Structure Refinement at Low Resolution

in

X-ray Crystallography
Cryo-electron Microscopy

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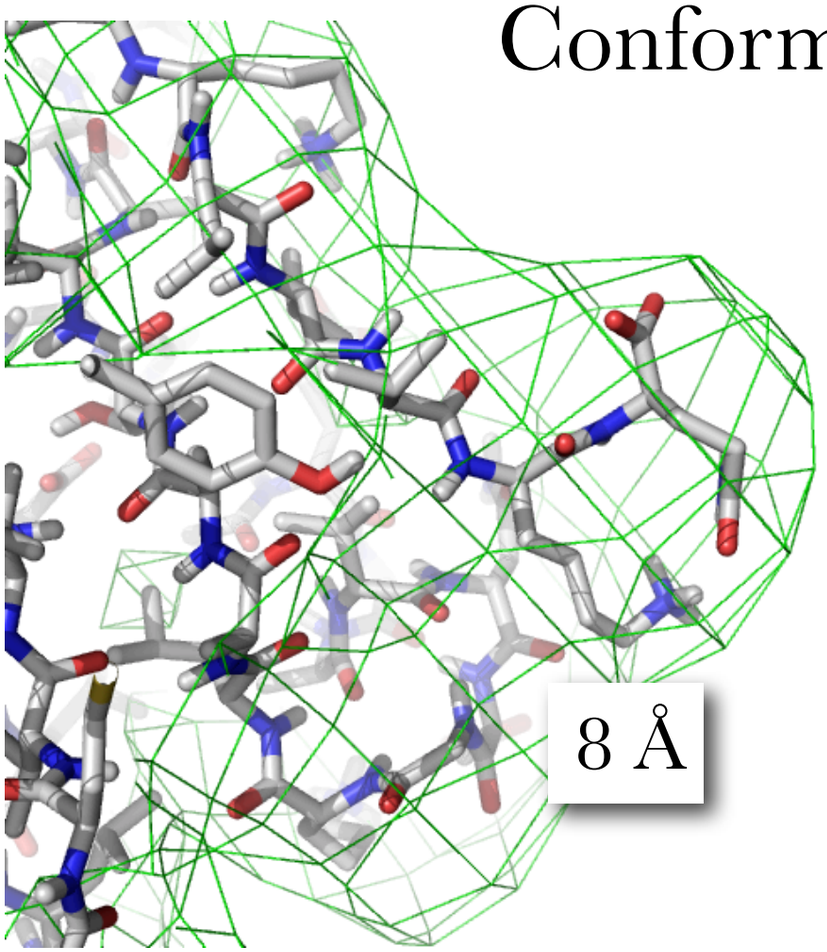
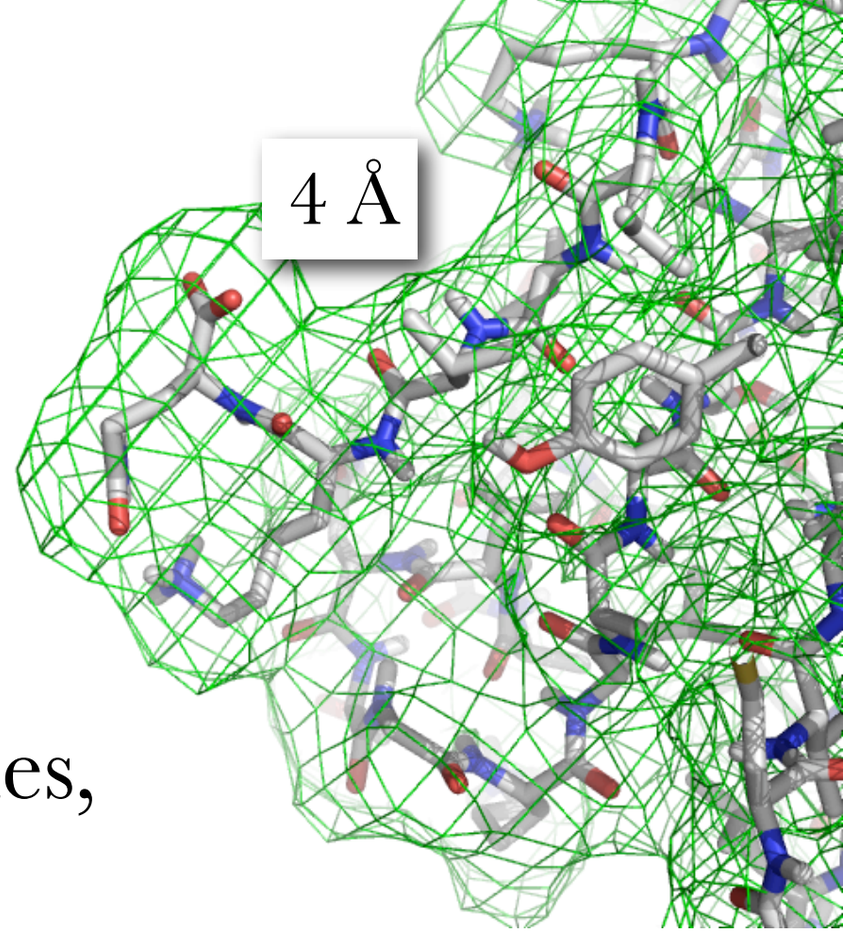
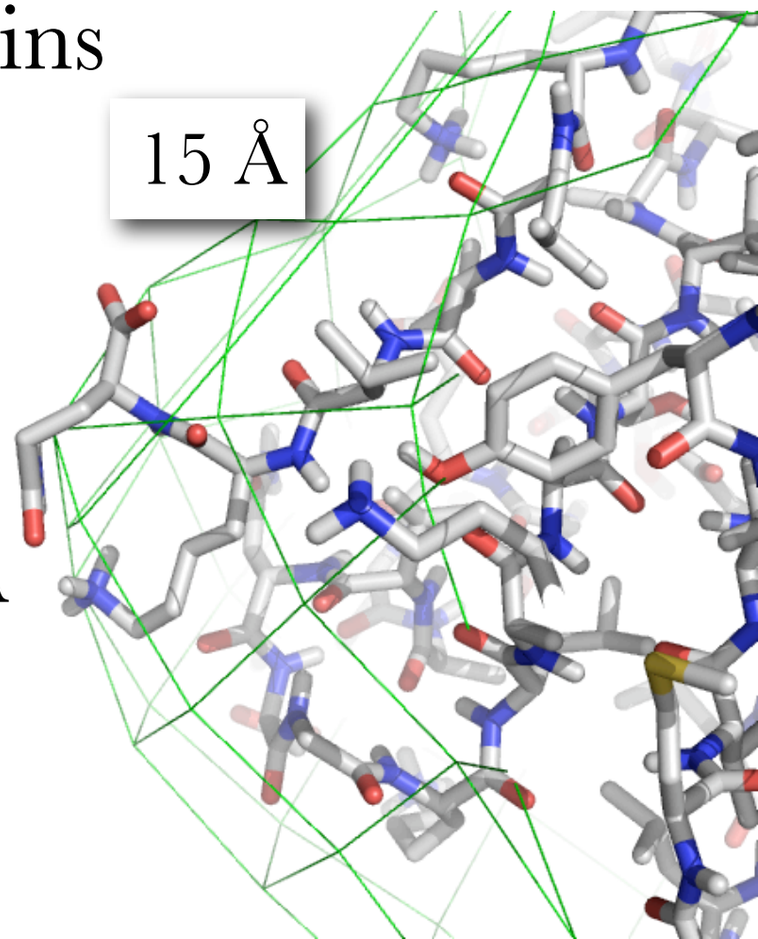
Low Resolution

Conformational heterogeneity
and flexibility





Large Macromolecules,
Protein Complexes
or Membrane Proteins



X-ray Crystallography

1 2 3 4 5 10 20 30 Å

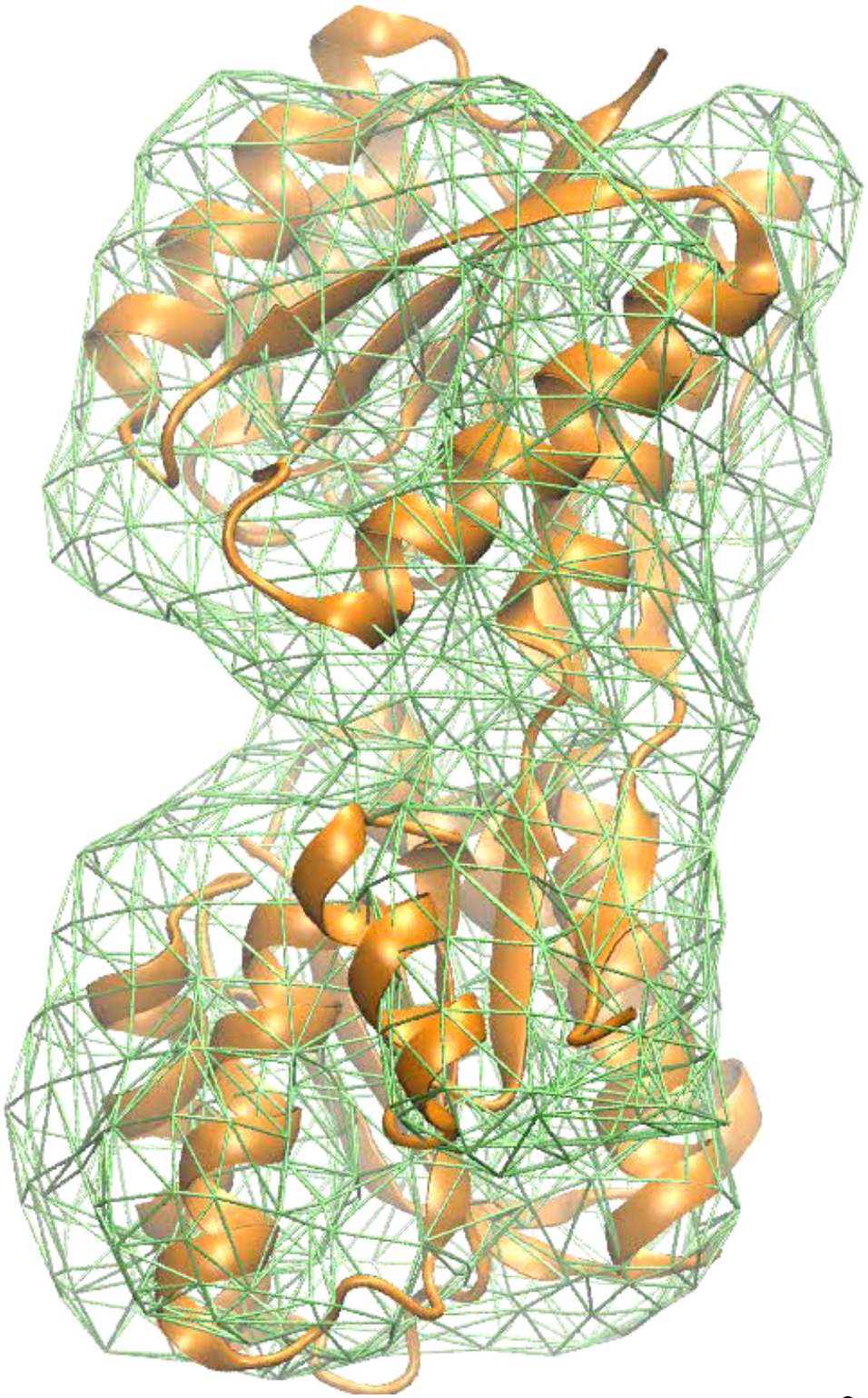
Cryo-electron Microscopy

Structure Refinement at Low-resolution

Assume: High-quality starting structure (known structure in different conformation or good homology model)

Standard refinement yields a bad structure

How to use prior structural information during the refinement?



10 Å

Deformable Elastic Network (DEN)

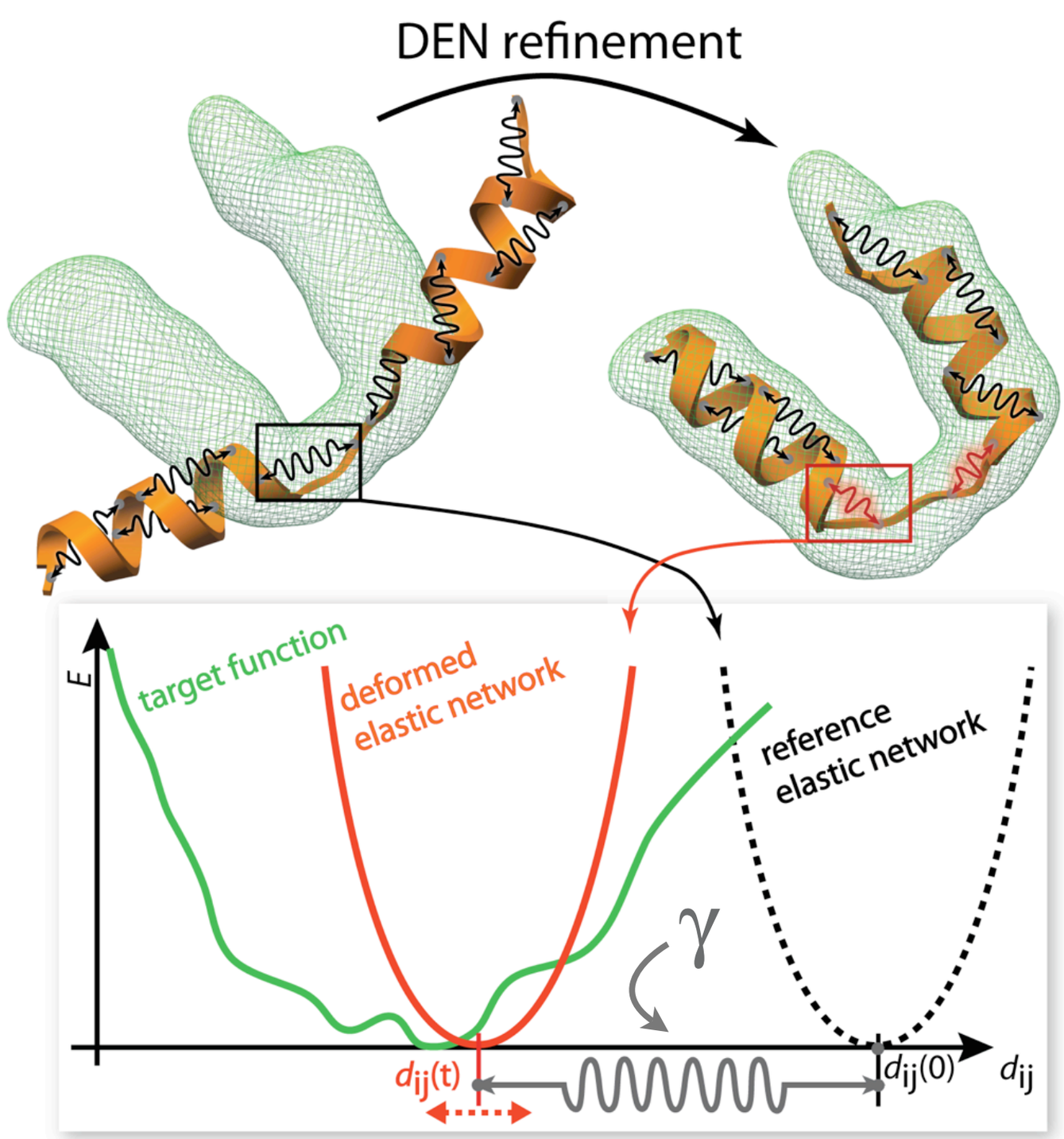
Refine only those degrees of freedom
that need to be refined to fit the data,
but not more.

Find only the relevant degrees of
freedom for which the data actually
provide information

Only input:
Reference Model and Experimental Data

Schröder, Brunger, and Levitt, *Structure* (2007) **15**:1630

The γ-parameter weights between reference model and experimental data

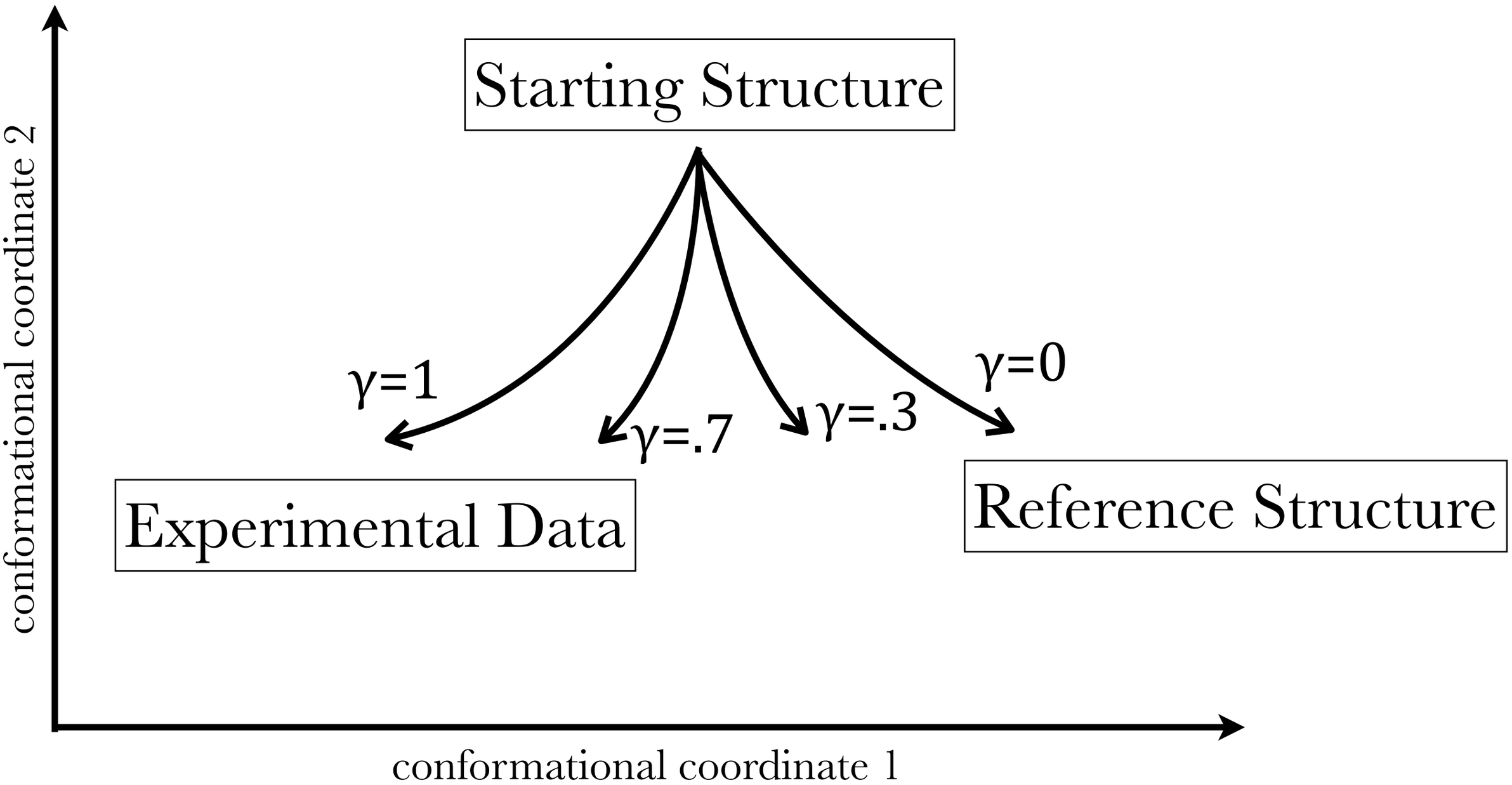


increasing deformability →

reference model ⟷ **experimental restraints**

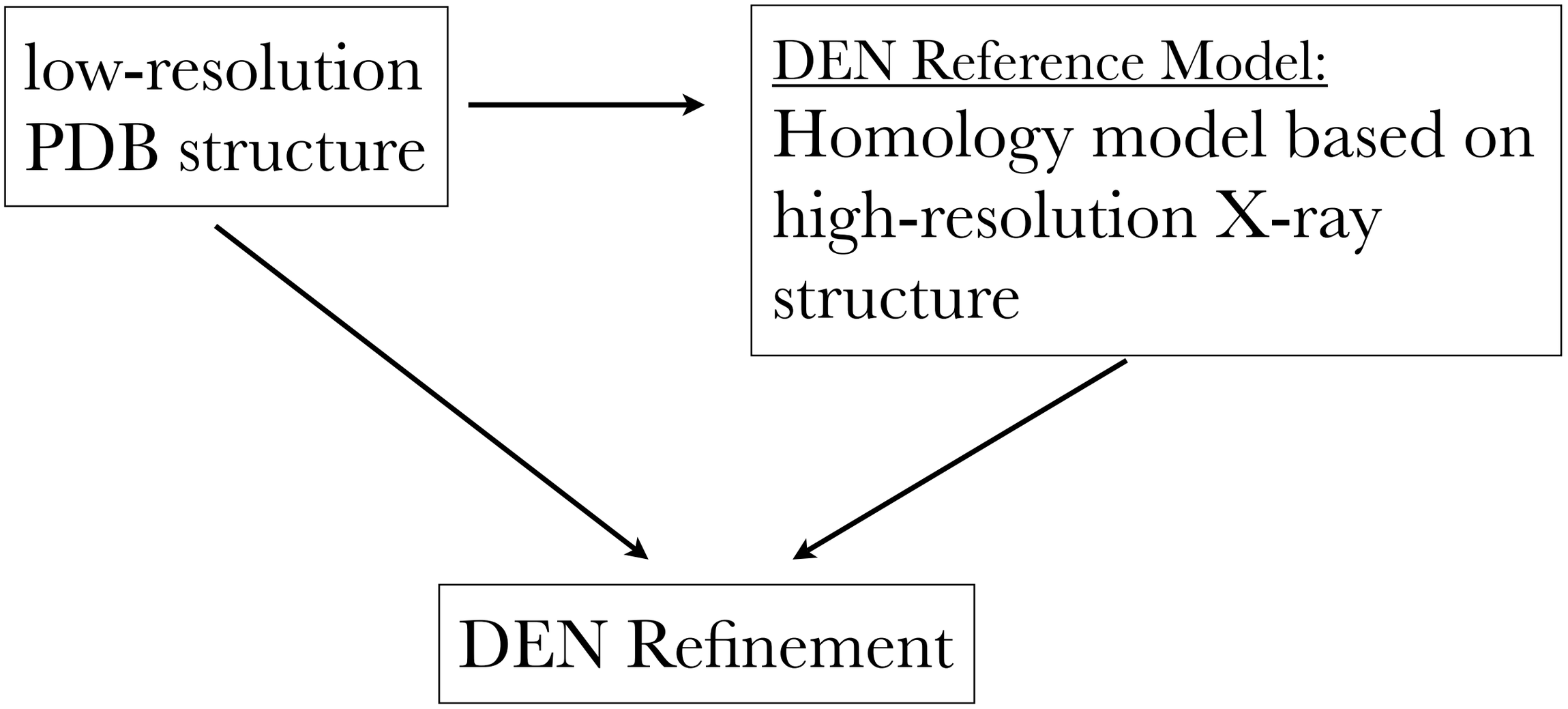
$\gamma = 0$ $\gamma = 1$

Reference Structure can be Different from Starting Structure



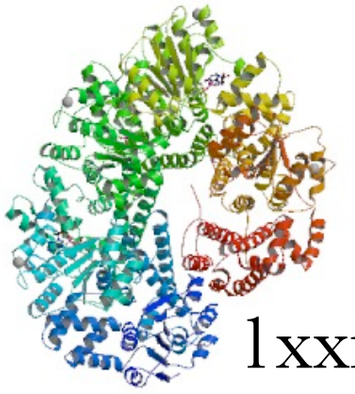
Application to X-ray Crystallography:

Improve existing low-resolution PDB structures

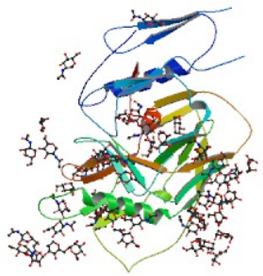


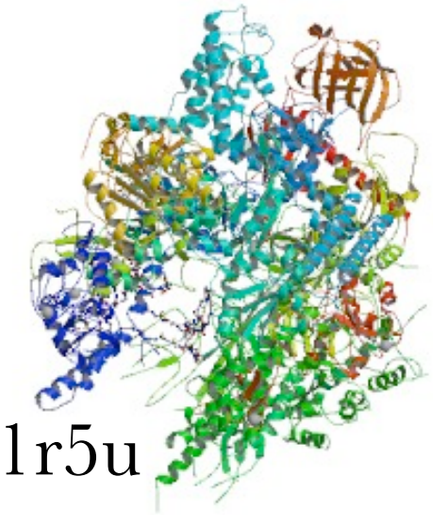
Application to a Set of 19 Low-Res Structures

resolution ≥ 4 Å


1xxi

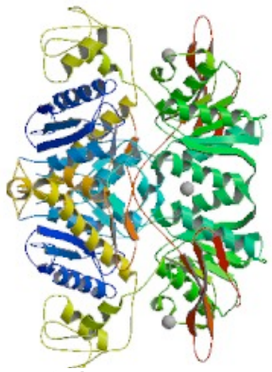

1xdv


2bf1


1r5u


1pgf


1jl4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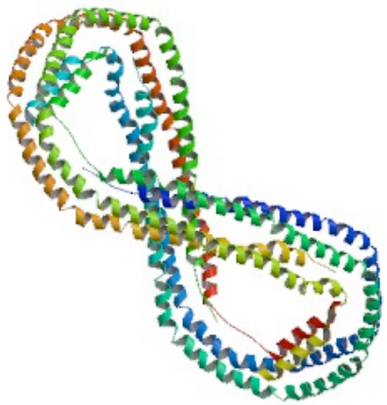
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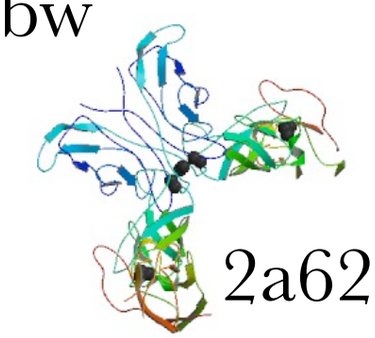
3bbw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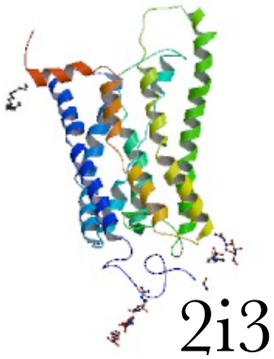
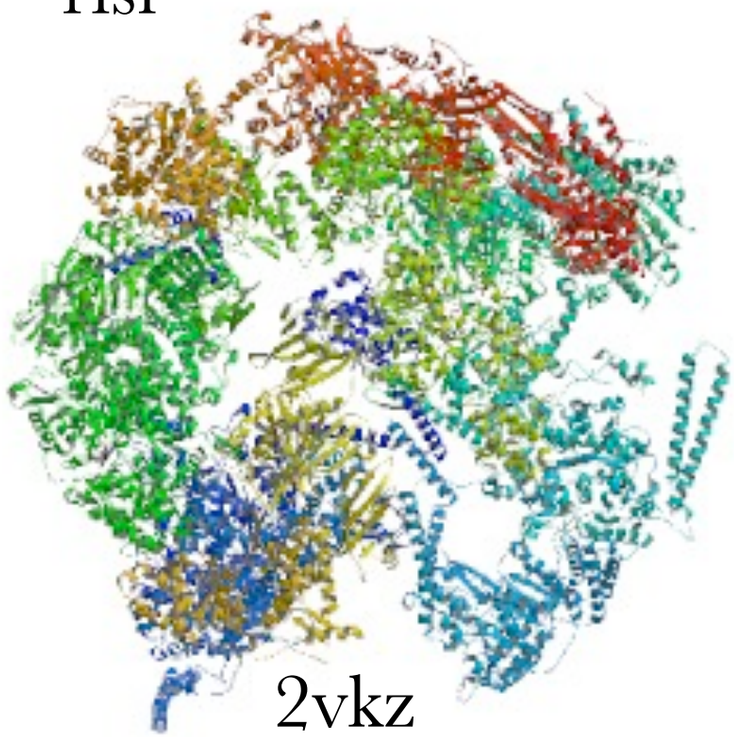
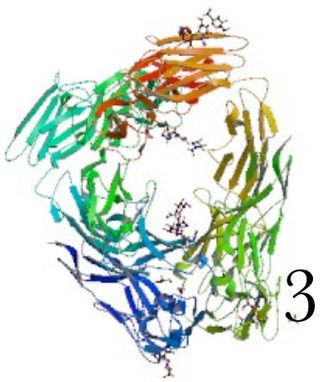

2i36

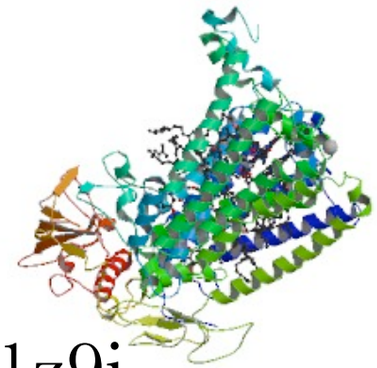

3crw

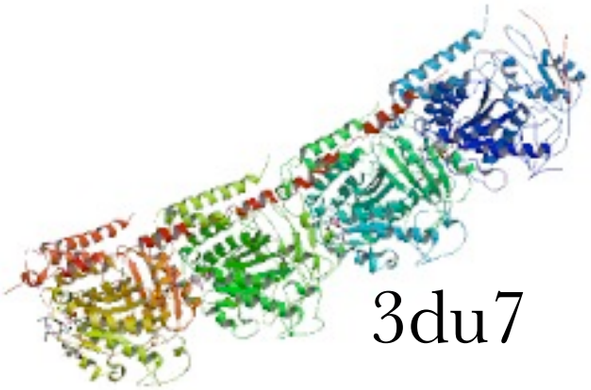

1ye1


2vkz


1yi5


3dmk


1z9j


3du7


2qag

Application to a Set of 19 Low-Res Structures

DEN method was built into the CNS refinement software.
(will be available with the next official CNS release)

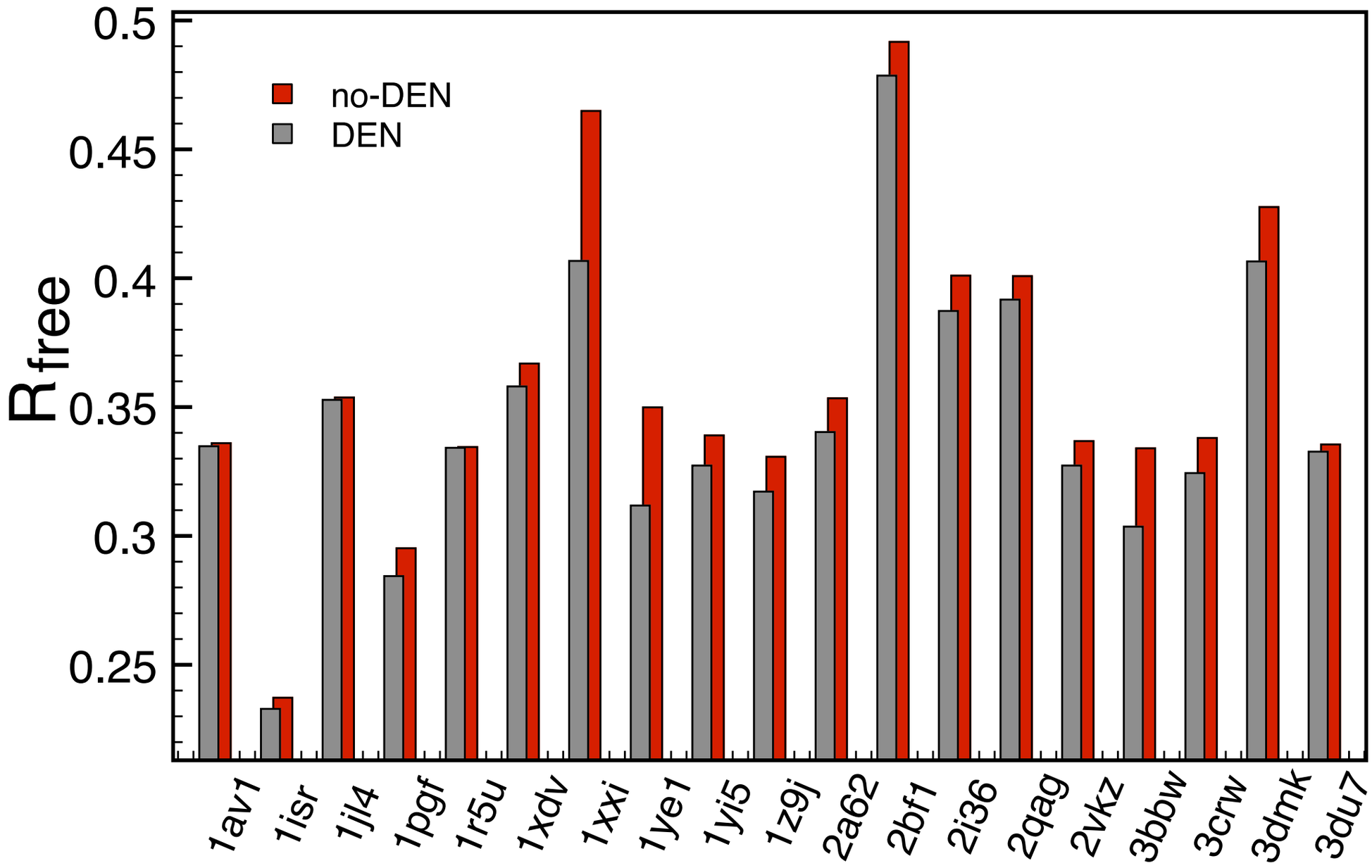
1) Standard simulated annealing protocol (control)

no-DEN

2) Standard simulated annealing with DEN restraints

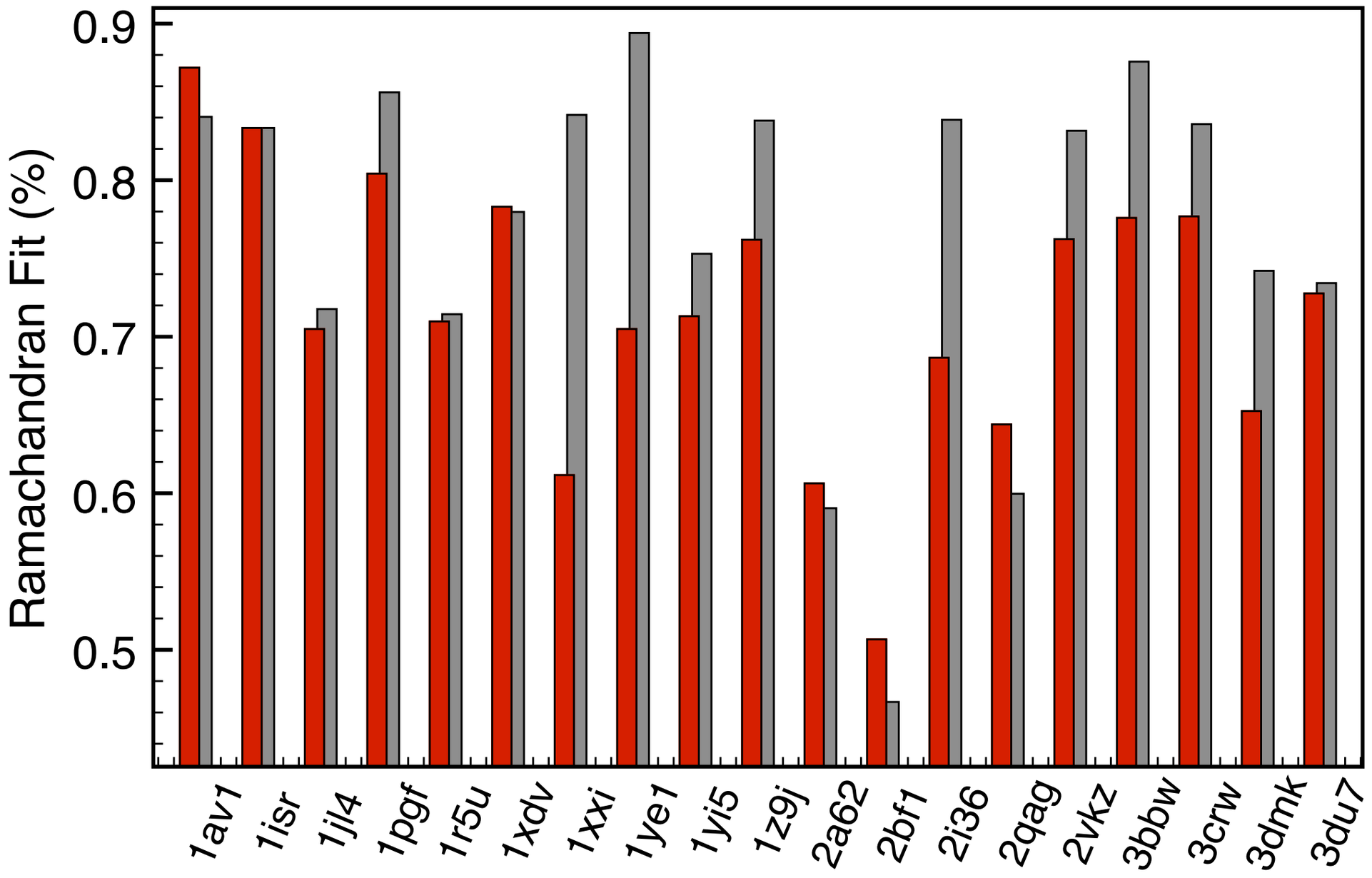
DEN

DEN improves R_{free}



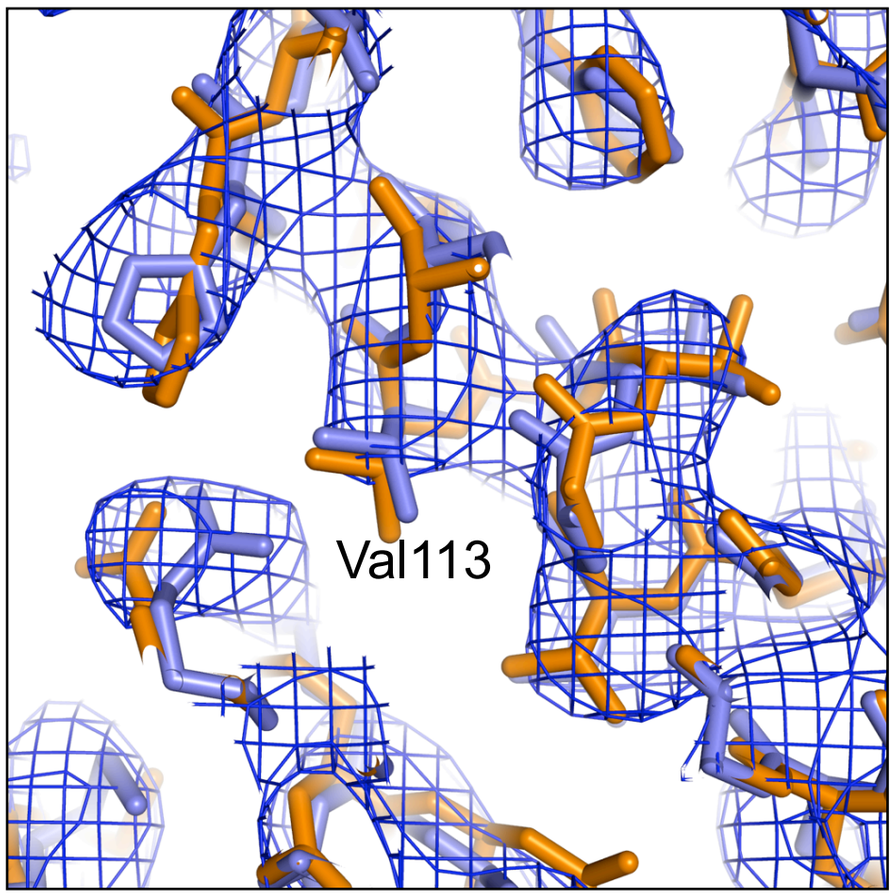
R_{free} measures how well the model fits the data

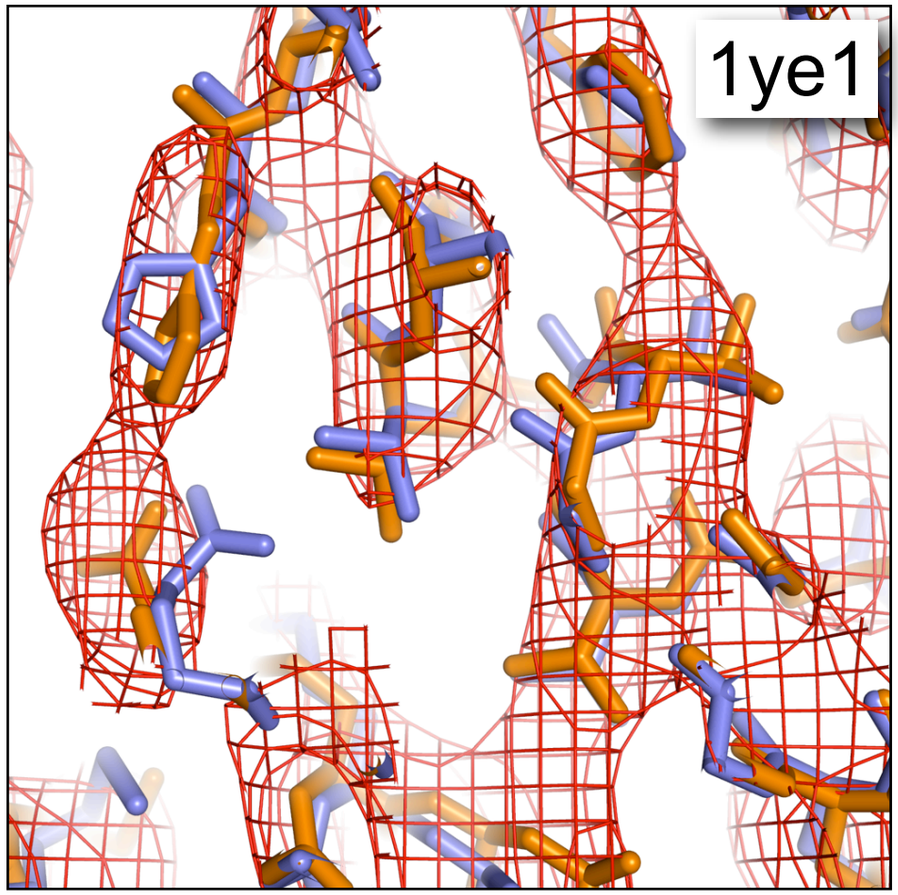
DEN improves Ramachandran statistics



% residues in favored region of the Ramachandran plot as determined by Molprobity

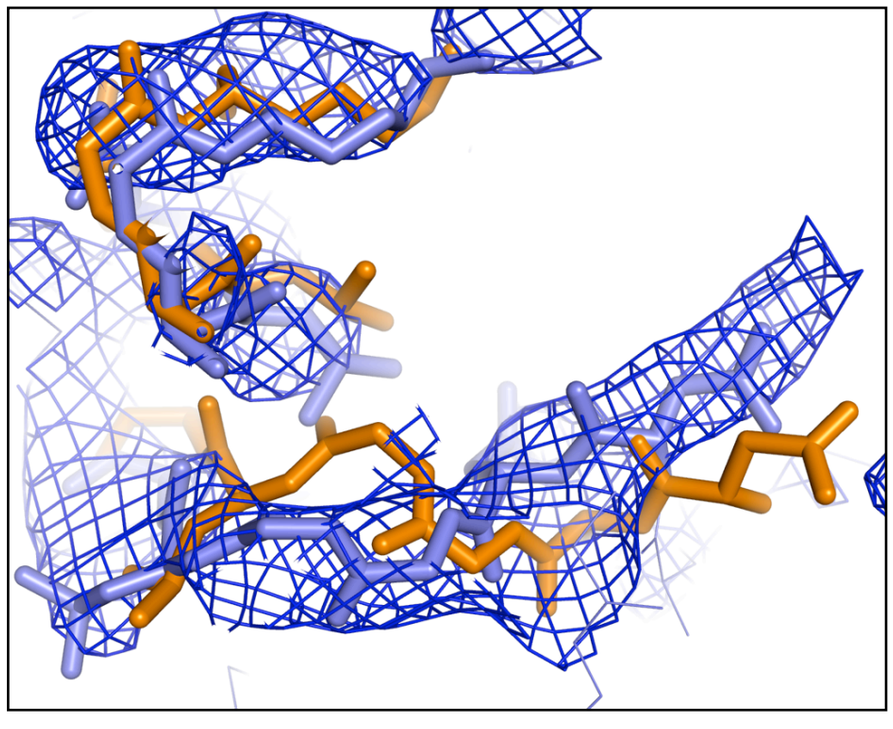
Phase and Density Map Improvement

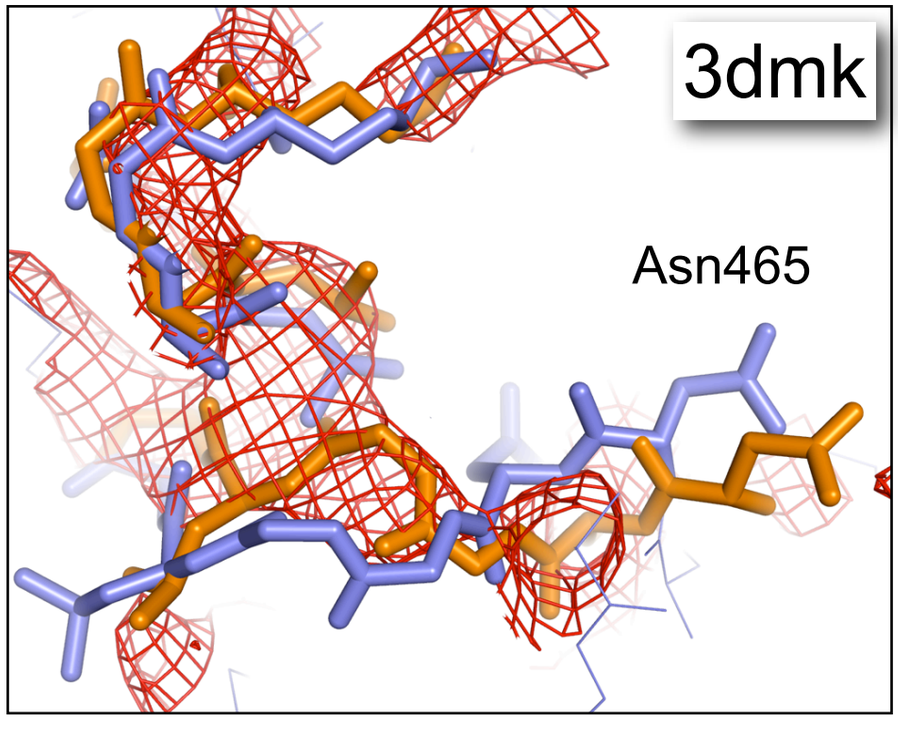




R_{free}

DEN 31.2
no-DEN 35.0

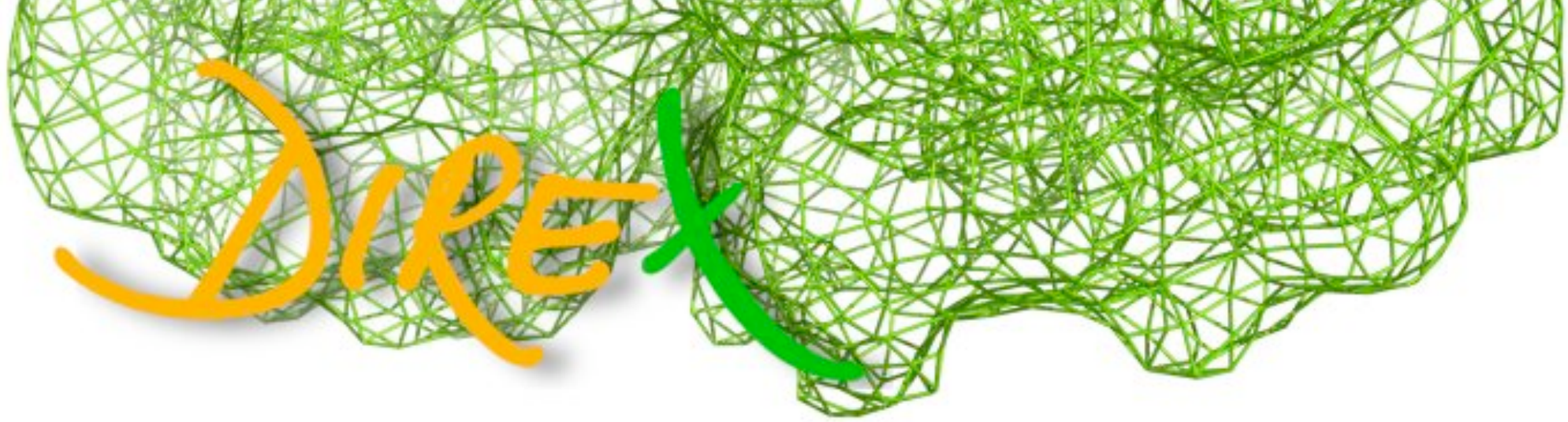




DEN 40.7
no-DEN 42.8

Schröder, Levitt, and Brunger, *submitted*

Application of DEN to Real-space Structure Refinement using density maps from single-particle Cryo-EM



- Geometry-based conformational sampling (based on Concoord)

- Forces
  1. Density map
  2. Deformable Elastic Network (DEN)

  3. Distance restraints
  4. Position restraints

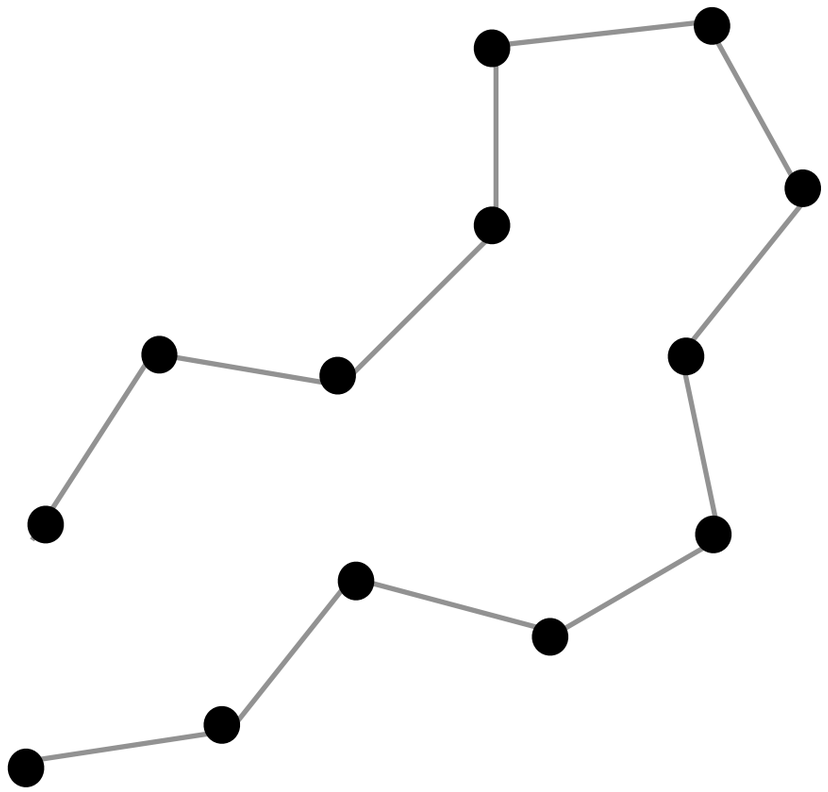
http://simtk.org/home/direx/
http://www.schroderlab.org/software/direx/

DireX: Geometry-based conformational sampling

based on CONCOORD

B.L. de Groot, et al. Proteins 29: 240-251 (1997)

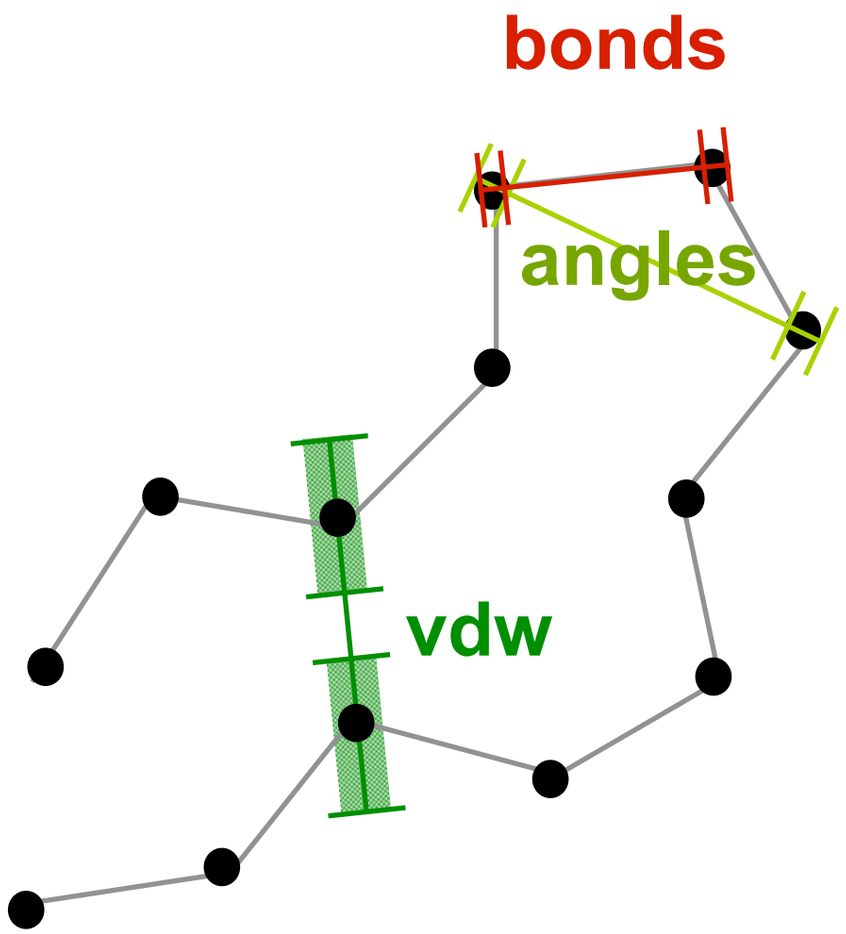
1. Initial model



DireX: Geometry-based conformational sampling

based on CONCOORD

B.L. de Groot, et al. Proteins 29: 240-251 (1997)



1. Initial model

2. Generate list of
   distance restraints (intervals)

DireX: Geometry-based conformational sampling

based on CONCOORD

B.L. de Groot, et al. Proteins 29: 240-251 (1997)

1. Initial model

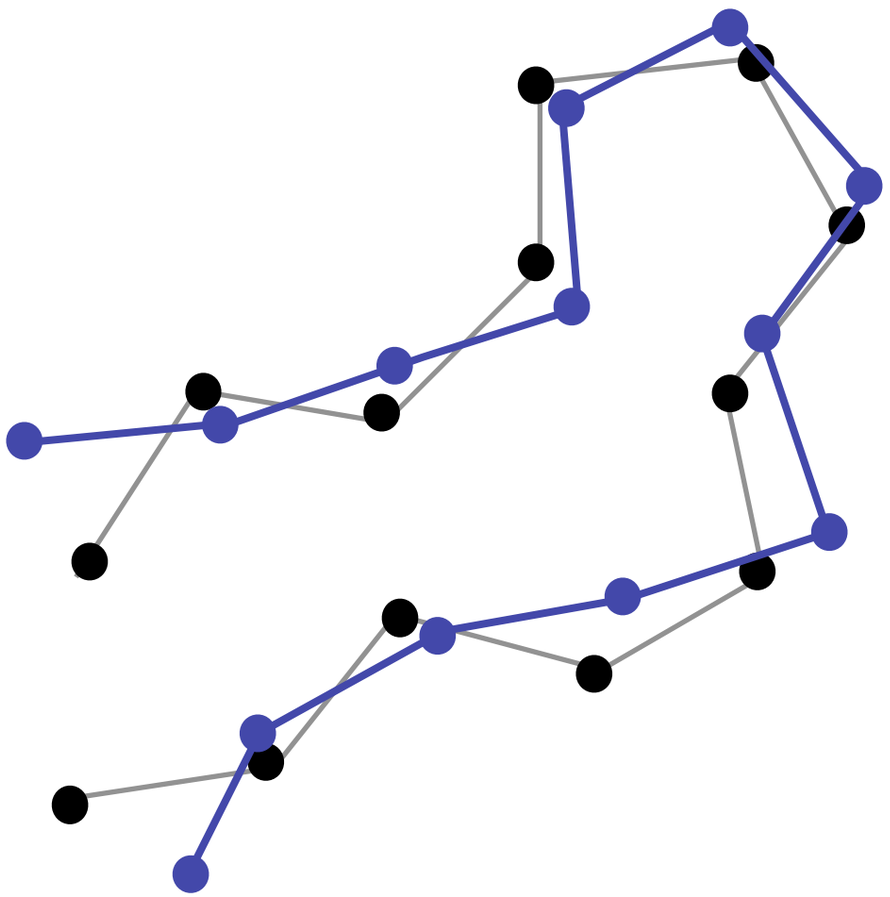
2. Generate list of
   distance restraints (intervals)

3. Perturb coordinates

DireX: Geometry-based conformational sampling

based on CONCOORD

B.L. de Groot, et al. Proteins 29: 240-251 (1997)



1. Initial model

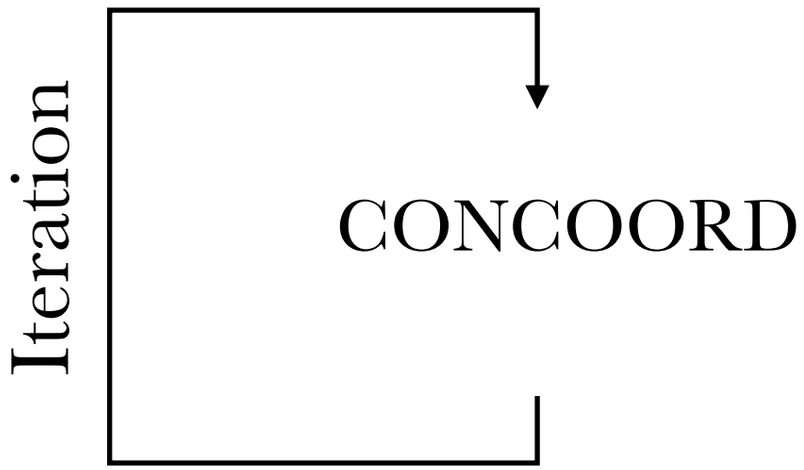
2. Generate list of
   distance restraints (intervals)

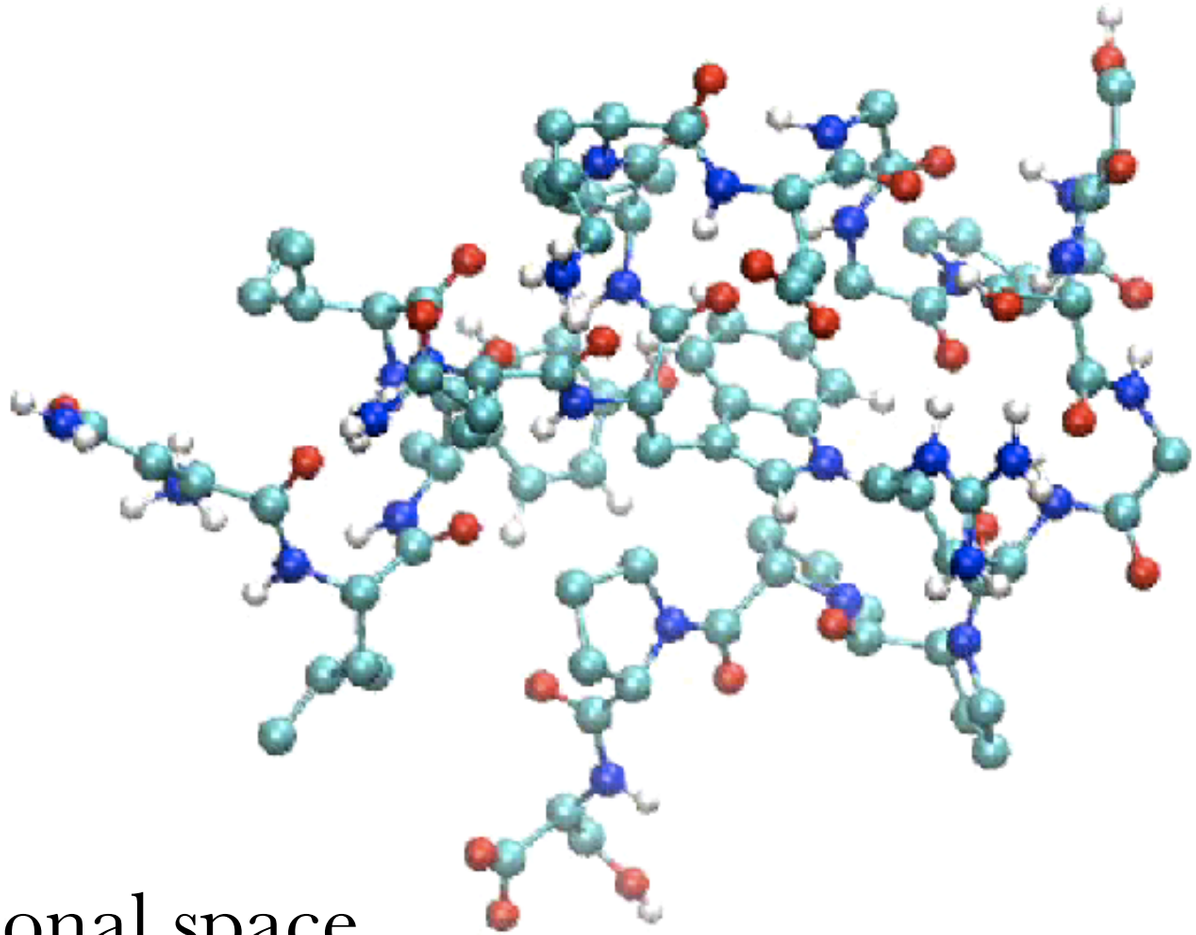
3. Perturb coordinates

4. use CONCOORD algorithm to obtain
   a new structure which also obeys
   all distance restraints

CONCOORD: correct distances
iteratively in a random order

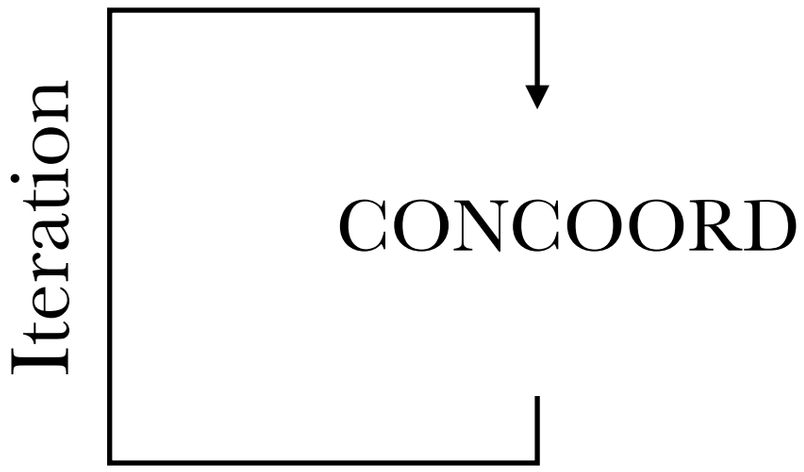
DireX: Geometry-based Conformational Sampling

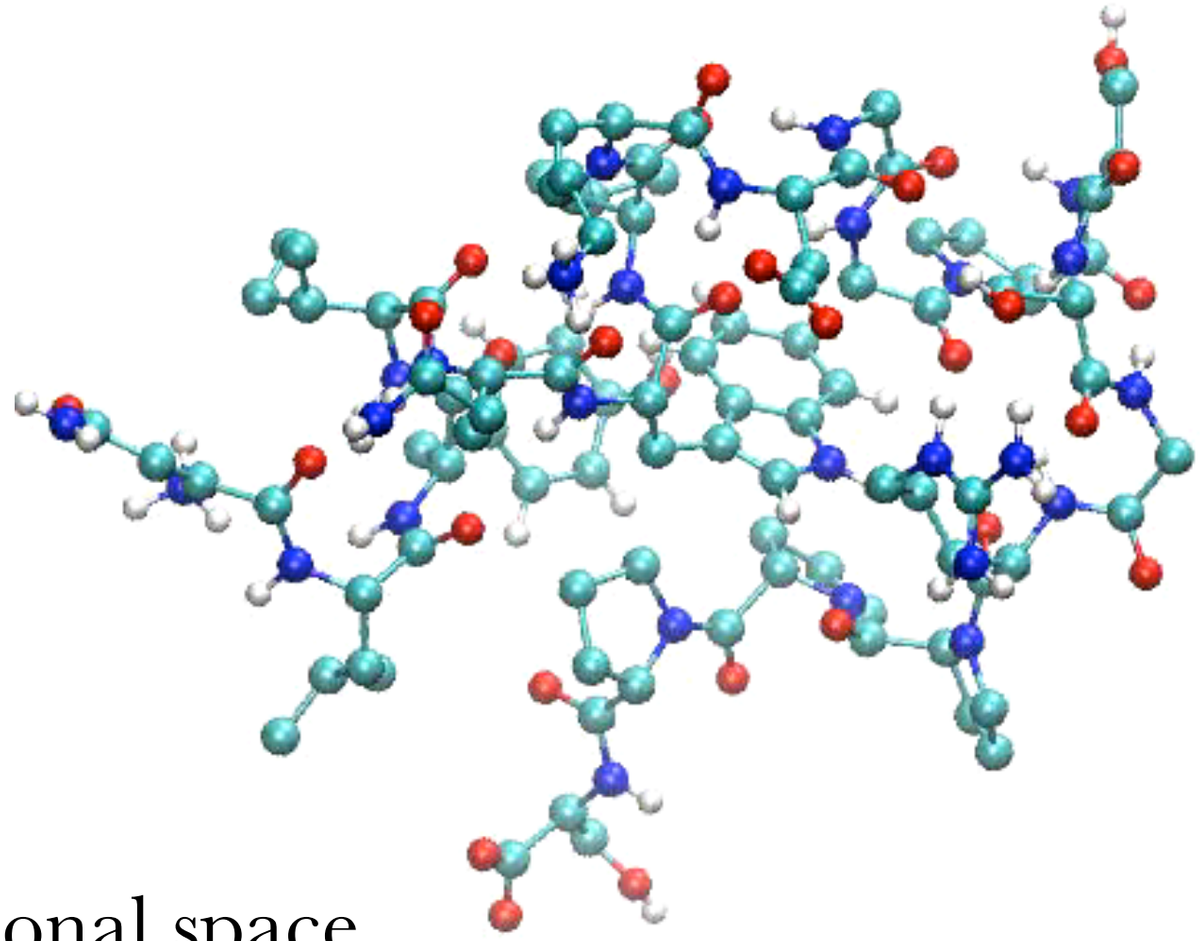




Random walk through conformational space
while maintaining correct stereochemistry
and avoiding atom clashes

DireX: Geometry-based Conformational Sampling



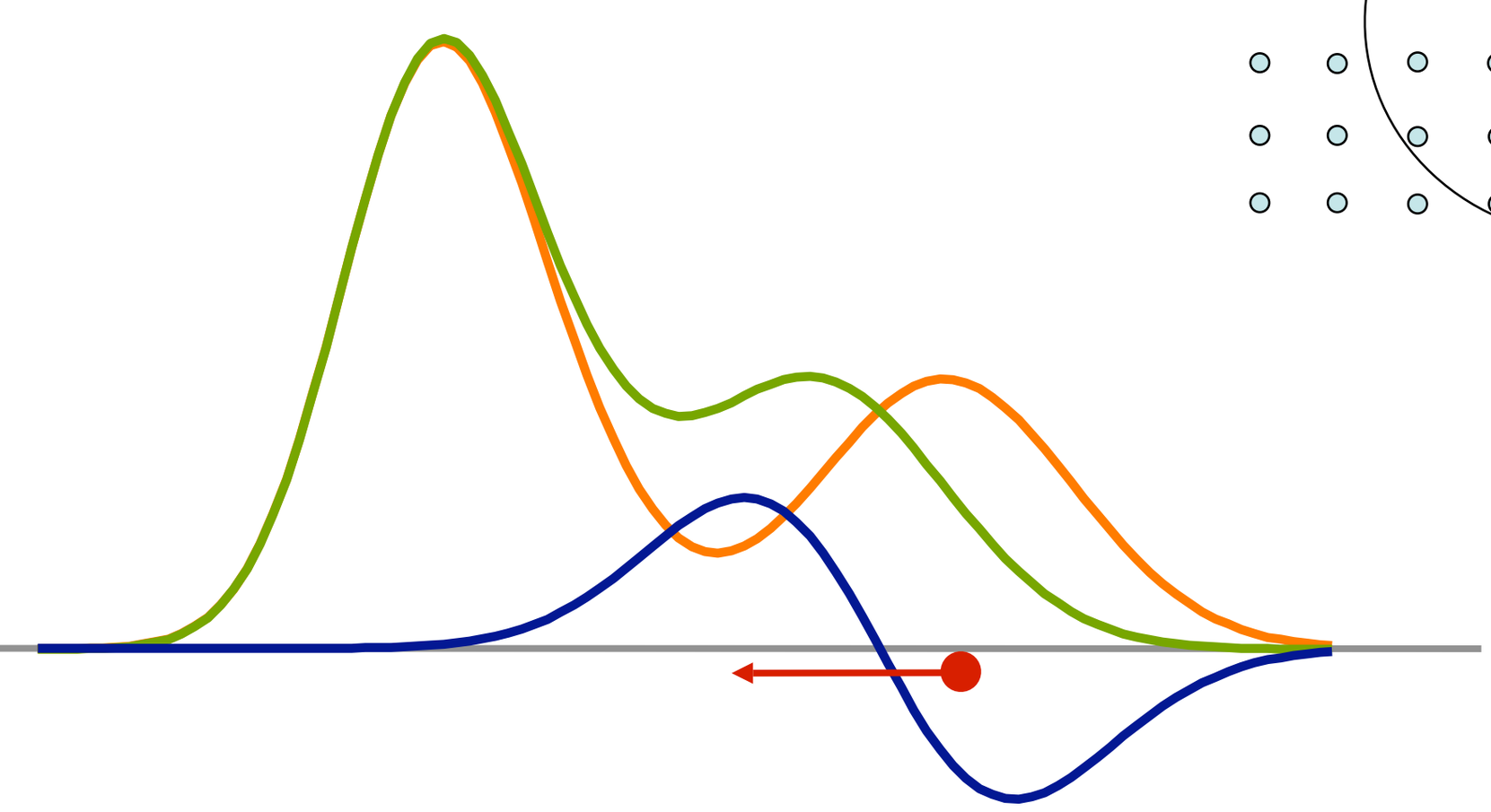


Random walk through conformational space
while maintaining correct stereochemistry
and avoiding atom clashes

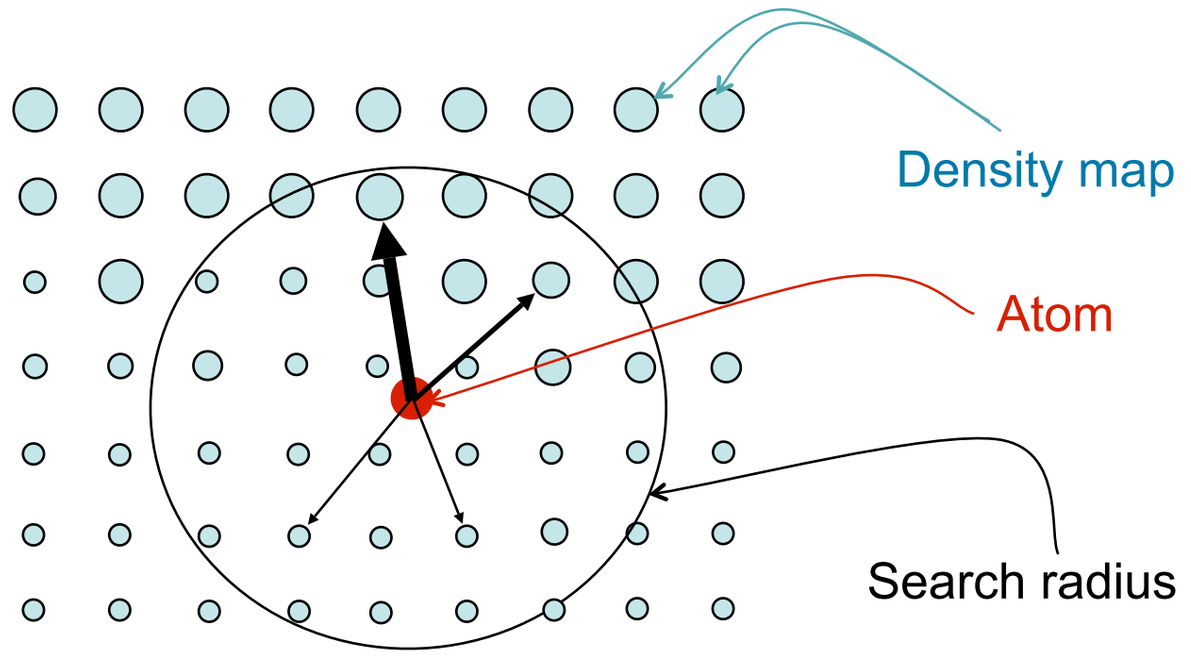
DireX: Forces derived from a density map

$$\rho_{difference}(x) = \rho_{target}(x) - \lambda\, \rho_{model}(x)$$

Target density
Model density
Difference



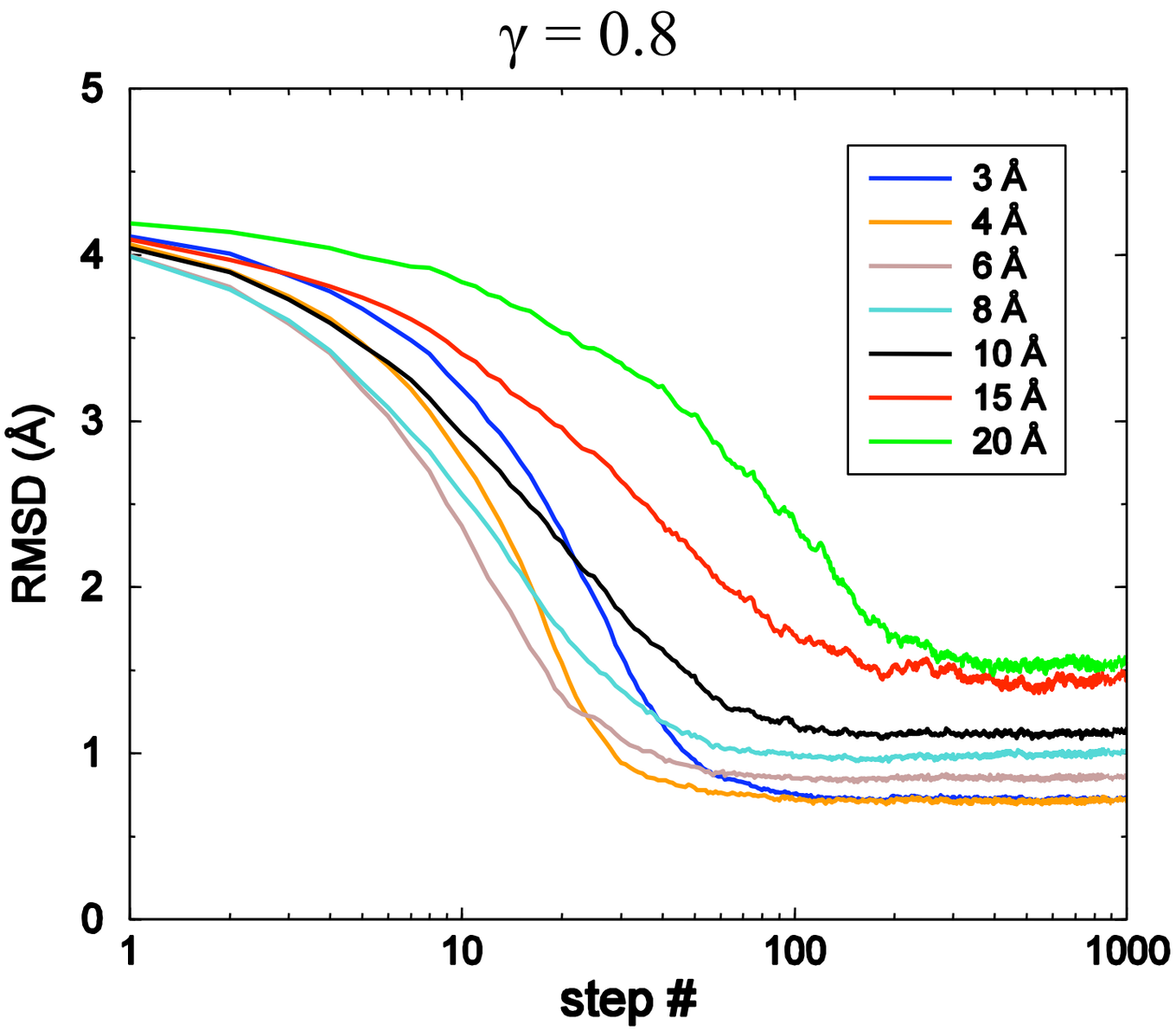
Stochastic gradient to move atoms into high difference-density regions

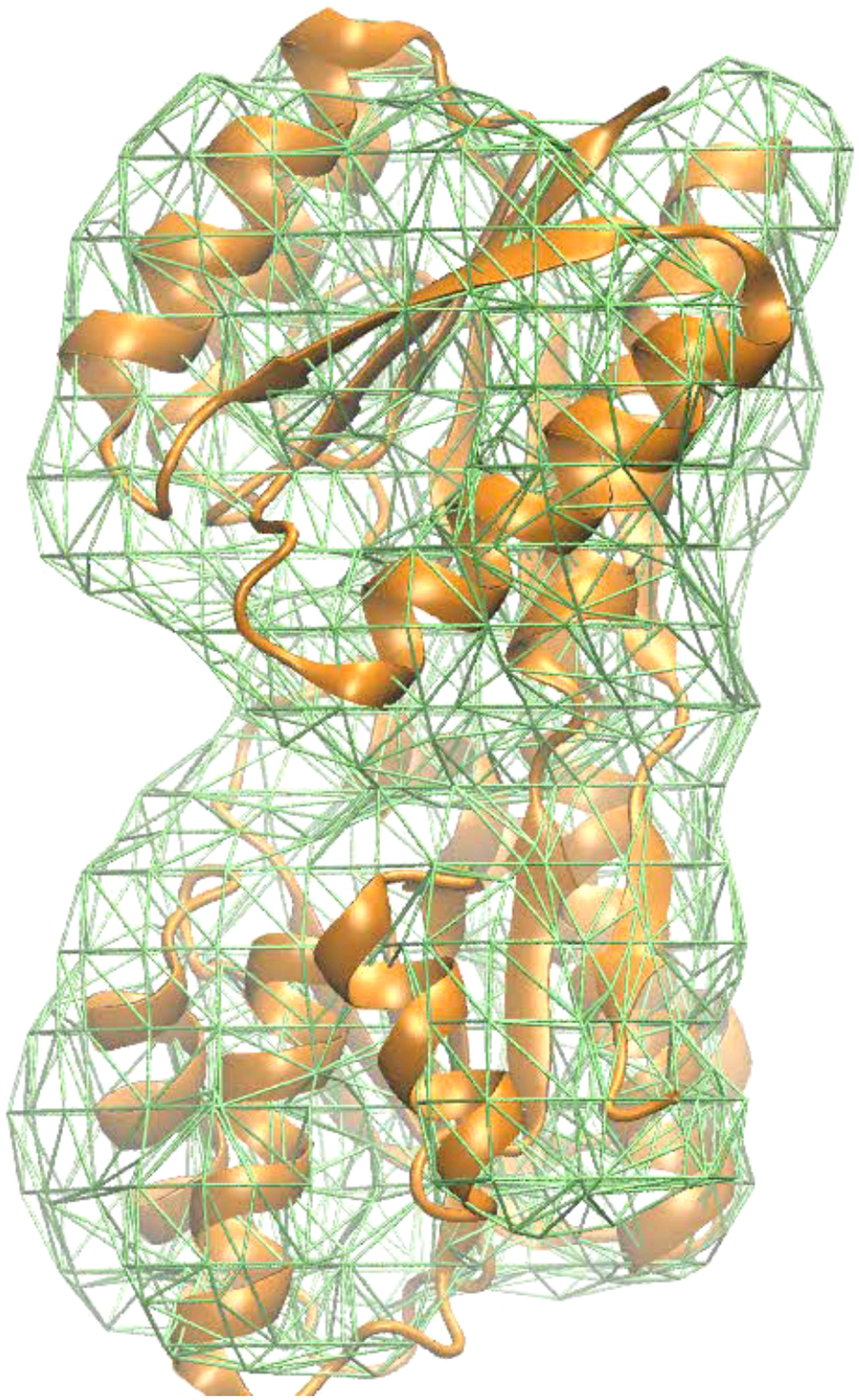


For each atom:
average over 10 randomly chosen vectors weighted by density difference

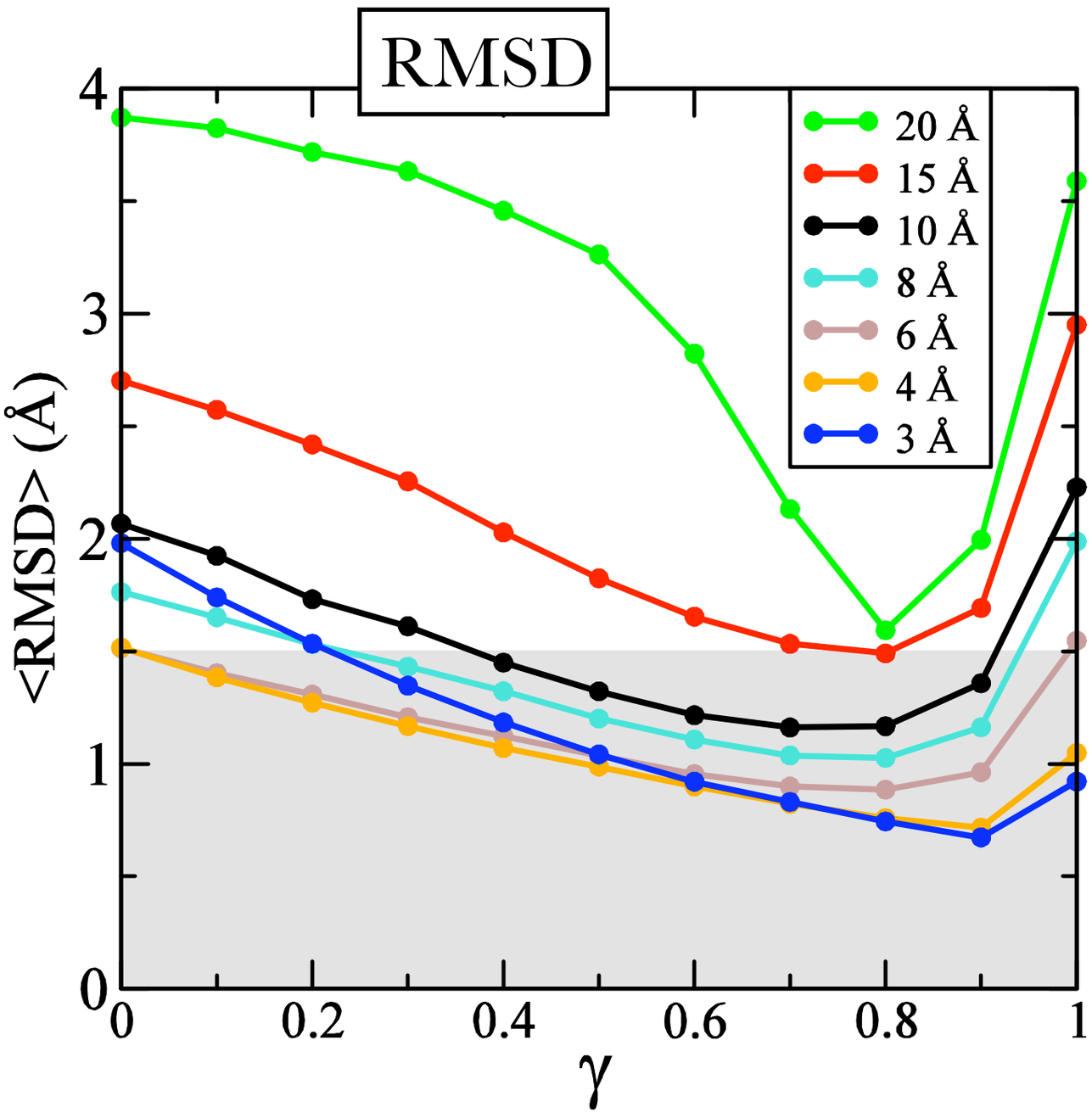
Test system: Ribose-binding protein

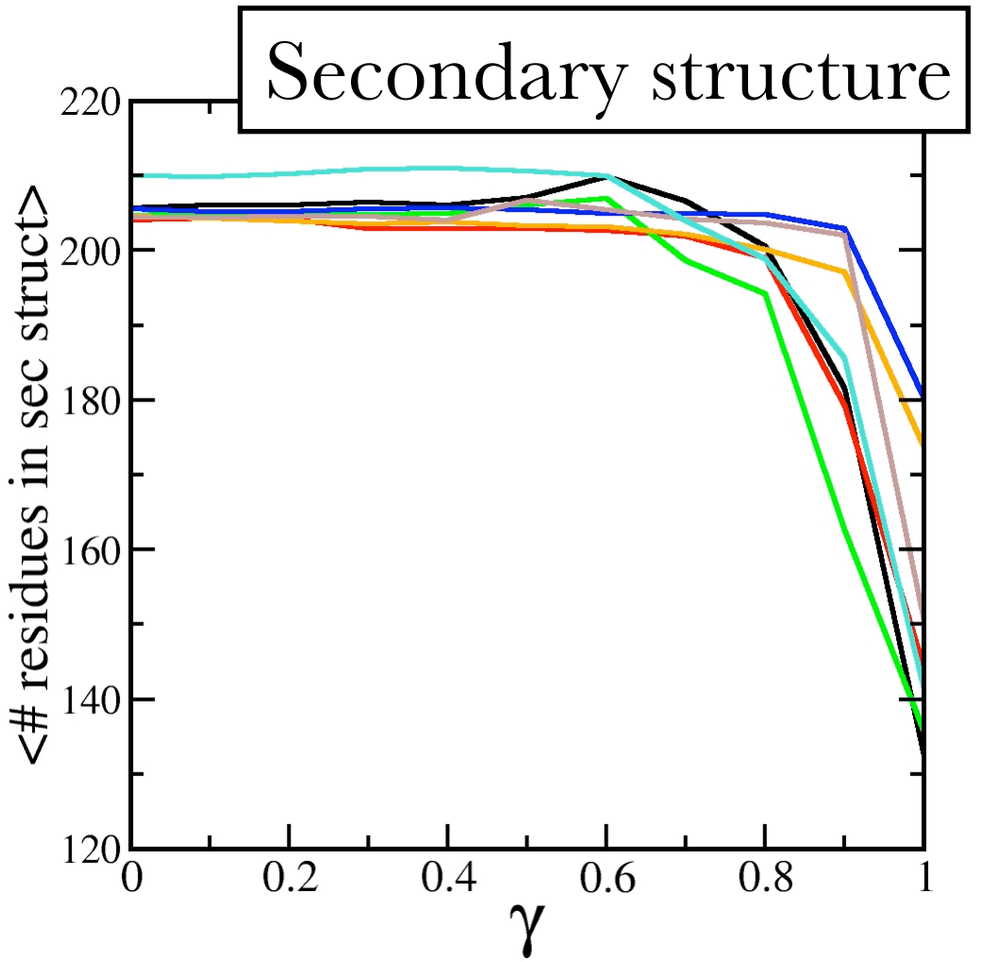
DEN





Deformable Elastic Network: Effect of γ





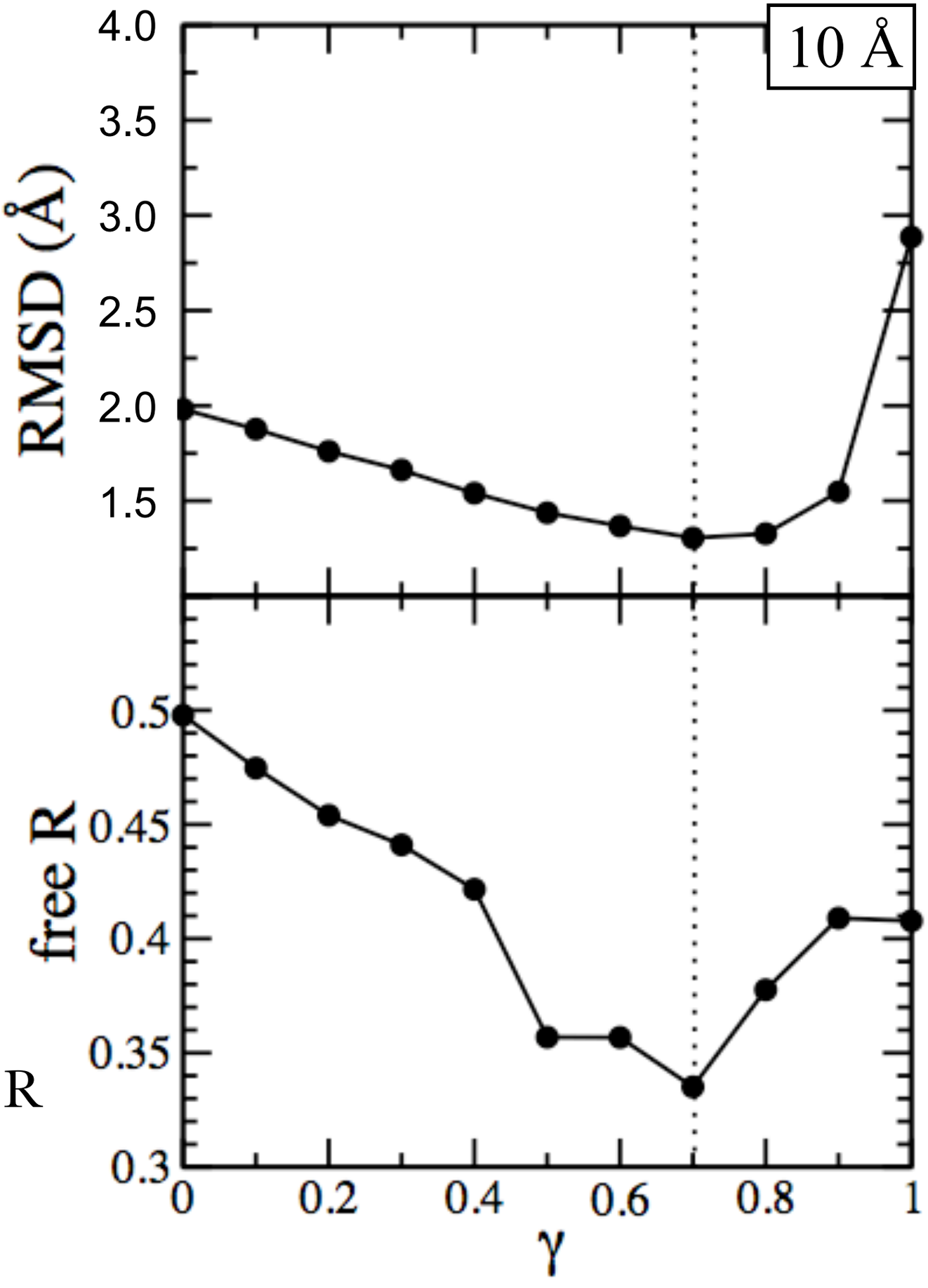
Cross-validation

. 10% of the structure factors in the 'test set'

$$R = \frac{\sum \left| |F_{obs}| - |F_{calc}| \right|}{\sum |F_{obs}|}$$

R_{free}

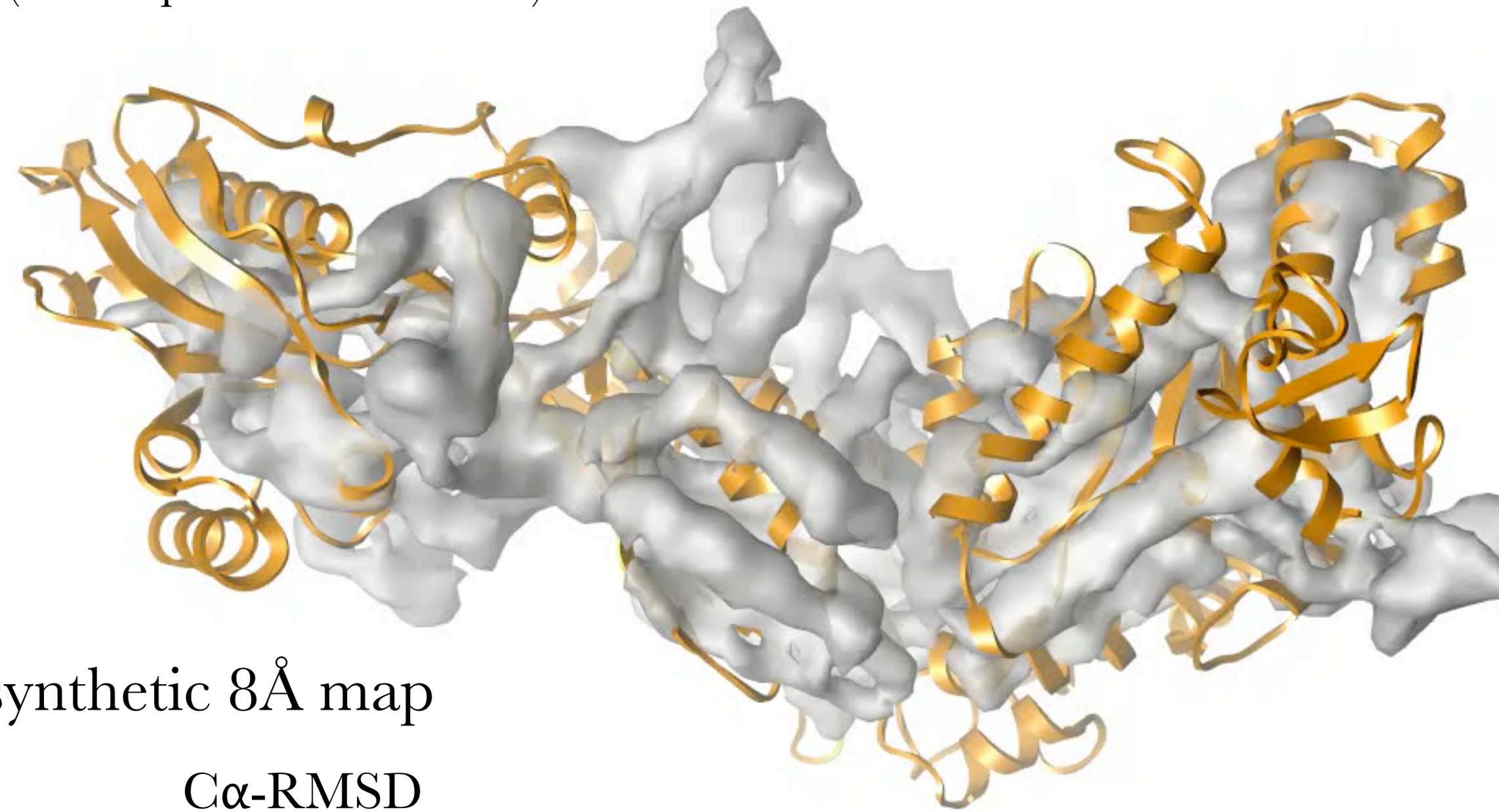
. Minimum of RMSD can be determined from minimum of free R



DireX

Example: Elongation Factor 2 (EF-2)

(will be part of the tutorial)



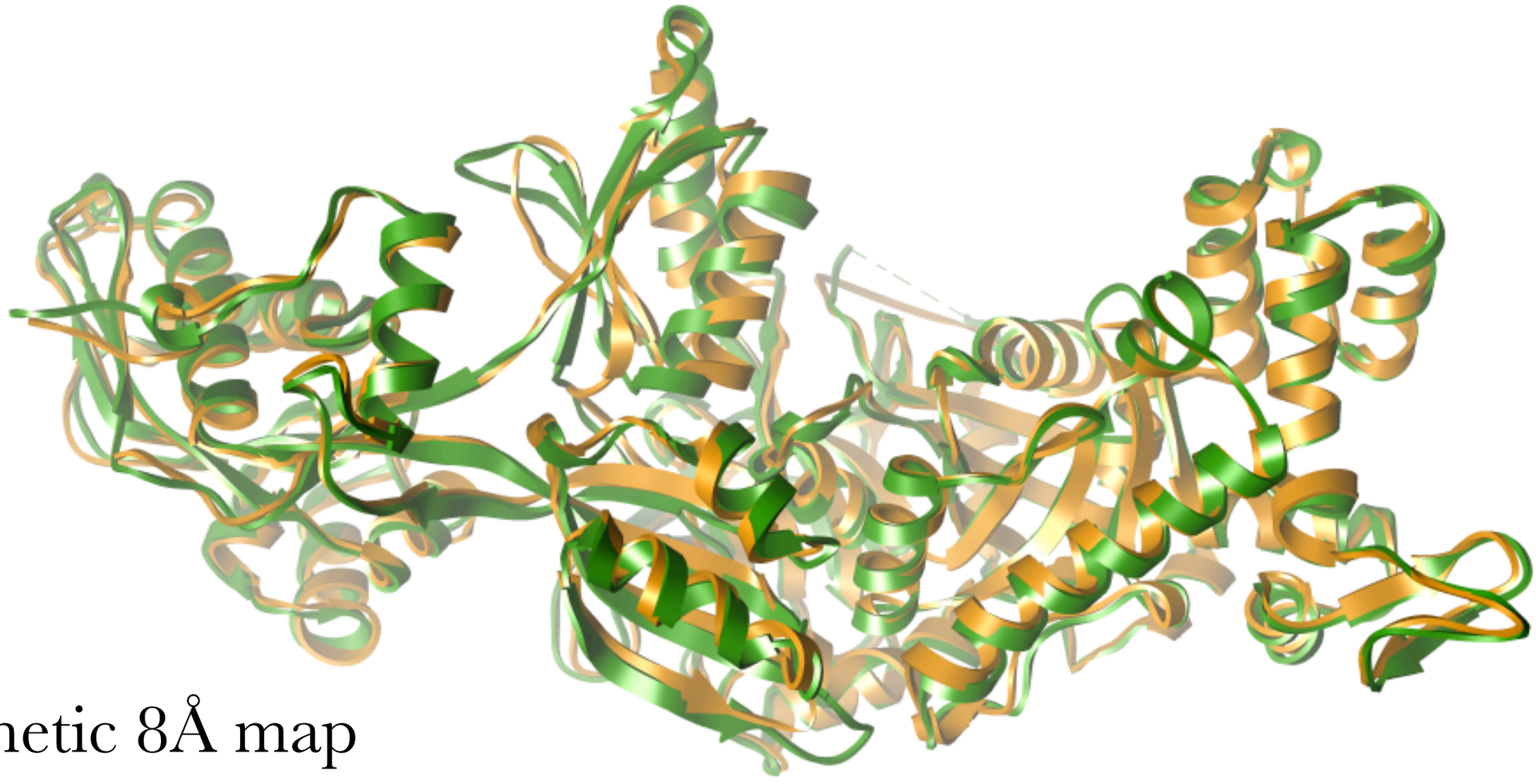
synthetic 8Å map

	Cα-RMSD
initial	13.6 Å
final	0.8 Å

6300 atoms, 14 steps/min
(3.5 hrs for 3000 steps)

DireX

Example: Elongation Factor 2 (EF-2)



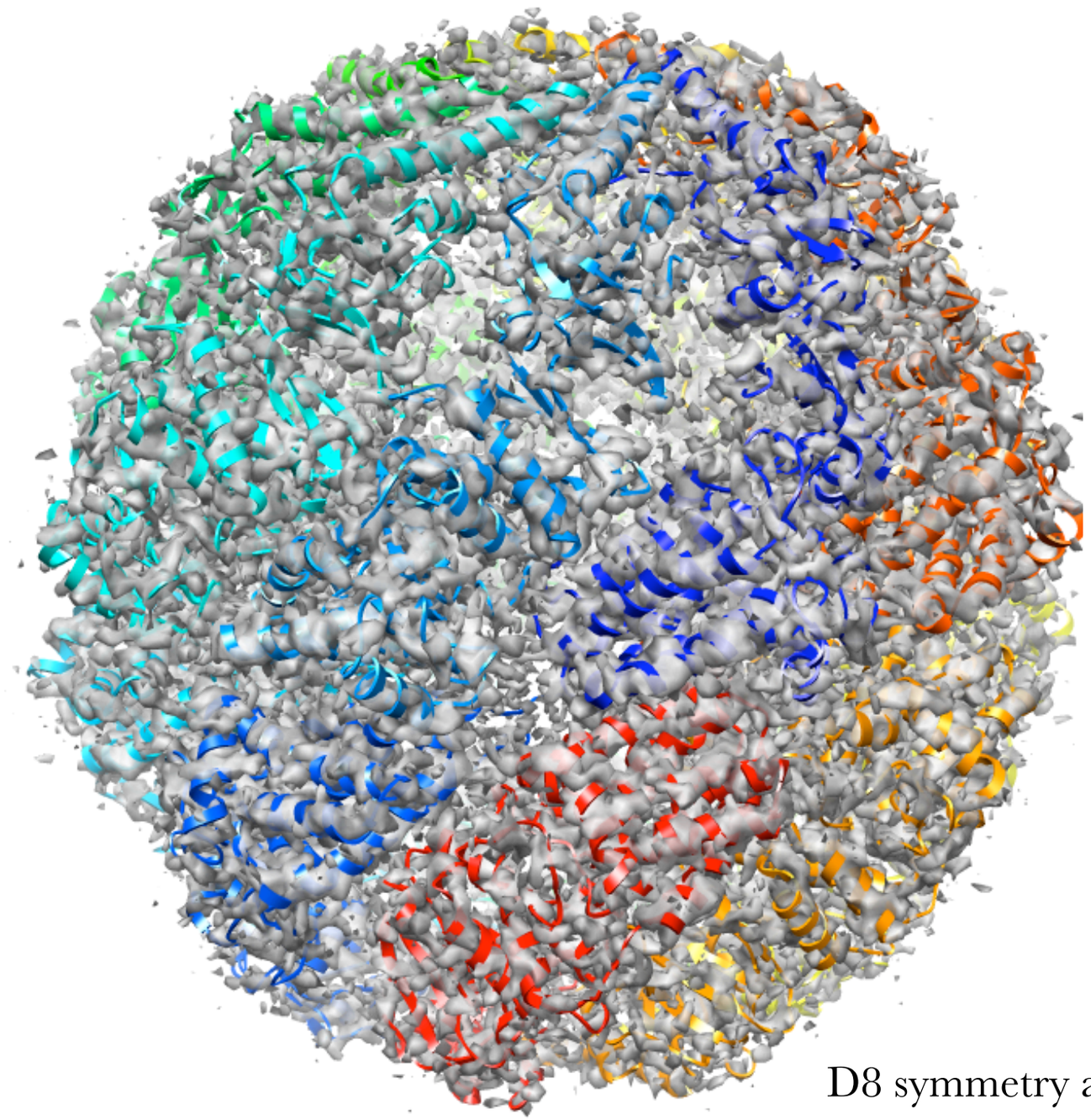
synthetic 8Å map

	Cα-RMSD
initial	13.6 Å
final	0.8 Å

6300 atoms, 14 steps/min
(3.5 hrs for 3000 steps)

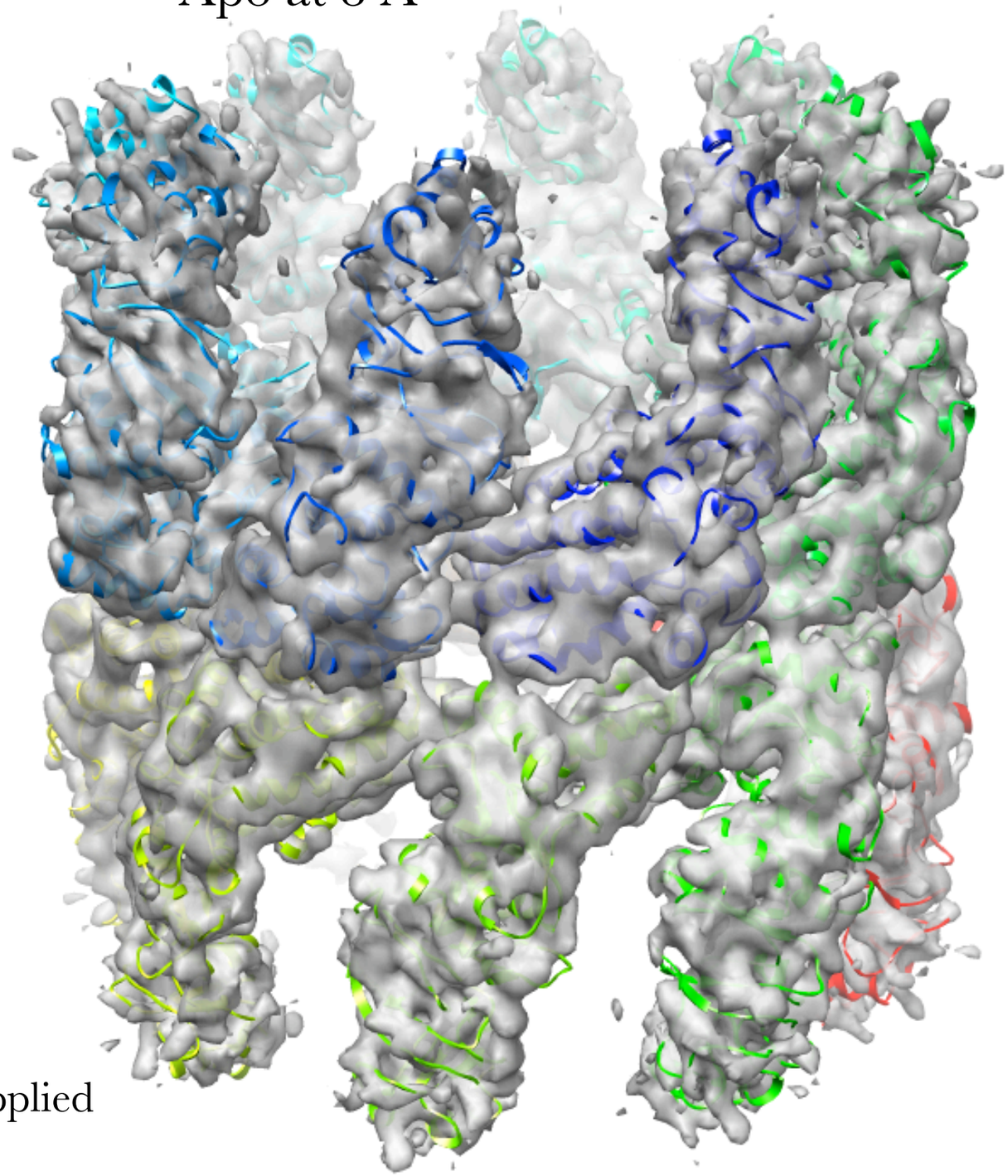
Mm-cpn
Closed conformation

ATP-AlFx at 4.3 Å



Mm-cpn
Open conformation

Apo at 8 Å



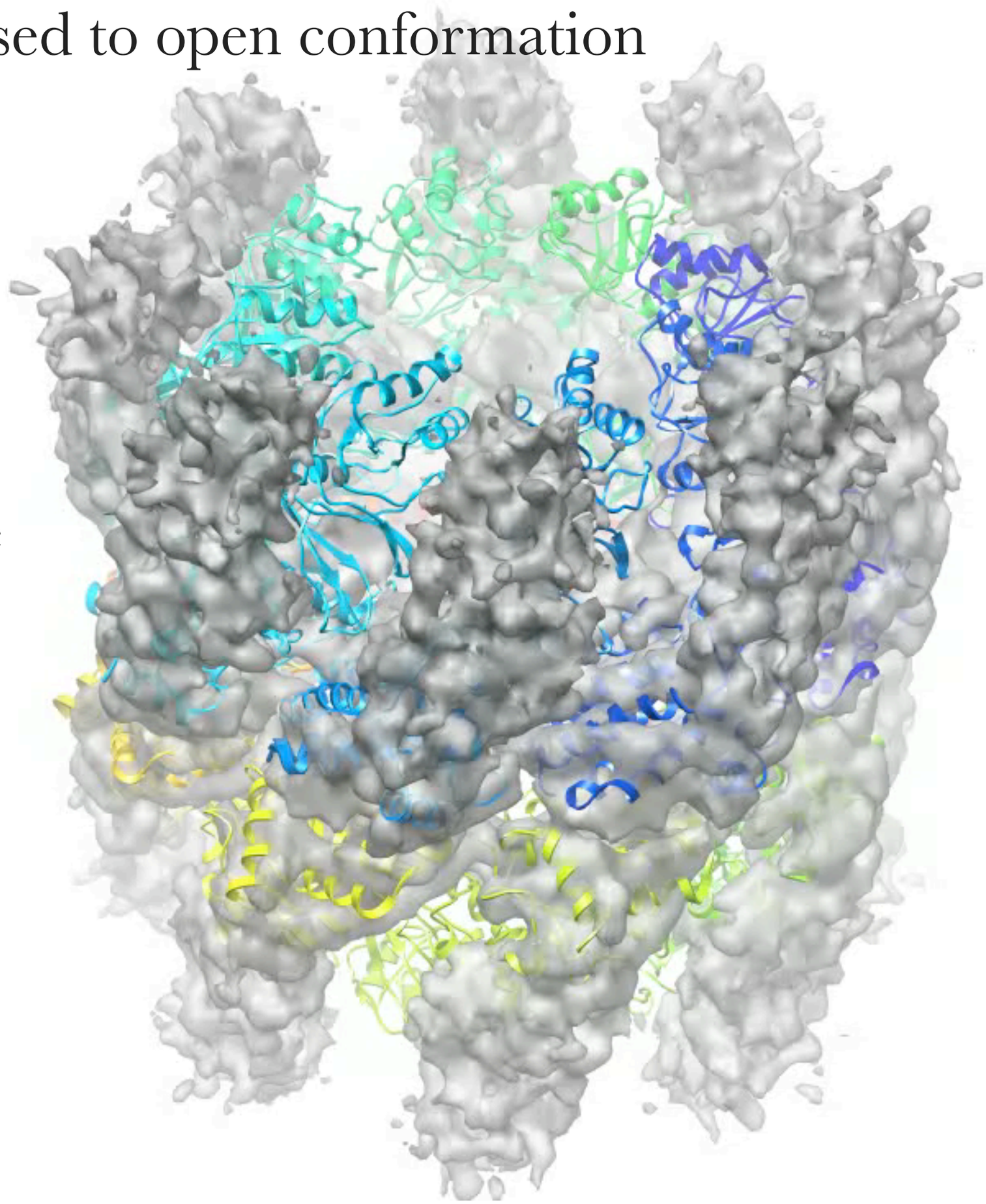
D8 symmetry applied

Zhang, et al., *Nature*, accepted.

Transition from closed to open conformation

Mm-cpn

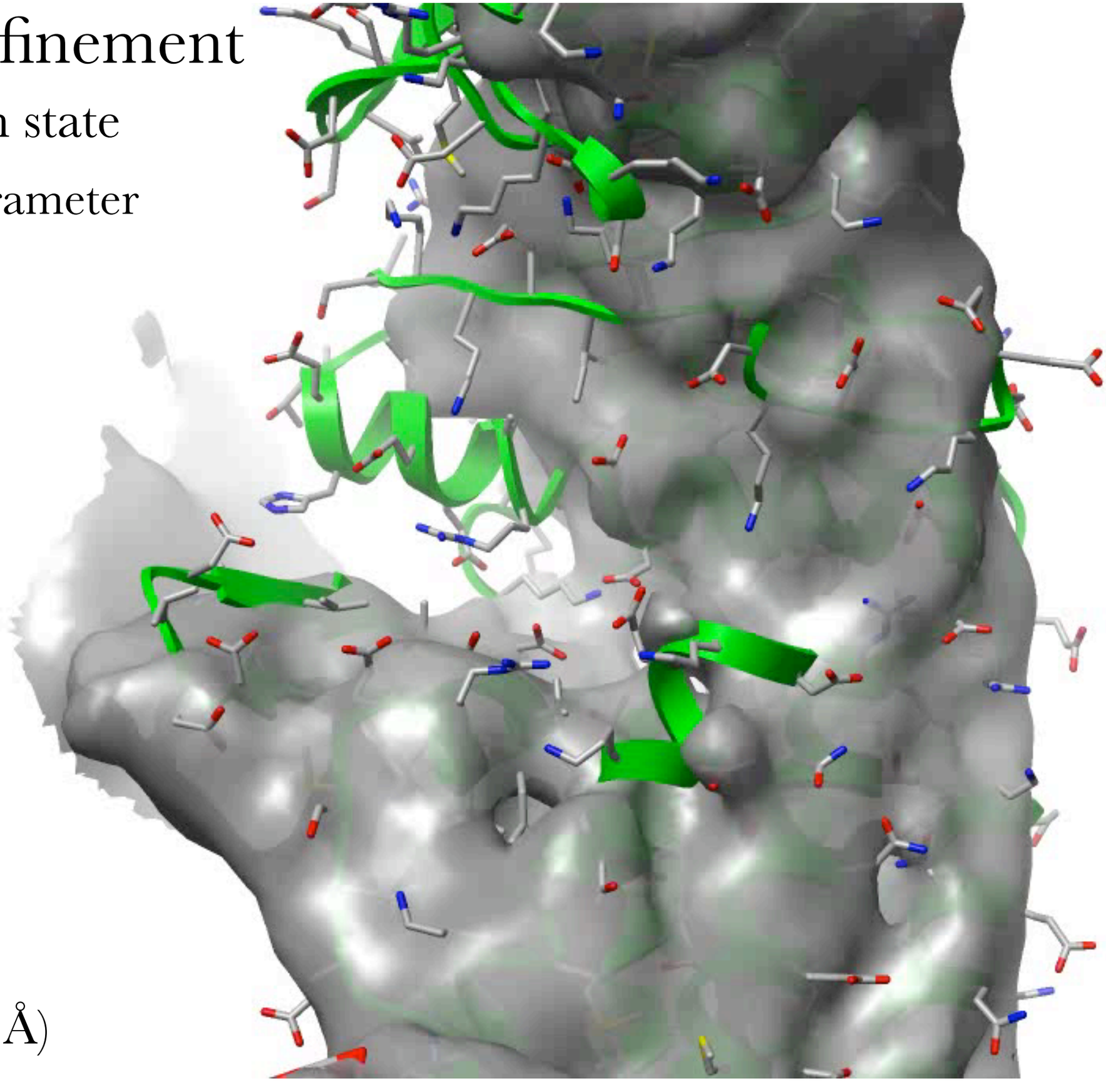
First step of
DireX refinement
using non-deformable
network ($\gamma = 0$)



DireX/DEN Refinement

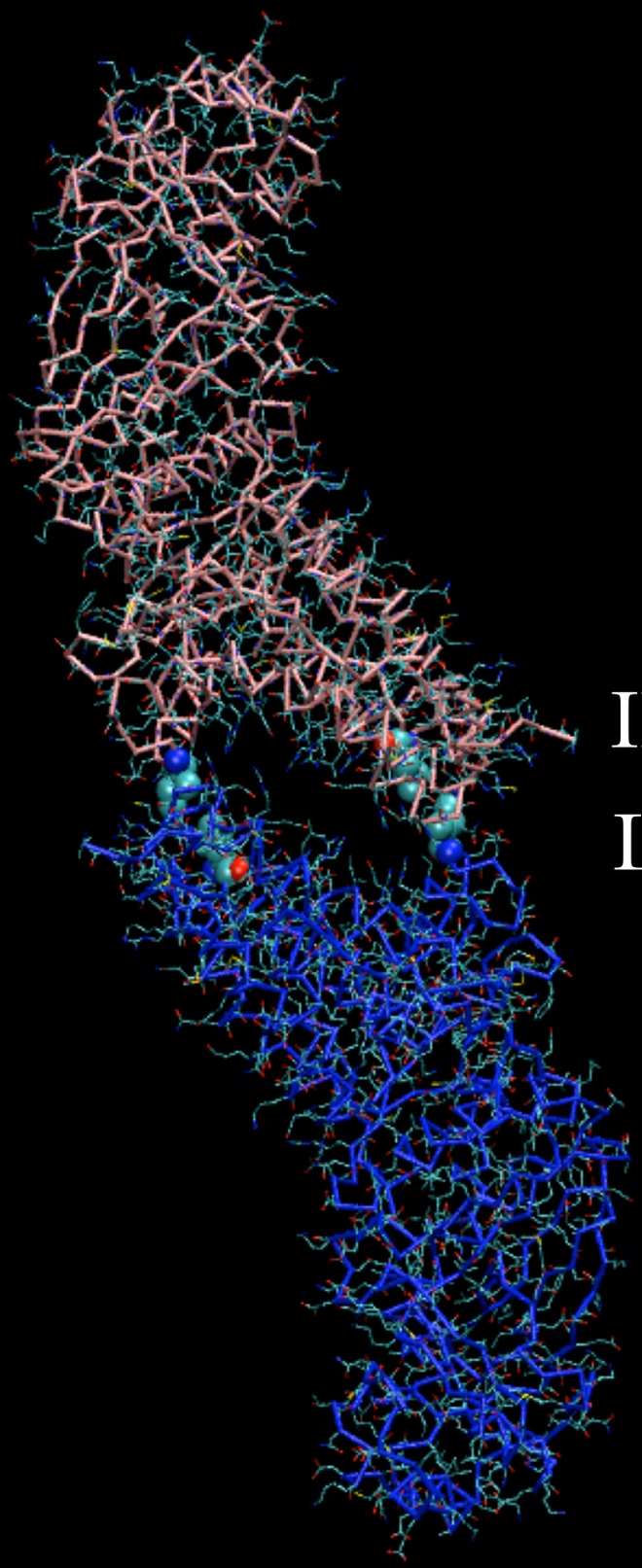
of Mm-cpn into open state

using optimized γ -parameter



Apo (8 Å)

Hydrophic inter-ring contact
forms a hinge

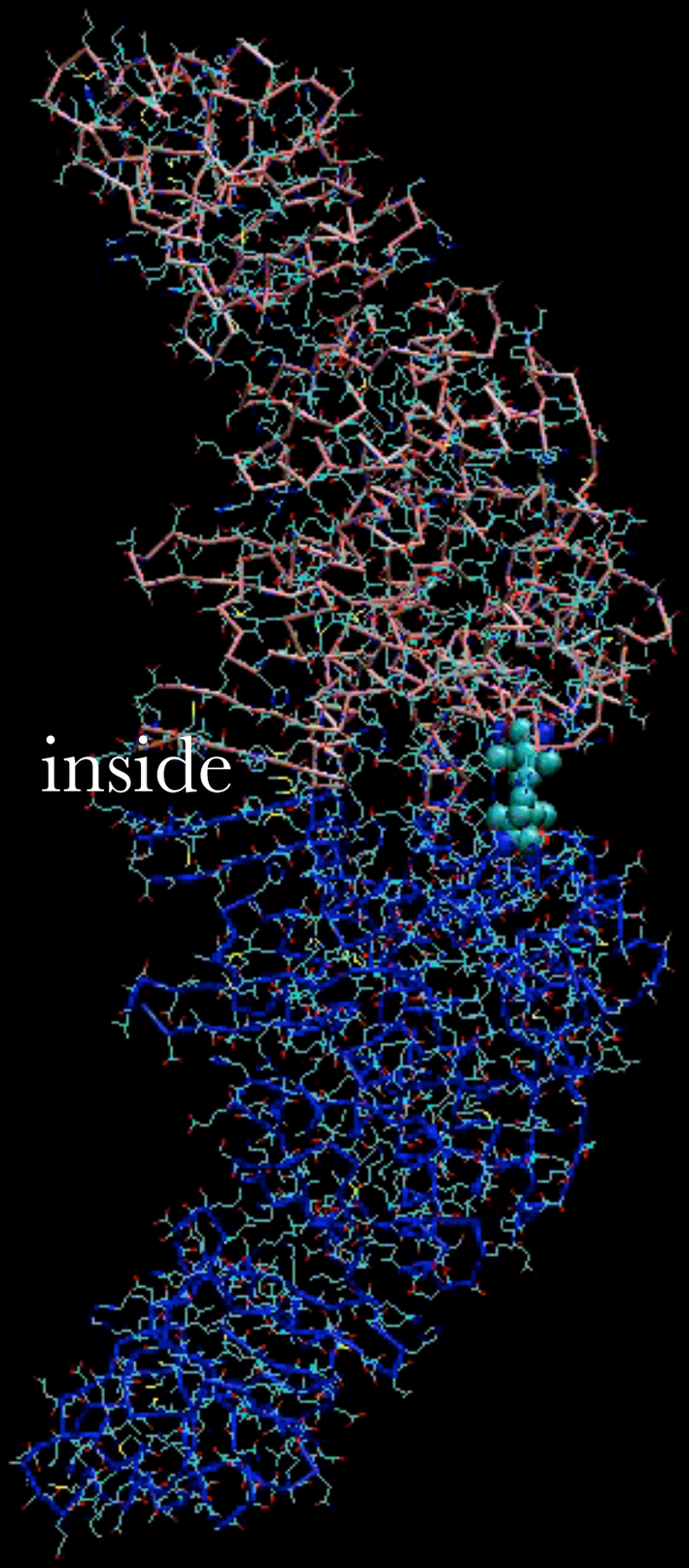


90°

ILE

LEU

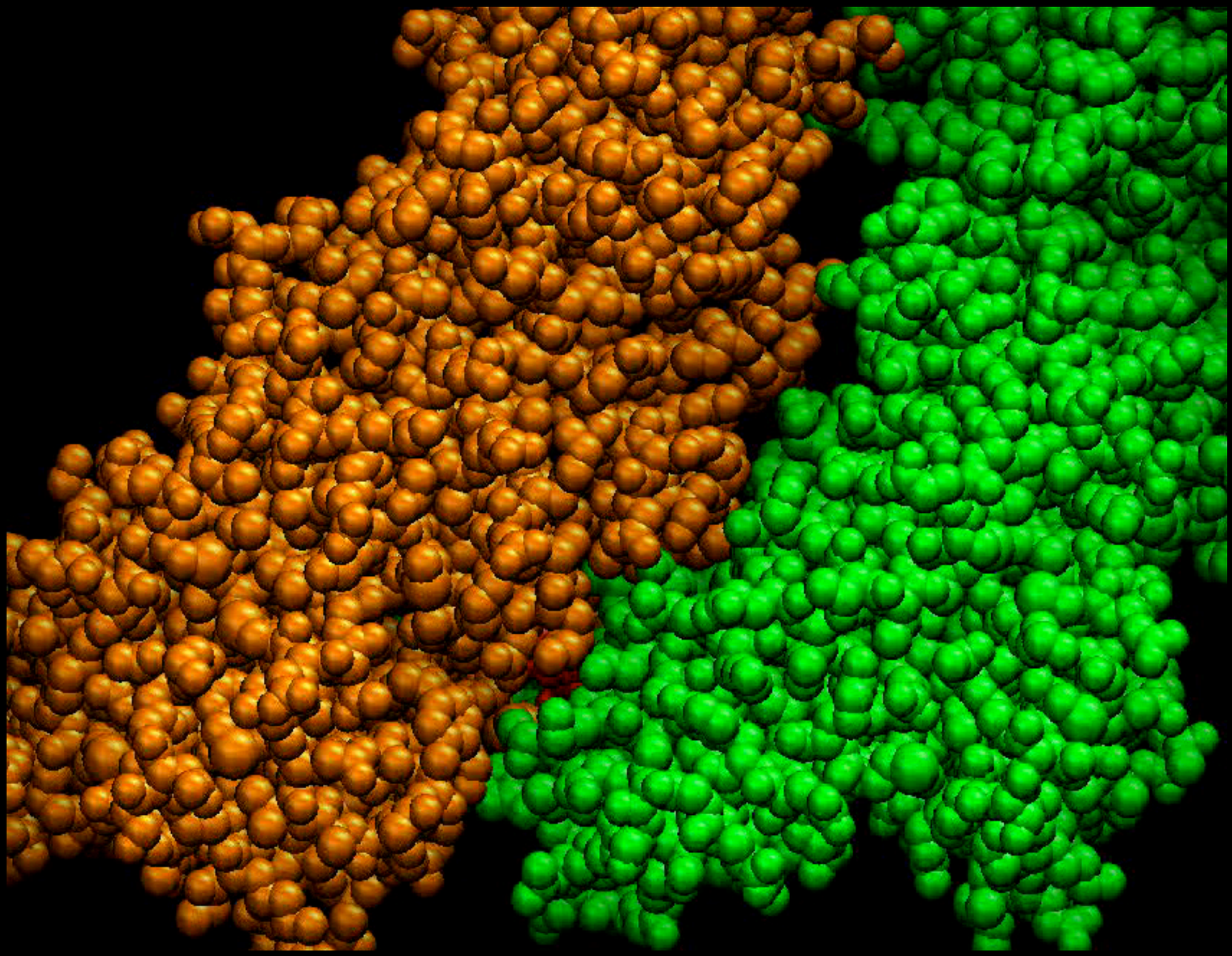
inside



outside

Intra-ring contact

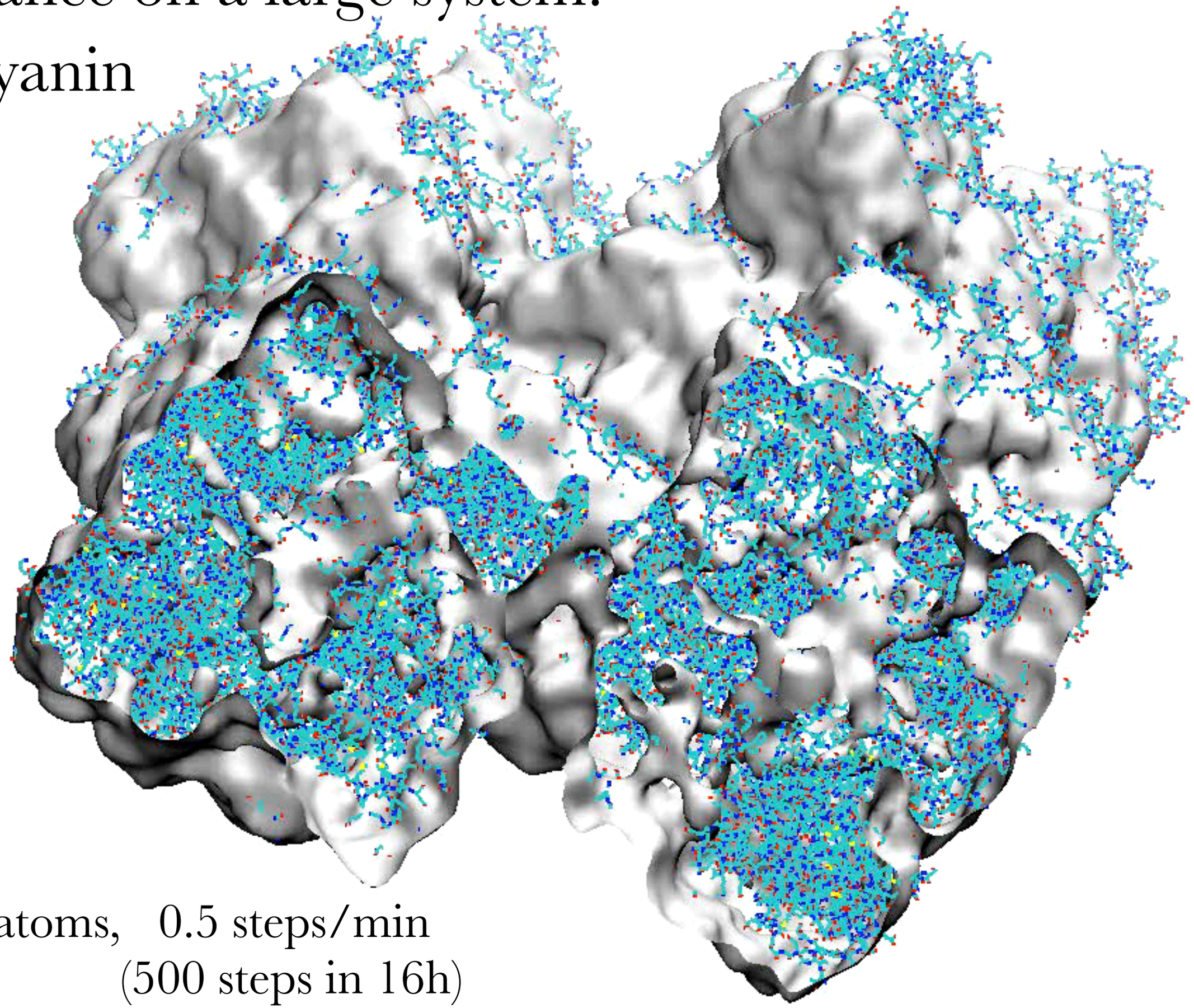
- Arg489



Performance on a large system:

Hemocyanin

at 9 Å



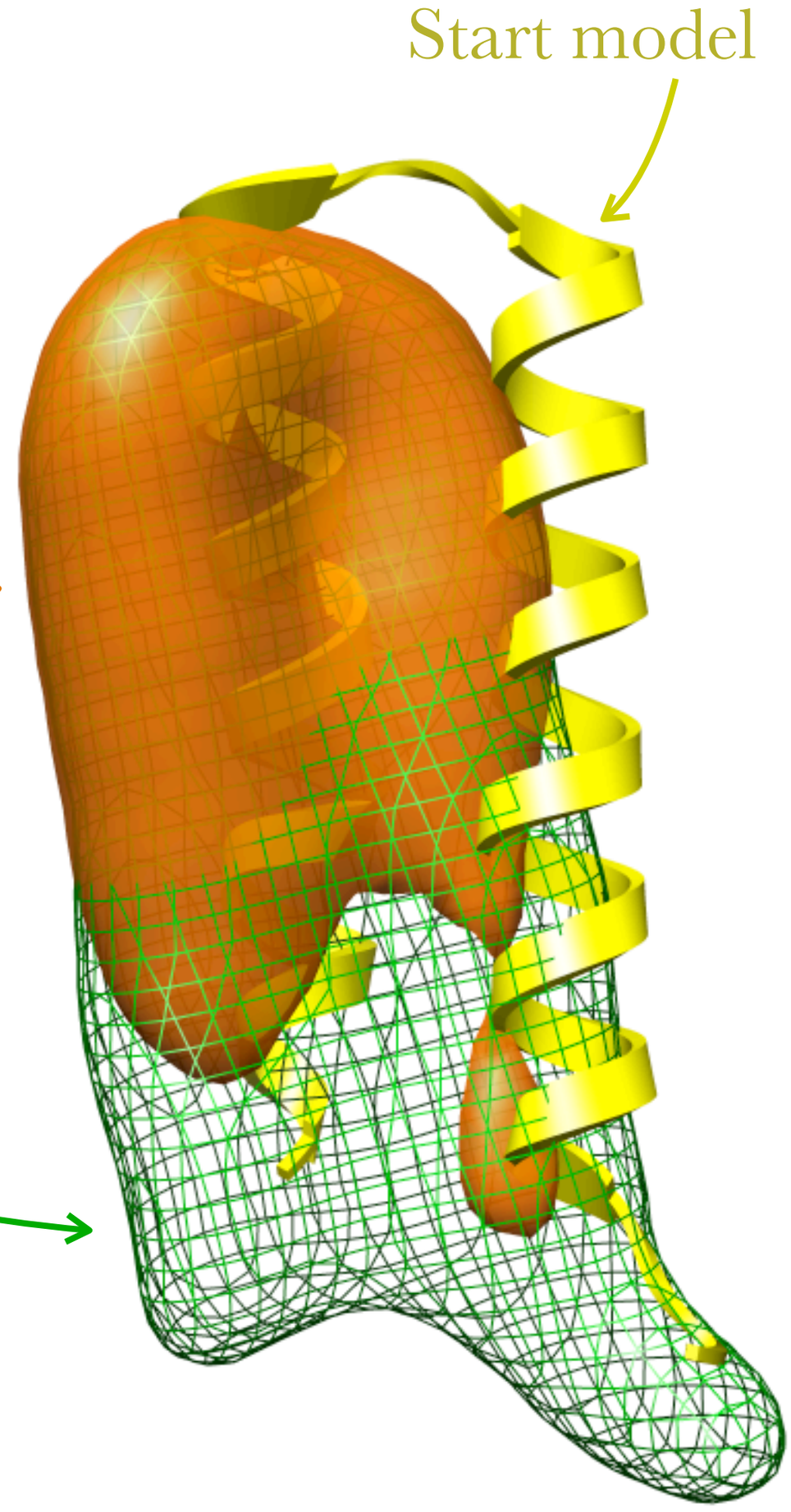
120.000 atoms, 0.5 steps/min

(500 steps in 16h)

“B-factor” Refinement

implemented in DireX.

In fact, we are refining the occupancy, i.e. atomic density is scaled by a factor [0..1]



Test with synthetic data at 10Å

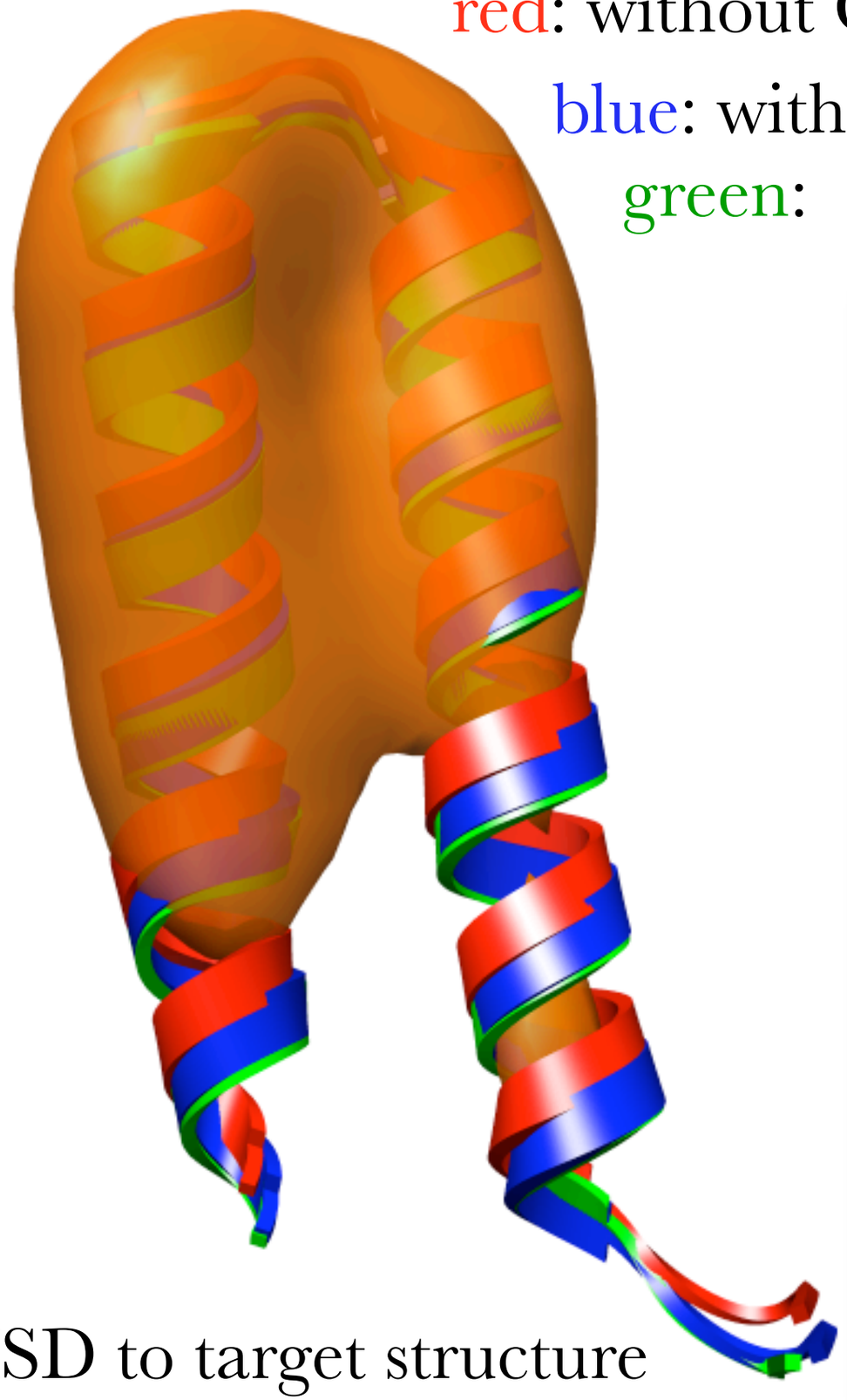
map computed from target model with modified occupancy values

map computed from full target model

red: without Occ refinement the structure is shifted

blue: with Occ refinement

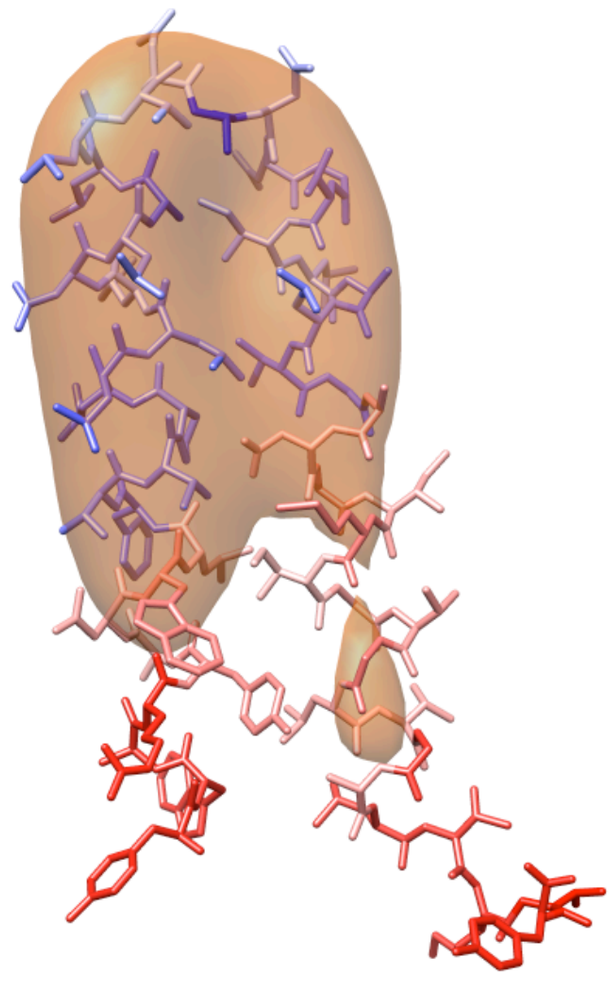
green: target (correct) structure



RMSD to target structure

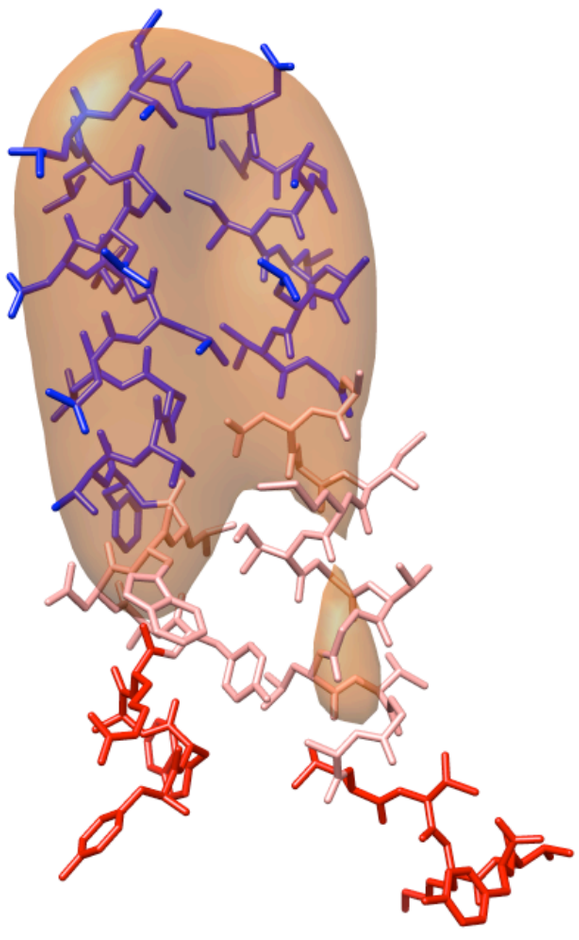
1.6 Å without Occ. refinement

0.3 Å with Occ. refinement



refined occupancy values

Map correlation 0.93



input occupancy values

Map correlation 0.99

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DFG

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To download DireX and Tutorial files:

www.simtk.org/home/direx