Low-resolution refinement

DLS-CCP4 Workshop

2019

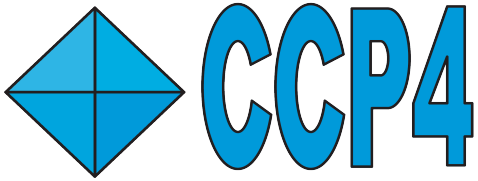
Diamond Light Source, Oxfordshire, UK

Oleg Kovalevskiy

oleg.kovalevskiy@stfc.ac.uk



Science and Technology Facilities Council



Restraints

How to improve the observation:parameter ratio.

1. Reduce number of parameters

2. Increase number of restraints

Particularly useful at low-resolution:

- Reflection intensities often noisy
- Limited data – poor observation:parameter ratio
- Refinement becomes unstable
- Overfitting – R-factors diverge

Increase number of restraints to regularise refinement

Jelly Body Restraints

Regularisers without a target:

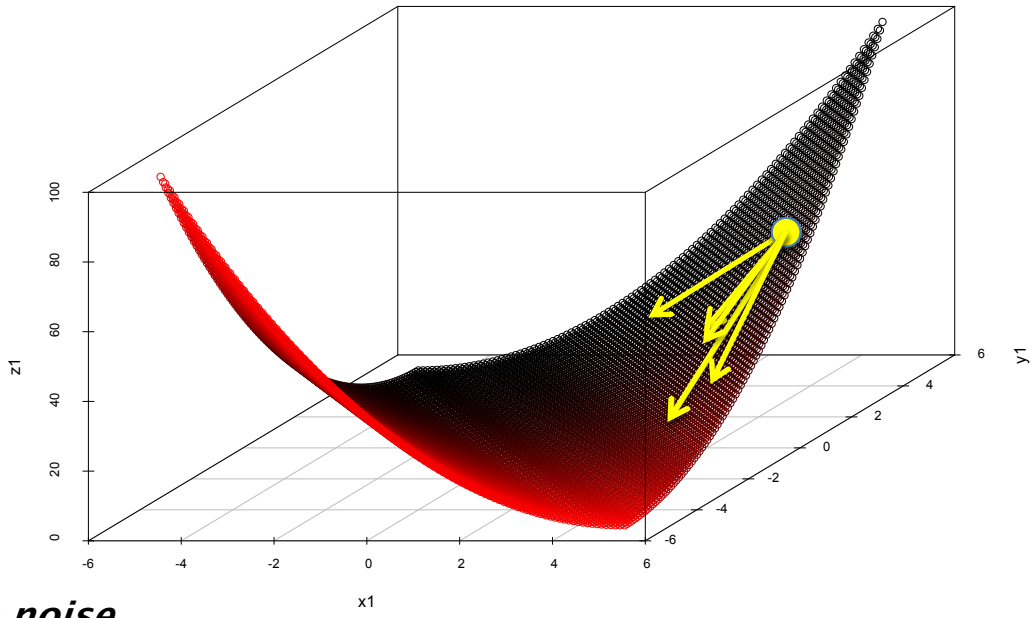
$$f = \sum_{\text{close atom pairs}} \frac{1}{\sigma^2}\left(d - d_{\text{current}}\right)^2$$

d : interatomic distance

d_{current} : current interatomic distance

σ : restraint standard deviation





Does not change likelihood function.

Does not change derivative.

Does change 2nd derivative – curvature.

Model should be less prone to fitting into noise

Should only work if parameters are near the minima (model is good)

Typical: σ = 0.01–0.02

Distance threshold: 4.2Å

ProSMART

Injection of prior knowledge to aid new structure determination

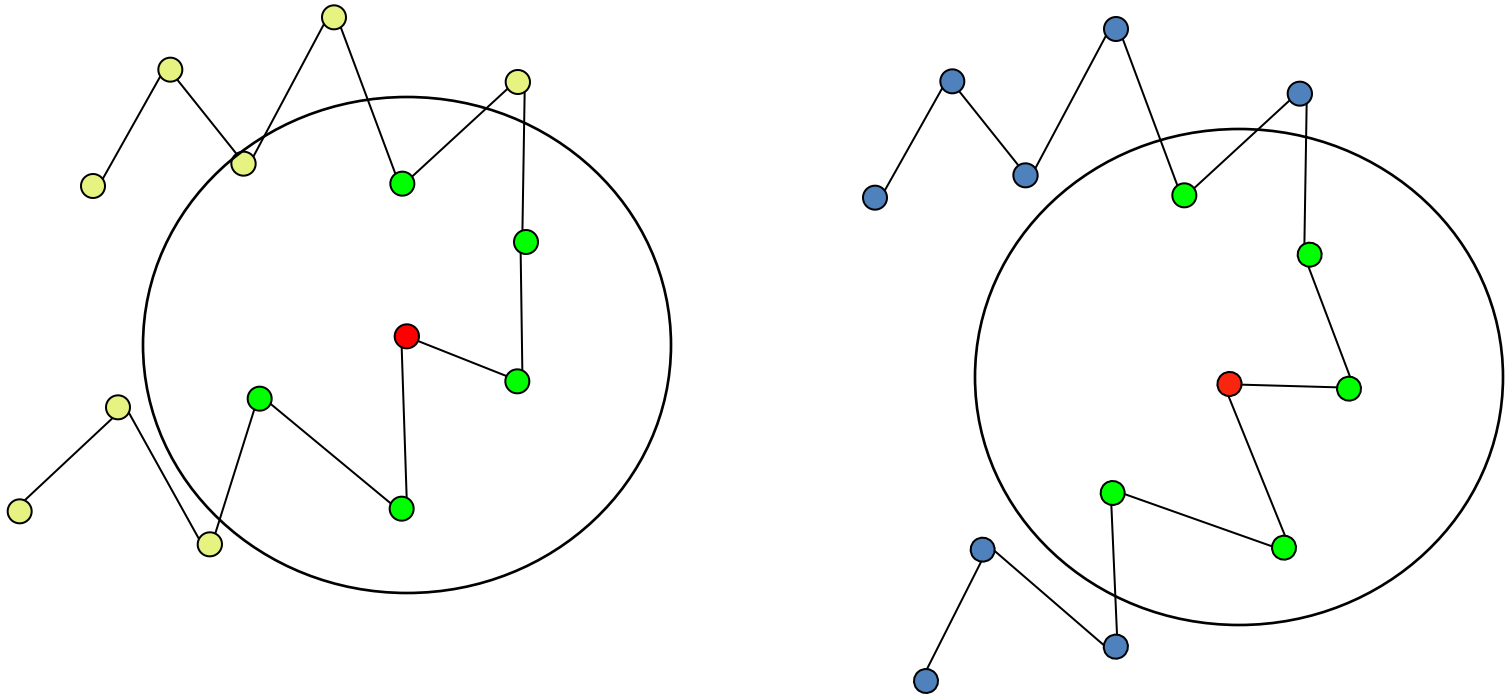
- **External Restraints from homologous structures**
  - Protein or nucleic acid chains
- **Hydrogen bond restraints**
  - Protein backbone
- **Generic self-restraints**
  - Everything – protein, nucleic acid, ligand, water
- **Structure analysis**
  - Alignment & comparison - helps analyse differences between models

Independent of global conformation

External Restraint Generation

structure to be refined

known similar structure (prior)

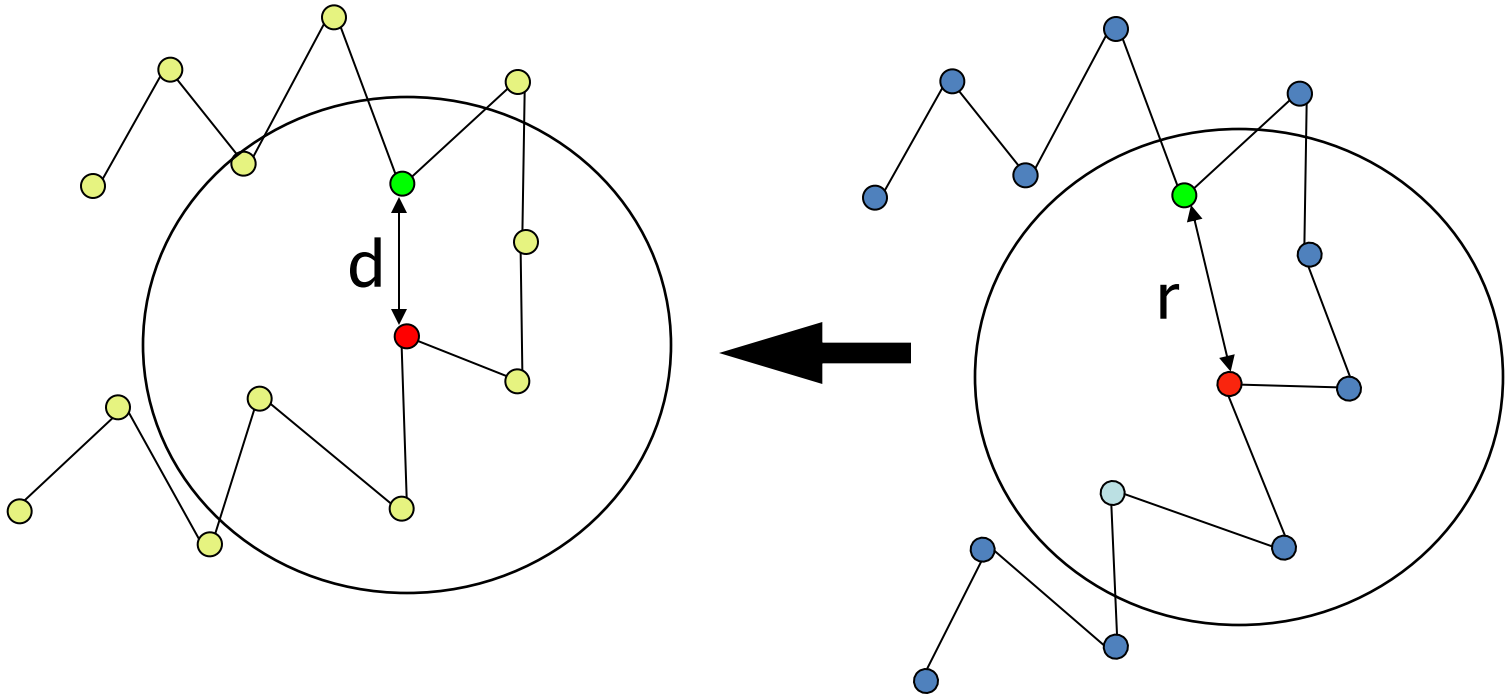


(abstract representation of an atomic model; circles = atoms)

External Restraint Generation

structure to be refined

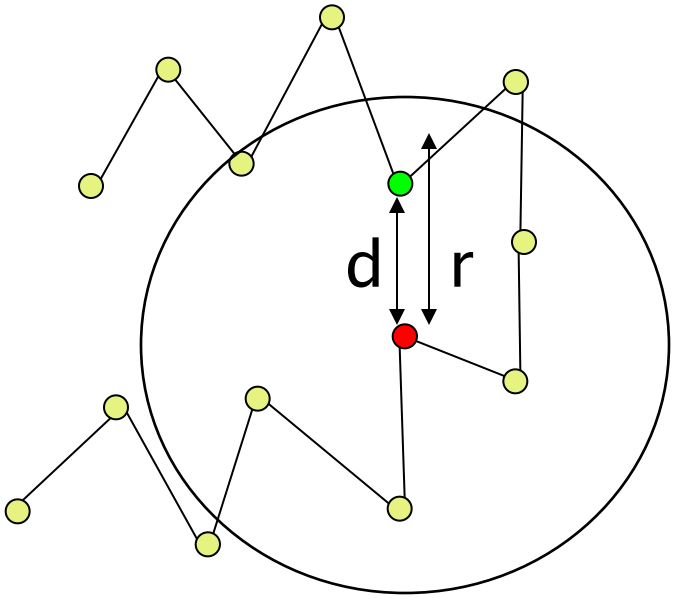
known similar structure (prior)



(abstract representation of an atomic model; circles = atoms)

External Restraint Generation

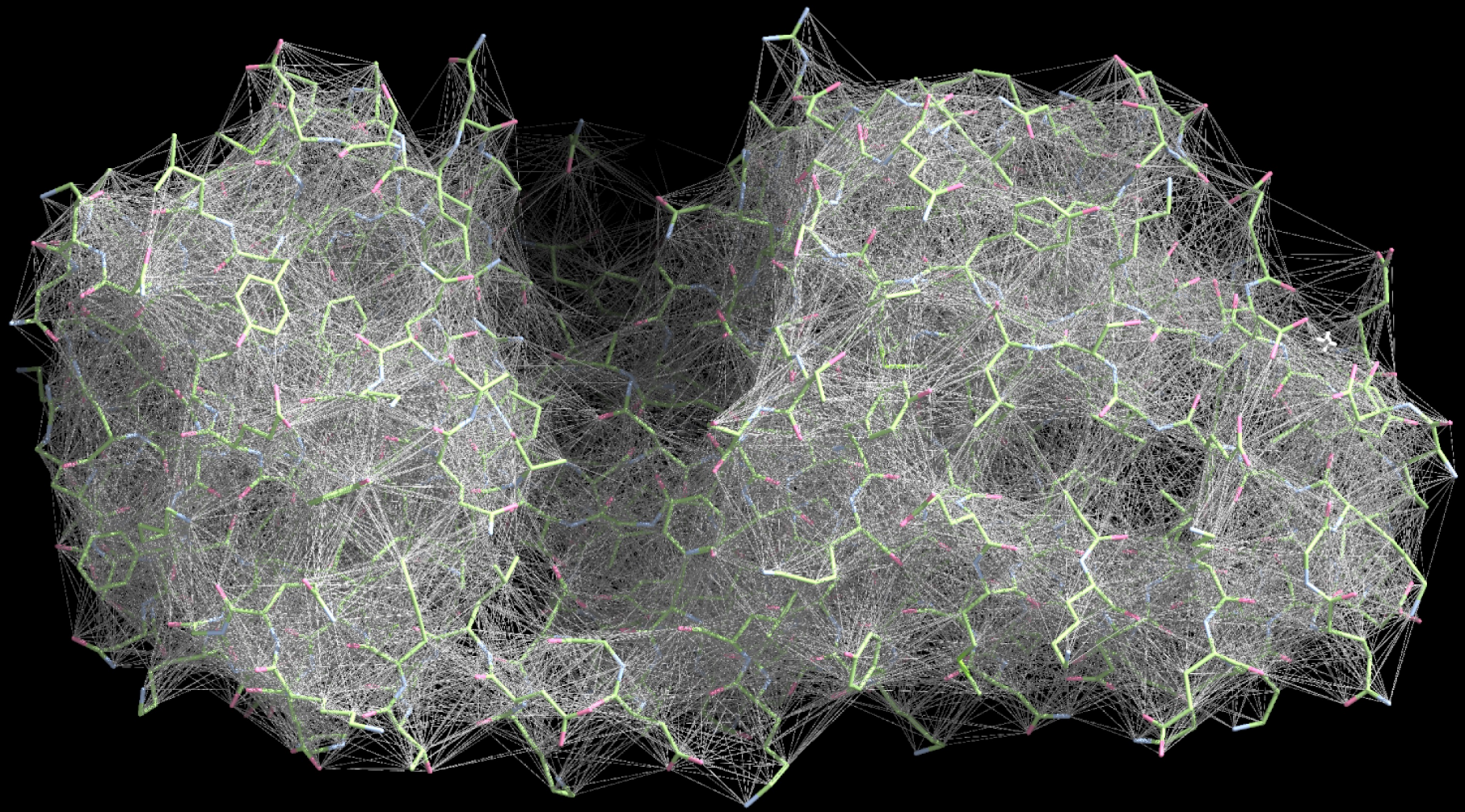
structure to be refined



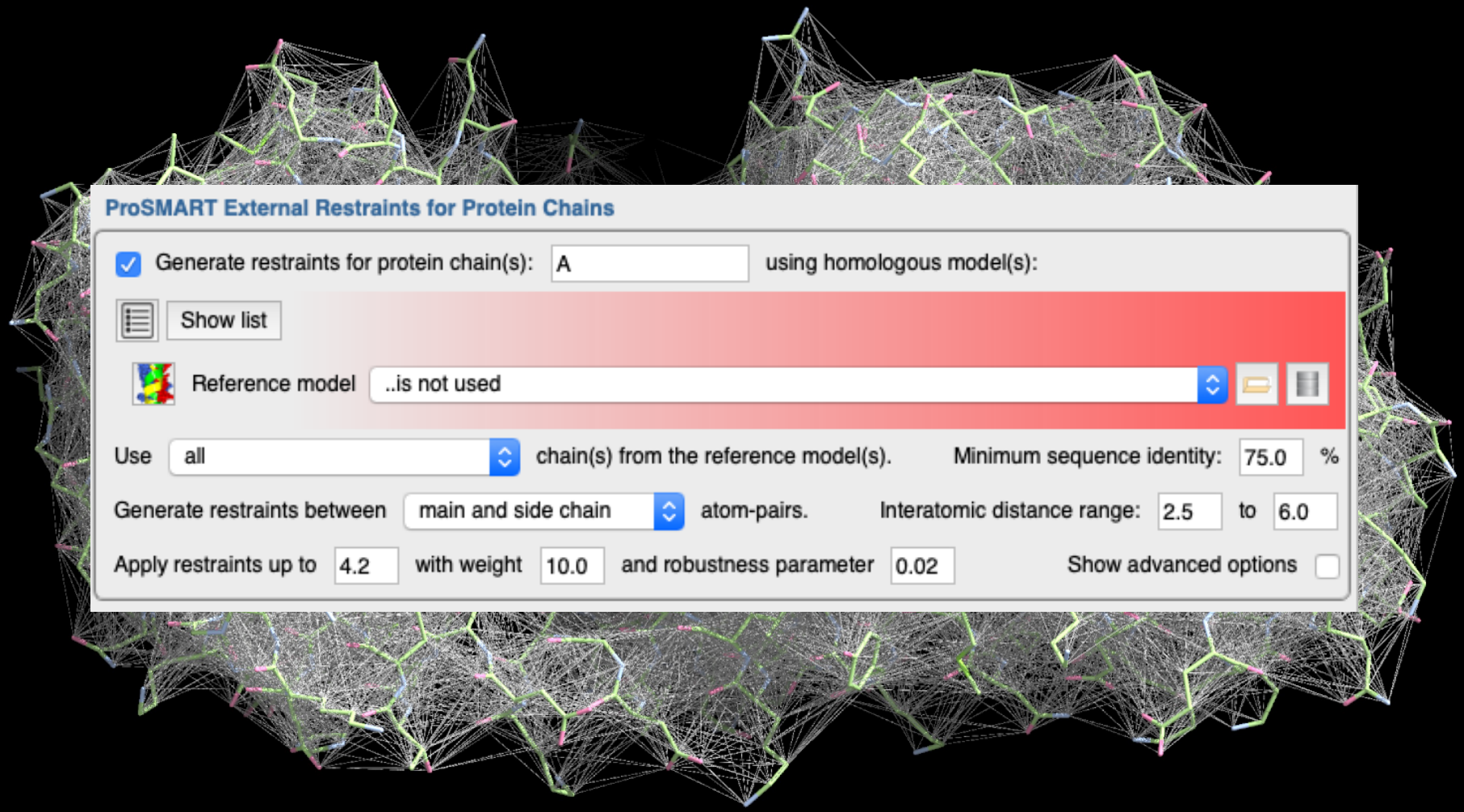
$d \sim N(r,\sigma^2)$

(abstract representation of an atomic model; circles = atoms)

ProSMART Restraints



ProSMART Restraints



Motivational Example

Ovotransferrin



1ryx – 3.5Å

Low-resolution refinement:

Weak signal
Noisy data

↓

Unstable refinement

Result:
Poor quality model

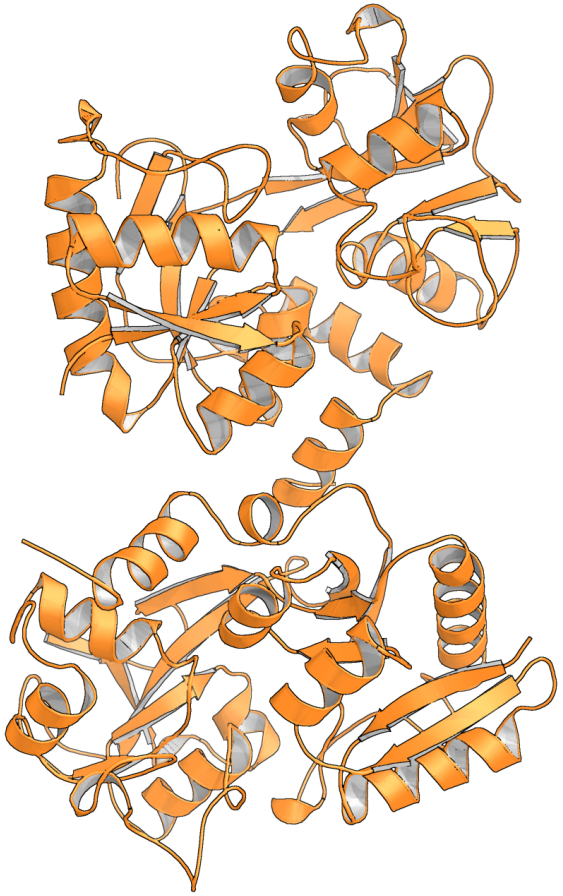
Motivational Example

Ovotransferrin



1ryx – 3.5Å

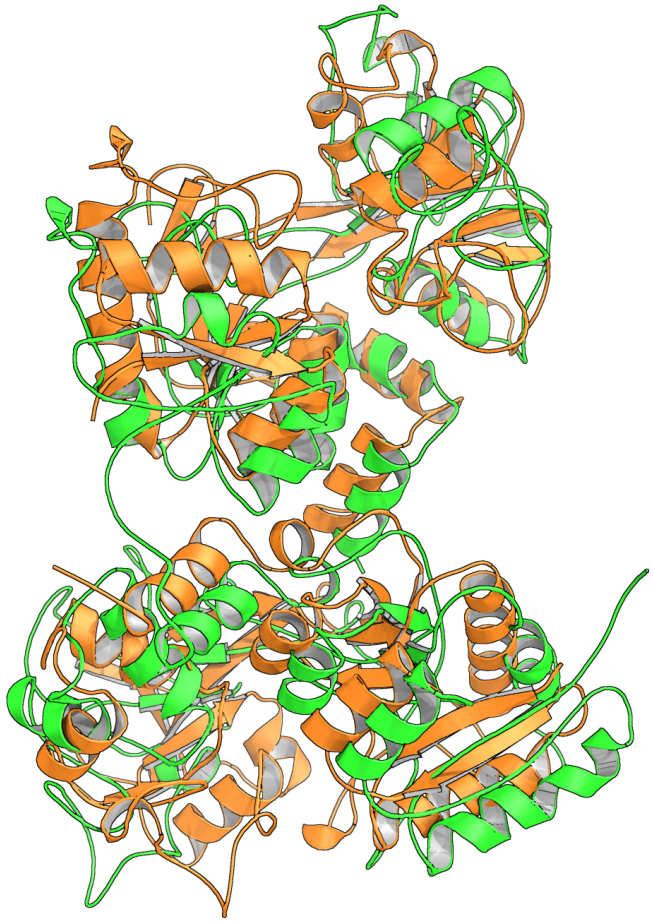
High-resolution homologue



2d3i – 2.15Å

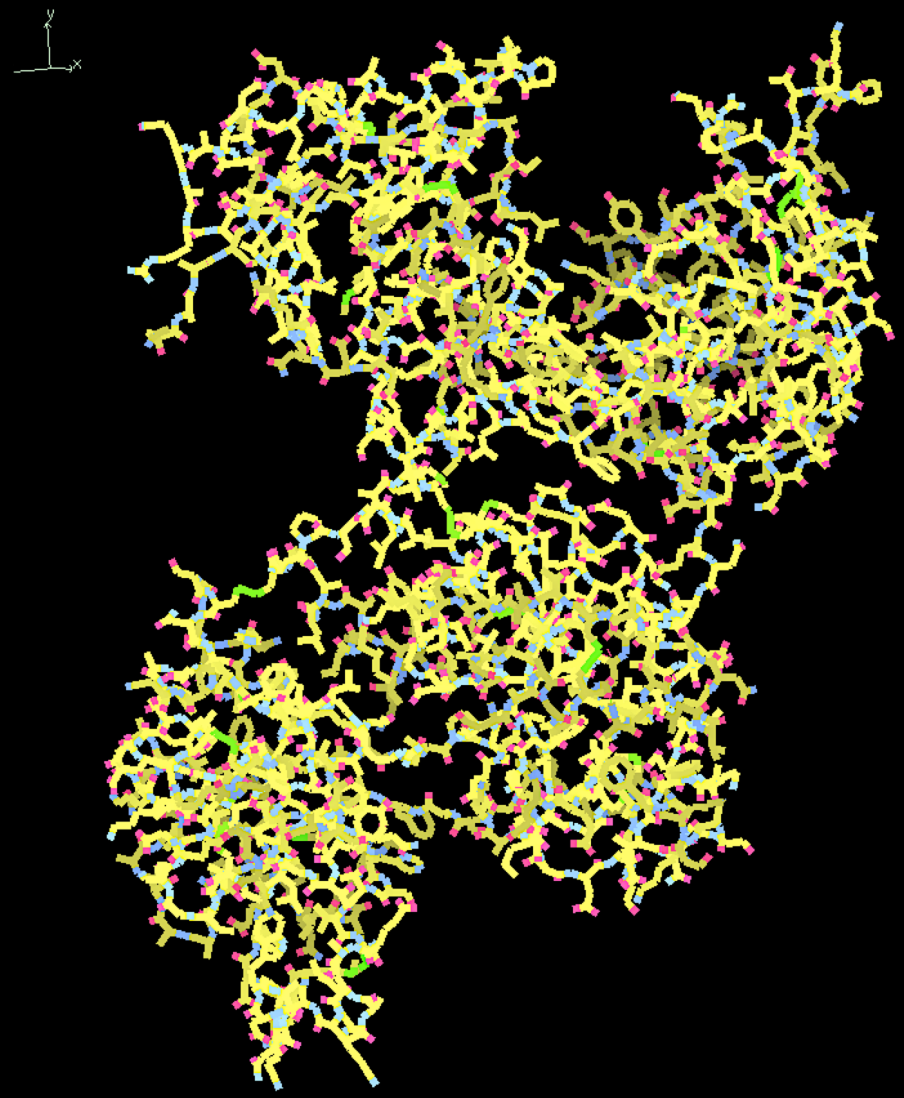
Motivational Example

Ovotransferrin



Models don’t superpose well

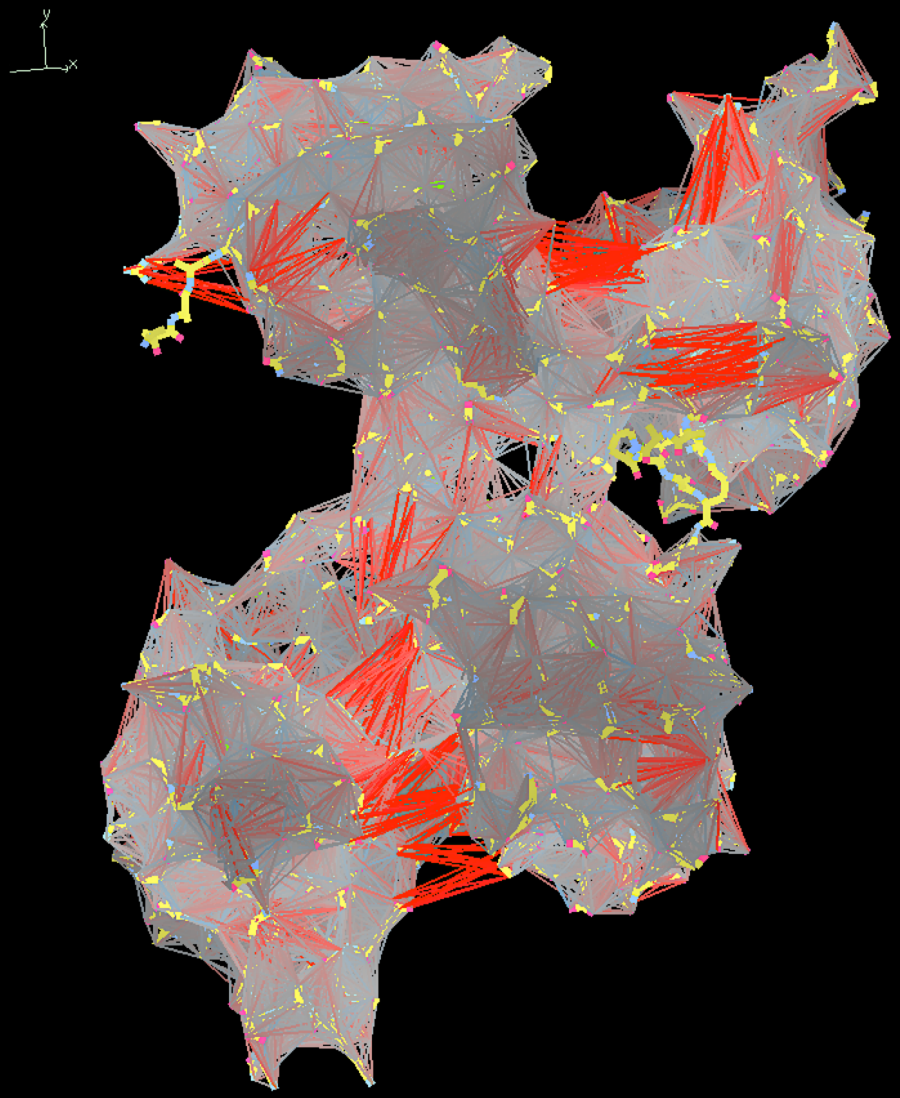
Example: Ovotransferrin



1ryx (3.5Å)

Example: Ovotransferrin

Restraints:
Backbone
Side chains



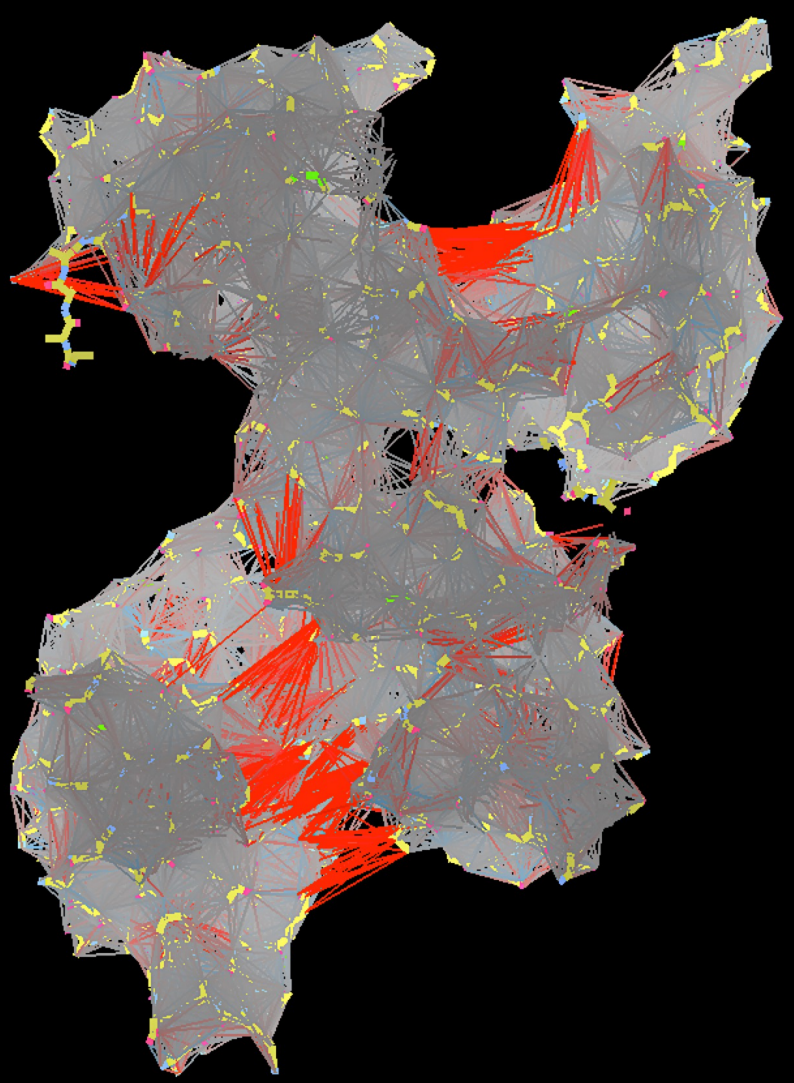
1ryx (3.5Å)
restrained to
2d3i (2.15Å)

Red: long
Grey: similar
Blue: short

Example: Ovotransferrin

Restraints:
- Backbone
- Side chains

After re-refinement

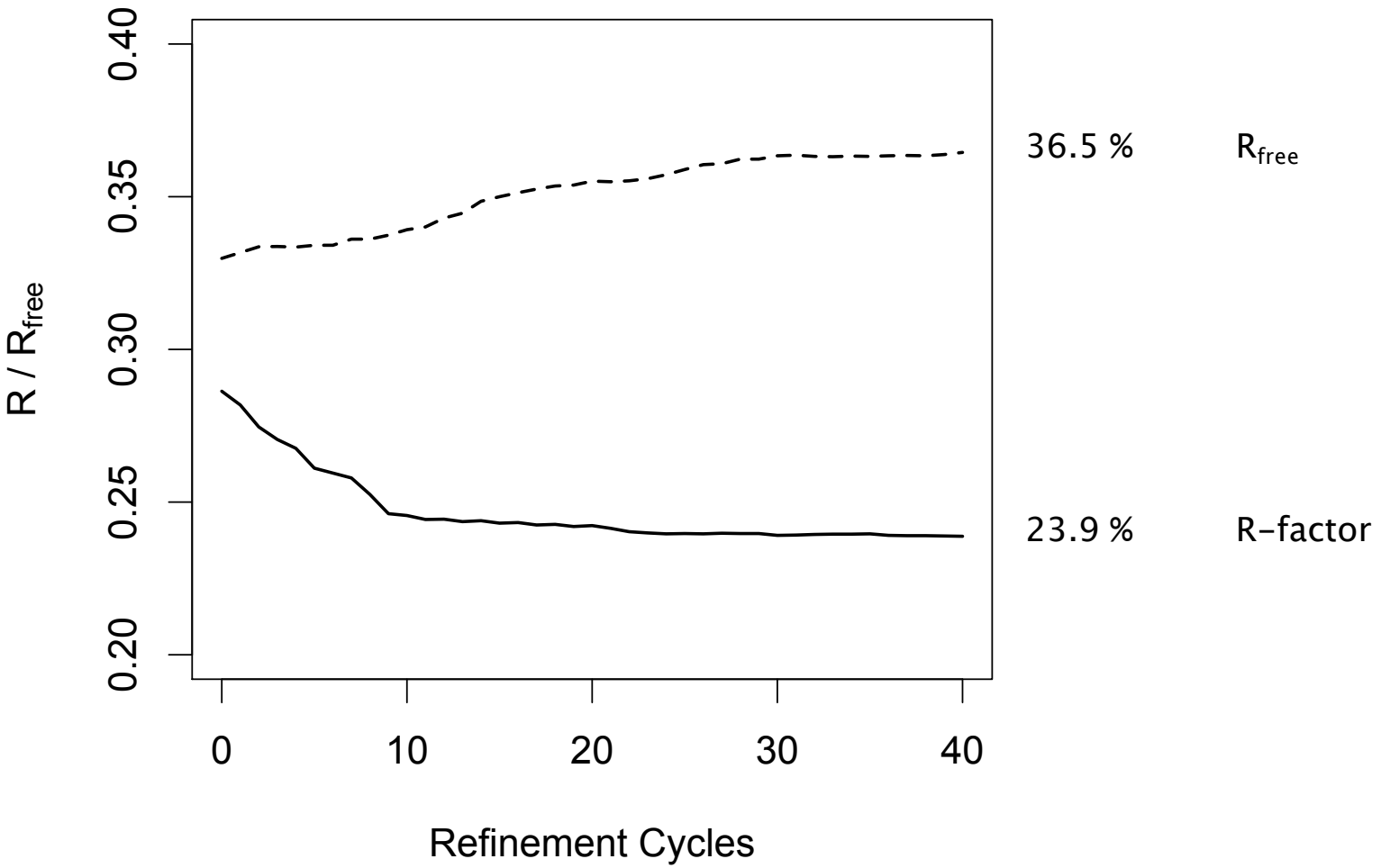


1ryx (3.5Å)
restrained to
2d3i (2.15Å)

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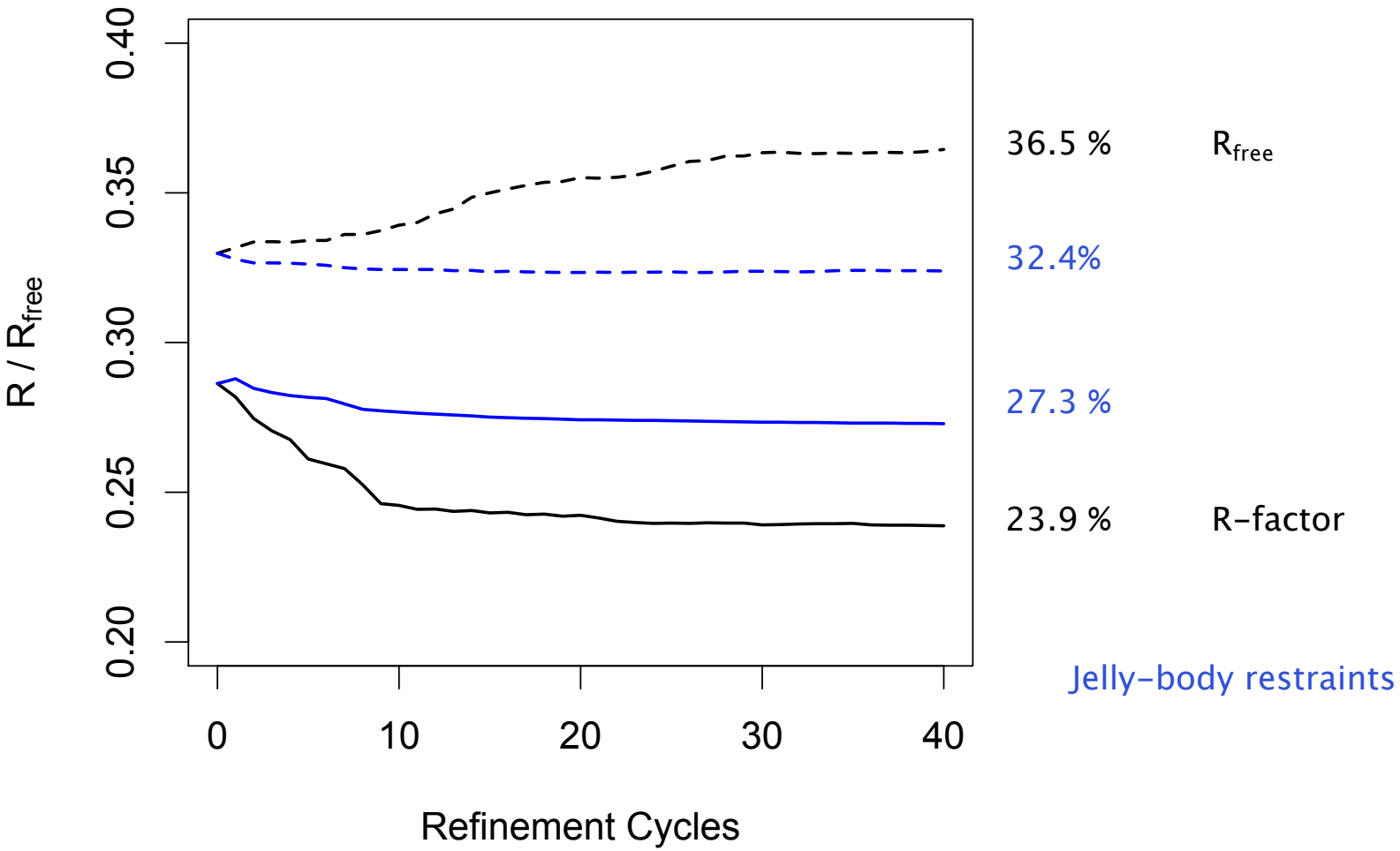
External Restraints

Ovotransferrin



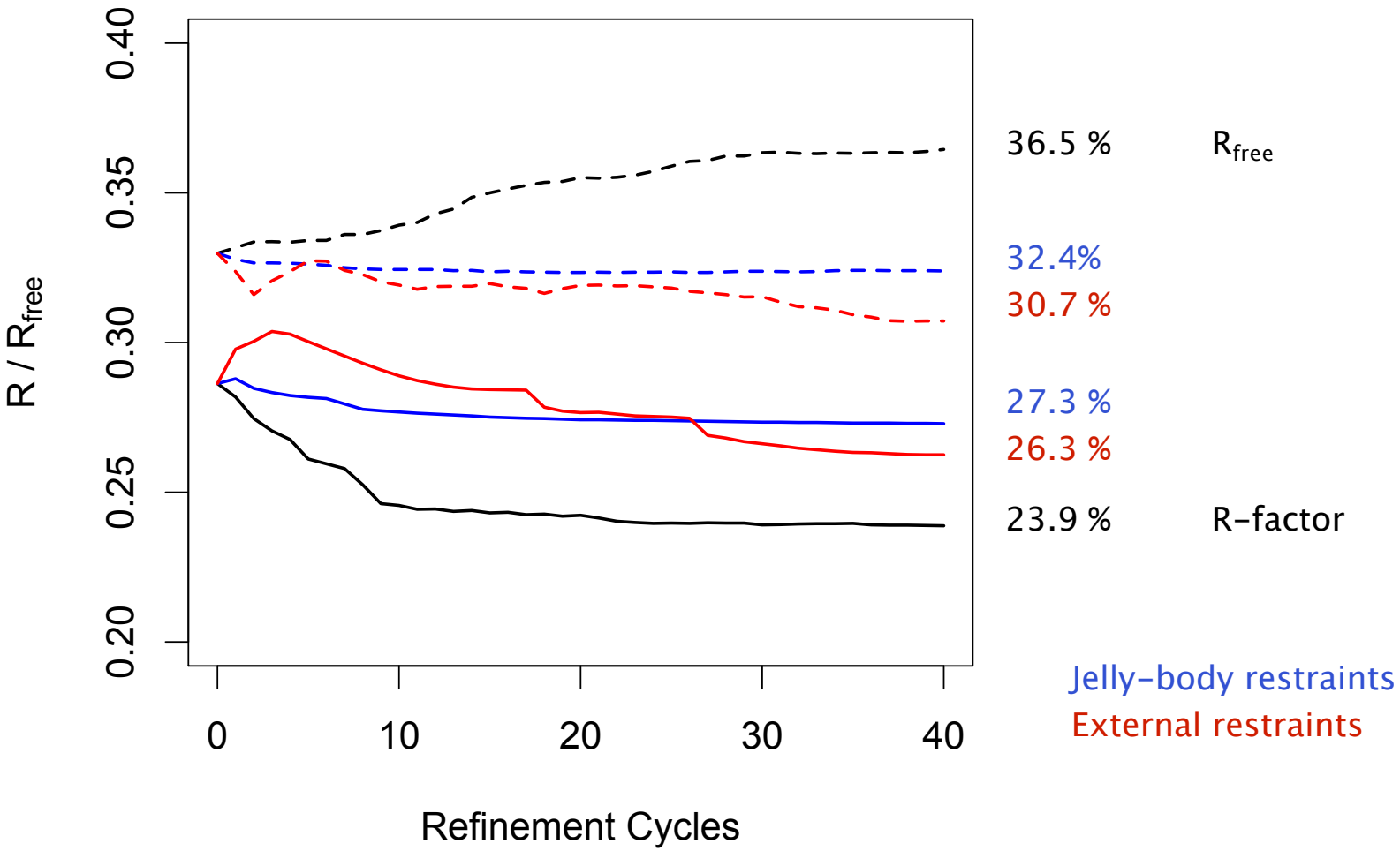
External Restraints

Ovotransferrin



External Restraints

Ovotransferrin

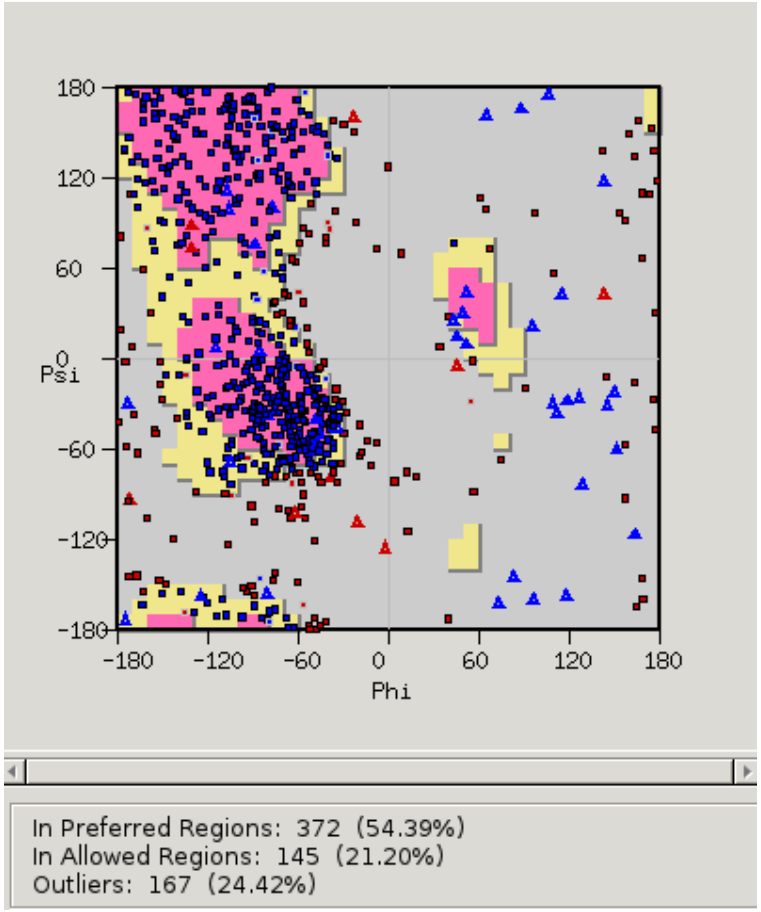


External Restraints

Ovotransferrin

Original Structure

R/R_{free} : 0.286/0.330

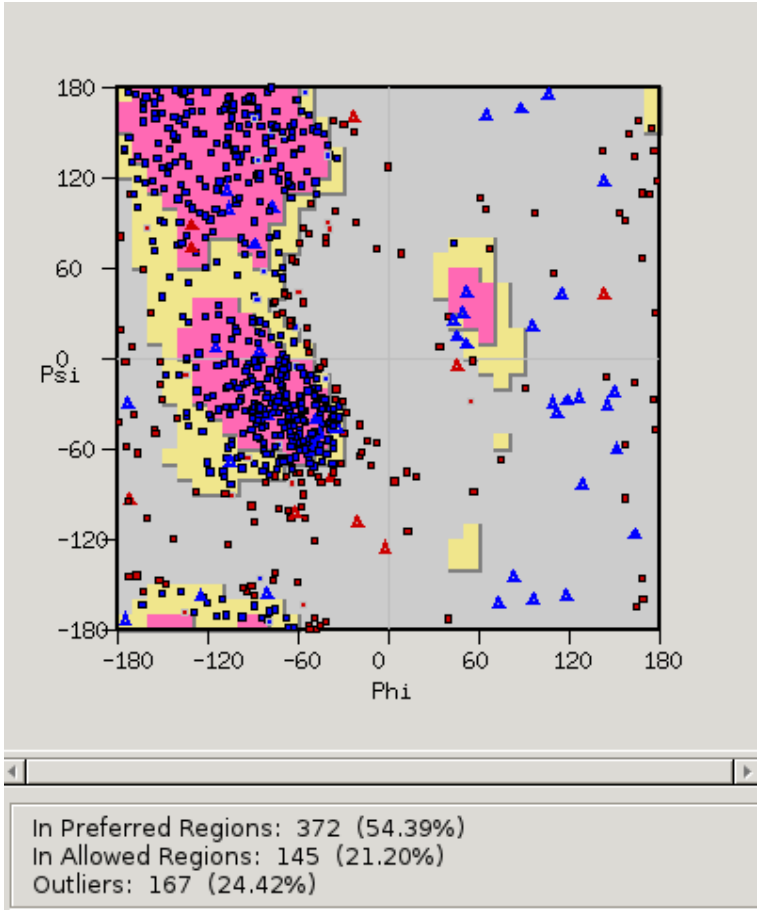


External Restraints

Ovotransferrin

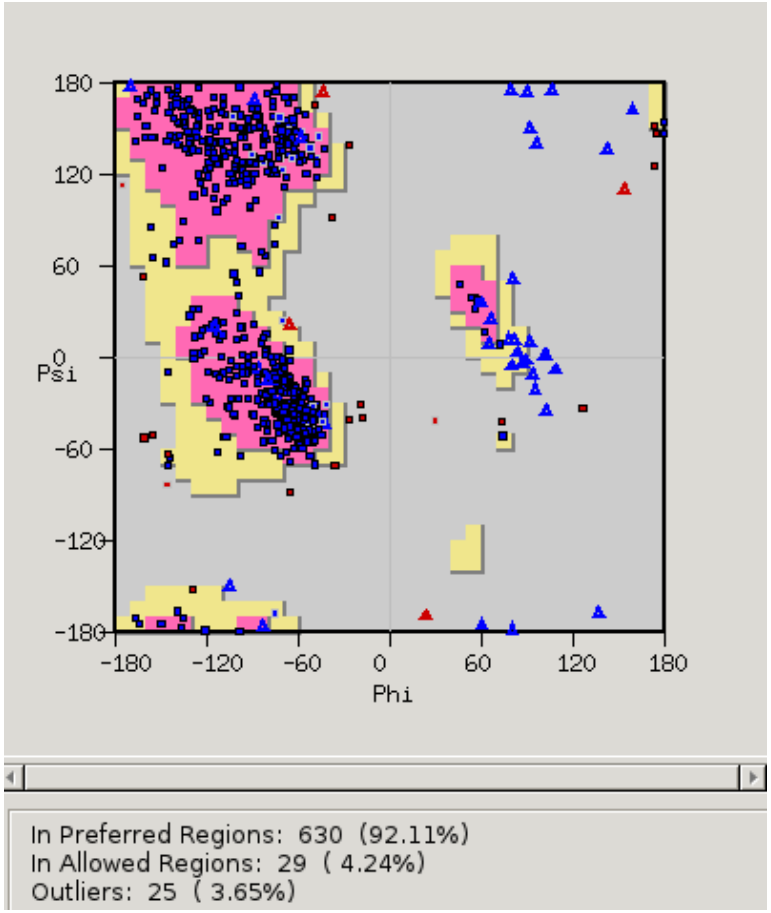
Original Structure

R/R_{free} : 0.286/0.330

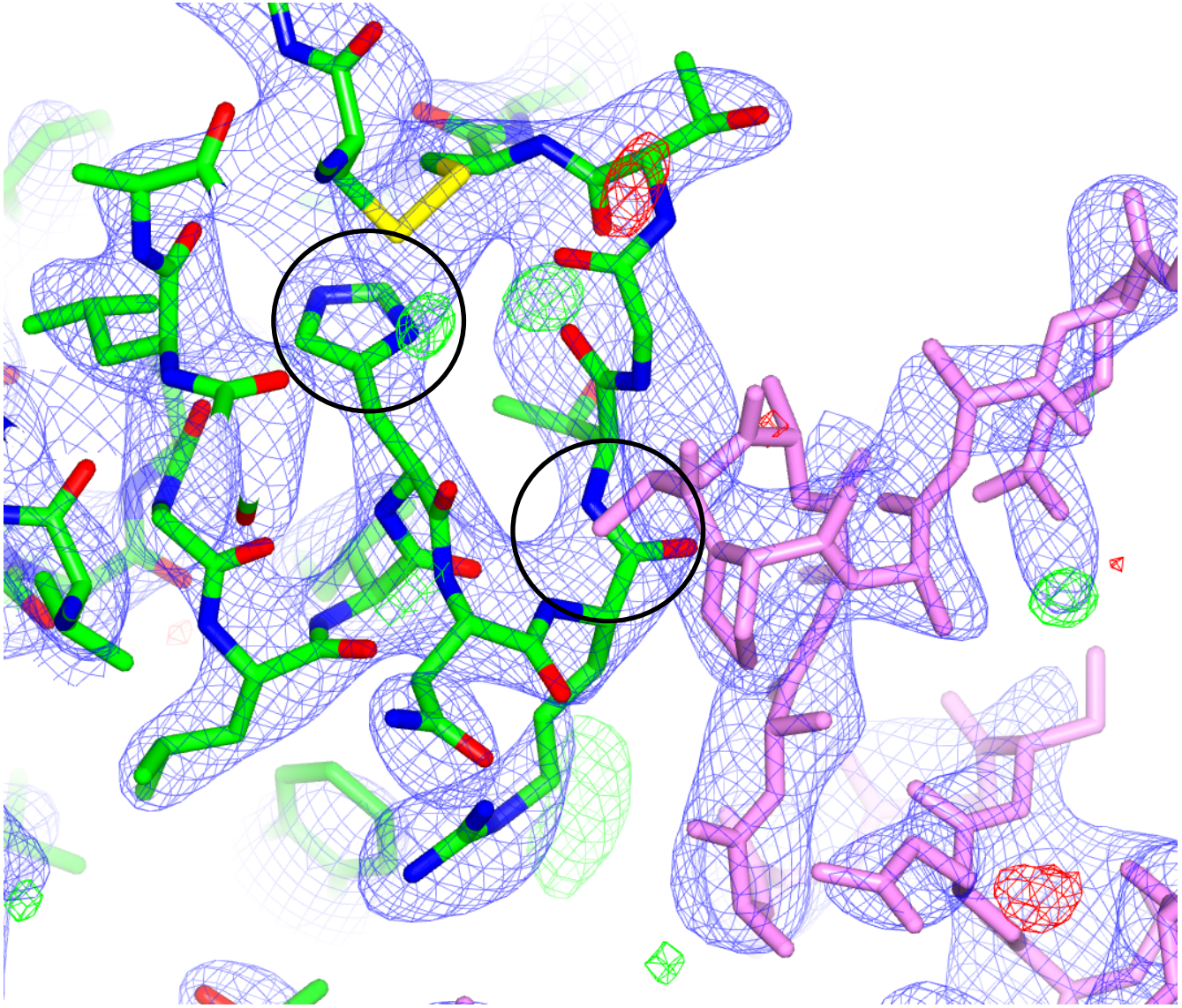


Re-refined with External Restraints

R/R_{free} : 0.263/0.307



External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

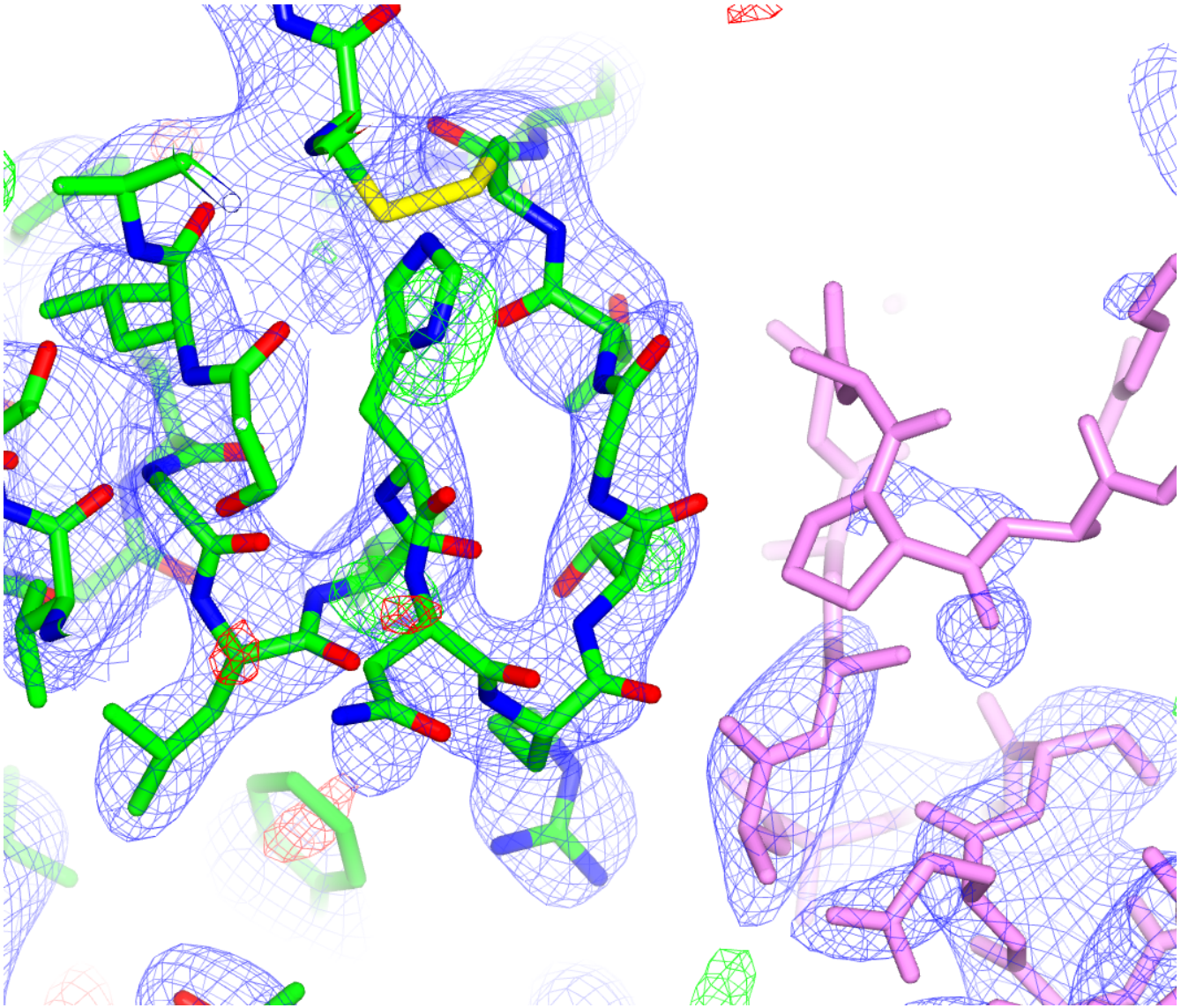
↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

↓

Modify

Real Space Refine

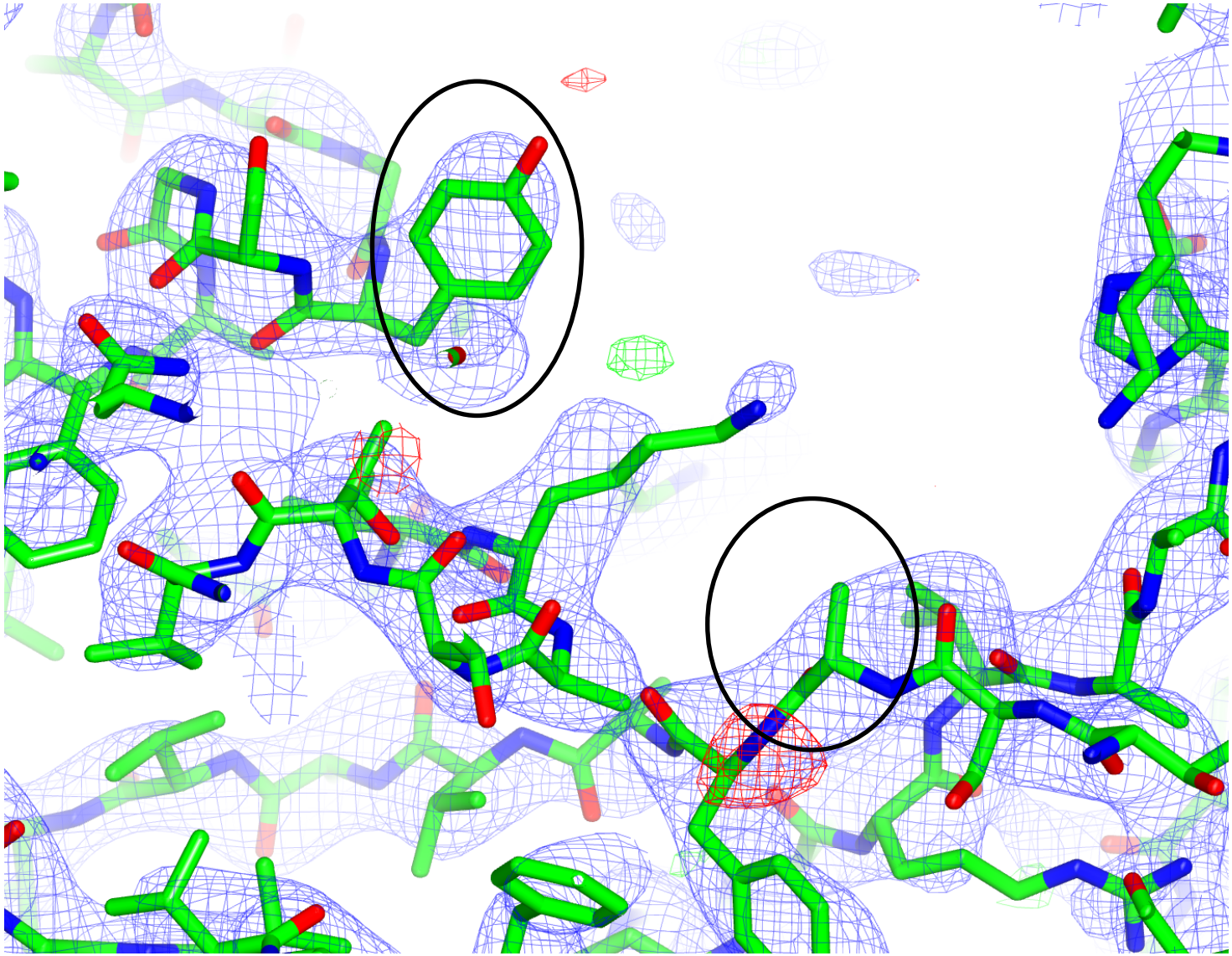
↓

Jelly body

(40 cycles)

R/R_{free} : 0.253/0.304

External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

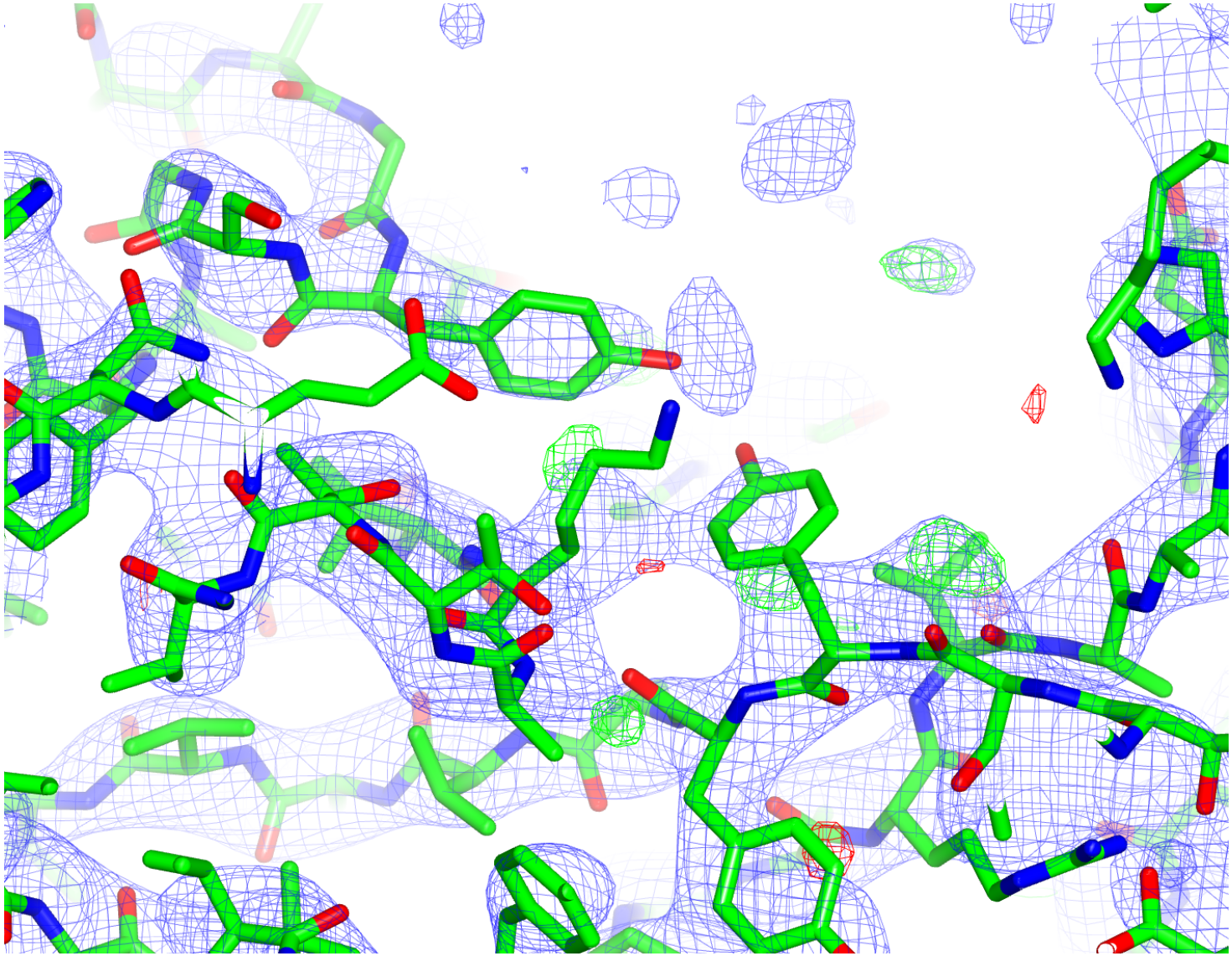
↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

External Restraints



1.3σ

Original Structure

R/R_{free} : 0.286/0.330

↓

External restraints

(40 cycles)

R/R_{free} : 0.263/0.307

↓

Build TYR92

Modify LYS209

↓

Jelly body

(40 cycles)

R/R_{free} : 0.252/0.307

Refinement At Low Resolution

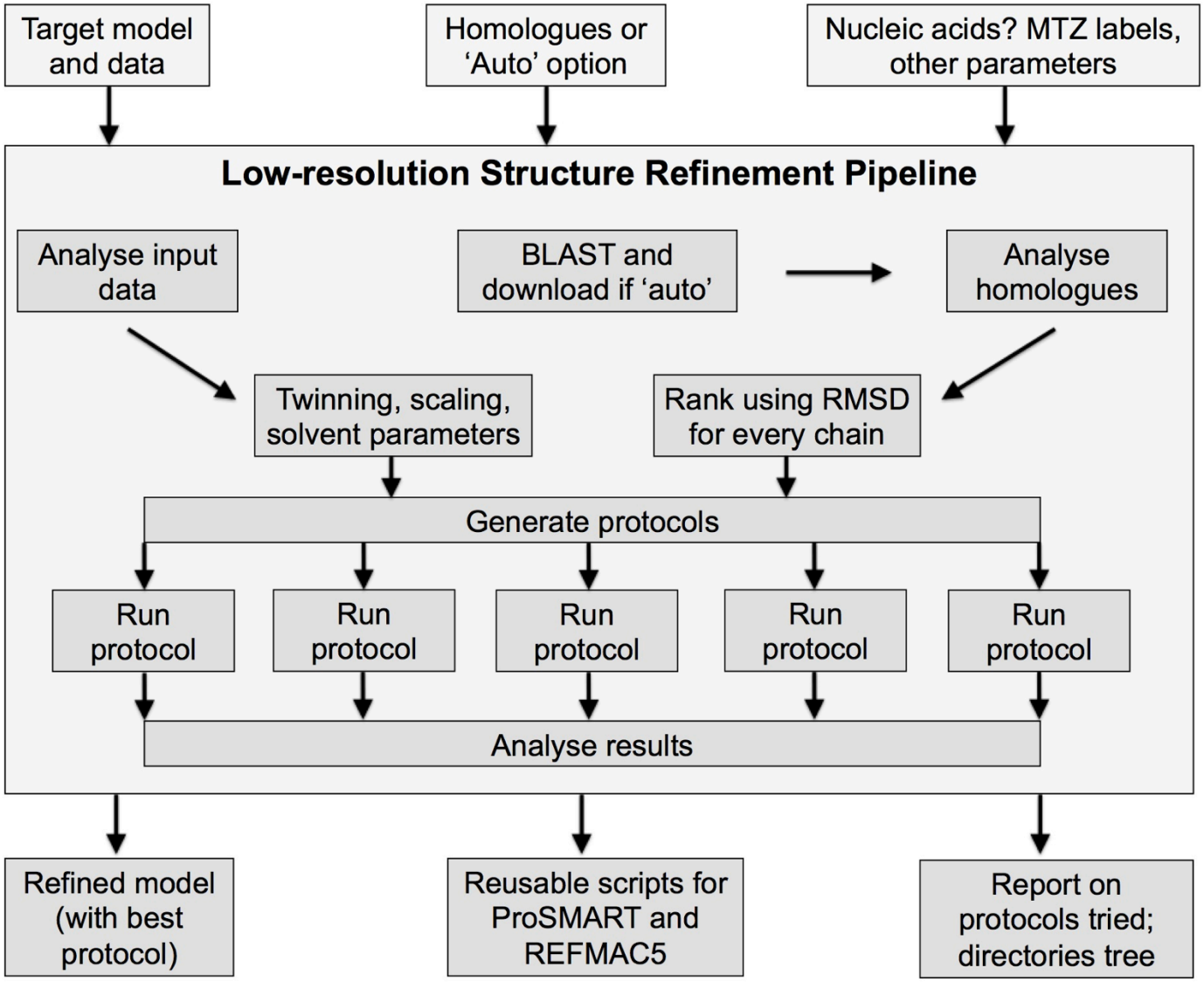
- Efficiency of ProSMART-generated restraints greatly depends on the homologues used
- If several homologues are available, substantial manual effort is required to find their optimal combination
- Other refinement parameters (scaling, solvent, etc) also affect efficiency of the process

Solution:

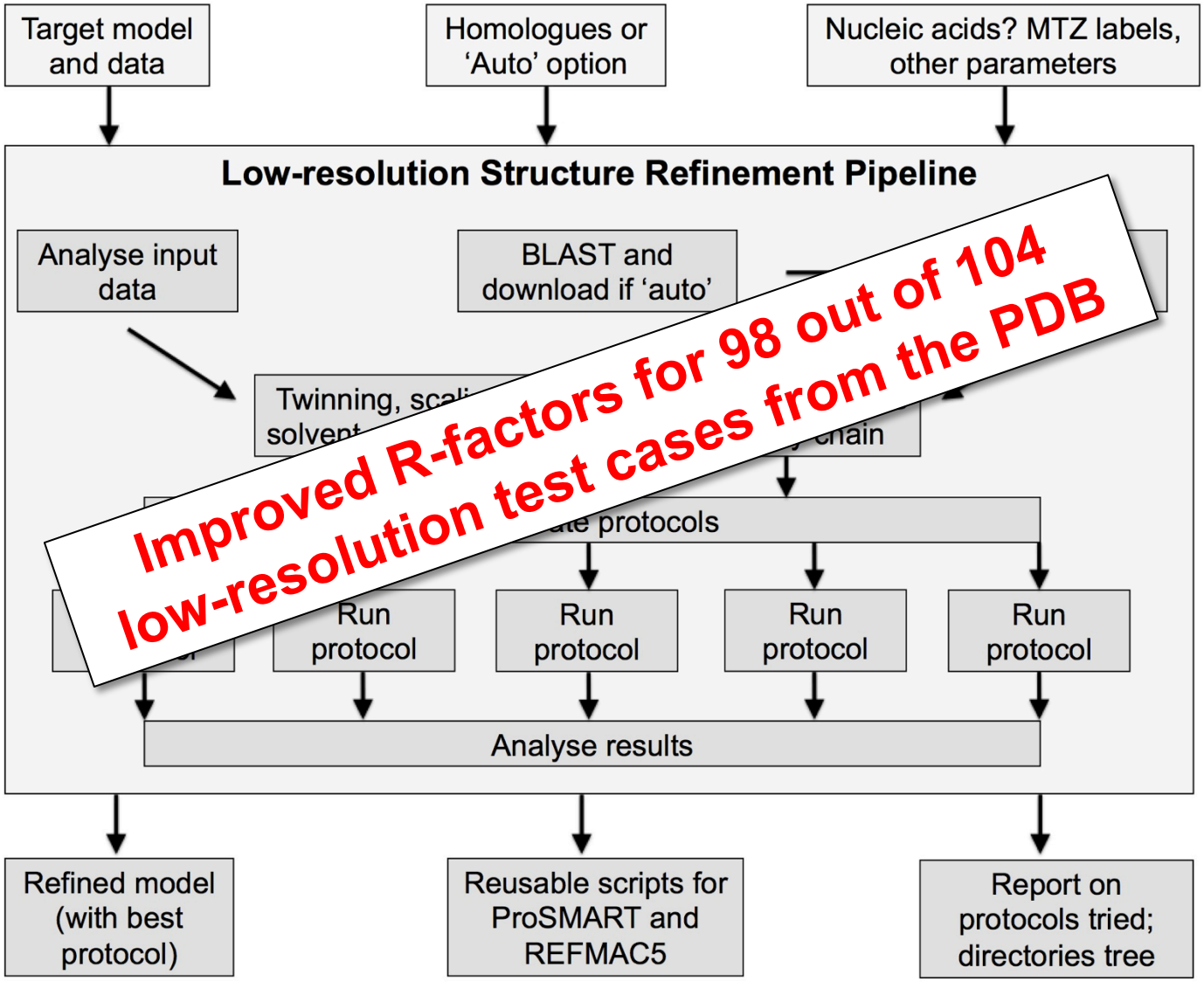
LOw-**RE**solution **ST**ructure **R**efinement

(LORESTR)

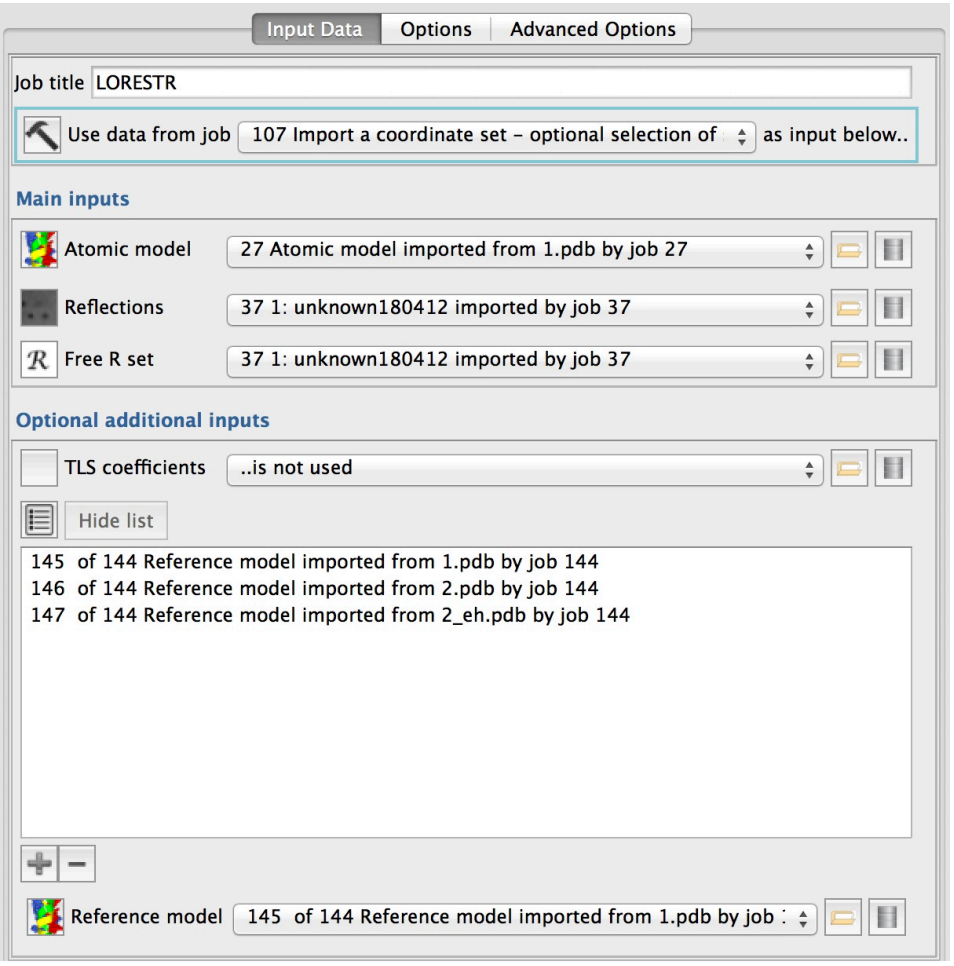
Refinement At Low Resolution

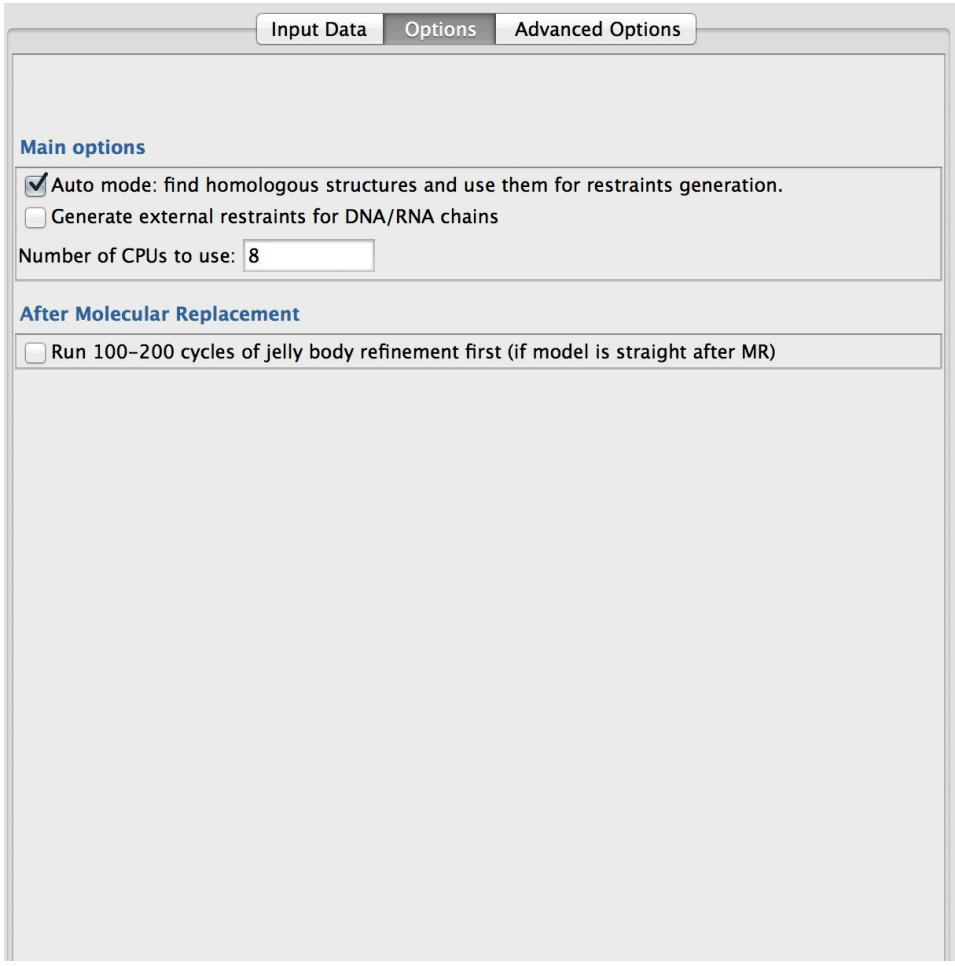


Refinement At Low Resolution



Refinement At Low Resolution

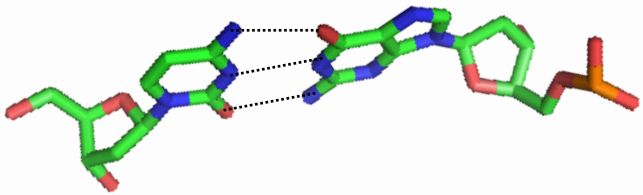


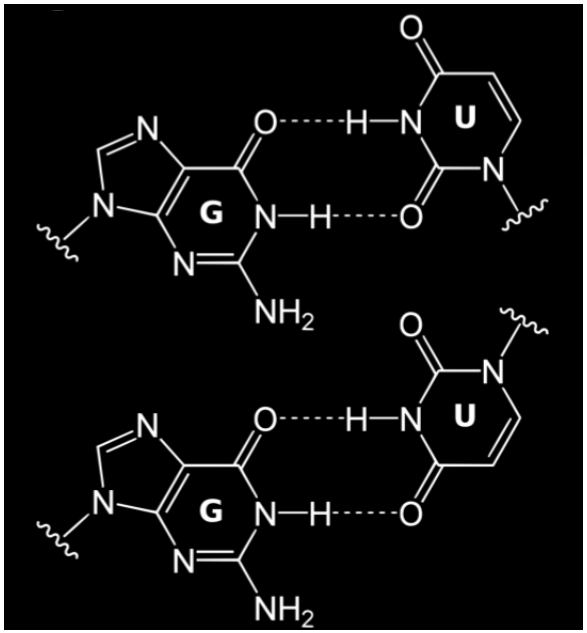


CCP4 7.0.044

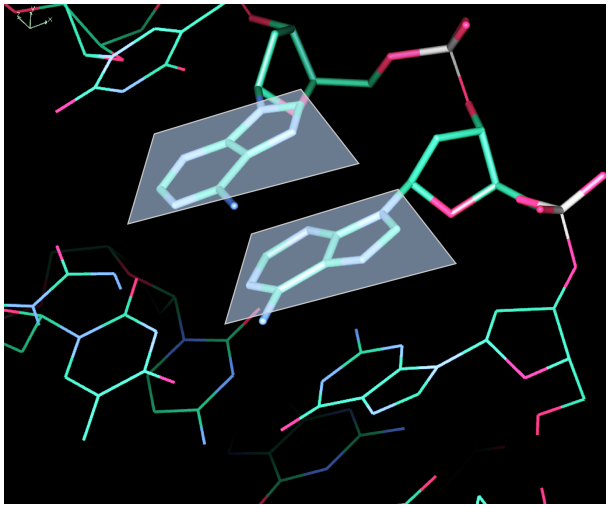
LibG Nucleic Acid Restraints

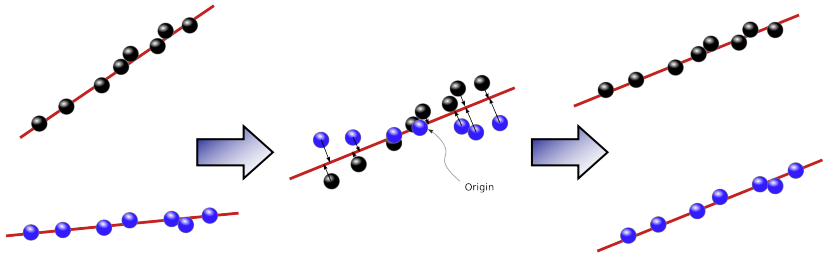
Base-pair restraints



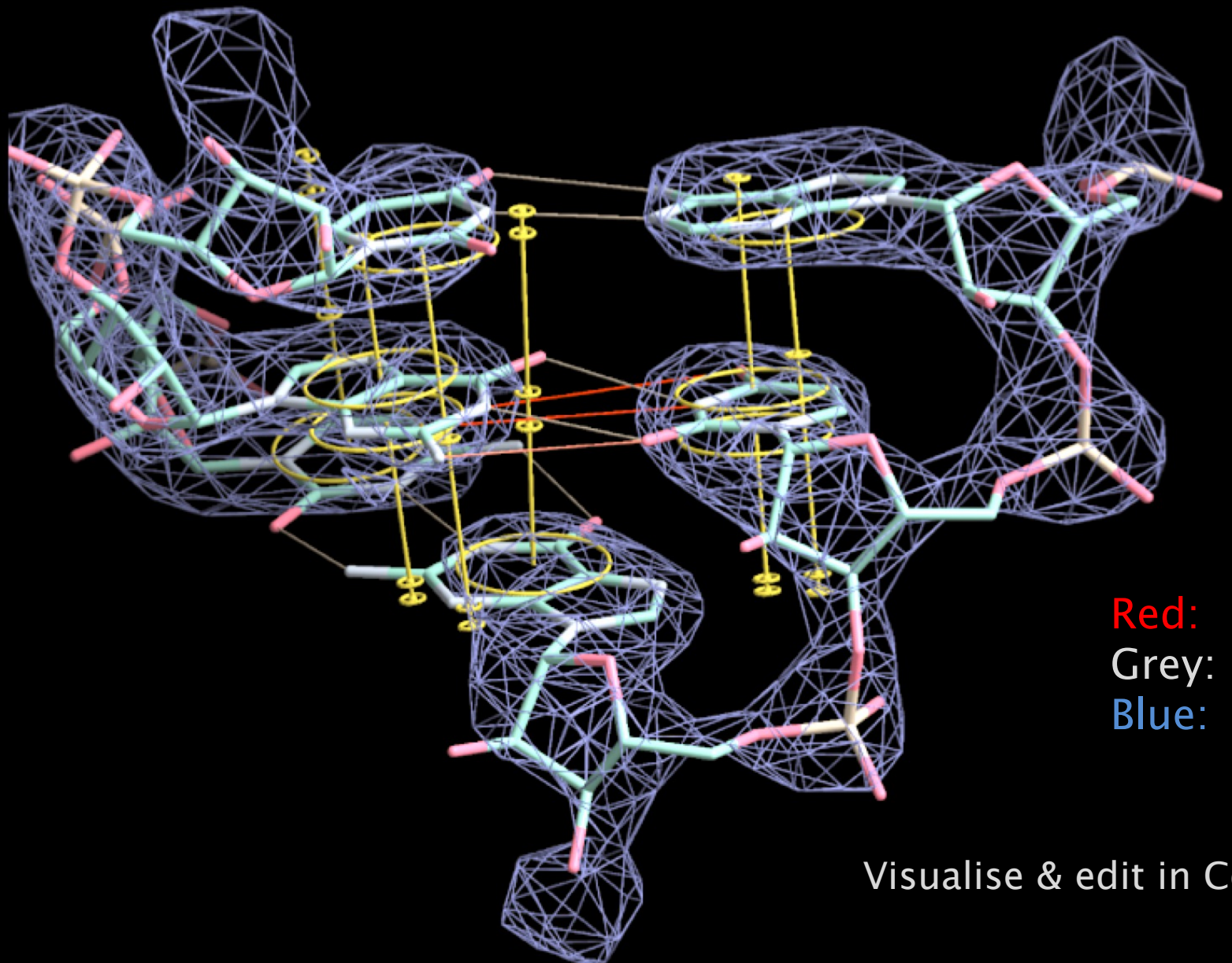


Parallel plane restraints





LibG Nucleic Acid Restraints



Red: long
Grey: similar
Blue: short

Visualise & edit in Coot

External Restraints

When refining at low resolution, check:

- Refinement statistics – ***Not always conclusive***
- Geometry – ***Not always conclusive***
- Electron density – ***Not always reliable***

Conclusion: **At low resolution, everything has to add up!**

Take care; reflect

Quality of prior information is important **– consider manual re-refinement**

– PDB_REDO is useful

Summary

Tools to help with model building and refinement:

REFMAC: Refinement, jelly body restraints, map sharpening/blurring

ProSMART: External restraints, comparative analysis

LIBG: Nucleic acid restraints

LORESTR: Automated low-resolution pipeline

ACEDRG: Ligand dictionary and conformer generation

COOT: Model building, real space refinement, restraint visualisation, map blurring, model morphing, jiggle-fit…

Many tools are applicable to cryo-EM as well as to MX

What and When – Low-Res MX

Early stages (e.g. straight after MR)

- Rigid body refinement
- Jelly body – sometimes up to 200 cycles

Medium stages – during model building

- Auto local NCS – wherever possible
- External restraints (40 cycles) – homologue available
- Otherwise, jelly body… but not together
- H-bond and DNA/RNA restraints – no homologue available
- Secondary structure conformation restraints – model building tool
- Add hydrogens (?)

Medium-final stages

- TLS – at medium resolutions
- Twin refinement – only if you are sure

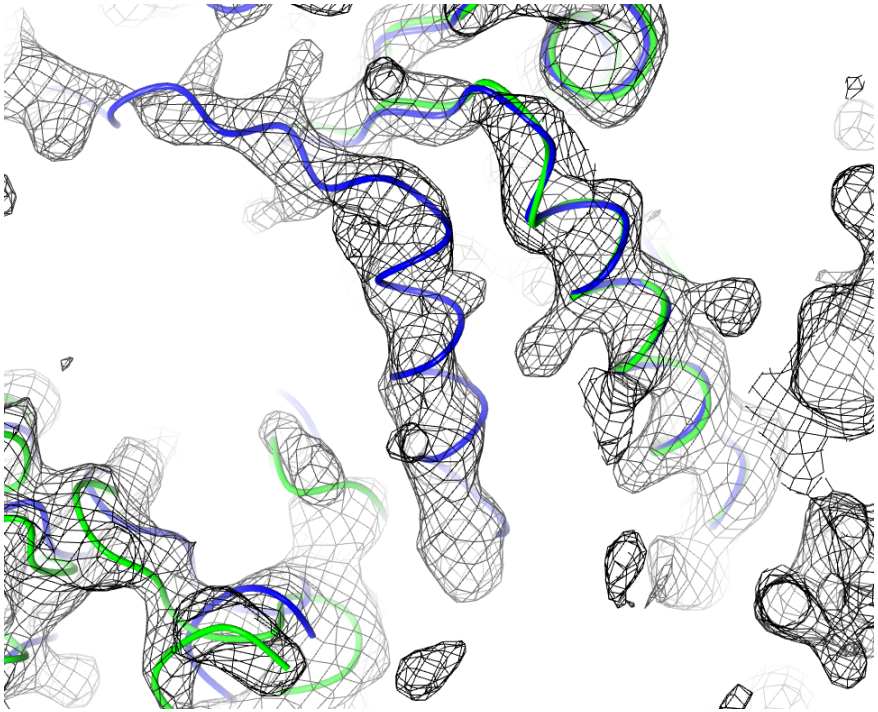
Final stages of refinement

Jelly body – around 20 cycles

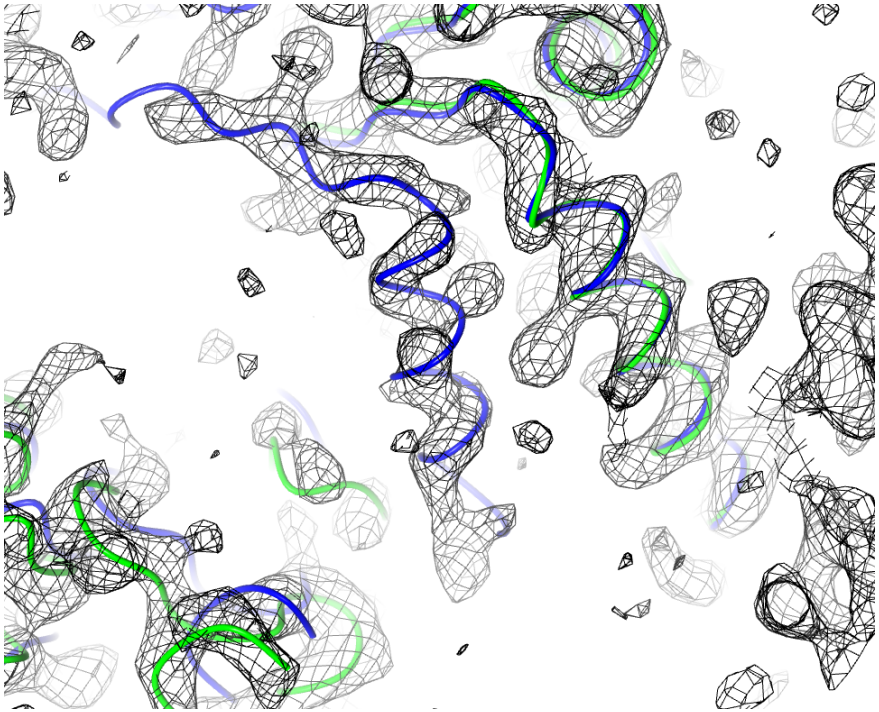
REFMAC Anisotropic Map Sharpening

Idea – remove an overall B value

Original Map



Sharpened map from REFMAC



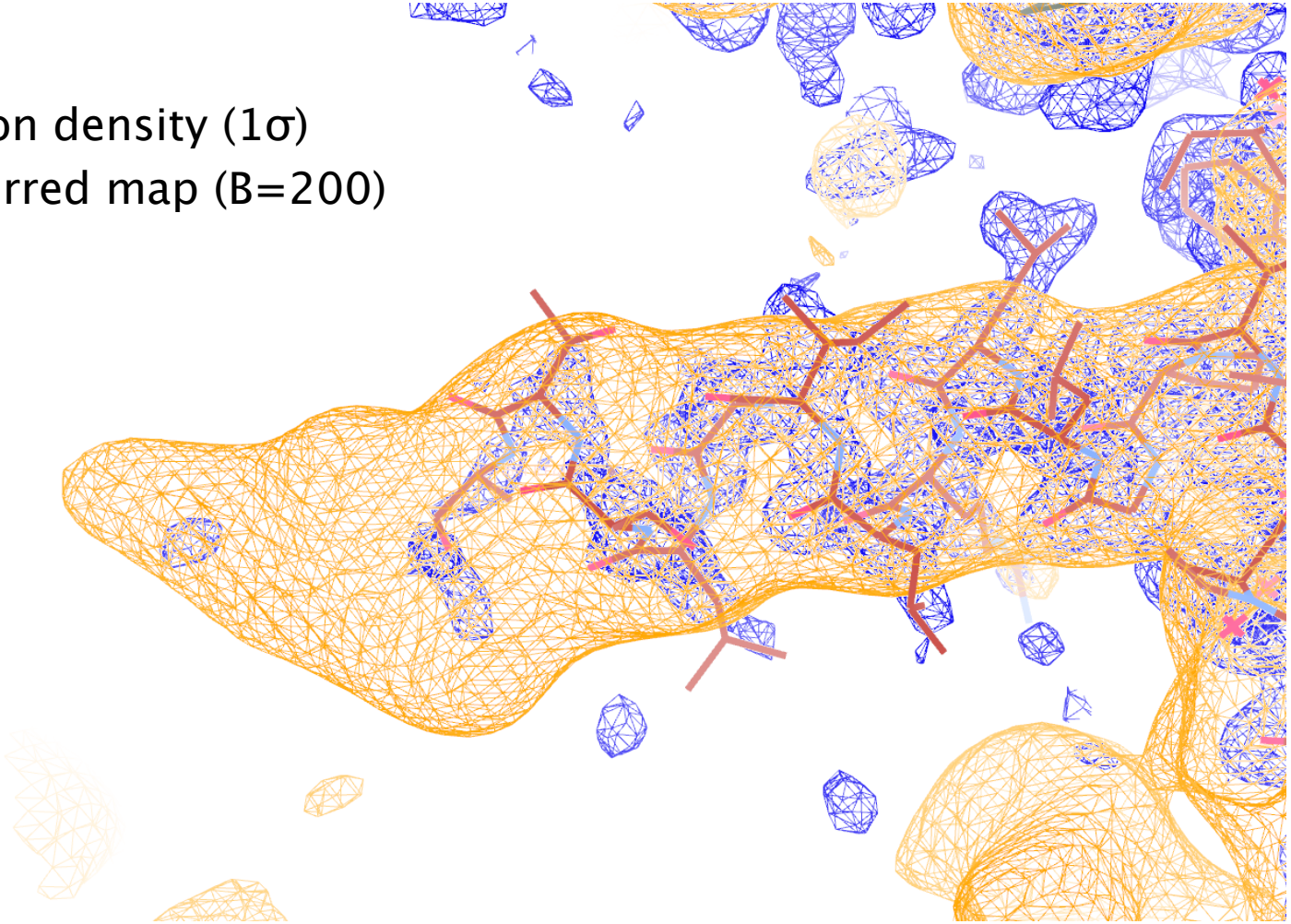
Green: original structure 2r6c (4.0Å) – helix unmodelled

Blue: homologous structure 2r6a (2.9Å)

Map Blurring

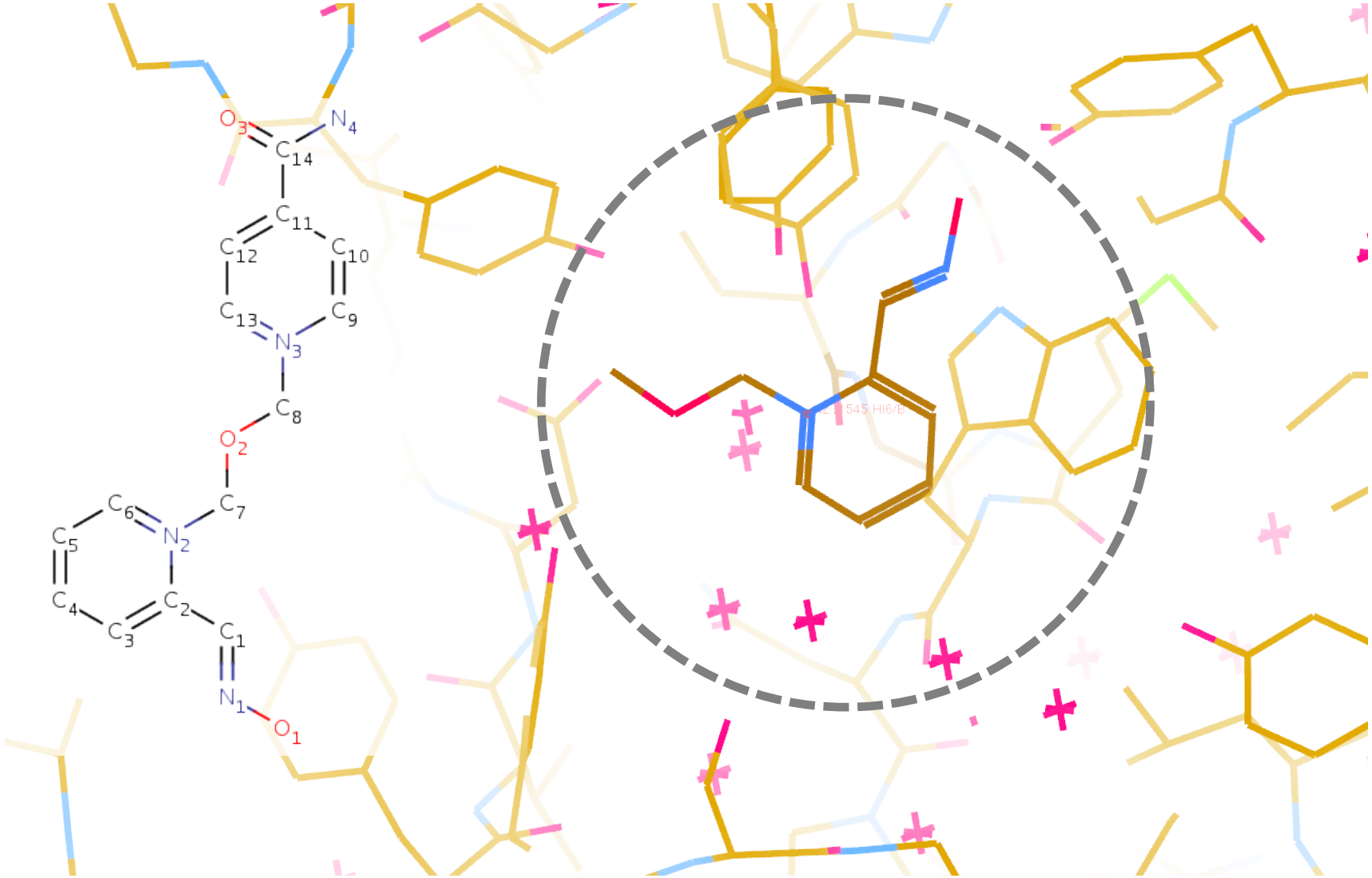
Idea – apply an overall B value

Blue – electron density (1σ)
Orange – blurred map (B=200)



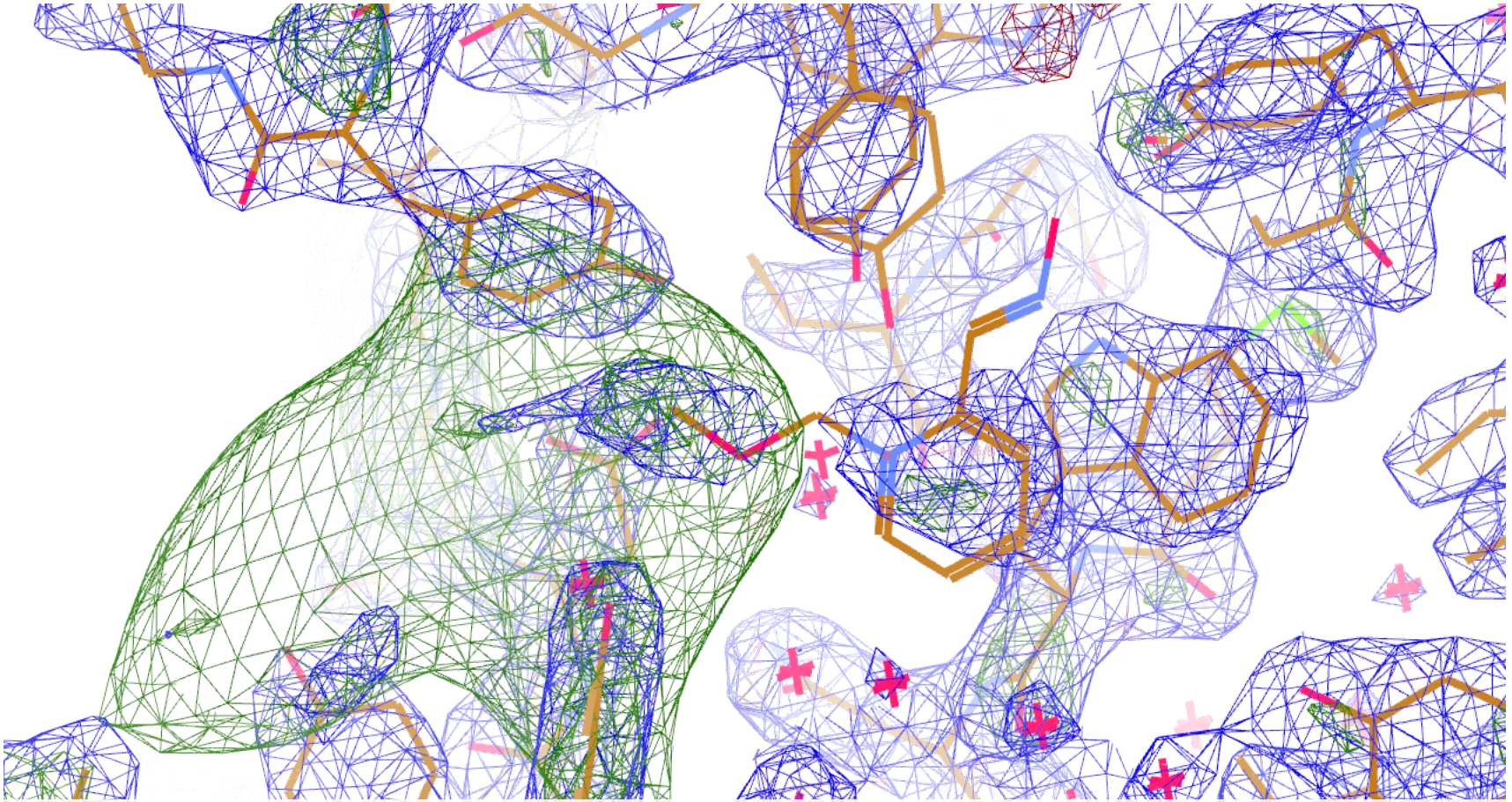
1s3e

Map Blurring



2whq

Map Blurring



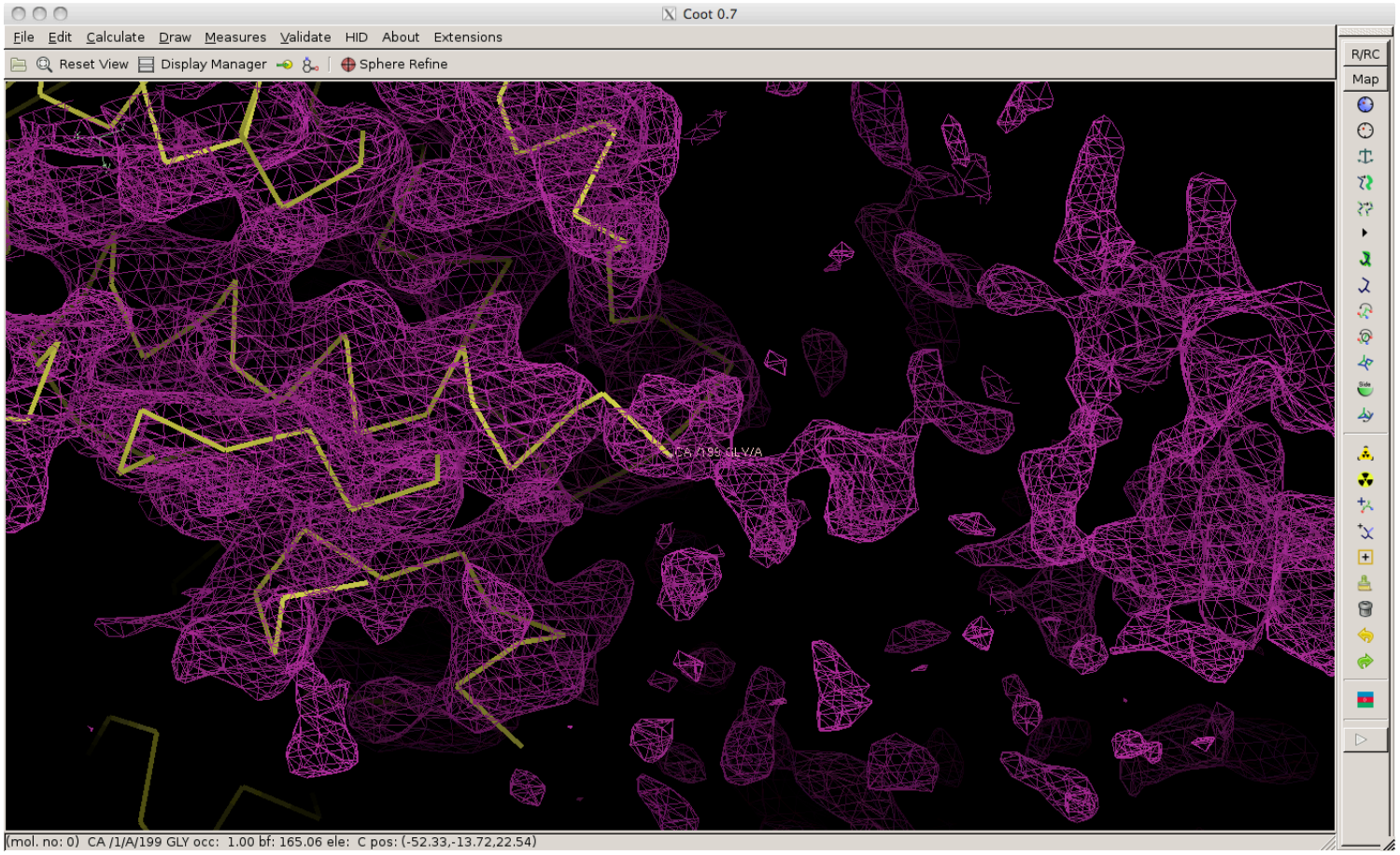
Electron density at 1.7σ; Residual density at 3.5σ
Blurred residual map, B = 200

2whq

Map Sharpening/Blurring

Output Array of Sharpened Maps

Standard:

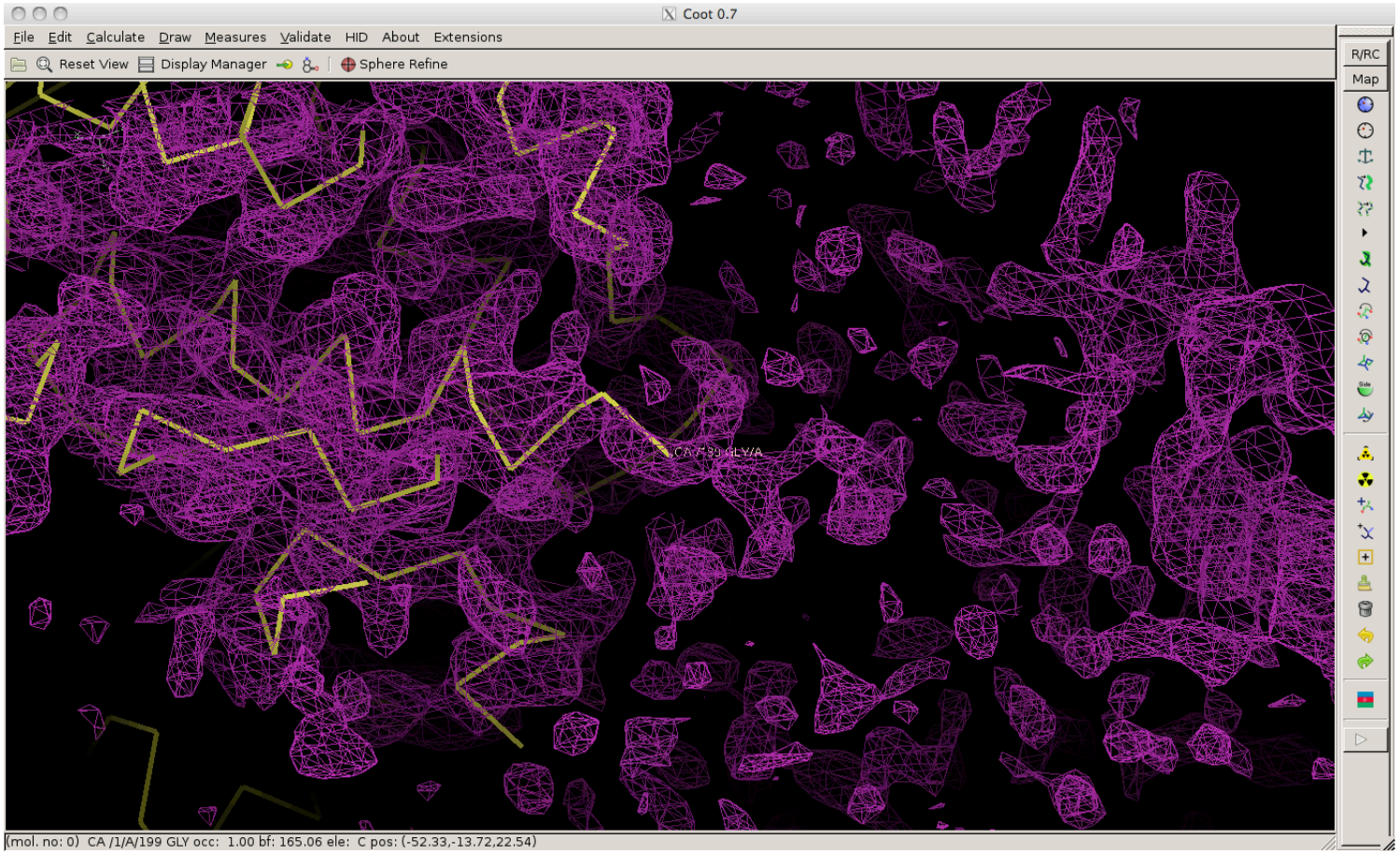


4iwo (2.6Å)

Map Sharpening/Blurring

Output Array of Sharpened Maps

Sharpen 50:

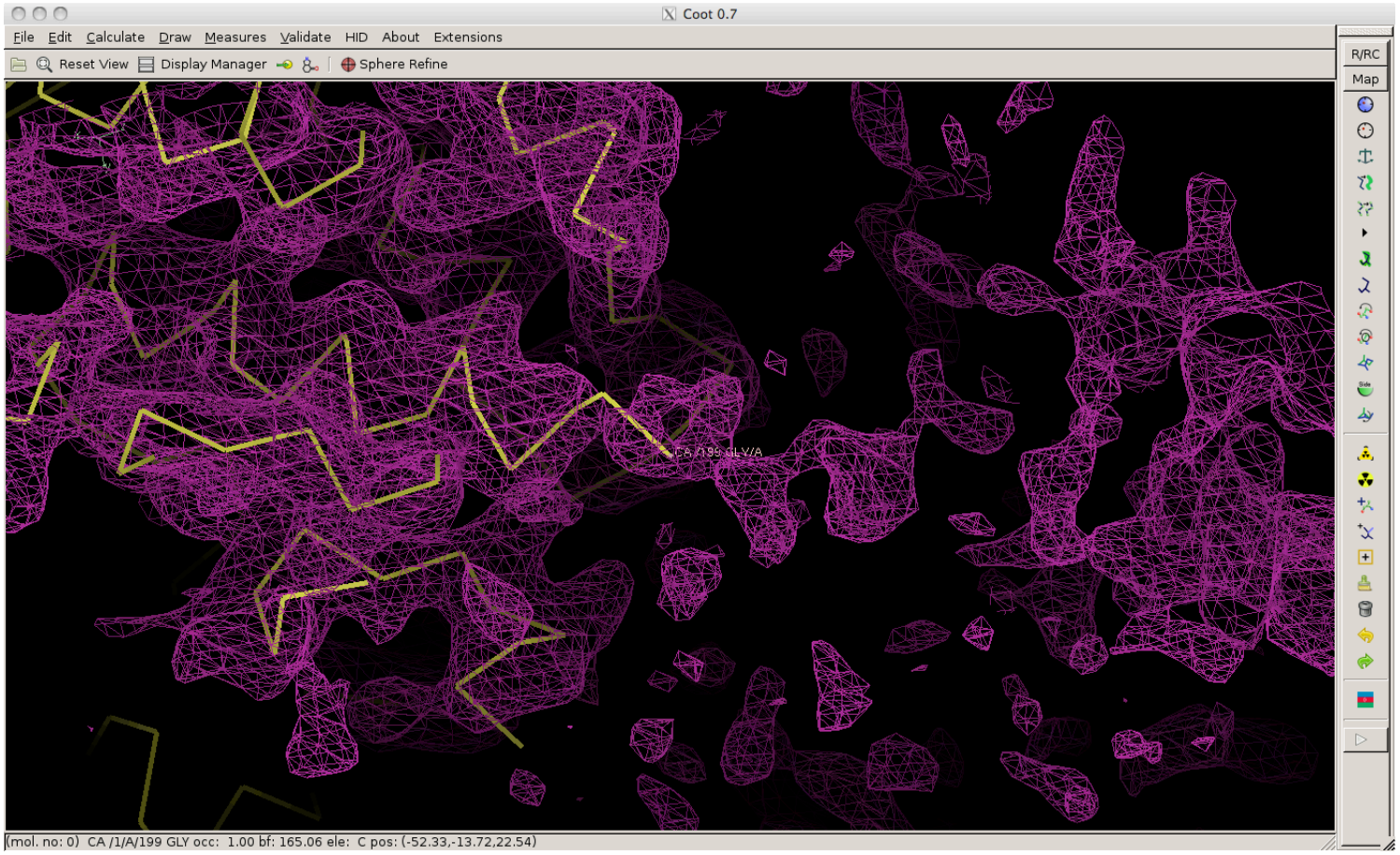


4iwo (2.6Å)

Map Sharpening/Blurring

Output Array of Sharpened Maps

Standard:

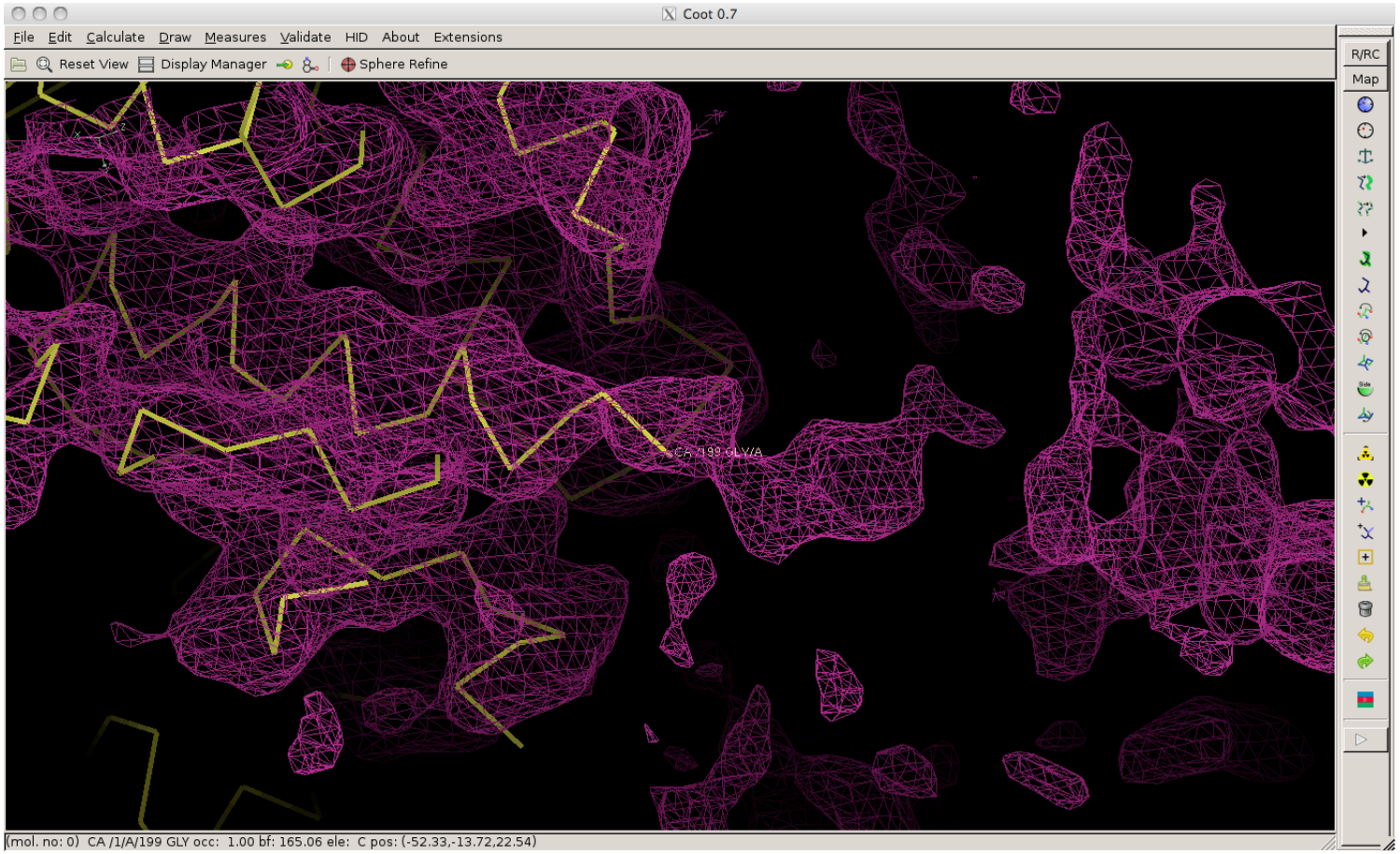


4iwo (2.6Å)

Map Sharpening/Blurring

Output Array of Sharpened Maps

Blur 50:

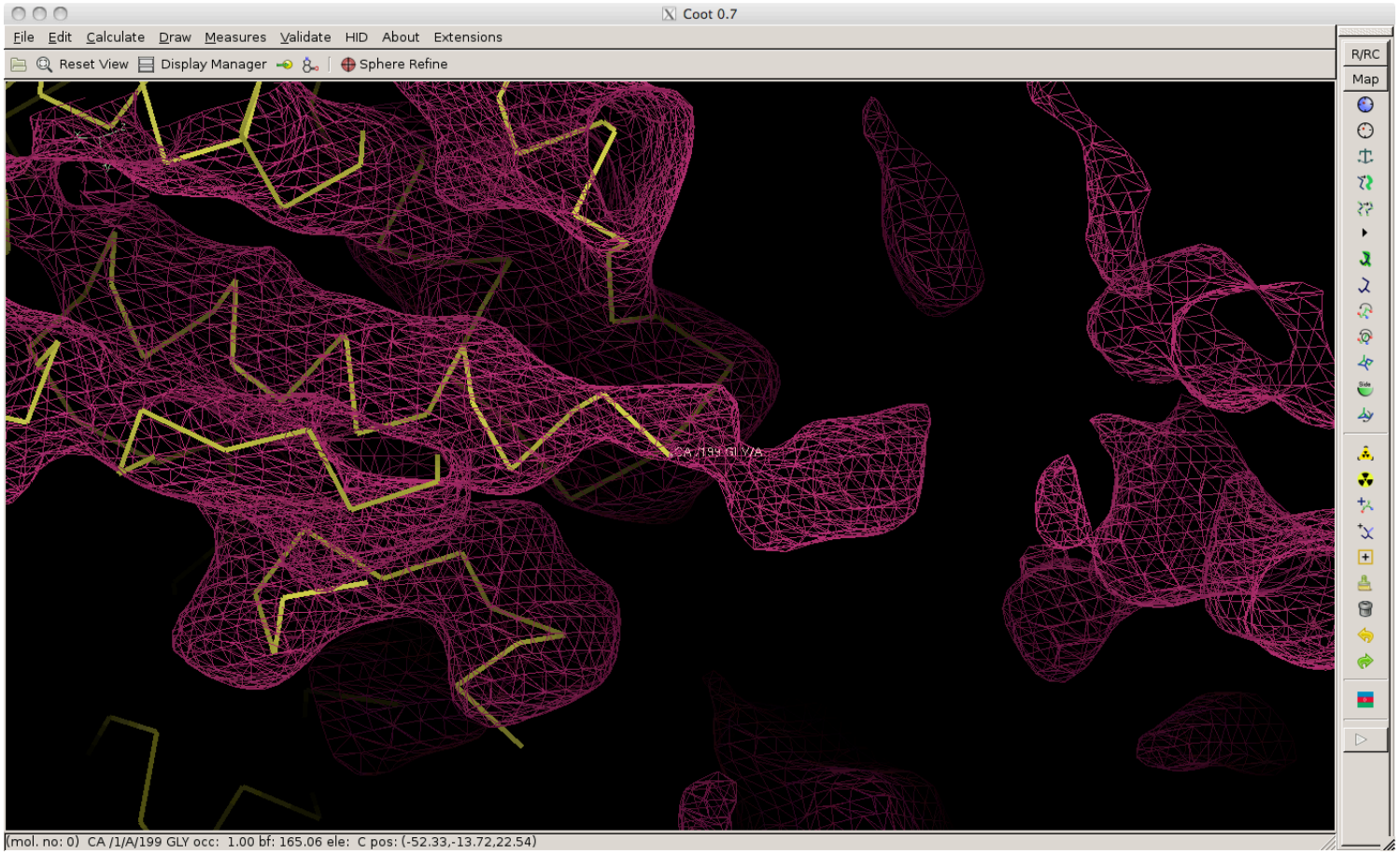


4iwo (2.6Å)

Map Sharpening/Blurring

Output Array of Sharpened Maps

Blur 150:



4iwo (2.6Å)