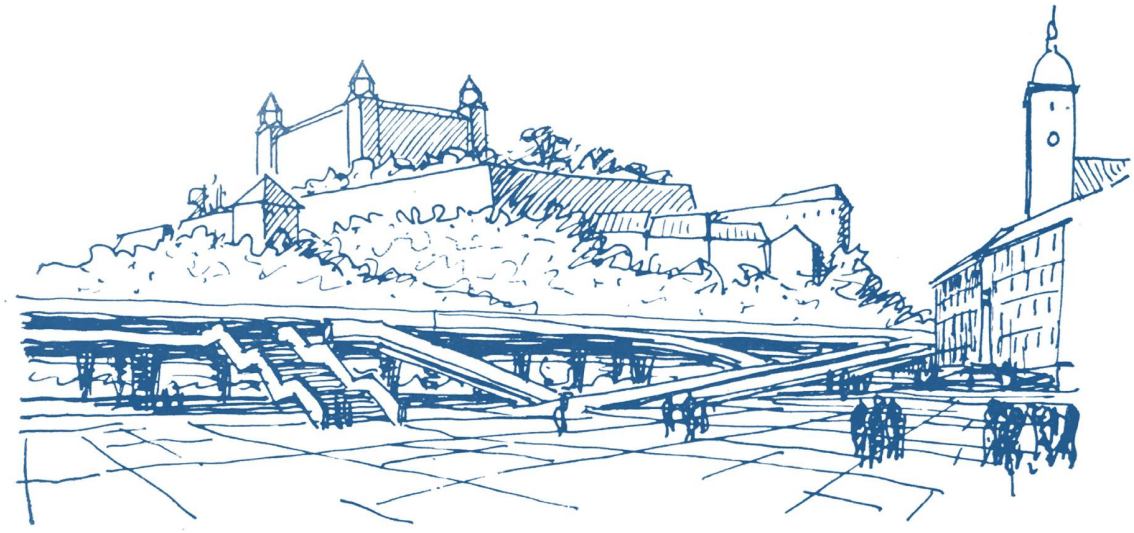
Maria Sklodowska Curie Actions - Research and Innovation
Staff Exchange

H2020-MSCA-RISE-2015 - FORMILK

International Workshop

Advances in analytical methods for monitoring food quality and safety

Program and Abstracts



Bratislava, November 10, 2019

Picture at the cover page: View on the Bratislava castle and St. Martin Cathedral.
Author: prof. Emanuel Hruška

International workshop

Advances in analytical methods for monitoring food quality and safety

Bratislava, Slovakia
November 10, 2019

Program and Abstracts

Organized by Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava
http://www.fmph.uniba.sk

in framework of

Marie Sklodowska-Curie Actions (MSCA)
Research and Innovation Staff Exchange (RISE)
H2020-MSCA-RISE-2015
FORMILK, Project No. 690898
http://www.formilk.fmph.uniba.sk

The goal of the workshop is to provide a venue for advancing characterization of the proteases activity in milk using various physical and analytical chemistry methods as well as on the novel analytical approaches for monitoring food quality and safety through series of lectures and discussions. The short oral contributions will present overview of the achievements obtained during the work on the FORMILK project. The workshop brings together experts in food analysis, fundamental and applied aspects of acoustic, optical and electrochemical methods. The results of surface nanofabrication and molecular modeling of the casein micelles will also be presented. Workshop is organized in framework of the project FORMILK funded by European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 690898.

ORGANIZING COMMITTEE

Tibor Hianik - chairman
Maria Hianiková
Veronika Šubjaková
Zuzana Garaiová
Lenka Bábelová

PROGRAM

09:00-10:00 **Registration**

10:00-10:05 **Welcome and Introduction (Tibor Hianik)**

10:05-10:50 **Paulo J. Oliveira,** University of Coimbra, Portugal: Over- and under-nutrition during pregnancy as a remodeling factor for liver and heart fetal mitochondria

10:50-11:15 **Coffee break**

11:15-12:00 **Michael Thompson,** University of Toronto, Canada: Prevention of bacterial adhesion on substrates with relevance to the food production industry

12:00-12:45 **Ilia N. Ivanov,** Oak Ridge National laboratories, USA: Advancing characterization of materials and interfaces with multi-modal, multi-scale approaches, and AI-centered analytics

13:00-14:30 **Lunch**

14:30-14:45 **Robert Kocsis,** Hungarian Dairy Research Institute, Mosonmagyarovar, Hungary: The role of H2020 project: Investigating of non-compliant dairy products based on industrial needs

14:45-15:00 **John Byrne,** Crosscare Ltd, Dublin, Ireland: Research means business!

15:00-15:15 **Mark Dizon,** University College Dublin, Ireland: Real-time monitoring of enzymatic hydrolysis of milk proteins by High-Resolution Ultrasonic Spectroscopy

15:15-15:30 **Sopio Melikishvili,** Comenius University in Bratislava, Slovakia: Application of high-resolution ultrasonic spectroscopy for real-time monitoring of trypsin activity in β-casein solution

15:30-15:45 **Marcela Morvova,** Comenius University in Bratislava, Slovakia: Determination of lactase activity by absorption spectroscopy

15:45-16:00 **Attila Hucker,** Hungarian Dairy Research Institute, Mosonmagyarovar, Hungary: Determination of plasmin in milk using ELISA and QCM methods

16:00-16:15 **Marek Tatarko,** Comenius University in Bratislava: Application of acoustic methods to study proteolytic degradation of caseins by plasmin

16:15-16:30 **Zoltán Varga**, Research Centre for Natural Sciences, Budapest, Hungary: Plasmin concentration measurement on interfacial beta-casein layers by means of tensiometry and EMPAS

16:30-17:00 **Coffee break**

17:00-17:15 **Adam Vass**, Research Centre for Natural Sciences, Budapest, Hungary: Effect of surface roughness on the availability of surface confined enzyme substrates - implications for sensor responses

17:15-17:30 **Tamás Szabó**, Research Centre for Natural Sciences, Budapest, Hungary: Biochemically directed metallic cluster formation and its application in enzymatic cleavage detection

17:30-17:45 **Martin Donoval**, Powertec Ltd, Bratislava, Slovakia: Development of portable potentiostat for sensing applications

17:45-18:00 **Miroslav Novota**, Powertec Ltd, Bratislava, Slovakia: Development of potentiostat for specific applications: expectations, requirements, and threats

18:00-18:15 **Michal Hanic**, Powertec Ltd, Bratislava, Slovakia: Participation of seconded researchers on EMPAS development

18:15-18:30 **Stefanos Karapetis**, National Technical University of Athens, Greece: Development of electrochemical biosensor for detection aflatoxin M1 in a milk

18:30-18:45 **Lida Bousiakou**, IMD Laboratories, Athens, Greece: TiO_2-based electrodes for enzymatic sensing: a case for glucose oxidase

18:45 **Concluding remarks**

19:00-21:00 **Dinner**

Abstracts of Plenary Lectures

Over- and under-nutrition during pregnancy as a remodeling factor for liver and heart fetal mitochondria

Paulo J. Oliveira

CNC – Center for Neuroscience and Cell Biology, UC-Biotech, University of Coimbra, Cantanhede, Portugal

Programming can be defined as responses to challenges in critical fetal time windows that alter life course phenotype in multiple physiological systems. Maternal obesity (MO) in pregnancy is increasing as an epidemic worldwide. MO increases offspring risk of heart and metabolic disease (e.g. diabetes), shortening offspring life. Mitochondrial bioenergetics play a crucial role in the function of all cell types, with respirasomes being critical in the process of energy production by oxidative phosphorylation. By using ewes as a model of MO, we demonstrated differences in fetal liver respirasome activity, especially in the left fetal liver in the MO group, as well as alterations in redox regulation. The results suggest hepatic fetal mitochondrial remodeling induced by MO that may significantly impact the offspring liver mitochondrial capacity in a lobe-dependent manner, potentially predisposing to metabolic diseases in adulthood. We will also show nutrient maternal restriction (NMR) also causes disruption of mitochondrial complex activity, as well as alterations of transcripts and proteins related with oxidative phosphorylation and mitochondrial fission and fusion. We will show here that a delicate balance is needed in terms of maternal nutrition during pregnancy in order to avoid loss of mitochondrial capacity and transmission of an energy deficient state to adulthood. We will present evidence in a rodent model that exercise during pregnancy reduces some of the effects caused by a high-fat, high-sugar diet.

Acknowledgments
These projects received funding from the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 722619 (FOIE GRAS) and Grant Agreement No. 734719 (mtFOIE GRAS). Also funded by FEDER funds through the Operational Programme Competitiveness Factors - COMPETE and national funds by FCT - Foundation for Science and Technology (grants PTDC/DTP-DES/1082/2014, POCI-01-0145-FEDER-016657; UID/NEU/04539/2019) and by NIH (R01HD070096-01A1).

Prevention of bacterial adhesion on substrates with relevance to the food production industry

Michael Thompson

Department of Chemistry and Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada

The adherence of various bacteria to metal containers and transfer systems employed in food and milk processing can result in serious contamination of the product involved. These are the principal reasons why a great deal of research has been conducted into antimicrobial surfaces over many years. Modification of surface topography, a plethora of coatings, surface covalent alteration and use of nanoparticles are among a number strategies that have been employed in attempts to effect resistance of bacterial adhesion. In own relevant work, we have demonstrated the successful prevention of protein adsorption on various polymers and stainless steel via the surface modification of these materials by covalently – attached monolayeric species. The surface modifier is a 0.5 nm long trichlorosilane containing a PEG backbone. (PEG has been the subject of intense study for many years, although its precise role in enhancing biocompatibility has remained elusive.) The structure of water intercalated in the adlayer appears to be crucial and has been studied in our lab by a number of surface analysis techniques including neutron reflectometry and MD calculation.

The aforementioned surface chemistry has now been employed in experiments involving exposure of bare and surface modified steel, Iridium (used for sensor technology), polyvinyl chloride, polyurethane and polyethylene to bacteria. These materials are used widely in food production and for the fabrication of catheters, conduits and other devices in contact with biological fluids. We have worked primarily with samples containing relatively high concentrations of pseudomonas and staphylococcus aureus both in static and dynamic experiments. The results of these experiments involving extensive microscopy show a dramatic reduction in bacterial adhesion caused by the ultra-thin covalently-attached monolayer. Moreover, the surface-modified polymers can be subjected to standard sterilization protocols without suffering damage.

Advancing characterization of materials and interfaces with multi-modal, multi-scale approaches, and AI-centered analytics

Ilia N. Ivanov[1], Ivan Sukuba[2], Eric S. Muckley[1], Monojoy Goswami[1], and Tibor Hianik[2]

[1]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory P.O. Box 2008, Oak Ridge, TN 37831-6496, USA
[2]Department of Nuclear Physics and Biophysics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia

Biological processes are thought of as the most complex, yet energy efficient phenomena, known to man. Learning this complexity presents a challenge due to a broad range of chemical, physical and catalytic phenomena and also multivariable dependency of these processes. Coupling of molecular dynamic simulation with the experiments opens an opportunity for better understanding of complex, multivariable phenomena on continuous scale and lead to the development of novel analytical tools and bio-inspired transformation technology.

In this presentation we will review our approach and current research activity on correlation of experiment and modeling of protein aggregation in solutions and at the interfaces as a function of pH, ionic strength and temperature. We will discuss advantages of synchronous and asynchronous correlation approaches for mixed-modal analytical techniques.

We will analyze a path forward for fundamental research and development effort which could have a direct impact on applied science, enabling intelligent analytical instruments and low-cost hand devices for control of food safety.

Acknowledgments
This research was conducted at the Center for Nanophase Materials Sciences, which is a US Department of Energy Office of Science User Facility. IS,TH acknowledge funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 690898.

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Abstracts of Short Oral Presentations

The role of the H2020 project: Investigating of non-compliant dairy products based on industrial needs

Attila Hucker[1], Katalin Szabó[1], Judit Süle[1], Zsófia Keresztes[2], Barbara Csupor[2], Róbert Kocsis[1]

[1]Hungarian Dairy Research Institute Ltd, 24 Lucsony Street, 9200 Mosonmagyaróvár, Hungary
[2]Research Centre for Natural Sciences, Magyar tudósok körútja 2, 1117 Budapest, Hungary

The aim of this study was to clarify the causing agents for exceptionable quality properties of four commercially available UHT-milks supplied by two different dairy companies (Hungarian and Slovakian). Bitterness of two samples was detected by sensory evaluation (the other two samples were used as control). In order to eliminate this quality problem microbiological-, physical-chemical- and later enzymatic investigations were carried out. Results showed that neither the microbiological nor the physical-chemical parameters were responsible for the quality deficiency. In order to prove the role of plasmin enzyme further investigations were needed. Therefore, plasmin was exactly determined quantitatively by spectrophotometric method. Change of absorbance was measured at 405 nm wavelength of a series of Spectrozyme.

The results confirmed that it has been a difference between control and non-compliant UHT-milk samples in the quantitative and qualitative characteristics of plasminogen enzyme.

Acknowledgments: This work was financially supported by European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 690898.

Research means business!

John Byrne

Crosscare Ltd., Dublin, Ireland

This presentation contains a short introduction to Crosscare Limited and an overview of the inward and outward secondment units undertaken by Crosscare Limited (Cross). The general experience of FORMILK workshops and conferences and some personal work experiences where research meets business are also considered.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015.

Real-time monitoring of enzymatic hydrolysis of milk proteins by High-Resolution Ultrasonic Spectroscopy

Vitaly Buckin and Mark Dizon

School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland

Milk proteins are rich source of functional protein hydrolysates which are widely exploited by the food industry. The most common way to manufacture these peptides is by enzymatic hydrolysis [1]. Their functional properties depend on the extent and fine control to which the proteins are hydrolysed [2]. Control of the enzymatic hydrolysis requires efficient and non-destructive analytical methods for real-time monitoring the hydrolytic process in native media under processing conditions, which is a difficult task for majority of existing bioanalytical techniques due to the structural complexity of the media, opaqueness, and other factors.

Recently, we have demonstrated that High-Resolution Ultrasonic Spectroscopy (HR-US) [3] can be applied for precision real-time measurements of concentration of peptide bonds hydrolysed by enzymes in solutions and complex systems [3, 4]. This poster demonstrates the application of HR-US technique for real-time monitoring of enzymatic hydrolysis of two major milk proteins, ''rheomorphic'' bovine β-casein and globular β-lactoglobulin, by serine proteases under various reaction conditions. The ultrasonic profiles of hydrolysis were compared with complementary techniques such as 2,4,6-trinitrobenzenesulfonic acid (TNBS) assay, pH method, and reversed-phased high-performance liquid chromatography (RP-HPLC) technique. As a result, ultrasonic time profiles were translated into the concentration of peptide bonds hydrolysed. These were used to characterize the activity of enzymes towards milk proteins, and to generate advanced hydrolytic profiles such as real-time evaluation of the average degree of polymerization and molar mass of milk protein hydrolysates during the hydrolysis,

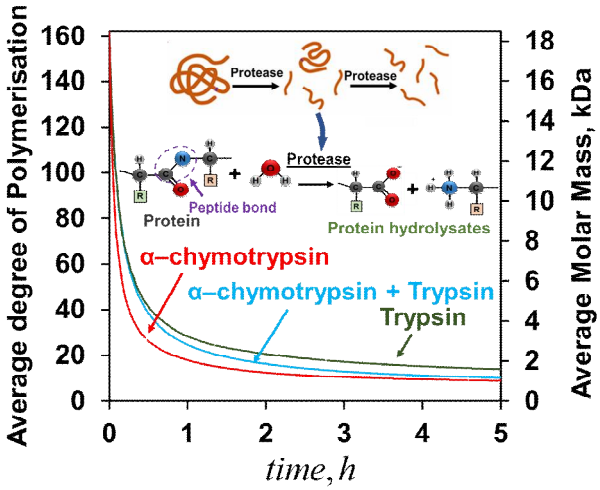

Fig. 1: Ultrasonic monitoring of hydrolysis of β–lactoglobulin (0.547 mmol/kg) by different enzymes (4 μmol/kg) in 0.1 M phosphate buffer pH 7.8 at 25 °C

Acknowledgements

This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015.

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Application of high-resolution ultrasonic spectroscopy for real-time monitoring of trypsin activity in β-casein solution.

Sopio Melikishvili[1], Mark Dizon[2], Vitaly Buckin[2], and Tibor Hianik[1]

[1]Department of Nuclear Physics and Biophysics, Comenius University, Bratislava, Slovakia
[2]School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland

Proteolysis, the enzymatic hydrolysis of a protein, plays an important role in various fields of bioscience and biotechnology. Technologically, there are broad applications of proteolysis in food processing [1]. For instance, the proteolytic activities in milk affect the texture and flavor of dairy products [2]. Proteolytic activities have also been linked with the release of Caseinophosphopeptides (CPPs) the phosphorylated bioactive peptides from milk protein casein which can be used as supplements for fortifying foods, with a view to improving mineral bioavailability [3]. Therefore detection and quantification of protein activity in milk has important industrial impacts.

We have applied high-resolution ultrasonic spectroscopy (HR-US) for real-time non-destructive monitoring of trypsinolysis of β-casein at various conditions (concentration of enzyme, temperature, pH). The addition of enzyme into the β-casein solution was accompanied by the increase of ultrasonic velocity in the reaction mixture caused by cleavage of peptide bonds. 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) assay was employed in order to convert a change of ultrasonic velocity in the reaction mixture caused by hydrolysis of β-casein to the change in concentration of covalent bonds hydrolyzed. It was demonstrated, that temperature has the significant effect on the performance of enzyme, showing optimal activity at 45°C and negligible activity at 15°C. The dependence of enzyme activity on pH was investigated in β-casein solution. The highest activity of trypsin has been observed at pH 7.4. In consideration of the obtained results we believe that HR-US constitutes a useful tool for nondestructive real-time assessment of the enzyme activity in complex formulations.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015.

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Determination of lactase activity by absorption spectroscopy

John Byrne,[1] and Marcela Morvová Jr.[2]

[1]Crosscare Ltd., Dublin, Ireland
[2]Department of Nuclear Physics and Biophysics, Comenius University, Bratislava, Slovakia

Lactase is essential to the complete digestion of whole milk, because it hydrolyses lactose into glucose and galactose. Lacking innate lactase, a person consuming dairy products may experience the symptoms of lactose intolerance [1]. This is also true for infants experiencing transient lactase deficiency. In infants it is especially critical as babies rely on breast milk or formula feeds for their complete nutritional requirements and lactose is present in breast milk and the majority of formula feeds. Therefore, the appropriate application of lactase enzymes allows infants to avail of this valuable natural carbohydrate as a nutritional source and allow adults and children to enjoy dairy based foods.

The ONPG Lactase (neutral) Activity Assay is based on a time dependent hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol (ONP) and galactose at constant pH and temperature. The purpose of this study was to compare results obtained by two different methods for determination of lactase (neutral) activity. Firstly the 1-minute hydrolysis at pH 6.5 and 37°C was performed and ONPG unit was calculated. The second procedure for lactase (neutral) activity is based on a 10 minutes hydrolysis of an o-nitrophenyl-β-D-galactopyranoside (ONPG) substrate at 30.0°C ± 0.1°C and at pH 6.50 [2]. Two ONPG substrates were also subjected to this method to see if any variation in the results could be observed.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015.

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Determination of plasmin in milk using QCM and ELISA methods

Alexandra Poturnayova[1,2], Katalin Szabo[3], Attila Hucker[3], Tibor Hianik[1]

[1]Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia
[2]Institute of Animal Biochemistry and Genetics, Center of Biosciences SAS, Dúbravská cesta 9, 840 05 Bratislava, Slovakia
[3]Hungarian Dairy Research Institute, 24 Lucsony Street, 9200 Mosonmagyarovar, Hungary

We report a quartz crystal microbalance (QCM) biosensor for detection of the serine protease plasmin (PLA), in different milk samples. The changes in a frequency of piezoelectric transducer were monitored in a PLA concentration range of 0.1-40 nM. The sensor was validated in raw milk as well as in the spiked milk samples. The universality of the sensor response has been verified also by other proteases such as trypsin or chymotrypsin. Sensor achieved a limit of PLA detection (LOD) 167.16 pM at pH 7.4 and 173.84 at pH 8.9. Similar LOD 121.98 pM was confirmed by ELISA test applied for the same samples. Atomic force microscopy (AFM) confirmed cleavage effect of PLA on the surface covered by β-casein.

Acknowledgments
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Application of acoustic methods to study proteolytic degradation of caseins by plasmin

Marek Tatarko[1], Eric Muckley[2], Ilia N. Ivanov[2], Michael Thompson[3], Loránd Románszki[4], Katalin Szabo[5], Atilla Hucker[5], Tibor Hianik[1]

[1]Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
[2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, USA
[3]Department of Chemistry, University of Toronto, Lash Miller Laboratories, Canada
[4]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Budapest, Hungary
[5]Hungarian Dairy Research Institute, Mosonmagyaróvár, Hungary

Our work is focused on the development and validation of the acoustic biosensor based on the thickness shear mode principle (TSM) able to detect proteolytic activity of the proteases on covalently and non-covalently bound casein layers. We applied TSM principle for monitoring of higher harmonic frequencies of the quartz crystal that also allowed us to study the adsorption processes of various non-covalently bound caseins types. While α-casein layer was removed from the surface after washing, β-casein and κ-casein produced stable layers. We also tested the pH stability of the adsorbed layers. Change of the pH into more alkaline value caused serious stripping of the β-casein layer, while acidic pH did not cause significant layer damage. Multi-frequency TSM was able to monitor protease activity at subnanomolar concentrations and allowing distinguishing between trypsin and plasmin. Cleavage of the κ-casein layer by these proteases caused similar kinetic changes in acoustics characteristics, corresponding to the one cleavage site available on the molecule. We were also concentrated on the detection of regulatory effects of plasmin protease system such as inhibition or autolysis using traditional TSM method. We have shown, that the frequency changes resulted from casein cleavage by plasmin were equivalent to the signal decrease caused by plasmin inhibitors. Monitoring of the plasmin activity on hour and daily basis revealed existence of the autolysis that caused decrease of the active plasmin in freshly prepared sample. We repeated the inhibition measurement by using the plasmin isolated from spoiled milk. After plasmin isolation by centrifugation and filtration, sample was used for measurements by SARK device, that allows multifrequency TSM measurements and it also monitors layer energy dissipation. Inhibited and uninhibited samples were applied on β-casein layer and we observed difference in frequency response. These measurements have presented high potential of acoustic biosensors as valuable tools for protease detection.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015.

Plasmin concentration measurement on interfacial beta-casein layers by means of tensiometry and EMPAS

Loránd Románszki[1], Zoltán Varga[1], Judith Mihály[1], Zsófia Keresztes[1]
Marek Tatarko[2], Tibor Hianik[2], Mengchi Jiao[3,4], and Michael Thompson[3]

[1]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Budapest, Hungary.
[2] Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia
[3]Department of Chemistry, University of Toronto, Canada.
[4]Nanjing University, People's Republic of China

We have proposed two different approaches for active plasmin determination as alternatives for the existing methods. In both arrangements, monolayers of β-casein adsorbed either to air/water, or to water/quartz interface were used as substrate for the determination of active plasmin.

In the first configuration, a conventional surface pressure meter - Langmuir Trough - was used. After the substrate was spread to the interface, plasmin was injected into the subphase and decay of the surface pressure caused by the digestion and disappearance of the substrate from the interface was followed. The maximum surface pressure decay rates were plotted against the plasmin concentration and calibration lines with slopes depending on the substrate concentration were obtained, with a limit of detection (LoD) in the 10 nM range.

In the second configuration, the substrate was adsorbed to the quartz resonator disc of an innovative device, the electromagnetic piezoelectric acoustic sensor (EMPAS) [1], operated at ~ 1 GHz. The frequency increase of the resonator as a result of the digestion and washout of the bound substrate by the enzyme solutions were recorded. By plotting the maximum rates of frequency shift against the plasmin concentration, a calibration curve was obtained with a LoD in the 10 pM range [2].

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015, the National Competitiveness and Excellence Program Hungary (NVKP_16-1-2016-0007), and the BIONANO_GINOP-2.3.2-15-2016-00017 project.

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Effect of surface roughness on the availability of surface confined enzyme substrates - implications for sensor responses

Ádám Vass[1], István Bakos[1], Zsófia Keresztes[1], Eric S. Muckley[2] and Ilia N. Ivanov[2]

[1]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Budapest, Hungary
[2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, USA

Formation of nanostructured gold layer on bulk gold electrode surface was elaborated by galvanostatic deposition at RCNS. The electrochemically active surface of the modified electrodes was characterized with cyclic voltammetry by defining the charge belonging to the oxygen reduction on the gold surface.

Surface confined, electrochemical reporter conjugated enzyme substrate was adsorbed on electrodes and the relationship between the electrode surface and the adsorbed substrate amount was determined by taking into account the oxidation peak related charging of the electrochemical reporter on the substrate. Substrate cleavage rate was also measured in order to define the effect of adsorbed substrate amount on the initial rate of enzymatic reaction.

In order to scale-up the nanostructured surface preparation procedure, a 3D printed cell was designed at CNMS to have a versatile device for electrochemical modification and subsequent examination of gold thin films deposited on silicon, quartz and glass substrates prepared by electron-beam physical vapor deposition technique. Electrochemical modification was carried out by using different parameters: different current densities, different sulfuric acid concentrations with different gold content, and different deposition times. In addition to electrochemical characterization, the modified gold surfaces were examined by microscopic (SEM, AFM) techniques as well.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015. and the BIONANO_GINOP-2.3.2-15-2016-00017 project. A part of this research was conducted at the Center for Nanophase Materials Sciences, which is a DOE Office of Science User Facility.

Biochemically directed metallic cluster formation and its application in enzymatic cleavage detection

Tamás Szabó[1], Ilona Felhősi[1], Barbara Csupor[1], Mária Pávai[1], Zsófia Keresztes[1], Mark Dizon[2], Breda O'Driscoll[3], Vitaly Buckin[2], Itthipon Jeerapan [4] and Joseph Wang[4]

[1]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungary
[2] School of Chemistry, University College Dublin,Dublin 4, Ireland
[3]Sonas Technologies Ltd., Ireland
[4] Department of NanoEngineering, University of California, San Diego, USA

Metallic nanocluster formation can be achieved with protein mediated process, when specific sequence of amino acids can serve with reducing and stabilizing functionality. Metallic nanoclusters have been prepared subnano to 50 nm size as conjugates bearing enzyme cleavage specific structures. The nanoparticle size distribution has been verified with transmission electron microscopy and the size dependent photoluminescent and UV-Vis absorbance characteristics have been evaluated with spectroscopic methods.

High resolution ultrasonic spectroscopy (HR-US) has been applied to follow the effect of nanocluster formation on hydrolytic cleavage rate in different protein environment.

As a supporting analytical method for detection of enzyme activity resulting in hydrolytic fragmentization of proteins, various electrochemical methods were tested. These methods rely on the detectability of noble metal ions or nanoparticles through their reduction and/or oxidation *via* certain current-potential functions like cyclic voltammetry and its more sophisticated variants: differential pulse voltammetry, square wave voltammetry or combined techniques like anodic stripping voltammetry. The application of metallic species in conjugation with the enzyme substrates provides a well-detectable electrochemical signal. In the course of enzymatic hydrolysis, the labelled substrate fragments detach from the original protein/peptide structure, which allows to record electrochemical feedback of the removed or the remaining probes, respectively.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015 and the BIONANO_GINOP-2.3.2-15-2016-00017 project.

Development of portable potentiostat for sensing applications

Martin Donoval, Martin Weis, Ľuboš Černaj, Ján Šubjak

Powertec, Ltd., Drotárska 19a, 811 04, Bratislava, Slovakia

Powertec has developed the second generation of a miniature portable potentiostat, suitable for reliable in-field measurements. The device properties have been designed in accordance with requirements of the experiment conditions defined by project partners and as well include improvements over the first prototype of the device, such as sensitivity, limit-of-detection, etc. The second generation of a miniature potentiostat was designed using the programmable system on chip (SoC) from Cypress semiconductor, combining high-precision and programmable analogue and digital peripherals with ARM Cortex M3 processor in a single chip. Since the potentiostat setup is done through the USB port, the device is during the experiment controlled by a single button only what enables make it smaller and handheld. Current on working electrode (WE) is changed on voltage by the transimpedance amplifier and measured by 20-bit Delta-Sigma ADC with 2000 samples per second (SPS). The cyclic voltammetry of ferrocene-modified peptide self-assembled monolayer was done to prove the device suitability and sensitivity. The redox voltammogram was compared with commercially available desktop setup (electrochemical workstation Im6eX, Zahner) and identical results have been obtained.

Acknowledgments
This work was supported by the European Commission within the project FORMILK under grant agreement number 690898/H2020-MSCA-RISE-2015.

Development of potentiostat for specific applications: expectations, requirements, and threats

Miroslav Novota, Michal Mičjan, Martin Weis, Martin Donoval

Powertec, Ltd., Drotárska 19a, 811 04, Bratislava, Slovakia

In recent years, there are few new studies based on plasmin detection in milk. Several techniques of measuring plasmin activity exist [1-3], but all of them require to use rather large and expensive equipment, not suitable for quick in field measurements. The ambition, however, is to provide the possibility to use these methods of measurements to ordinary farms simply, quickly, cheaply and at any time. The miniature potentiostat was designed using programmable SoC from Cypress semiconductor, combining high-precision analog and digital peripherals with ARM Cortex M3 processor, what enables make it smaller and handheld. The potentiostat performance and measurement reliability in comparison with a common desktop potentiostat was tested by a cyclic voltammetry study on ferrocyanide/ferricyanide redox couple in PBS solution with gold working electrode and Ag/AgCl reference electrode. We identified a potential risk in low sensitivity of cyclic voltammetry measurement. If the plasmin detection doesn't reach required level, the differential pulse voltammetry (DPV) could be used for measurements to achieve higher sensitivity. In addition, the low pass FIR filter can be optimized and/or DAC can be modified to 100 kSPS to improve resolution. Utilizing 3D printing technology new potentiostat enclosure and socket for screen-printed electrodes have been prepared.

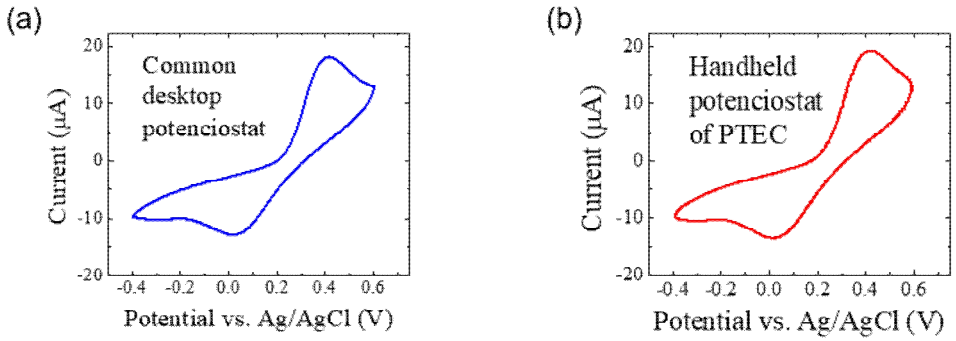

Fig. 1: Cyclic voltammetry study of 10mM ferrocyanide/ferricyanide redox couple in PBS at a scan rate of 100 mV/s recorded by (a) common desktop potentiostat (FRA2 µAUTOLAB type III) and (b) miniature handheld potentiostat designed by PTEC.

Acknowledgments

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Participation of seconded researchers on EMPAS development

Michal Hanic, Tomáš Debnár, Martin Weis, Martin Donoval

Powertec, Ltd., Drotárska 19a, 811 04, Bratislava, Slovakia.

Main research activity was focused to analyze the Electromagnetic Piezoelectric Acoustic Sensor (EMPAS) system, from a constructional point of view during real time measurements. EMPAS is used to detect the content of analyte such as enzymes, proteins and other substances in food industry and medicine, for example diagnosis of various types of cancer. It's an impedance measurement method in the Ultra High Frequency (UHF) range with a resonant frequency up to 1GHz and a modulation frequency in the range of tens of kHz. The motivation is to compare EMPAS with a commercial system such as ELISA, focusing on comparing the sensitivity, repeatability, and cost of measurements with respect to commercial applications. In cooperation with Department of chemistry at the University of Toronto during the real-time measurements were identified some issues. It was necessary to identify the problematic parts of the system and make recommendations how to improve their efficiency. The current version of the system consists of separate laboratory measuring devices such as RF generator up to 2 GHz, lock-in amplifier, narrow-band selective UHF amplifier, adjustable gain amplifier, power source, RLC crystal (quartz) resonant circuit and PC with GPIB card.

If the EMPAS to operate under real conditions, the measurement is accompanied by different factors, that cause signal interference and have a negative effect on the measurement results. The factors causing the interference were electrical and mechanical. Poor electrical contacts, cold connections due to mechanical stress, unshielded conductors of wrong lengths caused undesirable interference. Mechanical failures were caused by a poor downforce setting, which caused a change in the defined coupling between the crystal and the resonant circuit coil.

The basic improvements include correct modification of electrical connections in places of high mechanical stress, the coaxial section of UHF should have the correct length with respect to λ/2 as well as the capacity, which have a significant impact on the quality of the transmitted signal. The power supply wires from the selective UHF amplifier need to have a suitable length with shielding, to avoid the making of an antenna, which would be a source of interference. From a mechanical point of view, it is necessary to ensure that the pressure on the crystal is precisely defined.

In consideration of the identified issues, the best option would be to integrate the existing system into a small, compact, one-device platform. This approach would eliminate the effect of electrical interference on the output signal, but also the negative mechanical factors. It is also important to note that, the measurement in the current state, it's not very convenient, that there is movement around the UHF part, which would result in the formation of disturbing artifacts in the final signal and the final measurement would be distorted.

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Development of electrochemical biosensor for detection aflatoxin M1 in a milk

Stefanos Karapetis[1,2] **, Dimitrios P. Nikolelis**[3] **and Tibor Hianik**[1]

[1]*Department of Nuclear Physics and Biophysics, Comenius University, Bratislava, Slovakia*
[2]*Laboratory of Inorganic & Analytical Chemistry, School of Chemical Engineering, Department of Chemical Sciences, National Technical University of Athens, Greece*
[3]*Laboratory of Environmental Chemistry, Department of Chemistry, University of Athens, Greece*

We performed a comparative analysis of the sensitivity of aptamer-based biosensors for detection mycotoxin aflatoxin M1 (AFM1) depending on the method of immobilization of DNA aptamers and method of the detection. Label-free electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) for ferrocene labeled neutravidin layers were used for this purpose. Amino-modified DNA aptamers have been immobilized at the surface of polyamidoamine dendrimers (PAMAM) of fourth generation (G4) or biotin-modified aptamers were immobilized at the neutravidin layer chemisorbed at gold surface. In the first case the limit of detection (LOD) has been determined as 8.47 ng/L. In the second approach the LOD was similar 8.62 ng/L, which is below of allowable limits of AFM1 in milk and milk products. The aptasensors were validated in a spiked milk samples with good recovery better than 78%. Comparative analysis of the sensitivity of immuno- and aptasensors was also performed and showed comparable sensitivity.

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Index of Authors

Edited by

Prof. Tibor Hianik, Faculty of Mathematics, Physics and Informatics,
Comenius University in Bratislava, Mlynska dolina F1, 842 48 Bratislava, Slovakia

Reviewers

Mgr. Zuzana Garaiová, PhD., Faculty of Mathematics, Physics and Informatics,
Comenius University in Bratislava, Mlynska dolina F1, 842 48 Bratislava, Slovakia

RNDr. Peter Rybár, PhD., Faculty of Mathematics, Physics and Informatics,
Comenius University in Bratislava, Mlynska dolina F1, 842 48 Bratislava, Slovakia

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