

BE.320 Spring 2006

Problem Set #2 - Solutions

Due Feb 27, 2006 (by 5 pm)

① Calculate ΔH_{app} first: $\Delta G = \Delta H - T\Delta S \Rightarrow \Delta H = \Delta G + T\Delta S$

$$\Delta G = -5.18 \frac{\text{kcal}}{\text{mol}}$$

$$\Delta S = 10 \frac{\text{cal}}{\text{mol} \cdot \text{K}} = 0.01 \frac{\text{kcal}}{\text{mol} \cdot \text{K}}$$

$$T = 298 \text{ K (remember to convert to Kelvin)}$$

$$\Delta H = -5.2 \frac{\text{kcal}}{\text{mol}}$$

which we assume to be ΔH_{app}

Calculate heat released on 2nd injection:

$$\text{From lecture: } Q_1 = V \cdot \Delta H_{app} ([C]_i - [C]_{i-1})$$

$$\text{for 2nd injection: } Q_2 = V \cdot \Delta H_{app} ([C]_2 - [C]_1)$$

We know V (1.5 mL) and ΔH_{app} , so we need to calculate $[C]_2, [C]_1$.

This is a fractional saturation problem... can we "cheat" and use pseudo first-order approximation? well... $[P]_0 > [L]_0$! so no

CANT use pseudo first-order approx, use full equation:

$$y = \frac{[C]}{[P]_0} = \frac{([L]_0 + [P]_0 + K_d) - \sqrt{([L]_0 + [P]_0 + K_d)^2 - 4[P]_0[L]_0}}{2[P]_0}$$

into we need: $[L]_0, [P]_0, K_d$ for injections 1 & 2

Let's find K_d first:

$$\Delta G = \ln K_d (RT) \Rightarrow \ln K_d = \frac{\Delta G}{RT}$$

$$T = 298 \text{ K}, R = 1.987 \times 10^{-3} \frac{\text{kcal}}{\text{K} \cdot \text{mol}}, \Delta G = -5.18 \frac{\text{kcal}}{\text{mol}} \Rightarrow K_d = 1 \mu\text{M}$$

To calculate ~~[L]₀~~ [L]₀, [P]₀ for each injection, we need to know the AMOUNT of ligand added per injection:

per injection: $10 \mu\text{L} \times \frac{1 \text{ L}}{10^6 \mu\text{L}} \times \frac{300 \mu\text{mol}}{\text{L}} = 0.003 \mu\text{mol}$ ligand added per injection

protein amount: $20 \times 10^{-6} \frac{\text{mol}}{\text{L}} \times 1.5 \text{ mL} = 3 \times 10^{-8}$ moles of protein

injection #1: Volume = 1.5 mL + 10 μL

$$[L]_{01} = \frac{0.003 \mu\text{mol}}{1.5 \text{ mL} + 10 \mu\text{L}} = 1.987 \mu\text{M}$$

$$[P]_{01} = \frac{3 \times 10^{-8} \text{ mol}}{1.5 \text{ mL} + 10 \mu\text{L}} = 19.87 \mu\text{M}$$

$$K_d = 1 \mu\text{M}$$

$$[C]_1 = \frac{([L]_{01} + [P]_{01} + K_d) - \sqrt{([L]_{01} + [P]_{01} + K_d)^2 - 4[P]_{01}[L]_{01}}}{2}$$

$$= 1.88 \mu\text{M} = 2.8 \times 10^{-9} \text{ mol}$$

injection #2: Volume = 1.5 mL + 2(10 μL)

$$[L]_{02} = \frac{2(0.003) \mu\text{mol}}{1.5 \text{ mL} + 2(10 \mu\text{L})} = 3.94 \mu\text{M} \quad [P]_{02} = \frac{3 \times 10^{-8} \text{ moles}}{1.5 \text{ mL} + 2(10 \mu\text{L})} = 19.74 \mu\text{M}$$

$$K_d = 1 \mu\text{M} \quad [C]_2 = 3.72 \mu\text{M}$$

Now, we have everything we need to calculate ~~the~~ Q_2 :

$$Q_2 = V \cdot \Delta H_{\text{app}} ([C]_2 - [C]_1)$$

$$= (1.52 \text{ mL}) \left(-5.2 \frac{\text{kcal}}{\text{mol}} \right) (3.69 \mu\text{M} - 1.88 \mu\text{M}) = 1.45 \times 10^{-5} \text{ cal of heat released}$$

2. Amount of protein we need:

ideally, " ϵ " value for an experiment is between 10 and 100, so let's pick 50.

$$\epsilon \cdot 50 = K_a [P]_T, \quad K_a = \frac{1}{K_d} \rightarrow [P]_T = 50 K_d = 1 \times 10^{-6} \text{ M}$$

$$\text{in } 1.5 \text{ mL: } P = 1 \mu\text{M} (1.5 \text{ mL}) = 1.5 \times 10^{-9} \text{ moles of protein}$$

$$P_{\text{mass}} = P_{\text{molar}} \times P_{\text{MW}}$$

$$= 1.5 \times 10^{-9} \text{ mol} \times 50 \text{ kDa} = \boxed{75 \mu\text{g protein}}$$

Amount of ligand:

we want $[L] < 10-20$ times $n[P]_T$, $n=2$ (2:1 final molar ratio)

$$[L] = 15(2)(1 \mu\text{M}) = 30 \mu\text{M} \text{ in Volume of cell}$$

Assume 25 injections, ~~total~~ $V_{\text{cell}} = 1.5 \text{ mL} + 25(20 \mu\text{L}) = 2 \text{ mL}$

$$\frac{30 \mu\text{M} \times 2 \text{ mL}}{0.5 \text{ mL}} = \boxed{1.2 \times 10^{-4} \text{ M}} \text{ of ligand in injection well}$$

(0.5 mL ligand solution total)

$$3. \quad \frac{[C]_{L,I}}{[C]_L} = \frac{K_d(\text{ligand}) + [L]}{K_{dL} \left(1 + \frac{[I]}{K_{dI}}\right) + [L]} \quad \text{" \% inhibition"}$$

$[C]_{L,I}, [C]_L, [I]$ are actually equilibrium conditions. We're going to assume pseudo first-order approximation, which may not necessarily be valid... For this prob, we're just concerned a/b you understanding inhibition effects, so we'll make our lives easier and assume PFOA!

$$\left. \begin{array}{ll} [L] = 3 \text{ mM} & K_{dL} = 75 \mu\text{M} \\ [I] = 1 \mu\text{M} & K_{dI} = 20 \text{ nM} \\ \text{(max due to toxicity)} & \end{array} \right\}$$

$$\boxed{\% \text{ inhibition MAX} = 45\%}$$

6.8% if you used $[ATP] = 200 \text{ nM}$

4. Pseudo first order approximation: $y = \frac{[L]_0}{[L]_0 + K_d}$

Full: $y = \frac{([L]_0 + [P]_0 + K_d) \pm \sqrt{([L]_0 + [P]_0 + K_d)^2 - 4[P]_0[L]_0}}{2[P]_0}$

$[L]_0 = 5[P]_0$: PFOA: $y_a = 0.8333$
Full: $y_f = 0.807$ % error = $\frac{|y_a - y_f|}{y_f} = 3\%$

$[L]_0 = 50[P]_0$: PFOA: $y_a = 0.980392$
Full: $y_f = 0.98000$ % error = 0.04%

$K_d = 100 \mu M$: $[L]_0 = 5[P]_0$: PFOA: $y_a = 0.99800$
 $y_f = 0.997$ % error = 0.05%

In general, error decreases with increasing $[L]_0$
" " " " with improved K_d .

```
1  % BE.320 Problem Set #2
2  % Spring 2006
3  % Due: February 27, 2006
4  % Problem #5 Solution
5
6  close all;
7  clear all;
8  load 'pset2_data';
9
10 % *****Define Constants*****
11 R = 1.987e-3; % gas constant, units = kcal/K-mol
12 T = 300; % temperature, units = Kelvin
13 P = 0.019e-3; % concentration of protein, units = molar
14
15 % *****PART A*****
16 x = pset2_data(:,1);
17 y = pset2_data(:,2);
18 y = y./1000; % convert cal to kcal
19 plot (x, y, 'o');
20 xlabel ('Molar Ratio');
21 ylabel ('kcal/mole of injectant');
22 title ('Part A');
23
24 % *****PART B*****
25 % Assume: top of curve is saturated
26 % calculate dH by subtracting last point from first point
27
28 Q_high = max(pset2_data(:,2));
29 Q_low = min(pset2_data(:,2));
30 dH = Q_low - Q_high % VALUE OF dH = -7.34 kcal/mol
31
32 % *****PART C*****
33 % Assume: smallest Q value is the bottom of the curve
34 % We want to find the value of the molar ratio when  $Q_{1/2} = 1/2*dH$ 
35 [numrows, numcols] = size(pset2_data); % get the size of the data array
36 Q_half = abs(dH)/2 + Q_low;
37
38 % loop through to find the closest value to Q_half, and store the index of
39 % that value
40 Kd_i = 0;
41 Kd = 0;
42 difference = abs(Q_half-Q_low)
43 for i = 2:numrows,
44     d = abs(Q_half-pset2_data(i,2));
45     if (d < difference)
46         difference = d;
47         Kd_i = i;
48         mr = pset2_data(i, 1); % molar ratio
49     end
```

```
50 end
51
52 Kd_i % = 8
53 error = abs(pset2_data(Kd_i,2)-Q_half)/Q_half % %error from Q_half is 3.57%
54 Kd = mr * 0.019e-3 % = 12.4 uM
55 dG = R*T*log(Kd) % dG = -6.7346 kcal/mol
56 % NOTE: The best way to do this is an interpolation (fitting) algorithm that
57 % finds an interpolated value of [L]_T/[P]_T at exactly Q_half. But that's
58 % a bit complicated, and we weren't focusing on fitting for this
59 % assignment, and a 3.57% error isn't too bad. So we can make our lives
60 % easier and just pick the [L]_T/[P]_T associated with the Q that is
61 % closest to our target Q_half.
62
63 % *****PART D*****
64 dH = dH/1000;
65 dS = (dH - dG)/T % dS = -2 cal/mol-K
```

Part A

