Model Refinement: X-ray vs cryo-EM

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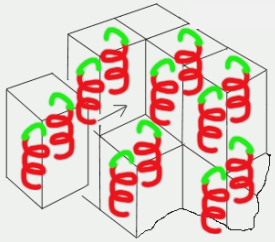
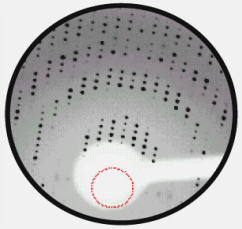
BNL

Refinement

Crystallography





Initial model

Experimental data

A priori knowledge

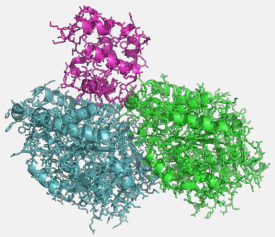
Score

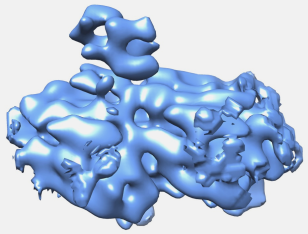
Modify model parameters

Improved model

phenix.refine

Cryo-EM





Initial model

Experimental data

A priori knowledge

Score

Modify model parameters

Improved model

phenix.real_space_refine

Atomic model refinement: crystallography vs cryo-EM

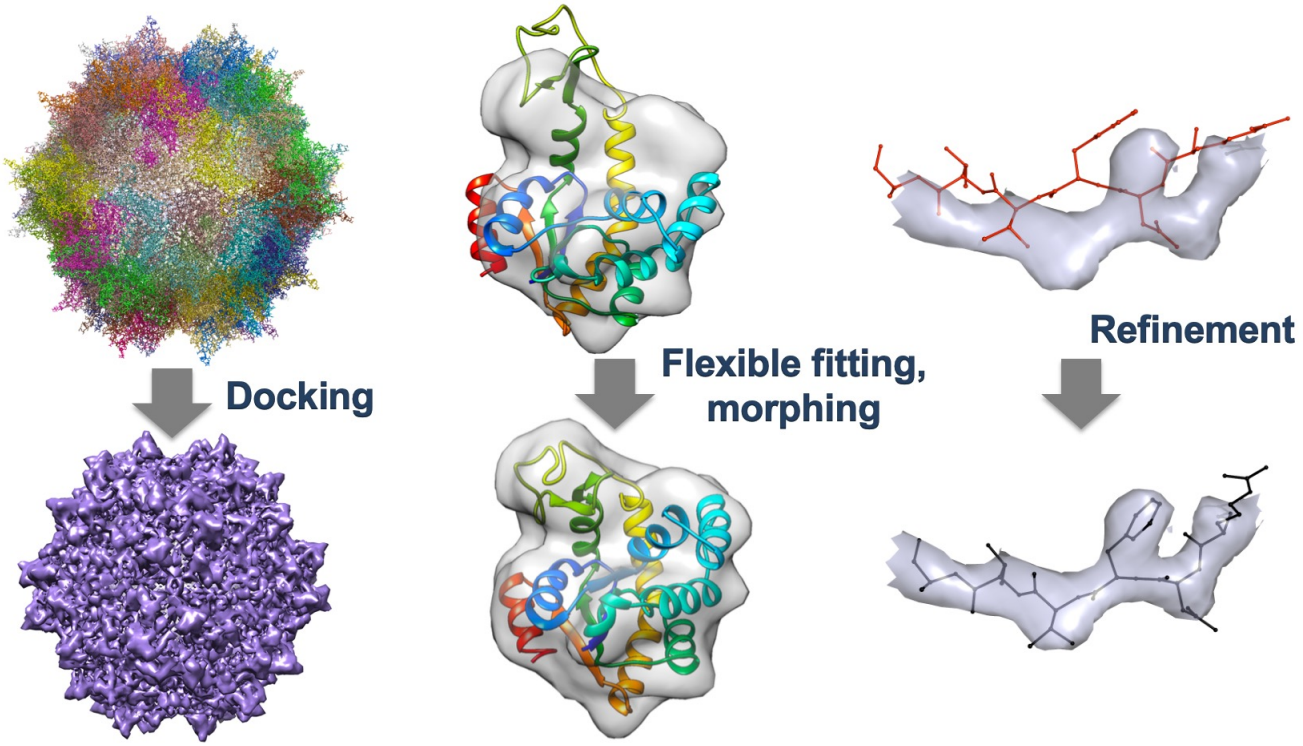
Crystallographic refinement

- 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

Not all model-to-data fitting is refinement



- Docking, flexible fitting, morphing are ***not*** refinement
- Refinement is to fine-tune an already fine atomic model
  - Refinement does only small changes to the model (within *convergence radius of refinement*, ~ 1Å)

Atomic model refinement: *phenix.real_space_refine*

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

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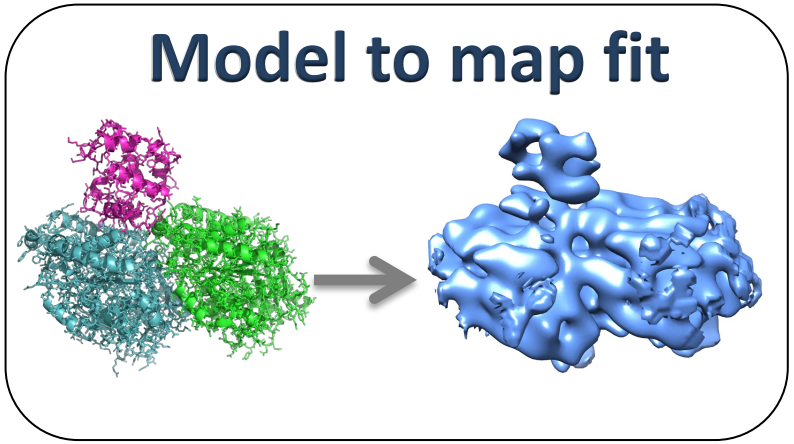
Model-to-map fit validation: CC_{MASK}



$$CC_{MASK} = \frac{\sum \rho_{obs}\, \rho_{calc}}{(\sum \rho_{obs}^{2}\ \sum \rho_{calc}^{2})^{1/2}}$$

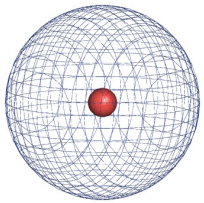
ρ_{obs} = experimental map
ρ_{calc} = model calculated map

- Easy interpretation: -1: anticorrelation, 0: no correlation, 1: perfect correlation
- Uses all atomic model parameters (XYZ, B-factors, occ, atom type)
- Not specific to map type (any map: x-ray, neutron, electron, cryo-EM, …)
- Can be calculated locally (per atom, residue, chain, molecule, whole box, …)
  - Local resolution can be trivially taken into account

Metric	Expected value
CC_{MASK}	Poor: < 0.3 So-so: 0.3-0.6 Good: > 0.6

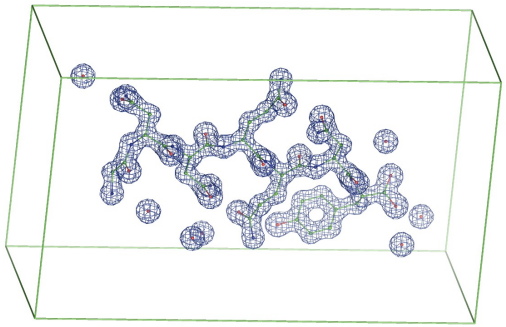
Model-to-map fit validation: CC_{MASK}

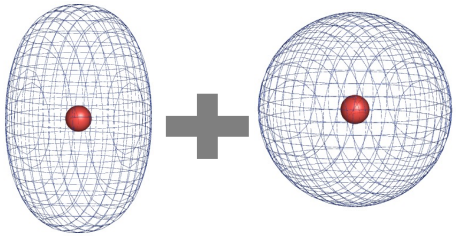
- Gaussian IAM (Independent Atom Model)



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

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

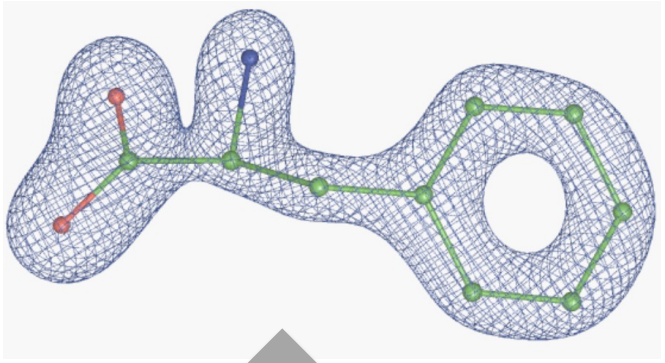




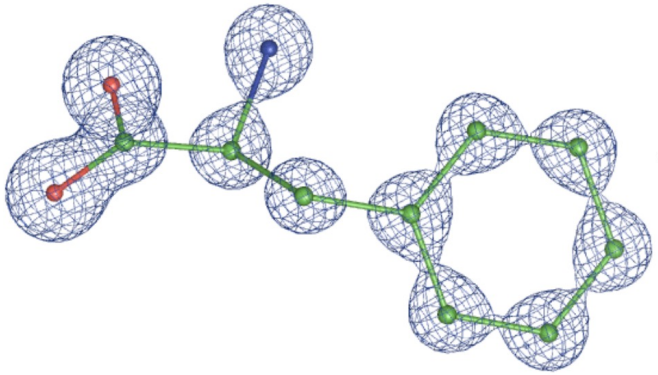
$$\rho_{MODEL}(\mathbf{r}) = \sum_{i=1}^{Natoms} \rho_{atoms}(\mathbf{r})$$

Model-to-map fit validation: CC_{MASK}

3Å model-calculated map



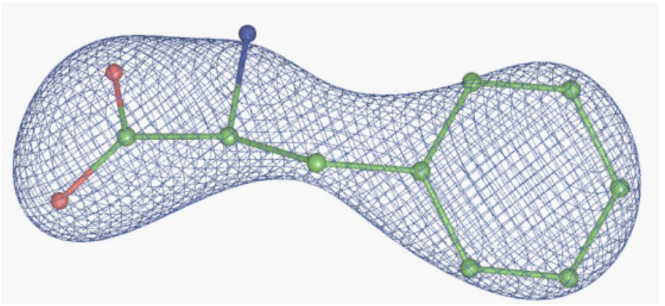
Exact model map



CC_{MASK}

CC_{MASK}

3Å experimental map



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

- FT exact model map
- Remove terms up to specified resolution
- FT back to real space to get a Fourier image = “Model map”

Atom inclusion

- **Atom inclusion**: fraction of atoms inside molecular envelope contoured at a given level

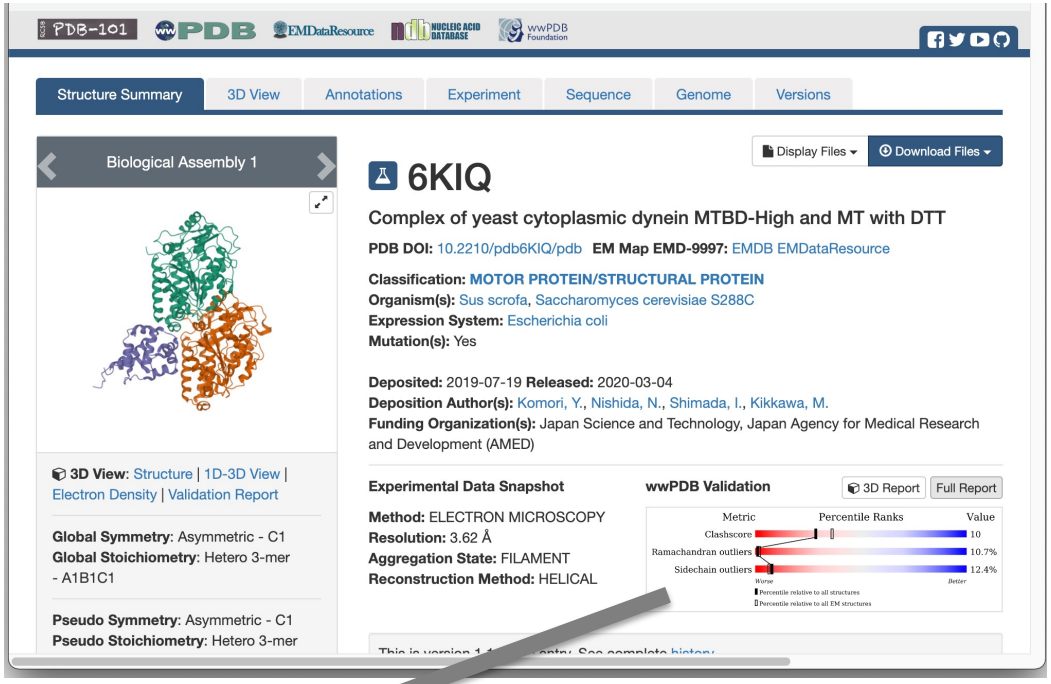
  - Contouring threshold (Arbitrarily? What is optimal level?)
  - No use of atomic model parameters such as ADP, occupancy, atom type, …
  - Does not use shape of density
  - Does not account for missing atoms
  - Does not use map type
  - Unaware of alternative conformations

Q-Score

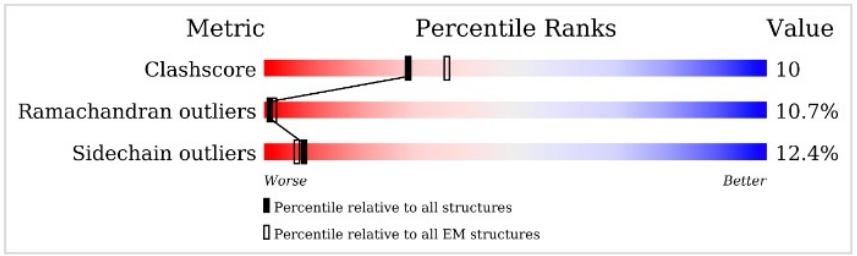
- **Q-score**: measure the resolvability of individual atoms in a cryo-EM map, using an atomic model fitted to or built into the map

  - Does not use ADP, occupancy, atom type, …
  - Does not use shape of density
  - Does not account for missing atoms
  - Alternative conformations are ***not*** handled
  - Anisotropic atoms are ***not*** handled

Validation reports (RCSB): only Q-score and atom inclusion



wwPDB Validation 3D Report Full Report



9.5 Map-model fit summary

The table lists the average atom inclusion at the recommended contour level (0.125) a for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.9062	0.4550
M	0.5810	0.3210
a	0.9659	0.4790
b	0.9656	0.4730

Model-to-map fit statistics is insufficient and very well hidden!

Refinement: practical considerations

- Final stages
  - Refine B-factors (Atomic Displacement Parameters)
    - Group B factor or individual
  - Refine occupancies
  - Use Hydrogen atoms (and keep them in the final model!)
  - Add water (phenix.douse: command line and GUI):

    Also available in ChimeraX

Variability refinement

Treasuring conformational changes

Contents lists available at ScienceDirect

BBA - Biomembranes

journal homepage: www.elsevier.com/locate/bbamem

Review

Conformational space exploration of cryo-EM structures by variability refinement

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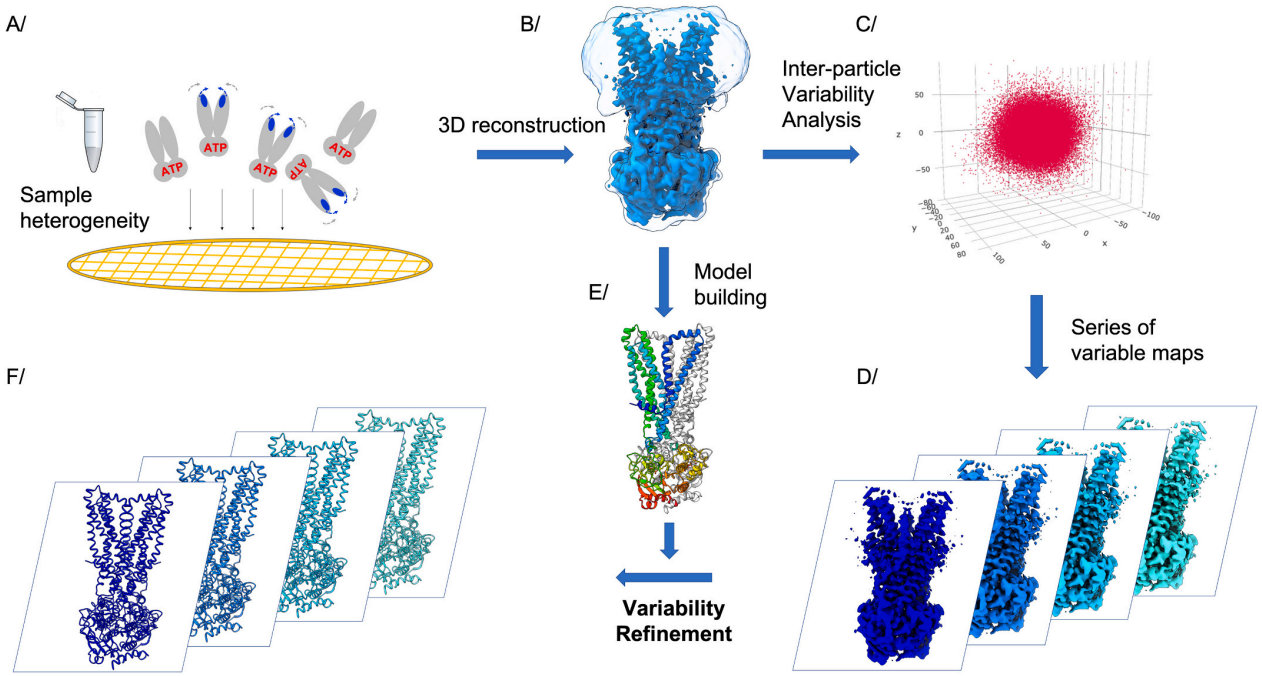
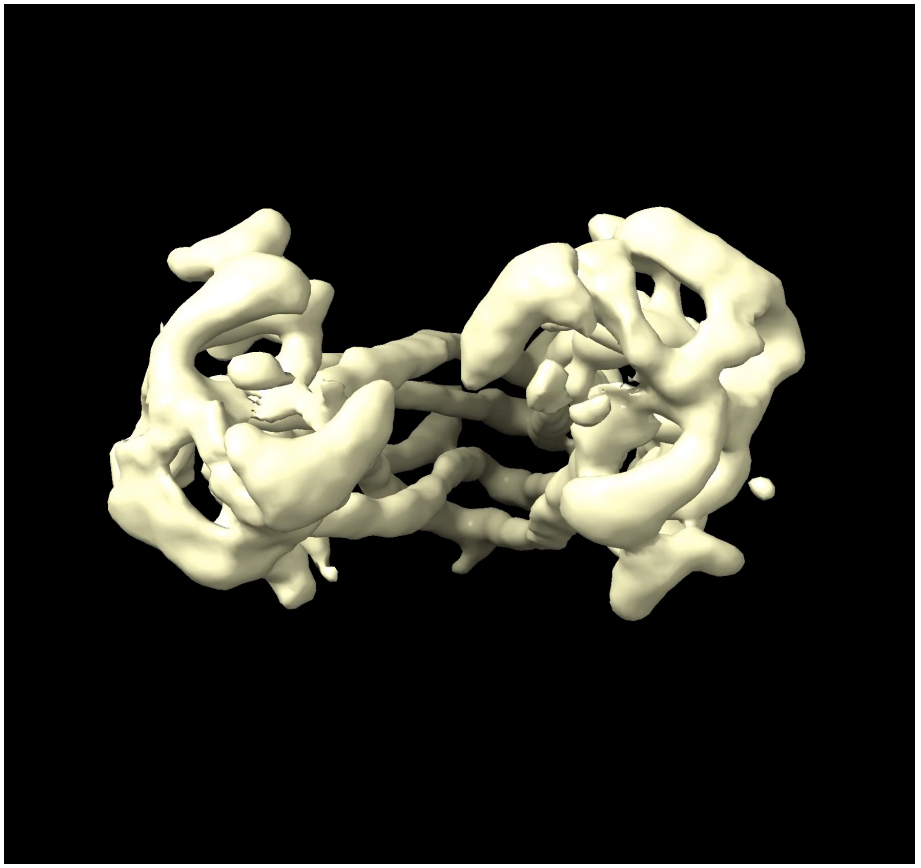
[b] Molecular Microbiology and Structural Biochemistry, UMR5086 CNRS University Lyon1, 7 passage du Vercors, 69007 Lyon, France

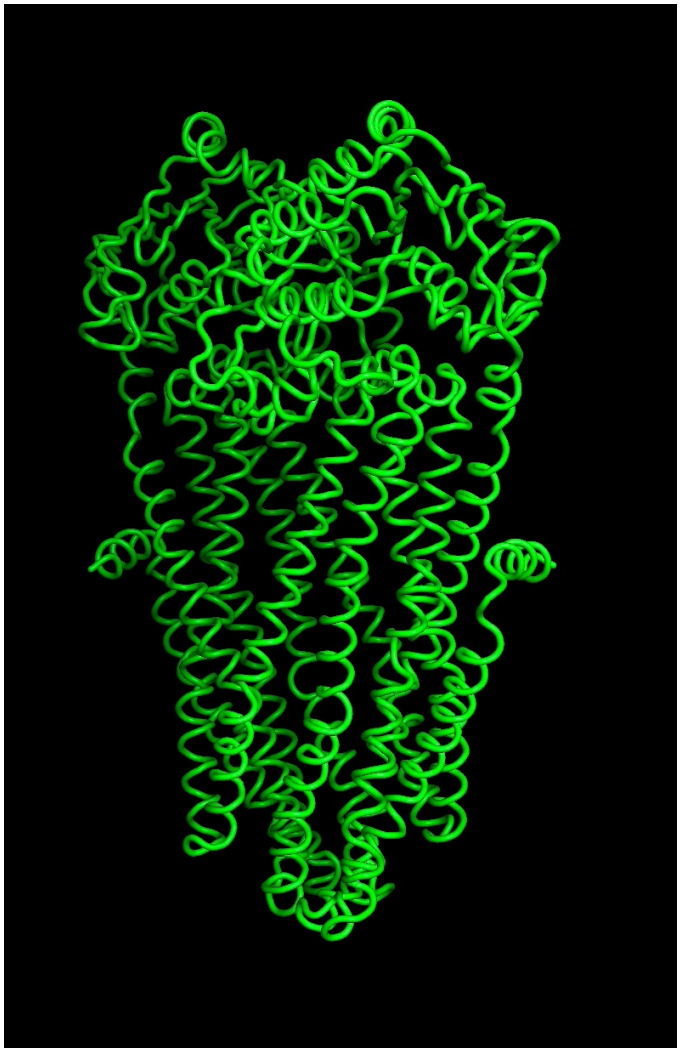


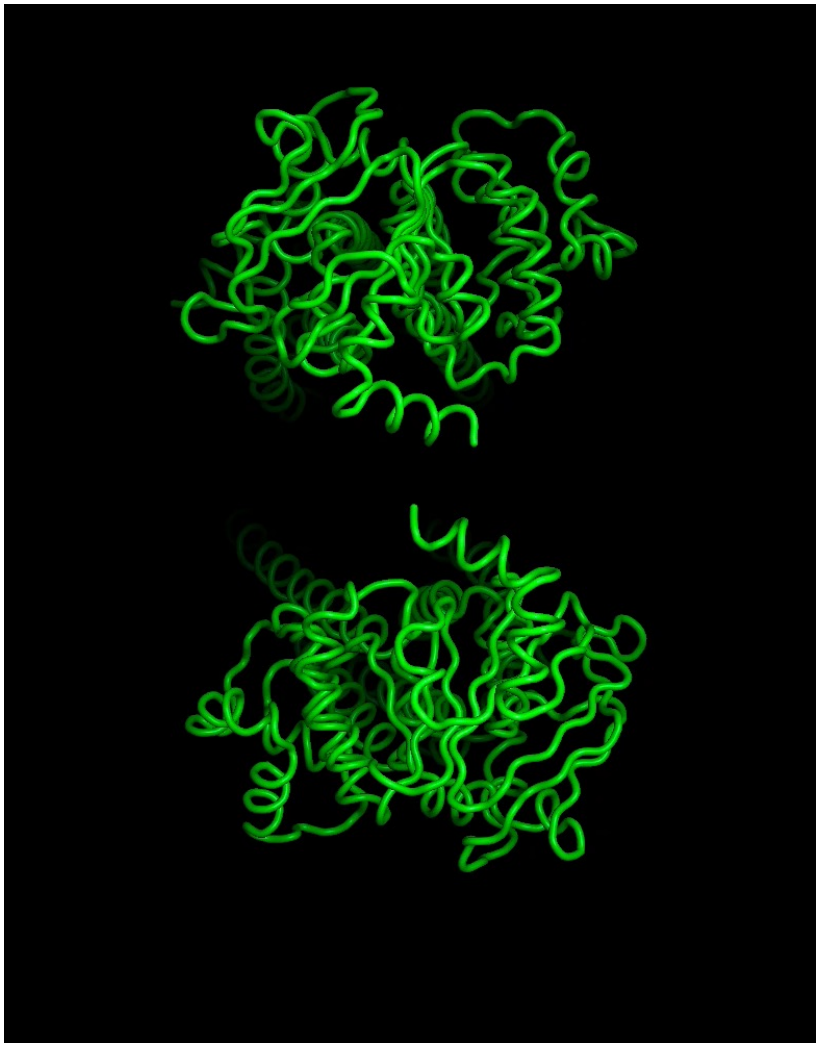
Maps

ABC transporter BmrA (unpublished!)



Refined ensembles of models





***phenix.varref* – Phenix tool to represent ensemble of maps with ensemble of atomic models**

phenix.varref

map1.mrc … mapN.mrc

model.pdb

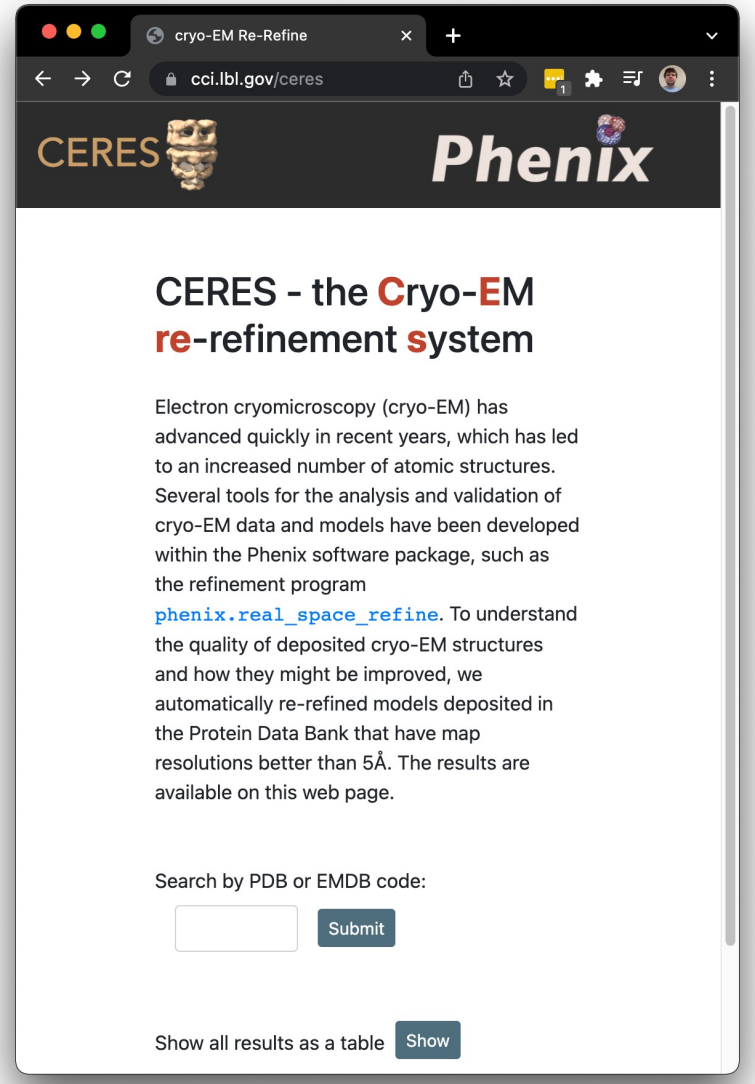
resolution=3

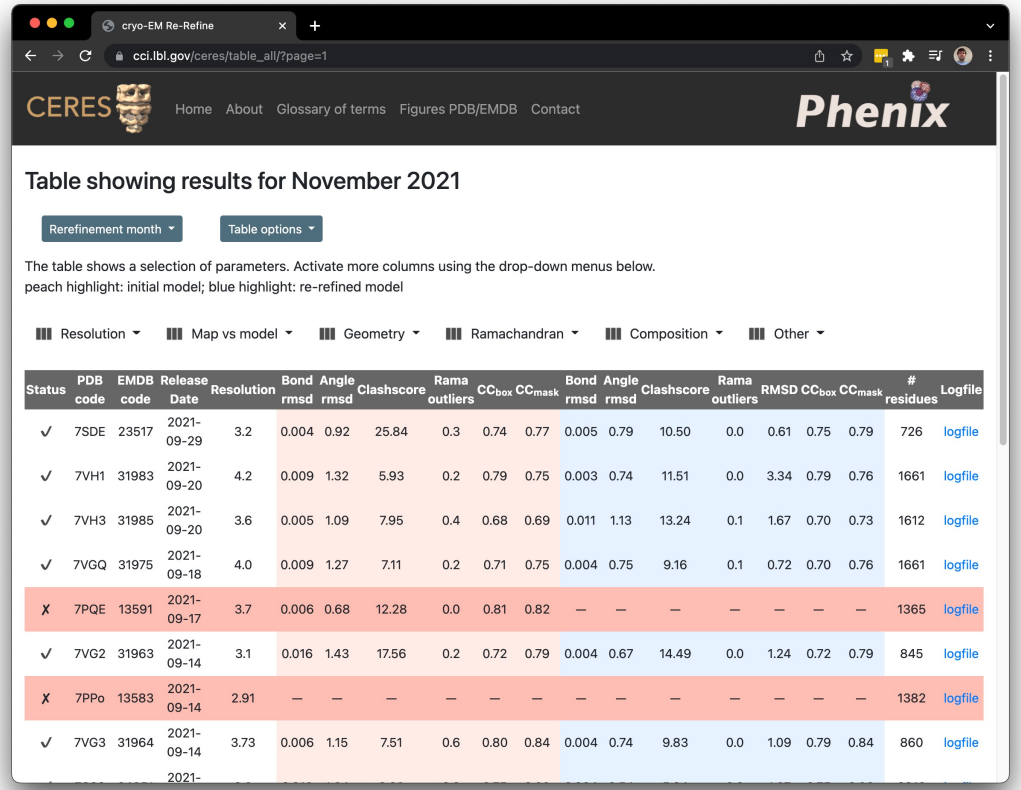
nproc=100

models_per_map=100

Output: ensemble of refined models that represents all maps

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