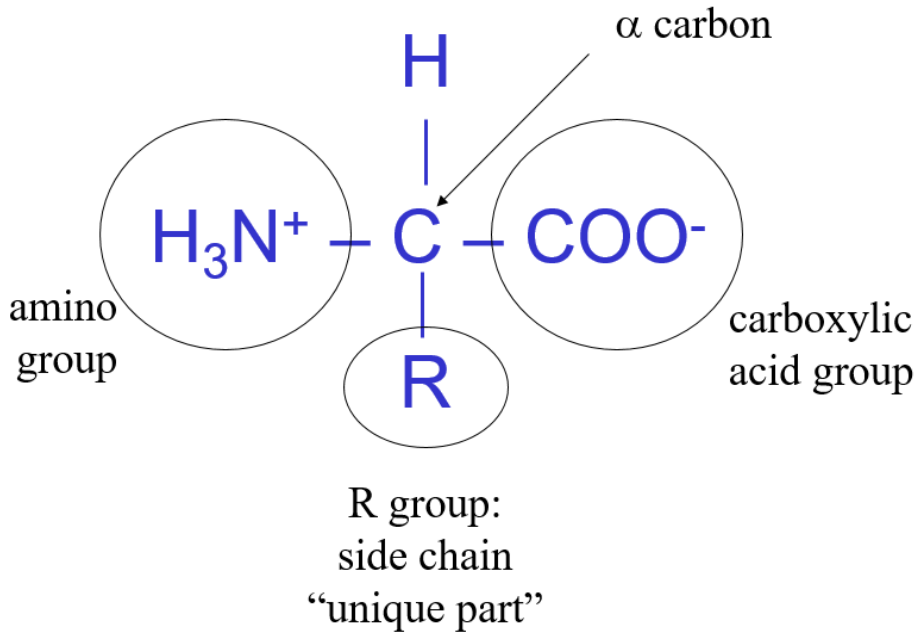
Proteins/Enzymes

Proteins

- Proteins are the most **versatile molecules** in the living system.
- Serve crucial functions in essentially all biological processes.
- Function as **catalysts**, transport and store (oxygen), provide mechanical support (intermediate filaments), immune protection (antibodies/immunoglobulins), generate movement (axoplasmic/motor), create nerve impulses (Na+ protein pumps) and control growth/ differentiation (Ras/Foxo proteins).
- Proteins are found **everywhere** in nature.
- Central dogma of molecular biology (Francis Crick 1958): **DNA → RNA → PROTEIN**
- All proteins are made of amino acids.
- There are **20 standard biological** (proteinogenic) amino acids. (More non-proteinogenic AAs exist, however not incorporated in proteins, and usually occur from post-translational modifications).

General Structure of an amino acid

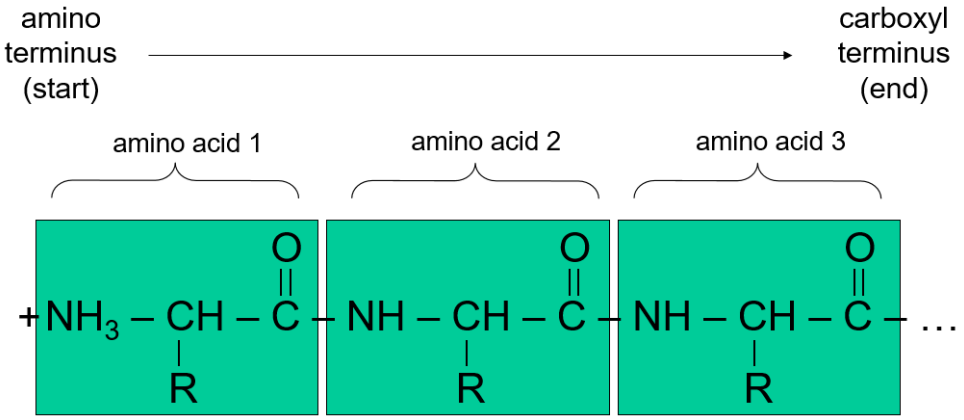


- AAs exist in mirror forms (L and D).
- In nature, **only L-amino acids** are found due to the Founder effect.
- AAs are **classified by their R groups**.
- AAs have different roles in proteins.

Amino Acid Groups

Acidic	Aspartic Acid, Glutamic Acid
Basic	Arginine, Histidine, Lysine
Polar (but uncharged)	Cysteine, Serine, Threonine, Tyrosine, Glycine, Asparagine, Glutamine
Non-Polar (hydrophobic)	Alanine, Proline, Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, Methionine

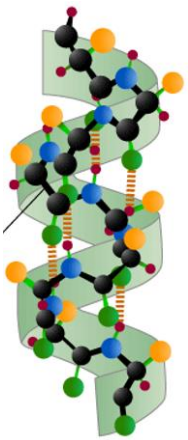
- AAs are joined by **peptide bonds**.
- Peptide bonds form between alpha-carboxyl group and alpha-amino group. Water is lost (condensation reaction).
- A protein is considered to have more than 50 joined amino acids.
- **Peptides and proteins have polarity and direction**
- Amino terminus is the start.
- Carboxyl terminus is the end.

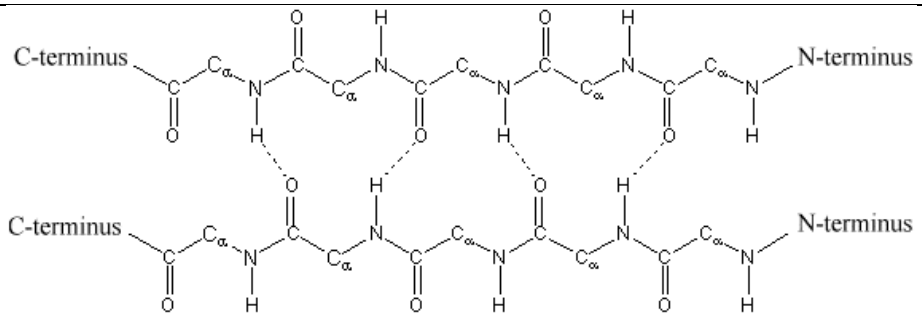


Protein Structure

Primary	• Protein Sequence: the order amino acids occur. • I.e. Gly – Ile – Val – Glu – Gln – Cys – Cys – Ala – Ser – Val – Cys – Ser … • Can be represented with 1 Letter: GIVEQCCASVCS…

Secondary	• Folding formation of amino acids.
	• Involves the backbone of amino acids and not the R groups.
	• Two main types: **Alpha helix and beta sheet**.
	• Loops and turns exist between these major structure types. Loops > turns.
	• **The Alpha Helix:**
	  ○ Involves one polypeptide chain.
	  ○ Stabilized by internal hydrogen bonds.
	  ○ R Groups project outwards from the helix.
	• **The Beta Sheet:**
	  ○ Involves one or more polypeptide chain.
	  ○ R groups project outwards from the sheet, Alternatively (up down).
	  ○ Hydrogen bonds exist only between adjacent sheets.



Tertiary	• The 3-D representation of all atoms of a protein.
	• A description of all secondary structural elements.
	• Dependent on amino sequence.
	• Two major types: **fibrous and globular.**
	• **Fibrous** are long, extended and rod-like.
	  ○ **E.g.** collagen, fibroin (spider silk), titin, keratin (hair protein).
	  ○ Insoluble in water.
	  ○ Contains mostly alpha-helices.
	• **Globular** are compact and fold back on themselves.
	  ○ **E.g.** Hemoglobin proteins.
	  ○ Are usually soluble.
	  ○ Have extremely complex structures
	  ○ Can contain alpha helices and beta sheets.
	  ○ Hydrophobic core and hydrophilic surface.

Enzymes

- Enzymes are catalysts of biological systems.
- They enhance reaction rates by lowering the activation energy.
- Typically proteins, but few are RNA.
- They can bind a stunning variety of molecules, however each enzyme binds to **only one or two** particular molecules, with very **high specificity.**
- **E.g.** Lysozyme protein found in phagocytes, breaks down bacterial cell wall.
- Enzyme nomenclature: named for their substrates and the reaction they catalyze.
  - E.g. ATPase breaks down ATP.
  - ATP synthase synthesizes ATP.
- Substrates are **reactants** in enzyme mediated reactions.
- They become converted into **new products**.
- Products themselves can be substrates for new reactions.
- Substrate specificity is dependent on protein 3D structure of its active site.
- Active Sites not purely made up of adjacent amino acids.
- Active sites are a small part of the enzyme.
- In the form of **clefts** or **crevices**.
- Binding occurs from: **van der Waals forces**, **hydrogen bonding** and **ionic forces**.
- **Catalysis:** the acceleration of the reaction rate of a chemical reaction.
- Catalyst is not consumed by the reaction.

- The catalyst can catalyze the same reaction many times.
- Catalysis **does not make new chemical reactions possible**. It only speeds up existing reactions.
- **Concept of free energy**: amount of energy in a system that can be converted into work.
- Enzyme substrates and products have free energy.
- The difference in free energy between substrate and products (ΔG) account for the reaction equilibrium.
- If the product has a **lower free energy** than a substrate the reaction can occur **spontaneously**. But it may be very slow.
- **Activation energy**: energy hurdle required for a reaction to take place. This is **independent** of a reactions change in free energy (ΔG).
- Enzymes lower activation energy. Provides lower energy pathway. Increases the likelihood of a reaction to occur. Thus speed it up.
- Enzymes **do not change the reaction equilibrium**.
- They lower activation energy by forming one for more **reaction intermediates** which cannot be formed without the catalyst. These intermediates are **transition states,** found at the “Tip of the hurdle"
- Enzymes can be inhibited by **transition-state analogs**, which have a slightly different structure to the intermediate, and act as a competitive inhibitor.
- **Induced fit model of catalysis**: suggest the active site changes shape with the binding of a substrate. Change in active site favors the transition state. Enzyme resumes normal shape after catalysis.
- Enzymes require specific conditions.
    - pH: most enzymes have optimal activity at physiological pH.
    - Temperature: 37 for mammals, extreme temperatures for extremophiles.
    - Co-factors: metals, vitamin-associated cofactors. Reason why you need a balanced diet.
- Enzymes that use the same co-enzymes are usually mechanistically similar.

Enzyme Kinetics

- The role of enzymes is to enhance reaction rates so that they are compatible with the needs of an organism.
- **Importance:** for biology, economical importance in industry, central to pharmaceutical design.
- Enzyme **reaction velocity is affected by substrate concentration**.
- **The Michaelis-Menten constant, Km**, describes the substrate concentration at which enzyme reaction velocity is half its maximal rate.
- Enzymes have different Km Values.
- Higher Km indicates faster enzyme kinetics.
- Velocity max is directly related to turnover values (rate of conversion of substrate to product).
- Enzymes are specific to what they can cut, however can cut the ‘next best side chain’ at a slower rate.

- Km is related to the rate constants of the steps of catalysis.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

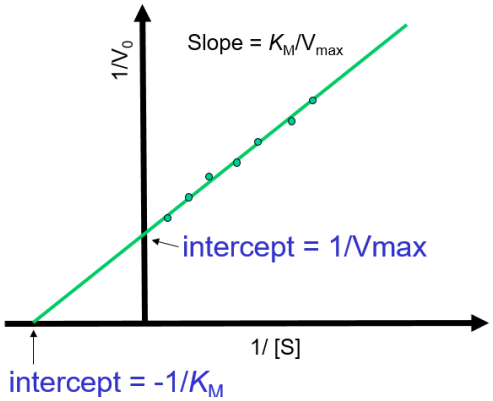
$$K_M \text{ can be calculated by : } K_M = \frac{k_{-1} + k_2}{k_1}$$

- Km measures the affinity of the enzyme for the substrate when K-1 is much larger than K2. In this case Km = K-1/K1 which estimates the dissociation constant of the ES complex.
- Lower Km indicates strong binding. High Km indicates weak binding.

The Michaelis-Menten Equation

$$V_0 = Vmax \frac{[S]}{([S] + K_M)}$$

- V_0 represents reaction rate. [S] represents substrate concentration.
- When [S] is much smaller than Km, V_0 is proportional to [S].
- When [S] is greater than Km, V_0 is equal to Vmax.
- **Issue with this equation:** Vmax is approached asymptotically which makes it difficult to measure. Especially due to issues:
    - solubility/saturation
    - small solution samples to test reactions
    - diffusion of molecules
- **Lineweaver-Burk Plots** is the double reciprocal of Michaelis-Menten Plots.
- A plot of $1/V_0$ versus 1/[S].

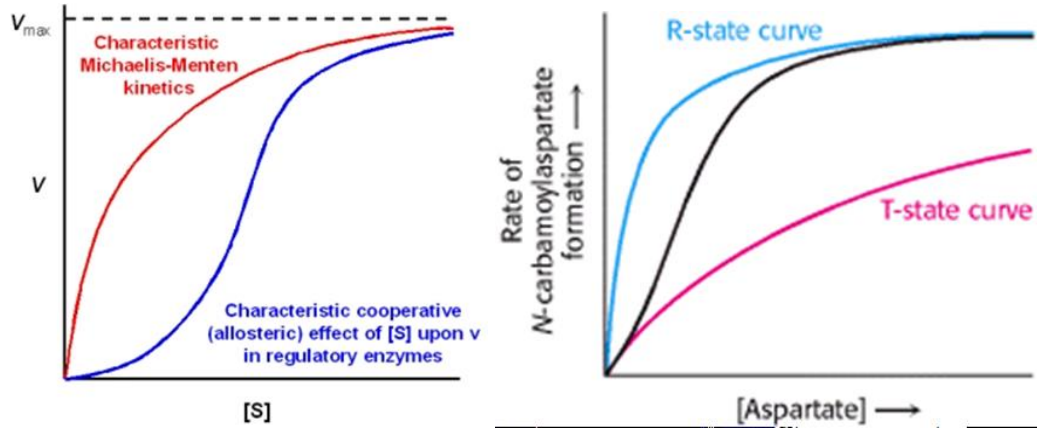


- A **perfect enzyme** is one where its catalytic rate is limited only the the rate at which it encounters substrates. In this case catalysis rates reflect diffusion. E.g. superoxide dismutase (removal of ROS), acetylcholine esterase (neural transmitter), triosephosphate isomerase (glycolysis).

- Understanding enzyme kinetics can help us engineer then to make them better.

Inhibition and regulation

- **Competitive inhibition:** when the inhibitor molecule occupies the active site.
- Inhibition will depend of the concentration of substrate and inhibitor.
- Results in a higher Km, as more substrate is required for significant catalysis to occur.
- Vmax remains the same, as with increased [S] inhibitor binding will not happen very frequently.
- On the Lineweaver Burk Plot the slop increases due to increasing Km.
- **Non-competitive Inhibition**: when inhibitor is binding to another site of the enzyme changing the conformation of the active site, which effects the ability of substrate binding.
- When an inhibitor bound, substrate may still be bind but with a hindered reaction rate.
- This lowers Vmax, as it prevents a portion of enzymes to convert substrate efficiently.
- Same Km as uninhibited enzymes can still work at the same rate.
- On the Lineweaver Burk Plot, same X intercept but higher slope, due to decreased Vmax.
- **Allosteric enzymes** contain distinct regulatory and active sites.
- Small molecules can bind to regulatory sites to cause conformational changes to the active site.
- **Feedback inhibition** is where the product acts as an inhibitor. This usually occurs in the first enzyme of a metabolic pathway. E.g. ATCase, first enzyme in pyrimidine biosynthesis. Inhibited by cytidine triphosphate, the final product in the pathway.
- **Cooperativity** in allosteric enzymes is when activity at one active site stimulates activity in adjacent active sites.
- Initial in a tense inactive state. Binding with substrate causes a relaxed active state.
- Due to this reason **allosteric enzymes do not show Michaelis-Menten kinetics**.



- **Zymogens** – are inactive precursors of enzymes.
- Many enzymes are only active after folding into their 3D shape. Other enzymes are made in an inactive state (zymogen).
- A **once cleave off** event will activate the enzyme. This allows enzymes to be active only where they are needed.
- They have signal sequence to get out of the cell and as activation peptide.
- **Phosphorylation** serves as a molecular switch which can turn enzymes off or on.
- Proteins are phosphorylated by kinases.

- Proteins are dephosphorylated by phosphatases.
- Phosphorylation occurs on **Serine, Threonine and Tyrosine.**
- Phosphorylation adds two negative changes to a protein which can alter the structure/function.
- Important for sending or amplifying signals.