Chapter 4 Odd Solutions

1. The text already gives some examples:

Airport procedure	Cell procedure
Tagging of baggage with bar codes	Signal peptides (usually removed once correct destination is reached!)
Physical separation of arrivals and departures, with streaming to avoid crossing of paths	Physical separation of export and import machinery, with streaming based on motor proteins and microtubules, etc.
Arrival passengers pick up baggage near exit	Exported molecules packaged into separate vesicles that are exported separately or fused close to cell membrane
Passengers move onto plane on buses	Proteins packaged into vesicles

Other possible examples are:

Moving walkways to speed movement	Movement along microtubules and myosin
Several checkpoints for passports, tickets, customs, security	Several checkpoints for foldedness and correct signal
Airline-specific staff and desks	In some cases, different sets of protein machinery for different exit routes
Gates for passengers waiting for a plane	Vesicles for proteins waiting for export
Physical separation of departures and arrivals only in large airports	Physical separation of export and import much less marked in prokaryotes
Customs check on arrival	Not really, although ABC transporters export unwanted imports
Physical barriers in the form of walls, gates, etc.	Physical barriers in the form of cell membrane, cytoskeleton, etc.

3. No, because (a) there would be too high a risk of the protein aggregating over the long lifetime of the protein, (b) housekeeping proteins tend to be present at very high concentration, which would markedly increase the risk of aggregation, and (c) intrinsically disordered proteins tend to be short-lived, which contrasts with the long lifetime required for housekeeping proteins.

5. Addition of dextran causes molecular crowding of the unfolded lysozyme. Because the unfolded protein has a significantly larger excluded volume than the molten globule state, crowding encourages folding to the molten globule state. The concentration of dextran needed to reach a roughly 50% molten globule state is the point of inflection in the curve, or about 230 g l^{-1}, corresponding to about 6.5 mM. The radius of gyration of a folded protein is only about 10% less than that of a molten globule, so it would need a much higher concentration of dextran to fold the protein to the native state; almost certainly so much dextran that it would not remain soluble. A polymer of lower molecular mass would have a stronger effect

because of its volume would be smaller, and in particular smaller than lysozyme (molecular mass ≅ 13 kDa), and should therefore start to fold the protein earlier.

7. See, for example, M.P. Williamson [*Biochem. J.* 297:249–260, 1994]. In addition to being found widely in signaling systems, proline-rich regions are also found in the regulation of assembly of actin (profilin, etc.) and activation of myosin, in a range of membrane and cell wall assemblies, in the machinery for import across the mitochondrial double membrane, and in a wide range of hinges between domains. So they are not universal but they are certainly widespread. Proline-rich regions are particularly useful where rapid on- and off-rates are required, as in all the examples above.

9. The key requirement for the formation of amyloid is that the native state should be capable of unfolding at least transiently, providing a high enough concentration of unfolded protein to allow it to self-associate into amyloid. Thus, in most experiments used to persuade proteins to form amyloid, one has to use rather harsh conditions such as low pH or a high concentration of denaturant. The argument of Dobson is that once such a state is achieved, all proteins are probably happy to self-assemble into amyloid, because the cross-β structure is essentially a property of the protein backbone rather than the side chain sequence. Given the fact that even highly helical proteins can be induced to form amyloid, this could indeed be true. But one has to bear in mind that the conditions needed to achieve a sufficient degree of partial unfolding could well be so unphysiological as to be irrelevant. Fortunately, we do not need to worry that all proteins may be able to form amyloid under native conditions.

N1. If the diameter is 0.65 μm, the radius is 0.325 μm and therefore the volume is $2 \times \pi \times 0.325^2 = 0.66\,\mu m^3$. One μm is 10^{-6} m, and 1 dm is 10^{-1} m; therefore there are $(10^5)^3$ or $10^{15}\,\mu m^3$ in $1\,dm^3$. Therefore the volume of an *Escherichia coli* cell is about 0.66×10^{-15} l.

N3. It will take 85×10^{-6} s^{-1} or roughly 90 μs. This feels rather slow in the context of a bacterial response, but of course this is the time required if only a single channel opens; normally we would expect many more than a single channel per cell.

N5. Following the calculations in Section 4.2.3, the relative rates are R/r, $\ln(R/r)$, and 1, where R is 50 μm, and r is 10 Å, namely 50,000:11:1. That is, there should be a gain in speed by a factor of nearly 5000 arising from search in two dimensions. This implies that there would be almost no free volume at all in plant cells and everything should occur along membranes. In practice this is not true, because (a) many plant cells contain large vacuoles which occupy much of the volume, so the accessible volume is therefore much smaller than would appear simply from the size of the cell, and (b) many plant cells make use of cytoplasmic streaming to mix the cell contents, as discussed in Chapter 7. The gain in going from two dimensions to one dimension is less substantial but still significant.