Low Resolution Refinement

ECM32 Vienna

August 2019

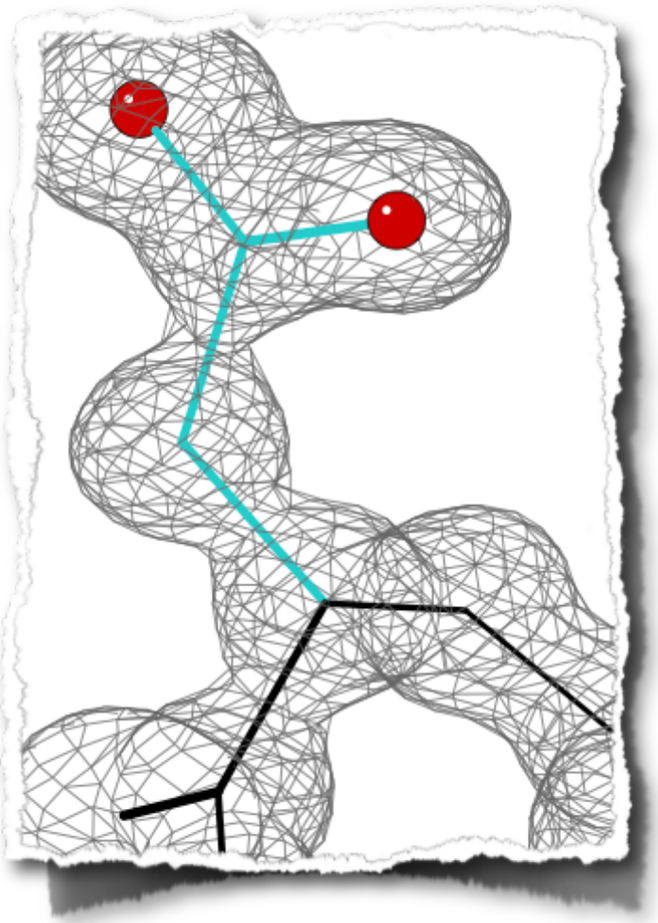
Paul Adams

Lawrence Berkeley Laboratory and

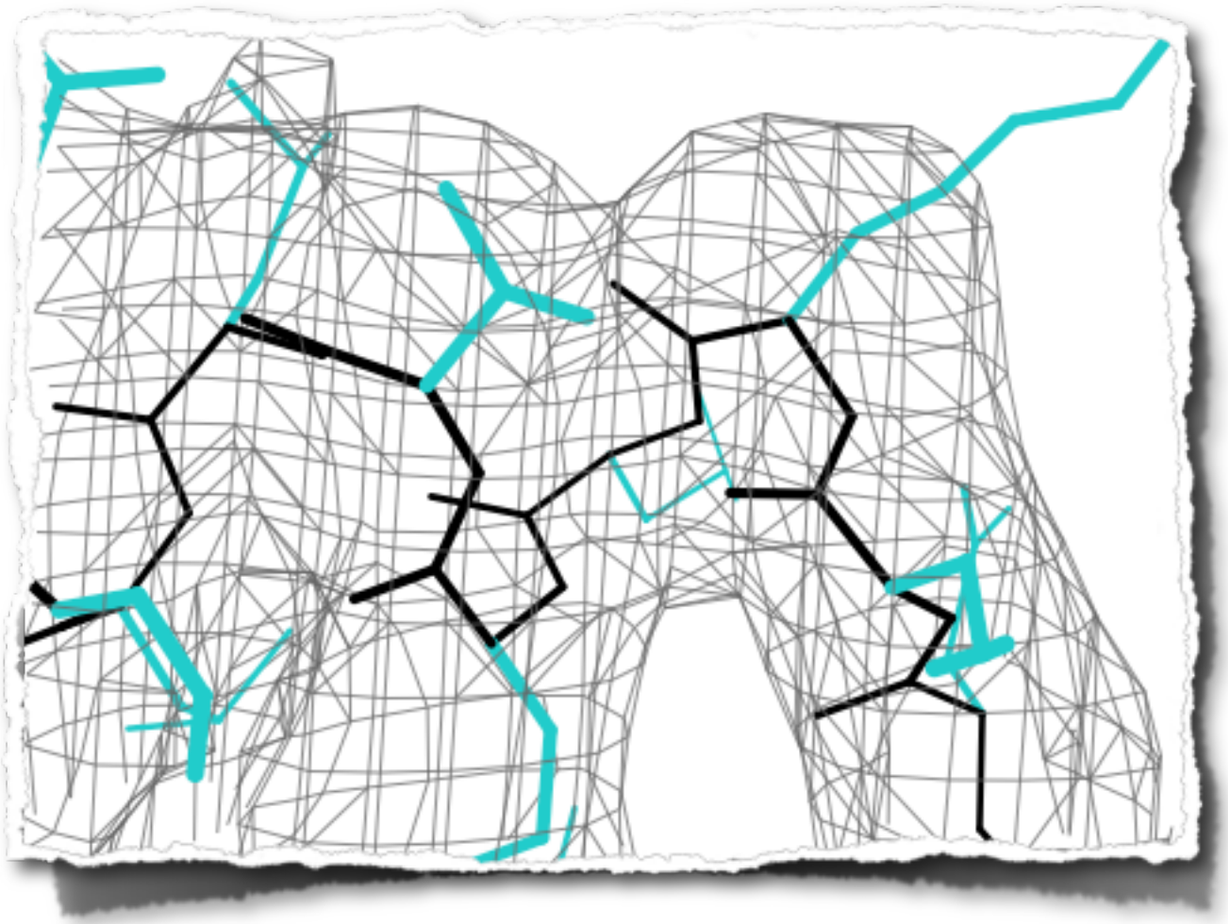
Department of Bioengineering UC Berkeley

Macromolecular Crystallography

PDBID: 2gkg
Resolution: 1.00Å



PDB ID: 3k7a
Resolution: 3.80Å



- Many challenges, but low resolution data is increasingly an issue:
  - How to interpret “featureless” maps (pattern matching, chemical constraints)
  - How to optimize models with sparse data (prior information)

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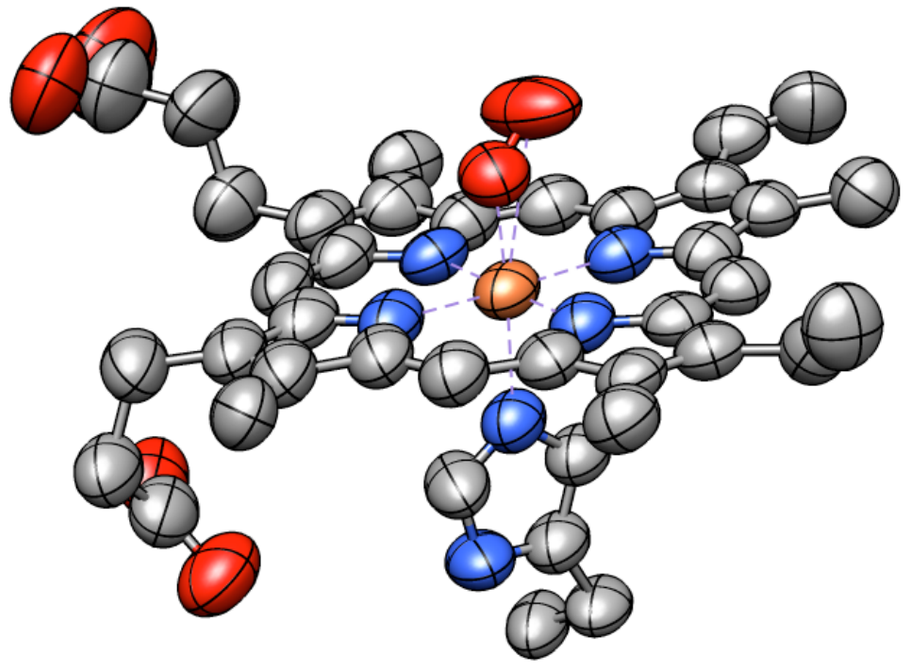
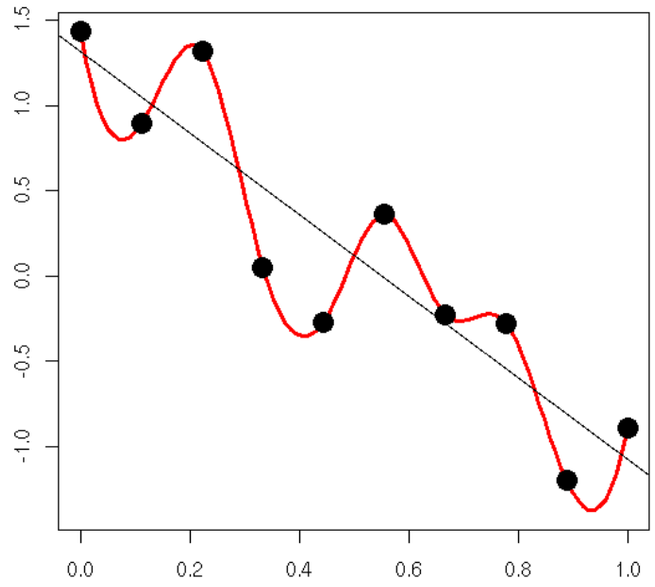
The Challenge of Too Few Data

- With only low resolution data we typically have too many parameters to optimize
  - Atomic coordinates, displacement parameters
- Underdetermined optimization problems lead to overfitting of the data
- To help address overfitting we can:
  - Add prior information to reduce the number of effective parameters
  - Remove parameters
- Current refinement methods do not define a reasonable chemical result in the absence of data





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Improving the Observation to Parameter Ratio

- To make refinement practical the observation to parameter ratio is increased using restraints and constraints:
- Restraint
  - Model property ~ ideal value
  - Adds prior observed information (reduces the number of parameters refined)
  - Inclusion of chemical information in the objective function
- Constraint
  - Model property = ideal value
  - Removes one or more parameters from the model

Methods in Phenix for Improving Models

- Using prior structural knowledge as additional restraints:
  - Secondary structure
  - Protein mainchain conformations (Ramachandran)
  - Related high resolution structures as restraints
  - Multiple copies of the same molecule as restraints *(c.f. local NCS restraints in SHELX)*
- Automated correction of models during refinement using prior knowledge of stereochemistry:
  - Fixing of rotamers
  - Flipping of side chains

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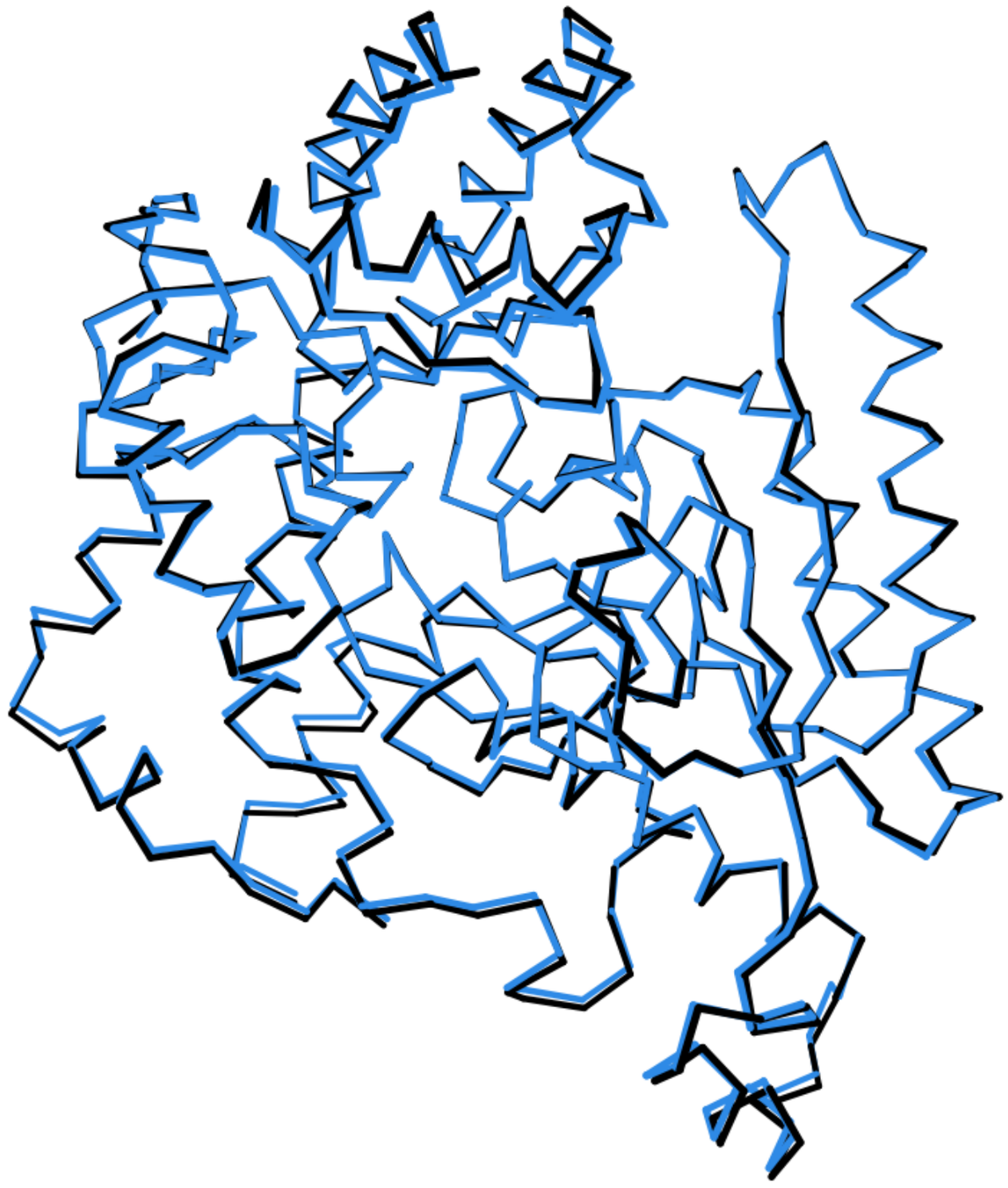
Reference model restraints

(Jeff Headd)

1GTX and 1OHV

1GTX: 3.0 Å

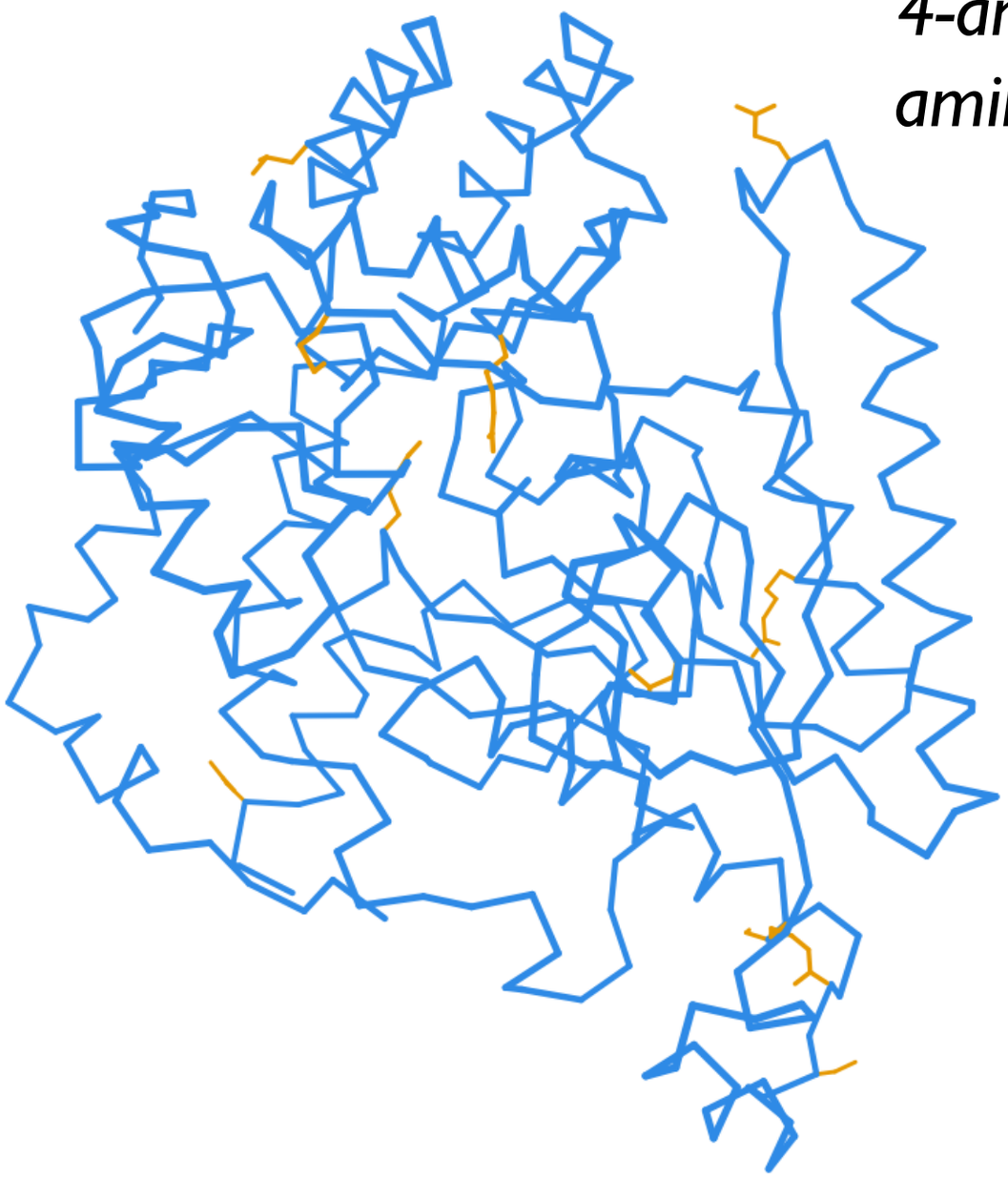
1OHV: 2.3 Å

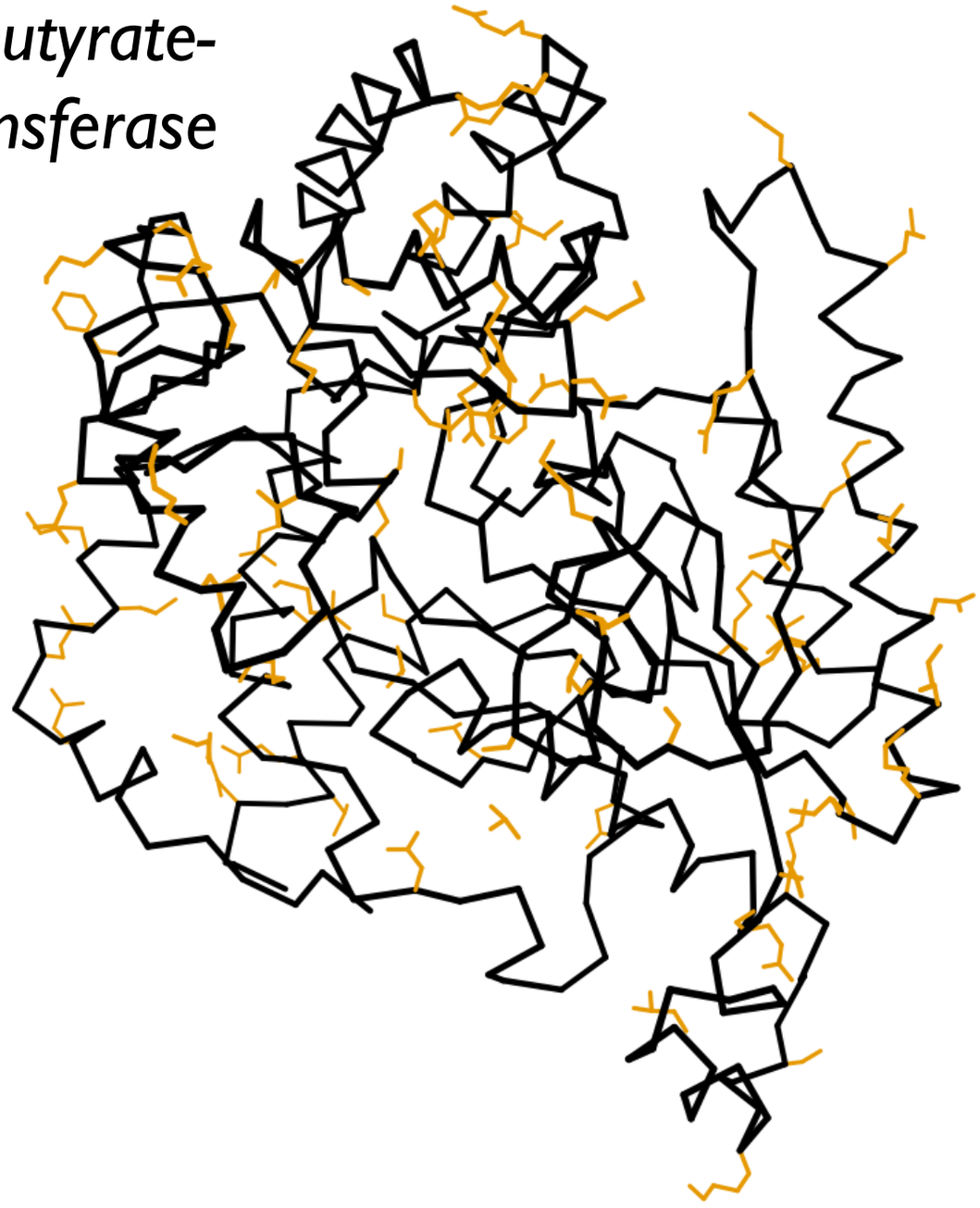


4-aminobutyrate-aminotransferase

1GTX and 1OHV

4-aminobutyrate-aminotransferase





1OHV: 2.3 Å

1GTX: 3.0 Å

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Reference Model Restraints

Combines two concepts:

- Pre-correct rotamer outliers
  - Set rotamer outliers in the model to match the torsion angles of the reference model if the reference model has an acceptable rotamer at that position and there is no significant clash or density mismatch
- Generate reference torsion restraints
  - Restrain each torsion angle in the working model to the corresponding torsion angle in the reference model
    - Chains are aligned using SSM alignment to allow for sequence differences
  - Restraints take the form of a modified harmonic 'top-out' potential that allows for structural differences

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Headd JJ et al., 2012, *Acta Cryst.* **D68**:381-390

Reference model restraints

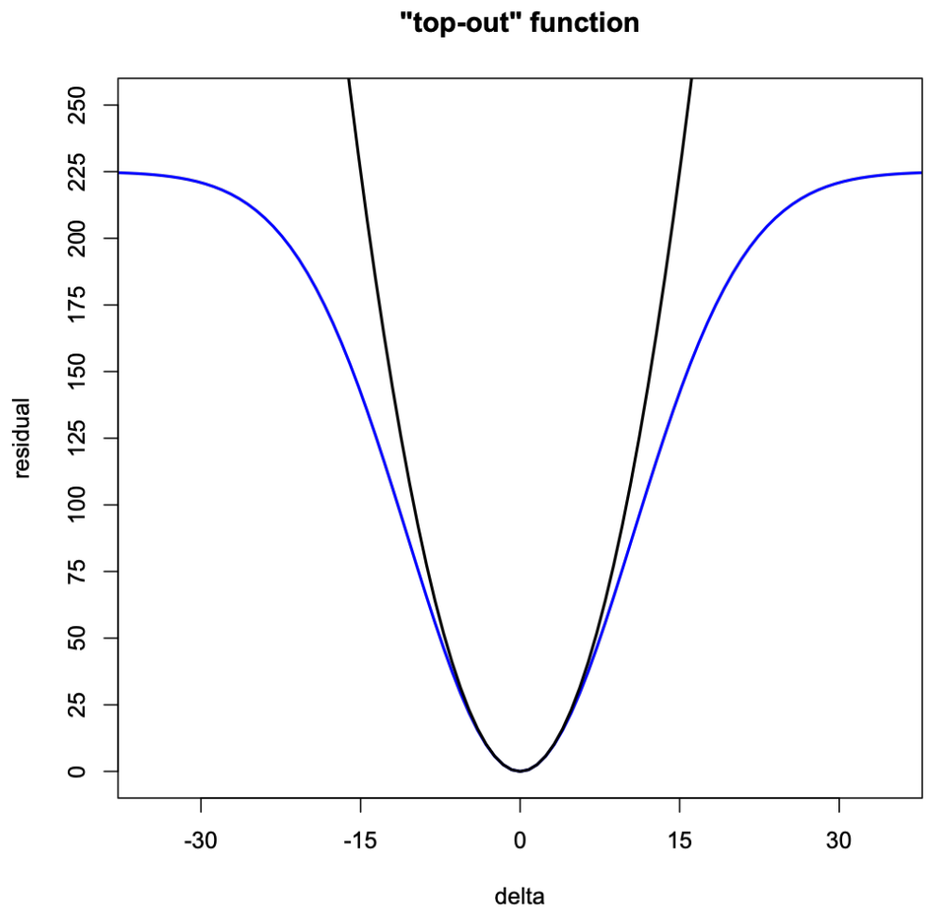
$$E_{total} = \sum_{i=1}^{n} E_i$$

Simple harmonic potential: $E_i = w\Delta_i^2$

'Top-out' potential: $E_i = \tau(1.0 - e^{\frac{-\Delta_i^2}{l^2}})$

$$\tau = wl^2$$

$$w = \frac{1}{\sigma^2}$$



Similar potentials are used in REFMAC5 and BUSTER - Geman-McClure robust estimator function

where σ is the ESD, Δ is the difference between the model dihedral and reference dihedral, and l is a 'limit' parameter that limits how far the model dihedral may vary from the reference dihedral before being shut off.

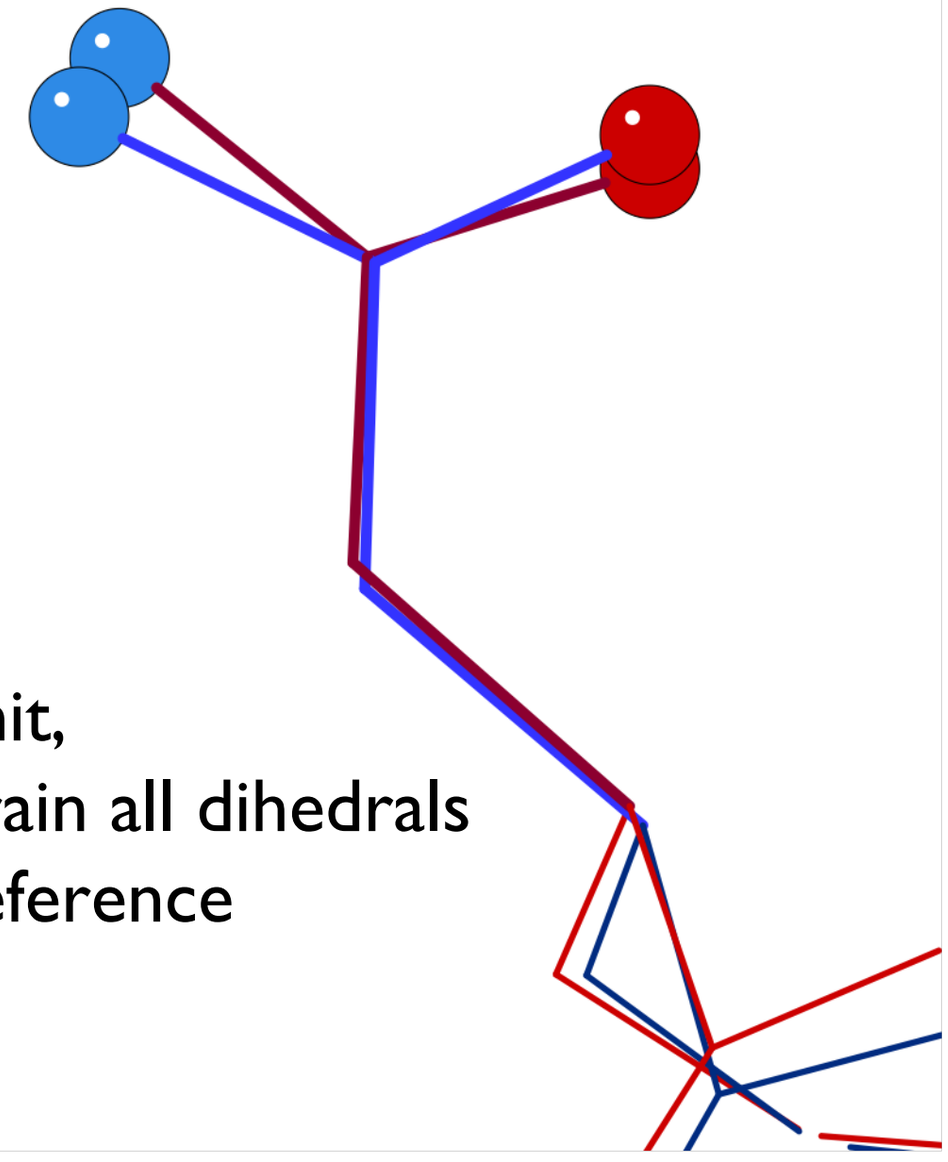
developed by Ralf Grosse-Kunstleve

default: limit = 15.0°

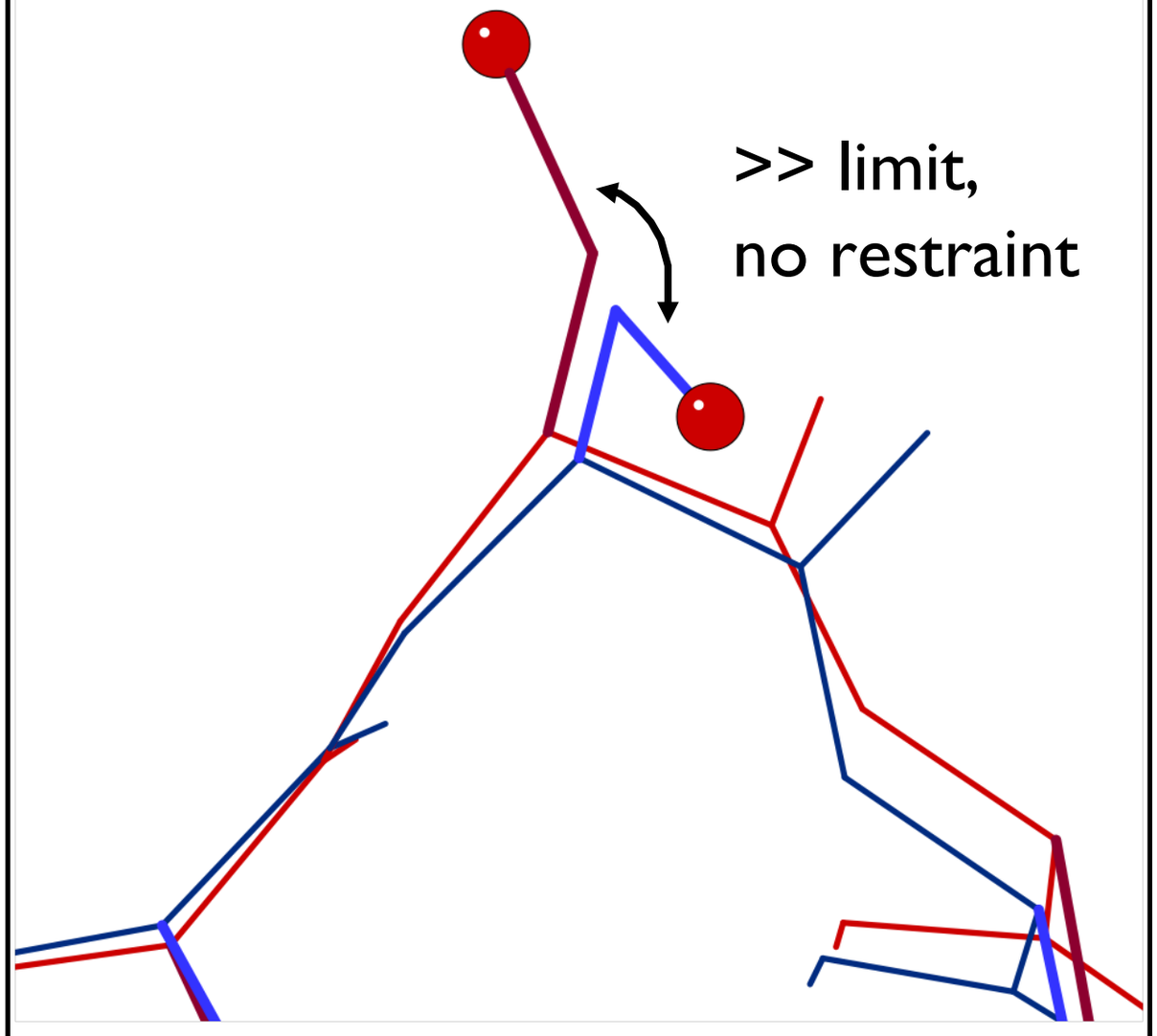
The ‘limit’ parameter

default: limit = 15.0°

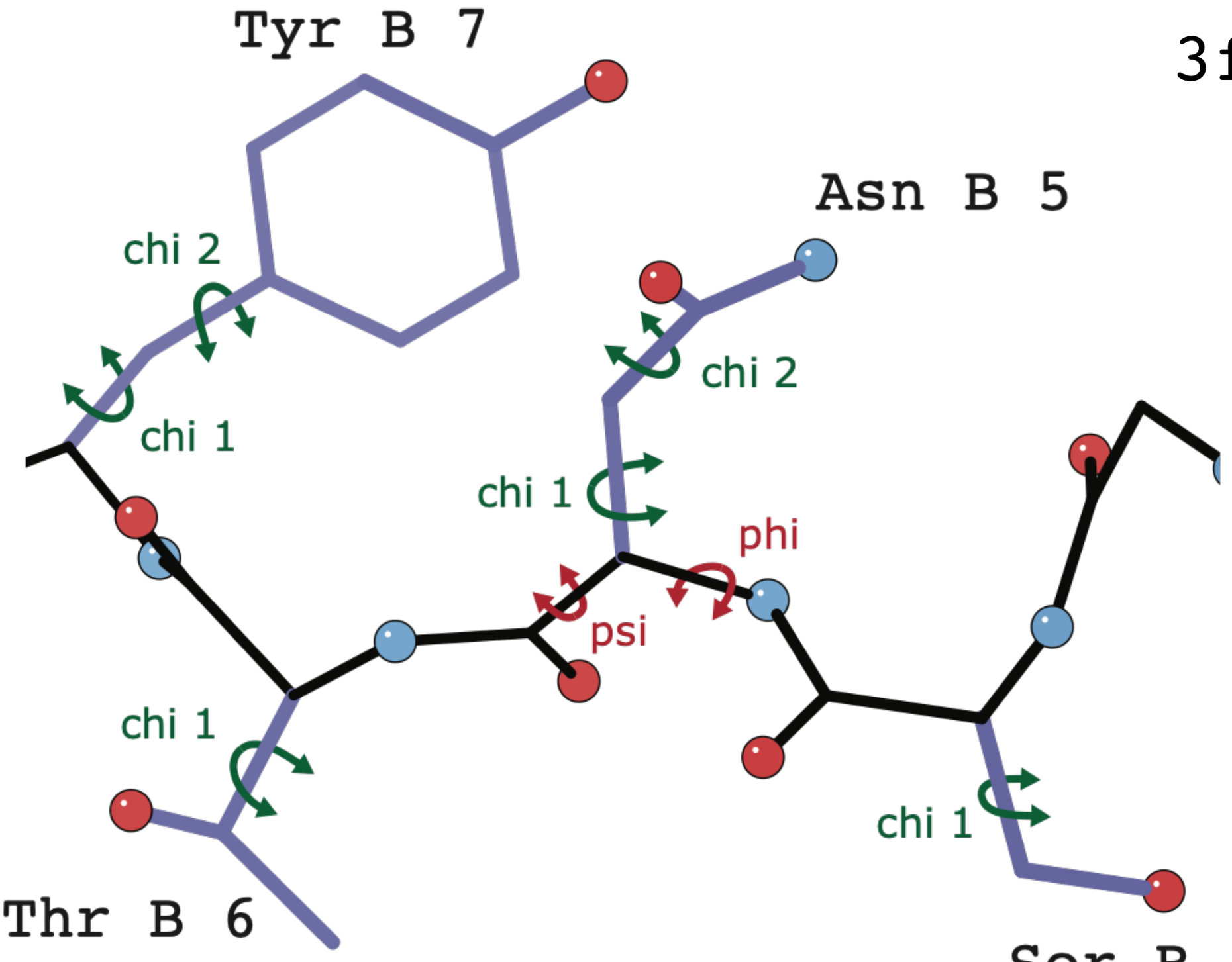
< limit,
restrain all dihedrals
to reference



>> limit,
no restraint

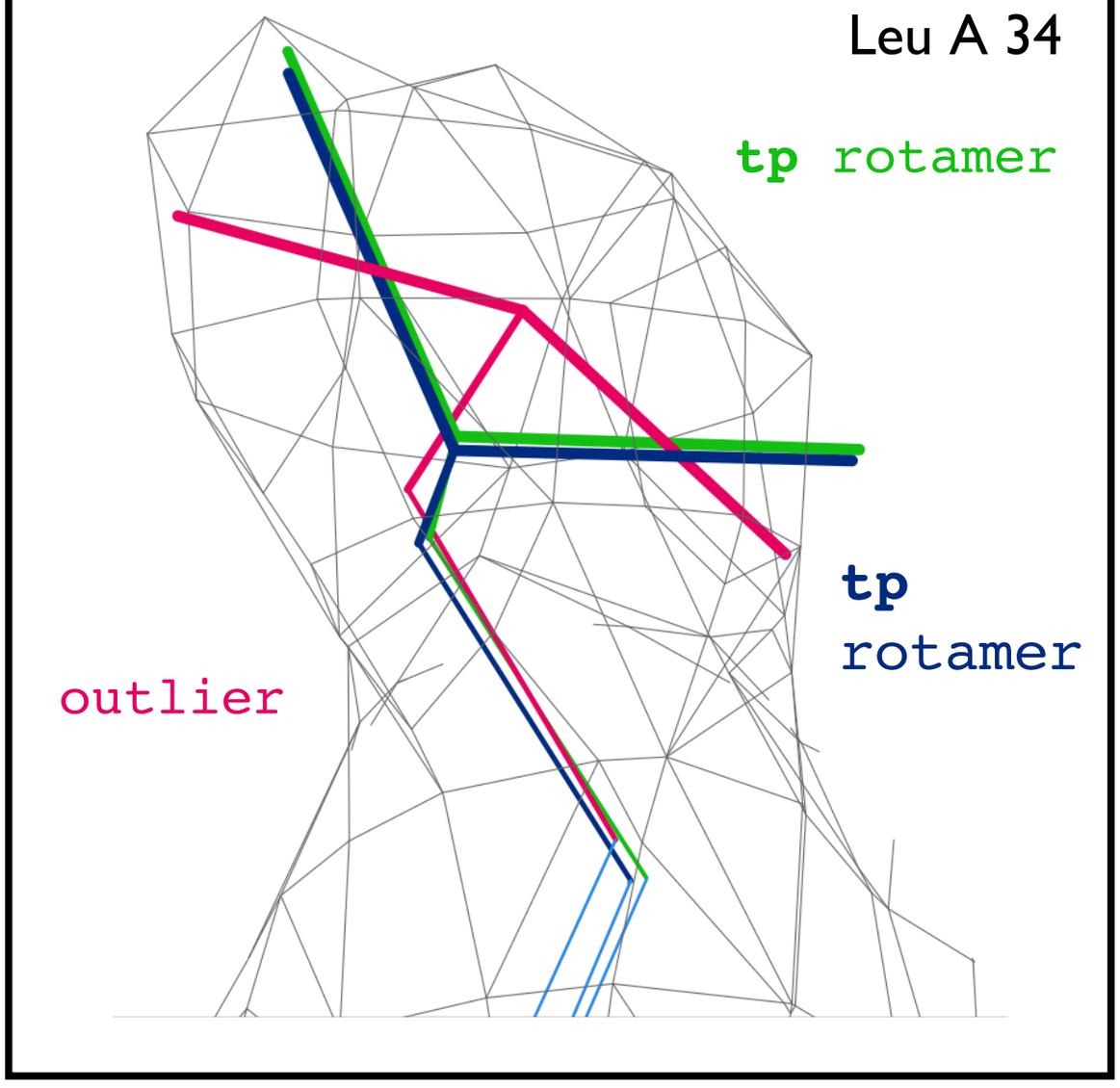


Why torsion angles?

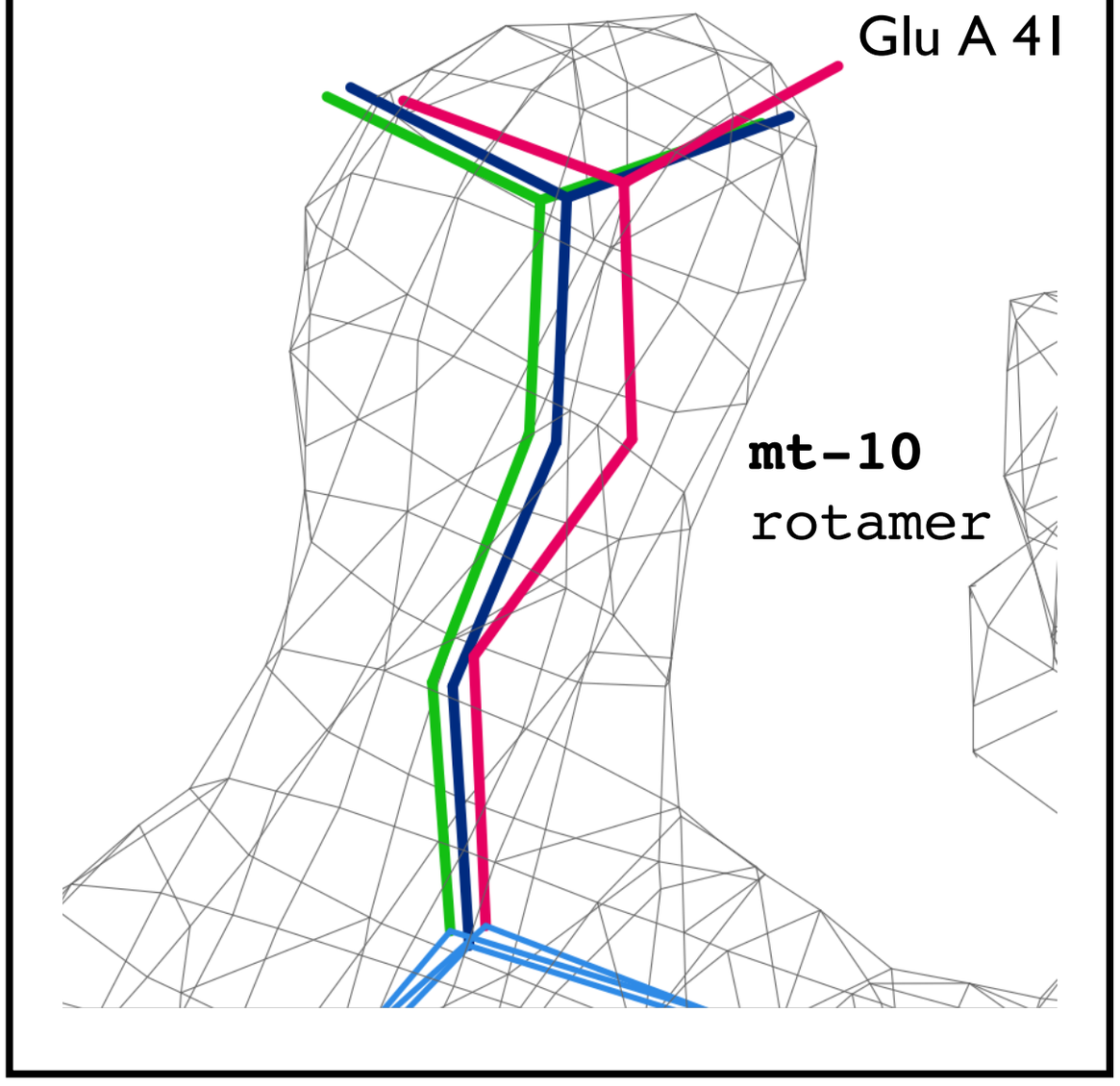


1GTX/1OHV reference example

outlier correction



restrained refinement



1GTX (3.0Å) 1OHV (2.3Å) 1GTX w/ 1OHV reference

5 macrocycles of *phenix.refine*
w/ reference restraints →

R_{free}: 0.2379 → 0.2186
ΔR: 0.833 → 0.60
MolProbity: 64th → 96th

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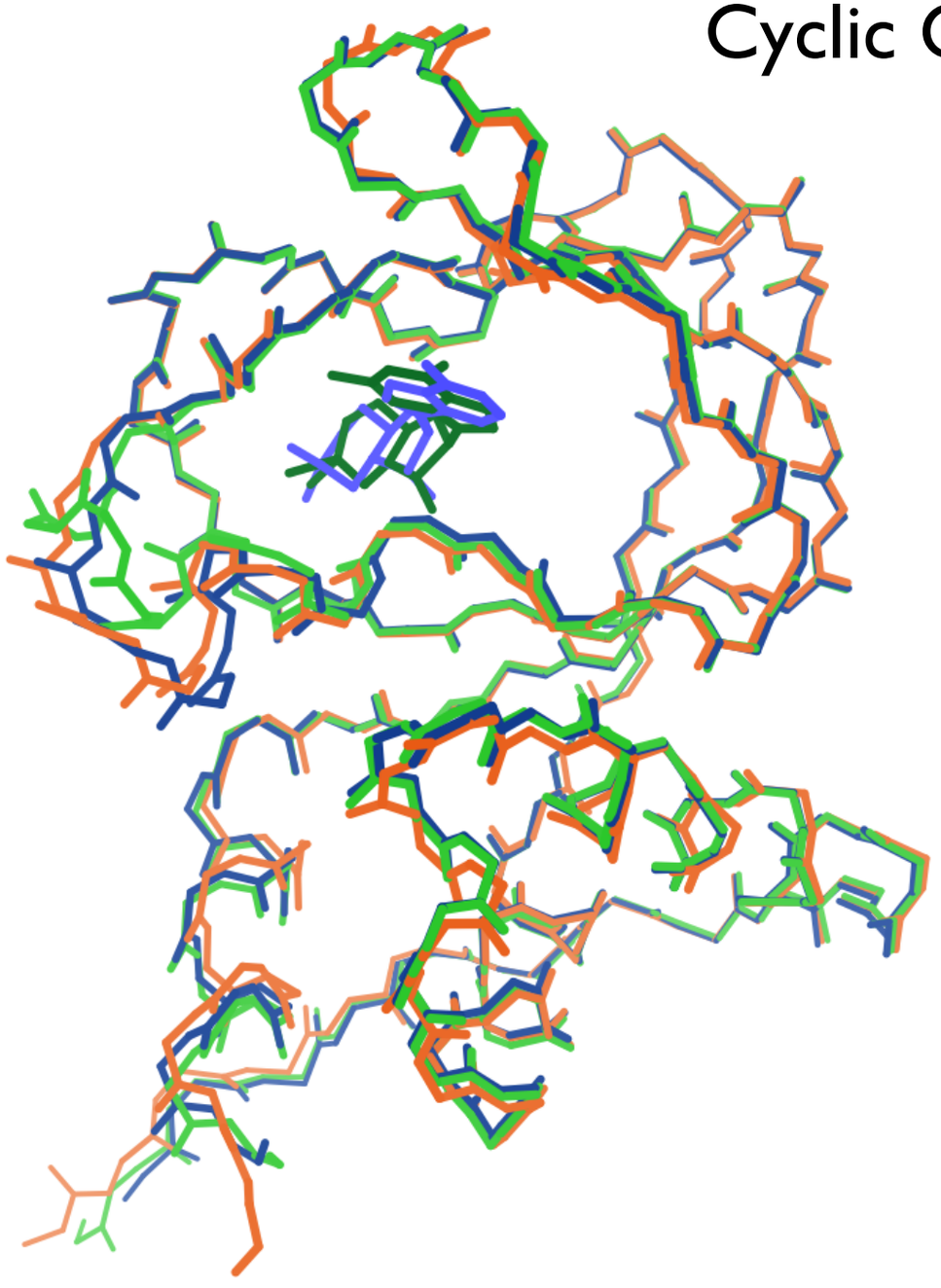
Practical Example

Cyclic GMP-dependent protein kinases (PKG's)



cAMP bound: 2.49Å

cGMP bound: 3.20Å

APO form: 2.69Å

JJ Kim et al. (2011) Crystal structures of PKG Iβ (92-227) with cGMP and cAMP reveal the molecular details of cyclic nucleotide binding. *PLoS ONE*.

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Cyclic GMP-dependent protein kinase

cAMP bound: 2.49Å

cGMP bound: 3.20Å

APO form: 2.69Å

	Validation Criteria	cAMP bound
All-Atom Contacts	Clashscore, all atoms:	16.53
	Clashscore percentile	81st
Protein Geometry	Poor rotamers:	2.61%
	Rama outliers:	0.00%
	Rama favored:	98.80%
	Cβ dev. > 0.25Å:	0
	MolProbity score:	2.04
	MP score percentile	95th
	Res w/ bad bonds:	0.00%
	Res w/ bad angles:	0.00%
Residual	R-work	0.1960
	R-free	0.2264

cGMP bound
56.57
15th
18.58%
2.02%
85.48%
23
3.84
12th
2.38%
5.95%
0.2102
0.2582

→

cGMP bound
24.56
87th
4.00%
0.40%
96.00%
0
2.61
96th
0.00%
1.18%
0.1980
0.2397

APO
28.52
46th
10.53%
3.19%
89.02%
3
3.29
12th
0.79%
0.98%
0.2205
0.2612

→

APO
19.5
15th
3.66%
0.60%
96.61%
0
2.43
89th
0.00%
0.20%
0.2166
0.2525

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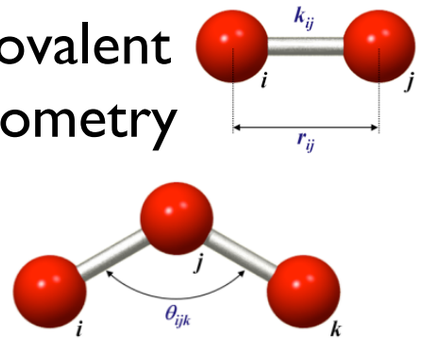
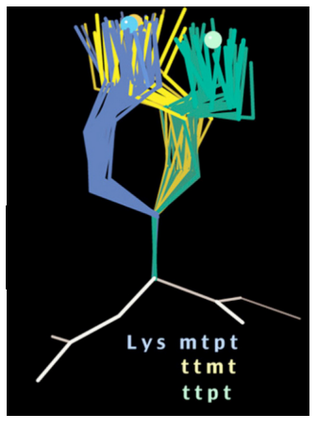
Sources of Prior Information

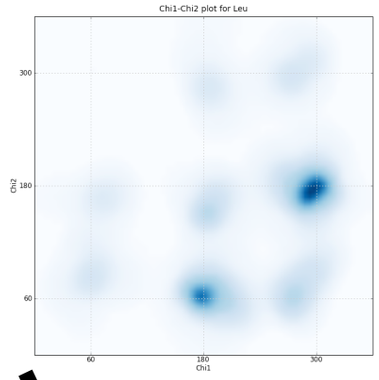
Covalent geometry

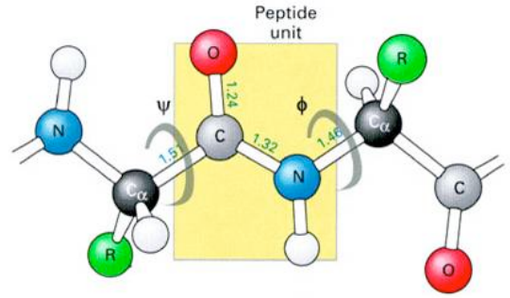


Images from PumMa web site (http://www.pumma.nl)

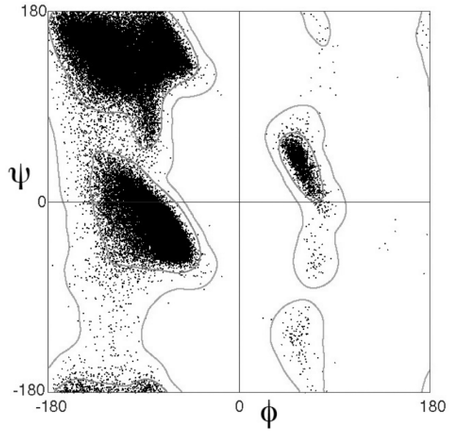
Sidechain distributions

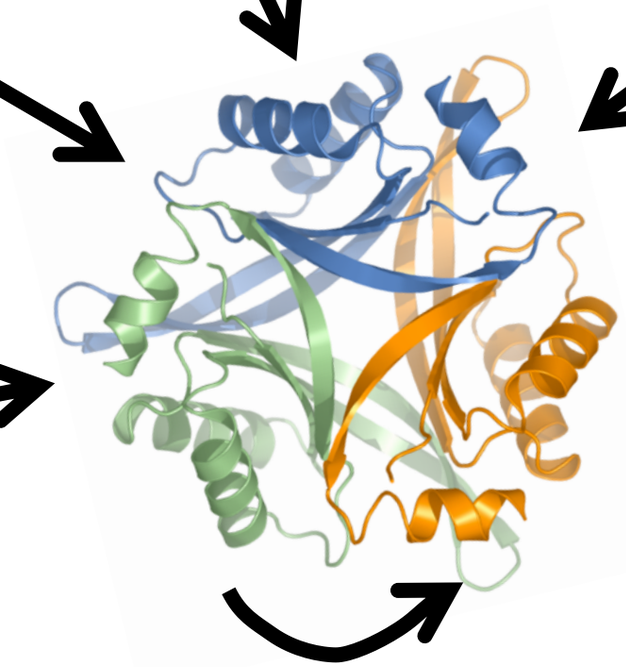


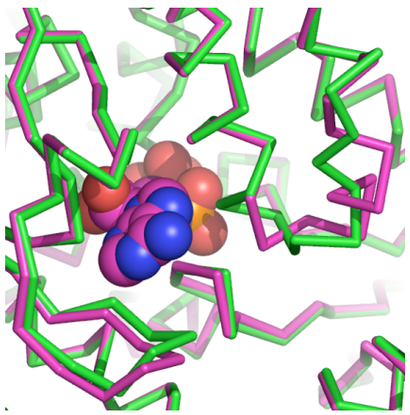




Mainchain distributions

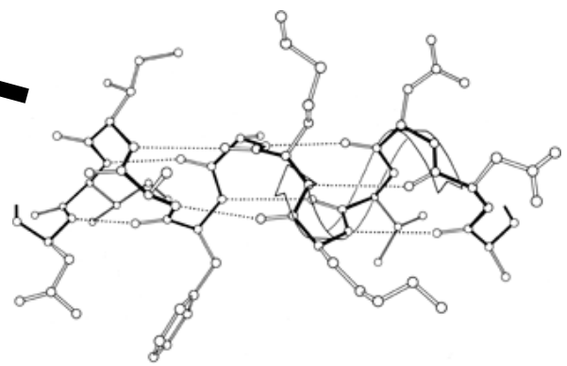






Related structures

Internal symmetry



Secondary structure

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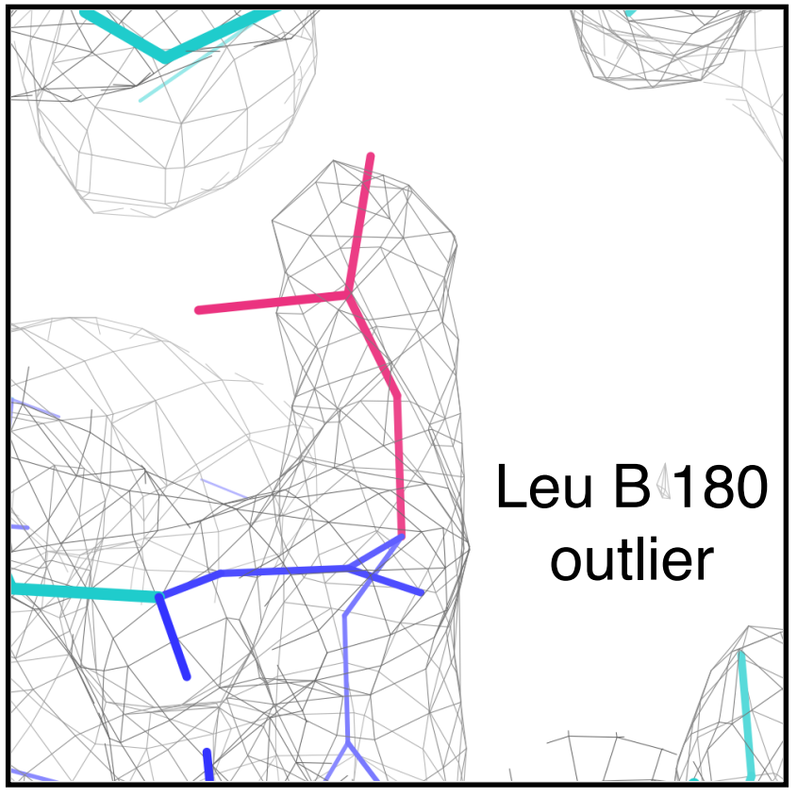
Torsion space NCS restraints

(Jeff Headd)

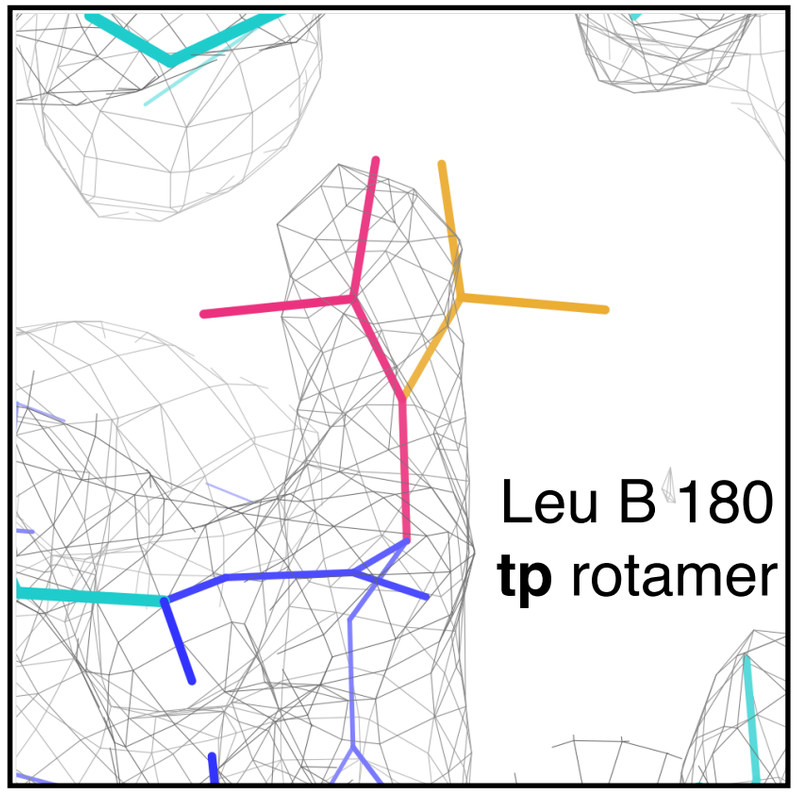
1b04: 2.8 Å DNA ligase

rotamer outlier correction

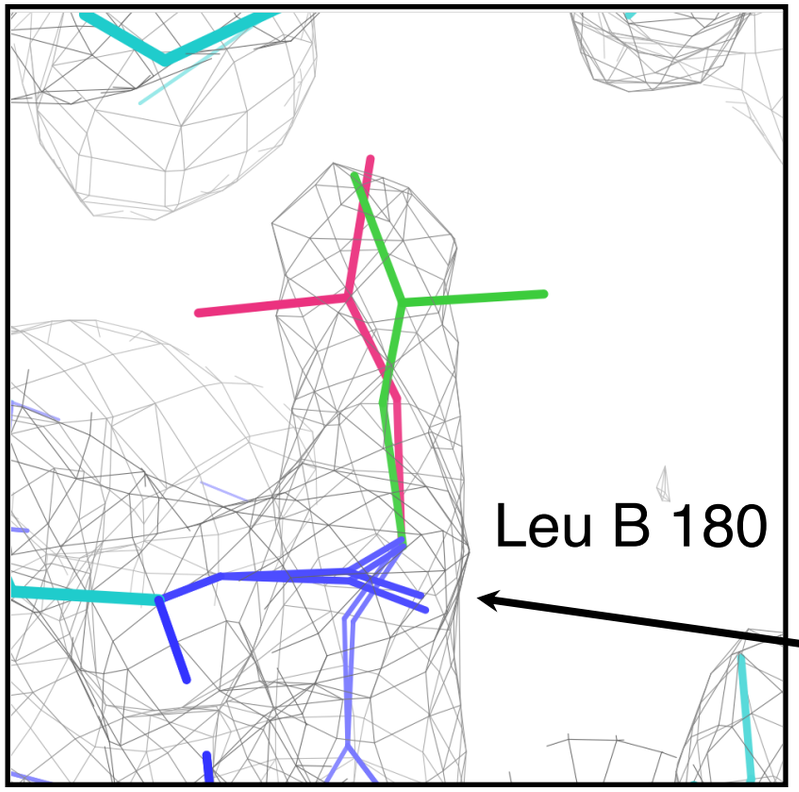
1. Identify rotamer outlier



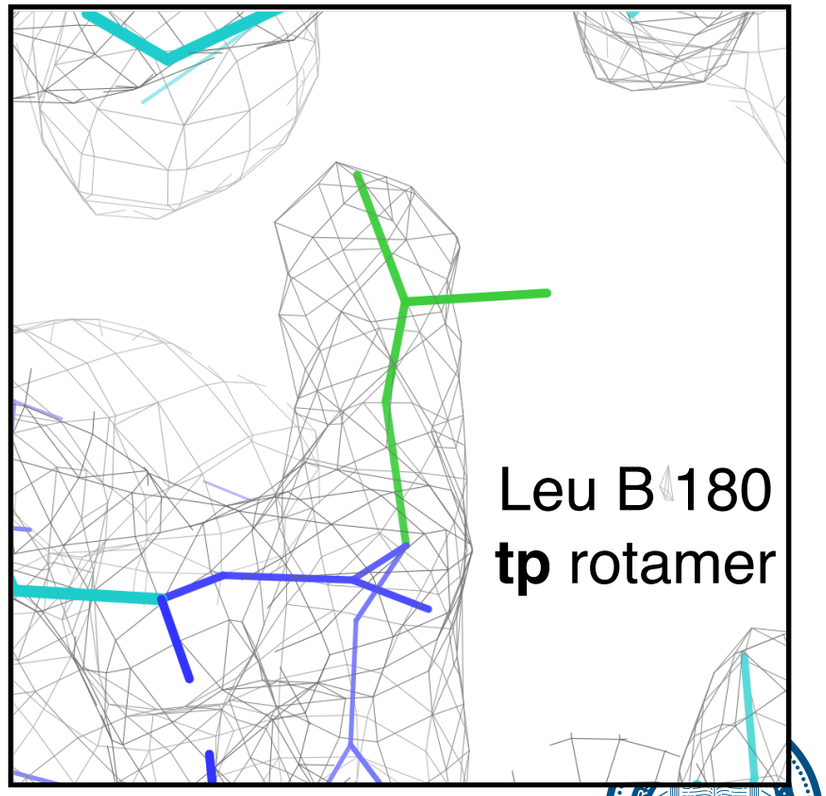
2. correct to corresponding rotamer in NCS-related chain by matching χ angles



3. ‘backrub’ search, then limited χ angle torsion search



4. verify rotamer is still correct match



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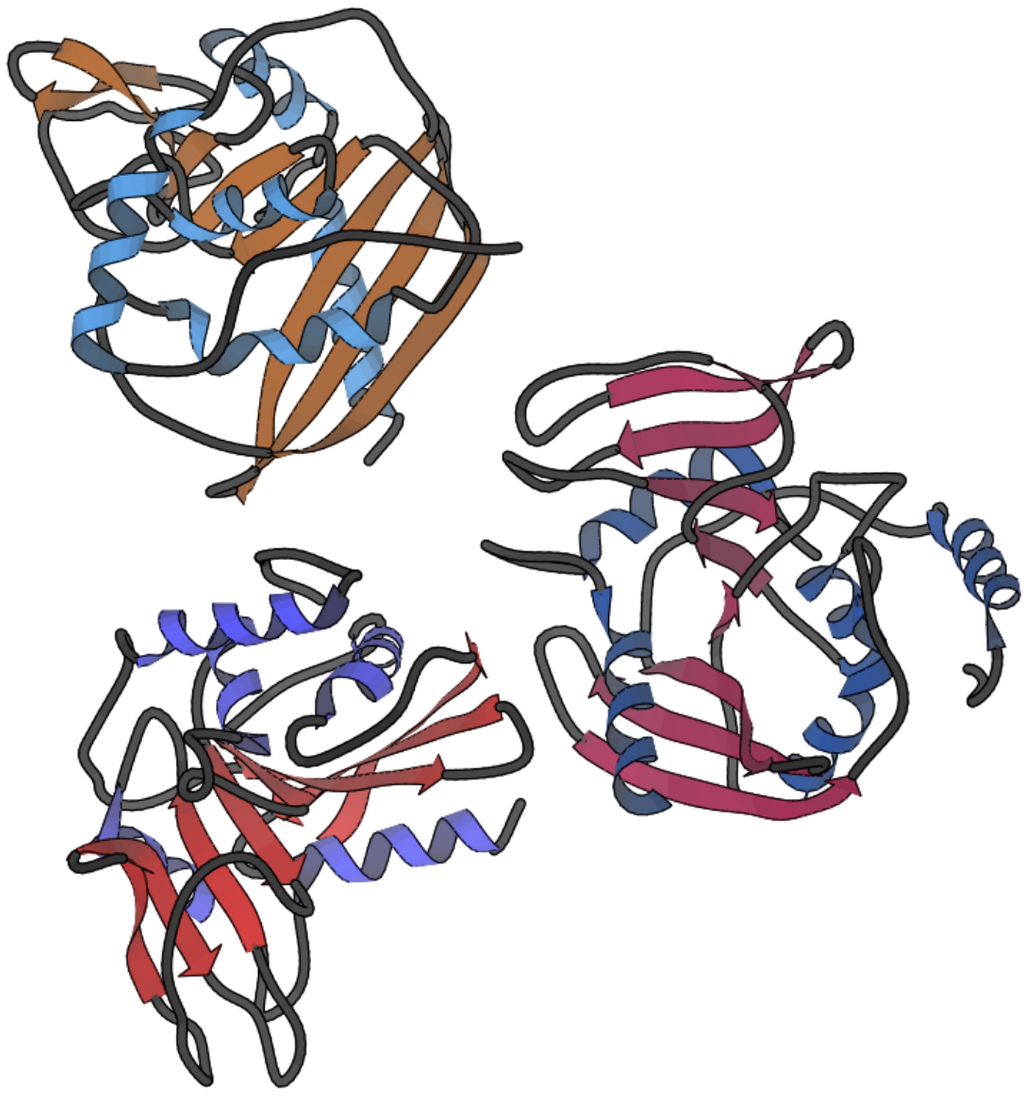
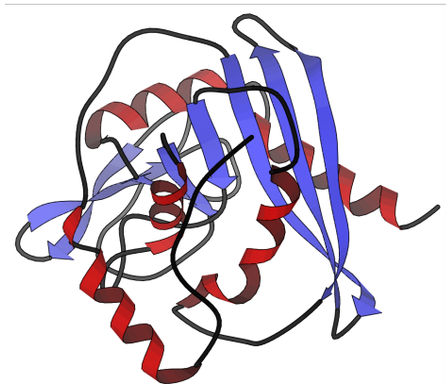
molecular replacement ⟶ refinement

3hd0: 2.70 Å
endonuclease



MR w/ Phaser



2w35
2.15 Å

R_{work} = 0.3844

AutoBuild

- Rebuild in place
- NCS on for rebuilding
- NCS off for refinement
- No water picking

R_{work} = 0.1895
R_{free} = 0.2745

R_{gap} = 0.085

phenix.refine

- 10 macrocycles
- optimize weights
- No NCS, Cartesian NCS, torsion NCS w/ and w/o rotamer correction

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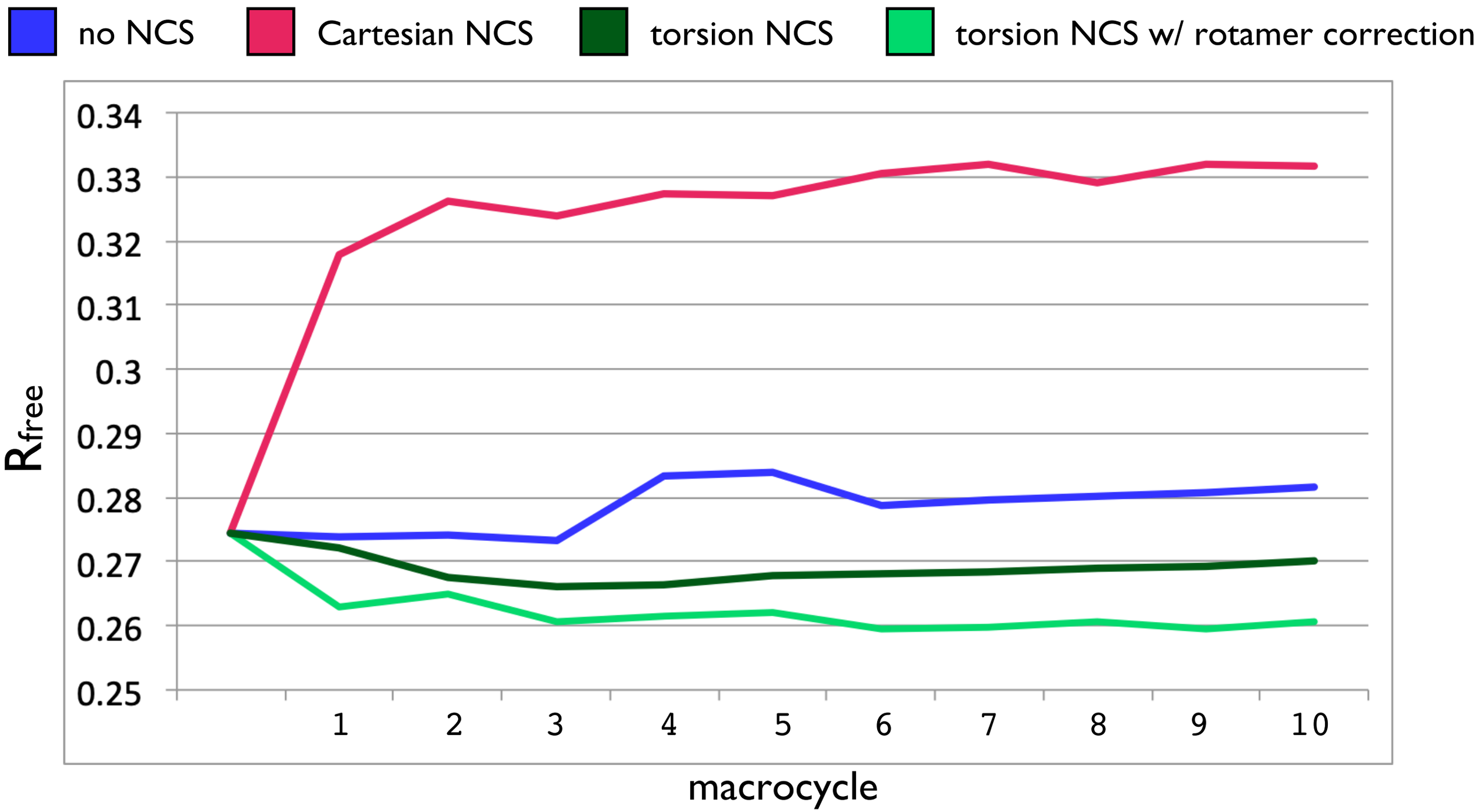
3hd0 refinement



torsion NCS w/ rotamer correction ⟶ $R_{work} = 0.2040$ $R_{free} = 0.2606$ $R_{gap} = 0.056$

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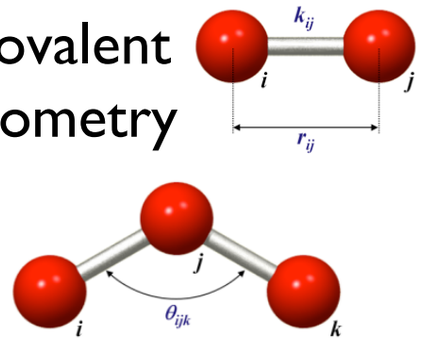
Sources of Prior Information

Covalent geometry



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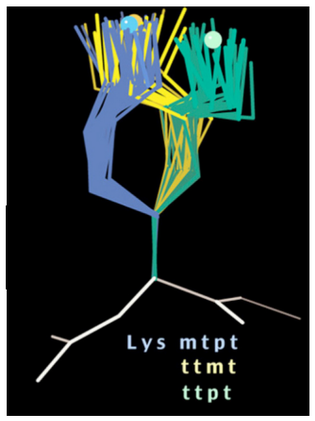
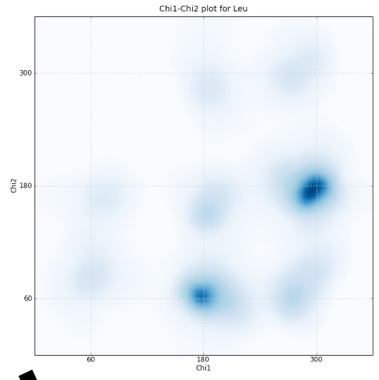
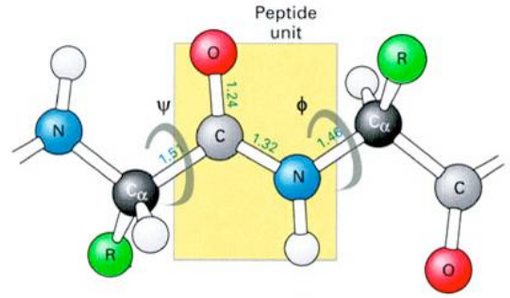
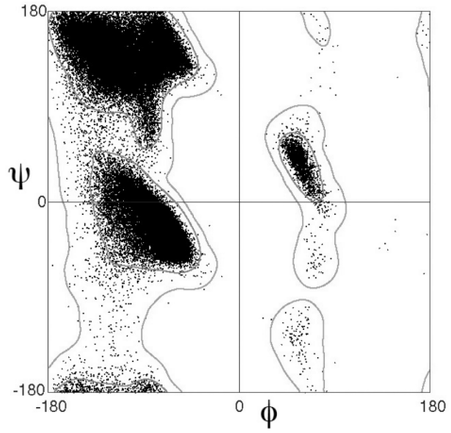
Sidechain distributions

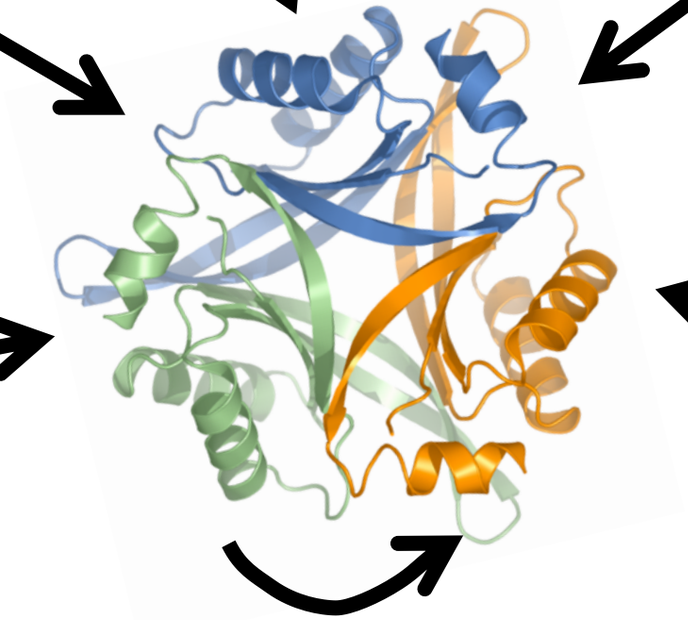


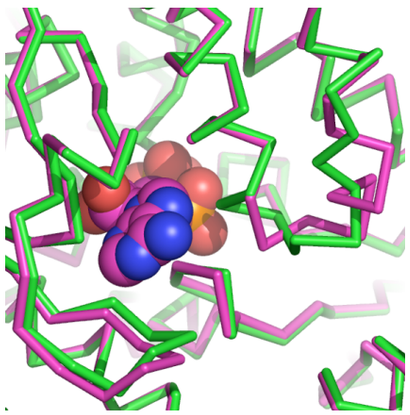




Mainchain distributions

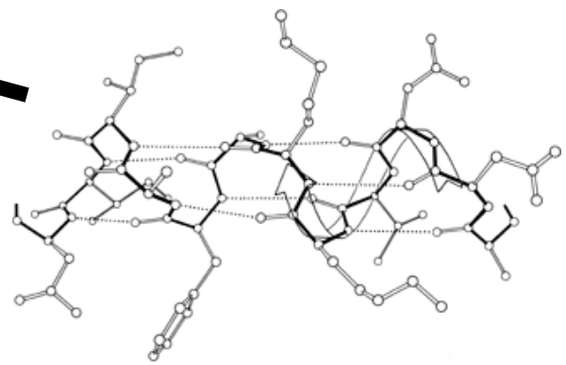






Related structures

Internal symmetry



Secondary structure

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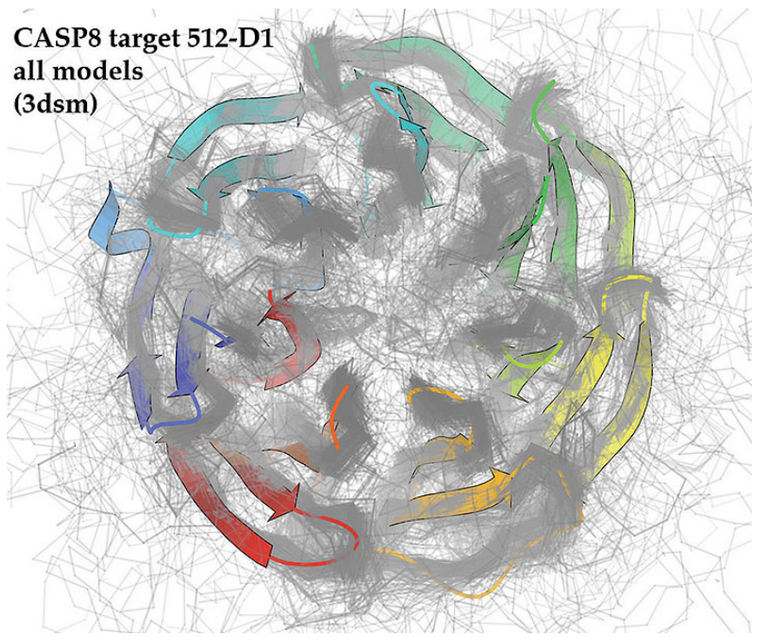
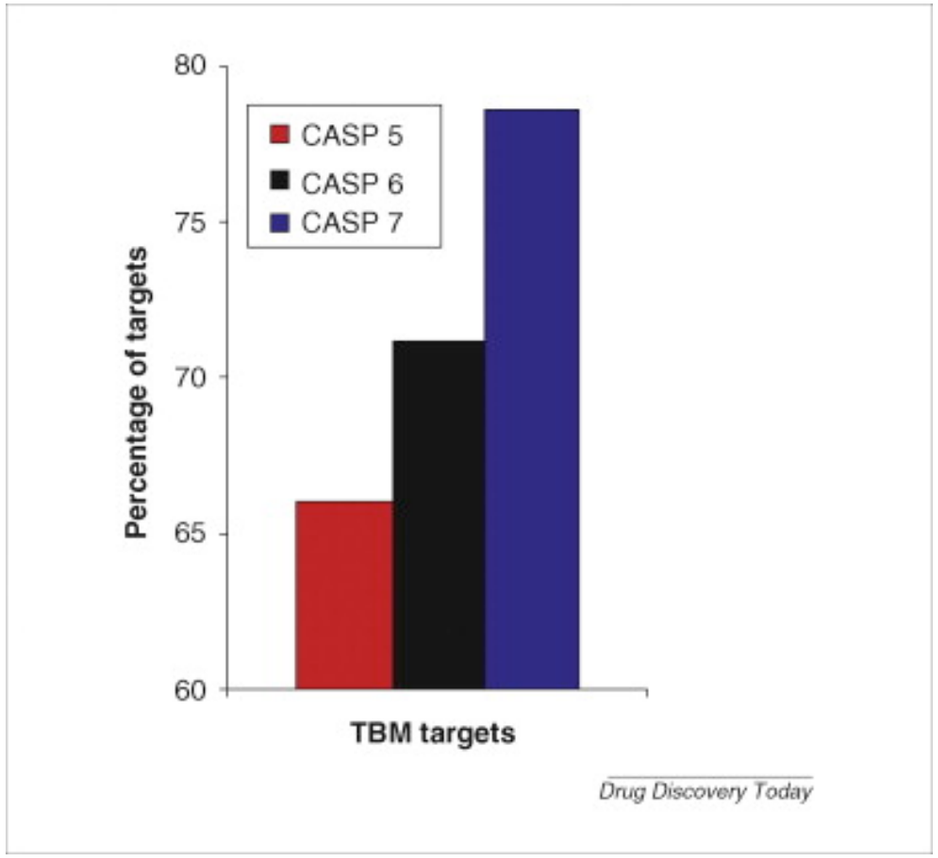
More Prior Information

- As the number of observations decreases we need to increase the amount of prior information we include (or the number of constraints we apply)
- At the extreme - what if we had no data?
- Other fields have been trying to address this problem:
  - Structure prediction
  - Homology modelling
  - Protein folding



http://www.predictioncenter.org



From: Kryshtafovych & Fidelis, Drug Discovery Today, 2009, 14:386–393

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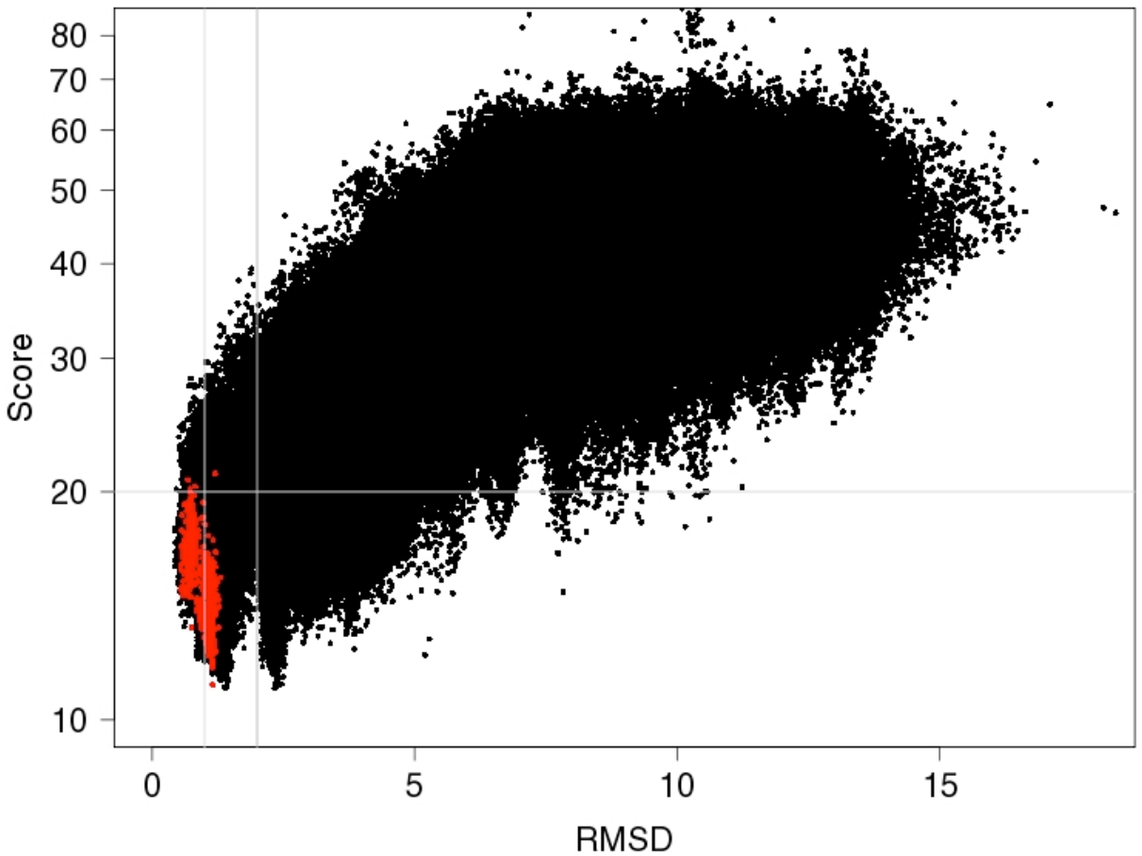
Physically Realistic Potentials (Rosetta)

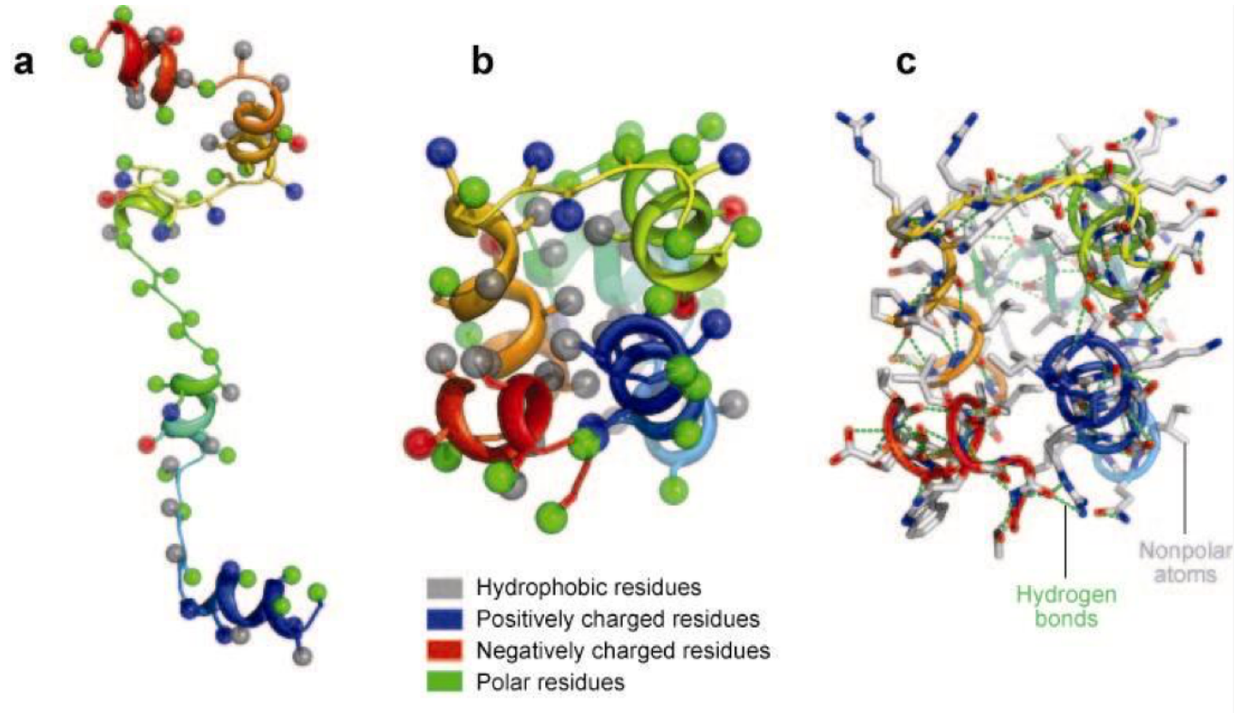
(Nat Echols & Frank DiMaio)

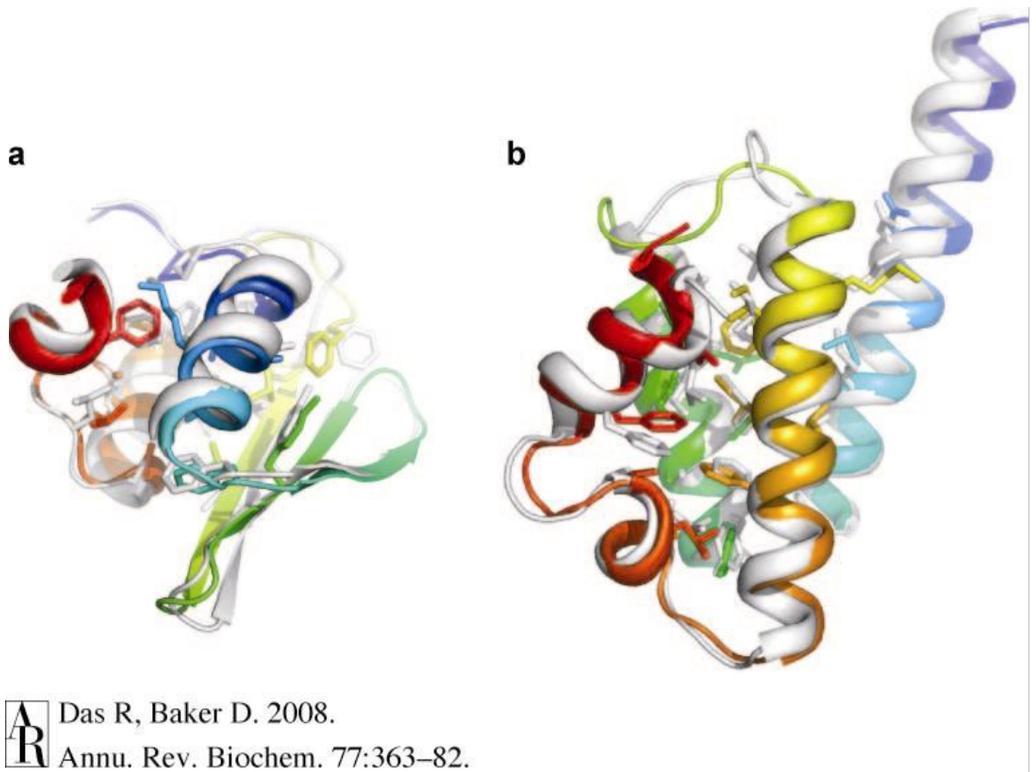
Rosetta

- *ab initio* model generation and model optimization
- Requires extensive computational sampling



Black - Rosetta *ab initio* models, Red - Crystal structure after Relax protocol





Das R, Baker D. 2008.
Annu. Rev. Biochem. 77:363–82.

Why Rosetta

- Designed to recognize near-native structures among many possible models; combines empirical and physical potentials
  - All-atom force field, incorporates solvation effects, attractive forces, hydrogen bonds, knowledge-based dihedral restraints
- Can yield chemically realistic *ab initio* models without experimental data to guide assembly
  - Occasionally good enough for molecular replacement
- Shown to be useful for NMR structure determination with sparse data (CS-Rosetta), MR solution improvement (MR-Rosetta), RNA structure refinement (ERRASER)

Kuhlman et al. (2003) *Science* **302**:1364-8
Rohl et al. (2004) *Methods Enzymol.* **383**:66-93
Keedy et al. (2009) *Proteins* **77**:29-49

https://www.rosettacommons.org

Complementary Algorithms

Phenix

- Reciprocal space X-ray target functions (ML, MLHL, LS-twin)
- Bulk solvent correction
- B-factor refinement (including TLS)
- Map calculation
- Density modification (using RESOLVE)

Rosetta

- Physically realistic potentials
- Repacking to remove steric clashes and building rotameric sidechains
- Torsion-angle minimization
- Real-space target (refinement against electron density)
- Fragment-based rebuilding (optional, not currently used)

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Python/C++ architecture facilitates combination

Low Resolution Protocol

- Sidechain repacking (using density)
- Coordinate refinement (reciprocal space torsion angle minimization and reduced nonbonded penalty)
- B-factor refinement

3 Cycles

- Sidechain repacking (using density)
- Coordinate refinement (real space and reciprocal space torsion angle minimization)
- B-factor refinement

5 Cycles

- Sidechain repacking (using density)
- Coordinate refinement (reciprocal space minimization with restrained bonds and angles)
- B-factor refinement

2 Cycles

Protocol run 5 times in parallel and the best model selected based on R-free

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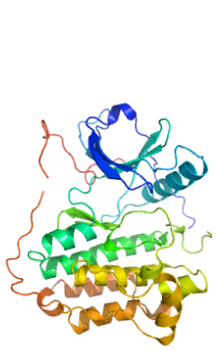
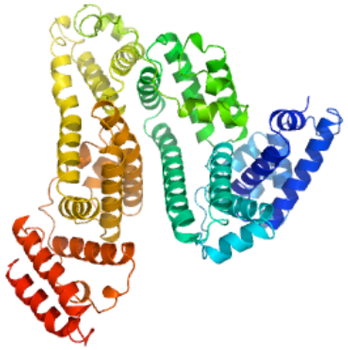
Test Cases

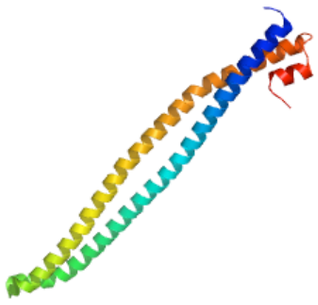
Solved by molecular replacement with same protein from another deposition at higher resolution



2j5f (3.0Å)



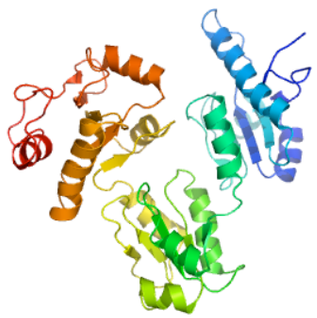
1bke (3.15Å)



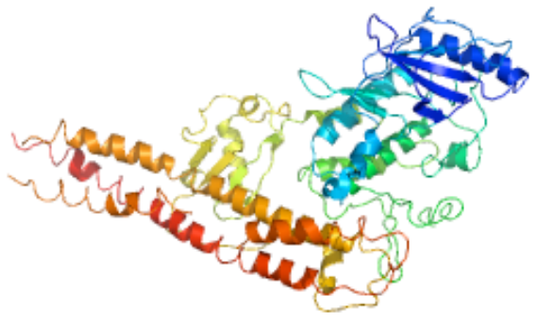
3mtt (3.3Å)

1kct (3.46Å)

3snh (3.7Å)

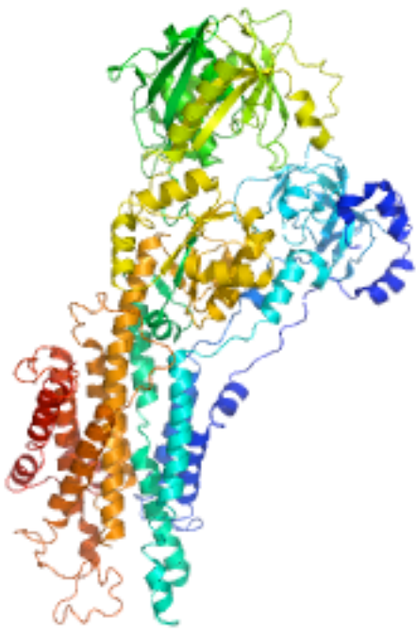


2vaf (3.8Å)

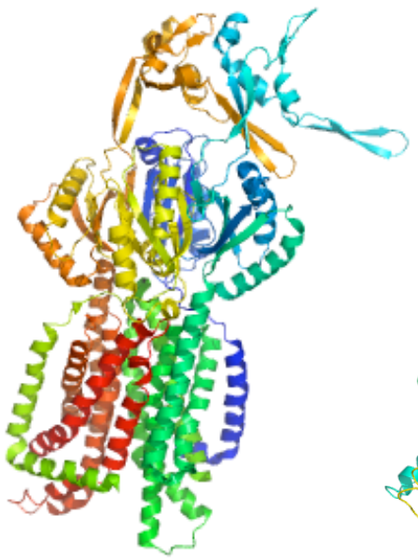


3rzf (4.0Å)

Membrane Proteins

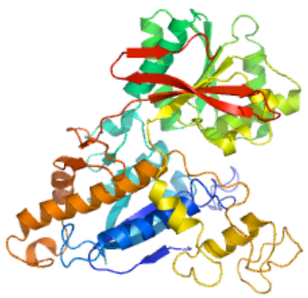


3fps (3.2Å)



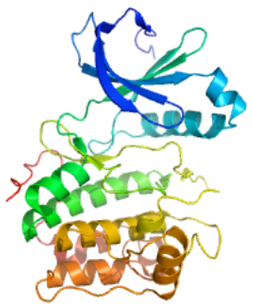
3k07 (3.52Å)

2x79 (3.8Å)

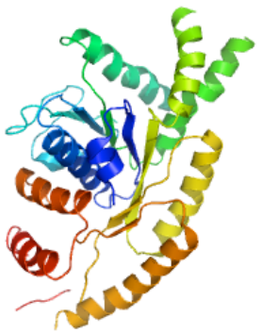


1isr (4.0Å)

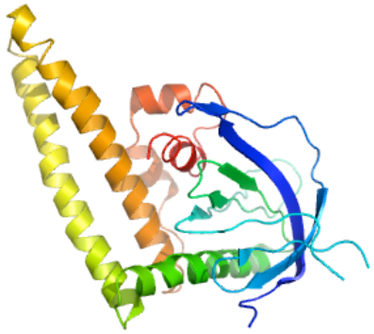
Solved using homologous proteins



3pwy (3.5Å)



3idq (3.7Å)



3a8n (4.5Å)

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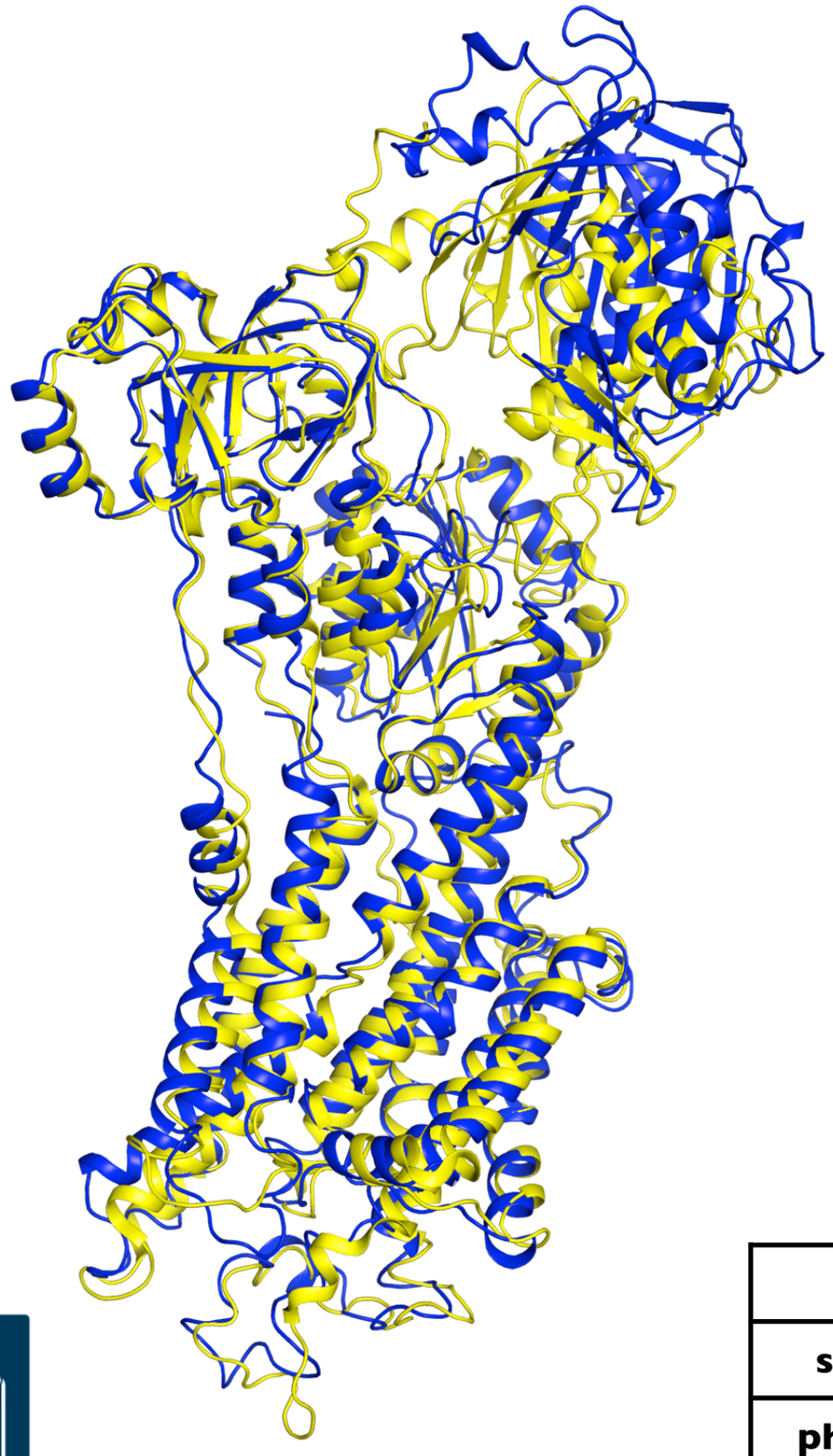
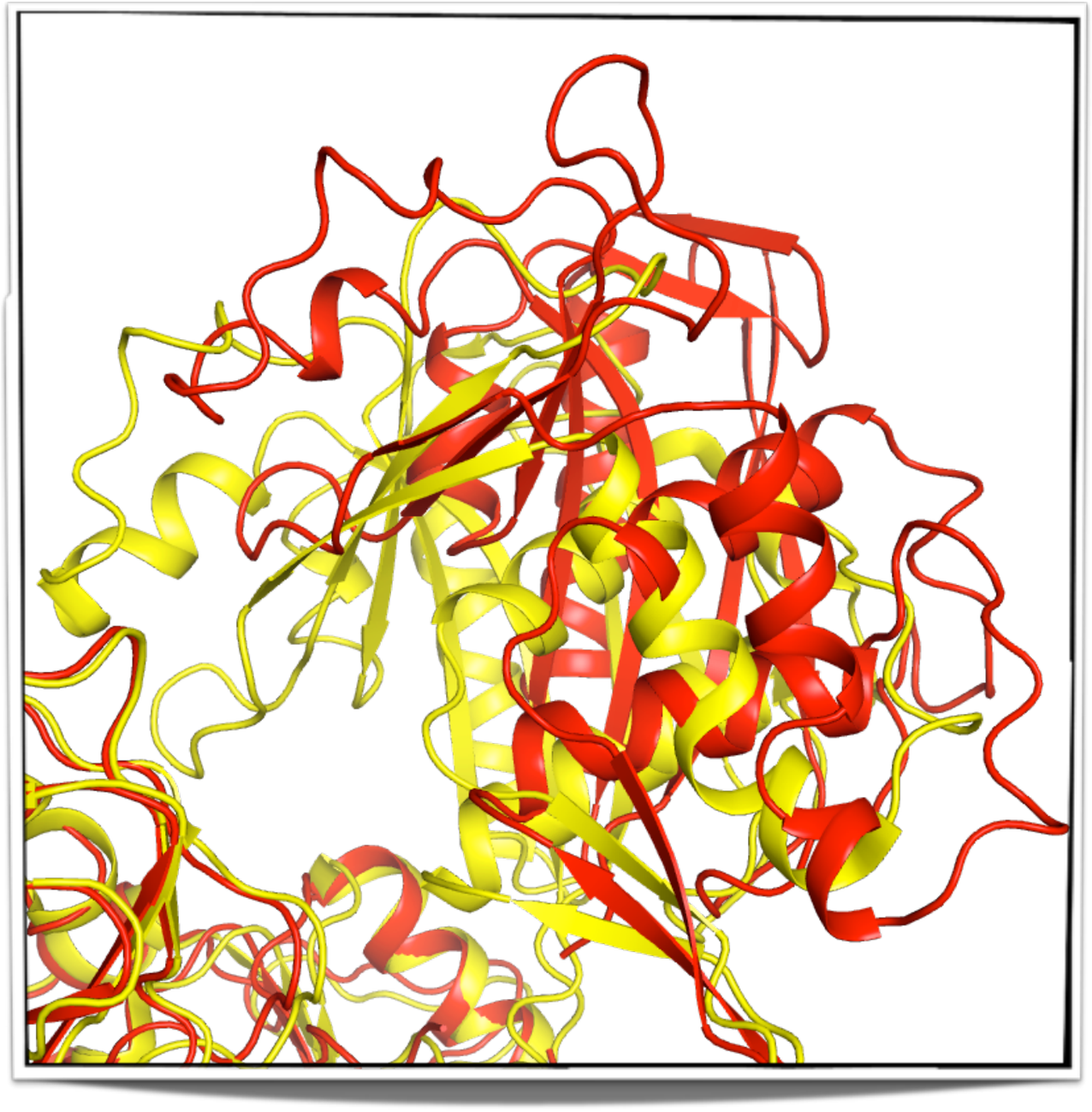
Calcium ATPase - phenix.refine





	R	R-free	mp score	RMSD
start	0.47	0.51	3.21	6.1
phenix	0.43	0.48	2.66	6.2

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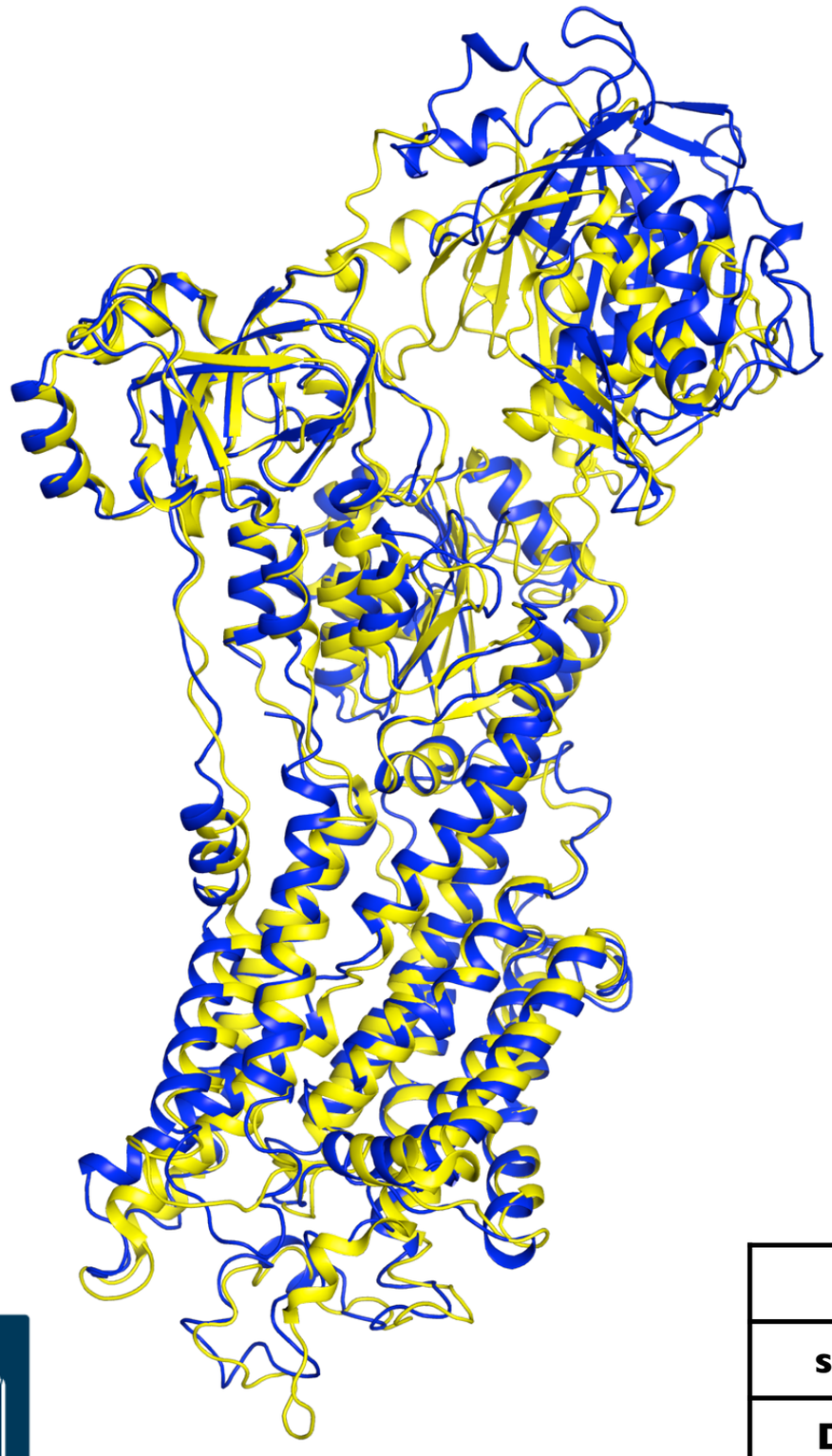
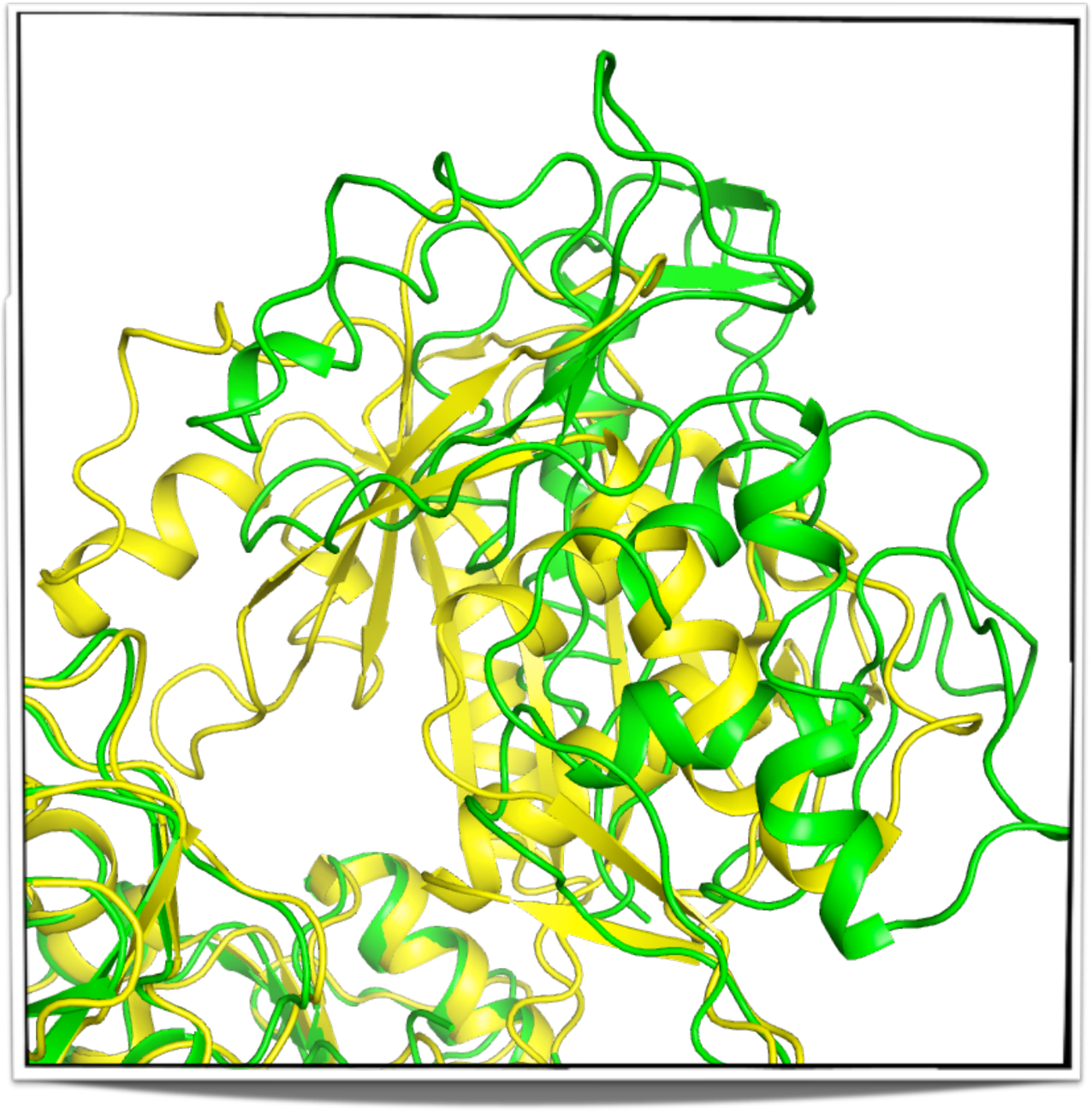
Calcium ATPase - DEN





	R	R-free	mp score	RMSD
start	0.47	0.51	3.21	6.1
DEN	0.38	0.44	3.79	6.1

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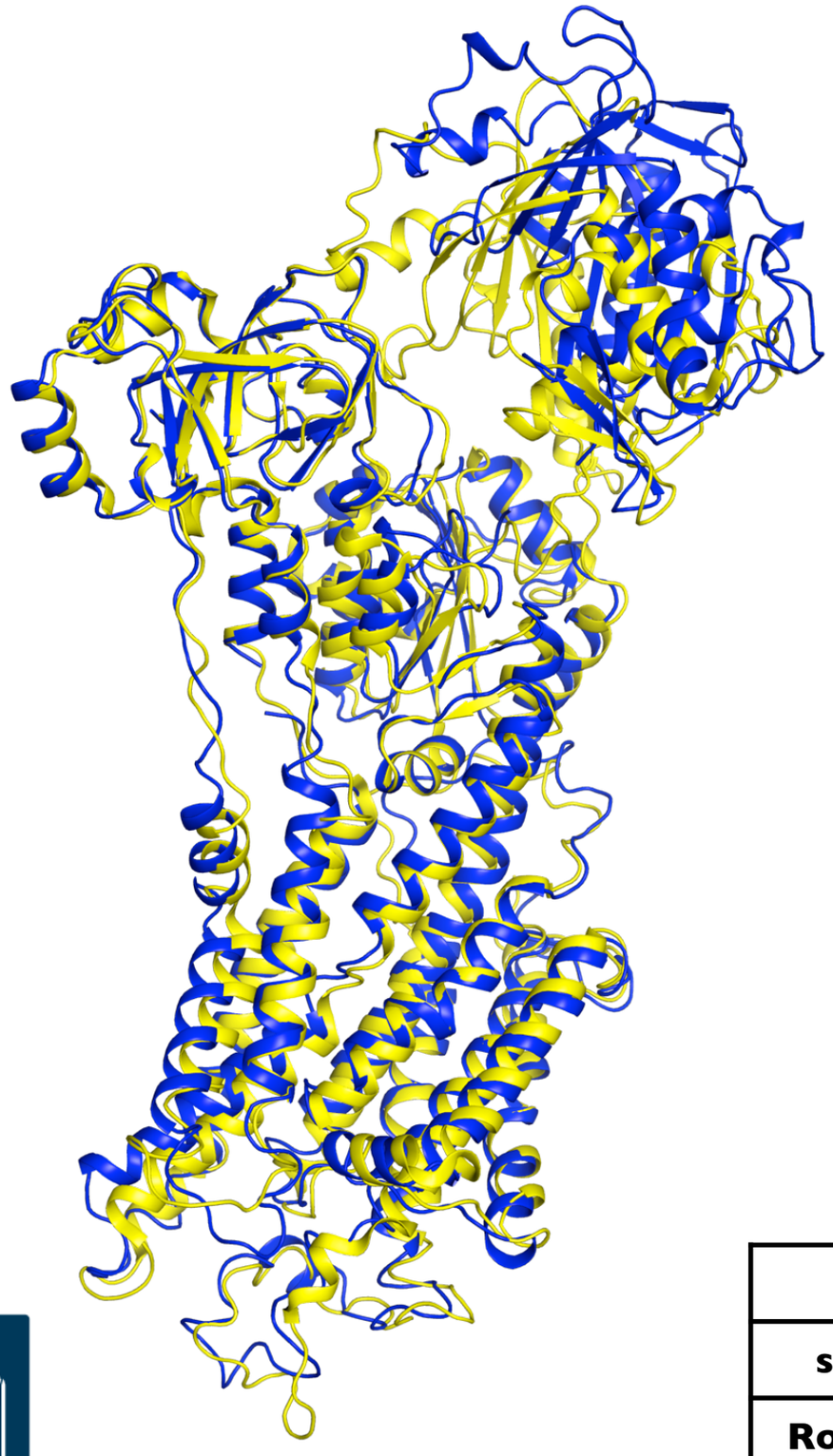
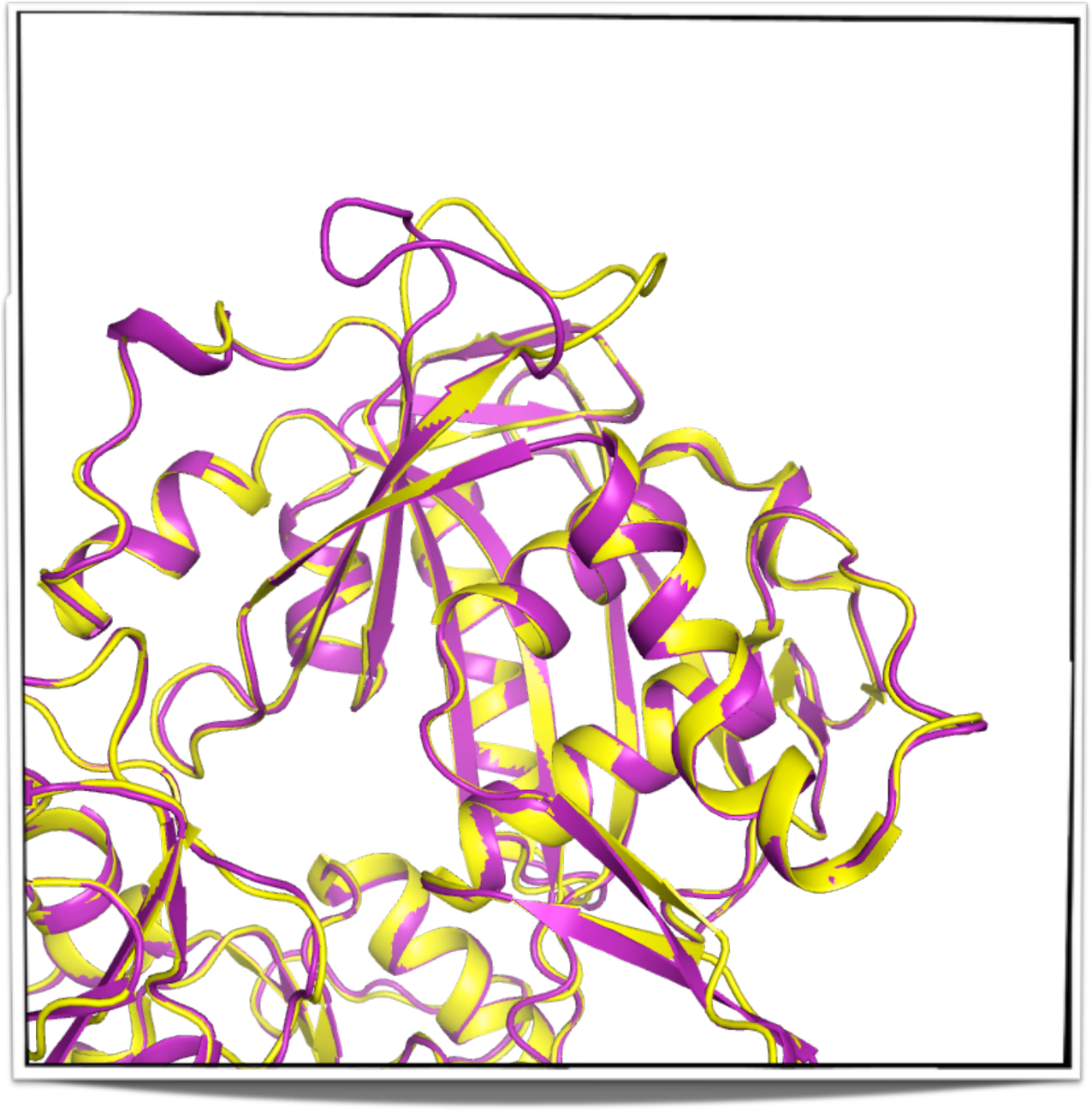
Calcium ATPase - Phenix-Rosetta





	R	R-free	mp score	RMSD
start	0.47	0.51	3.21	6.1
Rosetta	0.24	0.28	1.55	1.7

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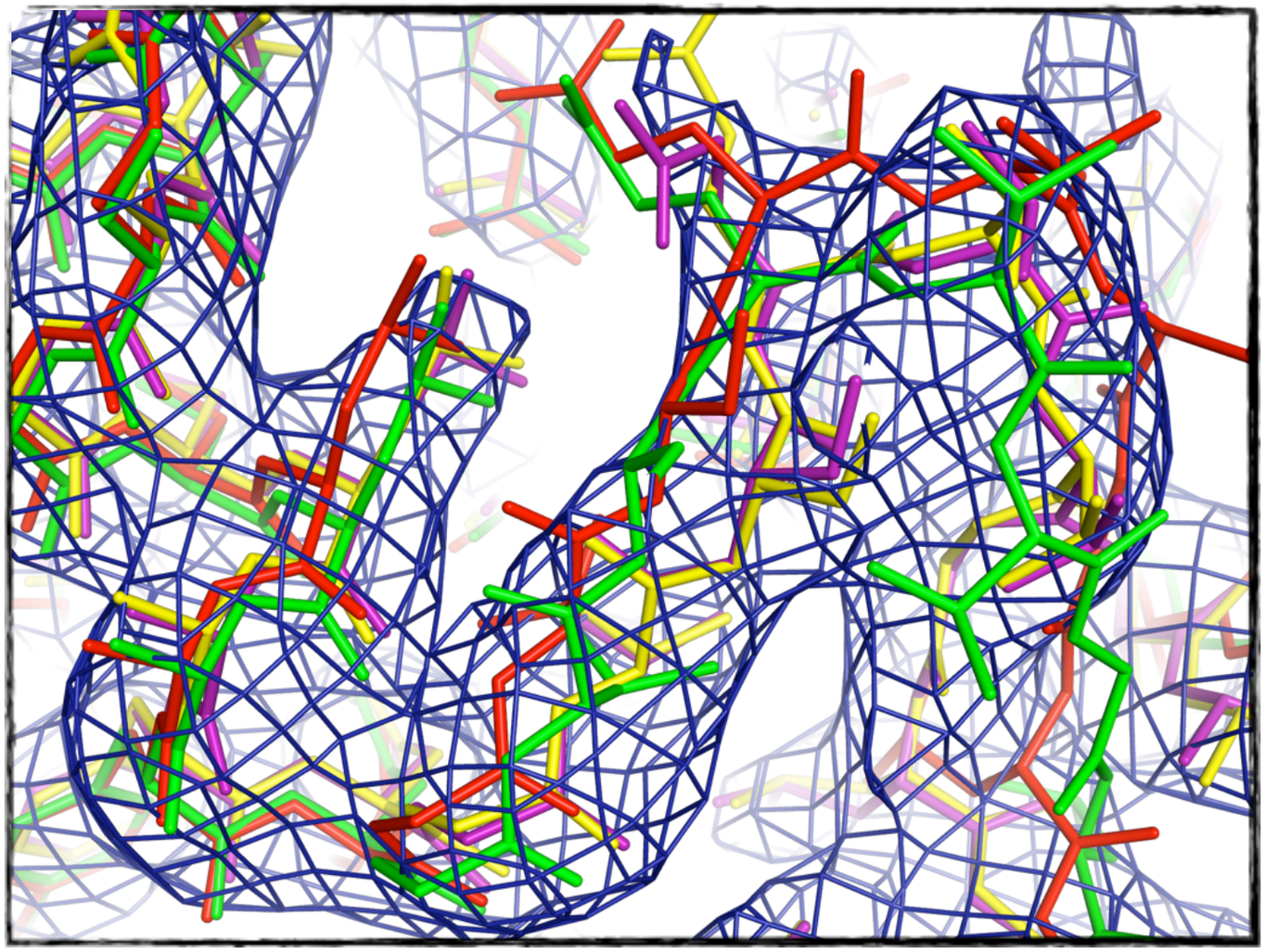
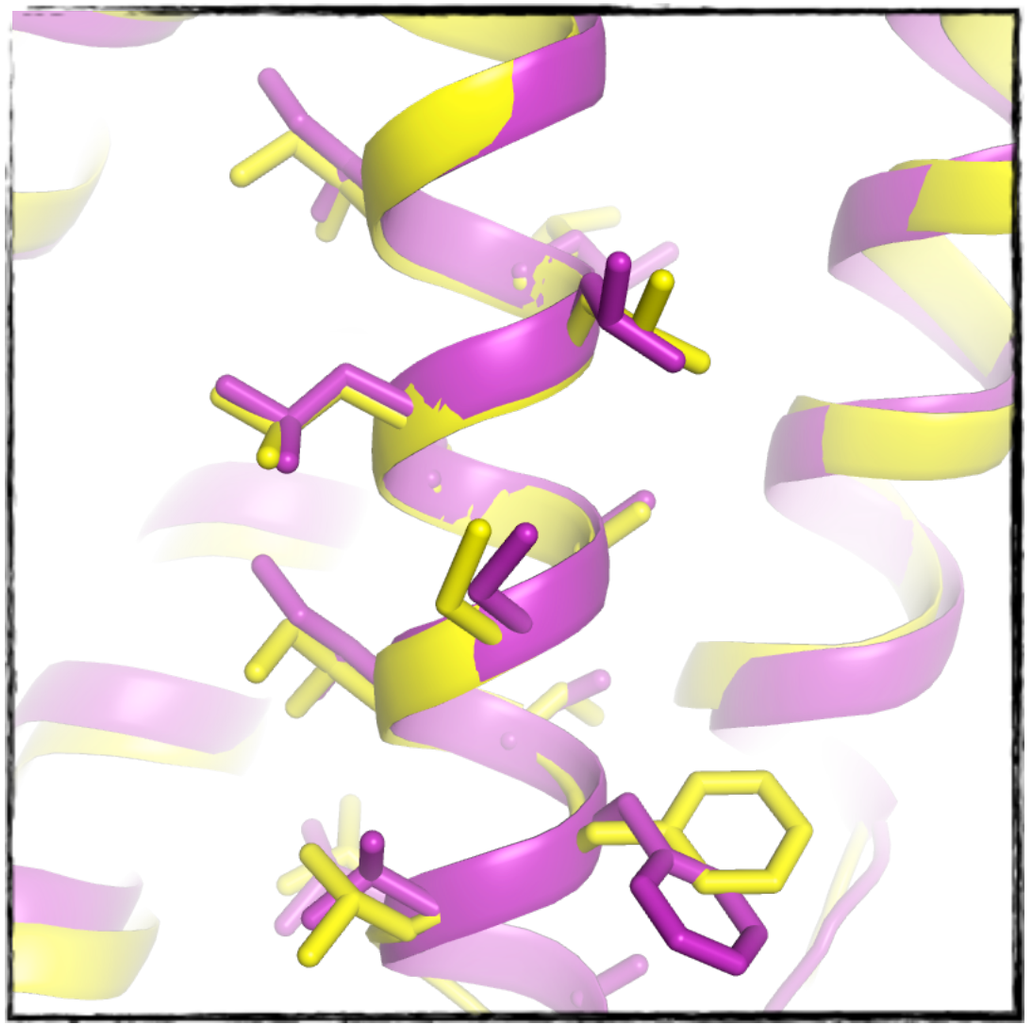
Calcium ATPase - Detail





- Phenix-Rosetta model is very close to the deposited structure (even at the level of side chains) with better fit to density

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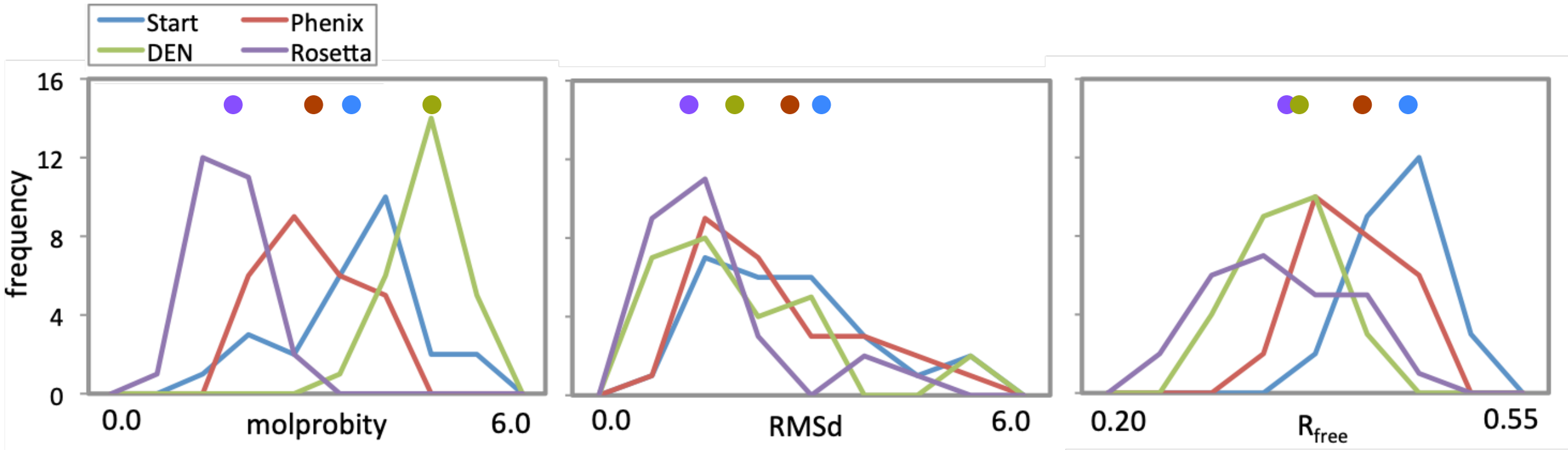
Improved Models



- Phenix-Rosetta typically has improved fit to the crystallographic data and models are closer to the known structure
- Phenix-Rosetta always has improved model quality, as judged by Molprobity
- Generally similar to DEN results but with much improved geometry, and generally faster

DiMiao et al., 2013, *Nature Methods* ***10****:1102-1104*

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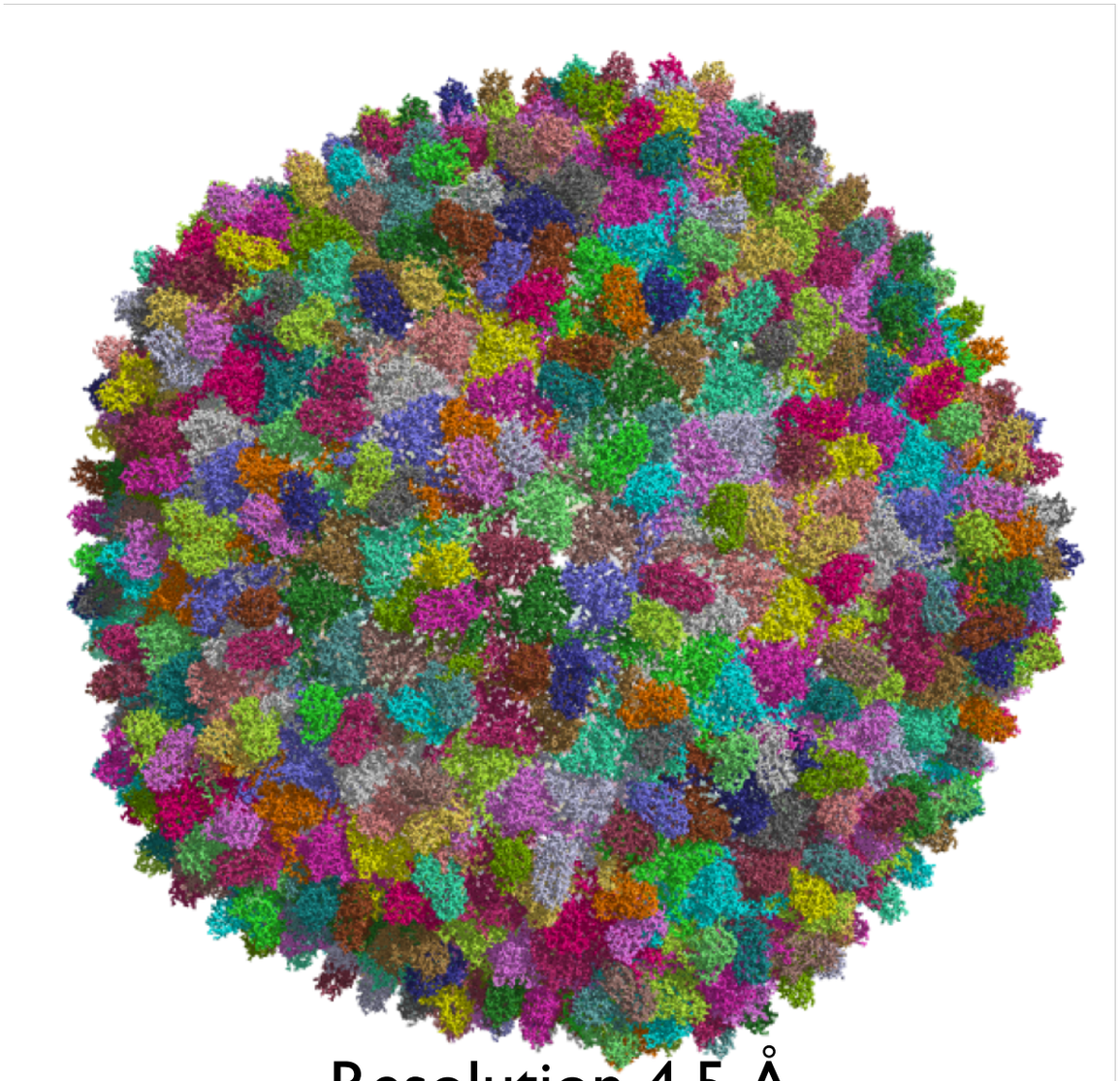
Cryo-EM Atomic Model Optimization

Pavel Afonine, Oleg Sobolev, Nat Echols, Jeff Headd, Nigel Moriarty
Lawrence Berkeley National Laboratory
Tom Terwilliger
Los Alamos National Laboratory

Phenix

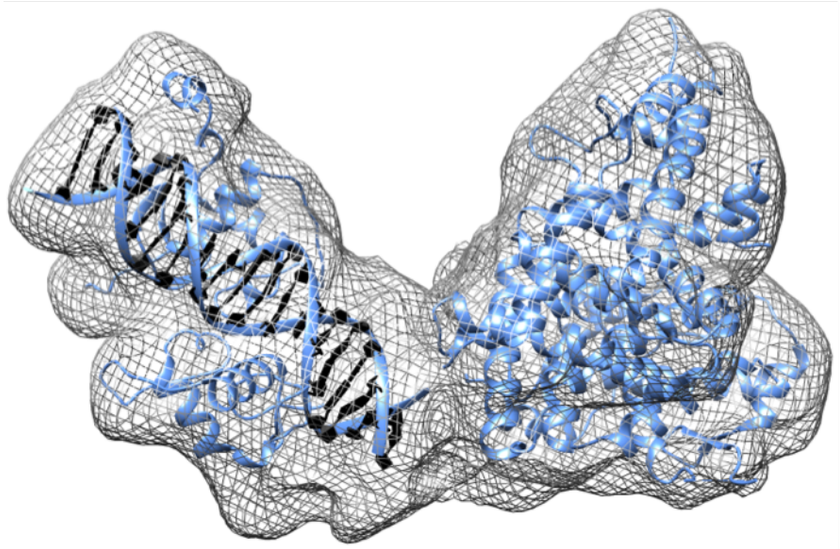
Challenges



Resolution 4.5 Å

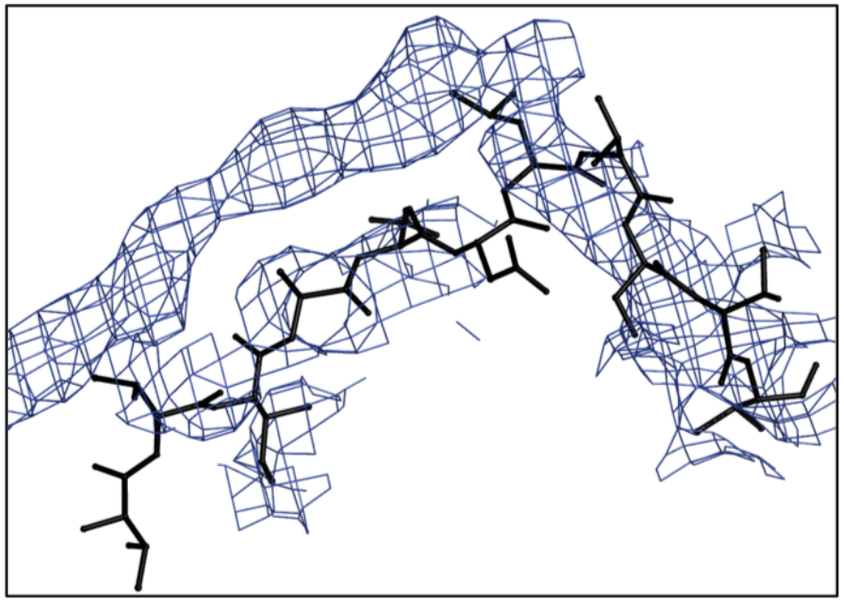
840 chains, 187,320 residues 1,443,960 atoms

Size



Resolution: 11.6 Å

Wide Resolution Range



User data, resolution: 3.8 Å

Poor Initial Fit

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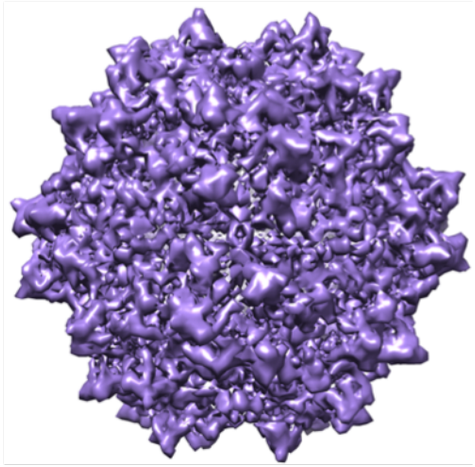
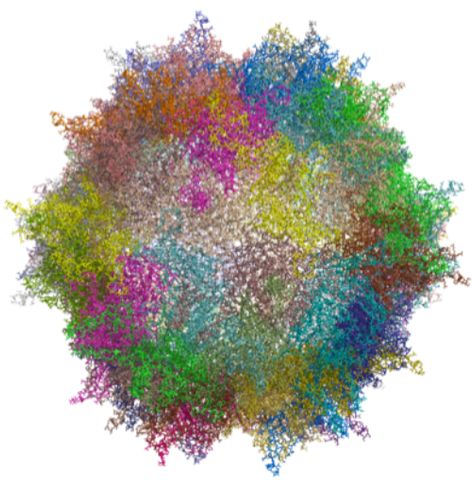
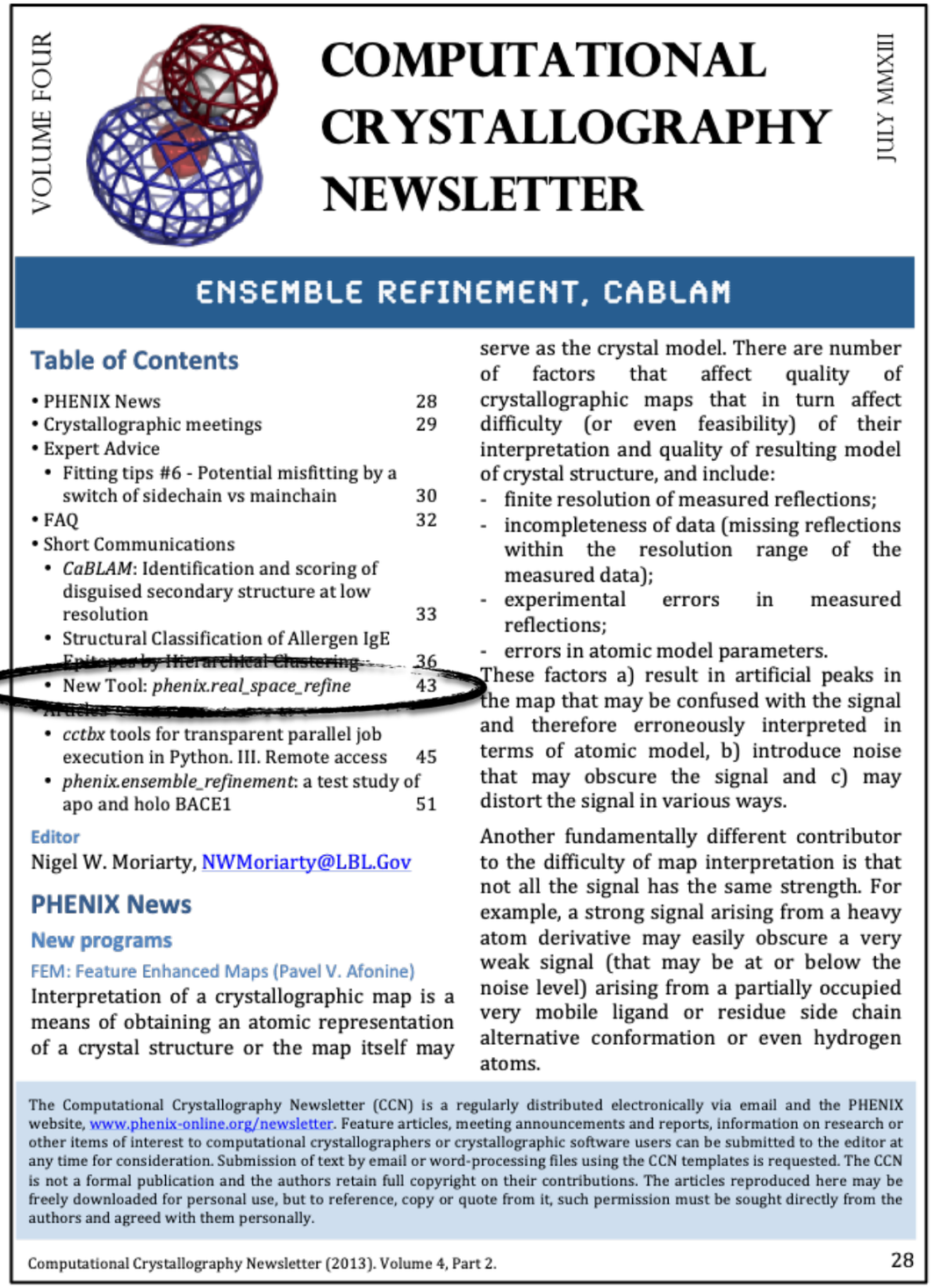
Direct Refinement Against the Map



Real space refinement





VOLUME FOUR

COMPUTATIONAL CRYSTALLOGRAPHY NEWSLETTER

JULY MMXIII

ENSEMBLE REFINEMENT, CABLAM

Table of Contents

Editor

Nigel W. Moriarty, NWMoriarty@LBL.Gov

PHENIX News

New programs

FEM: Feature Enhanced Maps (Pavel V. Afonine)

Interpretation of a crystallographic map is a means of obtaining an atomic representation of a crystal structure or the map itself may serve as the crystal model. There are number of factors that affect quality of crystallographic maps that in turn affect difficulty (or even feasibility) of their interpretation and quality of resulting model of crystal structure, and include:

- finite resolution of measured reflections;
- incompleteness of data (missing reflections within the resolution range of the measured data);
- experimental errors in measured reflections;
- errors in atomic model parameters.

These factors a) result in artificial peaks in the map that may be confused with the signal and therefore erroneously interpreted in terms of atomic model, b) introduce noise that may obscure the signal and c) may distort the signal in various ways.

Another fundamentally different contributor to the difficulty of map interpretation is that not all the signal has the same strength. For example, a strong signal arising from a heavy atom derivative may easily obscure a very weak signal (that may be at or below the noise level) arising from a partially occupied very mobile ligand or residue side chain alternative conformation or even hydrogen atoms.

The Computational Crystallography Newsletter (CCN) is a regularly distributed electronically via email and the PHENIX website, www.phenix-online.org/newsletter. Feature articles, meeting announcements and reports, information on research or other items of interest to computational crystallographers or crystallographic software users can be submitted to the editor at any time for consideration. Submission of text by email or word-processing files using the CCN templates is requested. The CCN is not a formal publication and the authors retain full copyright on their contributions. The articles reproduced here may be freely downloaded for personal use, but to reference, copy or quote from it, such permission must be sought directly from the authors and agreed with them personally.

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Real Space Refinement

- Has a long history in both X-ray crystallography and cryo-EM
  - Early crystallographic refinement programs (Diamond)
  - Alternative to reciprocal space refinement, then applied to EM maps (TNT, RSRef)
  - Regularly used in model building (O, Coot)
- New structure fitting approaches make use of real space refinement
  - Molecular dynamics flexible fitting (MDFF)
  - Deformable elastic network fitting (DireX)
  - Rosetta model building and model refinement

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Refinement

- An *optimization* algorithm is used to minimize a *target function* by changing the *parameters* of the model
- Parameters:
  - coordinates, atomic displacements, occupancies
- Optimization algorithm:
  - minimization, simulated annealing
- Target function (Objective function):
  - Function based on electron density (real-space refinement)
  - Function based on structure factors (reciprocal-space refinement)

$$E = E_{chem} + w_a \sum_{hkl} \frac{1}{\sigma^2} \left(|F_o| - |F_c| \right)^2$$

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Goal for Cryo-EM Model Refinement

- Stable refinement against any density map (Cryo-EM or X-ray)
- End result should be an improvement in the model
- Large radius of convergence
- Final models with good fit to density and physically reasonable geometry (Ramachandran distribution, rotamers, packing)
- Fast: no more than one second per residue

For Ethan: $E = E_{chemistry} + w \sum (d_{obs} - d_{ideal})^2$

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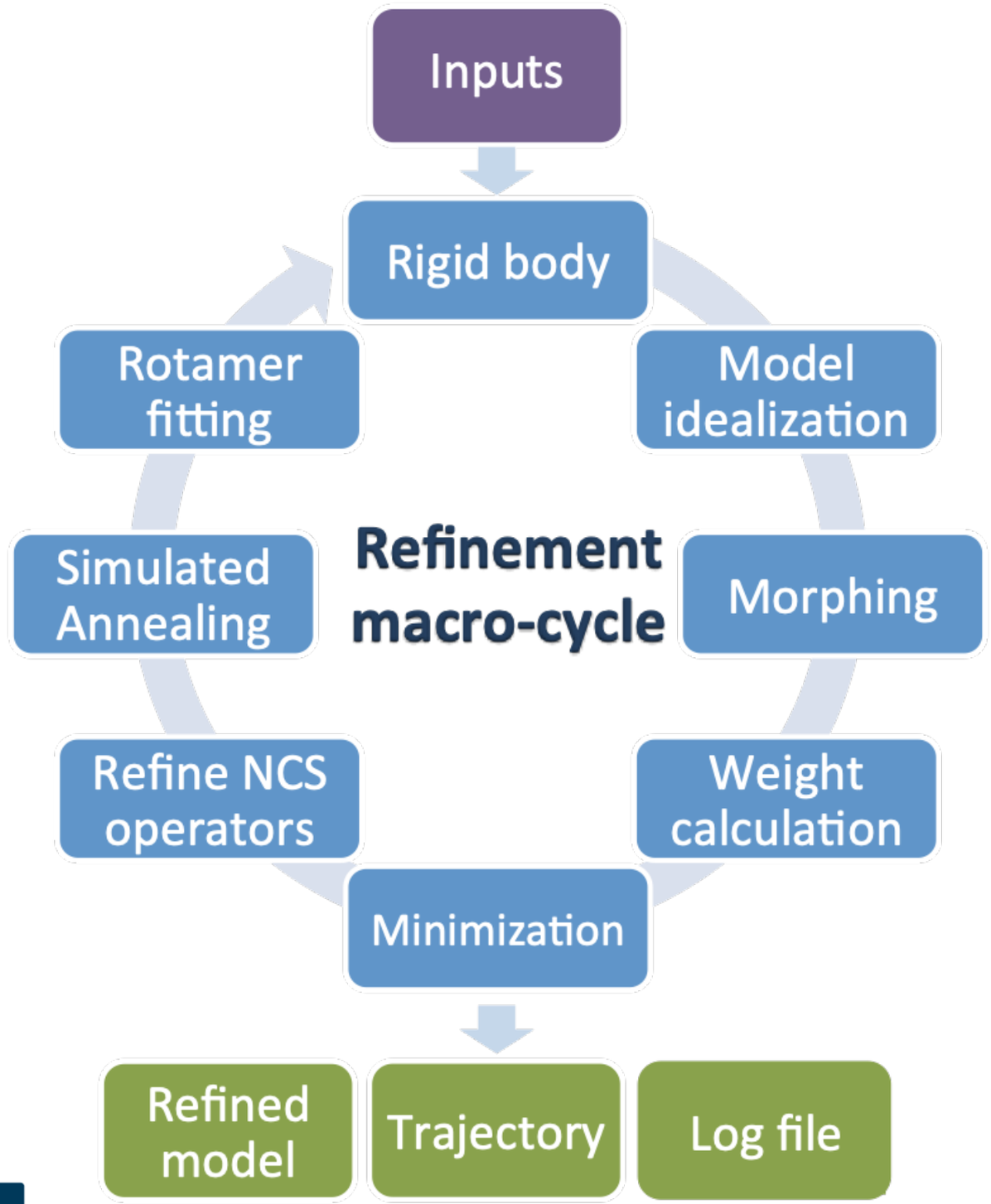
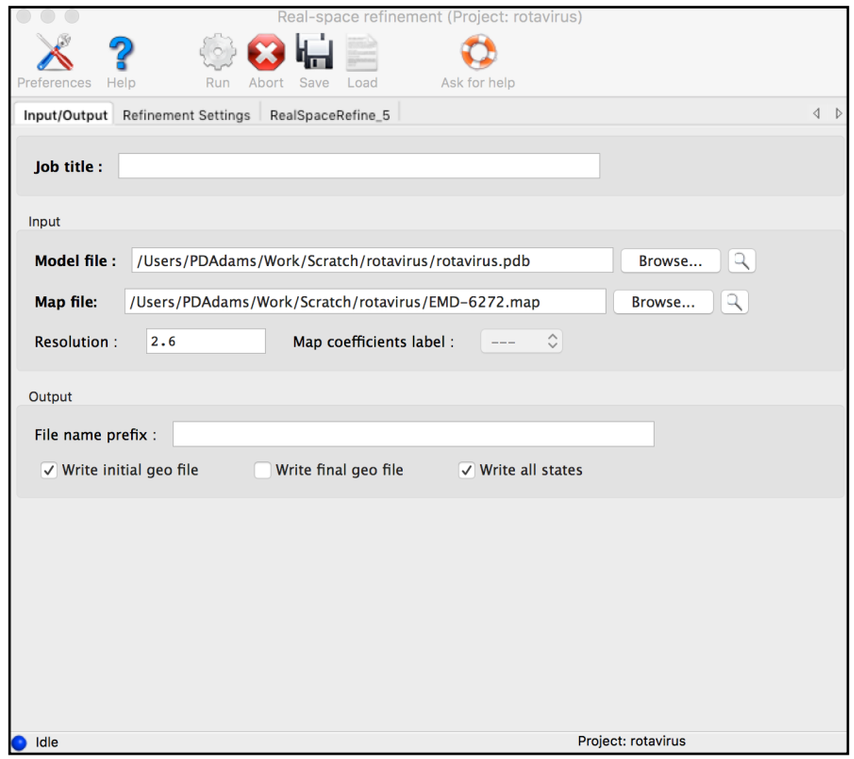
Real Space Refinement Procedure



- `phenix.real_space_refine`



Pavel Afonine, Oleg Sobolev, Billy Poon (LBNL), Tom Terwilliger (LANL)

Hryc et al. Accurate model annotation of a near-atomic resolution cryo-EM map. *Proc Natl Acad Sci U S A* 2017, **114**:3103-3108.

Afonine et al. Real-space refinement in PHENIX for cryo-EM and crystallography. *Acta Cryst* 2018, **D74**:531-544.

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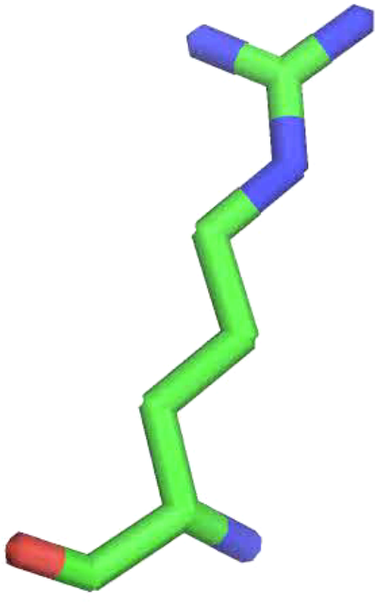
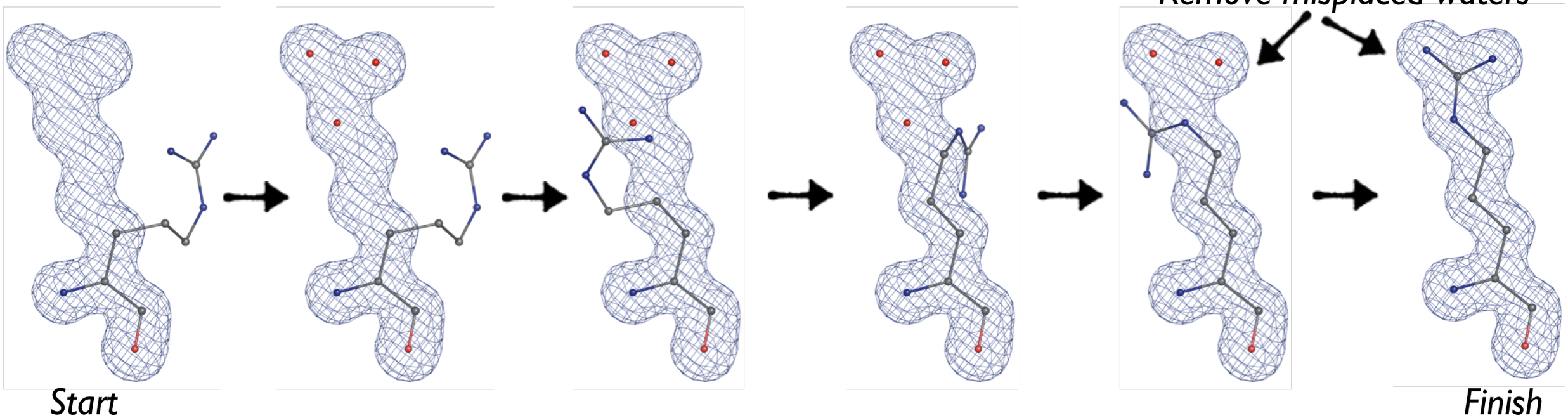
Systematic Searching of Rotamers



- In a protein structure 99% of the side chains obey known rotameric conformations
- Often errors are fixed manually but can now be fixed automatically following structure validation
- A systematic search through rotamer space is combined with a fit-to-density score



Fast: 0.01 – 1 second per residue

Pavel Afonine, Jeff Headd, Nat Echols

Phenix

Afonine et al., Acta Cryst. 2012, **D68**:352-367

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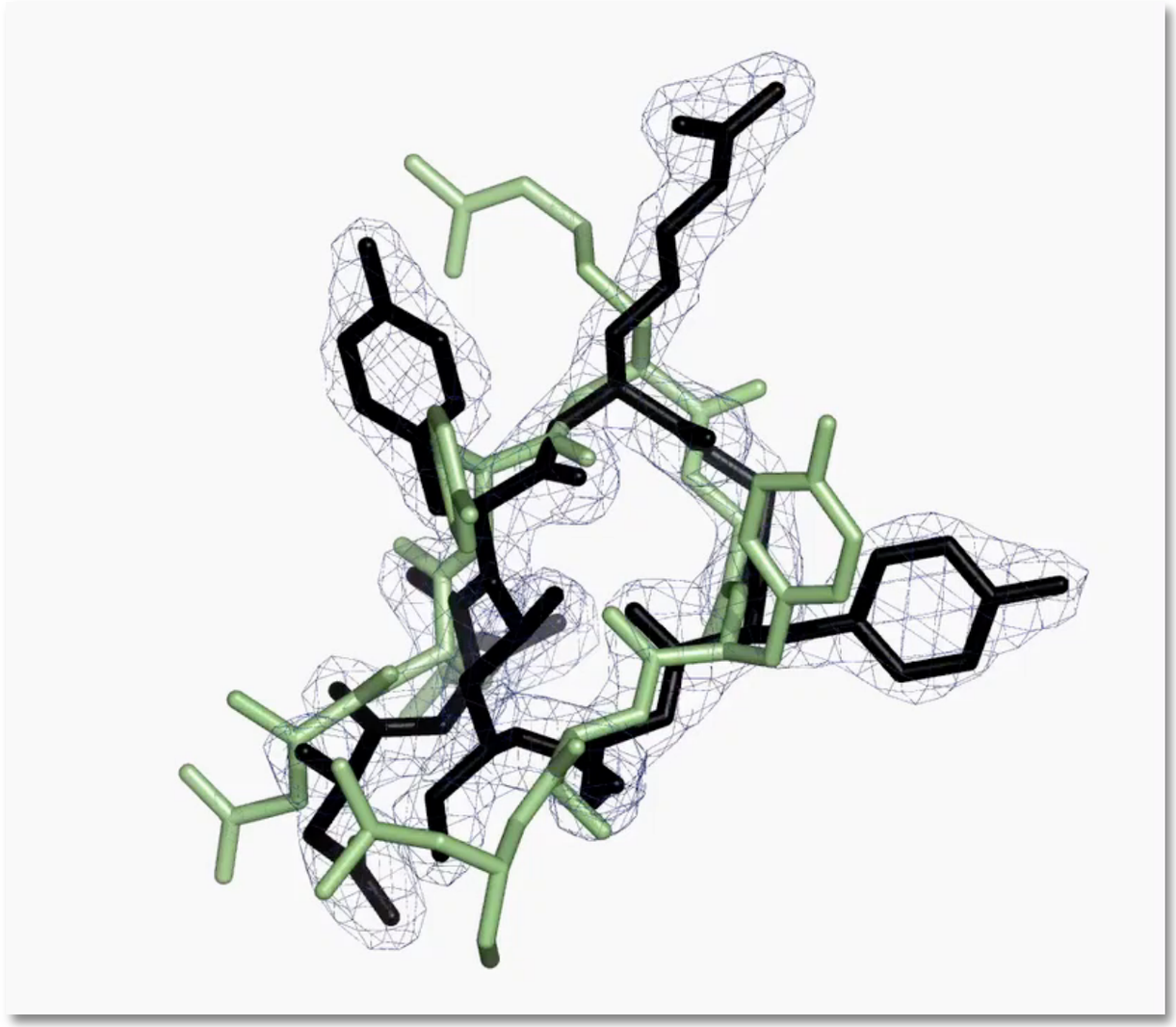
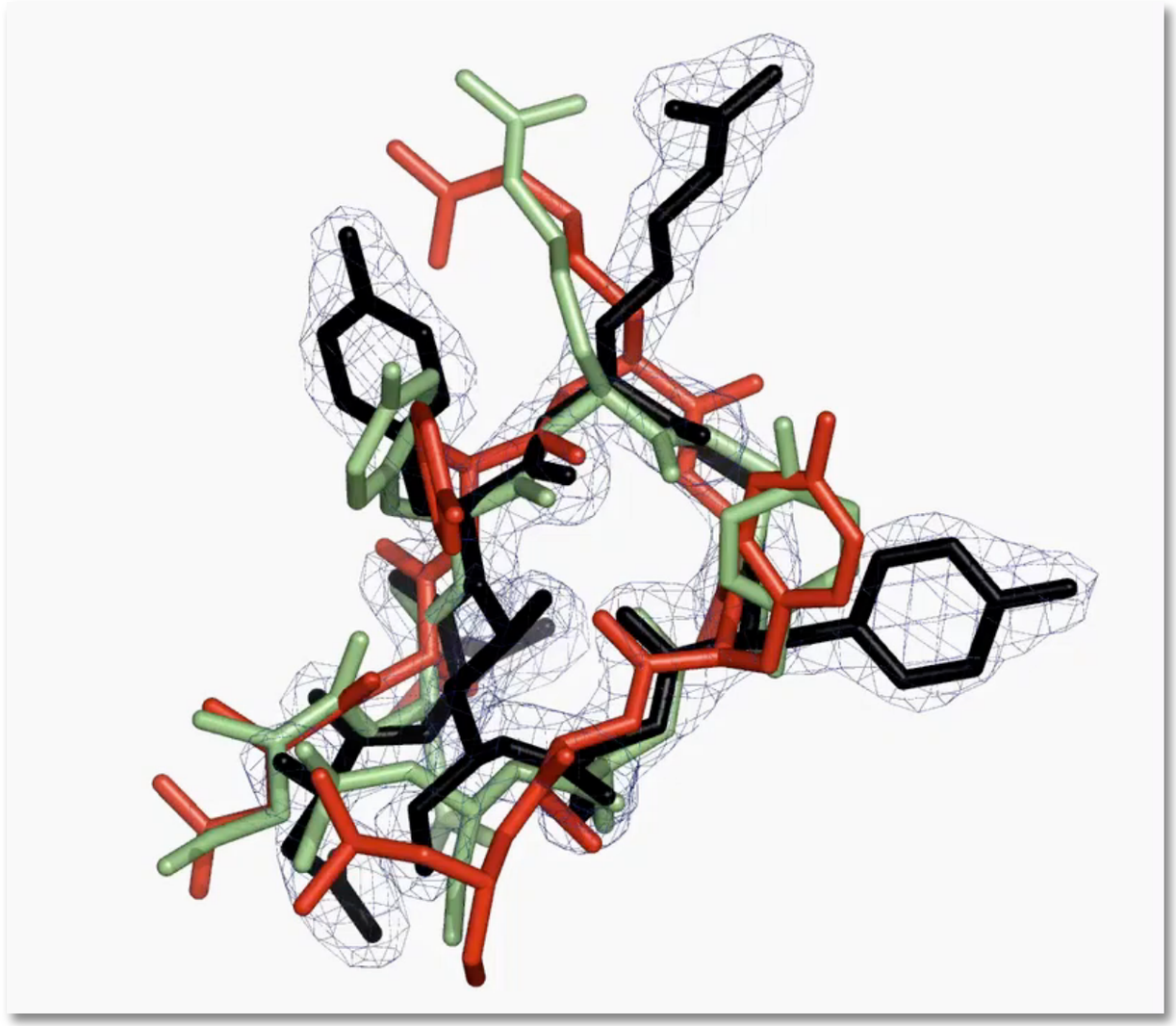
Optimization In Real Space

- Refinement against a map using minimization or other optimization method
- Minimization can get caught in local minima
- Simulated annealing is a method used to escape minima



Minimization



Simulated Annealing

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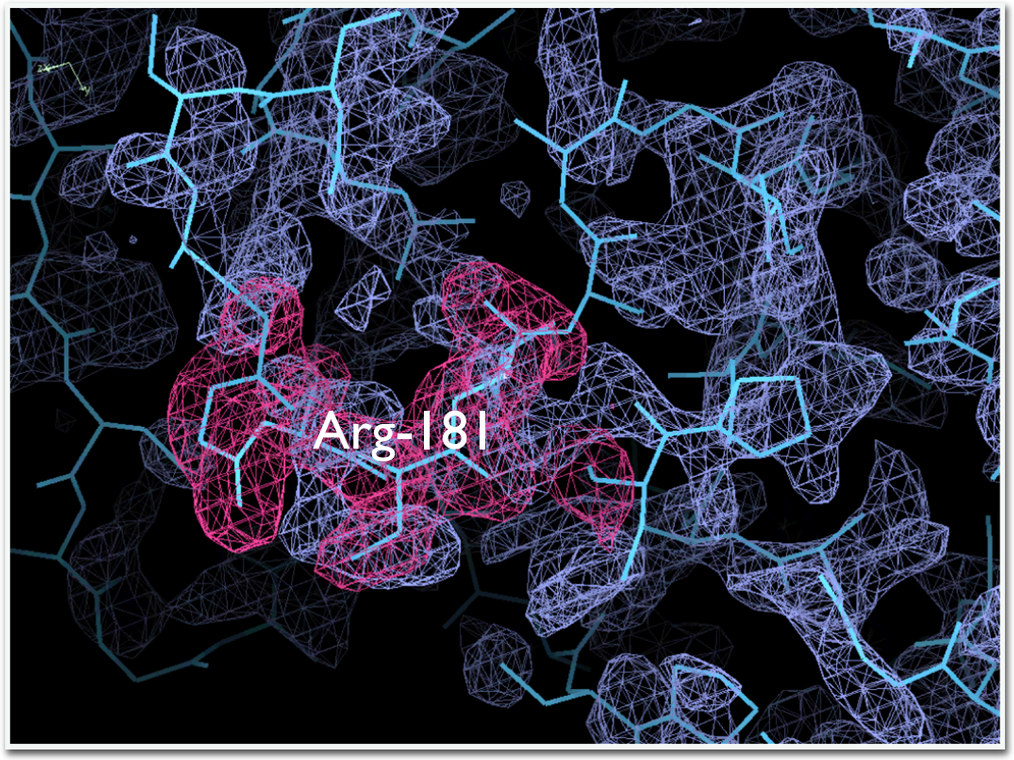
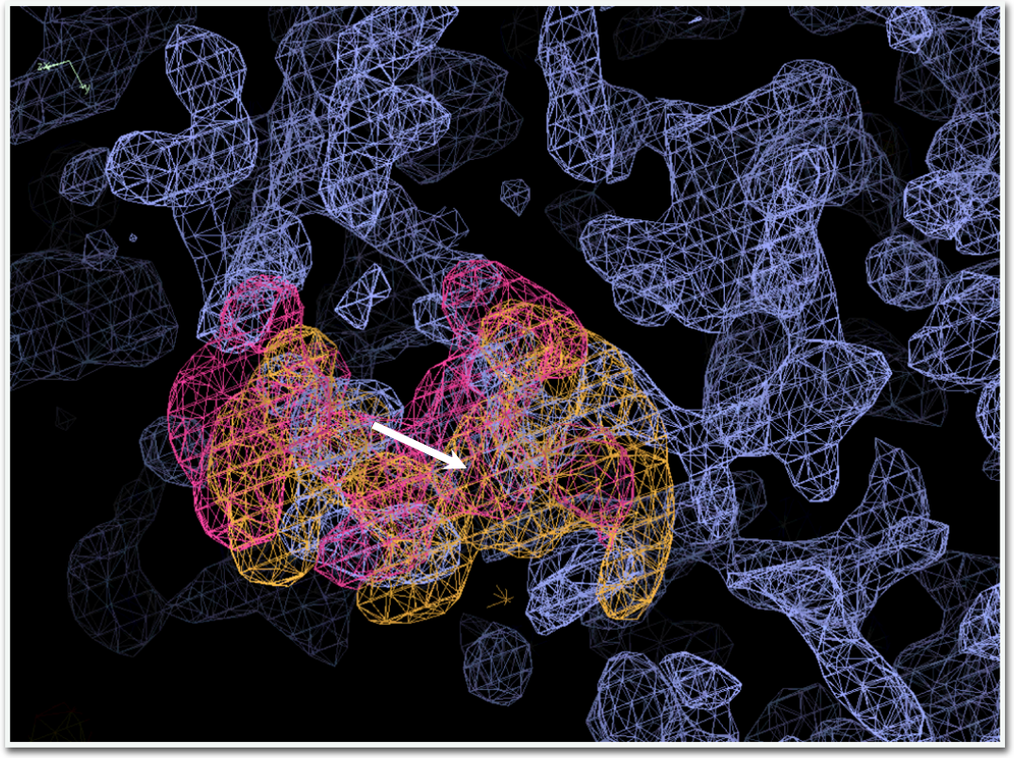
Pavel Afonine (LBNL)

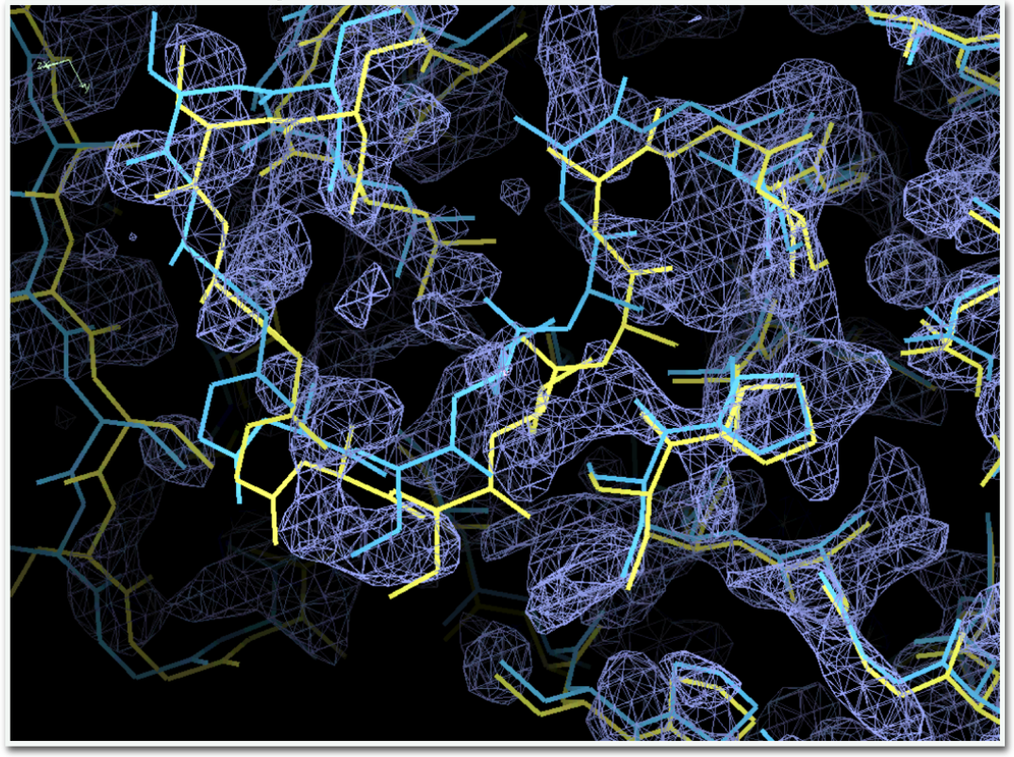
Morphing



- Identify local translation to apply to one $C\alpha$ atom and nearby atoms
- Smooth the local translations in window of 10 residues
- Apply the smoothed translation to all atoms in the residue

Tom Terwilliger, Los Alamos National Laboratory





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Terwilliger et al., Acta Cryst. 2012, **D68**:861-870

Phenix

Terwilliger et al., Acta Cryst. 2013, **D69**:2244-2250

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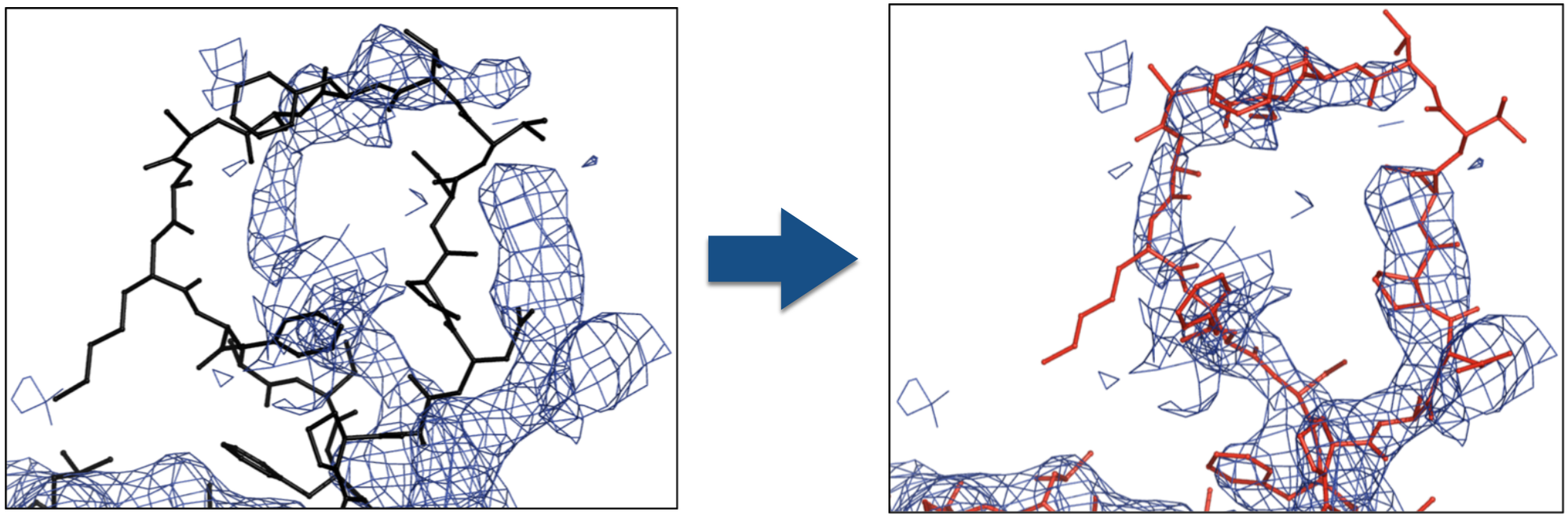
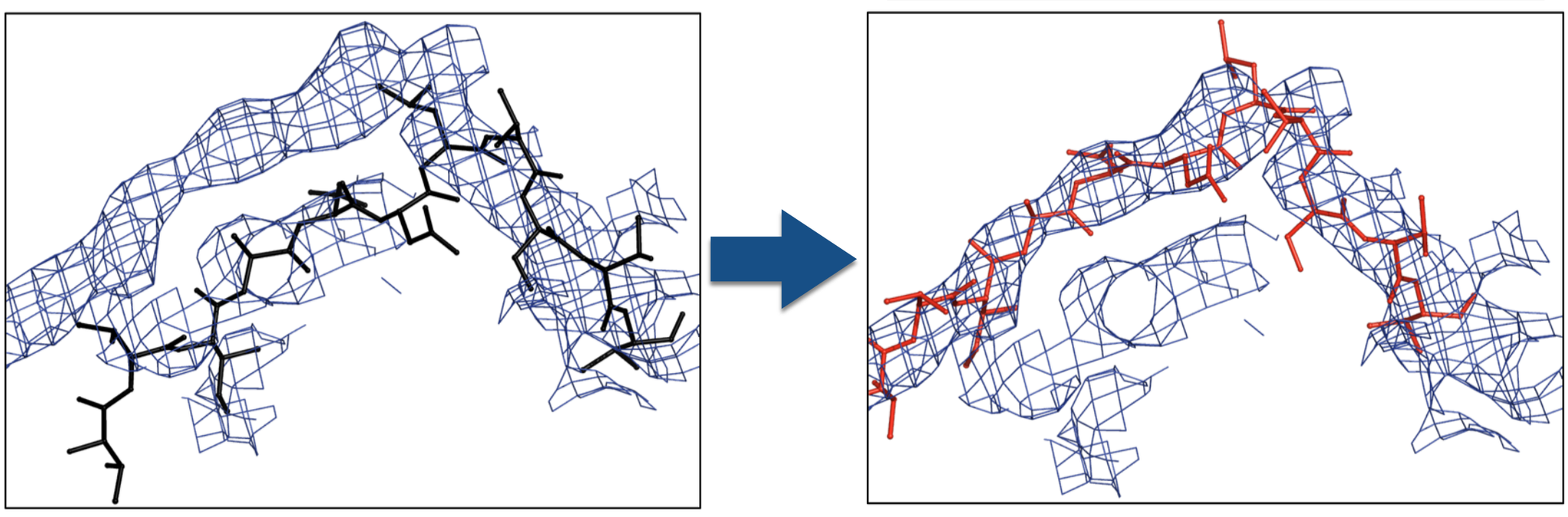
Real Space Refinement Improves Fit to Data

- Models are moved to better fit the Cryo-EM map





Phenix

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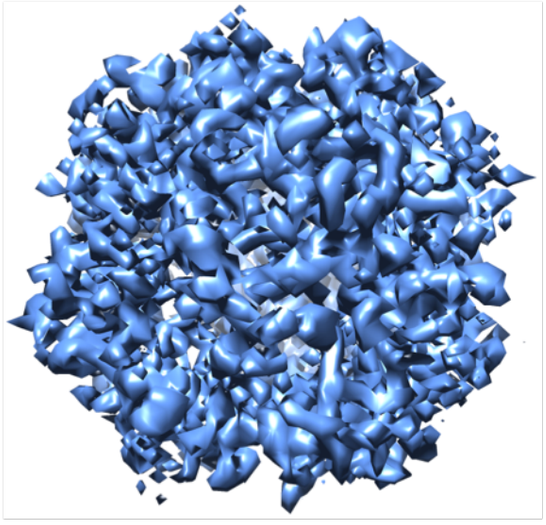
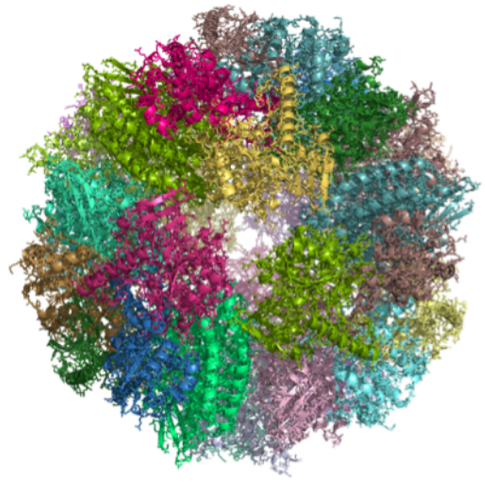
Typical Results at Higher Resolution

Resolution: 3.36 Å



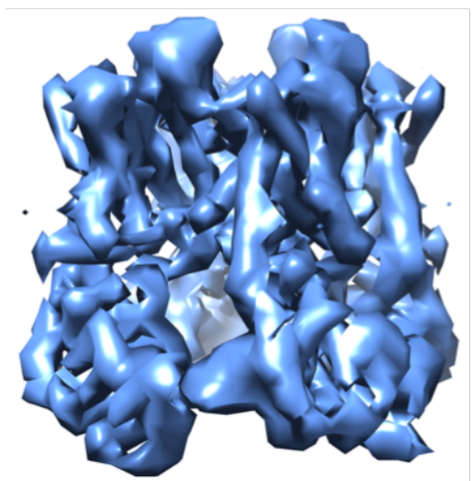


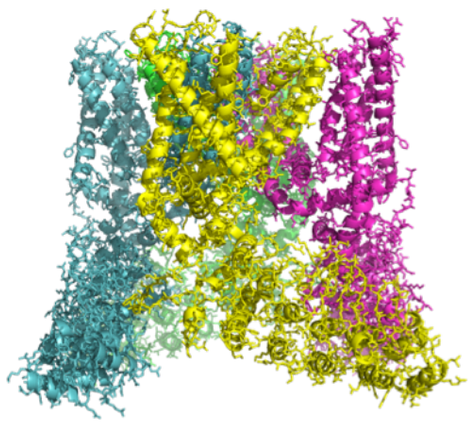
METRIC	Original	*Phenix*
Map CC	0.645	0.783
RMSD (bonds/angles)	0.02/2.05	0.01/1.21
Clashscore	117.1	18.79
Rama. outl., %	0.11	0.11
Rotamer outl., %	35.51	0
C-beta deviations	24	0

Residues/atoms: 10,716/82,404

Refinement: 173 min

Resolution: 3.8 Å





METRIC	Original	*Phenix*
Map CC	0.650	0.714
RMSD (bonds/angles)	0.01/1.34	0.01/1.31
Clashscore	100.9	32.84
Rama. outl., %	0.52	0
Rotamer outl., %	27.99	0
C-beta deviations	0	0

Residues/atoms: 2,324/17,424

Refinement: 20 min

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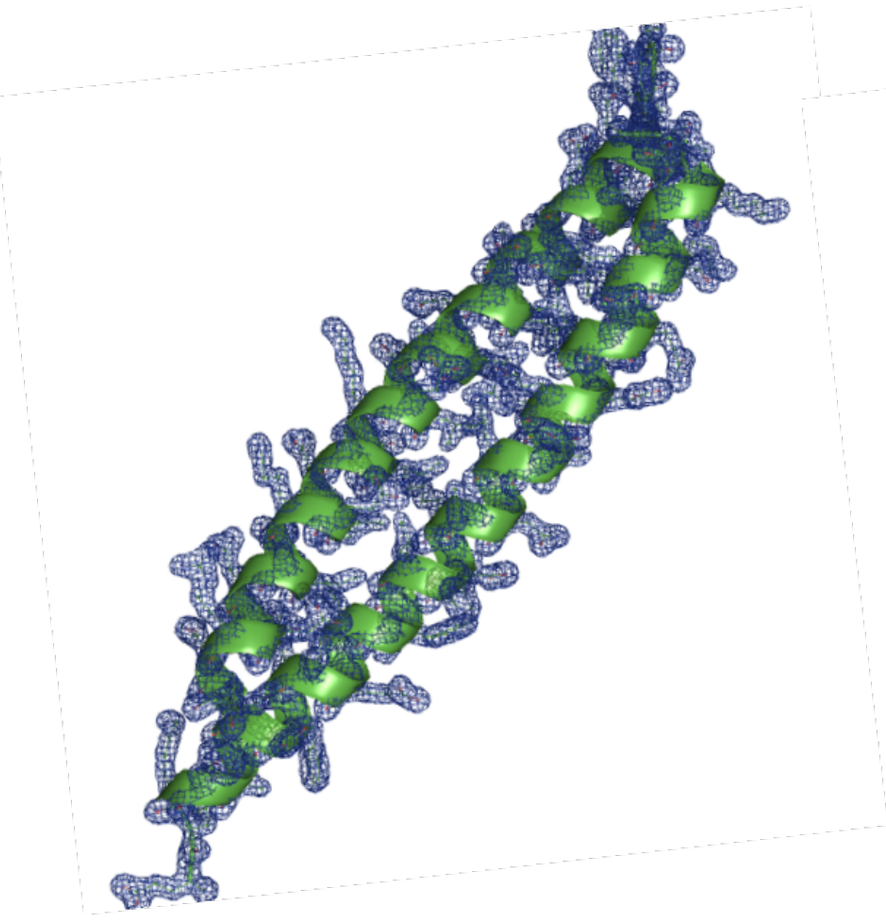
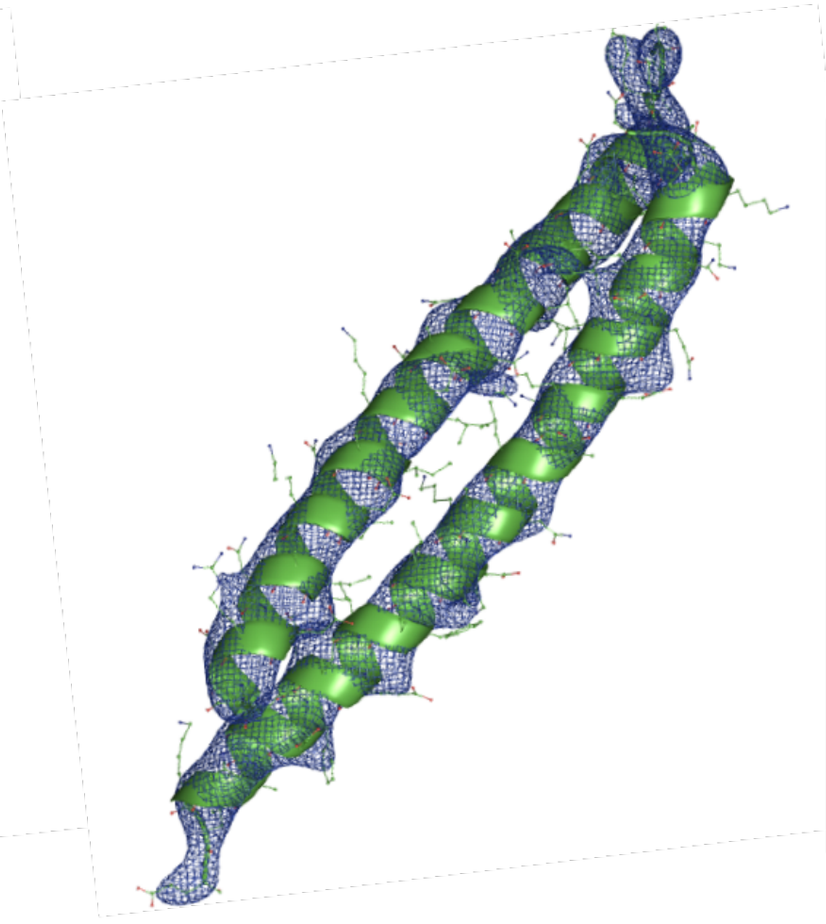
Lower Resolution Requires Additional Information

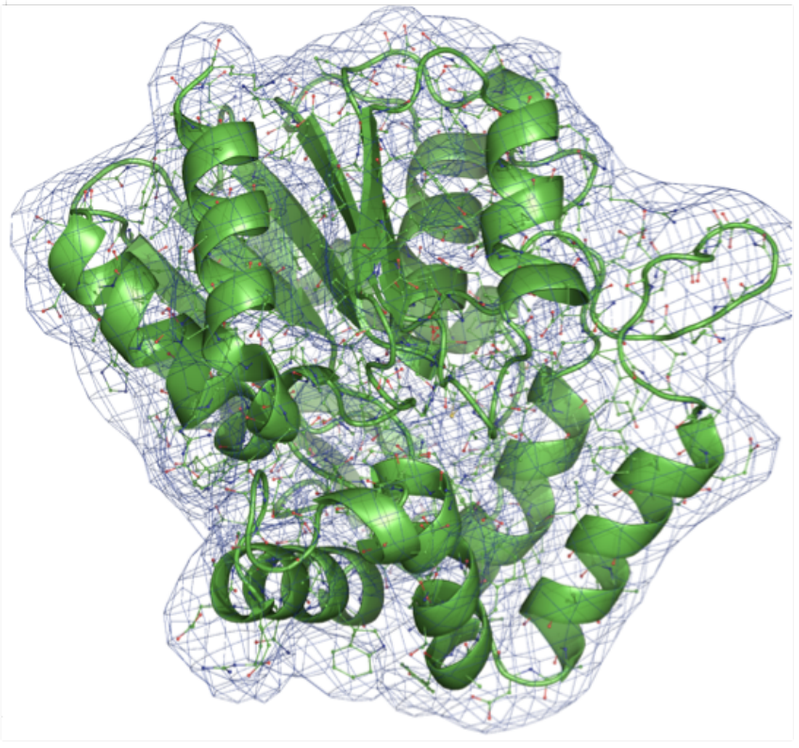
High Resolution **Low Resolution**



Side chains



Secondary Structure



Molecule

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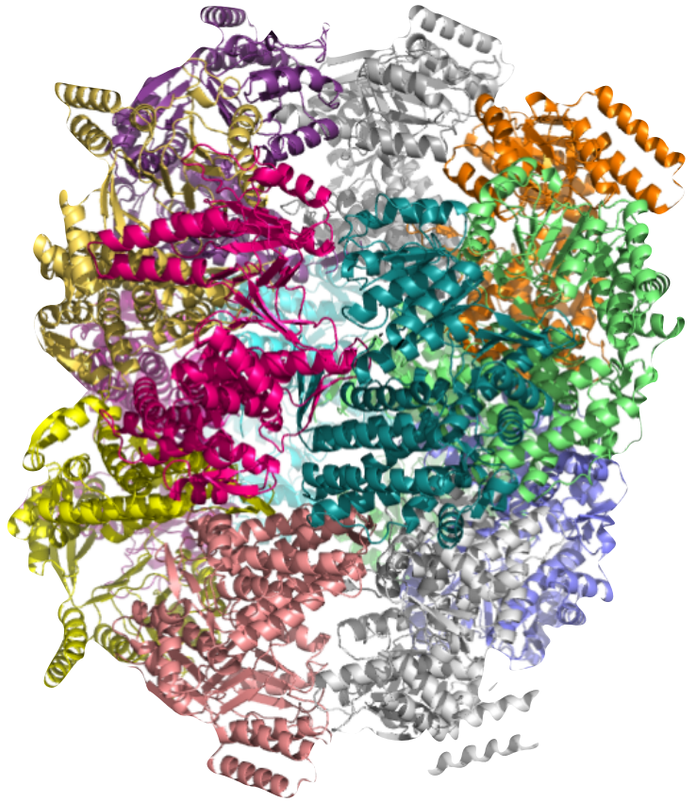
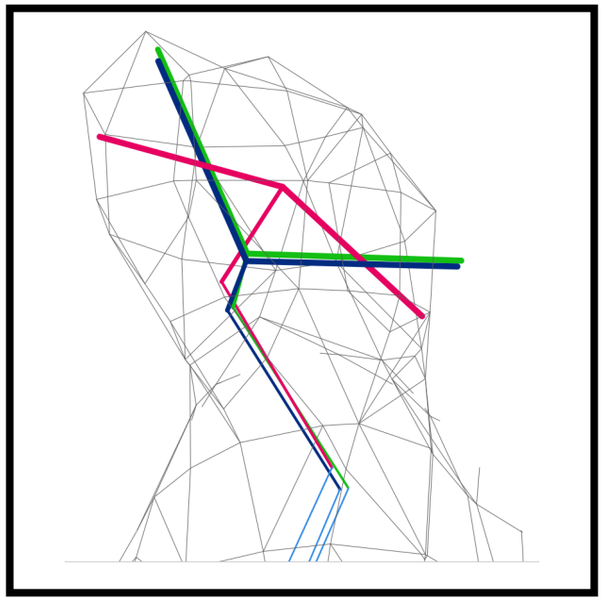
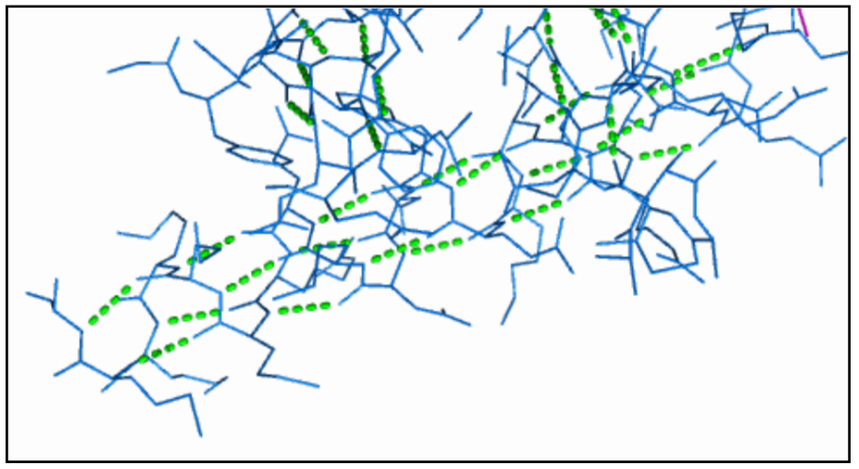
Model Restraints



- Symmetry constraints
- Multiple symmetry groups
- Optimization of NCS operators (w.r.t density)
- Automatic expansion of monomer from MTRX records

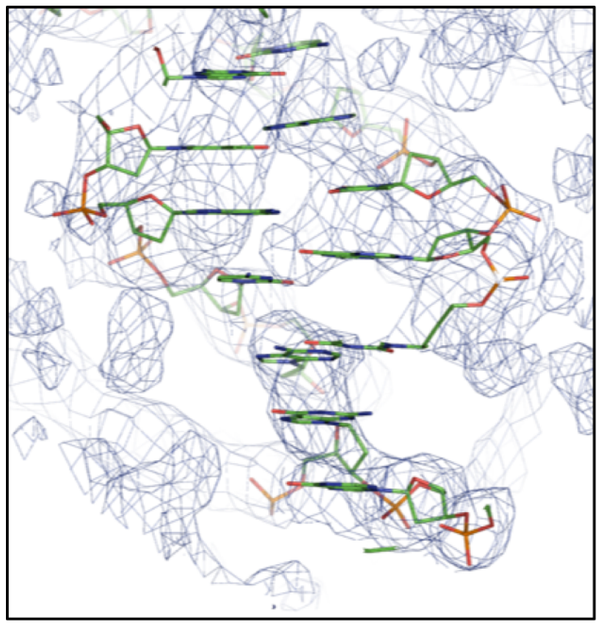


Reference model torsion angle restraints



Secondary structure restraints

Base pairing restraints



Parallelity restraints

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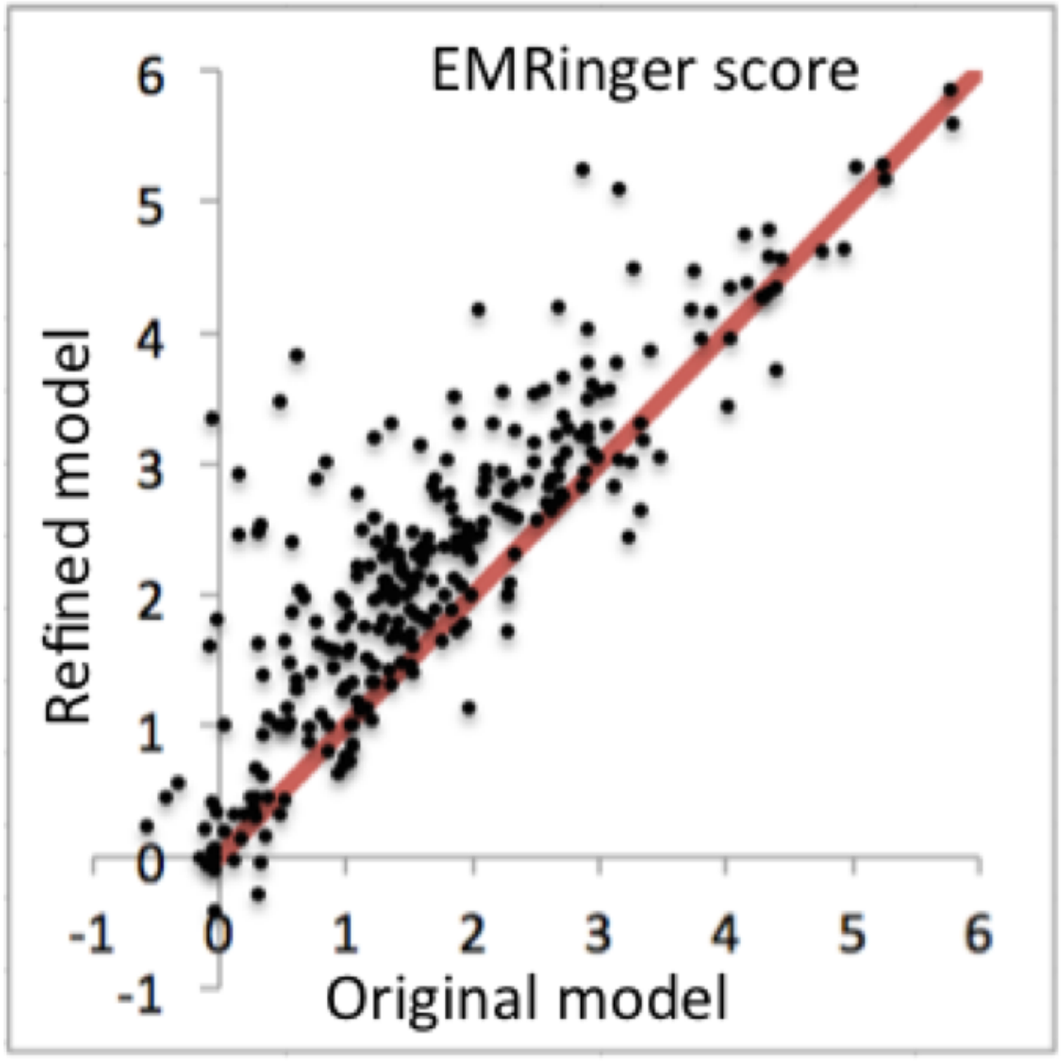
Improved Models from Real Space Refinement



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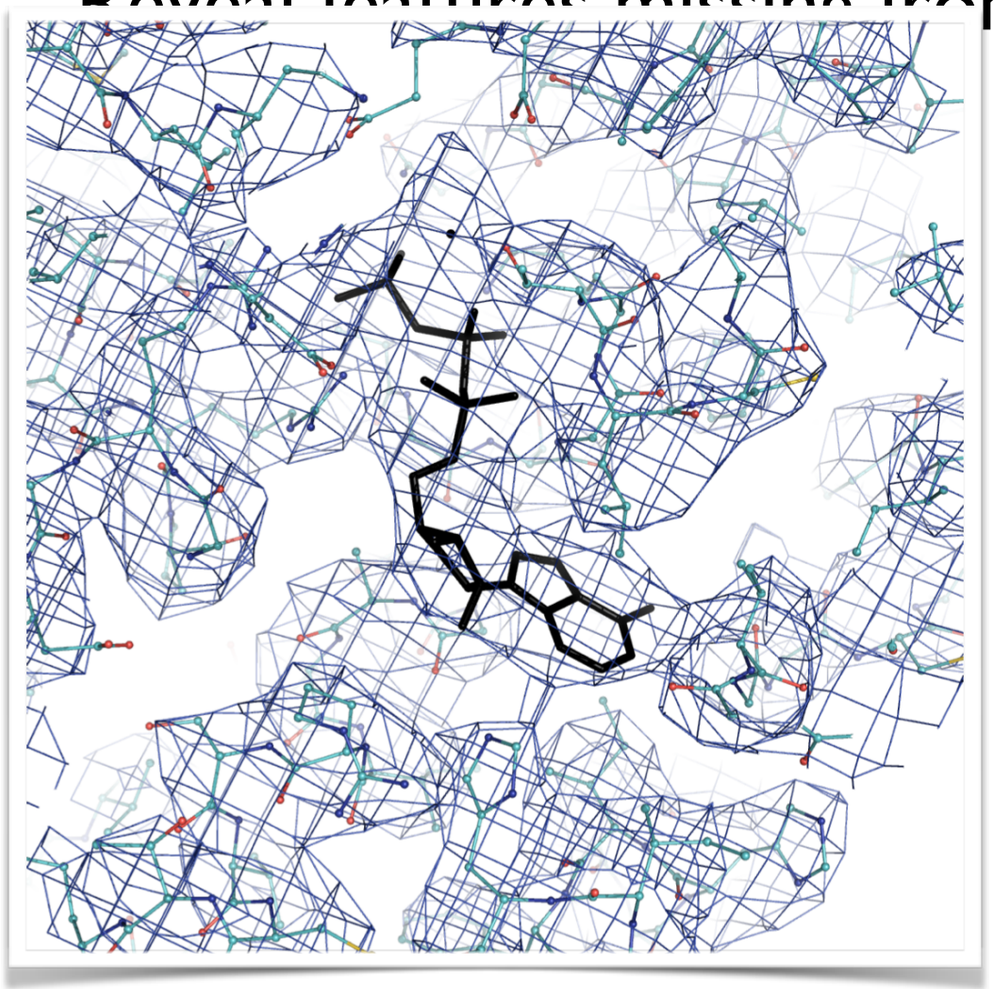
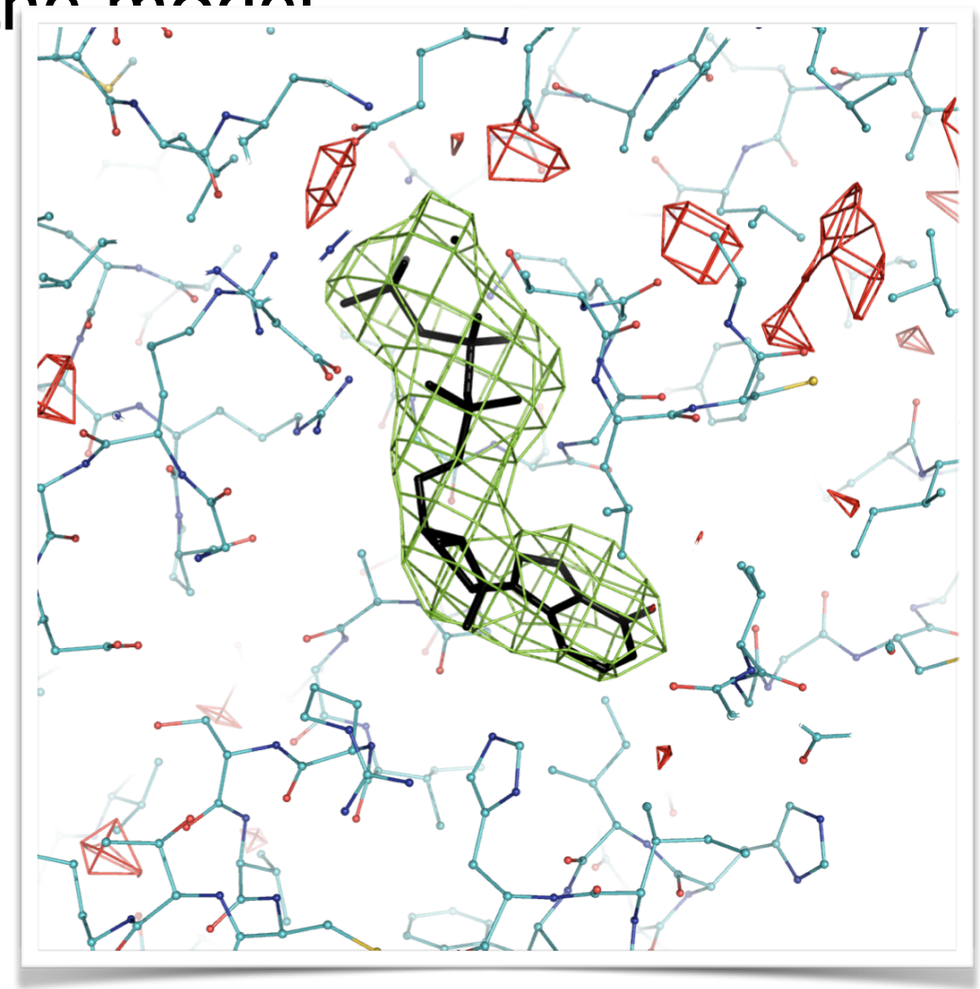
Difference Maps

- Local scaling of map and model density, real space subtraction
- Reveal features missing from the model





```
phenix.real_space_diff_map model.pdb map.ccp4 resolution=3.5
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Conclusions

- The application of prior or complementary information can improve refinement at low resolution for X-ray and Cryo-EM structures
  - Real space refinement is particularly powerful
- Methods from structure prediction provide additional information to improve models
  - Powerful combination of Rosetta and phenix.refine
- It is now feasible to generate good quality models even with low resolution data
  - Challenges still remain in arriving at initial models in the absence of related structures
- Many challenges remain:
  - Reliably accounting for uncertainty in magnification
  - Local variation in resolution leads to uncertainties in interpretation
  - Efficiently accounting for atomic displacements in models

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