A Strategy for Determining the Structure of Surface Bound Proteins

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Statement of Purpose: Information about protein structure and function at interfaces on the molecular level is crucial in drug design, biosensor applications and biomaterial engineering. Proteins on surfaces are an integral part of many biomedical applications (implanted biomedical devices, diagnostic arrays, tissue engineering scaffolds, cell cultures, etc.) and in biomimetic material design strategies. This importance has stimulated research towards developing techniques to assess the structure, activity, and surface interactions of immobilized proteins. Recent advances in sum frequency generation (SFG) spectroscopy now provide detailed information about surface bound proteins and peptides. Combining SFG measurements with near-edge X-ray absorption fine structure (NEXAFS) spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS) provide a particularly powerful approach for investigating protein structures on surfaces.

Methods: Short, well-designed peptides were used as model systems to develop the methods for detailed analysis of protein-surface interactions at the molecular level. Leucine-lysine (LK) peptides bound to a variety of surfaces ranging from self-assembled monolayers (SAMs) on gold to spin-coated polymers to liquid surfaces were investigated. LK peptides are amphiphilic and depending on the hydrophobic periodicity of the amino acid sequence can form α-helix, 3^{10}-helix and β-strand secondary structures. SFG is a coherent nonlinear optical process: spectrally broad or tunable infrared and fixed visible laser pulses with high power are overlapped in time and space at an interface to generate photons at the sum of the incident beam frequencies. The signal is enhanced for IR frequencies in resonance with SFG-active vibrational modes at the interface yielding vibrational spectra. SFG is a second order process, so no signal is generated in isotropic, randomly ordered or inversion symmetric media. Consequently, the SFG signal is generated at the interface, where inversion symmetry is broken. According to the 'selection rules' of SFG, only molecular groups with a net order will contribute to the measured signal. NEXAFS, a technique where the x-ray energy is scanned through an absorption edge, provides spectra that exhibit characteristic resonances related to electronic transitions from an atomic core levels to unoccupied molecular orbitals. Thus, NEXAFS is sensitive to the order and orientation of molecular bonds. For proteins studies, amide bonds are probed using the nitrogen *K*-edge resonance near 400 eV. ToF-SIMS, which provides both high chemical specificity and surface sensitivity, bombards a surface with a high-energy primary ion beam that sputters molecular fragments off the surface. A fraction of the sputtered fragments carries a net charge and can be extracted through a time-of-flight mass analyzer. The result is a mass spectrum of the secondary ions ejected from the interface that can used be to investigate the identity, concentration, conformation and orientation of surface bound proteins.

Results: Figure 1 summarizes the SFG sampling geometry, as well as showing examples of SFG spectra in C-H stretching region and a schematic of how the leucine groups in an α-helical LK peptide interacts with a methyl terminated SAM. By deuerating the iso-propyl group of a given leucine side and collecting SFG spectra at different polarization combinations the orientation of that side chain with respect to the surface can be determined. Similarly SFG spectra of the amide I band can be used to determine the overall orientation of the peptide with respect to the surface. On hydrophobic surfaces the backbone of the helical LK peptides were found to be oriented parallel to the surface with the leucine side chains oriented at angles ranging from 30 to 80° from the surface normal. For the β-strand LK peptides NEXAFS showed the backbones were parallel to the surface.

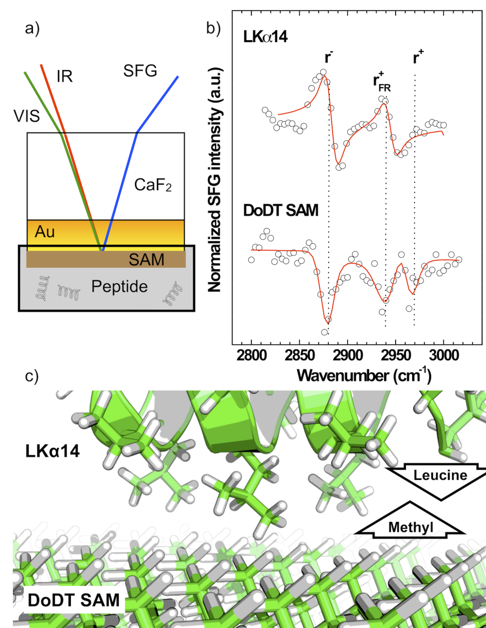

Figure 1. SFG results for α-helical LK peptide adsorbed onto a methyl terminated SAM.

We have also successfully expanded this approach to the B1 domain of the Protein G to determine secondary structure and backbone orientation of this protein immobilized either covalently or electrostatically onto appropriately functionalized surfaces.

Conclusions: SFG is a powerful tool to probe proteins on surfaces, but its full potential can only be realized when combined with complementary techniques. However, the combination of surface analytical tools alone can still not provide atomic structures of entire proteins. This requires the integration of computer modeling and stimulations with the experimental methods.

References: Weidner & Castner, PCCP 2013;15:12516.

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