LECTURE 9

BB 101: MODULE II

PHYSICAL BIOLOGY

Review

- Proteins and their structures
- Proteins are free energy minimizers
- Microstate and Macrostate
- Relations $G = H - TS$ and $G = -k_B T ln Z$
- $\mathrm{S} = k_B \ln W$
- HP model and a Toy model of protein folding
- Some aspects of real protein folding

Gene-Expression

- All these cells of a human body have EXACTLY the same DNA i.e. cells that form your eye, cells that form your skin, cells that form your bone

- Same “genetic code” but works differently, how?

- We roughly know that each cell uses slightly different parts of DNA i.e. Cells in your eye “expresses” (reads) a set of different “genes” from cells in your skin

Gene-Expression

- Cells can “regulate” packaging and reading of DNA depending on many factors, including the external environment

- There are many proteins involved in regulating this; these proteins bind onto DNA to regulate “gene expression” (reading of genes)

- We can again use free-energy minimization to understand dna-protein binding and its dynamics

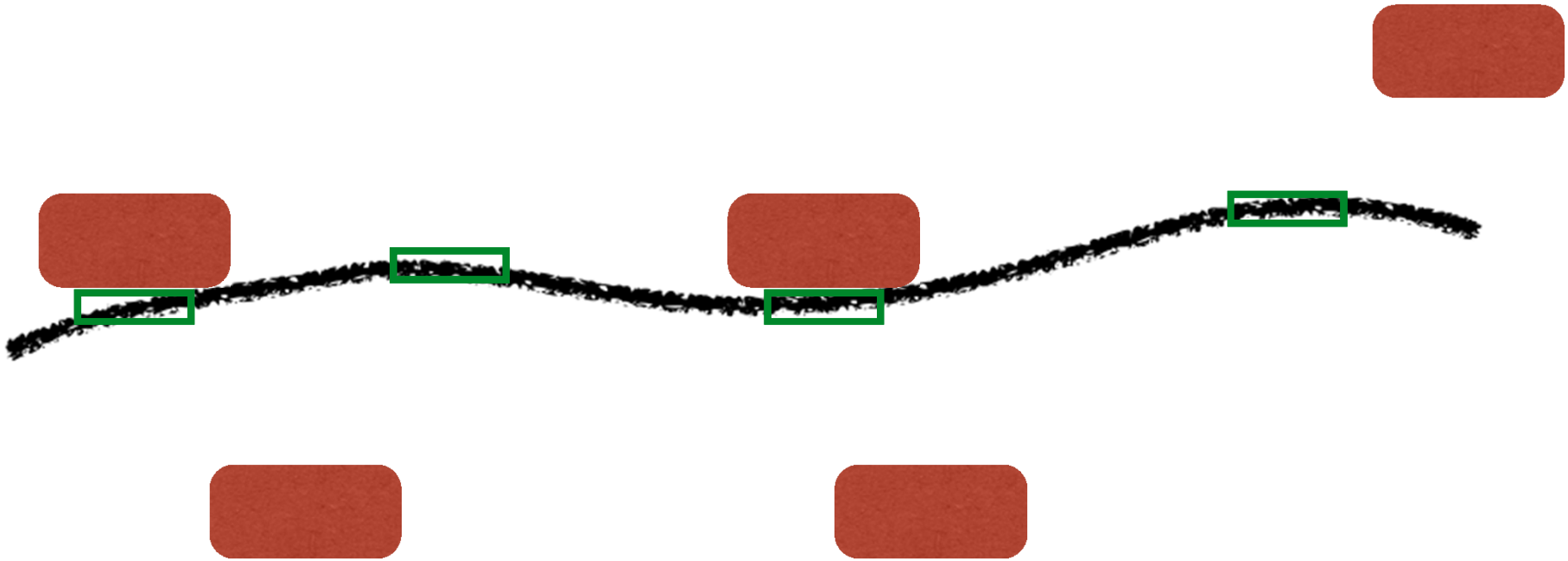
Protein-DNA Binding

- Typically, proteins and DNA are oppositely charged

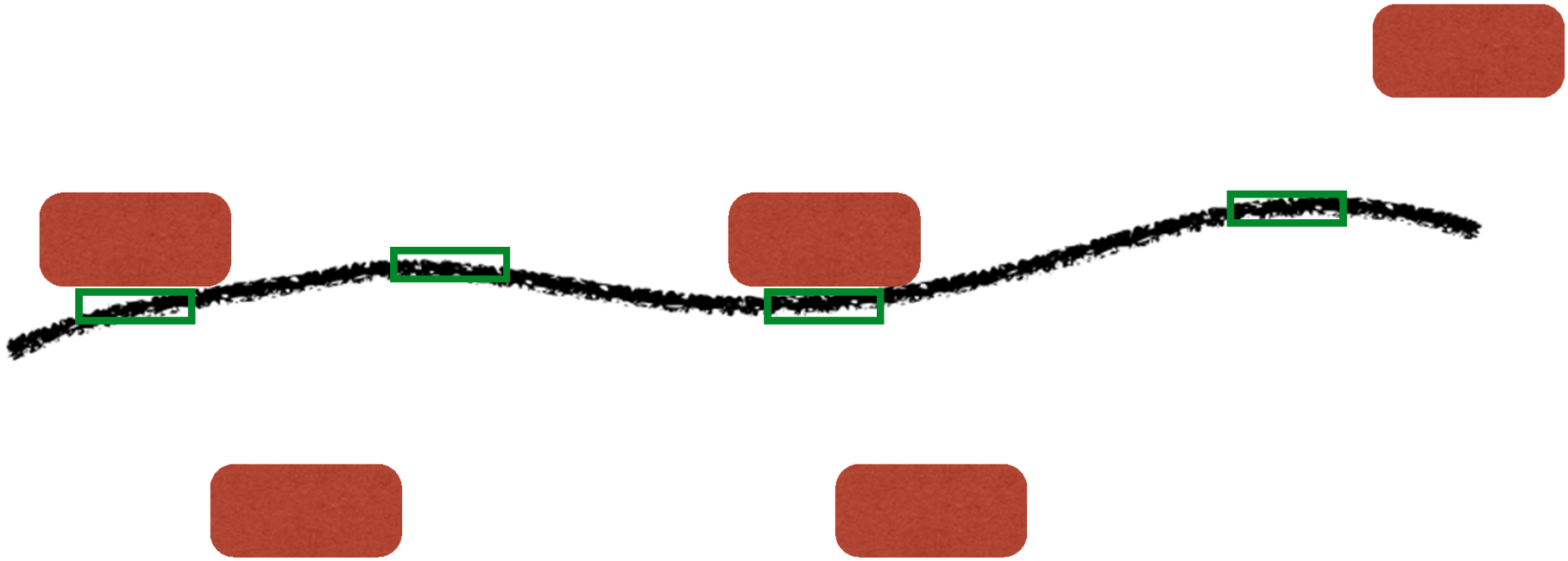
- Interaction energy favors binding; just like positive and negative charges to come together

Protein-DNA Binding

Imagine a DNA with N binding sites (locations) where a certain protein can bind with high affinity

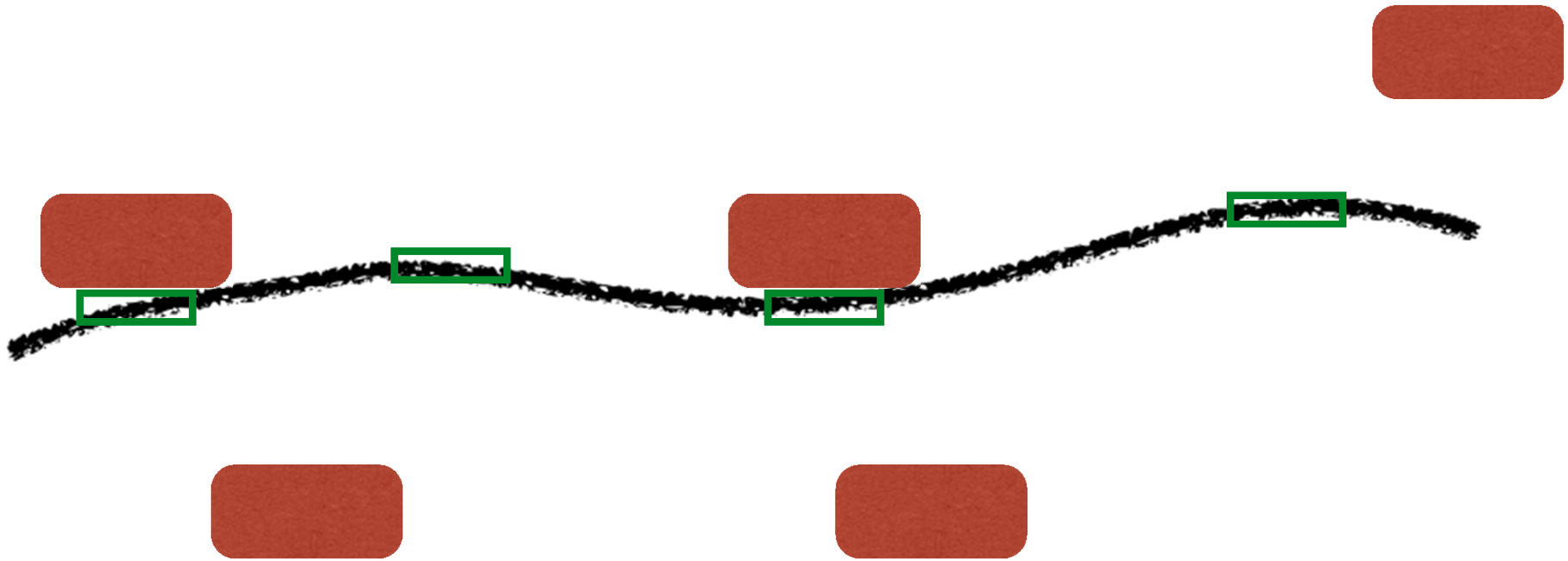


Protein-DNA Binding



If you do an experiment, how many of those “locations” will be occupied by proteins?

Protein-DNA Binding



Imagine a “state” with m proteins bound. (m<N)

In this picture m=2, N=4

Protein-DNA Binding

- If m proteins are bound then What is the total energy gain?
- Assume each protein binding gives a constant energy change $-\varepsilon k_B T$

Protein-DNA Binding

$$U = -m\varepsilon k_B T = -N\rho\varepsilon k_B T$$

In other words, ε is the binding energy of a protein: energy it gains by binding

Densityof proteins $\rho = \frac{m}{N}$

Protein-DNA Binding

Imagine a “macro-state” with m proteins bound. ($m<N$)

What is the entropy?

Protein-DNA Binding

What is the entropy?

“m” proteins, “N” binding locations

Number of arrangements (“micro-states”)?

Protein-DNA Binding

"m" proteins, "N" binding locations

Number of arrangements (number of "micro-states")

$$W = \frac{N!}{m!\,(N-m)!}$$

Protein-DNA Binding

$$S = k_B \, lnW = k_B \, ln\left(\frac{N!}{m!\,(N-m)!}\right)$$

Use Sterling's Approximation

$$\ln p! \approx plnp \; - p$$

Protein-DNA Binding

With Stirling's approximation, one can rewrite entropy as

$$S = -k_B N[\rho ln\rho + (1-\rho)\ln(1-\rho)]$$

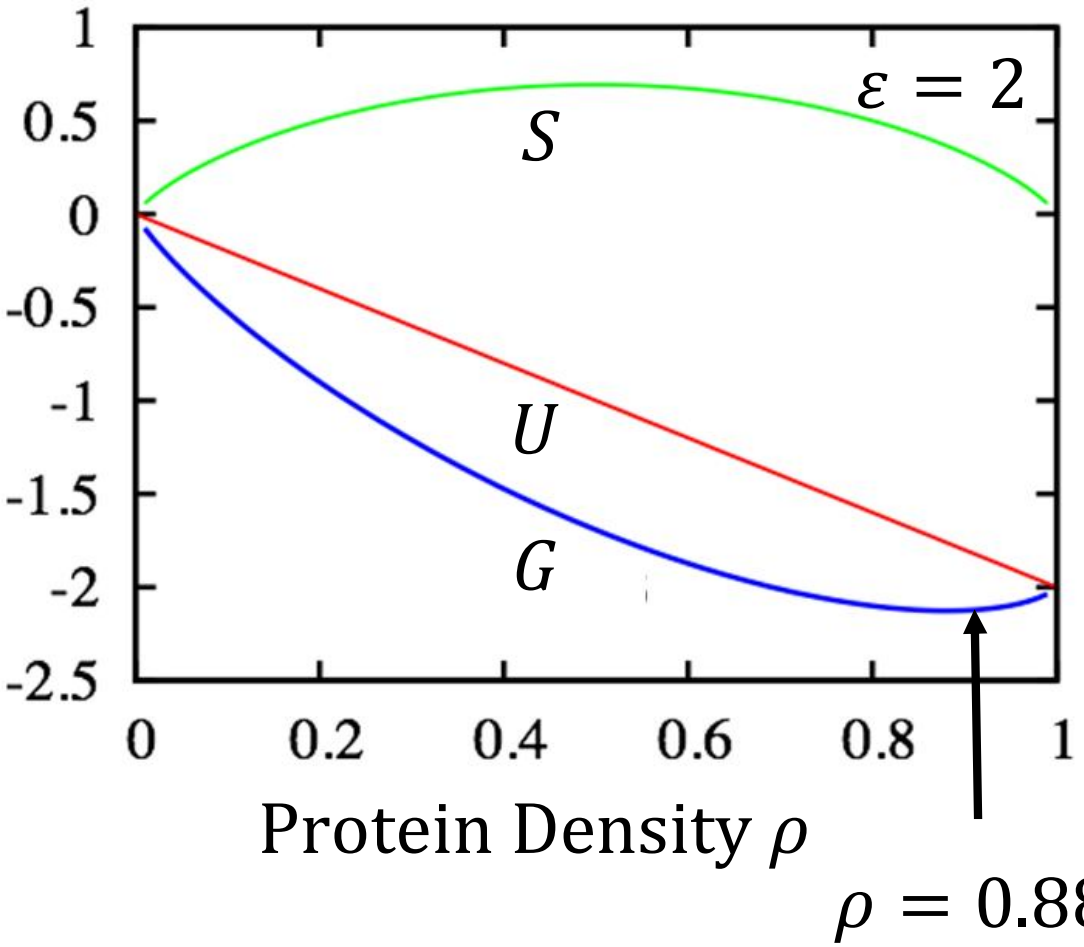
$$G = U - TS$$

$$G = -N\rho\varepsilon k_B T - k_B T N[\rho ln\rho + (1-\rho)\ln(1-\rho)]$$

$$\boxed{\frac{G}{Nk_B T} = -\rho\varepsilon - \rho ln\rho + (1-\rho)\ln(1-\rho)}$$

Protein-DNA Binding

The protein-DNA system would like to go to its minimum free energy "macro-state"



$$\frac{\partial G}{\partial \rho} = 0$$

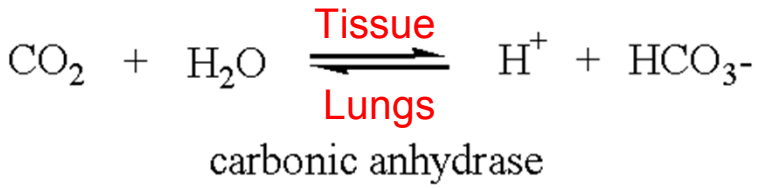
$$\rho = \frac{e^{\varepsilon}}{1 + e^{\varepsilon}}$$

ENZYMES

Why Enzymes are required?

Enzyme	Nonenzymatic $t_{1/2}$*	k_{non}* (s^{-1})	k_{cat}† (s^{-1})	k_{cat}/K_m† (s^{-1} M^{-1})	Rate enhancement (k_{cat}/k_{non})	Catalytic proficiency $[(k_{cat}/K_m)/k_{non}]$ (M^{-1})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	5.6×10^{7}	1.4×10^{17}	2.0×10^{23}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	1.0×10^{7}	5.6×10^{14}	5.9×10^{19}
Adenosine deaminase	120 years	1.8×10^{-10}	370	1.4×10^{7}	2.1×10^{12}	7.8×10^{16}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	5.0×10^{5}	6.0×10^{12}	5.0×10^{16}
Cytidine deaminase	69 years	3.2×10^{-10}	299	2.9×10^{6}	1.2×10^{12}	9.1×10^{15}
Phosphotriesterase	2.9 years	7.5×10^{-9}	2100	4.0×10^{7}	2.8×10^{11}	5.3×10^{15}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	6.6×10^{6}	1.9×10^{11}	2.2×10^{15}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66000	3.0×10^{8}	3.9×10^{11}	1.8×10^{15}
Triosephosphate isomerase	1.9 days	4.3×10^{6}	4300	2.4×10^{8}	1.0×10^{9}	5.6×10^{13}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.1×10^{6}	1.9×10^{6}	4.2×10^{10}
Carbonic anhydrase	5 s	1.3×10^{-1}	1×10^{6}	1.2×10^{8}	7.7×10^{6}	9.2×10^{8}
Cyclophilin, human	23 s	2.8×10^{-2}	13000	1.5×10^{7}	4.6×10^{5}	5.3×10^{8}

*Nonenzymatic reaction rate constants were obtained for OMP decarboxylase and staphylococcal nuclease from the present work, for adenosine and cytidine deaminases from (5), for AMP nucleosidase from (25), for phosphotriesterase from (26), for carboxypeptidase A from (3), for ketosteroid isomerase from (27), for triosephosphate isomerase from (28), for chorismate mutase from (4), for carbonic anhydrase from (2), and for cyclophilin from (3). †Enzyme reaction rate constants were obtained for OMP decarboxylase from (7), for staphylococcal nuclease from (29), for adenosine deaminase from (30), for AMP nucleosidase from (31), for phosphotriesterase from (26), for carboxypeptidase A from (32), for ketosteroid isomerase from (33), for triosephosphate isomerase from (34), for chorismate mutase from (4), for carbonic anhydrase from (35), and for cyclophilin from (36).



~10^7 fold enhancement in rate with enzyme

Radzicka A1, Wolfenden R., Science., Vol. 267, 90-93 (1995)

Enzymes

- Enzymes speed up the events which are thermodynamically very unfavorable such as Breaking covalent bonds, forming covalent bonds and moving large structures

Biological systems do so through use of Enzymes

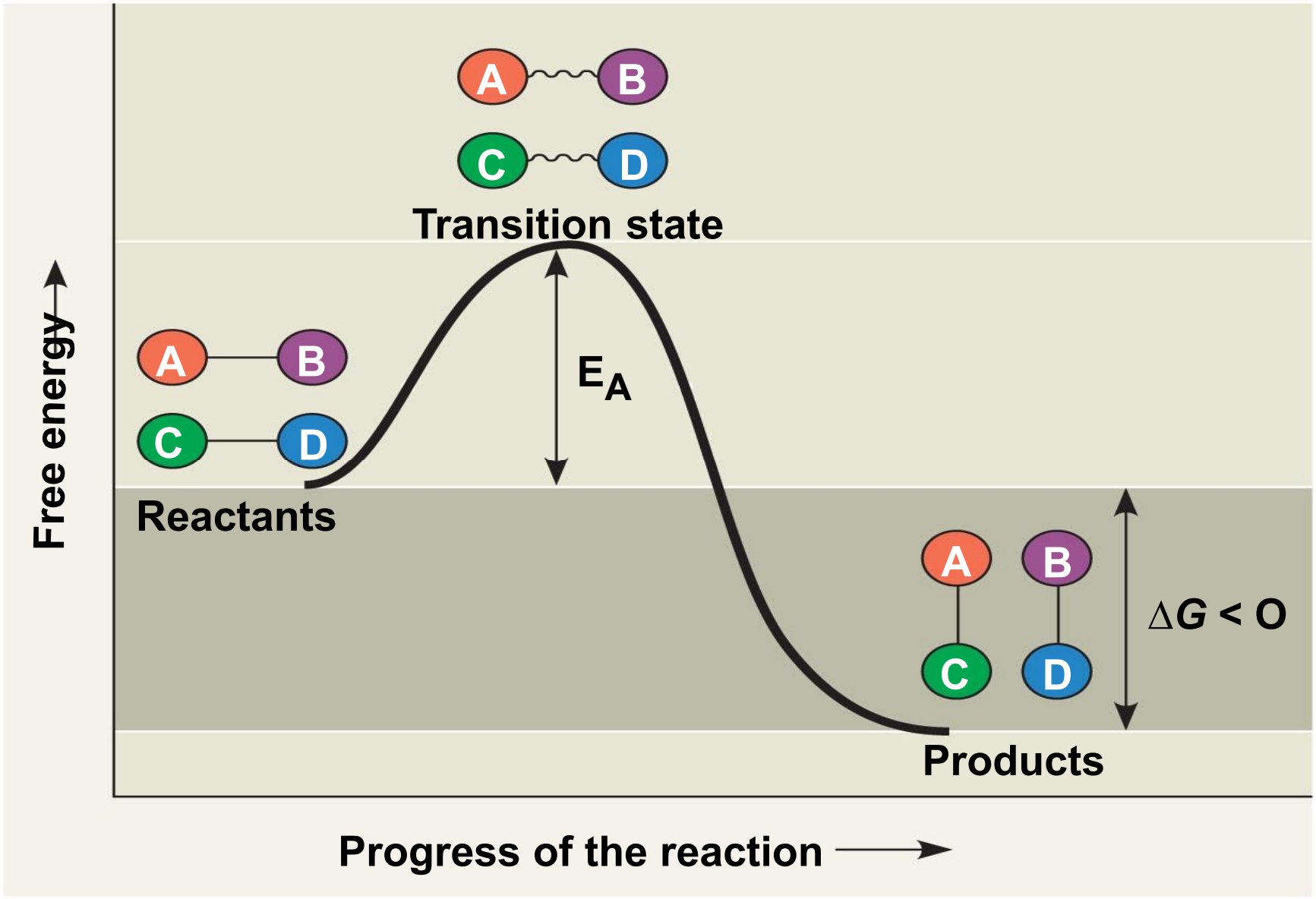
- **Enzymes** are **catalytic proteins**

- **catalyst** is a chemical agent that speeds up a reaction without being consumed by the reaction

The Activation Energy Barrier

- Every chemical reaction between molecules involves bond breaking and bond forming
- The initial energy needed to start a chemical reaction is called the free energy of activation, or activation energy (E_A)
- Activation energy is often supplied in the form of heat from the surroundings

The Activation Energy Barrier

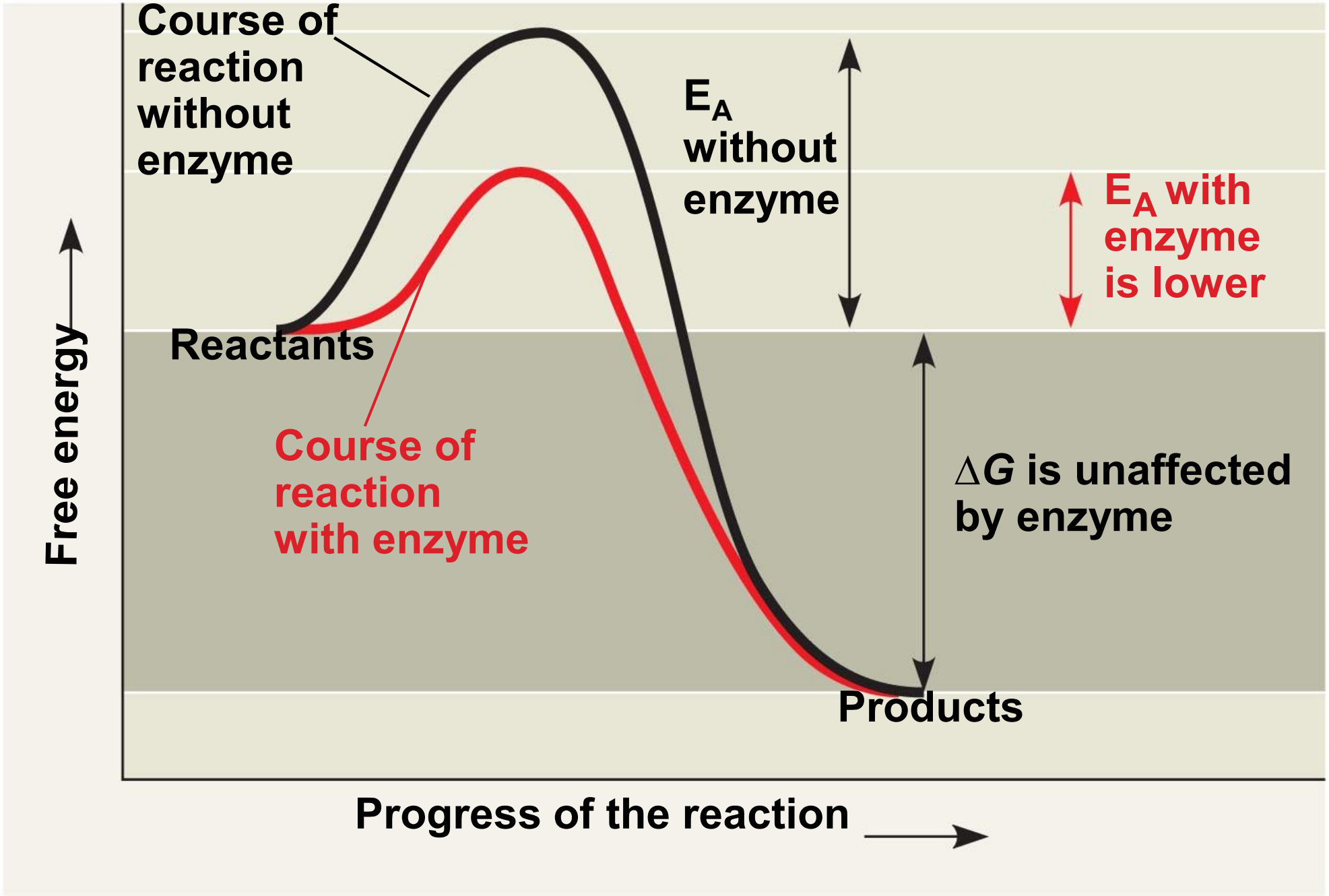


How Enzymes Lower the Activation Energy Barrier

- Enzymes catalyze reactions by lowering the E_A barrier

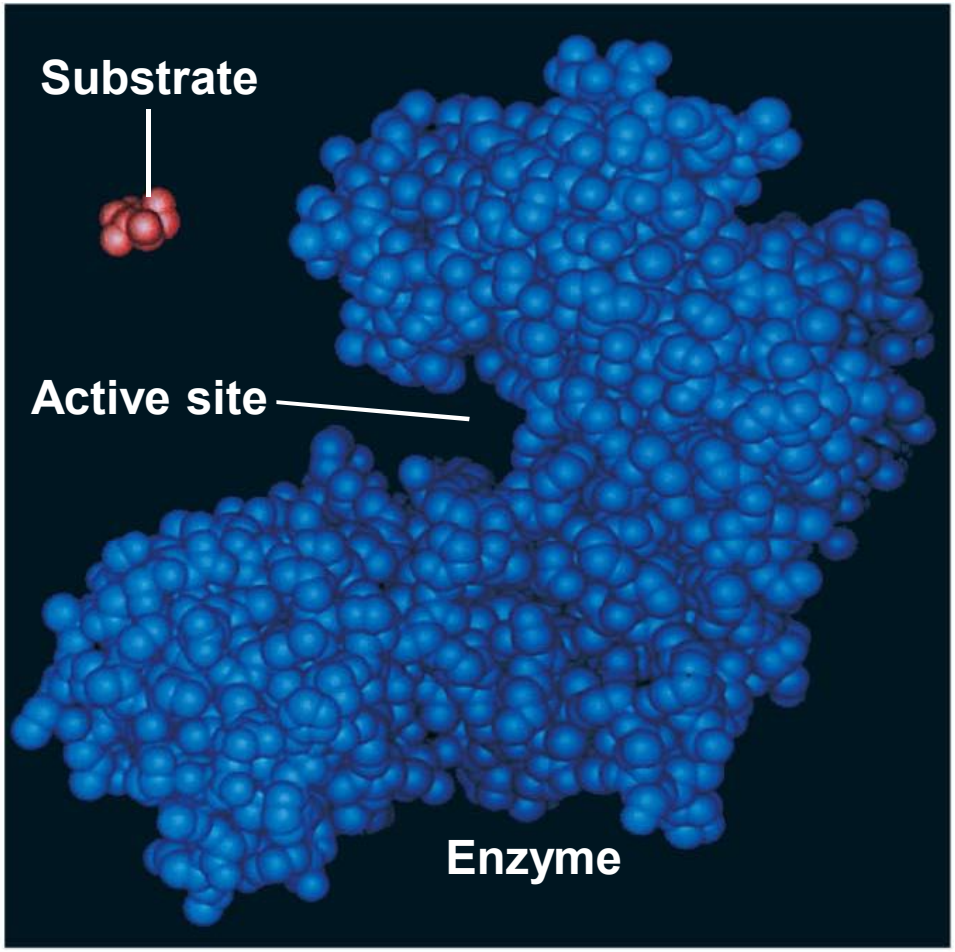
- Enzymes do not affect the change in free energy (ΔG); instead, they hasten reactions that would occur eventually

How Enzymes Lower the Activation Energy Barrier

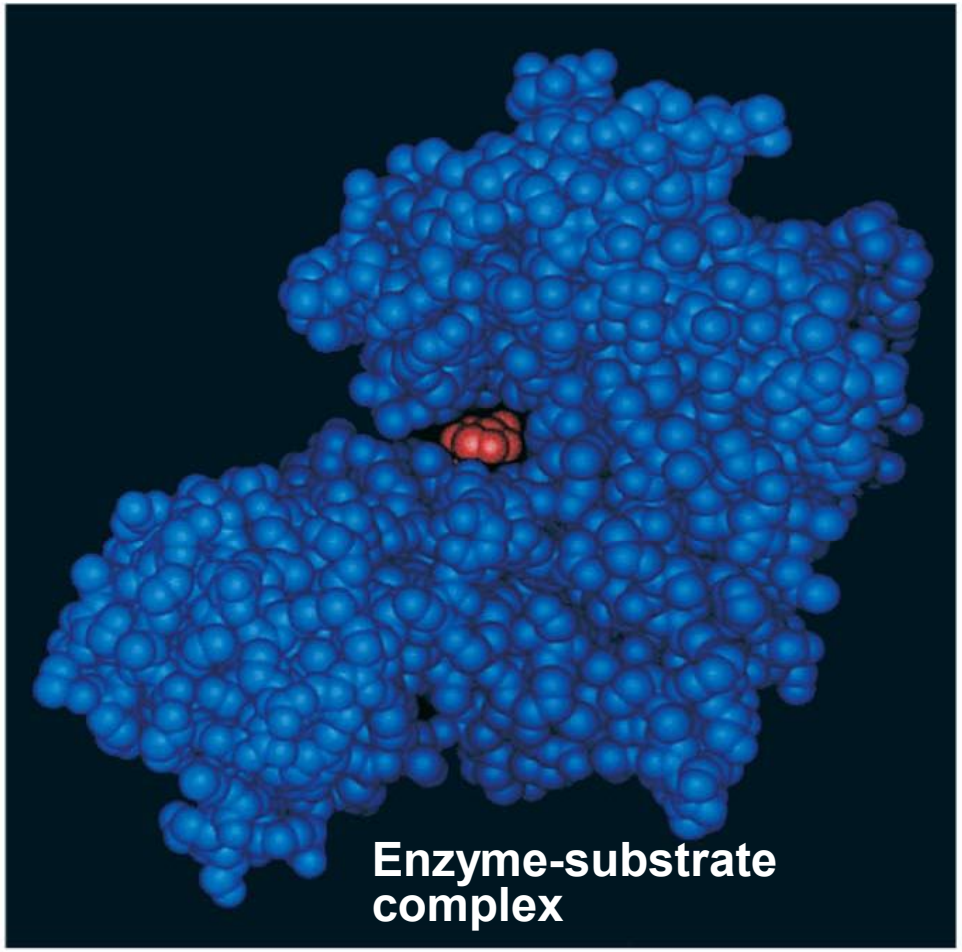


Substrate Specificity of Enzymes

- The reactant that an enzyme acts on is called the enzyme's **substrate**
- The enzyme binds to its substrate, forming an **enzyme-substrate** complex
- The **active site** is the region on the enzyme where the substrate binds
- **Induced fit** of a substrate brings chemical groups of the active site into positions that enhance their ability to catalyze the reaction



(a)



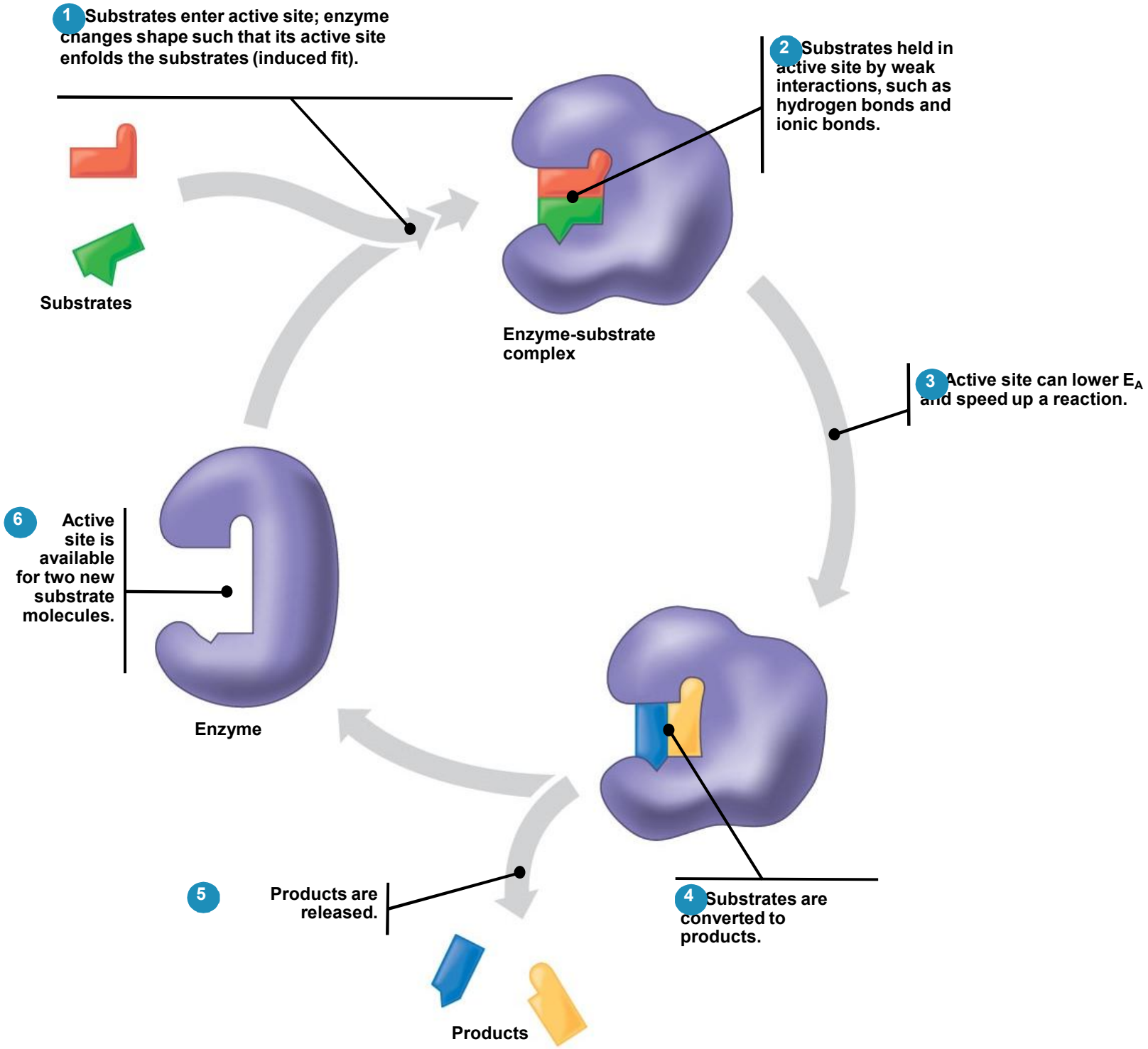
(b)

Enzymatic reaction and Lowering of Activation Barrier

- In an enzymatic reaction, the substrate binds to the active site of the enzyme

- The active site can lower an E_A barrier by

1. Orienting substrates correctly
2. Straining substrate bonds
3. Providing a favorable microenvironment
4. Covalently bonding to the substrate



Enzymes in Action

Watch animation of enzyme joining two substrate molecules on following link:

https://www.youtube.com/watch?v=r1ryDVgx0zw

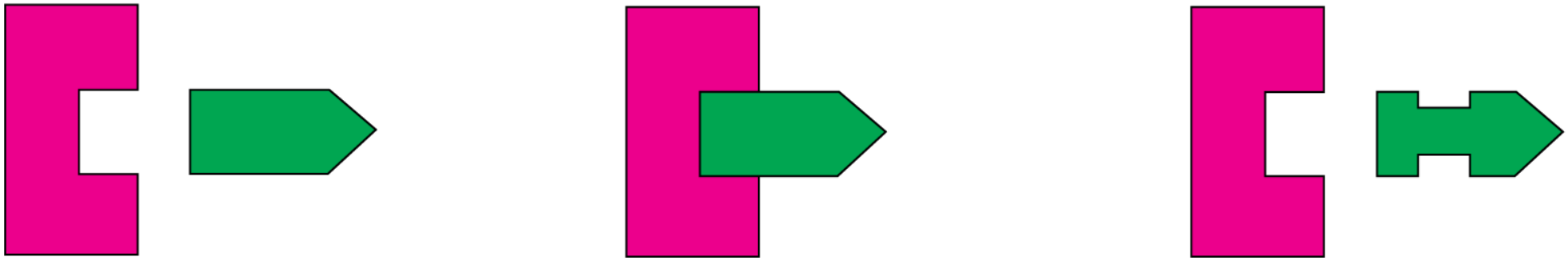
Watch animation of enzyme breaking a substrate molecule on following link:

https://www.youtube.com/watch?v=6Nw6XOqKuWg

A simple model of Enzyme Kinetics

L. Michaelis and M. Menten, proposed a mathematical model of single-substrate enzyme reaction

$$E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$



Variables

[E]: free enzyme molecules
[S]: free substrate molecules
[ES]: enzyme-substrate complexes
[P]: free product molecules

Parameters

k_f , k_r , k_{cat} : reaction rates

Michaelis-Menten Kinetics

A simple model of Enzyme Kinetics

Master equation for $[ES]$: $$\frac{d[ES]}{dt} = k_f[E][S] - k_r[ES] - k_{cat}[ES]$$

In steady state: $$\frac{d[ES]}{dt} = 0$$

$$[ES] = \frac{[E][S]}{\frac{k_r + k_{cat}}{k_f}} = \frac{([E]_0 - [ES])[S]}{\frac{k_r + k_{cat}}{k_f}} = \frac{([E]_0 - [ES])[S]}{K_m}$$

$$[ES] = \frac{[E]_0[S]}{K_m + [S]} \qquad K_m = \frac{k_r + k_{cat}}{k_f}$$

A simple model of Enzyme Kinetics

Velocity of reaction tells you how rapidly production is being formed

$$v = \frac{d[P]}{dt} = k_{cat}[ES] = \frac{k_{cat}[E]_0[S]}{K_m + [S]}$$

$$\boxed{v = \frac{v_{max}[S]}{K_m + [S]}}$$

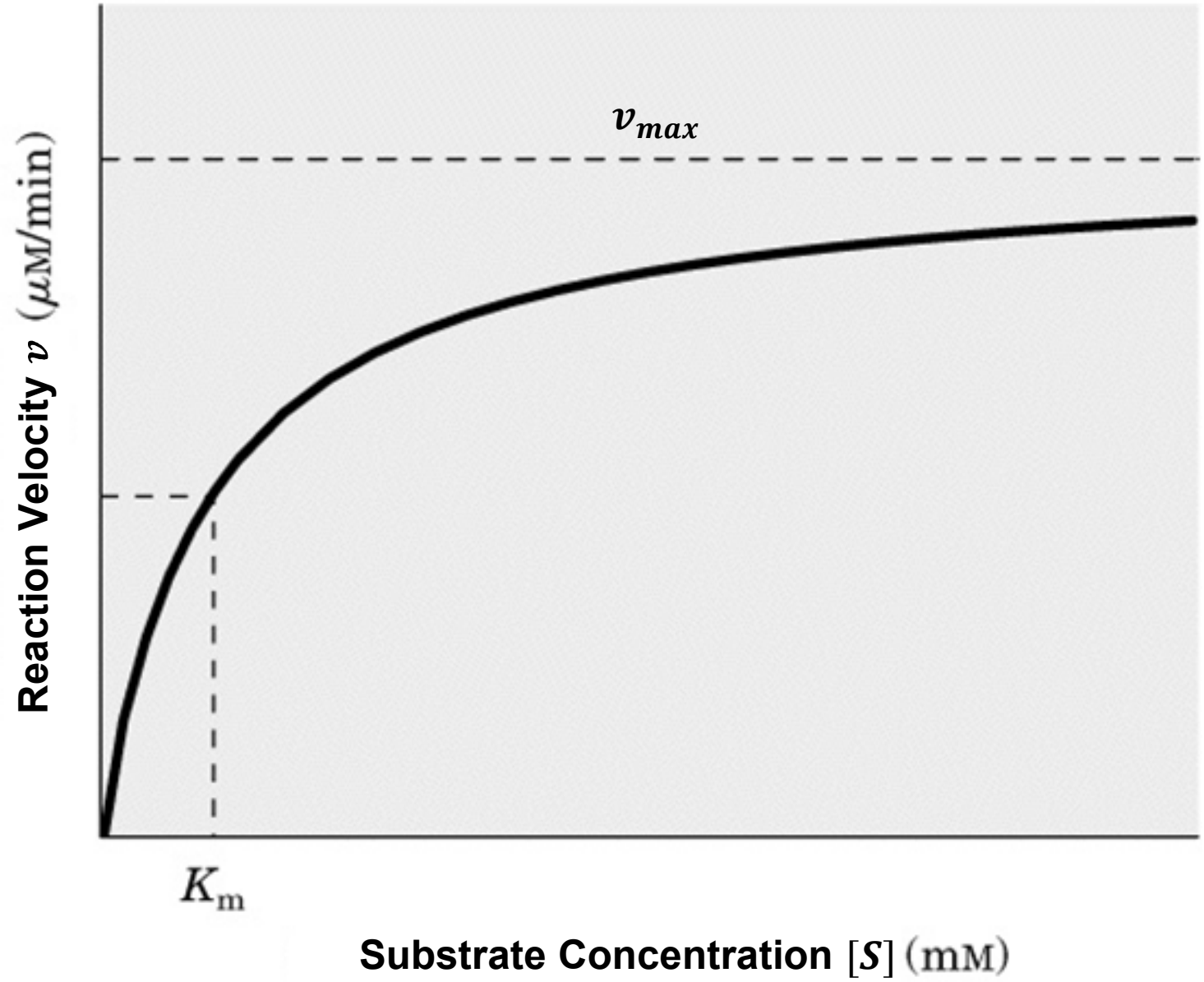
Michaelis-Menten Rule

$$v_{max} = k_{cat}[E]_0$$

Where $K_m = \frac{k_r + k_{cat}}{k_f}$ is called Michaelis Constant

A simple model of Enzyme Kinetics

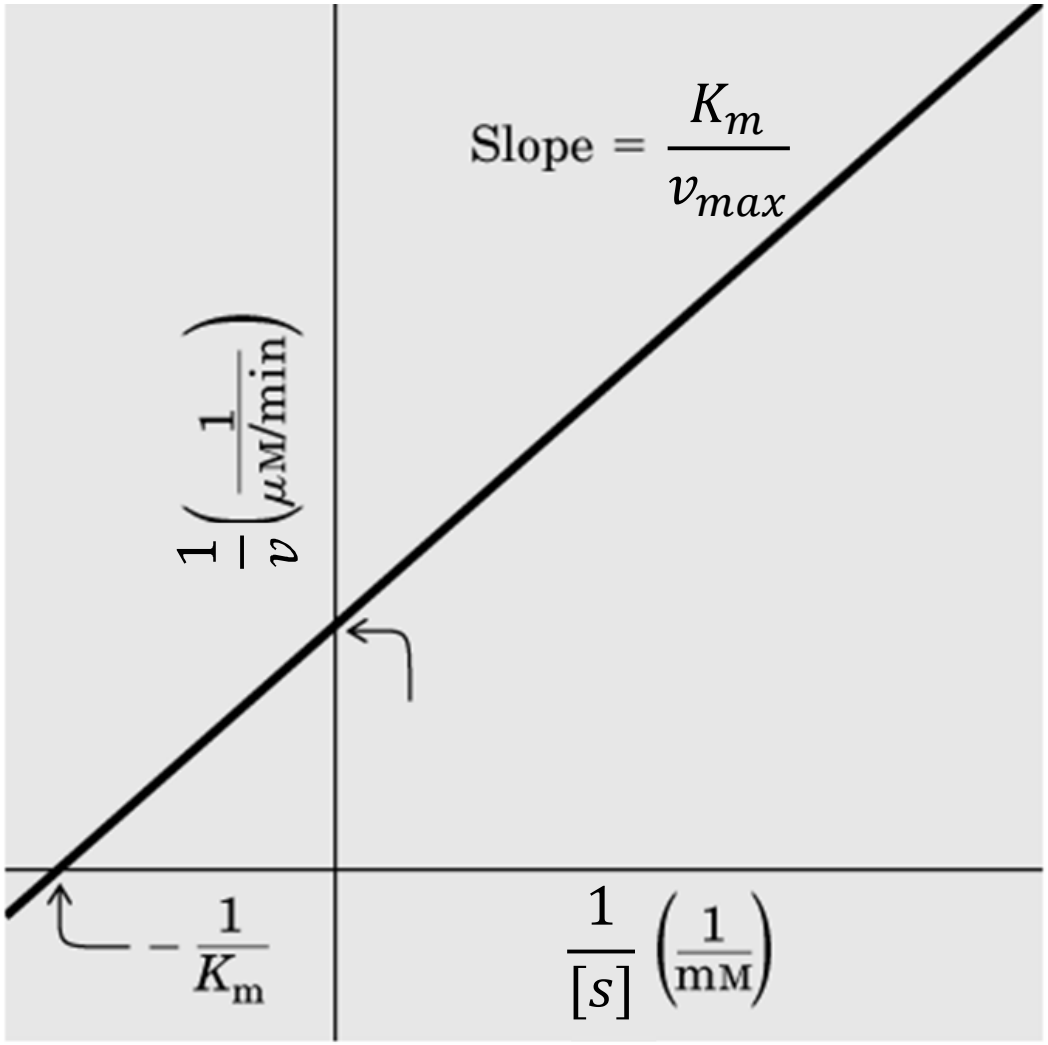
$$v = \frac{v_{max}[S]}{K_m + [S]}$$



A simple model of Enzyme Kinetics

Lineweaver-Burk Plot

$$\frac{1}{v} = \frac{K_m}{v_{max}}\frac{1}{[S]} + \frac{1}{v_{max}}$$



End of Module II

Physical biology or Biophysics exciting

you realized that we can use the physics and mathematics you learned, to think about biological problems!

End of Module II

Every time you see a biological phenomenon, think how to use your science/engineering knowledge to understand it

We know very little about what is going on in many biological processes

So, there is a great opportunity for you to go make important discoveries!!!

Enjoy Next Module

Summary

- Proteins that bind on to the DNA control the "gene" expression in each cell
- Protein-DNA system minimizes its free energy
- Number of proteins bound to DNA will depend on the free energy of the protein-DNA system
- Enzymes and their mechanism of action
- A simple model for enzyme kinetics: Michaelis-Menten Kinetics