MASARYKOVA UNIVERZITA

Protein expression and purification

II. Calculation in the molecular biosciences

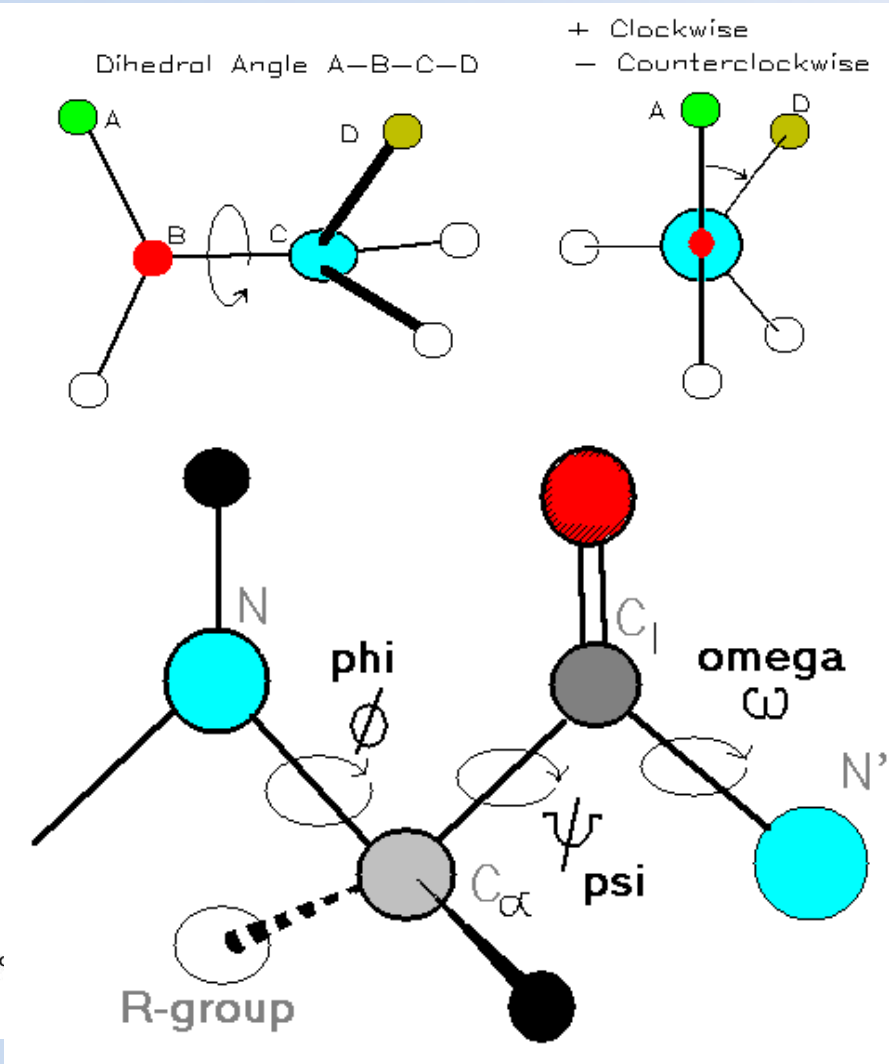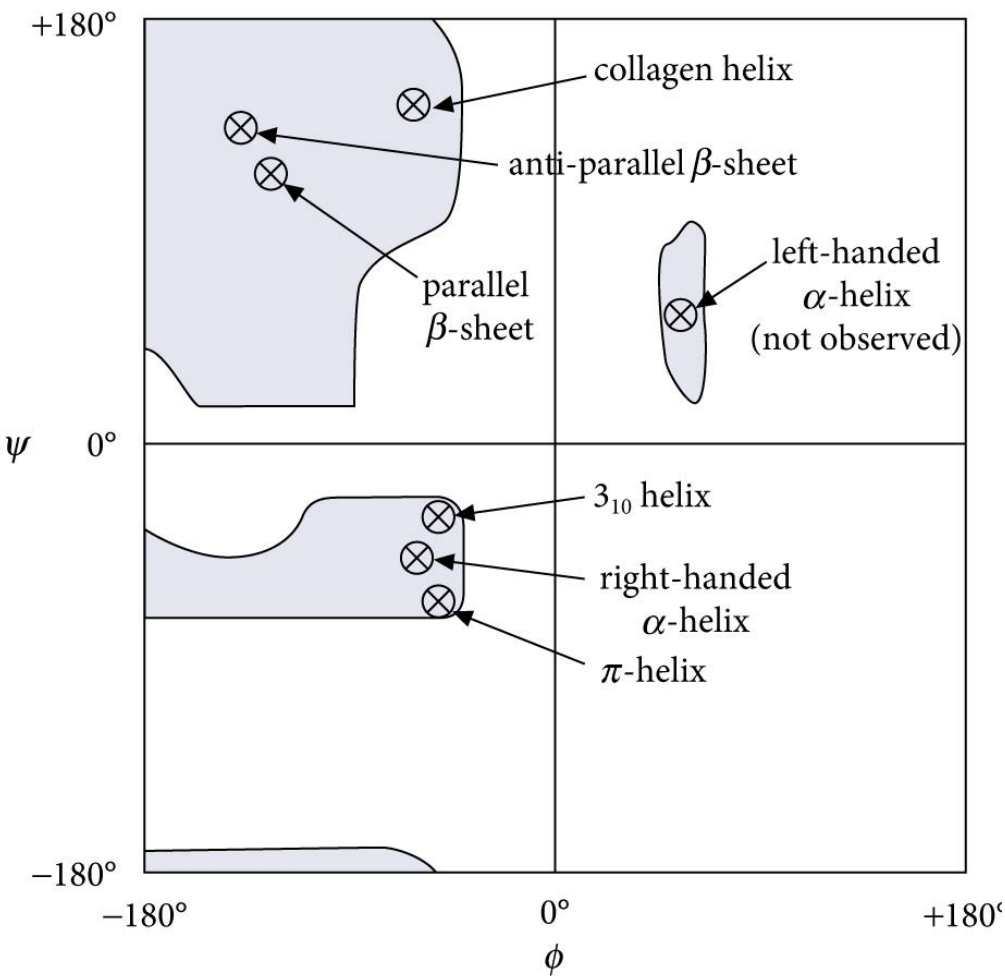Jozef Hritz, Lubomír Janda, Blanka Pekárová
Radka Dopitová

EVROPSKÁ UNIE

MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání
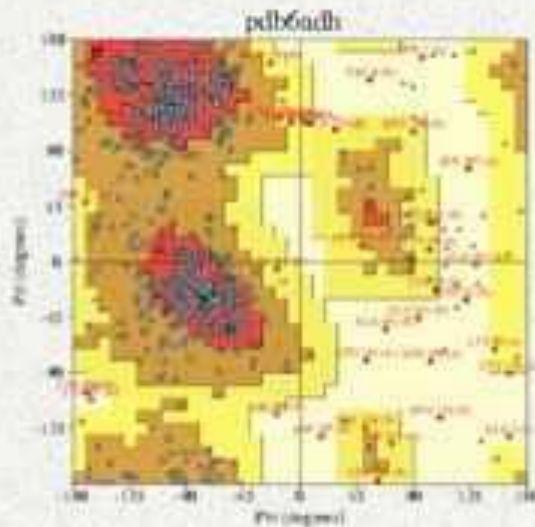pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

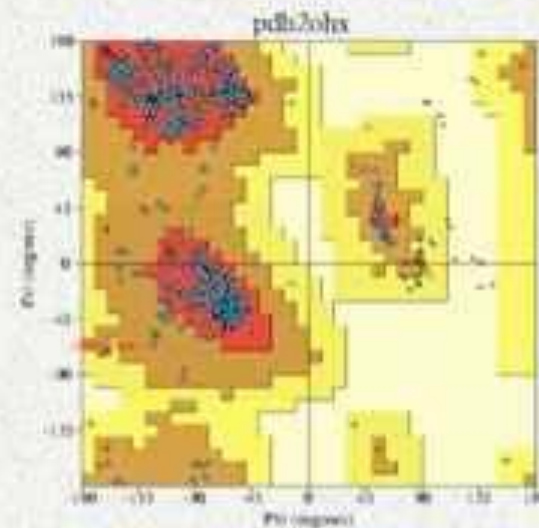## 1.4. The secondary structure of proteins

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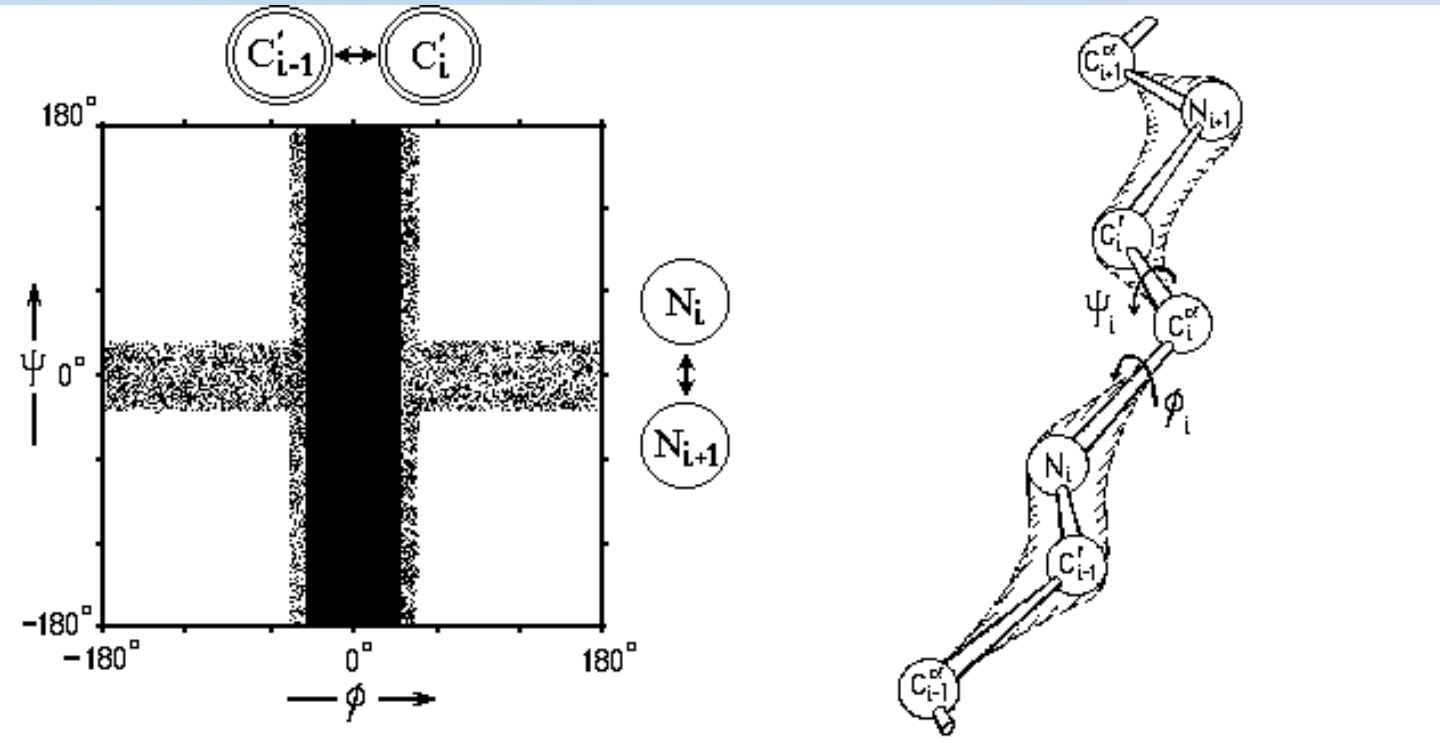Ramachandran plot for a bad (left) and good (right) quality structure

Low resolution (2.9 Å)   High resolution (1.8 Å)
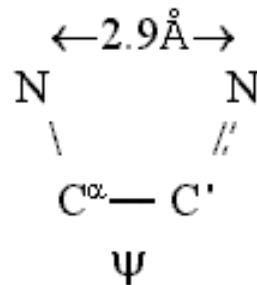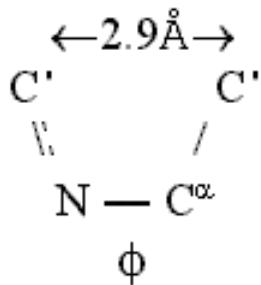
1.4. The secondary structure of proteins

Energetically unfavorable conformational areas

$$2.9\text{Å} < r_{min}(C...C)=3.0\text{Å} \qquad 2.9\text{Å} > r_{min}(N...N)=2.7\text{Å}$$

Atom	σ, Å	ε, kcal/mol
O	2.96	0.210
N	3.25	0.170
C in C=O	3.75	0.105
Other C	3.50	0.080
H on N	0.00	0.000
H on C	2.50	0.050

1.4. The secondary structure of proteins

Additional energetically unfavorable conformational areas because of the presence of carbonyl oxygen

Glycine – less sterically restricted because of lacking the side-chain

Atom	σ, Å	ε, kcal/mol
O	2.96	0.210
N	3.25	0.170
C in C=O	3.75	0.105
Other C	3.50	0.080
H on N	0.00	0.000
H on C	2.50	0.050

1.4. The secondary structure of proteins

Additional energetically unfavorable conformational areas because of the presence of C$^\beta$

Alanine

Atom	σ, Å	ε, kcal/mol
O	2.96	0.210
N	3.25	0.170
C in C=O	3.75	0.105
Other C	3.50	0.080
H on N	0.00	0.000
H on C	2.50	0.050

1.4. The secondary structure of proteins

1.4.3. Other structural features in proteins
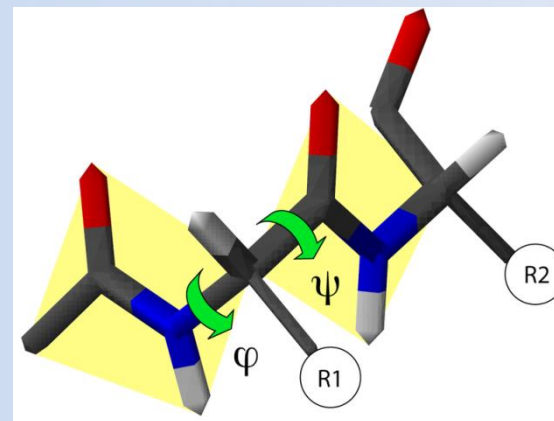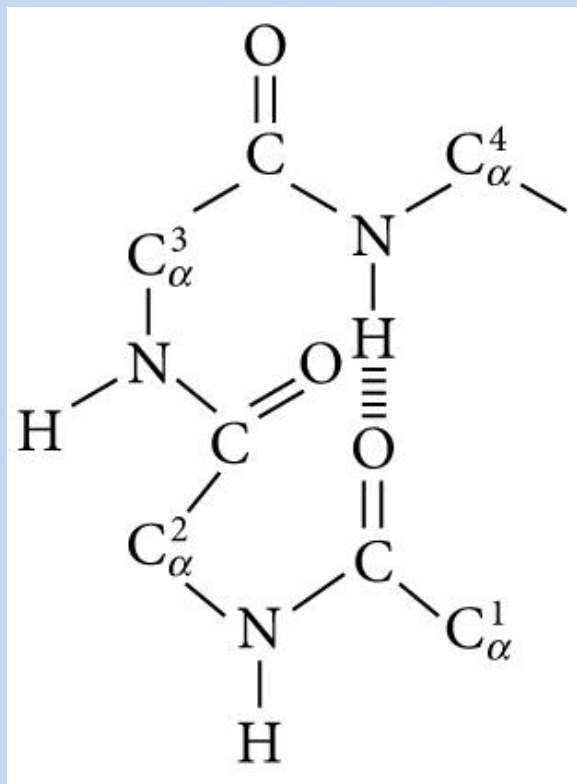
Structural preferences of the different amino acids

Met, Glu, Leu,Ala	+	alpha - helices
Pro, Gly, Tyr	-	alpha helices
Val, Ile, Phe	+	beta sheets
Pro, Asp	-	beta sheets
Pro, Gly, Asp	+	beta turn
Met, Val, Ile	-	beta turn

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1.6. The quaternary structure of proteins
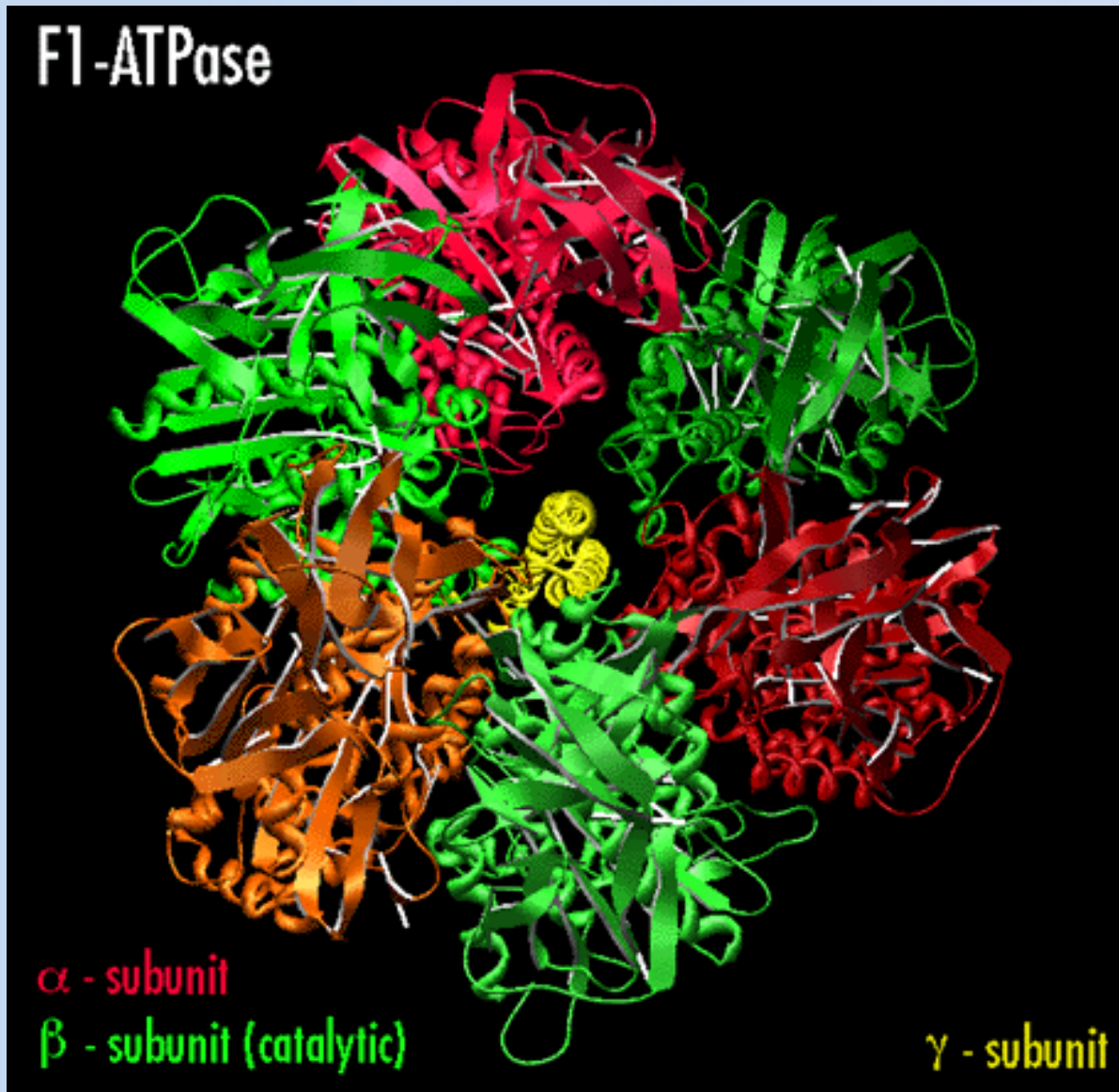
1.3. The primary structure of proteins

1.3.2. Information available from the amino acid sequence of a protein

1.3.2.1. Exact molecular mass   1.3.2.2. Isoelectric point

1.3.2.3. Absorption coefficient   1.3.2.4. Hydrofobicity

http://www.expasy.ch/tools/pi_tool.html
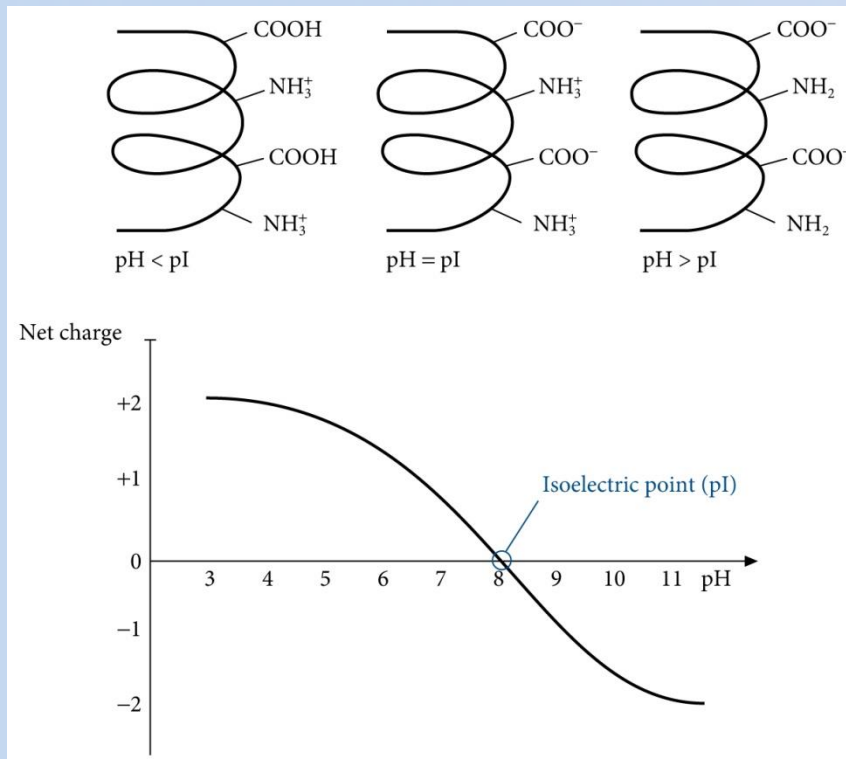
Beer–Lambert law $\quad\quad$ **A = ε * c * l**

$\quad$ **A** is the absorbance at a particular wavelength (without units; merely a ratio):

$$A = \log (I_0/I_t)$$

$\quad$ I_0 is the intensity of the incident light (light striking the cuvette).

$\quad$ I_t is the intenisty of the transmitted light (the light leaving the cuvette).

ε is the absorption coefficient (degree of absorption) (M^{-1} cm^{-1}).

l is the path length of the cuvette (cm).

c is the concentration of the solution (M).

Linear form of Beer-Lambert law is valid only for lower values of A.

Please solve a problem.

Question 1:   How many mg of protein you have in 5 ML of purified protein if its molar extinction coefficient is 15000 $M^{-1}cm^{-1}$; molecular weight 20 kDa. Advice – units!

Measured absorption in cuvette with 1 cm optical path-length is 0.3 at 280 nm.   10 points

Mass concentration equals to the molar concentration multiplied
by molecular weight of the protein.   8 points
Unit **M** correspond to the **mol.L^{-1}**   6 points

            $g.L^{-1}$ = $mg.ml^{-1}$ 4 points

Solution:

$A = \varepsilon c l$

$c\,[M] = A/\varepsilon l = 0.3\,/\,(15000\ M^{-1}cm^{-1}\,{}^*1\ cm) = 0.00002\ M = 0.02\ mM$

$C\,[g.L^{-1}] = 0.00002\ mol.L^{-1} * 20000\ g.mol^{-1} = 0.4\ g.L^{-1} = 0.4\ mg.ml^{-1}$

$m\,[mg] = C*V = 0.4\ mg.ml^{-1} * 5\ ml = \underline{2\ mg}$

1.7. Forces contributing to the structures and interactions of proteins

Bond strenght

1000   100   10   1

kJ/mol

Covalent bond energy

C=C
610

C-C
415

C=O
740

O-H
465

C-O
360

S-S
250

Ionic bond
10-20

Hydrophobic interaction
5-15

Hydrogen bond
5-10

Van der Waals interaction
0.5-5
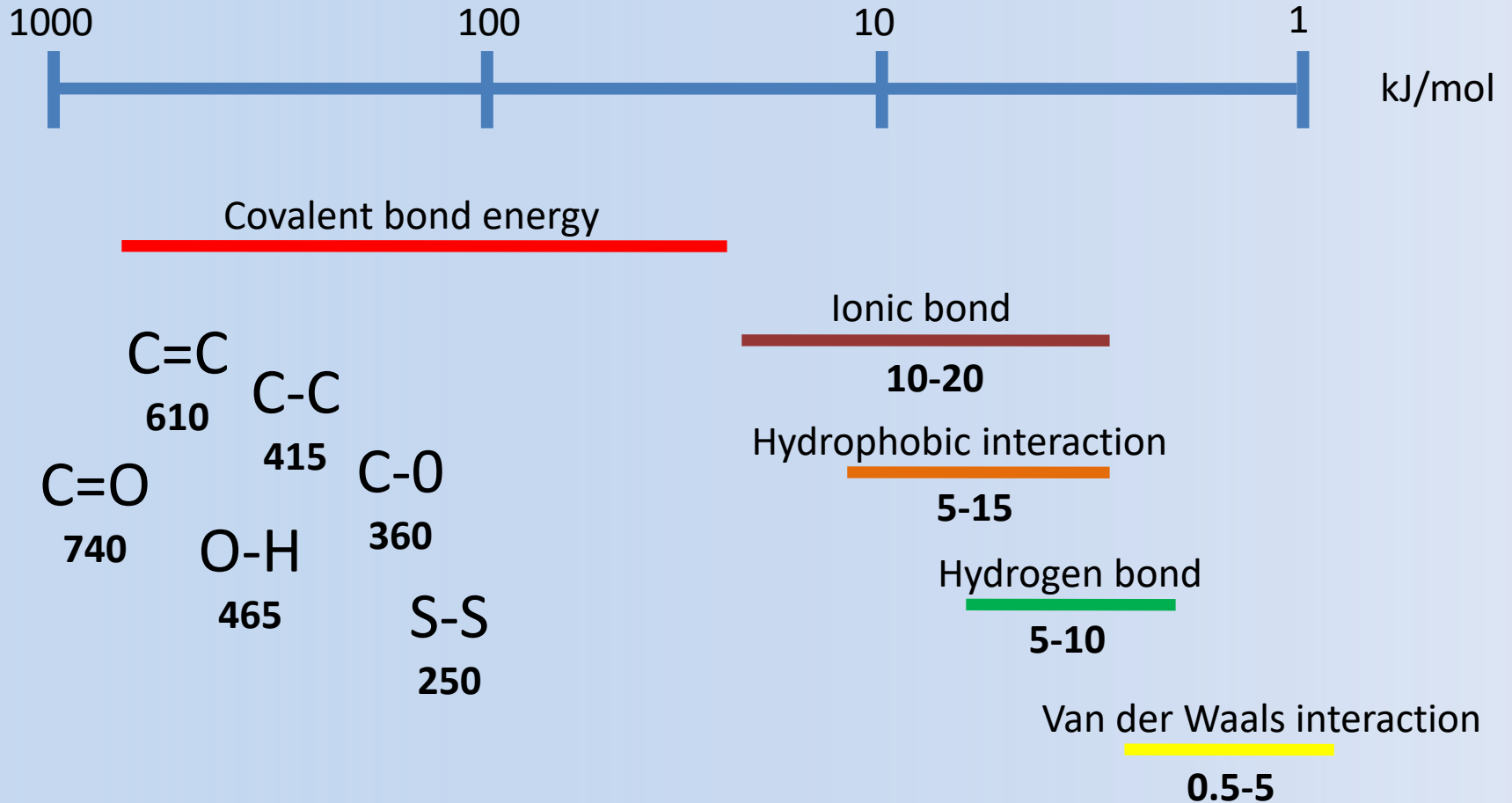
1.7. Forces contributing to the structures and interactions of proteins
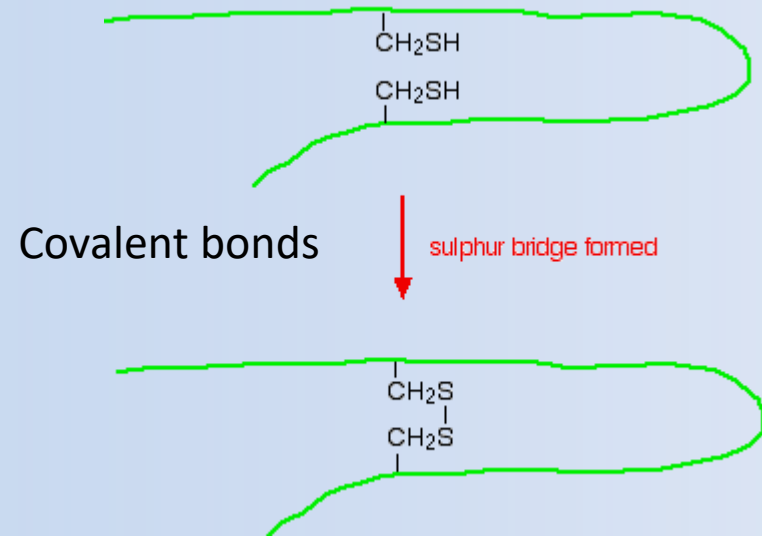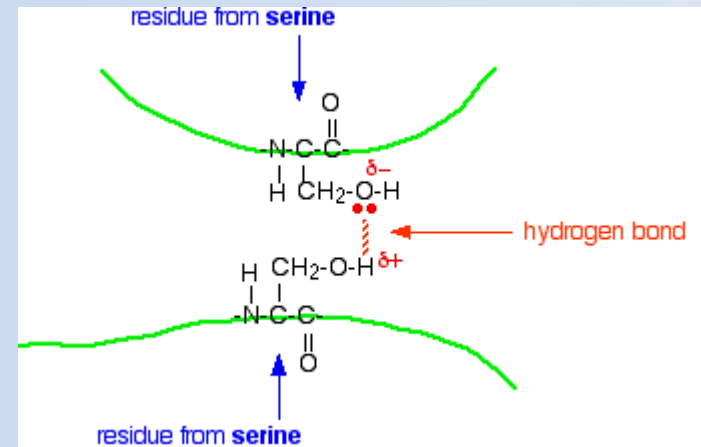
1.7.1. Ionic (electrostatic) interactions
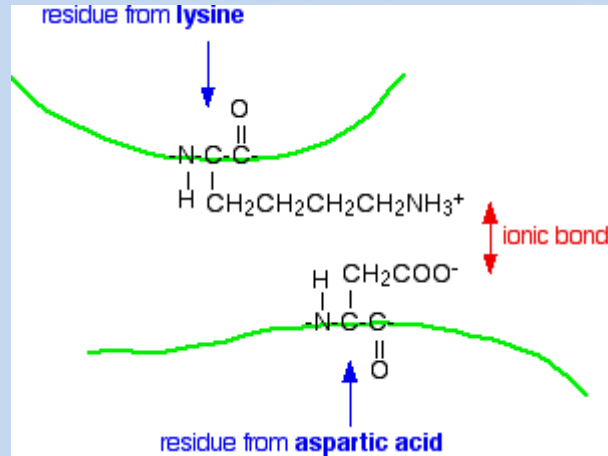
Covalent bonds

1.7.2. Hydrogen bonds
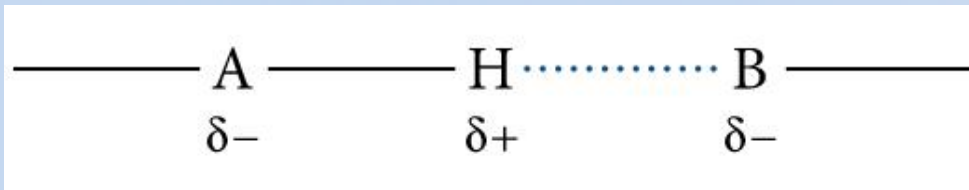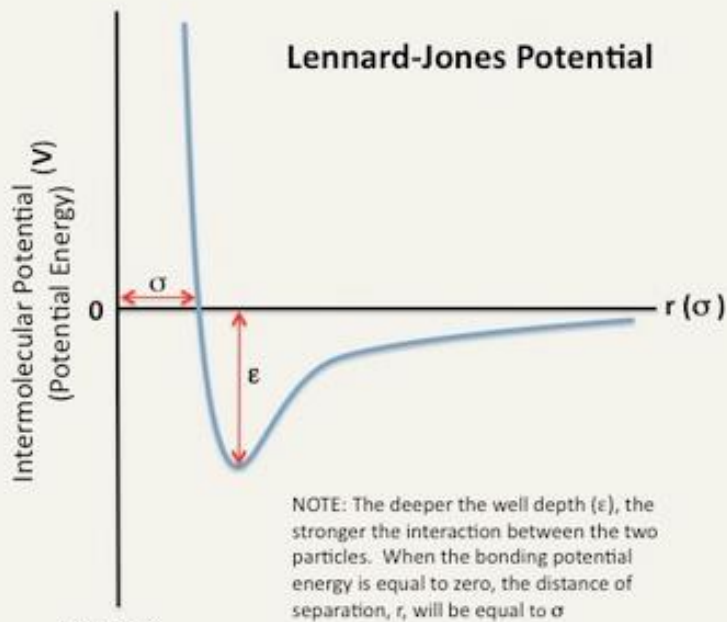
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Lennard-Jones Potential

Atom	σ, Å	ε, kcal/mol
O	2.96	0.210
N	3.25	0.170
C in C=O	3.75	0.105
Other C	3.50	0.080
H on N	0.00	0.000
H on C	2.50	0.050

NOTE: The deeper the well depth (ε), the stronger the interaction between the two particles. When the bonding potential energy is equal to zero, the distance of separation, r, will be equal to σ

Figure B

Combination rules for Lennard-Jones potential

$$S_{ij} = \frac{S_{ii} + S_{jj}}{2}$$

$$e_{ij} = \sqrt{e_{ii} e_{jj}}$$

$$E_{ij}^{LJ} = 4e_{ij}\left[\left(\frac{S_{ij}}{r_{ij}}\right)^{12} - \left(\frac{S_{ij}}{r_{ij}}\right)^{6}\right]$$

Electrostatic interactions at different media

$\varepsilon_r = 80$

$\varepsilon = ?$
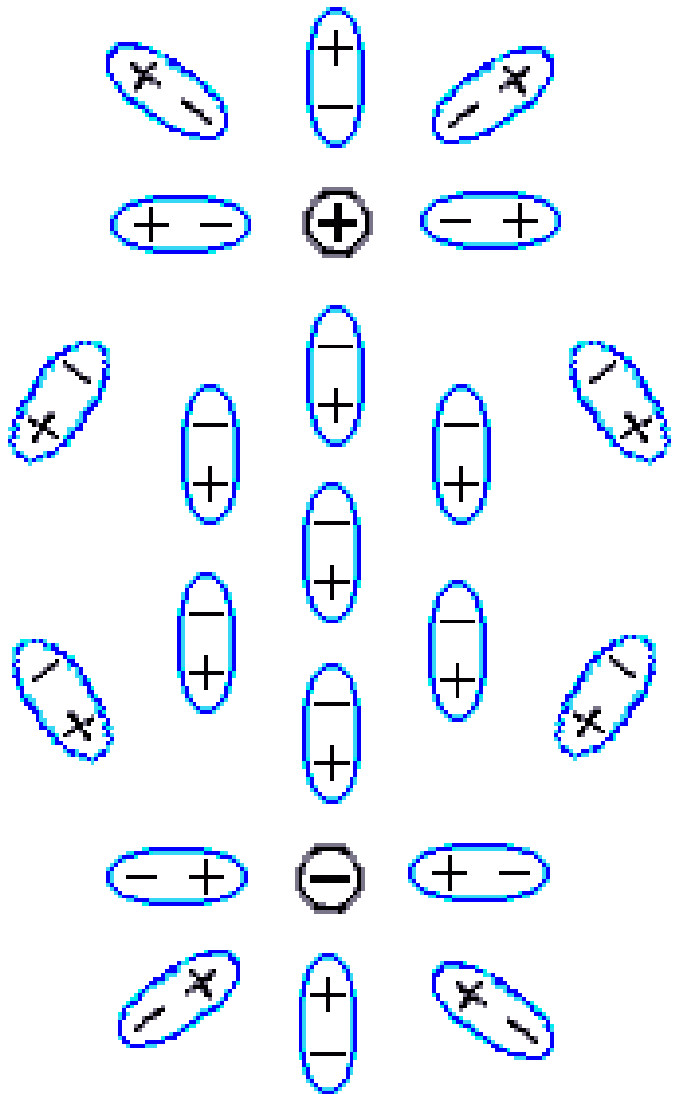
$$E_{ij}^{el} = \frac{1}{4\pi e_0 e_r} \frac{q_i q_j}{r_{ij}}$$

intermed.
"vacuum", ε~1?
but absence
of intermed.
dipoles can
only increase
interaction…
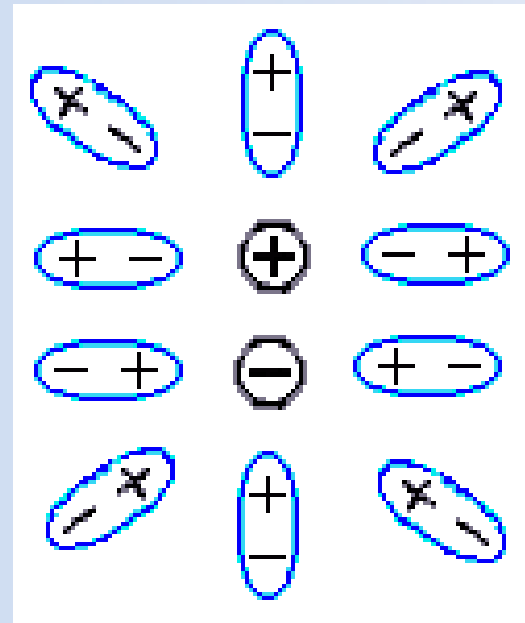
$\varepsilon_0 = 8.85 * 10^{-12}$ C*N^{-1}*m^{-2}, q=1.6*10^{-19} C

Relative permittivity:
- vacuum: $\varepsilon_r = 1$
- water: $\varepsilon_r = 80$
- protein $\varepsilon_r \sim 4$

$$E_{ij}^{el} = \frac{1}{4pe_0 e_r}\frac{q_i q_j}{r_{ij}}; k_e = \frac{1}{4pe_0}$$

$$E_{ij}^{el} = \frac{k_e}{e_r}\frac{q_i q_j}{r_{ij}}$$

$$E_p^{total} = \sum_{i,j} E_{ij}^{el} + E_{ij}^{LJ}$$

Useful Physical/Chemical Constants

Constant	Value
Avogadro's Number	$N_A = 6.022 \times 10^{23}$ mol^{-1}
Faraday Constant	$F = 96\ 485.33$ C mol^{-1}
Molar Gas Constant	$R = 8.314$ J mol^{-1} K^{-1}
Coulomb's Constant	$k_e = 8.987 \times 10^9$ N m^2 C^{-2}
Speed of Light (Vacuum)	$c = 299\ 792\ 458$ m s^{-1}
Boltzmann Constant	$k_b = 1.38 \times 10^{-23}$ J K^{-1}
Charge on a Proton/Electron	$e = 1.602 \times 10^{-19}$ C
Standard acceleration of gravity	$g = 9.8$ m s^{-2}
Planck's Constant	$h = 6.6 \times 10^{-34}$ m^2 kg / s

Please solve a problem.

Question 2:   What is the total potential energy of system consisting of salt ions Na$^+$ and Cl$^-$ in kJ/mol?For the electrostatic energy contribution consider the permittivity of water environment

	Mw[g/mol]	σ[Å]	ε[kJ/mol]
Na$^+$	23	2.3	0.5
Cl$^-$	35	4.3	0.5

Ions are separated by 0.28 nm distance.   20 points

$$S_{ij} = \frac{S_{ii} + S_{jj}}{2}; e_{ij} = \sqrt{e_{ii}e_{jj}}$$ 18 points

Coulombic energy term correspond to the energy per particle, not per mol   16 points

N.m = J   12 points

Be careful, which energy term is positive and which negative
8 points

Solution:

$$E_{ij}^{el} = \frac{k_e}{\varepsilon_r} \frac{q_i q_j}{r_{ij}} N_A = \frac{9 * 10^9 Nm^2 C^{-2}}{80} \frac{[-(1.6 * 10^{-19}C)(1.6 * 10^{-19}C)]}{0.28 * 10^{-9}m} 6.022 * 10^{23} mol^{-1} =$$

$$= -6.2 * 10^3 Jmol^{-1} = -6.2 kJmol^{-1}$$

$$E_{ij}^{LJ} = 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^6\right] = 4*0.5 kJ.mol^{-1}\left[\left(\frac{3.3A}{2.8A}\right)^{12} - \left(\frac{3.3A}{2.8A}\right)^6\right] =$$

$$= 2 kJ.mol^{-1}[7.29 - 2.7] = 9.18 kJ.mol^{-1}$$

$$E_p^{total} = E_{ij}^{el} + E_{ij}^{LJ} = -6.2 kJmol^{-1} + 9.18 kJmol^{-1} = 3 kJmol^{-1}$$

According what principle there should be the melting of an ice?

The crude meaning of entropy and free energy

Where is the higher probability to find one particular molcule of air here in our classroom (p_c) or in astronomy observatory at Lomnicky Stit (p_L)?

$p_c/p_L = [V_c exp(-E_c/kT)] / [V_L exp(-E_L/kT)]$
$\qquad = [exp(-(E_c-T*k*ln(V_c))/kT)] / [exp(-(E_L-T*k*ln(V_L))/kT)]$

where $S = k*ln(V)$ is entropy;
more general: $S = k*$[logarithm of the number of accessible states of the particle]
and $F = E – T*S$ is (Helmholtz) free energy (for system at constant volume)

$p_c/p_L = exp(-F_c/kT) / exp(-F_L/kT) = exp(-(F_c-F_L)/kT)$

Difference between Helmholtz and Gibbs free energy

T=const:

Helmholtz free energy	Gibbs free energy
V=const	P=const
F=E-TS	G=H-TS=F+PV
E	enthalpy H=E+PV
	chemical potential: μ=G/N
probability $\propto$ exp(-F/kT) ➔max F ➔min	probability $\propto$ exp(-G/kT) ➔max G ➔min

Typical value of PV for liquids at atmospheric pressure is very small in comparison with the thermal energy of the body. Therefore very often the term "

Let's estimate the work against the external atmospheric pressure related to the insertion of 1M of water.

Let's estimate the work against the external atmospheric pressure related to the insertion of 1M of water.

1M of water: 18 g*mol^{-1}, 18cm^3*mol^{-1}

at atmospheric pressure pV = 10^5 Pa * 18 * 10^{-6} m^3*mol^{-1} = 1.8 J*mol^{-1}

It is negligible value in comparison to the thermal energy.

G=H-TS; dG=dH-TdS-SdT

At T=const (dT=0)
at equilibrium, G=min, dG=0, dH-TdS=0

It implies:
T = dH/dS

Definition of heat capacity: $C_p=(dH/dT)_{P=const}$

$S=-(dG/dT)_{P=const}$

Free energy – driving force

Typical Examples:
(1) Conformational changes
(2) Affinity of the ligand binding
(3) Solubility
(4) Lipophilicity
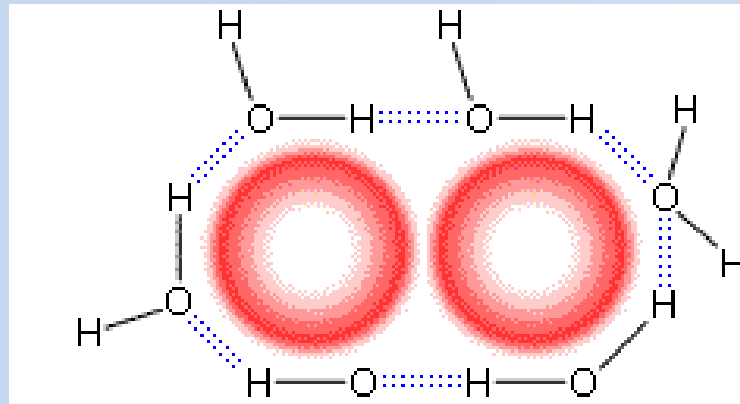(5) Protein in folded vs unfolded state

In the computational simulations it is easy to calculate the potential or kinetical energy of the system. However, it is much more challenging to calculate the free energy differences.

Thermodynamic cycle for binding affinities
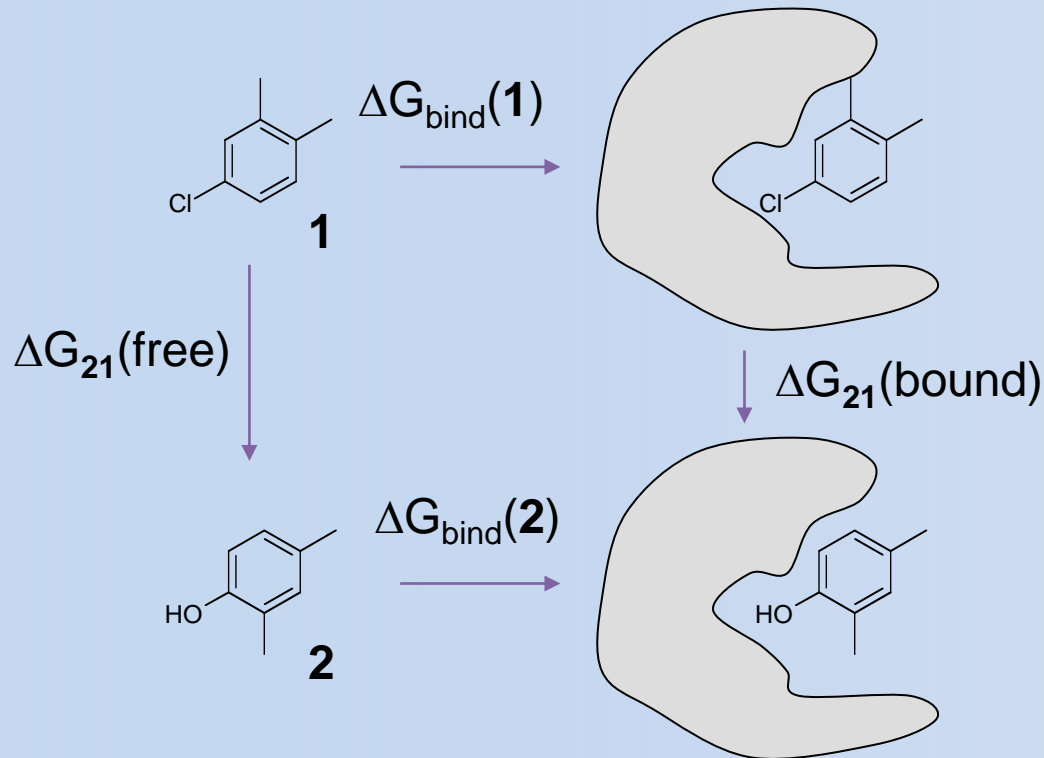
$$\Delta\Delta G_{bind} = \Delta G_{bind}(\mathbf{2}) - \Delta G_{bind}(\mathbf{1})$$
$$= \Delta G_{\mathbf{21}}(free) - \Delta G_{\mathbf{21}}(bound)$$

Hydrophobic bond between non-polar particles

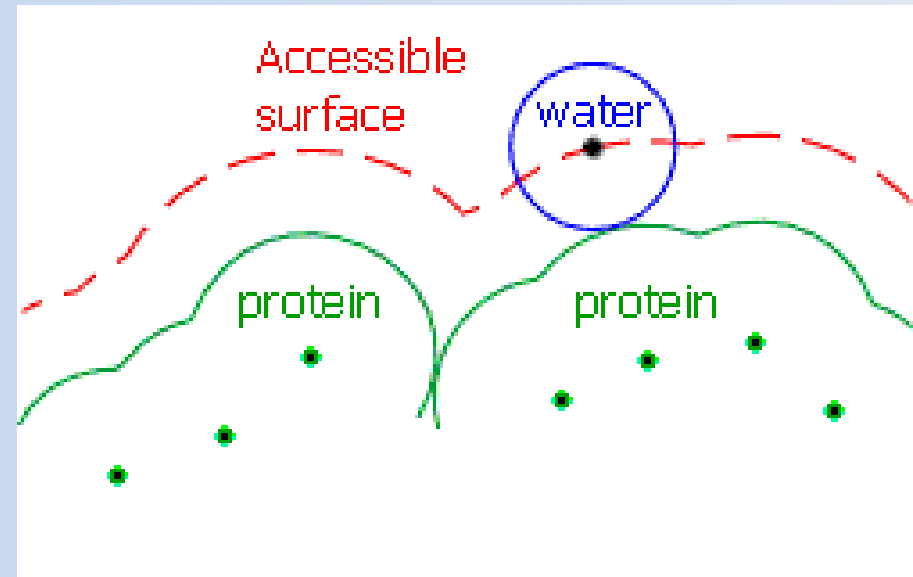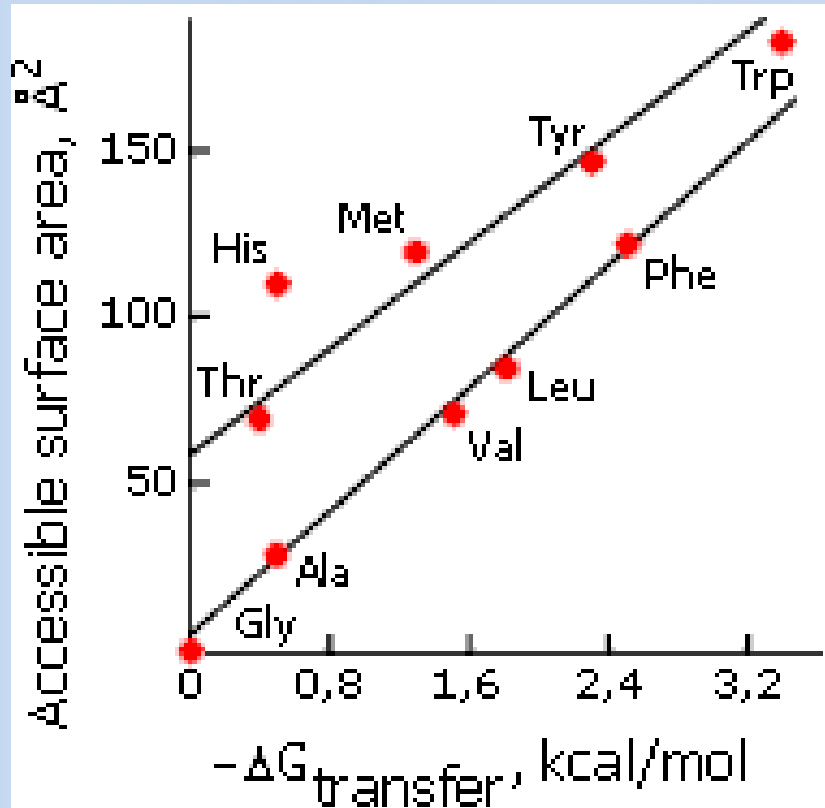Hydrophobic free energy increases almost proportionally to the accessible surface area of the non-polar molecules.

Its value is ~85-105 $J*mol^{-1}*Å^{-2}$

The accessible surface area of amino-acid side-chains and their hydrophobicity

~ -50 Å^2 for each polar atom

Adopted from Schulz G.E., Schirmer R.H. Principles of protein structure. 1979

Hydrophobic effect is the principle component of the protein 3D structure formation/stability.

Second look on the hydrogen bonding
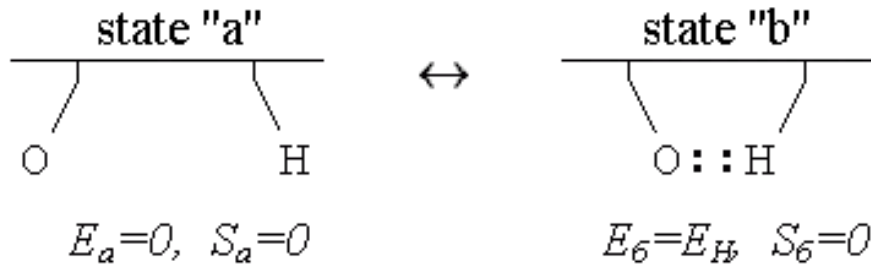
Role of water

H-bond energy is ~5kcal*mol^{-1}=21kJ*mol^{-1}

in water H-bond energy is only ~5-6 kJ*mol^{-1}

Large difference in increase of protein stability by forming of intraprotein H-bond in vacuum vs. water!!!

In vacuum:

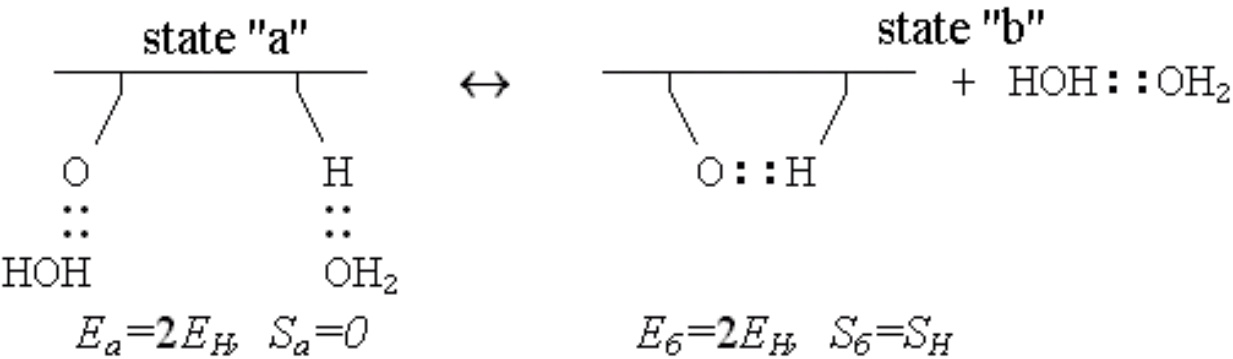state "a"   ⟷   state "b"

O   H   O::H

$E_a=0, \ S_a=0$ $E_b=E_H, \ S_b=0$ $E_b-E_a=E_H \quad S_b-S_a=0$
$$F_b-F_a= (E_b-E_a)-T(S_b-S_a) = E_H$$

In water:

state "a"   ⟷   state "b" $+ \ \ HOH::OH_2$

O   H   O::H
::   ::
HOH   OH₂

$E_a=2E_H, \ S_a=0$ $E_b=2E_H, \ S_b=S_H$ $E_b-E_a=0, \quad S_b-S_a=S_H$
$$F_b-F_a= (E_b-E_a)-T(S_b-S_a) = -TS_H$$

Difference in entropy of water molecule.
At the melting temperature 273 K
increase in entropy is compensated by decrease in energy

Melting heat of ice: 334 $J*g^{-1}$ (80 $cal*g^{-1}$)
334 $J*g^{-1}$ * 18 $g*mol^{-1}$ = 6.012 $kJ*mol^{-1}$ (1.5 kcal * mol^{-1})

Conclusions

- Free energy difference is the most important determinant of behavior in solutions
- Free energy has enthalpic and entropic part
- The increasing importance of entropic part with the increasing temperature
- Probability of certain state is given by Boltzmann formula
- Binding affinity depends on both – bound and unbound state in water

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