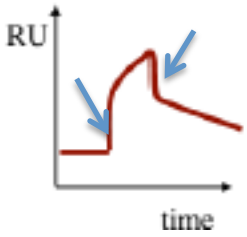
BMB/Bi/Ch 173 – Winter 2017
Problem Set 8.1: CD and SPR– Assigned 2-28-17. Due 3-7-17 by 10:30am
TA: Wen Zhou (201 Kerckoff, office hour: Fri 3/3 5-6pm, Mon 3/6 5-6pm or by appointment
100 points total

1. **(25 points) SPR Basics**
    a. (10 points) Draw a typical schematic of a Biacore SPR instrument and explain how signal is measured to produce affinity data for a biomolecular interaction.

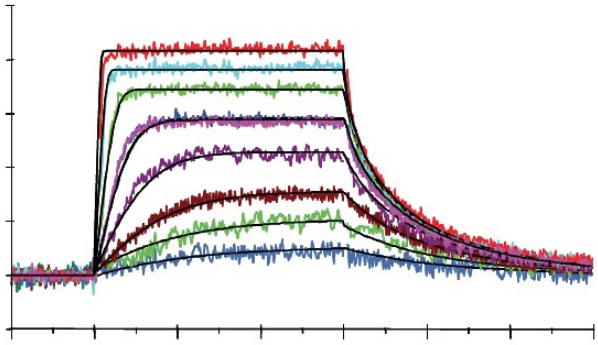
    b. (6 points) Why do SPR experiments include a surface regeneration step?

    c. (9 points) Given the raw experimental sensorgram shown below, you noticed that it didn't look right because of the sudden jumps in the curve (pointed by the arrows). Explain why and how to correct for it using the concept of "bulk refractive index".

Raw experimental



2. **(30 points) Analyzing SPR Data:** You carry out an SPR experiment with the growth factor and its antagonist to produce the sensorgram below.



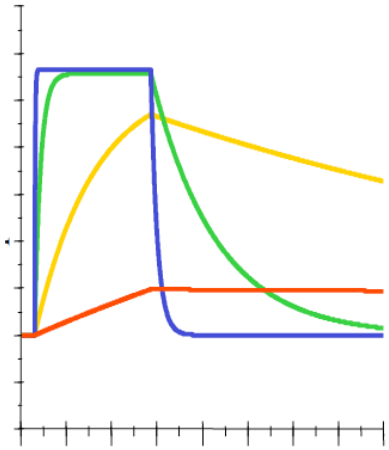
a. (4 points) Label the axes with appropriate units.

b. (6 points) There are three distinct experimental phases in this sensorgram. Label each phase and explain what is happening during that part of the experiment.

c. (8 points) What rate constants contribute to the curve at each of the phases in part (b)? How can the association and dissociation constants (k_a and k_d) be determined?

d. (5 points) How do you determine the binding affinity, often measured as equilibrium dissociation constant K_d? What needs to be true about the interaction in order to attain this constant?

e. (7 points) You move on to screening the library of small molecule inhibitors of the growth factor. Four molecules look promising, producing the four curves shown below. All four molecules have the same binding affinity K_d and were used at the same concentration but display very different SPR curves. Explain this observation.



3. **(25 points) CD Basics**
   a. (7 points) What is the difference between circular dichroism and circular birefringence?

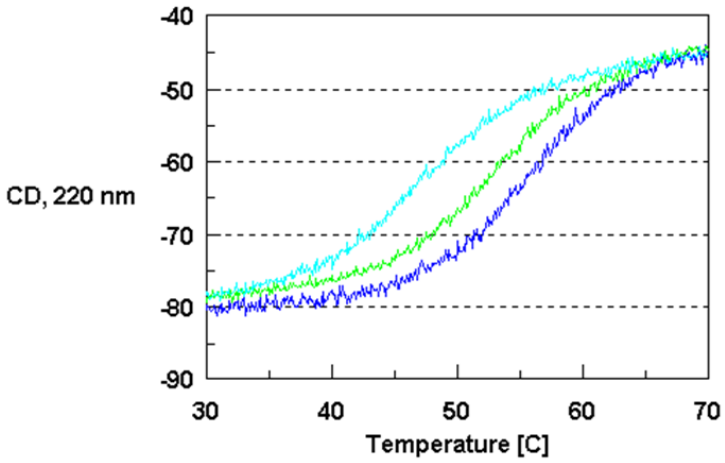
   b. (8 points) If you shine linearly polarized light at a circularly dichroic material, what would be its polarization as it emerged? What if the material were circularly birefringent? Why? (Diagrams maybe helpful)

   c. (10 points) What information does a CD spectrum provide? What are the advantages and disadvantages of CD in comparison to other structural techniques such as NMR or crystallography?

4. **(20 points) Using CD in the lab:** You work at a biotech company that has just discovered a protein that can be used as a protein therapeutic.
    a. (6 Points) The protein originates from a fungus, but your company wants to mass produce the protein in mammalian cells. How can CD be used to compare the native and recombinant proteins?

    b. (6 points) You suspected that your recombinant protein has altered folding than the native one. To thoroughly compare the folding and folding kinetics of the native and recombinant proteins using CD, what variables might you test? (hint: factors affect folding kinetics)

    c. (8 points) You want to increase the shelf---life of your product, so you examine the stability of the protein in different buffers using CD. You produce the data below, where each curve represents a different buffer:



- (3 points) Describe the experiment.
- (2 points) What information does the CD measurement at 220 nm provide?
- (3 points) Which buffer is best for the protein?