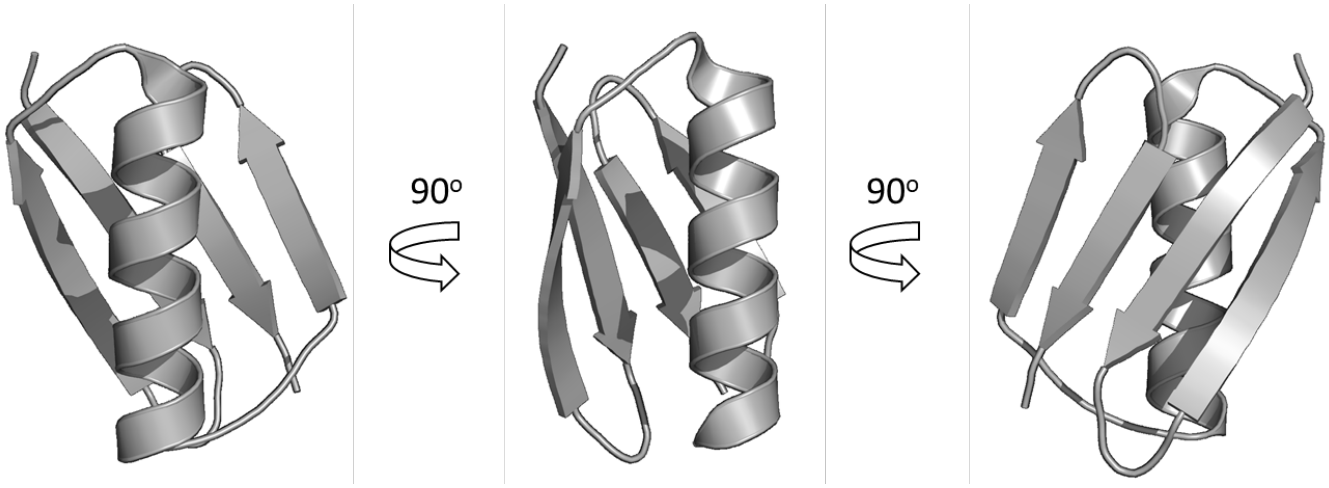
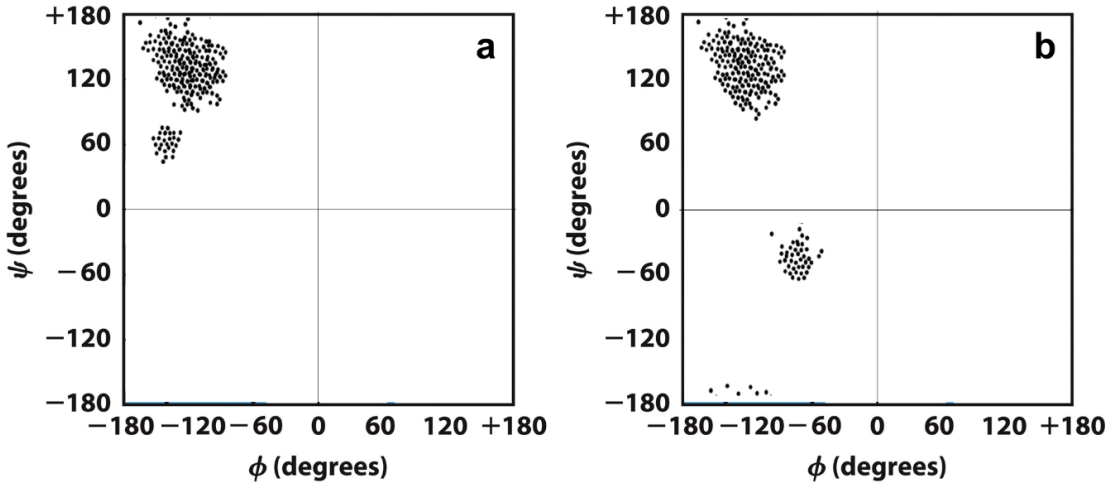
Problem 1.
Here is a cartoon representation of the tertiary structure of a protein GB3. The pictures represent consecutive 90° rotations of the protein about the vertical axis.



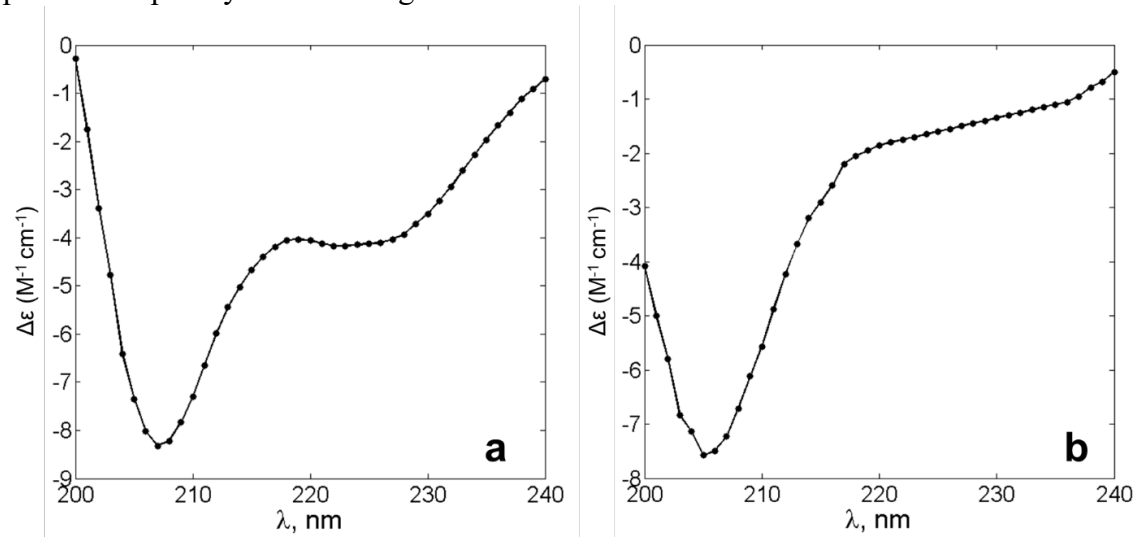
A. List all elements of secondary structure (and how many) that are present in this protein. Be as specific as possible: for a beta-sheet, what type of sheet and how many strands, for a helix, what type of helix and how many turns.

B. On the structural cartoons shown above, sketch backbone-to-backbone hydrogen bonds that you expect in this protein (just draw lines to schematically show hydrogen bonding patterns, no need to show individual atoms connected by these bonds). Explain briefly why you drew the lines the way you did.

C. Which of the two Ramachandran maps shown below cannot correspond to this protein? (The dots represent pairs of ϕ, ψ angles for each residue.) Explain your reasoning.



D. Which of the two circular dichroism (CD) spectra shown below likely corresponds to this protein? Explain your reasoning.



Problem 2.

You discovered an unknown protein and – naturally – you want to characterize its structure. The protein has molecular weight of 45 kDa, and, based on your biochemical data forms a homodimer. The analysis of its primary structure suggests that the protein might contain several independently folded domains connected by unstructured regions. Consider all methods for structure characterization that we covered in class. Answer the following questions.

A. What method allows fast determination of the secondary structure content of a protein even though it provides no details regarding the actual structure?

B. What method can provide information about the overall shape of your protein although without revealing atomic-resolution structure?

C. What features of your protein could potentially create problems for determining its structure using X-ray crystallography?

D. What features of your protein could potentially create problems for determining its structure using solution NMR spectroscopy?

E. What features of your protein could potentially create problems for determining its structure using cryoEM?

Problem 3.

When studying ligand binding to a protein X, you found that at ligand concentration of 35 μM 35% of the protein is in the bound state. Answer the following questions. Show your calculations and briefly explain your reasoning and assumptions.

A. What fraction of the protein can you expect to be bound at the ligand concentration of 70 μM?

B. You performed measurements at the ligand concentration of 195 μM and found that 95% of the protein was in the bound state. Compare your result with what you would expect at this ligand concentration. If your experimental result agrees with your expectation, what does this tell you about the binding? If there is a disagreement, what could be a possible reason for it?

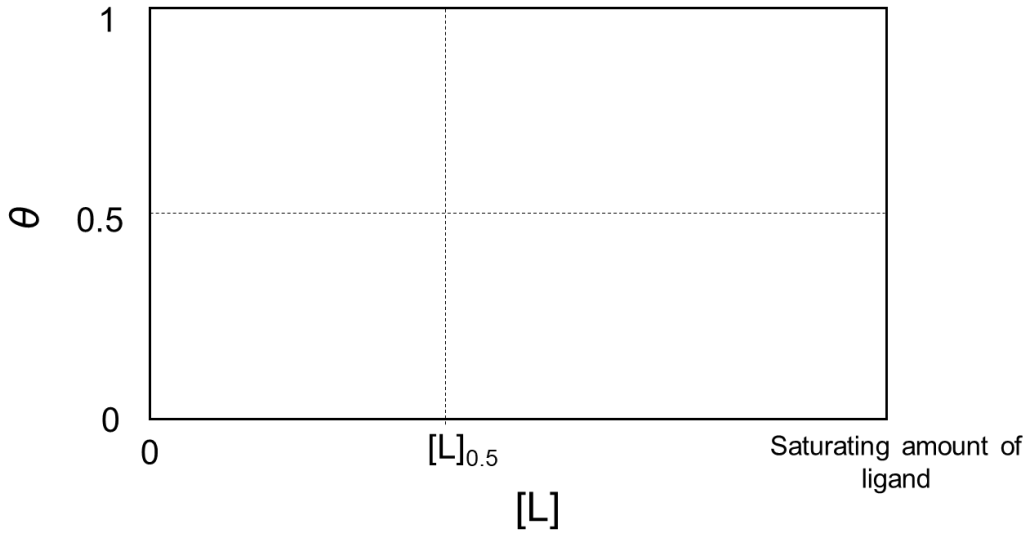
Problem 4.

A hypothetical allosteric protein has several identical subunits, each of which can bind ligand **L**. Each subunit can be in two states, T and R. The T state binds ligand **L** weaker than the R state. In the absence of ligand the T state is more stable. The protein fits the concerted (MWC) model for cooperativity, where all subunits undergo the transition from one state/conformation to another simultaneously. Ligand binding to the T state makes a transition to the R state more likely. Each subunit of this multimeric protein has a second binding site, to which other substances, **X** and **Y,** can bind. **X** binds only to subunit in the T state and stabilizes this state, while **Y** binds only to the R state and stabilizes this state.

A. Which of the three ligand molecules that can bind to this protein (**L**, **X**, or **Y**) is a *negative heterotropic modulator* for this protein? Explain your reasoning.

B. Which of the three ligand molecules is a *positive heterotropic modulator* for this protein? Explain your reasoning.

C. Using the axes below (ligand-bound fraction of protein (θ) versus molar ligand concentration), first draw the binding curve for ligand binding to your protein. Then sketch on this plot how the binding curve in the presence of a certain amount of **X** or **Y** (separately) should look like. Explain why you drew the curves the way you did.



Problem 5.

The enzyme ribonucleotide reductase binds several different nucleoside-triphosphate ligands, with a different affinity for each one. Listed below are the K_d values for three select ligands and their concentrations in cells at 25 °C.

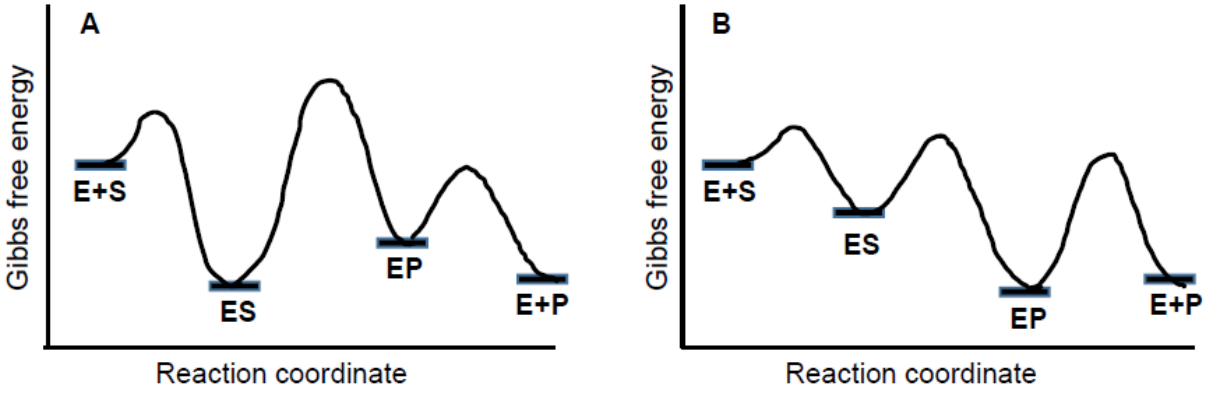
Ligand	K_d (μM)	[L] in cells (μM)
ATP	100	1000
CTP	1	1
GTP	10	1

A. Determine the standard free energy of binding (ΔG'°) for each of the nucleotide ligands. Show your work.

B. Assume that the concentration of the enzyme is much lower than the concentration of any of the ligands in the table above. Which enzyme-ligand complex is present at the highest concentration in the cell? Explain how you arrived at your answer.

Problem 6.

A reaction S ↔ P is catalyzed by two enzymes, A or B. The reaction coordinate diagrams for these enzymes are shown below. Answer the following questions.



A. Which of the two enzymes binds tighter to the substrate? Explain your reasoning

B. Which step in each reaction is the rate-limiting step for the forward reaction? Explain your reasoning

C. Which step in each reaction is the rate-limiting step for the reverse reaction? Explain your reasoning

D. Which of the two enzymes has higher turnover number? Explain your reasoning

E. Which of the two enzymes is a better catalyst? Explain your reasoning

Some equations and formulae that might or might not be useful:

$\Delta G = \Delta H - T\Delta S$

$\Delta G = \Delta G'^{0} + RT\ ln\{[\text{products}]/[\text{reactants}]\}$

$K_{eq} = [\text{products}] / [\text{reactants}]$

$\Delta G'^{0} = - RT\ ln\{K_{eq}\}$

$K_{eq} = exp(-\Delta G'^{0}/RT)$

$R = 8.31$ J/K/mol

$k_B = 1.38 \times 10^{-23}$ J K^{-1}; $h = 6.62 \times 10^{-34}$ J s;

$$k = \frac{k_B T}{h} \exp\left(-\frac{\Delta G^{\pm}}{RT}\right)$$

$$V_o = V_{\max} \frac{[S]}{[S] + K_m};\ \frac{1}{V_o} = \frac{K_m}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}}$$

$K_d = 1/K_a = exp(\Delta G'^{0}/RT)$

$K_d = [\text{P}][\text{L}]/[\text{PL}]$

$$\theta = \frac{[L]}{[L] + K_d}$$

$$\theta = \frac{[L]^{n_H}}{[L]^{n_H} + K_d};\ K_d = ([L]_{0.5})^{n_H}$$

$$\log\left(\frac{\theta}{1-\theta}\right) = n_H \left(\log[L]\right) - \log\left(K_d\right)$$

$$\frac{k_{catalyzed}}{k_{uncatalyzed}} = \exp\left(\frac{\Delta G^{\pm}_{uncat} - \Delta G^{\pm}_{cat}}{RT}\right)$$

$V_{\max} = k_{\text{cat}} [\text{E}_{\text{total}}]$; $K_m = (k_{-1} + k_2)/k_1$
