

Overview of Biomolecular Drug Targets

Chem4315/6315: Organic Medicinal Chemistry



Drug Target Distribution (2000)



- Cellular Receptors: 45%
- Enzymes: 28%
- Hormones and Factors: 11%
- Ion Channels: 5%
- DNA: 2%
- Nuclear Receptors: 2%
- Unknown: 7%

Proteins: 80%

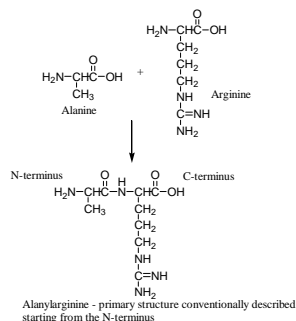
Brief Overview of Protein Structure



Protein Structure can be Described at Several Levels

- Primary
 - Linear sequence of amino acids in protein chain
- Secondary
 - Three-dimensional local conformation
- Tertiary
 - Overall fold of an entire protein chain
- Quaternary
 - Overall shape of a multi-chain protein

Protein Primary Structure



Primary Sequence Sources



- GeneBank: www.ncbi.nlm.nih.gov/Entrez/
- Protein Databank: www.rcsb.org (not limited to primary structure)
- Swiss-Prot: www.expasy.ch/sprot/

Protein Secondary Structure



- Examples
 - Alpha Helix
 - Beta sheet
 - Beta turn
- Major stabilizing contributions
 - Hydrogen bonding
 - Relief of steric crowding

Useful MOE Tools



- Sequence Window
 - Display menu allows you to highlight actual secondary structures (red=helix, yellow=sheet)
 - Display menu allows you to highlight hydrogen bonding (only for the backbone secondary structures)
- Main Window
 - Render->Draw menu allows you to show hydrogen bonds and protein ribbon diagram

3D Protein Structure Sources



- Protein DataBank (www.rcsb.org)
- BioMagResBank (<http://www.bmrb.wisc.edu/>)
- ExPASy Molecular Biology Server (<http://us.expasy.org/>)
- Membrane Protein Structures (http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html)

Class Exercise



- Download a protein structure from the Protein Databank (choose something related to your project if you've identified a relevant protein yet) and add hydrogens (Edit->Add Hydrogens)
- Isolate an alpha-helical secondary structure
 - Examine hydrogen-bonding in that region
 - Examine spacing of the amino acid sidechains
- Isolate a beta-sheet secondary structure (you will need non-contiguous parts of the sequence) and examine similarly

Protein Tertiary Structure



- Covalent stabilization:
 - Disulfide bond formation
- Non-covalent stabilization:
 - Hydrophobic sequestration from water (entropy driven)
 - Salt bridge formation (enthalpy driven)
 - Hydrogen bonding (enthalpy driven)

Class Exercise



- Show all atoms of your protein as a space-filling model
- Select all atoms that are part of hydrophobic residues
- Visually decide how well they are screened away from the solvent

pH, pKa and Ionizability



- Electrostatic (charge) interactions are an important feature of protein structure and activity – drug action often depends on ionizability and charge
- Ammonium pKa ~9, thus will remain cationic in water - this generalizes to amine groups in proteins
 - Carboxylic acid pKa's ~5, thus will lose their protons in water - this generalizes to carboxylic acid groups in proteins
 - Check out the section on amino acids in a biochemistry or organic text for other groups to be aware of

Receptors (Read Section 3.1)

- Types
 - G protein-coupled receptors (GPCR)
 - Ligand-gated ion channels (Section 3.2F)
 - Voltage-gated ion channels
 - Tyrosine kinase receptors
 - Nuclear receptors
- Subcellular localization
 - Nuclear receptors – intracellular
 - Others – cell membrane



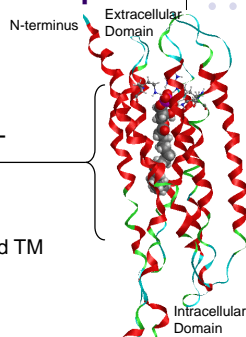
Receptor Ligand Terminology

- Agonist – A compound that interacts with the receptor and elicits a biological response
- Antagonist – A compound that interacts with the receptor and blocks agonist-induced biological responses
- Inverse Agonist – A compound that interacts with the receptor and reduces basal biological response



G Protein-Coupled Receptors

- Largest superfamily
- ~600 human genes
- Important drug targets
- 7 transmembrane alpha-helical domains (7TM)
- Ligand binding families
 - Family A: TM
 - Family B: N-terminus and TM
 - Family C: N-terminus



Class Exercise

- Download the x-ray structure of rhodopsin (a GPCR) from the Protein DataBank
- Delete the second protein chain in the sequence editor (dimer appears in the crystallographic unit cell – not necessarily relevant to biological function)
- Determine whether rhodopsin is a member of the A, B or C subfamily of GPCR based on the position of retinal



GPCR Activation Mechanism

- GPCR exist in a dynamic equilibrium between active and inactive conformational states
- Inactive state
 - Associated with α subunit of GDP-bound G protein
 - Promoted by inverse agonist binding
- Active state
 - Promotes exchange of GTP for GDP in G protein
 - Promotes dissociation of G protein subunits
 - Promoted by agonist binding



Question

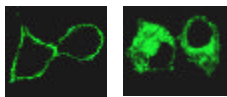
- Considering the equilibrium of GPCR states and the definition of an antagonist:
 - What effect do you think an antagonist has on the equilibrium?



A Student Question

- What does receptor desensitization mean? Does it have anything to do with overdose or drug resistance?
- GPCR activation results in receptor internalization and desensitization. It is an important (and temporary) part of ending signal transduction.

Untreated cells Agonist-treated cells



Ligand-Gated Ion Channels

- Quaternary structure usually involves multiple subunits, either identical (homomeric) or not (heteromeric)
- Ligand binding induces conformational change, opening a pore through the membrane
- Pore is ion-specific (Na^+ , Ca^{2+} , K^+ or Cl^-)

Tyrosine Kinase Receptors

- Topology
 - Extracellular agonist binding domain
 - One transmembrane segment
 - Intracellular domain
- Activation Mechanism
 - Ligand binding brings dimeric kinase domains together
 - Kinase domains autophosphorylate tyrosine residues
 - Intracellular proteins recruited and phosphorylated
 - Protein cascades ultimately regulate transcription factors

Nuclear Receptors

- Agonists are generally small lipophilic molecules (often hormones)
- Activation involves
 - Receptor dimerization
 - Co-activator binding
 - Action as transcription factors
- Located either in cytoplasm (and translocate to nucleus on activation) or in nucleus

Enzymes (Read 4.1 and 5.1)

- Proteins that serve as reaction catalysts
- Names generally end in -ase
- Numbered using Enzyme Classification (EC) system (EC class.subclass.subsubclass.index)
 - EC1.x.x.x Oxidoreductases
 - EC2.x.x.x Transferases
 - EC3.x.x.x Hydrolases
 - EC4.x.x.x Lyases
 - EC5.x.x.x Isomerases
 - EC6.x.x.x Lyases

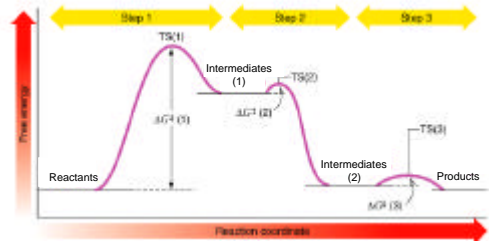
Catalyst Properties I

- What properties define a catalyst?
 - Increase the rate of a reaction
 - Are not depleted during a reaction

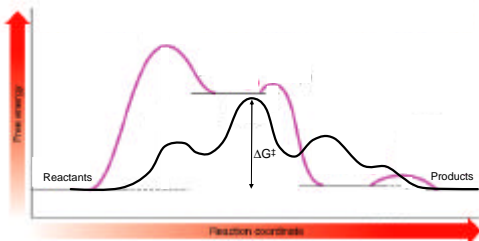
Catalyst Properties II

- Draw an energy-level diagram for a hypothetical uncatalyzed reaction
 - Label reactant, product, transition state(s), and intermediate(s) if any
 - How many mechanistic steps does your hypothetical reaction have?
- Draw an example of how the diagram might look for the corresponding enzyme-catalyzed reaction
 - Label the same species
 - Identify the number of mechanistic steps

Reaction Energetics



Catalyzed Reaction Energetics



Enzymes as Drug Targets

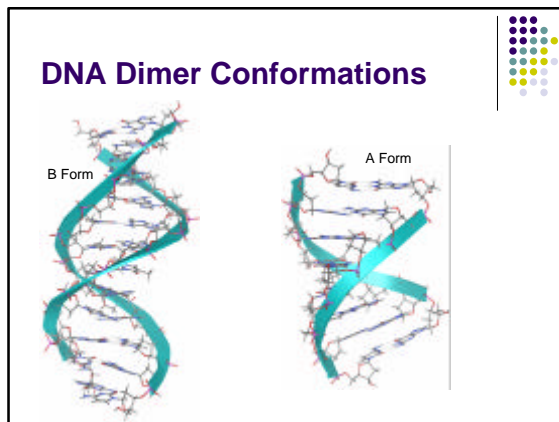
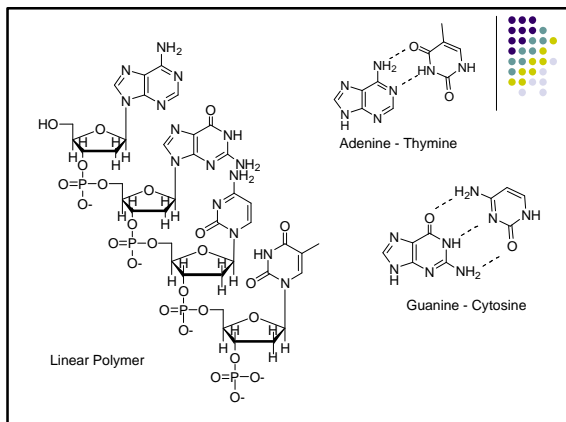
- Most clinical agents targeting enzymes are inhibitors
- Inhibitors can:
 - Interfere with substrate binding by directly blocking a substrate binding site (competitive inhibition)
 - Interfere with catalysis by causing a conformational change in the active site from a distant (allosteric) site (noncompetitive or uncompetitive inhibition)
 - Prevent interaction between proteins necessary to form a catalytically competent system
 - Form covalent bonds with the enzyme (suicide inhibition)

HIV-1 Reverse Transcriptase

- Reverse transcriptase (RT) is the enzyme responsible for transcription of RNA message into DNA in all retroviruses
- RT was the first clinical target in HIV therapy (AZT first FDA-approved drug)
- Using ProteinDatabank entries 2HMI and 1HNI, determine whether the inhibitor bound in 1HNI is competitive with DNA
 - 2HMI: RT is chains 1&2, DNA is chains 4&5
 - 1HNI: RT is chains 1&2, inhibitor is chain 3

DNA Structure (Section 6.2)

- DNA is a polymer of 2'-deoxyribonucleotides
- Alternating phosphate/sugar units comprise chain
- Pendent nitrogenous bases are attached at anomeric position of sugar
- DNA most commonly is found as a dimer of complementary strands interacting by Watson-Crick hydrogen bonding between the bases
- DNA dimer has several possible double-helical conformations (A form, B form...)



Drug Interactions with DNA (Section 6.1, 6.3)

- Covalent modification
 - Alkylating agents
 - Cross-linking agents (cis-platin: PDB # 1a2e)
 - Strand scission-inducing agents
- Non-covalent Interactions
 - Intercalation (ditercalinium: PDB # 1d32)
 - Minor groove binding (distamycin: PDB # 1JTL)
- Complementation (Anti-sense)

Related Reading

- The Organic Chemistry of Drug Design and Drug Action, Second Edition (ISBN 0-12-643732-7)
 - See notes inserted throughout
 - Problems: 4.6 number 1 and 6.6 numbers 2-6
- Textbook of Drug Design and Discovery, Third Edition (ISBN 0-415-28288-8)
 - Chapter 1
 - Section 6.2
 - Section 12.1
 - Sections 17.1.x.x (but not .x.x.x)