

BMB 173 – Winter 2017

Homework Set 5 (200 points) – Assigned 2/9/17. Due 2/16/17 (9:30a)

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Office Hours:

Monday 2/13/17 from 11am-12pm in Spalding B123 or by appointment

Wednesday 2/15/17 from 11am-12pm in Spalding B123 or by appointment

1. X-Ray Crystallography Basics (25 points)

- a. (10 points) When using X-rays, why are we limited to observing the diffraction pattern of an object? Recall that with EM we can observe either the magnified image or the diffraction pattern, depending on the plane to which the detector is conjugate.

Electron microscopy uses lenses to collect and focus electrons scattered from the sample. There is no reasonable X-ray lens to use in X-ray crystallography, so we can't focus X-rays and therefore we can only observe the diffraction pattern caused by scattering.

- b. (15 points) Briefly discuss three reasons why crystals rather than single molecules are used in protein X-ray diffraction experiments.

Immobilize protein in a single low-energy conformation
Enhance weak signal from weak x-ray scattering
Overcome radiation damage

2. Units and Symmetry in Crystals (30 points)

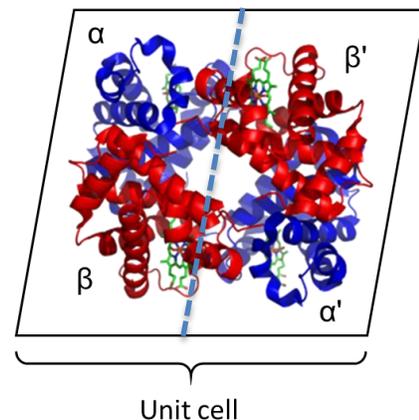
- a. (5 points) What is the difference between an asymmetric unit and a unit cell?

An asymmetric unit is the smallest part of a unit cell that lacks internal symmetry. A unit cell is the smallest part of a crystal lattice that can produce the lattice by translation.

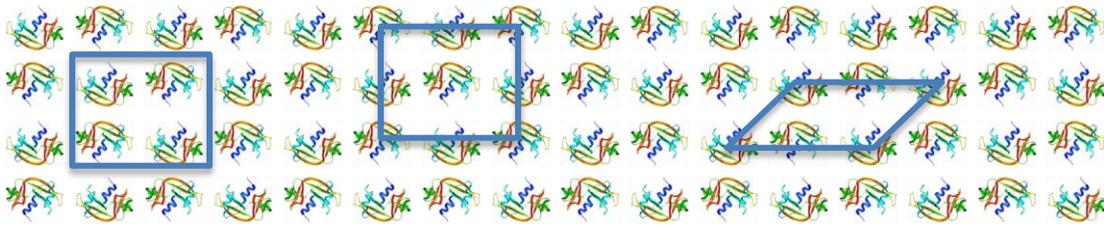
- b. (5 points) Hemoglobin, shown on the right, is a protein responsible for transporting oxygen. Its molecule comprises four subunits, two α -subunits (in blue) and two β -subunits (in red).

If we assume that this complex as a whole is the unit cell, how many asymmetric units does this unit cell contain? Please label them on the figure.

2 asymmetric units, separated by dashed line.



- c. (10 points) On the lattice of ribonuclease molecules shown below, outline three unique unit cells. Are these primitive or non-primitive cells? Briefly explain.



Non-primitive cells because they contain more than one complete protein in each unit cell.

- d. (10 points) Crystallographic symmetry operations describe the symmetry of the unit cell as well as of the entire crystal. In biological systems, what symmetry operations are not allowed? Why is this?

Mirror planes and inversion centers.

In biology we can't have this because biological systems are chiral, meaning they have a handedness, so they cannot generate mirror planes or inversion centers.

3. Fourier Transformations (35 points)

- a. (10 points) How does the convolution theorem relate to the formation of the diffraction pattern of a lattice?

$$\begin{array}{c}
 \mathbf{A} * \mathbf{G} = \mathbf{C} \\
 \downarrow_{\text{FT}} \quad \downarrow_{\text{FT}} \quad \downarrow_{\text{FT}} \\
 \mathbf{L} \times \mathbf{P} = \mathbf{M}
 \end{array}$$

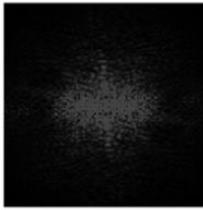
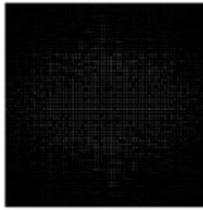
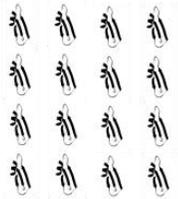
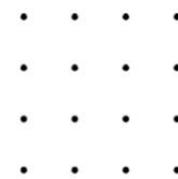
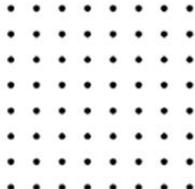
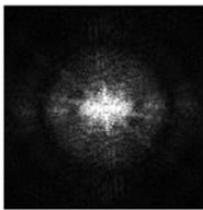
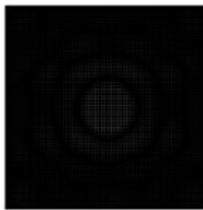
The convolution theorem allows us to understand how an object that is arranged in a lattice diffracts: in reciprocal space, the FT of the lattice is multiplied by the FT of the object, giving rise to a diffraction pattern. By knowing something about the lattice (in any dimension) and measuring the diffraction patterns, we can reconstruct the structure of the object.

- b. (5 points) In general, what is the relationship between the separation of lattice points in real space and the separation of points in reciprocal space?

They are inversely proportional. Closer lattice spacing in real space gives rise to more dispersed patterns in reciprocal space (Fourier transformations).

- c. (20 points) Given this trend as well as other key features, match each image below with its Fourier transformation. Briefly explain how you made each match.

Note: These images may not print clearly and are best viewed on a computer screen. You may also need to zoom in to distinguish important features.

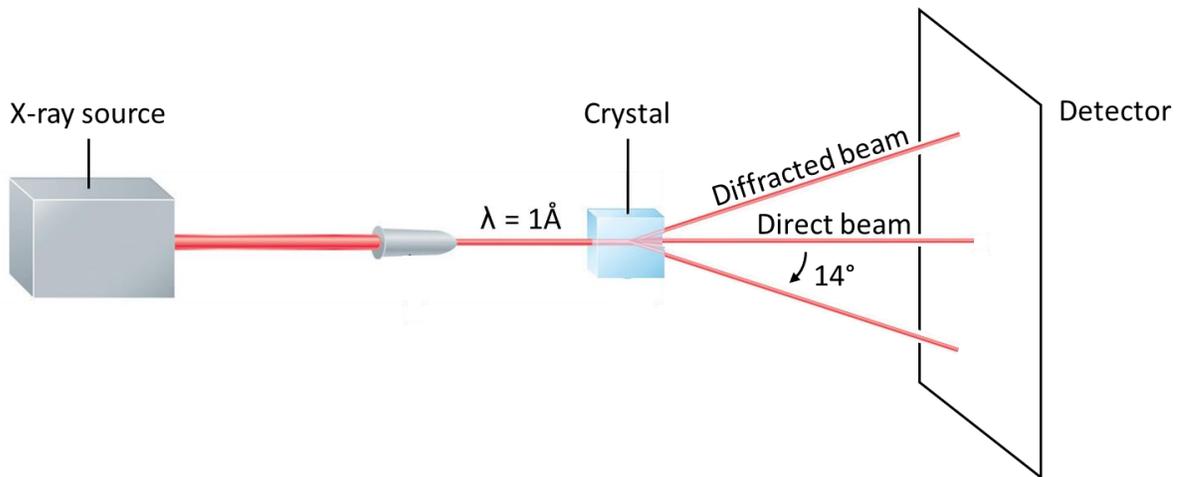
| Images | | Fourier Transformations | |
|--|--|---|--|
| A  | E  | I  | M  |
| B  | F  | J  | N  |
| C  | G  | K  | O  |
| D  | H  | L  | P  |

To make each assignment, recognize that the Fourier transform of a circle looks like a circle. The Fourier transformation of the ribbon structure must be the other pattern. Closer lattice spacing produces more dispersed patterns in reciprocal space. A key trend is that a lattice of objects produces the same overall pattern as a single instance of that object, but the pattern of dots is dependent on the lattice structure.

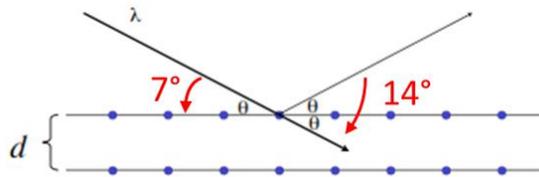
A=L, B=I, C=M, D=J, E=K, F=N, G=P, H=O

4. **Bragg's Law** (25 points)

The figure below shows the basic setup of an x-ray crystallography experiment. A beam of x-rays passes through the crystal to a detector. To keep things simple, one particular Bragg reflection is shown. The angle between the direct x-ray beam and the diffracted beam for this Bragg reflection is 14° .



- a. (10 points) Sketch a diagram of how Bragg's law is satisfied for this primary reflection, showing the relevant angles. Make sure to label the Bragg plane spacing.



- b. (10 points) Assuming the crystal is well-ordered, what is the resolution of the diffraction data that you can measure?

$$d = \frac{n\lambda}{2 \sin \theta} = \frac{1 \cdot 1 \text{ \AA}}{2 \sin(7^\circ)} = 4.1 \text{ \AA}$$

Resolution (d-spacing) of 4.1 Å for the primary Bragg reflection (n=1).

- c. (5 points) X-ray diffraction data consists of many images representing different rotations in reciprocal space. Why are multiple images required?

Individual images represent only a 2-dimensional slice of the diffraction pattern. To reconstruct a 3-dimensional object from a 3-dimensional diffraction pattern, multiple 2-dimensional images to reconstruct reciprocal space in three dimensions.

5. Radiation Sources (20 points)

- a. (5 points) How does a synchrotron generate x-rays?

In a synchrotron, magnets are used to deflect electrons in a ring. High energy X-ray radiation is emitted tangent to this ring.

- b. (5 points) What is the typical range of wavelengths for x-rays emitted from a synchrotron source?

Typically 0.5-2 Å

- c. (5 points) Suppose that the experiment in Problem 4, part 'b' is repeated, but this time the incident beam consists of neutrons instead of x-rays. What must the wavelength of the neutrons be in order to produce reflections at the same angle as those produced by the x-rays?

$$\lambda_{neutrons} = \lambda_{x-rays}$$

- d. (5 points) Given this, what must the neutron velocity be in order to produce reflections at the same angle? Remember that $\lambda = h/p$, where h is Planck's constant and p is the momentum.

$$\lambda_{neutrons} = \frac{h}{p} = \frac{h}{mv} \rightarrow v = \frac{h}{m\lambda} = \frac{6.626 \times 10^{-34} \text{ m}^2\text{kg/s}}{(1.675 \times 10^{-27} \text{ kg}) \cdot (1 \times 10^{-10} \text{ m})} = 3956 \text{ m/s}$$

6. **Lattice Madness** (25 points)

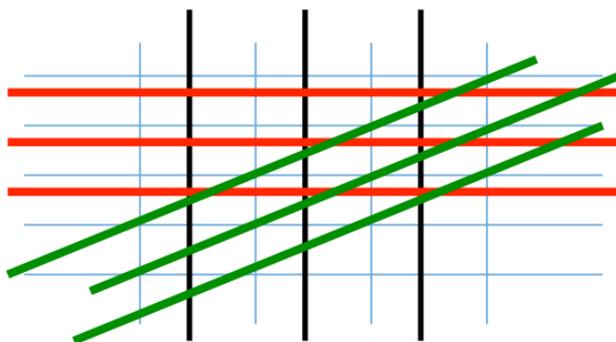
Consider a 2D rectangular lattice with unit cell dimensions 5x3 nm along the a- and b-axes, respectively.

- a. (5 points) Sketch an accurate picture of the lattice (to scale), giving at least nine lattice sites.

See below

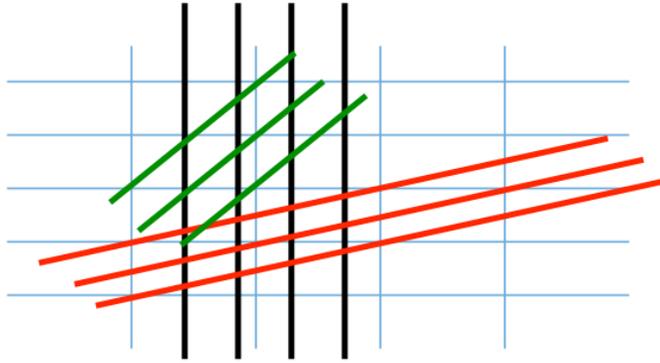
- b. (5 points) On the drawing from A, sketch and label three representative Miller indices (h,k) = (1,0). Repeat this for the (0,1) and (1,1) planes.

a,b:



Black – (1,0)
Red – (0,1)
Green – (1,1)

- c. (5 points) Make another drawing of the lattice. This time sketch and label the (2,0), (1,2), and (2,1) planes.



Black – (2,0)
 Red – (1,2)
 Green – (2,1)

- d. (5 points) What are the distances between the planes in the six cases above?

Using basic trigonometry and 5/3 ratio between the sides of the unit cell, we obtain the following distances:

(1,0): 5 nm
 (0,1): 3 nm
 (1,1): 2.572 nm
 (2,0): 2.5 nm
 (1,2): 1.437 nm
 (2,1): 1.921 nm

- e. (5 points) If we use 0.154 nm radiation, what will be the first order angle of the (1,0), (0,1), (1,1) and (1,2) reflections?

Using Bragg's law, $\theta = \sin^{-1} \left(\frac{\lambda}{2 d_{hkl}} \right)$, we can compute the following values:

(1,0): 0.882 deg
 (0,1): 1.47 deg
 (1,1): 1.71 deg
 (1,2): 3.07 deg

7. Protein crystallization (40 points)

- a. (10 points) Give two arguments for why crystallization might yield protein structures that are biologically relevant. Also give one argument for why crystallization might yield structures which do not reflect a protein's functional state.

Arguments *for* include:

Protein density in a crystal is approximately equivalent to the inside of a cell.

Water content in protein crystals is approximately equivalent to that of cells.

Protein crystals are catalytically active.

Arguments *against* include:

Solvents and buffers used to grow crystals might alter the protein fold.

Crystal packing might force proteins to adopt unnatural conformations.

Heavy atoms for phasing might affect the fold

- b. (5 points) For optimal diffraction, protein crystals must be well ordered; in other words, each unit cell should be identical in layout and orientation, and thermal motions should be kept to a minimum. What are two protein properties that make protein crystals less ordered than small molecule crystals?

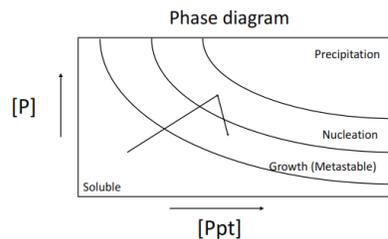
Many possible answers. For example,

High backbone flexibility

Proteins are usually solvated and will usually lose structure if solvent is removed.

Shells of solvent around proteins can lead to weaker interactions around molecules.

- c. (5 points) In the hanging drop or sitting drop methods for crystal formation, water evaporates from a protein solution until the equilibrium vapor pressure is reached. With the aid of a phase diagram, explain how this slow evaporation of water can promote crystal formation.



The evaporation of water slowly increases the concentration of protein and precipitant in the drop. Once the nucleation concentration is reached, the protein concentration can decrease as protein crystallizes out of solution. The hanging and sitting drop methods allow a crystallographer to gently change the protein conditions over time, and the equilibrium between concentration by evaporation and dilution by precipitation lets the protein solution find its own crystallization conditions.

- d. (10 points) You have unsuccessfully tried to crystallize a protein with the amino acid sequence below. Knowing that disorder can prevent crystallization, propose a N-terminally truncated version of your protein for use in additional crystallization trials. Use the secondary structure prediction server below to help design your construct:
<http://www.compbio.dundee.ac.uk/jpred/>

N terminus-

MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQW
FTEDPGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQKTYQGSY
GFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCVPQLWVDSTPPPGTRVRA
MAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNT

FRHSVVVPYEPPEVGS DCTTIHYNM CNSSCMGGMNRRPILTIITLEDSSGNLL
GRNSFEVRVCACPGRRRTEENLRKKGEPHELPPGSTKRALPNNTSSSPQP
KKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPGGSRAHSSHLKS
KKGQSTSRHKKLMFKTEGPDS D-Cterminus

JPred predicts that almost all of the first 108 residues lack any secondary structure. Any cut in this region will be accepted.

- e. (10 points) In late 2007 several X-ray structures of human β -adrenergic receptor were reported. All of these structures required some modification of the protein prior to crystallization. Describe the modifications made in the following paper, as well as their rationale.

Rosenbaum, et al. "GPCR Engineering Yields High-Resolution Structural Insights into β 2-Adrenergic Receptor Function" Science (2007) 318:1266-1273.

There were two changes made. First, residues were removed from the C-terminus. Second, T4 lysozyme, a small soluble protein, was introduced into ICL3, a flexible loop connecting transmembrane helices. This was done to remove disorder associated with ICL3 and reduce motion within the transmembrane domain, as well as provide additional polar surface area that can be used for lattice contacts.