

Welcome to the SDSU Structural Biology Program!

Wednesday, November 12, 2008

7:00 - 8:00 p.m.

GMCS 305

<http://sci.sdsu.edu/sbp/>

Protein structure determination by X-ray crystallography

- Derive a source of material for study
 - Cloning, expression, purification
- Grow single crystals of subject
 - Crystallization
- Collect x-ray diffraction data
 - X-ray source, experimental design
- X-ray diffraction data processing
 - Convert spots to numbers

Protein structure determination by X-ray crystallography

- Solve the “phase problem”
 - MIR
 - MR
 - MAD/SAD
- Build and refine model
 - Computer graphics workstation
- Analyze structure
 - Propose and then test structure-based functional hypotheses

Protein structure determination by X-ray crystallography

- Build and refine model
 - Computer graphics workstation

The electron density map

- From last week we learned that one can build electron density maps from a relatively complete and accurate set of structure factors
- Structure factors contain amplitudes (taken from measured reflection intensities) and phases (derived experimentally by one of three methods)

A real space point in the unit cell

Summation

Phase

$$\rho(\mathbf{x}, \mathbf{y}, \mathbf{z}) = (1/V) \sum |F_{hkl}| e^{-2\pi i(hx + ky + lz - \alpha_{hkl})}$$

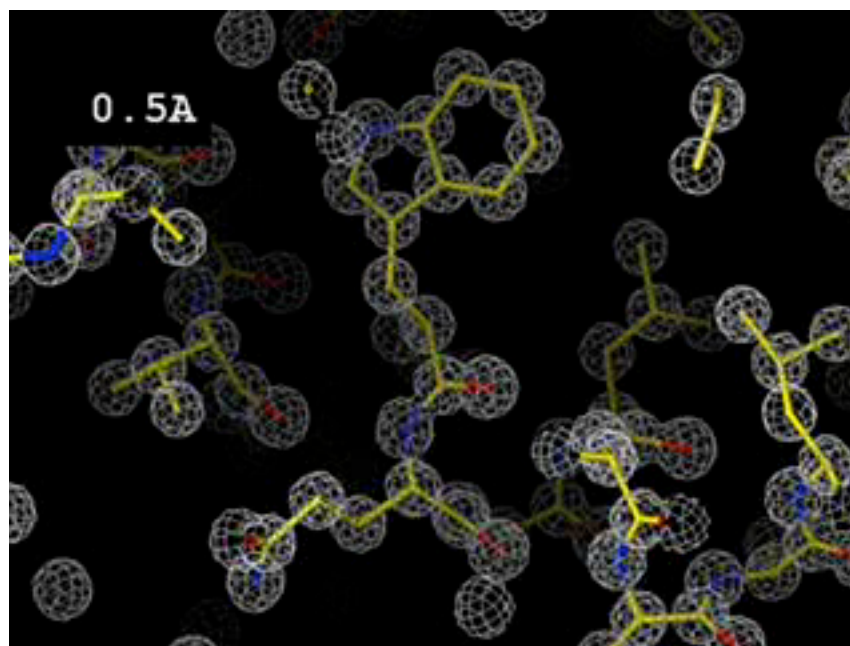
Electron density

Unit cell volume

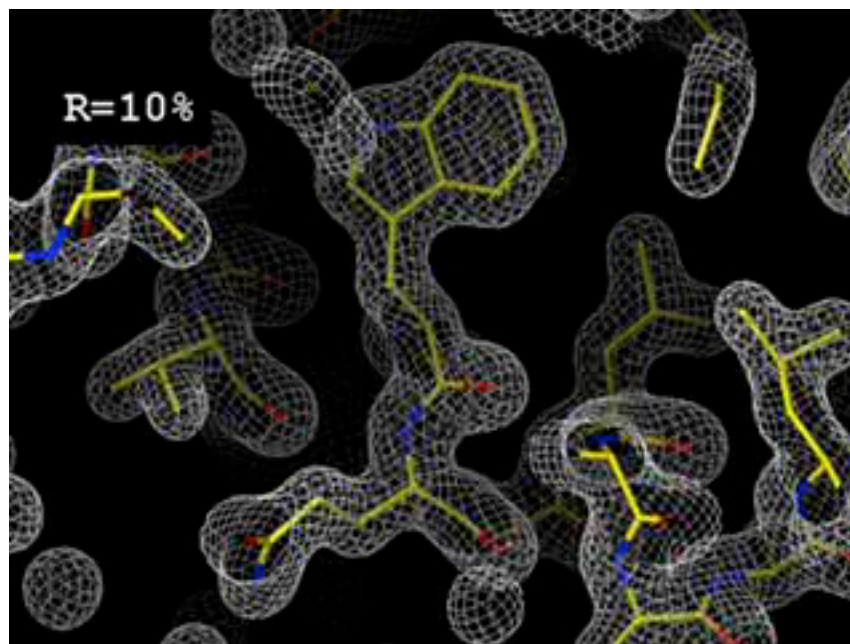
Amplitude

Miller indices

Increasing data resolution increases the amount AND the quality of structure information in the map synthesis

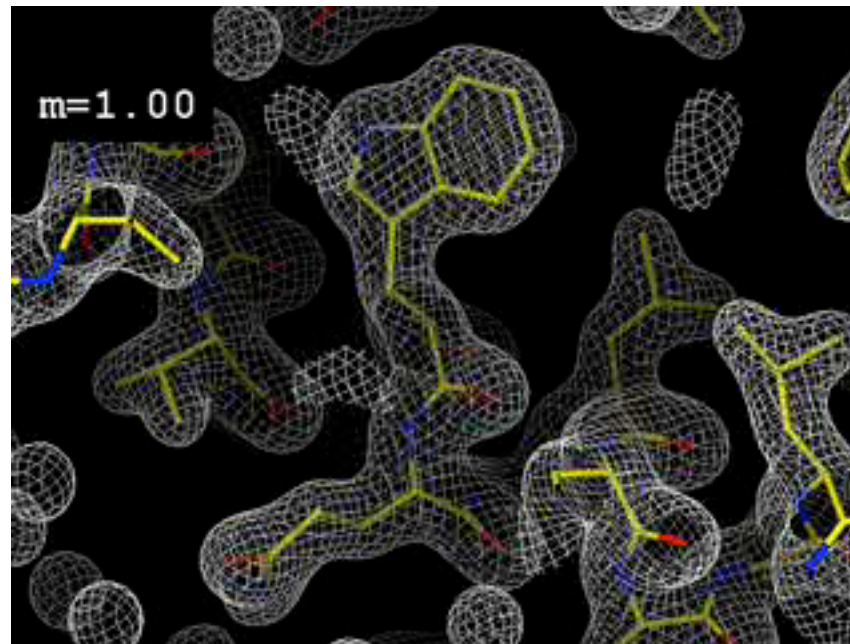


***Increased error in structure factor amplitudes
detrimentally affects map quality***



Phases remain perfect. Error in structure factor amplitudes is being calculated as an R-factor. Most completed refined structures at 1.5 Å resolution will have R-factors of <20%.

Increased error in structure factor phases detrimentally affects map quality



Amplitudes remain perfect. Data is merged with random phases and the cosine of the error (a number from 1 to 0 called “figure of merit”) is given as m.

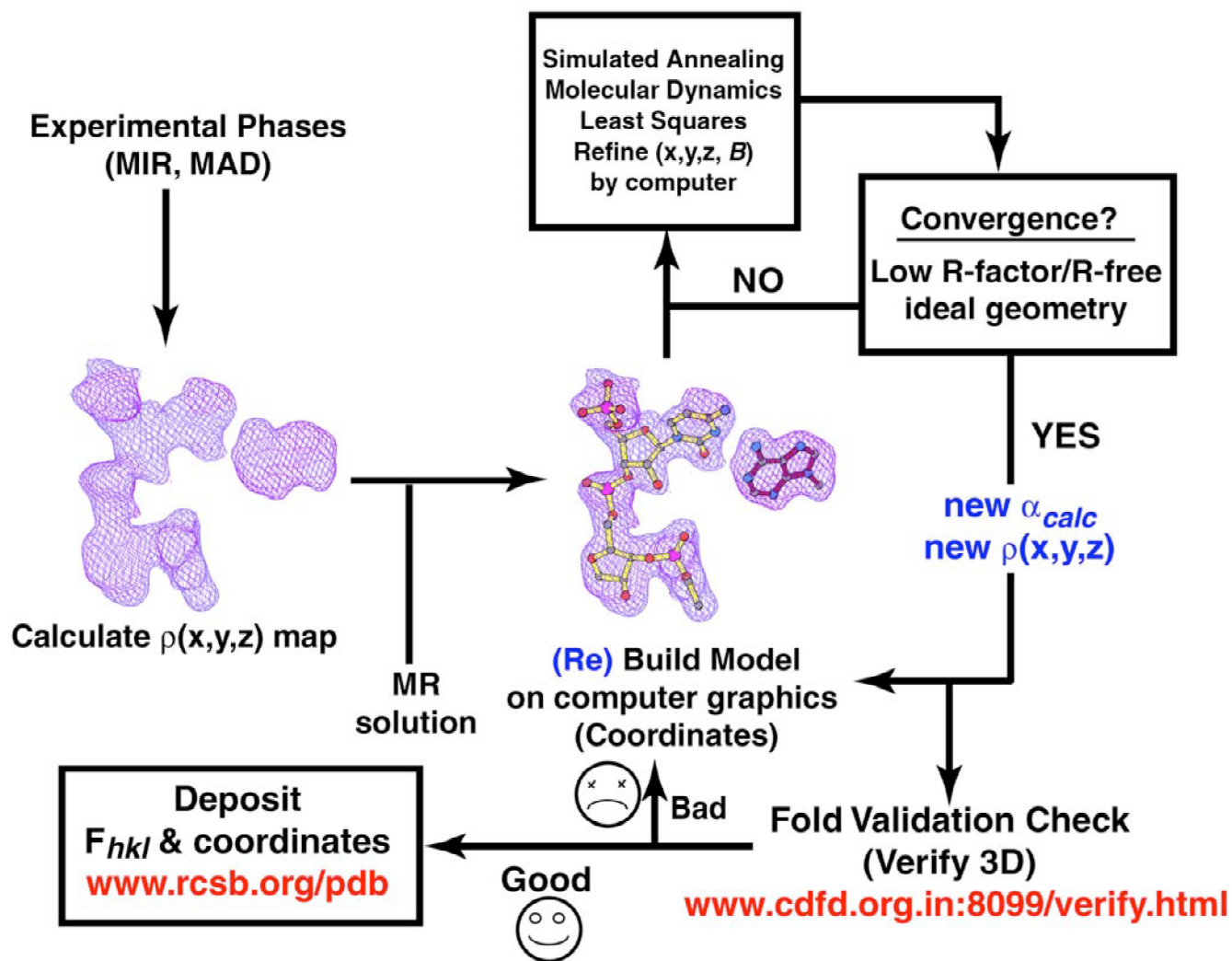
What does this teach us?

- Of the two values, correctness of phase has more consequence on the final map quality than amplitude
- However, our initial phases (derived experimentally by MIR, MR, MAD, or some combination of the three techniques) contains significant error
- As a result, we typically find ourselves working with relatively low quality initial experimental electron maps

Refinement

- An ITERATIVE (repeated where the output of one cycle becomes the input for the next) process of positioning atoms (the model) into an electron density map, using computational methods to improve upon the model, and then calculating a new set of phases from the model.

Crystallographic Refinement Flowchart



R-factor

- After each cycle of model building and refinement, a calculation of the error in the observed and calculated structure factors is measured.

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

This is known as the R-factor

Problems with R-factors

- During refinement, a crystallographer can “cheat” but putting atoms in every single blob of electron density that appears in a map whether or not it really represents anything in the crystal
- This quickly raises the number of fitted parameters (atomic positions) relative to the constant number of observables (reflections) and drives down R-factors

R-free

- Prior to any refinement a significant amount (5-10%) of reflection data are removed at random and not used during refinement
- The addition of new atoms and/or changes in model structure is accepted only if it results in a lowering of R-factor measured against both the working set (R-work) and the test set (R-free) of reflections

Assignment

- Go to the Swiss-Pdb Viewer website at:
<http://spdbv.vital-it.ch>

Download yourself a copy of the software
and a tutorial

- Go to the Biology Workbench at:
<http://workbench.sdsc.edu>
and register for your FREE account