

# Solutions to problems for Chapter 10

- 10.1** From the Hill plot for haemoglobin Howick, the values of  $h$  and  $K$  are 1.3 and 1.27, respectively; the latter is derived from the  $(pO_2)_{50}$  of 1.2 mm Hg and the value of  $h$ . Comparison with the values for normal haemoglobin ( $h = 2.8$ ,  $K = 9160$ ,  $(pO_2)_{50} = 26$  mm Hg) shows that the mutation has markedly increased the affinity for  $O_2$  and decreased the cooperativity between the  $O_2$ -binding sites. These changes result from the increased tendency of the tetrameric form of the mutant protein to dissociate into dimers (Brittain, T. (1994) *Biochem. J.* **300**, 553–6).
- 10.3** The data are analysed as follows. The maximum change values are converted to saturation (mol molybdate bound per mol protein) values by noting that 100% change corresponds to two molybdate bound per ModE ( $r = 2$ ). Since the  $[ModE]_{\text{present}} = 0.6 \mu\text{M}$ , the  $[molybdate]_{\text{bound}}$  can be calculated (100% saturation corresponds to  $1.2 \mu\text{M}$  bound ligand). Hence the free  $[molybdate]$  can be determined by subtraction from the  $[molybdate]_{\text{total}}$ . A Scatchard plot ( $r/[molybdate]_{\text{free}}$  vs  $r$ ) can be used to determine  $K_d$ . The slope of the Scatchard plot is  $-1.25 \mu\text{M}^{-1}$ ; hence  $K_d = 0.8 \mu\text{M}$ .
- 10.5** The concentration of enzyme octamer ( $2.2 \text{ mg mL}^{-1}$ ) =  $2.2/280\,000 \text{ M}$ , i.e.  $7.86 \mu\text{M}$ . The concentration of bound  $Zn^{2+}$  can be found by subtracting the concentration outside the bag from that inside. From this, the values of  $r$  and  $r/[Zn^{2+}]_{\text{free}}$  can be calculated to allow a Scatchard plot ( $r/[Zn^{2+}]_{\text{free}}$  vs  $r$ ) to be constructed. The plot is a straight line with an  $x$ -axis intercept of 8. There are thus eight binding sites per enzyme (i.e. 1 per subunit), which are equivalent and independent. From the slope of the plot, the  $K_d = 80 \mu\text{M}$ .
- 10.7** In the absence of cAMP, CAP does not bind to the promoter. However, addition of cAMP leads to the formation of a cAMP–CAP–promoter complex. The concentrations of bound and free promoter are 0.17 and  $0.03 \mu\text{M}$ , respectively, and the concentration of free CAP is  $0.9 - 0.17 \mu\text{M}$ , i.e.  $0.73 \mu\text{M}$ . Hence the  $K_d = ((0.73 \times 0.03)/0.17) \times 10^{-6} \text{ M} = 0.13 \mu\text{M}$ . The L8 mutant promoter does not form a complex with CAP or cAMP–CAP.
- 10.9** The factor by which  $V_{\text{max}}$  is reduced is  $(1 + ([I]/K_{\text{ESI}}))$ -fold. For  $0.5 \text{ mM L-Phe}$ , this is 1.923-fold; for  $1 \text{ mM L-Phe}$ , this is 2.857-fold. At both concentrations,  $K_{\text{ESI}}$  can be calculated to be  $0.54 \text{ mM}$ .