BMB170b Homework #4, Part 1, Due 2/8/11

In this problem set, you will use isomorphous replacement to determine possible phases of a single X-ray reflection from a protein crystal. For simplicity, you will only consider a two-dimensional lattice and the unit cell will not have any symmetry operators.

# Patterson Maps

You have collected the structure factor amplitudes of a two-dimensional protein crystal. You have also collected the structure factor amplitudes of a derivative crystal containing three mercury atoms bound to the protein. The Patterson map shown below is a difference map between the structure factor amplitudes for the mercury derivative, |**F**PHg(*hk*)|, and the native protein crystal, |**F**­P(*hk*)|, for the reflection at *h* = 5, *k* = 6 in the crystal diffraction pattern. In other words, it is a map of |**F**PHg(5 6)| − |**F**P(5 6)|. Assume that the mercury atoms do not perturb the protein structure, so that the peaks correspond only to mercury inter-atomic vectors.



The coordinate of the six peaks on the map are:

* *u* = 0.3, *v* = 0.7
* *u =* 0.9, *v* = 0.6
* *u* = 0.7, *v* = 0.3
* *u* = 0.4, *v* = 0.1
* *u* = 0.1, *v* = 0.4
* *u* = 0.6, *v* = 0.9

1. (20 points) Following the steps below, use this Patterson map to calculate the real space positions of all three mercury atoms in terms of *x* and *y*, the fractional unit cell coordinates.

**Things to know about Patterson maps:**

1. If an atom a1 is present at the position (*x*, *y*) in real space, and a second atom a2 is present at position (*x* + *u*, *y* + *v*), then in the Patterson function there will be a peak at (*u*, *v*), representing the vector a2 – a1, and a second peak at (–*u*, −*v*), representing the vector a1 – a2.
2. Everything is considered in fractional coordinates. For example, a value of −0.3 is equal to 0.7 in an adjacent unit cell.

**How to solve the Patterson map:**

1. There are no symmetry operators in this problem, so there are no peaks between symmetry related atoms. The choice of origin is arbitrary, which means the position of the first atom, a1, can be set to *x* = 0, *y* = 0. Note that the choice of atoms 1, 2, and 3 is also arbitrary. The work you show will depend on your starting peak, but you will get the same three atomic positions regardless of your choice.
2. Designate a peak on the Patterson map to be the vector between the first and second atom (a2 – a1). If a1 is at *x* = 0, *y* = 0, then a2 is at *x* = *u*, *y* = *v*. Then, from the remaining peaks, determine which one represents the vector from the second atom back to the first (a1 – a2).
3. There are four peaks remaining: two relating the first atom to the third atom (a3 – a1 and a1 – a3) and two relating the second atom to the third atom (a3 – a2 and a2 – a3). Guess and check to assign the remaining peaks. Guess which peak corresponds to the first and third atom vector (a3 – a1), and then predict the coordinates for the third to first atom vector (a1 – a3). If no peak is present at the predicted location, guess again. Repeat this process for the second to third (a3 – a2) and third to second (a2 – a3) vectors.
4. The model is complete when all Patterson peaks are accounted for. Please show your assignments for what each peak corresponds to (a3 – a1, a2 – a3, etc).

2. (10 points) Calculate the amplitude and phase of **F**Hg(5 6), the scattering from the heavy atom substructure alone. For this reflection, suppose the atomic scattering magnitude of mercury is 70. You can use the equation below to calculate scattering from *j* number of atoms:

$$F\left(hk\right)=\sum\_{}^{}f\_{j}e^{2πi\left(hxj+kyj\right)}$$

 To add up the exponential terms, you can use the following relationship (**in radians!**):

$$re^{iθ}=r\left(\cos(θ+i\sin(θ))\right)$$

 The first equation gives a vector in the form of *x* + *iy*. To solve for the amplitude and phase, plot this vector on a graph where the *x*-axis represents the real component and the *y*-axis represents the imaginary component. The phase of **F**(*hk*) is the angle between the *x*-axis and the vector, and the amplitude of **F**(*hk*) is the length of the vector.

3. (12 points) The amplitude of the native structure factor at *h* = 5, *k* = 6 is given by
|**F**P(5 6)| = 66.57. The mercury derivative amplitude is |**F**PHg(5 6)| = 60. Draw a Harker construct to demonstrate the two possible phase angles for the *h* = 5, *k* = 6 reflection. Calculate the two possible angles using the following equation, where α is the phase angle in radians:

$$α=α\_{Hg}\pm cos^{-1}\left[\left(F\_{PHg}^{2}-F\_{P}^{2}-F\_{Hg}^{2}\right)/\left(2\left|F\_{P}\right|\left|F\_{Hg}\right|\right)\right]$$

 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.

4. (4 points) In Problem 3, you solved for two possible phase angles, represented in your Harker diagram as the intersection of two circles. What additional experiment can you do to determine which of these two phases is correct?

5. (4 points) While you are solving the structure of your protein, the structure of a homolog is published. The homolog is a tetramer with each domain showing 90% similarity to your protein’s monomeric structure. What phasing technique could be performed with this information to solve the structure of your protein?