**Biological Molecules are Modular**

- **Protein**
  - Linear chain of amino acids
  - e.x. Amide bonds
- **Nucleic Acids**
  - Linear chain of nucleotides
  - e.x. Phosphate esters
- **Polysaccharides**
  - Linear chain of sugars, some are branched
  - e.x. Acetals
- **Lipids**
  - Linear chains of acetate or propionate
  - Chain is modified so the assembly units are "hidden"
  - e.x. Reduced aldol (1,3-dicarbonyl)

**Modular Construction Makes Life Possible**

- Easily assemble complex structures using simple molecule components
- Easily dis-assemble complex structures and regenerate parts for re-use
- Only need 1 enzyme system for each biomolecule type and function
  - Proteins made by ribosomes
  - Protein disassembled by proteasome

**Drugs Produce Effects by Binding to Biomolecules**

- Proteins are most common target
  - Nucleic acids less common
- Drugs produce effects by binding to biological molecules

**Biomolecules Have Well Defined 3D Shapes**

- Create 3D chemical environments
- Drugs interact with biological molecules in 3D way
- Shape and pattern of electron density determines binding
  - Non-covalent interactions
- Some drugs react chemically with biological molecules
  - Covalent bonds

**Protein Made of Amino Acids**

- 20 different amino acid types are used
  - Occasionally some proteins contain unusual amino acids
- Amino acids share same backbone and stereochemistry
- Differ from each other by sidechain (R)
  - Chemical properties of side chains vary

**Peptides Are Linear Chains of Amino Acids**

- Proteins are long peptides folded into a particular shape

**Nucleophilic Substitution of Carboxylates**

- Addition of the nucleophile breaks the weakest bond which is the pi bond
- If nucleophile leaves, the starting material is regenerated
- If X leaves, the product is formed

**Primary Structure of Proteins**

- Sequence of amino acids in a protein

    - This is the only information specified by a gene)
- Amino Acids are joined by peptide bonds (amide bonds)

**Primary Structure is a List**

- By convention, amino acids in a protein are listed in order from the N-terminus toward the C-terminus

**Secondary Structure**

- Regions of local order in the backbone chain
- Form small-scale structures
    - Alpha-helix
    - Beta-sheet
    - Loop Turn
- Represented in ribbon structures

**Origin of Secondary Structure**

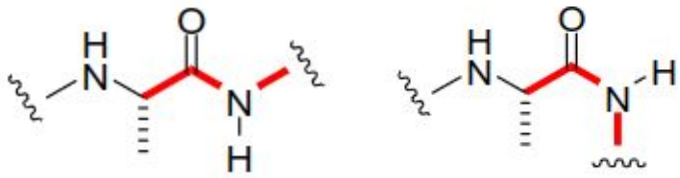
- Conformational restrictions in amide bonds
- Conformational restrictions between amide and alpha-carbon
- Interactions between amide bonds
    - Intermolecular forces acting in an intramolecular way
    - Same forces that control solubility
- Side chain interactions within a region of the chain

**Amide Bond has Double-Bond Character**

- C-O bond and C-N bond act like double bonds
- The 3rd resonance structure contributes a lot to the overall structure
    - The C-N bond of an amide therefore has a lot of double-bond characters
        - C-N bond is single but acts like a double

**s-CIS and s-Trans Conformations**

- 2 large groups close together in cis conformation generate steric interference that raise the energy
- Trans conformation is more stable
- Amide "prefers" s-trans conformation
- S-trans s-cis

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**SIde Chain Interactions**

- Negative charges attract positive charges
- Hydrogen bonding between side chains and backbone
- Non-polar side chains interact with other non-polar chains
- Result of the chemical interactions is the folding of the amino acid chain to produce large-scale structures

**Alpha Helix**

- Alpha helical backbone
- Hydrogen bonding between peptide bonds
- Position of side chains

**Beta Structures**

- Beta strand

        - Backbone atoms are coplanar or “flat”
- Several strands can associate together
    - Sheets (beta-sheet)
        - Can be parallel or antiparallel
    - Large sheets curl around themselves forming cylinders
        - Beta-barrel

**Beta Sheets can be Parallel or Antiparallel**

- Parallel - N→ C attached N→ C
- Antiparallel - N→ C attached C→ N

**Loops**

- Areas with no defined secondary structure
- Represented by “spaghetti” on ribbon diagrams

**Turns**

- Several types
- May not be explicitly represented on ribbon diagrams
- Look for areas where the chain changes direction by a large amount

**Tertiary Structure**

- Overall 3D shape of a protein
- Result of interactions between non-adjacent regions
    - Amino acid side chains
    - 2 secondary structures (2 helixes)
- Contains regions of order
    - Secondary structure
- Contains less ordered regions
    - Loops

**Tertiary Structure and Attractive Forces**

- Attractions between secondary structures cause the secondary structures to fold back on themselves
- Mostly non-bonding interactions
- Occasional covalent bonds

**Van der Waals Interactions are Very Important**

- Polar Side chains on the outside of the protein
- Nonpolar side chains on the inside of the protein

**Dipole Interactions and H-bonds Stronger inside Hydrophobic Environment**

- Non polar environment critical to holding protein together
    - Dipoles and hydrogen bond attraction waker in water
    - Electrostatic attraction is weaker in water
    - Electrostatic attraction is strong in non polar environment
    - Dipoles and hydrogen bond attraction strong in non-polar environment

**Some Proteins Form Quaternary Structures**

- Two or more proteins bind together
- Sub modules can be the same or different

**Protein-Protein Interactions are very Strong**

- Lots of surface constant area
- Lots of chemical interactions
- Exclusion of water from space between

- Proteins stick together well
- Difficult to separate some proteins

**Overall Structure Determines Function**

- Most of the molecule is a scaffold
- Only a small part is normally "functional"

**Types of Protein Target for Drugs**

- **Enzymes**
  - Drug usually stops the enzyme from working
  - Drug exerts by preventing the production of the enzyme product
- **Receptors**
  - Drug can activate (agonist) or (antagonist) the receptor
  - Some drugs perform both functions
  - Drug exerts effect by amplifying or suppressing receptor function
- **Structural Proteins (uncommon)**
  - Drugs interfere with the assembly or disassembly of certain protein structures

**Biological Mechanism of Top 50 Drugs**

- Enzyme inhibitor 38%
- Receptor antagonist 24%
- Receptor agonist 12%
- Ion channel Modulator 8%
- Unknown or Misc 18%

**Enzyme Catalyze Reactions**

- Provide a "designer solvet" for the transition state of a reaction
- Enzymes bind very tightly to transition states

**Designer Solvent for Transition State**

- Best interaction is with reaction transition state

**Enzyme Active Site Relatively Small**

- Most of the enzyme is a scaffold
- Only a small part of the enzyme touches substrate

**Enzyme (E) Binds to Substrate (S)**

- Forms the enzyme-substrate complex (ES)
- Equilibrium
- Enzyme normally changes shape to accommodate substrate
- Substrate also changes shape

**Reaction Occurs**

- The components in the ES complex (ES) now is correct alignment to facilitate reaction
- Substrate is converted into the product in the active site
- Enzyme may change shape during this process

**Product is Released**

- The components in the ES complex (ES) now is correct alignment to facilitate reaction
- Substrate is converted into the product in the active site
- Enzyme may change shape during this process

**Theories of Enzymatic Conformational Change**

- Lock and key (older)
  - Enzyme binding pocket is the exact shape for the substrate

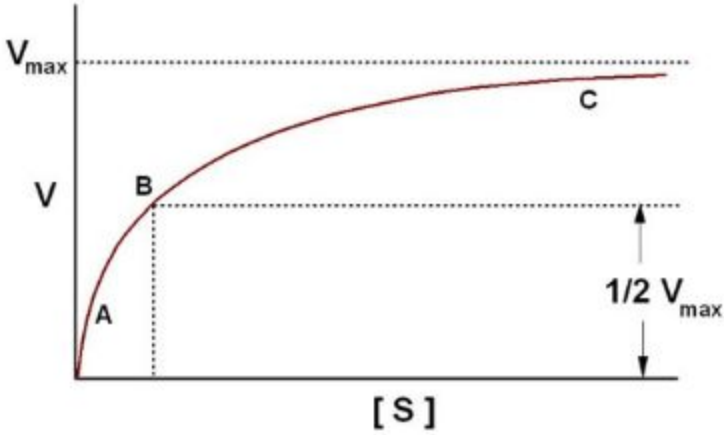
- Induced fit
    - Binding of substrate or other molecule changes conformation of the enzyme
    - Conformational change “activates” or “deactivates” the enzyme
    - Remember that the substrate is also flexible and will change conformation during binding

**Molecules are not Static**

- We draw them using fixed shapes
- Enzymes and substrates are constantly flexing
- Shape changes are not instant nor always symmetrical
- They never bind the same way

**Kinetics of Simple Enzyme Function**

- Michaelis-Menten Kinetics
- $E + S \leftrightarrow ES \rightarrow E + P$




- E - enzyme, S - Substrate, ES - enzyme substrate complex, P - product

**$K_{cat}$ and $K_m$**

- $K_{cat}$ = Turnover Rate
    - Measure of how efficiently ES complex produces product
    - Larger $K_{cat}$ = easier reaction
    - Higher the number the faster the reaction
- $K_m$ =[S] at ½ $V_{max}$
    - Ratio of $K_{cat}/K_m$ used to index enzyme efficiency
    - High ratio = more efficient enzyme
    - Also used as specificity constant
    - Compare ratios for different substrates
- $K_d$ = dissociation constant = $K_r/K_f$
    - Measure of binding between E and S
    - Small $K_d$ = tight binding

**Rearranging the Equation gives a Linear Relationship**

- Lineweaver - Burk Plot
- $1/V = K_m/V_{max}[S] + 1/V_{max}$

**Enzyme Inhibition**

- **Competitive Inhibition**
    - Inhibitor molecule competes with substrate molecule for active site
    - Binding is in the active site

        - Substrate cannot bind
- **Non-Competitive Inhibition**
    - Inhibitor binds to enzyme but not in active site
    - Substrate binds to active site
    - Inhibitor binding preventing ES complex from forming
        - prevents or alters conformational change
- **Uncompetitive Inhibition (very rare)**
    - Inhibitor binds to ES complex
    - Binding destroys catalytic ability of ES complex

**Competitive Inhibitor**

- Inhibitor molecule competes with substrate molecule for active site
- Binding is in the active site
    - Substrate cannot bind
- Enzyme is deactivated when inhibitor is bound

**Competitive Inhibitor Changes the Slope**

- $V_{max}$ is not changes, so intercept is unchanged
- Competitive inhibitor changes slop but not y-intercept
- This can help identify the comp. Inhibitor

**Disulfiram (Antabuse) is Competitive**

- Blocks aldehyde dehydrogenase
- Generates rapid and severe hangover when drinking
- Drug used to treat alcoholics
    - Makes the hangover worse
- Liver see alcohol as a poison
    - The liver goes through 2 reactions to make alcohol to make acetic acid
    - One byproduct called acetaldehyde dehydrogenase is what give you a hangover

**Non-Competitive Inhibitor**

- Inhibitor binds to enzyme but not in active site
- Substrate bind to active site
    - Inhibitor binding preventing ES complex from forming
        - Prevents or alters conformational change
- Inhibitor can bind to the enzyme whether or not the substrate is present
- When it binds, the active site is slightly changes, changing how the substrate reacts with the enzyme

**Non-Competitive Inhibitor Changes the Slope and Intercept**

- $K_m$ appears unaltered
- Slope and intercept change

**Fluconazole is Non-Competitive**

- Blocks Cytochrome $P_{450}$2C9 (CYP2C9)
- Does not bind to active site
- Changes the active site when bound to another part of enzyme

**Uncompetitive Inhibition (very rare)**

- Inhibitor binds to ES complex
    - Binding destroys catalytic ability of ES complex
- Can only bind to enzyme after substrate binds

**Uncompetitive Inhibitor Changes the Intercept**

- $V_{max}$ and $K_m$ decreased
- Changes both x and y intercept

**Lithium is Uncompetitive**

- Blocks inositol monophosphatase
- Used to treat manic depression
    - Exact mechanism of action unknown
- Interacts with certain enzymes in brain

**Irreversible Inhibitors**

- Also known as suicide inhibitors
- Bond covalently to enzyme
- Inhibit by altering conformations or disabling functional groups
- Specific inhibitors usually bond in the active site
- Enzyme is permanently disabled
- Inhibitor chemicaly changes and prevents function of enzyme

**Penicillin is an Irreversible Inhibitor**

- Blocks transpeptidase, enzyme used to finish cell wall construction

**Penicillin Reacts Covalently with Transpeptidase**

- OH group required for catalytic activity
- OH group replaced by an ester cannot act as a catalyst
- OH group acts as a nucleophile

**Messengers Work by Inducing Shape Changes**

- Chemical interactions between messenger (ligand) and receptor change the shape of the receptor
- Impossible to make a drug today to turn on an enzyme
- Carry information from the outside of the cell to the inside
- Molecule make contact with receptor and receptor changes shape
    - When a change is made on one side, the other side is also changes
    - When this occurs, a message is sent inside the cell

**Chemical Messaging Vis Binding**

- Chemical interactions between messenger and receptor
- Shape change allows second messenger to bind or to be released
    - Can either have a release of a chemical or allow a chemical to bind

**Chemical Massaging via Catalysis**

- Chemical interactions between messenger and receptor change the shape of the receptor
- Shape change allows creates/destroys catalytic function

**Agonists "Simulate" the Normal Messenger**

- Agonist binding induces shape change that results in transmission of a signal
- Usually bind at same location (active site) as messenger
- SOme agonists bind at other locations (allosteric sites)
    - Allosteric modulators
- Stimulate the function of a chemical already present in the body

**Asthma Drugs are Adrenaline Agonists**

- Adrenaline receptors in lungs stimulate bronchial opening when activated
- Binds to the active site of the noradrenaline receptor
- Noradrenaline has the ability to open us the lungs in asthma patients

**Benzodiazepines are Allosteric Modulators**

- Binds allosterically to $GABA_A$ ion channel
    - GABA is an inhibitory neurotransmitter
    - In presence of benzodiazepines the channel opens more readily and stays open longer
        - Lower concentration of GABA open the channel
- Adjust the ability of the ion channel in the way it should
- Changes the active site so that the GABA messengers ability is amplified

**Allosteric Modulator of Ion Channel**

- Block the function of messanges and bind to receptors to shut off normal signal

**Antagonists Block Normal Receptor Function**

- Antagonist binding induces abnormal shape change that results in no signal transmission
- May bind at sam location (active site) as messenger
- May bind at other locations (allosteric sites)

**Tagamet is an Active Site Antagonist**

- Binds to a histamine binding site
- Distance between main binding domains is larger than in histamine
    - "Stretches" the active site and produces antagonism

**Antagonist Binding Produces Abnormal Shape**

- When the 2 groups are far apart, the shape of the receptor-messenger complex produces a "signal"
- When the 2 groups are further apart, the shape of the receptor-drug complex cannot produce a "signal"
- Tagamet has similar binding groups
- Receptor only binds to the 2 groups
    - This changes the shape of the receptor

**Allosteric Antagonists**

- Can bind near or even partly inside the active site
- Can bind in a distal location
- Binding changes the shape so much it destroy binding pocket ad cannot accommodate the receptor anymore

**Partial Agonists (2 Possibilities)**

- Agonist binds to receptor and produces non-ideal conformational change
    - Weak signal is sent (short duration)
- Agonist capable of binding to receptor in more than one way
    - One binding mode gives agonism
    - Other binding mode gives antagonism

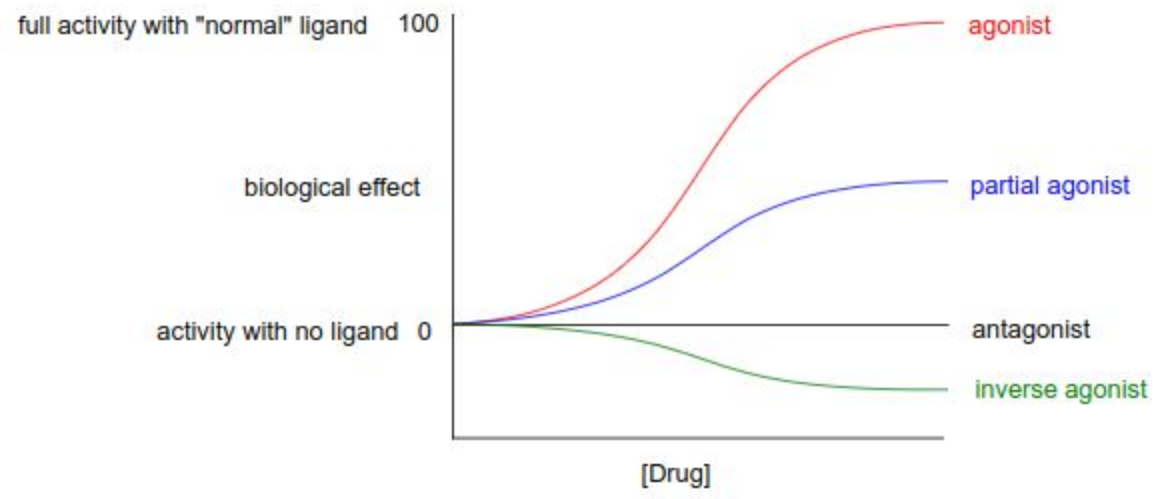
**Buphrenophine is a Partial Agonist of Opioid Receptor**

- Partial agonist effect
- Used to block the effects of opioid poisoning

**Inverse Agonist**

- Some receptors have a "background" activity
- Receptor shows weak function in absence of messenger
- Antagonist changes normal conformation, shutting off this background activity
- Results in an apparent reversal in response
    - GABA agonists produce relaxation
    - GABA inverse agonists produce agitation

**Effects on Biological Activity**




- Ligand = messenger molecule
- Note: inverse agonist goes in opposite direction as agonist

**Inverse Agonist Produces Apparent Reversal**

- At a molecular level, the inverse agonist is the same as an antagonist
- Drug acts as antagonist since is does not fold the same way
    - Thus turns off signal

**Clozapine shows Inverse Agonist Behaviour**

- Clozapine - Drug to treat schizophrenia
- Originally thought to be a weak $D_2$, (dopamine receptor) agonist
- Recently found to be an inverse agonist of $D_2$

**Measuring Drug Behaviour**

- Use a biological assay
- Qualitative (yes/no)
- Quantitative (number)
- In Vitro
    - Using biological chemicals, cells or tissues
- In Vivo
    - Tests done in living animals
- In vitro methods are ALWAYS preferred

**General Assay Types**

- High throughput screening - qualitative
- Routine SAR work - quantitative
- Kinetics or special studies - quantitative
    - 1 property measure
- Cell-Based
    - Antibiotics, antivirals, anti-cancer, metabolism studies
    - Result of several properties
- Tissue based
    - Permeability
    - Complex
    - All based on cancer cells

**HIgh Throughput Screens**

- Emphasis on speed
- Usually qualitative
    - yes/no at set concentration
    - Fully automated
    - Preferred detection method to measure effects
    - Usually performed on 384 or 1536 well plates

**Assays for Routine Work**

- Emphasis on accuracy
- Usually quantitative
    - Concentration or rate
- Semi-automated or manual
- Preferred detection method is fluorescence
- Some assay may use radioactivity to measure effects

**Cell-Based Assays**

- Usually quantitative
- Require skilled technicians
- Usually slow
- Sometimes difficult to interpret results
    - "Black box"
- Industry uses some standardized cell-based assays
    - Metabolism (liver microsomes)
    - Carcinogenicity/mutagenicity (ames)
    - Toxicity
    - Permeability (CACO-2) this actually uses an artificial tissue