Questions and Exercises

Chapter 1

1. All living organisms are made of cells. Suggest reasons for this phenomenon.

2. Explain the advantages of molecular complexity, and how living organisms obtain it.

3. How many unique sequences can construct a 60 amino-acid protein?

4. Suggest a reason why evolution has led to the stabilization of proteins *via* non-covalent interactions, rather the much stronger covalent bonds.

5. Provide a short, qualitative description of all intermolecular forces attracting an amino group (NH_3^+) to a carbonyl group (C=O).

6. Use your own words to describe the van der Waals interaction plot (Figure 1-16).

7. Does the hydrophobic effect involve a real inter-atomic force? Explain.

8. Two proteins bind each other non-covalently in an aqueous solution, with a total interaction surface (i.e. the interface on both binding partners) of 500 $Å^2$. A pH change leads to conformational changes in both proteins, which results in a decrease of their interface to 300 $Å^2$. Estimate the resulting change in the nonpolar interaction energy, using the empirical method described in the text.

9. Write down the mathematical expressions describing the corresponding energies of electrostatic (Coulomb) and van der Walls interactions. What can you deduce from the expressions on the range of each interaction?

10. Explain in short the significance of the dielectric constant to electrostatic interactions.

11. Describe the two main differences between ionic interactions and hydrogen bonds.

Chapter 2

1. A. Specify the two basic types of hetero-groups in proteins.

   B. Explain the general function of hetero-groups.

   C. Give three examples to hetero-groups and describe in short their specific function.

2. A. Specify the chemical groups in the amino acid arginine, which may undergo protonation/de-protonation.

   B. Draw the titration curve of arginine's side-chain with NaOH.

3. What is the main criterion used to separate natural amino acids into groups? Explain why.

4. Cysteine is considered a polar amino acid, and yet, it is often found inside the protein core. Explain why.

5. Two residues, arginine and lysine, are positioned 2 Å from each other in water.

A. Estimate their electrostatic interaction energy. Explain why this calculation is only an estimate.

B. Would you expect the pKa of the two residues' side chains in isolation to change as a result of their proximity? Explain why and calculate the extent of the change (in pKa units).

C. Is the pKa change calculated in *B* large enough to alter the residues' charging state? If not, suggest a way to accomplish this alteration.

6. The enzyme *hypothetase* hydrolyzes a covalent bond in the substrate using a nucleophilic attack. Taking other known hydrolases as example, which of the 20 natural amino acid residues is most likely to serve as the enzyme's nucleophile?

7. Explain in short the two basic mechanisms used by organisms to fight oxidative damage.

8. Explain the mechanisms allowing the appearance of amino acids derivatives inside proteins.

9. When the ϕ and ψ values of residues in experimentally determined proteins are collected, some of them reside outside the 'allowed' regions of the Ramachandran plot (Figure 2-12d). Explain why.

10. What are the two most popular secondary elements in proteins? Explain why.

11. What are the respective functions of backbone hydrogen bonds and nonpolar interactions in α-helices?

12. During the folding of a metalloenzyme, a zinc ion (Zn^{2+}), originally surrounded by water, is trapped inside the protein core. Assuming that the cation is a sphere of radius of 1.4 Å, and that the dielectric constant of the protein core is 2, estimate the change in electrostatic energy accompanying the process.

13. Predict which secondary structure the following sequence is most likely to acquire:

Ala-Leu-Met-Glu-Gln-Ile-Ala-Arg-Met-Gln-Leu-Glu

14. Explain how each of the secondary elements in the immunoglobulin motif fulfils its functional role.

15. Explain how proteins with different sequences may still possess a similar three-dimensional structure.

16. What are the main evolutionary advantages of quaternary structure?

17. Name two or three post-translational modifications of proteins, which have been implicated in the development or behavior of cancerous cells.

18. Explain in general terms how phosphorylation may change protein activity.

19. Specify the main features, which make fibrous proteins distinct than globular proteins.

20. List a few of the characteristic roles of fibrous proteins.

21. Explain the distinction between structural and fibrous proteins.

22. Compare between α-keratin and collagen in terms of source tissue, sub-cellular localization, structure and function.

23. Explain the molecular basis for the difference between soft and hard keratins.

24. How would you treat a man or woman suffering from scurvy?

Chapter 3

1. Describe the main differences between x-ray diffraction and NMR spectroscopy. Refer to both method and quality of results.

2. Explain why neutron diffraction is used for finding the orientation of water molecules in proteins.

3. What uses are there for NMR spectroscopy, other than structure determination?

4. Explain how low-resolution images produced by electron microscopy are used for protein structure determination. Why are these used and not low-resolution images produced by x-ray diffraction?

5. Explain the main differences between NMR and EPR spectroscopies.

6. Explain the main advantages and disadvantages of explicit vs. implicit descriptions employed by structure-prediction methods.

7. A. Why are energy-minimization methods unable to predict the native structure of proteins when starting from an unfolded state?

B. What solution has been developed for this problem, and did it work?

8. Describe the principles of the continuum-solvent model approach, its advantages and disadvantages in describing protein-related systems, and its current uses.

9. Why is homology modeling currently considered to be the best structure prediction approach?

10. Protein X has the following sequence:

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MVFSDQQLFEKVVEILKPFDLSVVDYEEICDRMGESMRLGLQKSTNEKSSIKMFPSYVT
KTPNGTETGNFLALDLGGTNYRVLSVTLEGKGKSPRIQERTYCIPAEKMSGSGTELFKY
IAETLADFLENNGMKDKKFDLGFTFSFPCVQKGLTHATLVRWTKGFSADGVEGHNVAEL
LQTELDKRELNVKCVAVVNDTVGTLASCALEDPKCAVGLIVGTGTNVAYIEDSSKVELM
DGVKEPEVVINTEWGAFGEKGELDCWRTQFDKSMDIDSLHPGKQLYEKMVSGMYLGELV
RHIIVYLVEQKILFRGDLPERLKVRNSLLTRYLTDVERDPAHLLYNTHYMLTDDLHVPV
VEPIDNRIVRYACEMVVKRAAYLAGAGIACILRRINRSEVTVGVDGSLYKFHPKFCERM
TDMVDKLKPKNTRFCLRLSEDGSGKGAAAIAASCTRQN
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A. Using the Internet resources mentioned in Chapters 1-3, find the name of the protein, its function, and the organism from which it was obtained.
B. Has the three-dimensional structure of the protein determined experimentally? If so, answer the following questions:
    I. Which method was used to determine this structure?
    II. Does the structure include a ligand?
    III. Does the structure contain any hetero-atoms?
    IV. Which secondary structure does the structure include?

Chapter 4

1. A chemical reaction in which a substrate A is turned into the product B involves a change of -5 kcal/mol in the standard free energy. When the reaction reaches equilibrium, the concentration of A is 0.01 mM. Calculate the equilibrium concentration of B.

2. A chemical reaction was carried out in 25° C (room temperature) and under constant pressure. At equilibrium, the change in free energy was measured as -3 kcal/mol, and the

reaction released 5 kcal/mol of heat, as measured by calorimetry. Calculate the change in entropy that accompanied the reaction.

3. Protein folding is a favorable process. Yet, it decreases the entropy of the polypeptide chain, in contrast to Nature's tendency to increase entropy. Explain how this alleged disagreement is possible.

4. What is the source of disagreement between scientists regarding the favorability of electrostatic interactions between protein core charges?

5. Is the loss of entropy upon protein folding equal among all parts of the protein? Explain.

6. Explain the mechanism through which high temperature disrupts the folded structure of proteins. How did evolution solve this problem for hyperthermophilic organisms?

7. A. List a few industrial uses of enzymes.
   B. Pick one example and elaborate.

8. Suggest ways to stabilize the structure of a mesophilic protein in acidic environments.

Chapter 5

1. Describe protein folding in terms of conformation, free energy, entropy, and kinetic barriers.

2. Explain the main differences between the framework, hydrophobic collapse, and nucleation-condensation models of protein folding.

3. Is the native structure the most stable form of a protein? Explain your answer.

4. Describe the process of amyloid fibril formation.

5. A. List the main types of molecular chaperons.

   B. What are the unique features of chaperonins like GroEL-GroES?

6. Explain how small structural changes in the folded conformation of the protein can lead to dramatic changes in its activity.

7. List the three main factors, which may affect the folded-state dynamics of proteins.

8. A. Explain how ligand binding can allosterically affect protein activity. Base you answer on the MWC, KNF and 'population shift' models.

   B. Do the models explain the function of both protein activators and inhibitors? How?

9. A. How does Max Perutz's 'stereo-chemical model' explain the well-known phenomenon of positive cooperativity in hemoglobin action?

B. Explain in short the main structural features underlying this phenomenon.

C. Does it also explain the long-known ‘Bohr effect’ of hemoglobin?

Chapter 6

1. Explain how Intrinsically Unstructured Proteins (IUPs) can be so common and yet so few of them are present in the Protein Data Bank.

2. Use the example of the Nuclear Pore Complex to explain how IUPs may carry out certain functional roles in cells more efficiently than structured proteins.

3. Which local structure(s) can be often found in IUPs? Explain its/their compatibility to the roles assigned to IUPs.

4. Explain the principles of the ‘fly casting’ mechanism.

Chapter 7

1. Explain why the interest in membrane proteins far exceeds their relative proportion in the cell.

2. The lipids found in biological membranes differ significantly from each other in chemical structure and composition. What is it that makes them all suitable as membrane building blocks?

3. Unlike other membrane lipids, cholesterol has a bulky, rigid structure. In your opinion, how would this structure affect membrane properties?

4. Suggest reasons why membranes of different organelles, cells and organs differ so significantly in their lipid composition.

5. A. Estimate the number of encounters between a divalent cation and the plasma membrane, which are needed for the cation to cross the membrane successfully. Assume that the radius of the cation is 1Å, and that the dielectrics of the membrane and cytoplasm/extracellular matrix are 2 and 80, respectively.

B. If each unsuccessful cation-membrane encounter lasts 10^{-12} sec, how much time may be needed for the system to achieve a successful encounter?

6. Predict the general location of the following amino acids in an integral membrane protein: Val, Trp, Arg, Pro, and Asp. Explain your prediction.

7. What are the main differences between the driving forces for the folding of globular proteins and those of integral membrane proteins?

8. Explain the following observations of membrane proteins that function in the transport of ions:

I. Channels contain a water annulus, yet make the passing ions lose their solvation shell for a short part of their way.

II. Carriers do not contain a constant water annulus linking the bulk solvent on both sides of the membrane.

9. Peripheral membrane proteins require more than a single type of non-covalent interaction to bind to the membrane. Explain the underlying advantage(s).

10 List the different ways with which the protein-membrane system can use to ameliorate the energy cost of positive hydrophobic mismatch.

11. Explain how activation of adrenergic receptors serves the ‘fight-or-flight’ response in animals.

12. Unlike other class 1 GPCRs, rhodopsin has no baseline activity in the absence of agonist. Which structural features of GPCRs have been proposed to explain this phenomenon?

13. List the structural features of GPCR activation, which have emerged from the study of rhodopsin’s active and inactive structures.

Chapter 8

1. List four specific biological processes that rely on protein-ligand binding, and explain for each how the binding serves its biological role.

2. Describe the three basic models proposed for protein-ligand binding and suggest which one is the most realistic.

3. Explain the phenomenon known as ‘electrostatic steering’.

4. Which branches of the animal nervous system include the neurotransmitter acetylcholine:

a. Central (brain and spinal cord).

b. Somatic.

c. Sympathetic.

d. All of the above.

5. Organophosphates are chemical agents that inactivate the enzyme acetylcholine esterase by binding covalently to its catalytic serine residue. Why, then, are some organophosphates (e.g. Sarin) far more dangerous than others (e.g. Parathion)?

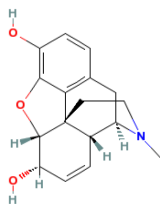
6. A. List some of the different criteria used to classify protein-protein complexes.

B. Are there significant differences between the interfaces of the different protein-protein complexes?

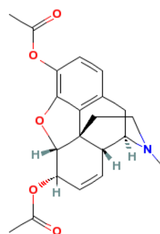
7. Explain the phenomenon known as ‘antibody maturation’.

8. Explain the differences in qualitative and quantitative contribution to binding, of different residues in protein-protein interfaces.

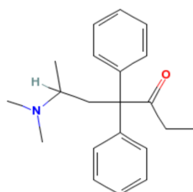
9. Morphine, heroin, methadone and papaverine are members of the opiate group of drugs:



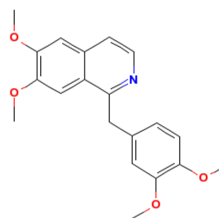
Morphine



Heroin



Methadone



Papaverine

A. Suggest the chemical-structural features of these drugs, which you believe are required for their activity.

B. Are there other features you would consider as important?

10. Explain the main differences between *N*-carboxy-alkyl dipeptide and keto-based ACE inhibitors.

Chapter 9

1. a. Prove mathematically that the half-life time of the zero-order reaction of Equations 9.1.1 and 9.1.2 is given by $[A]_0/2k$, where $[A]_0$ is the initial concentration of the substrate, and k is the reaction rate.

(b) Prove that the half-life of the first-order reaction of Equations 9.1.4 and 9.1.5 is given by Eq. 9.1.8, and that the half-life time of the second order reaction of Equations 9.1.9 and 9.1.10 is given by Eq. 9.1.12.

2. Which of the following describes correctly the dependency of the initial reaction rate (velocity V_0) of an enzyme on substrate concentration, according to the Michaelis-Menten model, and when the substrate concentration is very low?

a) V_0 depends linearly on the substrate's concentration, with a rate coefficient of $\frac{k_{cat}}{K_M}$ $[E_f]$.

b) V_0 depends linearly on the substrate's concentration, with a rate coefficient of $\frac{K_m}{k_{cat}}$.

c) V_0 depends cooperatively on the substrate's concentration.

d) V_0 is constant at low substrate concentration, and equals to the concentration of the enzyme ($[E_t]$).

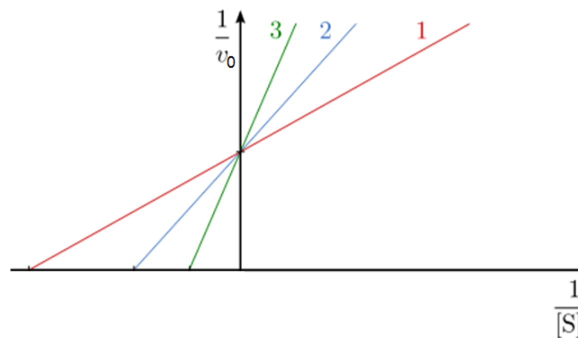
3. The initial velocity of an enzymatic reaction (V_0) was measured under the following conditions:

<u>Condition 1</u>: with 2 mM of the enzyme's natural substrate (S).

<u>Condition 2</u>: with 2 mM of the enzyme's natural substrate (S) and 0.5 mM of an inhibitor (I).

<u>Condition 3</u>: with 2 mM of the enzyme's natural substrate (S) and 1 mM of the inhibitor (I).

The dependency of $1/V_0$ on [S] under these three conditions was as follows:



How can the inhibitor's effect be lifted?

a) Only by removing the inhibitor from the reaction mixture.

b) Either by removing the inhibitor or by raising the substrate's concentration to high values.

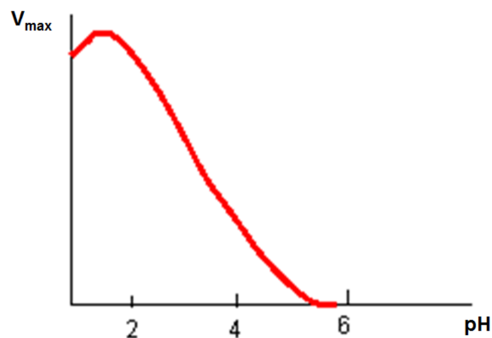
c) By removing the inhibitor and chemically reverting the enzyme's catalytic residues to their original form.

d) The inhibition is irreversible and cannot be lifted.

4. Under which condition can K_M be considered as a measure for the affinity of the enzyme to its substrate(s)?

5. Two isozymes, A and B, deaminate alanine to pyruvate. Their K_M values are 0.5 mM and 4 mM, respectively. When the two isozymes at 3 mM concentration were incubated with 10 mM of alanine under the same conditions, their rates (V_0) were 15 $\frac{mM}{sec}$ and 300 $\frac{mM}{sec}$, respectively. Which isozyme is more efficient?

6. The activity of an enzyme was measured under saturation and different pH values. The following dependency was obtained:



Then, the amino acids in the active site of the enzyme were systematically replaced (mutated), and the activity was measured again. Which of the following mutations is most likely to be deleterious (i.e. lead to loss of activity)?

a) Alanine → glutamine

b) Glutamate → valine

c) Arginine → aspartate

d) Phenylalanine → tryptophan

7. How does coenzyme A activate metabolites for subsequent condensations?

8. The following enzymatic cofactors are involved in group transfer. Connect between the cofactor and the group it transfers.

Cofactor	Group
a) S-adenosyl methionine	i. Acyl
b) Thiamine pyrophosphate	ii. Methyl (CH_3)
c) Coenzyme A	iii. Amino (NH_2)
d) Pyridoxal phosphate (PLP)	iv. CO_2
e) Biotin	v. Aldehyde

9. Which of the following cofactors carries out reactions that involves radicals?

a) Tetrahydrofolate (THF)

b) NADH

c) Coenzyme B_{12}

d) Coenzyme A

10. What is the common mechanistic aspect of the enzymatic cofactors pyridoxal phosphate (PLP) and thiamine pyrophosphate (TPP)?

11. The following enzyme activities were measured in the lab:

[S] (mM)	V_0 (mmol/min)
0	0.0
1	3.0
2	5.0
4	6.6
8	7.0
12	6.2
15	5.0

Does the enzyme present a Michaelis-Menten kinetics? How can you explain the activity values measured at high substrate concentrations?

12. Two enzymes from two different organisms catalyze the oxidation of glucose. Which of the following parameters would you expect to be the same in the two catalyzed reactions?

a) V_{max}

b) K_M

c) K_{eq} (equilibrium constant)

d) Optimal temperature

e) Optimal pH

13. An enzyme has a K_M of 2 mM. At which substrate concentration will the enzyme's activity be ¼ than V_{max}?

14. The activity of two enzymes was measured at different substrate concentrations:

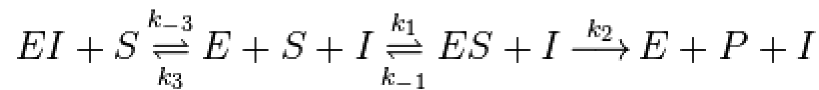
[S] (nM)	V_0 (nmol/min)	V'_0 (nmol/min)
1	150	82
2	256	150
10	600	450
30	770	670
50	818	750

a) Show that both enzymes follow Michaelis-Menten kinetics, and estimate their K_M values?

b) Assuming that the concentration of the enzymes are 0.2 nm and 0.5 nm, what are their turnover numbers?

15. The non-enzymatic decomposition of $2NO_2$ to $2NO$ and O_2 is a second-order reaction. If the initial concentration of NO_2 is 10mM and the catalytic rate of the reaction is 0.4 $mM^{-1}sec^{-1}$, what is its half-life?

16. The binding of a competitive inhibitor (I) to its target enzyme (E) can be described by the following scheme:



$$EI + S \underset{k_3}{\overset{k_{-3}}{\rightleftharpoons}} E + S + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES + I \xrightarrow{k_2} E + P + I$$

Based on the scheme, derive the Michaelis-Menten Equation for cases of competitive inhibition.

17. In an experiment, the same enzyme in three batches was incubated with 5 mM substrate and 2 mM of inhibitor. In each batch the inhibitor was of different type:

<u>Batch 1</u>: competitive inhibitor.

<u>Batch 2</u>: uncompetitive inhibitor.

<u>Batch 3</u>: non-competitive inhibitor.

Assuming that $V_{max} = 80 \frac{mM}{sec}$; K_M = 8 mM, and that the K_I of all three inhibitors was 10 mM, which of the inhibitors was the most efficient (i.e. had the strongest effect on V_0)?