Genomics and Proteomics
(BIOT 3014)
Unit 3:
PART-II
Physical interaction that determine property of protein

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What are molecular interactions?

- Molecular interactions are attractive or repulsive forces between molecules and non-bonded atoms.
- Molecular interactions are also known as non-covalent interactions or intermolecular interactions.
- A molecule is a set of atoms that associates tightly with covalent bond and it does not dissociate or lose its structure, when it interacts with its environment.
- Covalent bonds remain intact, when proteins unfold. The processes of unfolding are not chemical reactions (no covalent bonds break or form) and involve only changes in molecular interactions.
- Therefore, molecular interactions are important in protein folding and drug design.

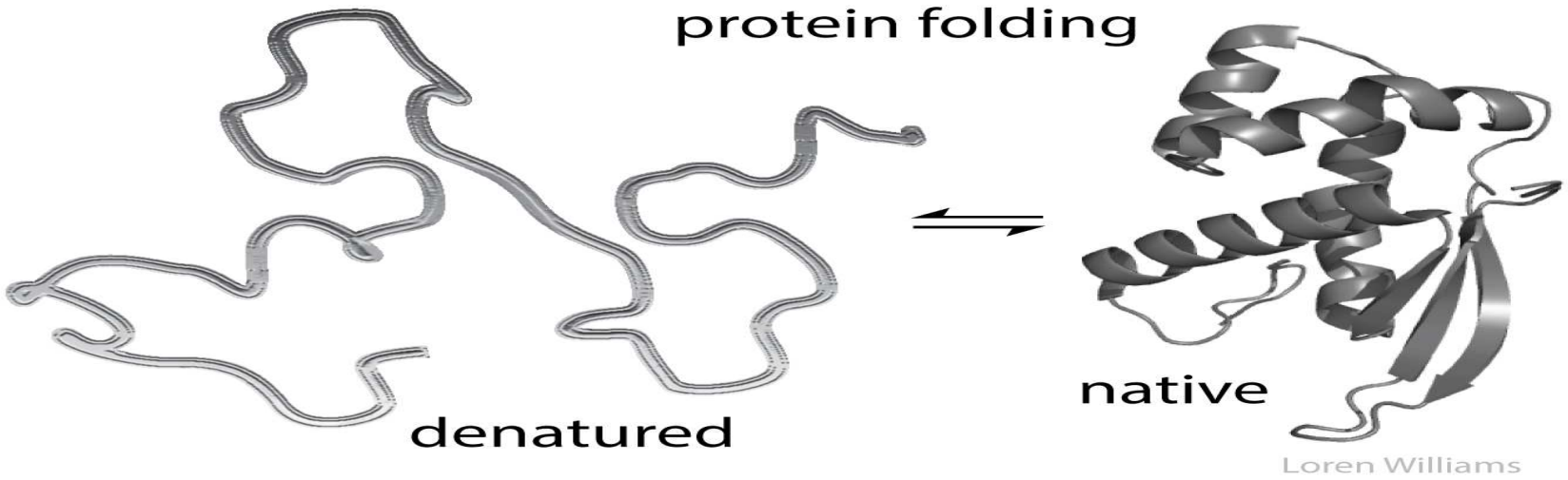
Native states

- When proteins fold into 3D globular structures, they are called in native states.

- The proteins native state and their assemblies are stabilized by several molecular interactions viz. electrostatic forces, Van der Waal interactions, hydrogen bonds and hydrophobic interactions.

- In biological systems, proteins assemble with DNA, RNA, membranes and with other proteins to perform specific function.

Fig. : Protein folding. It is the conversion from a denatured state (a random coil) to a native state. When a protein unfold (denature), then interior regions become exposed to the surroundings, which are mostly water and ions.



On the right-hand side the arrows are β-strands and the coils are α-helices.

Balance between native and denatured state

- Molecular interactions stabilize both the folded state and denatured state (the random coil.
- Large numbers of intra-molecular interactions within a protein native state are opposed by intermolecular interactions in the denatured state with surrounding water molecules.
- On balance, native biological macromolecules and assemblies are marginally stable.
- A small perturbations (e.g., change in pH or temperature or a single mutation) can tip the balance from folded state to unfolded state.
- Native states have low conformational entropy compare to denatured state .

Types of short-range molecular interaction

- All molecular interactions are fundamentally electrostatic in nature and can be described by some modification of Coulomb's law.
- The term electrostatic interaction is used to describe interactions between formally charged species.
- Interactions between partial charges are given other names like hydrogen bond, van der Waal (vdw) interaction.
- When two atoms are close together the occupied orbitals on the atom surfaces overlap, causing electrostatic repulsion between surface electrons.
- This repulsive force between atoms acts over a very short range, but is very large when distances are too short.

Electrostatic interactions

- Electrostatic interactions occurs amongst cation and anion species with formal charge of -2,-1,+1,+2 etc..
- It can be either attractive or repulsive, depending on the signs of the charges.
- Like charges repel while unlike charges attract.
- When cation and anion are close together, within a protein, this interaction could be considered as electrostatic/ionic interactions.
- Electrostatic forces fall off gradually with distance ($1/r^2$, where r is the distance between the ionic species).
- The terms dipole-dipole interaction is used to describe interactions between partial charges (discussed later).

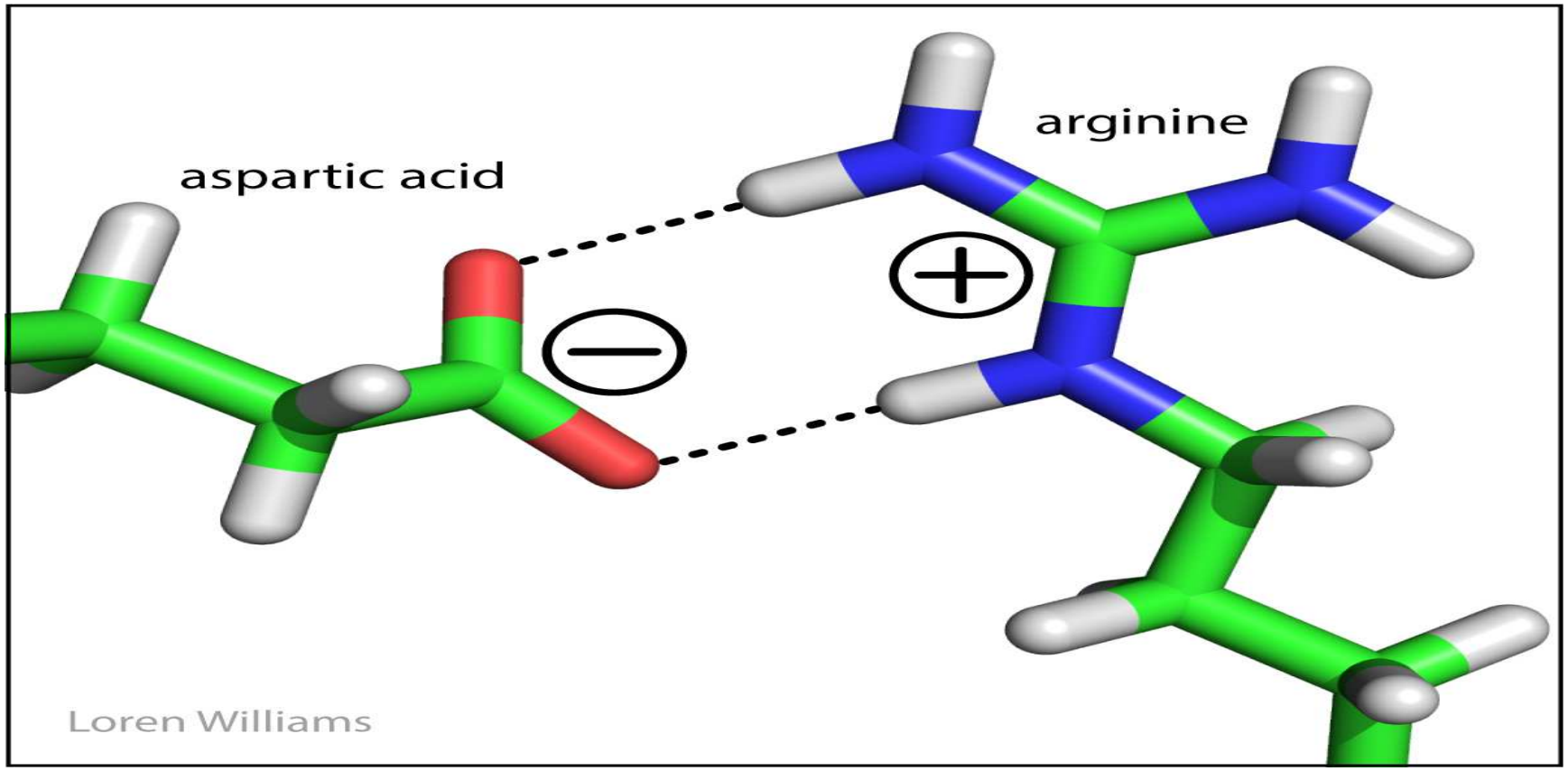
Ion pairs in proteins

- In proteins, electrostatic interactions between paired anionic and cationic amino acid side-chains are much frequent.

- Ion Pairs, sometimes called salt bridges, are formed when the charged group of a cationic amino acid (like lysine or arginine) is around 3.0-5.0 Å from the charged group of an anionic amino acid (like aspartate or glutamate).

- The charged groups in an ion pair are generally linked by hydrogen bonds, in addition to electrostatic interactions.

Fig. Ion pairing within a folded protein. An anionic aspartic acid (-1) interact with cationic arginine (+1) through attractive electrostatic forces. The dashed lines are representing the formation of hydrogen bonds.



Partial Charges

- A molecule consisting of atoms of different electro negativities i.e., the atom with lowest electro negativity hold partial positive charge (δ+) and the atom with highest electro negativity hold partial negative charge (δ-).
- A large difference in the electro negativities of two bound atoms causes the bond between them to be more polar, and the partial charges on the atoms to be higher in amount.
- In biological systems, oxygen is the most electro negative atom, carrying the largest partial negative charge (δ-).

Fig.: The partial charges and dipole moment of a water molecule. The electronegative oxygen atom pulls electron density away from the hydrogen atoms. The oxygen carries a partial negative charge and the hydrogen atoms carry partial positive charges. Bond dipoles (center) and molecular dipoles (right) can be represented as vectors from positive to negative charge.

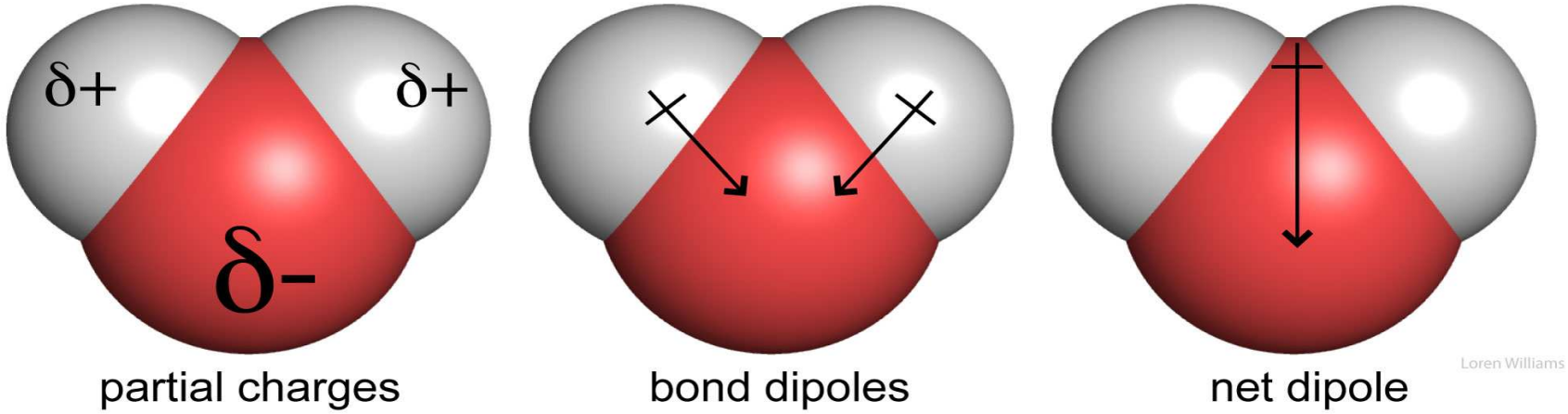
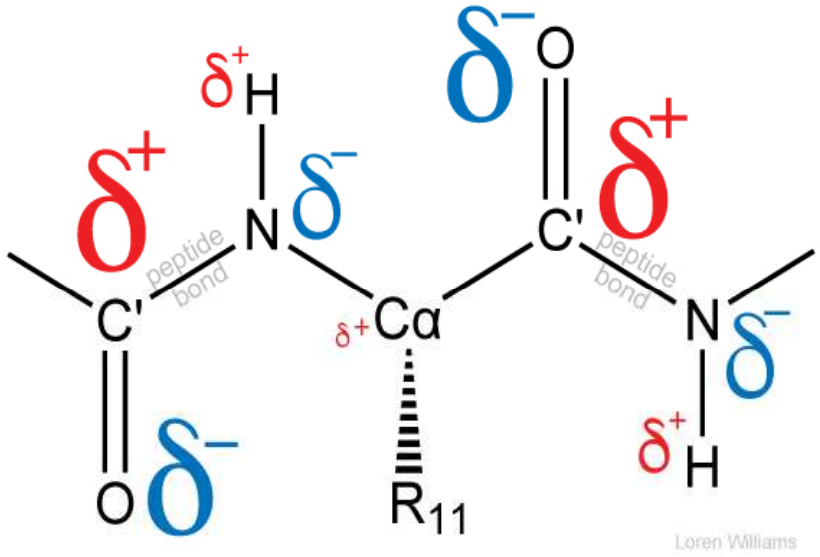


Fig. shows the partial charges within a polypeptide. The symbol size (δ) is scaled to the magnitude of the partial charge.



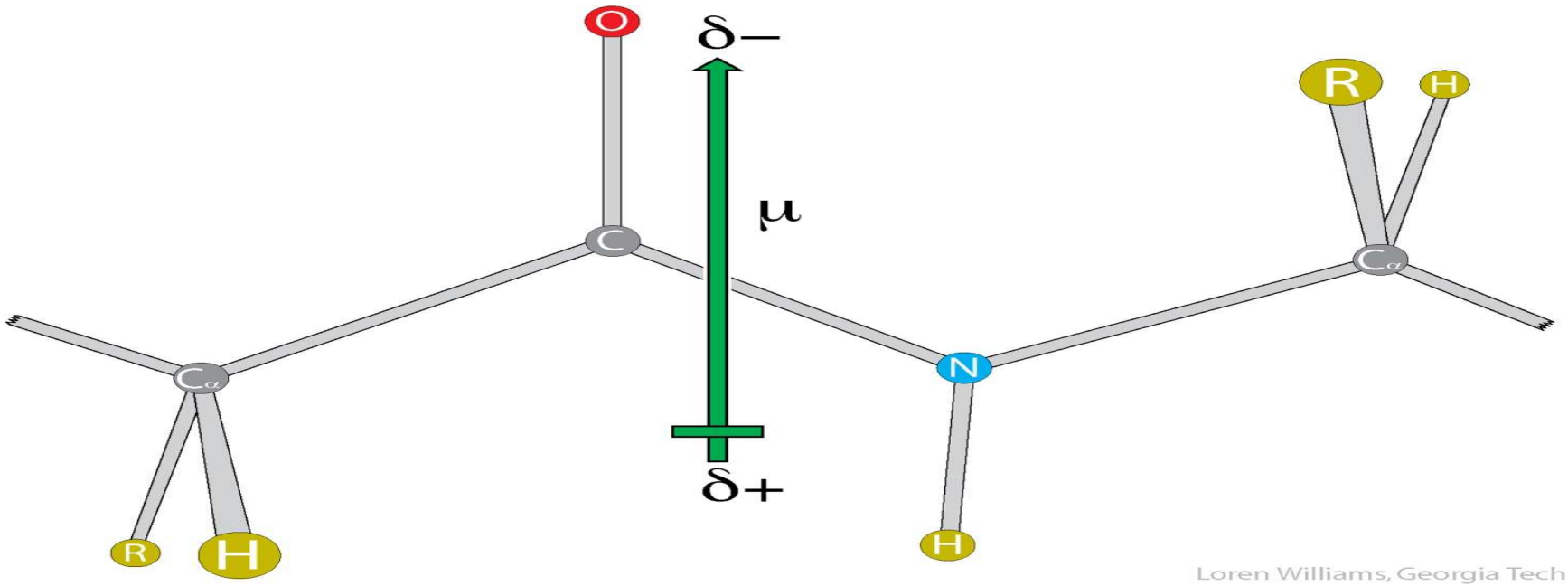
-- Partial Charges on the Atoms of a Peptide --	
Atom	partial charge (e^-)
N	-0.42
HN	+0.27
Cα	+0.12
C'	+0.60
O	-0.57

Dipole moments

- The extent of charge separation within a molecule is characterized by the dipole moment μ.
- The μ is determined by the magnitude of the partial charges (expressed in term of esu) and distance between them (in cm).
- The dipole moment of an electron and a proton separated by 1 Å equals:

  4.8×10^{-10} esu x 10^{-8}cm = 4.8×10^{-18} esu cm= 4.8 Debye.
- The dipole moment of water is 1.85 Debye.

Fig. : The orientation of the dipole moment of a peptide is approximately parallel to the N-H bond and is around 3.7 Debye. The large dipole moment of a peptide bond indicate that dipolar interactions are important in protein conformation and interactions.



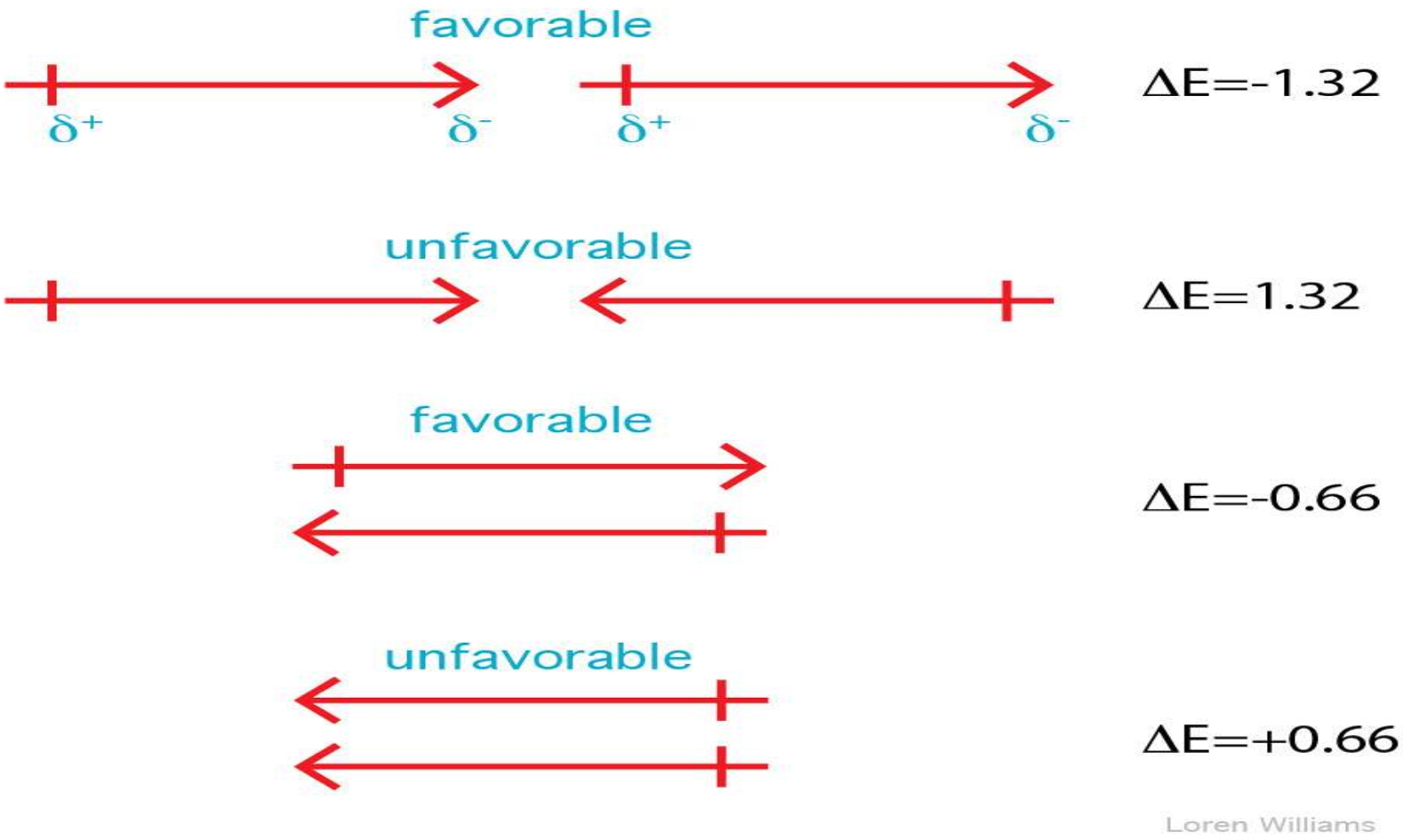
Multi-species interaction

- A dipole is surrounded by an electric field, which influence the nearby charged and partially charged species.
- Interactions between dipoles and ions are called ion-dipole Interactions.
- Dipoles also interact with other dipoles (called dipole-dipole interactions) that induces charge redistribution (polarization) in surrounding molecules i.e., dipole-induced dipole interactions.

Dipole-dipole interactions (Keesom interactions)

- The positive end of the first dipole is attracted to the negative end of second dipole and is repelled by positive end.
- The strength of a dipole-dipole interaction depends on the size of both dipoles and their proximity as well as orientations.
- The net interaction energy between two dipoles can be either positive or negative.
- Parallel end-end dipoles attract while anti-parallel end-end dipoles repel.

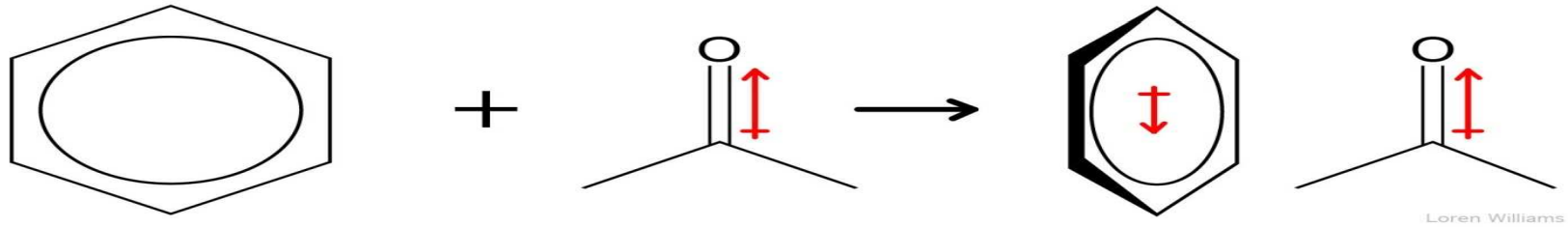
Fig. shows how dipole-dipole interactions with different orientations of the dipoles that fall off with $1/r^3$.



Dipole-induced dipole interactions (Debye interactions)

- Any molecule with a dipole moment (or any ion) is surrounded by an electrostatic field.
- This electrostatic field shifts the electron density (alters the dipole moments) of nearby molecules.
- A change in the dipole moment of one molecule by another (or by any external electric field) is called polarization.
- The ease with which electron density is shifted by an electronic field is called polarizability.
- Amino acid side-chains with π electrons, such as phenylalanine and tryptophan, are more polarizable than side-chains such as isoleucine, which lack π electrons.

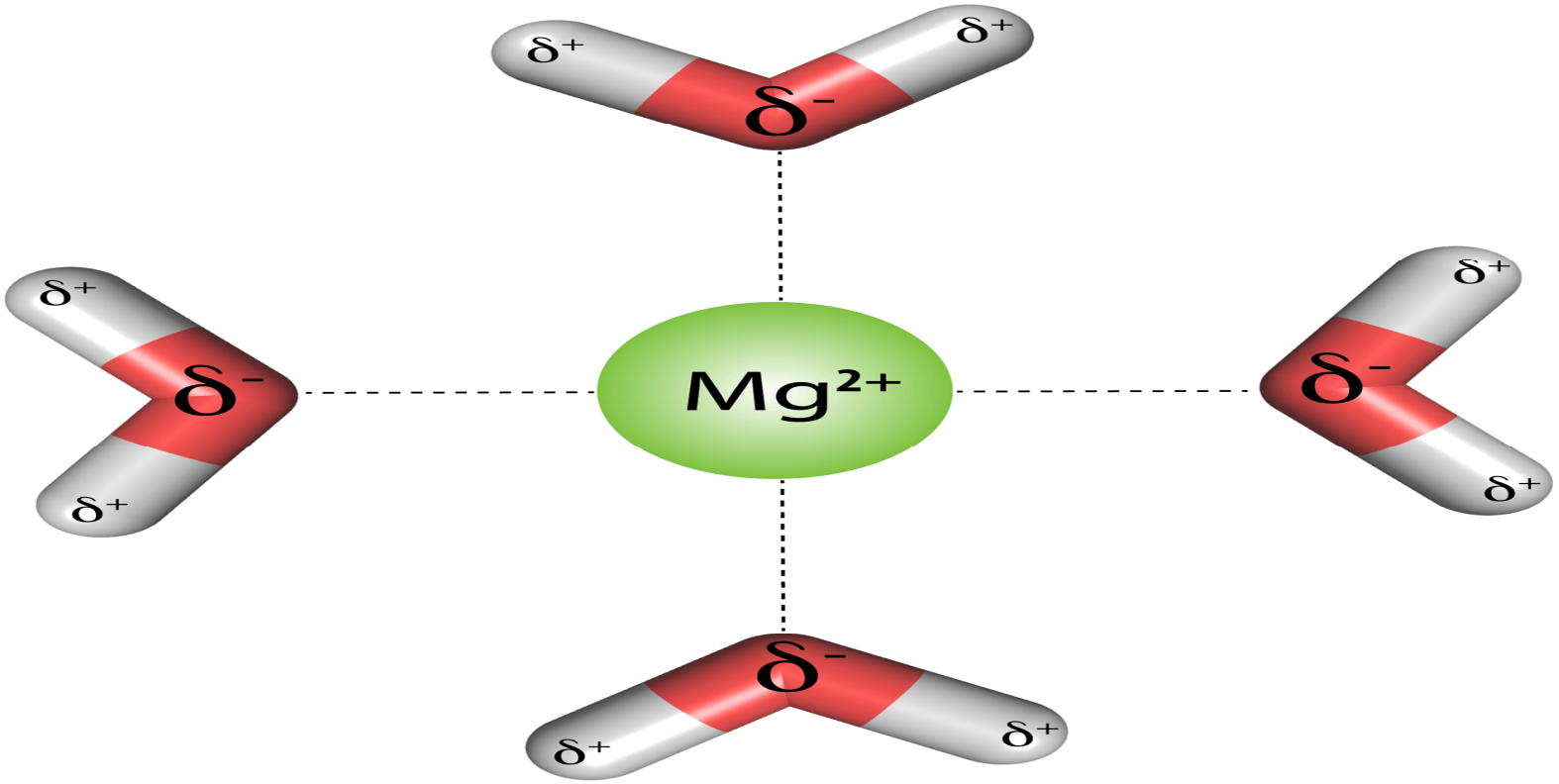
Fig. shows how a static dipole can induce a dipole in an adjacent molecule. When two isolated molecules (left) come together in a liquid or solid (right), the static dipole 'polarizes' the adjacent molecule. The strength of a dipole-induced dipole interaction depends on the size of the dipole moment of the first molecule and on the polarizability of the second molecule. π electrons are more polarizable than σ electrons.



Dipole-induced dipole interactions are always attractive and fall off with $1/r^4$. The charged species (Mg^{2+}, $-COO^-$, etc.) also polarize nearby molecules and induce favorable dipoles. The resulting interactions, called charge-induced dipole interactions and are also important in protein structure.

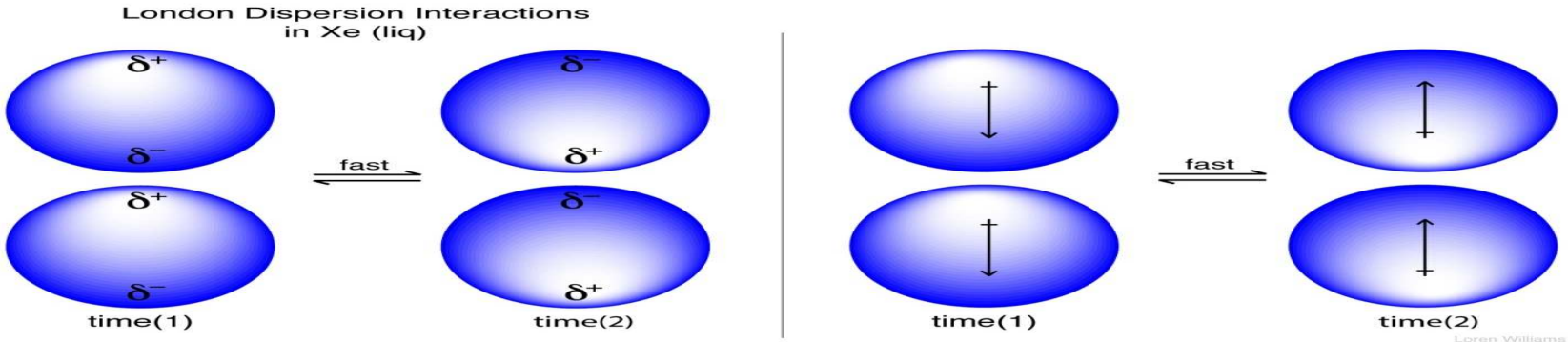
Charge-dipole interactions

A molecule with a permanent dipole can interact favorably with cations and anions.



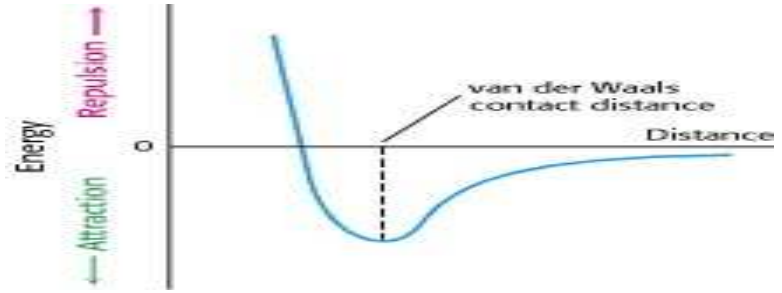
van der Waals interactions

Electrons, even in a spherical atom like Xenon, fluctuate over time according to the natural resonant frequency of the atom. As electron density fluctuates, dipole moments also fluctuate very fast in femto second. Therefore, all molecules and atoms contain oscillating dipoles. When molecules are close to each other, oscillating dipoles coupled and experience attractive electrostatic interaction known as dispersive interactions. It occurs between any pair of molecules, polar or non-polar that fall off with $1/r^6$.Darker blue indicates higher electron density.



van der Waals interactions

- The basis of a van der Waals interaction is the distribution of electronic charge around an atom changes with time.
- At any instant, the charge distribution is not perfectly symmetric.
- This transient asymmetry in the electronic charge around an atom acts through electrostatic interactions to induce a complementary asymmetry in the electron distribution around its neighboring atoms.
- The resulting attraction between two atoms increases as they come closer to each other, until they are separated by the van der Waals contact distance.
- At a shorter distance, very strong repulsive forces become dominant because the outer electron clouds overlap.

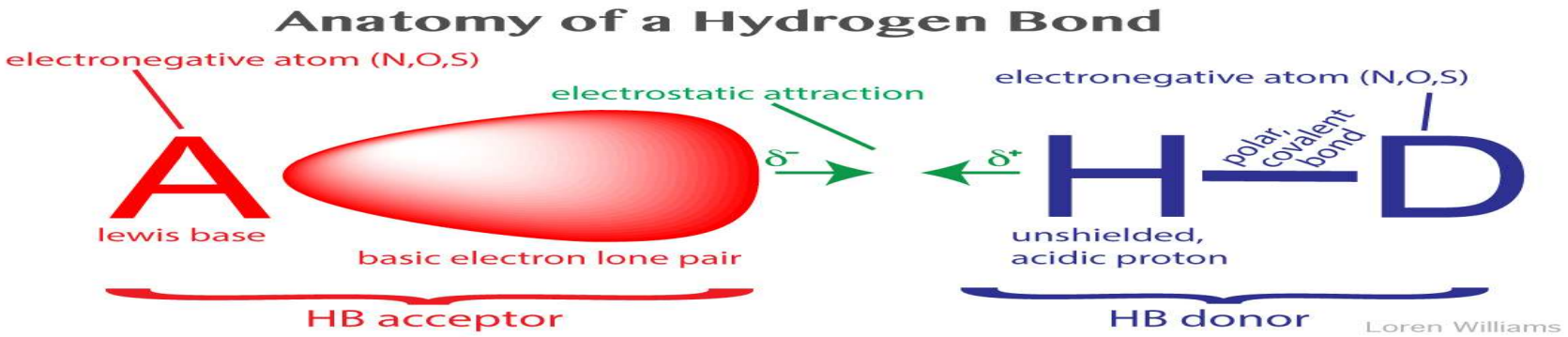


Dipsersive interactions in proteins

- The total number of pair-wise atom to atom dispersive interactions within a folded protein is vast, so dispersive interactions can make large contributions to protein stability and the strength of interaction is related to polarizability.
- Tryptophan, tyrosine, phenylalanine and histidine are the most polarizable amino acid side-chains, and form the strongest dispersive interactions in proteins.

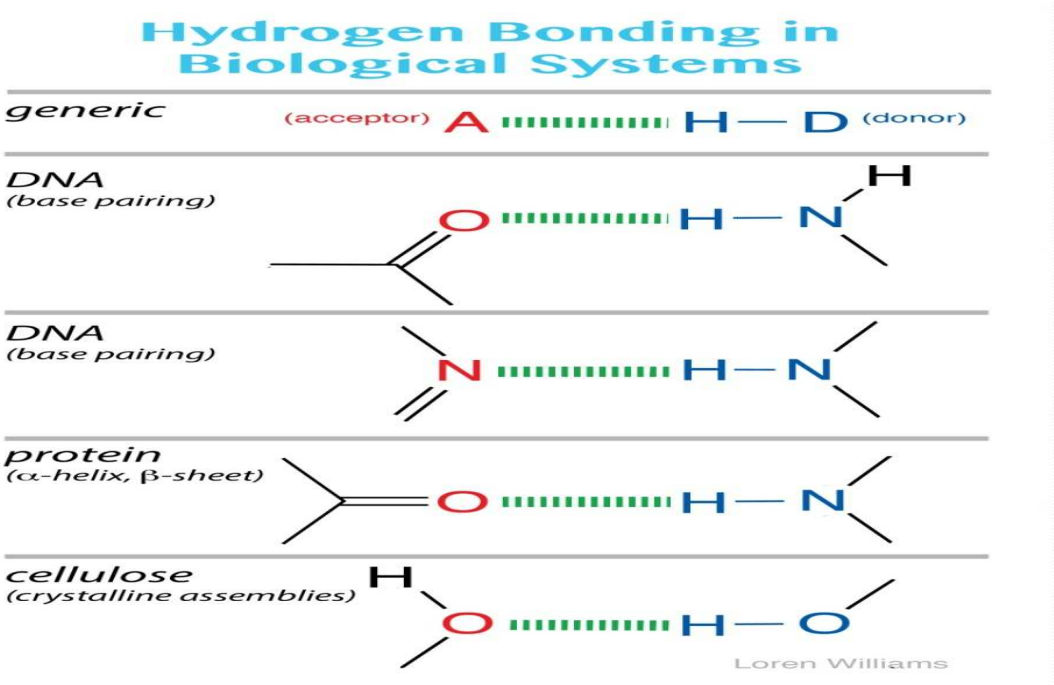
Hydrogen Bonding

A hydrogen bond is a favorable interaction between an atom with a basic lone pair of electrons (a Lewis Base) and a hydrogen atom that has been partially stripped of its electrons because it is covalently bound to an electronegative atom (N, O, or S). In a hydrogen bond, the H^+ is partially transferred from H-D to A, but H^+ remains covalently attached to D.



Hydrogen bonds are essentially electrostatic in nature, although the energy can be decomposed into additional contributions from polarization, exchange repulsion, charge transfer, and mixing.

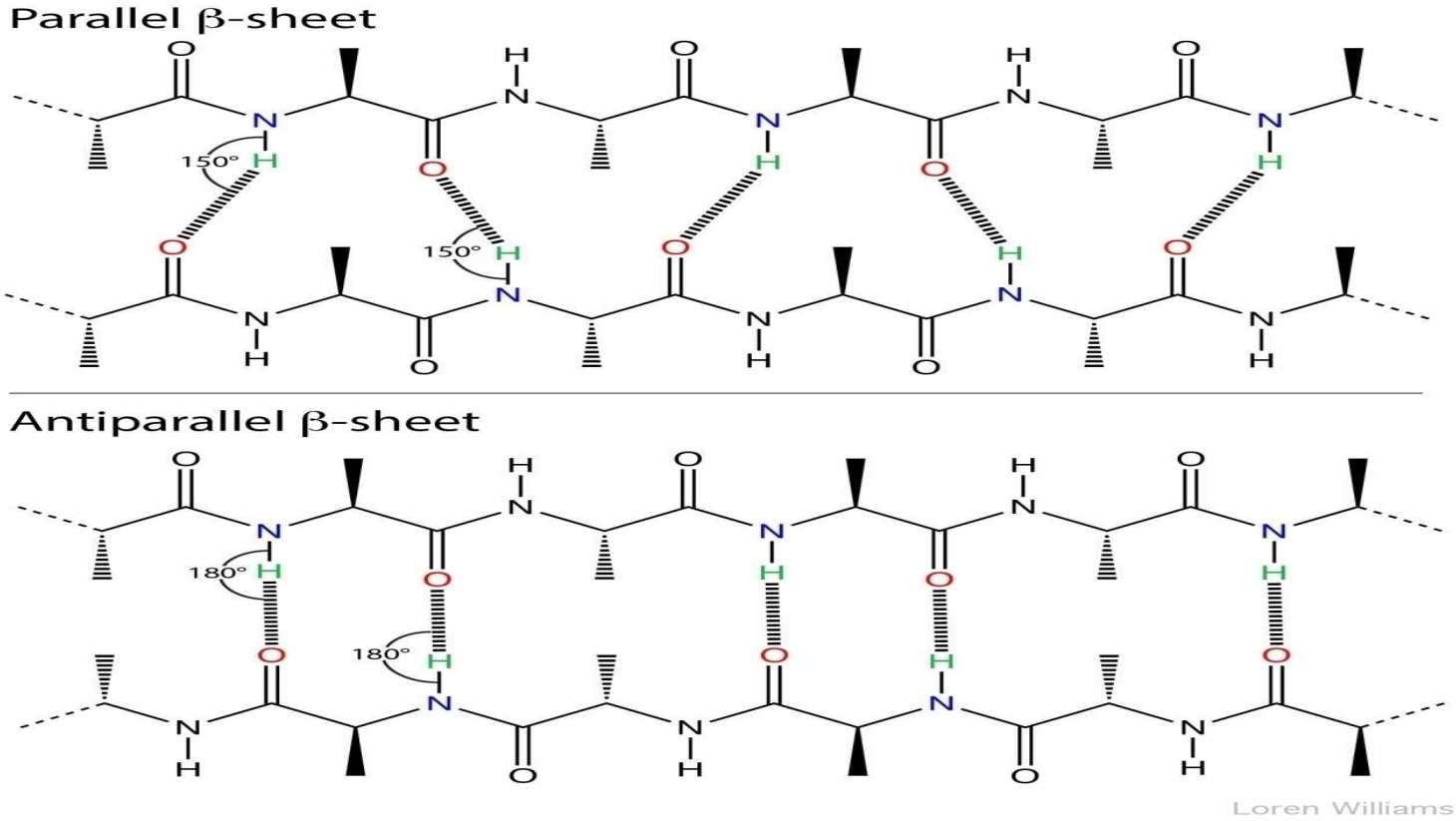
A hydrogen bond is a molecular interaction (a non-bonding interaction).



Hydrogen bond strengths form a continuum. Strong hydrogen bonds of 20-40 kcal/mole, generally formed between charged donors and acceptors. Weak hydrogen bonds of 1-5 kcal/mole formed with carbon as the proton donor. Moderate hydrogen bonds (most common) are formed between neutral donors and acceptors are from 3 - 12 kcal/mole.

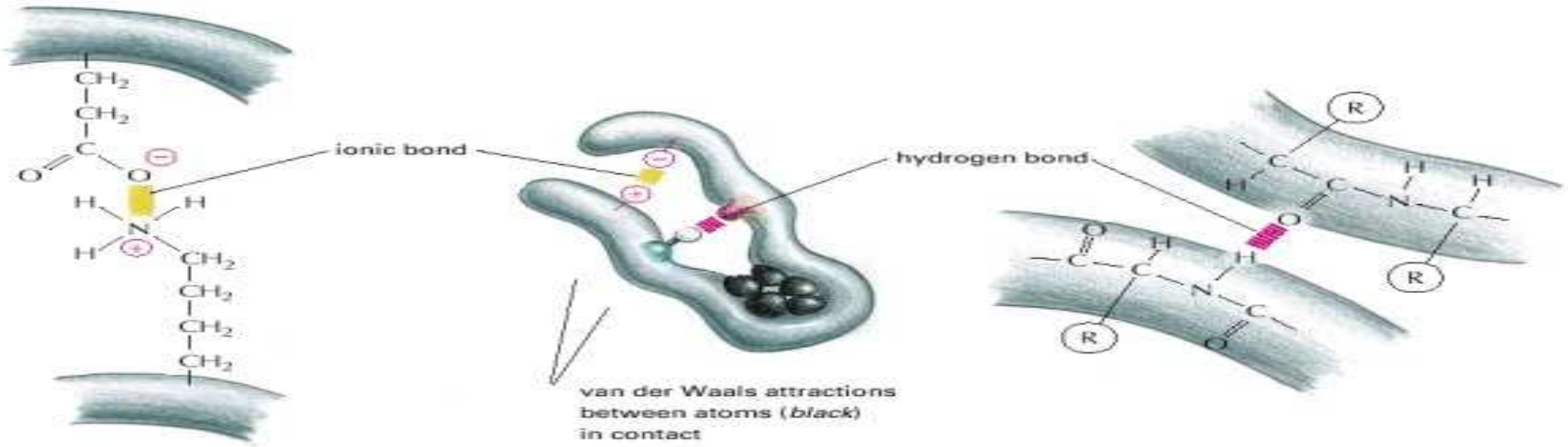
Geometry of hydrogen bonds

The geometry of a hydrogen bond can be described by three quantities, the D to H distance, the H to A distance, and the D to H to A angle. The most stable hydrogen bonds are close to linear (D to H to A angle of 180°). The hydrogen bonds in antiparallel β-sheets are linear, while the hydrogen bonds in parallel β-sheets are non-linear.



Hydrogen atoms are not observable by x-ray crystallography as applied to proteins.

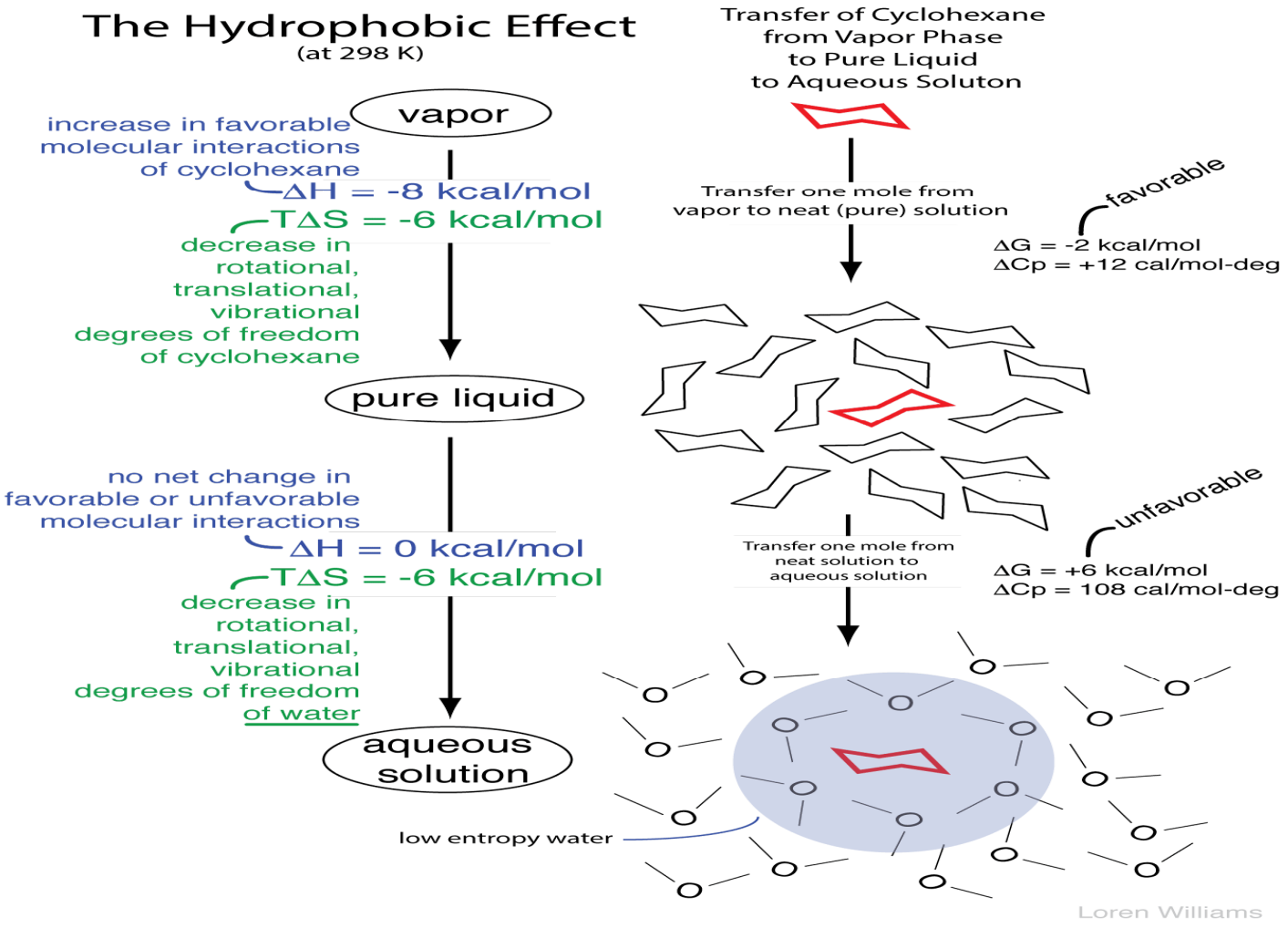
Non-covalent interactions that help proteins fold



Hydrophobic effect

- The hydrophobic effect is the insolubility of oil and other non-polar substances in water.
- Hydrophobic substances are those that are soluble in non-polar solvents (such as CCl_4 or cyclohexane, or olive oil or hydrocarbons).
- Attractive forces between non-polar solutes originate from the water, not from direct interactions between the solute molecules.
- The hydrophobic effect is an indirect consequence of strong directional interactions between water molecules (cohesive properties) and the complementarity of those interactions.
- The cohesive interactions between water molecules are not disrupted by dissolved hydrocarbon (or other non-polar molecules).
- In bulk solution a water molecule can rotate and still maintain hydrogen bonding interactions.
- At a hydrophobic interface the interactions are anisotropic (directional) because the hydrocarbon does not form hydrogen bonds.

Thermodynamic basis of the hydrophobic effect

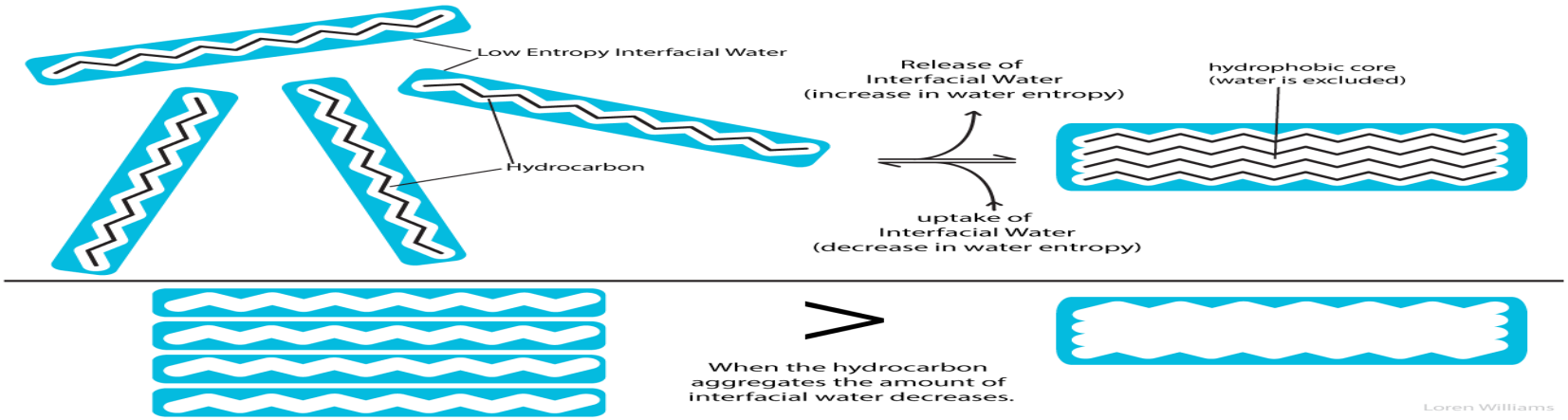


Hydrophobic effect Cont…

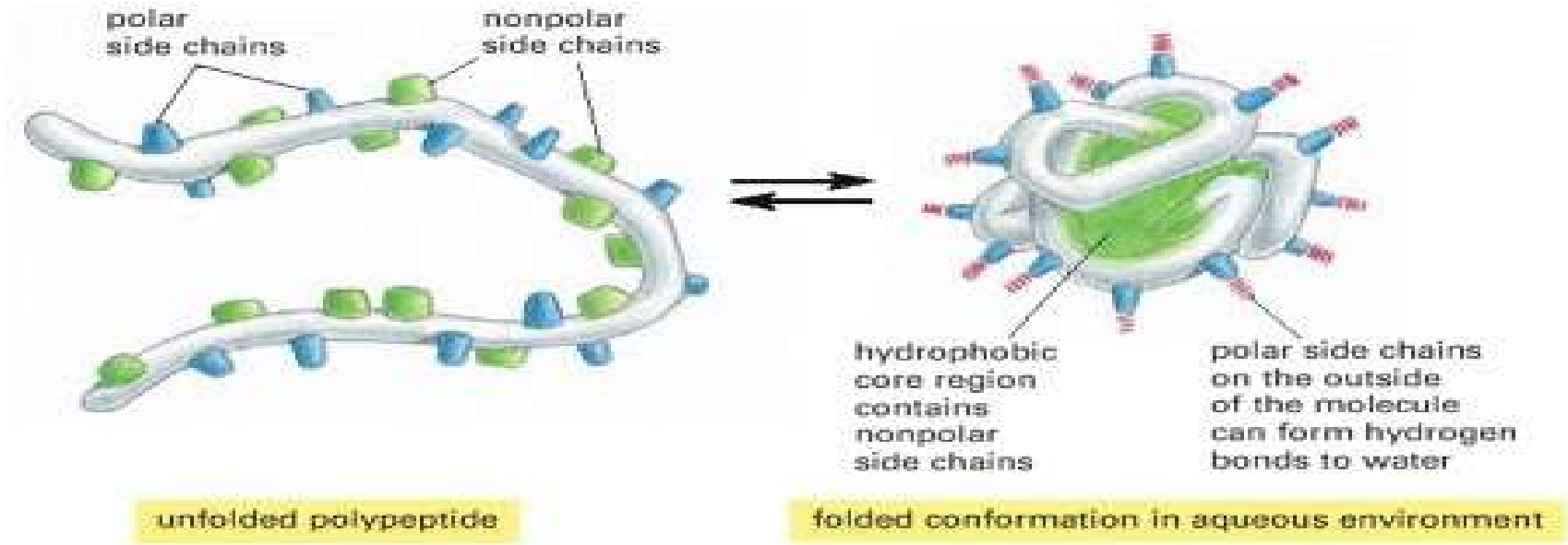
- Water drives non-polar substances out of the aqueous phase.
- This hydrophobic effect can be understood by the thermodynamic parameters enthalpy (ΔH, indicates changes in molecular interactions) and entropy (ΔS, indicates changes in available rotational, translational, vibrational states, etc).
- The strong directional cohesive interactions between water molecules are maintained, but at a high entropic cost.
- So, an entropic effect leads to an unfavorable free energy of mixing oil and water ($\Delta G=\Delta H-T\Delta S > 0$)

Hydrophobic effect Cont…

In the aqueous phase a region of relatively low entropy (highly order) water forms at the interface between the aqueous solvent and a hydrophobic solute. When hydrocarbon molecules aggregate in aqueous solution, the total volume of interfacial water decreases. Thus the driving force for aggregation of hydrophobic substances arises from an increase in entropy of the water (+TΔS). The driving force for aggregation does not arise from intrinsic attraction between hydrophobic solute molecules. Similarly, a protein may appear to have greater entropy in a random coil than in a native state.



How a protein folds into a compact conformation?



The side chains of polar amino acid tend to gather on the outside of the protein, where they can interact with water and the side chains of nonpolar amino acid are buried on the inside to form a tightly packed hydrophobic core of atoms hidden from the water molecule.

Summary

- Non-covalent interactions determine the shape of many large biological molecules (e.g., Proteins) and stabilize protein-protein complexes.
- There are four main types of non-covalent interactions in biological systems: ionic bonds (electrostatic interactions), van der Waals interactions, hydrogen bonds and hydrophobic bonds.
- Ionic bonds result from the electrostatic attraction between the positive and negative charges of ions.
- The weak and relatively nonspecific van der Waals interactions are developed when any two atoms/molecule approach each other closely. They result from the attraction between transient dipoles associated with all molecules.
- Hydrophobic interaction occur between non-polar molecules, such as hydrocarbons, in an aqueous environment. It result mainly because aggregation of the hydrophobic molecules necessitates less organization of water into “cages”.
- The bond energies for these interactions range from about 1 to 5 kcal/mol.
- Although any single non-covalent interaction is quite weak, several such interactions between molecules or between the parts of one molecule can stabilize the three dimensional structures of proteins.

References

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Thank you.

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