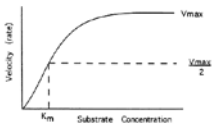
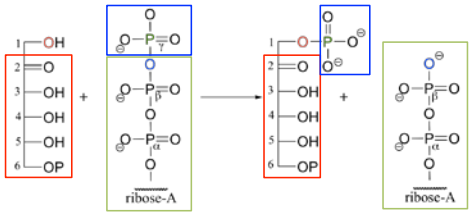




MCB 135 Lecture 6
Enzyme Kinetics

Recap: moiety conservation restricts how concentrations change together



fructose 6-phosphate ATP fructose 1,6-bisphosphate ADP

[F6P] + [F1,6BP] = constant

[ATP] + [F1,6BP] = constant

[ATP] + [ADP] = constant

Follow-up: systematic method for identifying moieties

General principle: if a set of equations are all true, then any linear combination of them is also true

$$\begin{aligned} ax + by &= c \\ dx + ey &= f \\ 2(ax + by) + dx + ey &= 2c + f \end{aligned}$$

Follow-up: systematic method for identifying moieties

You may have employed this technique to solve systems of equations in an algebra course.

$$\begin{aligned} -5x - 5y &= -10 \\ 5x - 7y &= 34 \\ -12y &= 24 \implies y = -2, x = 4 \end{aligned}$$

Follow-up: systematic method for identifying moieties

In matrix form, this technique is called Gauss-Jordan elimination.

$$\begin{aligned} x_1 + 2x_2 + 3x_3 &= 9 \\ 2x_1 - x_2 + x_3 &= 8 \\ 3x_1 - x_3 &= 3 \end{aligned} \longrightarrow \left[\begin{array}{ccc|c} 1 & 2 & 3 & 9 \\ 2 & -1 & 1 & 8 \\ 3 & 0 & -1 & 3 \end{array}\right] \downarrow$$

$$\begin{aligned} x_1 + 2x_2 + 3x_3 &= 9 \\ x_2 + x_3 &= 2 \\ x_3 &= 3 \end{aligned} \longleftarrow \left[\begin{array}{ccc|c} 1 & 2 & 3 & 9 \\ 0 & 1 & 1 & 2 \\ 0 & 0 & 1 & 3 \end{array}\right]$$

Follow-up: systematic method for identifying moieties

$$\begin{aligned} \frac{dA}{dt} &= k_{-1}B^2 + k_2D + k_4BE - k_1A - k_{-2}AC \\ \frac{dB}{dt} &= 2k_1A + k_3D - 2k_{-1}B^2 - k_4BE \\ \frac{dC}{dt} &= k_2D + k_4BE - k_{-2}AC \\ \frac{dD}{dt} &= k_{-2}AC - k_3D - k_2D \\ \frac{dE}{dt} &= k_3D - k_4BE \end{aligned}$$

If we could find a linear combination of ODEs that sums to zero, e.g.

$$\frac{dC}{dt} + \frac{dD}{dt} + \frac{dE}{dt} = 0$$

then integration w.r.t. time would give us a conservation rule:

$$[C] + [D] + [E] = \text{constant}$$

Follow-up: systematic method for identifying moieties

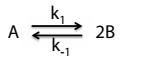
Step one: number the reactions

r_1 : $A \rightleftharpoons 2B$

r_2 : $D \rightleftharpoons A + C$

r_3 : $D \longrightarrow B + E$

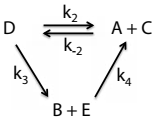
r_4 : $B + E \longrightarrow A + C$



Step two: construct a stoichiometry matrix

$$\begin{matrix} r_1 & r_2 & r_3 & r_4 \end{matrix}$$

$$\begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{matrix} A \\ B \\ C \\ D \\ E \end{matrix}$$



Follow-up: systematic method for identifying moieties

Step three: express the rates of change for each molecular species using the stoichiometry matrix

Found last time using the law of mass action

$$\begin{aligned}
\frac{dA}{dt} &= k_{-1}B^2 + k_3D + k_4BE - k_1A - k_{-2}AC \\
\frac{dB}{dt} &= 2k_1A + k_3D - 2k_{-1}B^2 - k_4BE \\
\frac{dC}{dt} &= k_2D + k_4BE - k_{-2}AC \\
\frac{dD}{dt} &= k_{-2}AC - k_3D - k_2D \\
\frac{dE}{dt} &= k_3D - k_4BE
\end{aligned}$$

Rewritten in matrix form

$$\frac{d}{dt}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} k_1A - k_{-1}B^2 \\ k_2D - k_{-2}AC \\ k_3D \\ k_4BE \end{pmatrix}$$

Follow-up: systematic method for identifying moieties

Step three: express the rates of change for each molecular species using the stoichiometry matrix

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$$\begin{aligned}
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\frac{dD}{dt} &= k_{-2}AC - k_3D - k_2D \\
\frac{dE}{dt} &= k_3D - k_4BE
\end{aligned}$$

Notice that these are the "net forward reaction rates" (we'll call them v_i)

Rewritten in matrix form

$$\frac{d}{dt}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} k_1A - k_{-1}B^2 \\ k_2D - k_{-2}AC \\ k_3D \\ k_4BE \end{pmatrix}$$

Follow-up: systematic method for identifying moieties

$$\frac{d}{dt}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} k_1A - k_{-1}B^2 \\ k_2D - k_{-2}AC \\ k_3D \\ k_4BE \end{pmatrix}$$

Rewrite in an equivalent form:

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix} \frac{d}{dt}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

Follow-up: systematic method for identifying moieties

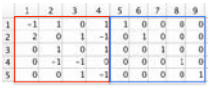
Our goal: apply row reduction (aka Gaussian elimination) so that some rows of the stoichiometry matrix become all zeros.

Then, the left-hand side will tell us that some combination of the derivatives sums to zero.

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix} \frac{d}{dt}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

Follow-up: systematic method for identifying moieties

Input the stoichiometry matrix (augmented by the identity matrix) into MATLAB



$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix} \frac{d}{dt}\begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 1 & -1 \\ 0 & 1 & 0 & 1 \\ 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

Follow-up: systematic method for identifying moieties

Input the stoichiometry matrix (augmented by the identity matrix) into MATLAB

Call the rref() function

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 \\ 2 & 1 & 0 & 2 & 1 \\ 0 & 0 & 1 & 1 & 1 \end{pmatrix} \frac{d}{dt} \begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 2 & -2 \\ 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

Follow-up: systematic method for identifying moieties

$$2\frac{dA}{dt} + 2\frac{dD}{dt} + \frac{dB}{dt} + \frac{dE}{dt} = 0$$

$$\frac{dC}{dt} + \frac{dD}{dt} + \frac{dE}{dt} = 0$$

$$2[A] + 2[D] + [B] + [E] = \text{constant}$$

$$[C] + [D] + [E] = \text{constant}$$

These are our two conserved moieties

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 \\ 2 & 1 & 0 & 2 & 1 \\ 0 & 0 & 1 & 1 & 1 \end{pmatrix} \frac{d}{dt} \begin{pmatrix} A \\ B \\ C \\ D \\ E \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 & 1 \\ 2 & 0 & 2 & -2 \\ 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}$$

Today's Outline

- Rate of product formation in enzyme catalysis
  - Recap of simplifying assumptions and enzyme catalysis
  - Michaelis-Menten kinetics
- Simple binding curves
  - Hyperbolic form
  - The switch-like behavior problem
  - Hemoglobin as a natural example of a sigmoidal form

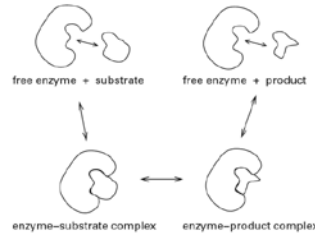
Enzyme catalysis is a composite reaction

$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow{k_3} \text{Fructose-1,6-bisphosphate} + \text{ADP}$$

When catalyzed by the enzyme phosphofructokinase, this reaction has several steps:

$$\text{Fructose-6-phosphate} + \text{Enzyme} \longrightarrow \text{Enzyme-F6P complex}$$
$$\text{ATP} + \text{Enzyme-F6P complex} \longrightarrow \text{Enzyme-F6P-ATP complex}$$
$$\text{Enzyme-F6P-ATP complex} \longrightarrow \text{Enzyme-F1,6BP-ADP complex}$$
$$\text{Enzyme-F1,6BP-ADP complex} \longrightarrow \text{Fructose-1,6-bisphosphate} + \text{ADP} + \text{Enzyme}$$

Enzyme catalysis is a composite reaction

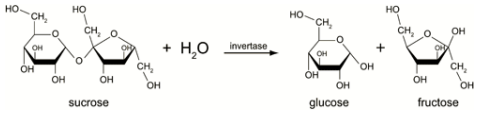


$$\underset{\text{Substrate}}{S} + \underset{\text{Free enzyme}}{E} \rightleftharpoons \underset{\text{Enzyme-substrate complex}}{C_1} \rightleftharpoons \underset{\text{Enzyme-product complex}}{C_2} \rightleftharpoons \underset{\text{Product}}{P} + \underset{\text{Free enzyme}}{E}$$

Reducing the model's complexity

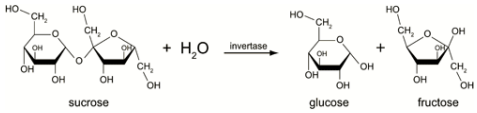
Leonor Michaelis

Maud Menten



sucrose glucose fructose

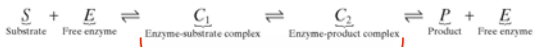
Reducing the model's complexity



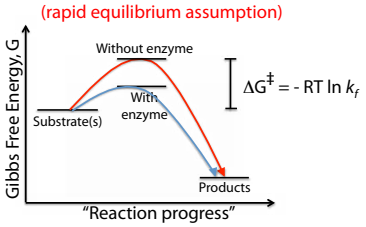
Recap: common simplifying assumptions

- A chemical's concentration has a particular constant value
- A chemical's concentration is at steady-state
  - The "quasi-steady-state" assumption
  - Can find an expression for the steady-state concentration from the chemical's ODE
- A particular reversible reaction has reached equilibrium
  - The "rapid equilibrium" assumption

Simplifying the enzyme catalysis model



Assume this interconversion is very fast (rapid equilibrium assumption)



Simplifying the enzyme catalysis model

Assume this reaction is effectively irreversible

Assume this interconversion is very fast (rapid equilibrium assumption)

Our simplified model:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E$$

Catalytic constant (also denoted k_{cat})

How are these species' concentrations changing with time?

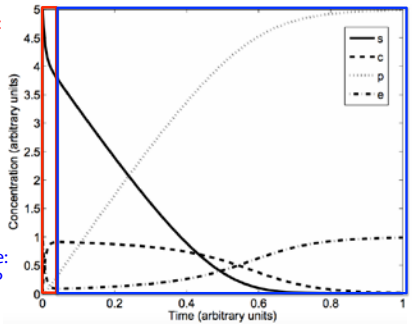
$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E$$

$$\begin{aligned}
\frac{dS}{dt} &= k_{-1}C - k_1SE \\
\frac{dE}{dt} &= k_{-1}C + k_2C - k_1SE \\
\frac{dC}{dt} &= k_1SE - k_{-1}C - k_2C \\
\frac{dP}{dt} &= k_2C
\end{aligned}$$

How are these species' concentrations changing with time?

Fast timescale: [C] rises to a quasi-steady-state

Slow timescale: S converts to P



Why does the fast timescale exist?

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E$$

1) $k_1, k_{-1} >> k_2$
2) [S] >> [E], [C]

Simplifying the model using moiety conservation

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E$$

$$\begin{aligned}
\frac{dS}{dt} &= k_{-1}C - k_1SE \\
\frac{dE}{dt} &= k_{-1}C + k_2C - k_1SE \\
\frac{dC}{dt} &= k_1SE - k_{-1}C - k_2C \\
\frac{dP}{dt} &= k_2C
\end{aligned}$$

Rewrite using a stoichiometry matrix:

$$\frac{d}{dt}\begin{pmatrix} S \\ E \\ C \\ P \end{pmatrix} = \begin{pmatrix} -1 & 0 \\ -1 & 1 \\ 1 & -1 \\ 0 & 1 \end{pmatrix}\begin{pmatrix} k_1SE - k_{-1}C \\ k_2C \end{pmatrix}$$

Perform row reduction, and find:

$$[S] + [C] + [P] = \text{constant}$$
$$[C] + [E] = \text{constant}$$

Simplifying the model using moiety conservation

$$[C] + [E] = \text{constant} \longrightarrow [E] = [E]_{tot} - [C]$$

$$\begin{aligned}
\frac{dS}{dt} &= k_{-1}C - k_1S[E_{tot} - C] &&= -k_1SE_{tot} + C[k_{-1} + k_1S] \\
\frac{dC}{dt} &= k_1S[E_{tot} - C] - k_{-1}C - k_2C &&= k_1SE_{tot} - C[k_{-1} + k_2 + k_1S] \\
\frac{dP}{dt} &= k_2C &&= k_2C
\end{aligned}$$

Exploiting the rapid approach of [C] to quasi-steady-state

$$\frac{dC}{dt} = k_1SE_{tot} - C[k_{-1} + k_2 + k_1S] = 0$$

$$C = \frac{k_1SE_{tot}}{k_{-1} + k_2 + k_1S} = \frac{SE_{tot}}{K_m + S}, \qquad K_m = \frac{k_{-1} + k_2}{k_1}$$

How fast is the product forming?

$$C = \frac{SE_{tot}}{K_m + S}, \qquad K_m = \frac{k_{-1} + k_2}{k_1}$$

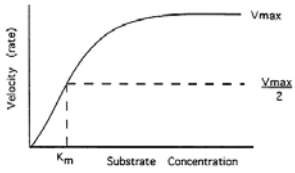
$$\frac{dP}{dt} = k_2C = \frac{V_{max}S}{K_m + S}, \qquad V_{max} = k_2E_{tot}$$

The Michaelis-Menten equation

The physical interpretation of K_m and V_{max}

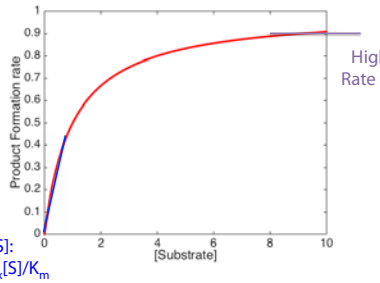
$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m + [S]}$$

$$\lim_{[S]\to\infty} \frac{d[P]}{dt} = V_{max}$$



$$\left.\frac{d[P]}{dt}\right|_{[S]=K_m} = \frac{V_{max}}{2}$$

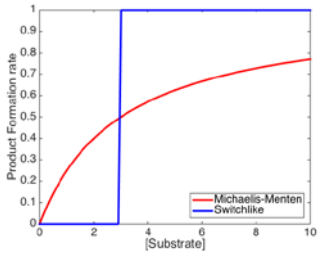
Approximations at two extremes



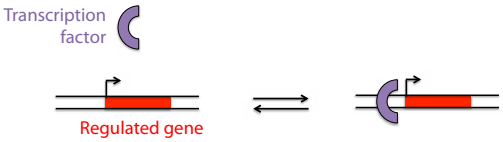
High [S]: Rate ≈ V_{max}

Low [S]: Rate ≈ $V_{max}[S]/K_m$

Hyperbolic, not switch-like, behavior

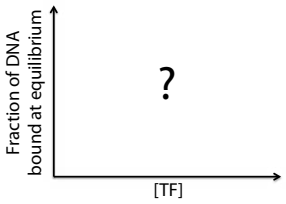


Not all biological reactions are enzyme-catalyzed



$$TF + DNA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Complex$$

Not all biological reactions are enzyme-catalyzed



$$TF + DNA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Complex$$

What is the fraction of bound DNA, $\frac{[Complex]}{[DNA]+[Complex]}$, at equilibrium?

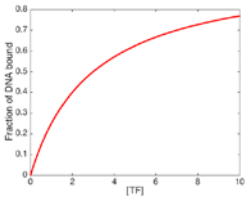
$$TF + DNA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Complex$$

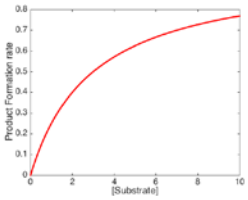
$$\text{At equilibrium, } k_1[TF][DNA] = k_{-1}[Complex]$$

$$\Longrightarrow [DNA] = \frac{k_{-1}[Complex]}{k_1[TF]}$$

$$\frac{[Complex]}{[DNA]+[Complex]} = \frac{[Complex]}{\frac{k_{-1}[Complex]}{k_1[TF]}+[Complex]} = \frac{[TF]}{K+[TF]}$$

Simple binding curves have the same form as Michaelis-Menten curves





$$\text{Fraction of DNA bound} = \frac{[TF]}{K+[TF]} \qquad \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_m+[S]}$$

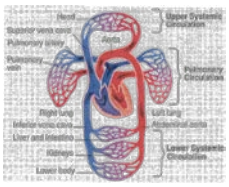
- Organisms follow algorithms to determine responses to "inputs" from their environments
  - Are these computations are inherently limited?
- A system can compute anything if it can implement Boolean logic and memory (Church-Turing thesis)
- Boolean logic requires all-or-none responses to binary inputs

→ Want to show that all living systems can implement Boolean variables

How can organisms make switch-like responses with binding/enzyme kinetics?

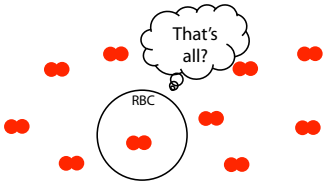
Oxygen transport

A few cells thick: diffusion suffices

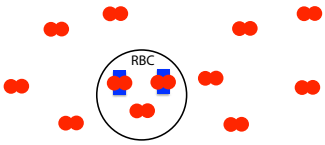


Much larger: circulatory and respiratory systems needed

Oxygen loading in the lungs can limit oxygen transport



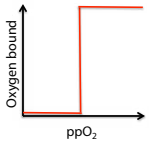
By forming complexes, oxygen-binding proteins can "hide" oxygen inside the red blood cell



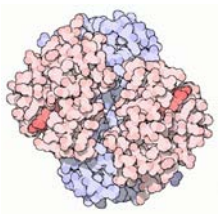
Ideal properties for an oxygen-binding protein

In the lungs, where ppO_2 is high, binds as much oxygen as possible

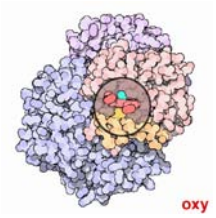
In other tissues, where ppO_2 is low, releases as much oxygen as possible



The protein hemoglobin has four subunits, each cradling a heme group

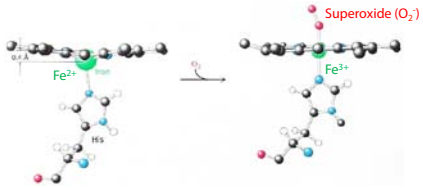


~ 4 nm



PDB May 2003 Molecule of the Month

Heme's absorption spectrum changes on oxygen binding

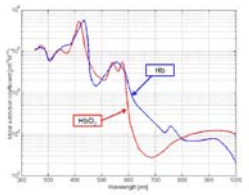


Dark red

Bright red

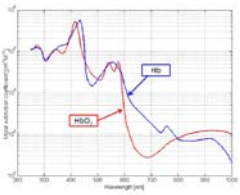
Stryer Fig. 7.2

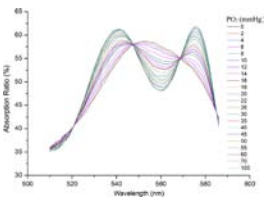
The fraction of hemoglobin bound by oxygen can be estimated from the absorption spectrum



Courtesy of Scott Prahl

The fraction of hemoglobin bound by oxygen can be estimated from the absorption spectrum

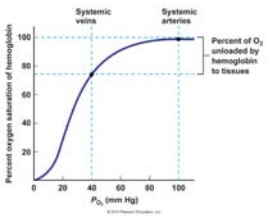


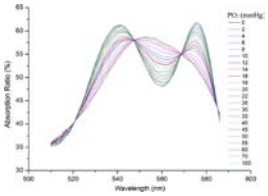


Courtesy of Scott Prahl

Liu et al., 2012

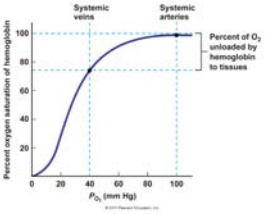
The fraction of hemoglobin bound by oxygen can be estimated from the absorption spectrum

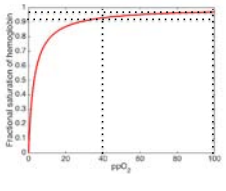




Liu et al., 2012

Hemoglobin has a sigmoidal, not hyperbolic, binding curve





Next time:
Cooperativity and inhibition