Better office hour time Monday 4:30-5:30pm?

Homework 3 due Thursday:

-> You can investigate grant abstracts from any grant-funding source, not just NIH

-> Identify two possible design projects: no need to explain how you will engineer protein, but explain how native protein is not ideal for proposed application and needs to be engineered to have a certain property

TODAY:
NMR and X-ray Crystallography
Structural Biology Techniques

- Tissues: 1 mm
- Cells: 100 μm
- Organelles: 10 μm
- (Macro)molecules: 1 nm, 0.1 nm

- Light microscopy
- Electron tomography
- Small-angle x-ray scattering
- Electron crystallography & Single-particle electron microscopy
- X-ray crystallography
- Nuclear magnetic resonance
Decided not to discuss...

- Electron microscopy (EM) and Cryo-EM

- SAXS
Crystallography Outline

- Protein crystallization
- Explain why crystals give diffraction spots
- How to collect diffraction data
- Relationship between diffraction data and electron density map
- Solving a structure
Overview of crystallography

1. Make protein form crystals
2. Shoot protein crystals with X-rays
3. Use data to compute 3D structure of HA protein
What is a crystal?

- A periodic arrangement of objects (i.e. proteins) repeating in two or three dimensions
- A typical crystal (0.1mm x 0.1mm x 0.1mm) contains $\sim 10^{15}$ protein molecules
Crystallization

Sugar crystallization:

1. Make a supersaturated solution
   Dissolve sugar in water with heat

2. Slowly concentrate sample
   Let cool. Change in temperature and evaporation of water causes sugar molecules to form crystals
Protein crystallization:
not that easy, but same principles

1. **Make a supersaturated solution**
   Concentrate protein and mix with a precipitant solution

2. **Slowly concentrate sample**
   Use vapor diffusion methods (see next slide) to slowly concentrate protein sample
Protein crystallization by vapor diffusion

1. Mix cocktail and protein on glass slide
2. Turn slide and seal well
3. Vapor diffuses into well, concentrations in drop increase
4. Observe for crystal formation

Well with crystallization cocktail (precipitants, additives, detergents, etc. – unlimited combinations possible)

(C) Bernhard Rupp 2010

Harvest and mount crystals
Protein crystallization: the zone between soluble and precipitated protein

Design of a Precipitant Screen

- SUPERSATURATED
- PRECIPITATE

[Protein] [Precipitant]
Protein crystallization trials

• Ideally: concentrated protein sample (~10mg/ml)
• >98% pure, monodispersed, homogeneous
• Crystallization robot: sets up 96 different crystallization conditions with 20ul protein in ~10min

What conditions make a protein crystallize?

UNPREDICTABLE!!!!

Test most common combinations of precipitants (ammonium sulfate, PEG), additives (metals, salts), pH, etc. that have previously yielded protein crystals
Preparing your crystals for data collection

• Crystals are ~50% water
  – Fragile, like jello squares!
• Crystals transferred to a cryo-protectant solution (add glycerol, PEG400, etc.)
  – Keeps samples clear, not ice, when frozen
• Crystals are looped and frozen in liquid N\textsubscript{2}
• Data collected in cryostream of liquid N\textsubscript{2}
  – Slows X-ray damage on crystals
Why do we use crystals when we’d like to see one molecule?

• We can’t focus enough x-rays into a small enough volume to “see” a molecule

• Even if we could focus them, the x-rays would burn up the molecule

• The crystal amplifies the signal
Overview of crystallography

1. Make protein form crystals
2. Shoot protein crystals with X-rays
3. Use data to compute 3D structure of HA protein
Relation between X-ray crystallography and light microscopy

Both: Light is scattered from the sample
Problem: no lens exists for X-rays
Why do we need X-rays for X-ray crystallography?

- We are trying to see atomic features:
  - C-C bond: 1.54 Å
  - C-N bond: 1.43 Å
  - C=O bond: 1.23 Å

- To see the atoms, we need to use light with a wavelength that is near to this distance

- X-rays have a suitable wavelength

- X-rays are scattered by the ELECTRONS on each atom
Sources of X-rays

- **“Home source” X-ray generator**
  - Electrons accelerated to copper anode: produces X-rays with 1.54Å wavelength
  - Low intensity: collect dataset in 12 hours

- **Synchrotron X-rays**
  - Electrons accelerated in a circular particle accelerator
  - Tunable to desired wavelength (necessary to experimentally solve “phase problem”)
  - X-ray beam size may be changeable (good for tiny crystals)
  - Robotics: quickly screen many crystal samples
  - Better, faster detectors
  - High intensity: collect dataset in 10 minutes
Synchrotron X-ray Use

- A lab applies for use of synchrotron
- Lab typically gets a 12- or 24-hour shift
- Lab gets use of 1 beamline for that shift
- Most
Data collection setup

- Crystal sample + sample holder (goniometer)
- Cryostream
- X-ray beam
- Detector
- Beamstop
- Camera
- Scientist on computer

Camera allows centering of X-ray beam on crystal
Let’s look at a series of images, each representing one degree of crystal rotation.
What did you observe?
What did you observe?

- Beam stop shadow
- Diffracted spots have varying intensities
- Ordered diffraction pattern
Why do crystals produce ordered diffraction patterns?
Why do crystals produce ordered diffraction patterns?

In both cases the repeating unit (Unit Cell) has the same AREA, or VOLUME for a three-dimensional crystal.
Why do crystals produce ordered diffraction patterns?

We can slice the crystal at lattice points: all planes pass through the same dot.
Why do crystals produce ordered diffraction patterns?

Think of a crystal as a set of planes.
Why do crystals produce ordered diffraction patterns?

Constructive interference

Diffracted waves are in phase

Destructive interference

Diffracted waves are out of phase

A crystal amplifies the intensity of the diffracted waves:
The bigger the crystal, the more planes, the larger the intensity of the diffracted waves.
Why do crystals produce ordered diffraction patterns?

- Constructive interference occurs **only** when all of the diffracted waves are **in phase**

- Parallel waves must go an extra $n\lambda$ ($n=$integer) in order to stay in phase

- Thus, we can learn about the distance $d$ between the planes of the crystal if we know the $\lambda$ of X-ray.
Bragg’s Law

\[
\sin \theta = \frac{x}{d}
\]

\[
x = ds \sin \theta
\]

\[
2x = 2d \sin \theta
\]

\[
n\lambda = 2d \sin \theta
\]

For constructive interference, we know that 2x must be an integer of our wavelength \(\lambda\).

We know the wavelength, we measure \(\theta\), so we can determine \(d\), the distance between planes.
Crystallography measurements

• Diffraction angle $\theta$
  – Bragg’s law: $n\lambda = 2dsin\theta$
  – Tells us information about the crystal unit cell dimensions

• Intensity of diffracted spots $I$
  – Tells us information about electron density inside unit cell: $I(h,k,l) = |F(h,k,l)|^2$
  – The structure factor is the Fourier transform of the electron density

\[
\rho(r) = \sum_{hkl} F(h,k,l) e^{-2\pi i(hx+ky+lz)}
\]
Data processing

**Identify diffraction spots**

**Determine crystallization lattice (space group)**

**Record intensity I(hkl)**

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The Phase Problem

• We’ve measured the intensity of diffracted spots $I$
  – $I(h,k,l) = |F(h,k,l)|^2$
  – The structure factor is the Fourier transform of the electron density

\[
\rho(r) = \sum_{hkl} F(h,k,l) e^{-2\pi i (hx + ky + lz)}
\]

• Phase information is lost but can be obtained by:

• Molecular replacement
  – Phases from a similar known structure

• Experimental methods (big atoms with lots of electrons scatter X-rays very well)
  - Se-Met crystals
  - Heavy atom soaks of crystals

We need phase for each of 10,000 – 1 million unique $F(H,k,l)$
X-ray crystallography data gives electron density maps
Solving a structure: 3D jigsaw puzzle
Visualizing a crystal structure
Visualizing a crystal structure
Visualizing a crystal structure
Understanding X-ray resolution
Thank you!

Questions?

DuBois Lab
0.95Å structure of virus protein