BIOC530 2012: Homework 1
Due 10/10

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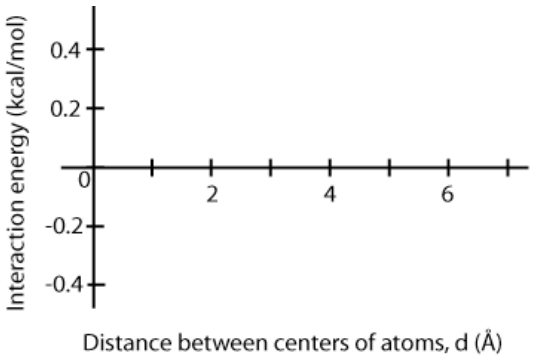
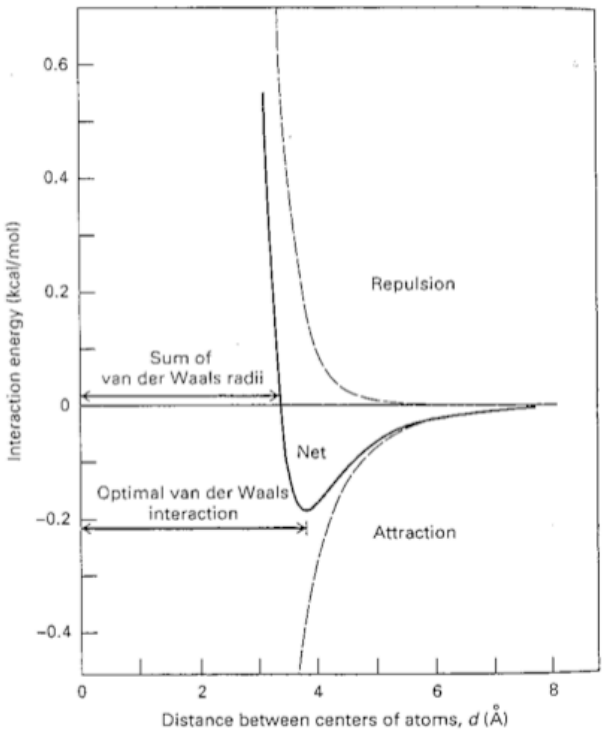
The following problems are based on David Baker's lectures of forces and protein folding. When numerical values are not specified, you should be able to find appropriate values in lecture notes.

1. Using the Lennard-Jones parameters from the lecture notes sketch out or plot the attractive and repulsive contributions (and their sum) to the interaction energy of a hydrogen and a oxygen atom as a function distance.



Solution:



2. What is the Lennard-Jones interaction energy between a nitrogen and an oxygen

when these are 6 Å apart? Use the parameter table from the lecture notes.

Solution:

The equation for Van der Waal's interaction energy is:
$EVdW = \varepsilon ij [(\sigma ij/rij)^{12} – 2(\sigma ij/rij)^{6}]$
$\varepsilon ij = sqrt(e_n \times e_o) = sqrt(0.24 \times 0.16) = 0.196$
$\sigma ij = r_n + r_o = 1.6 + 1.6 = 3.2$
rij = distance between the atoms = 8
$EVdW = 0.196[(3.2/6)^{12} – 2(3.2/6)^{6}]$
= -0.0088 kcal/mol

3. What is the electrostatic interaction energy of a lysine nitrogen and a glutamate oxygen when they are separated by 5 Å (Assume the charge on the nitrogen is +1 and the charge on the oxygen is -1.)
   (a) in water?
   (b) in the protein interior (assumed to be a homogeneous medium with dielectric constant 4.)

Solution:

$$E_{ES} = k\left(\frac{q_1 q_2}{\varepsilon r}\right)$$

Where r=5, q_1=1, q_2 = -1
k = 332 (kcal/mol) Å/e (this is just a conversion factor, given in the notes. It is not the same as the "k" in the entropy equations, which is Boltzmann's constant)

in water. $\varepsilon \approx 80$
E = 332(-1/80*5)
= -0.83 kcal/mol

in protein interior, $\varepsilon = 4$
E = 332(-1/4*5)
= -16.6 kcal/mol

4. Calculate the ΔG for moving two charges (+1 and -1) from an organic solvent with dielectric constant 1 into water, keeping their distance fixed at:
   a) r_{ij} = 3.5 Å
   b) r_{ij} = 7.5 Å

Solution:

The equation for electrostatic interaction energy between two charges is:

$$E_{ES} = k\left(\frac{q_1 q_2}{\varepsilon r}\right)$$

Where r=3.5, q_1=1, q_2 = -1, and remember that k = *332 (kcal/mol) Å/e*
Remember from the notes that "the energy associated with transferring a charge q from a vacuum (dielectric constant of 1) to a dielectric medium is thus:"

$(1/\varepsilon - 1)\, k\, q_2/2R$ (this is the solvation energy)

Moving the charges from organic solvent (ε=1) to water (ε=80):

$$\Delta G = k\left(\frac{q_1 q_2}{80r}\right) - k\left(\frac{q_1 q_2}{1r}\right)$$

But remember, we must add the solvation energy (for both charges):

$$\Delta G = \left(k\left(\frac{q_1 q_2}{80r}\right) - k\left(\frac{q_1 q_2}{1r}\right)\right) + \left(\left(\frac{1}{\varepsilon} + 1\right)k\left(\frac{q_1^2}{2R}\right)\right) + \left(\left(\frac{1}{\varepsilon} + 1\right)k\left(\frac{q_2^2}{2R}\right)\right)$$

Remember that R is the distance from the center of the ion to the solvent.
Solvation energy will be the same for both ions, so I double the term in the calculations below:

ΔG=332(-1/(80*3.5))-332(-1/(1*3.5))+2*(((1/80)-1)*332*((1)/(2*1.6))
ΔG=-111.2 kcal/mol

b)
Same equations, using r=7.5:
ΔG=332(-1/(80*7.5))-332(-1/(1*7.5))+2*(((1/80)-1)*332*((1)/(2*1.6))
ΔG=-161.2 kcal/mol

5. In mass spectrometers, proteins are dispersed into the gas phase. Would you expect proteins to be more or less stable in the gas phase? (Answer this by listing how the contributions to protein stability of each of the major forces would change for a protein in gas phase compared to a protein in water.)

Solution

Remember that folding intrinsically involves a decrease in entropy. When a disordered peptide

folds into an ordered structure, there is an entropic cost associated. The more "stable" a protein, the better this cost is offset by the other energies involved. There are four forces to consider:

a. Hydrogen bonds (dipole-dipole interactions):

In water, an unfolded protein has ideal hydrogen bonds, since the water is polar and it will interact favorably with any dipole in the protein. The folded protein will very rarely (if ever) have *ideal* hydrogen bonds because the process of folding will inevitably cause some of the polar groups within the protein to be in less-than-ideal environments where they don't interact with surrounding atoms as favorably as they would if they were surrounded with water. However, typical proteins fold such that *most* of the h-bonds between the water and the protein that are broken by folding are replaced by h-bonds within the protein. If there were too many h-bond donors & acceptors that were left unaccommodated within the protein interior, the protein wouldn't fold into a stable structure. So in water, h-bond interactions favor the unfolded state *slightly*, but are reasonably favorable in the folded state as well.

In gas, the unfolded protein would have no polar solvent to interact with, so hydrogen bonds are exclusive to the folded state. In gas, h-bond interactions favor the folded state much more than the unfolded state, so, considering only h-bonds, the fold is more stable in gas than water.

b. Electrostatics (interactions between fixed charges):

The same reasoning as with hydrogen bonds applies here. In water (or, more realistically, in a buffer that has salt ions dissolved in it), an unfolded protein has ideal electrostatic interactions with dissolved salts. The folded protein will very rarely (if ever) have perfectly *ideal* electrostatic interactions because the process of folding will inevitably cause a lessthan-perfect environment for some charges. However, typical proteins fold such that the charges that are internalized by folding are interacting *nearly* as well with their environments as they would in water so that the increase in energy of these charges as the protein folds is minimized; otherwise, the protein wouldn't fold! So in water, electrostatic interactions favor the unfolded state *slightly*, but are reasonably favorable in the folded state as well.

In gas, there is no solvent to interact with the charges of the unfolded protein, so favorable interactions that may have been going on in the interior of the folded protein will be lost (and replaced with nothing) when the protein unfolds. In gas, electrostatic interactions favor the folded state much more than the unfolded state.

c. Van der Waals interactions:

Again, we follow the same reasoning: VdW's are ideal in the unfolded state, less than ideal but almost as favorable in the folded state. In water, VdW's interactions are slightly more favorable in the unfolded state than in the folded state. In gas, VdW's favor the folded state much more than the unfolded state, since when the protein unfolds, there's no solvent for the protein to interact with. In gas, there is no solvent to interact with the charges of the unfold

d. "Hydrophobic effect"

The hydrophobic effect is the energy associated with opening a cavity in water and placing the protein into it. Cavities in water disrupt the H-bonding of the water – molecules that were next to each other are now separated and can't interact with each other. Furthermore, whereas the water molecules, if left to themselves, could adopt many favorable conformations, when they interact with protein, they have to align with the polar groups on

the surface of the protein (which exacts a significant entropy cost). By folding up, the protein sequesters its hydrophobic groups away from the water into the center of the protein, which decreases the disruption of H-bonding in the water. The hydrophobic effect therefore greatly favors the folded state to the unfolded state. **In fact, this is the primary driving force for the folding of the protein. The decrease in free energy associated with decreasing the interface between hydrophobic groups and water is the primary offset to the entropic cost of folding.**

In gas, of course, there is no hydrophobic effect. There is no water around to prefer the sequestration of hydrophobic groups into the center of the protein. The hydrophobic effect doesn't exist, and hence the primary driving force of folding doesn't exist. The entropic cost of folding in gas will be *slightly* offset by the other three forces listed above, but without the hydrophobic effect, the protein fold will be very unstable: the protein will denature in gas.

6. A lysine side-chain on the protein surface can adopt one of 81 possible rotamer conformations with equal probabilities. When the protein forms a complex with anther protein, only one rotamer is possible (the others would bump into the other protein). What is the free energy loss associated with this entropy loss at room temperature?

Solution:

The free energy change associated with entropy changes is:
$-T\Delta S$

Now all you need to know is the temperature (given as "room temperature" which is about 300K), and the change in entropy. The change in entropy is given in the lecture notes:

$\Delta S = k \ln(\Omega 2) - k \ln(\Omega 1)$

The Ω refers to the number of possible conformations; this goes from an initial value of 81 to a final value of 1. The constant *k* is Boltzmann's constant, given in the lecture notes as 0.00198. This is different from the conversion factor *k* used earlier!

$= 0.00198 \ln (1) - 0.00198 \ln(81)$
$= -0.0087$ kcal/molK

Since this is a negative number, the entropy decreased (i.e. the *disorder* decreased).When you apply the free energy equation, you see that the free energy change is positive, as it should be for a decrease in disorder.

$\Delta G = -T\Delta s = -300$ Å~ (-0.0087)
$= +2.61$ kcal/mol

7. If the free energy difference between unfolded state and folded state of protein X is 4.0 kcal/mol, what is the ratio of the two populations (the equilibrium constant) at 300K?

Solution:

According to the Boltzmann equation, the ratio of folded to unfolded molecules is:
$\Delta G = -k\ T\ \ln Keq$
Rearranging this to isolate Keq (which is the equilibrium ratio of one state over the other)
$K_{eq} = e^{(-\Delta G/kt)}$

8. A small protein has a ΔH of folding=3 kcal/mol and ΔS of folding=50 cal/K mol.
   a) Calculate the temperature at which the ratio of folded to unfolded protein is 10:1
   b) Calculate the melting temperature (the temperature at which half the protein will be folded and half will be unfolded).

Solution:

$K_{eq} = e^{-(\Delta G/kT)}$
Substituting in the Gibbs free energy equation:
Note: Remember that ΔG in this case will be for the protein going from the unfolded
state to the folded state, so you must reverse its sign.
$K_{eq} = e^{-(-\Delta G/kT)}$
$K_{eq} = e^{-((-(\Delta H-T\Delta S))/kt)=e^{-((T\Delta S-\Delta H))/kt)}}$
Solve for T:
$T=\Delta H/(\ln(K_{eq})K+\Delta S)$
Remember to change ΔS from cal to kcal:
ΔH=3 kcal/mol, ΔS=50 cal/K mol = 0.05 kcal/K mol, K=0.00198 kcal / K mol
a)
K_{eq}=folded/unfolded=10/1=10
$T=\Delta H/(\ln(K_{eq})K+\Delta S)$
$T=3/(\ln(10)*0.00198+0.05)$
T=54.98°K
b)
K_{eq}=folded/unfolded=1/1=1
$T=\Delta H/(\ln(K_{eq})K+\Delta S)$
$T=3/(\ln(1)*0.00198+0.05)$
T=60°K

9. A protein has three states:

Unfolded state--200 configurations, all with a +1 charge and a -1 charge 12Å apart, and two carbon atoms 10Å apart.

Intermediate state--50 configurations, all with a +1 charge and a -1 charge 8Å apart, and two carbon atoms 7Å apart.

Native state--1 configuration, with a +1 charge and a -1 charge 4Å apart, and two carbon atoms 4Å apart, and 3 hydrogen bonds worth 1kcal/M.

The charges are exposed to solvent in all three states.

Is the protein in the native state a) at 300K? b) at 400K?

Hint: "in the native state" means has a probability of >50%

Solution:

For each state Van Der Waals and Coulombic interaction energy needs to calculated using:

Solution:

The equation for Van der Waal's interaction energy in the native is:

$EVdW = \varepsilon ij\,[(\sigma ij/rij)^{12} - 2(\sigma ij/rij)^{6}]$
$\varepsilon ij = sqrt(e_n \times e_o) = sqrt(0.12 \times 0.12) = 0.12$
$\sigma ij = r_n + r_o = 2.1 + 2.1 = 4.2$
rij = distance between the atoms = 4
$EVdW = 0.12[(4.2/6)^{12} - 2(4.2/6)^{6}]$
= -0.106 kcal/mol

$$E_{ES} = k\left(\frac{q_1 q_2}{\varepsilon r}\right)$$

Where r=5, q_1=1, q_2 = -1
k = 332 (kcal/mol) Å/

in water. $\varepsilon \approx 80$
E = 332(-1/80*4)
= -1.00625 kcal/mol

Interaction energy(native) = -1.00625 + -0.106 + -3 (hydrogen bonds = -4.112 kcal/mol

Before partition function = configurations * exp(-interaction energy/kT)
= 1 * exp(4.112/(.6)) = 9.90E+02

But remember that this number is meaningless unless you use a partition function that includes all of the available states.

$Actual\_Prob_{native} = Prob_{native} / (Prob_{native} + Prob_{intermeidate} + Prob_{unfolded})$

So you have to calculate all of the probabilities for all the states:

300K	interaction energy	hydrogen bonds	configurations	before partition function	probability
unfolded	-0.33631	0	200	3.52E+02	0.24
intermediate	-0.514	0	50	1.18E+02	0.08
native	-4.112	3	1	9.90E+02	0.68

At 300K the probability of being in the native state is 68%

.68 = 9.90E+02/(9.90E+02 + 1.18E+02 + 3.52E+02)

And recalculated at 400K:

400K	interaction energy	hydrogen bonds	configurations	before partition funtion	probability
unfolded	-0.33631	0	200	3.05E+02	0.53
intermediate	-0.514	0	50	9.55E+01	0.17
native	-4.112	3	1	1.77E+02	0.31

At 300K the probability of being in the native state is 31%