

Graduate Programs in Physiology and Biophysics (M.A./Ph.D.)

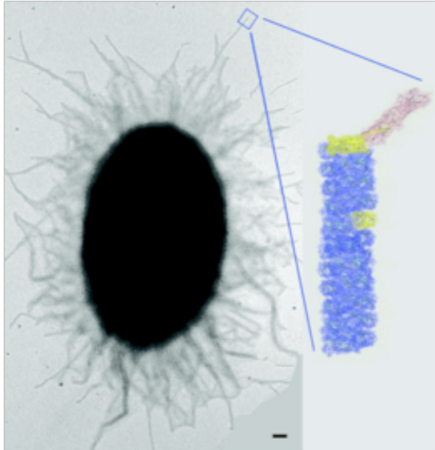
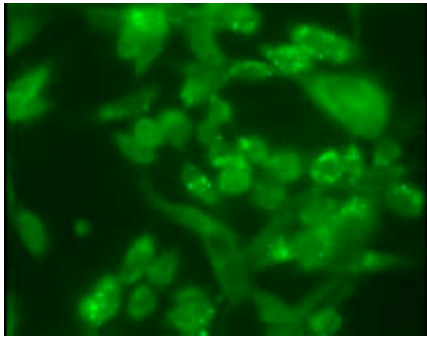
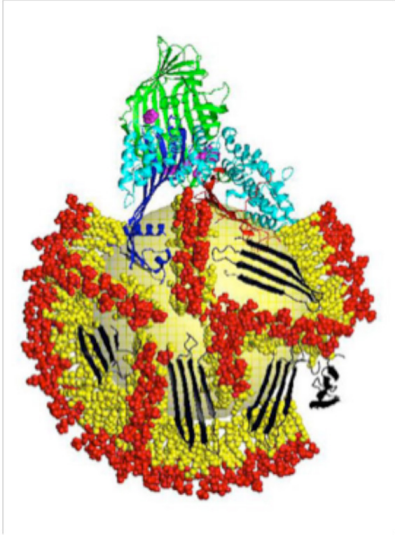


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Graduate Programs in Physiology and in Biophysics (M.A./Ph.D.)

I. Purpose and Background

The Department of Physiology and Biophysics, under the aegis of Dr. David Atkinson as chair, brings together 21 active faculty members to provide excellence in research and graduate education. Research interests in the Department span the modern areas of Cellular Physiology and Molecular Biophysics, with strong concentrations in Structural Biology, Vision Research, Muscle Physiology, and the Biology and Physical Chemistry of Lipids. The faculty is nationally and internationally recognized as leaders in their chosen areas of research. The Department provides flexible graduate programs with pathways either leading towards a degree in Physiology or in Biophysics. The department offers these two programs in a strong, collegial environment to encompass and promote the diverse overlapping research of all members of the Department.

The goal of a combined program in Physiology & Biophysics is to produce graduate students who understand the thermodynamic, chemical, electrical and structural aspects of biological systems at the atomic level and in the context of the cell and organism. This goal is particularly timely as it is in tune with the National Institutes of Health initiatives that encourage training across scientific and medical disciplines. The training will provide graduates the advantage of learning Physiology & Biophysics in the context of the regulation of homeostasis and pathogenicity of the cell, and of the organism as a whole. Graduates will be able to communicate and collaborate effectively with a broad range of disciplines ranging from medical personnel to chemists, engineers and physicists.

A set of required core courses will be taken by the students in the first year that will lead to a level of understanding of the two disciplines necessary for a degree in Physiology & Biophysics. After completing the core course requirements, students have flexibility to choose the appropriate additional course work for their interests, within the guidelines set out below. The courses for first year students are chosen with the guidance of members of the duly constituted Student Admissions and Affairs Committee (SAAC), and in the second year with input from their Masters or Ph.D. Advisor. Students who successfully complete the program are awarded either a Masters or Ph.D. in Physiology & Biophysics.

II. Administration of the Programs in Physiology and Biophysics

The Chairman of the Department of Physiology and Biophysics is the Director of the Graduate Programs and has the ultimate responsibility for administering the Graduate Programs in Physiology and in Biophysics. A Program Steering Committee oversees the Graduate Programs and advises the Chairman of the Student Admissions and Affairs Committee (SAAC) on specific needs of the Programs. Steering Committee members select faculty members to serve on the SAAC and oversee the appointment of committees that deal with developing new courses along with the review and updating of existing courses. The Steering Committee and the SAAC Chairman work jointly to supervise the appointment of faculty to run the Departmental Seminar Series and plan the Departmental Retreat. Student Seminar Days are organized by faculty members who are running the Department Seminar Series in a given year.

The Student Admissions and Affairs Committee (SAAC)

The SAAC oversees the day-to-day operations of the Graduate Programs, including: student recruitment, admissions, orientation, rotations, assignment of dissertation advisors and administering the qualifying examinations. The SAAC is comprised of 5-7 faculty members that adequately represent the diverse research interests within the Department. The SAAC serves many roles in the Department. Foremost is the recruitment, evaluation and acceptance of students into the Programs. The SAAC uses its experience and discretion to admit qualified students into the Programs based on the criteria outlined in the next section. Prospective students are interviewed on campus or by telephone. The SAAC also handles the orientation of new students into the Programs. The SAAC is charged with setting up graduate student research rotations, scheduling the Lab Fair (described below) and with administering the Qualifying Examination. The SAAC is also available to help with student problems and to mediate issues between students and Advisors.

III. Admission into the Programs in Physiology and in Biophysics

Student recruitment

Recruitment directly into the Programs in Physiology and in Biophysics is the preferred route of entry for Masters or Ph.D students. There is no strict formula for acceptance to the Programs and many factors go into the Admissions Committee's decisions. The Programs seek students from a wide range of backgrounds including Physics, Chemistry, Biochemistry, Biology, and Medical Sciences. For acceptance into the Program students should have outstanding grades in a rigorous curriculum and applicants should have completed Organic Chemistry, Physics and Physical Chemistry courses. The GRE general test is required and the subject test is recommended. Foreign students are required to take the TOEFL. We are especially interested in candidates with research experience. Finally, the letters from the applicant's references are extremely important, as is the applicant's personal statement. Underrepresented minorities are encouraged to apply.

Similar paths for Ph.D. and M.D./Ph.D. students

Requirements for Ph.D. and M.D./Ph.D. students are similar. Students in the Ph.D. Program take their qualifying examination in their second year. M.D./Ph.D. students enter after completing the second year Medical Curriculum and are therefore treated as post-masters students with a lower course and credit requirement (32 versus 64 total credits), and take their qualifying exam at the end of their first year in the Program. Other students entering into the programs with a Masters degree from within the USA are treated similarly and take their qualifying examination at the end of the first year. In both cases, post-Masters students are able to choose a suitable set of courses tailored to their backgrounds and research interests, with input from the SAAC during the first year and from their Advisor in following years. Transfer students are handled on a case-by-case basis, but may be able to take the qualifying examination at the end of their first year.

The M.A. programs

Masters programs in Physiology & in Biophysics are offered. In some cases, a qualified M.A. student may proceed into the Ph.D. program. This decision will be handled by the SAAC on a case-by-case basis. An

M.A. requires 32 credit hours (paid for by the student) and requires either a literature-based thesis or a short, laboratory based thesis with two readers from within the Department.

IV. The path of a graduate student

Special Topics Seminar Course (GMS BY 871, 872: 8 credits spread over 4 semesters)

In the first and second years, all students take the Special Topics Seminar course aimed at developing the student's ability to read the scientific literature and present the merits and/or deficits of a current research paper in front of other students and a proctoring faculty member. The students use a blackboard and computer projector during their presentations. This component of the course meets for 1 hour each week, as specified by the faculty member proctoring the class. Typically, all students present at least once each semester, and possibly more often, based on the number of enrolled students. An important complement to the student presentations is attendance at the weekly Physiology and Biophysics Seminar Series where the students are exposed to cutting edge research by outstanding speakers. Post-Bachelors students take 3 semesters (6 credits), while post-Masters, M.D./Ph.D., M.A., Cell and Molecular Biology (CMB) and Neuroscience students who enter into laboratories within the Department take 2 semesters (for a total of 4 credits). The Faculty member who teaches this course is rotated after each semester, to allow a fresh viewpoint and area of expertise to be covered. The Chairman of the SAAC, with input from the Program Steering Committee, selects 6-8 Faculty who are interested in teaching this course on a rotating basis.

Laboratory Rotations

The rotation program seeks to broaden the scientific and laboratory experience of new graduate students, while also providing the opportunity to evaluate possible Ph.D. Advisors. The guidelines for this process are presented in a natural sequence or timeline for the first year in graduate school:

1- Students are assigned a temporary desk within an appropriate laboratory that may be used as a home base during their initial 6-8 weeks in the Program. Laboratories are selected carefully by the SAAC, from a list of Faculty who have volunteered to act as hosts. This list is updated yearly.

2- To facilitate interactions between Faculty and new students, a “Laboratory Fair” is held within the first 6-8 weeks of the fall semester. The Fair starts in September and groups of Faculty (2-4) meet with the entire class of students for ~1-2 hours. Scheduling is coordinated by the SAAC and the entire Fair takes place over a period of 4-6 weeks, with 1-2 meetings per week. September and October are an intensive time for the students, as they will be attending classes and the Fair simultaneously. Thus, there is no formal rotation during the first half of the Fall semester.

3- Students participate in a Departmental Retreat, held at the end of September or beginning of October, in which faculty, students and post docs from individual laboratories give oral presentations and present posters that highlight the projects in their laboratories.

4- Generally, students participate in 3 rotations (~8 weeks each), with the possibility of a 4th, if they are undecided about the choice of a thesis Advisor after the first 3 rotations are completed. The SAAC strongly encourages new students to choose at least 1 rotation in an area that is distinct from their major area of research interest. For example, a student who chooses 2 rotations in structural biology laboratories (such as X-ray crystallography, EM or NMR) is encouraged to choose another rotation from an entirely different area within the Department. If this does not happen, then members of the SAAC meet with the student and try to work out a suitable rotation that fulfills this requirement. Where possible, the SAAC schedules rotations such that only one student is rotating in a laboratory in a given rotation slot.

Upon completion of the Laboratory Fair, students are asked to submit 3 names (rank ordered) for their first Fall rotation to the SAAC which then sets up a schedule that attempts to honor the first choice of most students. Near the end of the Fall semester, the students provide either 2 or 3 names for their Spring rotation(s). Students choose a Ph.D. Advisor by early June, at the end of the first year (unless a 4th rotation is required). The SAAC oversees this process.

A written thesis proposal

Success in science requires that students be able to express their thoughts both verbally and in written form. The necessary verbal skills are developed through participation in the Special Topics seminar course and

by giving presentations in yearly research seminars at the Student Seminar Day(s), laboratory group meetings, pre-thesis meetings, and the thesis defense seminar. Written skills are developed in required course work and in the writing of papers and the thesis. However, the latter may occur rather late in a student's research project when time is at a premium. Thus, students are required to prepare an 8-10 page research proposal on their thesis project using the NIH National Research Service Award (NRSA) or American Heart Association Research Training Grants and Fellowship format and forms, as appropriate. This exercise should allow the Advisor and student to clarify the proposed research and provides the student with a forum to learn basic scientific and grant writing skills, with input from the advisor. This proposal should be completed during the summer and/or early Fall, after passing the Qualifying Examination and is intended to help prepare the student for future writing of the dissertation, related papers, fellowships and grants. When the proposal is completed to the satisfaction of the advisor, it is given to members of the pre-thesis committee not less than two weeks prior to their first meeting, to serve as an introduction to the student's thesis project. A copy of the proposal is also submitted to the SAAC.

Student Seminars

In years 3-5, or starting with the Spring term after the Qualifying Examination has been completed, all graduate students will present a 20-30 minute seminar on their thesis research. These seminars form a logical extension of the Departmental Seminar Series and the Special Topics course and take place on a specified Student Seminar Day (or days) scheduled in April or May. Pre-thesis committee members for a presenting student take notes on the quality of the presentation and meet with the student within 2-3 days, on either a one-to-one basis or in small groups with other committee members, to provide feedback with the goal of improving the student's presentation skills.

Student Posters Presentations

Students who have completed their Qualifying Exams present a poster during Russek Day in the Spring of each year, to improve their organization and presentation skills. These students also present their posters at

the Departmental Retreat held each Fall. In addition, students are encouraged to enter their posters in the Science Day poster competition held at the Boston University Charles River Campus.

V. Course requirements

Incoming Ph.D. and M.A. students are required to take two semesters of Biochemistry (BI 755 & BI 756, 8 credits total) and four semesters of the Special Topics Seminar Course (BY 871 & 872, 8 credits total). To allow flexibility for the breadth of the fields of Physiology & Biophysics, yet insure a working knowledge of both, students are required to take a minimum of one Physiology course and one Biophysics course. The acceptable Physiology courses are: Cell Physiology (PH 842; 4 cr.), or Human Physiology A & B (PH 730 & PH731, 3 cr. each, total of 6 cr.). The acceptable Biophysics courses are: Biophysics of Macromolecular Assemblies (PH 771, 4 cr.) or Foundations of Biophysics and Structural Biology (BY 760, 4 cr.).

Elective coursework is chosen jointly by the student and their Advisor or thesis committee. To achieve a balanced curriculum for all students, the Ph.D. degree requirements of the Division of Graduate Medical Sciences include a *minimum* of 24 credits in formal course work. It is expected, although not required, that the formal course work credits are acquired prior to the Qualifying Examination at the end of the second year. Beyond 32 course work credits, students are encouraged to informally audit courses following consultation with their thesis committee. Students who have already taken an appropriate graduate-level course in the core curriculum are permitted to substitute an elective or electives in the first semester, as long as the total semester course credits remain 10.

A Sample curriculum

Fall semester, first year - all students

Foundations in Biomedical Sciences A, B, C (FC 701, 702, 703; 2 cr. each)

Foundations in Biophysics & Structural Biology (BY 760, 4 cr)

OR Human Physiology B (PH 732, 3 cr.)*

Special Topics Seminar Course (BY 871, 2 cr.)

Spring semester, first year

Foundations in Biomedical Sciences D, E (FC 704, 705; 2 cr. each)

Biophysics of Macromolecular Assemblies (BY 771) 4 cr.

OR Human Physiology B (PH 732, 3 cr.)*

Special Topics Seminar Course (BY 871, 2 cr.)

*Students fulfilling their Physiology requirement with Human Physiology A & B would fulfill their Biophysics requirement in their second year.

Remaining semesters, first & second years - all students

Special topics/student seminar (BY 871, 872; 4 cr. total)

Electives

Suggested electives:

Department of Physiology & Biophysics

Cell Physiology (PH 843) 4 cr.

Human Physiology A (PH 542) 4 cr.

Human Physiology B (PH 543) 4 cr.

Foundations in Biophysics & Structural Biology (BY 760) 4 cr.

Biophysics of Macromolecular Assemblies (BY 771) 4 cr.

NMR Spectroscopy in Biology & Biochemistry (BY 772) 2 cr.

Metabolism & Cellular Function of Complex Lipids (BY 774) 2 cr.

Special Topics (PH 745, 746) 2 cr. (e.g. Biology of Vision, Calcium & Cell Function)

Courses in other Departments

Biochemistry I, II (intermediary metabolism) (BI 755, 756) 4 cr.

Molecular Biology (BI 782) 4 cr.

Comprehensive Immunology (MI 713) 4 cr.

Molecular Neurobiology and Pharmacology (PM 700) 4 cr.

Structure & Function of Proteins (BI 783) 2 cr.

Enzyme Catalysis (BI 788) 2 cr.

Receptors and Signal Transduction (BI 790) 2 cr.

Physical Biochemistry (BI 789) 2 cr.

Gene Regulation and Pharmacology (PM 880) 2 cr.

Mass Spectrometry, Proteomics and Functional Genomics (BI 793) 2 cr.

Principles of Genetics and Genomics (GE 701) 4 cr.

Biostatistics (MS 700) 2 cr.

Molecules to Molecular Therapeutics (MM 710) 4 cr.

The Qualifying Examination

The Qualifying examination is given at the end of the second semester within the 2nd year for Ph.D. students. M.D./Ph.D., post-Masters and some transfer students in the Ph.D. Program have the option of taking the Qualifying Examination at the end of the second semester of the first year. Administration of the Qualifying Examination will be overseen by the SAAC. Program Faculty members serve on the Qualifying Examination committees on a rotating basis. The two-part exam consists of:

- 1- For the written qualifying examination students write a research proposal that correlates with, but may or may not include his/her planned thesis research. This document is judged by three members of the SAAC committee for the student's understanding of the relevant scientific literature and ability to propose testable scientific hypotheses.

2- An oral examination where the student is assigned 3 current research papers to read and is subsequently tested on their understanding of the material in front of an examining committee. This committee is comprised of 5 Program Faculty members. Papers are chosen by the committee and given to the students 2-3 weeks in advance of the oral examination. Thesis Advisors will not participate in the oral defense of students who are pursuing a Ph.D. in their laboratory.

Both the written and oral portion of the qualifying exam must be passed in order for a student to continue in the PhD. program. Students who fail either the oral or the written portion of the exam (or both) can retake that portion of the exam once in order to achieve a passing score. Masters students do not take the Qualifying Examination.

Pre-thesis meetings

After the student has passed the Qualifying Exam, a pre-thesis committee of at least five members must be established in the Fall of that year and submitted to the SAAC. Students submit their thesis proposal in grant form to the members of the committee 2 weeks before the first pre-thesis committee meeting. At least one member from outside the Department, and preferably from a different institution, should be included in the final thesis committee. An external committee member is not required for pre-thesis meetings, which will be held about every 10-12 months or ~3 times prior to graduation.

Thesis defense

The Ph.D. thesis defense consists of a public seminar followed by a closed door thesis defense with a minimum of 5 committee members, including 1 member from outside the Department

VI. Resources for the Programs

All Faculty of the Department of Physiology and Biophysics participate in the Graduate Programs in Physiology and Biophysics. The Faculty have well funded research programs and extensively equipped individual laboratories for carrying out research in Cellular Physiology and Biophysics. In addition, the Department maintains core facilities in Molecular Biology, Spectroscopy, X-ray crystallography, NMR and Structural Electron Microscopy that will be used by students carrying out their research. The Faculty of the

Department of Physiology and Biophysics and their research interests are listed below. A more in depth review of each Faculty members research can be obtained from the Department Web pages at <http://www.bumc.bu.edu/phys-biophys> .

Faculty Members Involved in the Training of Graduate Students	Research Interests
David Atkinson, Ph.D.	Plasma Lipoproteins: Structure & Biology
Christopher W. Akey, Ph.D.	Structural Biology of Channels and Chaperones
Esther Bullitt, Ph.D.	Bacterial Adhesion and Viral Replication
M. Carter Cornwall, Ph.D.	Visual Transduction and Adaptation
Christopher Gabel, Ph.D.	Neural Regeneration and Neurocircuitry in <i>C. elegans</i>
J. Fernando Garcia-Diaz, Ph.D.	Expression and Modulation of Ion Channels
Olga Gursky, Ph.D.	Protein Conformation, Structure and Energetics
James A. Hamilton, Ph.D.	Structural Biology of Membranes in Native and Pathological States
James F. Head, Ph.D.	Structural Studies of Protein-Protein and Protein-Ligand Interactions
Haya Herscovitz, Ph.D.	Assembly, Chaperone-Assisted Folding and Secretion of Lipoproteins
William J. Lehman, Ph.D.	Electron Microscopy and 3D Reconstruction of Actin Thin Filaments
Simon Levy, Ph.D.	Calcium Signaling in Nerve Cells
Assen Marintchev, Ph.D.	Eukaryotic Translation Initiation and NMR

C. James McKnight, Ph.D.	Protein Structure, Function, Folding and NMR Spectroscopy
Jeffrey R. Moore, Ph.D.	Cell Motility and Motor Proteins
Judith D. Saide, Ph.D.	Structural Proteins of <i>Drosophila</i> Flight Muscle
Barbara A. Seaton, Ph.D.	X-ray Crystallography and Protein-Membrane Interactions
G. Graham Shipley, Ph.D., D. Sc.	Membrane and Receptor Biology
Donald M. Small, M.D.	Lipids, Lipoproteins and Disease
Raphael A. Zoeller, Ph.D.	Functional Roles of Lipids in Cell and Membrane Biology

Appendix

A1. Course syllabi (Examples)

Cell Physiology GMS PH 843 (potential syllabus)

September 5	Electrodifffusion
September 7	Carriers, pumps, and channels
September 12	Ionic permeation in channels
September 14	Voltage-dependent gating
September 19	Electrical excitability and conduction
September 21	Electrophysiology of muscle cells
September 28	Ca measurement and regulation
October 3	Ca signaling
October 5	Cell communication I: gap junctions and neurosecretion
October 10	Cell communication II: post-synaptic mechanisms
October 12 S	Sensory transduction I: receptor physiology issues
October 17 S	Sensory transduction II: Photo- & chemo-transduction
October 19	Sensory transduction III: mechano- & thermal transduction
October 26	Exam 1
October 31	Historical view of muscle
November 2	Regulation of muscle contractile systems
November 7	General filament mechanics
November 9	Non-muscle myosins
November 14	Team-based learning of contraction
November 16	Structure and function of non-mysosin motors
November 21	Eukaryotic cilia and flagella
November 28	Regulation of motility in microtubule-based systems
November 30	Team-based learning of motors
December 5	Bacterial flagella I: structure
December 7	Bacterial flagella II: function and regulation
December 12	Bacterial flagella III: Motor switching, and current models for bacterial flagella
December 14	Exam 2

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY in BIOLOGY and BIOCHEMISTRY GMS BY 772

Course Director: James A. Hamilton, Ph.D.

Description:

This graduate level course provides an introduction to the basic theory and the fundamental measurements of NMR spectroscopy using the predominant biological nuclei, ^1H , ^2H , ^{13}C , and ^{31}P , and applications to structure and metabolism; NMR studies of pathological processes and NMR imaging.

Topics include:

Basic physical principles, Fourier transform NMR spectroscopy and instrumentation, with lab demonstration. Multinuclear NMR The biologically interesting nuclei Comparison of high-resolution NMR of ^1H , ^2H , ^{13}C , and ^{31}P Applications to lipids, membranes, and proteins Metabolism and pathology studies by NMR 2D NMR of peptides Magnetic resonance imaging (MRI) in medicine

Foundations of Biophysics And Structural Biology GMS BY 760

Course Director: David Atkinson, Ph.D.

Lecturers: Christopher W. Akey, Ph.D., Michael Rinkiewicz, Ph.D., Olga Gursky, Ph.D. C. James McKnight, Ph.D.

COURSE INTRODUCTION (Lecture I, David Atkinson Ph.D.) Lecture I. COURSE OVERVIEW

Aims and organization, introduction to faculty, examinations, text books

MACROMOLECULAR CONFORMATION AND THE PRINCIPLES OF SYMMETRY

Review of amino acid, and nucleic acid chemistry, protein and nucleic acid Conformation and structure. Principles of symmetry, symmetry operations, point groups and space group.

COMPUTATIONAL BIOLOGY (Lectures I-II, David Atkinson Ph.D.)

Lecture I. COMPUTATIONAL BIOLOGY

Overview of molecular mechanics, molecular graphics and data visualization.

Lecture II. STRUCTURAL DATABASE ACCESS

Software, searching, WWW access, Protein Data Bank. Molecular graphics, Molecular modeling, and graphic display of protein and nucleic acid structures.

FOURIER TRANSFORMS (Lecture I, David Atkinson Ph.D.)

Lecture I. FOURIER THEORY AND APPLICATION

Fourier series. Fourier and inverse Fourier Transforms.

Convolution operations.

Importance in structural biophysics.

PROTEIN THERMODYNAMICS AND SPECTROSCOPY (Lectures I-VI, Olga Gursky Ph.D.)

Lecture I. PROTEIN ENERGETICS-WHAT IS IT GOOD FOR?

Energetic-structure-function relationship in proteins. thermodynamic versus mechanical description of macroscopic systems. Absolute temperature. Units and dimensions. Statistical weight and probability. Extensive and intensive variables. Energy E as a state function. 1-st law of thermodynamics. Entropy S . Second and third laws of thermodynamics

Lecture II. A LONG WAY TO GIBBS-HELMHOLTZ EQUATION

Energy E and enthalpy H . Heat capacity C_p and C_v : Microscopic meaning, typical values. Protein unfolding as a 1-st order phase transition. Gibbs free energy ΔG as a measure of protein stability. Entropy-enthalpy compensation in globular proteins.

Lecture III. COOL AND HOT METHODS IN PROTEIN THERMODYNAMICS

Gibbs-Helmholtz equation. Low-temperature protein unfolding as a test for the Gibbs-Helmholtz equation. Differential Scanning Calorimetry – a direct method for thermodynamic analysis. Measurement and interpretation of the protein heat capacity. Instrumental design. Calorimetric and Van't Hoff enthalpy. Two-state transitions and cooperativity. Application to single and multidomain proteins and to protein folding intermediates. Advantages and limitations of DSC.

Lecture IV. SPECTROSCOPIC METHODS IN PROTEIN THERMODYNAMICS.

Gibbs distribution. Measuring protein stability ΔG by chemical unfolding. Van't Hoff plot. Measuring enthalpy ΔH by thermal unfolding. Indirect determination of ΔC_p by pH, thermal and chemical unfolding. Measuring small changes in stability of structurally similar proteins. Effects of mutations on the folded and unfolded

states. Entropic stabilization. Le Chatelier's principle. Application to protein thermodynamics.

Lecture V. CD SPECTROSCOPIC ANALYSIS OF PROTEINS.

Review of light polarization. Definition of ellipticity. Light absorption; linear and circular dichroism (CD). Physical origins of CD. Relation between CD and ellipticity. Far-UV CD spectra of pure secondary structures. Quantitative secondary structure analysis by spectral deconvolution. Tertiary structure analysis by near-UV CD. Using CD for protein thermodynamic analysis

Lecture VI. INFRARED (IR), RAMAN AND FLUORESCENCE SPECTROSCOPY.

IR spectroscopy: Molecular vibration, stretching and bending modes. Principal IR bands for peptide groups. Effects of secondary structure. Polarization. Raman spectroscopy: Stokes and anti Stokes scattering. Intensity and resolution. Raman spectroscopic analysis of macromolecules and their complexes. Instrumental design of FTIR. Secondary structure analysis by FTIR. Differential FTIR. Fluorescence spectroscopy: Absorption and emission spectra. Factors affecting fluorescence intensity. Steady state and kinetic modes. Fluorescent quenching as a probe for solvent accessibility of protein chromophores. Probing protein folding by fluorescent dyes. Fluorescent energy transfer.

X-RAY DIFFRACTION, SCATTERING AND CRYSTALLOGRAPHY

(Lectures I-III, David Atkinson Ph.D.),(Lectures IV-VII Hwai-Chen Guo Ph.D.)

Lecture I. X-RAY PHYSICS, INSTRUMENTATION AND GEOMETRICAL X-RAY DIFFRACTION

General X-ray physics: Energy spectrum, characteristic wavelengths. X-ray generators: Sealed source, rotating anode, synchrotron. X-ray detection: Film, proportional counters, position sensitive counters, multiwire area detectors, image plates, CCD detectors. Bragg's Law, the reciprocal lattice, von Laue conditions, Ewald sphere, Lorentz polarization factor, powder diffraction.

Lecture II. FOURIER ANALYSIS OF X-RAY SCATTERING AND DIFFRACTION

Point scatterers, atomic scattering, form factors, assemblies of scatterers, lattices, electron density, Patterson function, resolution, phases and phase problem, symmetry and systematic absences.

Lecture III. X-RAY DIFFRACTION AND SCATTERING OF PARACRYSTALLINE SYSTEMS

X-ray diffraction by membrane, lipid, and polymer systems. X-ray (neutron) scattering by macromolecular solutions: Hydrodynamic measurements, radius of gyration, shape factors, internal structure, distance distribution function. Fiber diffraction and helical diffraction.

Lecture IV. PROTEIN CRYSTALLIZATION

Preparing protein samples: Purification, concentrating, storage. Crystal growth: Principles and methods, solubility, saturation, nucleation, batch methods, vapor diffusion methods, dialysis methods, micro and macro seeding. Crystal storage and handling. Crystal soaking: Cryoprotectant, heavy atoms, substrates, ligands or inhibitors.

Lecture V. MACROMOLECULAR DATA COLLECTION AND PROCESSING

Data collection: Crystal mounting, radiation damage, cryo-techniques. Photography: Still, oscillation, precession, Laue, resolution, mosaicity. Data processing and reduction: Indexing, integration, error estimate, polarization correction Lorentz correction, absorption, space group determination, statistics.

Lecture VI. THE PHASE PROBLEM

Phase determination: Multiple isomorphous replacement, multiple anomalous dispersion, molecular replacement, direct methods. Phase improvement: Solvent flattening, histogram matching, non-crystallographic averaging.

Lecture VII. MODEL BUILDING AND REFINEMENT

Map calculation: Difference maps. Interpretation of electron density Maps: Model building. Refinement: Least squares, maximum likelihood, rigid body, group and individual B factor, positional, simulated annealing. Assessment: Conventional and free R-factors, real space correlation

STRUCTURAL ELECTRON MICROSCOPY

(Lectures I-V, Christopher W. Akey Ph.D.)

Lecture I. INTRODUCTION AND ELECTRON OPTICS

Introduction to Electron Microscopy and its use in Cell and Structural Biology (TEM and STEM). Comparison of electron and light optics. Phase contrast microscopy .

Lecture II. RADIATION DAMAGE, SPECIMEN PREPARATION AND THE PROJECTION THEOREM

Radiation damage and biology: Minimal dose and low temperature. Theory of specimen preparation for thin sections, negative staining and frozen-hydrated work. The Projection theorem and its application to 3D structural analysis of electron micrographs.

Lecture III. 3-DIMENSIONAL ANALYSIS OF SINGLE PARTICLES AND VIRUSES

Cross correlation and classification methods. Random conical tilt 3D reconstruction. Common lines methods in reciprocal or real space.

Lecture IV-V. ANALYSIS OF THIN TWO DIMENSIONAL CRYSTALS IN 2D AND 3D

2-dimensional plane groups. Cross correlation and Fourier methods of averaging. Merging data to form a 3D reconstruction. Electron diffraction.

STRUCTURAL NUCLEAR MAGNETIC RESONANCE (NMR)

(Lectures I-V, C. James McKnight Ph.D.)

Lecture I. INTRODUCTION TO FUNDAMENTAL ASPECTS OF NMR

Nuclear spin, Zeeman splitting, Boltzman distribution, precession of spins, Bloch equations, one pulse NMR experiment, spin relaxation, linewidth, chemical shifts, j-coupling, dipole-dipole interactions (NOE).

Lecture II. EXPERIMENTAL ASPECTS OF NMR

Sample considerations and conditions. Instrumentation: Tour of an NMR spectrometer, data acquisition, sampling theorem, quadrature detection, phasing, lock channel. Data processing: Fourier transforms, apodization, zerofilling, linear prediction, referencing, integration. Water suppression: Presaturation, gradient, jump-return, spinlocks, solvent deconvolution.

Lecture III. MULTIDIMENSIONAL AND HETERONUCLEAR NMR

Through bond experiments: COSY and TOCSY. Through space experiments: NOESY and ROESY. Heteronuclear experiments: HMQC, HCCH-COSY. Combining experiments: HMQC-NOESY, HMQC-TOCSY. Three dimensional experiments: 3D-HMQC-NOESY. Triple labeling: HNCA, HN(CO)CA . Deuterium labeling of large proteins.

Lecture IV. HYDROGEN EXCHANGE AND RELAXATION MEASUREMENTS

Hydrogen exchange rates and protection factors. Relationship of protection factors to ΔG . Pulsed HX and protein folding. Theory and mechanisms of relaxation (T1, T2, and NOE). The spectral density function. Experimental aspects to measure T1, T2 and NOE.

Lecture V. SEQUENTIAL ASSIGNMENT AND STRUCTURE CALCULATION

Proteins: Spin systems sequential NOEs, medium range NOEs, stereospecific assignments, direct methods with triple labeled samples. DNA: Spins systems, sequential NOEs. NMR distance, angle, and chemical shift restraints. Distance geometry, simulated annealing, and relaxation matrix back-calculation. Software packages. Judging the quality of NMR structures and comparison with X-ray.

TEXT BOOKS AND SELECTED READING

All textbooks will be available to students in the Department of Biophysics library. When necessary students will be provided with copies of required reading.

In addition, on-line interactive readings and tutorials will be made available through the Department's pages on the World Wide Web. These will include references to on-line manuals for computer software packages, for example programs for molecular visualization, which are discussed in the course.

General Texts

Biophysical Chemistry, Parts I-III, Charles Cantor and Paul R. Schimmel

W. H. Freeman and Company, San Francisco. 1980. Specifically, Part. II Techniques for the study of biological structure and function.

Principles of Physical Biochemistry, Kensal E. van Holde, W. Curtis Johnson and P. Shing Ho. Prentice Hall, New Jersey. 1998.

Selected Readings

Thermodynamics:

Biothermodynamics. The Study of Biochemical Processes at Equilibrium. J. T. Edsall and H. Gutfreund. J. Wiley and Sons, 1984.

Spectroscopic Methods:

Circular Dichroism and the Conformational Analysis of Biomolecules. G. Fasman, ed. Plenum Press, New York and London, 1996.

Structural Electron Microscopy:

Electron Tomography: Three-Dimensional Imaging with the Transmission Electron Microscope. Joachim Frank ed. Plenum Press, New York and London, 1992.

X-ray Diffraction and Crystallography:

Protein Crystallography. T. L. Blundell and L. N. Johnson, Academic Press New York, 1976. Methods in Enzymology Vol. 276,277. Macromolecular Crystallography Part A and B, Charles. W. Carter and Robert M Sweet eds., Academic Press Inc. 1997.

Structural NMR:

NMR of Proteins and Nucleic Acids. Kurt Wuthrich. John Wiley and Sons. 1986.

Protein NMR Spectroscopy: Principles and Practice. J. Cavanagh, W. J. Fairbrother, A. G. Palmer, and N. J. Skelton, Academic Press Inc., San Diego, 1996.

EXAMINATIONS

Students will be examined through two mechanisms.

Problem sets given either during or at the conclusion of each section of the course by each lecturer. These problem sets should be completed by the student individually not in study groups (honor system). The cumulative grades for the problem sets will contribute 25% of the final grade.

An end of course written examination will be given on all aspects of the course. This will be a 4-hour essay style examination designed to test the students' knowledge of individual aspects of the course and the integration of different methodologies. This examination will constitute seventy five percent (75%) of the final grade.

Biophysics of Macromolecular Assemblies –GMS BY 771

Instructor: Dr. G.Graham Shipley

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, $\alpha\alpha$, $\beta\alpha\beta$, $\beta\beta$ 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 theorem Negative 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 (hydrophobic 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
 $\Delta G_{t \text{ w} \rightarrow \text{o}} = RT \ln K_{w/o}$
- $\Delta G_{t \text{ w} \rightarrow \text{o}}$ 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- H₂O system
- The PC-C-H₂O, PC-CE-H₂O, PC-TG-H₂O 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, quinone
- 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 and *D. capsulatus* porins; 16-strand barrels, channel structure
- X-ray structure of OmpA; 8-strand barrel
- X-ray structure of FepA, 22-strand barrel, and OmpA (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⁺, Ca²⁺
- 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

- Prokaryotic 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
- Prokaryotic 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, quasiequivalence

Metabolism and Cellular Function of Complex Lipids GMS BY 774

Instructor: R. Andrew Zoeller, Ph.D.

This course describes the basics of complex lipid structure, biosynthesis and functions in animal cells. Emphasis will be on the role that specific lipid species play in cellular functions using a historical perspective of how these functions were discovered and using recent literature to update the status of the subject matter. Topics will include protein modifications, lipids in cell signaling, lipoproteins, as well as the role of gram-negative endotoxin in cell activation and sepsis. Techniques relevant to the study of each topic will also be discussed including techniques in somatic cell mutant isolation.

One 2 hour lecture will be given every week.

Prerequisites: Permission of the instructor

Credits: 2

Lectures:

Introduction: Lipid heterogeneity & the Biological Membrane (Zoeller)

Phospholipid biosynthesis I (Zoeller)

Phospholipid biosynthesis II (Zoeller)

Isolation of somatic cell mutants I (Zoeller)

Isolation of somatic cell mutants II (Zoeller)

Lipid transport (Zoeller)

Covalent modification of proteins by lipids (Zoeller)

MIDTERM EXAMINATION

Covalent modification of proteins by lipids (Zoeller)

Lipids and signaling (Zoeller)

Gram-negative endotoxin: Biochemistry & Pharmacology (Golenbock)

Cholesterol & Lipoproteins I (Zoeller)

Cholesterol & Lipoproteins II (Zoeller)