Phase Calculation

March 3, 2014

Solvent Content

- Fraction of the crystal volume occupied by solvent can be calculated from the crystal density and the partial specific volume
- partial specific volume of all proteins is close to 0.74 cm^3/g or 1.23 $Å^3/Da$
- Matthew's coefficient is the ratio of protein volume to solvent volume in the crystal.
- The volume of the asymmetric unit is estimated from the unit cell dimensions and the space group.
- Solvent content is usually between 40-60% (Matthew's coefficient between 1.5 and 0.66)

Calculation of Atomic Coordinates

- For each reflection has an amplitude and phase
- Amplitude depends on the scattering factor
- phase depends on the position of the atoms
- $F(hkl)=\sum_{j=1}^{n} f_j e^{i\alpha_j}$
- F(hkl)= structure factor for reflection h,k,l
- f_j = atomic scattering factor
- α = phase factor

The Phase Problem

- The diffraction patterns provides the intensity of each reflection but lost the phase information for the waves

- Intensity of the reflection = the structure factor for the molecule squared
- $I(hkl) = F(hkl)^2$
- Phases need to be experimentally determined or calculated

Methods to Determine Phases

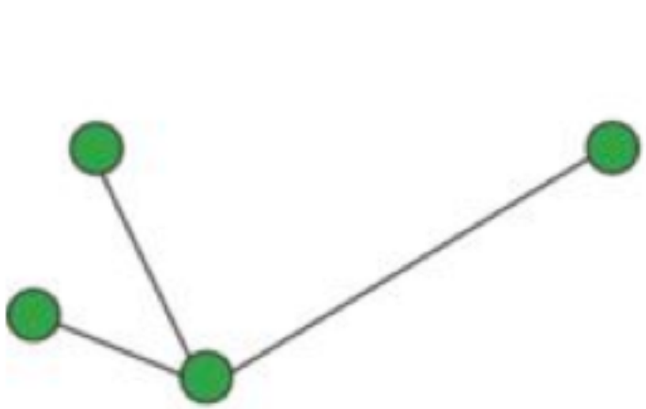
- There are several methods to determine the phases.
  - Direct methods
  - Isomorphous replacement
  - Multiwavelength Anomalous Diffraction - Single wavelength anomalous diffraction
  - Molecular Replacement

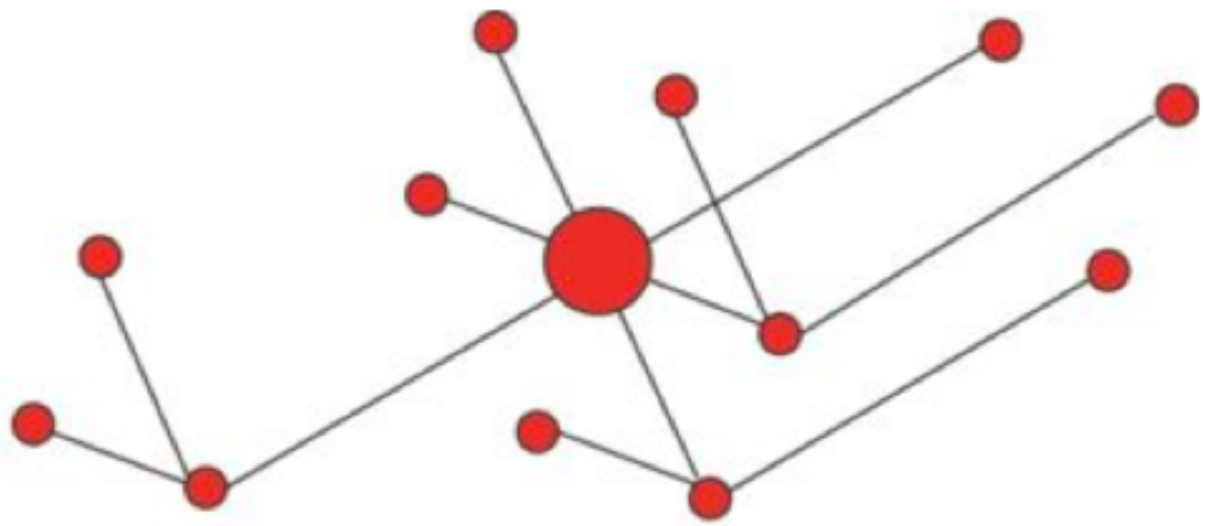
Patterson Function

- Can be used to deduce unit cell's components
- Patterson function *P*(*uvw*) does not rely upon phase information.
- Fourier transform of the intensity of structure factors, with their phases set to zero

$$P(uvw) = \frac{1}{V} \sum_h \sum_k \sum_l \exp\left[-2n(hu + kv + lw)\right]$$

- Patterson map's origin therefore contains the vector of each atom with itself, and the peaks throughout the map represent interatomic vectors of the crystal.





Direct Methods

$$F_{(h.k.l)} = \sum_{j=1}^{atoms} f_{(j)} \exp\left[2\pi \cdot i(hx_{(j)} + ky_{(j)} + lz_{(j)})\right]$$

- Guess the phases and try to calculate electron density. If you get negative values for electron density or random distribution it is wrong. Only certain values will give positive density
- If there are 100 atoms then there is 300 unknowns (100x3 for X,Y,Z of each atom)
- Another way - if we know the composition of the molecule then we can try a conformation, calculate the diffraction pattern and determine how well the calculated and experimental data correlate
- Routine method for small molecules (less than 200-300 hundred nonhydrogen atoms)
  - This becomes increasingly difficult as the size of molecule increases
- Computationally intensive

Multiple Isomorphous Replacement (MIR)

- Historically the most common technique for determining phases
- Attachment of heavy metals at specific locations in the crystal
- The scattering contribution to the INTENSITY is the square of the atomic scattering factor (scattering factor increases with atomic number). Just a few atoms can have a profound difference on the intensities
- Isomorphous replacement means that the protein in the native and derivative crystals has not changed much and the only difference is the addition of the heavy metal
- By comparing the different intensities between native and derivative data sets we can determine the position of the heavy metal sites.
- From these sites we can calculate the phases for the heavy metals
- These phases can be used to calculate phases for the entire molecule

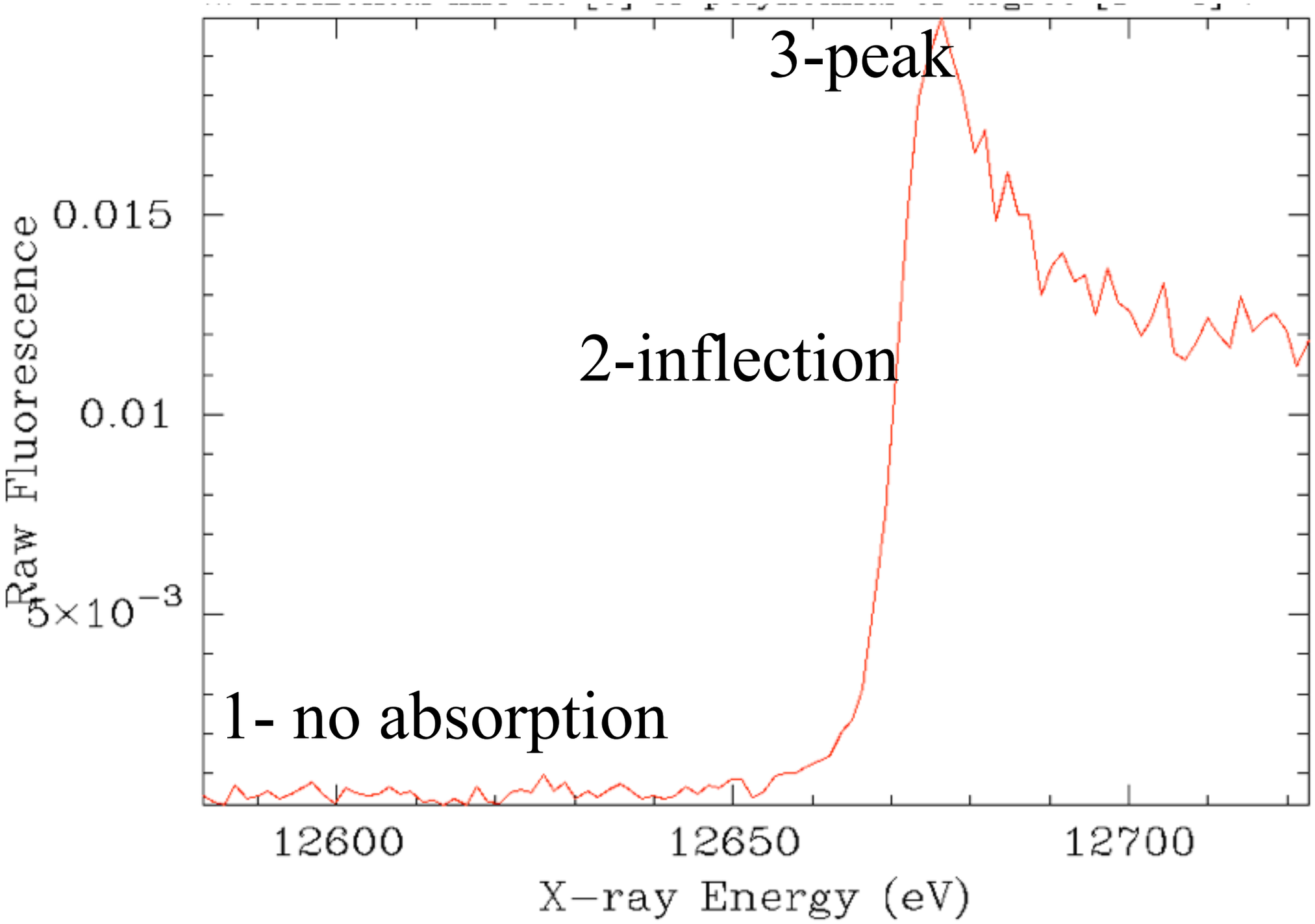
Disadvantages of MIR

- Require to have multiple (at least two) derivative data sets and a native data set
- Binding of heavy metals can be very difficult and result in nonisomorphous
- Too many binding sites cause changes to the protein
- Many partially occupied sites
- No binding

Multiwavelength Anomalous Diffraction

- The newest method to experimentally determine phases.
- Hendrickson Science 1991.
- Need to have a sample that is derivatized with a heavy metal
- Use incident wavelength of X-rays that are near to the absorption of the heavy atom
- This will cause the intensities to change depending on the wavelength of X-rays used.
- This change in intensities can be used to determine the sites of heavy metal attachment
- Atoms with atomic numbers greater than 20 (Ca and above) have absorption in the range of 0.3 to 3.0Å (wavelength)
- Absorption will create fluorescence which can be measured.

Selenium Fluorescence



- Collect 3 data sets at different wavelengths

MAD vs MIR

- MIR requires multiple crystals
- MAD can be considered a special case of MIR
- MAD requires one crystal and data is collected at several wavelength.
- Treat a data set where the atom does not absorb X-rays as “Native” and the data sets where the absorption occurs as “Derivatives”
- Since the data is collected from a single crystal, all data is isomorphous
- MAD data collection needs to be done at a synchrotron since it requires using many different wavelengths

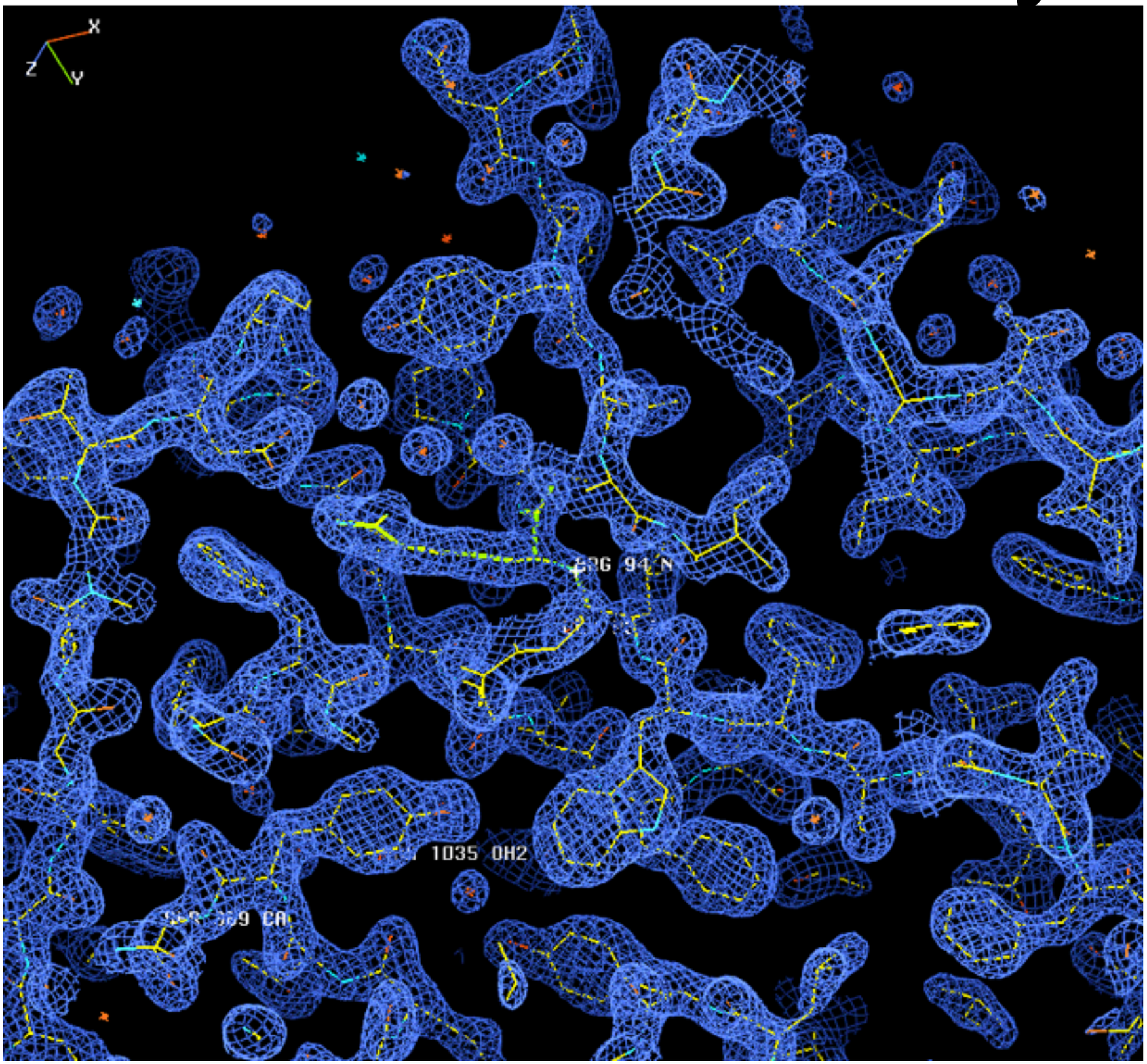
Molecular Replacement

- Know the structure of part or most of molecule
- Similar in many ways to direct methods
- translation is done first to localize the center of mass, then rotation to get the correct orientation
- Calculate phases using the known structure
- usefulness - if you have small molecule bound to a protein
- disadvantage - There can not be large changes in the structure that you using.

Electron Density

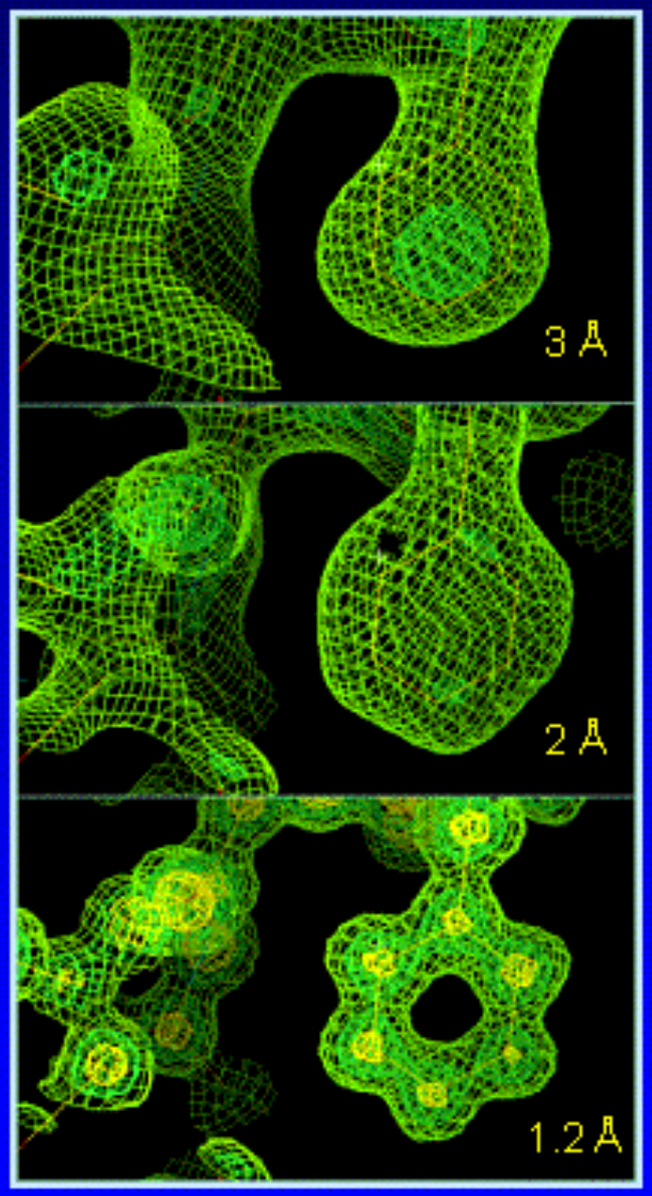
- Once you have determined the phases then use this information to calculate electron density
- Then build into electron density.
- Atomic resolution requires about 1-1.2Å resolution
- Most protein crystals do not diffract to this resolution
- For protein and DNA we do know the structure of the individual amino acids and nucleotides
- We can approximate their position in the electron density

Electron Density

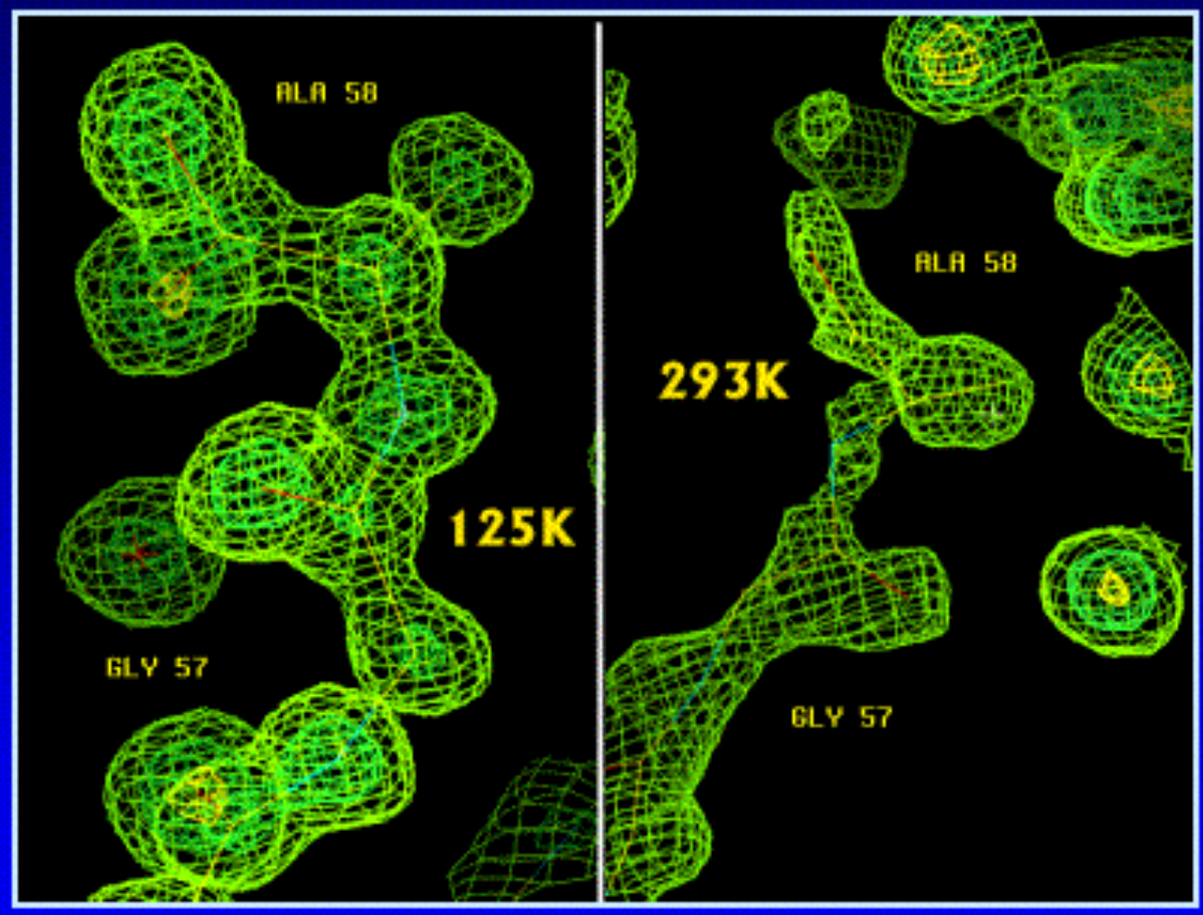


Advanced Methods in Modern Biomolecular Crystallography

Importance of resolution



Reduced disorder at low temperature



Dramatic improvements in the overall structure are likely to result from better definition of disordered regions regardless of resolution