

Refinement (Cryo-EM)

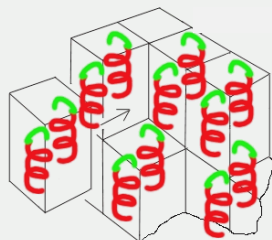
Pavel Afonine

Lawrence Berkeley National Lab, California, USA

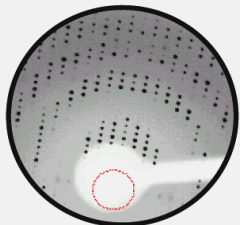
**July 29, 2022
ACA
Portland, Oregon**

Refinement

Crystallography



Initial model



Experimental data

A priori knowledge

Score

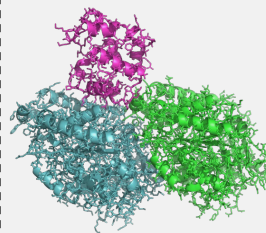
Modify model parameters

Improved model

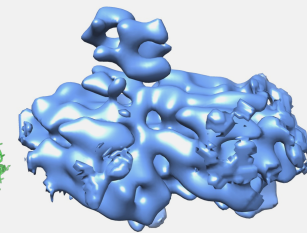
phenix.refine

Available since 2005

Cryo-EM



Initial model



Experimental data

A priori knowledge

Score

Modify model parameters

Improved model

phenix.real_space_refine

Available since 2013

Atomic model refinement: *phenix.real_space_refine*



STRUCTURAL
BIOLOGY

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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}

Atomic model refinement: crystallography vs cryo-EM

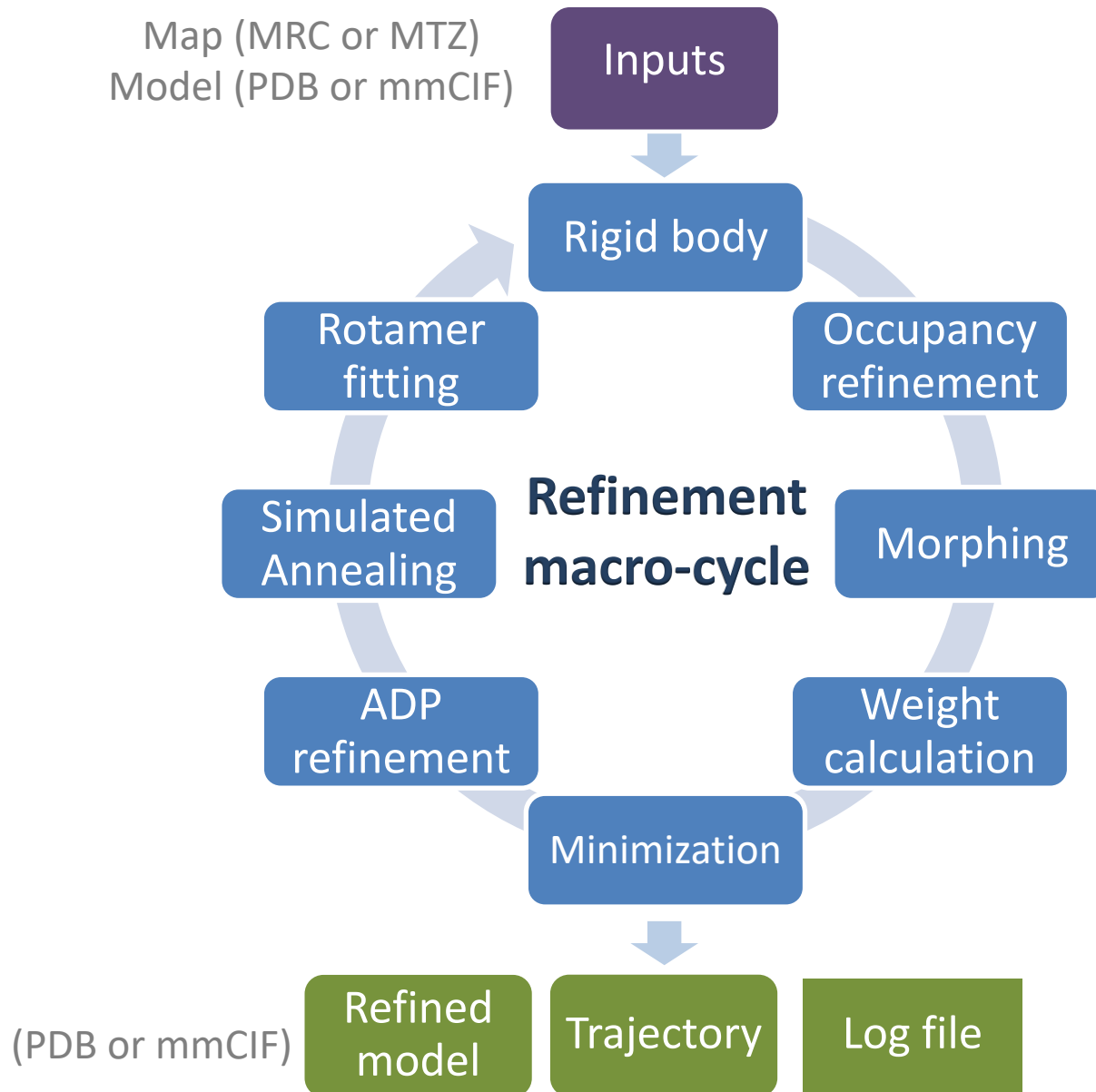
Crystallographic refinement

- Improving model improves map
 - (2mFo-DFc, Model phase), (mFo-DFc, Model phase)
 - Better model leads to better map
 - Better map leads to more model built
 - Improving model in one place lets build more model elsewhere in the unit cell
 - Refine all model parameters (XYZ, B) from start to end of structure solution
 - Build solvent (ordered water) early
- Experimental data never changed
- Data / restraints weight is global and time expensive to find best value
- Whole model needs to be refined

Cryo-EM refinement

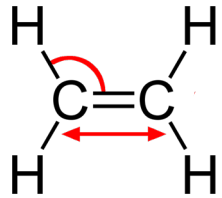
- Changing model does not change map
 - Build solvent (water) last
 - Get as complete and accurate model as possible before refining B factors and occupancies
- Experimental data changes a lot during the process (filtering, boxing, using maps with implied symmetry or not, etc.)
 - What map to use in refinement?
 - Refined B factors depend on map used
- Data / restraints weight can be local and is always optimal
- Boxed parts of the model can be refined

Refinement protocol

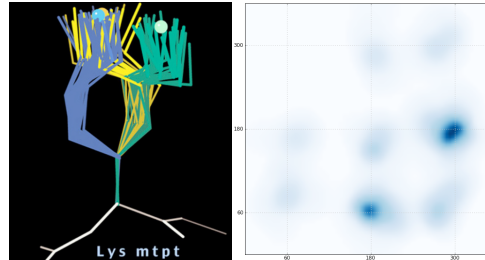


Restraints

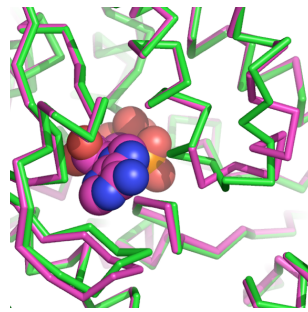
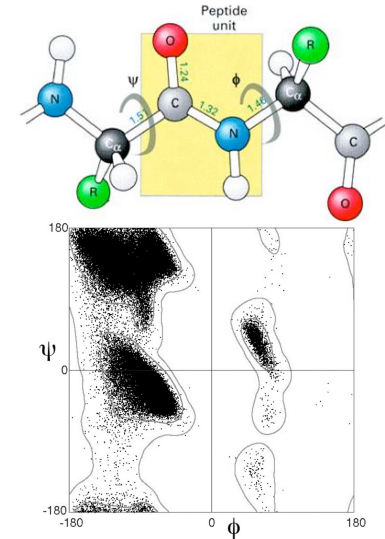
Covalent geometry



Side chain distributions



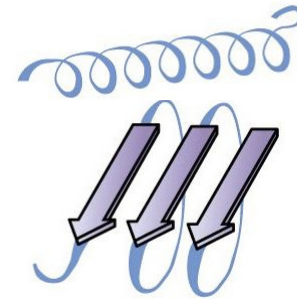
Main chain distributions



Similar (homologous) structures
(reference model restraints)

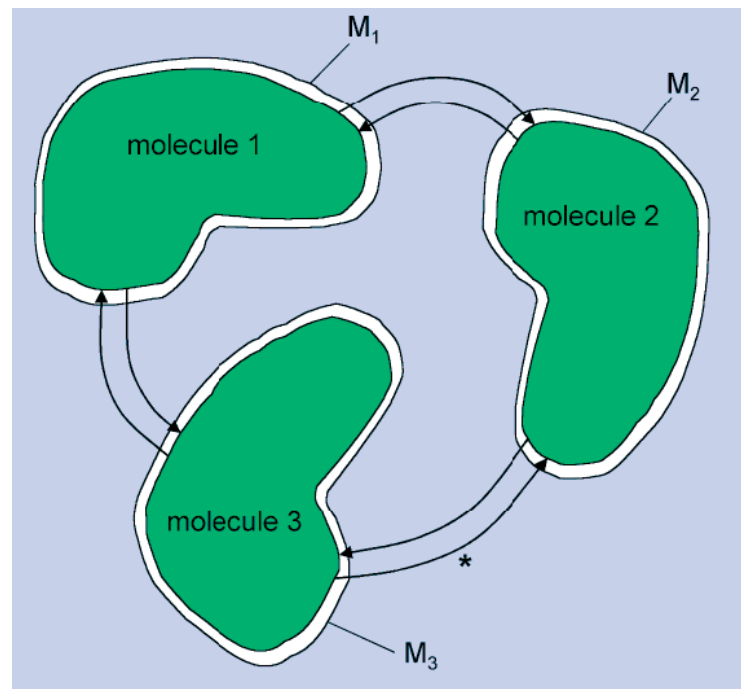
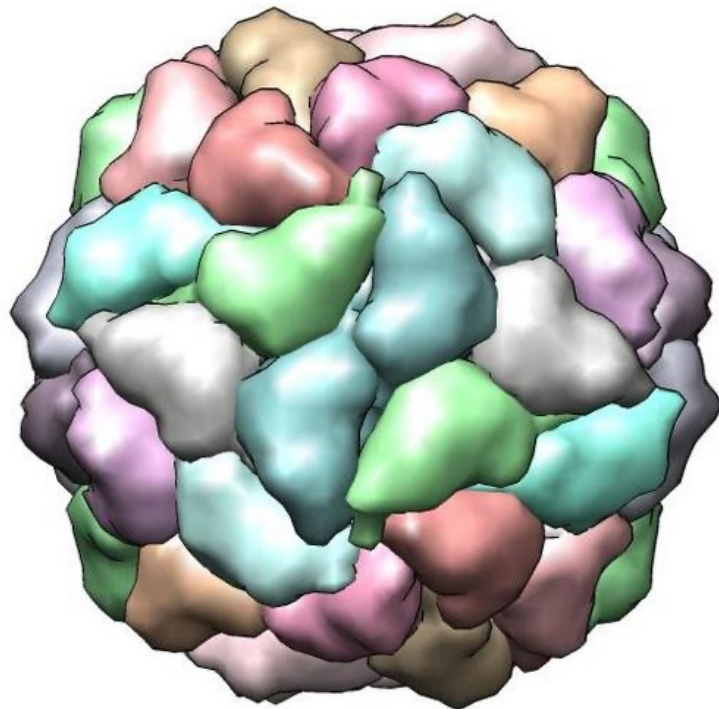


Internal
symmetry
(NCS)



Secondary structure

NCS (molecular symmetry)



Source: Internet

- **Constraints:** molecules 1, 2 and 3 are required to be **identical**
- **Restraints:** molecules 1, 2 and 3 are required to be **similar** but not necessarily identical

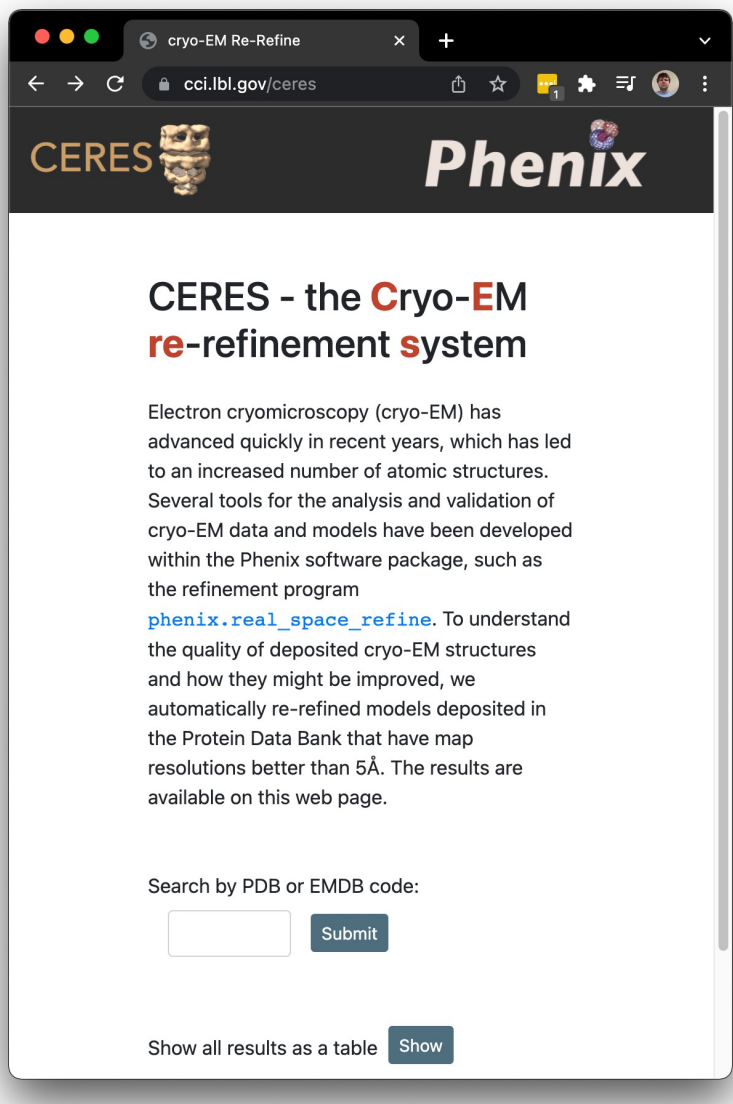
Real-space refinement – B-factors (new)

- Much faster than in previous versions
- Isotropic group or individual
- Symmetry-aware
 - Strict symmetry: B-factors are identical across symmetry copies
- Can use multiprocessing
- Data / restraints weight is always optimal
 - Ensures best model-to-map fit and physically meaningful B-factors
- Refine B-factors at the very last step
 - Because refined model does not feed back to the map
- Refined B-factor values depend on the map used (original vs sharpened or else manipulated)

Real-space refinement – occupancy (new)

- Symmetry-aware
 - Strict symmetry: B-factors are identical across symmetry copies
- Can use multiprocessing
- Rules are similar to *phenix.refine*:
 - Look for (phenix-online.org):
 - 13 typical occupancy refinement scenarios and available options in phenix.refine*
- Refine occupancies at the very last step
 - Because refined model does not feed back to the map

Automated re-refinement of deposited cryo-EM models



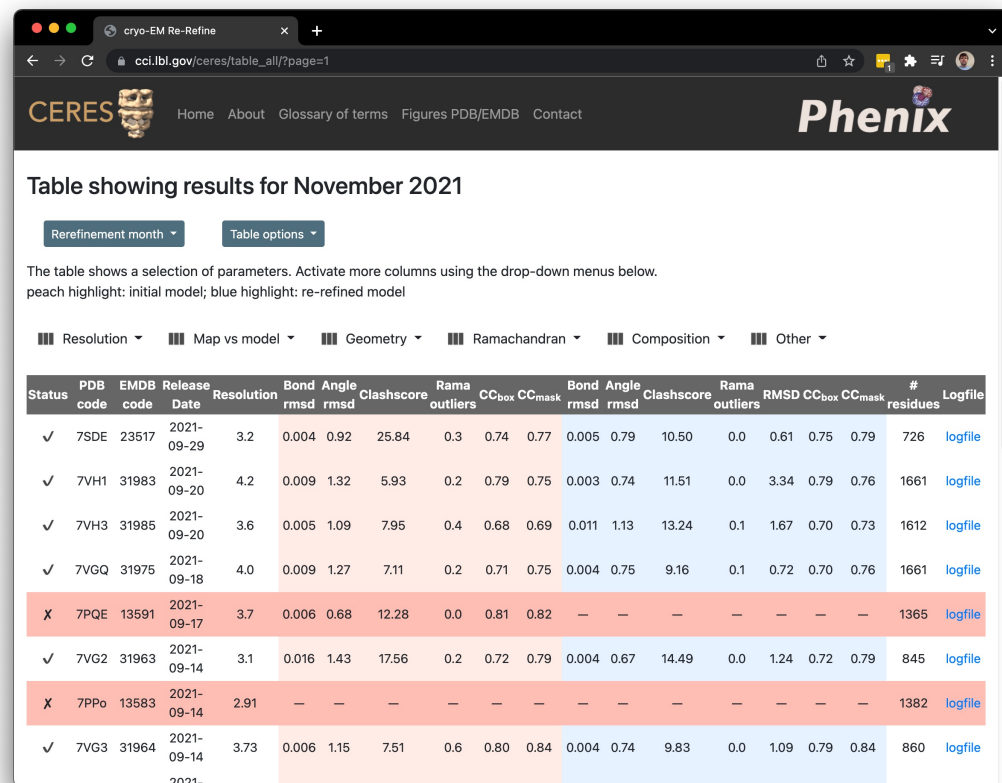
The screenshot shows the CERES website home page. The header features the CERES and Phenix logos. The main heading is "CERES - the Cryo-EM re-refinement system". The text describes the system's purpose: to improve the quality of deposited cryo-EM structures by automatically re-refining models with resolutions better than 5Å. A search bar is located at the bottom, with a "Submit" button and a "Show" button to view results as a table.

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



The screenshot shows a table of results for November 2021. The table has columns for 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, and Logfile. The table is color-coded: peach for initial models and blue for re-refined models. The table shows that re-refinement generally improves resolution and other quality metrics.

Table showing results for November 2021

Rer refinement month: Table options:

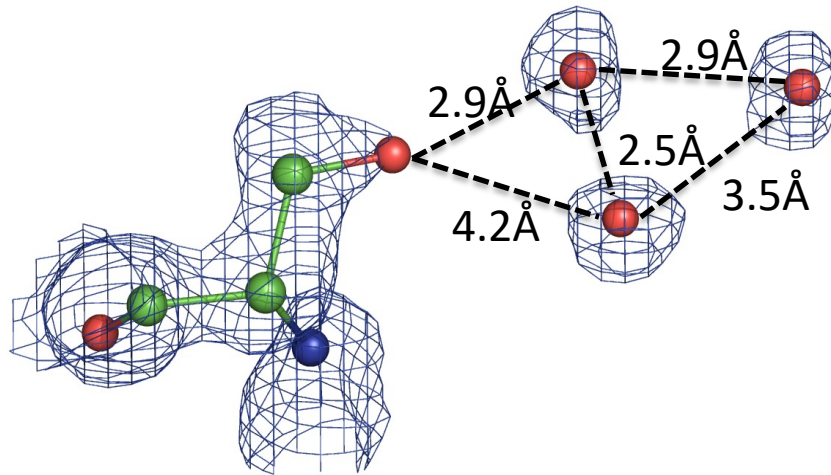
The table shows a selection of parameters. Activate more columns using the drop-down menus below.
peach highlight: initial model; blue highlight: re-refined model

Resolution | Map vs model | Geometry | Ramachandran | Composition | Other

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
X	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
X	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

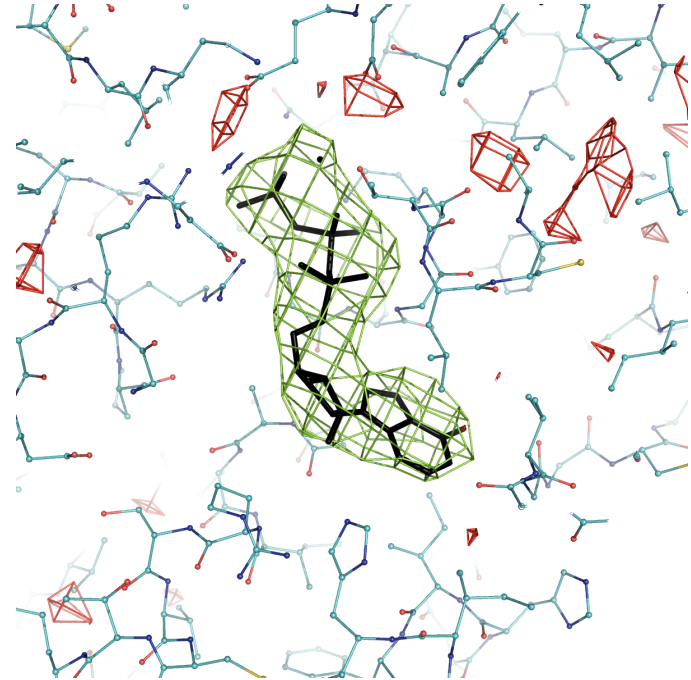
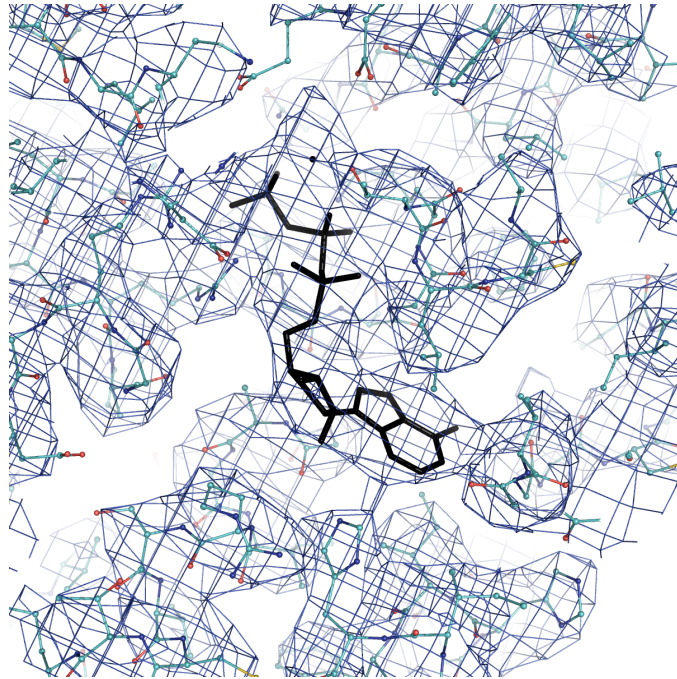
- Developers: helps track the impact of new methods and tools
- Users: lets to see how their models can benefit from improved methods and tools

Automated water building: *phenix.douse*



Difference maps

5L4g, EMDB 4002



User support

- **Feedback, questions, help**

Mailing list (all, developers and users): phenixbb@phenix-online.org

Bug reports (developers only): bugs@phenix-online.org

Ask for help (developers only): help@phenix-online.org

- **Reporting a bug or asking for help:**

- We can't help you if you don't help us to understand your problem
- Make sure the problem still exist using the latest *Phenix* version
- Send us all inputs (files, non-default parameters) and tell us steps that lead to the problem
- All data sent to us kept confidentially

The Project



Lawrence Berkeley Laboratory

Paul Adams, Pavel Afonine,
Dorothee Liebschner, Nigel
Moriarty, Billy Poon,
Christopher Schlicksup,
Oleg Sobolev



University of Cambridge

Randy Read, Airlie McCoy,
Tristan Croll, Claudia Millán Nebot,
Rob Oeffner



Los Alamos National Laboratory New Mexico Consortium

Tom Terwilliger, Li-Wei Hung



UTHealth

Matt Baker, Corey Hyrc



Duke University

Jane & David Richardson,
Christopher Williams,
Vincent Chen



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Liebschner D, *et al.*, Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*. *Acta Cryst.* 2019 **D75**:861–877