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Tutorial 3

Enzymes and Cell Biology: Enzymes, Inhibition, Membranes and Signalling

BMOL2201/6201

Tutorial 3 Aims

- Understand what **enzymes** are, what their **function** is, and how they're regulated through **inhibition**
  - ***Remember, 20% of proteins are enzymes!***
- Identify what makes a reaction **favourable** or **unfavourable**, and how the body counters this

---------- *upto here for in-semester test* ----------

- Describe the role of **membranes** in maintaining cells and allowing biochemical reactions to take place
- Understand the importance of **biochemical signalling**, and describe how this takes place through a few important examples

Enzymes: Biochemical Workhorses

- Enzymes help **catalyse** reactions and are essential to a lot of biochemical tasks

- Enzymes help **reduce the activation energy** of a reaction and therefore accelerate the reaction rate – often 10^6 – 10^{12} increase (millions to trillions of times faster!)

- If a certain reaction isn't favourable, adding an enzyme **doesn't change this** – it will just help the reaction go faster

Enzymes: Biochemical Workhorses

- Catalysts – so they are **regenerated** at the end of the reaction
- Agents of **metabolic change**
- 6 main types
- Accelerate biochemical reactions millions of times faster than the uncatalysed reactions
- Enzymes bind specific substrates and make specific products
- All this is done under physiological conditions

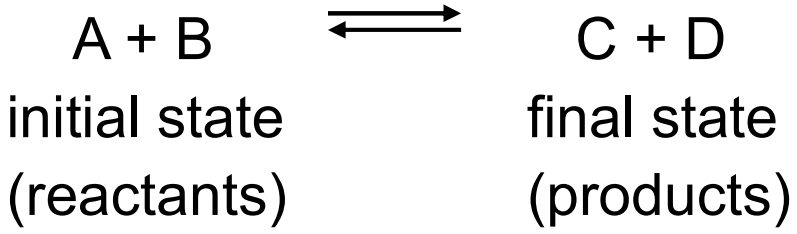
TABLE 11-2 Enzyme Classification According to Reaction Type

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

Table 11-2

Thermodynamics decides which reactions are favourable!

- A general reaction looks like:



- Free energy change of the reaction at constant T and P is called ΔG (i.e. final state – initial state)
  - If ΔG is 0, the reaction is at equilibrium.
  - If ΔG is negative, the reaction occurs spontaneously.
  - If ΔG is positive, the reaction does not occur spontaneously.

- What happens if an important reaction has a positive ΔG? Can we make it spontaneous somehow?

Reaction Coupling: How to beat thermodynamics

- We care about the ΔG of a reaction **overall** – if we couple an unfavourable reaction (**ΔG > 0**) to an even more favourable one (**ΔG << 0**), we can make the overall **ΔG < 0**:

$$\text{Glucose} + P_i \rightleftharpoons \text{glucose-6-phosphate} \qquad \Delta G = +13.8 \text{ kJ/mole}$$

$$+$$

$$ATP + H_2O \rightleftharpoons ADP + P_i + H^+ \qquad \Delta G = -32.2 \text{ kJ/mole}$$

$$ATP + \text{Glucose} \rightleftharpoons ADP + \text{glucose-6-phosphate}$$

$$\Delta G = -18.4 \text{ kJ/mole}$$

- *ΔG values from Biochemistry: AAM, for rabbit skeletal muscle*
- Coupling reactions to ATP like this is a very common biochemical strategy
- Can also have other coupling reactions, not just ATP!

Enzymes in Action

- Enzymes lower the activation energy, but **do not change ΔG**
- This speeds up the **reaction rate**
- To work out the new rate, need to look at **enzyme kinetics**

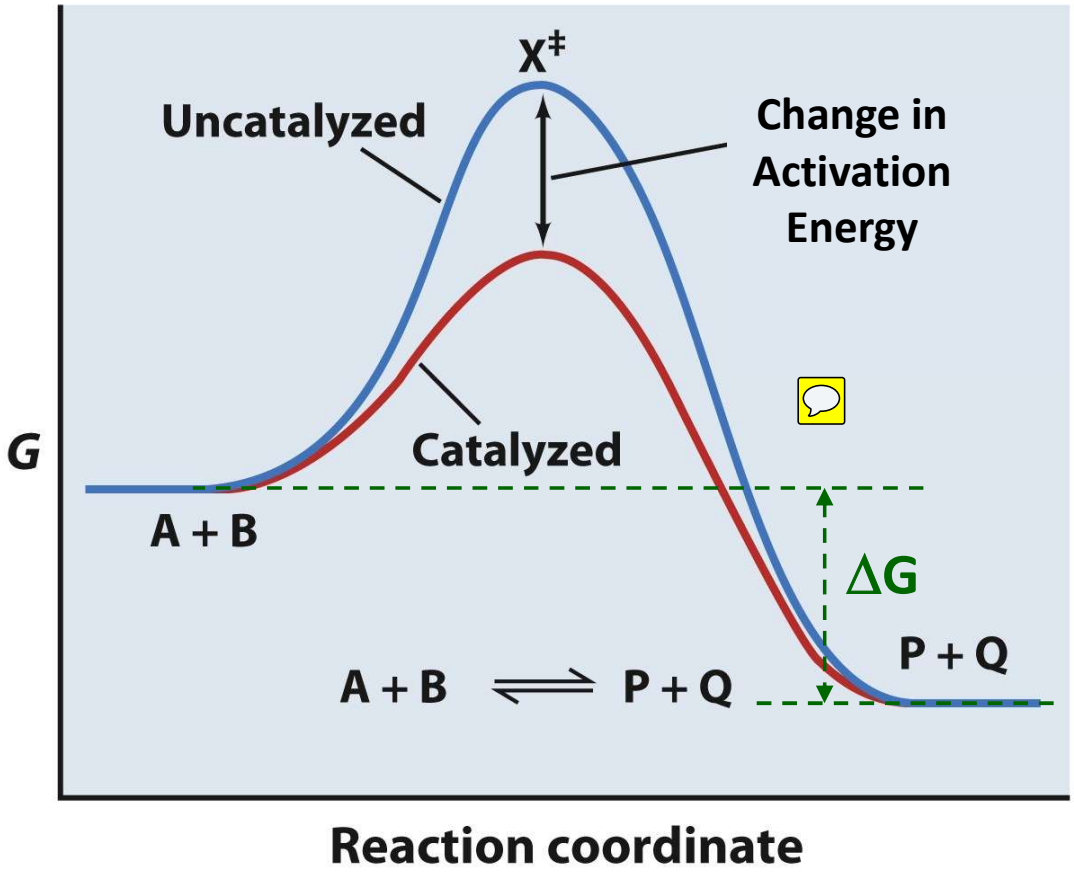


Figure 11-7

Question 1: Thermodynamics and Enzymes

a) How do we work out if a reaction is favourable or unfavourable?

b) If some reaction is unfavourable, how does the human body get around this?

c) True or false: Enzymes can change the energy values in a reaction

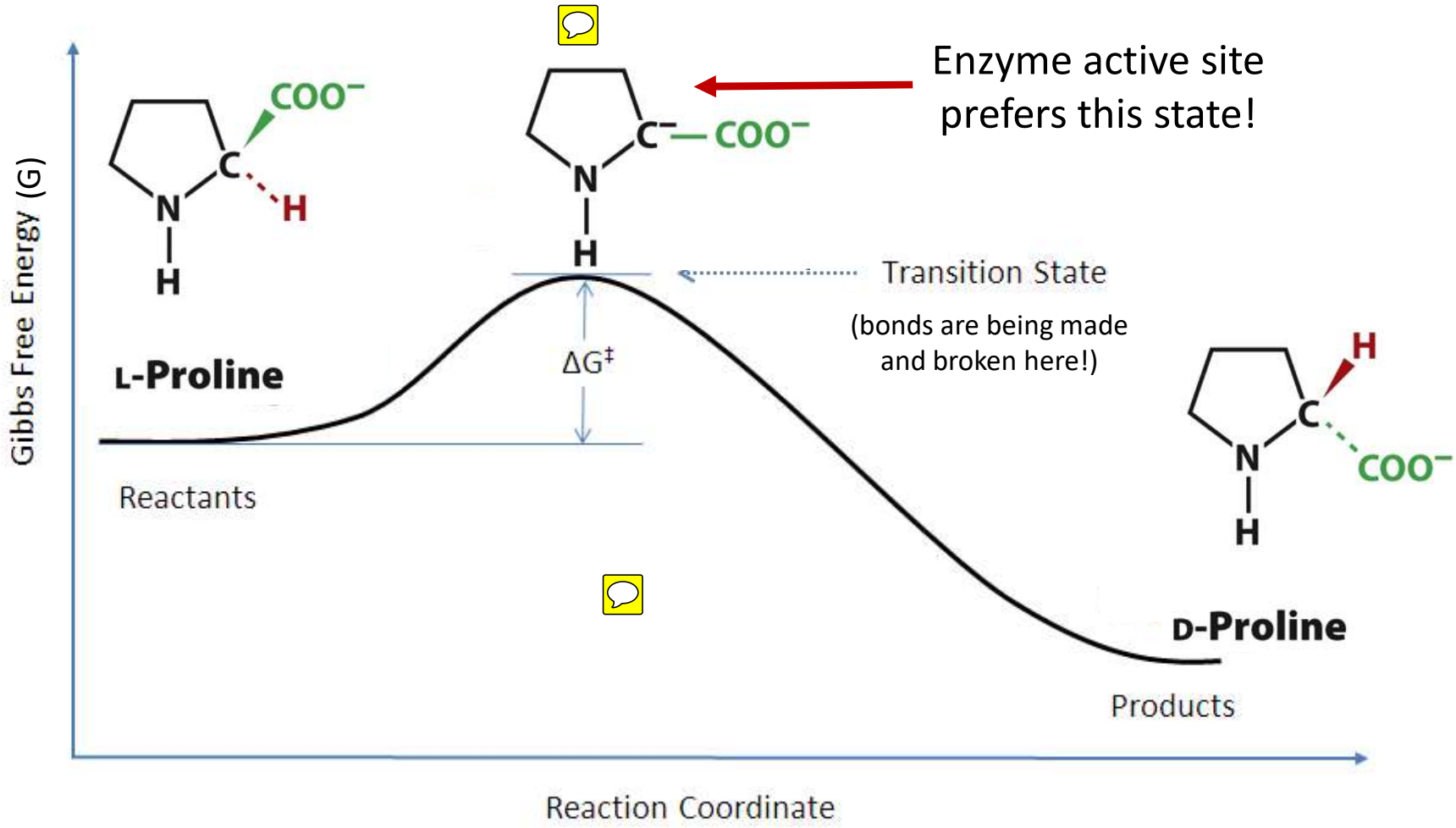
Enzyme Active Sites

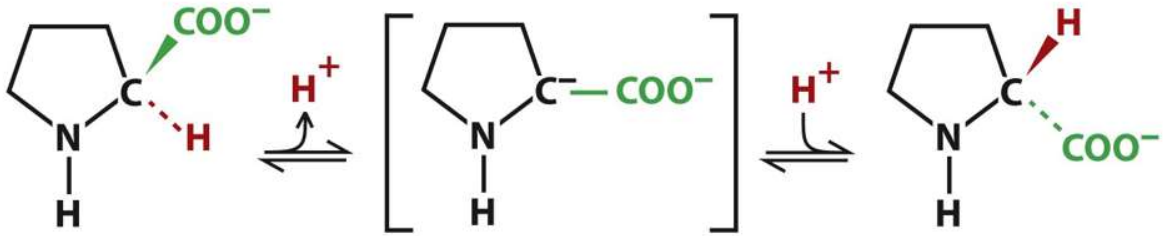
- Enzymes have **active sites** or **binding pockets** which the substrates bind to

- The amino acids in this binding pocket then help the reaction take place faster

- The active site prefers the **transition state** of the reaction the most, even more than the substrates or the products

Active Site and Transition State





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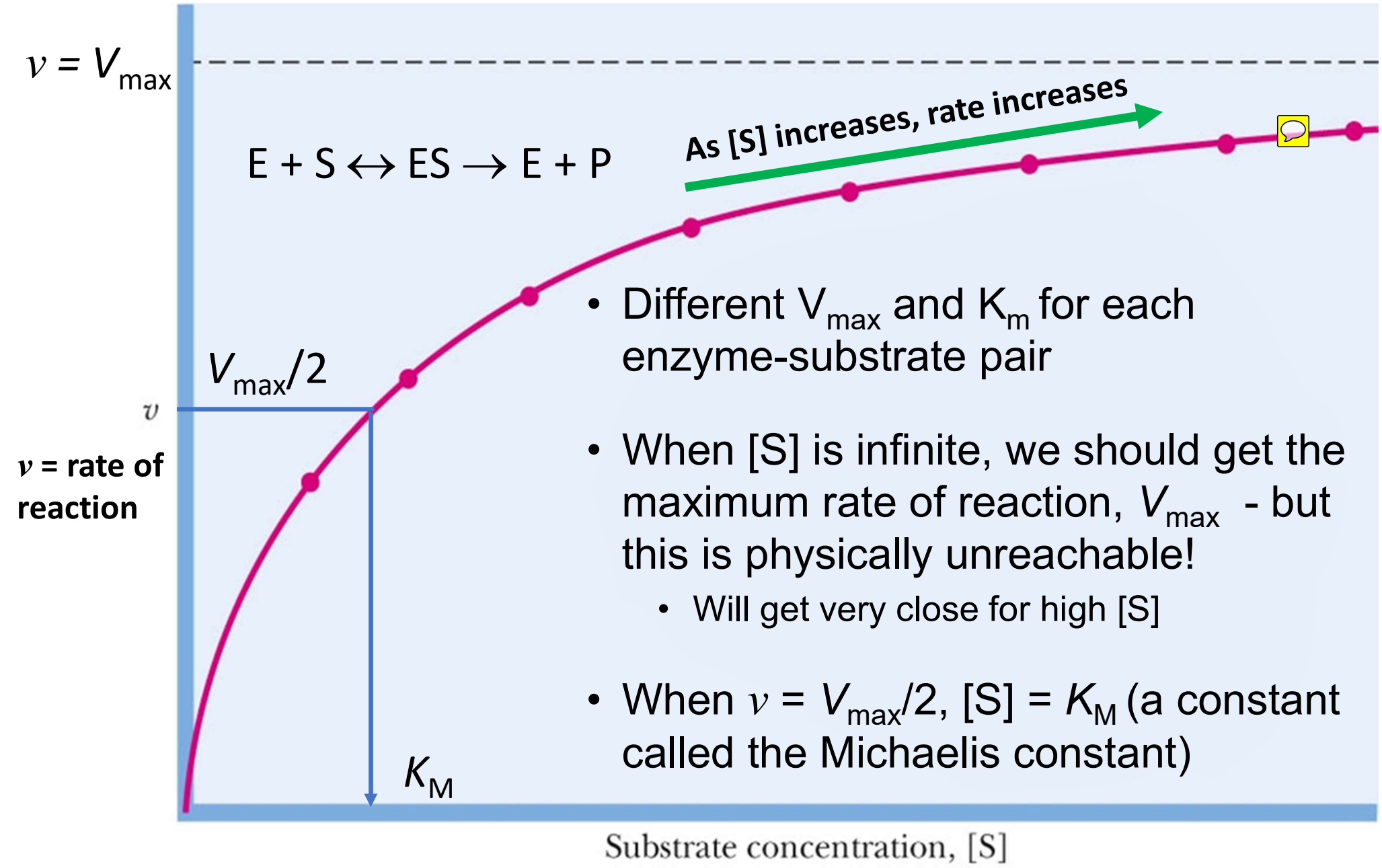
Enzyme Kinetics



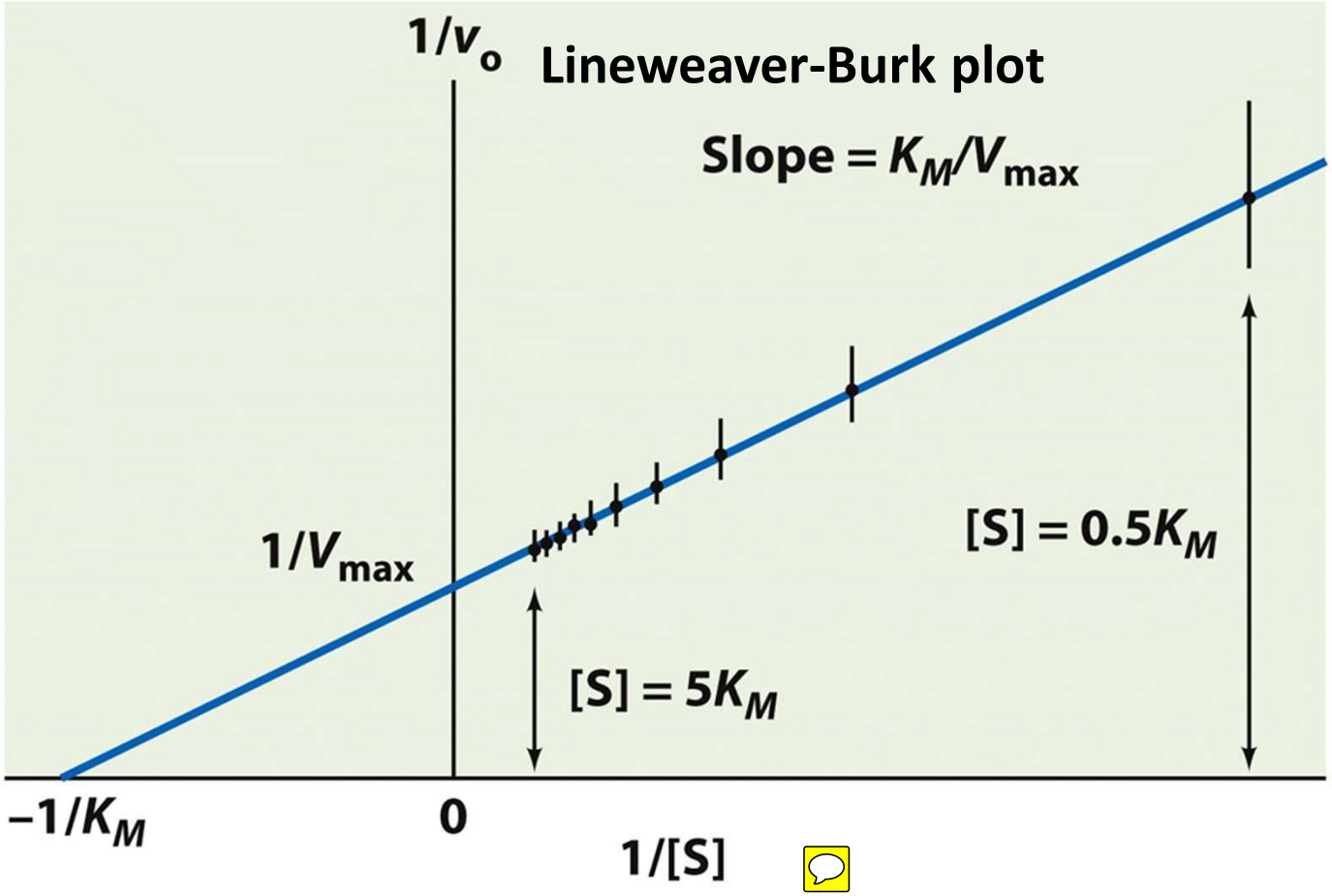
- Different V_{max} and K_m for each enzyme-substrate pair
- When [S] is infinite, we should get the maximum rate of reaction, V_{max} - but this is physically unreachable!
  - Will get very close for high [S]
- When $v = V_{max}/2$, [S] = K_M (a constant called the Michaelis constant)

Straightening the curve!

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

y = m x + b

- y intercept: $1/V_{max}$
- x intercept: $-1/K_M$
- Slope: K_M/V_{max}
- No need for large [S]

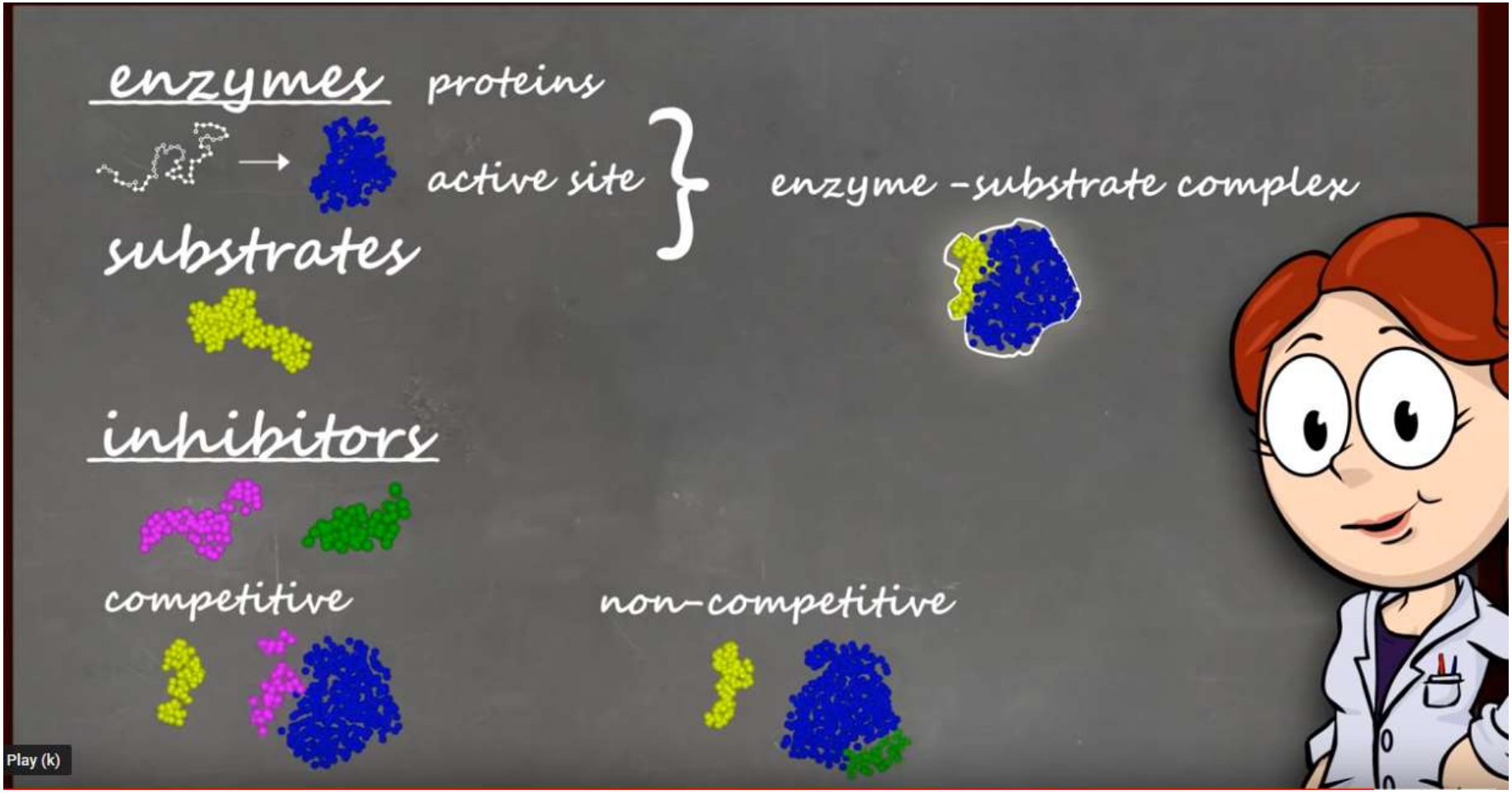


Enzyme Inhibition

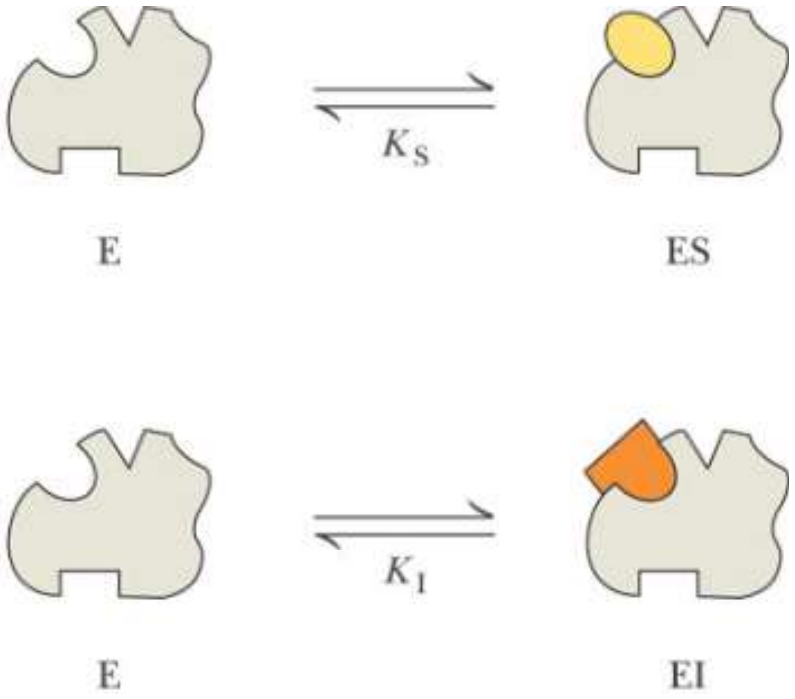
- An inhibitor is a small molecule that prevents product formation
  - The inhibitor can bind strongly to the **active site** and prevent the substrate from binding, **or**
  - It can bind to **some other site** on the enzyme and affect the reaction indirectly.

- Most drugs sold in pharmacies are enzyme inhibitors.

https://www.youtube.com/watch?v=c5j6ExHLFD8



Competitive inhibition



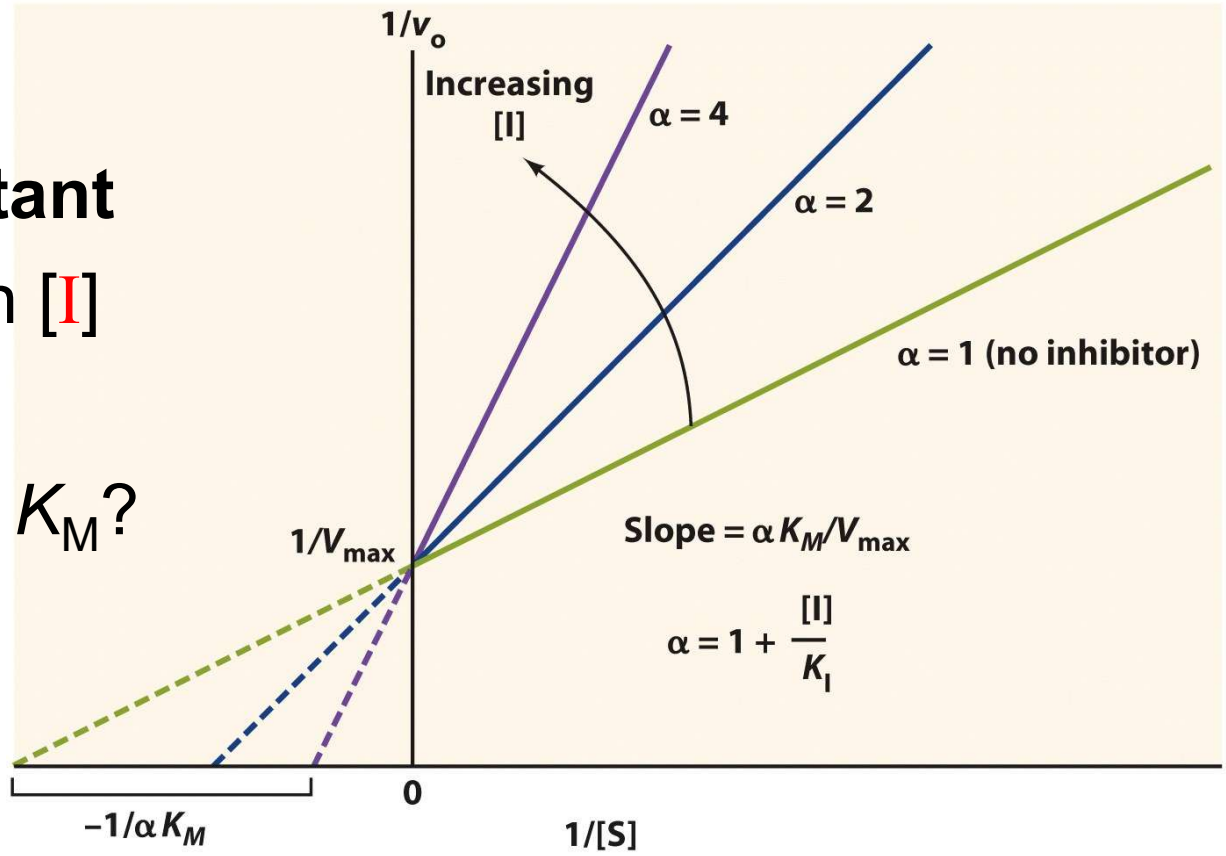
- **Inhibitor I** binds to the **active site:** usually a structural analogue
- Less enzyme E is available for binding S and producing P!
- Rate of reaction i.e. product formation goes down when **I** is present

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

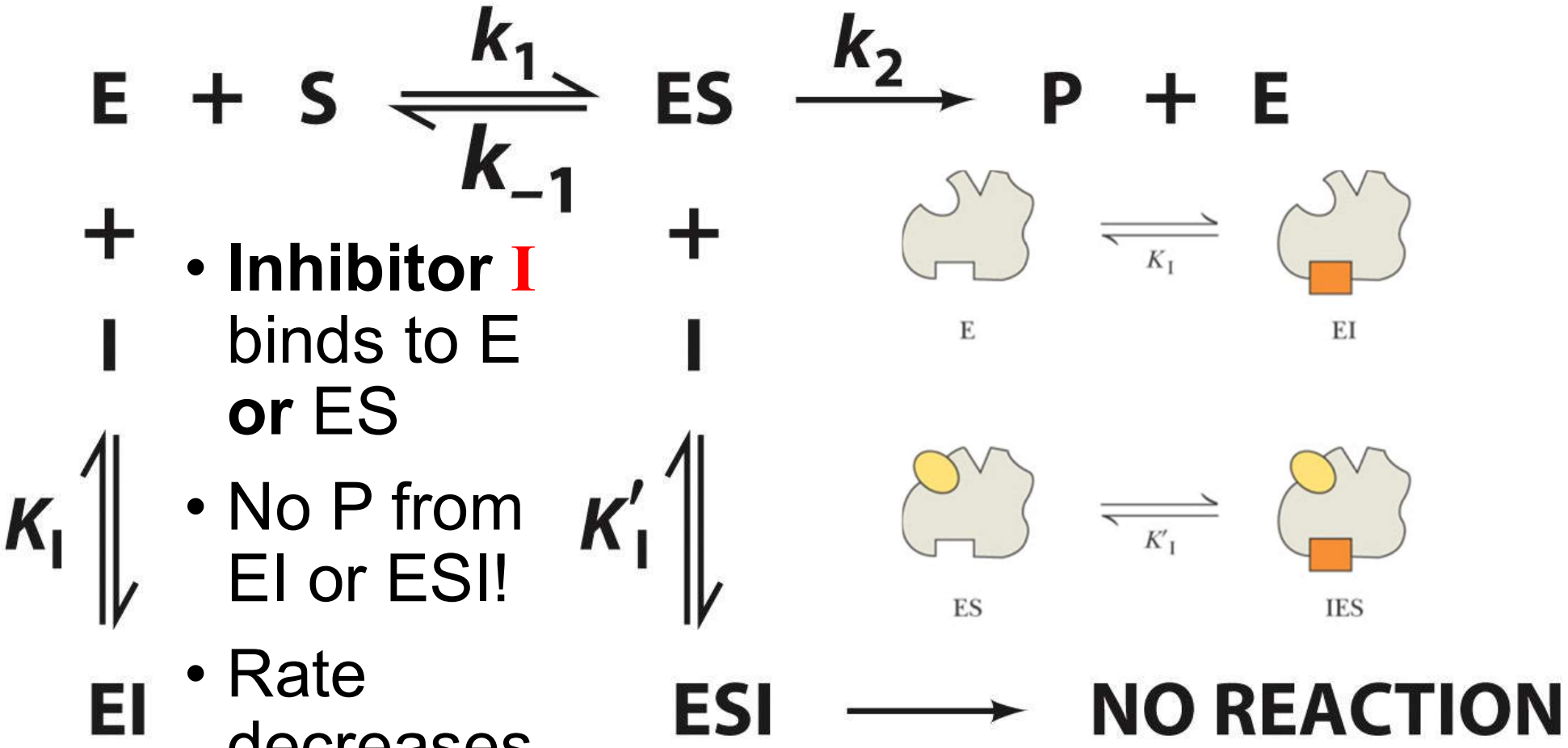
$$E + I \rightleftharpoons EI + S \longrightarrow \text{no products}$$

Competitive inhibition

- K_M changes
- **V_{max} remains constant**
- Slope increases with [I]
- $1/K_M$ decreases
- So what happens to K_M?



Non-competitive inhibition



- **Inhibitor I** binds to E **or** ES
- No P from EI or ESI!
- Rate decreases

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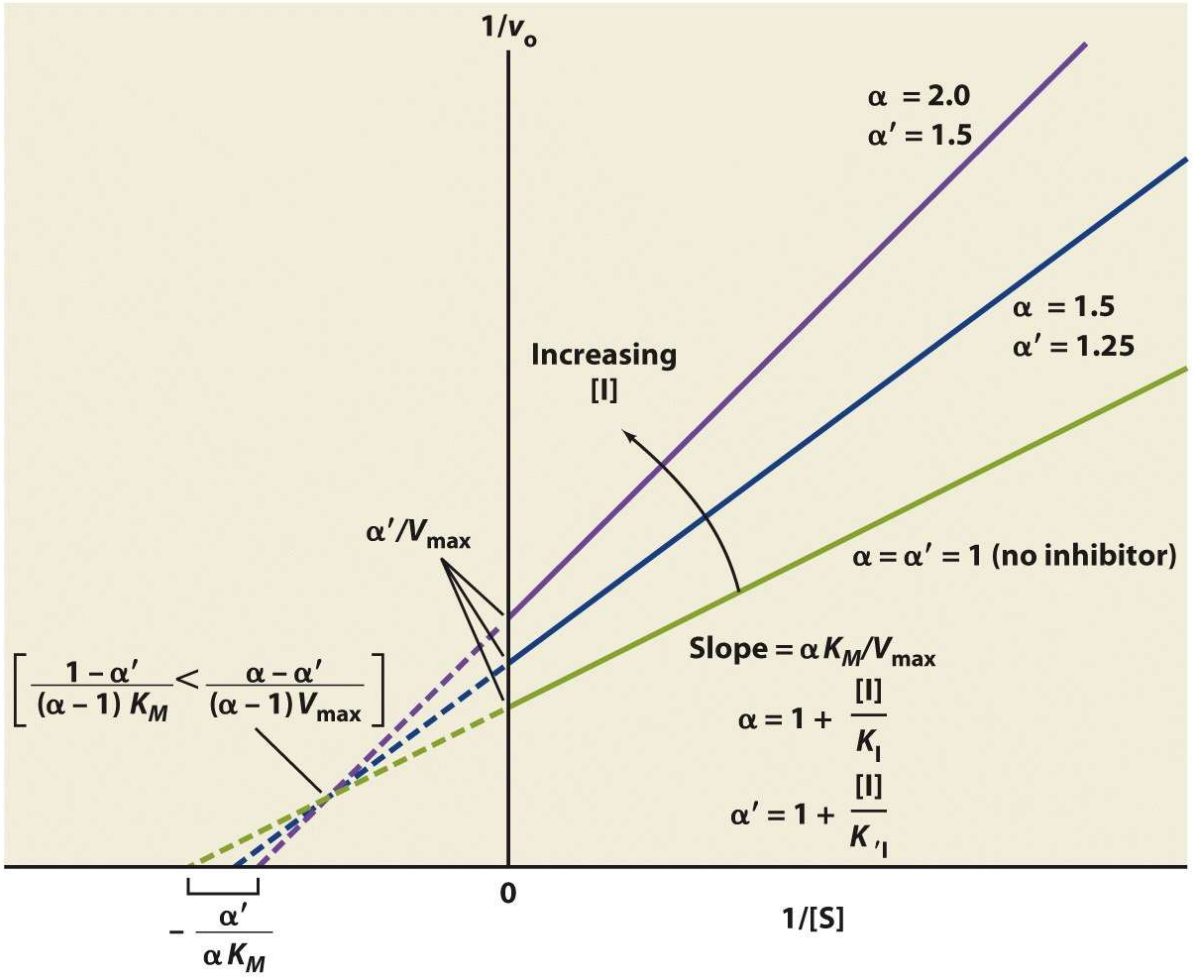
Non-competitive inhibition

- Inhibition by binding to a site other than the active site at any stage during catalysis
  - I does not compete with S for the active site
  - ***V*max changes** but K_M remains fairly constant
  - Both E and ES can bind I



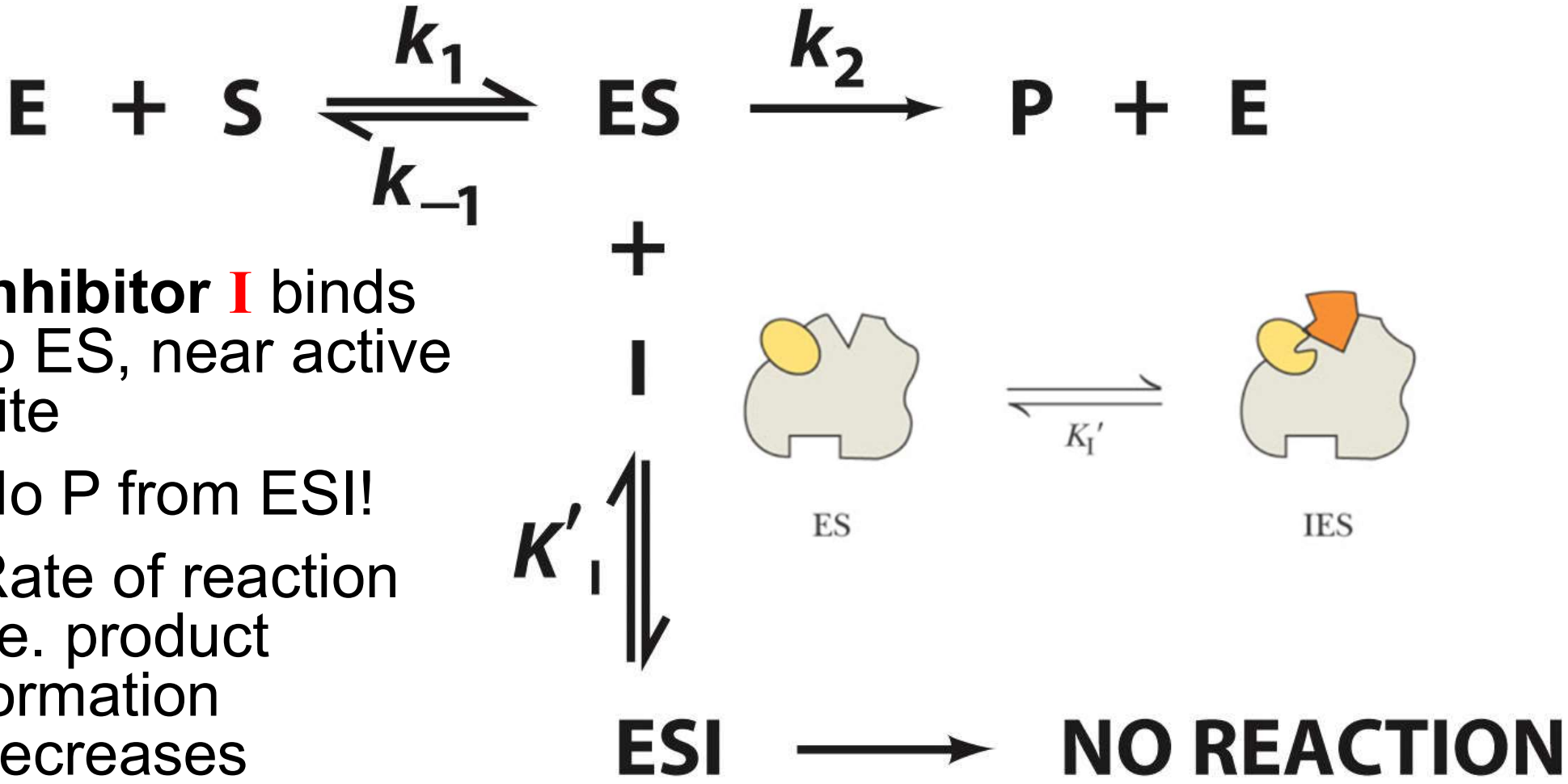
https://www.youtube.com/watch?v=jG20xIkiPFM

Uncompetitive Inhibition

Inhibitor binds only to enzyme-substrate complex

i.e. inhibitor loves "cooperation"

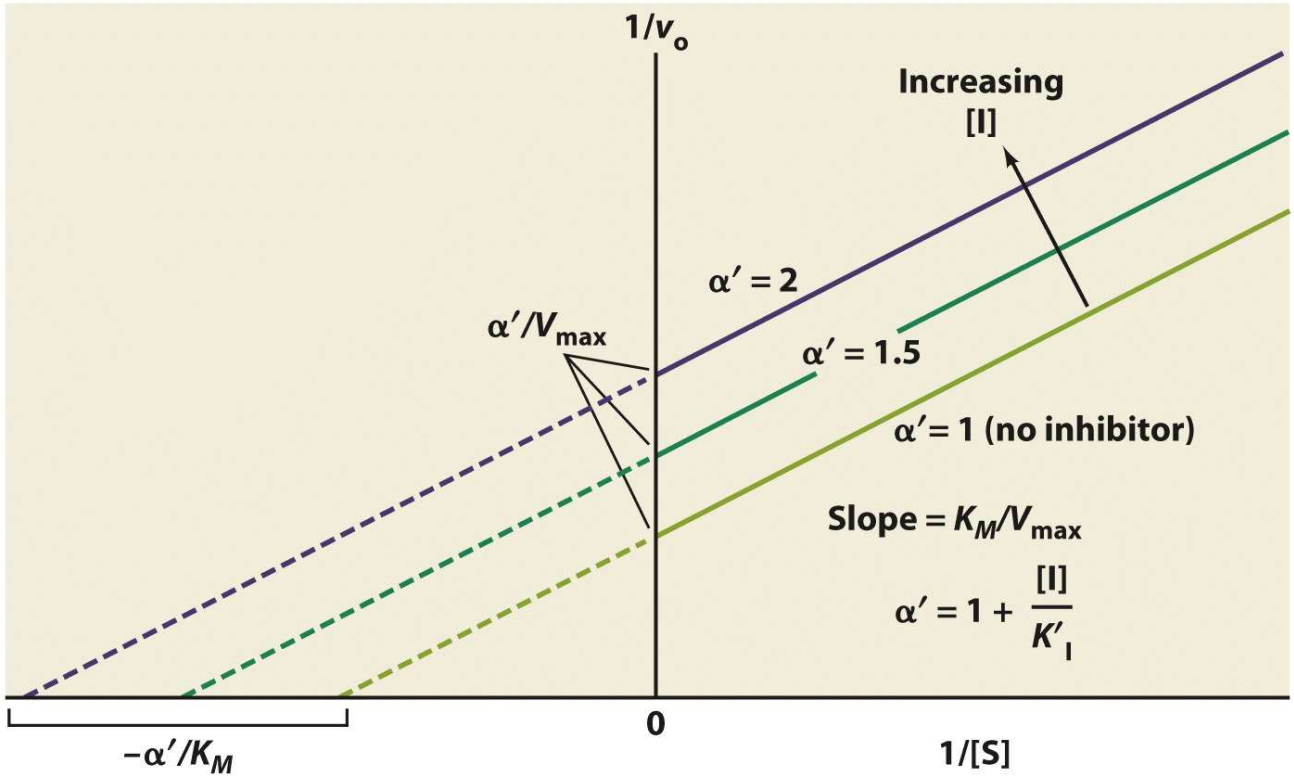
Uncompetitive inhibition



- **Inhibitor I** binds to ES, near active site
- No P from ESI!
- Rate of reaction i.e. product formation decreases

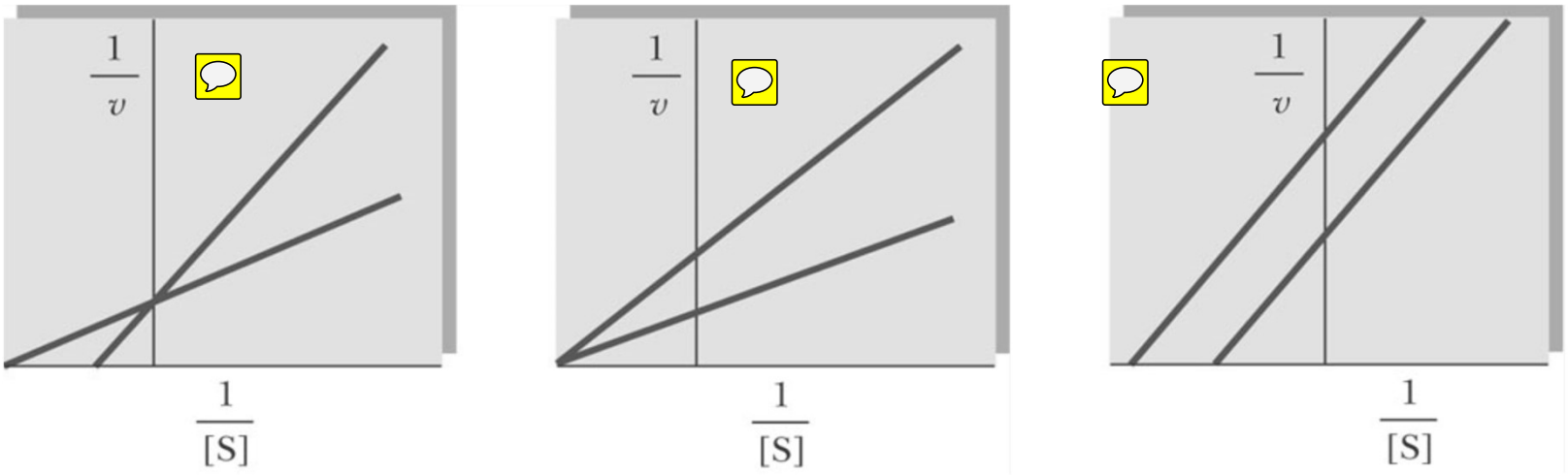
Uncompetitive inhibition

- Inhibitor **I** binds to enzyme <u>after ES has formed</u>
  - **I prevents product formation**
  - <u>Dead-end complex</u>
- Both <u>V_{max} and K_M change</u> in this case.
- **Slope (K_M / V_{max}) is the same (parallel lines!)**
- This kind of inhibition is also possible if **I** binds to enzyme-cofactor complex, preventing enzyme reaction.



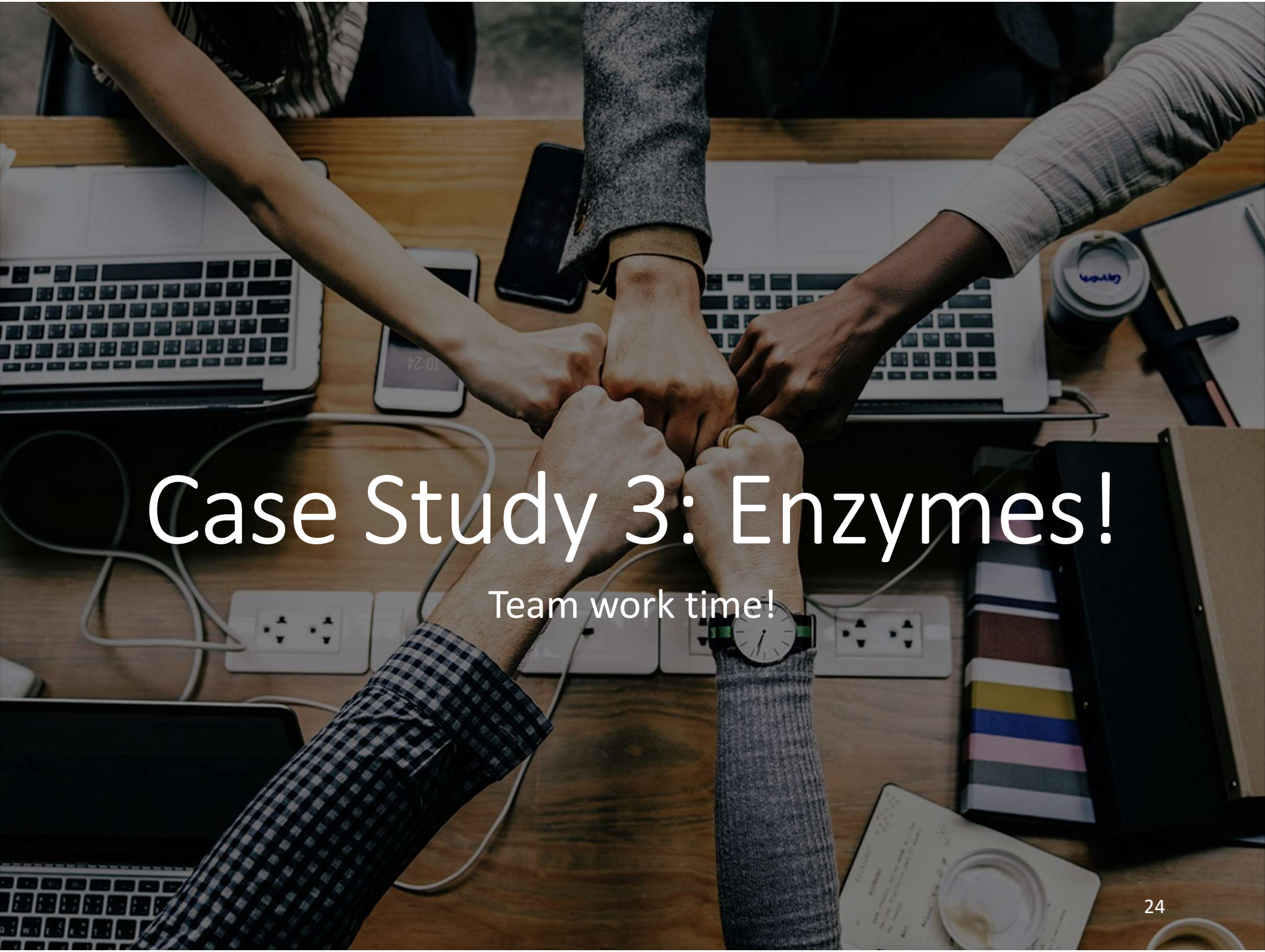
Question 2: Enzyme Inhibition

a) Inhibition types: which is which?



b) What happens to K_M and V_{max} in each case?

Part 2



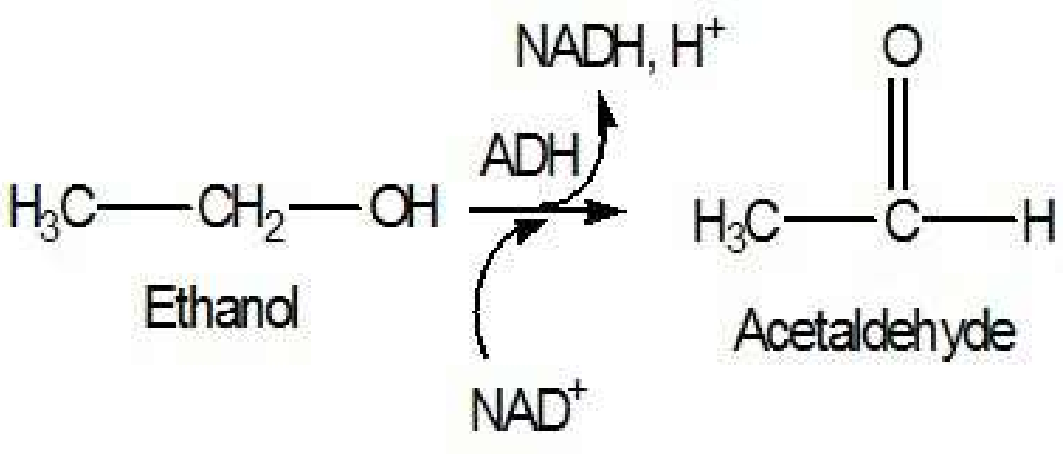
Case Study 3: Enzymes!

Team work time!

Case Study Q&A!

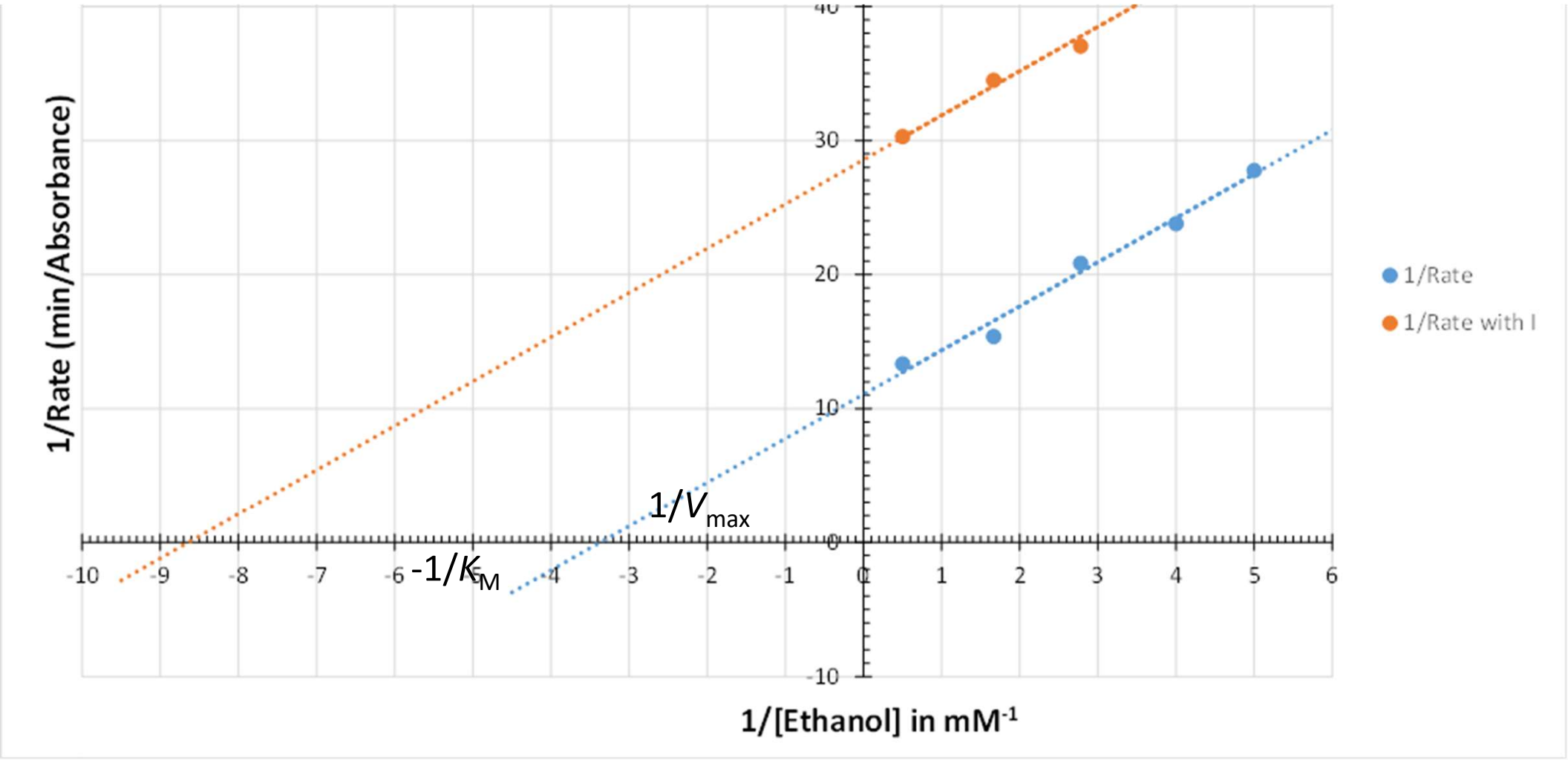
Case Study Question 1:

A treatment for methanol poisoning is to have the victim drink large amounts of ethanol. Why might this be an effective treatment?



Case Study Question 2:

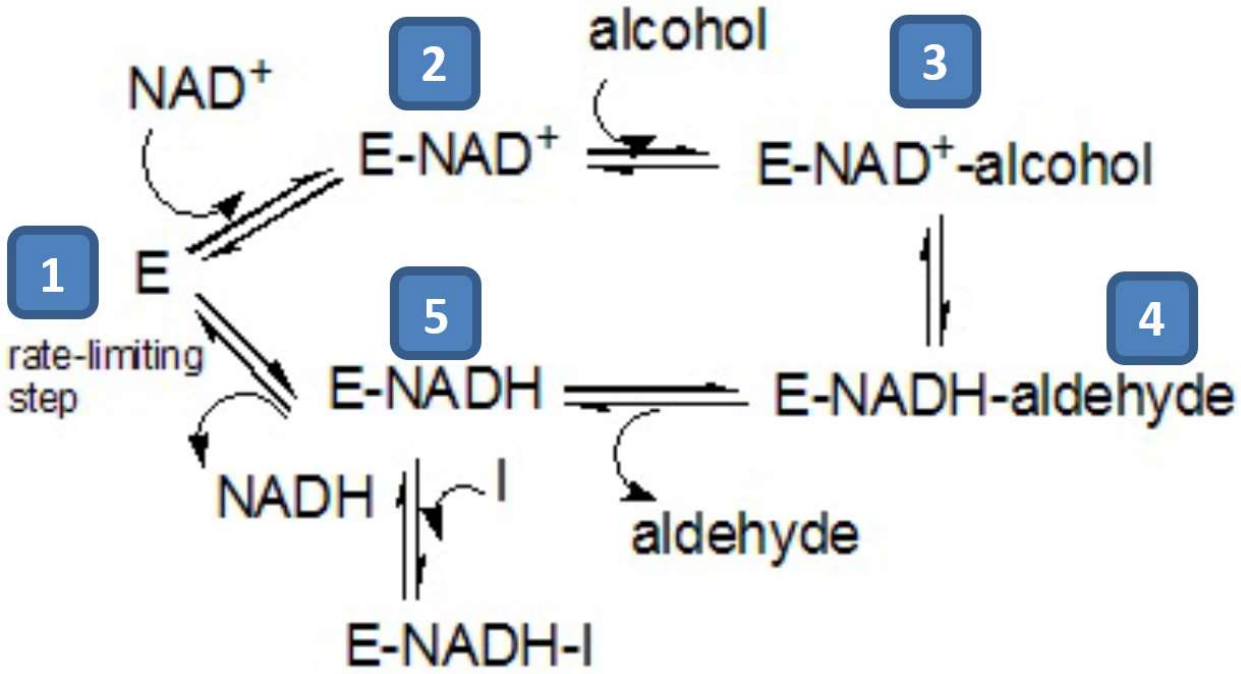
What are the K_M and V_{max} values for ADH in the absence of inhibitor and in the presence of the inhibitor? (I is *N*-1,5-dimethylhexylformamide)



Case Study Question 3:

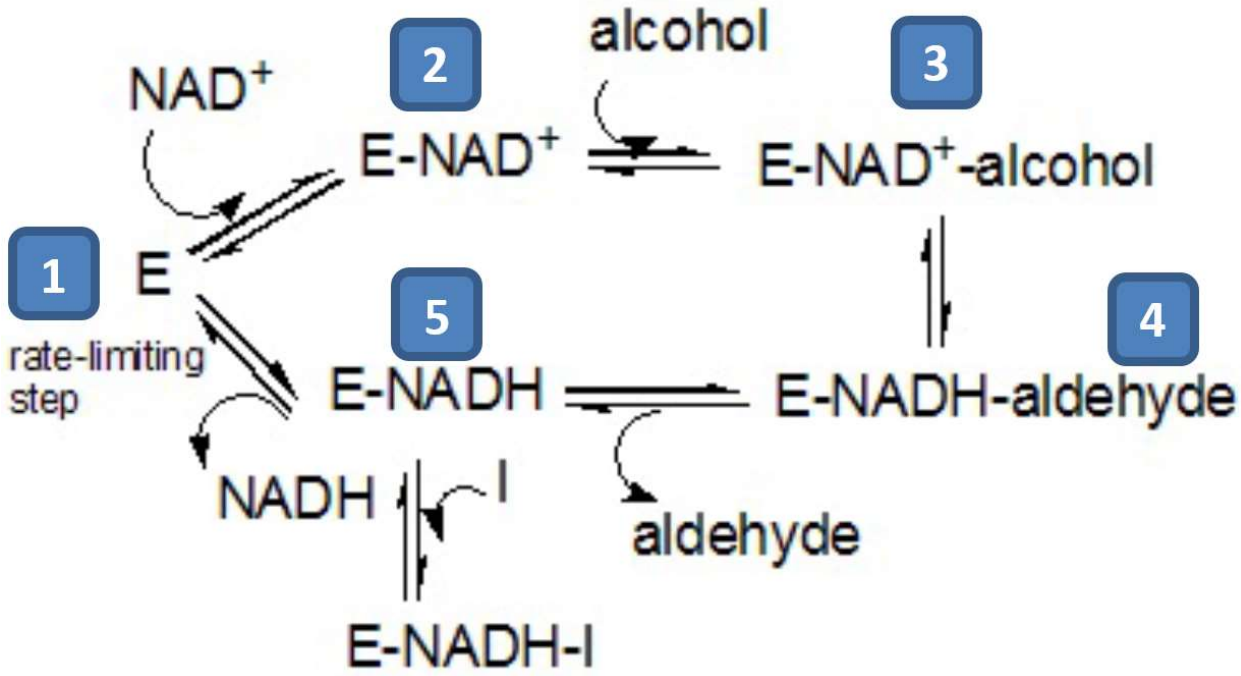
What type of inhibitor is N-1,5-dimethylhexylformamide? Explain.

(Hint: use the mechanism proposed and the kinetic data to support your answer).



Case Study Question 4:

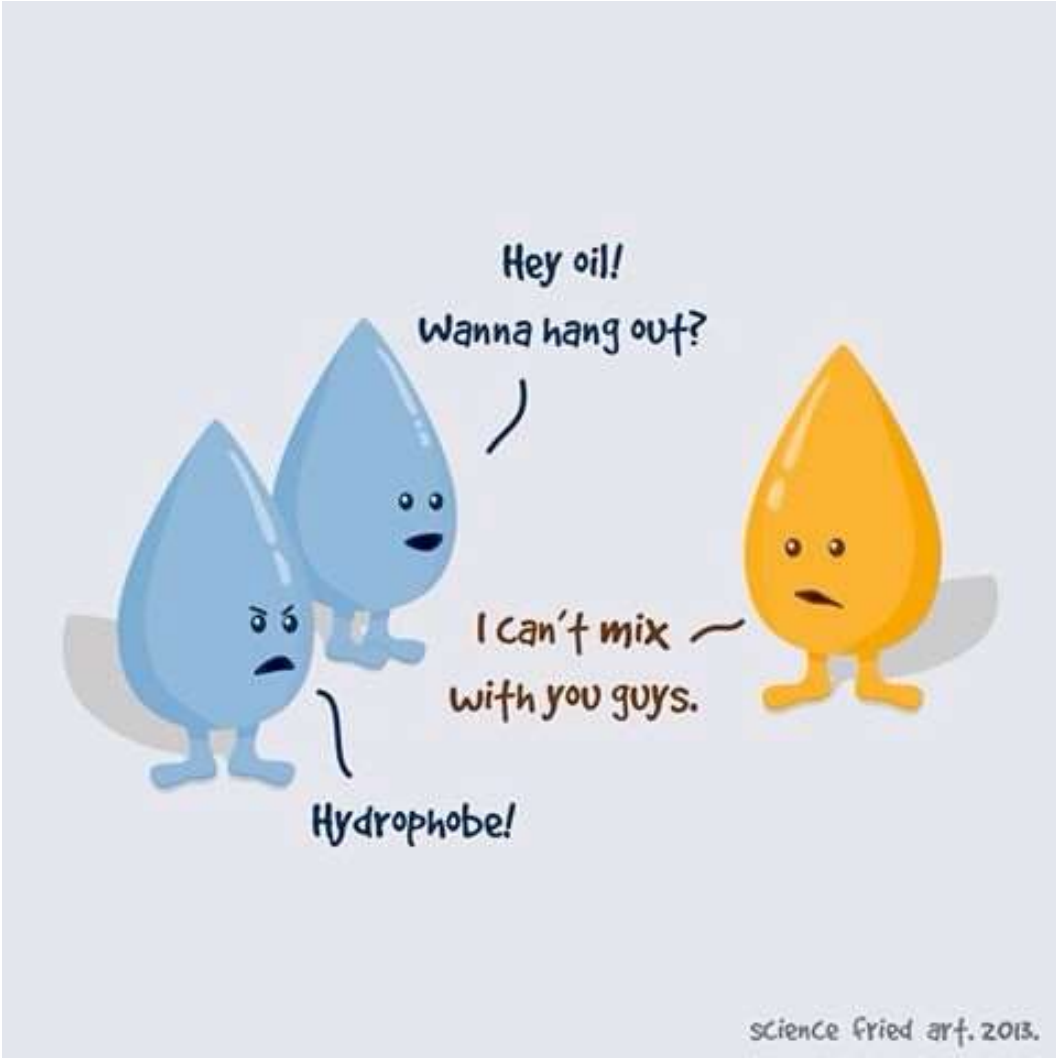
The scientists found that a class of compounds called pyrazoles were also inhibitors of ADH. These inhibitors bind to the E-NAD$^+$ complex, preventing the alcohol from binding. What kind of inhibitor are **pyrazoles**? Are these inhibitors the same or different to the **formamides**?



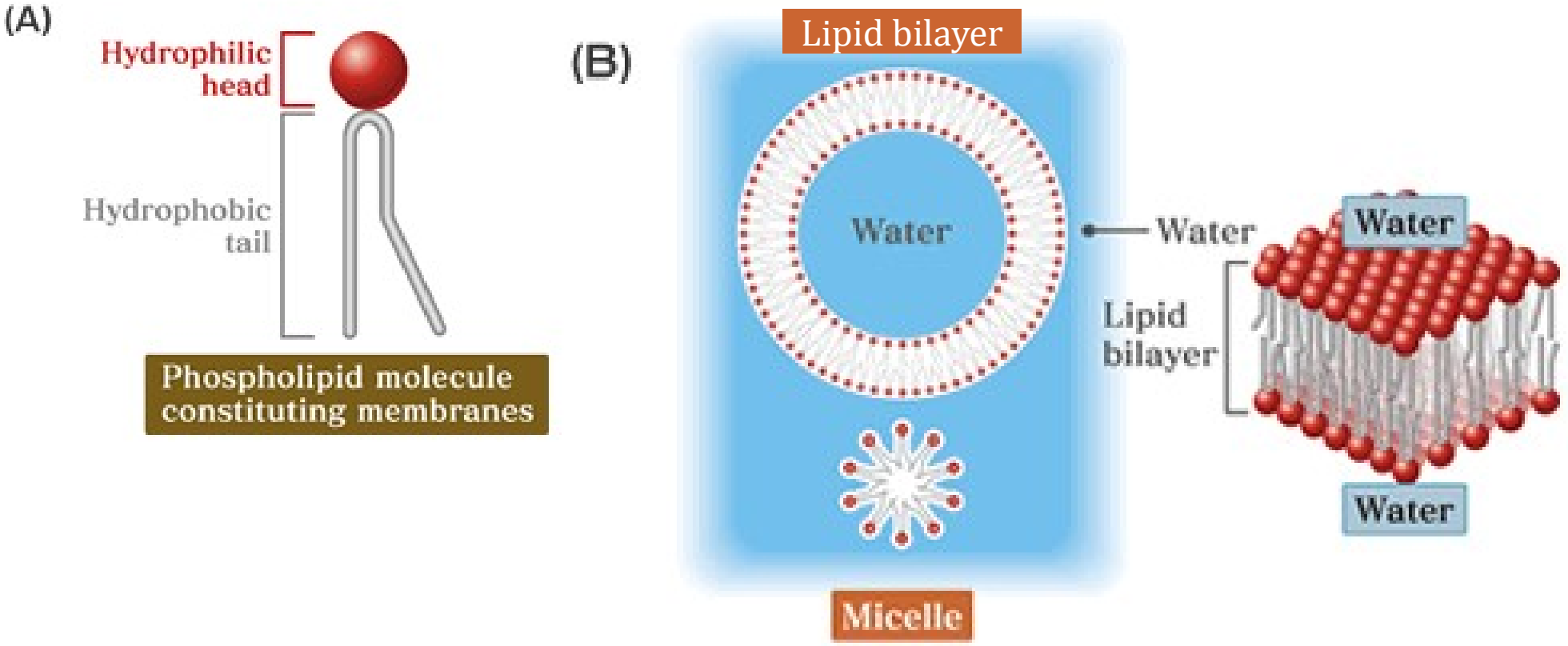
Part 3

Biological Membranes

- Membranes are made of **lipids** and proteins
- Lipids are molecules that are either completely hydrophobic or amphipathic (predominantly hydrophobic with a hydrophilic head group)

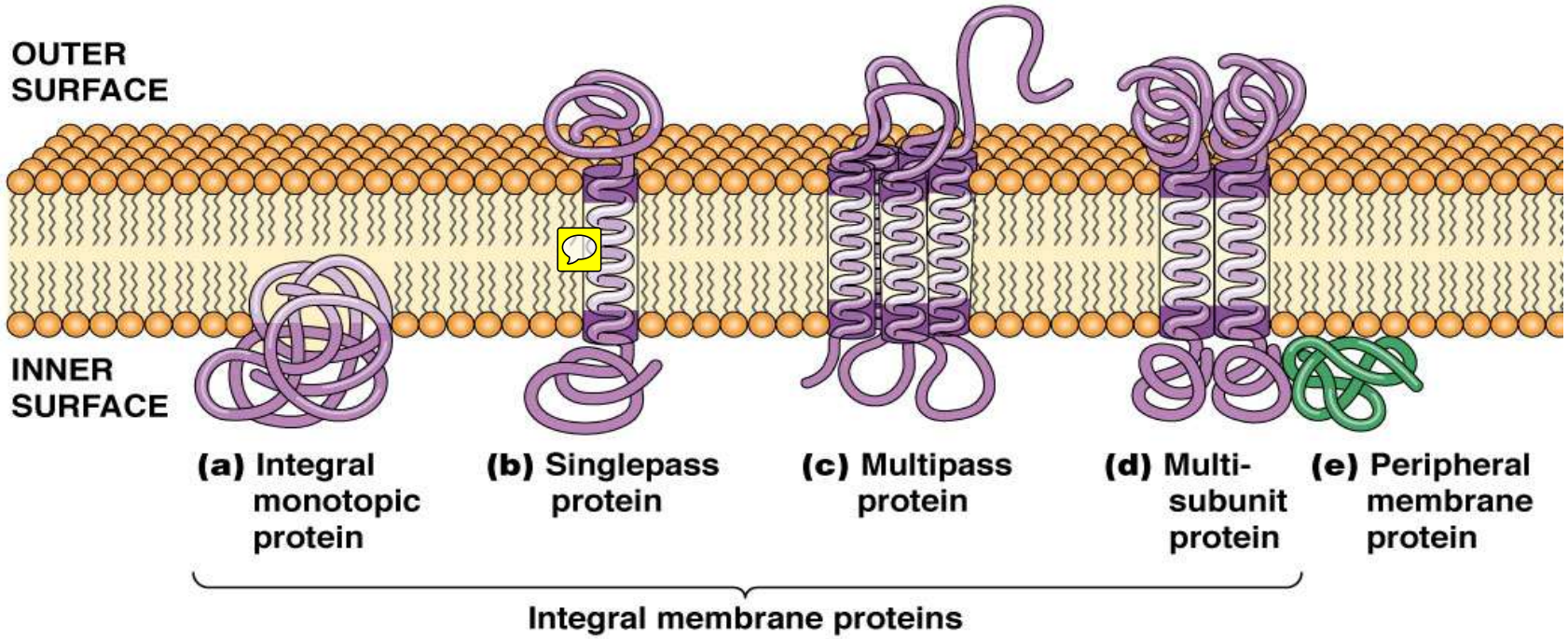


Biological membranes form lipid bilayers



Proteins in the membrane

- Integral and peripheral membrane proteins.
- Since membrane proteins are not evenly distributed, overall the biological membrane is asymmetric.



Question 3: Membranes

A biological membrane is **asymmetric**. Which of the following are true?

a. The lipids are not evenly distributed transversely in the membrane.
b. The proteins are not evenly distributed over the surface of the membrane.
c. Patches of cholesterol and other lipids occur on the surface of the membrane.
d. Integral membrane proteins show preferences for membranes with specific lipids.
e. All of the above.

Question 3: Membranes

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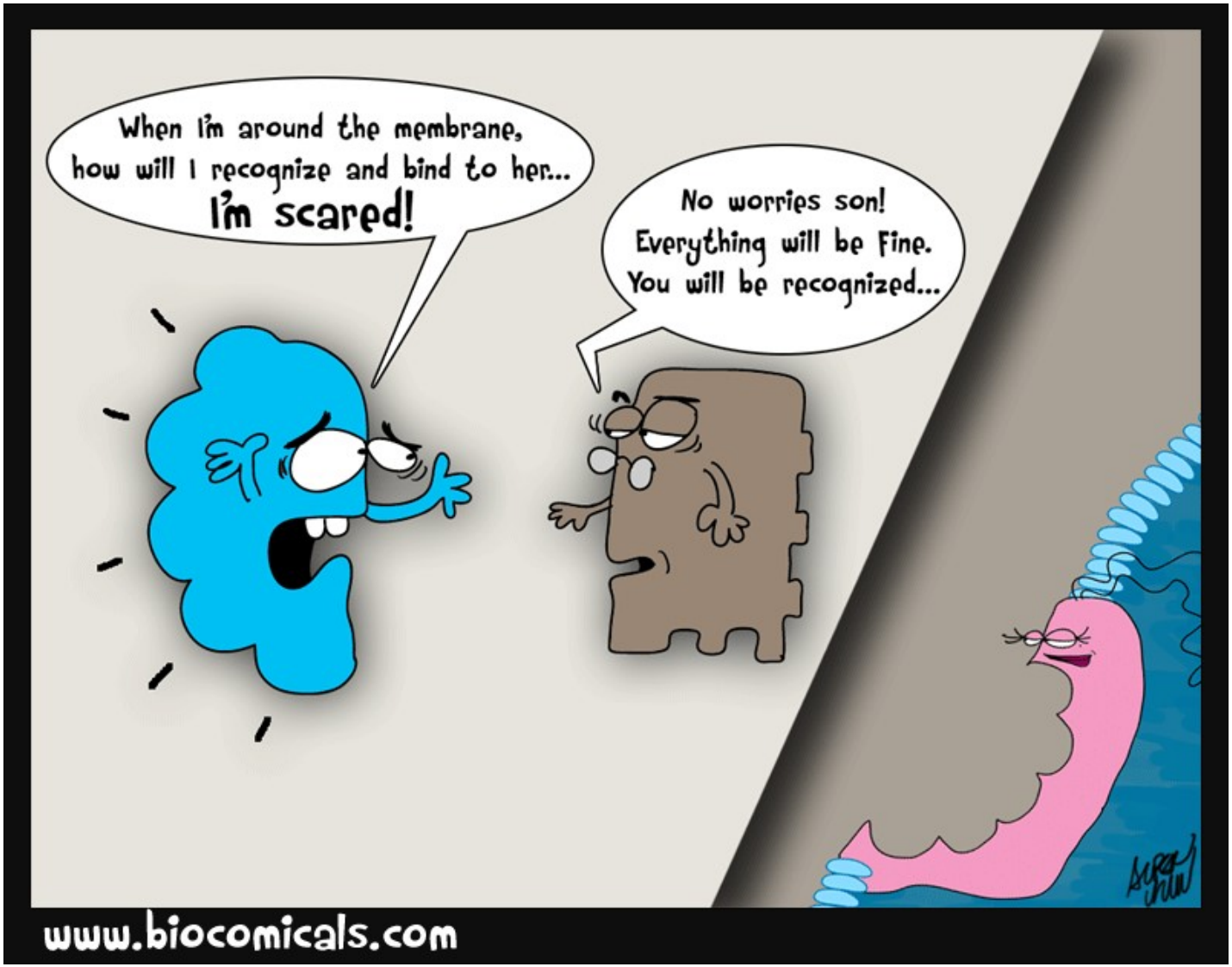
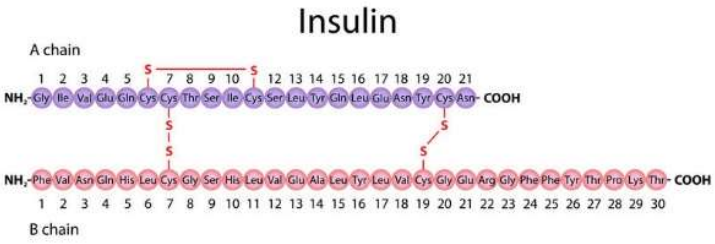
e. **All of the above.**

Biochemical signalling is specific

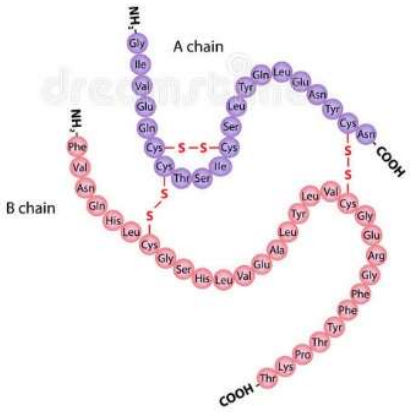


Pancreatic Islet Hormones Control Fuel Metabolism (a.k.a. Islet of Langerhans)



3 polypeptide hormones released by the Islet cells

1. α cells: **glucagon** (29 residues)
2. β cells: **insulin** (51 residues)
3. δ cells: **somatostatin** (14 residues)



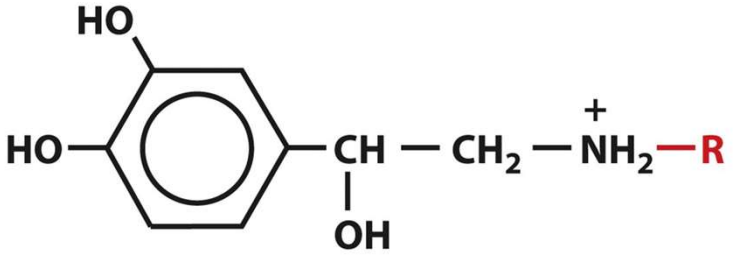
Glucagon and **insulin** have opposite effects on sugar metabolism:

Blood glucose too **low** ➔ **glucagon** released ➔ liver **releases** more glucose

Blood glucose too **high** ➔ **insulin** released ➔ liver **stores** more glucose

Somatostatin inhibits the release of both glucagon and insulin.

Epinephrine and Norepinephrine Prepare the Body for Action

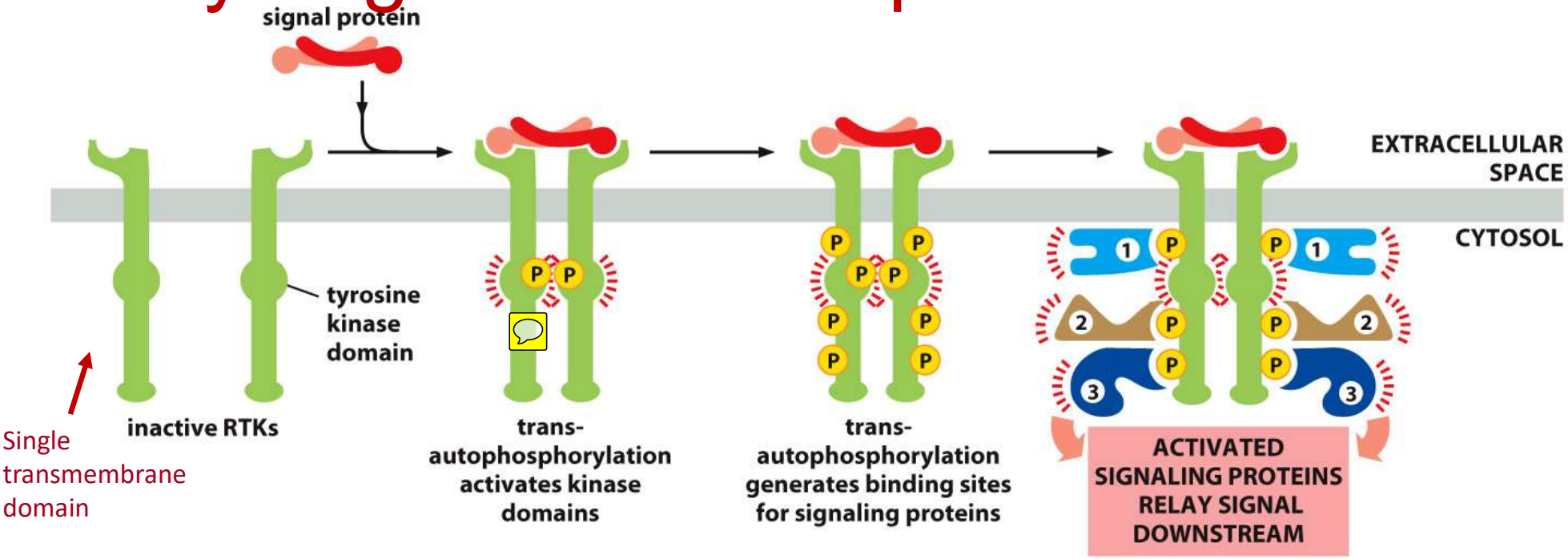


R = H **Norepinephrine (noradrenalin)**

R = CH_3 **Epinephrine (adrenalin)**

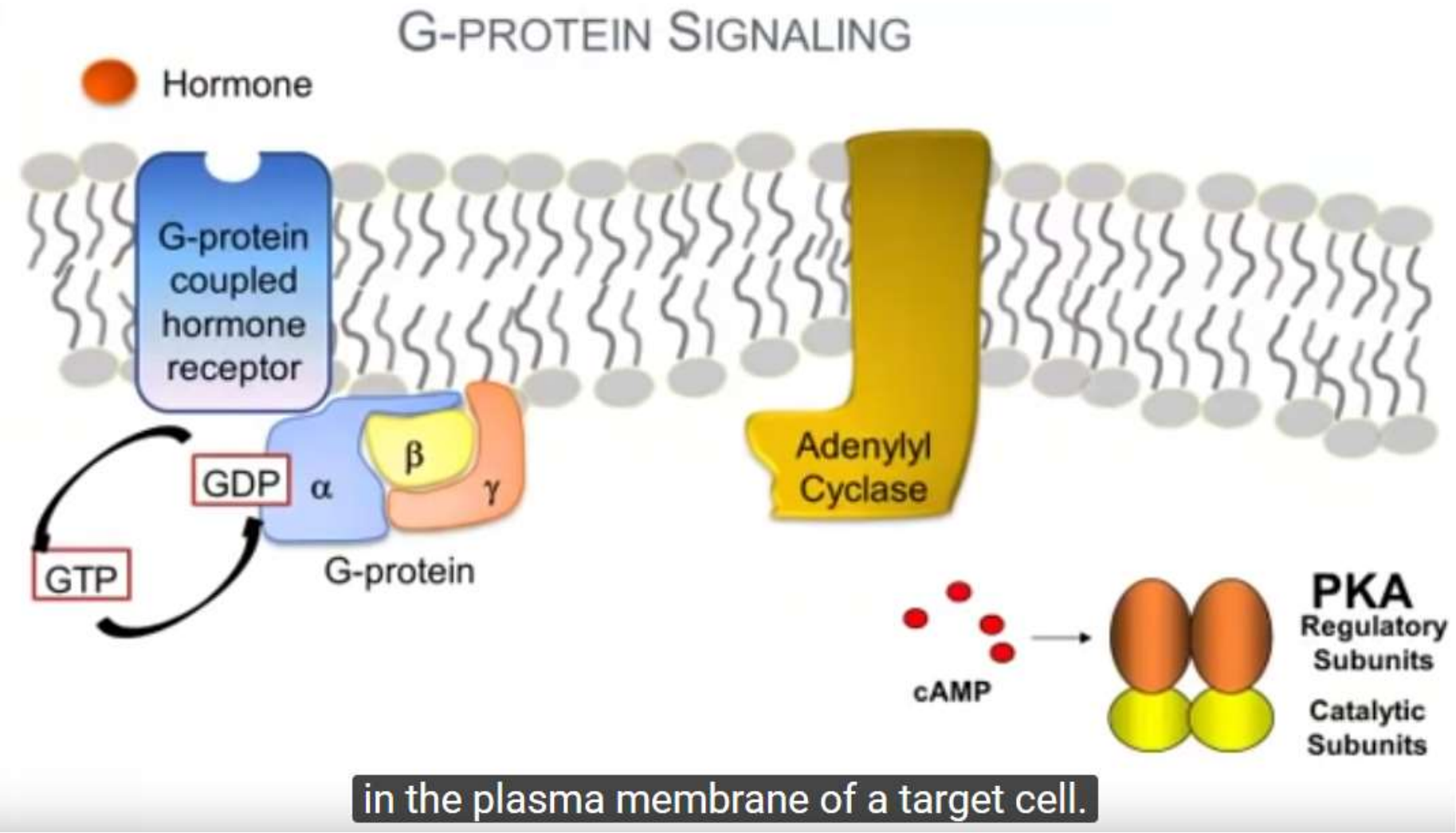
- The **medulla** (core) of the **adrenal glands** makes two ***catecholamine hormones***:
  1. **norepinephrine (noradrenalin)** and
  2. its methyl derivative **epinephrine (adrenalin)**
- These bind to membrane-bound α- and β-adrenergic receptors on the different tissues, leading mainly to smooth muscle contraction and smooth muscle relaxation respectively
- The main function of these hormones is to overcome normal regulation for “flight-or-fight” responses

Receptor Tyrosine Kinases (RTKs) Convey Signals using a phosphate relay: e.g. Insulin receptor



- Activation by dimerization and autophosphorylation.
- Adaptor proteins link RTKs with other signalling proteins, such as G proteins and additional kinases, to amplify the signal.
- After the signal is conveyed, deactivation is by protein phosphatases which remove phosphate groups from proteins.

GPCR signalling uses secondary messengers



https://www.youtube.com/watch?v=wC2_7Ror3qY

Question 4: Biochemical Signalling

The binding of ligands to many GPCRs leads to:

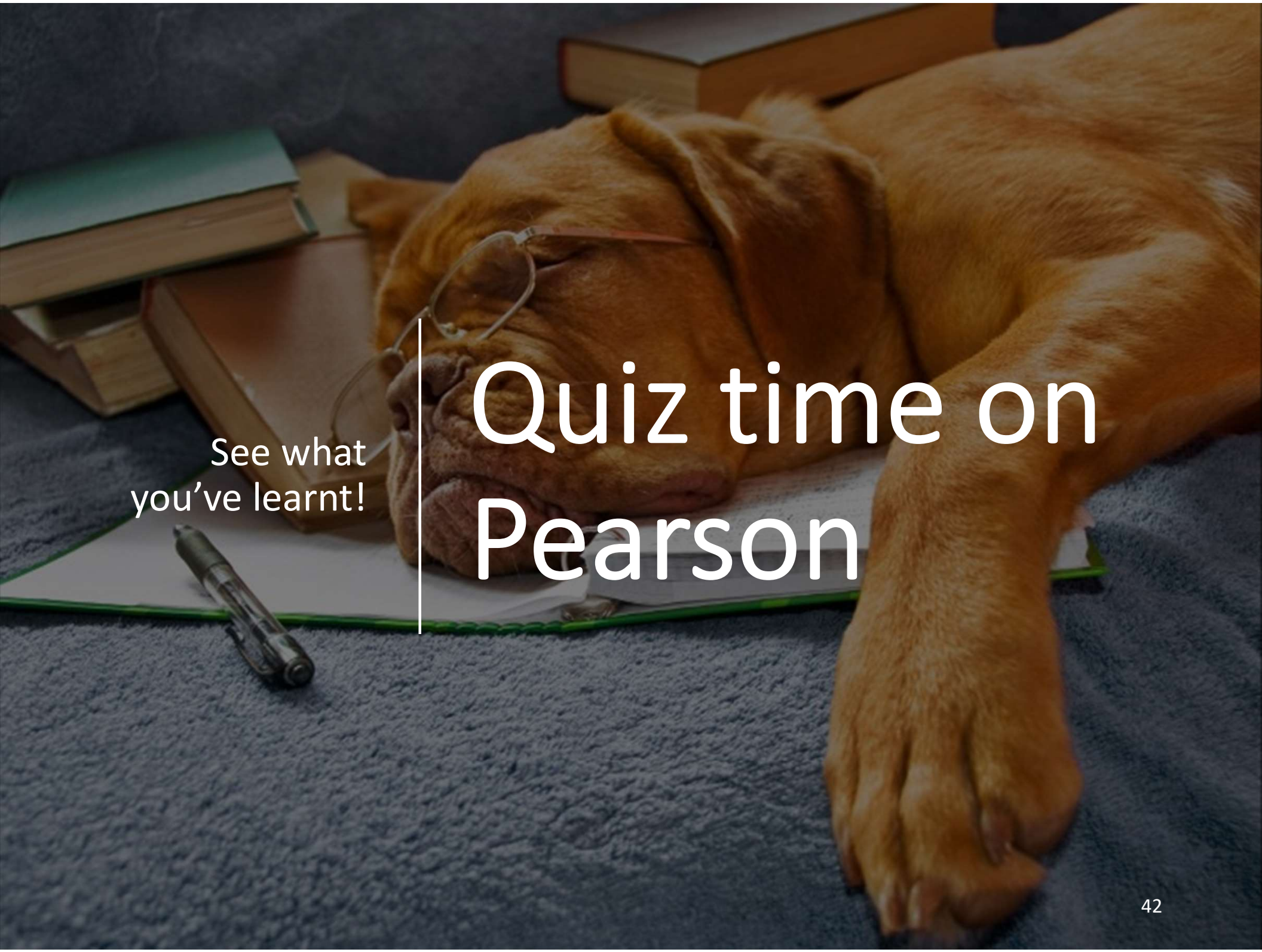
a. A decrease in concentration of intracellular signal molecules called secondary messengers.

b. An increase in concentration of intracellular signal molecules called secondary messengers.

c. A decrease in concentration of extracellular signal molecules called primary messengers.

d. An increase in concentration of extracellular signal molecules called primary messengers.

e. Does not involve primary or secondary messengers.


See what
you've learnt!
Quiz time on
Pearson