Biophysics final exam

1. Statistical thermodynamics and chemical kinetics. Consider a chemical system made of particles that can be in three energy levels: 0 kJ/mol, 1.2 kJ/mol and 2.4 kJ/mol.

- Calculate the population (probability) of particles corresponding to these energy levels at T= 25ºC. **(1 point)**

0 kJ/mol → 0 J/molecule
1,2 kJ/mol → $1.99 \cdot 10^{-21}$ J/molecule
2,4 kJ/mol → $3{,}98 \cdot 10^{-21}$ J/molecule

$E_0/KT = 0$ → $e^{-E_0/KT} = 1$
$E_1/KT = 0.48$ → $e^{-E_1/KT} = 0.62$
$E_2/KT = 0.97$ → $e^{-E_2/KT} = 0.38$

$q = 1 + 0.62 + 0.38 = 2$

$P_0 = ½ = 0.5$
$P_1 = 0.62/2 = 0.31$
$P_2 = 0.38/2 = 0.19$

- At what temperature will the population of the three states be the same? **(0.5 points)**

At infinite temperature all states become equally probable.

2. Protein folding and mutations. We want to predict qualitatively the effect of a mutation in a globular protein. This protein has the residues **E (glutamate)** and **K (lysine)** next to each other in the surface of the protein. We want to predict the effect of a mutation changing **E (glutamate)** by an **I (isoleucine)**.

- Fill the next table regarding the effect of the mutation in the **unfolded protein**. Use + to indicate contributions that increase the ΔG of the system and – to indicate contributions that decrease the ΔG of the system. Also, provide explanations for the results you include in the table. **(0.5 points)**

Unfolded protein	Wild type	Mutant	Overall ΔG
Polar interactions	0	0	0
Electrostatics	0	0	0
Van Der Waals int.	0	0	0
Solvation	--	++	++++
Total			++++

Since the protein is unfolded, we assume that no interactions are taking place between amino acids. This means that polar interactions, electrostatics and Van der Waals are zero.

For solvation, we have to imagine how the different amino acids behave in contact with water. The wild type (glutamate) is charged, meaning that will be very stable interacting with water and this will involve a reduction in ΔG (--). The mutant (isoleucine) is hydrophobic and it is not stable when interacting with water, involving an increase in ΔG (++).

In the unfolded state this mutation increases ΔG, thus reducing the stability of the unfolded state.

- Fill the next table regarding the effect of the mutation in the **folded protein**. Use + to indicate contributions that increase the ΔG of the system and – to indicate contributions that decrease the ΔG of the system. Also, provide explanations for the results you include in the table. **(0.5 points)**

Folded protein	Wild type	Mutant	Overall ΔG
Polar interactions	--	-	+
Electrostatics	---	0	+++
Van Der Waals int.	-	-	0
Solvation	--	++	++++
Total			+8

In the folded state we have interactions between amino acids:

- Polar interactions: the wild type amino acids can make hydrogen bonds with the main chain and the side chain (--), while the mutant can only make hydrogen bonds with the main chain because the side chain of isoleucine cannot make hydrogen bonds.
- Electrostatics: The wild type protein has two amino acids with opposite charge close to each other. This will create an electrostatic attraction, and since electrostatics are a strong interaction we put 3 minus signs (---). The mutant cannot make electrostatics because isoleucine is not charged (0).
- Van der Waals: the wild type and the mutant amino acids have similar sizes and contribute in the same way to the Van der Waals energy of the protein.

For solvation, since these amino acids are located in the surface of the protein, they are exposed to water molecules. Therefore, their solvation is the same as in the unfolded state, because in both situations the amino acids that we are analyzing are in contact with water.

The mutation also increases the ΔG of the folded protein, making the protein less stable.

- Use the results you obtained in the two tables you just filled to predict the effect of the mutation in the folding of the protein. Will it increase or decrease the stability of the protein? **(0.5 points)**

$$\Delta\Delta G_{A\rightarrow B} = \Delta\Delta G_{A\rightarrow B(F)}^{interP,AB} + \Delta\Delta G_{A\rightarrow B(F)}^{solvationAB} - \Delta\Delta G_{A\rightarrow B(U)}^{interP,AB} - \Delta\Delta G_{A\rightarrow B(U)}^{solvationAB}$$

ΔΔG = + 4 + 4 – 0 – 4 = 4

Since ΔΔG is higher than zero, we can say that this mutation is decreasing the stability of the protein. It is decreasing the stability of both, the folded and the unfolded state, but since this decrease is stronger in the folded state, this makes the folded protein less stable. This makes sense, since breaking an electrostatic interaction and putting a hydrophobic amino acid in the surface of a protein are two events that will decrease protein stability.

3. Protein-protein interactions. We perform an alanine scanning across the interface of a protein-protein interaction and obtain the following results:

Mutation	ΔΔG interaction (Kcal/mol)	ΔΔG solvation (Kcal/mol)
Lys244Ala	11.2	4.3
Val354Ala	0.2	0.3
Tyr278Ala	4.5	4.2

- What is the effect of each of these mutations in the stability of the interaction? What mutations are having a larger impact in this stability? **(0.5 points)**

$$\Delta G = \Delta G_{electrostatics} + \Delta G_{VanDerWaals} + \Delta G_{Solvation}$$

ΔΔGLys244Ala = 11.2 + 4.3 = 15.5 Kcal/mol

ΔΔGVal354Ala = 0.2 + 0.3 = 0.5 Kcal/mol

ΔΔGTyr278Ala = 4.5 + 4.2 = 8.7 Kcal/mol

All mutations destabilize the interaction, because all ΔΔG values are higher than zero. Here you can see the different mutations sorted from higher impact to lower impact in the stability of the interaction:

Lys244Ala > Tyr278Ala > Val354Ala

- Say if the following statements are true or false and explain why. Take into account the properties of the amino acids and how they can be involved in interactions and solvation. **(0.5 points)**

Lys244 is involved in an electrostatic interaction:

True. Lysine can make electrostatic interactions because is charged. Also, when we replace lysine by alanine (a hydrophobic amino acid that cannot make electrostatic interactions) we obtain a high positive ΔΔG. This means that a strong interaction interaction is lost due the mutation, and electrostatic interactions are usually strong.

Val354 interacts with the other protein by a hydrogen bond:

False. Valine cannot make hydrogen bonds with its side chain, which makes unlikely that makes a hydrogen bond with the other protein. Also, the disruption of this amino acid by the mutation to alanine has almost no effect in the ΔΔG of interaction. This indicates that this amino acid is not involved in a hydrogen bond.

Tyr278 is very unlikely that interacts with water molecules:

False. Tyrosine is a polar amino acid capable of interacting with water molecules and making hydrogen bonds with the OH group of its side chain. Also, when mutated to alanine (a hydrophobic amino acid) we see an increase in the ΔΔG of solvation. This means that the interaction with water is now less stable. If mutating to a more hydrophobic amino acid increases the ΔΔG of solvation, the only explanation for this is that this amino acid is interacting with water.

- Knowing that the experimental dissociation constant for the unmutated complex is 10.4 nM, calculate the dissociation constants for the mutants. **(0.5 points)**

$$\Delta\Delta G = R \cdot T \cdot \ln(K_D^{mut}) - R \cdot T \cdot \ln(K_D^{wt})$$

$$K_D^{mut} = K_D^{wt} \cdot e^{\Delta\Delta G/RT}$$

K_D(lys244) = 2366 M

K_D(val354) = $2.42 \cdot 10^{-8}$ M

K_D(tyr278) = 0.0246 M

4. Enzyme kinetics. We obtain the following results of reaction speeds for different concentrations of substrate. The concentration of enzyme is the same in all conditions.

	Reaction speed (µmol/s)	Substrate concentration (mM)
Experiment 1	3.8	0.1
Experiment 2	11.09	0.5
Experiment 3	14.6	1

Find the value of the maximum reaction speed. **(0.5 points)**

$$V = Vmax \cdot \frac{[S]}{[S] + KM} \rightarrow \quad KM = \left(Vmax \cdot \frac{[S]}{V}\right) - [S] \rightarrow$$

$$\left(Vmax \cdot \frac{[S]1}{V1}\right) - [S]1 = \left(Vmax \cdot \frac{[S]2}{V2}\right) - [S]2 \rightarrow$$

$$Vmax = \frac{[S]1 - [S]2}{\frac{[S]1}{V1} - \frac{[S]2}{V2}} \rightarrow \; Vmax = 41.9\ \mu mol/s$$

Find the value of the Michaelis constant. **(0.5 points)**

$$V = Vmax \cdot \frac{[S]}{[S] + KM} \quad \rightarrow \quad KM = \left(Vmax \cdot \frac{[S]}{V}\right) - [S] \quad \rightarrow \quad KM = 101.6\ \mu M$$

Compare the enzyme in this exercise to another enzyme whose Michaelis constant is 44 µM. Which of the two enzymes has more affinity for its substrate? If you couldn't calculate the Michaelis constant in the previous exercise, you can assume a Michaelis constant of 0.2 mM. **(0.5 points)**

The lower the KM, the higher the affinity between substrate and enzyme, because you need less substrate to saturate 50% of the enzyme molecules.

Then, the enzyme of KM = 44 µM is the one with higher affinity.

5) **(2 points)** Justify briefly the following sentences (all of them are true)

a. Protein stability can be evaluated experimentally by following changes in the heat capacity using differential calorimetry

Differential calorimetry measures the heat necessary to increase temperature. The heat absorbed in the unfolding process corresponds to ΔH (if constant pressure), and from that both ΔS and ΔG, giving the unfolding energy.

b. Replacement of ionic residues located at the protein surface by neutral polar residues is acceptable

At the surface the main interactions are with the solvent. Both ionic and neutral polar interacts well with water, so no major change should be expected.

c. Following the chemical mechanism of enzyme catalysed transformations requires the use of quantum mechanics methods

Chemical transformations involve changes in molecular structure, only QM can evaluate this.

d. Macromolecules' stable conformations are thermodynamic energy minima

If a given conformation is not an energy minimum, it will evolve towards lower energy states, so it is not stable. Only when the minimum is reached, the structure become stable.

e. Increasing the yield of protein folding "in vivo" requires stabilizing unfolded structures.

Unfolded structures may form irreversible structures. Keeping them stable prevents this behaviour and increases the yield of folding. Chaperones are the components in vivo responsible for that role.

f. Statistic potentials are the best choice to evaluate whether a protein fold is correct

Classical force-fields cannot distinguish between folded and unfolded structure. Statistical potentials however compare the structure with known protein structures and allows to assess whether the fold is comparable to normal proteins.

g. Molecular mechanics is the initial step on most simulation procedures

Molecular mechanics gives the energy minimum closest to the initial structure. It assures that the structure is a mathematical minimum in the used force-field, what avoids numerical errors in the simulation.

h. NPT and explicit solvent are the recommended ensemble in Molecular Dynamics simulations

Explicit solvent molecules (normally using PBC) and constant pressure and temperature, are the conditions most similar to experimental setups.

i. A hyperbolic shape in the plot of binding degree against ligand concentration indicates the participation of a protein in the process

   A hyperbolic shape is indictive that there is a limited number of binding sites that can be “saturated”.

j. During a MD simulation the structure visits more often conformations that correspond to lower energies.
   MD simulations gives population distributions equivalent to those in normal solutions. Therefore, the lowest energy states correspond to the most populated ones (following Boltzmann law)

6) **(1 point)** Regarding macromolecular energies Classify the following energy terms according to their influence in macromolecule stability (favourable (F) / unfavourable (U) / indifferent (I))

   i. __U__ Macromolecule conformational entropy
   ii. __F__ Stacking interactions between nucleic acid bases
   iii. __F__ Entropy of solvent
   iv. __F__ Electrostatic interaction between residues inside proteins
   v. __F__ Van der Waals energies

7) (1 point) Regarding transport processes identify pairs of concepts that are related

A) Fick’s law	a) Primary active transport
B) ATP hydrolysis	b) Secondary active transport
C) Concentration Gradient	c) Free diffusion
D) Membrane potential	d) Protein mediated transport
E) Hyperbolic saturation curve	e) Ionic concentration gradient

A – c
B – a
C – b
D – e
E – d