Molecular Structure Determination by X-ray Crystallography

BCH 6744C

Aim:
Provide theoretical and practical instruction on the methodology of X-ray crystallography

Credit: 1 or 2 hours
Instructor Contact Information:
Dr. Mavis Agbandje-McKenna (Labs)  
Office: LG-179, 2-5694  
e-mail: mckenna@ufl.edu

Dr. Robert McKenna (Lectures/Discussions)  
Office: LG-181, 2-5696  
e-mail: rmckenna@ufl.edu

Teaching Assistant: John Domsic  
e-mail: domsicj@ufl.edu
Teaching Times and Places:

Lectures (L):

Held Mondays (M), Wednesdays (W) and Fridays (F)
4th period (10:40 am to 11:30 am)
Mainly in LG-110A

Laboratory Practicals (P)

Held Thursdays
4th to 6th period (10:40 am to 1:30 pm) in LG-171
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Handouts:
http://www.mbi.ufl.edu/facilities/msg

Prerequisites:
BCH 6740 or equivalent or consent of instructor

Recommended Text:
And
Current structural biology literature
Tests and Grading:

**Lecture component** (1 credit)

Based on a take home final exam and course work
50% Assignments (30 minutes)
50% Exam (short answers/calculations)

**Laboratory component** (1 credit)

30% homework
70% completed lab. project report
Written in the form of crystal structure manuscript
Q: Why X-ray Crystallography?

Leads to atomic resolution structures

Understanding biological function (phenotype)

Future is bright (Human Genome project)

After grad School (Pharmaceutical, NASA etc.)
Biophysical techniques

1D  Low angle solution scattering
    viruses/macromolecular assemblages

2D  Fiber diffraction
    DNA/muscle

3D  Single crystal X-ray diffraction
    anything that can crystallize

Electron Microscopy (EM)
    Cryo-EM and 2D electron diffraction
    viruses/macromolecular assemblages/membrane proteins

Nuclear Magnetic Resonance (NMR)
    proteins/DNA 20-30kD
Why do you need crystals?

TWO REASONS:

1) Scattering techniques dependent on sample order. Degrees of freedom (rotational space) Crystals are ordered in 3D

2) Amplification of signal/ Multiply units all producing the same signal.
Low angle solution scattering
Sample in solution - free rotation

Result:
Size, shape, functional unit (averaged)
Can study conformation changes
Viruses/macromolecular assemblages
Fiber diffraction
Sample in solution - one axis of rotation fixed

(a)  (b)

layer lines

Molecules with helical symmetry
Fiber diffraction
Sample in solution - one axis of rotation fixed

Molecules with helical symmetry
Fiber diffraction
Sample in solution - one axis of rotation fixed

Molecules with helical symmetry
Example: DNA double helix

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Example: Fibrous Proteins

Collagen

Coiled-coil α helices keratin and myosin
Triple helix in collagen
β-sheets in amyloid fibers and silks

Spiders Webs
Single crystal X-ray diffraction
Sample in solid state - have to crystallize

Complete 3D structure
Millions of molecules giving simultaneous data
Why do you need X-rays?

Laws of Physics (from optics)

To distinguish objects they must be separated by at least half the wavelength of the radiation used (resolution)

\[ \frac{\lambda}{2} \]

Electromagnetic radiation is the media used

Visible light \[ \lambda = 4 \text{ to } 8 \times 10^{-5} \text{ cm} \]
X-ray \[ \lambda = 10^{-8} \text{ cm} \]

Atoms are separated on the order of \( 1 \times 10^{-8} \text{ cm} \)
C-c bond is 1.54 Å \( (1.54 \times 10^{-8} \text{ cm}) \)
Disadvantages:

Crystals (NOT BECAUSE IT IS IN THE SOLID STATE BUT BECAUSE THEY ARE HARD TO OBTAIN)

Amount of material (mg amounts)

Advantages:

Exact results (atom positions: X,Y,Z – WITH MEASURABLE ERRORS)

Structure leads to new research
Steps in determining a crystal structure

Expression & Purification
  ↓
Crystallization
  ↓
Data Collection
  ↓
Structure Solution
  ↓
Electron Density Map Interpretation - Model Building
  ↓
Structural Refinement - Validation
  ↓
Structural Analysis - Biological Implications
Concept

Reciprocal Space
\[ s = F(\rho) \]

Real Space
\[ \rho = F(s) \]
ASSIGNMENT:
History of crystallography:

Fourier, Jean Baptiste Joseph
RÖntgen, Wilhelm
Laue, Max von
Bragg, William Henry
Bragg, William Lawrence
Bernal, John Desmond
Hodgkin, Dorothy Crowfoot
Perutz, Max Ferdinand
Kendrew, John Cowdery