X-Ray Crystallography Resources

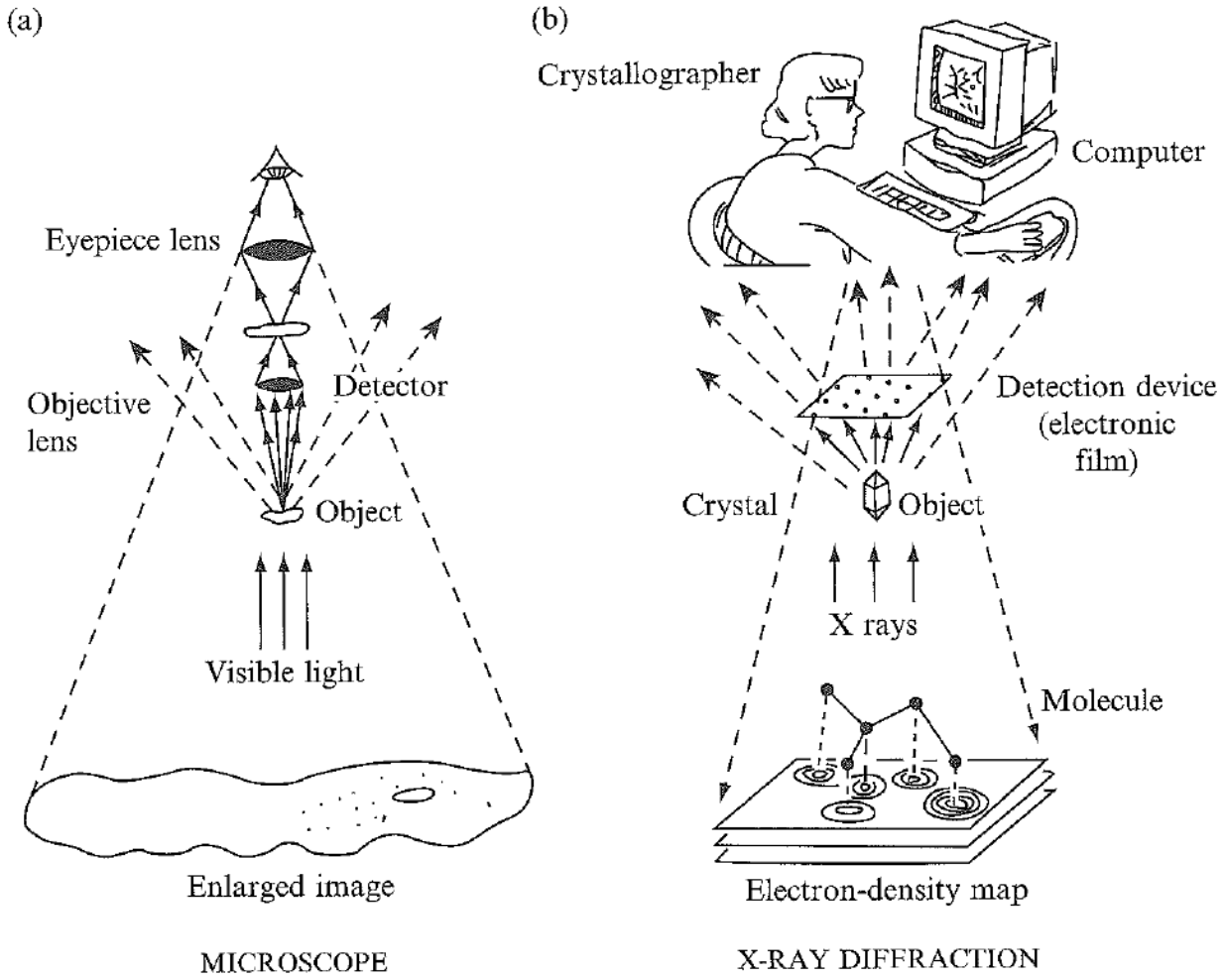
- Cantor and Schimmel, Chapter 13
  - Good, mathematical but dated
- Eisenberg and Crothers, Chapter 17
  - Good but also old
- Glusker and Trueblood, *Crystal Structure Analysis: A Primer*
  - Very good introductory text
- Rhodes, *Crystallography Made Crystal Clear*
  - Short text, intended for end 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?

Beyond the scope of this course

X-Ray vs. NMR

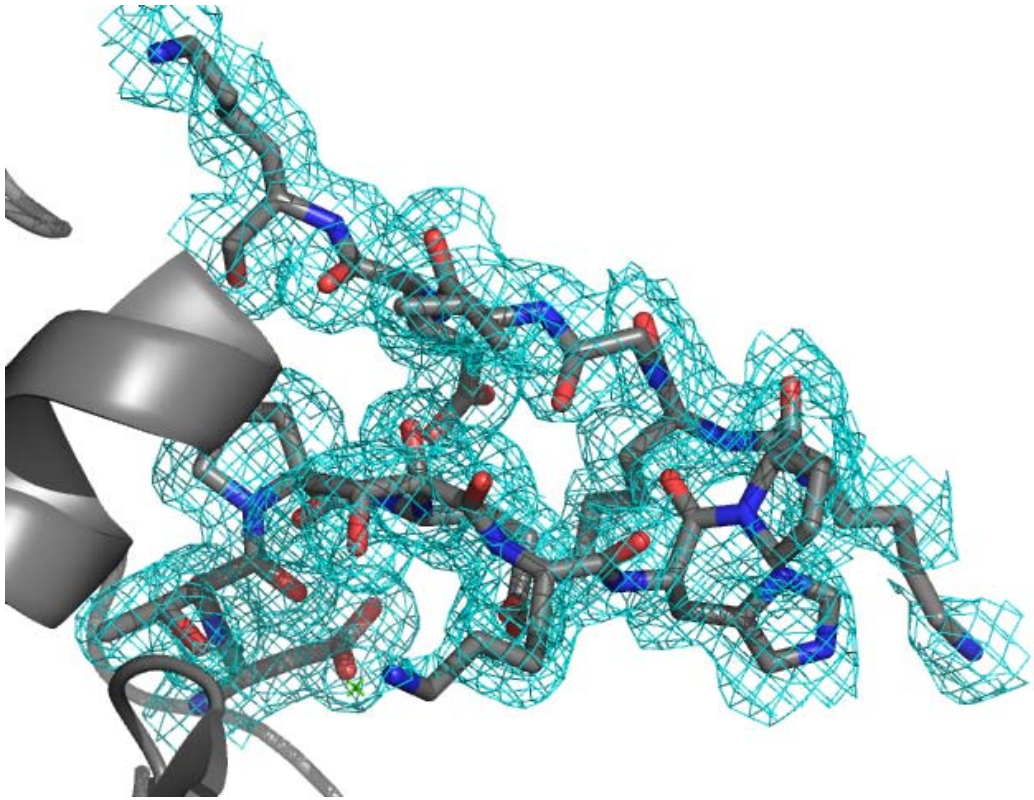
X-Ray Crystallography	NMR Spectroscopy
Pros • Large complexes • Highly accurate structures	Pros • Dynamics + structure • Detect changes in solution state
Cons • Crystal conditions • Static structures	Cons • Solution conditions • Less accurate structures • Size limited (but getting better)

Crystallography vs. Microscopy



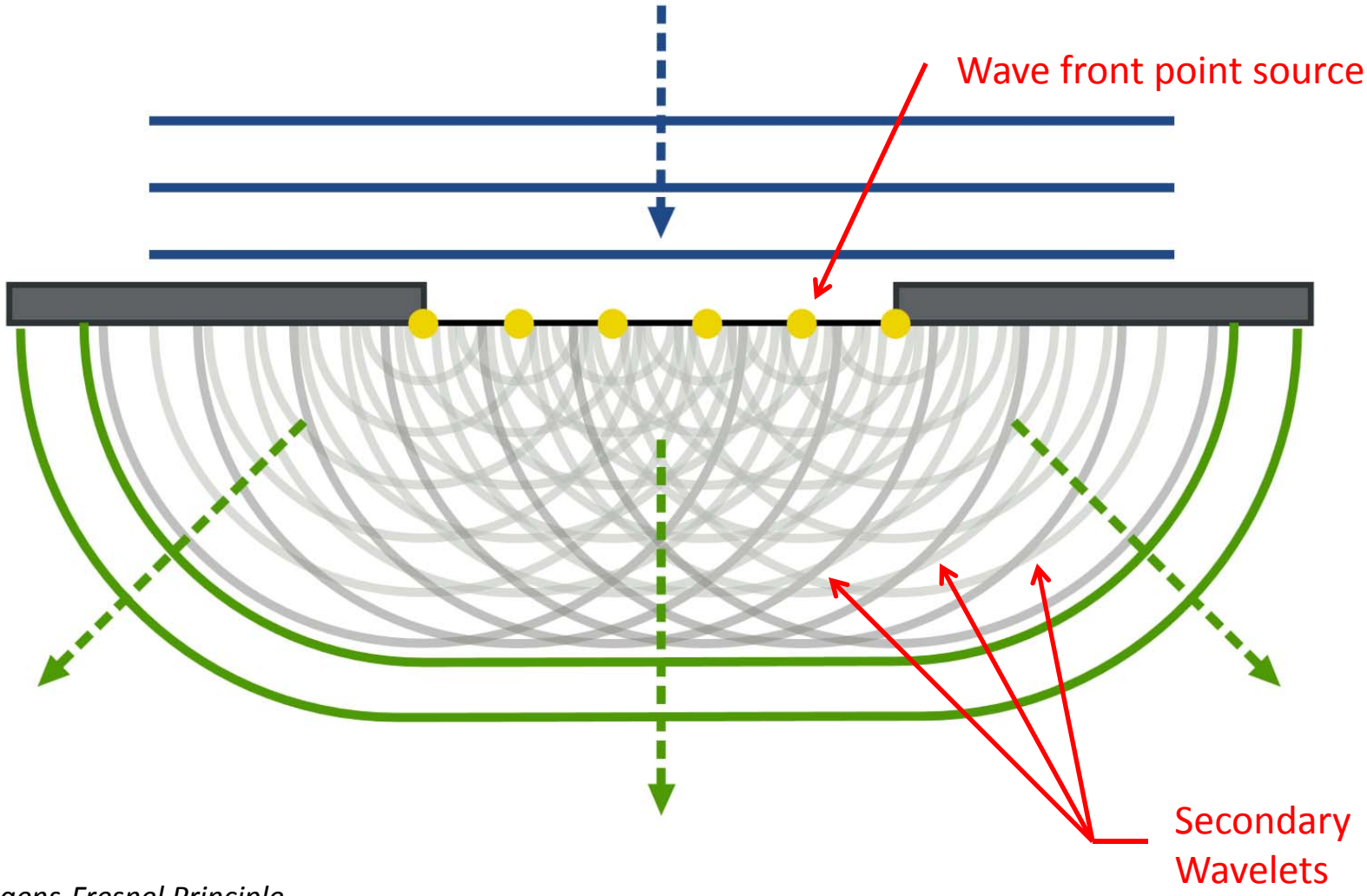
From *Crystal Structure Analysis: A Primer*
Glusker & Trueblood, Chapt. 1, p. 5

The End Result



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

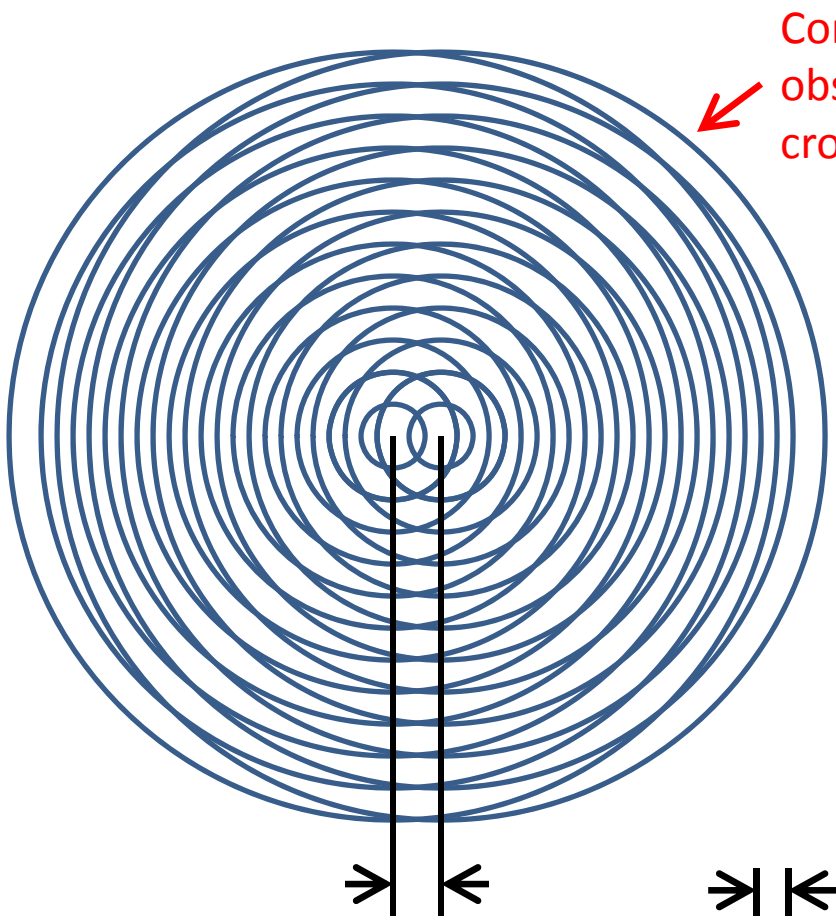
Huygens-Fresnel Principle



From *Huygens-Fresnel Principle*
http://en.wikipedia.org/wiki/Huygens-Fresnel_principle

Resolution and Wavelength

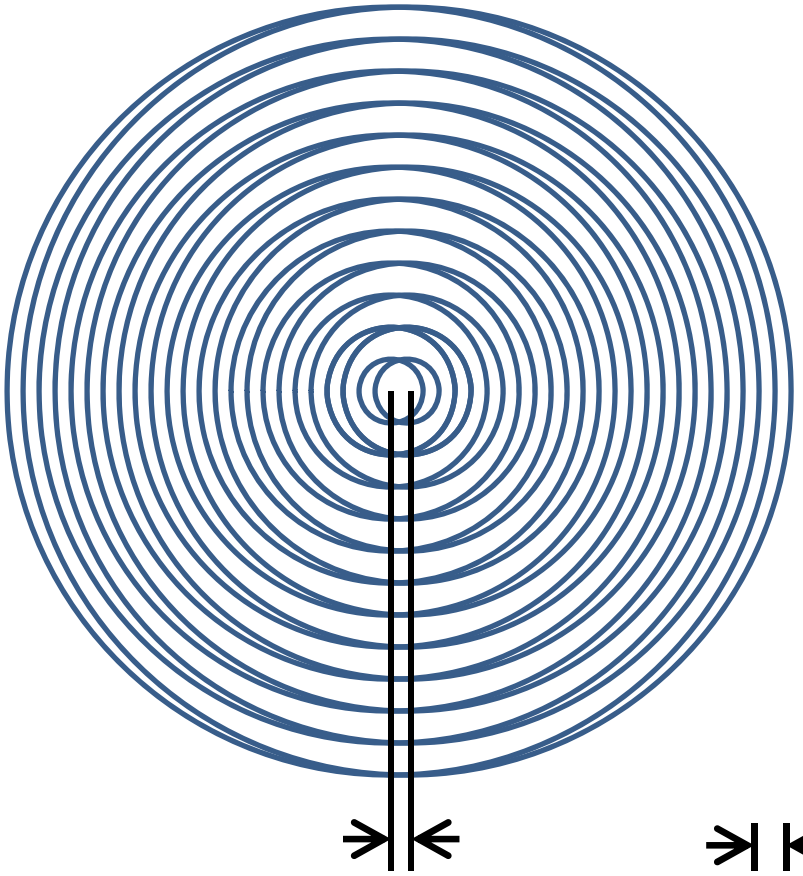
- As point sources become closer (less than λ), it becomes harder to see diffraction.
- Limit of resolution is approximately λ/2
- X-rays required to resolve bond lengths (1.2 Å)



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

Resolution and Wavelength

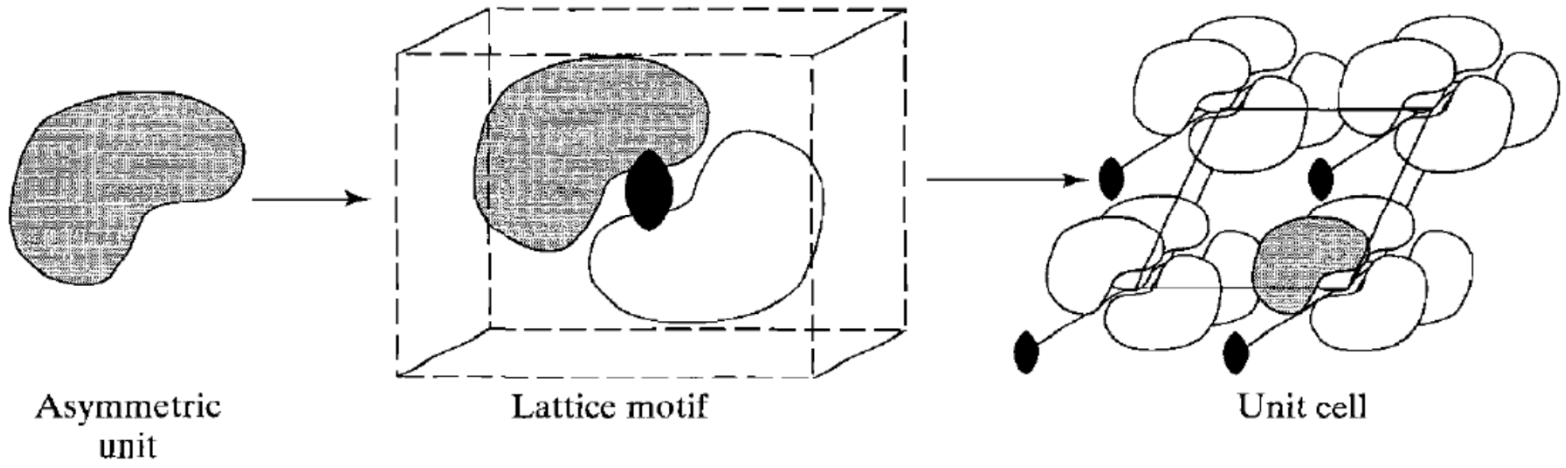
- As point sources become closer (less than λ), it becomes harder to see diffraction.
- Limit of resolution is approximately λ/2
- X-rays required to resolve bond lengths (1-2 Å)



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

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)

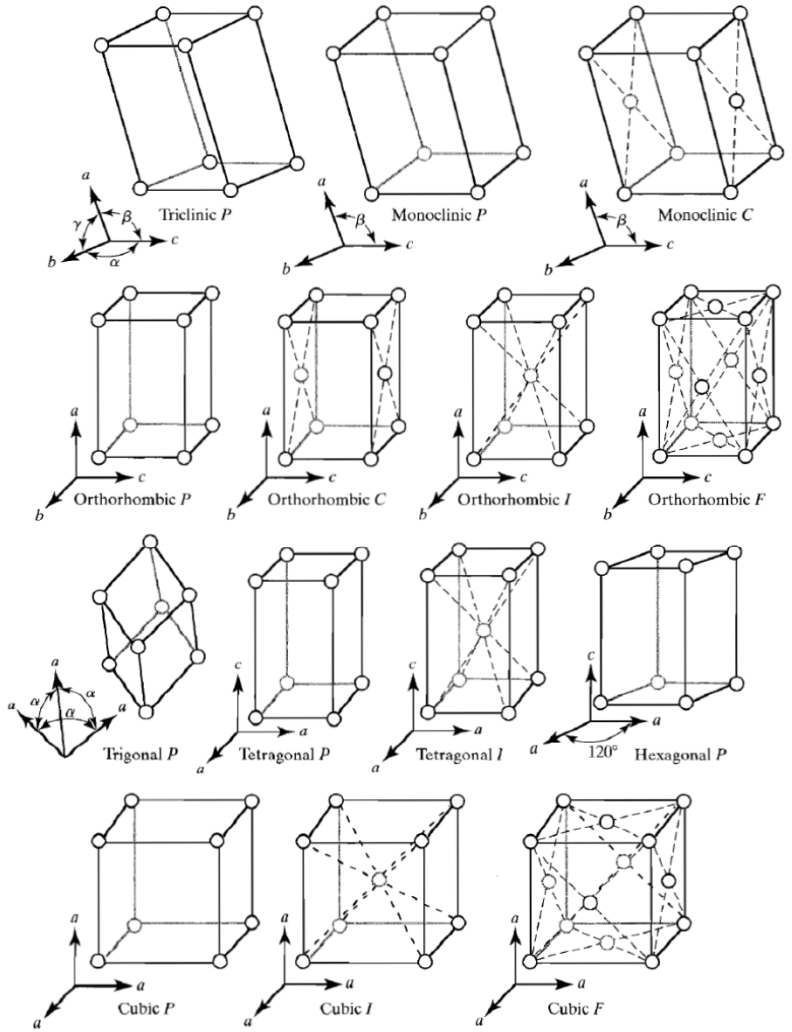
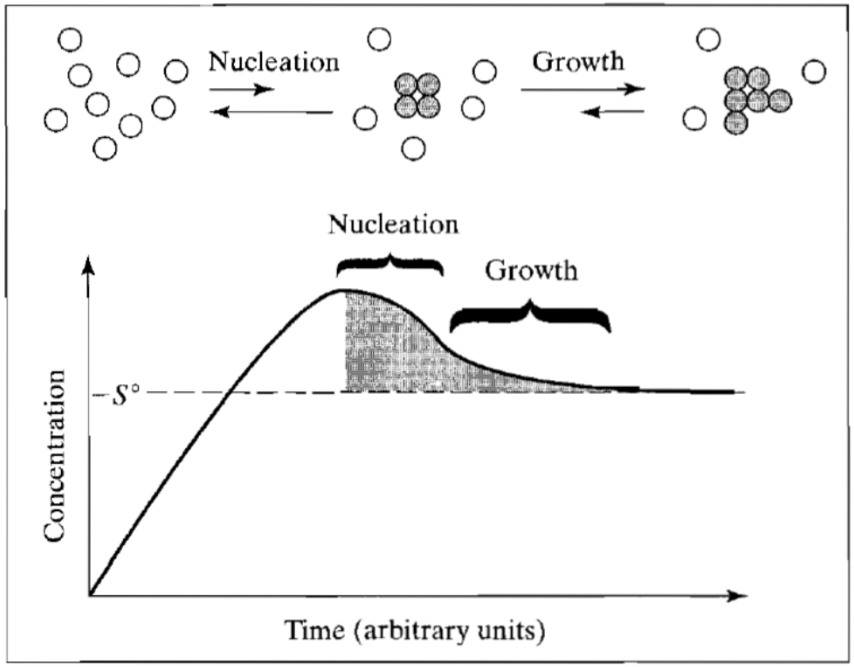


Figure 6.5 The 14 Bravais lattices in crystallography. [Adapted from G. H. Stout and L. H. Jensen (1989), *X-Ray Structure Determination, a Practical Guide*, 2nd ed., 50. John Wiley & Sons, New York.]

From van Holde, p. 282.

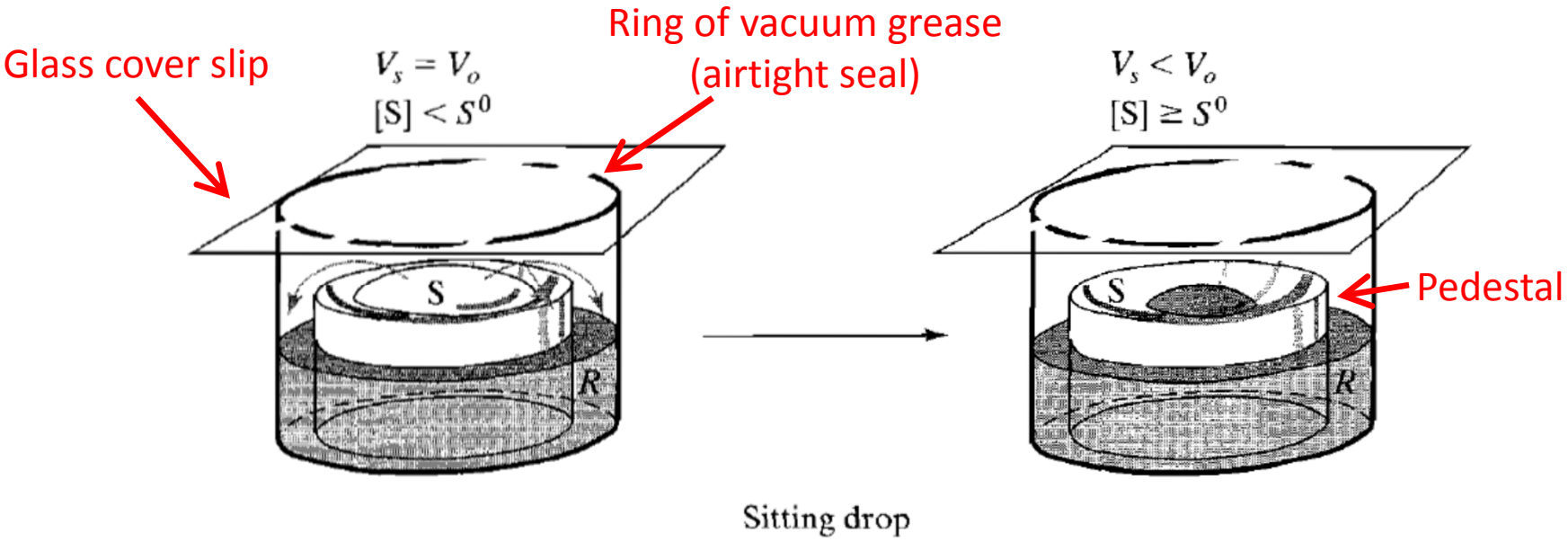
How to Grow Crystals

- Super-saturated protein solution (1 mM or 10 mg/mL or more)
- Precipitant is added to reduce solubility (e.g. $(NH_4)_2SO_4$)
- 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!

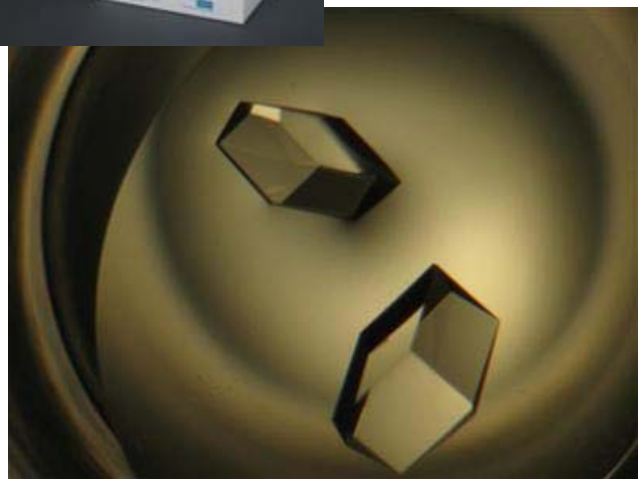
How to Grow Crystals



- 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