Combining Surface Analytical and Computational Techniques to Investigate Orientation Effects of Immobilized Proteins

Elisa T. Harrison[a,b], Gianluca Interlandi[c], and David G. Castner[a,b,c]
[a]Department of Chemical Engineering, University of Washington, Seattle, WA, 98195, USA
[b]National ESCA and Surface Analysis Center for Biomedical Problems, Seattle, WA, 98195, USA
[c]Department of Bioengineering, University of Washington, Seattle, WA, 98195, USA

Controlling how proteins are immobilized (e.g. controlling their orientation and conformation) is essential for developing and optimizing the performance of in vitro binding protein devices, such as enzyme-linked immunosorbent assays. The objective of this work is to develop new methodologies to study proteins and complex mixtures of proteins immobilized onto surfaces.

The focus of this study was to control and characterize the orientation of protein G B1, an 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 using a variety of surface analytical and computational techniques. The goal was to immobilize protein G B1 into well-ordered films with different orientations that control the accessibility of antibody binding sites.

The surface sensitivity of time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to distinguish between different proteins and their orientation by monitoring the changes in intensity of characteristic amino acid mass fragments. Amino acids with asymmetric distributions (Asn, Trp, Gly, Ala, and Thr) were used to calculate peak intensity ratios from ToF-SIMS data in the C- and N-terminus of protein G B1 to determine the orientation of five different cysteine mutants of protein G B1 covalently attached to a maleimide surface.

To study the effect of protein orientation on antibody binding, we formed multilayer protein films. Quartz crystal microbalance with dissipation monitoring (QCM-D) detected protein coverages of 69 - 130 ng/cm2. QCM-D and X-ray photoelectron spectroscopy (XPS) analysis revealed that packing density along with orientation affected the antibody binding process. Spectra from ToF-SIMS using large Ar gas cluster ion sources distinguished between different proteins in multilayer protein systems.

Additionally, development of computational methods to study proteins on surfaces can complement surface analytical data. A Monte Carlo algorithm was developed to predict protein orientation on surfaces. Two distinct orientations of protein G B1 adsorbed onto a hydrophobic surface were found and characterized as two mutually exclusive sets of amino acids on the outermost β-sheets contacting the surface. This prediction was consistent with sum frequency generation (SFG) vibrational spectroscopy results. In fact, theoretical SFG spectra calculated from an equal combination of the two predicted orientations exhibited reasonable agreement with measured spectra of protein G B1 on polystyrene surfaces. This method has been expanded to predict protein G B1 orientations on more complex surfaces, such as self-assembled monolayers.

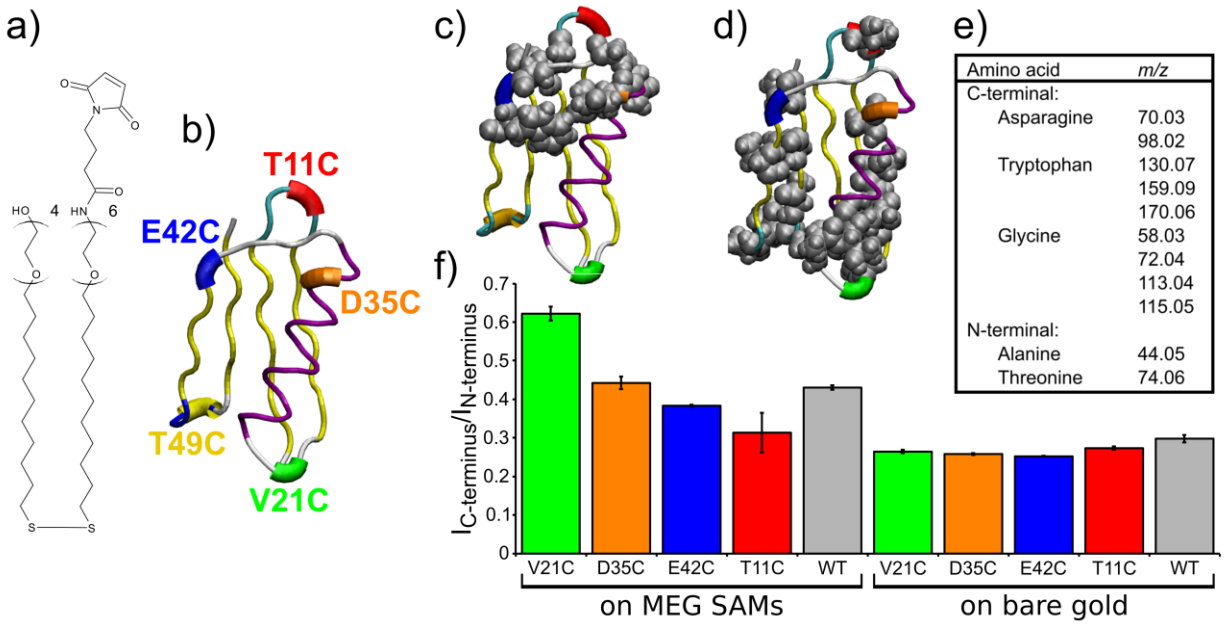

Figure: (a) Maleimide-oligo(ethylene glycol)-functionalized self-assembled monolayers (MEG SAMs). (b) Locations of cysteine mutations on protein G B1. Amino acids with asymmetric distributions used to calculate peak intensity ratios from ToF-SIMS data in the C-terminus (c) and N-terminus (d) are highlighted on the crystal structure of protein G B1 visualized by VMD and (e) listed in table shown. ToF-SIMS peak intensity ratios (f) were calculated as the sum of intensities of chosen amino acids from the C-terminus divided by the sum of intensities of chosen amino acids from the N-terminus.