

Problem 1. (25 points) You study ligand binding to two proteins, A and B. You measured the concentration, [PL], of the ligand-bound form of the protein at various ligand concentrations, [L]. The data are summarized in the two tables below. Note that you do not know the total concentration of the protein, but you know that adding more ligand did not noticeably change the bound-protein concentration.

Protein A	
[L], μM	[PL], μM
0	0.000
5	0.040
10	0.067
50	0.143
200	0.182
500	0.192
2000	0.198

Protein B	
[L], μM	[PL], μM
0	0.00
15	0.21
30	0.33
100	0.55
300	0.68
1000	0.74
3000	0.76

Based on these data, answer the following questions:

(1) Determine the K_d values for each of the proteins. Explain your assumptions.

Since adding more ligand did not change [PL], we can assume that a full saturation has been reached, therefore $[P_{\text{total}}] = [\text{PL}]$ at the end of the titration.

A: 20 μM ; B: 40 μM

(2) Which of the two proteins binds the ligand tighter? Explain your reasoning.

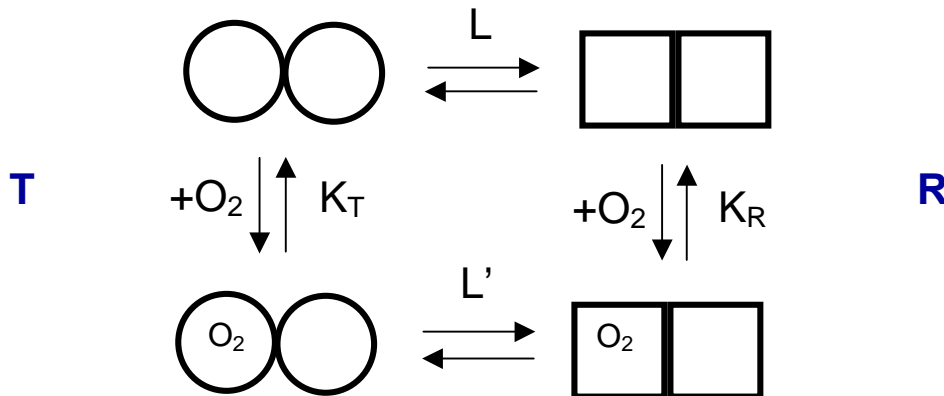
Protein A binds tighter: it has lower K_d , hence higher affinity.

(3) Is this binding cooperative or non-cooperative? How would you check it?

This binding is non-cooperative. This can be checked using a Hill plot, which will produce a slope of 1 (Hill coefficient $n_H = 1$). Alternatively, the non-cooperative character of the binding can be checked by verifying that the binding follows a hyperbolic curve: (1) by calculating K_d 's for several points, $K_d = [L](1 - \theta)/\theta$, and comparing them with each other or (2) by predicting [PL] for each value of [L] (assuming the hyperbolic curve and using the K_d value that you obtained) and comparing them with the actual values in the table.

Problem 2. (30 points)

Consider the concerted model for cooperative oxygen binding to hemoglobin. The simplest, two-subunit model for the protein is shown below:



Here K_T and K_R are the dissociation constants for O_2 binding to the T or R state, while L and L' are the equilibrium constants for $T \rightleftharpoons R$ and $T(\text{O}_2) \rightleftharpoons R(\text{O}_2)$:
 $L = [T]/[R]$ and $L' = [T(\text{O}_2)]/[R(\text{O}_2)]$

1. What should be the relationship between K_T and K_R and between L and L' in order to achieve positive cooperativity for O_2 binding in this model? (That is, for example, should K_T be smaller, greater than, or equal to K_R ?)

Positive cooperativity means that

(1) R state has higher binding affinity (lower dissociation constant) than the T state:

$$K_T > K_R$$

(2) binding of O_2 to the T state shifts the equilibrium towards the R state:

$$[T] / [R] > [T(\text{O}_2)] / [R(\text{O}_2)] , \text{ hence } L > L'.$$

2. CO_2 , H^+ , and BPG all act as heterotropic allosteric effectors for this process. Which of these four constants (if any) are affected by the presence of these compounds?

These heterotropic effectors bind preferentially to protein in the T-state, so they shift the $T \leftrightarrow R$ equilibrium towards the T-state: they will affect L and L' . They do not bind to the heme, so they are not expected to interfere with O_2 binding to the heme directly.

3. The conformation of deoxyhemoglobin is stabilized by ionic bonds between the subunits. In order to understand the role of ionic bonds in O_2 binding to Hb, you introduce specific mutations in the amino acid residues at the subunit interface. Mutation A introduces additional ionic bonds between the subunits while mutation B removes few of the existing ionic bonds.

How would each mutation affect each of the four constants compared to their magnitude in the normal, wild type Hb? (That is, would this constant increase, decrease, or remain unchanged?)

Mutation A stabilizes the T state, so it will increase L and L'. It should not directly change K_T or K_R .

Mutation B destabilizes the T state, so L and L' will decrease. It should not directly change K_T or K_R .

4. Which of these constants (K_T , K_R , L, L') could be affected by a mutation of the distal His to Ala?

Distal His is basically involved in creating sterical hindrance for molecules binding to the heme. It is not directly related to the allosteric transition between the T and R states. So, this mutation will change K_T , K_R , not the Ls.

5. Which of these constants (K_T , K_R , L, L') will be affected by a mutation of the proximal His to Ala?

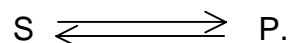
All of them.

(1) Mutation of the proximal His will change the coordination of Fe ion, hence it will affect heme binding properties: K_T , K_R

(2) Proximal His is directly involved in the translation of the change in the conformation of the heme (upon O_2 binding) into the rearrangement of the subunits in Hb that affects the $T \leftrightarrow R$ equilibrium. So, L and L' will also be affected.

Problem 3. (10 points)

Consider conversion of a substrate S into product P:



The rate of the uncatalyzed reaction at standard conditions is 10^{-5} M/sec. When enzyme was added, the rate increased to 1 M/sec.

1. Calculate the enzyme-induced change in the activation energy for the reaction. Show your calculations.

Rate constant k is related to the height of the activation barrier ΔG^\ddagger as

$$k = \kappa \exp(-\Delta G^\ddagger/RT).$$

Let $\Delta\Delta G$ be the enzyme-induced change in the activation energy:

$$\Delta\Delta G = \Delta G^\ddagger_{\text{uncat}} - \Delta G^\ddagger_{\text{cat}}.$$

Then $k_{\text{cat}}/k_{\text{uncat}} = \exp[-(\Delta G^\ddagger_{\text{cat}} - \Delta G^\ddagger_{\text{uncat}})/RT] = \exp(\Delta\Delta G / RT)$. From here we get

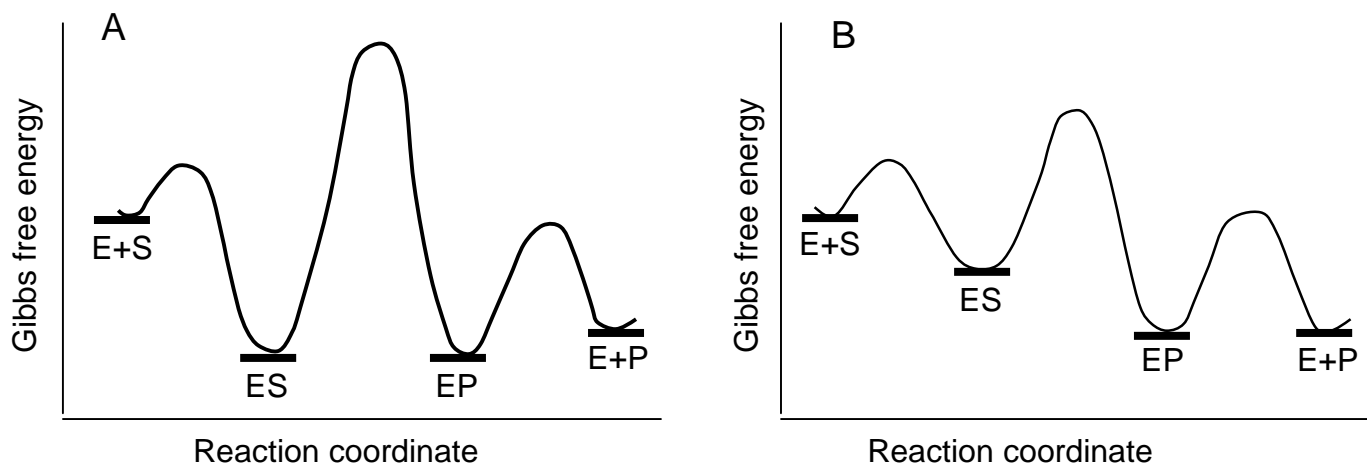
$$\Delta\Delta G = RT \ln(k_{\text{cat}}/k_{\text{uncat}}) = RT \ln(10^5) \approx 28.5 \text{ kJ/mol}$$

3. What is the induced change in the activation energy and in the rate of the backward reaction, $P \rightarrow S$?

The change in the activation barrier for the reverse reaction is the same: $\Delta\Delta G$. Therefore, the change in the rate of the backward reaction is the same: a 10^5 fold increase.

Problem 4. (15 points)

A reaction $S \rightleftharpoons P$ is catalyzed by two enzymes, A and B. The reaction coordinate diagrams for these enzymes are shown below.



1) Which of the two enzymes binds tighter to the substrate? Explain your reasoning

Enzyme A: it has a greater difference ΔG in the free energy between the substrate-free (E+S) and substrate bound (ES) states.

2) Which step in each reaction is the rate-limiting step? Explain

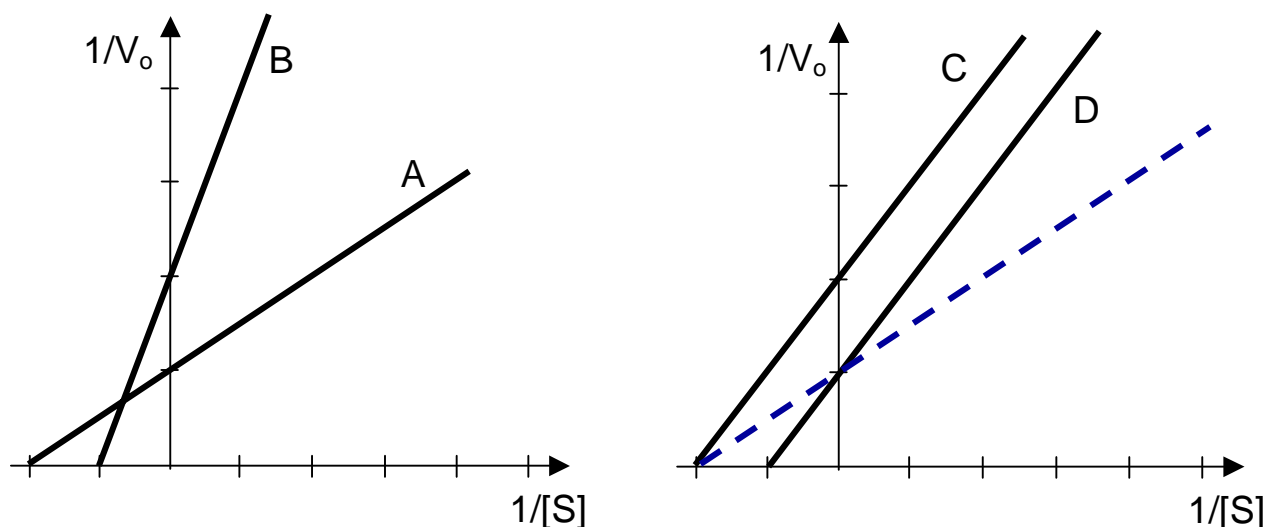
ES \rightarrow EP, because of all processes in this reaction it has the highest activation barriers.

3) Which of the two enzymes is a better catalyst? Explain your reasoning

Enzyme B: it has lower activation barrier for the ES \rightarrow EP transition, which is the rate-limiting step.

Problem 5. (20 points)

A double-reciprocal plot for two enzymes, A and B, is shown on the left plot below.



1. Given the concentrations of the two enzymes are the same, which of them has a higher turnover number? Explain

Enzyme A: It has higher V_{\max} compared to enzyme B. For the same $[E_{\text{tot}}]$, higher V_{\max} means higher turnover number, $k_{\text{cat}} = V_{\max} / [E_{\text{tot}}]$.

2. Which of the two has higher efficiency? Explain

**Enzyme A: The efficiency is determined by $k_{\text{cat}}/K_M = V_{\max} / (K_M [E_{\text{tot}}])$.
 $V_{\max}(\text{A}) = 2 V_{\max}(\text{B})$; $K_M(\text{A}) = \frac{1}{2} K_M(\text{B})$. \rightarrow Efficiency (A) = 4 • Efficiency (B).**

Answer questions 1 and 2 for enzymes C and D (right plot).

- 1. Enzyme D has higher turnover number: $k_{\text{cat}}(\text{D}) = 2 k_{\text{cat}}(\text{C})$.**
- 2. They have the same efficiency, because $K_M(\text{D}) = 2 K_M(\text{C})$.**

Draw how the plot for enzyme C will change if you increase its concentration by 2 fold. (you can draw on the same plot)

See the blue dashed line on the plot. $V_{\max} = k_{\text{cat}} [E_{\text{tot}}]$. An increase in $[E_{\text{tot}}]$ will increase V_{\max} but leave K_M unchanged.

Bonus (!) Problem. (15 points)

Carbon monoxide, an odorless gas, binds to hemoglobin to form CO-hemoglobin. Crystals of CO-hemoglobin are isomorphous with those of oxyhemoglobin, which suggests that CO-Hb has the same conformation as oxyhemoglobin. Each heme in Hb can bind one CO molecule, but O₂ and CO cannot simultaneously bind to the same heme. The binding affinity for CO is ~200 times higher than that for O₂. Exposure for 1 hour to a CO concentration of 0.1% in inspired air leads to the occupancy by CO of about half the heme sites in Hb, a proportion that is frequently fatal.

Here is the paradox: if the action of CO were simply to diminish the oxygen carrying power of the Hb, without other modifications of its properties, the symptoms of CO poisoning would be very difficult to understand in the light of other knowledge. Thus, a person whose blood is half-saturated with CO is practically helpless, but a person whose Hb percentage is simply diminished to half by anemia may be going about his work as usual. What is the key to this paradox?

CO binds to the heme much tighter than O₂ and causes a transition to the R state (according to the crystal data mentioned above). Because this binding is so tight, the equilibrium is shifted towards the R-state. As the result, Hb gets “locked” in the R state: it binds O₂ tightly and very efficiently, *but* it does not release it in the tissues.