Chapter 6 Odd Solutions

1. Clearly the answer cannot be yes: many of the internal motions that proteins undergo are small-scale random motions with no particular functional significance. It is still far from clear, however, whether slower and more correlated motions are necessarily functionally important. It seems inherently unlikely that all slower motions are functionally important, but several proteins have now been studied in detail, for which the major slow motion (described for example by the lowest energy normal mode, or by experimental measures such as NMR) does indeed look functionally significant. It could for example open the interface between two domains to allow substrate and product in or out, or it could uncover binding sites of various kinds. It may be too strong a statement to say that motion within a protein is selected by evolution to quite the same extent as structure is, but it is certainly clear that natural selection has refined the slower motions that proteins can undertake. Therefore it must be true that some amino acids in proteins tend to be conserved as much because of their importance for motion as for any (static) structural reason. In Chapter 6 I give examples of residues or chains of residues that seem to be conserved for precisely this reason. It is thus likely that analysis of conserved residues could provide evidence on motions, although disentangling this from structure is far from easy.

As the question notes, internal motions cover a very large range of timescales. In general, the faster motions are driven by ('slaved to') random thermal motion, and there is then a 'channeling' process by which a few slower motions are specifically selected. There must undoubtedly be a great number of motions that are functionally irrelevant, but so far the evidence is that if there is a single major slow motion, it does have functional importance. This may or may not extend to very slow (infrequent) motions slower than the turnover rate, in particular protein unfolding. For several proteins, the most common process leading to unfolding is essentially the same as the one responsible for function (see, for example, H.R. Kalbitzer et al., *J. Am. Chem. Soc.* 131:16714-16719, 2010 which postulates this as a general principle). Thus in cytochrome *c*, for example, the first stage in unfolding has been shown to be the loss of an interaction from a methionine side chain to the heme iron; this motion is also one of the lowest-energy motions in the native protein, and is a key part of the function of the protein [M.P. Williamson, *Proteins* 53:731–739, 2003]. However, in other proteins the unfolding pathway probably has no connection to functionally important motions. If the unfolding is much slower (less frequent) than the turnover rate, it is hard to see why the two should have any relationship.

3. There are a number of sites that display normal modes, one of which is http://igs-server.cnrs-mrs.fr/elnemo/examples.html. As described above, the normal mode is a low-energy correlated motion and the displayed normal modes are shown as nice-looking slow rocking motions, typically with domains rigid and the connections between them varying in a regular and harmonic oscillation. In real

proteins you would never see a harmonic oscillation like this, and it would be so obscured by all the faster random thermal motions that you could probably not identify anything remotely resembling a normal mode. This does not of course mean that normal modes are not useful or 'real'; they are, however, idealized.

5. Higher temperature means that the solvent has higher mobility. Because protein mobility is derived ultimately from the thermal motion of the solvent, at higher temperature the protein also has greater thermal energy and therefore a larger magnitude of high-frequency motions. Therefore all other motions will in general also have greater magnitude. Thus the slower motions will also occur faster and with higher probability; motions leading to denaturation will occur faster too and therefore denaturation is more rapid (and more likely). Almost exactly the opposite is true at low temperatures. The main difference is in protein stability. Protein stability tends to peak around ambient temperature, and becomes less at both high and low temperature. Therefore proteins can also be denatured by low temperature, although usually the temperature needed is so low as to be not physically achievable.

Pressure is not quite so straightforward. The thermodynamic consequence of high pressure is that motions that lead to an increase in volume are disfavored, while motions that lead to a reduction of volume are favored. The fast low-amplitude uncorrelated motions have almost no effect on molecular volume and are therefore not greatly affected by pressure. However, the slower and more correlated motions almost always increase protein volume, basically because they tend to produce an 'opening out' of the compact folded conformation. Therefore such motions are inhibited by high pressure.

The consequence for organisms is that organisms that live at high temperatures (thermophiles) have adapted their proteins to have the same amplitude of large-scale motion at their normal working temperature as those of mesophiles at more normal temperatures. This of course implies that at normal temperatures the large-scale motions of thermophilic proteins tend to be smaller. (This observation is good evidence for the suggestion that natural selection does act on protein mobility.) Conversely, organisms that live at low temperature (psychrophiles) have more dynamic proteins than mesophiles do. Similar conclusions probably hold also for organisms living at high pressure (which are called piezophiles or barophiles): their proteins probably have similar large-scale motion at high pressure as those of mesophiles at ambient pressure, or in other words more mobility at ambient pressure. However, there have been fewer observations of such proteins.

It is also worth adding that an unfolded protein has a smaller volume than a folded protein, basically because it is not possible to fold up an irregular shape like a polypeptide chain without leaving a few gaps or cavities here and there. Therefore pressure always leads eventually to protein denaturation, although the pressure required is rather strongly protein-dependent.

7. You would normally expect that the binding of a ligand to a protein would lead to a denser network of interactions between ligand and protein, and would therefore hold the protein together more and decrease the overall internal mobility within a protein. There is an energetic problem with this, namely that a decrease in mobility equates to a decrease in entropy. Via the relationship $\Delta G = \Delta H - T\Delta S$,

a decrease in entropy implies an increase in free energy; that is, weaker binding. In other words, any loss in mobility of the protein on binding weakens the binding. Therefore it is an attractive idea that binding could lead to increased mobility in some parts of the protein, to counteract the decreased mobility close to the binding site. There are several ways in which this could occur. For example, if the protein 'closes in' around a substrate, this could 'open up' the opposite side of the protein, permitting greater mobility.

The most obvious way to investigate this is probably by means of NMR relaxation, or by studies of amide proton exchange to deuterium (which can be done either by NMR or by mass spectrometry after peptide digestion). One can also perform computer simulations. There have been several such studies, but with no general conclusion: sometimes there is increased mobility on the opposite face of the protein, and sometimes (rather more often) there is not. Thus, this certainly does not represent a general solution to the problem of weaker binding, though it does happen sometimes.

N1. If each residue has three conformations, then the number of possible conformations is $3^{200} = 2.7 \times 10^{95}$. If they exchange every 10^{-12} seconds, then the total time to search all of them is $2.7 \times 10^{95} \times 10^{-12} = 2.7 \times 10^{83}$ s, or 8.4×10^{75} years. This is often compared with the age of the Universe, which is about 10^{10} years. The time taken is thus an unimaginable 10^{66} times longer than the age of the Universe. The real time taken to fold a protein is on the order of 100 ms, depending on the protein. This forms a very graphic illustration that protein folding cannot be a random process.