Name:

Rec. Exam Biophysics. January 2021
Bioinformatics degree. Part 1

1) (0.5 point) Consider the following two configurations with the same energy. Configuration A: {150, 150, 320, 180, 50, 25, 15} and configuration B: {160, 140, 340, 160, 40, 35, 15}. Calculate which configuration has the greatest weight. Discuss which configuration is the most probable.

$$lnW = NlnN - \sum n_i \, lnn_i$$

$$lnW_A = 1443$$

$$lnW_B = 1433$$

$$W_A > W_B$$

$S = k_B lnW$ Thus, $S_A > S_B$ and consequently the configuration A is the most probable

2) (1.5 points) Initially, we have two separated systems with CO_2 and O_2 molecules in two containers.
a) Calculate the kinetic energy of a single molecule of CO_2 in a container of 0.5 litres having $2 \cdot 10^{18}$ molecules of CO_2 at 35ºC; and calculate the kinetic energy of a single molecule of O_2 in a container of 0.6 litres having $3 \cdot 10^{18}$ molecules of O_2 at 55ºC.
b) Calculate the total energy of the CO_2 container, and also the total energy of the O_2 container.
c) Consider now that both containers are put together (having a global volume of 1.1 litre), mixing CO_2 and O_2 molecules and reaching the thermodynamic equilibrium. Calculate: I) the total energy of the global system; ii) the kinetic energy of each of a single CO_2 or O_2 molecule; iii) and the final temperature of the system. In all calculations, assume ideal gases having only kinetic energy.

Name:

$$v^{rms} = \sqrt{(3\,RT/M)} = \sqrt{(3\,k_B T/m)}$$

$$E_k = \frac{1}{2}mv^2 = \frac{3}{2}k_b T$$

The energy per molecule is:
$E_k(CO_2) = 6.3810^{-21}J \qquad E_k(O_2) = 6.8010^{-21}J$

$$E(CO_2 system) = NumCO_2 * E_k(CO_2) = 0.013J$$

$$E(O_2 system) = NumO_2 * E_k(O_2) = 0.020J$$

$$Etotal = NumCO_2 * E_k(CO_2) + NumO_2 * E_k(O_2) = 0.033J$$

$$Ek_{finalpermolec.} = E_{total}/(NumCO_2 + NumO_2) = 6.6310^{-21} Jpereachmolecule$$

$$T_{final} = \frac{E_{final} * 2}{3k_B} = 320K = 47C$$

Name:

3) (1.0 points) An enzyme with a flexible inner cavity could adopt three different macromolecular conformations: open, semi-open or closed conformations. One experiment carried out at 37°C determined the following populations 55%, 40% and 5%, for open, semi-open and closed conformations, respectively. a) Assuming that the degeneration of open and closed conformations are 1, and the degeneration of the semi-open conformation is 2, calculate the relative energy of these conformations.
b) Calculate the proportions of these conformations if we make a new experiment at 4°C.

$$p_i = g_i exp\,(-E_i/(RT))/q$$

$$q = \sum g_j\, exp\left(-E_j/(RT)\right)/q$$

$$g_0 = 1; g_1 = 2; g_2 = 1$$

$$E_0 = 0\,J/mol$$

$$q = exp\,(-E_0/(RT))/p_0 = 1/0.55 = 1.81$$

$$E_1 = -RTln(p_1 * q/g_1) = 2608\,J/mol$$

$$E_2 = -RTln(p_2 * q/g_2) = 6183\,J/mol$$

b) First, calculate the partition function for temperature of 4°C.

$$q = \sum g_j\, exp\left(-E_j/(RT)\right)/q = 1.71$$

Calculate the new proportions with $p_i = g_i exp\,(-E_i/(RT))/q$

$$p_0 = 0.58; p_1 = 0.38; p_2 = 0.04$$

Name:

4) (1.0 points) In the second order reaction A → Products, the concentration of A is [A] = 0.16 M at the initial time of the reaction, and [A]=0.08 M after 42 minutes. a) Determine the value of the rate constant.
b) Which will be the concentration of [A] at 3.5 h?

$$1/A_t = 1/A_0 + kt$$

$$k = 0.1488 M^{-1} s^{-1}$$

$$A_{3.5h} = 1/(1/A_0 + kt_{210min}) = 0.027M$$

Name:

5) (1.0 points) It is studied a reaction A+B→ P using different initial concentrations of the both reactants at several temperatures. A summary of the experiments is provided in the table.

a) Determine the partial order of A and B, and the rate constant at 30ºC

b) Determine the activation energy of this reaction.

Experiment	$[A]_0$ / (mol dm^{-3})	$[B]_0$ / (mol dm^{-3})	v_0 /(mol dm^{-3} s^{-1})	Temperature/ºC
1	0.252	0.142	1.35	30
2	0.504	0.142	5.43	30
3	0.504	0.213	5.44	30
4	0.252	0.142	2.79	70

$$v = k[A]^{\alpha}[B]^{\beta}$$

$$\alpha = ln\,(v_2/v_1)/ln\,(A_2/A_1) = 2$$

$$\beta = ln\,(v_3/v_2)/ln\,(A_3/A_2) = 0$$

From any experiment (from 1 to 3), we can obtain the value of the rate constant:

$$k_{30C} = 21.3\,L/(mols)$$

b) Using experiment 4, we can obtain the value of the rate constant at 70C:

$$k_{70C} = 43.9\,L/(mols)$$

Using Arrhenius $k = Aexp(-E_a/RT)$ and making a system with the two temperatures it can be obtained the activation energy:

$$E_a = 1596\,J/mol$$

Name:

Name:

Rec. Exam Biophysics. January 2021
Bioinformatics degree. Part 2

6) (2 points) Justify briefly the following sentences (all them are true)

a) A classical forcefield applied to a single structure cannot be used to evaluate solvation energy in water.

Classic forcefields cannot calculate entropy with a single structure (it can be calculated after generating a representative set of states). Since solvation energy has a strong entropy contribution, it cannot be obtained from a forcefield.

b) The choice of the simulation temperature in NVE molecular dynamics define the limits of the conformational space.

NVE corresponds to constant volume and constant total Energy. Once the temperature is set the total energy of the system cannot be changed. Since from that on only those conformations with an excess of kinetic energy can be visited, the selection of the temperature and total energy define which conformations are available

c) Flexible residues like Gly decrease protein stability

Flexible residues increase the degrees of freedom of the unfolded state, increasing its entropy and making it more stable, and folding less favourable.

d) In both proteins and nucleic acids, hydrogen bonds do not contribute to the stabilization energy.

The energy from a hydrogen bond is similar in a macromolecule (protein or NA) than in water, so there is no significant difference between the folded and the unfolded states.

e) Properly folded protein structures cannot be identified with classical forcefields only.

Classic forcefields define that the structure is "chemically correct", but has no terms to identify correct conformations. This can only be obtained by evaluating entropy (which is not available) or by comparison with known structures (using statistical potentials)

f) Protein folding in vivo is much more efficient than in vitro

In vivo, chaperones stabilize unfolded structures until they fold properly. This increases the efficiency of the folding (all synthetized proteins fold correctly).

g) NPT is the most realistic simulation ensemble

NPT corresponds to constant temperature and pressure, very much similar to laboratory experiments, so is the most realistinc ensemble,

h) The binding energy between the two components in a protein complex requires to estimate their solvation energies.

The binding energy can be obtained from the interaction energy and the change in solvation from the unbound components to the complex. Since solvation energy of the components of the complex is necessary

i) A hyperbolic curve when representing the degree of saturation in a binding process indicates that a protein is involved

A hyperbolic curve indicates a saturation process, i.e. from some concentration of the ligand the degree fo saturations does not increase. This is an indication of a limit in the amount of binding sites available, indicating that a "receptor" for the binding, i.e. a protein is required.

Name:

j) Folding “in vivo” is helped by accelerating the isomerization of Pro residues.

Pro residues can exist in both cis and trans backbone conformations, and the cis-trans isomerization is slow. Fast folding is helped by isomerases, enzymes able to catalyzed the isomerization of Pro residues,

7) (1 point) Indicate which of the following energy terms are Favourable (F), Unfavourable (U) or Indifferent (I) for protein folding

U Conformational entropy
F Solvent entropy
I Hydrogen bonds
F Electrostatic interactions inside the protein
I Electrostatic interactions in the surface of the protein
F Van der Waals interactions

Comments (not required):

U Conformational entropy
Folding implies a decrease in conformation entropy (selecting a specific fold from a lot of unfolded conformations). A decrease in entropy is unfavourable

F Solvent entropy
Most relevant driving force in folding is hydrophobic effect that is a consequence of solvent entropy.

I Hydrogen bonds
Hydrogen bonds contribute equally to the system energy in both unfolded and folded structures, so they are indifferent in energetics terms. They contribute to the proper orientation of macromolecular interactions.

F Electrostatic interactions inside the protein
An electrostatic interaction in an environment without solvent is the strongest interactions in energetic terms,

I Electrostatic interactions in the surface of the protein
In the surface of a protein all electrostatic interactions are reduced by the dielectric effect of water, no effect in the global energetics.,

F Van der Waals interactions
Vdw energies are small but always favourable, the accumulation of all of them in a protein makes a large contribution. They assure that the protein is compact, and all contacts are fulfilled.

Name:

8) (2 points) We wish to evaluate the influence of several amino acid residues in the stability of a protein-protein complex (R-L), by analysing the interaction energy change after mutations to Ala. The following table summarizes calculated changes in interaction and solvation energies due to the modification:

Mutation	ΔΔGelec.int (Kcal mol^{-1})	ΔΔGvdw.int (Kcal mol^{-1})	ΔΔGsolv (Kcal mol^{-1})
Glu 300 Ala	10.0	0.5	-3.0
Trp 260 Ala	-0.1	1.2	3.2
Val 310 Ala	0.0	0.1	0.3
Gln 302 Ala	0.7	0.5	-1.1

a) Order these residues from less to more important in the stability of the complex.

The importance of each residues is obtained from the global change in binding energy after mutation.

ΔΔGBinding is the sum of all contributions.

Mutation	ΔΔGbind (Kcal mol^{-1})
Glu 300 Ala	7.5
Trp 260 Ala	4.3
Val 310 Ala	0.4
Gln 302 Ala	0.1

Gln302 < Val 310 < Trp 260 < Glu 300

b) Suggest explanations for the observed energy changes. Consider that Glu is a negative charged residue while Gln es polar but neutral. Trp and Val are both hydrophobic but Trp is much larger than Val.

NOTE: the question was not about explaining the importance of the residues, but about explaining the energies obtained

-All mutants. Changes in Vdw energies a related to the change in the relative size of the side chains
-Glu 300.
It is a charged residue, a large change in electrostatic energy indicates a loss of an electrostatic interaction that was important for the complex so it should be buried in the interface. Change in solvation indicates an improvement since we move from a charged residue (better solvated in the unbound form) to a mildly hydrophobic (better solvated in the complex)
-Trp 260
Most change come from the solvation energy, which is compatible with a large change in ASA on binding. Trp 260 is probably buried in the complex interface.
-Val 310
Similar reasoning as Trp 260, but since the energies are almost negligible, there is no change in ASA on binding, so Val 310 is not buried in the interface.
-Gln 302 is polar. Change in solvation energy is negative (as it was in Glu300) but much smalled, hence indicating the Gln 302 is close but not in the interface. Since it is neutral electrostatic contribution is small, probably due to the loss of some hydrogen bonds.

c) Knowing that the experimental dissociation constant for the unmutated complex is 2.4 nM, evaluate

Name:

1. the expected dissociation constants for the mutants

$$\Delta G = RTlnK_D$$

$$\Delta\Delta Gwt \rightarrow A = \Delta G_A - \Delta G_{wt} = RTln\frac{K_D^A}{K_{D}{}^{wt}}$$

$$K_D^A = K_D^{wt} e^{\frac{\Delta\Delta Gwt \rightarrow A}{RT}}$$

UNITS:

$\Delta\Delta Gwt \rightarrow A \wedge Rshouldusethesameenergyunits$!!!

$K_D^A \wedge K_D^{wt}$ K_D use the same units.

2. the degree of complex formation at a concentration of L necessary for getting a 50% of the wild-type complex.

Saturation degree comes from $Y = \frac{L}{K_D + L}$

Value of L corresponds to the necessary to get a 50% of formation of the WT complex. By definition the concentration of L that gives a 50% saturation is K_D, = 2.4 nM = L. then the saturation degree for each mutant comes from the corresponding K_D value.

Mutation	ΔΔGbind (Kcal mol^{-1})	K_D (M)	Y
Glu 300 Ala	7.5	$7.6\ 10^{-4}$ (0.76 mM)	$3.26.10^{-6}$
Trp 260 Ala	4.3	$3.42.10^{-6}$ (3.42 µM)	$7.01.10^{-4}$
Val 310 Ala	0.4	$4.72.10^{-9}$ (4.72 nM)	0.34
Gln 302 Ala	0.1	$2.84.10^{-9}$(2.84 nM)	0.46

NOTE to check results: All ΔΔGbind are positive, so all K_D's should be larger (less binding) than WT, much larger as larger is ΔΔGbind (there a logarithmic relationship), and all saturation degrees should be smaller than 0.5 (the WT one)

Name:

Name:

Additional data and equations:

M(H)=1 g mol^{-1}; M(C)=12 g mol^{-1}; M(O)=16 g mol^{-1}; M(N)=14 g mol^{-1}
kB=$1.3806488 \cdot 10^{-23}$ J K^{-1}
R=1.987 cal K^{-1} mol^{-1} = 8.314 J K^{-1} mol^{-1} = 0.082 atm L K^{-1} mol^{-1}
NA=$6.022 \cdot 10^{23}$ mol^{-1}

$$v^{mp} = \sqrt{(2\,RT/M)}$$

$$\overline{v} = \sqrt{(8\,RT/(\pi M))}$$

$$v^{rms} = \sqrt{(3\,RT/M)}$$

$$f(v) = 4\pi\left(\frac{m}{2\pi k_B T}\right)^{3/2} v^2 e^{-mv^2/(2k_B T)}$$

$$\frac{1}{[A]_0 - [B]_0} ln \frac{[B]_0[A]}{[A]_0[B]} = kt$$

Electrostatic interaction

$$E_{pot} = 332.16 \frac{q_i q_j}{\varepsilon r_{ij}}$$

q_i, q_j: partial charges (e); ε: relative dielectric constant; r_{ij}: distance (Å). Resulting energy is Kcal mol^{-1}

VdW interaction energy between equal atoms

$$Epot = 4\epsilon\left(\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right)$$

ε: well depth (Kcal mol-1), σ: Distance at Epot=0 (Å)

Free energy related to dissociation constants

$$\Delta G = RTlnK_D$$

ΔG: Process free energy (units: energy mol^{-1}, depending on the R value used). R (gas constant), T: Temperature (K); K_D: Dissociation constant (Concentrations in M)

Saturation degree in a simple binding process

$$Y = \frac{L}{K_D + L}$$

Y: Saturation degree, K_D: Dissociation constant, L: Concentration of free ligand