a framework for understanding the structure of the spindle and its response to physical and molecular perturbations.

Workshop 4: Time-resolved AFM of Biological Systems

1978-Wkshp
Filming Dynamic Processes of Proteins by High-Speed Afm
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Directly observing individual protein molecules in action at high spatiotemporal resolution has long been the holy grail for biological science. High-speed atomic force microscopy (HS-AFM) which can perform this type of observations is now materialized. It opens up a new opportunity to directly observe the structure dynamics and dynamic processes of biological molecules in physiological solutions, at subsecond to sub-100 ms temporal resolution and ~2 nm lateral and 0.1 nm vertical spatial resolution. Importantly, the tip-sample interaction does not disturb their function. In fact, functioning molecules, such as myosin V walking on an actin filament and bacteriorhodopsin in response to light, have been successfully visualized. In the quest for the functional mechanism of proteins, inferences no longer have to be made from static snapshots of their molecular structures and dynamic behavior of optical markers attached to proteins. High-resolution molecular movies reveal the details of dynamic behavior of molecules in action, which can be interpreted without intricate analyses and interpretations. In this talk, I will highlight recent imaging studies and then describe the fundamentals behind the achieved high imaging rate and low-invasiveness to the sample.

1979-Wkshp
High-Speed Atomic Force Microscopy of Protein-Protein Interactions
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Protein-protein interactions are of major importance for biological function. Many proteins are oligomers and interact temporaly with other proteins. This is of particular importance in the membrane, where multiprotein assemblies act in important processes like signaling, respiration, photosynthesis, etc. High-speed atomic force microscopy (HS-AFM,[1]) offers unique possibilities to study protein-protein interactions in the membrane. HS-AFM allows not only visualization and tracking of single proteins but also their environment, hence describing the interaction energy between proteins (Fig.1A,[2]), their dynamic supramolecular assemblies (Fig.1B,[3]), and membrane crowding and interaction specificity (Fig.1C, [4]). The interaction profiles can be, though at different time scales, compared to molecular simulations [4]. The perspective of short-cantilever HS-AFM in force spectroscopy will be discussed: the high speed of short cantilevers allows measuring interaction forces at unprecedented loading rates and temporal resolution (Fig.1D).

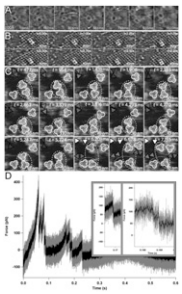

Fig. 1) Membrane protein interactions by HS-AFM. (a) Dimer interaction of ATP-synthase c-rings, (b) AQP0 array association/dissociation in eye lens membranes, (c) OmpF diffusion and interactions, (d) HS-AFM based force spectroscopy at 1MHz temporal resolution.

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1980-Wkshp
Nanoscale Dynamics of Protein-DNA Complexes
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Time lapse AFM is the technique with a great potential for molecular biophysics. Advances in the sample preparation techniques for AFM coupled with emerging of high-speed AFM (HS AFM) instrumentation led to the progress in the study of a number of protein-DNA complexes. The mechanism by which site-specific binding proteins accomplish the search of cognate sites on DNA is one of the important ones. The problem is more severe if the systems requiring interaction with two specific DNA sites (such as site-specific recombinases) are concerned. EcoRII restriction enzyme belongs to the family of such site-specific enzyme and with the use of HS AFM we were able to identify a novel process for the site search for this two-site binding protein. We have shown that EcoRII is able to bind to one site of DNA pulling another DNA duplex in the search of another site. The time-lapse AFM technique was also instrumental in understanding dynamics of nucleosomes. We demonstrated that nucleosomes are quite dynamic rather than static systems and answered a number of important questions related to their dynamics. Single-stranded DNA binding proteins are ubiquotous systems involved in DNA replication, recombination, transcription and various modifications of DNA. We have developed a novel approach for studies of ssDNA binding proteins with AFM in which a hybrid DNA substrate with desired structure and sequence is used. In such designs, ssDNA of a specific length is either attached to one of the ends of dsDNA or placed between the two DNA duplexes. Time-lapse HS AFM was applied to look at the DNA-protein interaction dynamics including dissociationa and association steps for both systems and A3G sliding along the DNA substrate.

1981-Wkshp
High-Speed AFM with a Light Touch
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There are three areas in which conventional AFM has limitations: (i) low imaging rate, (ii) probe-sample force interaction, and (iii) the planar nature of the sample.
We are developing two high-speed force microscopy techniques. One high-speed AFM (HS AFM) technique is a DC method in which the tip is in continuous contact with the specimen. This routinely allows video-rate imaging (30 frames per second, fps) and has achieved imaging at over 1000 fps, i.e., 100,000 times faster than conventional AFM. Damage to specimens resulting from this high-speed contact-mode imaging is surprisingly less than would be caused at normal speeds. The behavior of the cantilever and tip at these high velocities has been investigated and superlubricity is a key component in the success of this technique.
The other high-speed force microscope is a non-contact method based on shear-force microscopy (ShFM). In this HS ShFM, a vertically-mounted laterally-oscillating probe detects the sample surface at about 1 nm from it as a result of the change in the mechanical properties of the water confined between the probe tip and the sample. With this technique, very low normal forces are applied to the specimen.
Conventional AFMs require planar samples because the probe scans in a plane. It is as if the tip is only 'seeing' the sample from above. We have overcome this limitation be steering the tip of a nanorod in a three dimensional scan with six degrees of freedom using holographically generate optical traps such that it is possible to scan around a sample.