

Increasing the Sensitivity of Enzymatic Biosensors by Working at the Phase Transition Temperature

D. Grieshaber¹, E. Reimhult², J. Vörös¹

¹ *Laboratory of Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETH Zurich, Switzerland.* ² *Laboratory for Surface Science and Technology, Department of Materials, ETH Zurich.*

INTRODUCTION: Biosensors are highly selective measuring tools due to the high substrate specificity of the enzymes. They offer the possibility of real-time analysis which is important for the rapid measurement of body analytes. Their potential application lies in the clinical analysis for health care [1]. Glucose biosensors have successfully been used in measuring the glucose level of diabetes patients. Other applications of enzymatic biosensors are in the field of monitoring environmental pollution. A new possible application lies in multiplexed electronic detection systems for early cancer diagnostics. Based on the success of glucose sensors and ELISA assays [2] enzymatic biosensors are expected to play a key-role in clinical, biochemical and biotechnological analysis. However, at present their application in cancer diagnostics is limited because of the low number of existing cancer markers and their insufficient sensitivity. In the current work we present an approach towards a highly sensitive enzymatic biosensor.

METHODS: Biotinylated poly(L-lysine)-grafted-poly(ethylene glycol) (PPB) was adsorbed to an indium tin oxide (ITO) surface. Subsequently, single stranded biotinylated DNA, coupled to neutravidin (NA/bDNA), was adsorbed. Vesicles, tagged with complementary cholesterol-DNA (vesicle/cDNA), were added in a last step. The enzyme glucose oxidase was incorporated into the vesicles. Glucose was used as a substrate. All measurements were carried out in either 100 mM potassium chloride (KCl) or in a 160 mM buffer solution, consisting of 10 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid and 150 mM NaCl, adjusted to pH=7.4.

Adsorption on ITO was observed with a Quartz Crystal Microbalance with Dissipation (QCM-D). This instrument measures changes in the frequency (f) and dissipation factor (D) of an oscillating quartz crystal upon adsorption of a viscoelastic layer [3]. Another technique to monitor in situ adsorption is the Optical Waveguide Lightmode Spectroscopy (OWLS). Thereby, changes in the incoupling angle were monitored; the adsorbed mass was calculated according to de Feijter's

formula [4, 5]. Furthermore, simultaneous optical and electrochemical measurements were performed in an electrochemical flowcell.

RESULTS: The buildup of our biosensor prototype is shown in Figure 1. The three steps correspond to the adsorption of PPB, NA/bDNA and vesicle/cDNA.

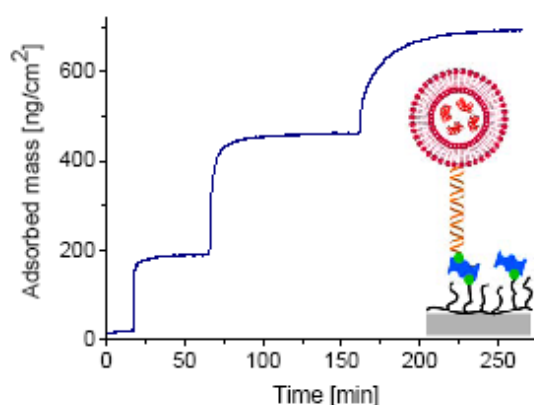


Figure 1: Adsorption curve of PPB, NA/bDNA and vesicle/cDNA. The insert shows a scheme of the biosensor that is built up.

Measurements at the phase transition temperature of the lipids showed a higher sensitivity. This is expected, because the coexistence of the two phases facilitates the diffusion of the substrate through the membrane.

DISCUSSION & CONCLUSIONS: We built a biosensor that allows for – with the currently used setup – detection of DNA hybridization. Once the sensitivity has been optimized, the next step will be to use an antibody/antigen system instead of the DNA-hybridization.

REFERENCES: ¹ A. Malhotra, *et al.* (2002) *Langmuir* **17**: 441-456. ² U.B. Nielsen *et al.* (2004) *J. of Immunological Methods* **290**: 107-120. ³ K. Marx (2002) *Bio Macromolecules*, **4**: 1090-1120. ⁴ J. Voros *et al.* (2002) *Biomaterials* **23**: 3699-3710. ⁵ J.A. De Feijter *et al.* (1978) *Biopolymers* **17**: 1759-1772

ACKNOWLEDGEMENTS: The authors would like to thank ETH Zurich for funding.