

NTA (nitrilotriacetic acid)-derivatized Poly(L-lysine)-g-poly(ethylene glycol): A Novel Polymeric Interface for Binding and Study of 6xHistidine-tagged Proteins

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INTRODUCTION: Interfaces are key elements in the design and fabrication of bioaffinity sensor chips with directed biological response. It has been reported that coating metal oxide surfaces with poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) provides an attractive option for producing stable surfaces that are protein-resistant¹. A novel NTA-functionalized PLL-g-PEG is presented that it can assemble on oxide surfaces as a monolayer, allows for the immobilization of proteins through NTA-Ni²⁺-histidine docking site chemistry.

METHODS: Graft copolymer PLL-g-PEG/PEG-NTA was synthesized with a fraction of the PEG chain terminus covalently functionalized with nitrilotriacetic acid (NTA) as a chelating ligand. The polymer was assembled from aqueous solution onto Nb₂O₅ coated optical chips, followed by coordination of Ni²⁺ to the surface-exposed NTA ligand. Subsequently, this sensing platform was used to specifically attach Histidine-tagged proteins, e.g., 6His-GFP (Green Fluorescent Protein) or enzymes, e.g., 6His-β-lactamase. Optical waveguide lightmode spectroscopy (OWLS) was used to monitor quantitatively and *in situ* for each step. Furthermore, the NTA-functionalized polymer was used to produce interactive micropatches in a non-interactive PLL-g-PEG background on Nb₂O₅ coated surfaces by a novel approach termed molecular assembly patterning by lift-off (MAPL)². The quality of the 6His-GFP patterns was evaluated by confocal laser scanning microscopy (CLSM).

RESULTS: OWLS studies of 6His-GFP bound to NTA-functionalized polymer modified surfaces proved that the binding of 6xHis-tagged proteins was stable and required the presence of Ni²⁺ attached to the NTA functionalities. The proteins could be fully removed by exposing the surface to imidazole or EDTA. Non-specific adsorptions of 6His-GFP and 6His-β-lactamase were below 2 ng/cm². Binding and desorption of 6xHis-tagged proteins was repeated in several cycles demonstrating the excellent regeneration capacity of the novel platform (Fig. 1). Fluorescence microscopy measurement proved that the patterning was successful and that surface-immobilized 6His-GFP was in an active conformation (Fig. 2).

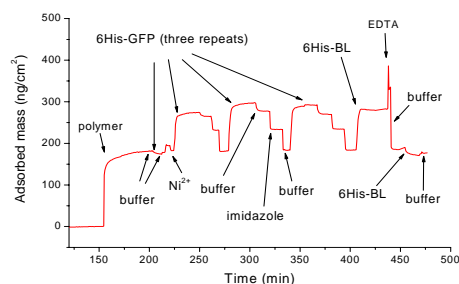


Fig. 1: Adsorbed mass measured by OWLS of the sequential adsorption of PLL-g-PEG/PEG-NTA, Ni²⁺, 6His-GFP and 6xHis-β-lactamase. Quantitative regeneration of the surface was achieved by adding either EDTA, which removes Ni²⁺ or imidazole, which removes only the His-tagged protein, but not Ni²⁺.

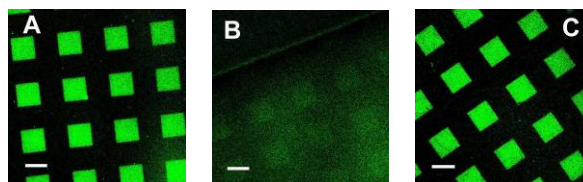


Fig. 2: Pattern of 6His-GFP on Nb₂O₅ analyzed by CLSM. The scale bar represents 60 μm. (A) 6His-GFP immobilized on MAPL patterned substrate after charging with Ni²⁺. (B) As A, rinsed with imidazole removing 6His-GFP. (C) As B, reloaded with 6His-GFP.

DISCUSSION & CONCLUSIONS: We have demonstrated that the novel PLL-g-PEG/PEG-NTA polymeric interface is a promising approach for the binding of 6xHis-tagged proteins in an oriented manner with active conformation. Furthermore, the combination of the MAPL patterning technique with the PLL-g-PEG/PEG-NTA system is considered to be a promising technique for the production of functional microarrays in the area of genomics and proteomics.

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