and nucleic acid

       biomarkers

14:35 **Julian Guercetti**, J-P. Salvador, A. Avino, R. Eritja, N. Pascual, P. Marco

O34   (Barcelona, Spain)

       DNA directed immobilization in a microarray immunoassay for multiplexed

       detection of antibiotics

14:50 Discussion

15:10 **Poster discussion** – session 3

17:00 End of the 3rd day

Friday 12.03.2021

08:45 Opening of the meeting room

09:00 Keynote lecture 6   Chair: **Gerald Urban** (Freiburg, Germany)

       Francesco Ricci (Rome, Italy)

       Electrochemical DNA-based nanodevices for IgG antibodies detection

09:25 **Julia Klüpfel**, S. Würstle, J. Mautner, M. Ungerer, U. Protzer, M. Elsner, M.

O35   Seidel (Munich, Germany)

       Automated, flow based chemiluminescence microarray immunoassay for the

       rapid multiplex detection of IgG antibodies to SARS-CoV-2 in human serum

       and plasma (CoVRapid CL-MIA)

09:40 **Michala Forinová,** A. Pilipenco, I. Visova, L. Frana, M. Houska, M. Vrabcová,

O36   J. Arnostová, V. Hönig, M. Palus, J. Sterba, J. Dostálek, N.S. Lynn, A. Dejneka,

       T. MacCulloch, N. Stephanopoulos, H. Vaisocherová-Lisalová (Prague, Czech

       Republic)

Rapid one-step quantitative detection of SARS-CoV-2 virus in crude samples using antifouling quartz crystal microbalance biosensor

09:55   Discussion

10:10   **Verónica Serafin**, A. Valverde, M. Gamella, E. Povedano, C.A. Razzino, M.
O37   Pedrero, M. Garranzo-Asensio, A. Montero-Calle, R. Barderas, M. Calero, A.O. Lobo, P. Yanez-Sedeno, S. Campuzano, J.M. Pingarrón (Madrid, Spain)
Hybrid nanostructures in dual immunosensing for determining candidate biomarkers in this century diseases

10:25   **Antonio Minopoli**, B. Della Ventura, B. Lenyk, F. Gentile, J.A. Tanner, A.
O38   Offenhäusser, D. Mayer, R. Velotta (Jülich, Germany; Naples, Italy)
Ultrasensitive plasmonic apta-immunosensor for detection of malaria biomarkers in human whole blood

10:40   **Eloy Povedano**, M. Gamella, R.M. Torrente-Rodriguez, A. Montero-Calle, M.
O39   Pedrero, G. Solis-Fernández, F. Navarro-Villoslada, R. Barderas, S. Campuzano, J.M. Pingarrón (Madrid, Spain)
Magnetic microbeads amperometric immunoplatform for the rapid and single-base sensitive detection of N6-methyladenosine residues to assist in metastatic cancer cells discrimination

10:55   Discussion

11:10   Coffee Break

                              Chair: **Laura Lechuga** (Barcelona, Spain)

11:30   **Rudolf J. Schneider** (Berlin, Germany)
O40   Environmental monitoring by high-throughput immunoanalytical methods and portable devices

11:45   **Greta Gaiani**, S. Leonardo, T. Tsumuraya, M. Rambla-Alegre, J. Diogène, C.K.
O41   O`Sullivan, C. Alcaraz, M. Campàs (Spain)
First electrochemical biosensor for the detection of ciguatoxins in fish and microalgal samples

12:00 **Claudy D´Costa**, M. Gesto, A. Heiskanen, A. Wolff, J. Emnéus (Kopenhagen,

O42   Denmark)

Online monitoring of cortisol in recirculated aquaculture systems

12:15 Discussion

12:30 Lunch Break

13:40 **Alvaro Luque-Uria**, R. Peltomaa, T.K. Nevanen, H. Arola, E. Benito-Pena,

O43   M.C. Moreno-Bondi, (Madrid, Spain)

Bioluminescent immunosensing of the immunosuppressant mycophenolic

acid with a recombinant peptide-mimetic luciferase

13:55 **Günther Proll**, P. Fechner (Tübingen, Germany)

O44   Functional immobilization of small molecules by carbene mediated photo-

linking for label-free detection

14:10 **Meryem Belkilani**, C. Farre, Y. Chevalier, A. Abdelghani, N. Jaffrézic-Renault,

O45   C. Chaix (Lyon, France; Tunis, Tunisia)

Liposome based surface plasmon resonance (SPR) sensor for mono

Rhamnolipid detection

14:25 **Siddharth S. Sahu**, P. Haag, A.E. Karlström, K. Viktorsson, R. Lewensohn, J.

O46   Linnros, A. Dev (Uppsala, Sweden)

Improved Electrokinetic Detection Of Extracellular Vesicles Through Surface

Charge Tuning

14:40 Discussion

15:00 Concluding remarks

15:10 **Poster discussion** – session 4

17:00 **Announcement of prize winners and outlook**

17:10 End of the conference

Poster register

Affinity Sensors and Biomimetica

Poster No.	Presenting Author / *Poster title*
P1	Bhargav D. Mansuriya *Enzyme-free electrochemical nano-immunosensor for early diagnosis of acute myocardial infarction*
P2	Ekin Sehit / Daniel Buchenau *Ultrasensitive nonenzymatic electrochemical glucose sensor based on gold nanoparticles and molecularly imprinted polymers*
P3	Muqsit Pirzada *Cancer biomarker detection in human serum samples using nanoparticle decorated epitope-mediated hybrid MIP*
P4	Tiziano Di Giulio *MIP based impedimetric sensor for a chronic disease marker*
P5	Sabrina Di Masi *Electrochemical sensor based on electrosynthesised ion imprinted polymeric film for Cd2+ ions determination in water samples*
P6	Dounia Elfadil *Rapid ultrasound-assisted synthesis of MIPs for sulfonamides*
P7	Marcus Menger *Binding affinity analysis and applications of DNA aptamers for therapeutic anthracyclines*
P8	Elisabetta Mazzotta *Electrosynthesis of a molecularly imprinted poly(metalloporphyrin) for the selective detection of carnosine*
P9	Patrick Severin Sfragano *An electrochemical assay based on a bicyclic peptide for urokinase-type plasminogen activator (uPA) determination*
P10	Wisnu Arfian Anditya Sudjarwo *Molecularly imprinted polymer nanoparticles for Human Serum Albumin (HSA) assay using Quartz Crystal Microbalance (QCM)*
P11	Cynthia Forier / Jean-Jacques Toulmé *Detecting pesticides with aptasensors.*

P12   Aysu Yarman

Strep-Tag II-Imprinted Polymer for the Recognition of Recombinant Proteins

Nucleic Acids and Sensing

Poster No.   **Presenting Author /** *Poster title*

P13   Lisa-Marie Broweleit

Specific and fast 16S rRNA-based detection of sepsis pathogens using PCR amplification and microarray hybridization

P14   Julián Cobos Suárez

Effect of the electrical transport properties of carbon nanomaterials applied to the design of electrochemical DNA biosensors.

P15   Kalogianni Despina

Gold nanoparticle-based biosensor for rapid liquid biopsy applications

P16   Michaela Domšicová

DNA-aptamers: a sensitive tool for detection of oncological diseases by quartz crystal microbalance.

P17   Sedigheh Falahi

A label free electrochemical biosensor for early detection of liver cancer biomarker miRNA-122, based on graphene oxide modified screen printed electrode

P18   Carolin Kornelia Fenzel

Isothermal multiplex amplification with microarray for the fast detection of multidrug-resistant pathogens in PoC-systems

P19   Dieter Frense

Improvements of an impedimetric aptamer-based biosensor for diclofenac by modifications of the aptamer used

P20   Vanessa Jungbluth

Improvement of PNA probe interactions for a sensitive SPR-based detection of gliomas-associated miRNA

P21   Raquel Sánchez-Salcedo

Non-invasive approach for the early diagnosis of prostate cancer by using an electrochemical platform

P22   Vera Shavokshina
          Poly (3,4- (1-azidomethylethylene) dioxythiophene) as an advanced interface
          for electrochemical detection of oligonucleotides

P23   Gerhard Schwaiger
          Quantification of Legionella spp. by viability heterogeneous asymmetric
          recombinase polymerase amplification (v-haRPA) on a flow-based
          chemiluminescence microarray

P24   Ivana Tomac
          Electrochemical DNA-based biosensor for the evaluation of antioxidant activity
          of some hydroxycinnamic acids

P25   Quoc Viet Vu
          One-step in-chip hybridization RPA for a fast and easy to use diagnostics
          platform

P26   Alissa Wieberneit
          Electrospun cationic nanofibers for nucleic acid extraction in paper-based
          analytical devices

Electrochemical Transduction and Electrokinetics

Poster No.   **Presenting Author /** *Poster title*

P27   Melinda David
          Detection of levothyroxine using nanostructured materials: an electrochemical
          assay

P28   Sheida Esmail Tehrani
          Enzymatic Histamine Biosensor Based On Prussian Blue-Modified 3D
          Pyrolytic Carbon Microelectrodes

P29   Aliyeh Hasanzadeh
          Design and development of electrochemical biosensors for bioprocess
          monitoring

P30   Yueh-Tien Hsu / Tzu-En Lin
          Electrochemical detection and cleaning of the contaminated contact lens by
          using scanning electrochemical microscopy (SECM)

P31   Andreas Hellmann
          Platinum black-modified microelectrodes for biomedically relevant hydrogen
          peroxide detection

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Abstracts

Keynote Lectures

Frontiers in Nanophotonic Biosensors

Hatice Altug[1] hatice.altug@epfl.ch

[1]Institute of Bioengineering, School of Engineering, Ecole Polytechnique Federal de Lausanne, Lausanne, Switzerland

Abstract: Emerging healthcare needs and initiatives, including global health crisis, personalized medicine, point-of-care applications are demanding breakthrough advancements in diagnostic tools. Biosensors play an essential role in diagnostics and bioanalytics, but traditional systems are lacking precision, affordability, and portability. Furthermore, they require long detection times, sophisticated infrastructure, and trained personnel, which limit their broader applicability. In our laboratory, we address these challenges by developing next-generation nanophotonic biosensors, bioimaging, and spectroscopy technologies. The expertise of our lab covers a variety of techniques, including nanophotonics, nanofabrication, microfluidics, surface chemistry, and data science. In particular, we exploit nanoplasmonics and metasurfaces, which can confine light below the fundamental diffraction limit and generate strong electromagnetic fields at nanometric volumes. We develop new nanofabrication methods for high-throughput and low-cost manufacturing of nanophotonic biochips. We integrate our sensors with micro/nanofluidic systems for efficient analyte trapping, sample manipulation, and automation. We also use smart data science tools with hyperspectral and bioimaging to achieve high sensor performance. In this talk, I will present some of our recent effort in these directions [1-15]. For example, I will introduce ultra-sensitive Mid-IR biosensors based on surface-enhanced infrared spectroscopy for chemical-specific detection of molecules, large-area chemical imaging, and real-time monitoring of protein conformations in aqueous environment. I will describe our effort to develop ultra-compact, portable, rapid, and low-cost microarrays and their use for early disease diagnostics in real-world settings. I will also highlight label-free optofluidic biosensors that can perform one-of-a-kind measurements on live cells down to the single-cell level, and provide their overall prospects in biomedical and clinical applications.

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Chemical and biochemical optical fibre sensing for invasive and intracellular application: past, present and future

Francesco Baldini

f.baldini@ifac.cnr.it

Institute of Applied Physics-CNR, Via Madonna del Piano 10, Sesto Fiorentino, 50019, Italy

Abstract: The general attitude of physicians is to undertake actions which can be easily tolerated by the patient and which introduce a minimum risk for the safety of the patient. From this point of view, non-invasive sensors are definitely preferable to invasive sensors. On the other hand, for several applications, the insertion of optical sensors inside the human body cannot be avoided and optical fibres has played an important role in medicine thanks to their invasive capabilities and unique performance that have allowed measurements inside the human body otherwise impracticable. At the end of the eighties, many efforts were carried out to develop an intravascular probe for the continuous monitoring of pH, partial pressure of oxygen (pO_2) and partial pressure of carbon dioxide (pCO_2). On the other hand, two main problems emerged during clinical trials on volunteers in critical care and on surgical patients: i) the so-called "wall effect" which primarily affects the oxygen detection (if the fiber tip is very close to or touches the arterial wall, it measures the tissue oxygen, which is lower than the oxygen in the arterial blood) and ii) the formation of a thrombus (clot) around the sensor tip which alters the value of all the analyte values. Even if these problems appeared to be overcome, the technology has not been able to replace standard blood gas monitoring based on intermittent blood sampling and analysis with laboratory analyzers [1]. Optical fibers sensors have been also proposed to monitor for gastro-esophageal applications and sensors for the continuous monitoring of carbon dioxide, bile, and pH in the stomach and/or in the stomach were developed, with the sensor for bile monitoring available on the market [2]. The measurement of gastric carbon dioxide can provide essential information on tissue perfusion, and its monitoring in intensive care units has been proven to be of particular relevance to patient morbidity and mortality. An optical fibre sensor using a single 600 µm glass fibre terminating with a pCO_2 probe was developed. The sensor is based on the measurement of the pH change induced by the CO_2 diffusion inside the probe, constituted by a plastic head containing the CO_2-sensitive layer consisting of an ion pair (cresol red and an organic quaternary hydroxide) dissolved in a thin layer of ethyl cellulose. The sensor was tested on critically ill patients demonstrating the superiority of the optical fibre approach with respect to the traditional one based on gastric tonometry. Bile-containing refluxes from duodenum into the stomach and into the esophagus have been demonstrated to be contributing factors to the development of several pathological conditions such as gastric ulcer, "chemical" gastritis, upper dyspeptic syndromes, and severe oesophagitis, thanks to the use of the optical fibre sensor Bilitec 2000. The sensor, which exploits the intrinsic optical properties of bilirubin, the main biliary pigment, is produced by Cecchi srl; it was distributed by Medtronic up to 2007 and now by EBNeuro. The damaging effects of acid gastroesophageal refluxes, traditionally measured with glass microelectrodes, are equally well-known. An optical fibre probe was developed for the detection of gastroesophageal pH immobilizing controlled pore glasses (CPGs) with methyl red at the distal end of 500 µm plastic fibres. Methyl red was shown to be able to cover the whole range of interest for gastric applications (1.0-8.0 pH units) after its covalent immobilization on CPG. The possibility of combining pH and bile measurement using a single fibre catheter is actually under study by reducing the dimension of the fibres for pH detection to 250 µm and integrating them in the same catheter containing the fibres for bile detection.

As for intracellular applications, tapering the fiber end down to nanometric size has given the possibility to optical fibre sensors to enter the cell and, by depositing a sensing layer on the fibre tip, to perform intracellular monitoring of specific molecules [3].

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Photoelectrochemical imaging for live cell monitoring and sensor applications

Steffi Krause, Bo Zhou, Anirban Das, Karin Hing

s.krause@qmul.ac.uk

School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London E1 4NS, UK

Abstract: Electrochemical imaging techniques are powerful tools for functional, label-free imaging of cellular processes with high resolution. This talk will focus on photoelectrochemical imaging at electrolyte/semiconductor structures that can investigate the properties of the substrate facing, basal side of living cells. The measurement technology for photocurrent imaging has primarily evolved from lightaddressable potentiometric sensors (LAPS), which are sensitive to local charges on the insulator surface of an electrolyte/insulator/semiconductor structure and can therefore be used for applications such as the measurement of local ion concentrations or for the detection of DNA binding events. Employing electrolyte/semiconductor substrates in a LAPS measurement setup for the imaging of photo-induced faradaic currents (Figure 1a) dramatically increases the functionality of this type of electrochemical imaging [1]. AC photocurrent imaging with ITO-coated glass has recently been shown to be sensitive to the surface charge of living cells [2].

Higher photocurrents, better stability and faster imaging speeds have now been achieved with hematite films as the semiconductor substrate. High-resolution LAPS imaging systems developed previously suffered from poor imaging speeds due the use of mechanical stages, while fast LAPS imaging suffered from poor lateral resolution, thereby limiting its use for the dynamic imaging of cell responses. To overcome these limitations, we have developed a novel photoelectrochemical imaging setup that combines, for the first time, fast imaging with micron resolution using an analogue micromirror combined with a precise optical setup to scan the focused laser beam across the semiconductor substrate. The new photoelectrochemical imaging system has been used to electrochemically image the basal side of living cells in culture in real time with excellent resolution and thereby monitor cell responses to a surfactant. Sample photocurrent images of B50 cells before and after exposure to 0.01% Triton X-100 (TX-100) show a clear increase of the photocurrent under the cells due to the permeabilization of the cell membrane (Figure 1c and d). Continuous mapping of the photocurrent demonstrated a recovery of the cells after the surfactant was removed, which was also confirmed by fluorescent images recorded in a parallel experiment. The advantage of the new photoelectrochemical imaging system is its capability to image multiple cells simultaneously and continuously at a microscopic level.
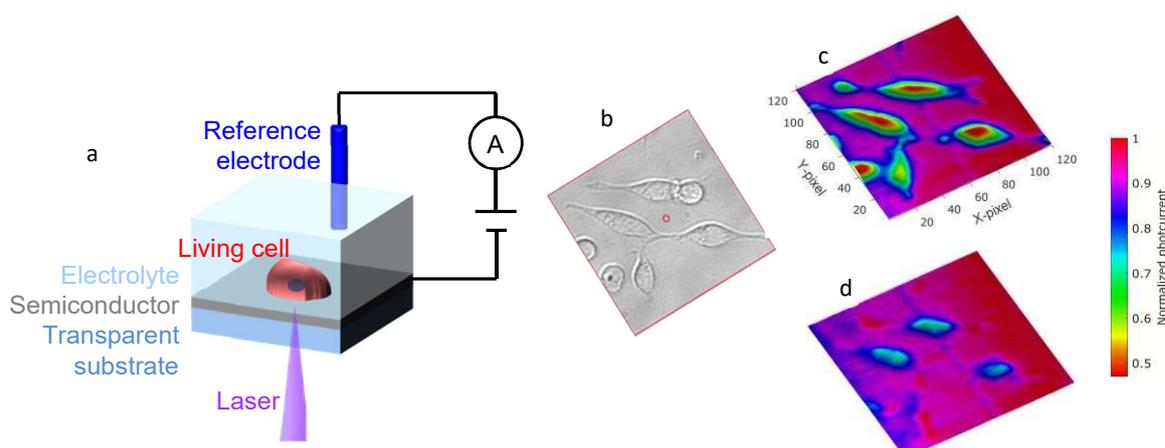
Figure 1: (a) Principle of photocurrent imaging; (b) 60 x 60 µm microscope image of B50 cells cultured on a hematite substrate; (c) Photocurrent image of cells shown in (a) in S-HEPES; (d) Photocurrent image of cells shown in (a) after exposure to 0.01% TX-100. Each 14.4k-pixel photocurrent image took 36 s to record.

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The role of non-specific binding in determining the limit of detection of diagnostically relevant biosensors

Janos Vörös voros@ethz.ch

Laboratory of Biosensors and Bioelectronics, ETH Zurich, Institute for Biomedical Engineering, Switzerland

One of the main pillars of modern diagnostics is the precise quantification of disease markers using biosensors. Non-specific binding (NSB) is the archenemy of such diagnostically relevant biosensors, because it is not only inevitable but it is also the main factor that determines the limit of detection (LOD) of most existing systems.[1] Real samples, e.g. blood, plasma, serum, saliva, synovial fluid, urine, tissue biopsy, cell lysate, etc., have many orders of magnitude higher concentration of background molecules than disease markers, which makes molecular diagnostics equivalent to "finding Nemo" among all the fish in the ocean. (Figure 1)

I will systematically introduce and define NSB, discuss the thermodynamic and molecular reasons behind it and show its consequences in diagnostics. In addition, I will provide a detailed recipe for correctly assessing the LOD of new sensor technologies and explain the most common mistakes that occur in literature.

Referencing is a common practice to deal with NSB-related problems. I will also explain why referencing is seldom a solution; and show how and why sub-micron-scale, coherent referencing is needed to substantially reduce NSB-induced and other environmental noise.[2] With the example of focal molography, I also show how diffraction based read-out along with coherent referencing enables real-time, label-free measurements of molecular binding in complex samples, such as plasma[3] or live cells[4].

Stochastic sensing deals with NSB by statistical means. Nanopore based biosensors not only provide the possibility of detecting single molecules, but the characteristic peaks in translocation events also enable their identification. Here, I will introduce a force-controlled nanopore sensor with scanning capabilities that allows analysing the content of live cells and the characterization of cell-cell communication.[5]
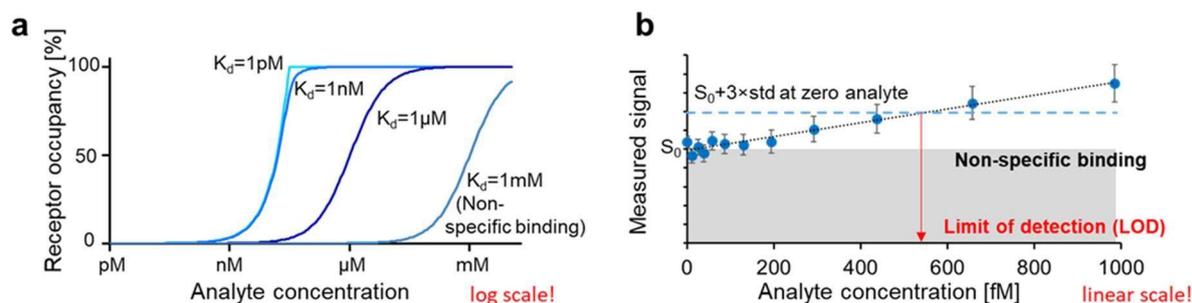
Figure 1: a) Dose response curves of a typical (ELISA) sandwich assay. The receptor occupancy saturates above the K_d value of the used receptors. b) The measured signal of typical biosensors is directly proportional to the amount of captured analytes. Here, the diagnostically relevant, linear part of a typical dose-response curve is shown along with the inevitable non-specific binding and the LOD. (Note the difference between the log and linear scale of the two curves.).

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CRISPR-powered electrochemical multiplexed nucleic acid testing

Richard Bruch[1,2], Midori Johnston[1,2], Julia Baaske[3], Wilfried Weber[3], Gerald A. Urban[2], Can Dincer[1,2]

dincer@imtek.de

[1]FIT – Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, Georges Köhler-Allee 105, 79110 Freiburg, Germany
[2]Laboratory for Sensors, Department of Microsystems Engineering – IMTEK, University of Freiburg, Georges Köhler-Allee 103, 79110 Freiburg, Germany
[3]Faculty of Biology and Signalling Research Centers BIOSS and CIBSS, University of Freiburg, Schänzlestraße 18, 79104 Freiburg, Germany

Abstract: Nucleic acid testing is fundamental for the prevention, diagnosis and treatment monitoring of many diseases. Recently, microRNAs (miRNAs), short non-coding RNAs, as biomarkers have gained substantial importance for diagnostics. The dysregulation of certain miRNA levels in human body fluids are linked to different diseases, such as various types of cancer[1]. Besides its broad application in gene editing, CRISPR technology features a powerful tool for the highly selective and sensitive detection of nucleic acids in a low-cost and easily scalable manner[2,3]. In our previous work, we introduced the first CRISPR/Cas13a-powered electrochemical microfluidic biosensor (CRISPRBiosensor) for on-site miRNA testing, without any nucleic acid amplification[4]. In this study, we expand our system for the multiplexed miRNA detection by dividing the microfluidic channel into subsections, implementing four novel chip designs of CRISPR-Biosensor X[5]. Using the sensor showing the best performance, we have successfully demonstrated the proof-of-concept of CRISPRpowered multiplexed detection of two different miRNAs (miRNA-19b and miRNA-20a) from the miRNA-17~92 cluster, which is dysregulated in the blood of pediatric medulloblastoma patients, along with their negative controls. Without any target amplification, CRISPR-Biosensor X offers a low-cost, easily scalable and multiplexed approach for on-site nucleic acid diagnostics. Our future work will include further optimization of the chip design and the clinical validation of our system.
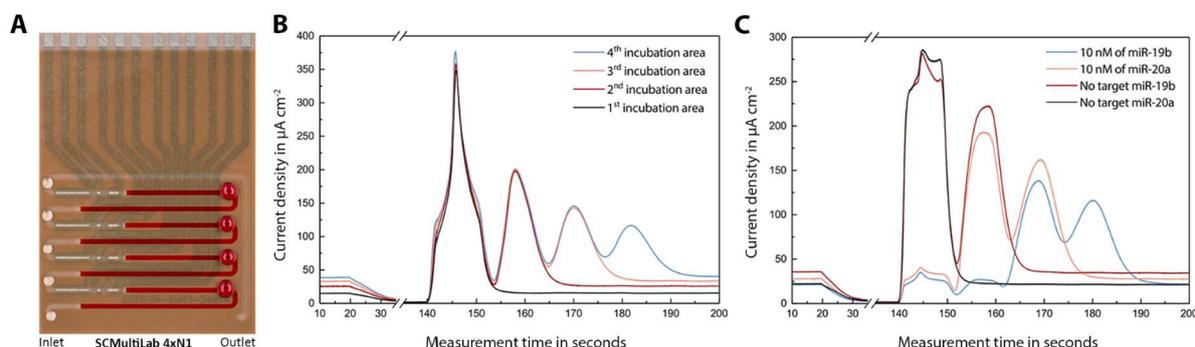
Figure 1: A) Electrochemical microfluidic multiplexed biosensor for the simultaneous measurement of 4 analytes, where each channel section (highlighted in red) has one incubation area and its own electrochemical cell. B) Chip design validation by a model assay, immobilizing 1 µg ml⁻¹ of avidin-glucose oxidase in all incubation areas. C) CRISPR/Cas13a-powered multiplexed miRNA detection of 10 nM of miR-19b and miR-20a along with their negative controls. All measurements are performed using a 2-min stop-flow protocol and at a flow rate of 5 µl min⁻¹.

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Electrochemical DNA-based nanodevices for IgG antibodies detection

Francesco Ricci[1]

francesco.ricci@uniroma2.it

[1] Dipartimento di Scienze e Tecnologie Chimiche, University of Rome, Tor Vergata, Via della Ricerca Scientifica, 00133, Rome, Italy

Abstract: DNA nanotechnology uses synthetic nucleic acid as a versatile material to rationally engineer tools and molecular devices that can find a multitude of different applications (e.g., in-vivo and in-vitro diagnostics, drug delivery, genetic circuits etc.). A wide range of DNA-based nanodevices have been reported to date that, in response to a specific molecular cue, can give a signal, release a cargo or perform a directional motion. The activity of these DNA-based nanodevices is usually triggered by a quite restricted class of molecular cues. These include environmental stimuli (like pH, temperature, etc) and chemical inputs that in the majority of cases are limited to DNA strands or small molecules and proteins. Despite their high diagnostic value as biomarkers and clinical importance as therapeutic tools it is quite surprising that only few examples have been reported to date where DNA-based nanodevices could be regulated by antibodies.

Motivated by the above considerations, I propose to exploit the "programmability" of DNA to rationally design a DNA-based electrochemical system for the detection of specific IgG antibodies. The synthetic DNA oligonucleotides act as versatile scaffolds for signalling labels and the antibody recognition elements (i.e. the antigens). The system is designed to give a current signal only in the presence of a specific IgG antibody through an antibody binding-induced increase in the effective concentrations of two antigen-conjugated DNA strands. Using this approach we demonstrate the rapid, quantitative detection of physiologically relevant, low-nanomolar concentrations of clinically relevant antibodies directly in complex clinical samples.

Abstracts

Oral presentations

Etched long period fiber gratings near turn around point for high sensitive label-free biosensing

Tanoy Kumar Dey[1], Sara Tombelli[2], Palas Biswas[1], Ambra Giannetti[2], Nandini Basumallick[1], Francesco Baldini[2], Somnath Bandyopadhyay[1], and Cosimo Trono[2]

c.trono@ifac.cnr.it (Corresponding e-mail address)

[1]Central Glass and Ceramic Research Institute, CSIR-CGCRI, 196 Raja S C Mullick Road, Kolkata 700032, India

[2]Institute of Applied Physics "Nello Carrara", CNR-IFAC, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy

Abstract: Long period fiber gratings (LPFGs) have been widely used for surrounding refractive index (RI) sensing throughout the last decades [1]. In this intrinsic optical fiber sensor the forward propagating optical core mode ($LP_{0,1}$ mode) couples with different higher order forward-propagating cladding modes at different resonant wavelengths ($\lambda\lambda_{res}$) that depend on the RI of the external medium [2]. Moreover, LPFG is able to measure the layer refractive index (LRI) variation and the thickness variation of the layer deposited on the sensor surface. For this property, LPFG is suitable for biosensing applications, where SRI remains constant (~1.33 RIU) and both LRI and thickness of the layer on the sensor surface vary due to a bio-molecular interaction. Different methodologies have been adopted to increase the sensitivity of the sensor. In this work, for the first time to the best of our knowledge, we use simultaneously two sensitivity enhancement approaches: i) design of the LPFG in order to work close to the turn around point (TAP) of the lowest possible cladding mode ($LP_{0,2}$) and ii) enhancement of the evanescent field by reducing the cladding diameter up to ~ 20 μm [3] (Figure 1 left). The sensor was characterized as RI sensor in the range RI 1.333 to 1.3335 by using NaCl water solutions. A label free immunoassay was then performed on the grating surface by using the IgG/anti-IgG interaction in a complex matrix consisting of human serum. The whole RI characterization and bioassay was performed inside a thermostated flow cell, for the temperature stabilization and for the protection and ease of handling of the sensor (Figure 1 right).
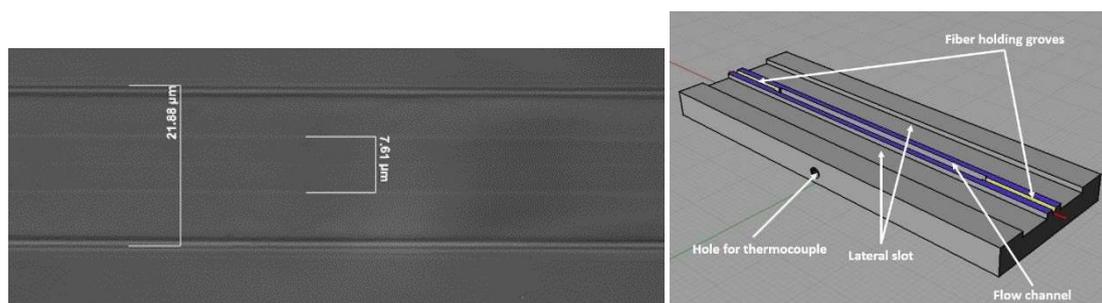
Figure 1: Left: Microscopic image of the etched fiber with 63× magnification. Right: Scheme of the upper part of the flow cell. The red line indicates the fiber, the two blue strips are the lateral borders of the flow channel and the yellow strips define the V-grooves where the fiber is glued.

The obtained bulk RI sensitivity was 8751 nm/RIU, with a resolution of 5 x 10^{-5} RIU. The bioassay limit of detection (LOD) was found out to be 0.16 ng/ml (1.06 pM). The real time kinetics of IgG/antiIgG was also observed in term of the $\lambda\lambda_{res}$ shift. We showed that the stability of the sensing apparatus and the reached sensitivity and resolution allow to obtain information not only about the reaction kinetics, but also about the fluidics behaviour.

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Flexible molecularly imprinted nanoparticles in plasmonic optical sensing

Alessandra Maria Bossi [1], Devid Maniglio [2], Nunzio Cennamo [3]

alessandramaria.bossi@univr.it

[1]University of Verona, Department of Biotechnology, Strada Le Grazie 15, 37134 Verona, Italy.
[2]University of Trento, Department of Industrial Engineering, BIOtech Research Center, Via delle Regole 101, Mattarello, 38123 Trento, Italy.
[3]University of Campania Luigi Vanvitelli, Department of Engineering, Via Roma 29, 81031 Aversa, Italy.

Abstract: Molecularly imprinted polymers (MIPs), given their exquisite selectivity, the robustness, the cost-effective synthesis, the integrability to electronics and the stimuli-responsiveness are interesting and versatile materials to exploit in optical sensing [1].

We studied the behavior of soft, protein-selective, nanometric-sized MIPs (nanoMIPs) when coupled to a metallized D-shaped plastic optical fibre (POF) [2] so to produce a nanoMIP-POF plasmonic sensing platform. The binding of the analyte was observed to led to the deformation of the nanoMIPs, that progressively increased their stiffness, as demonstrated by atomic force microscopy forcedistance curves. The optical effect of these analyte-induced nanoMIP-deformations resulted in significative plasmonic resonance shifts, so that the nanoMIP-POF attains ultralow sensitivity (attomol of analyte on the sensor) [3].

The analyte-induced nanoMIP-deformation represent a key effect to amplify the POF-plasmonic platform's sensitivity, offering an ultralow levels label-free detection.
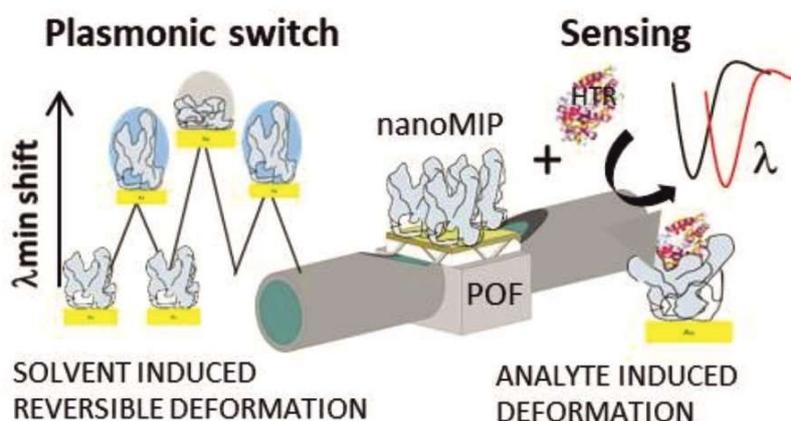
Figure 1: Soft nanoMIP coupled to the plasmonic plastic optical fiber (POF) can be deformed both by solvent, producing an optical switch, and by the analyte, providing an amplification of the optical shifts that results in ultralow detection limits (attomol on the sensor).

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Plasmonic-active nanostructured thin-films for sensing and biosensing. Towards lab-on-a-strip smart devices

Annalisa Scroccarello[1], Flavio Della Pelle[1], Dario Compagnone[1]

ascroccarello@unite.it

[1]Faculty of Bioscience and Technology for Food, Agriculture and Environment University of Teramo, Via Renato Balzarini 1, 64100, Teramo, Italy

Abstract: The daily evolution in the nanomaterials and nanotechnology science contaminates the (bio)sensoristic scenario, more and more paving the way for metal nanoparticles (MNPs) as bioanalytical tags and probes. Starting from the colloidal MNPs use in optical assays and analytical methodologies, nowadays, increasing demand for functional-MNPs immobilization/integration into point-of-care/need testing devices is on course.

Here, firstly will be presented a universal strategy to modify plastic rigid and flexible substrates (i.e., cuvettes, ELISA plates, micro-Tips, plastic pouches, etc.) with adhesive polymeric films integrating plasmonic-active gold/silver nanostructures [1]. Briefly, the strategy is composed of three steps: (i) the plastic surfaces are coated with a polydopamine film; (ii) the polydopamine layer is subsequently decorated with gold nanoparticles (AuNPs) self-assembled thanks to the catechol and amine residual moieties of the polymer; (iii) the as-produced nanocomposite film is further enriched with catechin acting as a reactive template for the formation of a plasmonic nano-silver network in the presence of silver precursor (Ag^+) [1].

To prove the nano-film potentiality, it was used as a plasmonic readout system on board of ELISA plates, for the oxidants capacity evaluation of bioanalytical interest compounds (i.e., H_2O_2, sodium hypochlorite, ABTS$\bullet^+$, etc.). In this case, the nanodecorated surface acts as an oxidative-power tester, where the oxidizing capacity of the analytes induces a proportional etching of the Ag nanonetwork, resulting in reducing localized surface plasmon resonance with consequent color changing, which is used as analytical signal. The proposed nano-decorated film allowed micromolar LODs, resulting selective and accurate also in complex media and presence of enzymes [1]. The developed bimetallic nanocomposite film proved to be robust and storable for several weeks, fully retaining the analytical performances, resulting particular useful for biosensoristic applications.

In our group's ongoing studies, nanodecorated films will represent the sensing elements of innovative point-of-need devices, in which micro-patterning of channels, reservoirs, and sampling-zone will be obtained fully by straightforward benchtop instruments by using low-cost flexible substrates (e.g, plastic sheets, paper, etc.). In particular, will be presented the preliminary results of a flexible device, in which the nanofilm act as the sensing element and the smart-design allows auto sampling. The proposed reagent-free 'analytical read-out system', aims to allow an in-situ oxidant-disinfectant agents monitoring, in the context of the SARS-CoV-2 pandemic management. The latter device aims to become a straightforward analytical tool for monitoring environmental disinfection treatments, able to return analytical-grade results just by a smartphone, resulting in inexpensive, portable, and within everyone's reach.

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Long period fiber grating-assisted optical biosensing for the realtime detection of C-reactive protein in serum

Francesco Chiavaioli[1], Flavio Esposito[2], Lucia Sansone[3], Anubhav Srivastava[2], Stefania Campopiano[2], Michele Giordano[3], Ambra Giannetti[1], Agostino Iadicicco[2], Francesco Baldini[1]

f.chiavaioli@ifac.cnr.it (Corresponding e-mail address)

[1]Institute of Applied Physics "Nello Carrara", National Research Council of Italy (CNR), 50019 Sesto Fiorentino, Italy; [2]Department of Engineering, University of Naples "Parthenope", 80143 Napoli, Italy; [3]Institute for Polymers, Composites and Biomaterials, National Research Council of Italy (CNR), 80055 Portici, Italy

Abstract: The development of biosensors able to guarantee simple, rapid and *in-situ* monitoring of molecular interaction is quite an actual topic, as occurring for SARS-CoV-2 infection. The commercially-available optical system for the detection of inflammatory biomarkers, such as Creactive protein (CRP), is based on the widespread ELISA method, with a limit of detection (LOD) of about 10 pg/mL. Other approaches rely on different techniques, such as immunoturbidimetric or immunonephelometry assay attaining a LOD of 0.03 µg/mL and 0.2 µg/mL, respectively [1]. The advantages of optical fibers, especially in light management, combined with nanotechnology can offer superior performances over other sensing platforms [2]. Different optical fiber biosensors have been proposed for the detection of CRP [3]. Among them, those based on surface plasmon resonance (SPR) are widely employed, attaining a LOD of 0.009 µg/mL using plastic optical fibers [4]. Here, we propose the development and testing of a label-free fiber-optic biosensing platform assisted with long period grating (LPG) for the real-time detection of CRP in serum [5]. It is based on the inscription of an LPG into a novel double cladding fiber (DCF), with a specific refractive index profile (Fig. 1a). A slight chemical etching of the fiber surface is used to optimize the device sensitivity taking the advantages of mode transition. A thin layer of graphene oxide (GO) is then deposited around the LPG region, to provide functional groups for the covalent immobilization of the biological recognition element. A large working range of CRP concentrations of clinical relevance (1 ng/mL – 100 µg/mL) is covered and a low LOD of 1.1 ng/mL is also attained with CRP spiked in serum (Fig. 1b).
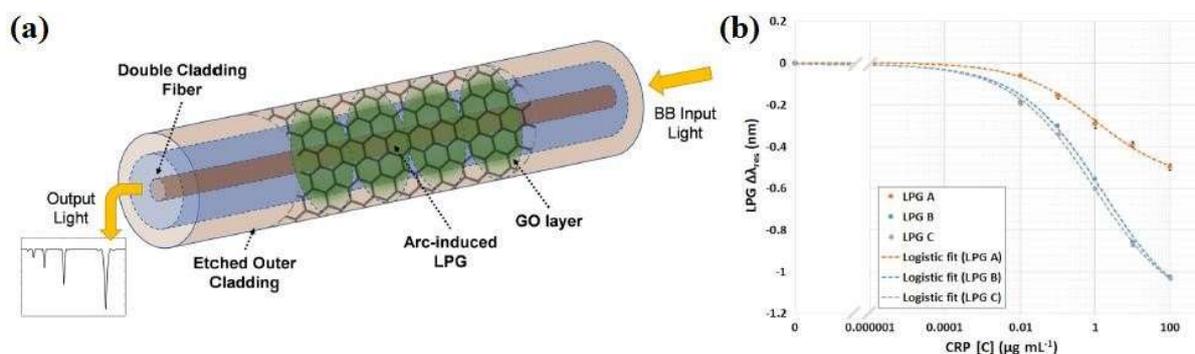
Figure 1: (a) Schematic view of the optical transducer based on LPG in DCF coated with GO. (b) Semi-log calibration curves achieved with different devices (LPG-A, orange; LPG-B, blue; LPG-C, grey). Each concentration of CRP (circles) is expressed in terms of the mean value and the respective standard deviation and the dashed curves represent the Logistic fitting function.

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A novel high-precision optical wound healing assay to analyze 2D cell migration

Stefanie Michaelis[1], Saphia Azzam[2], Maren Klein[2], Zeno von Guttenberg[3], Lea Tomasova[3], Joachim Wegener[1,2]

stefanie.michaelis@emft.fraunhofer.de

[1]Fraunhofer Research Institution for Microsystems and Solid State Technologies, EMFT, Cell-Based Sensors, Universitätsstr. 31, 93053 Regensburg, Germany

[2]Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitätstr. 31, 93053 Regensburg, Germany

[3]ibidi GmbH, Lochhamer Schlag 11, 82166 Graefelfing, Germany

Coordinated cell migration plays a key role in many physiological and also pathological processes. Among the most important forms of pathological migration is the dissemination of metastasizing tumor cells. Consequently, a detailed understanding of cell migration, its regulatory mechanisms and potential interventional strategies are of crucial importance in both basic and applied research. Cell migration is most commonly studied in a controlled laboratory environment using so-called *wound healing assays* based on using cultivated cells (*in vitro*). In such assays, a defined lesion (wound) is experimentally introduced into a continuous cell layer. Cells from the periphery of the wound can migrate into this lesion, documented by time-lapse video microscopy. The existing, established assays are functional but show a lack of automation or reproducibility in particular with respect to the dimensions of the wound. The latter is often crucial to the reproducibility of the entire assay.

A novel concept uses the precision of an optical microscope to generate highly reproducible lesions in a continuous cell layer down to the single cell level. The core component is a thin, light-sensitive yet biocompatible coating applied to an inert, transparent carrier material. When cells grown upon this composite material are exposed to visible light of a suitable wavelength, the light-sensitive layer generates highly toxic singlet oxygen that locally kills the cells growing on the surface of the substrate at the site of illumination. Vital cells from areas outside the field of view then grow into the wounded area, which is documented microscopically.
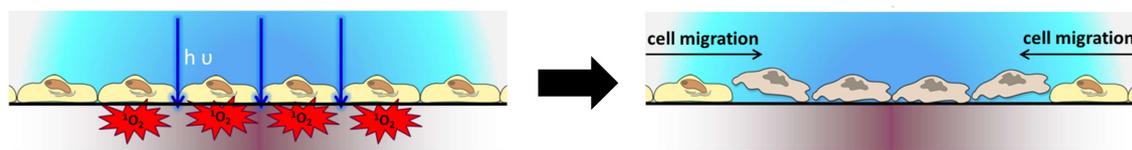
Figure 1: Principle of the optical wound healing assay. Cells are grown upon a light-sensitive composite material. Upon illumination with light of a suitable wavelength, highly cytotoxic singlet oxygen is generated, that locally kills the cells at the site of illumination.

The contactless introduction of a defined wound to a cell layer provides the basis for a wound healing assay with a high degree of automation and parallelization. In particular, the precision of the wound and its independence of the skills of the person conducting the experiment will significantly improve the reproducibility of this assay, making automation possible in the first place. Moreover, the concept allows application of optical wounding even in closed geometries like microfluidic devices as used in organ-on-chip approaches.

Towards Simultaneous Neurochemical Sensing and Electrophysiology *via* Aptamer-Based Bioelectronics

Nako Nakatsuka[1], Alix Faillétaz,[1] Krishna Vadodaria,[2] Ufuk Ilgin,[1] Fred H. Gage,[2] Dmitry Momotenko,[1] and Janos Vörös[1]

nakatsuka@biomed.ee.ethz.ch

[1]Laboratory of Biosensors and Bioelectronics, ETH Zurich, Institute for Biomedical Engineering, Zurich 8092, Switzerland
[2]Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA 91037, U.S.A.

Abstract: Fundamental understanding of human brain function necessitates biosensors that can probe neuronal communication at spatiotemporal scales that approach the resolution of information encoding. Neuronal signaling consists of both electrical and chemical interactions. To monitor the electronic action potentials from spiking neurons, we have developed precisely patterned *in vitro* neuronal networks on microelectrode arrays.[1] To extend our capability to measure the chemical flux that accompanies electrical firing between neurons simultaneously, we harnessed the molecular recognition mechanism of conformationally changing DNA aptamers (Figure 1). Aptamers are systematically designed oligonucleotide receptors that exhibit highly specific and selective recognition of targets. Aptamers that recognize small-molecule neurotransmitters, including serotonin and dopamine, have recently been isolated.[2] Upon reversible target binding, these aptamers undergo a rearrangement of the negatively charged backbone and these dynamic structural changes can be transduced as measurable electronic signals. We functionalized aptamers to the inner surface of quartz nanopipettes with ~10 nm pore openings. The ionic current flowing through the nanopipette under an applied bias was measured. Nanoscale confinement of ion fluxes, analyte-specific changes in molecular conformation of aptamer species, and related surface charge variations enabled highly sensitive detection of neurotransmitters, even in complex environments. We demonstrated the capacity to detect physiologically relevant differences in neurotransmitter amounts in media collected from human induced pluripotent stem cellderived neuron cultures. Validation of small-molecule detection in parallel with mechanistic investigations, demonstrates the potential of conformationally changing aptamer-modified nanopipettes as translatable nanotools for diverse biological systems.
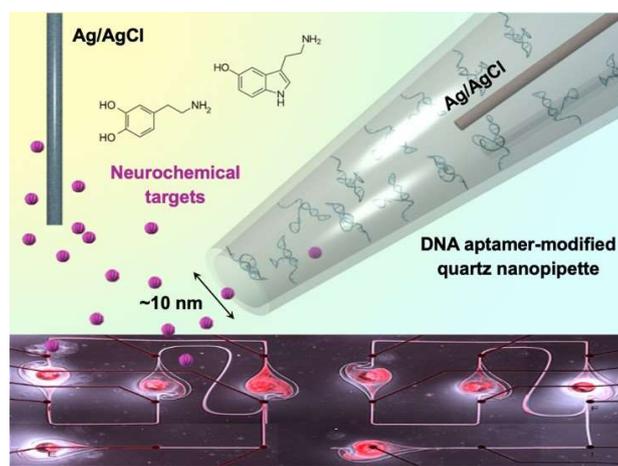
Figure 1: Neurochemical sensing in tandem with electrophysiology using microelectrode arrays on which live neurons are patterned. Quartz nanopipettes with nanoscale openings are modified with DNA aptamers that undergo conformational change upon target recognition for electronic signal transduction of neurochemical flux.

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Multiplexed redox gating measurements with a microelectrospotter. Towards electrochemical readout of molecularly imprinted polymer microarrays

Eszter Supala, Luca Tamás, Júlia Erdőssy, Róbert E. Gyurcsányi

supalaeszter@gmail.com

BME "Lendület" Chemical Nanosensors Research Group, Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Szt. Gellért tér 4, H-1111, Budapest, Hungary

Molecularly imprinted polymers (MIPs) emerged as alternatives of antibodies for selective protein recognition with clear advantages in terms of robustness and tailorable chemical synthesis. During the synthesis of MIPs, functional monomers prearrange around the template proteins and this structure is then fixed by the polymerization. The removal of the template leaves behind complementarily shaped and functionalized binding sites, which can be used to selectively detect it.

To overcome the main challenges in protein-imprinting, i.e. the slow diffusivity of the macromolecules in the polymer matrix and structural damage, surface imprinted nanofilms with thicknesses comparable with the diameter of the macromolecular templates (< 10 nm) can be electrosynthesized in aqueous media onto a solid support. [1]

Earlier we showed that MIP nanofilm microarrays can be electrosynthesized with the microelectrospotter for the surface plasmon resonance imaging (SPRi) coupled, high-throughput investigation of protein MIPs. [2] We realized however, that the microelectrospotting method can offer the multiplexed redox readout of these microarrays as well. As a proof of concept study, IgEimprinted polyscopoletin nanofilm microarrays were electrosynthesized with the microelectrospotter on gold SPRi chips. The concentration dependent binding of the target protein on the polymer spots was then followed by the suppressed electron-transfer of the ferrocenecarboxylic acid redox marker. As confirmation of the results, rebinding was followed with SPRi as well. The preliminary results suggest that microelectrospotting can provide a simple, cost-effective alternative to SPRi for the highthoughput readout of protein microarrays with results that are less influenced by out-of-cavity nonspecific binding. [3]

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Disposable paper-based biosensors for continuous remote sensing

Milad Eyvazi Hesar[1], Niloofar Seyedsadrkhani[1], Dibyendu Khan[1], Mateusz Piekarski[1], Sven Ingebrandt[1]

milad.eyvazi.hesar@rwth-aachen.de (Corresponding e-mail address)

[1]Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Sommerfeldstr. 24, 52074 Aachen, Germany

Abstract: In this paper, a low-cost disposable paper-based chemical sensor is implemented as an independent module for a modular near-field communication patch [1].

Limited supply of biosensors prevents efficient screening and control of the rapid transmission of diseases in the entire demographics. An inexpensive modular patch with a disposable electrode layer will provide us with an opportunity of rapid and efficient testing with minimal costs. It also provides more flexibility as a point-of-care testing unit and minimizes human contact, which is the core of reducing the rate of spread of the diseases. In this respect, we developed a chemical sensor based on disposable paper-based electrodes as independent module of a modular near-field communication based patch reported earlier [1]. The processing of the data and transmission is carried out by an ultralow power MCU using near field communication protocol [1]. The power required for operation of the patch is entirely harvested from the energy transmitted over RF from the reader. The circuit layer of the patch also utilizes a voltage regulator to supply regulated voltage to the electrode layer. Here, the MCU is programmed to record and transfer 256 samples per seconds.

The sensing electrodes were made with a very hydrophilic sensing area and the remaining nonsensing areas were passivated with wax paper. The electrodes were designed as interdigitated electrodes (IDEs) in a CAD software and then laser printed on a filter paper as a hydrophilic substrate. Then, this filter paper was laminated at 100°C with a very thin layer of copper/gold (4 μm) on top of it. Subsequently, the excess copper, which was not sticking to the polyester particles of laser toner outside the printed area, was wiped out. Then, this stack was aligned and sandwiched with wax papers from bottom and top for passivation. The top wax passivation layer was patterned beforehand by carving out the openings for the IDEs and contact pads from the wax paper using a conventional desktop cutter. These electrodes were connected with Z-axis conductive tape to the electronic readout system, which is embedded in the other layers of the modular patch. The metallic IDEs remaining after peeling off can be used for sensing concentration of different analytes in body fluids such as ion concentrations, glucose and lactate [2].
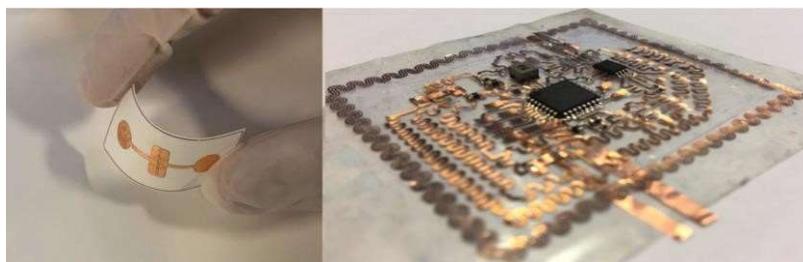

Figure 1: Disposable paper-based biosensing using the NFC-enabled patch. General purposed NFC-enabled patch was utilized to record signal from a disposable paper-based electrode structure in order to measure the concentration of electrolytes and glucose.

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Smartphone assisted PNA-based printed electrochemical biosensor for non-invasive coeliac disease diagnosis

Veronica Caratelli[1], Maria Moccia[2], Concetta Avitabile[2], Michele Saviano[2], Anna Lisa Imbriani[3], Danila Moscone[1], Fabiana Arduini[1,4]

Veronica.caratelli@uniroma2.it

[1] Department of Chemical Science and Technologies, University of Rome "Tor Vergata", Via Della Ricerca Scientifica 1, 00133, Rome, Italy
[2] Department of Chemical Sciences and Materials Technology, Institute of Crystallography, National Research Council, Via G. Amendola 122/O, 70126, Bari, Italy
[3] Biochemical Systems International S.p.A. Loc, Palazzo del Pero, 23, 52100, Arezzo, Italy
[4] SENSE4MED, Via Renato Rascel 30, 00133, Rome, Italy

Abstract: The Coeliac disease (CD) is a chronic inflammatory disorder of the small intestine, produced by the ingestion of dietary gluten products in susceptible people. It is a multifactorial disease, including genetic and environmental factors, which affects at least 1% of the population and evidence suggests that prevalence is increasing [1]. Villi biopsy is the gold standard method requiring an invasive procedure for the diagnosis of CD [2]. For this reason, in the past few years the attention was focused on alternative methods and techniques for a non-invasive fast and cost-effective diagnosis. Herein, we propose a miniaturised electrochemical biosensor connected to a smartphone assisted portable potentiostat able to detect a selected sequence of miRNA, recognised as biomarker in serum for celiac disease. The screen-printed electrode modified with gold nanoparticles was used as platform to immobilise the ad-hoc synthetised PNA probe for the selective detection of miRNA sequence. The electrochemical detection of miRNA was carried out using differential pulse voltammetry technique and ruthenium hexamine as electrochemical probe. The screen-printed electrode modified PNA sensing platform was able to detect the selective miRNA sequence in serum sample obtaining a linearity comprised between 10 and 100 nM with an association constant equal to 28 ± 6 nM and a limit of detection of 0.7 nM. The device was also tested in terms of selectivity measuring different concentrations of a fully random sequence. The presence of this non-complementary sequence had not a significant effect on the signal variation demonstrating the suitability and selectivity of the platform.

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Simultaneous online monitoring and sterility assurance in aseptic food processing based on a combined chemical/biosensor

Farnoosh Vahidpour [1], Torsten Wagner [1,2], Michael J. Schöning [1,2] vahidpour@fh-aachen.de

[1]Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, Heinrich-Mußmann-Straße 1, 52428 Jülich, Germany
[2]Institute of Biological Information Processing (IBI-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Abstract: Hydrogen peroxide is one of the most used sterilants for packaging in aseptic filling machines [1]. To validate the sterilization of the packages, spores (resistant microorganisms against the sterilant) are exposed to the sterilant, their kill rate is determined and the sterilization is concluded.

However, such method has the drawback of tedious physical work and slow response, as the results can be obtained only after 48 hours. To overcome this issue, a novel sensing platform, namely a spore-based biosensor has been previously introduced [2]. In this work, such spore-based biosensor is combined with a calorimetric H_2O_2 gas sensor on a single chip to evaluate the viability of spores and to determine gaseous H_2O_2 concentration and temperature. Here, interdigitated electrodes were operated as impedimetric sensors (see Fig. 1) at the top part of the sensor chip. Additionally, two meander structures were utilized as temperature sensors (see Fig. 1), at the bottom part of the sensor chip. A differential measurement set-up was used for both the calorimetric gas sensor and the sporebased biosensor. As for the former case, upon exposure to H_2O_2, a temperature difference between both temperature sensors could be correlated to the H_2O_2 concentration. Similarly, on the spore-based biosensor, changes in the morphology of the spores could be correlated to the viability of spores, after exposure to H_2O_2.

The calorimetric gas sensor responds to different concentrations of gaseous H_2O_2 with a linear dependence. The biosensor was investigated with different types of spores. Signal responses (impedance) of the spore-based biosensors at different, applied H_2O_2 concentrations were studied, also showing a correlation between H_2O_2 concentration and the spores' impedance. Figure 1 represents a schematic alteration of the impedance and calorimetric signals with regard to the H_2O_2 concentration. In conclusion, the synergy of these two sensor types as one combined sensor array allows a

considerably more specific multi-parameter-based fault indication in aseptic filling machines in comparison to isolated microbiological state-of-the-art- or hydrogen peroxide detection methods.
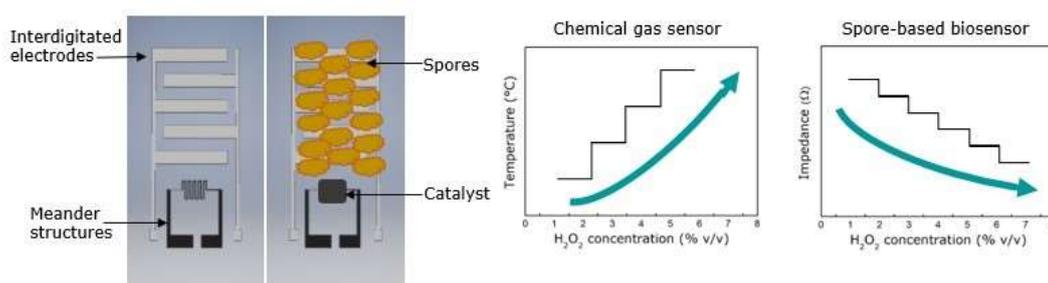
Figure 1: Combined sensor array consisting of a calorimetric H_2O_2 sensor and a spore-based biosensor (left) with their respective calorimetric and impedance responses (middle and right).

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Magnetic materials for electrodes and nanoparticles and their applications in biosensing

Victor C. Diculescu, Madalina M. Barsan, Teodor A. Enache, Elena Matei, Monica Enculescu, Nicoleta Apostol, Ionut Enculescu

victor.diculescu@infim.ro

National Institute of Materials Physics, Atomistilor 405A, Magurele, Romania

Abstract: The objective of this research was a new, simple architecture for the immobilization of biomolecules through magnetic forces. The methodology is based on the fabrication of metallic electrodes with magnetic properties where magnetic metallic nanoparticles can directly interact, without the use of an applied external magnetic field. In these conditions, it is envisioned that the magnetic electrodes play a dual role: as immobilization support for biological molecules and as transducers of the biological reactions, while, at the same time, the magnetic metallic nanoparticles can act as carriers for the biomolecules toward the immobilization support.

Both magnetic electrodes and nanoparticles were fabricated by simple and cost-effective methods: nickel electrodes by electroplating and nickel nanoparticles by the chemical reduction of a nickel salt. On another hand, palladium was employed for doping the nickel electrodes surface, in order to use the electrocatalytic properties of Pd in synergy with the magnetic properties of Ni for biosensors development.

For the magnetic characterization of the nickel electrodes and nanoparticles, SQUID measurements (with the superconductive quantum interference device) and micromagnetic simulations were performed. At the same time, structural characterization of the nickel electrode surface and nickel nanoparticles was performed by X-ray diffraction (XRD), the morphology investigated by scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX), while their chemical composition was determined by X-ray photoelectron spectroscopy (XPS).

Cyclic voltammetry and electrochemical impedance spectroscopy measurements were performed in order to determine the nature of the electrochemical process at Ni and Pd doped Ni electrodes. Both electrodes were applied as electrochemical sensors for: i) the detection of H_2O_2 in aqueous media, and ii) immobilization of the model enzyme glucose oxidase (GOx) functionalized with NiNP solely through magnetic interactions. The performance of the biosensor for glucose determination was investigated by fixed potential amperometry. The biosensor was able to operate at +0.05 V (vs. Ag/AgCl) with practically no interferences.

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Light-triggered biocatalytic reactions for multiplexed sensing

Marc Riedel [1], Adrian Ruff [2], Wolfgang Schuhmann [2], Fred Lisdat [1], Felipe Conzuelo [1]

felipe.conzuelo@rub.de

[1]Biosystems Technology, Institute for Life Sciences and Biomedical Technologies, Technical University Wildau, Hochschulring 1, D-15745 Wildau, Germany

[2]Analytical Chemistry – Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany

Towards the development of (bio)sensing platforms, the possibility for on-demand triggering of reactions provides advanced capabilities and possibilities for the simple fabrication of devices able to perform multiplexed detection of analytes. A particularly suitable approach in this regard is the use of light as stimulus to activate electrochemical reactions of interest. Rationally designed photoelectrochemical sensors can be used in combination with light-directed stimulation, making the spatially resolved analysis of sensing surfaces by sequential illumination of specific areas possible. As it has been shown before, the integration of redox enzymes with quantum dot (QD)-sensitized electrode architectures brings together the light switchable features provided by the semiconducting nanoparticles with the selectivity of the biocatalyst used as biorecognition element [1-3].

Photoelectrochemical detection of two model analytes has been realized as a proof-of-concept by means of a locally positioned light source [4]. The investigated bio-sensing platform consists of a three-dimensional inverse opal (IO)-TiO_2 structure modified with PbS QDs via a successive ionic layer adsorption reaction (SILAR) approach. As biocatalysts, the enzymes flavin adenine dinucleotidedependent glucose dehydrogenase (FAD-GDH) and lactate oxidase (LOX) are used for the detection of glucose and lactate, respectively. The enzymes are embedded in an Os-complex modified redox polymer (P-Os) enabling electrical wiring of the redox enzymes. In this way, illumination of the assembly results in the generation of electron-hole pairs at the QDs leading to the oxidation of polymer-bound Os-complexes by the generated holes and the concomitant injection of high energy electrons into the electrode substrate, resulting in the occurrence of an anodic photocurrent. Furthermore, enzymatic turnover enables amplification of the photocurrent response by the transfer of electrons from the enzymatic cofactor to P-Os. As a result, the enzyme substrate can be detected and quantified (Fig. 1a). Precise control of the light source positioned in close proximity to the biosensing platform allows the activation and subsequent read-out of the light-induced reactions as a function of the illuminated area (Fig. 1b).
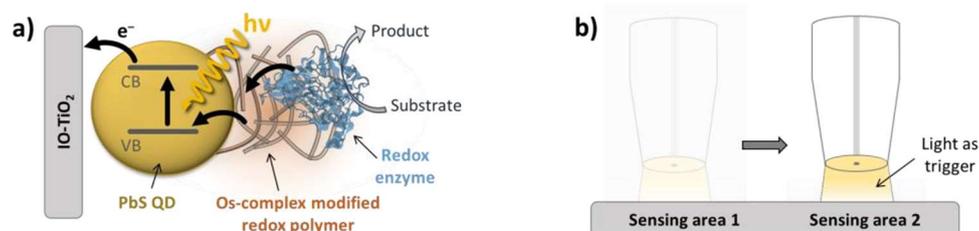

Figure 1: a) A quantum dot (QD)-sensitized inverse opal (IO)-TiO_2 electrode makes the light-control of biocatalytic reactions possible (CB: conduction band, VB: valence band). b) Local illumination enables the analysis of different modified surfaces on demand.

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A self-powered glucose sensor with optical readout

Jana M. Becker [1], Tim Bobrowski [1], Felipe Conzuelo [1], Adrian Ruff [1], Wolfgang Schuhmann [1]

Jana.Becker@rub.de

[1]Analytical Chemistry – Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Universitätsstraße 150, D-44780 Bochum, Germany

Due to its crucial role as main energy source, glucose levels are heavily regulated in the body. Therefore, a metabolic disorder like diabetes, which is linked to elevated blood glucose levels, can cause serious health issues for the people affected. Hence, on-site monitoring of the glucose levels using easy-to-apply point-of-care devices is highly desired. For this reason, substantial efforts have been put into the creation of miniaturized low-cost point-of-care devices[1], which can be handled by the patients themselves.

In this context, we report the fabrication of a self-powered glucose sensor with an optical readout system for simple and fast analyte detection with the bare eye. The sensing device consists of a glucose-powered biofuel cell compartment and an electrochromic reporter enabling the optical readout, both immobilized on a single screen-printed electrode chip (Figure 1). A glucose/ O_2 biofuel cell is used in which glucose is oxidized to glucono-δ-lactone by the oxygen-insensitive enzyme pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) that is wired to the electrode surface by the low potential redox polymer poly(1-vinylimidazole-co-allylamine)-[Os(dimethoxy-bipyridine)$_2$Cl]Cl[2]. A bilirubin oxidase (BOD)-based biocathode is used in direct electron transfer mode for the reduction of O_2 to H_2O. The biofuel cell is connected to the compartment for optical readout, which consists of an electrochromic window that gets oxidized by the glucose-limited current flow. Thereby, the color of the electrochromic reporter changes, which can be detected with the bare eye. Using the power output generated by the biofuel cell to oxidize the electrochromic film any necessity for external powering devices is excluded. Thus, the complexity of the system is reduced.
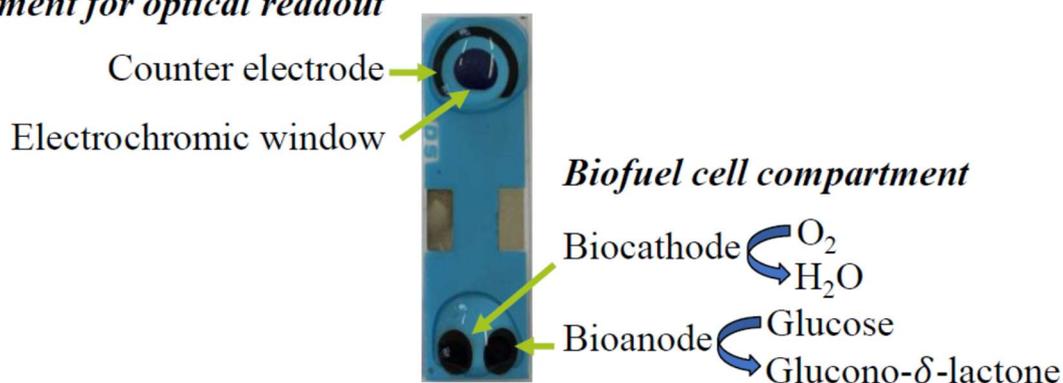
Figure 1: Representation of the modified screen-printed device for glucose detection with integrated biofuel cell coupled to an electrochromic reporter for optical readout. The electrodes assigned to both the biofuel cell compartment and the compartment for optical readout, as well as the involved key reactions, are indicated.

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Bioelectrocatalysis by PQQ-Glucose Dehydrogenase Enhanced by Electropolymerized Azines for Advanced Biosensors

Alexey K. Orlov[1], Andrew A. Galushin[2], Arkady A. Karykin[2], Maria A. Komkova[2]

Aleksei.Orlov@student.msu.ru

[1]Faculty of Material Sciences, Moscow State University, Moscow, Leninskiye Gori 1, 119234, Russia
[2]Chemisty Department, Moscow State University, Moscow, Leninskiye Gori 1, 119234, Russia

Abstract: Direct bioelectrocatalysis requires proper enzyme orientation, providing efficient communication between its active site or electron transport chain and the electrode surface [1]. We suggest conductive polymers of azine dyes for PQQ-Glucose Dehydrogenase (PQQ-GDH) wiring. On the one hand, displaying affinity to the active site, these polymers are expected to anchor the enzyme and facilitate electron transfer. On the other hand, rather low redox potentials of polyazines would allow to avoid interfering of reductants. Moreover, compared to biosensors based on freely diffusing azine dyes with inherently low operation stability [2], the use of polymer would provide advanced operational stability of the corresponding biosensors.

In order to check orientation effect of the polyazines on the PQQ-GDH we electropolymerized Azure A, Methylene Blue, Methylene Green, Coomassie Brilliant Blue and Toluidine Blue in cyclic voltammetry mode [3]. PQQ-GDH was immobilized on the polyazinesmodified electrodes by adsorption.

In the present study, characteristics of the developed biosensors were investigated in chronoamperometry mode. On an unmodified graphite electrode the catalytic currents were 0.2 $\mu A \cdot cm^{-2}$, that is more than 70 times lower than on biosensor based on poly(methylene blue). At the same time for poly(methylene green) electrocatalytic currents were 170 $\mu A \cdot cm^{-2}$ already at 0.0 V vs. Ag/AgCl, that is more than 10 times higher than for poly(methylene blue) and 700 times higher than on unmodified biosensor. The amount of adsorbed enzyme was the same in both cases. This value was estimated by the limiting catalytic currents in the presence of phenazine metasulfate. Accordingly, these studies allow us to conclude that poly(methylene green) exhibits an orienting effect in relation to the enzyme protein. Wherein, the maximal current for the biosensor based on poly(methylene green) – up to 220 $\mu A \cdot cm^{-2}$ - is only 5 times lower, than that in the presence of diffusing mediator phenazine methosulphate.

The use of polymer of azine dyes increases the operational stability. Thus, after 200 injections of 5 mM glucose solutions, the chronoamperometric responses reached 70% of the initial response.

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PQQ-Glucose Dehydrogenase-Calmodulin Chimera Enzyme: Different Triggered Activation for Multipurpose Biosensors

Paolo Bollella[a,d], Artem Melman[a], Zhong Guo[b], Selvakumar Edwardraja[c], Wayne Johnston[b], Kirill A. Alexandrov[b], Evgeny Katz[a]

pbollell@clarkson.edu

Department of Chemistry and Biomolecular Science, Clarkson University, Potsdam, NY 13699–5810, United States
[a]CSIRO-QUT Synthetic Biology Alliance, Queensland University of Technology 2 George st, Brisbane, 4001,QLD,Australia
[b]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, 4072, Australia
[c]Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", 70125 Bari, Italy

In the last decade, the rise of synthetic biology has driven the efforts to construct artificial allosteric protein switches in order to detected and quantify natural and artificial chemistries in vitro and in vivo. Typically, this involves construction of chimeric enzymes via insertion of a regulatory receptor domain into the biocatalytic reporter domain. Construction of such chimeric enzymes utilizes the recombinant DNA technology that is a core technology of protein engineering [1].

Herein, we report on the bioelectrocatalytic properties of pyrroloquinoline quinone-dependent glucose dehydrogenase fusion with calmodulin (PQQ-GDH-CaM). This protein is catalytically inactive in its ground form but can be activated by the addition of calmodulin binding peptide that induces its conformational transition and activation, as shown in Figure 1. The PQQ-GDHCaM was immobilized onto highly porous gold (hPG) produced electrochemically [2] by using a bifunctional linker, namely 4-mercaptobenzoic acid further activated through 1-ethyl-3-(3dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide (EDC/NHS) coupling to covalent bind the PQQ-GDH-CaM chimeric enzyme [3].

At first, we characterized the switchable features of PQQ-GDH-CaM by simply exposing it to the activating peptide. Subsequently we devised an approach to use proteolytic activation of a caged-peptide to generate activating peptide for PQQ-GDH-CaM. Furthermore, the system was utilized to study different pathways for PQQ-GDH-CaM triggered activation according to different logic gates in order to realize multipurpose biosensors (e.g., glucose detection, peptide detection, rapamycin etc.) [4].
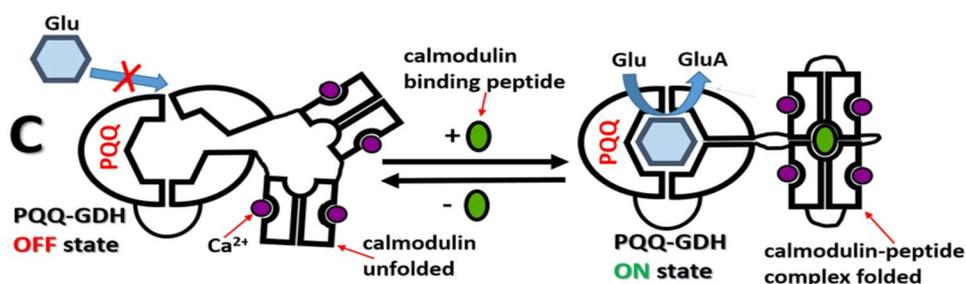

Figure 1. Activation mechanism of PQQ-GDH-CaM by the addition of calmodulin binding peptide

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Enzyme activity determination of human monoamine oxidase B (Mao B) by amperometric hydrogen peroxide detection

Soraya Höfs[1,*], A. Talke[2], F. Lisdat[1]

soraya.hoefs@bam.de, flisdat@th-wildau.de

[1] Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Hochschulring 1, 15745, Wildau, Germany

* present address: Department of Analytical Chemistry; Reference Materials, Bundesanstalt für Materialforschung and-prüfung (BAM), Richard-Willstätter-Straße 11, 12489 Berlin, Germany

[2] Biotez GmbH, Berlin Buch, Germany

Parkinson's disease (PD) is one of the most common neurodegenerative disorders worldwide. About 0.3 % of the global population and approximately 2 % of people older than 80 years are affected by PD[1]. Monoamine oxidase B (Mao B) is an enzyme, which is a drug target in Parkinson's disease (PD), since it is involved in dopamine metabolism. Several Mao B inhibitors are well established as medication for PD patients. However, the medical treatment is only little personalized since the monitoring of the patients Mao B activity is complex and requires sophisticated laboratory equipment. Here a sensorial Mao B activity determination system has been developed which has potential in the personalization of the medical PD treatment. The enzyme activity is quantified by amperometric detection of enzymatically produced H_2O_2 [2]. Therefore, the enzyme is enriched from the solution via cellulose particles which are functionalized with antibodies against human Mao B. The successful capturing of the enzyme can be verified by SDS-PAGE. For activity determination the enzyme is brought in contact with a suitable substrate - here benzylamine. Selectivity of the amperometric hydrogen peroxide detection in the presence of co-reactants has been verified. Within the time span of 30 min, a linear dependency of enzymatically produced H_2O_2 with the substrate incubation time can be observed. This allows the evaluation of the Mao B activity. The results have been correlated to an optical detection method. Furthermore, the method has been tested for different amounts of enzyme used in the experiments and found to be sensitive enough for Mao B analysis in blood samples.
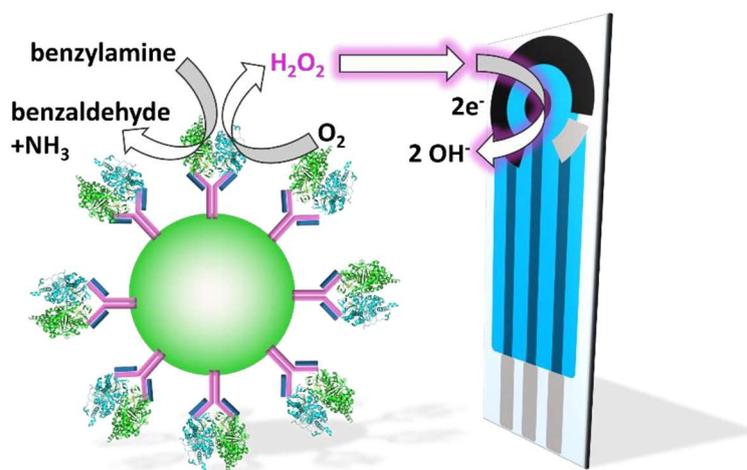

Figure 1: Schematic illustration of the concept of Mao B activity determination. Mao B is enriched from a sample on cellulose particles modified with anti-h-Mao B antibodies. The enzymatic reaction is quantified by amperometric detection of the enzymatically produced H_2O_2.

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Cell Secretion Measurements using Force-Controlled Interface Nanopores

T. Schlotter[1], T. Kloter, S. Weaver, D. Momotenko, M. Aramesh, J. Vörös, T. Zambelli, N. Nakatsuka

schlotter@biomed.ee.ethz.ch

[1]Laboratory for Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zurich
[2]Laboratory of Applied Mechanobiology, Department for Health Sciences and Technology, ETH Zurich

Introduction: Striving for more sensitive and selective single-molecule sensing techniques, nanopore devices have emerged as a promising and intriguing technology [1]. The development and commercialization of nanopore sensors is mainly stimulated by the recent advances in nanofabrication. However, on-demand pore size adjustability, durability, and the limitation in nanopore engineering remain bottlenecks for practical applications, such as proteomics. These shortcomings arise from the design of current nanopore sensors: a nanopore integrated into a thin membrane has limited accessibility and therefore does not allow for flexible engineering solutions such as serial nanopores, molecule-specific nanoconfinements, or selective pore functionalization.

Results and Discussion: To overcome those challenges, we first integrated a nanopore into an atomic force microscope (AFM) cantilever using 'ion-beam sculpting'. With these size-controlled nanopores, we could measure fibronectin (220 kDa) protein secretion from mouse embryonic fibroblasts [2]. To make the system more flexible and allow for the sensing of different biomolecules with one single nanopore, we developed the concept of a dynamic (*i.e.* pore size adjustable) AFM-controlled interface nanopore where a nanopore is formed between the micro-channelled AFM cantilever and a soft polymeric substrate [3]. By applying forces on the cantilever, different conformational states of biomolecules can translocate through the nanopore, which is recorded as changes in the ionic current. This technique was robust and stable to allow secretome monitoring in neuron cultures over several hours and to this end, enabled the detection and measurement of neural activity. To achieve functionality for single-cell proteomics, our current attempts extend the force-controlled nanopore system to surface functionalization with amino acid-specific aptamers for selective detection, manufacturing of serial nanopores and protein-tailored nanostructures.
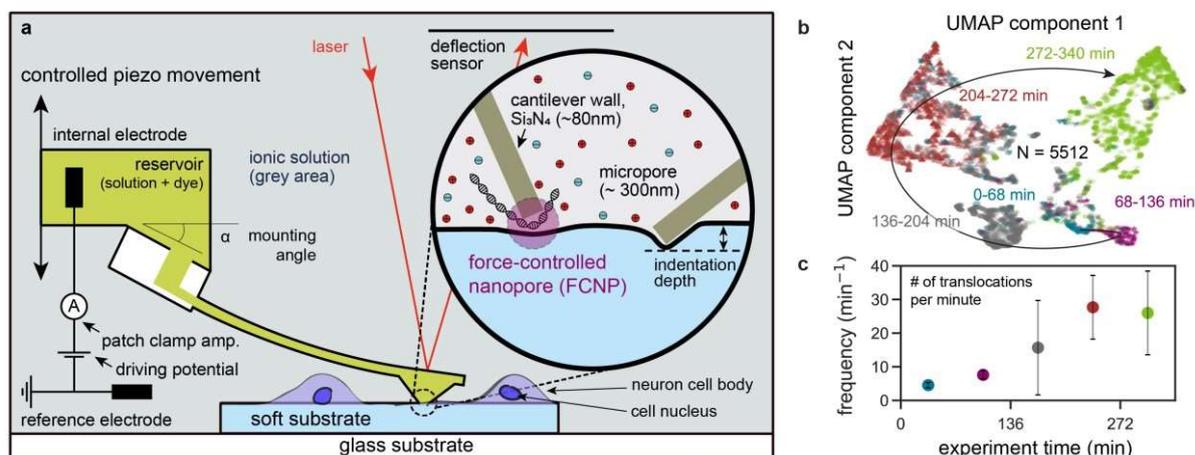

*Figure 1: Force-controlled nanopore (FCNP) concept applied for pore size specific and long-term detection of secretion from single neurons. **a**, Schematic of the setup, built on an atomic force microscope (AFM) together with a micro-channelled cantilever. Long-term measurement of a neurons' secretome. The events are clustered in bundles of 68 min. UMAP dimension reduction of 5512 single events in **b** shows clockwise clustering according to experiment time. **c**, Increase of cell signalling over time.*

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A transparent platform for cell capture and single cell isolation

Jiaxin Lian[123], Wenxian Tang[123], Ying Yang[123], Stephen G. Parker[123], Vinicius R. Gonçales[1], Richard D. Tilley[124], J. Justin Gooding[123]

Jiaxin.lian@unsw.edu.au; justin.gooding@unsw.edu.au

[1]School of Chemistry, [2]Australia Centre for NanoMedicine, [3]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, [4] Electron Microscopy Unit, The University of New South Wales, Sydney, NSW 2052, Australia

Abstract: The recognition of cellular heterogeneity has triggered the need to develop single cell isolation and analysis techniques, so the information for each single cell, rather than the whole population was obtained, which could help to better understand some biological behaviours.[1] This presentation will present the development of a transparent and easily constructed platform for target cell capture and single cell isolation, with circulating tumour cell (CTC) as a model system, as CTCs have tremendous values in early cancer detection and understanding the mechanism of metastasis.[2]

Inspired by the previous work,[3] the strategy to recover single cell in this work is to use a technique called 'light activate electrochemistry' (LAE) which makes use of the photo-responsive property of a semiconducting material.[4] The first step to build this single cell isolation platform is to construct a transparent semiconducting electrode which can perform LAE. This is achieved by forming amorphous silicon – indium tin oxide (aSi-ITO) heterojunction.[5] The electrochemical behaviours and optical properties of aSi-ITO are investigated and found a 20 nm aSi layer on ITO is transparent and its faradaic electrochemistry can only be switched on when it is illuminated.[6]

In order to capture the model CTCs and release them subsequently, the platform is designed to have some micron-sized Au islands deposited on the aSi-ITO. A surface engineering strategy which is able to functionalize the Au islands and the aSi area for local and selective CTC capture from blood mixture has been developed.[7] At the end, the cells being captured to the platform is then released with the assistant of a micromanipulator at single cell level when the platform is activated by a light from the microscope and simultaneously biased to desorb the cell binding molecules, as illustrated in Figure 1. The performance of the platform for single cell isolation is evaluated.
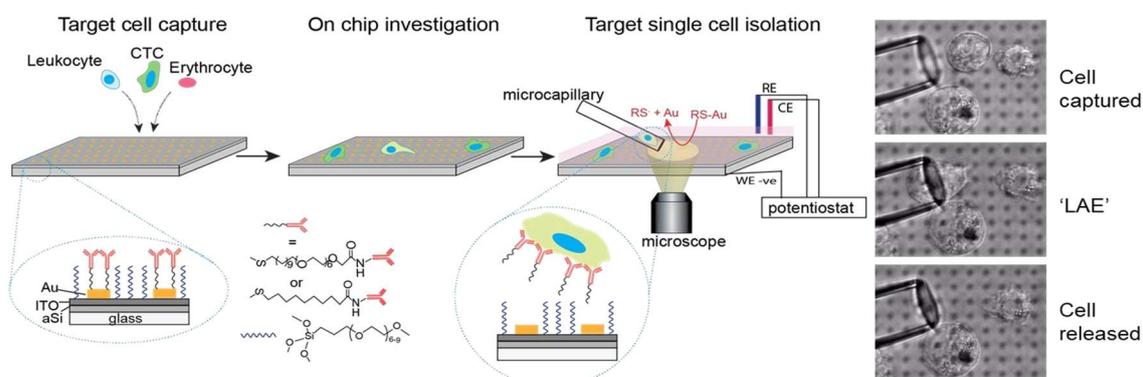
Figure 1: The schematic illustration and microscopy images of a single cell isolated from the platform.

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Electrochemical imaging of *E.coli* biofilms using Soft-Probe-Scanning Electrochemical Microscopy

Sorour Darvishi [1], Horst Pick [2], Emad Oveisi [3], Andreas Lesch [4], and Hubert H. Girault [1]

Sorour.darvishi@epfl.ch

[1]Ecole Polytechnique Fédérale de Lausanne (EPFL) Valais Wallis, Laboratory of Physical and Analytical Electrochemistry, Rue de l'Industrie 17, 1950 Sion, Switzerland.

[2]Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Protein and Cell Engineering, EPFL Station 15, 1015 Lausanne, Switzerland.

[3]Interdisciplinary Center for Electron Microscopy, École Polytechnique Fédérale de Lausanne (EPFL), CH-Lausanne, Switzerland

[4]University of Bologna, Department of Industrial Chemistry "Toso Montanari", Viale del Risorgimento 4, 40136 Bologna, Italy.

Microbial biofilms are involved in numerous subacute and chronic infections and cause the persistence of infections by microbial accumulation in the extracellular polymeric substance of the biofilm [1]. About 65% of all bacterial infections and 80% of chronic infections in the human body are linked to bacterial biofilm formation [2]. Understanding the mechanisms of dynamic biofilm growth is challenging, often because state-of-the-art optical characterization tools provide limited information. Therefore, in the battle against infections caused by biofilms, monitoring the growth and activity of biofilms is a key factor for understanding how harmful biofilms react against destructive treatments.

Herein, micrometric electrochemical imaging of *Escherichia coli* (*E. coli*) biofilms using Soft-ProbeScanning Electrochemical Microscopy (Soft-Probe-SECM) is presented as a complementary detection tool to overcome the limitations of various light microscopic techniques. Soft SECM probes are made of thin flexible insulating plastic films with embedded carbon microelectrode. They are brushed in a gentle contact mode over the surface of biofilms guaranteeing a constant working distance while the biofilm remains intact. The detection by SECM is based on the metabolic activity of living bacterial cells embedded in the biofilm, which reduces the oxidized form of a redox mediator. This enabled SECM feedback mode imaging (Figure 1). Biofilms of native as well as of ampicillin-resistant *E. coli* cells were grown for up to ten days. Qualitative monitoring with SECM and quantitative investigation with crystal violet colorimetric detection were performed. Finally, the degradation of an *E. coli* biofilm in the presence of different concentrations of the antibiotic gentamicin was investigated. Further, tape-stripping is presented as an efficient method for the collection of biofilm samples allowing the minimally invasive SECM analysis of biofilm samples taken from freely selectable areas and time-points of biofilm formation. The *in situ* SECM method is label-free and enables real-time monitoring of biofilm metabolic activity with spatio-temporal resolution [3].
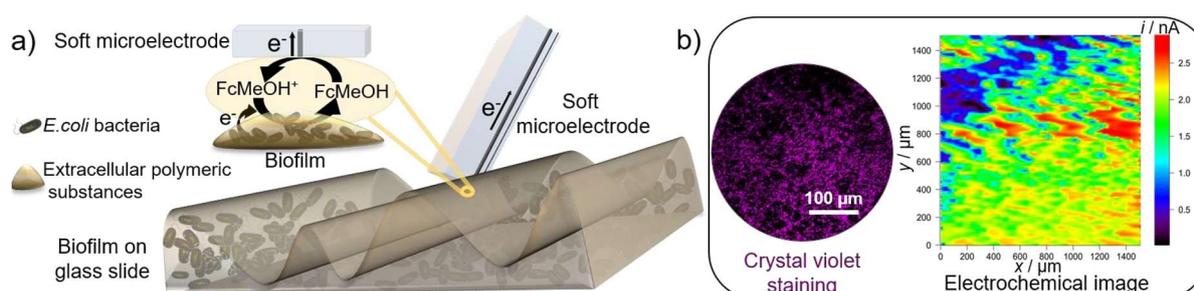

Figure 1: (a) Schematic representation of the SECM feedback mode of a biofilm using FcMeOH as redox mediator. (b) Crystal violet staining and SECM imaging of E. coli biofilms. Experimental details: E_T = 0.5 V, probe translation speed = 25 µm/s, step size = 10 µm, 2.5 mM FcMeOH in 100 mM PBS (pH = 7.4).

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Low-Cost Microfludic device based on Laser-induced Graphene Electrodes for highly sensitive Electrochemiluminescent Detection of Pathogens

Florian Gerstl [1], Uma Pongkitdachoti [2], Antje J. Baeumner [1]

Florian.Gerstl@ur.de

[1]Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany

[2]Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Bangkok 10330, Thailand

Abstract: There is a steadily growing demand for small, highly sensitive biosensors, which can be produced in large quantities at low costs. This can be achieved by combining the simple, scalable and inexpensive fabrication of laser-induced graphene (LIG) electrodes with the low limits of detection of electrochemiluminescence (ECL) in a simple microfluidic system. Here, this novel miniaturized ECL system in the form of a microfluidic chip is employed in an optimized version of the magnetic beadbased DNA hybridization assay described by Mayer et al.[1] Tris(2,2´-bipyridyl) ruthenium(II) (Ru(bpy)$_3$$^{2+}$) encapsulating liposomes are used as a signal enhancement strategy to facilitate the onchip detection of target DNA sequences derived from *C. parvum*, a waterborne parasite, with a remarkable limit of detection down to 22 pmol·L^{-1}. The assay requires only a single incubation step and can be done in less than 50 minutes. Selectivity studies showed a decrease in ECL intensity by already 58% for a single nucleotide polymorphism and > 98% for completely non-matching sequences from *E. coli* even in 10-times excess. *C. parvum* target DNA could be detected reliably in spiked real samples ranging from drinking water to complex matrices like soil extract.
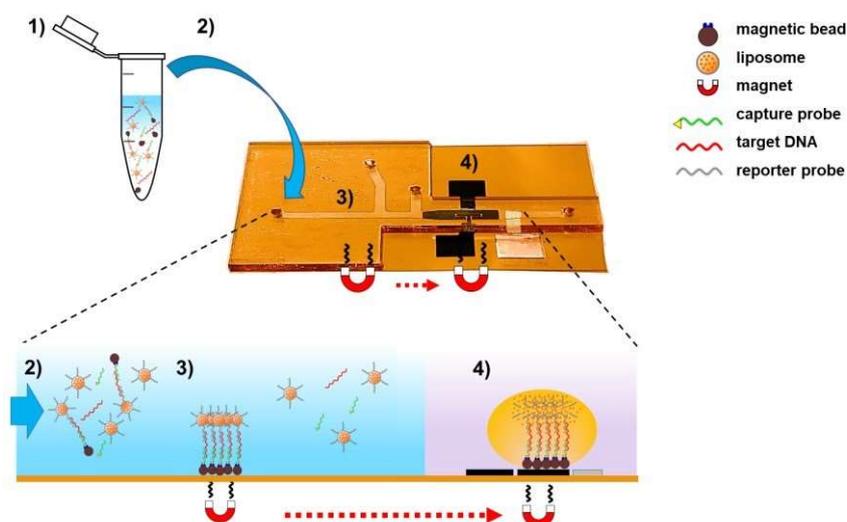
Figure 1: Schematic illustration of the miniaturized ECL system and the assay principle. 1) One-step incubation 2) Injection into the microfluidic chip 3) Separation of bound and unbound liposomes 4) Transfer onto the electrodes, lysis of liposomes and detection by ECL.

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Elastic electrospun polymer enabling cryo-preservation of a cellbased biosensor chip for ready-to-use on-site applications

Dua Özsoylu [1,2], Tuğba Isık [3,4], Mustafa M. Demir [4], Michael J. Schöning [1,5], Torsten Wagner [1, 5]

oezsoylu@fh-aachen.de

[1]Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, HeinrichMußmann-Straße 1, 52428 Jülich, Germany

[2]Medical Biology and Genetics, Graduate School of Health Sciences, Dokuz Eylül University, Mithatpasa Street 1606, Balcova, 35340 Izmir, Turkey

[3]School of Chemistry, University of Bristol, Great George Street 31, BS1 5QT Bristol, UK

[4]Department of Materials Science and Engineering, Izmir Institute of Technology, 35430 Izmir, Turkey [5]Institute of Biological Information Processing (IBI-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Abstract: There are only a few cell-based biosensors available on the market despite an increased number of publications and efforts, usually focusing on their capability to detect substances of interest such as pollutants [1], toxins [2], or viruses like SARS-CoV-2 [3]. As the sensor chip contains a 'living' component such as adherent mammalian cells, an efficient preservation and transport is still a major challenge in practical applicability and commercialization of these biosensors [1-3]. Thus, there is an urgent need for preservation tools and methods to enable ready-to-use on-site systems. Herein, we studied a strategy based on preserving the living component (adherent cells) by freezing them directly (-80 °C) on the biosensor surface. It is found that cryo-injury on cells occurs over a freezethaw cycle, which is most likely due to a mismatch in the coefficient of linear thermal expansion between the frozen cell membrane and the rigid sensor surface in contact. Therefore, the sensor surface was modified with elastic electrospun fibers composed of a polymer (polyethylene vinyl acetate), which has a high thermal expansion coefficient and low glass-transition temperature. The modified sensor chip is then integrated into a microfluidic system (Figure 1). This novel cryo-chip system is found to be effective for keeping cells viable during cryo-preservation as well as for postthaw detection of the extracellular acidification of CHO-K1 cells. Cryo-preservation of the chips containing cells at the manufacturing stage and transporting them using a cold-chain transport could open up a new possibility for ready-to-use on-site applications.
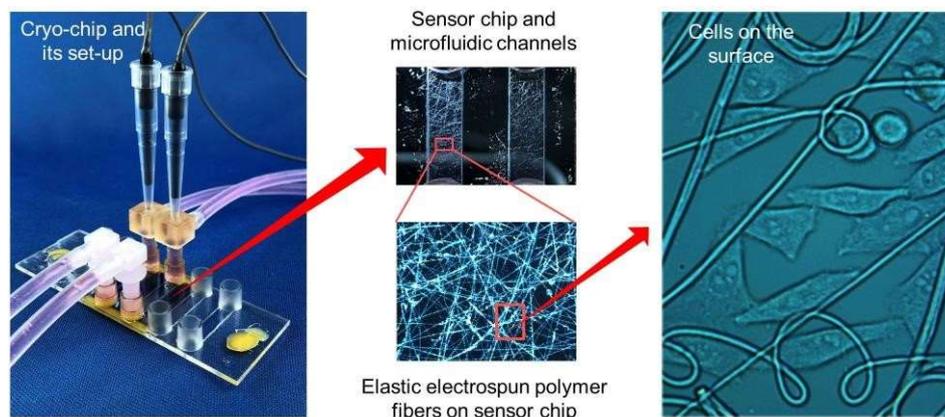
Figure 1: Elastic electrospun polymer fibers-modified light-addressable potentiometric sensor integrated into a microfluidic system (cryo-chip).

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Wireless sensing of biofilm formation in medically relevant bacteria based on Ag/AgCl redox conversion

Atefeh Shafaat [1,2], Jessica Neilands [3], Sebastian Björklund [1,2], Javier Sotres [1,2], and Tautgirdas Ruzgas[1,2]*

tautgirdas.ruzgas@mau.se

[1]Department of Biomedical Science, Faculty of Health and Society, Malmö University, 205 06 Malmö, Sweden
[2]Biofilms - Research Center for Biointerfaces, Malmö University, 205 06 Malmö, Sweden
[3]Department of Oral Biology, Faculty of Odontology, Malmö University, Malmö, Sweden

Abstract: Bacterial infection is one of the most severe problems particularly for patients after surgery, in cases of diabetes complication and even in elderly population. Infections hamper the wound healing by converting them in to chronic wounds and even leading to death (1). In the majority of infection related problems, growth of microbial biofilms are the biggest obstacle. In this study, we propose a battery-less sensor for wireless tracking of biofilm formed by some medically relevant bacteria.

The setup exploits an electrode modified with AgCl particles as a part of RFID passive tag antenna. This is then connected to an external electrode (glassy carbon or conductive fabric) for hosting growth of microorganisms (Fig. 1). Biofilm growth on the external electrode results in negative potential. We exploit this negative potential to convert AgCl to Ag as a part of tag antenna. The reduction changes the impedance of the antenna which is monitored wirelessly by the RFID setup (2). Some medically relevant microorganisms including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been assesed by OCP measurements (vs Ag/AgCl reference electrode) during their biofilm formation on the external electrode. The biofilm formed by all these types of bacterica resulted in negative potential ranging from -70 to -200 mV which was sufficient to convert AgCl to Ag particles. To approach a wireless monitoring of biofilm growth by wearable, wireless, battery-less biosensors in wound care the proposed biofilm sensor is currently assessed on skin, by using *in vitro* experiments.
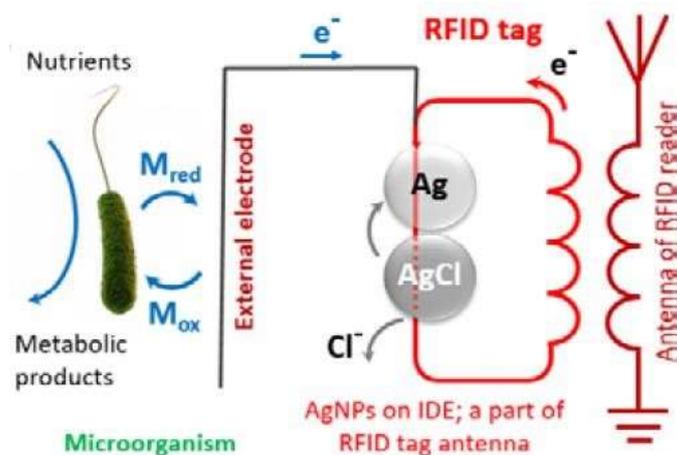
Fig.1. Schematic representation of biosensor-RFID tag for detection of bacterial growth on external electrode.

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Catalysis of oxygen electro-reduction by animal and human cells: a new platform for cell-based biosensors

Simon Guette-Marquet, Christine Roques, Alain Bergel

alain.bergel@toulouse-inp.fr (Corresponding e-mail address)

Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS 4 allée Emile Monso, 1432 Toulouse, France

Abstract: Animal cells from the Vero lineage (kidney tissue of a green monkey) and MRC5 human cells (fibroblastic cell lineage derived from human foetal lung) were checked for their capacity to catalyse the electrochemical oxygen reduction reaction (ORR). The cells were incubated in culture medium on carbon electrodes (37°C, 5 %CO_2) for different periods (4 to 72 hours) before ORR was analysed by cyclic voltammetry at 2 to 10 mV.s^{-1}.

The Vero cells needed 72 hours of incubation to induce significant ORR catalysis. The cyclic voltammetry curves were clearly modified by the presence of the cells with a shift of ORR of 50 mV towards positive potentials and the appearance of a limiting current of around 70 µA.cm^{-2}.

The MRC5 cells induced considerable ORR catalysis after only 4 hours of incubation with a positive potential shift of 110 mV (Figure 1), but with large experimental deviation. A longer incubation time, of 24 hours, made the results more reproducible with a potential shift of 90 mV. Modifying the electrode surface with carbon nanotubes or by pre-treatment with foetal bovine serum or poly-Dlysine with the objective to improve electron transfer and cell adhesion did not change the voltammetry records. This suggests that the electron transfer rate was controlled by some metabolic processes rather than the interfacial electron transfer rate.

These results are the first demonstrations of the capability of animal and human cells to catalyse the electrochemical ORR. The possible mechanisms are discussed on the basis of the literature available for ORR catalysis by bacterial cells. The involvement of proteins from the oxidative stress protection system is speculated, because of the catalytic effect observed with the supernatant in some cases. The ORR catalysis detected here could consequently be directly related to the oxidative stress protection system of the cells.

Oxidative stress is known to play key roles in diseases such as diabetes, neurodegenerative disorders, cardiovascular diseases and Alzheimer's troubles. It is consequently of high interest to develop any sensor that could assess the performance of the oxidative protective system of cells and to detect the impact of toxic agents or the efficiency of antioxidant strategies. The pioneering results presented here should pave the way for such cell-based biosensors.
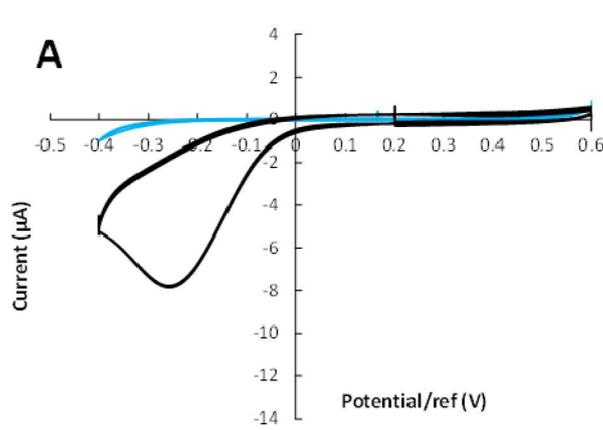

Figure 1: Cyclic voltammetry (10 mV.s^{-1}) showing catalysis of oxygen reduction by MRC5 cells after 4 hours of incubation on carbon electrodes. The blue curve is the control without cells.

Bifunctional Peptide Supports Designed for Electrochemical Signalling-off Detection of High-Molecular Weight Targets

Mihaela Puiu [1], Lucian-Gabriel Zamfir[1,2] Valentin Mirceski[3,4] and Camelia Bala[1,5]

elenamihaela.puiu@g.unibuc.ro

[1]Laboratory for Quality Control and Process Monitoring, University of Bucharest, 4-12 Elisabeta Blvd., 030018Bucharest, Romania

[2]ICUB, University of Bucharest, 36-46 Kogalniceanu Blvd., 050107 Bucharest, Romania

[3]Institute of Chemistry, Faculty of Natural Sciences and Mathematics, "Ss Cyril and Methodius" University, P.O. Box 162, Skopje, R. N. Macedonia;

[4]Department of Inorganic and Analytical Chemistry, University of Lodz, Tamka 12, Lodz, Poland

[5]Department of Analytical Chemistry, University of Bucharest, 4-12 Elisabeta Blvd., 030018-Bucharest, Romania

Abstract: Herein we report an electrochemical signalling-off biosensor for the detection of two structurally different high-molecular weight (HMW) targets, the anti-tumour-associated carbohydrate antigen (α-Tn) antibody and the growth hormone secretagogue receptor (GHS-R1a) using an affinity format. The affinity counterparts of the two HMW targets were specifically grafted on a redoxlabelled support peptide (SP) constrained to a helical conformation [1]. This particular grafting of the ligand between the redox-labelled $C_{terminus}$ residue and the thiol-functionalized $N_{terminus}$ end, immobilized onto the gold surface causes a significant decrease of the electron transfer (ET) from the redox label to the electrode surface, at the HMW target binding [2] (Figure 1). This biosensing format does not require a labelled ligand, but rather a redox-labelled bridge that act both as conductive layer and a self-assembled support. Square wave voltammetry (SWV) was used to estimate the ET rate constant from the redox label (methylene Blue, MB) to the electrode surface [3, 4], and to monitor the decrease of the peak current at the target binding. The signalling-off interrogation provided a 250 pM limit of detection (LOD) for GHS-R1a and a1.2 nM LOD for α-Tn antibody, while the 20 s^{-1} value of the ET rate constant indicate that the support peptide works as a molecular wire. Thus, short helical peptides may replace long-chain alkyl thiols as supporting layers, exhibiting conductive properties that alkyl thiols are lacking.
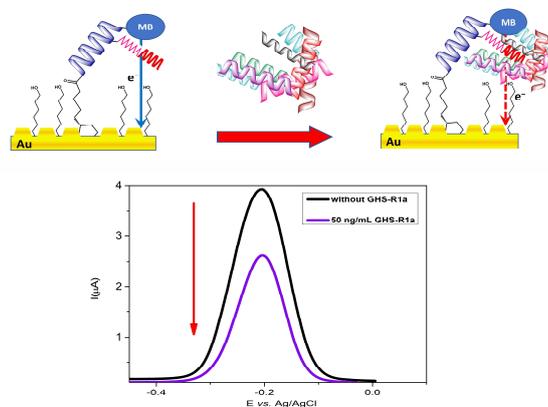
Figure 1: Detection principle of the signalling-off biosensor: decrease of the SWV signal at the target binding (here GHSR1a) due to the hampering of the ET rate from the redox label (Methylene Blue, MB) to the electrode surface

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Computationally determined epitopes and high-affinity synthetic protein binders for biosensor applications

Zeynep Altintas*, Raoul Tchinda, Anna Tutsch, Julia Drzazgowska, Bianca Schmid, Roderich Sussmuth

zeynep.altintas@tu-berlin.de (Corresponding e-mail address)

Technical University of Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany

Abstract: The conformation of biological molecules on surfaces or in solution environments strongly effects the successful implementation of biosensing platforms for the detection of target molecules as possible conformational changes lead to decreased sensing signals. To date, artificial protein binders have been developed using linear peptides with an unknown structure in epitope imprinting process. Despite successful outcomes obtained to some extent, most of these works lack of providing either high affinity, selectivity or sensitivity. We aim to address these problems by performing molecular dynamic calculations for the design of high affinity artificial protein binding surfaces for cancer biomarker recognition [1]. Computational simulations are employed to identify particularly stabile secondary structure elements. These epitopes are used for subsequent molecular imprinting, where surface imprinting approach is applied. The molecular imprints generated with the calculated epitopes of greater stability show better binding properties than those of lower stability. The average binding strength of imprints created with stabile epitopes is found to be around fourfold higher for the selected biomarker models [1]. The artificial protein binders can recognise the target molecules even in a complex medium including non-specific molecules at a high concentration [1, 2]. Moreover, certain amino acid modifications of the computationally selected epitope templates (e.g. addition of histidine to the peptide chain or cysteine modification on both terminal of the elongated peptide to form selfassembled monolayer bridges) further improve the performance of artificial protein binders [2,3]. Our novel and rational selection can be used for establishing epitope libraries for protein molecules by eliminating unsuitable epitopes and ranking the best candidates based on their stability analysis obtained from molecular dynamic simulations. The integrated approach has shown a good potential to contribute to some limitations of medical diagnostic field. Research disciplines that require recognition receptors can apply this technique for designing stable and efficient receptors.

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Combined thermal and electrochemical sensor platform employing a novel surface-imprinted polymer as receptor for the real time detection of *Escherichia Coli.*

Rocio Arreguin-Campos [1], Kasper Eersels [1], Hanne Diliën [1], Bart van Grinsven [1], Thomas J. Cleij [1]

r.arreguincampos@maastrichtuniversity.nl

[1] Sensor Engineering Department, Faculty of Science and Engineering, Maastricht University, P.O. Box 616,6200 MD Maastricht, The Netherlands

Abstract: Surface imprinted polymers (SIPs) are materials able to act as biomimetic receptors for different biological targets. The combination of SIPs as recognition elements with different transducing elements make these sensing platforms a versatile alternative detecting tool in comparison to conventional diagnostic techniques [1]. In this work, a novel polyurethane-urea SIP for the real time detection of pathogen Escherichia coli is presented. The ability of the synthetic receptor to bind the analyte was assessed optically as well as quantitatively. The integration of the SIP into a flow cell allowed the detection of the analyte in one simultaneous read-out platform that combines electrochemical impedance and thermal measurements derived from the interactions at the solid-toliquid interface. The results show that upon the exposure of the target in buffer within the flow cell, it re-binds to the polymer, resulting in an increase of the thermal resistance and a decrease of impedance, allowing the generation of a dose response curve for each transducer. This study supports that the prepared polyurethane-urea SIPs are suitable to be implemented into a combined thermal and impedometric platform. Moreover, the results highlight the possibility of detecting quantitatively in real time the pathogenic analyte with the proposed sensor. This device could possess relevance in fields in which bacterial testing is required, such as food safety and medical diagnosis.
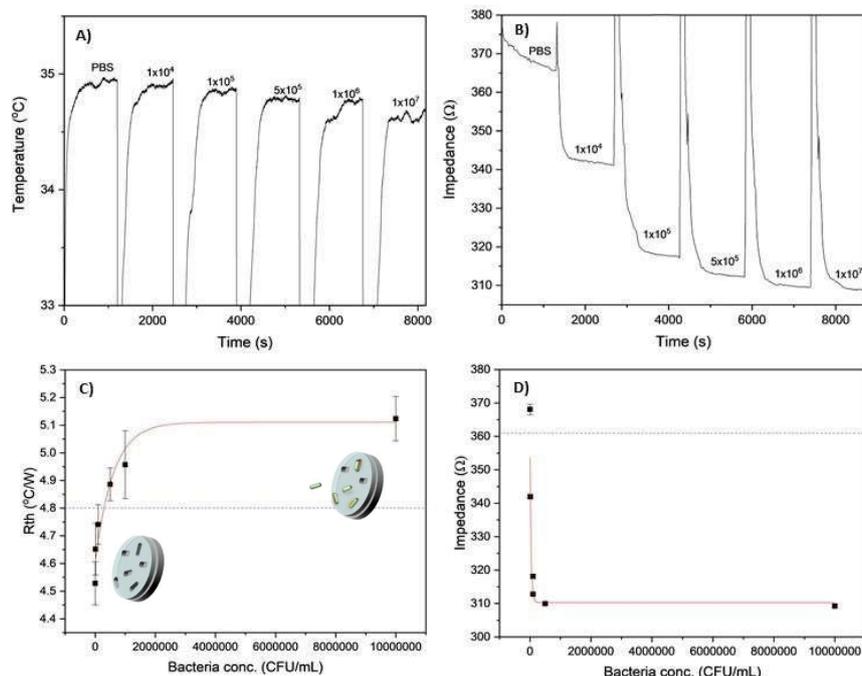
Figure 1: Simultaneous dose-response experiment performed on a polyurethane-urea SIP. The imprint was exposed to increasing concentrations (CFU/mL) of E. Coli in buffer. A) Temperature and B) Impedance response of the sensor in real time. An exponential fit is drawn throughout the obtained data of both transducers with a R^2 value of 0.94 for C) Temperature and 0.96 for D) Impedance readout. The dashed lines correspond to the limit of-detections, calculated as three times the highest error on each dataset.

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Nucleobase-Functionalized Molecularly Imprinted Polymer (MIP) Electrochemical Sensor for Determination of Heterocyclic Aromatic Amines

Viknasvarri Ayerdurai[1], A. Garcia-Cruz[1], Maciej Cieplak[1,*], Piyush Shindu Sharma[1], Francis D`Souza[2,*] and Wlodzimierz Kutner[1,3,*]

vayerdurai@ichf.edu.pl

[1]Institute of Physical Chemistry, Polish Academy of Sciences (IPC PAS), Kasprzaka 44/52, 01-224 Warsaw, Poland
[2]Department of Chemistry, University of North Texas, 1155 Union Circle, 305070, Denton, TX 76203-5017, USA
[3]Faculty of Mathematics and Natural Sciences, School of Sciences, Cardinal Stefan Wyszynski University in Warsaw, Woycickiego 1/3, 01-815 Warsaw, Poland

Quinoxaline heterocyclic aromatic amines (HAAs), are formed during meat and fish cooking, frying or grilling at high temperatures. HAAs are classified as potent hazardous carcinogens, even though the HAAs are usually generated at very low concentration (~ng per g of a food sample). This is because the HAA food contaminants effectively damage DNA by intercalation or strand break[1]. Hence, chronic exposure to HAAs, even in low doses, can cause cancers of lung, stomach, breast, etc. Currently, HPLC is used for determination of these toxins in food matrices. However, this technique is expensive, tedious, and time-consuming. Therefore, fast, simple, inexpensive, and reliable HAAs determination procedures, without need of separation of these toxins, in the protein food matrices are in demand. Molecularly imprinted polymers (MIPs) are excellent examples of bio-mimicking recognition materials[2]. Therefore, they have found numerous applications in selective chemosensing. Within the present project, we synthesized a nucleobase-functionalized molecularly imprinted polymer (MIP) as the recognition unit of a chemosensor for selective detection and determination of 2-amino-3,7,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (7,8-DiMeIQx) HAA. MIP-(7,8-DiMeIQx)film coated electrodes were sensitive and selective with respect to 7,8-DiMeIQx. The linear dynamic concentration range of the devised chemosensor extended from 12 µM to 0.4 mM 7,8-DiMeIQx and the imprinting factor was high, IF = 13.
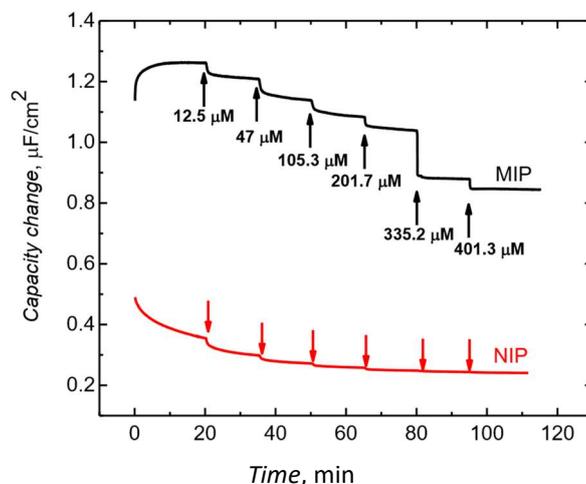

Figure 1: Capacity changes with time for the molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) film coated Au disk electrodes after addition of heterocyclic aromatic amines (HAA) of different concentrations.

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A polynorepinephrine-based molecular imprinting assay targeting a small peptide hormone in doping control analysis

Francesca Torrini[1], Pasquale Palladino, Simona Scarano and Maria Minunni

simona.scarano@unifi.it

[1]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy

Abstract: Molecularly imprinted polymers (MIPs), named "plastic antibodies", are synthetic affinity reagents with tailor-made binding sites mimicking the ability of natural receptors to bind a target molecule. The latest decade has witnessed a great advance in MIPs and nowadays soft and biocompatible polymers, e.g. polydopamine [1] and polynorepinephrine [2], represent a powerful tool to detect bio-molecules such as small peptides, proteins, glycoproteins, etc. Facile one-step synthesis, low cost, stability and reusability are some features that make the aforementioned polymers very attractive. Dopamine (DA) and norepinephrine (NE) are capable of self-polymerize in alkaline conditions leading to make adhesive nanolayers onto different surfaces. We recently started to investigate the potential application of PNE, in the biosensing field, which displays a markedly more hydrophilic and smoother surface [3] compared to PDA. In particular, our study aimed to design a highly specific PNE-based MIP assay to target a small peptide hormone of anti-doping interest, gonadorelin (MW = 1182.33 Da), by Surface Plasmon Resonance (SPR) transduction. Gonadorelin is improperly used by male athletes to improve their sports performances by stimulating the endogenous secretion of testosterone in the bloodstream. For this reason, gonadorelin was banned by the World Anti-Doping Agency (WADA) and its detection represents a new frontier in antidoping research. The specificity of the MIP was addressed by testing leuprolide, a second peptide of anti-doping interest, and random peptides with comparable molecular weight and number of amino acids to gonadorelin. Our findings corroborate the effectiveness of the imprinting strategy adopted in synthesizing MIPs for a small peptide hormone which are able to discriminate peptides differing for only one amino acid. The presence of specific recognition sites on the MIP was also confirmed by comparing the binding of the above peptides (target and random peptides) on a MIP *versus* a non-imprinted polymer (NIP) surface. Also, currently, we are deeply investigating the role and the synergic effect of gonadorelin' amino acids in the imprinting process. Then, a two-step MIP-based competitive assay to detect gonadorelin was designed by exploiting the competition between gonadorelin and a signal-enhancer competitor molecule with a high molecular weight, biotinylated gonadorelin tethered to streptavidin. Urine artificial samples spiked with gonadorelin were examined achieving a detection limit in the low ppb range, perfectly in line with the minimum required level (MRPL = 2 ppb) at which all WADAaccredited laboratories must operate in routine daily operations. Moreover, the ongoing work is focused to transfer the proposed strategy into multi-welled plates to establish a colorimetric assay that can be coupled with a smartphone application to read-out the signal for in-situ athletes' monitoring.
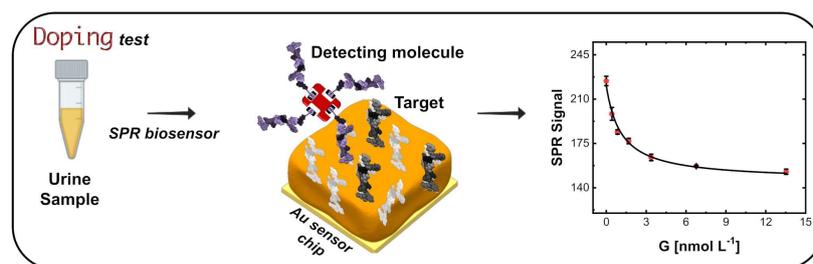
Figure 1: Sketched representation of the competitive MIP-based assay. Dose-response curve obtained for gonadorelin (G) in urine samples.

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Quantification of circulating microRNAs biomarkers using a novel isothermal amplification method in microfluidic devices

Noemi Bellassai[1], Meenu Selvaraj[1,2], Roberta D'Agata[1,3], Pierpaolo Greco[2], Giuseppe Spoto[1,3]

noemi.bellassai@unict.it

[1]Dipartimento di Scienze Chimiche, Università degli Studi di Catania, V.le A. Doria 6, 95125 Catania, Italy
[2]Scriba Nanotecnologie s.r.l., Via di Corticella 183/8, 40128 Bologna, Italy
[3]Istituto Nazionale di Biostrutture e Biosistemi, V.le delle Medaglie d'Oro 305, 00136 Rome, Italy

The development of novel technologies for effective microRNA (miRNA) detection is needed in clinical diagnosis since they have recently been recognized as significant and predictive biomarkers in liquid biopsy [1]. Based on polymerase chain reaction, the traditional nucleic acid detection methods do not achieve with simple approaches discrimination between miRNA family members with high sensitivity. Isothermal circular strand displacement polymerization (ICSDP) has recently emerged as a successful method for identifying nucleic acids with high sensitivity and specificity in miRNA detection. Isothermal amplification methods may exploit a molecular beacon (MB) fluorescent probe with a stem-loop structure complementary to the target sequence [2]. MB probe is a well-designed molecular system able to move between two different signalling conformations (close-state and openstate) depending on both the presence and the amount of the specific target sequence. The integration of bioassays for nucleic acid detection in microfluidic devices is of great importance for successfully detecting miRNAs where small sample volume is generally required [1,2].

Herein, we propose an innovative strategy for miRNA detection directly from biological fluids based on ICSDP amplification reaction with MB fluorescent probe. The integration of MB-based isothermal amplification in a microfluidic device allowed us to perform the bioassay using less than 1 microliter of the biological fluid and ensured cost-effective and single-step detection of target analytes directly in the complex media. We used the proposed method to detect miR-127 as target sequence in the synovial fluid of osteoarthritis patients. By using an innovative mutant polymerase and LNA bases in primer sequence, the MB-based isothermal amplification reaction achieves high sensitivity and specificity for miRNA discrimination at sub-picomolar concentration, according to the biomarker levels circulating into the synovial liquid related to osteoarthritis disease [3]. Moreover, the quantification of circulating miR-127 has also been performed in synovial fluid (less than 10 pmol/L), thus demonstrating that the proposed MB-based isothermal amplification can identify synovial fluid miRNAs as an useful tool for early diagnosis of osteoarthritis disease.
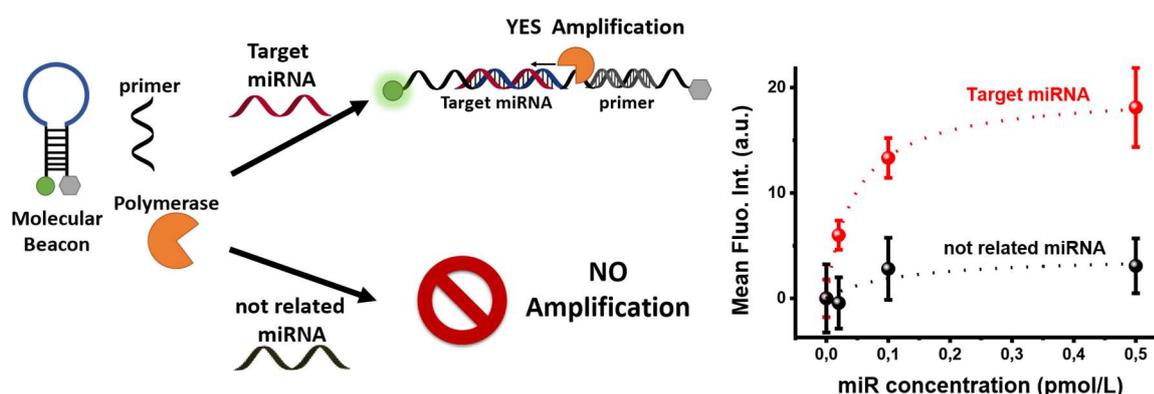

Figure 1: Rational design of MB-based isothermal amplification method for specific miRNA detection as circulating biomarker in liquid biopsy.

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Isothermal amplification methods as a versatile option for standard PCR in the field of lab automation and PoC testing

C. Warmt[1*], C. K. Fenzel[1,2], C. Yaslanmaz[1,2], S. Zwirner[1], L.-M. Broweleit[1,2], J. Henkel[1], F.F. Bier[2,3]

*corresponding author, e-mail: christian.warmt@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB), 14476 Potsdam, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: The polymerase chain reaction (PCR) is the most widely used tool for the amplification of DNA fragments in all fields of biology. Nevertheless, there are some crucial aspects that makes it difficult to use the PCR in the field of lab-on-chip or PoC testing. Above all is the necessity of fast and accurate temperature switching during the PCR cycles. This requires expensive equipment that is not useful for developing cheap and portable devices. Depending on the question and required setup there is also the need of PCR amplification times up to 2h and more.

Isothermal amplification methods such as recombinase polymerase amplification (RPA) or loopmediated isothermal amplification (LAMP) are promising tools to overcome these disadvantages. Amplification times of less than 20-30 min are possible and there is no need for expensive equipment. Combined with various subsequent detection means isothermal amplification is a promising tool for the detection of pathogens, especially in cases when analysis time is crucial like diagnostics of sepsis.[1,2] In our working group "laboratory and process automation" at the Fraunhofer IZI-BB, we develop methods to combine isothermal amplification with fast detection. In this presentation we show data on intercalating dyes for virus detection (Fig. 1A), on the fly labelling of amplicons or isothermal quantitative analysis (Fig. 1B) to detect different pathogens like *salmonella* or relevant genes such as carbapenem-resistance genes in single and multiplex processes (Fig. 1C). With these methods we are able to develop inexpensive, portable and easy to use PoC devices for fast and accurate target detections.
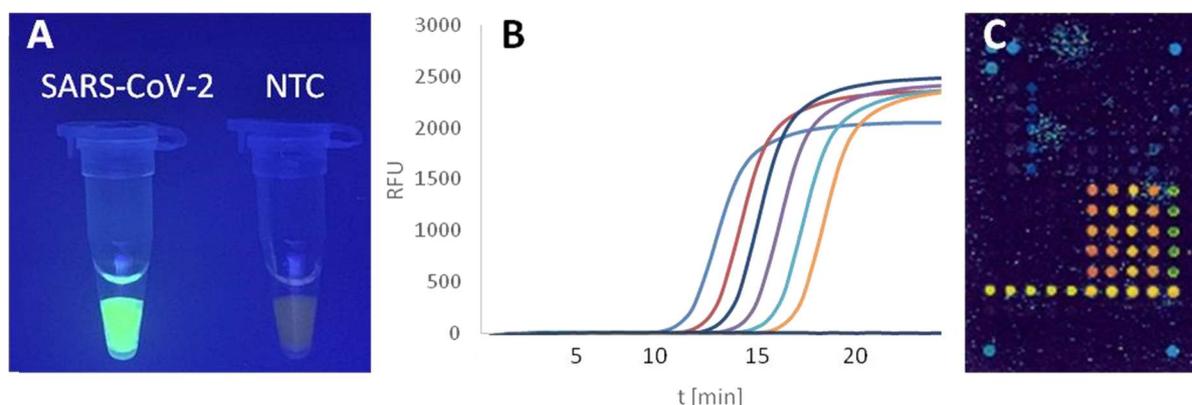
Figure 1: Diverse isothermal amplification-detection combinations for lab automation and point of care devices. (A) Optical LAMP assay for a fast and easy detection of corona viruses. (B) qLAMP detection of salmonella DNA (range 40 ng to 39 pg; 1:4 dilution). (C) Duplex RPA combined with microarray technology for the detection of multi resistance genes like KPC (white border) and CTX-M15.

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Programming DNA‑based Sensing Platforms through Effective Molarity Enforced by Biomolecular Confinement

Alessandro Porchetta[1], Marianna Rossetti[1], Francesco Ricci[1]

alessandro.porchetta@uniroma2.it

[1]Department of Chemistry, University of Rome Tor Vergata, Via della Ricerca Scientifica, 1, 00133, Italy

Abstract: Biological processes are underpinned by much more than a mere combination of uniformly distributed molecules reacting with one another in a diffusive regime. To control molecular encountering and promote effective interactions, nature places biomolecules in specific sites inside the cell in order to generate a high, localized concentration different from the bulk concentration.[1] Inspired by this mechanism, here we show how harnessing effective molarity enforced by biomolecular confinement has led to the development of proximity-based strategies to actuate artificial DNA-based functional systems for biosensing applications. Specifically, we employed antibodies as biomolecular actuators to drive the co-localization of antigen-labelled nucleic acid strands and generate a detectable signal output. In this regard, we first demonstrated a fluorescencebased programmable nucleic acid nanoswitch platform capable of detecting clinically relevant antibodies directly in human plasma in a one-step and no-wash format.[2,3] Motivated by the fact that the miniaturization of the fluorescence device and its use in off-laboratory settings could prove challenging, we also developed a signal-on, highly selective electrochemical DNA-based platform.[4] we demonstrated orthogonal multiplex detection of four different target antibodies by carrying out measurements in blood serum samples using an array of SPEs. our single-step direct sensing methods do not rely on any amplification step and so it cannot reach the sensitivity of standard serological clinical methods (e.g. ELISA/RIA methods). However, the good sensitivity, as well as the rapidity and the highly specific detection makes the platform highly advantageous in clinical and therapeutic drug monitoring applications. Our strategy is extremely versatile and can in principle be easily adapted to the detection of any biomolecule target whose recognition element can be coupled to a nucleic acid strand.
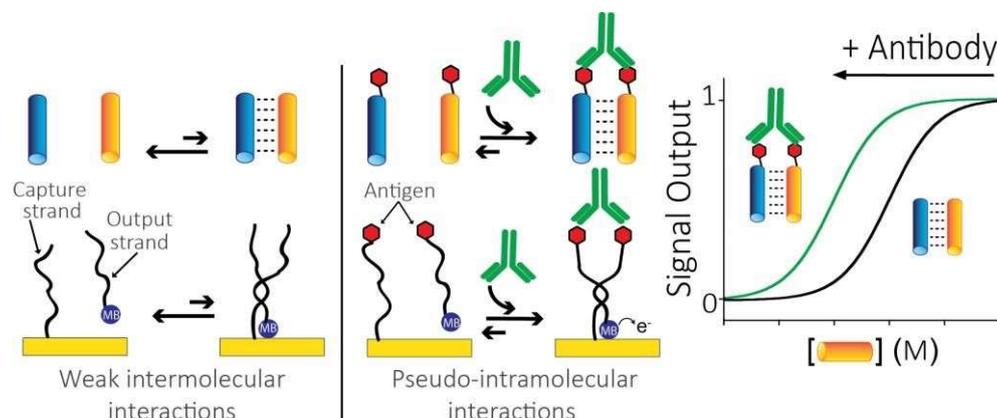
Figure 1: Principle of the electrochemical DNA-based sensing platform that employs effective molarity to detect antibodies. The co-localization of the antigenlabelled capture and output strands on the same target antibody generates an improvement of the observed binding affinity, and brings the redox-active tag near the gold surface thus generating an increase in the current signal.

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LSPR based detection based on Hybridization Chain Reaction for miRNA detection

Andrea Miti [1], Sophie Thamm [3], Philipp Müller [3], Andrea Csáki [3], Wolfgang Fritzsche [3], Giampaolo Zuccheri [1,2]

andrea.miti@unibo.it

[1]Department of Pharmacy and Biotechnology, University of Bologna, via San Giacomo 11, 40126 Bologna, Italy.
[2]S3 Center, Institute of Nanoscience of the Italian CNR.
[3]Institute of Photonic Technology, Albert-Einstein-Str. 9, 07745 Jena, Germany

Abstract: Circulating microRNAs are attracting great attention for their possible role as biomarkers in diagnosis and follow-up based on nucleic acids detection. DNA based biosensors can offer powerful tools at this purpose, overcoming the common drawbacks faced by the lab-based techniques. In DNA based sensing, isothermal amplifications such as Hybridization Chain Reaction (HCR) can lead to the improvement of the sensitivity through enhancement of the response. HCR is a completely isothermal and Enzyme-free self-assembling reaction involving a few species of hairpin-like DNA strands in solution [1]. HCR was previously reported as effective amplification method in the detection of DNA pathogens employing SPR technology [2]. Target detection and the self-assembly on the surface of metallic nanoparticles can be detected monitoring the shift in the LSPR peak due to a change in the refractive index [3]. Herein we report the combination of HCR and a LSPR method based on immobilized gold nanoparticles for the specific detection of microRNAs [4]. The specificity of the assay was obtained by employing a hairpin-like probe, able to efficiently trigger the amplification upon detection of the specific target. Time required for the overall detection was less then 1 h and the limit of detection achieved was improved by 3 orders of magnitude in presence of the HCR amplification. No successful examples of HCR combined with such LSPR based sensing are present in literature. The sensor we propose can offer a solid and robust alternative to the existing methods, does not require in principle specialized operators, components are easily available and cost-effective, allowing point-ofcare applications an large-scale production.
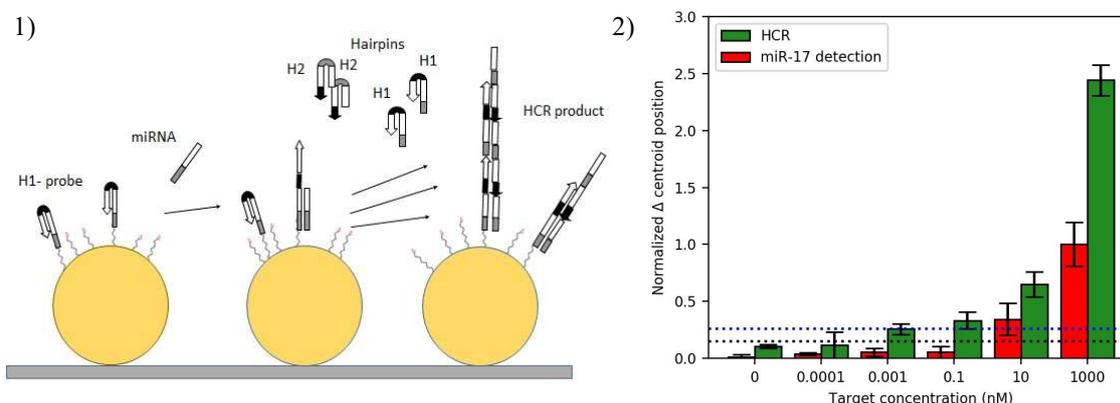
Figure 1: Scheme of the miRNA detection through HCR on immobilized gold nanoparticles. Figure 2: Bar plot showing the responses obtained injecting target sequence miR-17 (red bars) and performing HCR amplification (green bars).

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Multianalyte-Assays: Simultaneous detection of protein and nucleic acid biomarkers

Anna Brunauer[1], Anna-Sophia Kittel[2], René D. Verboket[3], Felix von Stetten[1,2], Susanna M. Früh[1,2]

anna.brunauer@imtek.uni-freiburg.de (Corresponding e-mail address)

[1]Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[2]Hahn-Schickard, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[3]Department of Trauma-, Hand- and Reconstructive Surgery, University Hospital Frankfurt, Johann Wolfgang Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

Abstract: The development of new molecular sensors that can detect multiple classes of biomolecules is highly desirable and will significantly improve a wide variety of areas including medical diagnostics. For example, it is beneficial to identify pathogens via nucleic acid analysis along with inflammatory protein markers of the host for the detection of infectious diseases. Traditionally, target biomolecules are detected by specialized molecular bioassays which require the use of various types of instrumentation. In contrast, multianalyte-assays allow the simultaneous detection of different biomolecule classes from a single sample using identical assay conditions. Thus, they (1) increase the density of information per sample volume, (2) save time and resources, and importantly, (3) enable a more informative assessment of biomarkers.[1,2] We developed a novel paper-based multianalyte sensor for the rapid and simple detection of infections. The multianlyte-assay detects interleukin-6 (IL-6) and *Pseudomonas aeruginosa* (*P. aeruginosa*) gDNA simultaneously from one sample. In a first step (Figure 1A), the target DNA was amplified via recombinase polymerase amplification (RPA), introducing antigenic labels into the amplification products. The isothermal reaction was performed at 37 °C and thus was compatible with protein analysis. Subsequently (Figure 1B and C), both target biomarkers were analyzed via lateral flow immunoassay (LFIA) using antibody-conjugated fluorescent microspheres. Furthermore, the validity of the test results was assessed by an internal amplification control (IAC) and flow controls (FCs). Previous results showed no cross-reaction with other pathogens.[3] This novel strategy has a turnaround time of 35 min with only two simple hands-on steps. Thus, our multianalyte sensor, which detects the pathogen and the local host immune response (IL-6), has tremendous potential to facilitate a fast therapy decision in time-critical infections.
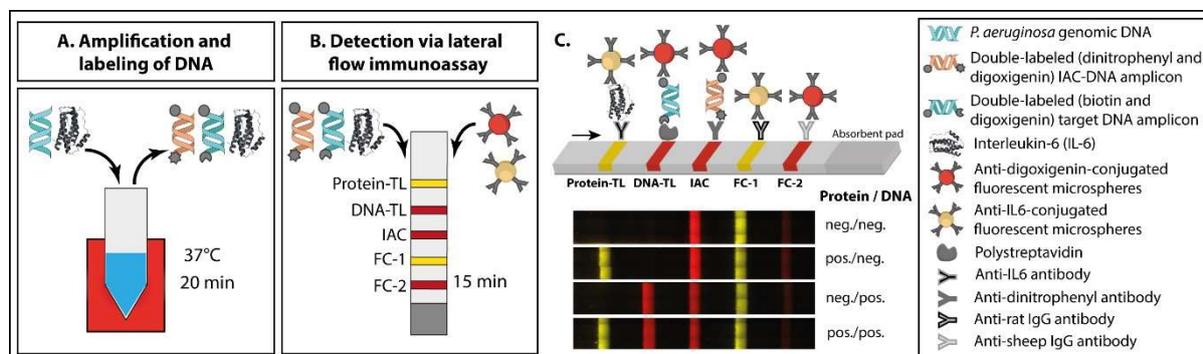
Figure 1: Principle of the multianalyte-assay for the simultaneous detection of DNA and protein biomarkers. (A) Isothermal amplification and labeling of the target DNA and IAC-DNA. (B) Detection of protein and DNA biomarkers via LFIA (C) Schematic drawing of the LFIA for multianalyte detection including FCs and IAC.

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DNA directed immobilization in a microarray immunoassay for multiplexed detection of antibiotics

J. Guercetti[1,2] J-P. Salvador[1,2], A. Aviñó[,2,3], R. Eritja[2,3], N. Pascual[1,2], P. Marco[1,2]

(Corresponding author: jpablo.salvador@iqac.csic.es)

[1]Nanobiotechnology for diagnostic group, Institute of advanced chemistry of Catalonia (IQAC) Spanish National Research Council (CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain.
[2]CIBER of Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Jordi Girona 18-26, 08034 Barcelona, Spain.
[3]Nucleic acid chemistry group, Institute of advanced chemistry of Catalonia (IQAC), Spanish National Research Council (CSIC), Jordi Girona 18-26, 08034, Barcelona, Spain.

Abstract:

The overuse of antibiotics in food producing animals is one of the main contributions to antimicrobial resistance and contamination of derivate products(1). The necessity of rapid, cost effective and multiplexed bioanalytical detection tools over the food chain is critical in order to ensure the quality of products and the health of consumers(2).

Therefore, the development of an antibody fluorescent microarray is presented exploiting DNA directed immobilization (DDI) strategies to detect three antibiotic residues in cow's milk. In first place the immobilization of the Oligo-down probe over a glass surface is required. Then, an incubation with the complementary strands conjugated to specific haptens allows the directed immobilization. A cocktail of monoclonal antibodies is utilized in an indirect competitive assay format to determine the presence of the target analytes. Furthermore, these are family-class antibodies and present selective profile for two types of Tylosins (A and B), more than 10 different Sulfonamides and a specific antibody for Chloramphenicol. The final step includes the addition of a secondary antibody labeled with a fluorophore to obtain the fluorescent signal.

This immunochemical approach allows the simultaneous detection of each analyte below the regulatory limits in less than one hour. Furthermore, a validation study was assessed in matrix following the EU guidelines and no sample pre-treatment was required.
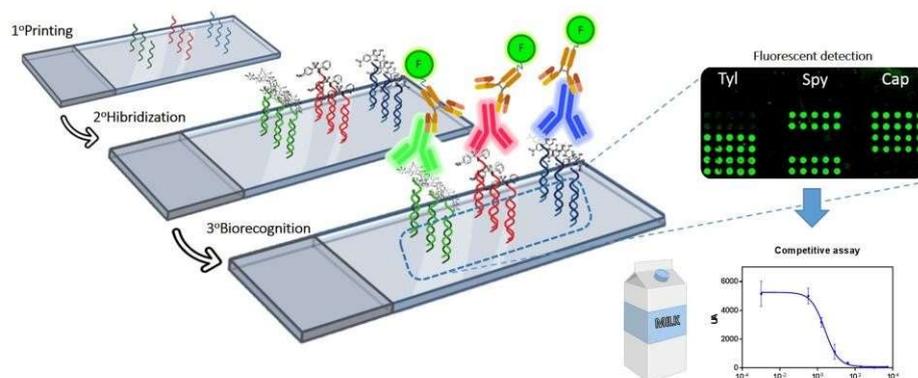
Figure 1. Steps of antibody fluorescent microarray based on DDI: 1:Printing of the oligo probe, 2:Incubation with a complementary strand, 3:Biorecognition with specific antibodies.

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Automated, flow based chemiluminescence microarray immunoassay for the rapid multiplex detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (CoVRapid CL-MIA)

Julia Klüpfel[1], Silvia Würstle[1], Josef Mautner[2], Martin Ungerer[3], Ulrike Protzer[4], Martin Elsner[1], Michael Seidel[1]

julia.kluepfel@tum.de

[1]Institute of Hydrochemistry, Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 Munich, Germany
[2]Helmholtz Zentrum München, Research Unit Gene Vectors, Marchioninistraße 25, 81377 München
[3]ISAR Bioscience GmbH, Semmelweisstr. 5, 82152 Planegg
[4]Institute of Virology, Technical University of Munich, Trogerstr. 30, 81675 München

Abstract: When first cases of infection with a formerly unknown respiratory virus, SARS-CoV-2, were reported in December 2019, it was not to be expected that one year later more than 1,800,000 people would have died from COVID-19 and about 85,000,000 would have survived an infection [1,2]. To assess the immune status of these reconvalescent patients, antibody tests are a valuable tool, and even more now, that first vaccines are available. The currently available antibody tests, however, have several drawbacks. Many of them are laborious and time-consuming or lack the required diagnostic specificity due to cross-reactivity and matrix effects, and they also lack diagnostic sensitivity when only one antigen is considered [3,4].

To overcome these drawbacks, we developed a rapid, flow-based chemiluminescence microarray immunoassay (CL-MIA). This CoVRapid CL-MIA allows for the multiplex analysis of IgG antibodies to three different SARS-CoV-2 antigens simultaneously in human serum and plasma. Specifically, it can probe for the nucleocapsid structural protein (N) that binds the viral RNA, as well as for a fragment of the spike protein (S1) and for the receptor binding domain (RBD, contained within the spike protein), which are responsible for receptor recognition and cell entry of the virus [5]. We succeeded in covalently immobilizing these three antigens on glass microarray chips in their native conformation applying established surface chemistry [6]. Subsequently, an indirect noncompetitive immunoassay is done on the microarray chips within the analysis device Microarray Chip Reader 3 (MCR 3) [7]. Sample as well as horseradish peroxidase-labelled detection antibody are flown over the chip, followed by luminol and hydrogen peroxide, leading to a chemiluminescence reaction that can be recorded with a CCD camera. With the CoVRapid CL-MIA, the measurement result is available in under eight minutes in a fully automated fashion.

To prove the diagnostic performance of the test, we performed measurements for a sample set of 32 SARS-CoV-2 serology negative and 33 positive samples. Cut-off values to classify samples as positive or negative were defined using receiver operating characteristic (ROC) curves. With these optimized cut-off values, all 65 samples could be classified correctly, resembling 100% diagnostic sensitivity and specificity. The CoVRapid CL-MIA even outcompeted two commercial tests applied to the same sample set.

We therefore have developed a powerful diagnostic tool for SARS-CoV-2 serosurveillance that also holds promise to better characterize immune responses and link them to disease patterns or vaccination efficiency for this new viral infection due to its multiplexing capability.

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Rapid one-step quantitative detection of SARS-CoV-2 virus in crude samples using antifouling quartz crystal microbalance biosensor

Michala Forinová[1], Alina Pilipenco[1], Ivana Víšová[1], Lukáš Frána[1], Milan Houska[1], Markéta Vrabcová[1], Judita Arnoštová[1], Václav Hönig[2,3], Martin Palus[2,3], Jan Štěrba[4], Jakub Dostálek[1,5], N. Scott Lynn[1], Alexandr Dejneka[1], Tara MacCulloch[6], Nicholas Stephanopoulos[6], Hana Vaisocherová-Lísalová[1]

Corresponding e-mail address: lisalova@fzu.cz

[1]Institute of Physics of the CAS, Na Slovance 2, 18221 Prague, Czech Republic
[2]Institute of Parasitology, Biology Centre CAS, Branisovska 31, 37005 Ceske Budejovice, Czech Republic
[3]Veterinary Research Institute, Hudcova 70, 62100 Brno, Czech Republic
[4]Faculty of Science, University of South Bohemia, Branisovska 31a, 37005 Ceske Budejovice, Czech Republic
[5]Austrian Institute of Technology GmbH, Konrad-Lorenz-Strasse 24, Tulln, Austria
[6]The Biodesign Institute, Arizona State University, 727 E. Tyler St., AZ 85281-5001 Tempe, USA

Abstract: The worldwide COVID-19 pandemic, caused by SARS-CoV-2, has led to 79 231 893 infected people and 1 754 574 deaths by the end of 2020 [1, 2]. Due to its relatively high infectivity, it is crucial to detect its presence within the shortest time possible. We introduce a novel technology based on the antifouling quartz crystal microbalance (A-QCM) powered by a high performance microfluidic system. This approach in contrast to the routinely used RT-qPCR, provides a faster response (less than 20 mins), is less expensive, and is suitable for POC detection of SARS-CoV-2 directly from clinical samples without any sample pre-treatment. This superior performance is enabled by antifouling polymer brush with a unique compostion combining zwitterionic and non-ionic moieties, functionalized with either specific antibodies against nucleocapsid protein or with a peptide recognizing the spike protein of SARS-CoV-2. We verified the fouling resistance of this polymer brush via exposure to a variety of crude clinically relevant biological samples (such as stool, oropharyngeal and nasopharyngeal swabs), including those containing other pathogenic microorganisms. We show that the A-QCM biosensor with an antibody and peptide-coated chip is capable of highly sensitive and rapid detection of SARS-CoV-2 in crude biological samples with a LOD of ~10^3 PFU/mL, and ~10^4 PFU/mL, respectively. Finally, the blinded study on a set of clinical nasopharyneal samples confirmed a high sensitivitvy of A-QCM biosensor compared to RT-qPCR.
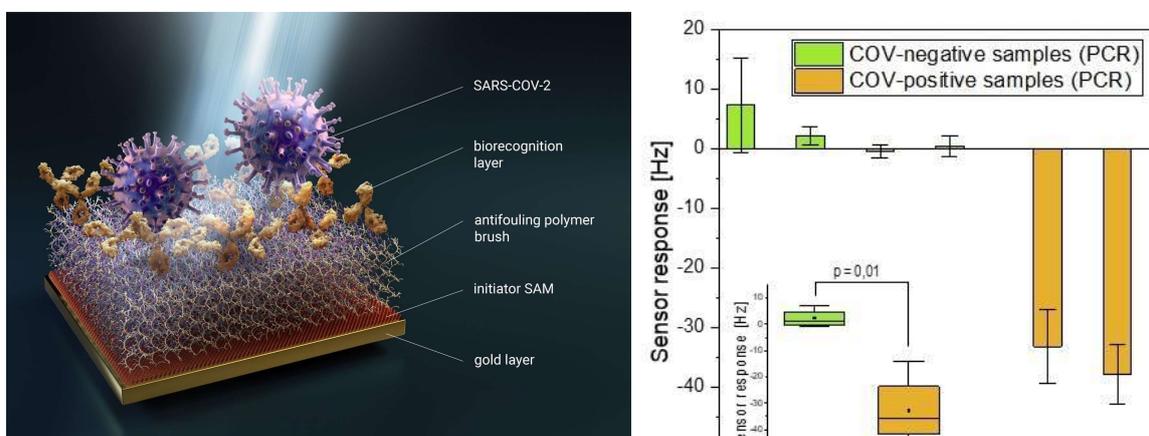

Figure 1: (left) Schematic representation of a functionalized polymer brush-based biochip for the detection of SARS-CoV-2 virus from complex solutions. (right) Selected data showing the sensor response from nasopharyngeal swabs taken from 8 patients (4 healthy, 4 Cov-positive).

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Hybrid nanostructures in dual immunosensing for determining candidate biomarkers in this century diseases

Verónica Serafín[1], Alejandro Valverde[1], María Gamella[1], Eloy Povedano[1], Claudia A. Razzino[1,2], María Pedrero[1], M. Garranzo-Asensio[3], Ana Montero-Calle[3], Rodrigo Barderas[3], Miguel Calero[3,4], Anderson O. Lobo[5], Paloma Yáñez-Sedeño[1], Susana Campuzano[1], José M. Pingarrón[1].

veronicaserafin@ucm.es

[1]Department of Analytical Chemistry, Faculty of Chemical Sciences, Complutense University of Madrid, 28040 Madrid, Spain, Country
[2]Institute of Research and Development, University of Vale do Paraiba, Sao Jose dos Campos, SP 12244000, Brazil Chronic Disease
[3]Programme, UFIEC, Carlos III Health Institute, Majadahonda, 28220 Madrid, Spain
[4]Alzheimer's Center Reina Sofía Foundation – CIEN Foundation and CIBERNED, Carlos III Institute of Health, Majadahonda, 28220 Madrid, Spain
[5]LIMAV – Interdisciplinary Laboratory for Advanced Materials, BioMatLab, Department of Materials Engineering, Federal University of Piaui, Teresina, PI 64049-550, Brazil

Abstract: Nowadays, neurodegenerative disorders (NDs) and cancer diseases, due to their increasing prevalence and prolonged duration, represent a major global health problem for worldwide population, and a tremendous financial impact associated with medical care for families and society. Therefore, the development of low-cost and accessible methods, compatible with point-of-care technology (POCT), for quantifying recently identified candidate biomarkers is of great interest and can help improving the accuracy of disease diagnosis, prognosis and, therefore, disease outcomes. In this context, the use of electrochemical immunosensors, due to their high sensitivity, simple use, rapid response, low cost, and compatibility with miniaturization and multiplexing is considered particularly suitable to develop POCT systems for on-site determination of these biomarkers.

Graphene quantum dots and multiwall carbon nanotubes (GQDs/MWCNTs) nanocomposites and dendrimer-encapsulated gold nanoparticles (AuNPs/PAMAM) are particularly attractive options to be exploited in the development of electrochemical biosensors due to their remarkable conductivity, catalytic ability, biocompatibility and high density of active groups for biomolecules immobilization. In this presentation, we summarize the most relevant aspects of the pioneering use of GQDs/MWCNTs [1] nanocomposite as nanocarrier for multiple detector antibodies and peroxidase (HRP) molecules and the performance of AuNPs/PAMAM dendrimer nanocomposites as electrode modifier for capture antibodies [2]. These nanostructures were used for the development of electrochemical immunosensing approaches for the simultaneous determination of candidate protein biomarkers in metastatic cancer such as Cadherin-17 and IL-13 Receptor α2 [1] and NDs biomarkers such as tau protein and TAR DNA binding protein 43 [2]. Both methodologies involve sandwich-type immunoassays performed on the surface of dual screen-printed carbon electrodes (dSPCEs) electrochemically grafted with p-aminobenzoic acid (p-ABA) and amperometric transduction using the H_2O_2/hydroquinone (HQ) system. Their potential to direct and accurate determine the endogenous target biomarker levels in complex samples such as human plasma, cancer cells lysates and tissues extracts from healthy and cancer or NDs-diagnosed patients, by simply placing a drop of the undiluted biofluid or a small amount of tissue extract (0.5−2.5 µg) and involving simple and short turnaround protocols (1-2 h), is demonstrated. These interesting features, along with the use of affordable, low-power requirement and portable instrumentation, make them competitive tools to further advance in the consolidation of candidate clinical biomarkers in metastasis and neurodegenerative processes, and their implementation in POCT devices to perform decentralized and rapid routine determinations.

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Ultrasensitive plasmonic apta-immunosensor for detection of malaria biomarkers in human whole blood

Antonio Minopoli[1,2], Bartolomeo Della Ventura[2], Bohdan Lenyk[1,3], Francesco Gentile[4], Julian A. Tanner[5], Andreas Offenhäusser[1], Dirk Mayer[1], Raffaele Velotta[2]

antonio.minopoli2@unina.it

[1]Institute of Biological Information Processing (IBI-3), Bioelectronics, Forschungszentrum Jülich, 52425 Jülich, Germany
[2]Department of Physics "E. Pancini", University Federico II, Via Cintia 26, 80126 Naples, Italy
[3]Department of Physics, University of Konstanz, 78457 Konstanz, Germany
[4]Department of Electrical Engineering and Information Technology, University Federico II, 80126 Naples, Italy
[5]School of Biomedical Sciences, University of Hong Kong, Hong Kong SAR, China

Abstract: Development of fast and reliable biosensors able to detect disease biomarkers at very low concentrations in a complex matrix such as human whole blood is still a challenge. In this regard, optical biosensors relying on plasmon-enhanced fluorescence (PEF) provide an effective strategy to push down the detection limits while holding low-cost production and easyto-use. In particular, honeycomb arrays of gold nanoparticle (AuNPs) realized through block copolymer micelle nanolithography (BCMN) stand out for their scalability and tunable plasmonic properties making them ideal substrates for fluorescence enhancement.

Figure 1: (a) Honeycomb array of AuNPs and (b) antibody-analyte fluorescently labelled aptamer sandwich configuration.

Here, we describe a PEF-based apta-immunosensor for the specific and ultrasensitive detection of Plasmodium falciparum lactate dehydrogenase (PfLDH) - a malaria biomarker - in whole blood.[1] Analyte capture is realized by oriented antibodies immobilized in a close-packed configuration onto the substrate via the photochemical immobilization technique (PIT), while the fluorescent labelling by a top bioreceptor layer of nucleic acid aptamers recognizing a different surface of PfLDH in a sandwich conformation. The combination of BCMN and PIT enabled maximum control over the nanoparticle size and lattice constant as well as the distance of the fluorophore from the sensing surface. Figure 1 shows (a) the geometrical parameters of the AuNP array and (b) the detection sandwich scheme used in this experiment.

The apta-immunosensor achieved a limit of detection (LOD) smaller than 1 pg/mL (< 30 fM) with very high specificity against PfLDH without any sample pretreatment. Figure 2 shows some fluorescence images recorded at different analyte concentrations spiked in human whole blood. Such a LOD is several orders of magnitude lower than that found in malaria rapid diagnostic tests or even commercial ELISA kits. Thanks to its overall dimensions, ease of use and high-throughput analysis, the device can be used as a substrate in automated multi-well plate readers and improve the efficiency of conventional fluoroimmunoassays.
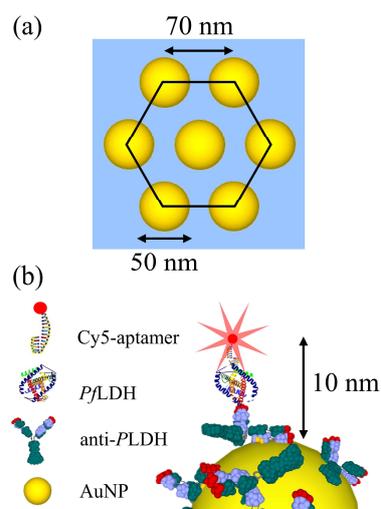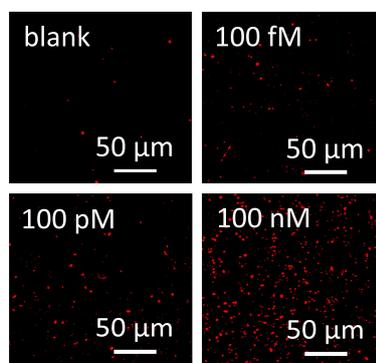

Figure 2: Example of fluorescence pictures acquired at different PfLDH concentrations spiked in whole blood.

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MAGNETIC MICROBEADS AMPEROMETRIC IMMUNOPLATFORM FOR THE RAPID AND SINGLE-BASE SENSITIVE DETECTION OF N[6]-METHYLADENOSINE RESIDUES TO ASSIST IN METASTATIC CANCER CELLS DISCRIMINATION

Eloy Povedano[1], María Gamella[1], Rebeca M. Torrente-Rodríguez[1], Ana Montero-Calle[2], María Pedrero[1], Guillermo Solís-Fernández[2], Fernando Navarro-Villoslada[1], Rodrigo Barderas[2], Susana Campuzano[1] and José M. Pingarrón[1]

elpove01@ucm.es

[1]Departamento de Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, 28040 Madrid (Spain)
[2]Chronic Disease Programme, UFIEC, Instituto de Salud Carlos III, 28220 Madrid (Spain).

Abstract: The term epigenetics, defined as the study of inherited changes in gene expression that occur independently of alterations in primary DNA sequences, involves different types of epigenetic modifications, including methylation processes in DNA and RNA. It has been recently demonstrated that human cancer cells exhibit global epigenetic abnormalities, which interact at all stages of cancer development and promote its progression. The fact that epigenetic modifications may be the key to the initiation and spread of some forms of cancer, coupled with the knowledge that they are potentially reversible, makes these alterations extraordinarily attractive and therapeutically promising.

The most common modification of RNA, which constitutes 80 % of total RNA methylations, is the methylation at position 6 of adenosine residues to produce N6-methyladenosine (m6A), which has a strong impact on the regulation of gene expression. Recent findings suggest that m6A is involved in various aspects of RNA metabolism in cancer cells, including proliferation and metastasis, differentiation of stem cells and homeostasis, which make m6A methylation a promising biomarker for detecting and preventing the occurrence of cancer.

In this sense, the present work describes the preparation of an immunoplatform for the simple, fast, sensitive, and selective determination of m6A involving, for the first time, the use of micromagnetic immunoconjugates to establish a direct competitive assay between the m6A target and a biotinylated RNA oligomer bearing a single m6A enzymatically labelled with a commercial conjugate of streptavidin-peroxidase as tracer [1]. The cathodic current change measured in the presence of the H_2O_2/hydroquinone system at screen-printed carbon electrodes upon capturing the magnetic bioconjugates on their working electrode surface is inversely proportional to the m6A target concentration. After evaluating the effect of key variables, the analytical characteristics were established for the amperometric determination of three different synthetic targets: the N[6]methyladenosine-5'-triphosphate ribonucleotide, a short synthetic RNA oligomer bearing a single m6A and a long RNA sequence containing 100 % of methylated adenosines. The obtained results show that this immunoplatform is competitive with other methods reported to date, achieving an improved sensitivity (limit of detection of 0.9 pM for the short synthetic oligomer) and excellent selectivity and reproducibility using a much simpler and faster protocol (~1 h) and disposable electrodes for the transduction. Moreover, the applicability for discriminating the metastatic potential of cancer cells by directly analysing a small amount (25-75 ng) of raw total RNA without amplifying or fragmenting was preliminary and pioneering assessed. The versatility offered by the methodology for targeting methylation bases in nucleic acids, simply by changing the specific capture antibody and the synthetic oligomer, offers a wide range of possibilities for multiplexing both types of methylated nucleic acids to provide a more accurate and complete molecular characterization and an earlier and more reliable disease diagnosis.

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Environmental monitoring by high-throughput immunoanalytical methods and portable devices

Rudolf J. Schneider

rudolf.schneider@bam.de

Bundesanstalt für Materialforschung und -prüfung (BAM), Richard-Willstätter-Str. 11, 12489 Berlin, Germany

Abstract: A vast number of emerging pollutants has been detected in the environment over the last decades. Analytical methods suitable for trace analysis are needed that are desirably also fast, inexpensive and, if possible, robust and portable. Immunoanalytical, i.e. antibody-based, methods which are available in a broad range of formats, can be profitably used here to analyse for the distribution and the trends of concentration levels of contaminants in the environment. Some of these formats are single-analyte but high-throughput methods. In order to use them wisely, indicator substances, sometimes called anthropogenic markers, should be selected and used in screening approaches. Other methods are suitable to be performed on portable instrumentation in the field (onsite) or in facilities such as wastewater treatment plants for on-line monitoring. Furthermore, there are array technologies that allow for parallel (multiplex) analysis of several analytes of interest.

The microtiter-plate based ELISA (Enzyme-linked Immunosorbent Assay) is the method of choice for the analysis of a large number of samples [1]. ELISAs are available to monitor for anthropogenic markers such as the antiepileptic carbamazepine, the analgesic diclofenac, the antihistaminic cetirizine, the steroid hormone estrone, the antimicrobial sulfamethoxazole, the stimulants caffeine and cocaine, the priority pollutant bisphenol A, and the bile acid isolithocholic acid. For on-site screening and monitoring, simpler formats, like mix-and-read assays, e.g. the Fluorescence Polarization Immunoassay (FPIA) [2] or Lateral-flow Immunoassays (LFIA) [3] are more suitable tools, the latter based on dipsticks or cassettes, that is why they are also called pregnancy test-like assays. The suitability of multi-analyte formats such as immunomicroarrays depends on the choice of a signal-producing system that provides small uncertainties and good reproducibility of the measurements. Bead-based ("suspension") arrays read out in flow cytometers are a powerful platform for multiplex assays [4]. Electrochemical formats run on portable devices provide additional advantages as no light source is required. They are most promising for stand-alone analysers and biosensors [5].

Figure 1: Some portable immunoanalytical devices suitable for environmental monitoring (Top: Polarimeters for FPIA, Bottom: LFIA cassette with available reading devices).

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First electrochemical biosensor for the detection of ciguatoxins in fish and microalgal samples

G. Gaiani[1], S. Leonardo[1], T. Tsumuraya[2], M. Rambla-Alegre[1], J. Diogène[1], C. K. O'Sullivan[3,4], C. Alcaraz[1], M. Campàs[1]

monica.campas@irta.cat

[1]IRTA, Ctra Poble Nou km 5.5, 43540, Spain
[2]Department of Biological Science, Osaka Prefecture University, 1-2, Gakuen-cho, Naka-ku, Sakai, 599-8570, Japan
[3]Departament d'Enginyeria Química, URV, Països Catalans 26, 43007 Tarragona, Spain
[4]ICREA, Pg. Lluís Companys 23, 08010, Spain

Ciguatera fish poisoning (CFP) is one of the most relevant seafood-borne diseases worldwide. It is caused by the ingestion of fish containing ciguatoxins (CTXs), lipophilic marine toxins produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa*[1] that accumulate into fish and through the food webs. CFP is characterized by severe neurological, gastrointestinal, and cardiovascular disorders and affects approximately between 50,000 and 500,000 consumers annually worldwide.[2]

Here, the first electrochemical immunosensor for the detection of CTXs is presented. Three different monoclonal antibodies (mAbs), two capture (3G8, 10C9)[3,4] and a detector (8H4)[4], were merged in a sandwich configuration for the combined detection of two main groups of CTX congeners (CTX1B and CTX3C). Magnetic beads were used as support to immobilize antibodies. Initially, the applicability of the immunosensor was demonstrated with the analysis of fish coming from La Réunion Island, enabling the discrimination between contaminated and uncontaminated samples. Obtained results correlate with mouse bioassay and cell-based assay. Then, fish coming from Mediterranean waters were analysed, giving promising results. Finally, extracts from *Gambierdiscus* and *Fukuyoa* were screened, allowing the separate detection of the two groups of CTX congeners at a cell concentration that can be found nature. Our results give new information regarding the toxin production of the genera, and demonstrate that our system is suitable for the analysis of field samples.

The developed bioanalytical tool is user-friendly and can help to mitigate ciguatera risk, contributing to the protection of consumers' health.
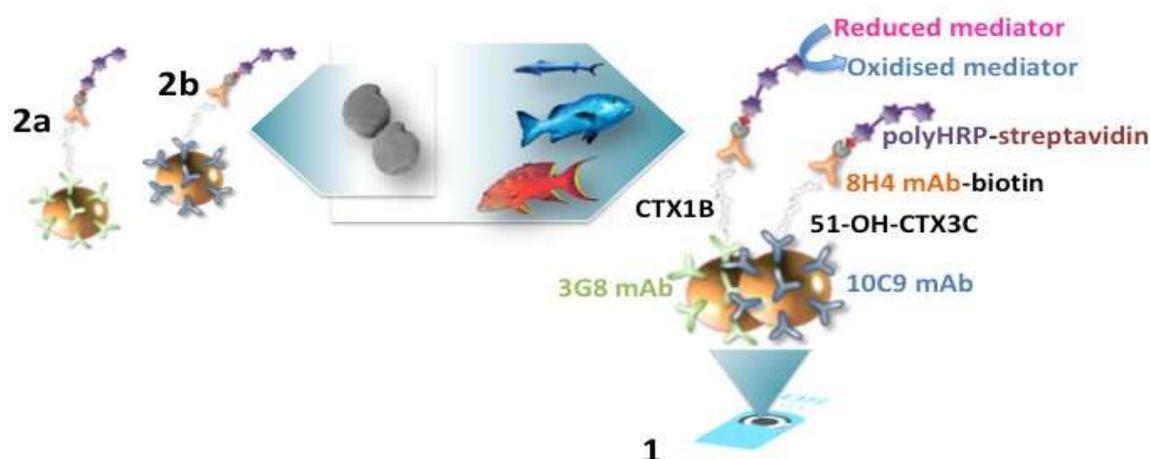

Figure 1. Experimental design. (1) Extracts from fish and algal samples were exposed to both capture mAbs at the same time. (2) Microalgal samples were also screened with the capture mAbs (2a: 3G8; 2b: 10C9) separately.

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Online monitoring of cortisol in Recirculated aquaculture systems

Claudy D'Costa[1], Manuel Gesto[2], Arto Heiskanen[1], Anders Wolff[1] and Jenny Emnéus[1]

jemn@dtu.dk

[1]DTU Bioengineering, Technical University of Denmark, Produktionstorvet, 2800, Denmark,
[2]DTU Aqua, Technical University of Denmark, Willemoesvej 2, 9850, Denmark

Abstract: The growing need of protein has accelerated interest in aquaculture systems since fish is a good source of protein [1]. Large scale production systems such as Recirculated Aquaculture System (RAS) provides a convenient environment for inland fish farming and also economical advantages, but it becomes important to ensure the welfare of the fish farmed in such systems. Cortisol, the stress hormone is an apt indicator to signal any variation from the optimal growing condition prevalent in the farm. Thus, continuous monitoring of cortisol can provide crucial information welfare, adaptation, and anthropological effects in aquaculture [2]. Cortisol can be measured from the blood plasma, mucus, scales, urine, faeces or from the release via the gills. Invasive measurement of cortisol may itself trigger a stress response. We here propose a method to address some of the challenges and develop a completely non-intrusive method of cortisol detection.
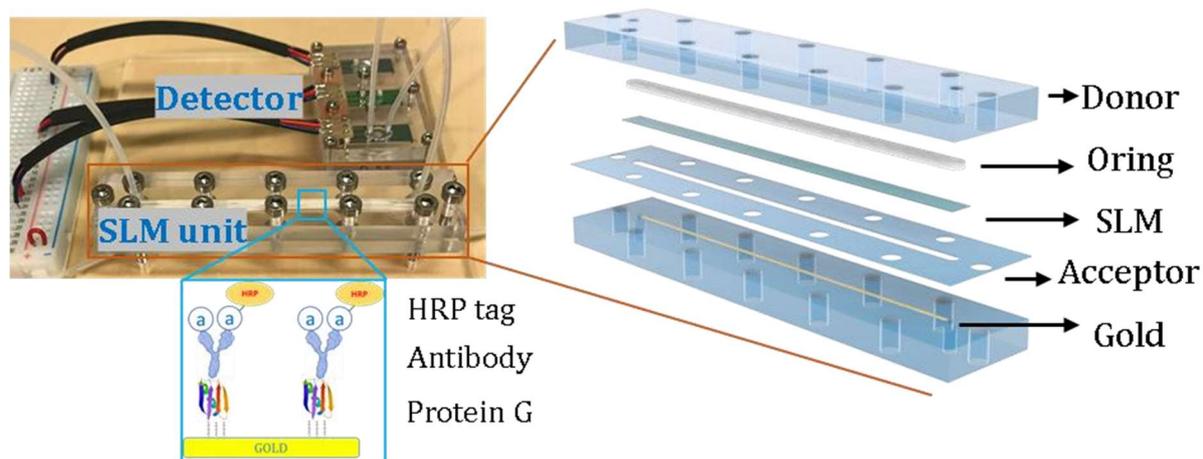
Figure 1: The flow injection system designed to collect and preconcentrate the sample and the electrode to measure the final product formed.

The very low level of cortisol, in the order of 2-80ng/L [3] detectable from the water in a closed farming system like the recirculating aquaculture system, would require adequate preconcentration steps before it can be measured. Here we report a method to non-intrusively collect water samples, preconcentrate the sample and measure the cortisol level all done using a programmable unit. An immuno-supported liquid membrane system [4] is used to eliminate charged particles and capture the target analyte there by enriching the same. A horse radish peroxidase labelled analyte is employed to fill the remaining capture sites as shown (Figure 1) and later forms an electrochemically active product in the presence of a suitable substrate. The product formed inversely corelates to the amount of cortisol present and is measured using the detector electrode. This signal can be fed into an existing control and monitoring system for corrective measures in the farm.

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Bioluminescent immunosensing of the immunosuppressant mycophenolic acid with a recombinant peptide-mimetic luciferase

Álvaro Luque-Uria[a], Riikka Peltomaa[a], Tarja K. Nevanen[b], Henri Arola[b], Elena Benito-Peña[a], María C. Moreno Bondi[a]

alvarluq@ucm.es

[a]Department of Analytical Chemistry, Faculty of Chemistry, Complutense University, Ciudad Universitaria s/n, 28040 Madrid, Spain
[b]VTT Technical Research Centre of Finland Ltd, Tietotie 2, FI-02150 Espoo, Finland

Abstract: Phage display is a commonly applied technique for identifying recombinant antibodies and epitope-mimicking peptides, also known as mimotopes.[1] Mimotopes have the exceptional ability to bind to the same antibody paratope as the antigen and can replace the labelled antigen in immunoassays or biosensors. This strategy is especially interesting for the analysis of low molecular weight target molecules, which present high toxicity, carcinogenicity, high price or are difficult to functionalize without altering its interaction with the antibody.

Mycophenolic acid (MPA) is a mycotoxin produced by Penicillium fungi, widely used as immunosuppressive drug to prevent organ rejection in transplanted patients. The analysis of MPA in biological samples is of interest for dose personalization in order to improve efficacy and reduce the side effects. In this work, a phage-displayed peptide library was used to select cyclic peptides that bind to recombinant antibody fragments (Fab)[2] selective to mycophenolic acid.

Several mimotope displaying phages have been isolated and tested to confirm their epitopemimicking nature in phage based competitive immunoassyas. The best peptide MPA-mimetic was selected to develop a recombinant fusion protein with a bioluminiscent enzyme which can be directly used as the tracer in competitive immunoassays without the need of secondary antibodies or further labelling. A bioluminiscent sensor, using streptavidin-coupled magnetic beads for the immobilization of the biotinylated Fab, has enabled the determination of MPA with a detection limit 0.70 nM and a IC_{50} of 9 ± 1 nM. The biosensor showed a good selectivity to MPA and has been applied to the analysis of the immunosuppressive drug in clinical samples, of both healthy and MPA-treated patients, followed by validation by HPLC-DAD.
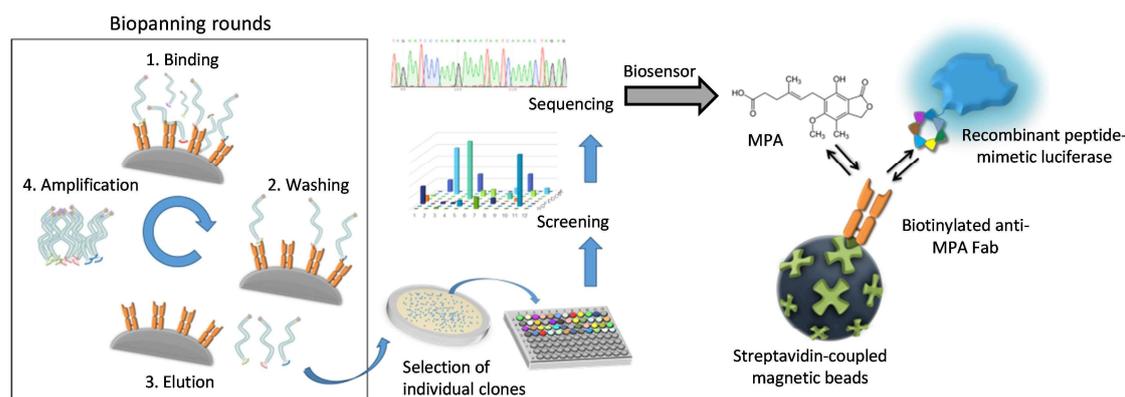
Figure 1. General scheme for phage display process and the developed biosensor

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Functional immobilization of small molecules by carbene mediated photo-linking for label-free detection

Günther Proll [1], Peter Fechner [1]

guenther.proll@uni-tuebingen.de (Corresponding e-mail address)

[1] Institute of Physical and Theoretical Chemistry, Eberhard Karls University Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

Abstract: The characterization of the interaction between small molecules and proteins (e.g. receptors) is a key bioanalytical question not only in drug discovery, but also in effect directed environmental analysis (Fechner, 2011). Huge effort has been made to develop label-free technologies capable for time-resolved measurement of small molecules binding to immobilized proteins to gain access to kinetic and thermodynamic constants. For most relevant applications in this field the time for analysis scales with the of small molecules being investigated rather than by the number of proteins/receptors, therefore multiplexing is mandatory. This led to the development of label-free biosensor devices, mainly based on surface plasmon resonance (SPR), offering several independent flow-cells to perform these biomolecular interaction studies in an automated way with reasonable throughput. However, screening of hundreds or even thousands of interactions is still very timeconsuming and challenging. To profit from the multiplexing capabilities of imaging-based versions of label-free biosensor like e.g. iSPR or SCORE(single colour reflectometry) it is necessary to switch the assay format towards immobilized small molecules detecting the binding of proteins out of the homogeneous phase. This logical development sounds very simple, but it comprises another technical challenge, which is the covalent immobilization of small molecules without destroying functional groups / altering their 3D-structere too much (resulting in a negative impact on the recognition/binding by the receptor).

Recently, scientists from the RIKEN institute proposed a carbene mediated photo-linking approach for the covalent immobilization of small molecules to e.g. microarray surfaces. The advantage of this chemistry is the covalent linkage of the small molecules to the biosensor transducer surface by introducing a C-C bond without attacking functional groups. As a result, the compounds are immobilized randomly in a "gentle" way maintaining their interaction capabilities with a specific receptor.
We present data from a SCORE based interaction study between small molecules and proteins/antibodies as a proof-of-concept to introduce this surface chemistry approach into emerging fields of bioanalytics.

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Liposome-based surface plasmon resonance (SPR) sensor for mono-Rhamnolipid detection

Meryem Belkilani[1,2,4]*, Carole Farre[1], Yves Chevalier[3], Adnane Abdelghani[4], Nicole Jaffrézic-

Renault[1], Carole Chaix[1]

*belkilanimeryem@hotmail.com

[1]Université de Lyon, CNRS, UCBL, Institut des Sciences Analytiques, Villeurbanne, France [2]Tunis University, ENSIT, Avenue Taha Hussein, Montfleury, 1008 Tunis, Tunisia.
[3]Université de Lyon, CNRS, UCBL, LAGEPP, Villeurbanne, France
[4]Carthage University, National Institute of Applied Science and Technology, Research Unit of Nanobiotechnology and Valorisation of Medicinal Plants, Bp 676, 1080 Charguia Cedex, Tunisia.

Abstract: Rhamnolipids are secondary metabolites naturally produced by bacteria Pseudomonas aeruginosa, with biosurfactant properties. They constitute the virulence factor of these bacteria and they are responsible of the fact that Pseudomonas aeruginosa causes chronic respiratory infections in cystic fibrosis and immunocompromised individuals [1]. Due to the numerous properties of rhamnolipids, there is a high need to describe the interaction of rhamnolipids with cell membranes. Therefore, this work aims to study the interaction between mono-rhamnolipids (mono-RLs) and biotinylated phospholipid based liposomes, immobilized on the gold surface of a chip dedicated to multi-parametric surface plasmon resonance (MP-SPR) technology. The efficiency of the liposome coating on the SPR gold chip was proven as a strong binding of the biotinylated liposomes on a streptavidin monolayer led to a SPR signal enhancement which was modeled through the MP-SPR method. A thickness of the liposome layer of ~79 nm was calculated by MP-SPR mathematic modeling, fitting with the average diameter of the liposomes. Then, the lysis action of the mono-RLs against the biotinylated phospholipid based liposome film was studied. The morphological change of the liposome layer was then described, based on the mathematical modeling of the SPR signals. We demonstrated the capacity of the MP-SPR technique to characterize the different steps of the liposome architecture evolution, i.e. from a monolayer of lipid vesicles to a single lipid bilayer induced by the interaction with mono-RLs, and then a lipid monolayer resulting from Triton X-100 (1%) treatment. At last, our method based on a biomimetic membrane coupled to a SPR measurement also proved to be a robust and sensitive analytical tool promoting the detection of mono-RLs with a detection limit of 2 μg mL^{-1}.
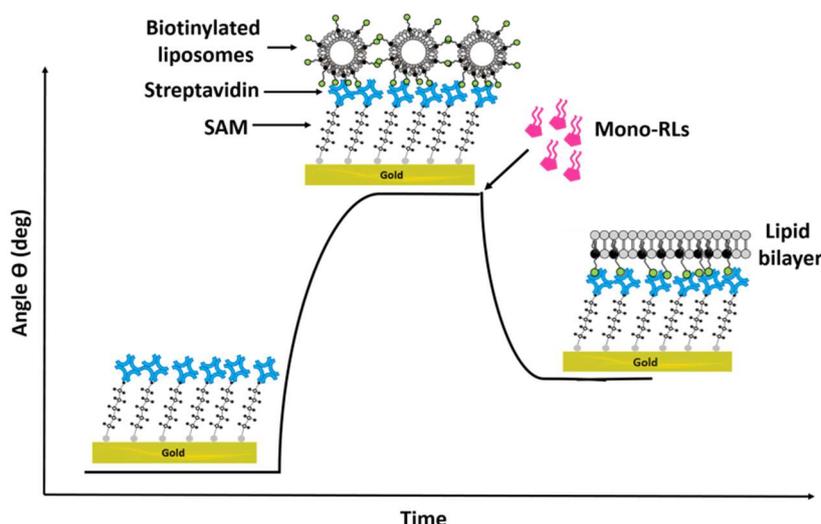
Figure 1: SPR signal corresponding to the different states of the sensor surface (immobilization of liposomes and lysis by mono-rhamnolipids)

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Improved Electrokinetic Detection Of Extracellular Vesicles Through Surface Charge Tuning

S. S. Sahu[1,*], P. Hååg[2], A. E. Karlström[3], K. Viktorsson[2], R. Lewensohn[2,4], J. Linnros[5], A. Dev[1,5]

*e-mail: siddharth.sahu@angstrom.uu.se

[1]Department of Electrical Engineering, Uppsala University, Uppsala, Sweden.
[2]Department of Oncology/Pathology, Karolinska Institute, Stockholm, Sweden.
[3]Department of Protein Science, KTH Royal Institute of Technology, Stockholm, Sweden
[4]Theme Cancer, Medical Unit head and neck, lung, and skin tumors, Thoracic Oncology Center, Karolinska University Hospital, Stockholm, Sweden.
[5]Department of Applied Physics, KTH Royal Institute of Technology, Stockholm, Sweden

Abstract: Small extracellular vesicles (sEVs), also known as exosomes, play a vital role in intracellular communication and have hence have attracted a lot of interest as a source of biomarkers in cancer diagnostics [1]. Detection of sEVs through streaming current method has already been demonstrated for sEVs from tumor cells using different surface markers [1]. However, a better understanding, through both theoretical and experimental means, of the role of surface charge of the sensor in electrokinetic biosensing [2,3] has opened up the scope of enhancing the signal from sEV detection. Here the charge contrast between the negatively charged sEVs and the sensor surface was increased by coating the sensor surface with biotinylated poly-L-lysine, followed by linkage to avidin, both of which are positively charged. This was followed by immobilization of anti-CD9 capture probes. A detection signal of ~23 mV was observed for the surface protein CD9 on sEVs derived from the non-small cell lung cancer cell line (NSCLC) H1975. This is nearly a 10-fold improvement as compared to the previously reported method in which the functionalization approach involved silanization and glutaraldehyde linkage to the capture probes [1]. To test the influence of surface charge further, negatively charged streptavidin was used as the linker instead of avidin. In this case, the signal was 7.7 mV. The baselines for the previous method and the new methods with streptavidin and avidin as the linkers was -32.5, -10.6, -2.5 mV respectively. Hence, the improvement in the signal is clearly a result of charge modulation of the sensor surface: the surface gets less negative before capturing negatively charged sEVs. This is consistent with theoretical predictions [2,3]. A series of concentration-dependent measurements is planned to determine the improvement in the limit of detection as compared to the previous results, along with probing the presence of other surface markers. Subsequently, this shall be used to monitor EGFR tyrosine kinase treatment responses in sEVs from NSCLC cells by comparing the expression level of surface markers in sEVs prior and post treatment.

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Abstracts

Poster presentations

Enzyme-free electrochemical nano-immunosensor for early diagnosis of acute myocardial infarction

Bhargav D. Mansuriya, Zeynep Altintas*

zeynep.altintas@tu-berlin.de (Corresponding e-mail address)

Technical University of Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany

Abstract: An ultrasensitive enzyme-free electrochemical nano-immunosensor, based on screen printed gold electrode (SPGE) modified with graphene quantum dots (GQDs) and gold nanoparticles (AuNPs), was engineered to detect cardiac troponin-I (cTnI) for the early diagnosis of acute myocardial infarction (AMI). The GQDs and in-house synthesized AuNPs were implanted onto SPGE and allowed for anti-cTnI immobilization, prior to quantifying cTnI. The biomarker could be determined in a wide concentration range using square wave voltammetry (SWV), cyclic voltammetry (CV), electron impedance spectroscopy (EIS) and amperometry.[1] The analyses were performed in buffer as well as in human serum in the investigation ranges of 1–1000 and 10–1000 pg mL^{-1}, respectively. The detection time ranged from 10.5–13 min, depending on the electrochemical method employed. The detection limit was calculated as 0.1 and 0.5 pg mL^{-1} for buffer and serum, respectively. The sensitivity of the immunosensor was found to be 6.81 µA cm^{-2} pg mL^{-1}, whereas the binding affinity was determined as < 0.89 pM. The sensor showed high specificity for cTnI with slight responses for the non-specific biomolecules. Each step of the sensor fabrication was characterized using CV, SWV, EIS and atomic force microscopy (AFM). Moreover, AuNPs, GQDs and their nanocomposites were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM).[1] This is the first immunosensor that represents the successful determination of an analyte using four different electrochemical techniques. Such a sensor would demonstrate a promising future for an on-site detection of AMI with its sensitivity, cost-effectiveness, rapidity and specificity.[1]
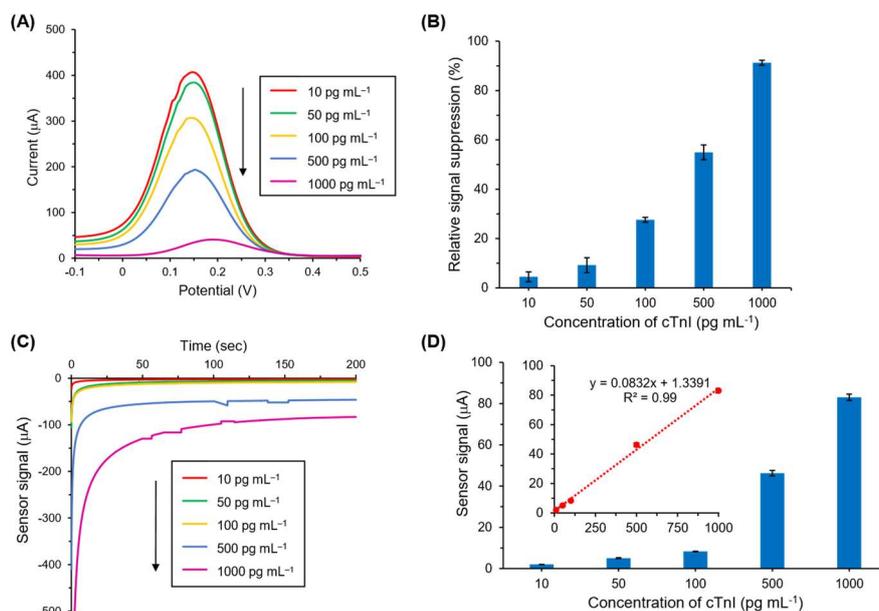
Figure 1: Square wave voltammetric (A, B) and amperometric (C, D) detection of cTnI by employing anticTnI/AuNPs@GQDs/SPGE sensor in human serum (n=3).

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Ultrasensitive nonenzymatic electrochemical glucose sensor based on gold nanoparticles and molecularly imprinted polymers

Ekin Sehit, Daniel Buchenau, Julia Drzazgowska, Zeynep Altintas*

zeynep.altintas@tu-berlin.de (Corresponding e-mail address)

Technical University of Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany

Abstract: A non-enzymatic electrochemical glucose sensor with high sensitivity and selectivity was developed using gold nanoparticles-decorated molecularly imprinted polymers (AuNP-MIPs). The AuNP-MIPs were synthesized on a gold surface by multistep amperometry using the optimized conditions and in-house synthesized gold nanoparticles in the presence of glucose as the template.[1] The electrochemical measurements for glucose binding on the AuNP-MIP sensor revealed a high affinity toward glucose with a dissociation constant (K_d) of $3×10^{-8}$ M whereas the MIPs without AuNPs could not detect even the highest concentration of the investigation range (1.25 nM–2.56 µM).[1] The comparative rebinding studies with AuNP-MIP and non-imprinted polymer exhibited an excellent selectivity toward glucose. The specificity of AuNP-MIP sensor was further investigated by studying with interfering compounds resulting in negligible cross-reactivity except for sucrose. The behavior of imprinted polymers in fluid solvents was also investigated by employing the atomic force microscopy for the first time. The sensor could detect glucose in human serum with a detection limit of 1.25 nM and preserved its stability up to around 95% during a storage time of 40 days.[1] Hence, such a sensor demonstrates a promising future for the detection of clinically relevant small molecules with its facile, cheap, and highly sensitive nature.
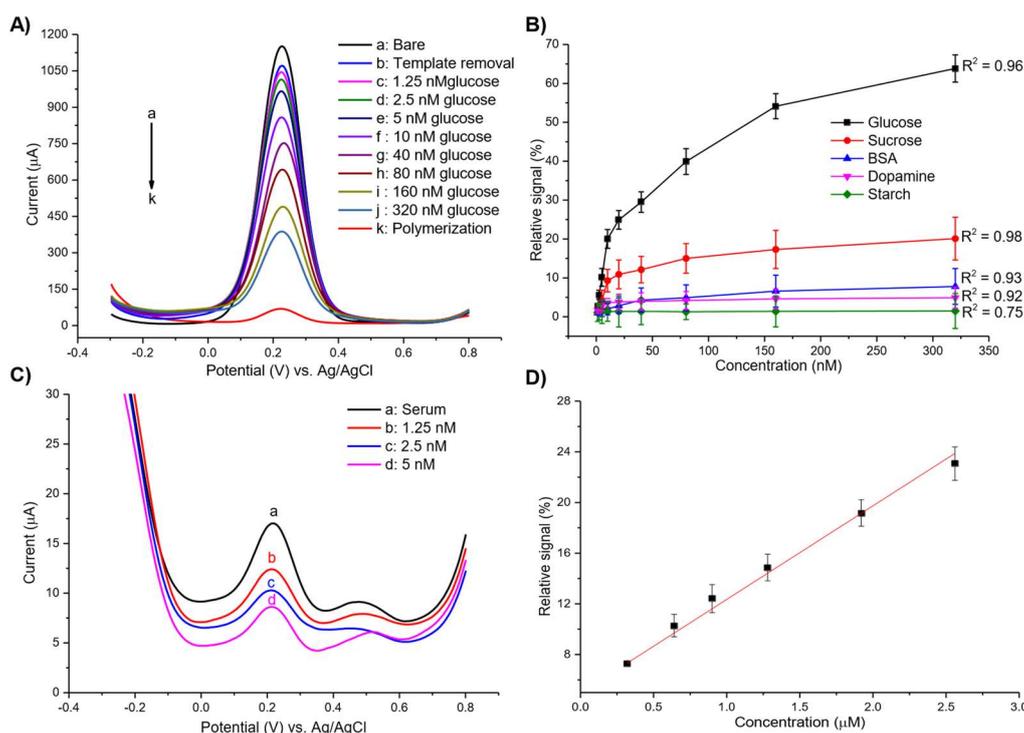
*Figure 1: **A)** Square wave voltammograms recorded for the concentration dependent recognition of glucose in PBS (pH =7.4). **B)** The cross-reactivity of reference molecules (dopamine, starch, sucrose and BSA) as well as glucose on the AuNP-MIP sensor in the concentration range of 1.25–320 nM (n = 6). **C)** The square wave voltammetry measurement of glucose in serum. **D)** The concentration dependent detection of glucose in serum (n = 3).*

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Cancer biomarker detection in human serum samples using nanoparticle decorated epitope-mediated hybrid MIP

Muqsit Pirzada, Ekin Sehit, Zeynep Altintas*

zeynep.altintas@tu-berlin.de (Corresponding e-mail address)

Technical University of Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany

Abstract: Early-stage diagnosis of diseases is possible by evaluating the concentrations of their specific biomarkers. Herein, an ultrasensitive electrochemical sensor based on hybrid epitope imprinting and nanomaterial amplification was developed by electropolymerization of the functional monomer in the presence of two computationally selected[1] and cysteine modified epitopes of neuron specific enolase (NSE) and gold nanoparticles (AuNPs).[2] The AuNPs decorated as well as the standard hybrid MIPs, were employed for the fabrication of electrochemical sensors to demonstrate the contribution of the AuNPs to the polymer network for sensing. The synthesis process of both sensors was studied with voltammetric, microscopic and spectroscopic techniques.[2] The NSE assay with the standard hybrid MIPs resulted in 2.5-fold higher sensitivity in comparison to single epitope imprints, whereas the AuNP-hybrid MIPs enhanced the sensitivity level to a great extent and facilitated NSE detection in human serum in the range of 25–4000 pg/mL.[2] Assays against a negative control resulted in an imprinting factor of 4.2, confirming the high target selectivity of AuNP-MIP cavities. Cross-reaction of the sensor with interferents was negligible. The AuNP hybrid epitope-MIPs have provided more desirable sensing platforms with high sensitivity, affinity and specificity in comparison to conventional epitope imprinting methods.
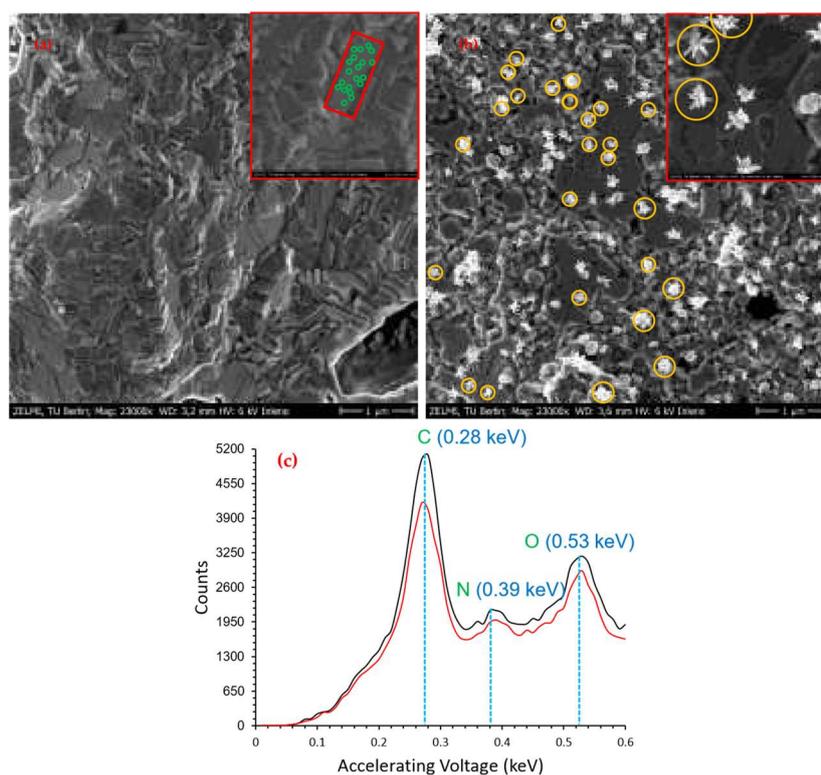
Figure 1: SEM images of (a) AuNP-MIP and (b) AuNP-NIP. The red box is an area on the polymer film in which the pills (green circles) were counted. The yellow circles represent AuNP aggregates. (c) EDX spectrum of AuNP–MIP before (black) and after (red) template removal.

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MIP based impedimetric sensor for a chronic disease marker

Tiziano Di Giulio, Elisabetta Mazzotta, Cosimino Malitesta,

tiziano.digiulio@unisalento.it (Corresponding e-mail address)

Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (Di.S.Te.B.A.), Università del Salento, Italy

Lysozyme (Lyz) is a small (14.6 kDa) but powerful antimicrobial enzyme commonly found in nature, able to damage the cell walls of susceptible bacteria by promoting the lysis of peptidoglycans, constituents of the external coating of these microrganisms. In humans it has been found in body fluids such as saliva, tears, sperm secretions, nasal mucus and urine. Changes in Lyz levels can be a symptom of a pathological condition. For example, there is an increase in lysozyme concentrations in case of oral infections, monocytic leukemia, Crohn's disease, sarcoidosis and renal tubular damage, resulting in elevated saliva, serum and urine levels [1]. For these reasons, it is essential to have systems capable of determining lysozyme in food, pharmaceutical and medical fields.

Herein we report the electropolymerization of a scopoletin based molecularly imprinted polymer (MIP) for the detection of Lyz. Molecular imprinting is a technique for the synthesis of artificial recognition sites in polymeric matrix which are complementary to the target molecules in their shape and the functional groups [2]. Two different approaches have been used for the imprinting of lysozyme based, respectively, on the use of a monomer-template mixture and on the covalent immobilization of the enzyme prior to polymer synthesis. In the second case, a multi-step protocol has been exploited with preliminary functionalization of gold electrode with amino groups, via 4aminothiophenol, followed by reaction with glutaraldehyde, to provide a suitable linker for lysozyme. Each step of surface electrode modification has been followed by cyclic voltammetry and electrochemical impedance spectroscopy (EIS). In both cases, MIP layer was synthesized via electropolymerization by multistep amperometry technique [3], applying 50 pulse pairs, starting with 0 V for 5 s and followed by 0.9 V for 1s. To obtain lysozyme-imprinted cavities, the template molecules were removed from the polymer matrix dipping the electrode consecutively in different aqueous solutions (5 mL) under stirring (200 rpm): i) 50 mM NaOH for 15 min; ii) SDS / acetic acid, 2.5% (w/v) and 5% (v/v) respectively, for 10 min; iii) ultra-pure water for 5 min. EIS has been employed to test the electrochemical responses of the developed MIPs. The sensors showed good ability to detect the enzyme at concentrations up to 292 mg/L (20 µM), but with different performances, depending on the used imprinting approach. An imprinting factor equal to 7.1 and 2.5 and a limit of detection of 0.9 mg/L (62 nM) and 2.1 mg/L (141 nM), have been estimated for MIPs prepared with and without enzyme immobilization, respectively. MIP selectivity was also tested by competitive rebinding experiments and tests in synthetic saliva demonstrated the potential application of the sensors in real matrices for clinical purposes.

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Electrochemical sensor based on electrosynthesised ion imprinted polymeric film for Cd^{2+} ions determination in water samples

Marco Costa [1], Sabrina Di Masi [1], Cosimino Malitesta [1]

sabrina.dimasi@unisalento.it (Corresponding e-mail address)

[1]Laboratorio di Chimica Analitica, Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Via Monteroni, 73100, Lecce (Italia)

Abstract: Imprinted polymers are devoted to artificial materials able to mimic recognition processes of such analytes, ranging from proteins, small molecules to ions. The recognition process results in the formation of ion-sized selective imprinted cavities, which are complementary to a specific template in terms of its functional groups. These materials can be easily applied to identify, monitor and remove the target ions in water environment [1]. In this view, the ion imprinted polymers (IIPs) can be introduced. The synthesis can be carried out both chemically and electrochemically, the latter producing the imprinted films, which are compatible in conjunction with transducers in sensor development. Our recent work reports the electrochemical synthesis of ion imprinted polymers and their application as sensors for metal ion detection [2]. With this regard, we propose the synthesis, optimisation, characterisation and subsequent application of an electrosynthesised IIPs for the electrochemical detection of Cd^{2+} ions in water. The proposed sensor (Cd^{2+}-IIP) was prepared by electropolymerisation of 4-aminophenylacetic acid (4-APA) monomer in presence of Cd^{2+} ions, which was the template. The screen-printed carbon electrodes (SPCE) were used as transducer during sensor development, whereas the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were selected as the electrochemical methods for the synthesis and Cd^{2+} ions sensing, respectively. The incubation of the developed sensor in NaOH 250 mM (3 min) involved into remove the template and the formation of specific recognition cavities into the polymer (Figure 1). FT-IR characterisation revealed the differences between the polymers in terms of functional groups, and it was also performed to ensure the template removal. SEM images of the proposed sensors confirmed differences between polymers. A multivariate optimisation was employed for studying the effect of three independent parameters on electrochemical performances of the sensor. The electrochemical characterisation of sensors was performed in ferrocyanide-ferricyanide redox couple, revealing redox properties from the polymeric film. The performances of sensors and the control (NIP) was observed in sodium acetate buffer (50 mM, pH = 5) over the Cd^{2+} concentration range 0.1 – 10 μM. An imprinting factor of almost 6 confirmed the high specificity of Cd^{2+}-IIP than NIP. Selectivity studies of the Cd^{2+}-IIP sensor against potential ion interferents were also carried out in order to confirm the selectivity of the imprinted cavities on the polymeric network.
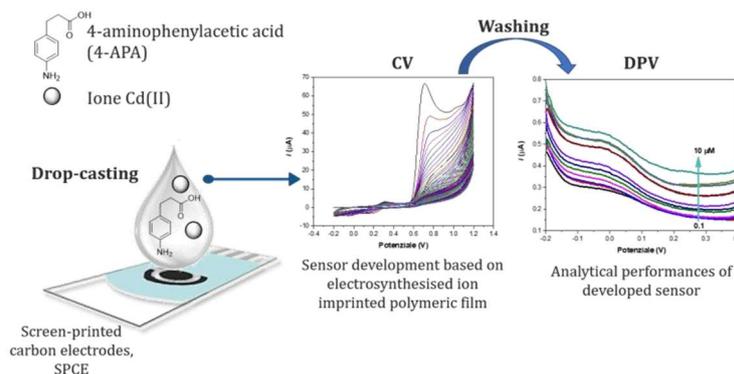
Figure 1: Scheme of preparation and electrochemical performances of Cd^{2+}-IIP sensor.

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Rapid ultrasound-assisted synthesis of MIPs for sulfonamides

Dounia Elfadil[1, 2], Flavio Della Pelle[1], Aziz Amine[2,*], Dario Compagnone[1,*]

(delfadil@unite.it)

[1]Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64023 Teramo, Italy.
[2]Laboratoire Génie des Procédés & Environnement, Faculté des Sciences et Techniques, Hassan II University of Casablanca,B.P. 146. Mohammedia, Morocco.

Molecularly imprinted polymers (MIPs) are synthetic materials used as biomimetic molecular recognition receptors [1]. In this work, we present a novel combination of magnetic molecularly imprinted polymers and enzymatic inhibition for sulfonamides determination.

In this study, we present a general method to prepare bulk and core-shell magnetic molecularly imprinted polymers (MMIPs) nanoparticles (NPs) for Acetazolamide (ACZ), and sulfamethoxazole (SMX). The synthesis of magnetic molecularly imprinted polymers is based on the use of high-power ultrasound using methacrylic acid, 4-vinylpyridine and methacrylamide as functional monomers, ethylene glycol dimethacrylate as a crosslinker, azobisisobutyronitrile as radical initiator and dimethylsulfoxide as porogen solvent was done in 5 min instead of 16 h (conventional synthesis).

The resulting Fe3O4@MIPs NPs displayed fast adsorption and high adsorption capacity with an IF of 2.7 for acetazolamide MIP, and can be applied in a selective solid-phase extraction method for sulfonamides determination.The developed Fe3O4@MIPs NPs will be combined with enzymatic inhibition method for the selective determination of ACZ. The ACZ can be determined using Fe3O4@MIPs NPs in solid-phase extraction, and sulfanilamide can be determined using ACZ@MIPs to remove selectively the ACZ form the sample.

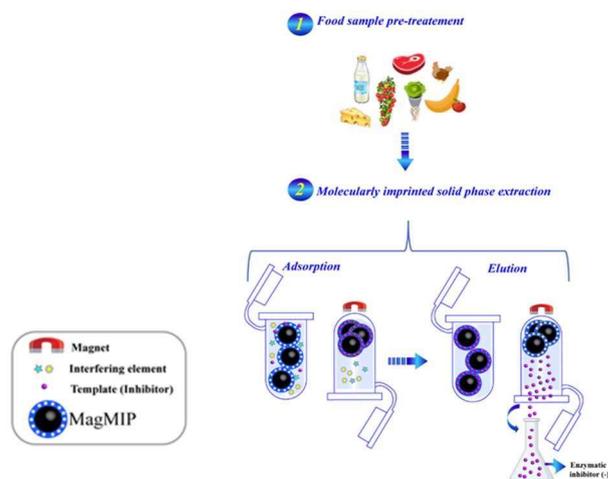Keywords: Molecularly imprinted polymer, Acetazolamide, solid-phase extraction, Sufonamides.

Figure 1: Overview on the application of Fe3O4@MIPs NPs in solid phase extraction of sulfonamides

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Binding affinity analysis and applications of DNA aptamers for therapeutic anthracyclines

Marcus Menger

Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Functional Nucleic Acids – Aptamers, 14476 Potsdam, Germany

Aptamers are single-stranded RNA or DNA nucleotides, which are generated using an iterative in vitro selection procedure (SELEX) and bind to target molecules with high specificity and high affinity due to their specific tertiary structures. Thus, aptamers are preferable interaction molecules in many different analytical systems like biosensors or lateral flow assays. Anthracyclines such as daunorubicin (DRN) and doxorubicin (DOX) are key chemotherapeutic substances in cancer treatment since decades. However, their chronic administration can induce severe side effects. The development of new nanocarriers for targeted drug delivery could probably minimize these side effects, and aptamers seem to be favorable molecular targeting agents. Here, we present the binding properties of a single-stranded DNA aptamer, which has been previously generated against DRN. For binding analysis methods like the label-free surface plasmon resonance (SPR) spectroscopy and immobilization-free microscale thermophoresis (MST) were used. Additionally, the aptamer could be transferred in applications like lateral flow device and electrochemical aptasensor.

Electrosynthesis of a molecularly imprinted poly(metalloporphyrin) for the selective detection of carnosine

T. Di Giulio, E. Mazzotta, A. Flemma, A. Barca, T. Verri, C. Malitesta

elisabetta.mazzotta@unisalento.it (Corresponding e-mail address)

Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (Di.S.Te.B.A.), Università del Salento, Italy

Carnosine is a dipeptide (β-alanine-L-histidine) which plays several homeostatic roles in human excitable tissues [1], including anti-oxidation, anti-glycation, anti-aging effects, pH buffering and chelation of divalent metal cations. Recent studies have shown that this small molecule acts as selective inhibitor of growth of neoplastic cell lines and it can aid the immune response against viral infections proving to be a promising tool in the treatment of patients affected by Covid-19 [2]. For the determination of carnosine various separative analytical techniques have been employed, which however, require equipped laboratories, long analysis time and additional sample pretreatment. Towards sensing of carnosine, only few works are reported so far [3-4].

Herein we propose the development of an electrochemical sensor for carnosine based on imprinted electropolymerized metallo-porphyrins. Molecular imprinting is a versatile technique for the synthesis of polymers with molecular memory (MIPs, Molecularly Imprinted Polymers), containing cavities able to selectively recognize target analytes. This work represents the first attempt to the electrosynthesis of MIPs for carnosine based on Zn- and Cu-porphyrin as monomers (namely, Zn(II)- and Cu(II)-5,10,15,20-tetrakis(4-aminophenyl)porphyrin), with the aim to get advantages from the chelating ability of carnosine towards Zn and Cu, thus achieving high-affinity MIPs. Metalloporphyrin layers are electropolymerized by Cyclic Voltammetry (CV). Different experimental conditions have been explored (scan number/rate, potential range, monomer:template ratio, electrolyte concentration, mixture of solvents) and the optimized ones consist in the application of a potential from 0 to 1.1 V vs SCE at 100 mV s^{-1} for 15 cycles in a solution of 0.1 mM metalloporphyrin containing 0.1 mM carnosine in ACN/H_2O (9:1, v/v). CV polymerization curves are slightly modified in the case of Zn-porphyrin in presence of carnosine, possibly due to the interaction with the target. The imprinted electrode is then washed in NaOH 50 mM, under stirring for 10 minutes, until the complete removal of carnosine from the polymeric matrix is achieved, as evidenced by CV and Electrochemical Impedance Spectroscopy (EIS) tests. Fourier transform infrared (FT-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS) were used to characterize the synthesized polymers, before and after the washing procedure. FT-IR results showed successful deposition of metalloporphyrin polymeric layers for both porphyrins and suggested higher affinity between carnosine and Zn-porphyrin, demonstrated by signals attributable to target in IR spectra of MIP film (typical peak centered at 1570 cm^{-1} associated with stretching frequencies of the imidazolium ring). Such evidences were further confirmed by XPS results, showing a chemical shift of the Zn signal in the polymer film containing carnosine. CV and EIS were used also for preliminary rebinding studies demonstrating MIP-sensor ability to detect carnosine at concentrations up to 1 mM.

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An electrochemical assay based on a bicyclic peptide for urokinase-type plasminogen activator (uPA) determination

Patrick Severin Sfragano [1], Francesca Brogi [1], Anna Rafaniello [2], Alessandro Angelini [2], Federico Polo [2], and Ilaria Palchetti [1,*]

*Correspondence: ilaria.palchetti@unifi.it

[1]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy.
[2]Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venice, Italy.

Abstract: Urokinase-type plasminogen activator (uPA) is considered an important biomarker for the diagnosis and prognosis of a variety of neoplasia. The uPA protein, together with the tissue plasminogen activator (tPA), the urokinase-type plasminogen activator receptor (uPAR) and plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2), compose the plasminogen activator system, an extracellular enzyme complex involved in physiological and pathological phenomena, which also comprehend tumour growth and development. uPA's main function is to convert plasminogen into plasmin, a proteolytical enzyme of the serine proteases family, able to degrade the extracellular matrix and promote the diffusion process of cancer inside the organism. Recent experimental evidence demonstrated that high levels of uPA are often associated with metastatic evolutions of the tumour, shorter survivability times and, in general, adverse prognoses for the patient. Our work aimed at developing a sandwich-type affinity electrochemical assay for the detection and quantification of uPA protein. The novelty is the use of a novel and *ad-hoc* synthesized bioreceptor for uPA: a biotinylated bicyclic peptide with two binding sites for uPA, located onto the two cycles that characterize its chemical structure. This bicyclic peptide was immobilized onto the surface of streptavidin-modified magnetic microbeads. After the immobilization, uPA protein was determined by using an anti-urokinase antibody, conjugated with alkaline phosphatase. The electroactive product of the enzymatic reaction, 1-naphthol, was determined by differential pulse voltammetry. The current measured is proportional to the concentration of uPA in the sample. Miniaturized screen-printed disposable sensors were used, and the operative conditions of the electrochemical assay were optimized. The described procedure allowed the quantitative determination of uPA protein in standard solutions at concentrations of clinical interest.

Molecularly imprinted polymer nanoparticles for Human Serum Albumin (HSA) assay using Quartz Crystal Microbalance (QCM)

Wisnu Arfian A. Sudjarwo[1], Mathias T. Dobler[1], Peter A. Lieberzeit[1]

wisnu.arfian.anditya.sudjarwo@univie.ac.at; peter.lieberzeit@univie.ac.at

[1]Universität Wien, Institut für Physikalische Chemie, Währinger Straße 42, 1090 Wien, Austria

Abstract: The level of Human Serum Albumin (HSA), which is the most abundant protein in human body, can be used as a marker for liver function: hypoalbuminemia, kidney disease and hepatocellular carcinoma are examples of diseases which lead to abnormal HSA levels[1,2,3]. Therefore, detection of HSA is an important aspect for diagnostics. Herein, we report on the synthesis of molecularly imprinted nanoparticles binding to HSA as probes for Quartz Crystal Microbalance (QCM) assays. The nanoparticle receptors were synthesized using the so-called "nano-MIP" technique published by the group of S. Piletsky[4]. The nanoMIPs were thus generated using solid phase synthesis. Commencing with anchoring HSA as a template in silica surface, the process involved polymerization to fabricate nanoparticles. Scanning Electron Microscope (SEM) and Dynamic Light Scattering (DLS) studies revealed nanoparticle diameters of 53±19 nm and 191±96 nm for nano-MIPs and nano-NIPs, respectively. Examining binding properties of nanoparticles using fluorescence spectroscopy was shown by Stern-Volmer plots in Fig.1 which reveal that the nano-MIPs bind HSA in a selective manner: the slopes of 0.0014 and 0.0008 between nano-MIPs and HSA as well as lysozyme, respectively. It is clearly seen that nano-MIPs are more selective to HSA than lysozyme, namely by a factor of 1.75, and more sensitive than nano-NIPs to HSA, namely by a factor of 14. Preliminary measurements with QCM show similar affinity patterns (Fig.2): the nano-MIPs bind to HSA molecules immobilized to the surface, but not to lysozyme. Neither do nano-NIPs lead to any measurable signal. This demonstrates that the approach is clearly feasible for developing an assay format toward those proteins.
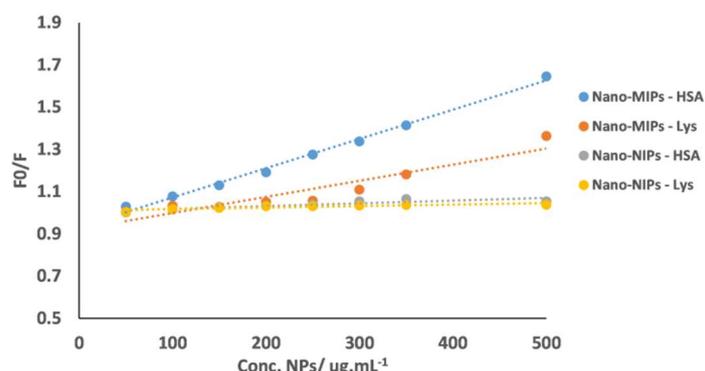
Figure 1. The Stern-Volmer plots of binding interaction between nanoparticles and HSA-Lysozyme.
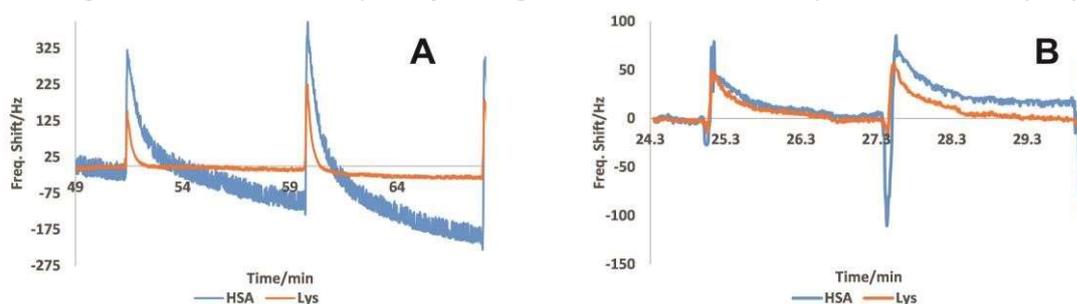

Figure 2. The investigation of affinity binding of nanoparticles toward protein. A) QCM measurement of nano-MIPs injection onto HSA and lysozyme- modified gold quartz, B) QCM measurement of nano-NIPs injection onto HSA and lysozyme- modified gold quartz, by injection of 100 and 200 ug/mL of nanoparticles

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Detecting pesticides with aptasensors.

Na Youn Cho[1], Cynthia Forier[1], Hubert Debreyne[1], Jean-Jacques Toulmé[1,2]

cynthia.forier@novaptech.com, Jean-Jacques.Toulme@inserm.fr

[1]Novaptech, Parc Scientifique Unitec 1, 2 Allée du Doyen Georges Brus, 33600 Pessac, France
[2]ARNA Laboratory, University of Bordeaux, 146 rue Léo Saignat, 33076, Bordeaux, France

Abstract:

The need to detect small molecules (<1000 Daltons) such as residues of pesticides or drugs, toxins, antibiotics or illegal drugs is of utmost importance for protecting the human health and the surrounding environment. It is important to detect these molecules by sensitive and rapid assays preferably on site. Standard detection methods imply complicated and time-consuming analysis carried out in remote laboratories by qualified scientists on expensive instruments. To overcome these limitations, biosensors offer a promising alternative. Aptamers are innovative recognition elements that display interesting characteristics and that can be easily included in sensing devices (aptasensors) [1,4].

Aptamers are single-stranded oligonucleotides that bind to their target with high affinity and specificity. They can be identified in vitro against a wide variety of targets including toxic molecules as their selection is performed in vitro. They are highly stable and are chemically synthesized ensuring guaranteed sourcing at an affordable price.

The increased use of pesticides has become a global issue worldwide. They persist for a long time in soil, get absorbed by plants entering the food chain, and contaminate water [5]. This is the case for widely used fungicides. We selected aptamers to two different but chemically related fungicides (BF1 and BF2).

Selection of DNA aptamers was carried out over 10 selection rounds against free BF1 and BF2 in solution. The selection against BF1 led to aptamers characterized by a Kd in the low micromolar range. These aptamers also recognized BF2 but with a lower affinity. We then performed a second selection against BF2 including a strong negative selection step against BF1, starting from one of the early oligonucleotide pool selected against BF1. Aptamers recognizing BF2 were thus obtained. A fluorescent BF1 and BF2 aptamer-based assay was designed for the specific detection of fungicides.

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Strep-Tag II-Imprinted Polymer for the Recognition of Recombinant Proteins

Aysu Yarman[1], Giorgio Caserta[2], Paloma Borrero[1], Stefan Frielingsdorf[2], Eszter Supala[3], Armel T. Waffo[2], Sevinc Kurbanoglu[4], Bettina Neumann[1], Ingo Zebger[2], Oliver Lenz[2], Róbert E. Gyurcsányi[3], Ulla Wollenberger[1], Frieder W. Scheller[1]

aysu.yarman@yahoo.de

[1]Universität Potsdam, Institute for Biochemistry and Biology, Potsdam, Germany
[2]Technische Universität Berlin, Institut für Chemie Berlin, Germany
[3]Budapest University of Technology and Economics, Budapest, Hungary
[4]Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey

Abstract: As new technique for surface structuring at molecular level, molecularly imprinted polymer layer (MIP) has emerged. MIPs are prepared by polymerizing one to six functional monomers (with or without cross-linkers) in the presence of template. Subsequent template removal from the polymer results in the formation of cavities that mirror the shape of the template. The application of MIPs using (bio)macromolecules as targets has been so far limited due to their size, instability, and conformational changes as compared to those for low-molecular-weight-targets. To overcome these drawbacks, small fragments of the (bio)macromolecules have been used as templates. This procedure is termed as epitope imprinting [1-3].

In this work, we used the Strep-tag II (ST) peptide as template. It is an eight amino acid containing affinity tag, which is commonly genetically added to the C- or N-terminus of a recombinant protein to enable its purification using Strep-Tactin® columns. Herein we explored two commonly applied procedures for the development of protein-imprinted polymers. In the first procedure, polymer nanofilms were prepared by a random single-step approach consisting of the electropolymerization of scopoletin and ST peptide mixtures. In the second procedure the MIP was prepared hierarchically via a two-step synthesis that involved electropolymerization of scopoletin after the chemisorption of the cys-extended ST.

All steps of MIPsynthesis and rebinding were analyzed by i) evaluating the permeation of the redox marker "ferricyanide" through the MIP nanofilm to the electrode via i) differential pulse voltammetry (DPV), ii) atomic force microscopy and iii) surface-enhanced infrared absorption (SEIRA) spectroscopy. DPV results demonstrated that the binding of ST to the MIPs prepared by random and hierarchical methods showed linear ranges up to 100 nM and 6 nM, respectively. Varying just a single amino acid of the ST sequence significantly reduced its affinity to the MIPs prepared by either of the two procedures. The effect proved stronger on the MIP prepared using the random approach. Likewise, the cross-reactivity tests studied with the MIP prepared hierarchically showed that removal of the terminal tryptophan resulted in an almost 20% lower signal suppression. On the other hand, substitution of glutamate with the uncharged glutamine reduced the peptide binding by 75 % underlying the importance of electrostatic interactions. The dipeptide aspartame, used as control, showed only a very low binding affinity.

Furthermore, the Strep-tag II tagged membrane-bound hydrogenase (MBH) from *Ralstonia eutropha* and the bacterial alkaline phosphatase were bound to the MIP and their enzymatic activities were measured by means of cyclic voltammetry and amperometry, respectively. SEIRA spectroscopy revealed a specific MBH binding to the MIP via its ST and only minute non-specific binding to the control non-imprinted polymer, which lacks cavities for the recognition of ST. Moreover, MBH carrying a his$_6$-tag in place of ST did not bind to the ST-imprinted MIPs, confirming the specificity of recognition.

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Specific and fast 16S rRNA-based detection of sepsis pathogens using PCR amplification and microarray hybridization

L.-M. Broweleit[1, 2], C. Warmt[1]*, J. Henkel[1], F.F. Bier[2, 3]

*Corresponding author, E-Mail: Christian.Warmt@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB), 14476 Potsdam, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: Sepsis is an acute life-threatening organ dysfunction caused by a misdirected immune response following an infection. It is one of the most serious complications of infectious diseases, which is usually triggered by bacteria. [1]

The diagnosis for the treatment of sepsis based on three pillars: the microbiological diagnosis to secure the pathogen, the focus search to identify the source of infection and the laboratory diagnosis to record the status of the patient. [1] An early microbiological diagnosis of sepsis and a rapid antibiotic initial therapy are of enormous importance, because there is a connection between the time of therapy start and mortality: every hour without medical care reduces the chance of survival by 7.6%.[2]

Our aim is the development of an acute diagnostic method for the intensive care medicine to guarantee the immediate and specific detection of eleven bacterial sepsis pathogens at the same time as a multiplex analysis.

For this purpose, specific fragments of the DNA, based on the 16S rRNA-Gen, were amplified using the Polymerase chain reaction (PCR) with the fluorescent dye Cy5, which is incorporated into the DNA during amplification. The resulting dye-labeled DNA amplicons were hybridized on complementary, highly specific probes, which are immobilized on the surface of a 3D-Epoxy Slide. More than 60 different probes (> 300 Spots in a 2.5x4 mm Array) were spotted on the microarrays we use (Figure 1(a)). It is currently possible to detect 6 out of 11 pathogens using this method. Figure 1 shows the detection of the S. aureus pathogen as an example. Only the specific probes for the S. aureus pathogen shows significantly higher fluorescence signals (Figure 1(b)).
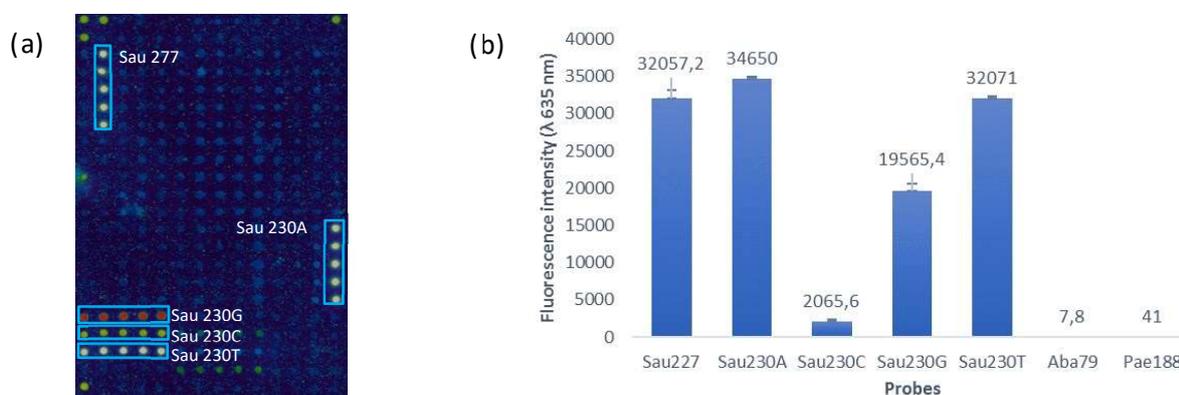

Figure 1: (a) Scan of the microarray slide. Shows the hybridization of the S. aureus pathogens. The Sau227 probe is specific for the pathogens S. aureus, S. epidermidis and S. warneri. Sau230A represents the high probe when S. aureus is present. (b) Probe comparison for the S. aureus pathogen. The mean fluorescence signals on the vertical axis are plotted against the probes on the horizontal axis.

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Effect of the electrical transport properties of carbon nanomaterials applied to the design of electrochemical DNA biosensors.

Julián Cobos-Suárez[1,2]. Wilfredo Valdivieso Quintero[1]. Viviana Sánchez-Torres[2]. J.A. Pedraza-Avella[2]. J.L. Ropero-Vega[1*].

jose.ropero@udes.edu.co

[1] Facultad de Ciencias Exactas, Naturales y Agropecuarias, Ciencias Básicas y Aplicadas para la SostenibilidadCIBAS, Universidad de Santander, Calle 70 No. 55-210, C.P. 680003 Bucaramanga Santander, Colombia.
[2] Grupo de Investigaciones en Minerales, Biohidrometalurgia y Ambiente – GIMBA, Universidad Industrial de Santander, Sede Guatiguará, Calle 8N, No. 3W-60 El Refugio, C.P. 681011 Piedecuesta Santander, Colombia.

The development of electrochemical DNA biosensors has been favorable in finding alternatives for measuring pathogens and other interest targets. Among these alternatives, it is worth noting the importance of nanomaterials in the design of biosensors on all their components due to their conductive properties on the electrode surfaces that provide high sensitivity and selectivity to the devices. Despite many reported studies for different electrochemical biosensors modified with nanomaterials, only a couple of publications are related to each nanomaterial electrochemical performance or even its synergy. This work presents different configurations in the deposition by adsorption of carbon nanostructures (graphene oxide (GO), multi-wall carbon nanotube (MWCNT)) on screen-printed electrodes to study their electrochemical performance for the detection of E. coli DNA (dsDNA). Gold nanoparticles (AuNPs) were obtained by chronoamperometry and used for the immobilization of capture probes (ssDNA) on the modified surface via thiol groups. The dispersion of the carbon nanostructures and the electrode modification process were characterized by Raman spectroscopy, UV-Vis spectroscopy, scanning electron microscopy (SEM), and electrochemical methods. The electrochemical measurements were obtained using Cyclic voltammetry (CV), Square wave voltammetry(SWV), and Electrochemical impedance spectroscopy (EIS) techniques; applied by VersaSTAT 3 potentiostat/galvanostat. The results showed that the biosensor with the incorporation of OG-MWCNT reached the highest electrochemical response than other configurations. This DNA biosensor can detect a DNA target in the range of 1.0×10^{-12} to 1.0×10^{-6} M. In addition, the DNA biosensor exhibits excellent selectivity of the complementary sequences from non-complementary sequences. These results reveal the impact of selecting a type of carbon nanomaterial to improve the biosensing parameters.
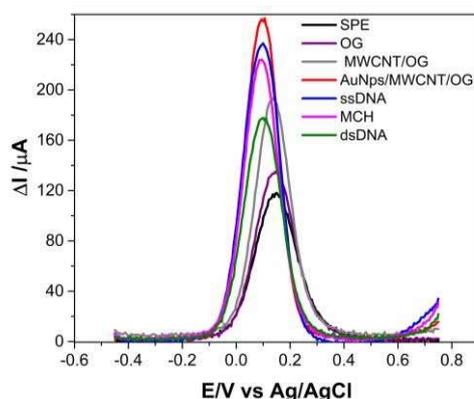
Fig 1. *Square-wave voltammograms obtained for 10 mM $[Fe(CN)6]^{3-/4-}$ in 0.1 M PBS, pH 7.4 at the surface of screen-printed electrodes modified, respectively. (Amplitude= 50 mV, SH= 5 mV, f=10 Hz).*

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Gold nanoparticle-based biosensor for rapid liquid biopsy applications

Kalligosfyri Panagiota[1], Nikou Sofia[2], Bravou Vasiliki[2], Kalogianni P. Despina[1]

kalogian@upatras.gr (Corresponding e-mail address)

[1]Department of Chemistry, University of Patras, 26504 Rio, Patras, Greece
[2]Department of Medicine, University of Patras, 26504 Rio, Patras, Greece

Abstract: Liquid biopsy is a non-invasive and easy alternative approach than that of tissue biopsy for cancer diagnosis and real-time monitoring of tumour progression. Liquid biopsies are represented by various biomarkers, such as blood circulating tumour cells (CTC), circulating tumour DNA (ctDNA), circulating microRNAs and exosomes that are secreted by the tumours. Body fluid samples, such as blood sample, are used in liquid biopsies for biomarker detection. The presence of circulating tumour DNA (ctDNA) or cell-free DNA (cfDNA) in blood circulation offers the capability of early cancer detection and disease monitoring for treatment guidance. The detection and quantification of cfDNA is a great analytical challenge due to its low molecular weight, its extremely low abundance in blood circulation and the need for the discrimination of ctDNA that carries specific tumour-related gene mutations from normal cfDNA. So, the discrimination of ctDNA from normal cfDNA is based on the detection of specific gene mutations that are located only at the tumour DNA and are usually singepoint mutations. In this project, a new gold nanoparticle-based DNA biosensor was developed for the detection of three major single-point mutations in the KRAS gene related to colorectal, as well as the normal cfDNA. The DNA biosensor was initially optimized using synthetic DNA targets and cell lines that express both the normal and the mutated KRAS. The biosensor was then applied to blood serum samples for cfDNA detection. The cell-free DNA that was isolated from the samples, was amplified by PCR, subjected to allele-discrimination reaction and analyzed with the biosensor. All three KRAS mutations, along with the normal KRAS allele were specifically detected by the biosensor with high reproducibility. In conclusion, the proposed biosensor offers a simple, costeffective and rapid alternative of liquid biopsy that is completed within 10 min, while the detection is accomplished by bare eye. The biosensor is universal and can be applied in the future for the detection and identification of several different kinds of cell-free or circulating tumour DNA molecules.
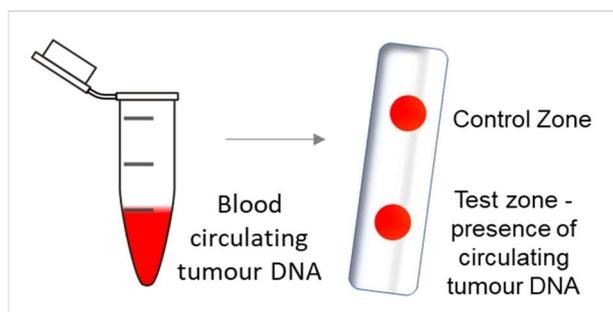
Figure 1: The principle of gold nanoparticle-based DNA biosensor for circulating tumour DNA detection

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DNA-aptamers: a sensitive tool for detection of oncological diseases by quartz crystal microbalance.

Michaela Domšicová [1*], Andrea Bábelová [2], Michal Šelc [2], Kristína Kopecká [2], Veronika Némethová [3,4], Filip Rázga [3,4], Iveta Oravcová [4], Alexandra Poturnayová [1]

michaela.domsicova@savba.sk

[1]Institute of Molecular Physiology and Genetics, Centre of Biosciences SAS, Dúbravská cesta 9, 840 05 Bratislava, Slovakia
[2]Cancer Research Institute, Biomedical Research Center SAS, Dúbravská cesta 9, 845 05, Bratislava, Slovakia
[3]Selecta Biotech SE, Istrijská 6094/20, 841 07 Bratislava, Slovakia
[4]Faculty of Medicine, Comenius University in Bratislava, Špitálska 24, 813 72   Bratislava, Slovakia

Abstract: Oncological diseases are the second most common cause of death in the world [1]. It includes leukemia, a form of cancer that affects both adults and children, responsible for the malfunction of the immune system. Human chronic myelogenous leukemia (CML), a malignancy of pluripotent hematopoietic cells, seriously affects a patient's bone marrow, peripheral blood and lymphatic system [2]. In recent years, many resources and scientific efforts are focusing on cancer research and treatment. In cancer treatment, early diagnosis proved to be the most effective prevention. Traditional methods for detecting CML include bone marrow examination, cytochemistry test, immunophenotyping, or minimal residual bone marrow disease examination. However, these methods have low specificity and cause great pain to the patient during the examination [3].

The aim is to develop and design a new, simple and rapid clinical diagnostic method for CML with high sensitivity, specificity and efficiency. Promising advances are being made with aptamer-based biosensors, where DNA or RNA aptamers are used to target cancer biomarkers on the cell surface. Aptamers are single-stranded nucleotide chains with highly specific recognition capabilities against proteins, can therefore be used to specifically recognize and bind cancer cells by creating a 3D structure under suitable conditions (pH, concentration, ion content) [4]. The ideal clinical diagnostic tool would be a biosensor, which can be used repeatedly. To achieve this, the possibility of regenerating the surface of the biosensor is required, ideally without loss of sensitivity to the target cells [5]. In this work, we used a DNA aptamer that can recognize leukemic cells and was covalently attached to the sensor surface. A highly sensitive acoustic method - quartz crystal microbalance (QCM) - was used to monitor the interactions between the aptamer and various leukemic cell lines (BV-173, K562). Non-specific reactions were prevented using non-specific aptamer or control cells. Cell-aptamer interactions were observed and quantified in real time by monitoring changes in the frequency and resistance of the oscillating sensor. This allowed the determination of binding kinetic constants between the aptamer and the target receptors. Specific interactions were visualized by confocal microscopy using a fluorescently labeled aptamer. The obtained results demonstrate the wide possibilities of using aptamers in clinical practice as a part of sensitive and specific systems for detection and visualization of cancer cells and their possible adaptability to other specific clinical needs.

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A label free electrochemical biosensor for early detection of liver cancer biomarker miRNA-122, based on graphene oxide modified screen printed electrode

Sedigheh Falahi[1] , Parvaneh Rahimi and Yvonne Joseph

parvaneh.rahimi@esm.tu-freiberg.de

Institute of Electronic and Sensor Materials, Faculty of Materials Science and Materials Technology, Technological University Freiberg, Akademie Str. 6, 09599 Freiberg, Germany

Abstract: Primary liver cancer is an aggressive, lethal malignancy that ranks as the fourth leading cause of cancer-related death worldwide. Up to now, small non-coding RNAs (miRNAs) have been extensively examined to serve as promising biomarkers for precise diagnostic of liver cancer. In this study, a novel, label-free electrochemical biosensor has been developed for miRNA-122 detection as a valuable early-stage liver cancer biomarker. For this purpose, the amino-modified inosine situated single stranded DNA (ss-DNA) was immobilized on the surface of graphene oxide modified screen printed electrode (GO-SPE). The detection system is based on the guanine oxidation signal as a result of hybridization between ss-DNA and miRNA-122 measured by differential pulse voltammetry (DPV). The oxidation signal of guanine is related to total miRNA-122 concentration. This biosensor is highly specific, and is able to discriminate between complementary target miRNA-122 and noncomplementary miRNA-let-7a. Consequently, the developed assay can serve as a promising portable platform for rapid, sensitive and specific early cancer diagnosis.
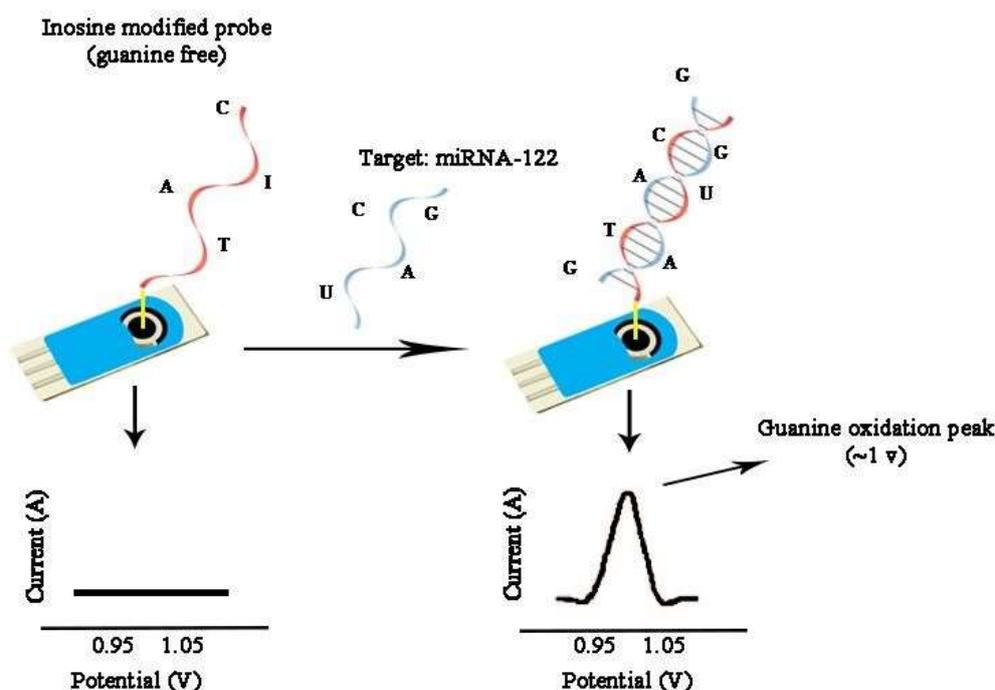
Figure 1: Illustration of label free electrochemical biosensor for early liver cancer biomarker –miRNA-122- detection based on inosine situated ss-DNA modified GO-SPE

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Isothermal multiplex amplification with microarray for the fast detection of multidrug-resistant pathogens in PoC-systems

C. K. Fenzel[1,2], A.-K. Hensel[1,2], C. Warmt[1]*, J. Henkel[1], F.F. Bier[2,3]

*corresponding author, e-mail: christian.warmt@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB), 14476 Potsdam, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: An infection with multidrug-resistant pathogens represents a serious problem for patients in hospitals. The process of diagnosis and finding an antibiotic treatment is time consuming. [1] [2] The increased appearance of antibiotic resistances is the result of the improper and frequent use of antibiotics over a long period. Furthermore, bacteria are able to insert numerous resistance genes, which leads to a multidrug-resistance. In Germany, there are about 500.000 nosocomial infections with pathogens in a hospital a year, about 35.000 are due to multidrug-resistant pathogens and about 2.400 people die in case of an infection.[1] [2]

Nowadays, clinical diagnostics of infectious diseases are operated in a half-automated or manual way, which consumes much laboratory effort and costs prolonged time before first results are available. To minimize time and effort of the detection it is helpful to develop a point-of-care diagnostic system. [1] [2] Nucleic acid amplification, especially Polymerase-Chain-Reaction (PCR) becomes a promising method in pathogen detection. Actually, Recombinase-Polymerase-Amplification (RPA) even provides more advantages. This isothermal method got popular in PoC-diagnostics because of its speed, sensitivity, specificity and simplified handling. Depending on target size, detectable levels are reached in 20-30 minutes. [3] [4] [5]

Our aim is a multiplexed pathogen detection system based on isothermal amplification combined with microarray technology for a more comprehensive assay. First results with three carbapenem-resistant genes (VIM, KPC, CTX-M15) are illustrated in Figure 1(a). We did RPA amplification with three specific primer pairs in one mastermix-reaction and added DNA-template of *Pseudomonas aeruginosa* and *Escherichia coli.* The detection via gel electrophoresis was carried out successfully, however unspecific amplicons appeared. Microarray technology as a promising tool in PoC-diagnostics convinced us as previous problems disappeared and we were able to detect four multidrug-resistant genes simultaneously (Figure 1b) without false positive signals.
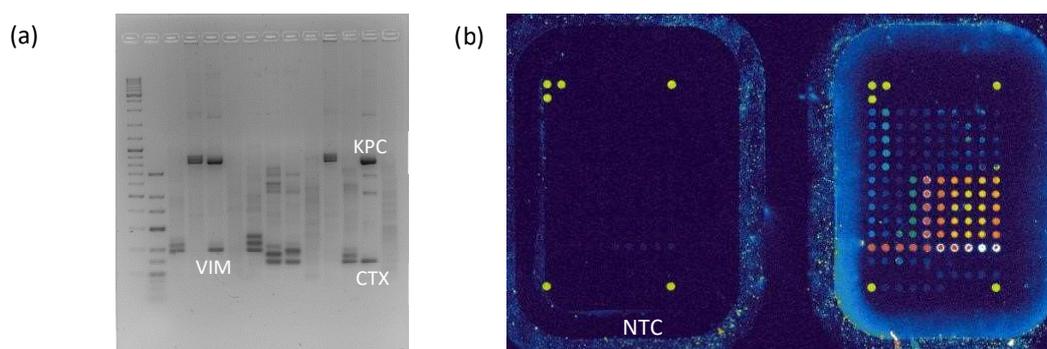

Figure 1: (a) Agarose gel electrophoresis based detection of three multiplexed multidrug-resistant genes (VIM, KPC, CTX-M15). (b) NDM, VIM, KPC, CTX-M15 multiplexed in one reaction via RPA and detected with Microarray

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Improvements of an impedimetric aptamer-based biosensor for diclofenac by modifications of the aptamer used

Dieter Frense, Katharina Schieke, Doreen Lisicki, Daniel Martin, Dieter Beckmann

Dieter.Frense@iba-heiligenstadt.de

Institute for Bioprocessing and Analytical Measurement Techniques e.V., Rosenhof, D-37308 Heilbad Heiligenstadt, Germany

Abstract: Diclofenac (DCF) is a non-steroidal anti-inflammatory drug and has anti-inflammatory, analgesic, antipyretic, and anti-rheumatic properties. It is freely available and is widely used to treat pain, including musculoskeletal disorders (rheumatoid arthritis, polymyositis, and toothache). Unfortunately, DCF belongs to the diverse group of micropollutants in water that sewage treatment plants (STP) [1] often do not adequately remove. Because of this fact and its widespread use, it is one of the most commonly detected pharmaceutically active drug residues in the water cycle, causing harmful environmental impacts for both fish and birds [2]. For this reason, state authorities such as the German Environment Agency are working out several recommendations for reducing micropollutants in water. The values for environmental quality standards (EQS) for DCF were set at 0.05 μg/L [3].

We describe a label-free electrochemical aptasensor for the detection of the drug DCF by monitoring the increase in charge transfer resistance (R_{ct}) due to the increase in DCF concentration. The DCFbinding aptamer was immobilized on the surface of a gold electrode [4] and examined with QCM, SPR, and impedimetric transducers (Fig. 1).

Figure 1: View and scheme of the measurement chamber developed and used for the investigations.

The modification of the DCF-binding aptamer by a 20 nt long polyadenine (poly-A) chain showed an improvement in the DCF detection. Under the optimized conditions, the sensor showed a reaction to the analyte in the low nM range (~ 1 μg/L), which was of interest at least for sewage treatment plants as a monitoring system in the inflow. The detection and quantification limits for DCF were 2.8 nM and 9.3 nM, respectively. The sensitivity increased from $1.5 \ \Omega * nM^{-1}$ of the unmodified aptamer to $40 \ \Omega*nM^{-1}$ of the poly-A-modified aptamer. Further improvements by modifying the electrode with nanoparticles are currently in progress.

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Improvement of PNA probe interactions for a sensitive SPR-based detection of gliomas-associated miRNA

Vanessa Jungbluth [1], Roberta D'Agata [1], Noemi Bellassai [1], Giuseppe Spoto [1]

vanessa.jungbluth@unict.it

[1]Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Viale Andrea Doria 6, 95129 Catania, Italy

Abstract: Gliomas are aggressive brain tumors with a low survival rate of 11-15 months postdiagnosis. Currently, gliomas are diagnosed only after the onset of symptoms via imaging methods (MRI, CT scan, PET, etc.), followed by an invasive surgical biopsy to obtain biological tissue used for disease prognosis. Liquid biopsy brain tumor detection tests do not exist, limiting patient options for important diagnostic and prognostic information to invasive procedures [1].

Small non-coding microRNAs (miRNA) have been demonstrated as promising non-invasive biomarkers for early disease detection, as altered expression levels of miRNA can be correlated to pathologies such as cancer and cardiovascular conditions. In particular, miR125b-5p has been suggested as a novel diagnostic biomarker for gliomas [2]. Conventional detection methods are only semi-quantitative, laborious, and expensive. Biosensor platforms offer rapid, highly sensitive, and specific detection of these circulating tumor markers obtained by non-invasive liquid biopsy approaches using detection probes immobilized to the sensor surface, complementary to target sequences [3]. Usually, DNA or RNA probes are used as capture probes for the detection of nucleic acids.

We describe a Surface Plasmon Resonance imaging (SPRI)-based biosensor detection of gliomasassociated miRNA miR125b-5p using synthetic nucleic acid analogous peptide nucleic acids (PNA) as detection probes. Compared to conventional detection probes, PNA probes exhibit a higher chemical and thermal stability and hybridize more sensitive and selective with target nucleic acids [4]. Due to its N-(2-aminoethyl)glycine backbone, PNA probes exhibit a neutral charge, making hybridization to target nucleic acids independent of ionic strength [5]. However, we demonstrate an improvement in sensitivity for the direct detection of glioma-associated miRNA when using selected metal ions in solution.

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Non-invasive approach for the early diagnosis of prostate cancer by using an electrochemical platform

Raquel Sánchez-Salcedo [1,2], Rebeca Miranda-Castro[1,2], Noemí de-los-Santos-Álvarez[1,2], María Jesús Lobo Castañón[1,2].

sanchezraquel@uniovi.es

[1]Departamento Química Física y Analítica, Universidad de Oviedo, Avenida Julián Clavería 8, 33006 Oviedo, Spain.
[2]Instituto de Investigación Sanitaria del Principado de Asturias, Avenida de Roma, 33011 Oviedo, Spain.

Abstract: The early detection of prostate cancer is decisive for the survival of the patients. To tackle this challenge current trends search for specific biomarkers with clinical validity released from tumor cells to body fluids. This is called liquid biopsy, which is characterized by minimal invasion and very few risks. Recent discoveries have demonstrated that among these biomarkers present in human fluids the long non-coding RNAs (lncRNAs) have gained popularity due to their participation in a wide range of biological processes.[1][2] Alterations in the levels of some of these lncRNAs have been related to different types of cancer. Prostate cancer antigen-3 or PCA3, a urinary biomarker, is the only one that has been approved by the US-FDA for the diagnosis of the prostate cancer. In fact, PCA3 is a good alternative to traditional serum PSA able to distinguish between aggresive and indolent tumours. [3][4]

In this work, we have developed a sandwich-type hybridization assay to detect the urinary biomarker PCA3. We have employed the strategy of using fluorescein-tagged hybridization assistant probes to favour the selective capture onto the sensing platform while incorporating multiple redox enzymes per target molecule, via the interaction between fluorescein and antifluorescein Fab fragment conjugated to peroxidase making possible to enhance assay sensitivity and to overcome the challenge of the low natural abundance and long size of this transcript as well as its internal secondary structure.

To exclude any non-cancer related variation, levels of PCA3 should be normalized with an internal RNA control of stable expression, which demands the development of multiplex tests. Therefore, an equivalent approach was also developed for the detection of PSA mRNA selected as the endogenous control. The usefulness of this multiplex electrochemical platform was demostrated by analysing RNA extracts from the prostate cancer cell line LNCaP and from urine samples of prostate cancer patients.

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Poly (3,4- (1-azidomethylethylene) dioxythiophene) as an advanced interface for electrochemical detection of oligonucleotides

Vera A. Shavokshina, Alexander A. Zarochintsev, Maria A. Komkova, Denis M. Melnik, Timofei S. Zatsepin, Egor A. Andreev, Arkady A. Karyakin

v.shavokshina@analyt.chem.msu.ru

Department of Chemistry, M.V. Lomonosov Moscow State University, 119991 Moscow, Leninskie gory, 1, build. 3, Russia.

In the past decades, electrochemical DNA sensors have attracted substantial attention owing to their high sensitivity, rapid response, portability, and low cost. Proper electrode surface modification is a principal stage in DNA sensors elaboration. Along with reliable immobilization of single-stranded DNA (ss-DNA) probes, a key requirement to surface coatings is to prevent nonspecific adsorption. However, modification of electrodes may lead to diminishing of electroactive properties. In this work we present conductive polymer material with improved electroactive properties and electrochemical DNA-sensor based on it.

We carried out electropolymerization of 3,4-(1-azidomethylethylene)dioxythiophene (azido-EDOT) by cyclic voltammetry in fully aqueous media. Other reports, on the contrary, rely on organic media, however, aqueous synthesis is preferable for biosensors application. Investigations of electroactive properties of azido-PEDOT modified electrodes were carried out with either anionic $[Fe(CN)_6]^{3-/4-}$ or cationic $[Ru(NH_3)_6]^{3+/2+}$ redox probe. Surface modification by azido-PEDOT improves electron transfer rate and provides a 2000-fold increase in standard heterogeneous rate constant (k^0) values for $[Fe(CN)_6]^{3-/4-}$ in pH 7.0 compared to bare electrodes (2.3×10^{-2} cm·s^{-1} versus 1.0×10^{-5} cm·s^{-1}). Even for surface insensitive $[Ru(NH_3)_6]^{3+/2+}$, azido-PEDOT gains an 3-fold enhancement of charge transfer rate. Furthermore, modified electrodes demonstrate improved kinetics for both mediators over pH range of 1.1-7.0.

Azido-groups of the polymer enable the covalent attachment of ss-DNA probes via alkyne-azide cycloaddition («click» reaction) [1]. SS-DNA probes were immobilized onto azido-PEDOT modified electrodes using «click»-chemistry. Targeting ss-DNA were labelled with electrocatalytic Prussian Blue nanoparticles [2, 3]. Hybridization resulted in increase of catalytic currents, that demonstrates the applicability of the developed DNA-sensor. The sensor could detect down to 5 nM target DNA. It was also noted that azido-PEDOT reduces nonspecific adsorption of oligonucleotides.

The azido-PEDOT combining a significant improvement of redox probe electroactivity with a simple route for immobilization is promising polymer material for elaboration of electrochemical (bio)sensors, especially of DNA-sensors.

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Quantification of *Legionella spp.* by viability heterogeneous asymmetric recombinase polymerase amplification (v-haRPA) on a flow-based chemiluminescence microarray

Gerhard Schwaiger [1], Michael Seidel[1]

Gerhard.Schwaiger@tum.de

[1]Institute of Hydrochemistry, Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 Munich, Germany

Abstract: *Legionella spp.*, a group of 2-20 μm large, gram-negative bacteria are found in all kind of freshwater systems. In the last years, increasing number of legionellosis outbreaks, a severe lung disease is a growing challenge for public health. Infection with *Legionella spp.* usually takes place via aerosols. Therefore, in addition to the shower, cooling towers, evaporative cooling systems and air filter systems can be a potential risk. For that reason, a reliable and fast detection method is needed for a proper risk evaluation. According to the EN ISO 11731:2017 the gold standard for the detection of *Legionella* is a culturing method. For that, after a long incubation time of 7 – 10 days on selective agar the colony forming units (CFU) can be determined [1]. Research has shown that this approach has a high risk for underestimating the number of infectious bacteria. The reason for that are viable but not culturable *Legionella*. That´s why upcoming alternative detection methods like qPCR are becoming increasingly popular. But these methods tend to overestimate the number of microorganisms, because they also amplify DNA from dead cells [2]. Additionally to that, usually a laborious experimental setup and a high expertise is needed to perform these measurements. For this reason, a flow-based chemiluminescence (CL) DNA microarray was developed, which is capable of the detection of only viable *Legionella.* For an easy and cheap experimental setup with the possibility for on site measurements, the isothermal amplification method recombinase polymerase amplification (RPA) was chosen. For the heterogenous asymmetric RPA (haRPA), *Legionella spp.* specific reverse primers targeting the 16S rRNA gene are immobilized on the microarray surface, allowing sitespecific amplification. After sampling, DNA from dead cells can be blocked with PMA and blue light. Then DNA extraction is performed and the amplification is done for 40 min at 39 °C on the microarray. After flushing the microarray with a mixture of horseradish peroxidase-labeled streptavidin (strep-HRP), binding to a biotin molecule from the modified forward primer added to the reaction and a 1:1 mixture of luminol and peroxide the CL reaction can be measured by a CCD camera. The assay principle for viable *Legionella* is described elsewhere in detail [3]. To improve the sensitivity, which was until now not suitable for real samples, the flow chamber of the microarray was reduced half in volume. Additionally, the incubation time of the haRPA was optimized. First results with these changes could achieve an LoD of 0.0019 CFU/mL and a linear working range from $0.911 – 3.2 \times 10^4$ CFU/mL with *L. spp.* DNA. In comparison with the previous assay, this is an improvement of the factor 10^3 for the LoD and the start of the linear working ranges starts 500 times earlier. According to German guidelines, e.g. the 42. BlmSchV, a suitable method needs to be able to detect at least 1 CFU/mL which is achieved with the new haRPA microchip format. Now the method can be adapted for real sample tests and the implementation of the viability approach will be done. To allow a differentiation of *Legionella* species, the microarray is suitable to immobilize more than one primer which can be specific for different *Legionella* species. Therefore, multiplex analysis with an *L. pneumophila* specific primer set is planned in the future.

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Electrochemical DNA-based biosensor for the evaluation of antioxidant activity of some hydroxycinnamic acids

Ivana Tomac [1], Marijan Seruga [1], Jan Labuda [2]

itomac@ptfos.hr

[1]Department of Applied Chemistry and Instrumental Methods, Faculty of Food Technology Osijek, F. Kuhača 18, 31 000 Osijek, Croatia

[2]Institute of Analytical Chemistry, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia

Abstract: An electrochemical DNA-based biosensor composed of the screen printed carbon electrode modified with carboxylated single-walled carbon nanotubes [1] and dsDNA, dsDNA/SWCNTCOOH/SPCE, was applied to assessment of the antioxidant activity of some hydroxycinnamic acids. Hydroxycinnamic acids are group of polyphenolic compounds widely spread in plants as fruits and vegetables represented mainly by caffeic, ferulic, and chlorogenic acids. They are present in the form of derivates of quinic acid. The electrochemical biosensor was first incubated in the mixture of a DNA cleavage agent created by the Fenton type reaction without or with the addition of some hydroxycinnamic acid. After the incubation, the degree of dsDNA deep degradation was determined applying cyclic voltammetry of the DNA redox indicator $[Fe(CN)_6]^{3-/4-}$ [2]. The antioxidant activity of hydroxycinnamic acids was evaluated using the normalized change of the indicator voltammetric responses ΔI_{rel} and $\Delta(\Delta E_{p,rel})$ [3,4]. The obtained results have shown that the addition of hydroxycinnamic acids to the cleavage agent significantly reduces the degree of DNA degradation. The investigated caffeic (CA) and dicaffeoylquinic acids, diCQAs, have exhibited the portion of survived DNA of about 70% comparing to 20% in their absence.
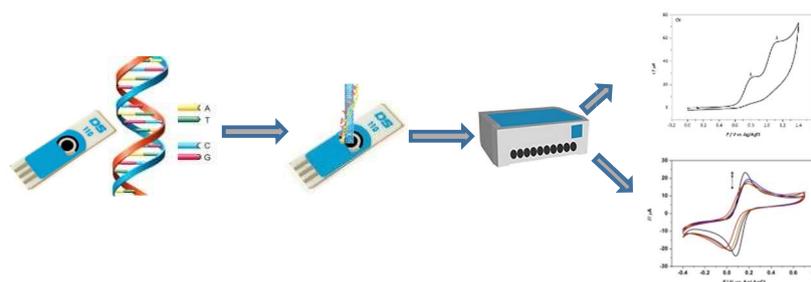
Figure 1: Preparation of the biosensor and the its response obtained for diCQA under investigation

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One-step in-chip hybridization RPA for a fast and easy to use diagnostics platform

Q. V. Vu[1], F. Ahmadi[1], C. Warmt[1*], J. Henkel[1], F.F. Bier[2,3]

*corresponding author, e-mail: christian.warmt@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB), 14476 Potsdam, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: The recombinase polymerase amplification (RPA) is a pretty new amplification method in contrast to the widely used polymerase chain reaction (PCR). It overcomes a lot of disadvantages, i.e. the necessity of fast and accurate temperature switching or expensive equipment, with a comparable sensitivity and specifity by processing the amplification at a constant temperature.[1]

For the detection and analysis of the amplicons there are a lot of methods published, above all the combination with some kind of lateral flow assays like dip sticks.[2] Quantitative RPA (qRPA) and the combination with CRISPR-Cas technologies are used as well as colorimetric RPA assays for PoC applications. Nevertheless, there are only a few publications combining RPA with microarray technologies.[3] Most frequently pre-labelled primer sets are used for the labelling of the amplicons. Depending on the detection method there are more or less elaborate post-RPA processes necessary. Furthermore, normally the labelling (i.e. primer labelling) as well as the RPA and the subsequent detection are unique and separated processes requiring manual intervention. Taking together, that is no meaningful requirement for an automated diagnostics platform or lab-on-Chip devices.

Here we report for the first time a one-step in-chip method that combines *I)* the recombinase polymerase amplification with *II)* an active on-the-fly fluorescence labelling followed by *III)* an hybridization process for the detection on a microarray *(Figure 1)*. With the combination of all three steps within a single process we were able to detect our targets in less then 1,5 h.
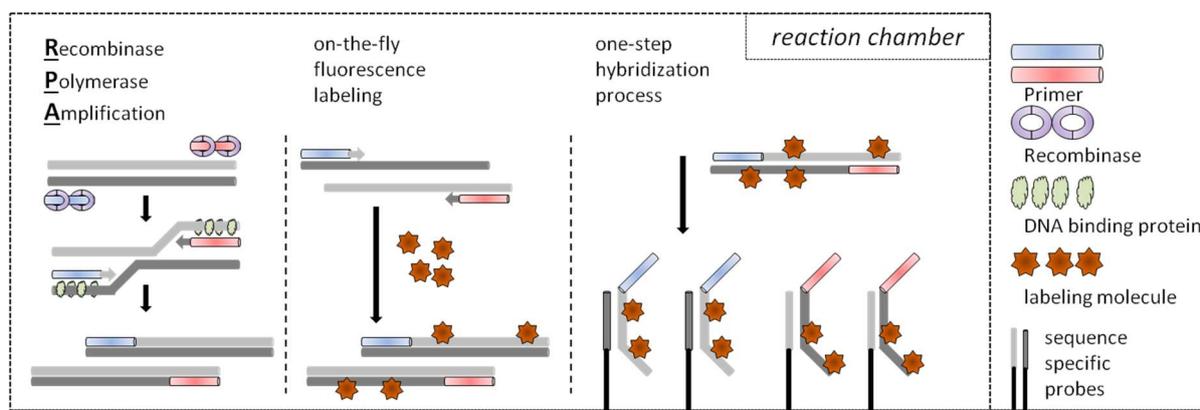

Figure 1: Schematic illustration of the RPA-hybridization process. The three steps consisting of RPA, labelling and hybridization can be carried out at the same time in the same reaction chamber without the need of changing reagents or reaction vessels.

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Electrospun cationic nanofibers for nucleic acid extraction in paper-based analytical devices

Alissa Wieberneit [1], Antje J. Baeumner [1], Nongnoot Wongkaew [1]

alissa.wieberneit@chemie.uni-regensburg.de

[1]Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, 93053 Regensburg, Germany

Abstract: Highly sensitive and yet rapid detection of pathogens such as HIV, Malaria and SARSCov-2 rely on the identification and quantification of their nucleic acid (NA), enabling the detection of the pathogen early on in the infection cycle. Mandatory is isolation of the nucleic acids from biological matrices to make it available for molecular amplification strategies such as PCR, LAMP, RPA and others [1]. For point-of-care devices, this is a true challenge, as the isolation and subsequent amplification of the DNA or RNA molecules have to be done with minimal effort, rapidly and yet very efficiently [2]. While most commercial systems are based on bench-top devices, we focus on the development of new materials for NA extraction in paper-based analytical devices (PADs) to be suitable for point-of-care in resource-limited settings [3]. Cationic nanofibers hold great promise as

Figure 1: Design of the PAD with an extraction zone, an amplification zone (RPA), Au-NPs as a label and a detection zone with three seperate spots.

they can easily adsorb the negatively charged NAs and offer a very large surface-to-volume ratio [4]. Since cationic polymers typically possess insufficient molecular weight to form fibers, they were thus generated from polymer blends based on one supporting polymer (polystyrene (PS), polylactic acid (PLA), and polyamide 6,6 (nylon)) and one polycationic polymer (polyaniline (PANI), polybrene (PB), and polyallylamine hydrochloride (PAH)) through electrospinning with low production and material cost. The as-spun nanofibers were characterized with respect to nanofiber diameter, porosity and fiber mat thickness. The nanofibers were then exposed for 5 minutes to genomic DNA from bacteria as model analyte, and adsorption yields were determined in different buffer systems. PSPANI and PLA-PANI nanofibers exhibited maximum adsorption efficiency of ca. 40-60% in citrate buffer (pH 4.2). In case of PB containing fibers, the adsorption efficiency as high as 95 % were obtained regardless of buffer systems and pHs, which is likely due to its quaternary amino groups. In contrast, the adsorption behaviour of nylon-PAH fibers depends strongly on the buffer's pH (97 ± 1 % and 30 ± 3 % for pH 4.5 and 7.5, respectively) due to their primary amino group. Up to 100 ng NAs could be strongly adsorbed on a mere 6 mm-circular shaped nylon-PAH fiber mat. Furthermore, it was found that PAH exhibits greater affinity for NA adsorption than that of PANI, independent from the supporting polymer. These findings suggest that nylon-PAH nanofibers are a good candidate for PADs that require NA releasing prior to detection or amplification whereas nylon-PB is highly suitable for in situ-based approaches. Further implementation of the as-developed nanofibers into PADs for NA extraction will pave the way for developing a fully automated system that can be utilized in point-of-care testing.
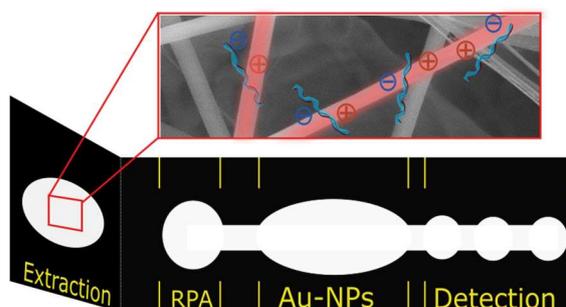
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Detection of levothyroxine using nanostructured materials: an electrochemical assay

Melinda David [1], Adrian Enache [2], Monica Florescu [1]

melinda.david@unitbv.ro

[1]Faculty of Medicine, Transilvania University of Brasov, Blvd. Eroilor 29, Brașov 500036, Romania [2] National Institute of Material Physics, Atomistilor 405A, 077125 Magurele, Romania

Abstract: The concentration of biomolecules, like thyroxine hormone, is linked to medical conditions, as hypothyroidism, where the best therapy is hormone replacement, by administration of levothyroxine (LT4) medication. The detection of these biomolecules still lacks a simple and reliable tool.

In this work characterization and optimisation of label-free nanosensors towards LT4 detection are described. Nanostructured materials such as graphene (G) and carbon nanotubes (CNT) were employed on the surface of electrochemical nanosensors since they are known as good conducting materials that assure an enhanced surface area and biocompatibility, and are able to catalyse specific reactions. Thus, the electrochemical oxidation of LT4 was studied using two configurations of electrochemical nanosensors: G- and CNT-modified carbon film-based electrodes. At both electrodes the oxidation of LT4 occurred in one step (O1) with the formation of electroactive product with a lower oxidation potential (O2). Optimizations of working parameters for LT4 detection were performed using cycling and differential pulse voltammetries. The most important parameter proved to be the pH of working electrolyte whose effect on electrochemical parameters of LT4 is presented in Fig. 1.
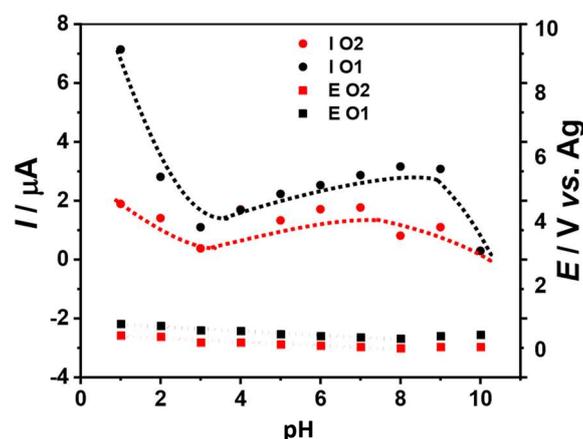

Figure 1: Variation of oxidation peak potentials (E - O1 and O2), respectively current (I - O1 and O2), as function of pH from DPVs in 20 μM LT4 solution prepared in 0.1 M various electrolytes at G- modified sensor. Pulse amplitude was 50 mV for 0.2 s, scan rate was 10 mV s^{-1}.

Electrochemical impedance spectroscopy was also used to highlight the detection mechanism of the nanostructured material-modified sensors in the presence of LT4. We found that CNT- modified sensor will easily interact electrostatically with the positively charged LT4 in acidic media, where, in the case of G- modified sensor, the possibility of molecular adsorption on its surface was suspected. The best analytical performances were obtained by DPV for the CNT-modified sensor in pH 4.0, with a sensibility S = 4.0 ± 0.06 μA cm^{-2} μM^{-1}, and a LoD of 30 nM (close to the physiological range).

Acknowledgments:

Enzymatic Histamine Biosensor Based On Prussian Blue-Modified 3D Pyrolytic Carbon Microelectrodes

Sheida Esmail Tehrani [1], Long Quang Nguyen[1], Bettina Margrethe Jensen[2], Jenny Emnéus[3], Tautgirdas Ruzgas[4], and Stephan Sylvest Keller[1]

shesme@dtu.dk (Corresponding e-mail address)

[1]DTU Nanolab, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
[2]Allergy Clinic, Gentofte Hospital, 2900 Hellerup, Denmark
[3]DTU Bioengineering, 2800 Kgs. Lyngby, Denmark
[4]Department of Biomedical Science, Malmö University, 205 06 Malmö, Sweden

Abstract: In allergic reactions, mast cells and basophils release 25-65 ng/ml histamine, which is about 200-600 nM [1,2]. Here, we propose an electrochemical enzymatic biosensor, with a novel method of measurement, which can enable detection of histamine in this range. Pyrolytic carbon working electrode (WE) with 3D microstructures is fabricated by photolithography with SU-8 photoresist followed by pyrolysis[3]. Thereafter, it is modified with Prussian Blue (PB) film by electrodeposition[4]. Next, diamine oxidase (DAO) in a solution containing BSA and glutaraldehyde is cross-linked on the WE. The mechanism of histamine detection is analogous to charge/ discharge cycles of a capacitor. First, the PB on WE is electrochemically reduced to form Prussian White (PW) resulting in charge accumulation in the PW/PB layer (charging). Second, the electrode is exposed to the solution containing histamine at an open circuit while the open circuit potential (OCP) is recorded. Histamine is oxidized to imidazole acetaldehyde and hydrogen peroxide (H_2O_2) is generated at the electrode by DAO. Subsequently, H_2O_2 oxidizes PW producing PB (discharging) imposing a positive shift in OCP. The amount of the produced PB is then assessed by recording chronoamperometric current by applying a step of reducing potential (recharging). The charge is obtained by integrating the area under the current. The obtained results shows that the combination of 3D pyrolytic carbon with the described two-step electrochemical method- i) open circuit potentiometry followed by ii) chronoamperometry- can practically provide the same sensitivity that is conventionally acquired from platinum microelectrodes in flow injection systems. Currently, we are optimizing the biosensor aiming for histamine detection in human LAD2 mast cells medium.

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Design and development of electrochemical biosensors for bioprocess monitoring

Aliyeh Hasanzadeh[a*], Babak Rezaei[b], Mogens Kilstrup[c], Krist V. Gernaey[a]

*Corresponding Author's E-mail: alhas@kt.dtu.dk

[a]Process and Systems Engineering Centre (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs, Lyngby, Denmark
[b]National Centre for Nano Fabrication and Characterization, DTU Nanolab, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
[c]Department of Biotechnology and Biomedicine, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

Abstract: Bioprocess design is still generally challenged by a lack of tools for process monitoring and control. Maximum bioreactor efficiency can only be established on the basis of targeted monitoring strategies through deep process understanding and targeted control strategies. For some physical and chemical parameters like temperature, pH, rotation speed, dissolved oxygen, pressure, liquid level, and viscosity, specific functional sensors have been developed, and control of these process parameters can be carried out via classic control strategies based on specific actuators (Moeller et al., 2011). On-line measurements of the crucial physiological and biochemical parameters such as biomass, key nutrient and metabolite concentrations are rare, and possible solutions are highly challenged by medium and process complexity. In this context, the development of novel (bio)sensors is pursued to provide a more detailed insight into bioprocesses. The combination of different sensors with knowledge-based process models opens up towards holistic and intelligent bioprocess monitoring and process control (Semenova et al., 2020).

Glucose is a major carbon and energy source in the fermentation industry and as such, evidently, monitoring and control of glucose concentrations during fermentation processes is beneficial for any feeding strategy, optimizing biomass production itself as well as the production of metabolites such as amino acids, alcohols, peptides and proteins. Generally, the measurements of glucose are performed by means of chromatographic techniques. These methods are considered resource and time intensive and as such not suitable for prompt analysis or continuous monitoring applications.

In this work, an electrochemical biosensor for on-line monitoring of glucose in the fermentation broth is presented. An electrochemical sensor based on polyaniline (PANi) and gold nanoparticles combination with glucose oxidase (GOx) has been developed. The successful application of the sensor in fermentation processes will be demonstrated. Moreover, in a traditional bioreactor set-up, data about each measured variable is collected by a single sensor that is mounted in a fixed position close to the reactor wall. In the novel concept, the developed sensors can be miniaturized and mounted on free-floating sensor particles that provide an unprecedented level of bioprocess monitoring throughout the bioreactor.

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Electrochemical detection and cleaning of the contaminated contact lens by using scanning electrochemical microscopy (SECM)

Yueh-Tien†, Hsu, Yen-Tzu†, Lin and Tzu-En, Lin*

E-mail: telin@nctu.edu.tw

Institute of Biomedical Engineering, National Chiao Tung University, Hsinchu 30010, Taiwan

†These authors contributed equally to this work.

Abstract: As the patients of myopia (near-sightedness) increase, the wearing rate of contact lens is raising drastically in recent years. Several millions of population wear contact lenses worldwide. However, improper wear and care of contact lenses can cause eye infections, such as Giant papillary conjunctivitis, and Acanthamoeba Kerratitis. Contact lens are usually made of transparent or colored porous polymers like polymethyl methacrylate (PMMA) hydrogels, or silicone, but the cleanliness of the lens cannot be identified easily. Bacteria, proteins or other impurities usually cannot be detected without antibody labeling or specific dyeing methods. For most consumers, they follow the protocol of cleaning lens: immersing conventional lens or monthly disposable lens into the commercial all-inone solution to clean them without knowing they are already cleaned or not.

As a result, the purpose of this experiment is to use the scanning electrochemical microscope (SECM) coupled with newly developed soft gold electrode which scanning in contact mode for the detection of the impurities on the surface of the lens as a more innovative and effective detection method. In order to investigate the new methodologies that can provide non-invasive, simpler, and precise detection, we discuss the methodologies using various electrochemical biosensors targeting the different kinds of biomarkers.[1] In previous study, SECM can be employed to improve oral cancer diagnosis. We utilized the SECM to map the distribution of biomarker for imaging different cancer stages on the tissue sections.[2]

According to the experimental results, we found out that the current signals over the contact lens samples soaked in proteins or bacterial solution were relatively higher than the clean ones. In this way, the "dirty region" of the contact lens can be identified. In the next step, we use electrochemical cleaning method to generate free radicals that may destroy the impurities on the contact lens. In future, SECM may help consumers distinguish the weekly or monthly disposable contact lens are still in a good status or not. And the electrochemical cleaning method may be a potential assay to clean the bacteria on the lens or even to clean the cases of contact lens.
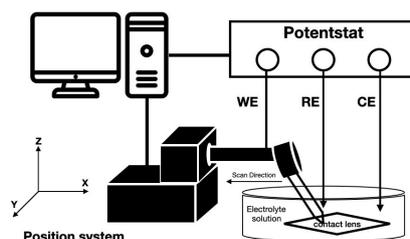

Figure 1: Scanning electrochemical microscopy (SECM) is a scanning probe technique that is composed of a three-electrode system that involves a soft probe scanning. And this is a simple model for the experiment.

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Platinum black-modified microelectrodes for biomedically relevant hydrogen peroxide detection

Andreas Hellmann[1], Sven Daboss[1], Denis Nalbantoglu[2], Sabine Vetorazzi[2] and Christine Kranz[1]

andreas.hellmann@uni-ulm.de

[1]Institute of Analytical and Bioanalytical Chemistry, Ulm University, Albert-Einstein-Allee 11, 89081 Ulm, Germany
[2]Institute of Comparative Molecular Endocrinology, Ulm University, Helmholtzstr. 8/1, 89081 Ulm, Germany

Abstract: Next to the free radical superoxide ($O^{2-}\cdot$) and hydroxyl radical (OH·), the more stable and non-radical hydrogen peroxide (H_2O_2) is one of the most extensively studied reactive oxygen species (ROS). ROS are responsible for protein oxidation, lipid peroxidation, and DNA damage but in dependence of the concentration, ROS are also crucial for signaling. H_2O_2 is generated from superoxide produced by mitochondria and NADPH oxidases and elevated H_2O_2 production leads to oxidative stress. Therefore, its detection is of particular interest since the involvement and concentration levels of H_2O_2 at the cellular level are not fully understood yet. H_2O_2 can be detected electrochemically at e.g., platinum electrodes at relatively high oxidation potentials (+0.6 V vs. Ag/AgCl). Co-oxidizable compounds such as ascorbic acid, catecholamines etc., frequently present in biomedically relevant samples, may lead to false positive results [1].

To overcome these challenges, strategies for electrochemical H_2O_2 detection at low potential will be presented avoiding such interference problems based on electrocatalytically modified microelectrodes. As these studies are targeted towards cell measurements (bone marrow macrophages from mice) at low sample volumes, microelectrodes will be used. Macrophages are phagocytic cells that produce and release ROS in response to phagocytosis or stimulation with various agents such as phorbol-myristate12-acetate (PMA) [2]. To enhance sensitivity for H_2O_2 detection, microelectrodes are modified with Platinum black (H_2O_2 detection at +0.3 V vs. Ag/AgCl) [3]. As concentration levels of H_2O_2 are expected in the low micromolar or nanomolar range, microelectrodes were positioned at close proximity (30 µm) to the cell surface via recording the current for oxygen reduction. Next to the thorough characterization of the microsensors, measurements alongside control experiments at bone marrow macrophages extracted from mice will be presented.

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AC electrokinetics on the nanoscale: immobilisation of nanoparticles and molecules

R. Hölzel[1], X. Knigge[1], E.-M. Laux[1], M. Noffke[1], S. Stanke[1], C. Wenger[2,3], F. F. Bier[4]

Ralph.Hoelzel@izi.bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Am Mühlenberg 13, 14476 Potsdam, Germany.
[2]IHP - Leibniz Institute for Innovative Microelectronics, Im Technologiepark 25, 15236 Frankfurt (Oder), Germany.
[3]Brandenburg Medical School Theodor Fontane, Fehrbelliner Straße 38, 16816 Neuruppin, Germany [4] Molecular Bioanalytics und Bioelectronics, University of Potsdam, Karl-Liebknecht-Str. 25, 14476 Potsdam, Germany.

Abstract: AC electrokinetic phenomena like dielectrophoresis (DEP) and AC electroosmosis have been applied to the spatial manipulation of biological cells over many years. They are increasingly exploited for the separation and immobilisation of nanoparticles and molecules in micro- and nanoelectrode systems. Especially the dielectrophoretic immobilisation of antibodies and enzymes on electrodes is important for a well controlled functionalisation of sensors [1].

With proteins being about three orders of magnitude smaller than cells, both the size dependence of DEP and that of thermal motion call for much higher field gradients when targeting molecules. Such an increase can be achieved by increasing the voltages applied, which, however, leads to heating and additional fluid flow that interfere with DEP action. A better approach is to use electrodes with sizes and curvatures reaching those of the target molecules.

For this purpose, we have developed different electrode types: Interdigitated electrodes with gaps below 1 µm, planar triangular electrodes with distances of around 100 nm, and regular arrays comprising up to 1 million pin-like electrodes with tip diameters reaching the size of proteins (< 10 nm). Successful immobilisation has been achieved for nano particles like polystyrene nanospheres [2], viruses and exosomes, as well as for dissolved molecules: single molecules of the autofluorescent protein R-phycoerythrin, horseradish peroxidase [3], antibodies [4] and small organic dye molecules [5]. Fluorescence microscopy shows that protein function is preserved in the course of DEP immobilisation. Localisation is controlled by scanning force, scanning electron and optical microscopy. Fluorescence polarisation microscopy reveals the immobilisation of eGFP in a properly aligned manner and allows to determine the orientation of the protein's fluorescing subunit in relation to the whole molecule [6]. Nanoparticles are immobilised as singles on each electrode tip following the array's regular arrangement [2]. The results are discussed in the light of recent hypotheses about the actual interaction mechanisms between dissolved molecules and polarising fields. [7]

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Ferrocene-functionalized multi-walled carbon nanotubes based solid contact ion-selective electrodes

József Kozma, Soma Papp, Róbert E. Gyurcsányi

kozma.jozsef@vbk.bme.hu

BME 'Lendület' Chemical Nanosensors Research Group, Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Szt. Gellért tér 4, H-1111 Budapest, Hungary

Solid contact ion-selective electrodes (SCISEs) are of great importance in the field of electrochemical sensors (e.g. wearable sensors) since they offer means for robust miniaturization and compact embodiments [1]. A wide range of materials expected to provide well defined phase boundary potential at their interface with the ion-selective membrane have been tested as solid contacts, including conducting polymers [2] and large surface area carbonaceous materials (e.g. graphene, carbon nanotubes, carbon black) with high double layer capacitance [3]. However, the reliable fabrication of such SCISEs, especially in the terms of extraordinary E^0 reproducibility and stability needed for "calibration-free" use, is still a challenge.

Here, we introduce ferrocene-functionalized multi-walled carbon nanotubes (Fc-MWCNT) as a new approach to combine the advantages of large capacitance nanomaterials and redox couples for ionophore-based solid contact ion-selective electrodes. Various methods were applied to improve the E^0 reproducibility, e.g. short-circuiting the SCISEs together in KCl solution (standard deviation (SD) of 1.85 mV). The redox functionalization of the nanotubes enables in principle the adjustment of the redox potential of Fc-MWCNT suspension and this eventuality was also preevaluated. The potential reproducibility of these SCISEs was remarkably good (SD of 1.77 mV), even before full conditioning. This is a major step towards the SCISEs with preadjustable standard potential, which is essential for mass production of high potential reproducibility SCISEs without any post-fabrication treatment. The ion-selective electrodes showed excellent selectivity and ideal Nernstian response in a wide concentration range, while no sensitivity for light and O_2 was detectable. The potentiometric water layer test confirmed that no aqueous layer was formed between the ion-selective membrane and the solid contact.
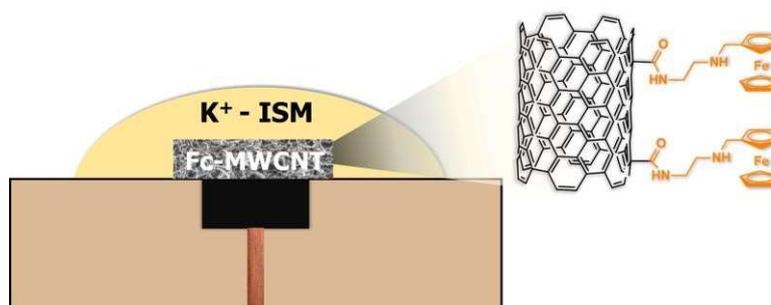

Figure 1: Schematic illustration of the ferrocene-functionalized carbon nanotube based K⁺-SCISE

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Improving the stability of redox polymers for bioelectrochemical applications

Anna Lielpetere [1], Julian Szczesny [1], Felipe Conzuelo [1], Tim Bobrowski [1], Adrian Ruff [1], Wolfgang Schuhmann [1]

anna.lielpetere@rub.de

[1]Analytical Chemistry – Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany

Redox polymers are often used in bioelectrochemistry to electrically wire the active site of the biocatalyst and provide an appropriate immobilisation matrix. Redox polymer hydrogels with a tailored redox potential provide a suitable solvated environment for the enzyme, making it possible to maximise the enzyme loading on the electrode surface while decreasing enzyme leaching. As a result, entrapping of enzymes into a polymer film also translates in a significantly enhanced stability of the modified electrode [1, 2].

One of the factors limiting a long-term use of a bioelectrode is the general stability of the redox polymer film over the electrode. To improve the stability of the polymer film, several strategies can be employed: chemical crosslinking [3, 4], layer-by-layer techniques [5], changing the surface properties of the electrode with an interlayer [6], by grafting or derivatization [7], or adding additional components like conductive nano-objects [8]. All these techniques aim to create stronger covalent or non-covalent interactions between parts of the polymer, polymer and enzyme, or polymer and electrode, thus increasing the stability of the system. For example, chemical crosslinking with bi- or multi-functional crosslinking agents forms a three-dimensional network that limits both leaching of the enzyme and disintegration of the film due to solubility of the polymer. However, the degree of crosslinking must be controlled the mobility of the redox-moiety containing segments and mass transport in the film, which would result in decreased currents. [2]

In this work, we investigate strategies to improve stability of redox polymer-modified electrodes by improving the design of redox polymers. Different functional groups with crosslinking capabilities, such as epoxy groups, primary and tertiary amines, activated carboxylic acids and hydrazides are introduced into polyvinylimidazole and polymethacrylic polymer backbones in varying ratios to evaluate their influence on the polymer film stability on the electrode with various bifunctional crosslinkers. Examples of functional devices such as nanobiosensors or biofuel cells with significantly improved performance and stability will be discussed.

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Sm$_2$O$_3$-SmO Modified Gold Electrodes: Development, Characterization and (Bio)sensing applications

Ricardo Leote[1,2], Monica Enculescu[1], Cristian M. Teodorescu[1,2], Victor Diculescu[1]

ricardo.leote@infim.ro

[1]National Institute of Materials Physics, Atomistilor 405A, Magurele, Romania
[2]University of Bucharest, 36 Mihail Kogălniceanu Bd, Sec. 5, Bucharest, 05010, Romania

Nanostructured metallic oxides have been applied as biosensor platforms due to enhanced electron transfer kinetics, high adsorptive properties and biocompatibility for immobilization of biorecognition elements, while being a more economical alternative to noble metals. Rare earth oxides are targets of extensive research for several applications given that their magnetic, electronic and luminescent properties [1]. Particularly, samarium oxide catalytic properties have been investigated over the years, demonstrating that this compound has outstanding performance as platform for gas sensors. Furthermore, its catalytic behaviour has been explored for alkene oxidation/hydrogenation and also as H$_2$O$_2$ sensors [2].
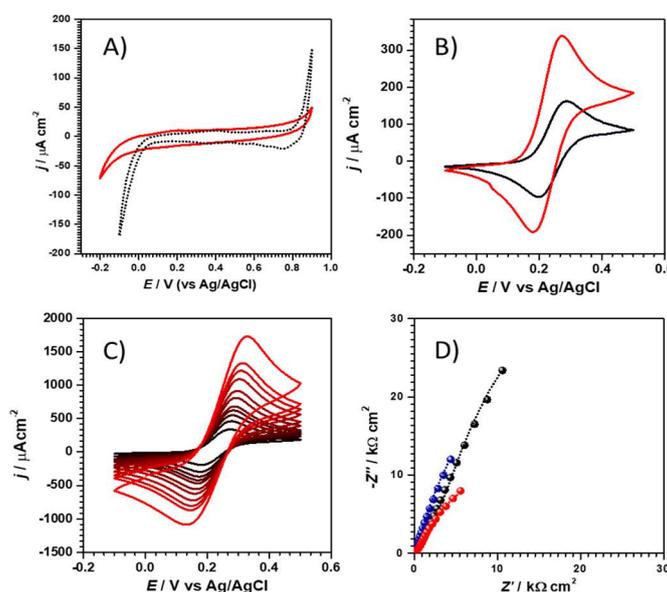
Figure 1: A-E) Cyclic voltamograms recorded with the Sm$_2$O$_3$-SmO/Au/Ti/SiO$_2$/Si (coloured lines) and Au/Ti/SiO$_2$/Si (dotted lines) in: A) 0.1 M KCl; B) at different scan rates in 0.1 M phosphate buffer pH 7.0. 5 mM K$_4$[Fe(CN)$_6$] in 0.1 M phosphate buffer pH 7.0 at C): 25 mV s^{-1}; D) EIS spectra recorded with the Sm$_2$O$_3$SmO/Au/Ti/SiO$_2$/Si in pH = 7.4 0.1 M phosphate buffer at applied potential values -0.30 (black), 0.00 (blue) and +0.20 V (red).

In this work, a nanostructured samarium oxide modified gold electrode was developed to be applied as a new platform for glucose oxidase immobilization, as a model enzyme. The electrode was thoroughly characterized by SEM and XRD to elucidate its morphological characteristics and XPS to investigate the nature of the chemical bonding and oxidation states of the newly prepared electrodes. Electrochemical phenomena were investigated by CV and EIS in order to provide a better insight of Sm$_2$O$_3$-SmO electrochemical properties. Experimental conditions such as pH and applied potential were assessed with the goal to enhance sensitivity to H$_2$O$_2$ and glucose detection. Furthermore, the analytical performance was evaluated by testing the selectivity and reproducibility of the newly developed device, and finally, the biosensor was applied to glucose determination in blood serum.

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Electrochemical Characterization of Conductive Ni(II)-Based Metal Organic Framework Films

Tyra Lewis [1], William Wallace[1], Dr. Sanela Martic*[1]

sanelamartic@trentu.ca

[1]Trent University, 1600 West Bank Dr., Peterborough, ON Canada K9L 0G2

Abstract: Metal organic frameworks (MOFs) are crystalline porous materials formed by the coordination of a metal ion with an organic linker/ligand.[1,2] MOFs have attracted attention in drug delivery, chemical separation, catalysis and sensing applications, due to their diverse structures, attainable high internal surface areas, and chemical functionalization.[2] Additionally, their reversible adsorption and high catalytic activity, make them particularly suitable for electrochemical sensing applications.[1,2] For instance, MOF-based electrochemical sensors have been employed in the detection of biomolecules such as dopamine and serotonin and heavy metal environmental contaminants.[3] With electrochemical sensors, MOFs are generally used as surface modifiers, but their low stability in aqueous solutions and poor electrical conductivity limit their uses in electrochemistry.[1] Thus, to further expand their applications in electrochemical sensing technology, determination of electrochemical characteristics of these materials is necessary. Herein, we synthesized MOF-TL1 using nickel(II) acetate with 2,3,6,7,10,11-hexahydroxytriphenylene.[3] A three-electrode cell comprising of a MOF-modified glassy carbon electrode (GCE), a platinum wire counter electrode and a Ag/AgCl reference electrode was used for all electrochemical measurements. The fabrication of MOF-TL1 films on GCE surfaces was optimized. The electrochemical behaviour of the MOF-TL1 films was assessed by cyclic voltammetry and electrochemical impedance spectroscopy in the presence of negatively charged or positively charged redox probe. The MOF-TL1 film stability was also tested in aqueous solutions at various pH values. The methods employed provide information on the redox properties, electrical conductivity, and stability of the synthesized MOF-TL1 and its films on surfaces.

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Investigating the effects of the contact metal to the characteristics of PEDOT:PSS based organic electrochemical transistors

Chunling Li[1], Hendrik Kupper[1], Vivek Pachauri[1], Sven Ingebrandt[1], Xuan Thang Vu[1]

chunling.li@iwe1.rwth-aachen.de; vu@iwe1.rwth-aachen.de

[1]Institute of Materials in Electrical Engineering 1 (IWE1), RWTH Aachen University, Sommerfeldstr. 24, 52074 Aachen, Germany

Abstract: Polymer-based organic electrochemical transistors (OECTs) have gained a lot of attention in recent years. They have already seen a wide variety of applications, including neural interfaces, biological and chemical sensing, printed circuits and neuromorphic devices. The use of organic materials also allows for their inclusion in flexible and wearable applications. A poly(3,4ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) OECT device consists of PEDOT:PSS in contact with metal drain and source electrodes. Au has been commonly used as electrode material mainly due to its process availability and its biocompatibility [1-2]. However due to a large difference in work function, the device shows high contact resistance, which limits the performance of the OECTs.

In this work, we study the effect different electrode materials to the performance of an OECT. Au, Pt, TiN, IrOx and ITO were chosen due to their process availability, their biocompatibility and their electronic properties. The OECTs were fabricated on 4-inch borosilicate glass wafers using standard microfabrication techniques (Figure 1). Every single device had a size of 7 mm x 7 mm containing 16 gate areas each. Interdigitated electrode (IDE) arrays and their contact lines were fabricated using either magnetron sputtering or electron beam evaporation, which depends on the selected electrode material, and the subsequent a lift-off processes in acetone and isopropanol. The contact lines were then passivated using Parylene C and the IDE arrays were opened by dry etching. An additional optical lithography was used to introduce a sacrificial photoresist layer for subsequent introduction of PEDOT: PSS to the IDE areas. A mixture of PEDOT: PSS solution (5 mL of PEDOT: PSS aqueous dispersion, 250μL of ethylene glycol, 50 μL of 3-methacryloxypropyl-trimethoxysilane (GOPS) [3]) was spin-coated over the wafers. The PEDOT:PSS on top of the sacrificial layer was then removed by dipping the wafer in acetone and isopropanol. The wafers were undergoing different heat treatment processes to stabilize the PEDOT:PSS on the IDEs. Chips were then separated from the wafer by dicing. Electrical characterization of the devices was carried out in Phosphate buffer solution (pH 7, 150 mM) using a Keithley 4200A-SCS parameter analyzer. In addition, the van der Pauw method was employed to characterize the electrical properties of the PEDOT:PSS film and extract the contact resistance values of the different electrode materials.
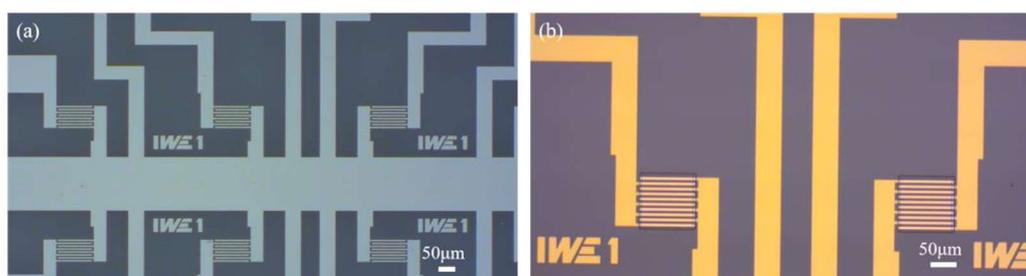

Figure 1: Schematic view of a PEDOT:PSS OECT with interdigitated electrode (IDE) structures. IDE arrays and their contact lines were fabricated from different electrode materials using lift-off technique. The contact lines were passivated by Parylene C. Optical images of two examples of electrode materials IrOx (a) and Au (b).

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Advanced 2D nanoscaled "MXene" interfaces as perspective immobilization platforms for design of (bio)sensors

Lenka Lorencova [1], Veronika Pinkova Gajdosova [1], Stefania Hroncekova [1], Tomas Bertok [1], Peter Kasak [2], Jan Tkac [1]

Lenka.Lorencova@savba.sk (Corresponding e-mail address)

[1]Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 845 38 Bratislava, Slovak Republic
[2]Center for Advanced Materials, Qatar University, P.O. Box 2713, Doha, Qatar

Abstract: The (bio)sensors based on functionalized surface nanostructures in combination with electrochemical methods allow us to achieve low limits of detection (LOD) and high specificity. The state-of-the-art 2D nanomaterials "MXenes" have appeared to be very promising and have become the subject of interest due to their specific properties and complex layered structure providing a number of alternatives in composition and subsequent utilization for various applications. In a pilot study, we examined the ability of the $Ti_3C_2T_x$ MXene (T: = O, -OH, -F) to detect electrochemically important analytes (O_2, H_2O_2 and NADH). The $Ti_3C_2T_x$ MXene showed remarkable electrocatalytic activity in H_2O_2 reduction with LOD at the nM level. In an effort to improve its stability and redox behavior, we subsequently modified MXene with Pt nanoparticles. The electrocatalytically active sensor based on the $Ti_3C_2T_x$/Pt nanocomposite was able to detect not only H_2O_2, but moreover small organic molecules (acetaminophen, dopamine, ascorbic acid, uric acid) at the nanomolar level[1]. Additionally electrochemical study confirmed a significant difference in the negative charge density on the surface of MXene and also in the electrocatalytic activity depending on the etchant (HF or in situ-generated HF from mixture of LiF and HCl) used in the preparation of MXenes[2]. In order to support the applicability of MXene-modified interfaces in biosensors, interfacial modification of the MXene should be implemented. To achieve this goal and as well to prevent non-specific binding, the modification of $Ti_3C_2T_x$ MXene interfaces by applying aryldiazonium-based grafting with derivatives bearing a sulpho-(SB) or carboxy-(CB) betaine pendant moiety was completed[3]. More than that our other concept consisted of $Ti_3C_2T_x$ MXene interfaced with chitosan for enzyme sarcosine oxidase immobilization. Such nanostructure-based biosensor determined sarcosine as a potential prostate cancer biomarker with LOD of 18 nM in a linear range up to 7.8 μM[4]. In the coming years, we suppose an exponential increase in the number of affinity biosensors based on MXene[1], while there is still a requiring demand to think about appropriate strategies for patterning the MXene interface and subsequent immobilization of target biomolecules. Furthermore, these designed MXene-based interfaces will expectantly excel in the progressive development of biosensors and in glycoprofiling of cancer biomarkers.

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A material-based approach for the development of wearable pH sensors

Federica Mariani [1], Isacco Gualandi [1], Francesco Decataldo [2], Luca Possanzini [2], Marta Tessarolo [2], Beatrice Fraboni [2], Domenica Tonelli [1], Erika Scavetta [1]

federica.mariani8@unibo.it

[1]Dipartimento di Chimica Industriale "Toso Montanari", Università di Bologna, Viale del Risorgimento 4, 40136 Bologna, Italy
[2]Dipartimento di Fisica e Astronomia, Università di Bologna, Viale Berti Pichat 6/2, 40127 Bologna, Italy

Abstract: Real-time and non-invasive monitoring of biological parameters by means of wearable chemical sensors holds great promise for next-generation technologies in personalised healthcare. However, such emerging applications pose several constraints to conventional electrochemical sensors, as conformability, robustness and simple architecture stand out as essential requirements. Organic electrochemical transistors (OECTs) are interesting candidates that can interface the biological domain providing intrinsic signal amplification, ease of miniaturisation and sensing capabilities without the need of a freestanding reference electrode. Moreover, the design of novel electrochemical transducers allows to develop selective OECT-based sensors and to realise innovative device architectures that well adapt to flexible substrates and textiles. In this contribution, we report a material-based approach for the development of wearable pH sensors based on the organic semiconductor poly(3,4-ethylenedioxythiophene) (PEDOT) doped with the pH dye Bromothymol Blue (BTB). Upon functionalisation of the gate electrode of the transistor with PEDOT:BTB, a flexible OECT-based pH sensor was realised on a plastic foil for the non-invasive monitoring of sweat pH [1]. Furthermore, a simpler, electrochemically gated device was designed based on PEDOT:BTB where a potentiometric mechanism allows to avoid the physically separated gate terminal, thus leading to a two-terminal pH sensor. The highly integrated configuration shows remarkable potential for sensor miniaturisation and the development of portable and wearable electrochemical probes. Indeed, the chemiresistor-like geometry based on flexible, all-polymeric materials is compatible with simple, high throughput and low-cost fabrication techniques, such as screen-printing, with potential integration into clothes or other real-life objects. In fact, the versatility and robustness of the twoterminal pH sensor architecture were demonstrated with the realisation of pH sensing textile yarns [2] and smart bioceramic fabrics [3] for real-time monitoring of body fluids.

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Gold nanostructured platform for lysozyme specific detection

Gheorghe Melinte[1,2], Oana Hosu[1], Geanina Ştefan[1,3], Diana Bogdan[4] Cecilia Cristea[1], Giovanna Marrazza[2]

Melinte.Gheorghe@umfcluj.ro

[1]Department of Analytical Chemistry, "Iuliu Hatieganu" University of Medicine and Pharmacy, 4 Pasteur Street, 400349 Cluj-Napoca, Romania
[2]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (Fi), Italy
[3] Robert Bosch SRL, Physical and Chemical Analysis Department (RBRO/EQV-A), Tetarom 3 Industrial Park,
Jucu Herghelie 407352, Cluj, Romania
[4]National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donath Street,
400293 Cluj-Napoca, Romania

Abstract: Lysozyme is an enzyme present in multiple organisms where it plays various vital roles. One of the most important relies on its antibacterial activity, being also called the body's own antibiotic. Despite its proven utility, lysozyme can potentially trigger allergic reactions in sensitive individuals, even in trace amounts, thus the need of continue monitoring of lysozyme in products rich in lysozyme like wine or egg white is of high importance [1].

In this work, an electrochemical aptasensor was designed for the analysis of lysozyme. First, poly-Llysine was electrodeposited at screen printed carbon electrodes (SPCE) in order to obtain a more structured platform with higher electroactive area. The best architecture was further chosen for sensor development. Next, gold nanostructures were electrodeposited from a mixture of $HAuCl_4$ and 10000 PEG solutions for enhanced electrocatalytic effect and to serve as immobilization platform for the thiolated aptamer. All platforms were electrochemically and morphologically characterized. For lysozyme detection, the 1st aptamer was immobilized within the thiol group from its 3'-end, followed by a blocking step of the remaining free sites of the gold nanotowers with 6mercaptohexanol. Next, after the lysozyme solution was dropped casted on the electrode surface and the aptamer-target reaction was performed, a 2nd aptamer, labelled with biotin, bound also lysozyme target to obtain a sandwich assay. Further, streptavidin-alkaline phosphatase (ALP) reacted with the biotin bound to the 2nd aptamer. For lysozyme quantification, ALP hydrolised the substrate (1-naphtil phosphate) and the signal obtained from the oxidation of 1-Naphtol was registered using differential pulse voltammetry technique.
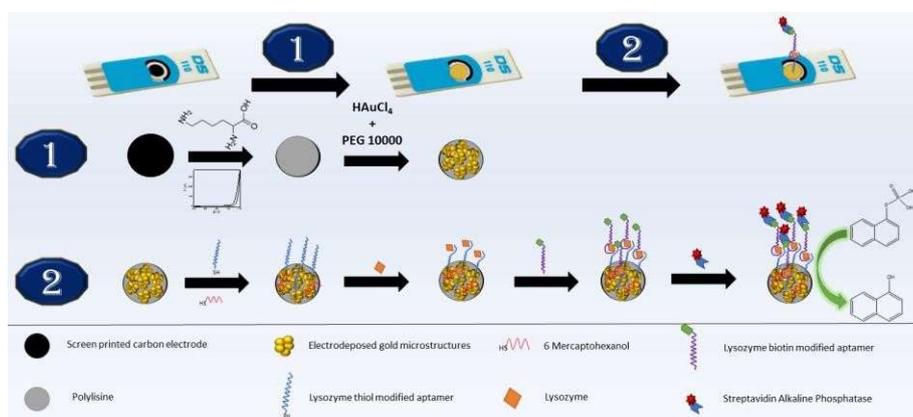

Figure 1: Schematic representation of the development of lysozyme specific aptasensor.

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Acknowledgement

Coating-free platinum nanoparticles for the electrocatalytic detection of hydrogen peroxide

E. Mazzotta[1], T. Di Giulio[1], N. Natalizio[1], V. Mastronardi[2], P. Pompa[2], M. Moglianetti[2], C. Malitesta[1]

elisabetta.mazzotta@unisalento.it (Corresponding e-mail address)

[1]Dipartimento di Scienze e Tecnologie Biologiche e Ambientali (Di.S.Te.B.A.), Università del Salento, Italy
[2]Istituto Italiano di Tecnologia (IIT), Genova, Italy

Over the last decades platinum nanoparticles (PtNPs) have found many increasing successful applications revealing excellent electrocatalytic activity mainly towards small molecules oxidation/reduction and oxygen reduction reaction [1], which has prompted their use in electrochemical sensing devices along with fuel cells design. Two key aspects in critically determining the beneficial exploitation of their high electron transfer and, in turn, their electrocatalytic activity are the adopted synthetic scheme and the approach for their integration with the electrode surface. It is in fact well-accepted that a correct understanding of the correlation between shape/surface structure and electrochemical reactivity indispensably requires the use of clean surfaces [2]. The use of effectively surface cleaned nanoparticles freed from capping agents represents an extremely important prerequisite to subsequently evaluate their electrocatalytic properties for any reaction of interest. Morever, in most cases, the use of composite systems integrating organic components and PtNPs is proposed for achieving nanoparticles anchoring to the electrode surface [3]. Among materials commonly used in combination with PtNPs in the design of electrocatalytic systems are carbon-based nanomaterials as reduced graphene oxide, graphite, and carbon nanotubes, conducting polymers, and polyanionic/cationic layers.

In the present work, we report an electrocatalytic application of PtNPs prepared by a simple and green method [4] producing size-tunable quasi-spherical nanoparticles, without the use of catalyst-poisoning reagents and/or organic coatings. These nanoparticles are stabilized by citrate molecules which is easily and completely removed by a simple and rapid step in NaOH, allowing to obtain cleaned coating-free nanoparticles after a simple washing in water. Here we demonstrate that PtNPs can be directly anchored to the electrode surface alone without any supporting material and can be effectively used for the electrocatalytic detection of H_2O_2, which has been selected as model molecule being well known the role of platinum as electrocatalyst in its redox processes [1] and also considering the significance of H_2O_2 in biological systems. PtNPs are analyzed by X-Ray Photoelectron Spectroscopy before and after citrate removal evidencing almost complete citrate elimination as well as high nanoparticles conductivity. PtNPs are deposited by a simple drop-casting method on the electrode surface guaranteeing no aggregation and homogeneous electrode coverage, as shown by Scanning Electron Microscopy analysis. Exploiting the fine tuning of size nanoparticles afforded by the adopted synthesis method, quasi-spherical nanoparticles with size as low as 4 nm and 18 nm are prepared, as revealed by Transmission Electron Microscopy analysis, and their electrocatalytic performances towards H_2O_2 reduction are compared and discussed.

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AC electric field mediated preparation of regular enzyme arrays and their functional characterisation

M. Noffke[1], C. Wenger[2,3], F. F. Bier[4], R. Hölzel[1]

Mareike.Noffke@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Am Mühlberg 13, 14476 Potsdam, Germany.
[2]IHP - Leibniz Institute for Innovative Microelectronics, Im Technologiepark 25, 15236 Frankfurt (Oder), Germany.
[3]Brandenburg Medical School Theodor Fontane, Fehrbelliner Straße 38, 16816 Neuruppin, Germany [4] Molecular Bioanalytics and Bioelectronics, University of Potsdam, Karl-Liebknecht-Str. 25, 14476 Potsdam, Germany.

Abstract: In this work, the enzyme Horseradish Peroxidase (HRP) is immobilized on nanoelectrode arrays by means of dielectrophoresis. Dielectrophoresis is the force acting on a polarizable particle caused by an inhomogeneous electric field. The force depends, amongst other factors, on the volume of the particle and the square of the gradient of the electric field [1]. Because of the small volume of the enzyme molecules, high field gradients are needed for their immobilization. To create sufficiently high field gradients, regularly arranged, vertical nanoelectrode arrays with different electrode tip sizes from 500 nm down to a few nm are used (figure 1A). The obtained enzyme nanoarrays are interesting tools for future biosensors or single enzyme molecule studies.

It was already shown that HRP can be immobilized on 500 nm tungsten cylinder electrodes (figure 1A and 1B) [2]. Now a method was developed to measure the activity of the HRP immobilizate on the whole nano-electrode array using the substrate Amplex Red and the detection of its oxidation to Resorufin by means of fluorescence microscopy (figure 1C). The use of even smaller electrodes is expected to lead to the deterministic singling of the enzyme molecules on the electrode tips [3]. For real-time monitoring of enzyme activity on individual electrodes, laser scanning microscopy and fluorescence correlation spectroscopy are used. For a quantitative interpretation of the results, experimental side effects like photooxidation and adsorption of fluorescent product molecules to surfaces have to accounted for. A microfluidic setup is being developed.
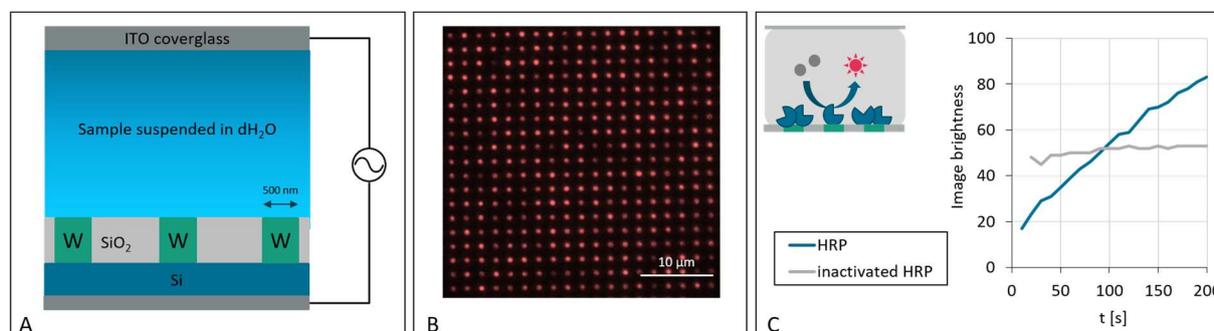
Figure 1: A) Schematic setup of the tungsten cylinder electrode array for protein dielectrophoresis. B) Fluorescence micrograph of Cy5-labeled HRP immobilized on nanoelectrode array.
C) Turnover of Amplex Red to Resorufin by immobilized HRP samples on nanoelectrode array, observed by fluorescence microscopy.

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Liquid-phase exfoliation of graphene by phytochemicals. A new source of redox-active nanostructured functional materials for (bio)sensing

Filippo Silveri[1], Flavio Della Pelle[1], Daniel Rojas[1], Qurat Ul Ain Bukhari[1], Dario Compagnone[1]

fsilveri@unite.it

[1]Faculty of Bioscience and Technology for Food, Agriculture and Environment University of Teramo, Via Renato Balzarini 1, 64100 Teramo, Italy

Abstract:

Polyphenol-rich natural products start to be quite employed to assist 2D nanomaterials 'synthesis'. However, a lack of studies on the phytochemical compounds involved and their ability to conferrer physicochemical properties for functional applications is evident.

In this work, a new sonochemical green and effective liquid-phase exfoliation strategy is proposed wherein, a flavonoid namely catechin (CT) exclusively assists graphite exfoliation in water-soluble graphene nanoflakes (GF). The obtained GF-CT flakes integrating well-defined electroactive quinone adducts, resulting in a redox-active nanostructured functional graphene.

The obtained few-layers graphene flakes intercalated with CT aromatic skeleton ensure tight electrical contact among graphene sheets, while the fully reversible quinonoid electrochemistry ($\Delta E= 28$ mV, $I_{p,a}/I_{p,c}= \sim1$) is attributed to the residual catechol moieties. The quinones functionalized nanoflakes results in stable (>8 weeks) and reproducible, not requiring activations or electropolymerization step. The GF-CT electrocatalytic boosting has been proven towards hydrazine (HY) and β-nicotinamide adenine dinucleotide (NADH), a pollutant and coenzyme, respectively. GF-CT-based electrodes demonstrated high sensitivity (nanomolar LODs) and extended linear ranges at low oxidation potential (+0.15 V), resulting significantly more performing compared with graphite commercial electrodes and graphene nanoflakes exfoliated with commonly used surfactants. By using the GF-CT nanoflakes, as functional materials for electrode modification, HY and NADH selective and accurate (recoveries between 104-95 %) quantification has been successfully achieved.

Moreover, exploiting the intrinsic conductivity of the GF-CT nanoflakes together with the electrocatalytic activity shown, our group is developing GF-CT exclusively-based films, combining smart low-cost benchtop technologies and flexible modular substrates. Thus, fully exploiting the intrinsic redox activity of the GF-CT-based nanofilms, first and second-generation biosensoristicplatforms are under development, in which the catechol-quinone moieties allow the communication with the biological element.

In our opinion, the proposed GF-CT elects itself as a cost-effective and sustainable nanostructured functional material, particularly promising in the nano(bio)sensing scenario.

From molecular docking to electrochemical detection of deoxynivalenol

G. Selvolini, G. Marrazza

giulia.selvolini@unifi.it

"Ugo Schiff" Chemistry Department, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy

Abstract: The presence of mycotoxins in foods poses serious threats to animal and human health, resulting in kidney and liver diseases, carcinogenic, mutagenic, hepatotoxic, teratogen effects, neurotoxic effects and even death [1]. Food safety is progressively becoming a global issue in the intensive agriculture and food industry growth scenario; the health reliability of food products must be determined in a quick, cost-effective and precise way [2]. This work proposes an enzyme-labeled voltammetric aptasensor to detect deoxynivalenol (DON) mycotoxin. Disposable screen-printed electrodes (SPEs) were modified with a nanocomposite material and used to develop a competitive format, which was revealed by an enzymatic labeling. The development steps of the aptasensor were partnered for the first time to a computational study, to gain insights onto the molecular mechanisms involved into the exploited competition. Thus, the molecular interaction between a thiol-tethered DNA aptamer (80mer-SH) and DON was investigated by a docking study, which allows to find the binding region of the oligonucleotide sequence and to determine DON preferred orientation in the binding event. A biotinylated complementary oligonucleotide sequence (20mer-BIO) of the aptamer sequence binding with DON was chosen to carry out a competitive format. Graphite screen-printed electrodes (GSPEs) were electrochemically modified with polyaniline and gold nanoparticles (AuNPs@PANI) by means of cyclic voltammetry (CV) [3] and worked as a scaffold for the immobilization of the DNA aptamer. Solutions containing increasing concentrations of DON and a fixed amount of 20mer-BIO were dropped onto the aptasensor surface: the resulting hybrids were labeled with an alkaline phosphatase (ALP) conjugate to hydrolyze 1-naphthyl phosphate (1-NPP) substrate into 1-naphthol product, detected by differential pulse voltammetry (DPV). According to its competitive format, the aptasensor response was signal-off, in accordance to the competitive format applied. Under optimized experimental conditions, a dose-response curve was obtained between 5.0 ng·mL^{-1} and 30.0 ng·mL^{-1} DON concentration range and a limit of detection (LOD) of 3.2 ng·mL^{-1} was achieved within a 1-hour detection time. Finally, preliminary experiments in maize flour samples spiked with DON standard solutions were also performed, retrieving good recovery values. The portability of SPEs and a remote-controlled device are coupled to make it suitable for a rapid and inexpensive screening analysis.
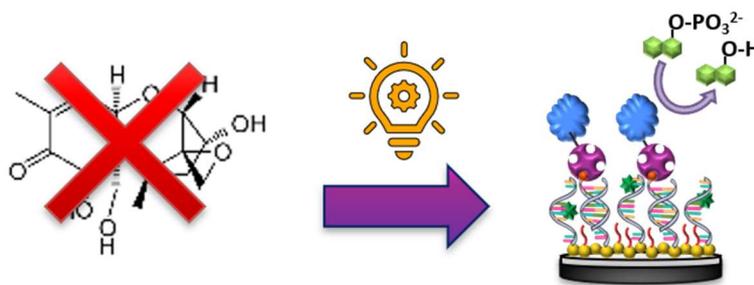
Figure 1: Scheme of the developed aptasensor for deoxynivalenol (DON) detection.

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Temperature-controlled silicon nanowire biosensor platform

Marcel Tintelott [1], Mohit Suranglikar [1], Fabian Brings [1], Vivek Pachauri [1], Sven Ingebrandt [1], and XuanThang Vu [1]

tintelott@iwe1.rwth-aachen.de; vu@iwe1.rwth-aachen.de

[1]Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Sommerfeldstr. 24, 52074 Aachen, Germany

Abstract: Silicon nanowire field-effect transistors (SiNW-FETs) are ultra-sensitive biosensors thanks to their high surface-to-volume ratio. It has been demonstrated that the detection limit of the biosensors based SiNW-FETs is down to attomolar and the sensors show a high dynamic detection range [1]. It is crucial that the measurement conditions during sensing have to be controlled to avoid cross-sensitivity, especially a stable temperature at which the measurements takes place. Temperature affects the characteristics of the SiNW-FETs and has a strong impact on the binding kinetic between target molecules and the probe molecules on the sensor surface for affinity sensing [2]. Temperature also has an impact on the chemical reaction in case the sensors are used to detect a chemical processes [3]. In this work, we present the realization of a portable temperature control unit integrated on a SiNW-FET sensor array based on surface integrated temperature sensors and a Peltier element combined with a PID controller. The SiNW sensors were fabricated based on a mix-and-match "topdown" approach combining electron beam lithography and optical lithography on a 4 inch silicon-oninsulator (SOI) wafer (SOITEC, France). The structuring of the top Si, doping and passivation of the contact lines, and formation of the gate oxide were adapted from our previous fabrication protocols [4]. Several micro-sized temperature sensors, which were fabricated from Pt and based on 4-point contacts were located close to the SiNW-FET sensors, which enable to probe the temperature at the SiNW-FETs precisely. The system was placed on top of a temperature control unit using a Peltier element that can precisely control and stabilize the temperature during the sensing procedure. We employed the system to investigate the behaviour of a chemical oscillator (the Briggs-Rauscher reaction) at different temperatures. The Briggs-Rauscher reaction is a reaction-diffusion system and is based on an oscillating chemical reaction between hydrogen peroxide and iodate in acidic solution [5]. The reaction changes the pH value of the solution due to the periodic consumption and production of H^+ ions. The change of the pH due to the oscillation was monitored using the SiNW-FET sensor arrays, while the temperature of the system was controlled and monitored closely during the reaction. Preliminary result is shown in figure 1 representing the change of the drain-source current of a SiNWFET over time during the Briggs-Rauscher reaction at room temperature. Further experiments will be carried out utilizing the SiNW-FET system to study the kinetic of the chemical oscillation at different temperatures. Furthermore, we will apply our temperature controlled SiNW-FET platform for future biosensing applications in which the dynamics of the reaction or binding event is strongly dependent on the temperature.
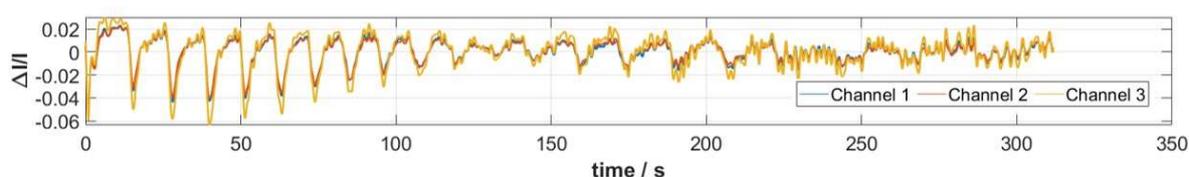
Figure 1: Electrical readout of the Briggs-Rauscher reaction over time at room temperature using a SiNW-FET array. A small reaction chamber (300ul) was integrated directly on the SiNW-FET array. The amplitude of the oscillation is decreasing over time and almost ended after 300 s.

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Quartz Crystal Microbalance Sensors Array based on Hairpin-DNA for the Detection of Volatile Organic Compounds.

[1] Sara Gaggiotti, [2] Marcello Mascini, [1]Angelo Cichelli, and [2] Dario Compagnone

sara.gaggiotti@unich.it

[1]Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy
[2]Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64100, Teramo, Italy

Abstract: The detection of gas molecules remaining a critical issue in several fields, such as environmental, chemical, agriculture, foodstuff, as well as in the medical diagnosis. Gas chromatography remain the reference method for the volatile compounds quantitative analysis (VOCs), but the use of gas sensors array is a new reality able to detect different aroma compounds and offer new opportunities in many fields. In particular, devices equipped with sensor arrays can return different information, compared to the gas chromatography, allowing a multi-target o totally un-target analysis. The latter sensors are powerful devices potentially able to take a fingerprinting of a sample or track an event evolution, in which a gas-pattern change occur. The use of Bio-compounds, as sensors recognition elements, is a widely exploited and consolidate strategy, however, in the gas sensing is a recent and challenging hot topic. Indeed, in the last ten years, the development of gas sensors devices based on the use of biomolecules as recognition/interaction element has allowed an unexpected advancement in VOCs sensing. Compagnone's group, for the first time, used piezoelectric sensors modified with HairpinDNA (HpDNA) sequences with unpaired bases as molecular binding element for the evaluation of the interaction versus four chemical classes (alcohols, aldehydes, esters and ketones) of volatile organic compounds (VOCs) [1]. Subsequently, the same sensor array developed in [1] was used by Gaggiotti et al. [2] to monitor volatiles profile change in carrots. In this work we used the HpDNA with heptamer loops as sensing elements versus 16 pure VOCs of different chemical class, and for analysing of real beer samples during fermentation. Data was elaborated by multivariate analysis. Principal component analysis (PCA), Hierarchical Cluster analysis (HCA), and partial least square discriminant analysis (PLS-DA) were used to classify the samples. The results obtained demonstrated the ability of the array based on heptamer loops to discriminate among chemical classes of VOCs, but more interestingly the sensors array was able to discriminate among beer samples at different times of fermentation. This work represents the starting point for the use of hpDNA-based gas sensors for food analysis.
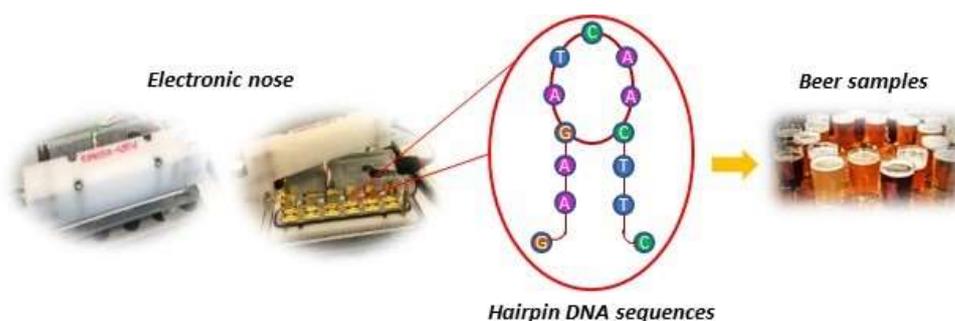
Figure 1: Electronic nose instrument and Hairpin DNA structures for food analysis.

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Paper-based electrodes for bioanalytical applications

M. Riedel,[1] G. Göbel,[1] O. Nagel,[2] W. Wirges,[2] V. Scherbahn,[3] C. Beta, R. Niedl[3] and F. Lisdat[1]

gero.goebel@th-wildau.de

[1]Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, 15745 Wildau, Germany
[2]Institute of Physics and Astronomy, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany
[3]Diamond inventics GmbH, Magnusstraße 11, 12489 Berlin, Germany

Medical and industrial demands have increased the need for easy and quick (bio)analytical detection devices with a high accuracy and low costs.[1,2] Particularly electrochemical biosensors provide a great promise for the utilization in point-of-care diagnostics since they are simple, cheap in operation and portable. For some years now, paper-based sensors have become more and more in focus of research and industry. The implementation electrochemical methods in paper-based set-ups of holds some interesting features, making them attractive as analytical tool. Paper is commercially available as a material in various compositions and thicknesses, biodegradable and environmentally friendly, and thus well suited for disposable systems. Due to the hydrophilicity and the high porosity, paper is nicely suited for the fabrication of microfluidic channels, which can be operated without external fluid control. Moreover, the paper surface can be easily modified by printing and coating of chemicals or (bio)molecules. This study will demonstrate some basic electrochemical characterization of paperbased electrodes needed for the elaboration of an analytical test system. Therefore, a carbon working electrode and a combined silver counter/reference electrode has been printed onto the paper fiber using a screen printing procedure. This 2-electrode arrangement gives rise to a stable charging current and a defined faradaic response, if a redox mediator is added to the paper. In order to investigate the possible application in antibody-based analysis, a model test system was tested on the paper-based electrodes. Therefore, a capture antibody was immobilized on the paper fiber and subsequently the binding of a signalling antibody (analyte) modified with peroxidase was monitored electrochemically. Initial experiments suggest that the paper-based electrodes can clearly discriminate between the analyte and control samples. The results provide insights in the development of paper-based electrodes and can be useful for analytical purposes.

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Interfacial behaviour of carbosilane dendrimers at the interface between two immiscible electrolyte solutions

Karolina Kowalewska[1], Tamara Rodriguez-Prieto[2,3,4], Slawomira Skrzypek[1], Jesús Cano[2,3,4], Rafael Gómez Ramírez[2,3,4]*, Lukasz Poltorak[1]*

karolina.kowalewska@chemia.uni.lodz.pl

[1]Department of Inorganic and Analytical Chemistry, Electroanalysis and Electrochemistry Group, Faculty of Chemistry, University of Lodz, Tamka 12, 91-403 Lodz, Poland.
[2]Department of Organic and Inorganic Chemistry, Chemical Research Institute "Andrés M. Del Río" (IQAR), University of Alcalá, 28805 Madrid, Spain.
[3]Ramón y Cajal Health Research Institute (IRYCIS), 28034 Madrid, Spain.
[4]Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 28029 Madrid, Spain.

Abstract: The Interface Between Two Immiscible Electrolyte Solutions (ITIES)[1] or polarized liquid – liquid interface is a young and still growing sub-field in electrochemistry. The ITIES signal transducing element is an ultra-thin interface formed between two liquids - hydrophilic and hydrophobic, which do not mix with each other. ITIES can be studied with all known electrochemical techniques. In the presented research, we have used Ion Transfer Voltammetry (ITV).[2] This technique is used to study the interfacial ion transfer phenomena occurring at the liquid – liquid interface under the influence of the applied potential. Five carbosilane dendrimers were tested using this method. Dendrimers are spherical structures with a core, branches and free internal spaces. Due to the latter feature, they have the ability to transfer various inorganic or organic compounds without reacting with them. As a result, these systems can be used in the medical sector for drug delivery applications. The experimental studies pursued in this work include: the effect of the dendrimer concentration initially present in the water phase and the value of the potential scan rate on the recorded electrochemical signals. Based on the obtained results, the following parameters were calculated: diffusion coefficients, limit of detection (LOD), detection sensitivity. This work has shown that all dendrimers are electrochemically active meaning that under the influence of the applied Galvani potential difference they undergo interfacial ion transfer reaction accompanied with adsorption/desorption to the liquid-liquid interface.[3]

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Roll-to-roll large-scale manufacturing of integrated microfluidics

Annukka Kokkonen[1], Sanna Aikio[1], Lauri Rannaste[1], Caglar Elbuken[1], Leena Hakalahti[1], Jussi Hiltunen[1]

Annukka.Kokkonen@vtt.fi

[1]VTT Technical Research Centre of Finland, Kaitoväylä 1, 90590 Oulu, Finland

Abstract: A key challenge for large-scale experiments and commercialization of disposable integrated microfluidic devices is the availability of manufacturing methods that are repeatable and low-cost, and have high throughput. To address this challenge, we have developed large-scale roll-toroll (R2R) manufacturing using 1) hot embossing, 2) laser patterning and 3) hybrid integration methods. Here, we discuss manufacturing of integrated microfluidics for immunosensor and electrochemical wearable sensor applications.

Roll of laser patterned microfluidics is shown in Fig 1a). The microfluidic channels and through holes for liquid application and air venting are cut into the tape and polymer layers that are laminated together. The laser cutting is done by a CO_2 laser integrated into a R2R conversion line (Delta) as shown in Fig 1b). R2R laser cutting enables manufacturing of thousands of microscope slide size microfluidic sensors in a single process run with high repeatability. Lamination of the cover layer can be done right after the cutting of the microfluidic channels that reduces the contamination risk. We have been studying the influence of the cutting parameters to the microfluidic channel quality, e.g. burr formation and sidewall roughness. A like laser cutting has been utilized in wearable electrochemical sweat sensors.[1]

Integrated immunosensor is shown in Fig. 1c). The device was designed to be easy to use with integrated sample handling functionalities such as plasma separation membrane for blood filtration, hot embossed microfluidics for sample transport and mixing, and blister for sample actuation. Cyclic olefin copolymer (COC) was chosen as sensor material. The device has been demonstrated to be capable to detect 2 µg/ml of C-reactive protein in the whole blood by using fluorescence based immunodetection.[2]
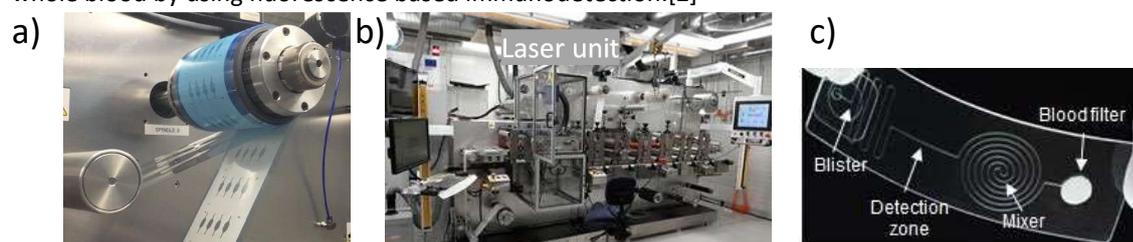

Figure 1: a) Roll of laser patterned microfluidics. b) Roll-to-roll conversion line with CO_2 laser unit. c) Integrated immunosensor with sample handling functionalities.

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Novel 3D-Printed Multiplanar Microfluidic Systems for Improved Biosensor Integration.

Benjamin Heidt[1], Renato Rogosic[1], Joseph Lowdon[1], Rocio Arreguin-Campos[1], Manlio Caldara[1], Kathia Jimenez-Monroy[1], Hanne Diliën[1], Kasper Eersels[1], Bart van Grinsven[1], Thomas J. Cleij[1]

Benjamin.Heidt@MaastrichtUniversity.nl

[1]Sensor Engineering Group, Faculty of Science and Engineering, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

Next to receptor, transducer and signal-processing elements, a key part of a sensing device is the sample handling system. Sample handling can take many forms, from simple flow-cells to intricate microfluidic circuits that form Lab-on-a-Chip devices. Due to the history of microfluidics, stemming from microchip manufacturing, the common construction techniques are of photolithographic nature[1]. Those traditional microfluidic construction methods are work- and time-intensive, require cleanroom manufacturing and are restricted to one plane.

The advent of 3D-printing created a new mode of cleanroom-free fabrication for microfluidic channels. 3D-printing is cheap, fast and significantly less work-intensive than traditional methods. Furthermore, it enables the construction of complex 3-dimensional microfluidic structures. However there are several challenges. Construction of microfluidic channels inside 3D-printed devices is difficult due to resin clogging, which gets more challenging, with more complex structures and smaller channels[2]. We present a novel, easy way to create microfluidic channels, with challenging structures in 3-dimensional form factors. Unlike conventional methods that depend on a cleanroom, this new method requires minimal investment of under 500 Euro to be set-up and minimal running costs due to commonly available consumables. The ability to cover 3-dimensional shapes makes this novel method very interesting for combination with established sensor-readout devices, as the shape can be easily adapted to any system.

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Tunable design of antifouling polymer brushes: from fouling molecular studies to biosensor applications

Markéta Vrabcová[1], Ivana Víšová[1], Milan Houska[1], Jakub Dostálek[1,2], Michala Forinová[1], Alina Pilipenco[1], Kateřina Mezuláníková[3], Alexandr Dejneka[1], Hana Vaisocherová-Lísalová[1]

lisalova@fzu.cz

[1]Institute of Physics of the CAS, Na Slovance 2, Prague, Czech Republic
[2]Austrian Institute of Technology GmbH, Muthgasse 11, Wien, Austria
[3]Department of Life Sciences, Imperial College London, London SW7 2AZ

Abstract: Recently, antifouling properties of artificial coatings have become increasingly important in all the fields dealing with real-world complex samples, such as bioanalytics, biomedicine, or food safety and security [1]. Particularly in biosensors and bioanalytics, it is critical to carefully balance the properties of the coatings in order to assure high loading capacity of biorecognition elements and preservring the resistance against nonspecific adsorption on the sensor surface at the same time.

Here we present an extensive study on tailoring of antifouling properties of different zwitterionic carboxybetaine-based (CB) polymer brush coatings documented by surface sensitive and vibrational methods, such as spectroscopic ellipsometry, infrared spectroscopy, SPR, QCM, and contact angle measurements. The optimum properties, such as swelling, hydration level and thickness of the polymer brush coatings are determined. Further, the impact of functionalization steps (including NHS/EDC activation, reaction with biorecognition elements, and subsequent deactivation) on the antifouling performance is evaluated. The optimized procedures of deactivation a significant improvement of post-functionalization resistance is determined. In addition, we investigated a longterm stability of antifouling CB-zwitterionic polymer brush coatings and determined the optimum storage conditions, preserving their fouling resistance and ability to be functionalized with biorecognition elements.

The above-mentioned research resulted in a design of a novel highly-stable polymer brush coating based on copolymerization of specific zwitterionic, and nonionic compounds, that combines high post-functionalization fouling resistance (below 5 ng/cm^2 from crude biological samples) with high biorecognition element loading capacity. Such coating architecture offers a versatile toolbox for a wide range of biosensor technologies for analytical applications. To demonstrate the performance of the new copolymer brush coating, the label-free QCM biosensor for direct detection of E.coli O157:H7 in crude hamburger samples in less than 20 minutes without any extra-preparation steps with the LOD as low as 3 CFU/mL is demonstrated.
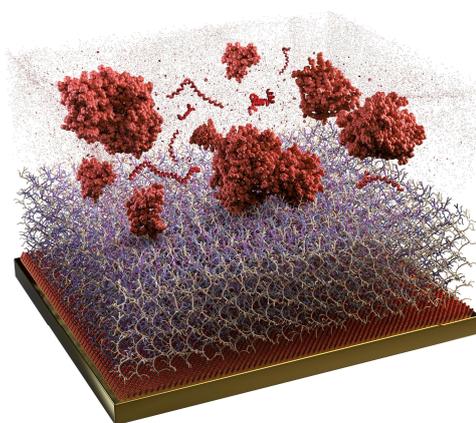

Figure 1: Ultraresistant polymer brushes enable specific detection from complex biological samples, such as undiluted blood plasma.

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Miniaturised microfluidic-based DBS sampling for therapeutic drug monitoring

Camilla Marasca [1,2,3], Michele Protti [1], Michael Seidel [3], Andrea Armirotti [4], Andrea Cavalli [2], Laura Mercolini [1]

camilla.marasca2@unibo.it / camilla.marasca@iit.it

[1]Research Group of Pharmaco-Toxicological Analysis, Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[2]Computational and Chemical Biology, Fondazione Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy
[3]Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 München, Germany
[4]Analytical Chemistry Lab, Fondazione Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy

Abstract: In recent years, the scientific research in the field of bioanalysis has focused an increasingly widespread interest on the miniaturisation of all processes involved in sampling, pretreatment and analysis. These miniaturised approaches are particularly promising to be able to streamline all the steps of the analytical workflow, while the reliability and soundness of the analytical results are improved. To this purpose, microsampling technologies represent an effective strategy able to combine the analytical lab needs with those of patients undergoing sampling, especially in the case of whole blood collection. Conventionally, whole blood is drawn by phlebotomy, an invasive and uncomfortable practice especially for delicate populations. For example, psychiatric patients often follow polypharmacy regimens and are subjected to frequent therapeutic drug monitoring (TDM), aimed at accurately measuring plasma levels of drugs and metabolites, to reduce adverse and toxic effects and improve clinical outcomes [1]. In order to improve whole blood collection in the clinical practice, an advanced and miniaturised whole blood sampling technology was developed by combining a microfluidic platform with dried blood spot (DBS) collection. This microfluidic-based DBS sampling allows to accurately collect 10 µL of capillary whole blood in a minimally invasive way, by means of a fingerprick. Once the microchannels integrated within the microfluidic chip are completely filled, the sample is transferred onto a cellulosic card generating 10µL fixed-volume DBS, independently from blood haematocrit (HCT) value. In fact, blood density can often be the main parameter affecting classic DBS volumetric accuracy and homogeneity, thus influencing analytical data reliability [2]. Moreover, this kind of blood microsamples are amenable to remote, point-of-care sampling as they can be dried, stored and transported at room temperature. These represent undoubted logistical advantages, while ensuring stability profiles often comparable to those of cryopreserved biological fluids and reducing biohazard risks. This original, miniaturised methodology based on a microfluidic chip for accurate DBS generation was designed for the monitoring of patients under therapy with phenytoin, an anti-epileptic drug chosen as a test compound and analytical target. A straight-forward and effective pretreatment combined with an original HPLCUV method was developed with the aim of obtaining fast and high-throughput analytical procedures.

The optimised workflow was validated, showing satisfactory results in terms of extraction yield (> 82%) and precision (RSD < 9.3%), then it was successfully applied for the TDM of patients undergoing phenytoin treatment. In order to demonstrate the reliability of quali-quantitative results obtained by the developed microsampling methodology, a reference plasma procedure was carried out for comparison, giving good agreement between the datasets (RE < 17%). The proposed microfluidicbased methodology proved to be a valid alternative to traditional, invasive venepuncture for in-tube whole blood withdrawal, allowing a volumetric capillary blood microsampling independent to HCT, while streamlining sample processing steps and simplifying storage and transport. The miniaturised approach proposed herein demonstrated to be suitable as a point-of-care sampling strategy for the TDM of patients treated with phenytoin, when compared to reference, routine procedures.

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Polyfluroalkyl substances sensing with serum proteins: transposing toxicological studies to biosensing strategies

Giulia Moro[1-2], Elise Daems[2], Matteo Trande[1], Lorenzo Maso[3], Sonia Covaceuszach[4], Alberto Cassetta[4], Laura Cendron[3], Frank Sobott[5], Alessandro Angelini[1], Ligia M. Moretto[1], Karolien De Wael[2]

giulia.moro@unive.it

[1]Department of Molecular Sciences and Nanosystems, Ca' Foscari University, Via Torino 155, Mestre, Italy.
[2]AXES research group, University of Antwerp, Groenenborgerlaan, 171, Antwerp, Belgium.
[3]Department of Biology, University of Padua, Via U. Bassi 58, Padova, Italy.
[4]Istituto di Cristallografia – CNR, Trieste Outstation, Italy.
[5]Univeristy of Leeds, Leeds LS2 9JT, UK.

The study of the interactions between per- and polyfluroalkyl substances (PFAS), a class of ubiquitous global contaminants, and serum proteins can improve the design of protein-based electrochemical biosensors for the on-site monitoring of these perfluorinated compounds in natural and industrial waters [1]. In the last decade, the affinity of PFAS for serum proteins (particularly albumins and globins) was subjected to extensive toxicological studies to explain their accumulation patterns, elimination rates and toxicity [2]. To transpose these findings in the design of bioreceptors, human serum albumin (hSA) was first considered. Untreated and delipidated hSA performances towards perfluorooctanoic acid (PFOA) binding were characterized with a multi-analytical approach. Isothermal titration calorimetry measurements allowed defining the stoichiometry and the affinity constants of the complexes showing a higher reproducibility of the binding event with the delipidated hSA. The stability of this latter was confirmed also by collision induced unfolding analysis performed by native nano-electrospray ionization mass spectrometry showing the potential of this latter in bioreceptor screening [3]. The molecular bases of PFOA interaction were clarified by the X-rays crystallography. Delipidated hSA was confirmed to be a more suitable bioreceptor than untreated hSA and so further applied in a proof-of-concept study for the development of an impedimetric biosensor (Fig.1) [4]. Aiming to design a portable device, the bioreceptor was immobilized at graphite screenprinted electrodes previously modified with pyrrole-2-carboxylic acid. The impedimetric data interpretation was supported by the small-angle X-ray scattering analysis of the bioreceptor-target complex. A parallel investigation of PFAS impact on globins activity was performed following the changes in the heme electroactivity in presence of perfluorooctane sulfonic acid (PFOS) and PFOA. The results suggested the potential of serum protein-PFAS interactions monitoring for analytical, toxicological as well as precision medicine applications.
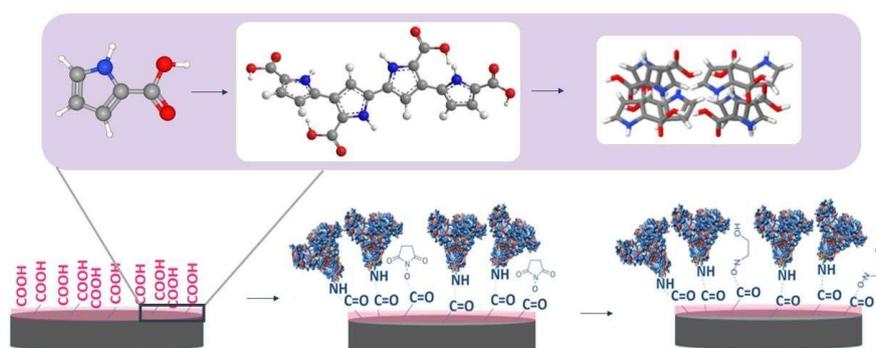

Figure 1. Schematic procedure optimized for hSA immobilization at graphite screen-printed electrodes modified with pyrrole-2-carboxylic acid for the impedimetric biosensing of PFOA.

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Real-time measurement of smart hydrogel swelling dynamics based on direct optical detection of cross-sectional area changes

Klaudia Rückmann [1], Guannan Mu [1], Hsuan-Yu Leu [2], Jules Magda [2], Florian Solzbacher [3], Christopher F. Reiche [3], Julia Körner [1,3]

Corresponding author: rueckmann@geml.uni-hannover.de

[1]Institute of Electrical Engineering and Measurement Technology, Leibniz University Hannover, 30167 Hannover, Germany.
[2]Department of Chemical Engineering, University of Utah, Salt Lake City, UT 84112, USA
[3]Department of Electrical and Computer Engineering, University of Utah, Salt Lake City, UT 84112, USA

In order to investigate the dynamic swelling-deswelling behaviour of stimuli-responsive (smart) hydrogels, reliable and robust characterization techniques are necessary. Hydrogels are viscoelastic three-dimensional networks of crosslinked hydrophilic polymers which contain a larger portion of their total weight or volume of water [1]. Stimuli-responsive hydrogels swell or shrink, i.e. absorb or release water, in response to an external stimulus or parameter change (e.g. ion concentration, light or pH value) [2]. The development of novel smart hydrogels requires methods to study their swelling dynamics. Especially real-time in-situ observation of small volumes poses a challenge, since many commonly used methods such as weighting only allow for discrete measurements. Furthermore, they may require the gel to be removed from their aqueous environment, leading to partial drying of the material.

Here, we present a method based on direct optical observation within a fluidic chamber environment which overcomes these difficulties and allows for real-time tracking of the hydrogel's area change. The optical setup is comprised of a 3D printed fluidic chamber equipped with a glass bottom on which the hydrogel sample is placed. A CMOS sensor underneath allows for continuous image recording in userdefined sequences. The fluidic chamber is additionally equipped with a liquid inlet and outlet for the use of different solutions and a top opening, which is necessary to ensure proper illumination (see Fig.

1A). The hydrogel's cross-sectional area is automatically calculated from the recorded images by a selfwritten Python-based script.

We have characterized the capabilities of this detection method with acrylamide-based smart hydrogels (thickness 400 µm and 800 µm) in varying concentrations of phosphate-buffered saline (PBS) solution in terms of reproducibility, (long-term) stability, sensitivity and detection limit. Fig. 1B exemplary depicts optical images (native and processed) for one PBS concentration and Fig. 1C shows a plot of the dynamic area change derived from the image processing. With this new detection method, it is possible to characterize any kind of stimuli-responsive hydrogel (composition and shape) in the desired solution environment with regard to its dynamic as well as steady state behaviour in a simple yet precise manner.
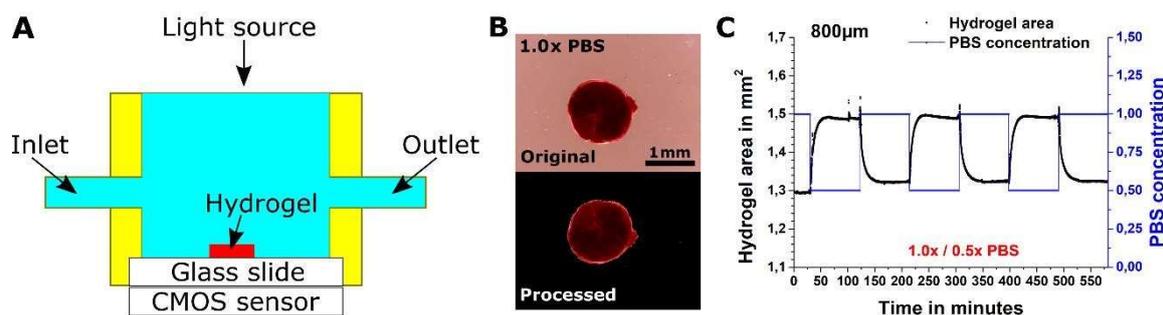

Figure 1: (A) Optical characterisation setup. (B) Recorded optical (upper) and processed (lower) image of an acrylamide-based smart hydrogel (thickness 800 µm) in 1.0x PBS solution. (C) Dynamic swelling response of another hydrogel sample in two different PBS concentrations derived from the processed optical images.

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Application of multiharmonic QCM method to study cytochrome c adsorption on lipid layers

Marek Tatarko, Tibor Hianik

tatarko4@uniba.sk (M.T.), tibor.hianik@fmph.uniba.sk (T.H.)

Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Mlynská Dolina F1, 842 48 Bratislava, Slovakia

Abstract: Detection of cytochrome c (cyt c) as well as study of the mechanisms of its interaction with lipid membranes is rather challenging due to important biological role of this protein in the cell apoptosis. Current research suggests improvement in cyt c detection by its binding to the specific DNA aptamers immobilized at the surface. Previous studies already used the lipid monolayers as a support for binding cyt c [1]. In our research, we prepared layers on the gold surface of the quartz crystal transducer with chemisorbed 1-octadecanethiol monolayer [2]. To produce the layers, we used liposomes made from mixture of lecithin and DMPG (dimyristoylphosphatidylglycerol). We used SARK-110 vector analyzer to monitor the formation of lipid layers and for study of the interaction of cyt c with supported lipid monolayers using measurement of fundamental and higher current harmonics. The method of multiharmonic quartz crystal microbalance (QCM) also allowed us to monitor energy dissipation of the layer. We studied the formation of the lecithin/DMPG layers in the different buffer solutions and with different ratio of lecithin and DMPG. We also analyzed the role of various cations on the lipid monolayer formation. 1:1 molar ratio of the lipids proved to be optimal for the cyt c adsorption on the surface of the crystal. Multiharmonic QCM can be also used for amplification of cyt c detection at surfaces by nanoparticles and nano/micromotors modified by DNA aptamers.

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Plasmonic biosensors fabricated by galvanic displacement reactions for monitoring biomolecular interactions in real time

Ruth Fabiola Balderas-Valadez[1], Claudia Pacholski[1]

cpachols@uni-potsdam.de

[1]Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Straβe 24-25, 14476 Potsdam, Germany

Abstract: Simple, fast and reproducible optical biosensors are prepared by the spontaneous reduction of gold ions on hydride-terminated porous silicon surfaces (galvanic displacement reaction). The resulting nanostructured gold layer on silicon shows an optical response in the visible range based on the excitation of localized surface plasmon resonance (LSPR) [1]. Variations in the refractive index of the surrounding medium result in a colour change of the sensor which can be observed by the naked eye (Figure 1). By monitoring the spectral position of the LSPR (a valley in the interference pattern at

⎕600nm in air), using reflectance spectroscopy, a bulk sensitivity of 296 nm ± 3 nm/RIU is determined. The ability to monitor biomolecular interactions with the plasmonic biosensor is studied through functionalization of its surface with protein A (PrA), a molecule capable of selectively binding to rabbit immunoglobulin G (IgG). For this purpose, PrA is deposited on the sensor either by physical adsorption or by covalent coupling. Both strategies are successfully tested by monitoring the specular reflectance spectrum and following the shift in the location of the LSPR (λ_P) on the wavelength scale, which is caused by the adsorption or binding of the biomolecules to the sensor surface (Figure 2). The optical response of the sensor is dependent on the concentration of the IgG that interact with the PrA attached to the plasmonic surface. The selectivity of the sensor is also probed by immersion of the sensor in bovine serum albumin (BSA) solution. In this case no optical response is detected. Furthermore, biomolecular interaction investigations facilitated the determination of equilibrium dissociation constants (K_D) for protein A/rabbit IgG: K_D=1.5×10⁻⁷M ± 2.7×10⁻⁸M and K_D=1.4×10⁻⁷M ± 2.8×10⁻⁸M for the sensors functionalized by covalent coupling or physical adsorption of PrA, respectively. These K_D values are in accordance with reported ones for similar studies [2]. These results demonstrate the potential of the developed optical sensor for cost-efficient biosensor applications.

Figure 1: Photographs of the plasmonic sensor. Change of the surface colour due to changes in the refractive index of the surrounding medium
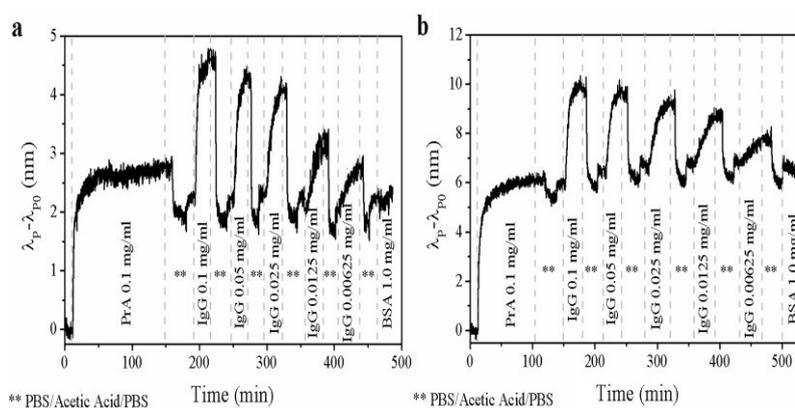

Figure 2: Optical response of plasmonic sensors to deposition of PrA and successive exposure to rabbit IgG solutions: **a** results for plasmonic sensor with covalently bound PrA, **b** results for plasmonic sensor with physically adsorbed PrA

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Data pre-processing of FTIR spectra from individual grass pollen grains embedded in paraffin

Sabrina Diehn[1], Boris Zimmermann[2], Valeria Tafintseva[2], Murat Bağcıoğlu[2], Achim Kohler[2], Mikael Ohlson[2], Siri Fjellheim[2] and Janina Kneipp[1]

diehnsab@hu-berlin.de (Corresponding e-mail address)

[1]Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Straße 2, 12489 Berlin, Germany
[2]Faculty of Science and Technology, Norwegian University of Life Sciences, 1432 Ås, Norway

Abstract: The fast and reliable identification and characterization of pollen species are of great interest in palaeoecology, allergology, and plant science. Since grass pollen grains have similar morphology, the identification by Fourier-transform infrared (FTIR) spectroscopy is more suitable than light microscopy and shows promising results for bulk experiments. Nevertheless, the acquisition of reliable FTIR spectra of single pollen grains is hindered by a strong spectral contribution from Mie scattering that occurs by measurements of micro-sized particles[1]. A promising approach for the measurement of reliable spectra is the embedding of the pollen grains in paraffin, which on the one hand reduces the scattering artifacts but on the other hand, it adds the spectral contribution from the paraffin to the spectra[2].

In this study, single grains from five different pollen species of the Poaceae family were embedded in paraffin, and their single grain spectra were obtained by FTIR microspectroscopy. Different data preprocessing approaches that enable digital suppression of the paraffin contribution in the spectra will be presented. In particular, using non-negative matrix factorization (NMF), the spectral contributions can be separated and the pollen spectra contribution can be analyzed individually. Another approach, a modified extended multiplicative signal correction (EMSC) algorithm allows the reduction of the spectral variation in the paraffin signals and enables classification of the pollen spectra using partial least square-discriminant analysis (PLS-DA) and machine learning[3].

The proposed data pre-processing strategies show the great potential of FTIR microspectroscopy for automated identification and characterization of single pollen grains and the analysis of micro-sized particles in general.

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Design, Simulations and Manufacturing of a Microring Resonator Biosensor Assisted by Dielectrophoresis

Anders Henriksson[1], Matthias Jäger[2], Laura Kasper[2], Peter Neubauer[1], Mario Birkholz[3]

henriksson@tu-berlin.de

[1]Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Straße des 17. Juni 135, 10623 Berlin, Germany

[2]Technische Universität Berlin, Department of High Frequency Technology/Photonics, Einsteinufer 25, 10587 Berlin, Germany

[3]IHP – Leibniz-Institut für innovative Mikroelektronik, Im Technologiepark 25, 15236 Frankfurt (Oder), Germany

Abstract: As biosensors are approaching the nanoscale, analyte mass transfer and bonding kinetics have been ascribed as a crucial factors that limit their performance. In the current work we have designed a system that applies dielectrophoretic forces to increase the mass transfer of a microring resonator-based biosensor. Dielectrophoresis, the migration of polarized dielectric particles in a non-uniform AC field, has previously successfully been applied to achieve a 1000-fold improved detection efficiency in nanopore sensing[1] and may potentially significantly increase the sensitivity also in microring resonator biosensors. The test chip design with two different electrode configuration, packaging strategies and electric field gradient simulations are presented. FEM simulations calculated for both electrode configurations displayed a ∇E^2 of 10^{16}-10^{20} V^2m^{-3} around the sensor areas. This is comparable to ∇E^2 previously reported for interaction with common analytes such as proteins and antibodies. The sensor chip was prepared by the SGH25_PIC technology at IHP. The position of the resonance peak of the realized ringresonator was confirmed to be linearly correlated to the refractive index of the environment, confirming its functionality as a sensor with a sensitivity of 4.9 ±0.03 nm/RIU (refractive index units) and a Q factor of approximately 1-1.5x10^5. Finally, as a proof of principle, the microalgae C. Cohni was successfully aligned onto the sensor surface by applying an AC field of 10 MHz and 10 Vpp[2].
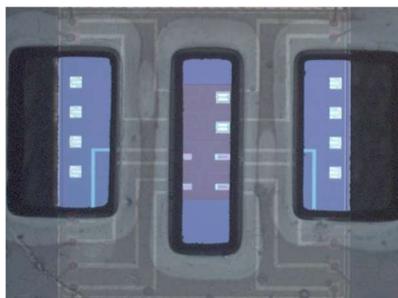
Figure 1: Microscope image of the packaged sensors chip (2.5 x 2.5 mm)

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Broad range amino acid identification via salt-concentration dependent gold nanoparticle aggregation

Stefan Leisten[1], Moustafa Saleem[1], Tetiana Kurkina[2], Parham Mohajerani[1], Satender Kataria[3], XuanThang Vu[1], Max Christian Lemme[3], Ulrich Schwaneberg[2], Sven Ingebrandt[1], Vivek Pachauri[1]

leisten@iwe1.rwth-aachen.de

[1]Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Sommerfeldstrasse 24, 52074 Aachen, Germany.
[2]Institute of Biotechnology, RWTH Aachen University, Worringer Weg 3, 52074 Aachen, Germany.
[3]Chair of Electronic Devices, RWTH Aachen University, Otto-Blumenthal-Strasse 2, 52074 Aachen, Germany.

Abstract: Gold nanoparticles (AuNP) are a versatile and well-studied material system that can be used for a plethora of different applications ranging from drug delivery to photothermal heating. The colour of these colloids is dominated by an absorbance band at around 520 nm that arises due to plasmon resonance, i.e. displacement and subsequent oscillation of the electron cloud surrounding particles due to incident electromagnetic radiation. The exact position of this band strongly depends on the size of particles, as well as the dielectric environment presented by potential surfactants and the solvent used. Recently, the tendency of certain analytes to induce nanoparticle aggregation has been used to determine their concentration based on the observed colour change upon mixing with AuNP. Structurally similar molecular systems, for instance amino acids (AA), can be difficult to distinguish using this approach. Martínez *et al.* proposed using nanoparticles dispersed in four different solvents to change the aggregation properties of AA that behave almost identically in the original solvent [1]. This 'barcode' then allowed for the unambiguous identification of 5 different amino acids.
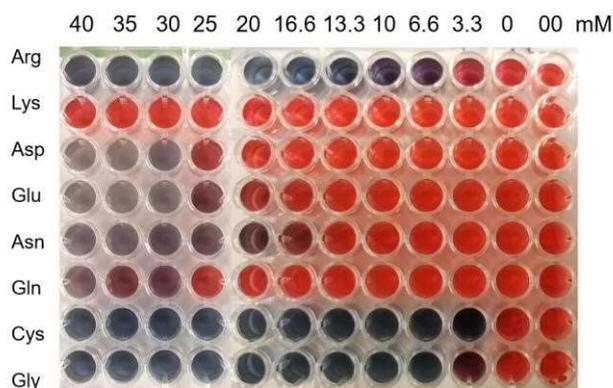
Figure 1: Microtiter plate containing a mix of AuNP solution (10nM), eight different AA (30mM) and varying salt (NaCl) concentrations, 10 min after AA addition. The salt concentration in each column is indicated in the top row. '00' denotes the control (no AA).

Literature indicates that certain salt concentrations are required to 'unlock' the ability of certain AA to aggregate AuNP [2]. With a similar goal in mind, we use different concentrations of sodium chloride (NaCl) to accelerate aggregation of AuNP via eight different AA. As demonstrated for a concentration of 30 mM (Fig. 1), this leads to a distinctive pattern for each amino acid that forms within 10 min. We are optimistic that further experiments with other AA concentrations will not only allow amino acid identification for one concentration but many different concentrations over a broad range.

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A novel Copper Nanoclusters-based platform for label-free detection of human serum albumin

Mariagrazia Lettieri [1], Pasquale Palladino, Simona Scarano*, Maria Minunni

*simona.scarano@unifi.it (Corresponding e-mail address)

[1]Department of Chemistry "Ugo Schiff", University of Florence, 50019, Sesto Fiorentino, FI, Italy

Abstract: The remarkable features of nanomaterials have revolutionized the existing detection strategies. Nowadays, most of bioanalytical detection techniques involve the use of nanomaterials, in particular nanoparticles. However, recently, nanoclusters became a new emerging class of nanomaterials apllied in different fields [1]. Principally, due to the large availability, cheapness and low toxicity as well as the outstanding fluorescent properties (*i.e.* high quantum yield (QY), photostability and large Stokes shifts), copper nanoclusters (CuNCs), have attracted more attention [2]. The stability and the easy fabrication of nanoclusters enabled to develop CuNCs-based platforms for the sensitive determination of various analytes, even in complex biological matrix. Therefore, in this study, the specific template-targeted CuNCs growth was exploited to quantitative detect human serum albumin (HSA) in urine and human serum. HSA is the body's predominant serum-binding protein which plays a crucial role in the maintenance of oncotic blood pressure. In addition, it acts as a carrier for many biological ligands such us hormones, fatty acids and steroids. The concentration of HSA, in body fluids, greatly influences the state of health of the patients. Taking into account these considerations, quantitative determination of human serum albumin is of clinical interest in early diagnosis of serious pathological conditions like as albuminuria and albuminemia. Here, we present a CuNCs-based assay in which copper nanoclusters were used as fluorescent signal indicators to detect serum albumin in complex biological matrix (Figure 1).
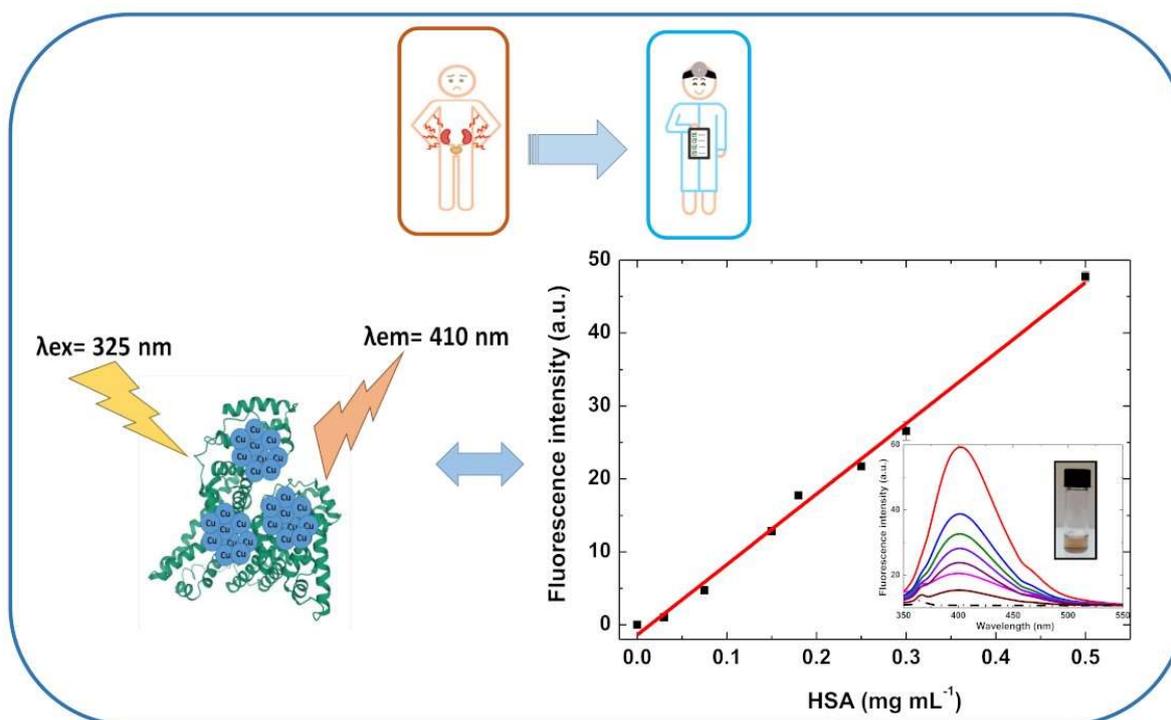
Figure 1: Detection strategy of CuNCs-based platform for label-free detection of albumin.

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Towards CMOS compatible materials for Surface enhanced Raman Spectroscopy (SERS)

C.L. Manganelli [1], HM. Ettehad[1], M. Masood[1], D. Spirito[1], C, Wenger[1,2]

manganelli@ihp-microelectronics.com (Corresponding e-mail address)

[1]IHP–Leibniz-Institut für innovativeMikroelektronik, Im Technologiepark 25, 15236 Frankfurt(Oder), Germany
[2]BTU Cottbus-Senftenberg, 03046 Cottbus, Germany

Abstract: The surface nano-engineering arena is now boosting the development of characterization techniques capable to reveal the structure properties of composite materials and also proteins. Among all the techniques with ultra-high sensitivity, high spectral resolution, and high spatial and temporal resolution, surface-enhanced Raman spectroscopy (SERS) is deemed as an effective technique to obtain optical fingerprint of few molecules at a µm or even nm scale. In SERS, the hurdle of the low Raman scattering rate is overcome by exploiting the huge local boost of the incident electromagnetic field obtained through the electromagnetic coupling between nanoparticles attached or in close vicinity to the analytes [1]. For plasmonics in the visiblewavelength range, silver and gold represent the favorite choice because of their low losses. However Au and Ag are not compatible with CMOS microfabrication processes and hence hinder the development of low cost and large scale plasmonic devices, because of their high cost and their ability to contaminate semiconductor materials. Going beyond silver and gold means developing high performance and cost effective substrates fully based on CMOS compatible techniques. Very recent studies investigated the surface plasmonic band in metal-Germanides in a spectral window ranging from the visible to near infrared [2]. Following this approach, we evaluated the optical performance of Silicide based micro-structures in the infrared.

A correct evaluation of SERS mechanism and an optimized design of the plasmonic structures requires a detailed analysis of the interaction of the electromagnetic field with suitably patterned plasmonic material. To this purpose, we propose a Finite Element Method (FEM) platform for calculations of electromagnetic field to optimize the CMOS manufactured nanostructures. The near-field enhancement studies are performed solving the scattered field of Maxwell's wave equations in a wavelength domain study with an incident excitation wavelength that reproduces the incident laser wavelength of Raman experiments. This study exhibits how the combination of FEM calculations and proper experimental setups for the evaluation of dielectric constants can act as a key tool to understand the plasmonic properties of CMOS compatible materials, aiming to a more straightforward nano-engineering of substrates for SERS in the mid-infrared.
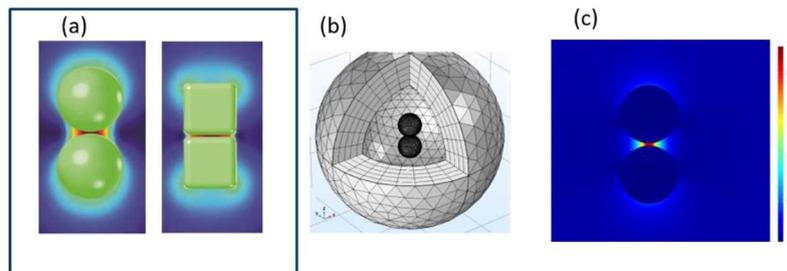

Figure 1: (a) ideal representation of patterned micro-structures that present electro-magnetic enhancement; (b) graphical representation of the FEM calculation for Silicide microstructures; (c) electro-magnetic enhancement for silicide microstructures.

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Fabrication and subsequent optimization of a plasmonic fiber optic sensor for biosensing applications

Nabarun Polley[1, 2]*, Peter Werner[1, 2], Ruth Fabiola Balderas Valadez[2], Claudia Pacholski[1, 2]

*nabapolley@uni-potsdam.de

[1]University of Potsdam, Physical Chemistry, innoFSPEC, Am Muehlenberg 3, 14476 Potsdam, Germany
[2]University of Potsdam, Institute of Chemistry, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

Abstract: The need of efficient biosensing for early disease detection, drug discovery and national security and defence is inevitable [1]. Plasmonic sensors have received significant attention as a potential solution due to its highly sensitive, reliable and rapid detection strategies. The plasmonic resonance created by the collective oscillation of free electrons at metal/dielectric interfaces has often been utilized for label free biosensing. However, bulky optical instrumentation of conventional plasmonic device limits the real-life applicability. As a potential alternative, optical fibers can be exploited for creating plasmonic sensors which facilitate the implementation of plasmonic sensors in real life applications.

Addition of plasmonic nano/microstructures on an optical fiber tip allows realizing a very compact optical sensor with several benefits. However, because of the inherent structure of the optical fiber, it is difficult to incorporate nano/micro structures. We have invented a fast-chemical approach to prepare gold nano-hole arrays on fiber tip. The efficacy of such plasmonic sensor as potential biosensor has been previously reported from our group [2]. However, the detection for small molecules remains a challenge at low concentration regime. A possible solution to overcome this limitation is either by improving the sensitivity of the sensor or by increasing the sensing volume.

Here we report a plasmonic fiber optic sensor with integrated microgel layer for improved overall sensor response by increasing the analyte loading capacity (2D surface to 3D volume) [3]. A process for depositing hydrogel microgel monolayers with high reproducibility on sensor surfaces has been developed. The produced monolayer was characterized by dark-field microscopy (Fig. 1a) and scanning electron microscopy (SEM, Fig. 1b). The method has high throughput and is perfectly suited for batch processing. The effect of incorporating multiple layers into the sensor has also been investigated (Fig. 1c). For this purpose, the sensing performance of the sensors concerning changes in temperature and in the refractive index of the surrounding medium (water-ethanol mixtures) has been studied. From the sensitivity studies it can be concluded that the multilayer approach has superior performance over a single layer approach. It is intuitive that our techniques and results demonstrated in the present work will open-up multiple avenues in the field of plasmonic biosensing.
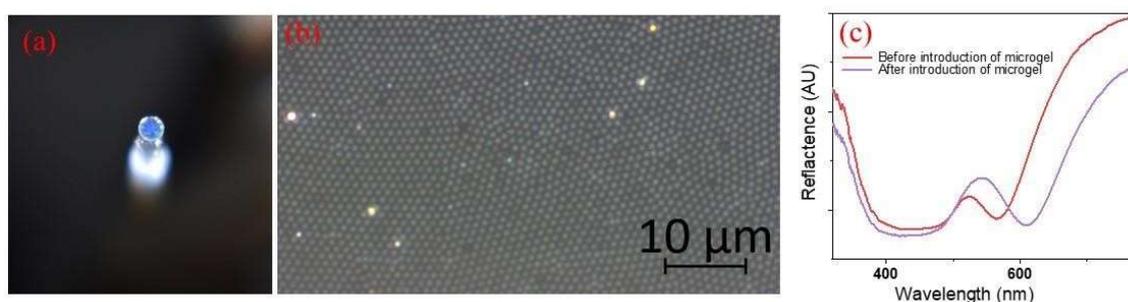

Figure 1: (a) Microgel monolayer on the tip of an optical fiber. (b) Dark field microscopic image of microgel monolayer. (c) Spectral response of fiber optic plasmonic sensor before and after microgel incorporation.

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Realization of a flexible SPR bioassays platform: Study of molecular interaction between HER-2 and novel NanoBodies

Daniele Storelli[1], Stefan Scholz, Xuan Thang Vu, Alessandro Angelini, Federico Polo, Joachim Knoch, Sven Ingebrandt, Vivek Pachauri

storelli@iwe1.rwth-aachen.de

[1]Institute of Materials in Electrical Engineering 1, RWTH Aachen University, Sommerfeldstraße 24, 52074 Aachen, Germany
[2]Institute of Semiconductor Electronics, RWTH Aachen University, Sommerfeldstrasse 24, 52074 Aachen, Germany
[3]Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venezia, Italy

Abstract: Surface Plasmon Resonance (SPR) is a widely used analytical technique to determine biochemical and biomolecular interactions. A necessary requirement for SPR to work reliably is the assembly of a smooth and clear optical system [2], which requires high standard materials and precision manufacturing. Bioassays in aqueous media require optical components such as prisms and SPR chips made of high refractive index (RI) glass, which increases the costs of production dramatically. As an alternative, optically transparent polyimide films present interesting physical properties for their use as a substrate for SPR chips. Characteristically high glass transition temperature (Tg), robust surface, low thermal expansion make them suitable for thin-film deposition [3], high RI value (>1.7) make them ideal as low-cost solution for SPR. Here, we demonstrate such a flexible SPR platform (figure 1a,b) based on the use of a transparent polyimide substrate. Such an SPR setup typically detects RI changes on the 50 nm gold film due to biomolecular interactions as small as 10^{-5} [1], which are then used to accurately determine the adlayer thickness and the kinetics of molecular or biomolecular interactions. The flexible SPR chips were thereby deployed to detect the kinetic interaction between the HER-2 breast cancer biomarker and two different epitopes specific nanobodies (NBs).

NBs are small polypeptides chains, which resemble the capturing moiety of monoclonal antibodies (mABs) and offer several advantages as receptor molecules. Given the simpler structure, NBs can be chemically modified in specific positions and suitably be bound on a sensing platform while keeping the proper orientation facing the sample solution, thus being more efficient in capturing the analyte. NBs can also be mass produced keeping the production costs low, thus making them available in large amounts. In the sensorgram of figure 1d, it is visible how the kinetic interaction between the HER-2 cancer biomarker and the nanobodies concentrations between 1 pM to 1 µM were successfully measured, validating the use of the polyimide layer as a substitute for the high refractive index glass.
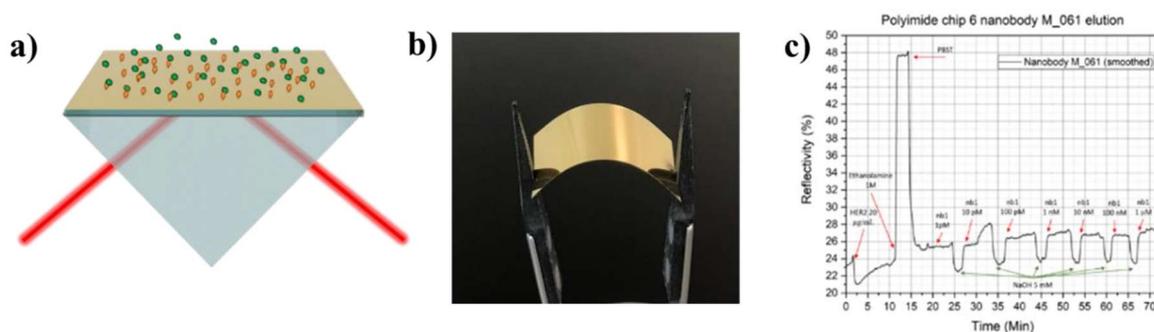

Figure 1: a) Representation of a Kretschmann setup of an SPR biosensor, b) image of a flexible polyimide SPR chip, c) kinetic sensorgram showing molecular binding of nanobodies with the HER-2 cancer biomarkers.

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Synthesis of gold nanoparticles in a 3D hydrodynamic focused microreactor and their application for online chemiluminescence

Yanwei Wang [1], Michael Seidel

Michael.Seidel@mytum.de (Corresponding e-mail address)

[1]Institute of Hydrochemistry, Chair of Analytical Chemistry and Water Chemistry, Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 Munich, Germany.

Abstract: Gold nanoparticles (AuNPs) have drawn big attention in chemiluminescence (CL) system with excellent properties, and the catalytic activity of AuNPs depends on size, shape and surface charge property.[1] The conventional batch synthesis of gold nanoparticles is well known, however it was shown, that microfluidic reactors are able to synthesize AuNPs with narrower size distributions and faster reaction rates.[2] In this work, a three-dimension (3D) microreactor was used to automatically synthesize AuNPs through a single-phase reaction in room temperature. Fouling was prevented by utilizing 3D hydrodynamic focusing flow through the simple assembly process of layering PMMA sheets and pressure sensitive adhesive (PSA) tape. A large variety of PMMA slides and PSA tapes can easily be designed and cut with a cutting plotter which allows a fast fabrication of microfluidic structures for different applications. The assembly of the devices was carried out manually with a simple alignment tool. No thermo-compression was required and only a soft compression by a roller was sufficient. The complete fabrication process from device design concept to working device can be completed in minutes without the need of expensive equipment. The synthesis was coupled directly with CCD camera for recording CL signals. All operations were performed in an automatic way. The property of AuNPs was easily controlled by tuning concentration of reagents in a 3D microreactor during synthesis. The synthesized AuNPs were used as catalyst for luminol-NaOCl CL system, and with optimized parameters of synthesis, the CL signal was enhanced hundreds of times. Without adding another stabilizer, AuNPs were stable for more than one month. By adding some salt, AuNPs aggregated and the catalytic activity was greatly enhanced. Glutathione was detected as an example and the CL signal was inhibited which was related to the concentration of glutathione. It offers a good way to confirm optimal synthesis condition of AuNPs for a certain CL system. For further application, synthesized AuNPs can be bind with antibody or aptamer for specific detection of analytes.[3]
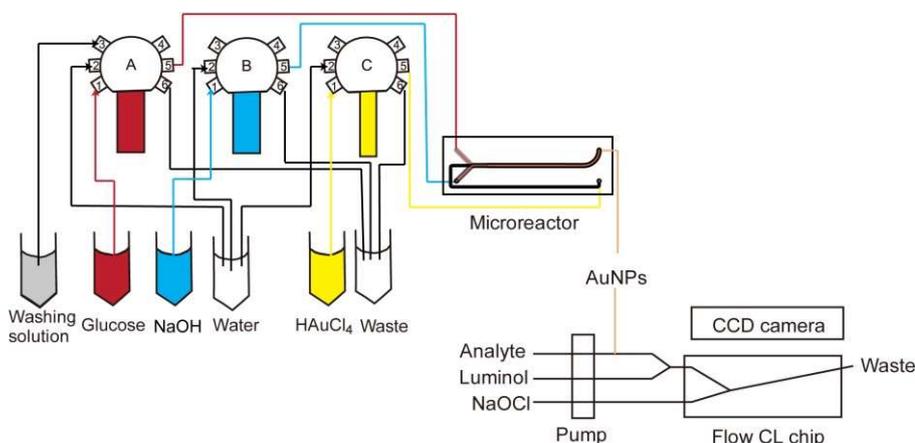
Figure 1: Scheme of online synthesis and CL measurement system. The synthesis was controlled automatically by Matlab. Reagents were supplied to the microreactor via three glass syringes connected to a 6-port valve. Synthesized AuNPs mixed with analyte, luminol and NaOCl in microchip to generate CL signal which was recorded by CCD camera.

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Light-addressable electrodes for the manipulation of biological systems in microfluidic channels

Rene Welden [1,2], Michael J. Schöning [1,3], Patrick H. Wagner [2], Torsten Wagner [1,3]

welden@fh-aachen.de

[1]Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, Heinrich-Mußmann-Straße 1, 52428 Jülich, Germany

[2]Laboratory for Soft Matter and Biophysics, KU Leuven, Celestijnenlaan 200D, B-3001 Leuven, Belgium

[3]Institute of Biological Information Processing (IBI-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Abstract: For the detection and manipulation of biological and chemical specimens in microfluidic systems, electrochemical electrodes can be used as sensors and actuators. Most frequently, noble metal electrodes are integrated for a wide range of applications. However, the geometry and location of these electrodes are defined during the design and fabrication process for the specific task and cannot be changed easily. Especially, in combination with biological systems this can be a drawback, as the exact location and geometry of the point of interest is only determined after e.g., cell culture cultivation and visual inspection. A different approach is the use of a semiconductor as electrode material, where charge carriers are generated by means of light [1]. The combination of these lightaddressable electrodes (LAE) with a digital light-processing (DLP) projector enables a flexible spatiotemporal adjustment of the conductive area.

In this work, a sol-gel fabrication method for a glass/SnO_2:F/TiO_2 LAE structure is presented and morphological and photoelectrochemical parameters are discussed. The structure of the LAE is depicted in figure 1. For the fabrication, a thin TiO_2 film was deposited on the glass/SnO_2:F substrate by a non-aqueous sol-gel process with subsequent spin-coating (2500 rpm) and heating steps (95 °C, 550 °C) [2]. The effect of different thicknesses of the TiO_2 for the application as an LAE were characterized by means of scanning electron microscopy and photocurrent measurements. Additionally, a microfluidic chamber was attached on top of the LAE, and fluorescence measurements with a pH-sensitive dye were performed to evaluate the spatio-temporal addressing of the LAE by visualising the local pH change inside this microfluidic chamber.

The results indicate the feasibility of the LAE, fabricated by a non-aqueous sol-gel approach, for the light-directed addressing inside a microfluidic system.
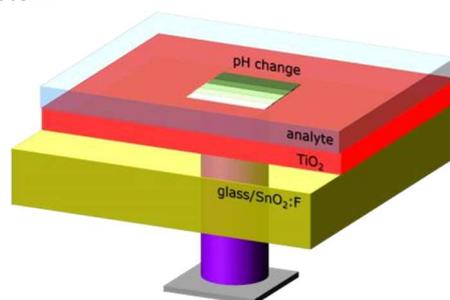
Figure 1: Schematic of the glass/SnO_2:F/TiO_2 structure with a local pH change at the illuminated area.

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Development and Optimization of an Optical Sensor Based on Reflectometric Interference Spectroscopy to Characterize Protein Kinase Inhibition

Viola Wurster, Günther Proll, Peter Fechner, Carolin Huhn

viola.wurster@uni-tuebingen.de

Institute of Physical and Theoretical Chemistry, Eberhard Karls Universität Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

Abstract: Protein kinase (PK) activity is vital for cell growth and proliferation. In the presence of tumor promotors PKs promote the growth of tumor cells. The most universal PK inhibitor, which can stop this process, is staurosporine, which -being toxic- is used for fundamental research on PK inhibition [1]. In this study, an optical, label-free and time-resolved method of reflectometric interference spectroscopy (RIfS) is developed to investigate kinase activities. When combined with a binding inhibition test, RIfS can be utilized both for kinase screening and inhibitor screening.

Sensor optimization included a coating of the sensor surface with biopolymer and immobilized inhibitor, regeneration and detection methods: Amino dextran was found to be a biopolymer preventing nonspecific interactions of the glass sensor with the kinases, while providing sufficient anchoring groups for a direct linking of staurosporine. The interaction of the immobilized inhibitor and kinases proved to be very strong, common media failed to regenerate the surface. Only tryptic digestion led to satisfactory results. We hypothesize, that irradiating white light during RIfS induced a photoreaction of staurosporine with the kinase to form covalent bonds on the sensor surface. This also prohibited binding inhibition tests due to UV absorption by the inhibitor and impairment of the binding signal of the PK. To circumvent these problems, lower wavelengths (under 400 nm) were eliminated by irradiating with green instead of white light; switching the detector method from RIfS to 1-lambda spectroscopy.

Using the optical sensor together with 1-lambda spectroscopy, kinase binding was monitored directly and binding inhibition tests using different inhibitors were performed.
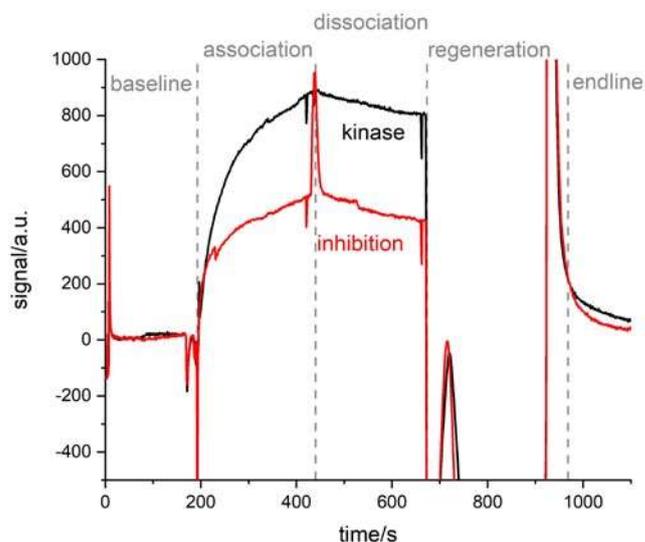

Figure 1: 1-lambda measurements using a sensor with a surface coated with amino dextran and immobilized staurosporine. Black: association curve of 5 µg protein kinase A in buffer. Red: association curve of 5 µg protein kinase A in buffer, inhibited with a 100fold molar excess of staurosporine.

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Development of a multiplex algae toxin immunoassay for the monitoring of algal blooms in surface water

Auernhammer A., Seidel M.

Institute of Hydrochemistry, Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 Munich, Germany

Algal blooms, the increased growth of cyanobacteria, have been observed more frequently in recent years. This phenomenon is favoured by climate change and the eutrophication of water bodies. It can be assumed that algal blooms will continue to increase as a result of excessive nutrient inputs to water bodies, heat waves and dry periods.[1] Due to an exponential growth spurt of cyanobacteria, the contamination of water bodies with cyanotoxins is increasing. Cyanotoxins are secondary metabolites formed intracellularly, which are released into the water as free toxins after cell death. This poses a threat to the affected ecosystem as well as to human health.

Currently, hazard assessment is based on experience, visual inspection of water bodies, and discrete sampling.[2] Contrary to these efforts, accidents continue to occur, as at Lake Mandicho in 2019 or at Lake Constance in 2020. To prevent accidents, comprehensive monitoring of potentially endangered waters is urgently needed. Therefore, an online monitoring system with cloud-based data processing is to be developed in cooperation with A.U.G. Signals Ltd., Toronto and Hydroisotop GmbH, Schweitenkirchen, as part of an AIF-ZIM project in order to be able to predict increased cyanobacterial growth and algal blooms at an early stage.

The online monitoring system is designed to collect information on certain parameters that can lead to excessive growth of cyanobacteria and to evaluate them online using an algorithm. These include nutrient concentrations of nitrate, phosphate and oxygen, as well as pH, solids content and redox potential. In addition, stress factors such as S- and Se-organic compounds that could induce an algal blooms will be monitored. In addition to the above parameters, it should also be possible to monitor free toxin concentrations in the water and intracellular toxins. For this purpose, an automated analysis platform is required, which, in addition to the online determination of cyanotoxins, also has an automated sample preparation, which includes the separation of the free and intracellular toxins, the lysis of the cyanobacteria, enrichment and lavation of the toxins.

The objective of the TUM is the development of an automated microarray immunoassay for the simultaneous quantification of different cyanotoxins with automated sample preparation and enrichment steps for free and intracellular toxins. The chemiluminescence-based biosensor will be tested on an algal reactor at laboratory scale and subsequently in the field. The measurement results will be integrated into a cloud-based online monitoring system at a later stage.

To establish the algal toxin immunoassay, a suitable immobilization strategy for the structurally diverse analytes must be developed first. Then, the sensitivity of the assay is determined and optimized by calibration experiments. The influence of the matrix on the immunoassay is investigated using cyanobacteria grown in shaking flasks. Similarly, the sample preparation and enrichment procedure is developed and optimized using cyanobacteria grown in shaking flasks. Subsequently, the automation of the sample preparation is elaborated and tested on an algae reactor.
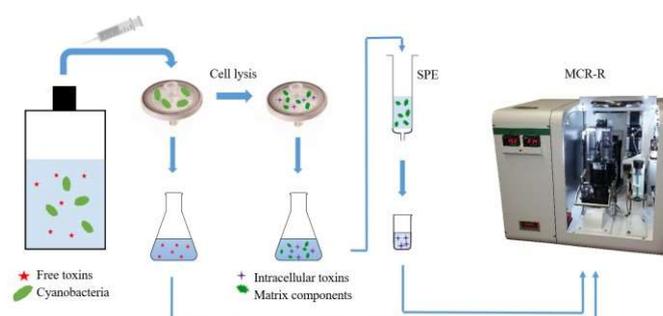

Figure 1: Schematic representation of sample preparation with sampling, separation of free toxins and cyanobacteria, cell lysis, purification of toxins exemplary with SPE and MCR-R as analysis platform.

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Development of a LAMP-Based Lateral Flow Assay for the Rapid Detection of SARS-CoV-2 infections

Saloni Agarwal [1]*, Christian Warmt [2], Joerg Henkel [2], Frank F. Bier [1,3]

*Corresponding author, E-Mail: agarwal@uni-potsdam.de

[1]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[2]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB)
14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: As COVID-19 caused by the SARS-CoV-2 virus, spreads rapidly in all the countries across the world, this emergency time calls for some rapid POC detection systems, with minimal equipment, better durability in extreme climates along with providing high sensitivity and high specificity, simultaneously.

Only PCR-based testing is sufficiently reliable and meets the requirement for sensitivity and specificity. Currently, antigen-based rapid tests in a Point-of-Care format are becoming widely adapted, however, these tests are much less reliable and need for an alternative to both PCR-testing and antigen tests is still prevalent. PCR testing has become gold standard, because of its unique sensitivity that has its reason in the use of nucleic acid based information. However, PCR needs laboratory equipment and is time consuming. Therefore, nucleic acid based detection combined with POC technology is required to solve the need of rapid and reliable testing at the point of need. The testing has to be affordable and should be performable everywhere and thus, making it easy for people in remote areas to be diagnosed.

Hence, we are investigating a lateral flow based detection system, combining the ease of handling with an appropriate nucleic acid amplification. Loop mediated amplification LAMP, has been shown to be the most effective isothermal amplification technique, that works reliably outside an established lab environment with ultimate sensitivity.1 The adaptation of LAMP to SARS-CoV-2 detection has been reported recently.[2] To improve performance, we adapted LAMP to the sequences of the N1-3 fragment of SARS-COV-2. This approach will detect virus nucleic acids with high sensitivity and specificity in less than 15 minutes (including the preparation time). The oligonucleotide sequences have been used in RT-LAMP and will serve to detect various fragments of N1-3 maintaining sensitivity and specificity.

The lateral flow nucleic acid detection follows the principle shown in figure 1, two probes of the oligonucleotides are labelled by fluorescein isothiocyanate (FITC) and biotin respectively. The assay works similar to a sandwich immunoassay and is performed on a lateral flow device. The signal is generated by change of colour, with the use of gold nanoparticles (GNPs). Once the GNP conjugated with the oligonucleotide captures the test sequence, the GNPs reach beyond a certain size and corresponds to the colour change and can be noted in few minutes. Instead of needing a fluorescence device or any other system to confirm the result, gold nano-particle based signal may simply be recognised with the naked eye. This technique could be an effective tool in this emergency time. To add to the precision, we will try to quantify the sample load with a smart-phone based data acquisition.
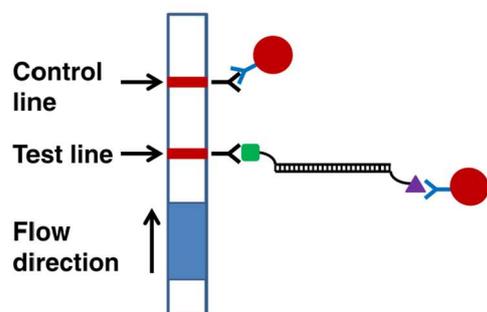

Figure 1: A simple idea for the Lateral Flow detection system used as a principle in the experiments. The test would be deemed positive, if we have both, the control and test bands coloured.

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Diagnose breast cancer and identify the most aggressive subtype by electrochemical immunosensing of matrix-metalloproteinase-9

Beatriz Arévalo[1], Amira ben Hassine[2], Alejando Valverde[1], Verónica Serafín[1], Ana Montero-Calle[3], Noureddine Raouafi[2], Jordi Camps[4], Meritxell Arenas[4], Rodrigo Barderas[3], Paloma Yáñez-Sedeño[1], Susana Campuzano[1], José M. Pingarrón[1]

beaare01@ucm.es

[1]Analytical chemistry department , Faculty of Chemistry, Complutense University of Madrid,Av. Complutense s/n, 28040 Madrid, Spain.
[2]University of Tunis El Manar, Tunis Faculty of Science, Sensors and Biosensors Group, Laboratory of Analytical Chemistry and Electrochemistry, 2092 Tunis El Manar, Tunisia.
[3]ChronicDiseaseProgramme, UFIEC, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid, Spain.
[4]Unitat de Recerca Biomedica, Hospital Universitari Sant Joan, Institutd'Investigació Sanitaria Pere Virgili, UniversitatRovirai Virgili, Reus, Spain.

Abstract: Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death among women worldwide. Extracellular matrix degradation, one of the causes of malignant tumor growth, invasion, metastasis and angiogenesis is mainly carried out by metalloproteinases, among which it can be highlighted the matrix metalloproteinase 9 (MMP-9). Recent studies have proven that an increase in circulating MMP-9 levels is closely related to the presence and aggressiveness of this disease, being considered nowadays a candidate biomarker to diagnose and classify this neoplasia.

For this reason, this communication describes a sandwich immunoassay for its electrochemical determination involving carboxylic acid-modified magnetic microbeads (HOOC-MBs) activated with EDC/NHSS chemistry, as suitable microsupports for capture antibody (cAb) covalent immobilization. The immunoconjugates so prepared allow the efficient capture of the target MMP-9 which is further recognized with a biotinylated detection antibody (biotin-dAb) enzimaticaly labelled with streptavidin-horseradish peroxidase (Strep-HRP). Detection was performed using amperometry at -200 mV (vs. Ag pseudoreference electrode) upon magnetic capture of the modified-MBs on the working electrode surface of the SPCEs and the hydroquinone/H_2O_2 system.

The developed bioplatform exhibits analytical characteristics in terms of sensitivity (LOD of 2.4 pg/mL) and selectivity for MMP-9 standards, attractive and suitable for endogenous determination of the target metalloproteinase in cell lysates and serum samples from patients diagnosed with different BC subtypes. Results achieved in serum samples, in agreement with those provided by the conventional ELISA methodology, demonstrate the possibility to discriminate clearly between healthy and BC-affected subjects, and reliably identify among these those with the very poor overall prognosis TNBC subtype.
The unique features in terms of simplicity (a single 30 min incubation step), sensitivity, disposable character, affordable cost and compatibility with use in decentralized environments, make this immunoplatform very attractive complement to clinically established methodologies for BC prediction and prognostic.
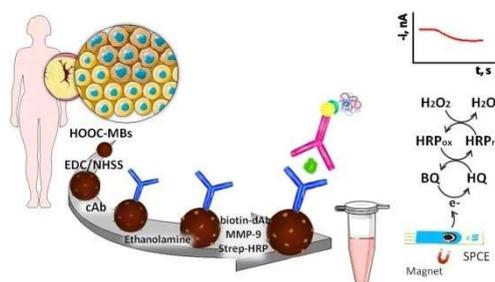
Figure 1: MBs-based immunoplatform for MMP-9 determination.

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A new amperometric 20S proteasome biosensor for proteasome activity and inhibitor screening

Madalina M. Barsan, Victor Diculescu

madalina.barsan@infim.ro

National Institute of Material Physics, 077125, Magurele, Romania

Abstract: The proteasome is an enzyme involved in the degradation of misfolded or oxidatively damaged proteins and it is currently a great focus in cancer research, which triggers the inhibition of the 20S for the development of new new cancer therapies[1,2]. Therefore, the determination of proteasome activity is crucial, in order to perfect such medical approaches. Beside the classical methods to measure proteasome activity (fluorimetry and bioluminescence), the electrochemical methods have already proven to be reliable and fast[3,4]. The principle is based on the electrochemical detection of the AMC released after proteolysis, which is electrochemically oxidized at positive potentials. The development of a biosensor for investigating the activity and inhibition of proteasome presents advantages such as faster and sensitive response, and reduced costs being more appropriate for fast screening of inhibitors and compounds with potential pharmaceutical applications.

The focus of this research is to evaluate electrochemically the activity of the proteasome 20S, immobilized at a GCE via monoclonal/polyclonal antibody-proteasome 20S interactions, the antibodies being immobilized prior at the GCE surface (see Figure 1).
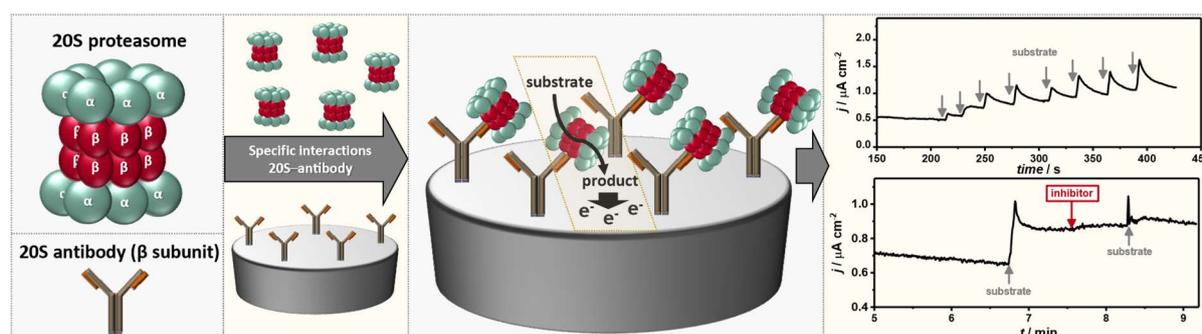

Figure 1 From left to right: Schematic representation of the proteasome biosensor, the structure of 20S proteasome and principle of biosensor electrochemical detection.

The influence of the antibody-proteasome interactions towards proteasome activity are evaluated by fixed potential amperometry. The same electrochemical procedure is able evaluate the tree different proteolytic activities of the proteasome: caspase-, trypsin- and chymotrypsin-like, by using 6 substrates, 2 for each activity.

The influence of two 20S inhibitors, one naturally occurring, epoxomicin, and the synthetic inhibitor bortezomib, on the biosensor response are also evaluated.

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Comparison of Evaluation Methods for Kinetic Analysis of Binding Events

M. Conrad [1], J. Hutterer[1], G. Gauglitz[1]

Guenter.Gauglitz@uni-tuebingen.de

[1]University of Tuebingen, Institute of Physical and Theoretical Chemistry, Auf der Morgenstelle 18, 72074 Tuebingen, Germany

Abstract: The analysis of binding events is an important tool to characterise kinetics and thermodynamics, but also error-prone due to the influence of the user. To determine how the best results can be obtained, data evaluation methods for pseudo-first-order kinetic behaviour were compared. The analysed binding curves were obtained through multiple reflectometric interference spectroscopy (RIfS) measurements with different anti-amitriptyline antibody concentrations flowing over a transducer on which the amitriptyline metabolite nortriptyline had been immobilised. For the calculation of the observed binding rate constant (k_{obs}), the fitting area of the association phase of the binding curve (Fig. 1a) needs to be carefully chosen. Furthermore, there are different methods to calculate k_{obs}: exponential fit of the detector response $\Gamma(t)$ with use of the integrated rate equation [1], linear fit of the derivative of the detector response $d\Gamma(t)/dt$ vs. $\Gamma(t)$ [2], or numerical integration of the detector response

and a subsequent linear fit of $\Gamma(t)/t$ vs. $(\int\Gamma(t)dt)/t$ [3]. To obtain the association rate constant (k_a) from k_{obs}, the k_{obs} linearization method was used as shown in Fig. 1b. The resulting values for k_a demonstrate that the choice of evaluation method greatly influences the calculated k_a. In case of deviations from pseudo-first-order kinetics and possible multiple k_a, a fit of the entire association phase will give an average rate constant. If k_a changes during the binding process, the results for k_a depend on the choice of fitting area. Since the derivative of the detector response uncovers different rate constants, the derivative should always be calculated to identify mass transport limitation or other inhomogeneity. On the basis of the observations in the derivative, the fitting area for the calculation of k_a should be chosen and different k_a during the association process can be distinguished. It was observed that the data evaluation of the chosen area by numerical integration gives the best results as it reduces noise which is especially important for noisy signals.
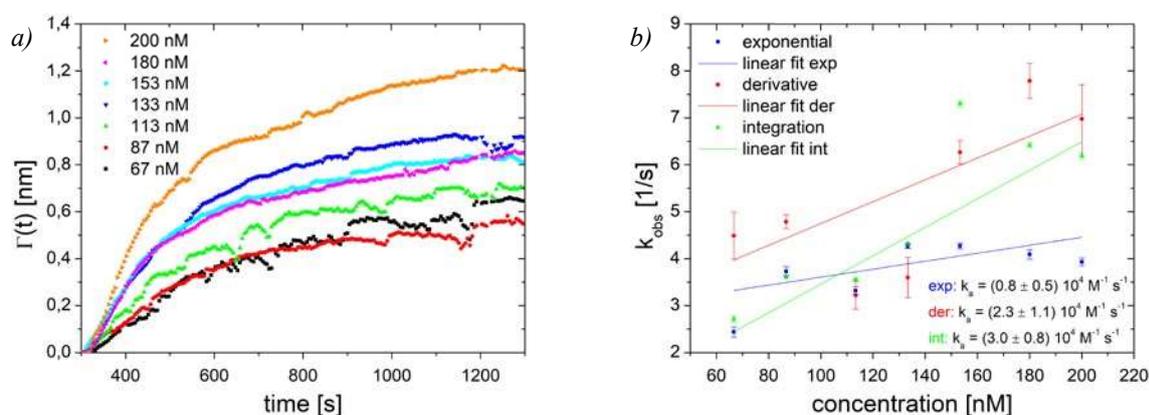
Figure 1: a) Association Phase of the Binding Curves. RIfS measurements of the association of multiple antiamitriptyline antibody concentrations on immobilised nortriptyline. b) k_{obs} Linearization. k_{obs} was obtained through different methods: exponential fit of the detector response $\Gamma(t)$, linear fit of the derivative $d\Gamma/dt$ vs. Γ, and linear fit of Γ/t vs. $(\int\Gamma(t)dt)/t$. k_a is the slope of k_{obs} vs. concentration.

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Ultra-sensitive immunoassay for Estradiol in saliva and drinking water

Omnia Elsayed[1], Nenad Gajovic-Eichelmann[1], Frank F. Bier[2]

oelsayed@uni-potsdam.de

[1]Fraunhofer Institute for Cell Therapy and Immunology, Institute Branch Potsdam: Bioanalytics and Bioprocesses, Am Mühlenberg 13,14476 Potsdam, Germany.

[2]Institute of Biochemistry und Biology, Potsdam University, Germany.

Abstract: A sensitive and simple method for monitoring of Estradiol in saliva and drinking water is presented. In non-pregnant women with normal menstrual cycles, the highest concentration of E2 is found immediately prior to ovulation. In contrast to the luteinizing hormone (LH), the best-known ovulation marker, E2 can be detected in saliva (peak value in saliva ca. 10-20 pg/mL) [1]. Saliva has many practical advantages as a diagnostic medium: Sampling is non-invasive, can be carried out without medical supervision and frequent sampling is easy, cheap, and painless. E2 is also an important marker for the quality control of drinking water: Being the most potent steroid hormone, with biological activity at the lower ng/L level, it cannot be removed by conventional water pretreatment techniques. Therefore, the European Union has suggested an acceptable limit of 1 ng/L E2 (1pg/mL) in drinking water. The current "gold standard" analytical method for low level estrogen detection is LC/MS-MS with pre-column chemical derivatization requires sample extraction and precolumn derivatization and is laborious, low-throughput and expensive [2]. Enzyme-linked immunosorbent assay (ELISA) is well suited for specific and inexpensive screening of E2, but the required LOD of ca. 1 pg/mL (= 1ng/L) poses a challenge and demands careful optimization of sample-preparation and all assay steps. Using a custom-made E2/ HRP conjugate, a novel patentpending surface chemistry (TruContact®) and real saliva-based calibration standards prepared by a proprietary protocol, we accomplished a highly sensitive and reproducible competitive ELISA. The work focuses on replacing the standard TMB-based detection reagent by the multimodal reagent Amplex Red®, allowing for simultaneous fluorescence and electrochemical detection [3]. Electrochemical detection is a promising alternative to optical methods for miniaturized low-cost read-out systems.
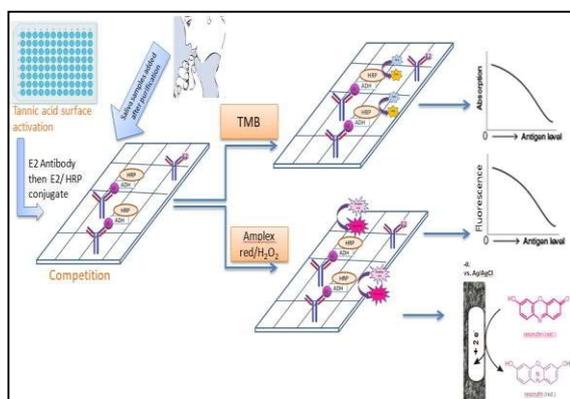
Figure 1: Principle of the competitive E2 ELISA: Spectrophotometric detection with TMB/H2O2 electrochemical and/or fluorimetric detection of Resorufin formed from the precursor Amplex Red®
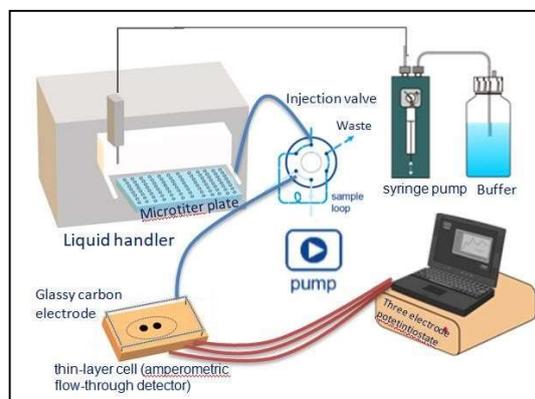

Figure 2: Prototype flow throught immunoassay setup. Detection at 3 mm glassy carbon electrode in a thin-layer flow cell. The samples are transfered from the 96-well microtiter plate using a Liquid handling robot.

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Magnetic Bead-Based Immunoassays for Online Sensing Applications

Alexander Ecke [1,2], Konrad Bohm [1,3], Rudolf J. Schneider [1,3]

alexander.ecke@bam.de

[1]BAM Federal Institute for Materials Research and Testing, Division 1.8 Environmental Analysis,
Richard-Willstätter-Straße 11, 12489 Berlin, Germany
[2]Humboldt-Universität zu Berlin, Department of Chemistry, Brook-Taylor-Straße 2, 12489 Berlin, Germany
[3]Technische Universität Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany

Abstract: The demand for powerful online sensors in the field of environmental analysis is rising with new and diversifying sources of pollution and contaminants. One aspect of this issue is the contamination of (drinking) water with pharmaceutical residues that comes with increased use of medication in human and veterinary medicine. Due to the insufficient degradation of these compounds in wastewater treatment plants or manure spreading in agriculture, the contaminants are discharged into surface and ground waters which serve as main sources of drinking water. In order to maintain control over drinking water quality and enable quick responses in case of contamination, fast and reliable online sensors are required. Here, immunoanalytical methods based on the binding of highly specific antibodies to the respective analyte can be helpful as they allow quick and cost-effective on-site (and potentially online) analyses.

In this context, we present the development of magnetic bead-based immunoassays (MBBAs) for two potential contaminants of drinking water: the nonsteroidal anti-inflammatory drug diclofenac (DCF) and the broad-spectrum antibiotic amoxicillin (AMX). Both have been listed on the EU watchlist of substances for a Union-wide monitoring in the field of water policy.[1,2] For these analytes, two different immunoassay formats were developed (see Figure 1), that are basically transferable to many other analytes and allow the implementation into an online system as the magnetic beads can be reversibly immobilized. Compared to the corresponding ELISAs (enzyme-linked immunosorbent assays) for both analytes, our MBBAs entail significant advantages such as improved measurement ranges with decreased limits of quantification, shorter assay duration (45-60 minutes vs. 4-5 hours) and less washing steps required (1 vs. 3-4). Strategies for the implementation of the MBBAs into an online system with electrochemical readout on a microfluidic chip will be presented.
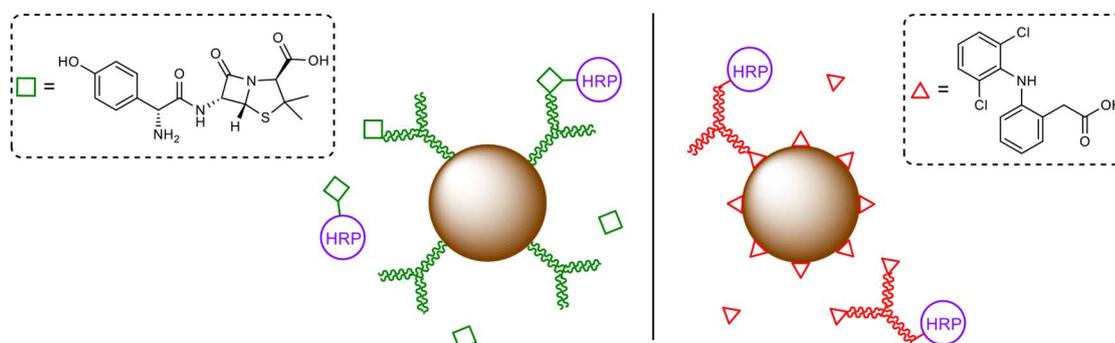

Figure 1: Schematic drawing of the MBBAs in the analytical step, i.e. the competition between the analyte and a tracer (enzyme-labelled analyte) for binding of immobilized antibody (for AMX, left) or the competitive binding of an enzyme-labelled antibody to the analyte in solution or on the bead surface (for DCF, right). The quantification of bound species is enabled by the enzyme activity via substrate addition and detection of converted substrate (optically or electrochemically).

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First electrochemical immunoplatform for the simple, sensitive and rapid detection of mustard in food extracts

María Gamella[1], Cristina Bueno-Díaz[2], Víctor Ruiz-Valdepeñas Montiel[1], E. Povedano[1], A. Julio Reviejo[1], Mayte Villalba[2], Susana Campuzano[1], José M. Pingarrón[1]

mariagam@ucm.es

[1]Departamento de Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, Avenida Complutense s/n, E-28040 Madrid, Spain

[2]Departamento de Bioquímica y Biología Molecular Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Avenida Complutense s/n, E-28040 Madrid, Spain

Abstract: Food allergies are a topic of increasing importance in public health. Mustard is among the many spices that lead to hypersensitivity reactions, having been included in the most recent European Directive concerning food safety (No. 1169/2011) which lists 14 main allergenic food ingredients forced to be declared by producers and manufacturers on food labels [1]. It has been reported that even small amounts of this spice are capable of triggering allergic reactions, fact that is aggravated by the high thermal resistance of some of its allergens [2]. Therefore, sensitive and selective analytical methods are required to verify the presence of mustard traces in foodstuffs. In this context, electrochemical immunosensors have emerged as a powerful alternative for the detection and quantification of allergenic proteins due to their high sensitivity and selectivity, low cost and compatibility with miniaturization and multiplexing, which make them applicable in decentralized settings.

Hence, we describe here the first electrochemical immunosensor reported so far for the determination of mustard residues through the determination of the Sin a 1 protein [3]. The designed sandwich immunoassay was implemented on the surface of magnetic microbeads (MBs), which provide improvements in assay time, sensitivity and minimization of sample matrix effects. Once immobilized the capture antibody (CAb) onto the MBs, the protein Sin a 1 is captured and then sandwiched by using a detector antibody (DAb) further tagged with a secondary antibody conjugated with horseradish peroxidase (HRP) and the amperometric detection at disposable screen-printed carbon electrodes (SPCEs) using hydroquinone (HQ) as electron transfer mediator and H_2O_2 as HRP substrate was performed. The resulting immunosensor exhibits a high sensitivity (LOD value of 0.82 ng mL^{-1}, which is three orders of magnitude lower than that obtained with the available commercial ELISA kits) and selectivity against other closely related allergenic proteins of the plant kingdom. The developed immnoplatform was applied to the quantification of Sin a 1 in food extracts in just 1 hour, providing results in agreement to those obtained using the ELISA method. Therefore, the developed immunosensor is highly competitive in terms of sensitivity, cost and compatibility with multiplexing and decentralized determinations, making it a very attractive and affordable biotool for on-site testing to minimize consumer-safety concerns and comply with increasingly enforcing labelling regulations and quality-assurance procedures.

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Smartphone-based amperometric detection of 3,3',5,5'tetramethylbenzidine (TMB) – An immunomagnetic Ochratoxin A assay

Soraya Höfs[1] and Rudolf J. Schneider[1]

soraya.hoefs@bam.de

[1]Department of Analytical Chemistry; Reference Materials, Bundesanstalt für Materialforschung undprüfung (BAM), Richard-Willstätter-Straße 11, 12489 Berlin, Germany

Abstract: The demand for miniaturized analytical devices monitoring important parameters in the food and medical industry has increased strongly in the past decades. With fast progress, smart technologies are finding their way into our everyday life. For the future, it is, therefore, a major goal to also link analytical methods with smart technologies to create user-friendly on-site devices. In food industry the monitoring of harmful substances such as dioxins, heavy metals or mycotoxins plays a key role, since the European Commission prescribes legal limits for various food products and beverages[1]. Therefore, companies often have their own laboratories and trained personnel. For one of the most abundant and toxic mycotoxins, Ochratoxin A (OTA) we want to present an electrochemical detection system in which the read-out can be performed with a smartphone connected *via* Bluetooth to a miniaturized potentiostat. The recognition of OTA is performed with specific antibodies in a competitive assay format. Anti-OTA-antibodies were captured on magnetic beads on which the competitive binding between OTA and an OTA horseradish peroxidase (HRP) tracer was performed. To quantify OTA, the enzymatic reaction of the tracer with 3,3',5,5'-tetramethylbenzidine (TMB) and H_2O_2 is employed. Oxidized TMB, which is enzymatically produced by the reduction of H_2O_2, is quantified by amperometry with screen-printed electrodes in a custom-made flow system. Since it is well-known that oxidized TMB can precipitate on electrode surfaces[2], we have studied pitfalls of the electrochemical detection of TMB. By cyclic voltammetry we have compared the stability of the electrochemistry of TMB at different electrode materials (gold and carbon) and pH values (pH 1 and pH 4). It was found that a stable response of the electrode could be achieved at pH 1 on gold electrodes. Thus, we applied these reaction conditions for amperometric detection of TMB in the OTA assay. The results of the electrochemical detection method are in good correlation with the photometric detection of TMB. To demonstrate the applicability, we tested our system with OTA-spiked beer and performed the measurement *via* smartphone.
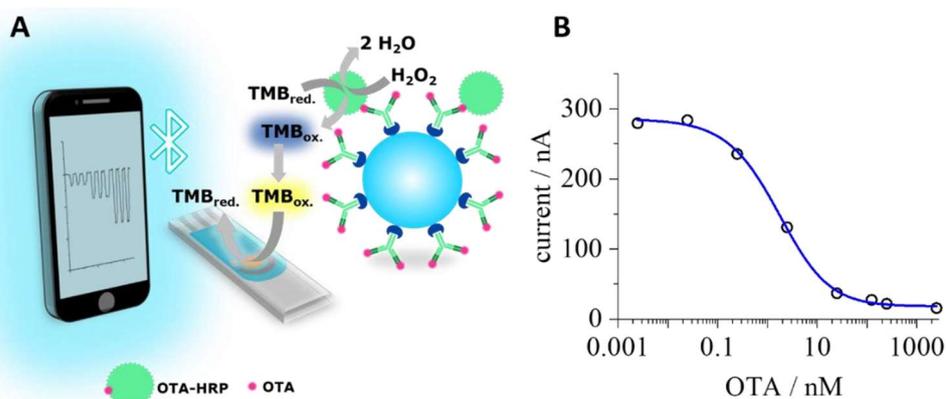
Figure 1: **A** *Schematic representation of the electrochemical detection system for Ochratoxin A. A competitive immunoassay is performed on antibody-decorated magnetic beads. Enzymatically oxidized TMB can be detected amperometrically at a screen-printed gold electrode with a smartphone connected via Bluetooth to a potentiostat.* **B** *results of the immunomagnetic assay with amperometric detection.*

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Developing a liposome-based lateral flow assay for the detection of SARS-CoV-2 neutralizing antibodies

Simon Streif [1], Anna Gebhard, Cornelia Hermann, Clemens Spitzenberg, Antje J. Baeumner

simon.streif@chemie.uni-regensburg.de

[1]Institute for Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany

Abstract: The current SARS-CoV-2 pandemic highlights the need for affordable, rapid point-of-care testing (POCT) devices for neutralizing antibodies in order to determine the immune status of large parts of the population. By binding to specific viral epitopes these antibodies neutralize its infectious properties. In the case of SARS-CoV-2 the neutralizing antibodies are thought of binding to the receptor binding domain (RBD) of the S protein, preventing the virus to attach to the angiotensin converting enzyme 2 (ACE2) present on human cells[1,2]. A lateral flow assay (LFA) based on ACE2tagged liposomes is being developed to quantify the presence of these neutralizing antibodies in a rapid manner outside of the lab. Specifically, the LFA easily separates ACE2-liposomes bound to virus from those that remain free, so that the resulting signal allows a direct correlation to the presence of neutralizing antibodies. Furthermore, the assay solely relies on the ability of the virus to bind to ACE2 as a mimic for its infectious nature and does not require analytical antibodies outcompeting neutralizing antibodies. This strategy is accomplished by lysing liposomes that were bound by virus through the activation of the serum inherent complement system. Therefore, signals obtained in the LFA present all non-virus bound liposomes and directly correlate to the presence of neutralizing antibodies. Here, we present initial studies of the development of human serum-stable liposomes, tagging with biotin and fluorescein for immobilization in test and control lines of an LFA, and tagging with ACE2 either through biotin-streptavidin or NTA-Ni-his$_6$-tag immobilization. LFAs for cationic and anionic liposomes were developed and complement-triggered lysis quantified via simple photo and imageJ analysis.
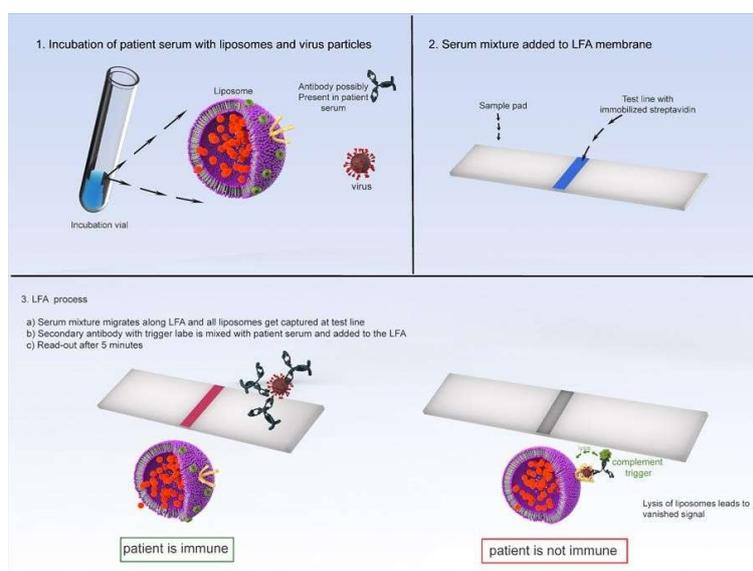
Figure 1: Principle of the envisioned SARS-CoV-2 neutralizing antibody lateral flow assay. Biotinylated liposomes (purple) are incubated with virus-like-particles (VLP's, black) and patient serum. The serum mixture is then added to the LFA sample pad and the liposomes are captured by the polystreptavidin test line. Addition of a secondary antibody with trigger label will then cause complement-induced lysis if the patient is not immune. If the patient is immune no lysis occurs, as the neutralizing antibodies prevented binding of the VLP's to the ACE2-receptor on the liposomes surface, subsequently preventing binding of the complement trigger.

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Electrochemical immunosensors for the sensitive determination of rheumatoid arthritis biomarkers

E. Sánchez-Tirado, A. González-Cortés, P. Yáñez-Sedeño, J.M. Pingarrón

esther.sanchez@ucm.es

Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Avda. Complutense s/n, 28040, Madrid, Spain

Rheumatoid arthritis is an autoimmune disorder characterized by persistent erosive synovitis, systemic inflammation and the presence of autoantibodies, particularly rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCPA) antibodies, which play an important role in inducing inflammation and joint damage, releasing pro-inflammatory cytokines from monocytes and macrophages [1, 2]. Besides, matrix metalloproteinase-3 (MMP-3), which is induced by inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) in rheumatoid synovium, degrades several extracellular matrix components of cartilage and plays central roles in rheumatoid joint destruction [3]. Thereby, monitoring serum RF, CCPA and MMP-3 levels is useful for predicting the disease activity in rheumatoid arthritis.

In this work, the construction and analytical performance of two electrochemical platforms for the individual and multiple detection of different rheumatoid arthritis biomarkers are described. The first amperometric immunosensor for the quantification of serum MMP-3 uses a specific capture antibody, which is covalently immobilized onto carboxylated-magnetic microparticles supported onto SPCEs, and a sandwich-type immunoassay with a biotinylated detection antibody and conjugation with peroxidase-labelled streptavidin. Amperometric measurements were carried out at –0.20 V by adding H_2O_2 solution onto the electrode surface in the presence of hydroquinone. The calibration plot allowed a range of linearity extending between 1 and 5000 pg·mL^{-1}, which is adequate for the determination of the enzyme in serum samples. The second configuration describes a dual immunosensor for the simultaneous determination of RF and CCPA antibodies. The optimized methodology involves the preparation of sandwich-type immunoassays with Fc fragments of IgG captured onto carboxylated-magnetic microparticles for the immobilization of RF and biotinylated-cyclic citrullinated peptide for the specific immobilization of CCPA, followed by the conjugation with the respective HRP-labelled antibodies. Electrochemical detection was performed by amperometry as previously. The dual biosensor exhibited high sensitivities for RF and CCPA with LOD values of 0.8 IU·mL^{-1} and 2.5 IU·mL^{-1}, respectively. Moreover, the simultaneous determination can be completed in about two hours, applying a simple protocol and a sample volume four times lower than that required by the ELISA method.

Figure 1: Assessment of disease activity from serum collection to the achievement of the final result

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Electrochemical bioplatform to unravel neurodegeneration and Alzheimer's disease through the determination of neurofilament light chain protein

A. Valverde[1], A. Montero-Calle[2], R. Barderas[2], M. Calero[2,3], P. Yáñez-Sedeño[1], S. Campuzano[1], J. M. Pingarrón[1]

alejaval@ucm.es

[1]Analytical Chemistry Dept., Faculty of Chemical Science, Complutense University of Madrid, Madrid, 28040, Spain.
[2]Chronic Disease Programme, UFIEC, Institute of Health Carlos III, Madrid, 28220, Spain.
[3]Alzheimer's Center Reina Sofía Foundation – CIEN Foundation and CIBERNED, Institute of Health Carlos III, Madrid, 28220, Spain.

Nowadays, neurofilament light chain (NfL) protein arises as one of the most promising blood biomarkers for early and accurate neurological disorders (NDs) diagnosis, but current available methodologies for its determination (ELISA kits or the single molecule array, Simoa™, assays) comprise multiple steps, have inadequate sensitivity or unaffordable cost, respectively, and are of limited application in centralized or high-resource environments. Since electrochemical biosensing technologies are demostrating to surpass these drawbacks, this work reports the first magnetic beads (MBs)-based electrochemical bioplatform developed so far for the rapid, sensitive (LOD value of 3.0 pg mL^{-1}) and accurate determination of NfL for assisting early diagnosis and reliable staging of NDs. The strategy, based on the formation of enzyme-labeled sandwich immunocomplexes with a secondary antibody conjugated to HRP on the surface of MBs and amperometric detection at SPCEs, allows the sensitivity required for the accurate NfL determination in human biopsies from both nondemented control subjects and patients diagnosed with NDs within 1 h and using minimal amount of samples (5 µL of plasma and 0.1 µg of brain tissue extracts) [1].

The developed electrochemical immunosensing strategy exhibits relevant practical advantages over other available methodologies for NfL determination providing a higher sensitivity than ELISA, thus allowing the determination in plasma, as well as making it possible quantification in solid biopsies using much smaller sample amount and in much shorter assay time than conventional dot-blot. Moreover, it has a much more affordable cost than the ultrasensitive Simoa™ assays. It is also important to highlight the feasibility of the bioplatform to perform the analysis in both solid and liquid biopsies, therefore improving the reliability in the diagnosis given the heterogeneity and temporal complexity of NDs. All these relevant features along with those intrinsic to electrochemical transduction in terms of portability and multiplexing capability make the NfL immunoplatform ideal for its translational progress beyond the well-controlled laboratory benchtop into field-portable and even "sample-to-result" devices for ambulatory and inpatient routine or even low-resource settings.
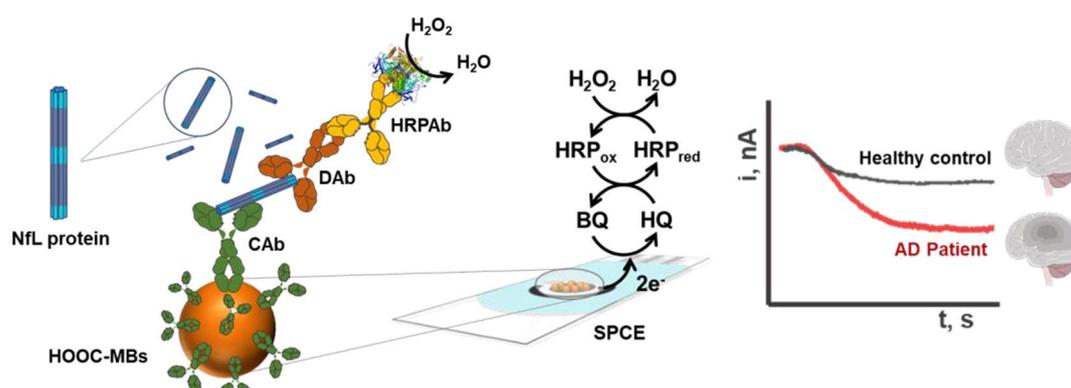

Figure 1: Scheme of the developed electrochemical bioplatform

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Smart and portable immunosensors for serological assessment of SARS-CoV-2 infection and rapid evaluation of immunity against SARS-CoV-2

Simone Fortunati[1], Chiara Giliberti[1], Angelo Bolchi[1], Davide Ferrari[1], Valentina Bianchi[2], Ilaria De Munari[2], Andrea Boni[2], Marco Giannetto[1], Maria Careri[1]

chiara.giliberti@unipr.it

[1]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, 43124, Parma, Italy.

[2]Department of Engineering and Architecture, University of Parma, Parco Area delle Scienze 181/A, 43124, Parma, Italy.

Abstract: The health and socio-economic impact of the SARS-CoV-2 pandemic revealed the need for rapid and efficient diagnostic devices suitable for non-hospital settings aimed at identifying and tracking positive subjects, but also at assessing the immunization status of the population. In such a context, we developed innovative voltammetric immunosensors based on disposable screen-printed electrodes functionalized with either nucleocapsid or spike viral proteins for the quantitative determination of the respective IgG and IgM antibodies, which can be possible biomarkers for evaluating natural immunity (nucleocapsid and spike proteins) and vaccine acquired immunity (spike protein) to COVID-19 [1]. The combined use of gold nanoparticles and carbon nanotubes allowed us to effectively immobilize the viral antigens through gold nanoparticles and to amplify the response exploiting the unique properties of carbon nanotubes. Immuno-absorbed antibodies were discriminated and detected by alkaline phosphatase-tagged anti-human IgG and IgM, as proven in a previous study [2]. The developed immunosensors were successfully validated in human serum, showing excellent performance in terms of limits of detection and quantification, making the current strategy promising for diagnostic purposes.
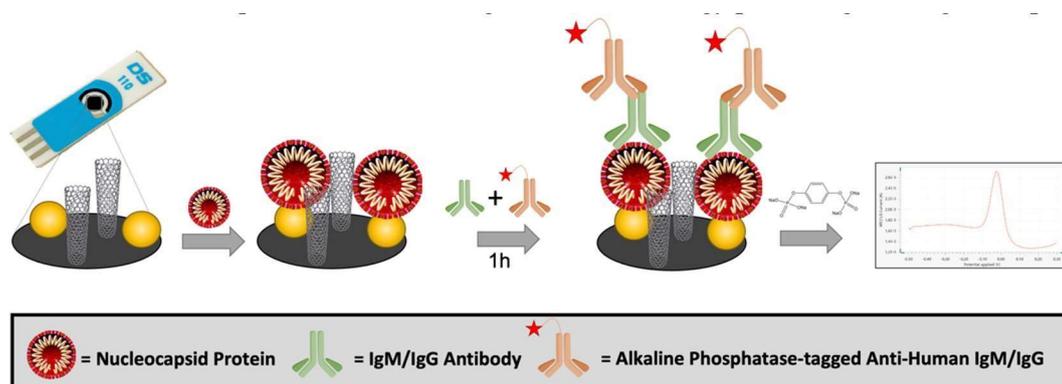
Figure 1: Schematic representation of the immunoassay setup.

Concerning the signal readout, an electrochemical portable and battery-operated IoT device equipped with a WiFi module and developed to maximize the measurement resolution was specifically designed for autonomous calibration and onboard data processing with cloud-based storage and sharing features [3]. The overall performance of the immunosensors and the reading device will allow to achieve higher reliability compared to the currently available rapid serological screening methods as lateral-flow immunochromatographic assays, also making this diagnostic platform promising for point-of-care testing in resource-limited settings.

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Development of a High-Throughput Cell-free Neutralization Test for SARS-CoV-2

Anna Gebhard [1], Clemens Spitzenberg, Cornelia Hermann, Simon Streif, Antje J. Baeumner

anna.gebhard@stud.uni-regensburg.de

[1]Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany

Abstract: The unprecedented vaccination effort to eliminate the threat of SARS-CoV-2 demands rapid low-cost testing for the presence of neutralizing antibodies in patients. A high-throughput cellfree neutralization test is being developed to determine the immunity status within hours using complement induced lysis of liposomes. Therefore, virus-like particles (VLPs) are added to patient serum, marker-filled liposomes labeled with recombinant angiotensin converting enzyme 2 (ACE2) and anti-VLP antibodies capable of activating the complement system (complement trigger) are added as well. Only immune patients have neutralizing antibodies, which prevent the VLPs from binding to the liposome-bound ACE2 receptor, otherwise liposome-VLP complexes will be formed[1]. Two assay formats, homogeneous and heterogeneous are under development, in the latter liposomes are coupled to streptavidin coated surfaces via a biotin-tag. Here, data will be presented for the development of serum-stable (stealth) liposomes, ACE2 and other protein immobilizations on liposomes and successful lysis of liposomes through a complement trigger[2]. Various coupling strategies for liposome modification with ACE2 proteins are investigated, where it is expected that as little as 50 ng of ACE2 per assay are sufficient for signal readout. In the future, the simple fluorescence readout of the encapsulated dye sulforhodamine B (SRB) allows highly sensitive and quantitative determination of an antibody titer, without the drawbacks of standard serum virus neutralization assays[3].
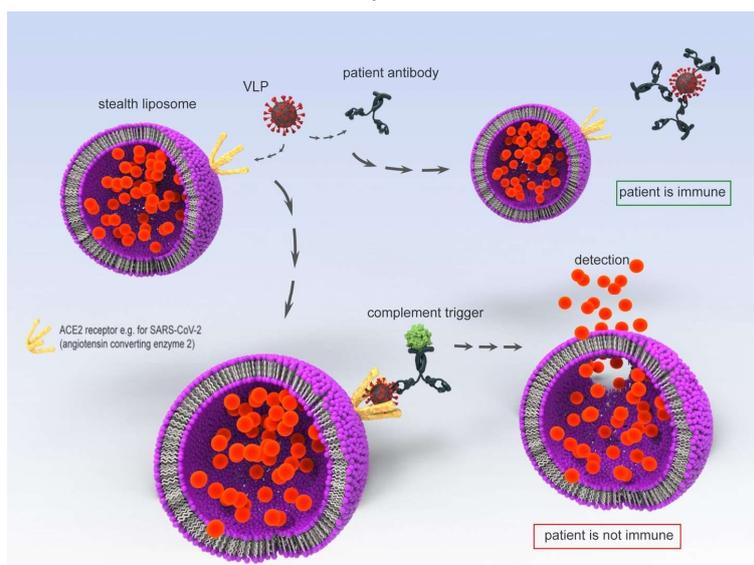
Figure 1: Principle of the homogeneous liposome-based assay to determine patient immunity against SARSCoV-2. First, virus-like particles (VLPs) are added to patient serum. Patient antibodies have a chance to bind to the virus and 'neutralize' it. Then, liposomes are added as shown here. Liposomes are filled with the fluorescence dye SRB and are tagged with ACE2. If the virus is neutralized by patient antibodies, it cannot bind to its receptor. If it is not neutralized, it will bind the ACE2-liposome complex. Upon subsequent binding of a trigger antibody, those liposomes are lysed by serum complement components.

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Studying the adsorption of *tobacco mosaic virus* particles on capacitive field-effect biosensors

Melanie Jablonski [1,2], Arshak Poghossian [3], Robin Severins [1], Michael Keusgen [2], Christina Wege [4], Michael J. Schöning [1,5]

m.jablonski@fh-aachen.de

[1]Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, Heinrich-Mußmann-Straße 1, 52428 Jülich, Germany
[2]Institute of Pharmaceutical Chemistry, Philipps University Marburg, Marbacher Weg 8, 35032 Marburg, Germany
[3]MicroNanoBio, Liebigstraße 4, 40479 Düsseldorf, Germany
[4]Institute of Biomaterials and Biomolecular Systems, University Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany
[5]Institute of Biological Information Processing (IBI-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Abstract: In (bio-)chemical sensor applications, plant viruses and especially *tobacco mosaic virus* (TMV) particles are seen as valuable nanoscaffolds. TMV particles are very attractive receptor nanocarriers due to their regularly arranged coat proteins, which exhibit thousands of sites on the outer surface of the virus, enabling the precisely positioned high-density immobilization of biomolecules, in particular enzymes [1]. In recent works, a TMV-based amperometric glucose biosensor as well as a potentiometric penicillin biosensor were introduced [2,3]. The sensitivity of such TMV-based biosensors is strongly influenced by the number of adsorbed TMV particles and the associated number of immobilized enzyme molecules. Hereby, the evaluation of sensor-surface functionalization is an important aspect during the fabrication. On the other hand, capacitive electrolyte-insulator-semiconductor (EIS) sensors are sensitive to pH- and surface-charge changes near to their gate-insulator surface: This way, they can detect the adsorption of charged molecules such as TMV particles [1].

In this work, different TMV-adsorption conditions have been systematically studied on capacitive EIS sensors of Al/p-Si/SiO$_2$/Ta$_2$O$_5$ by means of field-effect measurements and scanning electron microscopy (SEM). Additionally, the immobilization of penicillinase as a model receptor molecule on TMV particles was detected in a dynamic measurement mode, so-called constant capacitance measurements. TMV immobilization was performed from differently concentrated TMV solutions ranging from 0.005 μg/μL to 0.32 μg/μL, varying the incubation time between 1 h and 24 h. The achieved results will be discussed with regard to the potential of capacitive EIS sensors for label-free detection of TMV particles, as well as the integration of plant-viruses with field-effect devices as a attractive platform for label-free biosensing.

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Towards Measuring Multivalent Binding Interactions: Binding of Viruses and Peptides on DNA-nanoconstructs

Marlen Kruse[1], Christin Möser[2], David Smith[2], Ralph Hölzel[1], Frank Bier[3]

Marlen.kruse@izi-bb.fraunhofer.de (Corresponding e-mail address)

[1]Fraunhofer IZI-BB, Am Mühlenberg 13, 14476 Potsdam, Germany
[2]Fraunhofer IZI, Perlickstraße 1, 04103 Leipzig, Germany
[3]University of Potsdam, Karl-Liebknecht-Straße 25, 14476 Potsdam, Germany

Abstract: Viruses have shown to be a significant threat to humankind in history as well as recently [1]. Currently the coronavirus pandemic challenges global health care systems and economies. A deeper understanding of virus-receptor interactions can be beneficial for preventing future outbreaks or finding treatments for existing viruses. Many viruses bind multivalently to their target receptors. The monovalent interaction of the surface protein can be weak but the overall affinity gets enhanced by simultaneous binding of many surface proteins [2]. Using a measurement technique that is based on DNA-nanoconstructs [3], we present results of binding interaction measurements between viruses and receptors. As primary model system, the influenza A virus X31 was used. This virus carries a homotrimeric surface protein called Hemagglutinin on its surface that can bind to sialic acid residues in the lung for example. The receptor peptide PeB, that specifically binds Hemagglutinin [4], was attached to a DNA nanoconstruct that can carry one, two or three peptides [5]. Then the binding interaction of the X31 virus was measured by fluorescence proximity sensing. The results show a binding interaction of the virus to the peptides on the gold surface (see Figure 1).
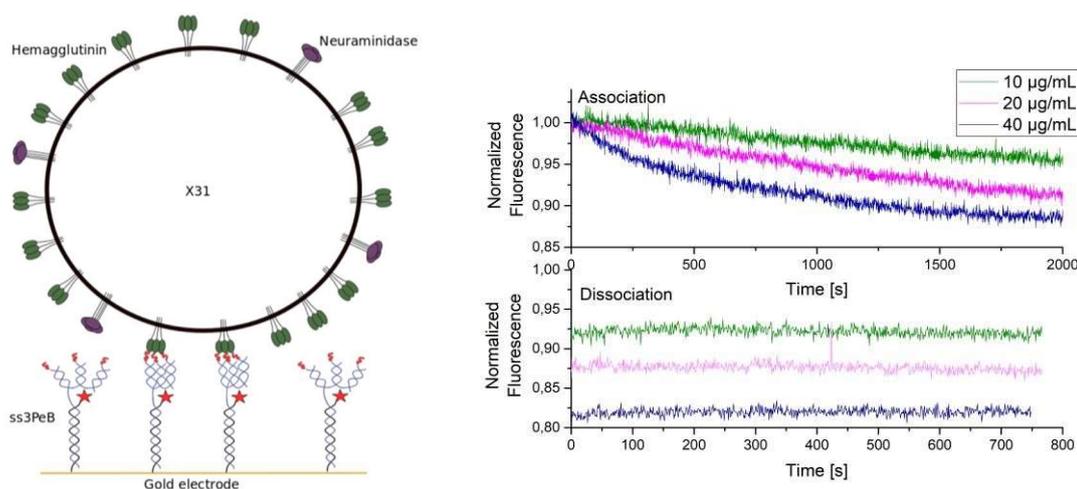
Figure 1: left: schematic overview of DNA nanostructures that carry the peptide PeB. They are immobilized on a gold sensor surface. The influenza virus X31 binds multivalently to the receptor. Right: Results show a strong signal change during the association. The dissociation indicates that viruses remain attached to the sensor surface during the measurement time.

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Flow-based chemiluminescence microfluidic chip for capturing bacteria with affinity ligands

Julia Neumair [1], Martin Elsner[1], Michael Seidel[1]

julia.neumair@tum.de

[1]Institute of Hydrochemistry, Chair for Analytical Chemistry and Water Chemistry, Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 Munich, Germany

Abstract: Research on rapid detection methods for pathogenic microorganisms is of general need because culture-dependent methods are too time-intensive. However, if the concentration of these pathogens is too low, detection methods can reach their limits. To avoid that, pathogens must be enriched. One option for this is monolithic affinity filtration where pathogens bind to ligands inside a porous filter, get removed from the sample and later can be eluted by changing salt and protein content, or the pH value [1]. In order to identify suitable ligands, we used a flow-based microfluidic chip setup. Captured bacteria are detected by chemiluminescence (CL) imaging. As a first ligand, the antibiotic Polymyxin B (PmB) was used, as it was already confirmed as an affinity ligand for *E. coli* [1]. PmB was immobilized via 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide and Nhydroxysulfosuccinimide (sNHS) on a polycarbonate (PC) surface by using succinylated Jeffamine® ED-2003 (sJeff) as a linker. This PC surface was assembled into a microfluidic chip consisting of a PMMA bottom plate with holes for inserting the reagents and a double-sided tape, in which the flow cell was cut. E. coli were biotinylated by using sNHS-biotin. On the microarray chip reader (MCR), the chip was incubated with the sample containing biotinylated *E. coli*. After stopped-flow incubation the chip was flushed and incubated with horseradish peroxidase-labelled streptavidin (strep-HRP). HRP catalysed a CL reaction exclusively at those places, where strep-HRP was bound. The CL signal was recorded with a CCD camera. Peskoller *et al.* showed a higher filtration efficiency of *E. coli* in tap water at pH 4 [1]. This could be confirmed with this screening chip: *E. coli* in tap water without acidification showed lower signals than at a pH of 4. Summarizing, it was possible to show that flowbased CL microfluidic chips can be used for capturing bacteria. In addition, the concept was proven that biotinylated viable bacteria can be used as testing agents for screening of various bacterial affinity ligands like antibiotics, antibacterial peptides, aptamers, or antibodies. This enables the choice of the best ligands for subsequent rapid enrichment of bacteria from diverse liquids.
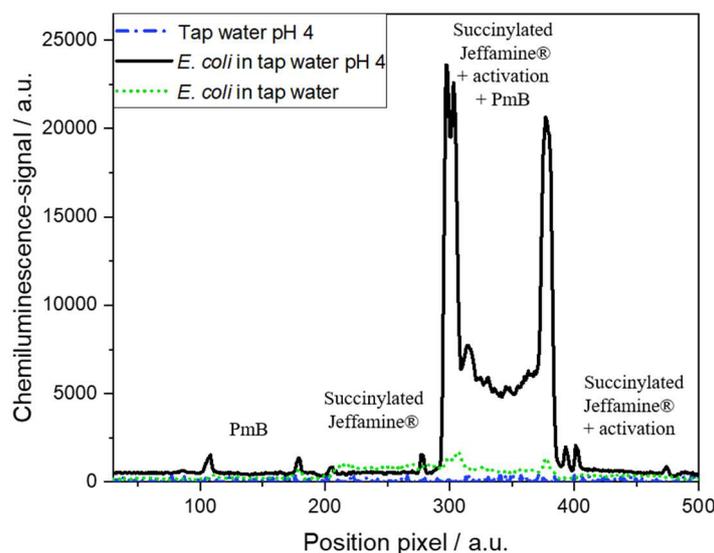
Figure 1: CL signal distribution for E. coli captured on immobilized PmB using succinylated Jeffamine as linker and on negative controls.

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AC field assisted deposition of influenza viruses on nanoelectrodes

Sandra Stanke [1,2], Christian Wenger[3], Frank F. Bier[2], Ralph Hölzel[1]

sandra.stanke@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalysis and Bioprocesses (IZI-BB), Am Mühlenberg 13, 14476 Potsdam, Germany

[2]University of Potsdam, Karl-Liebknecht-Straße 24 – 25, 14476 Potsdam, Germany

[3]IHP GmbH, Leibniz Institute for High Performance Microelectronics, Im Technologiepark 25, 15236 Frankfurt (Oder), Germany

Abstract: A rapid characterization of viruses and virus subtypes is of great biomedical interest. Here we present the use of AC electrokinetic forces, like dielectrophoresis and AC electroosmosis, as a simple and fast method to functionalize nanoelectrode arrays as a potential biosensor. The permanent immobilization of polystyrene nanoparticles, antibodies and other proteins on electrodes has already been demonstrated [1, 2, 3]. The sensor itself consists of four individual arrays, each built up of 6256 tungsten nanoelectrodes with a diameter of 500 nm each. The immobilization, detection and characterization of influenza material is done without any prior chemical modification of the electrode surface.

The accumulation of virus material over time has been observed, showing that the largest amount has already been drawn to the electrodes within 60 seconds and reached a saturation after 180 seconds of applied AC electric field. Due to side effects such as fluid streaming, a concentration gradient is created decreasing from the outer to the inner electrodes. It has been demonstrated, that the virus material is permanently immobilized even after switching off the electric field.

Furthermore, each functionalized electrode can be considered as a single event. Comparing these single events it seems like the virus material is distributed randomly across the nanoelectrodes. But after deconvolving the fluorescence image and merging the images of around 100 electrodes it reveals that the major part of virus material is collected at the electrode edge. This is in line with theory, as this is the region of the highest field gradient, and thus here the AC electrokinetic forces have the greatest impact on the sample.

The universal chip design does not limit the application to influenza viruses but also works for different viruses, bacteria, parasites or any other object that can be manipulated by AC electrokinetic forces. Additionally, each electrode can be used as part of an on-chip resonant circuit, whose frequency changes with surface coverage of the electrode [4] and, hence, serves as a measure of the amount of viruses attached to the electrodes. So in future, the evaluation by fluorescence microscopy can be changed to an electrical evaluation. Thus, combined with microfluidics this chip has the potential for a small and rapid Point-of-care system.
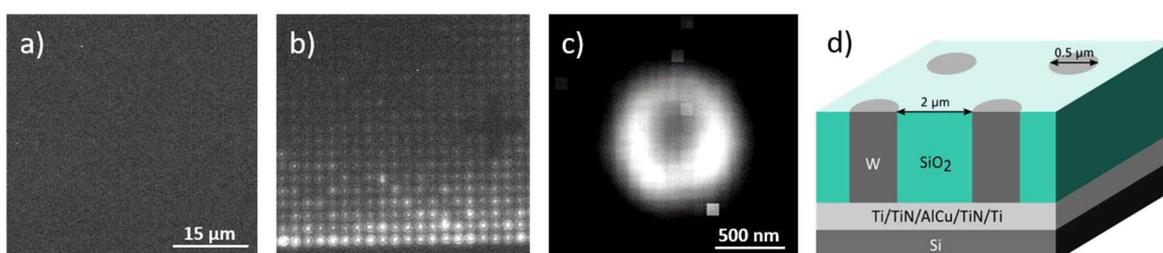

Figure 1: Fluorescence images of the time-depended increase of immobilized virus material on nanoelectrodes: after 0 sec (a) and 180 sec (b) of applied AC electric field. A concentration gradient is visible for the amount of immobilized material from the outer to the inner electrodes. c) Merging of around 100 single electrodes with accumulated virus material. d) Schematic figure of the electrode chip.

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Characterization and validation of screening methods for cultureindependent detection of Legionella in artificial water systems

Streich P., Seidel M.

philipp.streich@tum.de

M. Sc. Philipp Streich, Institute of Hydrochemistry, Chair of Analytical Chemistry, Technical University of Munich, Elisabeth-Winterhalter-Weg 6, 81377 Munich, Germany

Abstract: Legionella are gram-negative bacteria, which appear normally in our environment. Under certain circumstances they can also contaminate artificial water systems like cooling towers, evaporative coolers and wet separators. The species Legionella pneumophila and especially its serogroup (Sg) 1 is the most pathogenic and common danger for humans. Inhalation of Legionella contaminated bioaerosols can cause bronchial pneumonia called the legionnaires' disease with a mortality rate up to 10% [1]. Rarely, Legionella can also cause outbreaks like in Warstein (Germany) 2013 or Ulm (Germany) 2010. For these unfortune cases artificial water systems can be responsible, because they release Legionella contaminated aerosols over several kilometres, which can be inhaled by a variety of people [2]. Since 2017 the 42. BImSchV law is in Germany in force, which regulates the water quality of cooling towers, evaporative coolers and wet separators. These systems get screened in regular periods to determine the microbiological exposure with reference to the test and intervention values [3]. The most used method to analyse these water samples is the culture method with several disadvantages. Legionella colonies can be counted after 7 – 10 days growing time and this slight growth rate supports overgrowth with accompanying flora. In addition, Legionella can skip to a so called viable but non-culturable state (VBNC), while they are still pathogenic, but can't be detected via the culture method anymore. The industry is therefore looking for faster and culture-independent screening methods, which can describe the current statues of artificial water systems. In Germany, these methods are not standardized yet.

For this, our institute developed the chemiluminescence sandwich microarray immunoassay (CL-SMIA) which is an antibody dependent screening method and is performed on the Microarray-Chip-ReaderResearch (MCR-R) device. The CL-SMIA uses polycarbonate chips coated with several monoclonal antibodies as scavenger antibodies. The sample runs with a slow flowrate (1.0 µL/s) over the chip, that the Legionella can bind onto the chip. The bacteria own lipopolysaccharide chains (LPS) on their membrane, which act as antigens and are recognized by the antibodies. The LPS structures distinguish between each Sg and subgroup, wherefore a simultaneous detection and characterization is possible. The last step of the assay is the chemiluminescence reaction. Biotinylated detection antibody is directed over the chip and sets down on the scavenged Legionella. Afterwards, streptavidin conjugated with several horse radish peroxidase enzymes binds to the detection antibody and the enzymes catalyse the chemiluminescence reaction of luminol and H_2O_2, which can be detected with a CCD-camera.

The CL-SMIA was already evaluated for culture dependent characterization of Legionella pneumophila with a bacteria concentration over 10^5 CFU/mL. For this reason and culture independent utilization, the sensitivity of the assay must be improved by combining the CL-SMIA with the monolithic absorption filtration (MAF) as a suitable enrichment method for Legionella [4]. Therefore, macroporous filters with a diethyl amino ethanol (DEAE) functionalized surface will be used to ensure a higher binding affinity. For future applications the DEAE-MAF method will be optimized. The final aim will be a factor 1000 enrichment from a reasonable sample volume of 1.0 L without pre-treatment or acidifying of the sample.

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A Multiplexed Graphene-Based Telemedicine Platform for Rapid, Remote and Low-Cost COVID-19 Control and Monitoring

R. M. Torrente Rodríguez,[1,2] H. Lukas,[1] J. Tu,[1] J. Min,[1] Y. Yang,[1] C. Xu,[1] H. B. Rossiter[3] and W. Gao[1]

rebecamt@ucm.es

[1]Andrew and Peggy Cherng Department of Medical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

[2] Departamento de Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain

[3]Rehabilitation Clinical Trials Center, Division of Respiratory and Critical Care Physiology and Medicine, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA 90502, USA

Abstract: Nearly one year after the WHO characterized the COVID-19 outbreak as a pandemic, the global health crisis continues increasing with over 76.8 M confirmed cases and more than 1.5 M deaths worldwide.[1]

Despite the RT-PCR technique was employed in the clinical practice as the first option for COVID-19 pandemic diagnosis, the low stability of RNA material and highly experience required with these genetic tools is pushing these facilities to their limit.[2]

To address this, we propose a low-cost, ultrasensitive, portable, and wireless electrochemical POC platform for rapid and remote multiplex interrogation of relevant COVID-19-related biomarkers through the well-established sandwich and direct immunoassay configurations. Our SARS-CoV2 RapidPlex device, built-off on mass-producible laser-engraved graphene (LEG) electrodes simultaneously determinates viral antigen (nucleocapsid protein, NP), SARS-CoV-2 specific immunoglobulins (IgGs and IgMs), and the inflammatory biomarker C-reactive protein, with remarkable selectivity and within relevant physiological ranges (from pg mL^{-1} to µg mL^{-1} level). Truthful applicability of our SARS-CoV-2 RapidPlex platform was successfully demonstrated with RT-PCR confirmed COVID-19-positive and COVID-19 negative blood and saliva samples. As expected, significantly elevated levels of selected target molecules were observed in serum and saliva from COVID-19 infected patients. This, together with the rapid on-site evaluation of the COVID-19 disease severity by means of C-reactive protein in serum from patients clinically classified according to disease severity, place our platform as one of the pioneers POC devices able to provide comprehensive information on key aspects of COVID-19 disease: infection, immune response, and disease severity.[3]

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Development of a fast and reliable quantitative Loop-mediated isothermal amplification (qLAMP) assay for the detection of viral SARS-CoV-2 RNA (Cor(e)-LAMP)

C.Yaslanmaz [1,2], C. Warmt[1], J. Henkel[1], F.F. Bier[2,3]

*corresponding author, e-mail: christian.warmt@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB), Am Mühlberg 13, 14467 Potsdam, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: 2020 has been embossed by the global SARS-CoV-2 (Covid-19) pandemic. On 25th of November 2020 the World Health Organisation (WHO) reported 59.204.902[1] confirmed cases while the actual number is significantly higher.

The Polymerase Chain Reaction (PCR) test is used as the gold standard for the detection of SARSCoV-2 infections. Nevertheless, there are relevant limitations that have to be considered in this matter. PCR tests require specific equipment and have to be performed in clinical laboratories by qualified professionals. Additionally sample transportation and the duration of a conventional PCR test are not suitable for point-of-care diagnostics. Altogether these aspects cause long waiting times for test results. Therefore it is difficult to identify and to stop chains of infections in their early stages.

The Loop-mediated isothermal amplification (LAMP) is an amplification method for DNA amplification. With an additional reverse transcriptase enzyme (RT-LAMP), it can be used for RNA amplification as well[2]. The method usually operates at temperatures between 60-65°C yielding results within 15 minutes. Amplified products can easily be detected in real time with SYBR-Green as fluorophore.

The aim of this project is the development of a fast, reliable and sensitive quantitative LAMP assay that can be used in point of care diagnostic. Our most recent results allow the detection of concentrations up to 0,0035 pg/µl cDNA. The amplification evaluation can be realized by a nonspecific fluorophore (e.g. SYBR-Green) or by a specific fluorescence detection method. In *Figure 1* we show first results of qLAMP based on a 466 bp fragment of the N-protein coding gene of SARSCoV-2. We are able to detect cDNA targets with concentrations between 10,4 ng/1,5µl and 0,0053 pg/1,5 µl in less than 15 minutes with a negative no template control (NTC) .
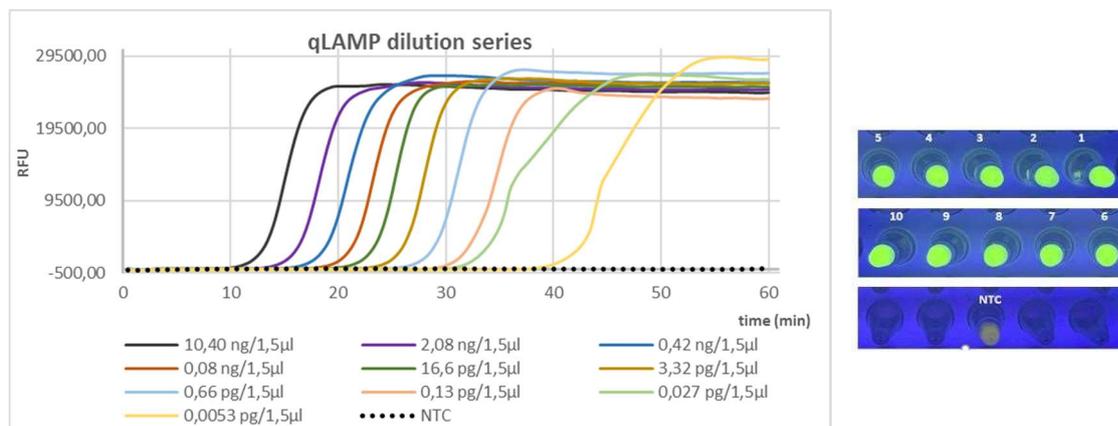

Figure 1: (Left) quantitative LAMP results: The relative fluorescence intensity is shown in relation to the reaction time in minutes. The graphs each represent a DNA dilution of the target fragment (N gene, 466bp). Dilutions are set between 10,4 ng/1,5 µl and 0,0053 pg/1,5µl including a no template control (NTC). (Right) Additional SYBR-Green detection after the qLAMP run (same run as in left graphic). 1µl of 1000X SYBR-Green was added to the reaction wells for visual detection. Sample labelling is equal to left figure

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On-chip detection of *Salmonella* in food, coupling the loop mediated isothermal amplification with microarray technology for increased specificity

S. Zwirner [1], C. Yaslanmaz[1,2], C. Warmt[1*], J. Henkel[1], F.F. Bier[2, 3]

*corresponding author, e-mail: christian.warmt@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology - Bioanalytics and Bioprocesses (IZI-BB), Am Mühlenberg 13, 14467 Potsdam, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology, 14476 Potsdam, Germany
[3]Institute for Molecular Diagnostics and Bioanalysis, IMDB, 16761 Hennigsdorf, Germany

Abstract: The rapid detection of *Salmonella* in food has great relevance even today, as *Salmonella* is still the leading cause of gastrointestinal infections. Symptoms of Salmonellosis include fever, diarrhoea, vomiting and abdominal cramps and appear within 6 to 72 hours after consumption [1]. The standard detection methods of *Salmonella* include pre-enrichment through bacterial culture followed by biochemical and serological tests such as polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA). These conventional detection methods are very expensive, time consuming and laborious. Therefore, more rapid and easy-to-use detection methods are required.

A biochemical method, which has gained increasing recognition, is the loop-mediated isothermal amplification (LAMP) [2]. It is fast, sensitive, easy to apply and can be carried out on site, which makes it suitable for point-of-care diagnostics. The amplification of DNA using LAMP can be performed in less than 60 minutes at a constant temperature using six primers and a *Bst* Polymerase. However, there are many reports of the occurrence of false positives using LAMP [3].

In this study, we amplified a target sequence of the invA gene from *Salmonella* using LAMP, and coupled it with microarray technology [4]. To our knowledge, this is the first time where the LAMP method is combined with a microarray. After 40 minutes of LAMP amplification, without any further processing, the products were hybridized on a modified glass slide with complementary probes. The specificity in detecting *Salmonella* has always posed a problem due to the similar genomic structure of *Salmonella* and *E. coli*. By coupling LAMP with a microarray, we were not only able to distinguish *Salmonella* from false positive negatives but also from false positive *E. coli* (Figure 1(b), 1(c)), creating a fast, sensitive and specific visual detection method for *Salmonella* in food samples.
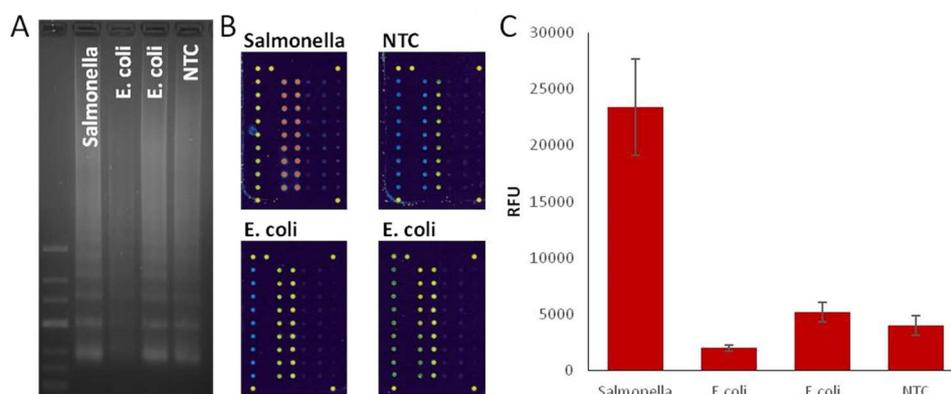

Figure 1: (A) Agarose gel electrophoresis of LAMP product before hybridisation. Lane 1: DNA Ladder, 2: Salmonella, 3: E. coli (strain 1), 4: E. coli (strain 2), 5: no template control. (B) Fluorescent visualisation of LAMP products after microarray hybridisation. Each row represents a probe, with row 2 and 3 detecting different target regions of the invA Gene in Salmonella (C) Relative fluorescence of microarray results of Salmonella, E. coli and the no template control.

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AC electrokinetic immobilization of K562 exosomes on nanoelectrode arrays

Eva-Maria Laux [1], Christian Wenger [2, 3], Ralph Hölzel [1]

eva-maria.laux@izi-bb.fraunhofer.de

[1]Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Am Mühlberg 13, 14476 Potsdam, Germany.
[2]IHP - Leibniz Institute for Innovative Microelectronics, Im Technologiepark 25, 15236 Frankfurt (Oder),   Germany.
[3]Brandenburg Medical School Theodor Fontane, Fehrbelliner Straße 38, 16816 Neuruppin, Germany

Abstract: Exosomes are vesicles that are secreted from most cell types, which usually contain a specific mixture of, e. g., proteins, lipids and nucleic acids. During the development of a disease, the composition of the exosome content can change significantly. Thus, exosomes can be used as markers for early detection, diagnosis and monitoring of diseases.

Here, fluorescently labelled exosomes isolated from K562, a myelogenous leukemia cell line, were immobilized on nanoelectrode arrays by a one-step AC electrokinetic method. The electrode tip diameter in the presented experiments varies from 30 nm to 50 nm, the typical size range of exosomes is 30 nm to 150 nm. Experimental parameters are tuned, aiming at immobilization of exosomes as singles on the tips of the nanoelectrodes.

Raman scattering will be used as additional detection and characterization method. The silicon nanoelectrodes used so far are not coated, but experiments with coated electrodes are envisaged, using various different plasmonic coating materials. Furthermore, the addition of plasmonic nanoparticles for co-immobilization or two-step immobilization is planned. Thus, the surface-enhancement effect for Raman measurements can be exploited for label-free detection and analysis of exosomes and their contents.
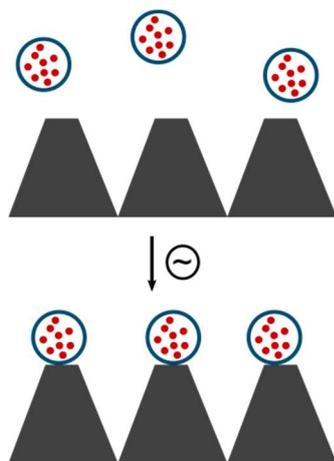
Figure 1: Attraction of exosomes towards nanoelectrode tips by AC electrokinetics; schematic side view.
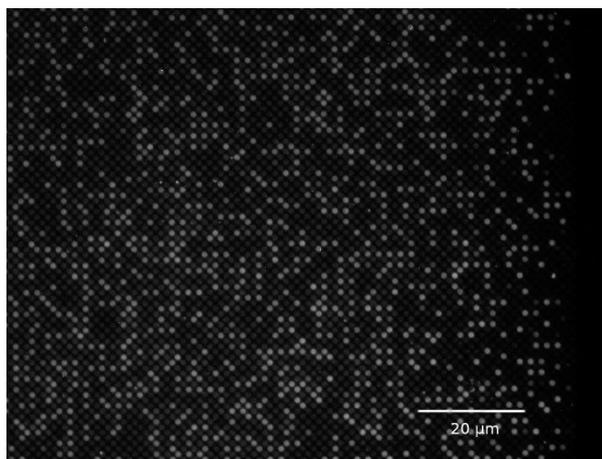

Figure 2: Fluorescently labelled exosomes, immobilized at the tips of nanoelectrodes; fluorescence micrograph of a section of the electrode array.

Whole cell biosensors for cytotoxicity and chemosensitivity assays

Karl-Heinz Feller , Monika Dubiak-Szepietowska, Ute Hofmann

karl-heinz.feller@eah-jena.de (Corresponding e-mail address)

Ernst-Abbe-University of Applied Sciences Jena, Institute of Microsystem and Precision Manufacturing Engineering Jena, Instrumental Analysis Group, Carl-Zeiss-Promenade 2, D-07745 Jena, Germany

Abstract: This contribution will deal with a new method for the complex description of cellular effects to investigate the toxic potential of substances to different cell model lines and biopsies in a real time, sensitive and high throughput manner [1-4]. In case of the cell lines the cells were transfected with a reporter/promoter plasmid construct as an early biomarker of stress induction (leading to GFP expression under stress). In this manner the measurements can be made under incubator-free conditions without any limitations. In 2D cell cultivation it was possible to show the cytotoxicity effect at various cell lines and in real time with plant extracts, chemicals as well as nanoparticles.

The development of 3D cell cultures with Matrigel scaffold and a hepatocyte cell line even more increases the relevance of the sensor towards the human skin or the behavior of the human organ (see Fig. 1). This is clearly shown in experiments comparing the toxic behavior of nanoparticles in 2D- and 3D- environment.
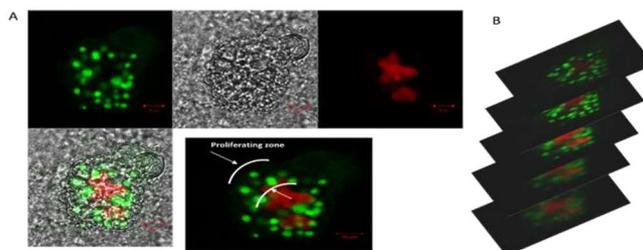

Figure 1: Cultivation of hepatocytes in 3D matrix (scaffold: Matrigel, green: living cells, red: dead cells)

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Characterization of cell adsorption on extracellular matrix proteins and peptides using RIfS and SCORE

J. Hutterer [1], G. Proll[1], G. Gauglitz[1]

johanna.hutterer@iptc.uni-tuebingen.de

[1]Institute of Physical and Theoretical Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

Abstract: The performance of an implant is closely related to the interaction of the surrounding cells with the material at the implant surface. It was shown that properties of the adherent cells – such as proliferation and migration - are affected by the type and strength of the initial cell adhesion [1]. In turn, the adsorption process is influenced by a variety of parameters like the chemical composition, the roughness and possible pre-coatings of the foreign material. Furthermore, each application involves the specific tailoring of the surface to the diverse requirements resulting from the interacting surrounding tissue. Considering the complexity of the cell adsorption process, a profound knowledge of these processes is needed. However, current state of the art methods are often limited by the use of marker molecules, strong influences by temperature fluctuations and the restriction so end-point analysis.

The reflectometric interference spectroscopy (RIfS) [2] offers a high potential to overcome the above stated problems. By its direct optical and temporally resolved operation principle, RIfS combines all advantages of other direct optical methods with far a less temperature dependency and a linear relation between the thickness of the forming film at the sensor surface and the readout signal over a film thickness of several µm. The Single colour reflectometry (SCORE) [3] – a method that evolved from the RIfS technology - additionally enables parallelized adsorption measurements on different surfaces.

Thus, RIfS and SCORE were adapted for measurements with cells. The integrated flow cell allows to pump different liquids across the sensor surface and thereby mimic - for example - the fluid dynamic conditions in the cardio-vascular system. The adsorption of HEK cells on glass surfaces coated with fibronectin, native and denatured collagen was recorded with RIfS and compared. These results are complemented with classical microscopic examinations to estimate the number of adherent cells and their morphology. Additionally, the adsorption of MEF cells on glass surfaces coated with fibronectin, native and denatured collagen, RGD peptides and a further cell adsorption promoting peptide was measured in parallel by SCORE.

It is concluded, that RIfS and SCORE are very well suited to study the adsorption processes of cells to surfaces. In future applications specific processes in the complex field of the foreign body reaction to implants can be investigated in more detail.

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Characterization and manipulation of yeast cells using microfluidicbased interdigitated biosensor

Honeyeh Matbaechi Ettehad [1], Christian Wenger [1,2]

matbaechi@ihp-microelectronics.com

[1] IHP–Leibniz-Institut für innovative Mikroelektronik, Im Technologiepark 25, 15236 Frankfurt/Oder, Germany [2] BTU Cottbus-Senftenberg, 03046 Cottbus, Germany

Abstract: Cell characterization and manipulation are critical when it comes to clinical and diagnostic applications. Immobilization and isolation of specific cells as a way to detect diseases [1], separation of live and dead cells as a means for early-stage disease diagnosis [2], as well as filtering and purification of cells, viruses, proteins, and micro/ nanoparticles [3] are essential examples in a variety of healthcare, biological and biomedical applications. This allows early treatment and increases the chance of saving a life. The development of lab-on-a-chip (LoC) devices such as microfluidic platforms has simplified the handling of complex and costly laboratory-based sample preparations and analyses. This relies on the blood sample's rapid analysis or other aqueous samples and detecting the disease using a singles device and can be replaced by the costly, labor-intense, and time-consuming traditional processes. CMOS technology with embedded electrodes has been demonstrated for various biosensing applications, such as cell immobilization and separation [4]. The integration of microfluidic channel with CMOS can scale down multiple-stage laboratory procedures all in a single chip and process micro and nano-liters samples within a fully isolated manner.

In this work, CMOS-based interdigitated electrodes (IDEs) is proposed and designed to characterize and manipulate the cells. Using the distinct differences of the cells, they can be trapped and detected or separated, employing dielectrophoretic (DEP) techniques. This IDE platform can be integrated with microfluidic channel and circuit by CMOS process line of IHP, for simultaneous immobilization, sensing, and detection of biological and non-biological particles. This work aims to enable the design of an IDE with the capability to manipulate biological particles. To this end capacitive biosensors (i.e. IDEs) were embedded in the microfluidic channel. Applying AC to these electrodes, a non-uniform EF is created. Depending on the relative permittivity of the cells and medium flown over IDEs as well as amplitude and angular frequency of the AC, cells can experience a translational DEP force in two opposite directions. Positive DEP force moves the cells toward the strongest EF locations (i.e., finger edges), and as a result, cells can be trapped there. Negative DEP repels the cells from the IDEs and moves them towards the lowest EF regions.

Various live and dead yeast cell suspensions were used during the measurements. Results showed that the device has a rapid response time, and trapping was obtained spontaneously after applying an electric potential. DEP force-frequency profile indicated that changing frequency influences the cell trajectory and gives rise to DEP force in both directions (positive and negative) and impacts the trapping yield (cell accumulation) at the IDEs. The increasing electric potential was found to be very influential in obtaining higher immobilization rates. The dead cell suspension in DIW, as well as live cell suspension in DIW, tap water, KCL and diluted PBS, showed positive DEP response in specific frequency ranges. As the medium conductivity increased, the pDEP spectrum got smaller, and trapping occurred at higher frequencies. The trapping trend for all the cases was almost similar. They exhibited a gradual increase in trapping yield by the transition of the real part from negative to positive, and the trapping yield gained peak by increasing frequency. By tuning the cell trapping, isolation and separation of specific cell types from a cell mixture was also achieved. IDEs have been shown suitable for the immobilization and separation of yeast cells within a CMOS-based microfluidic device.

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Development of a functional complement assay based on liposomes

Clemens Spitzenberg [1], Anna Gebhard, Cornelia Hermann, Simon Streif, Antje J. Baeumner

clemens.spitzenberg@chemie.uni-regensburg.de

[1] Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany

Abstract: The complement system is part of the innate immune system and is responsible for several important tasks as for example recognition and elimination of invading pathogens or connecting innate and adaptive immune responses[1]. The activation of the complement system follows mostly a similar and straightforward cascade that includes particle recognition and its opsonization, selfamplification, generation of effector molecules (for example the Membrane-Attack-Complex (MAC)) and induction of immune response[2]. Current research into the investigation of the functional roles of its participating proteins is limited, as most assays are based on erythrocyte lysis or on mere protein quantification via ELISAs. Thus, liposomes are developed here to function as biomimic instead of the erythrocytes and hence enable a highly targeted, specific and reliable functional assay. Initial studies focused on the development of liposomes that are not lysed when incubated with human serum. Lysis can easily be determined by the encapsulation of a fluorescent dye such as sulforhodamine B (SRB) as SRB self-quenches when inside the liposomes at high concentrations. By varying lipid composition and encapsulant concentrations, various stealth liposome compositions could be developed with anionic and cationic as well as polyethylene glycol surfaces. Secondly, the specific lysis of these liposomes by the MAC upon triggered activation of one of the three pathways of the complement system was studied. In a first proof of principle this was demonstrated by including lipopolysaccharides (LPS) in the liposomal membrane. Liposomes were specifically lysed by human serum and the presence of complement proteins was confirmed via a protein ELISAs. Further development of this proof-of-concept will lead to a point-of-care assay either for viral or immune status detection.
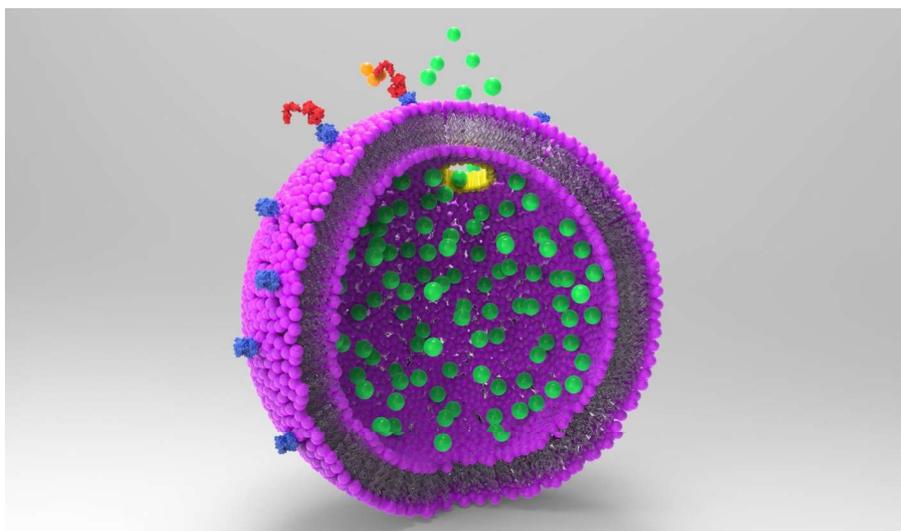
Figure 1: Schematic illustration of a liposome encapsulating SRB (green) in its cavity surrounded by the lipid bilayer (purple). Trigger modifications (e.g. LPS) on the liposome surface (blue, red, orange) enable pore formation (yellow) via the Membrane-Attack-Complex. Thus, SRB is released and an increase in fluorescence is detectable.

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Lab-on-a-chip system for developing and fluorescence imaging a three-dimensional model of pancreatic islets under flow conditions.

Patrycja Sokolowska[1,2], Kamil Zukowski[1,3], Justyna Janikiewicz[2]Elzbieta Jastrzebska[1], Agnieszka Dobrzyn[2], Zbigniew Brzozka[1]

psokolowska@ch.pw.edu.pl

[1] Chair of Medical Biotechnology, Faculty of Chemistry, Warsaw University of Technology, 00-661 Warsaw, POLAND
[2]Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, 02-093 Warsaw, POLAND
[3]CEZAMAT WUT, 02-822 Warsaw, Poland

Abstract: Diabetes mellitus is a group of metabolic diseases characterized by elevated blood sugar resulting from a defect in the production of insulin by pancreatic β-cells or tissue insensitivity for this peptide hormone [1]. Nowadays, there are approximately over 400 million diabetic adults in the world [2]. In our research, we focused on imitate the pancreatic islet structure and proper insulin secretion, which can be a universal model for testing the impact of various environmental factors on disease development. We present a *Lab-on-a-chip* system in which a pancreatic "pseudoislet" model will be developed. This system is composed of biocompatible, non-toxic and transparent materials, such as: poly(dimethylsiloxane) (PDMS) and thin glass. Thanks to this, it is possible to conduct cell culture and observe the results in various types of microscopes. The geometry of *Lab-on-a-chip* system consist of two elliptical cell culture chambers, one for the test sample and second for the reference sample. In in each of the chambers there are 15 round microtraps. Each of the microtraps is made of 7 micropillars. This solution forces the dense packing of cells and their aggregations. The geometry of the developed system is consistent with the culture wells on a standard multi-well plate, which allows proliferation measurements in a multi-well plate reader. All experiments were performed using two commercially purchased pancreatic islets cell lines: β-cells (INS-1E) and α-cells (α-TC1-6).
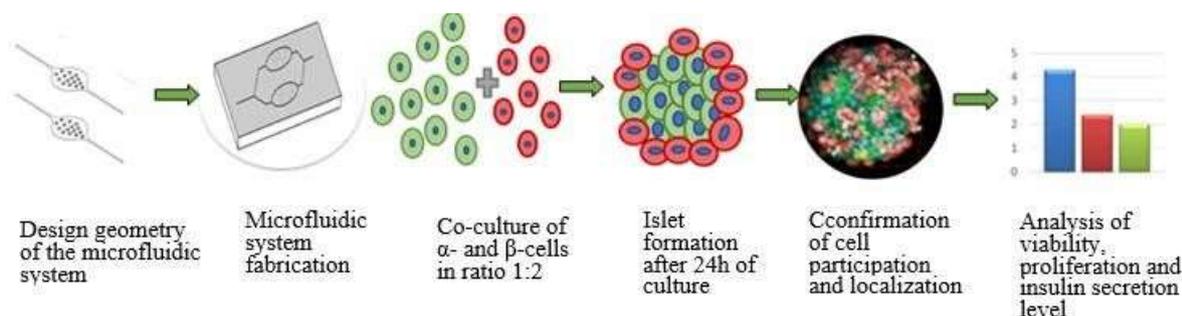
Figure 1: Diagram showing following stages of the research.

It was confirmed that 15 spherical α- and β-cell aggregates with diameters of 160-180 μm were obtained in one culture chamber of our *Lab-on-a-chip* system. As was expected the participation of β-cells in the core and α-cells on the periphery of the aggregate was confirmed by the use of immunostaining method. A high viability after 24, 48 and 72 hours of culture in the microsystem was confirmed. The cells maintained their morphology, function and high level of proliferation for up to 72 hours of culture. Moreover, in the developed model health and disease conditions were simulated and the level of insulin secreted from this model was determined using the ELISA test. At this stage, a research model corresponding to the model *in vivo* conditions was obtained. This study presents basic research and in the future, this model could be utilized to simulate diabetes, testing new drugs and therapy in diabetes mellitus treatment.

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Platelet Imprinted Polymers for rapid platelet function monitoring

Felix Thier [1], Hanna Troiss [1], Victoria Schober [1,2], Peter Lieberzeit [1]

peter.lieberzeit@univie.ac.at

[1]Department of Physical Chemistry, Faculty of Chemistry, University of Vienna, Währinger Straße 42, 1090 Vienna, Austria
[2]Department of Life Sciences, IMC University of Applied Sciences Krems, Piaristengasse 1, 3500 Krems, Austria

Abstract: The human haemostatic system involves a series of fine-tuned and complex processes that can be influenced by external factors (e.g. anti-inflammatory drugs) and internal malfunctions (e.g. clotting and bleeding disorders). Platelets (thrombocytes) play the key role in the haemostatic system. [1][2] Therefore, monitoring their normal function is not only important during surgery, but also during treatment of patients suffering from platelet disorders. This work presents first steps in creating a point-of-care sensor system for rapid platelet function assay. It relies on molecularly imprinted polymers (MIPs) selectively recognizing platelets: during activation, platelets change their shapes [3]. This can be made use of in the sensing approach (Figure 1a): when using resting platelets as the templates, only they can rebind into the imprinted recognition sites on the surface. Imprinting platelets in a resting, native state is therefore the first crucial step in designing the sensitive layer. Stampimprinting, which is a lithographic technique to generate surface imprints on polymer layers [4], turned out to be the most promising approach in creating recognition sites. Stamps were synthesized by immobilizing platelets on glass or polydimethylsiloxane (PDMS) substrates. As platelets tend to react to many external factors by being activated and aggregating, conserving their resting state prior and during imprinting is of importance. The use of low molar ratios of EDTA proved to be successful in prolonging platelet activation by chelating Ca(II) ions needed in the activation process. Applying NO-releasing S-Nitroso-N-acetylpenicillamine seems to further suppress platelet aggregation, which turned out useful during the drying of the generated stamps. First results indicate that it is in fact possible to imprint platelets in their native shape: Several different polymer layers (polyurethanes, polystyrenes and polyacrylates) were synthesized using polyaddition or free-radical polymerization (FRP), respectively, and imprinted via the stamp-imprinting approach. Atomic force microscopy (AFM) images of the imprinted polymer layers indicate that polystyrenes show very good imprint qualities (Figure 1b, left), but lack sufficient biocompatibility as plasma proteins tend to aggregate on the hydrophobic, low-wettable polymer surfaces [5]. Polyacrylates are the most promising matrix, as they comprise improved biocompatibility while still maintaining sufficient imprint quality (Figure 1b, right). Combined systems of the two polymers can lead to satisfying qualities of both respective parameters and additionally increase adhesiveness of the polymer film to the QCM substrate.
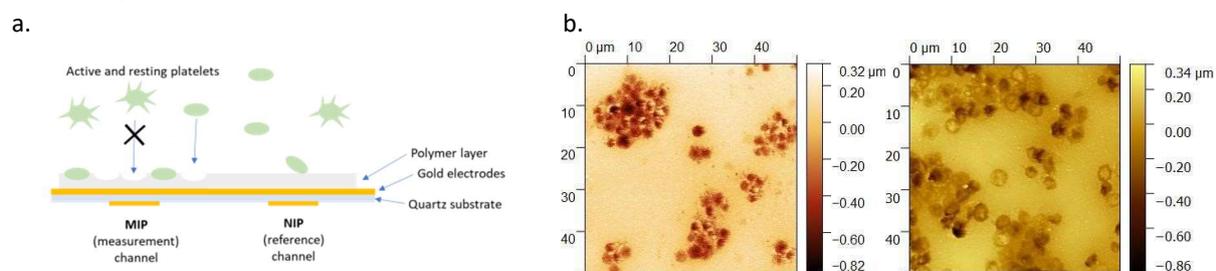
*Figure 1: **a:** Schematic representation of the generated sensing system. The platelet imprinted polymer can rebind resting platelets while active platelets stay in solution, making conclusions concerning their functional status possible. The reference channel compensates non-specific binding events on the polymer layer. **b:** The AFM images show platelet imprinted poly(styrene-co-divinylbenzene) (left) and poly(hydroxyethylmethacrylateco-ethylene glycol dimethacrylate) (right) films. Imprint shape and size match resting platelet dimensions.*

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Prussian Blue based nanozymes: electrocatalytic properties and applications for electrochemical (bio)sensors

Alexander A. Zarochintsev, Maria A. Komkova, Vera A. Shavokshina, Ksenia D. Andreeva, Arkady A. Karyakin

zarochintsev-aa@ya.ru

Faculty of Chemistry, Lomonosov Moscow State University, Leninskiye gory str., 1/3, 119234 Moscow, Russian Federation

Abstract: Inspired by nature and aiming to overcome the high cost and low stability of enzymes, nanozymes – nanomaterials mimicking enzymatic activity – have emerged as a distinct branch of biomimetics [1]. Nanozymes with peroxidase-like activity are of particular interest as horseradish peroxidase (HRP) is the most widely used enzyme in both biotechnology and medical analysis. Unfortunately, two essential criteria to be eligible for either application, catalytic selectivity and high activity in pH 7.0-7.4, aren't met by an overwhelming majority of known nanozymes. However, Prussian Blue nanoparticles (PBNPs) obtained through catalytic synthesis (by reducing $Fe[Fe(CN)_6]$ with H_2O_2) meet both criteria, surpassing turnover numbers of natural HRP by up to 4 orders [2].

Electrochemical properties of catalytically synthesized PB based nanozymes are notable. Simple drop-casting of their colloidal solution followed by annealing at 100 °C results in a ready-to-use H_2O_2 sensor. Its sensitivity, which reaches 0.85 A.M.cm^{-2}, exceeding that of PB film by 30%, allows detecting sub-micromolar concentrations of H_2O_2 [3]. This value of sensitivity apparently corresponds to the formation of a continuous monolayer of PBNPs: while being linearly dependent on the deposited amount of PBNPs at low concentrations, sensitivity plateaus as the concentration of PBNPs increases. Charge transfer resistance, which was calculated from electrochemical impedance spectra, linearly decays at low PBNPs concentration, also reaching its lower limit in the similar concentration region.

Achieved sensitivity of 0.85 A.M.cm^{-2} can be further increased with carbon black nanoparticles (CBNPs), resulting in record sensitivity of 1.15 A.M.cm^{-2}, almost doubling the sensitivity of PB film based sensors. Both glucose and lactate oxidases were co-immobilized with PBNPs-CBNPs mixture. The aforementioned drop-casting approach results in biosensors advantageous over conventional sensors produced upon layer-by-layer immobilization in terms of two times higher sensitivity and three times extended operation time.

Functionalization of catalytically synthesized PBNPs would allow using them as catalytic labels, significantly extending their potential applications. However, modifying the surface of PBNPs drastically decreases their catalytic activity, as the diffusion of substrates to their surface is disrupted. This problem is solved with a modification of catalytic synthesis of PBNPs, which allows their functionalization at the stage of synthesis while retaining their ultrahigh activity. This is achieved by swapping the reducing agent (originally H_2O_2) for monomers of conductive polymers. This modification of the "catalytic synthesis" protocol allows functionalization of PBNPs with amine, azide, boronate, sulphonate and carboxylate functional groups. Furthermore, drop-casting of polymermodified PBNPs results in considerably more operationally stable sensors. Such sensors retain 95% of their initial signal twice longer than those based on unmodified PB NPs.

Azide-modified PBNPs were successfully bioconjugated with alkene-modified HULC gene fragments through copper(I)-mediated 1,3-dipolar cycloaddition. Practical possibility of DNA hybridization detection, and thus, possibility of applying the noted PBNPs as electrocatalytic labels, was shown. The detection limit of oligonucleotides in model systems does not exceed 100 pM.

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Protease biosensor based on modification of gold nanoparticles and optical methods

Ivan Piovarci [1], Tibor Hianik[1], Ilia N. Ivanov[2]

piovarci.i@gmail.com

[1]Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska Dolina F1, 842 48 Bratislava, Slovakia
[2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

Abstract: Proteases play an important role in various biological as well as dairy industrial processes. In this work, we focused on measuring the activity of three proteases: chymotrypsin, trypsin and plasmin. These proteases are important in human digestion and also cleave milk proteins, which makes them useful in the study of the quality of dairy products like milk or cheese. In this work, we focused on detection of these proteases using spectrophotometric and dynamic light scattering (DLS) methods. To prepare biosensor sensitive to these proteases we use gold nanoparticles (AuNps) modified with their natural substrate - β-casein. In order to monitor spectrophotometric changes after protease addition we needed to modify the AuNps further with 1-mercaptohexanol (MCH) [1]. For DLS method AuNps modified with just β-casein were used. After addition of protease, we were are able to detect changes in absorbance (spectrophotometric method) and size (DLS method) of the modified AuNps. The scheme of modification and cleavage of gold nanoparticles is outlined in figure 1. We discussed the differences and calculated the limit of detection (LOD) for both spectrophotometric and DLS methods.
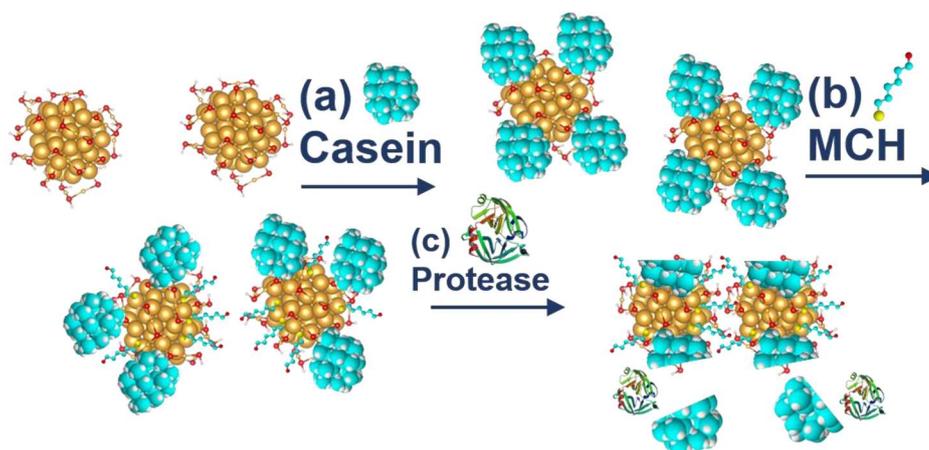
Figure 1: Scheme of gold nanoparticles (AuNPs) modification. (a) Modification with β-casein. (b) Modification with 1-mercaptohexanol – not used for DLS methods (c) cleavage by protease-plasmin, chymotrypsin or trypsin

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Voltammetric activity determination of the human catechol-Omethyl transferase at fluorine doped tin oxide

Gero Göbel [1], A. Talke[2], U. Ahnert[2], F. Lisdat[1]

ggoebel@th-wildau.de

[1]Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, Hochschulring 1, 15745 Wildau, Germany

[2]BioTeZ Berlin Buch GmbH, Robert-Rössle-Straße 10, 13125 Berlin, Germany

Morbus Parkinson is one of the most frequent disorders of the central nervous system. The loss of dopaminergic cells causes a lack of the neurotransmitter dopamine in the brain. This deficiency results in progressive movement disorders - typical symptoms of Parkinson's disease (PD). Because of its central role in the catecholamine metabolism numerous enzymes are involved in the control of the dopamine concentration, for example the catechol-O-methyltransferase (COMT). A medical treatment of PD occurs via a modulation of the COMT activity by the administration of COMT inhibitors (entacapone, tolcapone). For an improved personalized medication direct measurements of enzyme activity should complement the observation of the change of PD symptoms by the physician.

For this investigation differential pulse voltammetry (DPV) was used for the detection of the enzyme substrate dopamine. Fluorine doped tin oxide (FTO) – the applied electrode material - allows a clear discrimination between the COMT substrate dopamine and its conversion product methoxytyramine [1]. The linear dependency of the DPV signal on the dopamine concentration in the range of the maximum reaction rate of the COMT and the high signal stability during consecutive measurements are substantial requirements for the construction of reliable sensors. Nevertheless, in the complete activity assay with all essential components for enzyme action the dopamine oxidation signal was influenced by each of the added ingredients, even though none of the separate compounds causes a current signal at the FTO electrode. After changes of the assay composition and adjustments of the DPV potential range a reproducible determination of dopamine concentrations in the complete assay solution can be achieved. Incubations of substrate with COMT bound to agarose beads reveal clearly, that the voltammetric dopamine signal depends on the time of enzyme action. Furthermore, the change of the measuring signal correlates clearly to the activity of the captured COMT, demonstrating that enzyme activity can be followed by electrochemical measurements. Hence, FTO is a suitable electrode material for the voltammetric determination of the COMT activity. Alternative approaches can also be demonstrated.
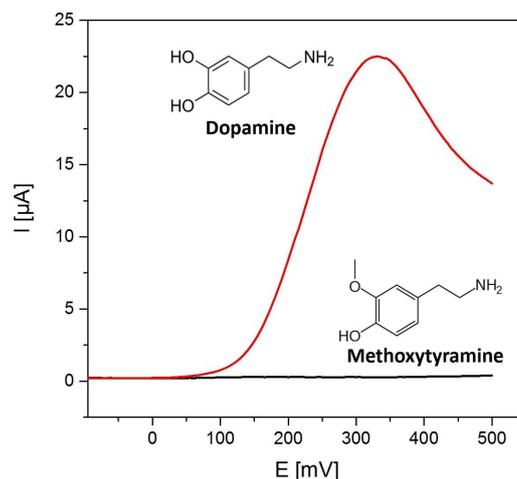

Figure 1: Voltammetric signal of 1 mM dopamine and 1 mM methoxytyramine in 20 mM potassium phosphate (pH 7.2) at FTO

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Detection of trypsin and plasmin using a QCM sensor based on βcasein immobilized on a hydrophobic surface

Sandro Spagnolo[1], Tibor Hianik[1], Ilia Ivanov[2]

sandrospagnolo1@gmail.com (Corresponding e-mail address)

[1]Faculty of Mathematics, Physics and Information, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia,
[2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

Abstract: Enzymatic hydrolysis of proteins is an important factor in food production and it can have various effects on taste development. However, unwanted proteolysis can adversely affect food quality. Proteolysis of milk proteins, such as caseins, caused by milk proteases, can change of the organoleptic and nutritional characteristics of milk [1]. One of the main proteases of milk is plasmin. The aim of this work is to develop a quartz crystal microbalance (QCM) biosensor for the detection of proteolysis of β-casein by plasmin and trypsin. Casein films seem to be a promising substrate for the study of the enzymatic activity of proteases. The casein aggregation mechanism was investigated by its absorption at low concentration (0.1 mg/mL) on a hydrophobic surface consisting of a selfassembled 1-dodecanthiol monolayer on the gold electrode of a quartz crystal. This architecture has been used for an analysis of trypsin and plasmin activity. After addition of enzymes, an increase in the resonance frequency of the piezoelectric crystal caused by a mass removal from the surface of the electrode occurs, and this variation can be correlated to the enzyme concentration (Figure 1). The kinetics of changes in the frequency of the crystal, after incubation of different concentrations of enzymes (0.1 - 15 nM), demonstrated that the effect of casein cleavage is very similar for both proteases. After a rapid increase in frequency in the region of relatively low protease concentrations (up to about 1 nM), there is a moderation in the frequency increase and saturation occurs. This approach can also be exploited to increase the sensitivity by forming an oligolayer; the negatively charged phosphoseryl clusters of β-casein, facing the bulk solution, can bind bivalent cations, making the layer capable of binding more casein molecules and casein-coated nanoparticles [2].
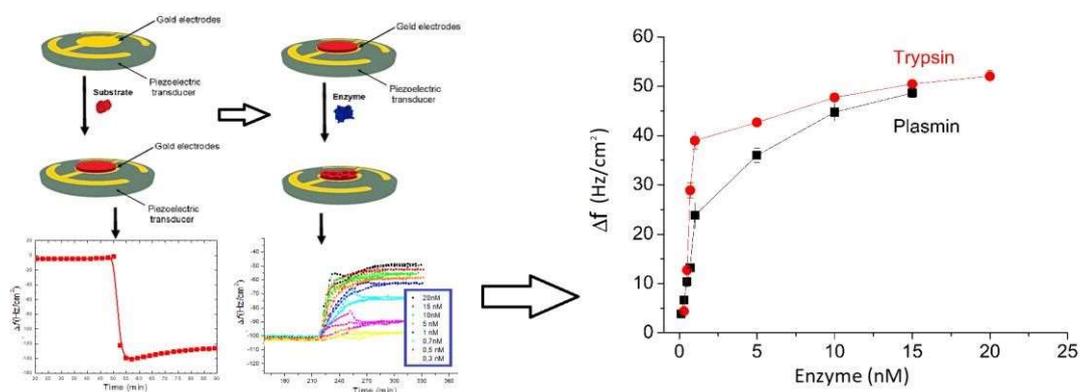
Figure 1: The scheme of the operation of the protease sensor. Left: the kinetics of changes in the fundamental frequency (8 MHz) of a crystal coated with dodecanethiol after the addition of casein and its cleavage by plasmin at different concentrations (0.1 - 15 nM). Right: a comparison of frequency changes after casein cleavage by plasmin and trypsin.

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Artificial heme-peroxidases for the construction of functional bionanoconjugates

Emilia Renzi,[1] Linda Leone,[1] Flavia Nastri[1] and Angela Lombardi[1]

emilia.renzi@unina.it

[1] Department of Chemical Sciences, University of Naples "Federico II", via Cintia, 21, 80126, Naples, (Italy)

Rapid progresses in the field of nanotechnology has offering numerous opportunities for the construction of enzyme/nanosupport hybrid biomaterials, with potential application in biotechnology, diagnostic and sensing.[1] Nanomaterials (NMs), as gold nanoparticles (AuNPs), offer excellent loading platforms for the development of bionanoconjugates, due to the high surface area-to-volume ratio, which allows for high biocatalyst loading, as well as versatile surface chemistry.[2] Furthermore, finetuning enzyme performances by design should open the way to the construction of a wide range of innovative bionanoconjugates tailored for specific applications. Artificial miniaturized heme-proteins, known as Mimochromes (MCs), revealed to be excellent candidates for this purpose. Indeed, preliminary results on Fe(III)-Mimochromes demonstrated their successful immobilization onto gold electrode surfaces and conjugation to AuNPs while retaining redox and catalytic properties.[3]

With the goal of preparing a catalytically active nano-construct, the work presented focused on the immobilization of the artificial heme-peroxidase Fe(III)-MimochromeVI*a (FeMC6*a) on AuNPs, chosen as a model support. For this purpose, "click chemistry" was exploited as a general and broad strategy for the fast-covalent immobilization of FeMC6*a on the target nanomaterial. The interest for click reactions, such as SPAAC (strain-promoted azide-alkyne cycloaddition), relies on their high selectivity and high reaction yields.[4] The SPAAC ligation strategy was employed by functionalising citrate-AuNPs with azide-exposing heterobifunctional linkers, and by derivatising the artificial catalyst through a site-specific reaction with a pegylated aza-dibenzocyclooctyne (DBCO) moiety, to give FeMC6*a-PEG(4)-DBCO. The conjugation between the two components afforded FeMC6*a-PEG(4)@AuNPs (**Figure 1**).
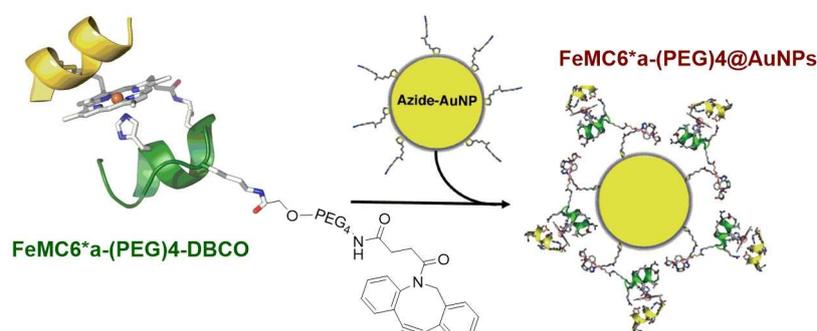

Figure 1*: Immobilization of FeMC6*a-(PEG)4-DBCO on azide-AuNPs, via SPAAC, to afford a catalytically active nanomaterial.*

The clicked biocatalyst retains its structural and, to some extent, its functional behaviours with respect to the freely diffusing enzyme.[5] Therefore, the adopted method is a proof of concept that the miniaturized artificial metalloprotein FeMC6*a is prone to be subjected to a synthetic procedure which ensures its stable anchoring on nanomaterials, with high degree of enzyme coverage and without loss of catalytic performances. In conclusion, the overall results strongly support the development of stable and functional heme-protein models bioconjugate with AuNPs, paving the way to future applications in biotechnology and catalysis.

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Direct bioelectrocatalysis of glucose dehydrogenase facilitated by carbon black: towards one-step fabrication of biosensors

Vera A. Shavokshina, Egor A. Andreev, Maria A. Komkova, Arkady A. Karyakin

v.shavokshina@analyt.chem.msu.ru

Department of Chemistry, M.V. Lomonosov Moscow State University, 119991 Moscow, Leninskie gory, 1, build. 3, Russia.

Direct enzymatic bioelectrocatalysis of pyrroloquinoline quinone dependent glucose dehydrogenase (PQQ-GDH) is exploited in glucose biosensing [1]. However, since enzyme active site is embedded inside the protein structure, various strategies for improving direct electron transfer (DET) are applied. The known systems rely on sophisticated matrixes such as graphdiyne [2], cryogels [3] etc. For industry and mass-production, on the contrary, simple, scalable, and reliable approaches are required. For example, conductive carbon black nanoparticles (CBNPs) are suitable modifiers for facilitating DET due to their biocompatibility, low cost, and large accessible electroactive area. In this work, we report on facile and scalable method of fabrication of third generation glucose biosensors based on self-made CBNPs.

The reported CBNPs were found to facilitate DET of adsorbed PQQ-GDH. In the presence of glucose, bioelectrocatalytic current was registered on biosensors in square wave voltammetry mode. In previous works, we presented an approach of enzyme immobilization from media with high content of organic solvent [4]. Application of the same approach to PQQ-GDH resulted in retainment of the enzyme activity. Water-organic media can provide favorable enzyme immobilization in one-step fabrication of glucose biosensors.

In contrast to existing protocols comprising different modification steps, biosensors were prepared by drop-casting a dispersion of CBNPs and PQQ-GDH in Nafion matrix. In comparison with nonmodified surface, CBNPs provide 35-fold raise in electrocatalytic current densities and increased signal to noise ratio. Limit of detection reaches 0.3 µmol per liter. This bioelectrocatalytic system has been also characterized by apparent Michaelis constant (K_m^{app}). K_m^{app} is practically independent of the amount of the CBNPs indicating good conductivity of the latter and favorable immobilization of the enzyme. In addition, storage stability of enzyme-containing dispersion has been assessed. It exhibits more than 50 % residual catalytic activity after 7 days of storage at −18°C. The possibility of dispersion storage is also beneficial for scale production.

The proposed approach of facile fabrication of third generation glucose biosensors is expected to have an impact on healthcare including non-invasive diagnostics such as sweat and breath condensate analysis enabling the measurement of ultralow glucose concentrations.

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Fructose dehydrogenase on self-assembled monolayers for fructose sensors

Xiaomei Yan[a], Su Ma[b], Jing Tang[a], David Ackland Tanner[a], Jens Ulstrup[a,*], Xinxin Xiao[a,*], Jingdong Zhang[a]

Corresponding authors: E-mail: xiyan@kemi.dtu.dk; ju@kemi.dtu.dk; xixiao@kemi.dtu.dk.

[a]Department of Chemistry, Technical University of Denmark, Kongens Lyngby 2800, Denmark
[b]Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, BOKU- University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria

Abstract: Fructose dehydrogenase (FDH) from *Gluconbacter japonicas* is a membrane-bound oxidoreductase with three subunits, subunits I (67 kDa), II (51 kDa) and III (20 kDa).[1] Subunit I has a flavin adenine dinucleotide (FAD) cofactor, serving as the catalytic center of two-electron fructose oxidation. Subunit II holds three heme c moieties as ET relays between FAD and electron acceptors. Subunit III has no redox center and constitutes for the enzymatic integrity. FDH has been widely developed to investigate the direct electron transfer (DET) and mediated electron transfer (MET) between an electrode and redox/catalytic center(s) in enzymatic bioelectrochemistry.[2] Favourable orientation and high enzyme loading on electrodes are paramount in DET-type bioelectrocatalysis to ascertain high catalytic activity and ET rate.[3] We present here systematic studies of the influences of both self-assembled molecular monolayers (SAMs) and nanoporous structure on DET-type bioelectrocatalysis of FDH. Two different SAMs with varying functional terminal-groups and alkyl chain lengths were investigated. FDH on a 2-mercaptoethanol (BME) SAM showed the maximum current density ($j_{max,det}$: 54.88 ± 3.03 μA cm^{-2}) and DET-capable fraction (χ_{det}: 13.33 %). This can be explained by the most favourable FDH orientation on the electrode surface. FDH on a 3mercaptopropanoic acid (MPA) SAM exhibited the best MET with Δj_{max} of 640.76 ± 7.65 μA cm^{-2} using ferrocenemethanol as the mediator due to the negatively charged surface efficiently adsorbing FDH. The FDH catalytic properties significantly decreased with increasing SAM carbon chain length. Porous gold structures not only improved the catalytic performance by increased enzyme loading and χ_{det}, but also increased notably the operational stability.
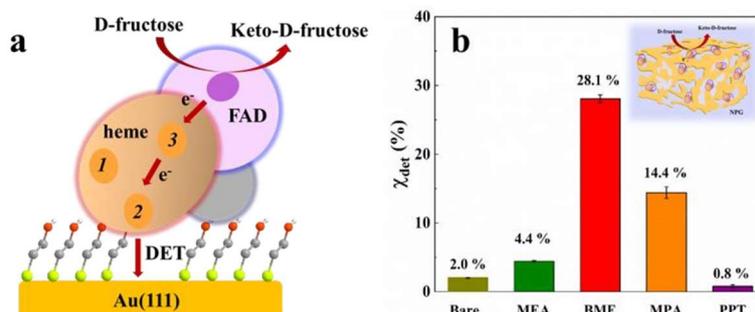
Figure 1. (a) Schematic illustration of possible DET pathway of FDH on Au(111) electrode. (b) DET-capable fraction (χ_{det}) of FDH on bare NPG and NPG modified with -NH₂ (MEA), -OH (BME), -COOH (MPA) and CH₃ (PPT).

Acknowledgment

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Epidermal sensing of H₂O₂: optical, Prussian blue based, visualisation of penetration pathways in skin

Skaidre Jankovskaja [1,2], Anaïs Labrousse [3], Léa Prévaud [4], Bo Holmqvist [5], Anders Brinte [5], Johan Engblom[1,2], Melinda Rezeli [6], György Marko-Varga [6], and Tautgirdas Ruzgas[1,2]

tautgirdas.ruzgas@mau.se

[1]Department of Biomedical Science, Faculty of Health and Society, Malmö University, 205 06 Malmö, Sweden
[2]Biofilms - Research Center for Biointerfaces, Malmö University, 205 06 Malmö, Sweden
[3]Department of Biological Engineering, Clermont Auvergne University, 63100 Aubiere, France
[4]Faculty of Sciences, University of Montpellier, 34085 Montpellier, France
[5]ImaGene-iT, Medicon Village, 223 81 Lund, Sweden
[6]Clinical Protein Science & Imaging, Biomedical Centre, Department of Biomedical Engineering, Lund University, BMC D13, 221 84 Lund, Sweden

Abstract: Elevated amount of reactive oxygen species (ROS), especially, hydrogen peroxide, H₂O₂, is produced in skin during different skin disorders. Epidermal sensing of H₂O₂ could, thus, be useful to monitor skin disorder progression and healing. In this work, we will present results of a study, which was devoted to evaluate epidermal sensing of H₂O₂ in vitro, by visualizing H₂O₂ permeation through the skin. The work adopted Franz cell setup, which is highly used in development and validation of transdermal drug delivery.

Skin membranes were placed in Franz cells and a suspension of Prussian white particles was pipetted on the top of stratum corneum side of the skin. During H₂O₂ permeation though the skin, Prussian white was oxidized to Prussian blue, giving a pattern of blue dots. Comparison of skin surface images with the dot patterns revealed that about 74% of the blue dots were associated with hairs. The conversion rate of the Prussian white to Prussian blue, in due course of the H₂O₂ penetration, strongly correlated with the reciprocal resistance of the skin membranes. All together, these results demonstrate that hair follicles are one of the major pathways of H₂O₂ penetration though skin. The study points out that the monitoring of hydrophilic biomarkers on skin would strongly benefit from targeting specific pathways by epidermal sensing. We would like to suggest that future development of epidermal biosensing approaches should allow micrometer resolution to enable biomarker detection at hair follicles.
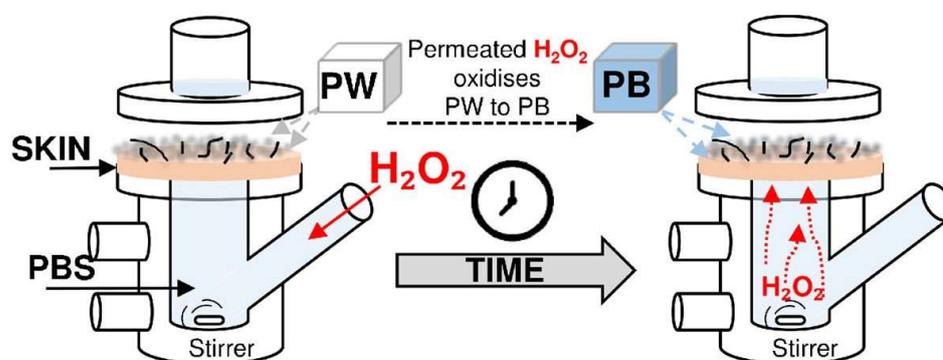

Fig.1. Schematic representation of in-vitro system for epidermal sensing of H₂O₂ penetration through skin.

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A photobioelectrochemical biofuel cell: exploiting light and biofuels for energetics

M. Riedel, S. Höfs, S. Morlock, and F. Lisdat

sascha.morlock@th-wildau.de

Biosystems Technology, Institute of Life Sciences and Biomedical Technologies, Technical University Wildau, 15745 Wildau, Germany

The coupling of photoactive materials with enzymes has gained much attention in the last decade for the modulation of electrochemical and biocatalytic reactions with light. This results in various fields of application, which can be divided into sensorial, synthesis and bioenergetic aspects. For the latter, there arise interesting properties regarding the potential behaviour. In previous work, we have already demonstrated the development of a photobioanode, which allows the collection of electrons from the sugar oxidation at extremely low potentials under illumination. Here a combination of two semiconducting materials (TiO_2 and PbS) with a biocatalytic reaction (FAD glucose dehydrogenase) has been exploited [1]. This approach has also been extended to photosystem 2 to improve the photoelectrochemical water splitting efficiency [2]. The present study is devoted to the investigation of $BiFeO_3$ as photocathode material for the construction of photobioelectrodes [3]. Therefore, a thin layer of $BiFeO_3$ has been prepared by a spin coating procedure on FTO slides, giving rise to cathodic photocurrents. The cathodic photocurrent is drastically enhanced in the presence of H_2O_2 and shows first signals at a rather positive onset potential of about 0.63 V vs Ag/AgCl. In contrast, in the dark no current response is obtained, which underlines the light-directed origin of H_2O_2 conversion. The generated photocurrent provides sufficient stability with millimolar hydrogen peroxide concentrations and a defined dependence on the H_2O_2 concentration in the range between 5 µM and 20 mM. The photocatalytic activity of the $BiFeO_3$ materials has been combined with glucose oxidase (GOx) for the construction of a photobiocathode. Here, GOx produces H_2O_2 under glucose consumption and thus supplies the photocathode with substrate. The photobiocathode has been finally coupled to a glucose consuming photobioanode for the elaboration of a photobioelectrochemical biofuel cell, which allows the light-driven generation of electricity under glucose consumption at the photobioanode and biocathode. This cell gives rise to quit large open circuit voltage of about 1 V under illumination and may illustrate the potential of coupling suitable semiconductor structures with biocatalytic conversions on electrode surfaces for application in bioenergetics, but also in biosensing.

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Electrochemical biosensing of specific proteases and hypoxia biomarkers to early identifying cancer aggressiveness

Cristina Muñoz-San Martín[1], Maria Gamella[1], María Pedrero[1], Ana Montero-Calle[2], Rodrigo Barderas[2], Susana Campuzano[1], José M. Pingarrón[1].

cmunoz04@ucm.es

[1]Departamento de Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, Pza. de las Ciencias 2, E-28040 Madrid, Spain

[2]UFIEC, Instituto de Salud Carlos III, Carretera Pozuelo Majadahonda Km 2, E-28220 Majadahonda, Madrid, Spain

Cancer is the second reason of death worldwide, the leading cause of this fatal outcome being due to the development of metastasis. This process is composed of a large number of complex mechanisms involving tumoral hypoxia and changes in cell-cell adhesion. In the tumour microenvironment, cells adapt to low oxygen levels (hypoxic conditions) by overexpressing certain proteins such as hypoxia inducible factor 1α (HIF-1α) and programmed dead ligand 1 (PD-L1). In addition, some proteases such as trypsin are also associated with cancer progression by activating matrix metalloproteinases, which are involved in the breakdown of cell-binding proteins. Therefore, the accurate determination of the endogenous levels of these three target biomolecules, found to be elevated in prevalent aggressive cancers, would allow early identification of metastatic processes and improve the therapy efficiency and cancer outcomes.

With this purpose, competitive electrochemical affinity biosensors have been developed for their accurate, simple and rapid determination [1,2]. All these bioplatforms are based on the use of magnetic particles as a solid microsupports for the implementation of the bioassay format (direct type and based on the use of a short peptide for the protease or sandwich type and based on specific antibodies for the hypoxia biomarkers) and amperometric transduction at screen-printed carbon electrodes (Figure 1). Once key experimental variables were optimized, the analytical characteristics of the developed biosensors were established for the amperometric determination of protein standards. These bioplatforms demonstrated a good analytical and operational performance, allowing the sensitive (LODs values lower than the cut-off levels established in serum of cancer patients) and selective determination of the target biomarkers to be completed within three hours. Moreover, their potential to perform the determination in cell lysates and serum samples from advanced colorectal cancer affected subjects was also demonstrated using simple protocols and reduced test time and sample quantity, which make them particularly attractive for their use in hospitals or outpatient oncology routine analysis.
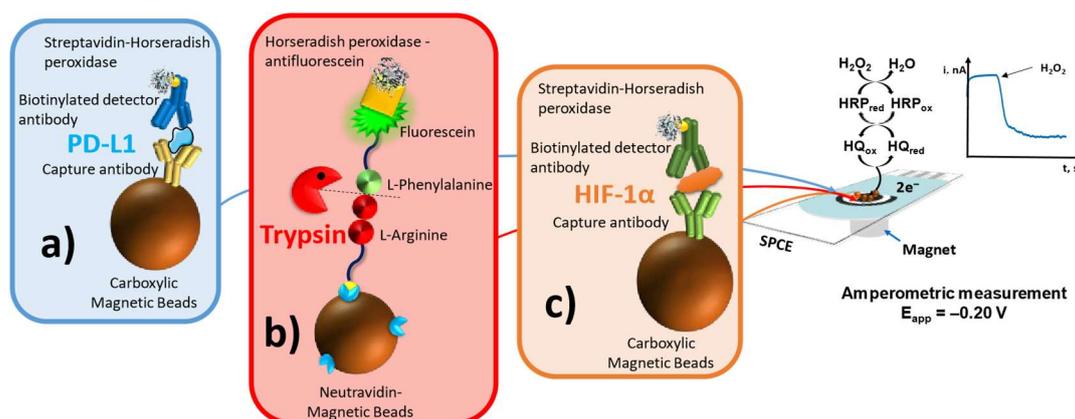

Figure 1: Schematic representation of the developed bioplatforms for the determination of PD-L1 (a), trypsin (b) and HIF-1α (c).

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