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Fluorescence imaging of the function and dynamics of single protein molecules

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Single-molecule fluorescence microscopy has evolved into a powerful methodology for the elucidation of biomolecular dynamics. Its main merit lies in its capability to directly measure the temporal trajectory of a functioning biomolecule. Such measurements can provide information on functional dynamics which cannot be gleaned by ensemble techniques. Further, single-molecule experiments can nicely separate subpopulations of biomolecules and measure the properties of each, instead of the weighted average obtained in most conventional experiments. Our lab develops and applies single-molecule fluorescence techniques to study several exciting biophysical problems, some of which are described here.

Single-molecule protein folding

The protein folding problem continues to escape full understanding even after half a decade of research. Folding kinetics are usually explained in terms of a deterministic sequence of intermediates connected by energy barriers. A new view which has emerged in recent years stresses the importance of the complicated and rugged 'energy landscape' of a protein for its folding mechanism. An important prediction of this new view is that the folding reaction will be very heterogeneous and will follow many different pathways. Studies of folding trajectories of single protein molecules should be able to provide an experimental test for this assertion.

How can we follow the folding reaction of individual molecules? One way to do this is to immobilize these molecules and measure their equilibrium fluctuations under conditions which promote frequent transitions between folded and unfolded conformations. A major problem that arises here is how to immobilize protein molecules without affecting their dynamics. In an effort to overcome this problem we devised a new technique, based on the confinement of biomolecules inside 100 nm surface-tethered lipid vesicles. We found that the number of molecules in each vesicle can be accurately determined from fluorescence time traces (see Figure 1), so that we can select only those vesicles strictly containing one molecule. We also used fluorescence polarization spectroscopy as a sensitive probe for the freedom of motion of trapped molecules, and thus for the environment they sample inside the liposomes. It was concluded that molecular motion within the vesicles is quite similar to free solution.

Vesicle entrapment is quite a general method for studying single protein dynamics, and can be used for both water-soluble and membrane proteins. We are currently using the technique to study the folding pathway of the enzyme adenylate kinase, in collaboration with Elisha Haas of Bar Ilan University. Fluorescence resonance energy transfer between two probes is used to directly measure structural fluctuations in each protein molecule. Careful analysis of the time scales involved in these fluctuations should provide us with the required information regarding the heterogeneity of the folding reaction.

There is also a 'natural' way to trap protein molecules and fold them, provided by the molecular chaperones, such as GroEL. This multi-subunit protein encapsulates unfolded polypeptides in its cavity and actively promotes their folding. It undergoes a complex sequence of conformational transitions, driven by ATP hydrolysis. The study of the allosteric nature of these transitions forms a lively field of research. In collaboration with Amnon Horovitz of the Structural Biology department we are studying allostery and protein folding reactions of single GroEL molecules attached to a surface. Again, a series of fluorescence techniques is used to follow both the fate of an encapsulated protein and the cycle of ATP-driven structural changes of the chaperone itself.

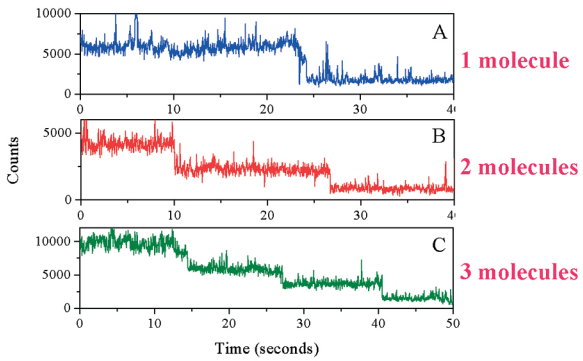

Fig. 1 *Time traces of the fluorescence of individual lipid vesicles encapsulating Texas Red-labeled adenylate kinase molecules. The number of sudden drops in fluorescence intensity, which are due to photobleaching, provides the number of molecules in each vesicle.*

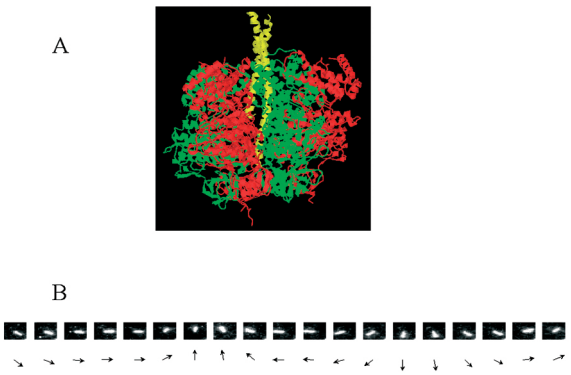

Fig. 2 *A. Structure of the enzyme F1-ATPase, with the gamma subunit in yellow. B. 30 ms snapshots of a rotating actin filament (~2 microns long) in the presence of CaATP. The arrows mimic the position of the filament.*

Mechanism of the smallest rotary motor, F1-ATPase

It is now well-established that the catalytic mechanism of the membrane protein ATP synthase involves an internal rotary motion, driven by proton translocation through the membrane. The rotational motion, first proposed by Boyer, has been directly observed by several groups, using single molecules of the soluble part of the enzyme, F_1-ATPase. However, little is known about the molecular steps involved in the coupling of rotation to ATP synthesis or hydrolysis. In collaboration with Zippora Gromet-Elhanan of the Biological Chemistry department and Mark Richter of the University of Kansas we are performing a series of studies that we hope will provide new insights into this issue.

The molecular system we study is comprised of recombinant subunits of the photosynthetic F_1-ATPase, which are assembled in-vitro, thereby allowing us to exert unique biochemical controls on the molecule. Rotational motion of surface-attached individual molecules of the enzymes is studied by following the motion of a fluorescent actin filament connected to the gamma subunit (Fig. 2), as well as by tracking changes in the fluorescence polarization of a single dye molecule labeling the same subunit. We have recently found the surprising result that rotational motion can be supported by CaATP, and not only by the 'natural' substrate, MgATP. This is interesting, because calcium is known to decouple ATP hydrolysis from proton translocation. It is now clear from our results that calcium does not do this through the induction of a "rotationless" form of catalysis. We are now using other effectors of the protein to learn more about this issue. We are also initiating experiments with fluorescently-labeled ATP molecules, where we will directly follow the interaction of ATP with the enzyme and correlate it with its activity.

Selected Publications

Boukobza, E., Sonnenfeld, A. and Haran, G. (2001) Immobilization in surface-tethered lipid vesicles as a new tool for single biomolecule spectroscopy. J. Phys. Chem. B, ASAP publication, web release date November 2, 2001.

Wang, Y., Astilean, S., Haran, G. and Warshawsky, A. (2001) Microenvironmental investigation of polymer-bound fluorescent chelator by fluorescence microscopy and optical spectroscopy. Anal. Chem., 73, 4096-4103.

Weiss, A. and Haran, G. (2001) Time-dependent single-molecule raman scattering as a probe of surface dynamics. J. Phys. Chem. B, ASAP publication, web release date November 15, 2001.

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For additional information see:

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