

Problem Set

Ligand Binding 9.1

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.

2. 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 (μM)	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

3. 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.)

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

$[\text{Ca}^{2+}]$ (μM)	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 $[Ca^{2+}]$ 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.)

- The velocities of the pyruvate kinase reaction obtained at a number of concentrations of phosphoenolpyruvate, at constant $[ADP]$ 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.

- 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 $0.5 \times 10^{-6} M^{-1}$. If the total Ca^{2+} content (the sum of free and bound) is $100 \mu M$, and the total $[EGTA]$ is $400 \mu M$, what is the free $[Ca^{2+}]$? What is $pCa = -\log [Ca^{2+}]$?
- 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?
- 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?

- 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 $[TBG] = 2 \text{ mg dL}^{-1}$.
 - What is TBG's half-life?
 - If TBG binds one molecule of T4, what is its binding capacity, expressed as $\mu g \text{ T4 dL}^{-1}$?
- 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.

- 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?
- The peak of progesterone during the luteal phase of the menstrual cycle is about 10 ng mL^{-1} .
 - If the molecular weight of progesterone is 314.5 g mol^{-1} , what is the peak concentration in nM?
 - Suppose that the daily production of progesterone is 20 mg day^{-1} during the luteal phase. What is the metabolic clearance rate?
 - If the volume of distribution of progesterone is 14 L, what is the fractional turnover?