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## *"Surface Characterization of Immobilized Peptides and Proteins"*

Biomedical surface analysis has undergone significant and numerous advances in the past 30 years in terms of improved instrumentation, introduction of new techniques, development of sophisticated data analysis methods, and the increasing complexity of samples analyzed. Comprehensive analysis of surfaces and surface immobilized biomolecules (peptides, proteins, DNA, etc.) with modern surface analysis instrumentation provides an unprecedented level of detail about the immobilization process and the structure of the immobilized biomolecules. Results from x-ray photoelectron spectroscopy (XPS or ESCA), time-of-flight secondary ion mass spectrometry (ToF-SIMS), near edge x-ray absorption fine structure (NEXAFS), surface plasmon resonance (SPR) biosensing, atomic force microscopy, and sum frequency generation (SFG) vibrational spectroscopy provide important information about the attachment, orientation, conformation, and specificity of peptides and proteins. This talk will present the latest experimental results on surfaces that range in complexity from well-defined, self-assembled monolayers (SAMs) to polymer coated glass slides. Understanding the interaction of proteins and peptides with engineered surfaces from first principles is essential for the design of biomaterials for antifouling, implant and sensor applications. NEXAFS and SFG vibrational spectroscopy were used to characterize the structure of α-helical and b–strand model peptides adsorbed onto SAMs and polymers. The formation of ordered peptide monolayers was quantified with XPS. The synthetic peptide contained hydrophilic lysine (K) and hydrophobic leucine (L) residues in a periodicity designed so that the hydrophobic and hydrophilic side-chains are on opposite sides of the peptide backbone. SFG spectra collected in situ in PBS buffer after peptide adsorption clearly shows that the leucine side chains are oriented towards hydrophobic surfaces such as methyl SAMs and polystyrene, while the lysine side chains are oriented towards the carboxylic acid terminated SAMs., SFG amide 1 spectra and NEXAFS nitrogen spectra were used to show the peptide backbones were oriented parallel to the surfaces. Recently this work as been extended to Protein G B1 variants with cysteines introduced at opposite ends of the protein or modified surface charge distributions. The cysteine mutants were immobilized onto both maleimide-oligo(ethylene glycol)-functionalized and bare gold substrates via the cysteine thiols. The charge mutants were immobilized onto amine and carboxylic acid terminated SAMS. ToF-SIMS, NEXAFS and SFG were combined to demonstrate the different orientations of the surface immobilized Protein G B1 mutants.