

Non-covalent interactions and how macromolecules fold

Lecture 3: Hydrogen bonds and water

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First-year Biophysics course

Objective:

Elicit the importance of hydrogen bonding in biology

What is a hydrogen bond and how can it be described?

What effect does the solvent have on the formation of hydrogen bonds?

What are the implications for the structures of biological macromolecules in water?

What are the different kinds of secondary structure found in proteins. How do hydrogen bonds affect the structure of DNA.

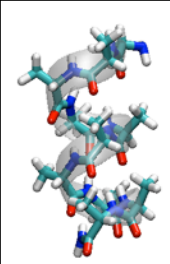
Summary:

Hydrogen bonds are very important in determining both the structure but also the function of biological macromolecules

They are partially covalent and therefore are not completely described by simple electrostatics and van der Waals forces

The formation of any hydrogen bond between groups exposed to water is unlikely since water will compete

Hydrogen bonds influence all elements of protein secondary structure and the structure of DNA



Importance of hydrogen bonding in biology

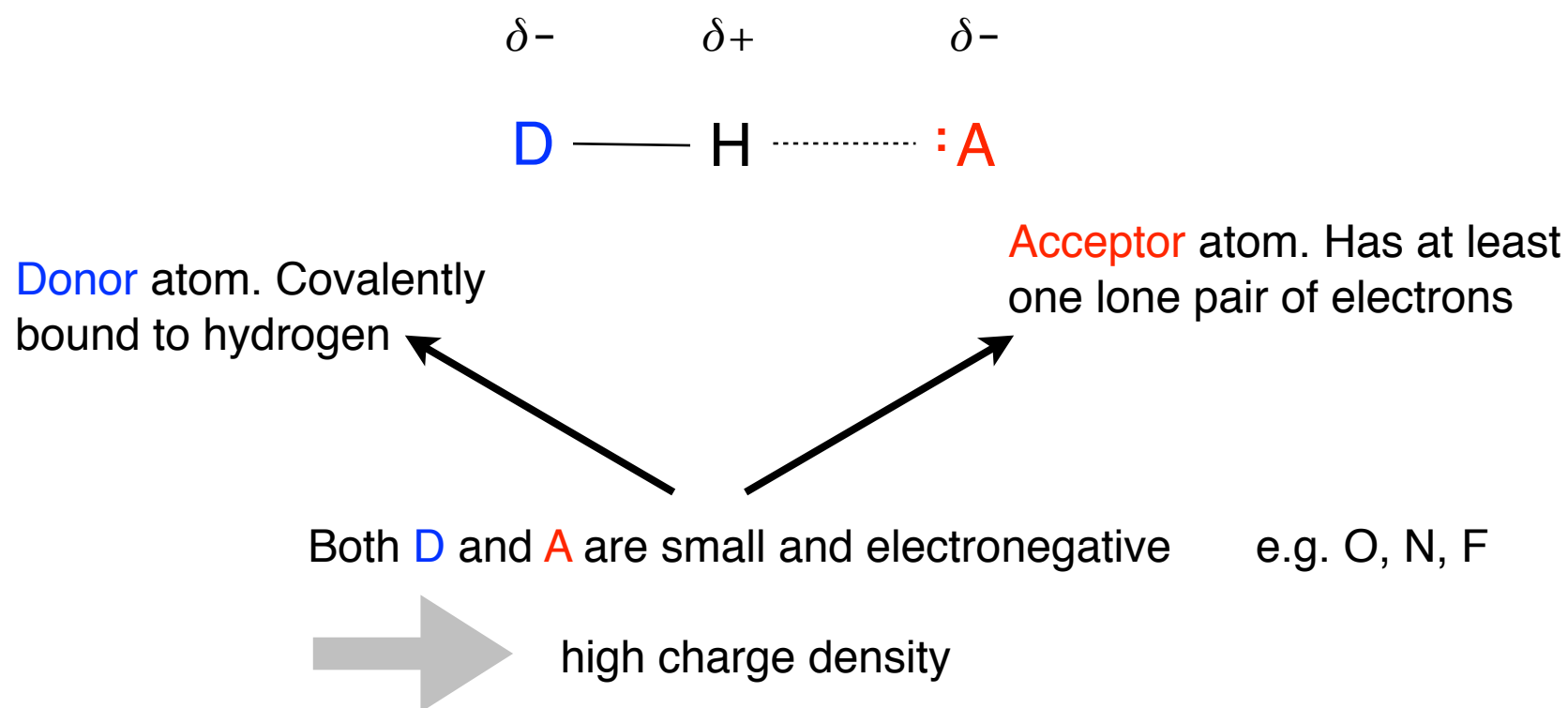
hydrogen bonding influences...

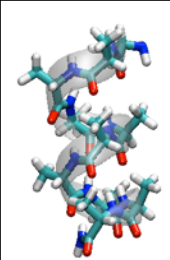
What is a hydrogen bond?

Hydrogen only has one electron

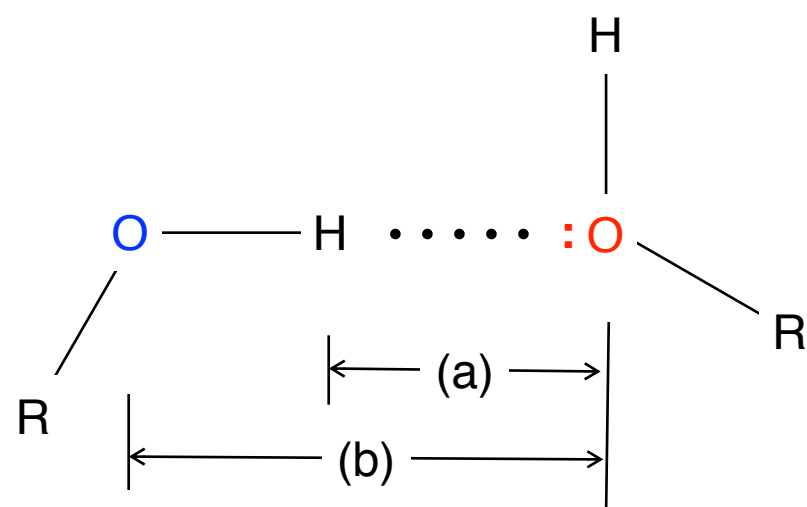
When hydrogen is covalently bound to an electronegative donor, its electron is predominantly found near the donor.

This proton is almost unshielded when viewed along the axis of the covalent bond and can interact strongly with other electronegative atoms.





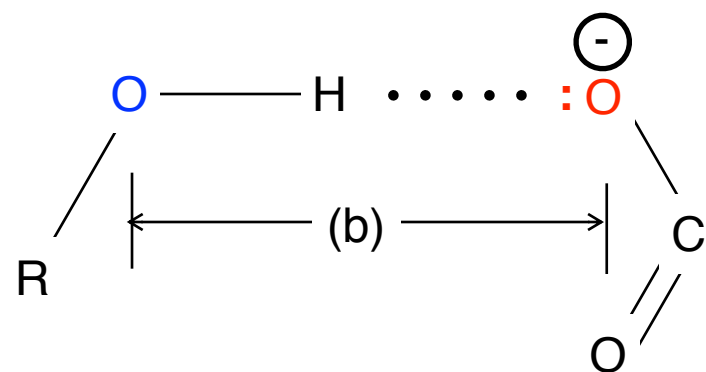
Observed hydrogen bond distances



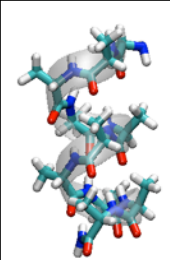
distance	observed (Å)	using vdW radii (Å)
(a)	~1.9	2.5
(b)	2.7	3.9

van der Waals radii (in Å)

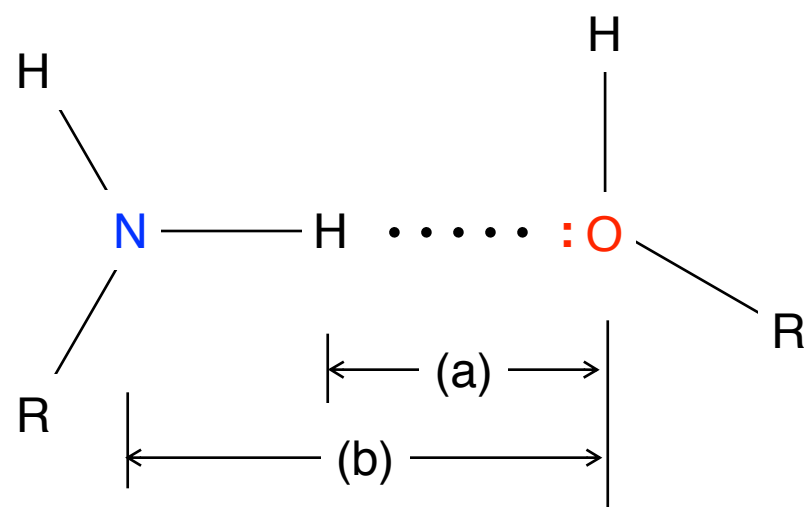
H	1.1
C	1.7
N	1.5
O	1.4



distance	observed (Å)	using vdW radii (Å)
(b)	2.6	3.9



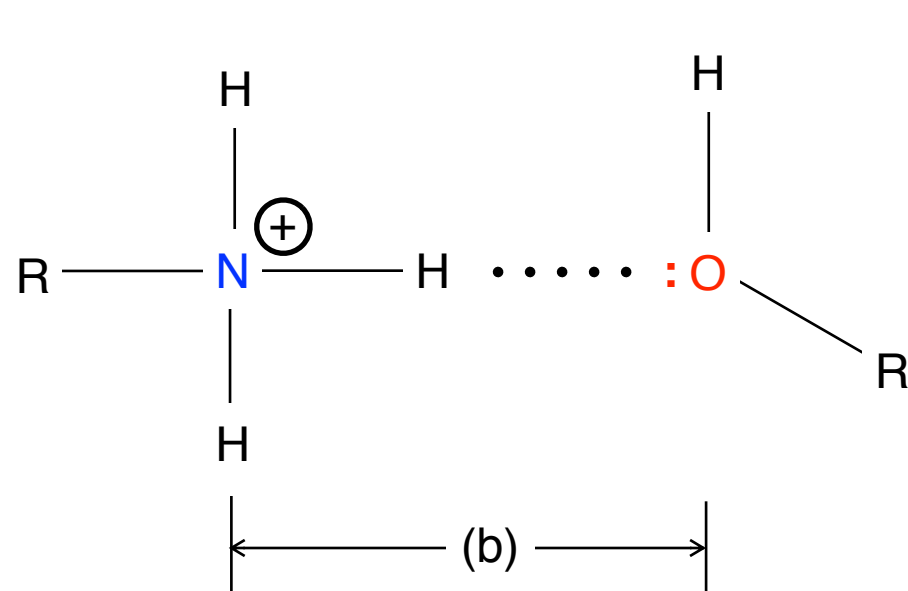
Observed hydrogen bond distances



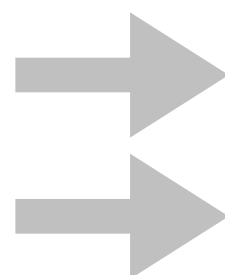
distance	observed (Å)	using vdW radii (Å)
(a)	2.0	2.5
(b)	3.0	4.0

van der Waals radii (in Å)

H	1.1
C	1.7
N	1.5
O	1.4



distance	observed (Å)	using vdW radii (Å)
(b)	2.9	4.0

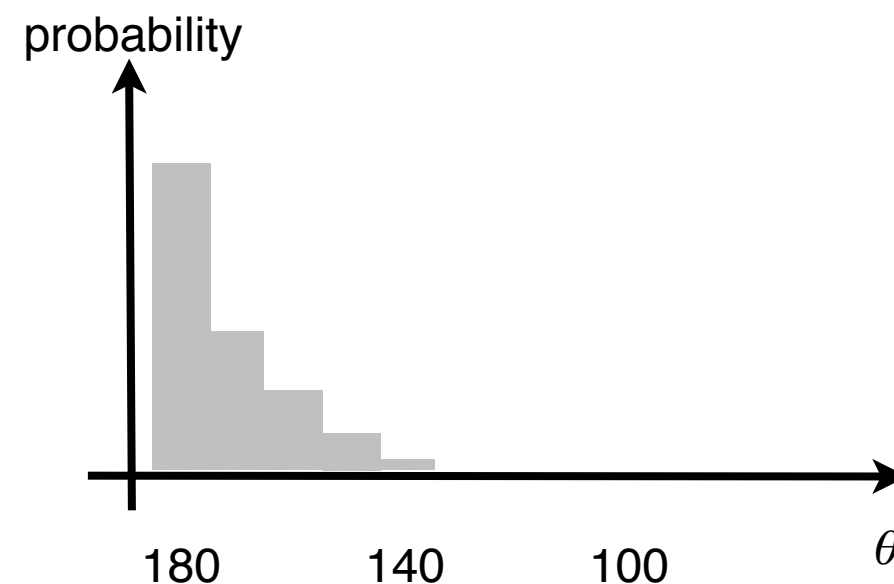
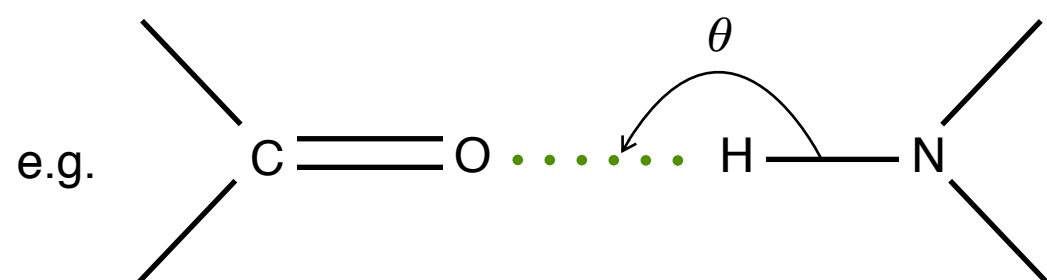


D and A approach closer than sum of the van der Waals radii
(approach closer still if donor or acceptor is charged)

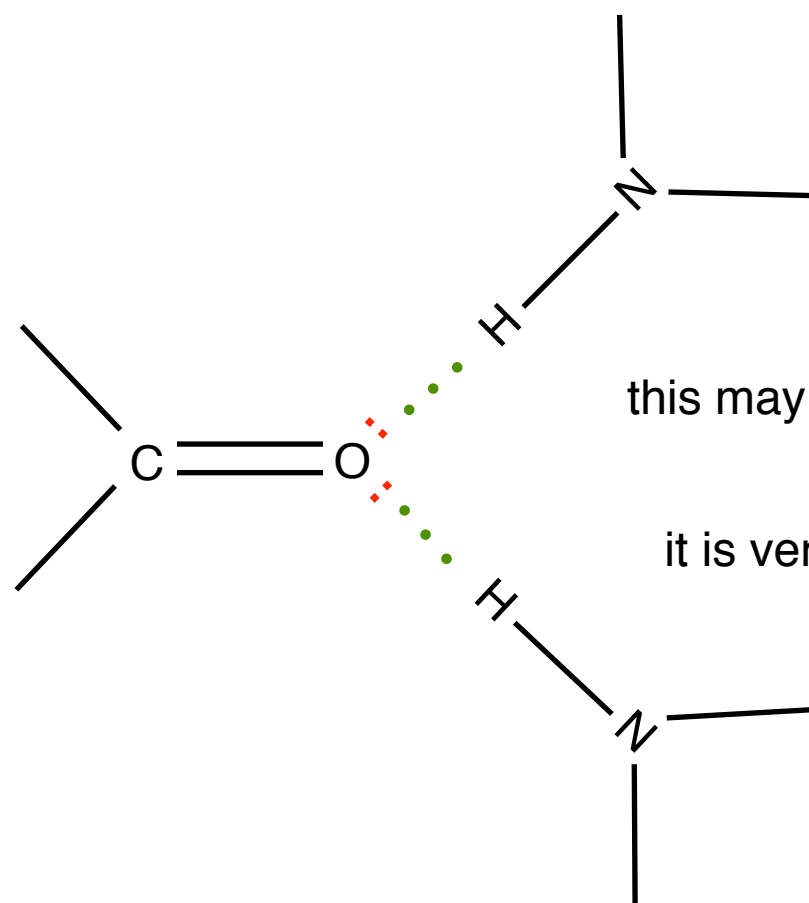
H-A distance shorter than expected

Observed hydrogen bond angles

The acceptor, donor and hydrogen are often observed to all lie on the same axis



when the acceptor is oxygen occasionally it is observed forming two hydrogen bonds



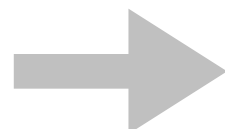
this may represent a tendency to point towards lone-pair on acceptor (oxygen)

it is very rare for 1 hydrogen atom to be shared by two acceptors

Hydrogen bonds

We observe that:

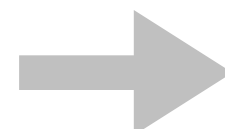
D and **A** approach closer than sum of the van der Waals radii



a hydrogen bond cannot be described as a van der Waals interaction; it is stronger

covalent bond ($\sim -450 \text{ kJ mol}^{-1}$) \gg hydrogen bond ($\sim -10 \text{ kJ mol}^{-1}$) $>$ dispersion interaction ($\sim -0.1 \text{ kJ mol}^{-1}$)

H-**A** distance shorter than expected



the hydrogen is partially shared between **D** and **A** so the hydrogen bond has some covalent character

observe as changes IR absorption spectra (which measures bond vibrational frequencies and bond stretching)

hydrogen bonds only observed for narrow ranges of orientation and distance

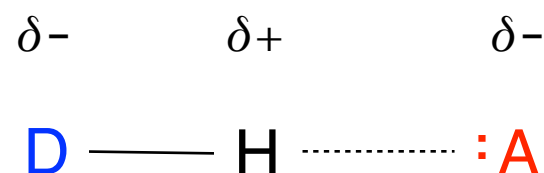


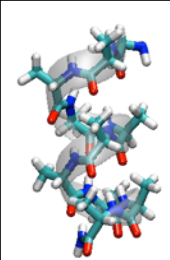
hydrogen bond is directional

Therefore describe the energy of a hydrogen bond as:

$\Delta U_{hbond} =$ electrostatic dipole + partial covalent character

note again this is at the limits of the classical approximation





Hydrogen bonding has important physical and chemical consequences

physical properties

gases are **non-ideal** (do not follow $pV = nRT$)

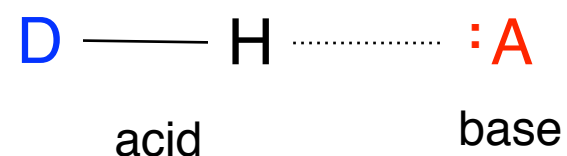
the boiling points are raised ← have to put in energy to break hydrogen bonds

the relative permittivity (dielectric constant) is affected

compound	boiling point	ϵ	hydrogen bonding
H ₂ O	100 °C	79.5	yes
CH ₄	-160 °C	1.3 @ 188K	no

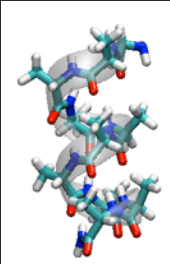
chemical properties

H**D** is a Lewis acid



therefore a hydrogen bond is an intermediate in the transfer of a proton an acid to a base:





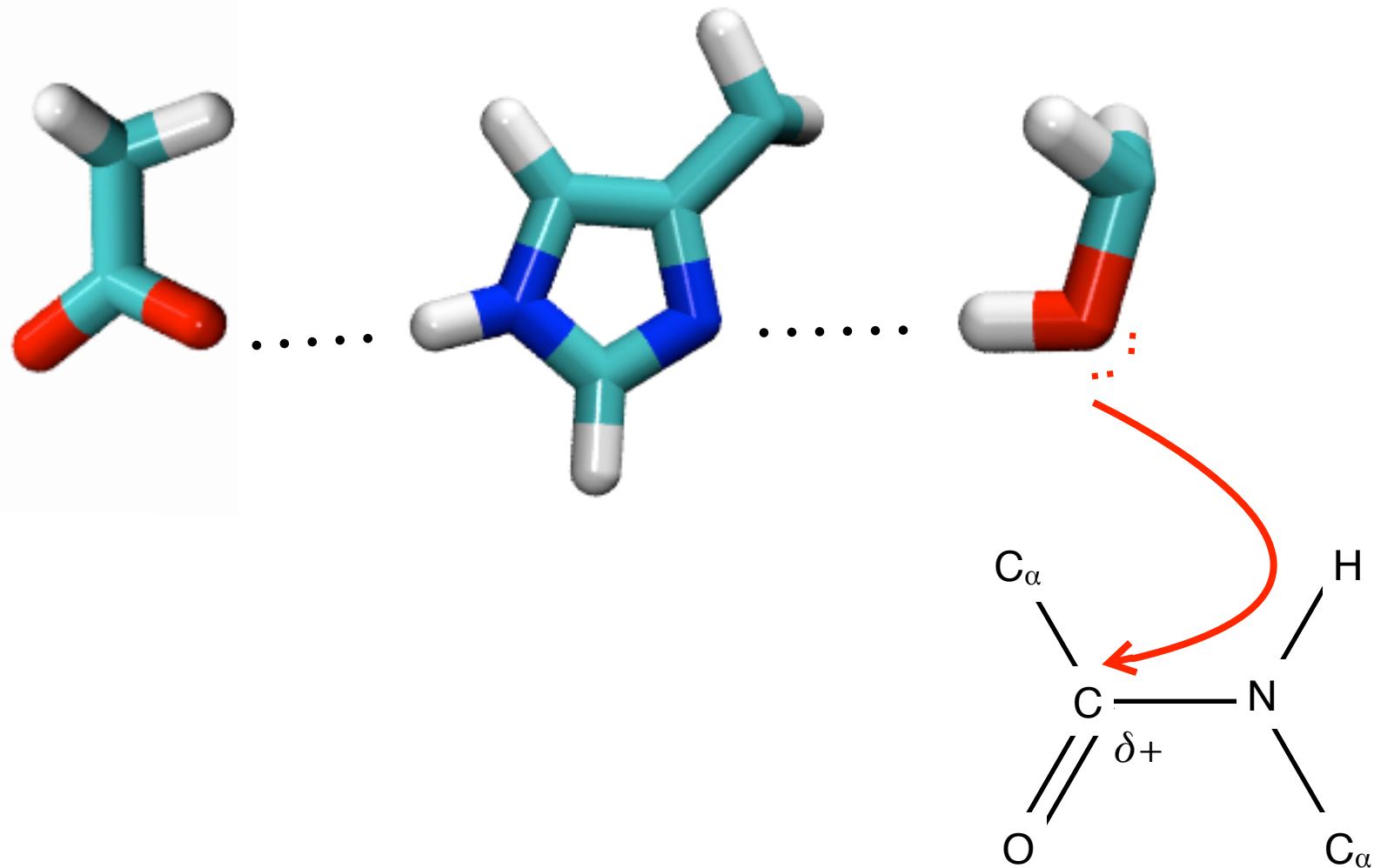
Example of importance of hydrogen bonds in enzymatic mechanisms

Catalytic triad of Chymotrypsin

Asp 102

His 57

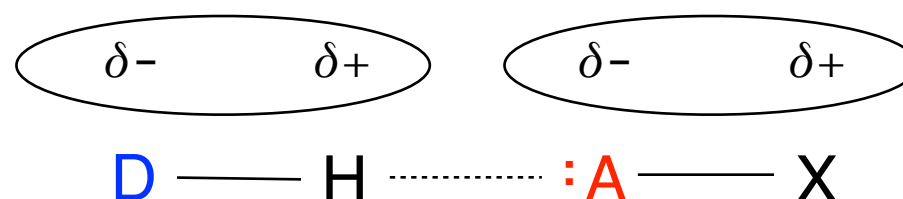
Ser 195



His 57 is abstracting a proton from Ser 195 and is therefore acting as a general base

His 57 orients Ser 195 for nucleophilic attack

Energy of a hydrogen bond



$$\Delta U_{hbond} = \frac{\text{electrostatic dipole} + \text{partial covalent character}}{}$$

$$\Delta U_{hbond} = \frac{1}{4\pi\epsilon\epsilon_0} \sum_i \sum_{j<i} \frac{q_i q_j}{r_{ij}} + \frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}$$

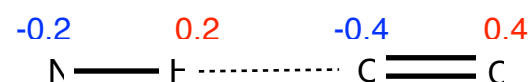
values of C_{ij} and D_{ij} depend on donor and acceptor

electrostatic dipole

repulsive term

empirical attractive term
(note falls off faster with distance than dispersion interaction)

Example: protein backbone hydrogen bonding



simple electrostatics yields (see lecture 1):

$$\Delta U_{hbond} = -6.2 \text{ kJ mol}^{-1}$$

additional 10-12 potential increases this by 2-8 kJ mol⁻¹

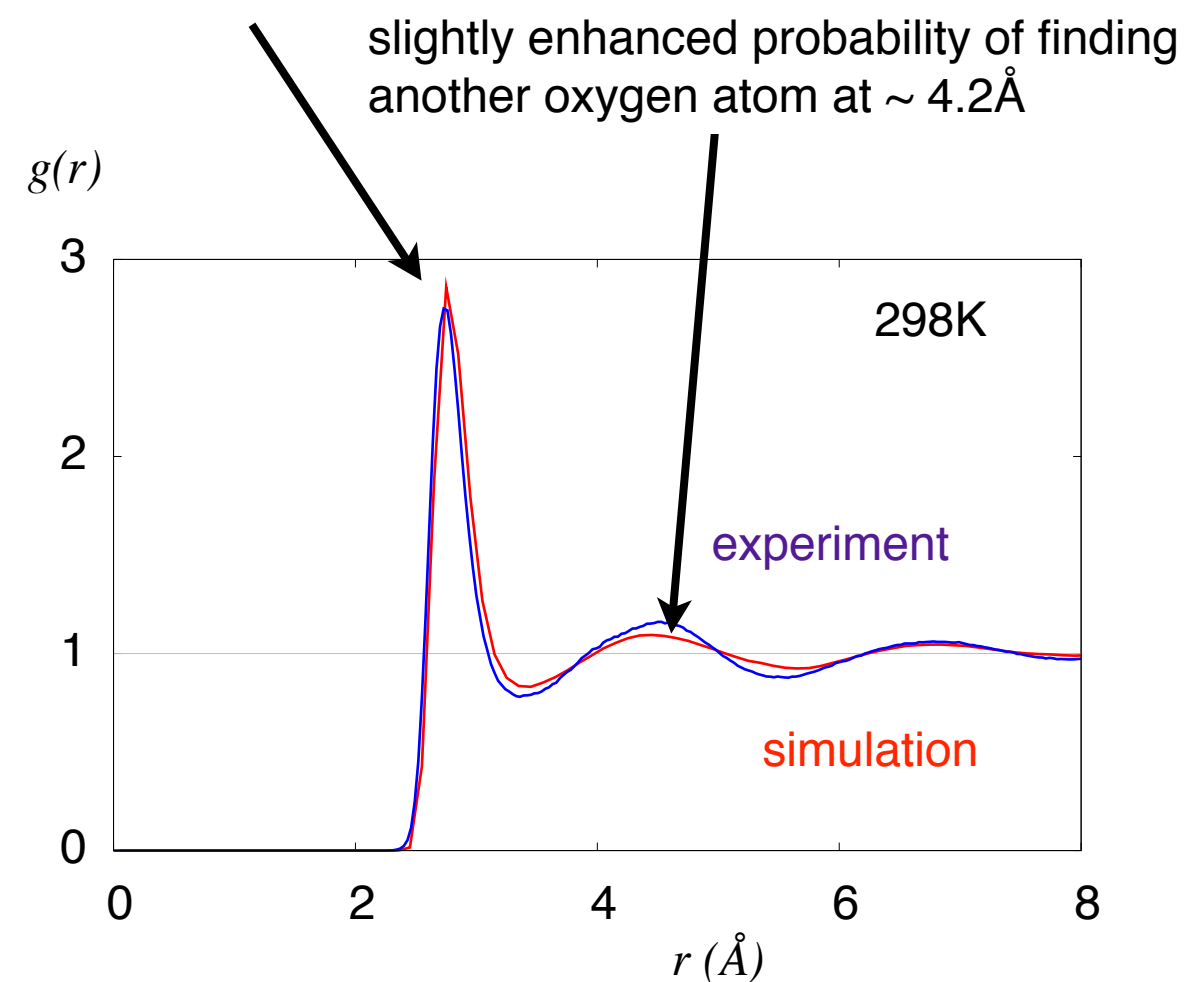
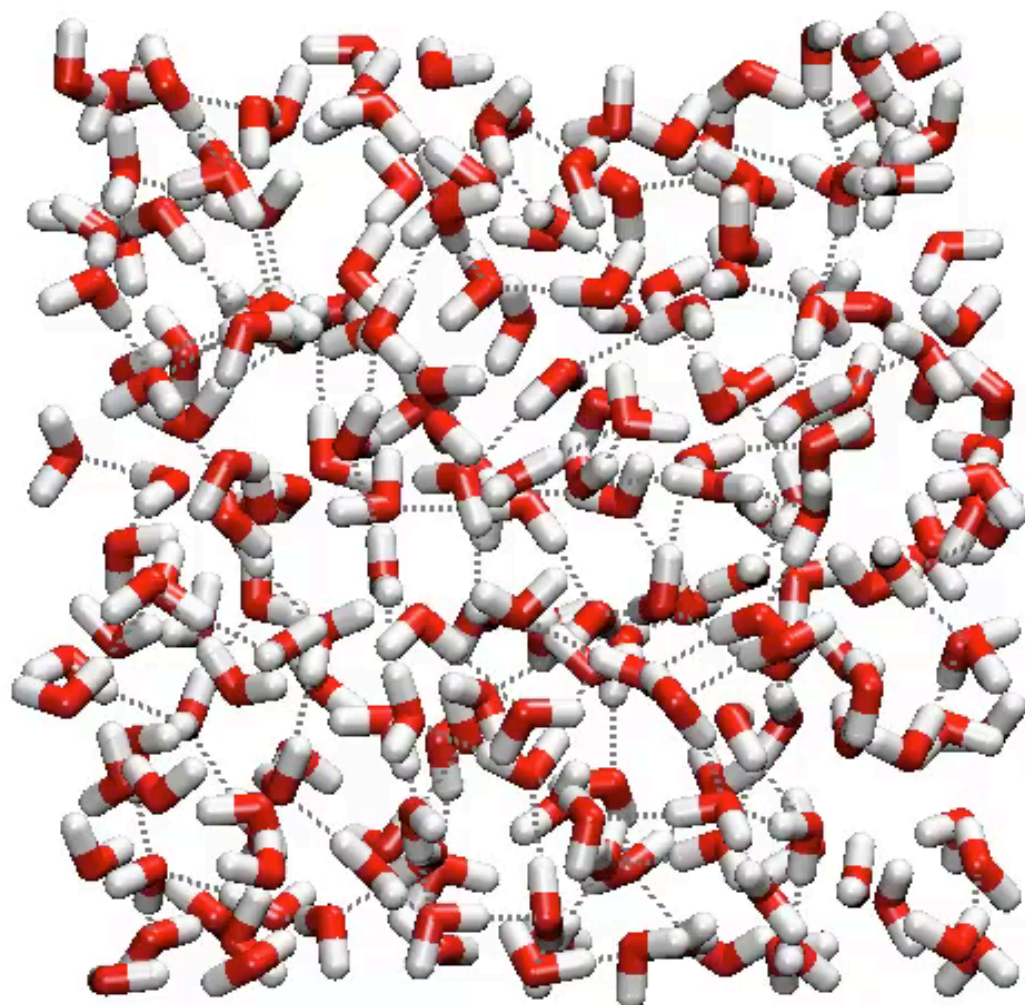
The energy of a hydrogen bond varies depending on the atoms involved and the geometry of their arrangement:

$$-48 < \Delta U_{hbond} < -4 \text{ kJ mol}^{-1}$$

This wide range also reflects our how poor our understanding of hydrogen bonds is still

Radial distribution function of water

enhanced probability of finding another oxygen atom at $\sim 2.7\text{\AA}$ due to hydrogen bonding between waters



zero probability of finding another oxygen atom at $< 2.2\text{\AA}$ due to repulsive part of van der Waals potential

Strength of a hydrogen bond

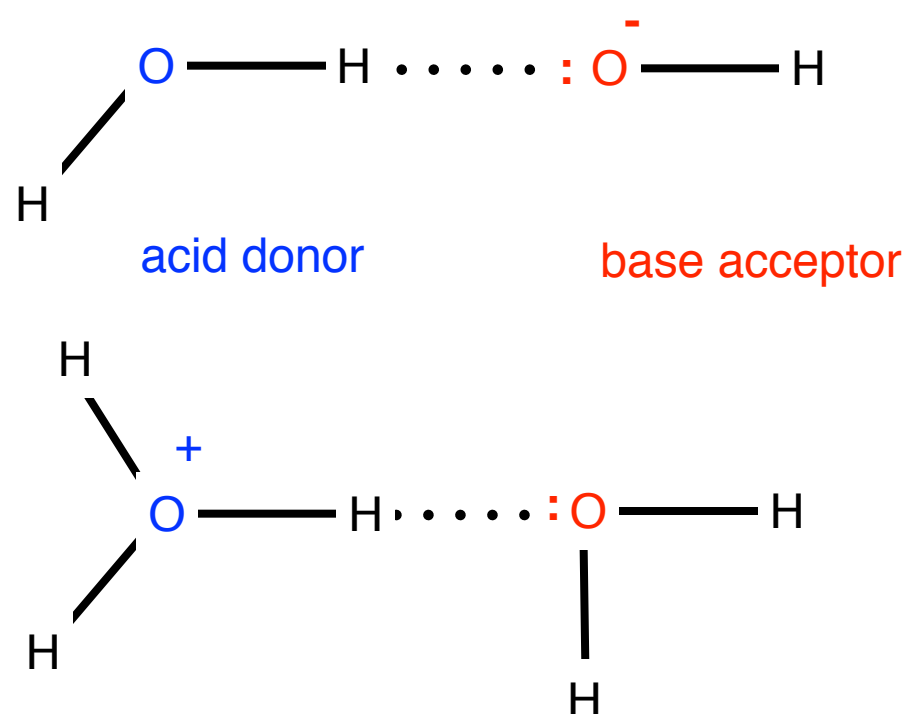
If $pK_a(\text{donor}) - pK_a(\text{acceptor})$ is large

or $pK_a(\text{acid}) - pK_a(\text{conjugate acid of base})$ is small



Hydrogen bond is strong

Example: water



$pK_a(\text{acid}) = pK_a(\text{conjugate acid of base})$

water forms strong hydrogen bonds with itself

Example: amino acids

Arg Asp

12.5 — 3.9 = 8.6

Arg-Asp is stronger*

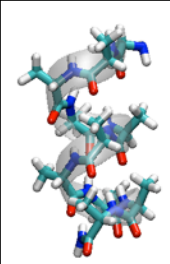
Glu Lys

10.8 — 4.3 = 6.5

Table: pK_a values of different sidechains in water

Residue	pK
Aspartic acid	3.9
Glutamic acid	4.3
Histidine	6
Cysteine	8.3
Tyrosine	10.9
Lysine	10.8
Arginine	12.5

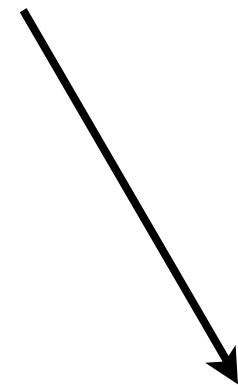
* but remember the precise protonation state will be determined by the pH and the environment



Back to free energy and entropy...

amount of work that can be extracted

Gibb's free energy
(i.e. constant temperature and pressure)



Temperature

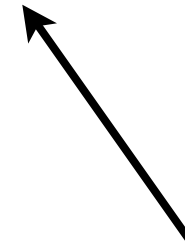


$$\Delta G = \Delta H - T\Delta S$$

Enthalpy



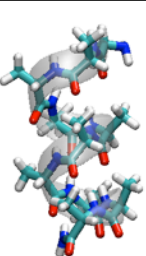
Entropy = *energy dispersal*



$$H = U + pV$$

covalent bonds, charged interactions, salt bridges, hydrogen bonds, van der Waals interactions

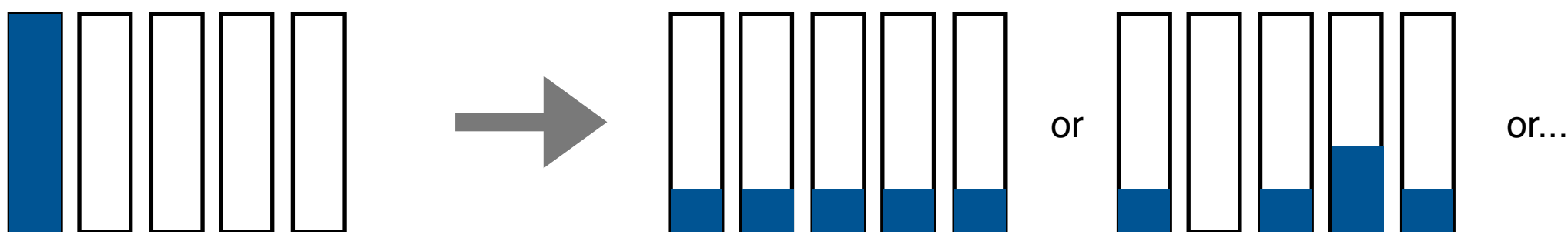
* we are ignoring the possibility of a large kinetic barrier here



What do we mean energy dispersal?

Macroscopic level: entropy measures how dispersed the energy is (usually between molecules)

Molecular level: entropy measures how dispersed the energy is (including within a molecule)



each bar could be

individual molecules

energy states within individual molecules

(e.g. translational, rotational, vibrational, electron states)

Entropy (J/K)

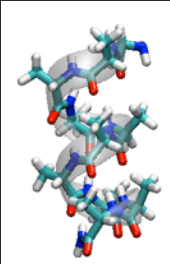
Boltzmann's constant
 $k = 1.38 \times 10^{-23} \text{ J/K}$

to determine how dispersed the energy is imagine
 counting the number of ways the energy could be
 arranged

$$S = k \ln W$$

fewer ways = more ordered





In which of this is the entropy increasing*?

bursting a balloon

dropping a china plate onto the floor

shuffling a deck of cards

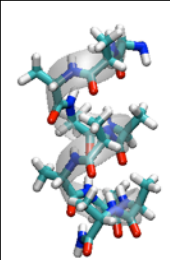
combustion of hydrogen

a ring-closing reaction

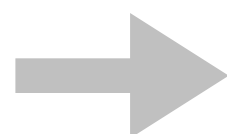
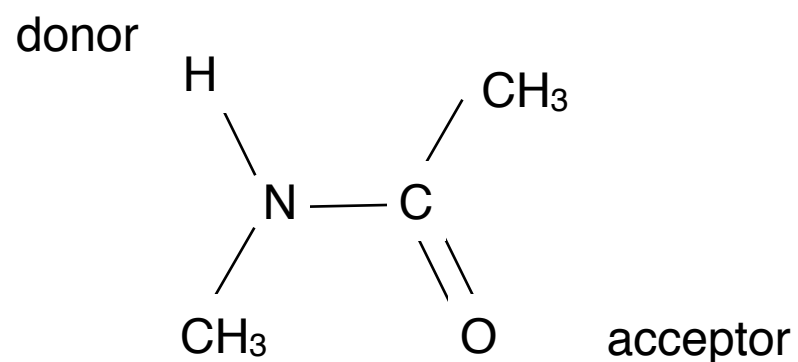
hydrolysis of ATP

a protein folding

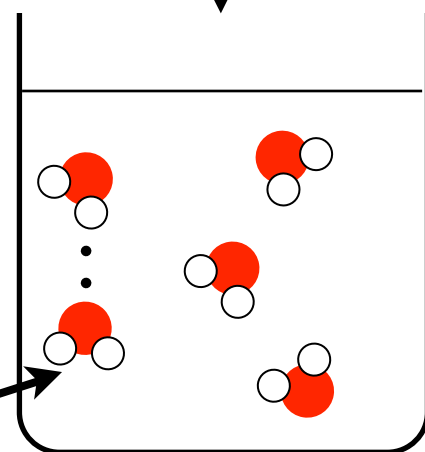
* the entropy of the universe always increases



N-methylacetamide is a good model for protein backbone hydrogen bonding

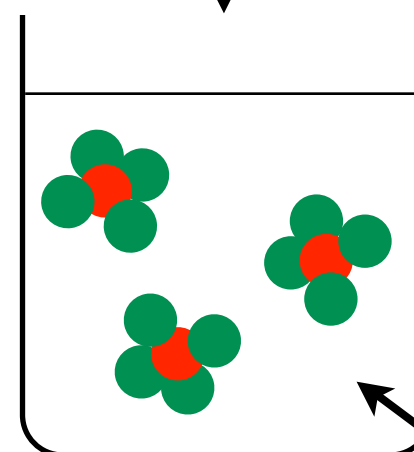


in which solvent will NMA form a dimer?



protic solvent
(can form hydrogen bonds)

water

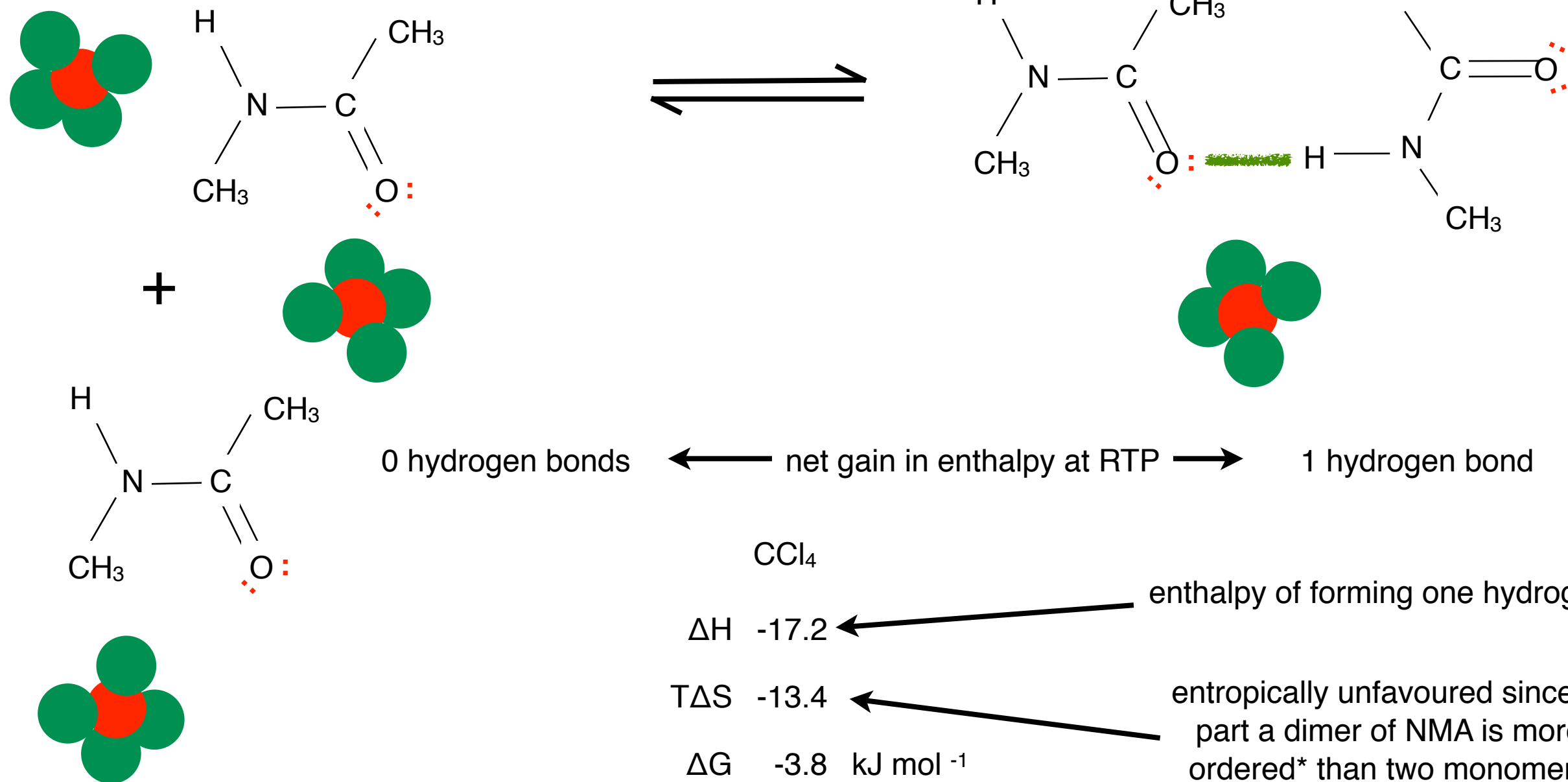


CCl₄

aprotic solvent
(cannot form hydrogen bonds)

also known as NMA

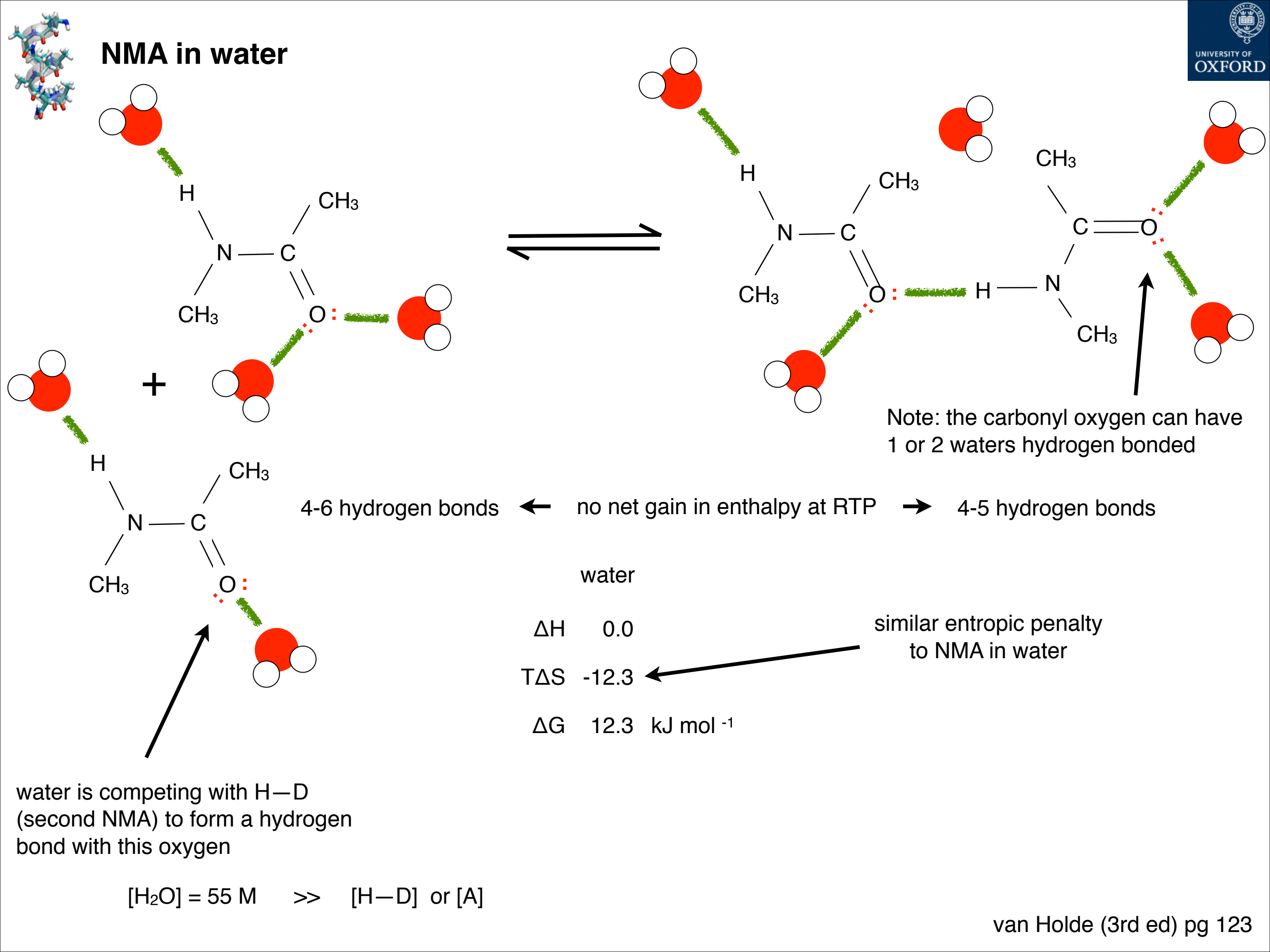
NMA in CCl₄



	μ (D)	α'
CCl	0	10.5
H	1.85	1.48

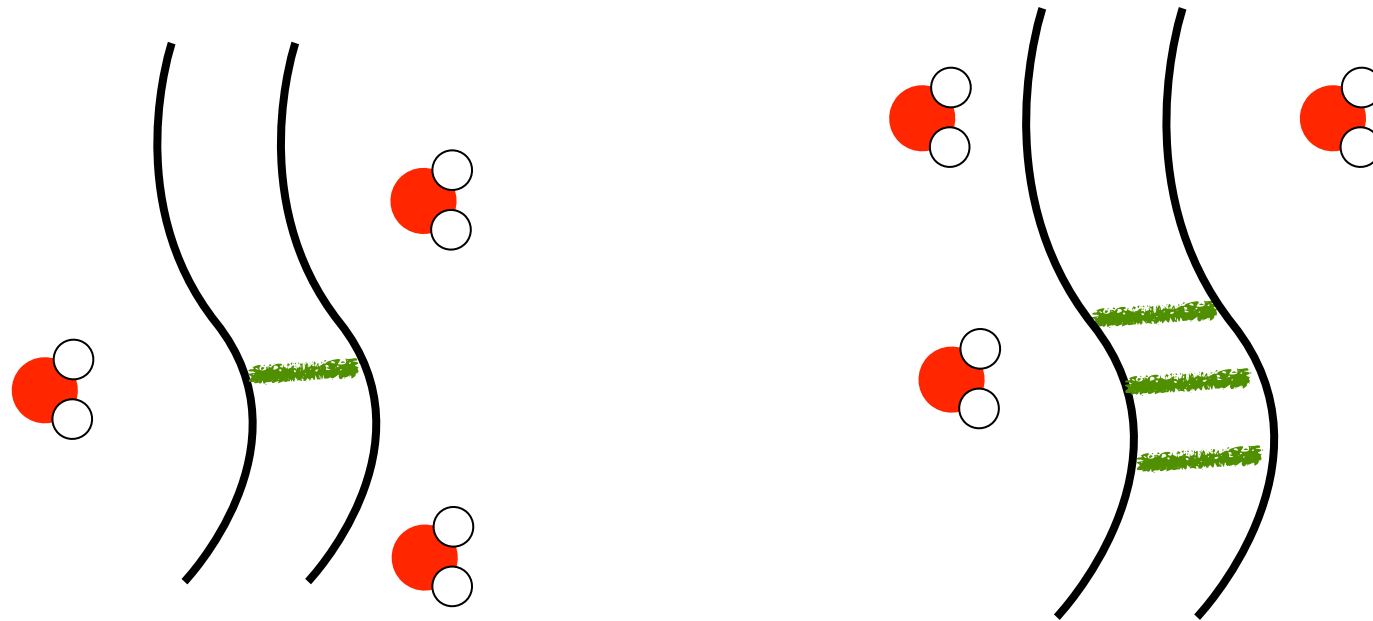
* has fewer ways to disperse energy

NMA in water



Hydrogen bonds in biology

hydrogen bonding between macromolecules

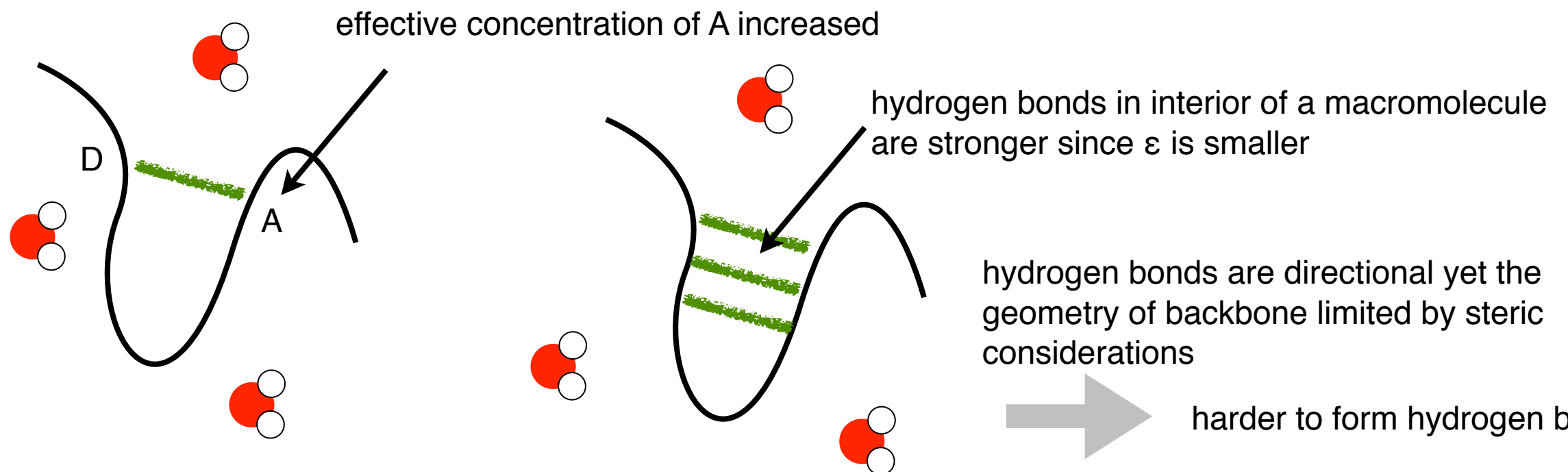


entropically unfavourable due to
reduction in number of energy states

additional hydrogen bonds don't reduce
the number of energy states much (i.e.
entropic penalty already been 'paid')
and can potentially compensate

➔ *cooperativity* (Lecture 5)

hydrogen bonding within a macromolecule

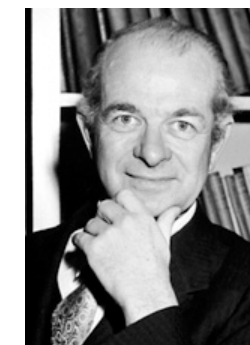


hydrogen bonds are directional yet the
geometry of backbone limited by steric
considerations

➔ harder to form hydrogen bonds

The 3.6₁₃ helix (or α -helix)

α -helices form when protein backbone groups hydrogen bond



There is more than one kind of helix that can form as a result

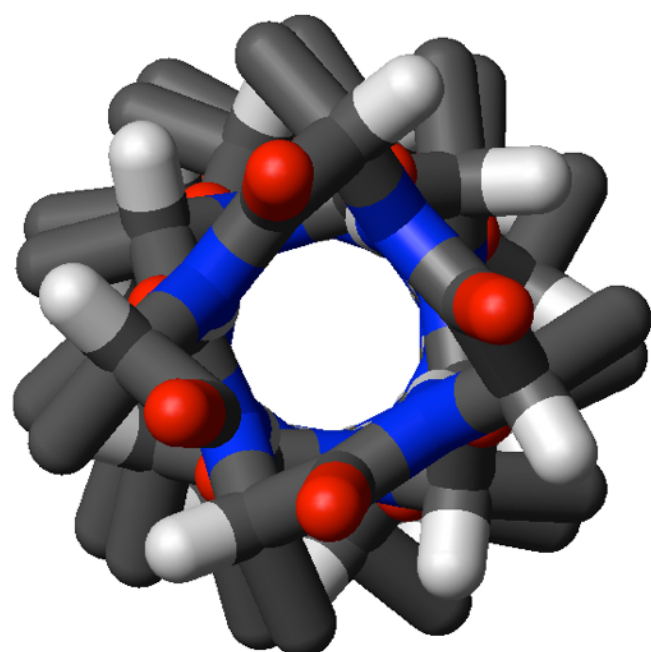
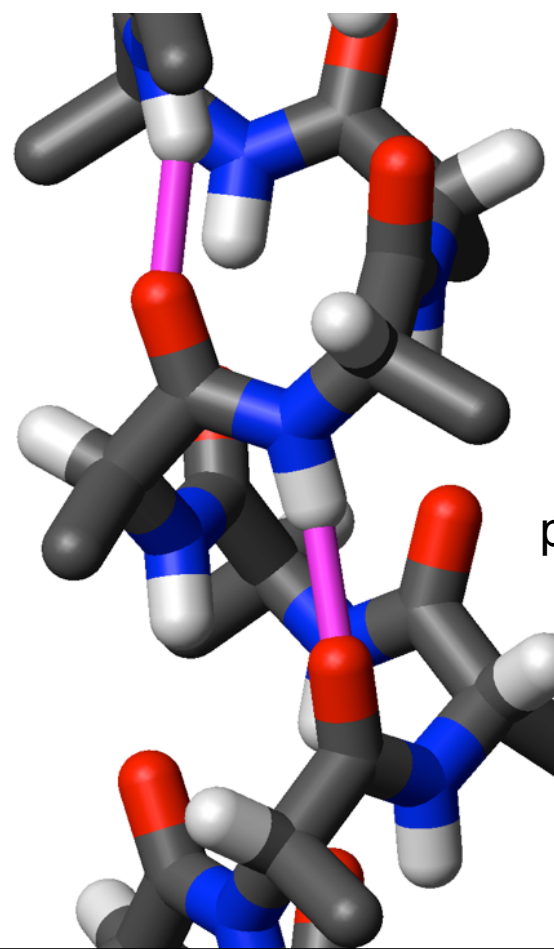
The α -helix has 3.6 residues per turn and 13 atoms

Pauling talking about discovering the alpha helix
<http://www.youtube.com/watch?v=yh9Cr5n21EE>

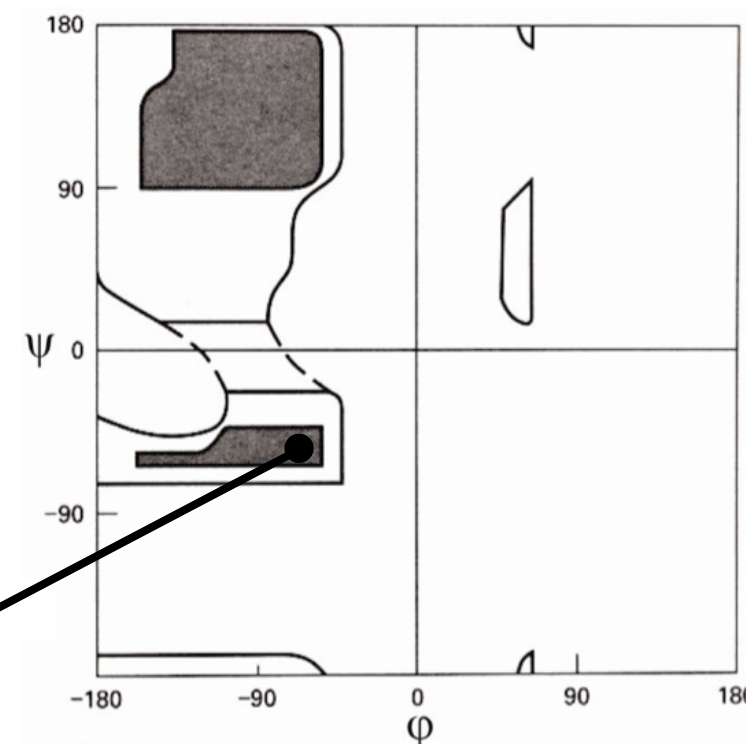
$$3.6_{13} \equiv n_N$$

number of atoms

number of residues per turn



$\phi = -57^\circ$, $\psi = -47^\circ$



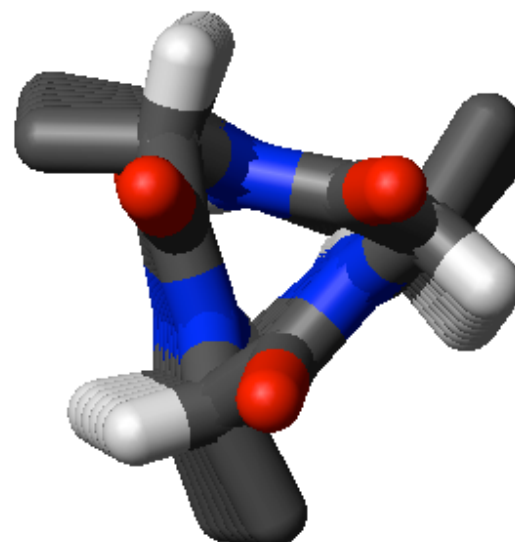
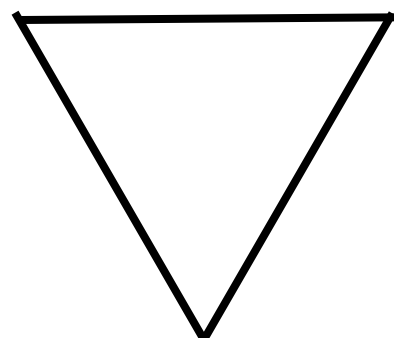
The 3₁₀-helix

has a smaller diameter than an α -helix

3₁₀

number of residues per turn

number of atoms including hydrogen in the ring that is closed by the hydrogen bond

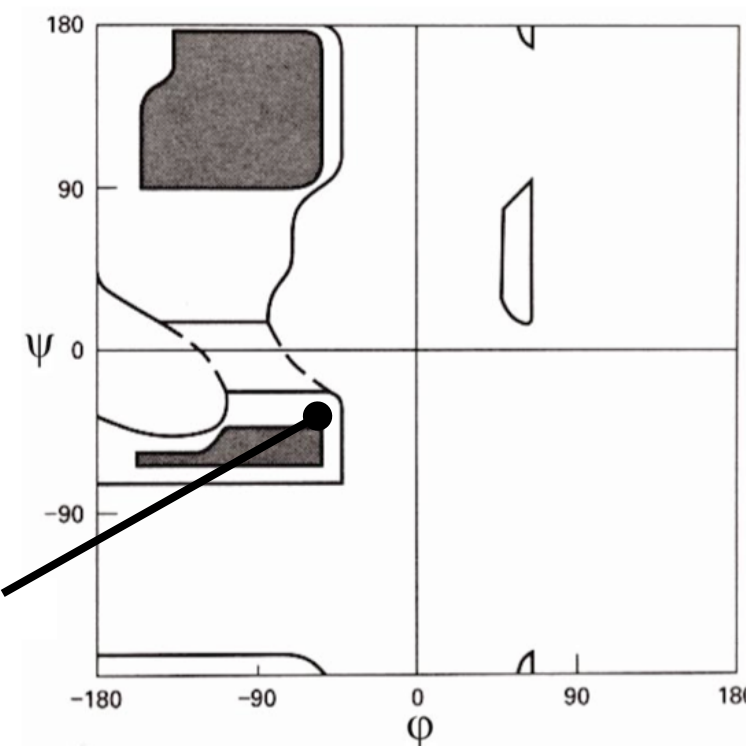


The side chains experience greater steric clashes with the main chain of the helix.

The 3₁₀ helix is in a mildly forbidden region of the Ramachandran plot

$\phi = -49^\circ$, $\psi = -26^\circ$

It is uncommon and is often short (1 or 2 turns long)



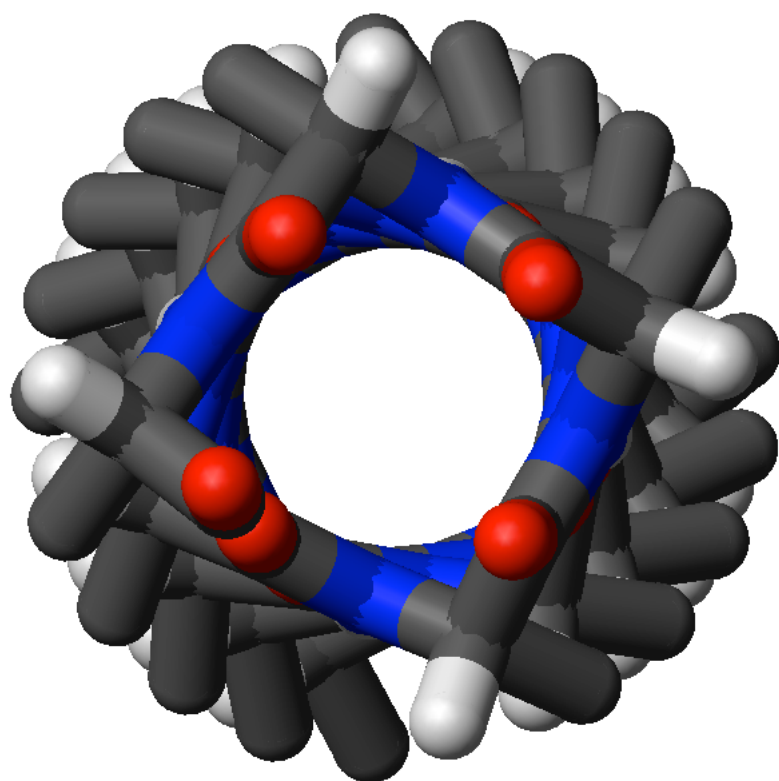
The 4.4₁₆-helix (or π helix)

4.4 residues per turn

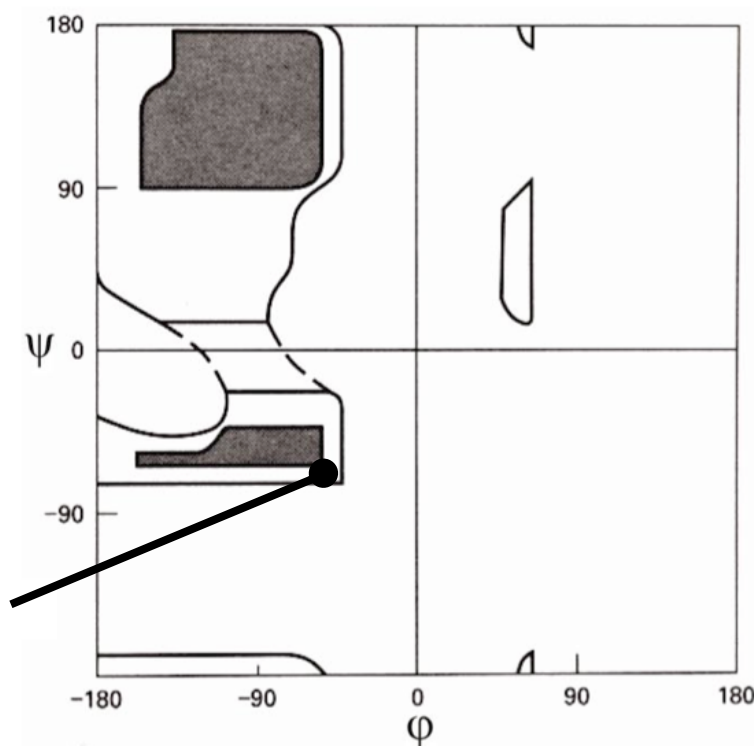
larger in diameter and shorter than an α -helix

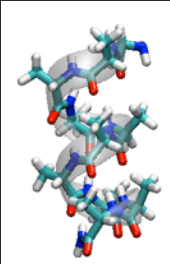
axial hole which results in poorer van der Waals packing and therefore the helix is less stable

has been observed at the ends of a few α -helices



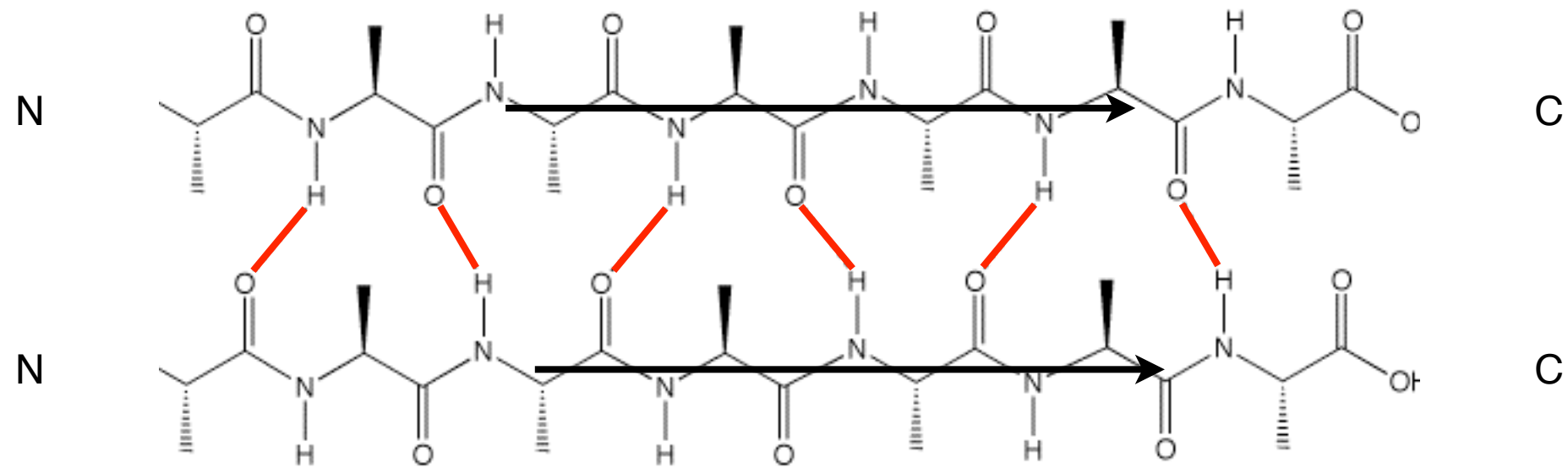
$\phi = -57^\circ$, $\psi = -70^\circ$



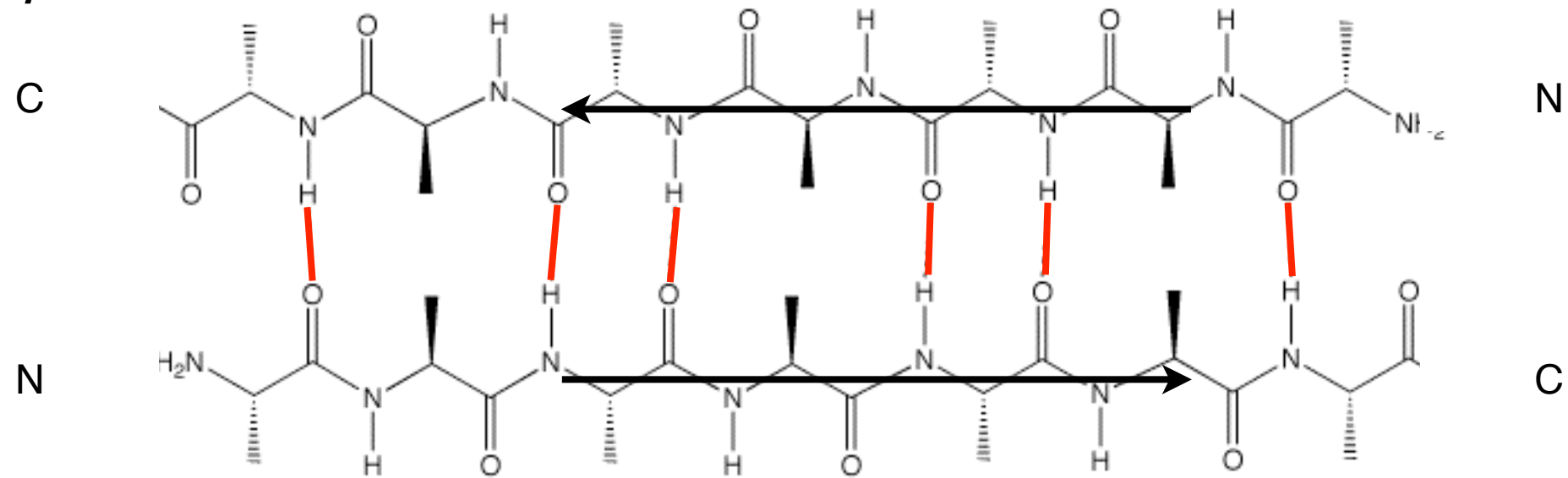


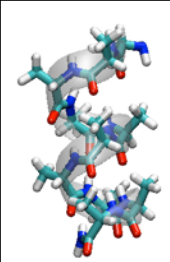
β sheets

(a) Parallel β sheet



(b) Anti-parallel β sheet





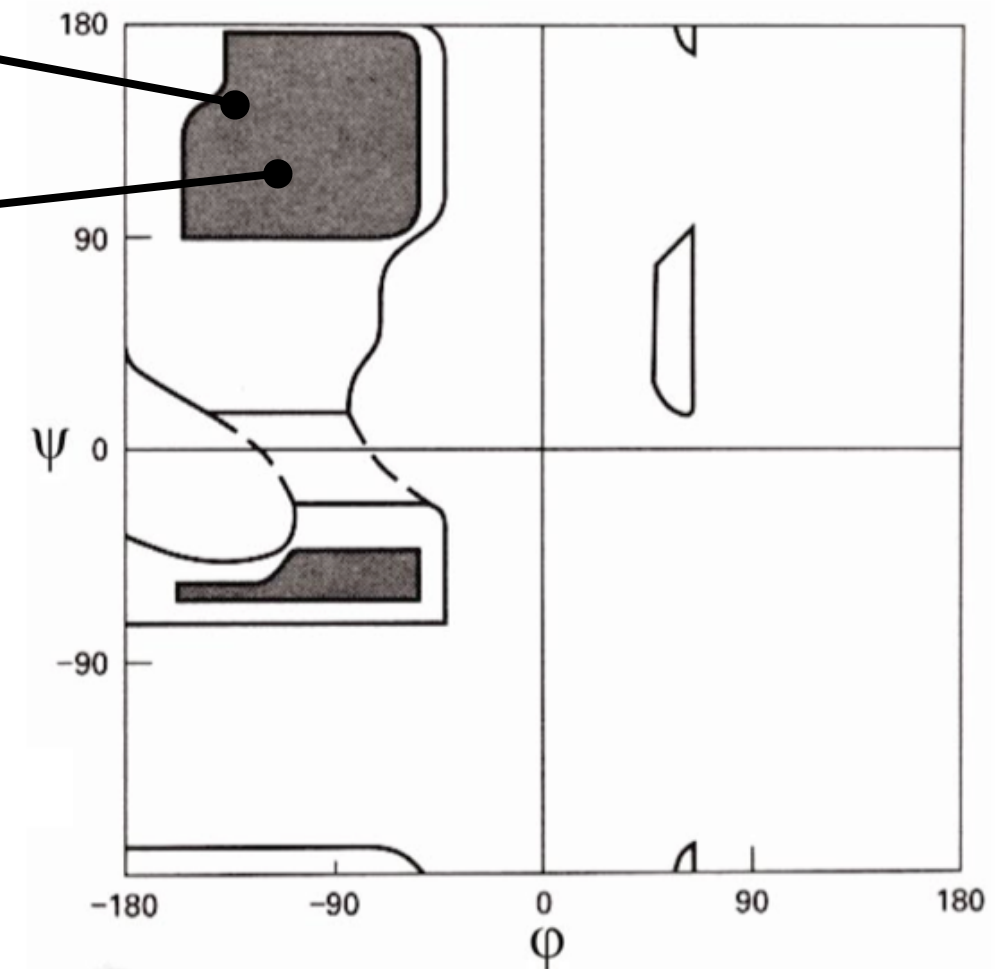
β sheets

anti-parallel β sheets

$\phi = -139^\circ$, $\psi = -135^\circ$

parallel β sheets

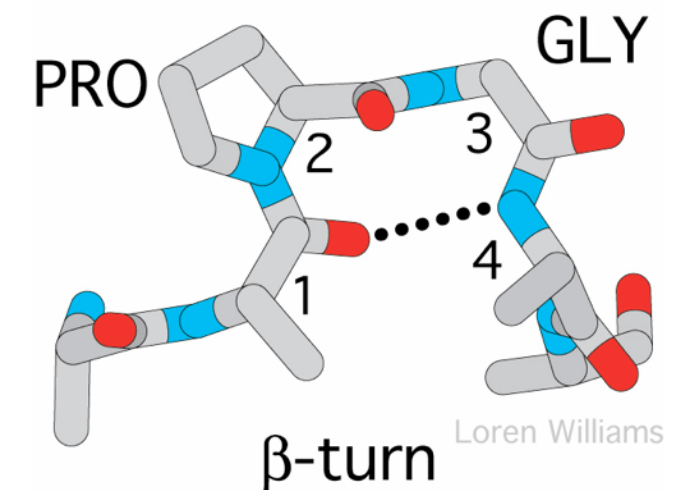
$\phi = -119^\circ$, $\psi = -113^\circ$



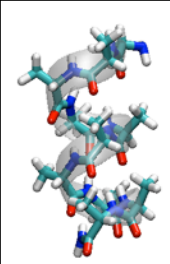
β turns

connect elements of secondary structure

any structure that has a hydrogen bond between the acyl oxygen of the first amino acid in the turn and the nitrogen-hydrogen bond of the amide of the fourth amino acid

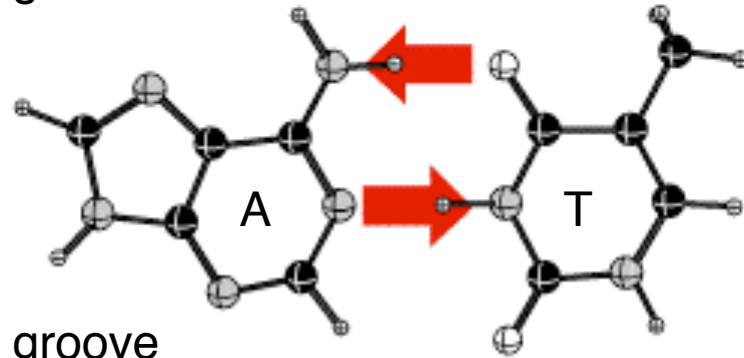


This is a Type-II turn. What does a Type-I turn look like?



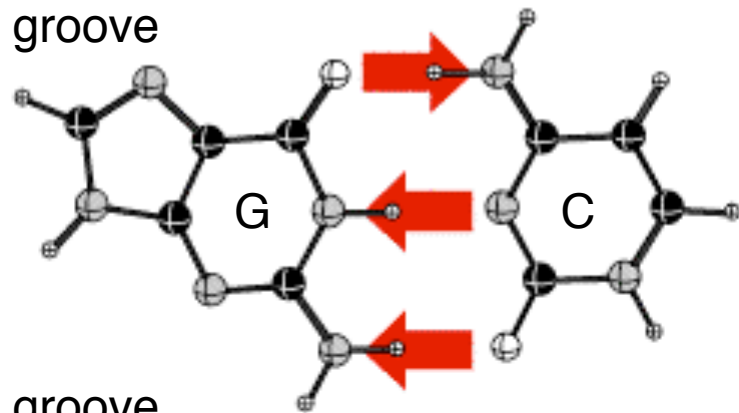
DNA

major groove



minor groove

major groove



minor groove

In the double helix of B-DNA there are Watson-Crick base pairs between complementary nucleotides.

As the sequence varies there is no change in the pattern of hydrogen bond donors and acceptors exposed in the narrow groove

Hence proteins (transcription factors, endonucleases) which recognise specific sequences bind to the exposed bases in the major groove of DNA

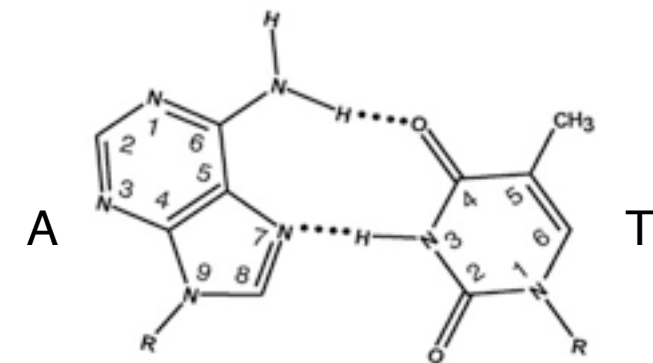
DNA polymerases detect allowed Watson-Crick base pairing by the hydrogen pattern within the minor groove. This increases the fidelity of polymerase reactions.

Free nucleotides also form Hoogsteen base pairs

e.g. between A and T for individual nucleotides

More stable than Watson-Crick base pairing. But steric factors make Watson-Crick base pairing more stable within a double helix.

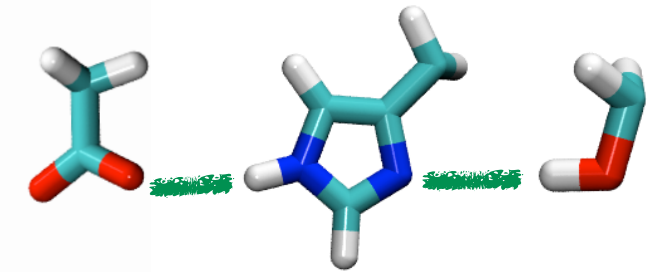
Hoogsteen base pairing also occurs within t-RNA molecules where it stabilises the structure



What other Hoogsteen base pairs are there?

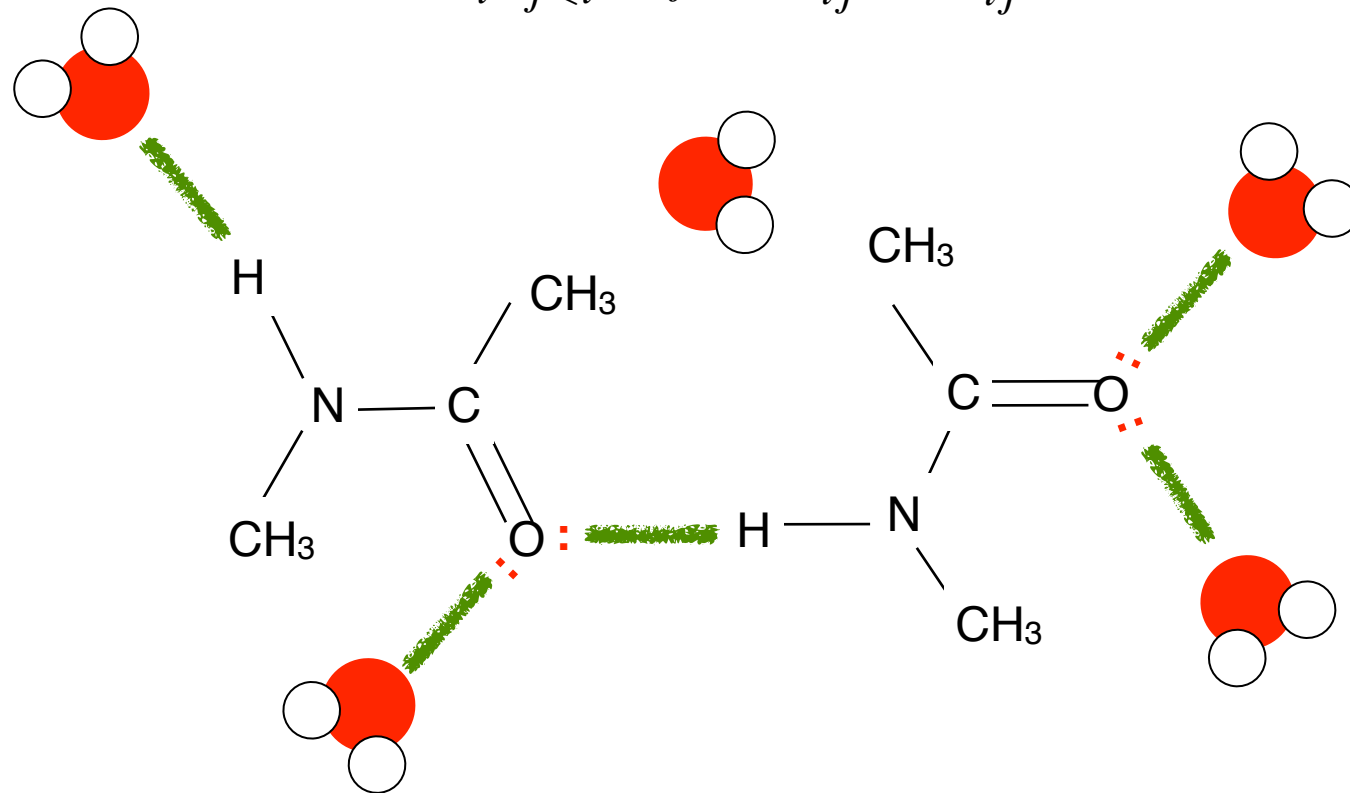
Lecture 3: Summary

Hydrogen bonds are very important in determining both the structure but also the function of biological macromolecules



$$\Delta U_{hbond} = \frac{1}{4\pi\epsilon\epsilon_0} \sum_i \sum_{j<i} \frac{q_i q_j}{r_{ij}} + \frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}$$

They are partially covalent and therefore are not completely described by simple electrostatics and van der Waals forces



Entropy as energy dispersal

The formation of any hydrogen bond between groups exposed to water is unlikely since water will compete

Hydrogen bonds influence all elements of protein secondary structure and the structure of DNA

