Name: Date:

Biophysics 2021-22
Bioinformatics degree
Recovery Exam. Part I

1) (1 point) Consider a four level system that could be populated with two different instantaneous configurations. Configuration A: {420, 60, 24, 11} and configuration B: {310, 110, 60, 35}.
a) Calculate which configuration has the greatest weight.
b) Calculate the associate entropy of these configurations.

a)
We can estimate the weight of both configurations using the Stirling's approximation.

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

For both configurations, the total number of particles, N=515

$$lnW_A = 330$$

$$lnW_B = 550$$

Thus, $W_B > W_A$

b)

$S = k_B lnW$

$$S_A = 4.5610^{-21} J/K$$

$$S_B = 7.6010^{-21} J/K$$

It is also valid to use R constant and to give the entropies in terms of J/Kmol.

Name: Date:

2) (2 points) An experiment determined that a transmembrane macromolecule could adopt three different conformations (A, B and C) at 37ºC degrees. The corresponding populations for A, B and C were determined to be 40%, 35% and 25%. Assuming that the degeneracy of the energetic level corresponding to the conformation B is 2, and the degeneracy corresponding to the energetic levels of both conformations A and C are one, determine the populations of the three conformations of the macromolecule at 5ºC.

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

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

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

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

$$q = exp\,(-E_0/(RT))/p_0 = 1/0.40 = 2.50$$

$$E_1 = -RTln(p_1 * q/g_1) = 2132\,J/mol$$

$$E_2 = -RTln(p_2 * q/g_2) = 1212\,J/mol$$

b) First, calculate the partition function for temperature of 5°C.

$$q = \sum g_j\, exp\left(-E_j/(RT)\right)/q = 2.39$$

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

$$p_0 = 0.42; p_1 = 0.33; p_2 = 0.25$$

Name: Date:

3) (1 point) In the second order reaction, A →Products, the concentration of [A] = 0.42 M at the initial time of the reaction, and [A]=0.26 M after 15 minutes at 25ºC.
a) Determine the value of the rate constant.
b) Deduce the analytical expression of the half life for this reaction, and calculate its value.
c) At which time the concentration of [A] will be 0.005 M?
d) Which will be the concentration of [A] at 7.5 h?

a)
With $1/A_t = 1/A_0 + kt$ we can obtain the value of k=0.098 $M^{-1}min^{-1}$.
b) With $2/A_0 = 1/A_0 + kt_{1/2}$ we obtain $t_{1/2} = \frac{1}{kA_0} = 24min$
c) t=2023 min
d) [A]=0.02 M

Name: Date:

4) (1 point) For a second order reaction in which the initial concentration of the reactant is 0.26 M, the rate constant is double when the temperature increases from 20ºC to 55ºC.
a) Which is the activation energy of the reaction?
b) Determine the kinetic rate constant at 80ºC if the half live at 20ºC is 3.5 seconds.

a) Using Arrhenius $k = Aexp(-E_a/RT)$ and making a system with the two temperatures (20ºC and 55ºC) it can be obtained the activation energy:

$$E_a = 15824\, J/mol$$

b) Using the expression of the half live for a second order reaction, $t_{1/2} = \frac{1}{kA_0}$, we can obtain the value of the rate constant at 20ºC, k(20ºC)=1.1 s^{-1} M^{-1}

The Arrhenius expression could be used with the previous value of the rate constant at 20ºC to determine the preexponential factor.
A=727 M^{-1} s^{-1}

Using again the Arrhenius expression for a temperature of 80ºC the value of the rate constant is:

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

Name: Date:

Biophysics 2021-22
Bioinformatics degree
Final Exam. Part II

5) (2.5 points) Justify the following statements (All of them are **true)**

a. The experimental evaluation of protein stability can be done by analysing protein properties along denaturation by guanidium chloride

The analysis of a protein property that differs between the Folded and unfolded states allow to evaluate the U/F ratio and hence the ΔG of unfolding. This data at several concentrations of denaturant allows to extrapolate the ΔG without denaturant that show protein stability

b. A larger content of buried hydrophobic residues increases the protein stability

Buried hydrophobic residues contribute favourably to folding due to the unfavourable solvation in the unfolded state that is proportional to surface. The increase of hydrophobic surface results in an increase of the hydrophobic effect.

c. Only quantum mechanics can be used to evaluate kinetic rate constants theoretically.

To evaluate rate constants the analysis of transition states and possibly the reorganization of bonds is required. Classic forcefields assume equilibrium structures and a fixed chemical sructure, and cannot be used. Only QM allows for reorganization of bonds and non.equilibrium structures

d. The protein folding process implies finding a preferred kinetic pathway from the unfolded to native states.
To evaluate all possible conformations is not feasible due to the large degrees of freedom. To achieve a folding in normal times requires a preferential pathway.

e. Protein folding is more efficient in the presence of chaperones.

Chaperones stabilize unfolded structures and allow them to retry folding, therefore the help to increase the efficiency of folding.

f. Knowledge of protein structures is required to evaluate whether a protein fold is correct

The definition of "correct fold" depends on the comparison with known structures as forcefields cannot distinguish between folded and unfolded structures

g. An initial relaxation of the structure is required before starting a simulation calculation.

To avoid numerical instability during simulation the initial structure should be in a mathematical minimum according to the forcefield to be used. An initial relaxation of the structure using molecular mechanics allow to achieve this.

h. The most realistic ensemble for a macromolecular simulation is the NPT one.

NPT refers to constant particles, temperature and pressure, that are the standard conditions in laboratory experiments and in vivo.

Name: Date:

i. The proper evaluation of binding energies in macromolecular complexes require the evaluation of hydrophobic solvation.

The evaluation of binding energies should incorporate a solvation term, the difference between the solvation energy of the complex and that of separate components. The evaluation of solvation energy includes hydrophobic solvation.

j. In an enzyme process a concentration of substrate of 100nM gives a reaction rate that corresponds to the 50% of the rate obtained at 10mM concentration of the same substrate. This finding confirms that 100nM is a good estimate of the K_M kinetic parameter.

As 10mM is much larger than 100nM, it can be assumed that corresponds to the màximum velocity. Hence the concentration of substrate giving 50% of such corresponds to the K_M

6) (0,5p) Identify terms of the left column with the methodologies (right) that are most appropriate to evaluate them theoretically.

A. Interaction energy between polar residues and solvent	A. 12-6 potentials
B. Hydrophobic solvation	B. Accessible Surface Areas (ASA)
C. Van der Waals interaction energy	C. Quantum mechanics
D. Entropic effects	D. Generalized-Born equation
E. Kinetic rate constants	E. Molecular Dynamics/Statistical Mechanics

A-D. Generalized-Born is a measure of the electrostatic component of solvation
B-B ASA can be used to estimate the hydrophobic component of solvation
C-A 12-6 potentials are the usual type of equations used to evaluate VdW energy
D-E Only simulation data (in combination with statistical mechanics) can evaluate entropy
E-C Only QM can evaluate chemical transformations and hence rate constants

Name: Date:

7) (2 p) The analysis using FOLDX of some sequence variants found on SARS-Cov-2 Spike protein from the Omicron strain regarding to the binding energy between RBD domain and ACE2 has given the following results. (Note: $\Delta\Delta G$ corresponds to $\Delta G_{Mut} - \Delta G_{Wt}$):

Variant	ΔΔGvdw (kcal/mol)	ΔΔGelec (kcal/mol)	ΔΔGsolv (kcal/mol)
Lys417Asn	0.56	2.23	-1.71
Glu484Ala	0	0	-0.23
Gln493Lys	-0.44	0.84	-0.20
Gly496Ser	-0.12	-0.85	0.35
Gln498Arg	-1.63	1.75	-0.24
Asn501Tyr	4.94	0.61	-2.08
Tyr505His	0.14	1.05	1.02

T = 298K, R = 1.987 cal/mol.K.

a) From the data provided, which would be the **order of stability** of the ACE2-RBD complex of the corresponding single-variant mutants. Considering that Omicron contains all such variants, which is the expected stability of Omicron'RBD-ACE2 (Note: Assume that variants effect is additive)

Total ΔΔGbind for each individual mutant is the sum of all energy components

Considering the Omicron protein contains all mutations, and that their effects are additive the total ΔΔGbind correspond to the sum of ΔΔGbind for all variants.

The Ratio between binding constants comes from

$$\Delta\Delta G_{bind} = \Delta G_{mut} - \Delta G_{wt} = -RT \ln K_{Bind}^{Mut} + RT \ln K_{Bind}^{WT} = -RT \ln \frac{K_{Bind}^{Mut}}{K_{Bind}^{WT}} = +RT \ln \frac{K_D^{Mut}}{K_D^{WT}}$$

Note that the ratios deduced from K_{bind} are the inverse from ratios deduced from K_D. Both were correct as long there were correctly indicated.

Variant	$\Delta\Delta G_{vdw}$ (Kcal/mol)	$\Delta\Delta G_{elec}$ (Kcal/mol)	$\Delta\Delta G_{solv}$ (Kcal/mol)	$\Delta\Delta Gbind_v$ (Kcal/mol)
Lys417Asn	0.56	2.23	-1.71	1.08
Glu484Ala	0	0	-0.23	-0.23
Gln493Lys	-0.44	0.84	-0.20	0.2
Gly496Ser	-0.12	-0.85	0.35	-0.62
Gln498Arg	-1.63	1.75	-0.24	-0.12
Asn501Tyr	4.94	0.61	-2.08	3.47
Tyr505His	0.14	1.05	1.02	2.21

The order of COMPLEX STABILITY from most to least stable is

Gly486Ser > Glu484Ala > Gln498Arg > Gln493Lys > Lys417Asn > Tyr505His > Asn501Tyr

b) Calculate the ratio of the complex binding constant of Omicron's (all variants) with respect to WT Spike

The total ΔΔGbind for the Omicron RBD-ACE2 is **+5.99 Kcal/mol**, the corresponding ratio in Kbind is 4.04 10^{-5}, and the ratio in K_D is 2,44.10^4

Name: Date:

c) Indicate (considering the energy values) whether the following statements regarding the above variants are **True of False (justify briefly)**

a. Gln493Lys shows a Vdw collision due to increased sidechain
False. $\Delta\Delta G_{vdw}$ is negative. A collision should give a positive values

b. Gln493Lys, Gly496Ser, and Gln498Arg show an improved side-chain packing
True, all them have negative $\Delta\Delta G_{vdw}$ indicating that side chains are better packed

c. Asn501Tyr shows an increase in the hydrophobic component of binding.
True. $\Delta\Delta G_{solv}$ is negative indicating an increased unfavourable solvation energy before binding, as expected from the amino acid change.

d. Lys417Asn shows an increase in the hydrophobic component of binding.

False. $\Delta\Delta G_{solv}$ is negative but none of the involved amino acids is hydrophobic, so the change should correspond to a decrease in the electrostatic solvation (as can be expected from a change to a charged residue to a neutral one)

e. Tyr505His shows loss of electrostatic interaction

True. $\Delta\Delta G_{elec}$ is positive. As none of the amino acids is charged the value should correspond to differences on hydrogen bond patterns.

Additional data and equations:

$k_B = 1.3806488 \cdot 10^{-23}$ 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}
$N_A = 6.022 \cdot 10^{23}$ mol^{-1}