X-Ray Crystallography Resources

- Cantor and Schimmel, Chapter 13
  - Good, mathematical but dated
- Eisenberg and Crothers, Chapter 17
  - Good but also old
- Glusker and Trueblood, *Crystal Structure Analysis: A Primer*
  - Very good introductory text
- Rhodes, *Crystallography Made Crystal Clear*
  - Short text, intended for end users (not crystallographers)
- Blundell and Johnson, *Protein Crystallography*
  - Classic text, but very old at this point
- Rupp, *Biomolecular Crystallography*
  - Maybe a modern replacement for Blundell and Johnson?
## X-Ray vs. NMR

<table>
<thead>
<tr>
<th>X-Ray Crystallography</th>
<th>NMR Spectroscopy</th>
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<tbody>
<tr>
<td><strong>Pros</strong></td>
<td><strong>Pros</strong></td>
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<tr>
<td>• Large complexes</td>
<td>• Dynamics + structure</td>
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<tr>
<td>• Highly accurate structures</td>
<td>• Detect changes in solution state</td>
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<tr>
<td><strong>Cons</strong></td>
<td><strong>Cons</strong></td>
</tr>
<tr>
<td>• Crystal conditions</td>
<td>• Solution conditions</td>
</tr>
<tr>
<td>• Static structures</td>
<td>• Less accurate structures</td>
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<tr>
<td></td>
<td>• Size limited (but getting better)</td>
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Crystallography vs. Microscopy

From *Crystal Structure Analysis: A Primer*
Glusker & Trueblood, Chapt. 1, p. 5
The End Result

- Staphylococcal nuclease (1SNC) using map from electron density server (EDS) and PyMOL
Huygens-Fresnel Principle

From Huygens-Fresnel Principle
Resolution and Wavelength

• As point sources become closer (less than $\lambda$), it becomes harder to see diffraction.

• Limit of resolution is approximately $\lambda/2$

• X-rays required to resolve bond lengths (1.2 Å)

Constructive interference observed when waves cross

Here, the wavelength is approximately equal to the separation; if we go much smaller, we won’t observe constructive interference (diffraction).
Resolution and Wavelength

- As point sources become closer (less than $\lambda$), it becomes harder to see diffraction.
- Limit of resolution is approximately $\lambda/2$
- X-rays required to resolve bond lengths (1-2 Å)

Separation is less than the wavelength; only constructive interference is directly above and below point sources (no diffraction).

We will describe this mathematically later on.

(separation (resolving distance))

(wavelength)
Requirement: Crystals

- **Signal to noise:** need lots of molecules in the same orientation
- **Regular arrangement:** prevent isotropic averaging

From van Holde, p. 280.
Crystal Packing

- 32 crystallographic point groups (symmetry operations compatible with 3D crystals)
- 14 Bravais lattices
- 230 space groups, but only 65 lack mirror planes (remember that proteins/DNA are chiral)

From van Holde, p. 282.
How to Grow Crystals

• Super-saturated protein solution (1 mM or 10 mg/mL or more)

• Precipitant is added to reduce solubility (e.g. (NH₄)₂SO₄)

• Salting in/Salting out

• **Purity is key:** both chemical and structural (think about dynamics)

This is an art as much as it is a science!
How to Grow Crystals

- Vapor pressure draws solvent from sample solution (S) to reservoir (R)
- Concentration increases as volume decreases
Crystal Screens

• High-quality buffers and established precipitants

• Standardized, and (mostly) reproducible

• What if it doesn’t work?

(lysozyme)
Summary

• X-rays probe distances on the order of their wavelengths (~1 Å)
• Crystallography produces higher resolution structures than NMR
• Many steps required for structure refinement: result is electron density map (not an image)
• Crystals needed to signal average identically oriented molecules
• Protein/DNA crystals require meticulousness, good biochemistry, and little luck