Refinement

Pavel Afonine

April 30, 2026
Lincoln, NE

More training events

MCCS-2026 Macromolecular

Ask Gemini

xtal.iqf.csic.es/MCCS2026/

Macromolecular Crystallography & Cryo-EM School

MCCS-2026 23-27 March 2026

CBE CSIC

c/ Serrano 119 E-28006 Madrid (Spain) Telf: +34 91 561 94 00 Fax: +34 91 564 24 31

Thursday, 30 April 2026

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These pages are regularly updated. If you find any error or incoherence, please, let us know

Last update: 01 Apr. 2026

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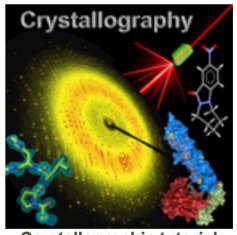
- Accomodation
- Participants
- Previous editions



Crystallographic tutorial (Spanish & English)

CSIC

*Macromolecular Crystallography & Cryo-EM School - **MCCS-2026** (if you don't see a menu on the left, use this link)*

We are pleased to announce the thirteenth edition of the ***Macromolecular Crystallography & Cryo-EM School - MCCS-2026***, to be held between ***23-27 March 2026*** at the Department of Crystallography and Structural Biology of the Institute of Physical-Chemistry "Blas Cabrera" (old "Rocasolano") at the CSIC, Spanish National Research Council, Madrid.

The workshop is meant for 25 graduate students or researchers with previous expertise in crystallography and/or CryoEM who need a deeper insight into the most advanced crystallographic and CryoEM techniques to carry out their research projects. The program traditionally covers aspects such as sample preparation, structure solution, model building, crystallographic refinement, validation, and analysis of the structural results, as well as an overview of the newest structural biology technologies. On top of these subjects, the program will include those aspects related to the impact of Artificial Intelligence methods on structure solution and low-resolution refinement. In addition, we will cover dynamic crystallography at XFEL and synchrotron and ligand building for drug discovery.

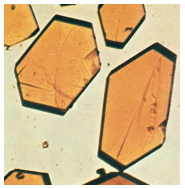
If you don't see a menu on the left, use this link.

For further information, please contact: *Dr. Armando Albert* / *Dr. Juan Hermoso* / *Dr, Lourdes Infantes* / *Dr. Maria J. Sánchez-Barrena*

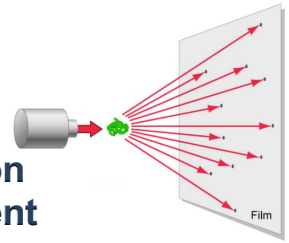
Solving structure - crystallography

Pure sample

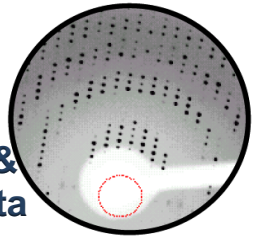
Crystals



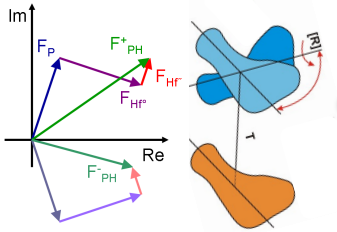
Diffraction experiment



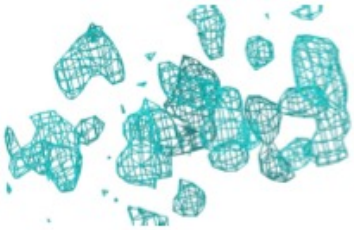
Process & check data



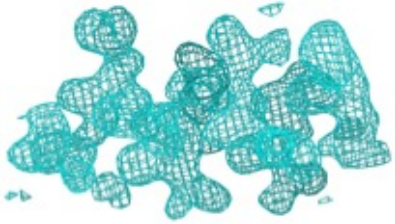
Solve phase problem



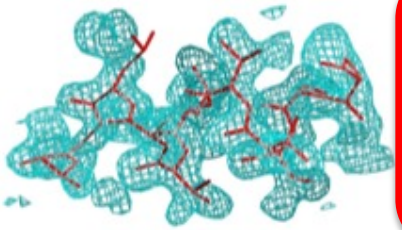
Initial map



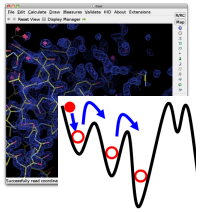
Improve d map



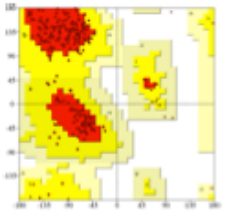
Build model



Refine model



Validate model



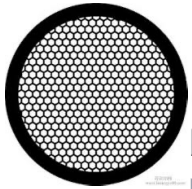
Publish & deposit

- Process is not as ‘linear’ as shown
- Each step has numerous sub-steps
- Crystals may not grow or exhibit pathologies
- Stuck solving phase problem

Solving structure by electron cryo-microscopy

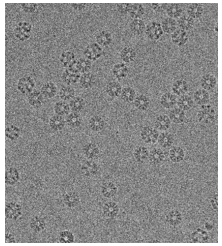
Pure sample

Grid

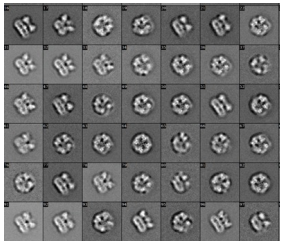


Electron microscope

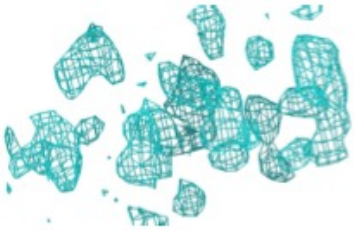
Electron microscope



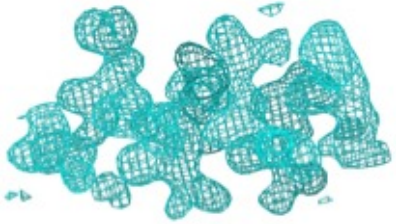
Pick, align & average particles



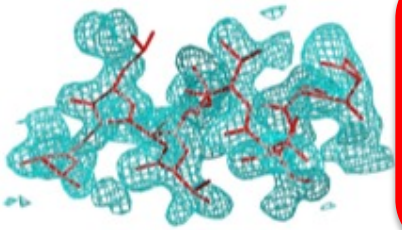
Initial map



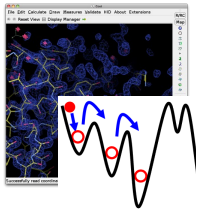
Improved map



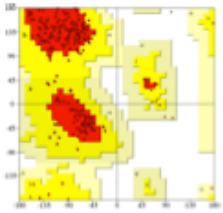
Build model



Refine model



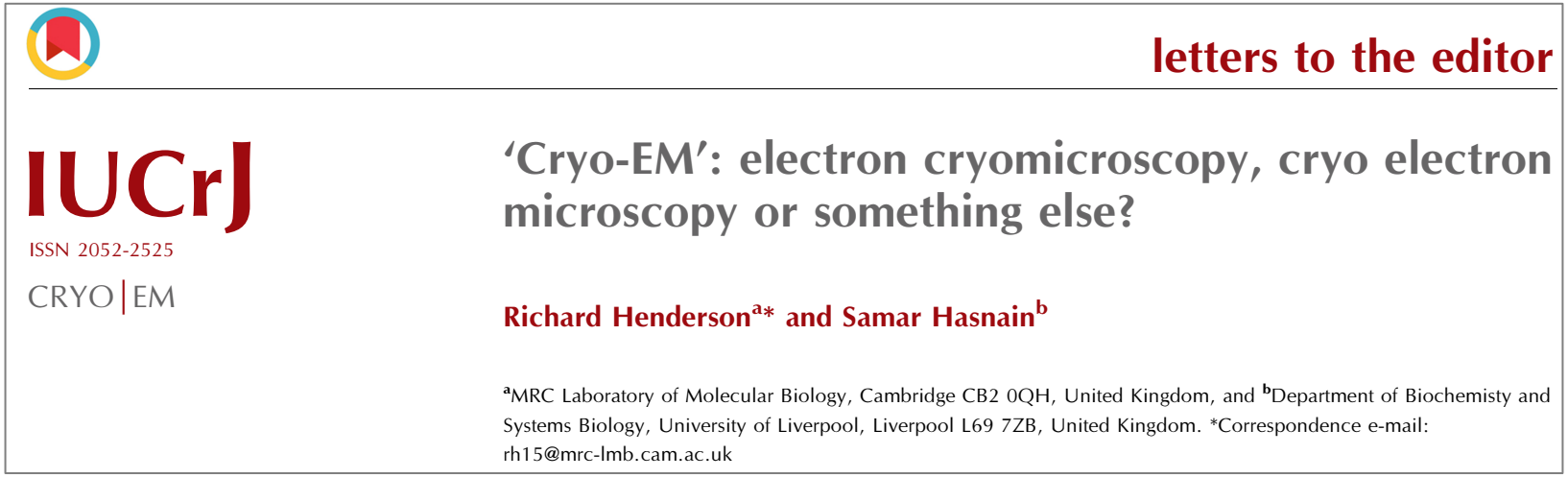
Validate model



Publish & deposit

- Process is not as ‘linear’ as shown
- Each step has numerous sub-steps
- Conformational heterogeneity, size, contamination, sample homogeneity, low contrast, weak signal

A note on naming



letters to the editor

IUCrJ

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CRYO | EM

'Cryo-EM': electron cryomicroscopy, cryo electron microscopy or something else?

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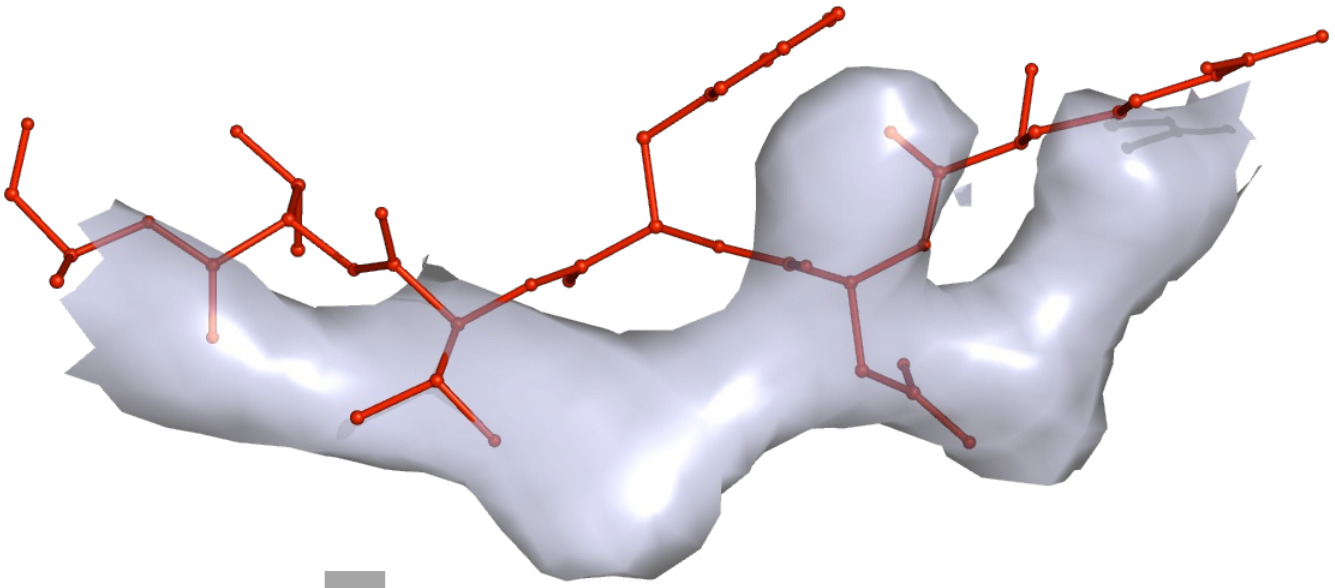
Key takeaway:

cryogenic-sample **E**lectron **M**icroscopy (cryoEM)

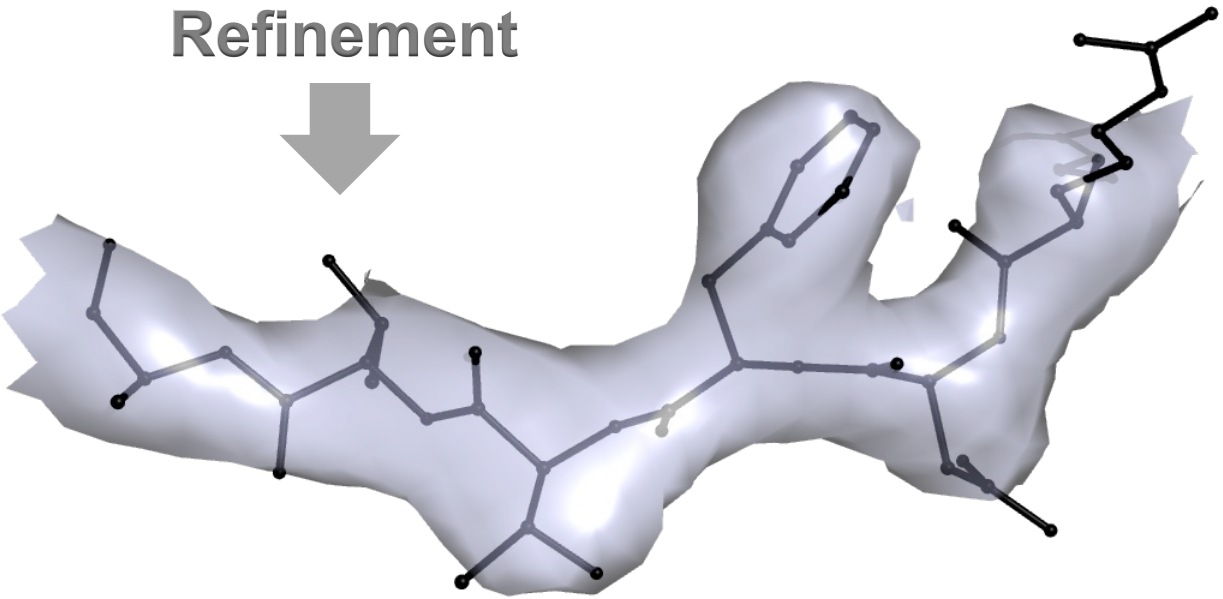
Model refinement

Initial (poor) model

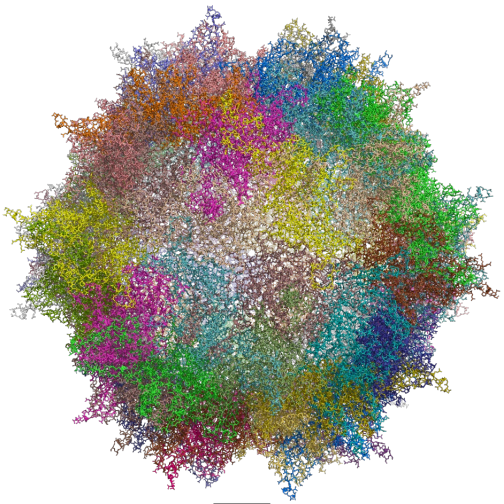


Refinement

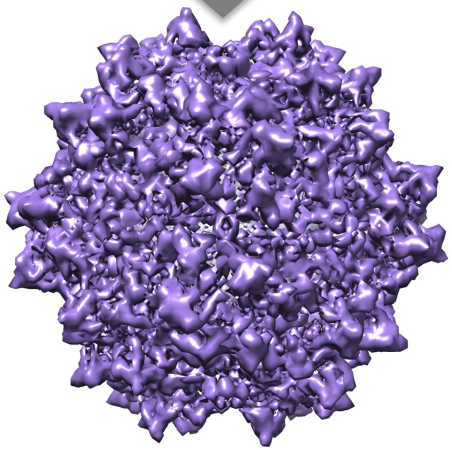
Improved (refined) model

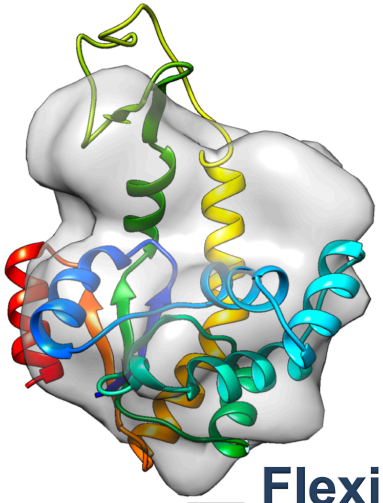


Model refinement vs other model fitting tools

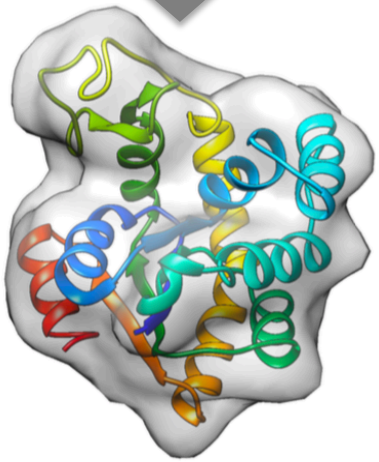


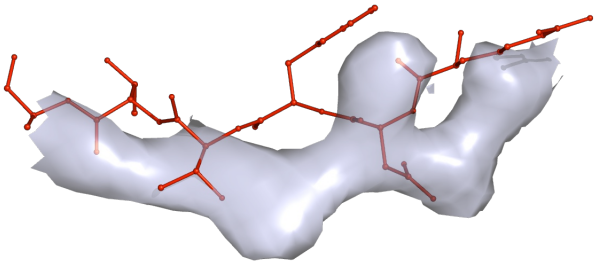
Docking



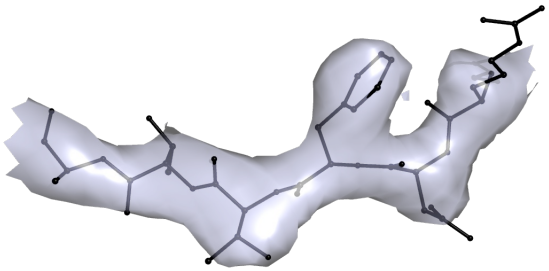


Flexible fitting, morphing



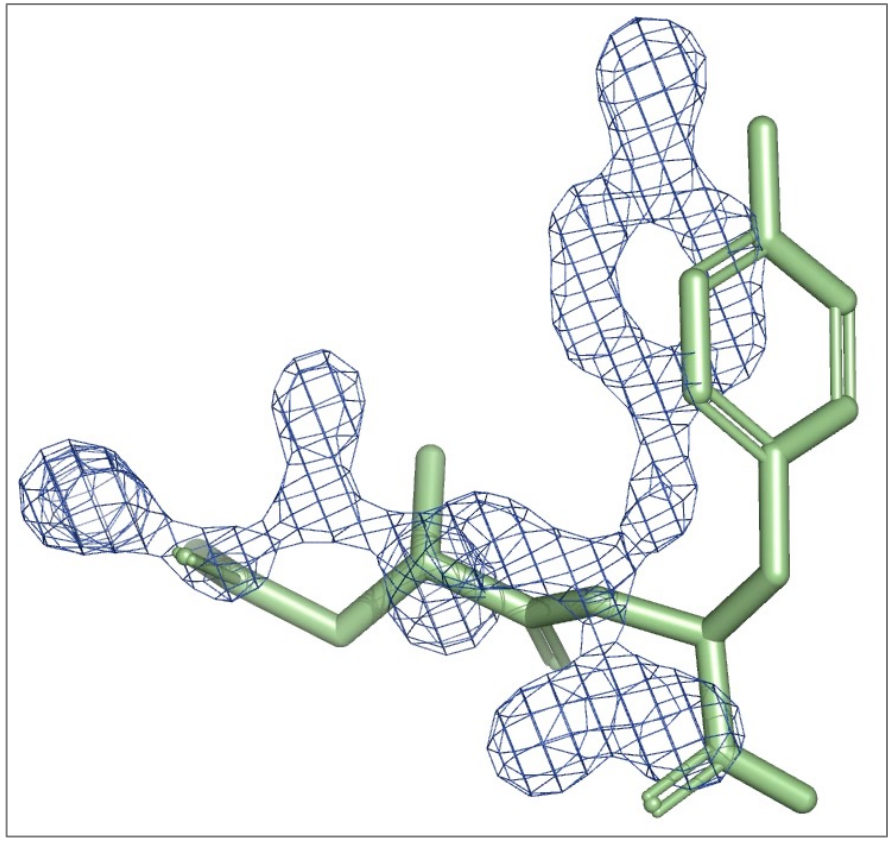


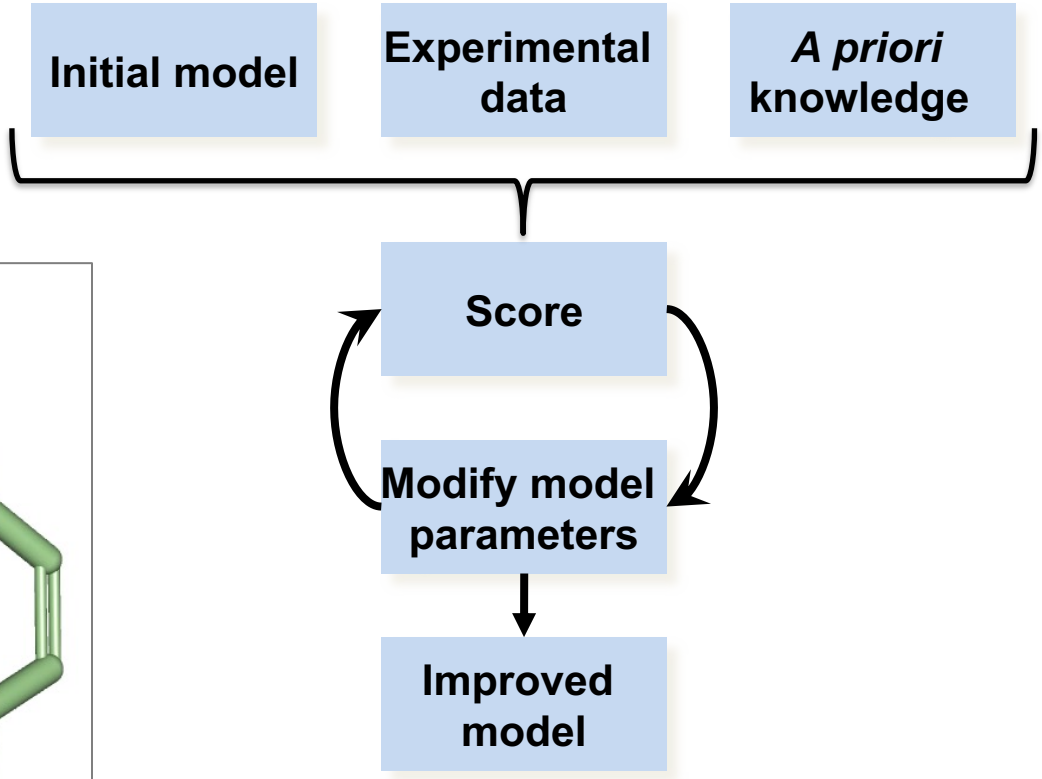
Refinement



- All the above move model to the map. The difference is: by how much

Model refinement

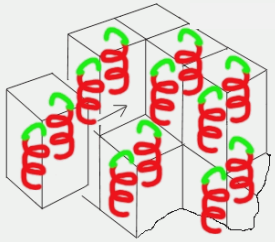


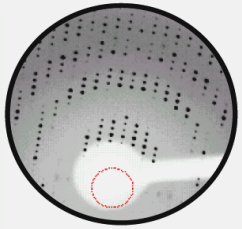


Optimization process of fitting atomic model parameters to experimental data

Refinement

Crystallography





Initial model

Experimental data

A priori knowledge

Score

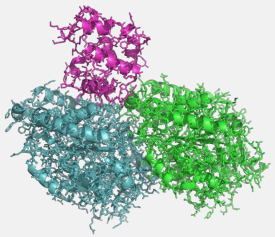
Modify model parameters

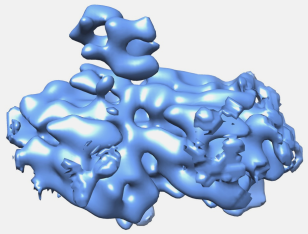
Improved model

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: crystallography vs cryo-EM

Crystallographic refinement

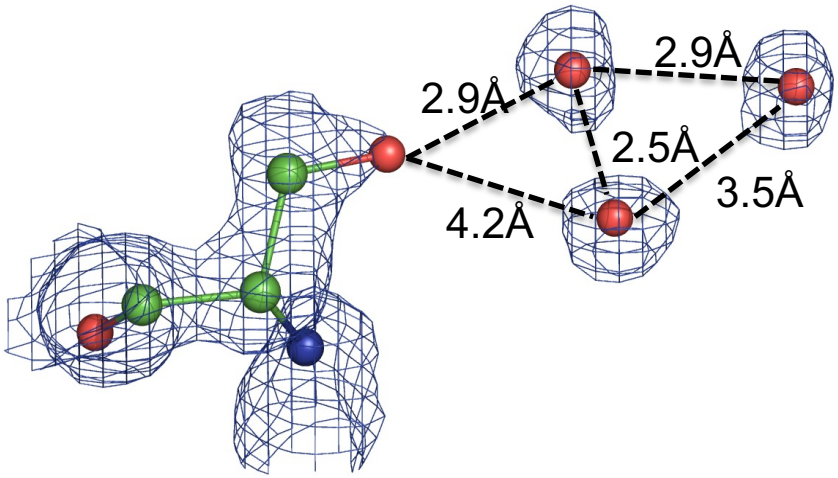
- Improving model improves map
  - (2mFo-DFc, Model phase), (mFo-DFc, Model phase)
  - Better model leads to better map
  - Better map leads to more model built
  - Improving model in one place lets build more model elsewhere in the unit cell
  - Refine all model parameters (XYZ, B) from start to end 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

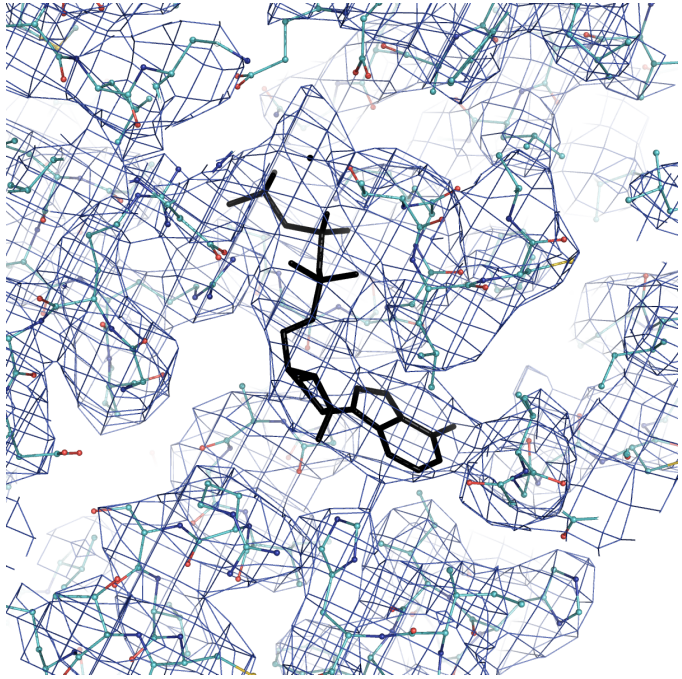
- Changing model does not change map
  - Build solvent (water) last
  - Get as complete and accurate model as possible before refining B factors and occupancies
- Experimental data changes a lot during the process (filtering, boxing, using maps with implied symmetry or not, etc.)
  - What map to use 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

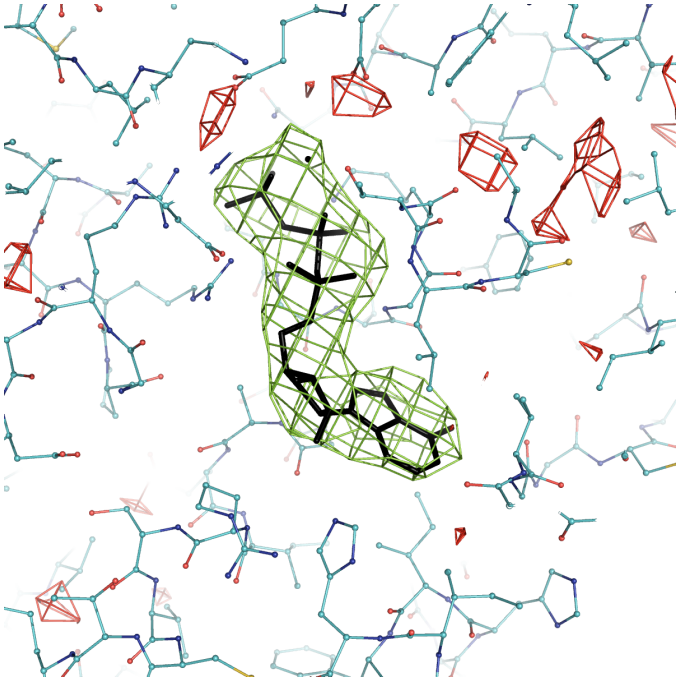
CryoEM

Build water, refine B and occupancy towards the end



Maps and refinement





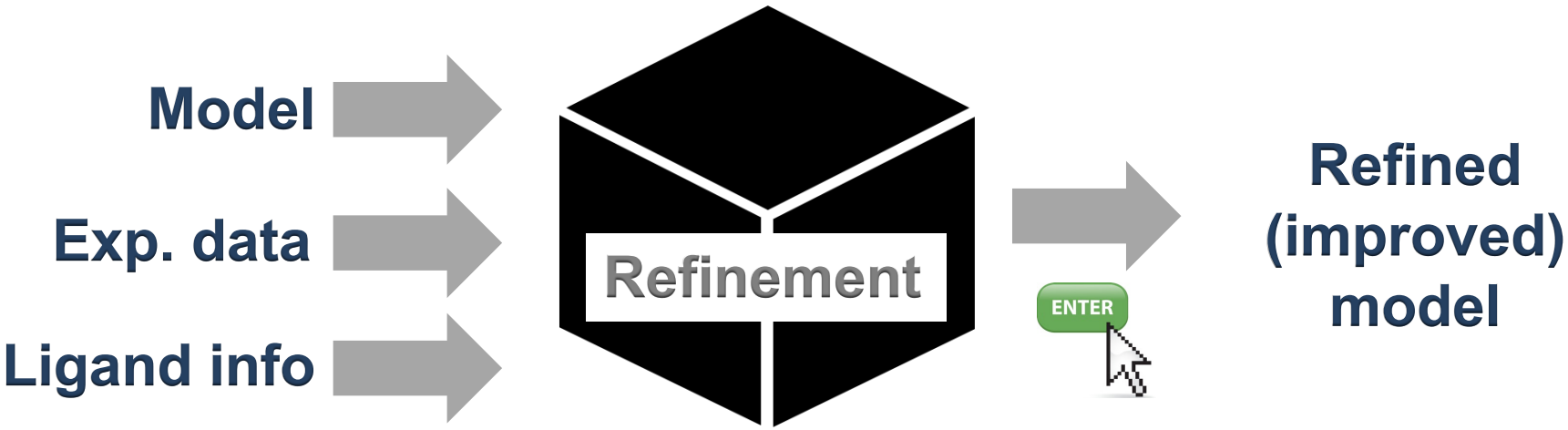
- Analogue of crystallographic Fo-Fc map
- Requires well-refined model (including B factors)

Solving structure in the past

- From many months to years
  - Spend days on graphics (manual atomic model building)
  - Run computations overnight

Solving my first structure back in 1997

Structure refinement: black box



Structure refinement: black box

- Does it always work? No.
- Is it always as easy as *poor model in, better model out*? No.
- Why? Because:
  - Refinement parameterization too complex
    - ~ 2 Å resolution data, model reasonably fits data
  - Default settings suit most common scenario
  - Less typical situations need customizations
    - E.g., very Low- or high-resolution data need special attention
- How you know: refined worked? Correctly? Time to stop?
  - Do Validation throughout!

Structure refinement: lots of jargon

Reference model?

TLS?

Rotamer fixing?

AltLocs?

Group B vs individual?

Local minima?

ADP?

Clashes?

NCS?

IAS?

tNCS?

Grid search?

Weights?

CDL?

SA?

Minimization?

Rama plot restraints?

f' & f''?

Hydrogens?

Restraints?

Bulk-Solvent?

Rigid body?

Rama-Z?

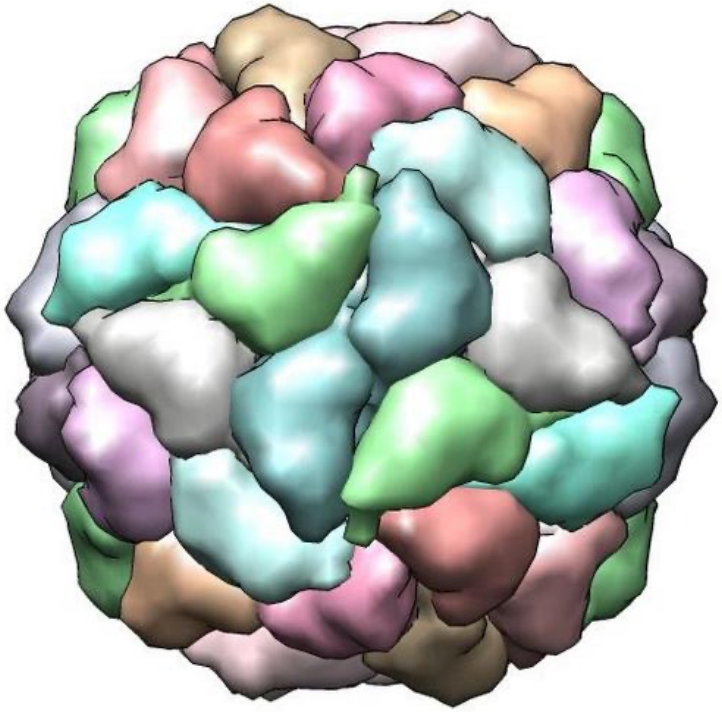
Anisotropy?

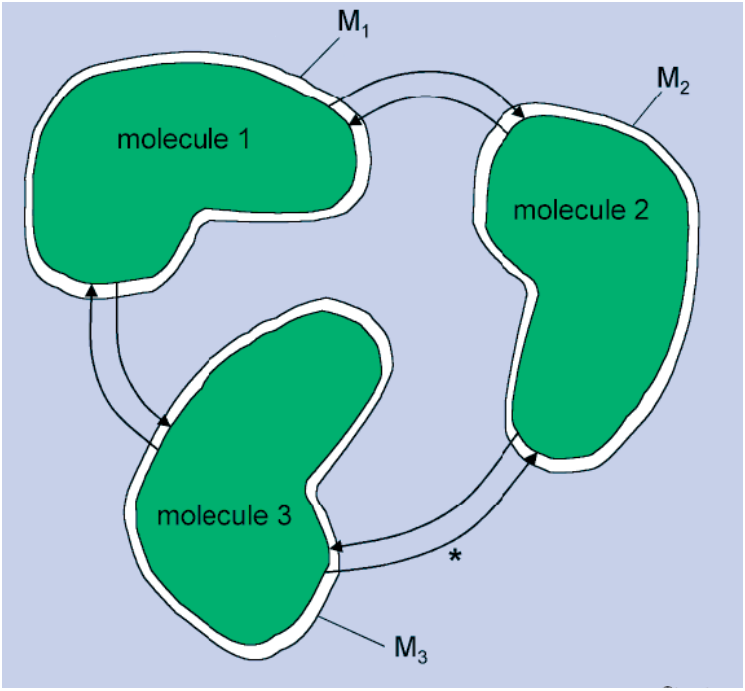
NQH flips?

SS restraints?

Twinning?

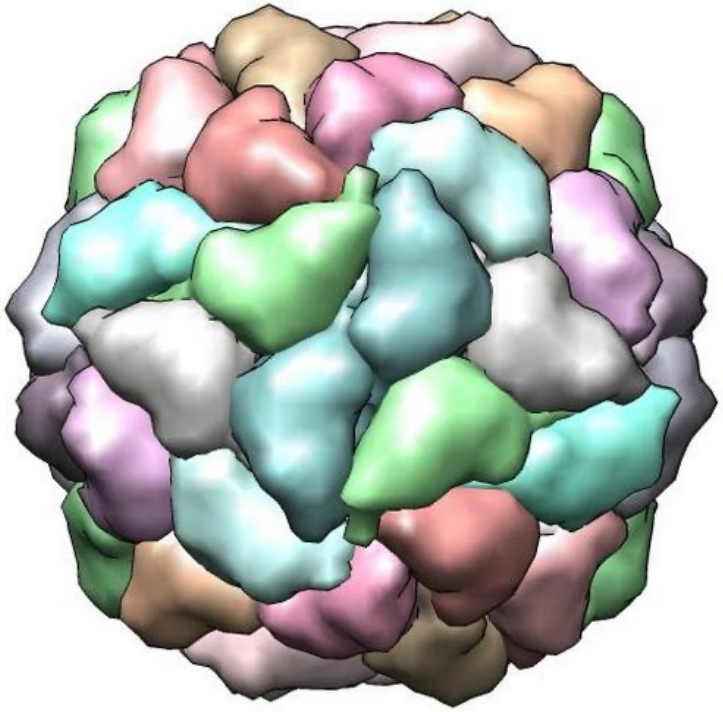
NCS (internal symmetry): constraints vs restraints

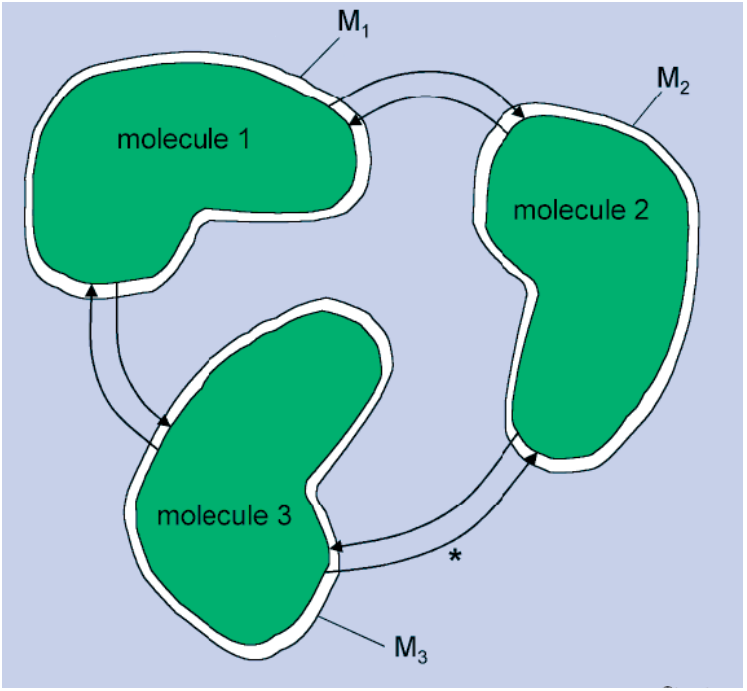




Source: Internet

NCS (internal symmetry): constraints vs restraints





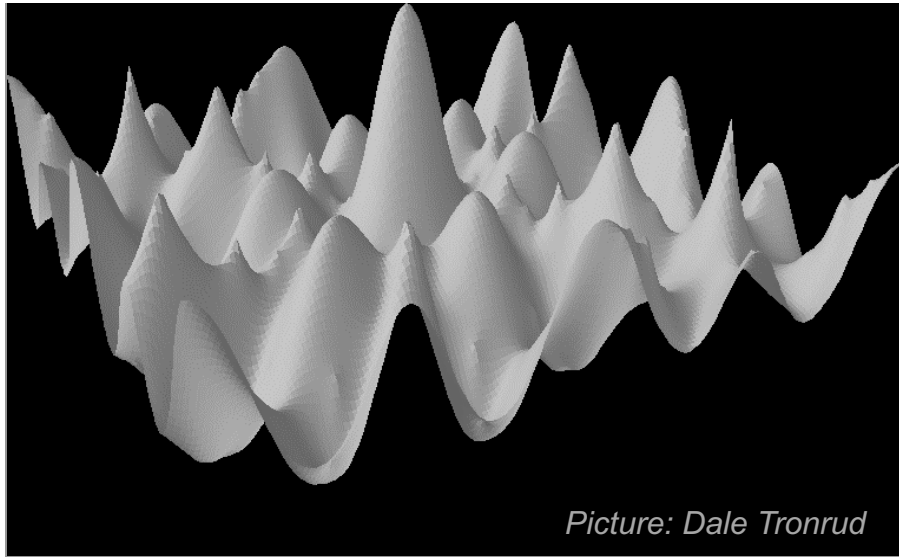
Source: Internet

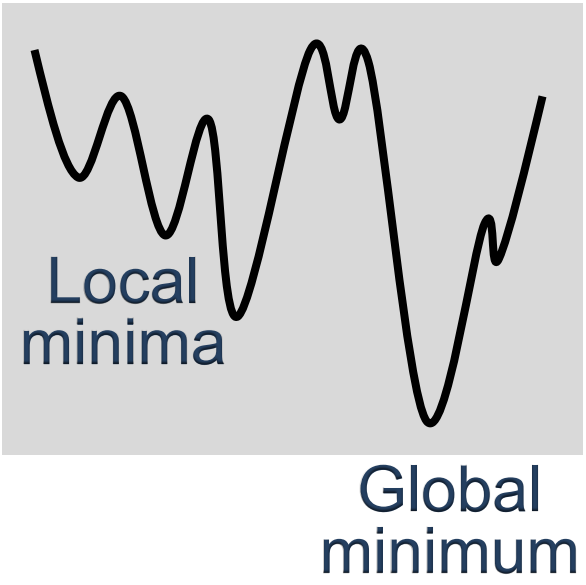
- **Constraints**: molecules 1, 2 and 3 are required to be identical

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

Complexity of refinement target

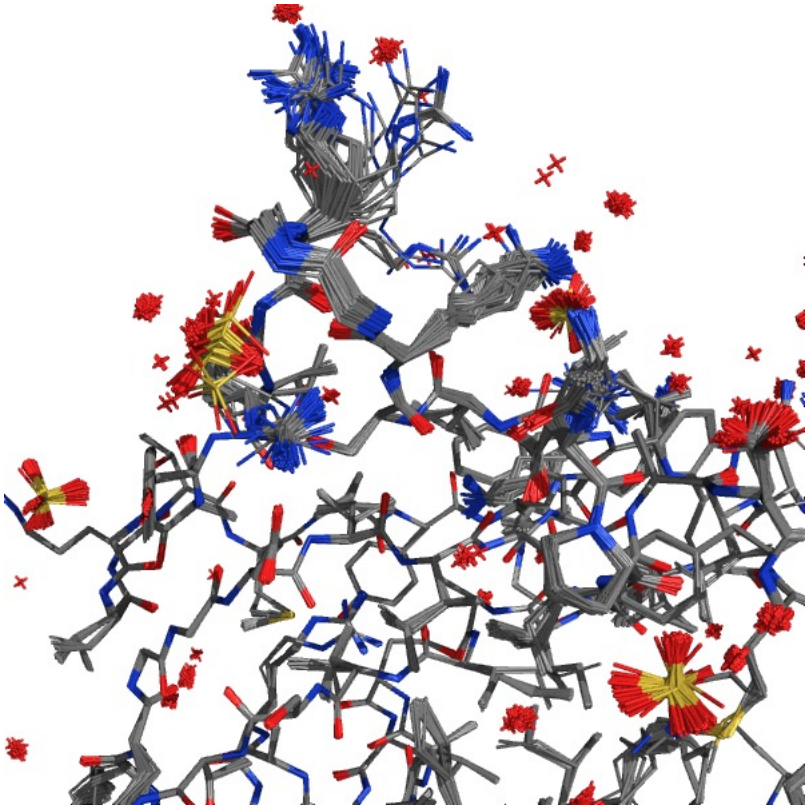
- Refinement target function (score) has very complex multi-dimensional profile

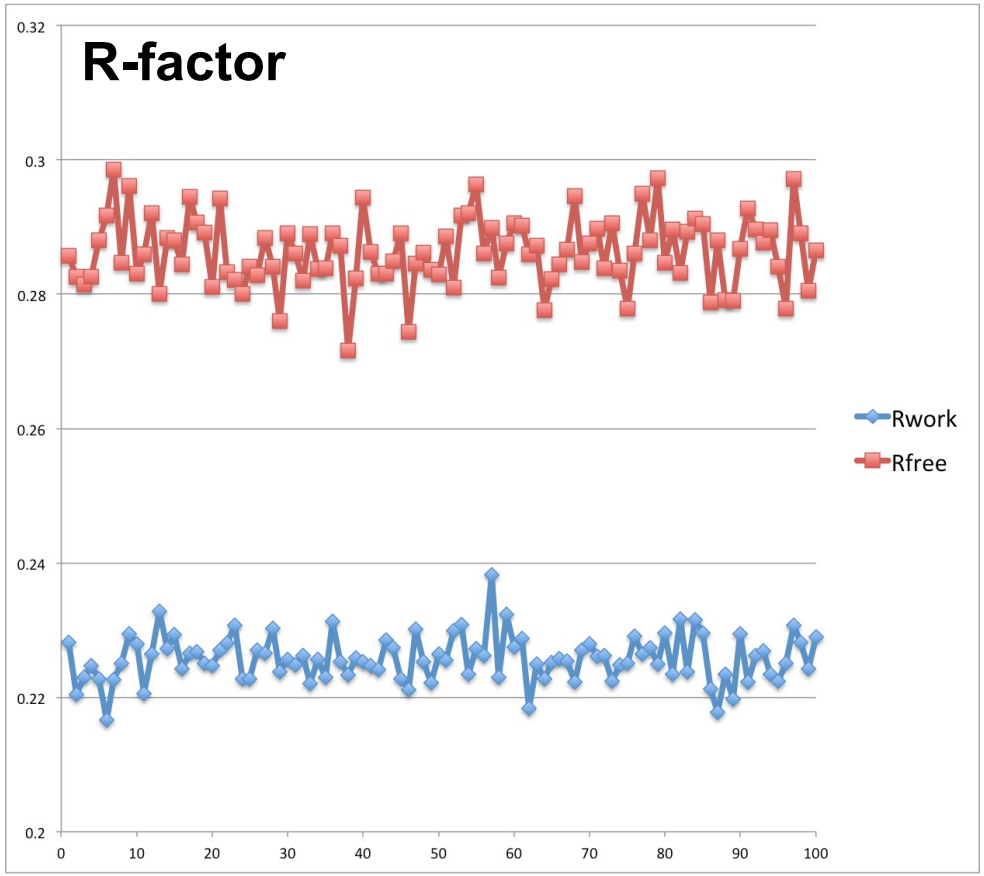




Estimating and using uncertainty

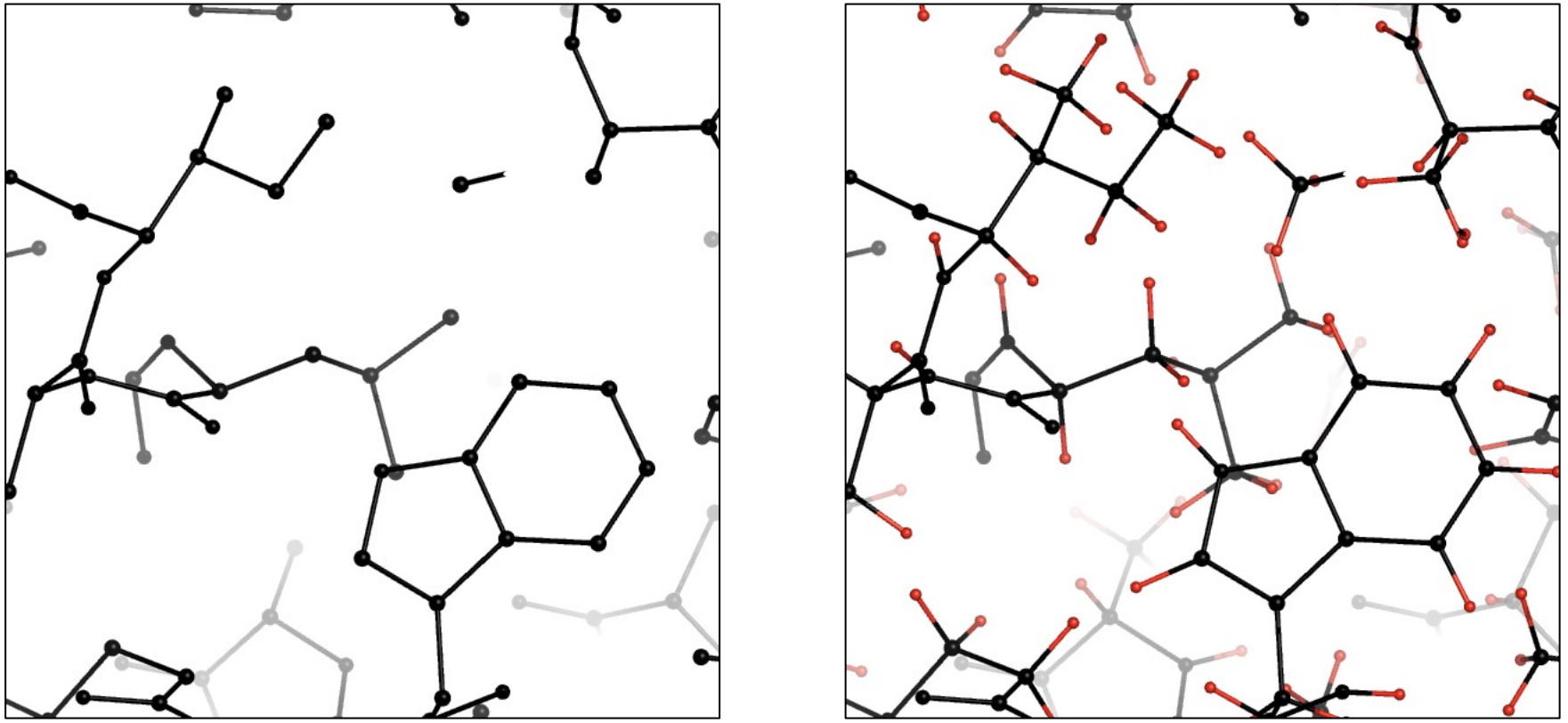
100 identical refinement runs each one starting with slightly perturbed model





Use Hydrogen atoms

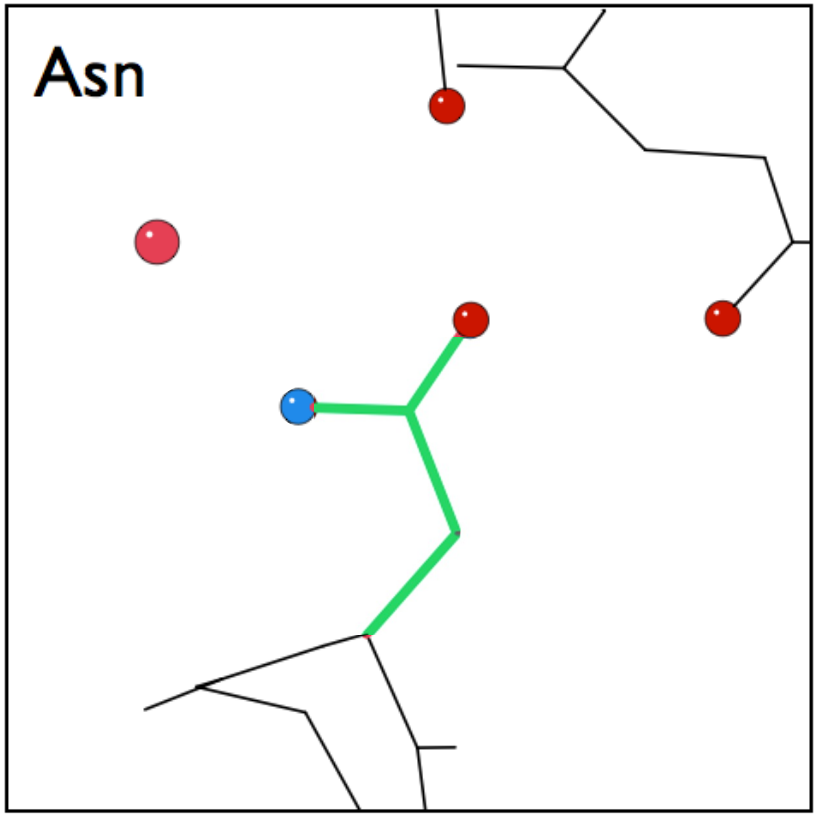
- Half of the atoms in a protein molecule
- Make most interatomic contacts
- Add to model towards the end, data resolution does not matter
- Once added, do not remove before the PDB deposition
- H do contribute to R-factors (expect 0.1-2% drop in R)

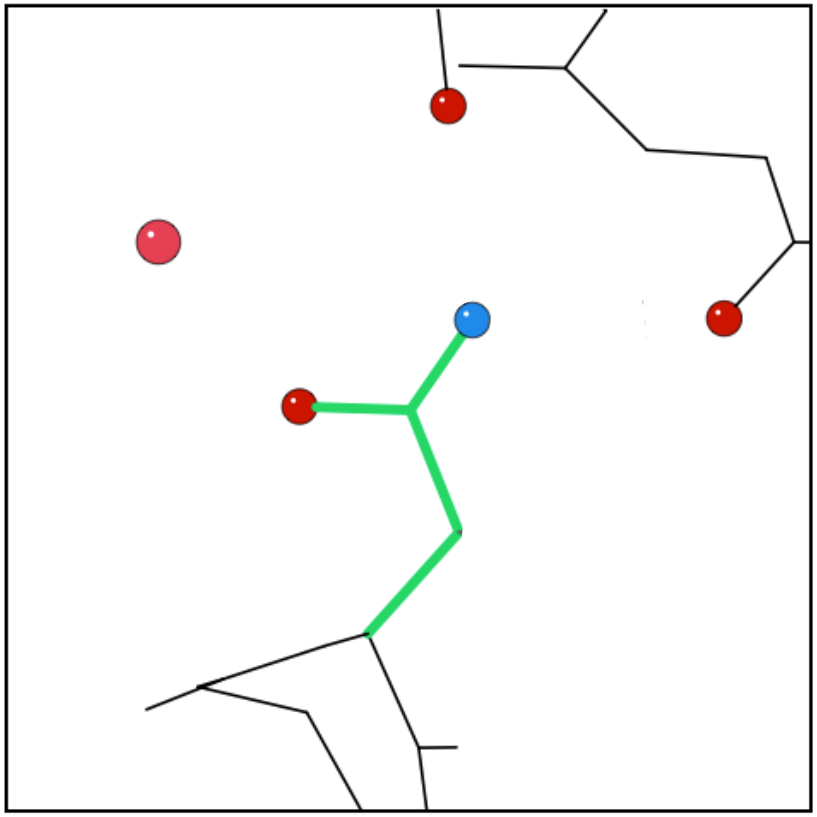


A structure without (left) and with (right) hydrogen atoms

Use Hydrogen atoms

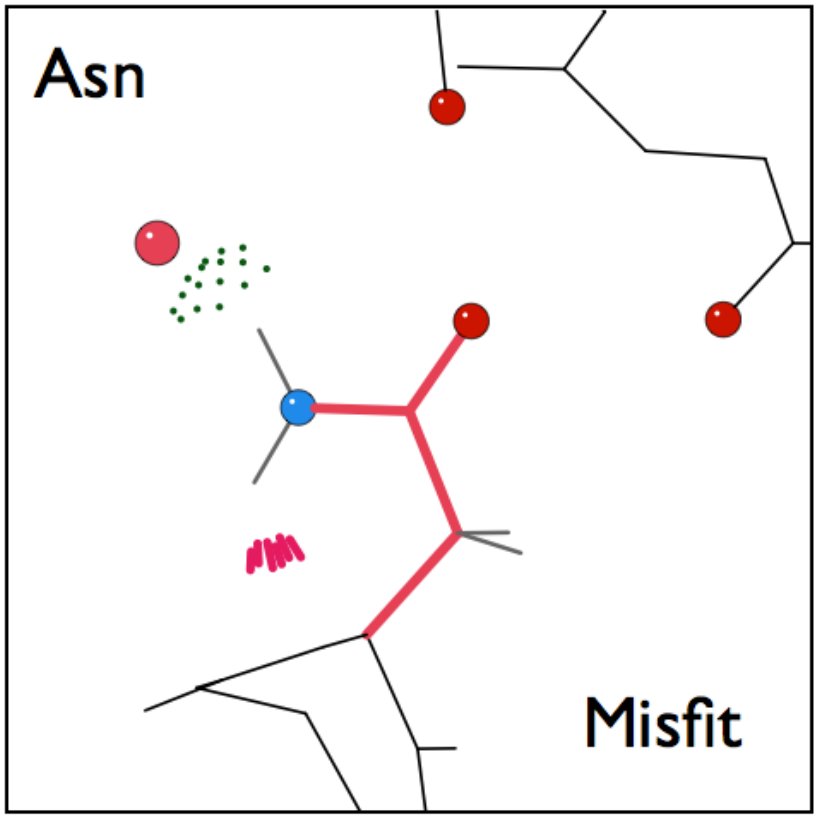
- N/Q/H flips (asparagine/glutamine/histidine)
  - Based on clash analysis
  - Requires H present

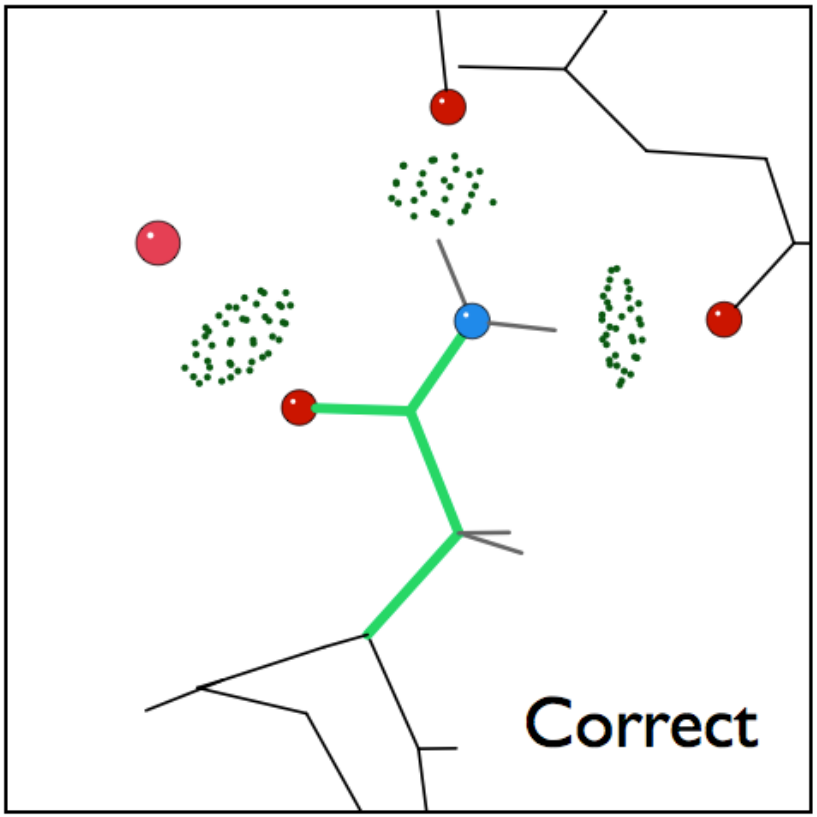




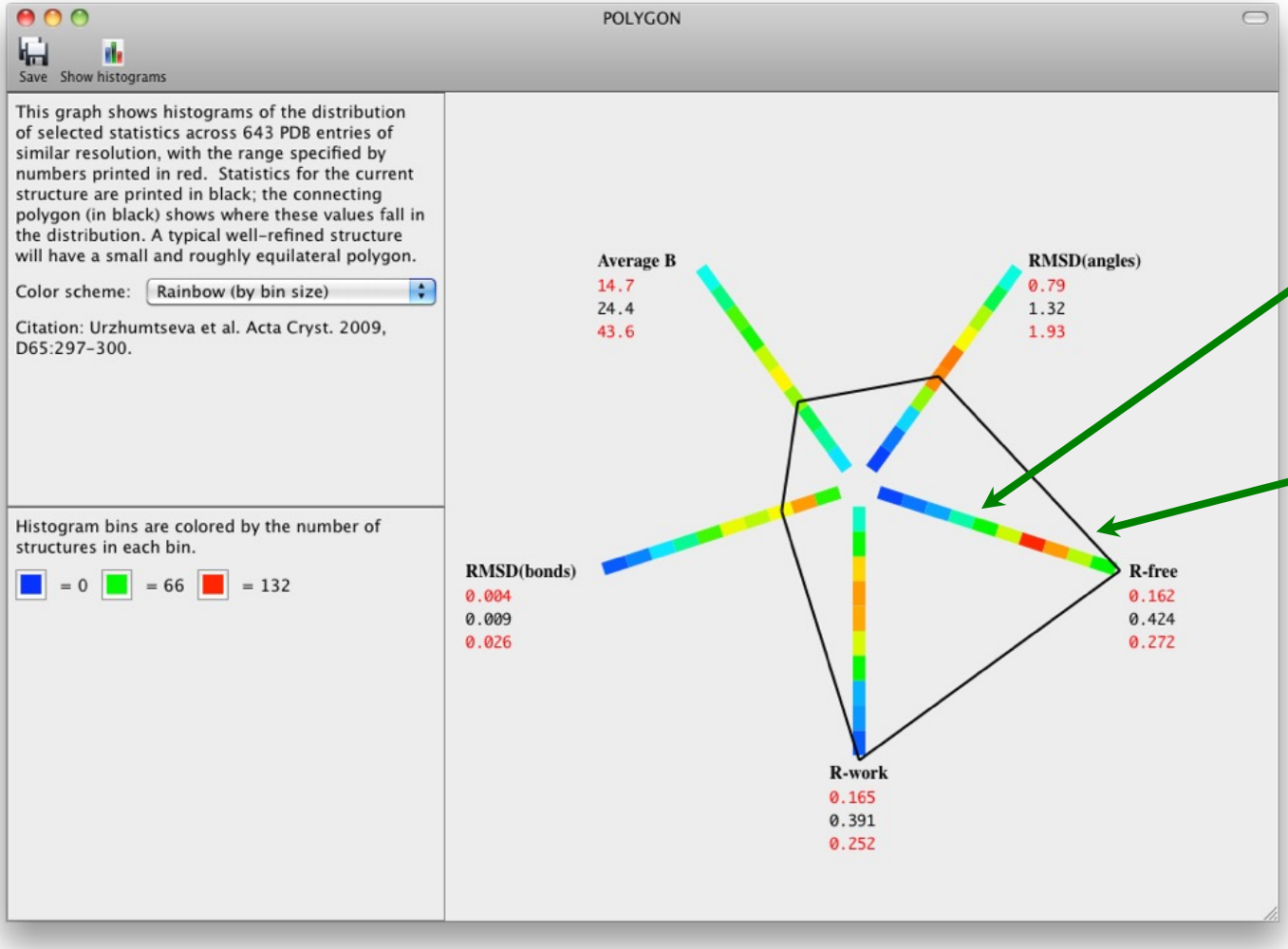
Use Hydrogen atoms

- N/Q/H flips
  - Based on clash analysis
  - Requires H present





Know when to stop



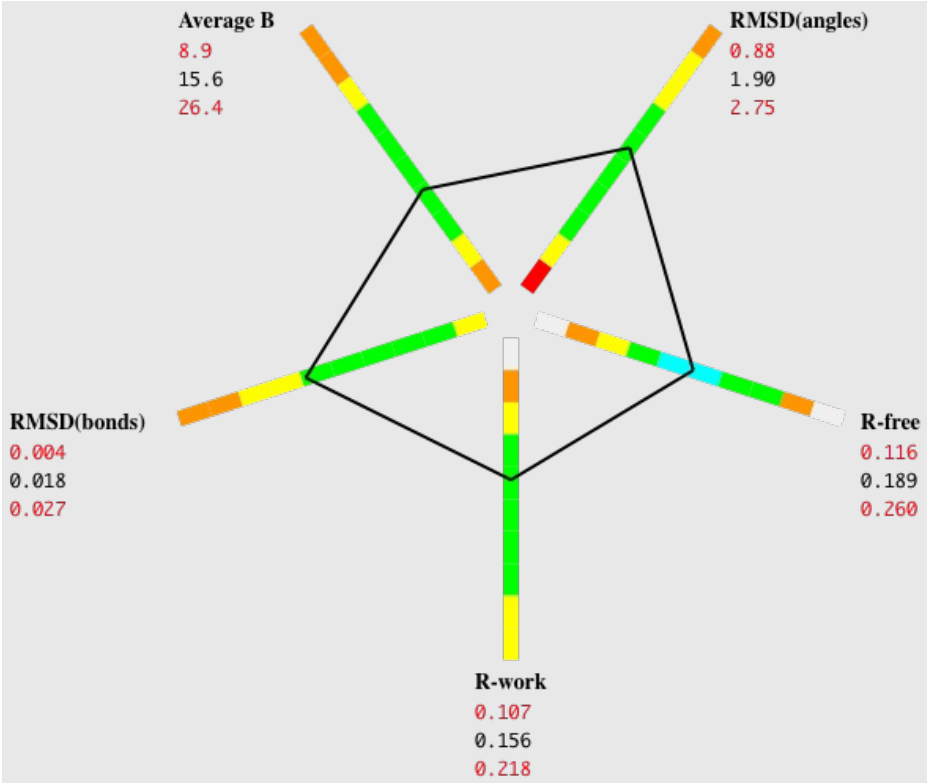
Colored bars are histograms showing distribution of values for structures at similar resolution

The black polygon shows where the statistics for the user's structure fall in each histogram

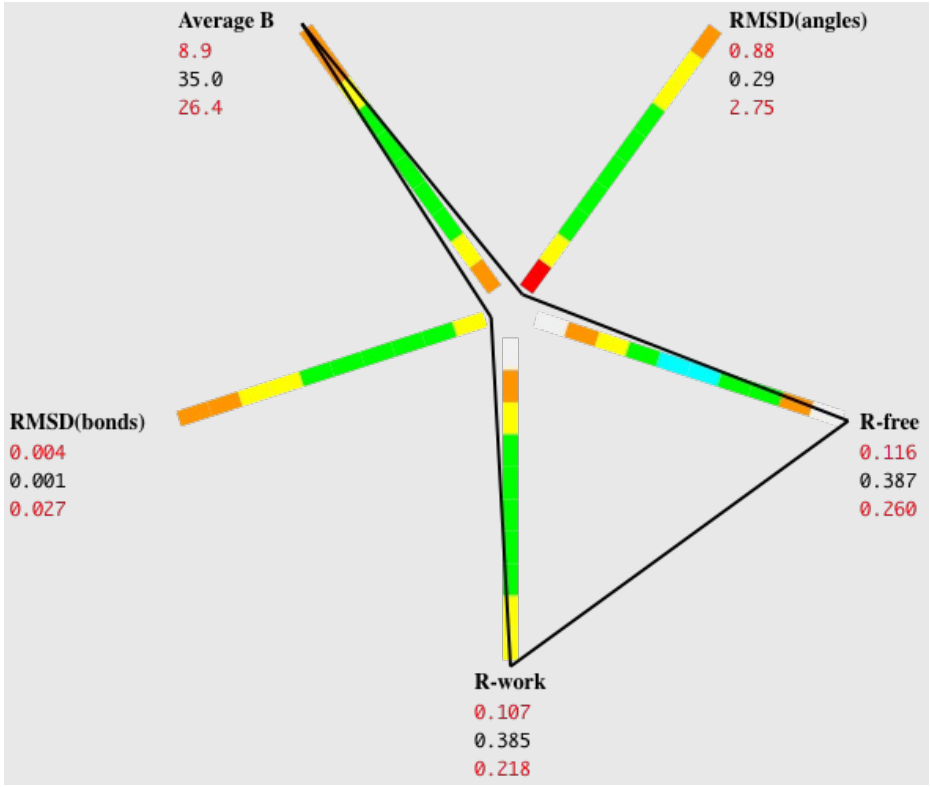
Crystallographic model quality at a glance.
L.Urzhumtseva, P.V.Afonine, P.D.Adams & A.Urzhumtsev. *Acta Cryst*. **D**65, 297-300 (2009)

Know when to stop

Likely overall good model

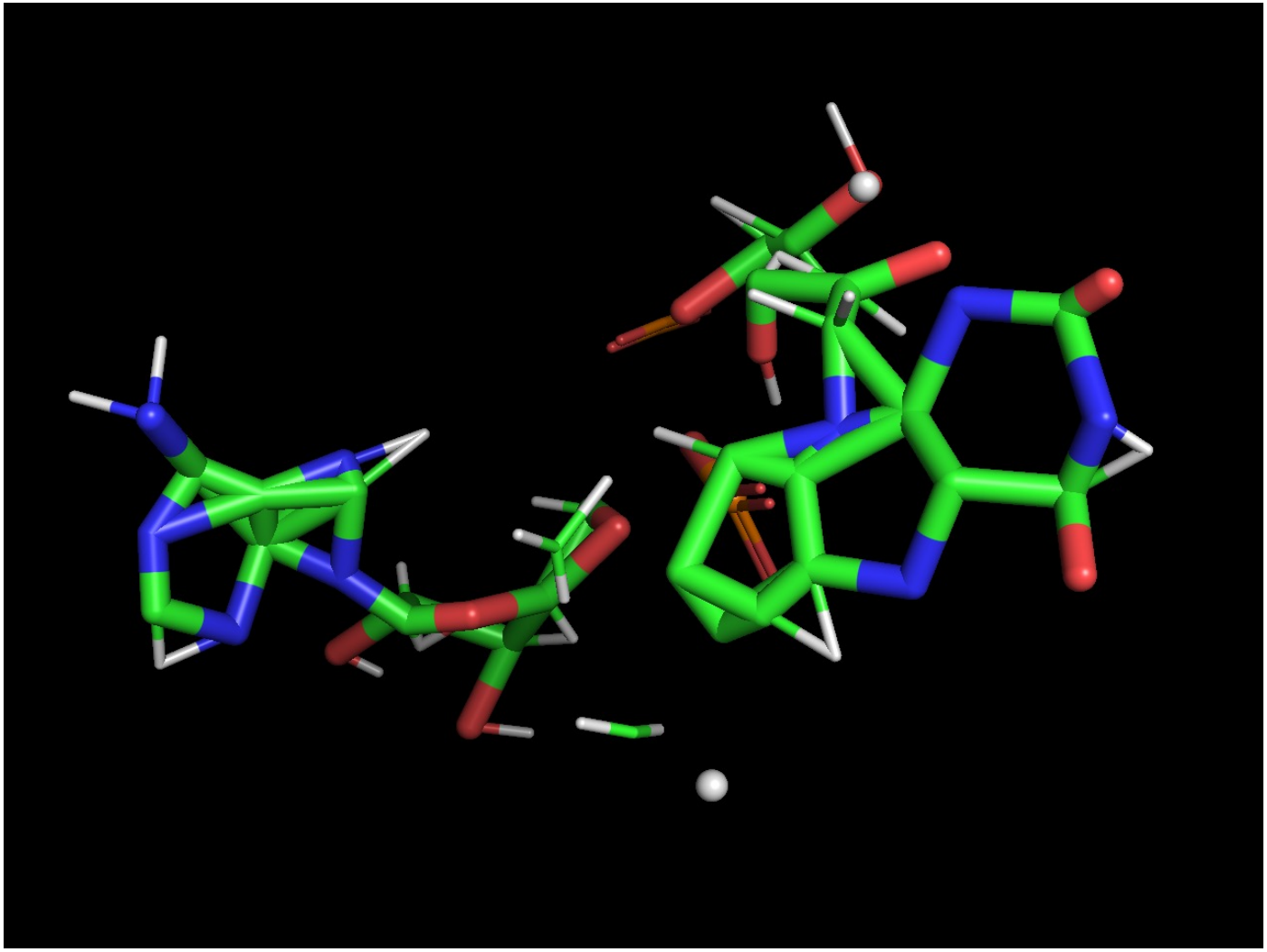


Clearly there are problems

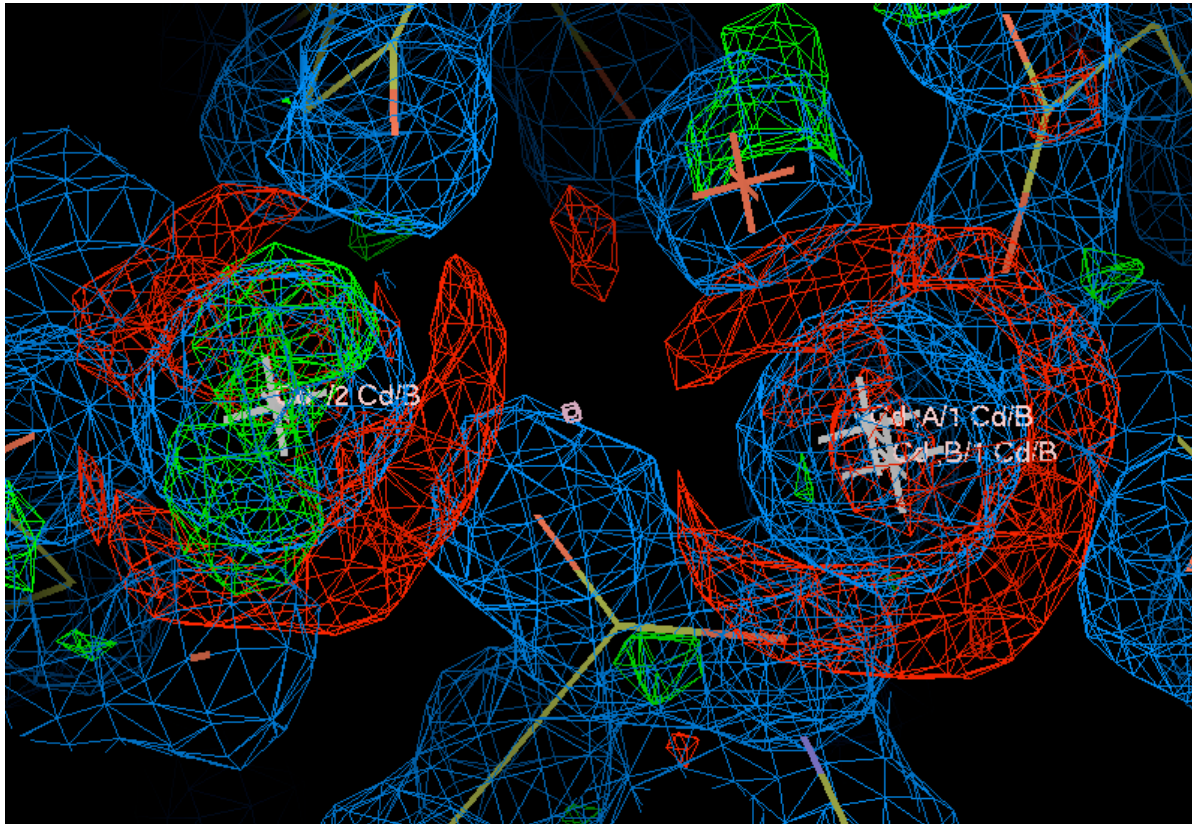


Local *vs* Global

- R_{WORK}/R_{FREE} , bond/angle RMSDs etc do not report on local errors



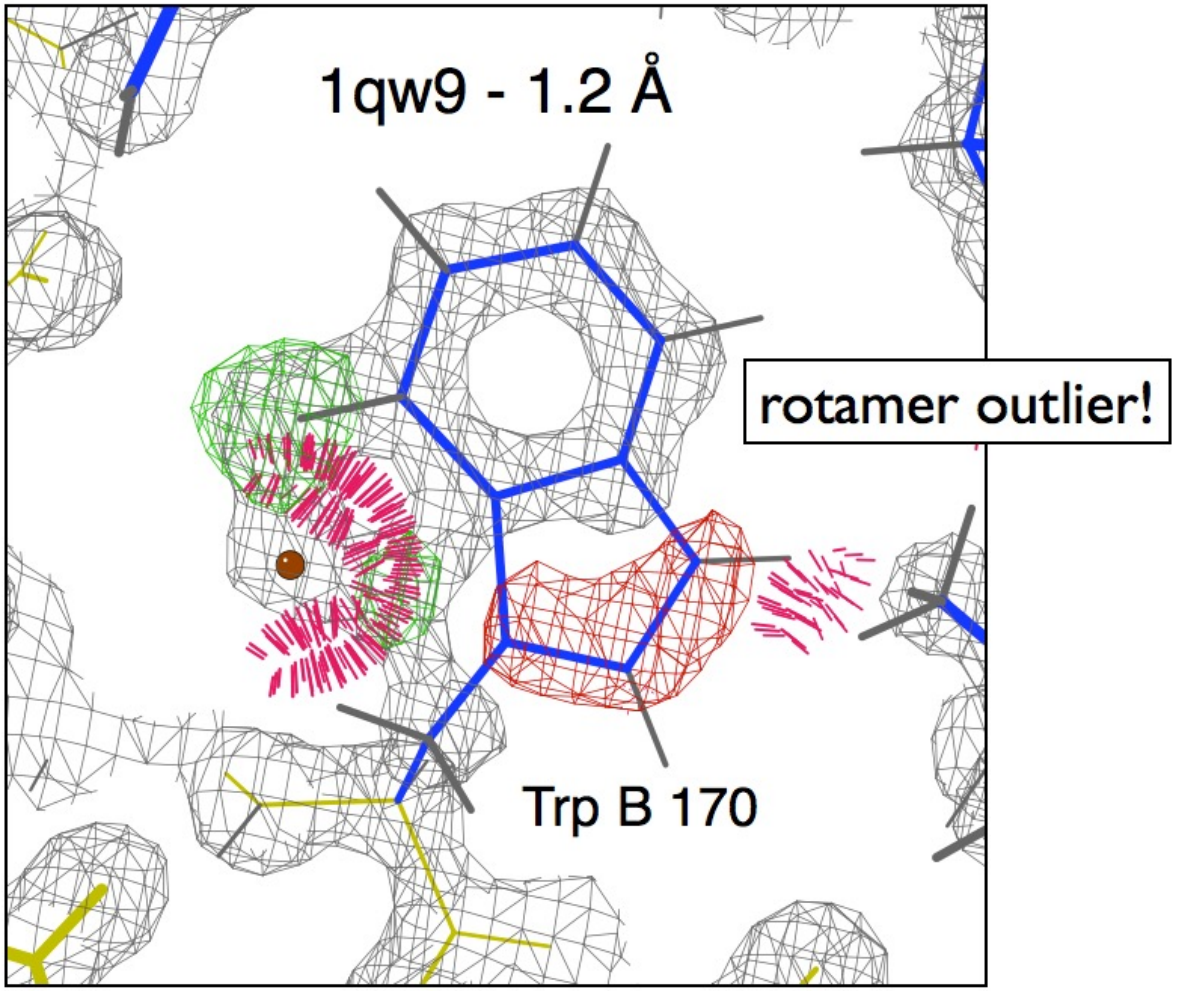
Map and model errors



Reasons for +ve/-ve density:

- Suboptimal xyz, occupancy, ADP, anomalous f’ & f’’, charge
- Refinement has not reached convergence
- Wrong atom (ion)
- Suboptimal ADP (B-factor) type: isotropic vs anisotropic

Not all modeling errors can be fixed by refinement



Low resolution (3Å or worse)

- Use:
  - Ramachandran plot restraints
  - Secondary structure restraints
  - Reference model restraints (if quality homology model is available)
  - NCS (restraints or constraints)

Aggressive optimization methods

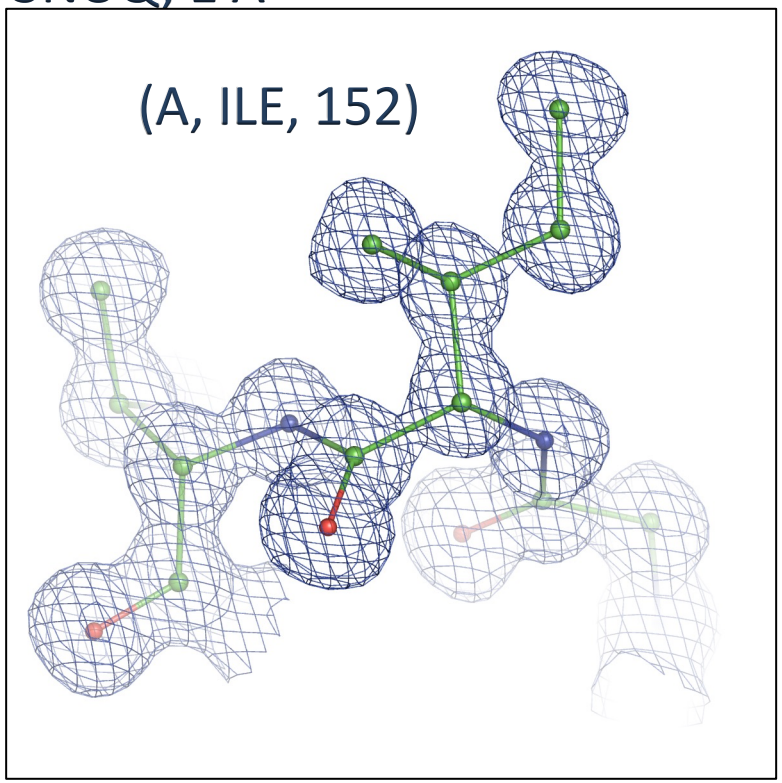
- Simulated annealing (SA)
- Model morphing
  - Only use if model has gross errors (correction requires large movements)
  - Do not use if model is relatively good and only needs small corrections

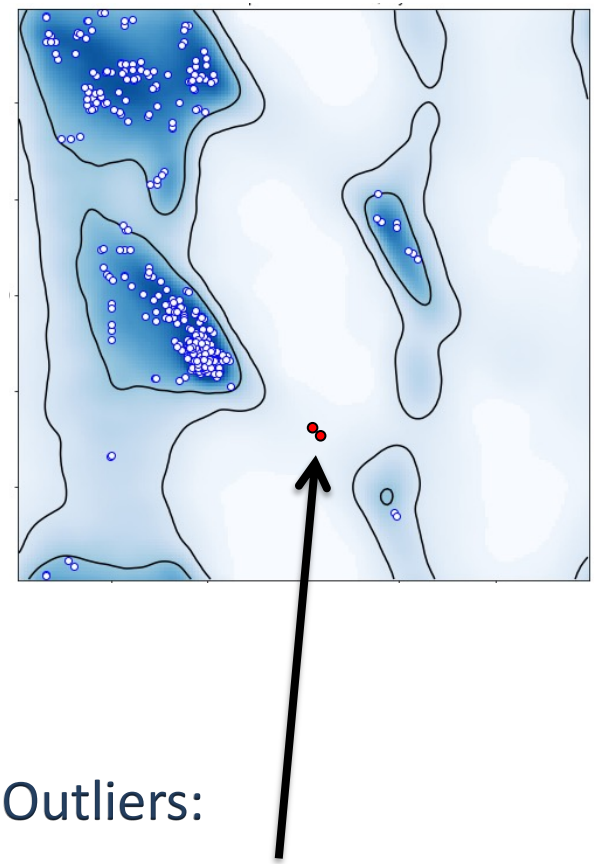
Ramachandran plot restraints

- Likely need at about 3Å and worse
- Better than 3Å: use if needed (preserve good initial model from deterioration)
- Check Ramachandran plot regularly
- Don’t use to fix outliers. Fix outliers first (manually), then use Ramachandran plot restraints to stop re-occurring outliers

An outlier ≠ wrong

3NOQ, 1 Å





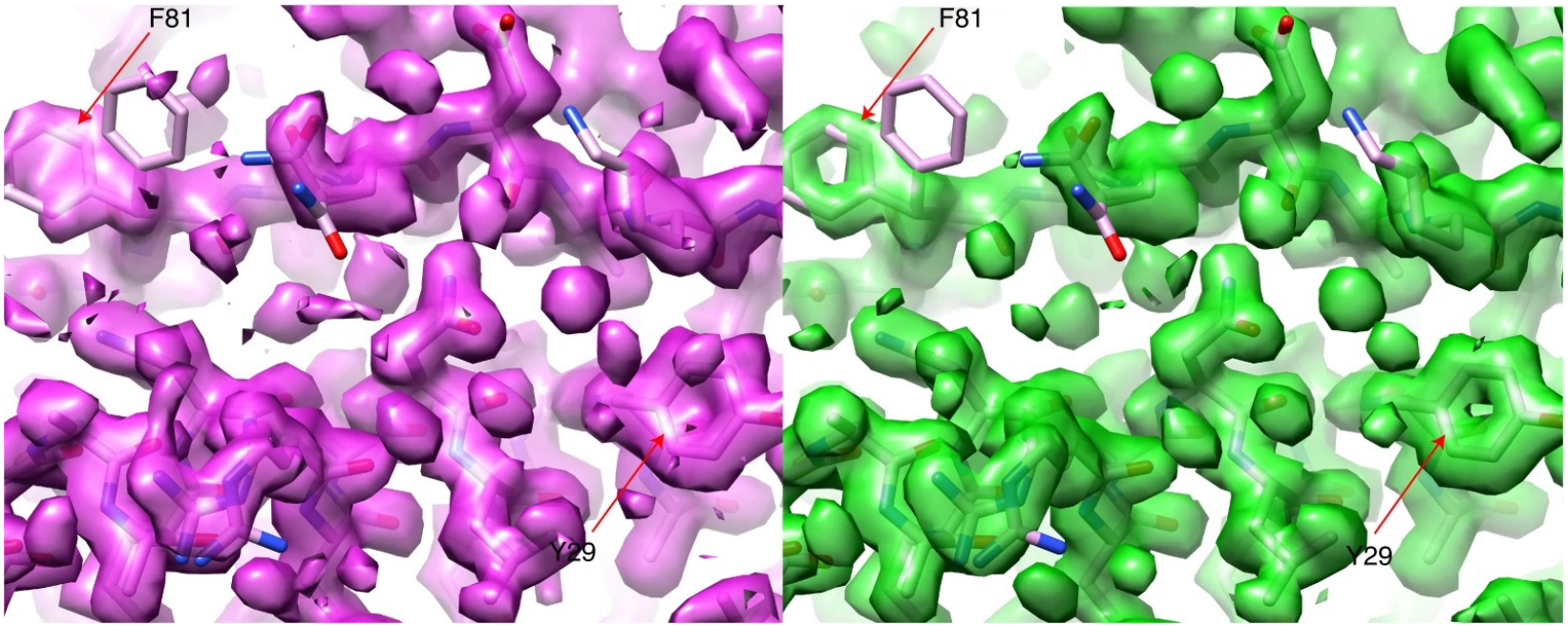
Outliers:

(A, ILE, 152), (B, ILE, 154)

- All outliers need to be explained (supported by the data)

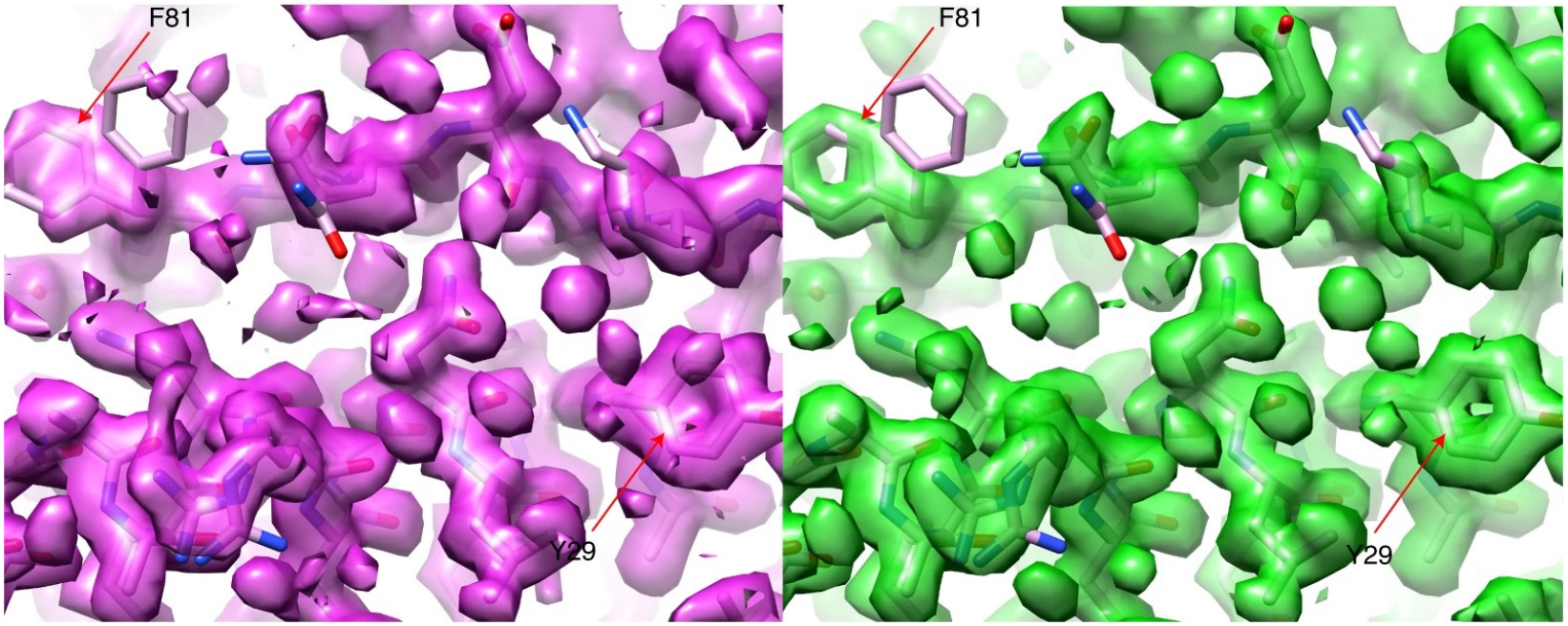
Map comparison

Need to compare (meaningfully) maps side-by-side?



How you choose contouring levels for both maps?

Map comparison

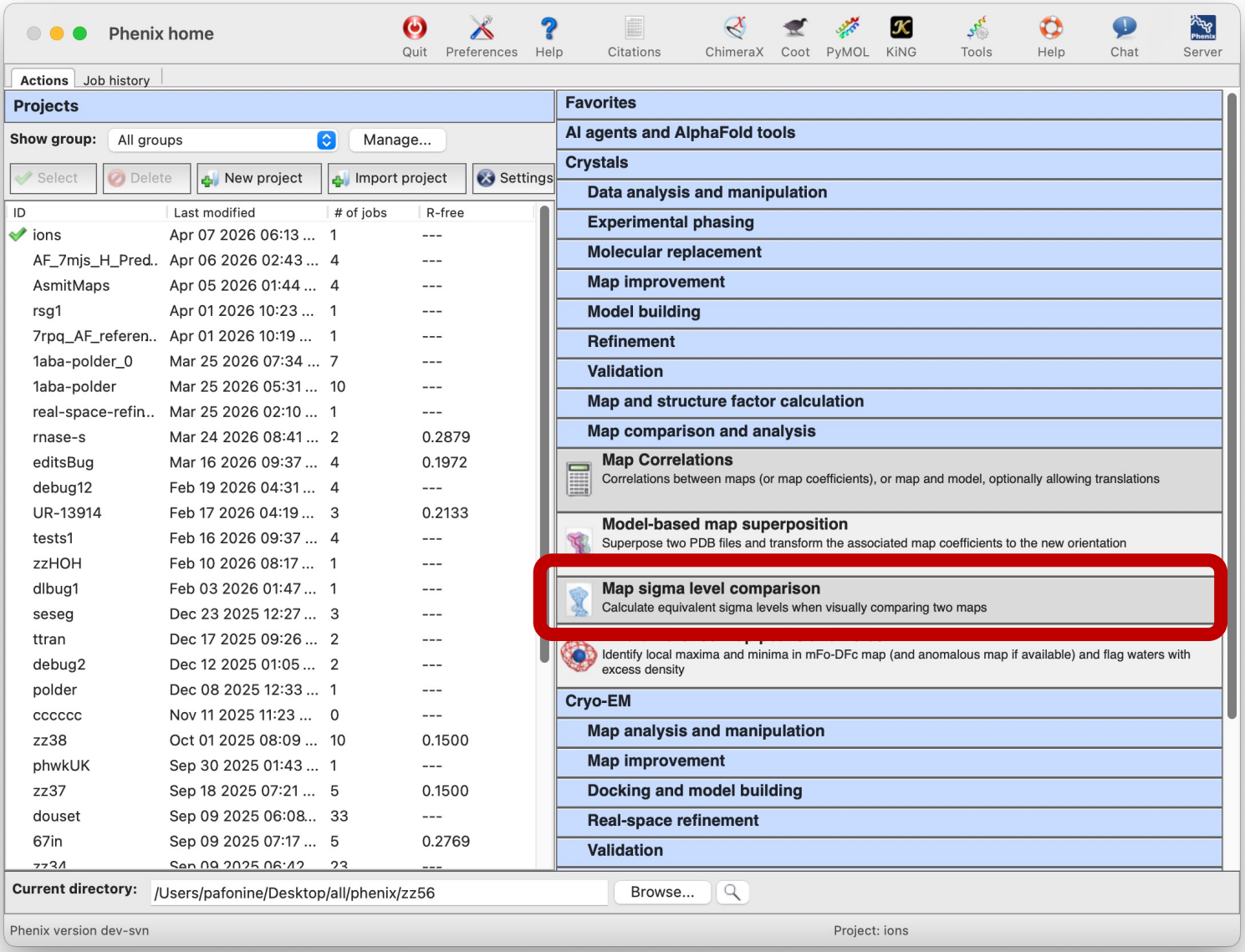


Use contours that enclose equal volumes.

Reason: contour levels then represent the same amount of displayed signal—independent of different noise levels or overall scaling.

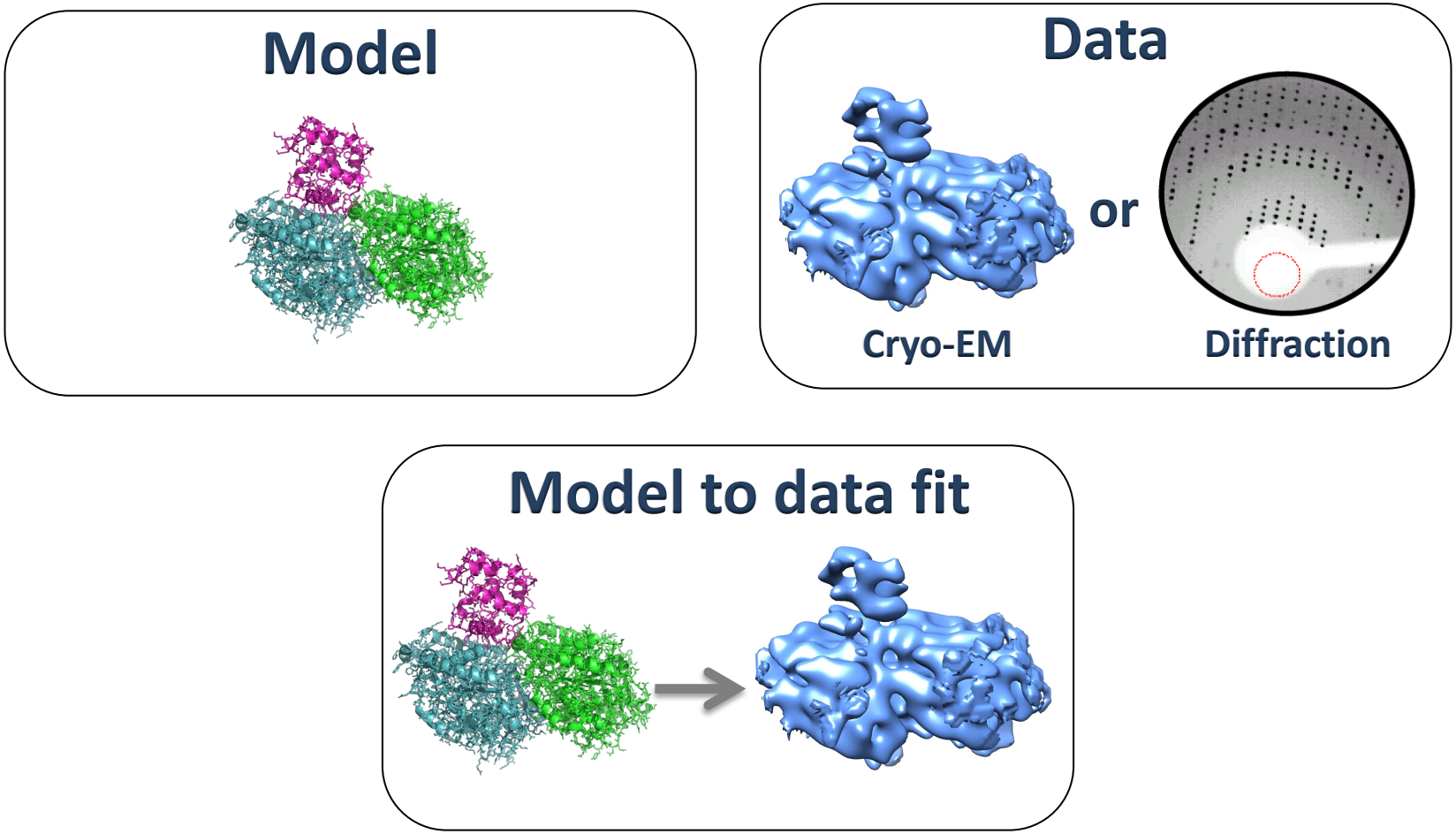
Result: side-by-side visuals show real differences in where density is and how strongly it's supported, not artifacts from different threshold choices.

Map comparison

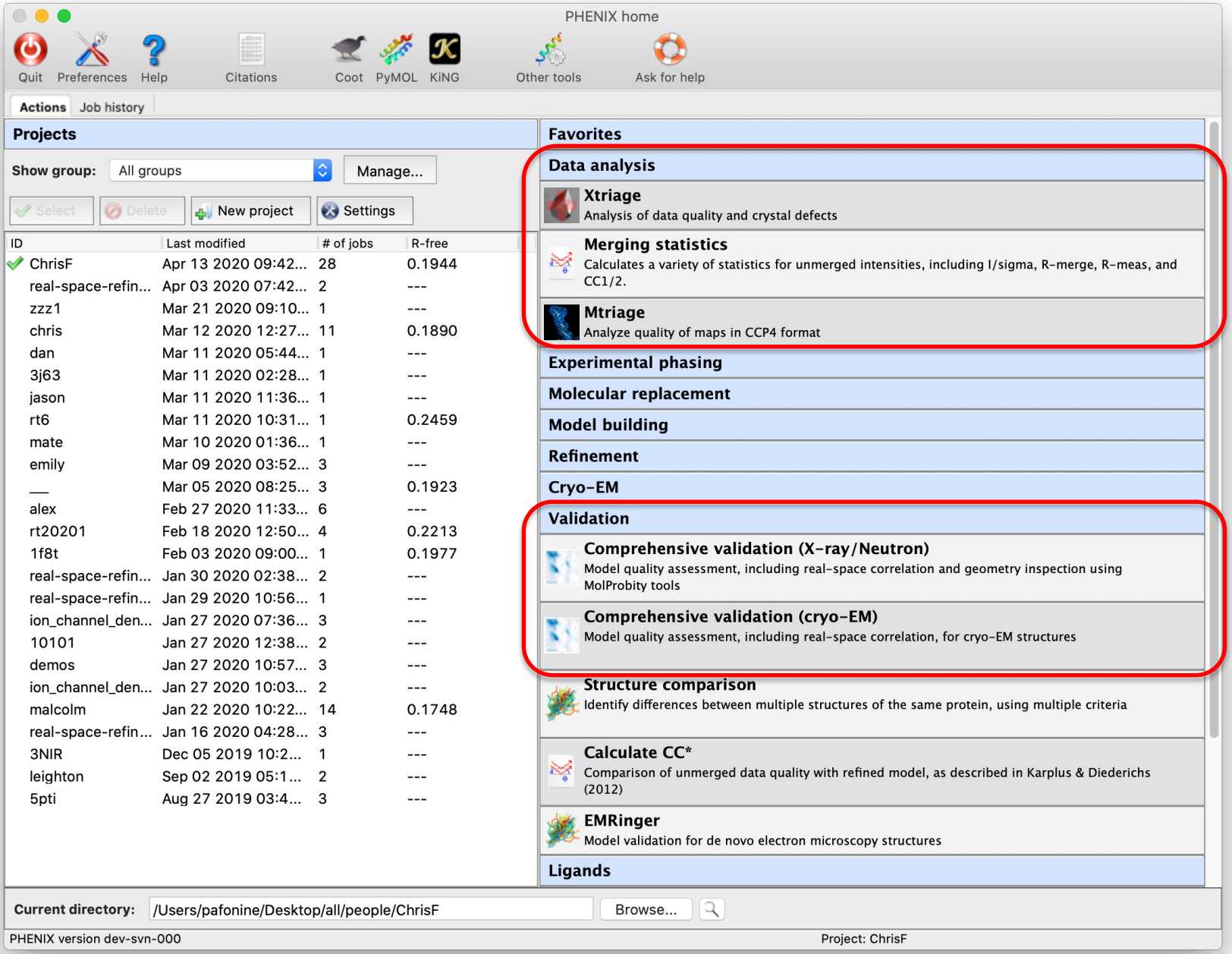


Refinement success is function of data quality

- Do validation

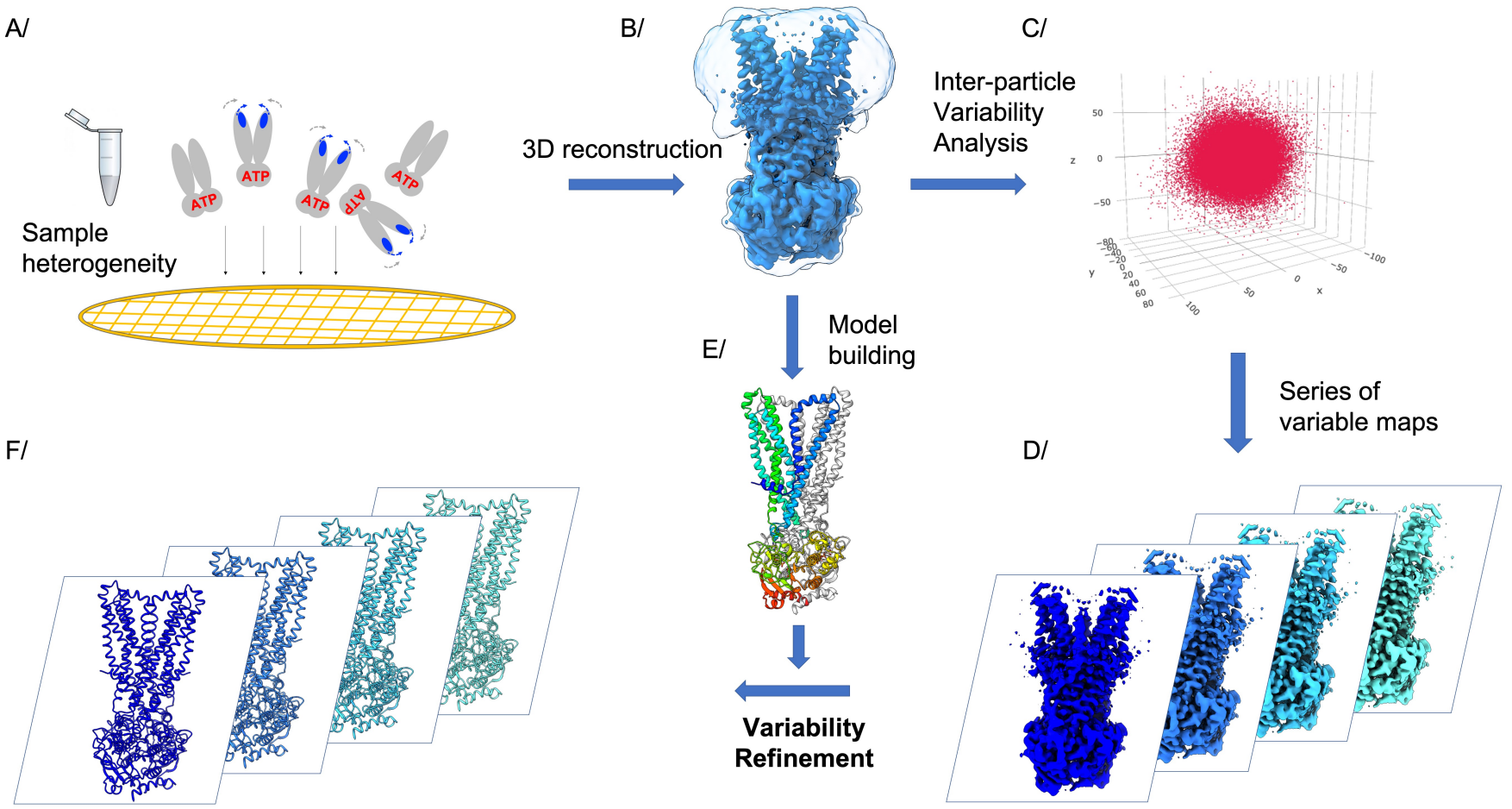


Validation tools in Phenix



Phenix is feedback & need driven

Variability refinement: treasuring conformational changes



Feedback & need driven – Example

Vincent's post on phenix mailing list (phenixbb)

From vincent Chaptal <vincent.chaptal@ibcp.fr> Reply | Reply List | Forward | Archive | Junk | Delete | More

To PHENIX user mailing list <phenixbb@phenix-online.org> 1/11/22, 04:48

Subject **[phenixbb] refinement of an ensemble of structures -> cryoEM variability**

Hi Phenix-ers,

I thought to ask for something that I believe you have already implemented, but I'm not sure of the best tool to use.

I have a cryoEM map where I refine my "high resolution" structure. I also have the 3D variability of this map that shows several maps varying around the consensus high-res map. I want to refine an ensemble (20) of structures, one for every 20 maps around the consensus map.
Is there a tool in phenix to do this?

I could refine individually the high-res structure into each map incrementally; since every map differs a little from the original one, Real-space-refinement could move the structure a little at a time. Then I could combine all the PDBs in an ensemble?
A tool to refine variability would be very useful. Input could be a PDB and an ensemble of maps, and output would be all the PDBs combined?

Thank you.

All the best
Vincent

--
Vincent Chaptal, PhD
Director of GdR APPICOM
Drug Resistance and Membrane Proteins Lab

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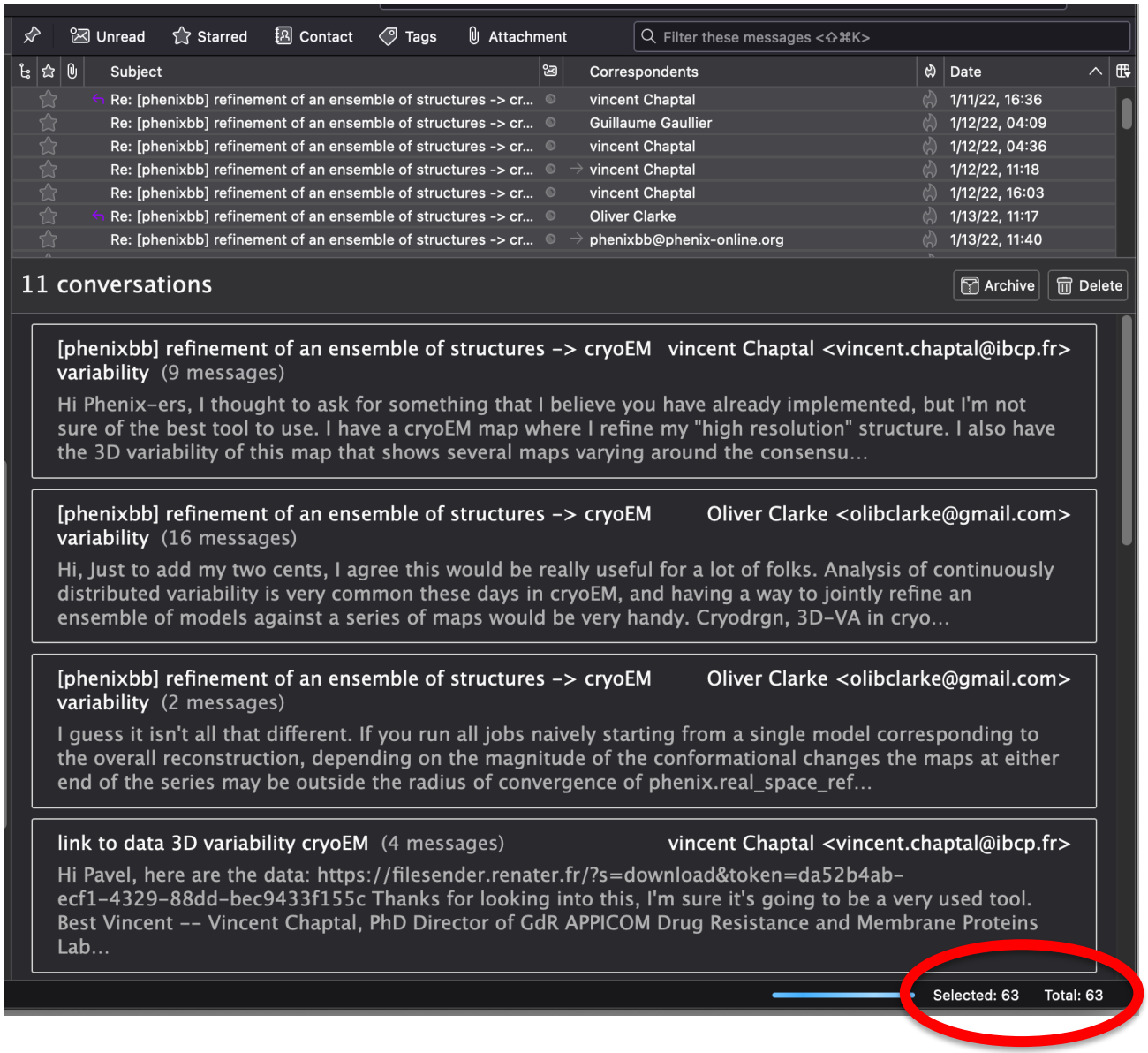
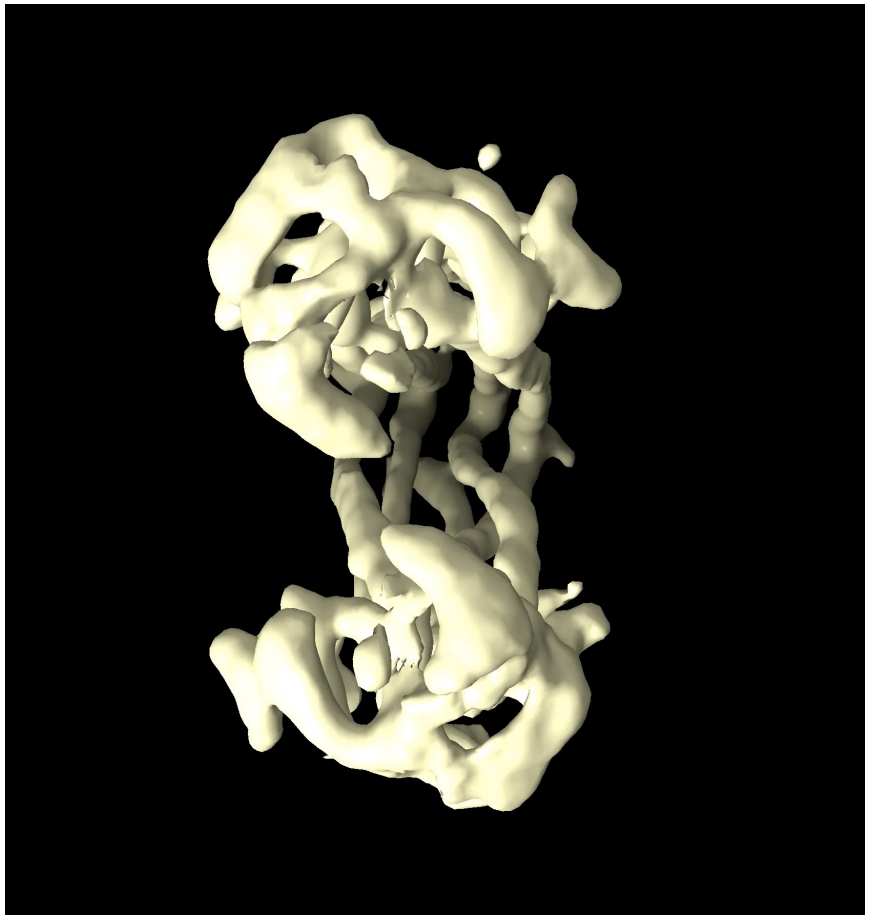
Feedback & need driven – Example

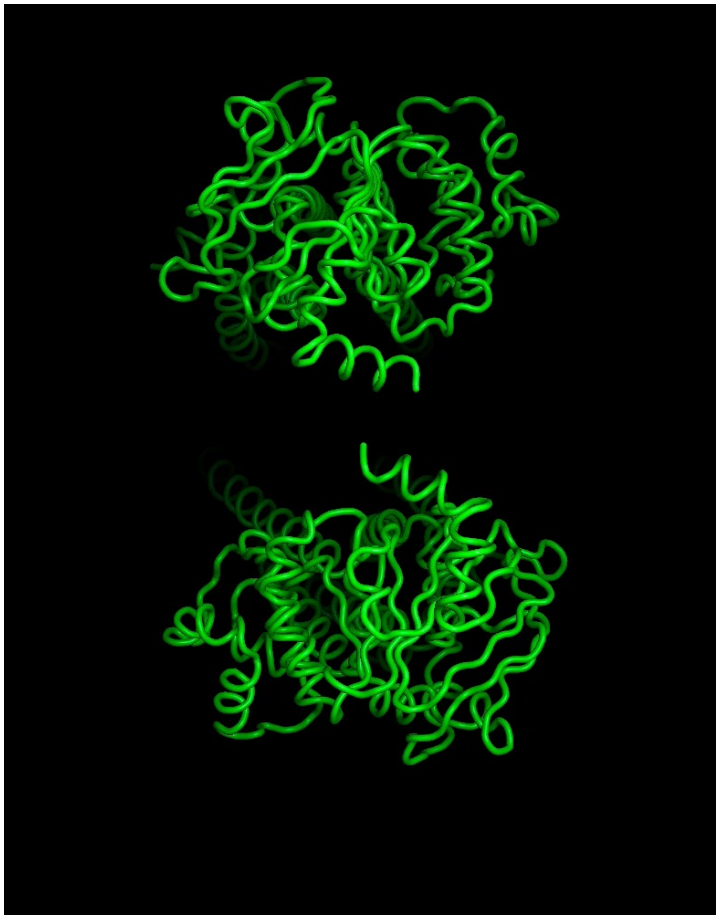


63 emails later….

Feedback & need driven – Varref (Variability Refinement)

… 63 emails later, we came up with a tool to effectively model ensemble of maps with ensemble of atomic models in a fully automated manner…





Feedback & need driven – Varref (Variability Refinement)

… and wrapped that into a publication and a user-accessible tool

Contents lists available at ScienceDirect

BBA - Biomembranes

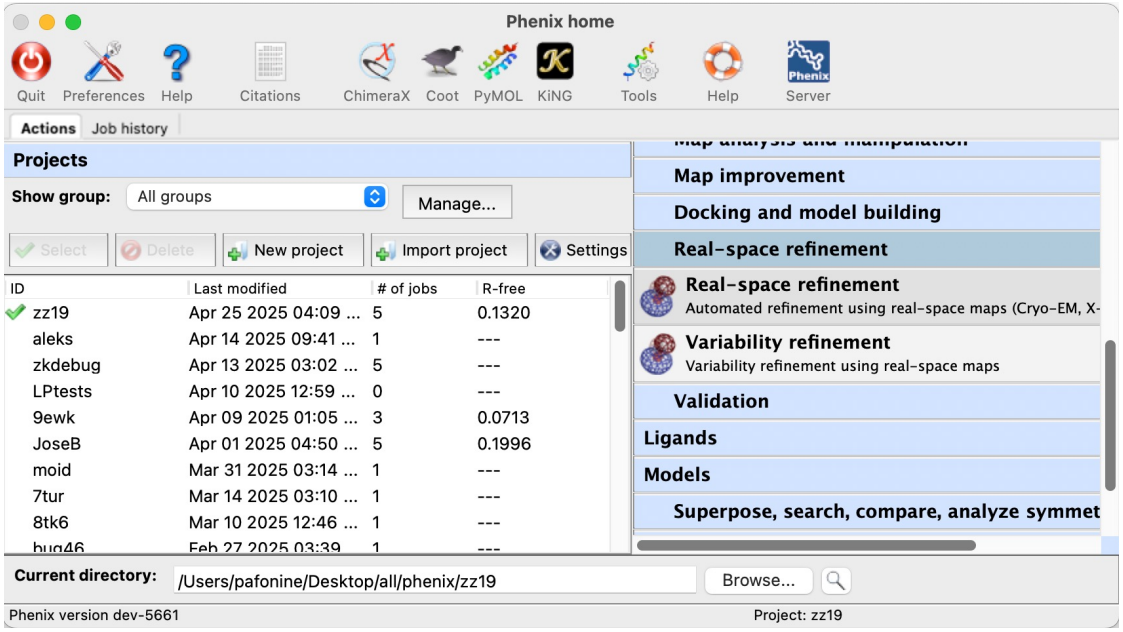
journal homepage: www.elsevier.com/locate/bbamem

Review

Conformational space exploration of cryo-EM structures by variability refinement

Pavel V. Afonine [a,*], Alexia Gobet [b], Loïck Moissonnier [b], Juliette Martin [b], Billy K. Poon [a], Vincent Chaptal [b,*]

[a] Molecular Biosciences and Integrated Bioimaging, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA
[b] Molecular Microbiology and Structural Biochemistry, UMR5086 CNRS University Lyon1, 7 passage du Vercors, 69007 Lyon, France



Feedback & need driven – Varref (Variability Refinement)

nature communications

Article

https://doi.org/10.1038/s41467-024-55439-9

Conformational diversity in class C GPCR positive allosteric modulation

Giuseppe Cannone[1,7], Ludovic Berto[2,7], Fanny Malhaire[2], Gavin Ferguson[2], Aurelien Fouillen[2], Stéphanie Balor[3], Joan Font-Ingles[4], Amadeu Llebaria[4], Cyril Goudet[2], Abhay Kotecha[5], Vinothkumar K.R.[6] & Guillaume Lebon[2]

Received: 23 November 2023

Accepted: 10 December 2024

Published online: 13 January 2025

nature communications

Article

https://doi.org/10.1038/s41467-025-56849-z

Rhodamine6G and Hœchst33342 narrow BmrA conformational spectrum for a more efficient use of ATP

A. Gobet[1,7], L. Moissonnier[2,7], E. Zarkadas[3], S. Magnard[2], E. Bettler[4], J. Martin[5], R. Terreux[4], G. Schoehn[6], C. Orelle[2], JM Jault[2], P. Falson[2] & V. Chaptal[2]

Received: 5 April 2024

Accepted: 3 February 2025

Published online: 18 February 2025

www.nature.com/scienti

scientific reports

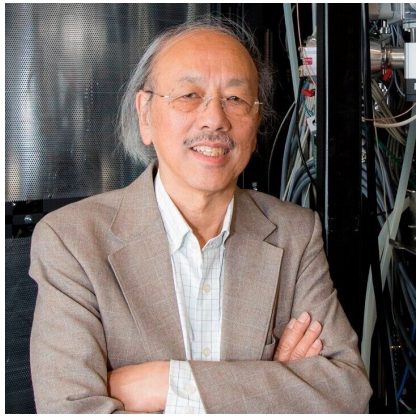
OPEN

Conformational landscape of soluble α-klotho revealed by cryogenic electron microscopy

Nicholas J. Schnicker[1,2,5], Zhen Xu[1,5], Mohammad Amir[3,5], Lokesh Gakhar[1,4] & Chou-Long Huang[3]

Feedback & need driven – Real Space Refinement

Around 2015: cryoEM resolution revolution about to happen!



- More and more high-resolution cryoEM maps, lack of model building and refinement tools!
- Birth of *phenix.real_space_refine* – refinement program in Phenix to refine atomic models into cryoEM maps

PNAS

Accurate model annotation of a near-atomic resolution cryo-EM map

Corey F. Hryc[a,1], Dong-Hua Chen[b,1,2], Pavel V. Afonine[c], Joanita Jakana[b], Zhao Wang[b], Cameron Haase-Pettingell[d], Wen Jiang[e], Paul D. Adams[c], Jonathan A. King[d], Michael F. Schmid[a,b], and Wah Chiu[a,b,3]

[a]Graduate Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030; [b]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030; [c]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [d]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and [e]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

Contributed by Wah Chiu, February 2, 2017 (sent for review December 7, 2016; reviewed by Terje Dokland and Jack E. Johnson)

Feedback & need driven – Real Space Refinement

feature articles

STRUCTURAL BIOLOGY

ISSN 2059-7983

Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*

Dorothee Liebschner,[a] Pavel V. Afonine,[a] Matthew L. Baker,[b] Gábor Bunkóczi,[c]‡ Vincent B. Chen,[d] Tristan I. Croll,[c] Bradley Hintze,[d]§ Li-Wei Hung,[e] Swati Jain,[d]¶ Airlie J. McCoy,[c] Nigel W. Moriarty,[a] Robert D. Oeffner,[c] Billy K. Poon,[a] Michael G. Prisant,[d] Randy J. Read,[c] Jane S. Richardson,[d] David C. Richardson,[d] Massimo D. Sammito,[c] Oleg V. Sobolev,[a] Duncan H. Stockwell,[c] Thomas C. Terwilliger,[e,f] Alexandre G. Urzhumtsev,[g,h] Lizbeth L. Videau,[d] Christopher J. Williams[d] and Paul D. Adams[a,i]*

Received 26 July 2019
Accepted 15 August 2019

Edited by K. Diederichs, University of Konstanz, Germany

Pixel size calibration (refinement)

Reply | Reply List | Forward | Archive | Junk | Delete | More

From Oliver Clarke

To phenixbb@phenix-online.org 6/3/25, 08:19

Subject **[phenixbb] Refine pixel size for EM map?**

Tags Important

List-ID Phenix user mailing list <phenixbb.phenix-online.org>

Hi,

I remember asking about this some time ago, but I am not sure if it was ever implemented.

Is there a way to refine just pixel size for an EM map in phenix.real_space_refine (or another phenix utility)? Even just automatically rigid-body fitting a crystal structure and calculating the pixel size with max correlation would be handy. We do this at the moment using Chimera/ChimeraX, but an automated way to do it in phenix would be handy.

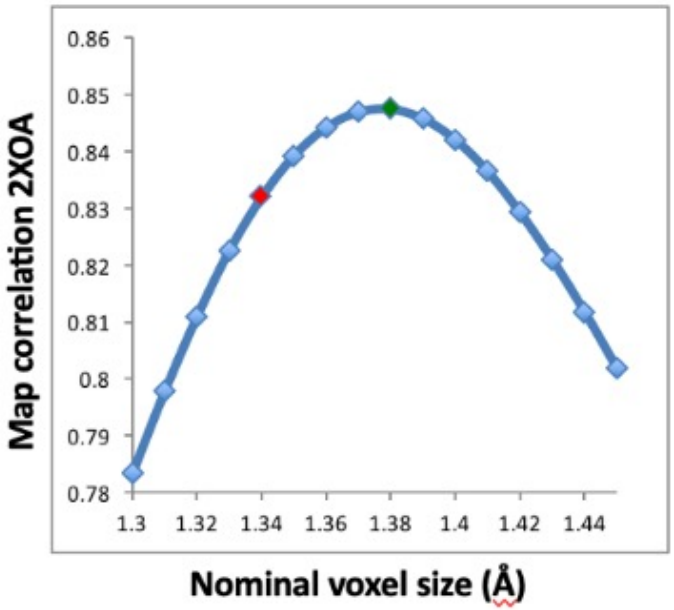
In the absence of a crystal structure, I wonder if directly refining pixel size during real space refinement would be possible/reasonable at higher resolution (<2Å)?

Cheers
Oli

Pixel size calibration (refinement)

Using UCSF Chimera for voxel size calibration (of your map and others)

- Voxel size generally requires calibration against a crystal structure.
- Once calibrated, generally stable between samples/datasets at same magnification.
- Can calibrate by fitting in Chimera at range of nominal voxel sizes and measuring correlation.
- Incorrect voxel sizes are common in deposited maps - **be aware of this when comparing structures**. E.g. here there is a 3% difference – affects structural alignment, reported resolution (3.8 vs 3.9Å).



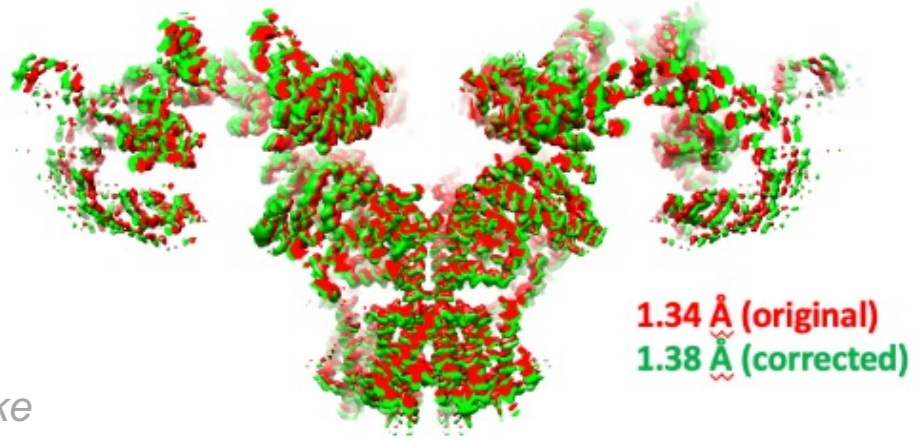
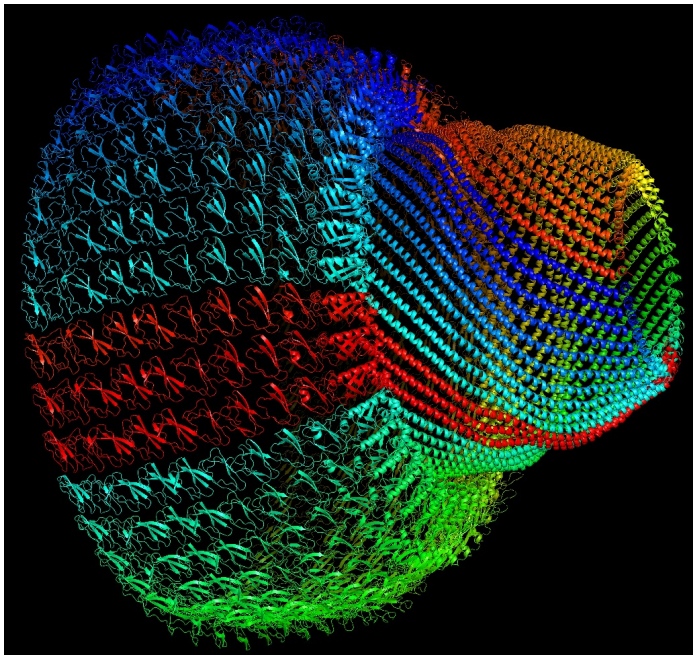


Figure: Oliver Clarke

MagRef: Pixel size calibration (refinement)

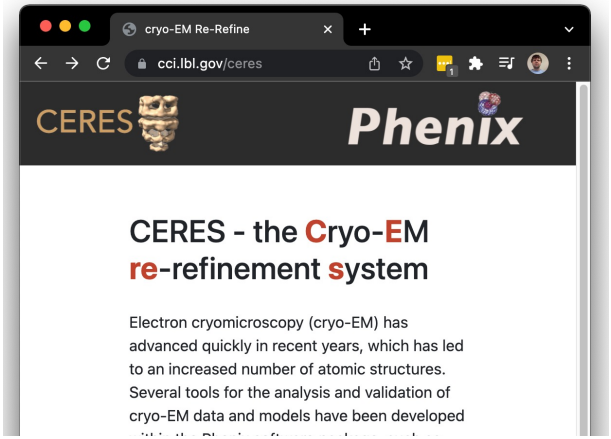
```
phenix.magref map.mrc model.pdb resolution=3.4
```

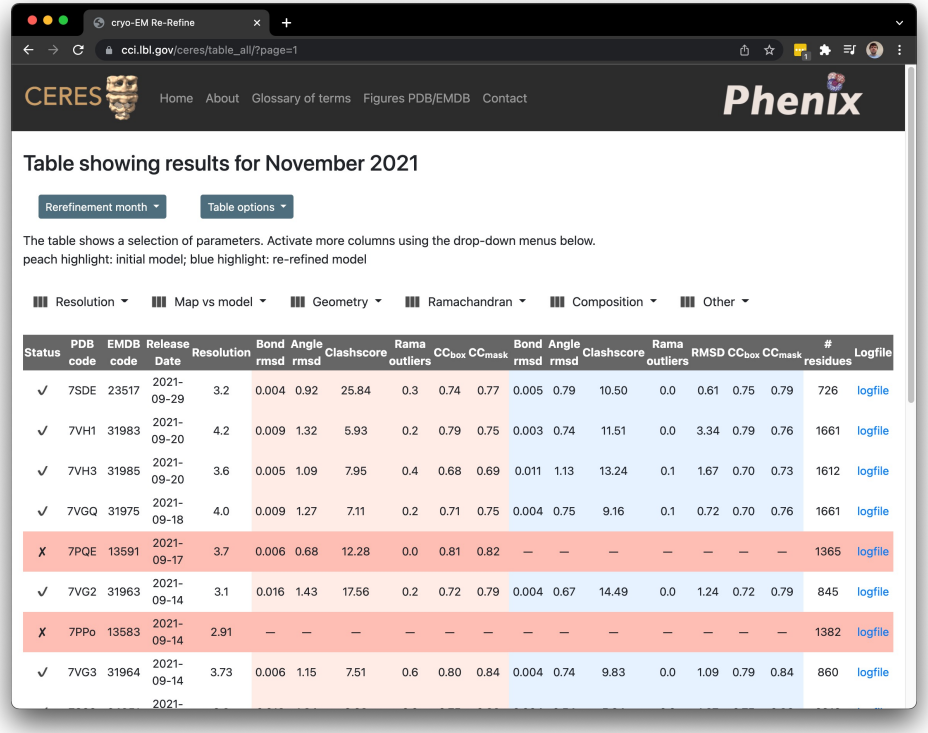
PDB: 4hl8
3.5 Å, 250,000 atoms



phenix.magref takes about 4 minutes on a laptop
(~100 of rigid-body refinements)

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
			2021-																

- Developers: track the impact of new
- Users: see how your models benefit from improved methods and tools

research papers

Acta Cryst D STRUCTURAL BIOLOGY
ISSN 2059-7983

CERES: a cryo-EM re-refinement system for continuous improvement of deposited models

Dorothee Liebschner,[a]* Pavel V. Afonine,[a] Nigel W. Moriarty,[a] Billy K. Poon,[a] Vincent B. Chen[b] and Paul D. Adams[a,c]

User support

- **Feedback, questions, help**

  Mailing list (anyone signed up): phenixbb@phenix-online.org
  Bug reports (developers only): bugs@phenix-online.org
  Ask for help (developers only): help@phenix-online.org

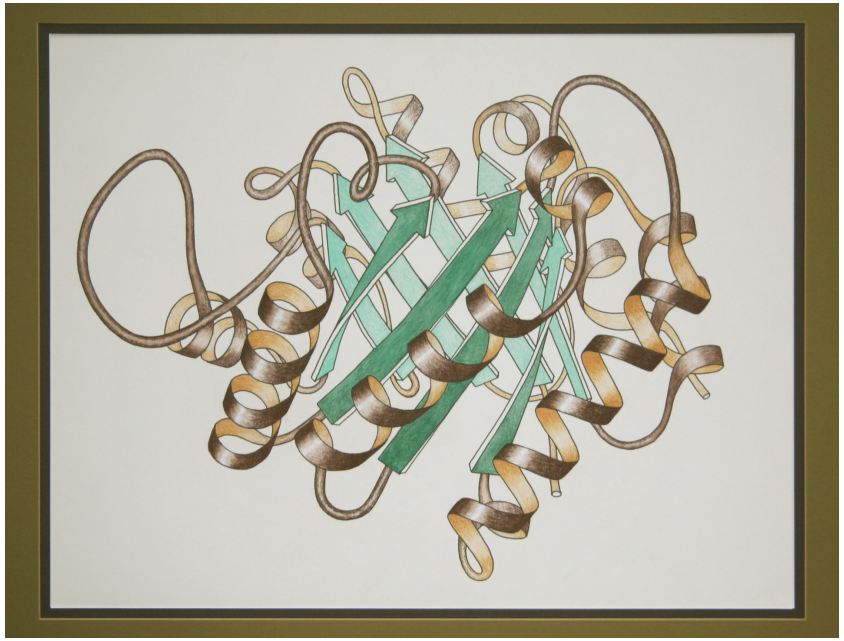
- **Reporting a bug or asking for help:**
  - We can't help you if you don't help us to understand your problem
  - Make sure the problem still exist using the latest *Phenix* version
  - Send us all inputs (files, non-default parameters) and tell us steps that lead to the problem
  - All data sent to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

A structure

Q: Who invented this method of drawing a structure (ribbon diagram)?

Ribbon diagram

Ribbon schematic of triose P isomerase monomer (PDB: 1TIM)



Hand-drawn by J. Richardson, 1981