BIOPHYSICS OF MACROMOLECULAR ASSEMBLIES
BY771

OUTLINE OF INTRODUCTION SECTION

Lecture 1: Introduction

- Course Outline
- Cell structure; nucleus endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, membranes, etc.
- Protein structure/assemblies
- Lipids
- Membranes; membrane protein classes
- Protein-nucleic acid assemblies; chromatin, ribosomes, viruses
- "Double Helix;" BBC film of Crick, Watson, Wilkins, Franklin, etc. and structure of DNA

OUTLINE OF PROTEINS SECTION

Lecture 2: Protein Folding Motifs and Quaternary Assembly

- Introduction to general aspects of protein assembly
- Aims of this section of course
- Structural hierarchy, secondary structure, loops, motifs and domains
- Folding hierarchy, super-secondary motifs, αα, βαβ, ββ motifs
- Tertiary motifs
- Alpha structures, helix packing, helix dipole, helix-turn-helix motif, amphipathic helices, packing geometry, helix bundles and globin fold
- Beta structures, parallel vs. anti-parallel sheet, crossovers, loops, sheet topology diagrams, the Greek key
- Beta-alpha-beta structures, helical crossovers
- Folding domains, sequence characteristics, repeated sequence domains
- Domain vs. quaternary assembly
- Fold classification, the CATH database

Lecture 3: Hemoglobin

- Myoglobin structure
- Hemoglobin quaternary assembly, symmetry of packing, cooperativity
- Hemoglobin/myoglobin sequence and secondary structure differences
- Subunit contacts and interactions
- Quaternary structure changes on oxygen binding
- Oxy vs. deoxy tertiary structure changes on oxygen binding
- Salt bridge interactions, role of penultimate Tyrosine, oxygen binding to iron
- Sequential tertiary structure changes, quaternary changes and cooperativity

Lecture 4: Clathrin

- Overview of the endocytic pathway, receptors, fuzzy coat, coated pits and vesicles
- Structure of fuzzy coat, transition to pits and vesicle, symmetry of vesicle formation, 5 vs. 6 fold packing symmetry, Euler's theoremNegative stain EM of vesicle and cage structure
- Clathrin molecule, triskelion, domain organization
- Clathrin packing and assembly in vesicles
- Cryo-EM of cages and coats, location of clathrin domains, adaptor proteins, receptor interactions
- Clathrin molecular structure from x-ray crystallography, proximal leg and N-terminal domain

Lecture 5: Spectrin

- Proteins of the erythrocyte membrane, cytoskeletal components
- Spectrin, Ankyrin, 4.1/4.2, Actin general features and interactions
- Assembly of Spectrin, repeated sequence domains, head-to-head vs. head-to-tail assembly
- Building the cytoskeleton, interactions
- Spectrin molecular details from x-ray structures, comparison with Actinin, structure of repeated unit
- Spectrin flexibility and conformational changes

Lecture 6: Actin, Myosin and Tubulin

- Review of striated muscle, supramolecular structure, sliding filament model
- Symmetry of sarcomere and filaments
- Assembly of myosin, repeating motifs of tail region, coiled-coil structures
- Myosin ATPase domain, molecular details from x-ray crystallography, domain structure, helix tail, ATP binding
- Assembly of Actin
- Molecular details from crystallography, domain structure
- Filament assembly, subunit interactions
- Actin-Myosin interactions

- Overview of cilia and organization of axoneme, protein of the axoneme
- Supramolecular structure of microtubules, helix geometry
- Tubulin dimmers, GTP/GDP binding
- Molecular structure, EM of tubulin sheets, subunit interfaces
- Molecular details, EM-crystallography, domain structure, nucleotide binding, subunit interfaces

OUTLINE OF LIPIDS SECTION

Lecture 7: Introduction

- Definitions and chemical classification of lipids
- Overview of lipid functions in cell and organisms
- Lipids – molecules with dual physical properties. HLB (hydropholic lipophilic balance); water and hydrocarbon (oil) solubility

Lecture 8: The Free Energy of Transfer from Water to Hydrocarbons

- Lipid distribution (partition) between water and hydrocarbon
- The partition coefficient, $K_{w/o}$
- Data obtained from varying the number of $-CH_2-$ in a chain
- The free energy of transport from water to oil
  $\Delta Gt_{w \rightarrow o} = RT \ln K_{w/o}$
- $\Delta G_{r\ w \rightarrow o}$ for methylene groups, ($-CH_2-$) methyls ($-CH_3$), double bonds ($-CH = CH-$) and hydrophilic groups

Lecture 9: Surface Behavior of Lipids

- Surface tensions and energy of cohesion
- Insoluble lipids – spreading and non spreading
- The spreading pressure
- Stable monolayers – Pockels – Langmuir balance
- Surface pressure/molecular area isotherms
- Unstable monolayers of soluble lipids
- Micelle formation and solubilization of hydrocarbons

Lecture 10: Structure of Lipids

- Classification of lipids based on interaction with H_2O
- Structure and packing of aliphatic chains in lipids
- Phase transitions in lipids; aliphatic chain transitions
- Effects of polar substitution on phase transitions
- The non ideal liquid state of lipids; the concept of fluidity

Lecture 11: The Mesomorphic State, a 4th State of Matter. Liquid Crystals

- Discontinuous changes in specific heat and volume between the solid crystalline state and the liquid state
- Definition of liquid crystals
- Structure of liquid crystals
- Classification of liquid crystals and ordered fluids
- Molecular motions, translations and translocation of lipids

OUTLINE OF LIPOPROTEINS SECTION

Lecture 12: The Lipids of Lipoproteins

- Introductions and historical prospective
- The classes of lipoproteins: chylomicrons (CM), VLDL, LDL, HDL, albumin
- The lipids of lipoproteins – classification and physical properties –phosphatidyl choline (PC), triacylglycerol (TG), cholesterol (C), cholesterol esters (CE)
- The interaction of lipids in lipoproteins

Lecture 13: Phase Behavior of the Lipoprotein Lipids

- The phase rule
- The PC- H_2O system
- The PC-C-H_2O, PC-CE-H_2O, PC-TG-H_2O system
- The lipoprotein lipid phase diagram
- Location of phases within a lipoprotein

Lecture 14: The Apoproteins

- The major apoproteins AI, AII, AIV, B, C, CI, CII, CIII and E
- Exchangeable and non exchangeable apoproteins
- Secondary and tertiary structure of apolipoproteins, amphipathic alpha helices (AAH) and amphipathic B strands (ABS)
- Interaction of apoproteins with lipids

Lecture 15: Lipoprotein Assembly, Plasma Conversions and Uptake – Physical Considerations

- Synthesis and secretion of apoB containing lipoproteins CM, VLDL
- Plasma conversions of VLDL to LDL – LDL uptake
- Formation of HDL

OUTLINE OF MEMBRANES SECTION

Lecture 16: Cell Membranes

- Review of overall membrane organization
- Cell membranes; plasma membrane, organelle membranes
- Membrane functions
- Membrane structure; average structure, localized structural domains
- Membrane composition; lipids, proteins
- Membrane lipids; organization, bilayers, distribution, dynamics
- Membrane proteins; organization, dynamics, functional classes

- Structural motifs of membrane proteins?

Lecture 17: Bacteriorhodopsin

- Halobacteria, H. Halobium, energetics, purple membrane
- Bacteriorhodopsin, light activated proton pump
- Early studies; isolation, chemical characterization
- Electron microscopy, x-ray diffraction, hexagonal arrangement
- Electron crystallography; Henderson/Unwin, 2D and 3D, transmembrane alpha helical bundles
- Labeling, neutron diffraction
- Retinal location, orientation
- Helix connectivity
- High resolution studies; electron and x-ray crystallography
- Proton channel, photocycle and pump mechanism

Lecture 18: Photosynthetic Reaction Center

- Plants, bacteria, energy transduction, photosynthesis
- Structure of R. viridis reaction center; L, M, H and cytochrome subunits
- Arrangement of prosthetic groups; heme, bacteriochlorophyll, bacteriopheophytin, carotenoid, quinine
- Electron flow
- Structure of bacterial light-harvesting complex; protein and pigment organization
- Structure of mitochondrial cytochrome bc1 complex; protein subunit structure

Lecture 19: Porins

- Porins in Gram-negative bacterial and mitochondria
- E. coli porins; PhoE, OmpF and OmpC
- Early structural studies; electron microscopy of OmpF and PhoE, trimers
- Transmembrane beta-barrel structures
- X-ray structures of OmpF, PhoE an Dr. capsulatus porins; 16-strand barrels, channel structure
- X-ray structure of OmpA; 8-strand barrel
- X-ray structure of FepA, 22-strand barrel, and Ompla (12 strand barrel
- Structure of maltoporins; 18-strand barrel, role of loops, structure of pore, sugar transport

Lecture 20: Bacterial Toxins

- S. aureus alpha hemolysin; cell lysis, oligomerization
- Structure of alpha hemolysin; monomer structure, heptamer structure
- Pore structure; 14-strand beta-barrel
- Anthrax toxin; subunit structure, mechanism of action

- Structure of protective antigen; domain structure of monomer, heptameric assembly, 14-strand beta-barrel?
- Structure of cholera toxin

Lecture 21: Influenza Virus Hemagglutinin

- Influenza virus; structure and mode of action
- Influenza virus hemagglutinin; receptor binding and membrane fusion activities
- Influenza virus hemagglutinin structure; bromelain treatment, trimeric assembly, coiled-coil domain, HA1 and HA2, receptor binding site, glycosylation sites, fusion activation site
- Influenza epidemics and pandemics; antigenic drift and shift, relation to structure
- Low pH structure of influenza virus hemagglutinin; conformational changes, fusion model

Lecture 22: Potassium Channel

- Membrane ion channels, $Na^{+,}$ K^{+}, Ca^{2+}
- Potassium channels; voltage-gated and ligand-gated
- Structure of KcsA K^{+} channel pore; selectivity filter, ion conduction
- Structure of calcium-gated MthK K^{+} channel; gating model
- Structure of chloride channels

OUTLINE OF PROTEIN-NUCLEIC ACID INTERACTIONS SECTION

Lecture 23: Chromatin

- Procaryotic and eukaryotic chromatin
- DNA structure; review double helix structure, higher order folding
- Eucaryotic chromatin; DNA/histone complexes
- Histones; sequence, structure, evolution, assembly
- Nucleosomes; "beads on a string," nucleosome core particle, histone octamer
- Nucleosome/core particle structure; electron microscopy, x-ray diffraction
- Nucleosome core particle; x-ray crystallography (Klug/Richmond), DNA-histone interactions, DNA superhelix, histone octamer

Lecture 24: Ribosomes

- Ribosomes and protein synthesis
- Procaryotic and eukaryotic ribosomes; subunits, ribosomal RNS and ribosomal proteins, disassembly/reassembly (Nomura)
- E coli ribosome structure; cross lining, electron microscopy, immunoelectron microscopy
- E coli 30S and 50S subunits; neutron scattering, distance measurements, triangulation

- E coli ribosome structure; cryoelectron microscopy, 3D crystals
- High resolution x-ray structures of 50S and 30S subunits; structure of complete ribosome
- Mechanism of protein synthesis

Lecture 25: Viruses

- Introduction; examples of DNA/RNA ss/ds viruses, overall structure, nucleic acid, protein capsid, membrane envelope
- Virus shape; spherical, rod-shaped, complex
- Virus structure; core/shell model, symmetric protein shells (Crick/Watson)
- Tobacco mosaic virus; rod-shaped RNA virus, electron microscopy, helical structure, RNA and protein helices
- TMV protein; disks, cylinders, helix, TMV protein disk structure
- TMV structure; x-ray fiber diffraction, RNA helix, disk-helix transition, RNA-protein interactions
- TMV assembly; initiation sequence, disk-helix transition

Lecture 26: Spherical Viruses

- Spherical viruses; cubic symmetry, polyhedra, icosahedral symmetry
- Examples; adenovirus, herpes virus, polyoma virus, etc.
- Icosahedral symmetry; simple (T=1) and complex (T-3) icosahedra, Caspar/Klug quasi-equivalence
- Satellite tobacco necrosis virus (STNV); T=1 capsid structure, symmetry
- Tomato bushy stunt virus (TBSV); T=3 capsid structure, symmetry, quasi-equivalence