Student Name : _____ 2013-11-07
Student ID : _____

Instructions:

a) Write neatly and clearly using complete words. Cross out with a single line any material you do not wish to have marked.

b) Marks will be deducted for incorrect statements.

c) Students must work independently and may not knowingly utilize resource materials or share resource materials with other students.

d) Students may use pens, pencils, erasers and calculators only.

e) Electronic devices including cell phones, personal information managers and audio devices are prohibited.

Question	Mark	Total Marks
1		12
2		8
3		6
4		7
5		6
Total		**39**

(1) In the space provided, give short answers to the following questions: (12 marks)

(a) What is the 'Molten Globule'?

Folding intermediate (most proteins) that rapidly forms when folding is initiated. Contains most secondary structures and has a compact shape similar to the final folded state. Precise orientation of secondary structures and side-chains varies.

(b) What is context dependent folding?

Folding only occurs in the presence of an external factor(s), such as another macromolecule, a prosthetic group or specific physical condition.

(c) What is Circular Dichroism and what does it detect?

Differential absorption of right- and left-handed circularly polarized light. Detects chiral structures and is typically used to the detect/quantify the relative amounts of secondary structures present.

(d) What are the properties of a folding mutant?

Fraction of polypeptide that folds (or folds rapidly) is significantly reduced while the physical properties (*eg*. thermal stabilitly) and biological function of proteins that do fold are unchanged.

(e) How does protein disulfide isomerase assist protein folding efficiency and rates?

PDI breaks accessible disulfide bonds. As correct disulfide inevitably occur within the hydrophobic core they are not substrates for PDI. Incorrect disulfides almost always are accessible on the surface of partially folded proteins and their reduction allows another opportunity for to polypeptide to fold to its native state. This increases the number of correctly folded polypeptides per time.

(2) Consider the following balanced chemical equation describing the reversible binding of calcium ion to the Parvalbumin protein. (8 marks)

$$\text{Parvalbumin} + Ca^{2+} \leftrightarrow \text{Parvalbumin:}Ca^{2+}$$

(a) Derive the fundamental equation for experimentally determining the K_d of calcium ion binding.

$\theta = [\text{Parvalbumin:}Ca^{2+}] / ([\text{Parvalbumin}] + [\text{Parvalbumin:}Ca^{2+}])$ and

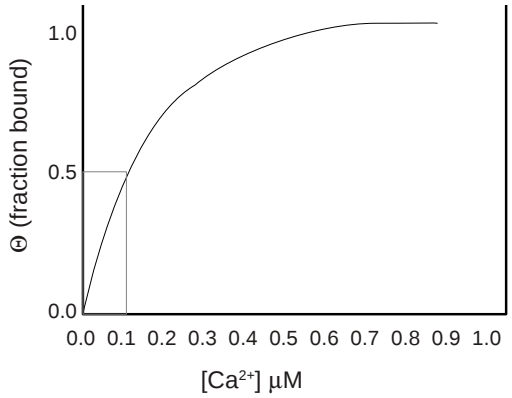
$K_{eq,a} = [\text{Parvalbumin:}Ca^{2+}] / ([\text{Parvalbumin}] [Ca^{2+}])$

Substituting for $[\text{Parvalbumin:}Ca^{2+}]$ in our fractional binding equation yields

$\theta = (K_{eq,a} [\text{Parvalbumin}] [Ca^{2+}]) / ([\text{Parvalbumin}] + K_{eq,a} [\text{Parvalbumin}] [Ca^{2+}])$ which simplifies to

$\theta = [Ca^{2+}] / ((1/K_{eq,a}) + [Ca^{2+}])$ or $\theta = [Ca^{2+}] / ((K_{eq,d}) + [Ca^{2+}])$

(b) Draw and label a reasonable binding curve that indicates Parvalbumin binds the calcium ion with an equilibrium dissociation constant of 0.1 μM.



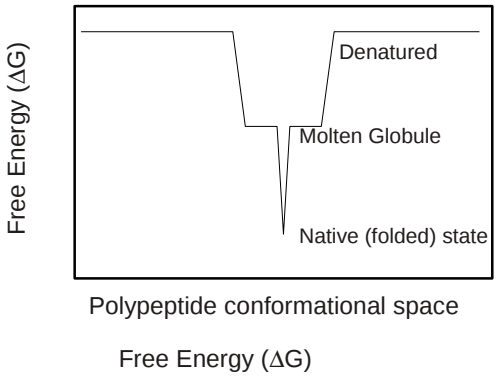
3 marks – 1 for labels, curve and K_d

(c) Assuming each Parvalbumin macromolecule actually binds 3 calcium ions with identical equilibrium dissociation constants of 0.1 μM, how would your answers to questions 2(a) and 2(b) change?

2a) $\theta = [Ca^{2+}]^3 / ((K_{eq,d}) + [Ca^{2+}]^3)$ 2b) no change

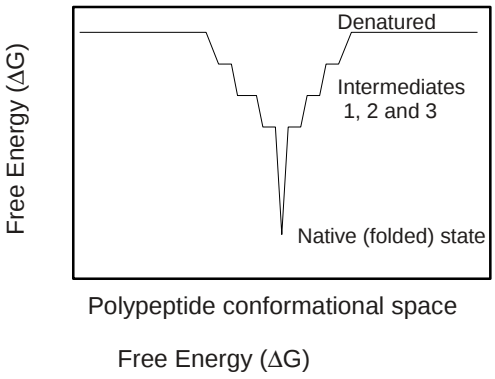
(3) Energy Landscape diagrams are convenient tools for describing the folding of macromolecules. (6 marks)

(a) Draw and label a 2D energy landscape diagram describing the folding of a typical globular protein.



Axis 1 mark
Molten Globule 1 mark
Denatured/Folded 1 mark

(b) Several small, disulfide rich proteins (eg. Bovine Pancreatic Trypsin Inhibitor) are believed to fold via several discrete intermediates. Draw and label a 2D energy landscape describing a folding pathway that involves three discrete intermediates.



Intermediates 2 mark
Denatured/Folded/Axis 1 mark

(4) GroEL/ES is a large protein complex that strongly interacts with over 300 proteins in *E. coli*. Answer the following questions regarding the function of GroEL/ES. (7 marks)

(a) What role or roles does ATP serve within the GroEL/ES catalytic cycle?

ATP binding to cis-ring

facilitates binding of misfolded protein to apical domain of GroEL in cis ring

triggers binding of GroES and internalization of misfolded protein

ATP hydrolysis in cis-ring

stimulates or starts refolding of internalized, misfolded protein

ATP binding to trans-ring

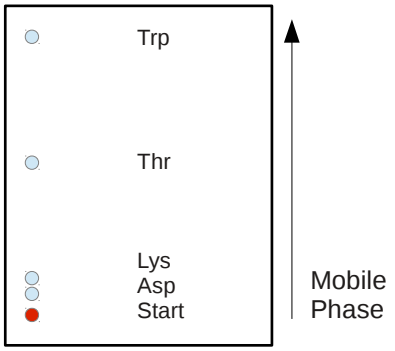
stimulates release of ADP, GroES and now folded protein

(b) What is the "Iterative Annealing Model" of GroEL/ES function and how does it improve protein folding efficiency?

ATP hydrolysis disrupts misfolded intermediate and allows 'second chance' at folding

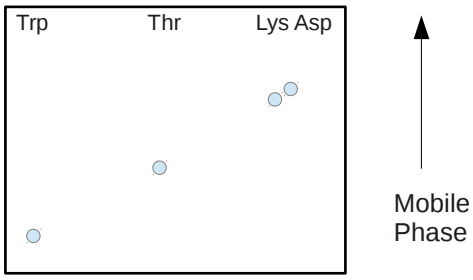
(5) You wish to verify the identity of free amino acids isolated from a particular organism. You have standard (known) amino acid and have chosen to use Thin-Layer Chromatography to separate the amino acids (known and unknown) and ninhydrin to detect the amino acids after separation. (6 marks)

(a) In your TLC experiment, you use the relatively non-polar acetone as the mobile phase. Draw a reasonable diagram of the TLC plate after separation and staining. Indicate the direction the mobile phase travels and identify where the following amino acids would expect to be detected on the TLC at neutral pH - Lys, Thr, Asp, Trp.



1 mark for mobile phase, 0.5 each for relative position

(b) In order to improve the separation of the amino acids you now run a 2D TLC experiment. You run the first dimension as in part (a), then rotate the TLC plate 90° and run a second dimension using a polar aqueous mobile phase. Draw a reasonable diagram of the TLC plate after separation and staining. Indicate the direction the mobile phase travels and identify where the following amino acids would now expect to be detected on the TLC at neutral pH - Lys, Thr, Asp, Trp.



1 mark for mobile phase, 0.5 each for relative position