UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: **BIC8X02 PROTEIN BIOCHEMISTRY**
SEMESTER	: First
EXAM	: June 2021

DATE : 14 JUNE 2021 **SESSION**: 08:30-11:30

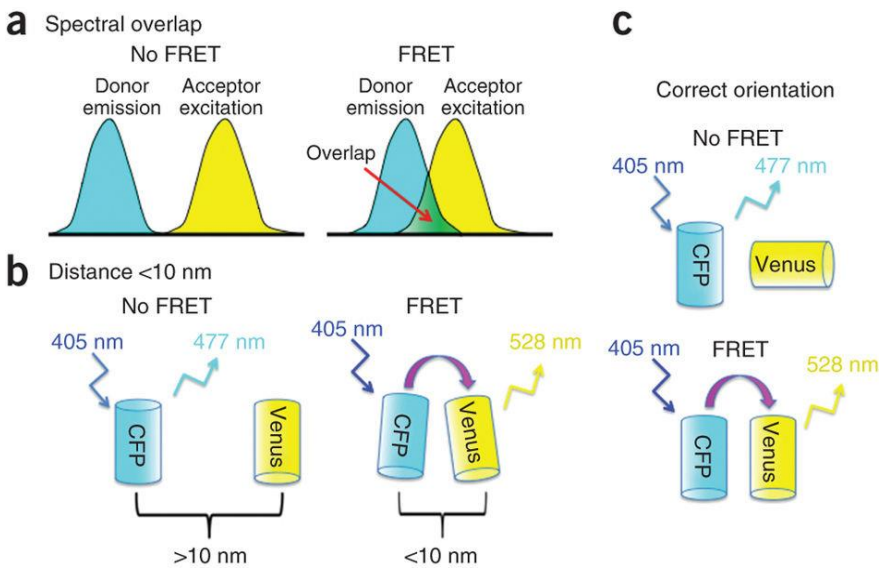
ASSESSOR : PROF AP KAPPO

MODERATOR : DR T MULAUDZI-MASUKU

DURATION : 3 HOURS **MARKS**: 100

INSTRUCTION : ANSWER ALL QUESTIONS

QUESTION 1:

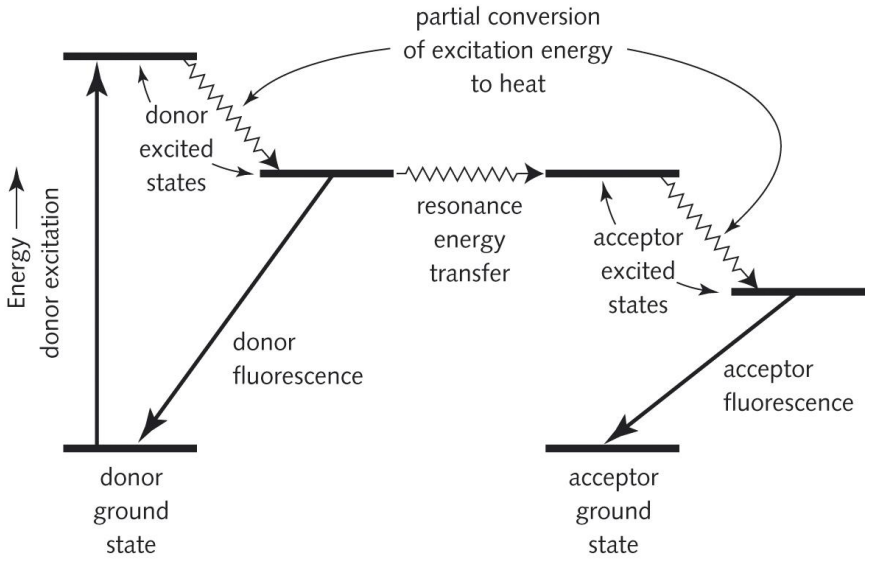


(i) The figure labelled **a, b & c** is a FRET diagram, which is explored by molecules during spectroscopic experimental measurements. Give a detailed description of how this mechanism is used to effect excitation and de-excitation [10 Marks]

In the figure above, spectroscopic measurements using FRET is possible only when there is enough spectral overlap between two proteins whereby at the same wavelength, the donor emission and the acceptor excitation overlap sequentially. More so, FRET can be used as spectroscopic measurement for two proteins if the distance between the two proteins even at different wavelengths is so close enough that it is not more than 10nm. In the diagram above, the distance between CPF and Venus proteins are less than 10nm and hence, FRET can be applied. Finally, the correct orientation of the two proteins (in this case, CPF and Venus) will allow FRET to be used if the two are orientated along the same axis even though their wavelengths of emission and excitation are very different.

The mechanism by which FRET works is that of excitation and de-excitation. This is a molecular biophysics principle whereby the structural conformation, dynamics and interactions of proteins and supramolecular complexes are spectroscopically-assessed before structural determination. As shown in the diagram below, during excitation, the molecule at the ground state acquire light energy and hence, becomes excited. The energy in the excited molecule is quickly released so the molecule returns to its ground state. It is often said that excitation involves a “short-term loan” of energy. However, the return of the molecule back to the ground state known as

de-excitation can be made possible via various means such as the conversion of excitation to heat, fluorescence, phosphorescence, energy transfer, and photochemical reaction. These can all be explained further by the students.

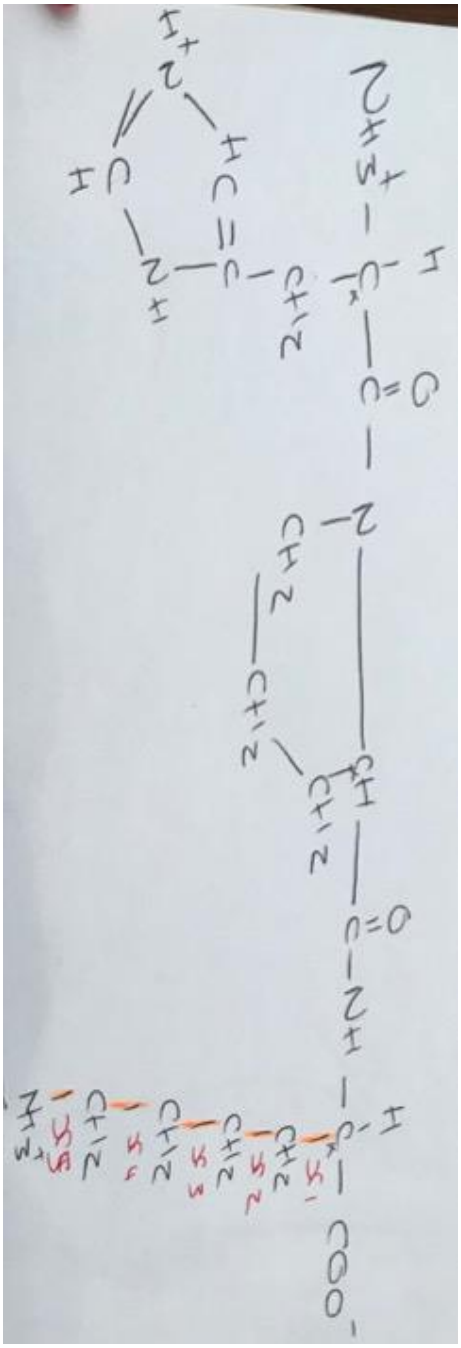


(ii) An inference from the central dogma of molecular biology is that DNA sequences of genes and amino acids of proteins are one-dimensional. Explain in detail how proteins achieve their three-dimensional biologically active states [5 Marks]

When proteins are newly synthesized and immediately released from the ribosomes, their three-dimensional biologically active states are acquired through spontaneous folding. However, protein native conformations are marginally stable over narrow ranges of physiological conditions of solvent and temperature; outside these narrow boundaries, the protein undergo denaturation and thus lose their biological active states. Therefore, in order to maintain this 3D conformation and remain active, electrostatic interactions, H-bonding, salt bridges and hydrophobic interactions between the various amino acid residues in the protein are elicited.

(iii) Draw the chemical structure of the tripeptide: **H-P-K** (assuming the peptide preceding Pro is in the *cis* configuration) and indicate how many angles of internal rotation are there in the amino acid residue **K** [10 Marks]

In the event that the amino acid preceeding Proline (P), which is Histidine (H) is in the cis configuration, the number of internal angles of rotation within Lysine (K) will be **5**. Below is the chemical structure of the tripeptide.

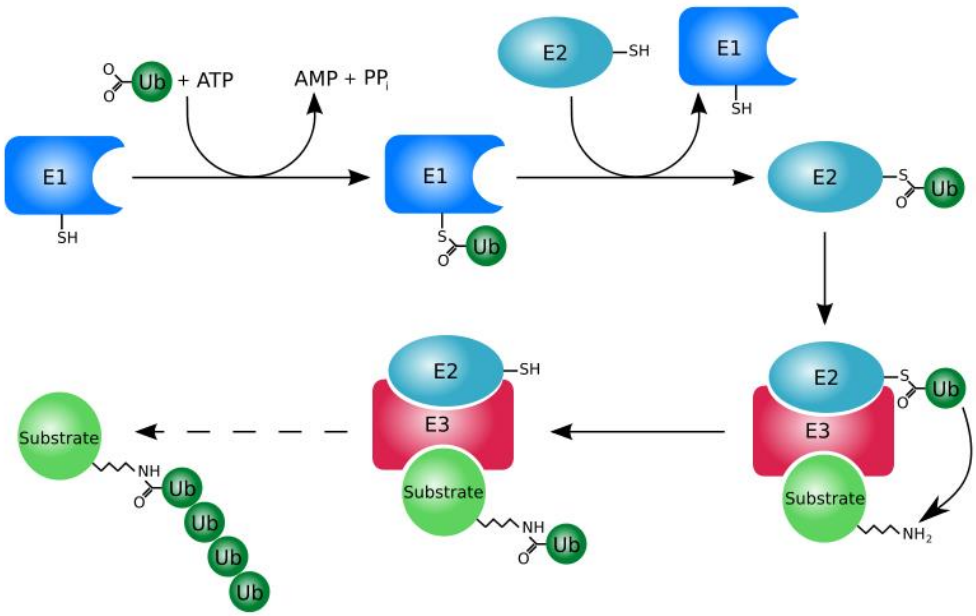


QUESTION 2:

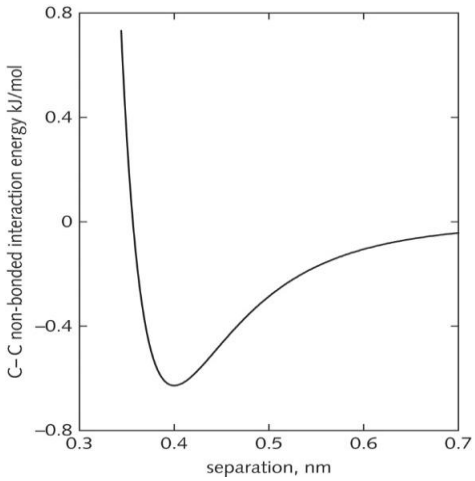
(a) Chaperones such as Hsp70, Hsp90 and others helps to unfold misfolded proteins in order for the protein to be folded into their 3D structure properly. However, some of

the proteins remain misfolded; hence experience a different fate in cells. Explain this biochemical phenomenon [15 Marks]

Protein turnover is the controlled degradation of proteins coordinated by a complex macromolecular particle called the 26S proteasome. Proteins at the end of their natural life-span such as those involved in glycolysis, DNA recognition, cell division and a host of others whose signals needs to be turned off after it has been received becomes unfolded and in some cases, misfolded. The chaperone protein system is the first port of call for these proteins to be refolded back to their 3D shape but if this fails, the proteins are then shunted to the alternative 26S proteasome via the ubiquitylation system. (The Ub-Proteasome system can be explained much further).



(b) The distance dependence of the van der Waals interaction between two non-bonded carbon atoms is a classical example of how nature stabilizes the native state of proteins. Using the figure below, give a detail explanation of this phenomenon [10 marks]



The dense packing of protein interior satisfies the hydrogen bonding potential hence, the closer the non-bonded carbon atoms of the amino acid residues come together, the stronger the van der Waals forces of interaction become until they are so close the forces almost become repulsive. Thus, from the graph it can be seen that the closer the non-bonded carbons of the residues become, the higher the van der Waals forces of interatomic interactions become and the lower the energy and hence the more stable the protein and the more stable its native state. The wider the distance between the non-bonded carbons of different residues the lower the VDW forces, the higher the energy the more unstable. (The energy trade-off between protein and water is initially too high and that is why the trade-off between the non-bonded carbons between residues is used).

QUESTION 3:

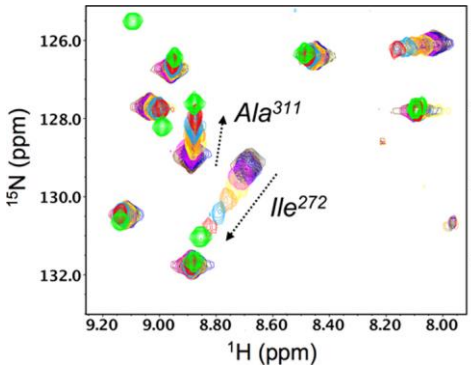
(a) Structural conformation of a protein can be measured by probing the fluorescence emitted and its recovery using a spectroscopic method called FRAP. Give a detail description of this mechanism and describe how it is different from FRET [20 Marks]

Refer to Introductory Slides (Lecture 1), Slides 27 & 28 for figures.

FRAP (Fluorescence Recovery After Photobleaching) - GFP tagged proteins are bleached by a beam of light. This technique measures the regain of fluorescence at a particular cellular site after localized destruction of Tags (bleaching whereby the GFP stops giving off fluorescence) by a laser beam. It is a time-specific study. This technique reveals the relative mobility of a protein.

FRET (Fluorescence Resonance Energy Transfer) – Provides structural information about supra macromolecular complexes (proteins and ligands bound together) whose determination are beyond that of X-ray crystallography. It is a spectroscopic method which uses proximity to neighboring groups to map out the groups in large macromolecular complexes in order to detect conformational changes in a molecule (the donor and acceptor must be in close proximity to each other).

(b) Biomolecular NMR spectroscopy is an inherently weak method of protein structure determination requiring milligram quantities of pure homogenous protein. What structural information can be acquired/inferred from the diagram shown below [5 Marks]



1. Assessment of protein interactions by chemical shifts and intensity changes

2. Assessment of the spontaneity of reactions i.e., whether reactions are slow or fast.

The 2 points listed above can be appropriately explained using Slide 11 of the Protein determination slides

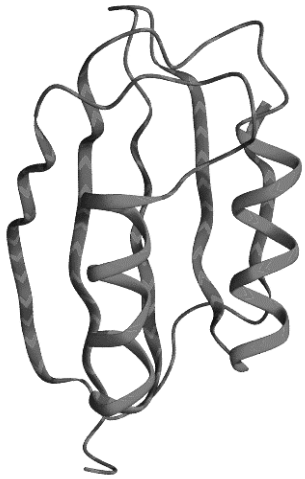
QUESTION 4:

(a) Describe the process of determining the structure a recombinant *Plasmodium falciparum* 15kDa Phosphoribosyltransferase enzyme, cloned into a pGEX-6P-2 protein expression vector using macromolecular X-ray crystallography [22 marks]

The structure of a recombinant P. falciparum Phosphoribosyltransferase enzyme can be determined using X-ray crystallography by first growing the protein crystals either

by sitting-drop or by hanging-drop method (Refer to Slide 15 of the Protein structure determination slides for a simplified diagram of the process).

(b) Identify the super-secondary structures occurring in the acylphosphatase enzyme structure as shown below [3 Marks]



Refer to Slide 35 of Lecture 2 on Protein Structure.