Quantifying Surface-Absorbed Protein Structure by Hydrogen-Deuterium Exchange

Marcus T. Cicerone, Jack R. Smith, Curtis Meuse
NIST 100 Bureau Drive, Gaithersburg, MD 20899-8543

Statement of Purpose: Proteins mediate the interaction of cells with materials to which they are absorbed. Thus, engineering cellular response to biomaterials via manipulation of surface chemistry or morphology requires understanding how protein adsorption changes with surface properties. The purpose of this work is to develop and apply a method for quantifying aspects of adsorbed protein conformation.

Methods: We have developed an assay to measure relative conformational changes of surface-adsorbed proteins. This method tracks hydrogen-deuterium exchange (HDX) at surface-accessible amides through infrared absorbance spectroscopy (IR). A monolayer of protein is adsorbed to thin polymer surfaces supported on gold through solution exposure and subsequent gentle washing.

The protein–on–polymer construct is immersed in deuterated buffer solution for a prescribed time period, dried, and evaluated with reflection-mode IR.

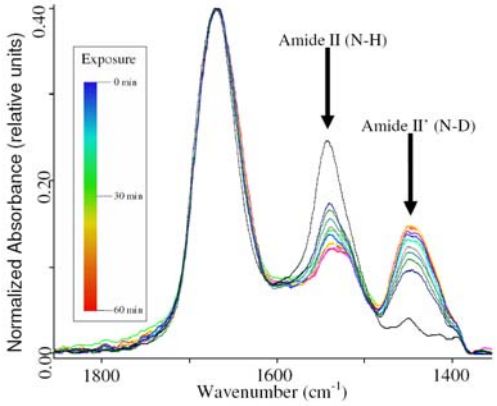

Figure 1. The change in amide spectrum of a BSA adsorbed to gold with exposure to D_2O over the course of 1 h.

We define a time-dependent fractionation number (*f*) as the ratio of the integrated areas of the amide II and amide I bands in the IR spectrum (due to N-H in plane bend and amide C=O stretch respectively). The quantity *f* is directly related to the time-averaged number of solvent-accessible amides; increase in *f* indicates either unfolding of the protein or increase in conformational fluctuations.

Results/Discussion: We have measured surface-adsorbed HDX fractionation rates for three proteins [Bovine Serum Albumin (BSA), Porcine Trypsin (PT) and Human Fibronectin (HFn)] and three surfaces [Au, polystyrene and PDLLA]. The time-dependent changes in *f* are characterized by three time-scales, < 1min, O(10 min), and > 100 min. We cannot resolve dynamics on the fastest time scale with the current approach. Also, we did not extend the measurements to sufficiently long times to quantify dynamics on the longer timescale. Experimental curves for BSA in solution, on gold, polystyrene, and PDLLA are shown in Figure 2.

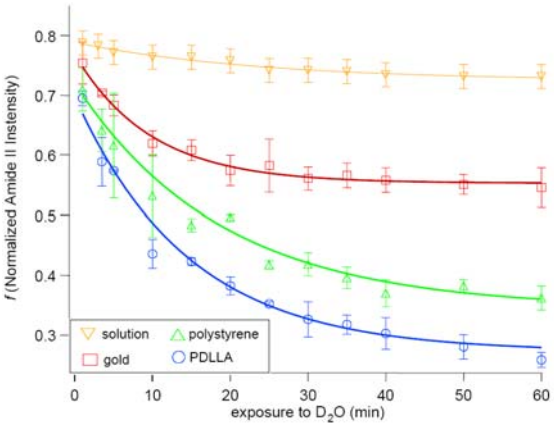

Figure 2. Changes in *f* with time of exposure to D_2O. Data points represent the average of five separate spectroscopic measurements. The lines are single exponential fits. Error bars reflect average of three experimental trials.

The solution result confirms the expectation that the globular, native conformation of albumin is effective at shielding its interior amide linkages from solvent on the intermediate timescale. The surface-adsorbed proteins show a time-averaged conformation that has more amides exposed to solvent, i.e. is more "open." This agrees with the bulk of the literature showing that BSA unfolds as it sticks to hydrophobic surfaces. We expected, due to the range of in air-water contact angle (θ: 76 – 110º) of the substrates, to see a range of HDX rates. We did not expect, based on contact angle, to see the ranking of rates as it is. The fact that BSA shows the most open conformation on adsorption to PDLLA, cannot be justified with empirical relationships between protein conformation and macroscopic surface hydrophobicity.

Conclusions: We have demonstrated that we can quantify aspects of surface-adsorbed protein conformation, and that factors other than surface-water contact angle appear to be important for determining the time-averaged conformation.