Phenix tools for cryo-EM

Pavel Afonine

phenix-online.org   lbl.gov   qrefine.com

Missoula, MT

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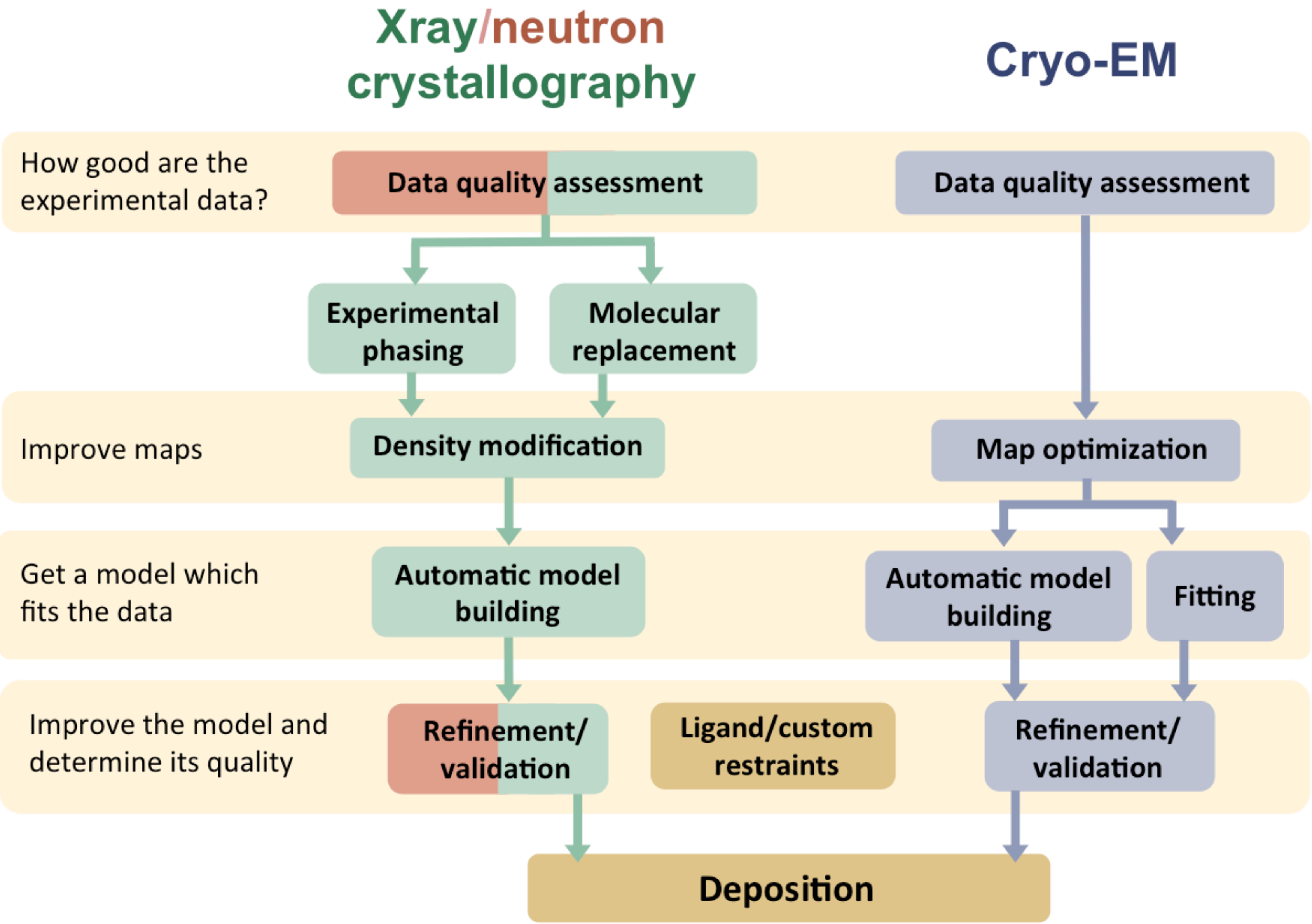
Phenix: tools for crystallography and cryo-EM



Xray/neutron crystallography

Cryo-EM

How good are the experimental data?

Data quality assessment

Data quality assessment

Experimental phasing

Molecular replacement

Improve maps

Density modification

Map optimization

Get a model which fits the data

Automatic model building

Automatic model building

Fitting

Improve the model and determine its quality

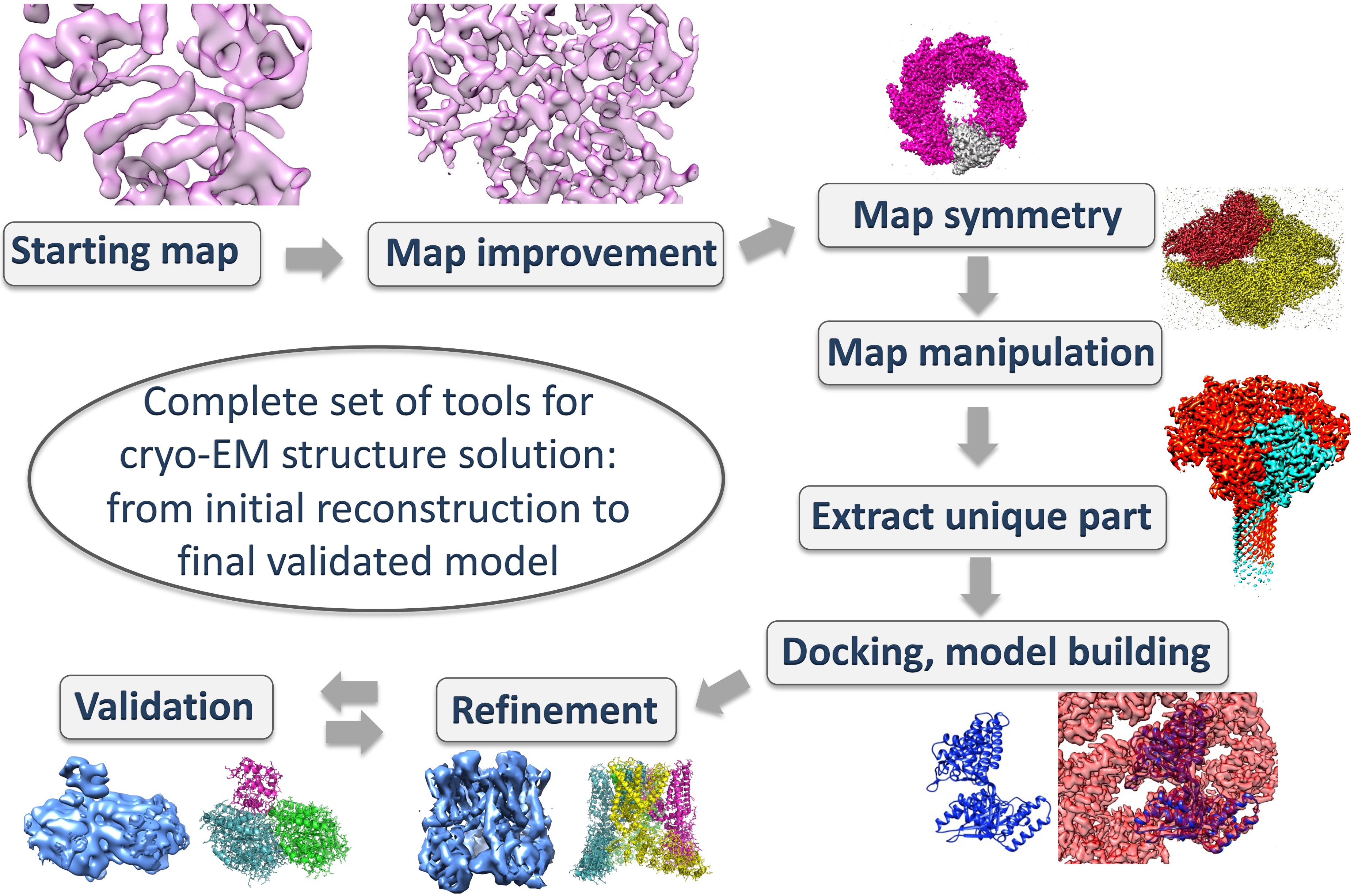
Refinement/ validation

Ligand/custom restraints

Refinement/ validation

Deposition

Phenix tools for cryo-EM



Starting map

Map improvement

Map symmetry

Map manipulation

Extract unique part

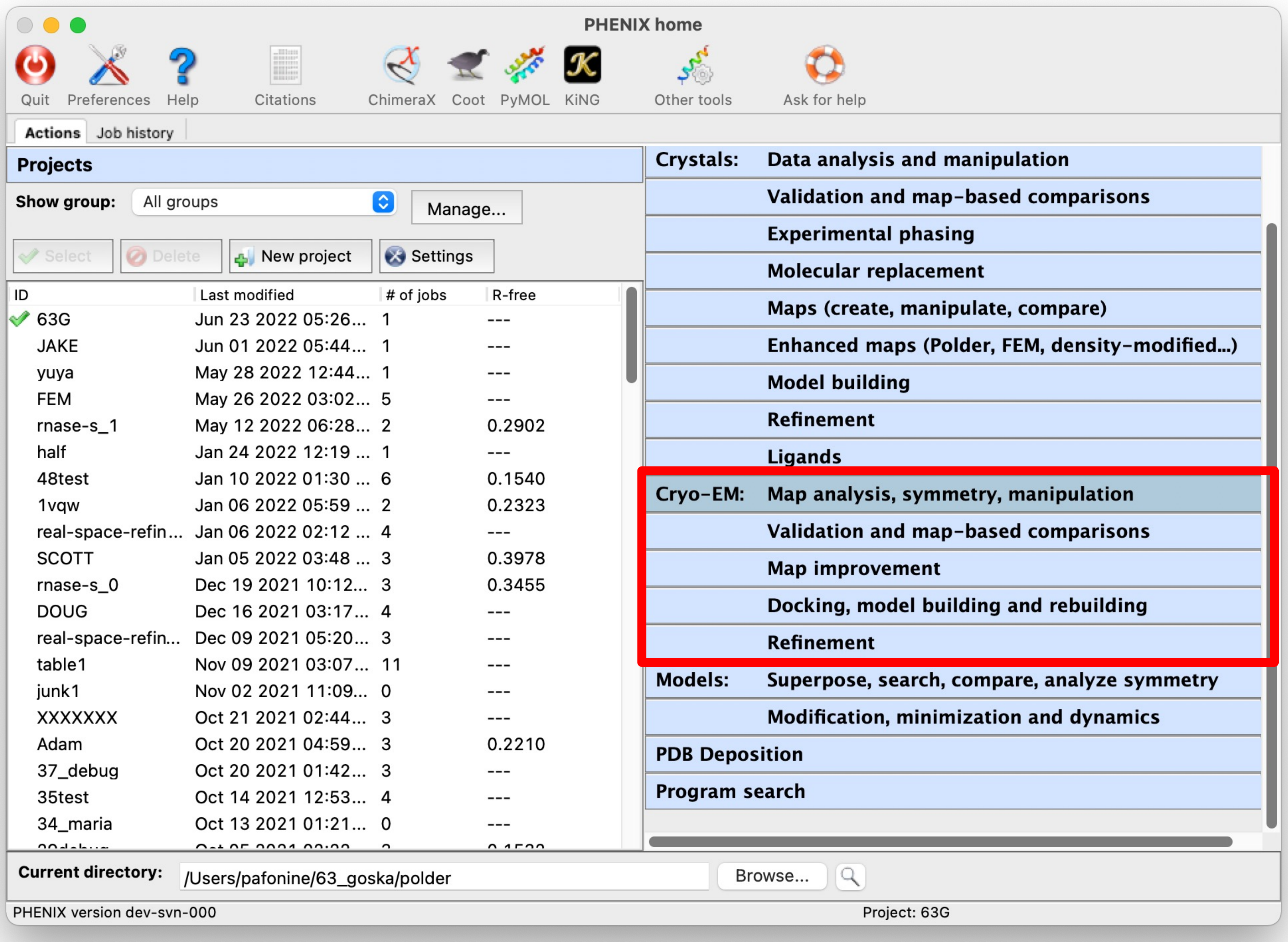
Docking, model building

Refinement

Validation

Complete set of tools for cryo-EM structure solution: from initial reconstruction to final validated model

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



Show group: All groups

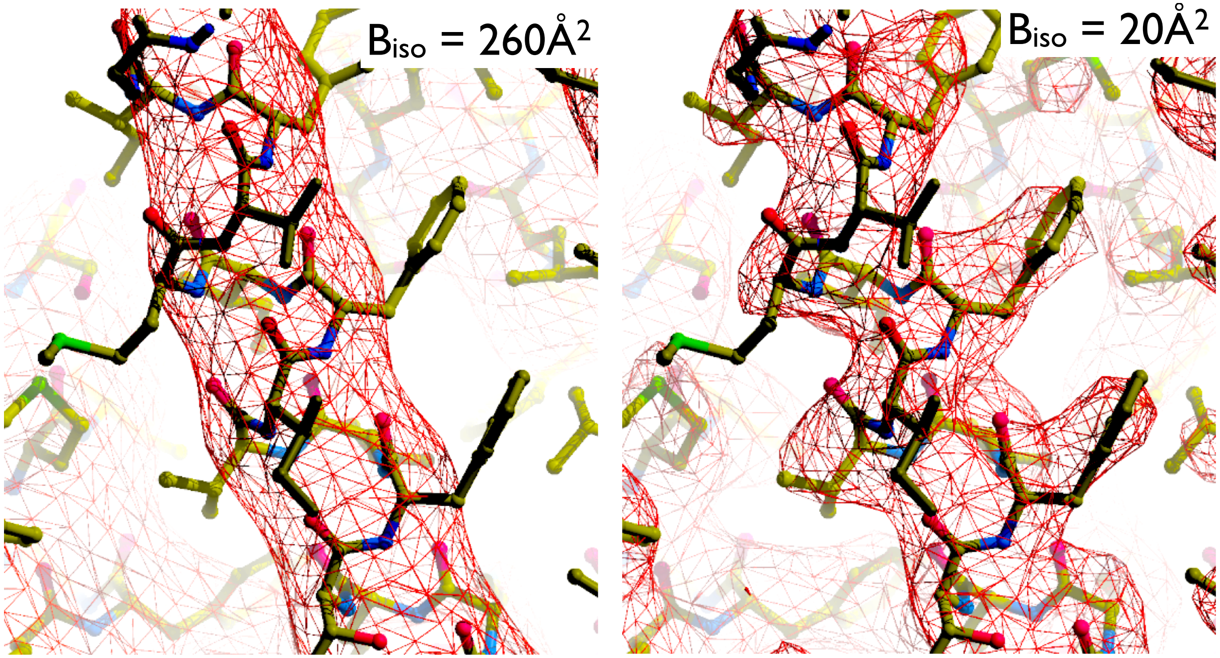
ID	Last modified	# of jobs	R-free
✓ 63G	Jun 23 2022 05:26...	1	---
JAKE	Jun 01 2022 05:44...	1	---
yuya	May 28 2022 12:44...	1	---
FEM	May 26 2022 03:02...	5	---
rnase-s_1	May 12 2022 06:28...	2	0.2902
half	Jan 24 2022 12:19 ...	1	---
48test	Jan 10 2022 01:30 ...	6	0.1540
1vqw	Jan 06 2022 05:59 ...	2	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	---

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

PHENIX version dev-svn-000

Project: 63G

Automated map sharpening: *phenix.auto_sharpen*

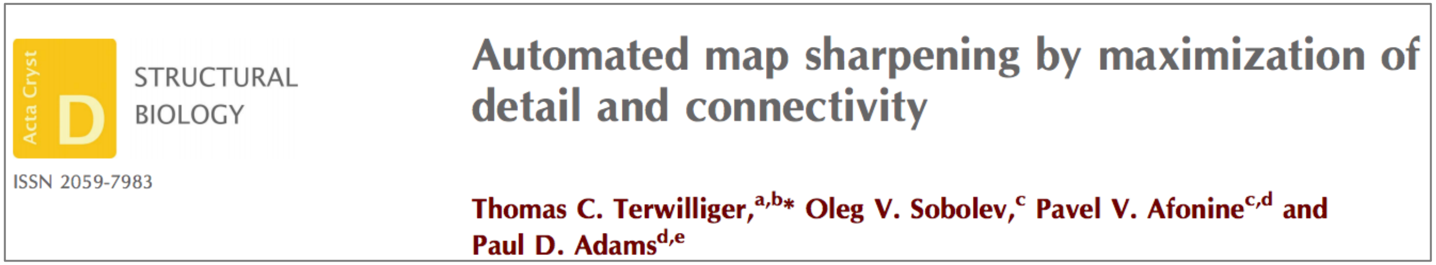


Deposited Map *Autosharpened Map*

EMDB: 8414, PDB: 5tji

Fully automatic:

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

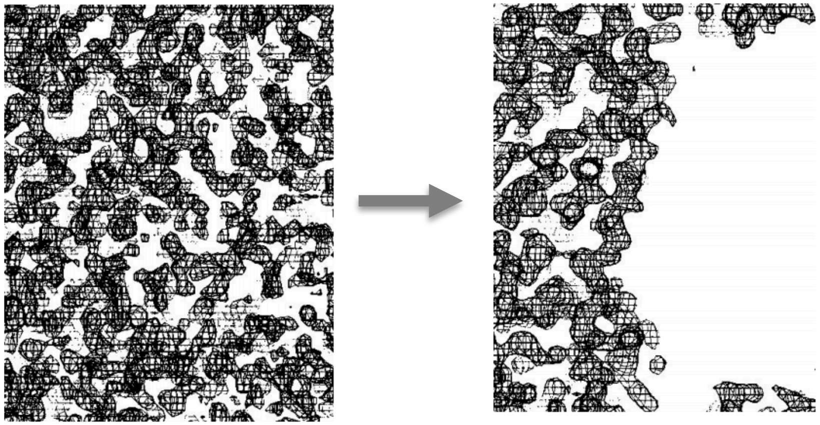
Density modification: *phenix.density_modify_cryo_em*

Similar principals for crystallography and cryo-EM:

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

Crystallography

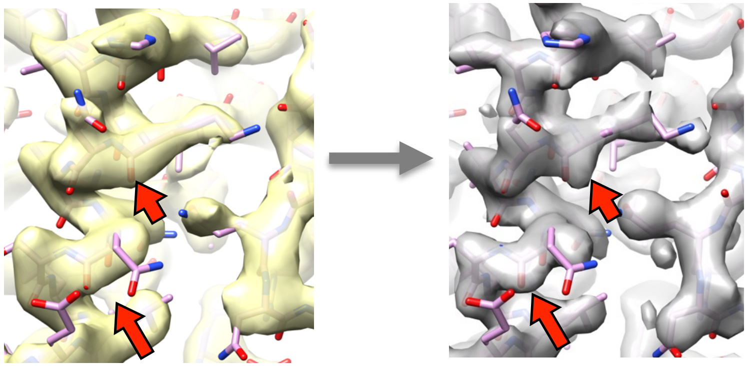
From uninterpretable to interpretable map

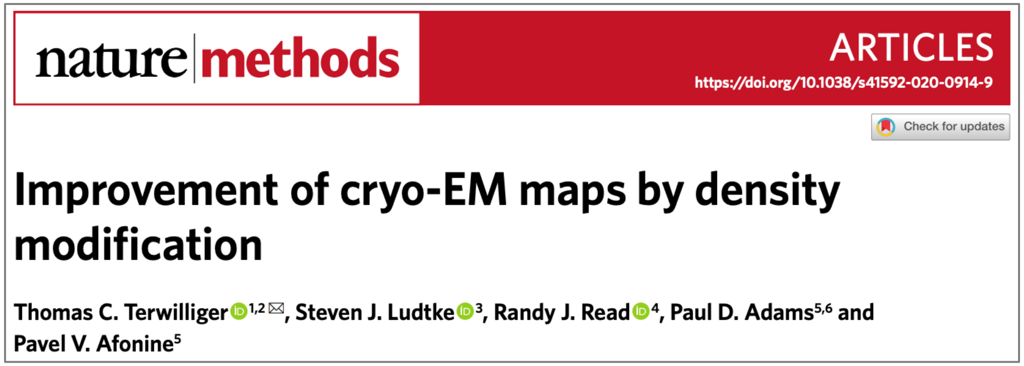


Cryo-EM

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

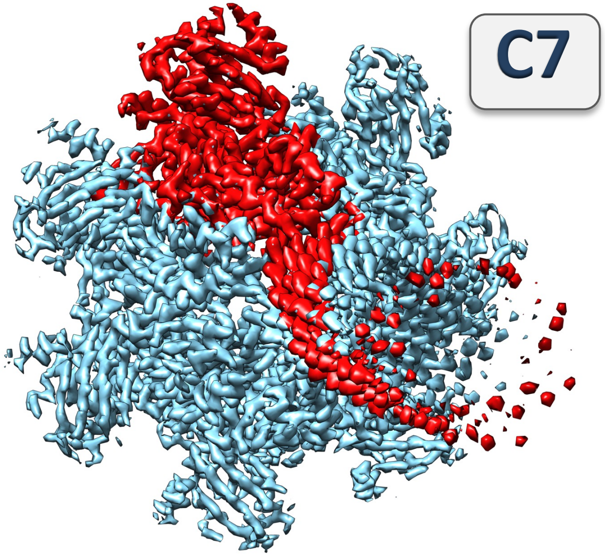
https://doi.org/10.1038/s41592-020-0914-9

Check for updates

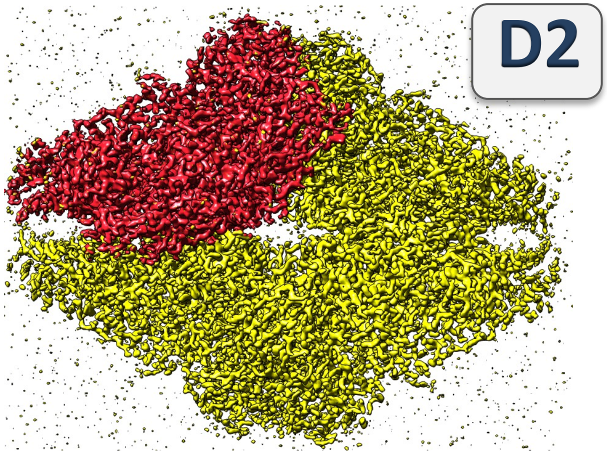
Improvement of cryo-EM maps by density modification

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

Finding map symmetry: *phenix.symmetry_from_map*



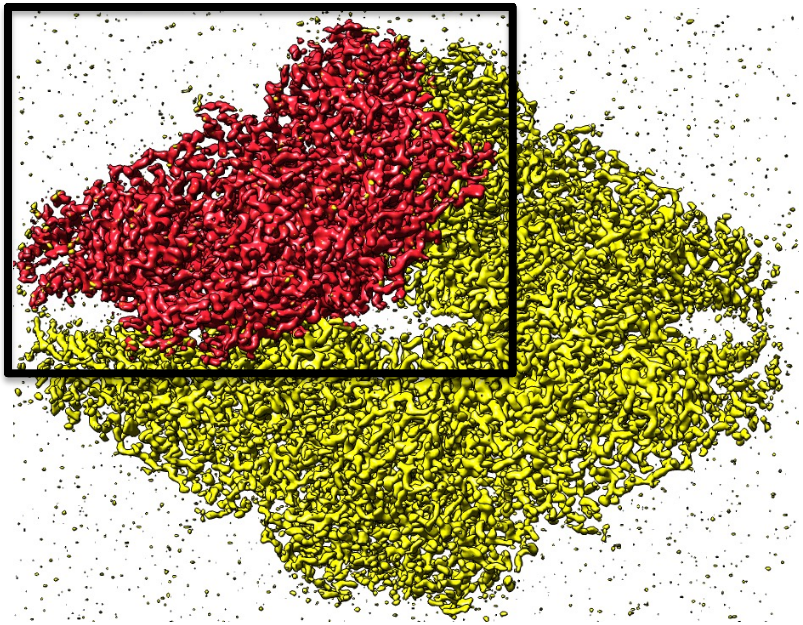
C7



D2

http://phenix-online.org/newsletter/
Tools for interpreting cryo-EM maps using models from the PDB

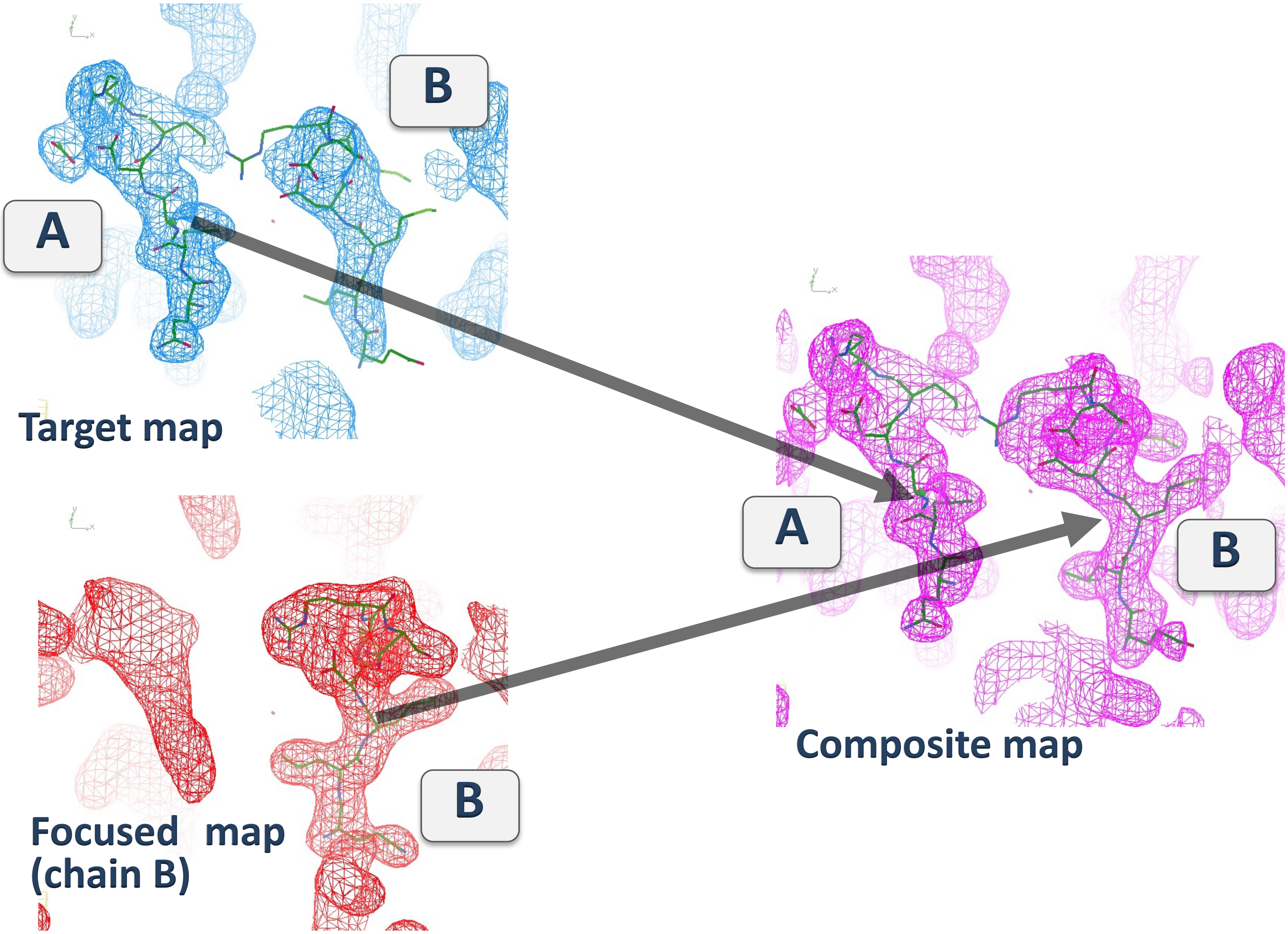
Extracting unique part of map using *phenix.map_box*



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*

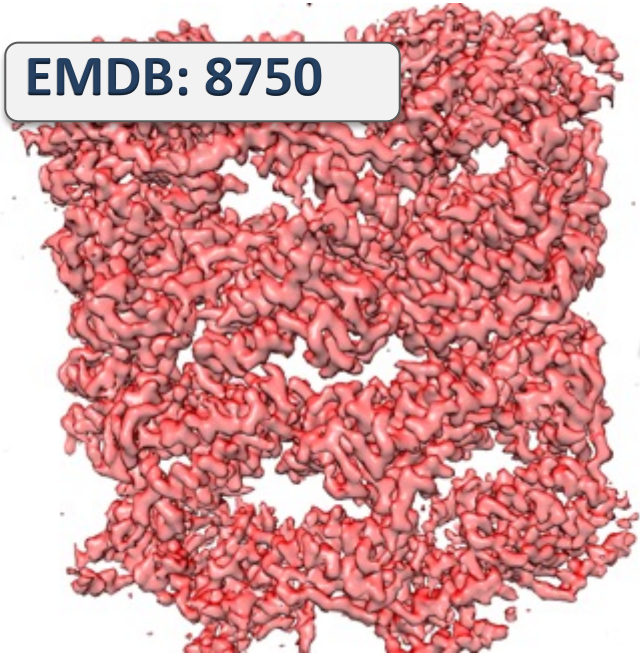


Target map

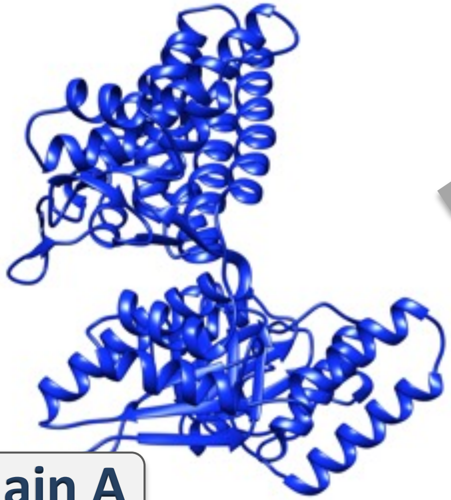
Focused map
(chain B)

Composite map

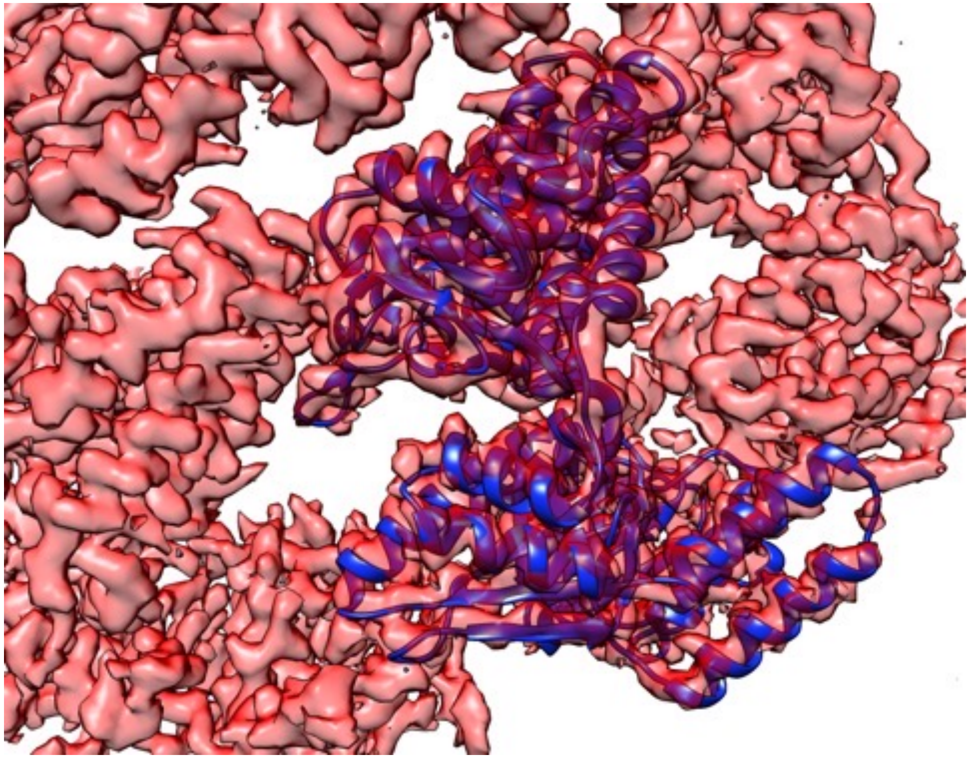
Docking models with *phenix.dock_in_map*



EMDB: 8750

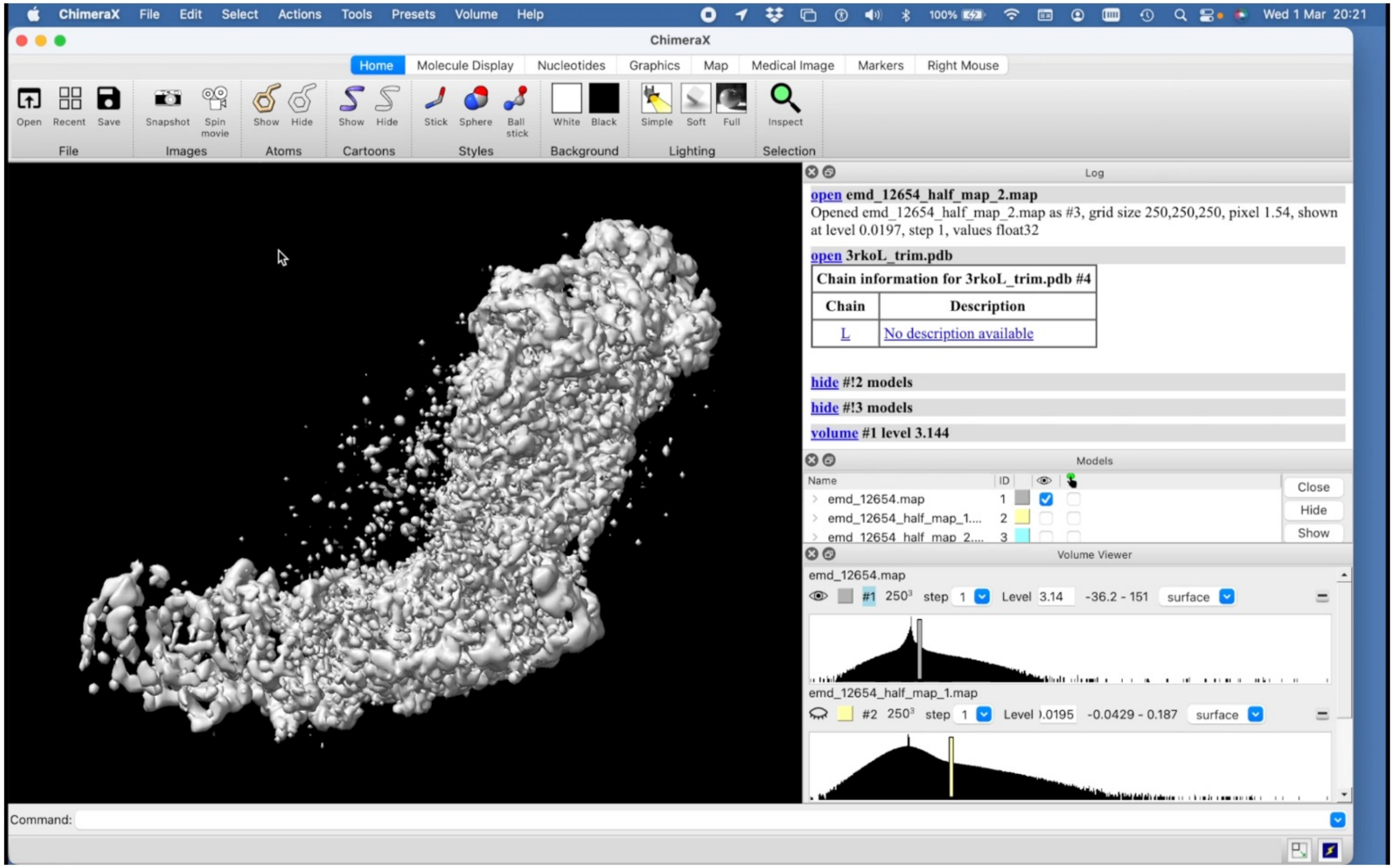


1ss8 chain A



Chain A docked in map

Integration of docking with ChimeraX



```
open emd_12654_half_map_2.map
Opened emd_12654_half_map_2.map as #3, grid size 250,250,250, pixel 1.54, shown at level 0.0197, step 1, values float32
open 3rkoL_trim.pdb
```

Chain information for 3rkoL_trim.pdb #4	
Chain	Description
L	No description available

```
hide #!2 models
hide #!3 models
volume #1 level 3.144
```

Name	ID
emd_12654.map	1
emd_12654_half_map_1....	2
emd_12654_half_map_2....	3

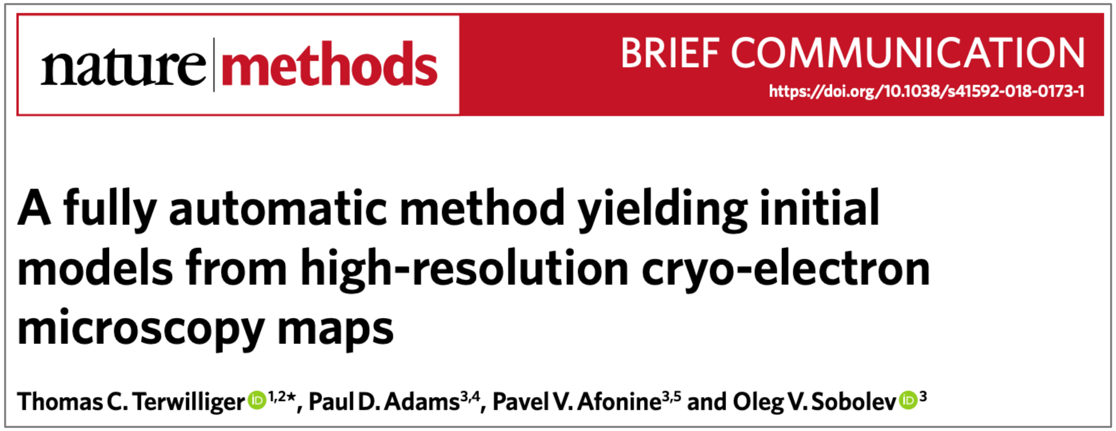
emd_12654.map
#1 250³ step 1 Level 3.14 -36.2 - 151 surface

emd_12654_half_map_1.map
#2 250³ step 1 Level).0195 -0.0429 - 0.187 surface

- 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

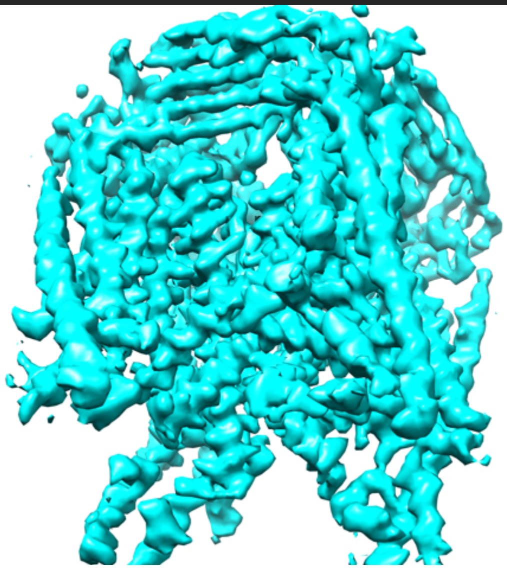
https://doi.org/10.1038/s41592-018-0173-1

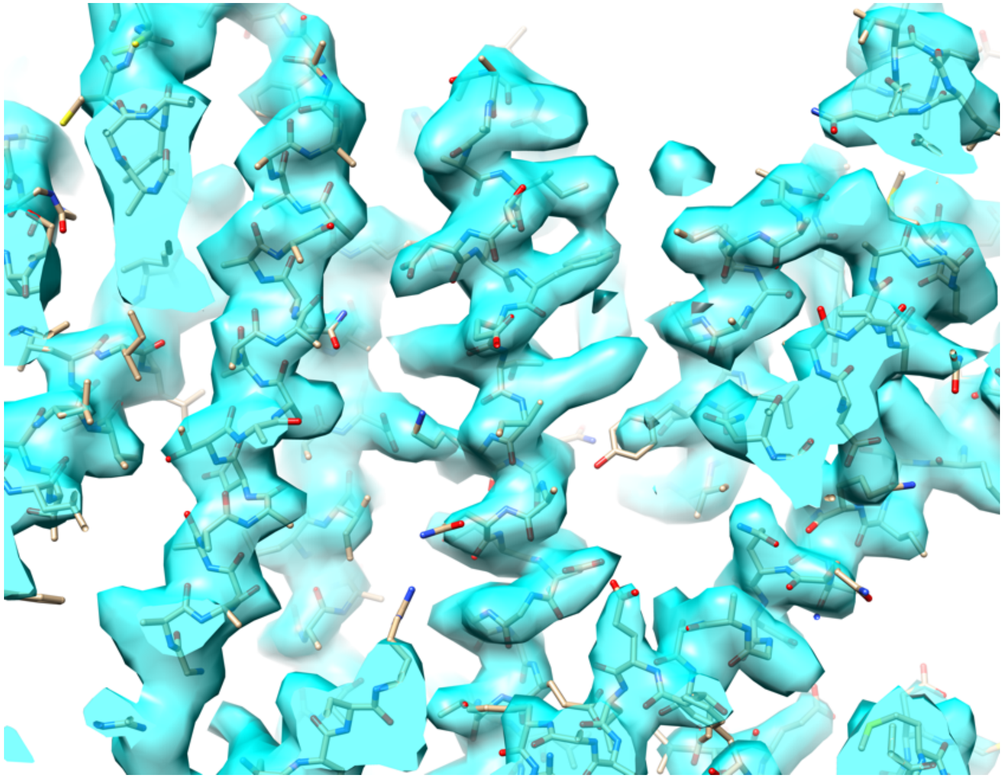
A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps

Thomas C. Terwilliger[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)

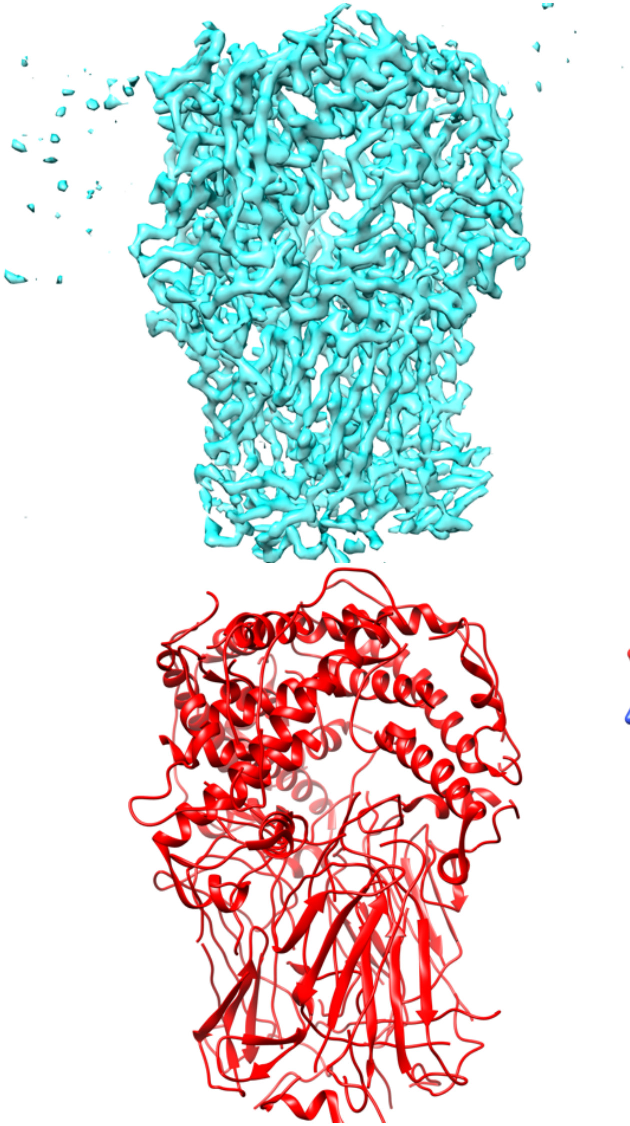


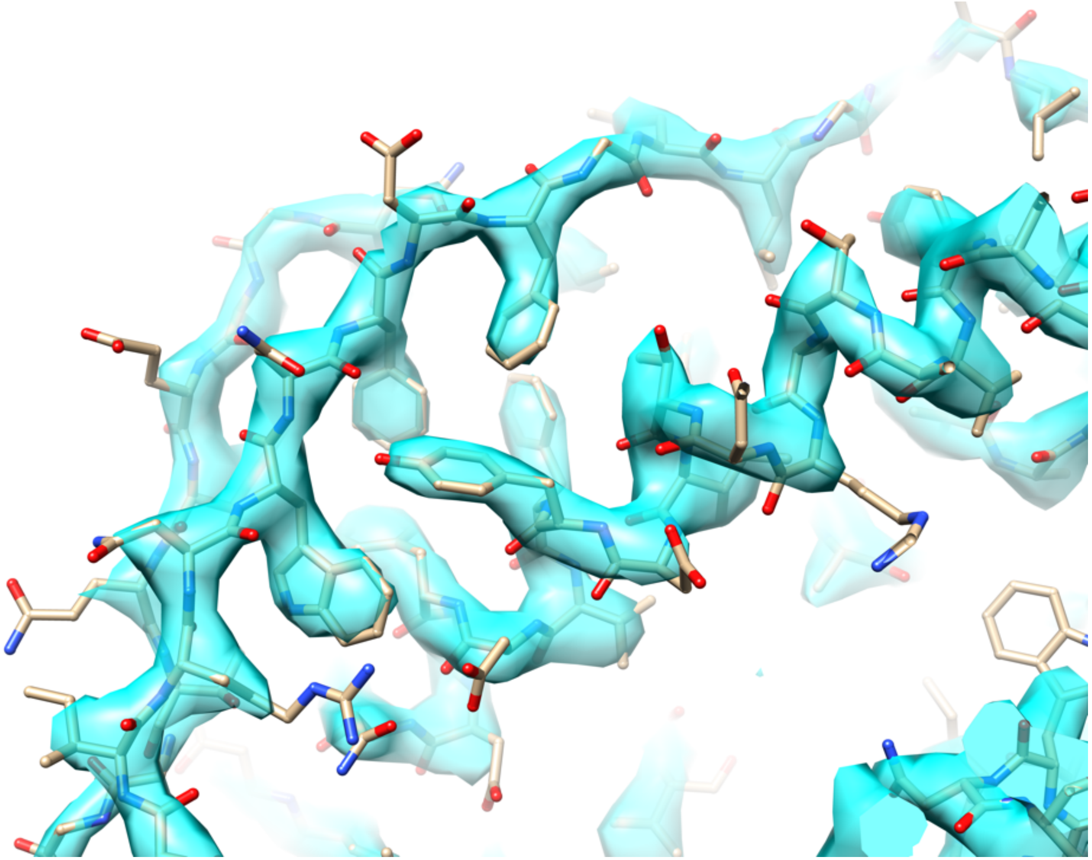


Data from Zhou, X. et al. (2017) Nat. Struct. Mol. Biol. 24: 1146

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

Automated model building: *phenix.map_to_model*

Automated model building, facts:

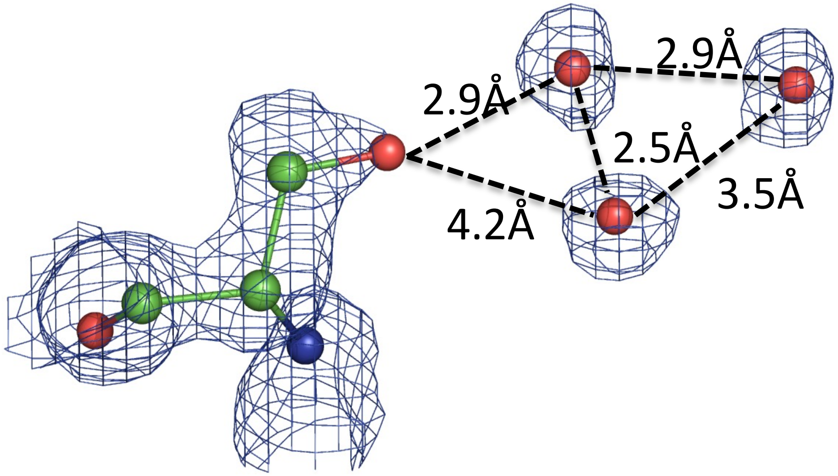
- No automated model building produces 100% complete and accurate model
- Produces initial model for further manual building
- The lower the resolution, the less complete and accurate the auto built model

Manual model building steps

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

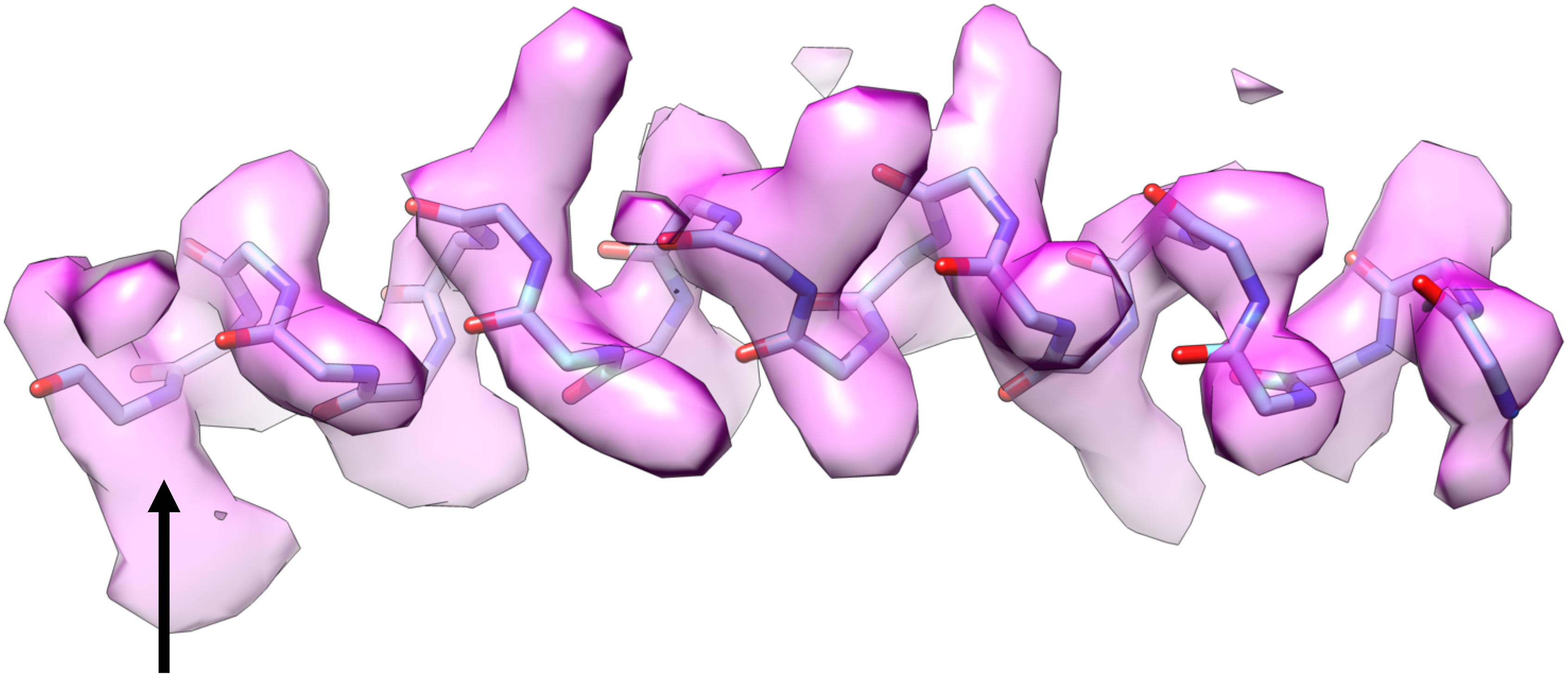
- Auto-sharpen the map
- Run Map Symmetry to obtain symmetry
- Run Map Box to obtain asymmetric unit (using symmetry)
- Run Map to Model on asymmetric unit
- Run Apply NCS Operators on model, with the trim overlap option (supplying the full map)

Automated water building: *phenix.douse*



Available in ChimeraX!

Sequence from map: *phenix.sequence_from_map*

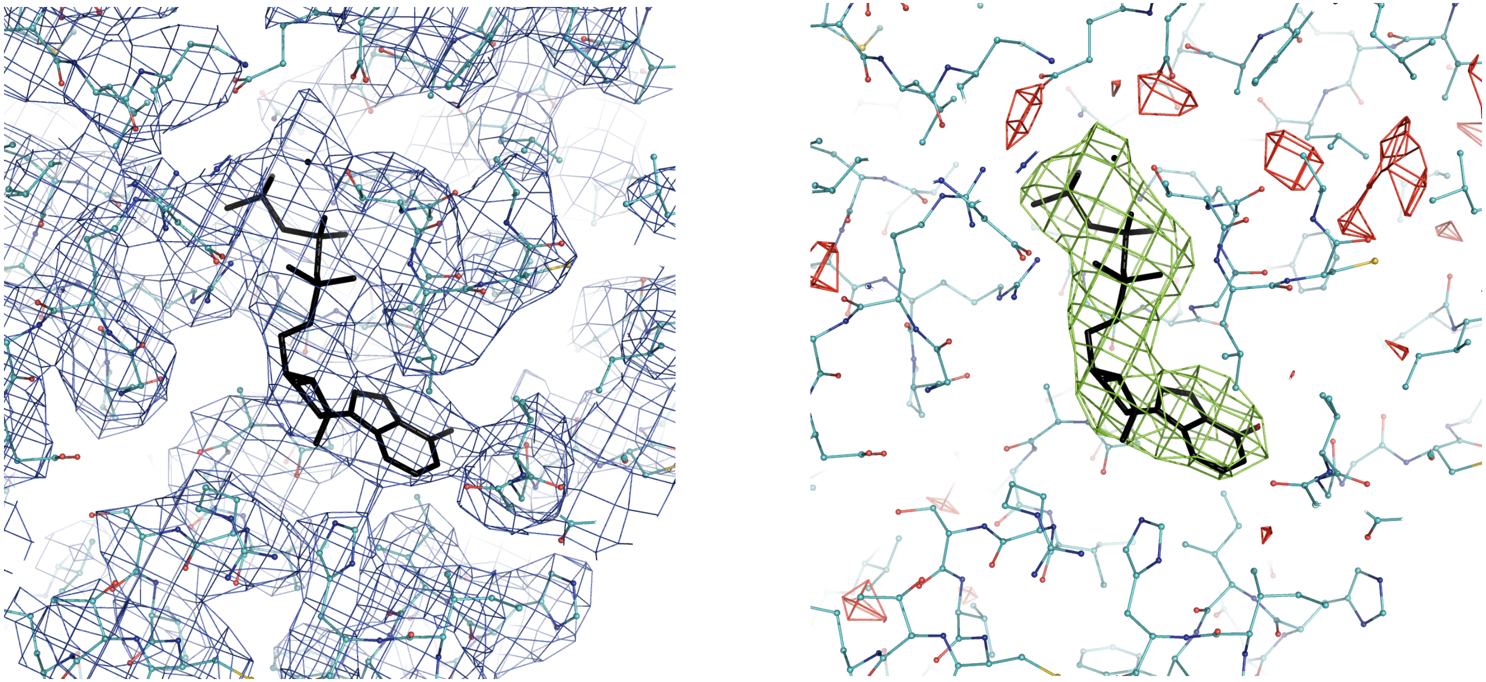


Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

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

Difference maps: ***phenix.real_space_diff_map***

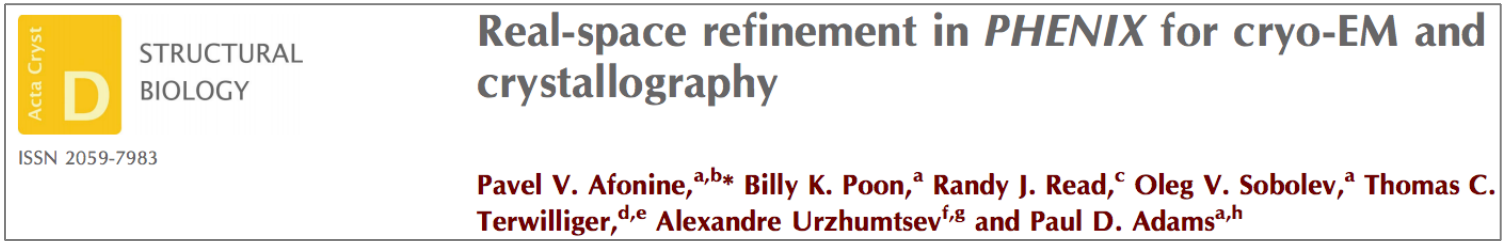
5L4g, EMDB 4002



- 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



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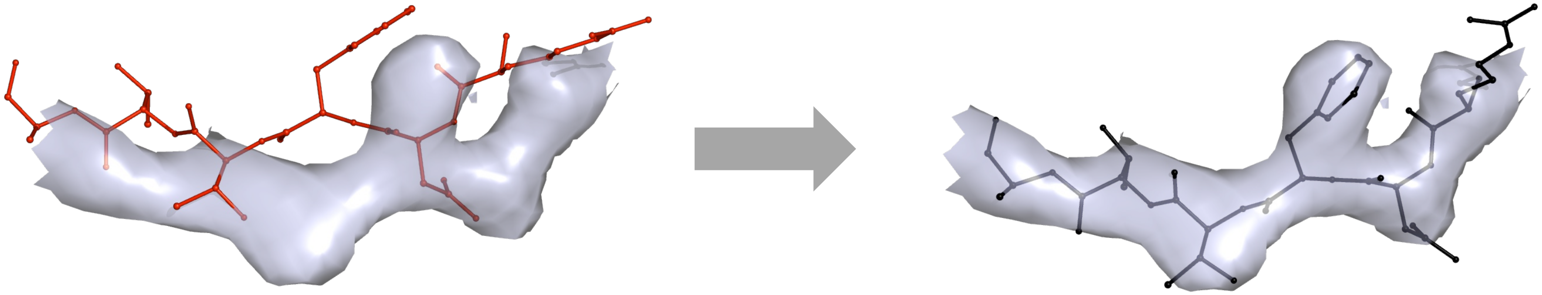
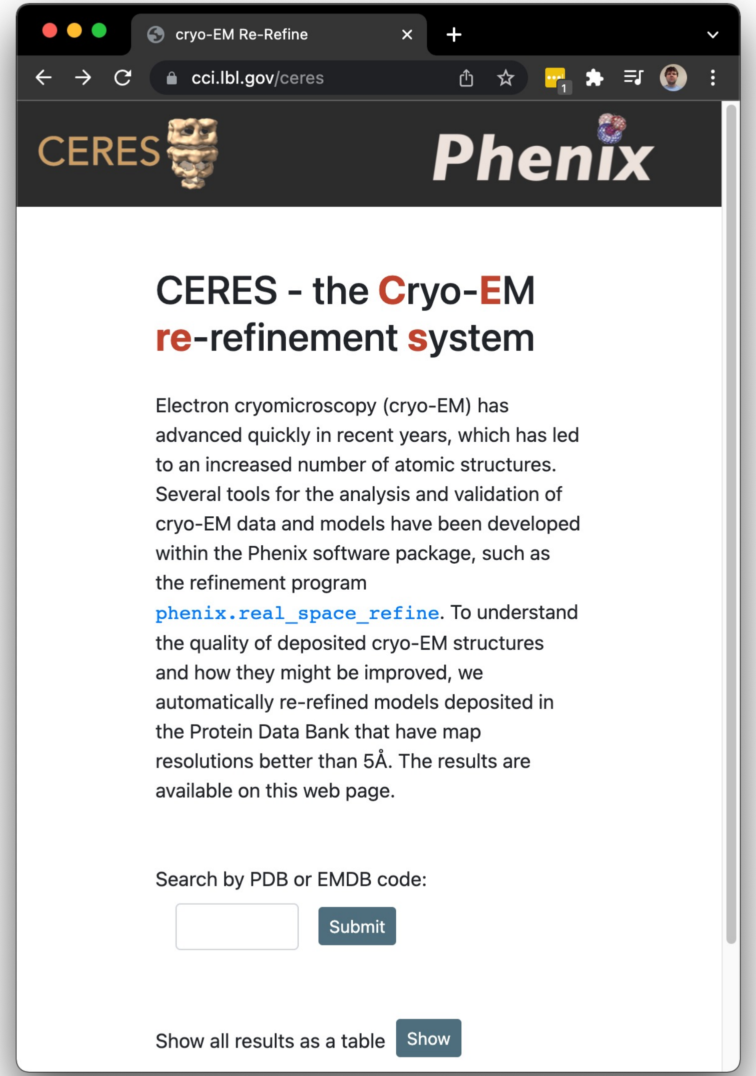
Real-space refinement in *PHENIX* for cryo-EM and crystallography

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



Automated re-refinement of deposited cryo-EM models



CERES - the Cryo-EM re-refinement system

Electron cryomicroscopy (cryo-EM) has advanced quickly in recent years, which has led to an increased number of atomic structures. Several tools for the analysis and validation of cryo-EM data and models have been developed within the Phenix software package, such as the refinement program `phenix.real_space_refine`. To understand the quality of deposited cryo-EM structures and how they might be improved, we automatically re-refined models deposited in the Protein Data Bank that have map resolutions better than 5Å. The results are available on this web page.

Search by PDB or EMDB code:

Show all results as a table

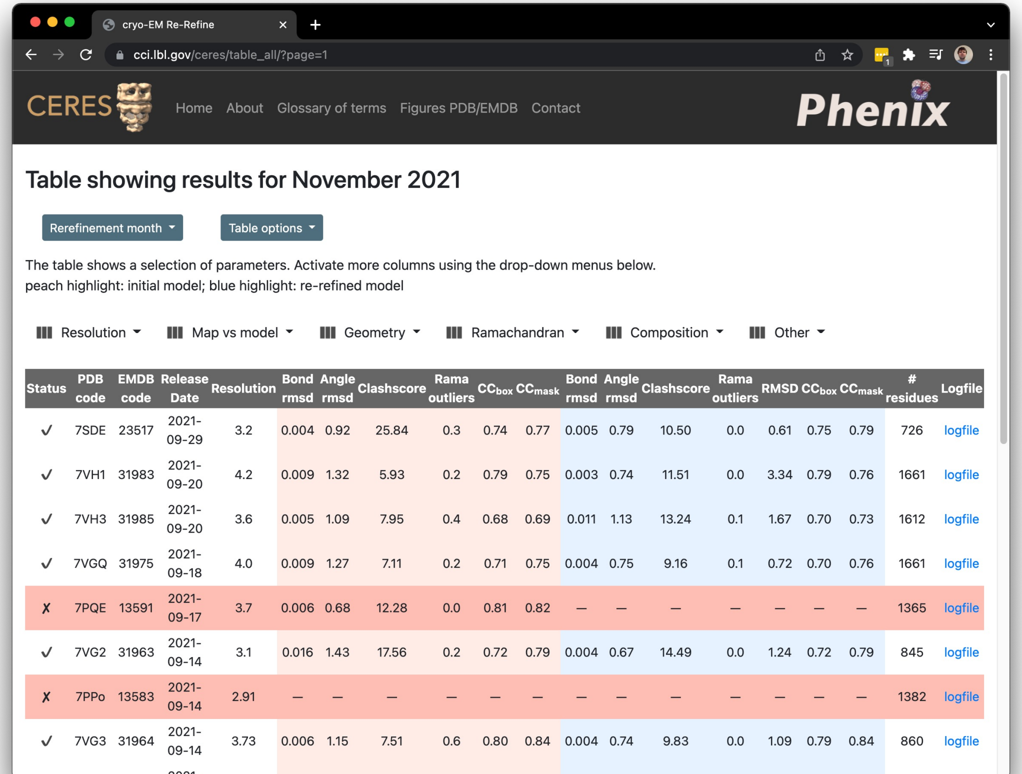


Table showing results for November 2021

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

Status	PDB code	EMDB code	Release Date	Resolution	Bond rmsd	Angle rmsd	Clashscore	Rama outliers	CC_{box}	CC_{mask}	Bond rmsd	Angle rmsd	Clashscore	Rama outliers	RMSD	CC_{box}	CC_{mask}	# 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	logfile
✓	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	logfile
✓	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	logfile
✓	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	logfile
✗	7PQE	13591	2021-09-17	3.7	0.006	0.68	12.28	0.0	0.81	0.82	—	—	—	—	—	—	—	1365	logfile
✓	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	logfile
✗	7PPo	13583	2021-09-14	2.91	—	—	—	—	—	—	—	—	—	—	—	—	—	1382	logfile
✓	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	logfile
			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