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Single-molecule biophysics: from protein folding to cytoskeleton dynamics

Should we make an effort to measure the noisy signal of a single molecule when we can study a billion molecules at once? The answer to this question is of course a resounding YES: single-molecule biophysical experiments have unraveled in recent years information which had been completely hidden in studies of large ensembles of molecules. Single molecule studies shed light on the functional dynamics of biomolecules on one hand, and help

the dynamics of proteins, as well as the relation between dynamics and function. Below we describe some of the current projects in the lab.

Single-molecule folding

The elucidation of the way by which proteins are able to spontaneously find their stable three-dimensional structures in a short time is still an important challenge. It has become clear in recent years that understanding

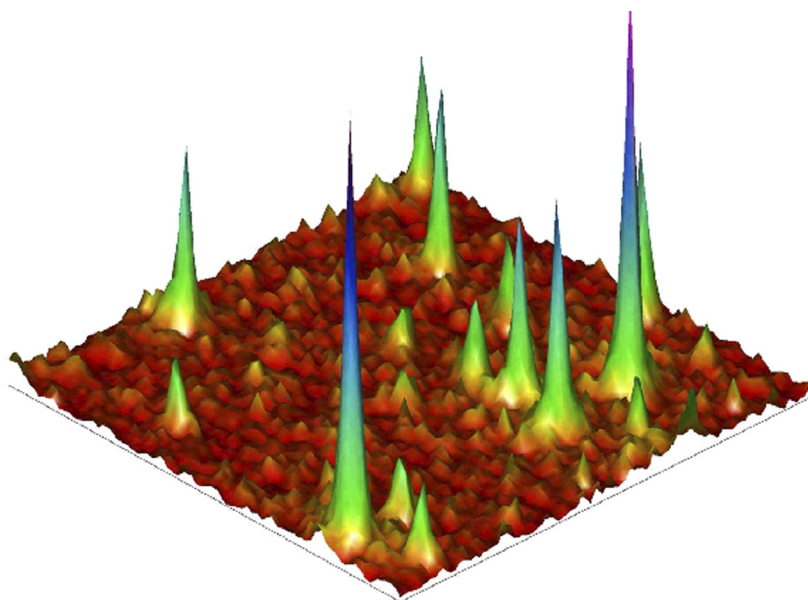


Fig. 1 An image of single fluorescent molecules on a surface. The spikes represent the intensity of emission from each molecule.

creating novel imaging methods on the other hand. The tool of choice for such studies is the fluorescence microscope, equipped with laser excitation sources and ultra-sensitive detectors. Our lab is interested in employing single-molecule spectroscopic methods to understand

the ensemble of conformations collectively forming the denatured state of a protein is very important for deciphering the mechanism of folding. The folding process itself may be quite heterogeneous and can involve various intermediate states and sub-

populations. A long-term objective of our research is to employ single-molecule fluorescence spectroscopy as a powerful tool for mapping the structure of the free energy landscape of proteins, from the denatured state to the folded state.

We have developed a set of original experimental tools that allow us to address the question of protein folding on the single-molecule level. First, we need to understand how a denatured protein behaves and how it changes its properties as a function of external conditions. We have developed the methodology needed in order to answer this question (Sherman and Haran, 2006). We find that in a highly-concentrated denaturant solution the protein is highly expanded, sampling many conformations (Figure 2A). As the denaturant concentration is lowered, the protein becomes more compact and globular in shape (Figure 2B) before it folds to the native structure (Figure 2C). This change in shape is termed the collapse transition, and can be modeled with theories borrowed from polymer physics. The collapse transition is due to a balance between the intramolecular attraction within the protein and the conformational entropy of the chain. Our analysis of this process teaches us about the energetics of the collapse, which are of prime importance for the folding process itself, but cannot be obtained in any other way.

A second question we ask is about the nature of the folding transition. When is folding very simple, involving only two states (either unfolded or folded) and when is it complex and heterogeneous, involving many intermediate states? To answer this question we need to follow

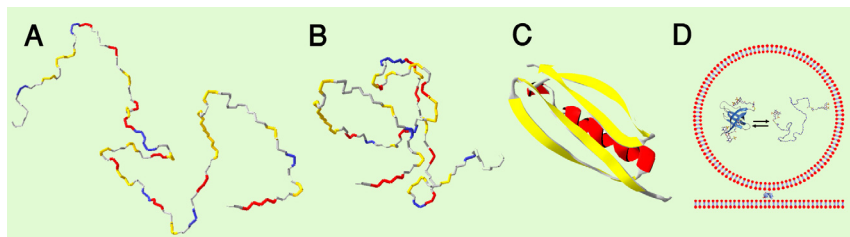


Fig. 2 From denatured to collapsed to folded. Under denaturing conditions a protein is in an expanded, random coil conformation (A). On the way to the folded conformation (C), a protein first collapses to a globular random structure (B). Single molecule studies of this process are facilitated by vesicle encapsulation of individual molecules (D). The vesicle is tethered to a surface via a biotin-avidin link.

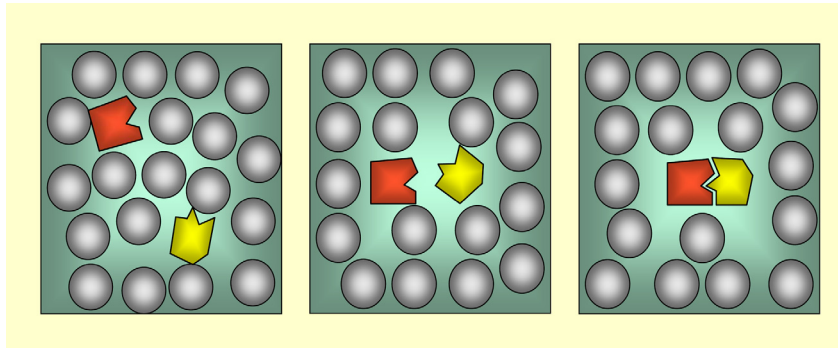


Fig. 3 Protein association in a crowded solution. The two proteins find each other by diffusion. When they are close enough together they feel the osmotic pressure exerted by the other macromolecules in solution, which increases their association rate.

the fate of individual protein molecule for long periods of time and explore the various states they visit. This requires a method for trapping these molecules, but we need to be sure that as we trap them we do not interfere with their dynamics. An approach developed in our lab to tackle this problem involves encapsulation of molecules in surface-tethered lipid vesicles (Figure 2D), which facilitates the measurement of trajectories of folding and unfolding transitions of these molecules under equilibrium conditions (Rhoades et al., 2003). Using this approach, we were able to show that the folding process of the protein adenylate kinase is extremely heterogeneous. Thus, instead of jumping from a completely unfolded conformation to a completely folded one, adenylate kinase molecules prefer making many small steps which gradually guide them

from one conformation to the other. This is a radically different picture of folding than is common to smaller and simpler proteins. Our current research attempts to understand quantitatively this heterogeneity and map the folding process in great detail based on our single-molecule fluorescence data.

Protein association- the effect of crowding

Association of proteins is a necessary step in many cellular processes, such as various signaling cascades. It is important to understand the effect of the dense and crowded cellular milieu on association kinetics. We are studying protein association together with Prof. Gideon Schreiber of the Biological Chemistry department (Kuttner et al., 2005). The rate of association depends both on translational diffusion, which brings two protein together, and rotational diffusion, which allows them to orient correctly for interaction. Our approach involves measuring translational and rotational diffusion in addition to association kinetics in each solution. The crowded intracellular environment is simulated by using concentrated polymer solutions. We employ the theory of diffusion-limited association to connect all the measured properties. We find that, surprisingly, the association rate depends only mildly on solution viscosity over a broad range of conditions. We attribute this to the 'correcting' influence of the polymer solution, due to the fact that it exerts osmotic pressure on the two proteins, enhancing their association (Figure

3). Thus a physical property of macromolecular solution is found to be unexpectedly important for a very biological process- the association of proteins.

Molecular machines

Single-molecule techniques are also useful for studying the dynamics of molecular machines and connecting conformational changes to function. Together with Prof. Amnon Horovitz of the Structural Biology department we have been studying the chaperonin GroEL as a paradigm for a molecular machine (Kipnis et al., 2007). This multimeric protein undergoes a cycle of concerted conformational changes. The relation between the allosteric transitions of the chaperone and the folding of an encapsulated protein was the focus of one recent experiment. In another experiment we devised an optical switch into the structure of the chaperone- a fluorescent probe that is completely quenched in one conformation, but becomes strongly fluorescent in the other conformation. This switch now allows us to investigate the conformational cycle of GroEL on the single-molecule level.

Cytoskeletal dynamics of the red blood cell

Shape changes of red blood cells (RBCs) have fascinated biophysicists for many years. The morphological flexibility of RBCs are due to the cytoskeleton, which is localized under their membrane, and is comprised of a network of spectrin filaments, each anchored at its two ends to transmembrane proteins. It has been postulated that energy-dependent detachment events of the filaments from their anchors may lead to functionally-important transient changes in the elastic properties of RBC membranes. Together with Dr. Nir Gov of the Chemical Physics department we study the dynamics of spectrin filaments in RBC membrane patches (Figure 4). We develop methods to label the filaments with fluorescent probes and to follow their temporal fluctuations using single-molecule imaging techniques.

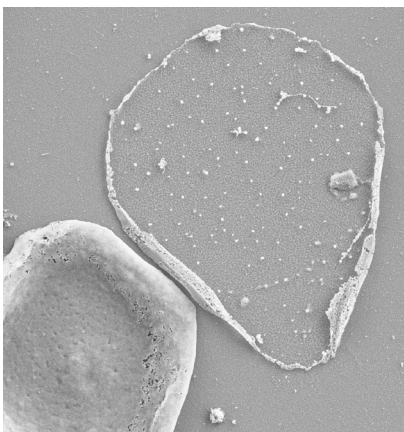


Fig. 4 SEM image of red blood cells. An osmotic shock treatment has led to the exposure of the cytoskeleton of the central cell.



Selected publications

Rhoades, E., Gussakovsky, E. and Haran, G. (2003) Watching Proteins Fold One Molecule at a Time. *Proc Natl Acad Sci U S A*, 100, 3197-3202.

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Sherman, E. and Haran, G. (2006) Coil-globule transition in the denatured state of a small protein. *Proc Natl Acad Sci U S A*, 103, 11539-11543.

Kipnis, Y., Papo, N., Haran, G. and Horovitz, A. (2007) Concerted ATP-induced allosteric transitions in GroEL facilitate release of protein substrate domains in an all-or-none manner. *Proc Natl Acad Sci U S A*, 104, 3119-3124.

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