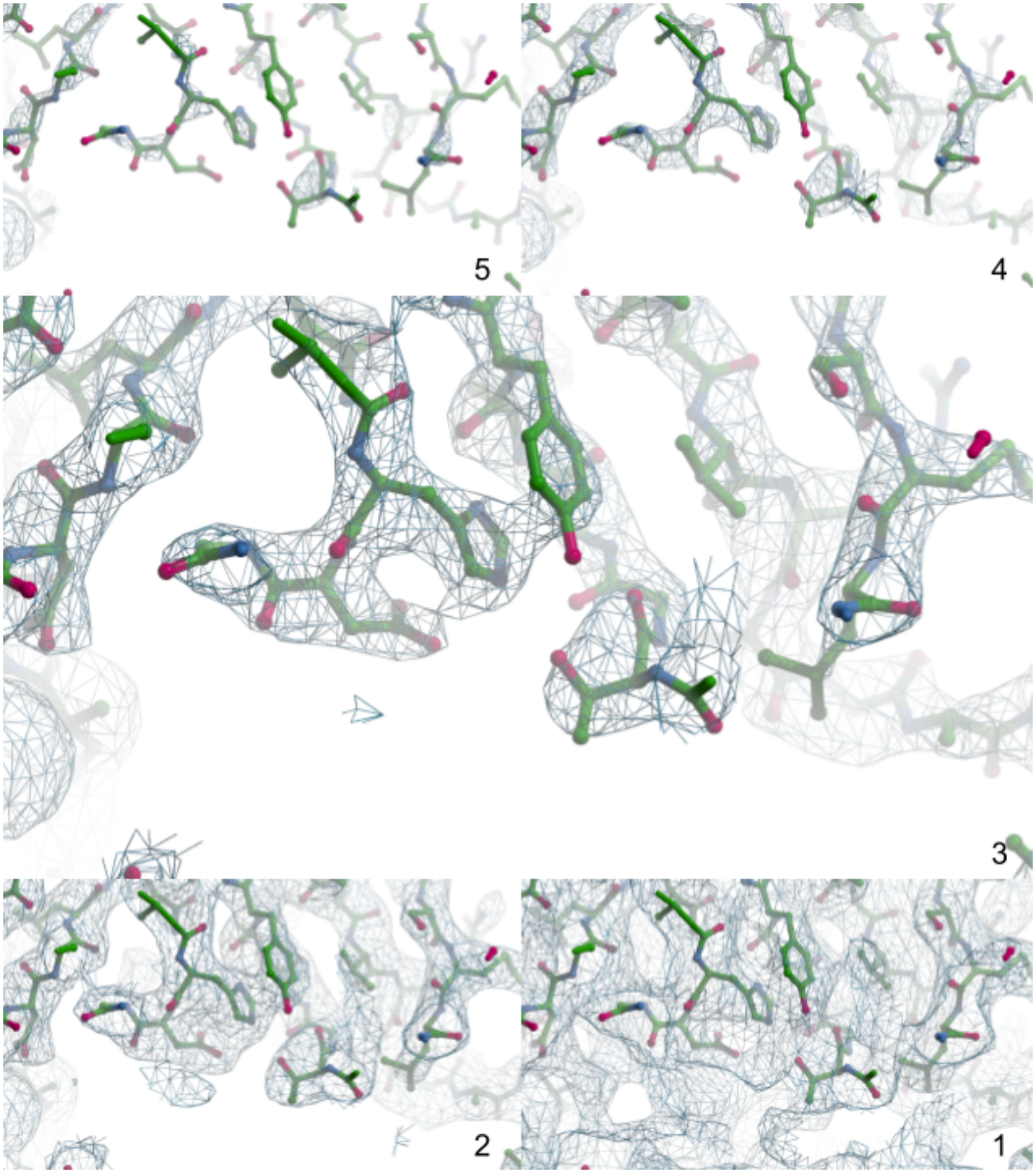
BMB/Bi/Ch 173 – Winter 2018
Homework Set 6.1 – Assigned 2-13-18, due 2-20-18 by 10:30 a.m
TA – Taylor Stevens tstevens@caltech.edu
Office hours – Friday Feb 16 3:00pm - 5:00pm (in SFL 220) and Monday Feb 19 4:00pm - 5:30pm (in SFL 229), or by appointment

1) From diffraction to electron density. (27 points)

a) A diffraction pattern gives us two important types of information needed to compute the electron density map of the crystal unit cell. Describe both types of information. What information is needed that is missing from the diffraction pattern? (6 points)

b) What is the quantity we measure from a single diffraction spot? Describe how it is measured. How does this quantity relate to **F(hkl)** (the structure factor)? (9 points)

c) Electron density maps are typically displayed with a surface or mesh representation that forms a shell around the structure. A cutoff value must be specified to generate this representation. To illustrate the effect of the cutoff value, figures of a structure and electron density map with decreasing cutoffs are shown below: (5 is the highest cutoff value, and 1 is the lowest)



What is the benefit of displaying electron density in this way? What would make a particular cutoff value appropriate for displaying electron density? Explain why it's important to avoid using an inappropriate cutoff value. (12 points)

2) Heavy metals: it's just a phase. (73 points)

a) MAD and MIR are two different experimental phasing methods, and the M stands for multi- in each case. What are there multiples of in MAD and in MIR? (4 points)

b) List two advantages of MAD phasing methods over MIR phasing methods. List one advantage of MIR phasing methods over MAD phasing methods: (6 points)

c) Caveats: (12 points)
i) What is the major caveat of isomorphous replacement related phasing methods?

ii) What is one potential caveat and one limitation of molecular replacement?

iii) It is common to use protein that has incorporated multiple selenomethionines in place of methionine residues for phasing via anomalous diffraction. What feature must a protein have in order be labelled with selenomethionine? If a protein does not naturally contain this feature, we must engineer it into the protein somehow. Explain how this could be a problem.

d) To demonstrate the process of determining phases via MIR we will go over a simplified example in 2D. Let's assume we have already used patterson methods to find the location of three mercury atoms in a unit cell (in fractional coordinates*): (0,0), (0.3,0.7), (0.4, 0.1). Calculate the amplitude and phase (in radians) of F_{Hg}(5 6), the structure factor of the mercury atoms alone. For this reflection, use a value of 70 for the atomic scattering magnitude of mercury (f_j). You can use the equation below to calculate scattering from j number of atoms:

$$F(hk) = \sum_j f_j e^{2\pi i(hx_j + ky_j)}$$

where f_j is the atomic scattering magnitude (70), *h* and *k* are the Miller indices (5,6) and *x* and *y* are the positions of each atom. To express F_{Hg}(5 6) as a single complex number, use euler's formula:

$$e^{ix} = cos(x) + isin(x)$$

To solve for the amplitude and phase, plot F_{Hg}(5 6) on the complex plane. The phase of *F*(*hk*) is the angle between the x-axis and the vector, and the amplitude of *F*(*hk*) is the magnitude of the vector. (24 points)

*Fractional coordinates describe the positioning of atoms in terms of the unit cell dimensions, *e.g.* an atom that is directly in the center of the unit cell will have the fractional coordinates (0.5, 0.5, 0.5)

e) The amplitude of the native structure factor at (*h*,*k*) = (5,6) ($|F_P(5,6)|$) is 66.57, and the amplitude of the mercury derivative structure factor ($|F_{PHg}(5,6)|$) is = 60. Draw a Harker construct to demonstrate the two possible phase angles for the $F_P(5,6)$. Calculate the two possible angles using the following equation, where α is the phase angle in radians:

$$\alpha = \alpha_{Hg} \pm cos^{-1}\left[(|F_{PHg}|^2 - |F_P|^2 - |F_{Hg}|^2)/(2|F_P||F_{Hg}|)\right]$$

Your Harker construct should illustrate F_P, the structure factor of the native protein, F_{Hg}, the structure factor of the mercury atoms, and F_{PHg}, the structure factor of the mercury derivative protein. (24 points)

f) What additional experiment can you do to determine which of these two phases is correct? (3 points)

g) (5 points extra credit) Explain how the above process can be applied to the whole diffraction dataset to generate an initial electron density map.