Chapter 11 Odd Solutions

1. This paper is discussed by A.M. Lesk [Introduction to Protein Science: Architecture, Function, and Genomics, 2nd ed. Oxford: Oxford University Press, 2010].

The most important conclusion to be drawn from this study is that making a mutation in a protein does not produce effects that are easy to interpret or predict! One might expect that it would be possible to mutate an arginine, which is positively charged and at least partly buried, to a lysine without major change in protein stability, and this is true. One might reasonably expect that mutation to a negatively charged glutamate would be very destabilizing, but this is not so, probably because the protein can rearrange itself enough to allow it. Mutation to aspartate is, by contrast, not well tolerated, probably because the negative charge is buried too deeply for the protein to be able to compensate by minor structural changes. Mutations to large hydrophobic residues or to proline are very unfavorable, which makes a lot of sense because they require major protein rearrangements. Mutations to similar-sized hydrophobic or polar (but uncharged) residues are tolerated fairly well: again, this is not unexpected, although it is a little surprising that glutamine is tolerated so well. Glutamine has a fairly long hydrophobic side chain with a polar part on the end, and it seems very likely that the hydrophobic region makes significant favorable interactions.

The change in free energy parallels the change in melting temperature remarkably well. The enthalpy changes, on the other hand, make very little sense. There is an overall correlation, but in detail the results are widely varying. This is the sort of result discussed in Section 1.2.10. Enthalpy/entropy compensation means that enthalpy and entropy can fluctuate wildly without affecting the free energy much. It is hard to rationalize the enthalpy changes, even with the benefit of detailed crystal structures.

3. As suggested by the question, mass spectroscopy (MS) can be used for all of these modifications. It is, however, not the best or most common method for all of them. (a) The most common mass spectroscopic method for looking at proteins is to digest the protein, for example by using trypsin, which cleaves after Lys and Arg, and then running the peptide fragments. As long as the three phosphorylation sites come in different peptides, it should be possible to use MS to detect where phosphorylation has occurred. MS is, however, not very quantitative: the relative intensities of different peptides can be unpredictably variable. There are several ways round this, particularly variations of a method called SILAC that uses stable isotope labeling as an internal standard, but they introduce extra complications. Many common peptides have antibodies specific for phosphorylated and unphosphorylated forms, so it is often simpler, at least in the first instance, to use an immunological method such as Western blotting. (b) This is a similar problem to (a), with the obvious complication that modification of a lysine makes trypsin digestion not a good method. Again, antibodies are often good, or proteins known to be specific for certain modifications. (c) You could of course use

MS again, but the change in mass is so large that SDS-PAGE is the obvious first method. (d) SDS-PAGE of glycosylated proteins usually results in a smear. MS could be tried. NMR is also possible but needs more material. If you know roughly what glycosylation to expect, then specific recognition proteins such as lectins (for example with secondary antibodies to visualize the binding) would work. (e) Because sulfation of tyrosine creates a characteristic UV signature, this would be a good method.

5. One of the major drawbacks with NMR is its low sensitivity. Cold probes and cryoprobes are a way of improving sensitivity in detection of the NMR signal. One of the main sources of noise in NMR experiments is thermal noise in the detector coil, and in cold probes and cryoprobes the detector coil is cooled by helium to about 30 K, which reduces the noise by a factor of roughly $(300/30)^{1/2}$ or about 3, and thus increases the signal-to-noise ratio by the same amount. This does not sound like a great gain, but in fact it is extremely useful (and a very cost-effective way of increasing sensitivity), and an increasing number of protein spectrometers are now fitted with such devices.

7. No. With any new crystal structure, one always needs to check whether the structure matches the biochemical data: that is, whether the structure 'looks right.' Sometimes the structure is clearly not that of an active state; for example, domains may be rotated so as to separate residues that are known to be adjacent in the active protein. However, this does not make the structure nonphysiological, because it is perfectly possible that this conformation is present *in vivo*. In general, detailed comparisons of crystal structures with biochemical data, and with for example NMR or cryo-electron microscopy structures, provide a remarkably good match.

Crystallization is a slow process, and the regular lattice packing that produces a crystal does sometimes distort the structure locally. It is not uncommon to find surface-exposed loops that are in different conformations in two different crystal forms, or differing from NMR structures.

The effects of temperature and ionic strength are remarkably small. Early crystal structures were obtained at ambient temperature, whereas nearly all modern structures are obtained at very low temperature. There is, however, almost no difference, other than a slight thermal expansion (and increasing disorder) as the temperature is raised. A detailed study of protein crystal structures at different temperatures found no other significant changes [R.F. Tilton, J.C. Dewan and G.A. Petsko, *Biochemistry* 31:2469–2481, 1992]. It is even more remarkable that the crystallization conditions seem to have almost no effect on the protein structure. Crystal structures often contain electron density attributable to metal ions or to the cosolvents (low-molecular-mass alcohols, for example) used in crystallizations. However, the number of such molecules is relatively small compared with their concentrations in the crystallization solution, and it seems that they interact weakly enough with the protein to have little overall effect. A solution of high ionic strength disrupts electrostatic interactions as a result of **electrostatic screening (*4.4)**, so one might expect that crystal structures would have fewer salt bridges than are normally present in less ionic solutions. However, the reverse seems to be true: a recent study showed that salt bridges present in crystal structures are not present in solution

[J.H. Tomlinson, S. Ullah, P.E. Hansen and M.P. Williamson, *J. Am. Chem. Soc.* 131:4674–4684, 2009]. Presumably this is because crystallization favors an ordered structure and therefore encourages the formation of salt bridges.

N1. At 500 MHz, the energy difference is (from the first equation) 3.3×10^{-25} J, and therefore the ratio of the populations in the two states is 1:0.999921. In other words, there is a relative excess in the lower energy state of 7.9×10^{-5} or one in 13,000. At 900 MHz the equivalent figure is one in 7000, which is close to an improvement by a factor 900/500. Thus, the population difference increases roughly in proportion to field strength. However, the sensitivity of detection of an NMR signal increases by a factor considerably more than this, because at higher field both the precession frequency and the detection sensitivity are increased, as explained in many NMR texts.

N3. As given in *11.11, NA = $\eta \sin\alpha$, where $\eta = 1.4$. Thus, at an incident angle of 60°, the numerical aperture is $1.4 \sin(60°) = 1.21$. The maximum resolution is (*11.11) $0.61\lambda/NA$, which (for blue light for example with a wavelength of 475 nm) is $0.61 \times 475/1.21 = 250$ nm, or 2500 Å.

N5. The molecular mass of the protein is 7250 Da, so one mole weighs 7250 g, and one molecule weighs $7250/(6 \times 10^{23})$ g. It therefore has a volume of $7250/(1.4 \times 6 \times 10^{23})$ ml (cm^3). We want to compare this with the unit cell from question N4 so we should convert the volume to cubic nanometers; 1 nm is 10^{-9} m and 1 cm is 10^{-2} m, so there are 10^7 nm in 1 cm, or $(10^7)^3 = 10^{21}$ nm^3 in 1 cm^3; that is, in 1 ml. Therefore the volume of one molecule of protein is roughly $7250 \times 10^{21}/(1.4 \times 6 \times 10^{23}) = 8.63$ nm^3. The volume of the unit cell calculated in question N4 is 55.93 nm^3, which means that, remarkably, the protein occupies only 8.63/55.93 = 0.15 (15%) of the volume of the unit cell (and therefore of the crystal), the rest being water. (Actually, a small fraction will also be vacuum: the small cavities inside the protein that are too small for water to fill are completely empty. These, however, constitute a rather small fraction of the total volume.) The rest is water (and ions and cosolvents: but as noted in Problem 7 of this chapter, these usually are not very numerous). Water therefore occupies $55.93 - 8.63$ $nm^3 = 47.3$ nm^3.

Following the same sort of arguments as used above, at 20°C there are 0.998 g of water in 1 ml. One mole of water weighs 18 g, so 1 ml contains 0.998/18 moles. Therefore 1 nm^3 contains $(0.998/18) \times 10^{-21}$ moles or $(0.998/18) \times 10^{-21} \times 6 \times 10^{23}$ molecules = 33.3 molecules. Therefore 47.3 nm^3 (one unit cell, less the protein) contains $47.3 \times 33.3 = 1574$ water molecules.

This means that in the crystal structure, 89 water molecules of the roughly 1574 were identified. The remaining 1485 (94% of the water molecules) were not seen.

This is a rather typical sort of figure. As we have seen, the protein itself occupies only 15% of the volume of the unit cell. Typically, up to two layers of water around each protein molecule are sufficiently ordered that they can be seen as peaks of density in the electron map and can therefore be located as being water molecules. Beyond this, the water molecules are sufficiently disordered that no useful peaks of electron density can be seen; there is therefore a smeared-out average density, and it makes no sense to try to put water molecules here (see Section 11.4.6).