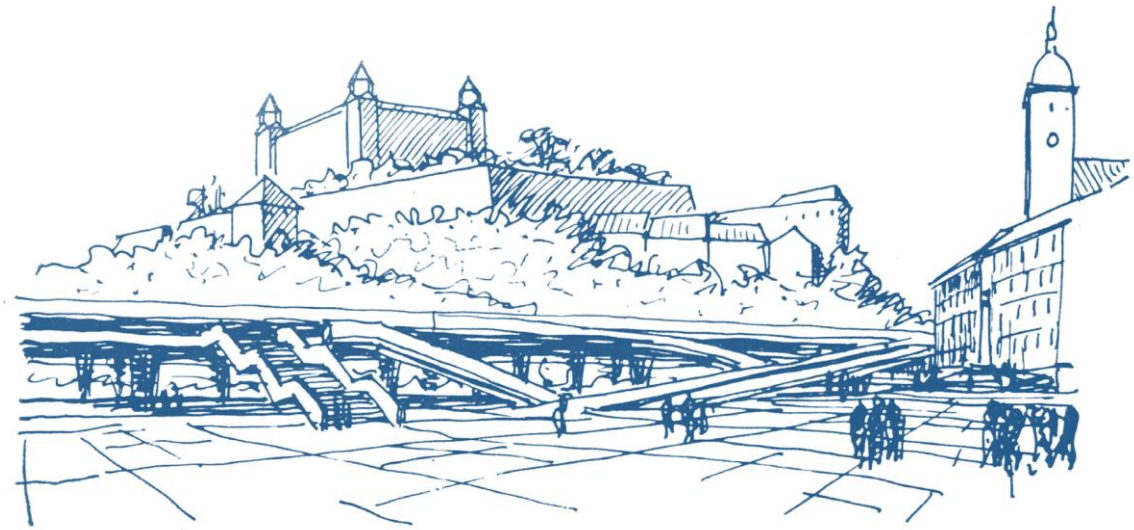
Maria Sklodowska Curie Actions - Research and Innovation
Staff Exchange

H2020-MSCA-RISE-2020 - SAFEMILK

International Workshop

Acoustic methods in the study of affinity interactions at surfaces

Program and Abstracts



Bratislava, November 16-17. 2021

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

International workshop

Acoustic methods in the study of affinity interactions at surfaces

Bratislava, Slovakia
November 16-17. 2021

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-2020
SAFEMILK, grant agreement No. 101007299
https://www.safemilkproject.com

The goal of the workshop is to provide a venue for advancing characterization of the surfaces using progressive acoustic methods. These label-free methods allowing detection of affinity interactions at surfaces with high sensitivity. The plenary lectures provide detailed explanations of the principles of molecular acoustics to study various phenomena. Short oral contributions present overview of the achievements obtained in particularly during the work on the SAFEMILK project. The workshop brings together experts in food analysis, medical diagnostics, fundamental and applied aspects of acoustic, optical and electrochemical methods. Workshop is organized in framework of the project SAFEMILK funded by European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 101007299.

Organizer

Prof. Tibor Hianik
Faculty of Mathematics, Physics and Informatics
Comenius University
Mlynska dolina F1
842 48 Bratislava
Slovakia
E-mail: tibor.hianik@fmph.uniba.sk

PROGRAM

16.11.2021

14:00-14:05 **Welcome and Introduction** (prof. Tibor Hianik)

14:05-14:50 **Prof. Electra Gizeli,** Department of Biology**,** University of Crete, Greece: *Employing acoustic wave biosensors for applications in cancer diagnosis with liquid biopsy*

14:50-15:35 **Prof. Diethelm Johannsmann,** Institute of Physical Chemistry, Clausthal University of Technology, Germany: *The modulation QCM enhances the information derived from QCM experiments, based on electroresponsivity and its kinetics*

15:35-15:50 **Break**

15:50-16:05 **MSc. Christian Leppin,** Institute of Physical Chemistry, Clausthal University of Technology, Germany: *A sensory osmium redox copolymer based on allylamine and vinylimidazole studied with a fast modulation EQCM*

16:05-16:20 **Mgr. Ivan Piovarči,** Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia**:** *Detection of protese activity by colorimetry and acoustics methods*

16:20-17:05 **Prof. Joseph Wang**, Department of Nanoengineering, University California San Diego, USA: *Wearable electrochemical sensors: Toward biochemical lab on the body*

17.11.2021

14:00-14:05 **Welcome and Introduction** (prof. Tibor Hianik)

14:05-14:50 **Prof. Michael Thompson**, Chemistry Department, University of Toronto, Canada: *Acoustic wave appraisal of the non-specific adsorption issue in biosensor technology*

14:50-15:35 **Prof. Gordon Hayward**, University of Guelph, Canada: *Piezo-acoustic sensor electronics*

15:35-15:50 Break

15:50-16:35 **Prof. Ilia N. Ivanov**, Center for Nanophase Materials Sciences,Oak Ridge National Laboratory, USA: *Acoustic and electro-optical probing of environmental response of thin film response*

16:35-16:50 **Dr. Gábor Mészáros**, Institute of Materials and Environmental Chemistry Research Centre for Natural Sciences, Budapest, Hungary: *Improvements in the measuring technique of EMPAS*

16:50-17:05 **Mgr. Sandro Spagnolo,** Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia: *Application of electromagnetic piezoelectric sensors for development of aptasensors with antifouling properties*

17:05-17:20 **Dr. Marek Tatarko,** Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia: *Application of multiharmonic QCM for detection of bacterial pathogens using DNA aptamers as receptors*

17:20 **Conclusion** (prof. Tibor Hianik)

Abstracts of Plenary Lectures

Employing acoustic wave biosensors for applications in cancer diagnosis with liquid biopsy

Nikoletta Naoumi[1,2], Kleita Michaelidou[3], George Papadakis[2,4], Agapi E. Simaiaki[1], Román Fernández[5,6], Maria Calero[6], Antonio Arnau[5,6], Achilleas Tsortos[1], Sofia Aggelaki[3,7], Electra Gizeli[1,2*]

[1] Department of Biology, University of Crete, Vassilika Vouton, Heraklion, 70013, Greece
[2] Institute of Molecular Biology and Biotechnology-FORTH, 100 N. Plastira Str., Heraklion 70013, Greece
[3] Laboratory of Translational Oncology, School of Medicine, University of Crete, Heraklion, Vassilika Vouton, 70013 Crete, Greece
[4] BIOPIX DNA TECHNOLOGY PC, Science and Technology Park of Crete, 100 N. Plastira Str., Heraklion 70013, Greece
[5]Advanced Wave Sensors S. L., Algepser 24, 46988 Paterna, Spain
[6]Centro de Investigación e Innovación en Bioingeniería, Universitat Politècnica de València, 46022 Valencia, Spain
[7]Department of Medical Oncology, University General Hospital of Heraklion, Vassilika Vouton, 71500 Crete, Greece
**Presenting author: gizeli@imbb.forth.gr*

Acoustic wave devices are used as sensitive biosensors for the detection of a wide range of substances and in several areas, e.g., healthcare, environmental monitoring and food/plant-safety. Commonly used acoustic devices are the Quartz Crystal Microbalance (QCM) or surface acoustic wave (SAW) geometry, the latter normally combined with a waveguide layer for enhanced sensitivity (Love wave geometry). SAW devices are also available in a single, dual or array format and can be integrated with microfluidics in a lab-on-chip platform [1].

Here we present a new type of QCM device based on a high fundamental frequency QCM (HFFQCM) array biochip operating at 150 MHz; this device, in combination with a dedicated measuring platform, can be used for the analysis of 24 samples per chip. In this work, the above technology is applied to the detection of cancer mutations through dissipation signal monitoring. Specifically, the biochip array is employed for the screening of the *BRAF* V600E and *KRAS* G12D cancer mutations in spiked-in and clinical samples. Each neutravidin-functionalized sensor surface can capture biotinylated double stranded DNA amplicons produced following enzymatic amplification. An impressive limit of detection of 1 mutant copy in the presence of 10^4 wild type cell free DNAs is demonstrated thanks to the use of liposome-particles as dissipation signal amplifiers. The acoustic assay was validated using tissue and plasma samples obtained from melanoma, colorectal and lung cancer patients. The high sensitivity and technology-readiness level of the methodology, together with the ability for multiple sample analysis (24 array biochip), cost-effectiveness and compatibility with routine work-flow, hold promise for the implementation of the acoustic methodology in clinical oncology-labs ιδανικά σχετικά με φυτά as a tool for tissue and liquid biopsy.

Acknowledgement: This research was supported by the European Union's Horizon2020-FETOPEN-1-2016-2017 under grant agreement No. 737212 (CATCH-U-DNA).

References

[1]. G. Papadakis, et al., Micro-nano-bio acoustic system for the detection of foodborne pathogens in real samples. *Biosens. Bioelectr*. 111 **(**2018) 52-58, https://doi.org/10.1016/j.bios.2018.03.056.
[2] N. Naoumi, et al., Acoustic array biochip combined with allele-specific PCR for multiple cancer mutation analysis in tissue and liquid biopsy. *Biorxiv* (2021). https://www.biorxiv.org/content/10.1101/2021.09.16.460590v1

The modulation QCM enhances the information derived from QCM experiments, based on electroresponsivity and its kinetics

Christian Leppin, Arne Langhoff, Diethelm Johannsmann*

Institute of Physical Chemistry, Clausthal University of Technology,
Arnold-Sommerfeld-Str. 4, 38678 Clausthal-Zellerfeld, Germany
**Presenting author: johannsmann@pc.tu-clausthal.de*

As an instrument to be used for biointeraction analysis (BIA), the QCM is less sensitive than surface plasmon resonance (SPR) spectroscopy and also has inferior baseline stability. The modern QCM's are superior to SPR spectroscopy in information content (determination of softness, in particular). The talk will discuss a further addition to the information content.

The modern QCM's (also called QCM-D for QCM with dissipation monitoring) tend to be slow in data acquisition, typical rates being 10 readings per second. The rate can be increased with comb measurements as implemented in the multi-frequency lockin amplifier (MLA, Intermodulation Products SE, Stockholm). Exciting the resonator with up to 32 frequencies at the same time, the MLA routinely reaches a data acquisition rate slightly below the resonance bandwidth. The MLA does not beat the QCMs fundamental noise limits, though.

As always, precision can be improved with accumulation and averaging [1]. We advertise a special mode of accumulation, which we term the modulation QCM [2-4]. The modulation QCM changes the shift of the frequency (Δf) and the shift of the half bandwidth ($\Delta\Gamma$) by some stimulus. A convenient stimulus is electric potential of the front electrode. Technically speaking, this device is an electrochemical QCM (EQCM), but the processes at the resonator surface may or may not involve charge transfer and redox reactions. The measurement of frequency and bandwidth is synchronized with the periodic stimulus. If the process under study is repetitive, one may accumulate and average without losing the time resolution, which is in the millisecond range. The QCM's drift is largely avoided because the modulation interval is short and the average of Δf over the modulation period is subtracted from $\Delta f(t)$. Accumulation overnight brings the rms noise down into the mHz range and even below (depending on the number of cycles entering the averaging process). This noise level rivals the noise of SPR spectroscopy (but is only possible in the modulation format).

Operated this way, the QCM reports electroresponsivity and its kinetics. We find just about all samples to display some electroresponsivity. The challenge is in the interpretation, the measurement is easy. The talk discusses examples (amino acids, bovine serum albumin). Both types of samples display two separate responses ("fast" and "slow"), but the underlying physics presumably differs between the two cases.

References

[1] S. Bourkane, C. Gabrielli, M. Keddam, *Electrochimica Acta* 34 (1989) 1081-1092.
[2] C. Leppin, A. Peschel, F.S. Meyer, A. Langhoff, D. Johannsmann, *Analyst* 146 (2021) 2160-2171.
[3] C. Leppin, A. Langhoff, H.-F. Poggemann, A.S. Gödde, D. Johannsmann, *Analyst* 146 (2021) 6005-6013.
[4] A. Gödde, C. Leppin, F.S. Meyer, A. Langhoff; J. Hartl, P. Garidel, D. Johannsmann, *Biointerphases* 15 (2020) 021004.

Wearable electrochemical sensors: Toward biochemical lab on the body

Joseph Wang*

Department of Nanoengineering, University California San Diego, CA 92093, US
**Presenting author: josephwang@ucsd.edu*

Wearable sensors have received a major recent attention owing to their considerable promise for monitoring the wearer's health and wellness [1,2]. These devices have the potential to continuously and non-invasively collect vital health information from a person's body and provide this information in a timely fashion. This presentation will discuss our recent efforts toward filling the gaps toward obtaining biochemical information, beyond that given by common wrist-watch mobility trackers. Such real-time molecular information is achieved using advanced wearable electrochemical biosensors integrated directly on the epidermis or within the mouth. The fabrication and applications of such wearable electrochemical sensors will be described, along with their current status and future prospects and challenges.

References

[1] A.J. Bandodkar, I. Jeerapan, J. Wang, Wearable chemical sensors: Present challenges and future prospects. *ACS Sensors,* 1 (2016) 464.

[2] J. Kim, A.S. Campbell, B. Esteban-Fernández de Ávila, J. Wang, Wearable biosensors for healthcare monitoring. *Nature Biotechnology*, 37 (2019) 389.

Acoustic wave appraisal of the non-specific adsorption issue in biosensor technology

Michael Thompson*, Brian De La Franier, Jack Chih-Chieh Sheng, Aries Delica

Department of Chemistry, University of Toronto, Canada.
**Presenting author: m.thompson@utoronto.ca*

It is mandatory that operation of a biosensor requires a device surface that is both highly selective towards the target analyte and is minimally subject to fouling by species present in a biological fluid such as blood, serum or urine, if application in clinical biochemistry is envisaged. We are employing both the traditional thickness shear mode (TSM) and electromagnetic piezoelectric (EMPAS) acoustic wave devices to examine surface chemistry strategies for enhancing device signals *in tandem* with reduction of interfacial fouling. In this research, the effect of surface modification of the silica surface of the EMPAS device and gold surface of the TSM sensor are performed in order to study responses to solutions containing biological moieties.

In previous work on silica surfaces we have demonstrated that dramatic decreases in fouling from serum can be achieved by modification with a covalent, ultra-thin SAM which contains both distal -OH and intra-chain glycol groups [1,2]. This advance was attributed to the role played by interstitial water in SAM molecular chains as shown by neutron reflectometry and MD studies [3,4]. Here, using the EMPAS sensor, we describe an investigation of the influence of chain length and number of glycol moieties. A second aim involves the employment of a diluent to space out surface linker molecules in a deliberately mixed SAM. The purpose of this system is to relieve steric hindrance around the linker head function in order to facilitate probe attachment together with the combined possibility of reducing non-specific adsorption. Experiments with three distinct potential SAM diluents of variable chain length were performed to investigate the role of molecular packing [5]. Employment of the diluent exhibiting maximal anti-fouling behaviour together with a linker resulted in a 8-fold reduction in fouling. In an analogous study with surface modification of the gold electrode of the TSN sensor we show that the lack of capability to instigate SAM intra-chain water severely diminishes anti-fouling behaviour [6].

Acknowledgement: This research was supported by the Natural Sciences and Engineering Research Council of Canada, MITACS, Vancouver, Canada and Econous Systems Inc. of Toronto.

References

[1] S. Sheikh, D.Y. Yang, C. Blaszykowski, M. Thompson, *Chem. Commun.* 48 (2012) 1305-1307.
[2] S. Sheikh, J.C-C. Sheng, C. Blaszykowski, M. Thompson, *Chem. Sci*.1 (2010) 271–275.
[3] N.M. Pawlowska, H. Fritzsche, M. Vezvaiem, C. Blaszykowski, S. Sheikh, M. Thompson, *Langmuir* 30 (2014) 1199-1203.
[4] S. Sheikh, C. Blaszykowski, D. Thompson, M. Thompson, *J. Coll. Interface Sci,* 437 (2015) 197-204.
[5] J.C-C. Sheng, B. De La Franier, M. Thompson, *Materials* 14 (2021) 472-487.
[6] B. De La Franier, M. Thompson, *Materials* 14 (2021) 4158-4166.

Piezo-acoustic sensor electronics methods

Gordon Hayward*

School of Engineering, University of Guelph, Guelph, Ontario, Canada
**Presenting author:* ghayward@*uoguelph*.ca

Piezo-electric sensors have been used for more than half a century since the publication of the technique by Sauerbrey who showed that the resonant frequency of a quartz crystal is affected by mass deposited onto the crystal surface. Later, others have included the effects of the surrounding media and the visco-elastic properties of attached surface layers on both the crystal resonant frequency and its effective resistance. These measurements have provided the bases for many chemical and biochemical sensors. Several electronic systems have been developed to provide resonance data, each with its advantages and disadvantages.

The first to operate in liquids were oscillators. Here, the operation of the crystal sensor is to control the frequency of a self sustaining feedback loop. The Barkhausen criteria must be met so the loop must have sufficient gain to overcome the energy lost from the sensor and must maintain an overall zero phase shift.

To overcome these limits, external excitation can be used. Systems of this type include phase-locked loops where the operation is held at the natural series resonant frequency of the crystal, ring down systems which stop the excitation and measure the oscillation decay and network analyzers which scan the sensor impedance across a range of frequencies.

By applying electromagnetic excitation without electrodes, the frequency of operation can be increased to higher harmonics, giving a corresponding increase in sensor sensitivity. Other high frequency systems use surface acoustic waves, where the wave velocity depends on the surface and its surroundings. Here, as well as network analyzers, delay line oscillators can be used. If the delay is too long, however, some data is missed by the oscillators. To obtain this data, the phase shift of a modulating signal may be measured.

Acoustic and electro-optical probing of environmental response of thin film response

Ilia N. Ivanov*

Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, USA
**Presenting author: ivanovin@ornl.gov*

A question of what modality should be selected to enable better response and lowest limit of detection for a particular thin film sensor is difficult to answer. Should it be electrical, electrochemical, optical, gravimetric, viscoelastic, or may be mixes modality? Despite potential benefits of multimodal detection and due to proprietary nature of instrument-specific data processing approaches the measurement of multimodal response to environment is still a difficult logistical task. The task is commonly solved through independent measurements, separate data processing and manual correlation of the results, which is very time-consuming and inefficient approach. Development of understanding of thin film multimodal response to environment is critical for broad range of advanced technologies, including broad range of biosensors, bio-degradable polymers, nano composites. These novel technologies could benefit from the automated multimodal explement.

We will review recent application of acoustic sensors and the development of multimodal acoustic- electro-optical system to study environmental response of thin film on macro and nano scales in one experiment [1] could help with the selection of the best detection modality in a single experiment. We will show that integration of additional modalities in machine learning modeling can improve prediction accuracy of thin film response. We will discuss how environmental response of composite thin films can be used for continuous scale functional- structural correlations, from macro- down to nano-scales [2].

Acknowledgement: This research was conducted at the Center for Nanophase Materials Sciences, which is a DOE Office of Science User Facility.

References

[1] E.S. Muckley, L. Collins, B.R. Srijanto, I.N. Ivanov, Machine Learning-Enabled Correlation and Modeling of Multimodal Response of Thin Film to Environment on Macro and Nanoscale Using “Lab-on-a-Crystal.” *Adv. Funct. Mater.* 30 (2020) 1908010. https://doi.org/10.1002/adfm.201908010

[2] L.A. Galuska, E.S. Muckley, Z. Cao, et al. SMART transfer method to directly compare the mechanical response of water-supported and free-standing ultrathin polymeric films. *Nat Commun* 12 (2021) 2347. https://doi.org/10.1038/s41467-021-22473-w.

Abstracts of Short Oral Presentations

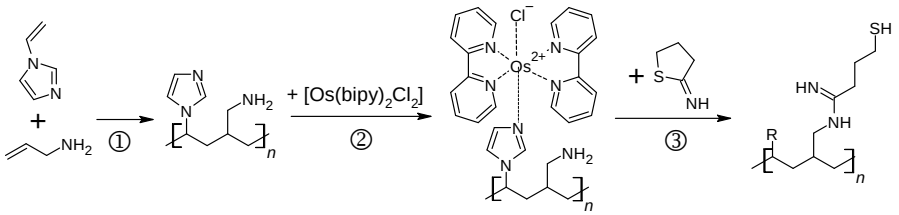
A sensory osmium redox copolymer based on allylamine and vinylimidazole studied with a fast modulation EQCM

Christian Leppin*, Arne Langhoff, Diethelm Johannsmann

Institute of Physical Chemistry, Clausthal University of Technology,
Arnold-Sommerfeld-Str. 4, 38678 Clausthal-Zellerfeld, Germany
**Presenting author: christian.leppin@tu-clausthal.de*

Similar to the conventional EQCM(D), the fast-modulation EQCM quantifies interactions between the surface and the sample in response to an electrical potential. However, the modulation EQCM achieves a time resolution and a frequency resolution superior to the conventional QCMs. The interrogation scheme is based on a multifrequency lockin amplifier (Intermodulation Products AB, Stockholm) and is referred to as a "comb measurement" [1]. The resonator is driven by simultaneous excitation with up to 32 sine waves with different frequencies. The resonance frequency, f_{res}, and the resonance bandwidth, Γ, are obtained on several overtones by fitting phase-shifted Lorentzians to the admittance traces. The time resolution is given as inverse frequency spacing, Δf_{cmb}, between two members of the comb. Therefore, a time resolution in liquids of down to 1 ms is feasible. Accumulation and averaging were used to improve the frequency resolution. The high precision, the evaluation of both frequency and bandwidth, and the comparison of data acquired on different overtones allows distinguish between gravimetric and non-gravimetric effects [2].

This instrument was applied to study an Os redox copolymer as used for amperometric biosensing e.g., for glucose detection [3,4].



The synthesis is depicted above. The copolymer backbone was synthesized in step ①. The imidazole groups in the polymer backbone were modified by a redox active $[Os(bipy)_2Cl_2]$ complex in step ②. Amine groups were modified in step ③ by Traut's reagent to prepare a tightly adsorbed monolayer on the resonator's Au electrode.

In cyclic voltammetry with parallel QCM detection, the modulation EQCM responds to changes of the polymer's charge with a frequency shift, Δf, of a few Hz and an even smaller bandwidth shift, $\Delta\Gamma$. The overtone scaling shows small but systematic deviations from Sauerbrey behavior (the latter being $-\Delta f/n \approx$ const. with n the overtone order, $-\Delta f \approx \Delta\Gamma$). The small noise in Δf enables for computing the time derivative $\mathrm{d}/\mathrm{d}t(\Delta f)$ and direct comparison to the current. The peaks in current density occur at similar potentials as the peaks in $\mathrm{d}/\mathrm{d}t(\Delta f)$. Both show the same dependence on sweep rate.

References

[1] F. Meyer, A. Langhoff, A. Arnau, D. Johannsmann, I. Reviakine, *Rev. Sci. Instrum.* 90 (2019) 115108.
[2] C. Leppin, A. Langhoff, H.-F. Poggemann, A.S. Gödde, D. Johannsmann, *Analyst* 146 (2021) 6005.
[3] A. Badura, D. Guschin, B. Esper, T. Kothe, S. Neugebauer, W. Schuhmann, M. Rögner, *Electroanalysis* 20 (2008) 1043-1047.
[4] K. Habermüller, A. Ramanavicius, V. Laurinavicius, W. Schuhmann, *Electroanalysis* 12 (2000) 1383-1389.

Comparative analysis of protease detection by acoustics and colorimetry methods

Ivan Piovarci[1*], Ilia N. Ivanov[2], Tibor Hianik[1]

[1]Faculty of Mathematics, Physics and Informatics, Department of Nuclear Physics and Biophysics, Comenius University in Bratislava, Slovakia.
[2]Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, USA
Presenting author: piovarci.i@gmail.com

Proteases present a large group of enzymes capable of cleaving proteins. They have a large utility in industry, medicine and food preparation. In this work we focused on digestive proteases also present in milk that affect its quality such as trypsin and chymotrypsin. We were able to measure the activity and kinetics parameters of these proteases using three distinct methods (Figure 1). The first method utilized the size of gold nanoparticles and measured the change in size after immobilization and cleavage of β-casein surface using Doppler Light Scattering (DLS) method. In the second method we utilized optical properties of gold nanoparticles (AuNPs) to measure the change in absorbance maximum of surface plasmon resonance (SPR) effect after modification with mercaptohexanol (MCH) and β-casein and their aggregation after the cleavage of β-casein layer. In the last, acoustic method, we used quartz crystal microbalance (QCM) to measure mass changes on top of the gold electrode covered quartz crystal after binding of β-casein and after its cleavage with the protease. All these methods are fast and cheap for detection of the activity of various proteases [1,2].

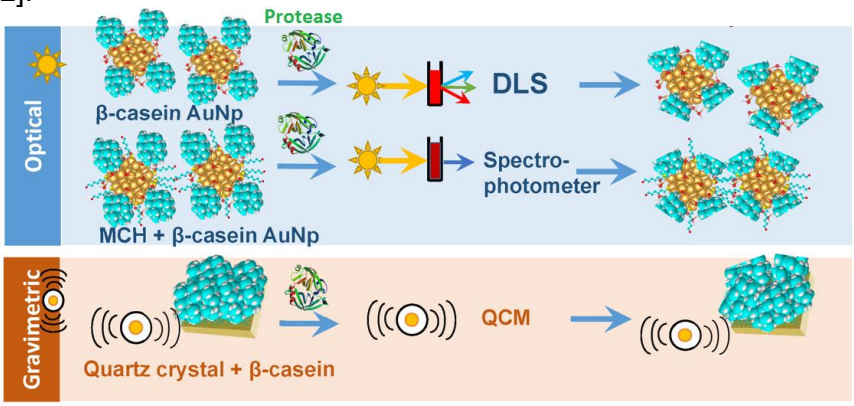


Figure 1. The scheme of the methods used for detection proteases using β-casein as a substrate.

Acknowledgement: A portion of this research was conducted at the Center for Nanophase Materials Sciences, which is a DOE Office of Science User Facility, project No. CNMS2018-293. This work was funded under European Union's Horizon 2020 research and innovation program through the Marie Skłodowska-Curie grant agreements No 690898, 101007299 and by Science Agency VEGA, project No. 1/0419/20.

References

[1] I. Piovarci, S. Melikishvili, M. Tatarko, T. Hianik, M. Thompson, *Biosensors* 11 (2021) 117.
[2] I. Piovarci, T. Hianik, I. N. Ivanov, *Biosensors* 11 (2021) 63.

Improvements in the measuring technique of EMPAS

Gábor Mészáros[1]*, Sanaz Akbarzadeh[2], Brian De La Franier[3], Zsófia Keresztes[1] and Michael Thompson[3]

[1]Institute of Materials and Environmental Chemistry,
Research Centre for Natural Sciences, Hungary.
[2]Department of Chemistry, Yasouj University, Yasouj, Iran.
[3]Department of Chemistry, University of Toronto, Canada.
**Presenting author: meszaros.gabor@ttk.hu*

EMPAS (electromagnetic piezoelectric acoustic sensor) has two advantages over the classical QCM technique, namely no electrodes are required on the surface of the quartz crystal and overtones of the basic mechanical resonance frequency even over 1 GHz can be detected [1]. The method is based on a high-frequency perturbation through a planar coil which is placed under the bare quartz crystal as close as possible. The high-frequency signal is stepped while at the same time it is frequency modulated by an audio frequency signal. Any structure in the impedance of the coil-crystal system will turn the frequency modulation to amplitude modulation as well. After demodulating the amplitude modulated signal, the resulting audio frequency response will be approximately proportional to the derivative of the |Z| vs. frequency curve of the coil-quartz system. In this derivative curve (also referred as to relative impedance) inflexion points will appear at the mechanical resonance frequencies of the quartz crystal. However, the resulting curve is not always clear due to the contribution of the coil to the overall impedance. As the main improvement, we also detect the higher harmonic components of the audio frequency modulating signal in the response of the system which permit the better detection of the resonance frequencies[2]. Namely, the in-phase component of the third harmonic vs. frequency exhibit a zero crossing at the mechanical resonances, while the second and forth harmonic components will show peaks. The ratio of those two peak values permits the calculation of the dissipation or quality factor of the corresponding mechanical resonance.

We also noticed that investigated *AT*-cut crystals were resonating in a different mode as compared to the QCM configuration. A crystal having a base resonance f_0 as a QCM will exhibit $2 f_0 (2k + 1)$, k=1,2,3… resonances in an EMPAS device. Testing different coil geometries, we could detect strong anisotropic behavior: in certain positions the crystals did not show any resonance while turning the crystals with 90º a maximum in the resonance was seen. This implies that in contrast to the QCM configuration, the component of the electromagnetic field parallel to the quartz sheet induces the resonance.

Acknowledgement: This work was supported by the European Union Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 690898, the Development and Innovation Office of Hungary (NKFIH) BIONANO-GINOP 2.3.2-15-2016-00017, and by the Natural Sciences and Engineering Research Council of Canada (grant to M.T.) Yasouj University, Yasouj, Iran is gratefully acknowledged for research leave and support awarded to S.A.

References

[1] Ch. Evers, A. Thorbjorn, M. Lund, M. Skepo, *Langmuir* 28 (2012) 11843-11849.
[2] G. Mészáros, S. Akbarzadeh, B. De La Franier, Zs. Keresztes and M. Thompson, *Chemosensors* 9 (2021) 58.

Application of electromagnetic piezoelectric sensors for development of aptasensors with antifouling properties

Sandro Spagnolo[1*], Brian De La Franier[2], Michael Thompson [2], Tibor Hianik[1]

[1]Faculty of Mathematics, Physics and Informatics, Department of Nuclear Physics and Biophysics, Comenius University in Bratislava, Slovakia.
[2]Department of Chemistry, University of Toronto, Canada.
**Presenting author: sandrospagnolo1@gmail.com*

The detection and quantification of bacteria in milk is of crucial importance to determine if it is safe for sale and consumption. With over 150 million tonnes of milk produced in the EU each year, it is vital to create a low cost in line sensor for milk production lines, which can quickly and accurately assay large quantities of milk for the presence of bacteria [1]. To test the feasibility of sensing bacteria in milk, an electromagnetic piezoelectric sensor (EMPAS) was used. This device uses thin quartz discs as a sensing surface, which operates at close to 1 GHz [2]. The discs were first modified with a silane monolayer of ((silyl)propoxy)propanoyl chloride (MEG-Cl), which provides both anti-fouling and linking capability [3]. This layer alone was found to prevent all fouling from bacteria in PBS solution, and was able to reduce the amount of fouling observed from bacteria contaminated milk by a significant amount. On new discs the MEG-Cl linker was extended with an aptamer that is specific to *E. coli* bacteria, which were then used for further testing. In PBS a linear change in frequency changes versus bacteria concentration was clearly observed, allowing for quantification of *E. coli* in solution to a limit of 100 CFU/mL. Additionally, the sensor was specific to *E. coli*, with no signal changes observed when high concentrations of *S. aureus*, or *P. aeruginosa* were used. Thus, this sensor can be used to accurately, and selectively quantify the concentration of *E. coli* in buffer. In ongoing milk studies, the sensor has been found to respond strongly to milk contaminated with *E. coli*, suggesting that detection and quantification of *E. coli* will be possible in raw milk using this combination of MEG-Cl and aptamer on the sensor surface.

Acknowledgement: This work was has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 101007299 and by the Science Grant Agency VEGA, project number: 1/0419/20.

References

[1] É. Vőneki, D. Mándi-Nagy, A. Stark, *Studies in Agricultural Economics* 117 (2015) 1-9.
[2] S. Sheikh, J. Sheng, C. Blaszykowski, M. Thompson, *Chemical Science* 1 (2010) 271-275.
[3] S. Spagnolo, B. De La Franier, T. Hianik, M. Thompson, *Biosensors* 10 (2020) 20.

Application of multiharmonic QCM for detection of bacterial pathogens using DNA aptamers as receptors

Marek Tatarko[1*], Veronika Oravczová[1], Sandro Spagnolo[1], Judit Süle[2], Attila Hucker[2], Robert Kocsis[2], Tibor Hianik[1]

[1]Faculty of Mathematics, Physics and Informatics, Department of Nuclear Physics and Biophysics, Comenius University in Bratislava, Slovakia.
[2]Hungarian Dairy Research Institute Ltd., 9200 Mosonmagyaróvár, Hungary
**Presenting author: tatarko4@uniba.sk*

Application of multiharmonic quartz crystal microbalance (QCM) provides possibility to study interaction between immobilized aptamer layer and bacteria. This method allowing label-free detection of bacteria with high sensitivity and specificity and can be used also for evaluation of viscoelastic properties of sensing layers. In experiments we used biotinylated or amino-modified aptamers specific to *Listeria monocytogenes.* The aptamers were immobilized at the gold layer of QCM crystals though neutravidin-biotin method or using NHS/EDC chemistry applied to the chemisorbed layer of mercaptoundecanoic acid (MUA). Addition of bacteria in the concentration range 5×10^3–10^6 CFU/mL resulted in a decrease of resonant frequency and in an increase of dissipation. Addition of *E. coli* at the surface of neutravidin as well as aptamer layers did not result in significant changes in frequency and dissipation. Using the Kelvin–Voight model, the analysis of the viscoelastic properties of the sensing layers was performed and several parameters such as penetration depth, Γ, viscosity coefficient, η, and shear modulus, μ, were determined following various modifications of QCM transducer [1]. The penetration depth decreased following adsorption of the neutravidin, which is evidence of the formation of a rigid protein structure. This value did not change significantly following adsorption of aptamers and *Listeria innocua*. Viscosity coefficient was higher for the neutravidin layer in comparison with the naked QCM transducer in a buffer. However, a further increase of viscosity coefficient took place following attachment of aptamers suggesting their softer structure. The interaction of *Listeria innocua* with the aptamer layer resulted in slight decrease of viscosity coefficient. The shearing modulus increased for the neutravidin layer and decreased following aptamer adsorption, while a slight increase of μ occurred after the addition of *Listeria innocua*. Further interactions of *Listeria innocua* and pathogenic *Listeria monocytogenes* with NH_2-modified aptamer were observed using similar method. Application of dead bacteria cells and aptamer layer regeneration were investigated as well.

Acknowledgement: This work was has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 101007299 and by the Science Grant Agency VEGA, project number: 1/0419/20.

References

[1] M. Tatarko, S. Spagnolo, V. Oravczová, J. Süle, M. Hun, A. Hucker, T. Hianik, *Sensors 21* (2021) 5585.

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

Prof. Július Cirák, Faculty of Electrical Engineering and Information Technology, Slovak University of Technology in Bratislava, Ilkovičova 3, 81ľ 19 Bratislava, Slovakia

Prof. Martin Weis, Faculty of Electrical Engineering and Information Technology, Slovak University of Technology in Bratislava, Ilkovičova 3, 81ľ 19 Bratislava, Slovakia

Acknowledgments

The publication of this Book of Abstracts was supported by European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 101007299

ISBN 978-80-8147-106-3