Public Abstract
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Title:Watching Biological Nanomotors at Work: Insights from Single-Molecule Studies

Recent decades have seen several complimentary biophysics tools emerge to study single protein macro-molecules. Most of these techniques use glass as a specimen support. The atomic force microscope, a vital tool in biophysics suited to study proteins in their near native environments, uses mica as a specimen support, as it is known for its extreme flatness and ease of use. Here we optimized glass as a specimen support for atomic force microscopy. This enables the combination of other single molecule techniques with atomic force microscopy to study the same protein macromolecular system in unison. Using bacteriorhodopsin from Halobacterium salinarum and the Sec-translocase (SecA/SecYEG) from Escherichia coli, we demonstrate that faithful images of 2D crystalline and non-crystalline membrane proteins in lipid bilayers can be obtained on common microscope cover glass following a straight-forward cleaning procedure. Repeated association and dissociation of SecA with SecYEG indicated that the proteins remain competent for biological processes on glass supports for long periods of time. This work opens the door for combining high resolution biological AFM with other powerful complementary single molecule techniques that require glass as a specimen support.

In the second part of this work we studied SecA-ATP hydrolysis and catalase enzyme dynamics. Both of these protein macromolecules were observed to be highly dynamic during catalytic turnover. Single molecule studies of catalase indicated that the enzyme undergoes significant dynamics including oligomeric state changes when exposed to H2O2. Conformational dynamics of the SecA-ATPase was visualized at the single molecule level and the protein macromolecule was observed to flicker between a compact and expanded state in the presence of ATP, indicating reversible conformational changes. Future studies in the lab will shed more light onto these important biological processes.