Combining surface plasmon resonance and quartz crystal microbalance to determine hydration of protein monolayers

K. Tokarczyk[1*], P. Komorek[1,2], I. Kralka[1,3], B. Jachimska[1]

[1]*J. Haber Institute of Catalysis and Surface Chemistry, PAS, Niezapominajek 8, 30-239 Cracow, Poland*
[2]*Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Lojasiewicza 11 30-348 Cracow, Poland*
[3]*Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Cracow, Poland*

**nctokarc@cyf-kr.edu.pl*

Proteins are a class of natural molecules that have unique functionalities and potential applications in both biomedical and material sciences. Controlling the interaction of proteins with different surface substrates is a fundamental challenge. Serum albumins have been widely used as model proteins. In this work, bovine serum albumin (BSA) has been selected as it belongs to the dominant group of mammalian blood plasma proteins. BSA is characterized by a relatively high molecular weight, three-domain structure and an asymmetric charge distribution. BSA is able to adopt different conformations, which are modified by changes in pH, concentration or ionic strength.
In our previous papers, we determined several basic physicochemical properties of bovine serum albumin, including the diffusion coefficient (expressed as hydrodynamic diameter), electrophoretic mobility, which made it possible to determine the isoelectric point, and the (electrically) non-compensated charge of the BSA molecule [1]. Measurements of dynamic viscosity were conducted to determine the conformational stability of BSA in the solution [2]. Based on the dynamic viscosity, the effective length of this molecule was determined as a function of the solution's pH.
The present study focuses on the protein monolayer structure on gold surface. Gold surface is one of the promising surface for protein controlled delivery due to its high stability and good biocompatibility. We used multi parametric surface plasmon resonance (MP-SPR) and a quartz crystal microbalance with dissipation energy monitoring (QCM-D) to investigate the conformational behaviour of the BSA molecules. The selected surface techniques made it possible to trace the process of protein adsorption and gave insight into the adsorption mechanism of this molecule [3]. Both the kinetics of protein deposition and the maximum surface concentration were determined.
From the combination of the QCM-D and MP-SPR data, with the assumption that the excess mass measured in QCM-D compared to the MP-SPR mass is due to trapped water molecules, we estimated the fraction of water in the protein film. The structure of the resulting films is strongly dependent on the deposition conditions. These results indicate that BSA form very hydrated films on gold surfaces and determine how the degree of hydration changes with pH and ionic strength. Additionally, the results confirmed the significant role of the highly anisotropic surface charge distribution of BSA molecules on the process of their adsorption.

Acknowledgements This work was supported by Grant NCN OPUS4 2012/07/B/ST5/00767.

[1] B. Jachimska, M. Wasilewska, Z. Adamczyk, *Langmuir*, 2008, **24**, 6866-6872.
[2] B. Jachimska, A. Pajor, *Bioelectrochemistry,* 2012, **87**, 138–146.
[3] B. Jachimska, K.Tokarczyk et al., *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2016, **489**, 163-172.