# Solutions to problems for Chapter 3

**3.1** The number of seconds in a year = 365 × 24 × 60 × 60 = 3.15 × $10^7$. The rate of hair growth = 0.18 m $year^{-1}$, i.e. 0.18/(3.15 × $10^7$) m $s^{-1}$ = 5.71 × $10^{-9}$ m $s^{-1}$, or 5.71 nm $s^{-1}$. The number of turns of α-helix produced per second = 5.71/0.54 turns, i.e. 10.6 turns. This is equivalent to 10.6 × 3.6, i.e. 38.2 peptide bonds $s^{-1}$. While this might sound an impressive rate of growth, it pales into insignificance by comparison with the activity of the silkworm, which can make a silk thread about 2000 m long in 3 days. Silk consists of the β-sheet protein fibroin and assuming that the span of the β-sheet is 0.33 nm per amino acid (see Chapter 1, section 1.4.2), this corresponds to a rate of synthesis of 2.3 × $10^7$ peptide bonds $s^{-1}$, some 6 × $10^5$-fold greater than that of hair growth.

**3.3** A 15-µM solution contains 15 × $10^{-6}$ × 545 mg $mL^{-1}$, i.e. 0.0082 mg $mL^{-1}$. Hence 3 mL contains 0.0246 mg.

**3.5** The concentration of polypeptide chains = 250/16 500 M = 0.0152 M (15.2 mM).

**3.7** The molecular mass of dimer = 88 kDa. Hence concentration = 13.6 × $10^{-6}$ × 88 000 mg $mL^{-1}$, i.e. 1.197 mg $mL^{-1}$.

**3.9** The amount of urea required is 10 × 60 mg $mL^{-1}$ (i.e. 600 mg $mL^{-1}$). For 15 mL we would require 9.0 g urea. This would be weighed out in a graduated tube and water added slowly, to ensure that the urea was dissolved, before being made up to the 15 mL mark.

**3.11** Applying the standard formula (eqn. 3.3) $V_S = C_W \times V_W/C_S$ where the concentrations are given in mg $mL^{-1}$ terms, and noting that $V_W$ = 1 mL, we find that $V_S$ = 0.2 × 1/4.3 mL = 0.0465 mL (46.5 µL). Our solution would therefore consist of 46.5 µL of protein and 953.5 µL buffer.

**3.13** The 0.5 M stock solution would be 0.5 × 238.3 mg $mL^{-1}$, i.e. 119.2 mg $mL^{-1}$. 5 mL of this solution would require 5 × 119.2 mg, i.e. 596 mg IPTG. This would be weighed out in a graduated tube and water added to give a final volume of 5 mL, ensuring that the IPTG is dissolved. The volume of this stock solution to be added can be calculated from the standard formula (eqn. 3.3) $V_S = C_W \times V_W/C_S$, giving $V_S$ = 1.2 × 50/500 mL = 0.12 mL. (Note that we have ignored the fact that this would give a small degree of dilution of the culture; this dilution would only amount to 0.24%).

**3.15** The concentration of the stock solution 3/709 M, i.e. 4.23 mM. To obtain 0.1 mM we use eqn 3.3; $V_S$ = 0.1 × 1.0/4.23 mL, i.e. 0.0236 mL (23.6 µL).

**3.17** The concentration of the protein solution after dilution was 0.237/0.66 mg $mL^{-1}$ = 0.359 mg $mL^{-1}$. This was a 25-fold (0.04 mL in a total of 1.0 mL) dilution of the original solution; the concentration of the original solution was therefore 0.359 × 25 mg $mL^{-1}$ = 8.98 mg $mL^{-1}$. This is the true concentration of the protein solution. It is less than that expected from the weight of protein taken (10 mg $mL^{-1}$); the most likely explanation for this is that there is a significant amount of moisture in the freeze-dried protein sample.

**3.19** The correct amounts of solid Tris ($50 \times 10^{-3} \times 121.1$ g = 6.06 g) and NaCl ($20 \times 10^{-3} \times 58.45$ g = 1.17 g) would be weighed out and dissolved in about 900 mL $H_2O$. This solution would be titrated to pH 7.5 with HCl of appropriate concentration and the solution would be made up to 1 L with $H_2O$.

**3.21** Using the approaches outlined, the table can be completed as follows:

| Step | Total protein (mg) | Total activity (units) | Specific activity (units $mg^{-1}$) | Yield (%) | Purification factor[¶] |
|---|---|---|---|---|---|
| Crude extract | 72 500 | 413 000 | 5.70 | 100 | 1.0 |
| Low pH | 18 700 | 365 000 | 19.5 | 88.4 | 3.4 (3.4) |
| Phosphocellulose | 290 | 223 000 | 769 | 54.0 | 39.4 (135) |
| Gel filtration | 77 | 200 000 | 2600 | 48.4 | 3.4 (456) |

¶ The figures represent the factor per step; those in brackets show the running total relative to the crude extract as 1.0 (e.g. $3.4 \times 39.4 = 135$).