

Lecture 4:

Hydrophobicity & Electrostatic INTs

Lecturer:

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Free energy of a protein immersion of into liquid H₂O

A transfer of a molecule from the state a to the state b

$$p_b : p_a = \exp(-(\mathbf{F}_b - \mathbf{F}_a) / k_B T) = \exp(-\Delta F / k_B T)$$

$$\mathbf{F} = \mathbf{E} - \mathbf{TS}$$

A generalized expression for the entropy S of one particle
(atom, molecule) of the system

$$S_1 = k_B \times \log(\# \text{ of accessible states})$$

or for one mol

$$S = R \times \log(\# \text{ of accessible states}) \ \& \ R = N_A k_B$$

Constant volume and temperature:

$F = E - TS \rightarrow$ Helmholtz free energy

Constant pressure and temperature (variable volume):

$G = H - TS \rightarrow$ Gibbs free energy, $H \rightarrow$ Enthalpy

$$H = E + pV$$

**(the work of the external pressure
against volume change)**

$$G = F + pV$$

pV is negligible in proteins: $pV = nRT$

**For one mol ($n=1$) of gas: $pV \sim 0.55-0.75$ kcal/mol
($T \sim 273, 373K$)**

$pV \sim k_B T \rightarrow G \sim F$ for proteins in solvent

BUT

→ $F = E - TS = \text{min for } V=\text{const.}$ (Helmholtz)

→ $G = H - TS = \text{min for } p=\text{const.}$ (Gibbs)

H & G scale with the number of particles, N
[E & F are NOT proportional to N]



→ $V=\text{const.} \rightarrow dF = dE - T dS$

→ $p=\text{const.} \rightarrow dG = dH - T dS$



$$T = dE/dS \Big|_{V=\text{const}} = dH/dS \Big|_{p=\text{const}}$$

Thermodynamic definition of temperature:

$$T = dE/dS \Big|_{V=\text{const}} = dH/dS \Big|_{p=\text{const}}$$

Chemical potential:

$\mu = G/N$ (Gibbs free energy per particle/molecule)

- molecules pass from the phase with a larger μ to the phase with a smaller μ (so that $G=\text{min.}$)
- in equilibrium, both phases the same μ

Definition of heat capacity C_p (at $p=\text{const.}$):

$$C_p = dH/dT \Big|_{p=\text{const.}}$$

Relationship between the entropy and the free energy:

$$(1) \quad dG = d(H - TS) = dH - S dT - T dS$$

(2) in equilibrium ($T=\text{const.}$ & $p=\text{const.}$), $G=\text{min}$

$$\rightarrow dG = dH - TdS = 0$$



$$S = - dG/dT \Big|_{p=\text{const.}} = - dF/dT \Big|_{V=\text{const.}}$$

Heat capacity can be calculated from G directly:

$$\begin{aligned} C_p &= dH/dT \Big|_{p=\text{const.}} = dH/dS \Big|_{p=\text{const.}} dS/dT \Big|_{p=\text{const.}} \\ &= T d/dT (- dG/dT) \Big|_{p=\text{const.}} = - T d^2G/dT^2 \Big|_{p=\text{const.}} \end{aligned}$$

Important: absolute values of S, H, F are not well-defined (depend on the frame or state of reference, e.g. sea level for gravitational potential energy) BUT the relative values and changes are: ΔS , ΔH , and ΔF

For example, entropy change for a particle between states a and b is: $\Delta S = k_B \ln(V_b/V_a)$.

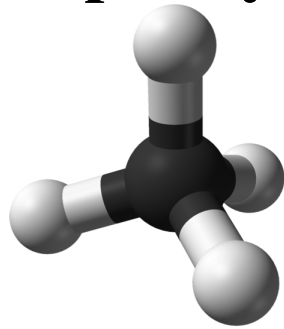
The free energy of transfer of a molecule from the non-polar liquid to water is (X_{np} and X_{aq} — equilibrium concentrations in np liquid and aq solution):

$$\Delta G_{np \rightarrow aq} = -RT \ln(X_{aq}/X_{np})$$

Hydrophobic Effect

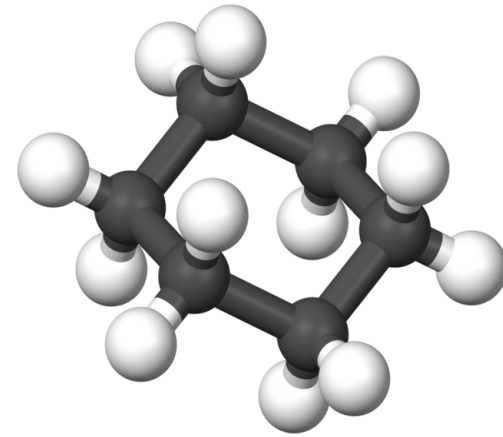
→ non-polar molecules are hydrophobic because they do not form hydrogen bonds with water molecules

→ all purely hydrocarbon molecules are hydrophobic:



← CH₄ – methane

(CH₂)₆ – cyclohexane →

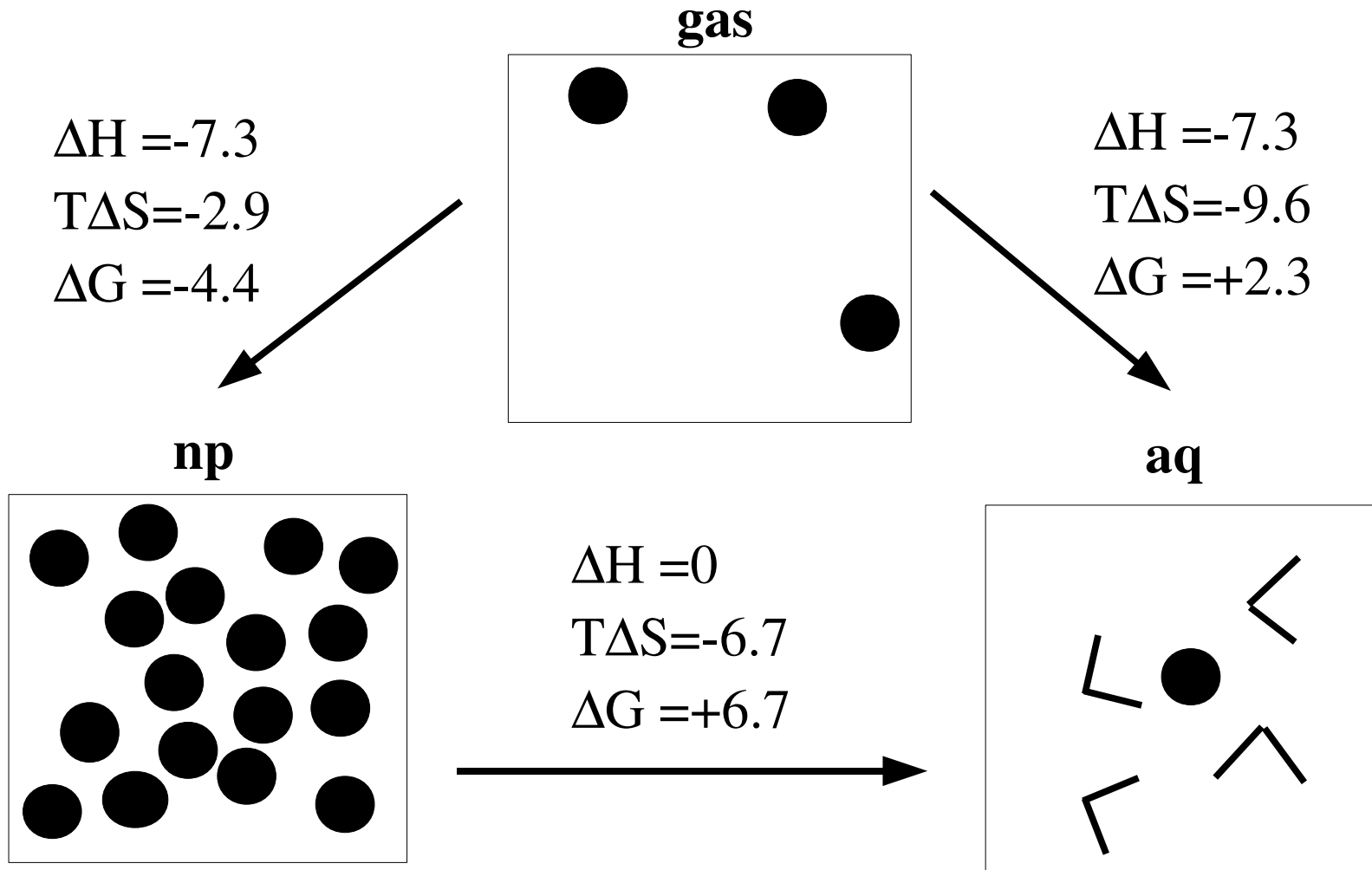


$$\rightarrow \Delta G_{np \rightarrow aq} = -RT \ln(X_{aq} / X_{np})$$

→ cyclohexane experiment (at 25°C and 1 atm. pressure):

$$X_{np} = 9.25 \text{ mol/l} \ \& \ X_{aq} = 10^{-4} \text{ mol/l} \ \rightarrow \ \Delta G_{np \rightarrow aq} = +6.7 \text{ kcal/mol}$$

**Consider three phases: gas, non-polar liquid (np)
& aqueous solution (aq)**



- ΔH (energy due to attraction) the same for
gas → np liquid
gas → aq solution
- $T\Delta S < 0$ (decrease of entropic contribution to G)
considerably larger loss of S on gas aq → solution



Hydrophobicity is of **entropic origin!**

Why are nonpolar hydrocarbon groups hydrophobic?



NO hydrogen bonding

→ both increase in energy H or decrease in the entropic TS contribute to increase of the free energy G



→ increase in the free energy G decreases the probability of a particle occupying the corresponding state/phase

Why does the entropy of water molecules decrease as a result of their contact with a nonpolar surface?

→ H_2O molecules cannot afford to lose HBs

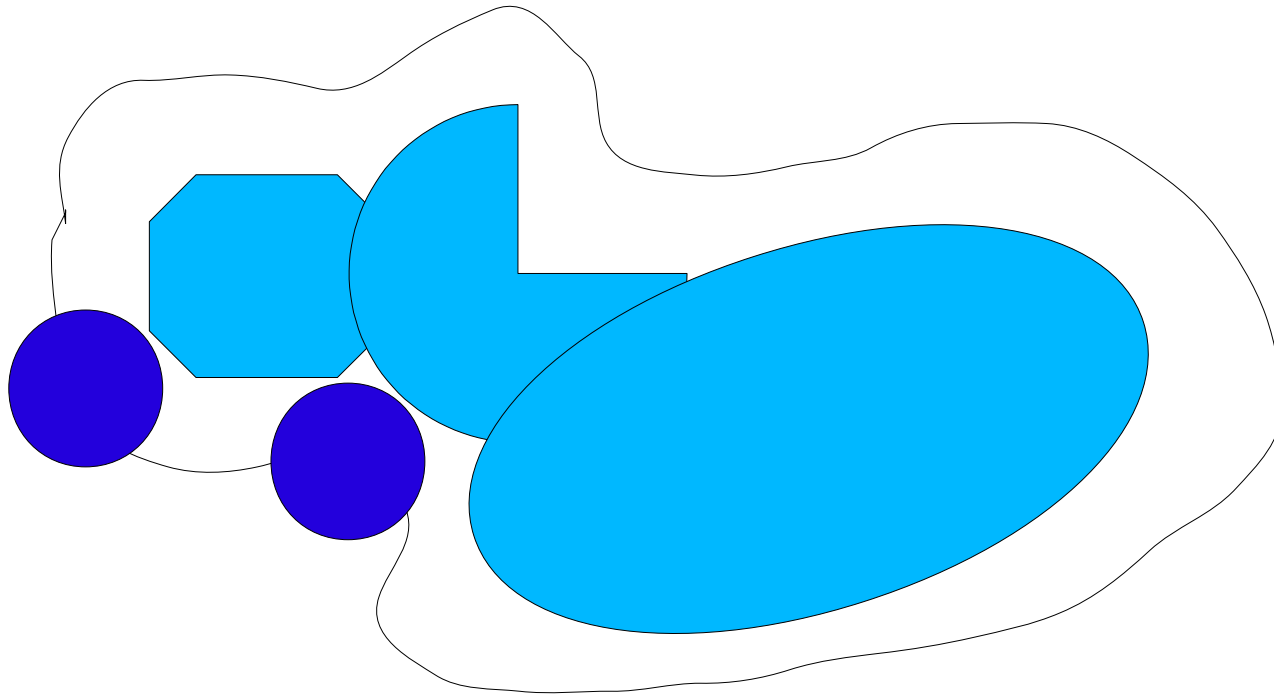
→ O–H bonds of H_2O molecules must be directed away from the hydrophobic surface → frozen conformations



→ H_2O molecules form a “frozen” layer around hydrophobic surfaces (as the energy of HB is very high 5kcal/mol)

- the layer of H_2O molecules does **NOT** have the 3D ice structure
- frozen 2D water shell around hydrophobic surfaces results in an anomalously high C_p of $(\text{CH}_2)_6$ in water (10-fold) due to the need to melt the frozen layer of H_2O molecules
- clathrate hydrate: a gas hydrate in which a lattice of water molecules encloses molecules of a trapped gas (e.g. methane naturally frozen in this form found under the ocean sea-bed)
- hydrophobic effect is temperature dependent: it **increases** with temperature up to $\sim 140^\circ\text{C}$ because ΔG increases (at low temperatures due to $T\Delta S$ decrease, at higher also due to loss of HBs in water near the hydrophobic surface)

→ ΔG increases with the **accessible surface area (ASA)** of the hydrophobic molecule (almost linearly)



→ **Hydrophobic free energy:** $\sim 0.02\text{-}0.025 \text{ kcal/mol/\AA}^2$

→ **ASA (benzene) = $200 \text{ \AA}^2 \rightarrow \Delta G \sim 4.6 \text{ kcal/mol}$**

→ **ASA (cyclohexane) = $300 \text{ \AA}^2 \rightarrow \Delta G \sim 6.7 \text{ kcal/mol}$**

Experimental measurements of individual hydrophobicities

- from equilibrium distributions of amino acids between water and slightly polar solvent (non-polar solvent does not solvate the polar amino acids) → $\Delta G_{\text{transfer}}$
- ASA (added up all accessible surfaces of nonpolar atoms within individual side chains, e.g. total ASA – 50 Å² for each polar atom) scales with $\Delta g_{\text{transfer}}$

Hydrophobicity responsible for formation of the molten globule, a compact structure, the first step in protein folding. Native contacts occur due to more specific INTs.

Electrostatic Interactions for Protein Globule in Water

→ potential energy between two elementary charges q_1 & q_2

$$U(r) = q_1 q_2 / 4\pi\epsilon\epsilon_0 r$$

$\epsilon = 80$ (in water) & $\epsilon = 2-4$ (in a dry protein)



Thus, for $r = 3 \text{ \AA}$: U (water) ~ 1.5 kcal/mol

U (protein) ~ 40 kcal/mol

Calculate the energy needed to transfer one charged atom from water into the protein interior (“dry” protein):

A sphere of charge q and radius R (medium permittivity ϵ) has the energy:

$$dU = q dq / 4\pi\epsilon\epsilon_0 R \rightarrow \text{integrate } q = [0, q_1] \rightarrow$$
$$U = q_1^2 / 8\pi\epsilon\epsilon_0 R$$

For a typical atomic radius of 1.5 :

$$U \sim 1.5 \text{ kcal/mol (in water, } \epsilon=80)$$

$$U \sim 40 \text{ kcal/mol (inside protein, } \epsilon=3)$$



Charged atoms are on the protein surface in contact with water.