Review: Diffraction from a Lattice

- One atom in each unit cell
- Scattering vector (**S**) is fixed to certain values:
  - Direction is perpendicular to Bragg plane defined by Miller indices
  - Magnitude is given by:

$$|\boldsymbol{S}| = \left(\frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}\right)^{1/2} = \frac{2\sin\theta}{\lambda}$$

Diffraction in a Protein Crystal

- Many atoms in a single unit cell (fixed position in each unit cell)

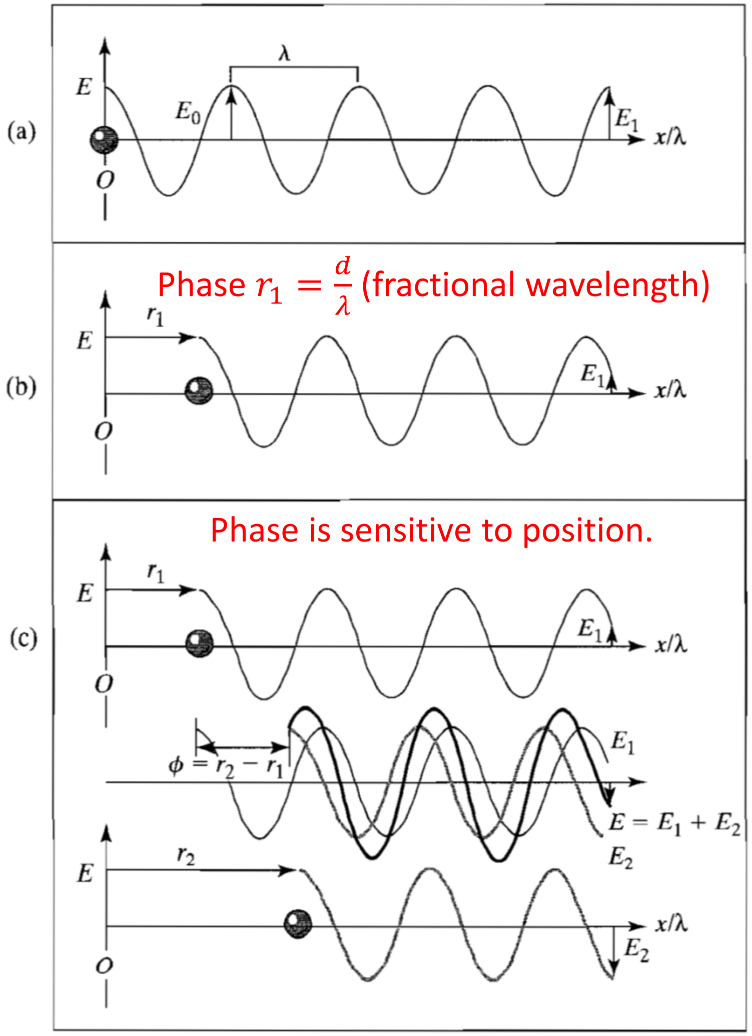
- Each atom contains multiple electrons

- **Simple case:** What happens when two electrons scatter (not in a crystal)?

- **Simpler case:** Scattering from a single electron

Single Point Scatterer

- Light scattering from a point source:
  $$|E| = E_0 \cos 2\pi \left(\nu t - \frac{x}{\lambda} + \phi\right)$$
  $$= E_0 \cos(\omega t - kx + \phi)$$
- Remember $c = \omega / k$
- Use Euler formula for convenience:
  $$|E|(\omega, t) = E_0 e^{i(\omega t - kx + \phi)}$$
- **Convention:** Ignore imaginary component



Phase $r_1 = \frac{d}{\lambda}$ (fractional wavelength)

Phase is sensitive to position.

van Holde, *et al.*, Chapt. 6, p. 310

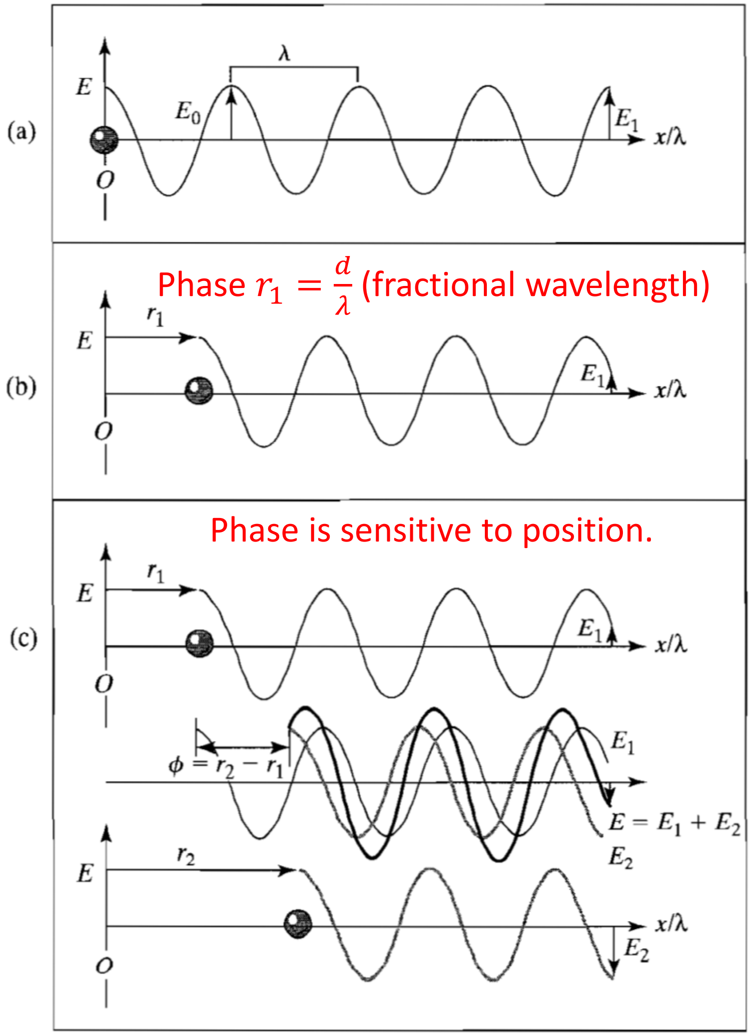
Single Point Scatterer

$$
\begin{aligned}
|E| &= E_0 \cos 2\pi\left(\nu t - \frac{x}{\lambda} + \phi\right) \\
&= E_0 \cos(\omega t - kx + \phi)
\end{aligned}
$$

- E_0 is dependent on *R* (distance to detector) and orientation, but at large distances is roughly constant

- **Electron at origin:** ϕ is spherically symmetric:

$$\phi = (kR - \omega t) + \frac{\pi}{2}$$



Phase $r_1 = \frac{d}{\lambda}$ (fractional wavelength)

Phase is sensitive to position.

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Add a Second Electron

- Phase shift:
  - Electron at origin:

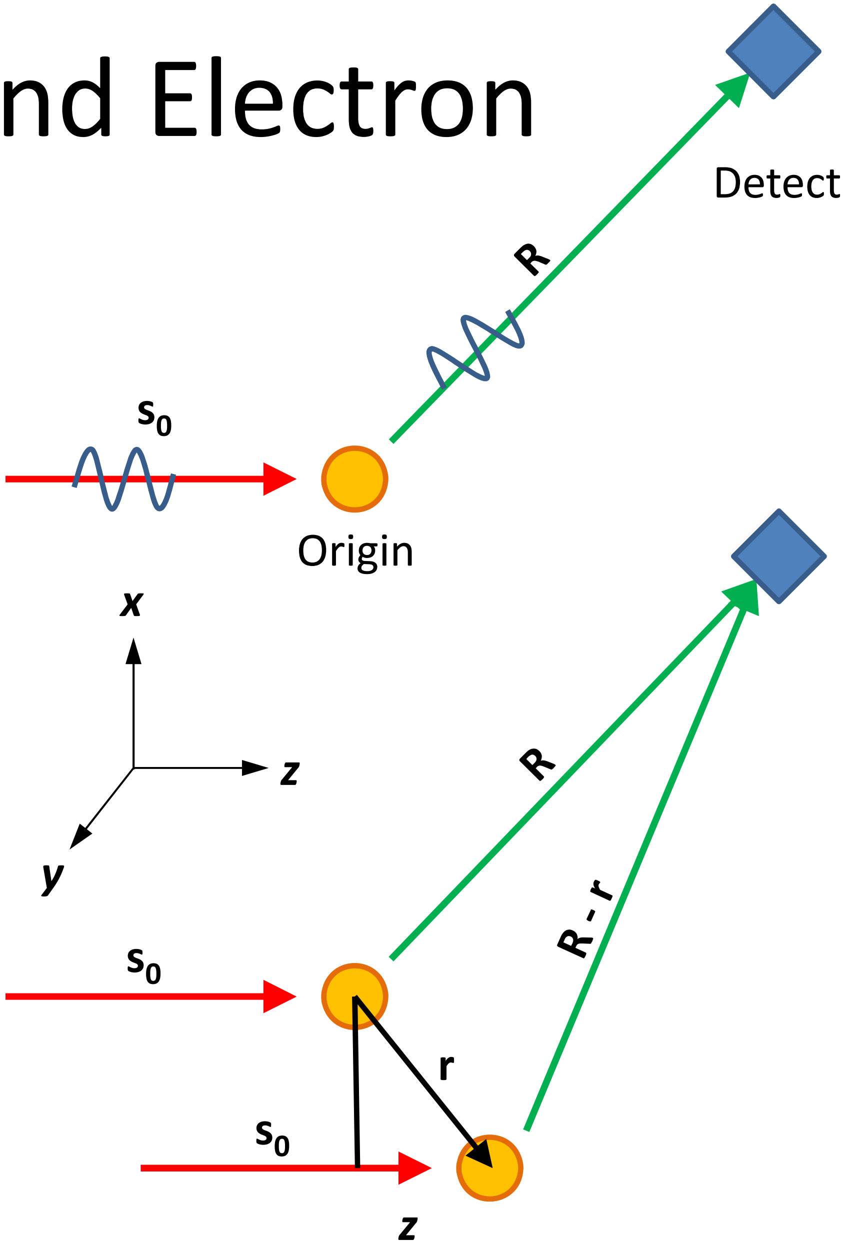
    $$\phi = (kR - \omega t) + \frac{\pi}{2}$$
  - Second electron:

    $$\phi = [k(z + |\boldsymbol{R} - \boldsymbol{r}|) - wt] + \frac{\pi}{2}$$

- Phase difference:

  $$\Delta\phi = k(z + |\boldsymbol{R} - \boldsymbol{r}| - R)$$

- No significant difference in E_0



Simplifying the Phase Difference

$$
\begin{aligned}
\Delta\phi \quad &= k\{z + |\boldsymbol{R} - \boldsymbol{r}| - R\} \\
&= k\left\{z + [(\boldsymbol{R} - \boldsymbol{r}) \cdot (\boldsymbol{R} - \boldsymbol{r})]^{1/2} - R\right\} \\
&= k\left\{z + [R^2 - 2(\boldsymbol{R} \cdot \boldsymbol{r}) + r^2]^{1/2} - R\right\} \\
&= k\left\{z + R[1 - 2(\boldsymbol{R} \cdot \boldsymbol{r})/R^2 + (r/R)^2]^{1/2} - R\right\} \\
&\approx k\{z - (\boldsymbol{r} \cdot \boldsymbol{R})/R\}
\end{aligned}
$$

Note that $r \ll R$, so $r/R \ll 1$, and:

$$\sqrt{1 - \frac{2(\boldsymbol{R}\cdot\boldsymbol{r})}{R^2} + \left(\frac{r}{R}\right)^2} \approx 1 - \frac{(\boldsymbol{R}\cdot\boldsymbol{r})}{R^2} - \frac{1}{2}\frac{r^2}{R} - \cdots$$

“Miller Index,” Wikipedia.

Add a Second Electron

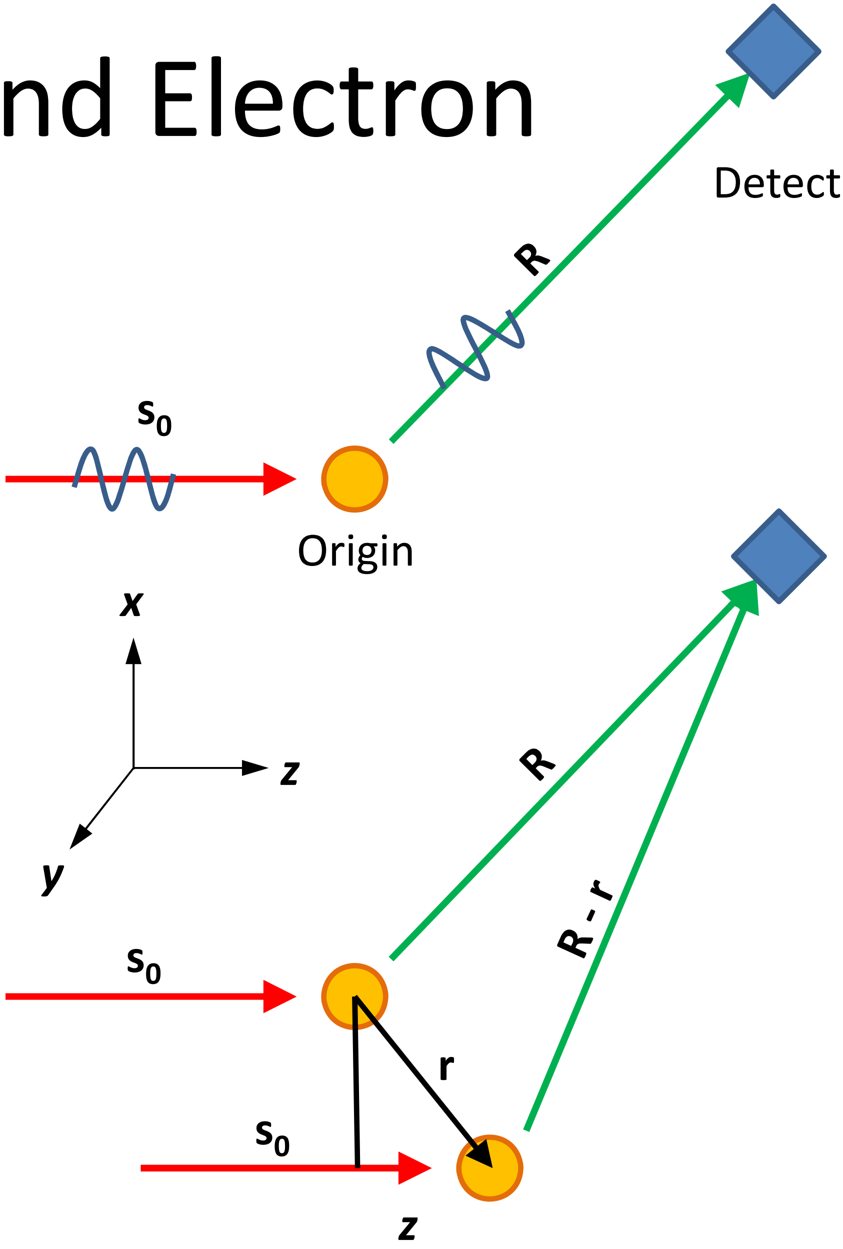
- Let $\mathbf{s}_0$ and $\mathbf{s}$ be unit vectors pointing along initial and scattering vector of light

- Then:

$$\begin{aligned}\Delta\phi &\approx k\{z - (\boldsymbol{r}\cdot\boldsymbol{R})/R\}\\ &= k[(\boldsymbol{r}\cdot\boldsymbol{s_0}) - (\boldsymbol{r}\cdot\boldsymbol{s})]\\ &= k\boldsymbol{r}\cdot(\boldsymbol{s_0} - \boldsymbol{s})\end{aligned}$$

$$\Delta\phi = -2\pi\,\boldsymbol{r}\cdot\boldsymbol{S}$$

- $\Delta\phi$ is dependent on the scattering vector and the position within the unit cell



Multiple Electrons

- Sum the contribution from each point scatterer:

$$E_{tot} = \sum_{j=1}^{N} E_0 \exp(i\phi_j)$$

$$E_{tot} = \underbrace{E_{0(\boldsymbol{r}=0)}}_{\text{Scattering from one electron}} \sum_{j=1}^{N} \exp(-i2\pi\Delta\phi_j)$$

- This initial term can be factored out to give scattering "relative" to electron at origin; E_{tot} increases with more electrons

Important Points

- Phase is mostly dependent on position of electrons

- Amplitude is mostly dependent on number of electrons

The Structure Factor

(Molecular Scattering Factor in your Book)

$$F(hkl) = F(\boldsymbol{S}) = \sum_{j=1}^{N_{atom}} f_j \, e^{-2\pi i(\boldsymbol{S}\cdot\boldsymbol{r})}$$

- Remember: only real part counts
- Crystal lattice makes **S** discrete (only see values for h, k, l)
- F is proportional to intensity at each reflection

The Structure Factor

(Molecular Scattering Factor in your Book)

$$F(hkl) = F(\boldsymbol{S}) = \sum_{j=1}^{N_{atom}} f_j(\boldsymbol{S})\, e^{-2\pi i(\boldsymbol{S}\cdot\boldsymbol{r})}$$

Atomic scattering factor

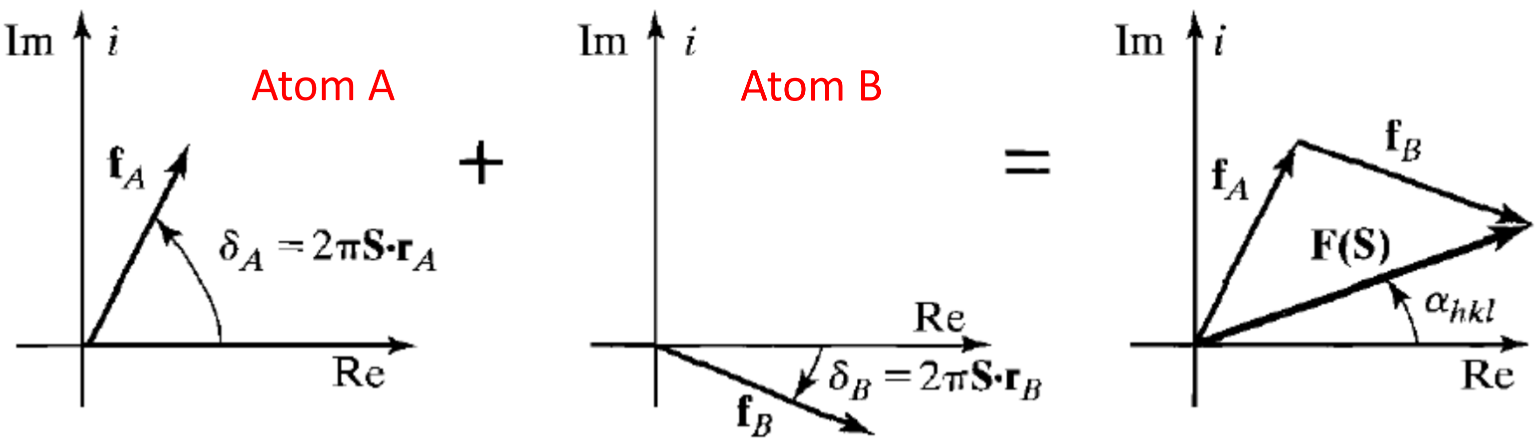
- f_j is linearly proportional to number of electrons in atom *j* (think about homework)
- Related to *electron density*

The Most Important Slide

$$\rho(\boldsymbol{r}) \propto \int_{V} F(\boldsymbol{S})\, e^{-2\pi i(\boldsymbol{S}\cdot\boldsymbol{r})} d\boldsymbol{r}$$

- Inverse Fourier transform is electron density
- If intensities *and phases* of structure factors were known, one could calculate $\rho(\boldsymbol{r})$

The Phase Problem



- Each reflection has an amplitude (observable) and phase difference (not observable on film)

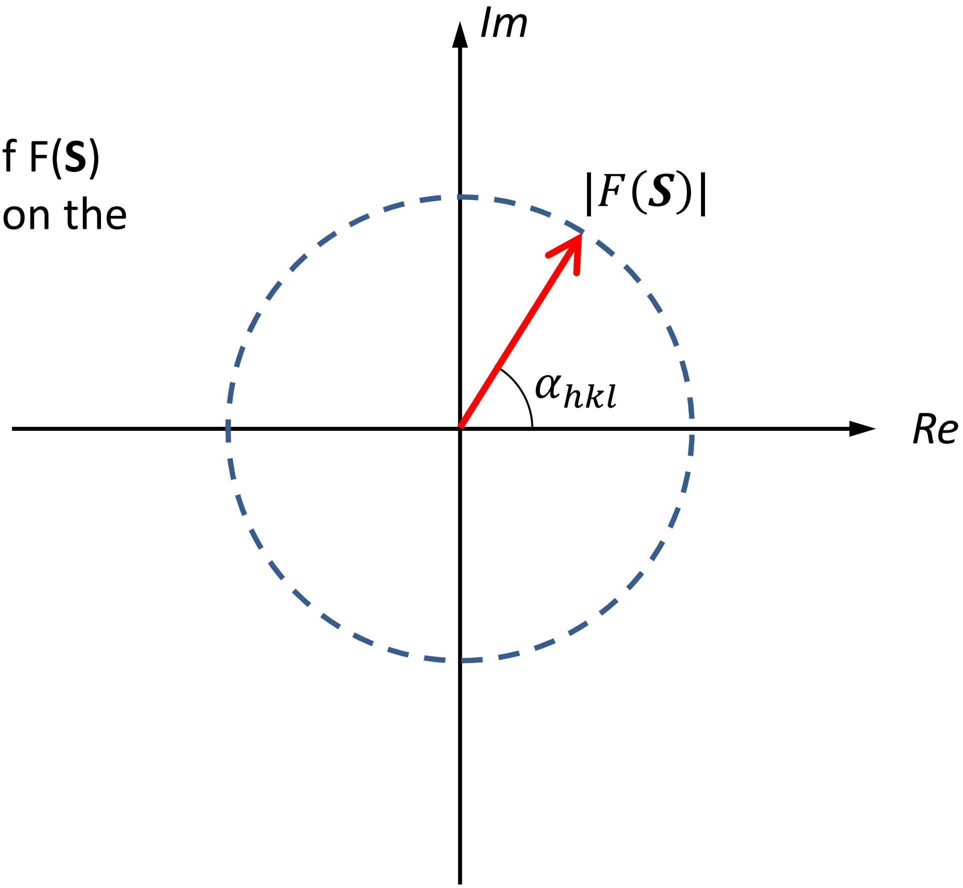
- Intensities are weakly dependent on phase, but dominated by crystal

- Argand diagrams are easy way to represent phase

van Holde, *et al.*, Chapt. 6, p. 314

The Phase Problem

The phase (angle) of F(**S**) could be anywhere on the circle.



Solving the Phase Problem

- Molecular replacement (if similar structure is known)
- Patterson maps (for small groups of atoms)
- Multiple isomorphous replacement
- Multiple-wavelength anomalous dispersion

Patterson Maps

- Appropriate for small numbers of atoms
- Fourier transform of intensities directly:

$$P(x,y,z)=\frac{1}{V}\sum_{h,k,l}|F(\boldsymbol{S})|^2 e^{2\pi i(\boldsymbol{S}\cdot\boldsymbol{r})}$$

- This is a density map that will plot every combination of possible distance pair in the unit cell (see next slide)

Patterson Maps

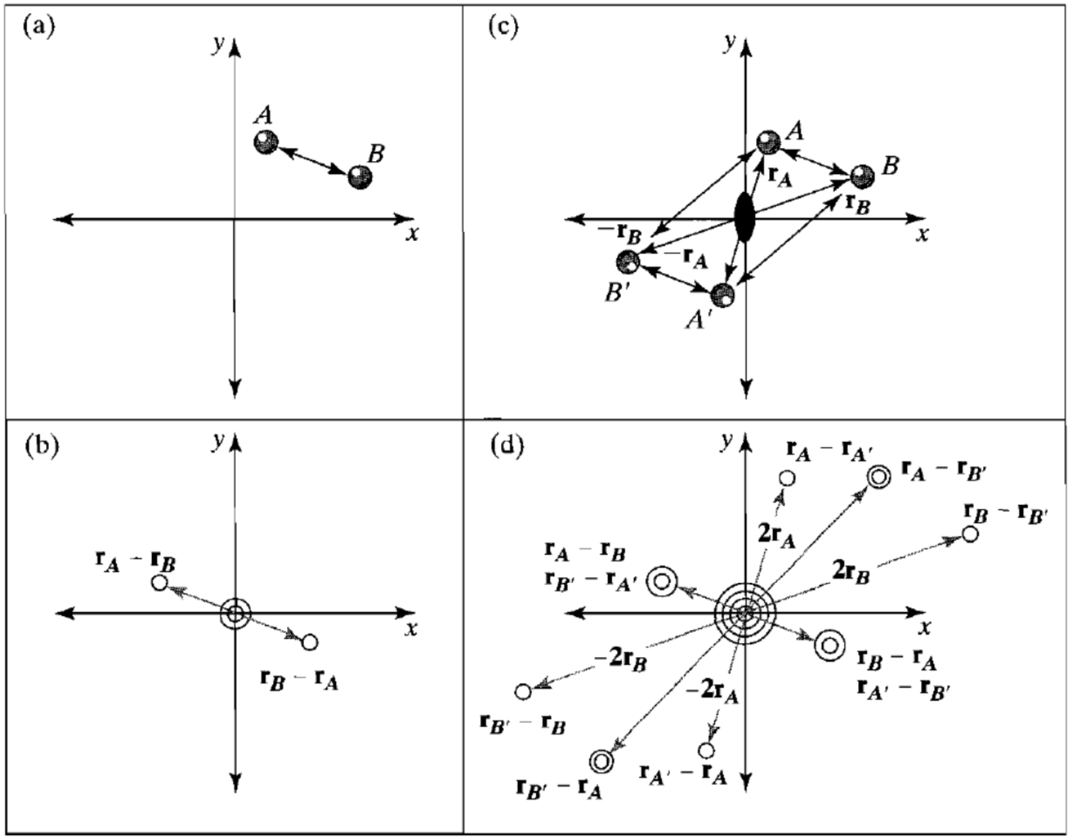
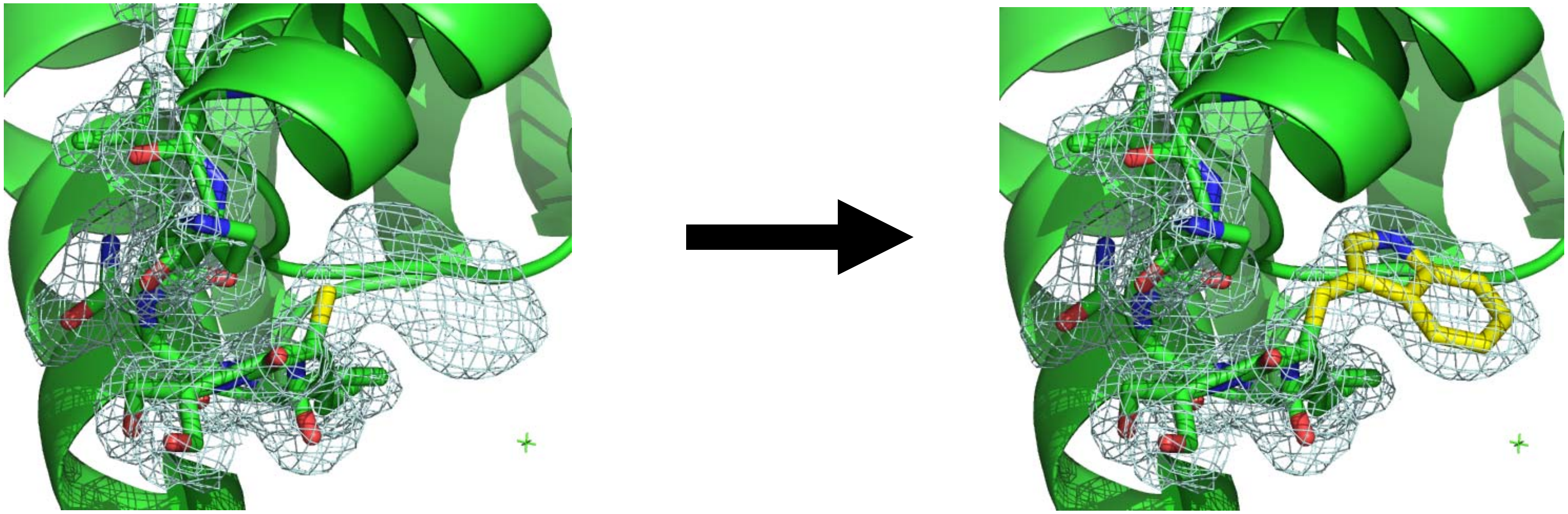


Figure 6.28 Patterson maps of two atoms in a unit cell. (a) Two unique atoms *A* and *B* in a unit cell are displaced from the origin by the distance vectors $\mathbf{r}_A$ and $\mathbf{r}_B$. (b) The Patterson map for the two atoms shows two cross vectors, one for the vector from *A* to *B* and the other from *B* to *A*. The two self-vectors (*A* to *A* and *B* to *B*) result in two contours at the origin of the map. (c) Two additional atoms, *A*′ and *B*′, are generated in a crystal with two-fold rotational symmetry. Although there are still only two unique atoms, there are now four additional cross vectors. If this is a Harker section in the Patterson map, the additional cross vectors are $2\mathbf{r}_A$ and $2\mathbf{r}_B$. This allows us to determine $\mathbf{r}_A$ and $\mathbf{r}_B$ (or the atomic coordinates of *A* and *B*) directly from the Patterson map.

van Holde, *et al.*, Chapt. 6, p. 324

Molecular Replacement



Wild-Type (Ala) phasing used on mutant reflections will reveal extra density, which can be fit mutation (to Trp). All atoms contribute to $\rho(r)$, not just those from Trp.

- **Idea:** Use a similar structure to generate phases, then refine based on rough structure
- Patterson map can be used to orient molecule

Multiple Isomorphous Replacement

- Incorporate heavy metals (selenium, mercury, etc.) into crystal
  - Cannot perturb structure/unit cell
  - Example: Selenomethionine

- Metal will scatter strongly; only a few per unit cell
  - Use Patterson to determine phase of metal scattering

Multiple Isomorphous Replacement

- Ideally, scattering will be additive:

$$\underbrace{F_{PH} = F_P}_{\text{Phases not known}} + F_H$$

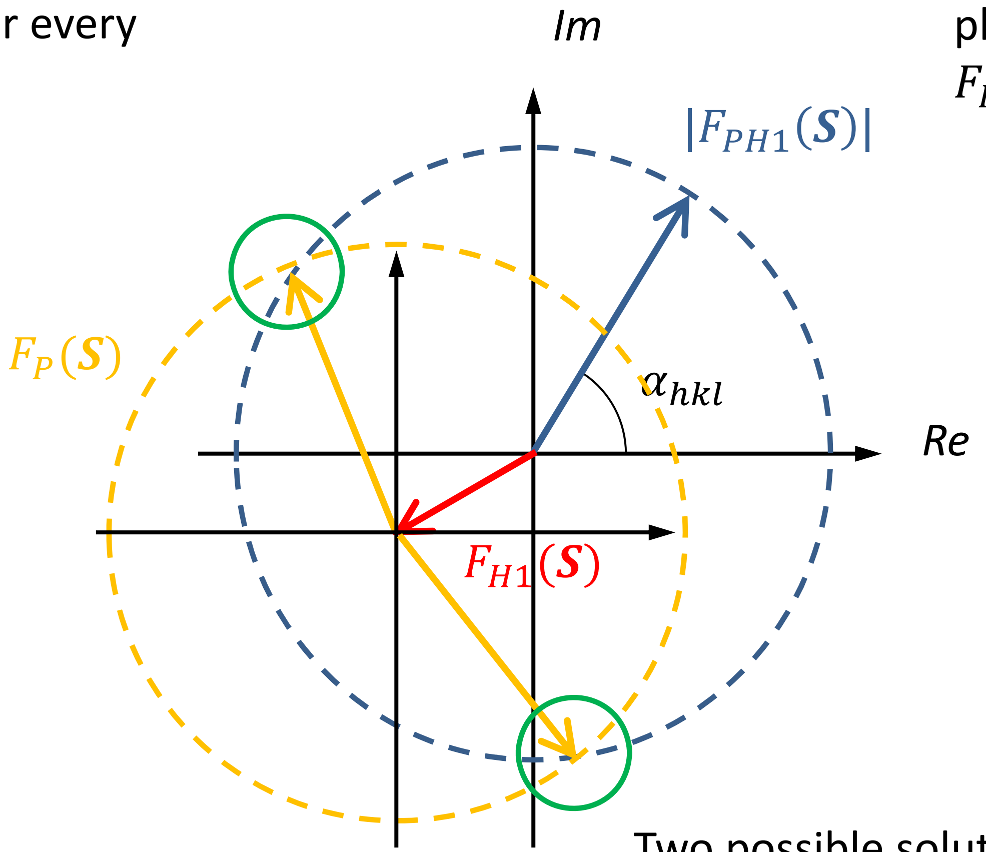
Phases known (from Patterson map)

- Use multiple heavy atoms to find phases (see next slide)

Multiple Isomorphous Replacement

Remember: the phase must be solved for every reflection. F(*hkl*)

Both intensity and phase are known for $F_{H1}(\boldsymbol{S})$

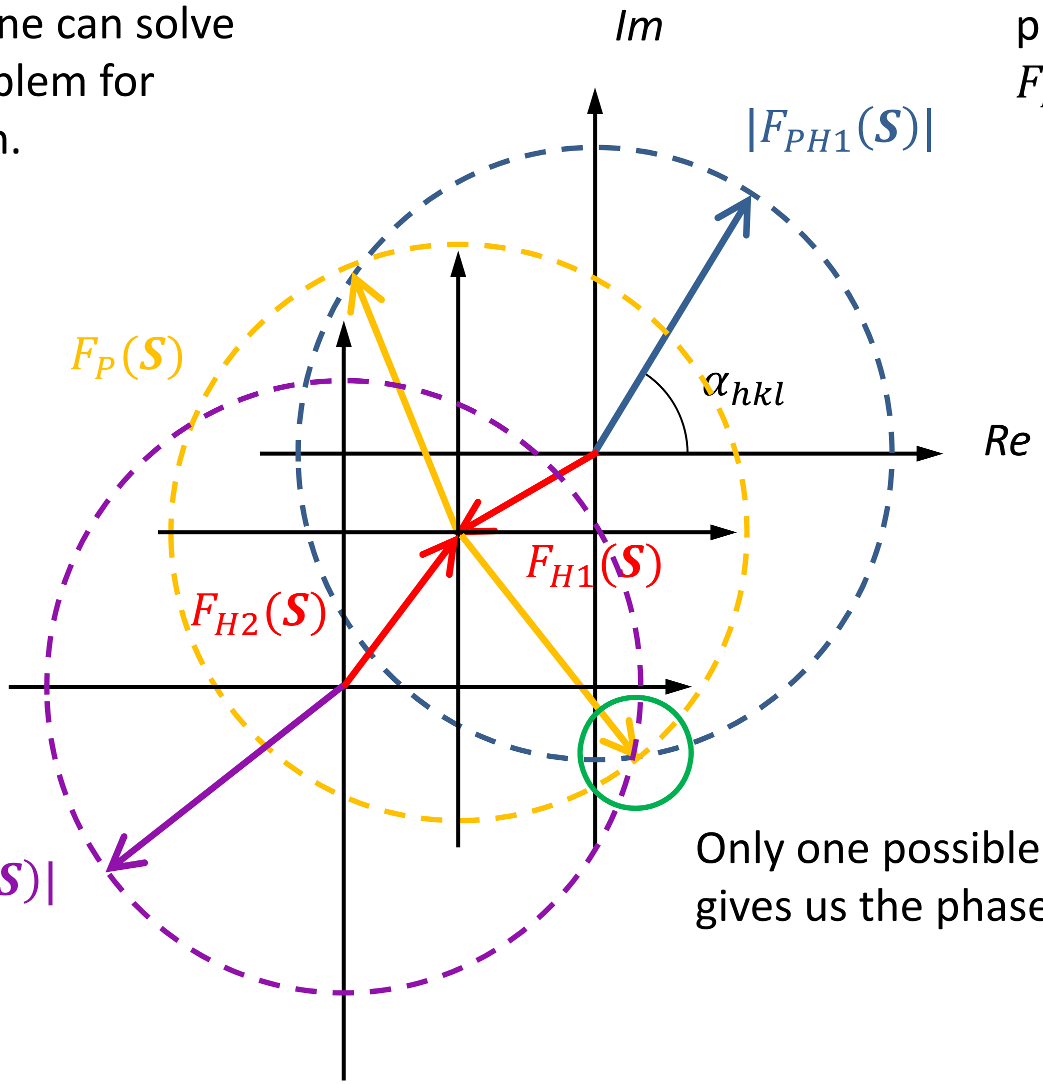


Two possible solutions at intersections (green circles)

Multiple Isomorphous Replacement

Using a second (or more) heavy atom, one can solve the phase problem for each reflection.

Both intensity and phase are known for $F_{H1}(\boldsymbol{S}), F_{H2}(\boldsymbol{S})$



Only one possible solution left; angle gives us the phase of $F_P(\boldsymbol{S})$

Multiple-Wavelength Anomalous Dispersion

- **Advantage:** Phasing with only one heavy atom derivative (often selenomethionine)

- **Disadvantage:** Usually requires tunable X-ray source (i.e. a synchrotron)

- Use X-rays near absorption edge of heavy metals to disrupt the relationship that $F(hkl) = F(\bar{h}\bar{k}\bar{l})$

Summary

- Scattering is convolution of diffraction from discrete *hkl* reflections (crystal) and electron density $\rho(r)$ (molecule)
- Phases are sensitive to position; amplitudes, not so much
- Inverse Fourier transform of structure factor intensities (reflections) and phases gives $\rho(r)$
- Phases not recorded on film/image plates/CCD
- Four main strategies to solve phasing problem