

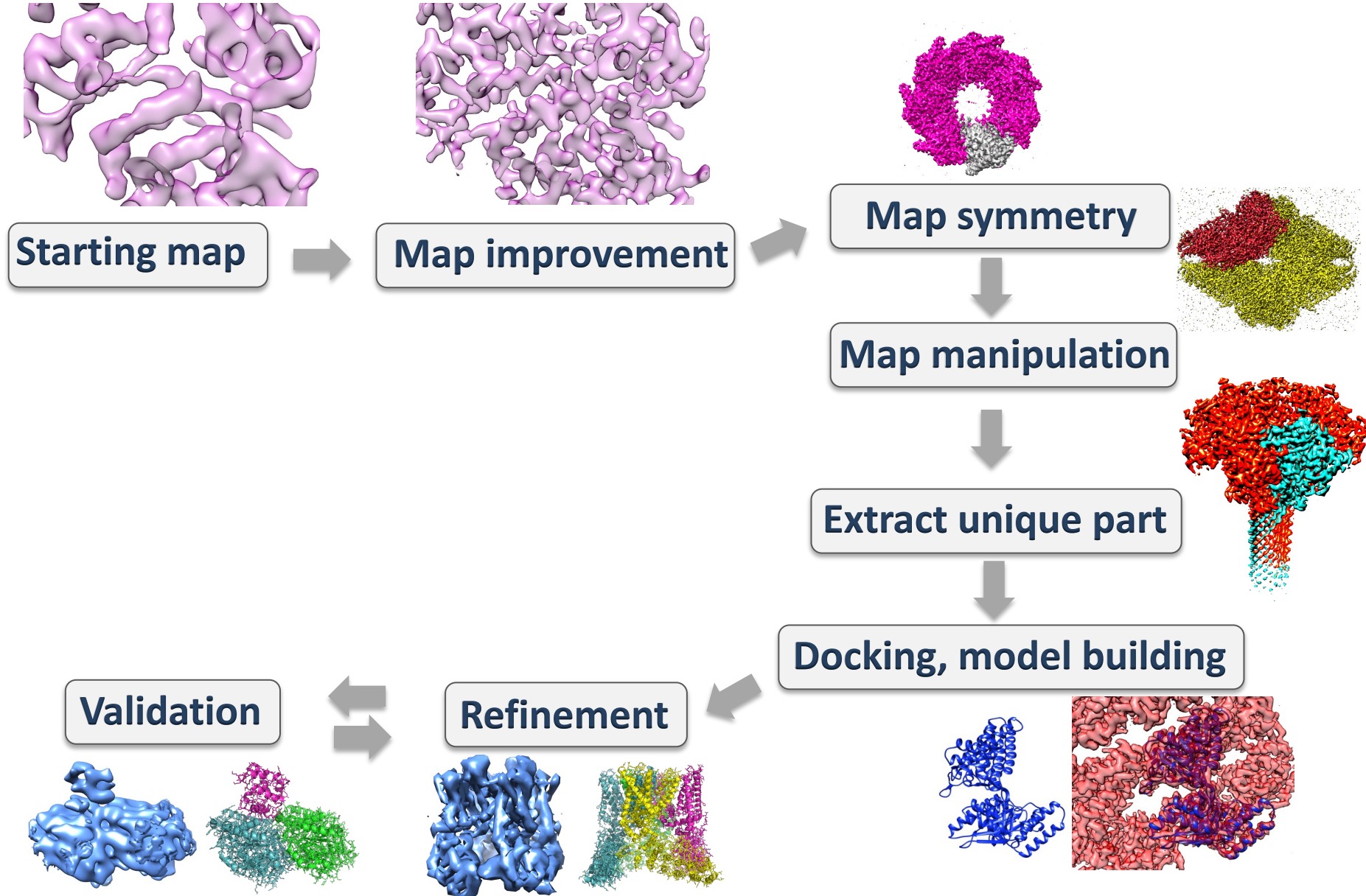
Phenix Map Tools

Pavel Afonine



October 28th, 2025
UTMB, Galveston, TX

Phenix tools for cryo-EM



Phenix tools for cryo-EM: *GUI and command line*

The screenshot displays the PHENIX home graphical user interface. At the top, there is a menu bar with icons for Quit, Preferences, Help, Citations, ChimeraX, Coot, PyMOL, KiNG, Other tools, and Ask for help. Below this is a toolbar with buttons for Actions, Job history, and a list of projects. The main area is divided into two panes. The left pane shows a table of projects with columns for ID, Last modified, # of jobs, and R-free. The right pane shows a list of tool categories, with the 'Cryo-EM' section highlighted by a red box.

PHENIX home

Quit Preferences Help Citations ChimeraX Coot PyMOL KiNG Other tools Ask for help

Actions Job history

Projects

Show group: All groups Manage...

Select Delete New project Settings

ID	Last modified	# of jobs	R-free
63G	Jun 23 2022 05:26...	1	---
JAKE	Jun 01 2022 05:44...	1	---
yuya	May 28 2022 12:44...	1	---
FEM	May 26 2022 03:02...	5	---
rnase-s_1	May 12 2022 06:28...	2	0.2902
half	Jan 24 2022 12:19 ...	1	---
48test	Jan 10 2022 01:30 ...	6	0.1540
1vqw	Jan 06 2022 05:59 ...	2	0.2323
real-space-refin...	Jan 06 2022 02:12 ...	4	---
SCOTT	Jan 05 2022 03:48 ...	3	0.3978
rnase-s_0	Dec 19 2021 10:12...	3	0.3455
DOUG	Dec 16 2021 03:17...	4	---
real-space-refin...	Dec 09 2021 05:20...	3	---
table1	Nov 09 2021 03:07...	11	---
junk1	Nov 02 2021 11:09...	0	---
XXXXXXX	Oct 21 2021 02:44...	3	---
Adam	Oct 20 2021 04:59...	3	0.2210
37_debug	Oct 20 2021 01:42...	3	---
35test	Oct 14 2021 12:53...	4	---
34_maria	Oct 13 2021 01:21...	0	---
30_debug	Oct 05 2021 02:22...	2	0.1522

Crystals: Data analysis and manipulation
Validation and map-based comparisons
Experimental phasing
Molecular replacement
Maps (create, manipulate, compare)
Enhanced maps (Polder, FEM, density-modified...)
Model building
Refinement
Ligands

Cryo-EM: Map analysis, symmetry, manipulation
Validation and map-based comparisons
Map improvement
Docking, model building and rebuilding
Refinement

Models: Superpose, search, compare, analyze symmetry
Modification, minimization and dynamics

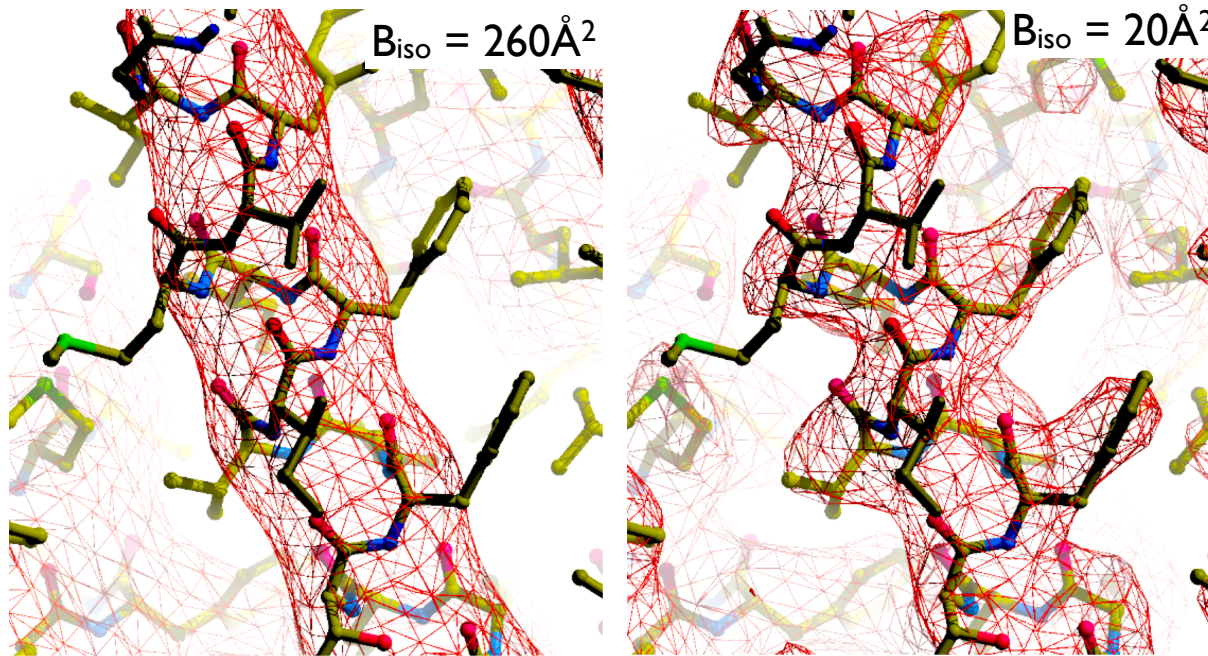
PDB Deposition

Program search

Current directory: /Users/pafonine/63_goska/polder Browse...

PHENIX version dev-svn-000 Project: 63G

Automated map sharpening: *phenix.auto_sharpen*



Deposited Map

Autosharpened Map

EMDB: 8414, PDB: 5tji

Fully automatic:

No manual trial-and-error | No parameters to adjust | Only inputs: map and resolution



STRUCTURAL
BIOLOGY

ISSN 2059-7983

Automated map sharpening by maximization of
detail and connectivity

Thomas C. Terwilliger,^{a,b*} Oleg V. Sobolev,^c Pavel V. Afonine^{c,d} and
Paul D. Adams^{d,e}

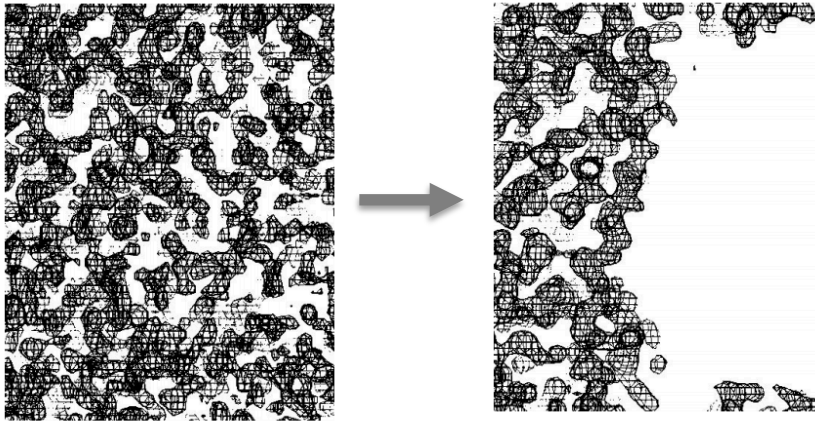
Density modification: *phenix.density_modify_cryo_em*

Similar principals for crystallography and cryo-EM:

change the map so that it is most consistent with what we know about macromolecules

Crystallography

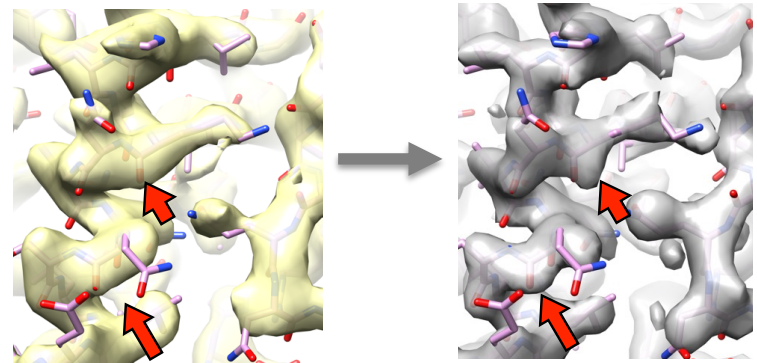
From uninterpretable to interpretable map



Cryo-EM

Effect is less dramatic as in crystallography

- Can increase map resolution (0.05-0.3 Å)
- Can improve map clarity for interpretation



nature **methods**

ARTICLES

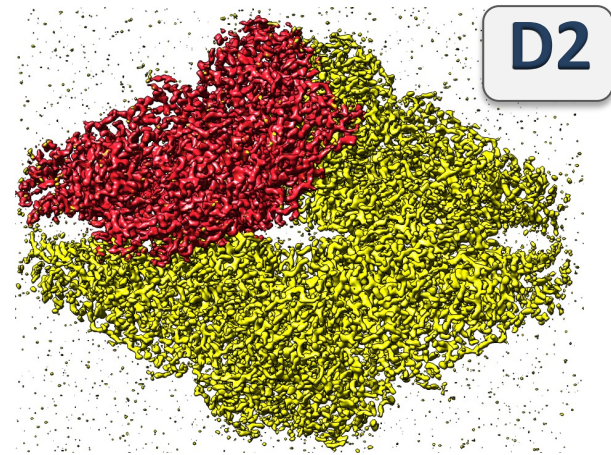
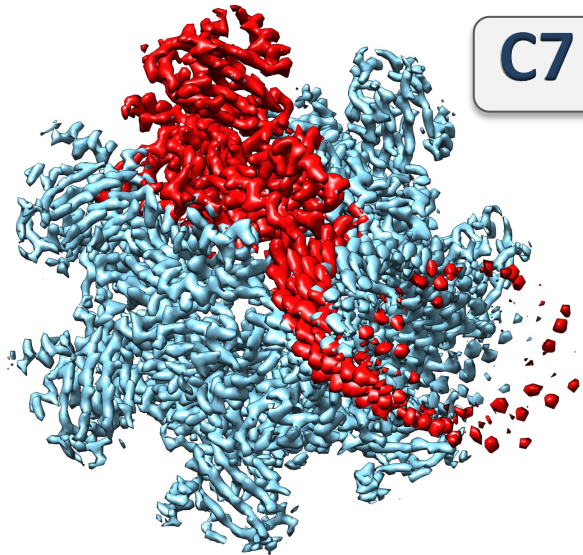
<https://doi.org/10.1038/s41592-020-0914-9>

Check for updates

Improvement of cryo-EM maps by density modification

Thomas C. Terwilliger^{1,2}✉, Steven J. Ludtke³, Randy J. Read⁴, Paul D. Adams^{5,6} and Pavel V. Afonine⁵

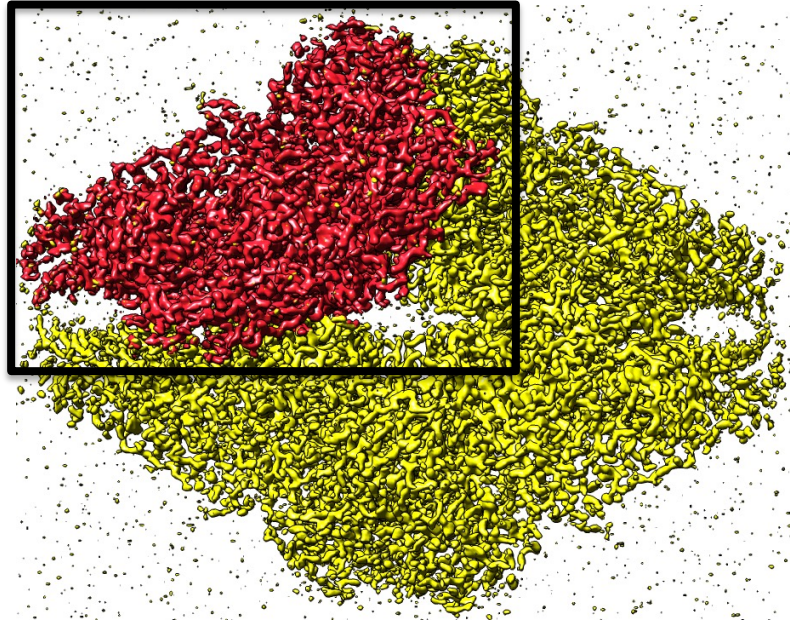
Finding map symmetry: *phenix.symmetry_from_map*



<http://phenix-online.org/newsletter/>

Tools for interpreting cryo-EM maps using models from the PDB

Extracting unique part of map using *phenix.map_box*

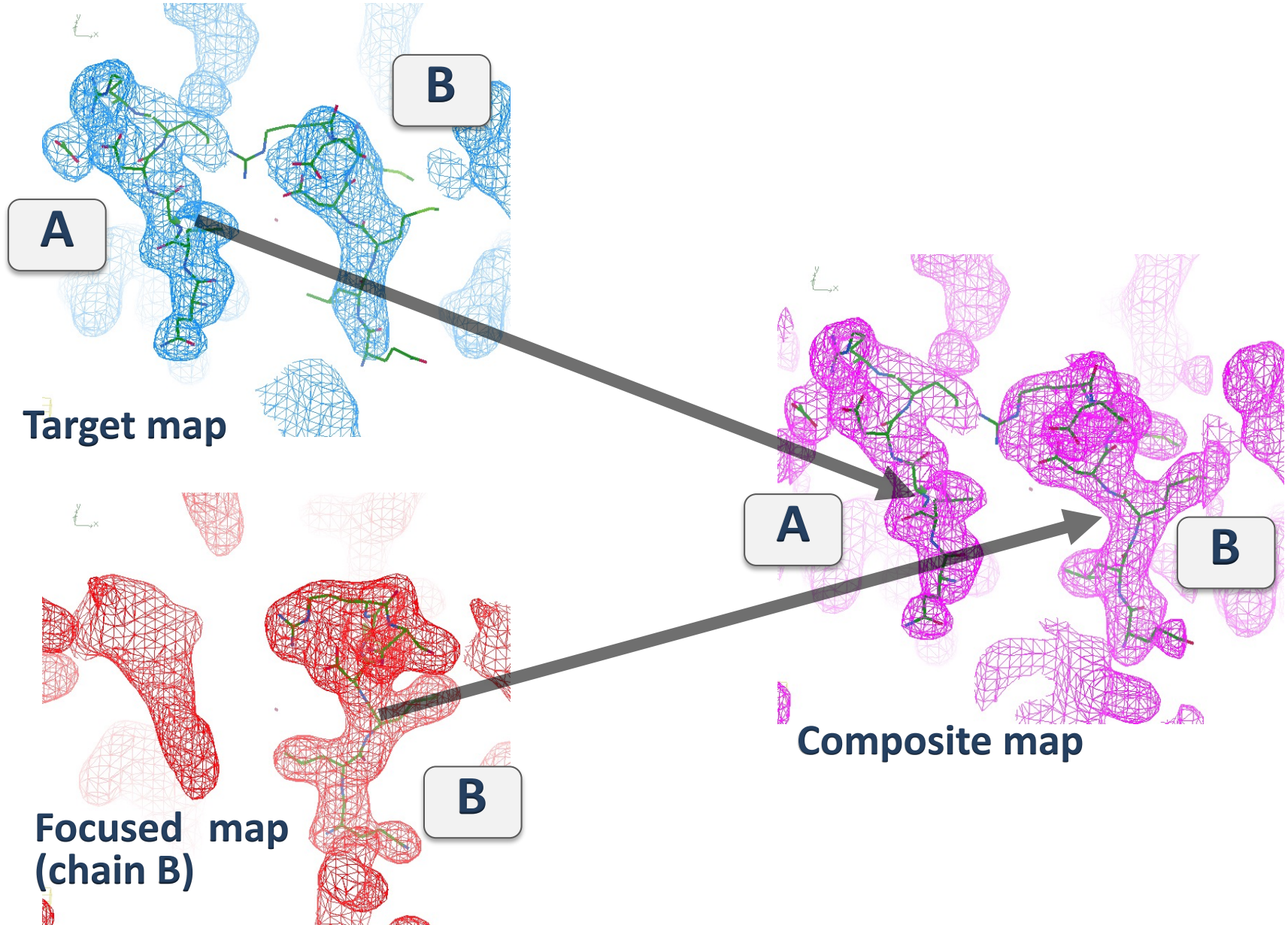


Lots of options: use map only, use model, use symmetry, mask boxed map, and many more!

<http://phenix-online.org/newsletter/>

Tools for interpreting cryo-EM maps using models from the PDB

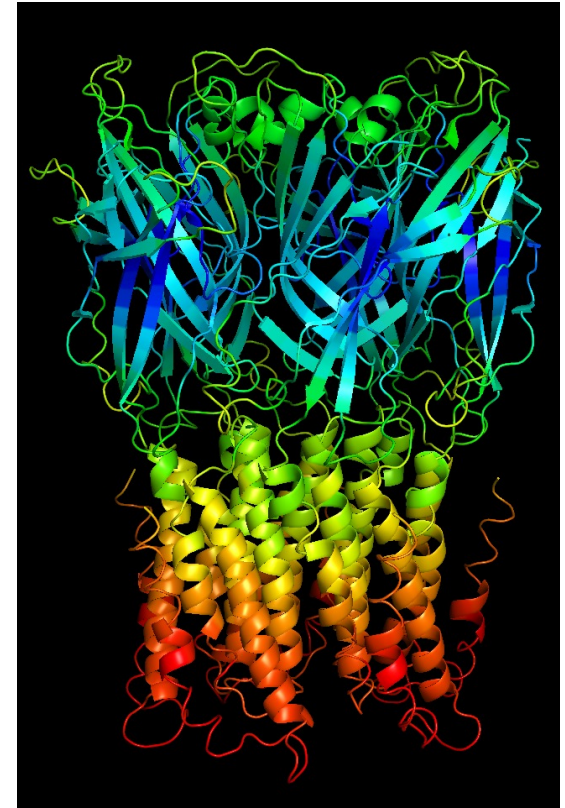
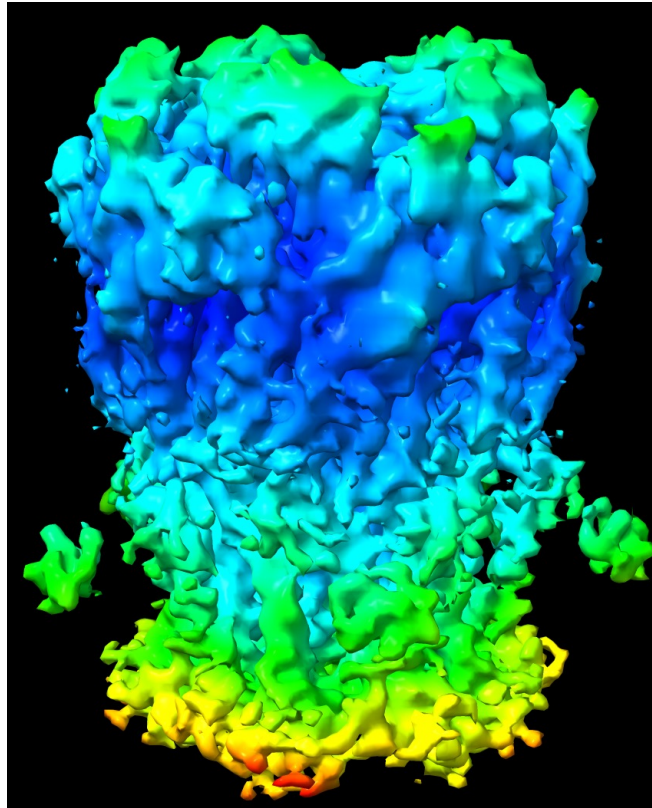
Combining maps with *phenix.combine_focused_maps*



Local resolution: *phenix.local_resolution*

EMDB: 20986

PDB: 6v0b



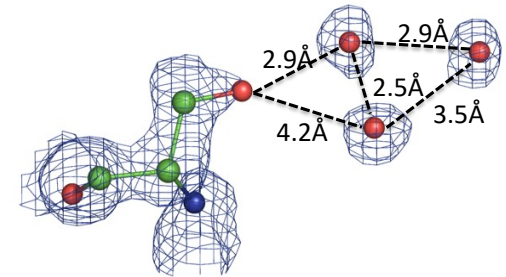
```
phenix.local_resolution half_map_1.mrc half_map_2.mrc
```

In ChimeraX load both maps and use

```
color sample #1 map #2 palette rainbow
```

to color map #1 based on the resolution values in map #2

Local EM map fitting



Loop Fitting

More is coming !

Automated model building: *phenix.map_to_model*

Automated model building, facts:

- No automated model building produces 100% complete and accurate model
- Produces initial model for further manual building
- The lower the resolution, the less complete and accurate the auto-built model

nature | **methods**

BRIEF COMMUNICATION

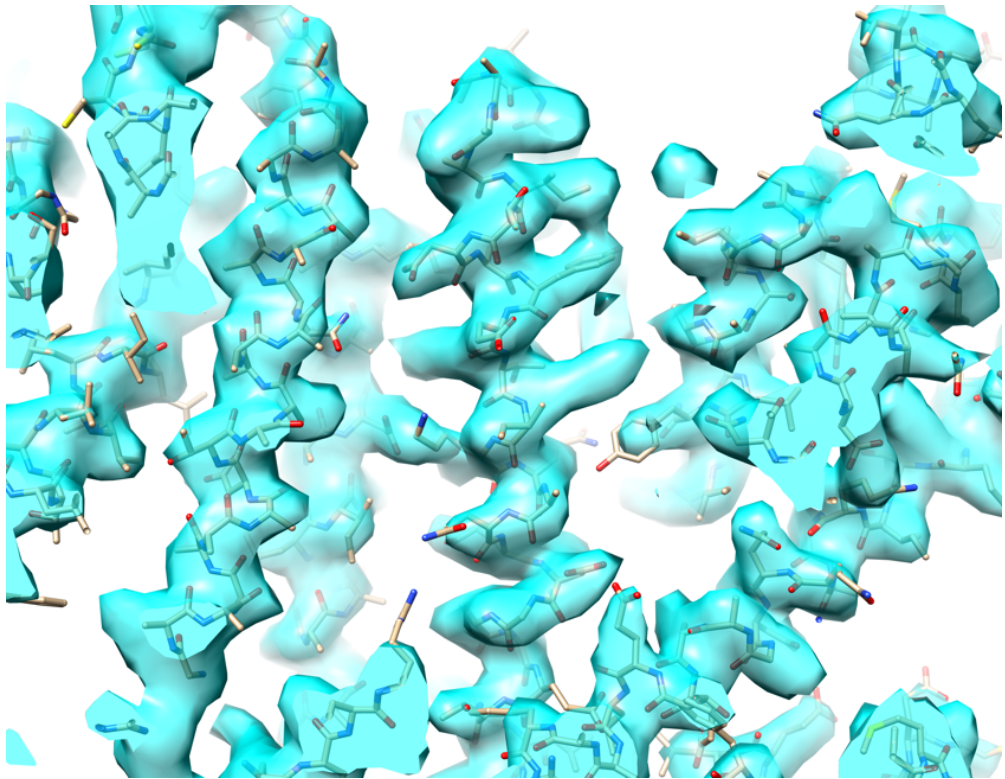
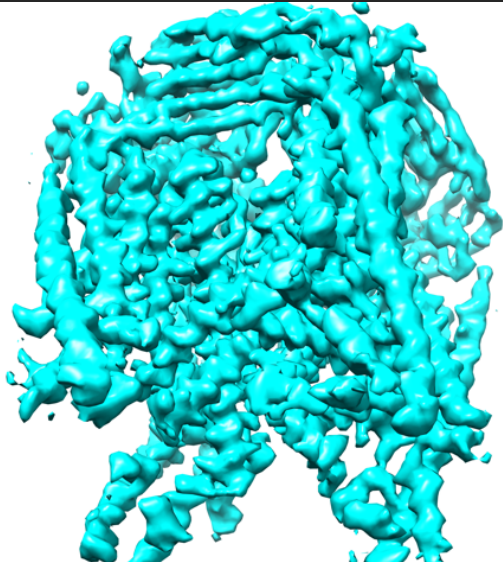
<https://doi.org/10.1038/s41592-018-0173-1>

A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps

Thomas C. Terwilliger ^{1,2*}, Paul D. Adams^{3,4}, Pavel V. Afonine^{3,5} and Oleg V. Sobolev ³

Automated model building: *phenix.map_to_model*

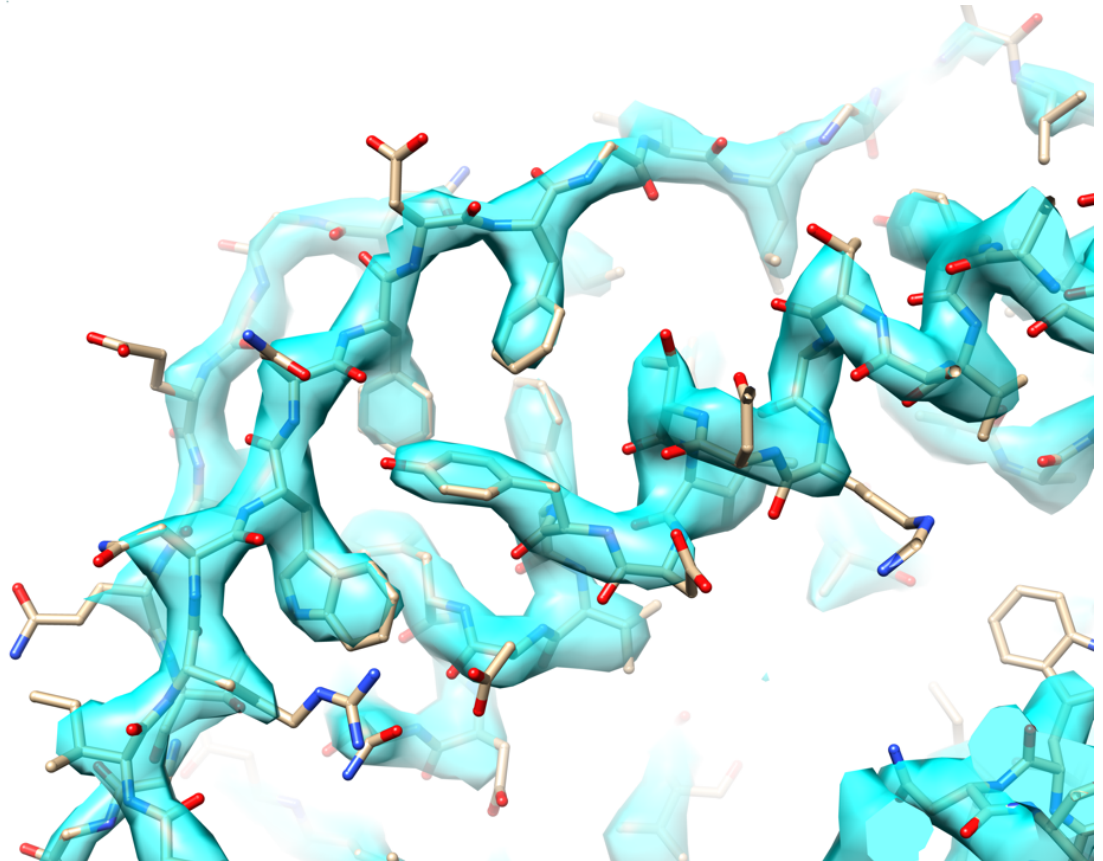
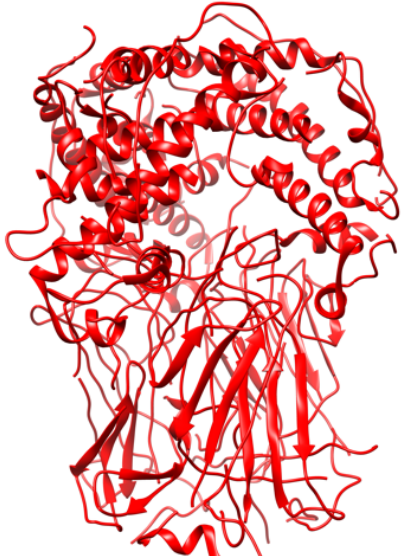
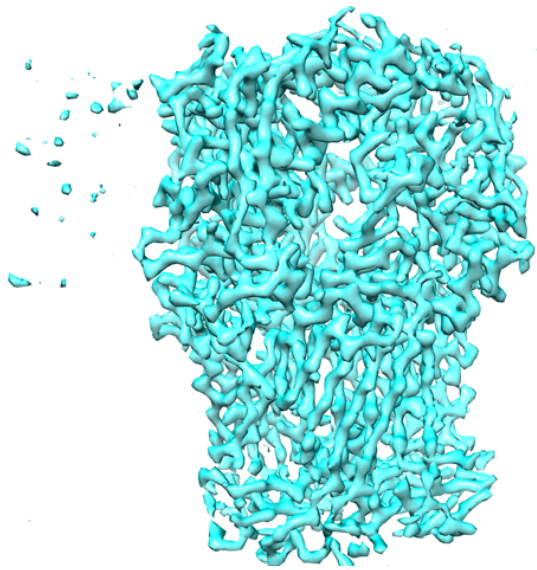
TRPML3 channel (4.1 Å, 78% built, 1.3 Å rmsd)



Data from Zhou, X. et al. (2017) Nat. Struct. Mol. Biol. 24: 1146

Automated model building: *phenix.map_to_model*

Rotavirus VP6 (2.6 Å, 100% built, 0.9 Å rmsd)



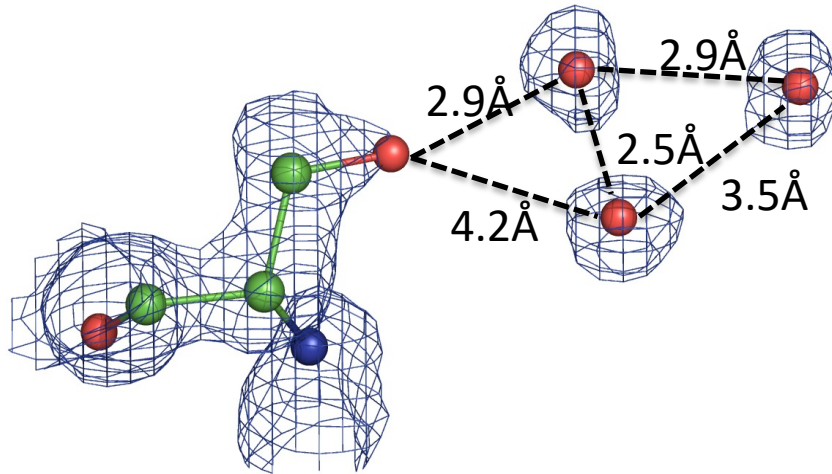
Data from Grant and Grigorieff, eLife 2015;4:e06980

Manual model building steps

If *phenix.map_to_model* fails or model is too big or else:

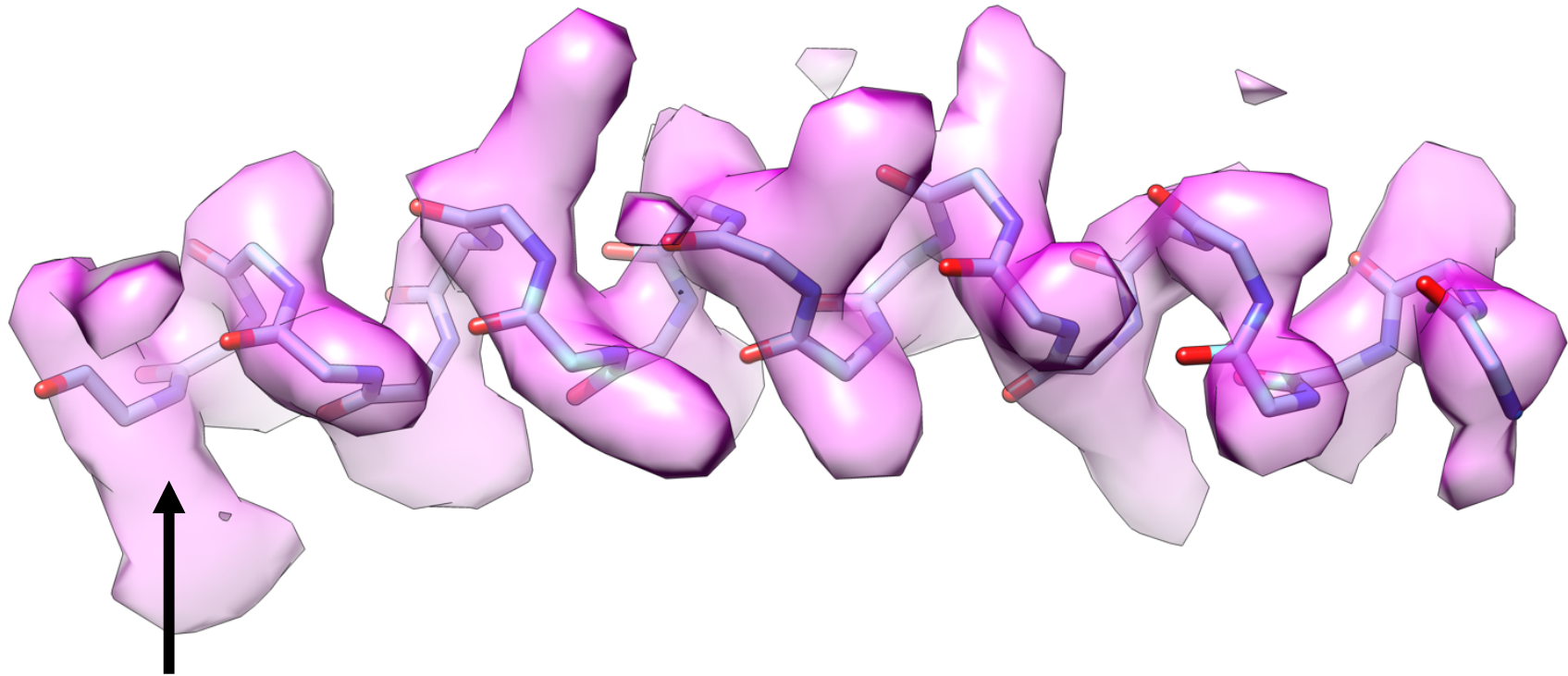
- Auto-sharpen the map
- Run Map Symmetry to obtain symmetry
- Run Map Box to obtain asymmetric unit (using symmetry)
- Run Map to Model on asymmetric unit
- Run Apply NCS Operators on model, with the trim overlap option (supplying the full map)

Automated water building: *phenix.douse*



Available in ChimeraX!

Sequence from map: *phenix.sequence_from_map*

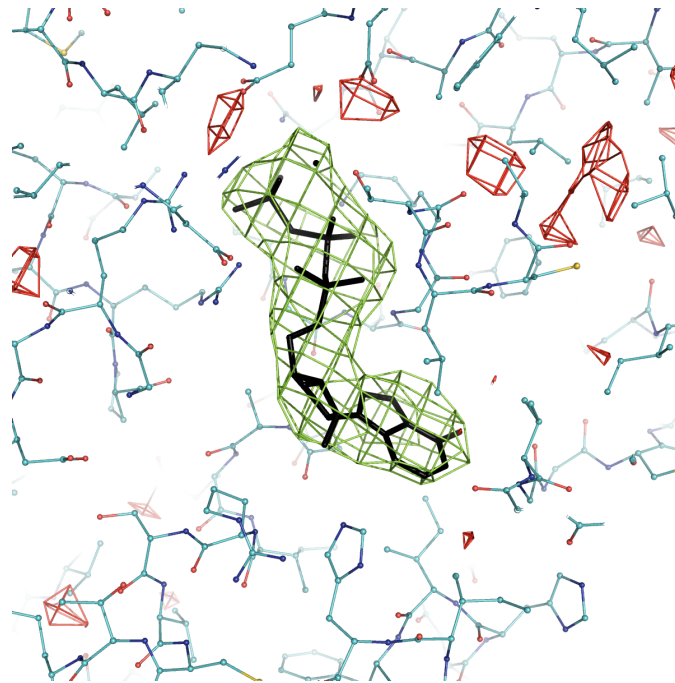
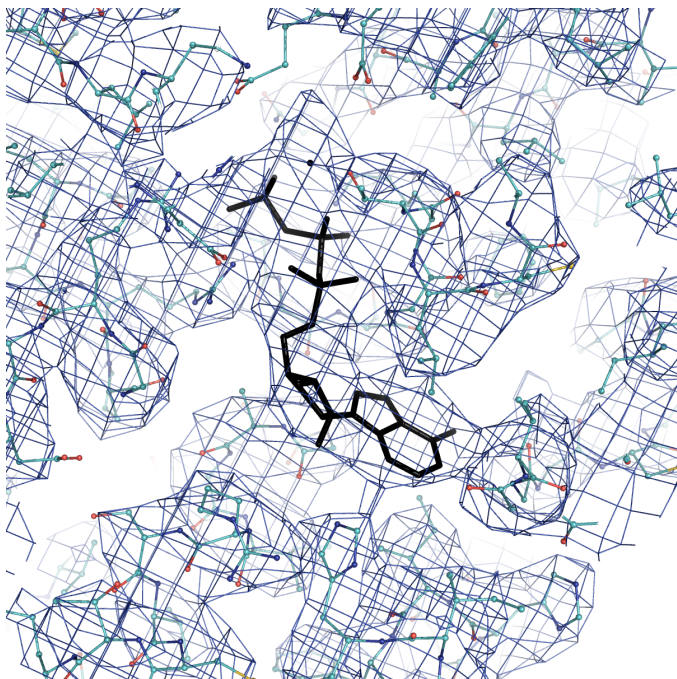


Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

- Determine probability of side chain at each C α
- Align sequence to maximize total probability for the chain

Difference maps: *phenix.real_space_diff_map*

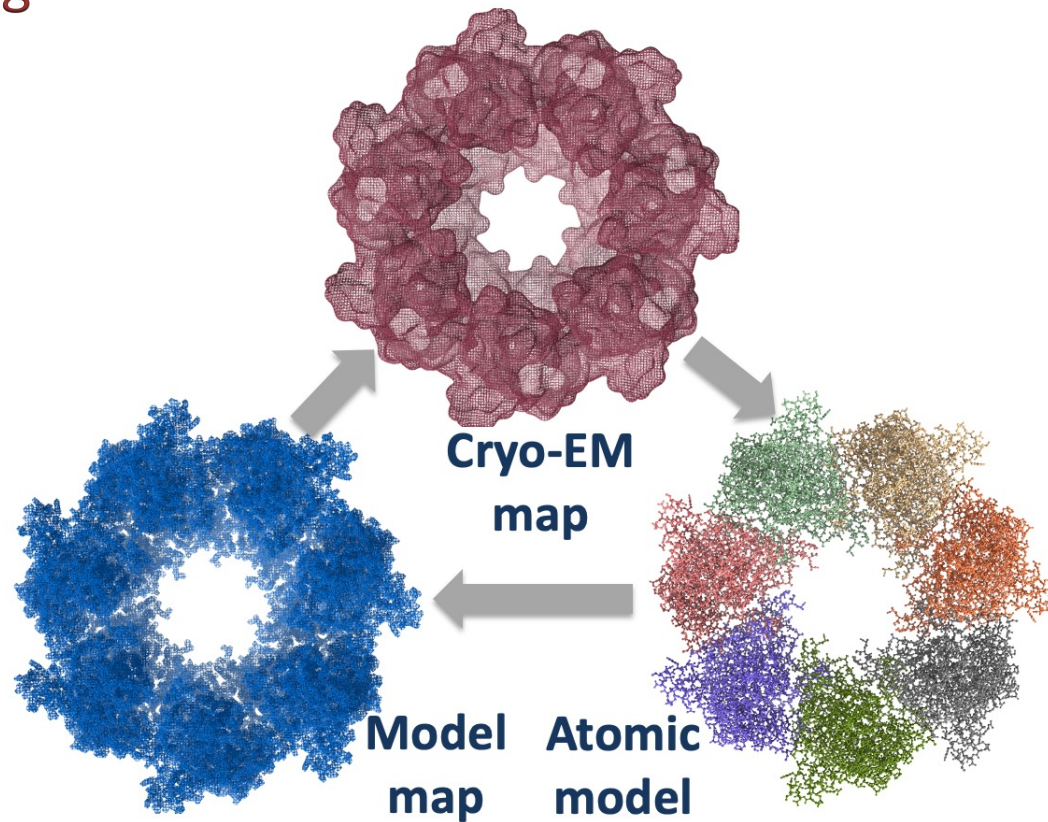
5L4g, EMDDB 4002



- Analogue of crystallographic Fo-Fc map
- Requires well-refined model (including B factors)

Model map

- Computing an adequate map from an atomic model is the key for:
 - Refinement
 - Model building
 - Validation
 - Ligands



Model map

- ChimeraX: (<https://www.rbvi.ucsf.edu/chimerax/docs/user/commands/molmap.html>)
 - Each atom is a 3D Gaussian distribution of width proportional to the resolution and amplitude proportional to the atomic number.
 - Tom Goddard (August 2025): *"I don't have a paper reference, I believe I copied what EMAN did 20 years ago"*

- CryoFit:

$$\rho(k, \mathbf{r}_j) = \exp \left[-\frac{1}{2} \left(\frac{\mathbf{r}_k - \mathbf{r}_j}{\sigma} \right)^2 \right]$$

2σ = resolution

- Struc2mapGAN, EMReady:

$$\rho(\mathbf{x}) = \sum_i^M \theta Z_i e^{-k|\mathbf{x} - \mathbf{r}_i|^2}$$

$$k = (\pi / (1.2 + 0.6R))^2$$

$$\Theta = (k / \pi)^{1.5}$$

R = resolution

- DEMO-EMfit:

$$\rho_M(\nu_i) = \sum_{j=1}^L m_j^3 \sqrt{\left(\frac{\pi}{(2.4 + 0.8R)^2} \right)^2} \exp \left(-\left(\frac{\pi}{2.4 + 0.8R} \right)^2 |\nu_i - x_j|^2 \right)$$

m = atomic mass
R = resolution

- Q-score

$$y = A e^{-\frac{1}{2} \left(\frac{x - \mu}{\sigma} \right)^2} + B$$

$$\sigma = 0.6$$

A, B ~ map mean and s.d.

Model map: two fundamental limitations

$$\rho(k, \mathbf{r}_j) = \exp \left[-\frac{1}{2} \left(\frac{\mathbf{r}_k - \mathbf{r}_j}{\sigma} \right)^2 \right]$$

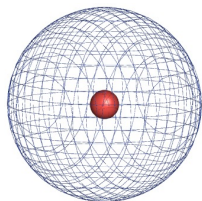
$$\rho(\mathbf{x}) = \sum_i^M \theta Z_i e^{-k|\mathbf{x} - \mathbf{r}_i|^2}$$

$$y = A e^{-\frac{1}{2} \left(\frac{x - \mu}{\sigma} \right)^2} + B$$

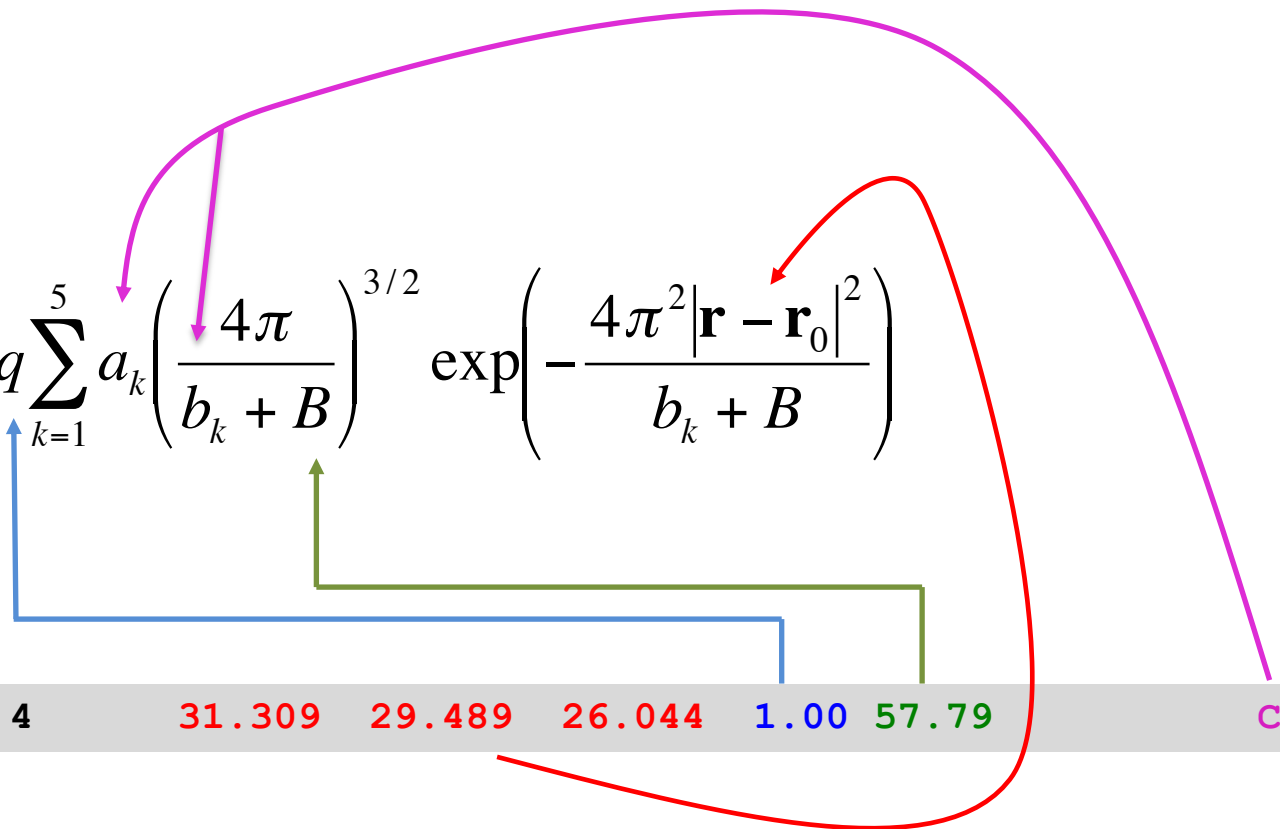
$$\rho_M(\nu_i) = \sum_{j=1}^L m_j^3 \sqrt{\left(\frac{\pi}{(2.4 + 0.8R)^2} \right)^2} \exp \left(-\left(\frac{\pi}{2.4 + 0.8R} \right)^2 |\nu_i - x_j|^2 \right)$$

- Ad hoc, intuitive formulations to mimic experimental density
 - No chemical element type
 - No charge
 - No occupancy
 - No B factors (isotropic, anisotropic)
- Resolution is not accounted for

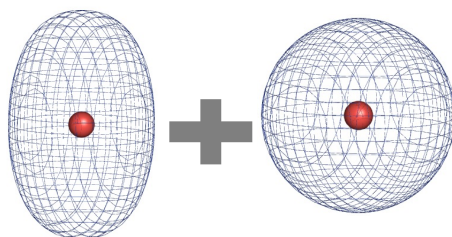
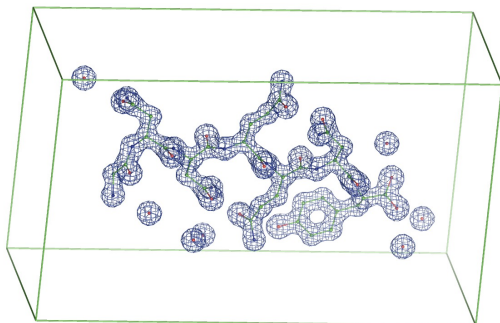
Model map: Independent Atom Model (IAM)



$$\rho_{atom}(\mathbf{r}, \mathbf{r}_0, B, q) = q \sum_{k=1}^5 a_k \left(\frac{4\pi}{b_k + B} \right)^{3/2} \exp \left(-\frac{4\pi^2 |\mathbf{r} - \mathbf{r}_0|^2}{b_k + B} \right)$$

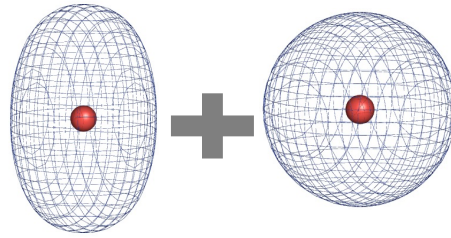
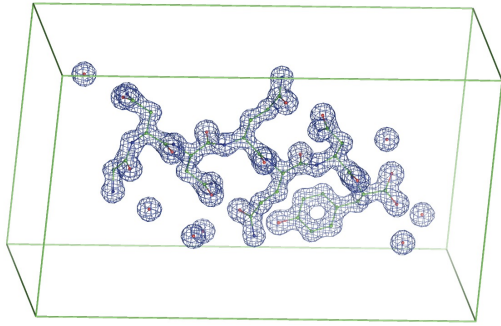


ATOM	25	CA	PRO	A	4	31.309	29.489	26.044	1.00	57.79	C
------	----	----	-----	---	---	--------	--------	--------	------	-------	---



$$\rho_{MODEL}(\mathbf{r}) = \sum_{i=1}^{N_{atoms}} \rho_{atoms}(\mathbf{r})$$

Model map



$$\rho_{MODEL}(\mathbf{r}) = \sum_{i=1}^{N_{atoms}} \rho_{atoms}(\mathbf{r})$$

This is not the map we need for refinement, validation, model building..

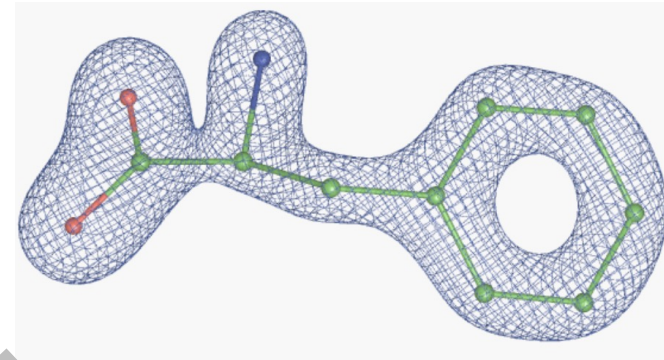
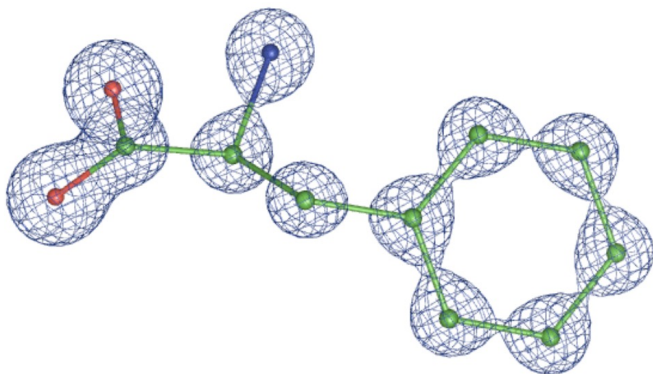
In practice, we rarely need this map at all!

This is because it is an “infinite resolution” (exact) map, which cannot be meaningfully compared with the experimental map.

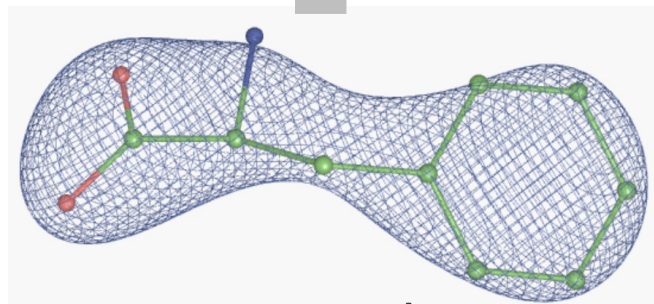
Model map suitable for calculations

Exact model map ρ_{calc}

Finite resolution model map $\widetilde{\rho_{calc}}$



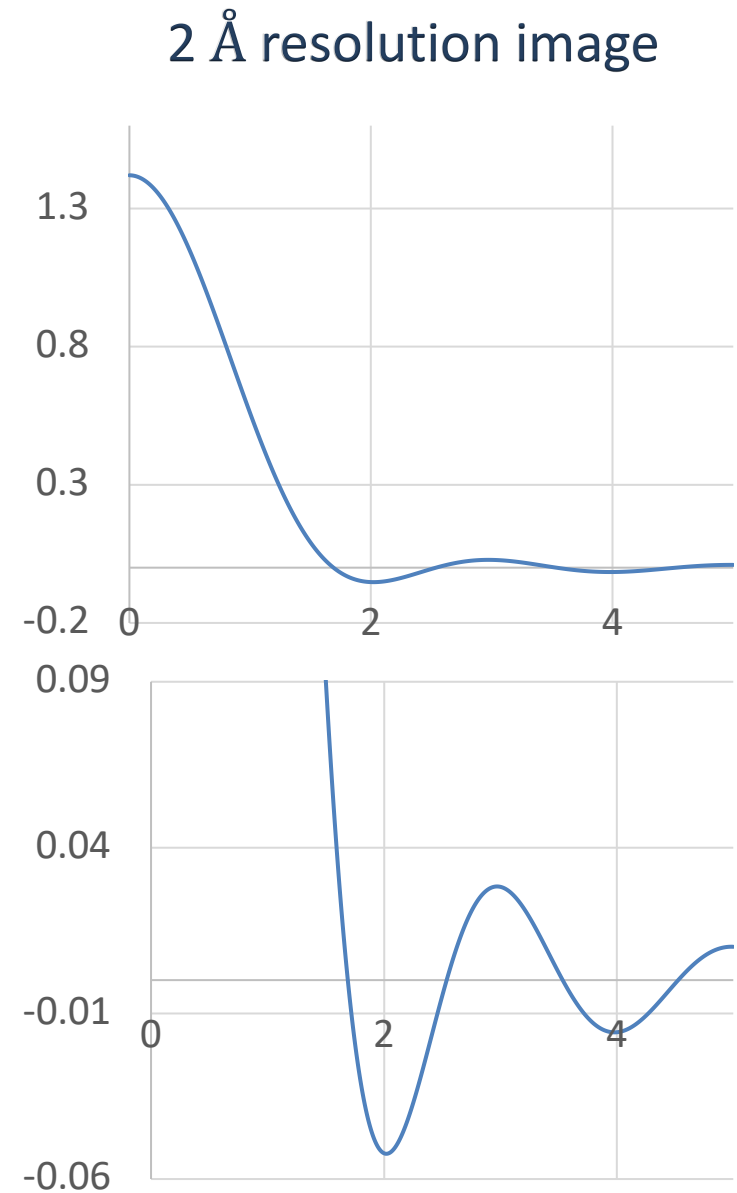
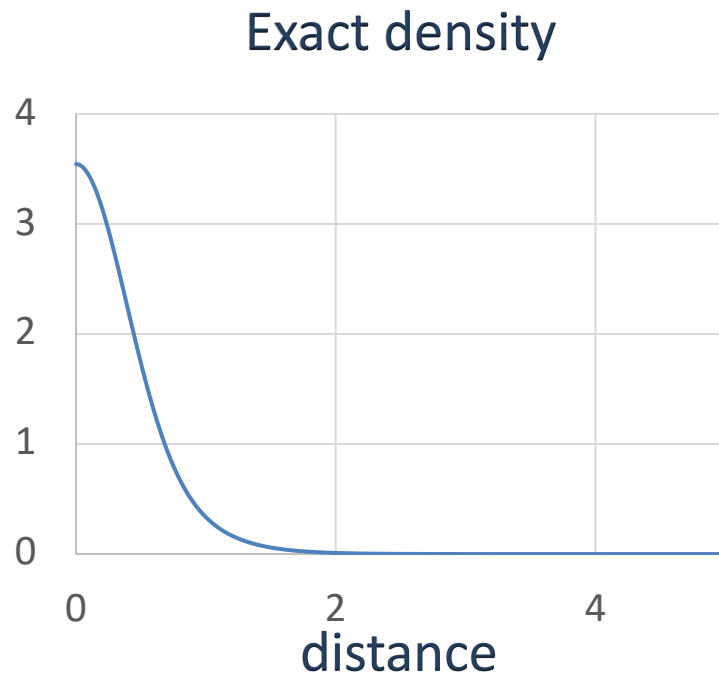
$$LS = \sum_{map} (\rho_{obs} - \widetilde{\rho_{calc}})^2 \quad CC = \frac{\sum \rho_{obs} \widetilde{\rho_{calc}}}{(\sum \rho_{obs}^2 \sum \widetilde{\rho_{calc}}^2)^{1/2}}$$



Experimental map

ρ_{obs} and $\widetilde{\rho_{calc}}$
must be the same
resolution

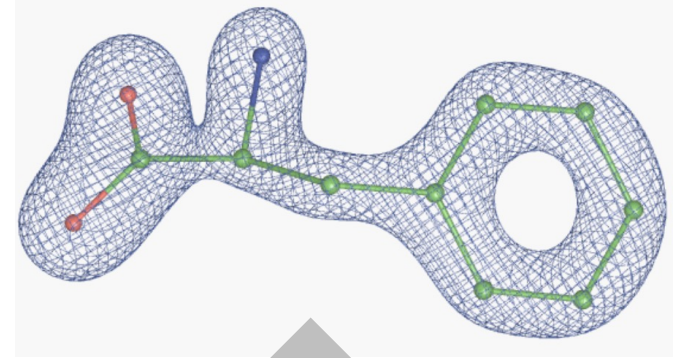
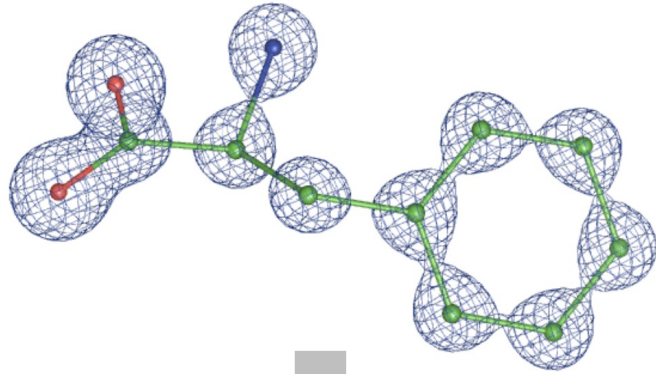
Model map illustrations for Carbon atom



Finite resolution model map calculation (Fourier map)

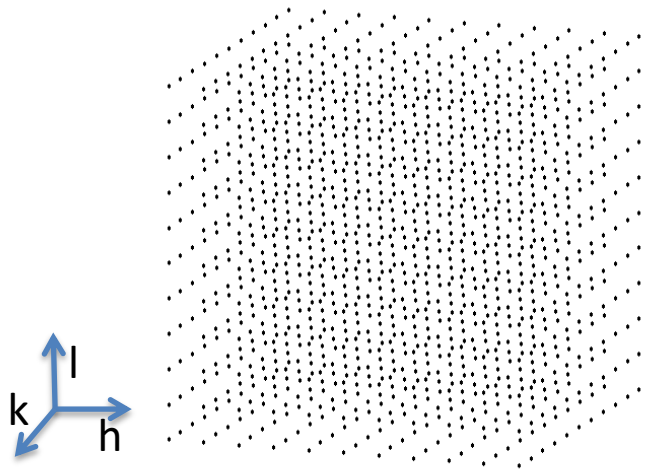
Exact model map ρ_{calc}

Model-calculated Fourier map $\widetilde{\rho_{calc}}$

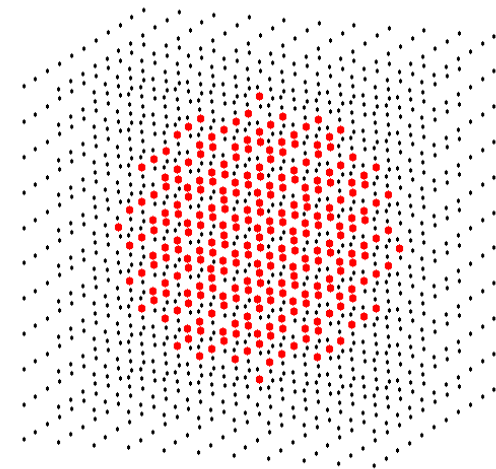


FT⁻¹

FT



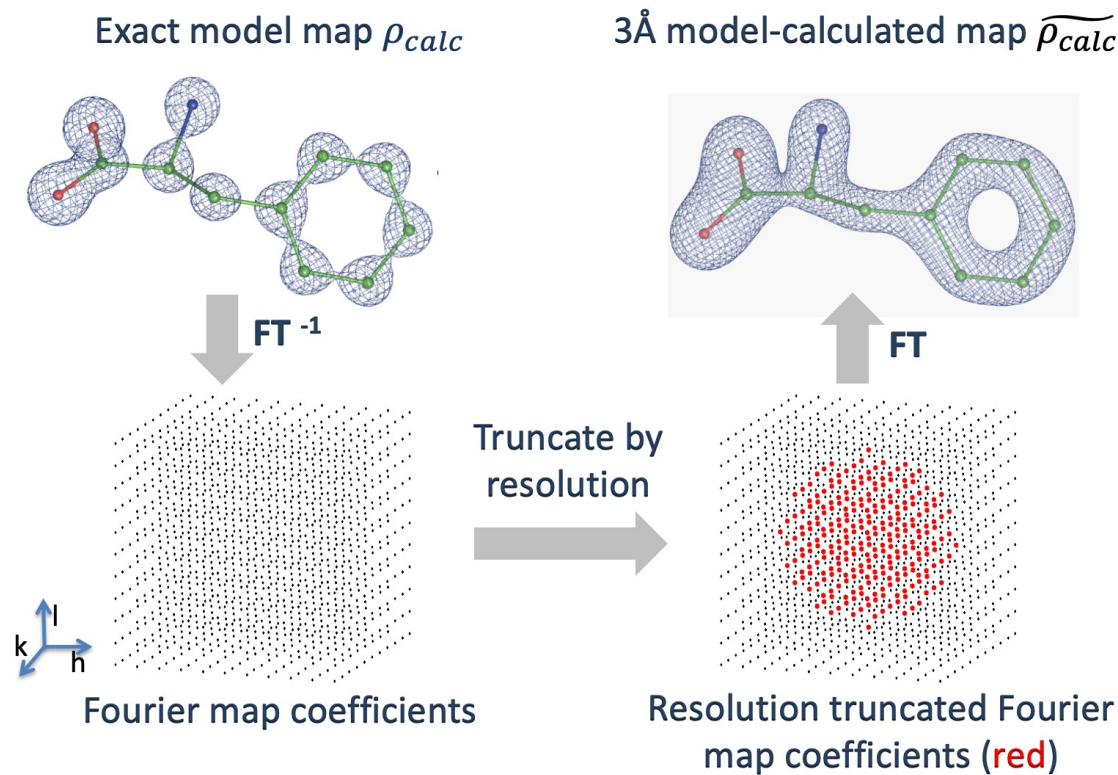
Truncate
resolution



Fourier map coefficients

Resolution truncated Fourier
map coefficients (red)

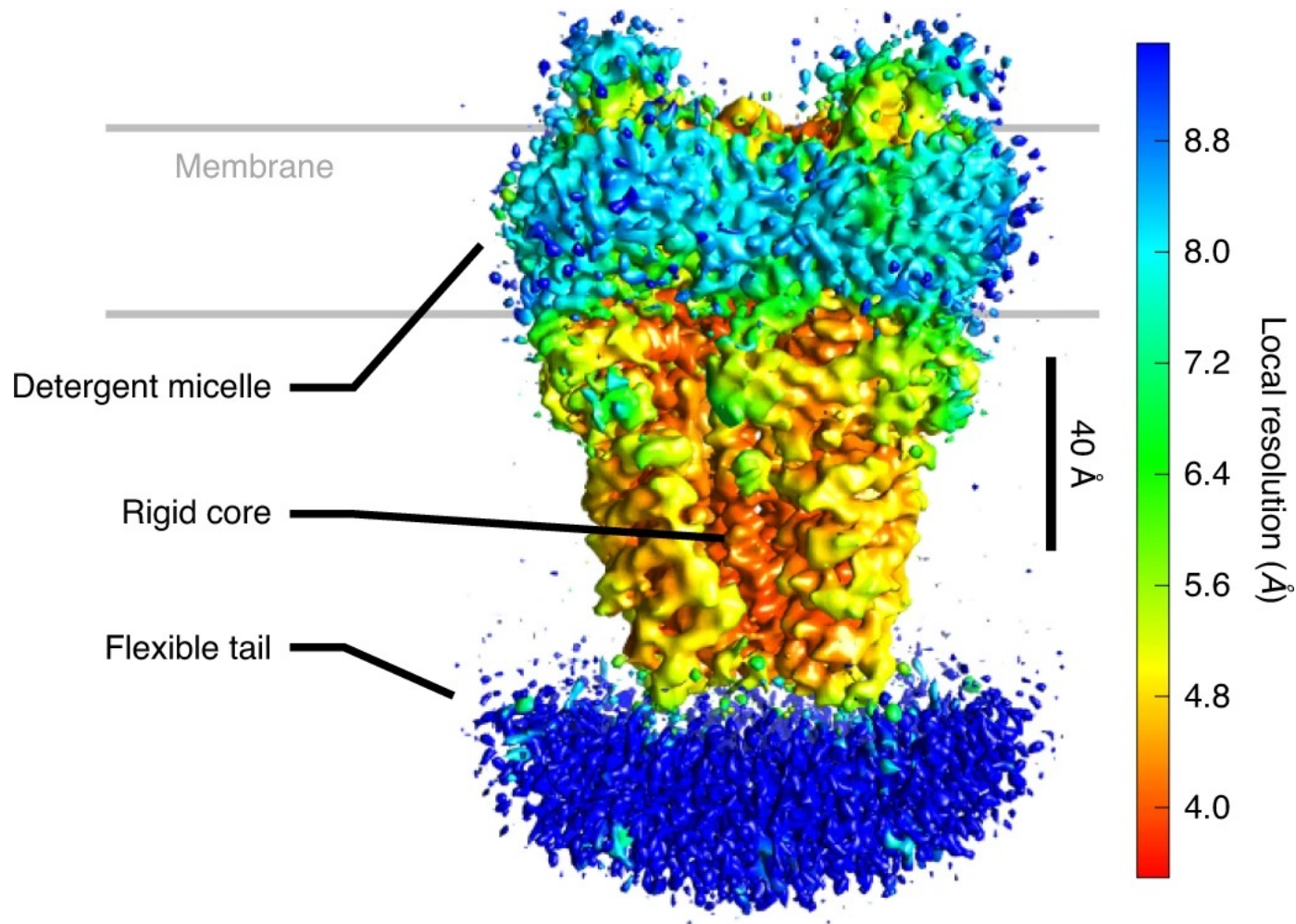
Finite resolution model map calculation (Fourier map)



Two problems with this approach:

1. This is a very indirect and heavy calculation
2. Resolution for all atoms is exactly the same

CryoEM: local resolution can vary a lot



Adopted from Zhang et al. *Nat Methods* **17**, 1214–1221 (2020).

Solution comes from this series of publications

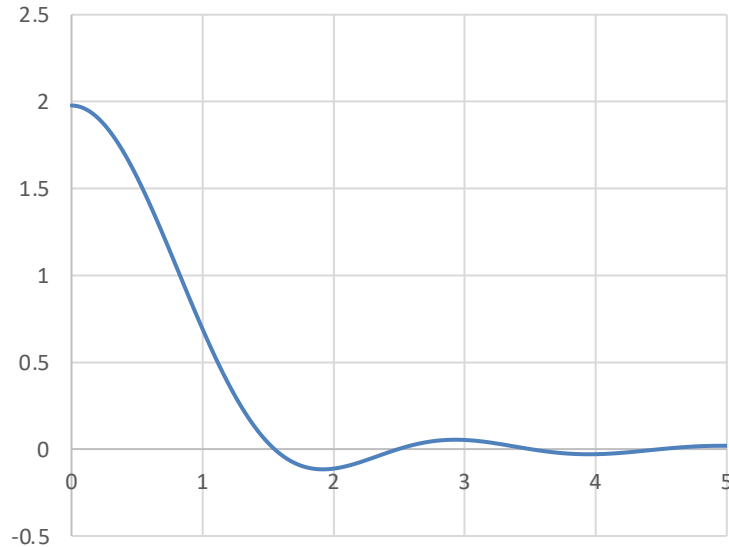
Urzhumtsev, A., Lunin, V.Y. (2022) "**Analytic modeling of inhomogeneous-resolution maps in cryo-electron microscopy and crystallography**". *IUCr Journal*, **9**, 728-734.

Urzhumtsev, A., Urzhumtseva, L.M., Lunin, V.Y. (2022) "**Direct calculation of cryo-EM and crystallographic model maps for real-space refinement**". *Acta Cryst.*, **D78**, 1451-1468.

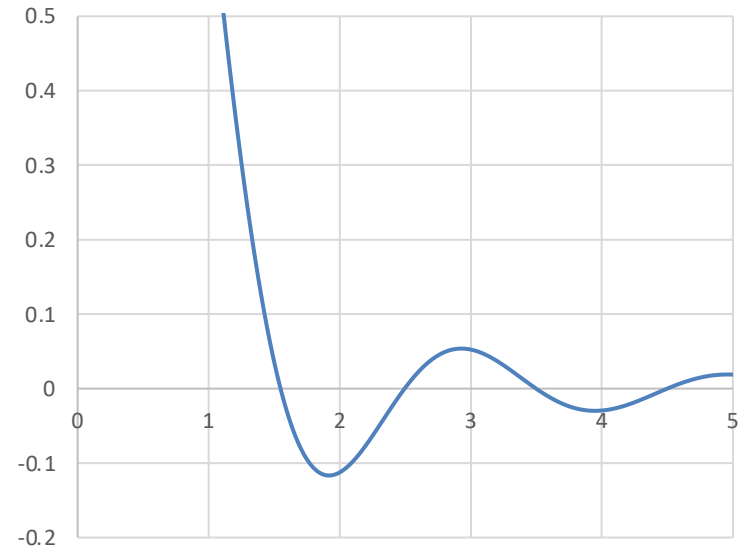
Urzhumtseva, L., Lunin, V.Y., Urzhumtsev, A. (2023) "**Algorithms and programs for the shell decomposition of oscillating functions in space**". *J. Appl. Cryst.*, **56**, 302-311.

Idea at a glance

3 Å resolution image of C atom (2D)



Zooming on ripples



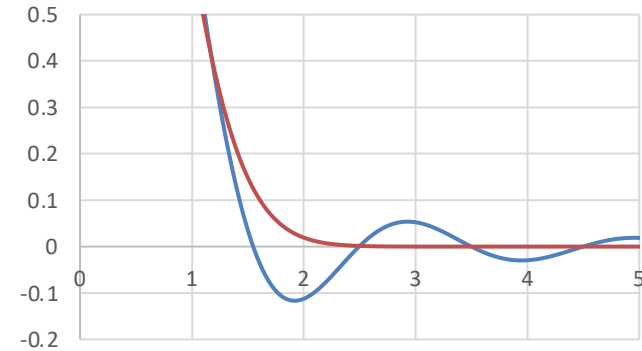
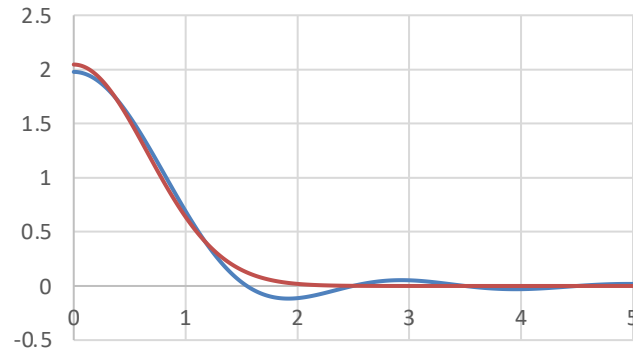
This image can be approximated with desired accuracy by this function

$$\sum_{m=1}^M C_m \Omega(\mathbf{r}; R_m, B_m)$$

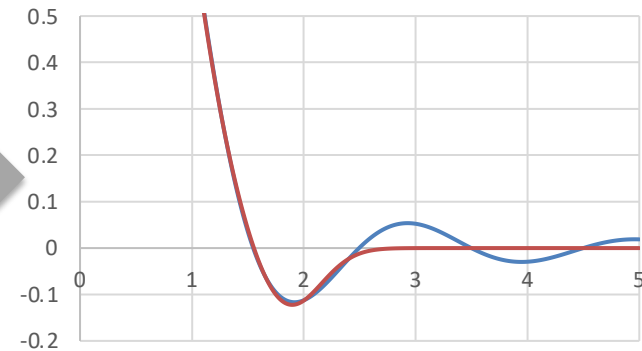
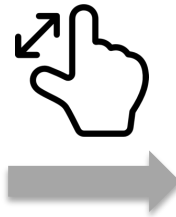
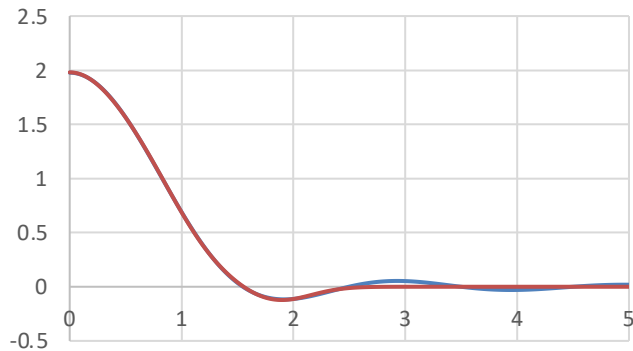
$$\Omega(\mathbf{r}; R, B) = \frac{1}{|\mathbf{r}|R} \left(\frac{1}{4\pi B} \right)^{1/2} \left[\exp\left(-\frac{4\pi^2(|\mathbf{r}| - R)^2}{B} \right) - \exp\left(-\frac{4\pi^2(|\mathbf{r}| + R)^2}{B} \right) \right]$$

More terms M = more accurate approximation

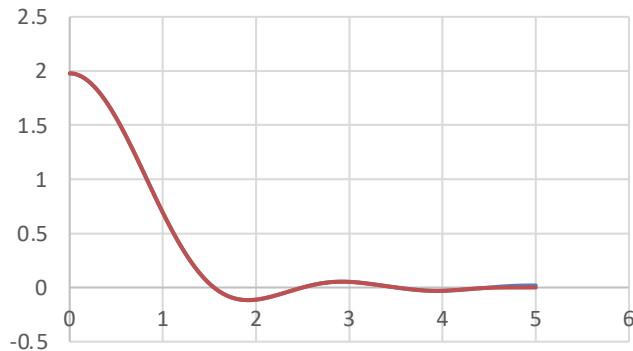
$$\sum_{m=1}^M C_m \Omega(\mathbf{r}; R_m, B_m)$$



1 term



2 terms



4 terms

Practicalities

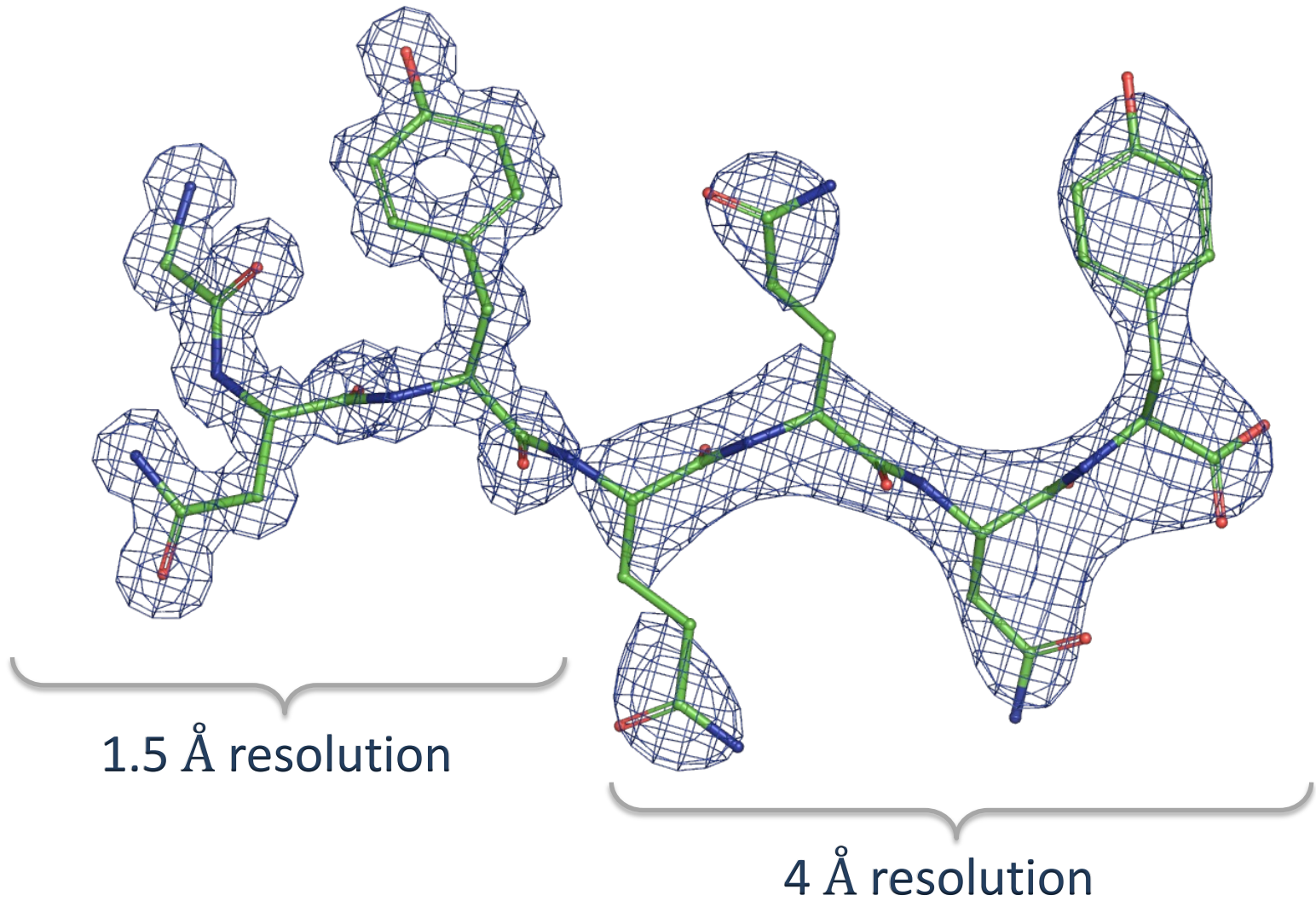
$$\rho_{calc} = \sum_{m=1}^M C_m \Omega(\mathbf{r}; R_m, B_m)$$

$$\Omega(\mathbf{r}; R, B) = \frac{1}{|\mathbf{r}|R} \left(\frac{1}{4\pi B} \right)^{1/2} \left[\exp\left(-\frac{4\pi^2(|\mathbf{r}| - R)^2}{B} \right) - \exp\left(-\frac{4\pi^2(|\mathbf{r}| + R)^2}{B} \right) \right]$$

- Precompute and tabulate B, C and R constants for all atoms from Periodic Table and all resolutions
 - This is done by fitting to actual Fourier maps
- To calculate the map, assign resolution to each atom in your model, then look up its parameters in precomputed tables, and compute the map

What can we do with this?

Fourier maps can be computed analytically for a model, allowing different atoms or regions (chains, domains, loops, etc.) to have distinct resolutions



What can we do with this?

- Difference maps (aka Fo-Fc in Xtal) can be calculated more accurately
- Refinement:
 - Analytic derivatives w.r.t. coordinates, occupancies, B factors
 - More accurate refinement target function
 - Local resolution aware
- Validation: CC_{MASK} aware of local resolution
- AI/ML based model building, ligand identification:
 - Fast and accurate generation of realistic density maps of given resolution for training purposes
- Accurate map-to-model fit evaluation (rotamer fitting)