LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

biophysikalische Chemie/molekulare Biophysik
Wintersemester 2020/2021

Homework III

Department Chemie
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Homework is due at the *beginning* of Lecture (8:15AM) on Thursday, January 21st, 2021.

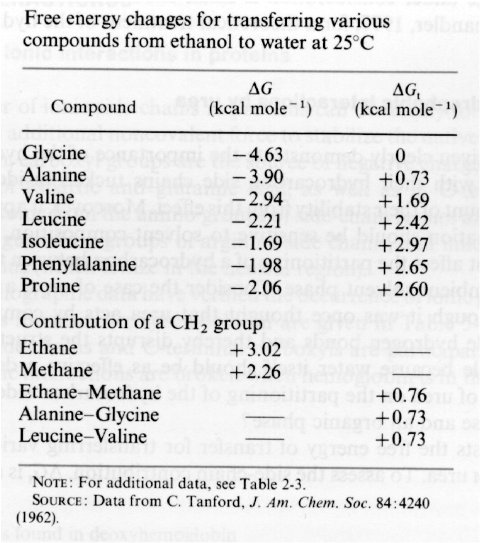
1. The Gibb's free energy of creating n imperfections to a crystal of N atoms is given by:

$$G(n) = G(0) + nH_p - nT\Delta S_{th} - k_b T \ln\left[\frac{(N+n)!}{N!\,n!}\right]$$

Using Sterling's approximation ($\ln x! \approx x \ln x$) and $n << N$, show that the fraction of impurities at the minimum Gibb's free energy is given by:

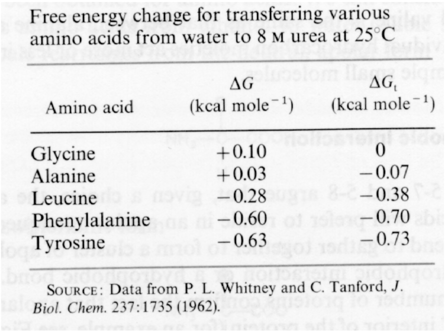
$$\frac{n}{N} = \exp\left(\frac{\Delta S_{th}}{k_b}\right)\exp\left(\frac{-H_p}{k_b T}\right)$$

2. A protein undergoes a subtle conformational change in which the most significant event is the movement of three closely packed amino acid side chains - leucine, alanine, and phenylalanine - from the ethanol-like interior of the protein to the exterior, where they are immersed in the solvent. Assuming that this is the only thermodynamically significant event in the conformational change, calculate the equilibrium constant for this change in water and in 8 M urea, at 25°C. Information in the adjacent tables may be of help.


Free energy changes for transferring various compounds from ethanol to water at 25°C

Compound	ΔG (kcal mole^{-1})	ΔG_t (kcal mole^{-1})
Glycine	−4.63	0
Alanine	−3.90	+0.73
Valine	−2.94	+1.69
Leucine	−2.21	+2.42
Isoleucine	−1.69	+2.97
Phenylalanine	−1.98	+2.65
Proline	−2.06	+2.60
Contribution of a CH_2 group		
Ethane	+3.02	——
Methane	+2.26	——
Ethane–Methane	——	+0.76
Alanine–Glycine	——	+0.73
Leucine–Valine	——	+0.73

NOTE: For additional data, see Table 2-3.
SOURCE: Data from C. Tanford, *J. Am. Chem. Soc.* 84:4240 (1962).


Free energy change for transferring various amino acids from water to 8 M urea at 25°C

Amino acid	ΔG (kcal mole^{-1})	ΔG_t (kcal mole^{-1})
Glycine	+0.10	0
Alanine	+0.03	−0.07
Leucine	−0.28	−0.38
Phenylalanine	−0.60	−0.70
Tyrosine	−0.63	−0.73

SOURCE: Data from P. L. Whitney and C. Tanford, *J. Biol. Chem.* 237:1735 (1962).

3. For single molecule measurements, molecules are often anchored to a substrate using a streptavidin-biotin linkage. The reason for tethering the individual molecules to the surface is to allow observation of the same molecules over several minutes. The dissociation constant, K_d, of biotin binding to streptavidin is ~0.005 pM (5×10^{-15} M). Assuming a diffusion limit on rate:

$$k = 4\pi r_0 (D_A + D_B) N_0 \times 10^{-3}\ s^{-1}M^{-1}$$

with a radius of interaction of 30 Å and composite diffusion constant of 90 µm^2/s, how quickly will the molecules dissociate from the surface (i.e. what is the off rate)? How quickly would the molecule dissociate if the dissociation constant were 5 nM?

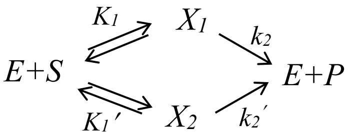
Note: In the given formula for diffusion limit rate constants, the unit of distance for the radius of interaction is cm, the diffusion coefficient is given in cm^2/s and N_0 is Avogadro's number.

4. Single molecule measurements on protein folding (Schuler et al, Nature 2002 **419**:743) estimated the enthalpy barrier for folding using Kramer's Equation:

$$\tau_{\mathrm{f}} = \frac{2\pi\omega_{\min}\tau_0}{\omega_{\max}}\exp\left(\frac{\Delta}{k_B T}\right)$$

Describe this equation using the framework of transition state theory.

5. Derive an expression for $V = d[\mathrm{P}]/dt$ for the mechanism:



Express V in terms of $[\mathrm{E}]_0$ = total enzyme concentration, and [S] = free substrate concentration. The following reactions may be assumed to be in equilibrium while P is produced:

$$E + S \rightleftarrows X_1$$
$$E + S \rightleftarrows X_2$$

Hence, you need not use the usual steady-state assumption. Is it possible to distinguish this reaction from the Michaelis-Menten expression for the following reaction:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

6. The absorbance cross-section of a molecule is defined as: $\sigma = \mathrm{P}\,\pi\,\mathrm{r}^2$
where P is the probability that light impinging on a molecule is absorbed.

a) Derive an equation for the extinction coefficient where ε is a function of σ.
b) Consider a chromophore with an absorbance cross-section $\sigma = 6.537 \cdot 10^{-14}\ \mathrm{cm}^2$. Determine the chromophore's extinction coefficient and the absorbance A of a 7 μM solution measured in a 1 cm long cuvette.

Hint: The fraction of light absorbed can be written as $\frac{dI}{I} = \frac{-P \cdot \pi \cdot r^2 \cdot c \cdot N_A}{1000} \cdot \mathrm{dl}$.