

20.320 — Problem Set # 1

September 17th, 2010

Due on September 24th, 2010 at 11:59am. No extensions will be granted.

General Instructions:

1. You are expected to state all your assumptions and provide step-by-step solutions to the numerical problems. Unless indicated otherwise, the computational problems may be solved using Python/MATLAB or hand-solved showing all calculations. Both the results of any calculations and the corresponding code must be printed and attached to the solutions. For ease of grading (and in order to receive partial credit), your code must be well organized and thoroughly commented, with meaningful variable names.
2. You will need to submit the solutions to each problem to a separate mail box, so please prepare your answers appropriately. Staples the pages for each question separately and make sure your name appears on each set of pages. (The problems will be sent to different graders, which should allow us to get the graded problem set back to you more quickly.)
3. Submit your completed problem set to the marked box mounted on the wall of the fourth floor hallway between buildings 8 and 16.
4. The problem sets are due at noon on Friday the week after they were issued. There will be no extensions of deadlines for any problem sets in 20.320. Late submissions will not be accepted.
5. Please review the information about acceptable forms of collaboration, which was provided on the first day of class and follow the guidelines carefully.

1 Surface Plasmon Resonance (SPR)

You are in charge of characterizing the pharmacokinetics of a set of 20 candidate drugs targeting the same cell surface receptor. In this problem, you will design experiments to characterize their binding kinetics by SPR and analyze the results.

- Under what circumstances is SPR a suitable experimental method for your purpose? List 3 advantages over other methods for measuring molecular binding parameters and 3 conditions for its applicability.
- In SPR, running just 1 experiment with your sample is *never* enough. List 3 controls to include and explain what possible artifacts they control for. Can you think of a fourth control which is useful to include if your ligand is itself a protein, but cannot be included if the ligand is a small molecule? Explain.
- What physical quantity does SPR measure? What unit is it commonly given in, and how can this unit be converted into the relevant SI unit? Provide a clearly labelled diagram of the expected data output, indicating where external interventions (e.g. changes in buffer composition) take place. What steps are required to extract the kinetic rate constants k_{on} and k_{off} from the resulting data *without curve-fitting*? Provide an equation and an explanation for each step.
- In one experiment, you flow your soluble species L through at 10 nM and read the following points off your sensorgram:

	Time / s	Signal / RU
Ligand flow begins	5	1
Ligand flow ends after the reaction has equilibrated	130	99
Run ends	300	1
Half-maximal signal is obtained	21.5	50
	147.3	50

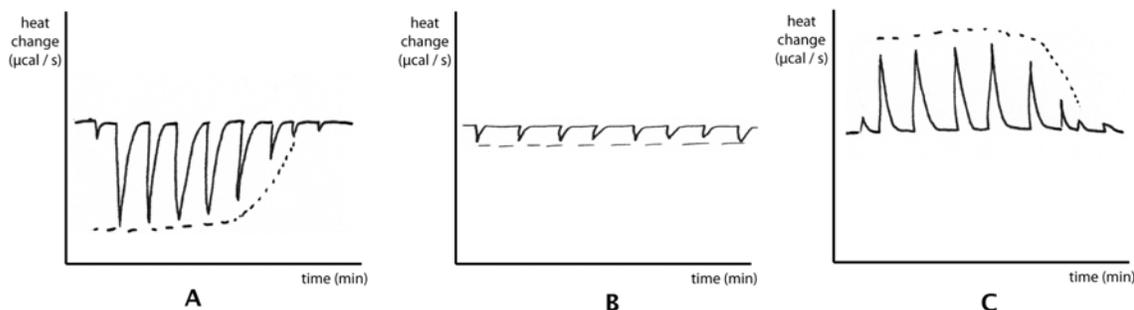
Using your equations above, calculate K_D . Show all work.

- If the concentration of the soluble species were increased to 50 nM, how long would take for the association phase reaction to reach exactly 99% of equilibrium binding?
- Assume your soluble ligand is the protein target of interest with a molecular weight of 450 kDa and that 450 fmol of your drug molecule (MW = 500 Da) have been immobilized on a 1 cm by 1 cm chip. In the scenario just described (i.e. $[L]_0 = 50$ nM), what is RU_{eq} ?

2 Isothermal Titration Calorimetry (ITC)

Your drug candidate performed well in kinetic testing by SPR — congratulations. Before commencing cell-based activity assays, you decide to control for artifacts of immobilization in your SPR data by also performing an ITC experiment. First, you will choose reasonable experimental parameters based on your prior knowledge. Then, you will simulate the experiment to ensure that the output is likely to be informative over a range of reasonable values for the key parameters.

- ITC is a powerful tool to extract a wide range of thermodynamic parameters without any need for modification or immobilization of the binding partners. Why did you not use it in initial screening? State two reasons.
- You may assume that the ΔH for your reaction is $\mathcal{O}(-10 \text{ kcal mol}^{-1})$. Which of the following graphs provides the best qualitative approximation to the expected raw data readout (dQ/dt vs. time) from your experiment? What do the other graphs represent? Extra credit for suggesting two qualitatively distinct interpretations of the peaks in (B).



- The K_D you determined by SPR is $2 \cdot 10^{-7} \text{ M}$, and you will use an ITC cell with $V_{\text{cell}} = 1 \text{ mL}$ and an injection volume of 10 uL per injection. What would be reasonable values for the protein concentration in the cell, $[P]_0$, and for the ligand concentration in the injected buffer solution, $[L]_0$? Justify your answer.
- To calculate the heat released or absorbed after each injection, you will require an expression for the fractional saturation of protein molecules with ligand at equilibrium,

$$[y]_{\text{eq}} \equiv \frac{[C]_{\text{eq}}}{[C]_{\text{eq}} + [P]_{\text{eq}}} = \frac{[C]_{\text{eq}}}{[P]_0} = \frac{[L]_{\text{eq}}}{[L]_{\text{eq}} + K_D}.$$

State and explain the pseudo-first order approximation. Does it apply for ITC? Justify why or why not. Write down an appropriate mathematical expression for the fractional saturation in terms of only the quantities for which values have been given or estimated in part c) of this problem.

- The heat absorbed or released after the i^{th} injection (i.e. the area under the i^{th} peak in the graphs provided for Problem 2b)) can be calculated as

$$Q_i = \Delta H \cdot V_{\text{cell}} \cdot ([C]_i - [C]_{i-1}),$$

where the concentration of bound complex is $[C] = y[P]_0$. Implement a function in which calculates Q_i from these parameters. Implement another function which calculates the fractional saturation from appropriate parameters. Use these functions and the parameter estimates above to plot a graph of the heat released or absorbed, Q , against the molar ratio of ligand and protein, $[L]/[P]$. You have been provided with skeleton code for this problem; edit it where indicated to implement your solution. Remember to label the axes with the relevant quantities and units.

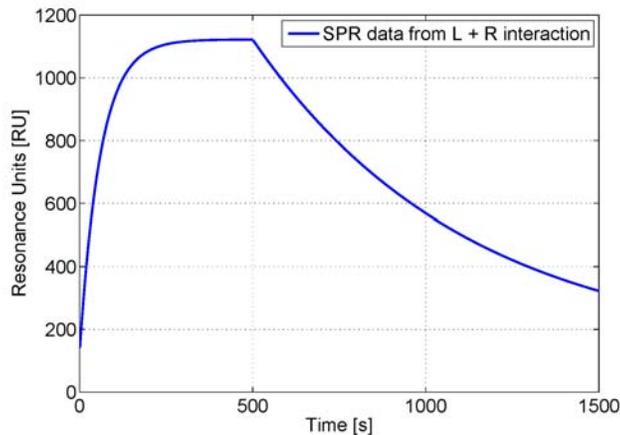
- f) Assuming that your initial parameter estimates were close and allowing 5 minutes per injection, how long at least should you let your ITC experiment run? Show how you derive your estimate from the simulation output. What would happen if you increased your ligand concentration tenfold? Plot the expected output and comment.

3 Special cases: Radioactive labeling measurement

A scientist is interested in quantifying the interaction between a ligand (L) and a receptor (R). A radioactive label is attached to the ligand and cells are incubated on tissue-culture polystyrene with the therapeutic at 4°C long enough to guarantee equilibrium.

Ligand / nM	Radioactive signal / AU
0.001	5.56 E-2
0.01	5.55 E-1
0.1	5.46 E+0
0.5	2.54 E+1
1	4.64 E+1
5	1.44 E+2
10	1.95 E+2
50	2.73 E+2
100	2.89 E+2
300	3.10 E+2
500	3.22 E+2
1000	3.99 E+2

- The experiment is to be conducted at 4°C. Does this have an impact on the K_D measurement, *i.e.* is it identical to the physiological K_D ?
- The data collected is shown in table 1 above. Using plot , plot this data using the appropriate representation and curve-fit a monovalent binding isotherm to extract K_D . Why does the data look unusual?
- Perform the necessary modifications to the data set so that a more accurate monovalent binding isotherm can be fitted. Curve-fit the modified data and extract the K_D . Explain qualitatively why the previously measured K_D was an over- or underestimate.
- The scientist would like to extract interaction kinetic constants (k_{on} and k_{off}) and performs an SPR experiment with 50nM ligand concentration. The data is shown in figure 1. Extract the values of k_{off} and k_{on} .



- Do these result corroborate the estimation obtained by radioactive labeling?

MIT OpenCourseWare
<http://ocw.mit.edu>

20.320 Analysis of Biomolecular and Cellular Systems
Fall 2012

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.