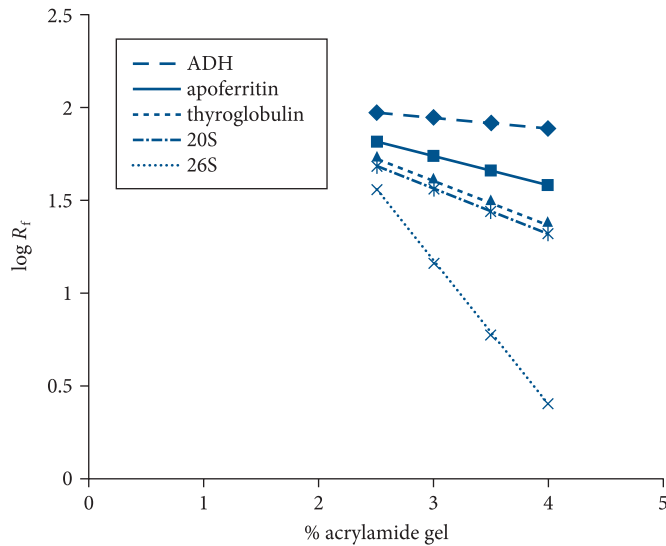
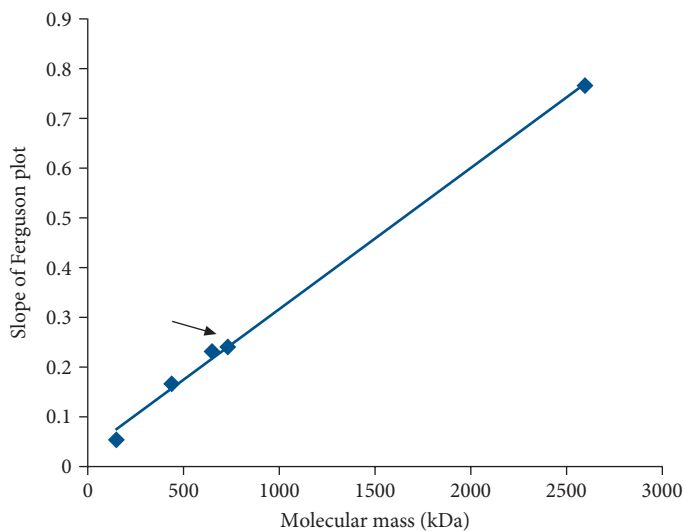


Solutions to problems for Chapter 8

8.1 A plot of $\log R_f$ values against % gel should generate a plot similar to that given below.



Calculation of the slopes from Ferguson plot above will allow you to construct a plot of these values against molecular mass, see below. The value for the 20S proteasome is indicated by the arrow and corresponds to a molecular mass of 700 kDa.



8.3 By plotting $\ln r$ vs. t , a straight line of slope $3.089 \times 10^{-5} \text{ s}^{-1}$ is obtained. This slope is equal to $\omega^2 s$. The rotor speed = 60 000 rpm., i.e. 1 000 rps; hence $\omega = 1\,000 \times 2\pi = 6\,283.2 \text{ rad s}^{-1}$. Thus $\omega^2 = 3.948 \times 10^7 \text{ (rad s}^{-1}\text{)}^2$, so that $s = 7.82 \times 10^{-13} \text{ s}$. Putting the values in the equation $M = RTs/(D(1 - \nu\rho))$, we obtain $M = 147\,200 \text{ g mol}^{-1}$ (i.e. the molecular mass is 147.2 kDa).

- 8.5** The difference between the two species identified by mass spectrometry is

$$15\,068.2 - 14\,865.9 = 202.3 \text{ Da.}$$

Assuming this loss represents the loss of a short peptide from N- or C-terminus, the mass of the resulting peptide will be $202.28 + 18.02$ (water) = 220.3 Da.

Knowing that the mass of an average amino acid is 110 Da, it is reasonable to assume the the loss of $220.3/110$ amino acids = 2 amino acids.

Looking at the sequence, the N-terminus and the C-terminus have the sequence methionine-alanine and alanine-methionine, respectively. If the full length subunit of ATPase has lost two amino acids from either the N- or C-terminus, there would be a reduction in mass by $71.08 + 131.20 = 202.28$ Da.

Thus it would appear the ATPase subunit has lost an alanine and a methionine residue (202.28 Da) giving rise to the species of molecular mass 14 865.9 Da. With the information provided we cannot determine whether these amino acids have been lost from the N-terminus or the C-terminus.

- 8.7** The difference between the experimental and theoretical mass of glycogen phosphorylase is $97\,280 - 97\,158 = 122$ Da. Using Table 8.14, which shows the mass changes associated with the more common post-translational modifications, a difference of 122 Da could be:

$$\text{either } 1 \text{ phosphorylation plus } 1 \text{ acetylation} = 80 + 42 = 122$$

$$\text{or } 1 \text{ phosphorylation plus } 3 \text{ methylations} = 80 + 3 \times 14 = 122$$

Further evidence to distinguish between 1 phosphorylation plus 1 acetylation and 1 phosphorylation plus 3 methylations would be provided by characterization of the glycogen phosphorylase tryptic peptides. If we calculate the mass differences, peptide 2–10 appears to be acetylated or triply methylated, whereas peptide 13–17 is phosphorylated. It is more likely that an acetylation has occurred in peptide 2–10, since this is a common modification to the N-terminal amino acid of native proteins. Further sequencing studies on this peptide would be required to confirm this.

Tryptic peptide	Position	Theoretical mass (Da)	Experimentally determined mass (Da)	Mass difference (Da)
SRPLSDQEK	2-10	1 059.5	1 101.5	42
QISVR	13-17	602.4	682.4	80

- 8.9** The quenching data are plotted in the form of Stern-Volmer plots (F_0/F) vs. $[Q]$; the slope of the line gives the Stern-Volmer constant (K_{SV}). The values of K_{SV} (in units of M^{-1}) are: NATA, 9.6; SK alone, 4.8; SK + shikimate, 1.8; SK + ADP, 4.4. From the relatively high value of the K_{SV} , it can be concluded that in SK the single Trp is significantly exposed to the solvent, a conclusion consistent with the high value of the wavelength of the emission maximum. On addition of shikimate, there is a considerable reduction in K_{SV} indicating that the Trp is at least partially buried. ADP has little effect on the exposure of the Trp. These findings are consistent with the structure of the enzyme which shows that Trp 54 is on one lobe of the enzyme close to the binding site for shikimate (Krell, T., Coggins, J.R. and Laphorn, A.J. (1998) *J. Mol. Biol.* 278, 985–997).

8.11 Using the approach outlined in section 8.6 (i.e. evaluating ΔG^0 at each [urea] and then extrapolating a graph of ΔG^0 vs. [urea] to give ΔG^0 (H_2O), the stability of the D76N mutant of RNase T1 is 10.8 kJ/mol. Comparison with the stability of the wild type enzyme in the worked example in section 8.6 shows that loss of the negative charge of the Asp 76 side chain has a major impact on the stability of the enzyme. Examination of the 3 dimensional structure of the enzyme shows that Asp 76 is buried in the enzyme and that it makes hydrogen bonds to the side chains of Asn 9, Tyr 11 and Thr 91 as well as to a bound water molecule which is conserved between structures of bacterial RNases; these interactions contribute to the stability of the folded state of the enzyme. Clearly replacement of the Asp 76 side chain by Asn weakens these interactions markedly and thus reduces the stability of the folded state (Giletto and Pace, 1999).