

# Phenix tools for cryo-EM

Pavel Afonine



[phenix-online.org](http://phenix-online.org)



[lbl.gov](http://lbl.gov)



[qrefine.com](http://qrefine.com)

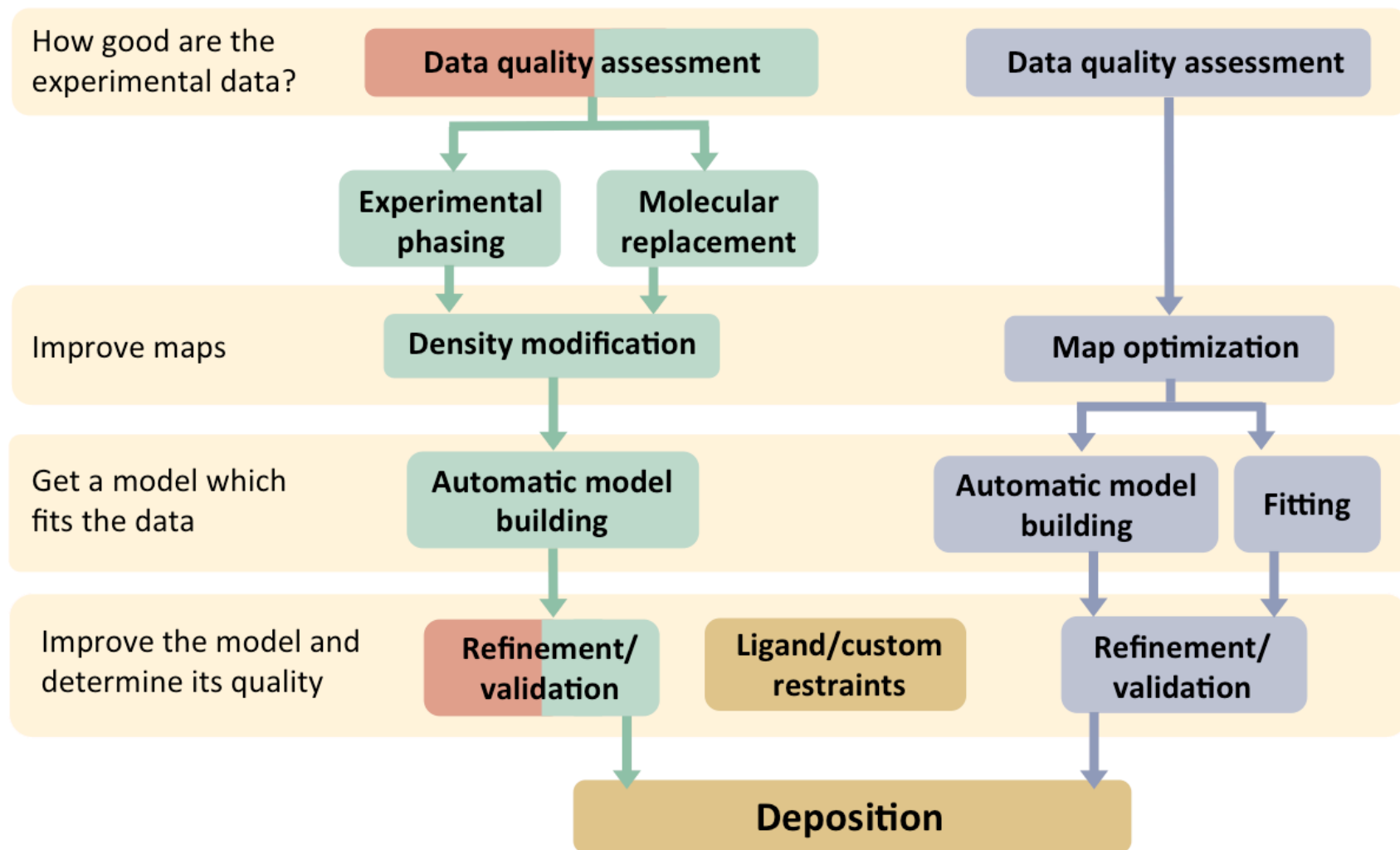
Hosted by the Oklahoma COBRE in Structural Biology

March 19<sup>th</sup> 2024

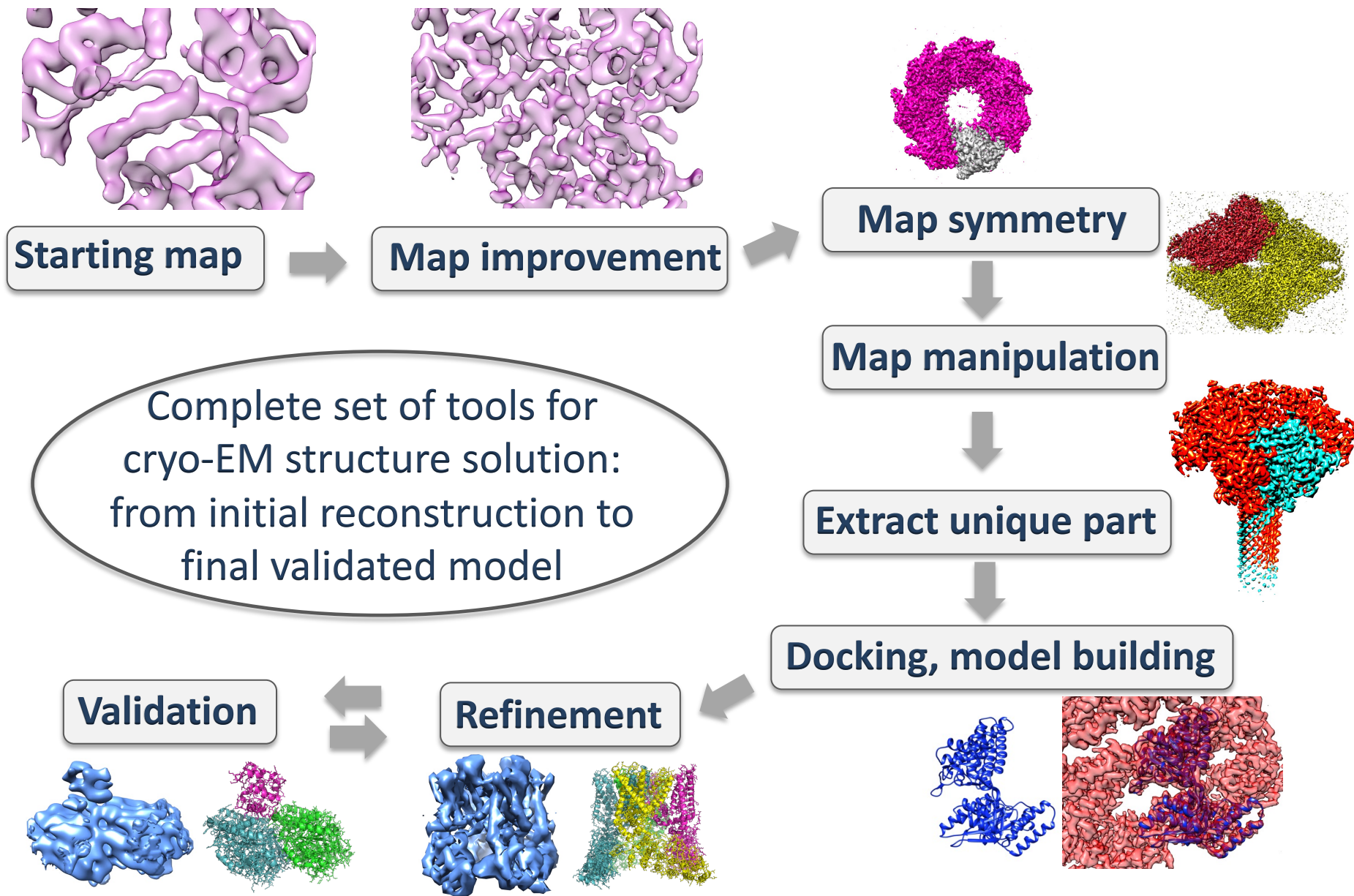
# Phenix: tools for crystallography and cryo-EM

## Xray/neutron crystallography

## Cryo-EM



# Phenix tools for cryo-EM



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

The screenshot displays the PHENIX home GUI. At the top, there is a toolbar with icons for Quit, Preferences, Help, Citations, ChimeraX, Coot, PyMOL, KING, Other tools, and Ask for help. Below the toolbar are tabs for 'Actions' and 'Job history'. The main interface is divided into two main sections: a 'Projects' table on the left and a navigation menu on the right.

**Projects Table:**

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

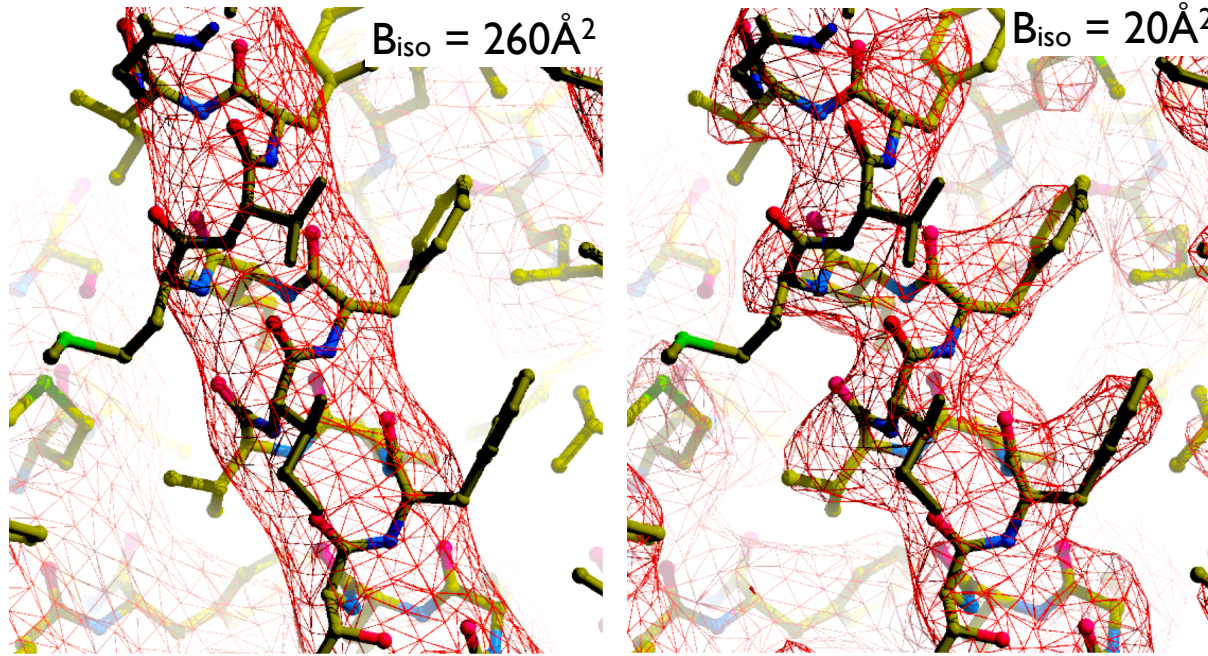
**Navigation Menu (Right Panel):**

- 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

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

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Automated map sharpening by maximization of  
detail and connectivity

Thomas C. Terwilliger,<sup>a,b\*</sup> Oleg V. Sobolev,<sup>c</sup> Pavel V. Afonine<sup>c,d</sup> and  
Paul D. Adams<sup>d,e</sup>

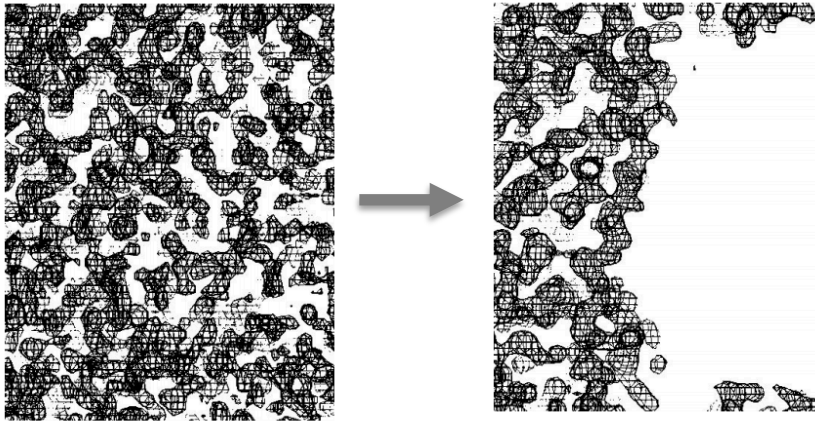
# Density modification: *phenix.density\_modify\_cryo\_em*

Similar principals for crystallography and cryo-EM:

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

## Crystallography

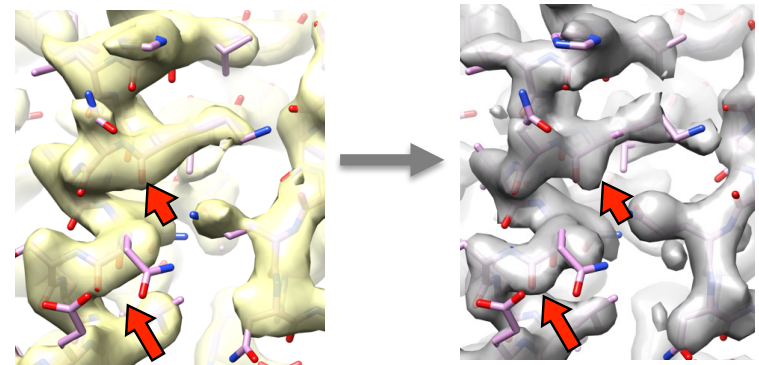
From uninterpretable to interpretable map



## 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

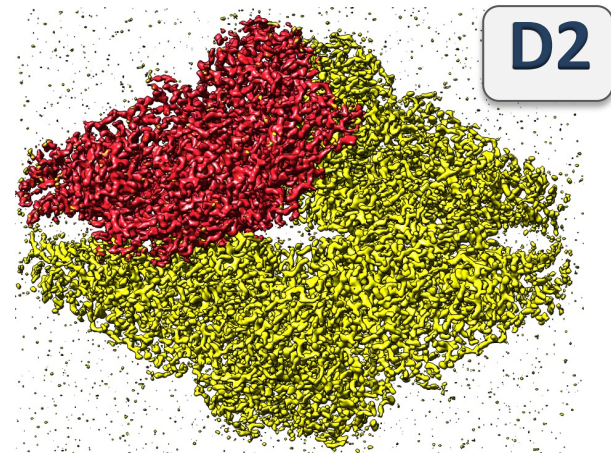
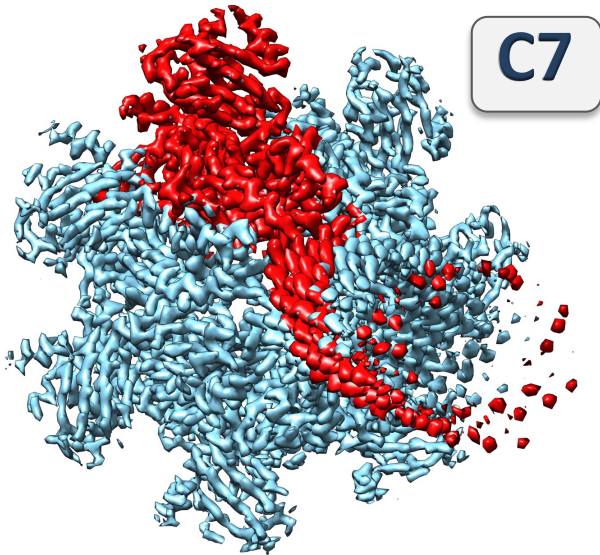
<https://doi.org/10.1038/s41592-020-0914-9>

 Check for updates

## Improvement of cryo-EM maps by density modification

Thomas C. Terwilliger<sup>1,2</sup>✉, Steven J. Ludtke<sup>3</sup>, Randy J. Read<sup>4</sup>, Paul D. Adams<sup>5,6</sup> and Pavel V. Afonine<sup>5</sup>

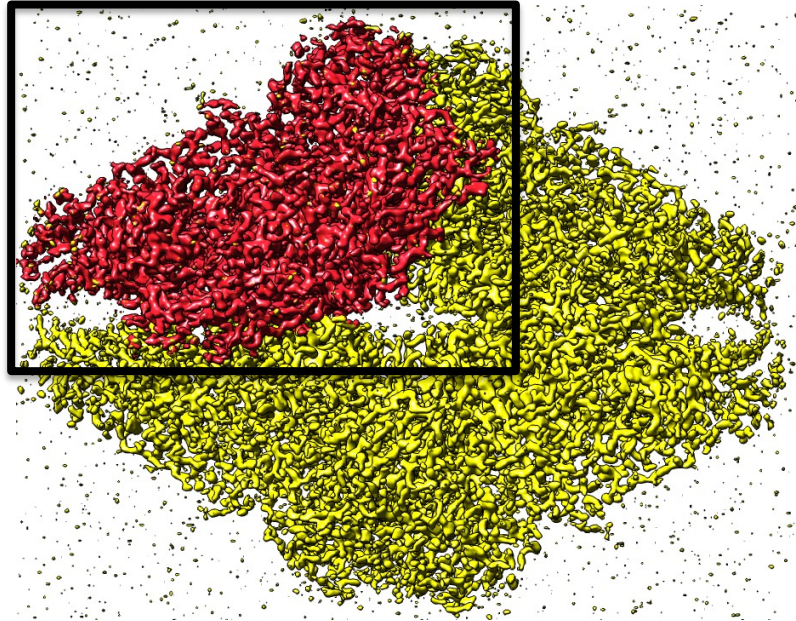
# Finding map symmetry: *phenix.symmetry\_from\_map*



<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*



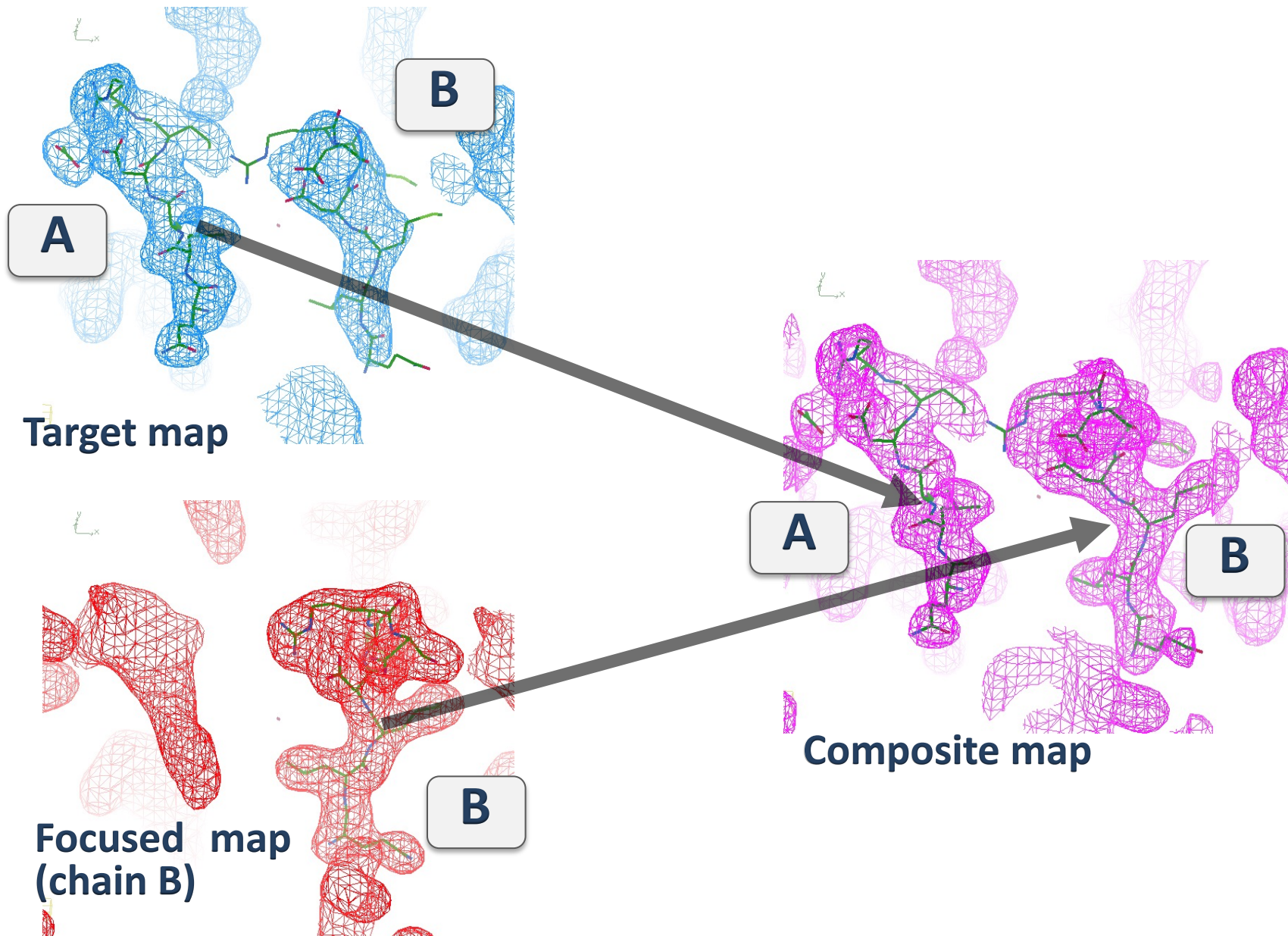
***Lots of options:*** use map only, use model, use symmetry, mask boxed map, and many more!

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

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

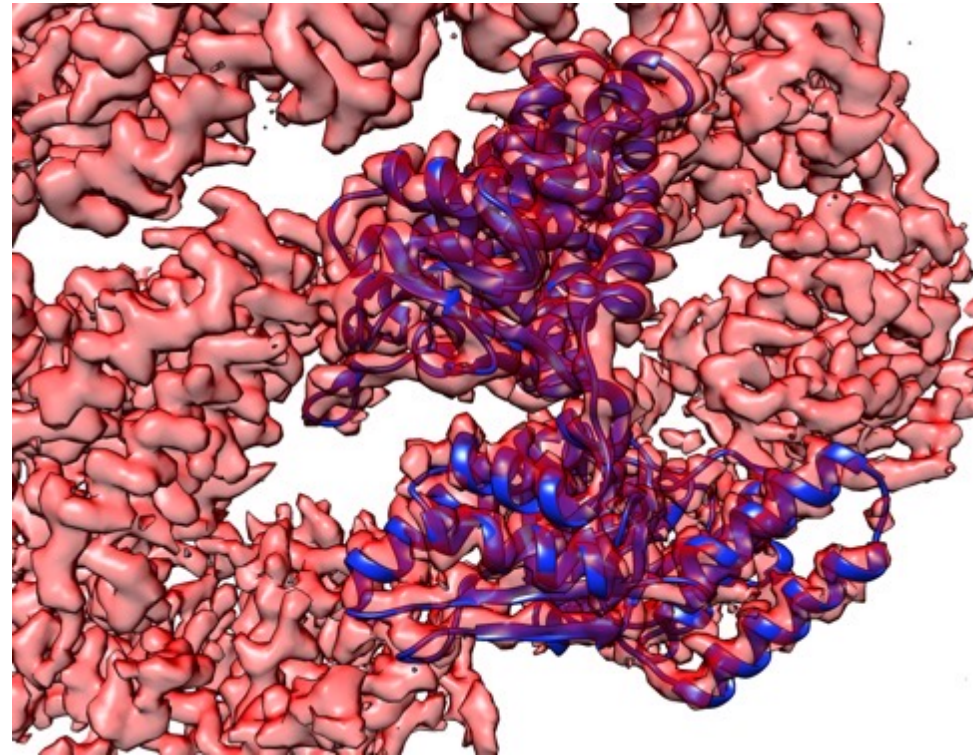
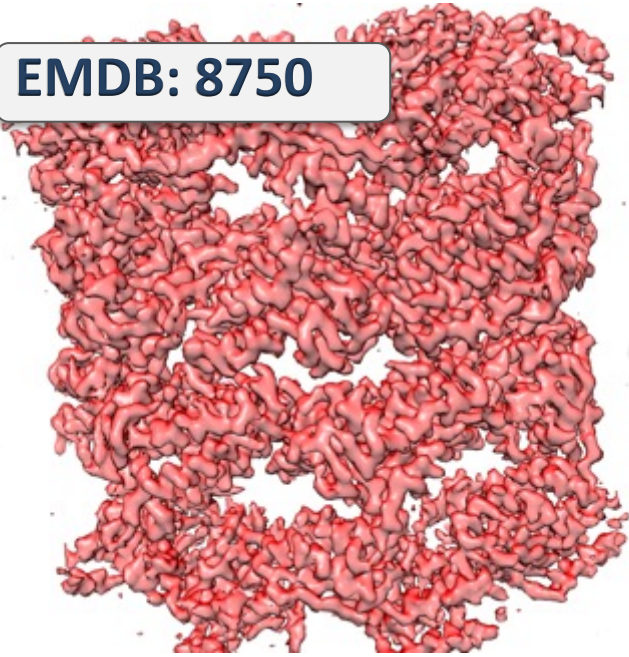


# Combining maps with *phenix.combine\_focused\_maps*



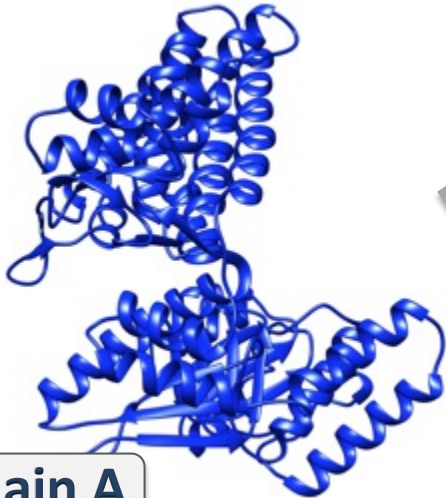
# Docking models with *phenix.dock\_in\_map*

EMDB: 8750

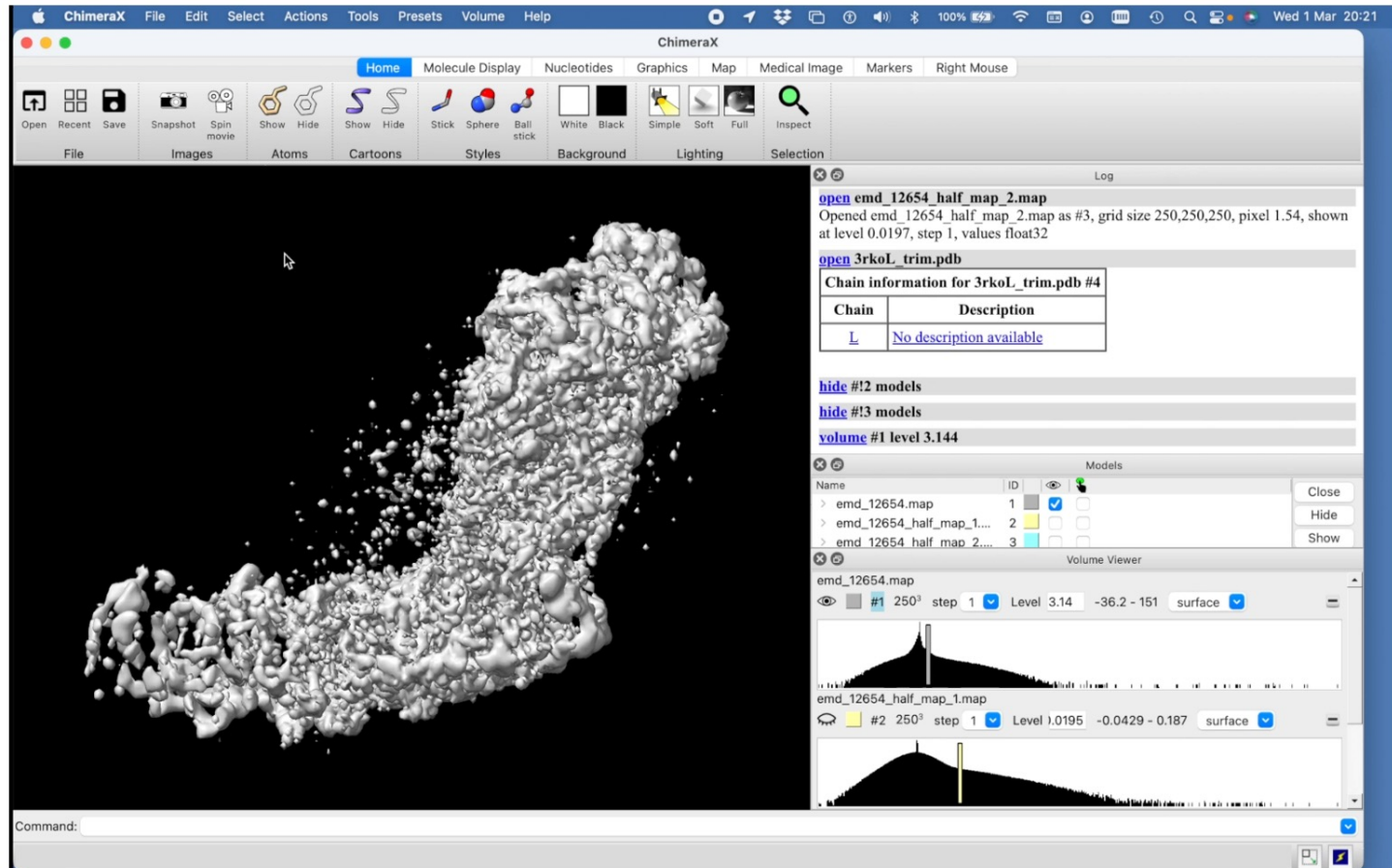


Chain A docked in map

1ss8 chain A



# Integration of docking with ChimeraX



- Read, Millán, McCoy & Terwilliger, “Likelihood-based signal and noise analysis for docking of models into cryo- EM maps”: BioRxiv, Acta Cryst. D (in press)
- Millán, McCoy, Terwilliger & Read, “Likelihood-based docking of models into cryo-EM maps”: BioRxiv, Acta Cryst. D (in press)



# Automated model building: *phenix.map\_to\_model*

nature|**methods**

BRIEF COMMUNICATION

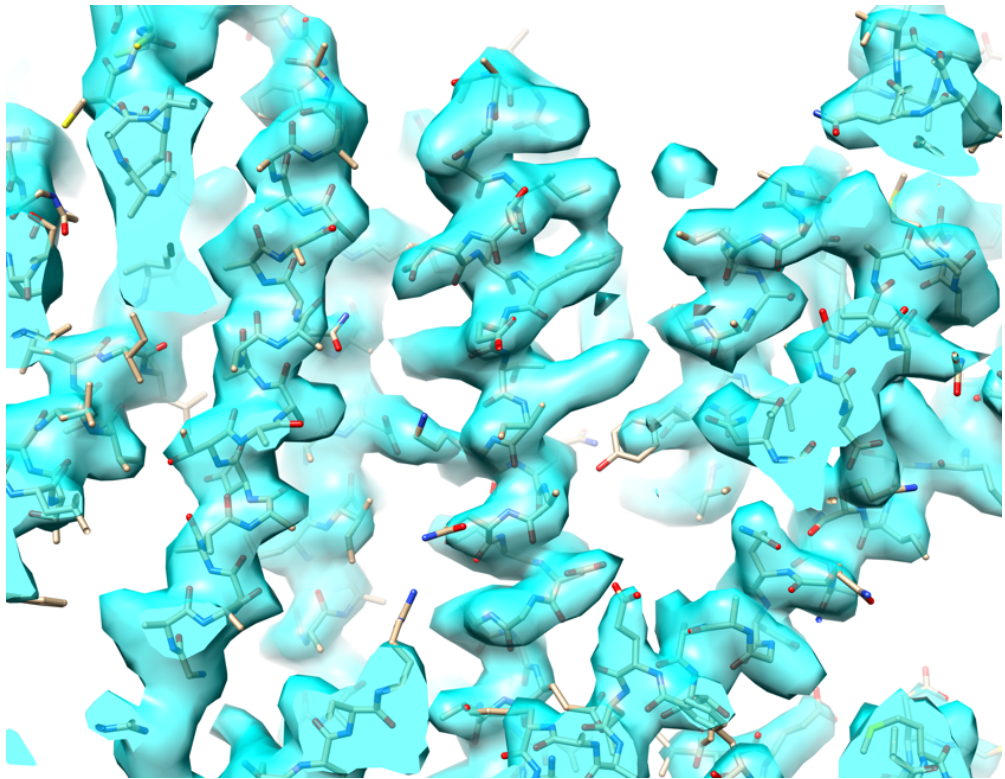
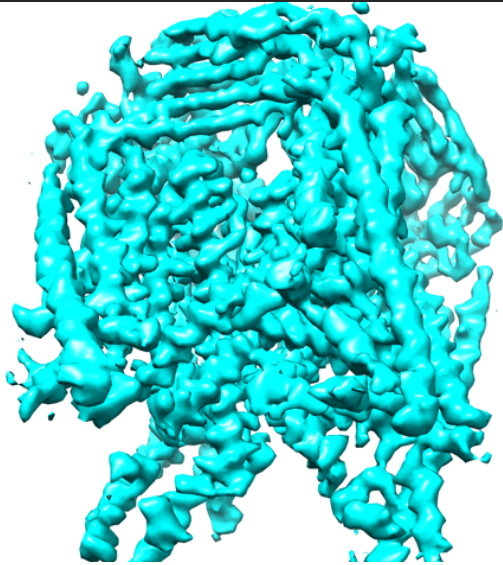
<https://doi.org/10.1038/s41592-018-0173-1>

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

Thomas C. Terwilliger <sup>1,2\*</sup>, Paul D. Adams<sup>3,4</sup>, Pavel V. Afonine<sup>3,5</sup> and Oleg V. Sobolev <sup>3</sup>

# Automated model building: *phenix.map\_to\_model*

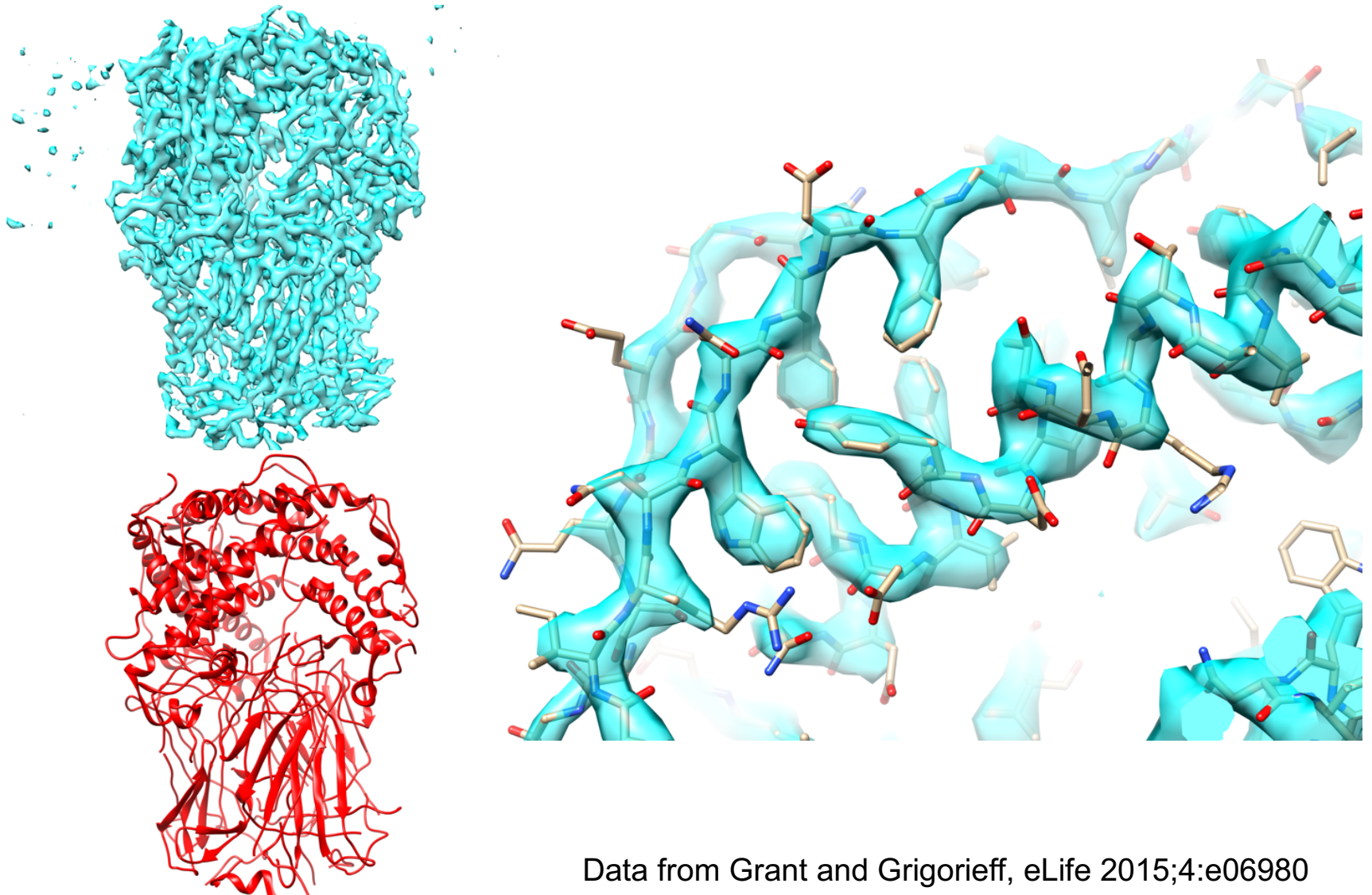
TRPML3 channel (4.1 Å, 78% built, 1.3 Å rmsd)



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

# 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

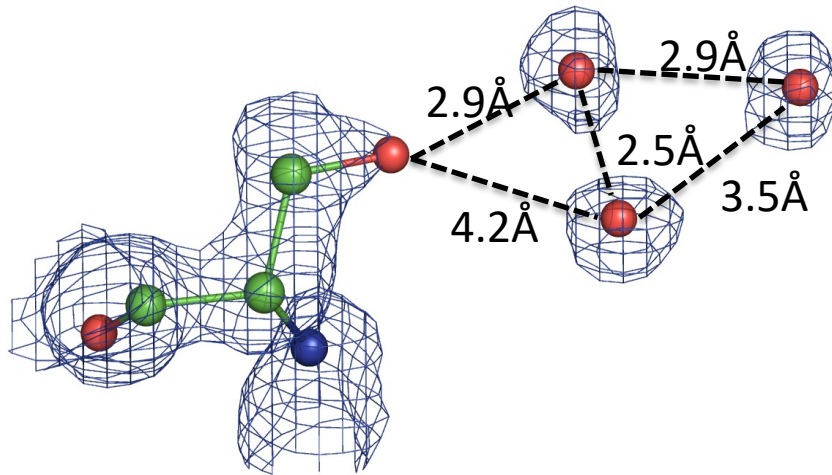
# Manual model building steps

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)

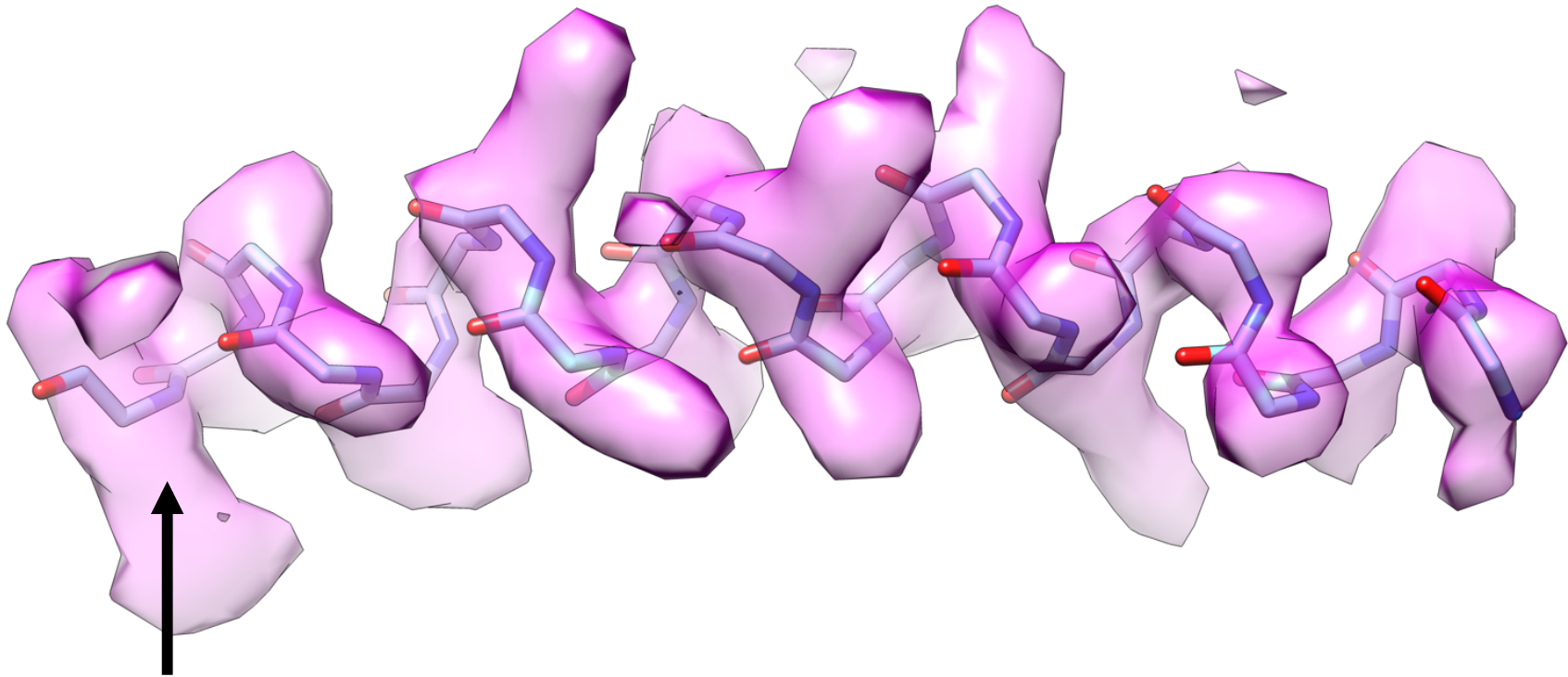


# Automated water building: *phenix.douse*



**Available in ChimeraX!**

# Sequence from map: *phenix.sequence\_from\_map*

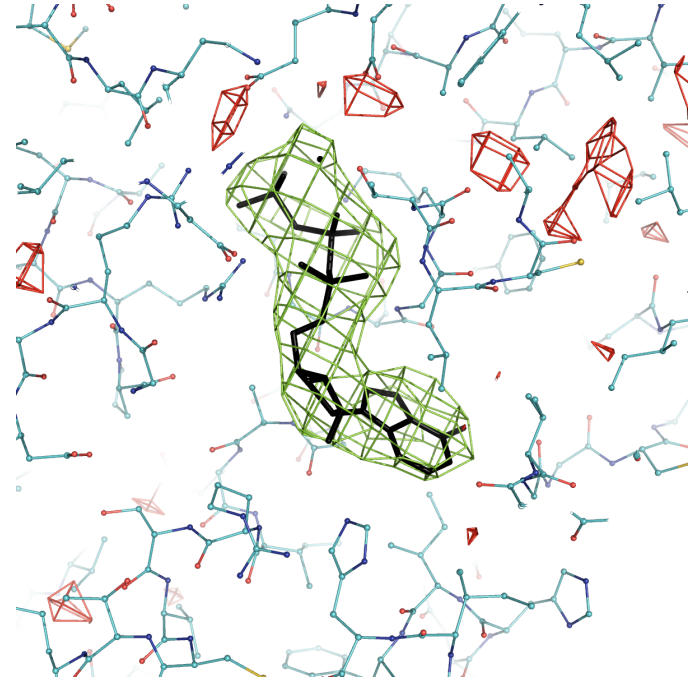
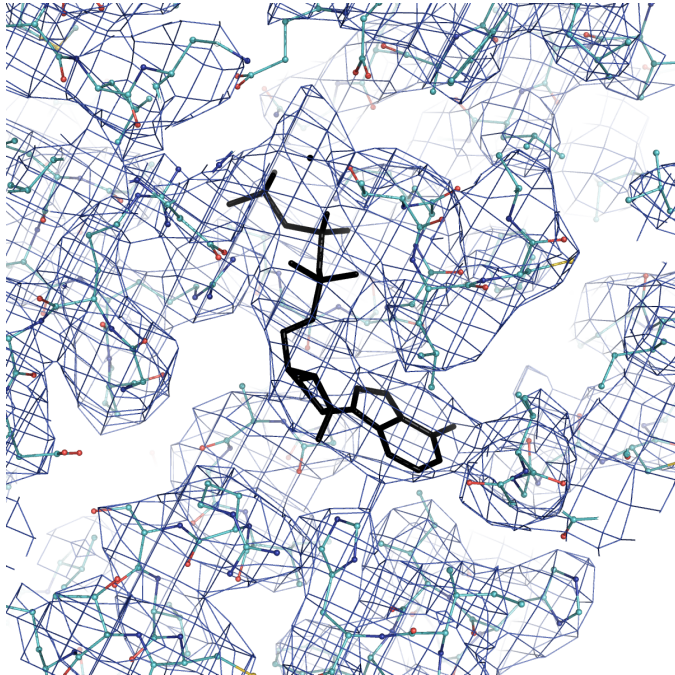


Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

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

# Difference maps: *phenix.real\_space\_diff\_map*

5L4g, EMDB 4002



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

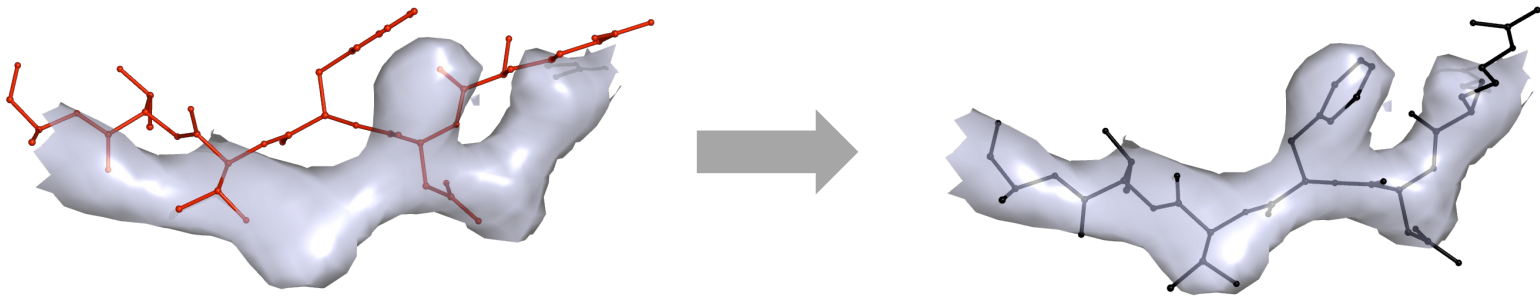
# Atomic model refinement: *phenix.real\_space\_refine*

## Direct refinement of atomic models against the map

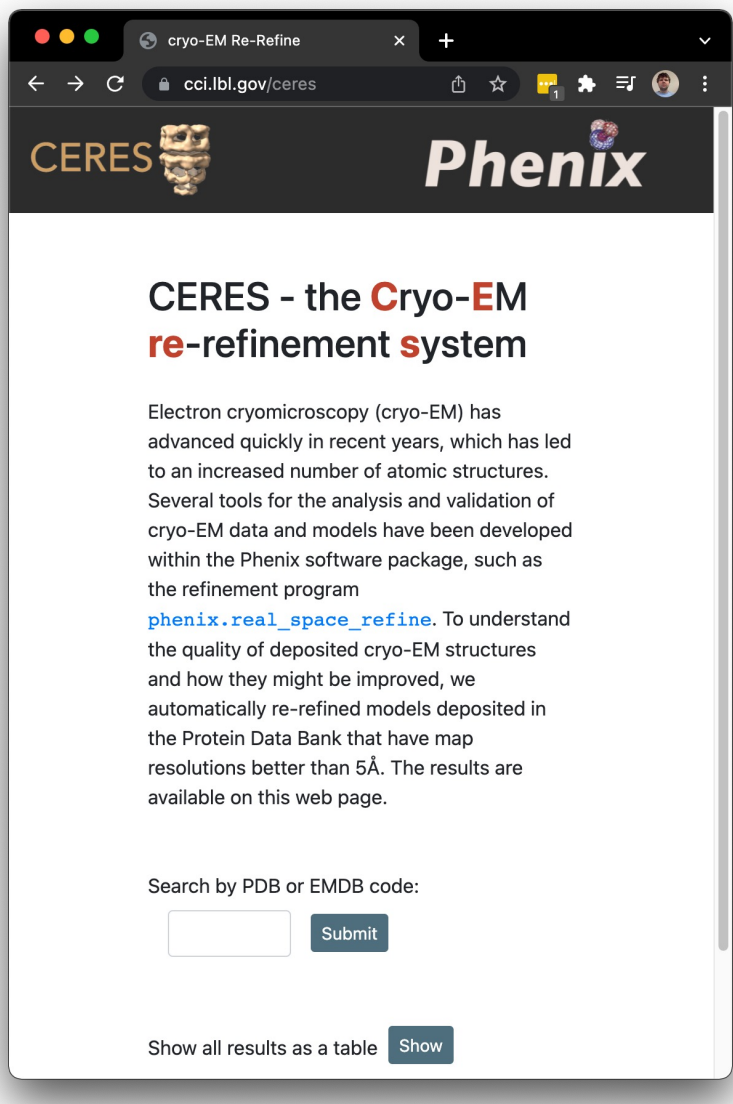
Acta Cryst  
**D** STRUCTURAL  
BIOLOGY  
ISSN 2059-7983

Real-space refinement in *PHENIX* for cryo-EM and crystallography

Pavel V. Afonine,<sup>a,b\*</sup> Billy K. Poon,<sup>a</sup> Randy J. Read,<sup>c</sup> Oleg V. Sobolev,<sup>a</sup> Thomas C. Terwilliger,<sup>d,e</sup> Alexandre Urzhumtsev<sup>f,g</sup> and Paul D. Adams<sup>a,h</sup>

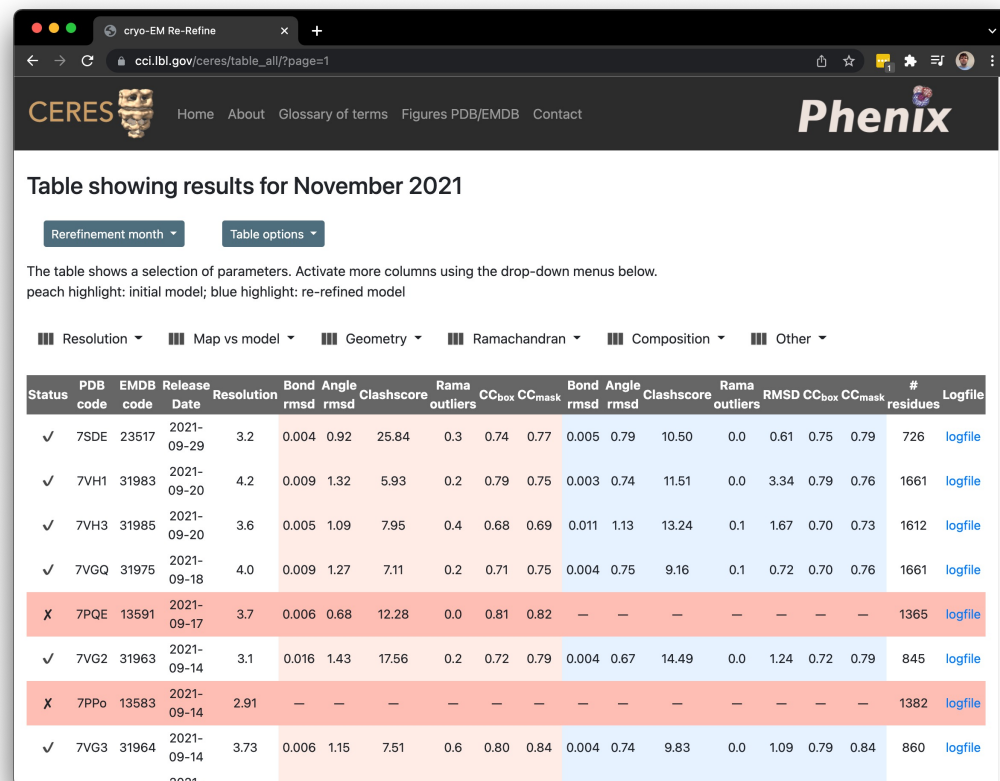


# Automated re-refinement of deposited cryo-EM models



The screenshot shows the CERES website home page. The header features the CERES and Phenix logos. The main heading is "CERES - the Cryo-EM re-refinement system". Below this, a paragraph explains that cryo-EM has advanced quickly, leading to more atomic structures, and that several tools for analysis and validation have been developed within the Phenix software package, such as the refinement program [phenix.real\\_space\\_refine](#). It states that the quality of deposited cryo-EM structures and how they might be improved is being addressed by automatically re-refining models deposited in the Protein Data Bank that have map resolutions better than 5Å. The results are available on this web page.

At the bottom, there is a search bar with the text "Search by PDB or EMDB code:" and a "Submit" button. Below the search bar, there is a link "Show all results as a table" and a "Show" button.



The screenshot shows the CERES website displaying a table of results for November 2021. The table is titled "Table showing results for November 2021" and includes a "Rerefinement month" dropdown menu and "Table options" button. Below the table, there is a note: "The table shows a selection of parameters. Activate more columns using the drop-down menus below. peach highlight: initial model; blue highlight: re-refined model".

The table has the following columns: Status, PDB code, EMDB code, Release Date, Resolution, Bond rmsd, Angle rmsd, Clashscore, Rama outliers, CC<sub>box</sub>, CC<sub>mask</sub>, Bond rmsd, Angle rmsd, Clashscore, Rama outliers, RMSD, CC<sub>box</sub>, CC<sub>mask</sub>, # residues, and Logfile. The table is filtered for November 2021 and shows 10 rows of data. The first 7 rows are highlighted in blue, indicating they are re-refined models. The 8th and 9th rows are highlighted in peach, indicating they are initial models.

Status	PDB code	EMDB code	Release Date	Resolution	Bond rmsd	Angle rmsd	Clashscore	Rama outliers	CC <sub>box</sub>	CC <sub>mask</sub>	Bond rmsd	Angle rmsd	Clashscore	Rama outliers	RMSD	CC <sub>box</sub>	CC <sub>mask</sub>	# residues	Logfile
✓	7SDE	23517	2021-09-29	3.2	0.004	0.92	25.84	0.3	0.74	0.77	0.005	0.79	10.50	0.0	0.61	0.75	0.79	726	<a href="#">logfile</a>
✓	7VH1	31983	2021-09-20	4.2	0.009	1.32	5.93	0.2	0.79	0.75	0.003	0.74	11.51	0.0	3.34	0.79	0.76	1661	<a href="#">logfile</a>
✓	7VH3	31985	2021-09-20	3.6	0.005	1.09	7.95	0.4	0.68	0.69	0.011	1.13	13.24	0.1	1.67	0.70	0.73	1612	<a href="#">logfile</a>
✓	7VGQ	31975	2021-09-18	4.0	0.009	1.27	7.11	0.2	0.71	0.75	0.004	0.75	9.16	0.1	0.72	0.70	0.76	1661	<a href="#">logfile</a>
X	7PQE	13591	2021-09-17	3.7	0.006	0.68	12.28	0.0	0.81	0.82	—	—	—	—	—	—	—	1365	<a href="#">logfile</a>
✓	7VG2	31963	2021-09-14	3.1	0.016	1.43	17.56	0.2	0.72	0.79	0.004	0.67	14.49	0.0	1.24	0.72	0.79	845	<a href="#">logfile</a>
X	7PPo	13583	2021-09-14	2.91	—	—	—	—	—	—	—	—	—	—	—	—	—	1382	<a href="#">logfile</a>
✓	7VG3	31964	2021-09-14	3.73	0.006	1.15	7.51	0.6	0.80	0.84	0.004	0.74	9.83	0.0	1.09	0.79	0.84	860	<a href="#">logfile</a>
			2021-																

- Developers: helps track the impact of new methods and tools
- Users: lets to see how their models can benefit from improved methods and tools