Protein Structure Determination '18

Lecture 3:

Growing crystals.
Bragg’s Law of Diffraction.
The color you see is “birefringence”, the wavelength-dependent rotation of polarized light.
**vapor diffusion method**

- Most popular.
- “Sitting drop” or “Hanging drop”.
- Volatiles (i.e. water) evaporate from one surface (drop) and condense on the other (reservoir).
- Drop has *higher* water concentration than reservoir, so drop slowly shrinks.
- Easier to access and mount crystals than the batch-under-oil method.

![Diagram of vapor diffusion method](image)
Other ways to grow crystals

Microdialysis
- Crystals grown in situ
- Not for cryo
- Reference solution, draws water out of capillary. Keeps protein in
- Protein + buffer + salts + precipitant.

Microbatch under oil
- Amenable to high-throughput
- No “reference solution”
- Easy to access and mount crystals
- Used by high throughput crystallization services
Crystallization robot

High-throughput crystallography labs use pipeting robots to explore thousands of “conditions”. Each condition is a formulation of the crystal drop and the reservoir solution.

Conditions can have different:

• protein concentration

• pH

• precipitant, precipitant concentration

• detergents

• organic co-solvents

• metal ions

• ligands

• concentration gradient

The Hauptman-Woodward Institute in Buffalo NY will screen 1500 precipitants for under $400.
Saturation and supersaturation

Crystal growth occurs between these two limits. Above the supersaturation limit, proteins form only disordered precipitate.

blue line = saturation of protein
red line = supersaturation limit
A precipitant (r) causes proteins (p) to stick to each other by competing for solvent.

\[ r = \text{EtOH, (NH}_4\text{)}_2\text{SO}_4, \text{methylpentanediol, polyethylene glycol, etc} \]
Crystal nucleation

Nucleation takes higher concentration than crystal growth.

After nucleation, the large size of a face makes the weak bond more likely.
Crystal growth

Determinants of the crystal dimensions

Bonds A, B are stronger than P, Q. Dimensions of crystal at equilibrium are proportional.

More on Periodic Bond Chain theory: http://www.che.utoledo.edu/nadarajah/webpages/PBC.htm
Crystal morphology

Growth in weak-bond directions increases proportional to the size of the face (collision theory).

Weak bonds in Z favor growth in XY, forming “plate” xtal.

Growing cross-section in XY favors growth in Z.

Ratio of cross sections is inverse to ratio of bond strength.
The end of crystal growth

Crystal growth depletes the surrounding solution of protein,

....slowing growth.

...preventing nucleations close to a growing crystal

...concentrating impurities on the surface of the crystal

Cobalt impurities in SiO$_2$ (amethyst) are concentrated in the part of the crystal that formed last (the tip).
mounting crystals the old way

Thin-walled glass capillaries (<1mm in diameter) are filled with "mother liquor" (the fluid in which the crystal was grown) and a crystal is carefully dropped in. The mother liquor is removed using filter paper cut to fine strips. The crystal sticks to the glass, immobilized.

The xtal remains in vapor diffusion contact with the mother liquor. If not it will dry out and crack.

Protein crystals are extremely fragile!!! They may break upon sudden contact with a solid object. Tiny pipets are used to pull crystals from drops.
**Freeze the crystals!**

Eliminates X-ray damage to crystal. Crystals do not “decay” during data collection.

Crystals, mounted on loops, are dipped in liquid N\(_2\) at –70°C.

*Crystals must be flash frozen in N\(_2\)* to prevent the formation of hexagonal ice. When freezing in liquid N\(_2\), water glass forms, not hexagonal ice. Water glass does not diffract.

Mounted xtal is attached to a goniometer head for precise adjustment.

Small wrenches fit here, here, here and here to adjust height, position, angle.

Pin mount. Swap for magnetic baseplate for cryo caps.

Cyro-cap. Base, pin, and nylon-loop. 1mm or smaller.
X-ray diffractometer
X-ray diffractometer
“machine center” is the intersection of the beam and the two goniostat rotation axes. The crystal must be at machine center.

To place crystal at machine center, rotate $\omega$ and $\kappa$ and watch the crystal. If it moves from side to side, it is off center.

If it is off-center, we adjust the screws on the goniometer head.
goniostat

κ geometry
Mounted crystal

**not freezing**

Xtal is mounted in a thin-walled glass capillary tube

**freezing (preferred)**

Xtal is mounted on a thin film of water in a wire loop. The loop is fixed to a metal or glass rod.

Mounted xtal is attached to a goniometer head for precise adjustment.

Crystal must be kept at proper humidity and temperature!! Very fragile!

- wax
- Must freeze immediately or film will dry out!
- eucentric goniometer head
- pin mount. Swap for magnetic baseplate for cryo caps.
- Small wrenches fit here, here, here and here.
• The crystal must rotate around machine center, in the beam.

• The beam must be able “see” the crystal from all angles.

• The orientation of the crystal axes must be known precisely.

• The beam is wider than the crystal, so the whole crystal is inside the beam.

• The interaction of the the crystal with the beam produces "reflection" spots.
X-ray source

Zooming in on the system...

X-ray beam

Typical beam width: ~0.20 mm

Typical crystal thickness: 0.10-1.00 mm
Typical protein unit cell: $\sim 100\text{Å} = 0.00001\text{mm}$
Typical protein molecule: \(~30\text{Å} = 0.000003\text{ mm}\)
Typical C-C bond distance: 1.52Å
Typical wavelength of X-rays: 1.5418Å
Angle of reflection range $= \theta$: $0-90^\circ$
Bragg plane separation distance range: $0.7-50\,\text{Å}$
Light wavelength must be on the same scale as electron density features.
How do you get reflection from a surface when the wavelength of the light is much smaller than the roughness of the surface?
X-rays are plane waves
(even though we sometimes draw them as arrows or lines)

Electrons (not nuclei) scatter X-rays.
Reflecting on mirrors

Why does light scatter at only one angle from a smooth surface?
Consider the following: plane waves hit a planar surface and bounce off.

The parallel lines represent the crests of waves. The arrow is the direction of travel.
This plane wave (frozen in time) sends out scattered waves from each point on the mirror, in all directions, but they are out of phase with each other.
The combined waves from nearby points on a plane interfere *destructively* in all directions but one, which is the *reflection angle*.
when a surface is rough, there is no reflection

If the points of scatter do not fall on a plane, then there is no consistent angle of constructive interference.
When the angle of incidence equals the angle of scatter, all rays travel the same distance.

...and at any other angle \( \neq \theta \), path lengths are different, therefore interference is destructive.
If the reflection angle = $\theta$

Then the scattering angle is $2\theta$
Integer path length differences leads to diffraction

If waves add, then path difference must be $n\lambda$. That is, an integer multiple of the wavelength.

Path difference = the two bold lines = $2d \sin \theta$

Let path difference equal integer multiple of the wavelength, and you get Bragg’s law, $n\lambda = 2d\sin\theta$
Points not on Bragg planes have non-zero phase

Path difference \( = 2(x^*d) \sin \theta \)

Phase difference \( = 2\pi \frac{2(x^*d) \sin \theta}{\lambda} \) radians

but, since \( d = \frac{\lambda}{2 \sin \theta} \)

therefore, phase difference in radians = \( 2\pi x \)
Mirrors reflect light because all points on the surface scatter in phase at only one angle, the reflection angle.

Bragg's law says that repeating planes of atoms separated by distance $d$ reflect monochromatic light of wavelength $\lambda$ at specific reflection angles $\theta$.

**Bragg's Law:** $n\lambda = 2dsin\theta$
Scattering from the Origin has phase 0 by definition

There is only one origin in the crystal (usually this is defined by crystal symmetry)

The wave travels a distance $a$ from the Xray source to the plane of the origin, and a distance $b$ from the origin to the detector.
The Fourier transform of Bragg planes

(1) All Bragg planes scatter in phase.
(2) Bragg planes through the origin have phase = 0
(3) The amplitude from Bragg planes is the sum of the density

Amplitude $\rho$ is proportional to the total number of $e^-$ on all of these planes.

planes extend throughout the crystal
Bragg planes *offset by x* have phase $2\pi x$

$\rho(x)$ is proportional to the total number of $e^-$ on these planes.
Integrating offset Bragg planes from $x=0$ to 1

The total $F$ is the wave sum over all offsets, $x$.

$$F = \sum_{x=0,1} \rho(x)e^{2\pi ix}$$

Amplitude as a function of phase (offset)

Integrate over phase.
The only Bragg planes *of interest are* crystal planes.

Only crystal planes scatter with non-zero amplitude. Other Bragg planes all have zero amplitude.

proof to follow...
Crystal planes are sets of parallel planes that pass through all of the unit cell origins.
Bragg Planes/Crystal Planes naming.

Planes are numbered according to how they intersect the crystal axes. Starting from the Origin and moving to the first Bragg plane, if it intersects the a axis at 1/h, the b axis at 1/k and the c axis at 1/l, then the Bragg planes are called the (h k l) reflection plane. Each set of Bragg planes defines a single diffracted spot, called a “reflection”. Reflections are also numbered using (h k l).

NOTE: h k and l must be integers!
How to draw Bragg planes on a lattice

1. Draw a **plane that intersects** the axes …
   - \( a \) at \( \frac{1}{h} \),  \( b \) at \( \frac{1}{k} \),  \( c \) at \( \frac{1}{l} \)

2. Then draw a **parallel plane through the origin**.

3. Draw equally spaced planes in both directions. Each origin has a Bragg plane going through it. The position of the \( hkl \) reflection in reciprocal space is normal to the Bragg planes at a reciprocal distance \( \frac{1}{d} \) from the beam.
If $hkl$ indeces are doubled, the reciprocal space distance is doubled and the Bragg real space distance $d$ is halved.

- All unit cell origins have phase zero. But not all phase-zero Bragg planes must go through a unit cell origin. For example, the $n=$odd Bragg planes for the 0 2 0 reflection does not touch a single unit cell origin.
3D Bragg planes/Crystal planes

(2 3 3) Bragg planes

(4 6 6) Bragg planes

Phase-zero planes intersect the cell axes at multiples of fractional coordinates

\((1/h,0,0), (0,1/k,0),(0,0,1/l)\)
Exercise. Draw crystal planes
Proof: The only Bragg planes that diffract X-rays are crystal planes.

If we see a reflection (spot) on the film, it corresponds to one set of crystal planes.
1. Bragg planes are either aligned with the Unit Cell Origins, or they are not.

**aligned**

If the Bragg planes all pass exactly through the Origins, the phase of every Origin is the same.

**not aligned**

If the Bragg planes don’t all go through the Origins, then phase of every Origin is different, depending on the where it is in the crystal.

Proof: All Bragg planes of phase zero pass through the Origins
2. All planes that pass through the Origins have the same number of electrons

The angle and intercept with the Unit Cell determine where atoms are on the plane.

3. All planes that pass through the Origins contribute the same amplitude.

...because amplitude is proportional to number of electrons, and (statement 2).
4. Total amplitude is the sum of the amplitudes of the planes if the planes have the same phase. Amplitude contributed by origin planes is 10K times the amplitude of one such plane, if there are 10K unit cells.

Proof: All Bragg planes of phase zero pass through the Origins

5. Total amplitude is approximately zero if the planes have different phases. Phase shifts by a constant for each unit cell. Vectors sum in a circle. Summed over 10K unit cells, vector length is small.
Conclusion: The only Bragg planes that diffract X-rays are the crystal planes.
Exercise. Draw crystal planes, calculate $\theta$, and draw diffraction geometry.
Crystal planes define a spot in reciprocal space

- Measure \( d \) = the distance between planes in Å.

- Calculate \( \theta \) using Braggs law.
  \[
  n\lambda = 2d \sin(\theta), \ n=1 \text{ if } d \text{ is 1 Bragg distance.}
  \]
  Therefore \( \theta = \sin^{-1}(\lambda / 2d) \)

- \( S = s - s_0 \)

- \( s \) and \( s_0 \) are the same length, so \( S \) is perpendicular to the Bragg planes.

- The length of \( S \), \( |S| = 2 \sin(\theta) / \lambda = 1/d \)
Exercise. Draw crystal planes and draw the direction and length of $\mathbf{S}$.
Homework 1

• due Mon Oct 29
Review

- Why does a smooth surface reflect light?
- What is Bragg’s Law?
- What are Bragg planes?
- What does it mean to talk about Bragg planes that are offset by some distance?
- How does the offset distance translate to a phase?
- What are crystal planes? How are they named?
- Are crystal planes Bragg planes?
- What happens to the total scattered amplitude if the Bragg planes are crystal planes?
- What happens to the total scattered amplitude if the Bragg planes are not crystal planes?