# Putting it All Together

- After initial phasing, we should have a rough idea of electron density

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

- Undoubtedly there will be errors: must refine structure, iteratively calculate phases, refine more, etc.

# Building a Structure

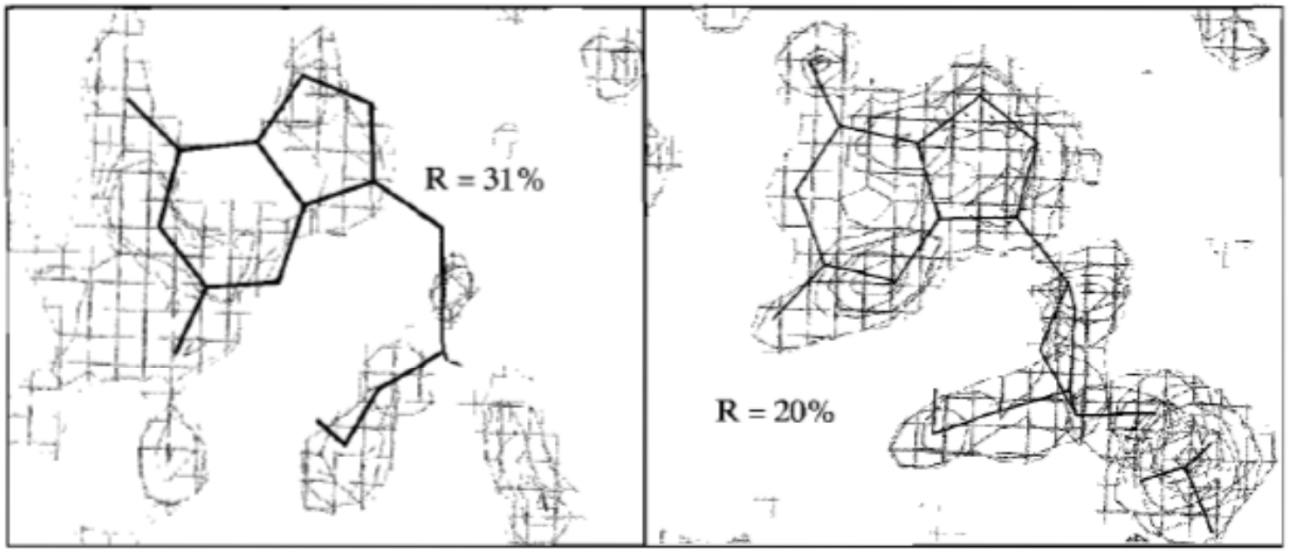



**Figure 6.31** Effect of refinement on structure. The guanine nucleotide of a DNA fragment is shown with its electron density map prior to refinement and after refinement. Prior to refinement, the *R* factor is 31%. The structure is refined against the data to an *R* factor of 20%, which is one criterion of a good fit of the model to the data.

- **At first:** look for gross structural features (helix, backbone), then add side chains
- Molecular mechanics are used to help refine positions

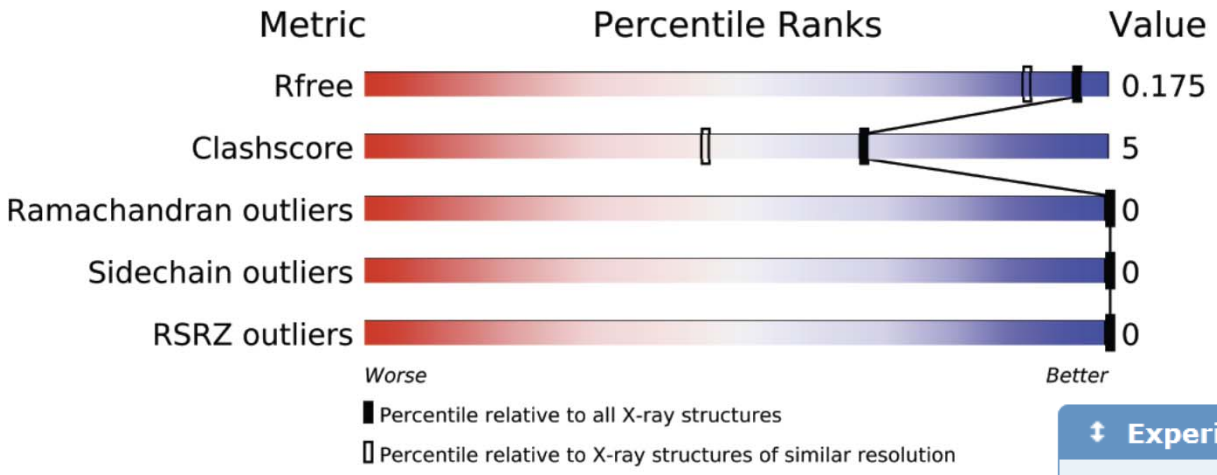
# Keeping Yourself Honest

- **Assessment:** Compare calculated intensities to observed intensities:

$$R = \frac{\sum ||F_{obs}| - |F_{calc}||}{\sum |F_{obs}|}$$

- **Better:** Leave some reflections out (5-10%) initially and compare those to computed values ($R_{free}$)
  - It's possible to fool yourself without independent validation

# Assessing a Crystal Structure: 3TJW




**Experimental Details** Hide

**Method:** X-RAY DIFFRACTION
**Exp. Data:**
Structure Factors
EDS

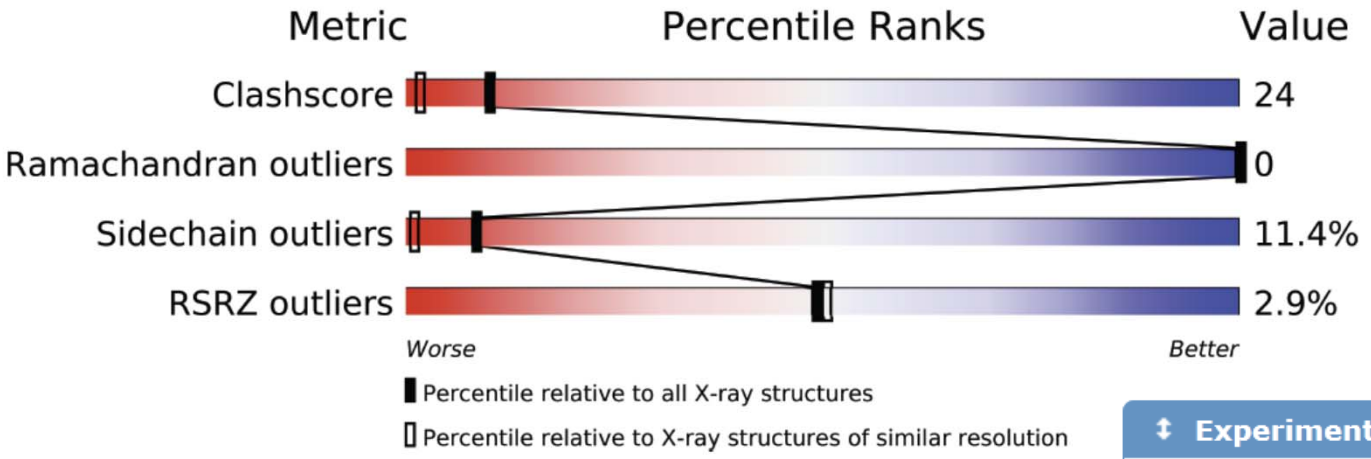
| | |
|---|---|
| **Resolution[Å]:** | 1.46 |
| **R-Value:** | 0.152 (obs.) |
| **R-Free:** | 0.190 |
| **Space Group:** | F 2 2 2 |

**Unit Cell:**

| Length [Å] | Angles [°] |
|---|---|
| a = 38.66 | α = 90.00 |
| b = 88.11 | β = 90.00 |
| c = 88.66 | γ = 90.00 |

- PDB contains a lot of useful information for determining how good a crystal structure is
- **Things to look at:** R, $R_{free}$, resolution, structure validation

# Assessing a Crystal Structure: 1SNC




**Experimental Details** Hide

**Method:** X-RAY DIFFRACTION
**Exp. Data:**
BMRB
Structure Factors
EDS

| | |
|---|---|
| **Resolution[Å]:** | 1.65 |
| **R-Value:** | 0.161 (obs.) |
| **R-Free:** | n/a |
| **Space Group:** | $P\,4_1$ |

**Unit Cell:**

| Length [Å] | Angles [°] |
|---|---|
| a = 48.00 | α = 90.00 |
| b = 48.00 | β = 90.00 |
| c = 63.50 | γ = 90.00 |

- Not every protein will have $R_{free}$; why?

# **The Ultimate Test:** Look for Yourself

- PDB recommends crystallographers submit all structure factors (intensities)
- Using the PDB structure, can calculate phases and density map
  - Not difficult, but not trivial either
  - Electron Density Server (EDS) does this for you
  - **For PyMOL:** $2F_o$-$F_c$ Map, CCP4 or CNS format
- Load map into PyMOL; does model hold up?

# Guidelines for Quality

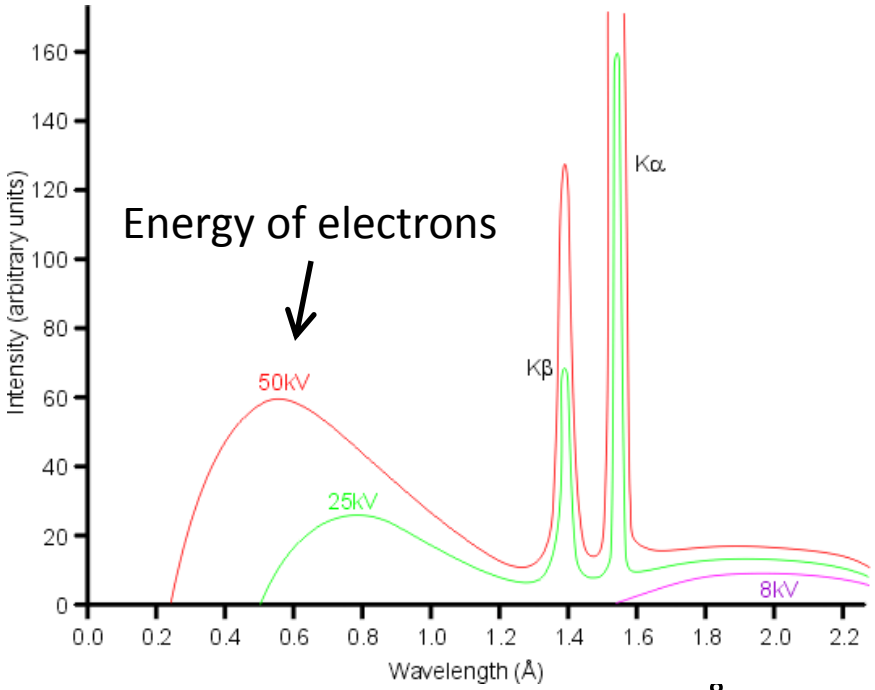
- **R-factor:** Less than 25% (ideally, less than 20%)
- **R-Free:** Bigger than R, but smaller than 25%
- **Resolution:** Less than 2.5 Å, but think about how much you need (1.5 Å usually very good)
  - At ~1 Å hydrogens become visible
- **Validation:** No clashes, good torsions, etc.
- **Water**: 2-5 molecules / kDa

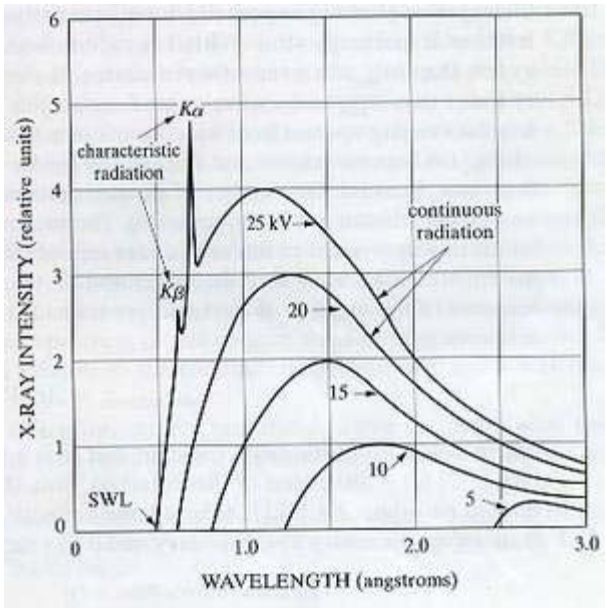
- Organic molecules will be considerably better

# Resolution

- What does it mean to have a structure solved to 1.5 Å?
- Smallest discernable d is determined by (n=1) $\lambda$ and $\theta$: $n\lambda = 2d \sin\theta$
- Practical: Detectors limited to $2\theta \approx 110^{\mathrm{o}}$

# X-Ray Radiation Sources



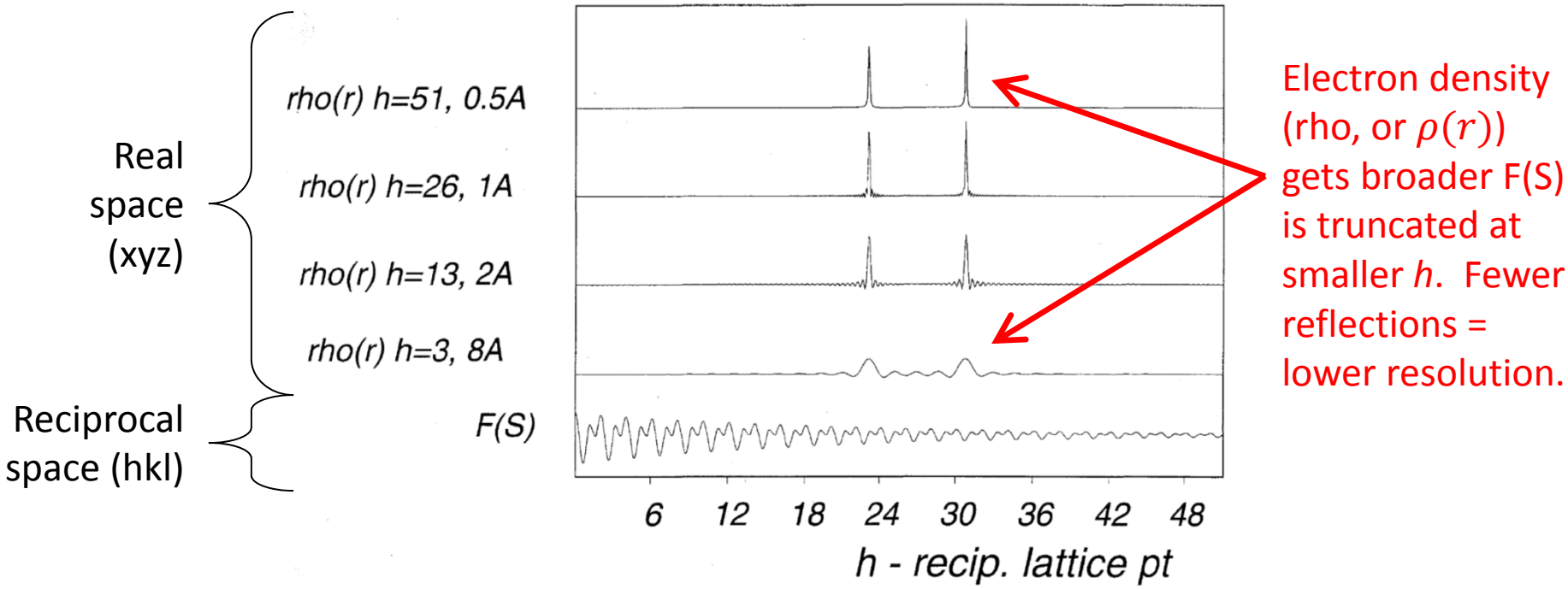

$CuK_\alpha$ Band: $\lambda = 1.54$ Å

$MoK_\alpha$ Band: $\lambda = 0.71$ Å

- Cu is better for proteins (reflections have finite size; Cu → more space between reflections)


"Generation of X-Rays." http://pd.chem.ucl.ac.uk/pdnn/inst1/xrays.htm
Varriano, John. "Physics III." http://facstaff.cbu.edu/~jvarrian/252/phys252.html

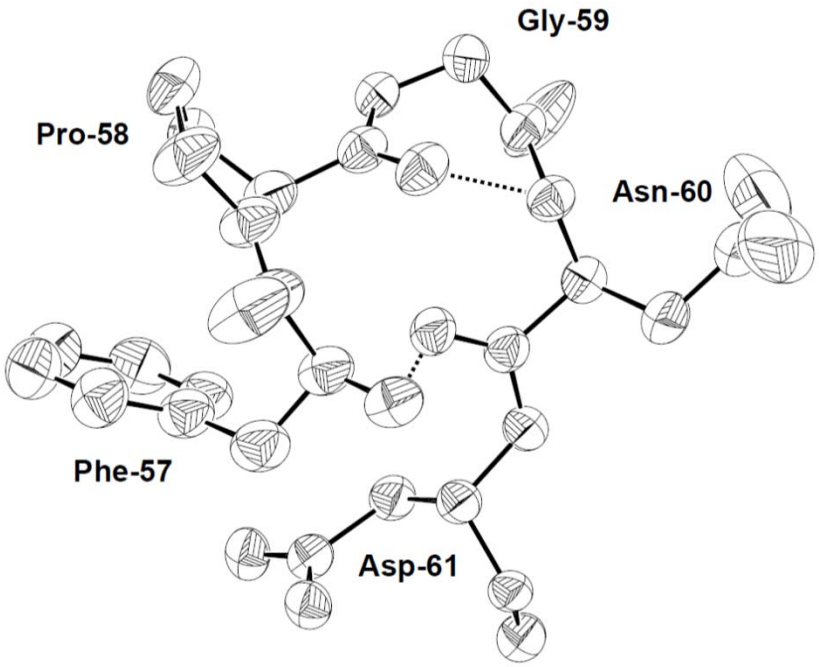
# How Many Reflections?




- "Truncating" number of reflections (e.g. $h < h_{max}$) will broaden electron density
  - Just like NMR, where fast decay of time signal leads to broader peaks in frequency domain

# **Uncertainty in x, y, z:** The B-Factor

- B-factor (temperature factor) accounts for broadening of electron density: thermal motion (and disorder)
- Ranges from 10-20 $Å^2$ (ordered) to 100+ $Å^2$ (disordered)
- Isotropic vs. anisotropic
  - High resolution required for anisotropic B-factors (< 1.5 Å)




Anisotropic Displacement Parameters (ADPs) represented graphically as ellipsoids

http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.112.4437&rep=rep1&type=pdf

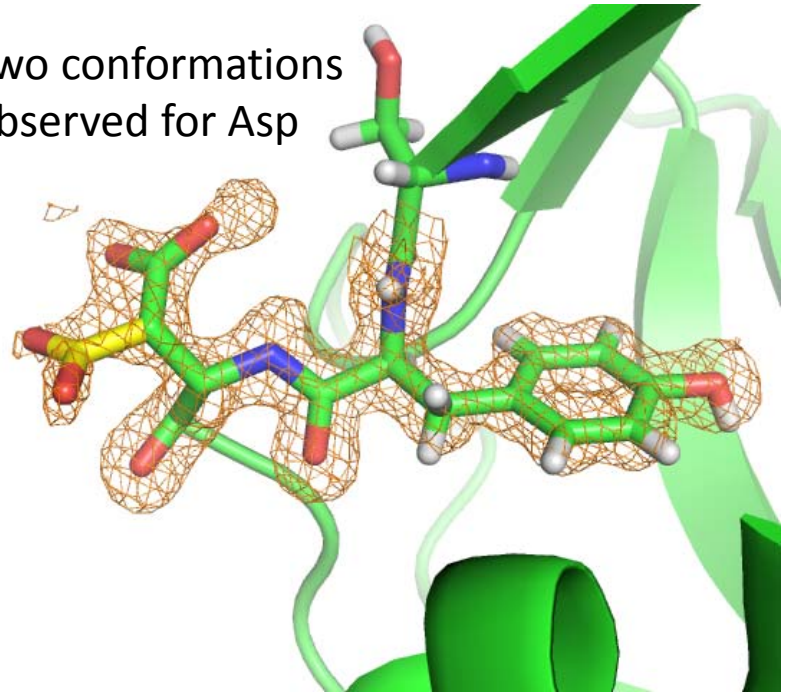
# Disorder in Crystals

- **Dynamic (Thermal) Disorder:** Atoms are rapidly moving around (especially loops), which "smears" electron density

- **Static Disorder:** Each unit cell has a different, fixed orientation

- Often difficult to distinguish these extremes!

Two conformations observed for Asp



Occupancy and "AltLoc" fields in PDB can resolve cases where multiple static conformations are observed

# Resolution: Implications

| Resolution Å | Structural Information |
|---|---|
| 4.0 | Global fold and some indication of secondary structure. |
| 3.5 | Secondary structure easily distinguished, large sidechains positioned |
| 3.0 | Most side chains are positioned, phi-psi angles are not well defined. May be possible to fit temperature factors |
| 2.5 | All side chains (except for the disordered ones!) are well defined, as are the phi and psi angles. Numerous water molecules are present in the model and the temperature factors are a reliable indication of disorder. |
| 1.5 | Backbone phi and psi angles are very well defined. Hydrogens can appear in the structure. Anisotropy can be detected in the temperature factors. |
| 1.0 | Hydrogens become apparent in the electron density map. |

Typical X-ray structures have a resolution of about 2.5 ÅProviding a considerable amount of biochemical information. There are a few structures that show a resolution of less that 1 Å.

# The Final

- **In class portion:** Friday, May 2 in HL 3324
  - Take as long as you like, but should be < 30 min.
- **Take home portion:** Distributed Friday, due Monday May 5
- **Not cumulative:** covers CD (March 5) through X-ray (April 30)

# Summary

- Refinement involves fitting atoms into density
  - Iterative, assisted by molecular mechanics
- Careful to validate structure: seeing is deceiving
- High resolution requires large scattering angle and many reflections
- Disorder can be both dynamic (B) and static