

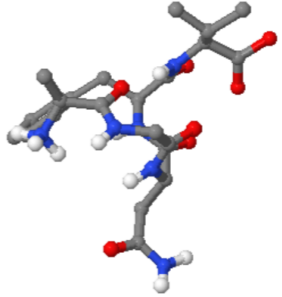
Lecture 2

Protein Structure and Function, Carbohydrates

- Protein Structure and Stability ✓
- Ligand Binding ✓
- Proteins as enzymes (PKU disease)
- Carbohydrates

✓ Prior to lecture 3, please review the lecture material on introduction to nucleic acids (slides and video link posted on course web site)

Problem Set 2 ✓



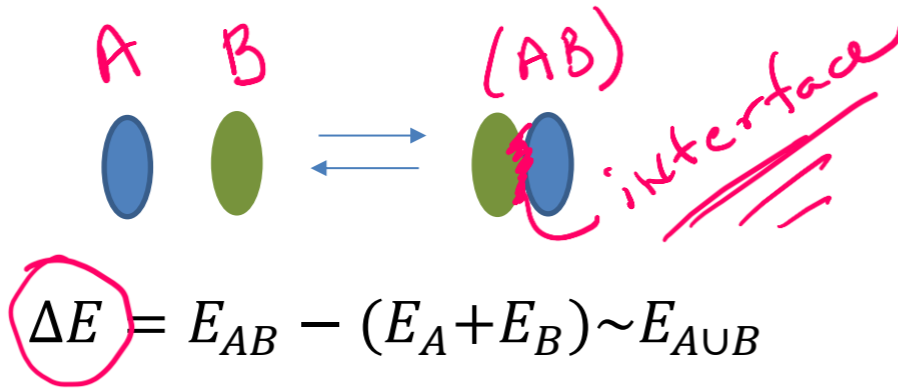
Color Scheme:
blue - nitrogen
red - oxygen
gray - carbon
yellow - sulfur
hydrogen - white

Some Useful Commands
left mouse - rotate
center mouse - zoom
right mouse - Jmol menu

white gray Lt blue black Spin Zoom
 +/- Hydrogen
 +/- H-Bonds
 +/- Sidechain
 +/- Residue Label

Note: In all of these structures all of the residues are labeled "LIU" (Look It Up). You will have to compare sidechains structures to identify the amino acid.

Molecular Interactions



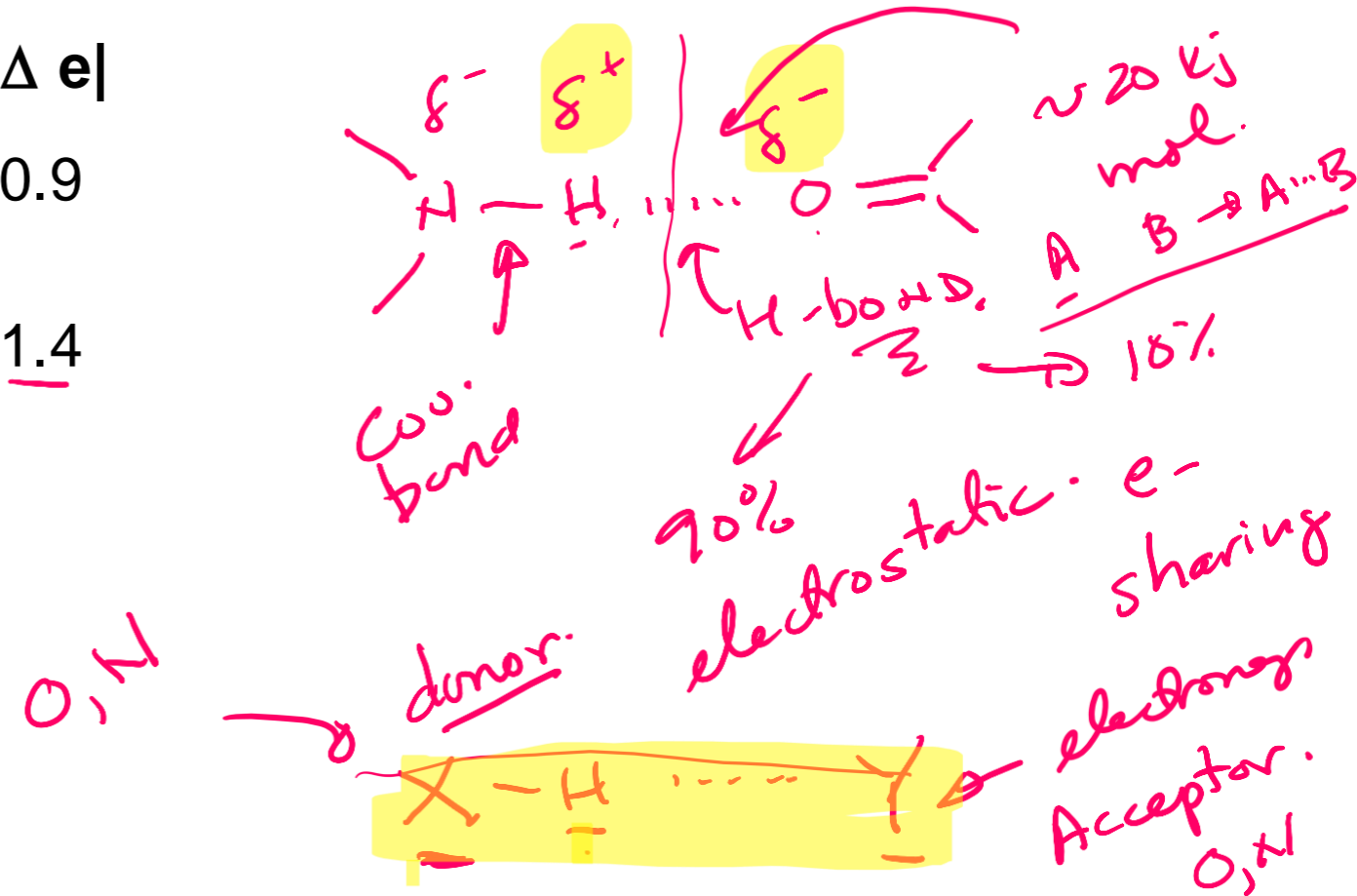
The energy change when two things come together can be approximated to be due to **new** inter-molecular interactions: E_{AUB} .

Interaction	Interaction	Energy (kJ/mol)
✓ Electrostatic interactions (in water)	Full charges	~5 kJ/mol/single interaction
✓ Van der Waals: <u>Dipole-Dipole</u>	Perm. partial charges	~0.05 kJ/A ² x 100 A ² = 5 kJ/mol for 100 A ²
✓ Van der Waals: <u>Induced-dipole</u>	Induced partial charges	~0.02 kJ/A ² x 100 A ² = 2 kJ/mol for 100 A²
<u>H-Bonds</u>	<u>Electrostatic + e sharing</u>	~20 kJ/mol gross, ~5 kJ/mol net

iv) Hydrogen Bonds

- H-bonds are primarily (90%) an electrostatic attraction between:
 - Electropositive hydrogen, attached to an electronegative atom is the hydrogen bond donor (i.e. NH).
 - Electronegative hydrogen bond acceptor (e.g. the lone pairs of oxygen, or C=O group of an amide).

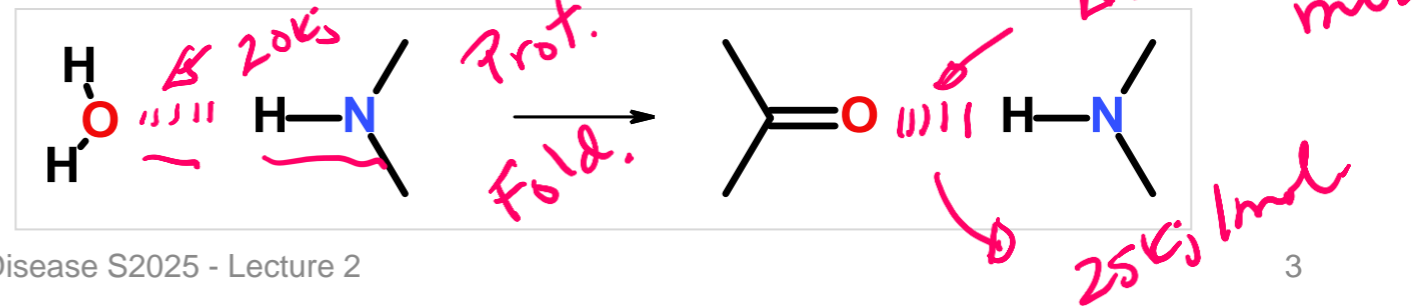
	δ^-	δ^+	$ \Delta e $
$\text{N}-\text{H}$	3.0	2.1	0.9
$\text{O}-\text{H}$	3.5	2.1	1.4



A "bond" implies electron sharing – about 10% of the electron is shared from one molecule to the next in the case of H-bonds

Note that the proton is **NOT** transferred to the acceptor, it remains covalently bonded to the donor atom. The Hydrogen Bond is the **interaction** between the X-H donor and electronegative acceptor.

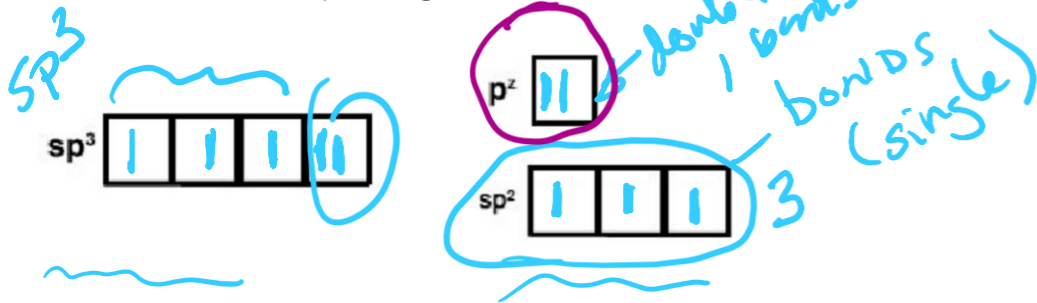
- The energy released when an H-bond forms depends on the distance and angle of the bond.
- Usually hydrogen bonds are exchanged, resulting in small **net** energy differences:



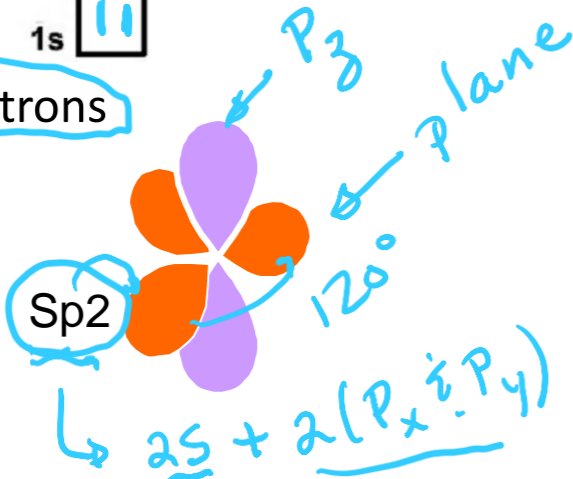
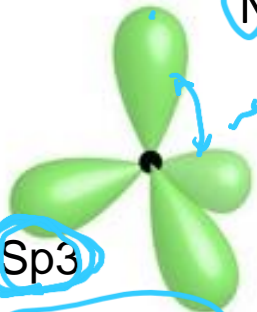
How to Identify Hydrogen Bond Donor and Acceptors

Exceptions, N in a delocalized system:

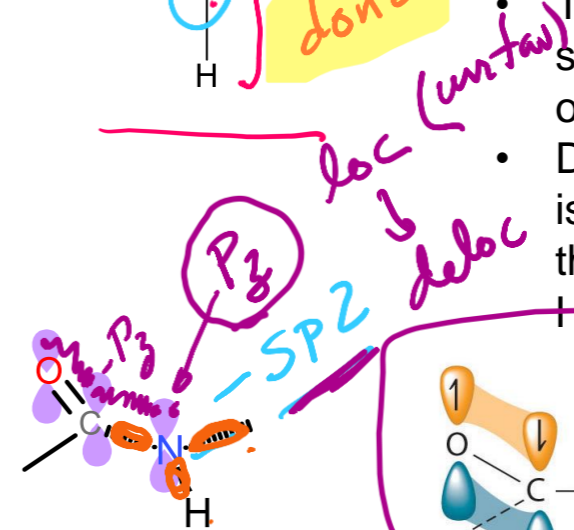
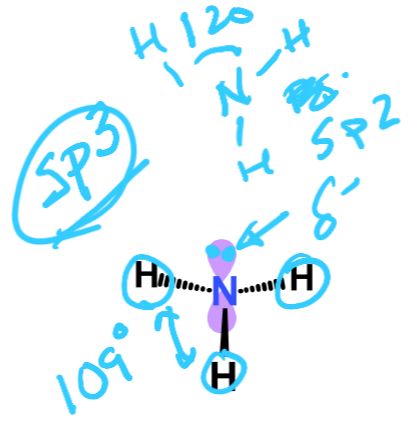
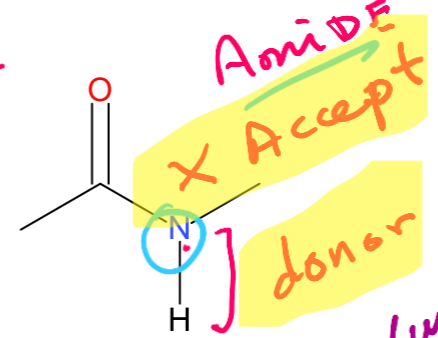
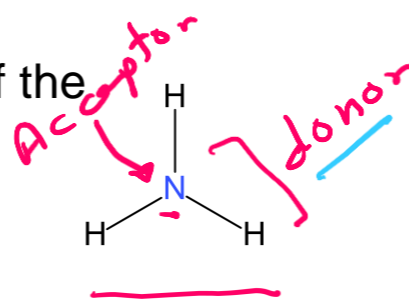
- Will not accept from above or below the plane of the system, because the lone pair is delocalized.
- Can accept in the plane of the ring if there is no attached hydrogen, via lone pair in sp^2 orbital



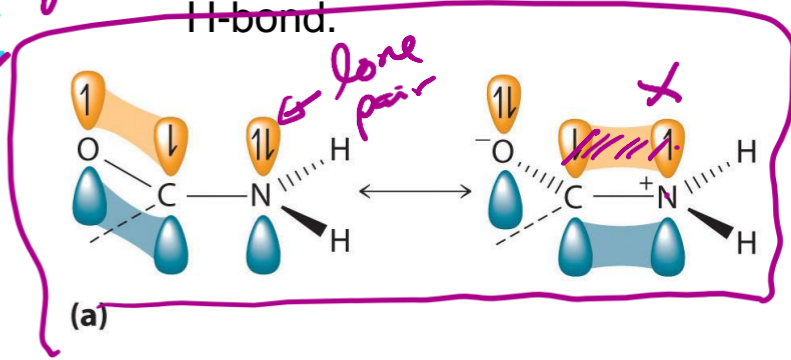
N - 7 electrons



Hybrid orbitals are a mixture of atomic orbitals. Nitrogen can form two types of hybrid orbitals, sp^3 (tetrahedral geometry) or sp^2 (planer) + p_z

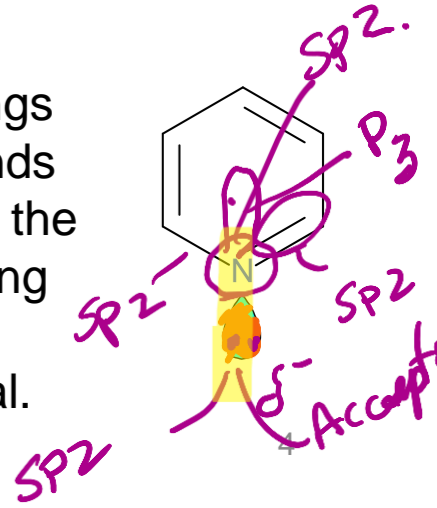


- sp^2 is used in amides, allowing favorable overlap of the full p_z orbital with the p_z on C and O
- The lone pair in the p_z is shared with the p_z electrons on carbon and oxygen.
- Due to electron sharing, there is only a slight neg. charge and the group does not accept an H-bond.

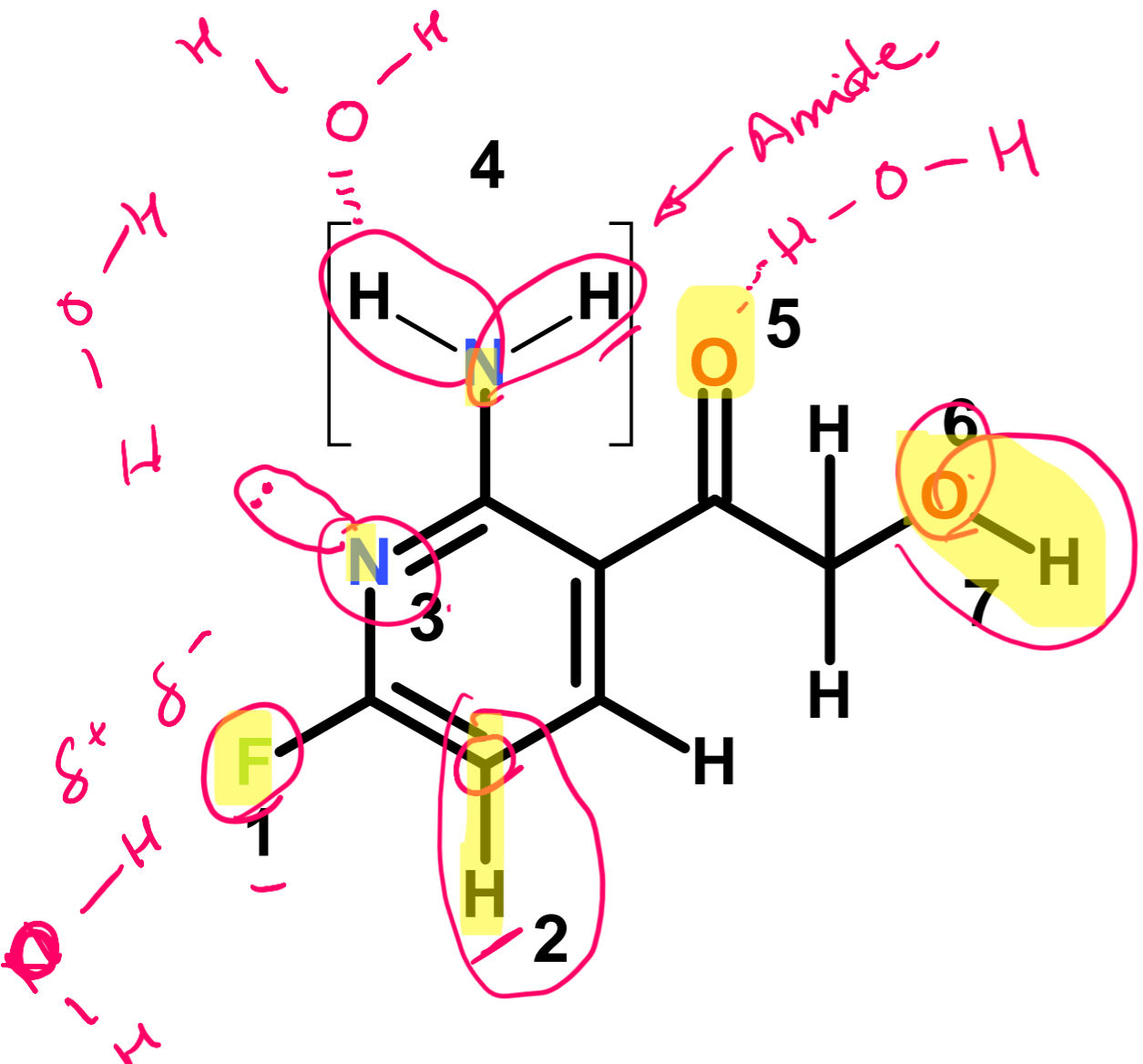


- sp^3 is used in ammonia, keeping the three hydrogen atoms as far from each other as possible. The fourth sp^3 orbital is full with two electrons (lone pair).
- The lone pair is an excellent acceptor.

- Nitrogen in rings with three bonds can **accept** in the plane of the ring due to a filled lone pair orbital.



1. Indicate which atoms could donate an H-bond and which could accept an H-bond



ATOM	Donor(D)?	Acceptor(A)?	Neither (N)
1 (F)	X	✓	
2 (C _{aro} -H)	X	X	✓
3 (N)	X	✓	
4 (-NH ₂)	✓ ✓	X	
5 (C=O)	X	✓	
6 (O)	X	✓	
7 (O-H)	✓	—	

Can you?

- Identify groups that can donate or accept hydrogen bonds?

Relative Energy of Interactions

Interaction	Interaction	Energy (kJ/mol)
Covalent Bond	Electron sharing	200-400 kJ/mol
Electrostatic interactions (in water)	Full charges	~5 kJ/mol/single interaction
VdW - Dipole-dipole (Keesom)	Perm. partial charges	~0.05 kJ/A ² x 100 A ² = 5 kJ/mol for 100 A ²
VdW - Induced dipole (London)	Induced partial charges	~0.02 kJ/A ² x 100 A ² = 2 kJ/mol for 100 A ²
H-Bonds	Electrostatic + e sharing	~20 kJ/mol gross, ~5 kJ/mol net

200 kJ/mol. C → C
C-H.

1. How does the energy of the last four interactions compare to covalent bonds?

1. Stronger 2. Weaker 3. The Same

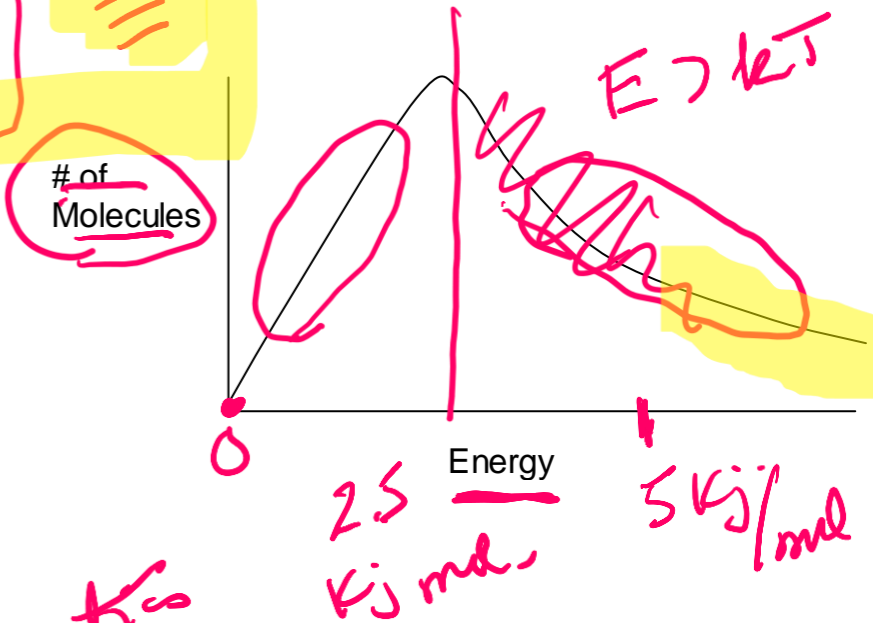


2. Which of these are closer to thermal energy, $kT = 2.5 \text{ kJ/mol}$ @ room temp.

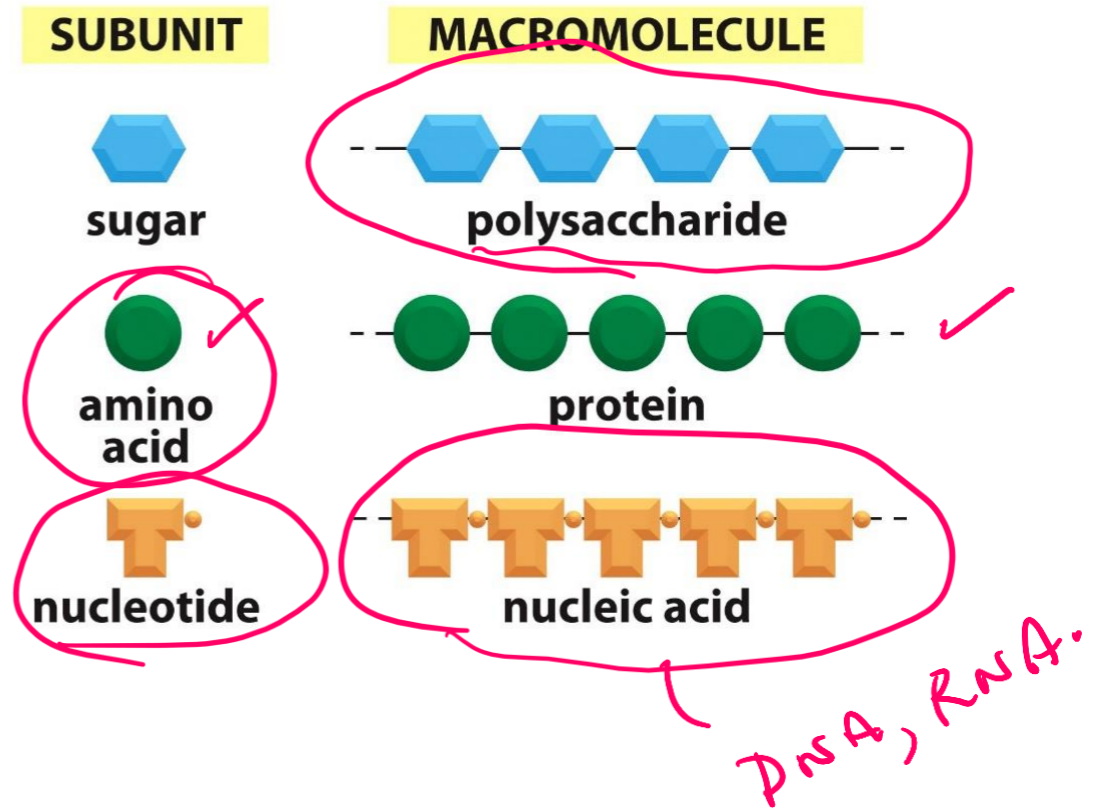
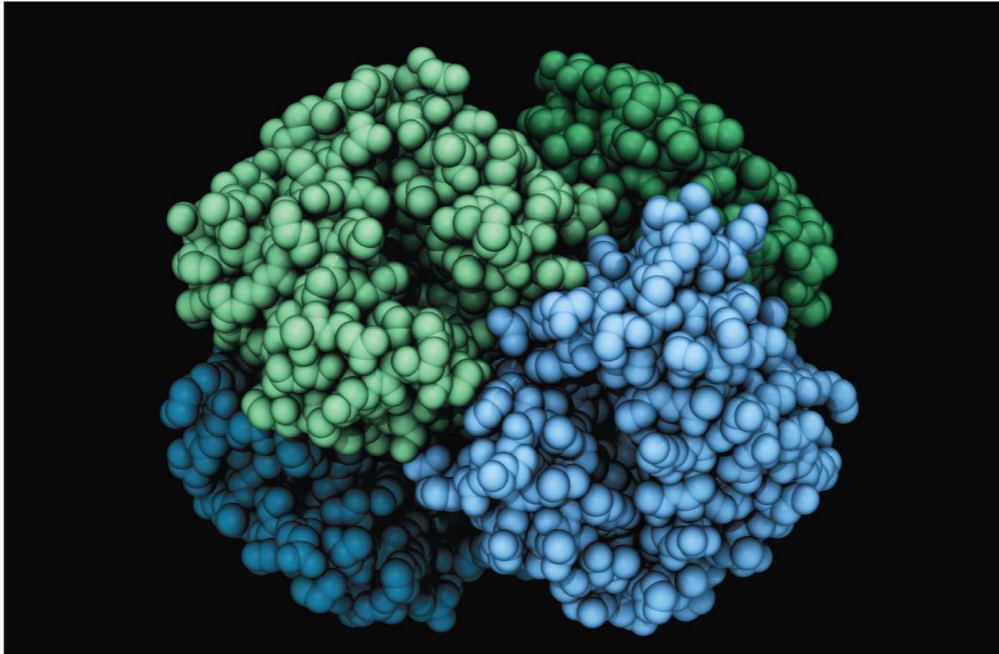
$E_{\text{last}} > kT$

3. Are there significant molecules with enough energy at room temperature to break the interaction?

Thermal energy ⇒ break weak interactions

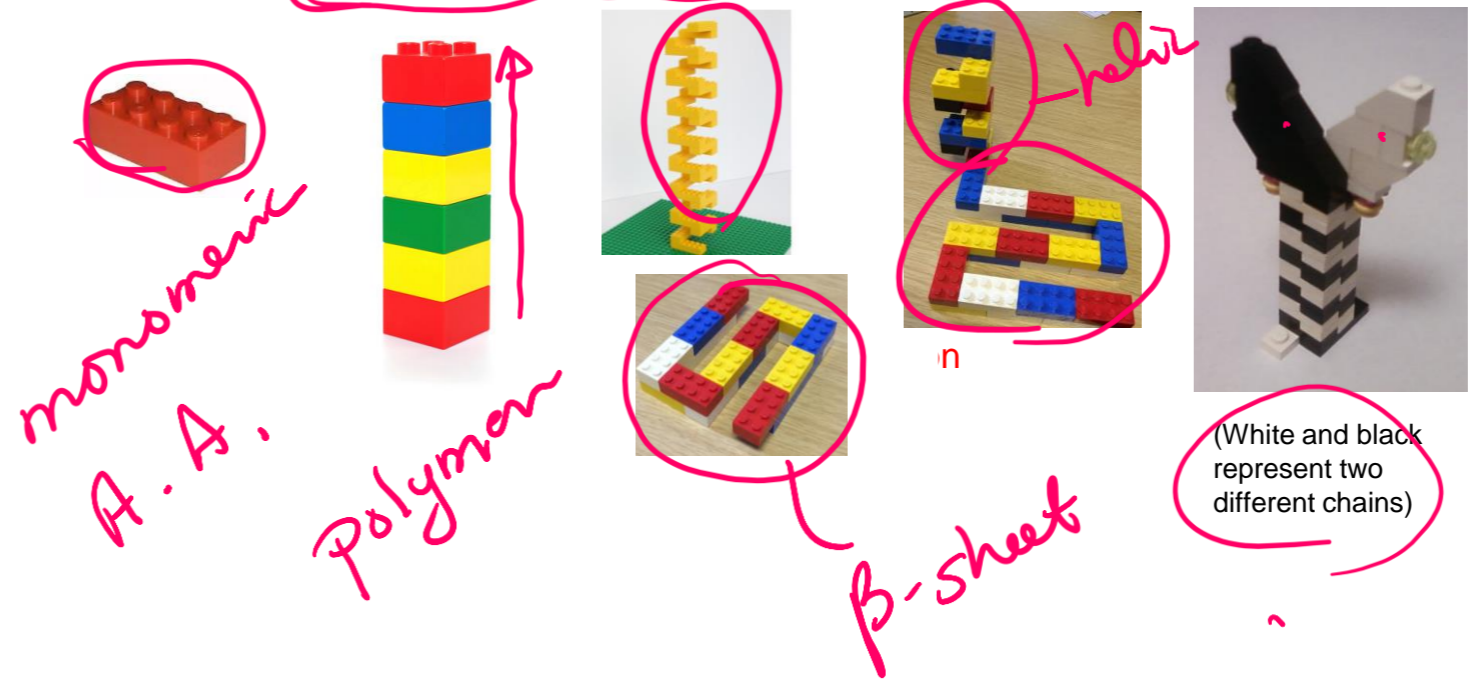
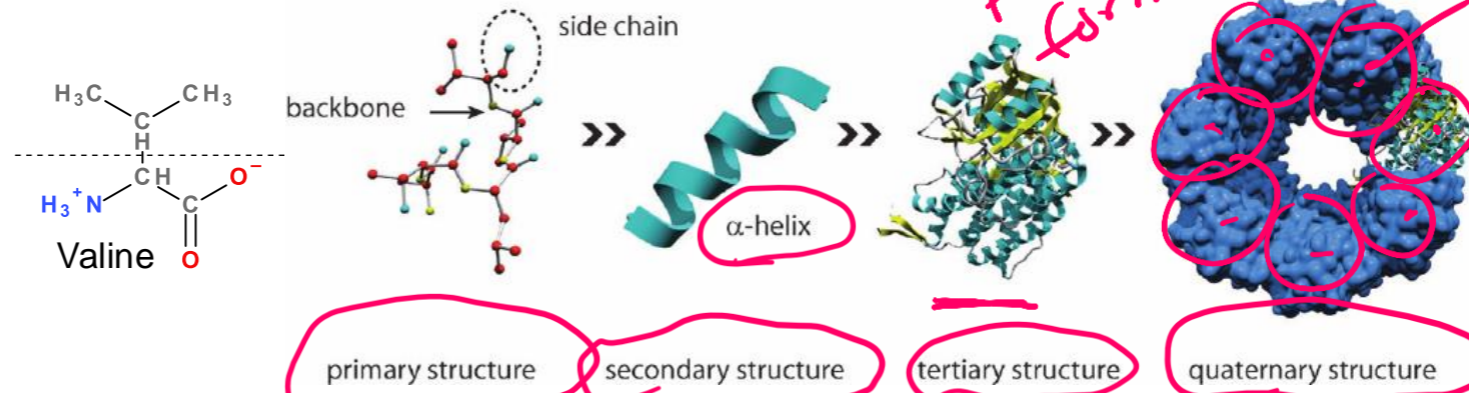


Proteins and Amino Acids



Structural Hierarchy of Proteins

- Primary - sequence of amino acids, no 3D structural information
- Secondary - local structural elements, only mainchain atoms involved
- Tertiary - 3D position of **all** atoms, functional form of many proteins.
- Quaternary - multiple chains – multiple chains often required for function.



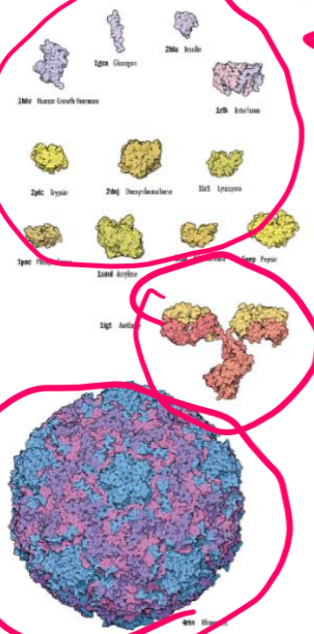
functional form
7 identical chains
multiple chains

MOLECULAR MACHINERY: A Tour of the Protein Data Bank

Living cells are filled with complex molecular machinery, a million times smaller than familiar machines like computers or automobiles. Cells use these tiny molecular machines to perform all the jobs needed for life. Some are molecular scissors that cut food into cellular-sized pieces. Some build new molecules when cells grow or when damaged tissues are repaired. Some are molecular bones and muscles that support cells and help them move and crawl. Some fight off attackers, defending against infection.

Researchers around the world are studying these molecules and determining their precise atomic structures. These structures are available on the Protein Data Bank (<http://www.pdb.org>), the central repository of three-dimensional molecular structures. A few of the thousands of structures held in the Protein Data Bank are shown here. In these pictures, the molecules are all drawn at a magnification of 3,000,000 times, and each atom is shown as a small sphere. Many of these structures are composed of several subunits, which are indicated by different colors. A tremendous range of sizes is shown here: the water molecule at the bottom is only three atoms and the ribosome shown below has thousands.

By David S. Goodsell, The Scripps Institution of Oceanography, La Jolla, California, USA
Graphic design by Gal Weinberg, San Diego Supercomputer Center



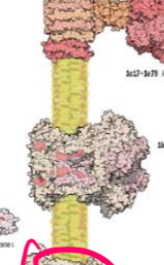
1 OUTSIDE THE CELL
Some molecular machines perform their jobs outside of cells. Many are compact, so that they can diffuse quickly to their site of action. This is a view of the four hemostases shown at the top: insulin and glucagon, which together regulate blood sugar levels; interferon, which carries signals in the immune system; and human growth hormone. The seven digestive enzymes (in red) are also small and very stable, so that they can survive the basic environment in the digestive tract. Each of these enzymes has a small groove in its surface that binds to a different target molecule and catalyzes its reaction. At the bottom is defensin, a protein that causes the common cold, and antibody, our major defense against viruses. Antibodies bind to viruses and prevent them from binding to cell surfaces. (See Molecular Biology.)

PROTEIN DATA BANK

<http://www.pdb.org/>
RESEARCH LABORATORY FOR STRUCTURAL BIOINFORMATICS
RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
SAN DIEGO SUPERCOMPUTER CENTER
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY

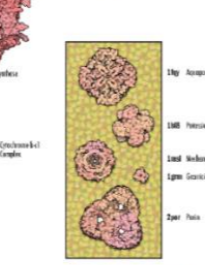
2 MEMBRANES

Cells are surrounded by a membrane made of lipids, like the phospholipid and cholesterol molecules shown at the top. Membranes keep the cellular machinery inside and unwanted material out. Many proteins are embedded in the membrane, performing a variety of essential tasks. ATP synthase is a rotary generator that produces ATP (adenosine triphosphate), the small molecule used for powering cells. The ion-transporter pumps in the membrane use energy from ATP hydrolysis to pump ATP hydrolysis, and the tiny protein cytochromes, a cluster of hemes, is found in membranes in the cytosol. The small rotator molecule inside of it changes shape when illuminated, causing the surrounding protein to send a signal to the brain. Cytochromes handle one of the molecules used in signal transduction—the cytochrome molecule here, however, is linked by two molecules of cytosine, shown inside in white. At the bottom are three molecules involved in photosynthesis, which capture energy from light and use it to power the synthesis of sugar in plant cells.



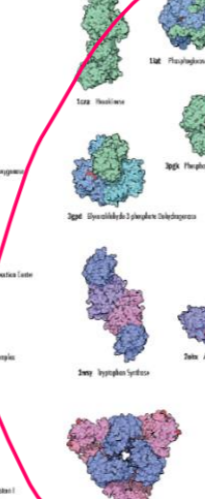
3 TRANSPORT AND STORAGE

Of course, a cell's membrane would be of little use to cells, because nutrients could not get in and wastes could not get out. The transport proteins involved in transport and storage of molecules, from hydrogen and oxygen to oxygen, various forms of fuel, and the like, are shown here. Serum albumin carries many different molecules in the blood.



4 CHEMICAL REACTIONS

Cells build a bewildering variety of molecules in their cytosol. At the top are the enzymes that perform glycolysis, the breakdown of sugar to form ATP. Below are several enzymes that perform different biosynthetic reactions. Dihydrofolate reductase is a key enzyme in the synthesis of nucleic acids. Riboflavin biosynthesis is the most common enzyme on the Earth, and performs a key step in the synthesis of carbon dioxide by plants to form sugar. The three enzymes and the membrane make different binding blocks for creating new molecules. Nitrogenase is essential to the ecosystem by converting nitrogen gas into a form that living cells can use.



5 DNA

Genetic information is stored in the DNA double helix, seen extending from top to bottom here. Many proteins are used to replicate, read, and move this information. DNA polymerase copies the information into a strand of DNA that will be used to direct the construction of new proteins. It is assisted by supercoiling, which reduces tension when the helix is wound and unwound, and gradually to appropriate starting points for the two protein complexes below. DNA polymerase replicates DNA semi-conservatively, the polymerase is filling a gap in the double helix. Some proteins, like the topoisomerase, grab DNA and bend it sharply, or even wrap it all the way around themselves, like the two nucleosomes at the bottom.



6 BUILDING NEW PROTEINS

New proteins are built by ribosomes—complex molecular factories that read the genetic code and use it to direct construction. Many molecular machines are needed to assist the process. Twenty different aminoacyl-tRNA synthetases are shown here, each the building block to new RNA. Each is shown as fitting a gap in the double helix. Several protein factors, shown below the ribosome, guide each tRNA into the proper spot. The three chapters protein shown at the bottom help each new protein fold into its proper shape.



7 BEAMS AND GIRDES

Cells are braced and supported by a complex infrastructure. This cytoskeleton is formed of sturdy filaments like actin and microtubules, composed of many subunits stacked like beads. Vimentin is a molecular motor that crawls along actin filaments, allowing the cell to move. Collagen, broken into two pieces here, is actually found outside of cells, where it forms connective tissue between cells.

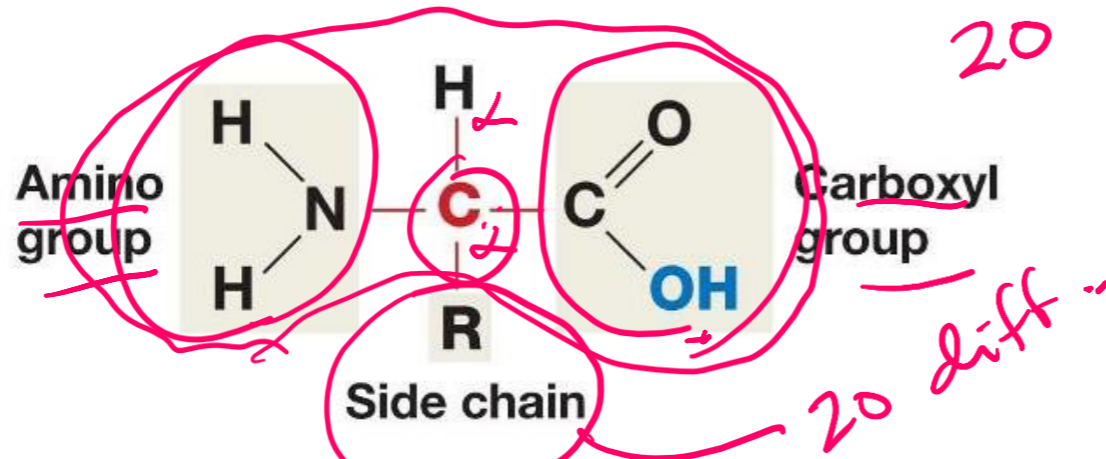
molecules
H-O-H

membrane

DNA

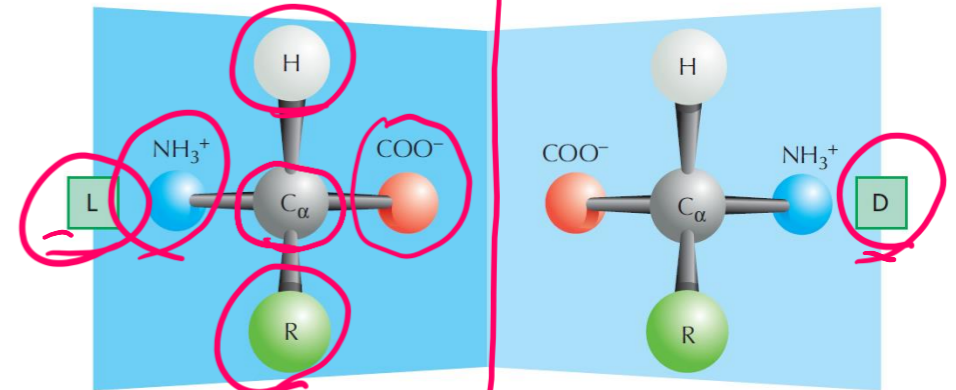
Ribosome

The Structure of Amino Acids and Proteins



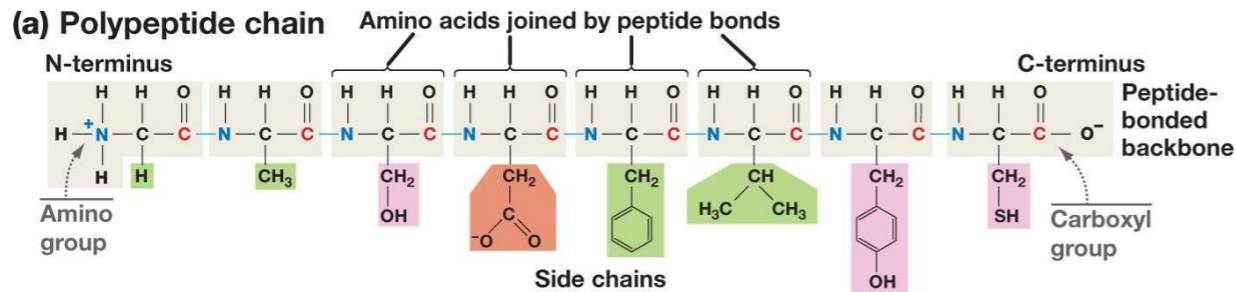
- The amino group, $C\alpha$ (and one hydrogen), and the carbonyl group are common to all amino acids
- The $N-C\alpha-C=O$ are the mainchain of the protein polymer.
- The R groups are different – there are 20 common R groups they are the sidechain of the protein polymer – their **sequence** defines the properties of the protein.

Is there a chiral carbon on amino acids?



Proteins consist exclusively of L-amino acids. (as does the ribosome that make them)

Structure
chiral center
L-form



Primary Structure

- Amino acids are joined together to form linear polymers by the formation of a **peptide bond** between the carboxyl of one amino acid and the amino group of the next.
- This reaction releases water: a **dehydration** reaction.
- The peptide bond can be broken (*lysis*) by the addition of water = **hydrolysis**.

Incorporated amino acid is called a **residue** (atoms are lost when the peptide bond is formed).

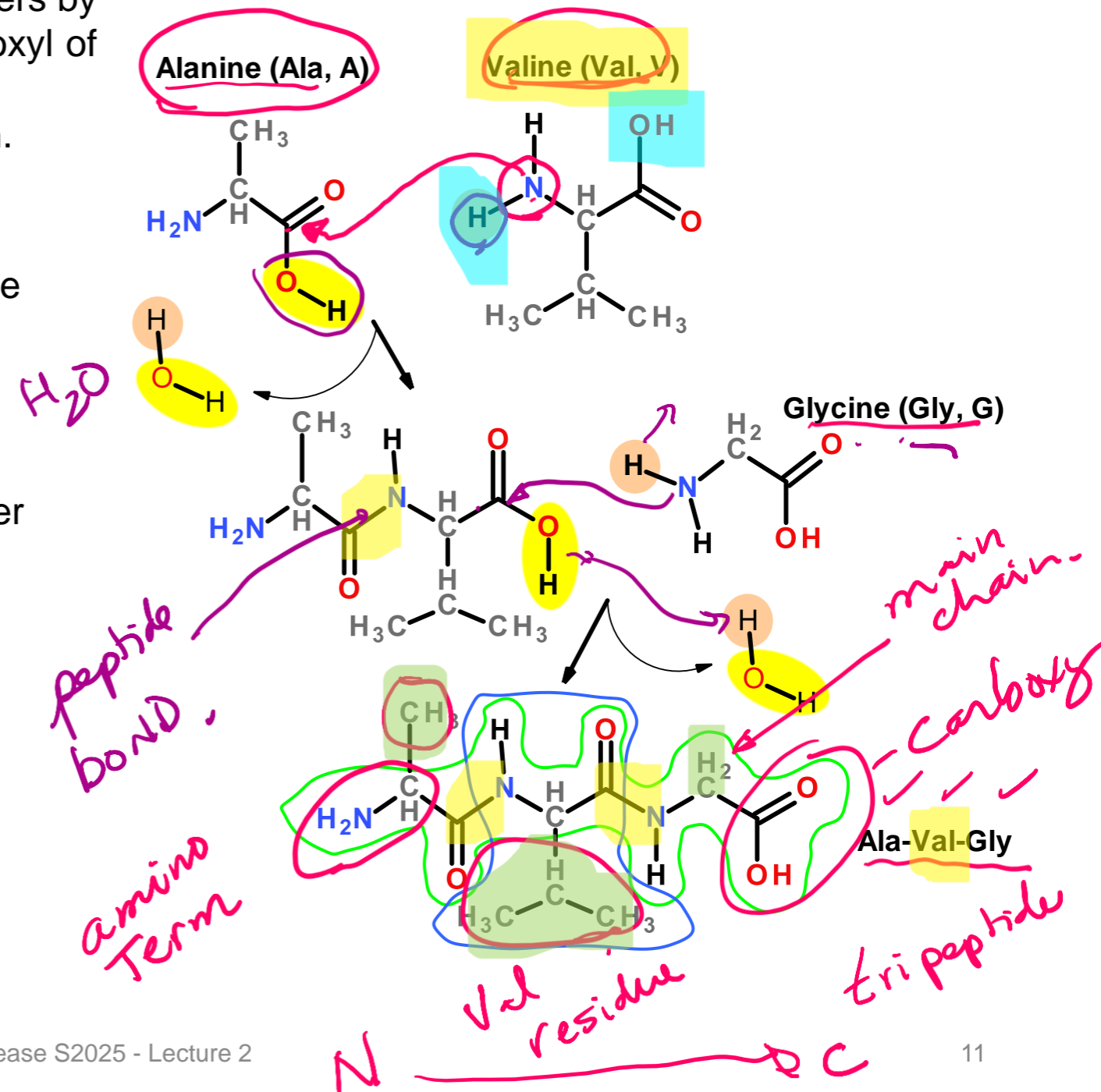
Polarity of chain direction – amino (N) terminus to carboxy(C) terminus = order of amino acids = **sequence** = **primary structure**

Mainchain (or backbone) – linear atoms of the polymer

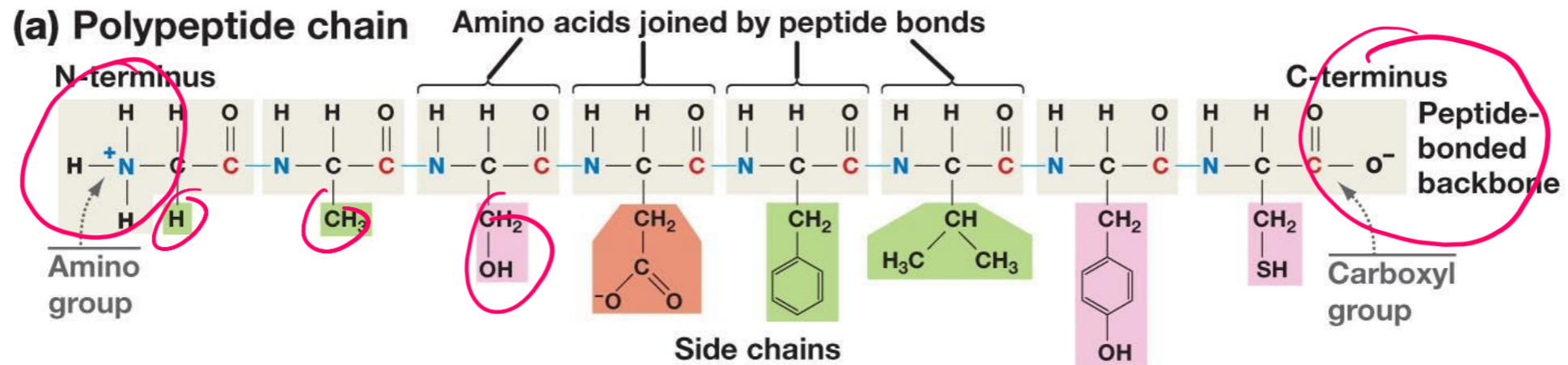
Sidechain – atoms off the Ca carbon

Primary Structure – Expectations

- Draw chemical structure given the sequence.
- Determine the seq. from chemical structure.
- Distinguish/identify:
 - **Mainchain** & **Sidechain** atoms,
 - **Residue** = aa in polymer,
 - N & C terminus,
 - Peptide bond(s).

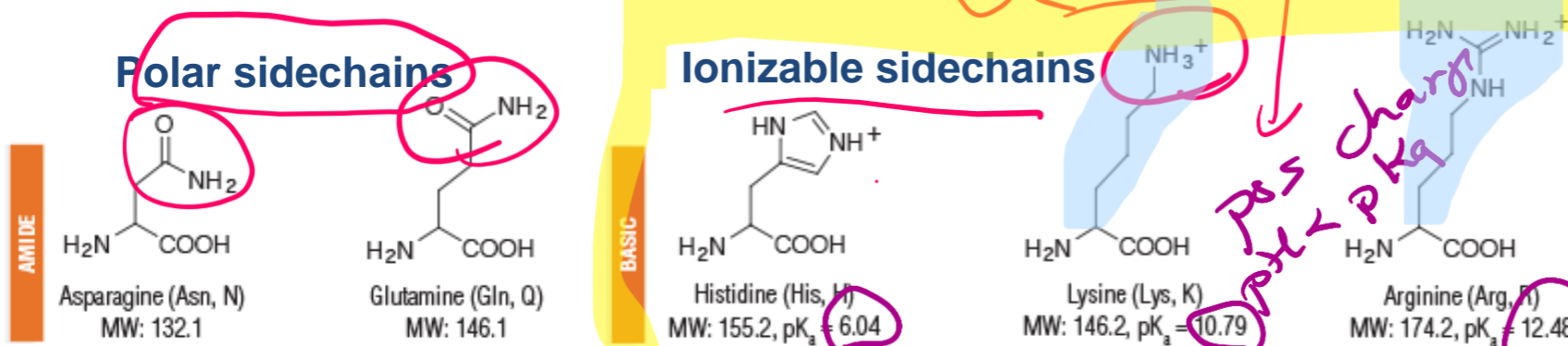
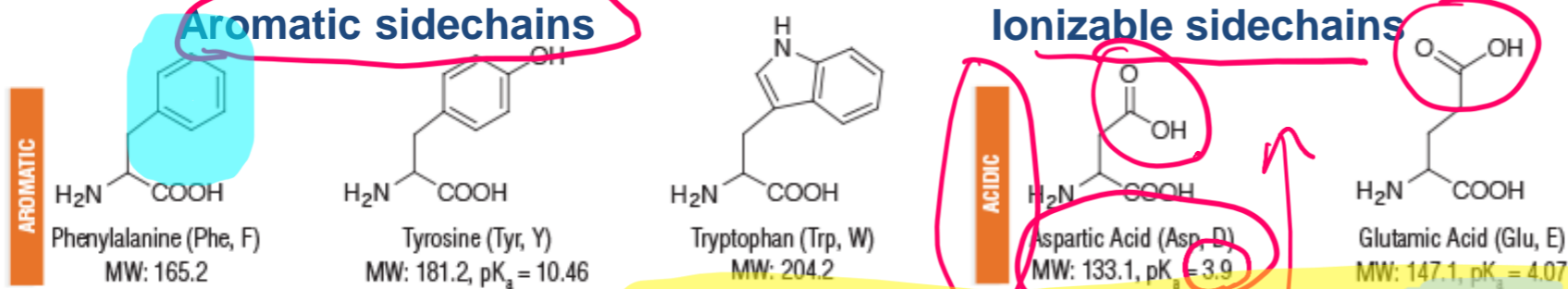
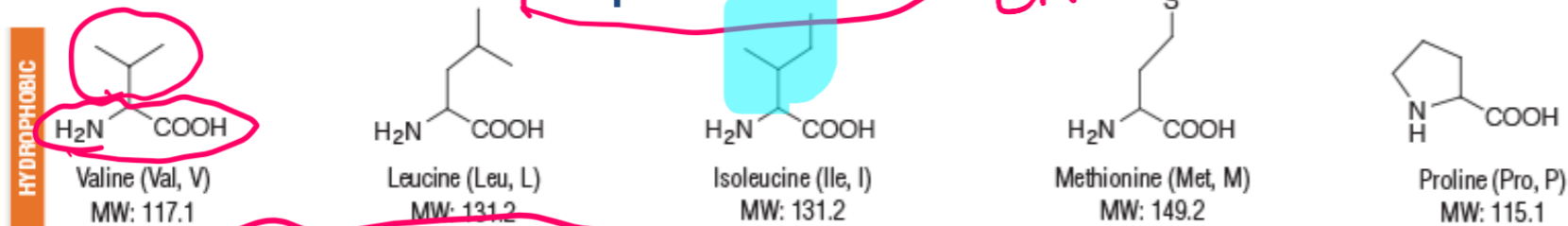
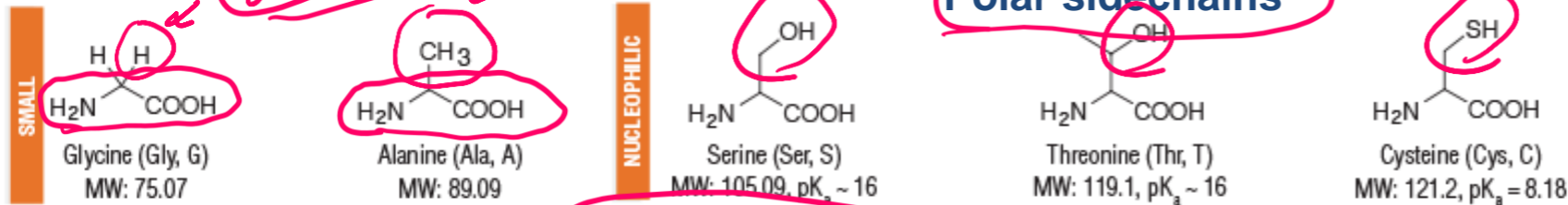


Sidechain *Functional* Groups Affect Behavior (and the order is important)



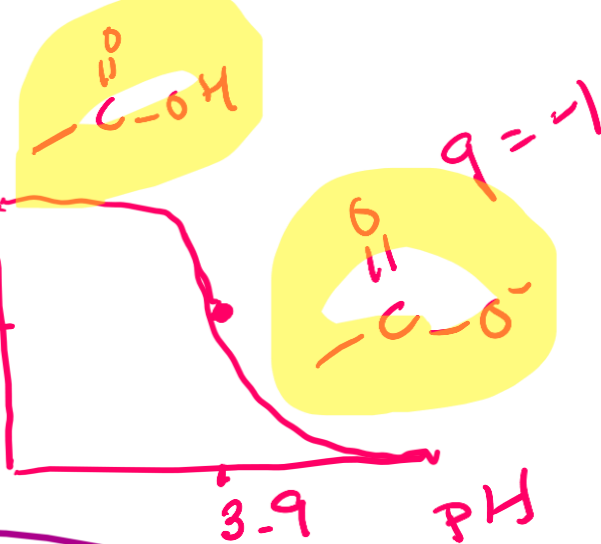
- Sidechains (R-groups) differ in their size, shape, reactivity, and interactions with water.
 1. Nonpolar Sidechains: **hydrophobic**; do not form hydrogen bonds; coalesce in water - typically form the core of folded proteins.
 2. Polar Sidechains: **hydrophilic**; form hydrogen bonds; readily dissolve in water
 3. **Ionizable** Sidechains: Can be charged at certain pH values. Interact strongly with water.

Amino Acids – Structure and Properties

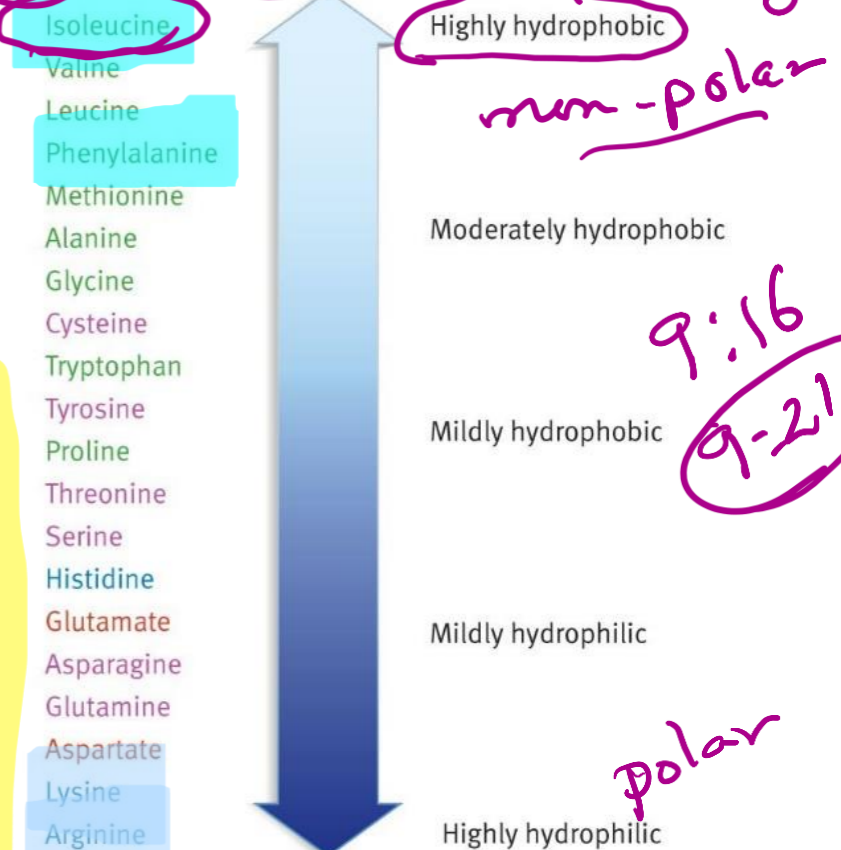


Charge

f_{H₂A}
0.5



Hydrophobicity

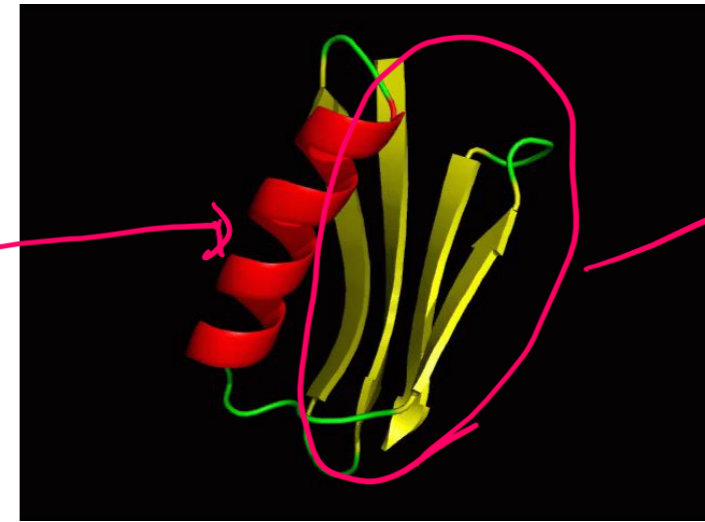


Secondary Structure

“Building blocks of proteins”

- **Hydrogen bonds** between the *mainchain* carbonyl group of one amino acid and the *mainchain* amino group of another form a protein's **secondary structure**.
 - A polypeptide must bend to allow this hydrogen bonding, forming:
 - **α -helices**
 - **β -pleated sheets**
- The large number of hydrogen bonds in a protein's secondary structure increases its stability - each hydrogen bond that is formed releases some energy.
- All amino acids can be incorporated into either secondary structure (However, some are found more frequently in one structure)

• main chain atoms.
 α -helix



β -sheet.

General Rule for Hydrogen Bonds:



X & Y are electronegative (N and O usually)

X-H = Donor of the hydrogen bond

Y = Acceptor of the hydrogen bond

Mainchain hydrogen bonds



The NH is the hydrogen bond donor.

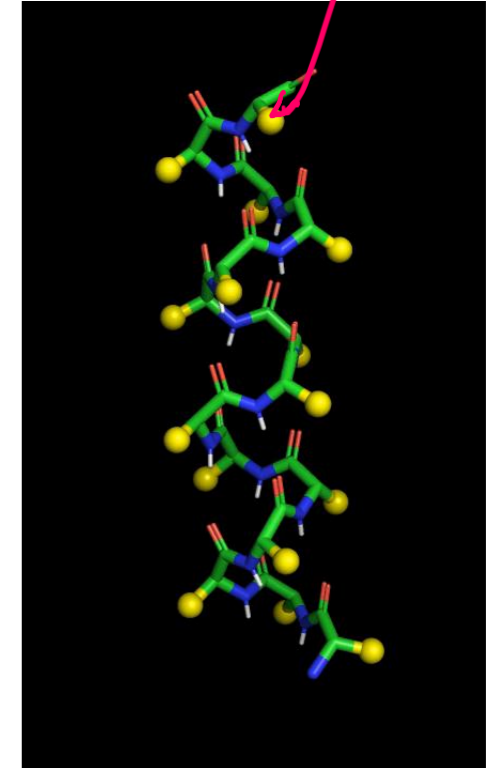
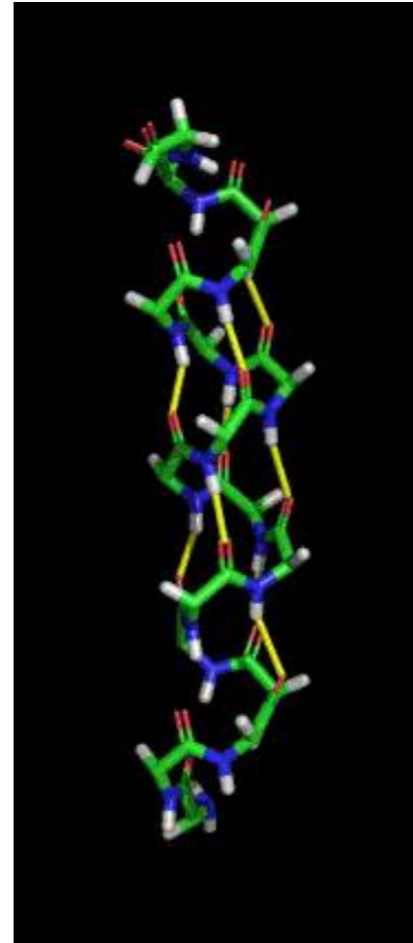
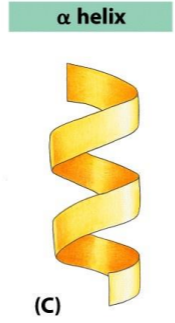
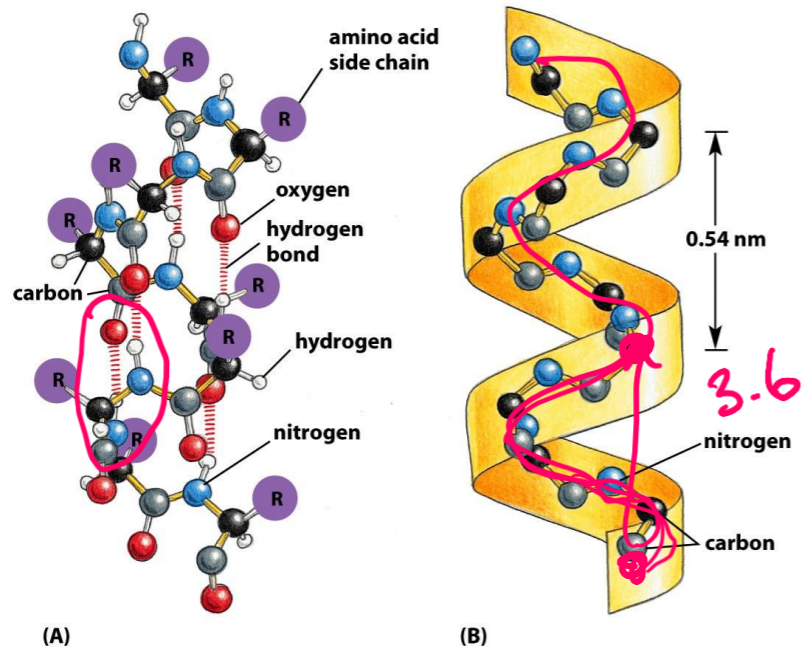
The C=O is the hydrogen bond _____.

acceptor.

Alpha Helix

H-bonds.

SIDE chains

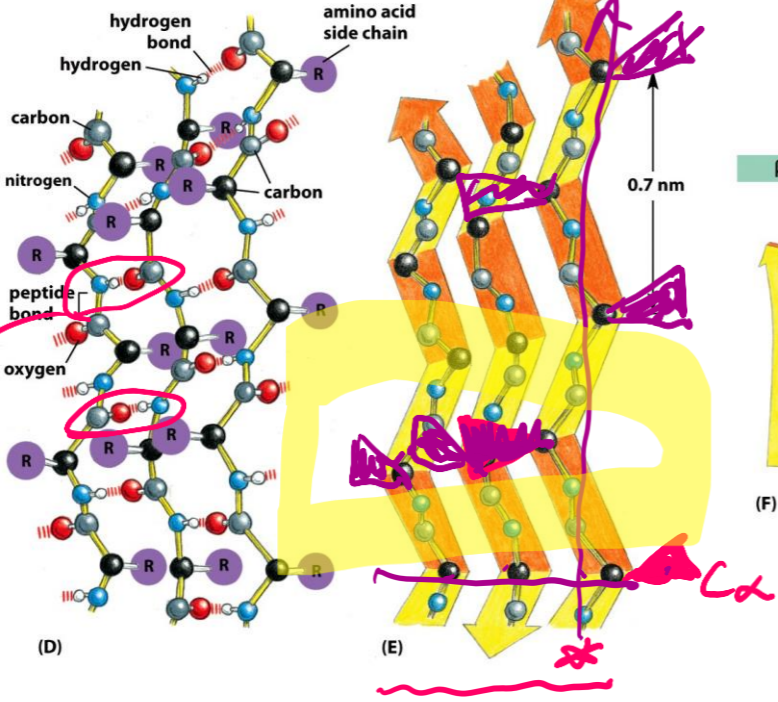


Spiral conformation (*helix*) in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of the amino acid four residues earlier:

✓ *Intra-strand H-bonds, parallel to helix axis.*

✓ *Side-chains project outwards.*

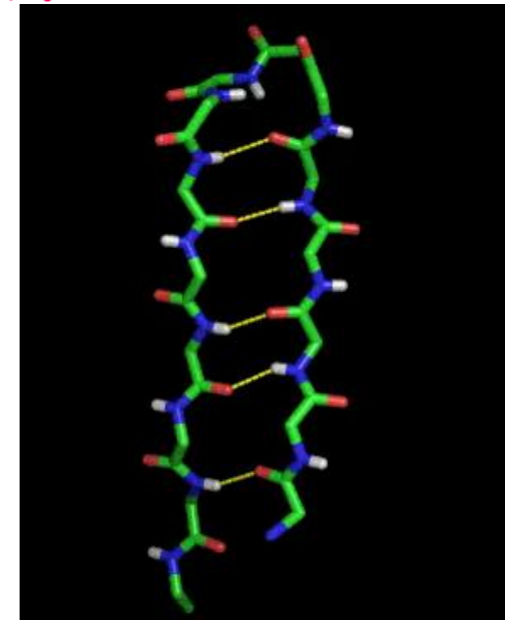
H Bonds



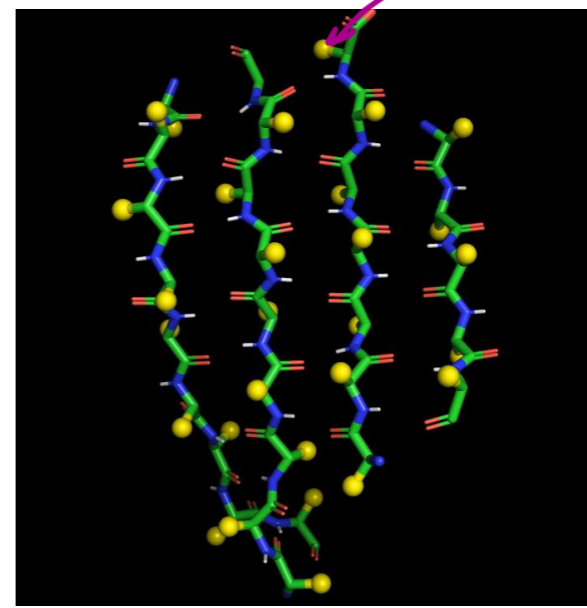
Beta Sheet

H bonds

1 strand. direction



2 strand.

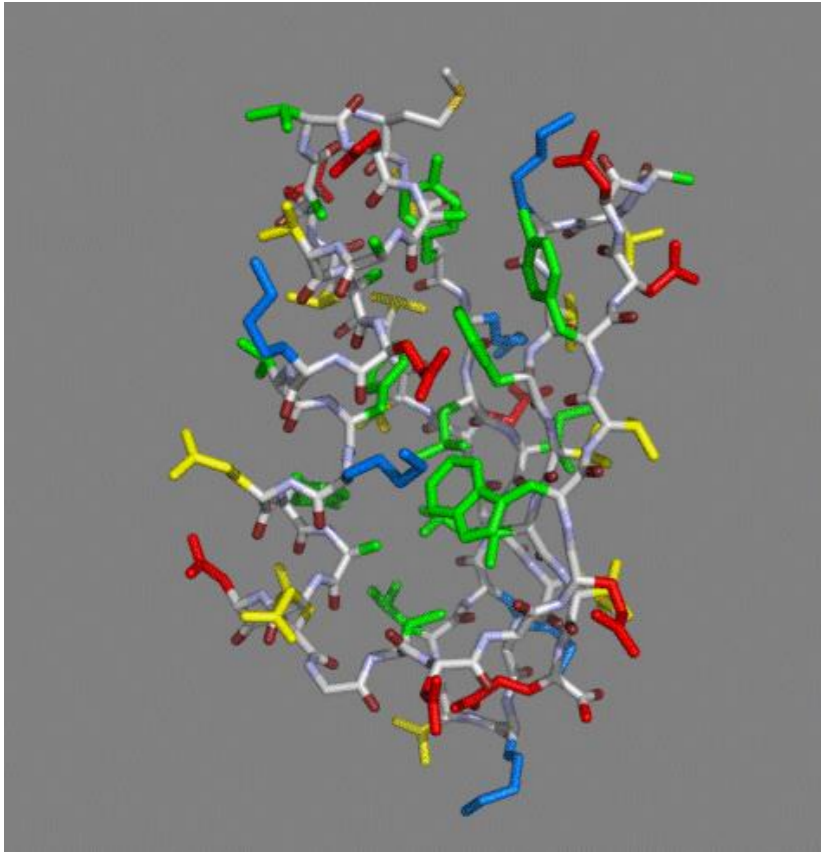


SIDE chain

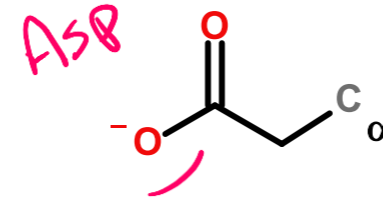
4-strand.

- Beta-Strands connected laterally by backbone hydrogen bonds that are perpendicular to the strand, forming a generally twisted, pleated sheet.
- Sheets can have two or more strands
- Side-chains:
 - project up and down along a strand.
 - project in the same direction going from strand to strand across the sheet.

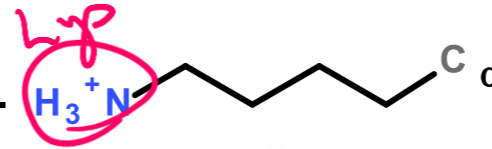
Tertiary Structure - Location of Residues in Globular Proteins



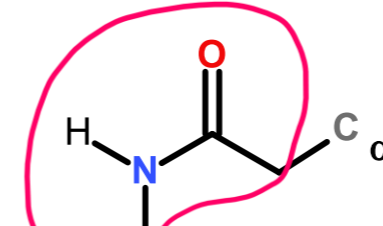
Red - amino acids with neg. sidechains (e.g. Asp)



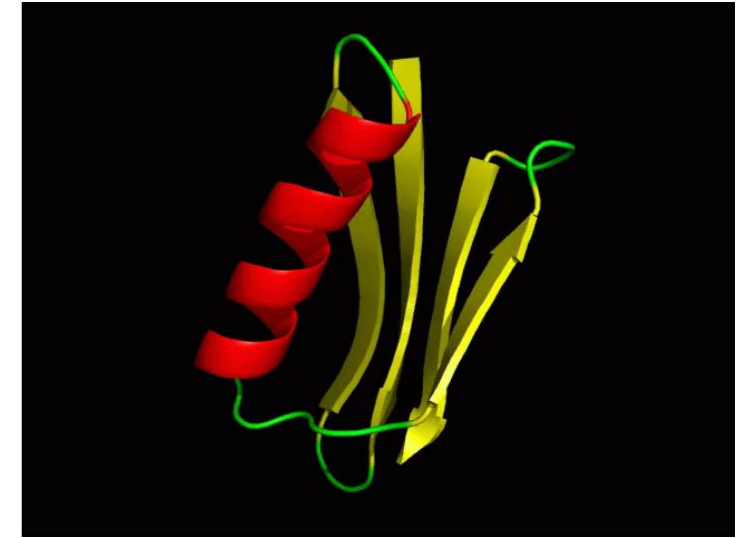
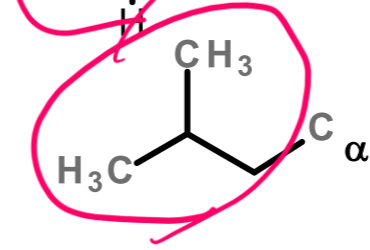
Blue - amino acids with pos. sidechains (e.g. Lys)



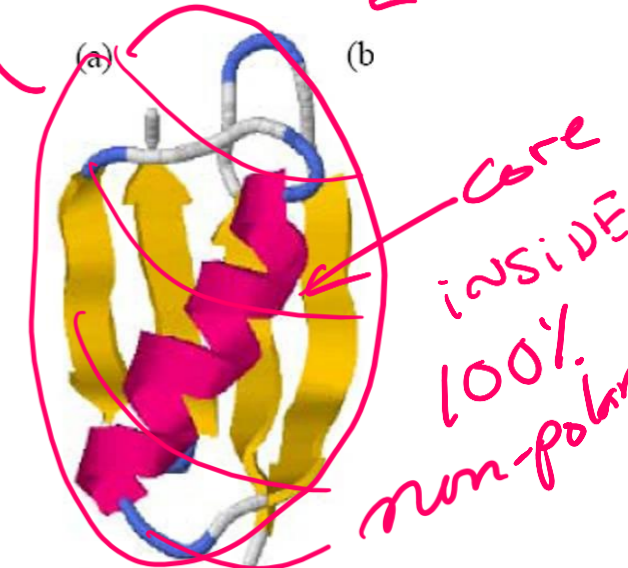
Yellow - amino acids with polar sidechain (e.g. Asn)



Green - amino acids with hydrophobic side chains (e.g. Leu)



Charged
polar
non-polar
surface



Amino Acid Type	Inside (I) <i>Core</i>	Surface (S) ✓
✓ Charged		✓
✓ Polar		✓
✓ Non-polar	100%	<i>also</i>

Protein Stability:

all shapes same
N.

H-bonds
van der Waals
Hydrophobic effect



Native

Unfolded

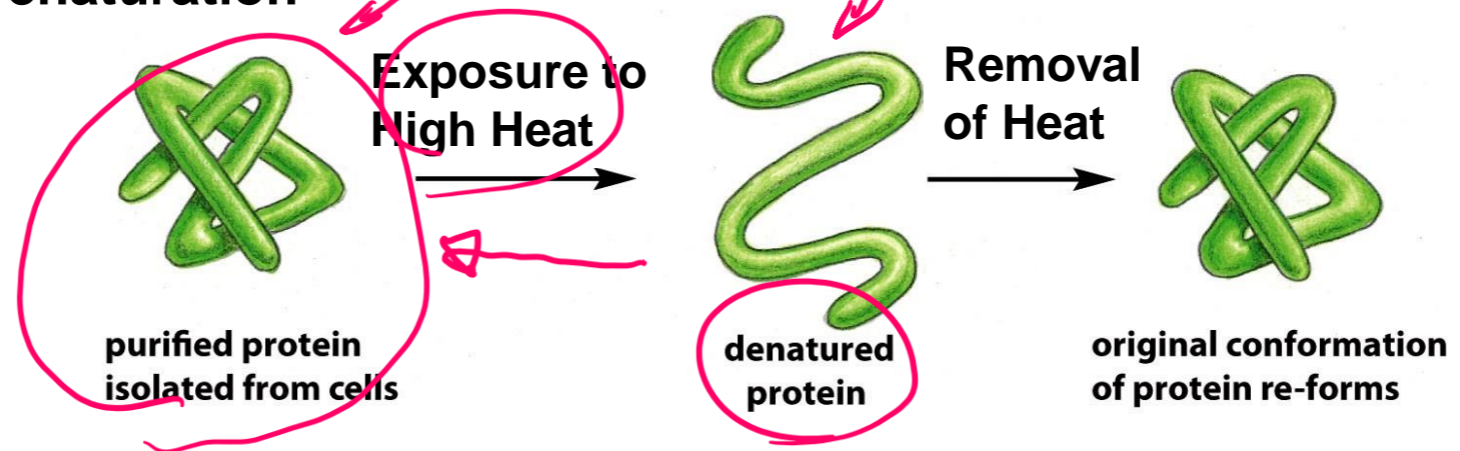
Chain disorder



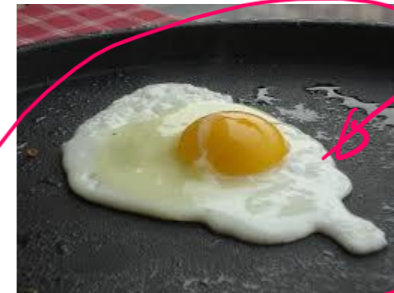
all diff. shapes

unfolded protein

Protein Denaturation

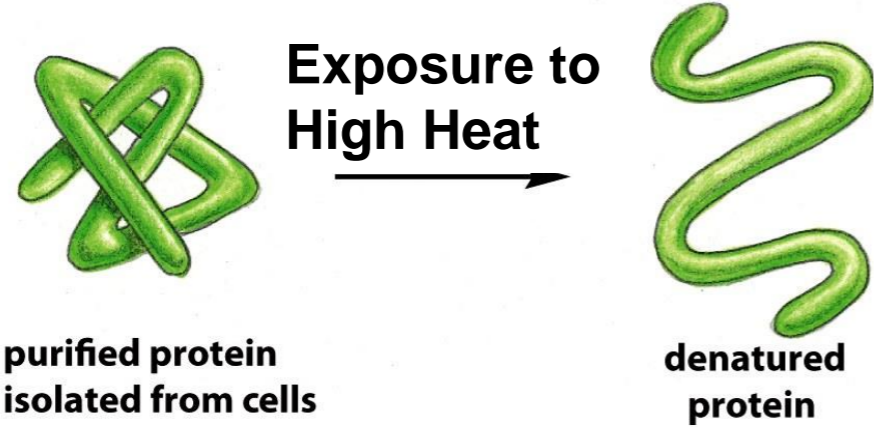


- Often, unfolded protein aggregate, which prevents refolding.

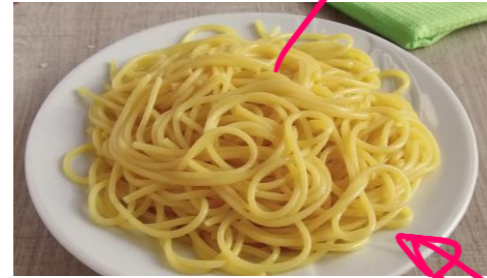
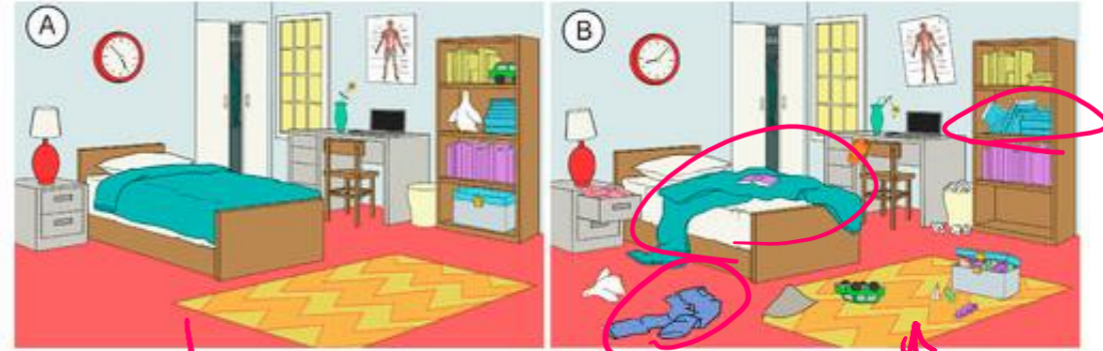


aggregation
to unfolded chains.

Unfolded Polypeptides Are Flexible – High Entropy stabilizes the Unfolded state



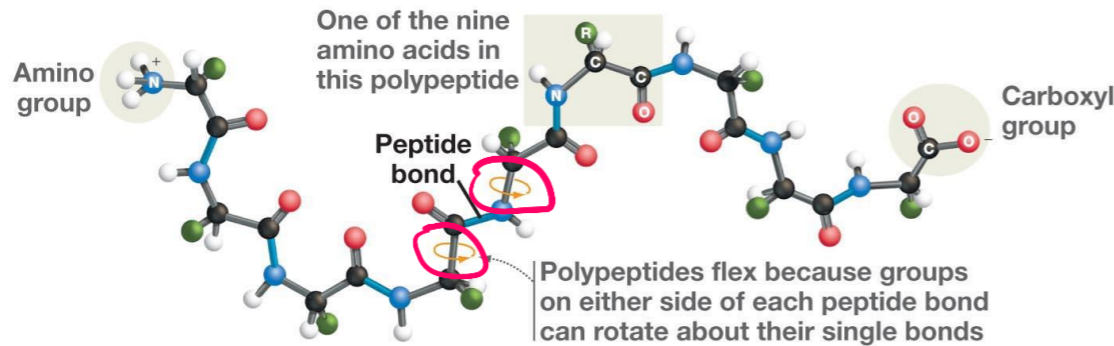
Energy and Entropy = disorder



ordered
more stable

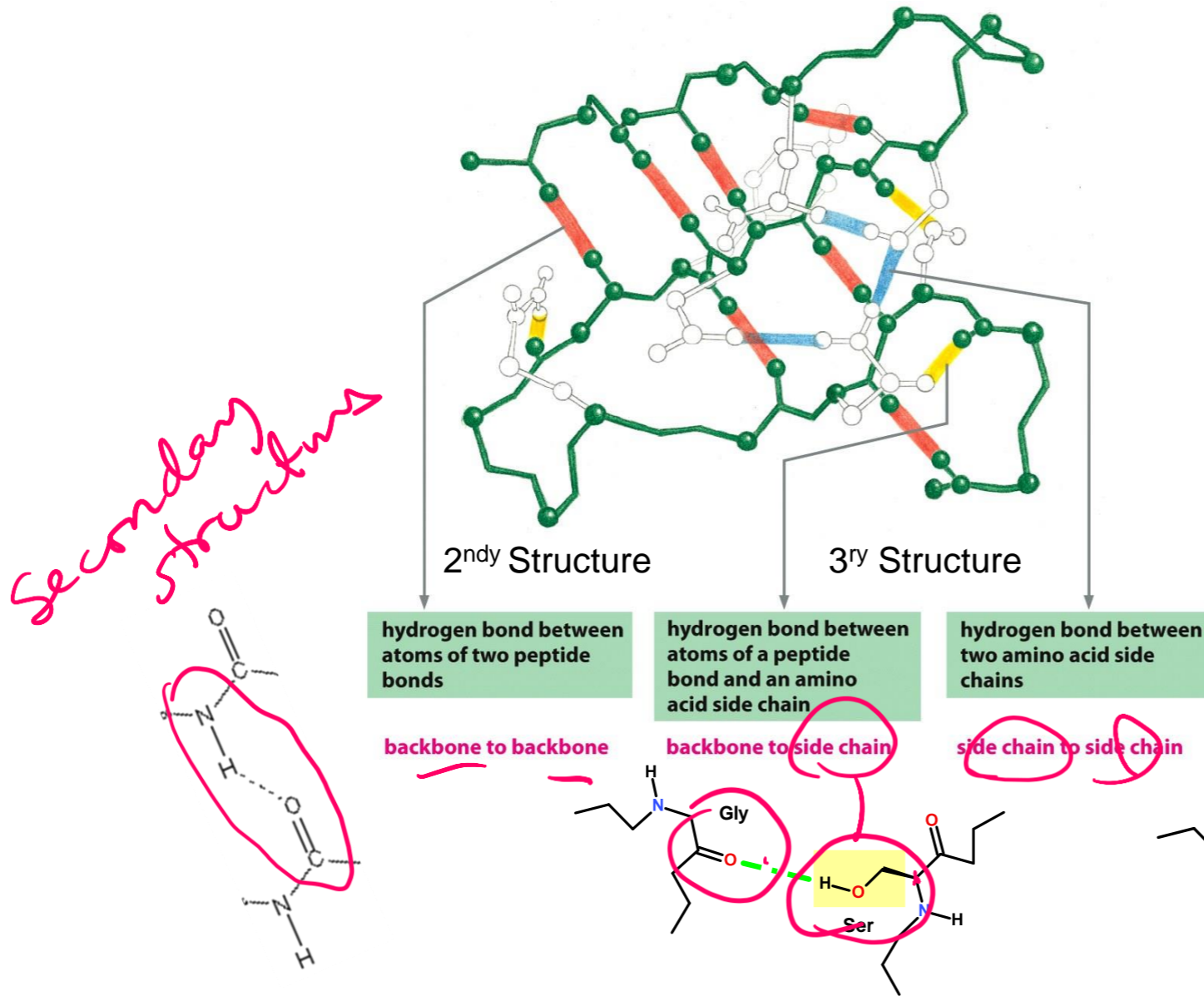
add energy

disorder



release energy
lower in energy more favorable

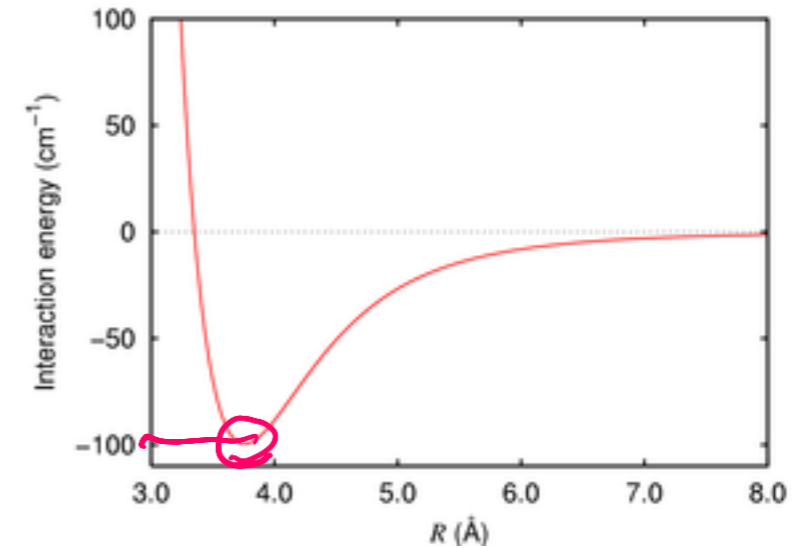
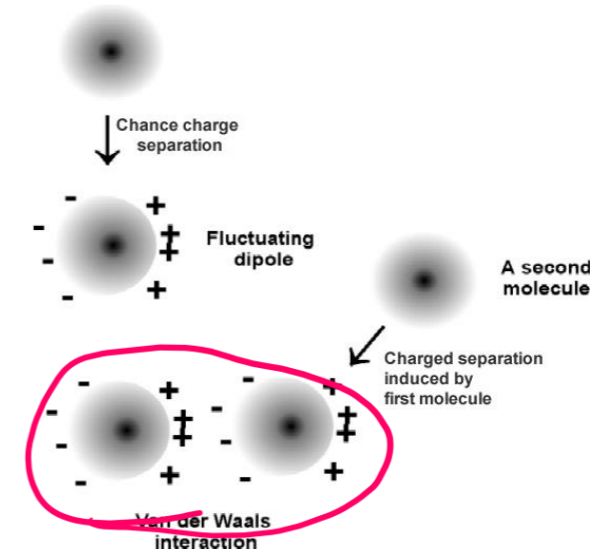
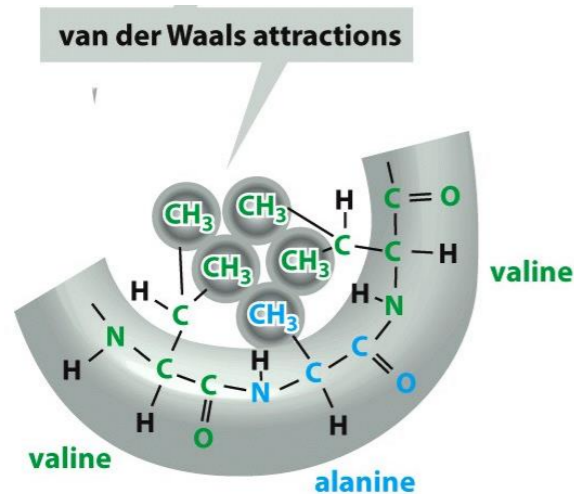
Hydrogen Bonding Stabilizes the Tertiary Structure



- **Hydrogen bonds** form between hydrogen atoms and the carbonyl group in the peptide-bonded backbone – secondary structure
- Hydrogen bonds are also found between hydrogen and electronegative atoms in side chains (sidechain-sidechain)
- Sidechains can form hydrogen bonds to the mainchain too.

Van der Waals (VdW) interactions Stabilize the Folded State

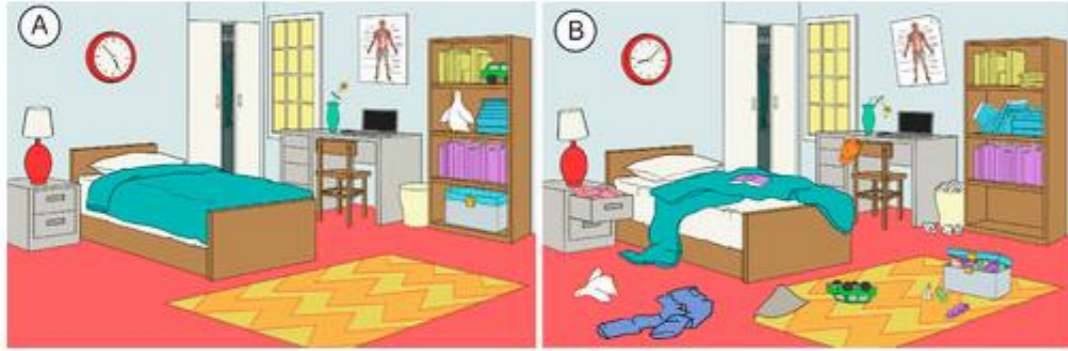
- VdW are weak electrostatic interactions between side chains due to temporary (fluctuating) charges.
- Attractive from long distance
- Distance at lowest energy is at the van der Waals radii of the atoms.
- Optimized in the core of folded proteins by “knobs fitting into holes”
- Strength proportional to contact area.



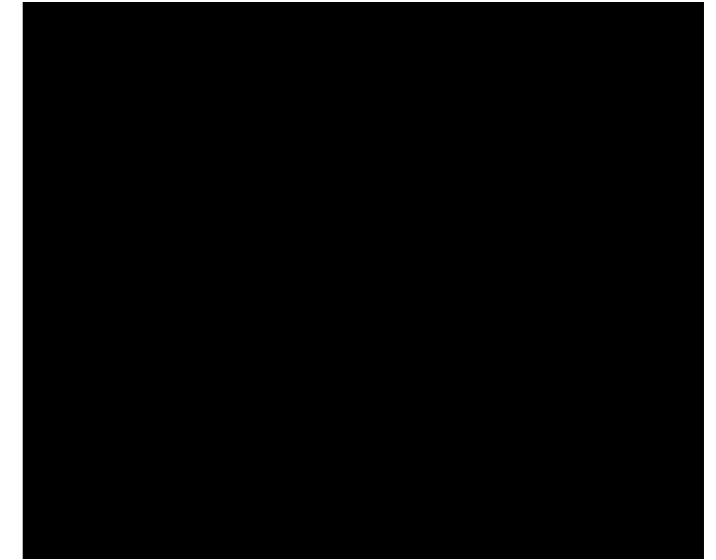
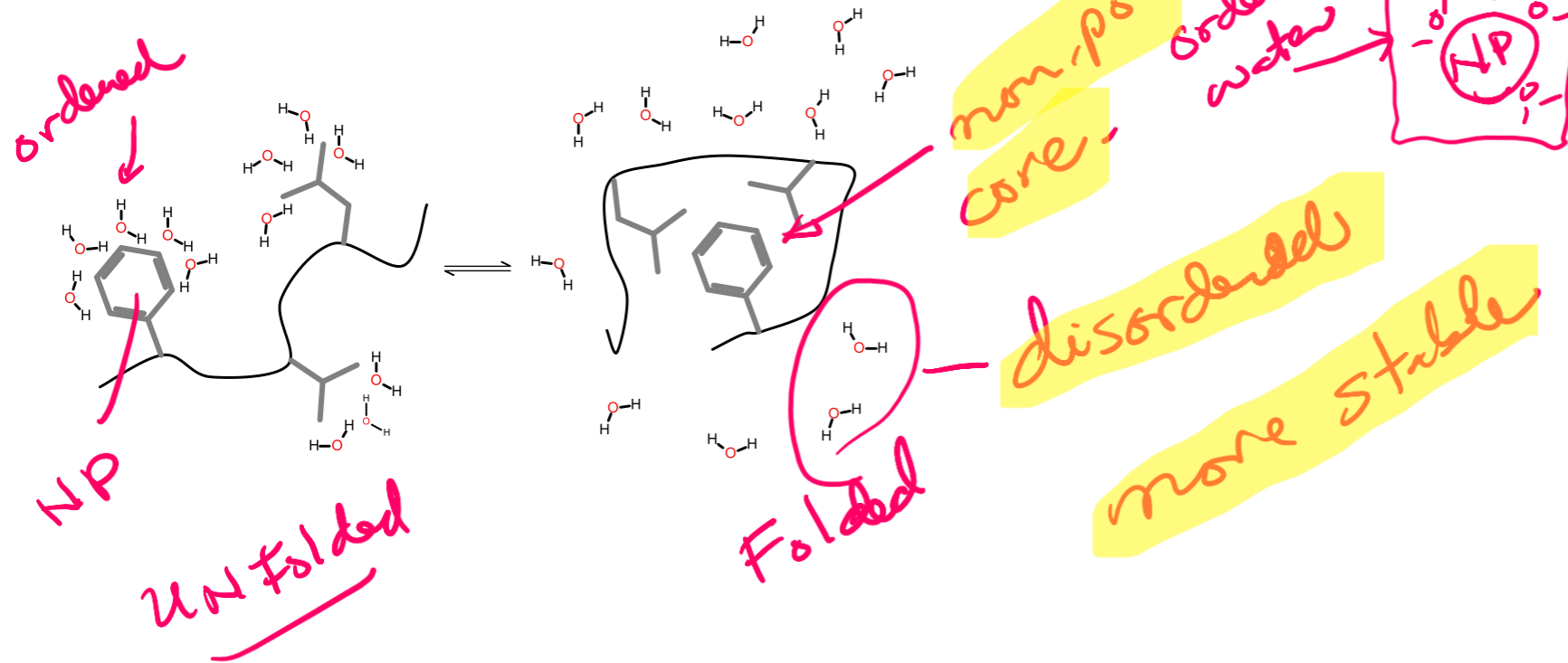
core ⇒ well packed ⇒ strong vdw

Hydrophobic Interactions are **Critical** for Stabilizing the Folded Structure

Energy and Entropy



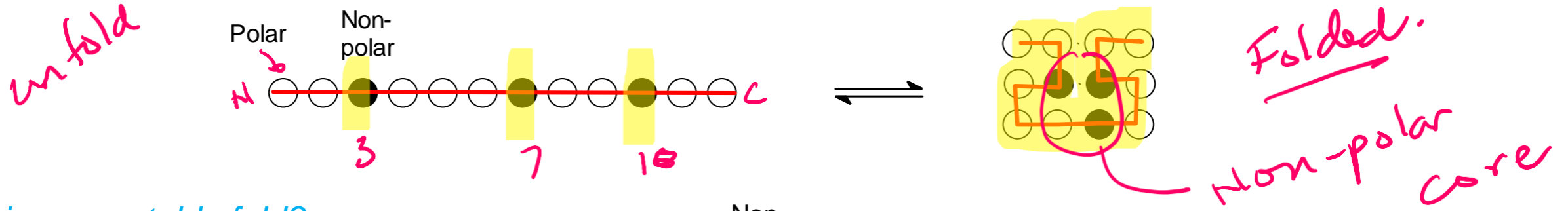
Ordered water hydrating a non-polar group



Hydrophobic interactions within a folded protein increase stability of the folded protein by releasing the ordered water that surrounded exposed non-polar groups in the unfolded protein. *Folding increases the entropy of the water – favorable.*

Fold Depends on Amino Acid Sequence

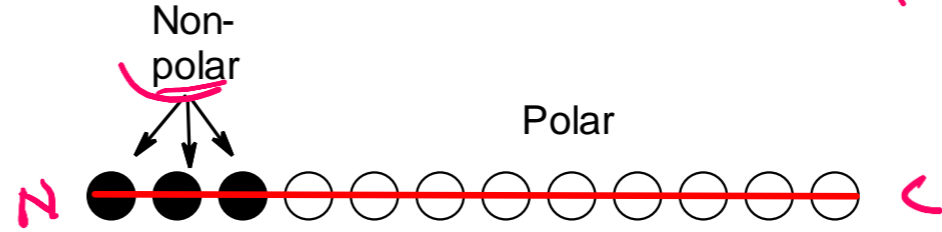
The *position* of non-polar residues (filled circles) mostly affects the final fold:



Which is more stable fold?

- A
- B
- C

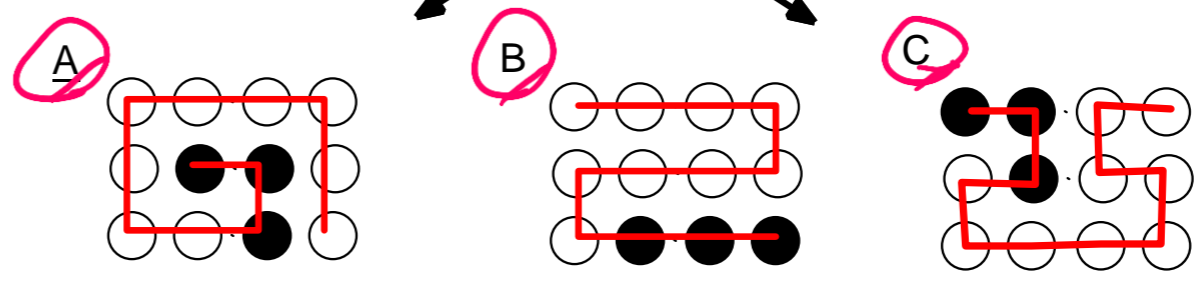
non-polar core



Which is the least stable fold?

- A
- B
- C

polar core exposed
non-polar exposed
still ordering water



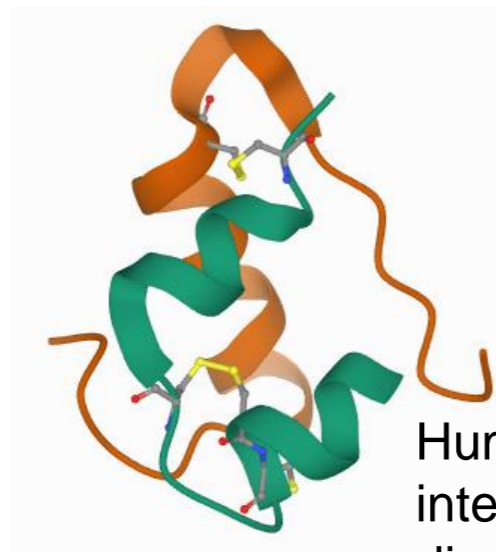
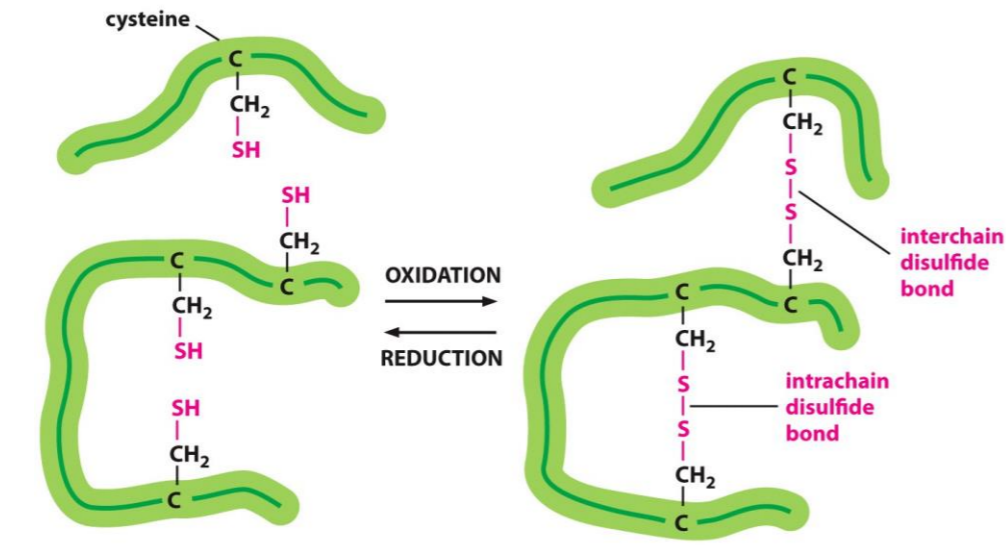
most

least

tertiary structure

Why?

Disulfide Bonds Stabilize Some Proteins Outside the Cell (and body)



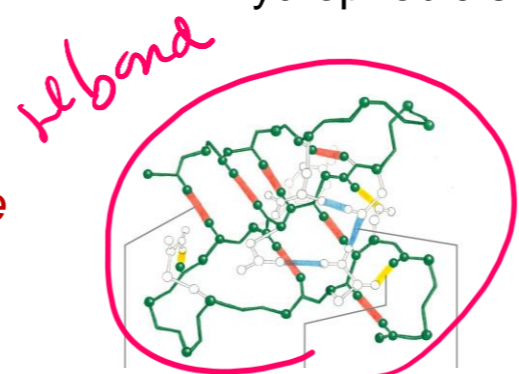
Summary - Interactions that Stabilize Folded Proteins.

- Hydrogen bonds** form between hydrogen atoms (NH) and the carbonyl group in the peptide backbone (mainchain), and between and donors and acceptors on sidechains. *Mainchain-mainchain H-bonds are responsible for secondary structures.*
- Hydrophobic interactions** within a protein increase stability of the folded state by *increasing entropy due to the release of water that was ordered by the exposed non-polar groups in the unfolded protein.*
- van der Waals interactions** are *optimized in the well packed core of the protein.*
- Covalent disulfide bonds** form **between sulfur-containing cysteine** residues **stabilizing them** (usually only exported, secreted proteins).

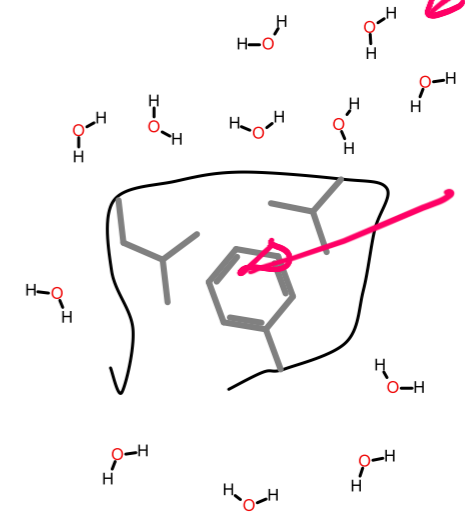
✓ H-bonds
 ✓ van der Waals
 ✓ Hydrophobic effect



Chain disorder ✓



many sharp disorder.
non polar



well packed core

