BMB/Bi/Ch 173 – Winter 2017

Homework Set 4.1 – Assigned 2/1/2017, Due 2/7/17 by 10:30am
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Office hours – Broad 3rd floor kitchen - Friday 2/3 11am-12pm, Monday 2/6 5:15pm-6:15pm, or by appointment

Problem 1 – (50 points) 3D Electron crystallography

Recently, the use of electron crystallography for small 3D protein crystals has gained traction (microED). Refer to this eLife article http://elifesciences.org/content/2/e01345 as you answer the following questions.

1.a. (5 points) Historically scientists have not used 3D crystals for electron crystallography. Why?

3D crystals get destroyed too quickly to take many diffraction patterns. "For every elastic scattering event that contributes to a diffraction pattern there are ~10 inelastic events that cause beam damage (Henderson, 1995). Therefore, large crystals are required to withstand the high levels of radiation damage received during data collection (Henderson, 1995)."

"Because electrons interact with materials more strongly than X-rays (Henderson, 1995), electrons can yield meaningful data from relatively small and thin crystals."

"A number of studies detail the difficulties associated with data collection and processing of diffraction data that originates from several hundreds of 3D crystals, limiting the ability to integrate and merge the data in order to determine a structure in such a way (Shi et al., 1998; Jiang et al., 2011)."

MicroED was partially developed to deal with micro 3D crystals that are a byproduct of crystallization trials. These small 3D crystals are too small to loop for traditional x-ray crystallography, and too large to do 2D crystallography (since they are not 2D). Often the layers of unit cells in a small 3D crystal aren't as regular as those in a larger 3D crystal, which complications solving the diffraction pattern.

1.b. (5 points) Routine x-ray crystallography experiments require large protein crystals (in part so that enough diffraction patterns can be collected before the x-ray beam destroys the crystal). Why are small crystals common in a protein crystallization experiment? What x-ray crystallography variant can tolerate the use of these small protein crystals to solve structures?

Some proteins don't crystallize, or only form crystals that are too small to withstand the x-ray beam in a conventional x-ray crystallography experiment

Specialized ultrashort x-ray pulses can be used to collect from these crystals, but this technology isn't as readily available as standard x-ray beamline time. Femtosecond x-ray crystallography also requires millions of small crystals

1.c. (5 points) Why can electron diffraction tolerate smaller crystals than routine x-ray crystallography (ignoring femtosecond x-ray crystallography)? What is the downside of this benefit?

Electron/crystal interactions are stronger than x-ray/crystal interactions, so you can get more data from a smaller crystal

Since these interactions are stronger, the crystal is destroyed quickly. This means you can't collect multiple diffraction patterns of the same crystal as you rotate it.

1.d. (10 points) Why did previous attempts to use electron diffraction for 3D crystals fail? Refer to the eLife article and the first two paragraphs of the introduction of this article http://www.sciencedirect.com/science/article/pii/S0022283698922835 to formulate your answer.

There were difficulties in data collection and processing that made it difficult to merge everything to solve structures

Hard to characterize the unit cell

Had to determine the geometry of the layers since the 3D crystals they tried were more like a few sheets of 2D crystals on top of each other

Irregular stacking patterns common

Previously microscopists used a higher electron dose that damaged the sample more quickly.

1.e. (5 points) Why can microED solve only small crystals? What thickness did the authors aim for in their experiments?

If the crystals are too thick the electron beam can't penetrate and produce a good diffraction pattern

The authors aimed for crystals that were less than 0.5 μm thick (like in tomography)

1.f. (5 points) How did Shi et al. 2013 adjust the electron diffraction protocol to facilitating imaging of small 3D crystals?

They reduced the electron dose by a factor of 200 and could collect 90 diffraction patterns before destroying their small 3D crystal

Collecting multiple diffraction patterns from a single 3D crystal simplified the data analysis process as data didn't need to be merged from slightly different crystals

1.g. (5 points) How did the authors evaluate if the electron dose delivered was too damaging to the sample?

They subjected a protein crystal to 12 e-/Å^2 over 10 seconds and tracked the intensity of three spots in the 2.9-4.6 Å resolution range. They didn't see significant deterioration of signal at these spots until ~9 e-/Å^2 so they decided that the radiation damage was low enough before that point.

1.h. (10 points) Watch video 1 from the eLife article. What does the spacing between the discrete spots represent? About 8 seconds in the discrete spots are much further apart than they were at the start of the video. Why?

The unit cell diffraction pattern is convolved with the lattice diffraction pattern. The spacing represents the lattice that the proteins are in. Each discrete spot represents the amplitude of a Fourier component describing the unit cell.

Diffraction spot spacing (called reflections in crystallography) vary reciprocally with lattice spacing in real space.

As the crystal is tilted, the spacing of the diffraction spots changes. They are far apart at high tilt, indicating that the proteins in the lattice appear closer together at high tilt angle

A side note that will be more interesting to you later: the phases were determined using molecular replacement. They didn't take imaging mode images of their crystals to determine the phase.