Spring 2003

BIOP 802: Advanced Macromolecular Crystallography

(3 credits)

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Tuesdays & Thursday; 2:00 - 3:30 pm Dept of Physiology Conference Room 4-25

Supplementary textbooks:

PRINCIPLES OF PROTEIN X-RAY CRYSTALLOGRAPHY Second Edition by J. Drenth

CRYSTAL STRUCTURE ANALYSIS, Second Edition by J.P. Glusker & K.N. Trueblood

INTERNATIONAL TABLES FOR CRYSTALLOGRAPHY, Vol. F. Crystallography of Biological Macromolecules.

Lecture 1 - January 16: History and Foundations of Chemical Crystallography (ZSD)

Historical roots of crystallography; chemical crystallography and crystal morphology; Bravais lattices, point groups and symmetry classes; space groups; definition of unit cell and asymmetric unit.

Lecture 2 – January 21: Discovery of X-rays and the Laue Experiment (RHK)

Mathematical representation of electromagnetic waves; complex algebra and Argand diagrams; phase and amplitude; the essence of the Laue experiment

Lecture 3 – January 23: Elementary Diffraction Physics (ZSD)

Diffraction of X-rays by a spherical atom; introduction of the S (diffraction) vector; Fourier transform and relationship between real and reciprocal space.

Lecture 4 – January 28: Elementary Diffraction Physics (ctd) (ZSD)

Diffraction of X-rays by an ensemble of spherical atoms; introduction of convolution theorem; Fourier transform of the convolution of functions; continuity of the Fourier transform of the molecular function; modulation of intensity in the diffraction pattern by the molecular structure.

Lecture 5 – January 30: Elementary Diffraction Physics (ctd) (ZSD)

Diffraction of X-rays by a crystal lattice and by a molecular crystals; discreet nature of the diffraction pattern, sampled by the crystal lattice; derivation of the Laue equations; Muller indices; temperature factors; structure factor. Bragg equations vs. Laue equations.

Lecture 6 – February 4: Practical implications of the Laue equations (ZSD)

Introduction of the reciprocal lattice; Miller indices; number of Bragg reflections from a given crystal lattice; resolution; Friedel's law; relationships between real and reciprocal space symmetries.

Lecture 7 – February 6: Diffraction by helical structures; fiber diffraction (RHK)

Lecture 8 – February 11: Protein chemistry and protein crystals (ZSD):

History of protein chemistry; discovery of amino acids; Fischer and the peptide bond; Sumner, Northrop and crystals of enzymes; Bernal and Hodgkin, diffraction of pepsin and insulin; Pauling, hydrogen bonds and the planarity of the peptide bond.

Lecture 9 – February 13: Protein crystallization (ZSD)

Uniqueness of protein crystals; basic concepts and methods of protein crystallization; solvent content and Matthews constant, large unit cells; consequences for data collection: resolution, diffraction geometry; computing requirements.

Lecture 10 – February 18: Data collection for protein crystals and X-ray diffraction laboratory (ZSD):

Single-counter methods and four-circle geometry; use of film and area detectors with the rotation (oscillation) method; data statistics; integrated data collection software packages. Rotating anode generators, image plate detectors and cryo-crystallography; combination of practical and theoretical aspects of operating X-ray diffraction equipment

February 20: Practical session

Practical training in groups with schedule depending on group size (ZSD & RHK): Preparation of specimen for X-ray diffraction experiments; crystallization; flash-freezing; preliminary diffraction analysis; autoindexing, identification of space group, etc.

Lecture 11 February 25: The Patterson function and the phase problem (RHK).

The nature of the phase problem; direct methods vs. Patterson methods; Patterson function and application to simple structures;

Lecture 12 February 27: The Patterson function and the phase problem (ctd) (RHK).

Patterson solution to heavy atom problems; difference Fourier analyses based on phase estimates.

Lecture 13 March 11: The MIR method (ZSD)

Isomorphous replacement for proteins; phase diagrams; phase probability distribution (Hendrickson-Lattman representation); figure of merit.

Lecture 14 & 15, March 13 & 18 Contemporary issues of macromolecular crystallography (ZSD)

Advances in molecular biology, automated protein crystallization, membrane proteins, macromolecular assemblies, synchrotron radiation, advances in computing sciences, structural genomics etc.

Lecture 16 March 20: Anomalous scattering and its use for phase determination (ZSD)

The nature of anomalous effects; anomalous scatterers and their diffracting power; resolution of phase ambiguity by anomalous signal (SIRAS); use of Se and halides for anomalous phasing; MAD vs. SAD phasing

Lecture 17 March 25: Density modification (ZSD)

Density modification by solvent flattening; phase ambiguity resolution for SIR and SAD experiments; solvent flipping and histogram matching; symmetry averaging

Lecture 18 March 27: Molecular Replacement (ZSD)

The nature of non-crystallographic symmetry and the origins of Molecular Replacement; the limited ensemble of protein folds; trial and error methods; the de-convolution of a six-dimensional search using a Patterson search; rotation *vs* translation functions

Lecture 19 April 1: Strategy of experimental phasing and phase enhancement by density modification (ZSD).

Comparison of phasing strategies including MIR, MIRAS, MIRAOAS, SIR, SIRAS, SIROAS, MAD and SAD; use of density modification including solvent flattening and symmetry averaging.

Lecture 20 April 3: Refinement (ZSD)

Mathematical background – least-squares refinement in non-linear environment; normal matrix; over- and underdetermined cases; Fast-Fourier algorithms; constraints vs. restraints; the use of molecular dynamics in protein refinement; the maximum likelihood target function; automated refinement using dummy-atom refinement; refinement at ultra-high resolution; modeling anisotropic vibrations.

Lecture 21 April 8: Crystallographic computing - an introduction (ZSD with David Cooper)

Introduction to CCP4 and CNS crystallographic computing systems. Introduction to SHARP. ARP/wARP automatic model building and refinement. Organization of the files, graphical user interfaces, strategies for computing.

Lecture 22 April 10: Validation of X-ray models (ZSD)

Validation of refinement using free-R factor; stereochemical criteria; specific cases of errors in structure determination; Refining atomic models using CNS, REFMAC and SHELX;

Lecture 23 April 15: Complementary diffraction methods: electron and neutron diffraction (RHK)

Lecture 24 April 17: The frontier: FEL and single molecule scattering (ZSD)

April 22 & 24: Discussion of selected research papers by students (ZSD & RHK)

April 29 make up lecture

May 1 Exam?