

CREATION OF SURFACE MACROMOLECULAR DOCKING SITES FOR THE REVERSIBLE IMMOBILIZATION OF PROTEINS IN ACTIVE CONFORMATION AND CONTROLLED ORIENTATION

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INTRODUCTION: Interfaces are key elements in the design and fabrication of bioaffinity sensor chips with directed biological response. At the same time, the knowledge of the conformation, specific activity and orientation of proteins bound to surfaces constitutes a crucial prerequisite for understanding the functional properties of proteins bound to surface and for the development and optimization of highly specific and sensitive biosensors.

METHODS: Two docking site platforms based on biotin-(Strep)avidin and NTA-Ni²⁺-histag linkage techniques, in combination with polycationic, PEG-grafted, biotinylated or NTA(nitrilotriacetic acid) functionalized copolymers were synthesized. One of the most efficient enzyme known β -lactamase served as the model protein. Five different variants of β -lactamase with single cysteine site-directed mutagenesis on the surface, one 6xHis-tagged β -lactamase and Green Fluorescent Protein (GFP) were engineered. The five β -lactamase variants were biotinylated at free thiol-group with a cleavable biotinylation reagent allowing for release of the surface-bound enzyme after adding DTT. The immobilization was achieved on niobium oxide surface coated with biotinylated Poly(L-lysine)-g-poly(ethylene glycol) monolayer. The biotinylated β -lactamase was subsequently bound to the surface via NeutrAvidin. The amount of immobilized β -lactamase on the chips was measured by two different techniques: OWLS and specific enzymatic activity via photospectroscopic detection of the turnover of the chromogenic substrate nitrocefin. Furthermore two protocols for the control of the amount of β -lactamase on chip are compared and investigated. Specific immobilization could be discriminated from non-specific adsorption. The catalytic activity of enzyme was firstly quantitatively compared when they are in solution and on chip. 6xHis-tagged GFP bound to NTA functionalized polymer modified surface were measured by OWLS and CLSM.

RESULTS: Michaelis kinetics of β -lactamase variants with and without biotinylation in solution are the same. No significant effects on the activity

of biotinylated β -lactamase variants for NeutrAvidin binding were found. Enzymatic study shows surface-bound β -lactamase lost 75% to 95% relative activity per molecule. OWLS studies of 6xHis-tagged GFP bound to NTA functionalized polymer modified surface showed that the binding was required the presence of Ni(II) on the surface and could be desorbed by treatment with 500 mM imidazole.

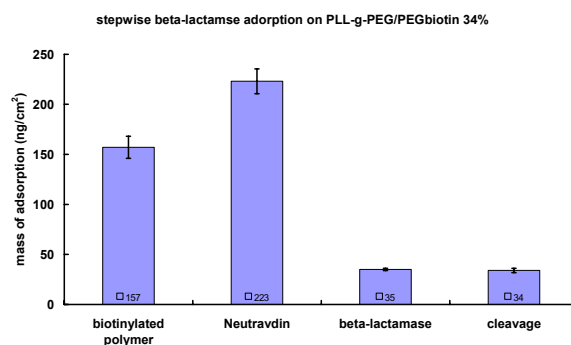


Fig. 1: Adsorption of PLL-g-PEG/PEGbiotin polymer, neutral avidin and biotinylated β -lactamase on chip (Nb_2O_5) measured with Optical Waveguide Lightmode Spectroscopy (OWLS).

DISCUSSION & CONCLUSIONS: OWLS shows that the different surface platforms are successfully build up and the mass adsorption of molecules were quantified. Enzymatic study shows surface-bound β -lactamase is less active than enzyme in solution because the reaction is extremely fast and diffusion becomes a limiting factor upon immobilization of the enzyme. NTA functionalized polymer shows the potential to be a new surface docking site interface for protein immobilization.

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