BMB/Bi/Ch 173 – Winter 2018
Homework Set 5.1 – Assigned 2-7-18, due 2-13-18 by 10:30 a.m
TA – Taylor Stevens tstevens@caltech.edu
Office hours – SFL 220, Friday Feb 9 12:00pm - 1:30pm and Monday Feb 12 1:00pm - 3:00pm, or by appointment

1) Crystallization of biological macromolecules (40 points)

a) i) Describe the chemical interactions that occur at the surface of a protein in solution. How does addition of a precipitant affect these interactions and lead to crystallization? (10 points)

Polar residues hydrogen bond with solvent water molecules to neutralize charges and form a hydration shell around the protein. Addition of a precipitant, in addition increasing protein concentration due to evaporation, reduces the amount of free water molecules that are available to form this hydration shell. Intermolecular interactions between proteins form to compensate for the loss in water, ultimately forming crystal contacts which drive crystallization.

ii) Briefly describe two physical details of the crystallization process that involve changes in entropy (ΔS) and enthalpy (ΔH). Also describe the overall changes in free energy (ΔG) resulting from both details. (12 points)

When proteins in solution pack into a crystal lattice, they can no longer freely diffuse throughout a solution, and they also lose some conformational flexibility, which increases order in the system, thus reducing entropy and increasing free energy. The formation of crystallization interfaces involve salt-bridges, hydrogen bonds, van der waals interactions, and hydrophobic interactions, thus reducing enthalpy and reducing free energy.

iii) For crystals to spontaneously form, crystallization must result in a negative free energy change. Describe a protein modification that can increase the probability of crystallization and explain which parameter (entropy or enthalpy) it will affect. (10 points)

- Deletion of loop regions which would reduce the entropy cost of crystallization
- Modification of surface residues to increase the strength of crystal contacts
- Addition of highly crystallizable domains such as lysozyme (which will allow new crystal contacts and reduce enthalpy)

b) Give two reasons for why crystal-derived structures are likely to be biologically relevant, as well as two reasons for why crystal-derived structures may not be biologically relevant. (8 points)

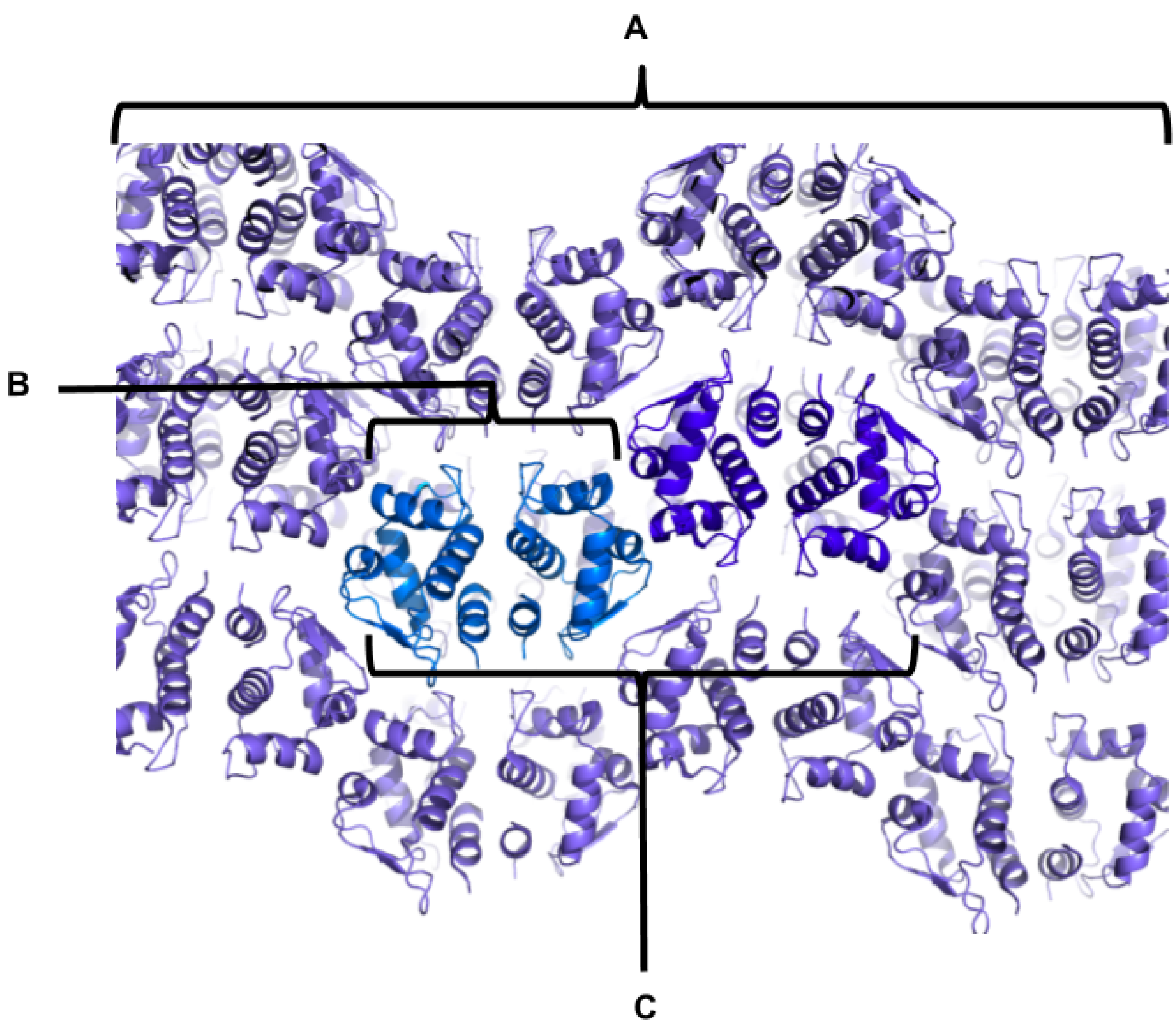
Arguments for include:
1. Structures obtained by X-ray crystal structures usually agree with those determined using other structural biology techniques (including NMR, which is performed in solution).

2. Enzymes in protein crystals are usually catalytically active.
3. Protein density in a crystal is approximately equivalent to the inside of a cell.
4. The process of crystallization is unlikely to cause significant changes to the folding of individual domains.

Arguments against include:
1. Solvents and buffers used to grow crystals might alter the protein fold.
2. Crystal packing might force proteins to adopt unnatural conformations.
3. Heavy atoms for phasing might affect the fold.

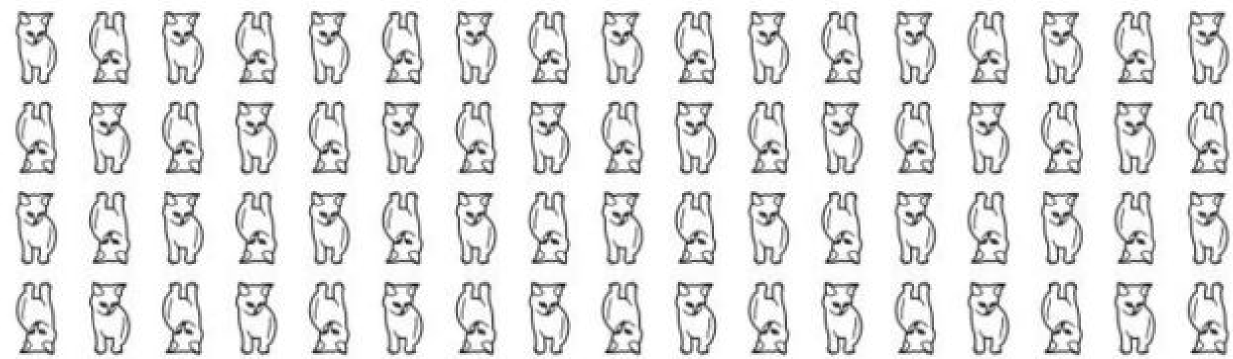
2) Lessons about lattices (40 points)

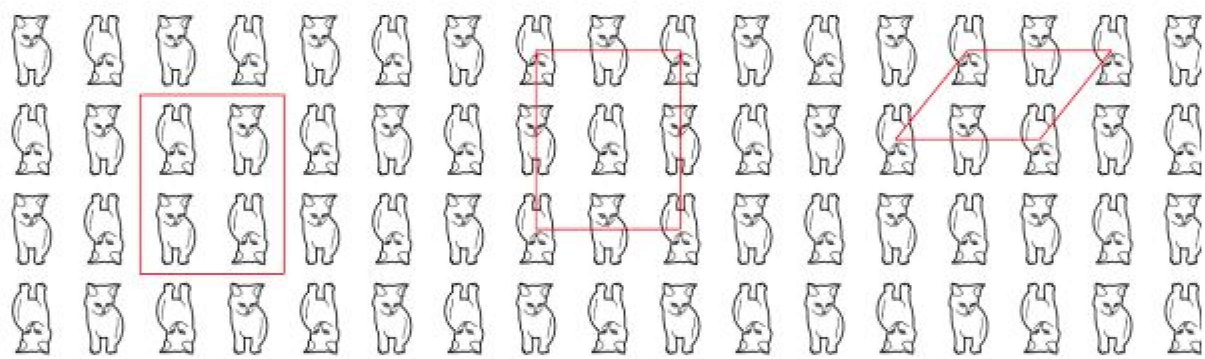
a) In the following figure, three features are highlighted. Determine which feature is the crystal lattice, the unit cell, and the asymmetric unit. How is the unit cell related to the crystal lattice? How is the asymmetric unit related to the unit cell? (15 points)



A represents a crystal lattice. B represents an asymmetric unit. C represents a unit cell. The asymmetric is the minimum object that can be used to generate the unit cell through symmetry operators. The unit cell is the minimum unit that can be repeated to generate the crystal lattice (through translation operations).

b) Outline three possible unit cells on the lattice shown below. (15 points)





What's important here is that any valid unit cell we draw can be repeated along either axis and you get the same exact contents in the same orientation.

c) What are space groups? How many space groups exist? Why are there not more? How many are available to biological macromolecules and why does this differ from the total number? (10 points)

Space groups specify a set of symmetry operations within a unit cell. Only, 230 exist, as certain symmetry operations cannot fill three-dimensional space. Due to the chirality of biological molecules, only 65 of these are available.

3) Properties of X-rays (40 points)

a) Why is X-ray radiation specifically used for structure determination as opposed to radiation with larger wavelengths (UV) or smaller wavelengths (gamma)? (12 points)

Diffraction data is limited to 1/2 of the wavelength of the photon source used. X-ray wavelengths go from lower angstrom to sub-angstrom values, while chemical bonds are normally 1-2 Å, therefore X-ray diffraction is able to resolve individual atoms. Lower wavelengths such as UV radiation cannot resolve individual atoms. The problem with gamma radiation is that the amount of energy in each photon is so high that it will quickly destroy biological samples.

b) You, as a potential Caltech crystallographer, have an advantage over many of your peers in the field in that you have convenient access to SSRL. List one advantage of using X-rays at this facility rather than from a home source? (10 points)

- Higher beam flux allows for faster data collection
- The collimator enables data collection with a very fine beam, allowing researchers to use smaller crystals, to reduce background scattering, and to avoid collecting data from local defects within a crystal
- Wide range of wavelengths allows for phasing with a variety of compounds

c) Why do proteins need to form crystals in order to produce diffraction data? (12 points)

- X-rays interact with matter weakly, therefore they must pass through a large cross-section of material to scatter
- Crystals can withstand a significant amount of radiation damage
- Repeating physical structure needed to generate a diffraction pattern

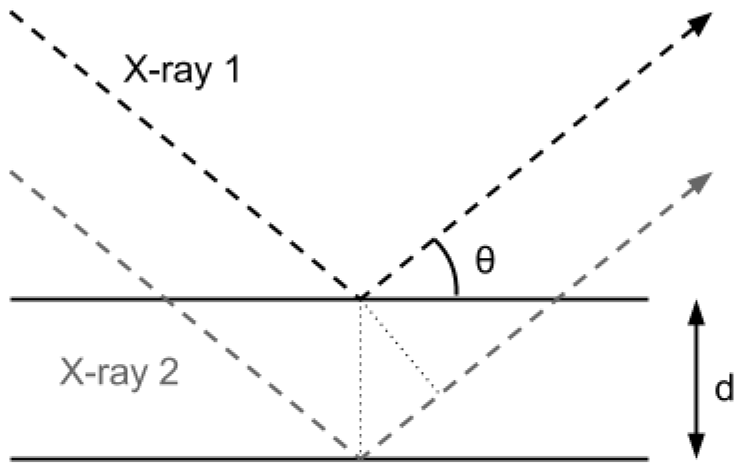
d) Why can we not do microscopy with X-rays. (6 points)

There are no x-ray lenses

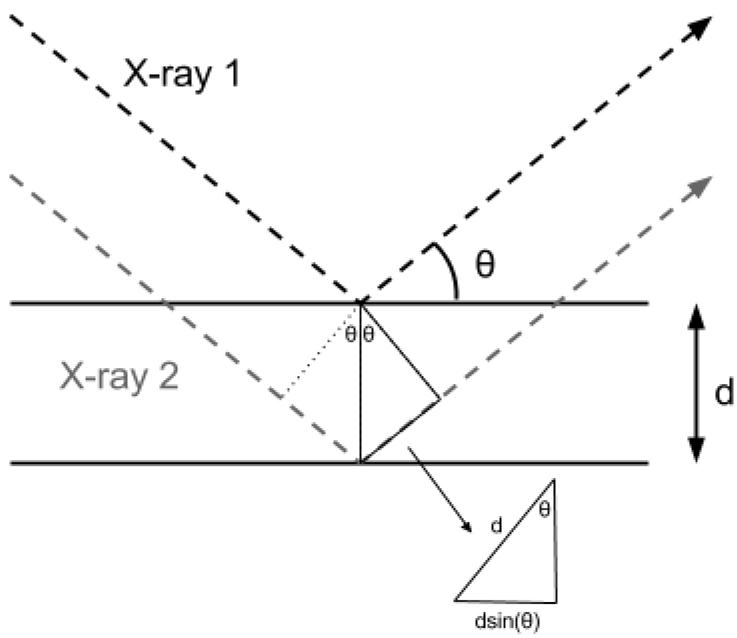
4) Diffraction theory (80 points)

a) Using the diagram below as a starting point, use simple geometry to derive Bragg's Law: $n\lambda = 2d\sin(\theta)$.

Hint: Use the right triangle that is drawn for you with a dashed line. (20 points).



To produce diffraction, two X-ray photons must constructively interfere at the detector (they must be in-phase). To be in-phase, X-ray 2 must travel an extra distance equal to some multiple of its wavelength. Using this condition that the extra distance X is equal to nλ, we just need to use the diagram to calculate X in terms of θ:

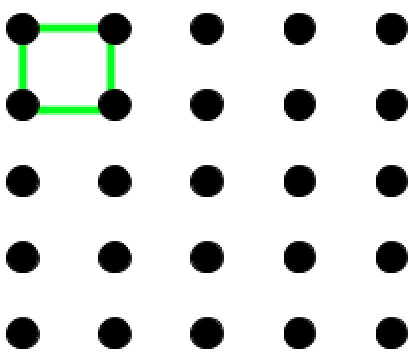


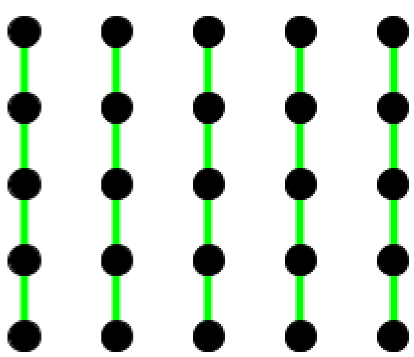
The above diagram shows that X/2 (half of the extra distance) is equal to the length of the triangle face opposite to the diffraction angle. Applying rules of trigonometry, we compute that $X/2 = d*\sin(\theta)$, therefore $X = n\lambda = 2d*\sin(\theta)$.

b) In Bragg's law, what set of numbers is n restricted to? In terms of the physical properties of the x-ray, what is n? (10 points)

Mathematically, n must be an integer to satisfy Bragg's law (again because otherwise the waves are out of phase). Physically, n is the number of x-ray wavelengths that it takes to traverse the extra distance between the planes.

c) Below is an example 2D lattice with the unit cell drawn in green. Assume that each point in the lattice is separated by 200 Å. We can draw a set of imaginary lines through this lattice by connecting each lattice point with a point that is k unit cells above and h unit cells across (these are referred to as bragg planes in 3D). Draw the bragg planes corresponding to (h,k) = (1,0), (1,1), and (1,2). For each of these cases, calculate the spacing between the bragg planes (d in bragg's law). Once you have calculated d for each set of lines, calculate the 3 smallest diffraction angles (in degrees) you will observe when using x-rays with a 1 Å wavelength. If the distance between each lattice point increases, how will this affect the diffraction angles (do not need to calculate)? (24 points)

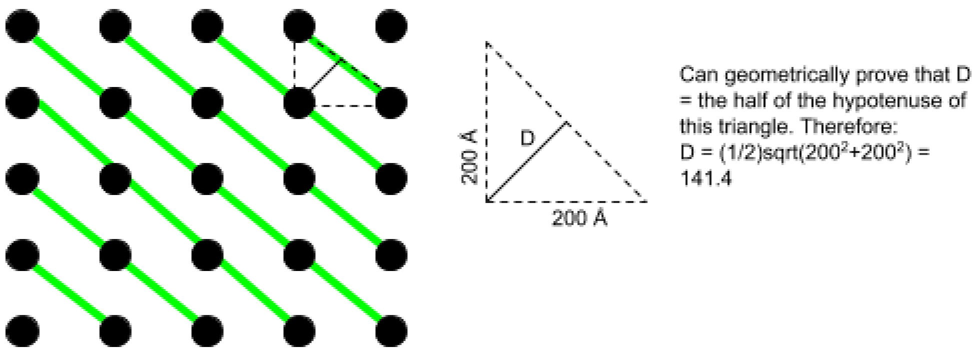




For (h,k) = (0,1) or (1,0), the spacing between each plane will be 200 Å, therefore the three lowest diffraction angles will be:

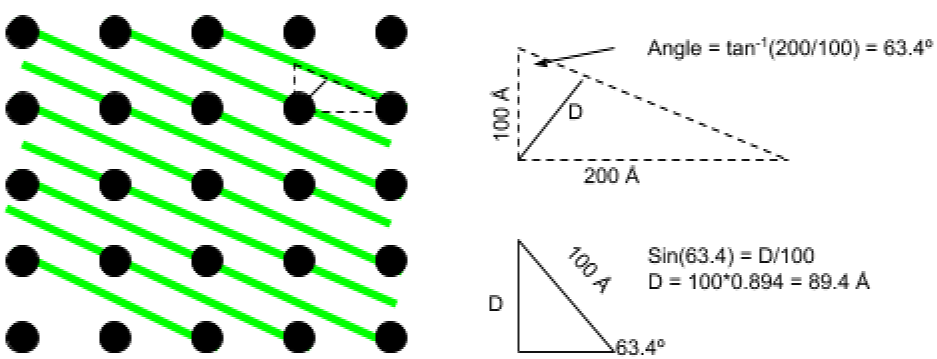
- $\sin^{-1}(1/400)$ = 0.14 degrees

- $\sin^{-1}(2/400)$ = 0.28 degrees
- $\sin^{-1}(3/400)$ = 0.42 degrees



For (h,k) = (1,1) the spacing between each plane will be 141.4 Å, therefore the three lowest diffraction angles will be:

- $\sin^{-1}(1/282.8)$ = 0.2 degrees
- $\sin^{-1}(2/282.8)$ = 0.4 degrees
- $\sin^{-1}(3/282.8)$ = 0.6 degrees



For (h,k) = (1,1) the spacing between each plane will be 89.4 Å, therefore the three lowest diffraction angles will be:

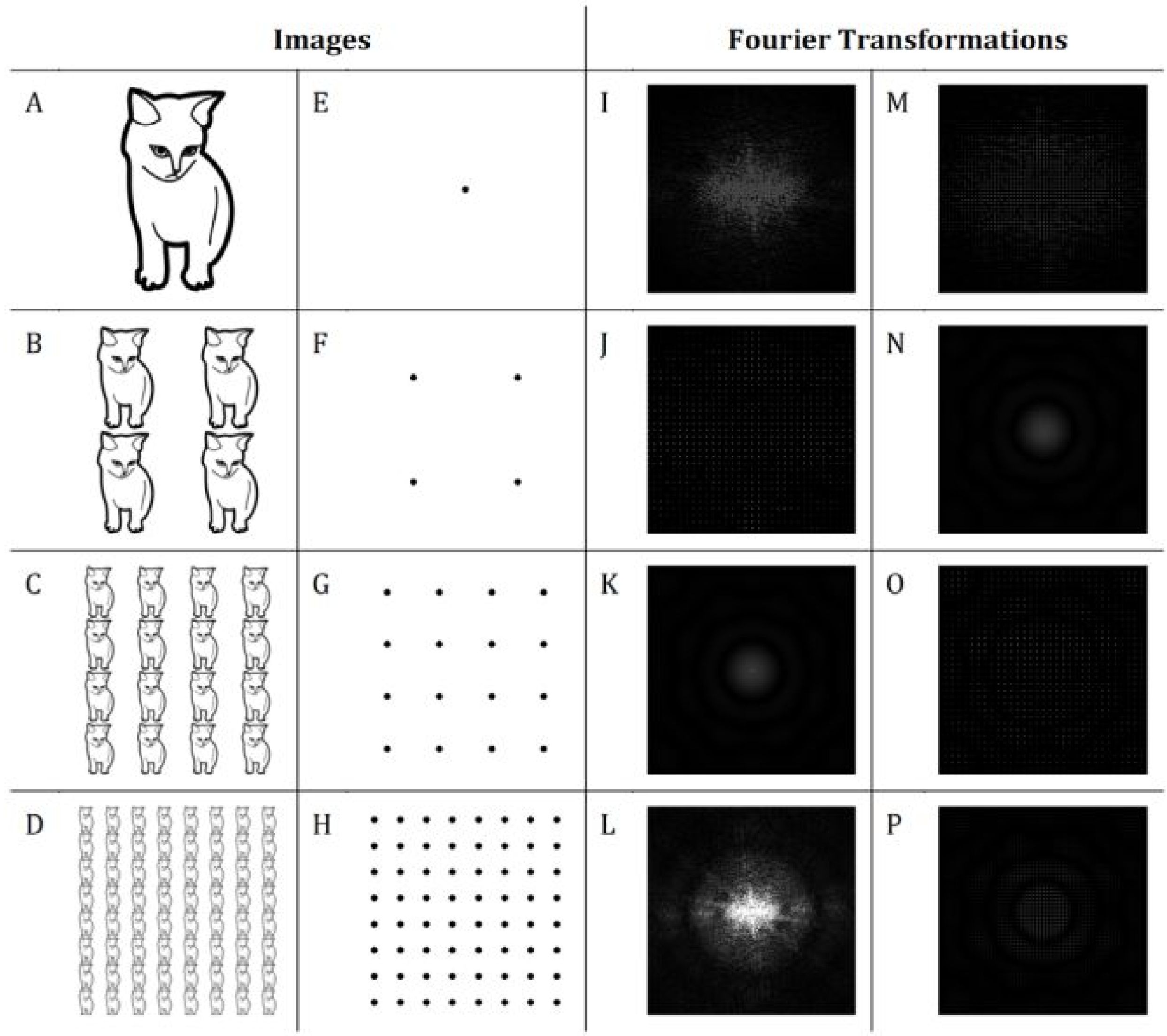
- $\sin^{-1}(1/178.8)$ = 0.32 degrees
- $\sin^{-1}(2/178.8)$ = 0.64 degrees
- $\sin^{-1}(3/178.8)$ = 0.96 degrees

If the distance between the lattice points increases, then the angles will decrease.

d) If two different proteins happen to crystallize in identical unit cells (same dimensions and space group), what would be different about the resulting diffraction data? (6 points)

Since the unit cell is the same the location of the diffraction spots on the detector will all be the same. Since the contents of the unit cell will be different, the electron density inside of the unit cell will be different, therefore the intensity of each diffraction spot should be different.

e) Match each image with its Fourier transformation. Briefly explain how you made each match, and discuss any trends that you see. (Note: These images may not print clearly and are best viewed on a computer screen. You may need to zoom in to distinguish important features) (20 points)



To make each assignment, recognize that the Fourier transform of a circle looks like a circle. The Fourier transformation of the cat must be the other pattern. Closer lattice spacing produce more dispersed patterns in reciprocal space (Fourier transformations). 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