JEPPIAAR ENGINEERING COLLEGE

JEPPIAAR NAGAR, CHENNAI – 119

DEPARTMENT OF BIOTECHNOLOGY

QUESTION BANK
ON

BT 6501– PROTEIN STRUCTURE FUNCTION AND PROTEOMICS

REGULATION - 2013

III YEAR & V SEMESTER

BATCH: (2016-2020)

VISION OF THE INSTITUTION

- To build Jeppiaar Engineering College as an institution of academic excellence in technological and management education to become a world class University

MISSION OF THE INSTITUTION

- To excel in teaching and **learning, research and innovation** by promoting the principles of scientific analysis and creative thinking.
- To participate in the production, **development and dissemination of knowledge** and interact with **national and international communities.**
- To equip students with **values, ethics and life skills n**eeded to enrich their lives and enable them to meaningfully contribute to the **progress of society.**
- To prepare students for **higher studies and lifelong learning,** enrich them with the **practical and entrepreneurial skills** necessary to excel as future professionals and contribute to **Nation's economy**

PROGRAM OUTCOMES (PO)	
PO 1	**Engineering knowledge**: **Apply** the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.
PO 2	**Problem analysis**: **Identify, formulate, review** research literature, and **analyze** complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences.
PO 3	**Design/development of solutions**: **Design solutions** for complex engineering problems and **design** system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations
PO 4	**Conduct investigations of complex problems**: **Use** research-based knowledge and research methods including **design** of experiments, **analysis** and **interpretation** of data, and **synthesis** of the information to provide valid conclusions.
PO 5	**Modern tool usage**: **Create**, **select**, and **apply** appropriate techniques, resources, and modern engineering and IT tools including prediction and modeling to complex engineering activities with an understanding of the limitations.
PO 6	**The engineer and society**: **Apply** reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.
PO 7	**Environment and sustainability**: **Understand** the impact of the professional engineering solutions in societal and environmental contexts, and **demonstrate** the knowledge of, and need for sustainable development.
PO 8	**Ethics**: **Apply** ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.
PO 9	**Individual and team work**: **Function effectively** as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.
PO 10	**Communication**: **Communicate effectively** on complex engineering activities with the engineering community and with society at large, such as, being able **to comprehend** and **write** effective reports and **design** documentation, **make** effective presentations, and **give** and **receive** clear instructions.

PO 11	**Project management and finance**: **Demonstrate** knowledge and understanding of the engineering and management principles and **apply** these to one's own work, as a member and leader in a team, to **manage** projects and in multidisciplinary environments.
PO 12	**Life-long learning**: **Recognize** the need for, and have the preparation and ability to **engage** in independent and life-long learning in the broadest context of technological change.

VISION OF THE DEPARTMENT	
To pursue excellence in producing bioengineers coupled with research attributes.	
MISSION OF THE DEPARTMENT	
M1	To **impart** quality education and **transform** technical knowledge into career opportunities.
M2	To **establish** a bridge between the **program and society** by **fostering** technical education.
M3	To **generate societal conscious technocrats** towards **community development**
M4	To **facilitate higher studies** and research in order to have an **effective career /** entrepreneurship.
PROGRAM EDUCATIONAL OBJECTIVES (PEOS)	
PEO - 1	To impart knowledge and produce competent graduates in the field of biotechnology
PEO - 2	To inculcate professional attributes and ability to integrate engineering issues to broader social contexts.
PEO - 3	To connect the program and community by fostering technical education.
PEO - 4	To provide a wide technical exposure to work in an interdisciplinary environment
PEO - 5	To prepare the students to have a professional career and motivation towards higher education.
PROGRAM SPECIFIC OUTCOMES (PSOS)	
PSO 1	**Professional Skills:** This programme will provide students with a solid foundation in the field of Biological Sciences and Chemical engineering enabling them to work on engineering platforms and applications in Biotechnology as per the requirement of Industries, and facilitating the students to pursue higher studies
PSO 2	**Problem-solving skills:** This programme will assist the students to acquire fundamental and problem solving knowledge on subjects relevant to Biotechnology thereby encouraging them to understand emerging and advanced concepts in modern biology
PSO 3	**Successful Career and Entrepreneurship:** Graduates of the program will have a strong successful career and entrepreneurial ability with the blend of inputs from basic science, engineering and technology, thereby enabling them to translate the technology and tools in various industries and/or institutes

BT6501 PROTEIN STRUCTURE FUNCTION AND PROTEOMICS LT P C 3 1 0 4
OBJECTIVES:

UNIT I BONDS, ENERGIES, BUILDING BLOCKS OF PROTEINS 9

Covalent, Ionic, Hydrogen, Coordinate, hydrophobic and Vander walls interactions in protein structure. Interaction with electromagnetic radiation (radio, micro, infrared, visible, ultraviolet, X-ray) and elucidation of protein structure. Amino acids (the students should be thorough with three and single letter codes) and their molecular properties (size, solubility, charge, pKa), Chemical reactivity in relation to post-translational modification (involving amino, carboxyl, hydroxyl, thiol, imidazole groups).

UNIT II PROTEIN ARCHITECTURE 9

Primary structure: peptide mapping, peptide sequencing - automated Edman method & mass- spec. High-throughput protein sequencing setup Secondary structure: Alpha, beta and loop structures and methods to determine Super-secondary structure: Alpha-turn alpha, beta-turn- beta (hairpin), beta-sheets, alpha-beta-alpha, topology diagrams, up and down & TIM barrel structures nucleotide binding folds.

UNT III TERTIARY STRUCTURE 9

Prediction of substrate binding sites, Tertiary structure: Domains, folding, denaturation and renaturation, overview of methods to determine 3D structures. Quaternary structure: Modular nature, formation of complexes, protein-protein interactions and methods to study it: Computer exercise on the above aspects

UNIT IV STRUCTURE-FUNCTION RELATIONSHIP 9

DNA-binding proteins: prokaryotic transcription factors, Helix-turn-Helix motif in DNA binding, Trp repressor, Eukaryotic transcription factors, Zn fingers, helix-turn helix motifs in homeodomain, Leucine zippers, Membrane proteins: General characteristics, Trans-membrane segments, prediction, bacteriorhodopsin and Photosynthetic reaction center, Immunoglobulins: IgG Light chain and heavy chain architecture, abzymes and Enzymes: Serine proteases, understanding catalytic design by engineering trypsin, chymotrypsin and elastase, substrate-assisted catalysis other commercial applications Computer exercise on the above aspects

UNIT V PROTEOMICS 9

Introduction to the concept of proteome, components of proteomics, proteomic analysis, importance of proteomics in biological functions, protein arrays, cross linking methods, affinity methods, yeast hybrid systems and protein arrays. Computer exercise on the above aspects

TOTAL (L: 45 + T: 15): 60 PERIODS

TEXT BOOKS:

- Branden C. and Tooze J., "Introduction to Protein Structured" 2nd Edition, Garlan Publishing, 1999.
- Creighton T.E. "Proteins" 2nd Edition. W.H. Freeman, 1993.
- Pennington, S.R and M.J. Dunn, "Proteomics: Protein Sequence to Function". Viva Books, 2002

REFERENCE:

- Liebler, "Introduction to Proteomics" Humana Press, 2002.

CO NO	COURSE OUTCOME
C301.1	To analyze the various interactions in protein makeup.
C301.2	To understand and familiarize with different levels of protein structure.
C301.3	To learn and acquire knowledge about functional proteins
C301.4	To understand the latest application of protein science in research
C301.5	To apply informatics and understand proteomics

BT 6501 PROTEIN STRUCTURE FUNCTION AND PROTEOMICS –LESSON PLAN

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	1	Covalent, Ionic, Hydrogen, Coordinate, hydrophobic and Vander walls interactions in protein structure.	1	3-12	Branded & Tooze
	2	Covalent, Ionic, Hydrogen, Coordinate, hydrophobic and Vander walls interactions in protein structure.	1	3-12	Branded & Tooze
	3	Interaction with electromagnetic radiation (Radio, Micro, Infrared, Visible, Ultraviolet, X-ray)	1	3-12	Branded & Tooze
	4	Interaction with electromagnetic radiation (Radio, Micro, Infrared, Visible, Ultraviolet, X-ray)	1	3-12	Branded & Tooze
	5	Elucidation of protein structure	18	374-392	Branded & Tooze
	6	Elucidation of protein structure	18	374-392	Branded & Tooze
	7	Amino acids	1	3-12	Branded & Tooze
	8	Amino acids -their molecular properties	1	3-12	Branded & Tooze
	9	Chemical reactivity in relation to post-translational modification	1	3-12	Branded & Tooze
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	11	Peptide mapping	18	374-392	Branded & Tooze
	12	Peptide sequencing - Automated Edman method & Mass- Spectroscopy	18	374-392	Branded & Tooze
	13	High-throughput protein sequencing setup	18	374-392	Branded & Tooze
	14	Secondary structure: Alpha, beta and loop structures	2	13-23	Branded & Tooze
	15	Methods to determine Super-secondary structure: Alpha-turn Alpha; Beta-turn-Beta (hairpin)	2	24-34	Branded & Tooze
	16	Methods to determine Super-secondary structure: Beta-sheets, Alpha-Beta-Alpha	2-5	36-87	Branded & Tooze
	17	Topology diagrams	2-5	36-87	Branded & Tooze
	18	Up and down & TIM barrel structures nucleotide binding folds.	2-5	36-87	Branded & Tooze
3	**TERTIARY STRUCTURE**				
	19	Prediction of substrate binding sites			Branded & Tooze
	20	Tertiary structure: Domains	7	141-165	Branded & Tooze
	21	Folding	6	89-104	Branded & Tooze
	22	Denaturation and renaturation	6	89-104	Branded & Tooze
	23	Overview of methods to determine 3D structures.	18	374-392	Branded & Tooze
	24	Quaternary structure	7	180-185	Branded & Tooze
	25	Formation of complexes	18	374-392	Branded & Tooze
	26	Protein-protein interactions and methods to study it	18	374-392	Branded & Tooze
	27	Protein-protein interactions and methods to study it	18	374-392	Branded & Tooze

4	**STRUCTURE-FUNCTION RELATIONSHIP**				
	28	DNA-binding proteins: prokaryotic transcription factors	19	463-469	Branded & Tooze
	29	Helix-turn-Helix motif in DNA binding, Trp repressor	8	129-150	Branded & Tooze
	30	Eukaryotic transcription factors, Zn fingers	9	151-174	Branded & Tooze
	31	Helix-turn helix motifs in homeodomain, Leucine zippers,	10	175-202	Branded & Tooze
	32	Membrane proteins: General characteristics, Trans-membrane segments, prediction,	7	157-164	Branded & Tooze
	33	Bacteriorhodopsin and Photosynthetic reaction center	12	223-248	Branded & Tooze
	34	Immunoglobulins: IgG Light chain and heavy chain architecture, abzymes, Enzymes: Serine proteases,	11 15	207-219 299-321	Branded & Tooze
	35	Understanding catalytic design by engineering trypsin, chymotrypsin and elastase	17	347-370	Branded & Tooze
	36	Substrate-assisted catalysis other commercial applications	11 17	207-219 347-370	Branded & Tooze
5	**PROTEOMICS**				
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	38	Components of proteomics			
	39	Proteomic analysis			
	40	Importance of proteomics in biological functions	18	374-392	Branded & Tooze
	41	Protein arrays	18	374-392	Branded & Tooze
	42	Cross linking methods	18	374-392	Branded & Tooze
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UNIT I BONDS, ENERGIES, BUILDING BLOCKS OF PROTEINS 9

Covalent, Ionic, Hydrogen, Coordinate, hydrophobic and Vander walls interactions in protein structure. Interaction with electromagnetic radiation (radio, micro, infrared, visible, ultraviolet, X-ray) and elucidation of protein structure. Amino acids (the students should be thorough with three and single letter codes) and their molecular properties (size, solubility, charge, pKa), Chemical reactivity in relation to post-translational modification (involving amino, carboxyl, hydroxyl, thiol, imidazole groups).

1. Define the term crystal lattice and unit cell. (November/December 2016)
   Crystal lattice is a geometric arrangement of the points in space at hich the atoms, molecules, or ions of a crystal or biomolecule occurs.

2. What are the primary and secondary bonds involved in protein structure? (November/December 2016)
   Primary bonds involves the covalent bonds inter-connecting two amino acids, whereas the secondary bonds involves ionic bonds such as hydrogen bonds stabilizing protein linear structures.

3. Define Post translational modification and mention the cellular sites for major post translational modification (May/June 2016).
   Post-translational modification generally refers to the addition of a functional group covalently to a protein as in phosphorylation and refers to proteolytic processing and folding processes necessary for a protein to mature functionally. The major sites are as follows;

Sites	Post translational modification
Ribosomes	N and O linked glycosylation
Ubiquitation	Lysosomes

4. What is Zwitterion? Give example (May/June 2016).
   Zwitterion ion is a neutral molecule with both positive and negative electrical charges. Example: Amino acids.

5. Define EMR and mention its importance with reference to protein engineering. (November/December 2015).
   Electromagnetic (EM) radiation is the movement of energy, through space or a medium, composed of both electric and magnetic waves. These waves oscillate at frequencies that can vary from a few cycles per second (hertz) to more than 10^20 hertz. Light, microwaves, X-rays, and cell phone transmissions are all examples of electromagnetic radiation.

6. Distinguish between preproproteins and proproteins (November/December 2015).
   Pre-sequences usually function as signal peptides for protein targeting, while pro-sequences play a crucial role in the folding of pro-proteins.

7. What are various chemical modifications during post translational modification?
   Various modifications include
   - Amino Group Modification
   - Carboxyl Group Modifications
   - Hydroxyl Group Modifications
   - Thiol Group Modifications
   - Imidazole Group Modification

8. What are chemical modifications at N-terminal end?
   Amino group modification involves the addition of a functional group at the N terminus of the amino acid includes
   - Acetylation
   - Pyroglutamate formation
   - Myristoylation
   - Methylation
   - Carbamylation

9. What are chemical modifications at C-terminal end?
   The C-terminus of a protein or polypeptide is the end of the amino acid chain terminated by a free carboxyl group;
   - Amidation
   - Prenylation
   - Ubiquitination
   - Sumoylation

10. How the carboxyl group different from hydroxyl group?
    The hydroxyl group is attached to carbonyl carbon atom in carboxyl group whereas in hydroxyl group, the hydroxyl group directly attached to carbon atom.

11. What is Post Translational Modification (PTM)?
    - Post-translational modifications are modifications that occur on a protein, catalysed by enzymes, after its translation by ribosomes is complete.
    - Post-translational modification generally refers to the addition of a functional group covalently to a protein as in phosphorylation and refers to proteolytic processing and folding processes necessary for a protein to mature functionally.

12. What are different types of post translation modification and its functions?

Modification	Charge-dependent change
Acylation	loss of a-amino positive charge
Alkylation	alteration of a- or e-amino positive group
Carboxylmethylation	esterification of specific carboxyl group
Phoshorylation	mainly modify Ser, Thr and Tyr
Sulfation	mainly modify Tyr
Carboxylation	bring negative charge
Sialyation	mainly on Asn, Thr and Ser
Proteolytic processing	truncation leads to change of pI

13. What is SUMO protein and sumolyation?
    - SUMOylation is another PTM known to modify a large number of proteins and plays a role in various cellular processes including: cell cycle regulation, gene transcription, differentiation and cellular localisation.
    - SUMOylation are reversible process.
    - SUMO proteins are 12 kDa proteins.
    - SUMO proteins are highly conserved from yeast to mammalian cells.
    - SUMOylation, the linkage of SUMOs to target proteins through isopeptide bonds.

14. What is Ubiquitylation?
    - Ubiquitylation, which involves attachment of the 76-residue protein ubiquitin (Ub) to other proteins, often targets the substrate protein for degradation by the proteasome.

15. Distinguish covalent bond from hydrogen ion bond.
    A Covalent bond is one in which one or more pairs of electrons are shared by two atoms, whereas a hydrogen bond is a force of attraction between a hydrogen atom in one molecule and a small atom of high electronegativity in another molecule.

16. Define hydrophobic interactions.
    Hydrophobic interactions describe the relations between water and **hydrophobes** (low water-soluble molecules i.e. nonpolar molecules and usually have a long chain of carbons that do not interact with water molecules).

17. Mention two uses of X-ray irradiation in protein research. (November/December 2011)
    - Protein structure prediction
    - Protein crystals morphology

18. Write a brief note on Vanderwaal's interactions in protein research. (November/December 2011)
    - Van der Waals forces are also known as London forces.
    - They are weak interactions caused by momentary changes in electron density in a molecule.
    - They are the only attractive forces present in nonpolar compounds.

19. Classify the amino acids based on chemical nature?
    The protein amino acids are classified according to the chemical nature of their R groups as aliphatic, aromatic, heterocyclic and sulphur containing amino acids.

20. What are the reactions due to amino groups?
    - Reaction with formaldehyde (Formal titration)
    - Reaction with nitrous acid
    - Reaction with ninhydrin

21. Account for high melting point of amino acids. (November/December 2011)
    Both acidic as well as basic amino groups are present in the same molecule of amino acids. In aqueous solutions, the carboxyl group can lose a proton and the amino group can accept a proton, thus giving rise to a dipolar ion known as zwitter ion. Due to this dipolar behavior, they have strong electrostatic interactions within them and with water. For this reason, the melting points and solubility of amino acids in water is higher than those of the corresponding halo-acids. In halo-acids, dipolar behavior is not exhibited.

22. List out any four molecular properties of amino acids. (November/December 2011)
    The molecular properties include charge, hydrophilicity or hydrophobicity, size, and functional groups.

23. Write the weak forces that are essential for the stability of protein structure. (May/June 2007)
    - Van der Waals : 0.4-4 kJ/mol
    - Hydrogen bonds : 12-30 kJ/mol
    - Ionic bonds : 20 kJ/mol
    - Hydrophobic interactions :<40 kJ/mol

24. Write the principle behind the UV absorption of protein at 280nm. (May/June 2007)
    At this wavelength, the absorbance of protein is *mainly* due to the amino acids tryptophan, tyrosine and cysteine with their molar absorption coefficients decreasing in that order. Of course, the molar absorption coefficient of the protein itself at 280 nm will depend upon the relative concentrations of these three amino acids.

25. Explain the significant features of a peptide bond in proteins. Why do most of the peptide backbones assume trans-conformation. (November/December 2008)

Features of peptide bond

- The peptide bond is rigid and planar
- The atoms in peptide bond is **C*α*-C-N-C*α*.**
- The peptide bond is coplanar, this indicated a **resonance** or partial sharing of two pairs of electrons between the carbonyl oxygen and the amide nitrogen.
- The 4 atoms of the peptide group (C, H, O, and N) lie in a single plane, in such a way that the oxygen atom of the carbonyl group and the hydrogen atom of the amide nitrogen are ***trans*** to each other.
- The peptide bond shows partial double bond character.

Peptide bond assumes trans conformation

Most of the 99% peptide bonds assume trans conformation because it confers stability, avoids steric hindrance and unwanted intermolecular interactions.

26. Classify proteins based on size and shape?
    - Globular proteins are mostly water-soluble and fragile in nature e.g., enzymes, hormones and antibodies.
    - Fibrous proteins are tough and water-insoluble. They are used to build a variety of materials that support and protect specific tissues, e.g., skin, hair, fingernails and keratin

27. Classify proteins classification based function?
    - Catalytic proteins (Enzymes)
    - Regulatory proteins (Hormones)
    - Protective proteins (Antibodies, Fibrin)
    - Storage proteins (Globulins and prolamins in cereals
    - Transport proteins (Haemoglobin, Myoglobin and Lipoprotein)
    - Toxic proteins (Ricin, Trypsin inhibitor, Lectin)
    - Structural proteins (Myosin, Keratin, Collagen)
    - Contractile proteins (Actin, Myosin)
    - Secretary proteins (Fibroin – spider)
    - Exotic proteins (Antifreeze proteins – Antartic species)

28. Classify the amino acids based on their charge. (November/December 2008)
    - Non Polar Amino acids have equal number of amino and carboxyl groups and are neutral (alanine, valine, leucine, isoleucine, phenyl alanine, glycine, tryptophan, methionine and proline)
    - Polar Amino acids (These amino acids do not have any charge on the 'R' group.)
    - Polar amino acids with positive charge have more amino groups as compared to carboxyl groups making it basic. (lysine, arginine and histidine)

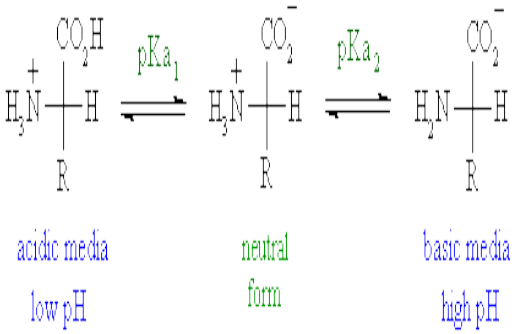
- Polar amino acids with negative charge have more carboxyl groups than amino groups making them acidic (aspartic acid and glutamic acid)

29. Below are five amino acids. Indicate all characteristics that apply to each. (November/December 2008)

Amino acid	Characteristics
Proline	The distinctive cyclic structure of proline's side chain gives proline an exceptional conformational rigidity compared to other amino acids. It also affects the rate of peptide bond formation between proline and other amino acids.
Methionine	Methionine, an essential amino acid, is one of the two sulfur-containing amino acids. The side chain is quite hydrophobic and methionine is usually found buried within proteins.
Tryptophan	Tryptophan, an essential amino acid, is the largest of the amino acids. It is also a derivative of alanine, having an indole substituent on the β carbon. The indole functional group absorbs strongly in the near ultraviolet part of the spectrum. The indole nitrogen can hydrogen bond donate, and as a result, tryptophan, or at least the nitrogen, is often in contact with solvent in folded proteins.

30. How do you calculated the pI of amino acids (Glycine)? (November/December 2009)

The isoelectronic point or isoionic point is the pH at which the amino acid does not migrate in an electric field. These amino acids are characterised by two pKas : pKa1 and pKa2 for the carboxylic acid and the amine respectively. The isoelectronic point will be halfway between, or the average of, these two pKas, i.e. pI = 1/2 (pKa1 + pKa2). This is most readily appreciated when you realise that at very acidic pH (below pKa1) the amino acid will have an overall +ve charge and at very basic pH (above pKa2) the amino acid will have an overall -ve charge. For the simplest amino acid, glycine, pKa1= 2.34 and pKa2 = 9.6, pI = 5.97.



31. Define Peptide bond.

In a peptide bond, the carboxyl group (COOH) of one amino acid bonds with the amino group (NH_2) of another, forming the sequence CONH and releasing water (H_2O).

32. Mention the different kinds of covalent and non-covalent interactions that stabilize the protein structure. (April/May 2011)

Covalent interactions	**Non-covalent interactions**
Single, double bond	Hydrogen bond
Disulfide bond	Hydrophobic interactions
	Electrostatic interactions
	Alternative hydrogen bonds
	Vander waal's interactions

33. Outline the property of protein which has been used for its estimation at 280nm. (April/May 2011)

    At this wavelength, the absorbance of protein is *mainly* due to the amino acids tryptophan, tyrosine and cysteine with their molar absorption coefficients decreasing in that order. Of course, the molar absorption coefficient of the protein itself at 280 nm will depend upon the relative concentrations of these three amino acids.

Part B

1. Explain the role of covalent and non-covalent bonds in protein structure, Pg.3-12, Cp.1; Nov 2012, 2013, 2015, May 2016
2. Discuss hydrophobic and van der waals interactions in protein structure, Pg.3-12, Cp.1;Nov-2014
3. Explain the post-translational modifications of amino, carboxyl, hydroxyl, methyl, thiol and imidazole rings,Pg.3-12, Cp.1; Nov-2014, 2013, 2012
4. Enlist and describe the molecular properties of amino acids, Pg.3-12, Cp.1; Nov-2014, 2013, 2012.
5. Write notes on Phosphorylation, Glycosylation, Sumolyation, Ribosylation Pg.3-12, Cp.1; Nov-2015.
6. Write brief notes on UV and Visible rays in protein structure analysis, Pg.3-22, Cp.2; Nov-2015.
7. What chromophores are responsible to UV absorption in a protein? Pg.3-22, Cp2; May 2016.
8. Explain in detail the elucidation of protein structure using NMR method, Nov 2016

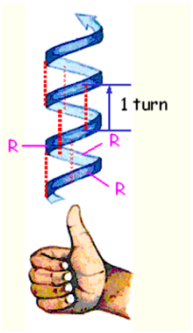
Part C

1. Explain Post translational modification and its biological significance, Pg.3-12, Cp.1; Nov-2014, 2013, 2012
2. Describe the protein structure elucidation, Nov 2016.
3. Write short notes on physical and chemical properties of amino acids? Pg.3-12, Cp.1; Nov-2014, 2013, 2012.

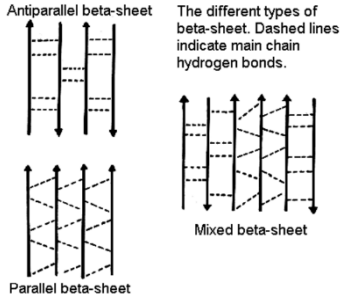
UNIT II PROTEIN ARCHITECTURE **9**

Primary structure: peptide mapping, peptide sequencing - automated Edman method & mass- spec. High-throughput protein sequencing setup Secondary structure: Alpha, beta and loop structures and methods to determine Super-secondary structure: Alpha-turn alpha, beta-turn- beta (hairpin), beta-sheets, alpha-beta-alpha, topology diagrams, up and down & TIM barrel structures nucleotide binding folds.

1. Show the diagrammatically the different structures of protein? (November/December 2016)



Alpha helix



1. Define the term dihedral or torsion angle (November/December 2016)
   In a polypeptide the main chain N-C_α and C_α-C bonds relatively are free to rotate. These rotations are represented by the torsion angles phi and psi, respectively.

2. What is meant by an Oligomer and a protomer?
   Proteins with more than one subunit are called oligomers, and their identical units are called protomers. A protomer may therefore consist of one polypeptide chain or several unlike polypeptide chains.

3. What is meant by PITC? Mention its use (May/June 2016).
4. What is Primary Structure?
   A carboxylic acid condenses with an amino group with the release of water molecule resulting in a peptide bond. The amino acid sequence is called as primary structure.

5. What is Top-down approach?
   In this strategy, separation of proteins by gel electrophoresis (GE) followed by in-gel proteolytic digestion and liquid chromatography (LC)–MS analysis of the peptides can be considered a top-down approach, i.e., intact protein ions or large protein fragments are subjected to gas-phase fragmentation for MS analysis.

6. What is Bottom-up approach?

In bottom-up proteomics, purified proteins, or complex protein mixtures, are subjected to proteolytic cleavage, and the peptide products are analyzed by MS.

7. What is Tryptic mapping or Tryptic digest?
   When trypsin is used as a protease for the digestion of proteins, the technique of peptide mapping is known as tryptic mapping.

8. Define Peptide mapping.
   Peptide mapping is an identity test for proteins, especially those obtained by rDNA technology. It involves the chemical or enzymatic treatment of a protein resulting in the formation of peptide fragments followed by separation and identification of these fragments in a reproducible manner. It is a powerful test that is capable of identifying almost any single amino acid changes.

9. What are the techniques for separation of peptides?
   - Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)
   - Ion-Exchange Chromatography (IEC)
   - Hydrophobic Interaction Chromatography (HIC)
   - Polyacrylamide Gel Electrophoresis (PAGE), nondenaturating
   - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
   - Capillary Electrophoresis (CE)
   - Paper Chromatography-High Voltage (PCHV)
   - High-Voltage Paper Electrophoresis (HVPE)

10. Define Peptide sequencing.
    Protein sequencing is a technique to determine the amino acid sequence of a protein, as well as which conformation the protein adopts and the extent to which it is complexed with any non-peptide molecules.

11. Write the difference between peptide mapping and peptide sequencing?

Peptide mapping	***Peptide sequencing***
Protein must be in the database	Sequence information
Extremely sensitive	Sensitive
Clean sample	Extremely clean sample
Easy data acquisition	More complex data acquisition
Easy data interpretation	More complex data interpretation
Fast	Can be time consuming
Easy to automate	More difficult to automate

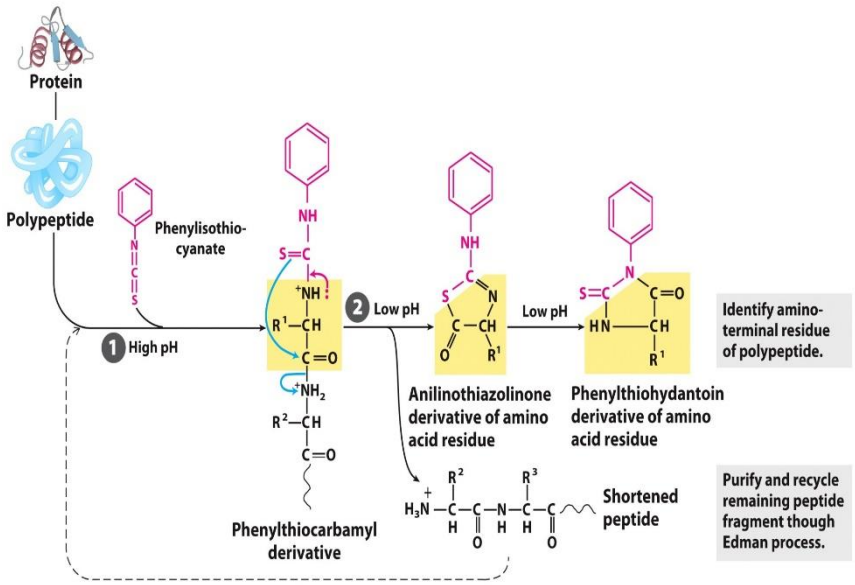
12. What are the enzymatic cleavage sites of peptides?

Type	Agent	Specificity
Enzymatic	Trypsin	C-terminal side of Arg and Lys
	Chymotrypsin	C-terminal side of hydrophobic residues (e.g., Leu, Met, Ala, aromatics)
	Pepsin	Nonspecific digest
	Glutamyl endopeptidase	C-terminal side of Glu and Asp

13. What are the chemical cleavage sites of peptides?

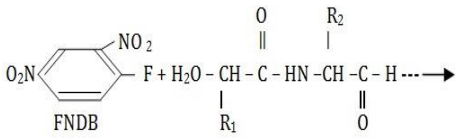
Type	Agent	Specificity
Chemical	Cyanogen bromide	C-terminal side of Met
	O-iodosobenzoic acid	C-terminal side of Trp and Tyr
	Dilute acid	Asp and Pro
	BNPS-skatole	Trp

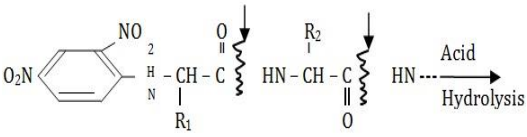
14. Write a note on Edman method of protein sequencing?

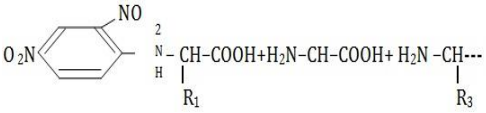


15. Brief out N-terminal method of determination of amino acid sequence (Sanger's method)
    - The N-terminal amino acid is identified by the reaction with 1-fluoro-2, 4-dinitrobenzene (FDNB) yielding dinitrophenyl residue of the N-terminal amino acid.

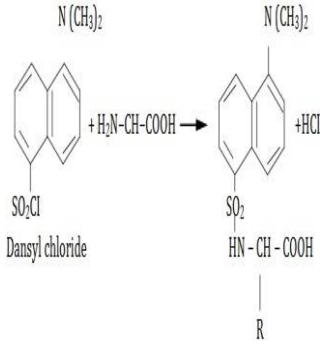
- The DNP-peptide is then hydrolyzed by 6N HCI so that all peptide bonds are cleaved leaving the DNP-derivatives.
- Since this method involves hydrolysis of peptide, it cannot be used for stepwise degradation



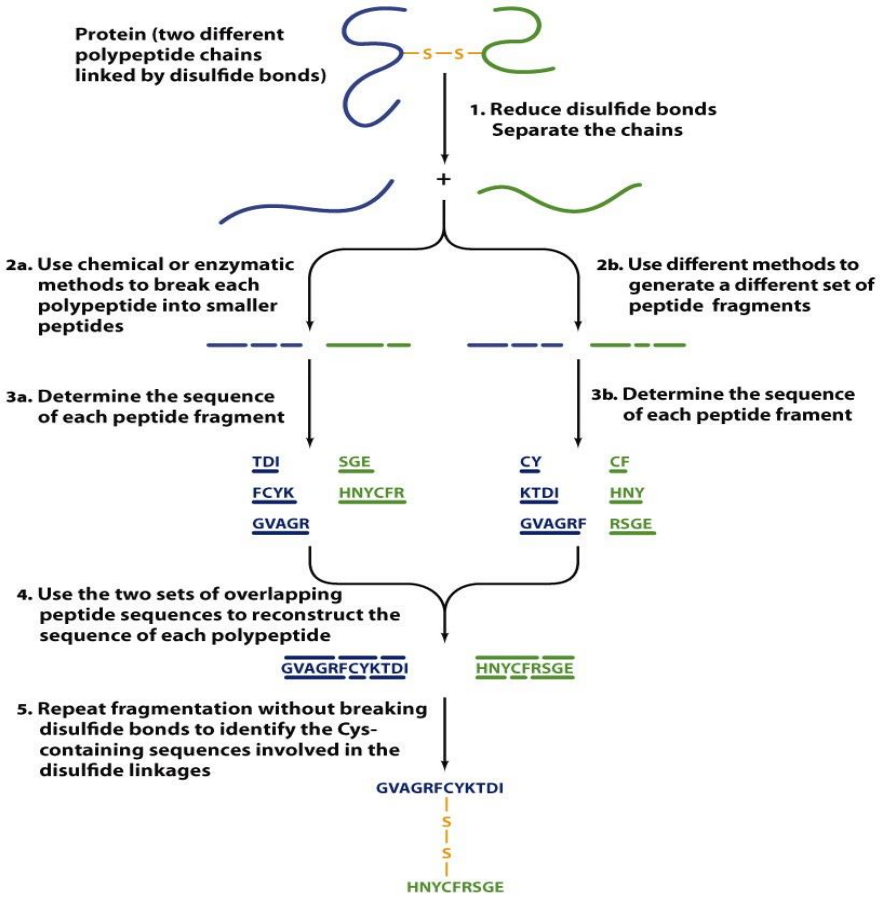




16. Explain the N-terminal method of determ ination of amino acid sequence (Dansyl chloride method)
    - Dansyl chloride regent is used for determination of N-terminal residue in alkaline condition.
    - The N-terminal residue forms a yellow fluorescent derivative which can be easily detected in minute amounts.
    - The derivative is also resistant to acid hydrolysis of polypeptides.



17. Outline protein sequencing protocol?



18. Write alternatives of Automated Edman method of protein sequencing?
    Automated Edman degradation as the primary method of peptide sequence analysis is now being increasingly replaced by mass spectrometric (MS) methods such as electrospray ionization combined with triple-quadrupole or ion-trap mass spectrometers and by MALDI-PSD.

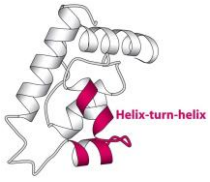
19. What are High throughput protein sequencing strategies?
    High throughput protein sequencing is a methodology of combination of various methods such as Edman degradation followed by identification of the components by advanced mass spectroscopic techniques. Example: Peptide mass finger printing, MALDI-TOF, -MALDI and SELDI. Various techniques used are as follows;
    - Peptide sequencing (Edman method)
    - Peptide mass finger printing
    - N-terminal high throughput protein sequencing
    - Mass spectroscopic techniques
        - MALDI-TOF
        - Tandem MS peptide sequencing
        - MALDI-PSD

20. What are Secondary structures? Give examples.
    Secondary structure is defined by the patterns of hydrogen bonds formed between amine hydrogen and carbonyl oxygen atoms contained in the backbone peptide bonds of the protein. Example: Alpha helix, Beta helix, Loops.

21. What are Super Secondary structures? Give examples.
    In a chain-like biological molecule, such as a protein or nucleic acid, a structural motif is a supersecondary structure. Example : βαβ, β meander, helix turn helix etc.

22. What are the methods available for the determination of secondary structures and super secondary structures?
    - Chou and Fasman method
    - GOR method
    - Nearest-neighbor method
    - Neural networks

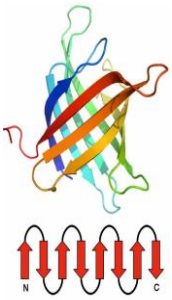
23. What is Alpha-turn alpha?



    - The helix-turn-helix (HTH) is a major structural motif capable of binding DNA.
    - It is composed of two α helices joined by a short strand of amino acids and is found in many proteins that regulate gene expression.
    - The helix-turn-helix (HTH) is a major structural motif observed in proteins capable of binding DNA. Eg. CAP and λ repressor (Cro).

24. What are reverse turns?

A reverse turn is region of the polypeptide having a hydrogen bond from one main chain carbonyl oxygen to the main chain N-H group 3 residues along the chain (i.e. O_i to N_{i+3}).



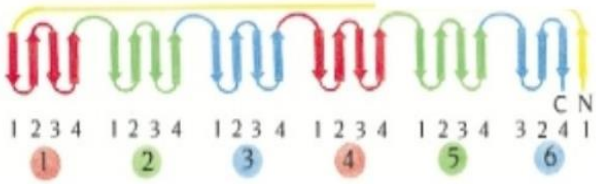
25. What are loops?

- α helices and β strands are connected by loop regions of various lengths and irregular shape. A combination of secondary structure elements forms the stable hydrophobic core of the molecule.
- The loop regions are at the surface of the molecule. The main-chain C=O and NH groups of these loop regions, which in general do not form hydrogen bonds to each other, are exposed to the solvent and can form hydrogen bonds to water molecules.

26. What are topology diagrams? Give examples.

Topology diagrams are the secondary structure layouparticulary with respect to β sheet, where the number of strands, their relative directions (parallel or antiparallel), and how the strands are connected. They are useful to compare β structures.

27. Write note on β-propeller?

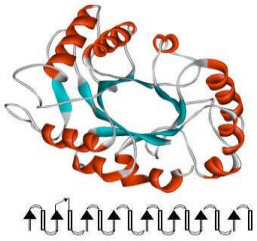


- The motif is a simple up-and-down anti-parallel β sheets of four strands.
- The directions of first and fourth strands differ by 90°.
- All the six motifs are arranged with six fold symmetry around an axis through the centre of the subunit. These six β sheets are arranged like six blades of a propeller.

28. Write a note on Up and down structures?

- The eight β strands are all anti-parallel to each other and are connected by hairpin loops.
- Beta strands that are adjacent in the amino acid sequence are also adjacent in the three-dimensional structure of up-and-down barrels.
- Example: The structure of human plasma retinol-binding protein (RBP) is an up-and-down β barrel.

29. What are TIM barrels?



- TIM barrel is a conserved protein fold consisting of eight α-helices and eight parallel β-strands that alternate along the peptide backbone.
- The structure is named after triosephosphate isomerase, a conserved metabolicenzyme.
- TIM barrels are one of the most common protein folds.
- TIM barrels are considered α/β protein folds because they include an alternating pattern of α-helices and β-strands in a single domain.

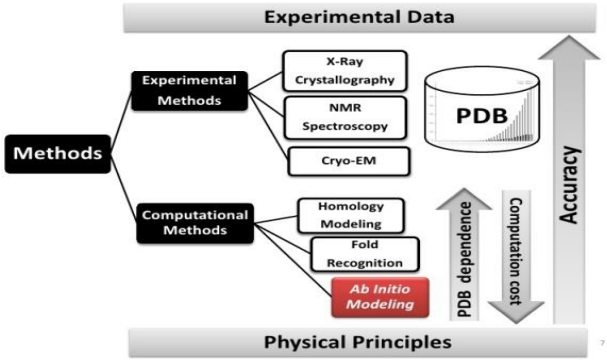
30. What are Domains?

A domain is defined as a polypeptide chain or a part of a polypeptide chain that can fold independently into a stable tertiary structure. Domains are also units of function, proteins may comprise a single domain or as many as several dozen domains. There is no fundamental structural distinction between a domain and a subunit.

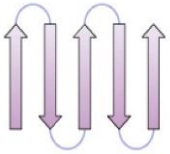
31. What is Rossmann fold?

The Rossmann fold is a protein structural motif found in proteins that bind nucleotides, such as enzyme cofactors FAD, NAD, and NADP. The structure is composed of up to seven mostly parallel beta strands. The first two strands are connected by an alpha-helix. 2 x βαβ motifs with the middle β shared between the two units. Example: 3-phosphoglycerate dehydrogenase beta sheet in the NAD binding domain. The two beta-strands that form the core of the Rossmann fold.

1. Sort the methods available for the determination of primary structure (November/December 2015).



32. Write a comment on β meander motif.



- A simple super secondary protein topology composed of 2 or more consecutive anti-parallel β-strands linked together by hairpin loops.
- This motif is common in β-sheets and can be found in several structural architectures including β-barrels and β-propellers.

Part B

1. Explain protein sequencing and its structural interpretation, Pg.374-92, Cp.18; Nov-2012.
2. Explain the role of peptide mapping in protein primary structure prediction, Pg.374-92, Cp.18; Nov-2013, May 2016.
3. Edman method of peptide sequencing and automatic peptide sequencing, Pg.374-92, Cp.18; Nov-2015.
4. Write short notes on Mass Spectroscopy and Tandem mass spectroscopy in primary structure determination, Pg.374-92, Cp.18; Nov-2015, Nov-2016.
5. Write notes on TIM barrel, Pg.36-87, Cp. 2-5, Nov-2015, Nov-2016.
6. Discuss with neat diagram and structure determination on supersecondary structures, Pg.24-34, Cp.2; Nov-2012, 2014, 2015, May 2016.

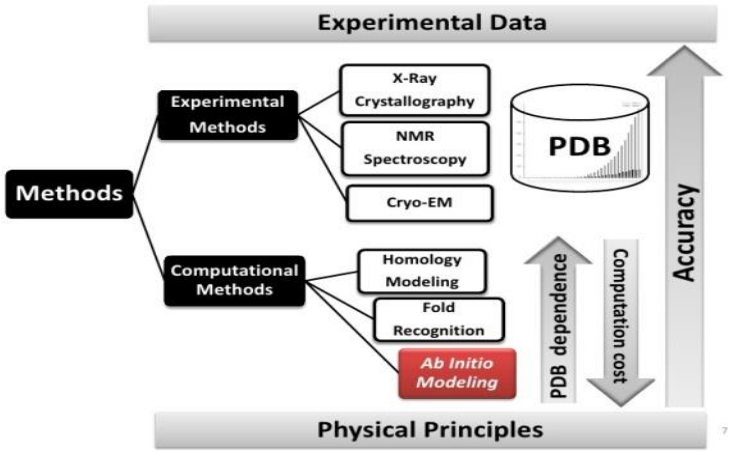
Part C

1. Describe and draw the supersecondary structures Pg.36-87, Cp. 2-5, Nov-2015, Nov-2016.
2. Elucidate the proteomic tools in protein structure prediction and analysis.
3. Enumerate the protein secondary structure prediction tools.

UNT III TERTIARY STRUCTURE **9**

Prediction of substrate binding sites, Tertiary structure: Domains, folding, denaturation and renaturation, overview of methods to determine 3D structures. Quaternary structure: Modular nature, formation of complexes, protein-protein interactions and methods to study it: Computer exercise on the above aspects

1. List the one advantages and one disadvantages of some of the 3D structure predicting techniques? (May/June 2016)

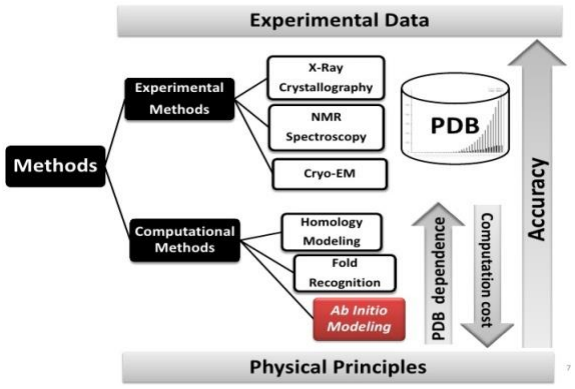


Give an example of a system exhibiting protein-protein interaction?
Yeast two-hybrid system
Mass spectroscopy

2. How to predict substrate binding sites?
   Substrate binding site or active sites can be predicted using experimental tool or by computational tools. Experimental tools such as X-ray diffraction, NMR, Peptide mass fingerprinting and site directed mutagenesis helps us to find the composition of the residues. Based on their input, computational tools assist in predicting the cavities.

3. What are the software used to predict active site?
   Castp, Qsite finder, Discovery studio etc.

4. What are the methodologies to determine 3D structure?

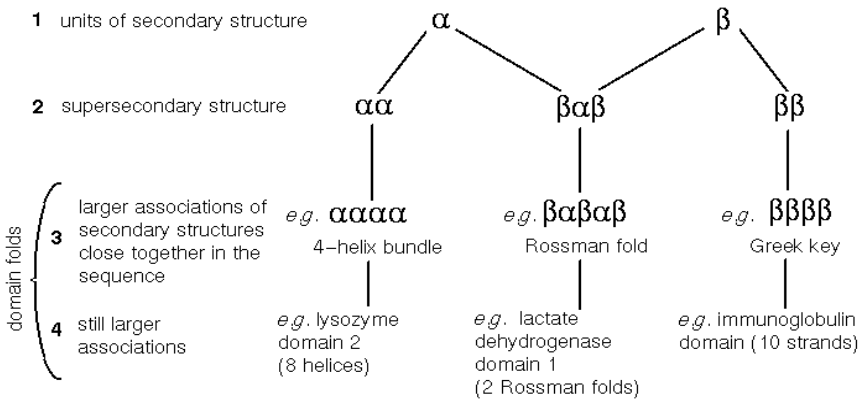


5. What is Tertiary structure of a protein? (November/December 2016)
   3-dimensional conformation of a whole polypeptide chain in its folded state (includes not only positions of backbone atoms, but of all the sidechain atoms as well) is referred to as Tertiary structure. Most water-soluble and membrane proteins are in tertiary structure only.

6. What are types of Tertiary structures?
   Tertiary structure describes how the secondary structure units associate within a single polypeptide chain to give a three-dimensional structure. Tertiary structures can be divided into three main classes:
   - a domain
   - b domains
   - a/b domains

7. Write a comment on Domains (November/December 2015).
   A domain is defined as a polypeptide chain or a part of a polypeptide chain that can fold independently into a stable tertiary structure. Domains are also units of function, proteins may comprise a single domain or as many as several dozen domains. There is no fundamental structural distinction between a domain and a subunit.

8. Give examples for Domains (Protein folds).



9. Give an example of a protein with quaternary structure.
   Insulin, Haemoglobin and Immunoglobulin

10. What is Protein folding?
    - Process in which a polypeptide chain goes from a linear chain of amino acids with vast number of more or less random conformations in solution to the native, folded tertiary (and for multichain proteins, quaternary) structure.
    - Protein folding is driven by its interaction with water as it emerges from ribosomal synthesis into the bulk aqueous phase of the cytoplasm.

- This particularly involves the hydrophobic interactions, so reducing their large surface area with water which otherwise would cause an unfavorable entropy decrease

11. Write a comment on Christian-Anfinsen experiment?
    Protein amino acid sequence determines the final shape a protein assumes in a water solution" was proven to be correct when Christian B. Anfinsen showed that if the enzyme ribonuclease was opened out into a linear chain and then allowed to reform, it reassumed the correct catalytic shape.

12. Write a note on Levinthal's paradox?
    Levinthal's paradox is that finding the native folded state of a protein by a random search among all possible configurations can take an enormously long time.

13. What are Protein folding models?
    The three Classic Models of Protein folding are
    - The classic Nucleation Model
    - The hydrophobic-collapse Model
    - Unified Nucleation-condensation Scheme

14. Write a note on folding funnel?
    The folding funnel highlights the many barriers to the preferred minimum energy structure on the folding pathway. There are numerous **local minima that might trap the protein in an inactive 3-D** molecular conformation.

15. What are the forces affecting Protein stability?
    Forces influencing protein stability;
    a. **Hydrophobic Forces**-The most important determining force of **secondary structure** of **tertiary structure**
    b. **Electrostatic Forces**
        i. **Ionic Interactions - Salt Bridge (bond)**: strong interactions but contribute little to stability because charged groups can be solvated by water in denatured form
        ii. **Dipole-Dipole Interactions - van der Waals forces:** weak but large number contributes significantly to stability
    c. **Disulfide Bonds**--not present in cytoplasm but form in extracellular secreted proteins to stabilize them.
    d. **Metal Ions** may stabilize proteins by cros-linking sidechains, e.g. **zinc finger motif**

16. Define Protein denaturation (November/December 2016)
    - Denaturation is a phenomenon that involves transformation of a well-defined, folded structure of a protein, formed under physiological conditions, to an

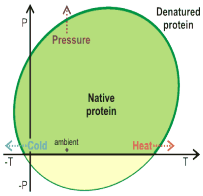
unfolded state under non-physiological conditions. It is a sudden, cooperative process and occurs over a narrow range.

- The free energy change on folding or unfolding is due to the combined effects of both protein folding/unfolding and hydration changes.

17. What are types of Protein denaturation.

Types of denaturation

- Temperature
- Organic solvents
- Surface
- pH
- Shear
- Pressure



18. What are Protein denaturants?

- High temperatures - cause protein unfolding, aggregation
- Low temperatures - some proteins are sensitive to cold denaturation
- Heavy metals (e.g., lead, cadmium, etc.) - highly toxic; efficiently induce the **'stress response'**
- Proteotoxic agents (e.g., alcohols, cross-linking agents, etc.)
- Oxygen radicals, ionizing radiation-cause permanent protein damage
- Chaotropes (urea, guanidine hydrochloride, etc.)-highly potent at denaturing proteins; often used in protein folding studies

19. Define Protein renaturation (November/December 2016)

Protein refolding is an important process by which proteins reform their native conformations. Renaturation or protein refolding is possible when denaturants are removed from the solvent system.

20. What are Molecular Chaperonins.

Chaperonins are proteins that provide favourable conditions for the correct folding of other proteins, thus preventing aggregation. Newly made proteins usually must fold from a linear chain of amino acids into a three-dimensional form. Chaperonins belong to a large class of molecules that assist protein folding, called molecular chaperones.

21. What are Molecular chaperones?

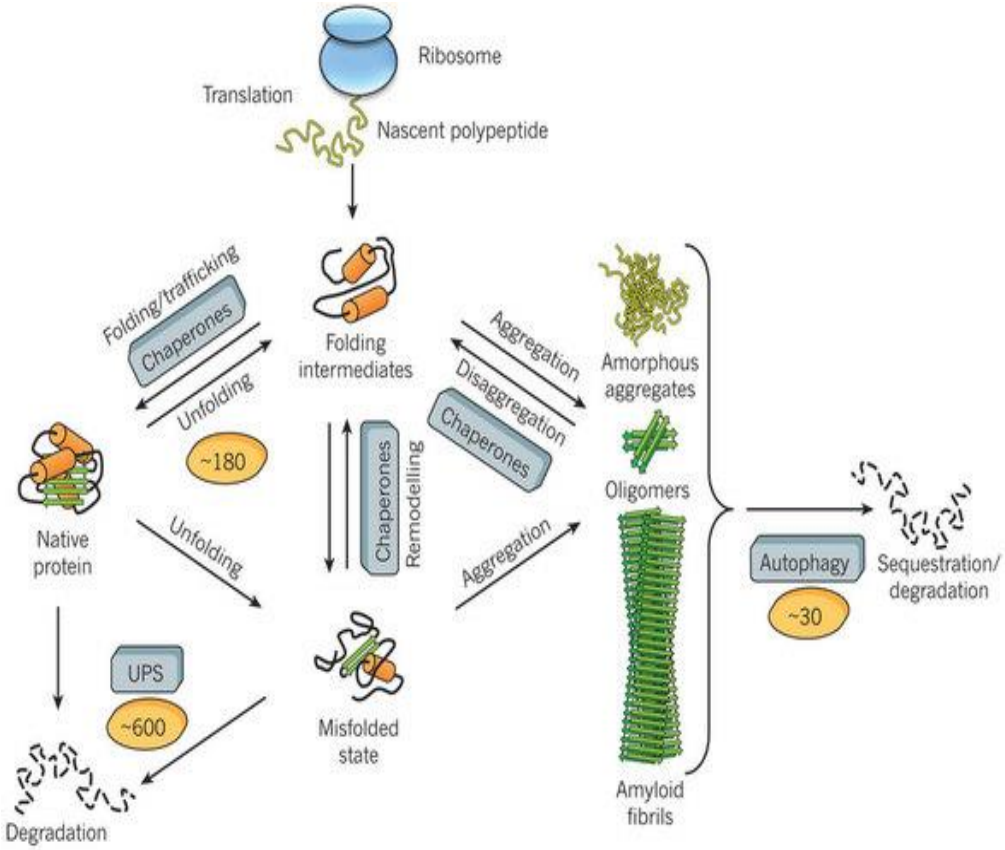
- Nascent polypeptides come off the ribosome and fold spontaneously, molecular chaperones are involved in their folding in vivo, and are related to heat shock proteins (hsp).
- The main hsp families are:
    - "Small hsp's" - Diverse "family" 10,000 - 30,000 MW (hsp26/27 - crystallins (eye lens))

- hsp40
- hsp60 (e.g. GroEL in *E. coli*)
- hsp70 (DnaK in *E. coli*)
- hsp90
- hsp100

22. Define Protomer.

A multisubunit protein may consist of identical or non-identical polypeptide chains. Proteins with more than one subunit are called oligomers, and their identical units are called protomers. A protomer may therefore consist of one polypeptide chain or several unlike polypeptide chains.

23. Outline general protein folding process.



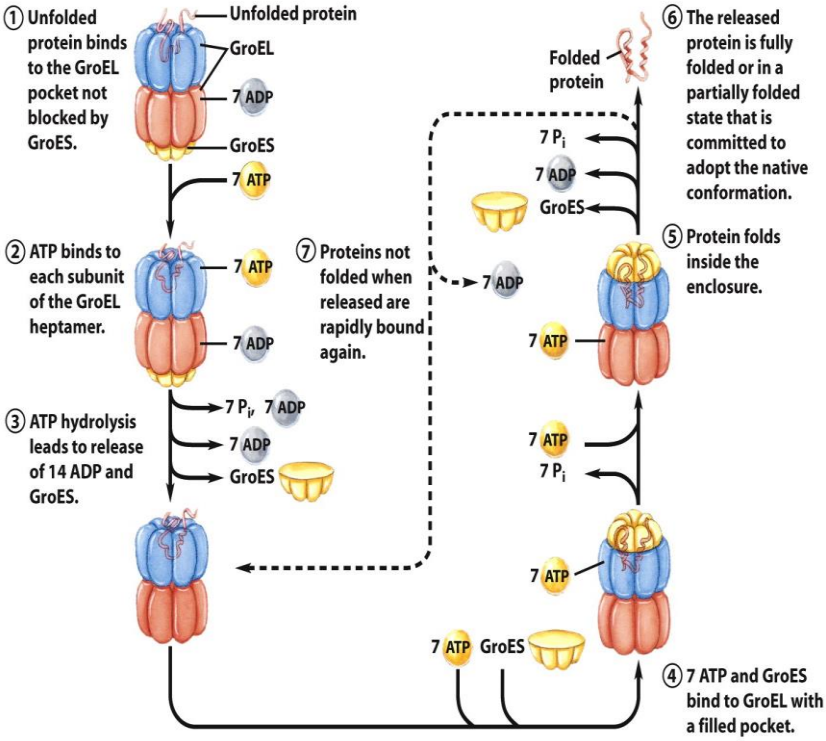
24. What are the functions of Molecular chaperones (Heat Shock proteins)?

- Minimize heat and stress damage to proteins (renaturation/degradation)
- Facilitate correct folding of proteins by minimizing aggregation and other misfolding
- Bind to nascent polypeptides to prevent premature folding
- Facilitate membrane translocation/import by preventing folding prior to membrane translocation
- Facilitate assembly/disassembly of multiprotein complexes

25. Write the significance of protein-protein interactions?

- Protein-protein interactions are intrinsic to virtually every cellular process.
- All modifications of proteins necessarily involve such transient protein-protein interactions. These include the interactions of protein kinases, protein phosphatases, glycosyl transferases, acyl transferases, proteases, etc., with their substrate proteins.
- Such protein-modifying enzymes encompass a large number of protein-protein interactions in the cell and regulate all manner of fundamental processes such as cell growth, cell cycle, metabolic pathways, and signal transduction.

26. Outline Protein folding mechanism?



27. What are the methods to study protein-protein interactions?

- Physical methods
  - Protein affinity chromatography
  - Affinity blotting
  - Immunoprecipitation
  - Cross linking
- Library based method
  - Protein probing (Mass Spec)
  - Phage display
  - Two hybrid method
  - Other library based method (Microarray)

28. What is Symmetry? What are the types of symmetry?

Symmetry is the concept of repetitive arrangements of similar objects in space. Various types of symmetry are rotational, cyclic, cubic, helical and dihedral symmetry.

29. Write a note on Phosphoprotein analysis?

- All processes regulated by protein phosphorylation are reversible and controlled by the combined action of two different classes of enzymes, namely protein kinases and phosphatases.
- These kinases and phosphatases, constitute about 2% of the human genome.
- Analysis of the entire cellular phosphoproteins panel, the so-called phosphoproteome, has been an attractive study subject since the discovery of phosphorylation as a key regulatory mechanism of cell life.

30. Define proteomics.

The term "proteomics" is a large-scale comprehensive study of a specific proteome, including information on protein abundances, their variations and modifications, along with their interacting partners and networks, in order to understand cellular processes. Proteomics has both a physical laboratory component and a computational component. These two parts are often linked together; at times data derived from laboratory work can be fed directly into sequence and structure prediction algorithms.

31. What are the techniques used to study Phosphoprotein interactions?

- Phosphoprotein detection and identification
    - 2D-Gel Electrophoresis
    - Immunoblot
    - Isotopic labelling –ICAT (Isotope Coded Affinity Tagging)
- Phosphoprotein enrichment
    - Immobilized Metal Affinity Chromatograpy (IMAC)
    - Specific Chemical derivatization
    - Immunoprecipitation
- Phosphoprotein identification
- Phosphorylation site mapping
    - Edman degradation and MS –Tandem MS
- Quantitative phosphoproteome analysis
- Phosphoprotein validation

Part B

1. Explain phosphoproteome analysis and techniques involved. Pg.374-92, Cp. 18.
2. Protein structure prediction by computational tools, Pg.374-94, Cp. 18; Nov-2014, May 2016.
3. Write notes on Domains, May 2016.
4. Write notes on Denaturation and Renaturation, Pg.89-104, Cp.6, Nov-2015.
5. Explain Protein folding process and mechanism, Pg.89-104, Cp. 6, Nov-2015, Nov 2016.
6. What is modular nature and complex formation in proteins, Pg.180-85, Cp. 7,
7. Protein structure prediction by x-ray crystallography and NMR, Pg.374-94, Cp. 18, Nov-2016, 2014, 2013.

Part C

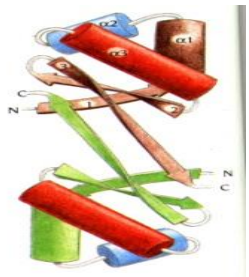
1. Write in detail the kinetics and re-kinetics pattern of protein folding? Pg.89-104, Cp. 6, Nov-2015, Nov 2016.
2. Describe the computational tools used in protein structure prediction.
3. Explain 2-D gel electrophoresis and its biological significance.

UNIT IV STRUCTURE-FUNCTION RELATIONSHIP **9**

DNA-binding proteins: prokaryotic transcription factors, Helix-turn-Helix motif in DNA binding, Trp repressor, Eukaryotic transcription factors, Zn fingers, helix-turn helix motifs in homeodomain, Leucine zippers, Membrane proteins: General characteristics, Trans-membrane segments, prediction, bacteriorhodopsin and Photosynthetic reaction center, Immunoglobulins: IgG Light chain and heavy chain architecture, abzymes and Enzymes: Serine proteases, understanding catalytic design by engineering trypsin, chymotrypsin and elastase, substrate-assisted catalysis other commercial applications Computer exercise on the above aspects

1. What is meant by Type I membrane proteins? (May/June 2016)
   Type I membrane protein has a single transmembrane span, anchored to the lipid membrane.

2. Show diagrammatically the binding site for protein in a DNA molecule (May/June 2016).



3. Give examples for serine proteases and a diagnostic test for identification of active serine group of serine proteases (November/December 2015).
   Various examples for serine proteases are Trypsin, Chymotrypsin, Elastase and Subtilisin. The diagnostic test to identification of active serine group of serine proteases is by site directed mutagenesis.

4. Write are Zn fingers?
   A zinc finger is a small protein structural motif that is characterized by the coordination of one or more zinc ions in order to stabilize the fold.

5. Mention the functions of membrane proteins (November/December 2015).
   - Membrane proteins are 25-35% of the genome.
   - Often important therapeutic targets: involved in signaling, transport, etc.
   - Can be anywhere from 25% (neurons) to 75% (mitochondria) by mass of the total membrane.
   - Under represented structurally

6. What are Transcription factors?

Transcription factors activate or repress transcription of target genes typically in response to an environmental or cellular trigger. These factors may be global or local depending on the number of genes and range of cellular functions that they target. The activities of both global and local transcription factors may be regulated either at a post-transcriptional level via signal-sensing protein domains or at the level of their own expression.

7. What are nucleotide binding proteins?
   - DNA-binding proteins are proteins that are composed of DNA-binding domains and thus have a specific or general affinity for either single or double stranded DNA.
   - Sequence-specific DNA-binding proteins generally interact with the major groove of B-DNA, because it exposes more functional groups that identify a base pair.
   - DNA-binding proteins include transcription factors which modulate the process of transcription, various polymerases, nucleases which cleave DNA molecules and histones which are involved in chromosome packaging and transcription in the cell nucleus.

8. What are forces involved in DNA-protein interactions?
   - Electrostatic forces: salt bridges
   - Dipolar forces: hydrogen bonds
   - Entropic forces: the hydrophobic effect
   - Dispersion forces: base stacking

9. Differentiate between activators and repressors.

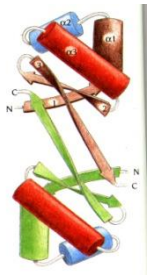
Repressors	Activators
Repressors bind tightly to the DNA at the promoter of a structural gene, preventing RNA polymerase from gaining access and hence blocking the initiation of transcription.	Activators work by binding next to the promoter and helping the polymerase to bind to the adjacent promoter thereby increasing the rate of transcription of the gene.

10. What are Eukaryotic transcription factor families
    - Helix-Turn-Helix proteins
    - Zinc finger proteins
    - Leucine zipper proteins
    - Helix-Loop-Helix proteins

11. Write a note on Helix-turn-helix DNA binding motif?
    - Helix-turn-helix motif is the most common DNA-binding motif in prokaryotes, present in many transcription repressors and activators

- One of the helices, DNA recognition helix, gets inserted in the major groove of DNA
- Helix-turn-helix proteins are often dimeric, with two recognition helices recognizing two adjacent DNA sequences

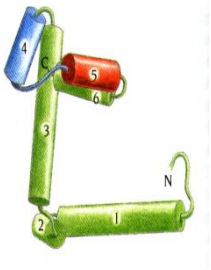
12. Write a note on Cro protein?



- Cro is a small protein that forms stable dimers in solution. Each subunit is a single polypeptide chain of 66 a.a. residues.
- It folds into three α helices and three strands of anti parallel β sheet.
- Belongs to α+β class of structures.
- α helix 2 and 3 form a unique HTH arrangement .
- Dimerization of Cro monomers depends primarily on interactions between β strands 3 from each subunit.

13. Write a note on Lamda repressor?
    - Lamda repressor protein is different from lamda Cro.
    - 236 a.a composed of two domains
    - The 92 N terminal residues is folded into five α helices connected by a loop regions.
    - α helix 2 and 3 form a unique HTH arrangement .
    - In spite of absence of C terminal domains, the DNA binding domains of lambda repressor form dimers in the crystals, as a result of interactions between the C-terminal helix number 5 C-terminal β strand.

14. Write a note on Trp repressor?

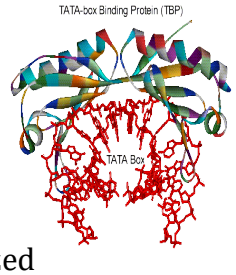


- The trp repressor controls the operon for the synthesis of L-tryptophan in E.coli by a simple negative feedback loop.
- In the absence of L-tryptophan, the repressor is inactive, the operon is switched on and the enzymes which synthesize L-tryptophan are produced.
- As concentration of L-tryptophan increases, it binds to the repressor and converts it to an active form so that it can bind to the operator region and switch off the gene.
- The trp respressor is a dimer (107 a.a), folded into six α helices.
- α helices 4-6 includes the HTH motif, form two “head” regions at the two ends of the molecule.
- α helix 3 connects the core to the head in both subunits.
- It has α-type structure like the lambda repressor, but the arrangement of the helices is quite different.
- The six helices do not pack to form a regular structure with a hydrophobic core.
- Stability is conferred by dimerization because two subunits fit together to give a functional molecule that, in contrast to its subunits, has a compact globular form.

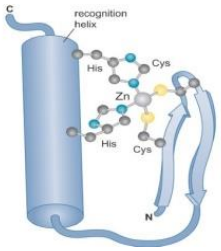
15. Write a note on Transcription Binding Proteins (TBPs)
    - TFIID contains several subunits
        - TATA-box binding protein (TBP)
            - Highly evolutionarily conserved
            - Binds to the minor groove of the TATA box
                - Saddle-shaped TBP lines up with DNA
                - Underside of the saddle forces open the minor groove
                - The TATA box is bent into 80° curve
        - TBP-associated factors (TAFs) specific for class II

16. Write a note on Homeodomain?
    - The homeobox, a DNA sequence of about 180 base pairs within the coding region of certain genes, was first discovered in the fruitfly Drosophila during studies of mutations that cause bizarre disturbances.
    - Homeoboxes have since been found in several hundred different genes from both vertebrates and invertebrates.
    - The characteristic homeodomain protein fold consists of a 60-amino acid helix-turn-helix (HTH) structure in which three alpha helices are connected by short loop regions.

17. What is Richardson diagram?
    Richardson-type diagram illustrates the structural similarity of the two domains. The loops that connect β strands is visualized as stirrups of this molecular saddle.



18. Write a note on Zinc fingers?
    - A **zinc finger** is a small protein structural motif that is characterized by the coordination of one or more **zinc** ions in order to stabilize the fold.
    - Zinc-binding repeats, known as zinc fingers (ZnF), are one such molecular scaffold.
    - This factor regulates transcription of ribosomal 5S RNA is isolated from Xenopus oocytes.



19. What are the types of Zinc fingers?
    Five types of zinc fingers are present in the system.

20. Write a note on Leucine zippers?
    - The **leucine zipper** is a protein–protein interaction domain consisting of amphipathic α helices that dimerize in parallel, either as homodimers or heterodimers, to form a coiled-coil. Introduction.

- Leucine zipper motif combines dimerization and DNA-binding surfaces within a single structural unit. Leucine-zipper-containing proteins often form heterodimers as well as homodimers.

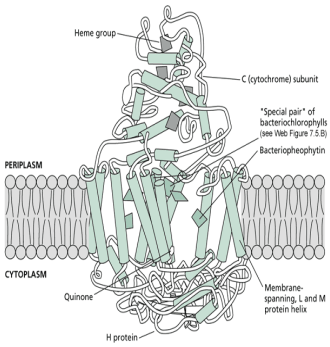
21. What is Homeodomain?

    The homeobox, a DNA sequence of about 180 base pairs within the coding region of certain genes, was first discovered in the fruitfly Drosophila during studies of mutations that cause bizarre disturbances. Homeoboxes have since been found in several hundred different genes from both vertebrates and invertebrates.

22. Brief out on Bacteriorhodopsin? (November/December 2016)
    - Bacteriorhodopsin is a proton pump found in Archaea, it takes light energy and coverts it into chemical energy, ATP, can be used by the cell for cellular functions.
    - Bacteriorhodopsin forms chains, which contain retinal molecule within, it is the retinal molecule that absorbs a photon from light, changes the confirmation of the nearby Bacteriorhodopsin protein, allowing it to act as a proton pump.
    - While chlorophyll based ATP generation depends on a protein gradient, like bacteriorhodopsin, but with striking differences, suggesting that phototrophy evolved in bacteria and archea independently of each other.

23. Write a note on Photosynthetic reaction center?
    - The reaction center is build up from four polypeptide chains, three of which are called L, M and H because they were thought to have light, medium and heavy molecular masses- based on electrophoretic mobility on SDS-PAGE it has three chains (H chain – 258 a.a, L chain – 273 a.a and M chain – 323 a.a).
    - The fourth subunit of the reaction center is a cytochrome that has 336 a.a
    - In addition to these four chains, the reaction centre contains a number of pigments. There are four bacteriochlorophyll molecules, two of which form the strongly interacting dimer called the special pair.
    - There is one Fe atom, a carotenoid, two quinone moleucles and two bacteriopheophytic molecules.

24. Write a note on structure of Photosynthetic reaction center?



    - The L and M subunits are firmly anchored in the membrane, each by five hydrophobic transmembrane α helices.
    - Cytochrome binds four heme groups.
    - The H segment has one such transmembrane helice AH and cytochrome has none.
    - No region of cytochrome penetrates the membrane, as this cytotchrome subunit is an intgral part of this reaction center.
    - Alpha helices D and E from the L and M subunits form the core of the membrane spanning part of the complex.

- These four helices provides a histidine side chain as ligand to the Fe atom- the role of Fe atom is probably to stabilize the structure of the four-helix bundle.

25. What are light harvesting particles?
    - Light harvesting complexes surrounds the reaction centers and increases photon capturing area. The reaction centers receive praclly all their light energy from such complexes.
    - The pigments are firmly bound to small hydrophobic protein molecules that are embedded in the membrane and which assemble into two types of multimeric complexes, called LH1 and LH2.

26. What are the functions of Immunoglobulins?
    - Igs- glycoprotein molecules produced by plasma cells in response to an immunogen and which function as antibodies.
    - **Antigen binding -**Each Ig actually binds to a specific antigenic determinant. It can result in protection of the host. The valency of Ab - no of antigenic determinants that an indi Ab molecule can bind. The valency of all Abs is at least 2 and in some instances more.
    - **Effector Functions -**Frequently the binding of ab to an ag - no direct biological effect. Rather, the significant biological effects - consequence of secondary "effector functions" of Abs.. Such effector functions include:
        - **Fixation of complement** - results in lysis of cells and release of biologically active molecules
        - **Binding to various cell types** - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind Igs. This binding can activate the cells to perform some function. Some Igs also bind to receptors on placental trophoblasts, which results in transfer of the Ig across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn

27. Differentiate between abzyme and enzyme?

Abzyme	Enzyme
An **abzyme** is an antibody that expresses catalytic activity. A single molecule of an antibody-enzyme, or **abzyme**, is capable of catalyzing the destruction of thousands of target molecules.	**Enzymes** are biological molecules (proteins) that act as catalysts and help complex reactions occur everywhere in life.
Eg.Catalytic antibody 28B4 abzyme catalyzes periodate oxidation of *p*-nitrotoluene-methyl sulfide to sulfoxide	Eg.Glucokinase catalyzes phosphorylation of glucose to give glucose-6-phosphate

28. What is Substrate Assisted Catalysis? (November/December 2016)
    - Substrate-assisted catalysis (SAC) is the process by which a functional group in a substrate contributes to catalysis by an enzyme.
    - In enzyme catalysis, the convention is that the enzyme supplies all the functional groups that are needed to convert a substrate into a product.
    - When a substrate provides one or more functional groups that actively participate in the catalytic process, are referred to as "substrate-assisted catalysis"

29. What are Proteinases? What are the types of proteinases?
    - Proteinases are widely distributed in nature.
    - Proteinases belong to one or other of four families: serine, cysteine, aspartic or metallo proteinases – based on functional criterion – prominent functional group in the active site.
    - Members are usually evolutionarily evolved

30. Write the mechanism of Serine proteinase?
    - The reaction proceeds in two steps
        - The first step produces a covalent bond between C1 of the substrate and the OH group of Ser residue of the enzyme- producing acyl-enzyme intermediate – negatively charged TS intermediate – tetrahedral geometry.
        - In the second step, deacylation takes places – hydrolysis of acyl-enzyme intermediate by water to release second peptide product with complete carboxy terminus and to restore the Ser-hydroxyl of the enzyme – negatively charged TS intermediate
31. What are the Structural features required for catalytic function of serine proteinases?
    - Catalytic triad: Asp-His-Ser. His – a general base that accepts the proton from the hydroxyl group of Ser, a nucleophile
    - Oxyanion hole: A pocket for negatively charged oxygen attached to C1
    - Main chain substrate binding: Nonspecific binding of substrates to the enzyme through their main-chain atoms of a loop region
    - Specificity pocket: A preference for a particular side chain before scissile bond

32. What is the basis of identifying trans-membrane helix in bioinformatics?
    Trans-membrane helix is identifying based on the presence of hydrophobic trans-membrane proteins either ased on neural networks. Example is TMPred based statistical methods.

Part B

1. Differentiate prokaryotic and eukaryotic transcription factors, Pg.463-69, Cp. 19, Nov-2016, 2014, 2013, May 2016.
2. Write a note on protein design and explain in detail Protein engineering, methodologies and advantages, Pg.347-60, Cp.17; Nov-2014.
3. Illustrate three major factors which may affect the stability of protein, Pg.347-60, Cp. 17; Nov-2013.
4. Demonstrate the photosynthetic reaction centre, Pg.223-48, Cp. 12; Nov-2012, Nov May-2016.
5. Explain Immunoglobulins and its fold in detail, Pg.207-19, Cp.11; Nov-2015, Nov-2016
6. Explain Serine proteases and its mechanism, Pg.299-21, Cp.15; Nov-2015, Nov, May-2016.

Part C

1. Describe the tools involved in drug designing.
2. Explain Substrate Assisted Catalysis and its biological significance. Pg.347-60, Cp.17; Nov-2014.
3. Describe the supersecondary structures involved in transcription factors, Pg.463-69, Cp. 19, Nov-2016, 2014, 2013, May 2016.

UNIT V PROTEOMICS **9**

Introduction to the concept of proteome, components of proteomics, proteomic analysis, importance of proteomics in biological functions, protein arrays, cross linking methods, affinity methods, yeast hybrid systems and protein arrays. Computer exercise on the above aspects

1. What is affinity purification? (November/December 2015), (May/June 2016).
   Affinity purification is based on the principle as follows; Protein sample is loaded into a column packed with Sepharose/sephadex materials. Then the protein are washed off readily using low salt conditions and are eluted using high salt solutions, chaotropic solvents or SDS.

2. Define proteome
   The term "proteome" refers to the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or a cellular system. This will vary with time and distinct requirements, such as stresses, that a cell or organism undergoes.

3. Define proteomics (November/December 2016) (May/June 2016)
   The term "proteomics" is a large-scale comprehensive study of a specific proteome, including information on protein abundances, their variations and modifications, along with their interacting partners and networks, in order to understand cellular processes. Proteomics has both a physical laboratory component and a computational component. These two parts are often linked together; at times data derived from laboratory work can be fed directly into sequence and structure prediction algorithms.

4. What is proteome analysis?
   Proteomic analysis (proteomics) refers to the systematic identification and quantification of the complete complement of proteins (the proteome) of a biological system (cell, tissue, organ, biological fluid, or organism) at a specific point in time.

5. What is the classification of proteomics?
   - Functional proteomics – Identification of protein-protein, protein-DNA and protein- RNA interactions affecting functions.
   - Structural proteomics- Identification of all interactions by metal ions, toxins, drugs etc affecting protein structure.
   - Differential proteomics – Determination of differences in protein expression

6. What are the unique challenges for proteomics compared to genomics?
   - One gene can encode more than one protein (even up to 1,000). The human genome contains about 21,000 protein-encoding genes, but the total number of proteins in human cells is estimated to be between 250,000 to one million.

- Proteins are dynamic. Proteins are continually undergoing changes, e.g., binding to the cell membrane, partnering with other proteins to form complexes, or undergoing synthesis and degradation. The genome, on the other hand, is relatively static.
- Proteins are co- and post-translationally modified. As a result, the types of proteins measured can vary considerably from one person to another under different environmental conditions, or even within the same person at different ages or states of health. Additionally, certain modifications can regulate the dynamics of proteins.
- Proteins exist in a wide range of concentrations in the body. For example, the concentration of the protein albumin in blood is more than a billion times greater than that of interleukin-6, making it extremely difficult to detect the low abundance proteins in a complex biological matrix such as blood. Scientists believe that the most important proteins for cancer may be those found in the lowest concentrations.

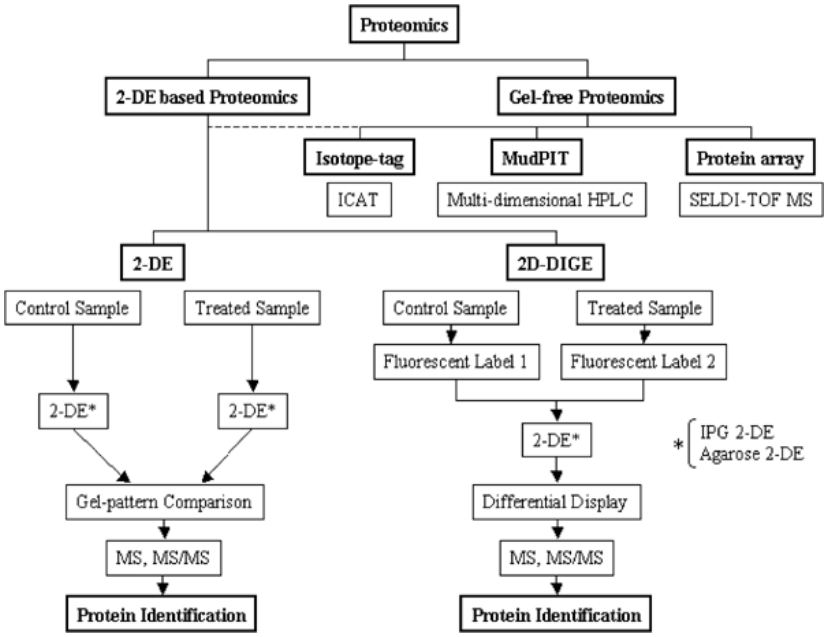
7. What are the applications of proteomics?
   - Discovery of protein biomarkers
   - Study of tumor metastatis
   - Study of neurotrauma
   - Disease diagnosis
   - Neurology
   - Nutrition research
   - Autoantibody profiling for the study and treatment of autoimmune disease

8. What are the levels of proteins?
   - Primary (1°): The amino acid sequence, containing members of a (usually) twenty-unit alphabet
   - Secondary (2°): Local folding of the amino acid sequence into α helices and β sheets
   - Tertiary (3°): 3D conformation of the entire amino acid sequence
   - Quaternary (4°): Interaction between multiple small peptides or protein subunits to create a large unit

9. What is Proteomic analysis?
   Proteomic analysis (proteomics) refers to the systematic identification and quantification of the complete complement of proteins (the proteome) of a biological system (cell, tissue, organ, biological fluid, or organism) at a specific point in time.

10. What are the tools used in proteomics?
    - Mass spectroscopy
    - Protein microarrays

11. Outline overall strategies of proteomic analysis.



12. General methodologies for protein detection during post translational modification
    - Glycoprotein detection methods
    - Phosphoprotein detection methods
    - Proteolytic modification detection methods
    - *S*-Nitrosylation detection method
    - Arginine methylation detection methods
    - ADP-ribosylation detection methods

13. What is the basis of identifying trans-membrane helix in bioinformatics?
    Trans-membrane helix is identifying based on the presence of hydrophobic trans-membrane proteins either by statistical or based on neural networks. Example is TMPred based statistical methods.

14. What are the methods to study protein-protein interactions?
    Physical methods
    - Protein affinity chromatography
    - Affinity blotting
    - Immunoprecipitation
    - Cross linking

    Library based method
    - Protein probing

- Phage display
- Two hybrid method
- Other library based method

15. Write a note on Protein Microarray and its types?

A **protein microarray** (or **protein** chip) is a high-throughput method used to track the interactions and activities of **proteins**, and to determine their function, and determining function on a large scale. Its main advantage lies in the fact that large numbers of **proteins** can be tracked in parallel. The various types are

- Large scale arrays of functional proteomics
- Antibody or capture arrays
- Reverse phase microarray

16. Write note on Immunoprecipitation?

- Immunoprecipitation (IP) is the small-scale affinity purification of antigens using a specific antibody, and one of the most widely used methods for antigen purification and detection.
- IP enables researchers to enrich for low-abundance proteins in order to improve downstream analyses, such as identifying the activation status, determining post-translational modifications, or capturing protein-binding partners (co-immunoprecipitation).

17. What is cross-linking? (November/December 2015, 2016)

When two or more proteins have specific affinity for one another that causes them to come together in biological systems, bioconjugation technology can provide the means for investigating those interactions. Cross-linking is used in two ways to deduce protein-protein interactions. First, it is used to deduce the architecture of proteins or assemblies that are readily isolated intact from the cell. Second, it is used to detect proteins that interact with a given test protein ligand by probing extracts, whole cells, or partially purified preparations.

18. Write a note on Yeast Two hybrid system?

- The yeast 2-hybrid (Y2H) assay is a well-established technique to detect protein-protein interactions. This is an extremely powerful tool for researchers and is often used alongside one or two other methods to examine the multitude of interactions that take place in cells.
- Principle
    - Y2H assay relies on the expression of a reporter gene (such as lacZ or GFP), which is activated by the binding of a particular transcription factor.
    - The transcription factor is comprised of a **DNA-binding domain (BD)** and an **activation domain (AD)**.
    - The query protein of interest fused with the **BD** is known as the **Bait,** and the protein library fused with the **AD** is referred to as the **Prey**.

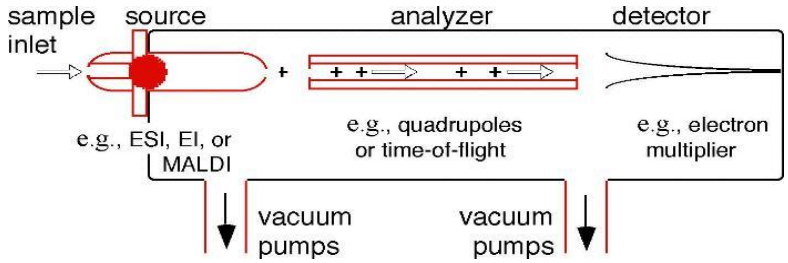
- In order to activate the reporter gene expression, a transcriptional unit must be present at the gene locus, which is only possible if Bait and Prey interact.

19. What are the techniques for separation of peptides?
    - Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)
    - Ion-Exchange Chromatography (IEC)
    - Hydrophobic Interaction Chromatography (HIC)
    - Polyacrylamide Gel Electrophoresis (PAGE), nondenaturating
    - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
    - Capillary Electrophoresis (CE)
    - Paper Chromatography-High Voltage (PCHV)
    - High-Voltage Paper Electrophoresis (HVPE)

20. Differentiate between MALDI-TOF and TANDEM MS

MALDI-TOF	**TANDEM MS**
Sample on a slide	Sample in solution
Spectra generate masses of peptide ions	MS-MS spectra reveal fragmentation patterns- amino acid sequence data possible
Protein Id by peptide mass fingerprinting	Protein identification by cross-correlation algorithms
Expensive	Very expensive
Good for sequenced genomes	Good for unsequenced genomic data

21. Draw the instrumentation of mass spectroscopy?



22. What are different types of mass spectroscopy?
    MALDI, MALDI-TOF, MS-MS, SELDI, GC-MS, LC-MS etc

23. What is matrix assisted laser desorption ionization (MALDI)?
    MALDI involved co-precipitation of large excess of a matrix material with the analyte molecule by pipetting a sub-microliter volume of a mixture of matrix and analyte onto a metal substance, where it is allowed to dry. The dried solid is then irradiated

by nanosecond nitrogen laser pulse with a wave length of 337 nm and there detected and analysed by MALDI TOF –MS.

24. Explain Phosphoproteomics.

    Phosphoproteomics is a branch of proteomics that identifies, catalogs, and characterizes proteins containing a phosphate group as a post-translational modification. Phosphorylation is a key reversible modification that regulates protein function, sub-cellular localization, complex formation, degradation of proteins and therefore cell signaling networks.

25. What is Recombinant Insulin?

    A form of insulin (trade name Humulin) made from recombinant DNA that is identical to human insulin; used to treat diabetics who are allergic to preparations made from beef or pork insulin.

Part B

1. What are the parameters which decide the protein-protein interactions? Explain in detail with example, Pg.347-92, Cp.18.
2. Explain Protein arrays and its applications, Pg.347-92, Cp. 18; Nov-2015, Nov-2016
3. Write note on Proteomic analysis and its application, Pg.347-92, Cp. 18; Nov-2015, May 2016.
4. Explain Yeast Two hybrid system in detail, Pg.347-92, Cp. 18; Nov-2015, May 2016.
5. Explain in detail the protein cross linking process and its application, Pg.347-92, Cp. 18.
6. What are the affinity methods in protein purification? Pg.347-92, Cp.18. Nov-2016
7. Write short notes on 2D gel electrophoresis? May 2016.

Part B

1. Explain the techniques and techniques for separation of peptides?
2. Describe the methodologies for protein detection during post translational modification
3. Explain the detail the microarray techniques in detail.

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Question Paper Code : 50194

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2017

Fifth Semester

Bio Technology

BT 6501 : PROTEIN STRUCTURE FUNCTION AND PROTEOMICS

(Regulations 2013)

Time : Three Hours | Maximum : 100 Marks

Answer ALL questions

PART – A **(10×2=20 Marks)**

1. Write note on the different kinds of non covalent interactions that stabilize protein structure.
2. Write the name and one letter code of the twenty amino acids.
3. Outline the principle of peptide mapping.
4. What is Ramachandran plot ? How it is involved in protein structure validation ?
5. Mention any two reagents used for denaturing the proteins and outline their mode of action.
6. Outline the approaches used to crystallize the proteins.
7. What are the main structural characteristics of membrane proteins ?
8. Outline the structural features of DNA polymerase.
9. What are phage antibodies how it is exploited in proteome analysis ?
10. Outline the principle of PSD-MALDI-MS method.

PART – B

(5×13=65 Marks)

11. a) Outline the interaction of proteins with electromagnetic radiation and their use in elucidation of protein structure.

(OR)

b) Explain the chemical reactivity of the following functional groups of the amino acids with suitable examples – Amino, carboxyl, thiol, hydroxyl and imidazole group.

12. a) Outline the steps involved in protein sequencing. Write a note on high-throughput protein sequencing setup.

(OR)

b) Explain the different types of supersecondary structures existing in proteins with suitable examples.

13. a) Explain any two methods used for determination of tertiary structure of proteins.

(OR)

b) Describe the Computational approaches used to understand protein – protein interactions.

14. a) What are the essential residues participate in catalysis of serine proteases and explain its catalytic mechanism ?

(OR)

b) Describe the structure of immunoglobulin and how it contribute to specificity of antigen binding.

15. a) How a snapshot of a proteome is obtained using 2D SDS PAGE ? Why it is considered as 'the tool of proteomics' despite its disadvantages ?

(OR)

b) Explain Yeast two hybrid system to analyze protein interaction.

PART – C

(1×15=15 Marks)

16. a) The following reagents are often used in protein chemistry :

CNBr	Performic acid	Phenyl isothiocyanate
Urea	Dabsyl chloride	Chymotrypsin
Mercaptoethanol	6 N HCl	
Trypsin	Ninhydrin	

Write the use of each reagent in protein structure analysis and outline their characteristic chemical reaction.

(OR)

b) Describe the steps involved in developing the antibody array and explain any two applications of antibody array.

———————

B.E/B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2017
Fifth Semester
BT 6501: PROTEIN STRUCTURE FUNCTION AND PROTEOMICS
(Regulation 2013)

Time : Three Hours **Maximum : 100 Marks**

Part A

Answer all the questions (2 × 10 = 20)

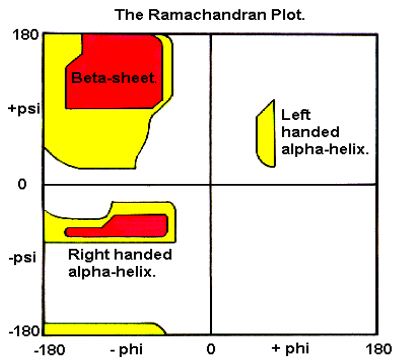
1. Write note on the different kinds of non-covalent interactions that stabilize protein structure? (November/December 2017)
   Non-covalent interactions are critical in maintaining the three-dimensional structure of large molecules, such as proteins. The different kinds of non-covalent interactions include electrostatic, π-effects, van der Waals forces, and hydrophobic effects.

2. Write the name and one letter code of the twenty amino acids (November/December 2017)

Amino acid (or residue in protein)	3-letter abbreviation	1-letter abbreviation	Mnemonic for 1-letter abbreviation
Glycine	Gly	G	Glycine
Alanine	Ala	A	Alanine
Valine	Val	V	Valine
Leucine	Leu	L	Leucine
Isoleucine	Ile	I	Isoleucine
Proline	Pro	P	Proline
Methionine	Met	M	Methionine
Phenylalanine	Phe	F	Fenylalanine
Tryptophan	Trp	W	tWyptophan (or tWo rings)
Tyrosine	Tyr	Y	tYrosine
Serine	Ser	S	Serine
Threonine	Thr	T	Threonine
Cysteine	Cys	C	Cysteine
Aspartic Acid	Asp**	D	asparDic acid
Glutamic Acid	Glu*	E	gluEtamic acid
Asparagine	Asn**	N	asparagiNe
Glutamine	Gln*	Q	Q-tamine
Histidine	His	H	Histidine
Lysine	Lys	K	(before L)
Arginine	Arg	R	aRginine

3. Outline the principle of peptide mapping (November/December 2017)
   Peptide mapping is an identity test for proteins, especially those obtained by rDNA technology. It involves the chemical or enzymatic treatment of a protein resulting in the formation of peptide fragments followed by separation and identification of these fragments in a reproducible manner. It is a powerful test that is capable of identifying almost any single amino acid changes.

4. What is Ramachandran plot? How it is involved in protein structure validation (November/December 2017)
   - In a polypeptide the main chain N-Calpha and C alpha-C bonds relatively are free to rotate. These rotations are



represented by the torsion angles phi and psi, respectively.

- A Ramachandran plot can be used in two somewhat different ways.
- One is to show in theory which values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein (as at top right).
- A second is to show the empirical distribution of data points observed in a single structure (as at right, here) in usage for structure validation

5. Mention any two reagents used for denaturing the proteins and outline their mode of action. (November/December 2017)
   Chaotropes such as urea, guanidine hydrochloride, etc, are highly potent at denaturing proteins; often used in protein folding studies. The mechanism of denaturation may be changes in the structure of the hydrogen bond network of water.

6. Outline the approaches used to crystallize the proteins (November/December 2017)
   - Hanging droplet method
   - Sitting method
   - Cryo-freeze method

7. What are the main structural characteristics of membrane proteins(November/December 2017)
   - Membrane proteins are 25-35% of the genome.
   - Often important therapeutic targets: involved in signaling, transport, etc.
   - Can be anywhere from 25% (neurons) to 75% (mitochondria) by mass of the total membrane.

8. Outline the structural features of DNA polymerase. (November/December 2017)
   - Posses super secondary structures such as helix-turn helix and helix loop helix
   - Participates in switch on and switch off mechanism
   - Has effective protein- nucleic acid binding sites

9. What are phage antibodies how it is exploited in proteome analyze? (November/December 2017)
   In this technique, a gene encoding a protein of interest is inserted into a phage coat protein gene, causing the phage to "display" the protein on its outside while containing the gene for the protein on its inside, resulting in a connection between genotype and phenotype. These displaying phages can then be screened against other proteins, peptides or DNA sequences, in order to detect interaction between the displayed protein and those other molecules. In this way, large libraries of proteins can be screened and amplified in a process called in vitro selection, which is analogous to natural selection.

10. Outline the principle of PSD-MALDI-MS method. (November/December 2017)
    - Post-source Decay (PSD) analysis is an extension of MALDI/MS that allows one to observe and identify structurally informative fragment ions from decay taking place in the field-free region after leaving the ion source.

- MALDI-PSD has been widely applied, mainly because of its high sensitivity for prepared sample amounts in the range 30-00 fmol and because of its high tolerance of sample impurities and sample in homogeneity.

Part B

Answer all questions (5×13 = 65)

1. Outline the interaction of proteins with electromagnetic radiation and their use in elucidation of protein structure **Pg.3-22, Cp.2; Nov-2017, 2015.** (OR)
2. Explain the chemical reactivity of the following functional groups of the amino acids with suitable examples – Amino, carboxyl, thiol, hydroxyl and imidazole group **Pg.3-12, Cp.1; Nov-2017, 2014, 2013, 2012.**
3. Outline the steps involved in protein sequencing. Write a note on high throughput protein sequencing set up. **Pg. 374-92, Cp.18; Nov-2017, 2012.** (OR)
4. Explain the different types of supersecondary structures existing in proteins with suitable examples. **Pg.24-34, Cp.2; Nov- 2017, 2015, 2014, 2012, May 2016.**
5. Explain any two methods used for determination of tertiary structure of proteins. **Pg.374-94, Cp. 18, Nov-2017, 2016, 2014, 2013.** (OR)
6. Describe the computational approaches used to understand protein-protein interactions. **Pg.347-92, Cp.18. Nov 2017**
7. What are the essential residues participate in catalysis of serine proteases and explain the catalytic mechanisms? **Pg.207-19, Cp.11; Nov-2017, 2016, 2015** (OR)
8. Describe the structure of immunoglobulin and how it contribute to specificity of antigen binding. **Pg.207-19, Cp.11; Nov-2015, Nov-2016**
9. How a snapshot of proteome is obtained using 2D SDS PAGE? Why it is considered as the tool of proteomics despite its disadvantages, **May 2016.** (OR)
10. Explain the yeast two hybrid system to analyze protein interactions, **Pg.347-92, Cp. 18; Nov-2017, 2015, May 2016.**

Part C

Answer all questions (1 ×15 = 15)

1. The following reagents are often used in protein chemistry
    a. CNBr
    b. Performic acid
    c. Phenyl isothiocyanate
    d. Urea
    e. Dansyl chloride
    f. Chymotrypsin
    g. Trypsin
    h. Mercaptoethanol
    i. 6 N HCl
    j. Ninhydrin

    Write the use of each reagent in protein structure analysis and outline their characteristic chemical reaction. **Pg.89-104, Cp.6, Nov-2017, 2015.** (OR)
2. Describe the steps involved in developing the antibody array and explain any two applications of antibody array. **Pg.347-92, Cp. 18; Nov-2015, Nov-2017, 2016**