Phenix tools for cryo-EM

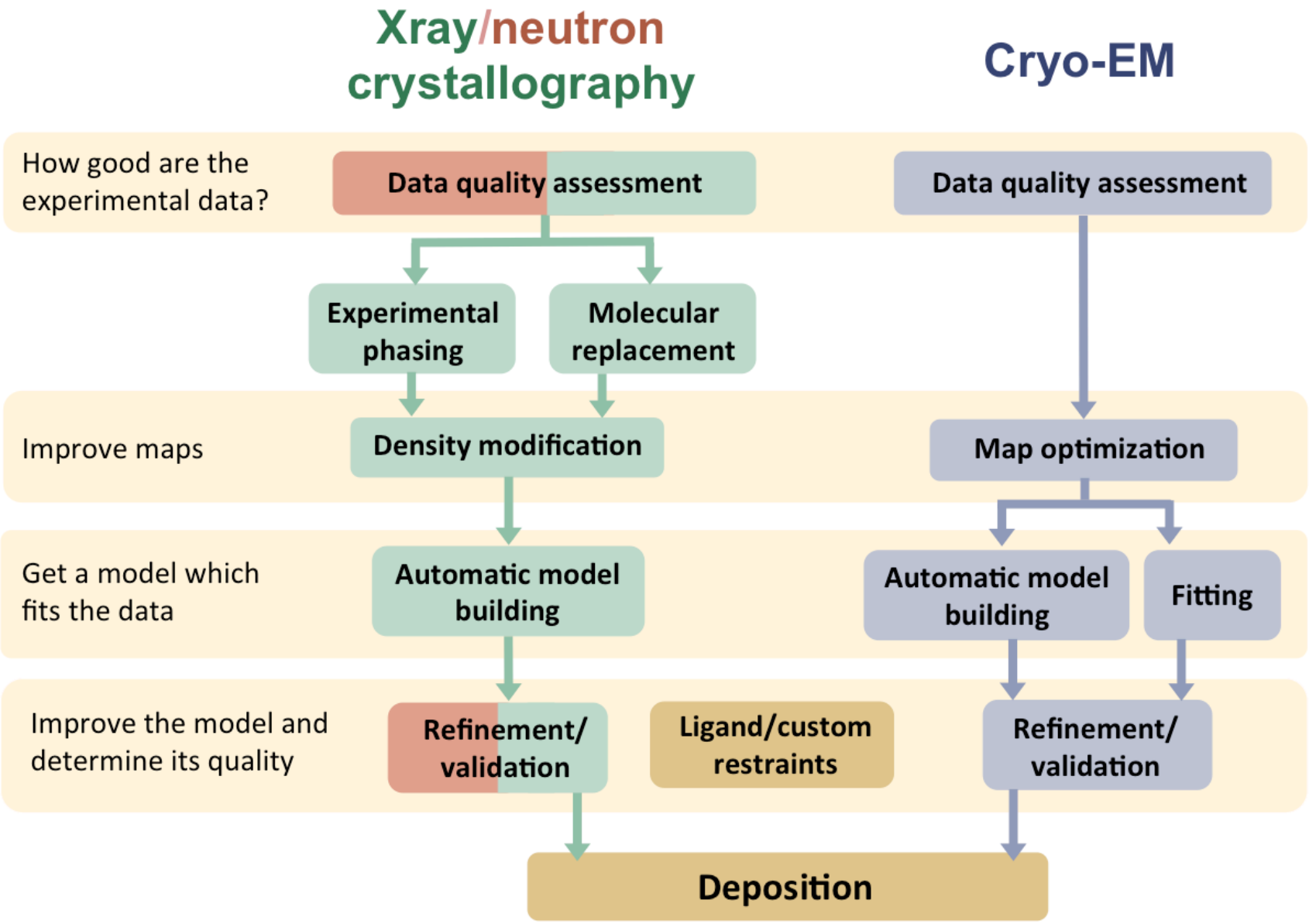
Pavel Afonine

`phenix-online.org` `lbl.gov` `qrefine.com`

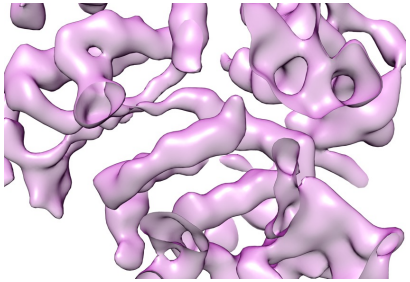
Hosted by the Oklahoma COBRE in Structural Biology

March 19th 2024

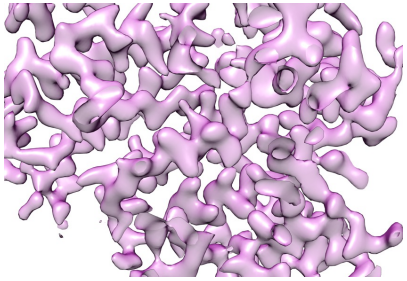
Phenix: tools for crystallography and cryo-EM



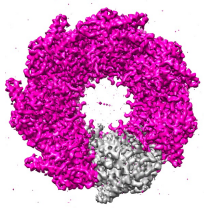
Phenix tools for cryo-EM



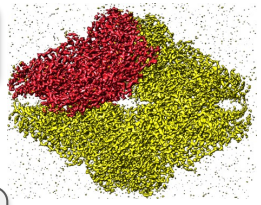
Starting map



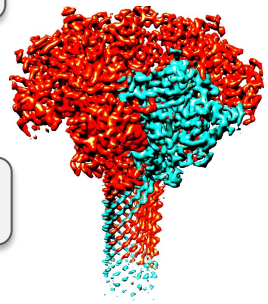
Map improvement



Map symmetry

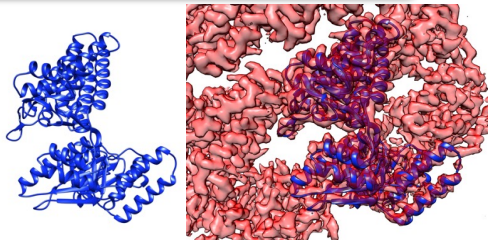


Map manipulation

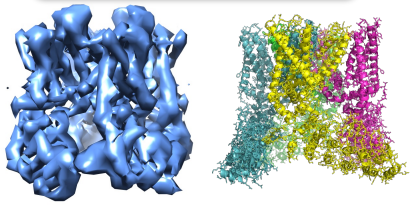


Extract unique part

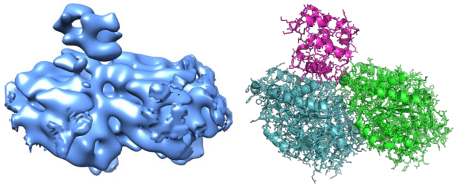
Docking, model building



Refinement

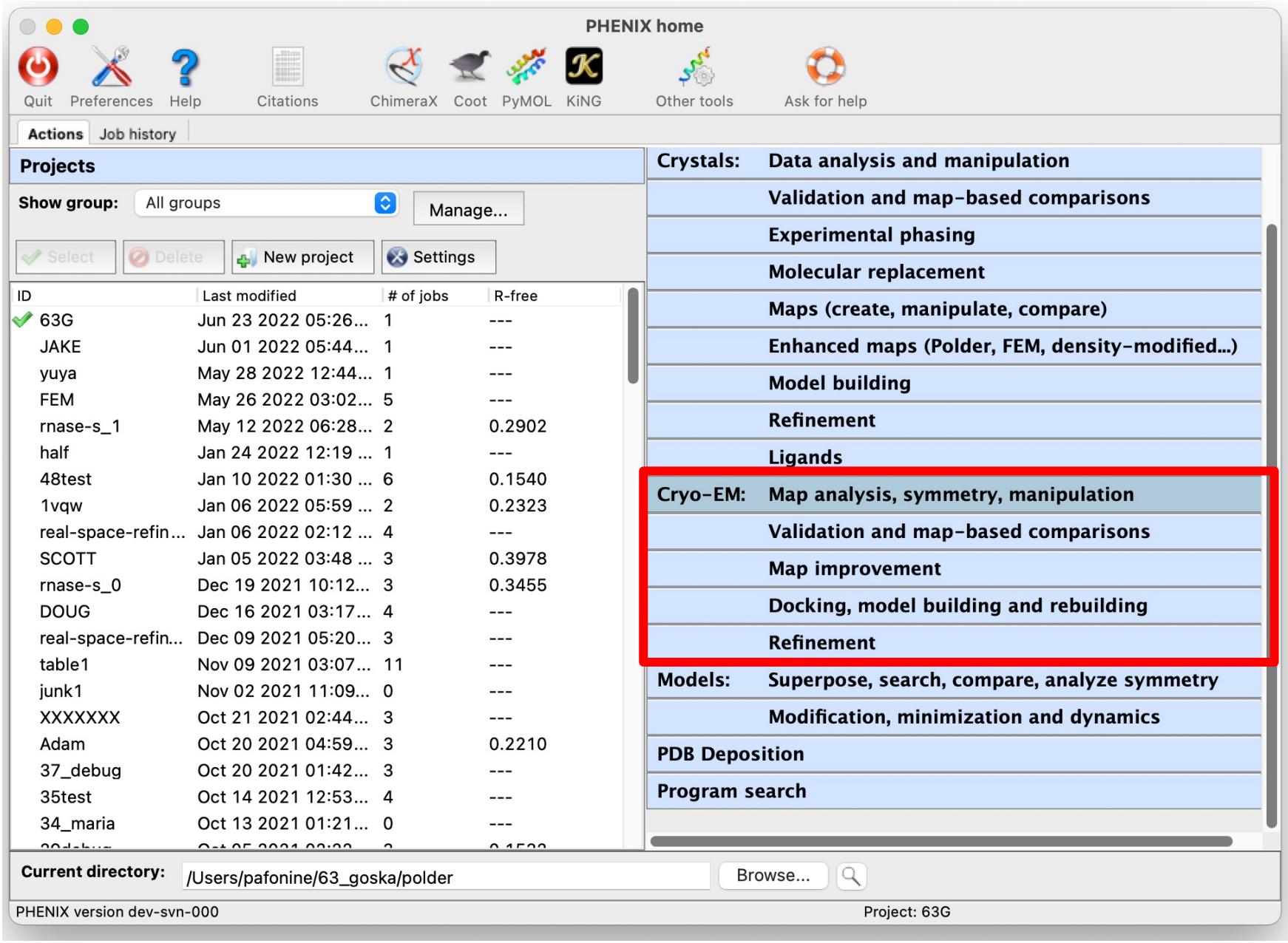


Validation

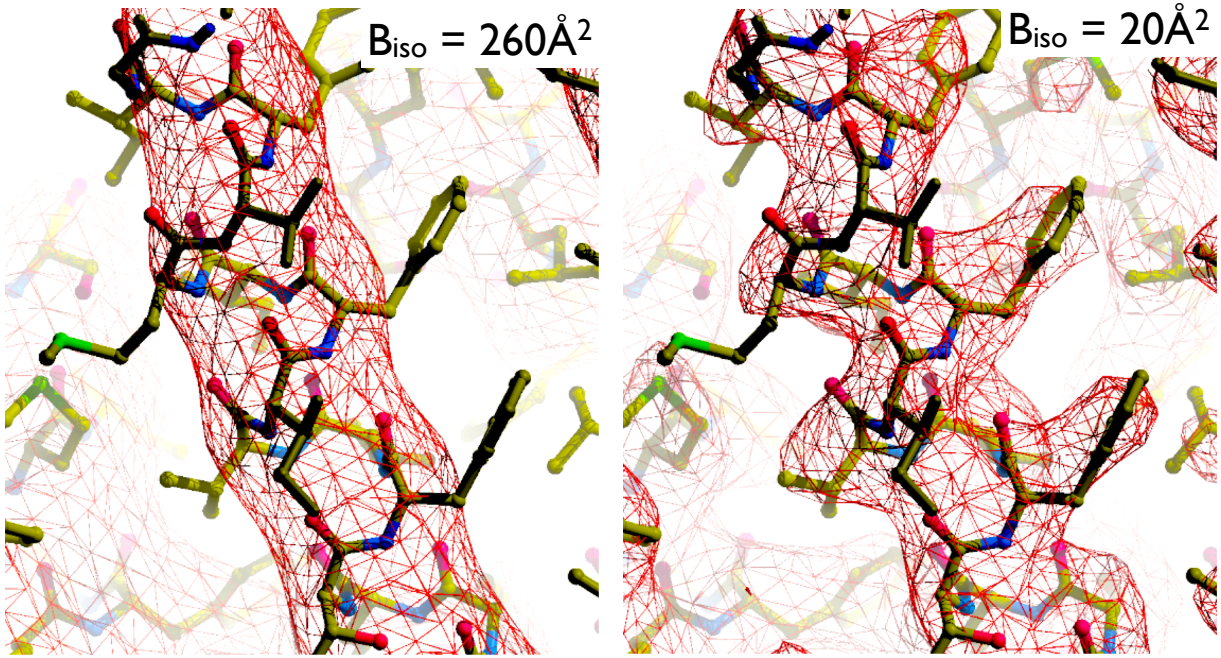


Complete set of tools for cryo-EM structure solution: from initial reconstruction to final validated model

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



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]

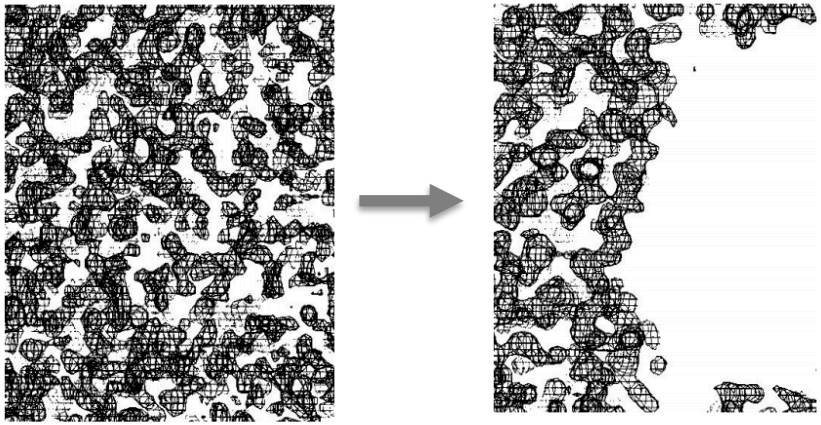
Density modification: *phenix.density_modify_cryo_em*

Similar principals for crystallography and cryo-EM:

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

Crystallography

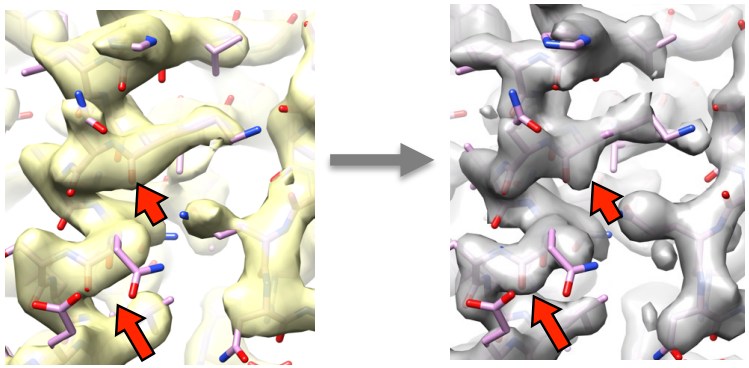
From uninterpretable to interpretable map



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

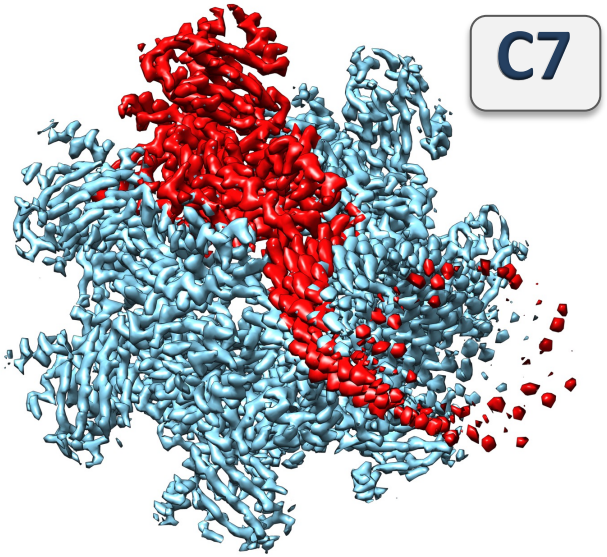
https://doi.org/10.1038/s41592-020-0914-9

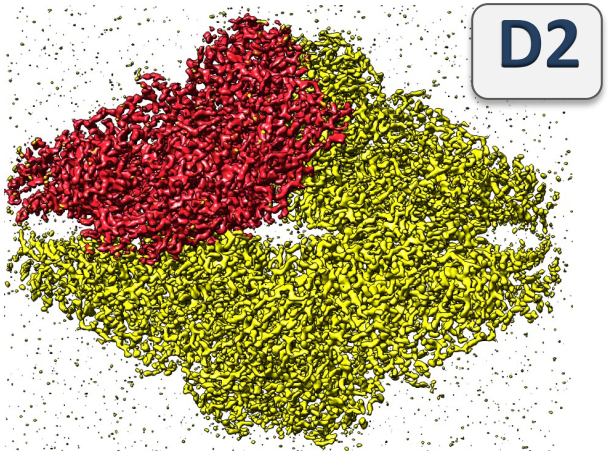
Check for updates

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*

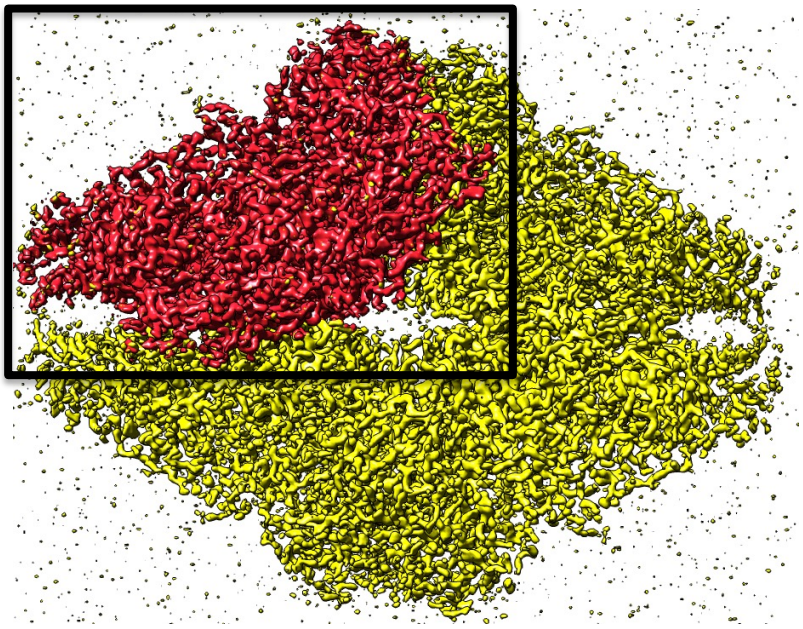




http://phenix-online.org/newsletter/

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

Extracting unique part of map using *phenix.map_box*

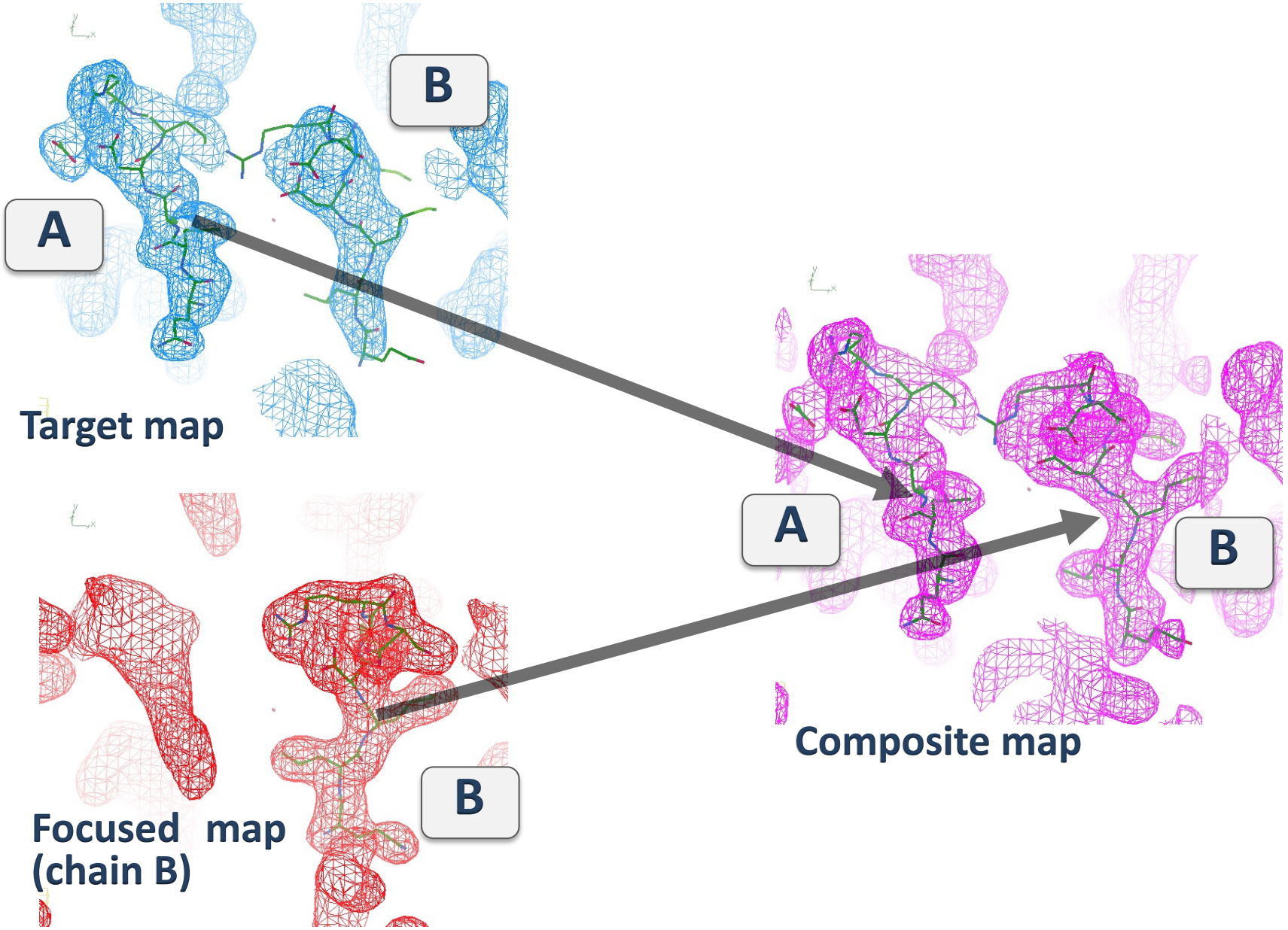


Lots of options: use map only, use model, use symmetry, mask boxed map, and many more!

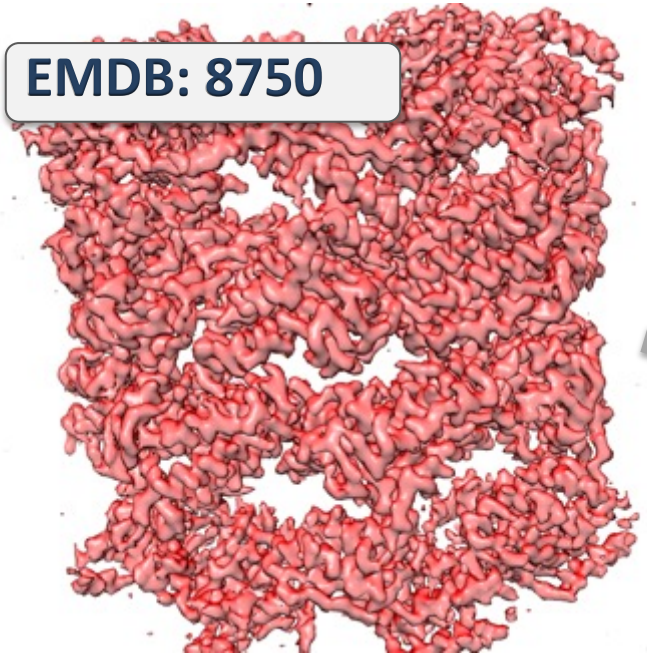
http://phenix-online.org/newsletter/

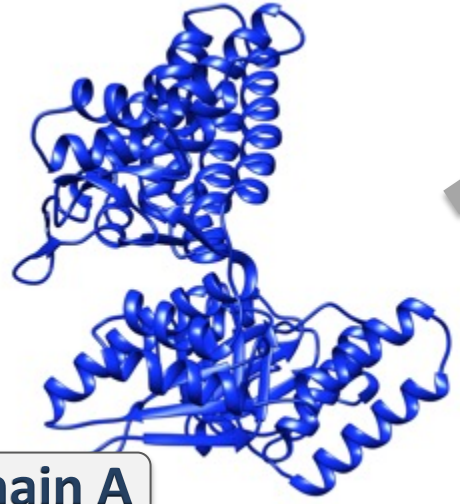
Tools for interpreting cryo-EM maps using models from the PDB

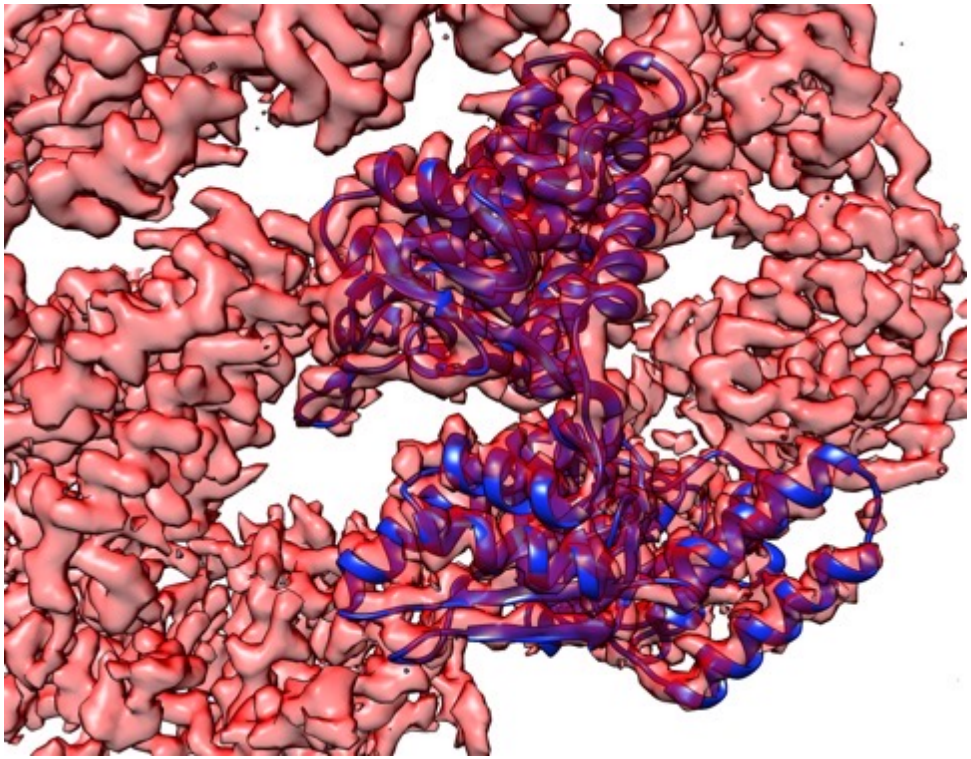
Combining maps with *phenix.combine_focused_maps*



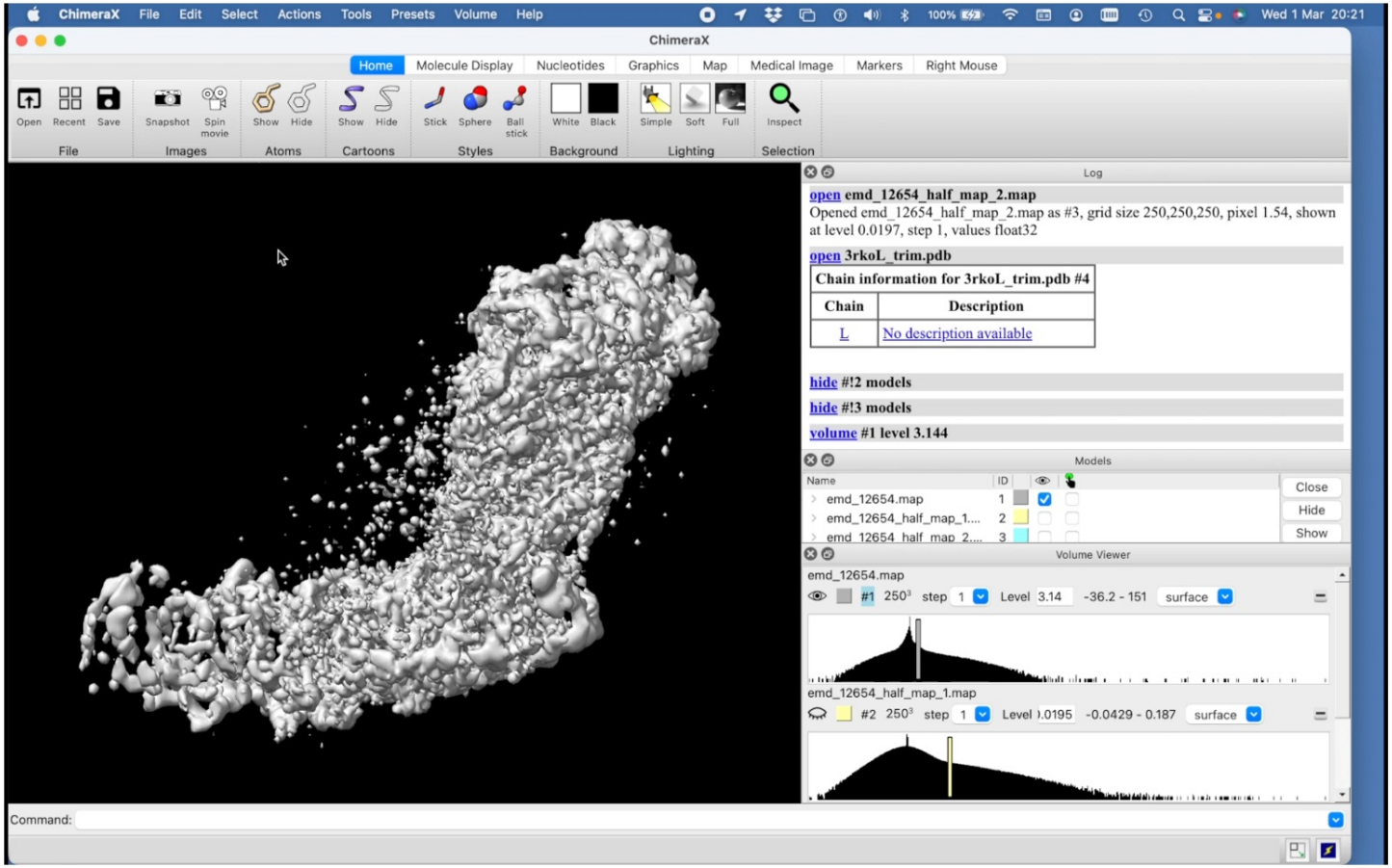
Docking models with *phenix.dock_in_map*







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

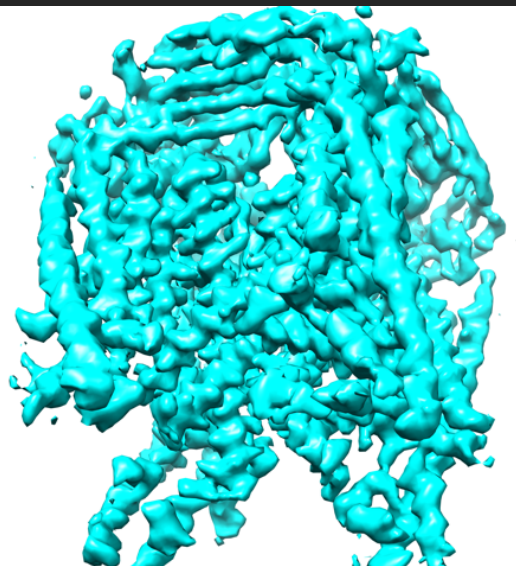
https://doi.org/10.1038/s41592-018-0173-1

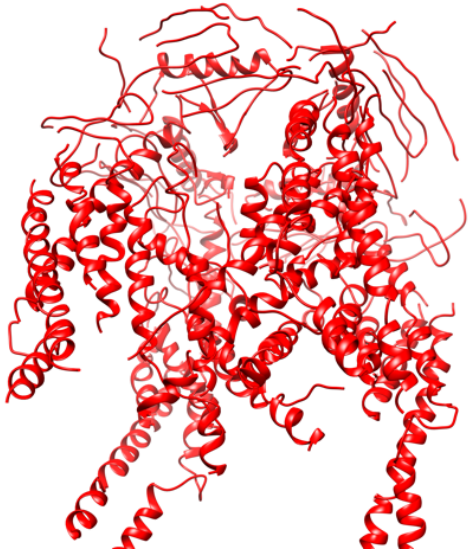
A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps

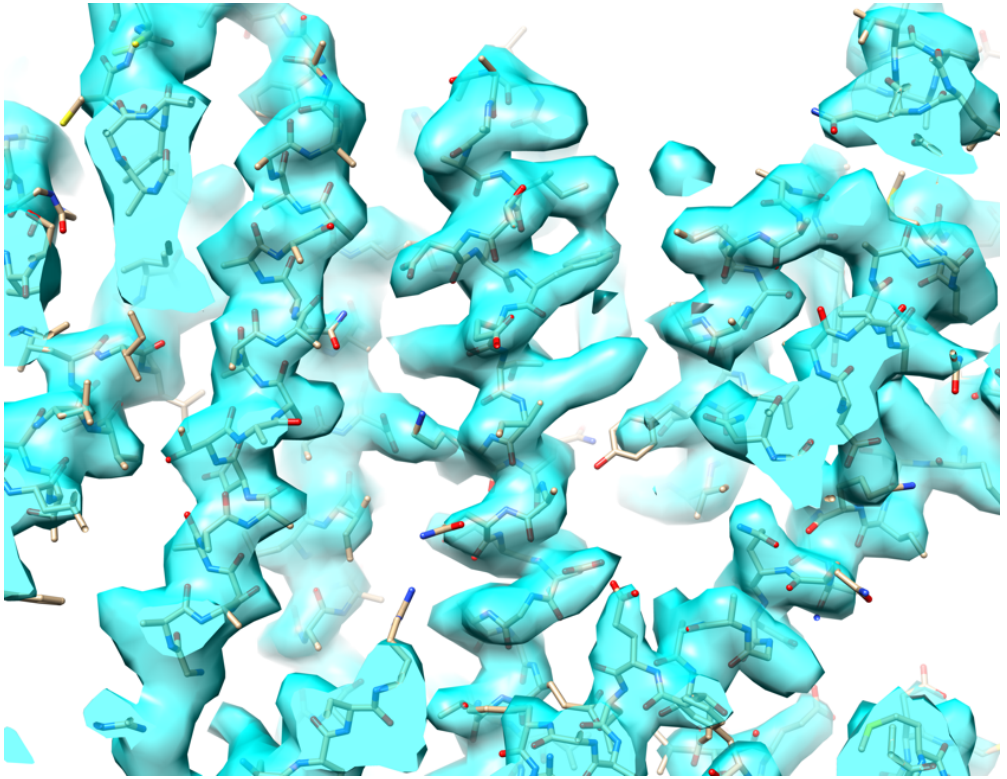
Thomas C. Terwilliger[1,2*], Paul D. Adams[3,4], Pavel V. Afonine[3,5] and Oleg V. Sobolev[3]

Automated model building: *phenix.map_to_model*

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



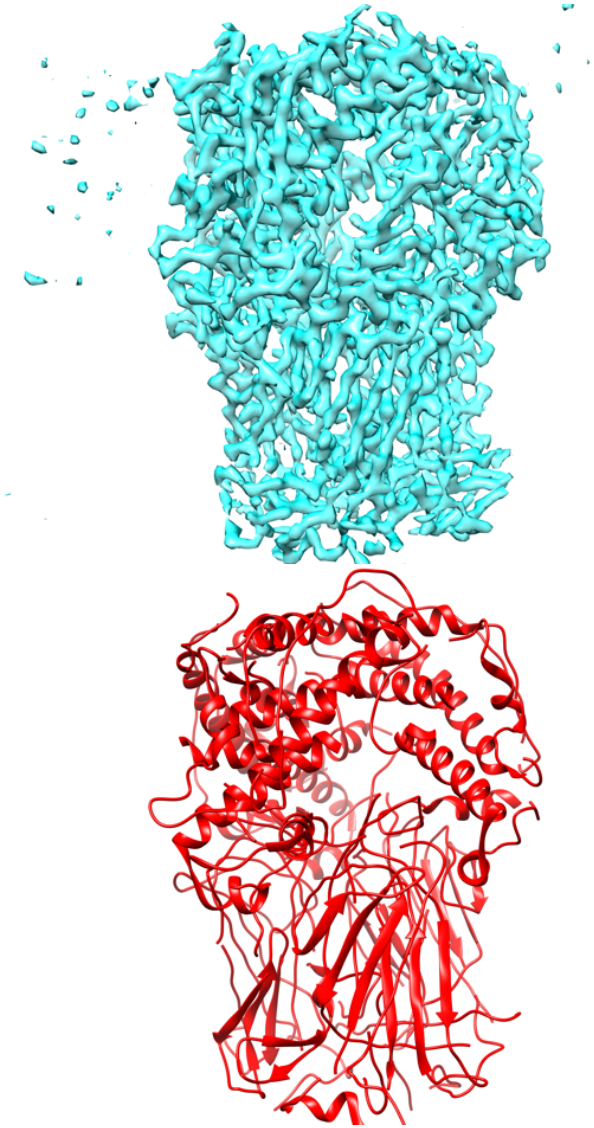


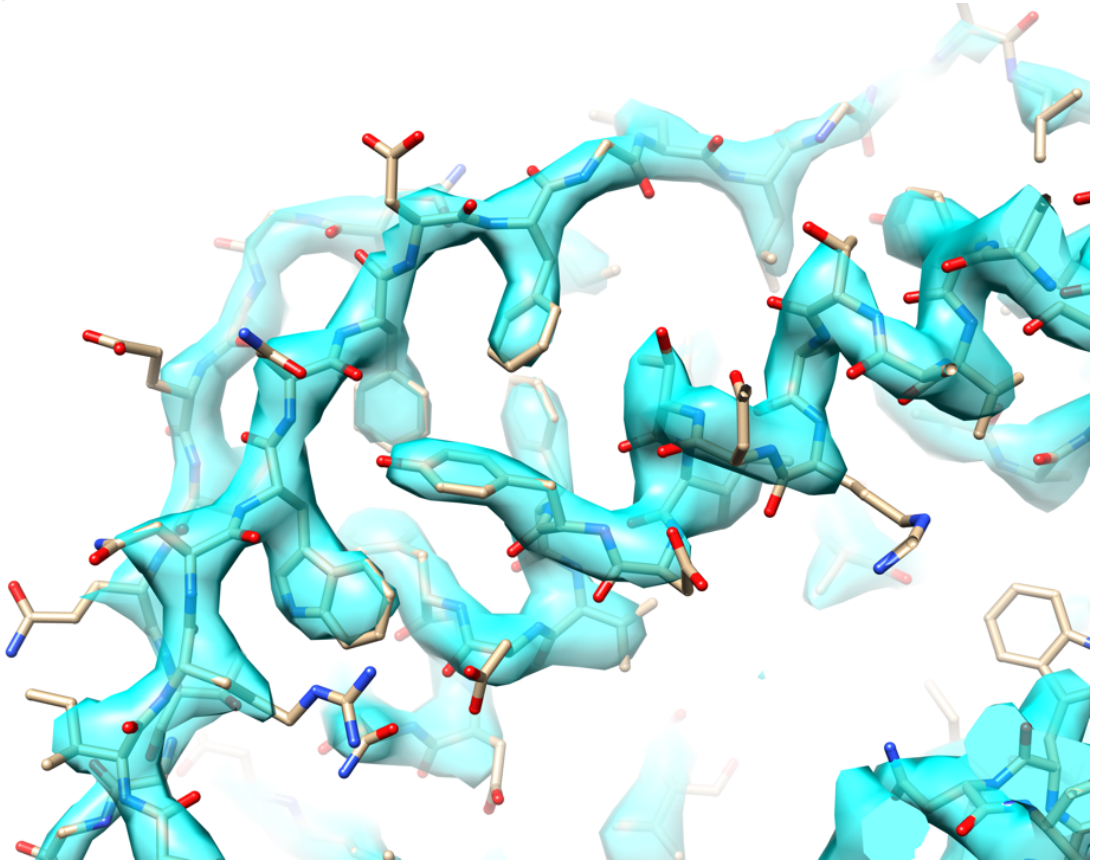


Data from Zhou, X. et al. (2017) Nat. Struct. Mol. Biol. 24: 1146

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:

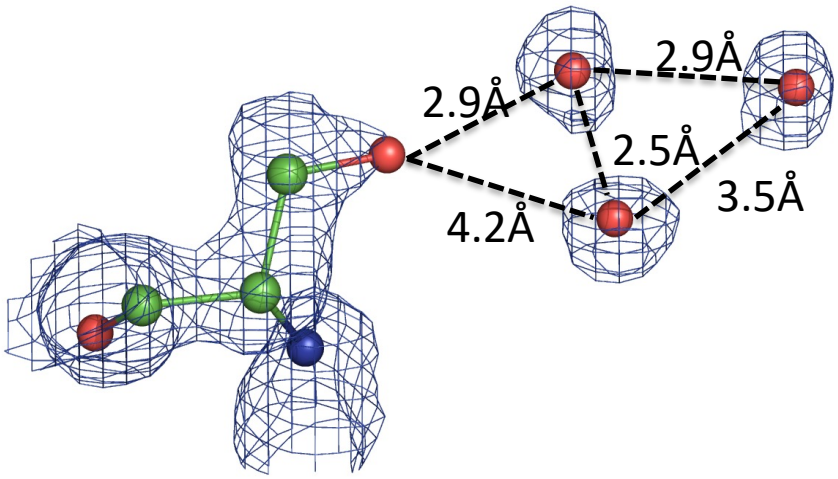
- No automated model building produces 100% complete and accurate model
- Produces initial model for further manual building
- The lower the resolution, the less complete and accurate the auto built model

Manual model building steps

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

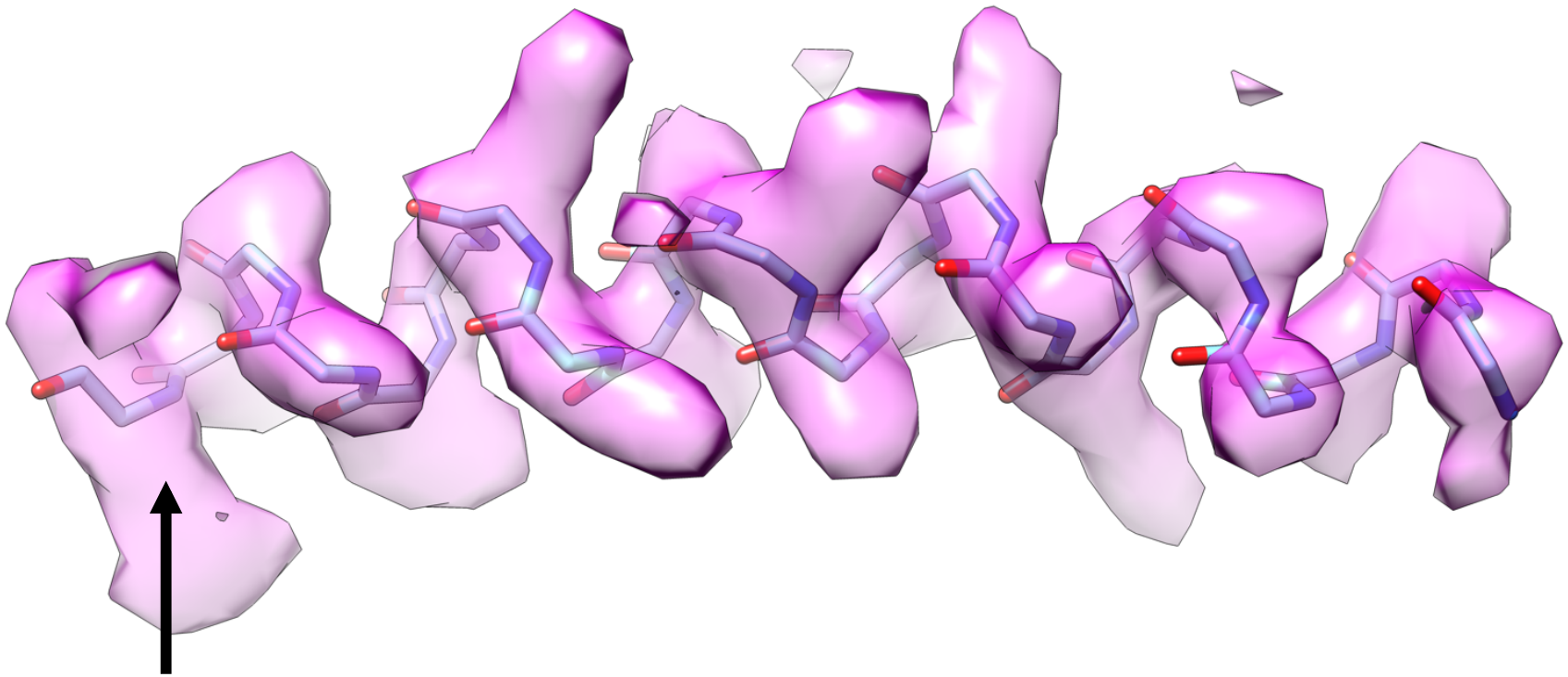
- Auto-sharpen the map
- Run Map Symmetry to obtain symmetry
- Run Map Box to obtain asymmetric unit (using symmetry)
- Run Map to Model on asymmetric unit
- Run Apply NCS Operators on model, with the trim overlap option (supplying the full map)

Automated water building: *phenix.douse*



Available in ChimeraX!

Sequence from map: *phenix.sequence_from_map*

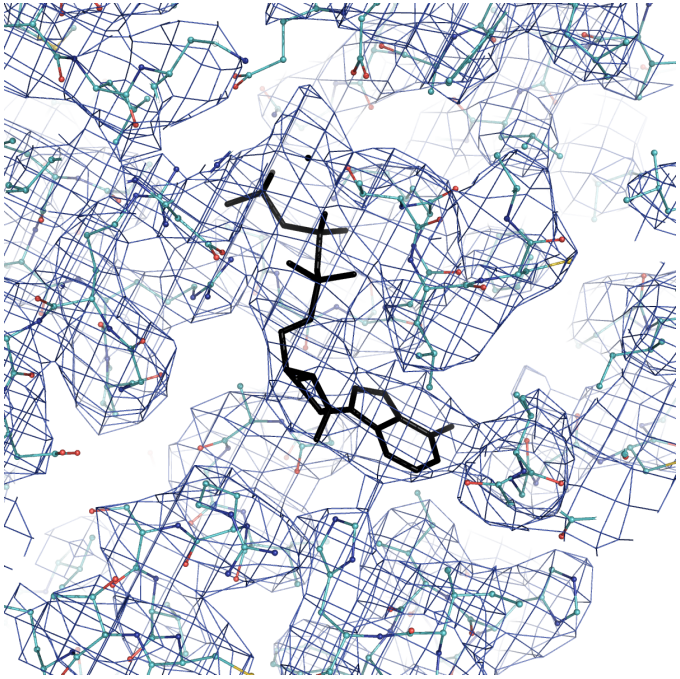


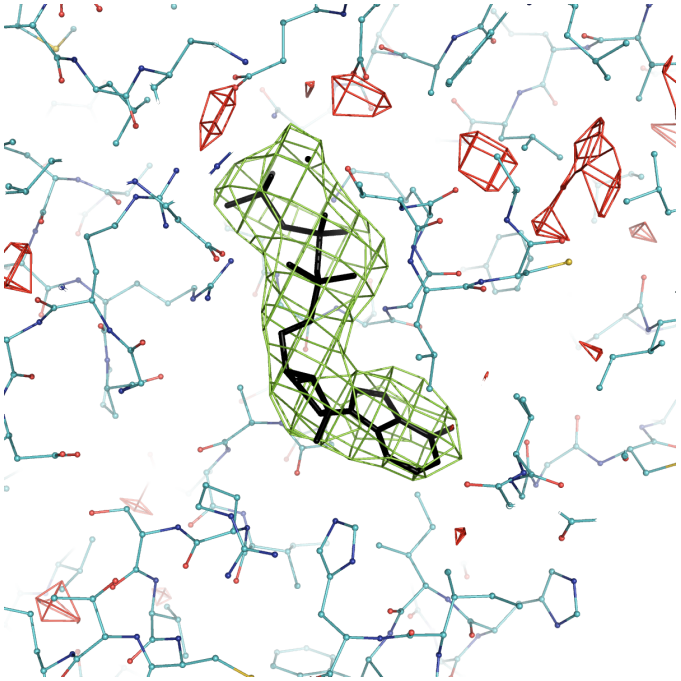
Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

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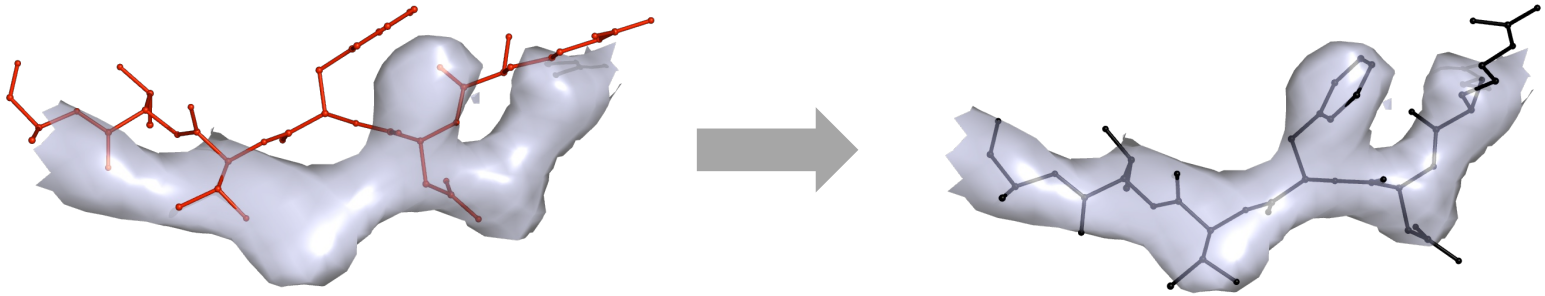
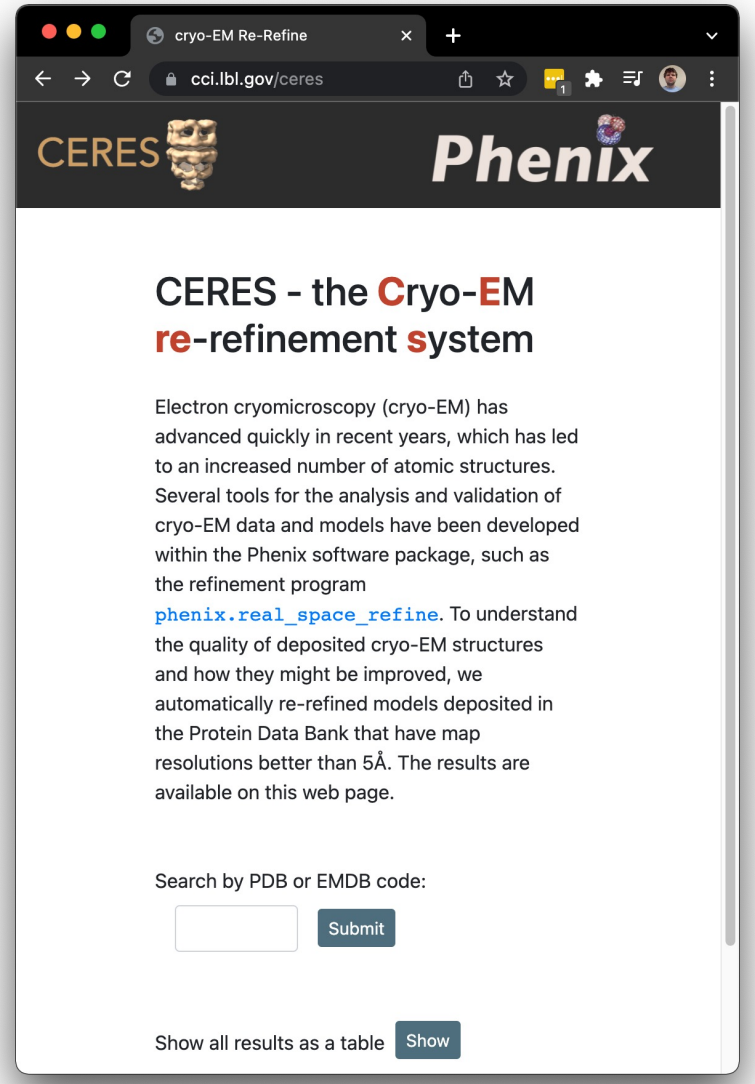
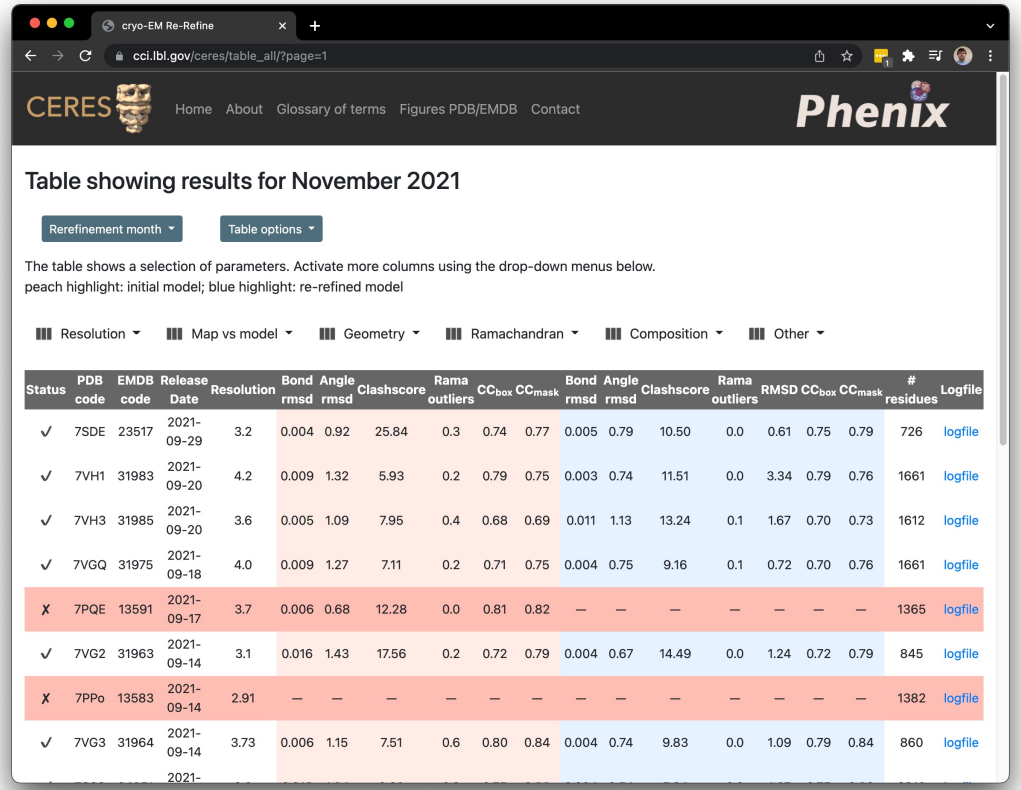
Real-space refinement in *PHENIX* for cryo-EM and crystallography

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





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

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