ToF-SIMS and XPS Characterization of Protein Films Adsorbed onto Bare and Sodium Styrene Sulfonate Grafted Gold Substrates

Rami N. Foster,[†] *Elisa T. Harrison*,[†] *David G. Castner*[*†‡]

[†] National ESCA and Surface Analysis Center for Biomedical Problems, Department of Chemical Engineering, University of Washington – Seattle, Seattle, WA 98195

[‡] National ESCA and Surface Analysis Center for Biomedical Problems, Department of Bioengineering, University of Washington – Seattle, Seattle, WA 98195

Corresponding Author

*castner@uw.edu

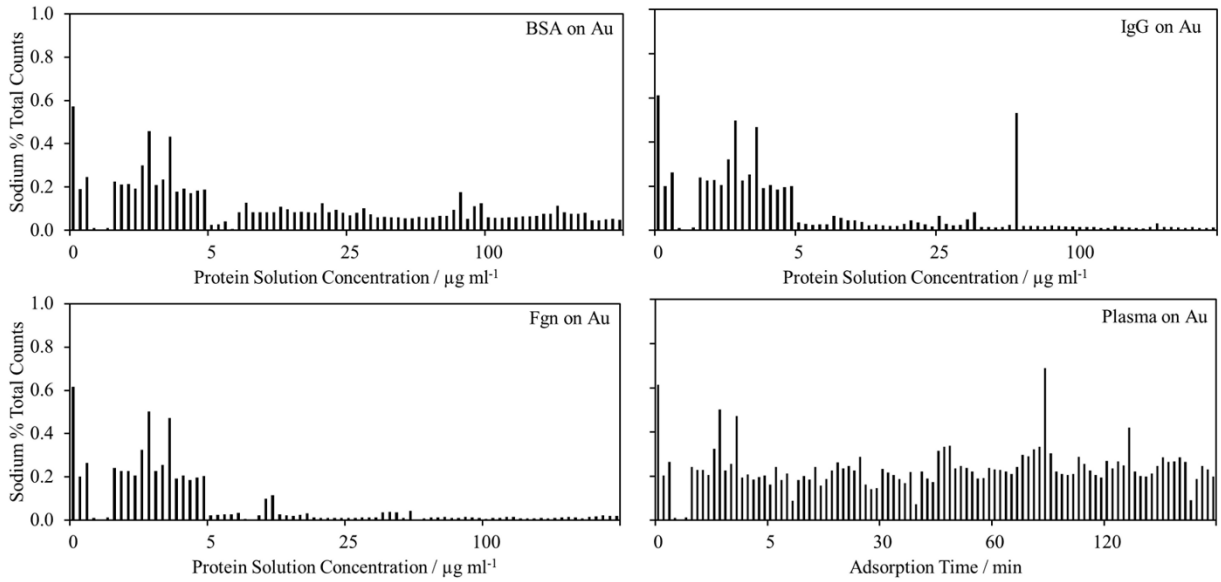

Figure S1. Percentage of the total secondary-ion counts accounted for by the sodium peak (m/z 22.99) for BSA, Fgn, IgG, and plasma adsorbed onto gold surfaces. Spectra containing sodium counts >1% of the total counts are typically removed from the dataset. The sodium counts in these set of spectra never exceed ~0.7%.

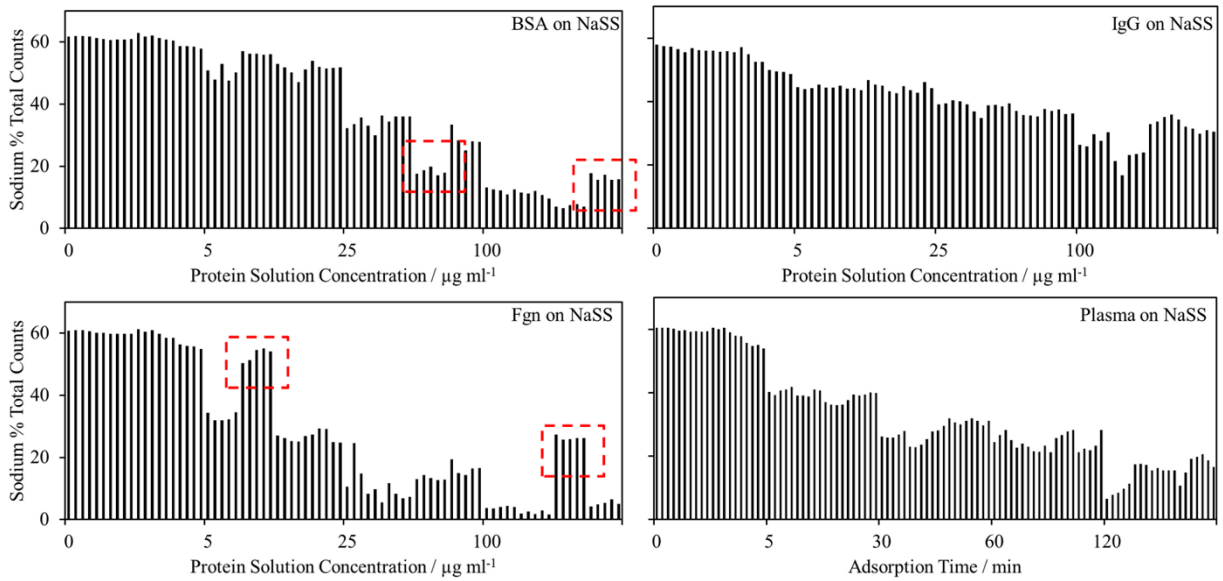

Figure S2. Percentage of the total secondary-ion counts accounted for by the sodium peak (m/z 22.99) for BSA, Fgn, IgG, and plasma adsorbed onto NaSS surfaces. Spectra containing sodium counts >1% of the total counts are typically removed from the dataset. The sodium counts in this set of spectra ranged from 2% – 60%. However, the sodium is part of the NaSS substrate and do not exhibit the same influence on the spectra that free buffer salts do. The samples highlighted with red boxes are the outlier groups discussed in the text.

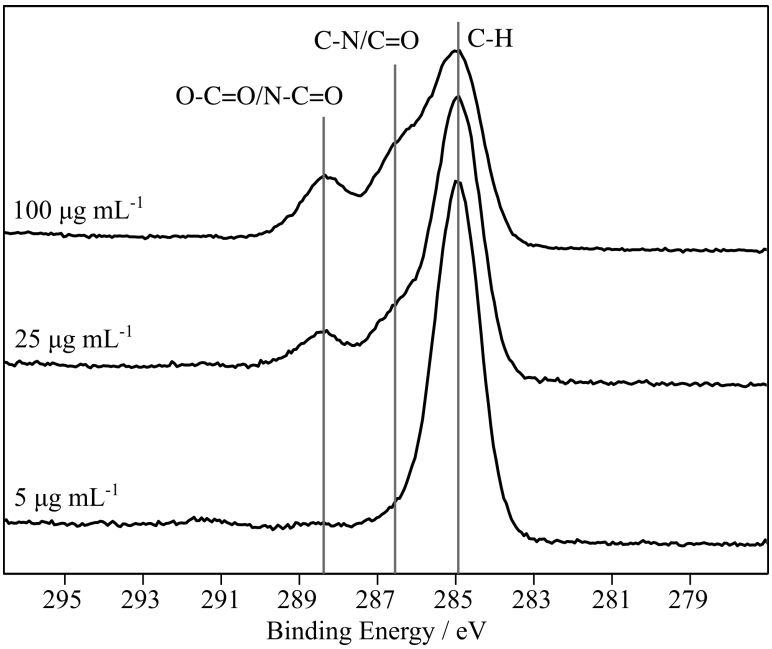

Figure S3. High-resolution XPS C 1s spectra of BSA adsorbed onto NaSS from solution concentrations of 5, 25, and 100 μg/mL.

PCA of All Positive and Negative Peaks

While the focus of this paper is how adsorbed protein surface structure changes between bare and NaSS-grafted gold substrates, this section briefly addresses some overarching trends observed in the ToF-SIMS data. The PCA results discussed below were obtained using peak lists containing all positive or negative peaks with intensity at least three times the background.

PCA of All Peaks on Gold

In both polarities, PCA clearly distinguishes the protein-free from the protein-containing surfaces (panels A through C of figures S3 – S8 in the supplemental information). However, in the positive polarity, clear separation between sample groups with different protein surface coverages is observed only for BSA. Better separation is observed in the negative polarity; where samples prepared at different solution concentrations clearly separate for Fgn and IgG, but not for BSA. Regardless of polarity, the variance in the data is dominated by differences between the protein-free and protein-containing samples, which separate along PC1. In the positive polarity, this separation appears to be based on small hydrocarbon peaks loading (negatively) with the protein-free samples, and amino acid fragments loading (positively) with the protein-containing samples. One notable exception is the arginine amino acid peak (m/z 73.06, $C_2H_7N_3$), which consistently loads with those samples that should be protein-free. Inspection of the raw spectra reveals that the arginine peak overlaps with a broad peak found only in the protein-free samples. A likely assignment for this peak is SiC_3H_9 at m/z 73.19, with poly(dimethyl silicone) (PDMS) contamination the likely source. Therefore, the m/z 73.06 peak was excluded from the protein structure analyses discussed below in the PCA of Amino Acid Mass Fragments section. Other arginine peaks (m/z 100.9, $C_4H_{10}N_3$; m/z 101.1, $C_4H_{11}N_3$; and m/z 127.1, $C_5H_{11}N_4$) were retained, minimizing the impact of the information lost. The small hydrocarbons detected on the protein-free samples are likely from adventitious carbon, which is then displaced by adsorbing protein. Thus, separation is based on substrate vs. amino acid peaks. This also appears to be the case in the negative polarity, where small hydrocarbon fragments, gold, and contaminants load (negatively) with the protein-free samples, and nitrogen-containing peaks load (positively) with the protein-containing samples. If there is any separation between the protein-containing treatment groups, it occurs along PC2. In certain cases, PC2 scores increase with increasing protein concentration. In other cases, the opposite is true. Therefore, few clear trends were observed in PC2.

PCA of All Peaks on NaSS

Despite being a more complex system, PCA is better able to separate between treatment groups on NaSS than on gold (panels D through F of figures S3 – S8 in the supplemental information). For all proteins, PC1 scores increase with increasing protein adsorption solution concentration. This is consistent our observations on gold. However, the increase in PC1 scores is more gradual since the increase in protein surface concentration is more gradual on NaSS versus gold, as

evidenced by the XPS isotherms. In both polarities, substrate peaks are associated with the samples prepared from the 0 and 5 μg/ml protein solutions. Interestingly, a number of the high loading substrate peaks in the positive polarity are sodium adducts of the same peaks observed in the negative spectra. And in both polarities, amino acid fragments are associated with samples prepared from the 25 and 100 μg/ml protein solution concentrations. Breaking with this trend in the negative polarity is the NaSS monomer ($C_8H_7SO_3$), which paradoxically loads (positively) with the samples prepared from the 25 and 100 μg/ml protein solution concentrations. There is some separation along PC2 in both polarities. However, there are no obvious trends in the loadings that lead to a physical explanation for the source of variance.

In summary, for both gold and NaSS surfaces, and for both positive and negative polarities, PC1 scores trend with protein surface concentration, as was discussed above in the XPS and ToF-SIMS isotherm section. This is confirmed by the loadings, which associate substrate peaks with the protein-free samples, and amino acid peaks with the protein-containing samples. Some separation is observed along PC2 between samples exposed to different protein solution concentrations, but not consistently. Also, no discernable trends are observed in the loadings to explain the separation. More information regarding adsorbed protein structure is likely to be obtained by narrowing the peak list to include only amino acid-derived mass fragments.

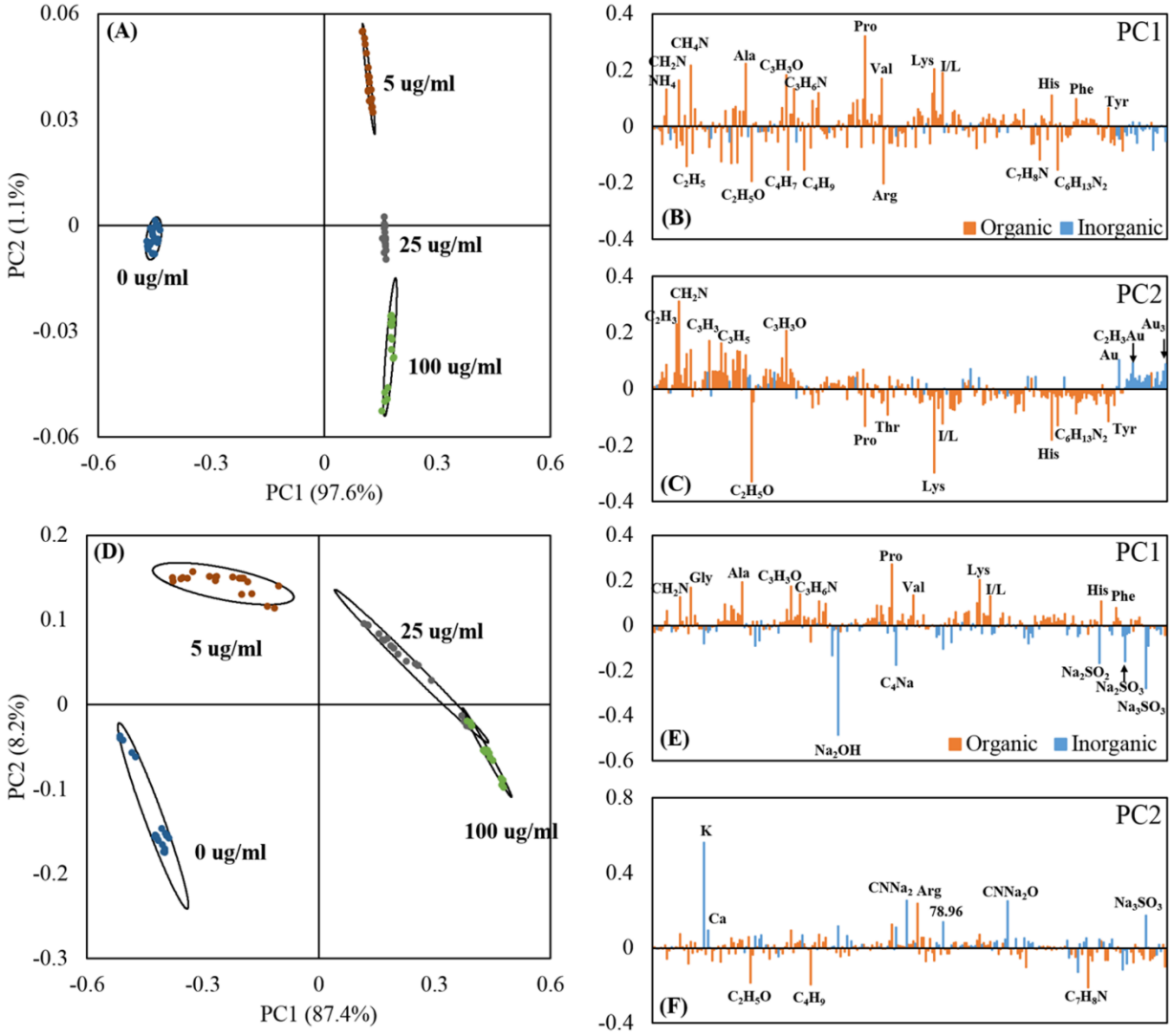

Figure S4. Adsorbed BSA PCA results using all peaks in the positive ion spectra. Panels A – C correspond to the scores and loadings plots for BSA adsorbed onto gold surfaces. Panels D – F correspond to the scores and loadings plots for BSA adsorbed onto NaSS surfaces.

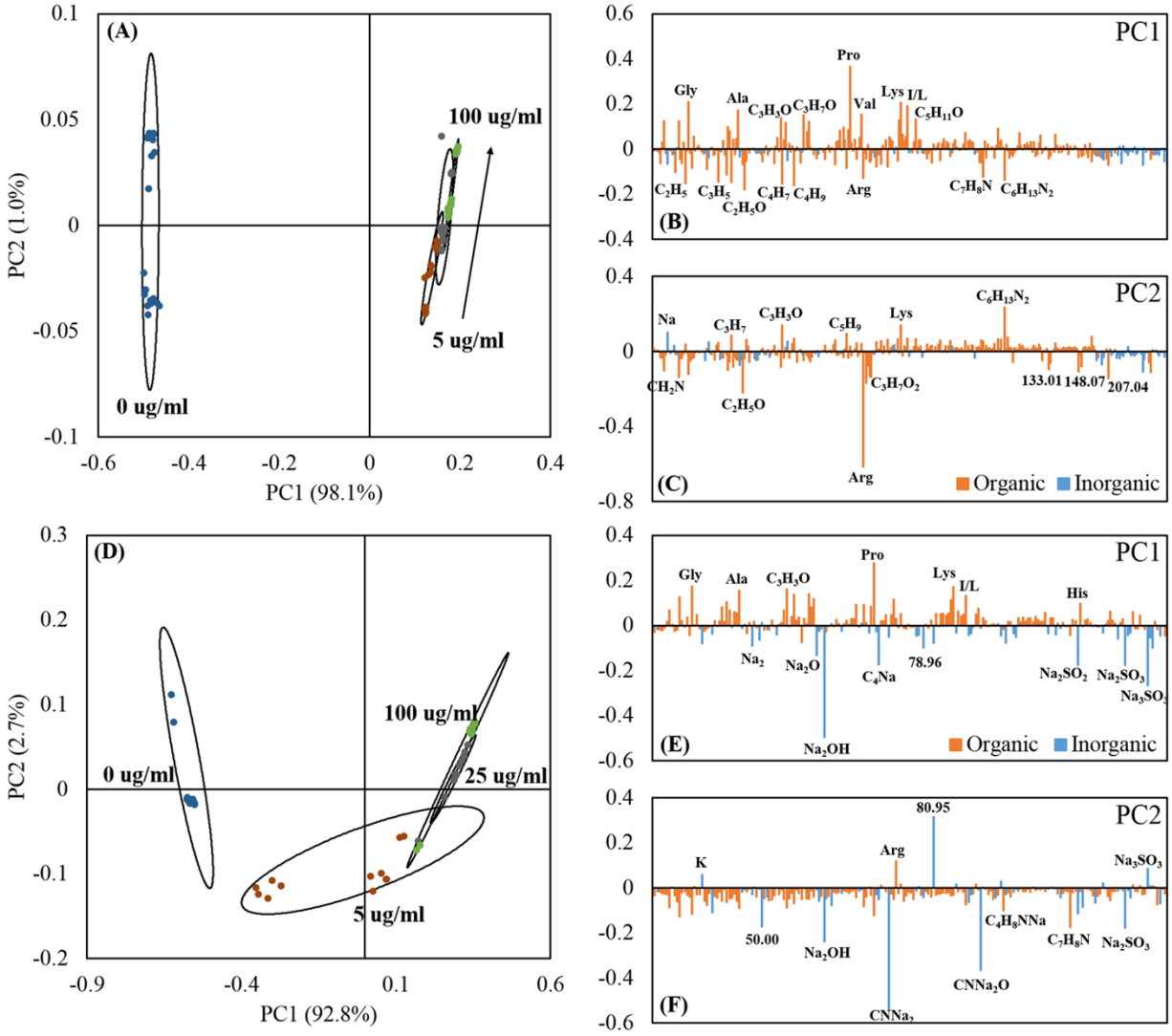

Figure S5. Adsorbed Fgn PCA results using all peaks in the positive ion spectra. Panels A – C correspond to the scores and loadings plots for Fgn adsorbed onto gold surfaces. Panels D – F correspond to the scores and loadings plots for Fgn adsorbed onto NaSS surfaces.

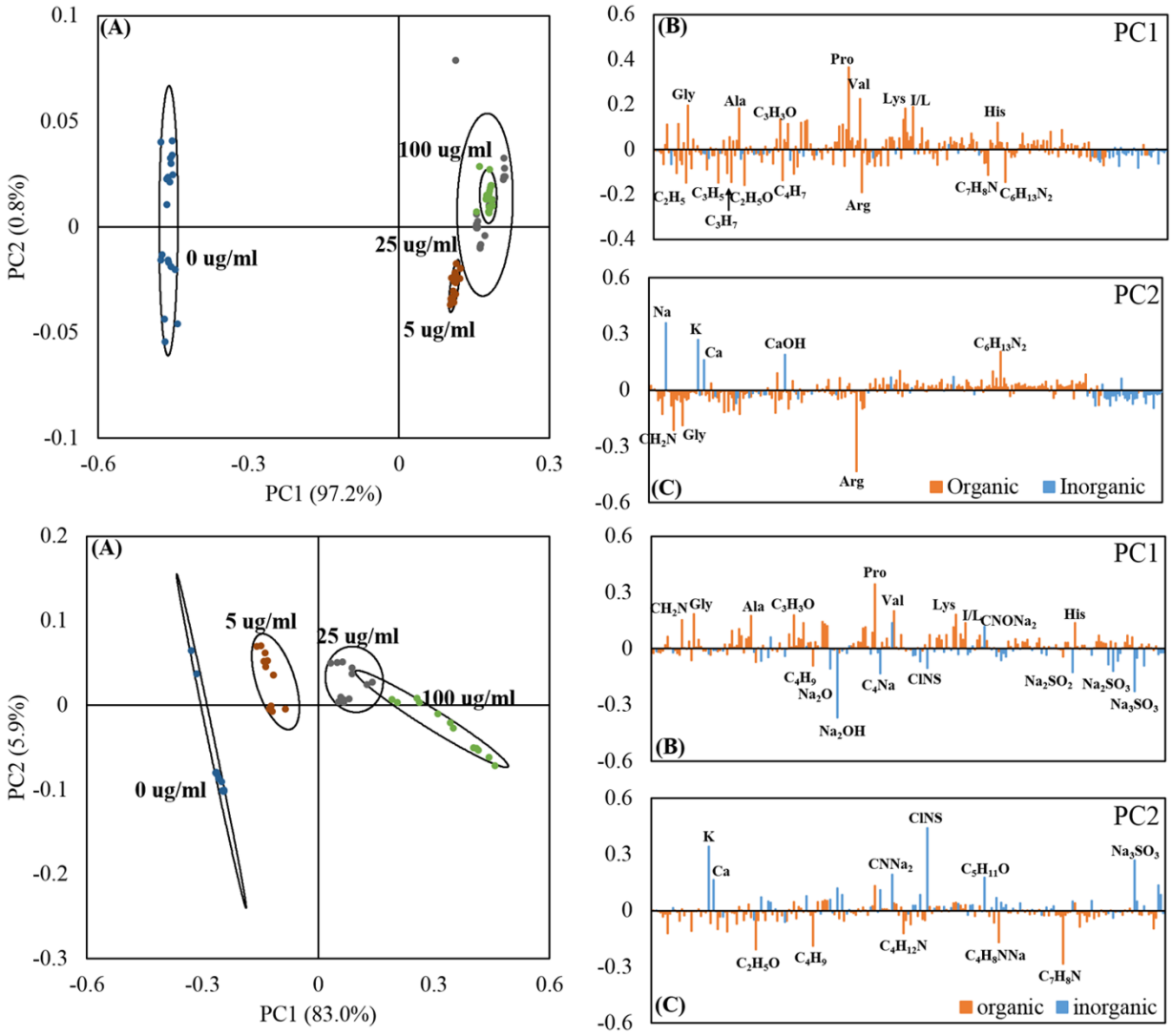

Figure S6. Adsorbed IgG PCA results using all peaks in the positive ion spectra. Panels A – C correspond to the scores and loadings plots for IgG adsorbed onto gold surfaces. Panels D – F correspond to the scores and loadings plots for IgG adsorbed onto NaSS surfaces.

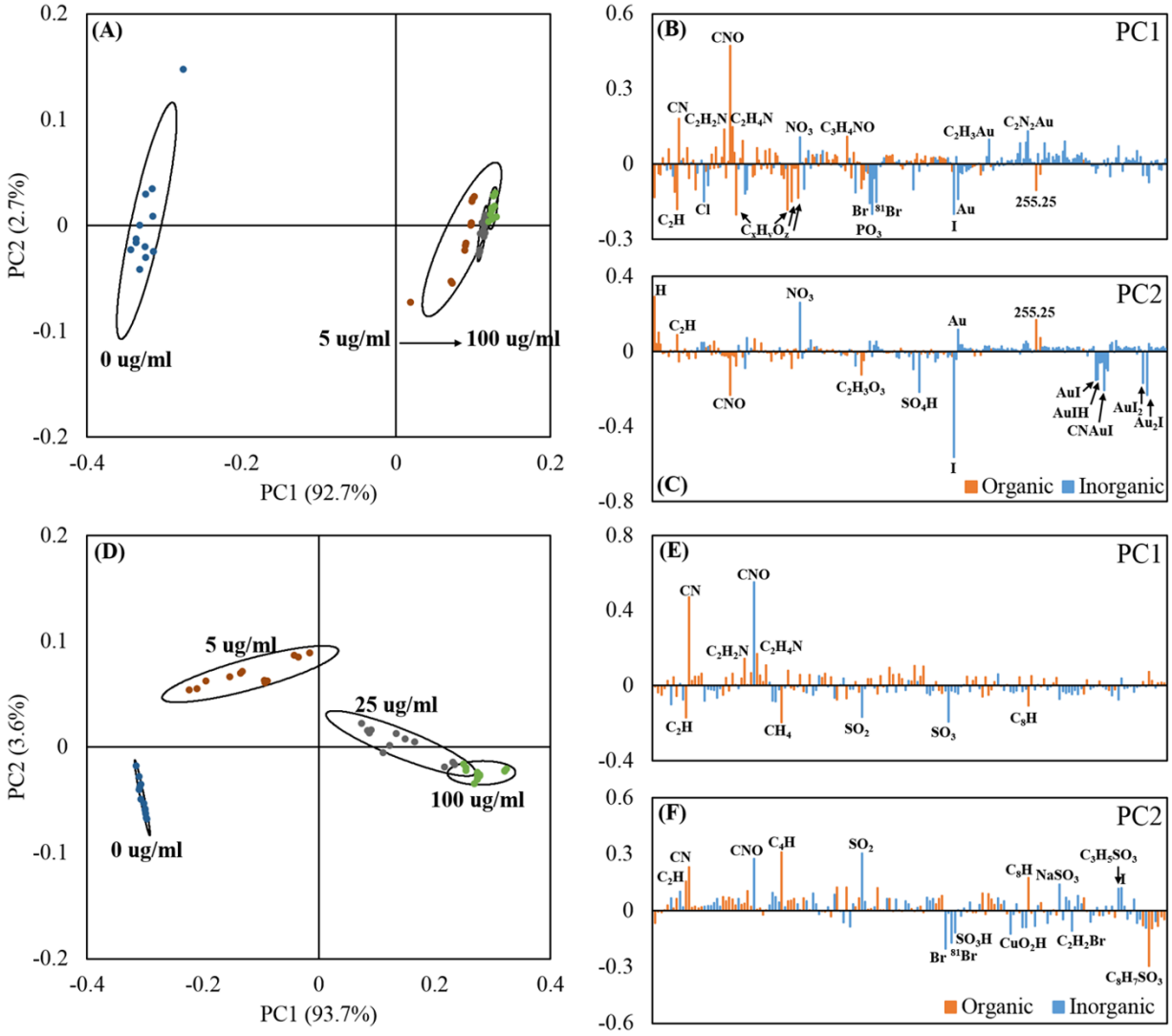

Figure S7. Adsorbed BSA PCA results using all peaks in the negative ion spectra. Panels A – C correspond to the scores and loadings plots for BSA adsorbed onto gold surfaces. Panels D – F correspond to the scores and loadings plots for BSA adsorbed onto NaSS surfaces.

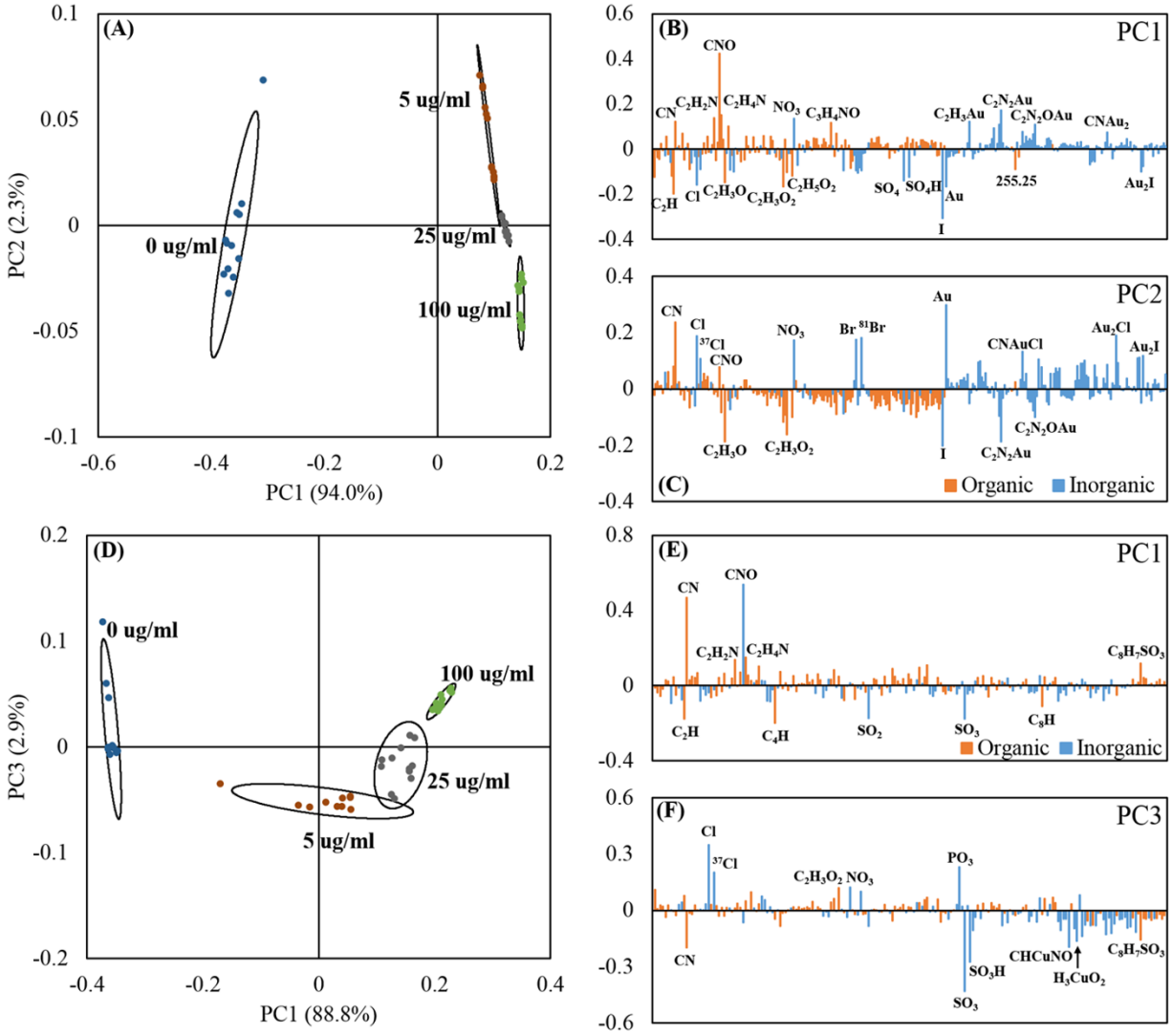

Figure S8. Adsorbed Fgn PCA results using all peaks in the negative ion spectra. Panels A – C correspond to the scores and loadings plots for Fgn adsorbed onto gold surfaces. Panels D – F correspond to the scores and loadings plots for Fgn adsorbed onto NaSS surfaces.

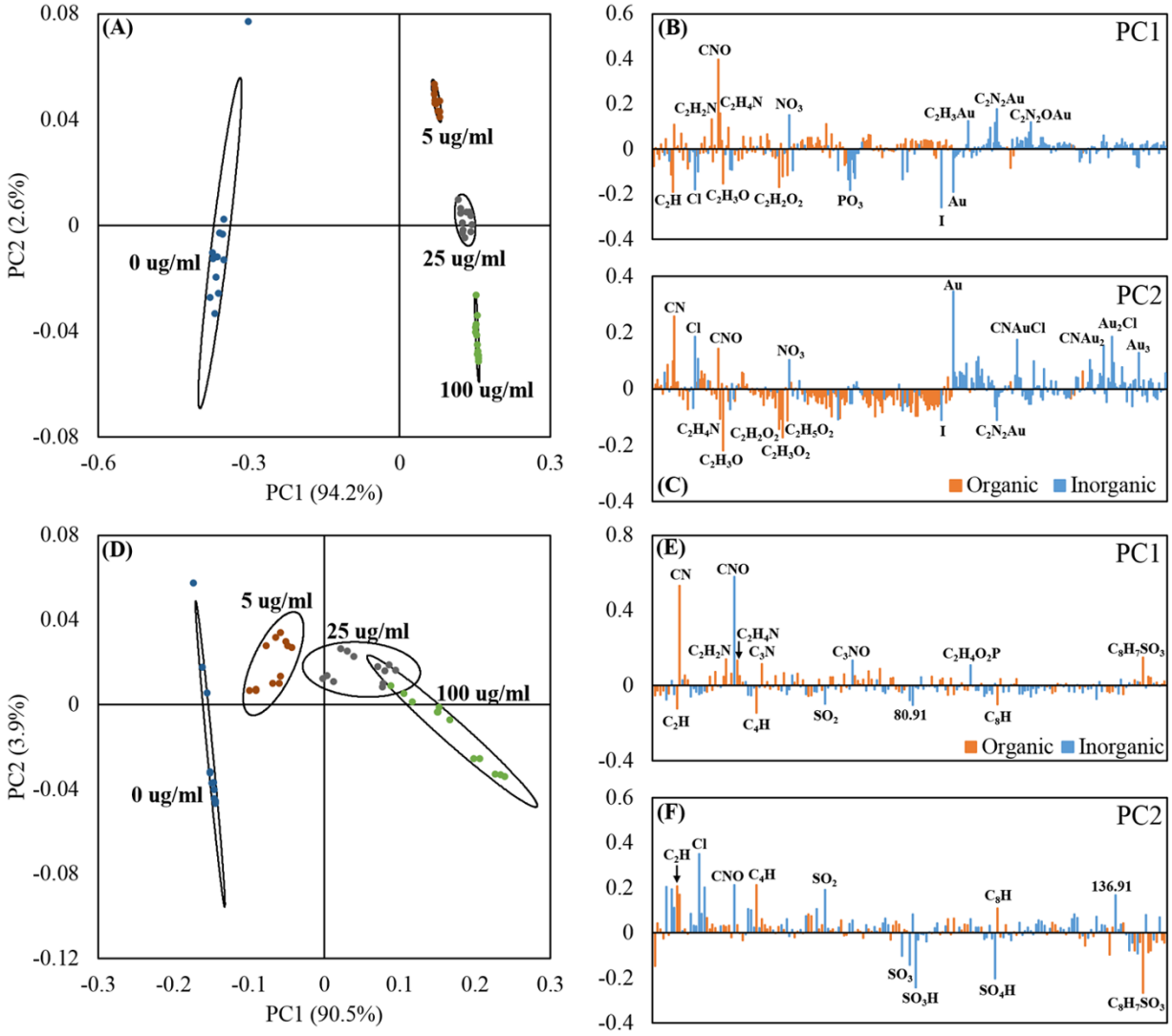

Figure S9. Adsorbed IgG PCA results using all peaks in the negative ion spectra. Panels A – C correspond to the scores and loadings plots for IgG adsorbed onto gold surfaces. Panels D – F correspond to the scores and loadings plots for IgG adsorbed onto NaSS surfaces.

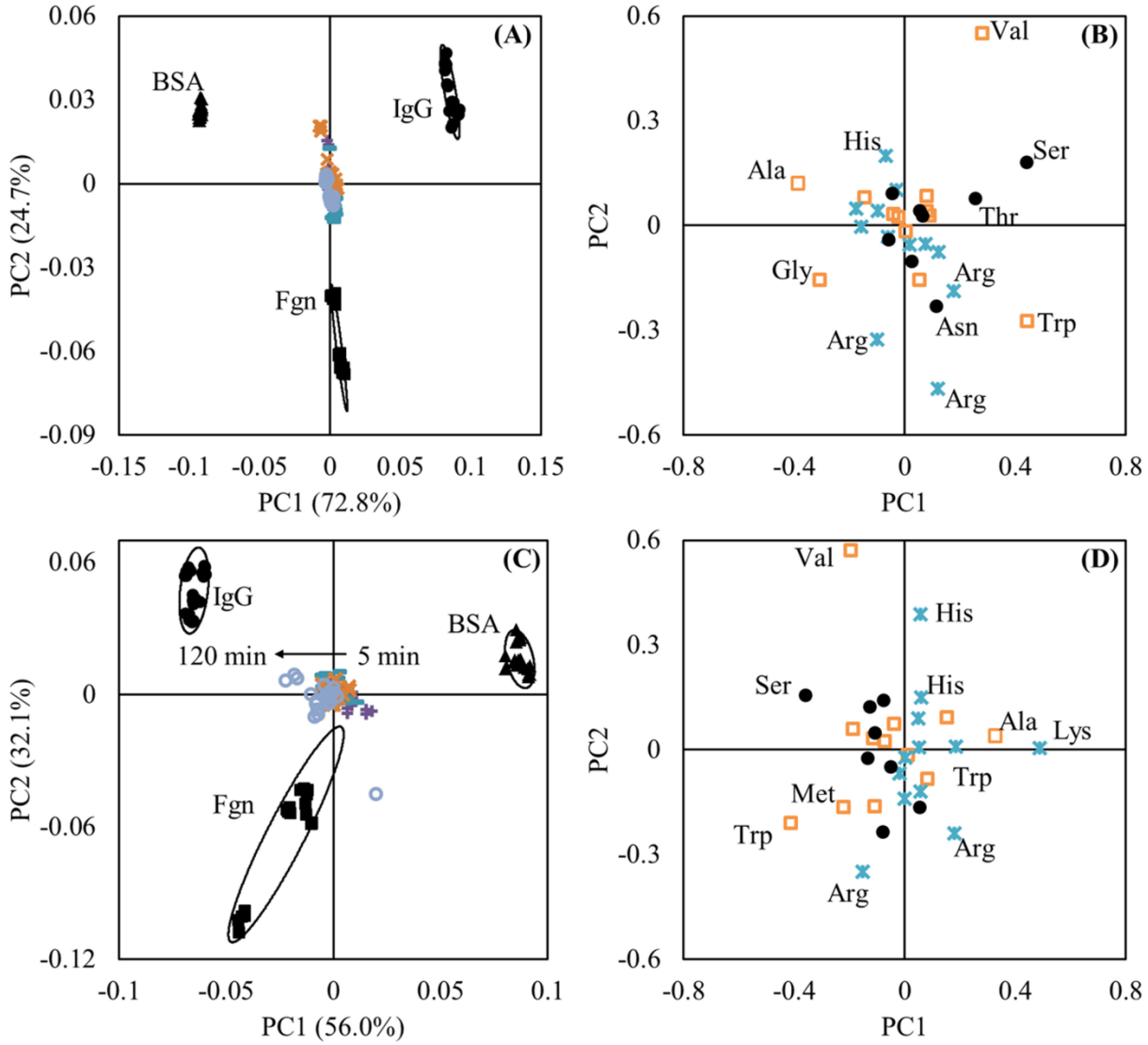

Figure S10. PC model constructed from the 100 µg/ml single-component protein adsorption ToF-SIMS spectra onto gold (A and B) and NaSS (C and D) surfaces. In panels (A) and (C) all black symbols represent the protein adsorption data used to construct the model: BSA (▲), Fgn (■), and IgG (●). Also in panels (A) and (C) the projected plasma data are represented as follows: (+) represent the 5 minute adsorption group, (—) represent the 30 minute adsorption group, (×) represent the 60 minute adsorption group, and (○) represent the 120 minute adsorption group.