

BMB/Bi/Ch 173 – Winter 2017

Homework Set 4.1 – Assigned 2/1/2017, Due 2/7/17 by 10:30am

TA - Sara Weaver sjweaver [a] Caltech.edu

Office hours – Broad 3rd floor kitchen - Friday 2/3 11am-12pm, Monday 2/6 5:15pm-6:15pm, or by appointment

Please keep problem sets 4.1 and 4.2 separated, as Sara will grade 4.1 and Emily will grade 4.2

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?

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?

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?

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.

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

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

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

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?