

Surface Structure and Activity of Immobilized Protein G Mutants

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Controlling how proteins are immobilized is essential for optimizing the performance of *in vitro* protein-binding devices. Comprehensive analysis of surface immobilized proteins provides the level of detail about the immobilization process and the structure of the immobilized biomolecules needed to develop optimized these devices. In particular, surface analysis methods such as XPS, ToF-SIMS, SFG, NEXAFS, SPR and QCM-D, when combined with Monte Carlo (MC) and molecular dynamics (MD) computation methods provide a powerful method for obtaining information about the attachment, type, orientation, conformation and spatial distribution of surface immobilized proteins. The focus of this work was to control and characterize the orientation of Protein G B1, an immunoglobulin (IgG) antibody-binding domain of Protein G, on well-defined surfaces as well as measure the effect of Protein G B1 orientation on IgG antibody binding.

A MC algorithm was developed to predict the most likely orientations of wild type (WT) Protein G B1 onto a hydrophobic surface. The MC simulations predicted that WT Protein G B1 is adsorbed onto a hydrophobic surface in two different side-on orientations. This prediction was consistent with SFG vibrational spectroscopy results. QCM-D experiments showed that WT Protein G B1 adsorbed onto polystyrene retained its IgG antibody-binding activity. Additional MD simulations provided further detail about the structure of WT Protein G B1 on hydrophobic surfaces.

To systematically vary the orientation of Protein G B1, five different cysteine mutants were immobilized onto maleimide oligo(ethylene glycol) (MEG) covered flat and nanoparticle gold surfaces. The amount of Protein G B1 immobilized along with its IgG binding activity was measured with XPS and QCM-D. The surface sensitivity of ToF-SIMS was used to distinguish between the different Protein G B1 mutant orientations by monitoring the changes in intensity of characteristic amino acid mass fragments from different locations in the Protein G B1 structure. ToF-SIMS results showed that the Protein G B1 orientation can be flipped by changing the location of the Cys from the C-terminus to the N-terminus, which has implications for IgG binding since the binding site for IgG is located near the C-terminus of Protein G B1. QCM-D measurements show that when a monolayer of Protein G is immobilized with the C-terminus facing outward it can bind a monolayer of IgG. Conversely, QCM-D measurements show that when a monolayer of Protein G B1 is immobilized with the N-terminus facing outward it binds very little IgG (~x30 decrease in binding capacity). ToF-SIMS also identified the Protein G B1 Cys mutant expected to bind in a side-on orientation.

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