BMB 173 – Winter 2017

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

TA: Emily Wyatt, ewyatt@caltech.edu
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

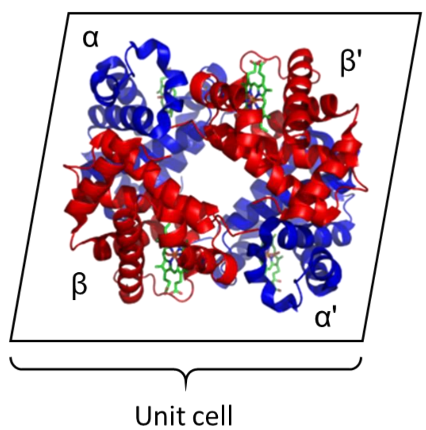
1. **X-Ray Crystallography Basics** (25 points)
   a. (10 points) When using X-rays, why are we limited to observing the diffraction pattern of an object? Recall that with EM we can observe either the magnified image or the diffraction pattern, depending on the plane to which the detector is conjugate.

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

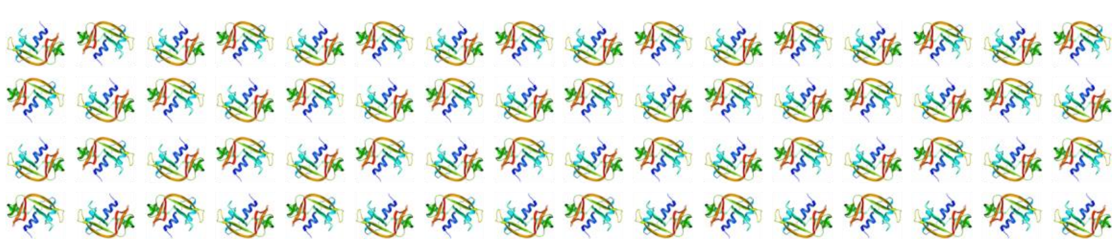
2. **Units and Symmetry in Crystals** (30 points)
   a. (5 points) What is the difference between an asymmetric unit and a unit cell?

   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.



   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.



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?

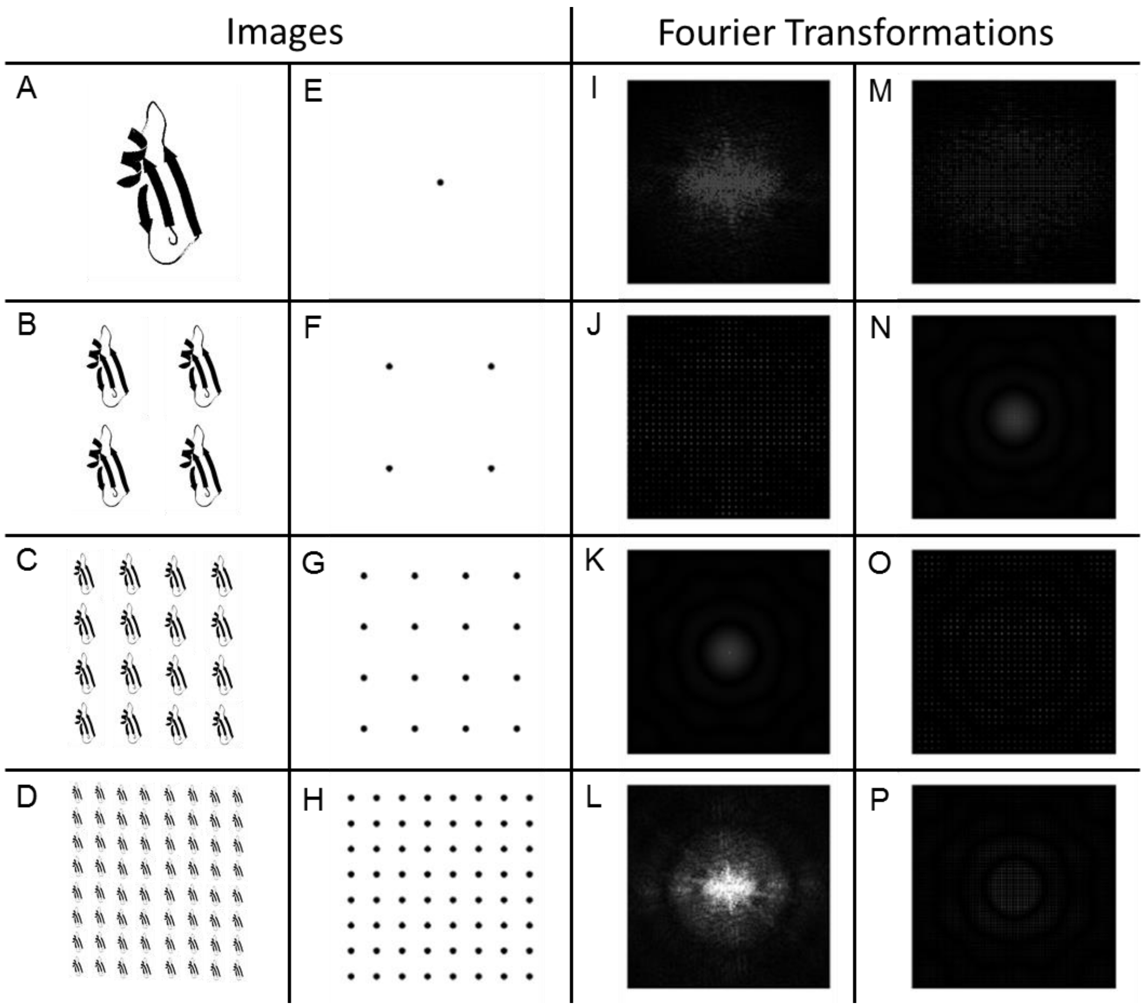
3. **Fourier Transformations** (35 points)

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

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?

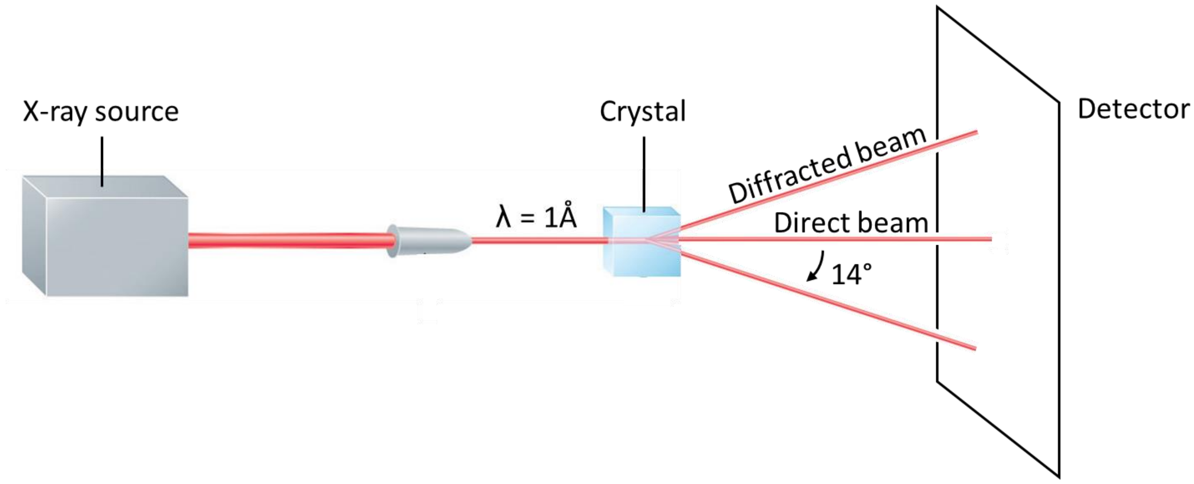
c. (20 points) Given this trend as well as other key features, match each image below with its Fourier transformation. Briefly explain how you made each match.

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.



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?

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

5. **Radiation Sources** (20 points)

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

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

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?

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.

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.

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) an (1,1) planes.

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

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

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?

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.

   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?

   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.

   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
      GFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRA
      MAIYKQSQHMTEVVRRCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNT
      FRHSVVVPYEPPEVGSDCTTIHYNYMCNSSCMGGMNRRPILTIITLEDSSGNLL
      GRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELPPGSTKRALPNNTSSSPQP
      KKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPGGSRAHSSHLKS
      KKGQSTSRHKKLMFKTEGPDSD-Cterminus

   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 β2-Adrenergic Receptor Function" Science (2007) 318:1266-1273.