Protein Dynamics Intro

From rigid structures to motions on energy landscapes

Do you all remember Anfinsen? What concept now associated with his name made Anfinsen famous? Right, it is the concept that the structure of a functionally folded protein represents the global conformational energy minimum of that protein. One minimum -> one structure.

Throughout the previous section of the course we always assumed that there was one single structure for the protein; i.e. a protein folds once and then stays in that folded state until it is degraded by the cell.

In the following few classes I hope I will convince you that proteins are by no means rigid structures and that motion is, in fact, just as important for protein function as adopting a stable structure. The picture that should emerge at the end of this section is that proteins achieve a fine balance between adopting a defined structure and maintaining a high degree of flexibility. A protein's structure has to be stable enough to organize and orient side chains into active sites or to form specific protein-protein interaction interfaces. At the same time the protein has to be flexible enough to let substrates into active sites or to under go conformational changes to transmit signals etc.

The Folding-Unfolding Reaction is a dynamic Equilibrium.

Lets think about an extreme case of protein dynamics first. We know that the stability of a protein is often on the order of 10 kcal/mol. What fraction of the molecules then is completely unfolded, if we have our protein sitting around at room temperature?

$$\Delta G = -RT \ln K_{eq}$$

so

$$K_{eq} = e^{\frac{-\Delta G}{RT}}$$

for

$$\Delta G = 10 kcal/mol$$

$$K_{eq} = \frac{1}{17 \cdot 10^6}$$

The calculation shows us that on average one in every 17 million molecules is completely unfolded. Given that the folding/unfolding process of many proteins takes place on the order of milliseconds, each of these protein molecules will visit the completely unfolded state within a few hours. Put a different way, while the energies that hold together a protein will lead the vast majority of molecules to adopt a conformation very close to the overall conformational energy minimum, thermal energy alone is sufficient to let proteins explore areas of conformational space that is very far from the global conformational energy minimum.

We can measure this unfolding/refolding process using hydrogen deuterium exchange and NMR. Many hydrogen atoms, like those bound to backbone-amide nitrogens, exist in a rapidly exchanging equilibrium in which the protons dissociate and re-associate with the protein. If the amid group is exposed to water the proton that dissociates is not necessarily the same proton that re-associates and a proton originally bound to the protein is rapidly exchanged with a proton from solvent.
If we take a protein and place it in deuterated water, then those hydrogen atoms that are chemically predisposed to exchange and are exposed to the water will be replaced by deuterium ions. NMR spectroscopy allows us to distinguish between normal hydrogen atoms and deuterium atoms. If we set our NMR spectrometer to record hydrogen data only, then we can follow the rate with which individual hydrogen atoms are replaced by deuteriums atoms.
The experimental observations we get from those hydrogen-deuterium exchange experiments agree quite well with the predictions from our unfolding/refolding picture. Small, fast-folding proteins exchange the majority of their amide hydrogens, even those that are completely buried in the protein's interior in the fully folded state, within a few hours. The only possible explanation is that each protein has sampled a completely unfolded conformation at least once during this time period.

Protein Motion a nuisance or a necessity
I hope this little example above convinces you that protein motions, up to and including the complete unfolding, are very real and commonplace. The question now is whether protein motion is just a nuisance, something the protein has to live with, or is protein motion actually essential for protein function? There are two lines if evidence that the latter is true, that protein motions are essential for protein function!

The first line of evidence comes from protein structure. The active sites of many enzymes are completely buried in the interior of the protein and are not accessible to solvent, let alone a substrate molecule. Myoglobin and Acetylcholine esterase are two classic examples. In both cases, the structure of the free protein gives no indication of how the substrate might possibly be able to get into the active site.

The second line of evidence is the temperature dependence of protein motions and of protein function. Most proteins undergo a glass transition around 200 deg. Kelvin. Above this temperature proteins display relatively large and concerted molecular motions, below this temperature molecular motions seem to be restricted to simple, small-scale, harmonic vibrations. It is possible to see this transition in protein dynamics by a variety of experimental methods including dynamic neutron scattering, Moesbauer spectroscopy, and hole burning spectroscopy as well as X-ray crystallography. Associated with the cessation of these larger-scale, concerted and non-harmonic motions is a dramatic drop off in protein function. Enzyme catalysis for example does not just slow down, it stops completely below this temperature. Greg Petsko and Dagmar Ringe have written a very nice review article on this topic. (Ringe D, Petsko GA. *Biophys Chem*. Vol. 105, pp. 667-80 (2003)).

Two physical pictures of molecular motion

Now that I have hopefully convinced you about the importance of protein motion, lets take a step back and think about the motions of individual atoms and see if this can teach us something about the way proteins move.

One way we can think about the motion of atoms in a protein is as a Newtonian particle, i.e. a particle that has a certain mass and a certain amount of kinetic energy and that this particle just keeps going in a straight line, until it experiences a force that alters its course. An alternative picture is that of a diffusing particle propelled by Brownian motion on a stochastic trajectory. Lets do some model calculations for both models.

Atoms as Newtonian Particles

Kinetic gas theory tells us that thermal and kinetic energy of a molecule is one and the same thing. Specifically, this theory predicts that the translational energy of a molecule is 3/2 kT. Lets use this information to calculate the speed with which a carbon atom moves at room temperature. How long does it take such an atom to travel the length of a good-sized protein (i.e. 50 Å)?

$$E_{kinetic} = \frac{1}{2}mv^2$$

$$E_{trans} = \frac{3}{2}kT$$

$$E_{kinetic} = E_{trans}$$

so

$$\frac{3}{2}kT = \frac{1}{2}mv^2$$

$$\frac{3}{2}kTN_A = \frac{1}{2}mN_Av^2$$

$$\frac{3}{2}RT = \frac{1}{2}Mv^2$$

$$\frac{3RT}{M} = v^2$$

$$\sqrt{\frac{3RT}{M}} = v$$

units

$$\sqrt{\frac{J \cdot K^{-1} \cdot mol^{-1} \cdot K}{kg \cdot mol^{-1}}} = \sqrt{\frac{kg \cdot m^2 \cdot s^{-2} \cdot K^{-1} \cdot mol^{-1} \cdot K}{kg \cdot mol^{-1}}} = \frac{m}{s}$$

Lets plug in some numbers. The molecular mass of carbon is 12g/mol, the temperature is 300 K. Then we get:

$$\sqrt{\frac{3RT}{M}} = v$$

$$v = 800 m \cdot s^{-1}$$

$$d = t \cdot v$$

$$\frac{d}{v} = t$$

$$\frac{50 \cdot 10^{-10} m}{800 m \cdot s^{-1}} = 6.25 \cdot 10^{-12} s = 6.25 ps$$

The calculation indicates that the atom will travel the 50Å length of the protein in 6.25 picoseconds.

Atoms as Brownian Particles

Alternatively we could think about molecular motion as diffusion. Lets see what "speed" we get for molecular motions when we think of them as diffusion processes. Specifically lets see how far, on average, a molecule will have diffused in the same 6.25 picoseconds it took the atom in the above example to move 50Å.

$$\sqrt{\langle r^2 \rangle} = rmsd = \sqrt{6Dt}$$

with

$$D = \frac{k_B \cdot T}{f}$$

$$rmsd = \sqrt{\frac{6k_B \cdot Tt}{f}}$$

with

$$f = 6\pi\eta a$$

$$a = radius = 1.5 \cdot 10^{-10} m$$

$$\eta_{H_2O} = viscosity_{H_2O} = 8 \cdot 10^{-4} kg \cdot m^{-1} \cdot s^{-1}$$

$$T = temperature = 300K$$

$$k_B = 1.38 \cdot 10^{-23} kg \cdot m^2 \cdot s^{-2} \cdot K^{-1}$$

$$rmsd = \sqrt{\frac{6k_B Tt}{6\pi\eta a}}$$

$$rmsd = \sqrt{\frac{6 \cdot 1.38 \cdot 10^{-23} \cdot 300 \cdot 6.25 \cdot 10^{-12}}{6 \cdot 3.14 \cdot 8 \cdot 10^{-4} \cdot 1.5 \cdot 10^{-10}}} = 2.6 \cdot 10^{-10} m = 2.6 Å$$

There is obviously a big disparity in the results we get from the two models for molecular motion. The Newtonian picture of molecular motion predicts that we will move 50 Å in the same time period in which a diffusing particle will move a mere 2.6 Å. Note that this disparity increases with time. The distance the particle diffuses depends on the square root of the time, while the distance traveled by the Newtonian particle is linearly proportional to time. How can we explain this disparity and which one of the two pictures of molecular motion is right?
The first calculation assumed that the atom would move in a deterministic fashion following a straight path, but we know that proteins as well as solvent are quite tightly packed; individual molecules or neighboring water molecules are in VDW contacts. As a result, an atom moving along at 800 m/s will slam into a neighboring atom very soon. The mean free path in the interior of a protein (i.e. the average distance between each atom and a neighbor) is ~0.2 Angstrom. Lets calculate how long an atom can move on a given trajectory before it will slam into a neighbor causing it to reverse its course.

If we consider events that are faster than those 10-100 fs it takes before an atom or a group of atoms (e.g. a phenyl ring or any other protein side chain) bangs into a neighbor, then it is appropriate to treat this motion as a simple, deterministic, Newtonian motion. The types of motions were such a treatment is appropriate are the very fast motions of bond breaking, photo-isomerization and very small-scale rattling in cages motions in which molecular groups bounce around in a cage formed by the surrounding groups and atoms.
Motions larger than a few tenths of an Angstrom in amplitude are not well described by Newtonian motion. Those motions are instead better described as diffusion like motions. These larger scale molecular motions can only take place, if the atoms forming the cage –and each undergoing rattling motions themselves– happen to adopt a constellation allowing the molecular group in the center to escape and slip into a neighboring cage. Waiting for constellations that allow molecular groups to jump from one cage to the next takes the bulk of the time in the diffusion process. In other words the molecular motion in a diffusion process appears so much slower than the Newtonian motion, because a molecular group spend a lot of time rattling back and forth in a local cage without any net change in its position.

Just as a little exercise, I would encourage you to do the two calculations we did above for a distance corresponding the mean free path in a protein (0.2Å) instead of 50Å. You should see that for very short distances the two equations give very similar results, just as they should.

Time scales for some protein motions

Bond breaking	10^{-14} s
Cis-trans photo isomerizations	10^{-13} s
Light-induced electron transfer	10^{-4} - 10^{-12} s
Side-chain rotations on surface	10^{-9} - 10^{-10} s
Side-chain rotations in interior	10^{-6} s
Turn over of fastest enzyme	10^{-6} s
Protein conformational changes	10^{-5} - 10^{0} s

Atomic vibrations, protein catalysis and the Mayflower

Ok. We now have some numbers for rate constants and time-scales and we know that anything involving motions larger than a few tenth of an Angstrom or taking longer than a few hundred femto seconds are bound to be stochastic, diffusion-like motions that are heavily dampened by viscous drag. However, if experience is any guide, many of you will have a hard time to develop an intuitive feel for the relative time-scales of molecular motions. In particular it will be hard to see how the time-scale of rapid Newtonian rattling in cages relates to the diffusion-like stochastic motions that characterize protein motions on longer time and distance scales. This problem is perfectly understandable; our day-to-day experience does little to prepare us to think about time-scales faster than a second. Sub consciously, we tend to glom everything faster than a millisecond into one category; "really fast".

Still thinking about microseconds and femtoseconds as being similar is a REALLY BIG MISTAKE that leads to many misconceptions about how proteins work.

On the one hand, we saw that atomic vibrations and breaking covalent bonds breaking occur on the femtosecond (10^{-15} sec) time scale. On the other hand, we learned that the very fastest enzyme (carbonic anhydrase) turns over at a rate of 1,000,000 per seconds (i.e. 10^{-6} sec per turn over). To understand how huge a difference in time-scales this is, lets relate the relative ratio of these two time-scales to the ratios of time scales more familiar to us.

Lets say it takes a person about 10 seconds to take a deep breath. Then one would take this many deep breath per second, minute, hour etc. :

second	0.1	
Min	6	x60
Hour	360	x60
Day	8640	x24
Year	3,150,000	x365
since Mayflower landing	$\sim 1 \times 10^{9}$	x(2004-1620)

The table shows us that the number of breaths taken by a person, if they had lived since the landing of the Mayflower, is also the number of femtoseconds in a microsecond. Remember, a microsecond is about the fastest turnover time for any enzyme, but it only takes a few fs to make or break chemical bond. So there is an incredible amount of thermal vibration, rattling in cages and diffusion like motions going on during each catalytic cycle of even the very fastest of enzymes. Proteins are **not** deterministic machines that stamp out reaction products the way metal forming press stamps out auto parts, with very few exceptions protein conformational changes required for function are stochastic, minimally biased random walks.