Refmac for single particle cryo-EM Model Refinement

CCP4 Crystallography School and Workshop

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Sao Carlos, Brazil

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University of London

with slides from **Rob Nicholls**

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Cryo-EM vs MX Refinement

MX refinement software can be repurposed for cryo-EM

Tools designed for low-resolution in MX can be used for high-resolution cryo-EM

Some relevant differences:

- In cryo-EM, the "Observations" are electrostatic potential maps
  - *In MX observations are diffraction spot intensities (typically converted to SF amplitudes)*
- Able to obtain phase information (though amplitudes and phases are noisy)
  - *map is not updated as model is refined*
- No crystallographic properties (e.g. space groups) or peculiarities/pathologies (e.g. twinning)
- No fixed unit cell - boundaries are not enforced; artificial boxes are used
- Concept of "resolution"
  - *quoted resolution in MX it is the diffraction limit (resolution of the largest Miller indices used)*
  - *possible to consider local resolution*
  - *local map quality varies greatly within and between reconstructions*

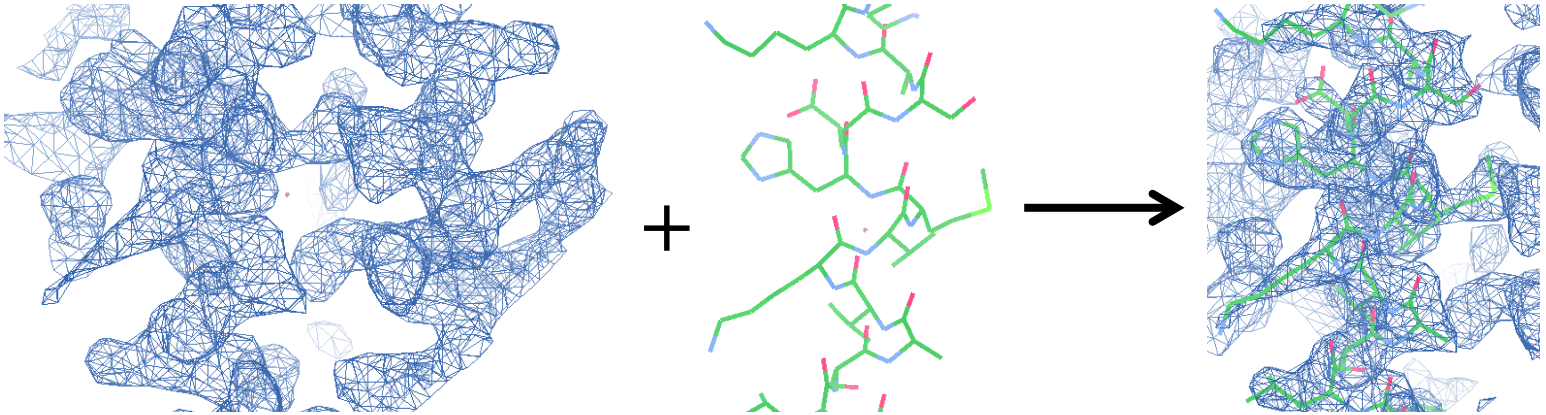
One similarity:

- Scattering: High-resolution information loss
  - *most methods developed can be transferred*

Cryo-EM Refinement

Data | Atomic model | Fit and refine

Cryo-EM



Cryo-EM refinement: structure factors (reconstruction/map) are treated as observations.

The map does not change during refinement.

Regularisation

Use of available information (prior knowledge):

Regularisers with a target value:

- Geometry restraints (chemical information)
- B-value restraints
- Local NCS restraints – where applicable
- External restraints (ProSMART/LibG) – where available

Regularisers without a target value:

- Jelly-body restraints

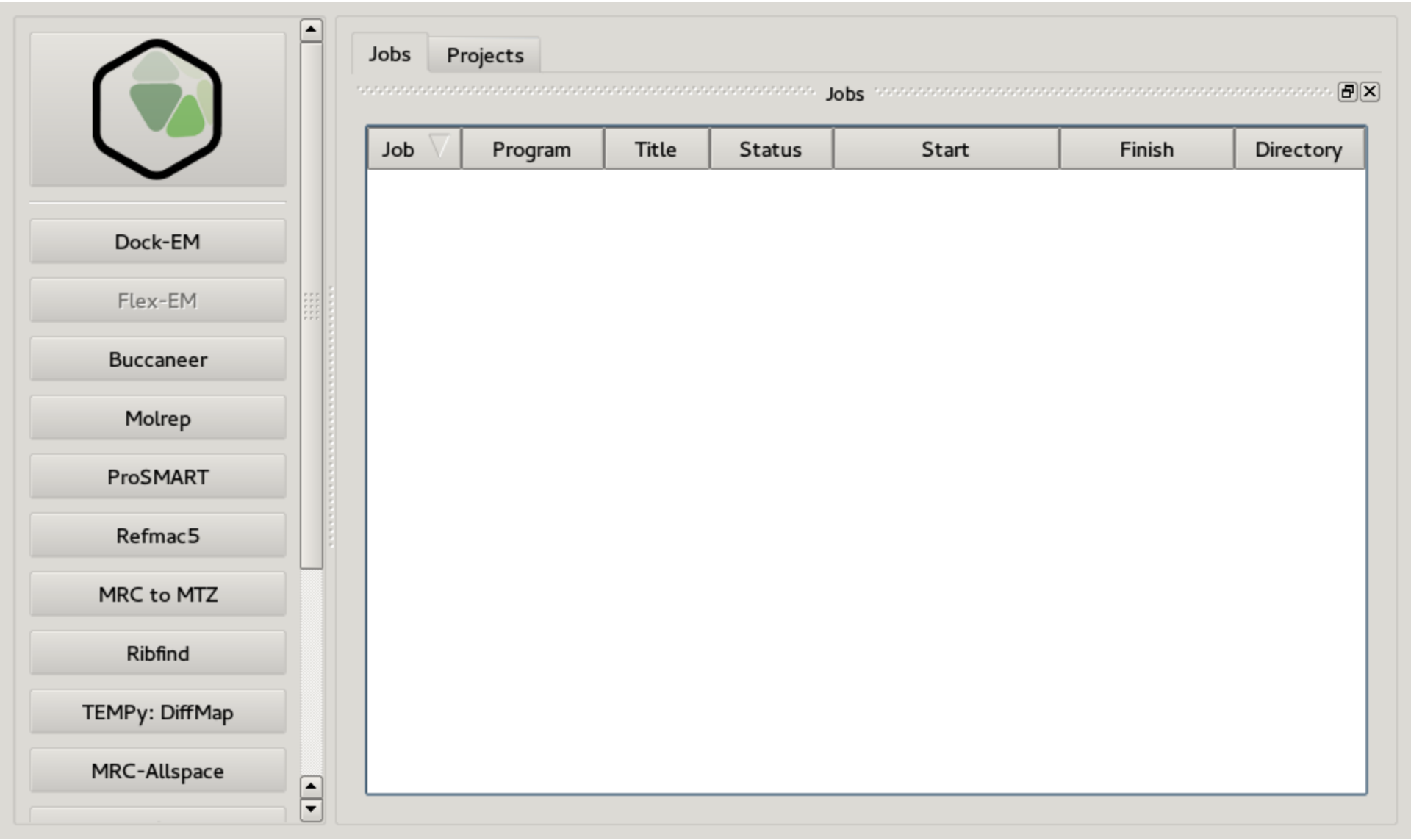
Cryo-EM

When refining ~~MX~~ models at low-resolution, check:

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

Check map sharpness?

CCP-EM


Jobs
Projects
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Program
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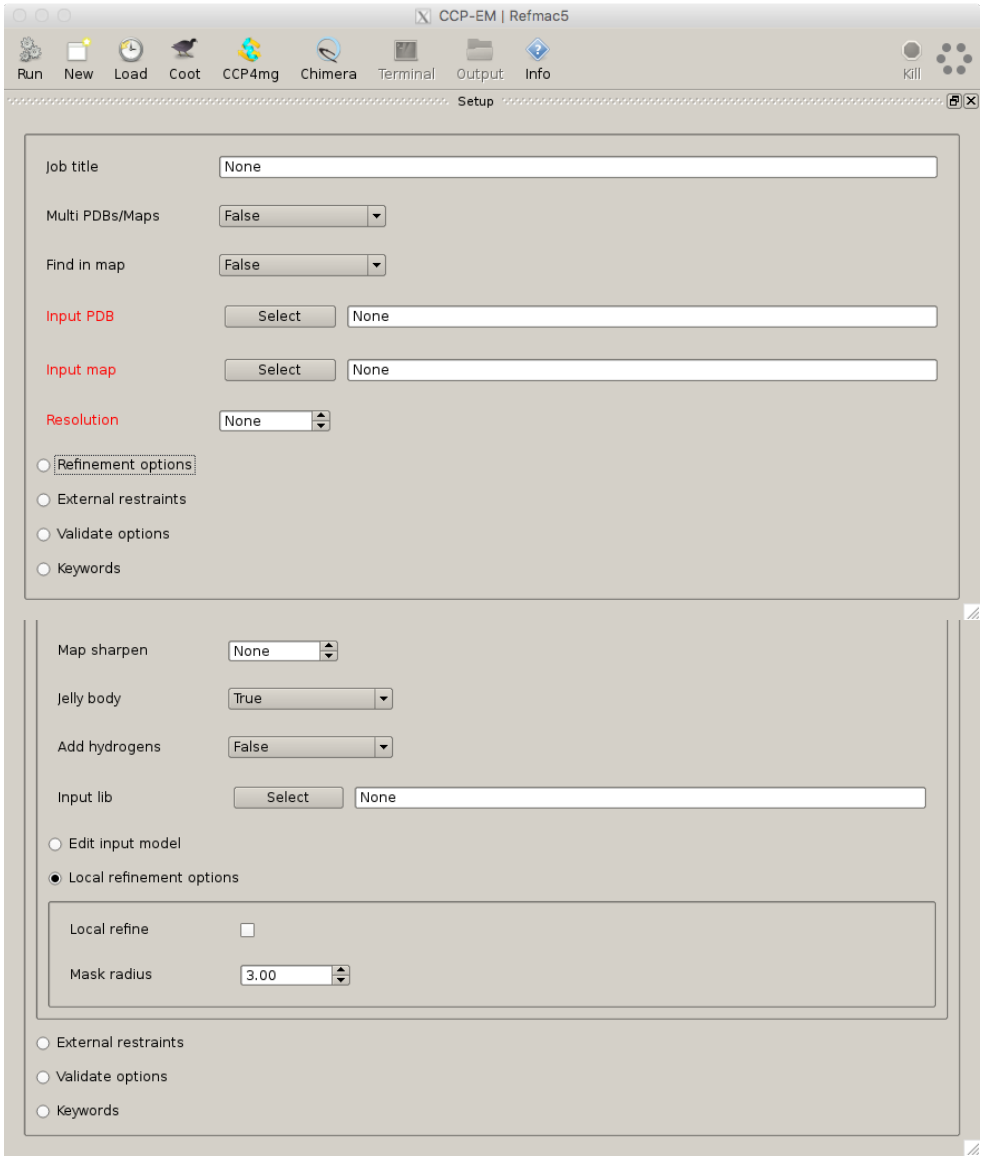
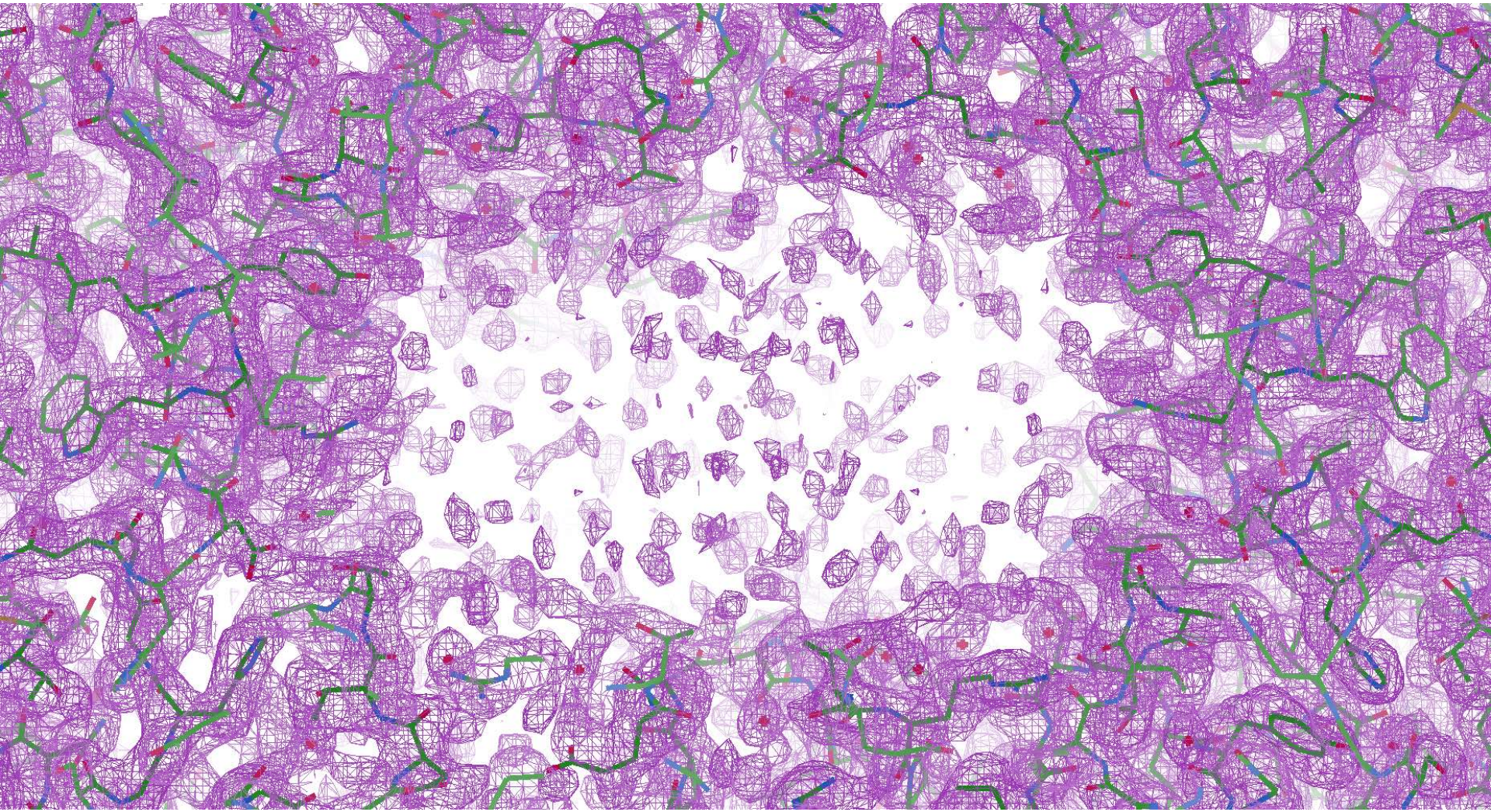
Finish
Directory
Dock-EM
Flex-EM
Buccaneer
Molrep
ProSMART
Refmac5
MRC to MTZ
Ribfind
TEMPy: DiffMap
MRC-Allspace

Jelly Body Restraints



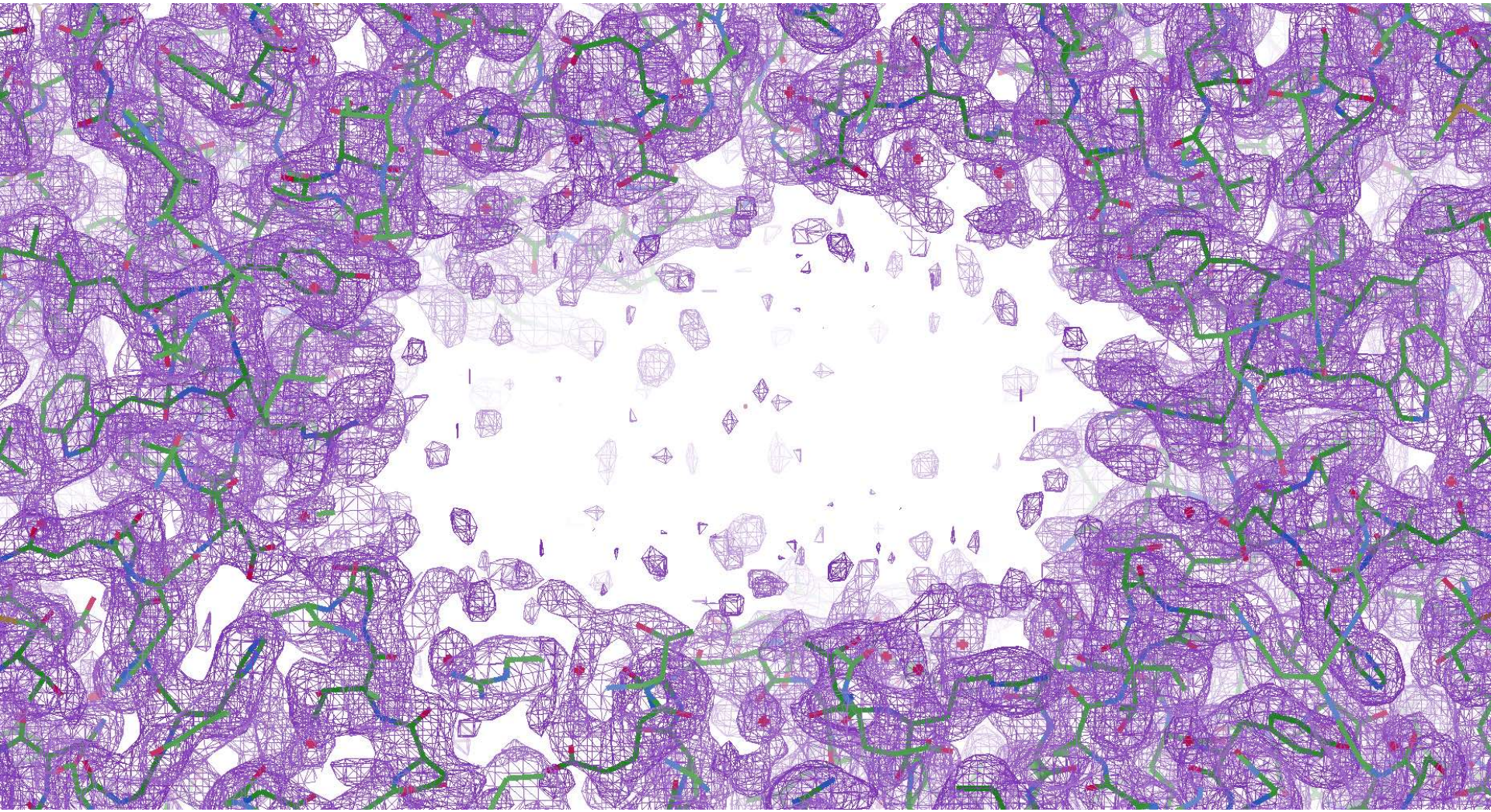
Map Sharpening/Blurring

Default map **5a1a (2.2Å)**



Map Sharpening/Blurring

Blur 20 Å^2 **5a1a (2.2Å)**



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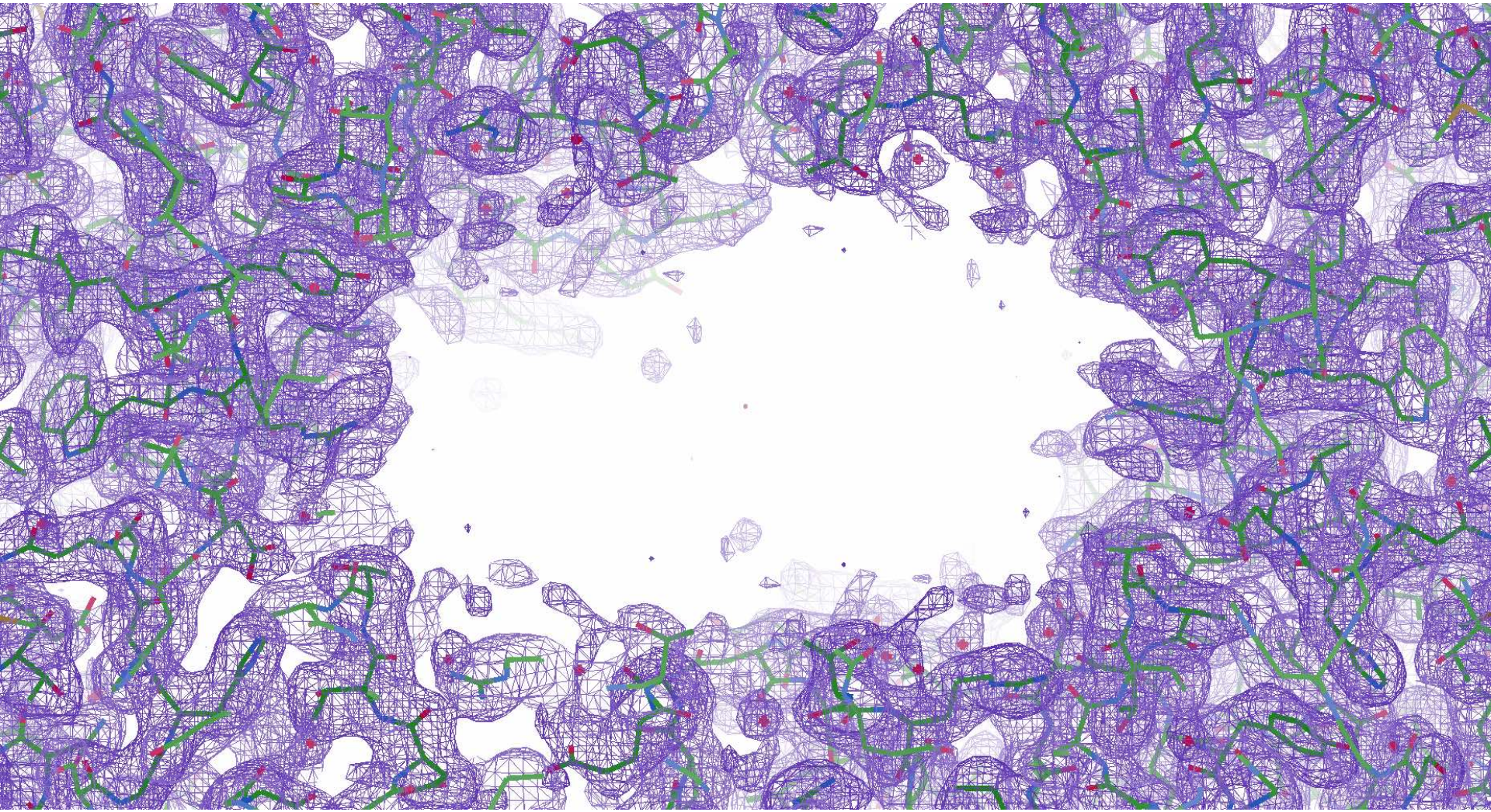

Map Sharpening/Blurring

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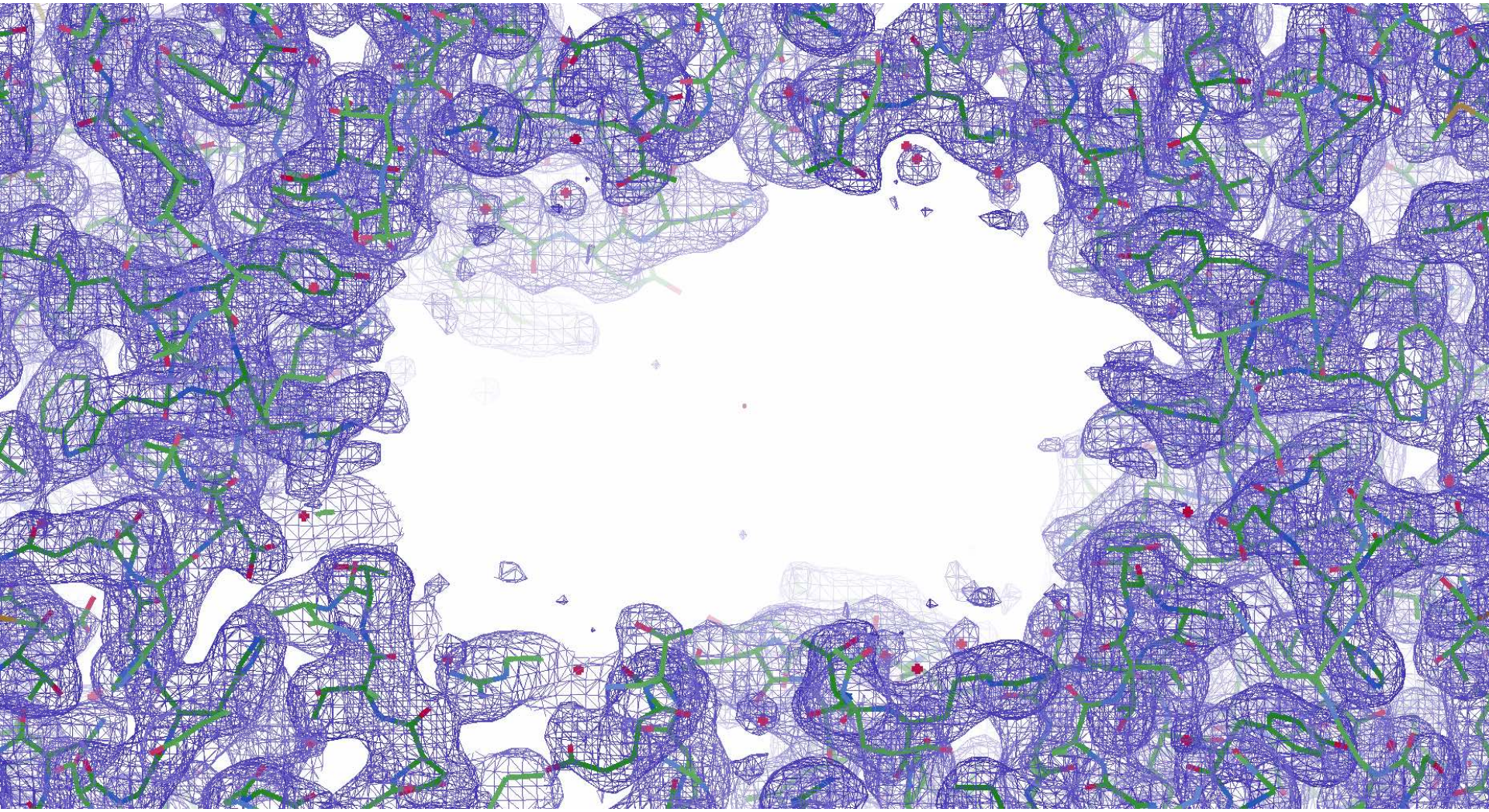

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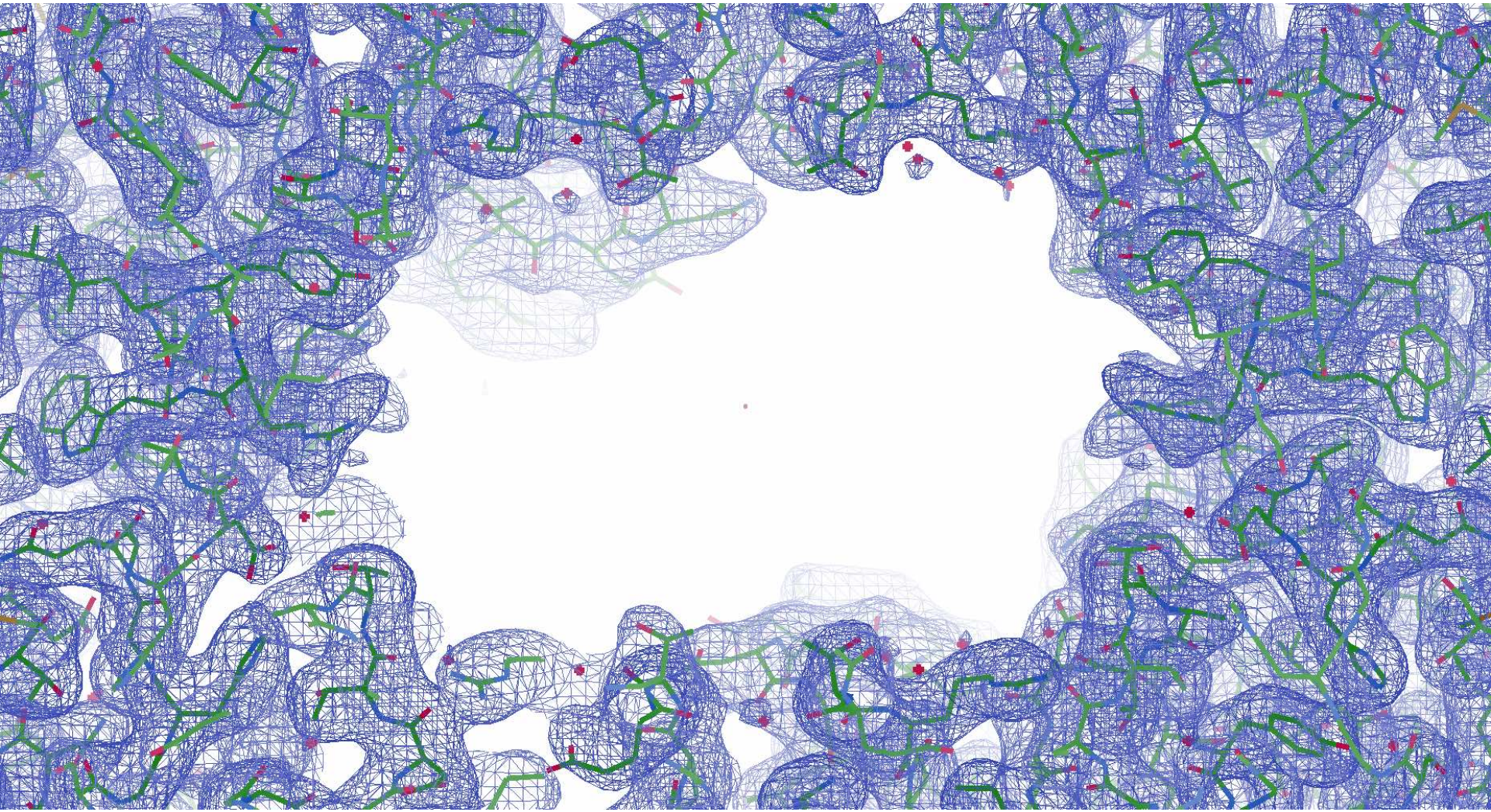

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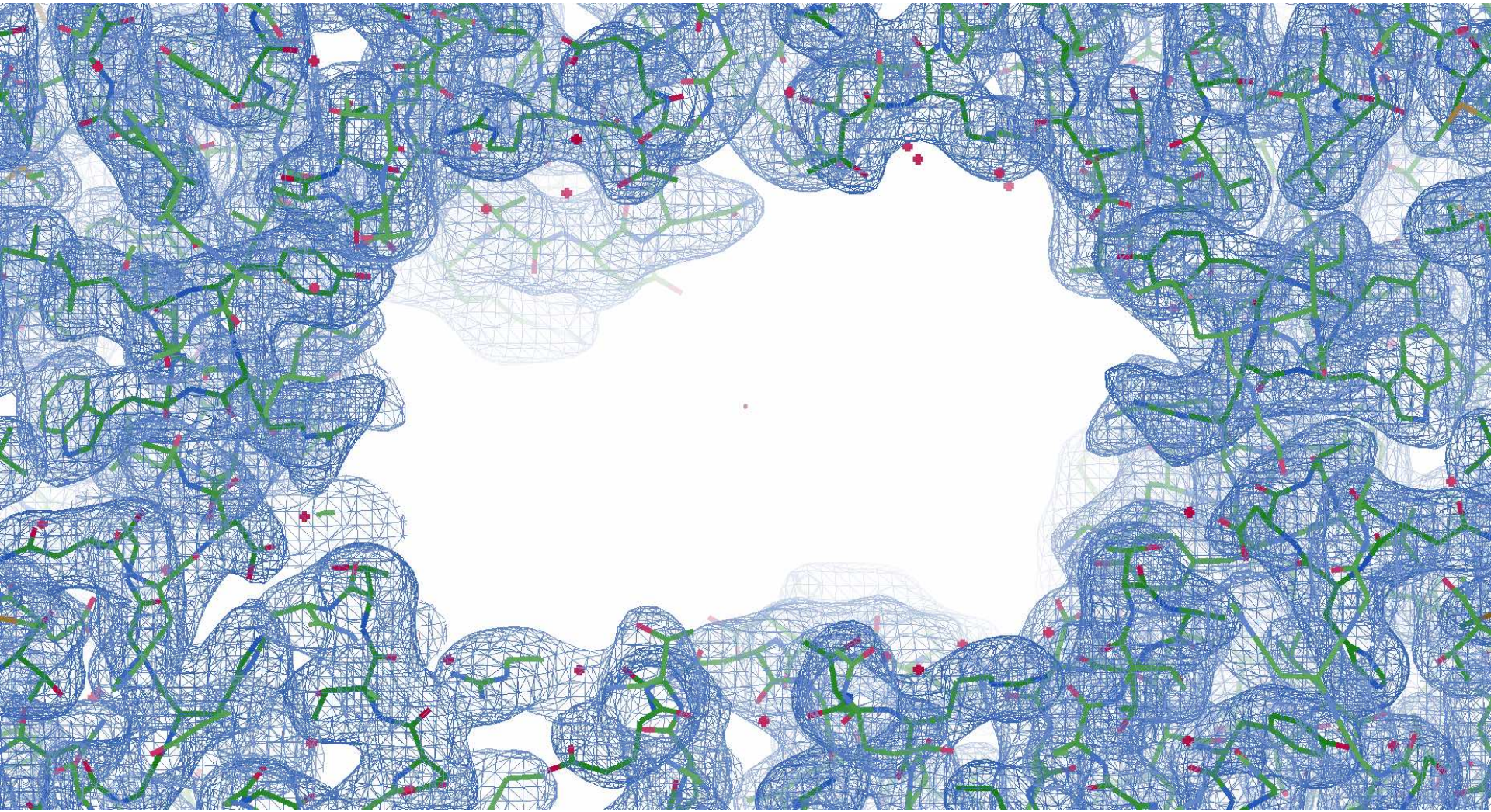

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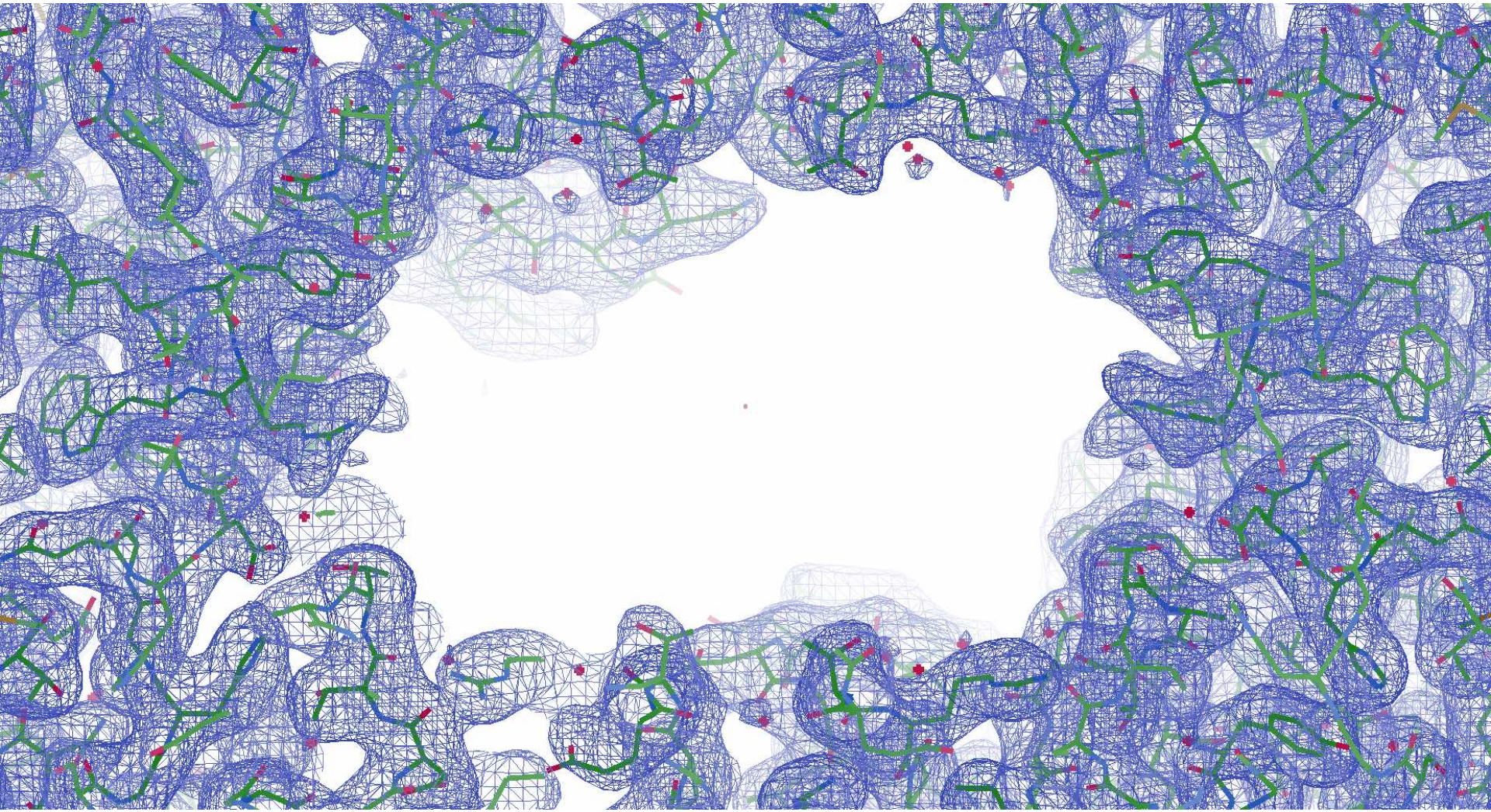

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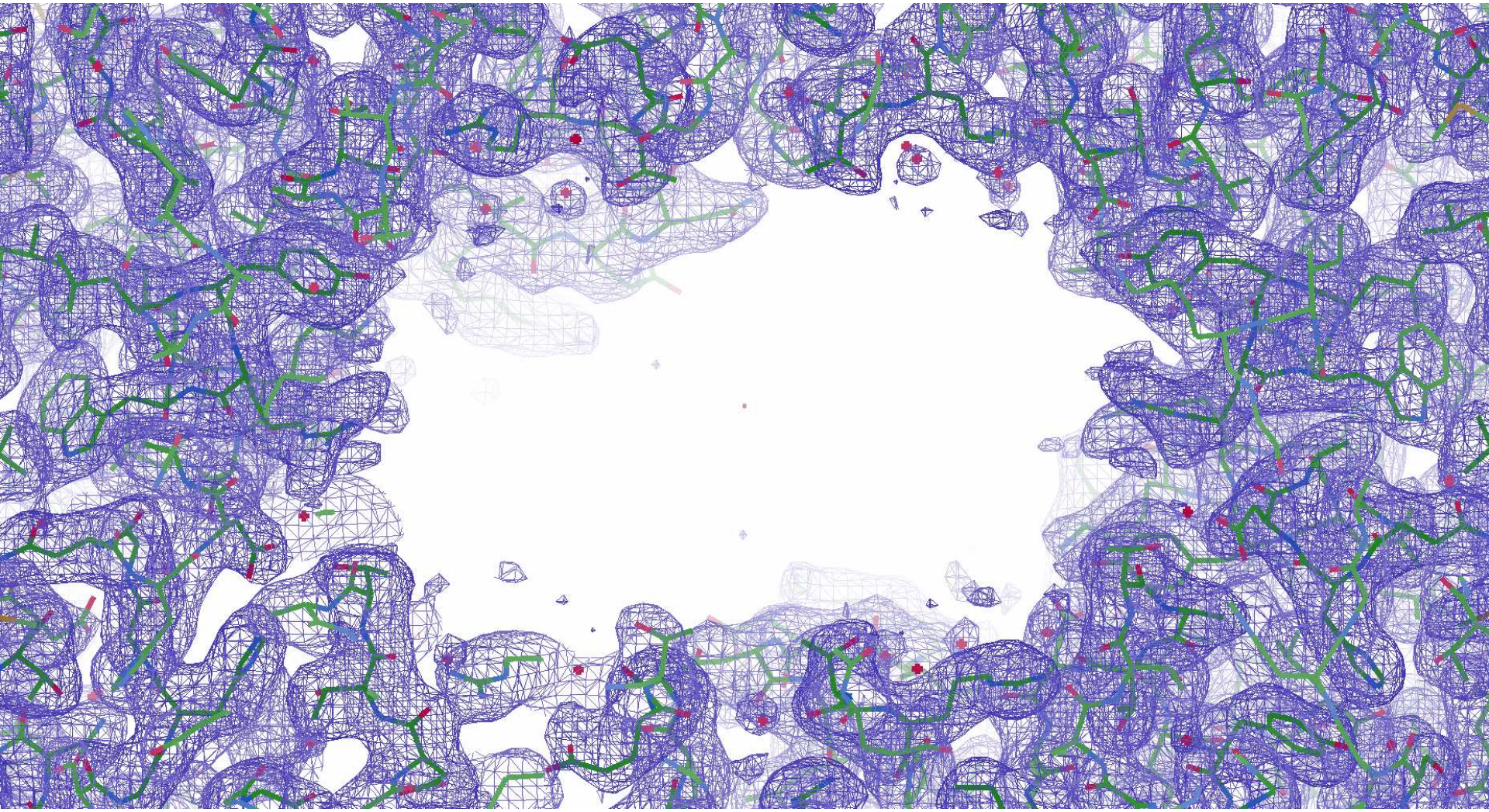

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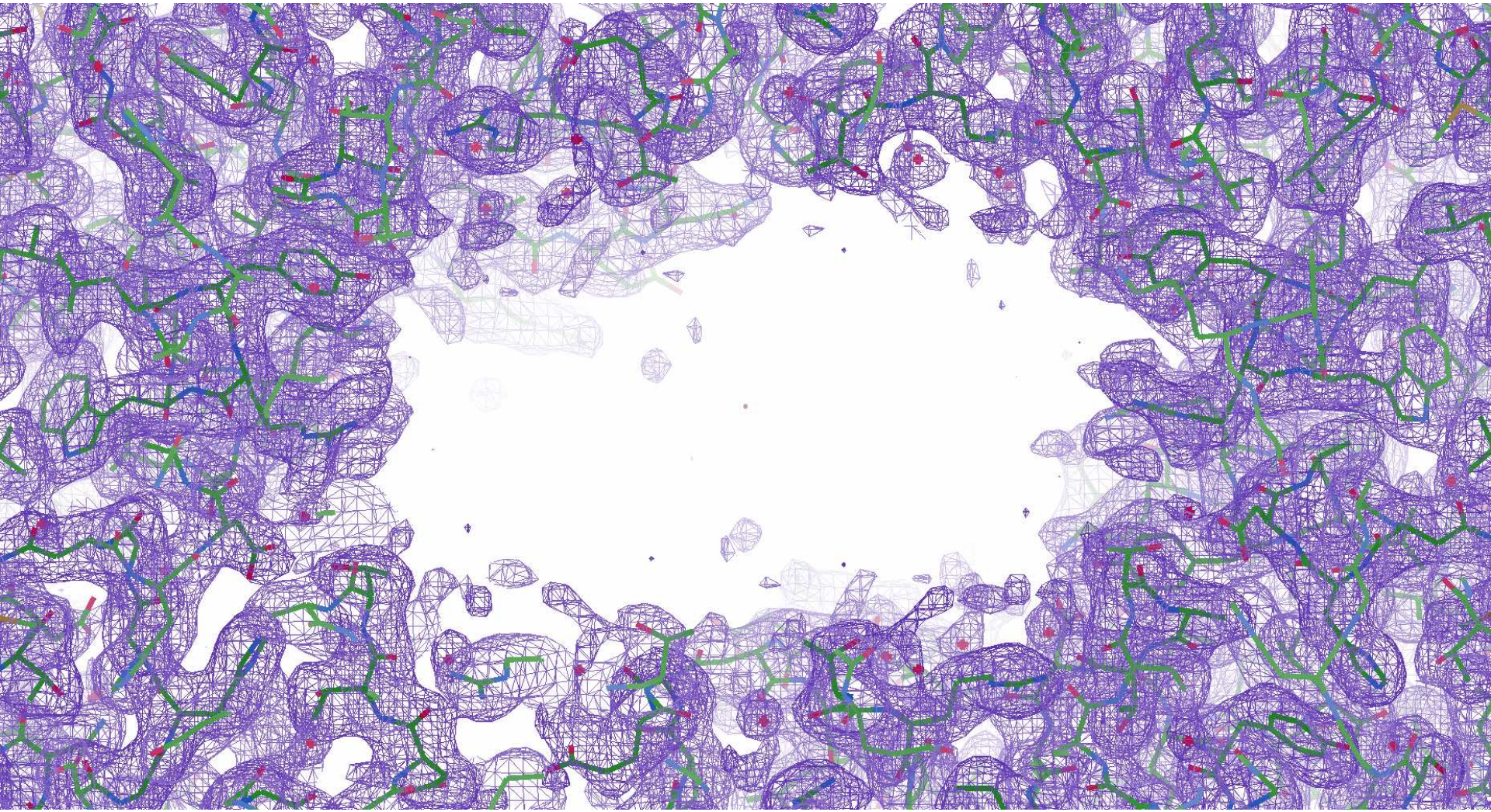

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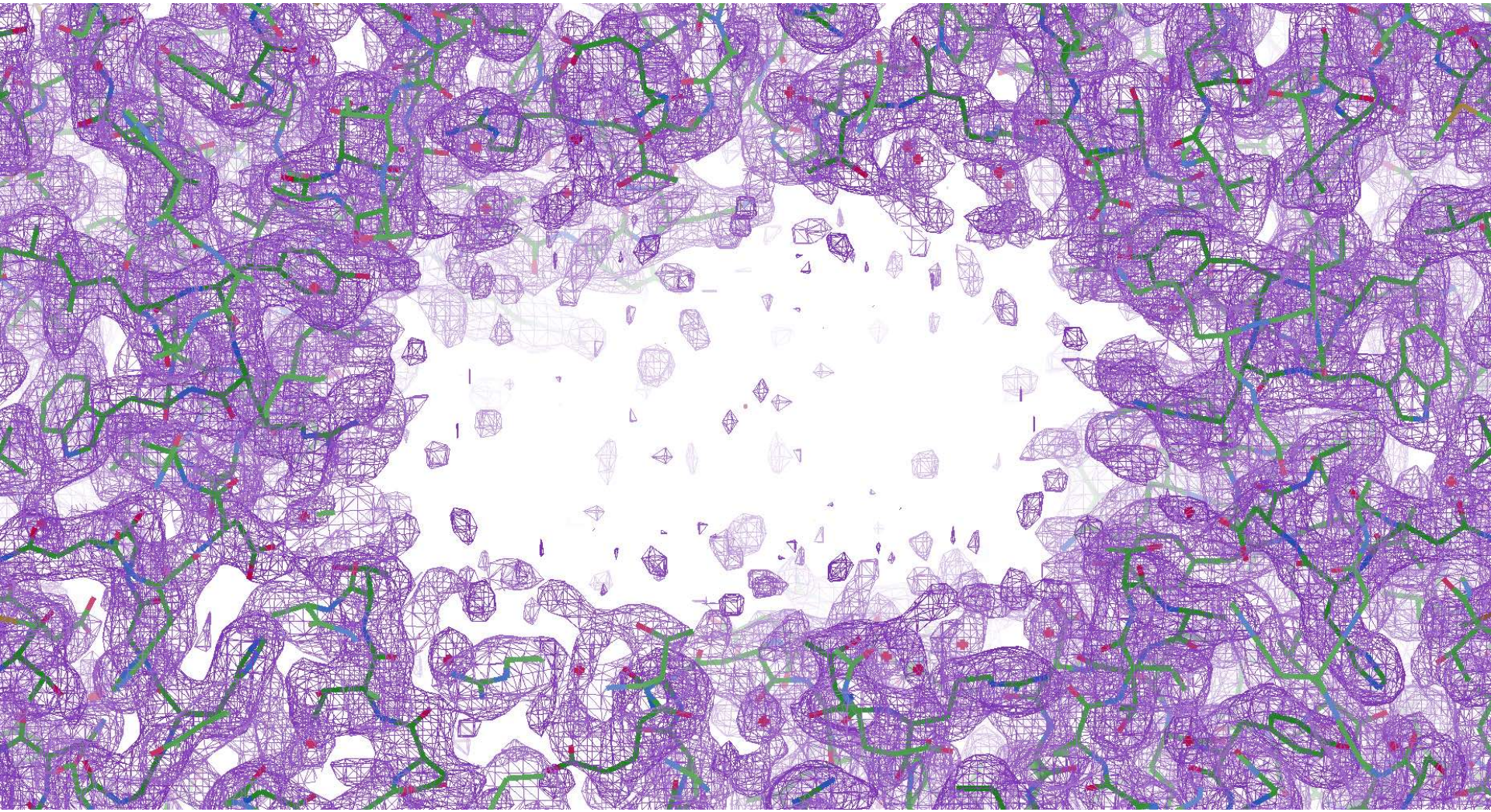

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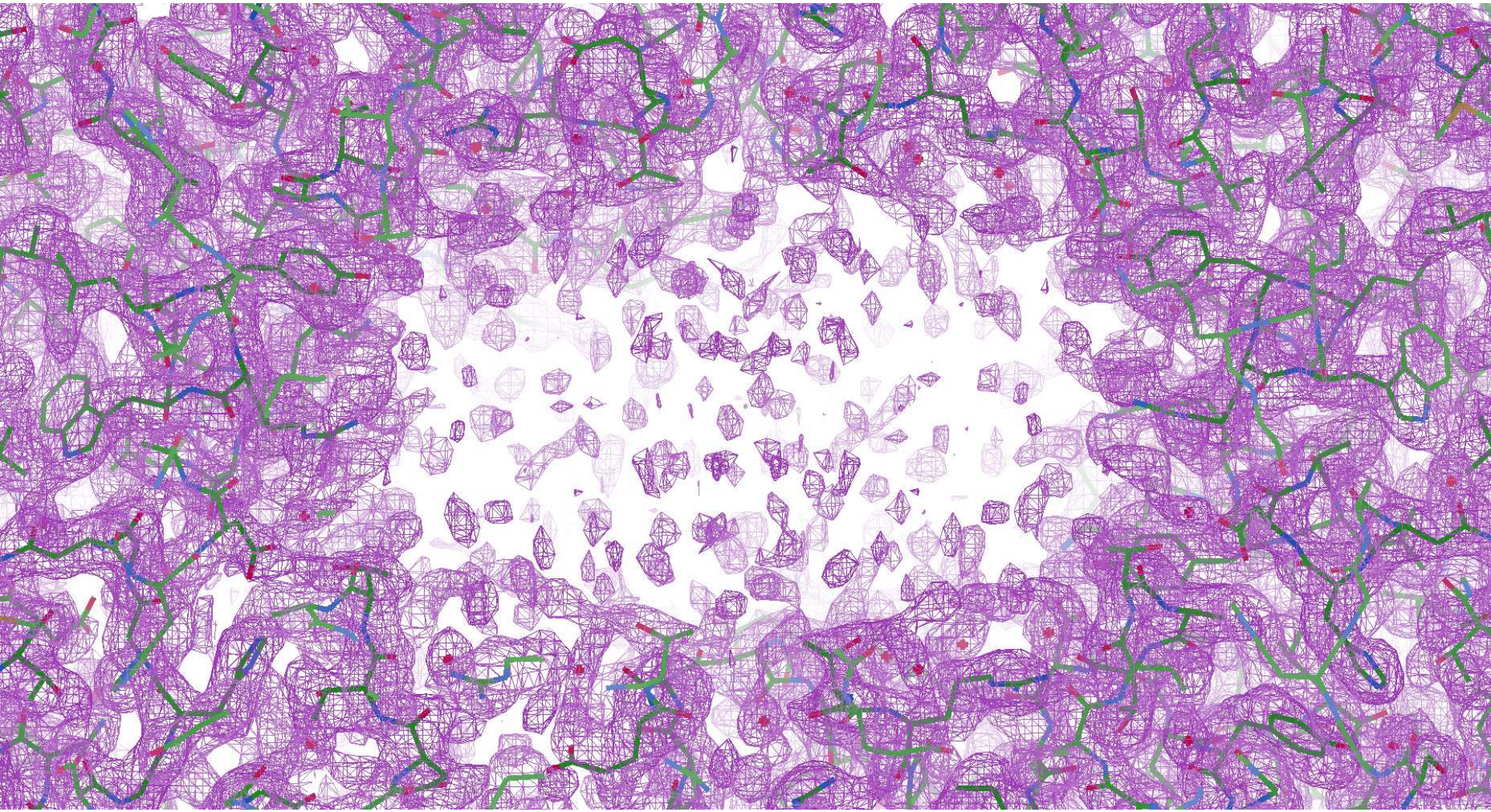

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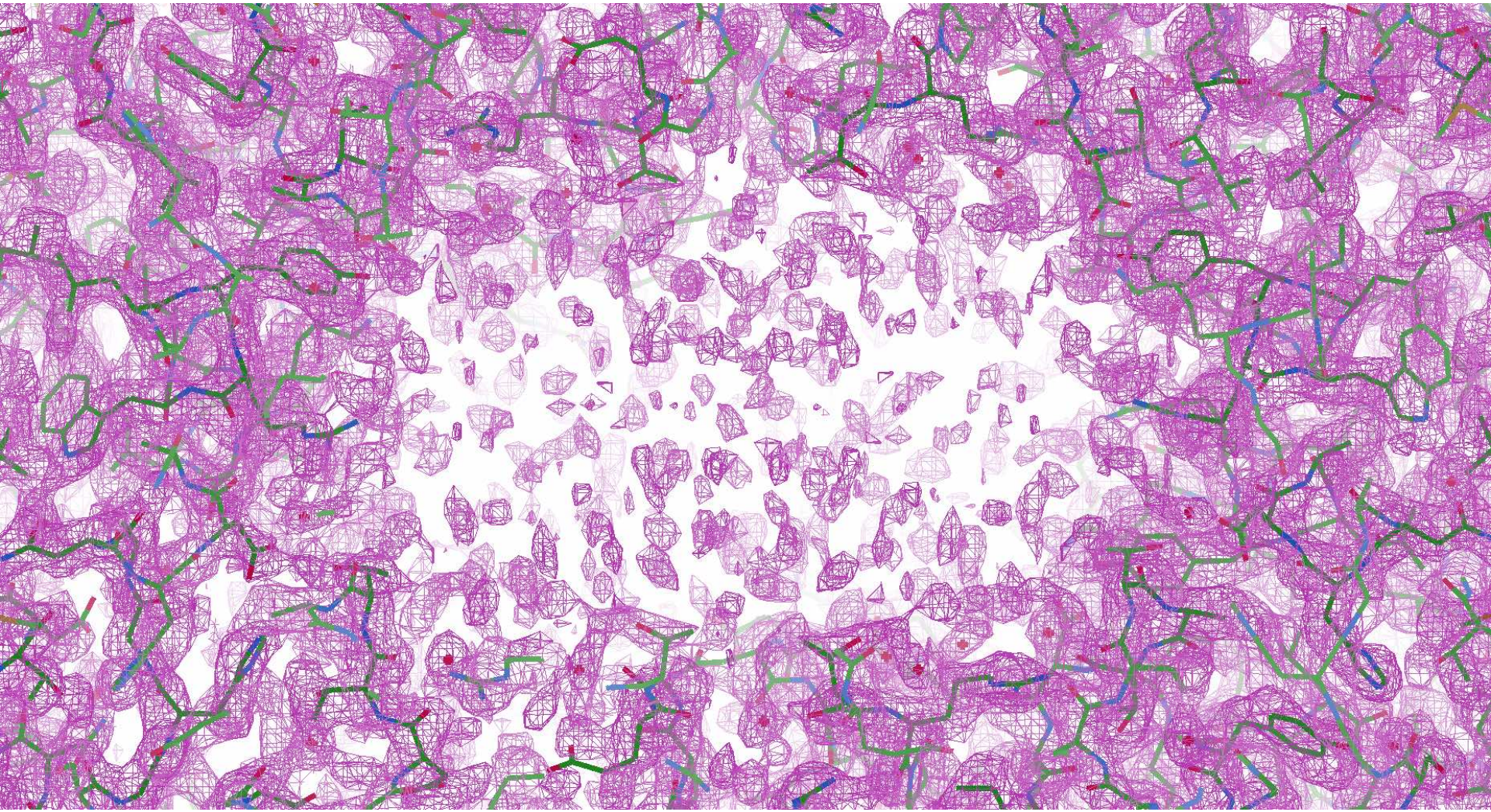

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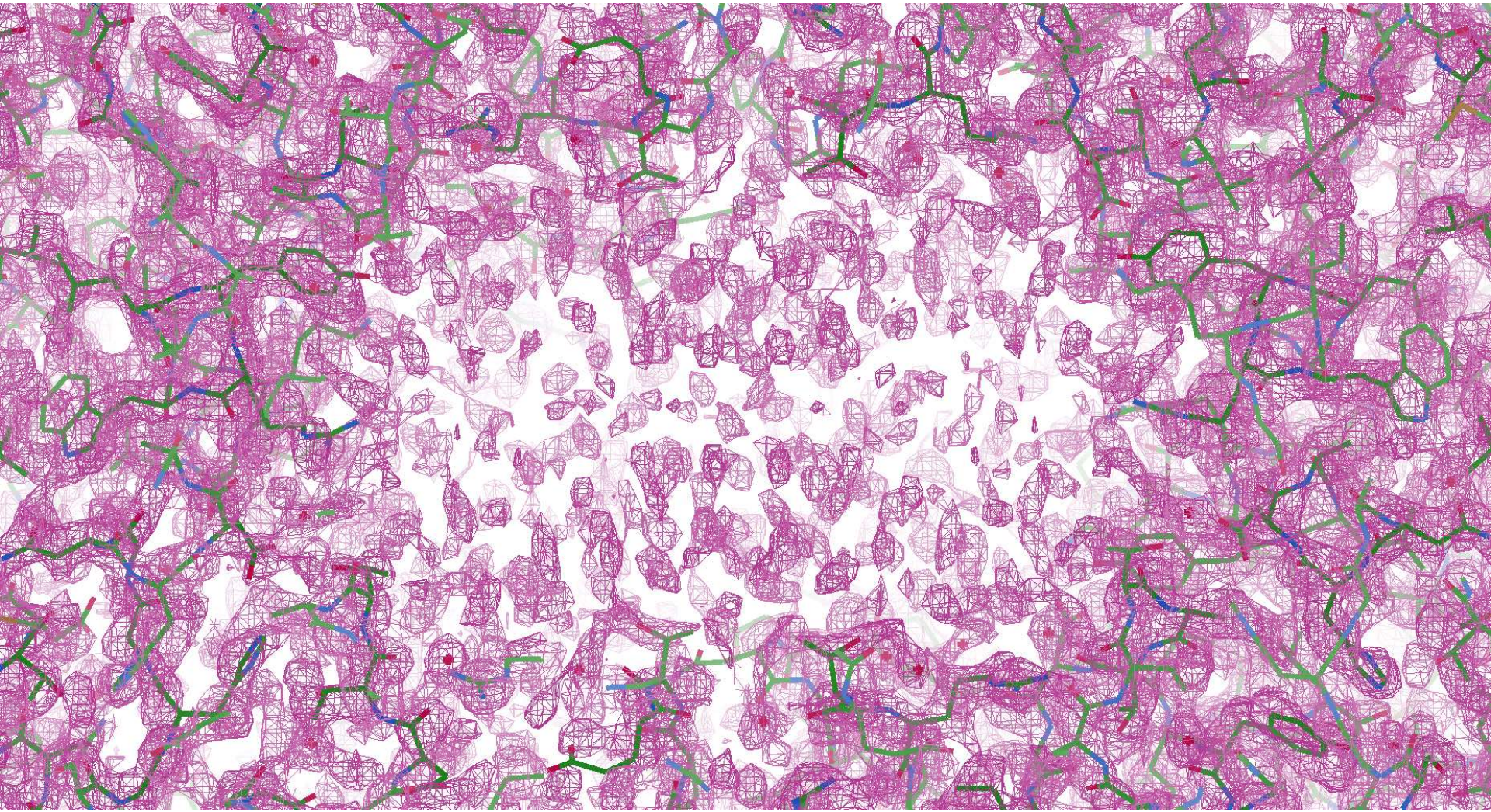

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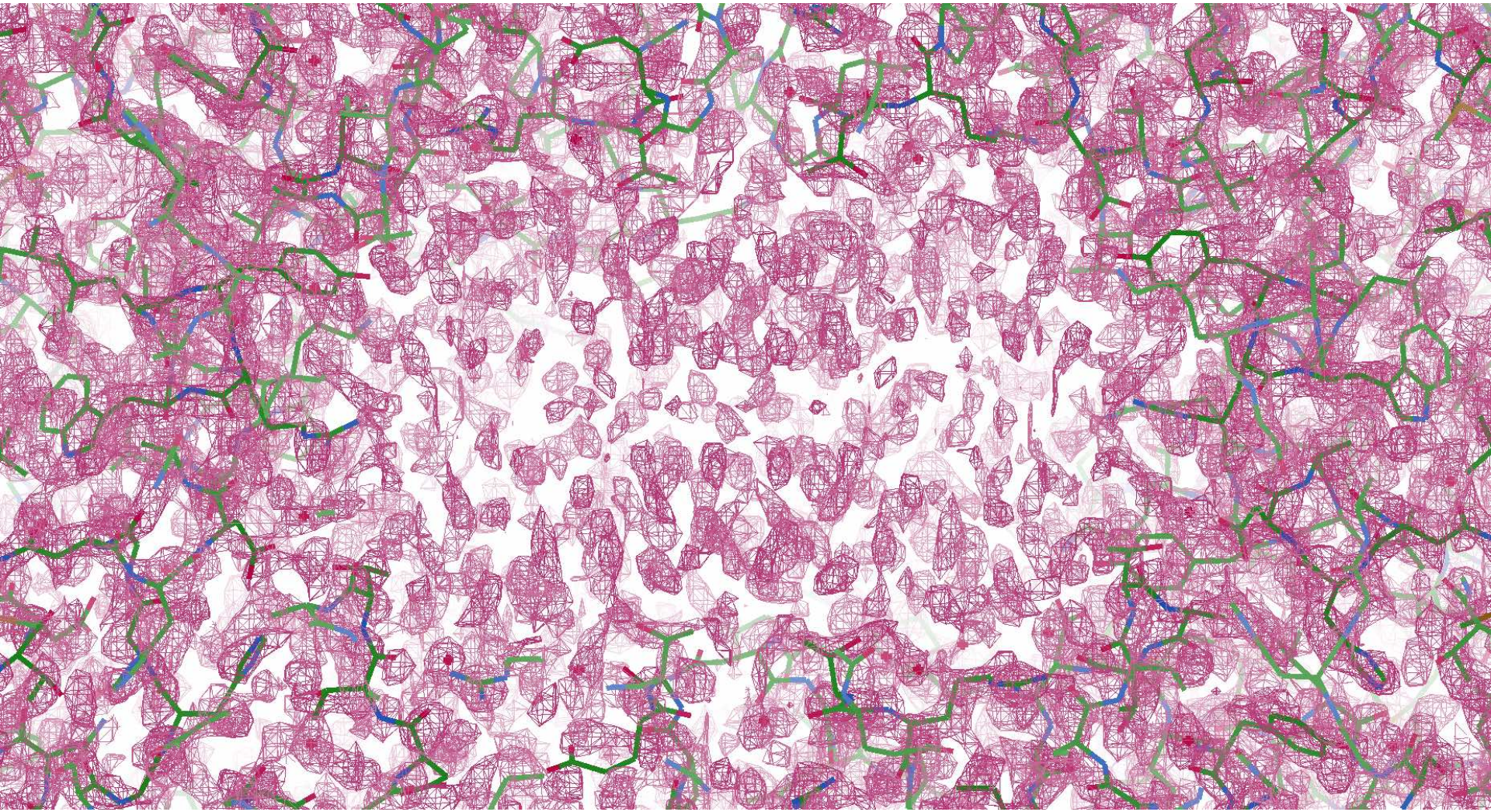
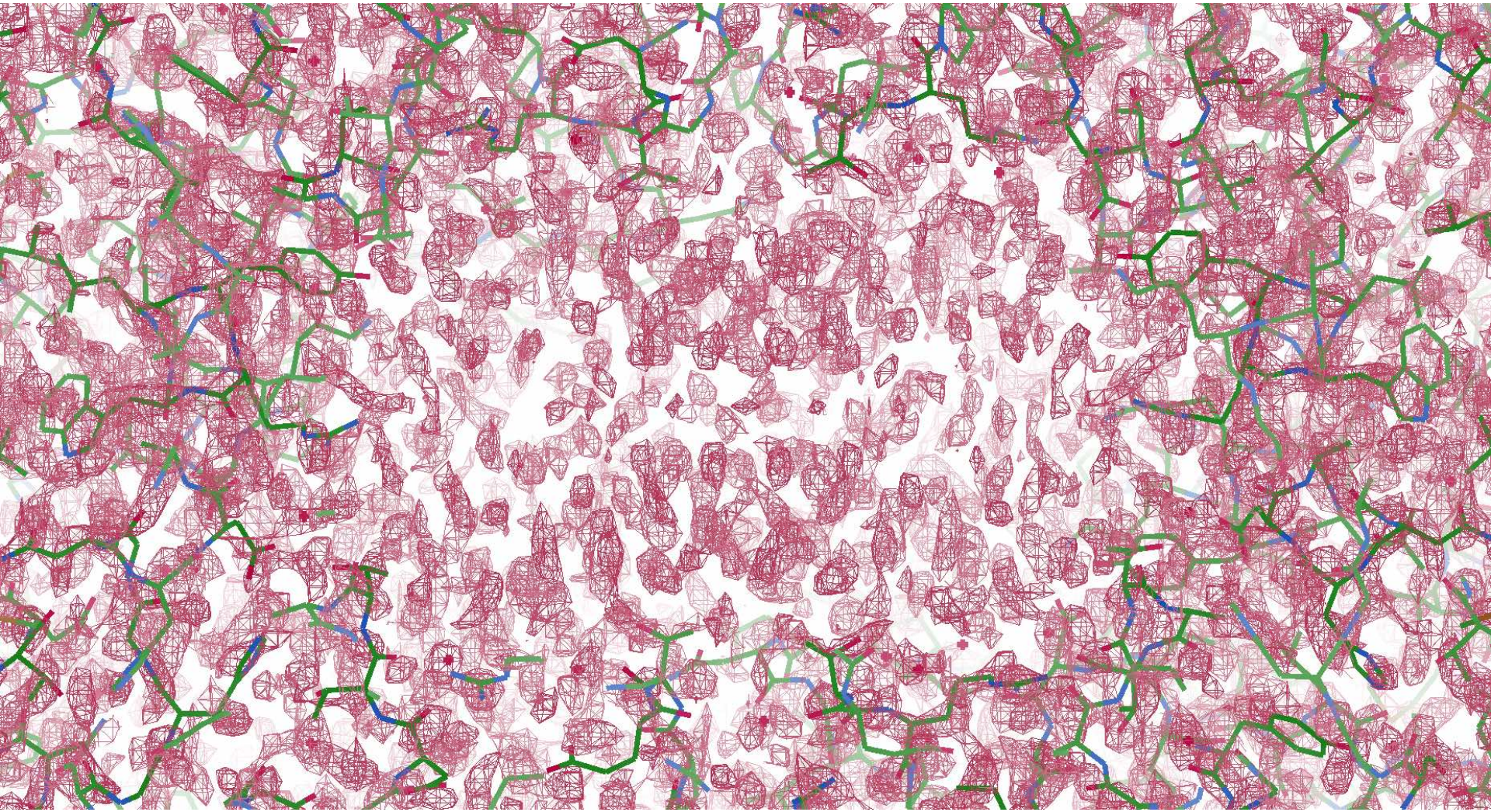


Map Sharpening/Blurring

Sharpen 80 Å^2 **5a1a (2.2Å)**



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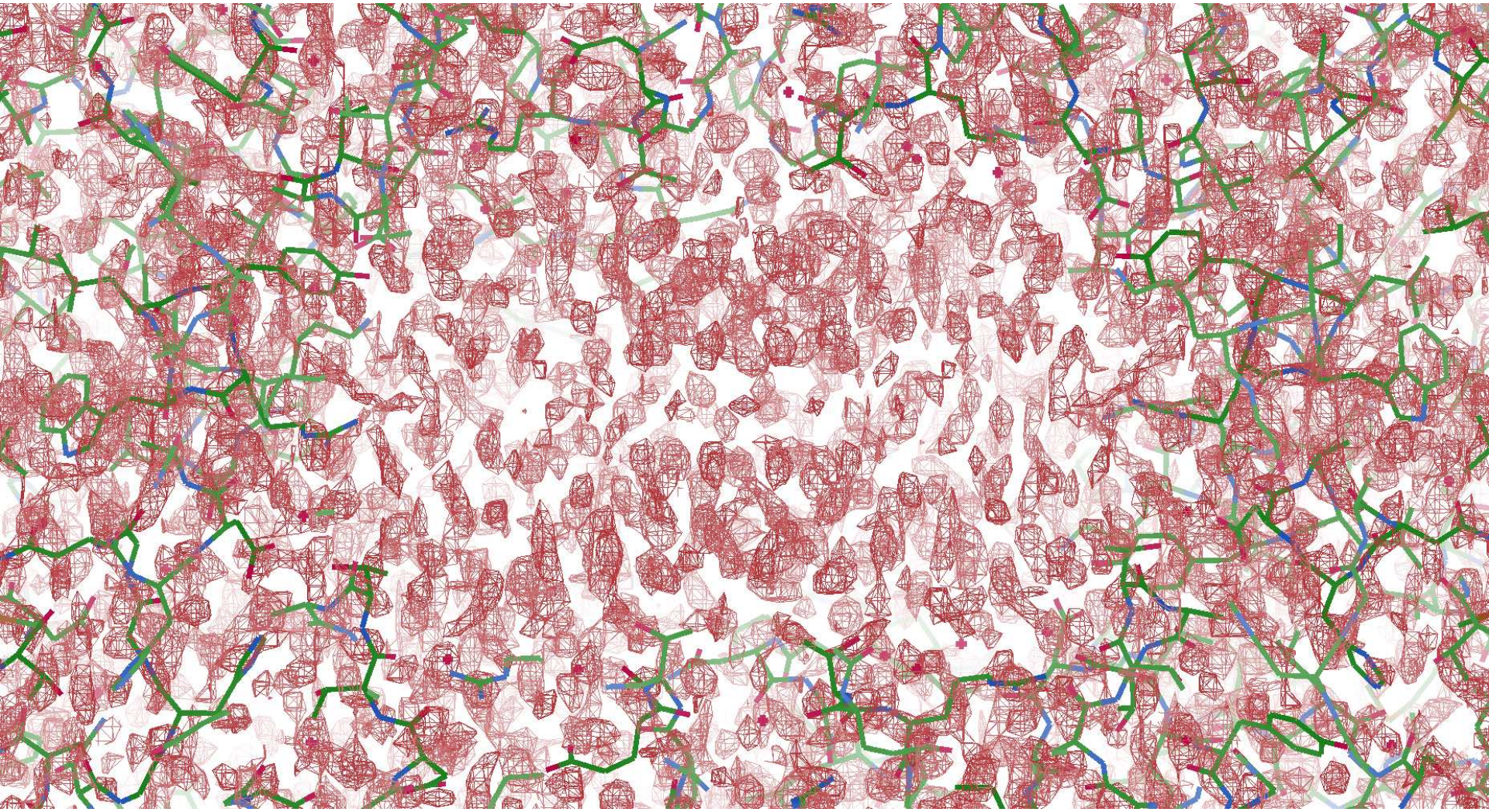

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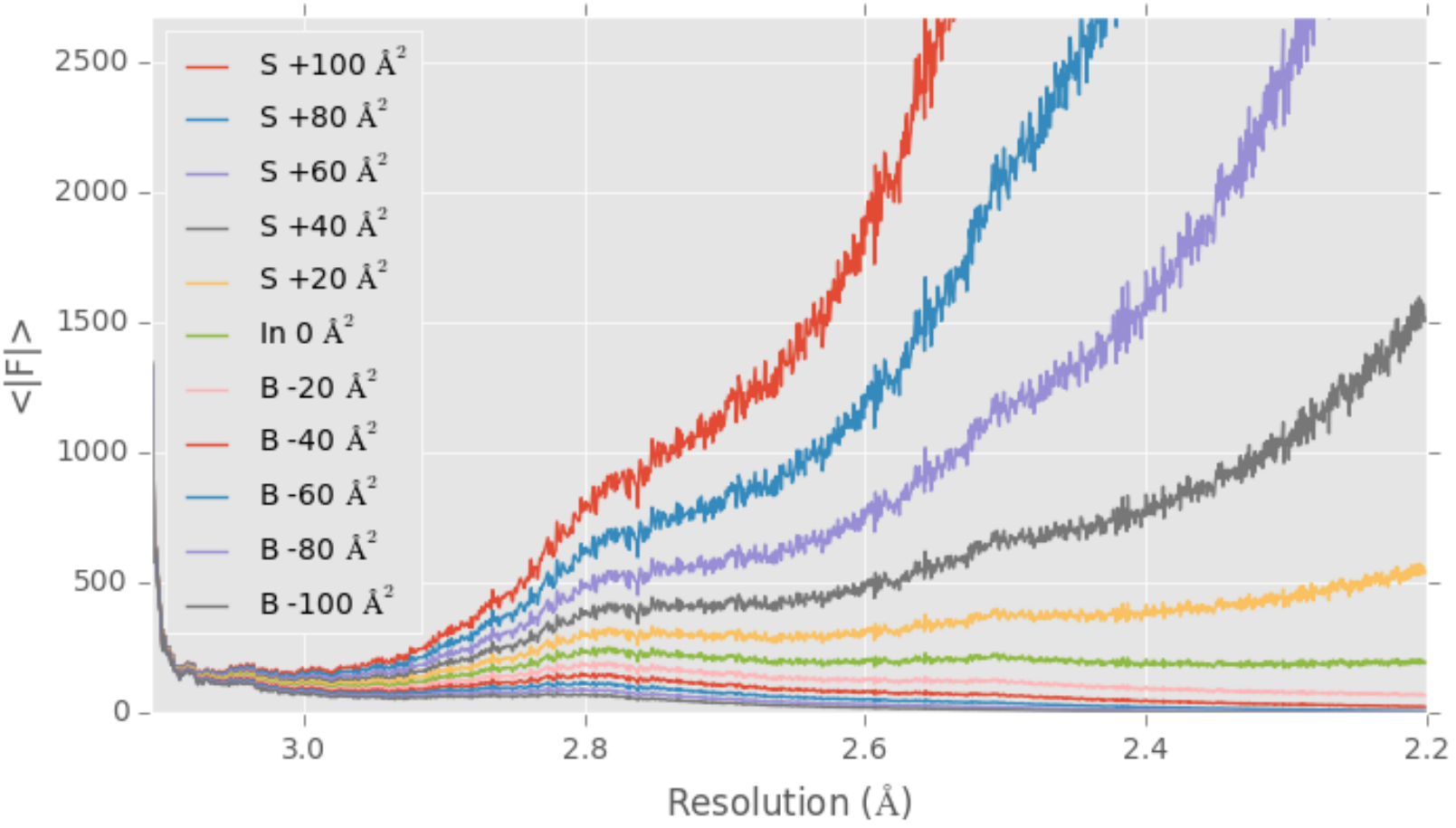

Map Sharpening/Blurring

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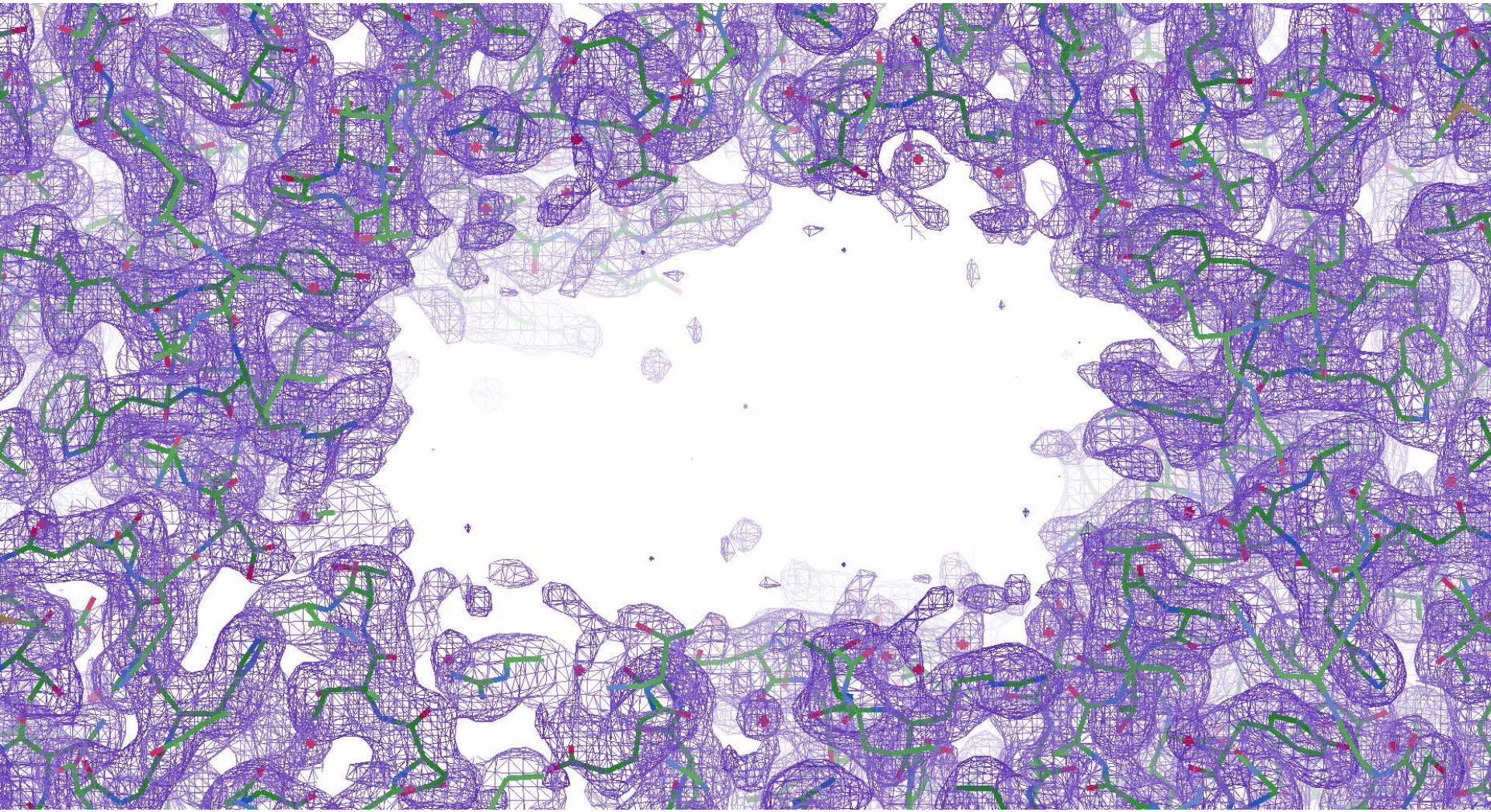
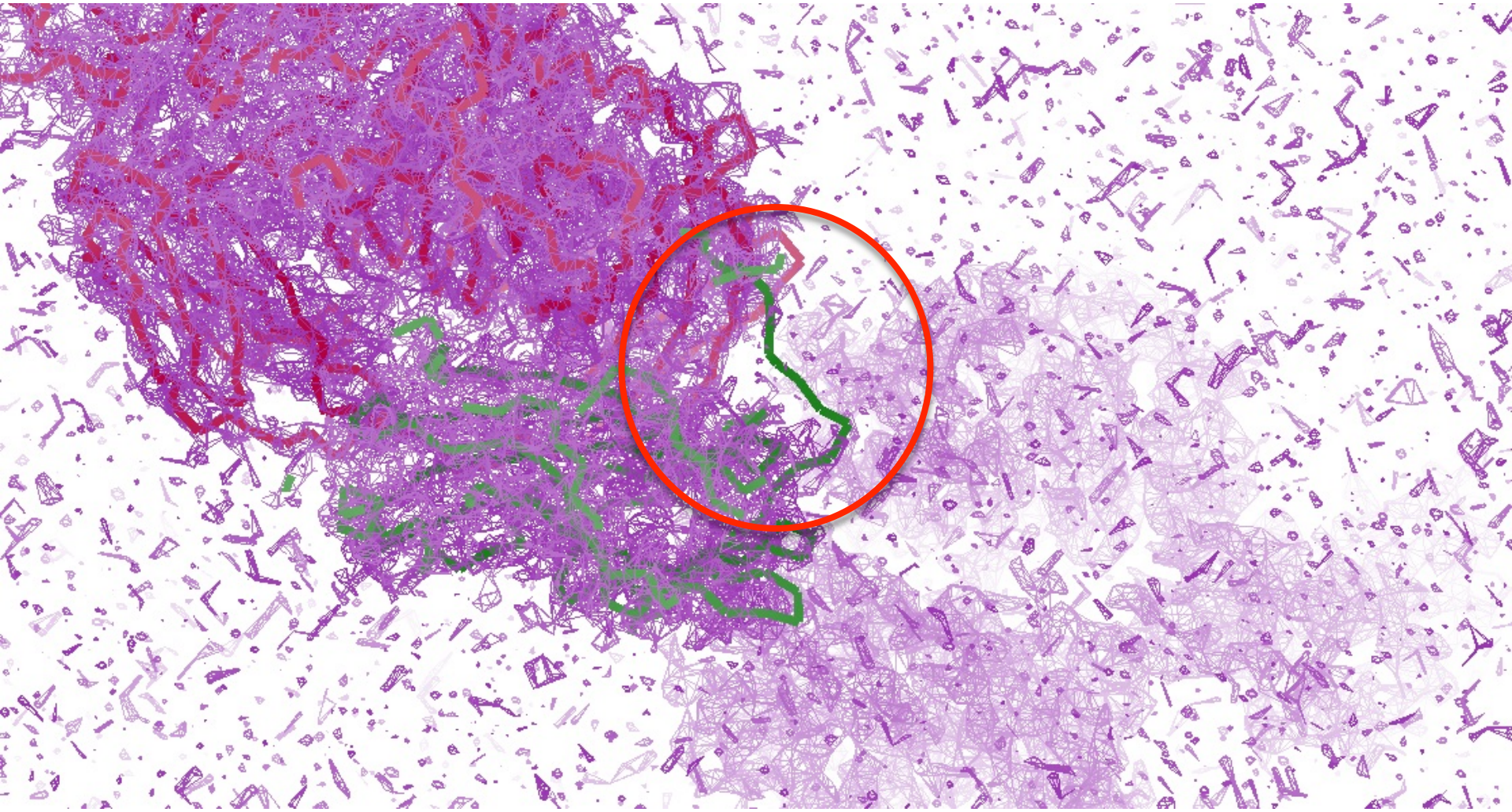
5a1a (2.2Å)



Blurring / Sharpening

Default map

5a1a (2.2Å)



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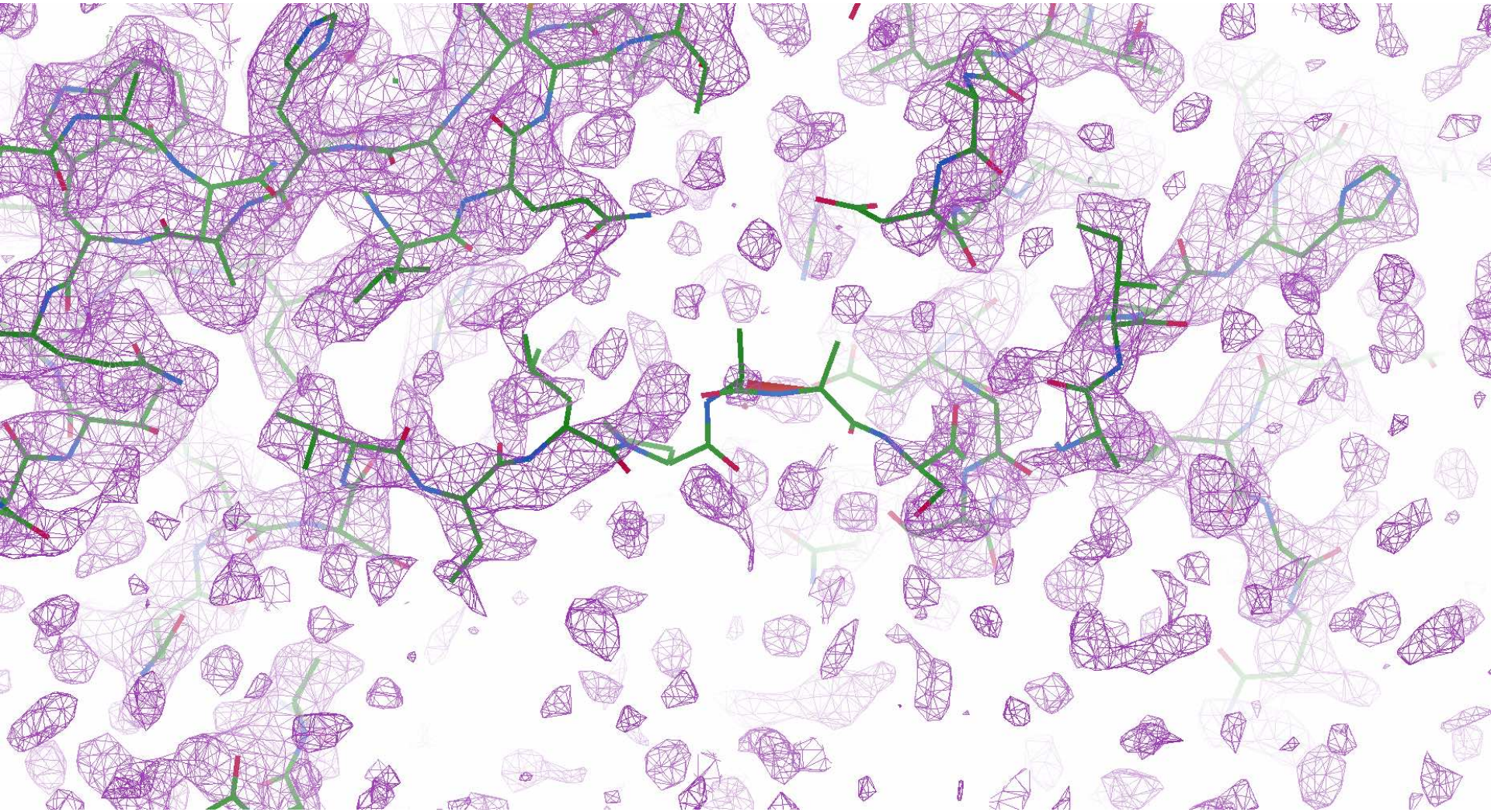
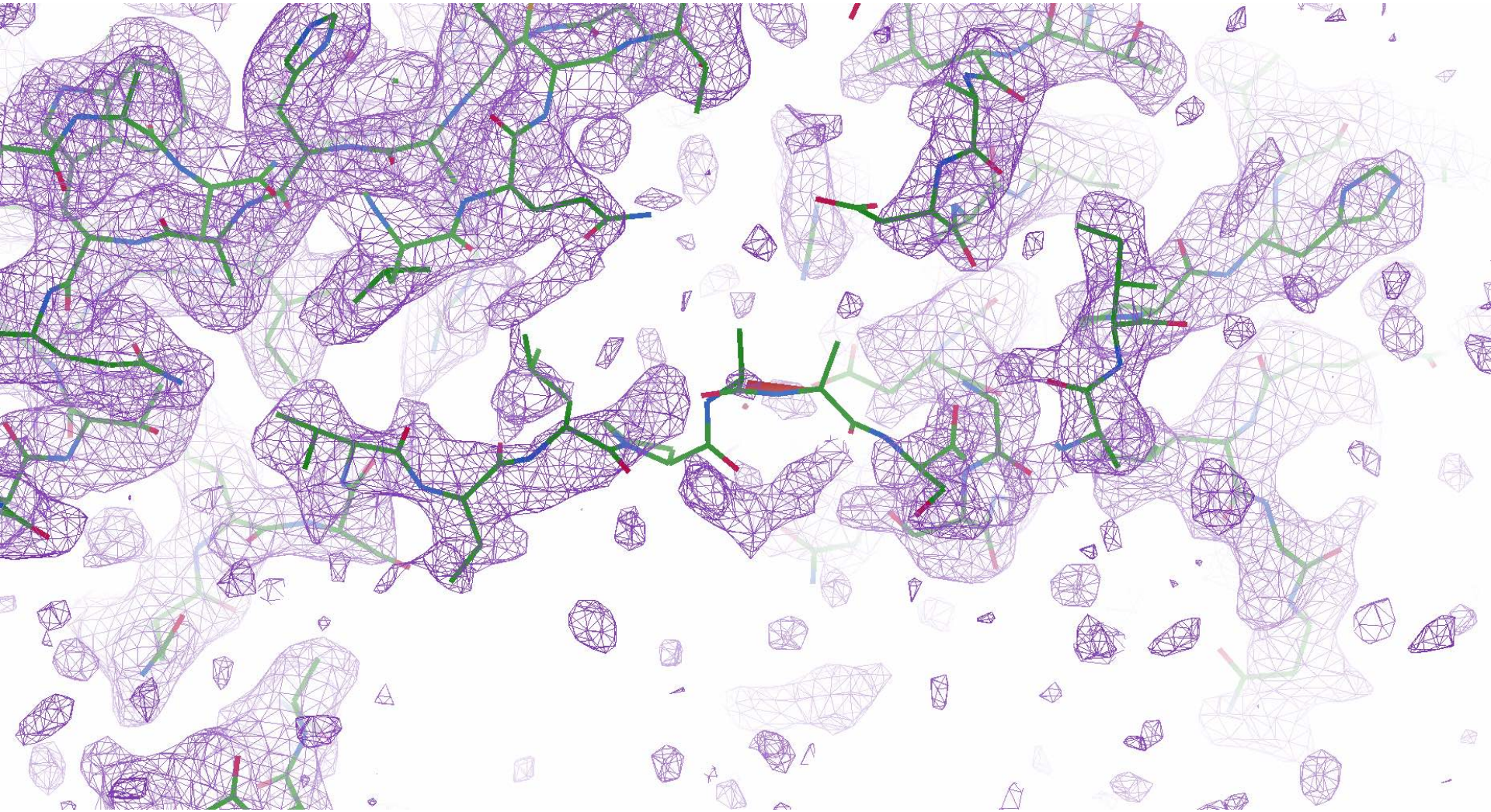


Blurring / Sharpening

Blur 20 Å^2

5a1a (2.2Å)



Blurring / Sharpening

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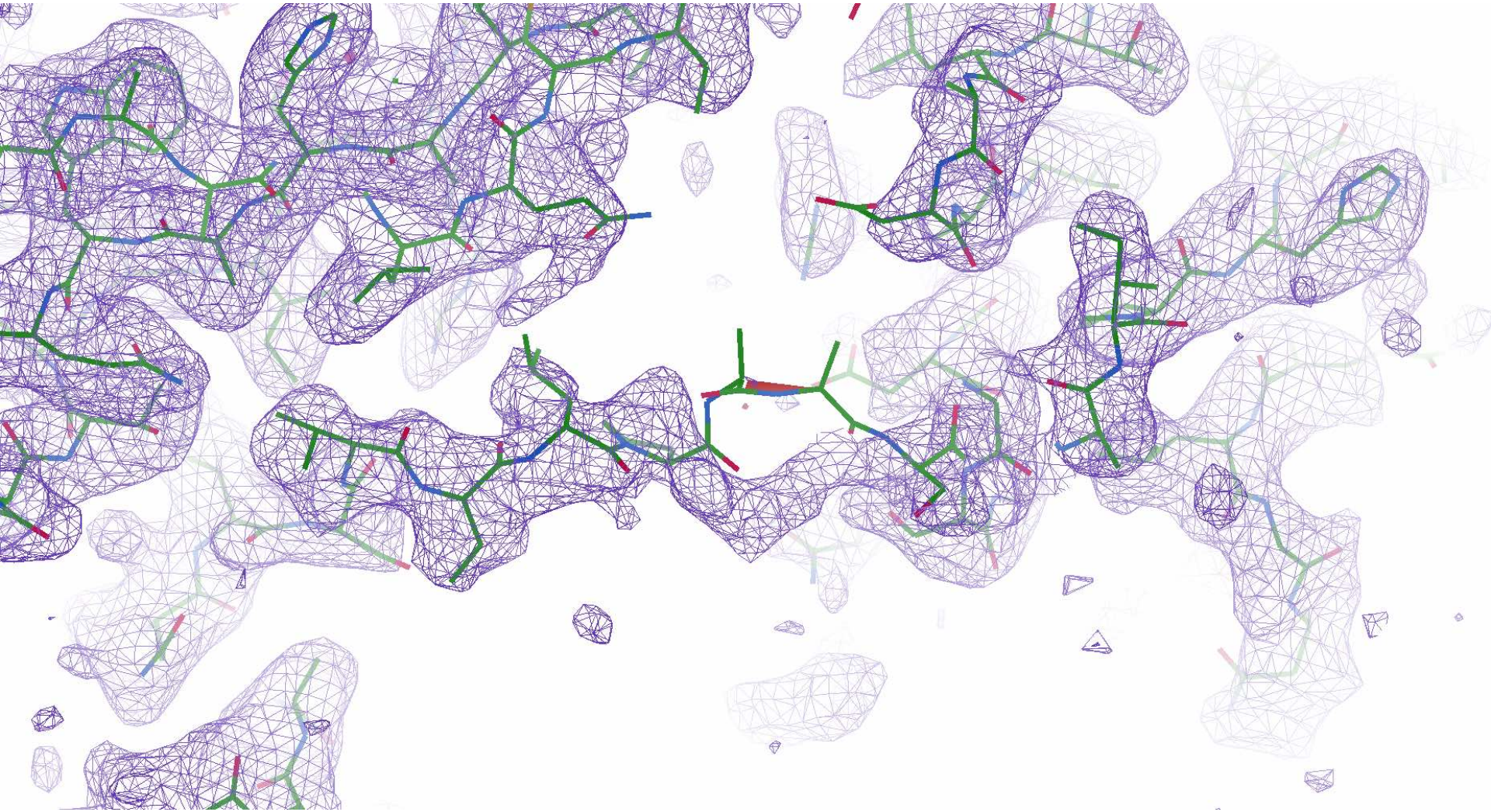

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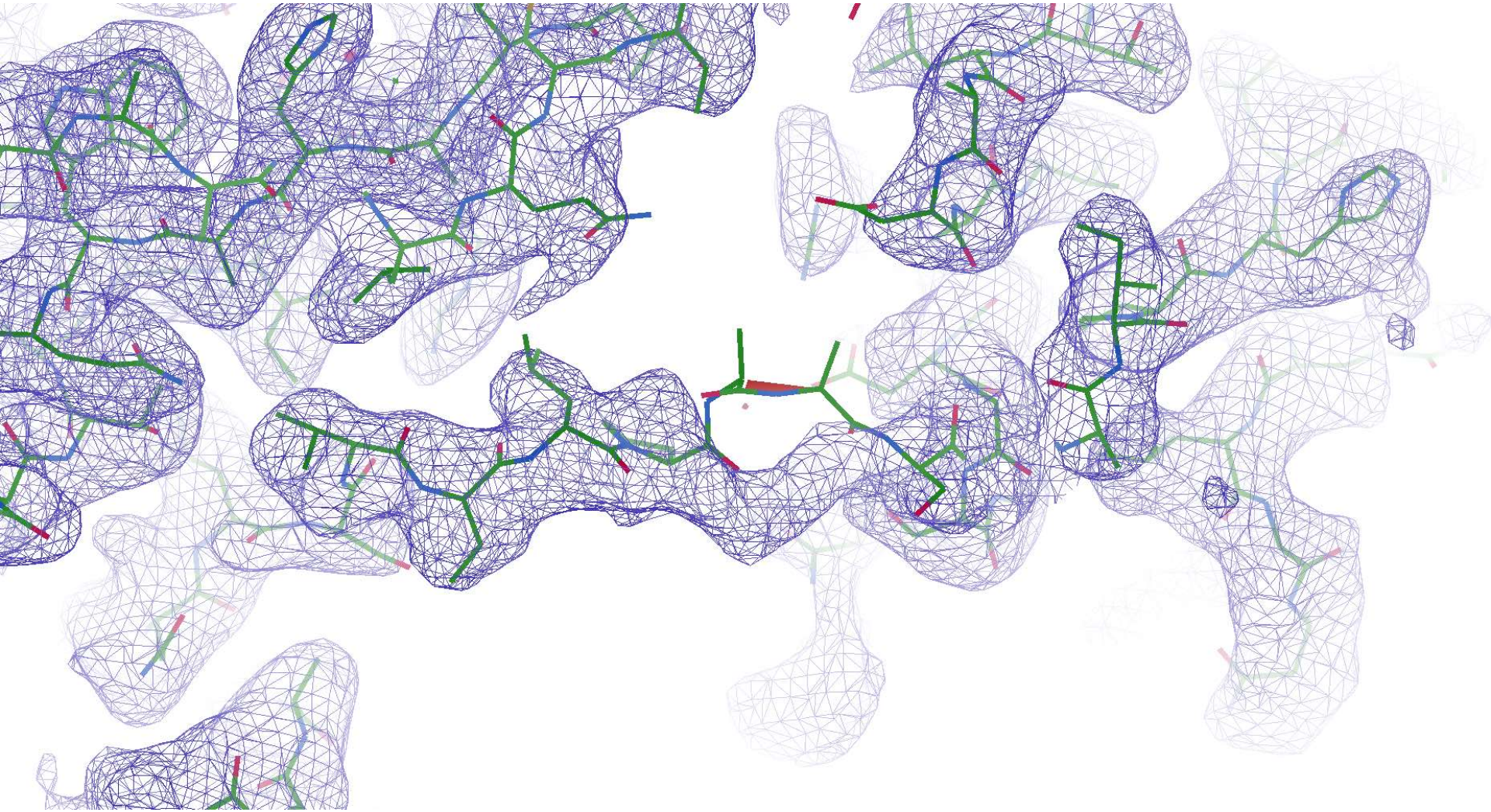

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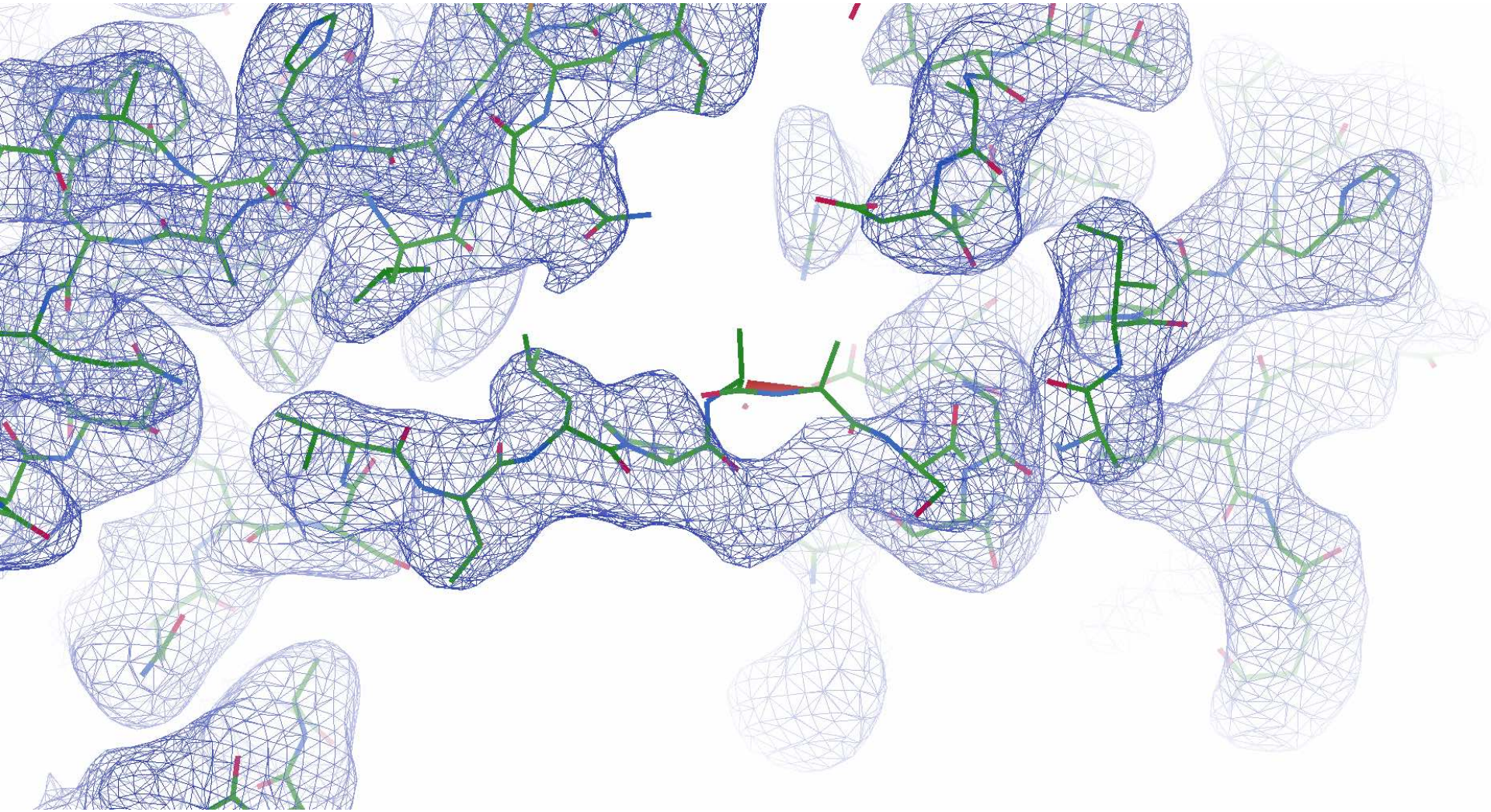

Blurring / Sharpening

Blur 100 Å^2

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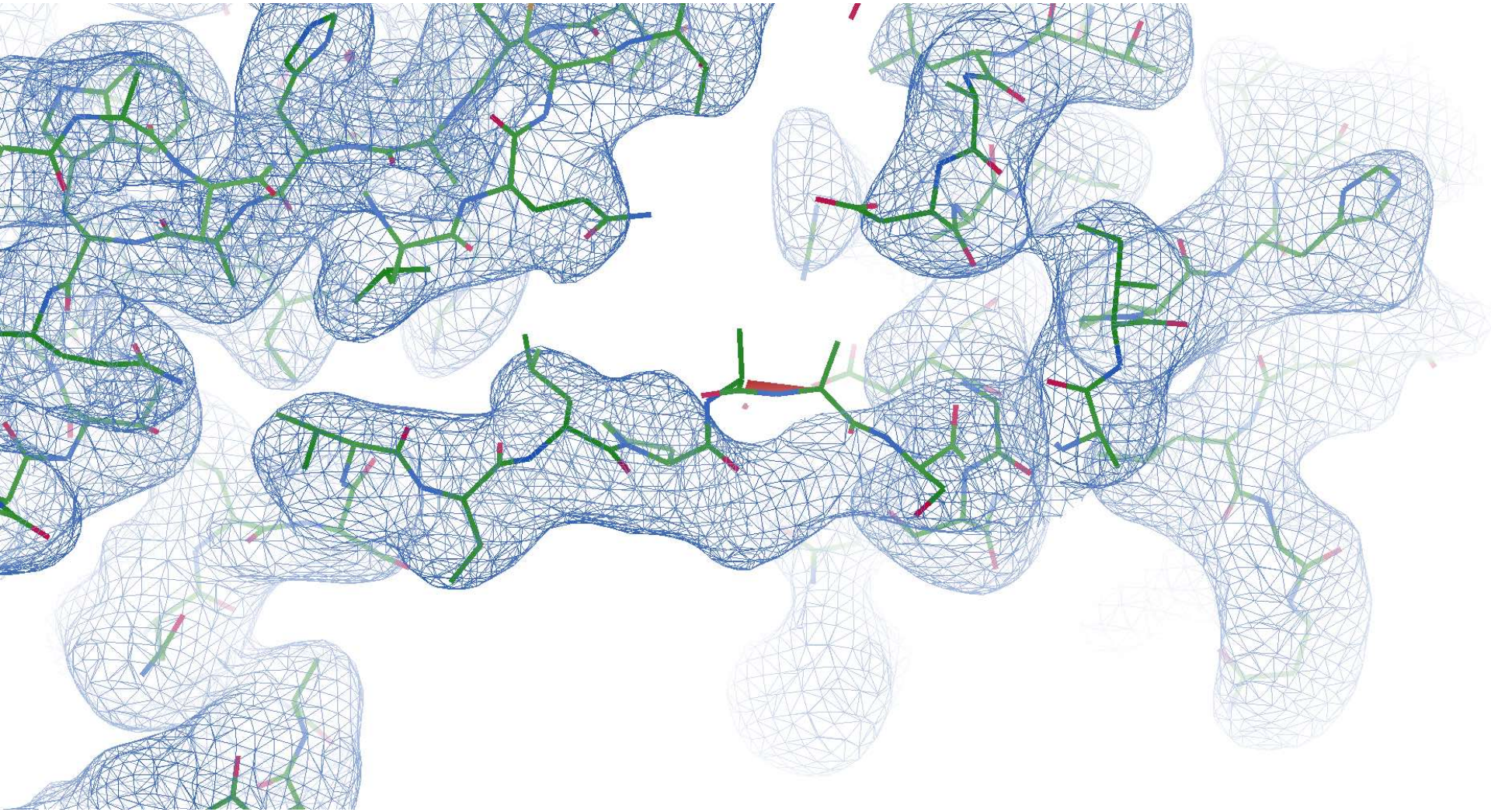
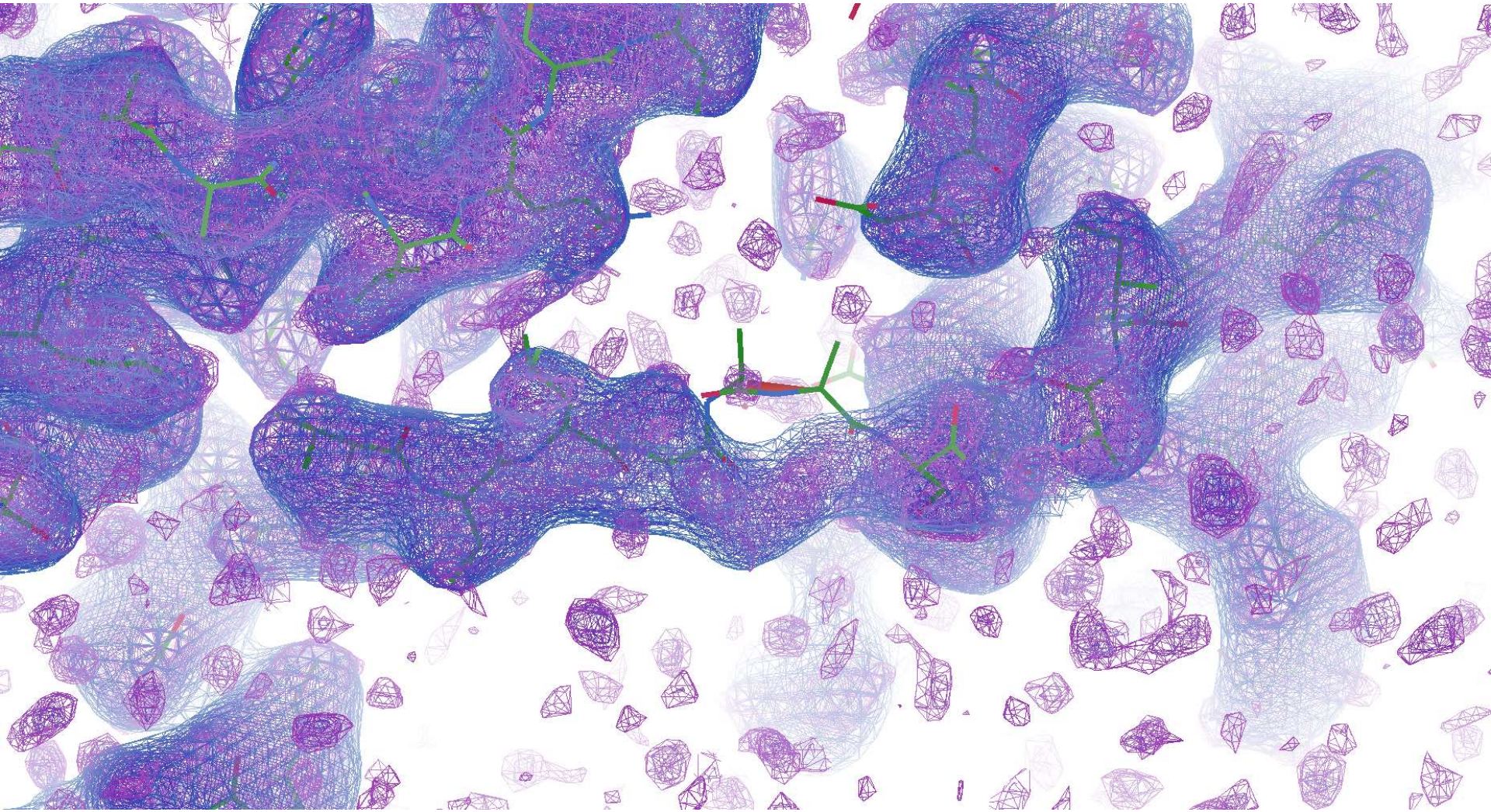


Blurring / Sharpening

Blur 0–100 Å²

5a1a (2.2Å)



Blurring / Sharpening

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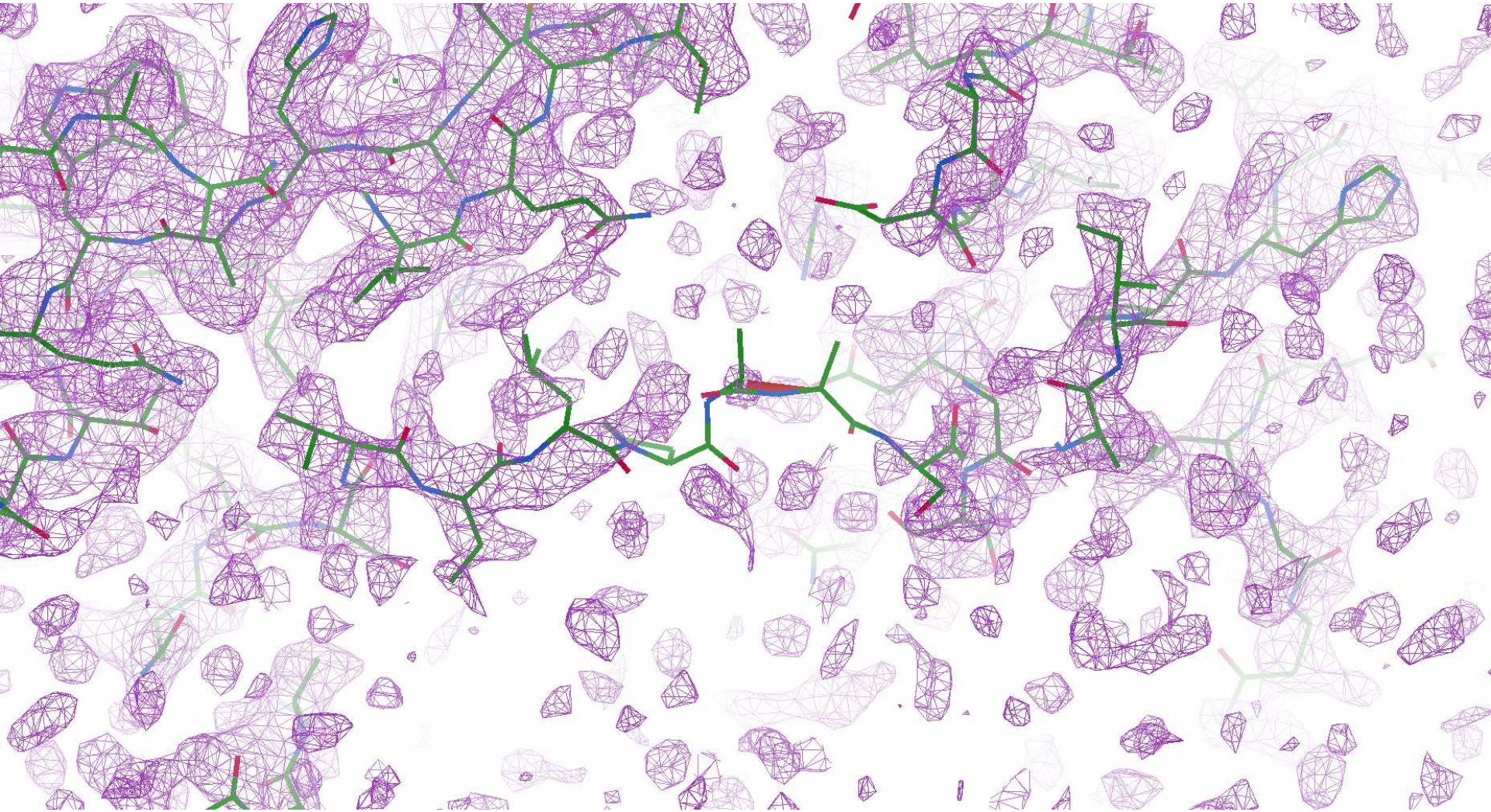


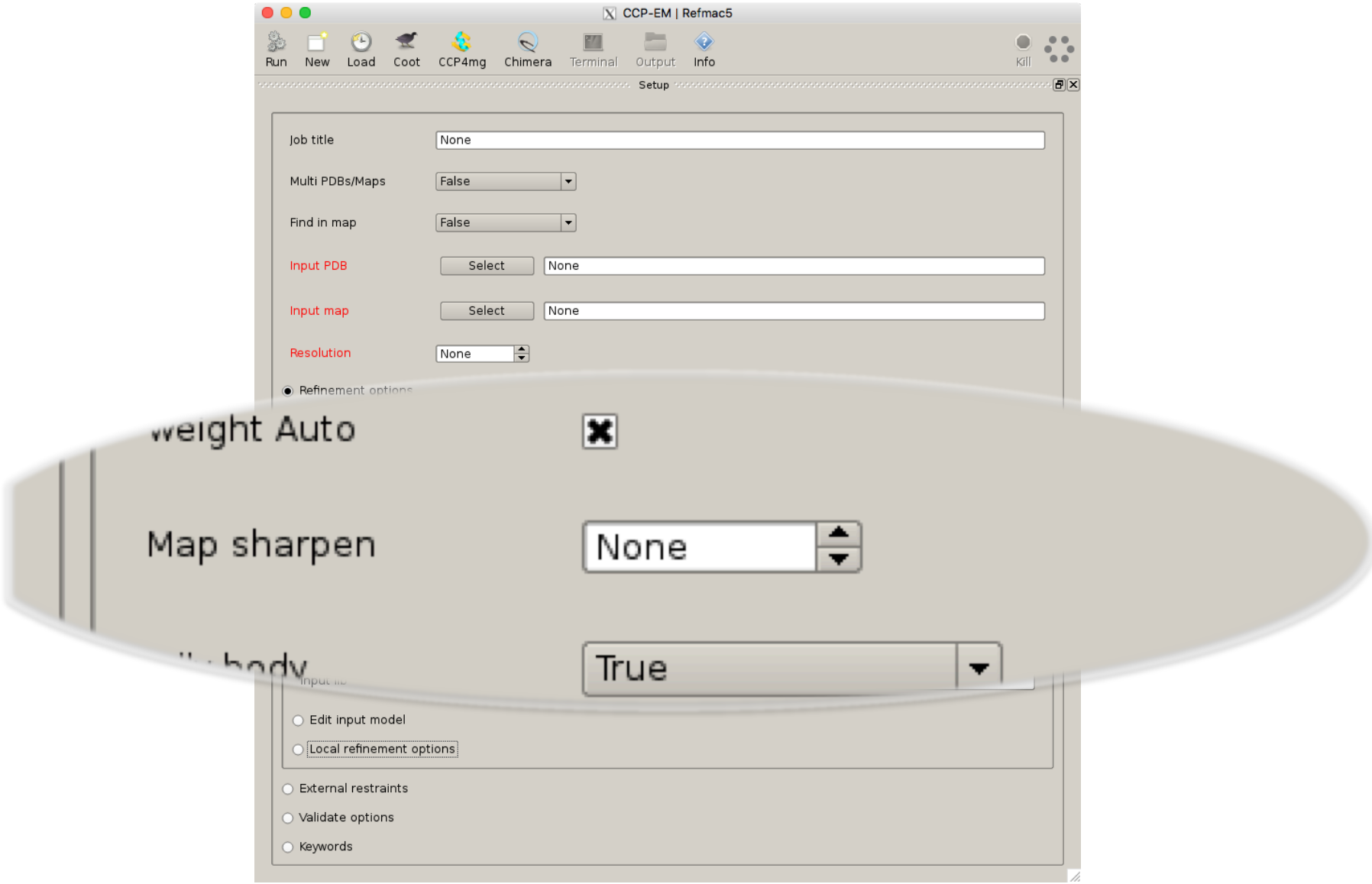
Blurring / Sharpening

Blurring/Sharpening is useful for visual interpretation

- In MX, map blurring/sharpening does not affect refinement

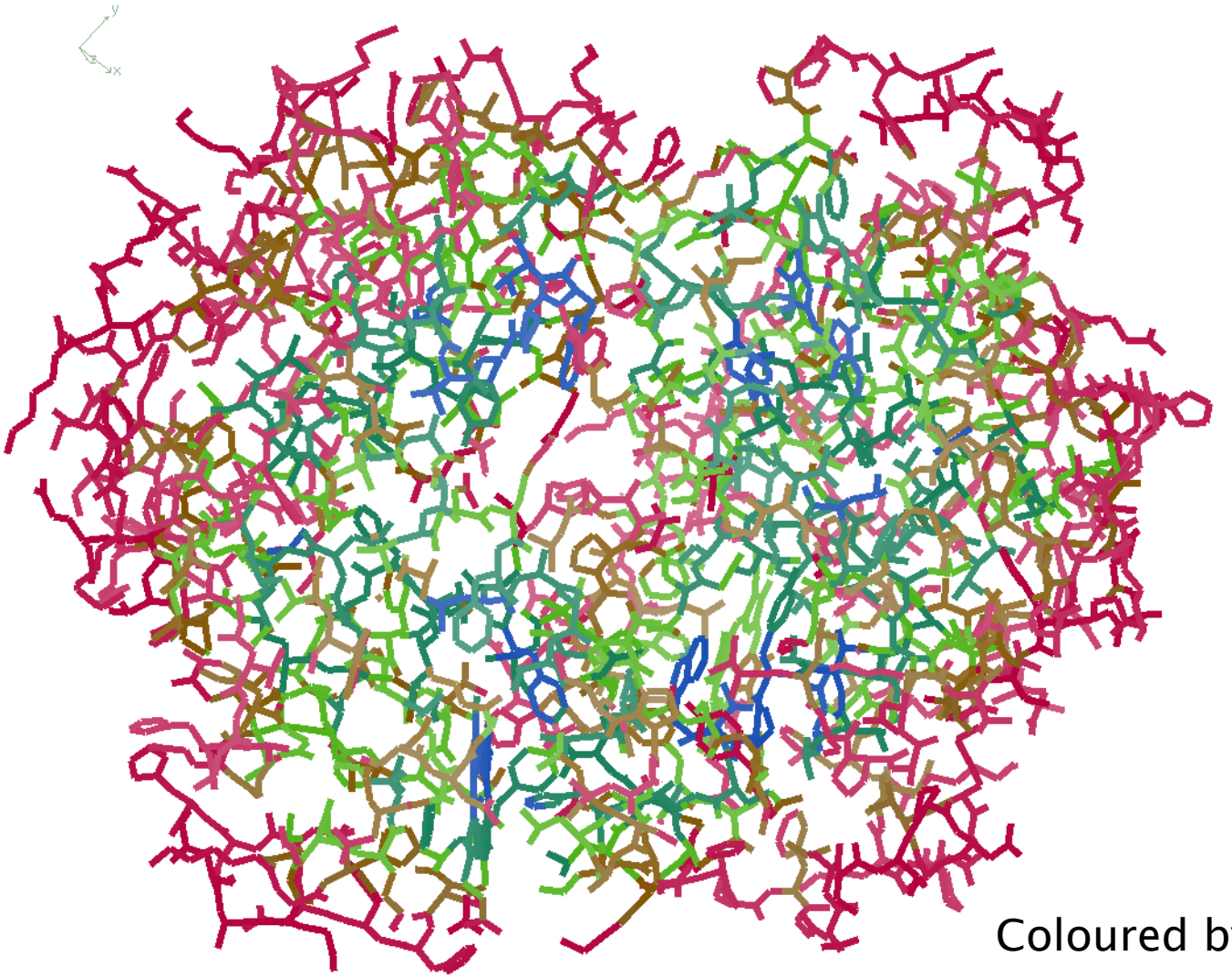
- In cryo-EM, map blurring/sharpening does affect refinement

Blurring / Sharpening



Blurring / Sharpening

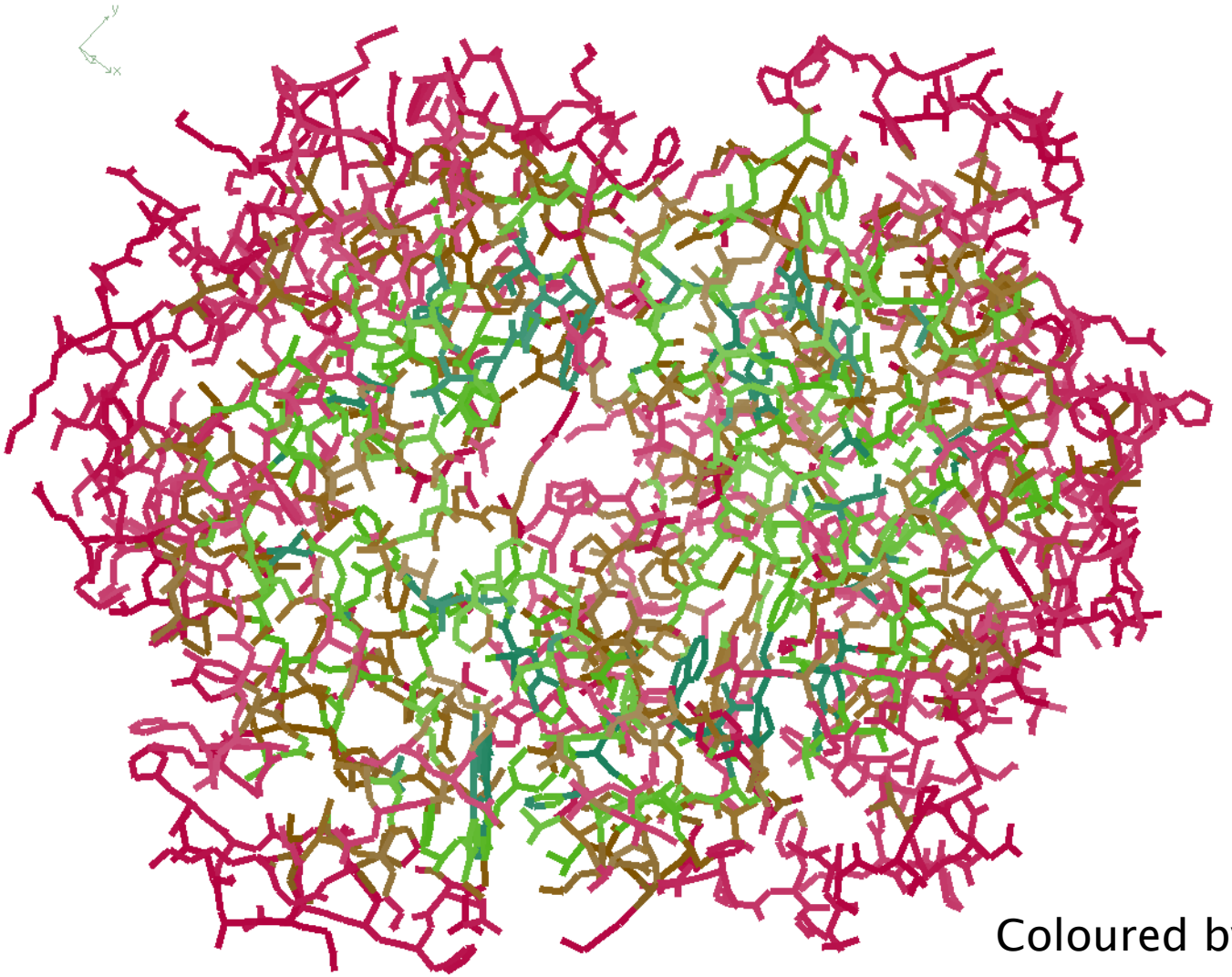
Default refinement – 10 cycles **5me2 (3.2Å)**



Coloured by B-factor

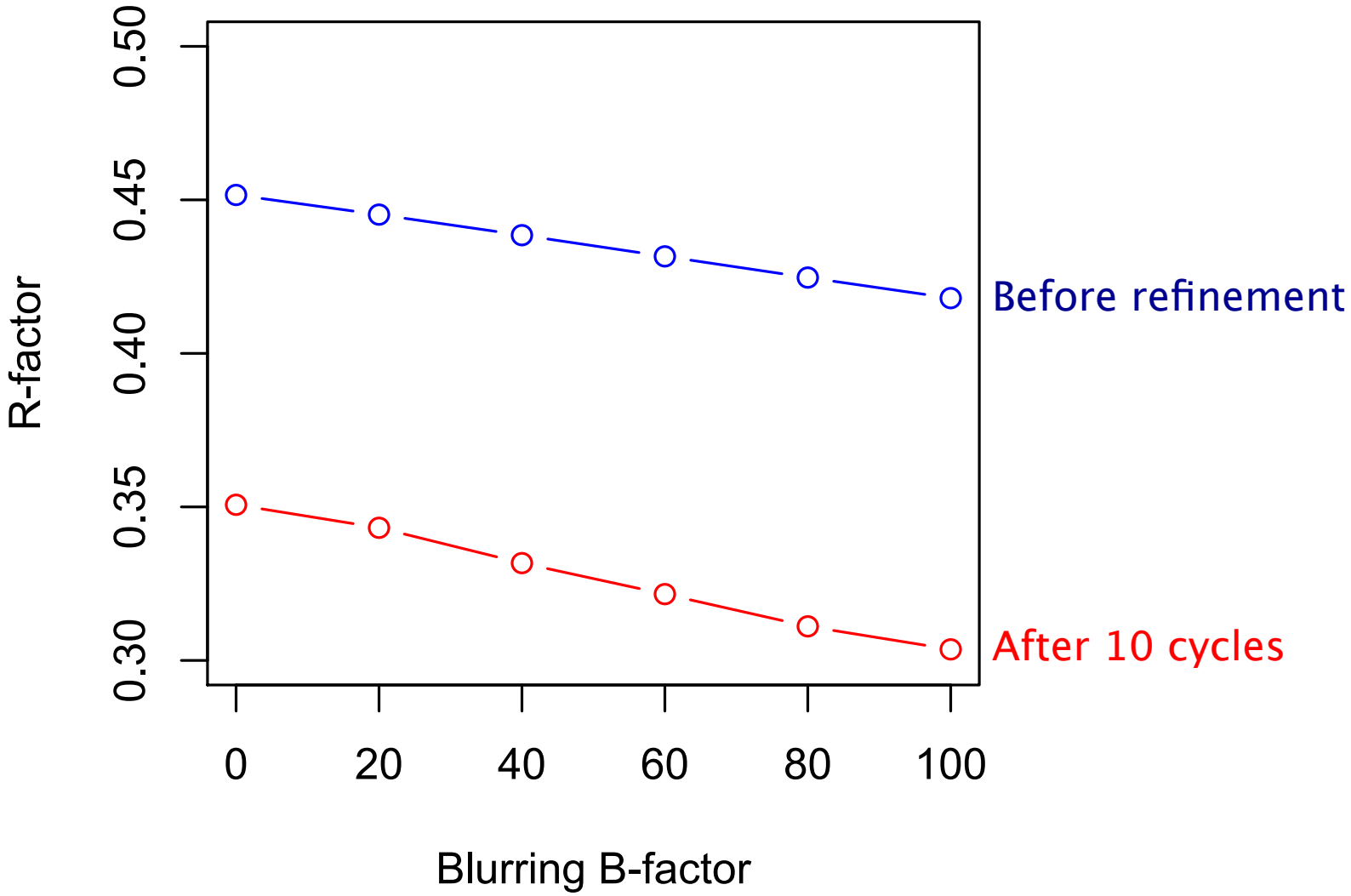
Blurring / Sharpening

Blur 40 Å^2 refinement – 10 cycles **5me2 (3.2Å)**

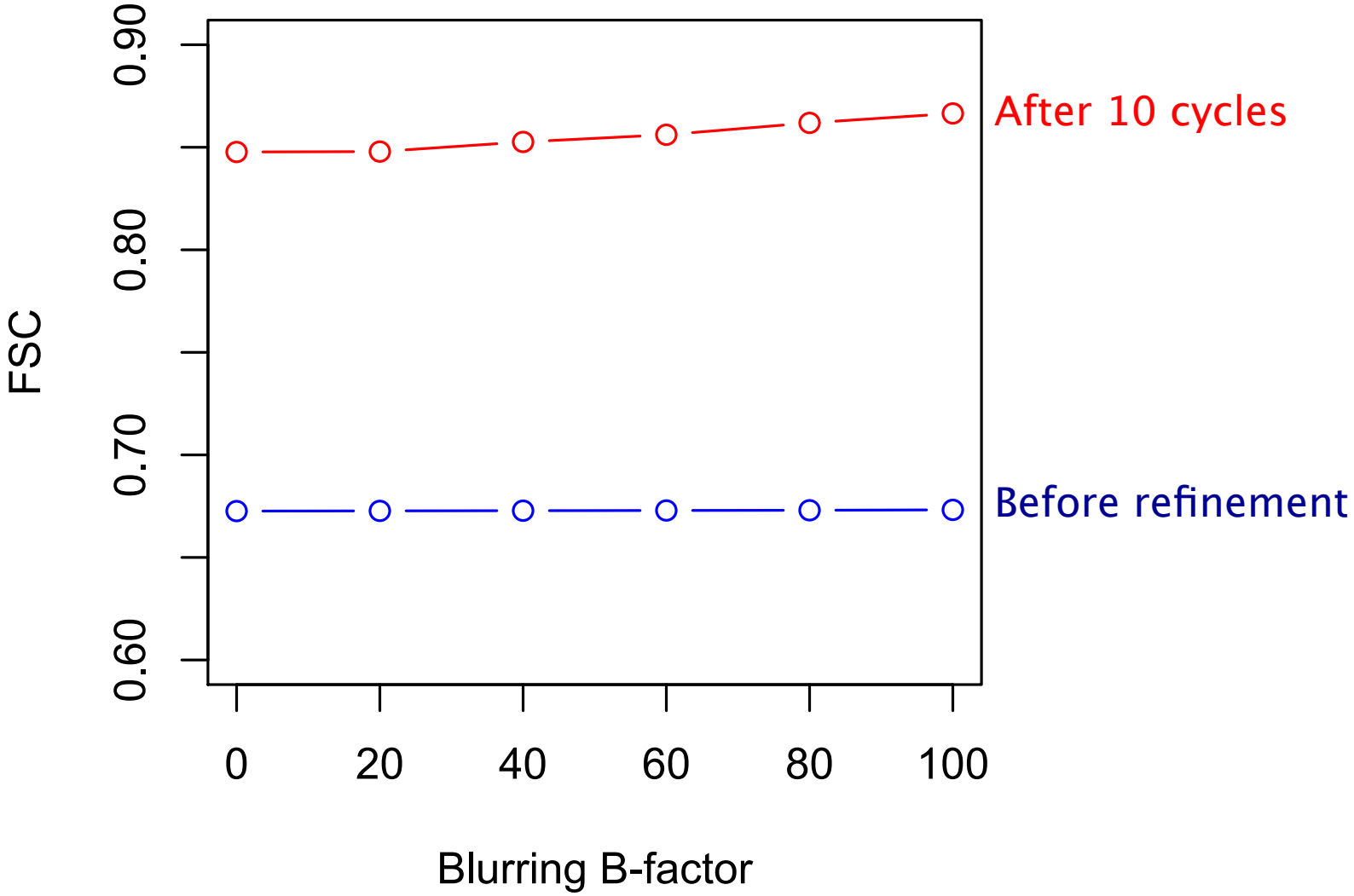


Coloured by B-factor

Blurring / Sharpening

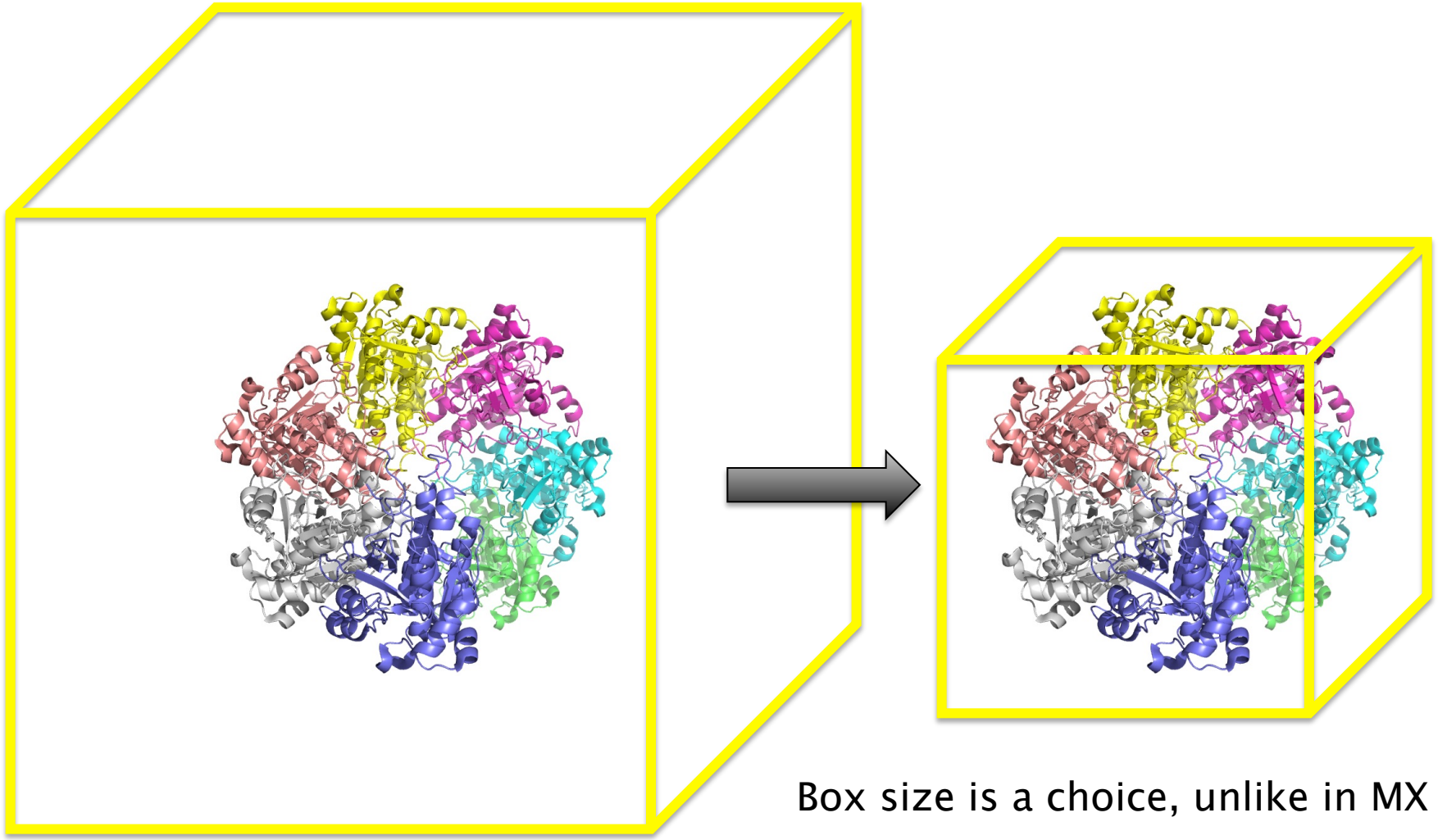


Blurring / Sharpening



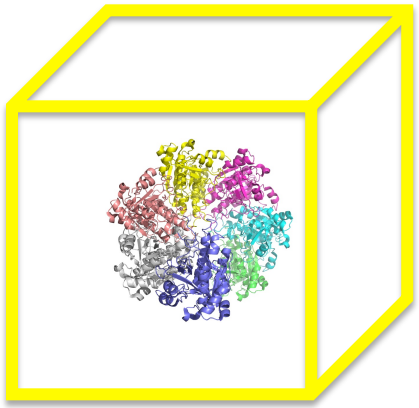
Auto Box Size – “local” refinement

Helical filament 5jzc (4.2 Å)



Box size is a choice, unlike in MX

Auto Box Size – “local” refinement



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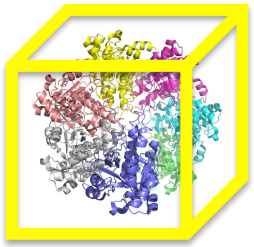
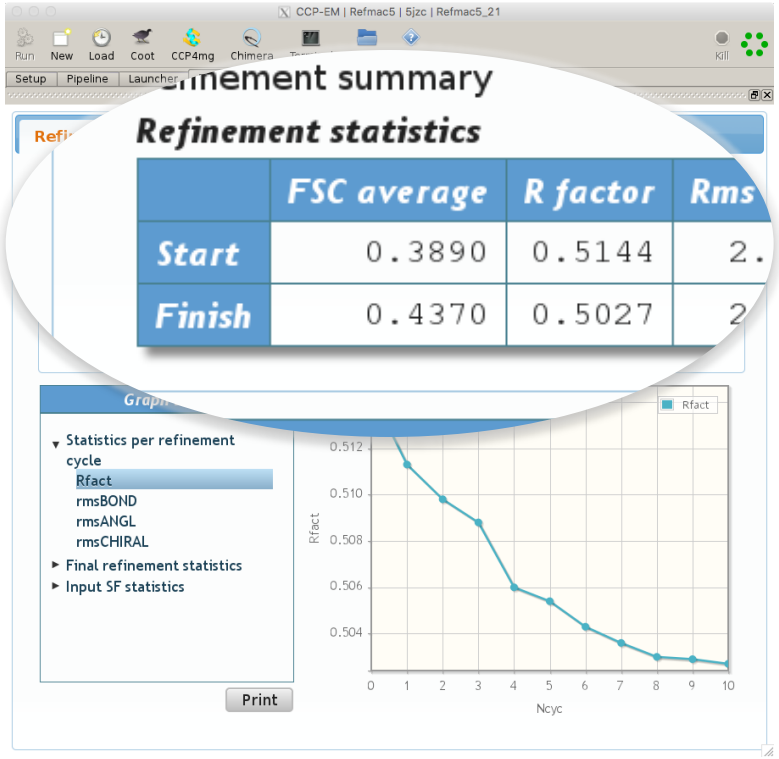
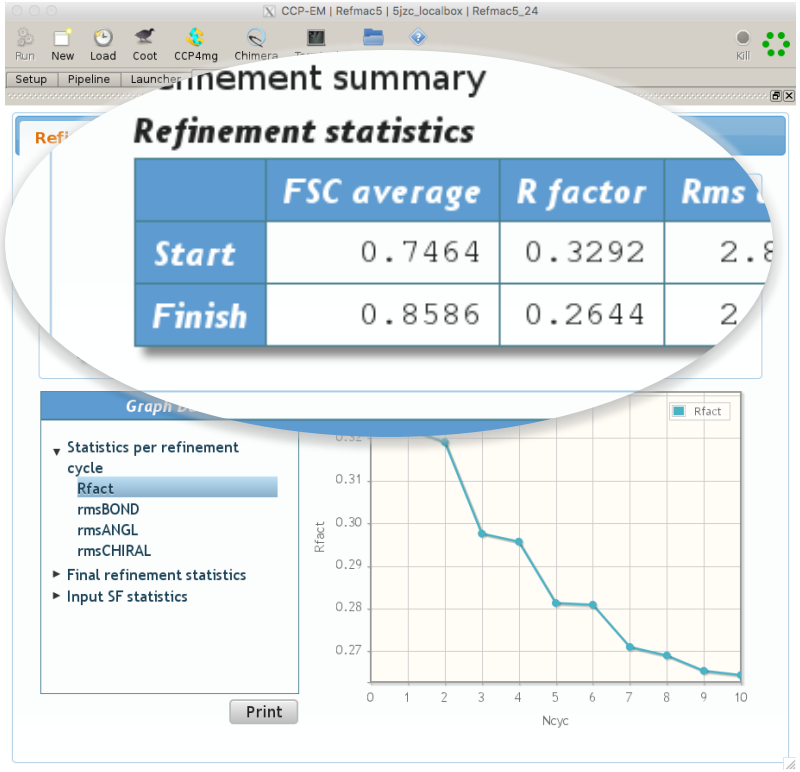
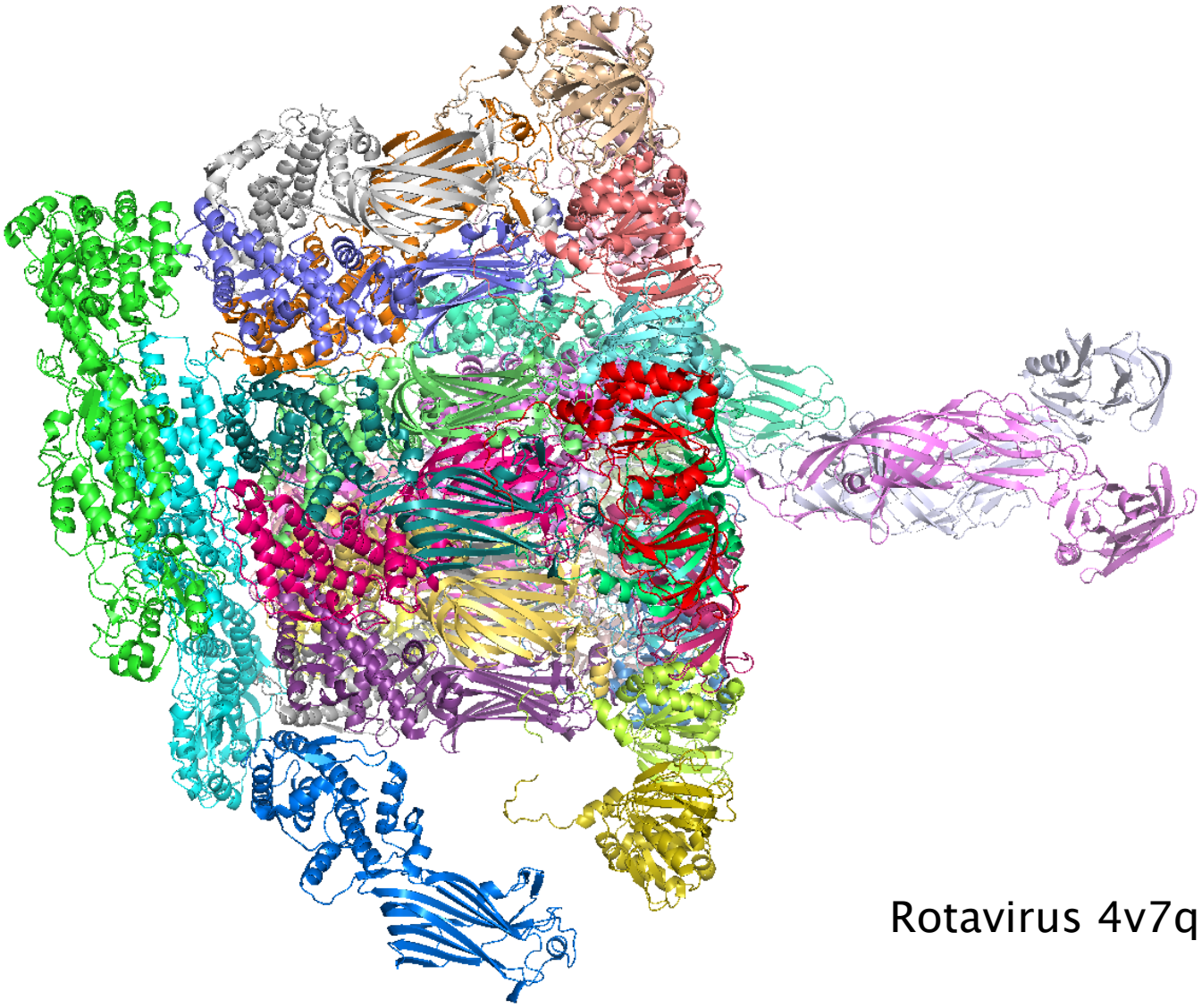




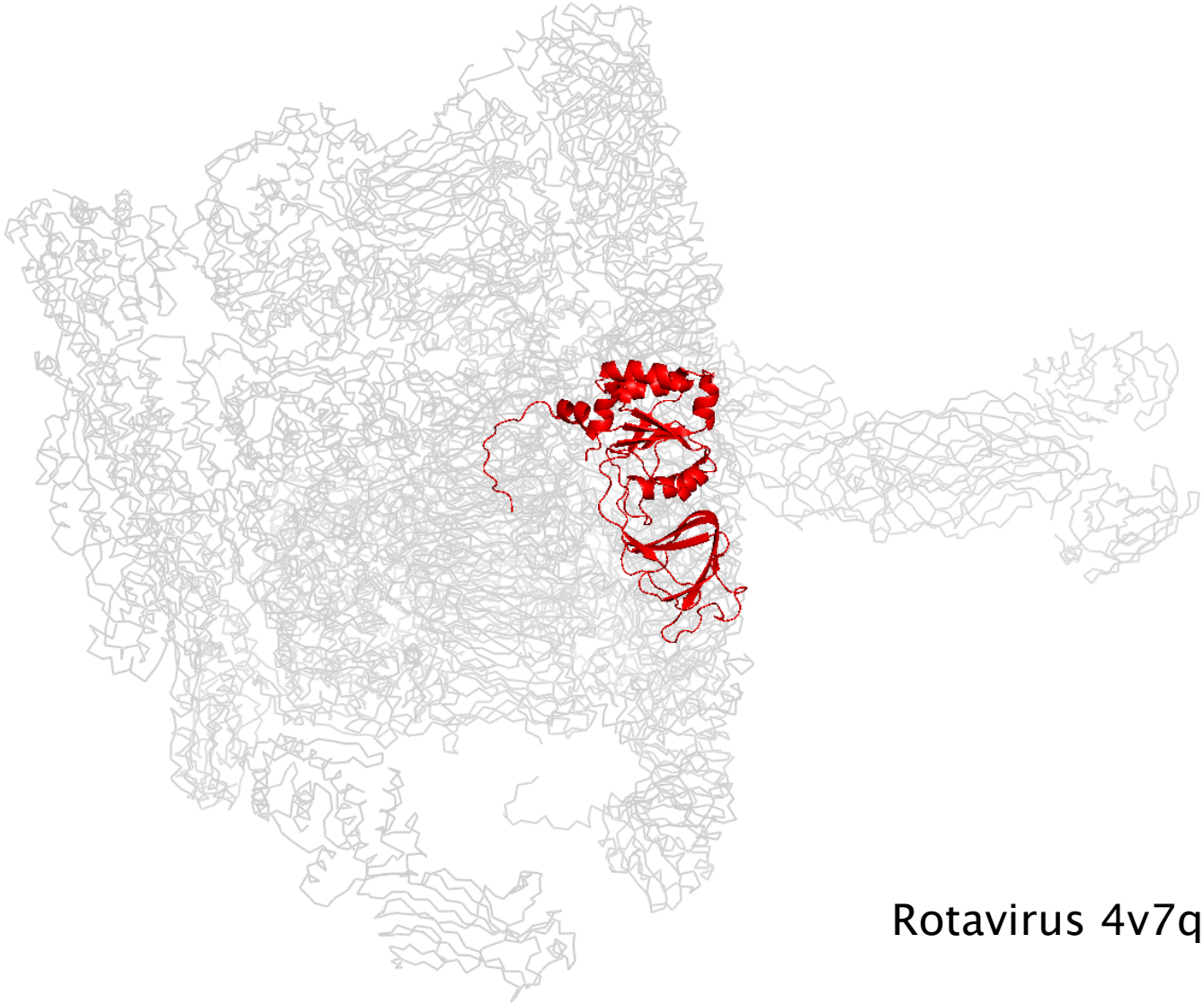


Divide & Conquer Pipeline



Rotavirus 4v7q (3.8 Å)

Divide & Conquer Pipeline



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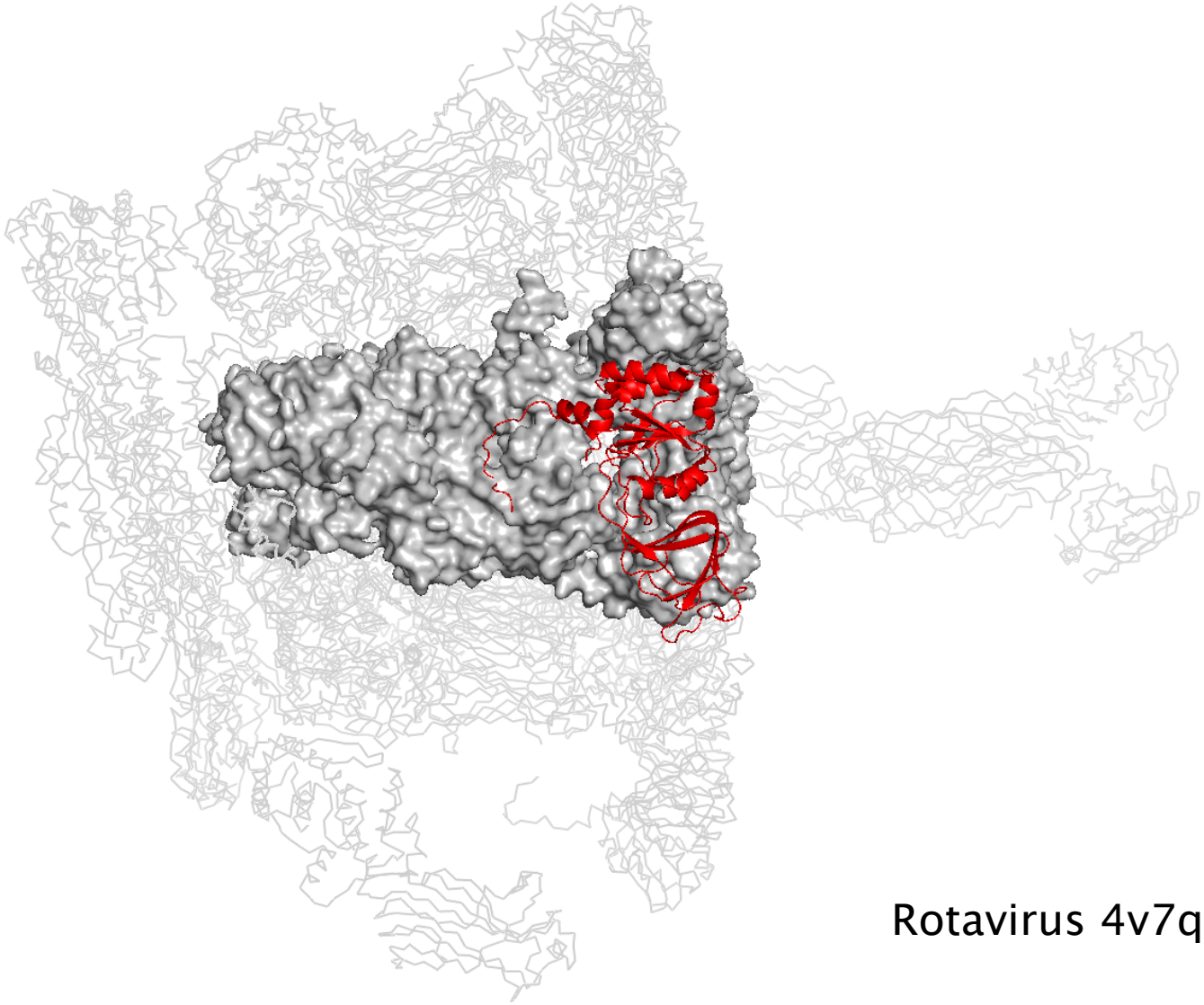

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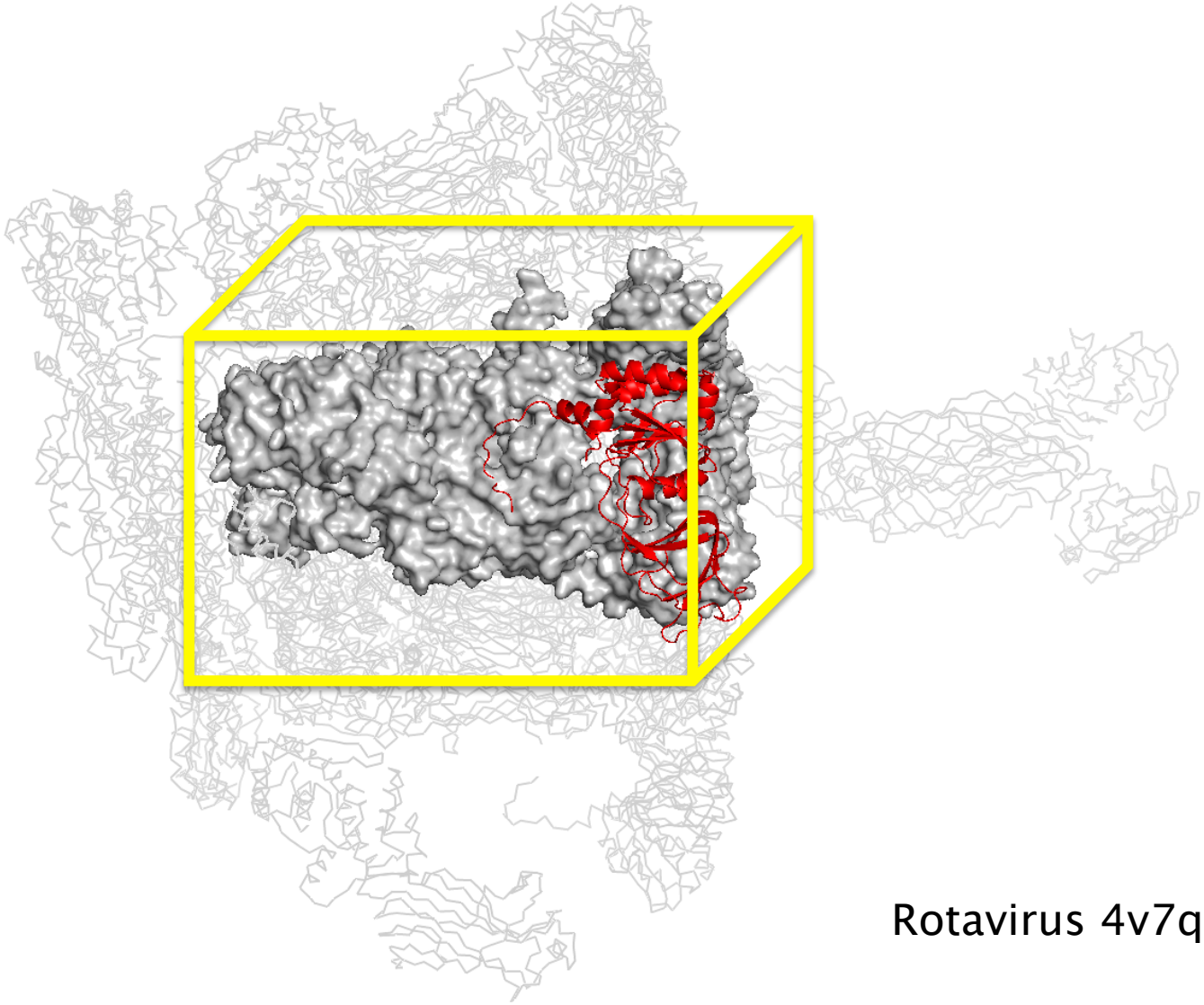

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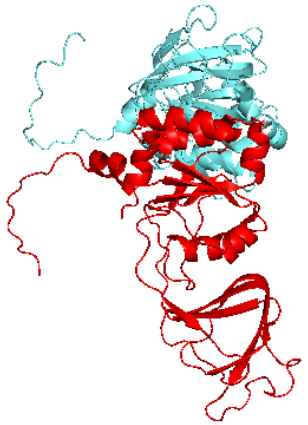
Parallel refinement



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Divide & Conquer Pipeline

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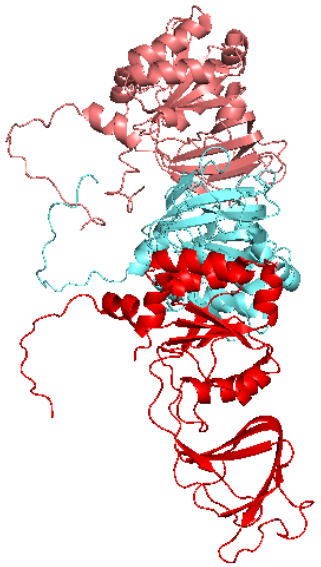

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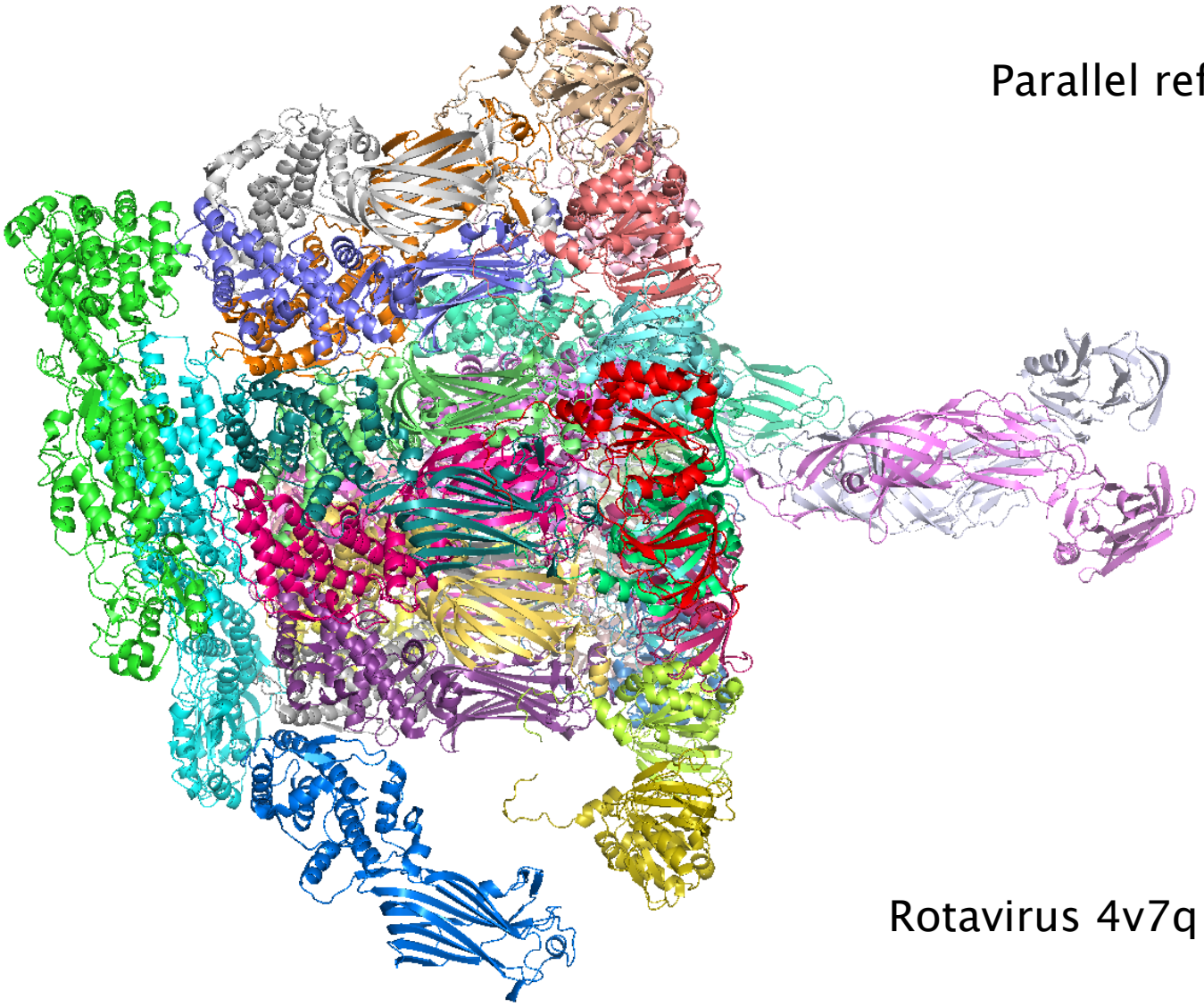


Parallel refinement

Rotavirus 4v7q (3.8 Å)

Summary

External restraints to homologous structures can be useful

- Used by REFMAC5 for full-model refinement
- Visualised in Coot, and used for real space refinement
- If homologs are not available, use:
  - Generic h-bond restraints for protein
  - Generic base-pair/stacking restraints for DNA/RNA

Restrained refinement can be used for low-res & cryo-EM refinement

- Need lots of “extra” restraints to regularise refinement
- Jelly-body restraints are almost always needed

Other things to think about in cryo-EM:

- Multiple levels of blurring/sharpening helps, but care is needed
- Box size should be selected appropriately
- Divide & conquer pipeline for large models

Summary

Tools to help with model building and refinement:

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

ProSMART: External restraints, comparative analysis

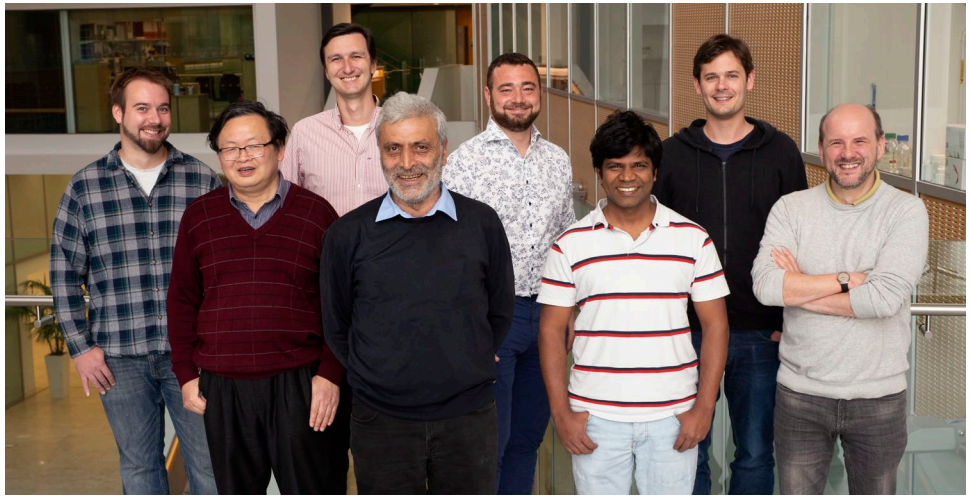
LIBG: Nucleic acid restraints

COOT: Visualisation & manipulation of restraints, map blurring
…also morphing, jiggle-fit, backrub rotamers…

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

Acknowledgements

MRC-LMB Computational Structural Biology Group



Left to right:

Rob Nicholls
Fei Long
Oleg Kovalevskiy
Garib Murshudov
Michal Tykac
Rangana Warshamanage
James Parkhurst
Paul Emsley

Many Thanks:

Marcus Fischer
Stuart McNicholas
Martin Noble
Tom Burnley
Martyn Winn
Alan Brown
Jude Short
Ana Casañal
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Users for feedback!

Tools for cryo-EM model fitting & refinement:

- Nicholls *et al.* (2018) Current approaches for the fitting and refinement of atomic models into cryo-EM maps using CCP-EM. Acta Cryst D74.
- Murshudov (2016) Refinement of atomic structures against cryo-EM maps. Methods in Enzymology, 277-305.
- Brown *et al.* (2015) Tools for macromolecular model building and refinement into electron cryo-microscopy reconstructions. *Acta Cryst.* D71.

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