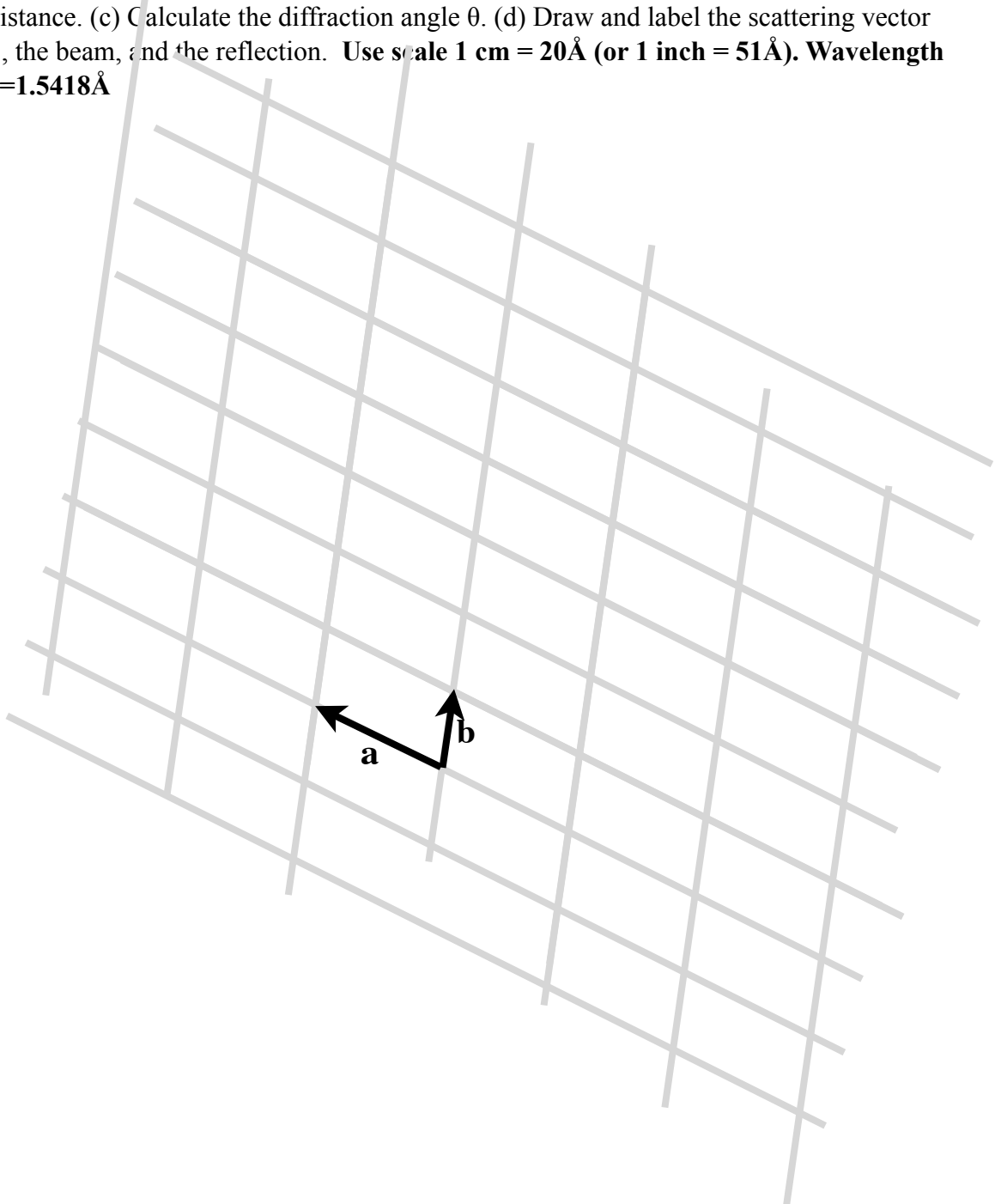


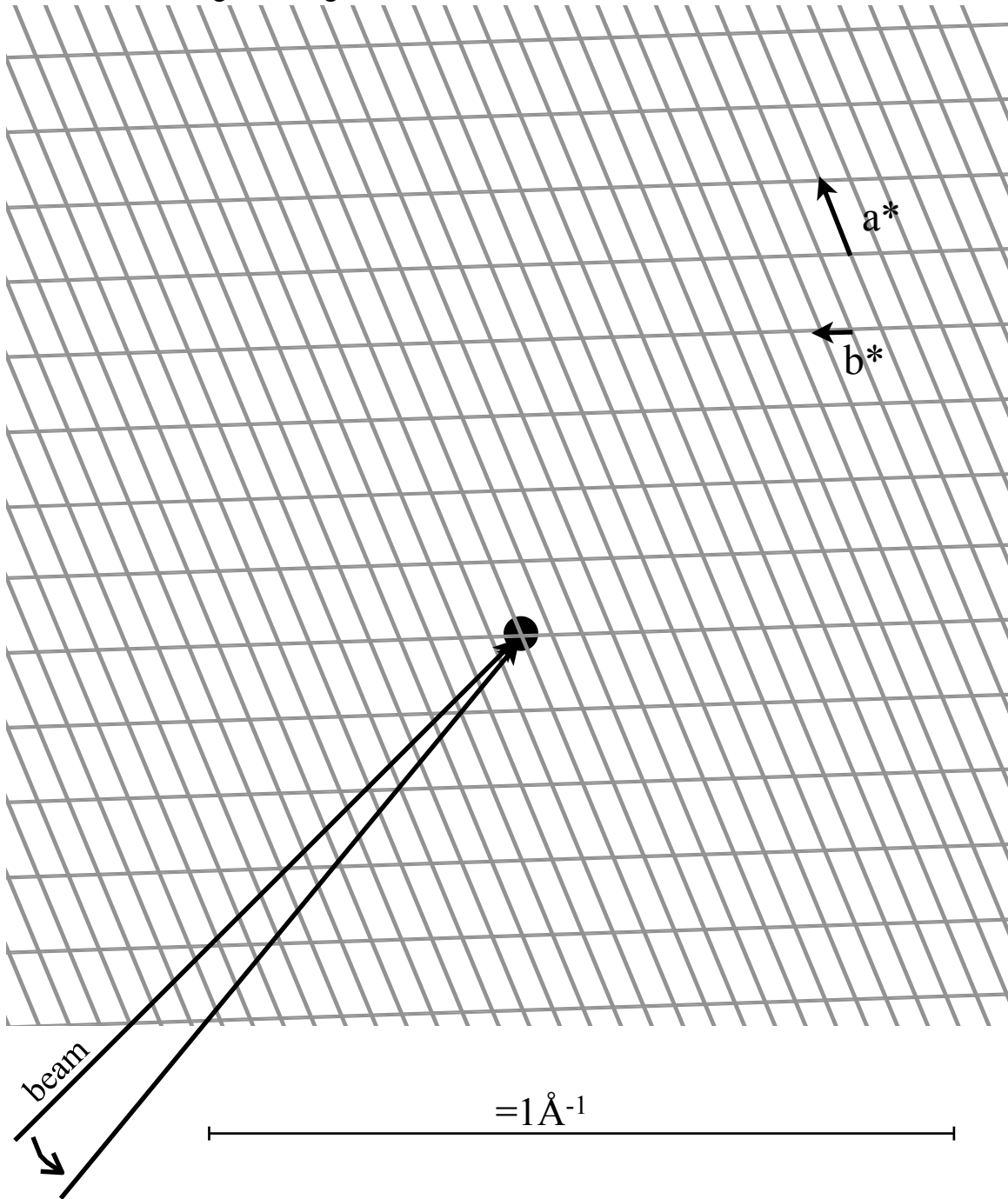
Past and possible exam questions: Xray Crystallography

The following exam questions have appeared in PSD exams (Xray part) over the past few years. Some may appear in this years exam, possibly in a modified form.

(1) Bragg planes. [15 pts] (a) **Accurately draw ten consecutive Bragg planes, $hkl=(1\ -3\ 0)$, on the real space lattice below.** (b) Measure the Bragg distance. (c) Calculate the diffraction angle θ . (d) Draw and label the scattering vector S , the beam, and the reflection. **Use scale 1 cm = 20Å (or 1 inch = 51Å). Wavelength $\lambda=1.5418\text{\AA}$**



(2) **Ewald sphere.** [15 pts] Accurately draw the Ewald sphere (in this case the Ewald "circle") on the reciprocal lattice below, **before and after rotating the beam** as shown. Use wavelength 1.5418\AA and the scale below to calculate the radius of the Ewald sphere. The black spot is the beam-stop (0 0 0). a^* and b^* vectors are shown. Label (using Miller indices $h k 0$) at least three reflections that will show up on the film as the beam rotates through the range.



(4) Patterson maps. [15 pts]

A heavy atom $F_{ph} - F_p$ Patterson map was calculated for a crystal with space group $P6_3$. Cell dimensions: $a = b \neq c$. $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$

Symmetry operators for $P6_3$ (equivalent positions):

- (1) x, y, z
- (2) $-y, x - y, z$
- (3) $-x + y, -x, z$
- (4) $-x, -y, z + 1/2$
- (5) $y, -x + y, z + 1/2$
- (6) $x - y, x, z + 1/2$

(a) Is this a polar space group? Circle one: **yes** **no**

(b) Where is/are the Harker section(s) for sym ops 2, 3, 4, 5 and 6 ?

Symop 2 _____ Symop 3 _____

Symop 4 _____ Symop 5 _____

Symop 6 _____

(c) There is a Patterson peak at (0.12, 0.26, 0.50). What sym op could have generated this peak? Circle all that apply **1 2 3 4 5 6**

(d) Using one of the sym ops from (c), determine the real space coordinates of one heavy atom. (show your work)

(e) Given the coordinates from (d), write the new coordinates after applying sym op 5.

(5) Multiple choice

Circle the **best answer**.

(a) Isomorphous crystals...

1. have cell dimensions that differ significantly
2. cannot be of the same space group
3. cannot be used as heavy atom derivatives
4. have proportionally equal sides
5. have identical cell dimensions

(b) The Matthews number is...

1. related to the figure of merit
2. related to R-factor
3. related to the crystal growth conditions
4. related to the solvent content
5. related to the phase
6. related to Bob Matthews

(c) Crystal planes are...

1. planes parallel to crystal faces
2. the only relevant Bragg planes
3. equally spaced planes passing through all unit cell origins
4. all of the above
5. none of the above

(d) Bragg planes are...

1. positions in space that scatter in phase
2. only relevant if they are also crystal planes
3. reflection planes
4. perpendicular to the scattering vector
5. all of the above
6. none of the above

(e) The Ewald sphere is...

1. the set of all possible wave vectors when the phase is unknown
2. the resolution limit in reciprocal space
3. the root-mean-square deviation of atomic positions
4. the part of reciprocal space you can see on the film
5. all of the above
6. none of the above

(f) A Scattering Factor is...

1. a reflection
2. a spot on the film
3. a wave
4. the integral of all scattered waves at one position in reciprocal space
5. all of the above
6. none of the above

(g) A Structure factor is...

1. a reflection
2. a spot on the film
3. a wave
4. the integral of all scattered waves at one position in reciprocal space
5. all of the above
6. none of the above

(h) When the Temperature factor...

1. is greater than 10, then the electron density is diffuse and weak
2. is less than 10, then the electron density is diffuse and weak
3. is greater than 60, then the electron density is diffuse and weak
4. is low, it implies poor crystal quality
5. is omitted in the Fourier transform, then the atoms are like points

(i) The free R-factor...

1. is a factor in the Fourier transform
2. does not require the data sets to be isomorphous
3. is scale-free
4. correlates with the phase error
5. results from over-fitting

(j) Molecular replacement...

1. is composed of a rotation function and a translation function
2. does not require a previously solved structure
3. requires heavy atom derivatives
4. requires at least two crystals
5. removes phase bias

(k) A Harker diagram is...

1. used to index reflections on film.
2. used to solve for the locations of heavy atoms.

3. used to solve for the phases.
4. used to find the Bragg planes.
5. None of the above.

(l) A Harker section is ...

1. a symmetry operator
2. a Patterson map
3. a plane in reciprocal space.
4. the solution to the phase problem.
5. None of the above.

(m) Centric reflections are...

1. found in centric space groups.
2. found in non-centric space groups.
3. found on 0-planes of reciprocal space.
4. reflections that have phase 0 or 180° .
5. identical in phase to their Friedel mates.
6. All of the above.
7. None of the above.

(n) Mosaicity is...

1. a property of atoms in a crystal.
2. a property of the crystal lattice.
3. a property of the Xray data.
4. a property of the model.
5. All of the above.
6. None of the above.

(o) The residual between scaled F_{obs} and F_{calc} is...

1. the B-value.
2. the amplitude error, Sigma.
3. used to calculate a difference map.
4. used to calculate the R-value.
5. the completeness.
6. None of the above.

(p) Ammonium sulfate, polyethylene glycol, and methyl pentane diol are examples of ...

1. small molecular crystals.
2. heavy atom derivatives.
3. solvents.
4. precipitants.
5. cryoprotectants.
6. None of the above.

(q) The distance between Bragg planes is...

1. always measured along the cell axes.
2. never an integer multiple of the wavelength.
3. always a multiple of the unit cell vectors.
4. always measured along the scattering vector S .
5. equal to the resolution squared.
6. All of the above.

(r) The highest resolution reflection data...

1. has the highest Miller indices
2. has the shortest scattering vector.
3. corresponds to the highest Bragg distance d .
4. corresponds to the smallest scattering angle θ .
5. All of the above.
6. None of the above.

(s) Waves may be represented as complex numbers according to...

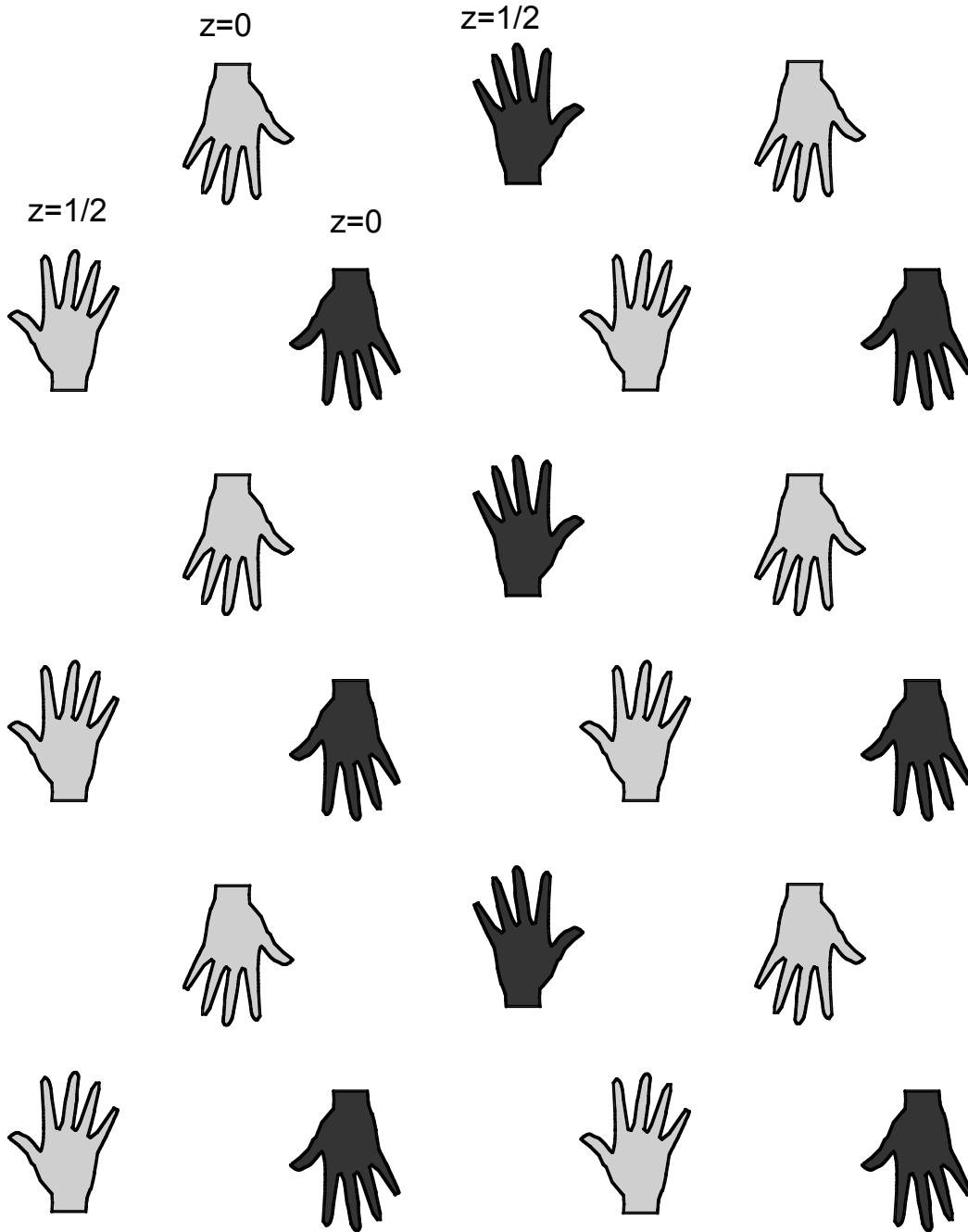
1. Euler's theorem
2. Bragg's Law
3. Friedel's law
4. the Fourier series
5. group theory

(6) Short essays [20 pts]
Answer as concisely as possible.

(a) Why do crystals diffract X-rays?

(b) What is the "phase problem" and how do we solve it?

(7) **Symmetry.** Draw **symmetry operations**, **unit cell boundaries**, and the **asymmetric unit**. All hands are *left hands*. Light grey hands are *palm up*, dark grey are *palm down*. X and Y axes are horizontal and vertical respectively. As noted below, the first column of hands is isomorphous at $z=1/2$, second and third columns are at $z=0$, 4th and 5th at $z=1/2$, 6th and 7th at $z=0$, and so on.



(8) [16 pts] Solve for the phase ϕ of F_p using a Harker diagram, for one reflection ($h\ k\ l$ not specified, since you don't need it). Show your work.

F_p is the amplitude of the native protein crystal. F_{ph1} is the amplitude of the structure factor for first heavy atom derivative. F_{ph2} is the amplitude of the structure factor for the second heavy atom derivative. F_{h1} is the structure factor for the first heavy atom. F_{h2} is the structure factor for the second heavy atom. Structure factors are given as amplitude and phase. Amplitudes are in arbitrary units. (*Use the back if you need more space.*)

$$F_p = 3.88$$

$$F_{ph1} = 3.11$$

$$F_{ph2} = 2.51$$

$$F_{h1} = (0.73, 37.2^\circ)$$

$$F_{h2} = (4.06, -1.1^\circ)$$

phase of F_p _____

(9) Solve a Patterson [16 pts]

A heavy atom Patterson map was calculated for a crystal with space group $P2_12_12_1$ (orthorhombic, 2-fold screw symmetry). Symmetry operators (equivalent positions):

(x,y,z) , $(x+1/2,1/2-y,-z)$, $(1/2-x,-y,1/2+z)$, $(-x,1/2+y,1/2-z)$

Symmetry-generated heavy-atom peaks were seen on two Harker sections at:

$(0.44, 0.12, 1/2)$, and **$(1/2, -0.38, 0.22)$** .

(a) Which symmetry operators generated the Patterson peak at

$(0.44, 0.12, 1/2)$? sym ops : _____ - _____

$(1/2, -0.38, 0.22)$? sym ops : _____ - _____

(b) **Solve (algebraically) for the real-space coordinates of the heavy atom.** NOTE: to get all three coordinates (x,y,z) you need to use *both* Patterson peaks .

(c) Apply space group symmetry to give **4 heavy atom equivalent positions.**

Heavy atom positions:

1. _____

2. _____

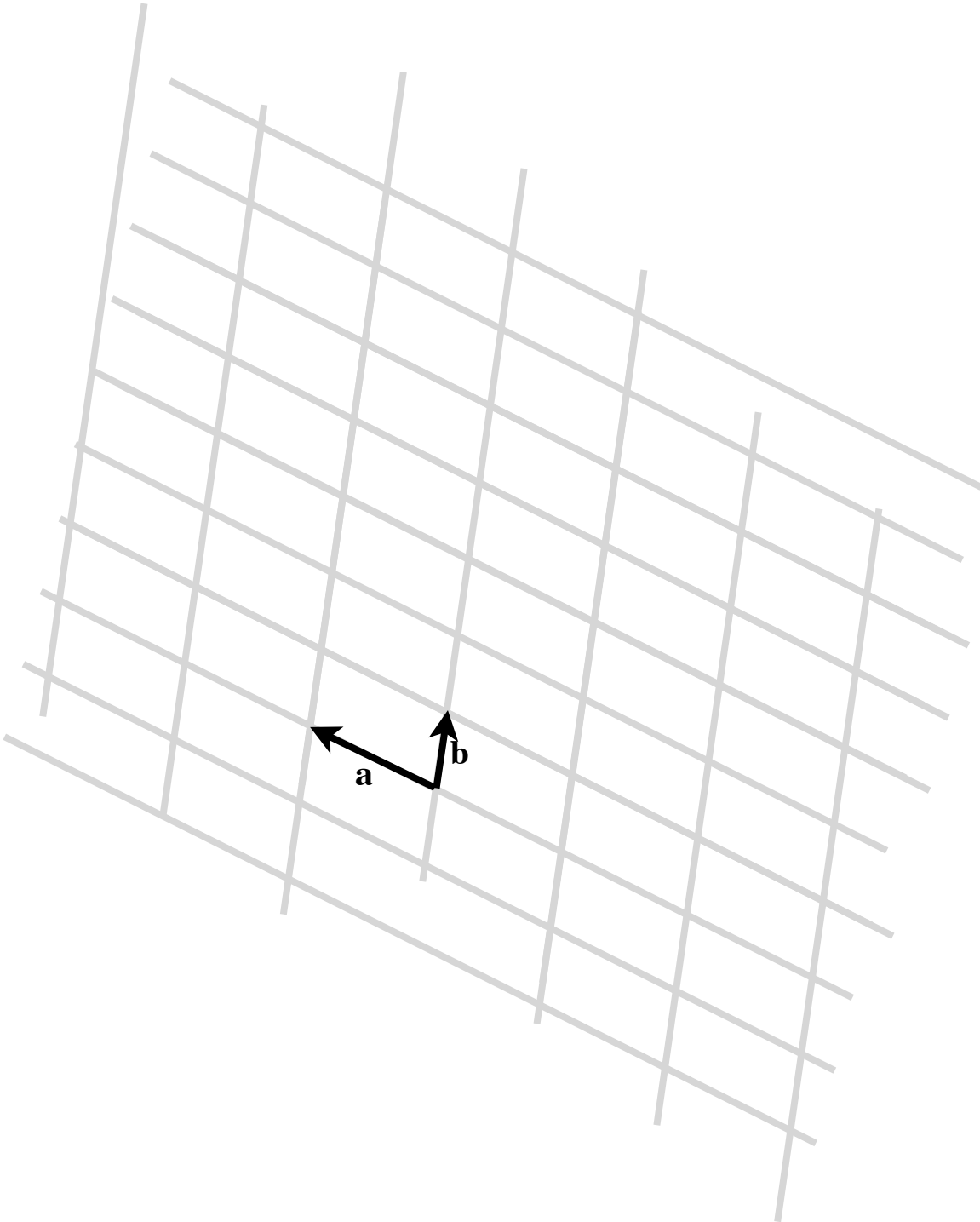
3. _____

4. _____

(10) [16 pts] **Draw the reciprocal lattice directly on top of the real space lattice below**, using the real space origin as the reciprocal space origin.. Draw and label \mathbf{a}^* and \mathbf{b}^* . The real space scale is $1\text{mm} = 1\text{\AA}$. For reciprocal space, use $1\text{mm} = 0.005\text{\AA}^{-1}$.

Draw and label the reciprocal space locations of reflections

$(0\ 2\ 0)$, $(-1\ 2\ 0)$, $(-2\ 2\ 0)$, $(-2\ 0\ 0)$



(11) Write the equations [16 pts]

Write the following equations. *Define the variables you use.*

(a) **The forward Fourier transform.** Structure factors from a set of atoms with atomic scattering factors and atomic B-factors.

(b) **The inverse Fourier transform.** Difference electron density from a set of observed structure factors with calculated phases and figure of merits.

(c) **Bragg's Law**

(d) **Laue's conditions** (all 9)

(12) CCP4 and COOT questions:

(a) Which columns of a data set are required to calculate a 2Fo-Fc map using FFT

- (1) H K L Fo Sigma
- (2) H K L Fo Sigma Fc Phic
- (3) H K L FOM Fo Sigma Fc Phic
- (4) H K L Fo Sigma Fc X Y Z
- (5) H K L Fo Fc

(b) Which columns of a data set are required to calculate a Patterson map using FFT

- (1) H K L Fo Sigma
- (2) H K L Fo Sigma Fc Phic
- (3) H K L FOM Fo Sigma Fc Phic
- (4) H K L Fo Sigma Fc X Y Z
- (5) H K L Fo Fc

(c) Circle the parameters that are refined by iMosFlm.

H K L Fo Io Sigma Crystal-to-film-distance Beam-position
Beam-intensity B-factors Background a b c alpha beta gamma mosaicity
phase model occupancy crystal-orientation-angles

(d) What does twinning mean, and how do you know a crystal is twinned?

(e) Molrep output. What do the labels mean? What units do they have?

RF _____
TF _____
theta _____
phi _____
chi _____
tx _____
ty _____
tz _____
TFcnt _____
wRfac _____
Score _____

--- Summary ---

	RF	TF	theta	phi	chi	tx	ty	tz	TFcnt	wRfac	Score
1	1	1	165.63	-151.22	69.36	0.253	0.208	0.010	20.39	0.606	0.336
2	2	1	165.84	-154.65	69.63	0.253	0.208	0.010	11.37	0.608	0.332
3	5	1	34.58	112.71	146.20	0.479	0.151	0.338	13.19	0.612	0.318
4	6	1	128.02	157.19	117.76	0.209	0.434	0.059	11.97	0.614	0.309
5	3	1	134.80	-140.63	160.01	0.216	0.451	0.148	9.28	0.619	0.302
6	7	1	143.18	-104.72	91.57	0.099	0.327	0.395	11.85	0.621	0.300
7	4	1	52.17	165.91	127.10	0.284	0.451	0.352	10.53	0.622	0.295
8	9	5	34.54	99.01	149.77	0.469	0.130	0.306	3.83	0.634	0.267
9	11	1	34.99	100.13	149.13	0.471	0.326	0.199	1.74	0.637	0.262
10	21	2	132.55	-43.86	147.40	0.792	0.433	0.443	2.38	0.638	0.258
11	17	6	65.04	150.16	44.84	0.186	0.255	0.219	2.25	0.636	0.257
12	8	3	80.43	143.77	46.62	0.259	0.444	0.431	1.34	0.640	0.257
13	15	1	14.15	-119.61	49.97	0.393	0.255	0.237	2.19	0.640	0.256
14	14	6	173.34	155.62	131.52	0.122	0.317	0.485	1.48	0.640	0.255
15	19	6	24.08	102.57	143.36	0.281	0.290	0.002	1.71	0.642	0.254
16	10	1	175.98	-128.29	69.45	0.105	0.321	0.477	2.34	0.639	0.254
17	12	11	136.99	-112.96	91.83	0.286	0.131	0.481	1.92	0.640	0.254
18	23	2	110.69	133.81	88.85	0.327	0.130	0.033	2.06	0.639	0.254
19	13	9	176.14	-140.85	68.98	0.980	0.324	0.038	1.81	0.641	0.254
20	16	1	45.33	148.93	33.04	0.069	0.377	0.496	1.97	0.638	0.252
21	22	1	23.36	101.53	145.28	0.491	0.262	0.209	1.70	0.642	0.252
22	18	1	137.45	-149.60	152.64	0.065	0.334	0.054	2.00	0.641	0.252
23	20	13	43.38	149.29	33.71	0.069	0.267	0.493	1.87	0.639	0.251

(f) What is the equation for a 2Fo-Fc map using molecular replacement phases?

(g) Which of the following is an example of real-space refinement?

- (1) Least-squares minimization of the R-factor as a function of the coordinates
- (2) Scaling F_o to F_c
- (3) Fitting a sidechain rotamer into density
- (4) Density modification using non-crystallographic symmetry
- (5) Density modification using solvent flattening

More problem solving

(13) Harker diagram

You have collected three datasets, F_P , F_{PH1} and F_{PH2} . Each reflection has been collected many times (using many syms) and a sigma value, the root-mean-square amplitude, has been determined. Using Patterson maps, you have determined the heavy atom positions (see homework 5) and calculated the F_{H1} and F_{H2} heavy atom vectors using the Fourier transform. Using the amplitudes and heavy atom vectors below (corresponding to just one non-centric reflection, i.e. $F(3\ 12\ 7)$) answer the following questions.

$$F_P = 50 \quad \sigma_P = 2.5$$

$$F_{PH1} = 46 \quad \sigma_{PH1} = 7.5$$

$$F_{PH2} = 57 \quad \sigma_{PH2} = 9.0$$

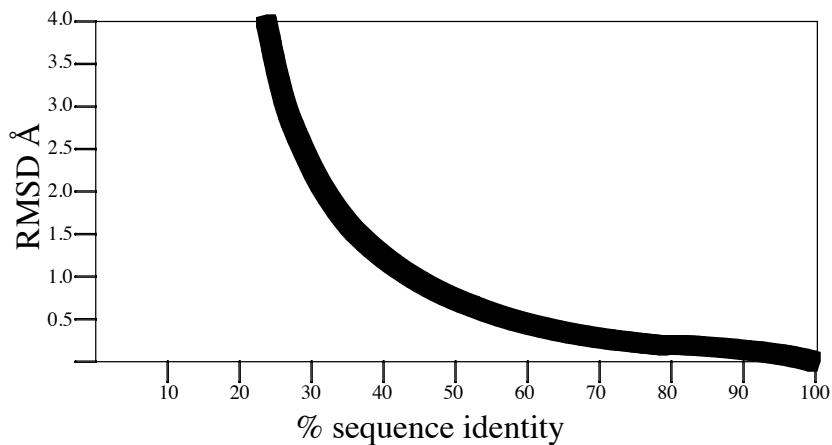
$$|F_{H1}| = 13.8 \quad \alpha_{H1} = -24.6^\circ$$

$$|F_{H2}| = 21.1 \quad \alpha_{H2} = 174.8^\circ$$

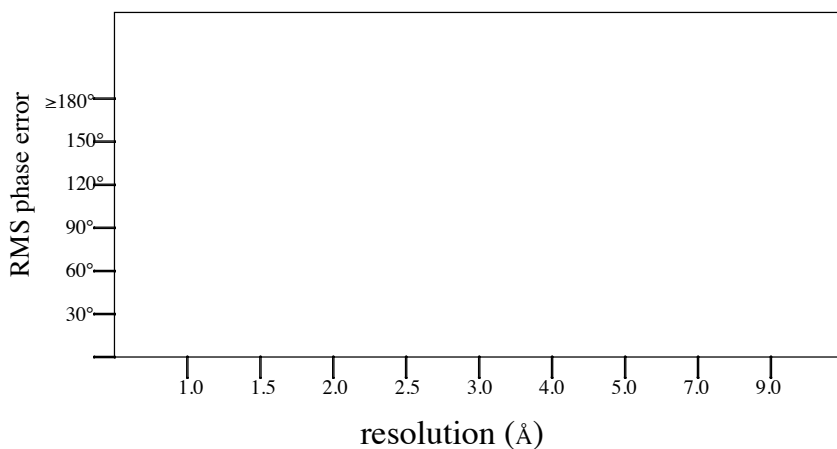
- (a) Draw the Harker diagram showing amplitudes, sigmas, the zone of solution vectors, and the *estimated* centroid of phase probability.
- (b) What is the most probable phase of F_P ?
- (c) What is the most probable phase of F_{PH1} ?
- (d) Plot the phase probability distribution.
- (e) What is the Figure of Merit, m ?
- (f) What is the best phase, α_{best} ?

(14) Molecular replacement -- how does coordinate error relate to phase error?

You have collected data for a protein that has a *homolog of known structure*. You want to use this homolog to calculate phases, using the molecular replacement method. Given the plot below of RMSD in atomic positions versus sequence identity, estimate and plot on the figure below the expected RMS phase error as a function of resolution for three molecular replacement models with 30% identity, 50% identity and 90% identity. What is the resolution cutoff (lowest d) you should use for each molecular replacement attempt, assuming you need 90° phase error or better to get a good map? What % identity do you need for a molecular replacement model if you need at least 3Å resolution data to solve the structure?



Plot 30%, 50% 90% phase error curves below.



What is the minimum % sequence identity for a successful 3Å resolution molecular replacement solution: _____

(15) Short essay questions. Write equations where appropriate.

(a) How are data sets scaled? Write the equation for the scale factor.

(b) What is non-isomorphism? Why can't we combine Xray data from non-isomorphous crystals, even if they contain the exact same molecule in the exact same orientation?

(c) In what three ways can phases be improved by density modification?

(d) What is the "free R-factor"? Why use it?

\

(e) What is the "figure of merit" (m, or fom)? How is it calculated? How is it used?

(f) Why calculate a 2Fo-Fc map?

(g) What is an "omit map"?

(h) Write the equation for how the coordinate error (in Å RMSD) relates to the phase error (in °). Use it to calculate the expected phase error for a 2.5Å reflection, given a 1.0Å RMSD atomic coordinates error.

(16) Calculate F_c , and then calculate the R-factor, for a three atom model listed below, using only the three reflections listed (only 3 reflections? what a tiny dataset!). The space group is P1. The scattering factor for each atom is 1.0 at all resolutions (This is unrealistic since it is the scattering factor of a point! But use it anyway.) Use global scaling. **Show your work.**

Fractional coordinates:

$$r_1 = (0.1, 0.2, 0.4)$$

$$r_2 = (0.15, 0.25, 0.25)$$

$$r_3 = (0.25, 0.10, 0.10)$$

Observed amplitudes:

$$F_o(2\ 3\ 1) = 20.$$

$$F_o(1\ 2\ 3) = 15.$$

$$F_o(-1\ 4\ 1) = 25.$$

Calculated amplitudes, unscaled:

$$F_c(2\ 3\ 1) = \underline{\hspace{2cm}}$$

$$F_c(1\ 2\ 3) = \underline{\hspace{2cm}}$$

$$F_c(0\ 4\ 1) = \underline{\hspace{2cm}}$$

Calculated amplitudes, scaled:

$$F_c(2\ 3\ 1) = \underline{\hspace{2cm}}$$

$$F_c(1\ 2\ 3) = \underline{\hspace{2cm}}$$

$$F_c(0\ 4\ 1) = \underline{\hspace{2cm}}$$

$$\text{R-factor} = \underline{\hspace{2cm}}$$