

**Foundations of Biophysics
And
Structural Biology
[GMS BY 760]**

Course Director: David Atkinson Ph.D.
Professor of Biophysics,
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Lecturers: Christopher W. Akey Ph.D.
Professor of Biophysics

Hwai-Chen Guo Ph.D.
Associate Professor of Biophysics

Olga Gursky Ph.D.
Associate Professor of Biophysics

C. James McKnight Ph.D.
Associate Professor of Biophysics

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

Lecture I. COURSE OVERVIEW

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

**MACROMOLECULAR CONFORMATION AND THE PRINCIPLES
OF SYMMETRY**

Principles of symmetry, symmetry operations, point groups and space groups

**THERMODYNAMIC
AND
SPECTROSCOPIC METHODS**
(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.
2-nd law, entropy S in spontaneous and equilibrium processes.
3-rd law 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 DG as a measure of protein stability.
Typical values of $D S$, DH and DG for globular proteins.
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 Gibbs-Helmholtz equation.
Differential Scanning Calorimetry (DSC)— a direct method for thermodynamic analysis.
Measuring protein heat capacity. Instrumental design.
Calorimetric and Van't Hoff enthalpy, cooperativity.
Application to single- and multidomain proteins and protein folding intermediates.
Advantages and limitations of DSC.

Lecture IV. SPECTROSCOPIC METHODS OF PROTEIN THERMODYNAMIC ANALYSIS

Gibbs distribution. Measuring protein stability ΔG by chemical unfolding. Van't Hoff plot; measuring enthalpy ΔH_v by thermal unfolding. Indirect determination of the heat capacity increment ΔC_p : heat unfolding at different pH, combination of thermal and chemical unfolding, low-temperature unfolding. Measuring small changes in stability of structurally similar proteins. Le Chatelier's principle, applications to protein thermodynamics.

Lecture V. CIRCULAR DICHROISM (CD) SPECTROSCOPIC ANALYSIS OF PROTEINS.

Review of light polarization. Definition of ellipticity. Review of light absorption; normal absorption, linear and circular dichroism. Physical origins of CD. Relation between CD and ellipticity. Typical values of protein ellipticity. Far-UV CD spectra of pure secondary structures. Thermodynamic analysis of α -helical proteins using CD. Spectral deconvolution and quantitative secondary structural analysis. Effects of protein tertiary structure on far-UV CD. Selection of the reference spectra.

Lecture VI. INFRARED, RAMAN AND FLUORESCENCE SPECTROSCOPY.

Infrared spectroscopy: molecular vibration, stretching and bending modes. Principal IR bands for the peptide group as a function of secondary structure. IR polarization. Instrumental design of Fourier Transform IR spectrometers. Secondary structural analysis using FTIR. Differential FTIR. Raman spectroscopy: Stokes and anti-Stokes components of scattering. Intensity, resolution, applications of Raman to macromolecules and their complexes. Fluorescent spectroscopy: relation between absorption and emission spectra. Factors affecting fluorescent intensity. Steady state and kinetic measurements. Fluorescent quenching as a probe for solvent accessibility of protein chromophores. Probing folding states by fluorescent dyes. Fluorescent energy transfer.

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.

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-HMCQ-NOESY.

Triple labeling: HNCA, HN(CO)CA . Deuterium labeling of large proteins.

Lecture IV. HYDROGEN EXCHANGE AND RELAXATION MEASUREMENTS

Hydrogen exchange rates and protections factors.

Relationship of protections 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.

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

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

Lecture IV. 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.

Lecture V. HELICAL 3D RECONSTRUCTION

Helical symmetry and the Fourier transform of a helix.
Indexing helical diffraction patterns and computational analysis (near and far side 2D projections).
Three-dimensional reconstruction using Fourier-Bessel techniques.

COMPUTATIONAL BIOLOGY

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

Lecture 1. COMPUTATIONAL BIOLOGY

Molecular mechanics and dynamics. Extension to crystallographic and NMR structure refinement.

Lecture II. COMPUTATIONAL BIOLOGY (continued)

TEXT BOOKS AND SELECTED READING

General Text 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.

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.

All text books 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 that are discussed in the course.

EXAMINATIONS

Students will be examined through two mechanisms

1. 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.
2. End of course written examination 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.

Foundations of Biophysics and Structural Biology
COURSE SCHEDULE (2002)

Time: 2:00 – 4:00 pm

Location: L705 (Physiology and Biophysics Conference Room)

	Week 1 14,16 Jan.	Week 2 21,23 Jan.	Week 3 28,30 Jan.	Week 4 4,6 Feb.	Week 5 11,13 Feb.	Week 6 18,20 Feb.	Week 7 25,27 Feb.	Week 8 4,6 Mar
Mon. 2hr.	Course Introduction Macromolecular Conformation and Principles of Symmetry	Martin Luther King Holiday	Thermodynamic Methods III	Spectroscopic Methods II	Fourier Transforms	Presidents' Day Holiday	X-ray Diffraction, Scattering and Crystallography III	Spring Recess
Wed. 2hr.	Thermodynamic Methods I	Thermodynamic Methods II	Spectroscopic Methods I	Spectroscopic Methods III	X-ray Diffraction, Scattering and Crystallography I	X-ray Diffraction, Scattering and Crystallography II	X-ray Diffraction, Scattering and Crystallography IV	Spring Recess

	Week 9 11,13 Mar.	Week 10 18,20 Mar.	Week 11 25,27 Mar.	Week 12 1,3 Apr.	Week 13 8,10 Apr.	Week 14 15,17 Apr.	Week 15 22,24 Apr.	Week 16 29 Apr, 1 May
Mon. 2hr.	X-ray Diffraction, Scattering and Crystallography V	X-ray Diffraction, Scattering and Crystallography VII	Structural Electron Microscopy and Image Processing II	Structural Electron Microscopy and Image Processing IV	Computational Biology I	Patriots' Day Holiday	Structural Nuclear Magnetic Resonance II	Structural Nuclear Magnetic Resonance V
Wed. 2hr.	X-ray Diffraction, Scattering and Crystallography VI	Structural Electron Microscopy and Image Processing I	Structural Electron Microscopy and Image Processing III	Structural Electron Microscopy and Image Processing V	Computational Biology II	Structural Nuclear Magnetic Resonance I	Structural Nuclear Magnetic Resonance III	Structural Nuclear Magnetic Resonance IV

Examination: Wednesday, May 8

