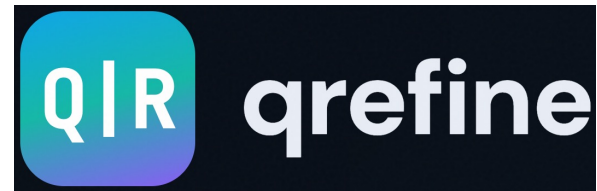


Refinement

Pavel Afonine



April 30, 2026
Lincoln, NE

More training events

Browser tabs: CBE MCCS-2026 Macromolecula... x +
Ask Gemini

Address bar: xtal.iqf.csic.es/MCCS2026/

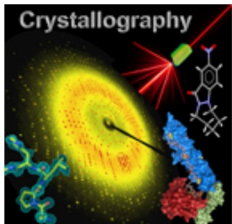
Macromolecular Crystallography & Cryo-EM School

MCCS-2026 23-27 March 2026


c/ Serrano 119 E-28006 Madrid (Spain) Telf: +34 91 561 94 00 Fax: +34 91 564 24 31 Thursday, 30 April 2026
These pages are regularly updated. If you find any error or incoherence, please, [let us know](#) Last update: 01 Apr. 2026

- Welcome
- Organisers
- Speakers
- Program
- Registration fee
- Sponsors
- Location map & Transport
- Accomodation
- Participants
- Previous editions

Crystallography



Crystallographic tutorial
(Spanish & English)



Macromolecular Crystallography & Cryo-EM School - MCCS-2026

(if you don't see a menu on the left, [use this link](#))

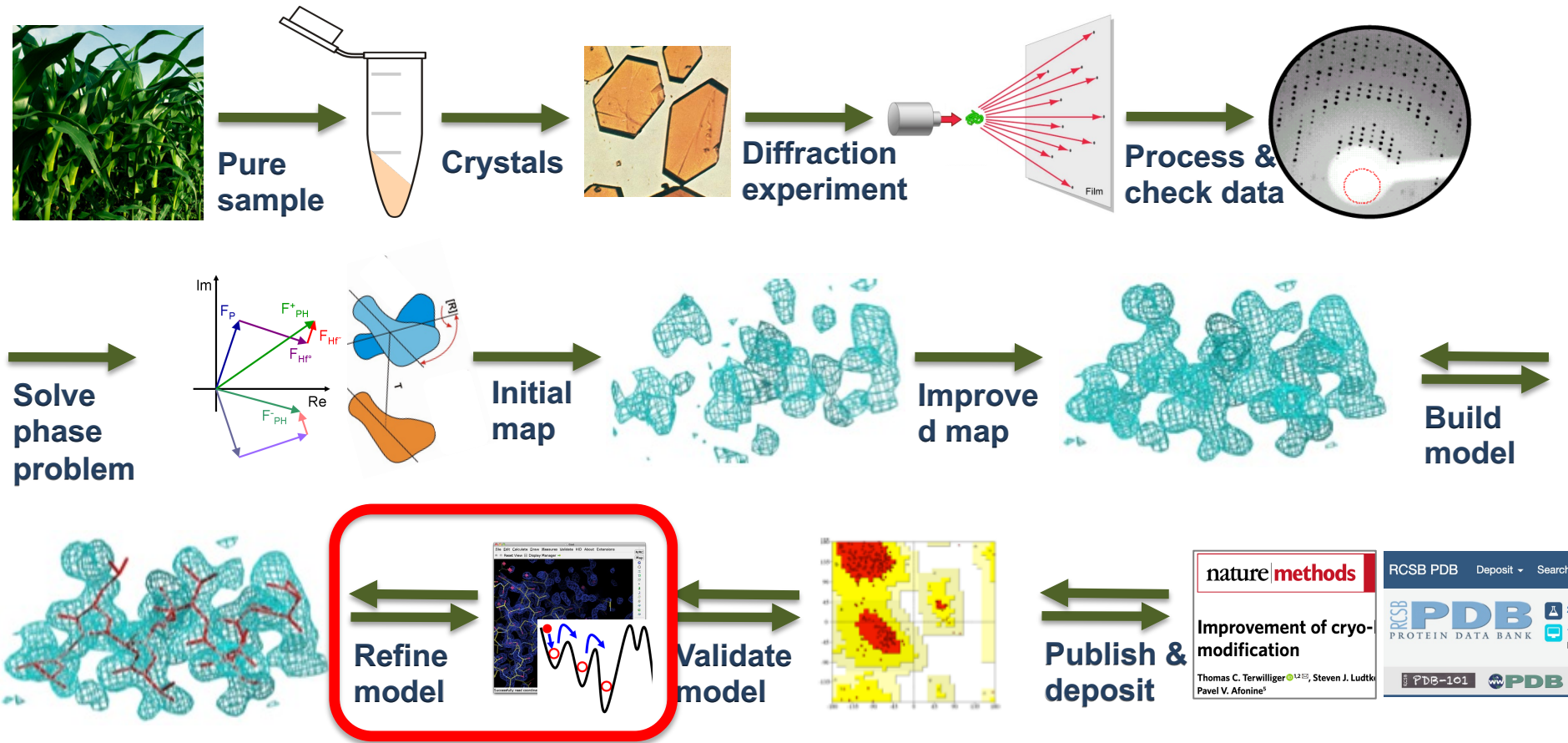
We are pleased to announce the thirteenth edition of the **Macromolecular Crystallography & Cryo-EM School - MCCS-2026**, to be held between **23-27 March 2026** at the [Department of Crystallography and Structural Biology](#) of the [Institute of Physical-Chemistry "Blas Cabrera"](#) (old "Rocasolano")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

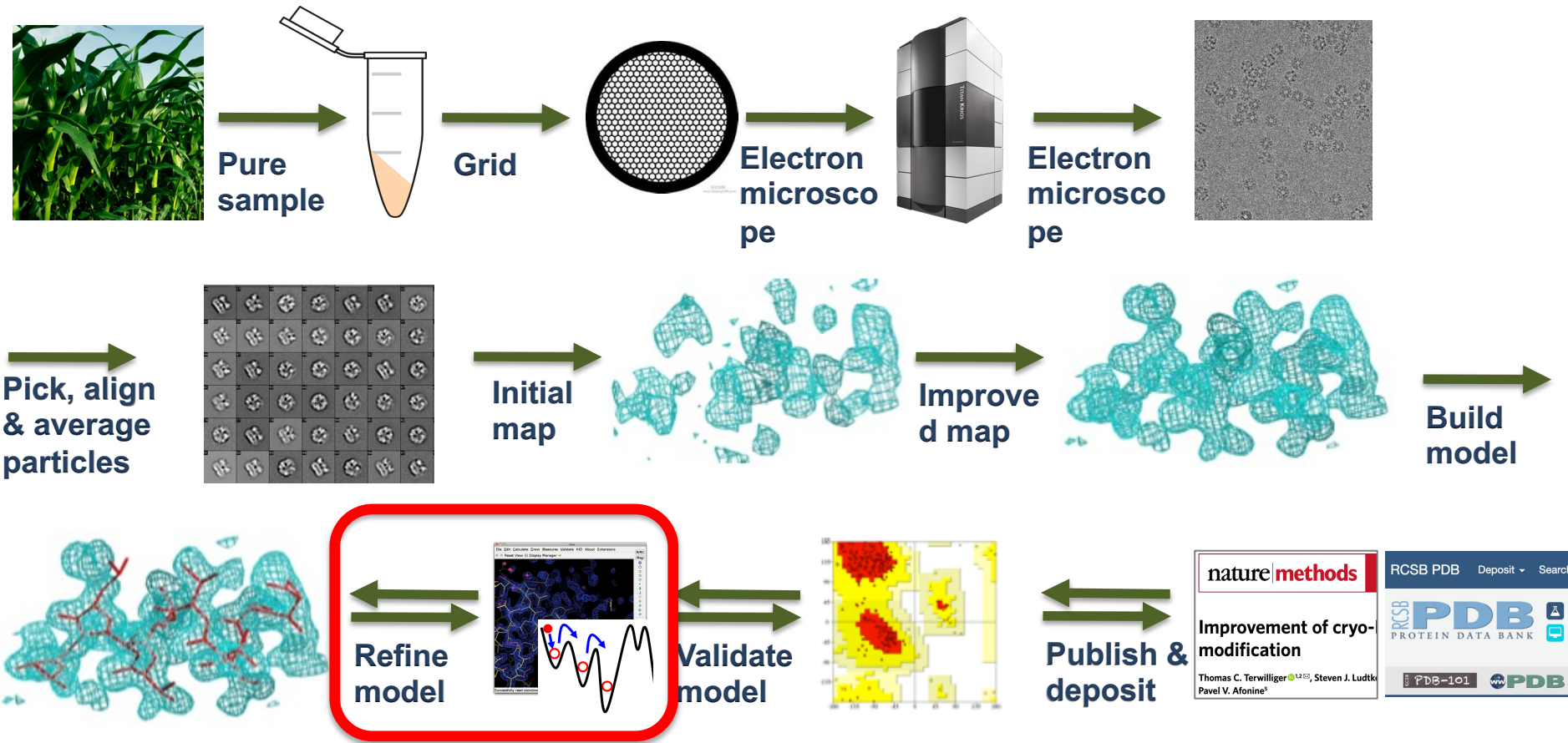
For further information, please contact: [Dr. Armando Albert](#) / [Dr. Juan Hermoso](#) / [Dr. Lourdes Infantes](#) / [Dr. Maria J. Sánchez-Barrena](#)

Solving structure - crystallography



- 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



- 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



IUCrJ

ISSN 2052-2525

CRYO | EM

letters to the editor

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

Richard Henderson^{a*} and Samar Hasnain^b

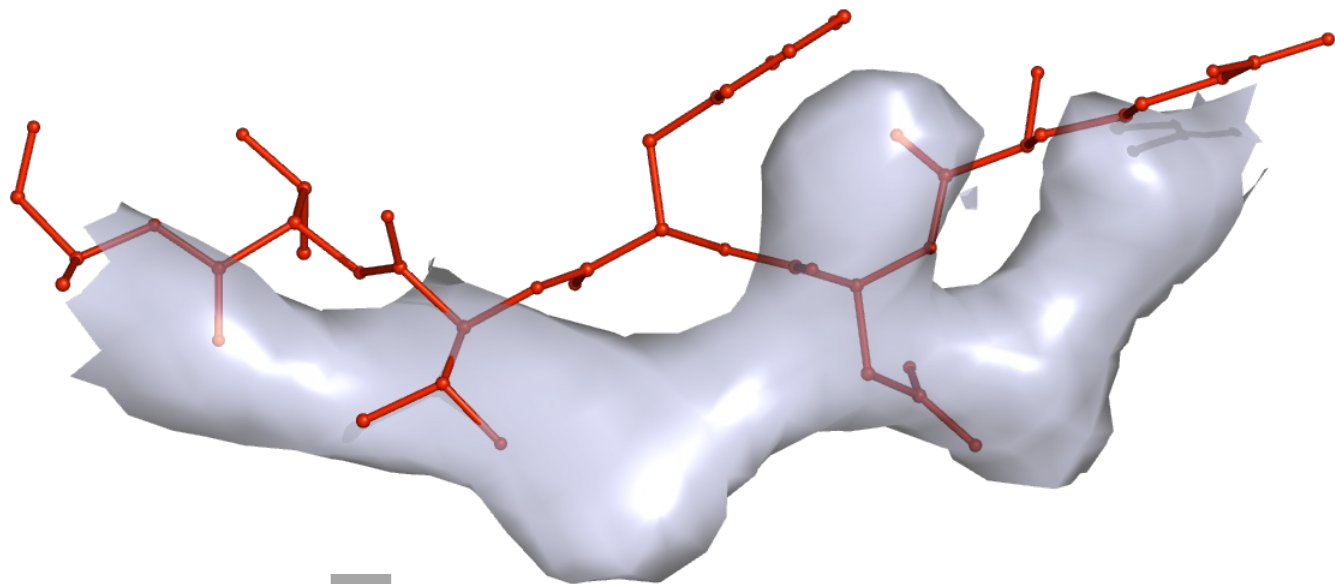
^aMRC Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom, and ^bDepartment of Biochemistry and Systems Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom. *Correspondence e-mail: rh15@mrc-lmb.cam.ac.uk

Key takeaway:

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

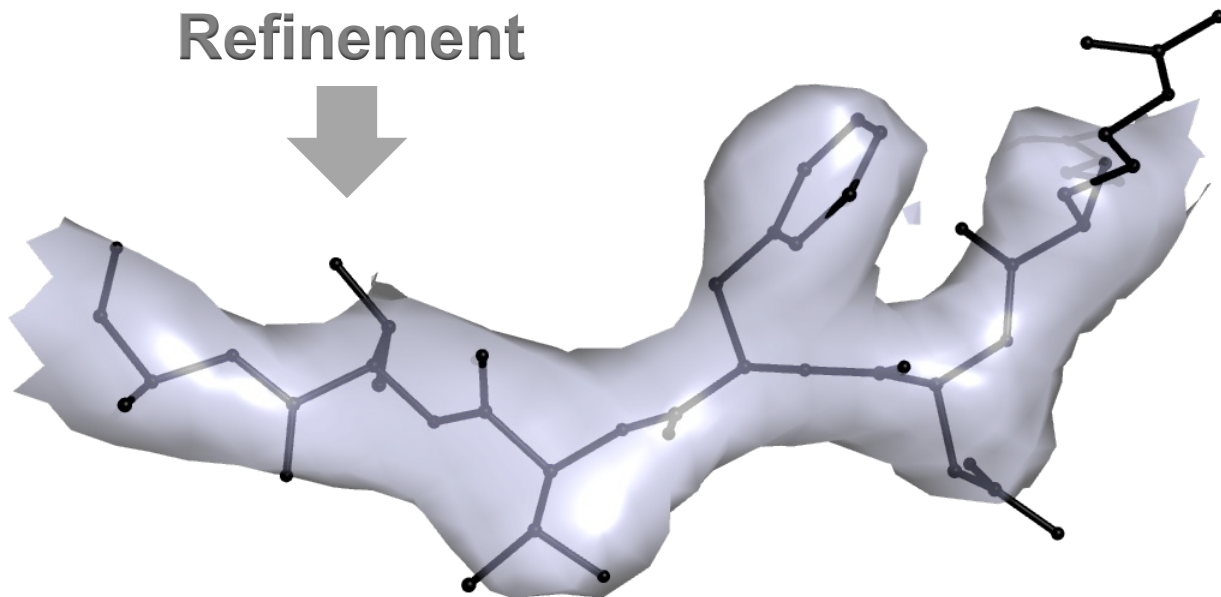
Model refinement

**Initial (poor)
model**

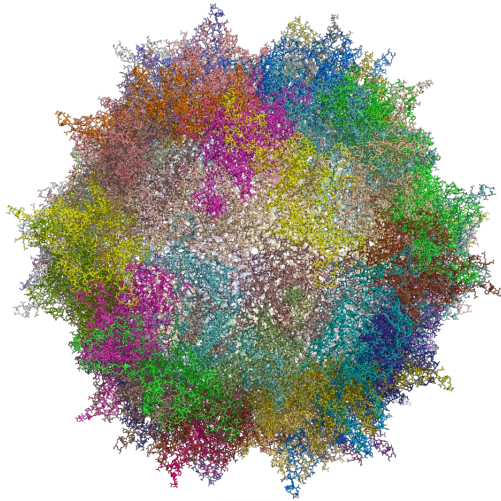


■
Refinement
↓

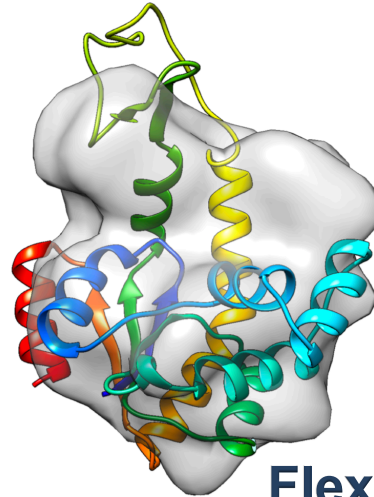
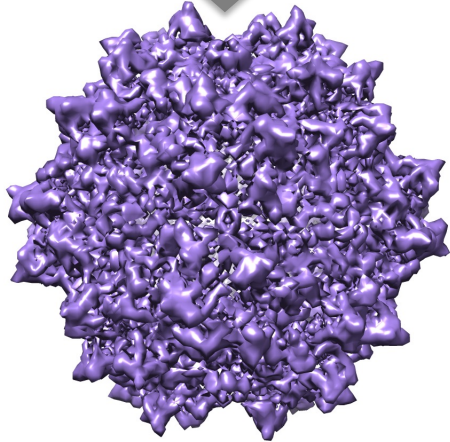
**Improved
(refined)
model**



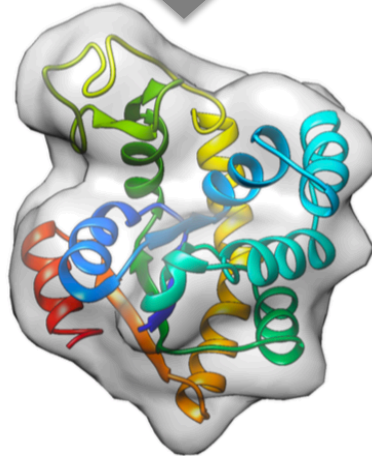
Model refinement vs other model fitting tools



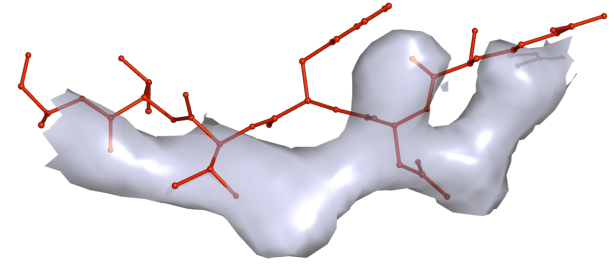
↓
Docking



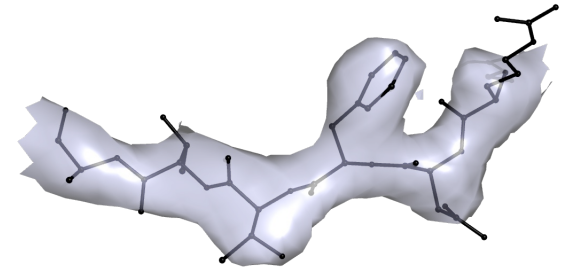
↓



**Flexible fitting,
morphing**



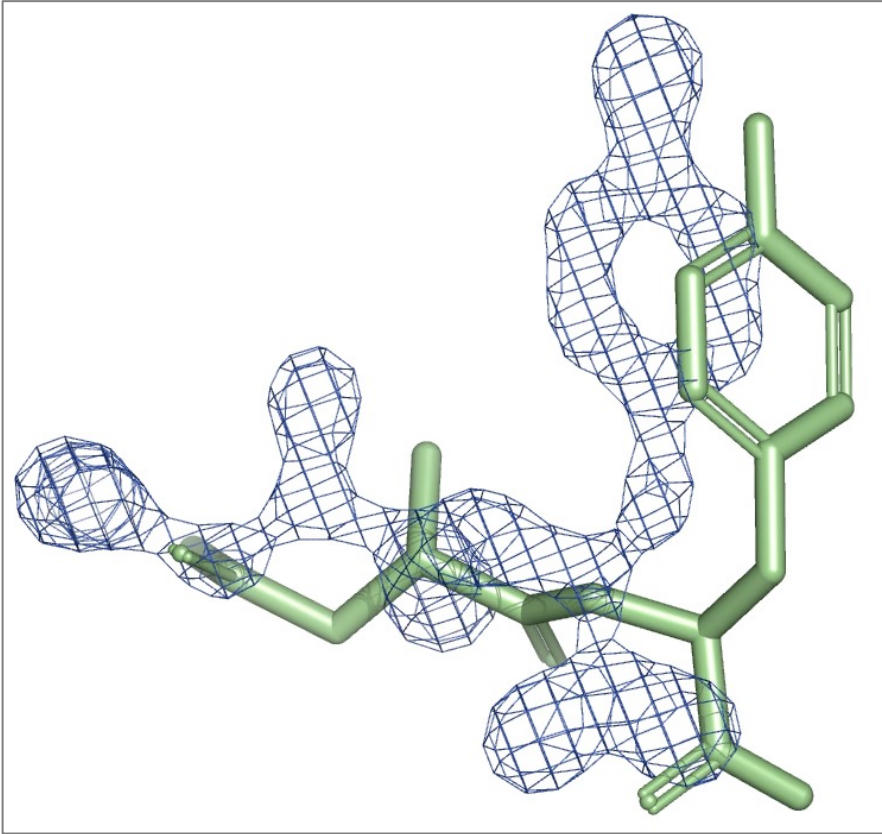
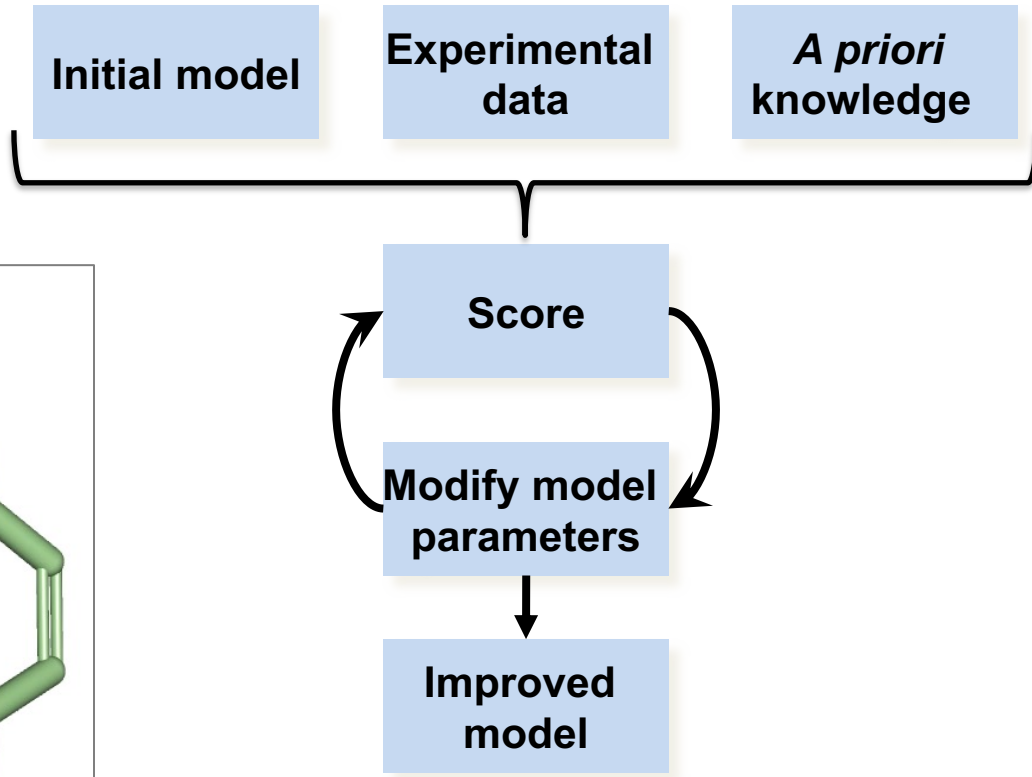
↓



Refinement

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

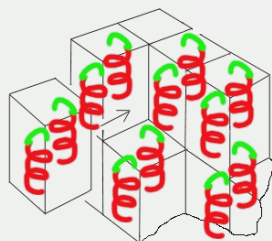
Model refinement



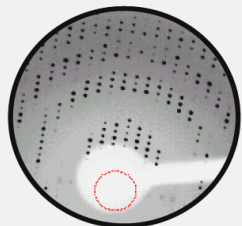
Optimization process of fitting atomic model parameters to experimental data

Refinement

Crystallography



Initial model



Experimental
data

A priori
knowledge

Score

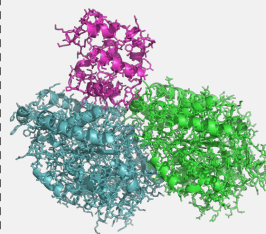
Modify model
parameters

Improved
model

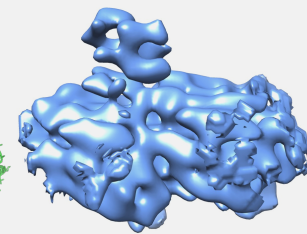
phenix.refine

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

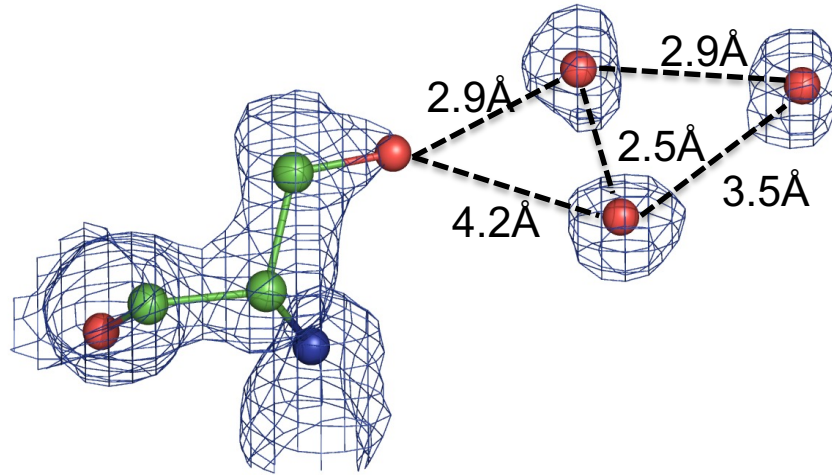
- Improving model improves map
 - (2mFo-DFc, Model phase), (mFo-DFc, Model phase)
 - Better model leads to better map
 - Better map leads to more model built
 - Improving model in one place lets build more model elsewhere in the unit cell
 - Refine all model parameters (XYZ, B) from start to end of structure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

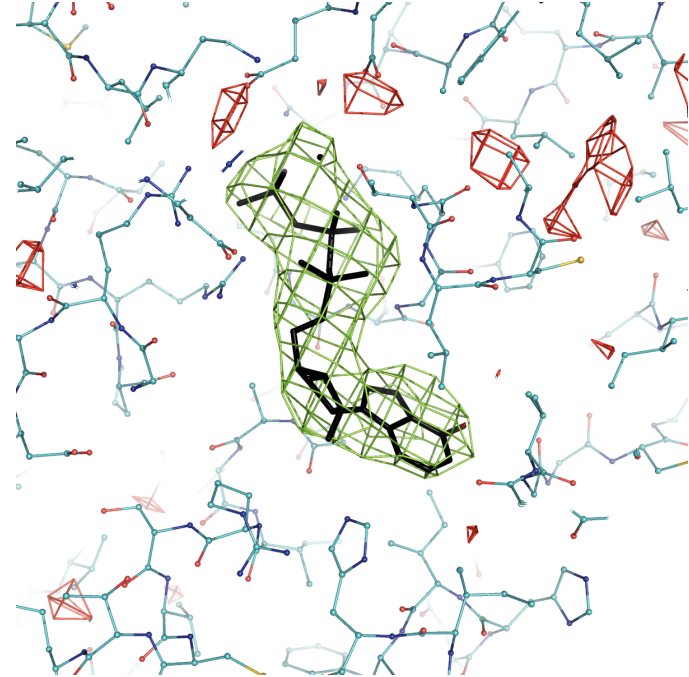
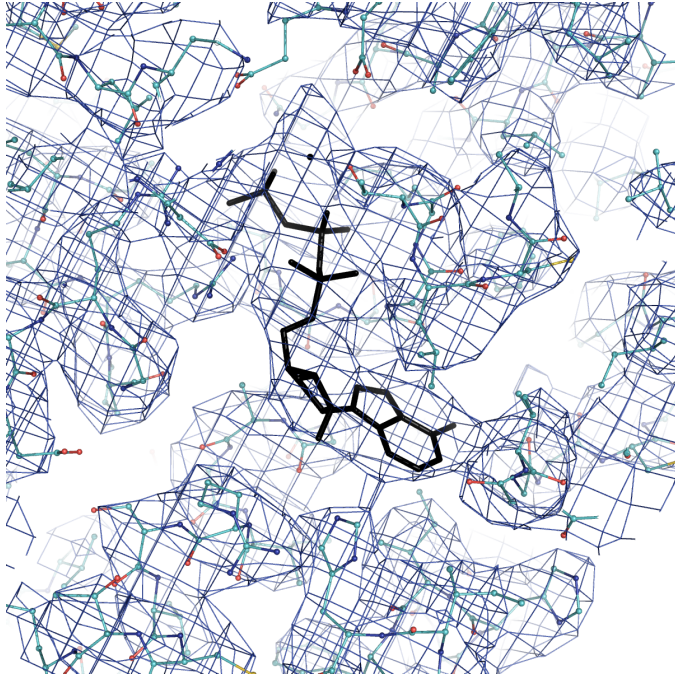
- Changing model does not change map
 - Build solvent (water) last
 - Get as complete and accurate model as possible before refining B factors and occupancies
- Experimental data changes a lot during the process (filtering, boxing, using maps with implied symmetry or not, etc.)
 - What map to use in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

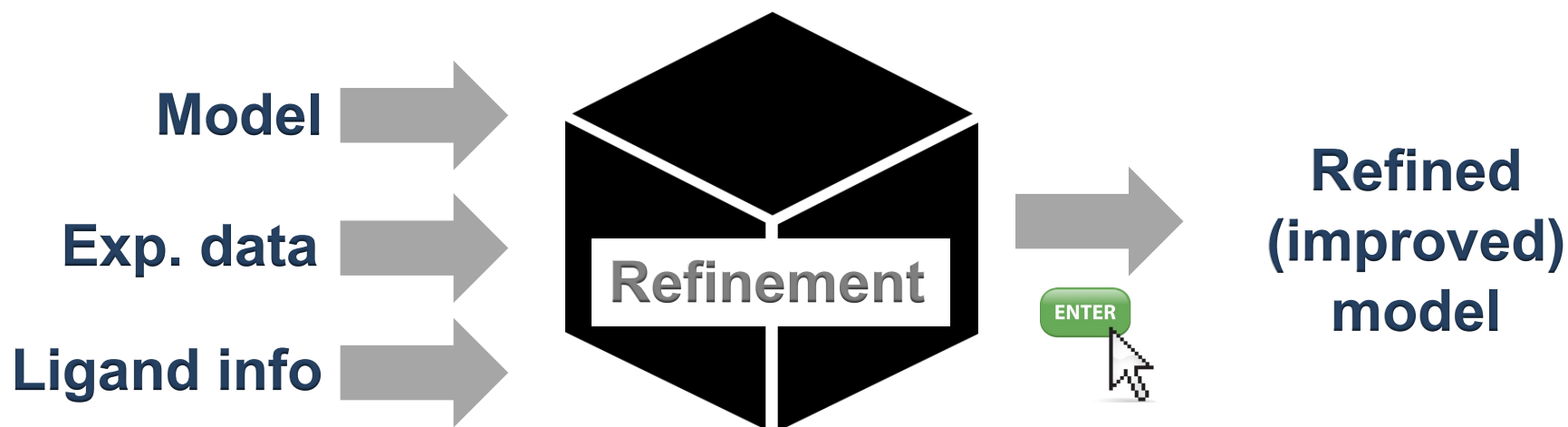
Solving structure in the past

- 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
 - $\sim 2 \text{ \AA}$ 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?

ADP?

Group B vs individual?

Local minima?

tNCS?

Clashes?

NCS?

IAS?

Weights?

CDL?

SA?

Grid search?



Minimization?

Rama plot restraints?

f' & f'' ?

Hydrogens?

Restraints?

Bulk-Solvent?

Rigid body?

Rama-Z?

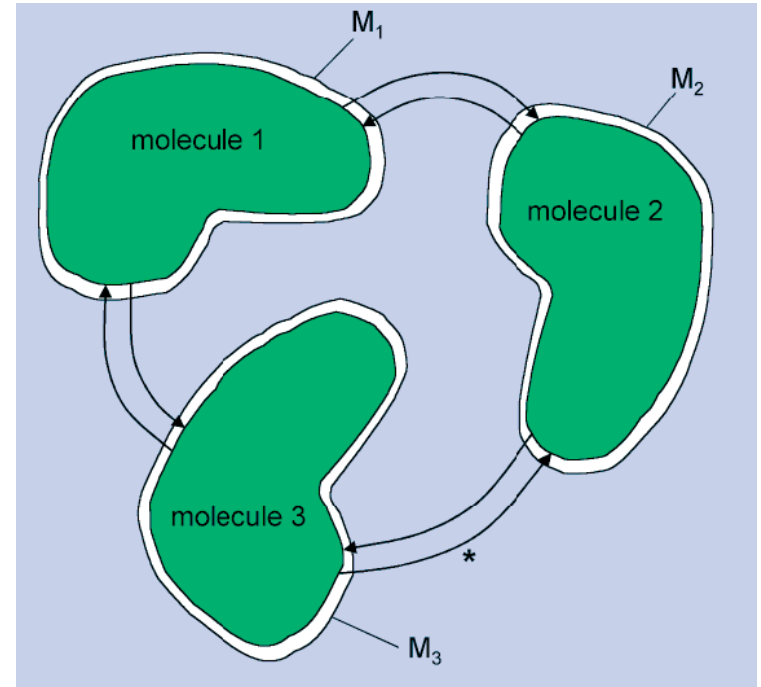
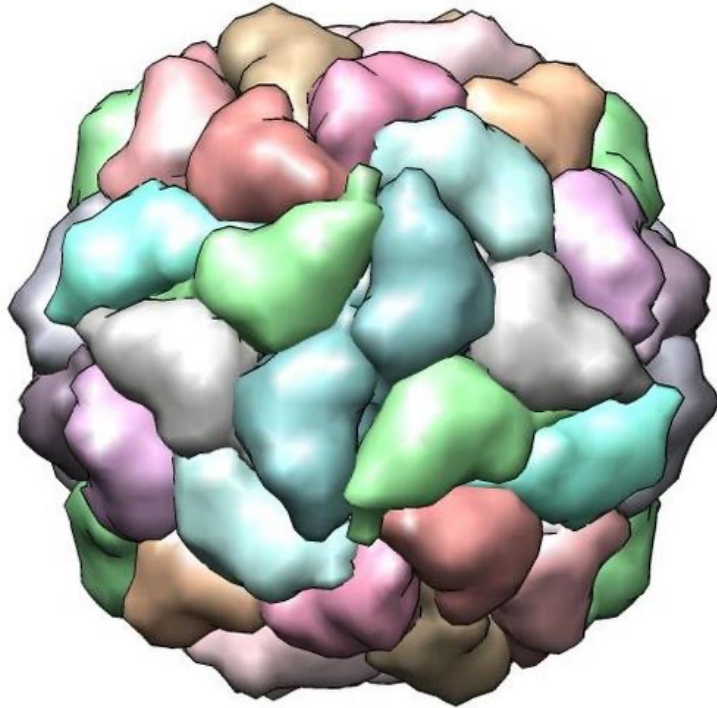
Anisotropy?

NQH flips?

SS restraints?

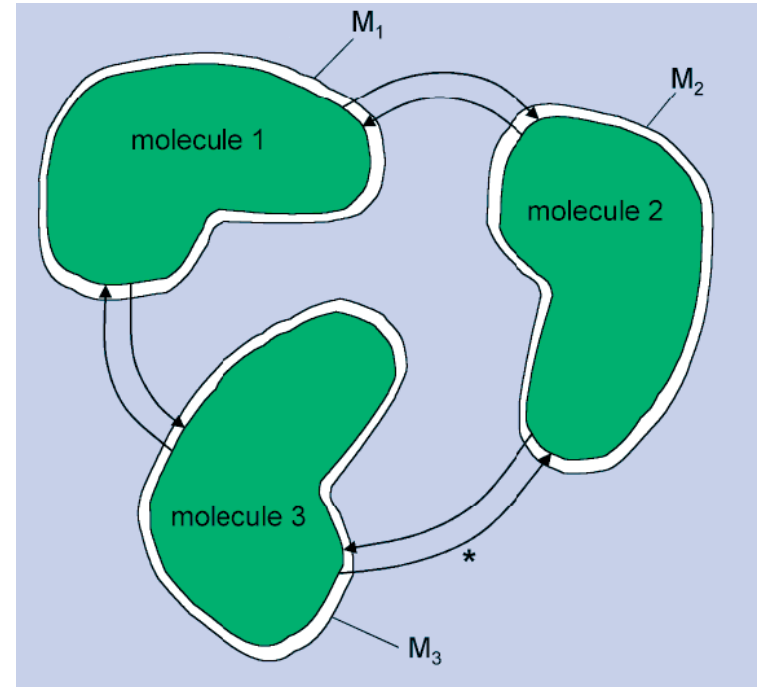
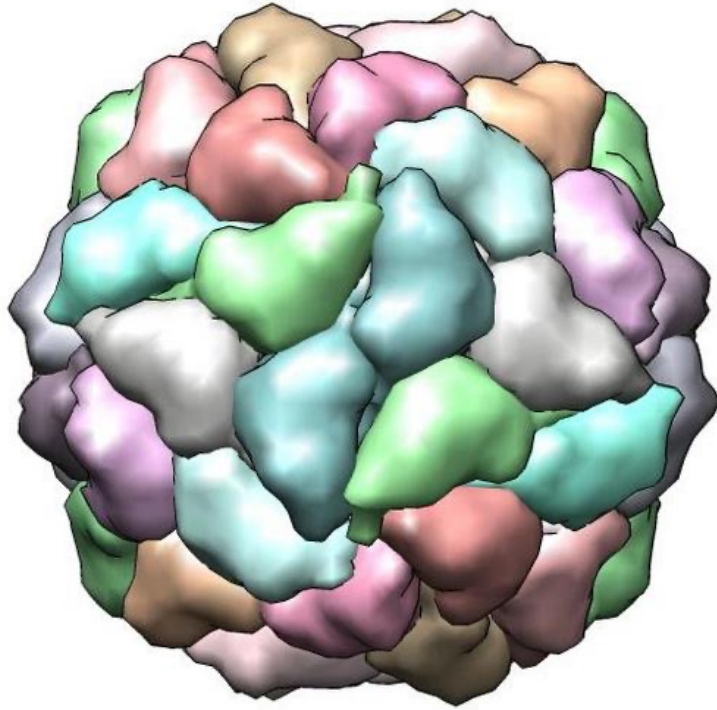
Twinning?

NCS (internal symmetry): constraints vs restraints



Source: Internet

NCS (internal symmetry): constraints vs restraints

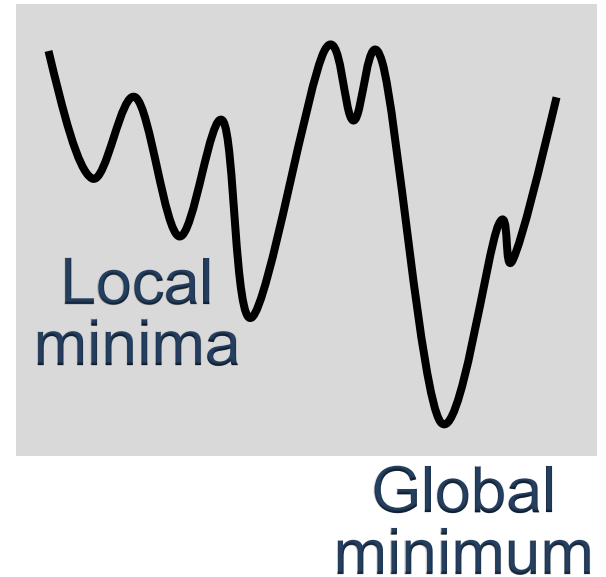
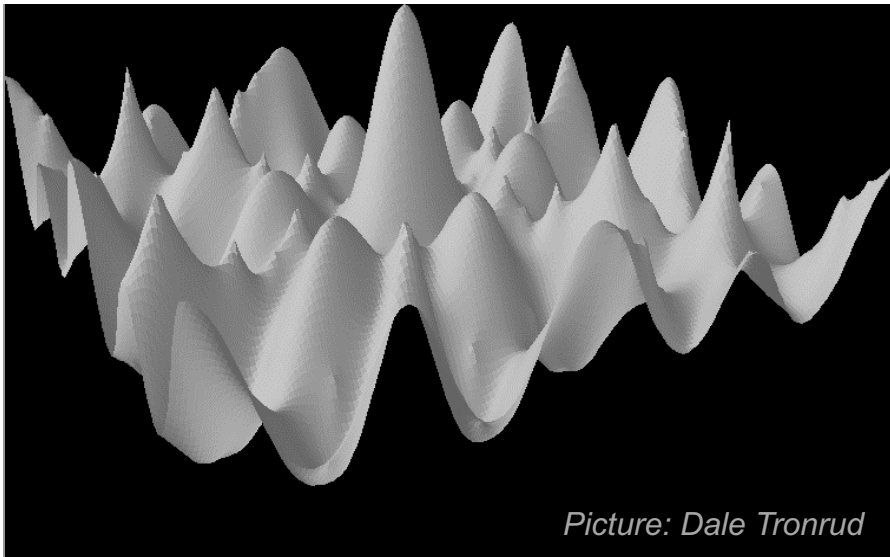


Source: Internet

- **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

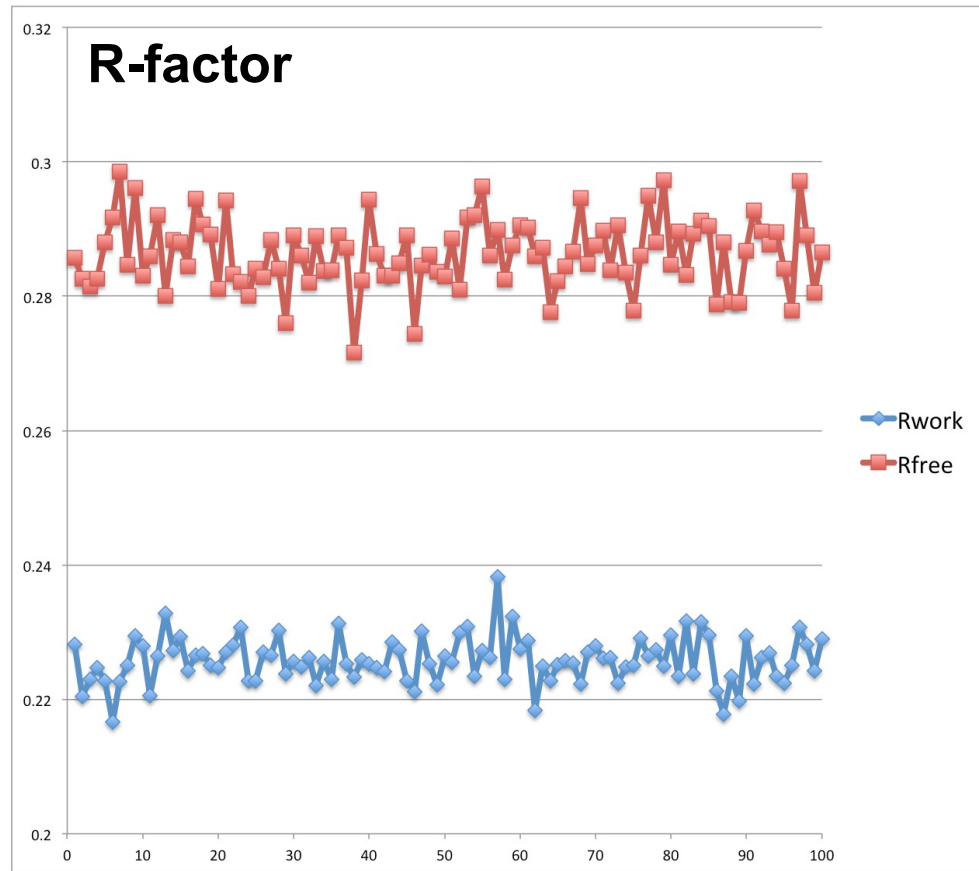
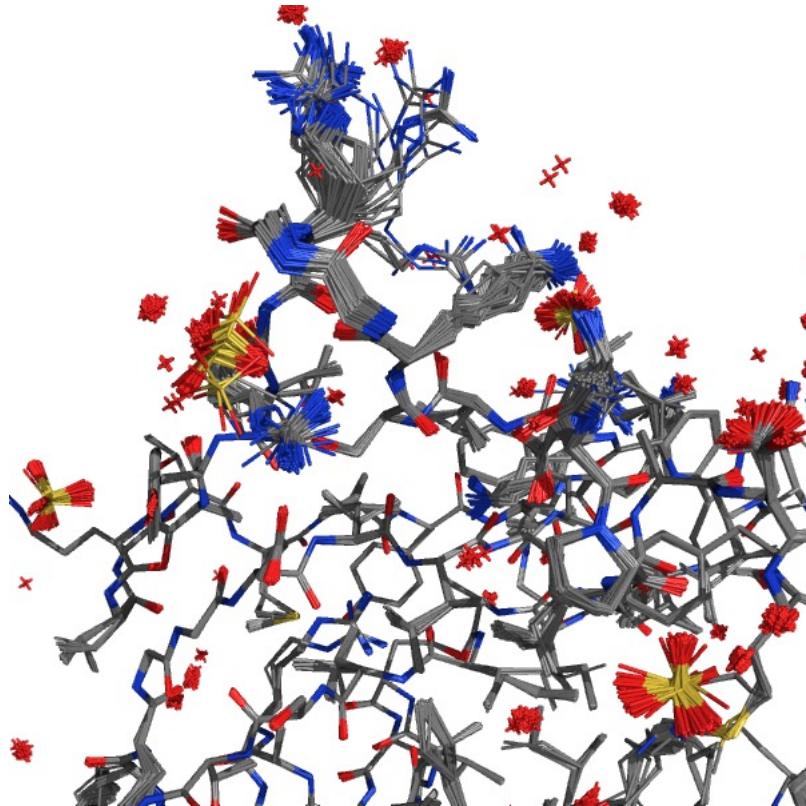
Complexity of refinement target

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



Estimating and using uncertainty

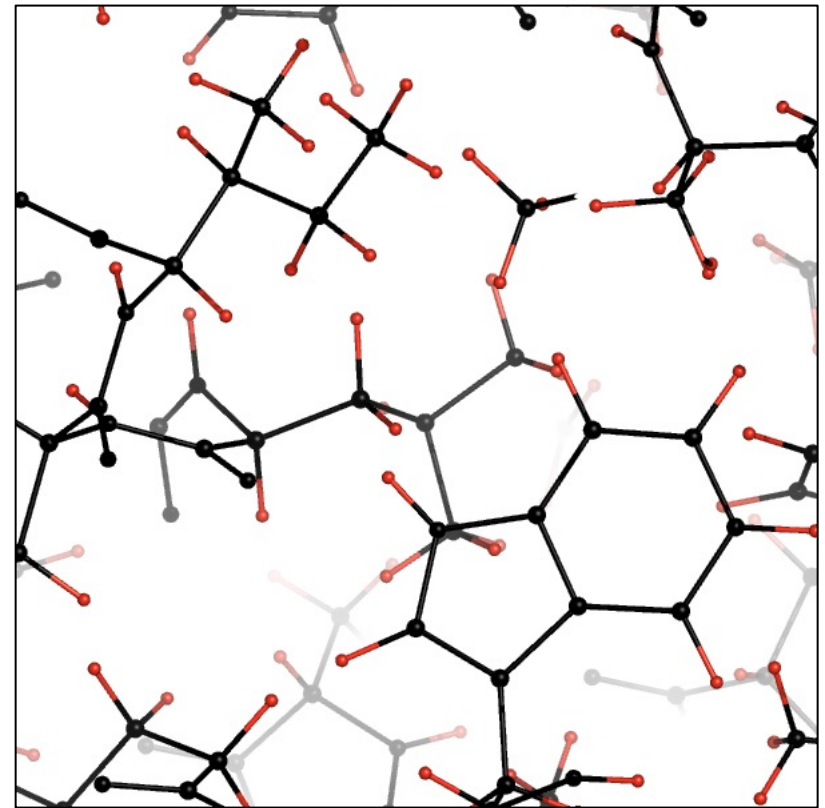
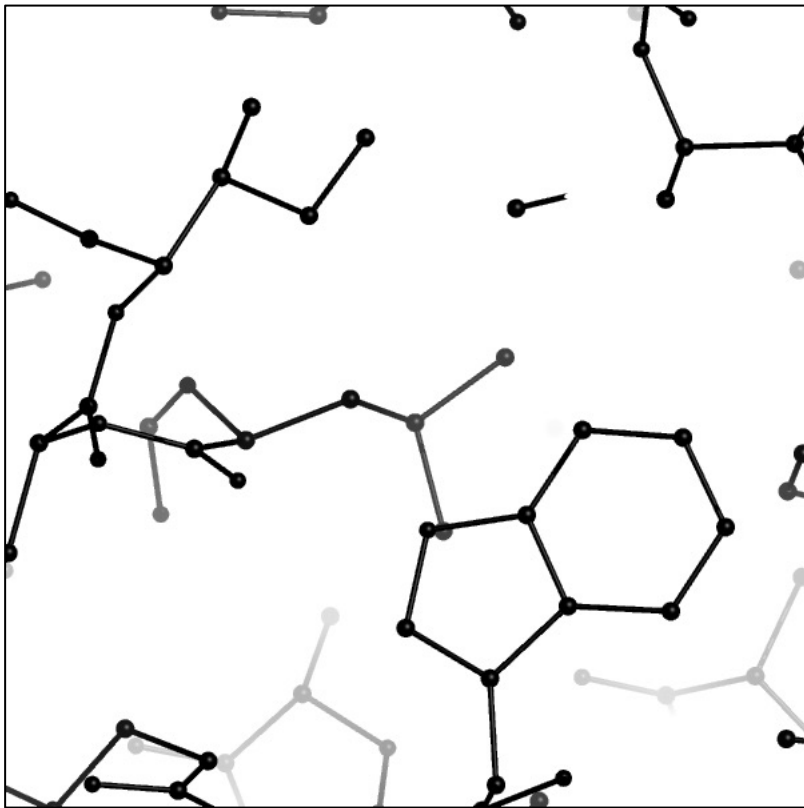
100 identical refinement runs each one starting with slightly perturbed model



Refinement run

Use Hydrogen atoms

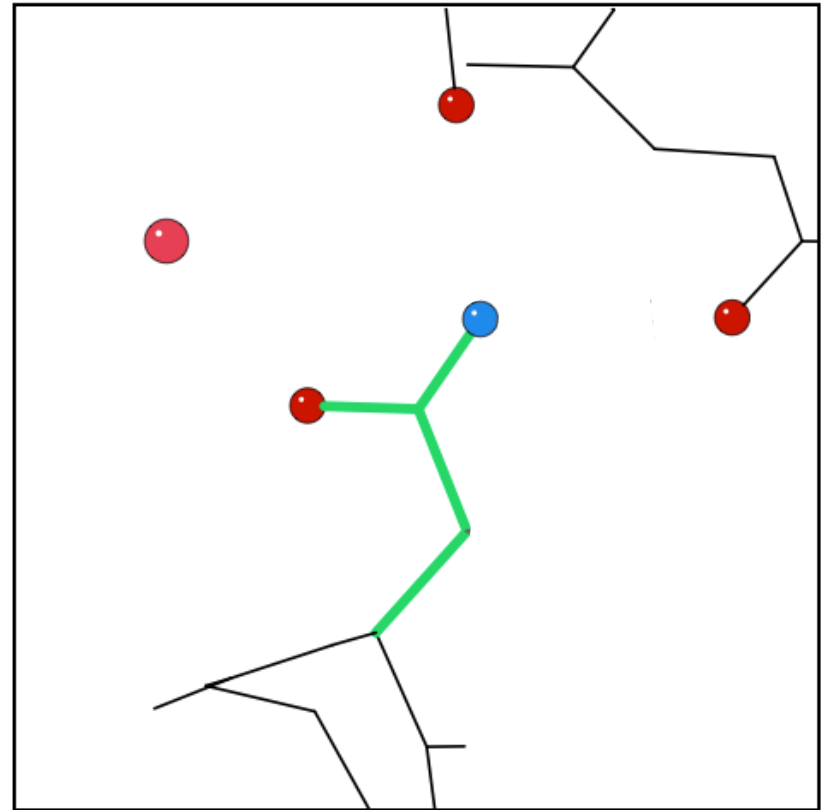
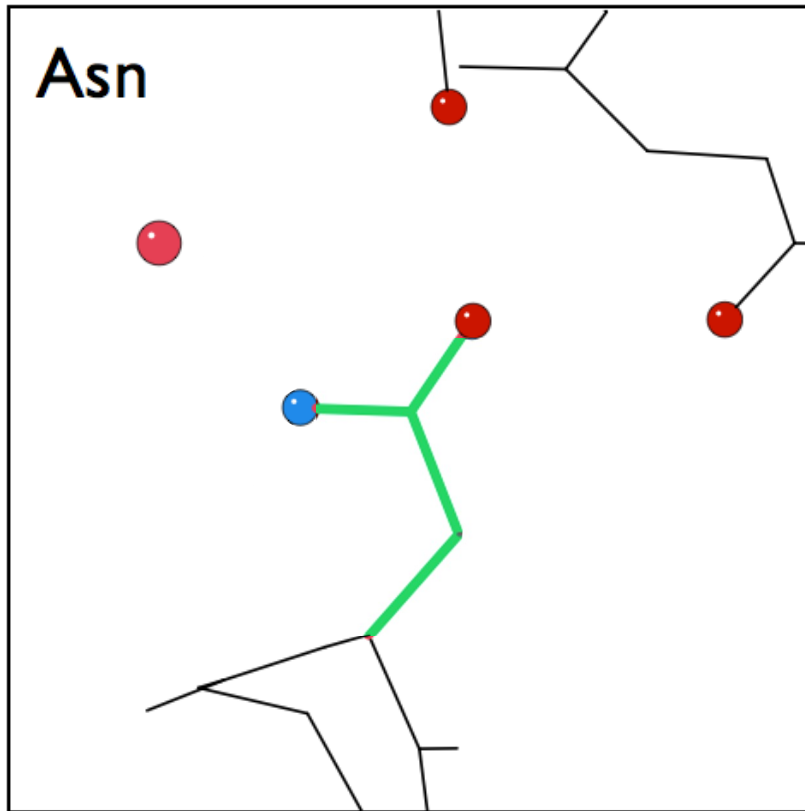
- Half of the atoms in a protein molecule
- Make most interatomic contacts
- Add to model towards the end, data resolution does not matter
- Once added, do not remove before the PDB deposition
- H do contribute to R-factors (expect 0.1-2% drop in R)



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

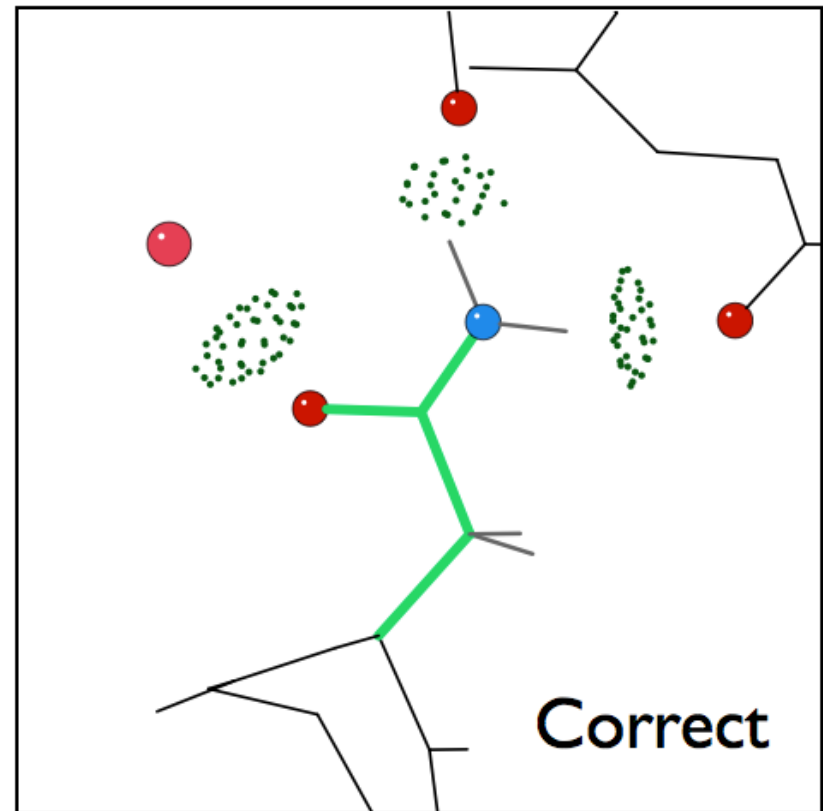
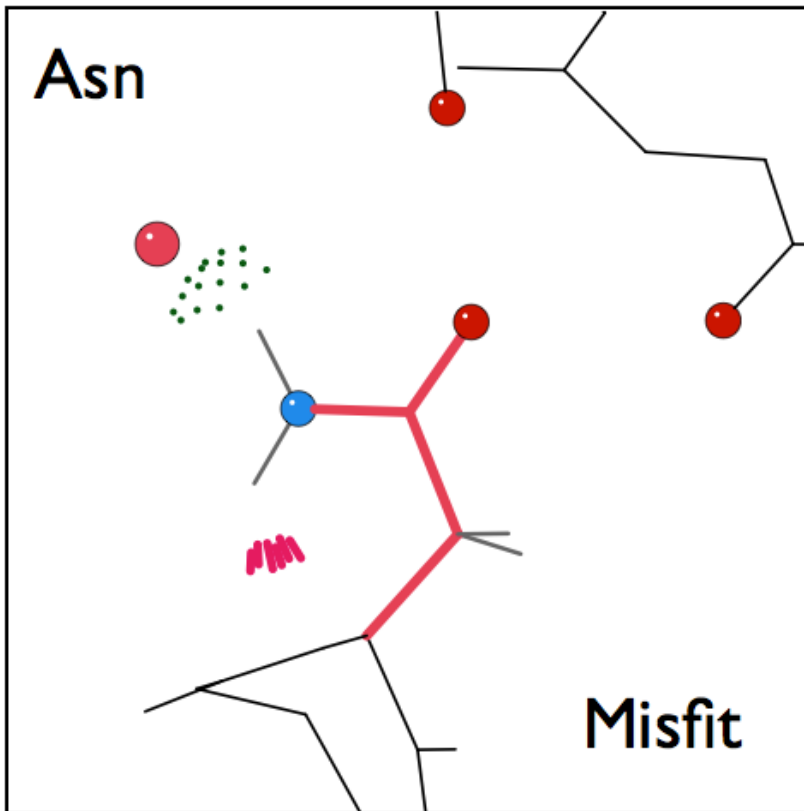
Use Hydrogen atoms

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

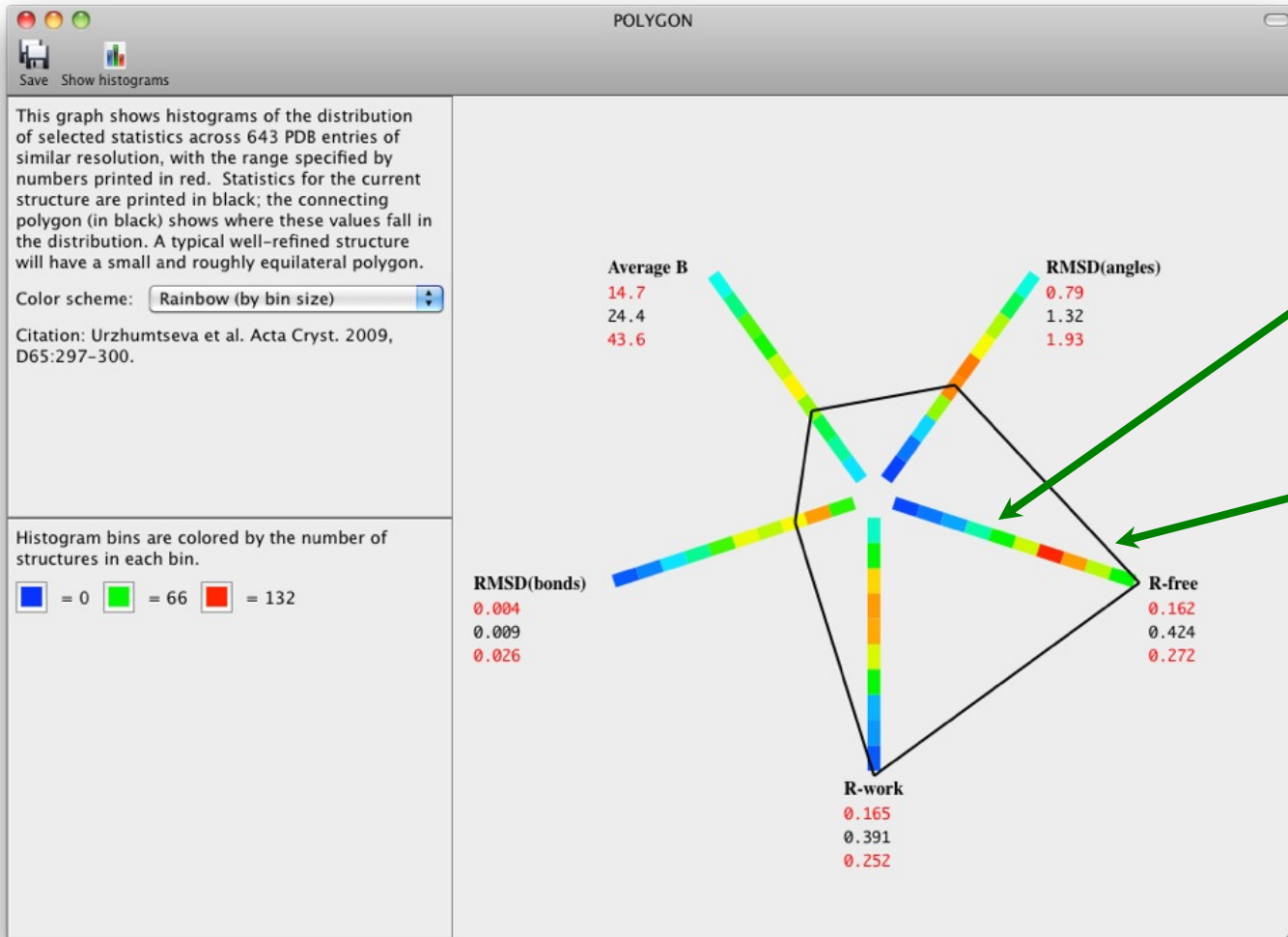


Use Hydrogen atoms

- 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

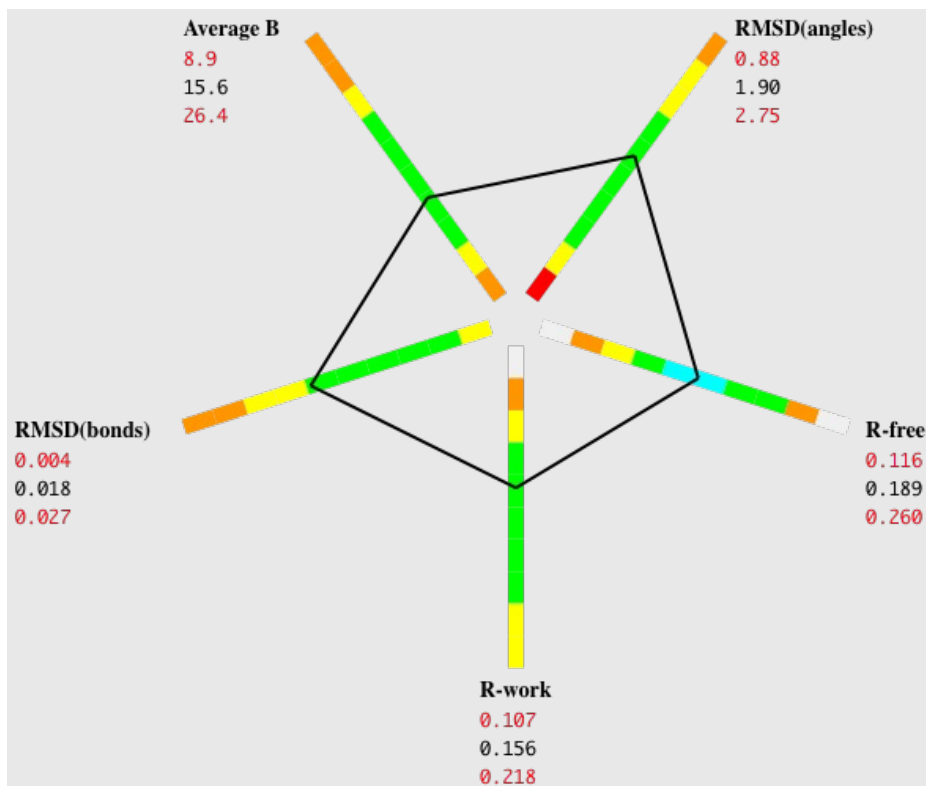
The black polygon shows where the statistics for the user's structure fall in each histogram

Crystallographic model quality at a glance.

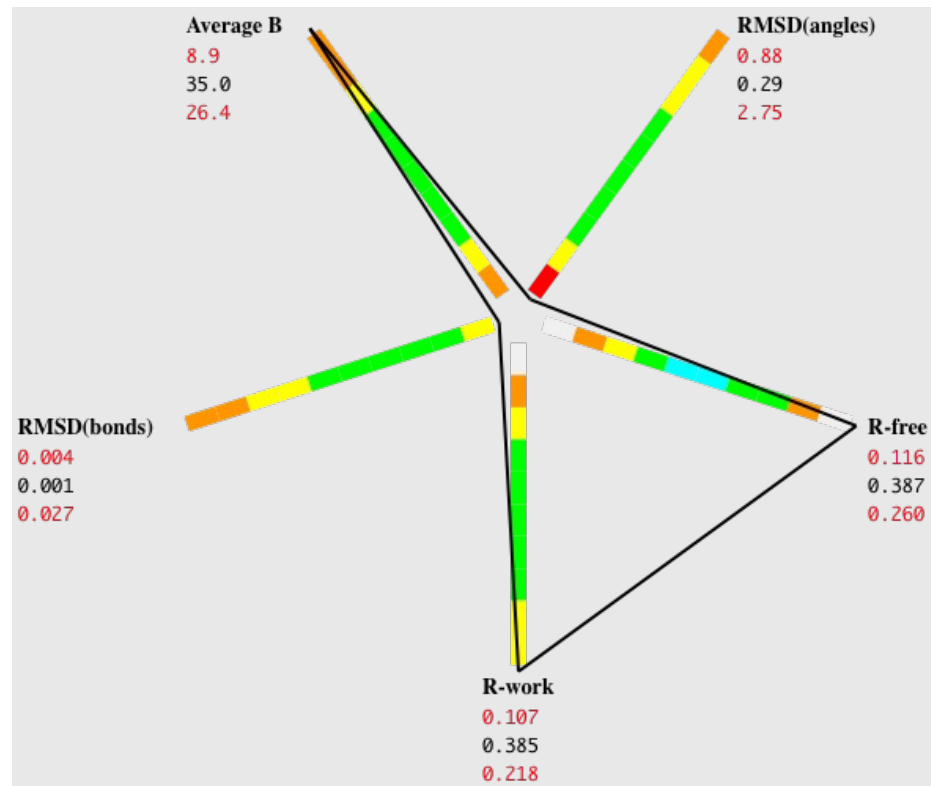
L.Urzhumtseva, P.V.Afonine, P.D.Adams & A.Urzhumtsev. *Acta Cryst.* D65, 297-300 (2009)

Know when to stop

Likely overall good model

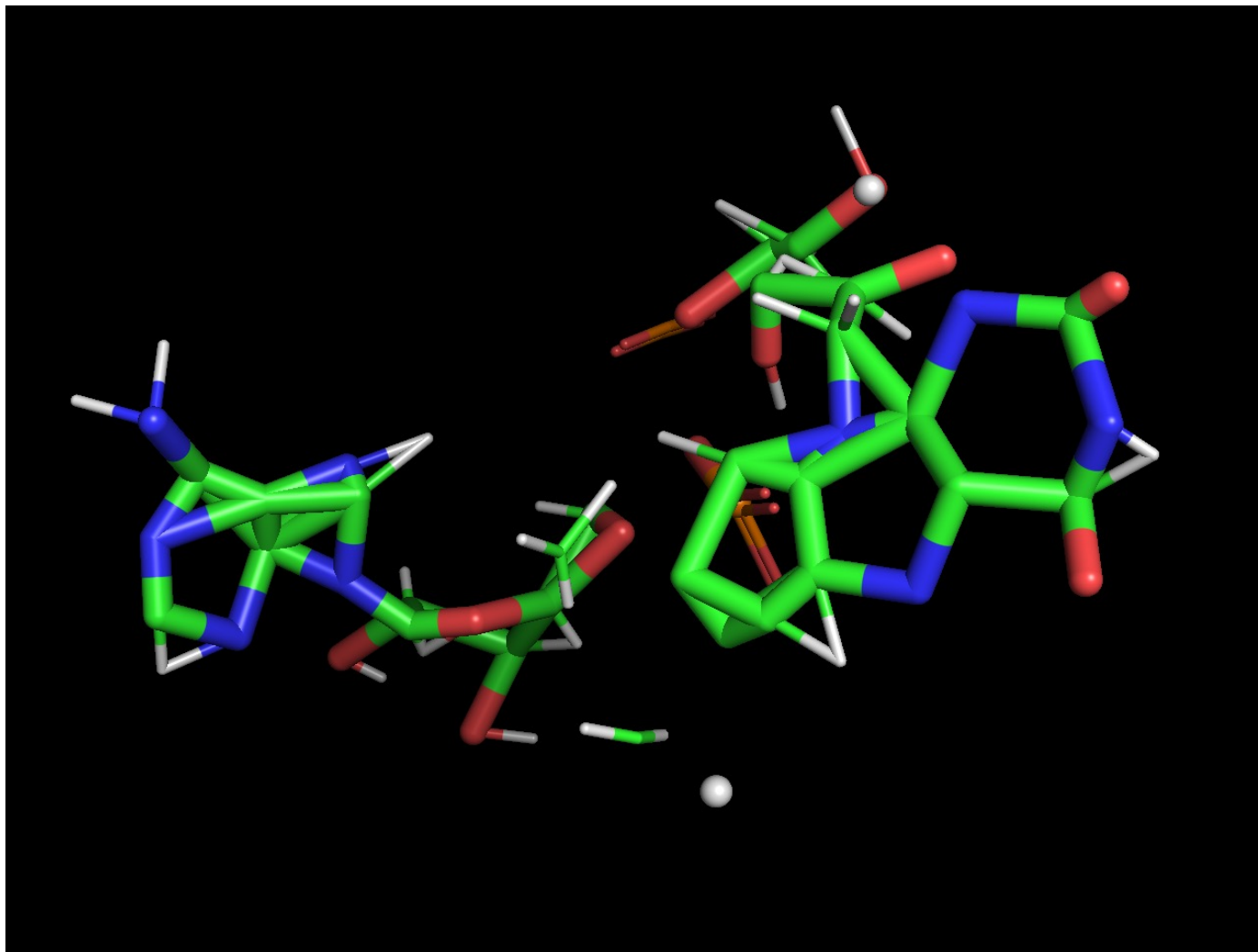


Clearly there are problems

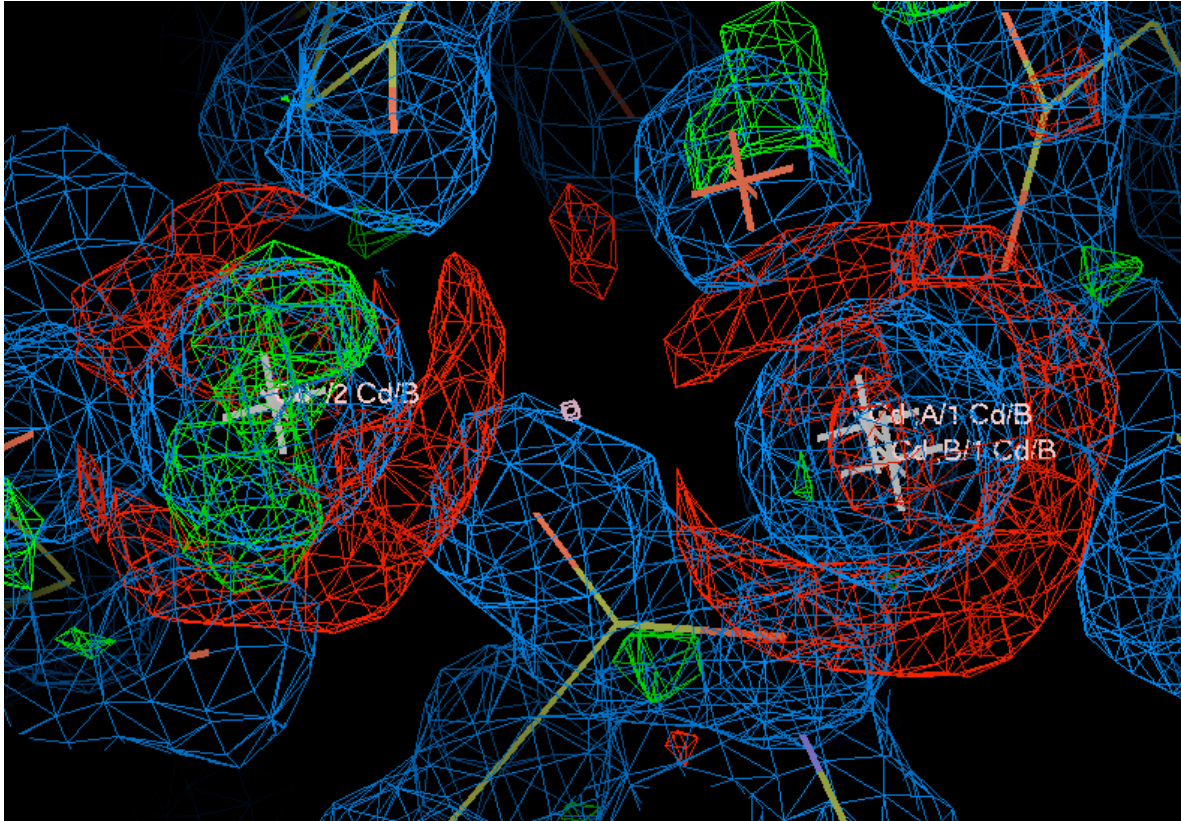


Local vs Global

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



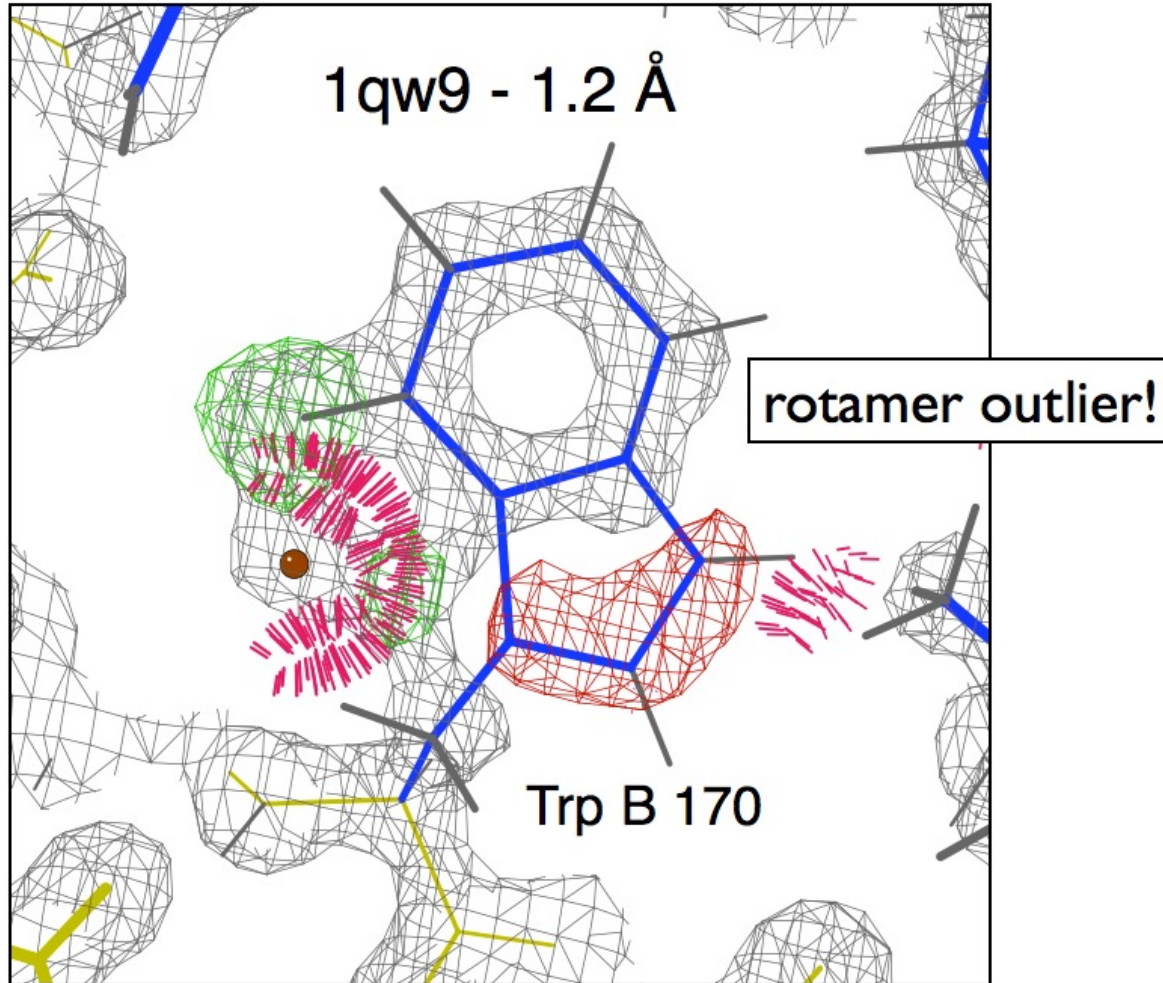
Map and model errors



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

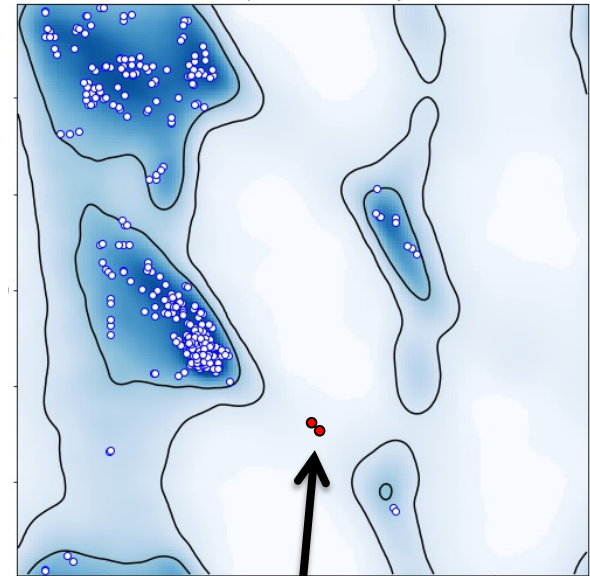
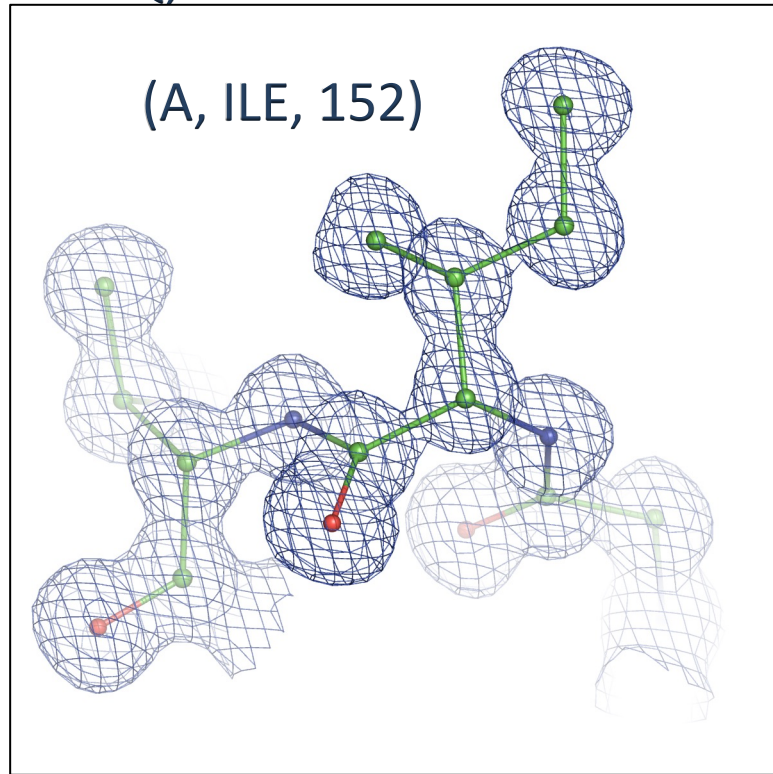
- Simulated annealing (SA)
- Model morphing
 - Only use if model has gross errors (correction requires large movements)
 - Do not use if model is relatively good and only needs small corrections

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 \neq wrong

3NOQ, 1 Å



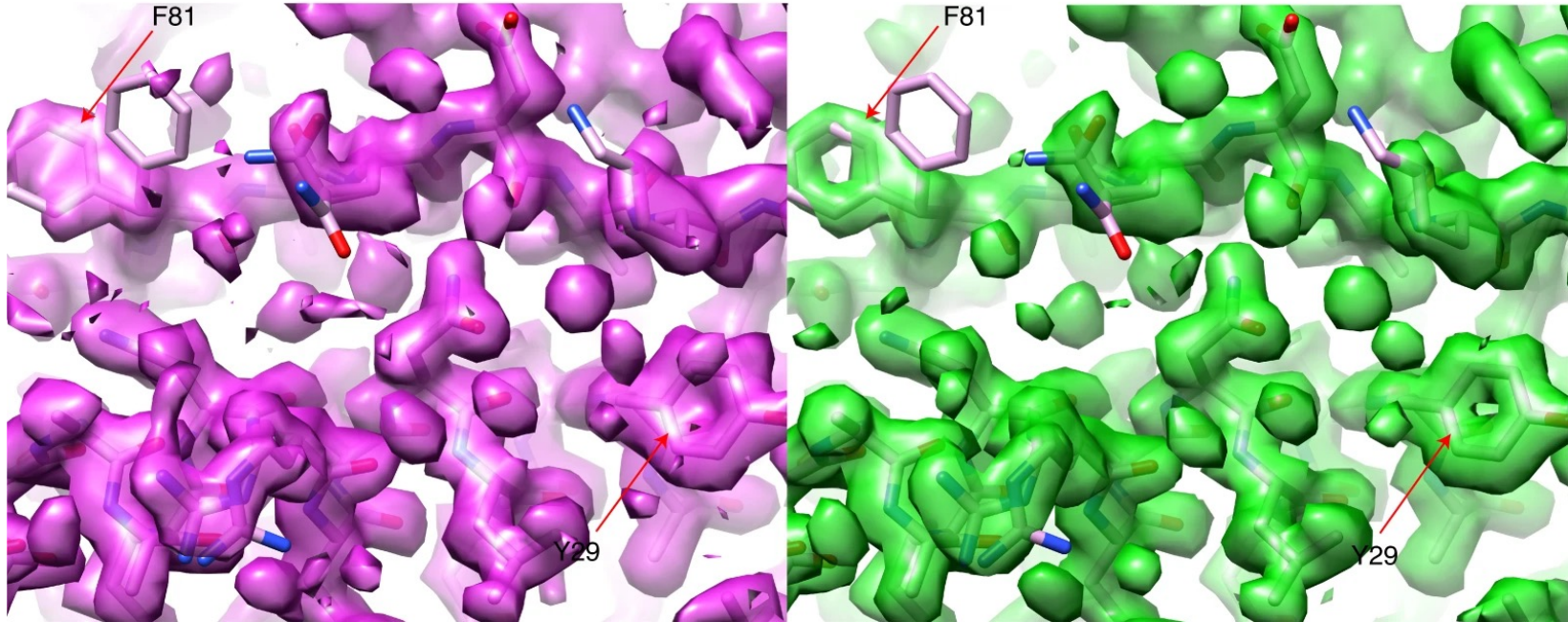
Outliers:

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

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

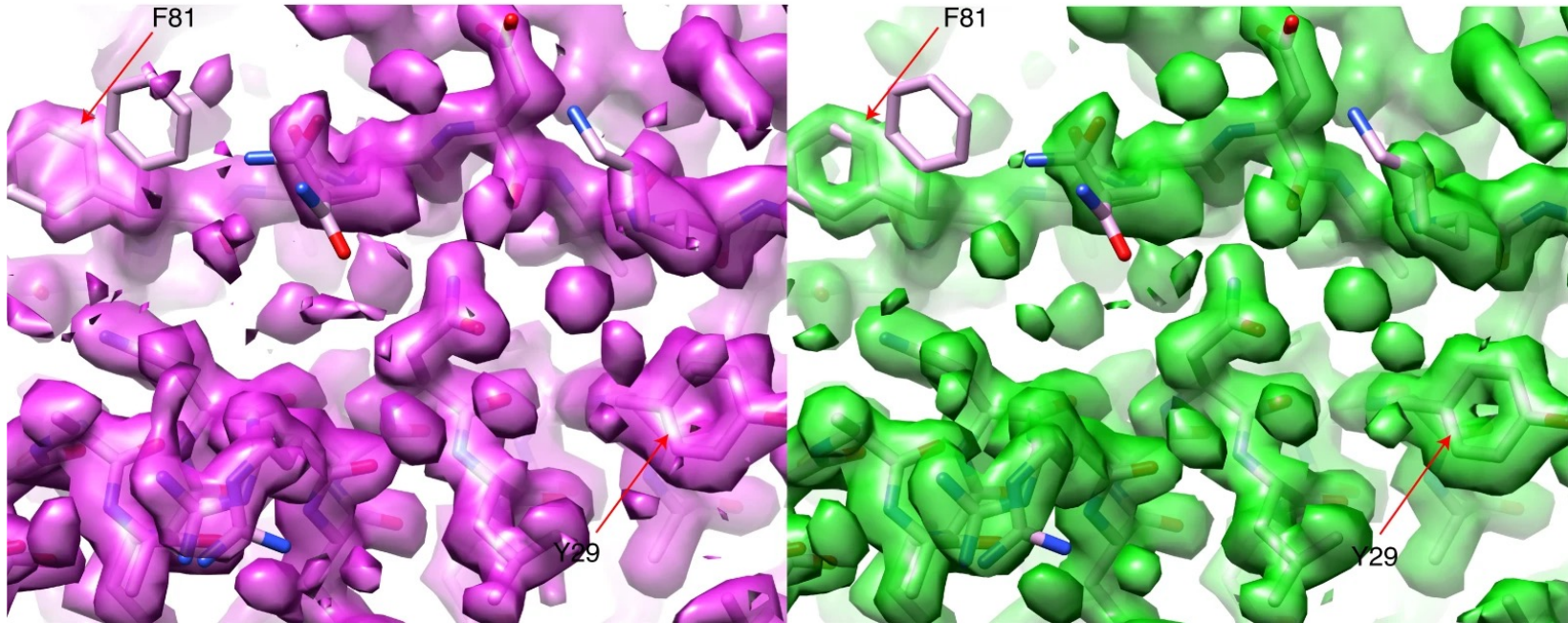
Map comparison

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

Phenix home

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Actions Job history

Projects

Show group: All groups Manage...

Select Delete New project Import project Settings

ID	Last modified	# of jobs	R-free
ions	Apr 07 2026 06:13 ...	1	---
AF_7mjs_H_Pred..	Apr 06 2026 02:43 ...	4	---
AsmitMaps	Apr 05 2026 01:44 ...	4	---
rsg1	Apr 01 2026 10:23 ...	1	---
7rpq_AF_referen..	Apr 01 2026 10:19 ...	1	---
1aba-polder_0	Mar 25 2026 07:34 ...	7	---
1aba-polder	Mar 25 2026 05:31 ...	10	---
real-space-refin..	Mar 25 2026 02:10 ...	1	---
rnase-s	Mar 24 2026 08:41 ...	2	0.2879
editsBug	Mar 16 2026 09:37 ...	4	0.1972
debug12	Feb 19 2026 04:31 ...	4	---
UR-13914	Feb 17 2026 04:19 ...	3	0.2133
tests1	Feb 16 2026 09:37 ...	4	---
zzHOH	Feb 10 2026 08:17 ...	1	---
dlbug1	Feb 03 2026 01:47 ...	1	---
seseg	Dec 23 2025 12:27 ...	3	---
ttran	Dec 17 2025 09:26 ...	2	---
debug2	Dec 12 2025 01:05 ...	2	---
polder	Dec 08 2025 12:33 ...	1	---
cccccc	Nov 11 2025 11:23 ...	0	---
zz38	Oct 01 2025 08:09 ...	10	0.1500
phwkUK	Sep 30 2025 01:43 ...	1	---
zz37	Sep 18 2025 07:21 ...	5	0.1500
douset	Sep 09 2025 06:08... 33	---	---
67in	Sep 09 2025 07:17 ... 5	---	0.2769
zz34	Sep 09 2025 06:42 ... 23	---	---

Favorites

- AI agents and AlphaFold tools
- Crystals
 - Data analysis and manipulation
 - Experimental phasing
 - Molecular replacement
 - Map improvement
 - Model building
 - Refinement
 - Validation
 - Map and structure factor calculation
 - Map comparison and analysis
 - Map Correlations
 - Correlations between maps (or map coefficients), or map and model, optionally allowing translations
 - Model-based map superposition
 - Superpose two PDB files and transform the associated map coefficients to the new orientation
 - Map sigma level comparison**
 - Calculate equivalent sigma levels when visually comparing two maps
 - Identify local maxima and minima in mFo-DFc map (and anomalous map if available) and flag waters with excess density
- Cryo-EM
 - Map analysis and manipulation
 - Map improvement
 - Docking and model building
 - Real-space refinement
 - Validation

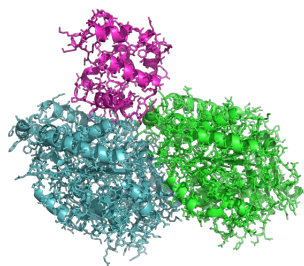
Current directory: /Users/pafonine/Desktop/all/phenix/zz56 Browse...

Phenix version dev-svn Project: ions

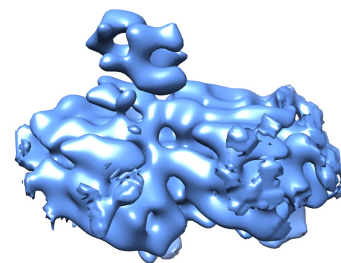
Refinement success is function of data quality

- Do validation

Model

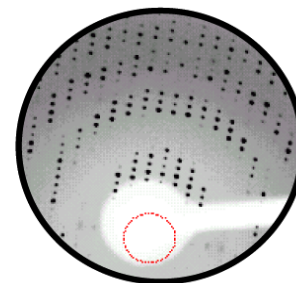


Data



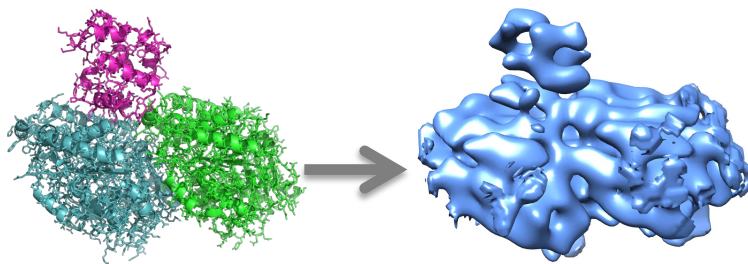
Cryo-EM

or



Diffraction

Model to data fit



Validation tools in Phenix

PHENIX home

Quit Preferences Help Citations Coot PyMOL KING Other tools Ask for help

Actions Job history

Projects

Show group: All groups Manage...

Select Delete New project Settings

ID	Last modified	# of jobs	R-free
✓ ChrisF	Apr 13 2020 09:42...	28	0.1944
real-space-refin...	Apr 03 2020 07:42...	2	---
zzz1	Mar 21 2020 09:10...	1	---
chris	Mar 12 2020 12:27...	11	0.1890
dan	Mar 11 2020 05:44...	1	---
3j63	Mar 11 2020 02:28...	1	---
jason	Mar 11 2020 11:36...	1	---
rt6	Mar 11 2020 10:31...	1	0.2459
mate	Mar 10 2020 01:36...	1	---
emily	Mar 09 2020 03:52...	3	---
—	Mar 05 2020 08:25...	3	0.1923
alex	Feb 27 2020 11:33...	6	---
rt20201	Feb 18 2020 12:50...	4	0.2213
1f8t	Feb 03 2020 09:00...	1	0.1977
real-space-refin...	Jan 30 2020 02:38...	2	---
real-space-refin...	Jan 29 2020 10:56...	1	---
ion_channel_den...	Jan 27 2020 07:36...	3	---
10101	Jan 27 2020 12:38...	2	---
demos	Jan 27 2020 10:57...	3	---
ion_channel_den...	Jan 27 2020 10:03...	2	---
malcolm	Jan 22 2020 10:22...	14	0.1748
real-space-refin...	Jan 16 2020 04:28...	3	---
3NIR	Dec 05 2019 10:2...	1	---
leighton	Sep 02 2019 05:1...	2	---
5pti	Aug 27 2019 03:4...	3	---

Favorites

Data analysis

- Xtrriage**
Analysis of data quality and crystal defects
- Merging statistics**
Calculates a variety of statistics for unmerged intensities, including I/sigma, R-merge, R-meas, and CC1/2.
- Mtrriage**
Analyze quality of maps in CCP4 format

Experimental phasing

Molecular replacement

Model building

Refinement

Cryo-EM

Validation

- Comprehensive validation (X-ray/Neutron)**
Model quality assessment, including real-space correlation and geometry inspection using MolProbity tools
- Comprehensive validation (cryo-EM)**
Model quality assessment, including real-space correlation, for cryo-EM structures

Structure comparison

Identify differences between multiple structures of the same protein, using multiple criteria

Calculate CC*

Comparison of unmerged data quality with refined model, as described in Karplus & Diederichs (2012)

EMRinger

Model validation for de novo electron microscopy structures

Ligands

Current directory: /Users/pafonine/Desktop/all/people/ChrisF Browse...

PHENIX version dev-svn-000 Project: ChrisF

Phenix is feedback & need driven

YOU

**CAN MAKE
AN IMPACT**

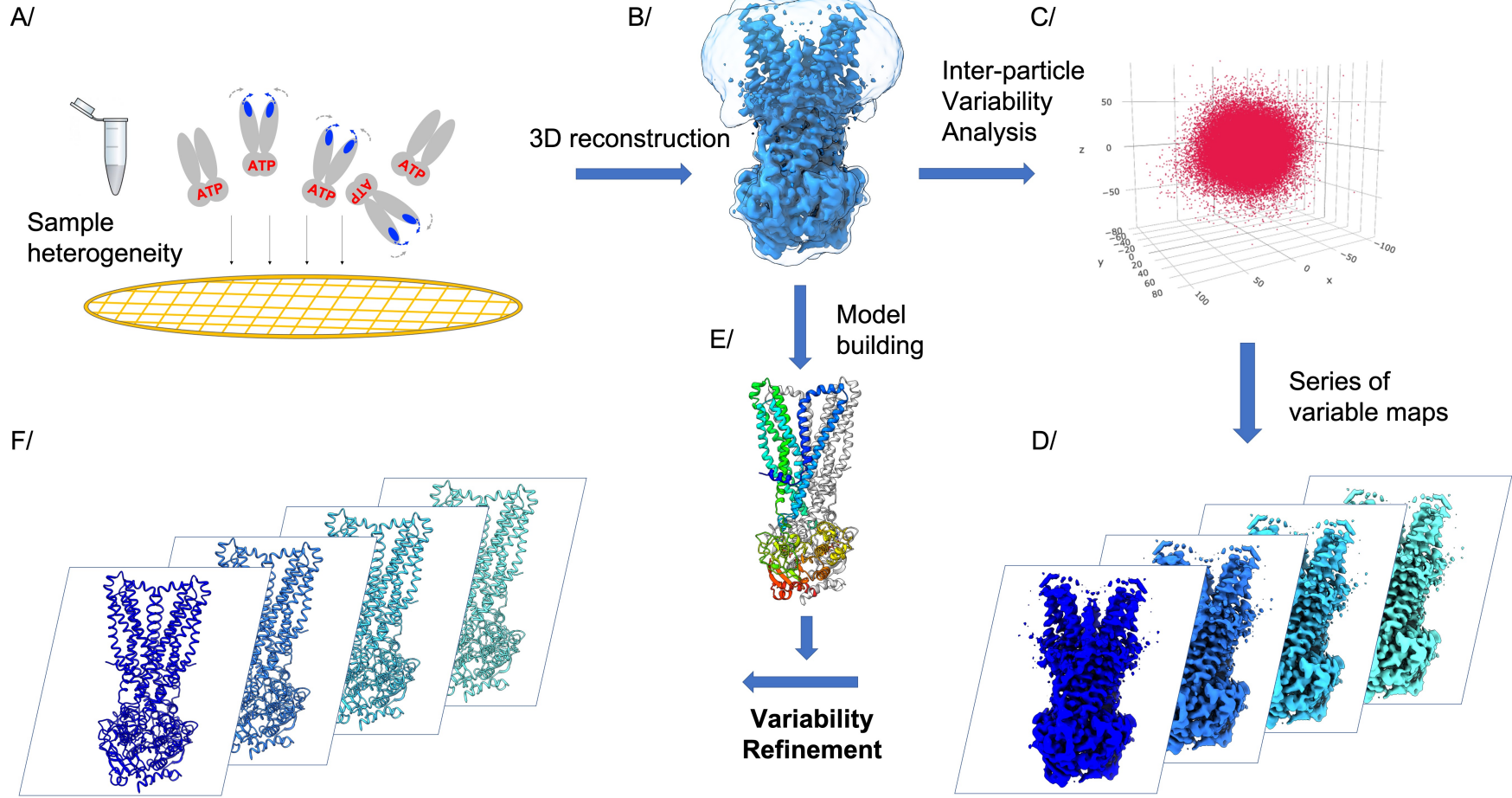
CAN LEAVE

YOUR FOOTPRINT





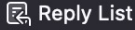






YOU


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

MMSB -UMR5086
7 passage du Vercors
69007 LYON
FRANCE
+33 4 37 65 29 01
<http://www.appicom.cnrs.fr>
<http://mmsb.cnrs.fr/en/>

Feedback & need driven – Example

The screenshot shows an email client interface. At the top, there are navigation icons for Unread, Starred, Contact, Tags, and Attachment, along with a search bar. Below this is a list of 11 conversations. The first conversation is selected and expanded, showing a thread of 9 messages. The sender is Vincent Chaptal. The subject is "[phenixbb] refinement of an ensemble of structures -> cryoEM variability". The message content discusses cryoEM maps and 3D variability. Below this, two other conversations are visible, one from Oliver Clarke and another from Vincent Chaptal. At the bottom of the interface, a status bar shows "Selected: 63" and "Total: 63", with the "Selected: 63" text circled in red.

Subject	Correspondents	Date
Re: [phenixbb] refinement of an ensemble of structures -> cr...	vincent Chaptal	1/11/22, 16:36
Re: [phenixbb] refinement of an ensemble of structures -> cr...	Guillaume Gaullier	1/12/22, 04:09
Re: [phenixbb] refinement of an ensemble of structures -> cr...	vincent Chaptal	1/12/22, 04:36
Re: [phenixbb] refinement of an ensemble of structures -> cr...	vincent Chaptal	1/12/22, 11:18
Re: [phenixbb] refinement of an ensemble of structures -> cr...	vincent Chaptal	1/12/22, 16:03
Re: [phenixbb] refinement of an ensemble of structures -> cr...	Oliver Clarke	1/13/22, 11:17
Re: [phenixbb] refinement of an ensemble of structures -> cr...	phenixbb@phenix-online.org	1/13/22, 11:40

11 conversations Archive Delete

[phenixbb] refinement of an ensemble of structures -> cryoEM vincent Chaptal <vincent.chaptal@ibcp.fr> variability (9 messages)

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 consensu...

[phenixbb] refinement of an ensemble of structures -> cryoEM Oliver Clarke <olibclarke@gmail.com> variability (16 messages)

Hi, just to add my two cents, I agree this would be really useful for a lot of folks. Analysis of continuously distributed variability is very common these days in cryoEM, and having a way to jointly refine an ensemble of models against a series of maps would be very handy. Cryodrgrn, 3D-VA in cryo...

[phenixbb] refinement of an ensemble of structures -> cryoEM Oliver Clarke <olibclarke@gmail.com> variability (2 messages)

I guess it isn't all that different. If you run all jobs naively starting from a single model corresponding to the overall reconstruction, depending on the magnitude of the conformational changes the maps at either end of the series may be outside the radius of convergence of phenix.real_space_ref...

link to data 3D variability cryoEM (4 messages) vincent Chaptal <vincent.chaptal@ibcp.fr>

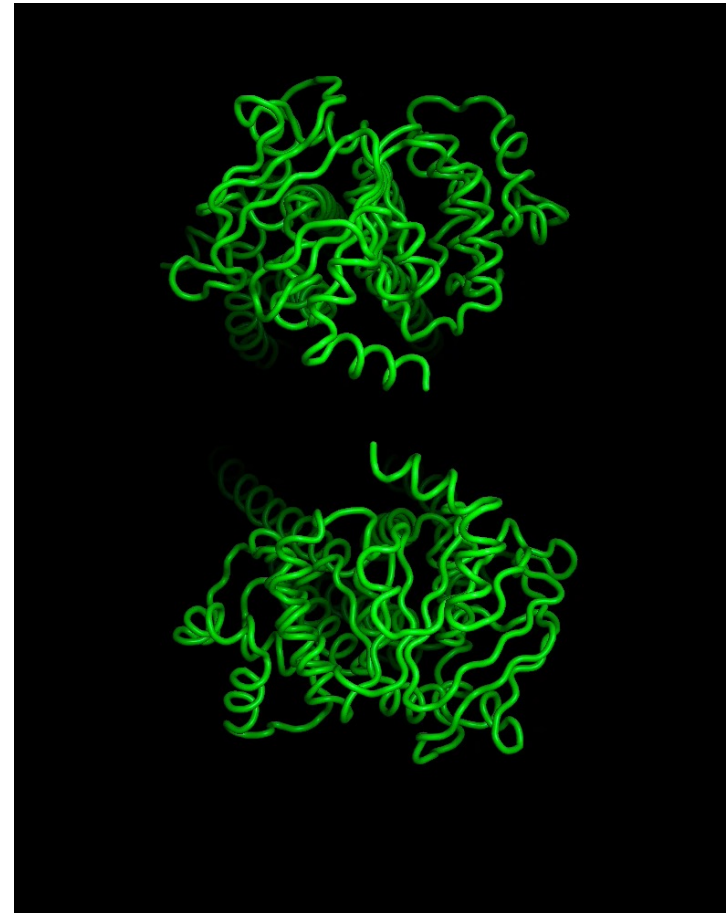
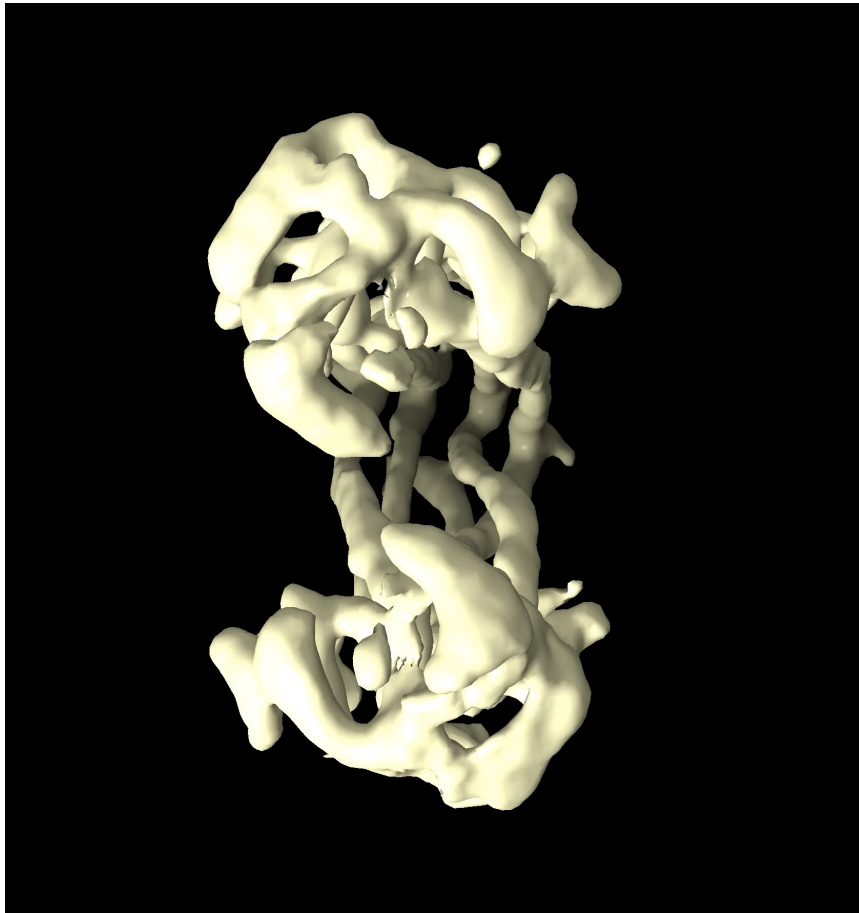
Hi Pavel, here are the data: <https://filesender.renater.fr/?s=download&token=da52b4ab-ecf1-4329-88dd-bec9433f155c> Thanks for looking into this, I'm sure it's going to be a very used tool. Best Vincent -- Vincent Chaptal, PhD Director of GdR APPICOM Drug Resistance and Membrane Proteins Lab...

Selected: 63 Total: 63

**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

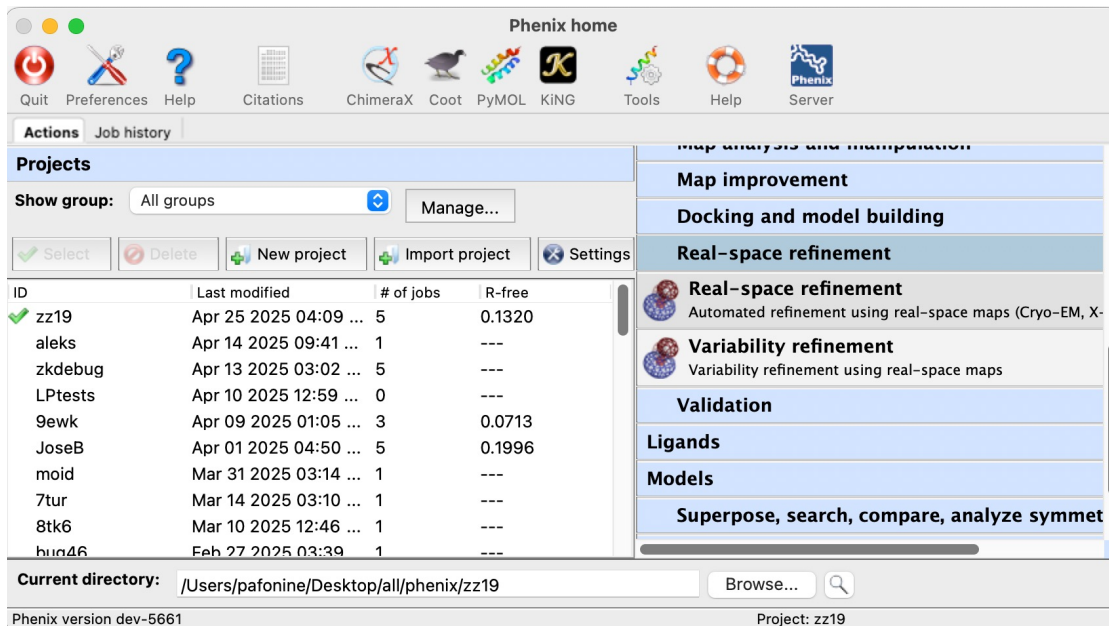



Review

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*



Phenix home

Quit Preferences Help Citations ChimeraX Coot PyMOL KING Tools Help Server

Actions Job history

Projects

Show group: All groups Manage...

Select Delete New project Import project Settings

ID	Last modified	# of jobs	R-free
✓ zz19	Apr 25 2025 04:09 ...	5	0.1320
aleks	Apr 14 2025 09:41 ...	1	---
zkdebug	Apr 13 2025 03:02 ...	5	---
LPtests	Apr 10 2025 12:59 ...	0	---
9ewk	Apr 09 2025 01:05 ...	3	0.0713
JoseB	Apr 01 2025 04:50 ...	5	0.1996
moid	Mar 31 2025 03:14 ...	1	---
7tur	Mar 14 2025 03:10 ...	1	---
8tk6	Mar 10 2025 12:46 ...	1	---
hna46	Feh 27 2025 03:39 ...	1	---

Current directory: /Users/pafonine/Desktop/all/phenix/zz19 Browse...

Phenix version dev-5661 Project: zz19

- Map analysis and manipulation
- Map improvement
- Docking and model building
- Real-space refinement
 - Real-space refinement
 - Automated refinement using real-space maps (Cryo-EM, X-)
 - Variability refinement
 - Variability refinement using real-space maps
- Validation
- Ligands
- Models
- Superpose, search, compare, analyze symmet

Feedback & need driven – Varref (Variability Refinement)

nature communications

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

Article Conformational diversity in class C GPCR positive allosteric modulation

Giuseppe Cannone^{1,7}, Ludovic Berto^{2,7}, Fanny Malhaire², Gavin Ferguson²,
Aurelien Fouillen², Stéphanie Balor³, Joan Font-Ingles⁴, Amadeu Llebaria⁴,
Cyril Goudet², Abhay Kotecha⁵, Vinothkumar K.R.⁶ &
Guillaume Lebon²

Received: 23 November 2023

Accepted: 10 December 2024

Published online: 13 January 2025

www.nature.com/scientificreports

nature communications

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

Article Rhodamine6G and Hoechst33342 narrow BmrA conformational spectrum for a more efficient use of ATP

A. Gobet^{1,7}, L. Moissomier^{2,7}, E. Zarkadas³, S. Magnard², E. Bettler⁴,
J. Martin⁵, R. Terreux⁴, G. Schoehn⁶, C. Orelle², JM Jault²,
P. Falson² & V. Chaptal²

Received: 5 April 2024

Accepted: 3 February 2025

Published online: 18 February 2025

scientific reports

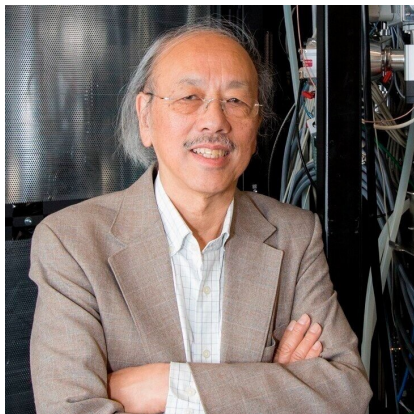
OPEN

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 Huang³

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



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}

^aGraduate Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030; ^bNational Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030; ^cMolecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; ^dDepartment of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; and ^eDepartment 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).

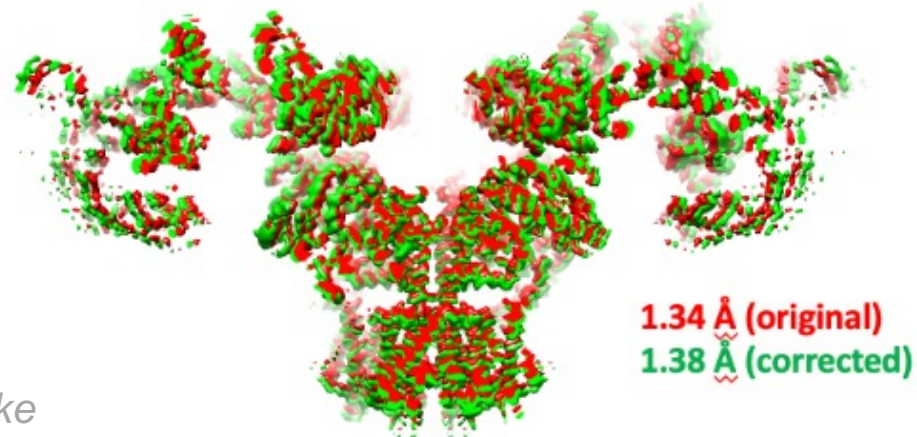
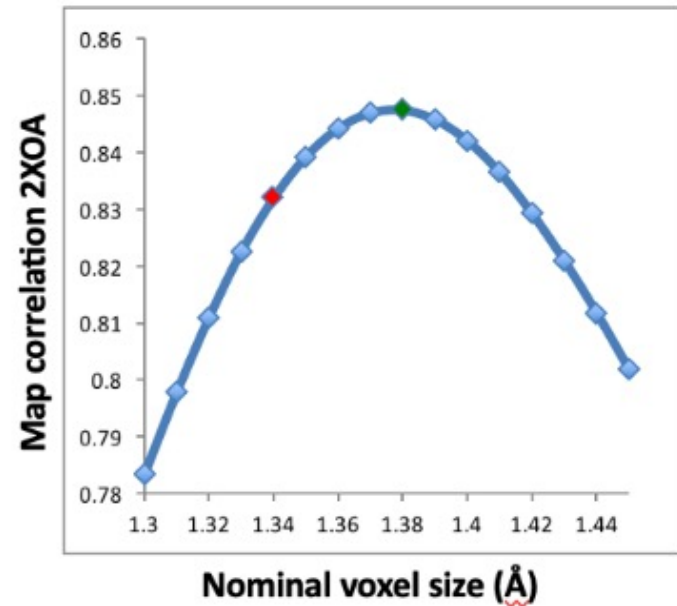
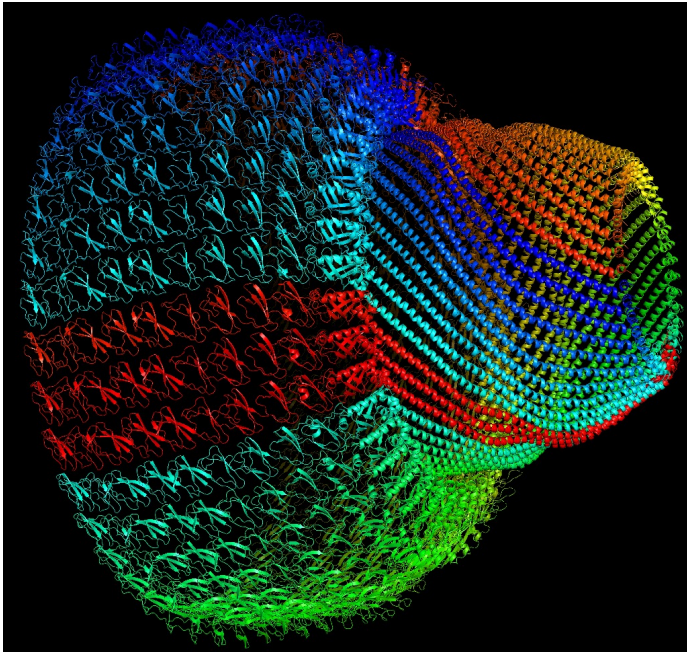


Figure: Oliver Clarke

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

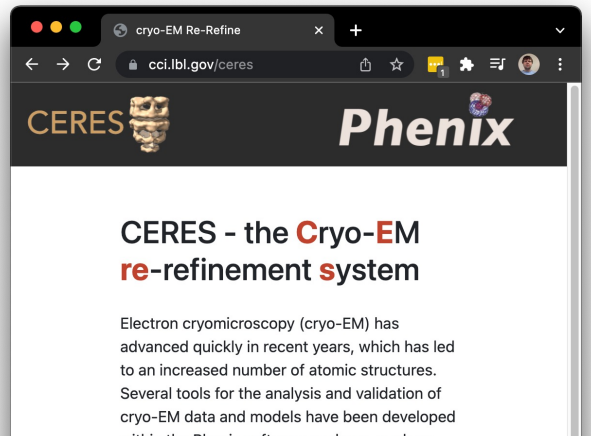


Table showing results for November 2021

Rerefinement month: [dropdown] Table options: [dropdown]

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 [dropdown] Map vs model [dropdown] Geometry [dropdown] Ramachandran [dropdown] Composition [dropdown] Other [dropdown]

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



STRUCTURAL
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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

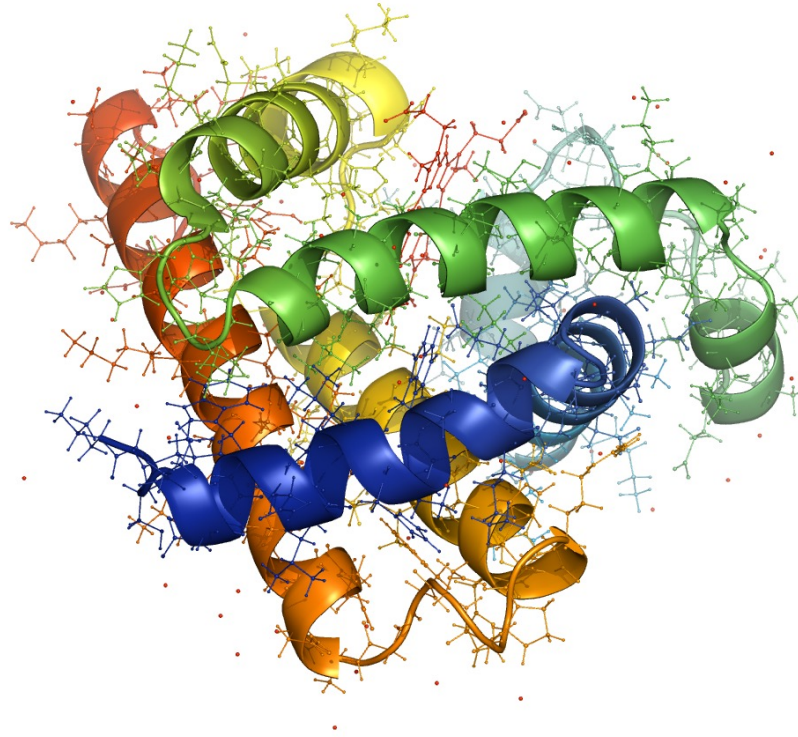
Bug reports (developers only): bugs@phenix-online.org

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

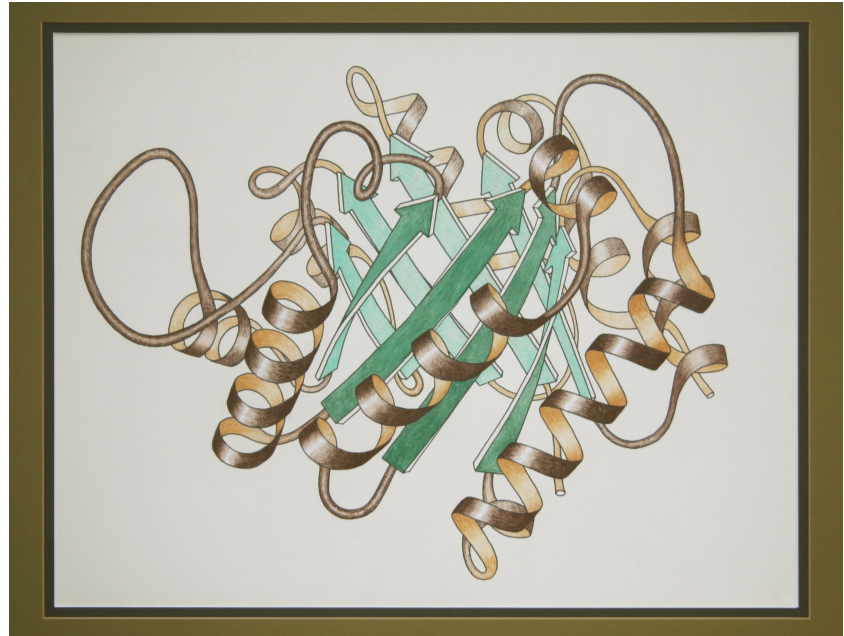


Q: Who invented this method of drawing a structure (ribbon diagram)?

Ribbon diagram



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



Hand-drawn by **J. Richardson,**
1981