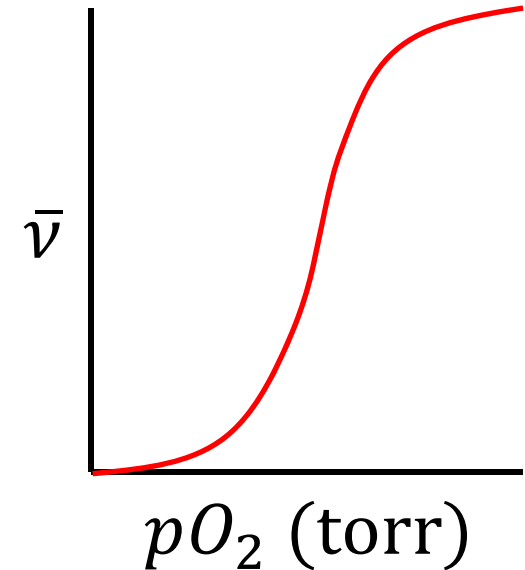
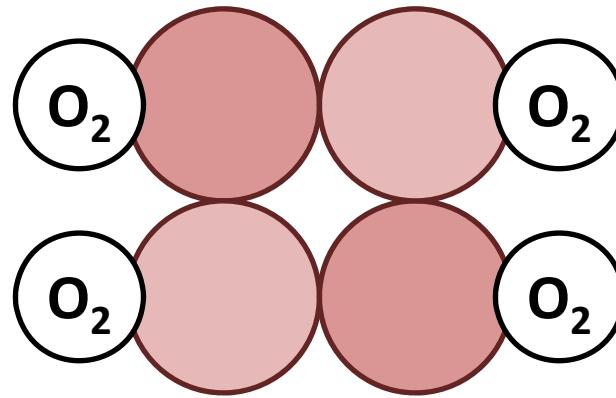
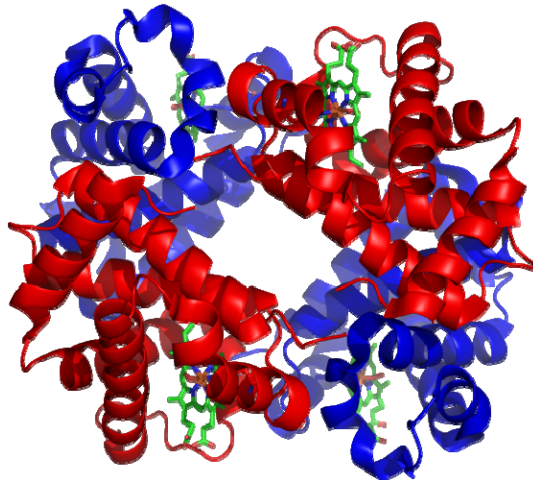


What About Hemoglobin?



- Oxygen binding is *cooperative*: binding one site makes adjacent binding more favorable

Allostery and Cooperativity

- **Allostery:** Binding at one site affects the binding at another site
 - It can become more or less favorable
- **Cooperativity:** Binding at one site increases affinity at another site (sometimes positive cooperativity)
- This behavior shows up in *many* biological systems:
 - Can we model it?
 - What is the molecular (physical) basis of allostery?

Statistical Weights

- Alternative approach to what we did last week
- **Idea:** The *weight* of a species (A) is its concentration relative to a reference concentration (X)

$$w_A = \frac{[A]}{[X]}$$

- More succinct (for species $i = 1, 2, 3, \dots$):

$$w_i = \frac{c_i}{c_{ref}}$$

Properties of Statistical Weights

- Reference concentration is arbitrary; you can pick any species you like
 - Some make more sense than others

- Mole fractions: $X_i = \frac{w_i}{\sum w_i}$. Why?

$$X_2 = \frac{w_2}{w_1 + w_2 + w_3} = \frac{\frac{c_2}{c_{ref}}}{\frac{c_1}{c_{ref}} + \frac{c_2}{c_{ref}} + \frac{c_3}{c_{ref}}}$$

Example: Binding

- Reaction: $AB \leftrightarrow A + B$
 - Suppose we know A_{tot} and weights (w_A and w_{AB})
 - What is $[A]$?

$$[A] = X_A[A_{tot}] = \frac{w_A}{w_A + w_{AB}} [A_{tot}]$$

- **On your own:** What is $[AB]$?
 - This works for more complicated binding, too (e.g., your homework!)

Properties of Statistical Weights

- Can relate to Gibbs energies (enthalpies, etc.)

$$X_f = \frac{1}{1 + K} = \frac{1}{1 + e^{-\Delta\bar{G}^0/RT}}$$

- Spectroscopic signals are often “weighted sums” of components:

$$\begin{aligned}\theta_{obs} &= \frac{w_{fold}\theta_{fold} + w_{unf}\theta_{unf}}{w_{fold} + w_{unf}} \\ &= X_{fold}\theta_{fold} + X_{unf}\theta_{unf}\end{aligned}$$

Example: Binding (Again)

- What about the degree of binding $\bar{\nu}$?

$$\bar{\nu} = \frac{[L_{bound}]}{[P_{tot}]} = \frac{[PL]}{[P_{tot}]} + \frac{2[PL_2]}{[P_{tot}]} + \frac{3[PL_3]}{[P_{tot}]} + \dots$$

$$\bar{\nu} = X_{PL} + 2X_{PL_2} + 3X_{PL_3} + \dots$$

$$\bar{\nu} = \frac{w_{PL} + 2w_{PL_2} + 3w_{PL_3} + \dots}{w_P + w_{PL} + w_{PL_2} + w_{PL_3} + \dots}$$

Last Lecture

(in one slide)

State	# Bound	Equivalent Configurations	Raw Weight	Weight with Equivalence
000	0	1	$\frac{[000]}{[000]} = 1$	1
100, 010, 001	1	3	$\frac{[100]}{[000]} = K[L] \equiv S$	$3K[L] \equiv 3S$
110, 101, 011	2	3	$\frac{[110]}{[000]} = K^2[L]^2 = S^2$	$3K^2[L]^2 = 3S^2$
111	3	1	$\frac{[111]}{[000]} = K^3[L]^3 = S^3$	$K^3[L]^3 = S^3$

- Sum of states (the **partition function**)

$$Z = \sum n_i w_i = 1 + 3K[L] + 3K^2[L]^2 + K^3[L]^3$$

Comparing to Last Time

- Previous expression:

$$\bar{v} = \frac{3K[P](1 + 2K[P] + K^2[P]^2)}{1 + 3K[P] + 3K^2[P]^2 + K^3[P]^3}$$

- Our expression (equivalent, since $S = K[P]$)

$$\bar{v} = \frac{3S(1 + 2S + S^2)}{1 + 3S + 3S^2 + S^3}$$

Three-Site Model With Cooperativity

State	# Bound	Equivalent Configurations	Raw Weight	Weight with Equivalence
000	0	1	$\frac{[000]}{[000]} = 1$	1
100, 010, 001	1	3	$\frac{[100]}{[000]} = K[L] \equiv S$	$3K[L] \equiv 3S$
110, 011	2	2	$\frac{[110]}{[000]} = \tau K^2[L]^2 = \tau S^2$	$2\tau K^2[L]^2 = 2\tau S^2$
101	2	1	$\frac{[101]}{[000]} = K^2[L]^2 = S^2$	$K^2[L]^2 = S^2$
111	3	1	$\frac{[111]}{[000]} = \tau^2 K^3[L]^3 = \tau^2 S^3$	$\tau^2 K^3[L]^3 = \tau^2 S^3$

Three Site Model With Cooperativity

- Partition Function (sum the column):

$$Z = 1 + 3S + (1 + 2\tau)S^2 + \tau^2 S^3$$

- Take the partial derivative:

$$\frac{\partial Z}{\partial S} = 3 + 2(1 + 2\tau)S + 3\tau^2 S^2$$

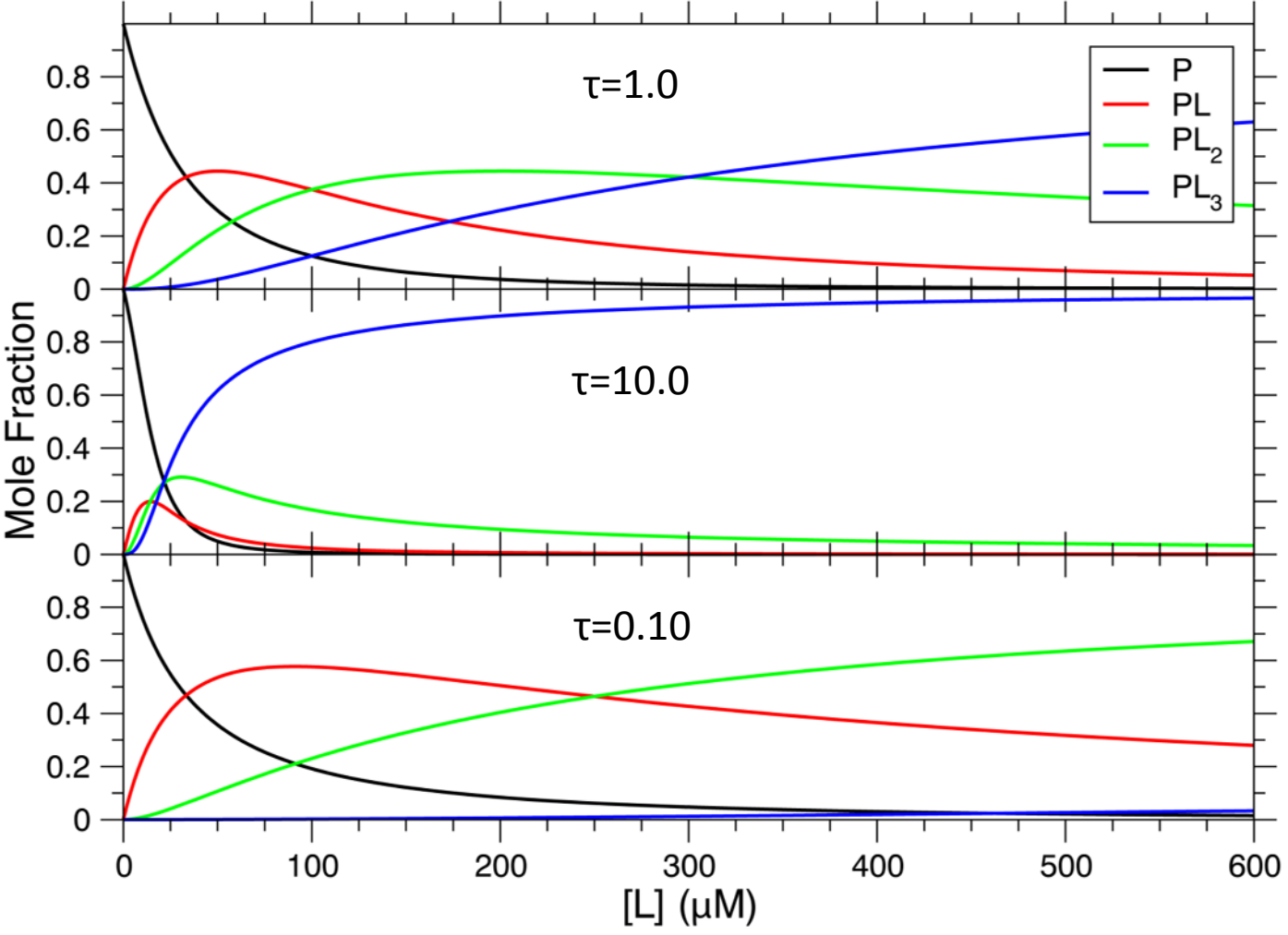
- Calculate the degree of binding:

$$\bar{v} = \frac{\partial \ln Z}{\partial \ln S} = \frac{S \frac{\partial Z}{\partial S}}{Z} = \frac{3S + 2(1 + 2\tau)S^2 + 3\tau^2 S^3}{1 + 3S + (1 + 2\tau)S^2 + \tau^2 S^3}$$

- You can also use the definition of \bar{v} :

$$\bar{v} = \frac{\sum n_i \text{ bound } w_i}{\sum w_i}$$

Three Site Model With Cooperativity



Extreme #1: All-Or-None Model

State	# Bound	Equivalent Configurations	Raw Weight	Weight with Equivalence
000	0	1	$\frac{[000]}{[000]} = 1$	1
100, 010, 001	1	3	0 (by definition)	0
110, 101, 011	2	3	0 (by definition)	0
111	3	1	$\frac{[111]}{[000]} = K^3[L]^3 = S^3$	$K^3[L]^3 = S^3$

- Partition function:

$$Z = 1 + K^3[L]^3 \text{ and } S \frac{\partial Z}{\partial S} = 3S^3 = 3K^3[L]^3$$

Extreme #2: Excluded Site Model

State	# Bound	Equivalent Configurations	Raw Weight	Weight with Equivalence
000	0	1	$\frac{[000]}{[000]} = 1$	1
100, 010, 001	1	3	$\frac{[100]}{[000]} = K[L] \equiv S$	$3K[L] \equiv 3S$
110, 011	2	2	0 (by definition)	0
101	2	1	$\frac{[101]}{[000]} = K^2[L]^2 = S^2$	$K^2[L]^2 = S^2$
111	3	1	0 (by definition)	0

$$Z = 1 + 3K[L] + K^2[L]^2$$

$$\frac{\partial Z}{\partial S} = 3 + 2S = 3 + 2K[L]$$

Hill Plots and Cooperativity

- Define the fractional saturation:

$$f = \frac{\bar{v}}{N} = \% \text{ sites occupied}$$

- For identical, independent sites:

$$f = \frac{\bar{v}}{N} = \frac{K[L]}{1+K[L]} \rightarrow \frac{f}{1-f} = K[L]$$

- For all-or-none model (maximum cooperativity):

$$f = \frac{\bar{v}}{N} = \frac{(K[L])^N}{1+(K[L])^N} \rightarrow \frac{f}{1-f} = K^N [L]^N$$

Hill Coefficients

- Hill Plot:

$$\log \frac{f}{1-f} \text{ vs. } \log [L]$$

- If the slope (n) of the linear region is:
 - $n \approx N$: Binding is nearly perfectly cooperative
 - $n \approx 1$: Binding is not cooperative (independent)
 - $n < 1$: Binding is anti-cooperative (approaching excluded site)

Summary: Binding Problems

- **Step 1:** Construct a table with all possible states; include degeneracy and interesting info (e.g. # of molecules bound)
- **Step 2:** Use chemistry to calculate raw weights; multiply by degeneracy to get final weights
- **Step 3:** Construct $Z = \text{sum of weights}$. Relate to observable (e.g., $\bar{\nu}$, fraction folded, etc.)