Part 7: Electron crystallography
Basic approaches in cryo-EM

- Tomography
- Single particle analysis
- 2D crystallography
Some proteins naturally assemble into 2D arrays

Example: bacteriorhodopsin

http://www.unitus.it/scienze/corsonew/lezionele11.html

Grigorieff et al., JMB 1995
Second example: aquaporin

Gonen et al., Nature 2004
Others can be crystallized in-vitro

Courtesy Roger Kornberg
After formation, crystals can be embedded in a sugar like trehalose and dried, or plunge-frozen.

Abeyrathne et al., MIE 2010

embedded in trehalose between two layers of continuous carbon film

adsorbed onto holey carbon film and plunge-frozen
2-D crystallography - Intro and sample prep
Concept check questions:

• What is a “2-D crystal”?

• When is 2-D crystallography the cryo-EM approach of choice?

• Describe a method for inducing a protein of interest to form a 2-D crystal.

• In addition to plunge-freezing, what other way have 2-D crystals been stabilized for EM imaging?
The Fourier transform of an asymmetric object

all pixels are significant
In the Fourier transform of a crystal, some pixels have significant non-zero values and others do not.
Object $\otimes$ lattice

$$\text{FT} \{ \text{obj} \otimes \mathcal{L} \} = \text{FT} \{ \text{obj} \} \cdot \text{FT} \{ \mathcal{L} \}$$
How the Fourier transforms of images of 2-D crystals sample the 3-D Fourier transform of the object.
Boisset et al.,
Ultramicroscopy 1998

Orlova and Saibil,
Chemical Reviews 2011
Fourier transform of a 2-D crystal
Concept check questions:

• Why does the Fourier transform of a crystalline object have discrete spots separated by pixels with near-zero amplitudes?

• What is the convolution theorem, and what does it have to do with crystallography?

• What does the Fourier transform of a 2-D crystal look like?

• What is the “missing cone,” why is it “missing,” and what effect does it have on 2-D crystallographic reconstructions?
Sample lens

Back focal plane

Magnified image

2D crystal

diffraction pattern on back focal plane
In electron crystallography, the best measurements of amplitudes come from diffraction patterns, but images are recorded to obtain phases.
Example images and diffraction patterns from aquaporin crystals

Aquaporin crystal

Electron diffraction pattern of an untilted crystal

Electron diffraction pattern of a crystal tilted to 70°

Gonen et al., Nature 2004
Example lattice line data (amplitude and phase) and curve fitting

Nogales et al., Nature 1998
Crystal “unbending”

Braun and Engel,
Encyclopedia of Life Sciences 2005
Challenges in 2D crystallography

- Hard to get well-ordered crystals
- Hard to get flat crystals
- Charging, beam-induced movement can blur images
- “Missing cone”
2-D crystallography - Data collection and reconstruction

Concept check questions:

• What is the difference between “imaging” and “diffraction” modes on an EM?

• Why are both images and diffraction patterns of 2-D crystals recorded in a 2-D crystallography project?

• Why are images of both untilted and tilted samples recorded?

• How is all the data from all these images and diffraction patterns merged to produce the reconstruction?

• What is crystal “unbending”? How and why is it done?

• Describe four common challenges in 2-D crystallography projects.
Helical tubes are “rolled up” versions of 2-D crystals, can be rolled up into different families of tubes with different pitches.

Miyazawa et al., JMB (1999)
Cryo-EM projection image of a helical tube of purified HIV CA protein

Power spectrum shows “layer lines”

3D reconstruction

Su Li et al. Nature 2000
Helical tubes
Concept check questions:

• How are helical tubes related to 2-D crystals?
• Why are helical tubes particularly good samples for cryo-EM reconstruction?
• What does the diffraction pattern of a helical tube look like?