Investigation of Protein Immobilization on Nitrilotriacetic acid (NTA)-terminated Self-assembled Monolayers by Time-of-Flight Secondary Ion Mass Spectrometry and Principal Component Analysis

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Statement of Purpose: Controlling the conformation and orientation of immobilized proteins is essential for achieving their optimal biological activity. The objective of this study is to develop methods for characterizing the orientation and conformation of immobilized proteins. Recently, static time-of-flight secondary ion mass spectrometry (ToF-SIMS) has the chemical selectivity and surface sensitivity to characterize of protein films on biomaterials.[1-3] The combination of ToF-SIMS with multivariate analysis methods provide a unique opportunity to understand the identity, composition, conformation and orientation of protein films.[4] In previous studies, successful classification of protein spectra was typically achieved by using amino acids peaks.[4] The immobilization of histidine-tagged proteins onto nitrilotriacetic acid (NTA)-terminated self-assembled monolayers (SAMs) on gold is a widely used model system to study protein orientation and interactions.[5] In this work immobilization of a histag anti-humanized lysozyme fragment (Fv HuLys) onto a NTA/OEG SAM was the model system for development of ToF-SIMS and principal component analysis (PCA) methods.

Materials and Methods:
NTA/OEG SAMs: Gold-coated wafers were immersed in an NTA-thiol (Prochimia) solution, and then immersed in OEG_4-thiol (Asemblon) solution for 0.5 hr. Protein immobilization: NTA/OEG SAMs were immersed in $NiSO_4$, and then were incubated in Fv HuLys and finally rinsed with buffer and water. Surface Analysis: XPS compositional survey and detail scans (C 1s, O 1s, Au 4f, N 1s and S 2p) were acquired on a Kratos Axis Ultra DLD instrument. Static ToF-SIMS positive ion spectra were acquired on a PHI Model 7200 instrument. PCA was utilized to analyze the positive ion spectra of the SAMs and protein films. For PCA, a "full" set of amino acids fragments was selected.[2,6] A discussion of PCA for ToF-SIMS analysis is available elsewhere.[3]

Results/Discussion: A mixed NTA/OEG SAM was generated for Fv HuLys immobilization. Figure 1 presents the XPS determined N and S surface concentrations for pure NTA, mixed NTA/OEG and pure OEG SAMs. After a 0.5 hr OEG backfill, the %N decreased and the %S increased, indicating addition of OEG to the NTA monolayer. The positive ion spectra of NTA/OEG SAMs before and after protein immobilization were clearly differentiated by the first principal component (PC1), which accounted for 99% of the variance in the dataset. Examination of the PC1 PCA loadings plot in Figure 2 showed how the various amino acid of fragments contributed to Fv HuLys immobilized (positive loadings) and NTA/OEG (negative loadings) surfaces. The prominent peaks with negative loadings indicated that peaks from SAMs made significant contributions to some of amino acid fragments in the "full" peak set. The fragments related to the underlying NTA SAM included Gly30, Cys 45 and 59, Met 61, Pro 68, Thr 69, Ser 71, Val 83, Lys 84, Asn 87, Asp 88 and Glu 102. These peaks were removed to form a reduced peak set for use in future experiments to examine the orientation and conformation of Fv HuLys on NTA surfaces.

Conclusions: NTA/OEG SAMs were characterized before and after protein immobilization using XPS and ToF-SIMS. Applying PCA to the ToF-SIMS data using a "full" peak set of amino acid fragments easily separated the positive ion spectra before and after protein immobilization. Some peaks related with NTA and OEG were observed and removed. The new peak set containing peaks unambiguously attributed to amino acids from the immobilized protein will be used for further PCA studies.

References:
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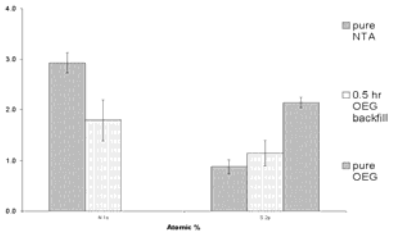

Figure 1. XPS determined N and S surface concentrations for pure NTA, mixed NTA/OEG and pure OEG SAMs.

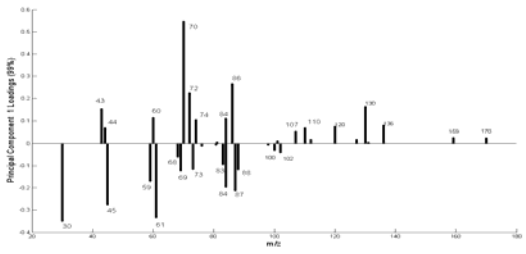

Figure 2 PC1 positive ion spectra loadings plot NTA/OEG SAMs before (negative scores) and after (positive scores) histag Fv HuLys immobilization.

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