

SURFACE DOCKING SITES FOR MACROMOLECULES: INTERFACE ARCHITECTURE BASED ON PLL-G-PEG/PEGBIOTIN-(STREPT)AVIDIN

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INTRODUCTION: Surface docking sites (nanoscale islands) are desirable for the specific adhesion of macromolecules, such as proteins and oligonucleotides, onto surfaces. Ideally, the surrounding areas of such docking sites should be non-adhesive so that the adsorbed macromolecules are prevented from denaturing after adsorption. We have mixed poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) and a variant of PLL-g-PEG in which some of the PEG chains are biotinylated (PLL-g-PEG/PEGbiotin) to form a novel polymeric interface, in order to tailor chip surfaces in terms of non-specific and specific analyte-surface interactions. We expect that such a platform could serve as a powerful tool for the investigation of molecular recognition effects.

METHODS: PLL-g-PEG derivatives were synthesized from poly(L-lysine) hydrobromide (mol wt ~20 kDa), N-hydroxysuccinimidyl ester of methoxy-poly(ethylene glycol) propionic acid (mol wt ~2 kDa) and α -biotin- ω -N-hydroxysuccinimidyl ester of poly(ethylene glycol)-carbonate (mol wt ~3.4 kDa) [1].

The Optical Waveguide Lightmode Spectroscopy (OWLS) technique [2] was used for the *in situ* monitoring of macromolecule adsorption, including polymeric interface formation based on mixtures of PLL-g-PEG and PLL-g-PEG/PEGbiotin in various ratios and sequential immobilization of streptavidin and biotinylated goat-anti-rabbit immunoglobulin (α RlgG-biotin) to sense the target molecule, rabbit immunoglobulin (RlgG). The OWLS technique is highly sensitive (i.e. $\sim 1\text{ng}/\text{cm}^2$) to adsorption up to a distance of 200 nm above the surface of the waveguide.

The distribution of surface docking sites was investigated by forming mixed polymeric layers, adding streptavidin and finally using biotinylated liposomes (as markers) for Atomic Force Microscopy measurements.

RESULTS: By means of optical waveguide lightmode spectroscopy (OWLS), streptavidin is shown to bind specifically to the biotin-functionalised PEG, while the resistance of the remaining PEG chains to protein adsorption yields a high specific-binding-to-non-specific-binding ratio, especially when using a buffer with

physiological ionic strength. Subsequent binding of biotinylated goat-anti-rabbit immunoglobulin (α RlgG-biotin) to streptavidin as a capture molecule allows the system to be used as an immunoassay for the target molecule, rabbit immunoglobulin (RlgG). Changing the ratios of PLL-g-PEG and PLL-g-PEG/PEGbiotin in the mixture changes the distribution of docking (biotin) sites on the interface and, thus, allows optimisation of the sensing response (see Fig.1). AFM images of the distribution of docking sites reflected by streptavidin and biotinylated liposomes also show a reasonable correlation among streptavidin, biotinylated liposomes and the surface biotin concentration.

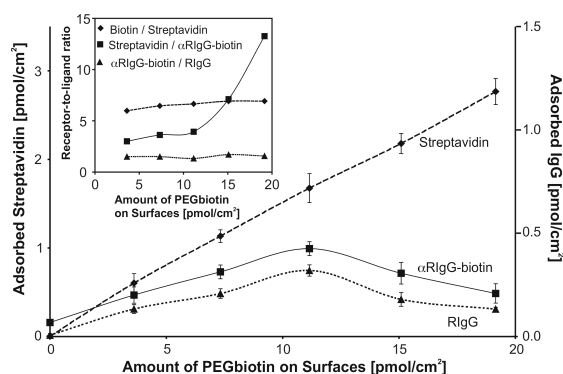


Fig.1 Results from OWLS measurements on the sequential adsorption of streptavidin, α RlgG-biotin and RlgG on mixed polymeric interfaces. The surface concentration of adsorbed streptavidin is plotted on the left axis, whereas the concentration of adsorbed IgG is indicated on the right. The inset shows the receptor-to-ligand ratio for each step of the bioaffinity assay.

DISCUSSION & CONCLUSIONS: The novel polymeric interfaces tailored by PLL-g-PEG and PLL-g-PEG/PEGbiotin are shown to be highly resistant to nonspecific adsorption from serum while still allowing for the specific surface binding of the linkage protein, streptavidin. The amount of immobilized streptavidin is shown to be closely related to the surface biotin concentration. The subsequent adsorption behaviour of α RlgG-biotin and RlgG, however, depends in a more complex

manner on each individual surface modification step and orientational and steric repulsion effects within the adlayers. The optimum interface can be found as a promising platform for bioaffinity sensing of proteins.

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² J. Vörös, J.J. Ramsden, G. Csucs, I. Szendrő, M. Textor, N.D. Spencer (2000) *J. Molecular and Cellular Biology*, submitted.

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