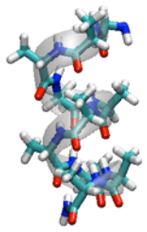


Non-covalent interactions and how macromolecules fold

Lecture 5: Cooperativity of non-covalent interactions

Dr Philip Fowler

First-year Biophysics course

Objective:

Check understanding of the hydrophobic effect.

Examine the thermodynamics of how a protein folds.

Determine what drives the formation of the folded, native structure.

Show how cooperativity works.

Investigate the consequences for protein folding.

Summary:

The hydrophobic effect causes an extended polypeptide chain to undergo ‘hydrophobic collapse’ and form a molten globule

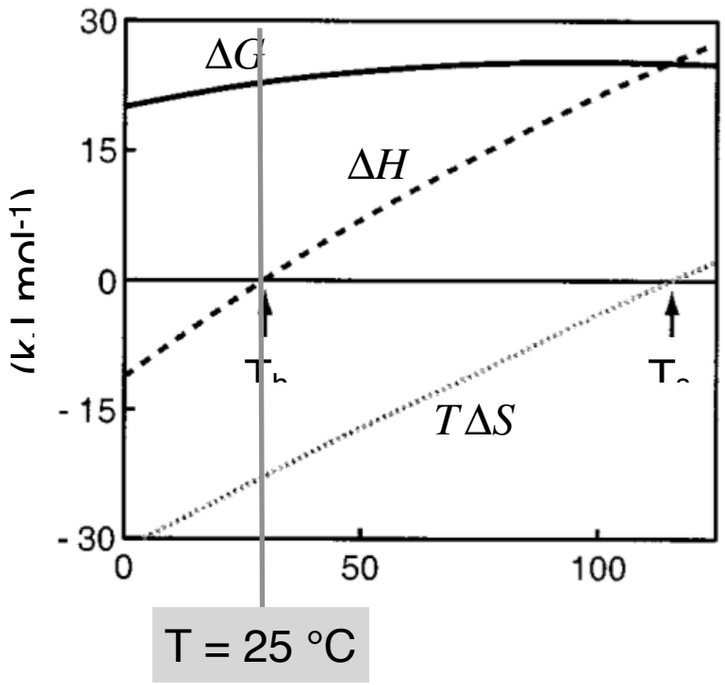
This is driven by the increase in the entropy of the water

Water cannot penetrate the molten globule and clusters of non-covalent interactions form cooperatively

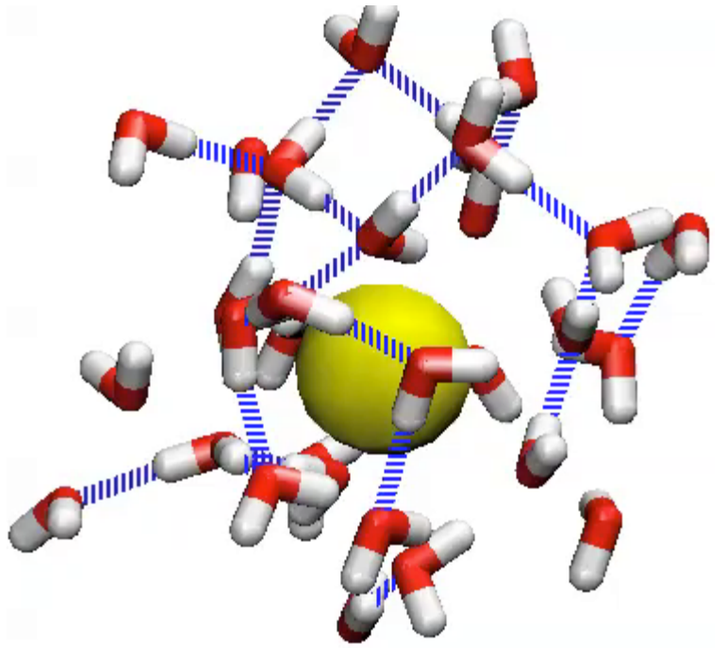
The change in enthalpy from forming these interactions drives the formation of the folded, native structure

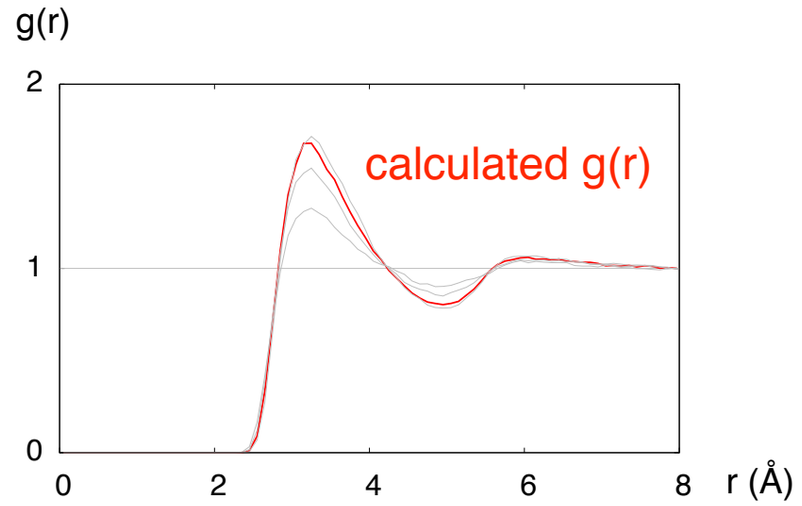
The cooperativity of this transition has many consequences for proteins

At physiological temperature the hydrophobic effect is entropically driven



Argon in water (25 °C)





Caution: the thermodynamic data is for neopentane and the simulations are of Argon

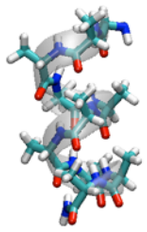
ΔH

1. no interactions between **solute** and **water**	0	
2. interactions between **solute** and **non-polar solvent** lost	> 0	☹
3. increase in the number of **water** hydrogen bonds	< 0	☺
2 and 3 have about the same magnitude therefore:	$\Delta H = 0$	

$T\Delta S$

1. the **water** orders itself around the **solute** as it cannot hydrogen bond to it	< 0	☹
	$T\Delta S < 0$	☹

$\Delta G > 0$ driven by the large decrease in entropy as water orders itself around the non-polar solute

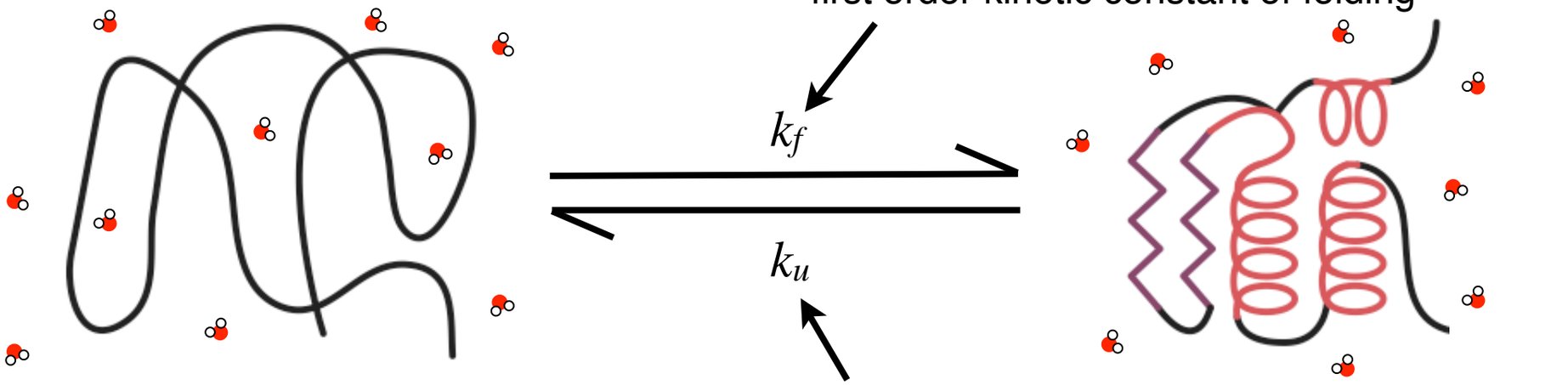


Protein folding is a two-state process

see Maths course

Folding is a **first-order process** i.e. the rate depends on the amount of unfolded left

first order kinetic constant of folding



unfolded, extended state

first order kinetic constant of unfolding

folded, compact native state

equilibrium constant of folding

$$K_f = \frac{k_f}{k_u}$$

$$K_f = \frac{[\mathrm{F}]}{[\mathrm{U}]}$$

folding free energy

$$\Delta G = -RT \ln K_f$$

gas constant

temperature (K)

if $\Delta G < 0$ then the reaction proceeds (equivalently if $K_f \ll 1$ then the equilibrium lies on the right hand side)

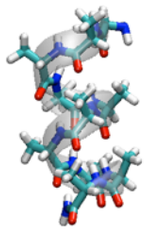
intermediates only exist transiently

folded state may adopt slightly different conformations:

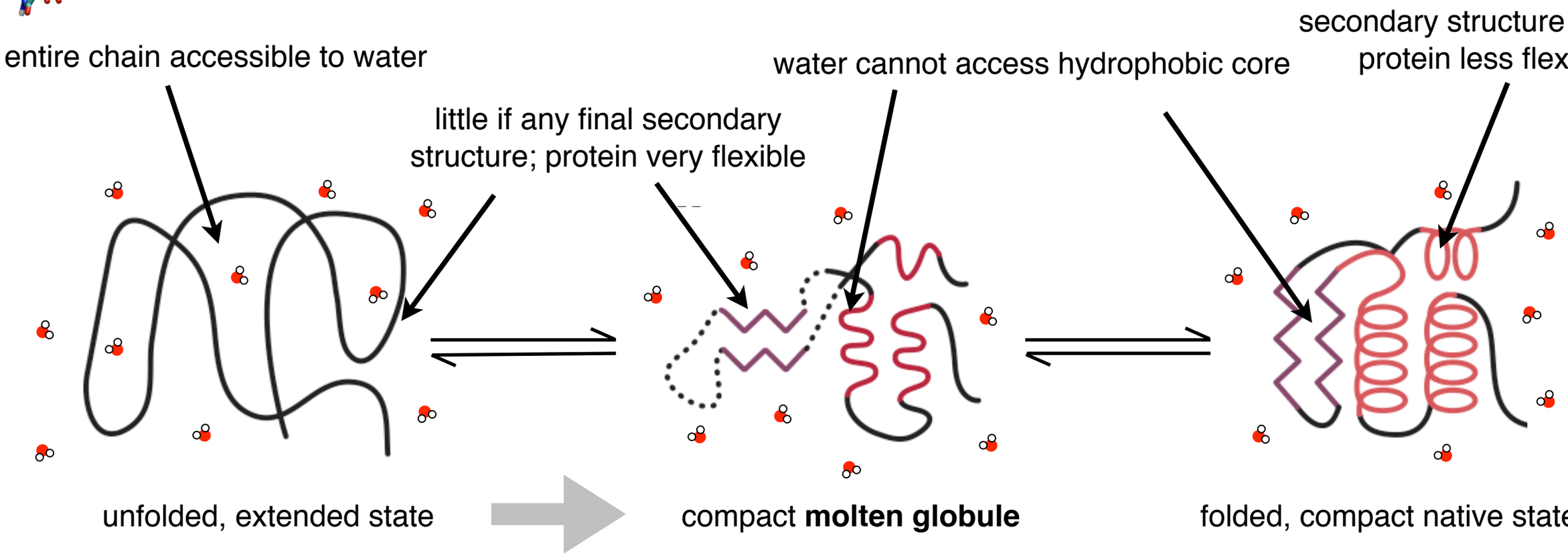
- tertiary: hinge bending (e.g. AdK)
- quaternary: rotation of subunits (e.g. hemoglobin)

the energy between these conformations may be low

Figure adapted from Nature (2003) **426** 900



Hydrophobic collapse is the first step



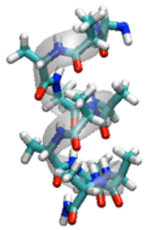
The hydrophobic effect causes the extended polypeptide chain to collapse and form a compact but dynamic **molten globule**

The molten globule is not an intermediate* but represents a typical compact conformation that the polypeptide chain must pass through in order to reach the folded, native state

It has few, if any, of the final non-covalent interactions

* this is not always true but for small globular proteins it often is

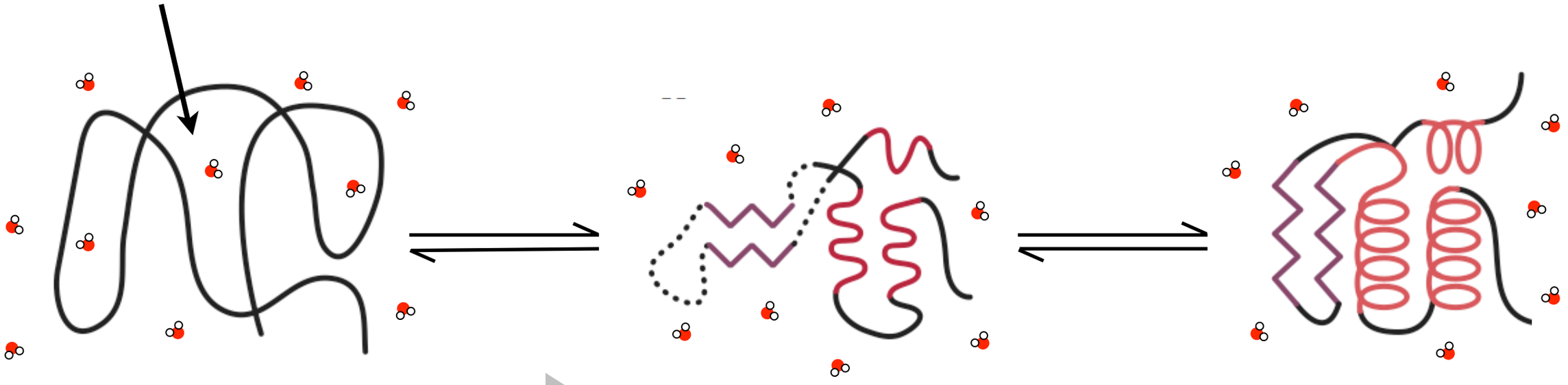
Price (3rd ed) pg 350



Thermodynamics of hydrophobic collapse

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

water ordered around non-polar residues to maximise the number of water—water hydrogen bonds



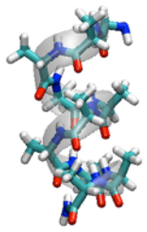
unfolded, extended state → compact **molten globule** → folded, compact native state

protein	ΔH	> 0	☹	fewer protein—water hydrogen bonds
	ΔS	< 0	☹	protein can adopt fewer conformations when a molten globule
water	ΔH	< 0	☺	more water—water hydrogen bonds (can form complete network)
	ΔS	> 0	☺ ☺	much less local ordering** of the water

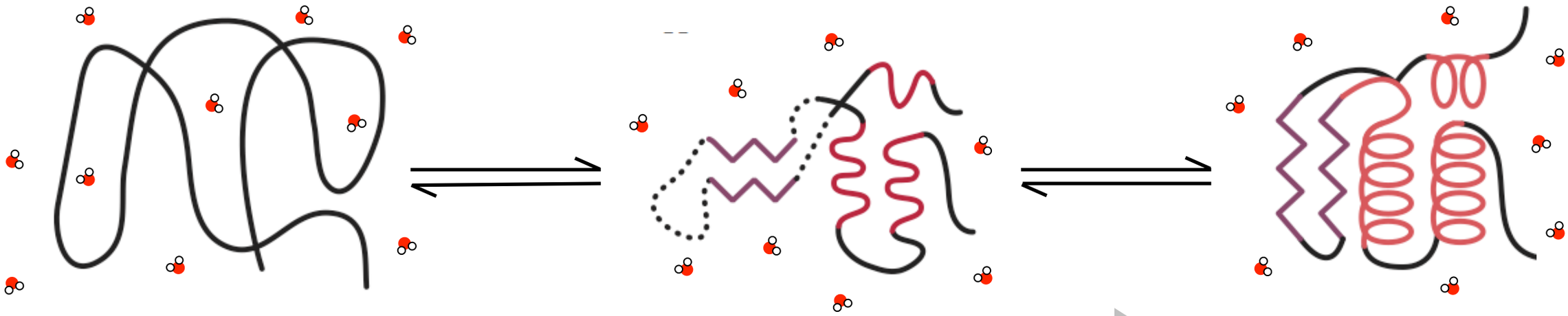
→ hydrophobic collapse is driven by the increase of entropy of the water*

** and therefore there are more ways that energy can be distributed

* at physiological temperatures



But what drives the second step i.e. formation of the native structure?



unfolded, extended state

compact **molten globule**

folded, compact native state

ΔG must be < 0 for the protein to fold

protein

ΔH	?
ΔS	< 0 ☹

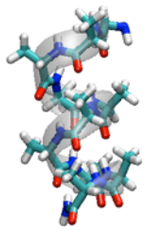
native fold is less flexible than molten globule

from the perspective of the solvent, there is little difference between the molten globule and the folded, native state

water

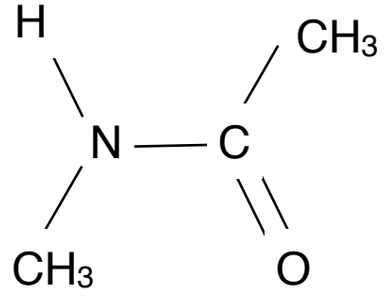
ΔH	≈ 0
ΔS	≈ 0

the enthalpy of forming additional non-covalent interactions not present in the molten globule must overcome the reduction in entropy of the protein



It is not favourable to form hydrogen bonds between molecules in water

donor NMA



acceptor

NMA forms a dimer in CCl_4 since water out-competes other NMA molecules for the hydrogen acceptors and donors

both pay an entropic penalty since there are fewer ways to arrange dimers of NMA than there are monomers...

water

ΔH 0.0

☹ TΔS -12.8

☹ ΔG 12.8 kJ mol $^{-1}$

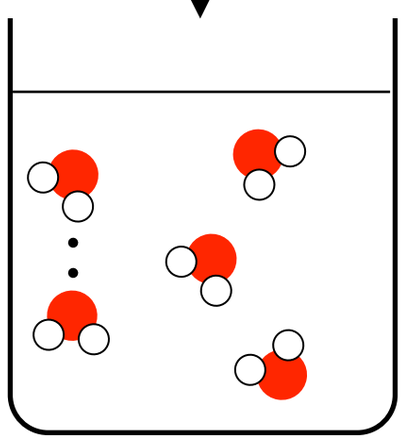
but this is compensated for in CCl_4 by the net formation of one hydrogen bond

CCl_4

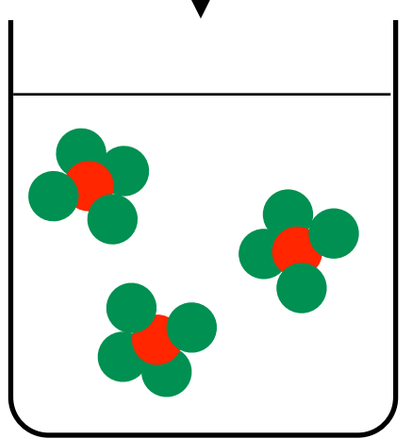
☺ ΔH -16.4

☹ TΔS -12.8

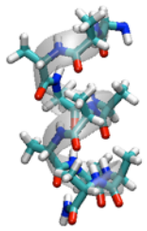
☺ ΔG -3.6 kJ mol $^{-1}$



water

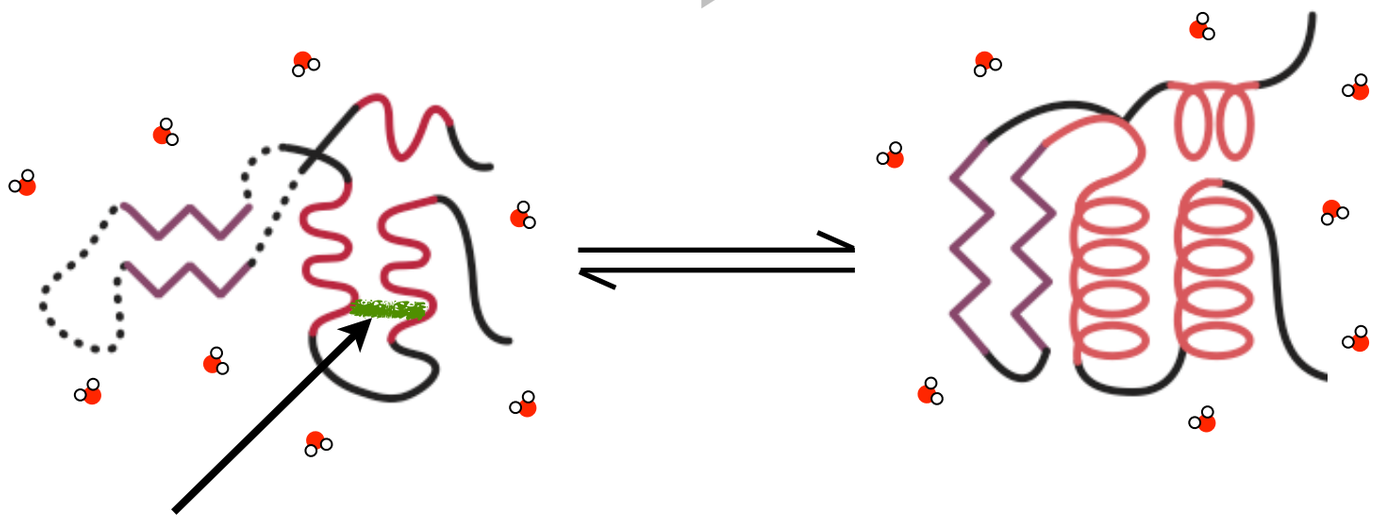


CCl_4



...but water cannot get into the interior of the molten globule

compact **molten globule** → folded, compact native state



consider a single intra-protein hydrogen bonding forming

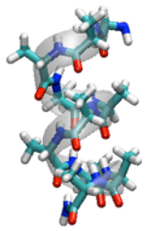
since water cannot reach the acceptor and donors it cannot compete → ☺ ΔH_1 < 0

but we have dramatically reduced the number of conformations available to the molten globule → ☹ ΔS_1 $\ll 0$

therefore for a single hydrogen bond ☹ ΔG_1 > 0

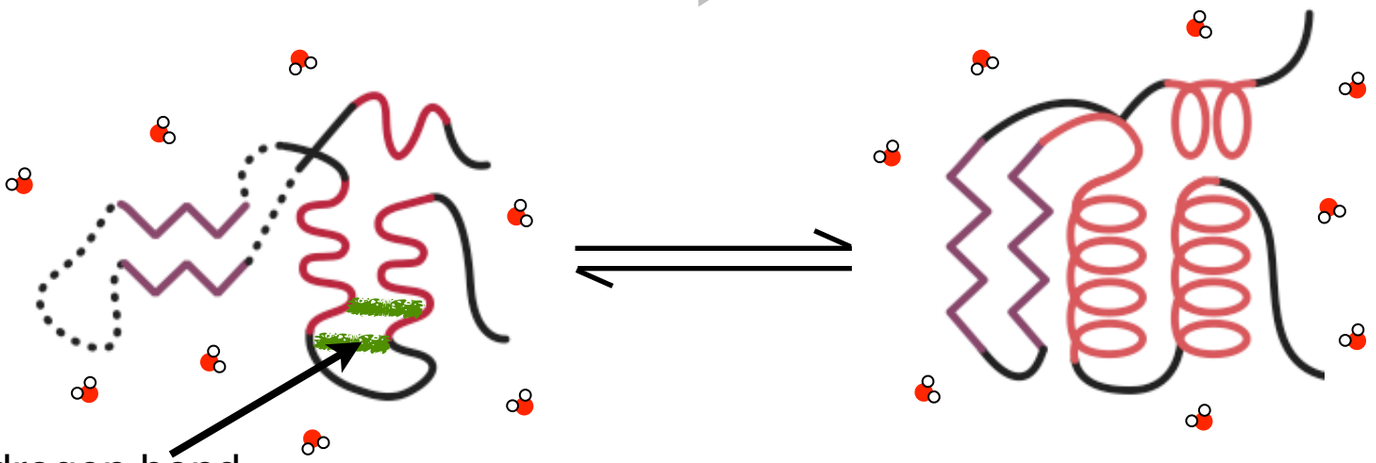
So how do the non-covalent interactions form that stabilise the folded, native state compared to the molten globule?

The answer is that the non-covalent interactions form in a **co-operative** manner



Consider adding a second hydrogen bond...

compact **molten globule** → folded, compact native state



the enthalpy of formation is still favourable since water cannot compete

for the **2nd** hydrogen bond

☺ ΔH_2 < 0

☹ ΔS_2 $< \Delta S_1$ $<< 0$ ← since the protein is **already partially constrained** by the first hydrogen bond, adding a second hydrogen bond reduces the entropy **by less than the first***

concept of **degrees of freedom**

☹ ΔG_2 $< \Delta G_1$ > 0 ← the free energy of adding the second bond is still positive but it is **less than** the free energy of adding the first

→ it gets easier and easier to add subsequent non-covalent interactions

each non-covalent interaction encourages the formation of subsequent non-covalent interactions

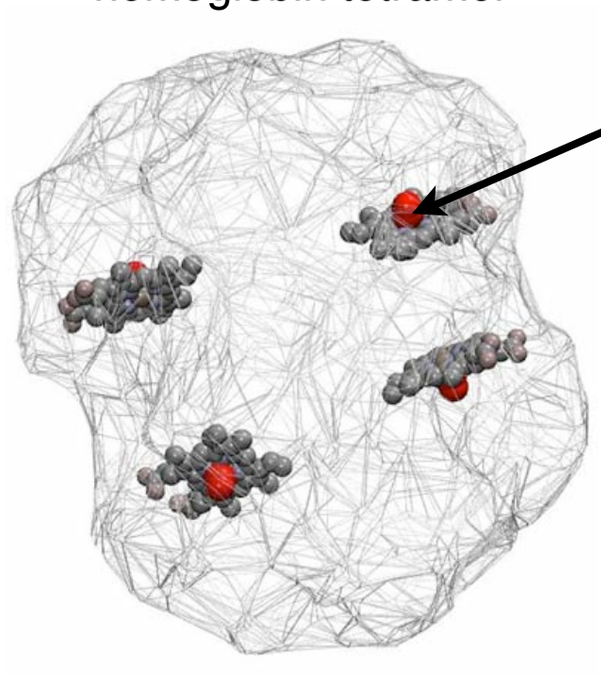
remember non-covalent interactions are always weak

*especially if it is close to the first

Hemoglobin: co-operativity in ligand-binding

from Biological Chemistry

hemoglobin tetramer



each hemoglobin monomer has one **heme** that can bind a molecule of O_2

the binding is **cooperative** i.e. if one oxygen binds this makes it easier for subsequent O_2 molecules to bind

so if n molecules of O_2 bind together:

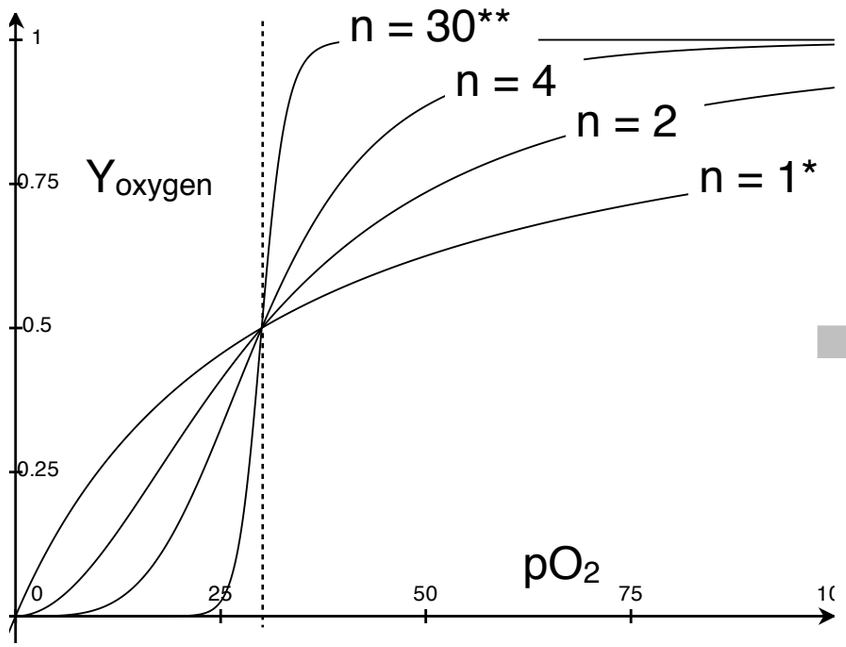
$$Hb + nO_2 \rightarrow Hb(O_2)_n$$

fraction of O_2 sites occupied $\longrightarrow$ $Y_{oxygen} = \dfrac{(pO_2)^n}{(p_{50})^n + (pO_2)^n}$

partial pressure of O_2

partial pressure at which hemoglobin is half-saturated

the measured value of n is the Hill coefficient which for hemoglobin is ~ 2.8

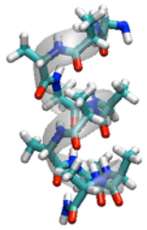


the greater the number of cooperative events, the steeper the transition from unbound to bound

these **sharp transitions** as the conditions are varied are **characteristic of co-operativity**

*no cooperativity

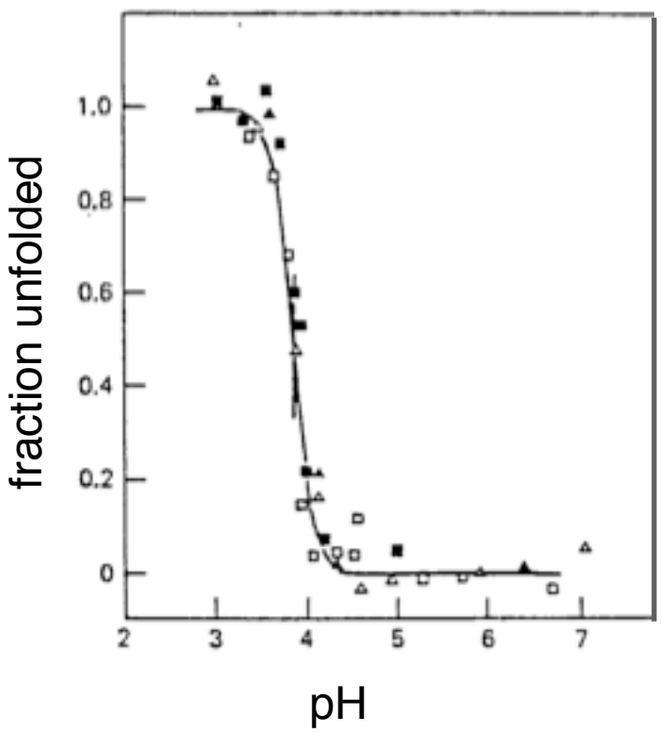
**not possible for hemoglobin but is shown for illustration



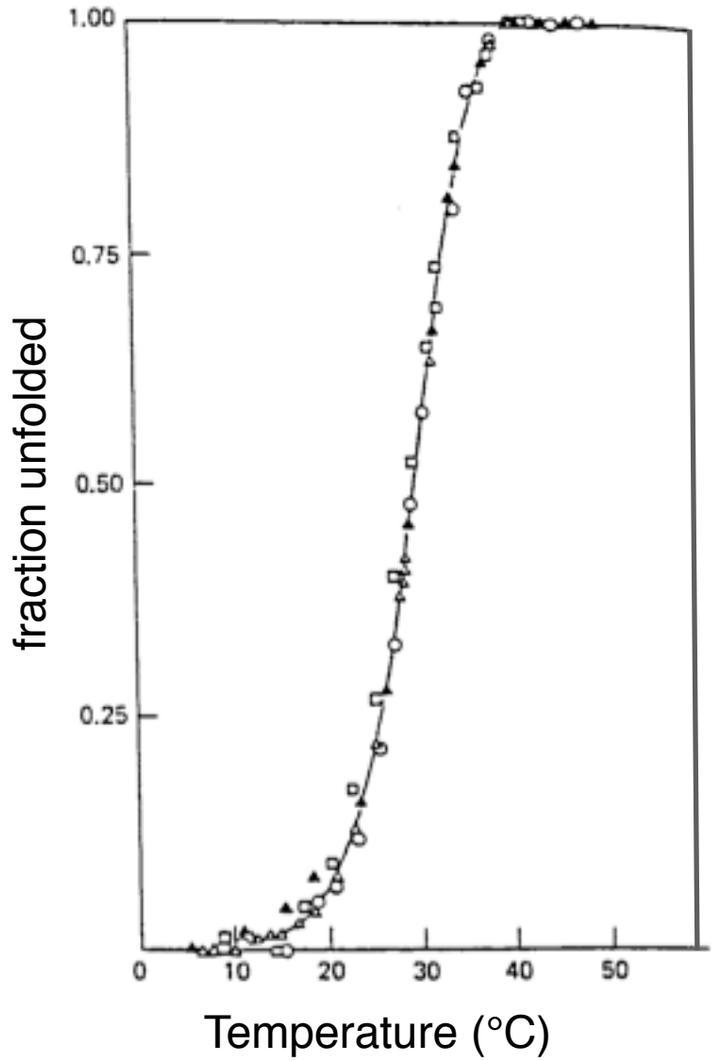
We observe sharp transitions in the folding of proteins

more on this next lecture

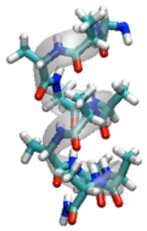
Staphlococcal nuclease A



Bovine ribonuclease A

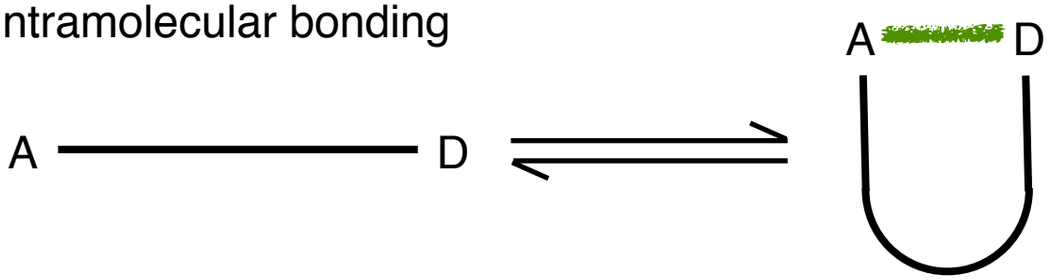


Creighton (2nd ed) Figure 7.11, pg 288



A more quantitive approach: proteins can bind to themselves or other proteins

(1) Intramolecular bonding



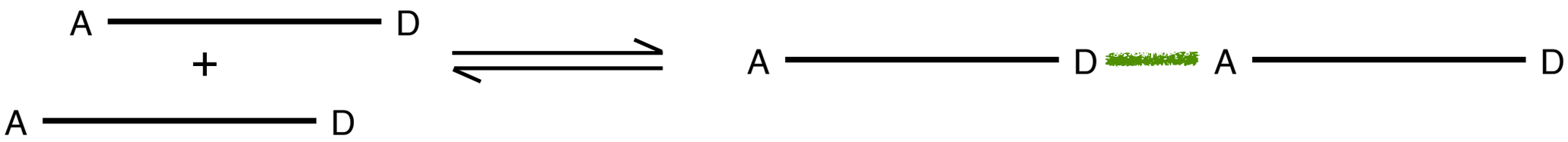
$$K_{intra} = \frac{[\text{bonded monomer}]}{[\text{monomer}]}$$

no units; dimensionless

$$K_{inter} = \frac{[\text{dimer}]}{[\text{monomer}]^2}$$

units: M^{-1}

(2) Intermolecular bonding



The ratio of the two equilibrium constants is known as the **effective concentration**

$$[AD]_u = \frac{K_{intra}}{K_{inter}}$$

units; M

Remember: $\Delta G = -RT \ln K$ and $\Delta G = \Delta H - T\Delta S$

so both enthalpic and entropic effects are described by the equilibrium constants

How do we increase the magnitude of the effective concentration*?

in both cases a single non-covalent interaction has formed → therefore the enthalpies are similar

hence the size of the effective concentration depends on the relative reduction in entropies upon formation of the non-covalent interaction

$\frac{K_{intra}}{K_{inter}}$

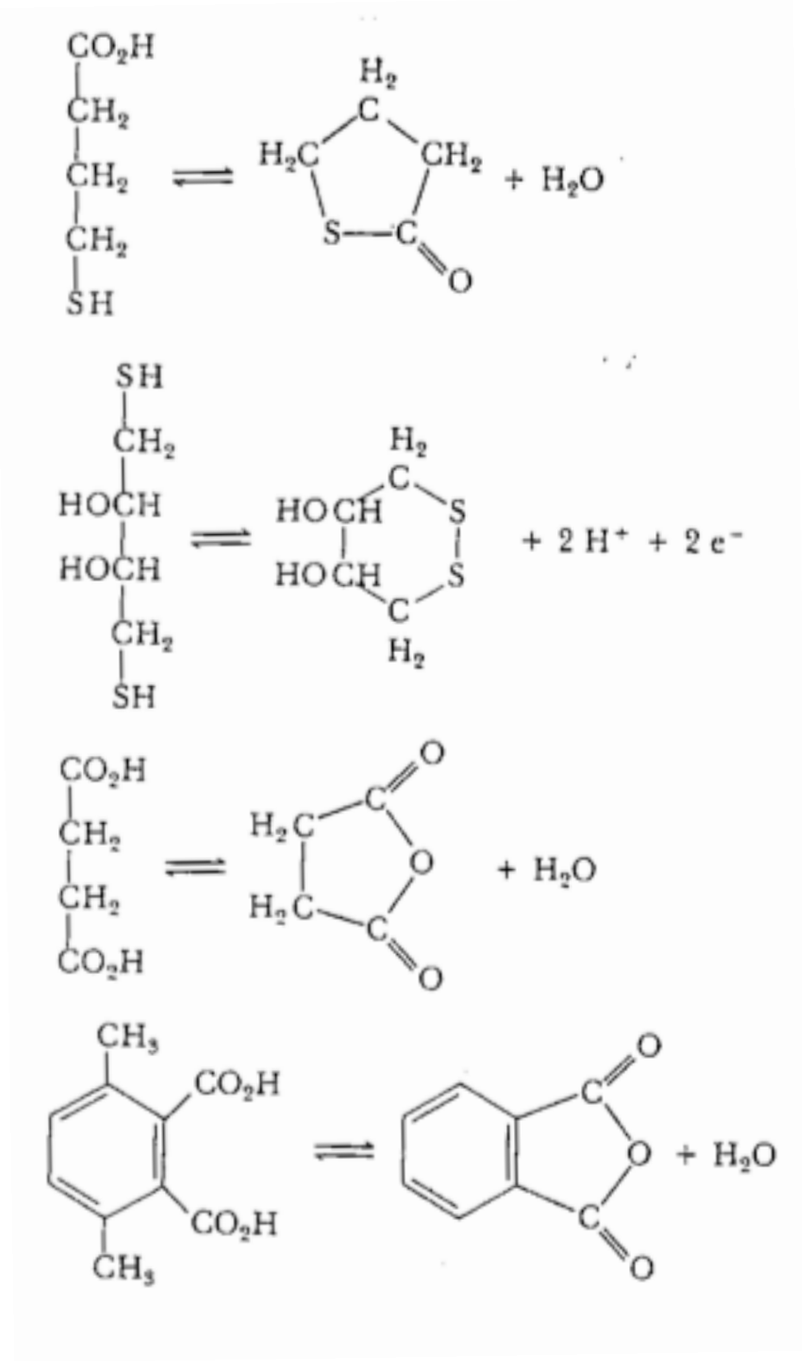
- reduce the loss in entropy upon formation of the intramolecular bond
- increase the loss in entropy upon formation of the intermolecular bond e.g. non-covalent interactions with more stringent geometries such as hydrogen bonds will lead to small values of K_{inter}

*$[AD]_u$ can be very small if the protein keeps A and D apart

Small molecule examples

Equilibrium reaction

Effective concentration (M)



3.7 x 10^3 ← can easily exceed the concentration of water (55 M)

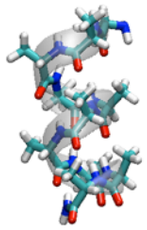
2 x 10^2

$$[AD]_u = \frac{K_{intra}}{K_{inter}}$$

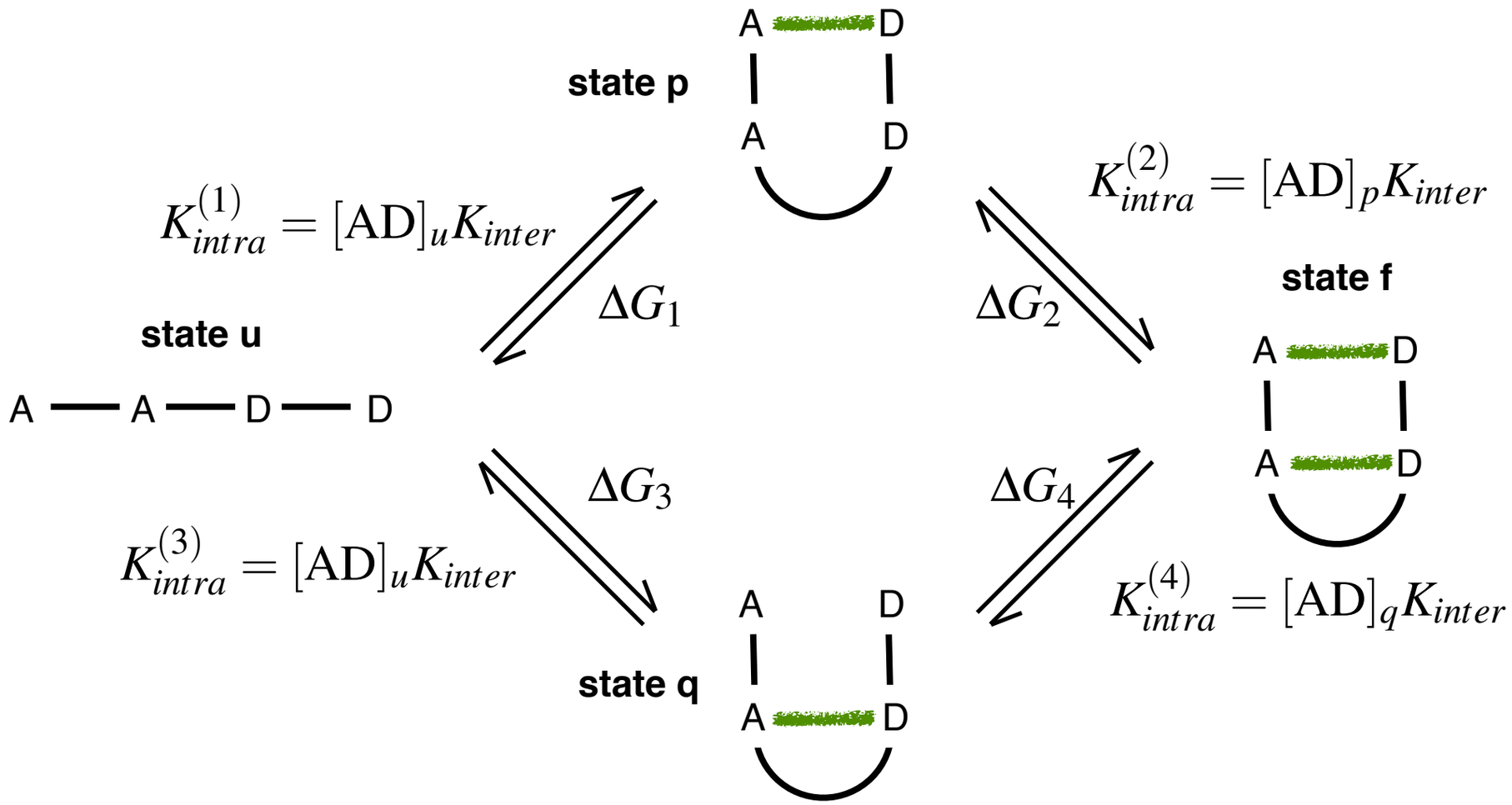
1.9 x 10^5

5.4 x 10^9 ← very large because
(1) a covalent bond is highly directional which decreases the entropy of the intermolecular reaction
(2) there is almost no loss of entropy in the intramolecular reaction

Creighton (2nd ed) Figure 4.11, pg 164



But what if two non-covalent interactions can be formed?



Consider traversing the top path:

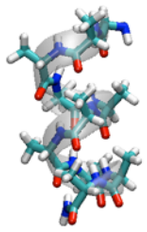
$$K_{intra}^{(1)} = [\mathrm{AD}]_u K_{inter} \qquad K_{intra}^{(2)} = [\mathrm{AD}]_p K_{inter}$$

but $[\mathrm{AD}]_p > [\mathrm{AD}]_u$ since the backbone is already partly restraining in state p (always > 1 if there is cooperativity)

the increase in the effective concentration is known as the **cooperativity factor** $C_f = \dfrac{[\mathrm{AD}]_p}{[\mathrm{AD}]_u}$

hence $K_{intra}^{(1)} = [\mathrm{AD}]_u K_{inter}$ $K_{intra}^{(2)} = C_f [\mathrm{AD}]_u K_{inter}$ i.e. $K_{intra}^{(2)} = C_f K_{intra}^{(1)}$

more sophisticated approach in Creighton (2nd ed) pg 165



What is the overall equilibrium constant?

$$K_{intra}^{(1)} = [\mathrm{AD}]_u K_{inter}$$

$$K_{intra}^{(2)} = C_f [\mathrm{AD}]_u K_{inter} \quad \text{or} \quad K_{intra}^{(2)} = C_f K_{intra}^{(1)}$$

$$K_{overall} = K_{intra}^{(1)} \times K_{intra}^{(2)}$$

$$K_{overall} = C_f \left(K_{intra}^{(1)} \right)^2$$

$$K_{overall} = C_f \left([\mathrm{AD}]_u K_{inter} \right)^2$$

We can generalise this to a situation with n cooperative non-covalent interactions if we assume that the cooperativity factor remains a constant

$$K_{intra}^{(1)} = [\mathrm{AD}]_u K_{inter}$$

$$K_{intra}^{(2)} = C_f K_{intra}^{(1)}$$

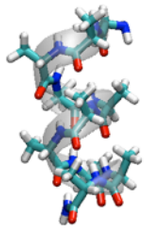
$$K_{intra}^{(3)} = C_f K_{intra}^{(2)}$$

$$\vdots$$

$$K_{intra}^{(n)} = C_f K_{intra}^{(n-1)}$$

$$K_{overall} = K_{intra}^{(1)} \times K_{intra}^{(2)} \times K_{intra}^{(3)} \times \ldots K_{intra}^{(n)}$$

Calculating $K_{overall}$ is an iterative process (there is no simple equation)



An example

$$K_{intra}^{(n)} = C_f K_{intra}^{(n-1)} \qquad K_{intra}^{(1)} = [\mathrm{AD}]_u K_{inter}$$

$[\mathrm{AD}]_u$ = 10^{-2} M

K_{inter} = 10^{-2} M^{-1}

C_f = 10

n	$K_{intra}^{(n)}$	$K_{overall}$	ΔG (kJ mol^{-1})
1	10^{-4}	10^{-4}	23.7
2	10^{-3}	10^{-7}	41.5
3	10^{-2}	10^{-9}	53.4
4	10^{-1}	10^{-10}	59.4
5	1	10^{-10}	59.4
6	10^{1}	10^{-9}	53.4
7	10^{2}	10^{-7}	41.5
8	10^{3}	10^{-4}	23.7
9	10^{4}	1	0.0
10	10^{5}	10^{5}	-29.7
11	10^{6}	10^{11}	-65.3

(each step: x C_f; $K_{overall}$ = $K_{intra}^{(n)}$ x $K_{overall}$ of the previous row)

$$\Delta G = -RT \ln K$$

$$K_{overall} = K_{intra}^{(1)} \times K_{intra}^{(2)} \times K_{intra}^{(3)} \times \ldots K_{intra}^{(n)}$$

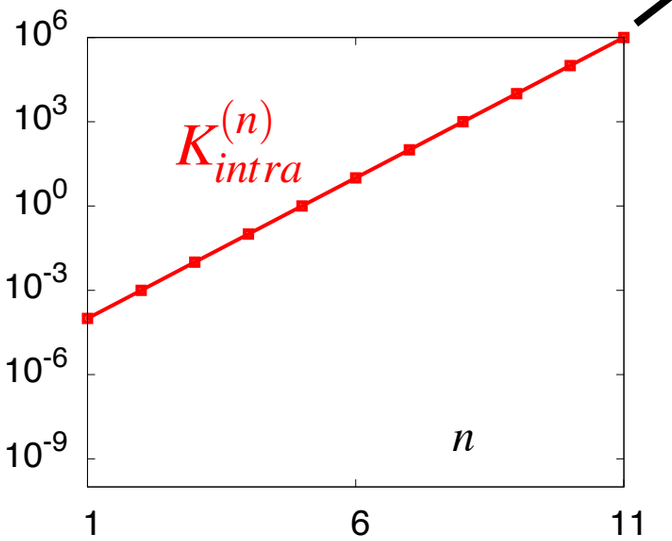
The first few interactions are not favourable and..

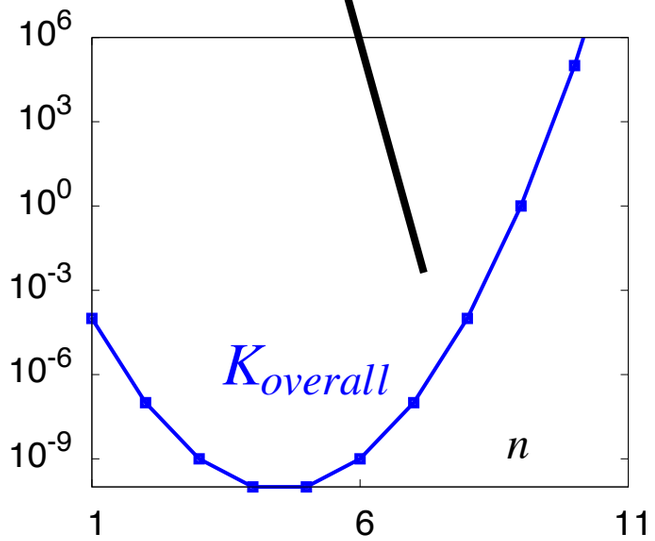
..a maximum in ΔG is reached when $K_{intra}^{(5)} = 1$.

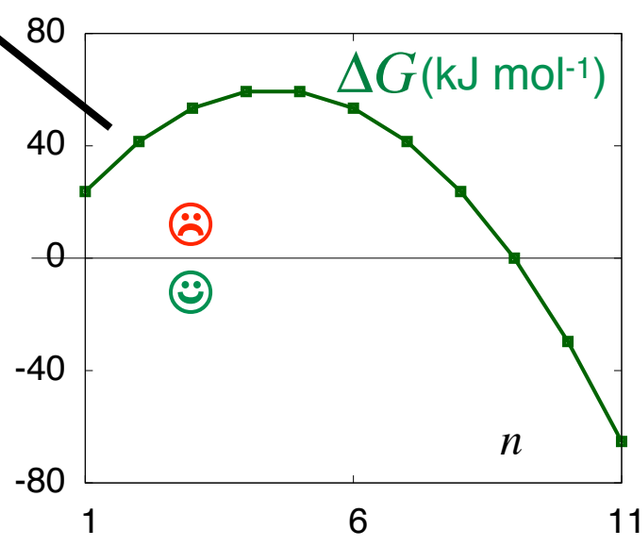
Adding further interactions reduces $K_{overall}$ ..

..until with 9 interactions $\Delta G = 0$..

..and the reaction becomes favourable for $n > 9$.







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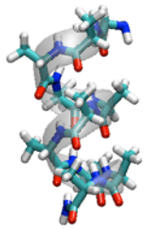
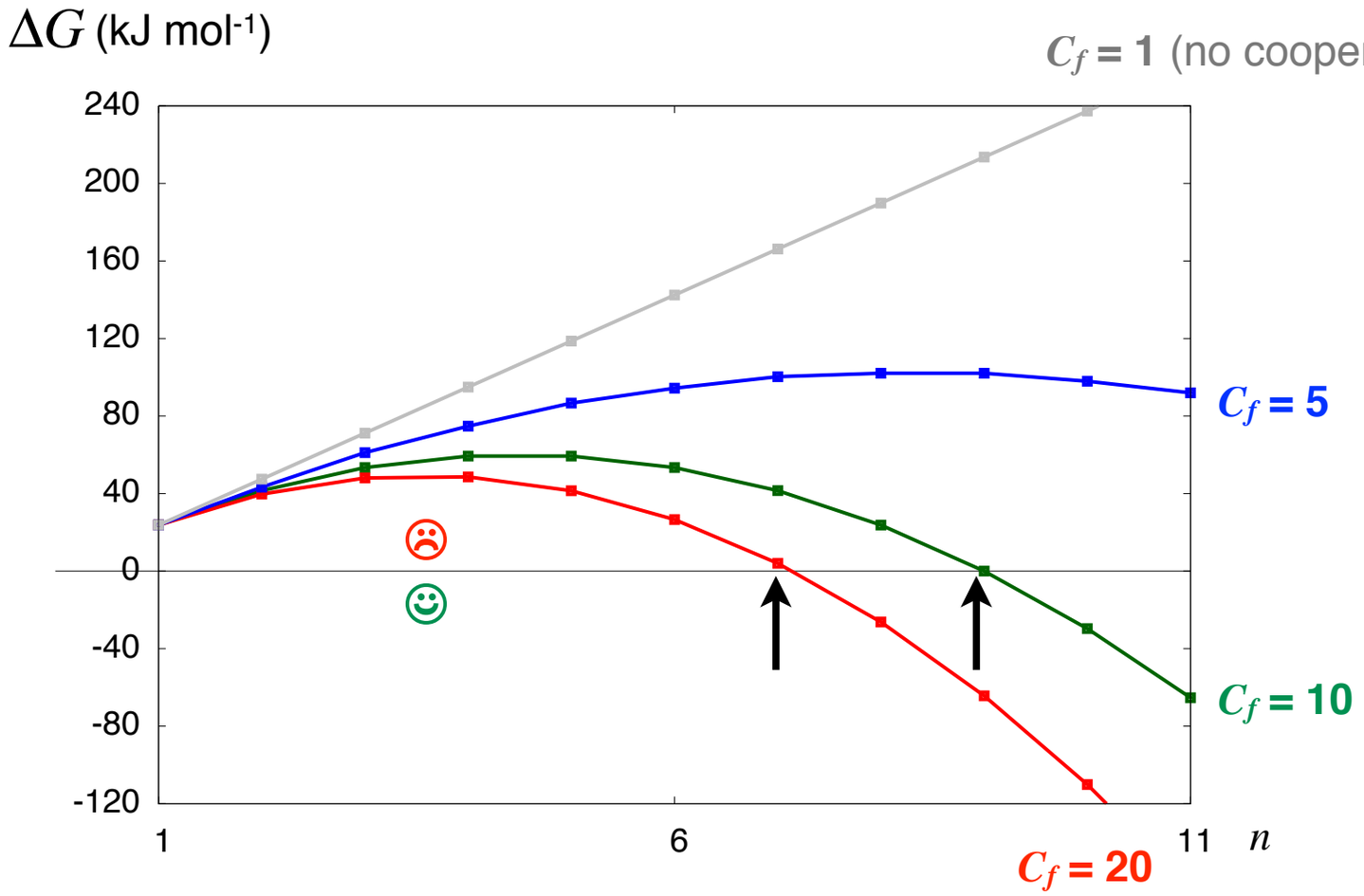


How does varying C_f affect the minimum number of interactions?



the larger the co-operativity factor, the fewer the non-covalent interactions required in each cluster

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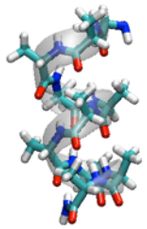
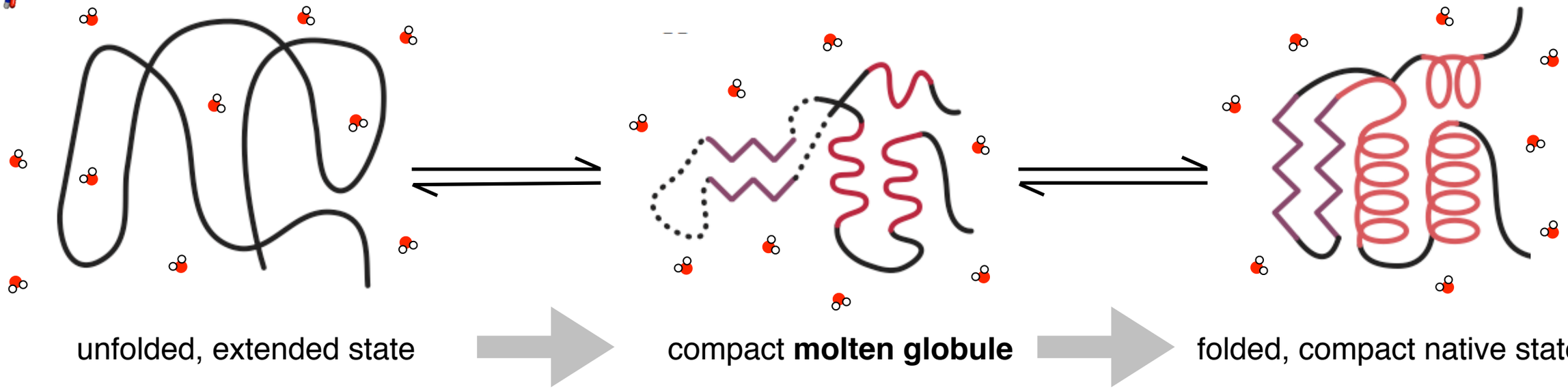


Protein folding...



fewer protein—water hydrogen bonds

more water—water hydrogen bonds (can form complete network)

clusters of non-covalent interactions form **cooperatively**

protein

$\Delta H > 0$ ☹

protein can adopt fewer conformations when a molten globule

$\Delta S < 0$ ☹

native fold is less flexible than molten globule

$\Delta H < 0$ ☺☺

$\Delta S < 0$ ☹

much less local ordering of the water

water

$\Delta H < 0$ ☺

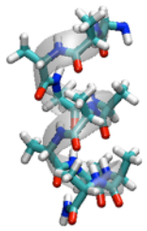
$\Delta S > 0$ ☺☺

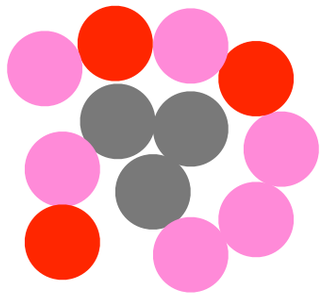
$\Delta H \approx 0$

$\Delta S \approx 0$

from the perspective of the solvent, there is little difference between the molten globule and the folded, native state

The **hydrophobic effect** causes the extended polypeptide chain to collapse and form a compact but dynamic **molten globule**. Clusters of **non-covalent interactions** within the protein then form **cooperatively**. This leads to both secondary structure and the dense packing of the hydrophobic interior.



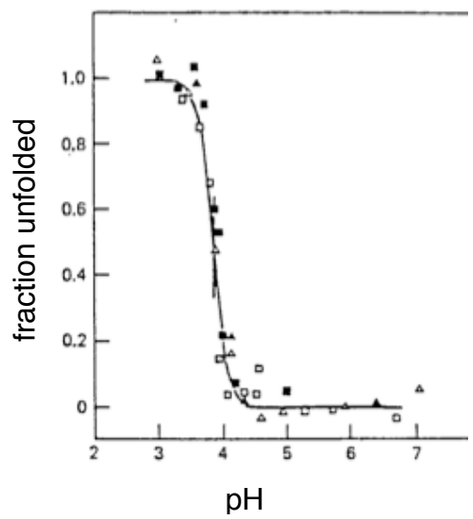


Proteins have to be large enough to be able to bury hydrophobic groups in their centre

The side-chains of polar and charged amino acids are found on the surface of proteins

The side-chains of hydrophobic amino acids are buried in the centre of proteins

Staphlococcal nuclease A



Proteins undergo very rapid, sharp transitions between the folded and unfolded states when conditions and/or the environment is altered

Consequences

The vast majority of protein acceptors and donors participate in hydrogen bonds

Secondary structure (e.g. α-helices)

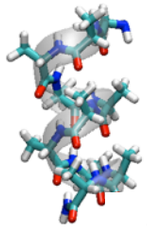
The peptide backbone can adopt relatively few combinations of torsional angles due to steric hindrance

The interior of proteins is very densely packed

a cavity probably has a function

The environment (especially the properties of water) plays a major role in determining the tertiary structure of a protein

by altering the environment we may be able to unfold (denature) a protein

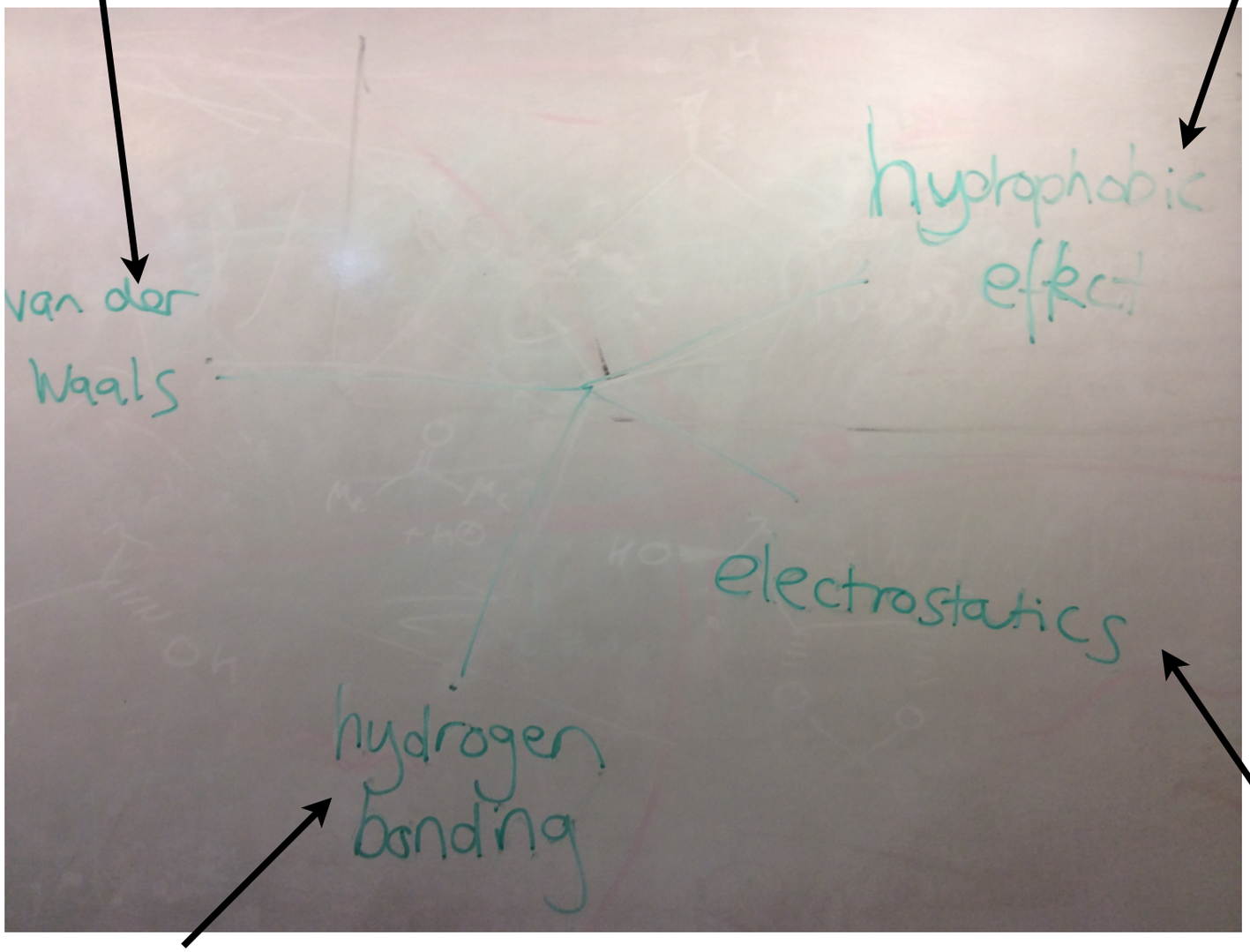


This is what we wrote in the first lecture...

van der Waals interactions, as modelled by the Lennard-Jones 6-12 potential, captures the attractive induced-dipole induced-dipole force and the repulsive force that occurs between atoms at very short range

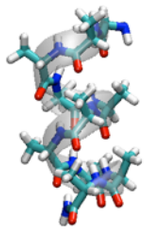
It is the entropy of the water that drives the hydrophobic effect

The entropy of the protein itself is also important in the folding of proteins



Hydrogen bonds are very important (e.g. in the secondary structure of proteins or in determining the behaviour of water)

Electrostatics is the fundamental attractive force and describes charge-charge and induced-dipole, induced-dipole (London dispersion) interactions. The latter gives us the attractive term in the Lennard-Jones 6-12 potential.



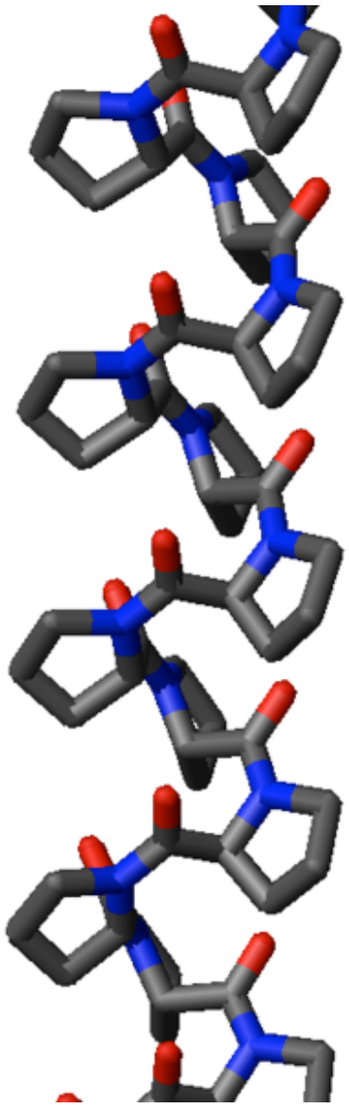
Poly-proline helices I and II

Proline cannot form a backbone hydrogen bond as its backbone amide is part of the ‘sidechain’

→ Prolines disrupt α-helices and β-sheets

Long chains of prolines can also form unusual structures

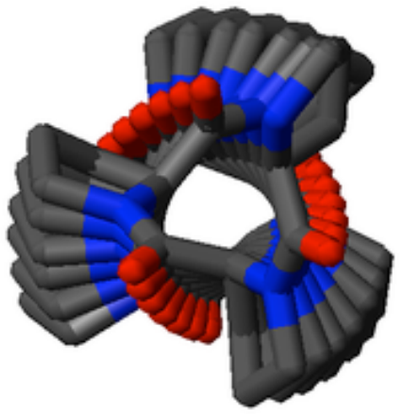
Type I poly-Proline helix



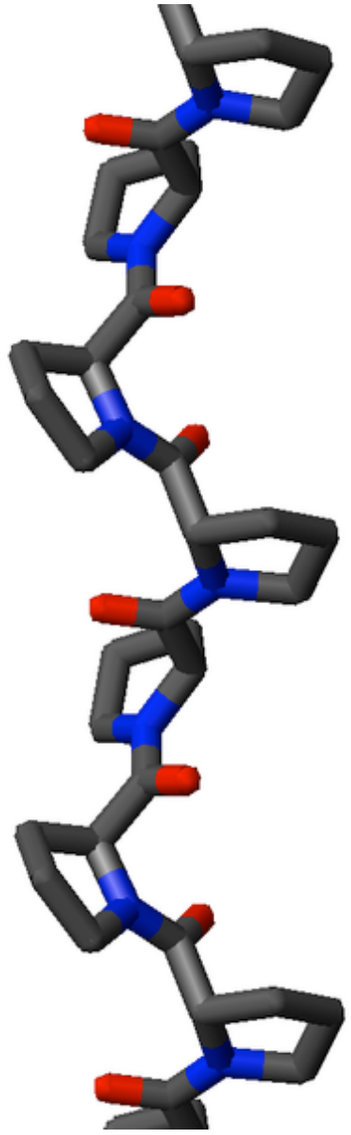
Proline is *cis* about the peptide bond

3.3 residues per turn

right-handed helix



Type II poly-Proline helix

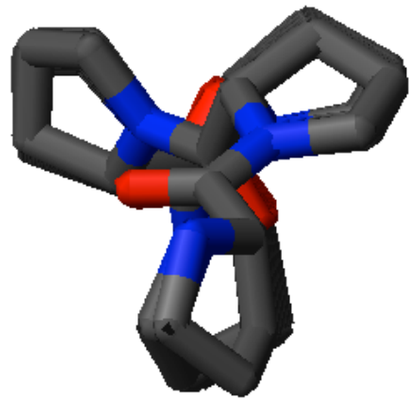


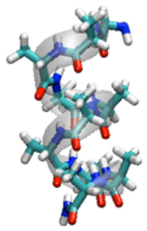
Proline is *trans* about the peptide bond

3.0 residues per turn

left-handed helix

phi = -83°, psi = -78°





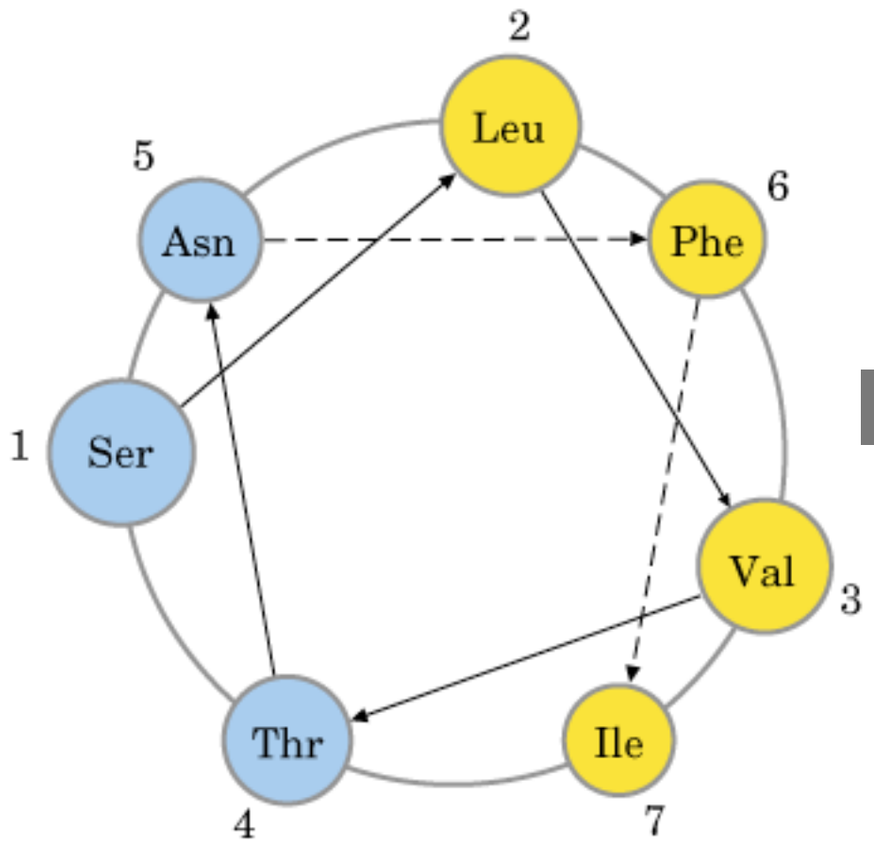
Amphipathic helices

When an α-helix traverses the surface of a protein the surface of the helix directed towards the interior of the protein is hydrophobic and the surface directed towards the water is hydrophilic. The helix is said to be **amphipathic.**

This asymmetry in hydropathy can be displayed by drawing the distribution of amino acids as one looks down the helical axis. This is known as a **helical wheel** and is similar to a Newman projection for covalent bonds.

Example of a helical wheel

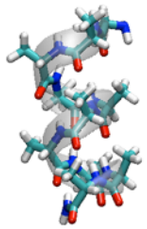
−Ser−Leu−Val−Thr−Asn−Phe−Ile−



since an α-helix has 3.6 residues per turn there are 100° between successive residues

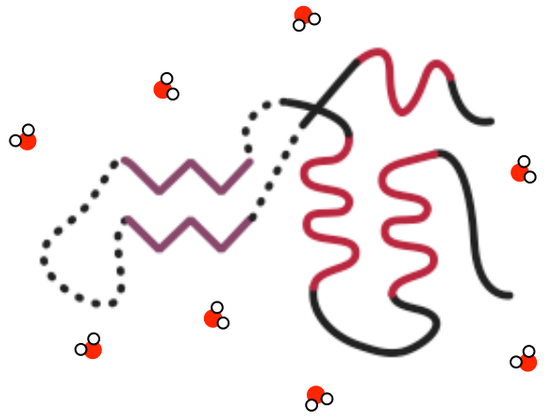
hydrophilic residues

hydrophobic residues

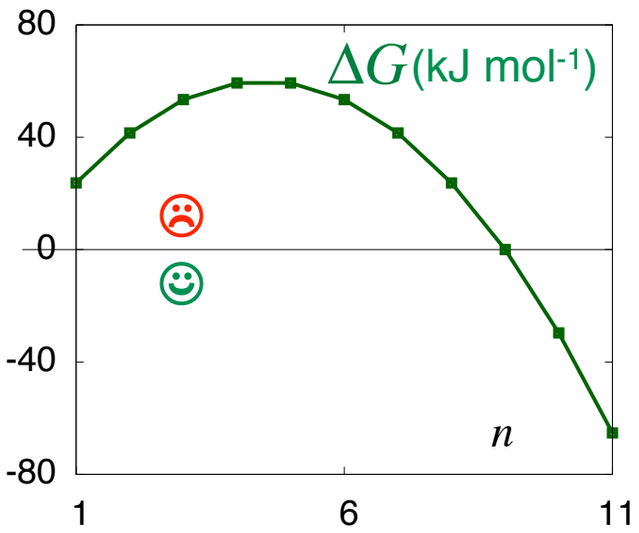


Lecture 5: Summary

The hydrophobic effect causes an extended polypeptide chain to undergo '**hydrophobic collapse**' and form a **molten globule**

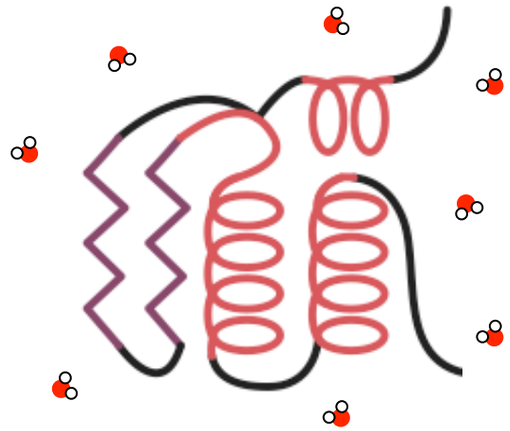


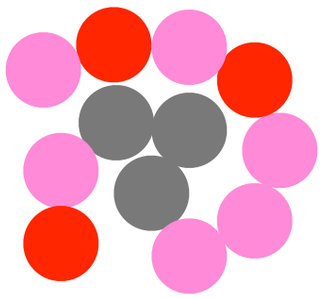
This is driven by the increase in the entropy of the water



Water cannot penetrate the molten globule and clusters of **non-covalent interactions** form **cooperatively**

The change in enthalpy from forming these interactions drives the formation of the folded, native structure





The cooperativity of this transition has many consequences for proteins