

## **Biophysics retake exam**

**1. Statistical thermodynamics.** It has been seen that a human transmembrane protein could make conformational changes to adopt an active or an inactive state. The estimated free energy for the active state of the protein is -164.5 kJ/mol. The free energy of the inactive state is -166 kJ/mol. Assuming that the human body temperature is 37 °C and that in each cell there are  $5 \cdot 10^6$  of these proteins, find out how many proteins per cell are in the active state and how many are in the inactive state. **(1 point)**

**2. Chemical kinetics.** For a second order reaction, the initial concentration of the reactant was 1.2 M. After 12 minutes, the concentration of this reactant was determined to be 0.84 M.

- Determine the value of the rate constant. **(0.5 points)**
- Using the integrated expression of the velocity, deduce the analytical expression of the half life for this reaction, and calculate its value. **(0.5 points)**
- At which time the concentration of [R] will be 0.18 M? **(0.5 points)**
- Which will be the concentration of [R] at 10 h? **(0.5 points)**

**3. Protein folding and mutations.** We want to predict qualitatively the effect of a mutation in a globular protein. This protein has the residues **L (leucine)** and **I (isoleucine)** next to each other buried in the core of the protein. We want to predict the effect of a mutation changing the **L (leucine)** by an **R (arginine)**.

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

Unfolded protein	Wild type	Mutant	Overall $\Delta G$
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  $\Delta G$  of the system and – to indicate contributions that decrease the  $\Delta G$  of the system. Also, provide explanations for the results you include in the table. **(0.5 points)**

<b>Folded protein</b>	Wild type	Mutant	Overall $\Delta G$
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)**

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

Mutation	$\Delta\Delta G$ interaction (Kcal/mol)	$\Delta\Delta G$ solvation (Kcal/mol)
Cys124Ala	20.3	1.2
Gly133Ala	0.3	0.4
Gln145Ala	0.4	-1.6

- 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)**

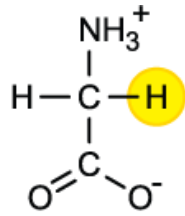
- Say if the following statements are true or false and explain why. Take into account the energy values in the table, the properties of the amino acids and how they can be involved in interactions and solvation. **(0.5 points)**

**Cys124 is involved in a disulphide bridge:**

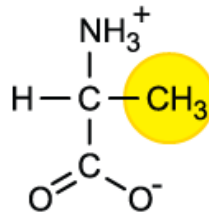
**Replacing Gly133 by an alanine increases the flexibility of the protein:**

**Gln145 is surrounded by hydrophobic side chains and has no interaction with water:**

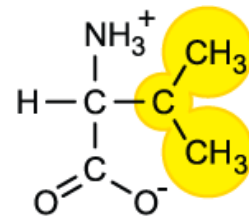
- Knowing that the experimental dissociation constant for the unmutated complex is 8.4 nM, calculate the dissociation constants for the mutants. **(0.5 points)**



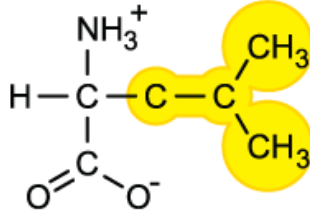
Glycine (G) - Gly



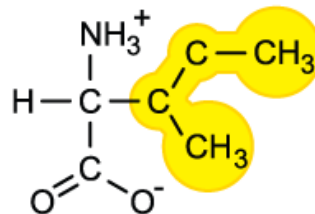
Alanine (A) - Ala



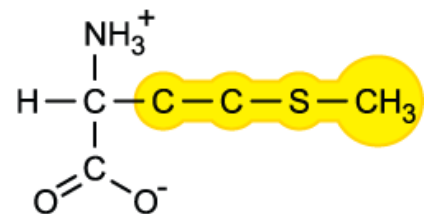
Valine (V) - Val



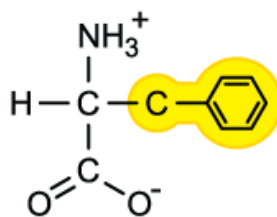
Leucine (L) - Leu



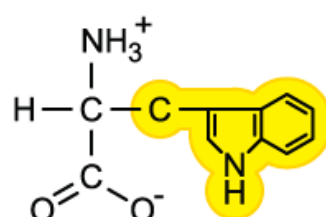
Isoleucine (I) - Ile



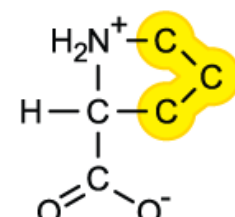
Methionine (M) - Met



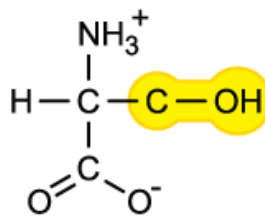
Phenylalanine (F) - Phe



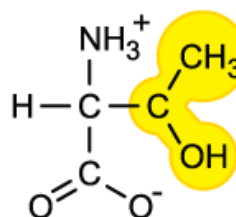
Tryptophan (W) - Trp



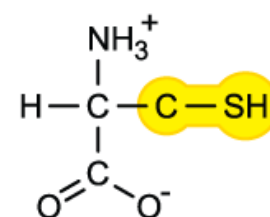
Proline (P) - Pro



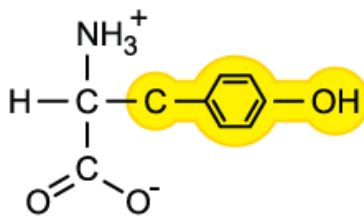
Serine (S) - Ser



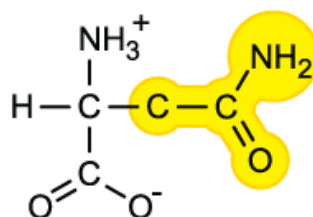
Threonine (T) - Thr



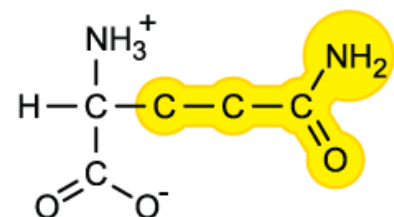
Cysteine (C) - Cys



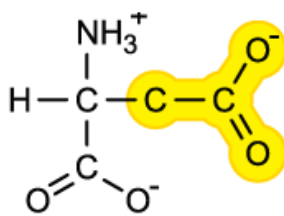
Tyrosine (Y) - Tyr



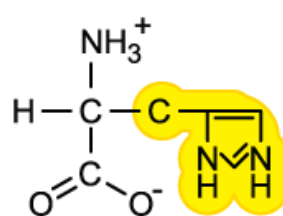
Asparagine (N) - Asn



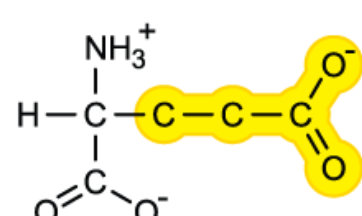
Glutamine (Q) - Gln



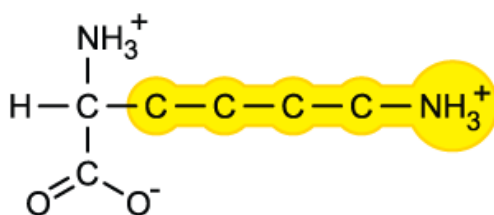
Aspartate (D) - Asp



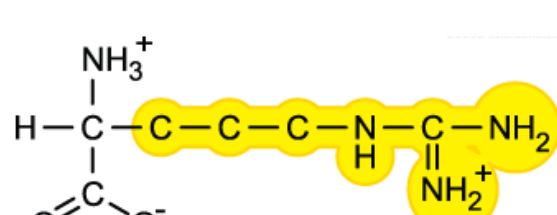
Histidine (H) - His



Glutamate (E) - Glu



Lysine (K) - Lys



Arginine (R) - Arg

## Formulas:

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

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

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

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

Order	Differential form	Integrated form	Half live	Units of k
0	$-\frac{d[A]}{dt} = k$	$[A] = [A]_0 - k_A t$	$\frac{[A]_0}{2k}$	M s <sup>-1</sup>
1	$-\frac{d[A]}{dt} = k[A]$	$[A] = [A]_0 e^{-k_A t}$	$\frac{\ln 2}{k}$	s <sup>-1</sup>
2	$-\frac{d[A]}{dt} = k[A]^2$	$\frac{1}{A} = \frac{1}{A_0} + kt$	$\frac{1}{[A]_0 k}$	M <sup>-1</sup> s <sup>-1</sup>
2'	$-\frac{d[A]}{dt} = k[A][B]$	$\frac{1}{[A]_0 - [B]_0} \ln \frac{[B]_0[A]}{[A]_0[B]} = kt$		M <sup>-1</sup> s <sup>-1</sup>

$$k = A e^{-E_a/RT}$$

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



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

$$K_D \text{(dissociation constant)} = \frac{[A][B]}{[AB]}$$

$$K_i \text{(Inhibition constant)} = \frac{[R][L]}{[RL]}$$

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

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

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

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

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

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

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

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

## Constants:

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

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

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

$$1 \text{ cal} = 4.184 \text{ J}$$

$$0 \text{ } ^\circ\text{C} = 273.15 \text{ K}$$