

## Analyzing and Protecting the Conformation and Orientation of Immobilized Proteins

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**Statement of Purpose:** Controlled immobilization of proteins onto solid surface is of interest in for developing biomaterials as well as biosensors. However, the immobilization of proteins often causes structural changes in the protein that results in altered physiochemical properties and biological responses from their behavior in solution. In addition, the drying of these proteins on the surface (such as for biosensor applications) can change their conformation. A thorough understanding of the adsorbed proteins (composition, concentration, orientation, conformation, activity, etc.) is key for understanding protein-surface interactions and the design of biocompatible materials and devices. Here surfaces are used that selectively anchor a protein while resisting non-specific adsorption of other proteins is prepared. Time of flight secondary ion mass spectrometry (ToF-SIMS) is used to study the orientation and conformation of the surface bound protein. ToF-SIMS is used to compare dried versus protected proteins (using the sugar trehalose to protect the protein conformation when dried).

**Methods:** Self-assembled monolayers on gold were prepared for the model substrates using a mixture of Nitrilotriacetic acid - terminated tetra(ethylene glycol) undecythiol (NTA thiol) and hydroxyl - terminated tetra(ethylene glycol) undecythiol (OEG thiol). Humanized anti-lysozyme Fragment variable (HuLys Fv) (25,574 Da) was used to immobilize to the NTA functionalized surface. ESCA was used to verify the SAM formation as well as quantify the protein immobilization. Positive and negative ion ToF-SIMS data were acquired with a Physical Electronics PHI 7200 reflectron time-of-flight secondary ion mass spectrometer using an 8 keV  $\text{Cs}^+$  primary ion source in the pulsed mode. The area of analysis for each spectrum was  $100 \mu\text{m} \times 100 \mu\text{m}$ , and the total ion dose was maintained below  $10^{12}$  ions/cm<sup>2</sup>. Mass resolution ( $m/\Delta m$ ) was typically above 5000 and 6000 for the ( $m/z$ ) 27 and 25 peaks in the positive and negative ion spectra, respectively. Calibration errors were kept below 10 ppm. Principal component analysis (PCA) was used to analyzed the ToF-SIMS results.

**Results:** The composition, structure, and order of the mixed NTA-OEG self-assembled monolayers designed for the controllable immobilization of his-tagged proteins have been characterized with ESCA, ToF-SIMS, and SPR1. Short assembly times of the NTA thiols resulted in incomplete monolayers. Subsequent exposure of these surfaces to OEG thiols resulted in the incorporation of the OEG into the monolayer forming a mixed NTA/OEG surface that also resulted in improved orientation of NTA headgroup. SPR showed that the maximum specific binding with slow dissociation rate of his-tagged protein was achieved on the highly packed pure NTA monolayer, after which the amount of specific binding decreased due to significant OEG dilution of the NTA headgroups. ToF-SIMS of the surfaces exposed to 200nM Fv fragment were compared using PCA for surface that had been

protected with trehalose before they were dried and those that had been exposed to trehalose after the protein was dried. The PCA results from the positive ion spectra of 200nM trehalose protected vs. unprotected Fv samples are shown in Figure 1. The first PC captured 48% of the variance and clearly separated the two sets of samples in the PCA scores plot. The loadings showed that the peaks at 43 (Arg), 60 (Ser), 107 (Tyr) and 120 (Phe) were more intense in the spectra of trehalose-unprotected Fv fragment, while, the peaks at 70 (Pro), 98 (Asn), 110 (His) 130 (Trp) were more prominent in the spectra of protected Fv fragment. These results indicate the protein does not have the same conformation/orientation after drying.

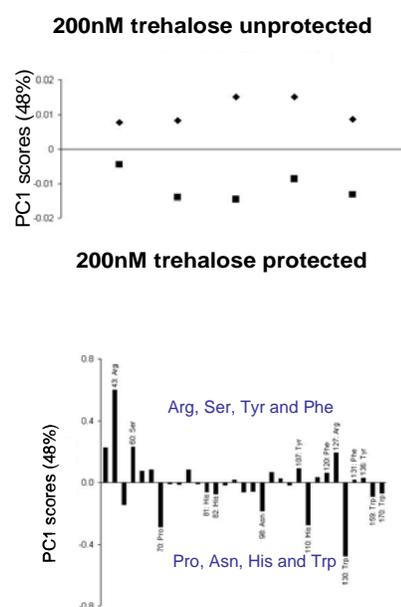


Figure 1. ToF-SIMS PCA results from the positive ion spectra of 200nM trehalose protected vs. unprotected Fv samples.

**Conclusions:** The results from this study provide detailed information and characterization methods that can be used to design novel bioactive films for controllable immobilization of proteins. The study also shows that protection of the adsorbed proteins is a critical step if samples are to be dried (e.g. for storage and shipping)

**References:** (References to published literature.)

1. "XPS, ToF-SIMS, NEXAFS, and SPR Characterization of Nitrilotriacetic Acid-Terminated Self-Assembled Monolayers for Controllable Immobilization of Proteins" F. Cheng, L.J. Gamble and D.G. Castner, Analytical Chemistry, in press, 2008.

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