X-Ray Crystallography Pt. II

For students of HI 6001-125
“Computational Structural Biology”

Sugoto Chakravarty, Ph.D.
Baylor College of Medicine

http://biomachina.org/courses/structures/03.html
Periodic Array of 2N+1 Identical Atoms

**Periodicity** \( a \)

\[
F_n(S) = e^{2i\pi s a} F(S)
\]

\[
F_{\text{Tot}}(S) = \sum_{n=-N}^{N} F_n(S) = F(S) \sum_{n} e^{2i\pi s a}
\]

\[
\sum_{n=-N}^{N} e^{2i\pi s a}
\]

Fringe function of the array
Atomic Form Factors

http://www.public.asu.edu/~awsmith
Periodic Array of $2N+1$ Identical Atoms

\[
F_{\text{Tot}}(S) = f(S) \frac{e^{2\pi i NS.a} (1 - e^{2\pi i (2N+1) S.a})}{(1 - e^{2\pi i S.a})}
\]

Multiplying both numerator and denominator by $e^{-\pi i S.a}$

\[
F_{\text{Tot}}(S) = f(S) \frac{e^{-\pi i (2N+1) S.a} - e^{\pi i (2N+1) S.a}}{e^{-\pi i S.a} - e^{\pi i S.a}}
\]

\[
= f(S) \frac{\sin[(2N+1)\pi S.a]}{\sin(\pi S.a)}
\]

\[
I_{\text{Tot}}(S) = \left| F_{\text{Tot}}(S) \right|^2 = \left| f(S) \right|^2 \left| \frac{\sin[(2N+1)\pi S.a]}{\sin(\pi S.a)} \right|^2
\]
Fringe Function and Diffraction

\[ I_{\text{Tot}}(S) = \left| F_{\text{Tot}}(S) \right|^2 = |f(S)|^2 \left\{ \frac{\sin[(2N+1)\pi S.a]}{\sin(\pi S.a)} \right\}^2 \]

Fringe function

Plots:
1. \( I = 0 \) everywhere except integral \( S.a \)
2. \( I_{\text{max}} \) only when \( S.a = 0 \)
i.e. \( S \) is in a plane \( \perp \) to the long atomic axis
Condition for Diffraction Maximum

\[ I_{\text{Tot}}(S) = |F_{\text{Tot}}(S)|^2 = |f(S)|^2 \left\{ \frac{\sin[(2N+1)\pi S.a]}{\sin(\pi S.a)} \right\}^2 \]

Usually, when \( 0.1 < |\sin(\pi S.a)| \leq 1.0 \)

the value of \( \sin[(2N+1)\pi S.a] \) oscillates between 0 and 1. Then

\[-10 \leq \frac{\sin[(2N+1)\pi S.a]}{\sin(\pi S.a)} \leq 10.\]
Condition for Diffraction Maximum

\[ I_{\text{Tot}}(S) = |F_{\text{Tot}}(S)|^2 = |f(S)|^2 \left\{ \frac{\sin[(2N+1)\pi S.a]}{\sin(\pi S.a)} \right\}^2 \]

But when \( \sin(\pi S.a) \to 0 \), using the expansion for \( \sin(x) \), the ratio becomes \( (2N+1) \) which is very large for macromolecules.

Thus, \( I_{\text{Tot}}(S) \) is large only when

\[ S.a = n \quad \text{where} \quad n \text{ is } 0, 1, 2... \]

von Laue condition
Miller Indices in a Lattice

Miller indices \((h,k,l)\)

Indices that characterize a set of parallel planes having intercepts \(a/h\), \(b/k\) and \(c/l\) on the three axes.
Two Conditions for Diffraction

1. $|S| = 2 \frac{\sin \theta}{\lambda}$

Geometrical interpretation

Scattering vectors that satisfy this condition diffract.
Two Conditions for Diffraction

2. Geometric interpretation of von Laue condition

\[ I_{\text{Tot}}(S) = \left| F_{\text{Tot}}(S) \right|^2 = \left| f(S) \right|^2 \left\{ \frac{\sin[(2N+1)\pi S \cdot a]}{\sin(\pi S \cdot a)} \right\}^2 \]

\[ S \cdot a = n \quad \text{where n is 0, 1, 2...} \]

von Laue condition
Visualizing the Two Conditions for Diffraction

**Visualizing the Two Conditions for Diffraction**

**Diffracted intensities are observed only when both the sphere of reflection and the von Laue conditions are satisfied together**

**Geometric interpretation**

The diagram illustrates the geometric interpretation of the two conditions for diffraction. The orientation of the crystal lattice and the positions of the diffracted beams are shown, highlighting the intersection of the two conditions for observing diffracted intensities.
Diffraction Pattern
Observable Part of Ewald Sphere

Satisfy both sphere of reflection and von Laue conditions

If the wavelength $\lambda$ and the incident direction $S_i$ are fixed, only a limited portion of the Ewald sphere can diffract. Limiting sphere: $2/\lambda$

To increase the diffracting region, the incident direction and/or the wavelength needs to be changed.

Leads to many experimental data collection strategies.
Scattering in 3 Dimensions

1-d: \[ F(S) = \sum_{n} f_n(S)e^{2\pi i S \cdot x_n} \] (Single summation over x)

3-d: \[ F(S) = \sum_{n} f_n(S)e^{2\pi i S \cdot (x_n a + y_n b + z_n c)} \] (Triple summation over x, y & z)

Using von Laue conditions \( S \cdot a = h \) etc.,

\[ F(S) = \sum_{n} f_n(S)e^{2\pi i (hx_n + ky_n + lz_n)} \] (Fourier series and not a transform)

\[ \rho (x,y,z) = \frac{1}{NV} \sum_{x} \sum_{y} \sum_{z} F(S)e^{-2\pi i (hx + ky + lz)} \]
Argand Diagram of Structure Factors

\[ |F| = \left[ A^2 + B^2 \right]^{\frac{1}{2}} \] : Measured experimentally

\[ \phi = \tan^{-1} \left( \frac{B}{A} \right) \] : Unknown

The circle indicates that \( F \) is a vector quantity with an amplitude and a phase.

Signs of both \( A \) and \( B \) important in determining the magnitude and quadrant of \( \phi \).
Crystal Lattice and Convolution

1-d convolution

\[
fg(u) = \int_{-\infty}^{\infty} dx f(x) g(u-x) = gf(u)
\]

If \( g(x) = \delta(x-a) \):

\[
f\delta(u) = f(u+a)
\]

Thus the convolution of \( f(x) \) with \( \delta(x-a) \) just shifts \( f(x) \) by a distance \( a \).
Crystal Lattice and Convolution

**Periodic lattice:**

Lattice function $L(x) = \sum_{n=-\infty}^{\infty} \delta(x-na)$

Crystal = $L \rho(u) = \sum_{n=-\infty}^{\infty} \rho(u+na)$

**3-d convolution**

$L(r) = \sum_{n=-\infty}^{\infty} \delta(r-na-mb-pc)$

Crystal = $L \rho(u) = \sum_{m,n,p=-\infty}^{\infty} \rho(u+na+mb+pc)$

Crystal = Convolution of unit cell with lattice
Unit Cell and Symmetry

Lattice: repetition of unit cells by pure translation

Contents of the unit cell:
Contents cannot be arbitrarily arranged but there must be an asymmetric unit that is rotated (and possibly fractionally translated) to generate the complete unit cell contents

Possible rotational symmetry among the asymmetric units

1, 2, 3, 4, 6-fold: 5-fold not allowed by translational symmetry
Mirror reflection not allowed in biological molecules
Fractional translations: \( \frac{1}{2}, \frac{1}{3}, \frac{2}{3}, \frac{1}{4}, \frac{3}{4}, \frac{1}{6}, \ldots \) of the unit cell dimensions
Unit Cell Types Determined by Symmetry

**Point groups:** All possible rotations and reflections among the asymmetric units

32 Point groups

**Crystal systems:** Point groups + translation symmetry restricts types of the unit cell possible (7 crystal systems)

Restrictions on the unit cell lengths and angles
### 7 Crystal Systems and 14 Bravais Lattices

<table>
<thead>
<tr>
<th>System</th>
<th>Lattice</th>
<th>Min symmetry</th>
<th>Unit cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclinic</td>
<td>P</td>
<td>None (1-fold)</td>
<td>a≠b≠c  (\alpha\neq\beta\neq\gamma)</td>
</tr>
<tr>
<td>Monoclinic</td>
<td>P</td>
<td>2-fold along b</td>
<td>a≠b≠c  (\alpha\neq\gamma\ \beta\neq90)</td>
</tr>
<tr>
<td>Orthorhombic</td>
<td>P, C, I, F</td>
<td>2-folds along a,b,c</td>
<td>a≠b≠c</td>
</tr>
<tr>
<td>Tetragonal</td>
<td>P, I</td>
<td>4-fold along c</td>
<td>a = b ≠ c</td>
</tr>
<tr>
<td>Trigonal/Rhombohedral</td>
<td>R, P</td>
<td>3-fold along c</td>
<td>a = b = c  (\alpha = \beta = \gamma \neq 90)</td>
</tr>
<tr>
<td>Hexagonal</td>
<td>P</td>
<td>6-fold along c</td>
<td>a = b ≠ c  (\alpha = \beta = 90\ \gamma = 120)</td>
</tr>
<tr>
<td>Cubic</td>
<td>P, I, F</td>
<td>3-fold along body diagonals</td>
<td>a = b = c</td>
</tr>
</tbody>
</table>

![Centering Diagram](image)
Different Lattices and Centering

- Triclinic & Monoclinic
- Orthorhombic
- Tetragonal
- Cubic

© http://www.uwgb.edu/dutchs/symmetry/bravais.htm
Applying a Screw Operation

2-fold through origin:
\[
\begin{pmatrix}
1 & 0 & 0 \\
0 & -1 & 0 \\
0 & 0 & -1
\end{pmatrix}
\]
x, y, z  x, -y, -z

2-fold screw axis \( \mathbf{2}_1 \)

Symbol when axis is perpendicular to the page

Symbol when axis is parallel to the page

\( \mathbf{2}_1 \) along [001] and passing through origin:
\[
(x, y, z) \rightarrow (-x, -y, \frac{1}{2} + z)
\]

\( \mathbf{2}_1 \) along [001] and passing through \((1/4, 0, 0)\):
\[
(x, y, z) \rightarrow (\frac{1}{2} - x, -y, \frac{1}{2} + z)
\]
Example of a Space Group

\( \frac{1}{2}, \frac{1}{2}, \frac{1}{2} \) from International tables (#19)

\[(x, y, z) \quad (\frac{1}{2} - x, -y, z + \frac{1}{2}) \quad (-x, y + \frac{1}{2}, -z + \frac{1}{2}) \quad (x + \frac{1}{2}, -y + \frac{1}{2}, -z)\]
Bragg’s Law of Diffraction

The atoms in a crystal can be considered as a series of parallel planes.

To observe diffraction, path difference between reflected beams from adjacent planes must be an integral number of wavelengths

\[ 2d \sin \theta = n \lambda \]
Phase Solution

Aim: Determine $\phi_p$ for each reflection

- Isomorphous replacement
- Anomalous scattering
- Molecular replacement

(Note: A.s. not explained here, similar to I.r., but using special wavelengths to break Friedel symmetry, see http://www.bmsc.washington.edu/scatter/AS_tutorial.html)
Isomorphous Replacement

\[
|F_{PH}| = |F_p| \pm |F_H|
\]

(Fig. a)

If the derivatized structure remains similar, hope to get vector relationships between \( F_p, F_H \) and \( F_{PH} \)

Simple collinear relationship between the structure factors holds for only centro-symmetric reflections whose phases are 0 or \( \pi \) (Fig. a)

Soak in heavy atoms into the crystal

No simple relationship between the structure factors for the general non-centrosymmetric reflections (Fig. b)
Patterson Functions to Locate Heavy Atoms

\[ \rho(r) = \int F(S) e^{-2\pi i S \cdot r} \, dr \]

\[ P = \int I(S) e^{-2\pi i S \cdot r} \, dr \]

\[ = \int F^*(S) F(S) e^{-2\pi i S \cdot r} \, dr \]

\[ = \rho(r) \rho(-r) \]

The Patterson is a convolution of the electron density with its image that is inverted through the origin.
Patterson are Inter-Atomic Distance Maps

\[ I_h \propto |F_h|^2 = \sum_{j} f_j e^{2\pi i h \cdot r_j} \sum_{k} f_k e^{-2\pi i h \cdot r_k} \]

\[ = \sum_{j} f_j f_k e^{2\pi i h \cdot (r_j - r_k)} \]

Thus, the Patterson functions, computed using the intensities as coefficients, map the inter-atomic vectors

In real space: \[ P = \int_{\text{unit cell}} \rho(r) \rho(r+u) d^3r \]
Pattersons Determine Heavy Atom Vectors

For large $N$, Patterson maps become uninterpretable

**Orthorhombic $P2_12_12_1$:**

$$(x,y,z); (\frac{1}{2}-x,-y,1/2+z); (\frac{1}{2}+x,1/2-y,-z); (-x,1/2+y, \frac{1}{2}-z)$$

**Difference Patterson:**

**Coefficients:** $|F_D|^2 - |F_N|^2$

**Peaks at inter-atomic vectors U,V,W:**

<table>
<thead>
<tr>
<th>U</th>
<th>V</th>
<th>W</th>
<th>Peak type</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.404</td>
<td>0.453</td>
<td>0.5</td>
<td>Harker</td>
</tr>
<tr>
<td>0.5</td>
<td>0.048</td>
<td>0.199</td>
<td>Harker</td>
</tr>
<tr>
<td>0.42</td>
<td>0.108</td>
<td>0.986</td>
<td>General (NH)</td>
</tr>
</tbody>
</table>

**Patterson peaks:**

| $\frac{1}{2}$ - $2x$, -2y, $\frac{1}{2}$ (H) |
| $\frac{1}{2}$, $\frac{1}{2}$-2y, -2z (H) |
| 2x, $\frac{1}{2}$, -$\frac{1}{2}$-2z (H) |
| -2x, $\frac{1}{2}$, $\frac{1}{2}$-2z (H) |
| -$\frac{1}{2}$, $\frac{1}{2}$+2y, -2z (H) |
| -$\frac{1}{2}$, -2x, 2y, $\frac{1}{2}$ (H) |

H: Harker section peak

Solve for $x,y$ & $z$ using the Harker peaks:

$x = 0.048; \ y = 0.774; \ z = -0.099 = 0.900$ (translate by 1.0)

These are the heavy atom coordinates
Patterson Heavy Atom Peaks May Solve Structure

Hypothetical 2 molecule structure:
(10 Carbon atoms + 1 Br atom) / molecule

Patterson peak $h \propto Z_A Z_B$

Br-Br peaks will be the highest

Determine the coordinate of Br

$$F_h = F_h^{\text{known}} + F_h^{\text{unknown}}$$

(Due to rest of structure)

(due to the heavy atoms in the unit cell)

Compute partial electron density map, fill up the missing density and iterate
Single Isomorphous Replacement (SIR)

For a given reflection hkl (Vector addition of Sfs)

Known: $|F_p|$, $|F_h|$ and $|F_{ph}|$

$|F_p|$ circle center: Origin O

$|F_h|$ : contribution of heavy atom to structure factor amplitude

$P_1$ and $P_2$, the points of intersection of the two circles, represent the two possible values of the phases for the given reflection hkl

$|F_{ph}|$ circle centered about the end of the vector $-|F_h|$
Resolving Phase Ambiguities in SIR

$P_1$ and $P_2$, the points of intersection of the two circles, represent the two values of the phases for the given reflection hkl.

How to choose the best phase?

◊ $P_1 + P_2$

(Large phase error if $F_h$ is relatively small)

Prepare multiple heavy-atom deriviatives
Multiple Isomorphous Replacement (MIR)

**Known:** $|F_p|$, $|F_{H1,2}|$ and $|F_{PH1,2}|$

**Contribution of 2 separate heavy atoms to the structure factor amplitude**

$P_1$ represents the only possible phase for the given reflection hkl

$|F_{PH1,2}|$ circles centered about the ends of the vectors $-|F_{H1,2}|$
Errors in MIR Phases

Assumptions:
1. Ideal isomorphism
2. Exact heavy atom positions

But
1. Random and systematic errors in measurement of intensities
2. Errors in estimation of heavy atom positions
3. Errors due to lack of isomorphism

How to treat these errors?
Errors in MIR Phases

Assumptions:
1. All errors are Gaussian
2. Errors in heavy atom position and that due to lack of isomorphism can be considered together

\[ \epsilon = |F_{ph}| - (|F_p| + |F_h|) \]

\( \sigma_{exp} : \text{Errors in experimental observations} \)
\( \sigma_{th} : \text{Combined errors in } F_h \text{ and lack of isomorphism} \)
Errors in MIR Phases

Total error in $F_{PH}$: $\sigma = \left[ \sigma_{\text{exp}}^2 + \sigma_{\text{fh}}^2 \right]^{1/2}$

$\sigma_{\text{exp}}$: Errors in experimental observations
$\sigma_{\text{fh}}$: Combined heavy atom position and lack of isomorphism error
Errors in MIR Phases

Probability distribution \( P(\phi) = C \exp \left(-\frac{\epsilon^2}{2\sigma^2}\right) \)

where \( C \) is a normalization constant such that

\[
\int_0^{2\pi} P(\phi) \, d\phi = 1
\]
Errors in MIR Phases

**Probability distribution** $P(\phi) = C \exp \left( -\frac{\epsilon^2}{2\sigma^2} \right)$

Using

$$\cos \phi = \frac{F_h^2 + F_p^2 - (F_{ph} + \epsilon)^2}{2F_h F_{ph}}$$

it is possible to calculate the probability distribution for each phase of each derivative.

For all derivatives

$$P(\phi) = \prod_{j} P_j(\phi) = C \exp \sum_{j} \left( -\frac{\epsilon_j^2}{\sigma_j^2} \right)$$
Best MIR Phase

Probability distribution of the MIR phases

\[ P(\phi) = \prod_j P_j(\phi) = C \exp \sum_j \left( -\frac{\epsilon_j^2}{\sigma_j^2} \right) \]

Best phase for a given reflection?

For a unimodal distribution, the phase angle \( \phi \) at which \( P(\phi) \) is maximum, is the best phase angle.
Best MIR Phase

Usually $P(\phi)$ is bimodal, and the centroid of the phase probability gives the best phase

$$\phi_{\text{best}} = \frac{\int_0^{2\pi} P(\phi) \exp(i\phi) d\phi}{\int_0^{2\pi} P(\phi) d\phi}$$
Figure of Merit of a Phase

\[ \phi_{\text{best}} = \frac{\int_{0}^{2\pi} P(\phi) \exp(i\phi) d\phi}{\int_{0}^{2\pi} P(\phi) d\phi} \]

The Figure-of-merit (m) of a phase is defined as the mean of the cosine of the phase error

\[ m = \frac{\sum P(\phi_i) \cos(\phi_i)}{\sum P(\phi_i)} = \langle \cos \Delta \phi_i \rangle \]

where \( \Delta \phi_i = \phi_{\text{best}} - \phi_i \)

The FOM weighted Fourier coefficient is \( mF \exp(i\phi_{\text{best}}) \)
Molecular Replacement

- Similar structure exists (sequence identity) MIR not required

- Orient the known structure as closely as possible to the unknown structure

- Place the known structure as correctly as possible

- Rotational and translational parameters will give a good set of starting phases
Molecular Replacement

Model
Human lysozyme

Data
Dinosaur lysozyme
Rotation Conventions

Rossman

Euler

\[ \Phi, \Psi \]
Patterson Rotation Functions

\[ R(\kappa, \phi, \psi) = \int_{r_{\text{min}}}^{r_{\text{max}}} P_{\text{data}}(u) P_{\text{model}}(\kappa, \phi, \psi, u) \, du \]

- **model**: Atomic model of known structure (Human lysozyme)
- **data**: Unknown structure (Dinosaurus lysozyme)

**Real space**: Rotation of inter-atomic vectors
**Reciprocal space**: Convolution of SF **2

Determine the best(\kappa, \phi, \psi) at which R shows a maximum

Orient the model through these angles
Orientation of Unknown Molecule

Model
Human lysozyme
One asymmetric unit in unit cell (P1 symmetry)

Data
Dinosaur lysozyme
Crystallographic symmetry

Position of unknown molecule wrt symmetry axes?
Position of Molecule: Translation Function

\[ T(u) = \int P_{\text{data}}(u) P_{\text{model}}(u+r) du \]

**model:** Atomic model of known structure (Human lysozyme)

**data:** Unknown structure (Dinosaursus lysozyme)

**Real space:** Rotation of inter-atomic vectors
**Reciprocal space:** Convolution of SF **2**

Determine the best position \( r \) at which \( T \) shows a maximum for the oriented model
Intra- and Inter-Molecular Vectors

Model
Human lysozyme
One asymmetric unit in unit cell (P1 symmetry)

Data
Dinosaur lysozyme
Crystallographic symmetry

Rotation fn: Match intra-molecular vectors
Translation fn: Match inter-molecular vectors
Another Translation Function

Model
Human lysozyme

One asymmetric unit in unit cell (P1 symmetry)

Packing function: Discrepancy between the calculated and observed SFs

Position which gives the minimum discrepancy factor is the best position of the molecule in the unit cell
Averaging Using Inherent Symmetry

No of faces: 12 (5-folds)
No of edges: 30 (2-folds)
No of vertices: 20 (3-folds)
T=3 Icosahedron
Averaging

Amplitude

Position

Noise

Signal

25 2 35
26 1 29
28 2 32
20 4 28
30 5 34
26 7 32
20 0 31
19 3 25
Averaging and Iterative Improvement of Phases

Amplitudes + initial phases $\rightarrow$ FT $\rightarrow$ Noisy initial Atomic map $\rightarrow$ Average $\rightarrow$ Cleaner atomic map

Better phases (Combine with Amplitudes) $\rightarrow$ FT $\rightarrow$ Better atomic maps $\rightarrow$ Average
Poor Initial Phases
Improved Phases after Cycles of Averaging
Display of Electron Density

Electron density maps are displayed as iso-contour figures

Electron density sections
Refinement of Atomic Model

Poor initial phases => $F_c$ does not agree with $F_{obs}$

Discrepancy or R-factor = \[
\sum_{h\ k\ l} \frac{|F_{obs}| - |F_c|}{|F_c|}
\] is high

- Adjust atomic model to the poor initial electron density
- Fourier invert $F_c$s and refined phases to get more well defined electron density
- Better $F_c$s, phases & R-factor
- Better phases
- Compute $F_c$ with the better atomic model
Refinement is Like Curve Fitting

Fit the best 2-parameter curve through 9 points

No. of observations: 9
No. of parameters: 2

Observational equations

\[ y_1 = a_{11} x_1 + a_{12} x_2 \]
\[ y_2 = a_{21} x_1 + a_{22} x_2 \]
\[ \ldots \]
\[ y_9 = a_{91} x_1 + a_{92} x_2 \]

Ax = y, where A is a (9x2) matrix that gives the best estimate of the parameters x to match the observational column vector y

Minimize \((y-Ax)'(y-Ax)\) to get the best estimates of the parameters x
Normal Equations in Refinement

Observational equations: \( A\psi = b \)

- \( A \): Matrix that calculates the \( F_c \)s from the positional and the thermal parameters \( \psi \)

- \( b \): Experimentally obtained structure factors \( F_{obs} \)

Residual vector: \( r = b - A\psi \)

Minimize the objective function \( M = r^\top r \)

\[
\frac{\partial M}{\partial \psi} = 0 \implies A^\top A\psi = A^\top b
\]

Normal equations

Observational equations are non-linear in parameters:
\( A(\delta\psi) = b \)

\[
A^\top A\delta\psi = A^\top b
\]

Conjugate Gradient
Full LSQ Refinement not Possible

Refinement parameters: Positional and thermal

Protein of 30kDa: ~300 residues => ~3000 atoms
(3 positional + 1 thermal parameter) / atom
12000 refinement parameters.

Unit cell: 80x70x40 Å => ~16000 reflections within
the 2Å Ewald sphere

Overdeterminacy ratio = # observations / # parameters
= 16K/12K ~ 1.4 (Inadequate for LSQ refinement)

Introduce geometric and energy restraints/constraints
(Restrained/constrained least squares refinement)
Anisotropic B-values

Thermal Ellipsoids

\[ B_{\text{isotropic}} = \text{const} \times \langle u^2 \rangle \]

http://www.crystalimpact.com/diamond/v2feature-ellipsoids.htm
**$R_{\text{free}}$ in Refinement of Atomic Models**

The **Discrepancy or R-factor** is given by:

$$\text{Discrepancy or R-factor} = \sum \frac{|F_{\text{obs}} - |F_c|}{|F_c|}$$

**Q:** How good does this model predict the data that it has not seen?

**Curve fitting analogy:** Does a 4th pt lie on a 3-pt quadratic curve?

**$R_{\text{free}}$:** Choose a random 5-10% test data sub-set and calculate R-factor for this test set.

**If the model is good, $R_{\text{free}}$ should closely follow R of the remaining data set.**
Refined Electron Density

Poorly defined ed

Lack of clear density

Uninterpreted density

Conformers in Lys, Asn...

http://www.usm.maine.edu/~rhodes/
Manual Editing Using Graphics

Disordered Lysine

Manual model editing is necessary
Concluding Remarks

- X-ray diffraction may be analyzed by FT/series. Alternative methods also reported

- Phases may be determined using several methods

- Phases may be improved by averaging and/or refining the atomic model with model fitting

- Well-refined models are essential to correctly interpret biological functions
Resources

