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Multi-technique Characterization of Adsorbed Peptide and Protein Orientation: LK3₁₀ and Protein G B1

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Abstract

The ability to orient biologically active proteins on surfaces is a major challenge in the design, construction, and successful deployment of many medical technologies. As methods to orient biomolecules are developed, it is also essential to develop techniques that can accurately determine the orientation and structure of these materials. In this study, two model protein and peptide systems are presented to highlight the strengths of three surface analysis techniques for characterizing protein films: time-of-flight secondary ion mass spectrometry (ToF-SIMS), sum-frequency generation (SFG) vibrational spectroscopy, and near-edge x-ray absorption fine structure (NEXAFS) spectroscopy. First, the orientation of Protein G B1, a rigid 6 kDa domain covalently attached to a maleimide-functionalized self-assembled monolayer, was examined using ToF-SIMS. Although the thickness of the Protein G layer was similar to the ToF-SIMS sampling depth, orientation of Protein G was successfully determined by analyzing the C₂H₅S⁺ intensity, a secondary ion derived from a methionine residue located at one end of the protein. Next, the secondary structure of a 13-mer leucine-lysine peptide (LK3₁₀) adsorbed onto hydrophilic quartz and hydrophobic fluorocarbon surfaces was examined. SFG spectra indicated that the peptide's lysine side chains were ordered on the quartz surface, while the peptide's leucine side chains were ordered on the fluorocarbon surface. NEXAFS results provided complementary information about the structure of the LK3₁₀ film and the orientations of amide bonds within the LK3₁₀ peptide.

Keywords

ToF-SIMS; SFG; NEXAFS; peptides; proteins; biointerfaces

MATERIAL NAMES

Protein G B1; LK3₁₀ peptides; maleimide-oligo(ethylene glycol); hexafluoropropylene

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I. Introduction

Recently, there has been an explosion of interest in producing biomolecule-based sensors and diagnostic microarrays.¹ The main hurdle that must be overcome in the design and construction of these devices is creating a reproducible, multiplexed surface that optimizes the affinity towards specific biomarkers or targets (protein, DNA oligomer, etc).^{2–4} A variety of schemes have been employed to optimize the orientation and activity of immobilized species, including attachment via bioactive ligands^{5–14}, charge-driven orientation^{15–17}, and physical adsorption through hydrophobic interactions.^{18–20} Schematics of these immobilization schemes are shown in Figure 1.

To optimize the performance of protein microarray and antibody diagnostic technologies, there is an ongoing need for techniques that provide a detailed, accurate determination of protein conformation and orientation at surfaces. One technique that can provide this information is time-of-flight secondary ion mass spectrometry (ToF-SIMS). ToF-SIMS offers both high chemical specificity (mass resolution of ~ 5000 m/m and higher) and extreme surface sensitivity (sampling depths typically 1–3 nm).^{21,22} The technique involves bombarding a surface with a pulsed primary ion beam that sputters fragments from the surface. The small fraction of these fragments (<1%) that are ionized – the secondary ions – are then extracted into a time-of-flight mass analyzer. Both positive and negative secondary ions can be analyzed. The result is a comprehensive mass analysis of all secondary ions ejected from the surface.^{22,23}

The mechanics of how secondary ions originate within the collision cascade is complicated, dependent on the type and size of primary ion, and is beyond the scope of this work.²⁴ However, what is relevant is that different materials have distinct spectral fingerprints.²⁵ Mantus *et al.*, examining thin films composed of amino acid homopolymers, showed that amino acids fragment into distinct patterns of secondary ions, creating a list of characteristic peaks for each amino acid.²⁶ Recently, other groups have further developed these lists by expanding the analysis to include different primary ion sources.^{27–29} With these lists it is now possible to use ToF-SIMS for characterizing the compositions of protein films.^{25,30–32} Taking advantage of the small sampling depth of ToF-SIMS, researchers have also begun probing the conformation and orientation of surface immobilized proteins.^{15,33–37} For example, as the conformation of a protein changes (e.g., unfolding as it denatures), different amino acid side chains are brought into the ToF-SIMS sampling region. Such conformational changes are observed as changes in intensities of secondary-ions from hydrophobic and hydrophilic amino acids.³⁸ Similarly, information about orientation is provided by the intensities of secondary ions originating from amino acids asymmetrically distributed in the protein's three dimensional structure.^{15,34,38,39}

To complement ToF-SIMS studies of protein conformation and orientation, techniques such as sum frequency generation (SFG) vibrational spectroscopy^{40,41} and near-edge x-ray absorption fine structure (NEXAFS) spectroscopy⁴² can be used to examine both the ordering of secondary structural elements within a protein and how specific side chains interact with a surface. SFG vibrational spectroscopy probes amide, N-H, C-H, and O-H

vibrational modes to identify specific secondary structures (α -helices, β -sheets and β -turns) and detect ordering of specific amino acid side chains. To date, SFG has been used to characterize a range of surface interactions, from single amino acids at a surface to monolayers of large proteins.^{43–49}

During an SFG experiment, two photon sources, one with a fixed frequency in the visible range and the other with a tunable frequency in the IR range, are overlapped in time and space at a sample interface.^{50,51} If the photon created from the sum of the incoming visible and IR beams has a frequency that matches a second order vibrational mode of a chemical species at the sample interface, the signal intensity of the reflected photons will change. One limitation that results from this second order process is that the molecule of interest must be both IR and Raman active. In addition, since SFG is a second order non-linear optical technique, no change in the intensity response will be observed in a medium with inversion symmetry (i.e., an isotropic medium). Fortunately for surface scientists, inversion symmetry is always broken at interfaces and surfaces, including the protein/substrate interface.

NEXAFS spectroscopy can supplement SFG data by providing information about the electronic structure of surface species.⁴² Polarized synchrotron x-rays are absorbed by the core levels of surface atoms, resulting in the emission of photo, auger and secondary electrons. By following the change in the x-ray absorption intensity as the incident angle of the x-rays is varied, the orientation and tilt angles of ordered molecular bonds can be determined. Recently, groups have used NEXAFS to characterize DNA oligomers, model peptides and proteins immobilized to a surface.^{11,12,52–54}

The work presented in this study describes two model systems created to further develop ToF-SIMS, SFG and NEXAFS for molecular level characterization of the chemical state, orientation, and structure of immobilized biomolecules. The first system is the rigid 6 kDa Protein G B1 domain, covalently attached to a maleimide-functionalized self-assembled monolayer in a well-defined orientation. The second system is a 13 amino acid model peptide of leucine (leu or L) and lysine (lys or K) residues designed to form a 3–10 helix (LK3₁₀) adsorbed onto hydrophobic fluorocarbon and hydrophilic quartz surfaces.

II. Experimental

a. Protein G B1 films

Protein G B1 wild type and a mutant with a single cysteine residue inserted into a loop near the C-terminus (T11C, threonine11 mutated to cysteine) were immobilized onto a maleimide-oligo(ethylene glycol) self-assembled monolayer on gold (MEG-SAM)¹². These monolayers were assembled following previously published protocols and the quality of the MEG-SAM was monitored by x-ray photoelectron spectroscopy (XPS). Mutagenesis was performed using a synthetic Protein G B1 gene and mutants were expressed using the T7 expression system in BL21(DE3) *E. coli* (Invitrogen, Carlsbad, CA). Expressed protein was purified by anion exchange chromatography and characterized using electrospray mass spectroscopy and amino acid analysis (AAA Laboratory, Mercer Island, WA). For adsorption, MEG-SAM substrates were submerged in a buffer solution (50 mM sodium carbonate, pH 9.5) and protein was added for a final concentration of 2 mg/mL. The reaction

proceeded for 3 h at room temperature. Samples were washed by serial dilution with buffer followed by submerging in a series of ultra-pure water baths. Samples were then air dried and stored under nitrogen.

Protein coverage was monitored using changes in atomic percent nitrogen as determined by XPS analysis. The XPS data was acquired on a Kratos AXIS Ultra DLD instrument (Kratos, Manchester, England) in the hybrid mode using a 0° take-off angle (defined as the angle between the surface normal and the axis of the analyzer lens) and a monochromatic Al $K\alpha_{1,2}$ X-ray source ($h\nu=1486.6$ eV). Atomic compositions were calculated from peak areas obtained from 0–1100 eV survey scans (C_{1s} , Au_{4f}) and selected region scans (524–544 eV, O_{1s} ; 390–410 eV, N_{1s} ; 155–173 eV, S_{2p}) acquired at analyzer pass energy of 80 eV.

b. ToF-SIMS

All positive secondary ion spectra were collected on a TOF.SIMS 5–100 (ION-TOF, Münster, Germany) system with a pulsed 25 keV Bi_3^+ ion source under static conditions (primary ion dose $< 10^{11}$ ions/cm²). For each sample, five spectra were collected from $100\ \mu m \times 100\ \mu m$ regions at mass resolutions (m/m) between 4000 and 8000. Positive ion spectra were mass calibrated using CH_3^+ , $C_2H_3^+$, $C_3H_5^+$, and $AuSCH_2^+$ where m/z corrections were typically below 20 ppm. To make comparisons across spectra, each spectrum was normalized to the total ion intensity.

c. Peptide Films

Amino acids used to synthesize LK3₁₀ and LK β 9 peptides were Fmoc-Leu-OH and Fmoc-Lys(Boc)-OH (Novabiochem). The peptides were synthesized on a PS3 solid-state peptide synthesizer (Rainin) on a Leu-Wang resin (Novabiochem). O-(Benzotriazole-N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) (Advanced ChemTech) was used as an activator, and the N-termini of the resin-bound peptides were capped following synthesis by acetylation with 3 mL of acetic acid. Peptides were cleaved from the resin, dried by overnight lyophilization and their purity was checked using mass spectrometry following Long *et al.*⁵⁵ The final peptide sequences were Ac-LLKLLKLLKLLKL-OH (LK3₁₀) and Ac-LKLKLLKLLKL-OH (LK β 9).

Fluorocarbon substrates were deposited from a hexafluoropropylene (HFP) RF glow discharge onto silicon substrates using a home-built, inductively-coupled RF reactor^{54,56}. Phosphate buffered saline (PBS, pH 7.4) for the peptide adsorption was obtained from Fischer Biotech. The samples for NEXAFS analysis were prepared by placing $1\ cm \times 1\ cm$ pieces of the fluorocarbon coated silicon into 20 $\mu g/ml$ of the LK3₁₀ or LK β 9 peptide PBS solution, avoiding the air-buffer interface. The adsorption was performed for 2 hours in 1.5 ml polystyrene cups. The samples were then washed with deionized water by dilution displacement, blown dry with nitrogen and then stored in plastic containers under inert gas until characterization.

d. NEXAFS Spectroscopy

The NEXAFS experiments were performed at the National Synchrotron Light Source (NSLS) U7-A beamline at Brookhaven National Laboratory. Details of the data acquisition

procedure are published elsewhere.⁵⁷ Briefly, the nitrogen and oxygen edge data were calibrated to the intense π^* amide features of poly(glycine) at 401.5 eV and a V₂O mesh, respectively. The NEXAFS spectra were normalized with the signal from an *in situ* gold coated 90% transmission grid placed in the path of the X-rays. Partial electron yield (PEY) was monitored using a bias voltage of -390 V and -280 V for the oxygen and nitrogen *K*-edges, respectively. The pre-edge was subtracted from each spectrum using a linear background and then each spectrum was normalized to a unity absorption jump height.

e. SFG Spectroscopy

SFG spectra were obtained by overlapping visible and tunable IR laser pulses (25 ps) in time and space at incidence angles of 60° and 54°, respectively.⁵⁷ The visible beam with a wavelength of 532 nm was delivered by an EKSPLA Nd:YAG laser operating at 10 Hz. Both beams were unfocussed and had a diameter of approximately 3 mm at the sample. The energy for both beams was 160 μ J per pulse. The spectra were collected with 400 shots per data point in 1 to 4 cm^{-1} increments. All spectra were recorded in the *ssp* (sum, visible, and infrared) polarization combination. FC-coated CaF₂ and cleaned bare SiO₂ windows were brought into contact with the peptide solution in a 1× PBS buffer solution and the interface was then probed going through the backside of the window.

III. Results and Discussion

a. Probing Protein G B1 Orientation with ToF-SIMS

ToF-SIMS has been used previously to characterize the conformation and orientation of absorbed proteins. In most of this previous work, the protein film characterized was significantly thicker than the ToF-SIMS sampling depth (e.g., a 10 nm protein layer versus a 1–3 nm ToF-SIMS sampling depth).^{15,34–37} In this study, the first model system involved using a $\sim 1.5 \times 1.5 \times 3.0$ nm rigid protein domain to test the limits of ToF-SIMS to determine the orientation of a small surface immobilized protein.

This model system is built upon three different forms of Protein G B1 immobilized onto MEG-SAM substrates (Figure 2). These included Protein G B1 wild type (WT), a variant with a cysteine residue substituted for threonine in a loop at one end of the protein (T11C), and an isotopically labelled form of T11C incorporating ¹³C-labeled methionine near the N-terminus (T11C*). Previously, these MEG-SAMs were used as an attachment point for thiolated DNA and for cysteine mutants of Protein G B1.^{12,58} The T11C cysteine was inserted to induce specific binding to the maleimide ring at the outer surface of the MEG-SAM. This produced a well-ordered protein layer ~ 3 nm in height. In this ordered state, the methionine near the N-terminus, which is located at the opposite end of the protein from the cysteine, is expected to be at the top of the immobilized protein layer (Figure 2A). In contrast, wild type Protein G B1 contains no cysteines and should non-specifically adsorb onto the MEG-SAM producing a protein layer consisting of random orientations (Figure 2B). In this “disordered” state, the methionine residue can be either buried or exposed. Therefore, secondary ion intensities from the methionine are expected to be higher for the well-ordered T11C samples than for the WT samples.

Figure 3 shows positive secondary-ion mass spectra for the most intense fragment originating from the methionine residue, the $C_2H_5S^+$ fragment at $m/z = 61.0112$ for the unlabeled proteins and the $^{13}CCH_5S^+$ fragment at $m/z = 62.0112$ for ^{13}C -Met-labeled T11C (T11C*).^{26,29} Spectra acquired from a MEG-SAM before protein immobilization and from MEG-SAMs after WT, T11C, and T11C* immobilization are shown. The first comparisons made were between unlabeled WT and T11C samples using the $C_2H_5S^+$ peak. There is a significant difference in the $C_2H_5S^+$ fragment intensity between these two samples. This difference cannot be attributed to different Protein G B1 surface coverages since XPS-determined compositions from both samples showed similar amounts of nitrogen (Table 1). The observed XPS nitrogen concentrations were consistent with monolayer coverage of Protein G B1.⁵⁸ Therefore, this ToF-SIMS intensity difference was a result of different protein orientations, consistent with a well-ordered T11C protein layer with methionine near the top of the film.

However, it is important to note that the spectrum from the MEG-SAM sample before protein immobilization has a significant $C_2H_5S^+$ peak since this peak also originates from the thiol group of the MEG-SAM. To differentiate the contribution of the substrate-derived MEG-SAM $C_2H_5S^+$ peak from the protein-derived methionine $C_2H_5S^+$ peak, ^{13}C -labeled methionine was incorporated into T11C (T11C*) to create a shifted peak. This labeling shifts the methionine $C_2H_5S^+$ peak by one mass unit to $m/z = 62.0112$. The $^{13}CCH_5S^+$ peak intensity from the T11C* sample (large peak on the right) is approximately equal to the $C_2H_5S^+$ ($m/z = 61.0112$) peak intensity from the T11C sample (large peak on the left). (Note: all spectra in Figure 3 have been normalized to the total ion intensity of each spectrum.) The WT/MEG-SAM sample exhibits a significantly smaller methionine-derived peak intensity than for either T11C or T11C*, despite a similar amount of protein immobilized based on % N from XPS analysis. This indicates that the methionine in the WT sample is, on average, more buried or further from the top of the protein film than in the T11C samples. This is consistent with an increased degree of orientation in the T11C samples.

b. Probing the orientation and secondary structure of 3_{10} -helical LK peptides with SFG and NEXAFS spectroscopy

Small synthetic peptides are ideal model systems to develop methods to probe surface bound proteins with molecular level detail.⁴⁶ Especially valuable model systems are amphiphilic peptides comprised of only two different amino acids. Hydrophobic and charged residues have successfully been used to elucidate basic principles of peptide immobilization.^{53,59} Studies of peptides made from hydrophobic leucine (L) and positively charged lysine (K), referred to as LK peptides, have been particularly successful in this context.^{60,61} The secondary structure of LK peptides can be tuned by varying the hydrophobic periodicity, i.e., the L and K positions within the sequence.^{60,61} To date, most SFG studies have used α -helix and β -sheet variants.^{59,62–64} Here, we report a detailed analysis of adsorbed LK peptides with 3_{10} -helical secondary structures (see Figure 4 for a drawing of the 3_{10} LK peptide structure). The 3_{10} -helix has a lower dihedral angle compared to the α -helix because the internal stabilization of the 3_{10} -helix structure occurs between the N-H group of an amino

acid residue with the C=O group of an amino acid three positions away in the primary structure, as opposed to four positions away in the α -helix.

Figure 5 shows SFG spectra of LK3₁₀ on hydrophobic fluorocarbon and hydrophilic SiO₂ surfaces recorded *in situ* in PBS. Note that the technique only detects ordered peptides at the interface, even though peptides are present in solution during the measurements. This is due to the “selection rules” of SFG spectroscopy.^{65,66} On the SiO₂ surface, the spectrum is dominated by a pronounced feature near 3300 cm⁻¹ and a shoulder near 3200 cm⁻¹ (evident from the asymmetry of the 3300 cm⁻¹ mode). These resonances are related to ordered lysine side chains⁶² and tetrahedrally coordinated water molecules at the interface.^{67–70} The spectra are similar to those observed for α -helical LK peptides, where positively charged lysines become ordered upon charge-charge interactions with negatively charged surfaces (the quartz surface is negatively charged at pH 7.4).⁶² Water molecules also become ordered at the quartz-water interface, and the OH modes observed in Figure 5 are also found in ice crystals. Whether the layer of coordinated water is located near the lysine-surface bonds or near the hydrophobic leucines cannot be determined from the data. Since tetrahedrally coordinated water molecules are typically not detected near hydrophobic surfaces such as gold, polystyrene and methyl terminated self-assembled monolayers,⁷¹ the first possibility is the more likely explanation.

Conversely, the spectrum of LK3₁₀ on hydrophobic FC surfaces (Figure 5, right panel) shows no feature at 3300 cm⁻¹, but sharp resonances are present below 3000 cm⁻¹ that are related to the leucine CH₃-modes⁷² as well as two broad OH resonances. The first OH mode near 3200 cm⁻¹ is also observed on SiO₂, while the second peak near 3400 cm⁻¹ is commonly assigned to ordered water molecules with a less-than-tetrahedral coordination (similar to liquid water).^{67–70,73} Because the leucine isopropyl moieties are the only source of CH₃ groups in the peptide, the presence of the CH₃ modes in the spectra indicates that the leucine side chains become ordered upon adsorption. The leucine side chains likely become ordered due to hydrophobic interactions with the fluorocarbon surface. This would expose the lysines located on the opposite face of the LK3₁₀ peptide to the surrounding water and lead to the formation of ordered water molecules. The pronounced water peaks observed in the spectrum are consistent with water molecules ordered by the lysine side chains, and the overall spectra are similar to those observed for α -helical LK peptides.^{49,53,54}

In addition to orientation, the secondary structure of the adsorbed peptides was probed by examining the amide I region of the SFG spectrum. Amide I modes are very sensitive to secondary structure and have been used successfully in IR⁷⁴ and SFG studies.⁴⁶ Figure 6 displays the amide I spectrum for the LK3₁₀ peptide adsorbed onto a fluorocarbon surface. The pronounced peak near 1655 cm⁻¹ can be assigned to a helical secondary structure.^{46,74} This indicates that LK3₁₀ maintains its helical secondary structure when adsorbed onto the surface. Details such as small variations of dihedral angles in the peptide backbone cannot be inferred from the SFG spectrum in Figure 6. Thus, we cannot exclude minor changes to the helical structure after adsorption. Since SiO₂ is not transparent to the IR laser light in this spectral region, SFG amide I spectra of peptides adsorbed onto the SiO₂ surface were not acquired.

The present study provides important details, such as the overall orientation and the secondary structure of LK3₁₀ on different surface chemistries. A more detailed analysis of the orientation of individual side chains within the peptides and their absolute orientation can be performed using isotopically labeled peptides and phase sensitive measurements.

NEXAFS spectra provide complementary information about the electronic structure of the LK3₁₀ films by sampling the electronic structure of the unoccupied molecular orbitals of the peptides.⁴² Nitrogen *K*-edge spectra for the LK3₁₀ films acquired at 90° and 20° x-ray incidence angles are shown in Figure 7 (left panel) along with NEXAFS spectra of a β -strand LK peptide variant for comparison. The spectra exhibit a narrow resonance near 401.5 eV, related to transitions from the N 1s to the amide π^* orbitals, as well as two broader resonances near 406.4 eV and 413.0 eV assigned to transitions into the N-H σ^* orbitals.^{75–78} The weak pre-edge feature near 398.8 eV can either be assigned to positively charged amine groups on the lysine side chains^{79,80} or traces of nitrogen contaminants in the underlying FC film.

NEXAFS spectra also provide information about the orientation and order of the film constituents. The intensities of NEXAFS resonances depend on the relative orientations of the electric field vector of the incoming x-rays and the transition dipole moment of the excitation from the atomic core levels into the respective molecular valence orbitals.⁴² This linear dichroism is typically monitored by calculating the difference between spectra acquired at normal (90°) and grazing (20°) x-ray incidence. The LK3₁₀ peptide exhibits a weak but discernible negative linear dichroism for the amide resonance near 401.5 eV. The weak dichroism can be explained by the intrinsically broad distribution of amide bond orientations in helical secondary structures.⁵³ However, the amide bond can be separated into two components: a radial component with an almost uniform orientation distribution (not leading to any NEXAFS dichroism) and a component along the backbone axis. The negative sign of the dichroism shows that the amide bond is oriented nominally parallel to the surface, since the transition dipole moment of the latter amide π^* component is oriented perpendicular to the plane of the peptide (CONH) bond.⁴² For LK β 9, the characteristic π^* and σ^* resonances show a strong negative dichroism (Figure 7). Since the amide π^* orbitals are oriented perpendicular to the amide bond, the observed intensity differences indicate β -strands are adsorbed in a conformation with backbones parallel to the FC surfaces. A similar trend has also been observed for α -helix 14-mer and β -strand 15-mer LK peptides on self-assembled monolayers and FC surfaces.^{53,57}

This orientational analysis is further supported by O *K*-edge NEXAFS spectra for 90° and 20° x-ray incident angles shown in Figure 7. The spectra consist of a sharp pre-edge feature near 531.8 eV related to transitions into the CO π^* orbital along with two broad σ^* resonances at higher photon energies.⁸¹ Consistent with the N *K*-edge data, the LK β 9 spectra exhibit a significantly stronger variation with x-ray incidence angle compared to the LK3₁₀ spectra. The pronounced negative dichroism of the CO π^* resonance for LK β 9 confirms the CO bond is nominally oriented parallel to the interface.

Conclusions

There are many techniques that allow surface scientists to study interfaces. However, few are routinely applied to probe biological interfaces. Here we describe three techniques (ToF-SIMS, SFG, and NEXAFS) that provide detailed information about the conformation, orientation, chemical state, and molecular structure of biological molecules immobilized onto a surface. ToF-SIMS was used to determine the orientation of the Protein G B1 domain covalently attached to a surface by tracking the intensities of specific secondary ions. SFG and NEXAFS were used to probe the ordering and orientation of secondary structures of a model leucine-lysine peptide adsorbed onto hydrophobic and hydrophilic surfaces. The systems described here are simple and far less complex than what is typically encountered in the real world. However, by first building and characterizing model systems, a suite of tools for characterizing the structure of surface bound biomolecules can be developed and used for designing microarray and diagnostic technologies.

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Figure 1.
Surface immobilization strategies used to control protein orientation.

A. T11C & T11C*

B. WT

Figure 2.

A) Schematic diagram of the Protein G1 B1 variant, T11C, with a cysteine introduced at one end of the protein and immobilized via the cysteine thiol onto maleimide-functionalized gold (MEG-SAM) substrates. T11C contains one methionine (black, spacefill), located at the opposite end of the protein from the cysteine. **B)** Schematic of the Protein G1 B1 wild type (WT) samples. Protein G1 B1 WT contains no cysteines and is expected to be randomly oriented on the MEG-SAM surface.

Figure 3.

Positive secondary ion spectra near $m/z = 61\text{--}62$ acquired from MEG-SAM-only (dashed line), WT (solid line), T11C (solid circles), and T11C* samples (open circles). The intensities of the $\text{C}_2\text{H}_5\text{S}^+$ and $^{13}\text{CCH}_5\text{S}^+$ peaks were used to demonstrate the T11C and T11C* samples were aligned in an upright orientation. (Note: all spectra in this Figure have been normalized to the total ion intensity of each spectrum.)

Figure 4. Schematic drawing of the LK₃₁₀ peptide used for the SFG and NEXAFS studies. The peptide consists of only two different amino acids, leucine (hydrophobic) and lysine (hydrophilic) that are located on opposite faces of the backbone helix, making it a promising model system for protein adsorption studies. While this peptide was designed to form a 3_{10} -helix, the methods used in this study do not allow us to completely distinguish between 3_{10} - and α -helical peptide secondary structures.

Figure 5.

SFG spectra of the LK3₁₀ peptide adsorbed onto hydrophilic SiO₂ and hydrophobic fluorocarbon surfaces. The spectra were acquired *in situ* with the surfaces exposed to the peptide solutions and peaks are only detected from ordered groups at the interface. The data show that different parts of the peptide become ordered on different surfaces – the hydrophobic leucines on the hydrophobic substrate and the positively charged lysines on the negatively charged substrate – likely because of different binding mechanisms and peptide orientations.

Figure 6.

SFG Amide I spectrum of LK₃₁₀ adsorbed onto a hydrophobic fluorocarbon surface. The mode detected near 1655 cm⁻¹ is related to the helical secondary structure of the adsorbed peptide.

Figure 7. Nitrogen and oxygen K-edge NEXAFS spectra (at both 90° (red) and 20° (blue) x-ray incidence angles) from the LK3₁₀ and LKβ peptides adsorbed onto the hydrophobic fluorocarbon surface.

Table 1

XPS determined compositions of the Protein G samples.

Sample	C(1s)	O(1s)	N(1s)	S(2p)	Au(4f)
MEG-SAM ^a	54.7 ± 0.7	16.3 ± 0.2	3.1 ± 0.2	0.9 ± 0.1	25.0 ± 0
WT ^a	56.7 ± 0.8	18.3 ± 0.4	6.2 ± 0.5	1.5 ± 0.0	17.4 ± 1
T11C ^a	58.1 ± 0.9	16.5 ± 0.2	7.2 ± 0.3	1.1 ± 0.1	17.1 ± 0
T11C* ^b	53	16.2	6.1	1.9	22.7

^{a)}Composition is the average of three spots taken from one sample.

^{b)}Composition is taken from just a single spot on one sample.