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)**

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

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			
Electrostatics			
Van Der Waals int.			
Solvation			
Total			

- 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			
Van Der Waals int.			
Solvation			
Total			

- 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)**

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)**

- 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:

Val354 interacts with the other protein by a hydrogen bond:

Tyr278 is very unlikely that interacts with water molecules:

- Knowing that the experimental dissociation constant for the unmutated complex is 10.4 nM, calculate the dissociation constants for the mutants. Assume a temperature of 25 ºC. **(0.5 points)**

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)**

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

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)**

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

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

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

d. Macromolecules' stable conformations are thermodynamic energy minima

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

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

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

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

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

j. During a MD simulation the structure visits more often conformations that correspond to lower energies.

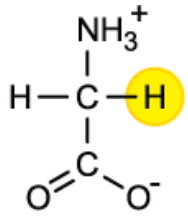
6) **(1 point)** Regarding macromolecular energies Classify the following energy terms according to their influence in macromolecule stability (favourable (F) / unfavourable (U) / indifferent (I))

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

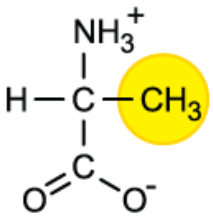
7) (1 point) Regarding transport processes identify pairs of concepts that are related

A) Fick's law
B) ATP hydrolysis
C) Concentration Gradient
D) Membrane potential
E) Hyperbolic saturation curve

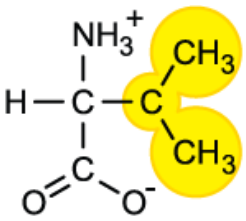
a) Primary active transport
b) Secondary active transport
c) Free diffusion
d) Protein mediated transport
e) Ionic concentration gradient



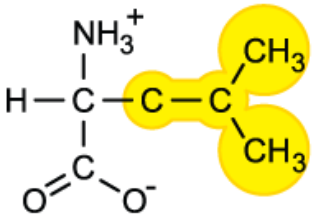
Glycine (G) – Gly



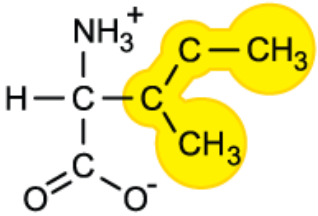
Alanine (A) – Ala



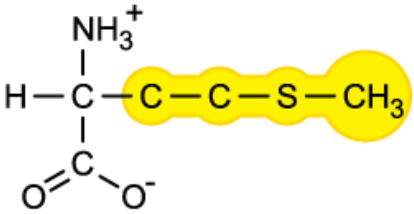
Valine (V) – Val



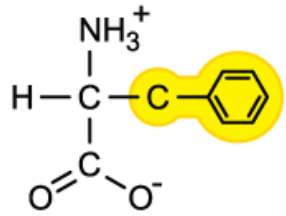
Leucine (L) – Leu



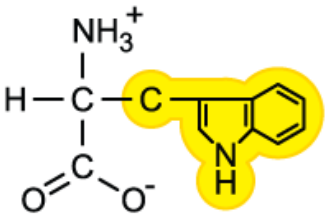
Isoleucine (I) – Ile



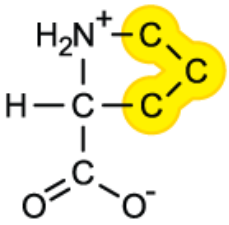
Methionine (M) – Met



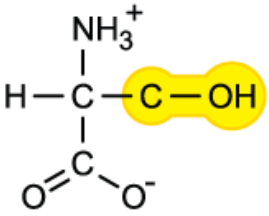
Phenylalanine (F) – Phe



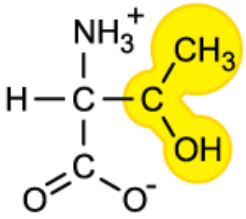
Tryptophan (W) – Trp



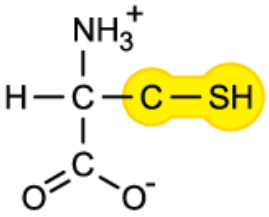
Proline (P) – Pro



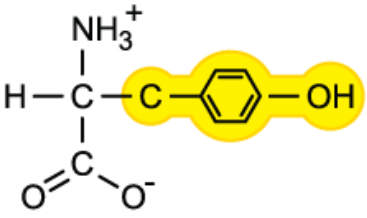
Serine (S) – Ser



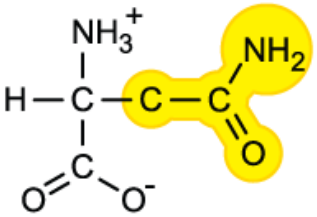
Threonine (T) – Thr



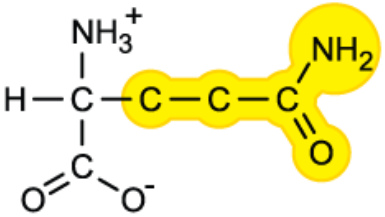
Cysteine (C) – Cys



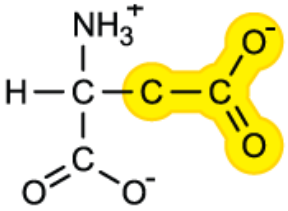
Tyrosine (Y) – Tyr



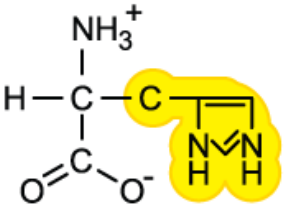
Asparagine (N) – Asn



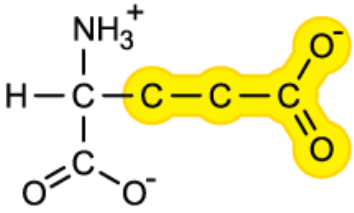
Glutamine (Q) – Gln



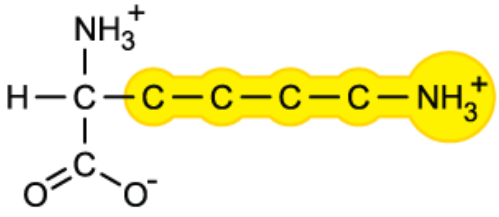
Aspartate (D) – Asp



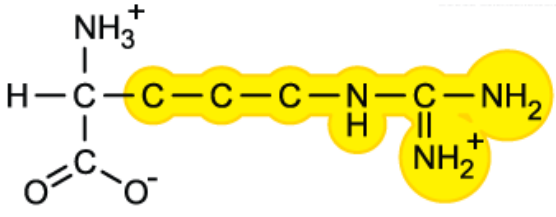
Hisidine (H) – His



Glutamate (E) – Glu



Lysine (K) – Lys



Arginine (R) – Arg

Formulas:

$\Delta G = \Delta H - T \cdot \Delta S$

$$W = \frac{N!}{N_0!N_1!N_2!N_i!}$$

$S = K_B \cdot \ln(W)$

$$\frac{N_i}{N} = \frac{e^{-E_i/kT}}{\sum_n e^{-E_n/kT}}$$

Order	*Differential form*	*Integrated form*	*Half live*	*Units of k*
0	$-\frac{d[\mathrm{A}]}{dt} = k$	$[A] = [A]_o - k_A t$	$\frac{[A]_0}{2k}$	$\mathrm{M\ s^{-1}}$
1	$-\frac{d[\mathrm{A}]}{dt} = k[\mathrm{A}]$	$[A] = [A]_o e^{-k_A t}$	$\frac{\ln 2}{k}$	$\mathrm{s^{-1}}$
2	$-\frac{d[\mathrm{A}]}{dt} = k[\mathrm{A}]^2$	$\frac{1}{A} = \frac{1}{A_o} + kt$	$\frac{1}{[A]_0 k}$	$\mathrm{M^{-1}\ s^{-1}}$
2'	$-\frac{d[\mathrm{A}]}{dt} = k[\mathrm{A}][\mathrm{B}]$	$\frac{1}{[\mathrm{A}]_0-[\mathrm{B}]_0} \ln \frac{[\mathrm{B}]_0[\mathrm{A}]}{[\mathrm{A}]_0[\mathrm{B}]} = kt$		$\mathrm{M^{-1}\ s^{-1}}$

$$k = Ae^{-E_a/RT}$$

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

$$K_{(equilibrium\ constant)} = \frac{[AB]^{ab}}{[A]^{A} \cdot [B]^{B}}$$

$$K_{D(dissociation\ constant)} = \frac{[A][B]}{[AB]}$$

$$K_{i(Inhibition\ constant)} = \frac{[R][L]}{[RL]}$$

$$\Delta G = R \cdot T \cdot \ln(K_D)$$

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

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

$$Y_{(saturation\ degree)} = \frac{L}{Kd + L}$$

$$V_{(reaction\ speed)} = k_2 \cdot [ES]$$

$$K_{M(Michaelis\ constant)} = \frac{[E] \cdot [S]}{[ES]}$$

$$V_{(reaction\ speed)} = k_2 \cdot [E]total \cdot \frac{[S]}{[S] + KM}$$

$$V_{max} = k_2 \cdot [E]total$$

Constants:

$$N_A = 6.022 \cdot 10^{23}$$

$$k_B = 1.38 \times 10^{-23}\ J \cdot K^{-1}$$

$$R = 8.314\ J \cdot K^{-1} \cdot mol^{-1}$$

$$1\ cal = 4.184\ J$$

$$0\ ºC = 273.15\ K$$