

## "Poking" Membranes with Sharp Needles: Biophysical Insights via Precision Atomic Force Microscopy

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The atomic force microscope (AFM) has emerged as an important complementary apparatus in the molecular biologist's toolkit. AFM is inherently a single-molecule technique. In contrast, bulk techniques yield ensemble averages, which can mask a range of unique, asynchronous activities. In addition to imaging, AFM can be used in force spectroscopy mode to unfold proteins mechanically. This provides a detailed view of the energy landscape that stabilizes macromolecular structure. Our laboratory has developed and applied precision AFM apparatus for imaging and force spectroscopy of membrane proteins and lipophilic peptides in near-native conditions. In one project we shed light on core components of the general secretory system of E. coli. We observed the dynamic structure of the integral membrane translocon SecYEG in a lipid bilayer as well as its interactions with peripheral ATPase SecA and precursor proteins. In another project we affixed lipophilic peptides to the AFM tip and directly observed the mechanical consequences of partitioning into the bilayer. Together with analytical modeling and simulations, the results represent a step towards a more detailed understanding the forces and kinetic pathways driving protein-bilayer partitioning. In this talk I will discuss these two projects and highlight a few of the unique aspects of our precision instrumentation along the way.

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Students, meet the speaker over coffee and cookies in the Bennett Conference room at 3:30 pm