University of Glasgow
Department of Chemistry

CMC-3 and Chem-3

Biophysical Chemistry

Biomolecular Interactions

Professor Alan Cooper

Rm. B4-20 (Joseph Black Building)

Lecture # 1

Aims: To discuss the theoretical and experimental basis of intermolecular forces in complex, condensed systems and how this applies to the stabilization of biomolecular structures and interactions.

Recommended reading/Chemistry Library reference/reserve shelf:

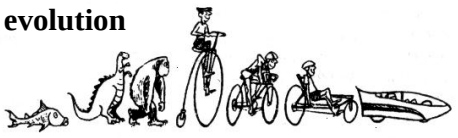
Physical Biochemistry: principles and applications, David Sheehan, Wiley
Biophysical Chemistry, C R Cantor and P R Schimmel, W H Freeman & Co.
Physical Biochemistry, D Freifelder, W H Freeman & Co.
Enzyme Structure and Mechanism, A Fersht, W H Freeman & Co.
Protein Structure - a Practical Approach, T E Creighton, IRL Press.
Introduction to Protein Structure, C Branden and J Tooze, Garland Publishing

\+ *new for 2004...*
Biophysical Chemistry, A. Cooper (RSC Tutorial Chemistry Text)

\+ website material (to come)

What is special (if anything) about biological molecules ?

- they are **big** (usually)
- they are **polymers** (usually) with specific **sequence**
- they have specific **structure** which determines **function**
- these structures are determined (mainly) by **non-covalent** forces
- they exist and perform in **water**
- they are the products of **evolution**



Proteins & Polypeptides

Proteins are polymers made up of specific sequences of L-amino acids linked together by covalent peptide (amide) bonds (Fig.1). Amino acids are chosen from a basic set of 20 building blocks differing in sidechain (Fig.2), with occasional special-purpose side chains made to order (e.g. hydroxyproline).

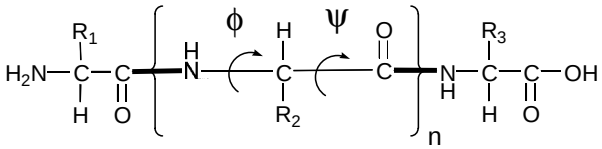


Figure 1: Polypeptide structure showing rotatable ϕ/ψ angles. The planar peptide (amide) bonds are shown in bold, and are usually *trans*.

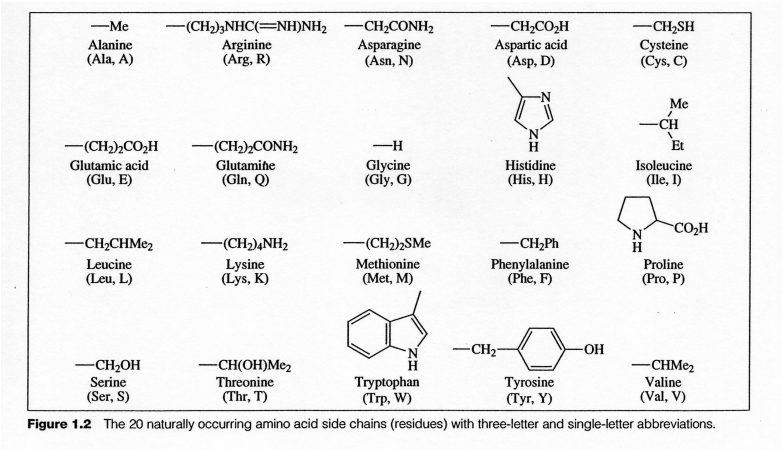


Figure 1.2 The 20 naturally occurring amino acid side chains (residues) with three-letter and single-letter abbreviations.

The 20 naturally-occurring amino acid side chains (residues) with standard 3-letter and single-letter abbreviations.

Primary structure: the sequence of amino acids in the polypeptide chain. This is unique to each protein, and is determined (primarily) by the genetic information encoded in the DNA of the relevant gene.

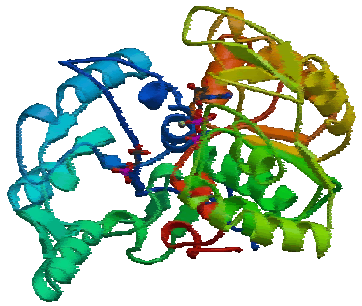
```
KVFERCELAR TLKRLGMDGY RGISLANWMC LAKWESGYNT
RATNYNAGDR STDYGIFQIN SRYWCNDGKT PGAVNACHCS
ASALLQDNIA DAVACAKRVV RDPQGIRAWV AWRNRCQNRD
VRQYVQGCGV
```

Primary structure of a 130-residue protein (human lysozyme) shown using the single-letter amino acid codes

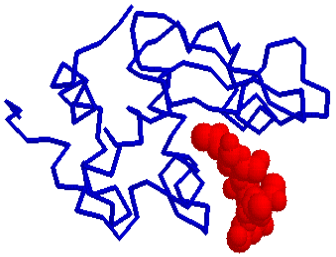
Secondary structure: regular, repeating structures such as α-helix, β-sheets, etc.

Tertiary structure: the three-dimensional arrangement of secondary structure elements that defines the overall conformation of the (globular) protein.

Examples of protein tertiary structure...

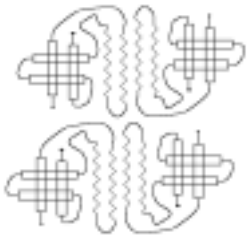


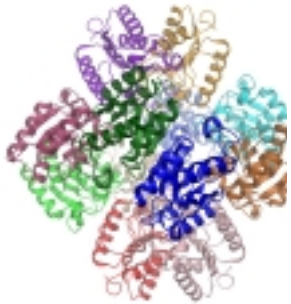
Phosphoglycerate kinase



Lysozyme (with bound inhibitor)

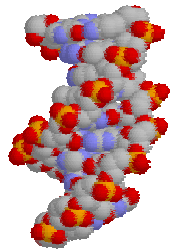
Quaternary structure: in multi-subunit proteins, the three-dimensional arrangement of the subunits.

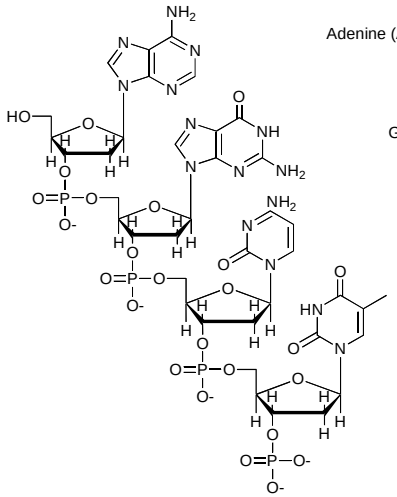




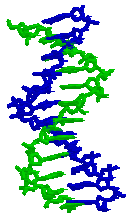
Polynucleotides

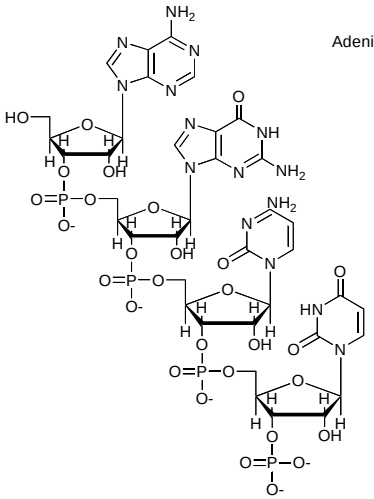
The genetic information which encodes protein sequences is found in DNA (deoxyribonucleic acid), and the transcription and translation process involves RNA (ribonucleic acid). Both are polynucleotides consisting of long sequences of nucleic acids made up of a phospho-ribose backbone, with a choice of four different purine or pyrimidine side-chains or “bases” attached.



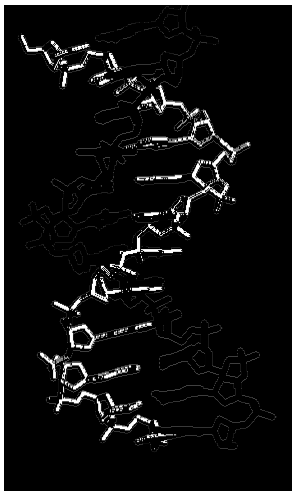


DNA structure illustrating the deoxyribose-phosphate backbone, to which may be attached purine (A, G) or pyrimidine (C, T) bases





RNA structure illustrating the sugar (ribose) -phosphate backbone, to which may be attached purine (A, G) or pyrimidine (C, U) bases



DNA double-helix with complementary base pairing…

purine-pyrimidine

A-T (A-U in RNA)

G-C

…genetic coding

Polysaccharides

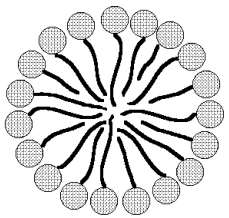
Complex polysaccharides such as starch, glycogen, cellulose, and so forth, play an important part in biochemistry both as energy stores and structural components. Many proteins are glycosylated ("glycoproteins"), with oligosaccharide chains (often branched) attached to specific amino acid residues, usually at the protein surface. The carbohydrate portion of glycoproteins is often involved in antigenicity, cell receptor and other molecular recognition processes.

Fats, Lipids and Detergents

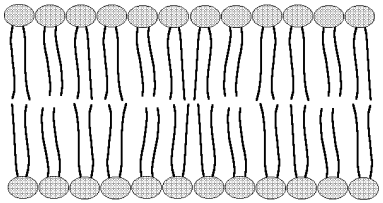
Fats and lipids are common terms for those bits of biological organisms that are insoluble in water but can be extracted with organic solvents such as trichloromethane (chloroform), ethers, etc. They generally consist of a polar head group attached to non-polar tails of unbranched hydrocarbons. This amphiphilic nature – hydrophilic head, hydrophobic tail – gives this class of molecule important properties that are exploited both by biology itself, and by biophysical chemists in studying such systems.

Broadly speaking, the number of hydrocarbon tails governs the behaviour in water.

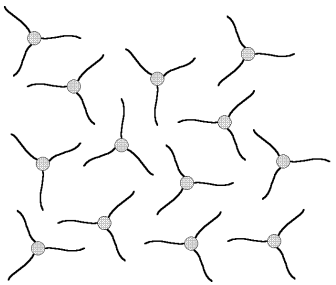
Detergents generally contain a polar head group attached to a single non-polar tail (or equivalent). This allows them to form *micelles* in water: roughly globular assemblies of a number of molecules clustered together, with their head groups exposed to water, while their non-polar tails are buried inside the cluster and away from direct contact with the surrounding water.



Lipids have two tails. This makes it difficult to pack the hydrocarbon chains effectively into a globular micelle structure, but they can form *lipid bilayers* instead. Here the molecules form into two-dimensional arrays or sheets, in which two layers of lipids bury their tails inside, leaving the hydrophilic heads exposed either side to the water. These lipid bilayers provide the basic structures of cell membranes.



Neutral fats or *triglycerides* commonly have three tails. This makes it difficult to form a compromise between the hydrophilic head and the bulky hydrophobic tails, so these substances tend to be very insoluble and just form an amorphous mass in water. This is what we commonly see as "fat".



Covalent *versus* Non-Covalent interactions...

Covalent

Definition: a covalent bond is an interaction between adjacent atoms involving sharing of an electron pair

Properties: Strong ($\Delta H^o_f \approx -400$ kJ mol^{-1} ; >> RT)

Difficult to break (except with enzymes, etc.)

Stiff (don't stretch or bend, but may rotate)

Short range

Responsible for: Primary structure of biopolymers...

DNA sequence

↳ RNA sequence

↳ Protein sequence...

Covalent *versus* Non-Covalent interactions...

Non-Covalent

Definition: a non-covalent interaction is any interaction (attractive or repulsive) not involving sharing of an electron pair.

Properties: Weak (≤ 50 kJ mol^{-1} ; ≈ RT)

Easily disrupted by thermal motion

Mobile, flexible, non-specific

Long range

Responsible for: Macromolecular conformations

DNA/RNA helices; protein secondary/tertiary/ quaternary structures; active site binding, subunit association… (liquid/solid properties)

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Lecture # 2

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- these structures are determined (mainly) by **non-covalent** forces
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- they are the products of **evolution**

Types of non-covalent interactions…

- Electrostatic
- Van der Waals
- Dispersion
- Hydrogen bonding
- Hydrophobic
- etc...

Reminder...

$$AH \rightleftharpoons A^- + H^+$$

acid dissociation constant, $K_A = [A^-][H^+]/[AH]$

and $pK_A = -\log_{10}K_A$

$$AH \rightleftharpoons A^- + H^+$$

pH < pK pH > pK

Group	*pH < pK_A*	*Typical pK_A*	*pH > pK_A*
C-terminus	-COOH	3	$-COO^-$
Glu, Asp	-COOH	4	$-COO^-$
His	$-Im-H^+$	6	-Im
N-terminus	$-NH_3^+$	8	$-NH_2$
Cys	-SH	8	$-S^-$
Lys	$-NH_3^+$	11	$-NH_2$
Tyr	-ϕ-OH	11	-ϕ-O^-
Arg	$-C(NH_2)_2^+$	12.5	$-C(NH)(NH_2)$
Phosphoglycerol, g = $CH_2(OH)CH(OH)CH_2$-O	g-$P(OH)O_2^-$	5.6	g-PO_3^{2-}

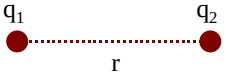
Typical pK_A and charge state for protein amino acid residues and other groups in water

Electrostatic Interactions

(A) Point Charges: "Coulomb Interaction"

Potential energy between two point charges, q_1 and q_2 :-

$$V_{qq} = q_1 q_2 / 4\pi\varepsilon_0\varepsilon_r r$$



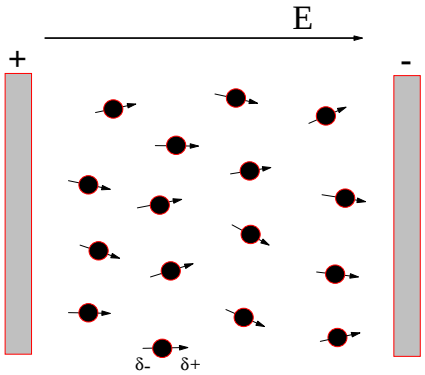
ε_0 = vacuum permittivity = 8.85×10^{-12} $C^2 J^{-1} m^{-1}$

ε_r = relative permittivity or "dielectric constant"

r = distance between charges (in metres)

q_1 , q_2 are the charges (in Coulomb)

(electronic charge: 1 e = 1.6×10^{-19} C)



The *dielectric constant* or *relative permittivity* (ε_r) of a substance is a measure of its polarizability in an electric field. For water at room temperature, $\varepsilon_r \approx 80$ (compared to 1 for a vacuum). This very high value arises because the dioplar water molecules tend to re-orient and align parallel to the electric field. This has the effect of partially cancelling the electric field, and results in a weakening of electrostatic interactions between charged groups.

Example: What is the electrostatic potential energy between Na^+ and Cl^- ions, 5 Å apart, in vacuum ?

$q_{Na+} = -q_{Cl-} = 1.6 \times 10^{-19}$ C ; $\varepsilon_r = 1$ *for vacuum*

r = 5 Å = 0.5 nm = 5×10^{-10} m

$V_{qq} = q_1 q_2 / 4\pi\varepsilon_0\varepsilon_r r = -(1.6 \times 10^{-19})^2 / (4\pi . 8.85 \times 10^{-12} . 5 \times 10^{-10})$

$= -4.6 \times 10^{-19}$ J *note minus sign, attractive interaction*

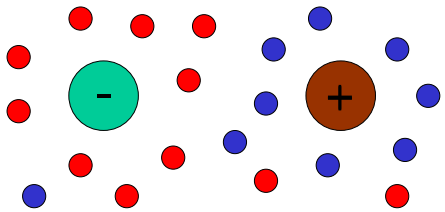
$\times N_A \equiv -276$ kJ mol^{-1}

Now add water… $\varepsilon_r = 80$

$V_{qq} = -3.5$ kJ mol^{-1} comparable to thermal energy

kT (per molecule) ≡ RT (per mole) ≈ 2.5 kJ mol^{-1}

Ionic screening...



Dissolved ions tend to cluster around fixed charges and partial cancel ("screen") electrostatic interactions.

Debye-Hückel limiting law for low ionic strengths…

$$V \approx V_{qq} \cdot \exp(-r/r_D)$$

Debye-Hückel limiting law for low ionic strengths…

$$V \approx V_{qq} \cdot \exp(-r/r_D)$$

r_D = "Debye length" = $(\varepsilon_0 \varepsilon_r RT / 2\rho F^2 I)^{1/2}$

ρ = density of water (10^3 kg m^{-3})

F = 1 Faraday (96,500 C)

I = "ionic strength" = $\frac{1}{2}\Sigma z_i m_i \equiv$ [salt] for 1:1 electrolytes

For 1:1 electrolytes, $r_D = 3.04/[\text{salt}]^{1/2}$ Å

Typical physiological ionic strength, I = 0.14

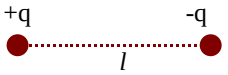
$r_D = (\varepsilon_0 \varepsilon_r RT / 2\rho F^2 I)^{1/2} = 8.2 \times 10^{-10}$ m = 8.2 Å

e.g. for previous calculation… (ions 5 Å apart)

$V = -3.5 \times \exp(-5.0/8.2) = -1.9$ kJ mol^{-1}

Electrostatic Interactions

(B) Dipole Interactions



Dipole moment:

$\mu = ql$

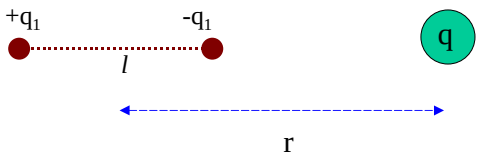
Units: 1 Debye = 1 D = 3.336×10^{-30} C m

e.g. $\pm e$, separated by 0.1 nm… μ = 4.8 D

Typical dipole moment ≈ 1 D for small molecules/groups

Interaction energies of dipoles with other charges may be calculated by summation of individual point charge interactions, or by formulas in special cases...

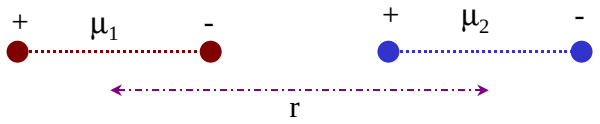
Charge-Dipole interaction



$$V_{q\mu} = - q\mu / (4\pi\varepsilon_0\varepsilon_r r^2)$$

for $r >> l$

Dipole-Dipole interaction

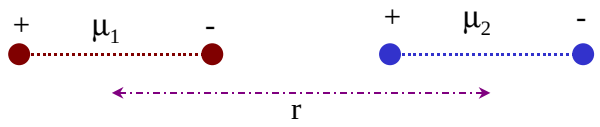


$$V_{\mu\mu} = - 2\mu_1\mu_2 / (4\pi\varepsilon_0\varepsilon_r r^3)$$

for $r >> l$

Note: sign (attractive or repulsive) depends on dipole orientations.

Dipole-Dipole interaction



$$V_{\mu\mu} = - 2\mu_1\mu_2 / (4\pi\varepsilon_0\varepsilon_r r^3)$$

Example: for two dipoles, 1 D each, 5 Å apart, in line, in vacuum…

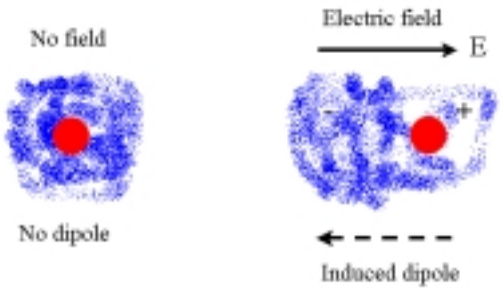
$V_{\mu\mu} = - 2(3.336 \times 10^{-30})^2 / \{4\pi . 8.85 \times 10^{-12} . (5 \times 10^{-10})r^3\}$

$= - 1.6 \times 10^{-21}$ J

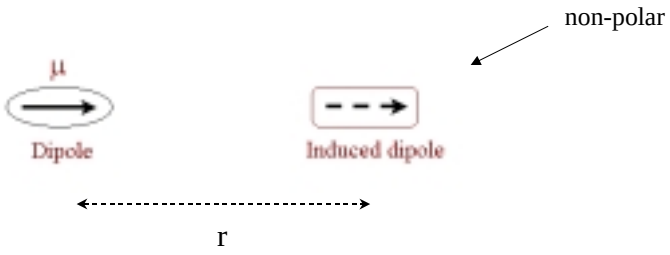
$\equiv - 0.96$ kJ mol^{-1} (x N_A)

Induced Dipole interactions

Electrostatic fields can *induce* dipole moments in polarizable molecules or materials - even when those groups are uncharged or non-polar...



Example: Dipole - Induced Dipole interaction...



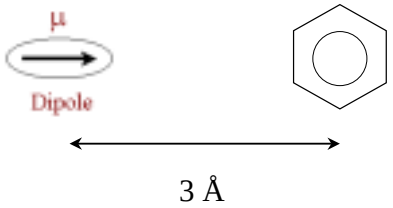
$V_{\mu I} = - C_1/r^6$ - always attractive

where $C_1 = \mu^2 \alpha / (4\pi\varepsilon_0)^2$ -- (corrected Nov 2004)

and α = polarizability of the non-polar group

Typical value…

… for a 1D dipole, 3 Å from a benzene molecule



$V_{\mu I} \approx - 0.8$ kJ mol^{-1}

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Lecture # 3

Previous lecture:-

Types of non-covalent interactions…

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- Hydrophobic
- etc...

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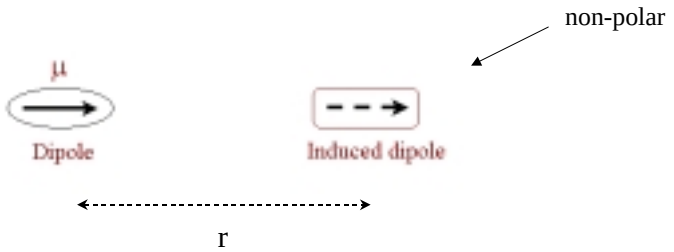
Reminder: Dipole - Induced Dipole interaction...



$V_{\mu I} = - C_1/r^6$ - always attractive

where $C_1 = \mu^2 \alpha / 4\pi\varepsilon_0$

and α = polarizability of the non-polar group

(London) Dispersion Forces

Transient dipole - Induced dipole interaction

All atoms or molecules will tend to attract each other due to a quantum mechanical effect...

Fritz London (1900-1954)

Heisenberg Uncertainty:-

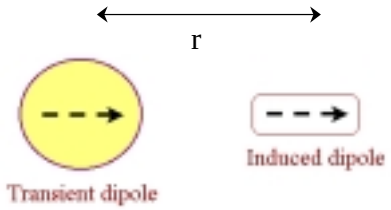
→ transient fluctuations in electron density

→ transient dipoles (even in totally non-polar groups)

→ transient induced dipoles in adjacent molecules

→ attractive "London dispersion forces"

(London) Dispersion Forces



$V_{Dispersion} = - C_2/r^6$ - always attractive

$V_{Dispersion} = - C_2/r^6$

where $C_2 = \frac{3}{2} \frac{\alpha_1\alpha_2}{(4\pi\varepsilon_0)^2} \cdot \frac{I_1I_2}{(I_1 + I_2)}$

I_1, I_2 = ionization energies of interacting groups

α_1, α_2 = polarizabilities of the interacting groups

$V_{Dispersion} = - C_2/r^6$

where $C_2 = \frac{3}{2} \frac{\alpha_1\alpha_2}{(4\pi\varepsilon_0)^2} \cdot \frac{I_1I_2}{(I_1 + I_2)}$

I_1, I_2 = ionization energies of interacting groups

α_1, α_2 = polarizabilities of the interacting groups

at high (optical) frequencies - since quantum fluctuations very rapid… hence "dispersion"

$V_{Dispersion} = - C_2/r^6$

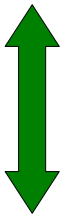
Typical value…

For 2 methane molecules, 3 Å apart

$V_{Dispersion} \approx - 5$ kJ mol^{-1}

Distance dependence of various interactions...

charge-charge	V_{qq}	$\propto$	r^{-1}
charge-dipole	$V_{q\mu}$	$\propto$	r^{-2}
dipole-dipole	$V_{\mu\mu}$	$\propto$	r^{-3}
dispersion & dipole-induced dipole	V_{Disp}	$\propto$	r^{-6}



long range

short range

Short-range repulsions...

Orbital overlap/Pauli exclusion at short range

→ very strong repulsion

→ “hard sphere” approximation

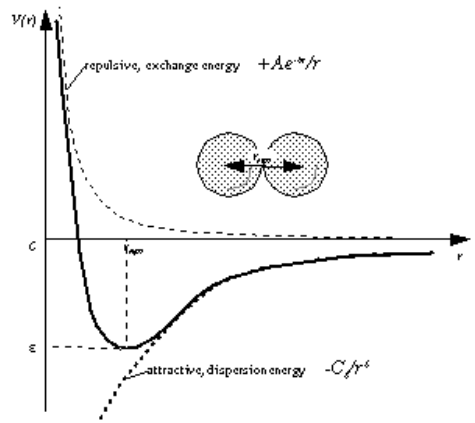
→ Van der Waals radii

“Van der Waals interaction” is the term (loosely) used to designate all these attractive/repulsive non-covalent interactions

Van der Waals/Lennard-Jones potential

$V(r) = (A/r)^{12} - (B/r)^6$

repulsive *attractive*



The Hydrogen Bond

Hydrogen "bonds" are extreme examples of dipole-dipole interactions…

δ- δ+ δ-
-A-H --- B-

where A and B are electronegative atoms such as F, N, O, and (to a lesser extent) Cl and S.

The Hydrogen Bond

Examples:

-OH --- O-	alcohols, water
-NH --- O=C-	amides, protein secondary structure
-NH --- N-	ammonia (liqu.), DNA/RNA base pairing

H-bonds may be inter- or intra-molecular

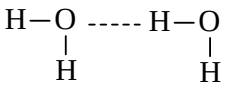
Evidence for Hydrogen Bonds

- High melting points
- High boiling points
- High ΔH of melting/boiling/sublimation…

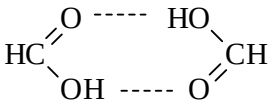
	Melting point K	*Boiling point K*	*ΔH(vap) kJ mol^{-1}*	*H-bond ?*
H_2O	273	373	40.3	Y
H_2S	190	212	18.8	N
HF	181	292	30.1	Y
HCl	161	189	16.2	N
NH_3	195	240	23.3	Y
CH_4	89	112	9.2	N

Evidence for Hydrogen Bonds

- High melting points
- High boiling points
- High ΔH of melting/boiling/sublimation…
- Gas phase dimers & higher aggregates



water vapour



methanoic (formic) acid

Evidence for Hydrogen Bonds

- High melting points
- High boiling points
- High ΔH of melting/boiling/sublimation…
- Gas phase dimers & higher aggregates
- Anomalous vibrational spectra

IR band shifts and broadening (e.g -OH)

Evidence for Hydrogen Bonds

- High melting points
- High boiling points
- High ΔH of melting/boiling/sublimation…
- Gas phase dimers & higher aggregates
- Anomalous vibrational spectra
- Crystal structures...

- atom-atom contacts closer than expected for Van der Waals radii, but longer than covalent bond lengths

How "strong" is a Hydrogen Bond ?

Measurements from gas-phase monomer-dimer equilibrium…

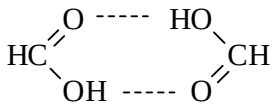
$$A\text{-}H + B \rightleftharpoons AH\text{---}B$$

$$K = [AH\text{---}B]/[AH][B]$$

$$\Delta G^o = -RT.\ln K = \Delta H^o - T.\Delta S^o$$

Example: formic (methanoic) acid dimers…

$K = 260\ atm^{-1}$ at 300K



From experiment:

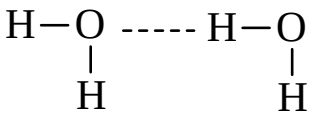
$\Delta G^o = -13.9\ kJ\ mol^{-1}$

$\Delta H^o = -59.0\ kJ\ mol^{-1}$

$\Delta S^o = -150\ J\ K^{-1}\ mol^{-1}$

} 2 H-bonds per dimer

For water dimers, in the gas phase...

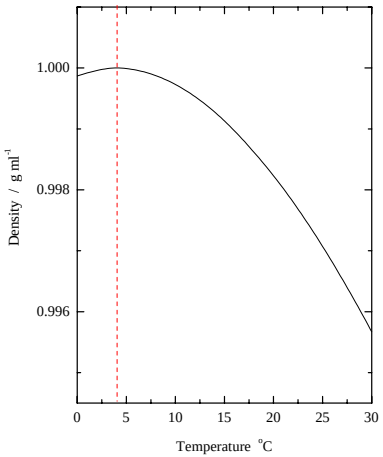


$\Delta H^o \approx -20 \text{ kJ mol}^{-1}$

Water

Anomalous properties (related to polarity/H-bonding):

- High m.p.
- High b.p.
- High heat of vaporization & sublimation - *but low heat of melting*
- Liquid more dense than solid - ice floats
- 4 °C maximum density of liquid
- High surface tension
- High heat capacity of the liquid
- …etc



Density of liquid water, showing 4 °C maximum

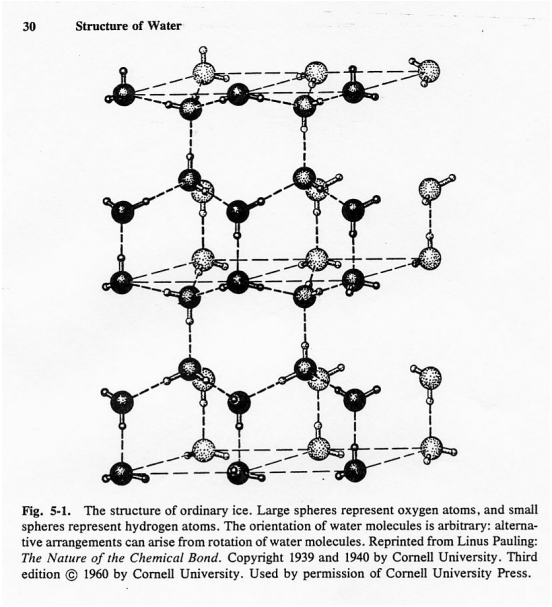

30 Structure of Water

Fig. 5-1. The structure of ordinary ice. Large spheres represent oxygen atoms, and small spheres represent hydrogen atoms. The orientation of water molecules is arbitrary: alternative arrangements can arise from rotation of water molecules. Reprinted from Linus Pauling: *The Nature of the Chemical Bond*. Copyright 1939 and 1940 by Cornell University. Third edition © 1960 by Cornell University. Used by permission of Cornell University Press.

University of Glasgow
Department of Chemistry

CMC/Chem-3

Biophysical Chemistry

Biomolecular Interactions

Professor Alan Cooper

Rm. B4-20 (Joseph Black Building)

Lecture # 4

Types of non-covalent interactions…

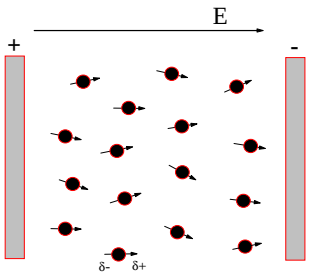
- Electrostatic
- Van der Waals
- Dispersion
- Hydrogen bonding
- Hydrophobic
- etc...

Effects of Water on Biomolecular Interactions

(1) Electrostatic interactions

High dielectric constant of water (ε ≈ 80)

Reduces interaction potentials coming from static charges

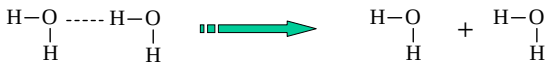


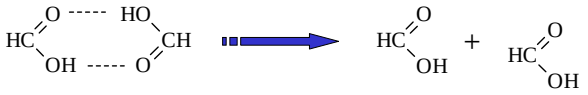
Note: This has much less an effect on high-frequency dispersion forces, since the water dipoles do not have sufficient time to re-orient during the lifetime of the quantum fluctuations

Effects of Water on Biomolecular Interactions

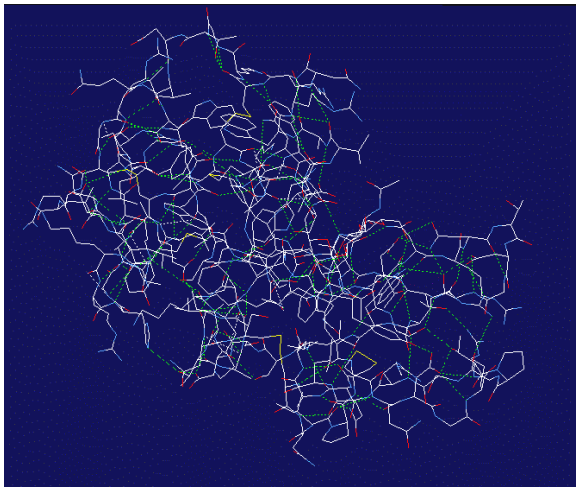
(2) Hydrogen bonding thermodynamics

Hydrogen bonds *in vacuum* are relatively strong…

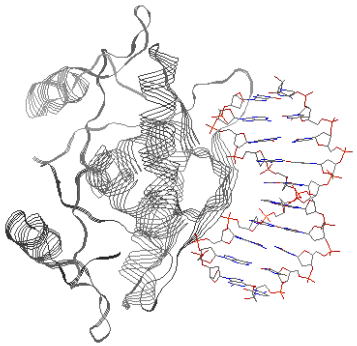




Biomolecular secondary & tertiary structures contain lots of H-bonds...



Lysozyme structure showing H-bonds



protein - DNA complex

α-helical secondary structure in proteins

CMC-3 Biomolecular Interactions

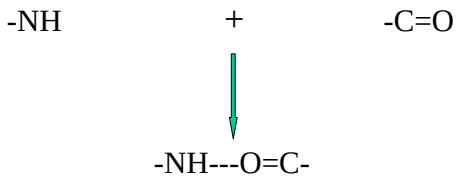
A paradox…?

Polar, hydrogen-bonding groups tend to be very soluble in water

Water molecules can form strong H-bonds with other polar groups (solvation/hydration)…

… so how come H-bonds in proteins and DNA (etc.) ???

e.g. H-bond formation during protein folding ?



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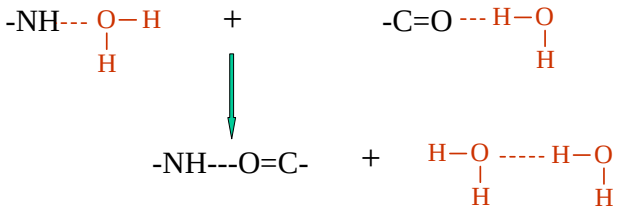
In water ?



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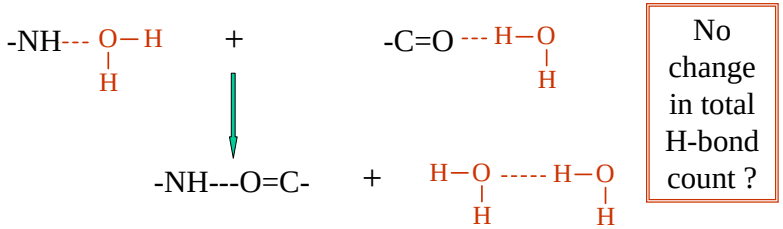


No change in total H-bond count ?

Thermodynamics of hydrogen bonding interactions in water

Experiments involving model compounds that mimic processes in larger biomolecules

Ref: Klotz & Franzen (1962) J.Am.Chem.Soc. 84, 3461.

Klotz & Farnham (1968) Biochemistry 7, 3879.

Eberhardt & Raines (1994) J.Am.Chem.Soc. 116, 2149.

Cooper (2000) Biophys. Chem. 85, 25.

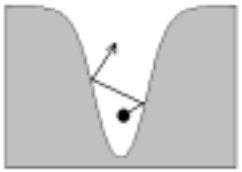
A bluffer's guide to Thermodynamic Equilibrium…

There is a natural tendency for all things (even atoms & molecules) to roll downhill - to fall to lower energy.

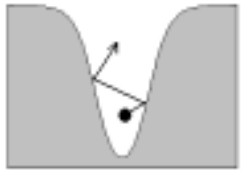
ΔH tends to want to be negative

This is opposed (at the molecular level) by the equally natural tendency for thermal/Brownian motion (otherwise known as "entropy") to make things go the other way…

…and this effect gets bigger as the temperature increases.



T.ΔS tends to want to be positive



Thermodynamic Equilibrium, expressed in terms of the Gibbs Free Energy change, reflects just the balance between these opposing tendencies…

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

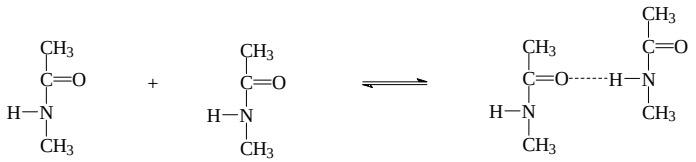
Equilibrium is reached when these two forces just balance ($\Delta G = 0$).

The standard free energy change, $\Delta G°$, is just another way of expressing the equilibrium constant, or affinity (K) for any process, on a logarithmic scale…

$$\Delta G° = -RT.\ln K$$

Association (dimerization) of N-methylacetamide (NMA) in solution:-

(Ref: Klotz & Franzen (1962) J.Amer.Chem.Soc. 84, 3461-3466.)



IR:- (NH stretch) Low conc. 6800 cm^{-1} ---> High conc. 6450 cm^{-1}

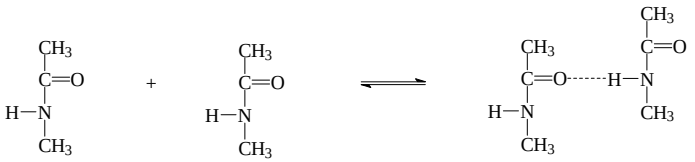
$K = [NMA_2]/[NMA]^2$

$\Delta G° = -RT.\ln(K) = \Delta H° - T.\Delta S°$

$d(\ln.K)/d(1/T) = -\Delta H°/R$ (van't Hoff equation)

Association (dimerization) of N-methylacetamide (NMA) in solution:-

(Ref: Klotz & Franzen (1962) J.Amer.Chem.Soc. 84, 3461-3466.)



Results (for 25 °C)

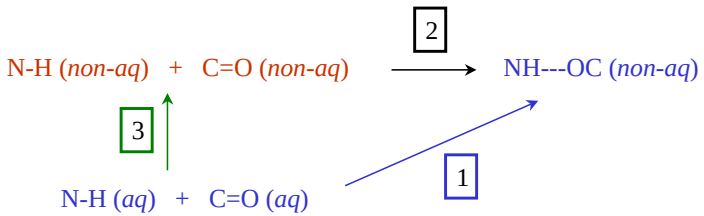
Solvent	K /M^{-1}	$\Delta G°$ /kJ mol^{-1}	$\Delta H°$ /kJ mol^{-1}	$\Delta S°$ /J K^{-1} mol^{-1}
CCl_4	4.7	- 3.8	-17.6	- 46
Dioxane	0.52	+ 1.6	- 3.3	- 16.5
Water	0.005	+ 13.1	0	- 44

Conclusion: Peptide (amide) H-bonding is thermodynamically favourable in non-polar solvents, but not in water.

Better model ?

N-H (*aq*) + C=O (*aq*) $\xrightarrow{1}$ NH---OC (*non-aq*)

Thermodynamic cycle:-

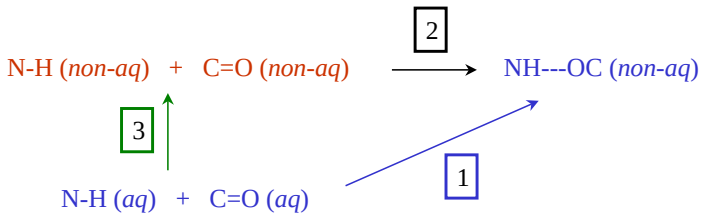


1 = 3 + 2

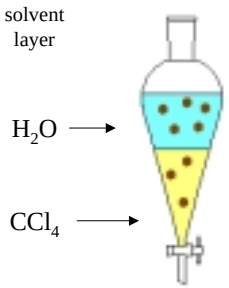
from partitioning of NMA between *aq* and *non-aq* solvents

from NMA in CCl_4

Thermodynamic cycle:-



Thermodynamics of transfer: partitioning experiments...



At equilibrium:-

$$D = \frac{[NMA]_{CCl4}}{[NMA]_{H2O}}$$

$= 1.1 \times 10^{-3}$ (from expt.)

$\Delta G°(3) = -RT.\ln(D) = +16.9$ kJ mol^{-1}

---

[1] = [3] + [2]

[3]: from partitioning of NMA between *aq* and *non-aq* solvents

[2]: from NMA in CCl_4

Thermodynamic cycle:-

N-H (*non-aq*) + C=O (*non-aq*) —[2]→ NH---OC (*non-aq*)

N-H (*non-aq*) —[3]→ N-H (*aq*)

N-H (*aq*) + C=O (*aq*) —[1]→ NH---OC (*non-aq*)

$\Delta G°$ [1] = [16.9] + [-3.8] = + 13.1 kJ mol^{-1}

[16.9]: from partitioning of NMA between *aq* and *non-aq* solvents

[-3.8]: from NMA in CCl_4

Thermodynamic cycle:-

N-H (*non-aq*) + C=O (*non-aq*) —[2]→ NH---OC (*non-aq*)

N-H (*non-aq*) —[3]→ N-H (*aq*)

N-H (*aq*) + C=O (*aq*) —[1]→ NH---OC (*non-aq*)

<u>Conclusion</u>: Even taking into account the transfer to non-aqueous environment, peptide-peptide H-bonding is still appears thermodynamically unfavourable in the presence of water.

From model compound experiments:-

N-H (*aq*) + C=O (*aq*) ⟶ NH---OC (*non-aq*)

$\Delta G°$ = + 13.1 kJ mol^{-1}

$\Delta H°$, $\Delta S°$ } both positive *(data not shown)*

release of bound water ?

?? Maybe the model compounds are wrong (or inappropriate models)

?? Maybe there are other interactions more important

University of Glasgow
Department of Chemistry

CMC/Chem-3

Biophysical Chemistry

Biomolecular Interactions

Professor Alan Cooper

Rm. B4-20 (Joseph Black Building)

Lecture # 5

Previous lecture...

Conclusion: Even taking into account the transfer to non-aqueous environment, peptide-peptide H-bonding is still appears thermodynamically unfavourable in the presence of water.

From model compound experiments:-

N-H (*aq*) + C=O (*aq*) ⟶ NH---OC (*non-aq*)

$\Delta G^{o} = +13.1$ kJ mol^{-1}

?? Maybe the model compounds are wrong (or inappropriate models)

?? Maybe there are other interactions more important

Types of non-covalent interactions…

- Electrostatic
- Van der Waals
- Dispersion
- Hydrogen bonding
- Hydrophobic
- etc...

Effects of Water on Biomolecular Interactions

(1) Hydrophobic Interactions

References: Cantor & Schimell I pp. 279-288

Tanford (1980) "The Hydrophobic Effect"

Kauzmann (1959) Adv. Protein Chem. 14, 1.

Oil and water don't mix.. Why not ?

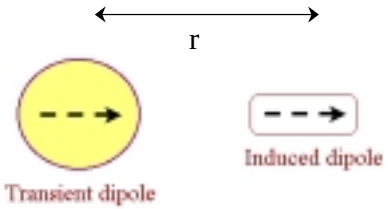
Oil and water don't mix.. Why not ?

Non-polar molecules & groups are insoluble in water…

Water does not wet non-polar surfaces (Teflon®, Gortex®, etc.)

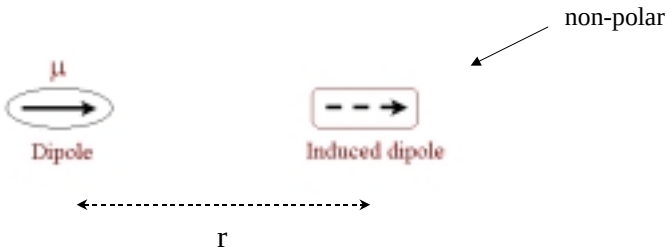
Water appears to repel non-polar molecules (and *vice versa*)

(London) Dispersion Forces



$V_{Dispersion} = - C_2/r^6$ - always attractive

Reminder: Dipole - Induced Dipole interaction...



$V_{\mu I} = - C_1/r^6$ - always attractive

So… water should attract non-polar groups (and does, in isolation). → But...

Because of polarity and strong H-bonding, water molecules have a greater affinity for each other than for other (non-polar) groups.

So, acting collectively in the bulk liquid, they will tend to bind preferentially to each other - to the exclusion of others: apparently repelling non-polar groups.

This is called the "Hydrophobic Effect"

Because of polarity and strong H-bonding, water molecules have a greater affinity for each other than for other (non-polar) groups.

So, acting collectively in the bulk liquid, they will tend to bind preferentially to each other - to the exclusion of others: apparently repelling non-polar groups.

This is called the "Hydrophobic Effect"

How can we measure this ?

Since protein folding, ligand binding, etc., (usually) involves burial of groups in a non-polar environment..

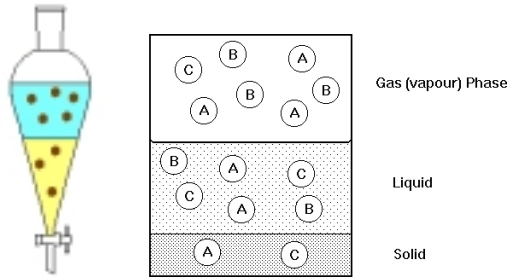
What is the difference in free energy (ΔG^o) and other thermodynamic parameters (ΔH^o, ΔS^o , etc.) between groups in an aqueous and non-polar environment ?

Partitioning of model compounds between solvents

Reminder from Chem-2X (Molecular Thermodynamics)

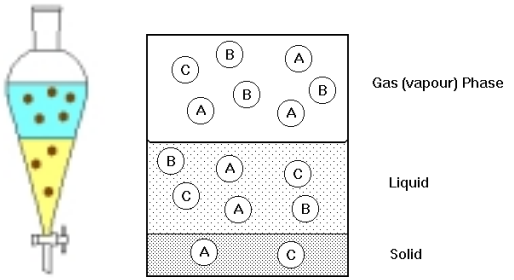
-- read the "Hitch-hiker's Guide"

What is the situation at equilibrium ?



Molecules (A say) will tend to move from regions (phases) of high chemical potential to lower μ (low probability to high), until an equilibrium situation is reached in which the chemical potential of A is the same in all phases...

What is the situation at equilibrium ?



The chemical potential of a particular molecule is the same in all phases...

i.e. at equilibrium

μ_A(in phase 1) = μ_A(in phase 2)

μ_B(in phase 1) = μ_B(in phase 2)

.. ..

... and so on.

(Important note: This does not mean that $\mu_A = \mu_B$, etc...)

Chemical potentials vary with concentration, etc…

In general: $\mu_i = \mu^o_i + RT.\ln a_i$

where μ_i is the actual chemical potential of species i

μ^o_i is the chemical potential of i under standard conditions

$a_i = \gamma_i[i]$ = activity of i

and the term $RT.\ln a_i$ takes account of the effects of concentration and intermolecular forces.

Rules for specific circumstances →

(a) For solutions:-

Chemical potential of component A in solution =

$$\mu_A = \mu^o_A + RT.\ln \gamma_A[A]$$

$$\approx \mu^o_A + RT.\ln [A]$$

if interaction effects can be ignored (i.e. if $\gamma_A \approx 1$). This is often the case in dilute solutions.

μ^o_A = chemical potential of A when [A] = 1 M

So the "standard state" for solutions is a concentration of 1 M.

Reminder: "activity" $a = \gamma_A[A]$ = concentration, corrected for intermolecular interactions

(b) For liquid mixtures:-

Composition of liquid mixtures is usually expressed in mole fractions (x_i)

$$\mu_i = \mu^o_i + RT.\ln \gamma_i x_i$$

Standard state is the pure liquid, for which $x_i = 1$ and $\gamma_i = 1$

(c) For gases:-

Composition of gas mixtures is usually expressed in terms of the partial pressures (Pi , in atm), and we can usually ignore activity corrections since the molecules are too far apart to interact (ideal gas assumption, $\gamma = 1$).

$$\mu_i = \mu^o_i + RT.\ln P_i$$

Standard state is when $P_i = 1$ atm.

Liquid-Liquid Equilibrium

← liquid 1

← liquid 2

Imagine two immiscible liquids (e.g. water & cyclohexane) in which test compound A is dissolved

What is the situation at equilibrium ? What is the concentration of *i* in each phase (layer) ?

At equilibrium, for any component A:

$$\mu_A(\text{liquid 1}) = \mu_A(\text{liquid 2})$$

$$\mu_A(\text{liquid 1}) = \mu_A(\text{liquid 2})$$

For A in liquid 1: $\mu_A(\text{liquid 1}) = \mu^o_A(\text{liquid 1}) + RT.\ln a_A(\text{liquid 1})$

For A in liquid 2: $\mu_A(\text{liquid 2}) = \mu^o_A(\text{liquid 2}) + RT.\ln a_A(\text{liquid 2})$

So, overall:

$$\mu^o_A(\text{liquid 1}) + RT.\ln a_A(\text{liquid 1}) = \mu^o_A(\text{liquid 2}) + RT.\ln a_A(\text{liquid 2})$$

Re-arrange:

$$\ln [a_A(\text{liquid 2})/ a_A(\text{liquid 1})] = -\{\mu^o_A(\text{liquid 2}) - \mu^o_A(\text{liquid 1})\}/RT$$
$$= -\Delta G^\circ_{transfer}(1 \rightarrow 2)/RT$$

where $\Delta G^\circ_{transfer}(1 \rightarrow 2)$ = standard free energy of transfer
$= \mu^o_A(\text{liquid 2}) - \mu^o_A(\text{liquid 1})$

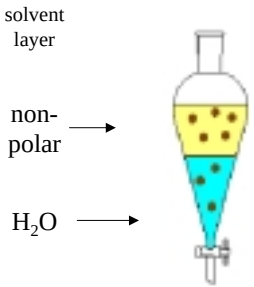
$$\Delta G^\circ_{transfer}(1 \rightarrow 2) = -RT.\ln [a_A(\text{liquid 2})/ a_A(\text{liquid 1})]$$

For <u>dilute</u> solutions: $a_A \cong [A]$

$$\Delta G^\circ_{transfer}(1 \rightarrow 2) \cong -RT.\ln D$$

where $D = [A]_2 / [A]_1$
$=$ "distribution" or "partition" coefficient

Thermodynamics of transfer: partitioning experiments...



At equilibrium:-

$$D = \frac{[A]_{non\text{-}polar}}{[A]_{H2O}}$$

where [A] = concentration or <u>activity</u> (more accurate) of model compound, A, in appropriate layer

$$\Delta G^\circ_{transfer}(H_2O \rightarrow \text{non-polar}) = -RT.\ln(D)$$

Thermodynamics from Solubility

$$\Delta G^\circ_{transfer}(1 \rightarrow 2) = -RT.\ln \{a_A(\text{liquid 2})/ a_A(\text{liquid 1})\}$$

<u>Special case</u>:

If the test molecule, A, is the liquid itself (e.g. liquid 2) - partially soluble in water (liquid 1)

$a_A(\text{liquid 2})$ = activity of pure liquid 2 (pure A)
$= 1$ (by definition)

$$\Delta G^\circ_{transfer}(1 \rightarrow 2) = -RT.\ln \{1/ a_A(\text{liquid 1})\}$$
$$\cong -RT.\ln\{1/[A]_1\}$$
$$= RT.\ln [A]_1$$

$[A]_1$ = solubility of A in solvent 1 (water)

$\therefore$ measure solubility to give $\Delta G^\circ_{transfer}$

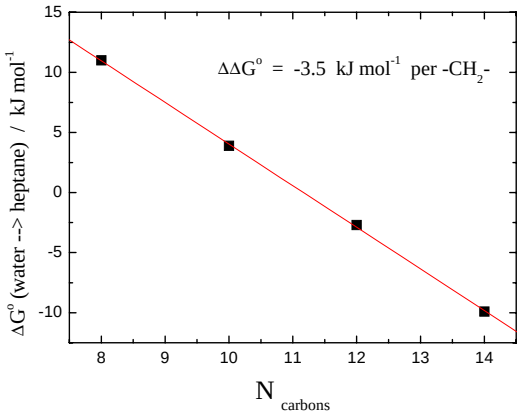
temperature dependence $\rightarrow \Delta H^\circ_{transfer}$ and $\Delta S^\circ_{transfer}$

Example: Partitioning of fatty acids between heptane and water (pH 7)

JACS 80, 3817 (1958)

Fatty acid	*N carbons*	*D*	*ΔG^o (water → heptane) / kJ mol^{-1}*
Octanoic	8	0.012	11.0
Decanoic	10	0.21	3.9
Lauric	12	3.0	-2.7
Myristic	14	54	-9.9

Example: Partitioning of fatty acids between heptane and water (pH 7)



- “linear free energy” / “group additivity” effects

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Biophysical Chemistry

Biomolecular Interactions

Professor Alan Cooper

Rm. B4-20 (Joseph Black Building)

Lecture # 6

Types of non-covalent interactions…

- Electrostatic
- Van der Waals
- Dispersion
- Hydrogen bonding
- Hydrophobic
- etc...

This lecture…

(a) Effect of temperature on hydrophobic interactions

(b) Biomolecular interactions in real systems

→ The “Protein Folding Problem”

Effect of temperature on hydrophobic interactions

Anomalous solubility of non-polar groups in water

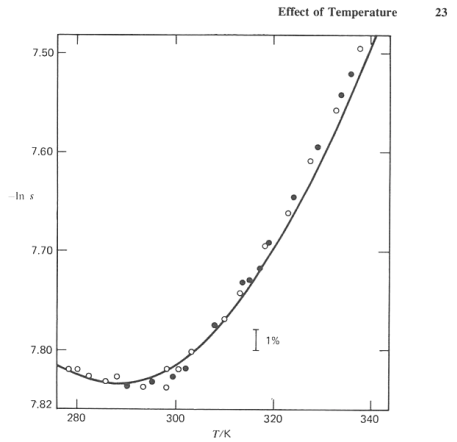


Fig. 4-1. The effect of temperature on the solubility of benzene in water, from Gill et al. (1976). Experimental data are from Arnold et al. (1958) and Franks et al. (1963), and the line is based on the thermodynamic paramaters for benzene in Table 4-1, assuming that the heat capacity change is independent of temperature. The small deviation of the experimental points from the curve at high temperature might reflect a slight temperature dependence of the heat capacity change. Reproduced with permission from the *Journal of Chemical Thermodynamics*. Copyright by Academic Press Inc. (London) Ltd.

Thermodynamics from Solubility

$\Delta G°_{transfer}(1 \rightarrow 2) = -RT.\ln \{a_A(\text{liquid 2})/ a_A(\text{liquid 1})\}$

Special case:

If the test molecule, A, is the liquid itself (e.g. liquid 2) - partially soluble in water (liquid 1)

$a_A(\text{liquid 2})$ = activity of pure liquid 2 (pure A)

= 1 (by definition)

$\Delta G°_{transfer}(1 \rightarrow 2) = -RT.\ln \{1/ a_A(\text{liquid 1})\}$

$\cong -RT.\ln\{1/[A]_1\}$

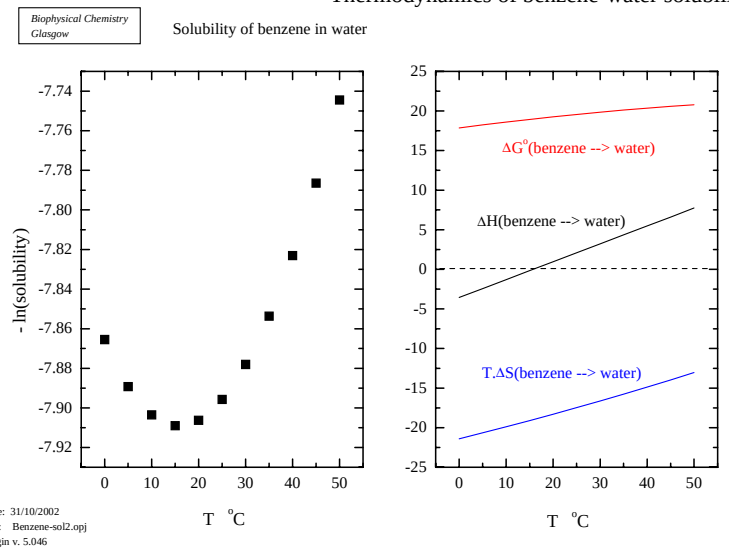
$= RT.\ln [A]_1$

$[A]_1$ = solubility of A in solvent 1 (water)

$\therefore$ measure solubility to give $\Delta G°_{transfer}$

temperature dependence $\rightarrow \Delta H°_{transfer}$ and $\Delta S°_{transfer}$

Thermodynamics of benzene-water solubility

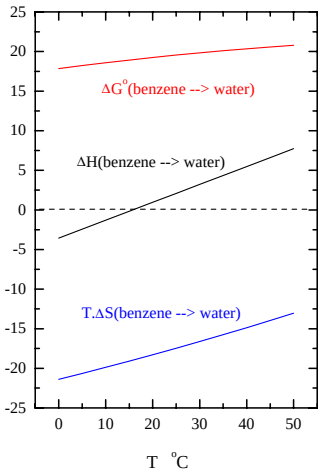


Thermodynamics of benzene-water solubility

$\Delta G°_{transfer}$(benzene → water) is positive (unfavourable)

$\Delta H°_{transfer}$(benzene → water) is exothermic at low temperatures, endothermic at high temperatures

$\Delta S°_{transfer}$(benzene → water) is negative (unfavourable), and varies with temperature



Thermodynamics of hydrophobic interactions…

Conclusions from model compound transfer experiments:

$\Delta H°_{transfer}$ and $\Delta S°_{transfer}$ depend on temperature

For breaking (pulling apart) hydrophobic interactions, $\Delta G°$ is positive, and both $\Delta H°$ and $\Delta S°$ increase with temperature

Heat capacity change

$\Delta C_p°_{transfer} = \partial(\Delta H°_{transfer})/\partial T = T.\partial(\Delta S°_{transfer})/\partial T$

is positive

Heat capacity

Both enthalpy and entropy are fundamentally related to the *heat capacity* (or *specific heat*) of an object.

$$\Delta H(T) = \Delta H(T_{ref}) + \int_{Tref}^{T} \Delta C_p \, .dT$$

and

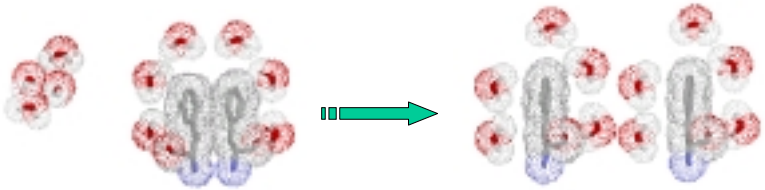
$$\Delta S(T) = \Delta S(T_{ref}) + \int_{Tref}^{T} (\Delta C_p / T).dT$$

where ΔC_p is the heat capacity change at constant pressure, which is related to the temperature dependence of both entropy and enthalpy:

$$\Delta C_p = \partial \Delta H / \partial T = T. \partial \Delta S / \partial T$$

Heat capacity is the quantity which tells us how much heat energy (H) we need to add to a system in order to increase its temperature. It is also related to entropy (S) because, if *w* is high, there are lots of different ways in which the added energy might be distributed without raising the temperature, and the heat capacity is consequently also high.

Interpretation in terms of water clustering...



- formation of H-bonded water clusters around non-polar groups can explain (in part) the thermodynamics

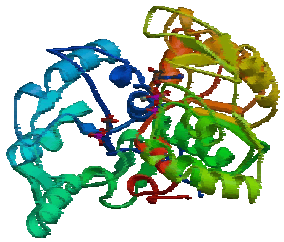
This lecture…

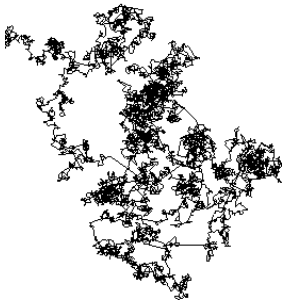
(a) Effect of temperature on hydrophobic interactions

(b) Biomolecular interactions in real systems

→ The “Protein Folding Problem”

So what’s the problem… ?





Biopolymers fold spontaneously to form specific structures

…synthetic polymers don’t

The structure is determined by the sequence
→ Anfinsen “thermodynamic hypothesis”

Christian B. Anfinsen

Nobel Prize in Chemistry 1972

"for his work on ribonuclease, especially concerning the connection between the amino acid sequence and the biologically active conformation"

Proteins are polymers made up of specific sequences of L-amino acids linked together by covalent peptide (amide) bonds (Fig.1). Amino acids are chosen from a basic set of 20 building blocks differing in sidechain (Fig.2), with occasional special-purpose side chains made to order (e.g. hydroxyproline).

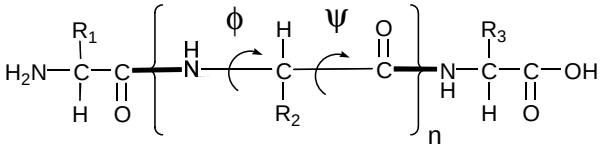


Figure 1: Polypeptide structure showing rotatable ϕ/ψ angles. The planar peptide (amide) bonds are shown in bold, and are usually *trans*.

Primary structure: the sequence of amino acids in the polypeptide chain. This is unique to each protein, and is determined (primarily) by the genetic information encoded in the DNA of the relevant gene.

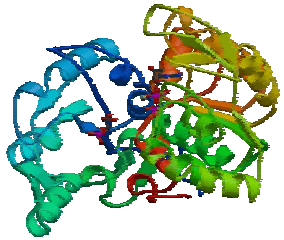
```
KVFERCELAR TLKRLGMDGY RGISLANWMC LAKWESGYNT
RATNYNAGDR STDYGIFQIN SRYWCNDGKT PGAVNACHCS
ASALLQDNIA DAVACAKRVV RDPQGIRAWV AWRNRCQNRD
VRQYVQGCGV
```

Primary structure of a 130-residue protein (human lysozyme) shown using the single-letter amino acid codes

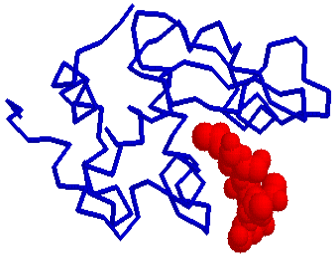
Secondary structure: regular, repeating structures such as α-helix, β-sheets, etc.

Tertiary structure: the three-dimensional arrangement of secondary structure elements that defines the overall conformation of the (globular) protein.

Examples of protein tertiary structure...



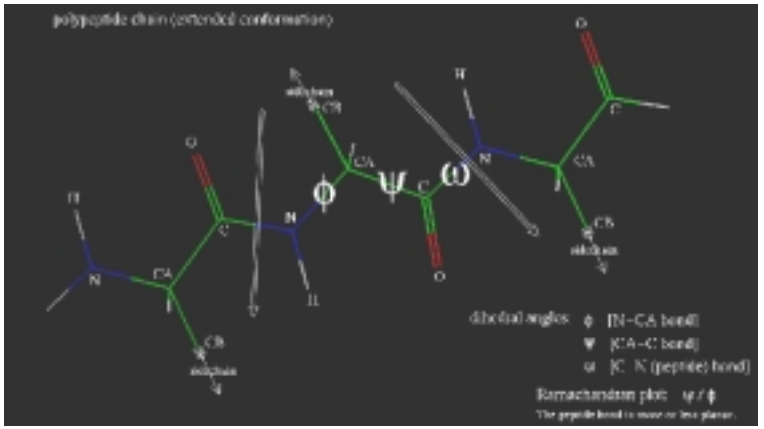
Phosphoglycerate kinase

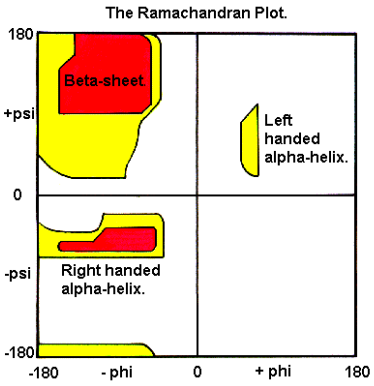


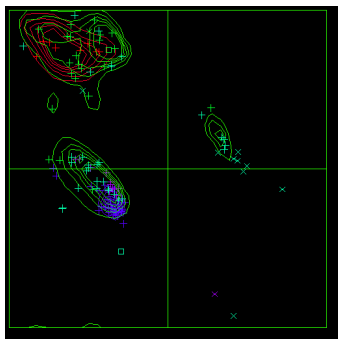
Lysozyme (with bound inhibitor)

Problem 1: How does a protein "find" the correct conformer ?

(Levinthal paradox)







- stereochemical constraints limit the number of Φ-Ψ angles available, but there are still many different possibilities

The Levinthal "paradox":

C. Levinthal, J. Chim. Phys. 65 (1968) 44; D.B. Wetlaufer, Proc. Natl. Acad. Sci. USA 70 (1973) 691.

Each ϕ or ψ angle in a peptide might have roughly three possible values, giving $3 \times 3 = 9$ possible conformers for each peptide (not counting side chain conformers).

How many possible conformers for a protein (polypeptide) of n residues ?

For n peptide units, no. of possible conformers = 9^n

Even for a small protein (n = 100), this is an enormous number of possible conformers…

$$9^{100} = 2.6 \times 10^{95}$$

… only one of which is "correct"

Problem 1: How does a protein "find" the correct conformer ?

(Levinthal paradox)

How long might it take to explore all possible conformations ?

Assuming (optimistically) that peptide conformations can switch on the femtosecond time scale (10^{-15} sec) -

Time taken = no. of conformers x time per conformer

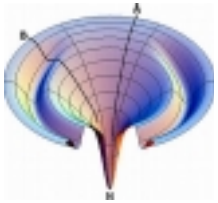
= $2.6 \times 10^{95} \times 10^{-15}$ sec (for n = 100)

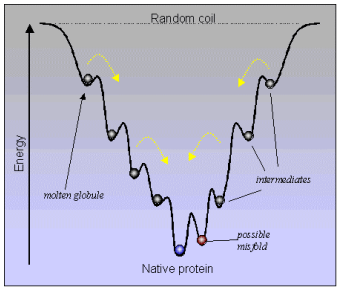
= 2.6×10^{80} sec

≈ 10^{73} years (1 year ≈ 3×10^{7} sec)

→ kinetic pathways for folding

- just like every other physical/chemical process...





- the "folding funnel"

Problem 2: Why does a protein fold ?

- what are the thermodynamic driving forces ?

Random coil conformations…

- much more probable than specific folded structure
- higher entropy
- intrinsically lower free energy

What is ΔS for folding ?

What is Entropy ?

The absolute entropy of any object is given by:

$S = k.\ln(w)$ per object (molecule)

or $S = R.\ln(w)$ *per mole* of objects

Ludwig Boltzmann (1844-1906)

For a polypeptide in the "random coil" state (in which all Φ,Ψ angles are possible)…

$$w \approx 9^n$$

$$S_{conf}(\text{random coil}) = R.\ln(w)$$

$$\approx nR.\ln(9) \approx 18n \text{ J K}^{-1} \text{ mol}^{-1}$$

$$\approx 1800 \text{ J K}^{-1} \text{ mol}^{-1} \text{ for n = 100-residue protein}$$

For "native" conformation…

$$w \approx 1$$

$$S_{conf}(\text{native}) = R.\ln(1) = 0$$

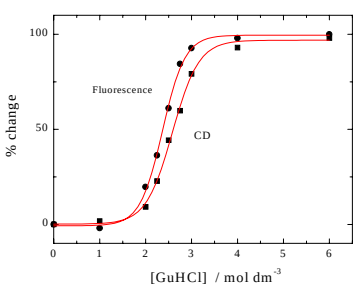
$$\Delta S_{conf}(\text{random coil} \rightarrow \text{native}) \approx -18n \text{ J K}^{-1} \text{ mol}^{-1}$$

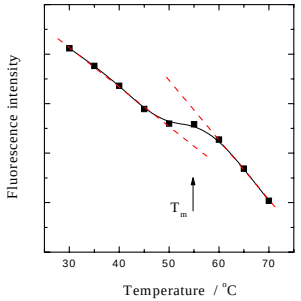
$$\Delta G^{o}_{conf}(\text{random coil} \rightarrow \text{native}) = -T.\Delta S_{conf}$$

$$\approx +540 \text{ kJ mol}^{-1} \text{ for 100-residue protein}$$

- thermodynamically unfavourable free energy change
- this must be overcome by more favourable interactions from other sources

How can we measure these forces in real proteins ?

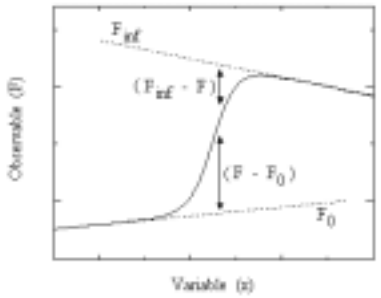




- most proteins are marginally stable, and can be unfolded by relatively small perturbations
- transitions can be followed indirectly through changes in spectroscopic and other properties

For a 2-state unfolding transition:

$$N \rightleftharpoons U \quad ; \quad K = [U]/[N]$$



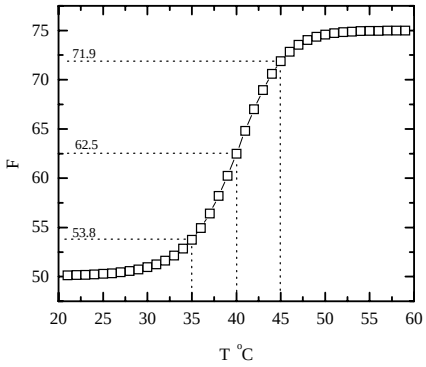
$$\Delta G°_{unf} = \Delta H°_{unf} - T.\Delta S°_{unf}$$

$$= -RT.\ln K$$

$$K = (F - F_0)/(F_{inf} - F)$$

Numerical example...

$F_0 = 50$



$F_{inf} = 75$

t / C	F	K	ΔG° / kJ mol^{-1}	ΔH°	ΔS° /kJ mol^{-1} K^{-1}
35	53.8	0.18	+4.4	255	0.81
40	62.5	1	0	300	0.96
45	71.9	7.1	-5.2	345	1.10

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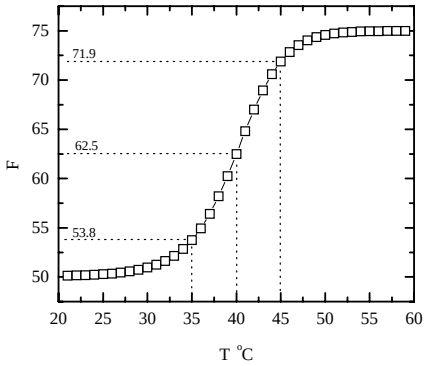
Lectures # 7 & 8

Numerical example...



F_{inf} = 75

F_0 = 50

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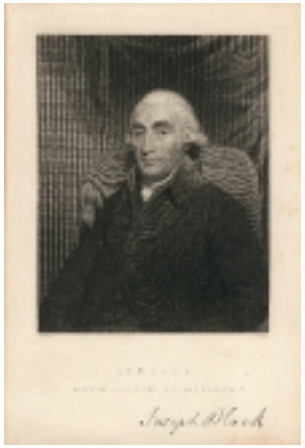
45	71.9	7.1	-5.2	345	1.10

Lectures # 7 & 8

- Microcalorimeter methods
- Protein unfolding thermodynamics
- Ligand binding
- Other experimental methods

Calorimetry



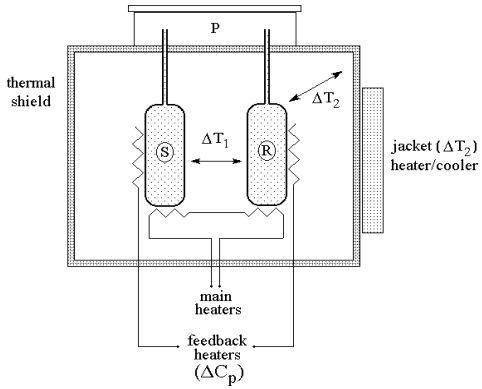
Calorimetry began with Joseph Black in Glasgow 250 years ago...

"He waited with impatience for the winter" in Glasgow so that he could do experiments on the freezing and melting of water and water/alcohol mixtures.

This led to the concept of latent heat and the earliest studies of the heat capacity of hydrogen-bonded networks.

Simpson, A.D.C. (1982) "Joseph Black 1728-1799: a commemorative symposium" (Royal Scottish Museum, Edinburgh, 1982)

Differential scanning calorimeter



Sample volume: 0.5 ml Temperature range: 10 - 120 °C

Sample concentration: 0.2 - 2 mg/ml

Worked Problem:

Q: What might be the temperature difference between sample and identical buffer reference solutions for a sample comprising 1 mg cm^{-3} of a protein of RMM 50000 undergoing a thermal transition with ΔH = 80 kJ mol^{-1} ?

A: 1 mg ≡ 1 x 10^{-3} / 50,000 = 2 x 10^{-8} moles of protein

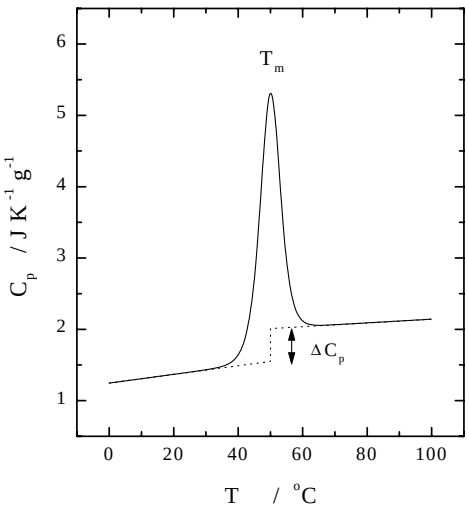
x ΔH ≡ 1.6 x 10^{-3} J heat energy uptake per mg of protein.

The specific heat capacity of water (assume identical for buffer and protein solution) = 4.2 J K^{-1} mol^{-1}

Assuming that all this heat energy is absorbed by the 1 cm^3 sample
ΔT_1 = 1.6 x 10^{-3} / 4.2 = 3.8 x 10^{-4} °C

(In practice, thermal transitions in biomolecules do not occur all at once, but take place over a finite temperature range. This means that temperature changes observed by DSC are usually very much smaller than this.)

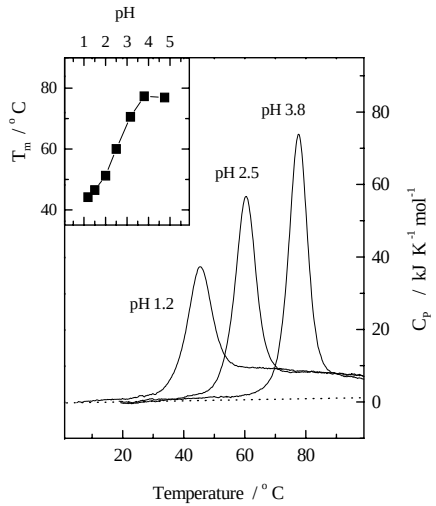
Protein (un)folding...



Typical data for the heat capacity increment (ΔC_p) observed upon thermal unfolding of a globular protein in aqueous solution

A.Cooper (1999). *Thermodynamics of protein folding and stability*. "Protein: A Comprehensive Treatise", Volume 2, pp. 217-270. (Editor: Geoffrey Allen. JAI Press Inc., Stamford CT, 1999)

Typical DSC data for the unfolding of a small globular protein (lysozyme) in solution at various pH values. The insert shows the variation in mid-point unfolding temperature (T_m) as a function of pH. The increase in area under each endotherm with higher T_m, and the higher heat capacity baselines after the unfolding transitions, are both indications of the significant positive ΔC_p commonly associated with such processes.



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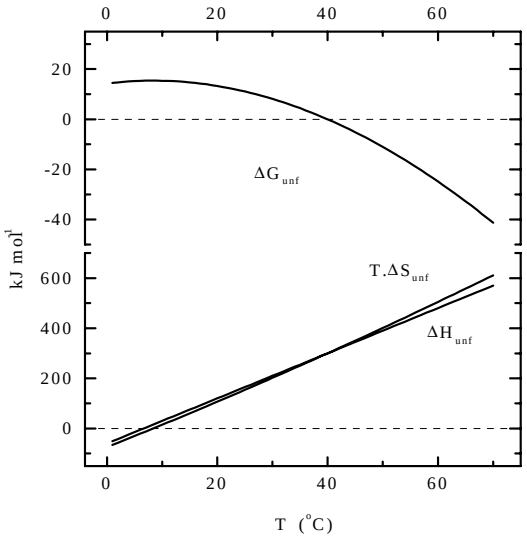
From: Cooper, Lakey, Johnson & Nollman (2001) - *Biophysical Chemistry* 93, 215-230.

Typical protein unfolding thermodynamics...

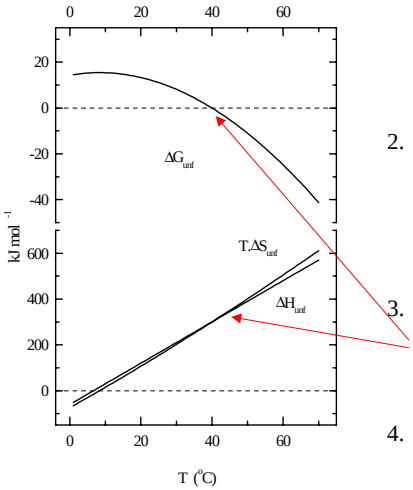


ΔH and T.ΔS compensate to give small changes in ΔG

Large variations in ΔH and ΔS

Positive ΔC_p

Traditionally characteristic of exposure of hydrophobic groups (Kauzmann)



1. Folded proteins are only marginally stable

   ΔG_{unf} << RT per residue

   ∴ needs cooperativity for stability

2. $\Delta G_{unf} = \Delta H_{unf} - T.\Delta S_{unf}$

   = small difference between large numbers

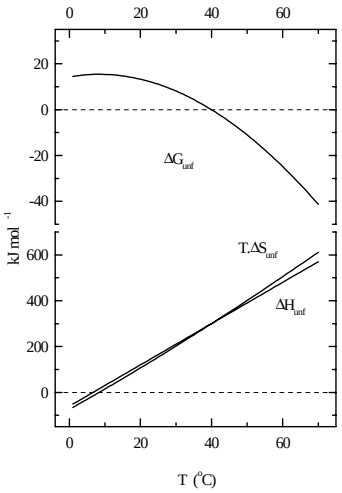
   ∴ very difficult to predict theoretically

3. Mid-point for thermal unfolding…

   $\Delta G_{unf} = 0$; $T_m = \Delta H_{unf} / \Delta S_{unf}$

4. Extrapolation suggests "cold denaturation"

   - unfolding at <u>low</u> temperatures (as well as high)



What does this suggest about the forces that stabilise the folded protein ?

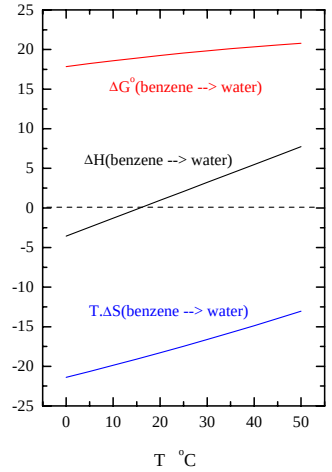
- compare to model compound data for hydrophobic interactions...

Thermodynamics of benzene-water solubility

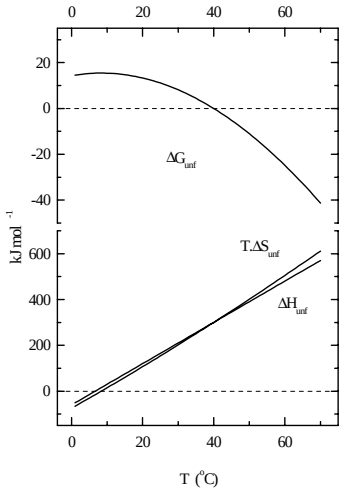
$\Delta G°_{transfer}$(benzene → water) is positive (unfavourable)

$\Delta H°_{transfer}$(benzene → water) is exothermic at low temperatures, endothermic at high temperatures

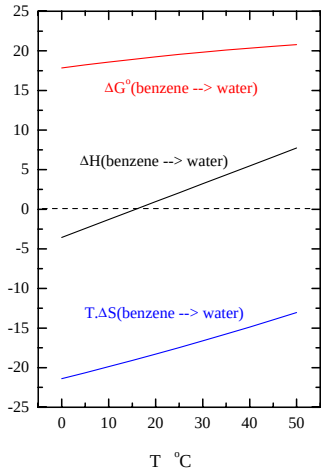
$\Delta S°_{transfer}$(benzene → water) is negative (unfavourable), and varies with temperature



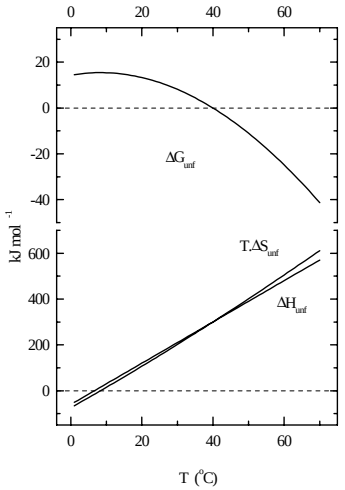
Protein unfolding



Transfer of non-polar groups to water



- suggests that hydrophobic interactions are (mainly) responsible for protein folding stability



What does this suggest about the forces that stabilise the folded protein ?

- compare to model compound data for hydrophobic interactions...

- suggests that hydrophobic interactions are (mainly) responsible for protein folding stability

Textbook version

Is there an alternative ?

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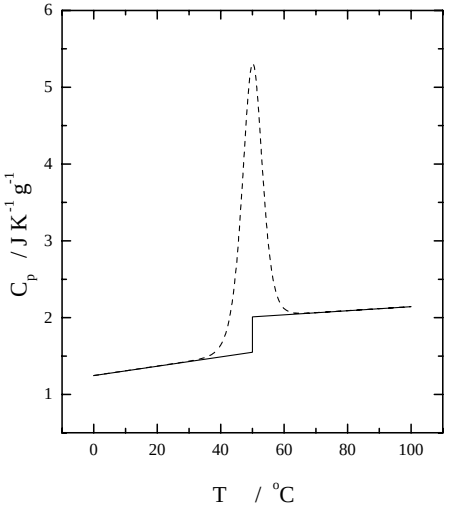
Positive ΔC_p for protein unfolding is normally attributed to exposure of hydrophobic groups.

However...

Typical data for the heat capacity increment (ΔC_p) observed upon thermal unfolding of a globular protein in aqueous solution



Cooper (2000) *Biophys. Chem*. 85, 25-39.

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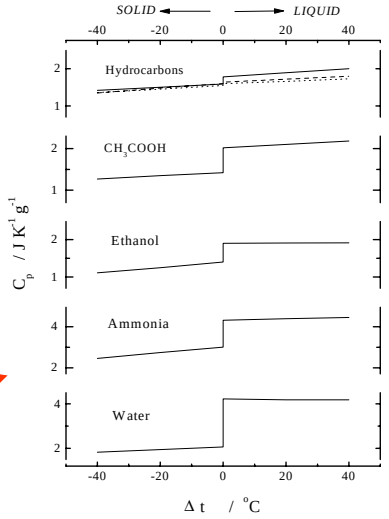
However...

Most, if not all, order-disorder transitions in hydrogen-bonded networks also take place with a positive ΔC_p

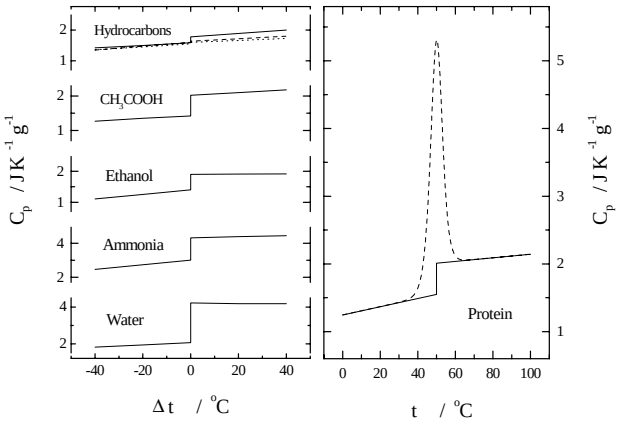
The melting of H-bonded lattices



Cooper (2000) *Biophys. Chem*. 85, 25-39.

Examples of absolute heat capacities for pure compounds as a function of temperature, plotted with respect to the normal melting point ($\Delta t = T - T_m$).

Positive ΔC_p is normal for any order-disorder transition
especially in hydrogen-bonded lattices ...



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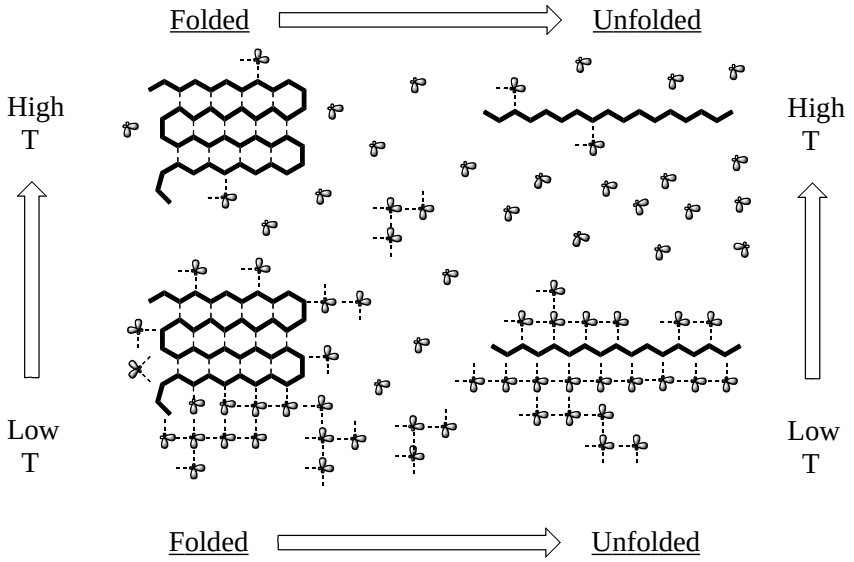
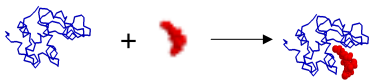
Cooper (2000) *Biophys. Chem.* 85, 25-39.



Ligand binding



$$P + L \rightleftharpoons PL$$

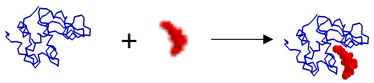
Association constant: $K_{Ass} = [PL]/[P][L]$

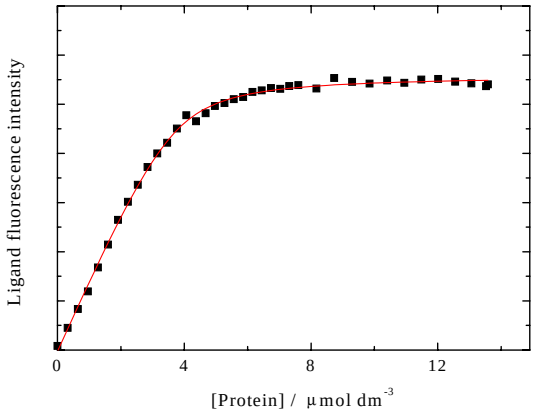
Dissociation constant: $K_{Diss} = [P][L]/[PL] = 1/K_{Ass}$

Note: $K_{Diss} = [L]_{50\%}$ (when [P] = [PL] , 50% of protein bound)

How can we measure this ?

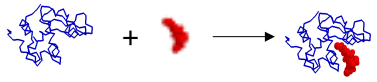
Ligand binding

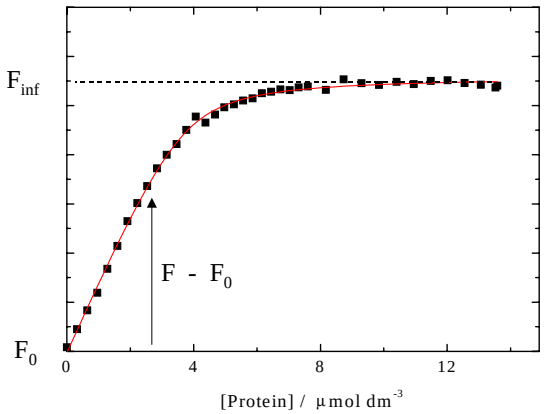




Indirect methods using spectroscopic changes (e.g. fluorescence)

Ligand binding





Fraction bound

= [PL]/([P] + [PL])

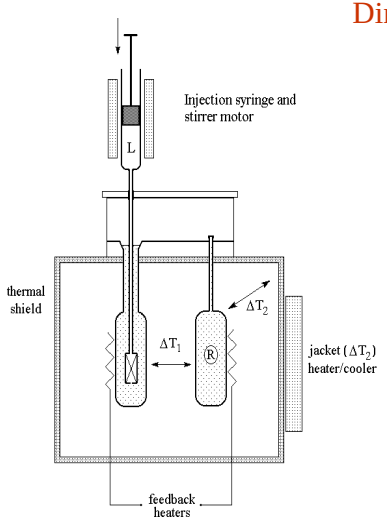
$$= \frac{F - F_0}{F_{inf} - F_0}$$

→ K , ΔG° , etc.

Indirect methods using spectroscopic changes (e.g. fluorescence)

Direct method...

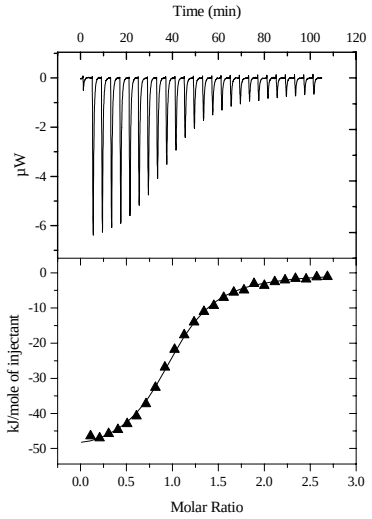
Isothermal titration microcalorimeter



Microcal VP-ITC

Sample volume: 1-2 ml ; [protein] ~ 5-50 µM

Typical ITC data for binding of a trisaccharide inhibitor (tri-N-acetyl-glucosamine; tri-NAG) to hen egg white lysozyme, in 0.1M acetate buffer, pH 5. Each exothermic heat pulse (upper panel) corresponds to injection of 10 µl of tri-NAG (0.45mM) into the protein solution (36µM). Integrated heat data (lower panel) constitute a differential binding curve that may be fit to a standard single-site binding model to give, in this instance, the stoichiometry of binding, N = 0.99, binding affinity, K_{ass} = 3.9 x 10^5 M^{-1} (K_{diss} = 2.6 µM) , and enthalpy of binding, ΔH = -51.7 kJ mol^{-1} .



From: Cooper, Lakey, Johnson & Nollman (2001) - *Biophysical Chemistry* 93, 215-230.

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Binding Equilibrium

Suppose we know the equilibrium constant for binding of a ligand to a protein. How do we know how much is bound under particular conditions? Typically we might know the total protein and total ligand concentrations, but how much is bound?

For protein-ligand binding (or anything equivalent):

$$P + L \rightleftharpoons PL$$

Dissociation constant: $K = [P][L]/[PL]$ ……{1}

Total ligand concentration: $C_L = [L] + [PL]$ ……{2}

Total protein concentration: $C_P = [P] + [PL]$

$= K[PL]/[L] + [PL]$ using {1}

$= K[PL]/(C_L - [PL]) + [PL]$ using {2}

Rearrange to give the quadratic equation for [PL]:-

$$[PL]^2 - (C_P + C_L + K)[PL] + C_P C_L = 0$$

Binding Equilibrium

For which the two solutions are:

$$[PL] = [(C_P + C_L + K) \pm \{(C_P + C_L + K)^2 - 4C_PC_L\}^{½}]/2$$

By inspection, the minus sign is the physically correct solution, giving the exact expression for protein-ligand complex formation, [PL], as a function of the total protein and ligand concentrations:

$$[PL] = [(C_P + C_L + K) - \{(C_P + C_L + K)^2 - 4C_PC_L\}^{½}]/2$$

If there are n binding sites per mole of protein, then $C_P = n.C_0$, where C_0 is the estimated protein concentration, giving:

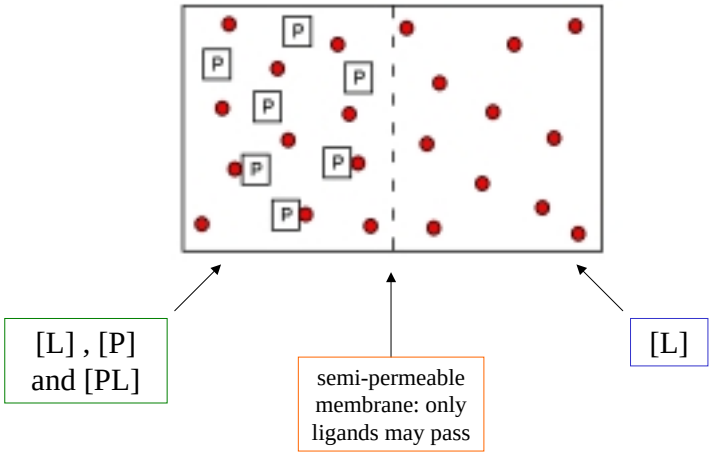
$$[PL] = [(n.C_0 + C_L + K) - \{(n.C_0 + C_L + K)^2 - 4nC_0C_L\}^{½}]/2$$

The fraction (ϕ) of sites occupied at any ligand concentration is given by:

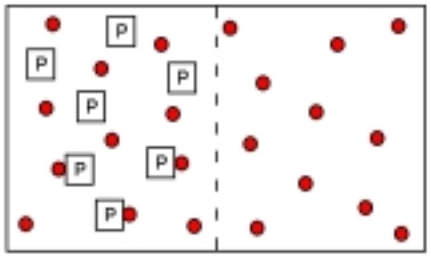
$$\phi(C_L) = [PL]/n.C_0$$

$$= [(n.C_0 + C_L + K) - \{(n.C_0 + C_L + K)^2 - 4nC_0C_L\}^{½}]/2nC_0$$

Equilibrium dialysis: ligand binding



Equilibrium dialysis: ligand binding



At equilibrium, measure…

(a) Total conc^n of L on RHS: C_L(right) = [L]

(b) Total conc^n of L on LHS: C_L(left) = [L] + [PL]

(c) Total conc^n of P on LHS: C_P(left) = [P] + [PL]

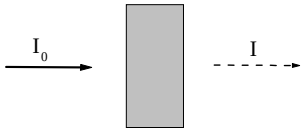
→ [PL] = (b) - (a) ; [P] = (c) - [PL]

K = [PL]/[P][L] ….. *directly from concentration measurements*

Lectures # 7 & 8

- Microcalorimeter methods
- Protein unfolding thermodynamics
- Ligand binding
- Other experimental methods

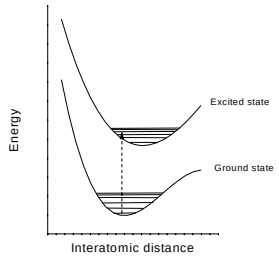
UV/visible absorbance spectroscopy



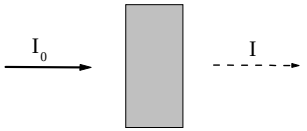
Absorbance $A = -\log_{10}(I/I_0) = -\log(T)$

$= \varepsilon c l$

(Beer-Lambert law)



UV/visible absorbance spectroscopy



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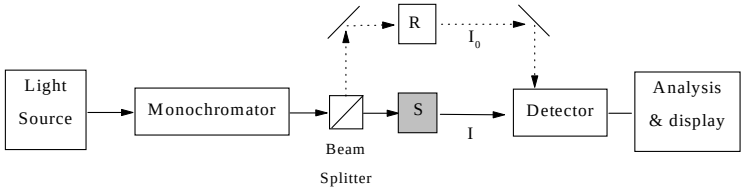
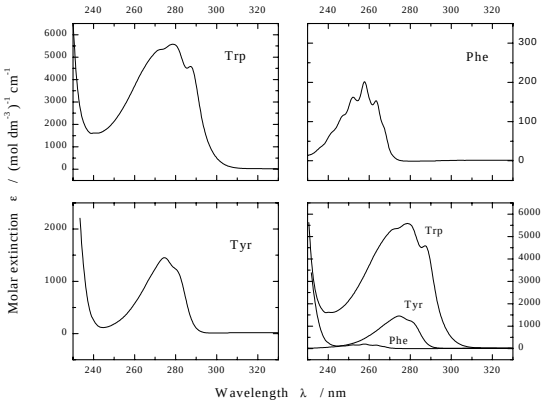


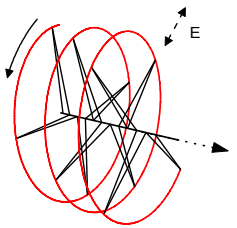
UV/visible absorbance spectroscopy



Near-UV absorbance spectra for aromatic amino acid side chains.
When superimposed on the same scale (bottom right panel), the dominant contribution from tryptophan and tyrosine residues becomes more obvious.

Circular Dichroism ("CD")

Differential absorption of left- and right-circularly polarized light by chiral molecules...

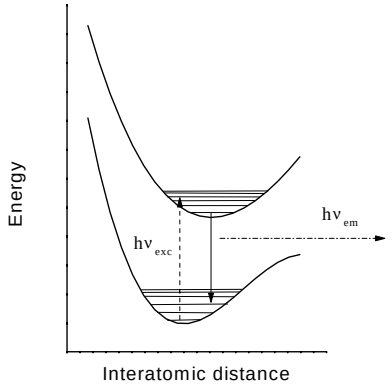


Circular dichroism is defined as the normalized difference in molar extinction:

$$\Delta\varepsilon = (\varepsilon_R - \varepsilon_L)/(\varepsilon_R + \varepsilon_L)$$

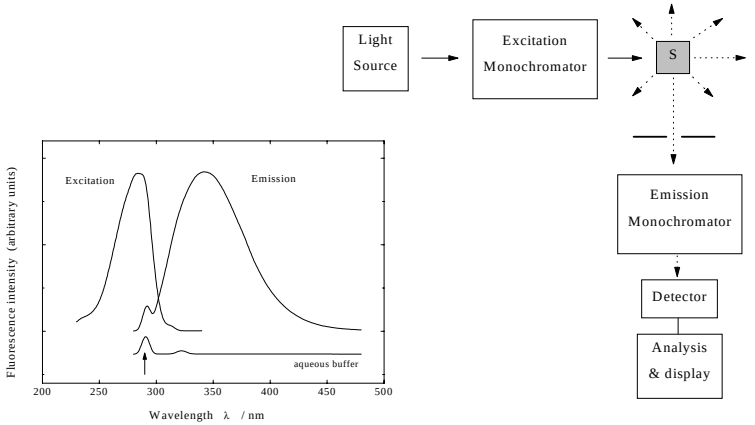
where ε_R and ε_L refer to the molar absorbance at a particular wavelength for right- or left-circularly polarized light, respectively.

Fluorescence spectroscopy



Energy level diagram illustrating electronic excitation followed by fluorescence emission. After initial (vertical) excitation from the ground state, the system rapidly relaxes to the energy minimum of the excited state.

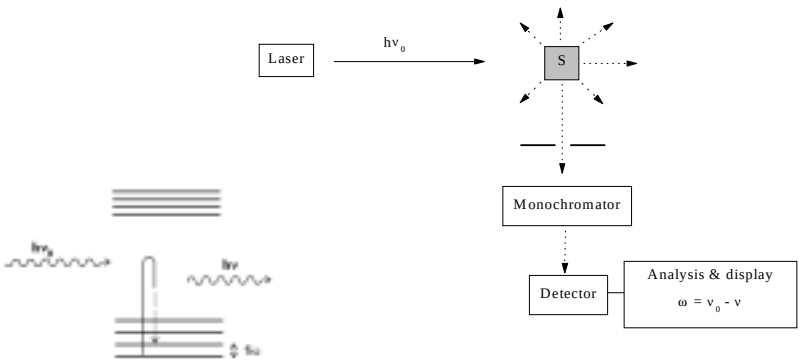
Fluorescence spectroscopy



Typical fluorescence excitation and emission spectra for a globular protein in aqueous buffer at room temperature. The excitation wavelength, λ_{exc}, is 290 nm (arrow). The excitation spectrum baseline measured with buffer in the absence of protein is shown offset for clarity.

Raman spectroscopy

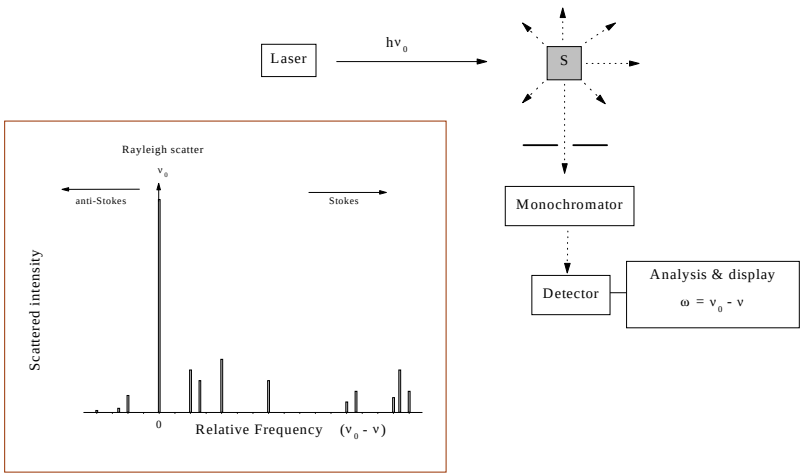
- vibrational spectroscopy using visible light



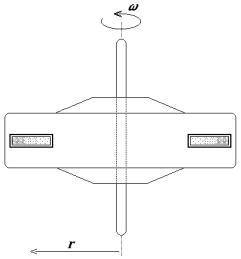
Raman (inelastic) scattering

Raman spectroscopy

- vibrational spectroscopy using visible light



Hydrodynamic methods: Analytical Ultracentrifuge



Analytical ultracentrifuge rotor

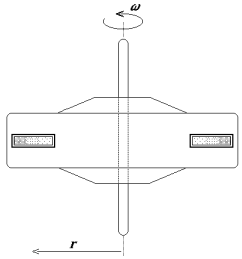
1. Sedimentation equilibrium

Boltzmann (barometric law) exponential concentration gradient at equilibrium.

$c(r_1)/c(r_2) = \exp\{-m'(r_1^2-r_2^2)\omega^2/2RT\}$

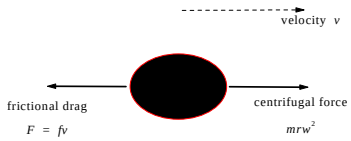
where m' is the *buoyant mass* of the molecule.

Hydrodynamic methods: Analytical Ultracentrifuge



Analytical ultracentrifuge rotor

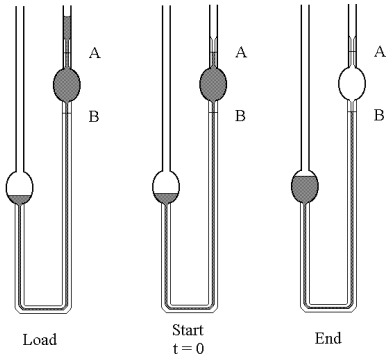
1. Sedimentation rate



Gives information about size and shape of macromolecules in solution.

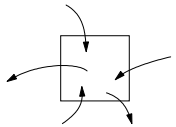
Hydrodynamic methods: Viscometry

The viscosity of a solution of macromolecules depends on their size and shape



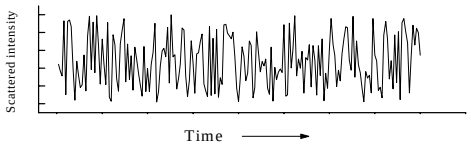
The rate of flow under gravity of a liquid through a capillary tube depends on a number of factors including the viscosity (η) and density (ρ) of the liquid as well as the size and shape of the tube. For a standard capillary viscometer (see Figure) the time taken (*t*) for a set volume of liquid to flow between points A and B is proportional to η/ρ so that, after appropriate calibration with known liquids, the viscosity of any sample can be determined from its flow time, *t*.

Hydrodynamic methods: Dynamic Light Scattering



The diffusional or Brownian motion of molecules in a liquid or gas gives rise to fluctuations in density or concentration that can be observed by optical methods.

This is the basis of the technique known as "*dynamic light scattering*" (DLS). A laser beam is passed through the solution of macromolecules, and the time dependence of the light scattered from a small volume within the sample is recorded.



Analysis of the shape and frequency of this flickering pattern gives the "*autocorrelation time*" (τ) which is related to the diffusion constant (D) of the molecules. This information can be used to determine the relative molecular masses and heterogeneities of macromolecular samples.