

ABE 580

Chapter 8 Enzyme Kinetics

Enzyme Kinetics

- Similar derivation to all chemical kinetics
- Enzyme kinetics has some special considerations

Enzyme Kinetics

- Aqueous environment
- Catalyst reactions
- Multiple substrates in single reaction common

Mechanistic Reaction Models

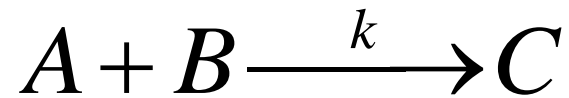
- Focus on mechanism of reaction
- Interaction of substrate(s) and catalyst(s)
- Interactions of product(s) and catalyst(s)

Use of Mechanistic Models

- Accurately captures interactions between enzymes and substrates
- Elucidates dynamics of enzyme binding and dissociation

Kinetic Theory

Collision Theory Of Reaction Rates



$$k = (Z_{AB})(F)$$

where

k = kinetic constant

Z_{AB} = frequency of collisions

F = Boltzman factor (fraction of collisions
giving rise to reactions)

Kinetic Theory

- Developed for dilute (low pressure) gases
- Reactions in liquid (or aqueous!) phase incoherent based upon first principles estimations for gases
- However, still widely used for developing mathematical expressions of biochemical reactions

Michaelis Menten Kinetics



Maud Menten

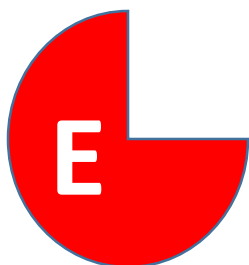
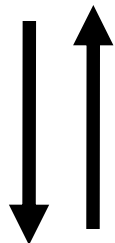
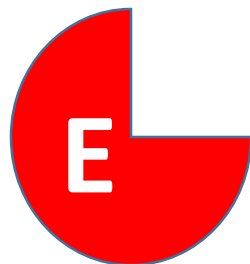
$$\frac{dP}{dt} = v = \frac{V_{max}[S]}{[S] + K_M}$$



Leonor Michaelis

Michaelis Menten Kinetics

- Mechanistic Model
- Based upon Law of Mass Action
- Key Assumptions
 - Homogeneous reaction
 - Substrate in excess $[S] \gg [E]$
 - (pseudo) steady-state or equilibrium



Enzymatic Reaction Rates

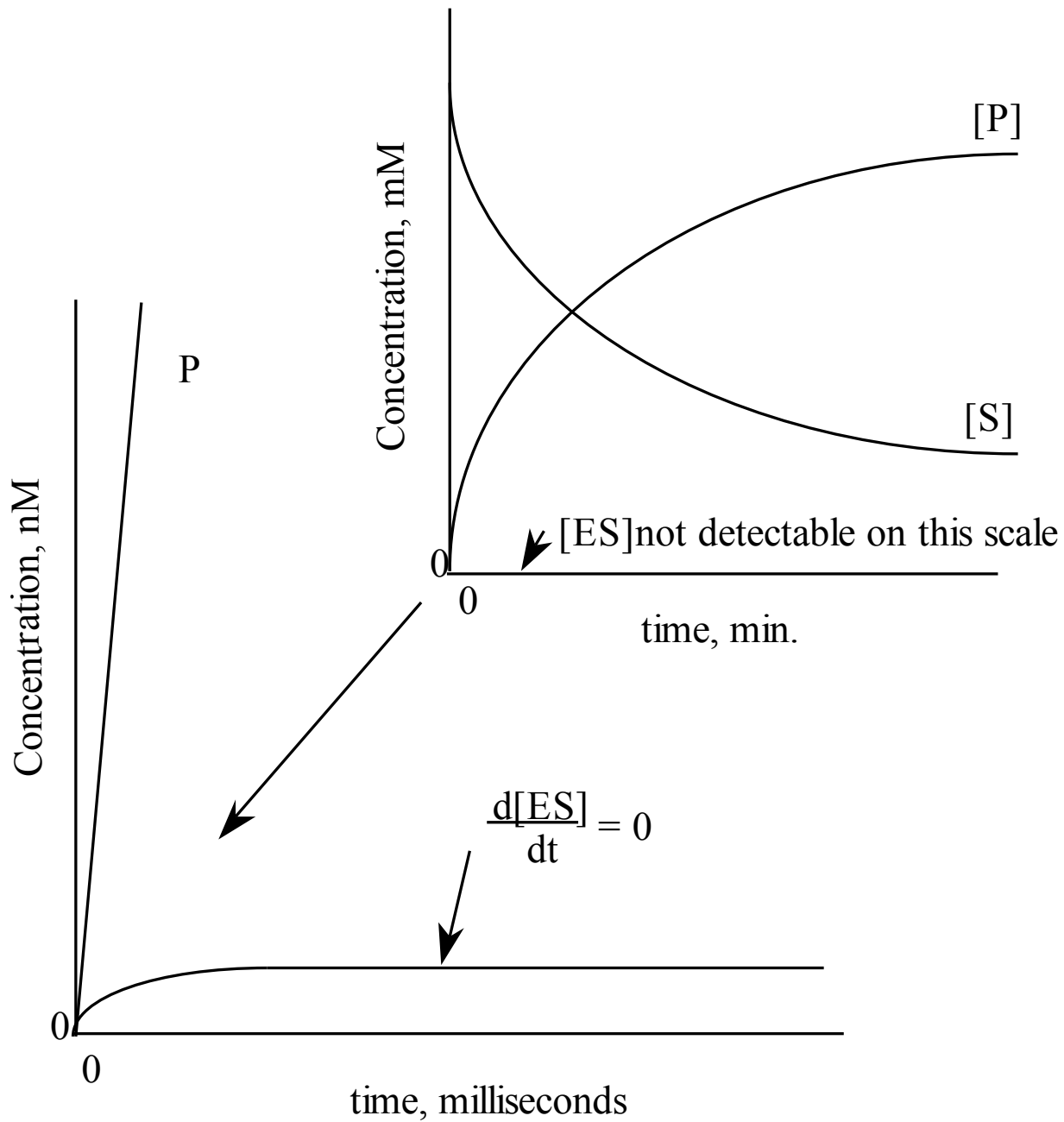
Michaelis-Menten



Assume k_1 and $k_{-1} \gg k_2$

$$\frac{d ES}{dt} = 0$$

Steady - State



Enzymatic Reaction Rates

Michaelis-Menten



$$\frac{dP}{dt} = k_2[ES] = k_2[E_T] \frac{[S]}{[S] + K_M}$$

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

Michaelis Menten Kinetics

$$\frac{dP}{dt} = k_2[E_T] \frac{[S]}{[S] + K_M}$$

$$\frac{dP}{dt} = v$$

$$V_{max} = k_2[E_T]$$

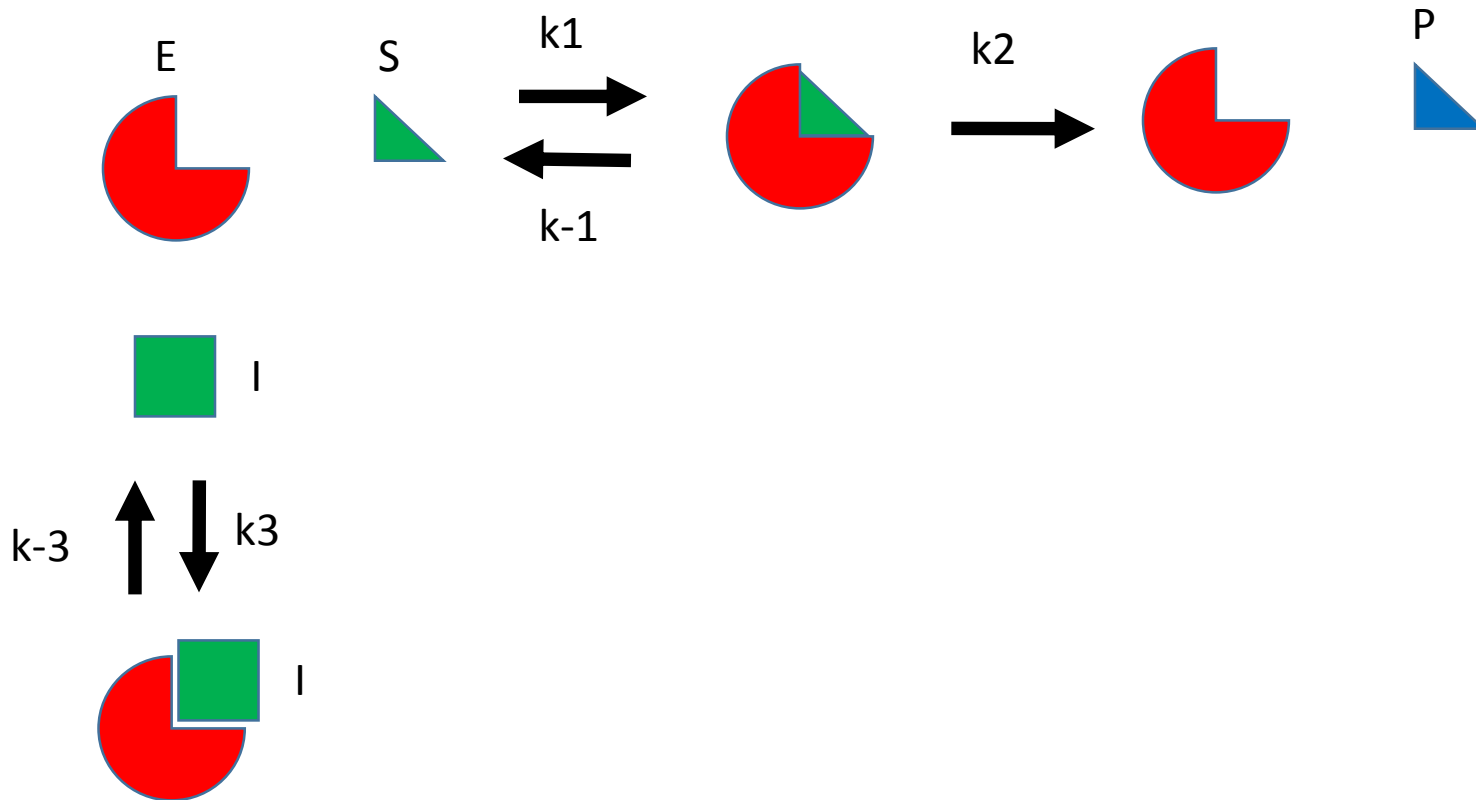
$$v = \frac{V_{max}[S]}{[S] + K_M}$$

Modulators (activators or inhibitors) can be factored in

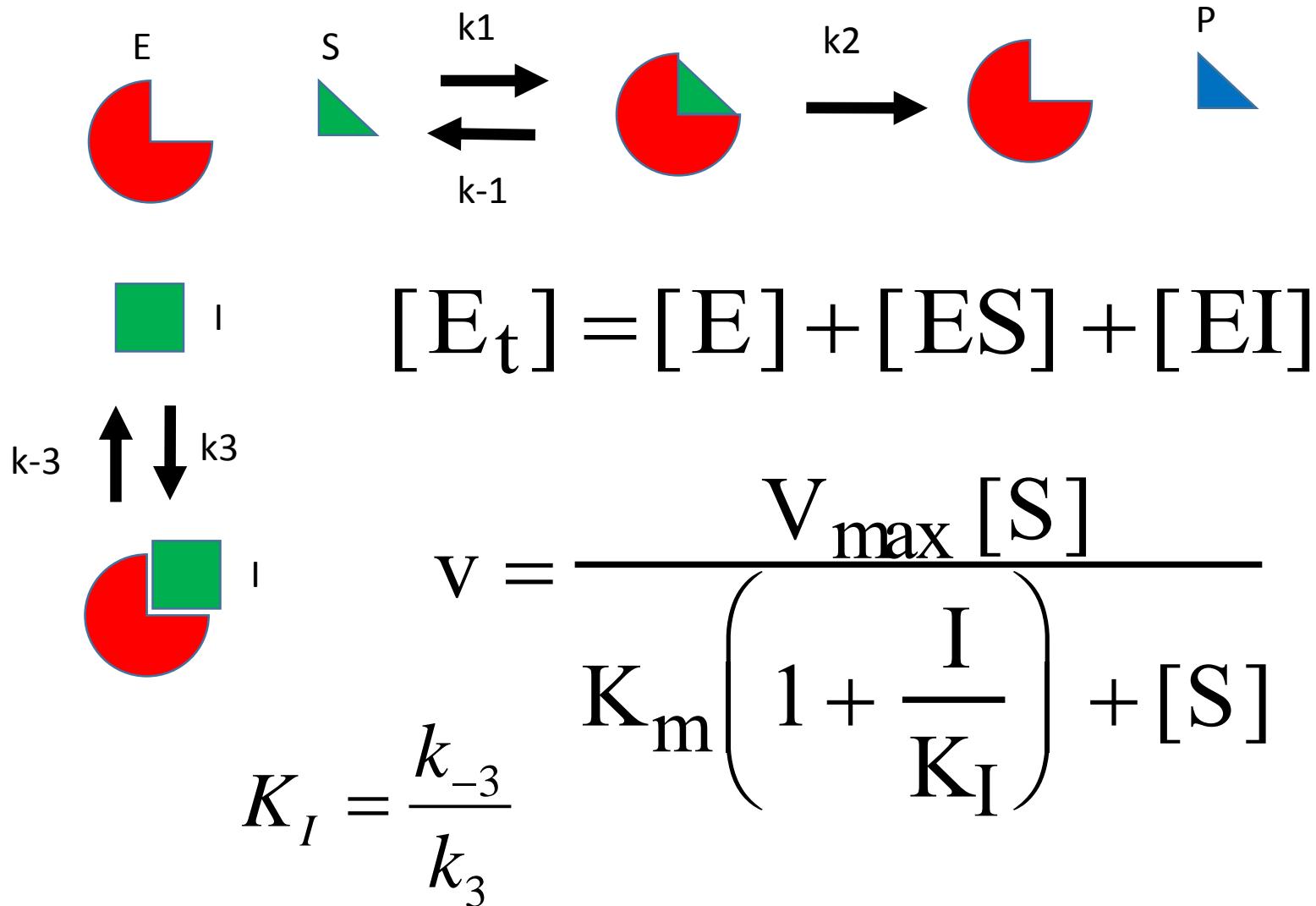
Inhibitors

- Classically, describes mechanism of interaction between
 - Enzyme
 - Substrate
 - Non-substrate small molecules
- Models impact of interaction of non-substrate small molecules on catalytic rate(s)

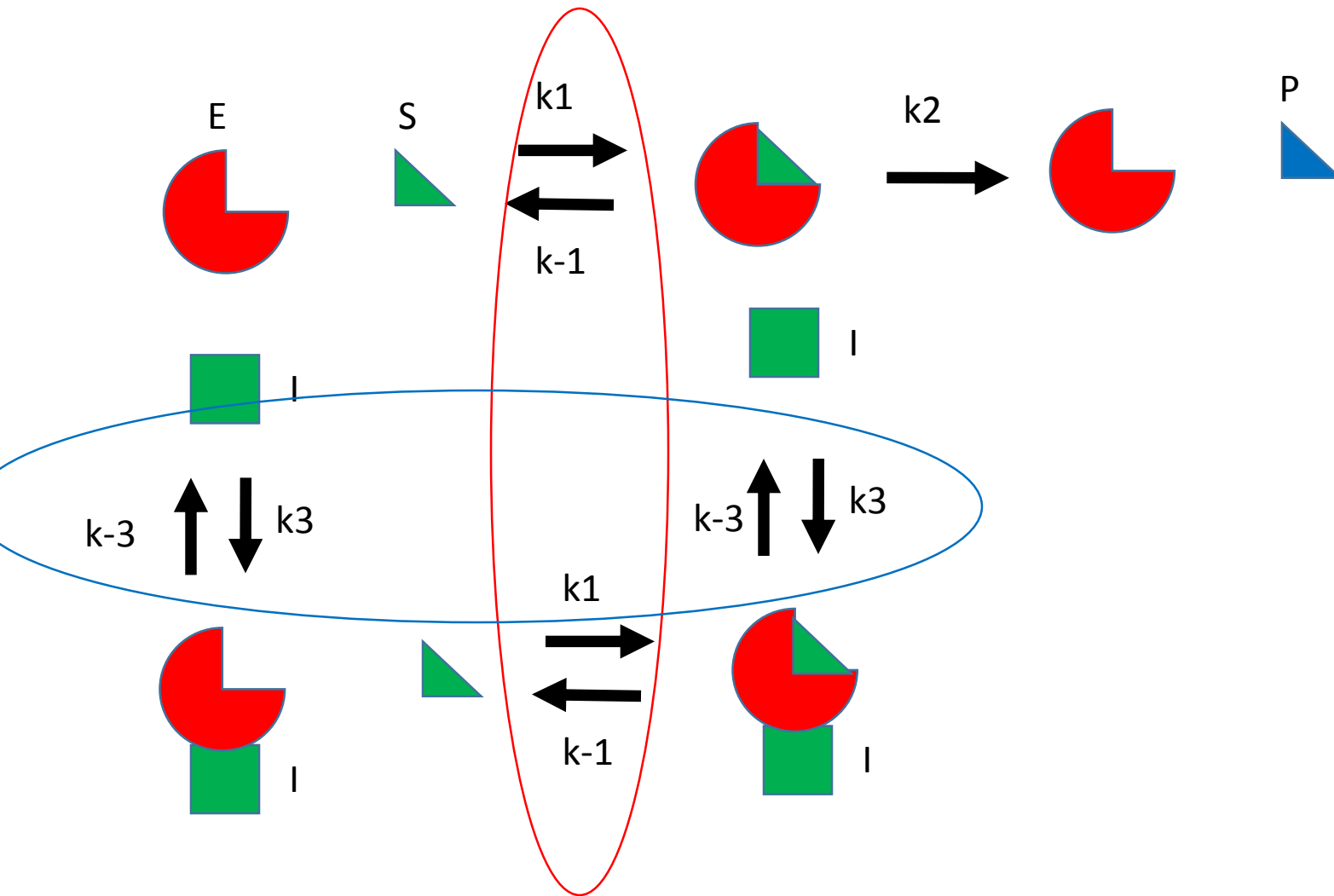
Classic Inhibition: Competitive



Classic Inhibition: Competitive



Classic Inhibition: Non-Competitive



$$v = \frac{V_{\max} [S]}{K_s \left(1 + \frac{[I]}{K_I} \right) + \left(1 + \frac{[I]}{K_I} \right) [S]} = \frac{V_{\max} [S]}{(K_s + [S]) \left(1 + \frac{[I]}{K_I} \right)}$$

$$= \frac{\frac{V_{\max}}{\left(1 + \frac{[I]}{K_I} \right)} [S]}{K_s + [S]}$$

$$K_I = \frac{k_{-3}}{k_3}$$

Determining Enzyme Kinetic Constants

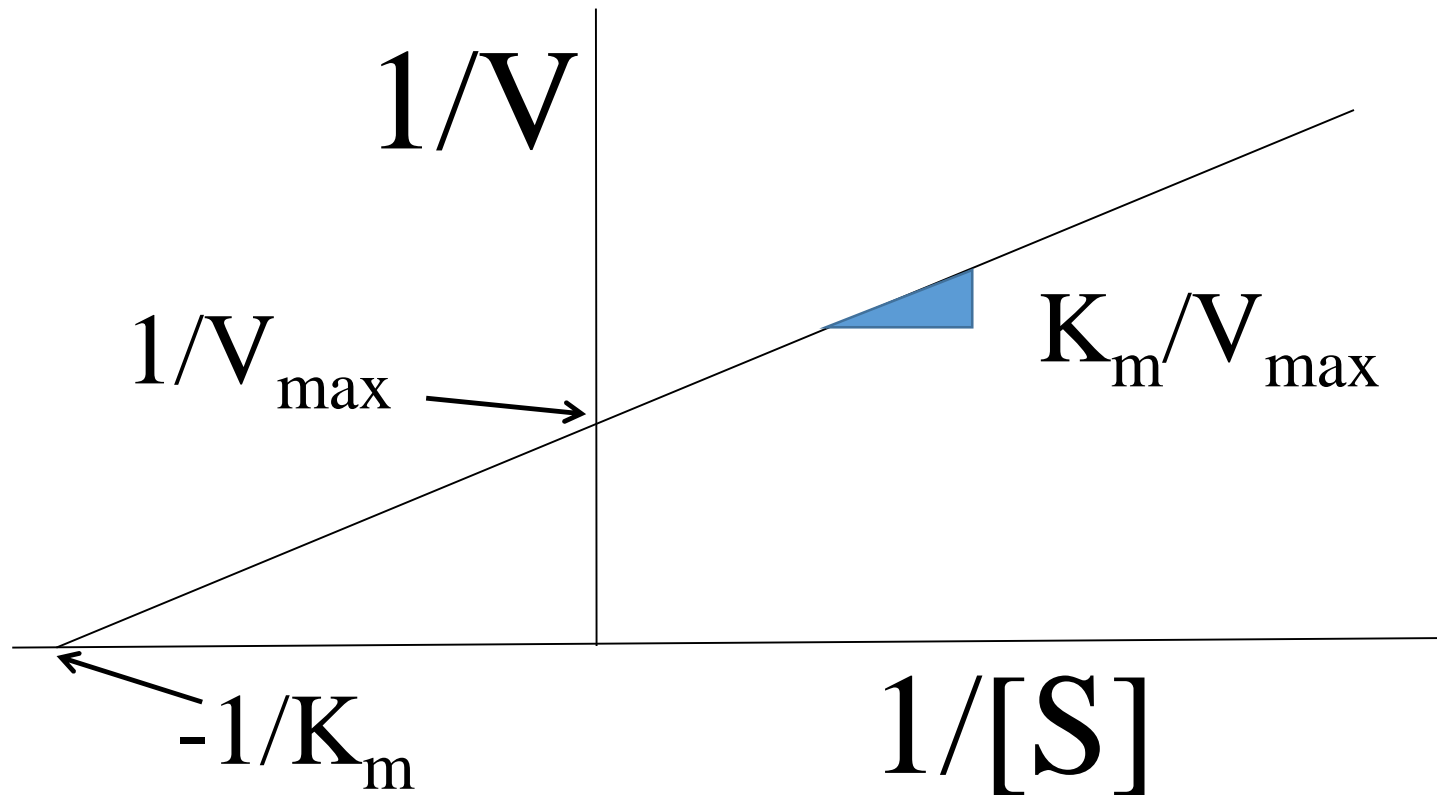
- Linearizing kinetic expression and using linear regression
- Non-linear optimization

Linearizing Michaelis-Menten Kinetics

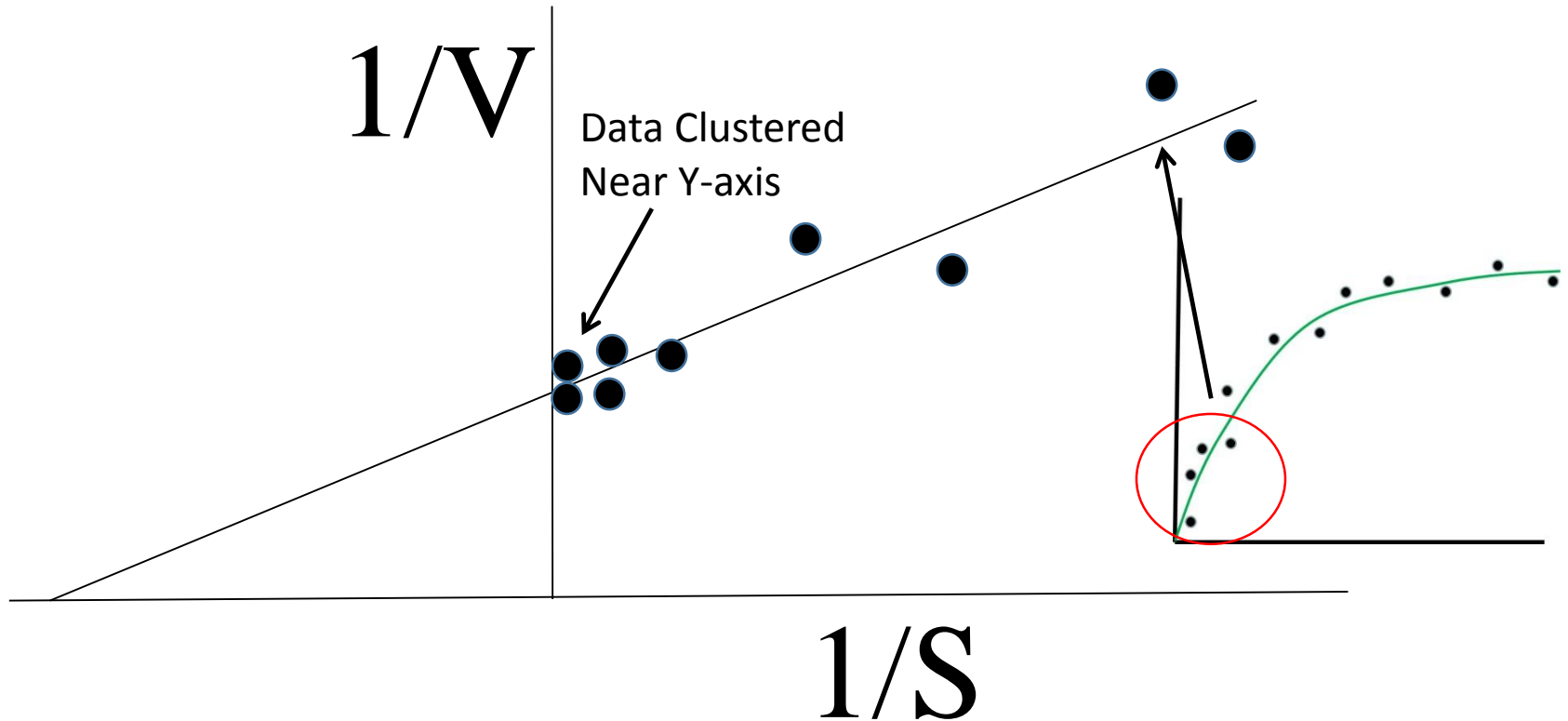
- Lineweaver-Burke
 - Double Reciprocal
- Eadie-Hofstee
 - Single Reciprocal
- Hanes-Woolf
 - Single Reciprocal

Lineweaver-Burke

$$v = \frac{V_{\max} \cdot [S]}{[S] + K_M} \longrightarrow \frac{1}{v} = \frac{K_m}{V_{\max}} \left(\frac{1}{[S]} \right) + \frac{1}{V_{\max}}$$

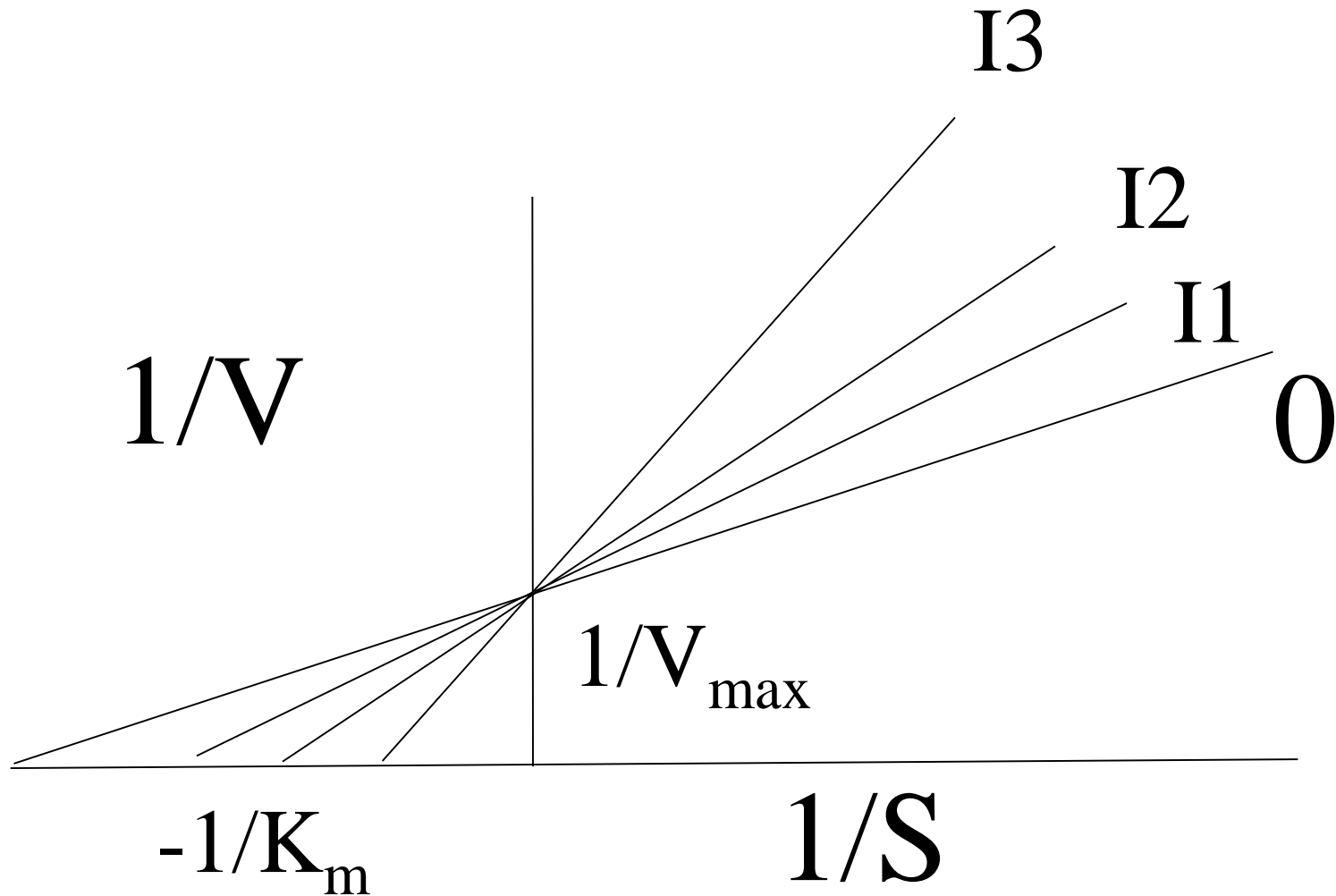


Lineweaver-Burke: Problems

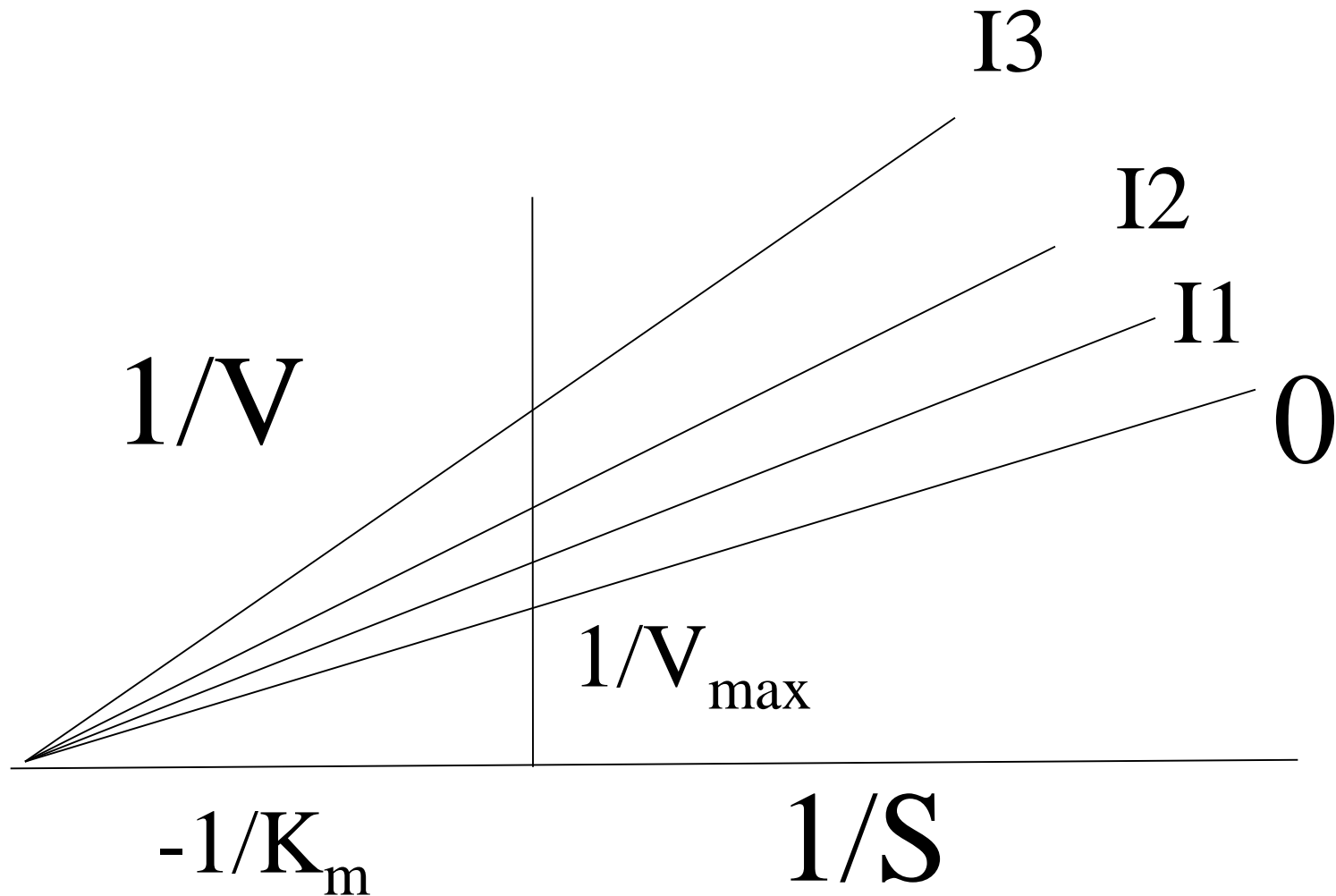


Small measurement errors = large parameter estimation errors

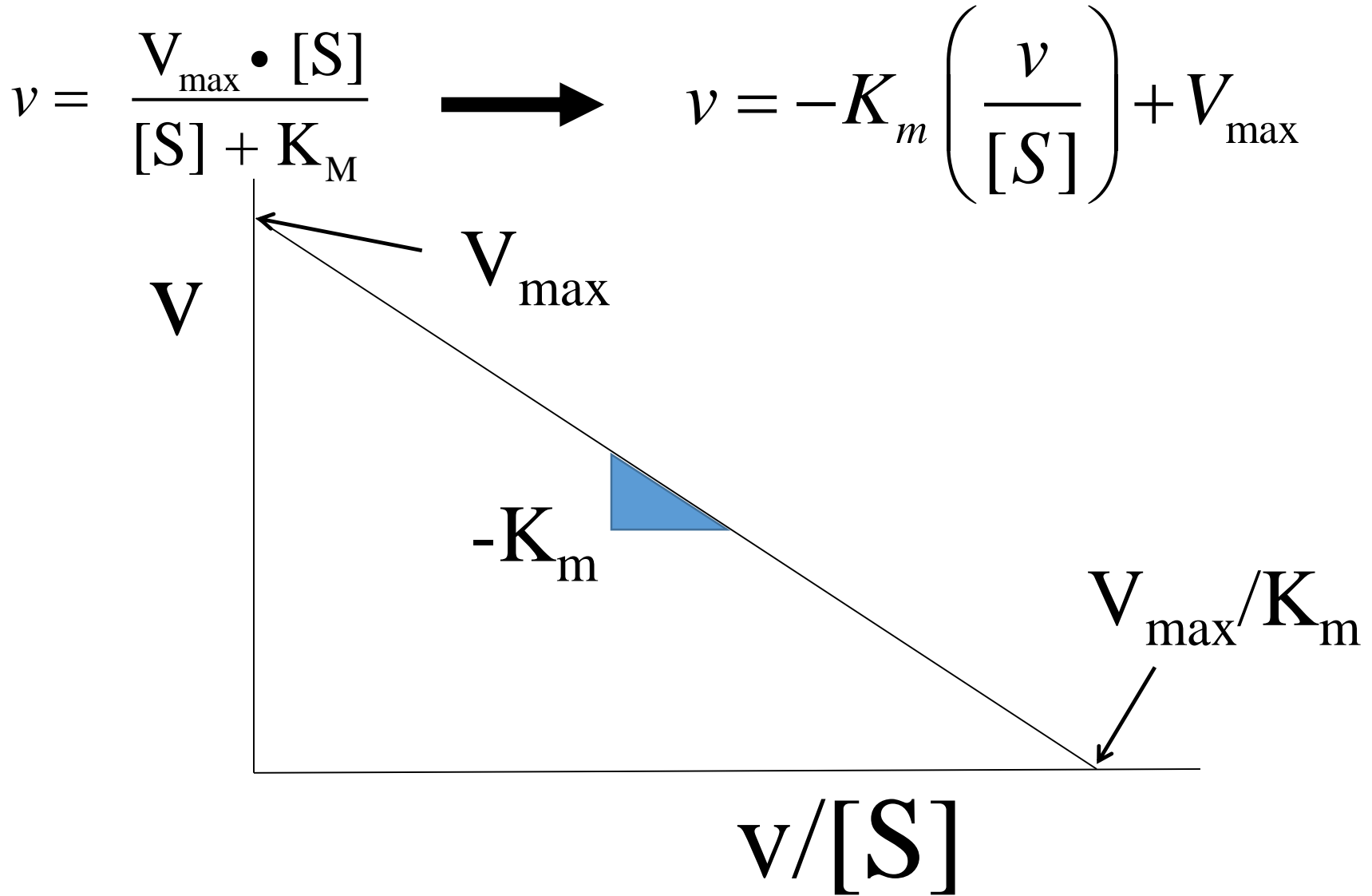
Classic Inhibition: Competitive



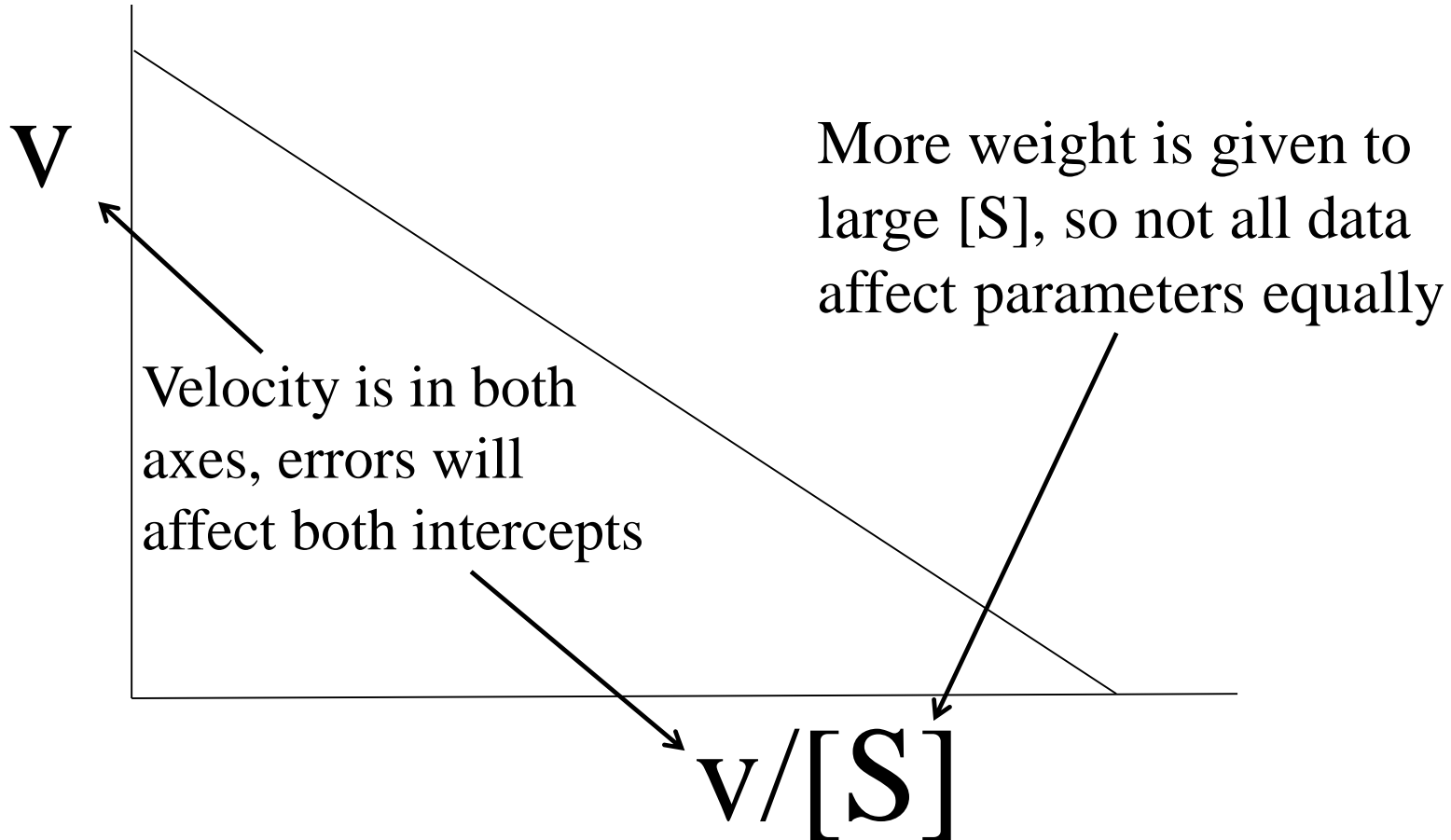
Classic Inhibition: Non-Competitive



Eadie-Hofstee



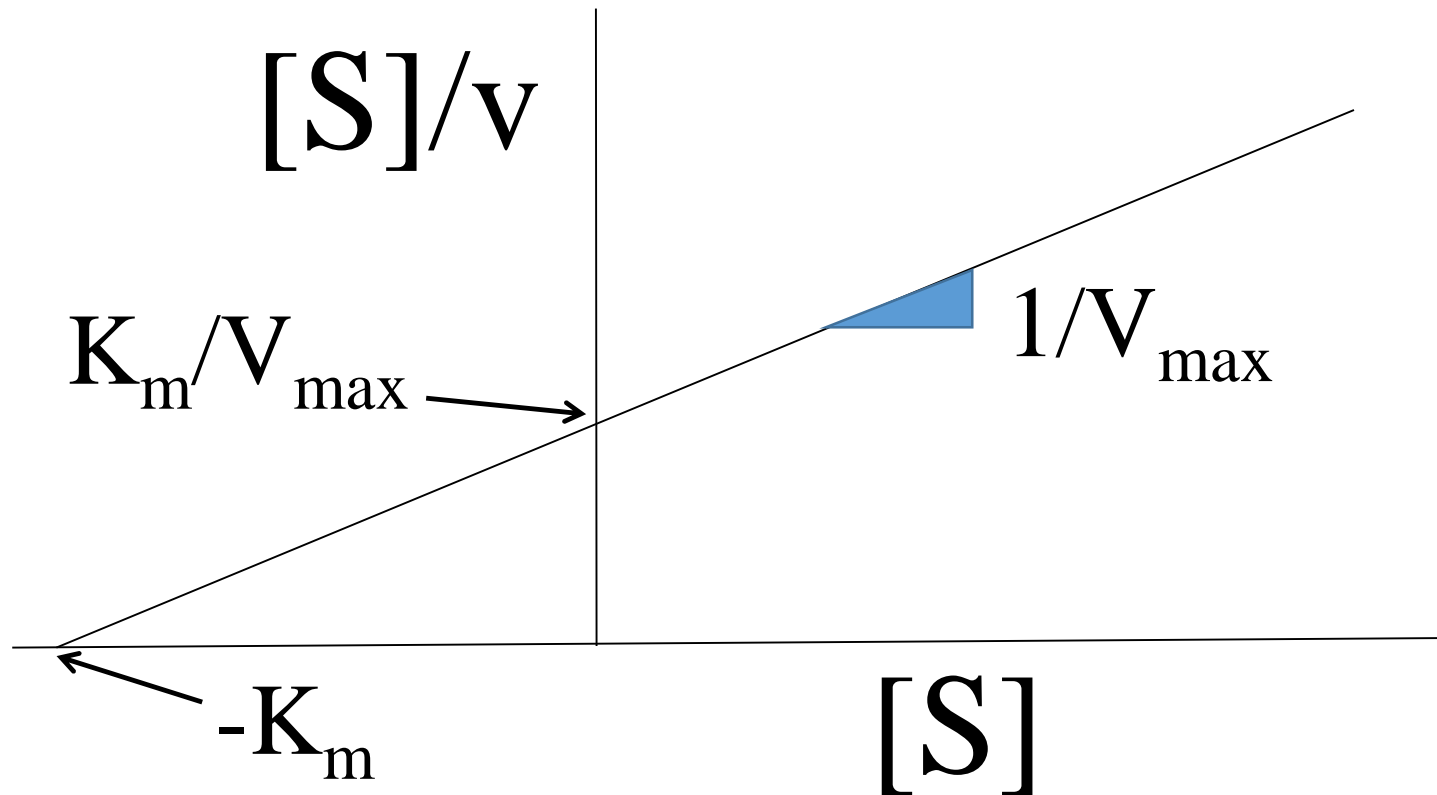
Eadie-Hofstee: Problems



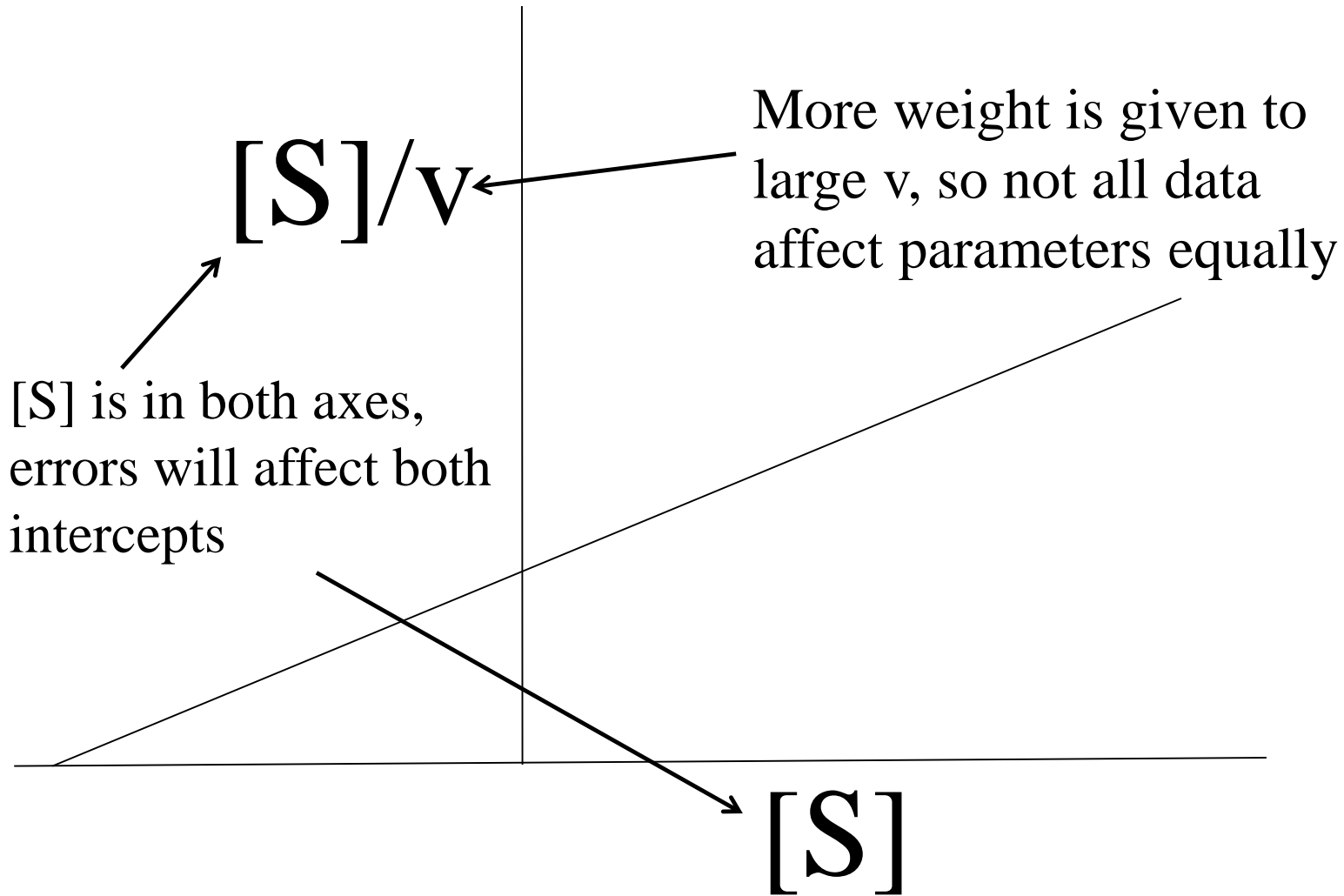
X-axis is not independent

Hanes-Woolf

$$v = \frac{V_{\max} \cdot [S]}{[S] + K_M} \longrightarrow \frac{[S]}{v} = \frac{1}{V_{\max}}[S] + \frac{K_m}{V_{\max}}$$



Hanes-Woolf: Problems



Non-Linear Regression

- Couples statistics (least squares) with modeling (computer simulation)
- Ordinary Least Squares
- Weighted Least Squares

Elements of Non-Linear Regression

- A model
- Initial “guesses” for parameters
- Experimental data
- A “fitness function”: $\min\{\text{SSE}\}$

Model

$$v = k_2[E_T] \frac{[S]}{[S] + K_M}$$

$$v = \frac{\frac{V_{\max,f}}{K_{m,s}} [S] - \frac{V_{\max,r}}{K_{m,p}} [P]}{1 + \frac{[S]}{K_{m,s}} + \frac{[P]}{K_{m,p}}}$$

$$v = \frac{V_{\max} [S]}{K_m \left(1 + \frac{I}{K_I} \right) + [S]}$$

Initial Guesses

$$V_{\max} = 0.22s^{-1}$$

$$K_m = 50mM$$

$$K_I = 150mM$$

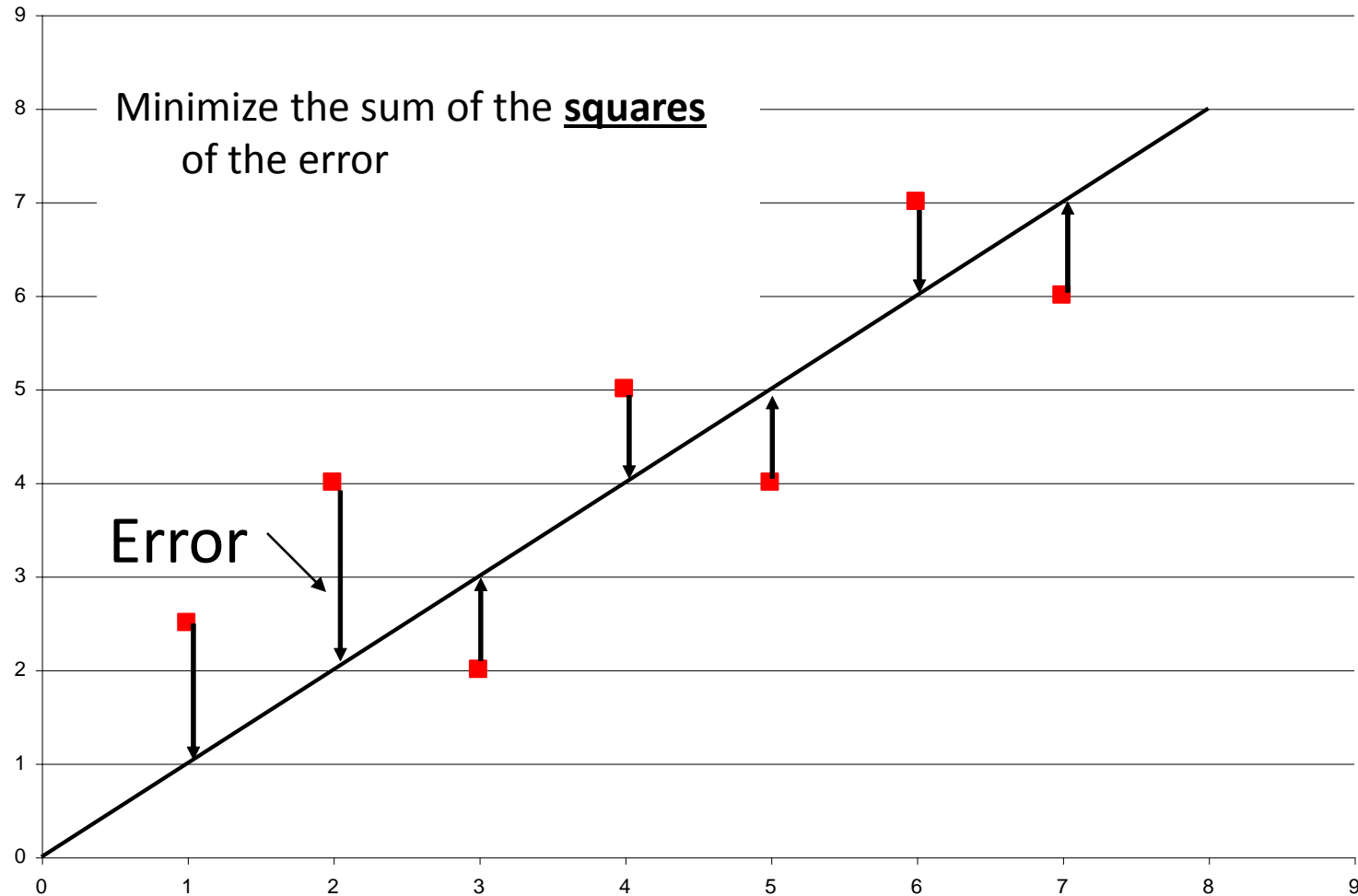
Experimental Data

S mM	V s ⁻¹
20	0.030
40	0.048
60	0.073
80	0.075
100	0.085
120	0.093
140	0.121
160	0.109
180	0.111
200	0.149
220	0.156
240	0.130
260	0.144
280	0.155
300	0.159
400	0.168
500	0.196

Fitness Function

- Function describing state of model with respect to experimental data
- Least Squares \Rightarrow sum of squares of errors (residuals)

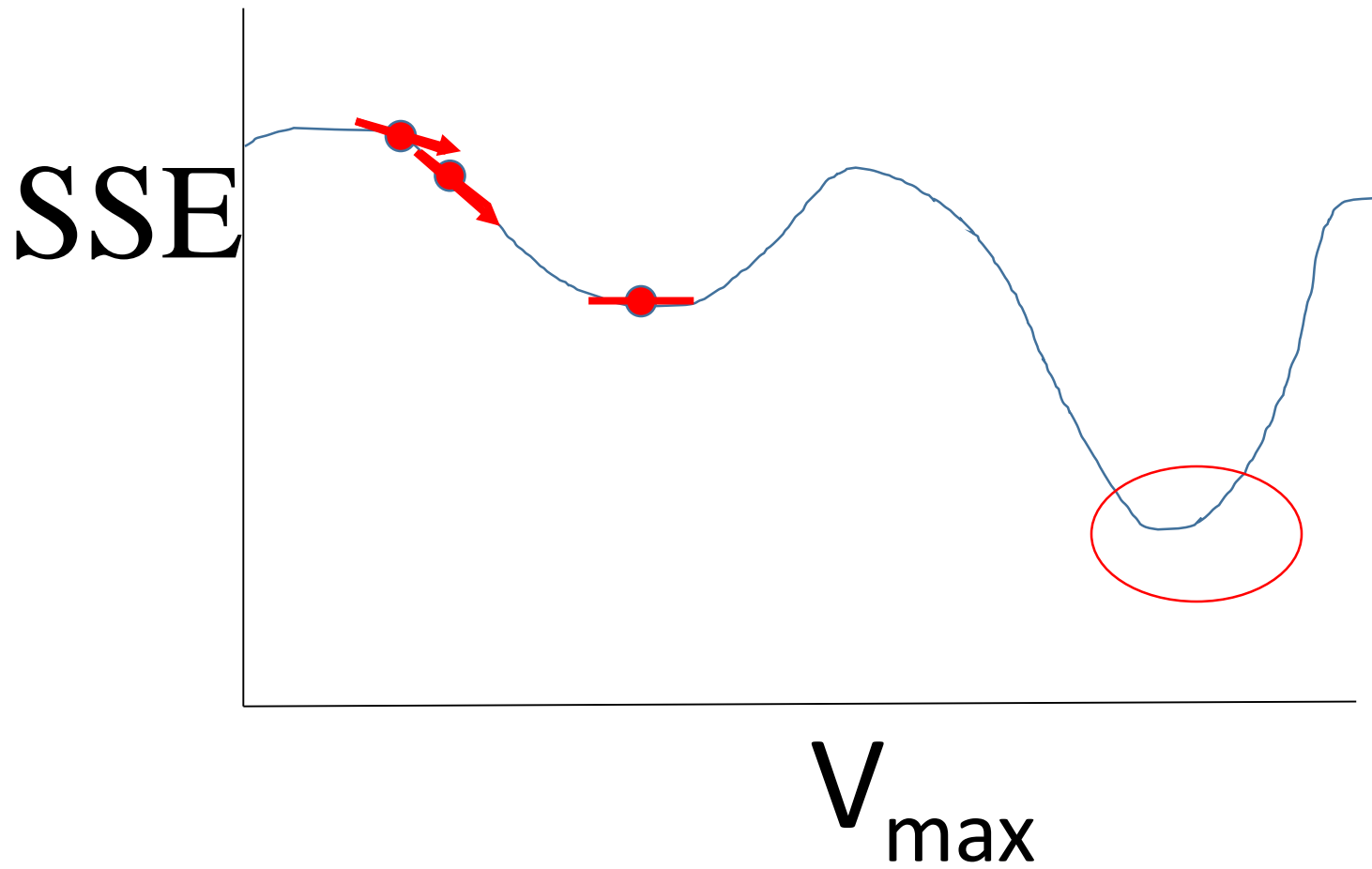
Similar to Linear Regression



GRG Non-Linear Solver in Excel

- Generalized Reduced Gradient Algorithm
- Good at finding local minimum, not global minimum
- Converts non-linear system to reduced linear system, follows the downward slope, then iterates

2-D Example



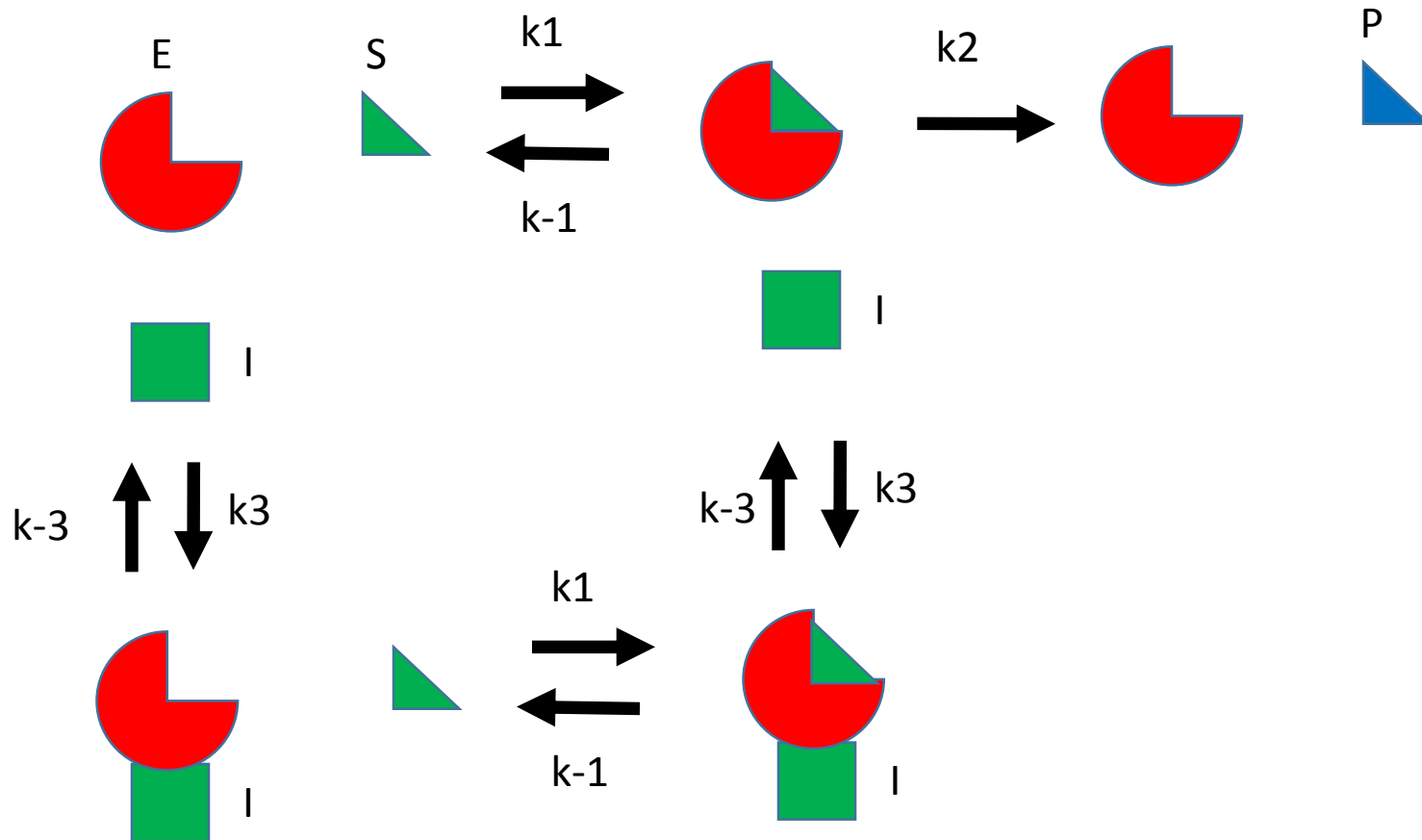
Classical Inhibition: Assumptions

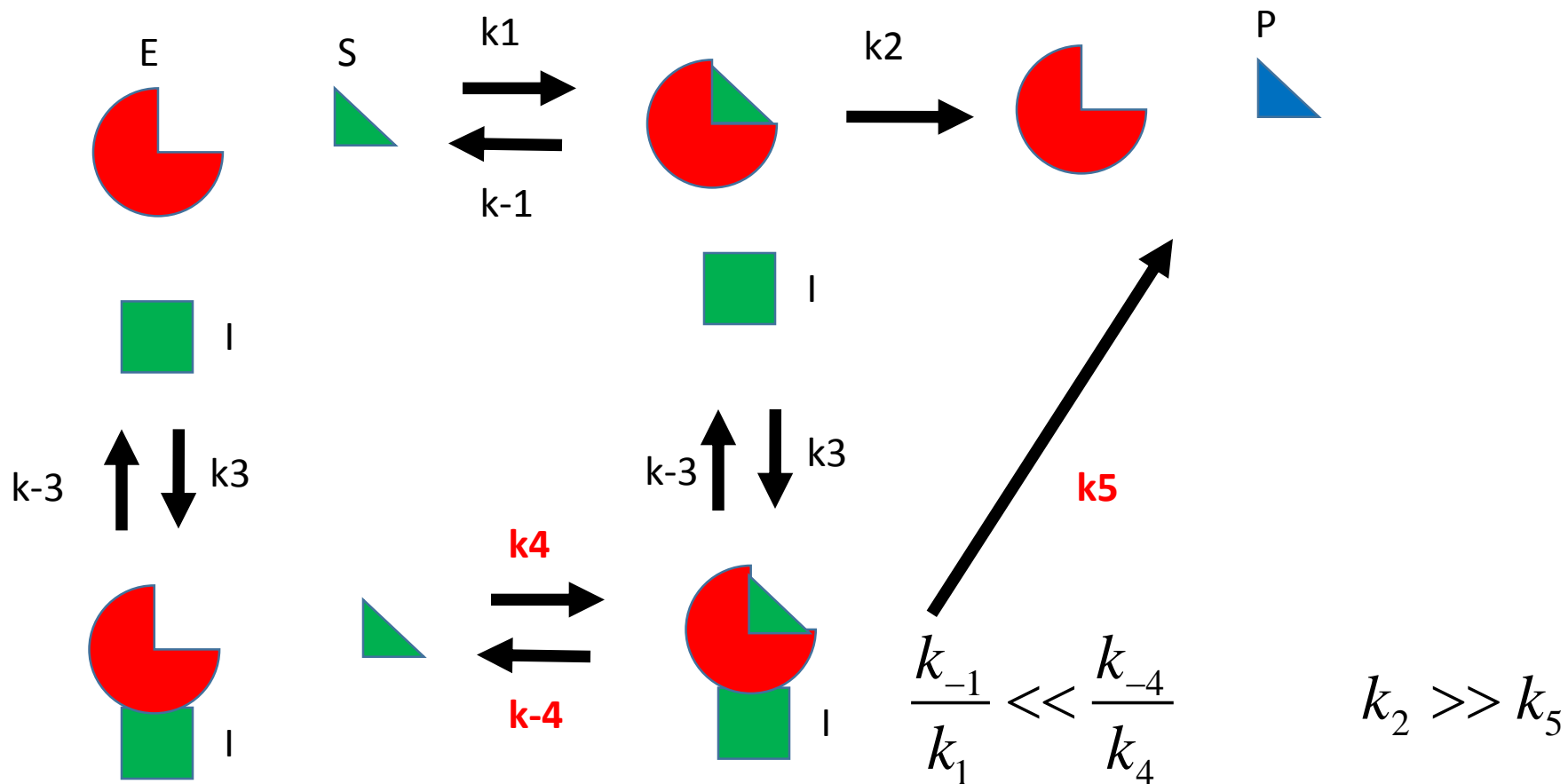
- Irreversible reaction
- Simplified effect of inhibitor
 - Competitive: blocks substrate binding
 - Non-competitive: blocks catalysis without affecting substrate binding

Where Assumptions Break Down

- Inhibitor affects binding kinetics/equilibrium without blocking binding or inhibiting catalysis
- Inhibitor affects catalysis kinetics without affecting binding of substrate
- Inhibitor affects both substrate binding and catalytic rate

Classic Inhibition: Non-Competitive



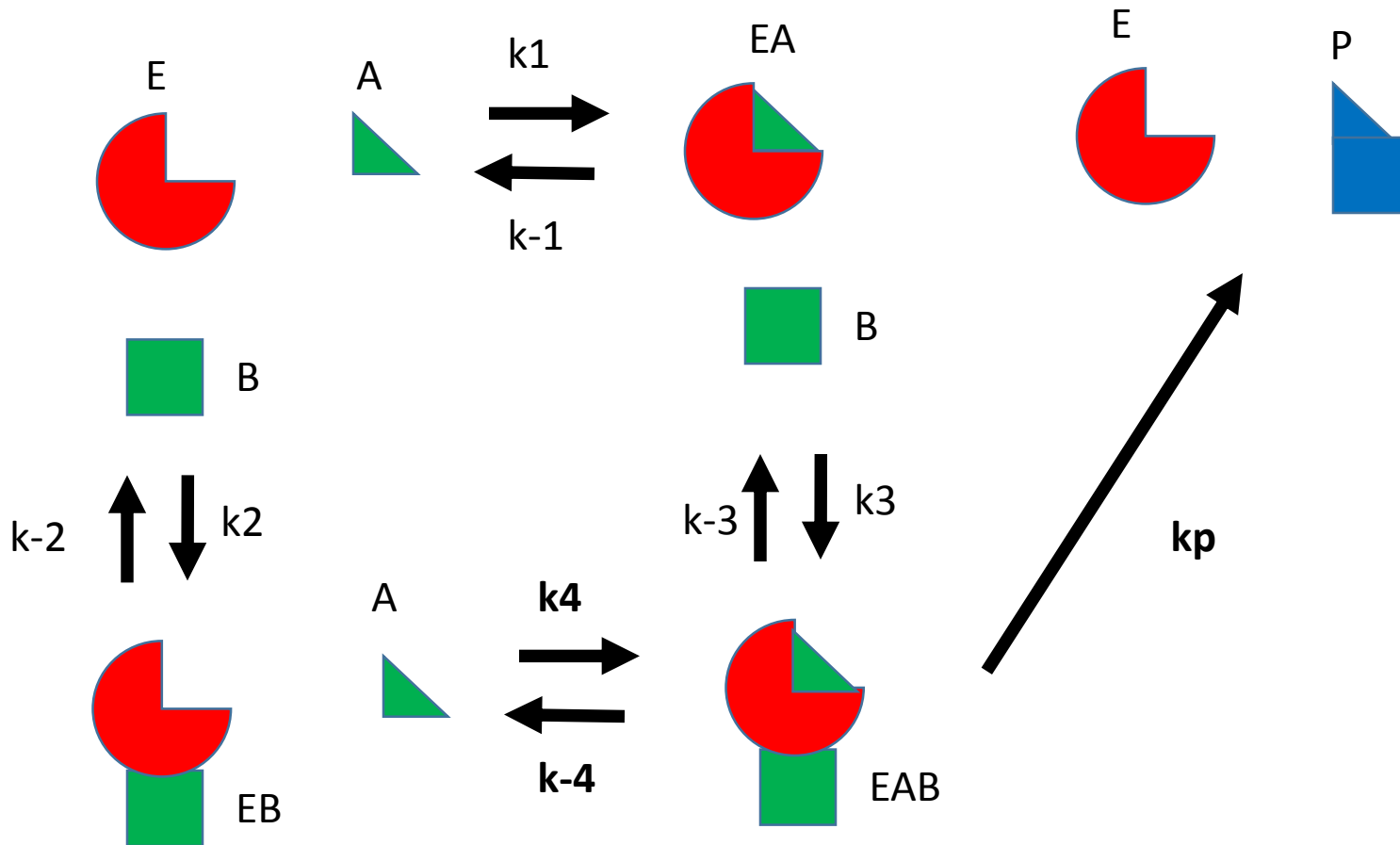


What About Multiple Substrates?



- Must both substrates be bound for reaction to occur?
- Does binding of A affect binding of B (and vice-versa)?
- Does reaction proceed via intermediate step?

Bi Mechanism

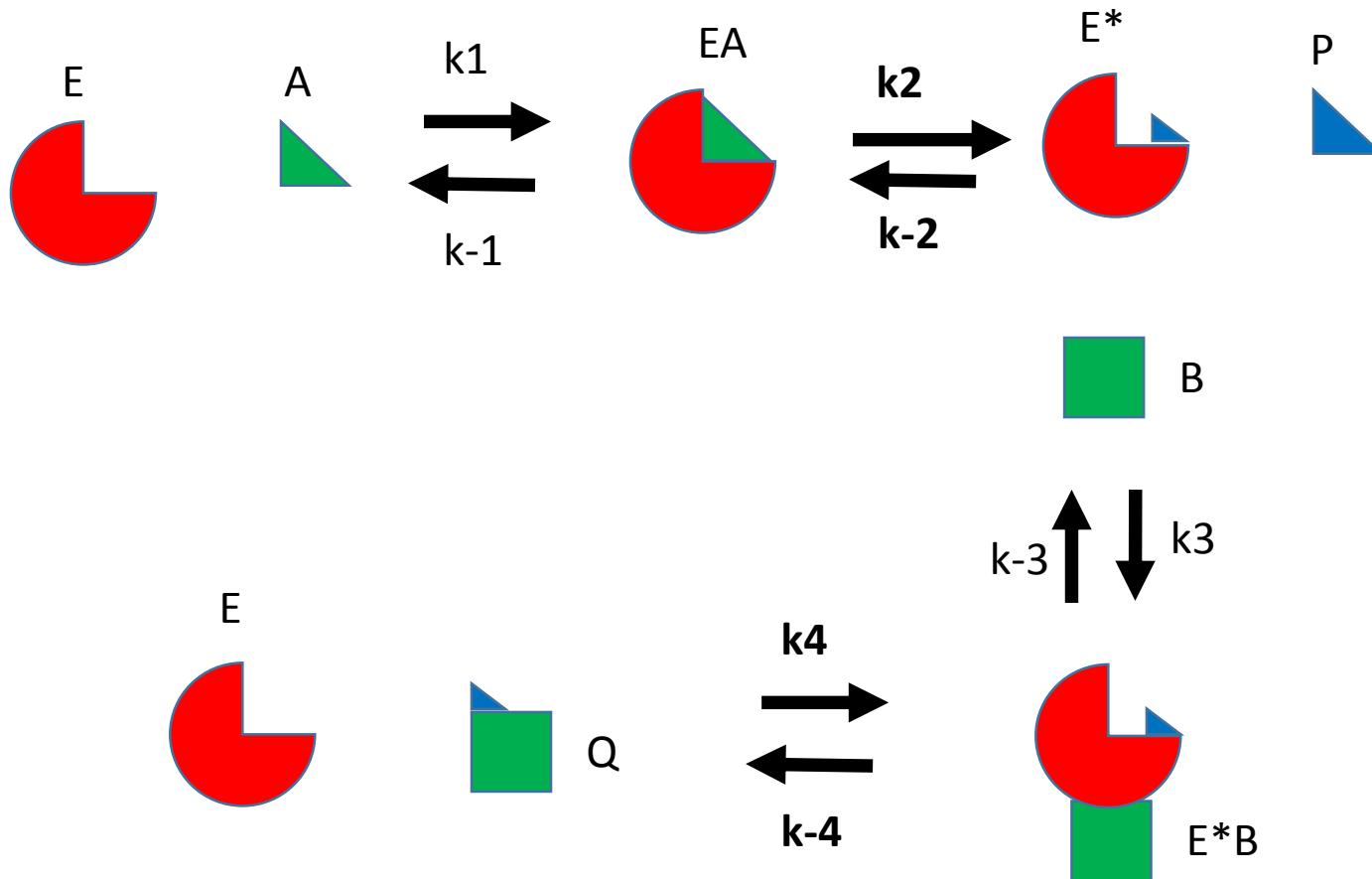


$$E_t = E + EA + EAB$$

A Question of Mechanism

- Bi
 - Two Substrates- One Product
- Bi-Bi
 - Two Substrates – Two Products
- Ping-Pong
 - Retaining mechanism

Ping-Pong Mechanism



$$E_t = E + EA + E^* + E^*B$$

King-Altman Method

- Method for deriving Michaelis-Menten-type rate expressions
- Application of Euler circuits

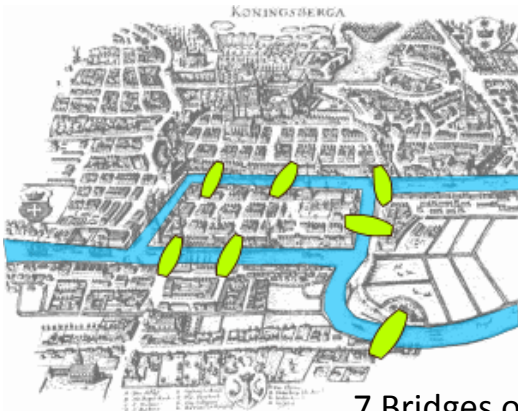


Leonhard Euler

Natural logarithm (e – Euler's number)

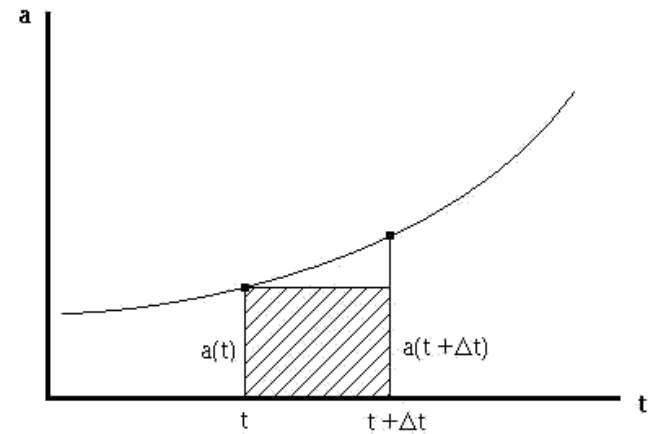
1707-1783

Graph Theory and Topology



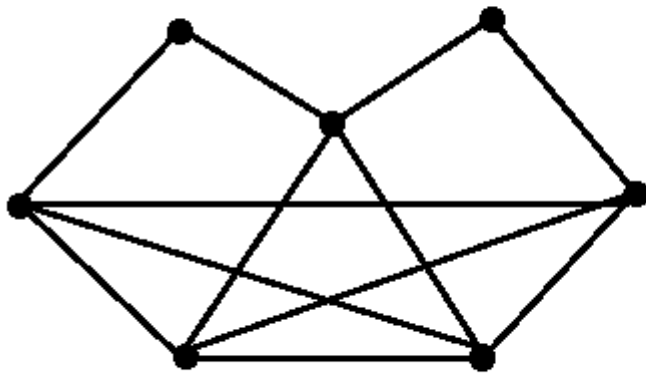
7 Bridges of Königsberg

Euler's method of integration

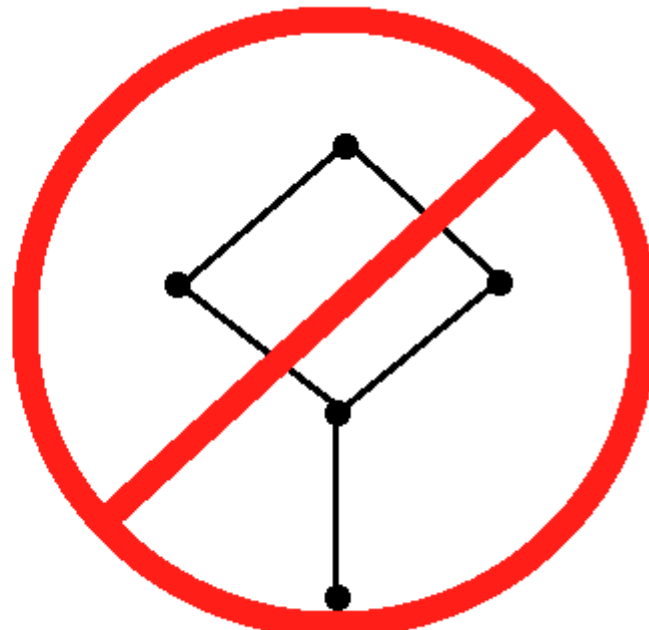


Euler Graph (Map): A graph with a closed trail (a walk with no repeated edges) that contains all edges of the graph.

Euler Circuit (Trail): A closed walk with no repeated edges that starts and stops at the same vertex.

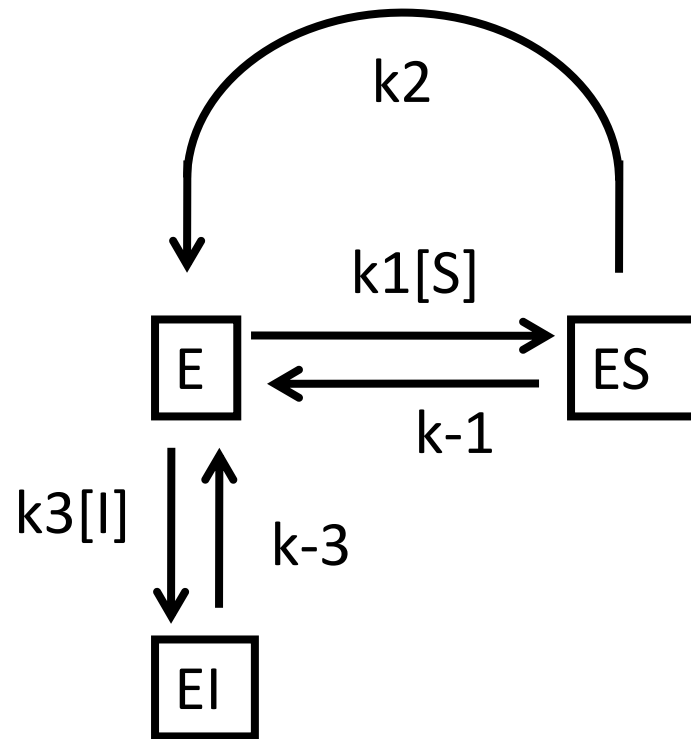


Eulerian



Not Eulerian

Application to Enzyme Kinetics: Competitive Inhibition

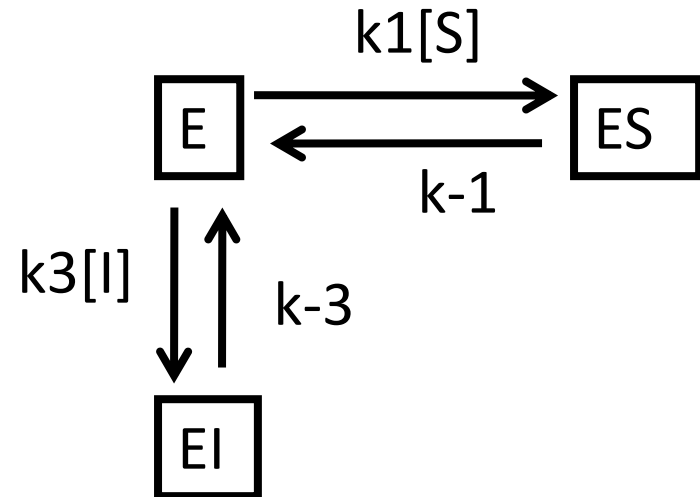
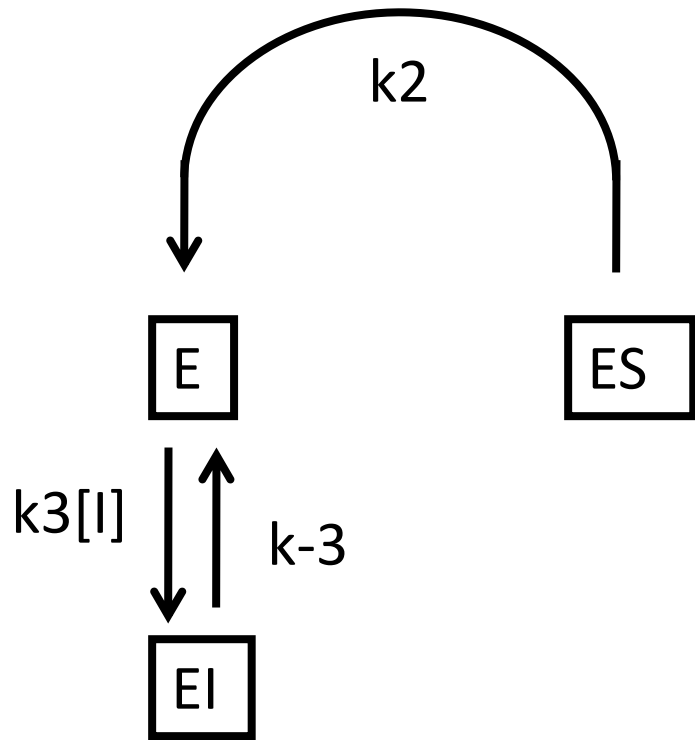


Rules:

Vertices = forms of enzyme

Edges/paths = valid enzyme reactions/interactions

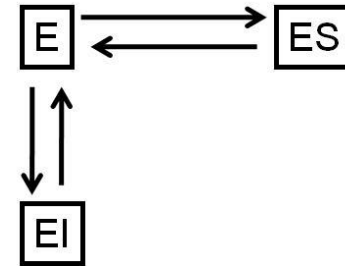
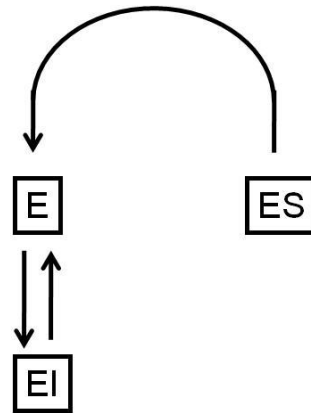
“Circuits”



Rule:

A continuous walk connecting all vertices,
not necessarily a closed Euler circuit

Kinetic Expressions



E	$k_2 k_{-3}$	$k_{-1} k_{-3}$
ES	$k_{-3} 0 = 0$	$k_{-3} k_1 [S]$
EI	$k_2 k_3 [I]$	$k_{-1} k_3 [I]$

Rules:

Matrix filled by products of edges (paths) for the given circuit (column) to a given vertex/destination (row).

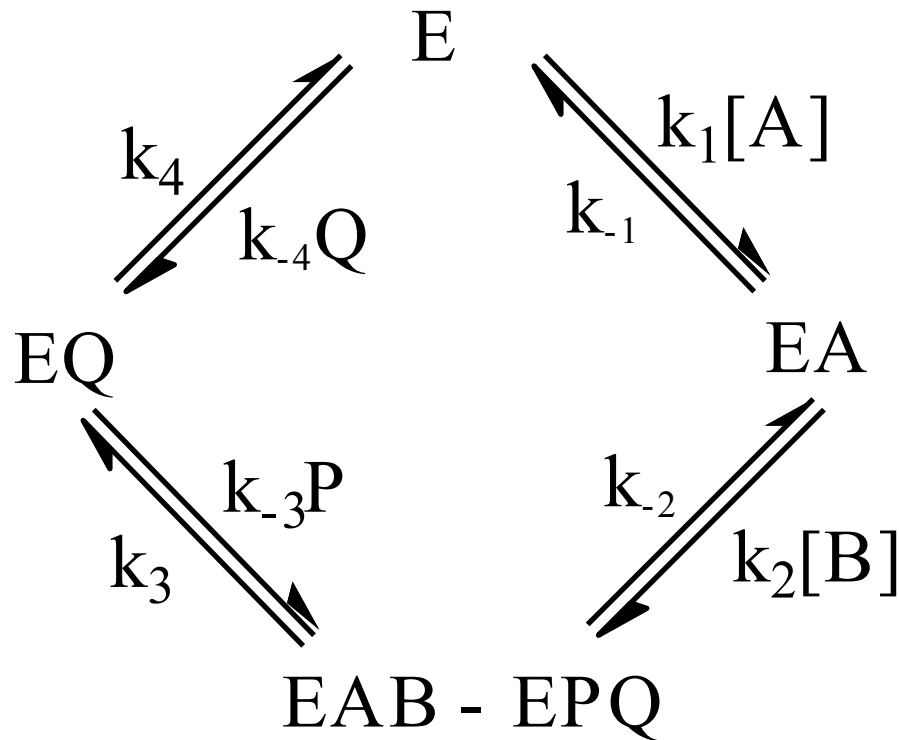
Why Does This Work?

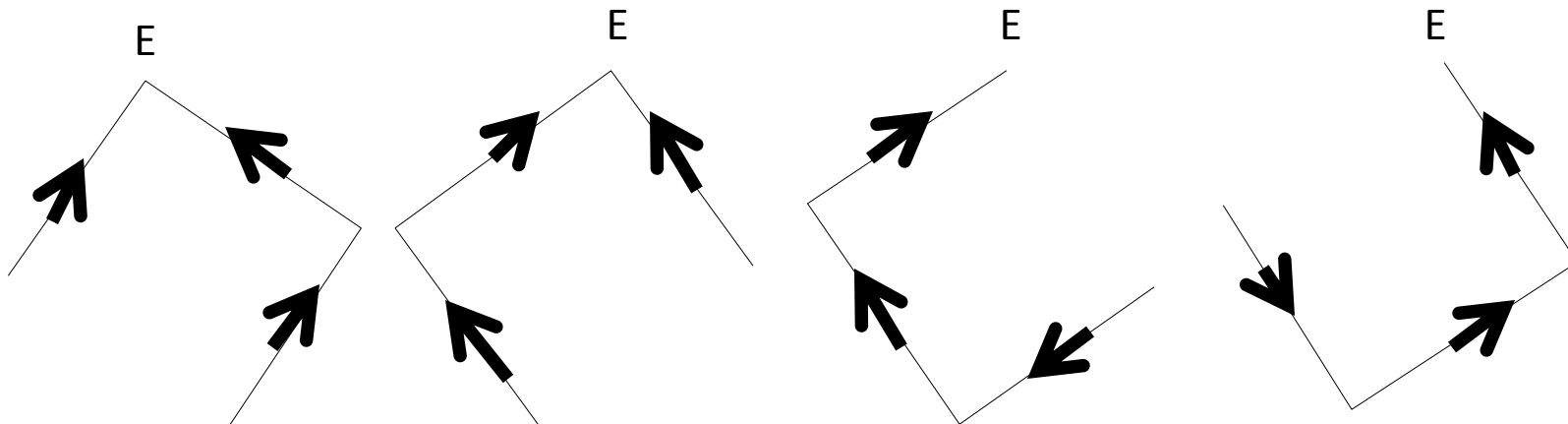
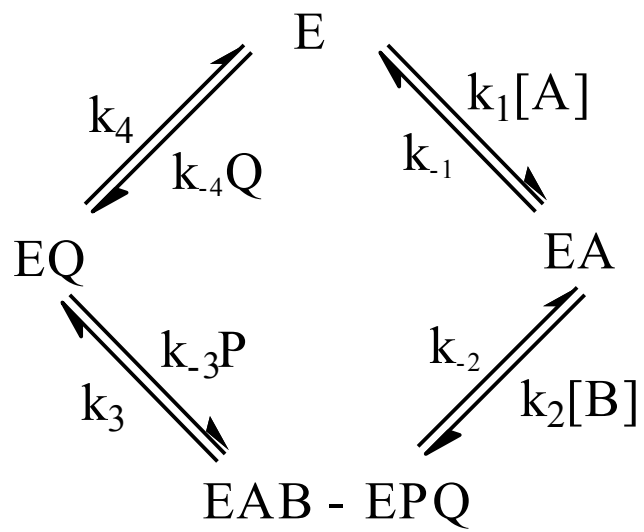
This is an application of Cramer's Rule for solving systems of linear equations

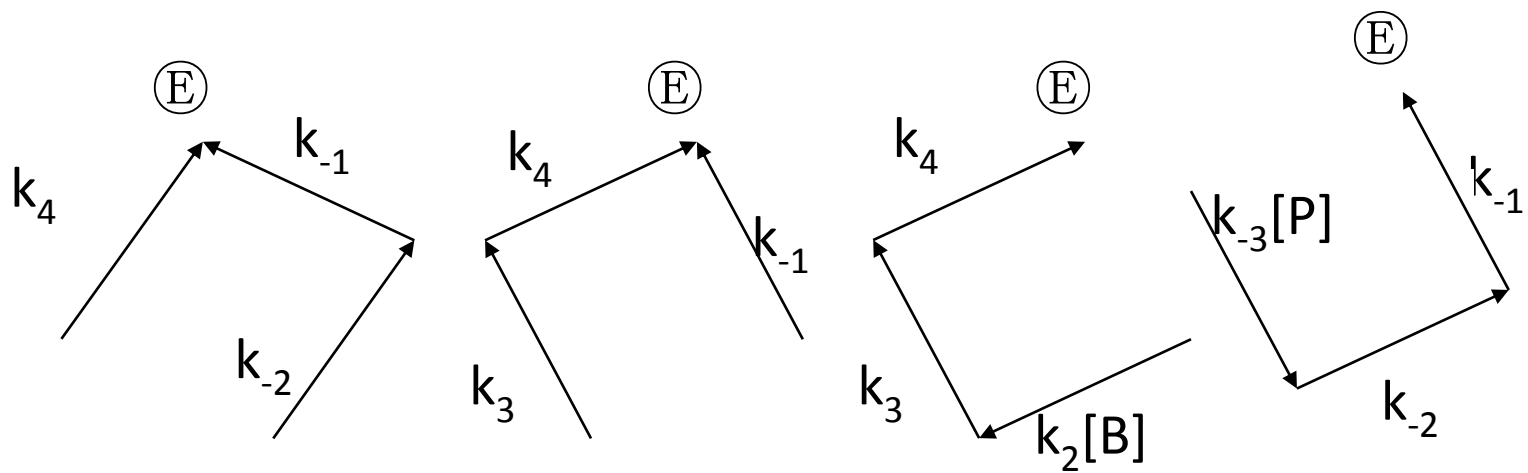
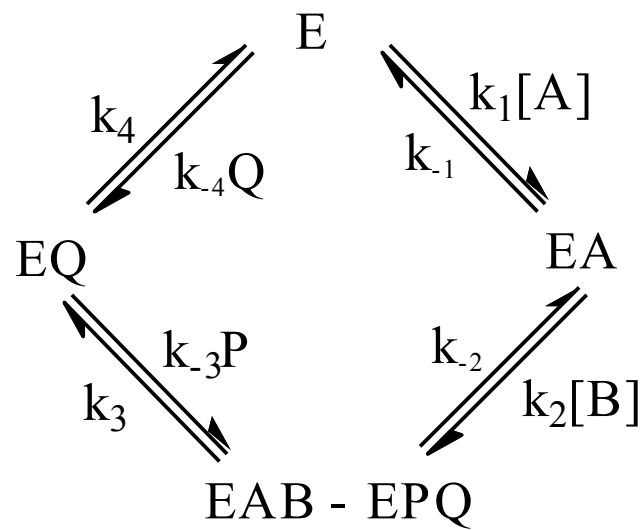
Ordered, Sequential Bi-Bi Reaction (Reversible)

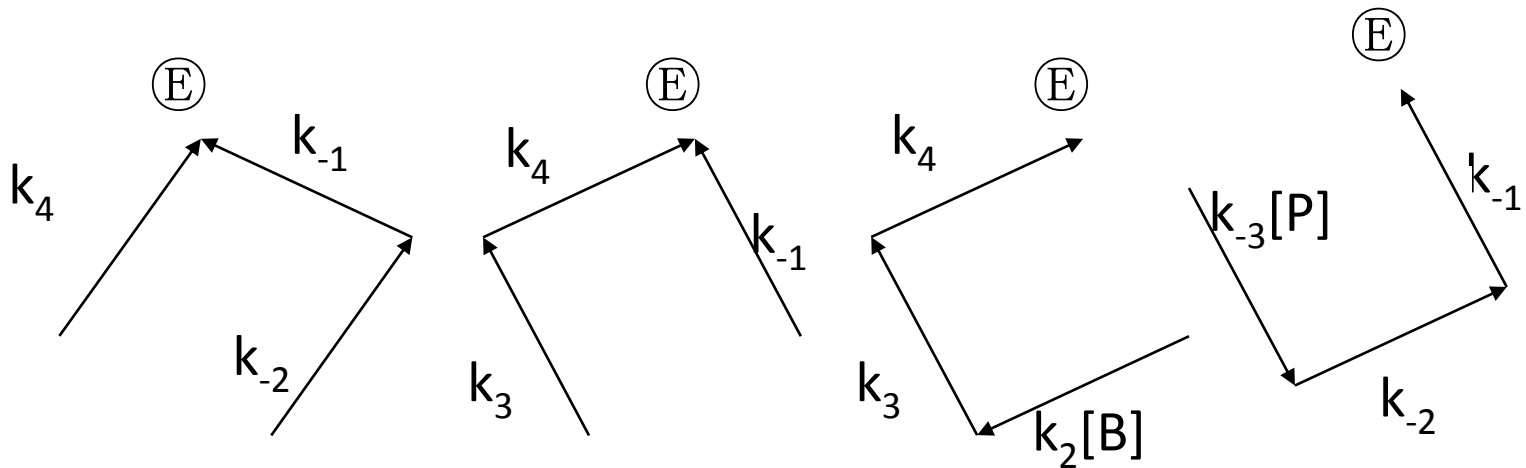


Ordered, Sequential Bi-Bi Reaction (Reversible)

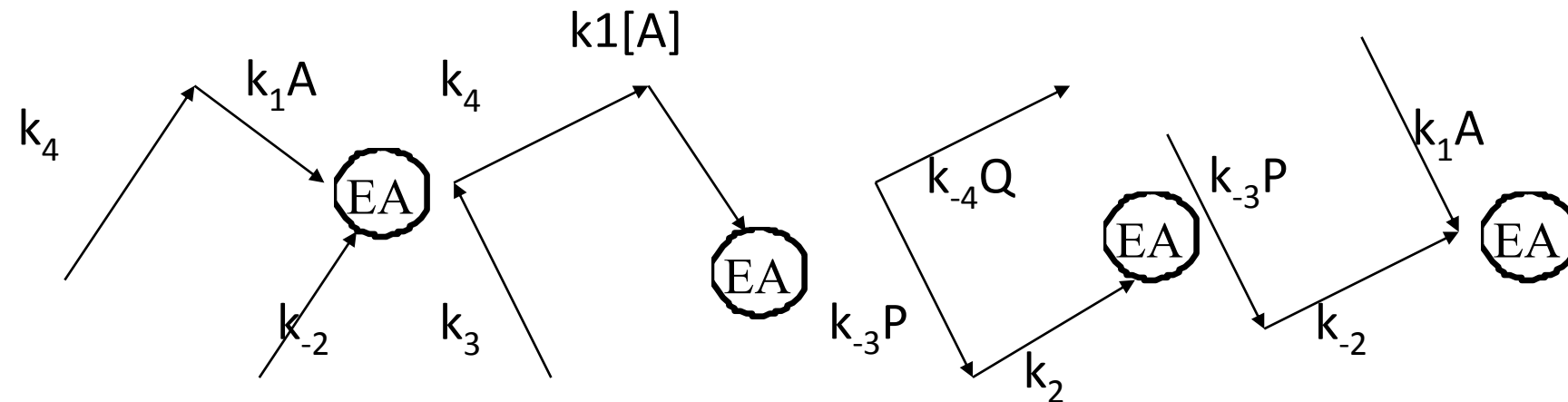








$$\frac{E}{E_T} = \frac{k_4 k_{-2} k_{-1} + k_{-1} k_3 k_4 + k_2 k_3 k_4 [B] + k_{-1} k_{-2} k_{-3} [P]}{E + EA + [EAB - EPQ] + EQ}$$



$$\frac{EA}{E_T} = \frac{k_1 k_{-2} k_4 [A] + k_1 k_3 k_4 [A] + k_{-2} k_{-3} k_{-4} [PQ] + k_1 k_{-2} k_{-3} [A][P]}{E + EA + [EAB - EPQ] + EQ}$$

$$\begin{aligned}
v = & \frac{V_{\max,f} V_{\max,r} [A][B] - \frac{V_{\max,f} V_{\max,r}}{K_{eq}} [P][Q]}{K_{i,A} K_B V_{\max,r} + K_B V_{\max,r} [A] + K_A V_{\max,r} [B] + V_{\max,r} [A][B]} \\
& + \frac{K_Q V_{\max,f}}{K_{eq}} [P] + \frac{K_P V_{\max,f}}{K_{eq}} [Q] + \frac{V_{\max,f}}{K_{eq}} [P][Q] \\
& + \frac{K_Q V_{\max,f}}{K_{i,A} K_{eq}} [A][P] + \frac{K_A V_{\max,r}}{K_{i,Q}} [B][Q] \\
& + \frac{V_{\max,r}}{K_{i,P}} [A][B][P] + \frac{V_{\max,f}}{K_{i,P} K_{eq}} [B][P][Q]
\end{aligned}$$

$$V_{\max,f} = k_3 k_4 / (k_3 + k_4) E_t$$

$$V_{\max,r} = k_{-1} k_{-2} / (k_{-1} + k_{-2}) E_t$$

$$K_{i,A} = k_{-1} / k_1$$

$$K_{i,B} = (k_{-1} + k_{-2}) / k_3$$

$$K_{i,P} = (k_3 + k_4) / k_{-3}$$

$$K_{i,Q} = k_4 / k_{-4}$$

$$K_A = k_3 k_4 / (k_1 (k_3 + k_4))$$

$$K_B = k_4 (k_{-1} + k_3) / (k_2 (k_3 + k_4))$$

$$K_P = k_{-1} (k_{-2} + k_3) / (k_{-3} (k_{-1} + k_{-2}))$$

$$K_Q = k_{-1} k_{-2} / (k_{-4} (k_{-1} + k_{-2}))$$

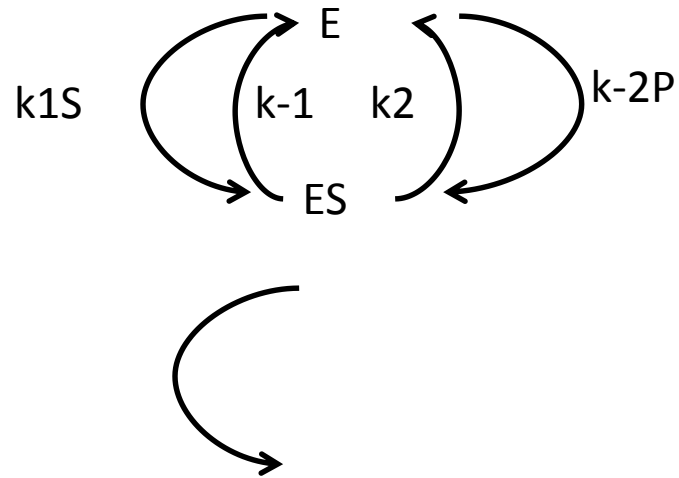
$$K_{eq} = k_1 k_2 k_3 k_4 / k_{-1} k_{-2} k_{-3} k_{-4}$$

Reversible Enzyme Expression



$$v = V_f - V_r$$

$$= k_2 ES - k_{-1} ES$$



E	K-1	K2
ES	k1S	K-2P

$$\begin{aligned}
 E_t &= E + ES \\
 &= (k_{-1} + k_2) + (k_1 S + k_{-2} P)
 \end{aligned}$$

$$v = k_2 ES - k_{-1} ES$$

$$\frac{v}{E_t} = \frac{k_2 ES - k_{-1} ES}{E_t}$$

$$= \frac{k_2 (k_1 S + k_{-2} P) - k_{-1} (k_1 S + k_{-2} P)}{(k_{-1} + k_2) + (k_1 S + k_{-2} P)}$$

$$v = \frac{dP}{dt} = \frac{\frac{V_{\max,f}}{K_{m,s}}[S] - \frac{V_{\max,r}}{K_{m,p}}[P]}{1 + \frac{[S]}{K_{m,s}} + \frac{[P]}{K_{m,p}}}$$

$$K_{m,S} = \frac{k_{-1} + k_2}{k_1}$$

$$K_{m,P} = \frac{k_{-1} + k_2}{k_{-2}}$$

$$V_{\max,f} = k_2 E_t$$

$$V_{\max,r} = k_{-1} E_t$$