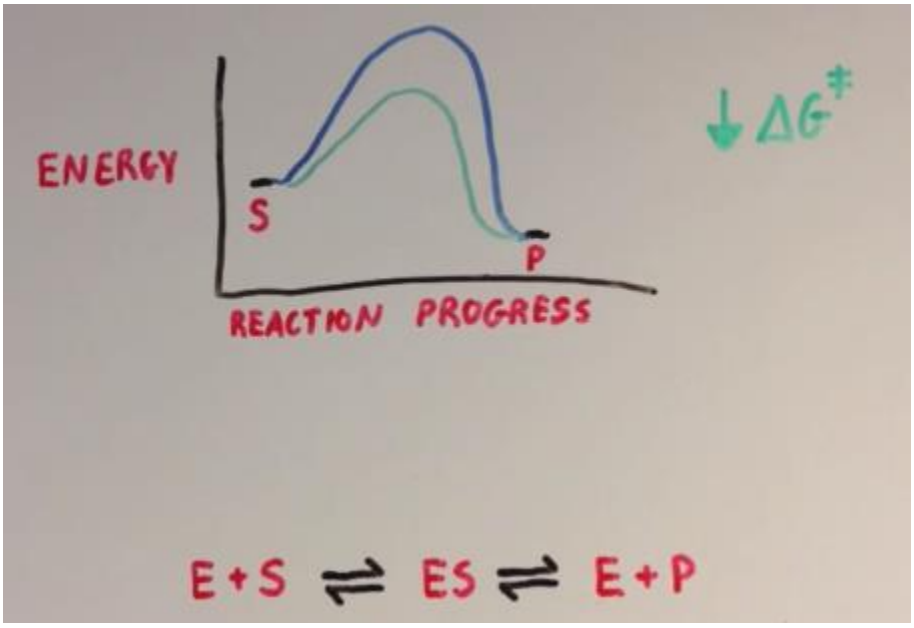
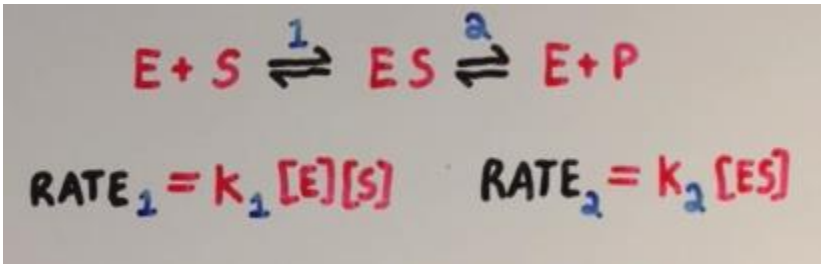
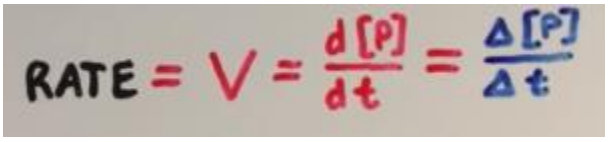
An introduction to enzyme kinetics



Rate at which a reaction proceeds is dependent on the constant 'k' and the concentration of the enzyme and substrate



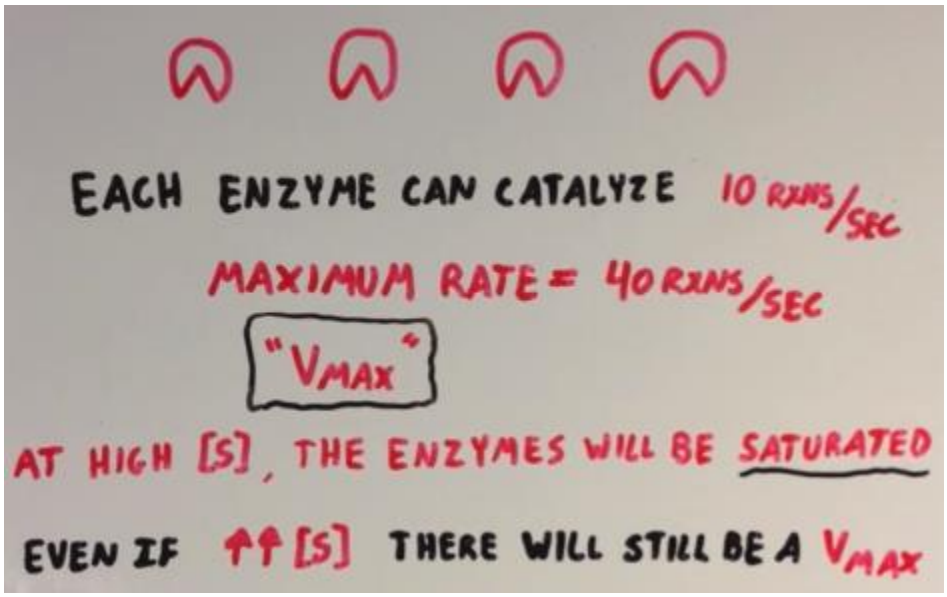
Rate is equal to the *rate of change* of the concentration of our product with respect to time



If we assume that k is constant, then the only way to increase the rate is to increase either [E] or [S]

Generally in the cell, total [E] is constant

- Ex: 4 enzymes, each of which can catalyze 10 reactions per second
- V_{max} is the maximum rate, the total number of reactions that can be catalyzed per second by the cumulative effort of the enzymes
    - When V_{max} is reached, even increasing [S] won't increase the number of reactions catalyzed per second, since the enzymes are working at full capacity



Note: not all enzymes are proteins. Non-protein enzymes include inorganic metals (ex. Mg^{2+}), small organic molecules (ex. flavin), and ribozymes (ex. RNase P)

ASSUMPTIONS

1. OUR SOLUTIONS ARE BEHAVING IDEALLY

$$E + S \underset{}{\overset{1}{\rightleftharpoons}} ES \overset{2}{\rightleftharpoons} E + P$$

$RATE_1 = k_1[E][S]$ $RATE_2 = k_2[ES]$

2. OUR CONSTANTS ARE INDEED CONSTANT

[E] can be influenced by PROTEIN SYNTHESIS/DEGRADATION

K can be influenced by ENVIRONMENTAL FACTORS

...but we are assuming that they're not being influenced, and are indeed constant

3. S → P WITHOUT ENZYME IS NEGLIGIBLE

SUMMARY

1. $E + S \underset{}{\overset{1}{\rightleftharpoons}} ES \overset{2}{\rightleftharpoons} E + P$

$RATE_1 = k_1[E][S]$ $RATE_2 = k_2[ES]$

2. IF [E] IS CONSTANT THEN THERE EXISTS A V_{MAX}

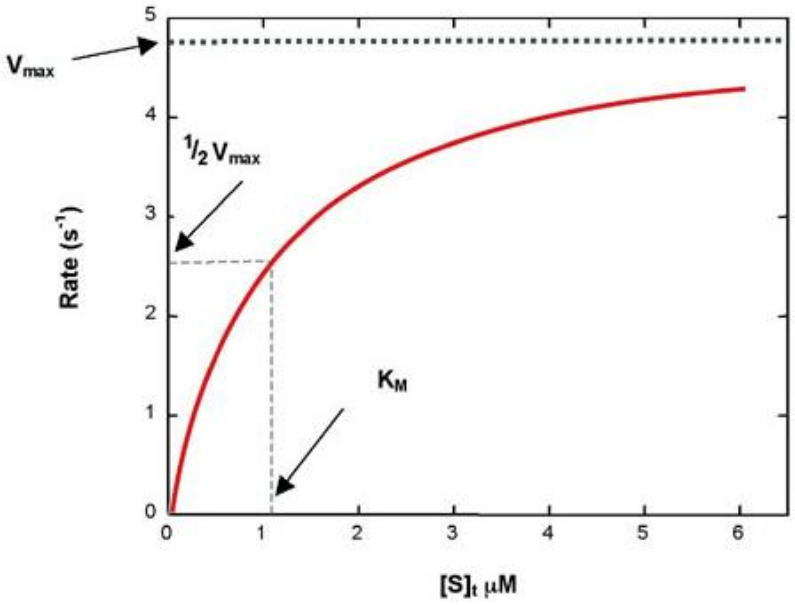
Steady states and the Michaelis Menton equation

Steady-state assumption – **[ES] is constant**, and therefore **formation of ES** = **loss of ES**

Michaelis-Menten equation:

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

- V_0 = initial velocity of the reaction
- V_{max} = maximum rate of the reaction
- [S] = concentration of substrate
- K_m = Michaelis constant
    - **An inverse measure of affinity** (so a higher K_m means a lower affinity)
        - The lower the K_m, the better the enzyme is at working when substrate levels are small (enzyme has more affinity for the substrate)
    - **K_m = [S] when V_o = ½V_{max}**
    - **K_m is the concentration of substrate which permits the enzyme to achieve half V_{max}.**



$K_{cat} = V_{max} / [E]_T$

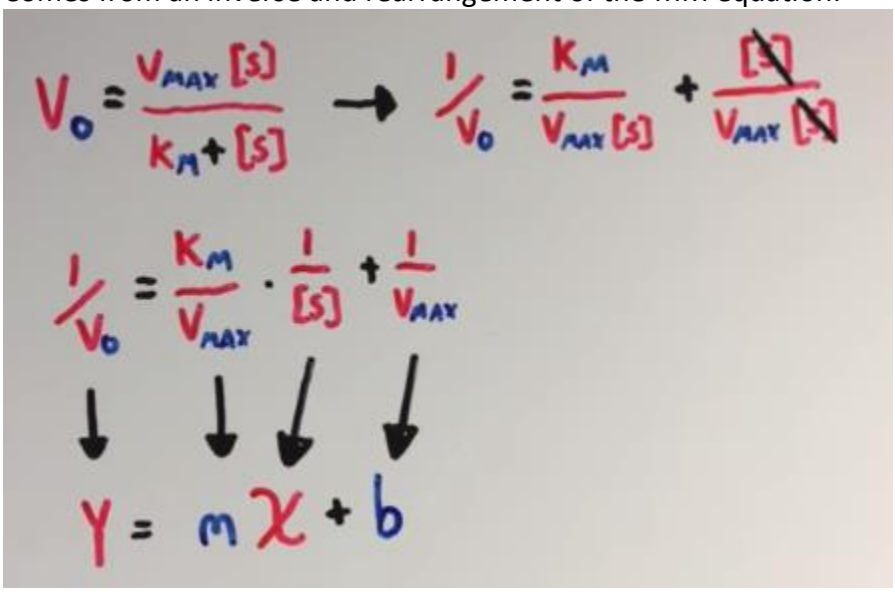
- K_{cat} = enzyme turnover number
    - How many substrates (S) an enzyme (E) can turn into product (P) per *one second* at its maximum speed.
    - Units: sec^{-1}
- V_{max} = maximum speed of the reaction
- $[E]_T$ = total concentration of available enzyme

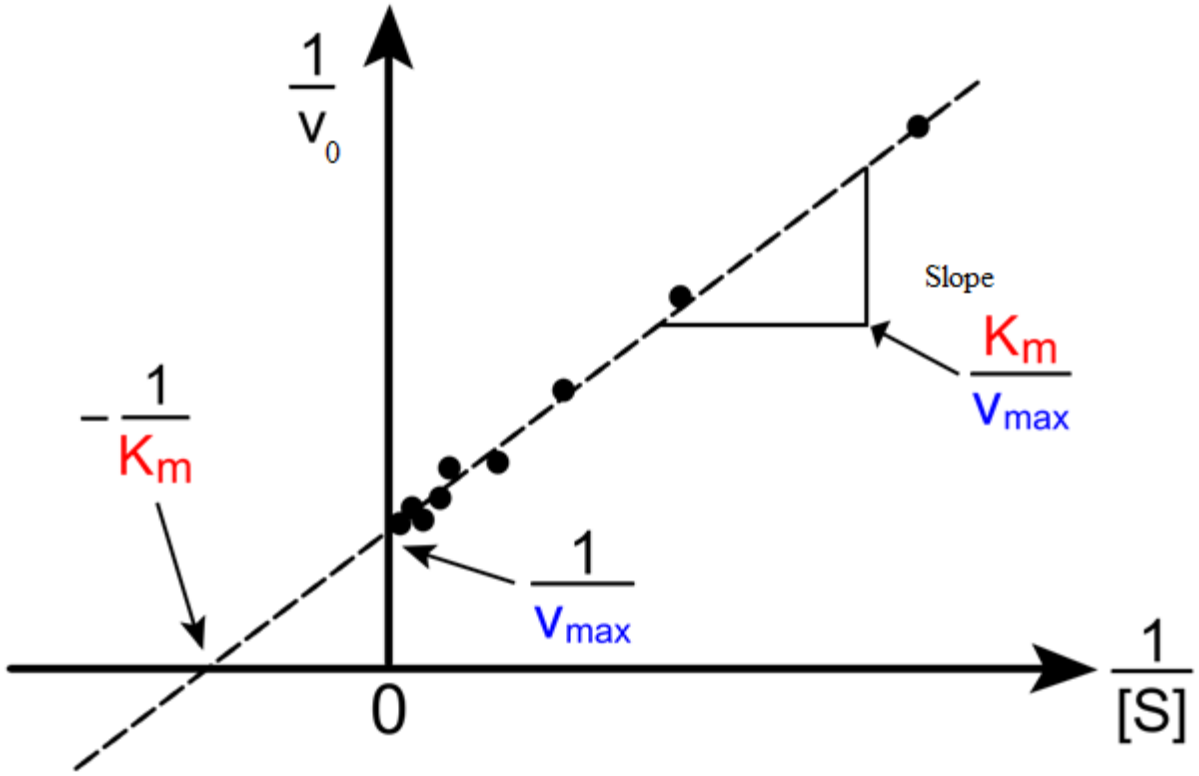
Catalytic efficiency = K_{cat} / K_m

Enzymatic inhibition and Lineweaver Burk plots

Lineweaver Burk plot

Comes from an inverse and rearrangement of the MM equation:

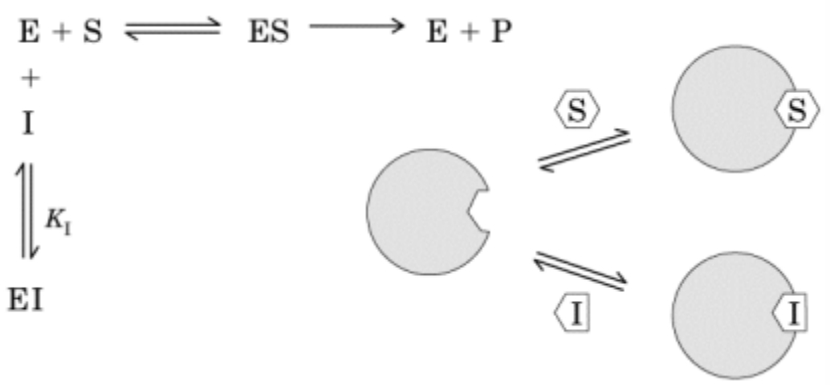




Inhibition (non-covalent)

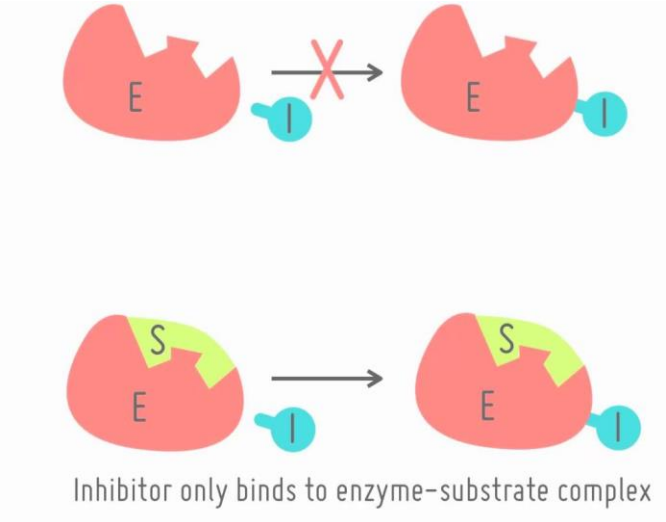
Competitive Inhibition – inhibitor resembles the substrate and will bind to the enzyme's active site, preventing the substrate from occupying the active site

- **K_m is increased**, since the affinity of the substrates has decreased because of the competition with the inhibitor
- **V_{max} stays the same,** because a high concentration of substrate can overcome inhibition (through probability).



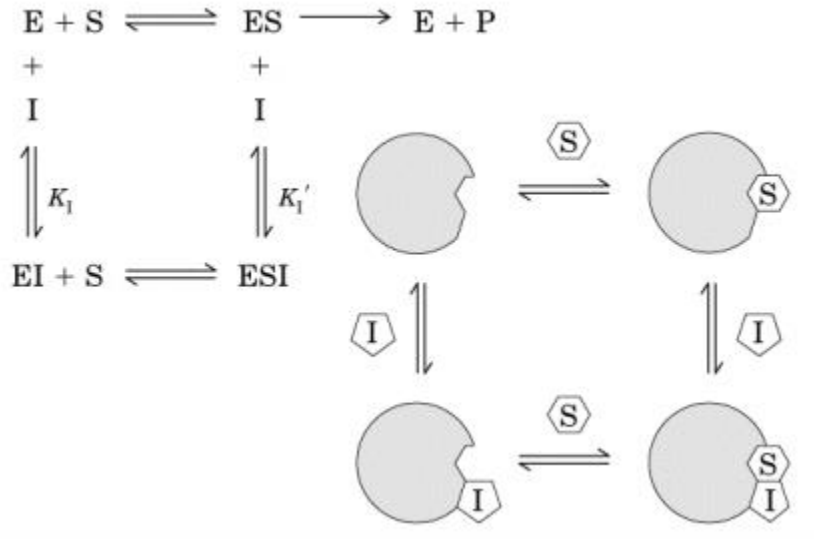
Uncompetitive Inhibition – binding of substrate to the active site of the enzyme causes a conformational change, opening up a new site (allosteric site) for the uncompetitive inhibitor to bind.

- Inhibitor can now bind to form the ESI
- **K_m is decreased**, because the substrate gets 'locked in' to the enzyme, and so in a sense, the enzyme has 'increased affinity' with the substrate, leading to.
- **V_{max} is decreased**, since this form of inhibition cannot be overcome by increasing susbtrate concentration.



Noncompetitive Inhibition – allosteric site is available to be bound by the inhibitor even *before* the substrate binds. Inhibitor can either bind to the enzyme *or* the enzyme-substrate complex.

- **K_m stays the same**
- **V_{max} is decreased**
- **K_{cat} (turnover number) is decreased**



Mixed inhibition – very similar to noncompetitive inhibition. The inhibitor can bind to either the allosteric site of the enzyme or the allosteric site of enzyme-substrate complex, but now has a *different affinity* for each of these states.

- If the mixed inhibitor ends up binding more readily to the enzyme, **K_m is higher**
- If the mixed inhibitor binds more readily to the enzyme-substrate complex, **K_m is lower**
- **V_{max} decreases** because increasing substrate concentration would not lead to more enzymes being available.

Summary:

Type	MoA	Effect
Competitive	Inhibitor binds in place of substrate in enzyme's active site	↑ Km
Uncompetitive	Inhibitor can only bind after substrate has bound and opened up the allosteric site	↓ Km ↓ Vmax
Non-competitive	Inhibitor can bind to allosteric site either before or after the substrate has bound	↓ Vmax
Mixed	Inhibitor can bind to allosteric site either before or after the substrate has bound, but has a higher affinity for one state over the other. • If the mixed inhibitor ends up binding more readily to the enzyme, **K_m is higher** • If the mixed inhibitor binds more readily to the enzyme-substrate complex, **K_m is lower**	↑/↓ Km ↓ Vmax

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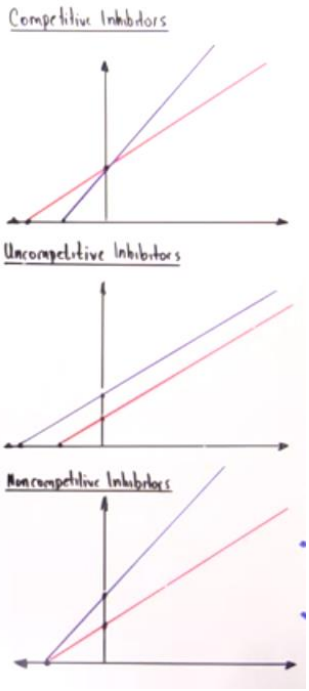


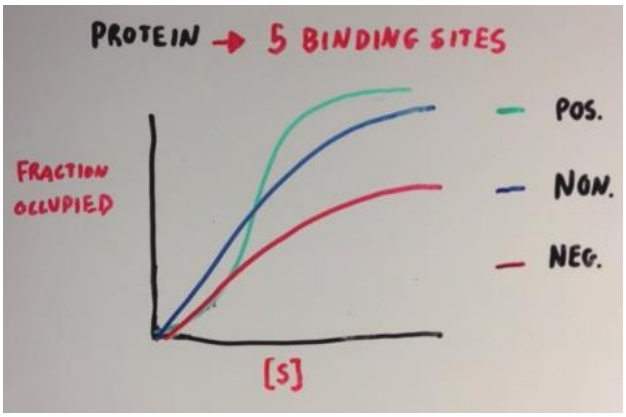
Cooperativity

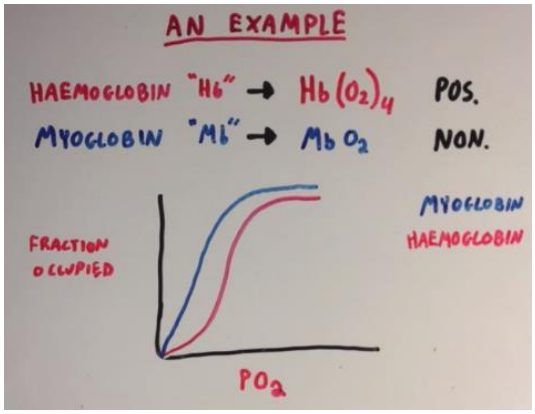
Some enzymes or proteins can house more than one substrate at the same time.

However, sometimes substrate binding changes substrate affinity (**cooperativity**):

- **Positive cooperative binding** – substrate binding increases affinity for subsequent substrates
    - Ex. Hemoglobin (Hb) $Hb(O_2)_4$
    - Sigmoidal graph shape

- **Negative cooperative binding** – substrate binding decreases affinity for subsequent substrates
    - Hyperbolic graph shape
- **Non-cooperative binding** – substrate binding does not affect affinity for subsequent substrates
    - Ex. Myoglobin (Mb) MbO_2
    - Hyperbolic graph shape





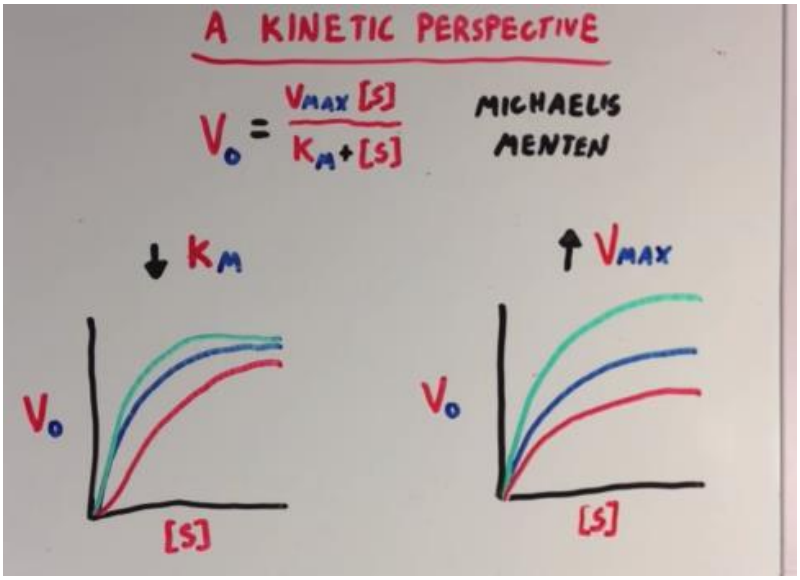
Hill coefficient – measure of cooperativity
(ex. Hb, where you see sigmoidal kinetic curves that don't following Michaelis-Menten)

n<1	n=1	n>1
Negative cooperativity	No cooperativivty (normal MM kinetics)	Positive cooperativity

Allosteric regulation and feedback loops

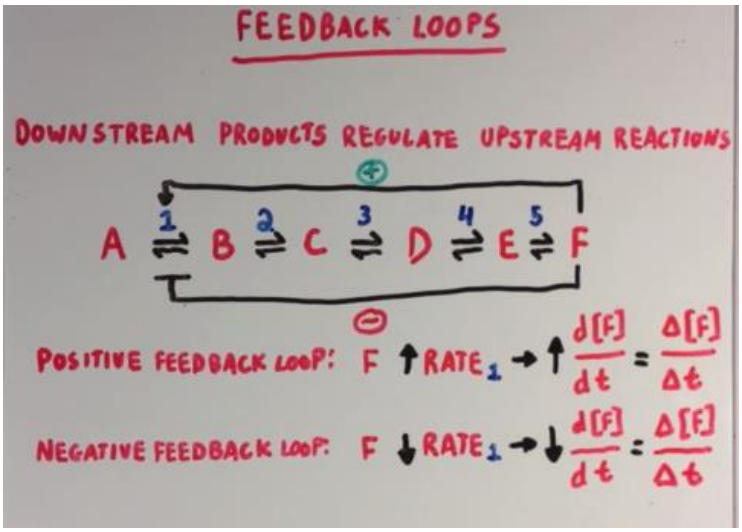
Allosteric regulators bind to **allosteric sites** (which exist anywhere on the enzyme) to regulate the activity of the enzyme

- **Allosteric activators** *increase* enzymatic activity
    - Either by *increasing* Vmax or *decreasing* K_m
- **Allosteric inhibitors** *decrease* enzymatic activity
    - Either by *decreasing* Vmax or *increasing* K_m



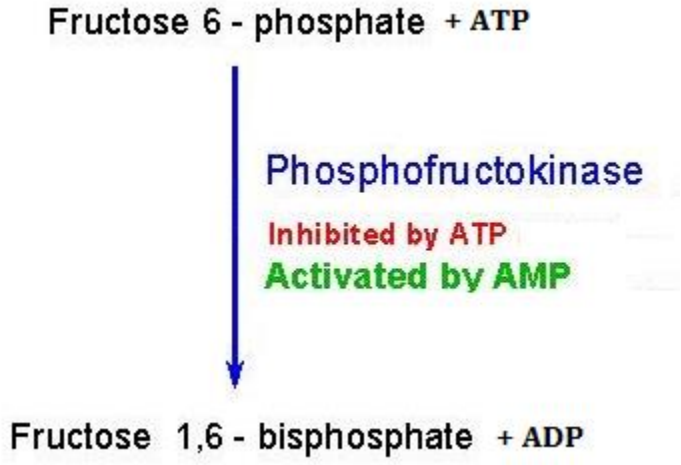
Feedback loop – downstream products regulate reactions upstream

- Positive feedback loop – a change that causes an even further change in the same direction
- Negative feedback loop – a change that causes a change in the opposite direction



Example:

Consider this step in glycolysis



Glycolysis is the process by which cells use to *make* ATP. Thus, ATP is a product of the pathway.

ATP is also an allosteric inhibitor of phosphofructokinase as part of a negative feedback loop to ensure that glycolysis is downregulated when ATP levels are adequate.

- However, this is an interesting case, because while ATP is the product that feeds back to inhibit the enzyme, it is also a *substrate* for the enzyme (see in the pathway above how it is a substrate too)
    - **Homotropic regulator** – a molecule that is both a substrate *and* a regulator

AMP is an allosteric activator of phosphofructokinase that turns on glycolysis when ATP levels are low.

- AMP is a regulator but not a substrate for the enzyme
    - **Heterotrophic regulator**

This reaction in particular has a $\Delta G^\circ = -4.5$ kcal/mol, meaning that *a lot* of energy is released from the reaction. This makes the reaction *more or less* a one way reaction, making it an excellent control point for all 10 steps of glycolysis

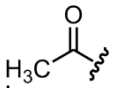
SUMMARY

1. ALLOSTERY - REGULATORS CAN BIND TO ALLOSTERIC SITES ON AN ENZYME.

2. REGULATORS CAN INFLUENCE AN ENZYME'S KINETICS BY ↑↓ K_M V_{MAX}

3. FEEDBACK LOOPS GOOD CONTROL POINTS ARE "COMMITTING" STEPS (VERY ⊖ ΔG°)

Covalent modifications to enzymes

Methylation – addition of a methyl group
Acetylation – addition of an acetyl group ——————— 

- Ex. acetylation of a lysine on its R group NH_3^+ can change acidic/basicity and electrostatic interactions of this AA.

Glycosolation – addition of a sugar molecule

Zymogen – inactive form of an enzyme that requires covalent modification to become active

- Ex. Trypsinogen ——*enterokinase*—> Trypsin
- Zymogen form of an enzyme has the suffix -ogen

Suicide inhibition – *covalently* bind to an enzyme and prevent it from catalyzing reactions.

Models of substrate binding – Lock & Key vs. Induced fit

Similarities

- Both require an enzyme and a substrate.
- Both state that only one substrate will work when it meets the active site of the enzyme.

Differences

- Lock and Key – states that there is no change needed and that only a certain type will fit.
    - Induced fit – states that the active site will change to help to substrate fit.
- Lock and Key – states that the active site has one single entry
    - Induced fit – states that the active site is made of two components.