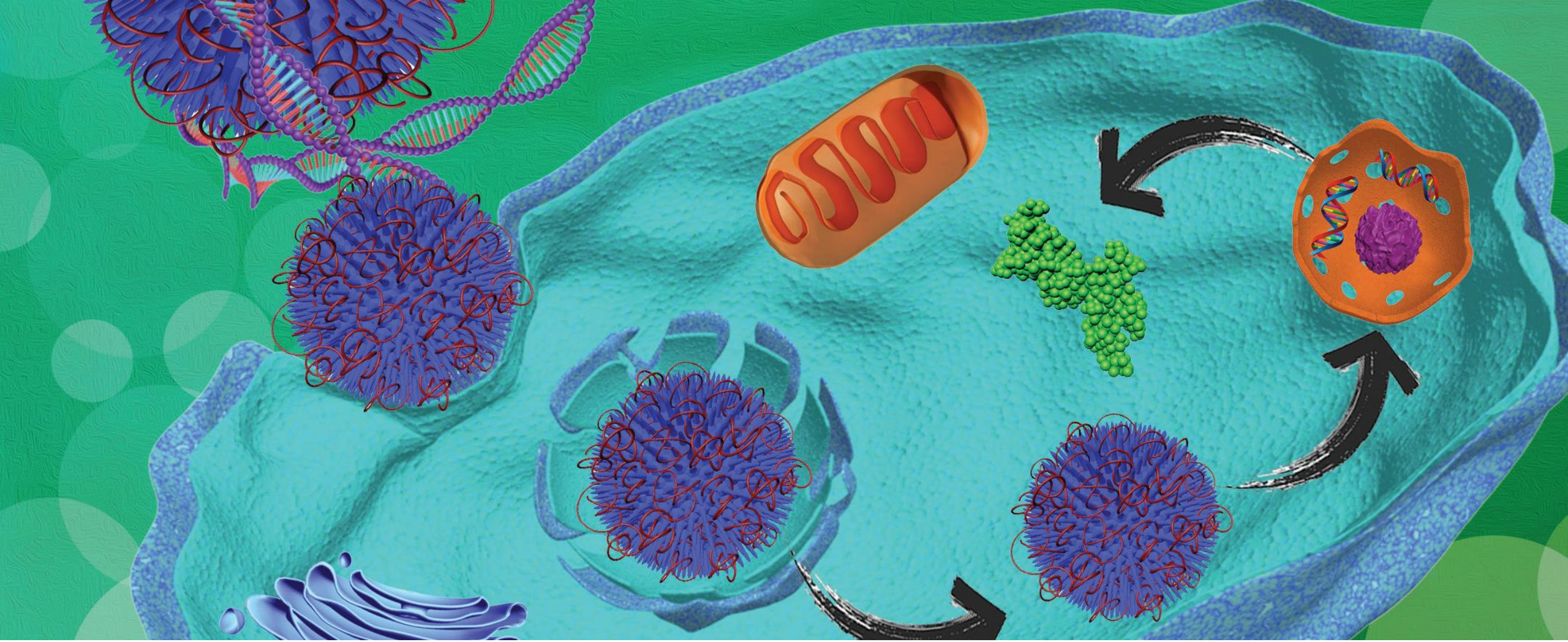


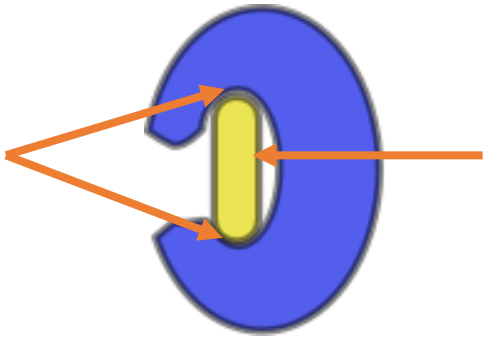
Enzyme regulation and mechanisms inside the cell

Prerequisite Knowledge:
How do enzymes increase reaction rates?

1) **Reducing the transition state energy** (i.e., barrier to instability) by **using binding energy** to stabilize the transition state.
2) Provide an alternate path for product formation.
3) Reduce entropy by binding and orienting multiple substrates.

$-\Delta G$

Stronger binding, would **release energy**



$+\Delta G$

High energy (transition state), **requires energy**

Strain, cage effect, acid-base, covalent, metal ion

Prerequisite Knowledge:

Quantifying enzyme activity:

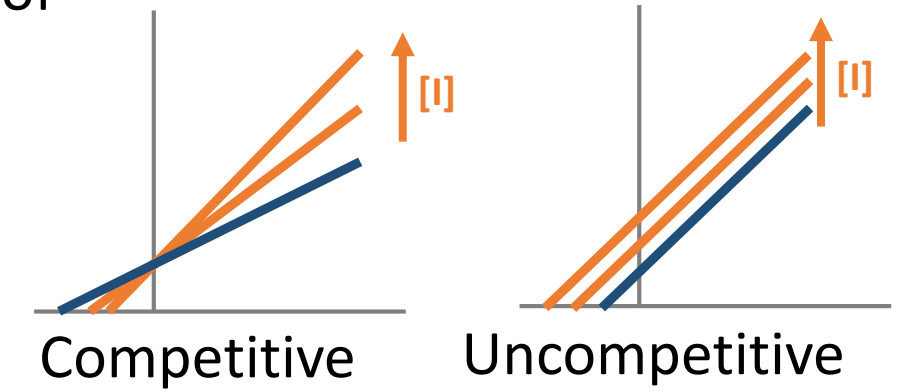
$$\frac{[E][S]}{[ES]} \neq K_m = \frac{k_{-1} + k_p}{k_1}$$

learned the Briggs-Haldane fix for M-M kinetics: K_m

k_{cat} (turnover), and the comparison of k_{cat}/K_m

How enzymes are frequently represented by "**Units**"

How to **plot kinetics** to easily determine the type of reaction, type of inhibition.



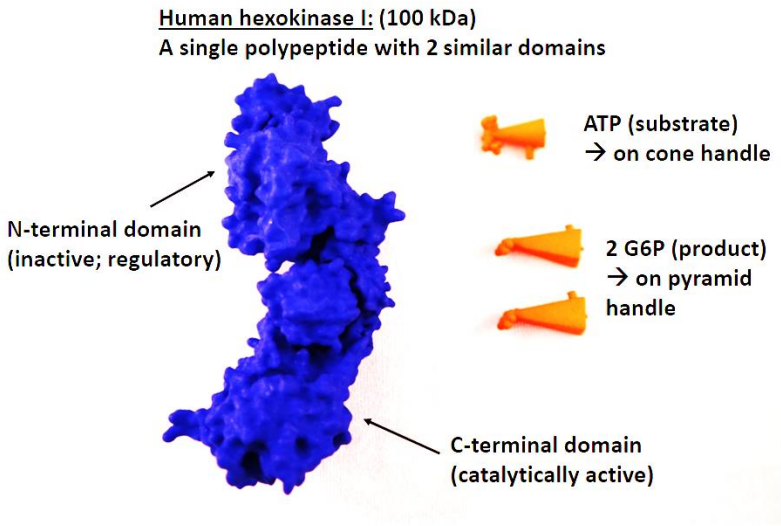
Enzyme Inhibition:

Competitive, **uncompetitive**, **mixed**

How to **distinguish** the effects on V_{max} and K_m.

Today: Enzyme mechanism:

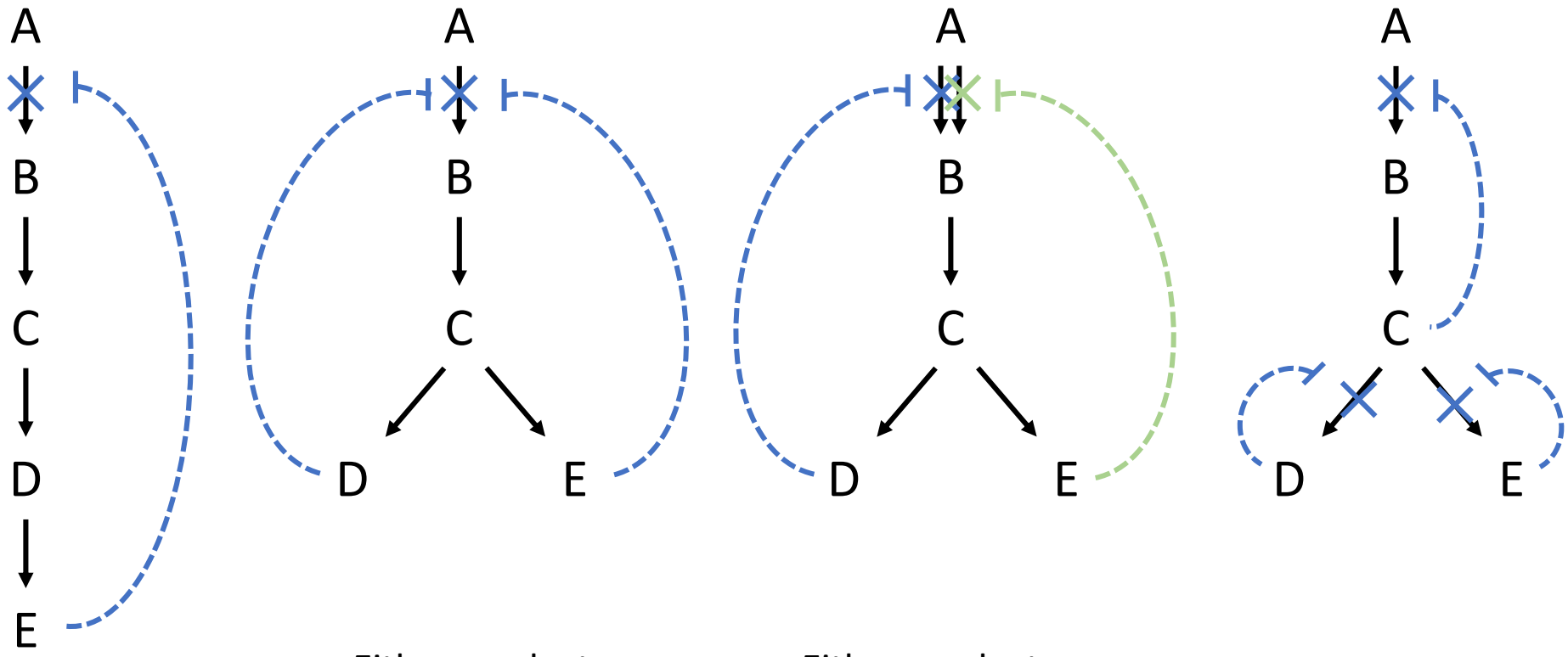
Internal regulation of enzymes (in class activity)



6 classes of enzymes

1. **Oxidoreductase** Transfer of electrons
2. **Transferase** Transfer of functional groups
3. **Hydrolase** Single bond cleavage (water)
4. **Lyase** Bond cleavage by elimination
5. **Isomerase** Intramolecular rearrangement
6. **Ligase** NTP-dependent bond formation

Feedback Inhibition, a common form of control



Neither intermediates or final product are made

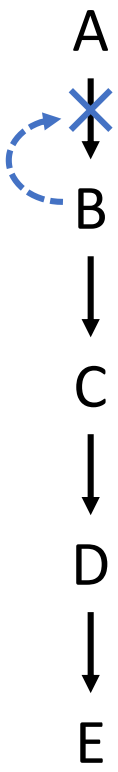
Either product building up inhibits production of both products

Either product building up reduces production of both products

Both products are independently controlled.

How does feedback inhibition occur?

Product inhibiton



When B builds up, it automatically “inhibits” production of more B.

Why?

$$S + E \underset{k_{-1}}{\overset{k_1}{\leftrightharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\leftrightharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\leftrightharpoons}} E + P$$

Some back reaction occurs, where product is converted to substrate.

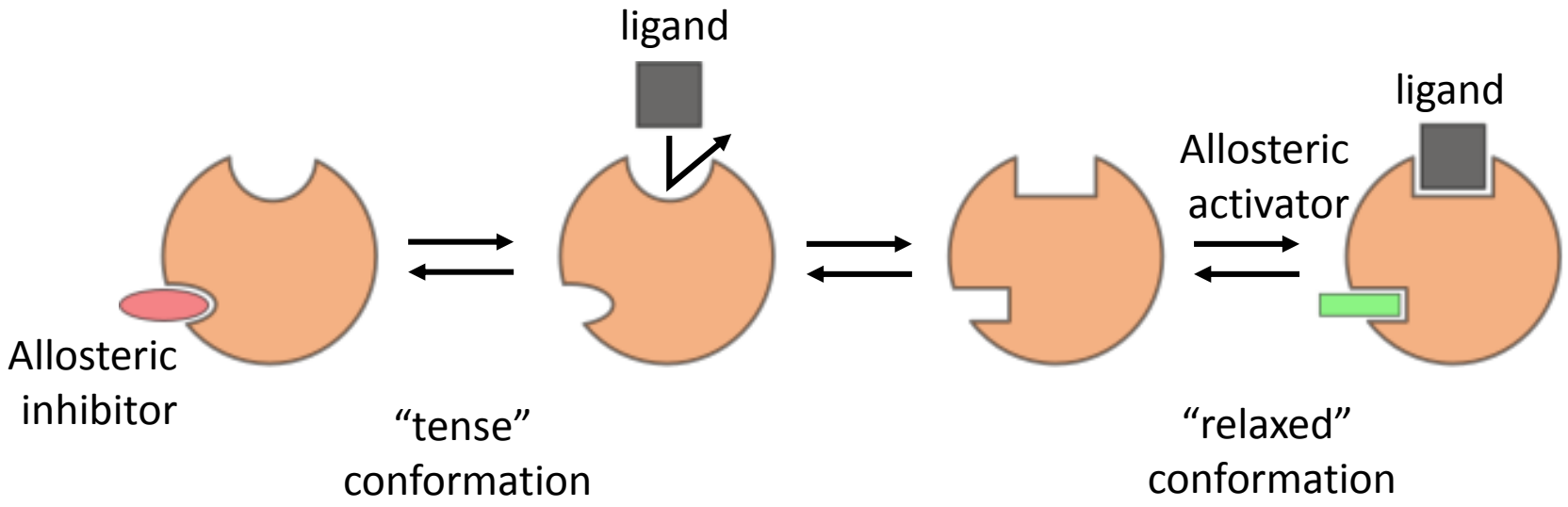
It binds to free enzyme. So free enzyme can’t be used to bind substrate.

How does feedback inhibition occur?

Feedback inhibition often uses the immediate product of the reaction (**product inhibition**), but can also use downstream products. How?

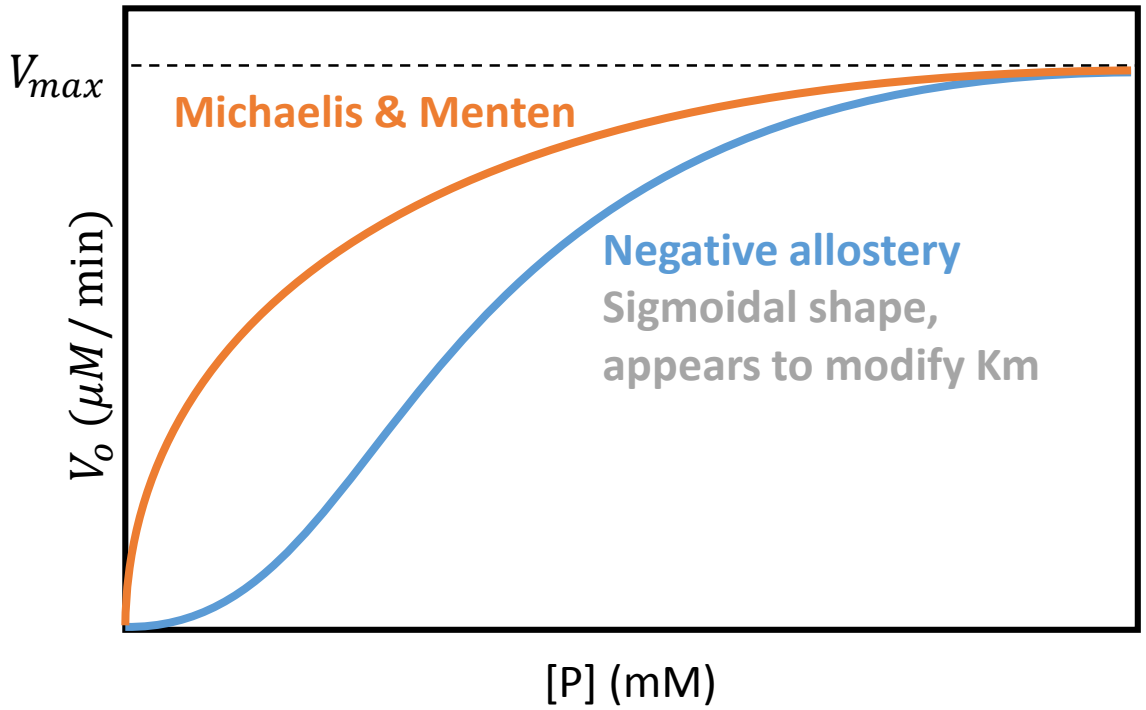
Inhibitors: (**Competitive**, **uncompetitive**, **mixed**)

Allosteric inhibitors and activators

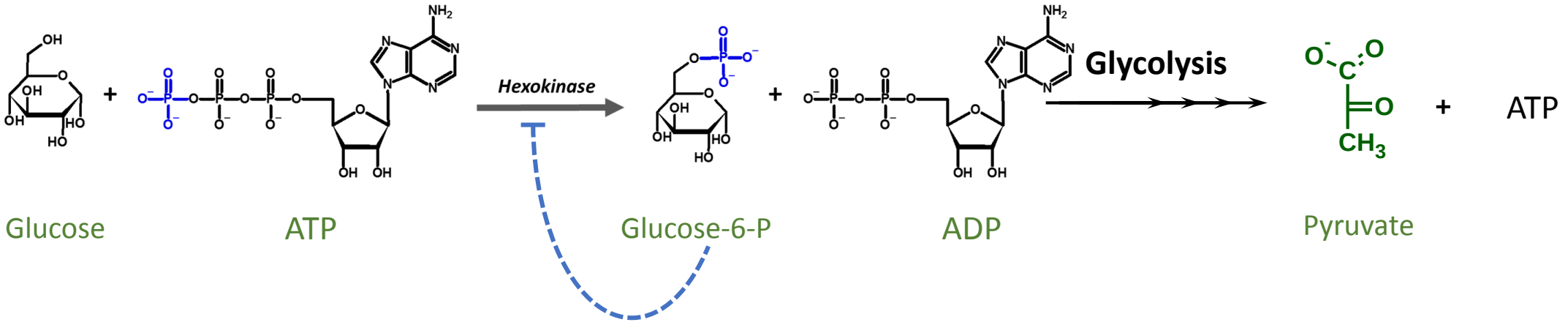


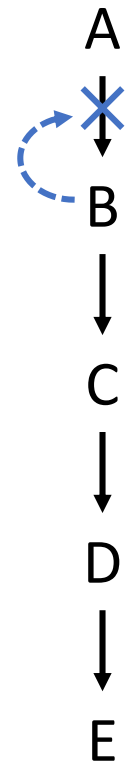
Noncovalent Modification: Allosteric Regulators

The kinetics of allosteric regulators **differ from Michaelis & Menten kinetics**.
Remember Hemoglobin vs. Myoglobin?



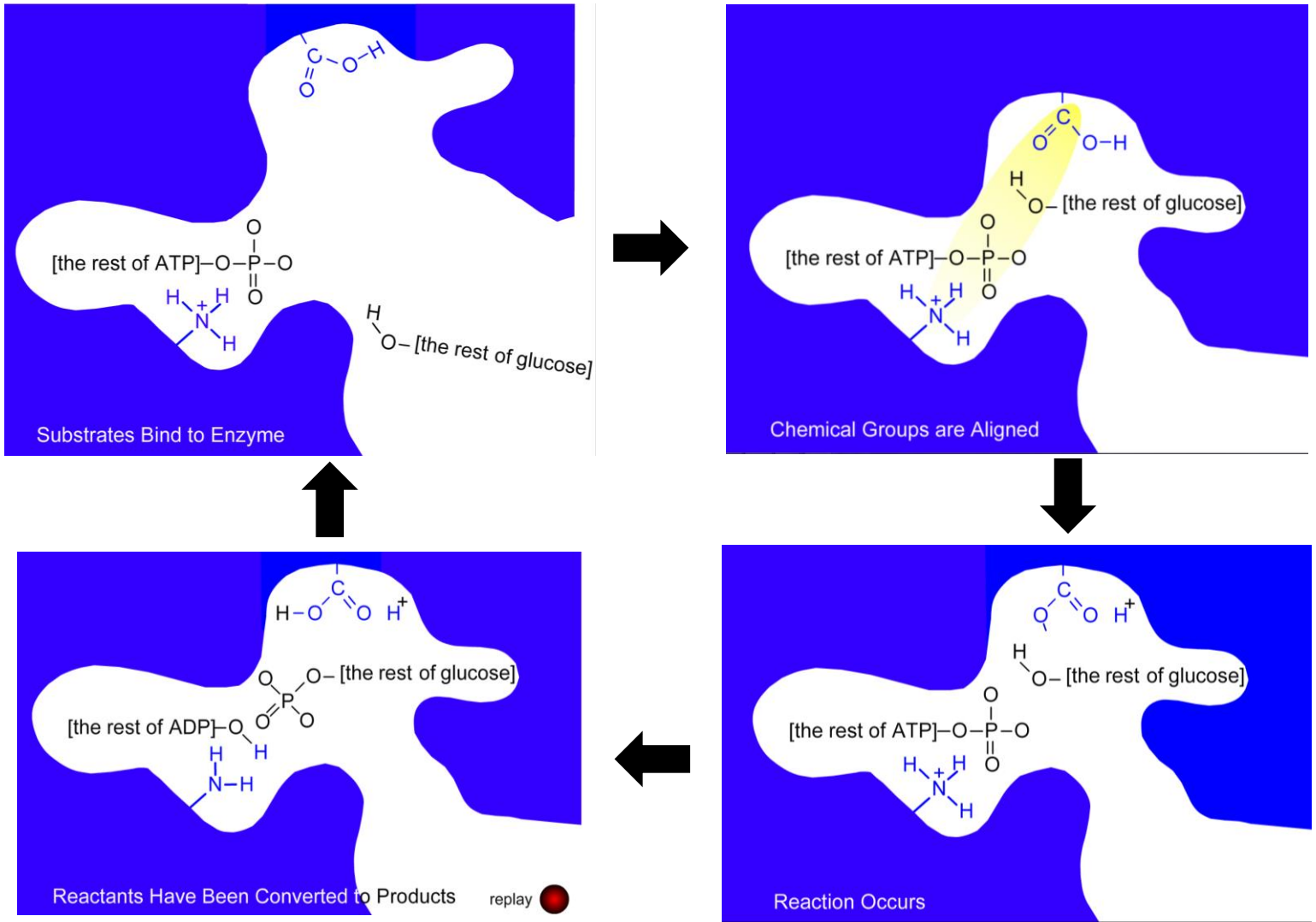
Use Hexokinase as an example





EC2. **Transferase**: Hexokinase mechanism

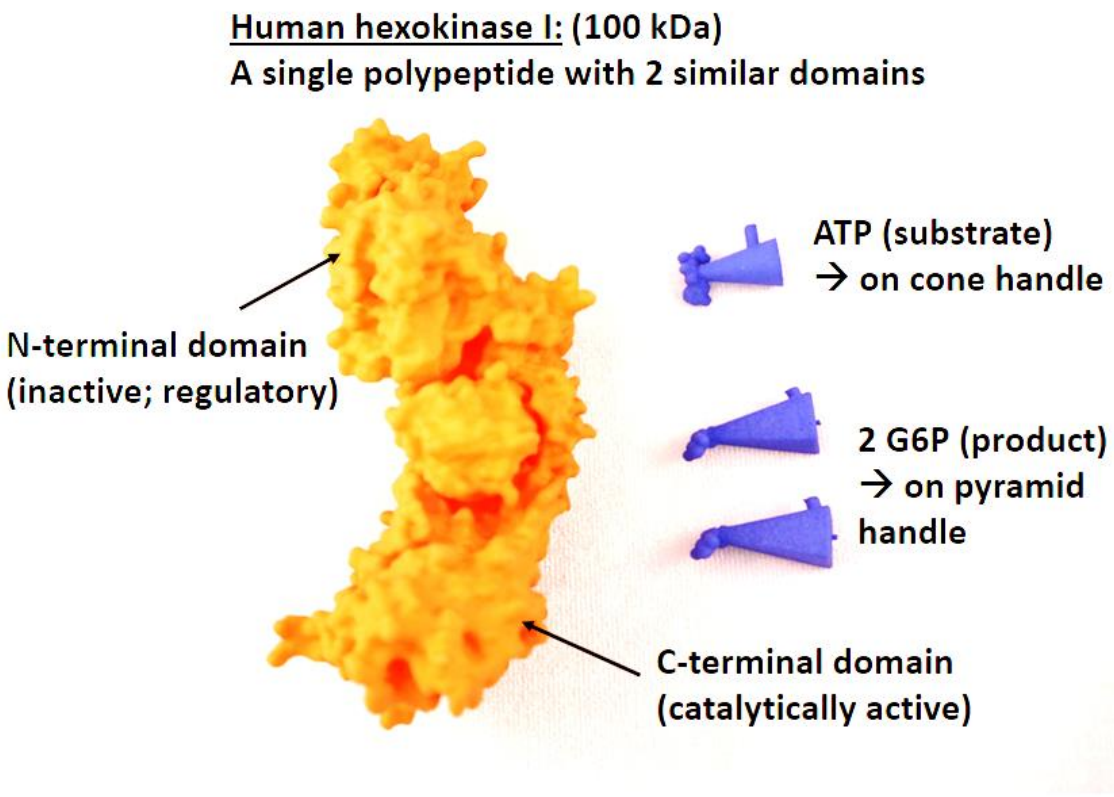
http://www.indiana.edu/~oso/animations/hexokinase.html

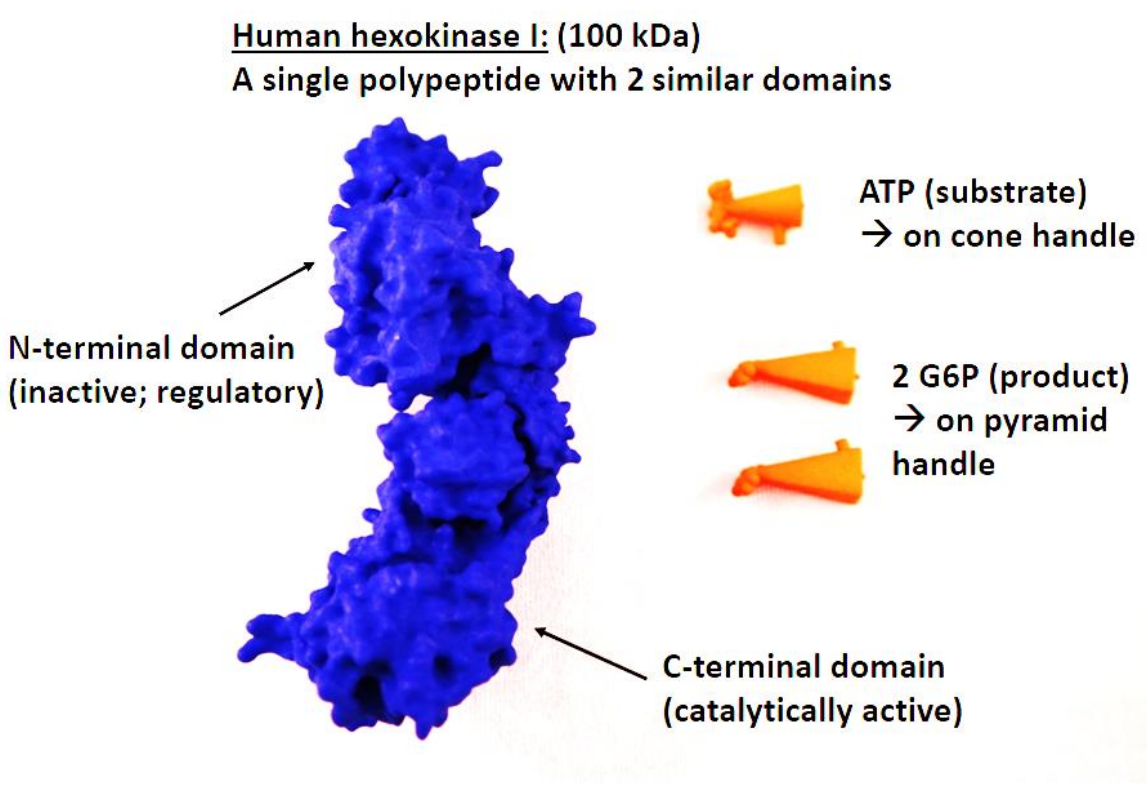


Cage effect: binding two substrates in the correct orientation.

Acid-base: give and take protons from/to substrate.

Discovering enzyme regulation with 3D models





Clicker Question

How does Glucose-6-phosphate regulate hexokinase I?

A. Competition for active site

B. Back reaction

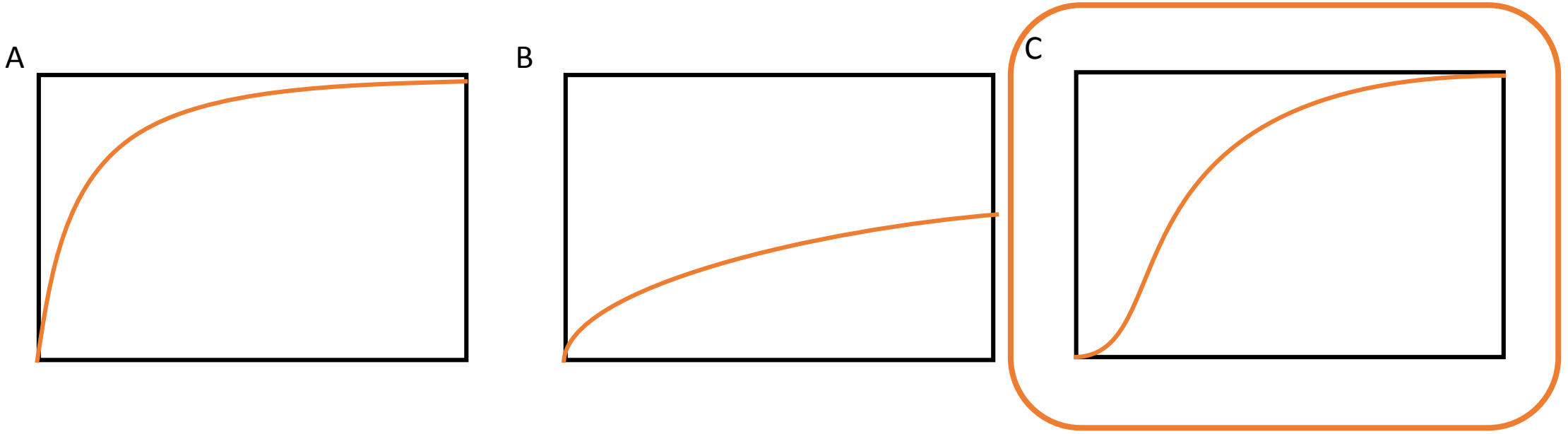
C. Allostery

D. A and B

E. A, B, and C

Clicker Question

Which of the following represent the activity of hexokinase I when Glucose-6-phosphate is present?



Summary: Internal Regulation of Enzymes

Within the body, enzymes are regulated by **noncovalent** or **covalent modification**, which can be **reversible** or **irreversible** at the protein level.

Common modifications include: allosteric regulators (especially **feedback inhibitors**), **phosphorylation**, alkylation, ubiquitination, and hydrophobic group addition/removal.

The cell can also alter conditions of small molecules (**pH**!) and **cofactor requirements** (Ca^{++}) to modulate enzyme activity.