

INTERFACIAL BINDING OF BETA-LACTAMASE TO DIFFERENT MATRICES MONITORED BY SURFACE-PLASMON SPECTROSCOPY

F.Xu¹, G.Zhen², E.Zobeley³, V.Eggli³ & R.Glockshuber³, M.Textor² & W.Knoll¹

¹Max-Planck-Institut fuer Polymerforschung, Ackermannweg 10, 55128 Mainz, Germany.

²Laboratory for Surface Science and Technology, ETHZ, Zürich, Switzerland.

³Institute of Molecular Biology and Biophysics, ETHZ, Hönggerberg, Switzerland.

INTRODUCTION: Surface-plasmon resonance spectroscopy (SPR) is a well-accepted analytical tool for the characterization of interfaces and thin films. One of the major advantages of surface-plasmon spectroscopy is the fact that it allows for the label-free detection of binding events. In this study, we used SPR to investigate the binding of biotin labelled β -lactamase.

METHODS: Three kinds of matrices were compared for the protein binding. Biotin-terminated Self-Assembly Monolayers (SAMs) were used for the binding of Neutravidin and further attachment of lactamase. On the other hand, we compared the binding of Biotin-PLL-PEG to carboxyl-terminated SAMs via electrostatic interaction and by covalent interactions (NHS ester activation), separately. In these two matrices, biotin-PLL-PEG was used for the binding of a pre-incubated Neutravidin and lactamase mixture. For all the systems, the non-specific binding and regeneration possibilities were tested. In order to calculate the amount of bound lactamase, DTT was used to release lactamase from the surface by opening the disulfide bond between lactamase and Neutravidin.

RESULTS: 1) Biotin-terminated SAMs. We found a layer thickness ($n=1.45$) for Neutravidin of 4.15nm and for Biotin labelled β -lactamase ($n=1.41$) of 2.78nm. For Neutravidin, this corresponds to the coverage of 60%. No non-specific binding of β -lactamase was found. These results are summarized in Table 1. **2) Carboxyl-terminated SAMs.** 3.63nm Biotin-PLL-PEG and 3.5nm Neutravidin/Biotin labelled β -lactamase bound on SAMs. HCl can be used to eliminate the electrostatic interaction between

SAMs and Biotin-PLL-PEG in order to regenerate the matrix. **3) Carboxyl-terminated (NHS ester) SAMs.** 3.2nm Biotin-PLL-PEG and 3.5nm Neutravidin/ Biotin labelled β -lactamase bound on SAMs.

DISCUSSION & CONCLUSIONS: Our results show that all three matrices are well suited for the binding of the protein, although quantitative differences are found and will be discussed. Furthermore, it is found that the data obtained by SPR are quite comparable to those derived from optical waveguide measurements.

Table 1. Comparison of data obtained by SPR and derived from optical waveguide measurements, respectively, based on Biotin-terminated SAMs

	Results from SPR		Results from Optical Waveguide measurements
	Thickness	Mass	Mass
Biotin thiol mixture	2.92 nm		
Neutravidin	4.15 nm	294 ng/cm ²	270 ng/cm ²
Biotin labelled β -lactamase	2.78 nm	134 ng/cm ²	130 ng/cm ²
Cutting amount by DTT	2.78 nm	134 ng/cm ²	130 ng/cm ²

REFERENCES: ¹J. Spinke, M. Liley, et al (1993) Langmuir 9: 1821-1825. ²E.K. Sinner, K. Kobayashi, et al (2003) biopolymers at Interfaces, 2nd Ed., Marcel Dekker (ed. 2003. Malmsten), 583-607. ³David M. Livermore (1995) Clinical Microbiology Reviews, 8: 557-584.