The Wonderful World of Maps

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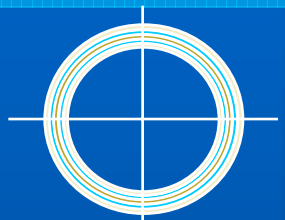
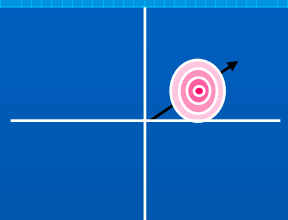
What Kinds of Maps are There?

- **Direct Maps**
  - **They are calculated from amplitudes and phases which have been inferred from the diffraction of the crystal or from a model.**
- **Difference Maps**
  - **Difference maps are used to highlight errors in your model.**
- **Composite Maps**
  - **These maps are a combination of a Direct Map and a Difference Map.**
- **Demonstration Maps**
  - **These special maps devised to prove some point. They emphasize freedom from bias at the expense, often, of clarity.**

Direct Maps

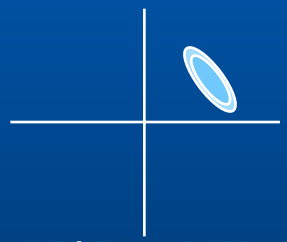


Fobs

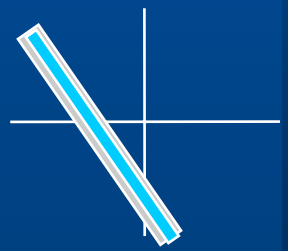


Fcalc

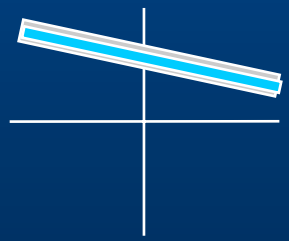
SIR



MAD or MIR

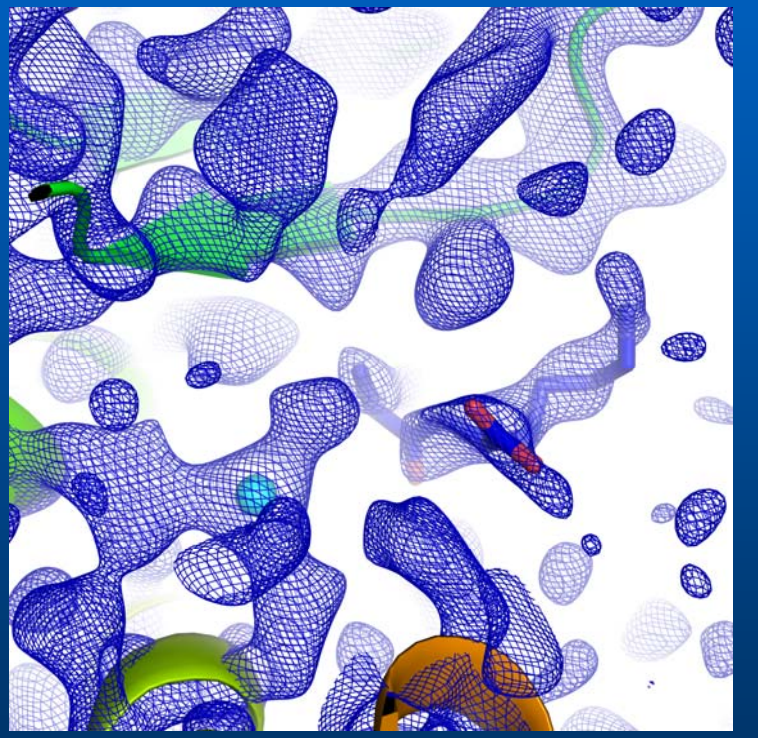


Solvent Flattening



NCS

Fourier Synthesis

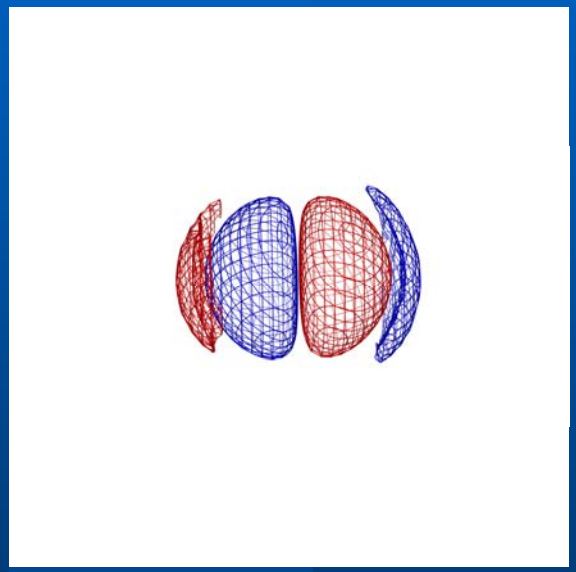


MIR Map: Thermolysin Active Site

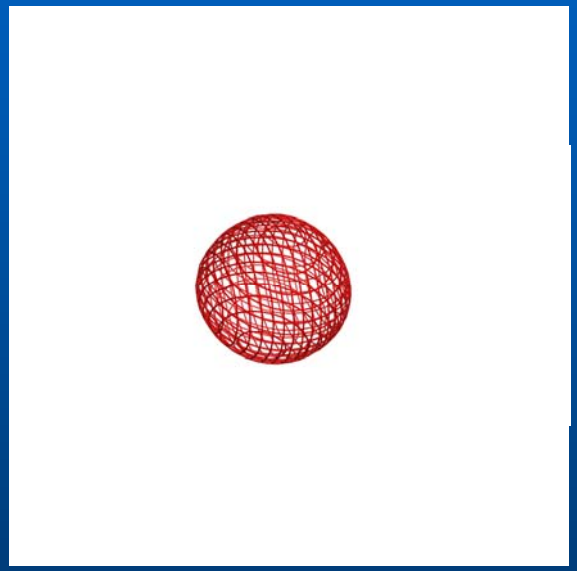
Difference Maps

- Provide a comparison between a model and the diffraction data.
  - They are the first step in finding its errors.
- They are intimately connected to your refinement program.
  - It uses difference maps to determine how to improve your model.
- The classic difference map is the Fo-Fc map. It is directly connected to the least-squares optimization function.
- They have positive density where your model should have more electrons and negative density where your model should have fewer electrons.
- All difference maps show the same patterns that indicate errors in your model.

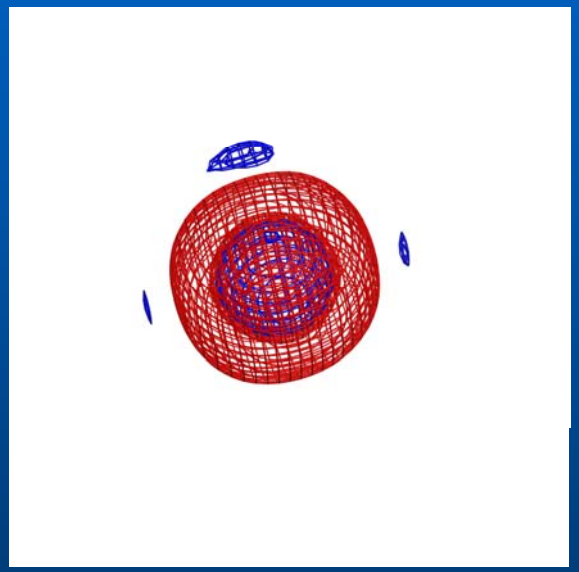
Difference Map Signatures of Model Parameter Errors



Error in Position



Error in Occupancy



Error in B Factor

Difference Map Features Look Like Atomic Orbitals

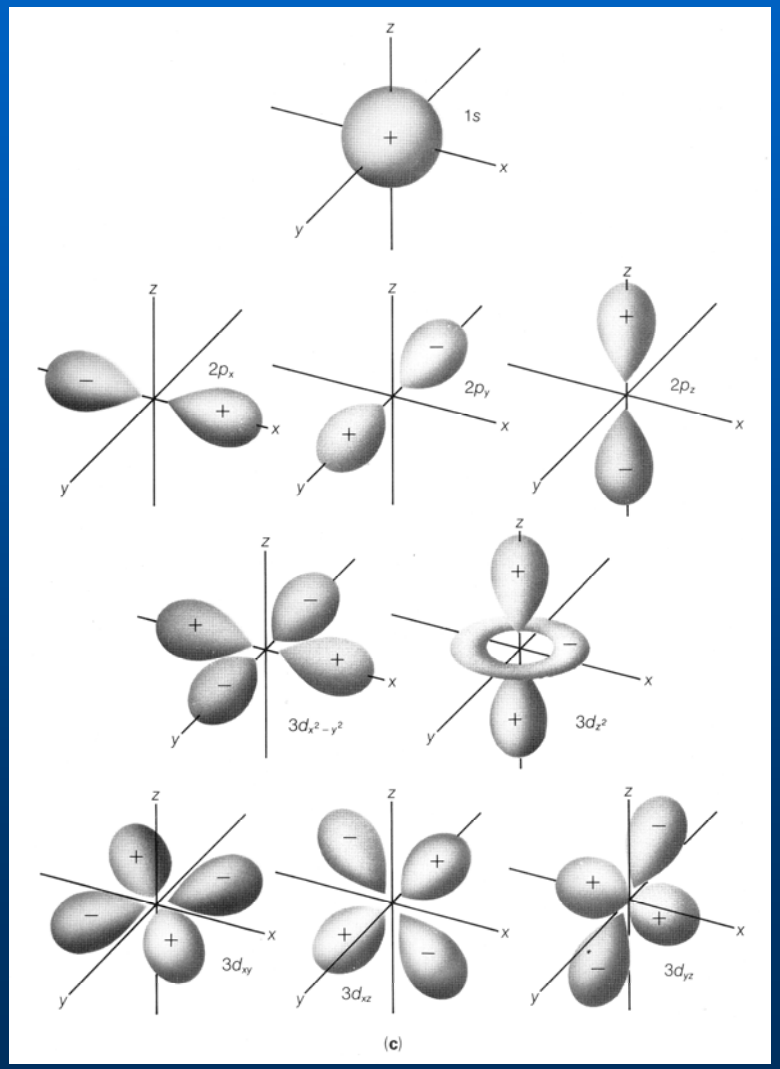
Occupancy errors look like 1s orbitals

B errors look like 2s orbitals

Positional errors look like 2p orbitals

Anisotropic B errors look like 3d orbitals

But only if you ignore series termination…



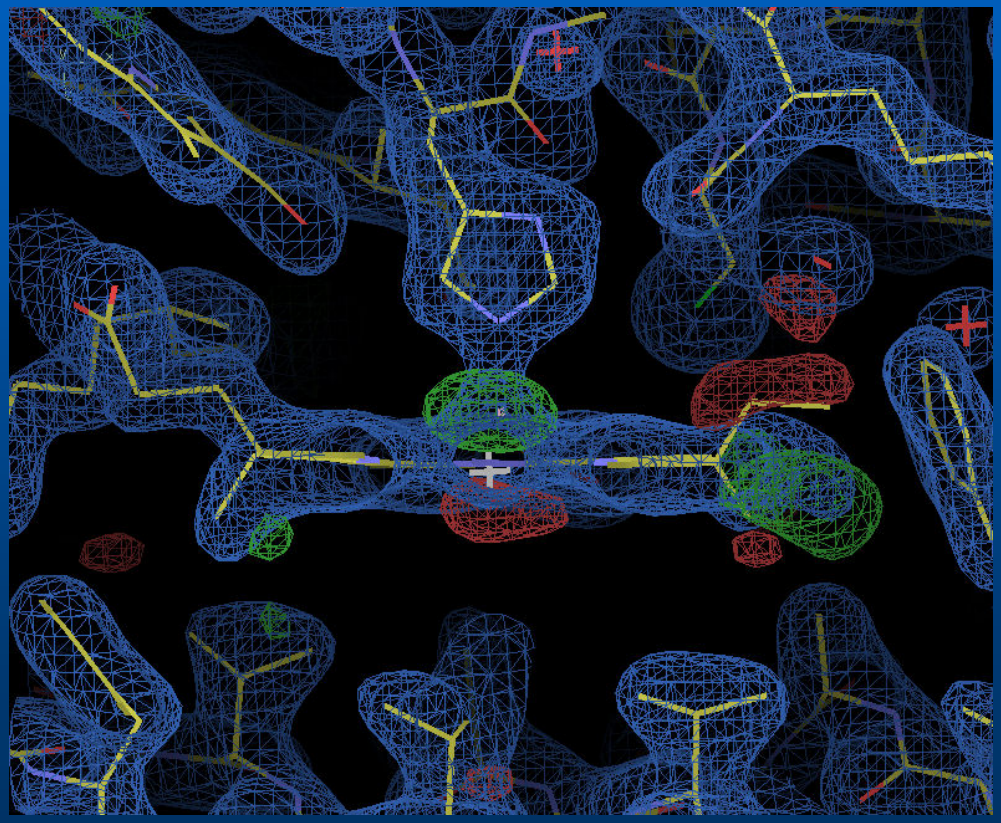
Eisenberg and Carothers – page 453

Interpreting Difference Maps

- **Following refinement you will see only those features the refinement program could not fix.**
  - **If you see a shift or B factor error signature in your map, you have to determine why the refinement program left it there.**
- **Positive peaks possibly indicate something is missing.**
  - **Probably water molecules could it also indicate alternative conformations, ions, or small molecules.**
- **Negative peaks are harder to interpret.**
  - **There should be none over atoms.**
  - **Otherwise, they could be due to scaling errors, poor bulk solvent model, series truncation, or just plain “noise”.**
  - **The average value of a difference map must be zero. There must be negative density to counter the positive peaks.**

A Difference Map with Positional Errors

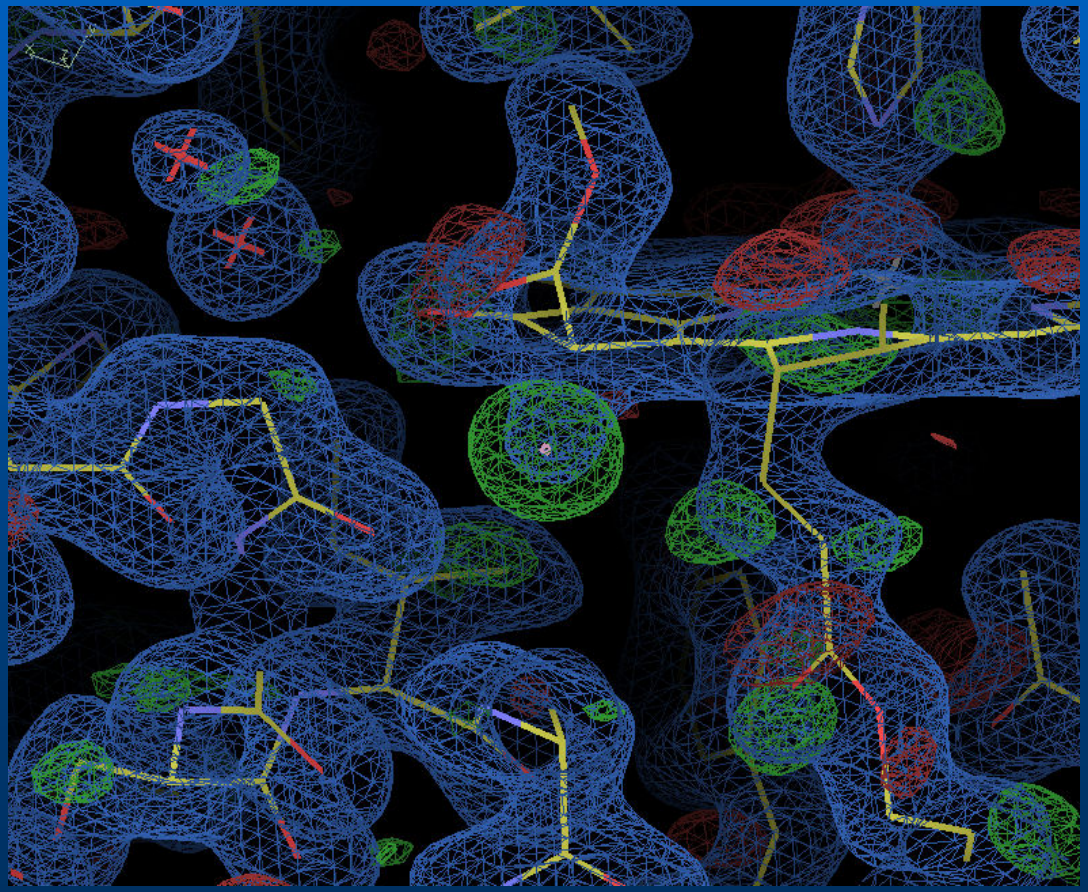
This map was calculated from PDB entry 1M50. The central magnesium atom was trapped on the wrong side of the Bchl-a molecule and refinement could not correct the problem.



1M50 has been replaced by 3ENI.

A Difference Map with Occupancy Error

This map was calculated from PDB entry 1RZH. There appears to be an extra atom covalently bound to this Bchl-*a* molecule.



Composite Maps

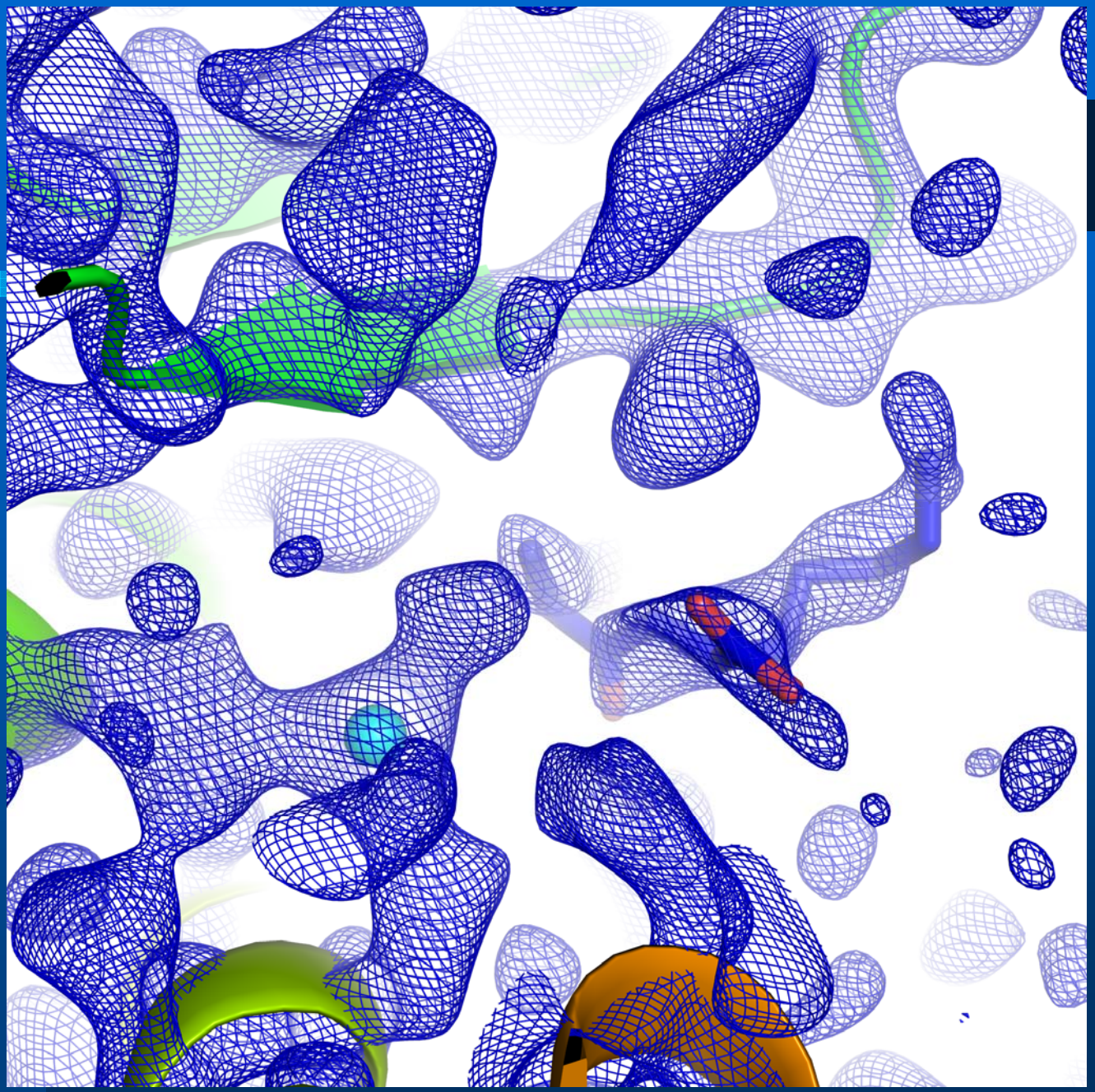
- One would like to use the improved phases from a refined model to calculate better maps than the Direct Map.
- To decrease this bias, a Difference map can be added to a Direct Map.
- The basic idea is to take an Fcalc map and add a Difference Map.

$$|Fcalc| \quad +(|Fobs| - |Fcalc|) \quad = |Fobs|$$

  - Actually, this does not work. Instead you have to add two difference maps for the correction in amplitude to overcome the lack of phase correction.

$$|Fcalc| \quad +2(|Fobs| - |Fcalc|) \quad = 2|Fobs| - |Fcalc|$$

- In maximum likelihood one would add the log likelihood gradient map to the sigma-a weighted Fcalc map. Your refinement program should do the calculation for you.



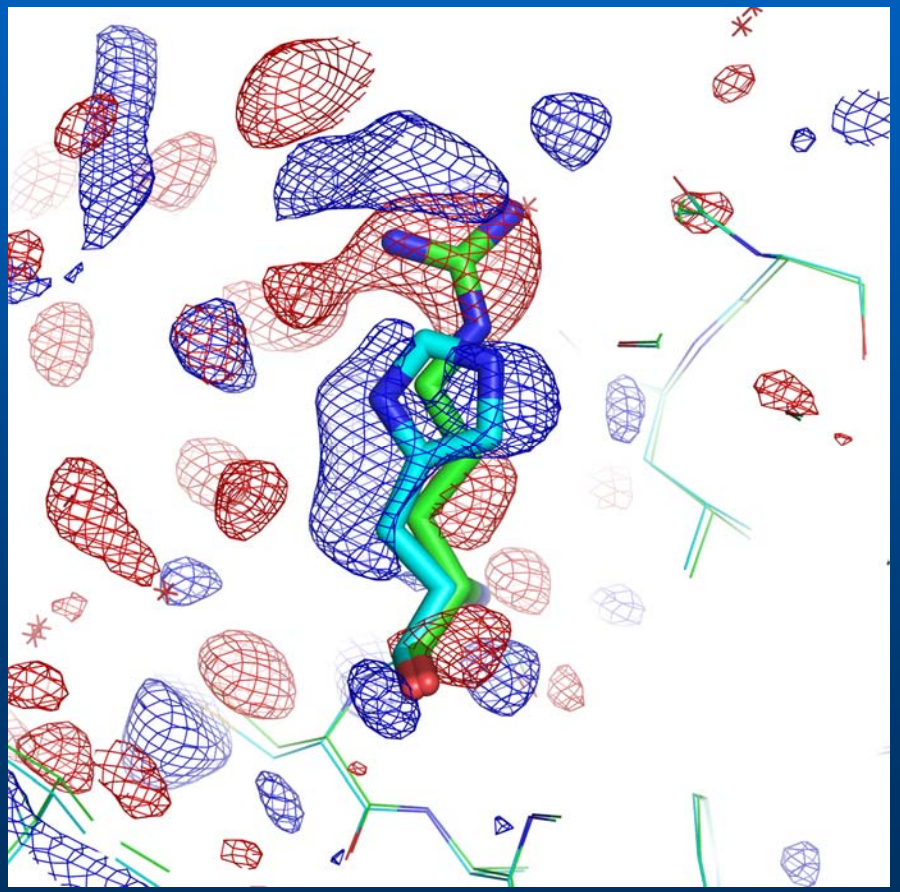
2Fo-Fc Map for Thermolysin at 1.6Å

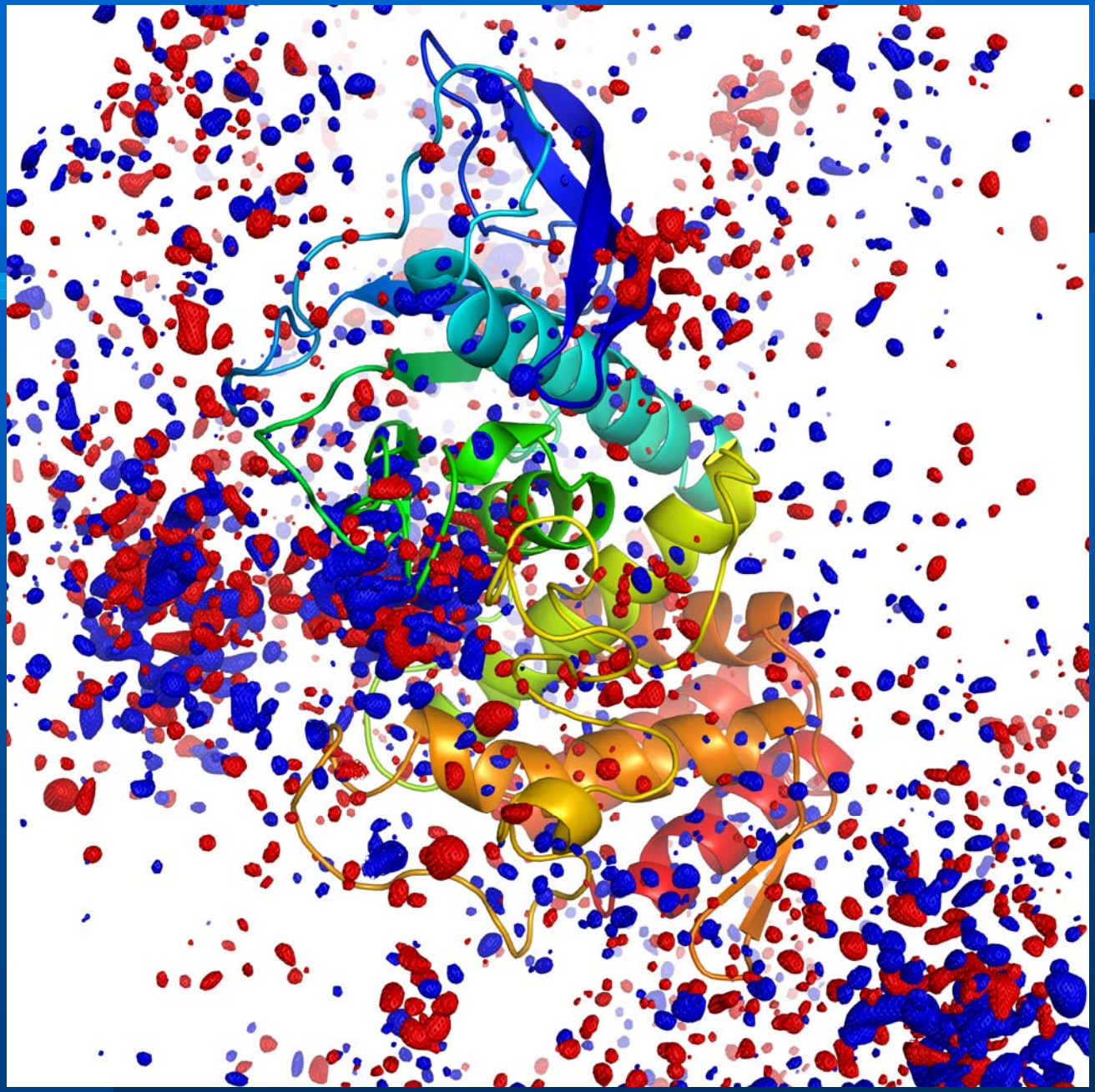
Demonstration Maps

- Sometimes you want a map to prove some structural feature, and avoid the charge of model bias.
- Principal examples are to prove that a compound has bound, or that you made the mutation you claim to have made.
- Often you will sacrifice interpretability for unbiasedness.
- The best is an “Fobs - Fobs” map
  - Fobs of the holo crystal minus Fobs of the apo phased either from experimental phases or calculated phases with the relevant part removed.
- The next best is an “Fobs - Fcalc” omit map.
  - Generally one refines the omit model to further remove bias.
- Additional effort can be made to reduce bias.
  - One strategy is to calculate a simulated annealing omit map.
  - Other methods to remove bias exist – Check your documentation!

Fo-Fo Map for T4 Lysozyme Mutant R96H

In the calculation of this map, Fo (blue) is the histidine mutant while Fo (red) is the wild type.





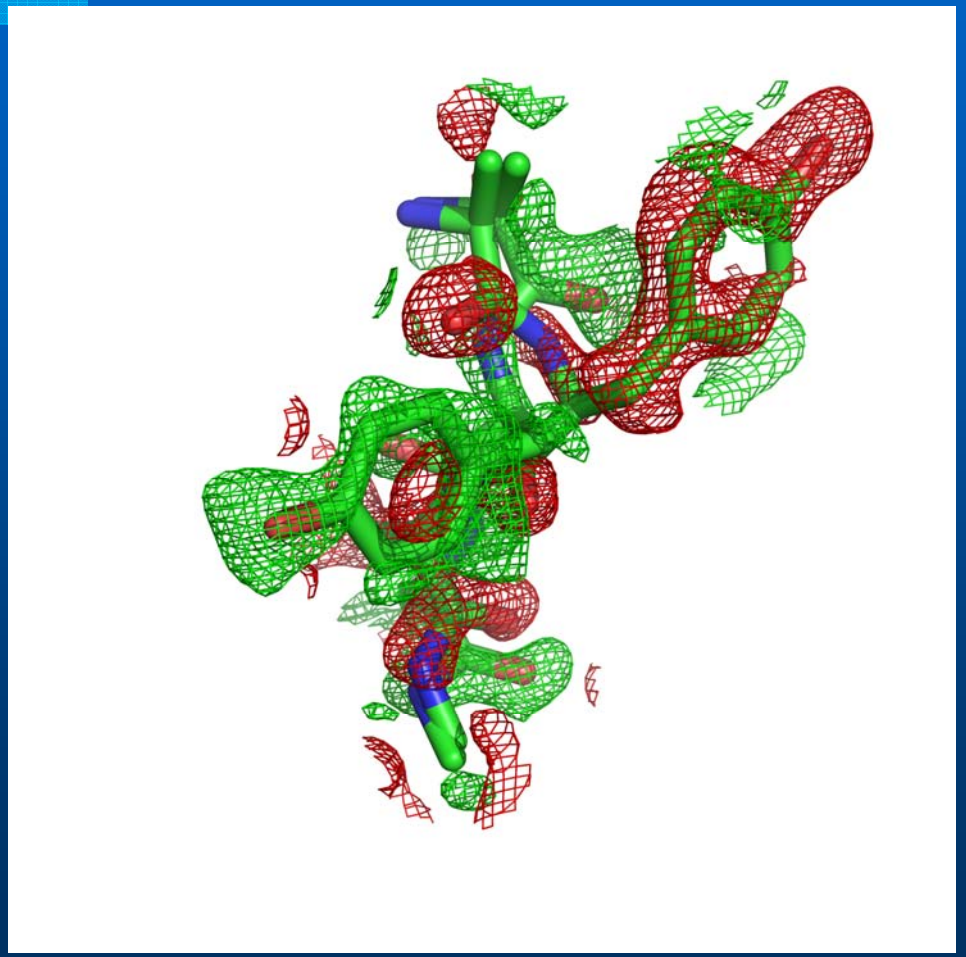
Check the entire map!

Fo-Fo Map for TLN:ZFPLA

Deconvoluting Complex Density

- **Density for structures with multiple conformations are difficult to interpret.**

- **The Fo-Fo map from two crystals with slightly different occupancies will provide detailed information about the deconvoluted electron density.**



Contour Levels

- The common practice of the field is to contour maps at 1 sigma and difference maps at ± 3 sigma.
- This habit throws away some significant information.
  - Once you have built a model, you can calculate maps on an absolute scale (*i.e.* electrons/Å^3). The absence of an atom should result in a peak of a particular height when expressed in e/Å^3, but will not be consistent when viewed in sigma's.
  - The sigma of a difference map will drop as refinement progresses, but that does not mean that peaks are becoming more significant.
  - The sigma of a density map will depend on the solvent fraction of the map calculated.

Final Point: Don’t Lie

- **If you are publishing a map, you should not mislead your readers.**
- **You must report exactly how the map was calculated, and what contour level was chosen.**
- **The “cover radius” or “carve” options can be used make the point of a figure clearer or to hide inconvenient truths.**