Introduction. The structure and bioactivity of adsorbed proteins on biomaterial surfaces is recognized to be a critical factor of cellular response; however, little is known about the actual molecular mechanisms involved. To address this limitation, we are working to develop molecular modeling capabilities and supporting experimental methods to enable protein adsorption to be understood at the molecular level. The objective of this research was thus to experimentally measure the effect of adsorption on the structure and bioactivity of proteins using small enzymes and functionalized surfaces as model systems that can be readily studied by molecular simulation for comparison with experimental results.

Materials and Methods. *Self Assembled Monolayers (SAMs)*: Bare-gold surface plasmon resonance (SPR) sensor chips (Biacore AB) and gold-coated bare quartz slides (Chemglass) for circular dichroism (CD) were treated with 1 mM alkanethiol solutions for a minimum of 12 h to form CH_3, NH_2, and COOH-SAMs. Contact angle and ellipsometry were used to characterize the SAMs.

Protein Adsorption and Bioactivity:

- Buffer: 0.01M phosphate buffered saline (pH ~ 7.4).
- Protein: Trypsin derived from porcine pancreas (TRP, 23.5 kDa, SIGMA).
- Substrate: 1 mM N-α-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (BANA, Acros Organics) in 8% (v/v) dimethyl sulfoxide.

Preliminary studies confirmed irreversible adsorption of TRP to each SAM surface, which is a necessary condition for assessment of the adsorbed protein's bioactivity and adsorbed-state structure by CD. TRP was injected over the various SAM surfaces followed by buffer until the SPR signal stabilized and no further desorption occurred. For the COOH and NH_2 SAMs, a 0.1% (v/v) Tween®-20 (Sigma) solution was injected over the SAM surfaces prior to TRP to block defects in the SAM structure that would tend to expose the underlying hydrophobic segments of the alkane chains. The BANA substrate solution was then flowed over the adsorbed TRP layer, recollected, and the absorbance was read at 405 nm and normalized by the amount of adsorbed TRP.

CD Studies: CD spectra of TRP adsorbed on each SAM surface were collected from 190 to 240 nm using a detachable window cell with a 1mm path length. G-factor analysis [1] was used to extract structural content from the spectra.

Results and Discussion. *Trypsin Bioactivity*: The activity of the adsorbed layer of TRP on both the COOH and CH_3 SAM surfaces was significantly lower than on the NH_2 SAM (Fig. 1). The results of the CD studies (Fig. 2) suggest that the reduction of activity on the COOH SAM was driven by orientation effects since the secondary structure of TRP is not significantly different on the COOH and NH_2 SAMs. Without blocking hydrophobic defect sites with Tween, TRP activity on both the NH_2 and COOH SAMs was substantially decreased, but this was not accompanied by conformational changes. This suggests that the activity loss on the CH_3 SAM is also primarily due to adsorbed orientation. Although TRP on the CH_3 SAM does show a significant decrease in its % α-helix, the % 3_{10} helix, which forms part of TRP's bioactive site, is not significantly lower than on the NH_2 and COOH SAM surfaces.

Figure 1. Activity of the absorbed TRP layer on the various SAM surfaces with and without Tween. (N=6, Mean ± 95% CI)

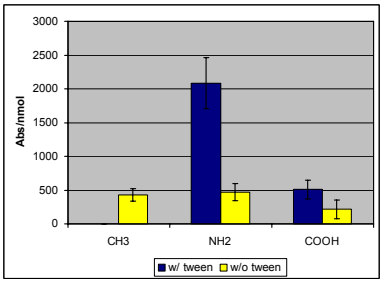
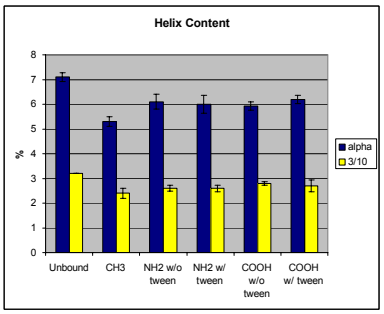


Figure 2. Secondary structure of unbound and adsorbed TRP determined by CD. (N=6, Mean ± 95% CI)



Conclusions. Experimental methods using SPR and CD have been developed in order to relate adsorption-induced changes in trypsin bioactivity with adsorption-induced orientation and/or conformational changes. The relatively small size of this enzyme (<25kDa) will enable molecular simulations to be readily performed to model the same protein-SAM interactions, with the simulations potentially providing additional insights into the molecular mechanisms involved.

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Ref.: 1) Baker B and Garrell R., Faraday Discuss., 126: 209-222 (2004).