Hydrogen attached directly to most electronegative atoms
• Partial positive charge of hydrogen
• Partial negative charge on the electronegative atoms as well as at least one lone pair

The partially positive hydrogen strongly attracted to the lone pair
• Interaction much stronger than dipole-dipole interaction

Ethanol (BP = 78.5°C)
• Methoxymethane (BP = -24.8°C)
• 100-fold elevation of BP

H-bond direction, lengths, angles, and strength
• H-bond possesses direction and is generally asymmetric
• Bond strength depends on angle and length
• Small changes from linearity (20°) have minor effect in strength
• H-bond strength, on the other hand, decreases exponentially with distance
• There is a tradeoff between H-bond and covalent bond strength
• Stronger the H····O bond, the weaker the O-H covalent bond, and shorter the O····O distance
• Bond strength depends on angle and length
• Cooperativity and anti-cooperativity in H-bonding

Hydrogen bonding in water
• H-bond partly electrostatic (90%), partly covalent (10%)
• Approximately by the following bonds:
  - Dipole-dipole interaction
  - X-Ray spectroscopic evidence suggests that these interactions shift within a femtosecond

Early evidence of hydrogen bonding
• Many elements form hydrides
• Most BP of hydrides of Group 4 elements increase as we go down a gp
  - Van der Waals forces
• There is a tradeoff between H-bond and covalent bond strength
  - H-bond strength, on the other hand, decreases exponentially with distance
  - Small changes from linearity (20°) have minor effect in strength
  - Bond strength depends on angle and length
• Partial positive charge of hydrogen

Hydrogen bonding in water
• H-bond partly electrostatic (90%), partly covalent (10%)
• Approximated by the following bonds:
  - Dipole-dipole interaction
  - X-Ray spectroscopic evidence suggests that these interactions shift within a femtosecond

How would you calculate the strength of an H-bond?
• Between 3 and 9 kcal/mol
• Dissociation rate constants of 4 X 10^10 to 2 X 10^6 s^-1 from transition state theory

\[
K_w = \frac{[\text{ROH}^+][\text{H}_2\text{O}^\cdot]}{[\text{H}_3\text{O}^+][\text{ROH}]} = \frac{[\text{H}_3\text{O}^+][\text{ROH}]}{[\text{H}_2\text{O}^\cdot][\text{ROH}]} = \left[\frac{K_a}{\Delta G}\right]
\]

If \( K_w = 1, \Delta G = 0; \) if \( K_w = 10, \Delta G = 1.3 \text{ kcal/mol}; \) if \( K_w = 100, \Delta G = 2.6 \text{ kcal/mol}. \)

But it is more complicated than this for biological systems in water
H-bond Inventory

- Hard to determine the exact strength because of H-bonding to water
- \( R-O-H \cdots ORH \rightarrow ROH + ROH \)
  - A typical H-bonding reaction
  \( E-XH - OH_2 + OH_2 - B-S \rightarrow E-XH - B-S + H_2O - H_2O \)

- Count the number of H-bonds on each side
- H-bond isoenthalpic
- Remember entropic advantage!

Complications

- \( A + B \rightarrow AB \)
  - \( A \) has 3 rotational and 3 translational Degree of Freedom (DoF)
  - \( B \) has 3 translational and 3 rotational DoF
  - \( AB \) only has 3 translational and 3 rotational DoF
  - Net loss of 3 degrees of freedom
  - For small molecules, 3 DoF equal 12-16 kcal/mol
  - Therefore, H-bonds are worth more than 3-9 kcal/mol because they have to overcome their 12-16 kcal/mol of freedom loss

Different types of H-bonds

- Common elements that form H-bonds
  - S, O, N, F
- CH H-bond
  \( ROH \cdots RCOH^+COR' \)
- \( \pi \)-cation interaction

Low Barrier Hydrogen bonds (LBHB)

- H-bond strength depends on its length, linearity, microenvironment, and the pK_a values of the the H sharing components
- H-bonds in water are relatively weak because of pK_a mismatch between \( H_3O^+ (-1.7) \) and \( H_2O (15.7) \)
  - The proton in the structure is tightly associated with the OH- as a water molecule
- In gas phase, dielectric constant is low
  - Hydrogen bonds between heteroatoms with matched pK_a values can be \( \approx 2.5 \text{ Å} \) and very strong (25-30 kcal/mol)

Detection of LBHB

- Low field proton NMR signal
  - O-H and N-H bond length gets longer, H gets deshielded
  - Chemical shifts are 18-22 ppm in LBHB
  - \( \delta \) in proton NMR spectra of rotation of cycloprene-1,1-diketobutyric acid
- Deuterium fractionation factor of the central hydrogen
  - O-H and N-H bond length increases, bond order decreases
  - Discrimination against deuterium increases 0.3-0.5

- Enz-H + D_{solvent} \rightarrow Enz-H + H_{solvent}
- \( \phi = [\text{Enz-D}] [\text{H_{solvent}}] / [\text{Enz-H}] [\text{D_{solvent}}] \)

LBHB in enzymes

- Change the substrate pK_a to match that of the amino acid
- H-bond between lactate (15) and His-195 (6)
  - Mismatch of 9 pH units
  - During the run, pK_a of lactate crosses that of histidine
  - LBHB forms and activation energy of the reaction is lowered
  - Elimination of mismatch of 9 pH units = 4.5 orders of magnitude acceleration of rate
Other examples

- Chymotrypsin
- Ketosteroid Isomerase
- Triose-Phosphate Isomerase
- Citrate Synthase
- Mandelate Racemase

Cleland et al., JBC, 1998, (273), 25529-25532

H-bonds in Proteins

I. H-bonds contributing to **structure and folding**

II. H-bonds contributing to **catalysis**

α-helix

- i+4, i+3, i+5

- Dipole moment
  - + charge at N-termini, - charge at COOH
  - Negative molecules (PO\(_4\)\(^2-\) groups) bind at N-termini

β-sheets

- Antiparallel β-sheets
  - 10-14 atoms in a ring
  - H-bonds linear
  - Stability?

- Parallel β-sheets
  - 12 atoms in a ring
  - H-bonds not 180°
  - Stability?

Peptide H-bonds

- Strength of H-bond ≈ 3-9 kcal/mol
  - Is this the energy that it provides towards protein folding?

- Remember, folded protein loses entropy
  - Entropic cost of freezing one aa residue is 1.2 kcal/mol
  - What then drives protein folding? H-bonds? Hydrophobics?

- Hydrogen bond inventory
  - About 0 ± 1 kcal/mol for peptide bond in water

- A protein H-bond network is complex
  - Protein forms ≈ 70% of all H-bonds
    - In a 100 aa protein this represents 70 kcal/mol of energy towards folding
    - Note: folded proteins are generally 5-10 kcal/mol more stable than unfolded ones

Thermolysin-inhibitor complexes

- Crystal structures of thermolysin
  - first with inhibitor A (has NH)
  - second with inhibitor B (has O)
  - Differ by only one H-bond

- Contribution of that one additional H-bond?
PON1 H-bonds (backbone)  

PON1 H-bonds (backbone + sidechain)  

H-bonds in HIV protease: Contributions to catalysis  

H-bonds in catalysis  

Hydrogen bond types  

Ligand binding H-Bonds  

Few papers you all might find enjoyable (or maybe not…)