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

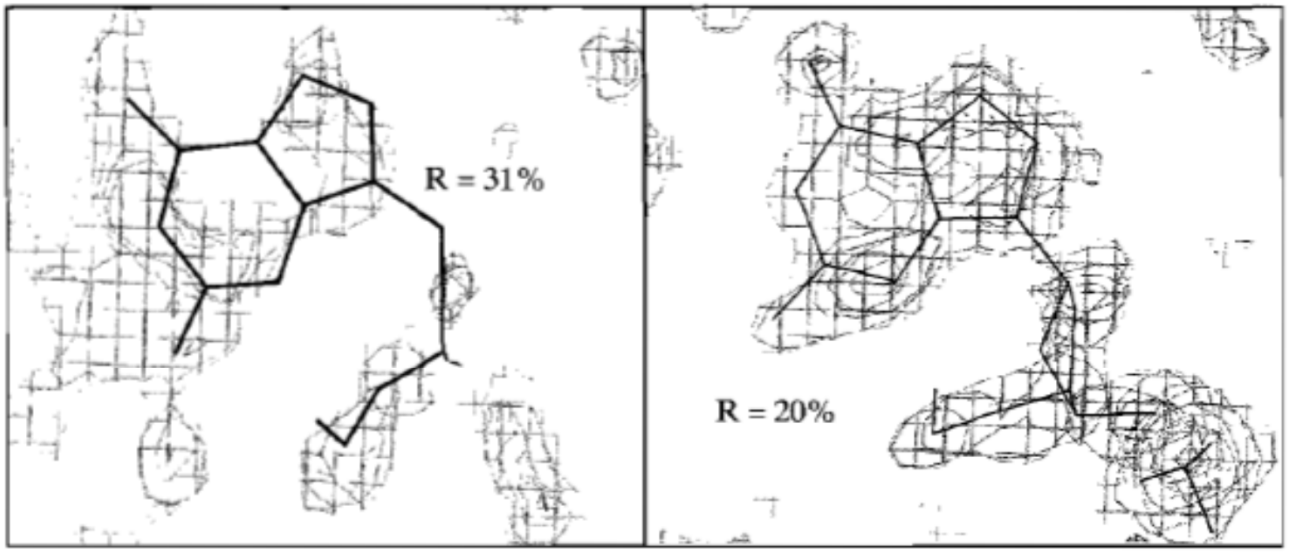


Figure 6.31 Effect of refinement on structure. The guanine nucleotide of a DNA fragment is shown with its electron density map prior to refinement and after refinement. Prior to refinement, the *R* factor is 31%. The structure is refined against the data to an *R* factor of 20%, which is one criterion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

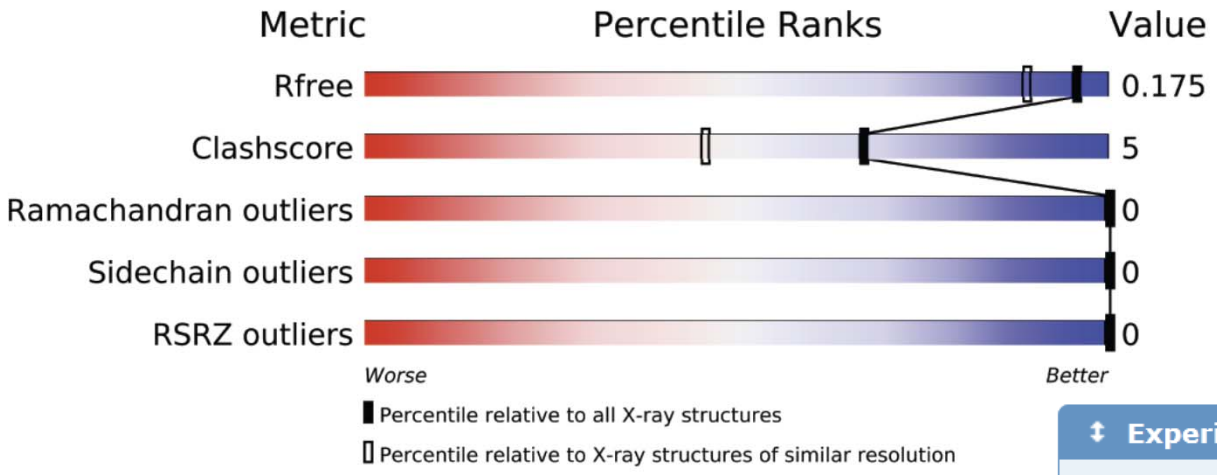
Keeping Yourself Honest

- **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

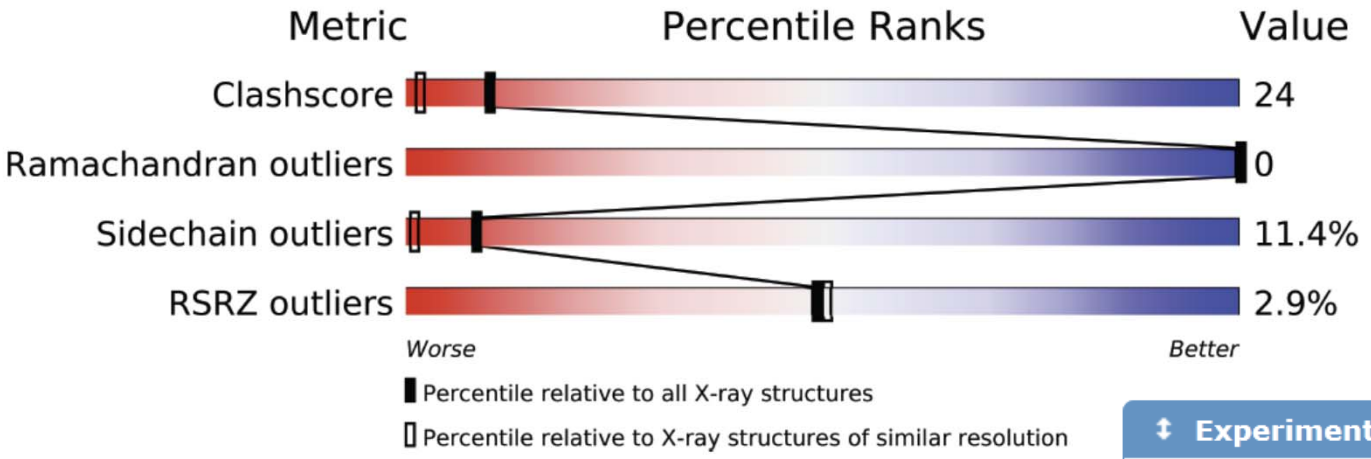
Resolution[Å]:	1.46
R-Value:	0.152 (obs.)
R-Free:	0.190
Space Group:	F 2 2 2

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

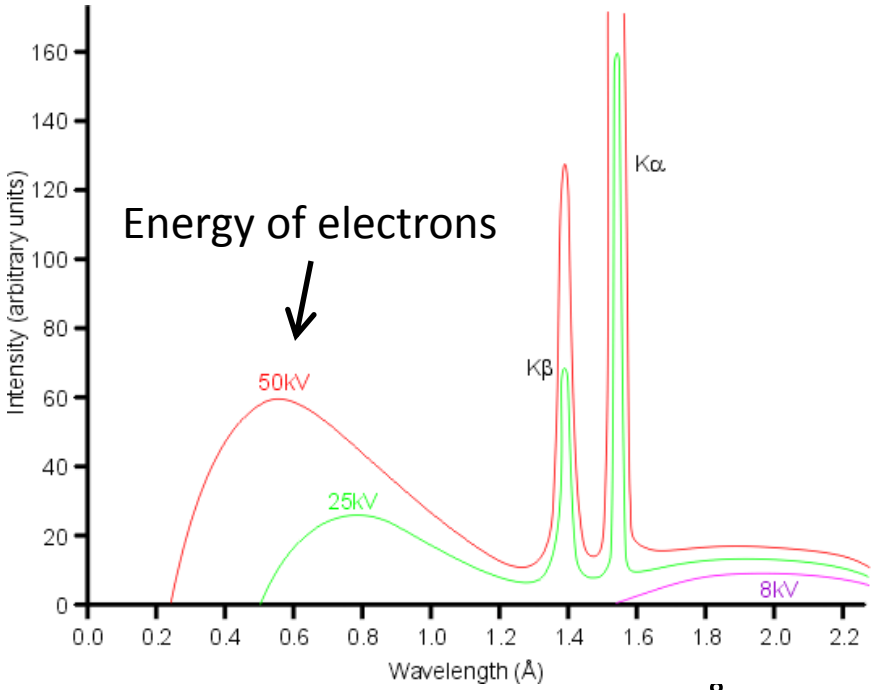
- **R-factor:** Less than 25% (ideally, less than 20%)
- **R-Free:** Bigger than R, but smaller than 25%
- **Resolution:** Less than 2.5 Å, but think about how much you need (1.5 Å usually very good)
  - At ~1 Å hydrogens become visible
- **Validation:** No clashes, good torsions, etc.
- **Water**: 2-5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

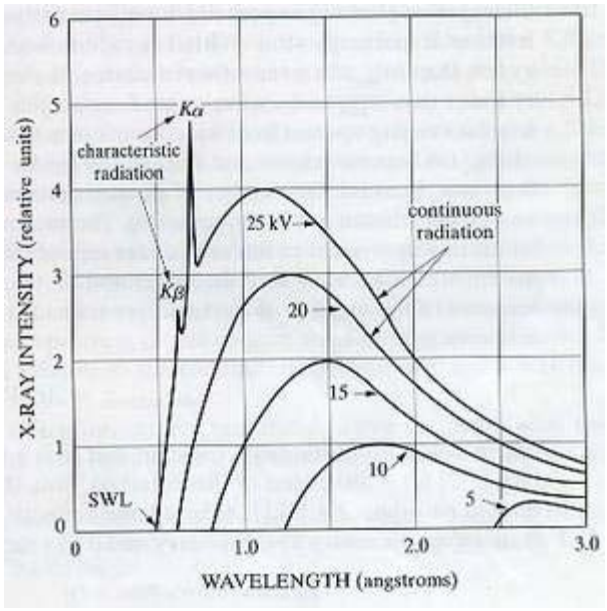
- Organic molecules will be considerably better

Resolution

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

X-Ray Radiation Sources





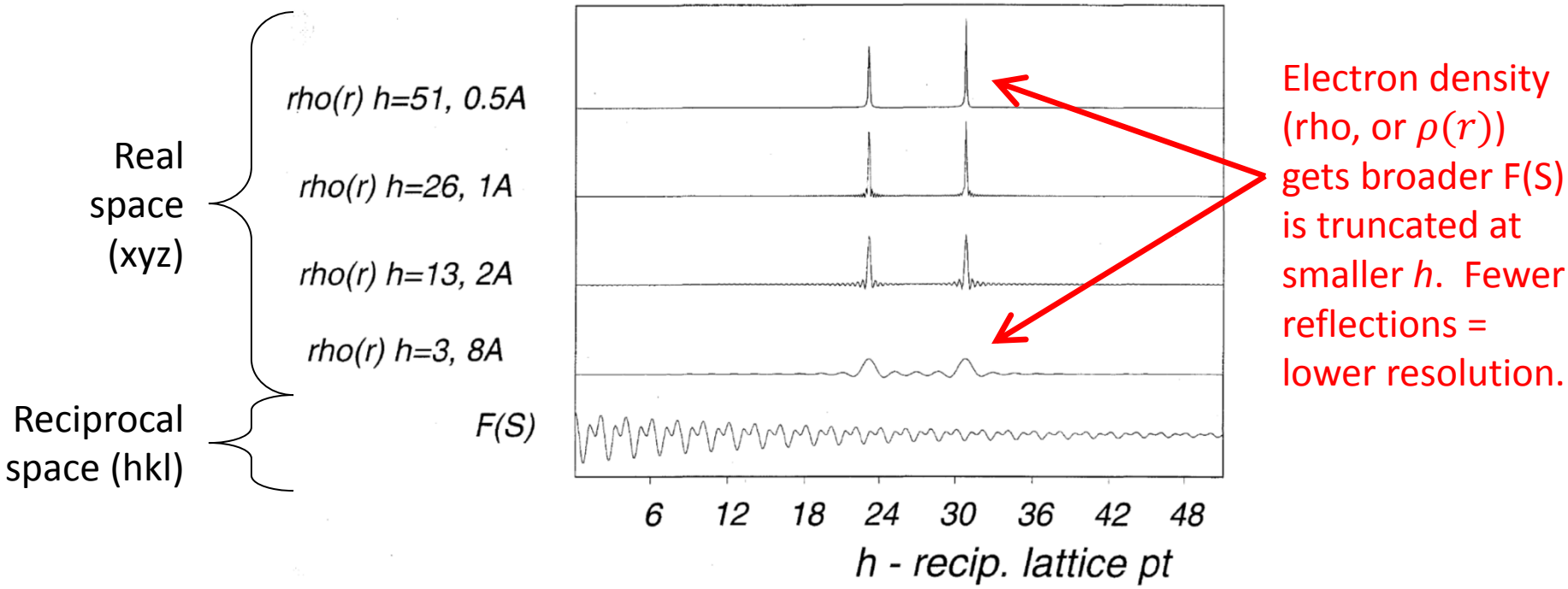
CuK_α Band: $\lambda = 1.54$ Å

MoK_α 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

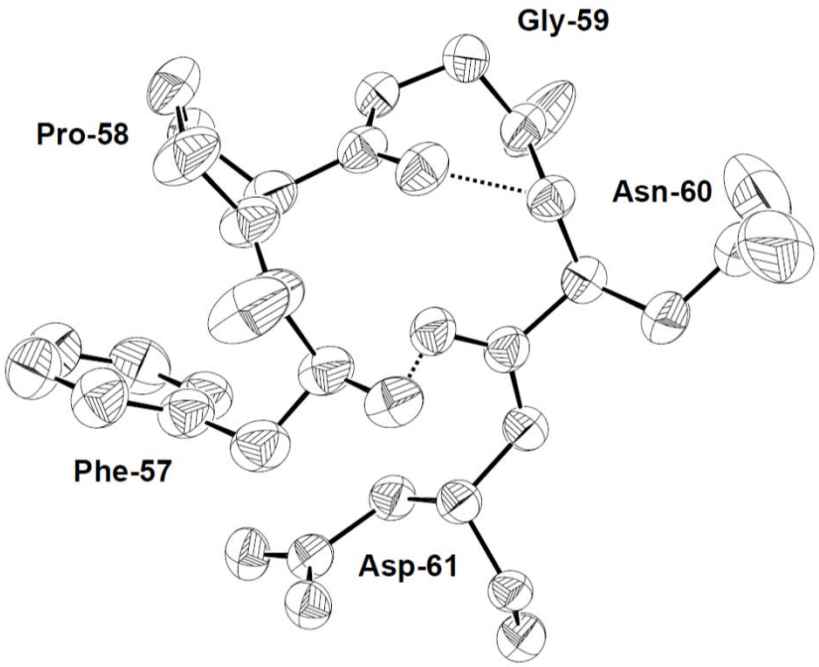
How Many Reflections?



- "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

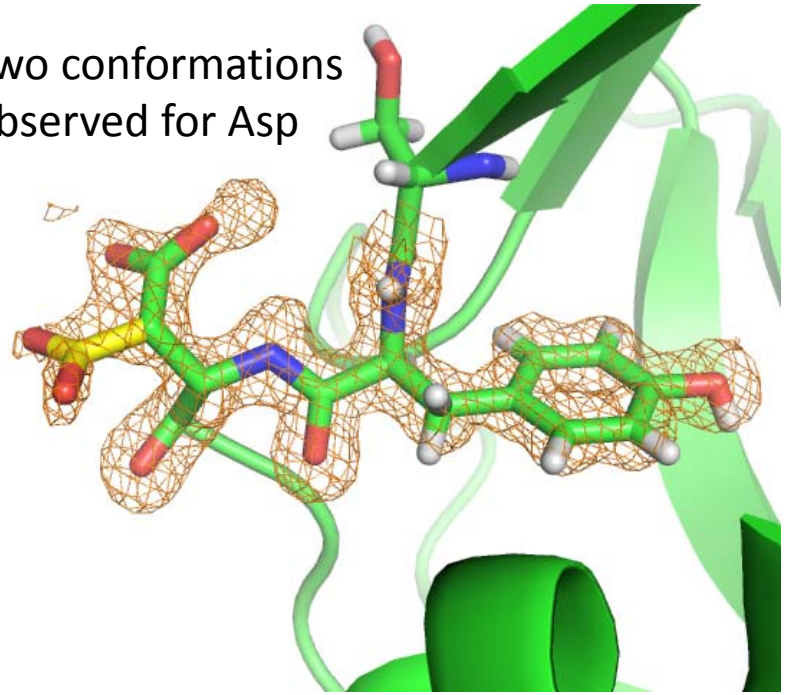
Disorder in Crystals

- **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

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