Charge Density Measurement of Individual Nanoparticles for the Purpose of Understanding Molecular Self-Assembly

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Statement of Purpose: Self-assembly of biologic and synthetic materials is largely controlled by electrostatic forces between their constituent molecules. Since most proteins are not easily crystallized, knowledge of the nanoscale location of charges on these molecules is lacking. Spatially resolved experimental measurement of the charge density on protein molecules would thus be useful in the development of models of extracellular matrix and biomaterial self assembly. Previously, we showed this might be accomplished using an atomic force microscope (AFM) to measure Debye screening above a graphite surface in solution.[1] We now seek to extend that result by measuring the charge density on a non-covalently bound charged polystyrene sphere resting on a graphite surface. Recovering the known charge density of this calibration object will provide confidence that subsequent measurements of unknown charge densities on proteins using our technique are accurate.

Methods: 40 nm carboxylate-modified polystyrene spheres with a known charge density were immobilized from solution onto the basal plane of highly oriented pyrolytic graphite, HOPG(bp). This was achieved electrophoretically. Such an immobilization strategy is expected to allow future analysis of proteins with minimal conformational change due to covalent linkages or antibody binding.

Immobilized spheres were imaged in the presence of bulk liquid using tapping mode AFM. After imaging, force-distance measurements between the AFM tip and an immobilized sphere and between the tip and a region of the HOPG adjacent to the sphere were performed. The measured AFM cantilever deflections were recorded at 10 MHz allowing collection of a significant number of data points in the "snap-to-contact region."

Results: A typical AFM image of an immobilized polystyrene sphere is shown in Figure 1. The force-distance experiments were performed at sites 1 and 2 in the image.
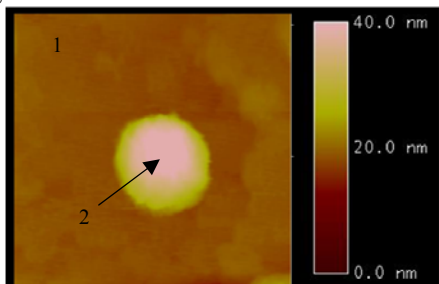

Figure 1: AFM image of an immobilized polystyrene sphere. The measured height of the sphere is 23 nm and the diameter is 70 nm. The large diameter is attributed to the tip broadening artifact.

The force-distance experiments revealed an attractive force between both the tip and the HOPG and the tip and the polystyrene sphere. This force resulted in cantilever deflections in the snap-to-contact region of $1.7 \pm .25$ nm for the case of the tip interacting with the sphere and $1.0 \pm .17$ nm for the tip interacting with the HOPG.

The average of 15 reconstructed force-separation curves for the tip-sphere and tip-HOPG experiments are shown in Figure 2. In time, the data is collected from right (large separation between tip and surface) to left in these curves. The last data point (at 0,0), is the point usually chosen in AFM force-distance curves to represent contact between tip and sample.
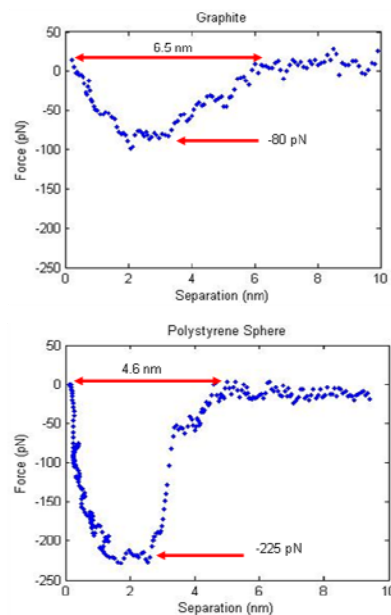

Figure 2: Top plot shows average force-separation curve between AFM tip and the HOPG surface. Bottom plot shows corresponding curve for the tip-sphere interaction. Maximum attractive force between tip and sphere is nearly 3 times larger than that between tip and HOPG. Net attraction between tip and sphere begins at a separation distance of 4.6 nm, compared to 6.5 nm for tip and HOPG.

Conclusions: The large attractive force between the tip and the sphere compared to the force between the tip and the HOPG suggests that electrostatic forces are being measured. A theory is currently being developed to model the electrostatic force between a charged sphere and an AFM probe. This model will be used to recover an experimental value for the charge density of the sphere that will then be compared to the expected value.

References: [1]Todd BA. Langmuir. 2004;20:4892-4897.