Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 14:

k_{cat}, K_m, K_d and Energy Profiles for Enzymatic Reactions

23 February 2023

©David P. Goldenberg
University of Utah
goldenberg@biology.utah.edu

A Closer Look at Binding and K_m: $K_m$versus K_d

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{E{\cdot}S} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P}$$

- K_m is defined in terms of the rate constants:

$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

- K_d is the equilibrium constant for dissociation.

$$K_d = \frac{[\mathrm{E}]_{eq}[\mathrm{S}]_{eq}}{[\mathrm{E} \cdot \mathrm{S}]_{eq}} = \frac{k_{-1}}{k_1}$$

A large K_d indicates weak binding.

K_m versus K_d

$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

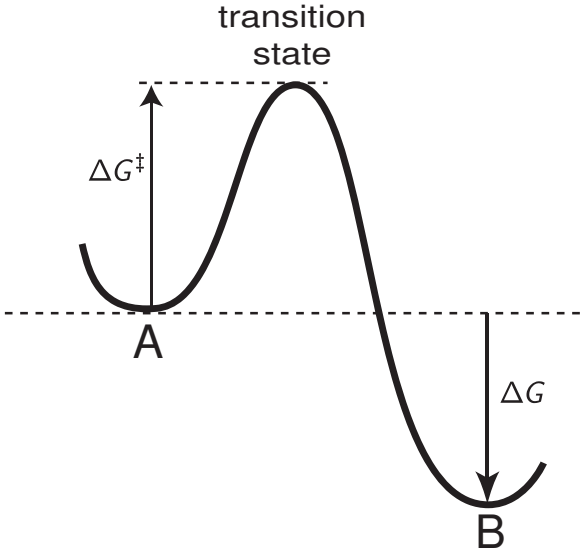
$$K_d = \frac{[E]_{eq}[S]_{eq}}{[E \cdot S]_{eq}} = \frac{k_{-1}}{k_1}$$

- If $k_{cat} \ll k_{-1}$, the E·S complex is more likely to dissociate than undergo catalysis:

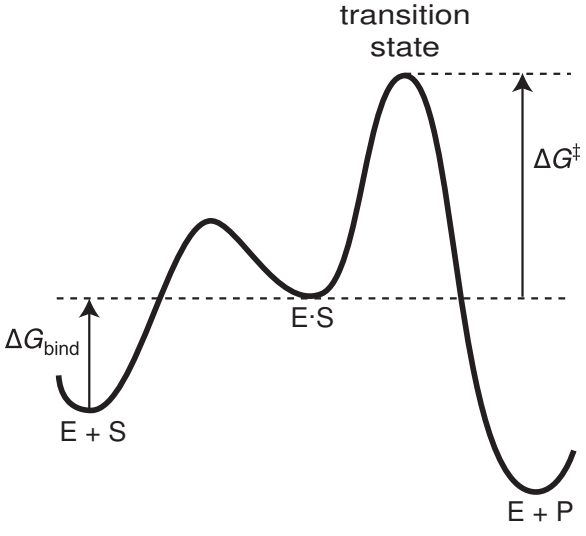
$$K_m \approx \frac{k_{-1}}{k_1} = K_d$$

- In general, $K_m \geq K_d$
- Strength of equilibrium binding may be greater than indicated by K_m.

Energy Profile for a Simple Chemical Reaction

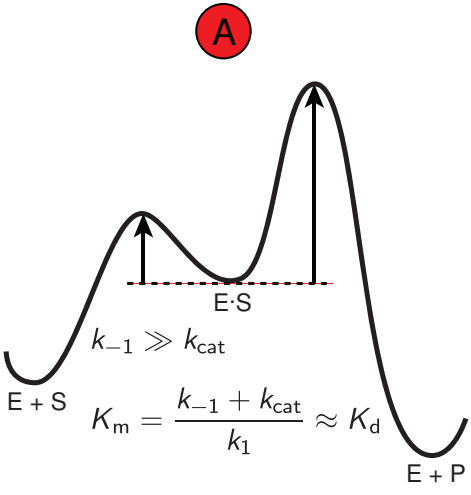


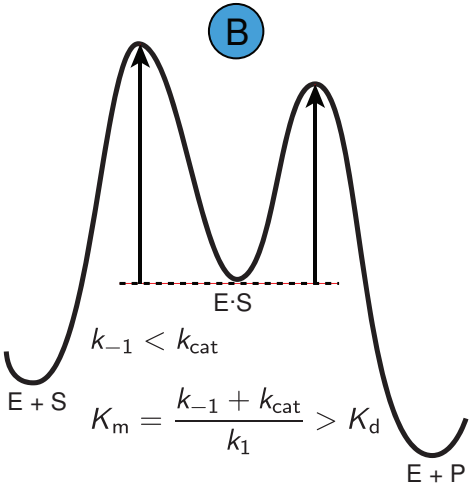
Energy Profile for an Enzyme-Catalyzed Reaction



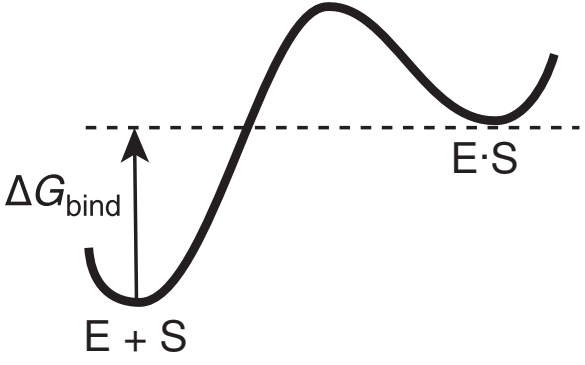
Clicker Question #1

For which enzyme-substrate pair is $K_m \approx K_d$?





How do we define ΔG_{bind}?



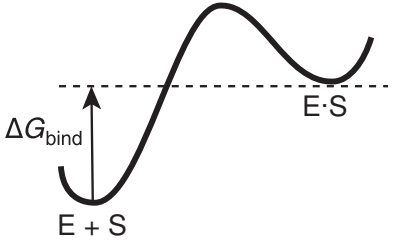
The Standard Free Energy Change and the Equilibrium Constant

$$\Delta G^\circ = -RT \ln K_{eq} \qquad A \rightleftharpoons B \qquad K_{eq} = \frac{[B]_{eq}}{[A]_{eq}}$$

- If $K_{eq} > 1$:
  - Reaction favors B over A.
  - $\Delta G^\circ < 0$
- If $K_{eq} < 1$:
  - Reaction favors A over B.
  - $\Delta G^\circ > 0$
- If $K_{eq} = 1$:
  - A and B have equal free energies.
  - $\Delta G^\circ = 0$

- ΔG° refers to reactant and product concentrations of 1 M.
- The free-energy change, ΔG, depends on the concentrations of products and reactants.
- Things get trickier when there are more than one reactant or product!

One Way to Define ΔG_{bind}: Focus on E and E · S

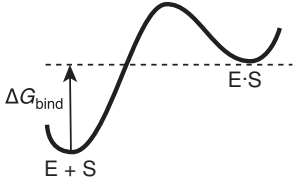


$$\Delta G_{\text{bind}} = -RT \ln \frac{[\text{E} \cdot \text{S}]}{[\text{E}]}$$

$$= -RT \ln \left(\frac{[\text{E} \cdot \text{S}]}{[\text{E}][\text{S}]} [\text{S}] \right)$$

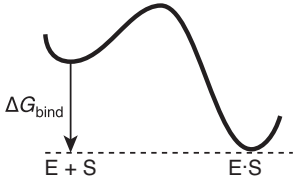
$$= -RT \ln \frac{[\text{S}]}{K_{\text{d}}}$$

ΔG_{bind} and Substrate Concentration



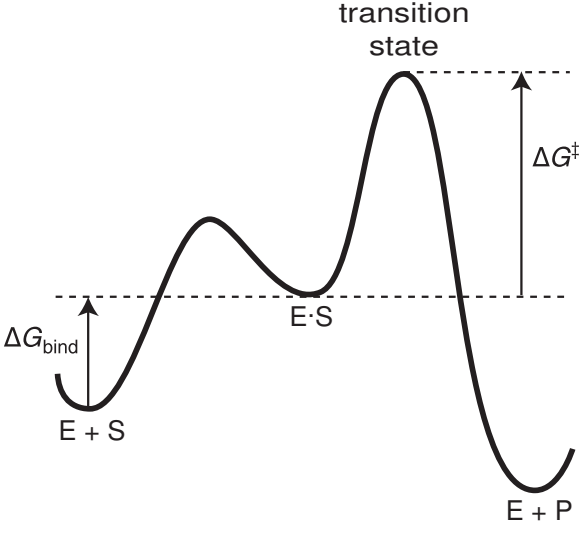
$$[\mathrm{S}] < K_\mathrm{d}$$
$$\Delta G_{\text{bind}} = -RT \ln \frac{[\mathrm{S}]}{K_\mathrm{d}} > 0$$

$$[\mathrm{S}] = K_\mathrm{d}$$
$$\Delta G_{\text{bind}} = -RT \ln \frac{[\mathrm{S}]}{K_\mathrm{d}} = 0$$



$$[\mathrm{S}] > K_\mathrm{d}$$
$$\Delta G_{\text{bind}} = -RT \ln \frac{[\mathrm{S}]}{K_\mathrm{d}} < 0$$

The Transition-state Free-energy Change

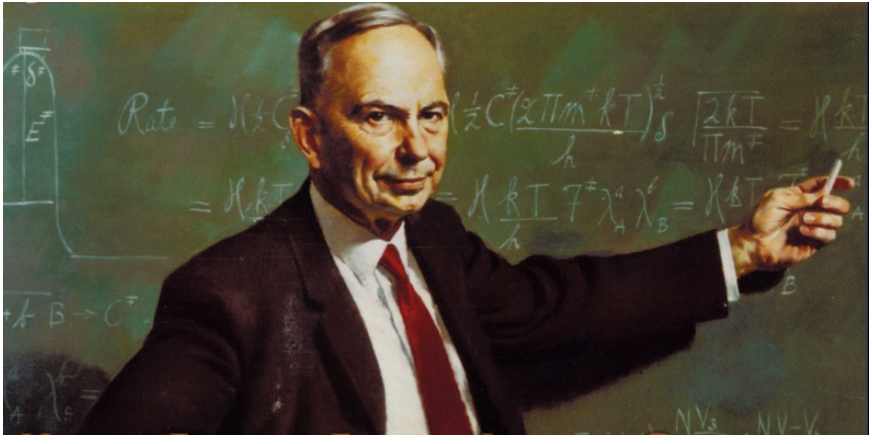


- Free-energy change from E·S complex to transition state:

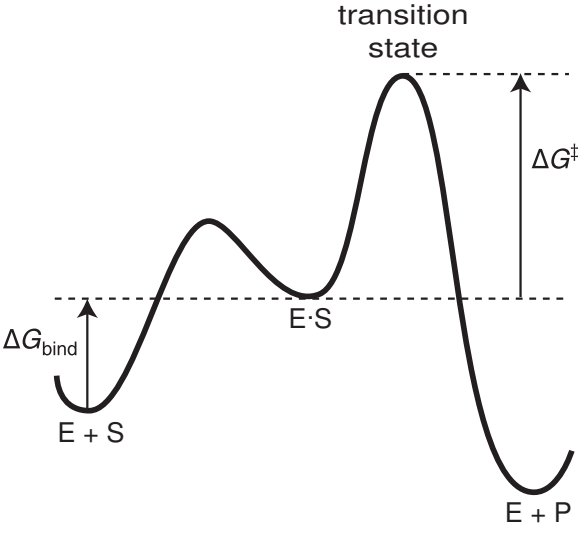
$$\Delta G^{\ddagger} = RT \ln \left(\frac{k_{\text{b}} T}{k_{\text{cat}} h} \right)$$

T = Temperature
R = Gas constant
k_{b} = Boltzmann constant
h = Planck constant

Henry B. Eyring: Founder of Transition-state Theory



Energy Profile for an Enzyme-Catalyzed Reaction



- Free-energy change for binding:

$$\Delta G_{\text{bind}} = -RT \ln \frac{[\text{S}]}{K_{\text{d}}}$$

$$\Delta G^{\circ}_{\text{bind}} = -RT \ln \frac{1\,\text{M}}{K_{\text{d}}} = RT \ln K_{\text{d}}$$

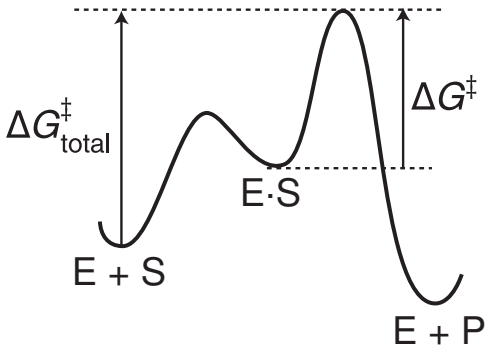
R = Gas constant
T = Temperature

- Free-energy change from E·S complex to transition state:

$$\Delta G^{\ddagger} = RT \ln \left(\frac{k_{\text{b}} T}{k_{\text{cat}} h} \right)$$

k_{b} = Boltzmann constant
h = Planck constant

The Significance of k_{cat}/K_m



- Free-energy difference between E + S and the transition state:

$$\Delta G^{\ddagger}_{total} = \Delta G^{\circ}_{bind} + \Delta G^{\ddagger}$$
$$= \underbrace{RT \ln\left(\frac{k_b T}{h}\right)}_{\text{Constant}} + RT \ln\left(\frac{K_d}{k_{cat}}\right)$$

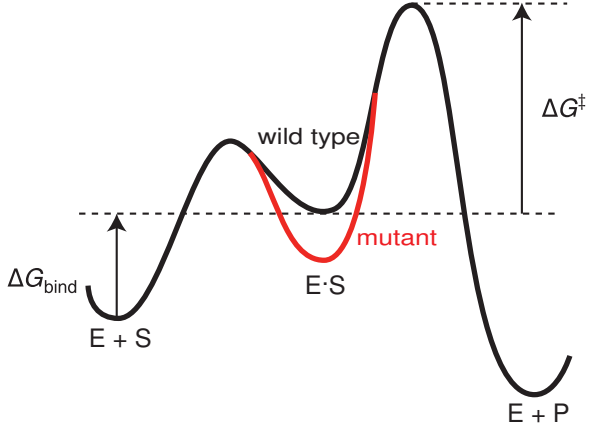
- If $k_{-1} \gg k_{cat}$, $K_d \approx K_m$:

$$\Delta G^{\ddagger}_{total} = C - RT \ln\left(\frac{k_{cat}}{K_m}\right)$$

- The ratio k_{cat}/K_m reflects the standard-state free-energy difference between E · S and the transition state. (Assuming $K_d \approx K_m$)
- k_{cat}/K_m is commonly interpreted as a measure of enzymatic efficiency.
- Catalytic efficiency is favored by a large value of k_{cat} and a small value of K_m.

Is a Low K_m Always Good?

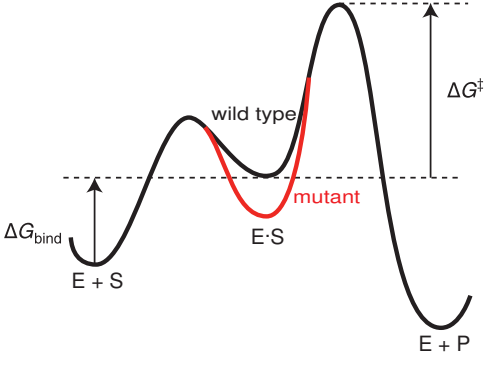
Suppose that we could design a mutant enzyme that forms a more stable complex with substrate.



This will lower K_m and k_{cat}, but leave k_{cat}/K_m the same.

Clicker Question #2

At **low** substrate concentration ([S] $\ll K_m$), will the velocity for the mutant enzyme be greater or less than that of the original enzyme?



A) Greater than the original enzyme

B) Less than the original enzyme

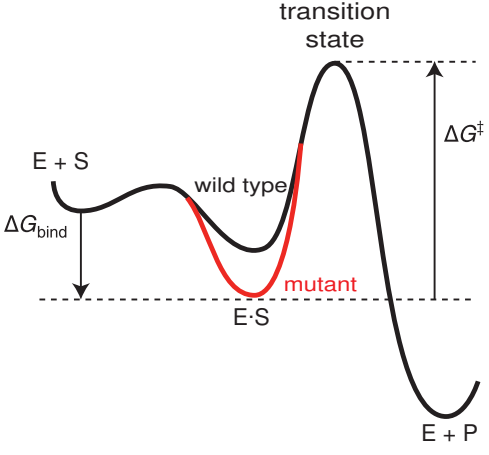
C) The same as the original enzyme!

$$V = \frac{[S][E]_T k_{cat}}{K_m + [S]}$$

$$V \approx \frac{k_{cat}}{K_m}[S][E]_T$$

Clicker Question #3

At **high** substrate concentration, will the velocity for the mutant enzyme be greater or less than that of the original enzyme?



A) Greater than the original enzyme

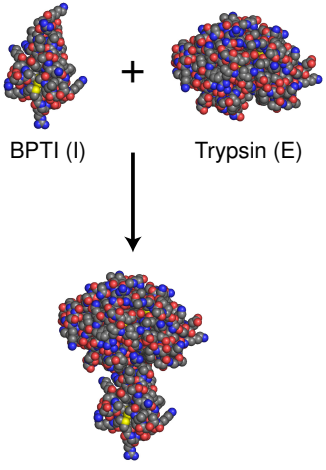
B) Less than the original enzyme!

C) The same as the original enzyme

$$V = \frac{[S][E]_T k_{cat}}{K_m + [S]}$$

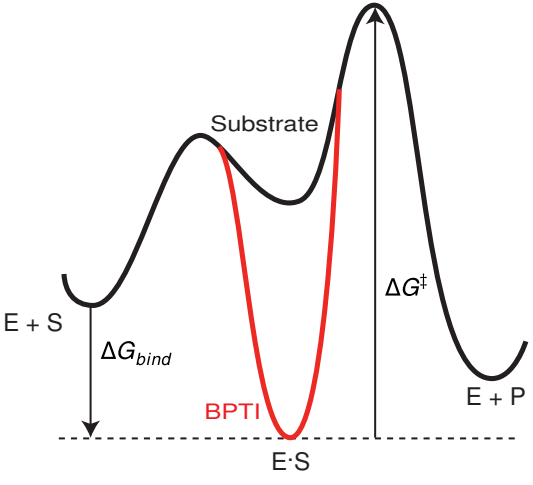
$$V \approx k_{cat}[E]_T = V_{max}$$

BPTI is an Extreme Example of a Low-K_m, Low-k_{cat} substrate

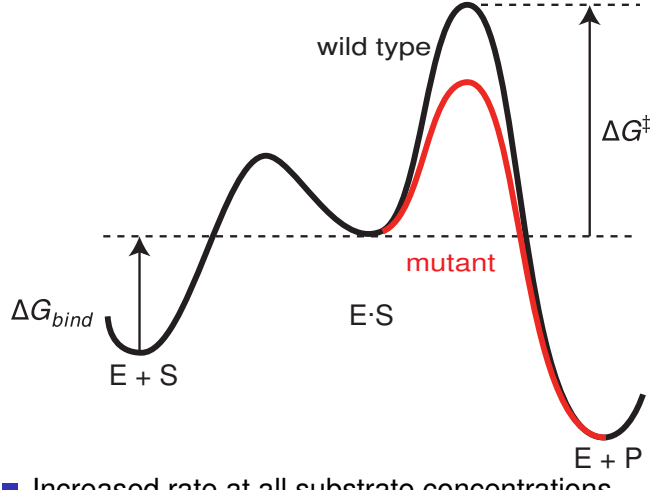


$K_d \approx 10^{-12}$ M

$k_{cat} \approx 0.03\,yr^{-1}$

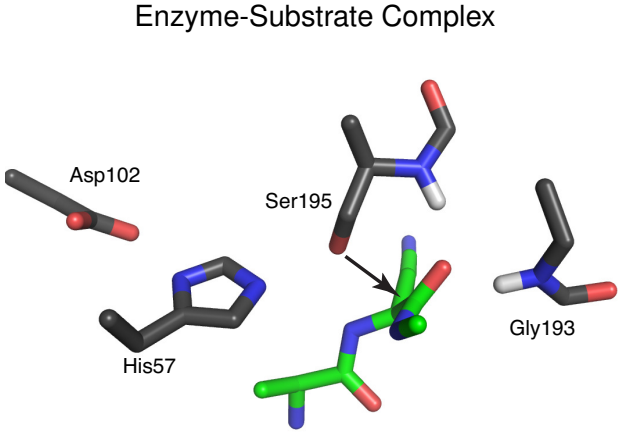


To Make a Better Enzyme (or Substrate), Stabilize the Transition State!



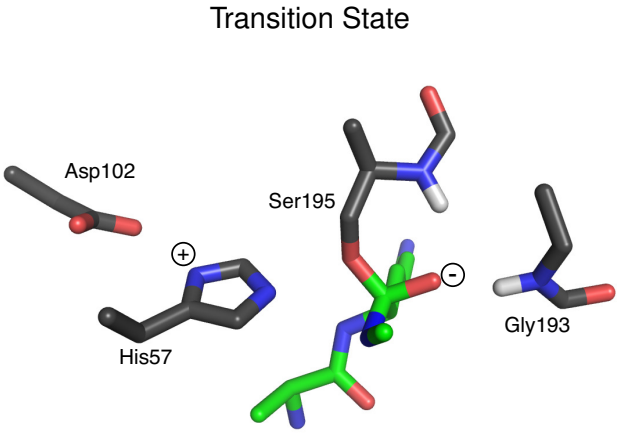
- Increased rate at all substrate concentrations.
- Easier said than done!

Transition State Stabilization in Serine Proteases



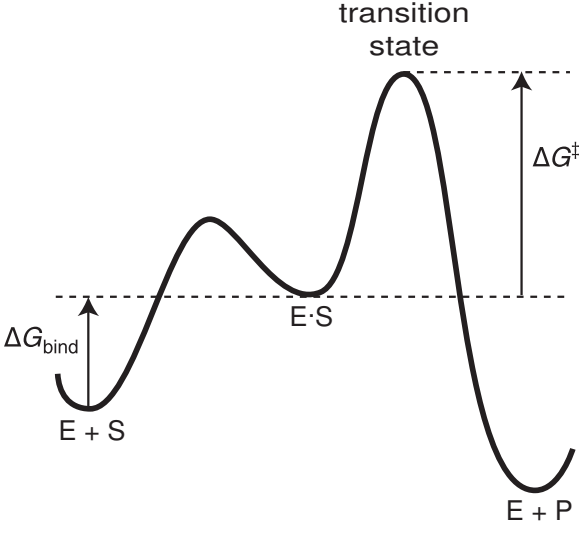
Substrate-enzyme model from structure of trypsin-BPTI complex (PDB entry 2FTL)

Transition State Stabilization in Serine Proteases



Transition state model from structure of trypsin with boronic inhibitor (PDB entry 1BZT)

What is Missing from this Energy Profile?



- A product-enzyme complex
- For the serine proteases: The acyl-enzyme intermediate.

A More Complete Energy Profile for Serine Proteases