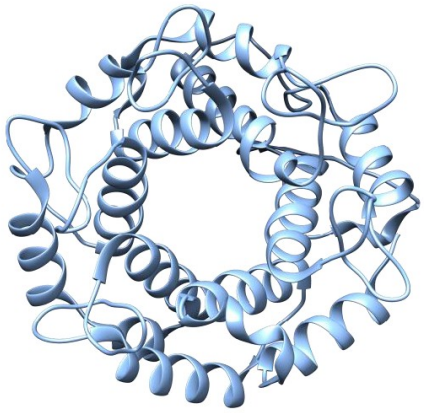
11TH INTERNATIONAL CONFERENCE

# STRUCTURE AND STABILITY OF BIOMACROMOLECULES

# SSB 2019

# *BOOK OF ABSTRACTS*



3 - 6 SEPTEMBER 2019, KOŠICE, SLOVAKIA


Department of Biophysics
Institute of Experimental Physics
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Book of Contributions, 11th International Conference Structure and Stability of Biomacromolecules
SSB2019, September 3 – 6, 2019, Košice, Slovakia
Editors: RNDr. Diana Fedunová, PhD., RNDr. Zuzana Bednáriková, PhD.,
RNDr. Vladimír Vaník
Reviewers: doc. MUDr. Marek Dudáš, PhD., doc. RNDr. Zuzana Gažová, CSc.


ISBN: 978-80-89656-25-7

EAN: 9788089656257

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Biomacromolecules:

- structural aspects
- aggregation
- activity
- factors influencing stability
- ligand binding and inhibition
- modifications and engineering
- bioinformatics, molecular and nanodesign
- applications in medicine, bio- and nanotechnologies
- experimental and computational strategies for structural and conformational studies

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# Application of acoustic methods for detection of casein degradation caused by milk protease systems

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Our work is focused on the development and validation of the acoustic biosensors based on the quartz-crystal microbalance principles (QCM) able to detect proteolytic activity of the proteases on covalently and non-covalently bound casein layers formed on a gold or $SiO_2$ surface [1]. We used a QCM with monitoring of higher harmonic frequencies of the quartz crystal that also allowed us to study of the adsorption processes of various non-covalently bound caseins. While α-casein layer was removed from the surface after washing, β-casein and κ-casein produced stable 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 QCM was able to monitor protease activity at subnanomolar concentrations and allowing distinguishing between trypsin and plasmin [2]. 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. Another sensor tested was electromagnetic piezoelectric acoustic sensor (EMPAS) utilizing extremely thin quartz disc and planar coil that generates electromagnetic field to produce piezoelectric oscillation of high fundamental frequency (20 MHz) [3]. The sensitivity of detection has been increased by measurement frequency changes at 49$^{th}$ harmonic frequency. β-casein was immobilized on hydrophilic or hydrophobic surfaces. Hydrophobic surface was more complicated to prepare, but it created more stable and suitable layer for detection of the plasmin cleavage. We were also concentrated on the detection of regulatory effects of plasmin protease system such as inhibition or autolysis using traditional QCM 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.

*Acknowledgement: Supported by the European Commission within the project FORMILK under grant agreement number 690898 /H2020-MSCA-RISE-2015, CNMS User project No. CNMS2018-293 and Comenius University grant No. UK/328/2019.*

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