Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 14:

A Bit More on Energy Profiles for Enzymatic Reactions and Introduction to Enzyme Inhibitors

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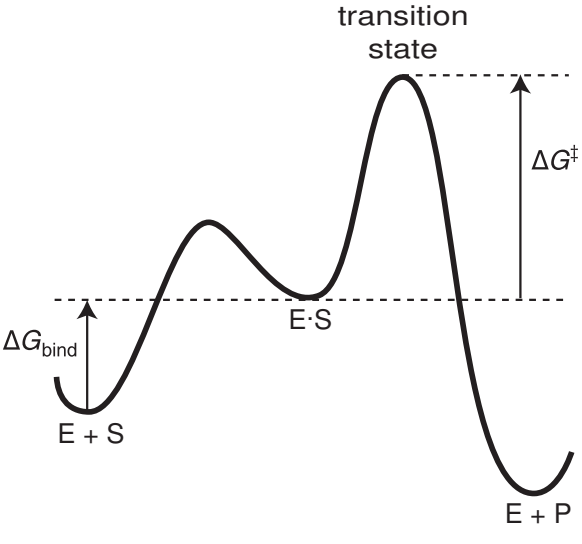
Energy Profile for an Enzyme-Catalyzed Reaction



- Free-energy change for binding:

$$\Delta G_{\text{bind}} = RT \ln K_{\text{d}}$$

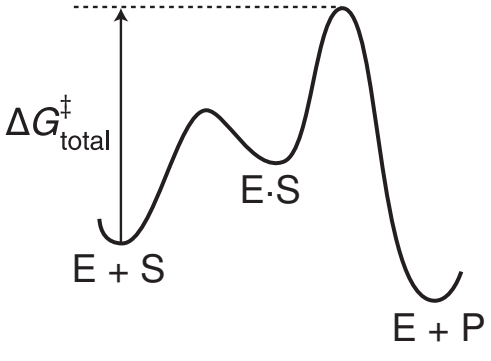
R = Gas constant
T = Temperature

- 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:

$$\Delta G^{\ddagger}_{total} = \Delta G_{bind} + \Delta G^{\ddagger}$$

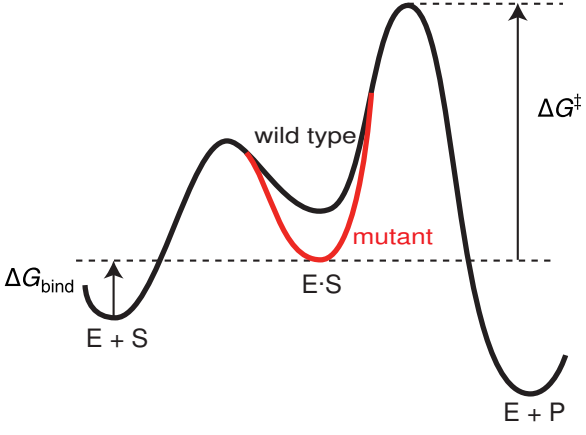
- If $k_{-1} \gg k_{cat}$, $K_d \approx K_m$:

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

- The ratio k_{cat}/K_m reflects the 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?

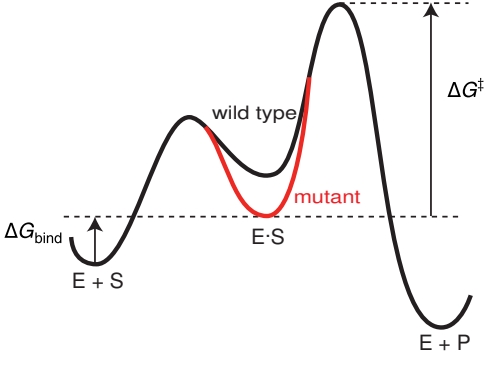
Suppose that we could design a mutant enzyme that forms a more stable complex with substrate.



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

Clicker Question #1

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?



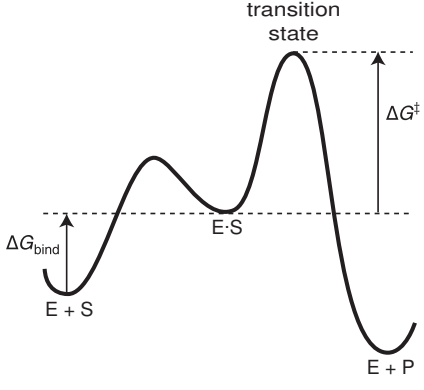
1. Greater than the original enzyme
2. Less than the original enzyme
3. The same as the original enzyme!

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

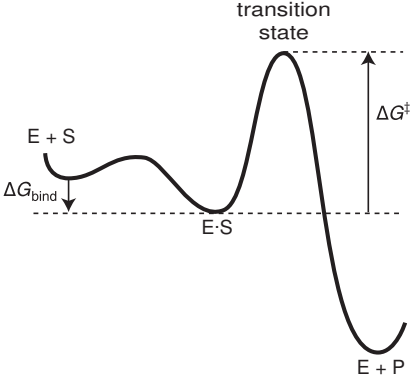
$$V \approx \frac{k_{cat}}{K_m}[S][E]_T$$

What About High Substrate Concentrations?

Low Substrate Concentration



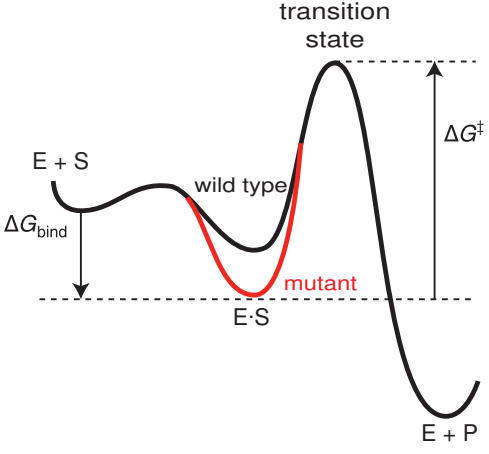
High Substrate Concentration



- When [S] ≫ K_m, the enzyme-substrate complex is favored with respect to the free enzyme.

Clicker Question #2

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

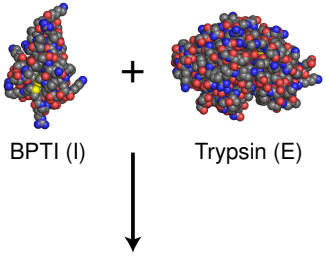


1. Greater than the original enzyme
2. Less than the original enzyme!
3. The same as the original enzyme

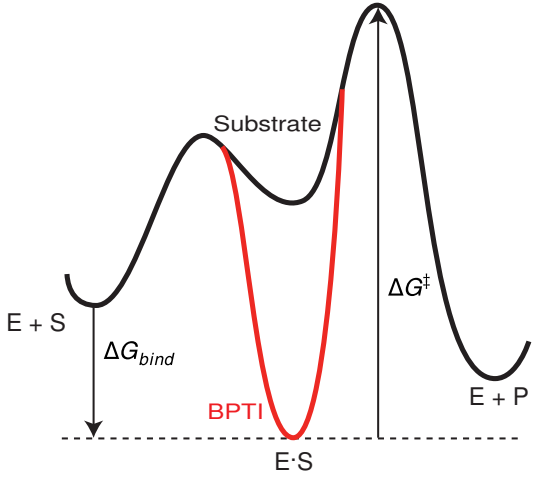
$$V = \frac{[S][E]_T k_{cat}}{K_m + [S]}$$

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

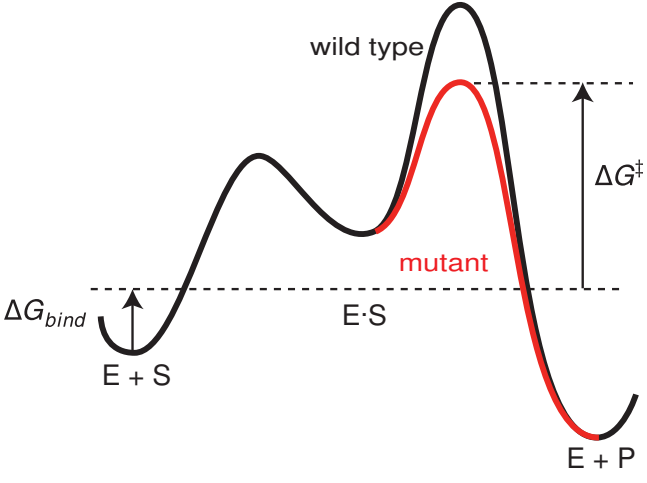
BPTI is an Extreme Example of a Low-K_m substrate



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



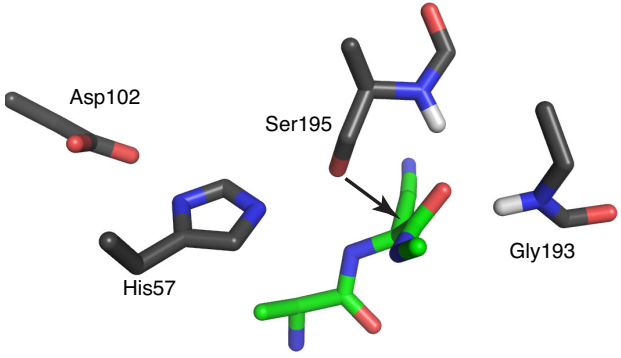
To Make a Better Enzyme, Stabilize the Transition State!



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

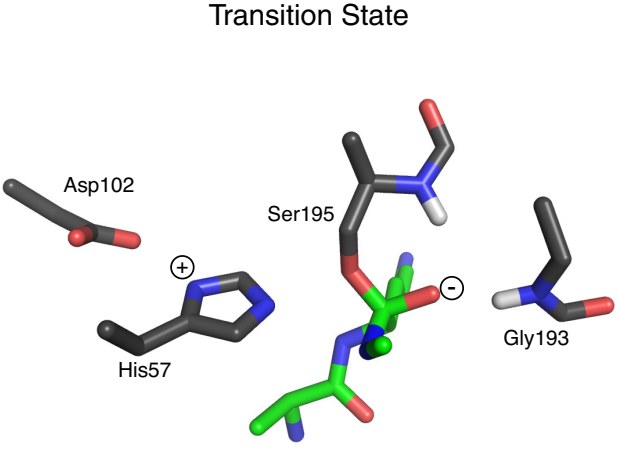
Transition State Stabilization in Serine Proteases

Enzyme-Substrate Complex



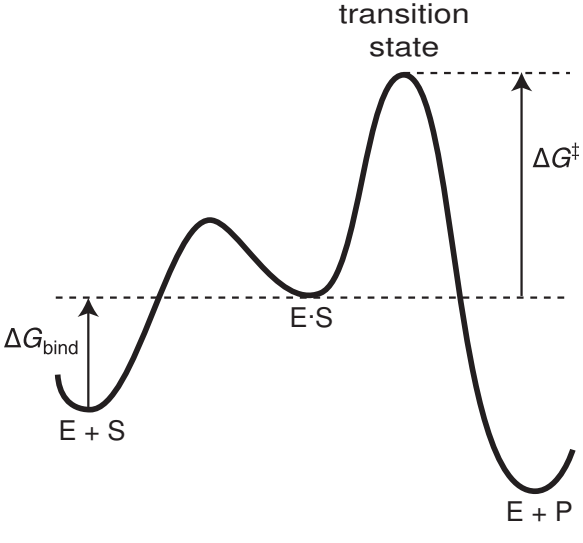
Substrate-enzyme model from structure of trypsin-BPTI complex (PDB entry 2FTL)

Transition State Stabilization in Serine Proteases



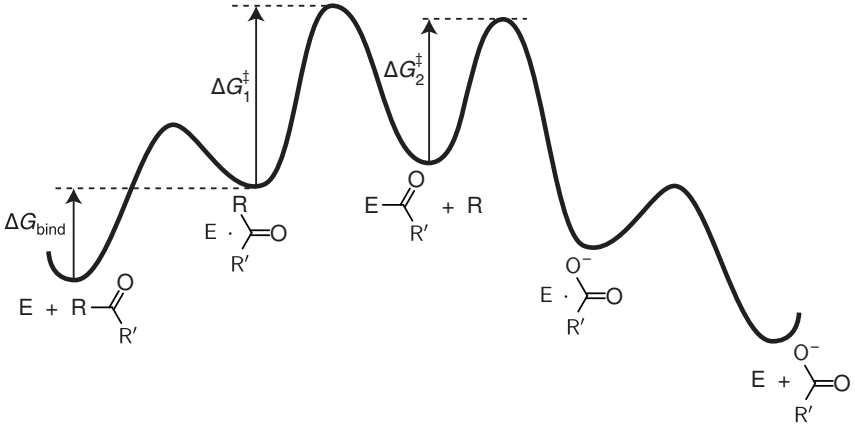
Transition state model from structure of trypsin with boronic inhibitor (PDB entry 1BZT)

What is Missing from this Energy Profile?



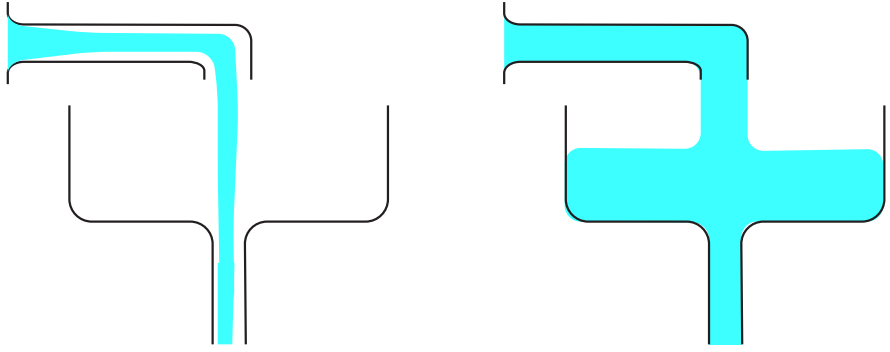
- A product-enzyme complex
- For the serine proteases: The acyl-enzyme intermediate.

A More Complete Energy Profile for Serine Proteases



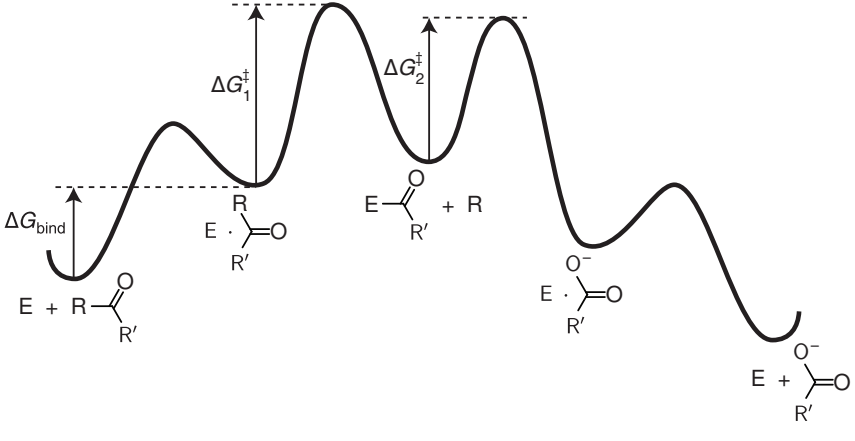
- Why do we use the much simpler model?

Enzymes as Drain Pipes



- If we only measure the flow out of the drain pipe, at steady-state, we never see how many twists and turns it has!

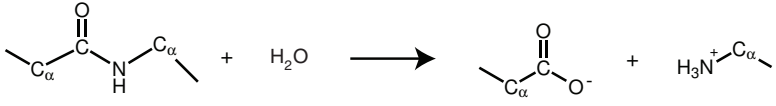
A More Complete Energy Profile for Serine Proteases



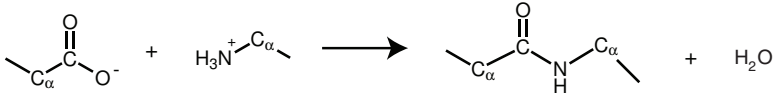
- Thermodynamics requires that enzymes catalyze reactions in both directions.
- Why don't we see reverse reaction with proteases?
- Could we make the reverse reaction more favorable?

Could We Make Peptide Bonds with a Protease?

- “Forward” reaction:



- “Reverse” reaction:



- How do we make peptide synthesis favorable?
- Lower the product concentrations (relative to reactants) for the synthesis reaction.

Warning!

Direction Change

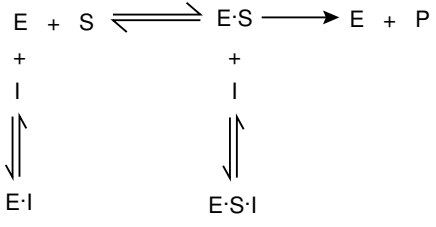
Enzyme Inhibitors

Enzyme Inhibitors

- Important aspects of enzyme inhibitors
  - Natural regulators of metabolism
  - Tools for studying enzyme mechanisms
  - Tools for studying other biological processes
  - Pharmaceuticals
- Major classes of inhibitors
  1. Reversible
     $E + I \rightleftharpoons E{\cdot}I$
     Usually form non-covalent interaction with enzyme
  2. Irreversible
     $E + I \rightarrow E\text{-}I$
     Usually form covalent bond with enzyme

Reversible Inhibitors

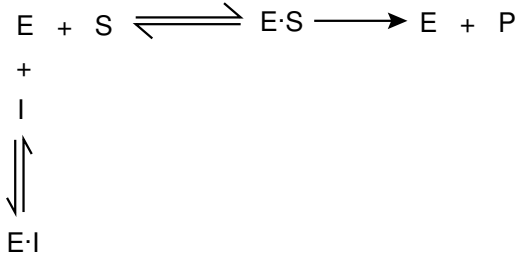
- Can bind to either free enzyme or enzyme-substrate complex



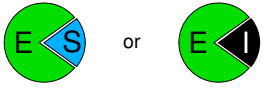
- Classification of reversible inhibitors:
  - Competitive: Inhibitor binds only to free enzyme
  - Noncompetitive: Inhibitor binds equally well to E and $E \cdot S$
  - Uncompetitive: Inhibitor binds only to $E \cdot S$
  - Mixed inhibition: Inhibitor binds to E and $E \cdot S$, but with different affinities.
- Different classes have different kinetic properties.

Competitive Inhibition

- Inhibitor binds only to free enzyme



- Competitive inhibitors often mimic the substrate.



- Inhibitor disfavors formation of the $E \cdot S$ complex.

Clicker Question #3:

How will a competitive inhibitor affect the Michaelis-Menten parameters?

1. Decrease V_{max}
2. Increase V_{max}
3. Decrease K_m
4. Increase K_m
5. Change V_{max} and K_m
6. Change neither V_{max} nor K_m

All answers count (for today)!