

Problem Set

Ligand Binding

9.1

- The specific ryanodine binding to isolated cardiac sarcoplasmic reticulum was determined and gave the following results:

Bound Ryanodine (pmol mg ⁻¹)	Free Ryanodine (nM)
0.31	0.19
0.73	0.52
1.23	1.27
1.67	2.08
2.01	2.99
2.53	7.47
2.82	17.18
3.12	26.88

What is the B_{\max} and K_d for ryanodine binding? Is the binding cooperative? Be sure to include any graphs that you might want to make. Mathematics without the graphics is a bad mistake because the human mind is a much better filter than mathematical constructs like the correlation coefficient.

- The following table shows the inhibition of ryanodine binding by ruthenium red. Is ruthenium red a competitive or noncompetitive inhibitor of ryanodine binding? Calculate the K_i for ruthenium red.

Ryanodine (nM)	Ruthenium Red (μ M)	Bound Ryanodine (pmol mg ⁻¹)
5	0	3.14
5	0.043	1.76
5	0.118	0.99
5	0.370	0.39
10	0	4.28
10	0.043	2.43
10	0.118	1.46
10	0.370	0.71
30	0	5.33
30	0.043	4.33
30	0.118	2.99
30	0.370	1.77

- The following data were obtained for the Ca^{2+} dependence of ryanodine binding to rat heart homogenates:

Bound Ryanodine (pmol mg protein ⁻¹)	pCa
0.025	7.5
0.048	6.3
0.087	6.0
0.185	5.8
0.329	5.6
0.480	5.4
0.547	5.2
0.552	4.7
0.568	4.3
0.543	3.4

Here $\text{pCa} = -\log [\text{Ca}^{2+}]$. The nonspecific binding of ryanodine, measured with excess cold ryanodine, averaged 0.025 pmol mg protein⁻¹ at pCa 7.5, 5.4, and 3.4. That is, the nonspecific binding did not depend on pCa. What is the Hill coefficient and K_{Ca} for this binding? (Hint: first plot the saturation curve. Determine B_{\max} for use in the Hill plot. Subtract $B_{\text{nonspecific}}$ from all values to obtain the specific binding and then perform the Hill analysis. Use for the analysis only those points on the Hill plot that are between 10% and 90% saturation.)

- The following data were obtained for the velocity of the SERCA2a Ca^{2+} -ATPase of cardiac sarcoplasmic reticulum as a function of the free $[\text{Ca}^{2+}]$:

$[\text{Ca}^{2+}]$ (μ M)	ATPase Rate (nmol min ⁻¹ mg SR protein ⁻¹)
61.6	519
25.8	519
6.9	82
4.3	395
3.07	311
2.37	271
1.92	195
1.09	115
0.66	61
0.04	19

What is the Hill coefficient and K for the Hill equation? (Hint: The value of the ATPase rate at very low $[\text{Ca}^{2+}]$ is due to contaminating Mg^{2+} -ATPase of unknown origin. Its value must be subtracted from all ATPase values prior to the Hill analysis. Plot v against pCa to convince yourself of this.)

5. The velocities of the pyruvate kinase reaction obtained at a number of concentrations of phosphoenolpyruvate, at constant $[\text{ADP}]$ and in the absence of modifiers, are given below:

[PEP] (mM)	Rate (a.u.)
0.0195	0.006
0.0325	0.012
0.065	0.031
0.195	0.094
0.325	0.114
Saturating	0.130

Determine the Hill coefficient and K for the Hill equation.

6. EGTA complexes Ca^{2+} and thereby allows for experimental buffering of solutions containing Ca^{2+} . Assume the association constant for 1:1 complex of Ca^{2+} with EGTA is $0.5 \times 10^{-6} \text{ M}^{-1}$. If the total Ca^{2+} content (the sum of free and bound) is $100 \mu\text{M}$, and the total $[\text{EGTA}]$ is $400 \mu\text{M}$, what is the free $[\text{Ca}^{2+}]$? What is $\text{pCa} = -\log [\text{Ca}^{2+}]$?
7. Suppose that PTH is destroyed only by the kidneys in a single pass. Suppose that the blood volume is 5 L. How much blood would have to flow through the kidneys to achieve a half-life for PTH of 2 min? Is it reasonable to suppose that only the kidney destroys PTH?
8. The half-life of insulin is about 5 min. What is its fractional turnover? If the blood volume is 5 L and the GFR is 120 mL min^{-1} , and assuming

a sieving coefficient of 1.0, is loss through glomerular filtration sufficient to explain the half-life of insulin?

9. Thyroxine binding globulin (TBG) has a M_r of 54 kDa. Its metabolic clearance rate is 800 mL day^{-1} and its volume of distribution is 7 L. The plasma $[\text{TBG}] = 2 \text{ mg dL}^{-1}$.
- A. What is TBG's half-life?
- B. If TBG binds one molecule of T4, what is its binding capacity, expressed as $\mu\text{g T4 dL}^{-1}$?
10. The following data are normal for humans:

Total T4	100 nM
Free T4	20 pM
Total T3	1.8 nM
Free T3	5 pM

The concentration of TBG is about 1.5 mg%. Its molecular weight is 54 kDa. Assume that 80% of the total T4 and T3 are bound to TBG. Estimate the association constant of TBG for T3 and T4.

11. The daily production rate of cortisol is about 15 mg day^{-1} (10–20). Its circulating half-life is about 95 min (70–120 min). If its volume of distribution is 7 L, what is the average total concentration in blood?
12. The peak of progesterone during the luteal phase of the menstrual cycle is about 10 ng mL^{-1} .
- A. If the molecular weight of progesterone is 314.5 g mol^{-1} , what is the peak concentration in nM?
- B. Suppose that the daily production of progesterone is 20 mg day^{-1} during the luteal phase. What is the metabolic clearance rate?
- C. If the volume of distribution of progesterone is 14 L, what is the fractional turnover?