Molecular Dynamics Flexible Fitting

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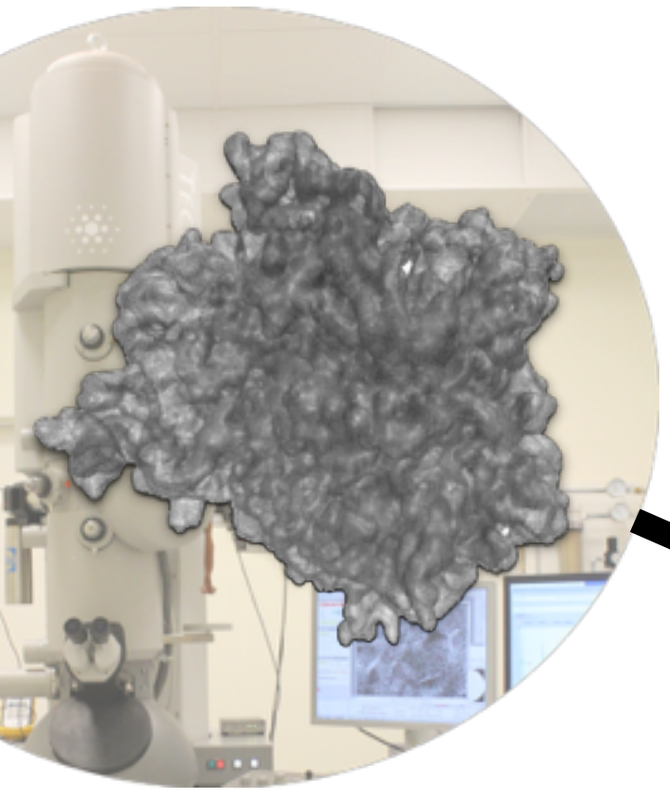
NIH Resource for Macromolecular Modeling and Bioinformatics

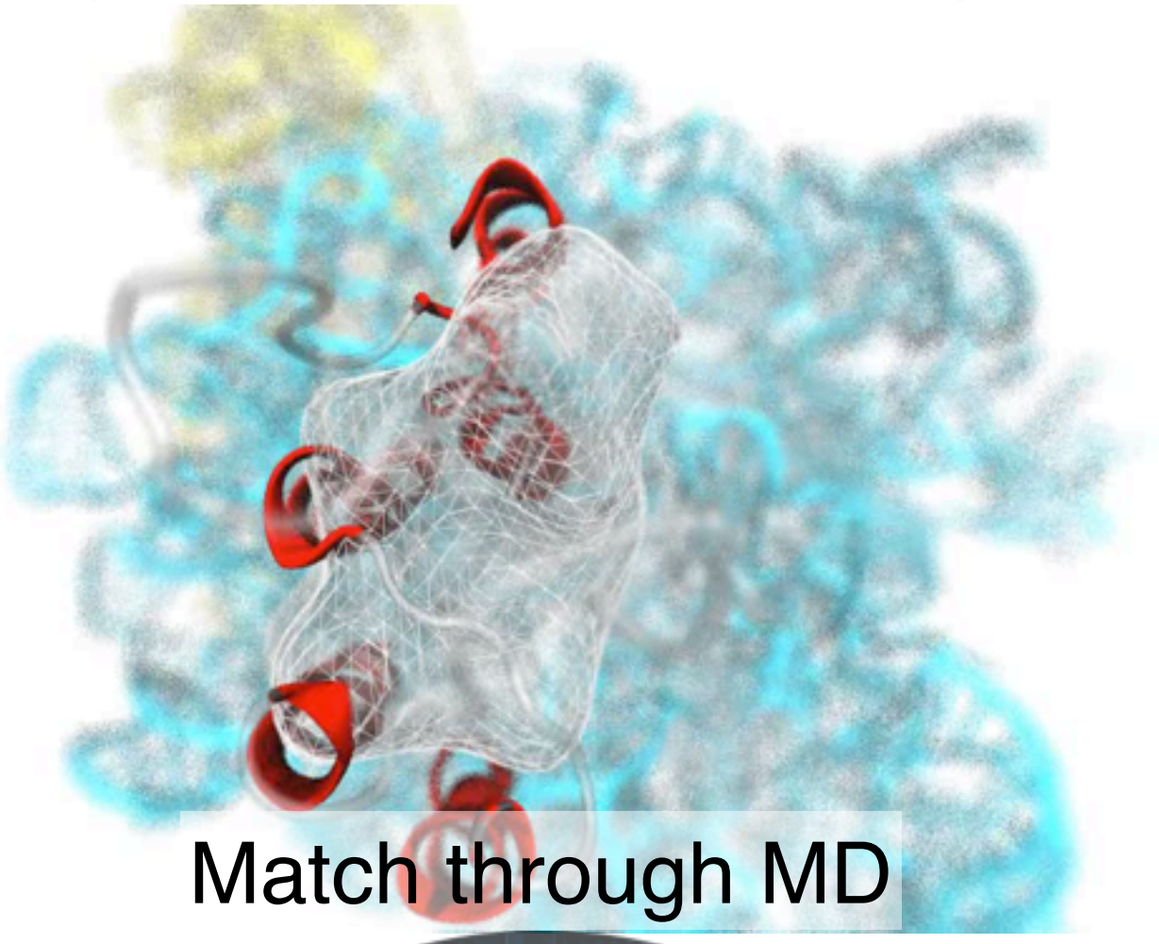
Molecular **D**ynamics **F**lexible **F**itting

(Ribosome-bound YidC)

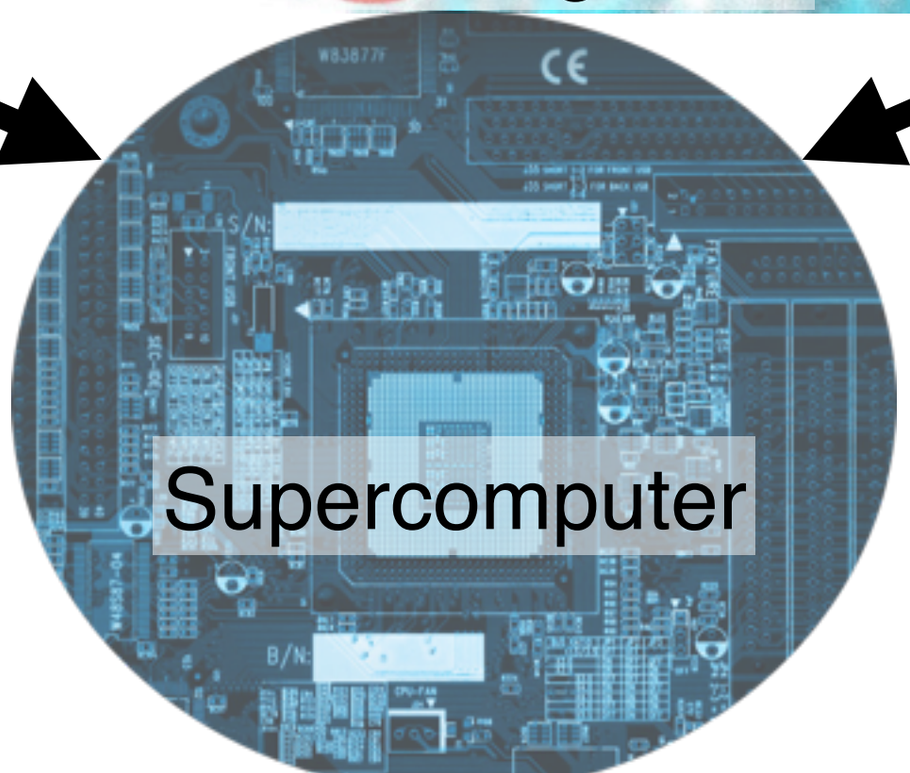
Electron Microscope



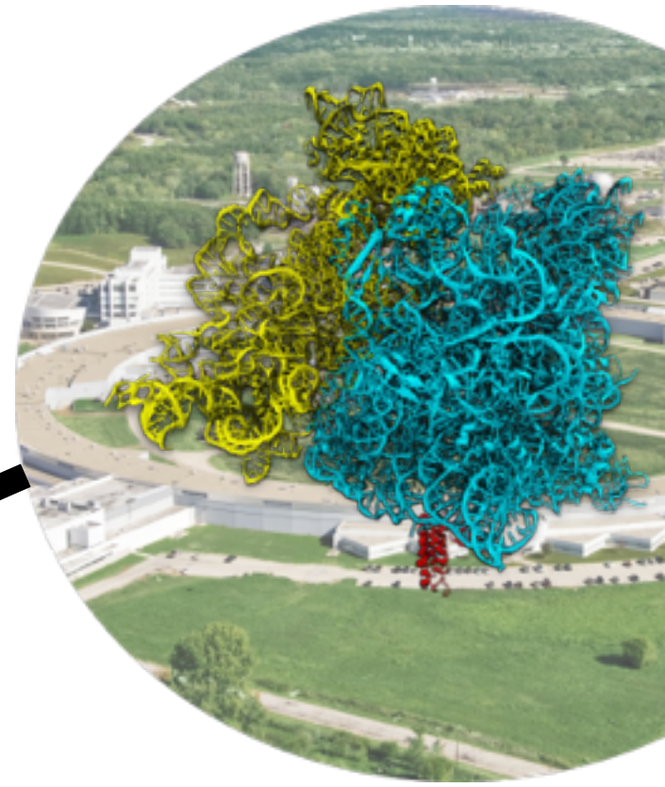
EM density map



Match through MD



APS Synchrotron



crystallographic structure

Molecular Dynamics Flexible Fitting - Theory

Two terms are added to the MD potential

$$U_{total} = U_{MD} + U_{EM} + U_{SS}$$

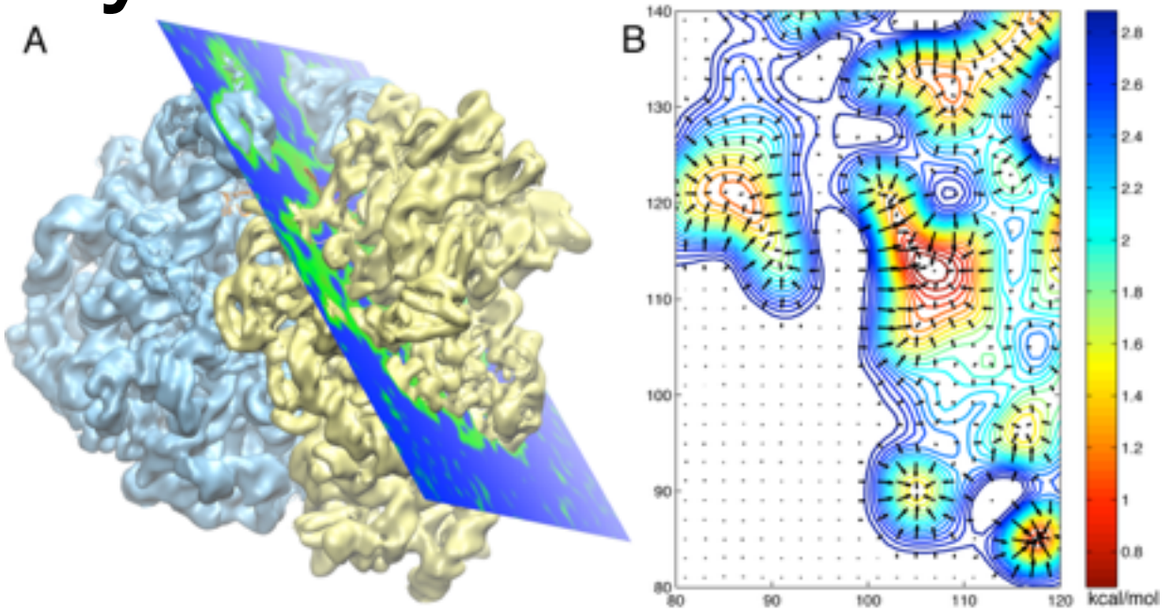
An external potential derived from the EM map is defined on a grid as

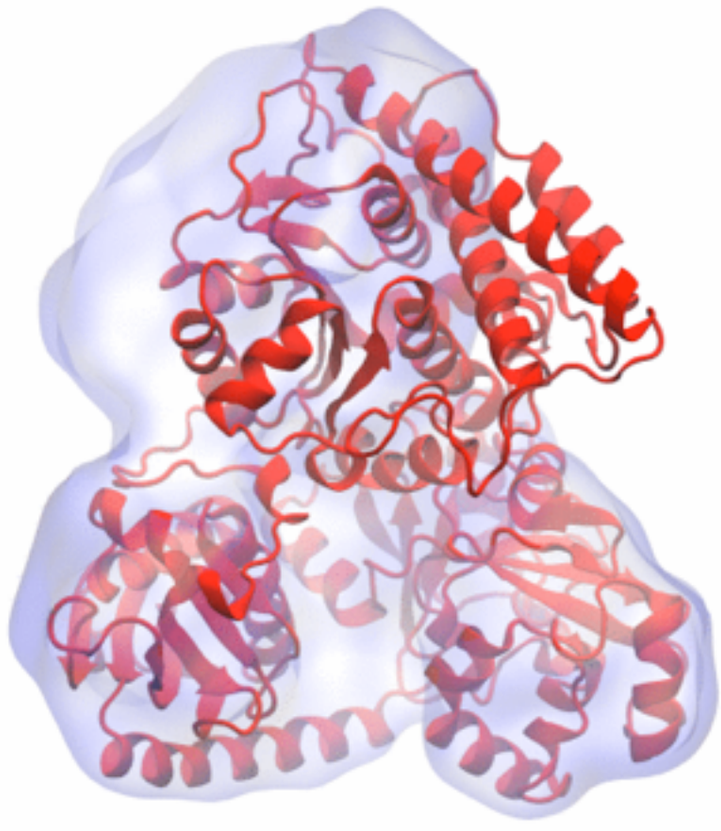
$$U_{EM}(\mathbf{R}) = \sum_j w_j V_{EM}(\mathbf{r}_j)$$

$$V_{EM}(\mathbf{r}) = \begin{cases} \xi \left(1 - \frac{\Phi(\mathbf{r}) - \Phi_{thr}}{\Phi_{max} - \Phi_{thr}}\right) & \text{if } \Phi(\mathbf{r}) \geq \Phi_{thr}, \\ \xi & \text{if } \Phi(\mathbf{r}) < \Phi_{thr}. \end{cases}$$

A mass-weighted force is then applied to each atom

$$\mathbf{f}_i^{EM} = -\nabla U_{EM}(\mathbf{R}) = -w_i \partial V_{EM}(\mathbf{r}_i)/\partial r_i$$





Acetyl – CoA Synthase

[1] Trabuco et al. *Structure* (2008) 16:673-683.

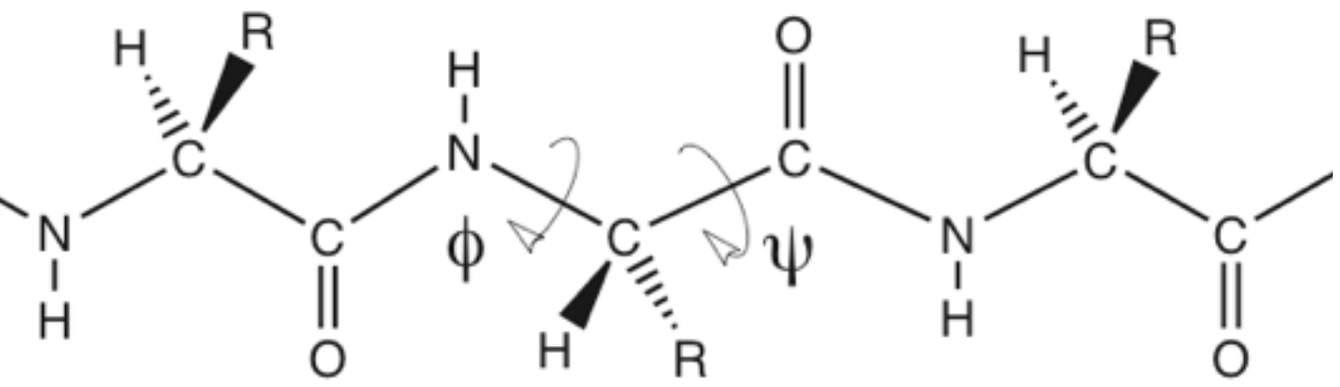
[2] Trabuco et al. *Methods* (2009) 49:174-180.

Secondary structure restraints

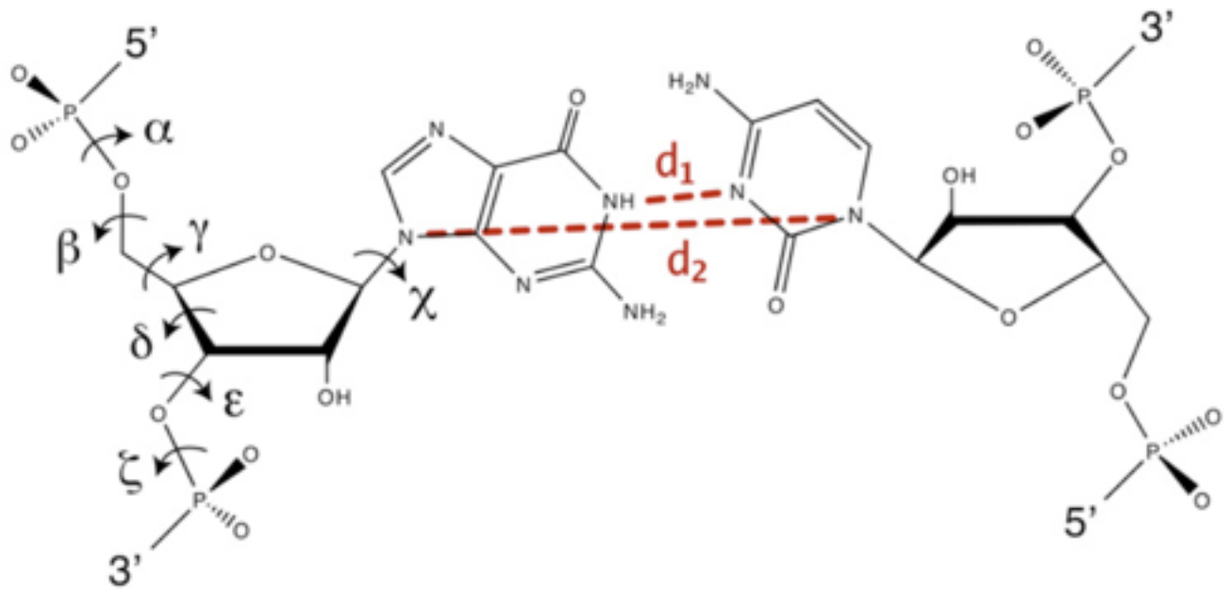
Harmonic restraints are applied to preserve secondary structure of proteins and nucleic acids, avoiding “overfitting.”

$$U_{SS} = \sum_{restraints} k_\mu(\mu - \mu_0)^2$$

For proteins, ϕ and ψ dihedral angles of residues within helices or beta strands are restrained.



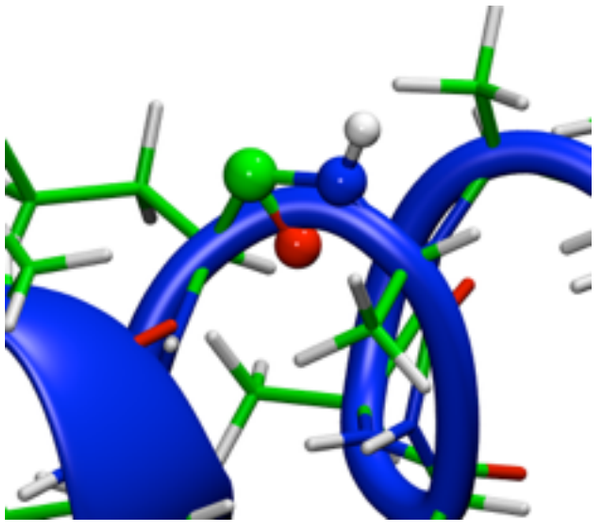
For nucleic acids, distance and dihedral restraints are applied to a selected set of base pairs.



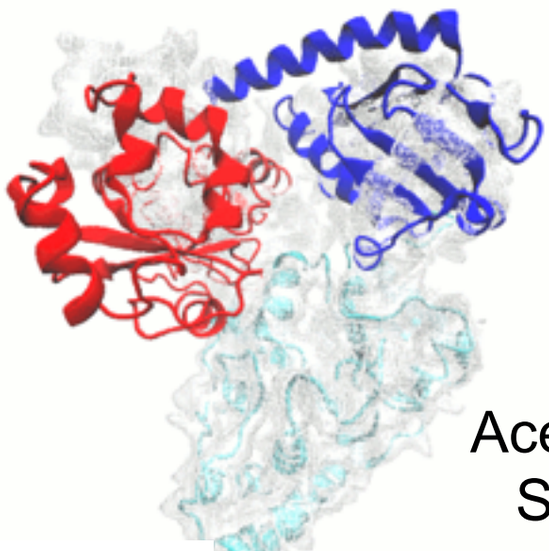
Additional Restraints

Cis-peptide and Chirality

Eduard Schreiner, et al. BMC Bioinformatics, 12, 190, 2011

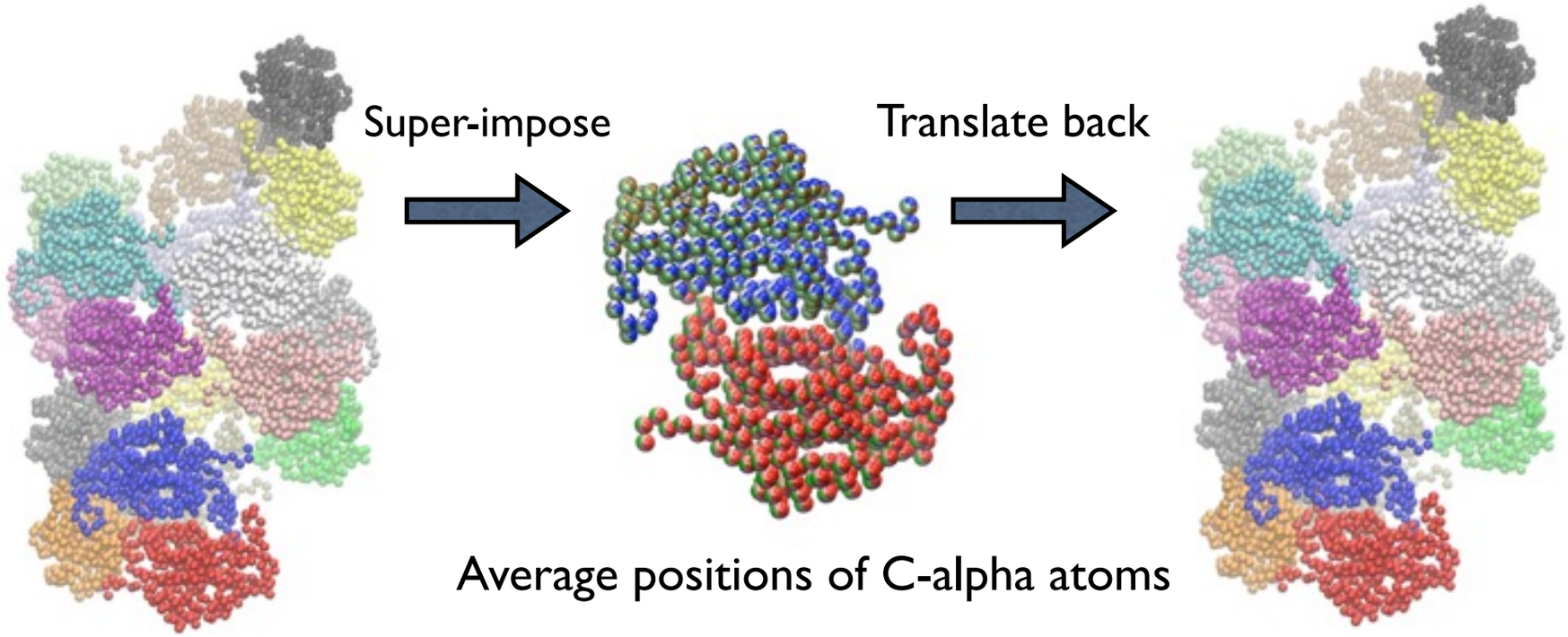


Domain-wise

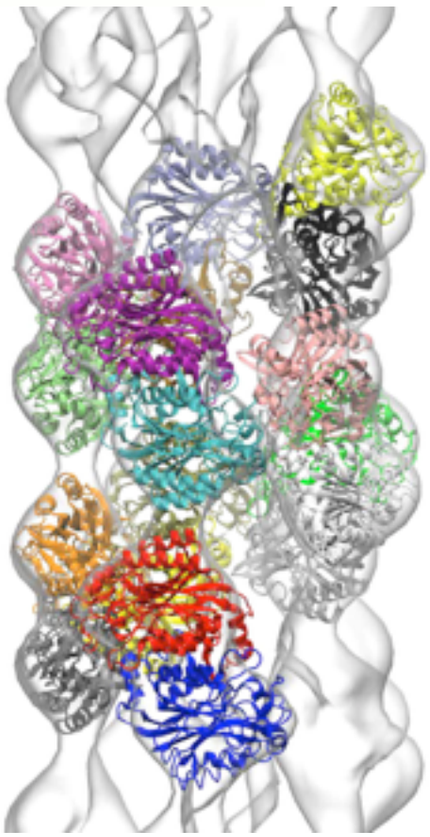


Acetyl – CoA Synthase

Symmetry



Kwok-Yan Chan, et al. Structure, 19, 1211-1218, 2011

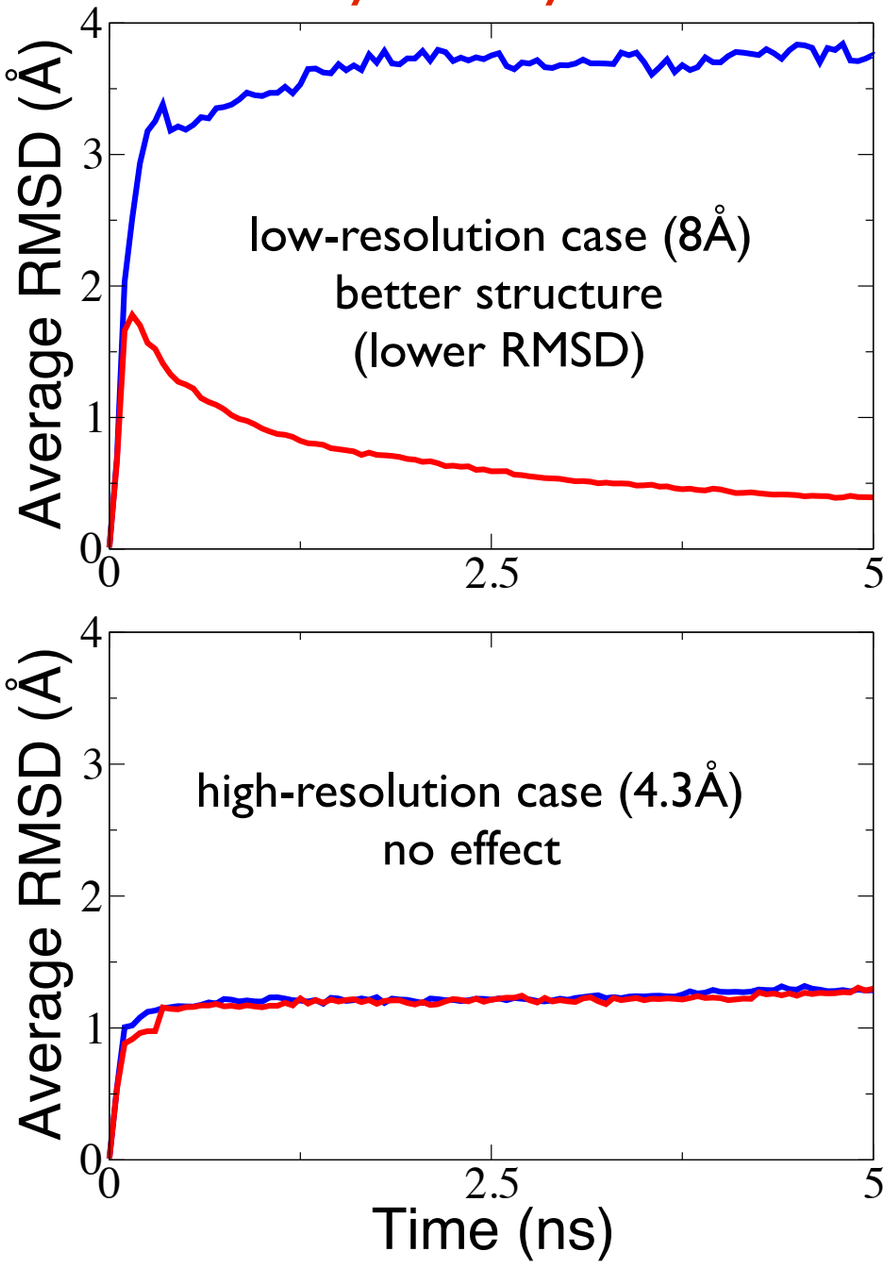


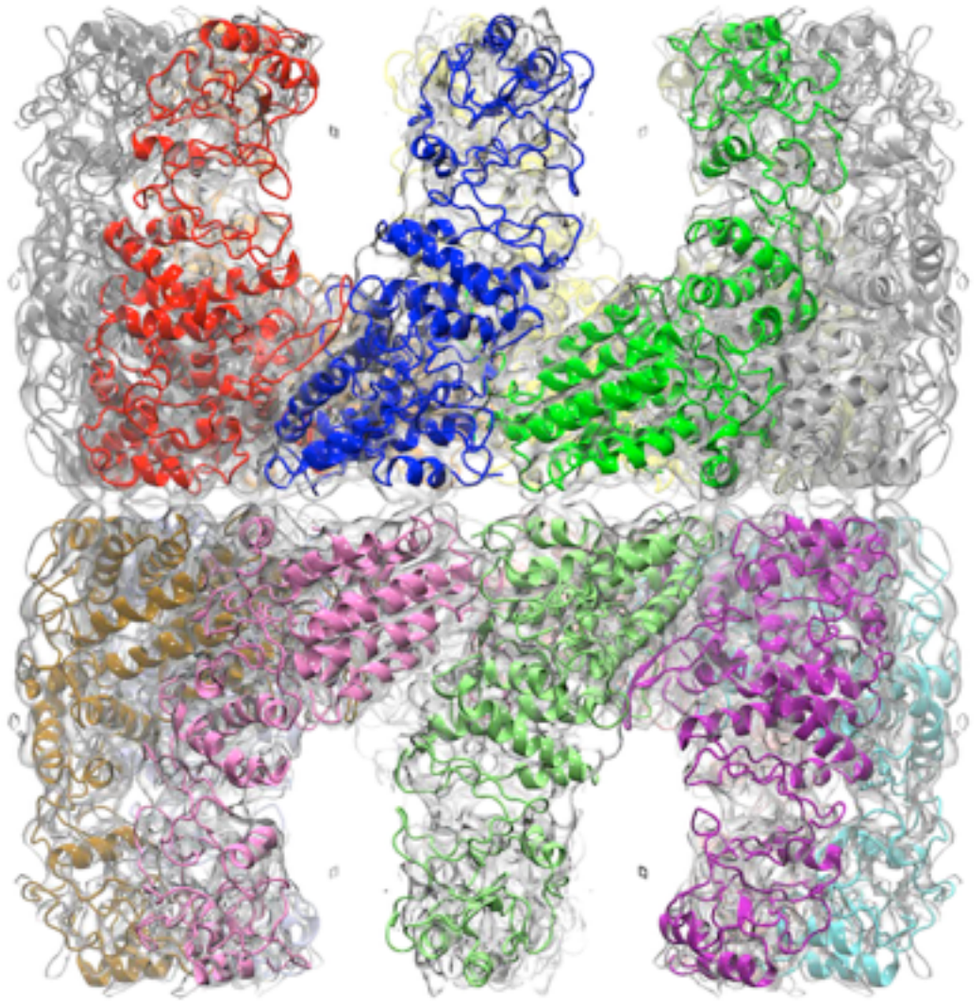
B. pumilus cyanide dihydratase

Symmetry restrained MDFF - Test Case 1
Improve quality of fit for low-resolution data

Blue: without symmetry restraints

Red: with symmetry restraints





Archaeal group II chaperonin from *M. maripaludis* (Mm-cpn)
8-fold rotational + 2 fold reflection symmetry
homology model (based on PDB 3LOS) fitted into EM map (EMDB 5140)

Chan et al. *Structure* (2011) 19:1211-1218.

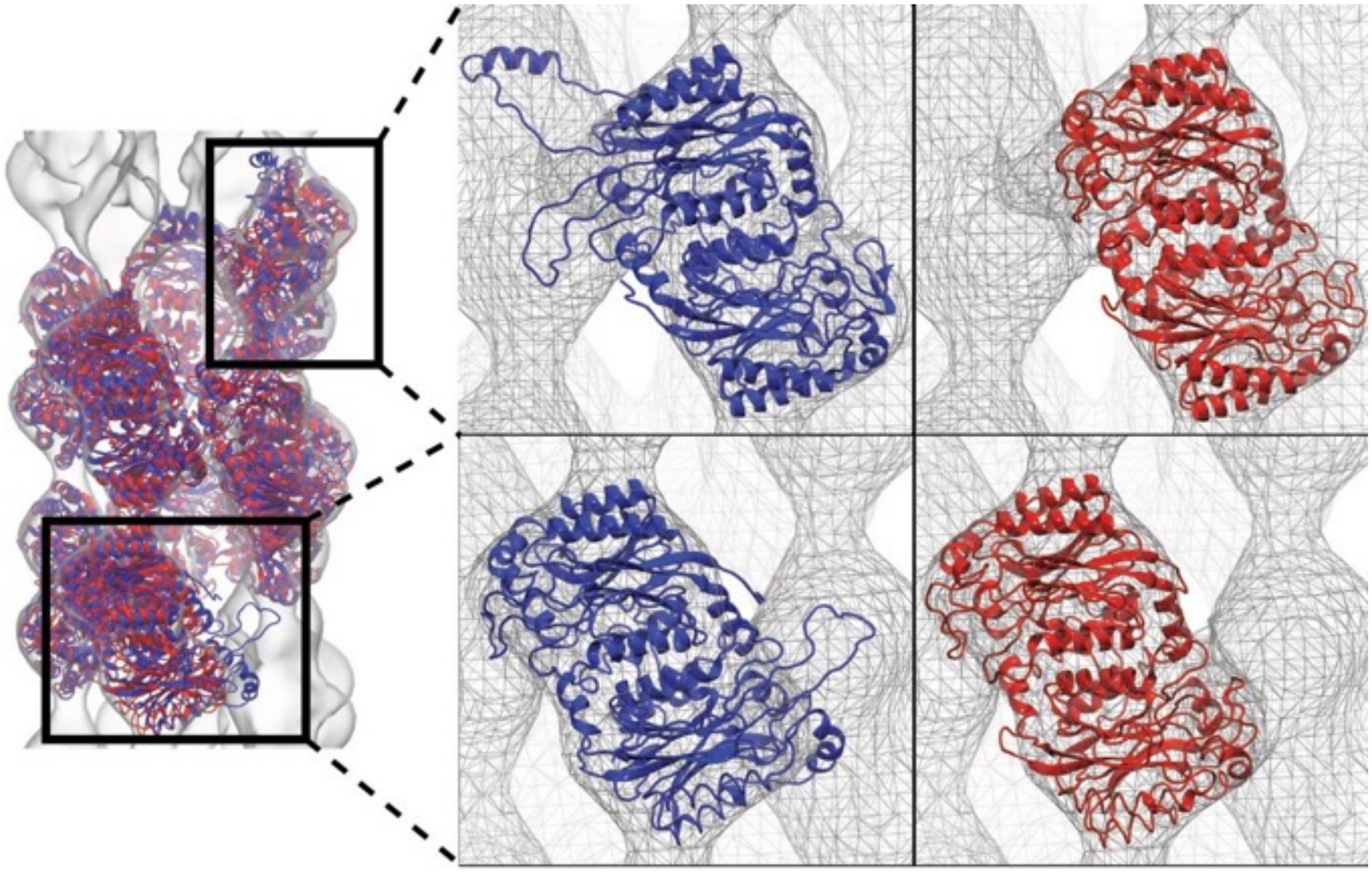
Symmetry restrained MDFF - Test Case 2
Prevent “edge distortion effect”

Finite-size Simulation
(9 dimers)

helical symmetry

Fitted models of J1 nitrilase
from R. *rhodochrous*

homology model and EM map (EMD 1313)
from collaborator T. Sewell, U. of Cape
Town



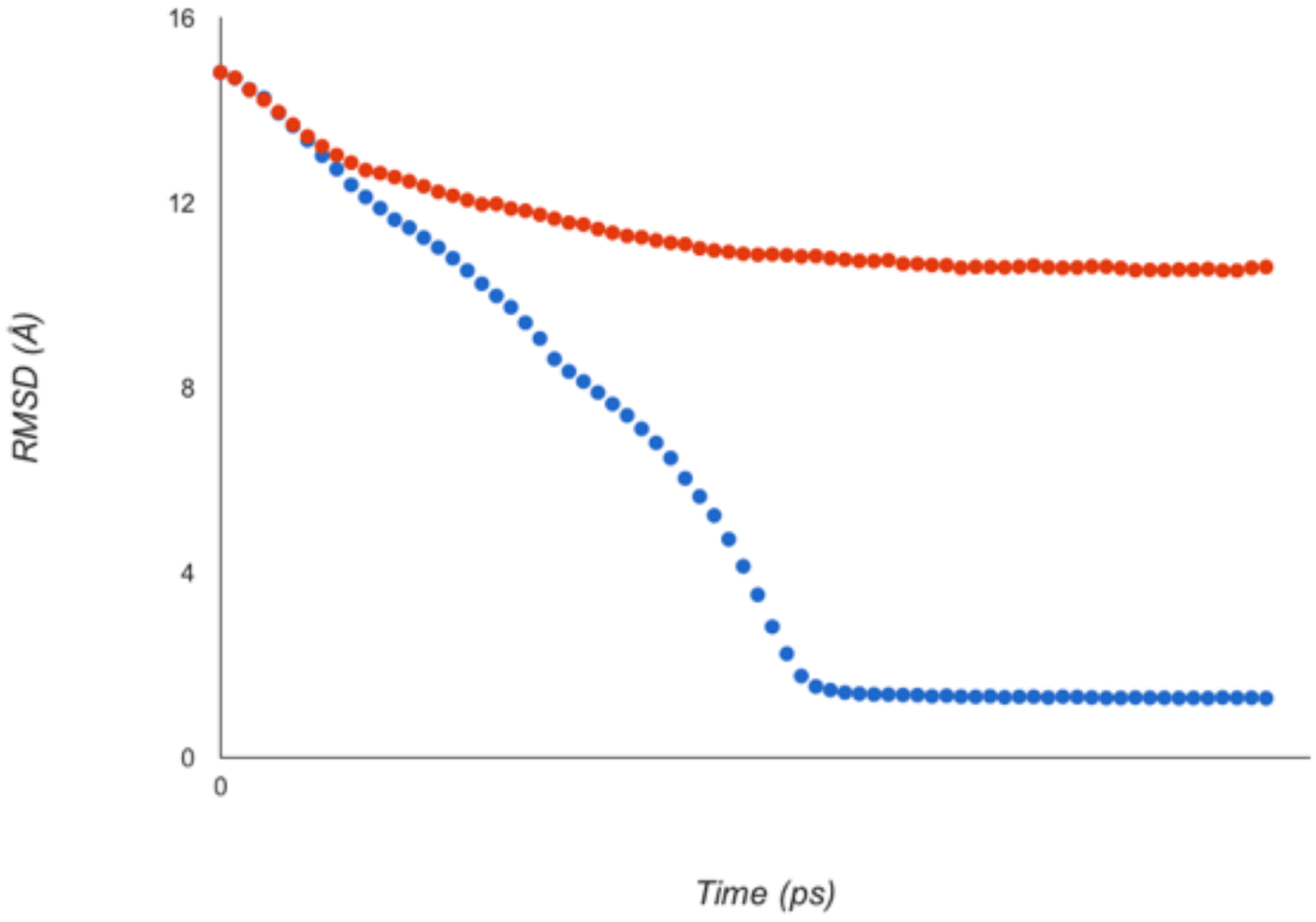
Without Symmetry Restraints

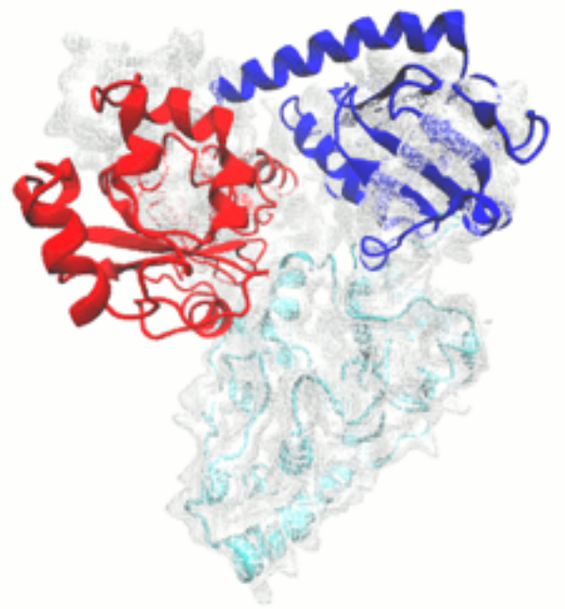
With Symmetry Restraints

Chan et al. *Structure* (2011) 19:1211-1218.

Domain restrained MDFF

Use Targeted MD (TMD) feature of NAMD to restrain non-overlapping groups of atoms to maintain rigid domains



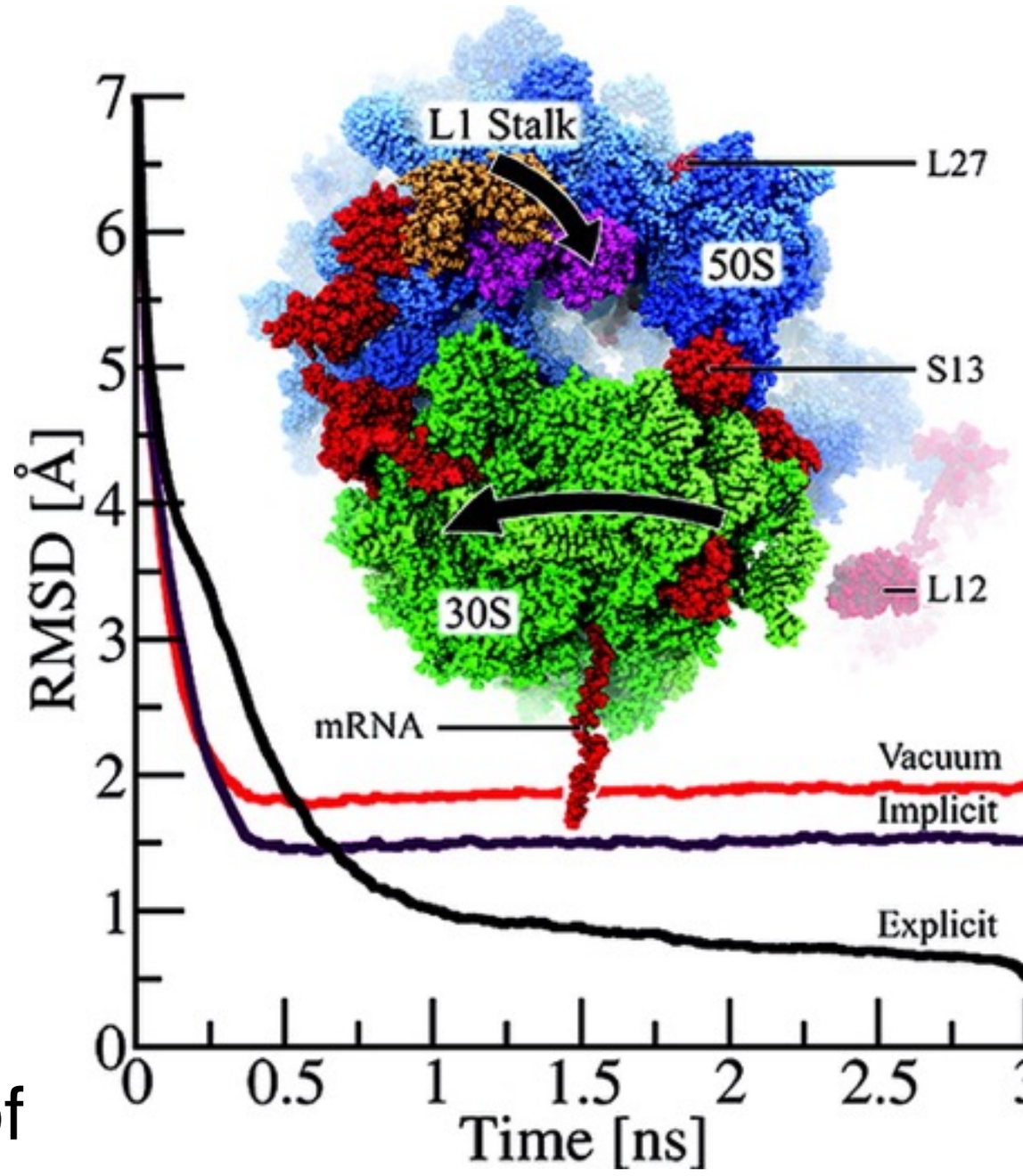


Acetyl CoA Synthase with two domains (red and blue) separately restrained

Simulation Environment

MDFF can be run in different environments:

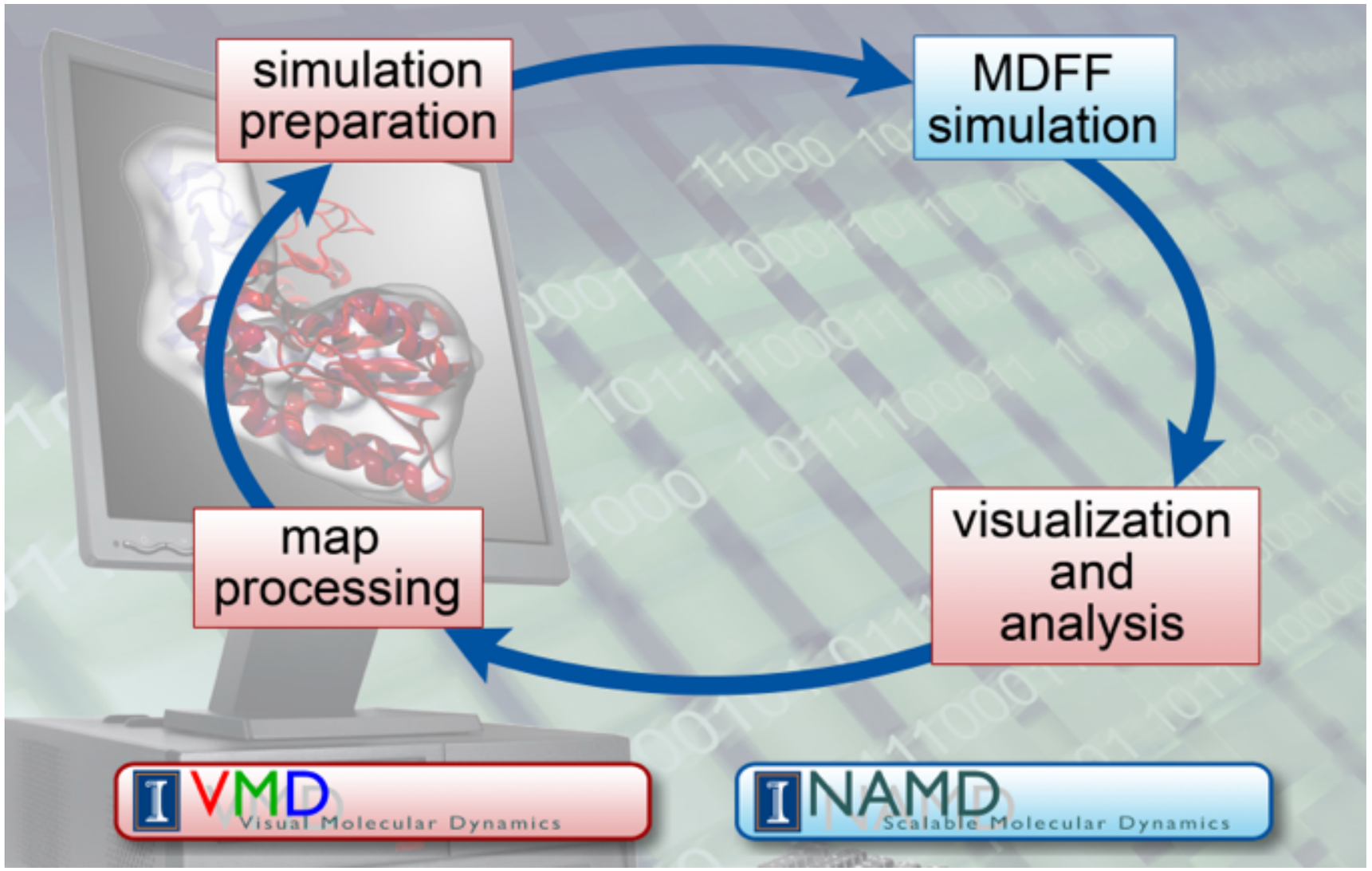
1. <u>Vacuum</u>
   - No water molecules
   - Fastest but potentially inaccurate
2. <u>Explicit Solvent</u>
   - Explicit atomic detail water molecules
   - Computationally slow and introduces effects of viscous drag
3. <u>Implicit Solvent</u>
   - generalized Born approximation of electrostatics
   - Compromise between speed and accuracy



Tanner, et al. *Journal of Chemical Theory and Computation* 7(11) 3635–3642, 2011.

MDFF Software Suite

- NAMD and VMD used together to run MDFF
- Every NAMD and VMD feature is available in MDFF



Fitting time is dependent on:

- system size
- map and structure quality
- Generally need ~ 1ns or less (much shorter than MD)

Input: MDFF only requires a PDB, PSF, and density map

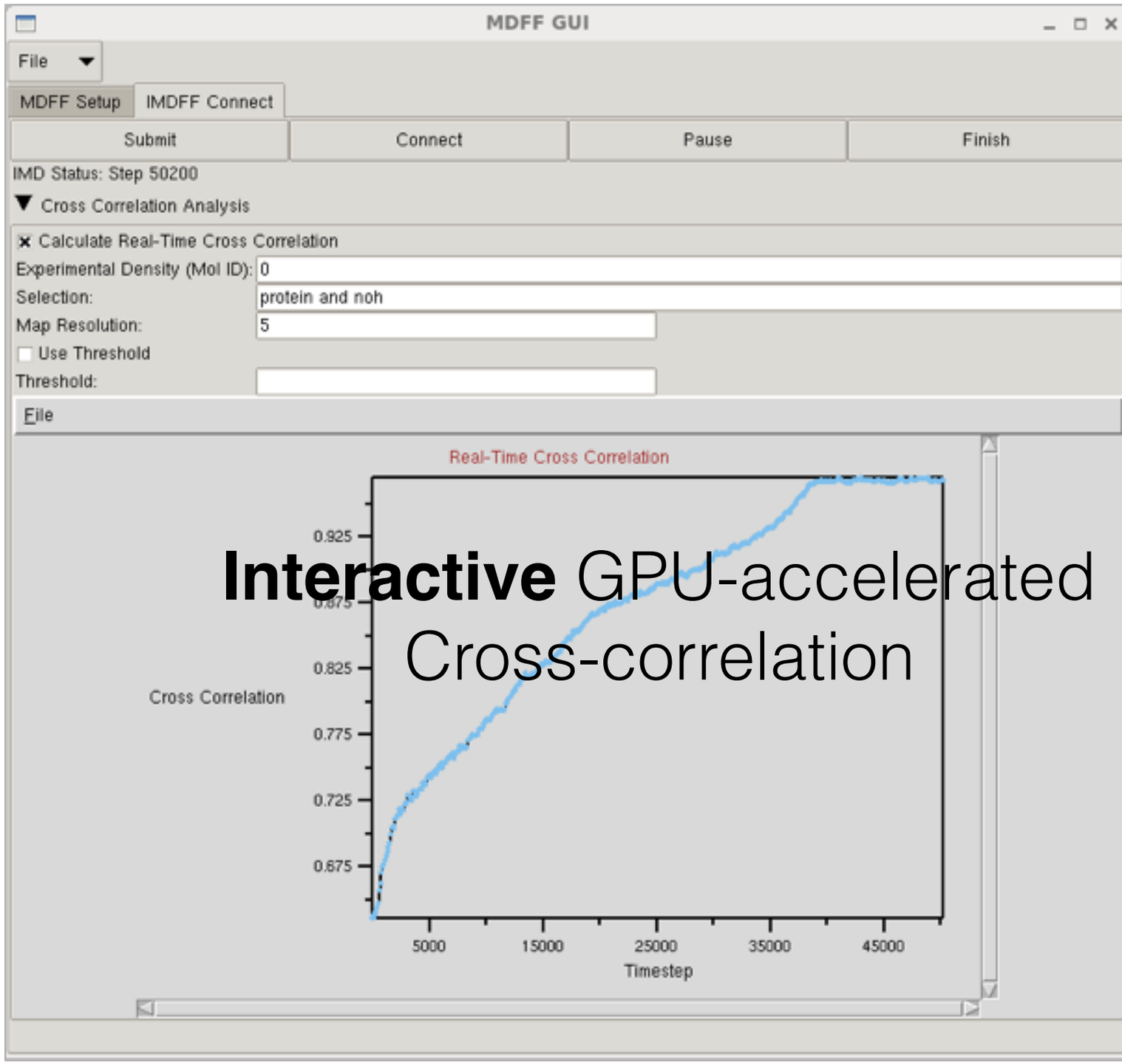
Output: produces simulation trajectory from which an ensemble of structures can be extracted

http://www.ks.uiuc.edu/Research/mdff/

MDFF Software Suite

- system sizes up to 100 million atoms (viruses, chromatophore)
- maps from 3 to 15 Å
- runs on laptops to petascale computing resources (Blue Waters, Titan)

New MDFF GUI (VMD 1.9.2) makes setting up, running, and analyzing fitting simulations even easier

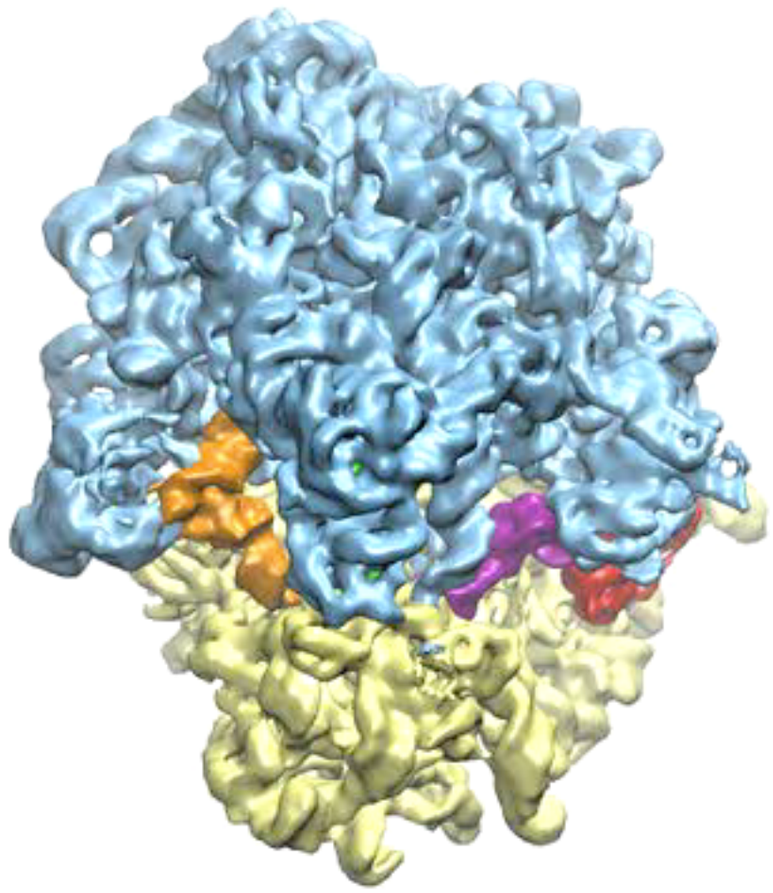


Interactive GPU-accelerated Cross-correlation

http://www.ks.uiuc.edu/Research/mdff/

Molecular Dynamics Flexible Fitting - Example

Cryo-EM map of the *E. coli* ribosome at 6.7-Å resolution



Villa et al. PNAS (2009)106:1063-1068.

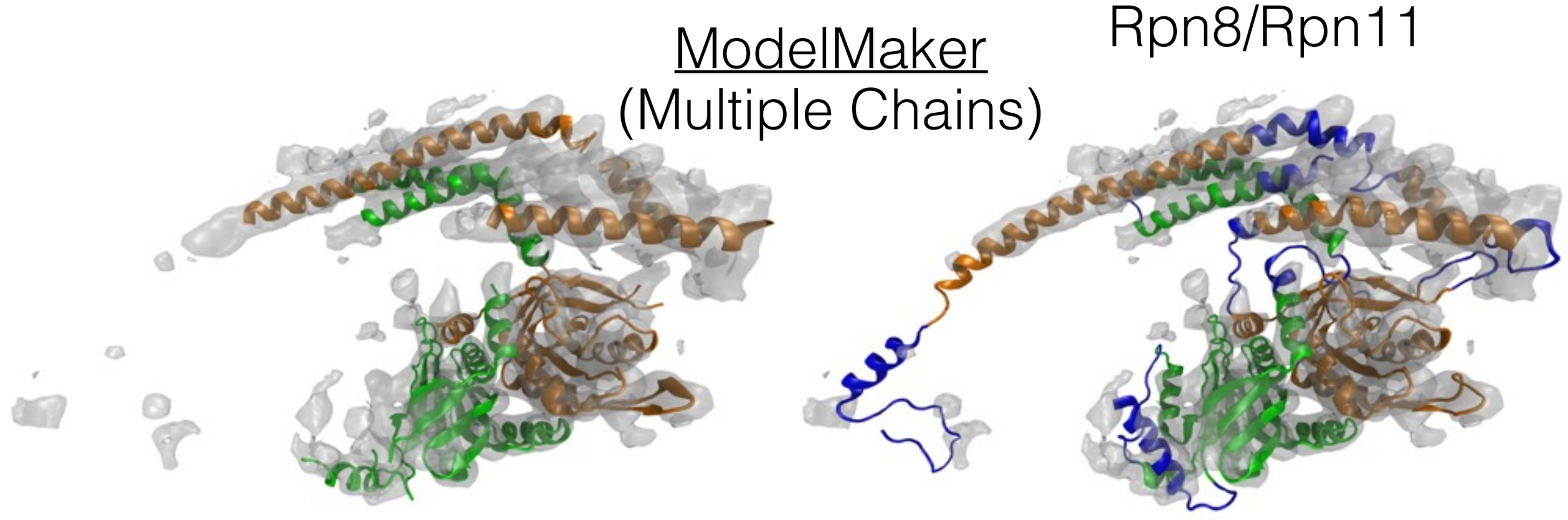
Obtaining Initial Structures

1. X-ray crystallography or NMR structures

2. Refine structures from low-res X-ray data with xMDFF

   Ryan McGreevy*, Abhishek Singharoy*,et al. Acta Crystallographica D70, 2344-2355, 2014

3. Homology or ab initio modeling with Modeller, Rosetta, MUFOLD (Ci-VSP, YidC, Holotranslocon)



Rosetta structure prediction to fill **missing pieces** and MDFF to filter, refine and validate candidate structures

ModelMaker Interactive Modeling

Combining structure prediction with the user's expertise to interpret densities

incomplete structural model deposited in the PDB

de novo structure prediction → energy ranking → model filtering → interactive MDFF of cryo-EM data

complete structural model that fits cryo-EM data

Rosetta

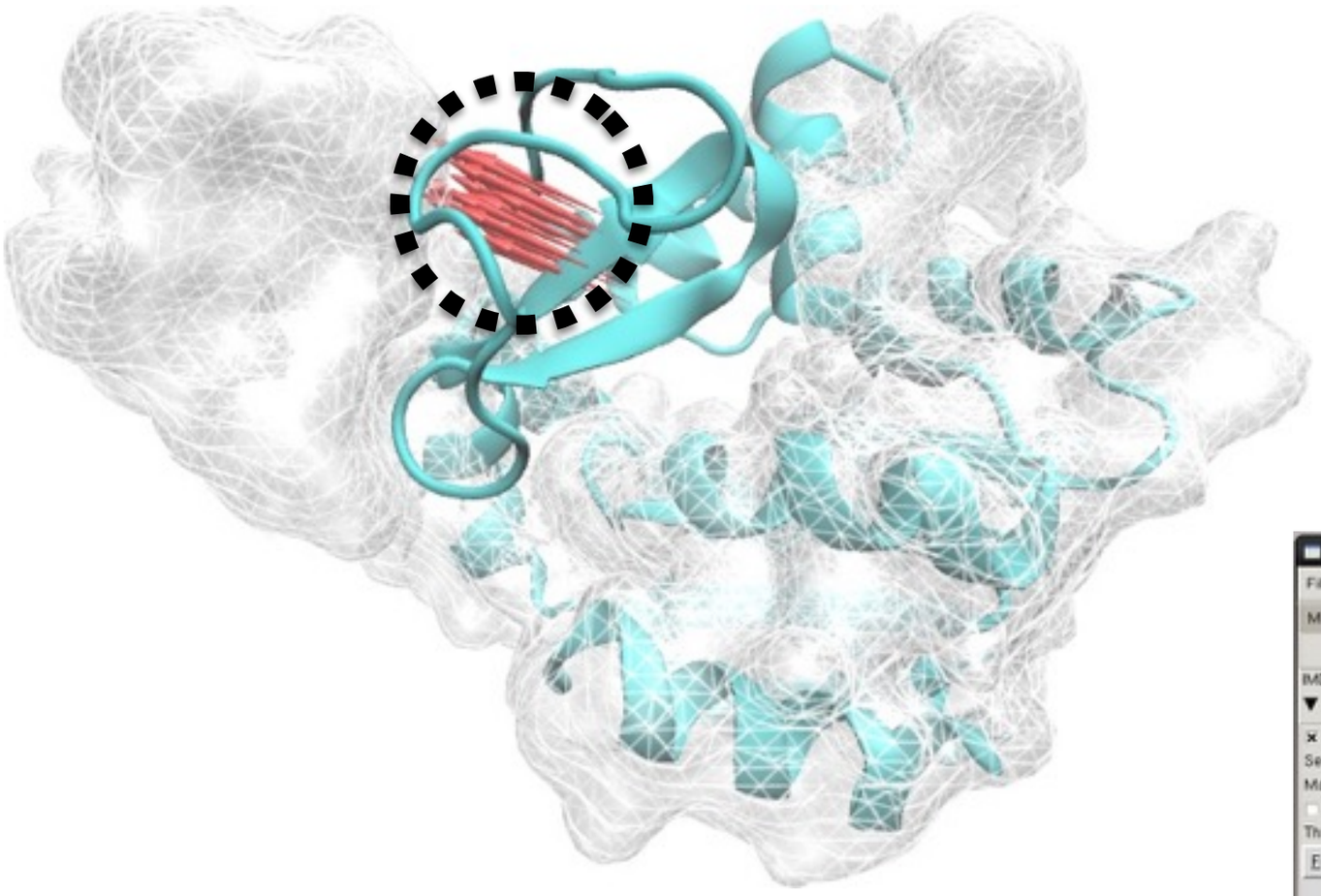
Leaver-Fay *et al.* Methods Enzymol. 2011
Porter *et al.* PLoS One 2015

VMD/NAMD

Humphrey *et al.* J. Mol. Graph. 1996
Philips *et al.* J. Comput. Chem. 2005

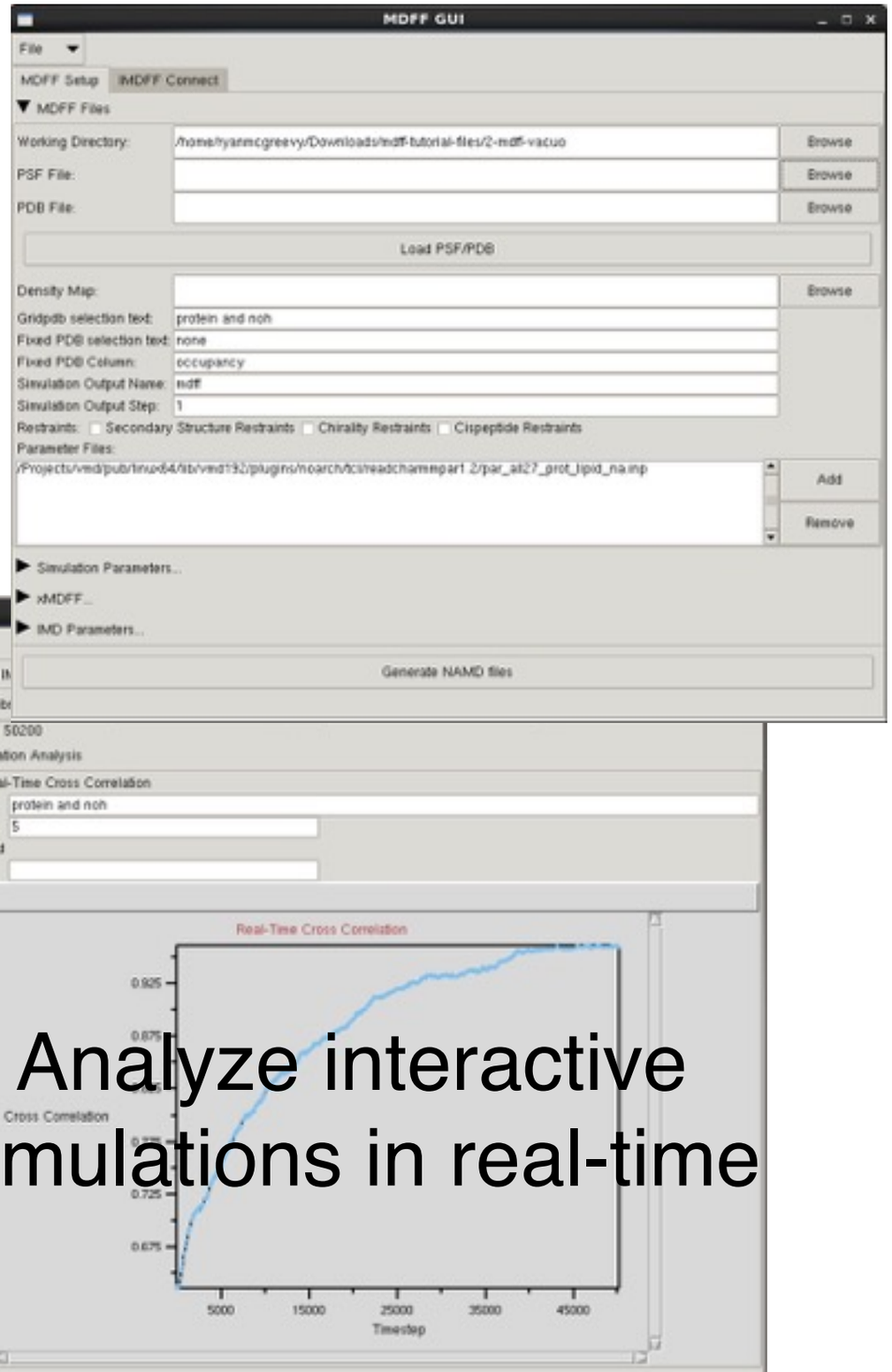
Interactive Modeling with MDFF GUI

- Apply forces to manually manipulate structure into the density
- Useful for difficult to fit structures with large conformational changes



New MDFF GUI in VMD 1.9.2

Set up and run interactive (or traditional) MDFF/xMDFF simulations

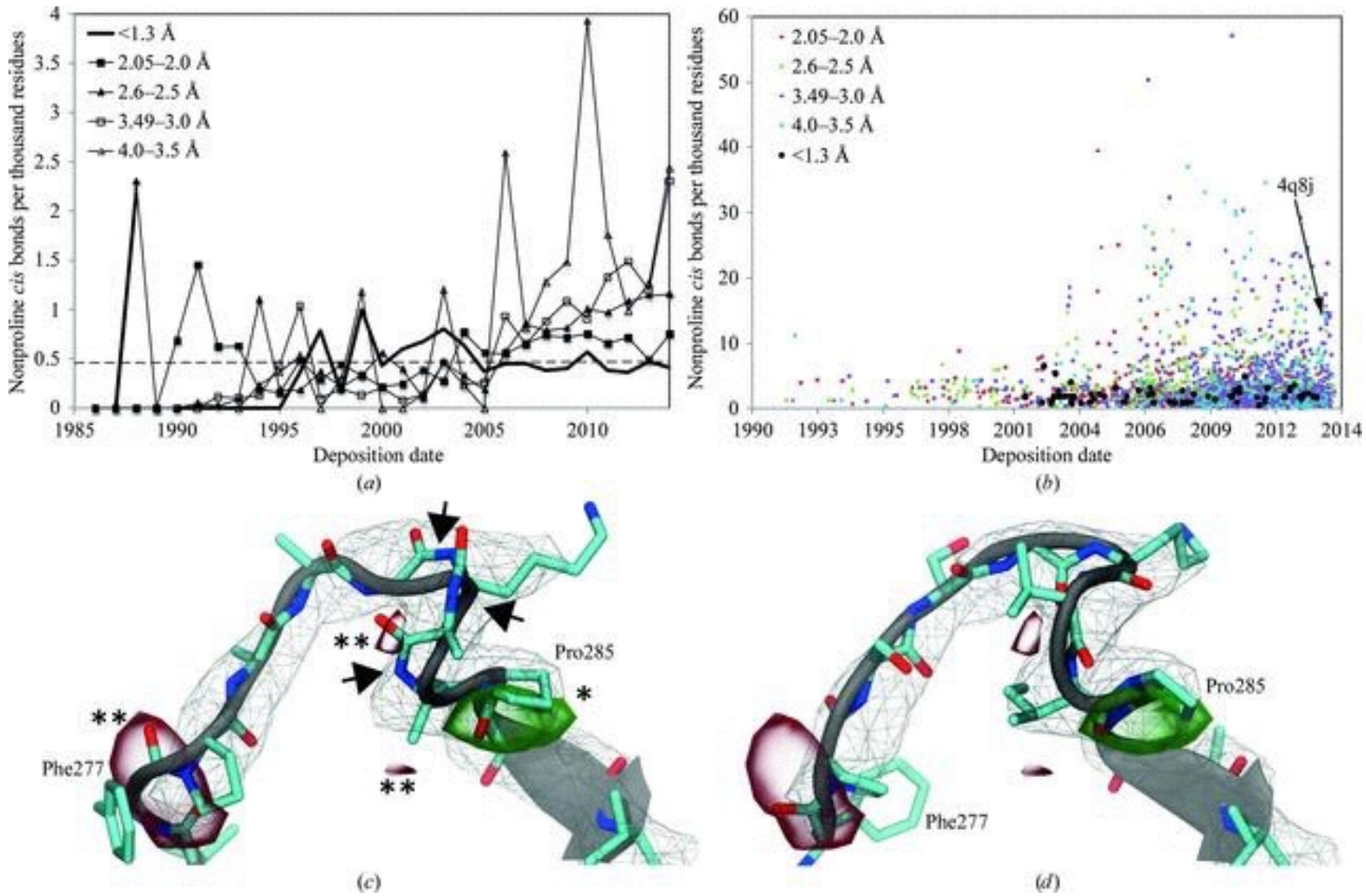


Analyze interactive simulations in real-time

Importance of Checking Initial Structure

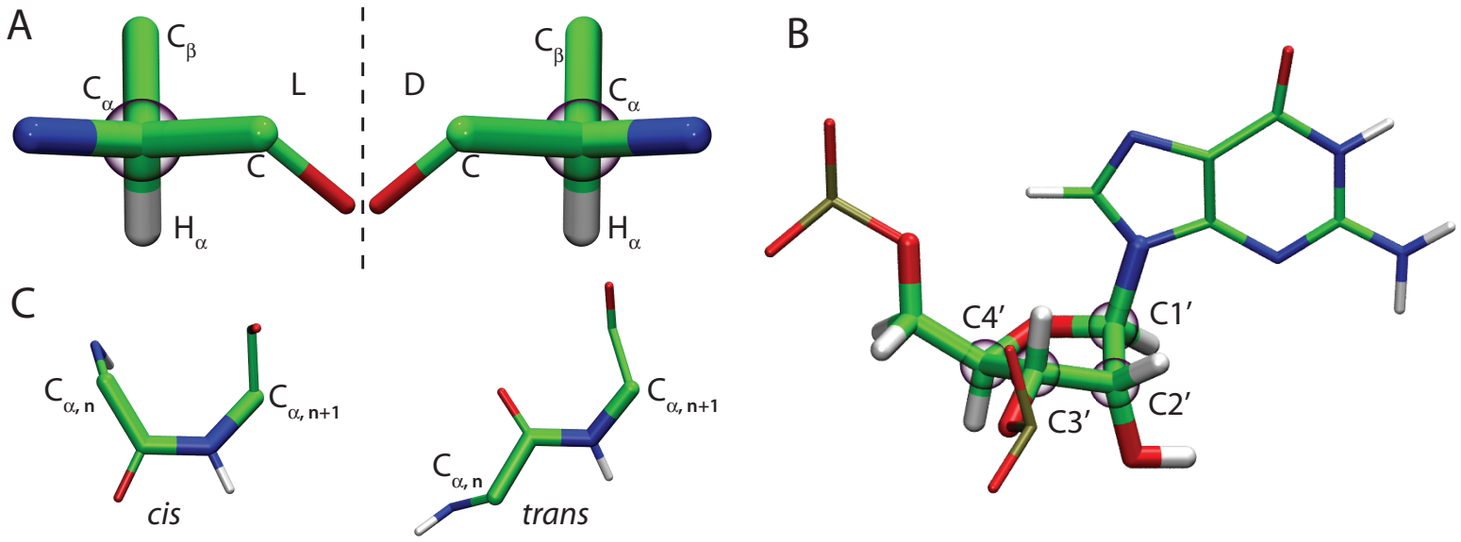
<0.05% non-proline bonds found in the cis conformation natively, however:

- The frequency of non-proline cis-peptide bond errors has been increasing for low-resolution
- These errors can hide issues in other parts of the structure



Tristan Croll. Acta Crystallographica D71, 706-709, 2015.

Structure Checking Plugins in VMD

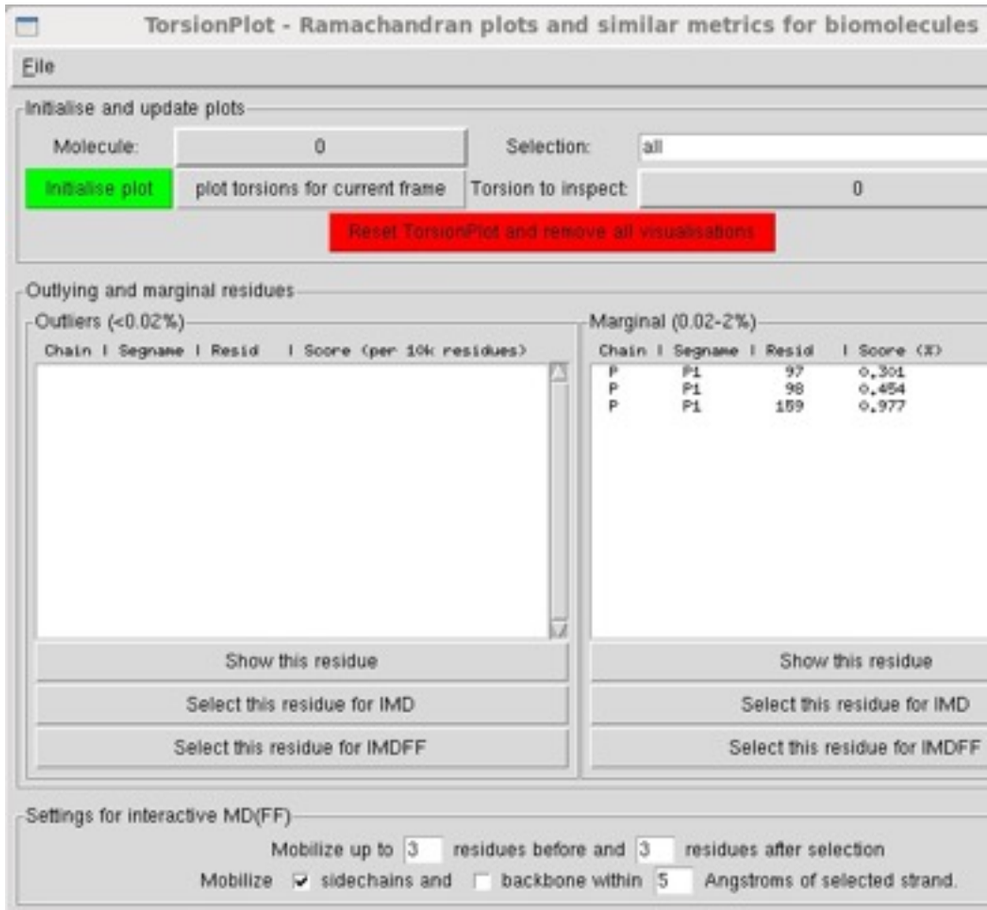


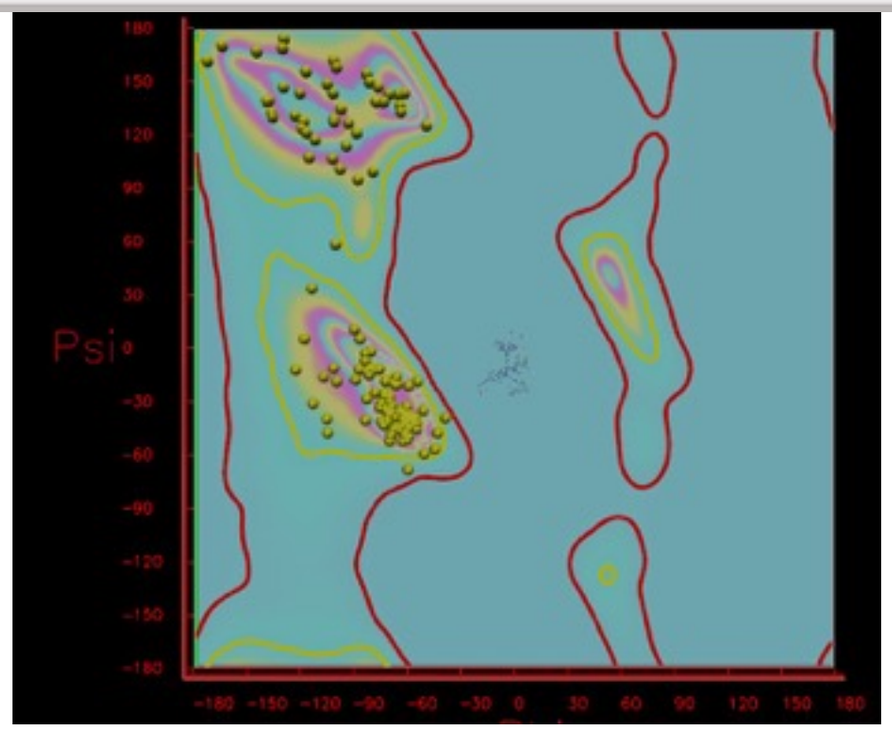
Eduard Schreiner, et al. BMC Bioinformatics, 12, 190, 2011

- Wrong chirality, cis-peptide bonds, and torsion angle outliers may arise during modeling

- VMD provides tools to check, visualize, and correct these errors

- These tools, together with MD force fields, produce models with good structural geometry

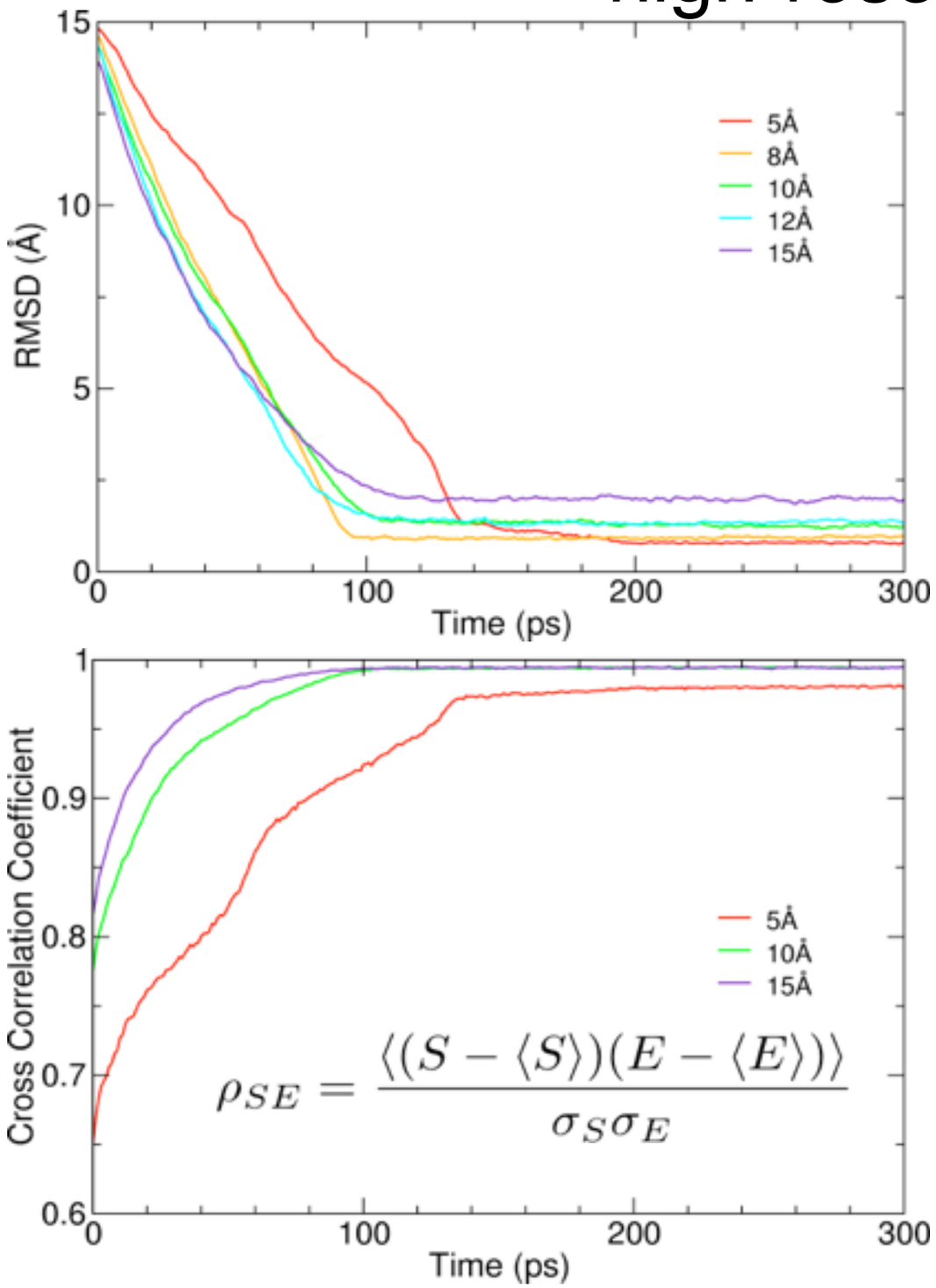


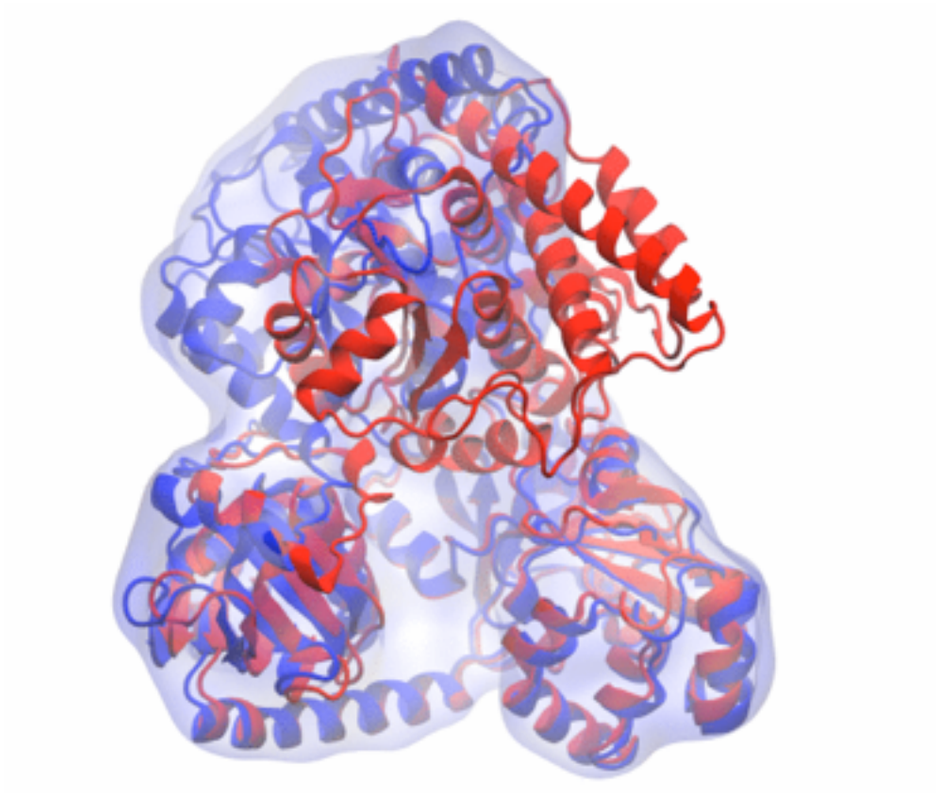


TorsionPlot Plugin new in VMD 1.9.3

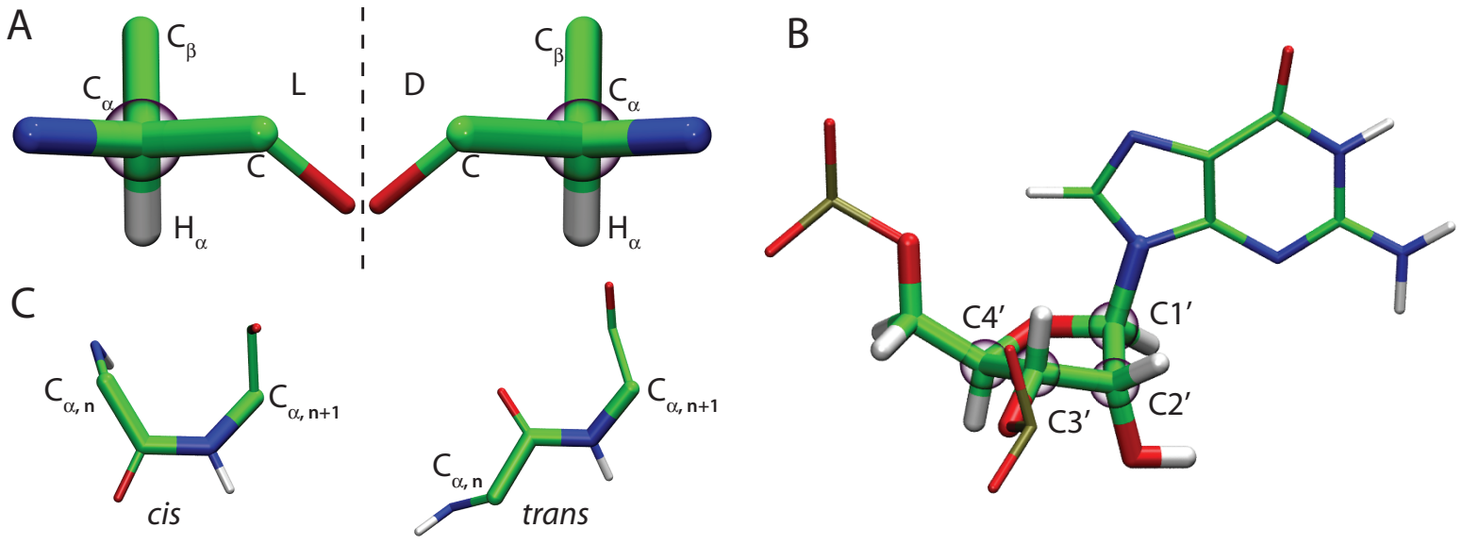
Analyzing MDFF Model Quality 0: **Known Structures**

MDFF has been validated against a wide-ranging set of known high-resolution structures





Analyzing MDFF Model Quality 1: **Structure Checking**



Eduard Schreiner, et al. BMC Bioinformatics, 12, 190, 2011

- Wrong chirality, cis-peptide bonds, and torsion angle outliers may arise during modeling
- VMD provides tools to check, visualize, and correct these errors
- These tools, together with MD force fields, produce models with good structural geometry

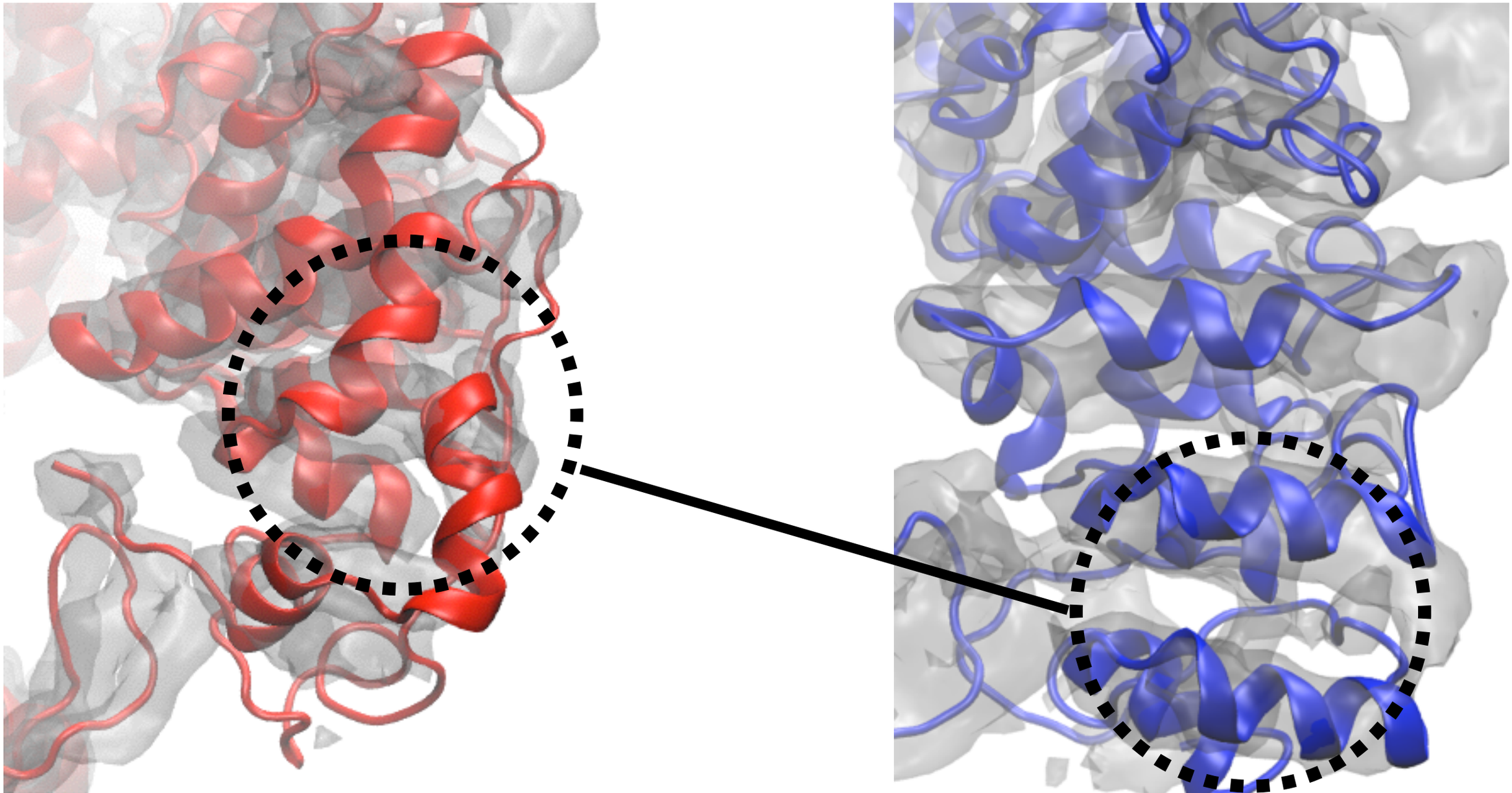
xMDFF refined structures

PDB ID	Molprobity initial (published)	final
1AV1	**3.72**	**1.94**
1YE1	**2.68**	**1.89**
1JL4	**3.24**	**1.47**
1AOS	**3.40**	**2.45**
1XDV	**2.87**	**2.01**
1YI5	**3.08**	**1.73**

Ryan McGreevy*, Abhishek Singharoy*,et al. Acta Crystallographica D70, 2344-2355, 2014

Analyzing MDFF Model Quality 2: **Global Cross Correlation**

Global CC is not always a good indicator of fit



CC = 0.728
RMSD(reference) = 6.23 Å

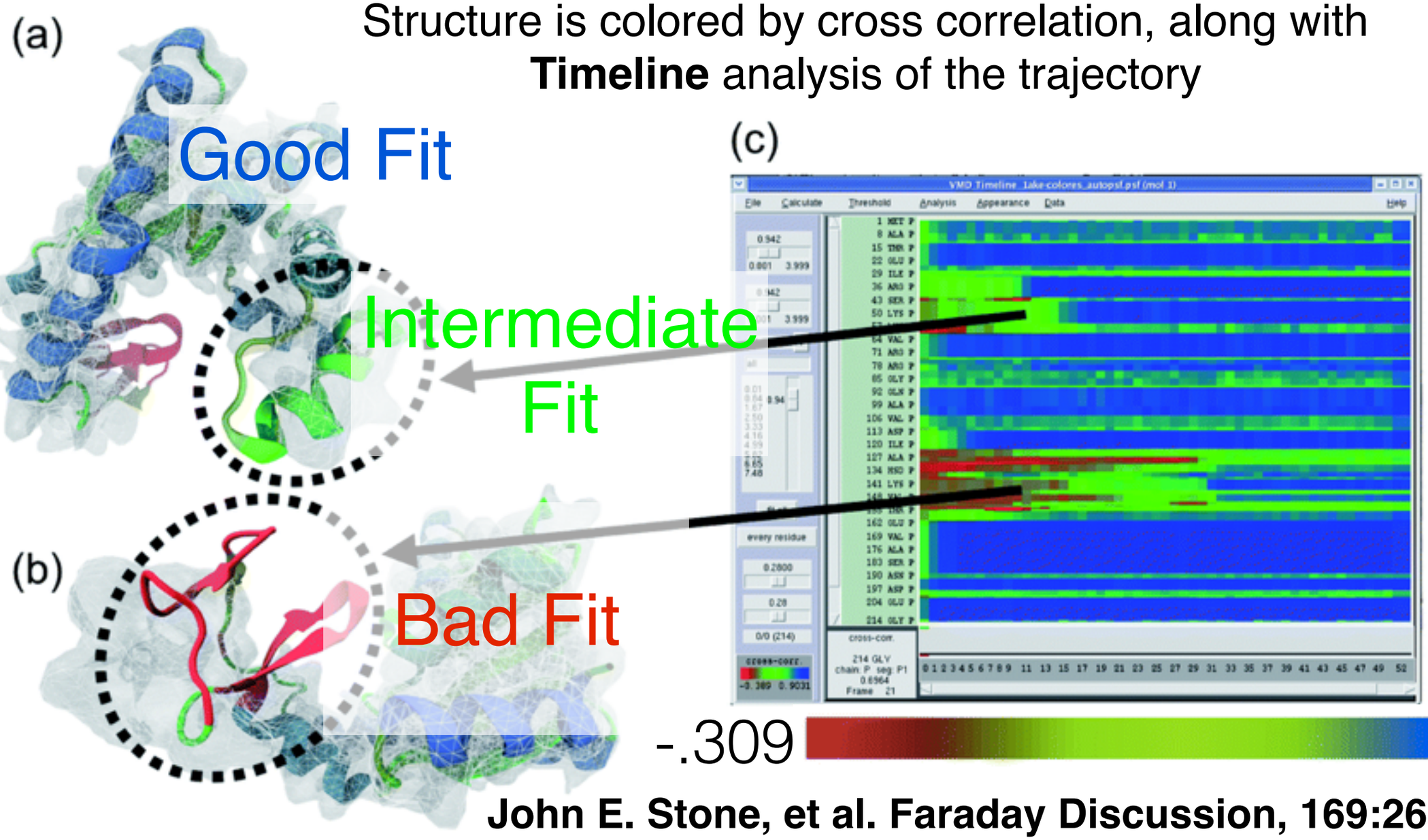
CC = 0.723
RMSD(reference) = 2.30 Å

TRPV1 EM map and structure from M. Liao, E. Cao, D. Julius, Y. Cheng, Nature 504, 2013.

Analyzing MDFF Model Quality 2: **Local Cross Correlation**

- Local cross correlation indicates quality of fit of specific regions across the entire structure
- New parallel CPU and GPU algorithms provide significant speed up (**25-50x speedup over Chimera**), allowing for fast computation along fitting trajectories

Structure is colored by cross correlation, along with **Timeline** analysis of the trajectory

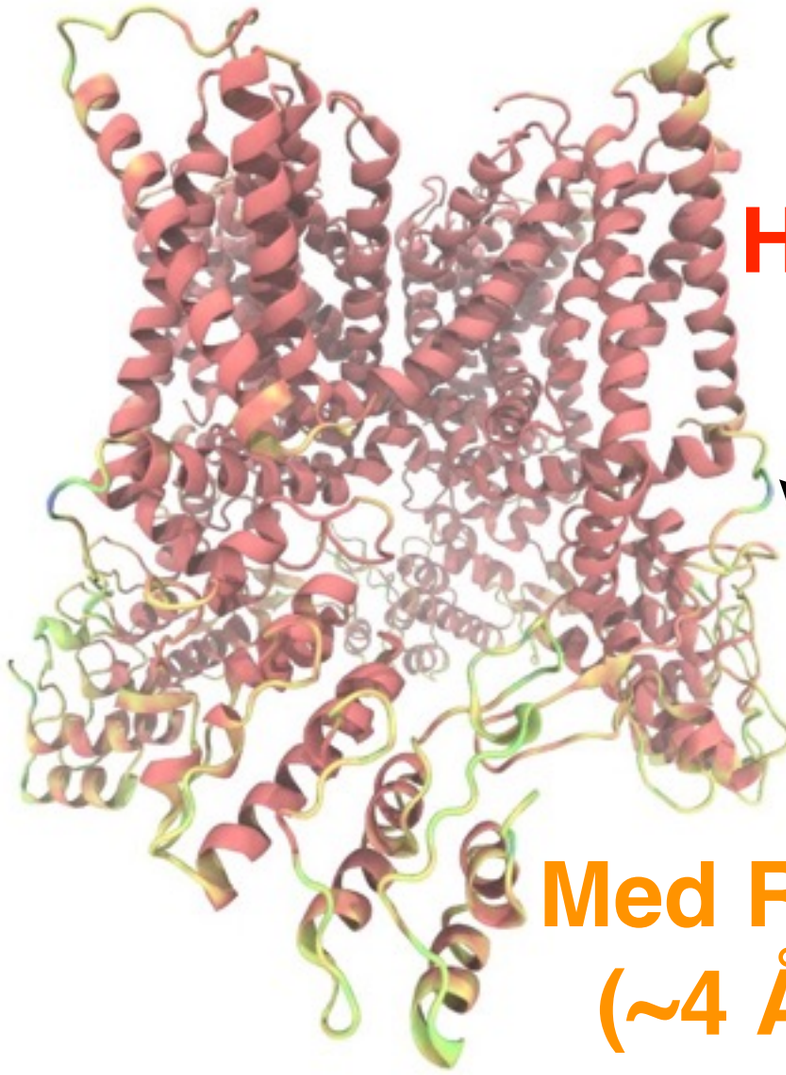


John E. Stone, et al. Faraday Discussion, 169:265-283, 2014

Analyzing MDFF Model Quality 3: **Local Resolution Analysis**

Local resolution of the experimental density from ResMap for error analysis and simulation parameterization

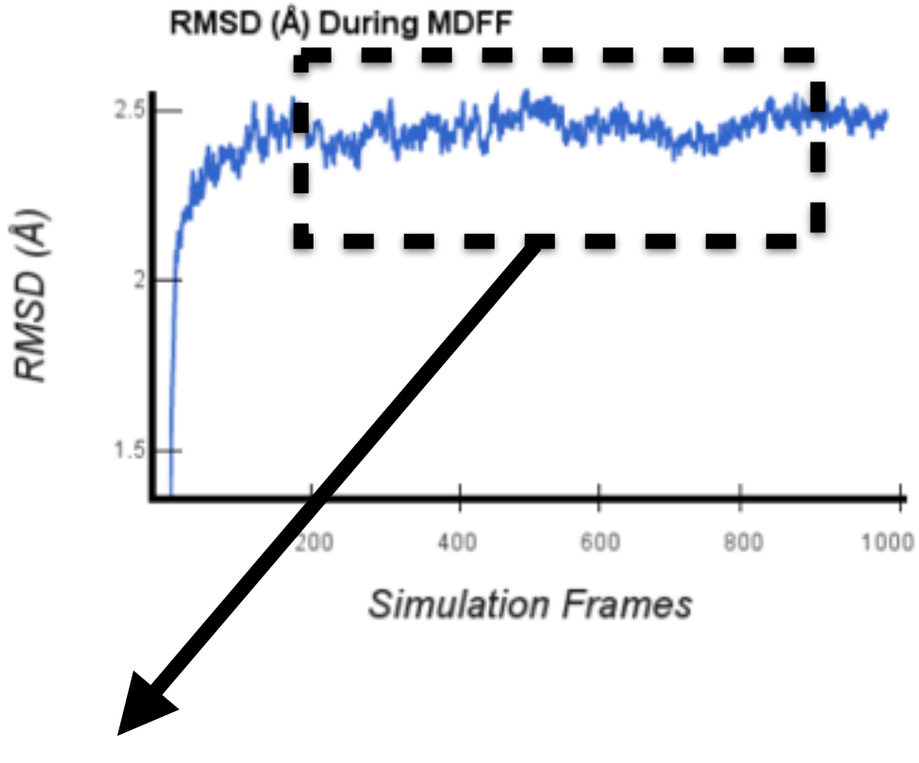
Root Mean Square Fluctuation (RMSF) correlates highly with local resolution

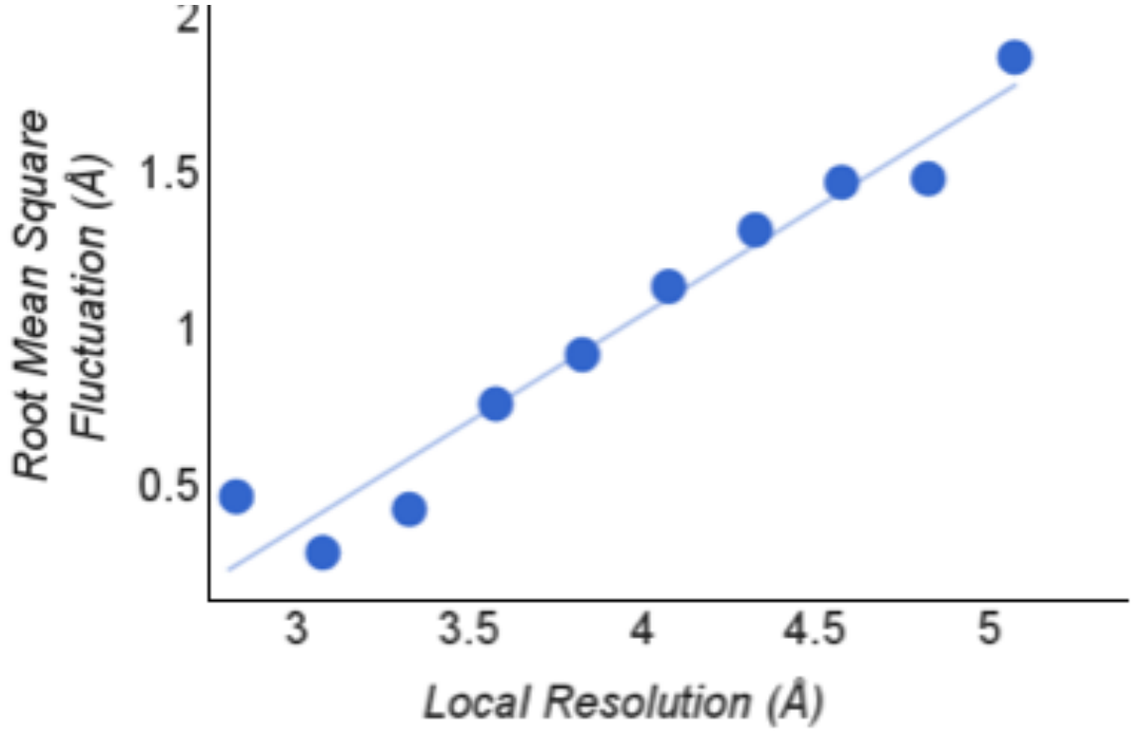


High Res (~3 Å)

Low Res (~5 Å)

Med Res (~4 Å)





A. Kucukelbir, F.J. Sigworth, H.D. Tagare, Quantifying the Local Resolution of Cryo-EM Density Maps, Nature Methods, Volume 11, Issue 1, Pages 63-65, 2014.

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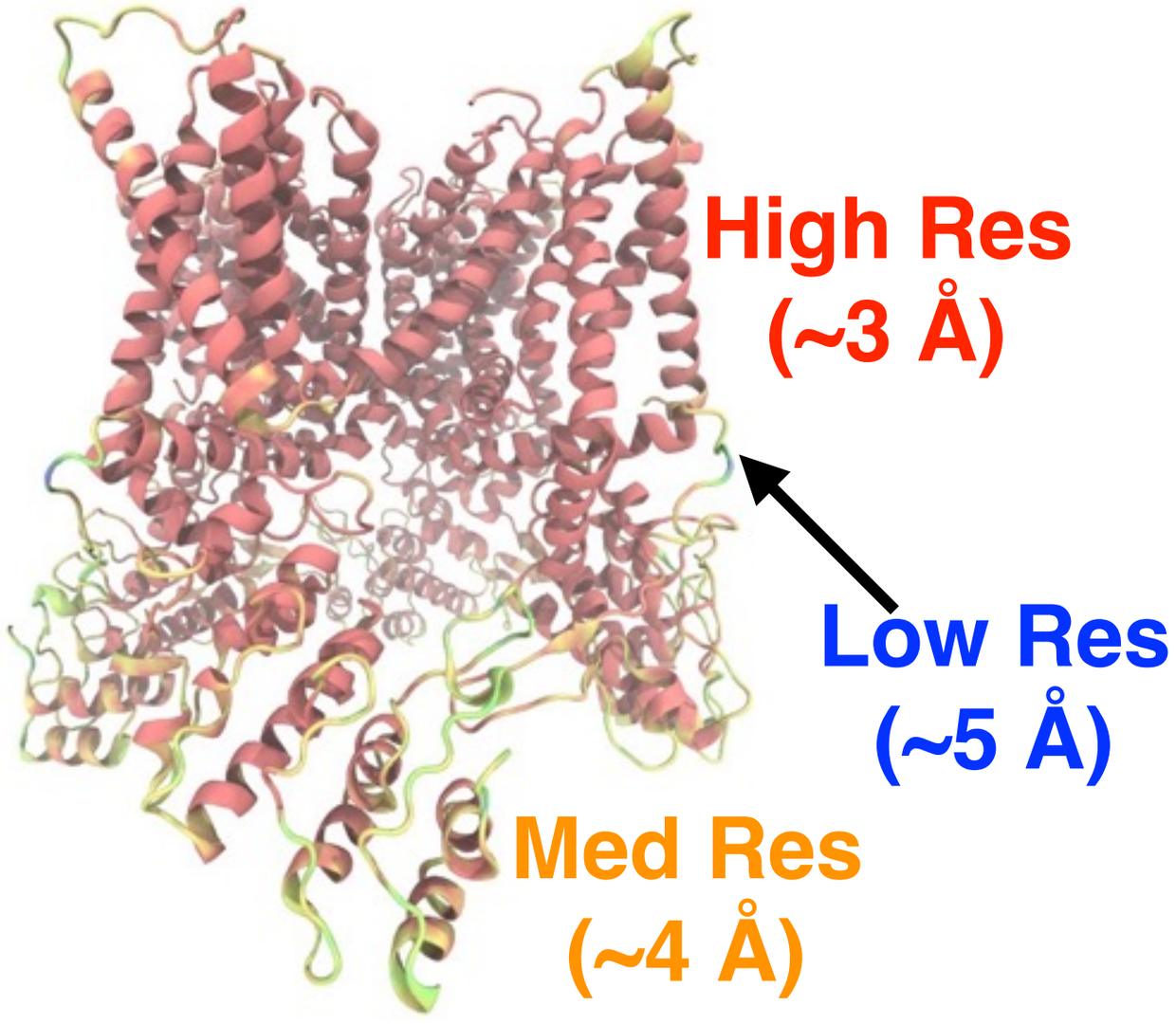
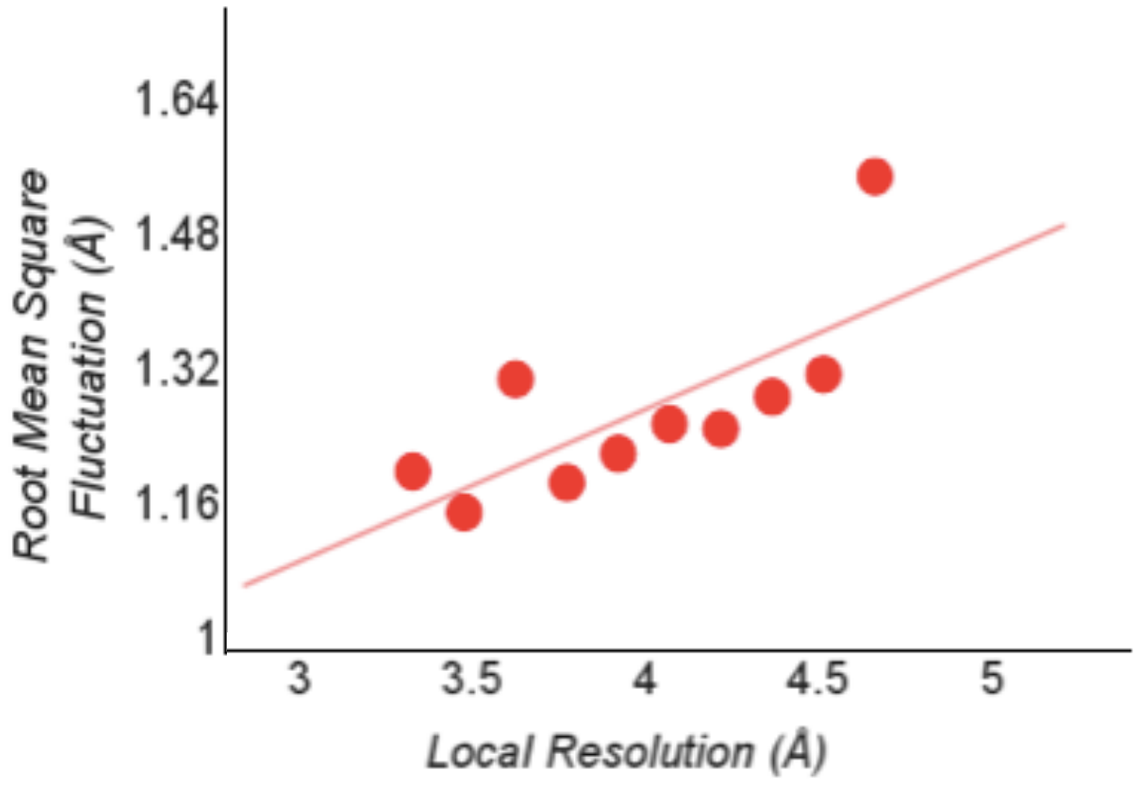


RMSF During Equilibration



A. Kucukelbir, F.J. Sigworth, H.D. Tagare, Quantifying the Local Resolution of Cryo-EM Density Maps, Nature Methods, Volume 11, Issue 1, Pages 63-65, 2014.

Analyzing MDFF Model Quality 4: **Cross-validation correlation**

Cascade and **direct** fitting structure to one half map and calculating the cross correlation to the other

CC w.r.t. / Fit to	Halfmap I	Halfmap II
Halfmap I	0.715 (0.686)	0.714 (0.685)
Halfmap II	0.716 (0.688)	0.716 (0.688)

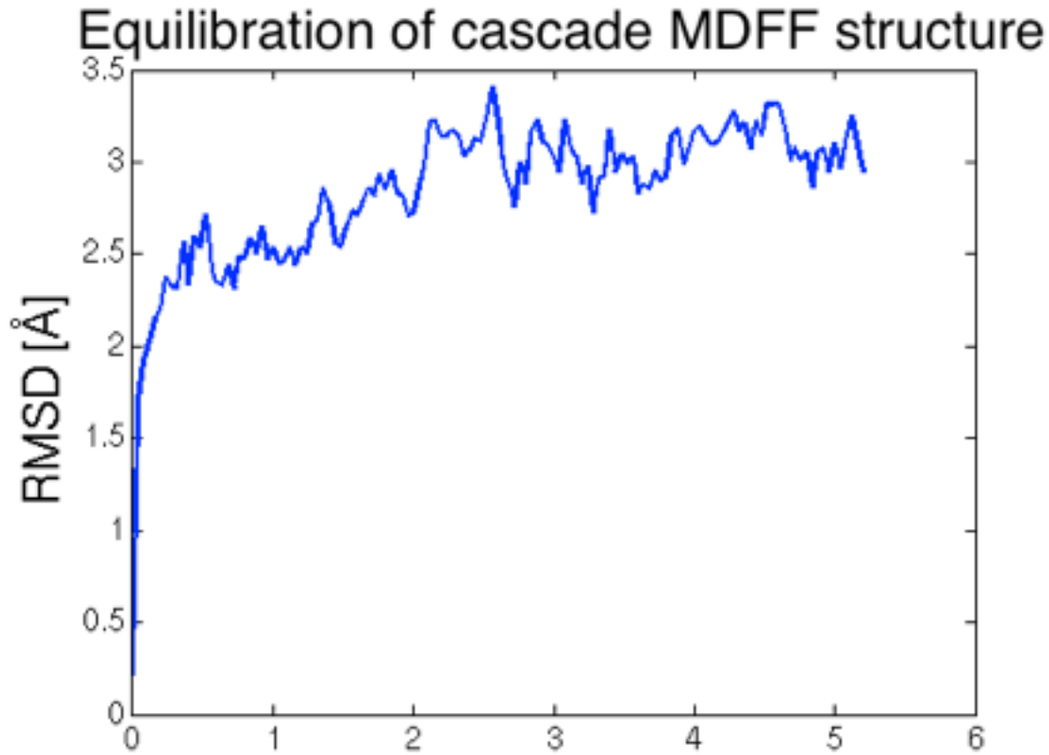
CC of reference structure w.r.t. each half map was 0.719

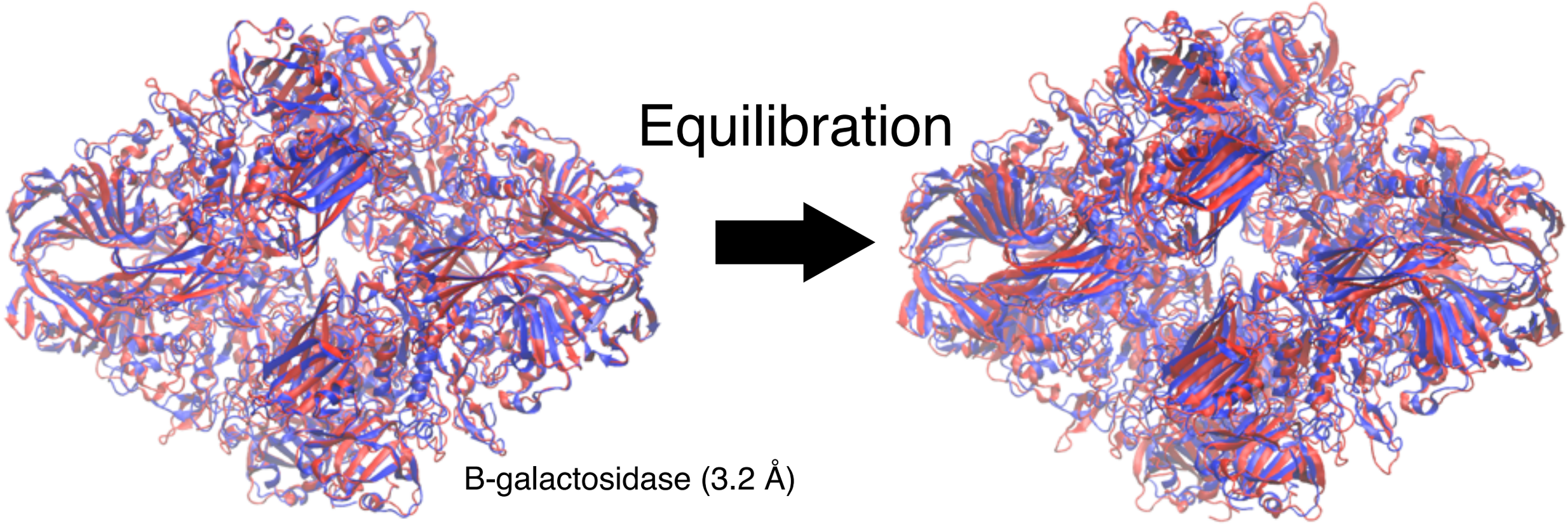
TRPV1 EM map and structure from M. Liao, E. Cao, D. Julius, Y. Cheng, Nature 504, 2013.

Analyzing MDFF Model Quality 5: **MD post-processing**

Stability of structure during equilibration

Deviation from fitted structure after equilibration is within map resolution (~3Å)

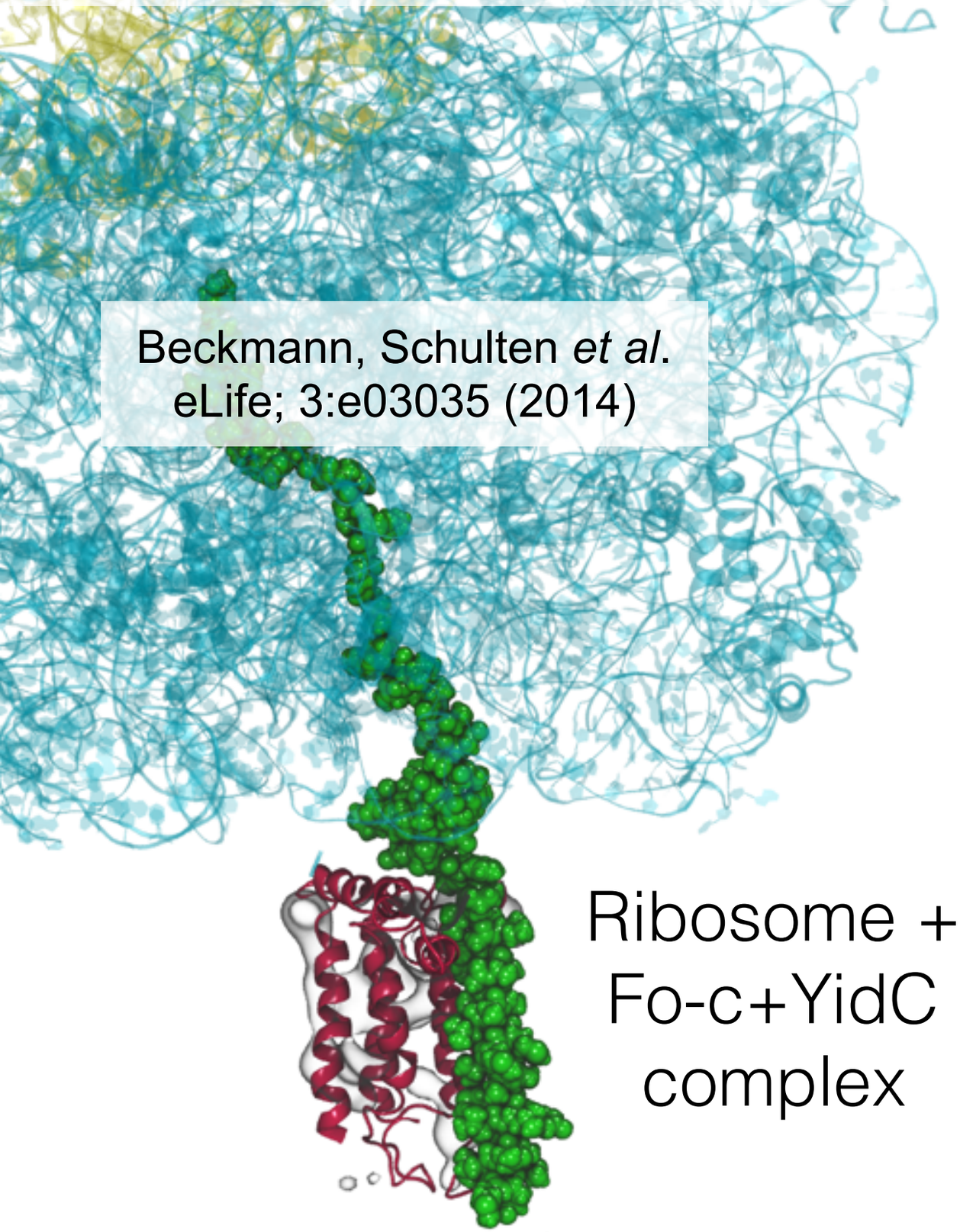




B-galactosidase (3.2 Å)

A. Bartesaghi, D. Matthies, S. Banerjee, A. Merk, S. Subramaniam, Proc. Natl. Acad. Sci. 111, 2014.

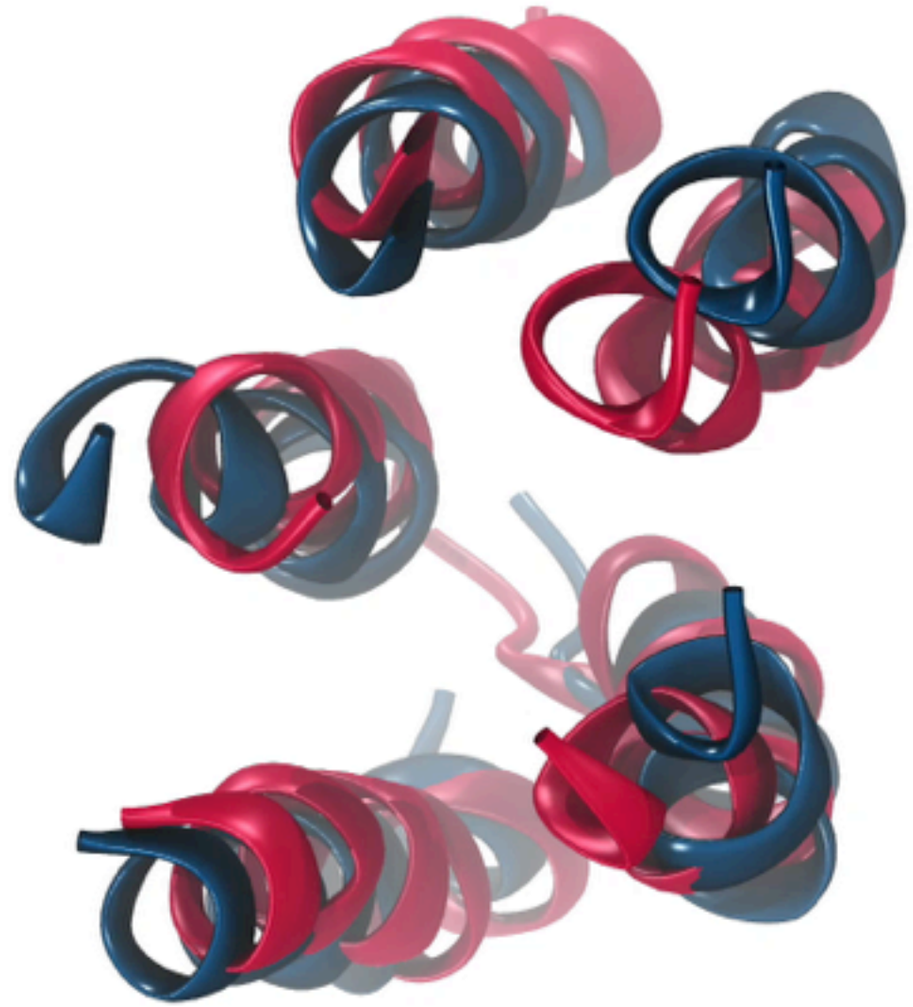
Analyzing MDFF Model Quality 6: **Agreement with Experiment**



Beckmann, Schulten *et al*.
eLife; 3:e03035 (2014)

Ribosome +
Fo-c+YidC
complex

Ribosome-bound structure predicted by MDFF from cryo-EM map ~ 7.5 Å



Crystal Structure (3WVF) 3.2 Å

Kumazaki *et al*. Nature (2014)

Nascent chain confirmed also by chemical cross-linking, gel filtration chromatography and mass spectroscopy.

MDFF Has a Wide Range of Applications

Over 60 reported MDFF applications:

- ***By intramural Researchers:***

Qufei Li et al. ***Nat. Struct. Mol. Biol***. (2014): Structural mechanism of voltage-sensing protein

Wickels et al. ***eLife*** (2014): Ribosomal insertase YidC

Zhao et al. ***Nature*** (2013): All-atom structure of HIV-1 Capsid

Agirrezabala et al. ***PNAS*** (2012): Ribosome translocation intermediates

Frauenfeld et al. ***Nat. Struct. Mol. Biol***. (2011): SecYE ribosome complex

- ***By extramural Researchers:***

Gogala et al. ***Nature*** (2014): Ribosome Sec61 complex

Unverdorben et al. ***PNAS*** (2014): 26S proteasome

Bharat et al. ***PNAS*** (2014): Tubular arrays of HIV-1 Gag

Park et al. ***Nature*** (2014)***:*** SecY channel during initiation of protein translocation

Hashem et al. ***Nature*** (2013): *Trypanosoma brucei* ribosome

Becker et al. ***Nature*** (2012): Ribosome recycling complex

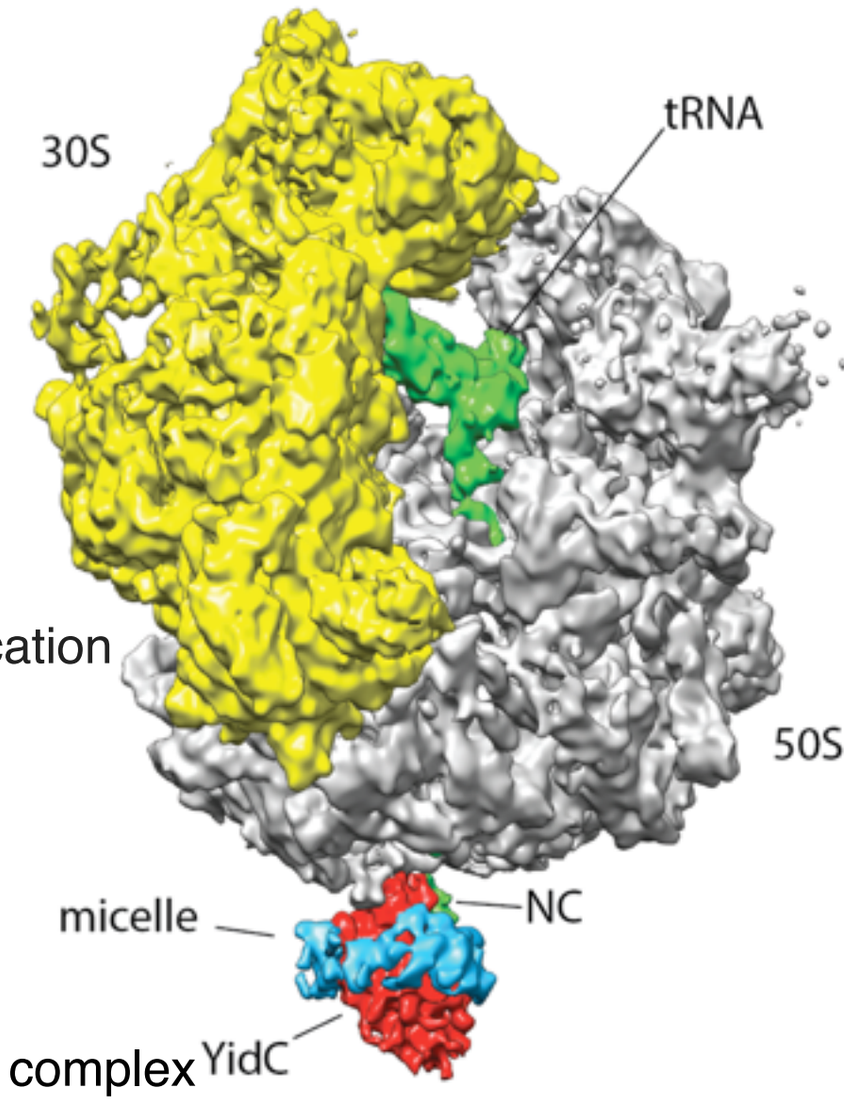
Lasker et al. ***PNAS*** (2012): Proteasome

Strunk et al. ***Science*** (2011): Ribosome assembly factors

Wollmann et al. ***Nature*** (2011): Mot1–TBP complex

Becker et al. ***Nat. Struct. Mol. Biol***. (2011) Dom34–Hbs1 stalled ribosome complex

Guo et al. ***PNAS*** (2011): RsgA GTPase on ribosomal subunit



8 Å resolution cryoEM density of ribosome-YidC complex. Wickels et al. ***eLife.*** 2014

<u>MDFF/xMDFF Methodological Articles:</u>

Singharoy, Teo, McGreevy, et al. *eLife* (2016)

McGreevy et al. *Methods* (2016) 100:50-60

Ryan McGreevy*, Abhishek Singharoy*, et al. Acta Crystallographica (2014) D70, 2344-2355

Trabuco et al. *Structure* (2008) 16:673-683.

Trabuco et al. *Methods* (2009) 49:174-180.

Acknowledgements and Further Information

Find out more about MDFF including:

- software downloads
- publications
- documentation
- **tutorials**

http://www.ks.uiuc.edu/Research/mdff/

http://www.ks.uiuc.edu/Research/mdff/vmdbeta/

Abhi Singharoy

Ivan Teo

Till Rudack

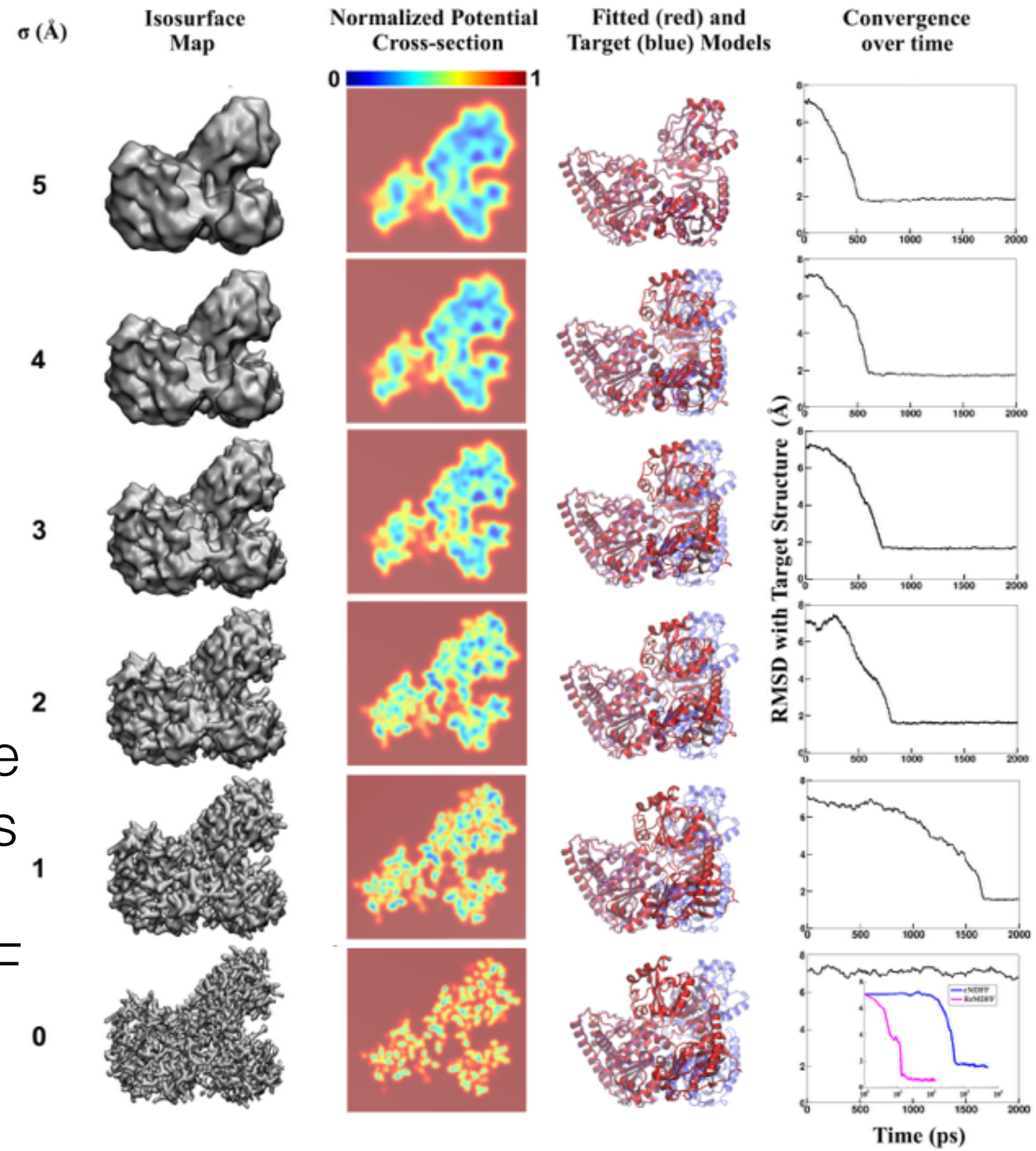
Molecular Dynamics Flexible Fitting
Advanced Techniques

Ryan McGreevy
Research Programmer

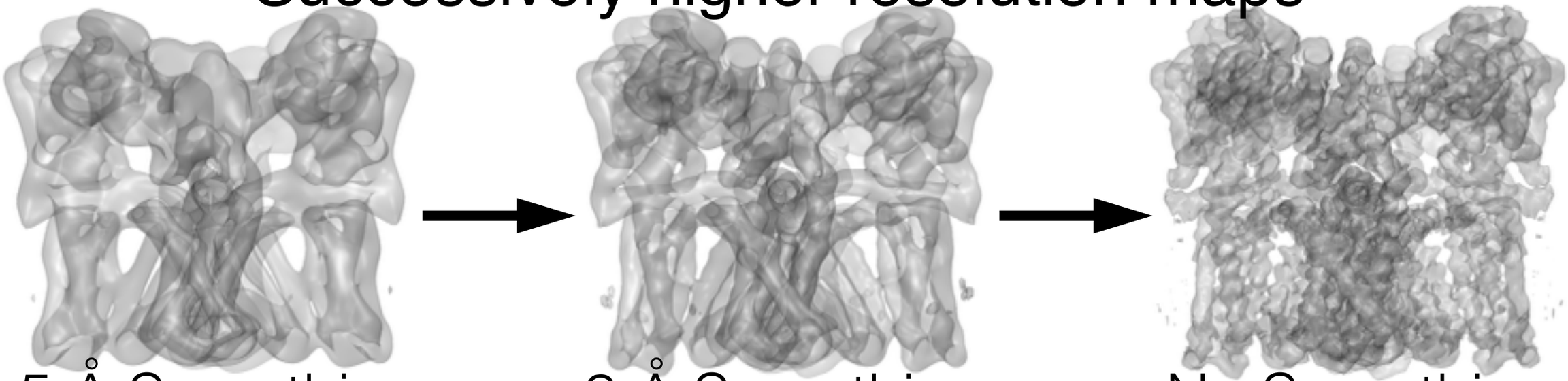
University of Illinois at Urbana-Champaign
NIH Resource for Macromolecular Modeling and Bioinformatics

MDFF for **high-resolution** cryo-EM

- MDFF made in a time of lower resolution (~8-15 Å) EM maps
- High-resolution (< 5 Å) now more easily obtainable
- Structure can become trapped in steep wells of high-resolution potential during MDFF



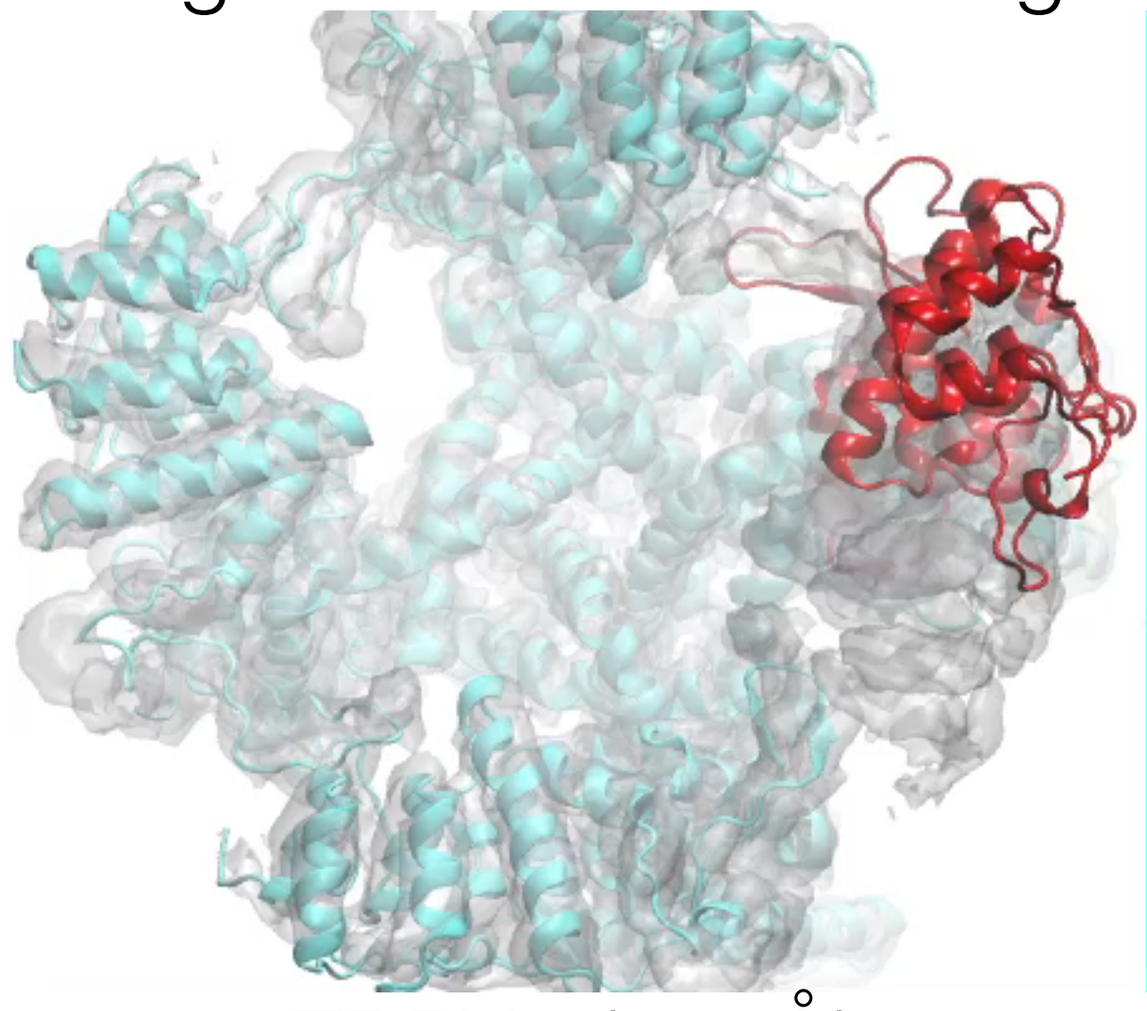
Cascade MDFF for **high-resolution** cryo-EM: Successively higher resolution maps



5 Å Smoothing | 3 Å Smoothing | No Smoothing

Protocol	Global correlation	RMSD (Å)
Reference	0.732	-
Direct	0.699	12.41
Cascade	0.724	2.30

Singharoy, Teo, McGreevy, et. al. eLife, 2016

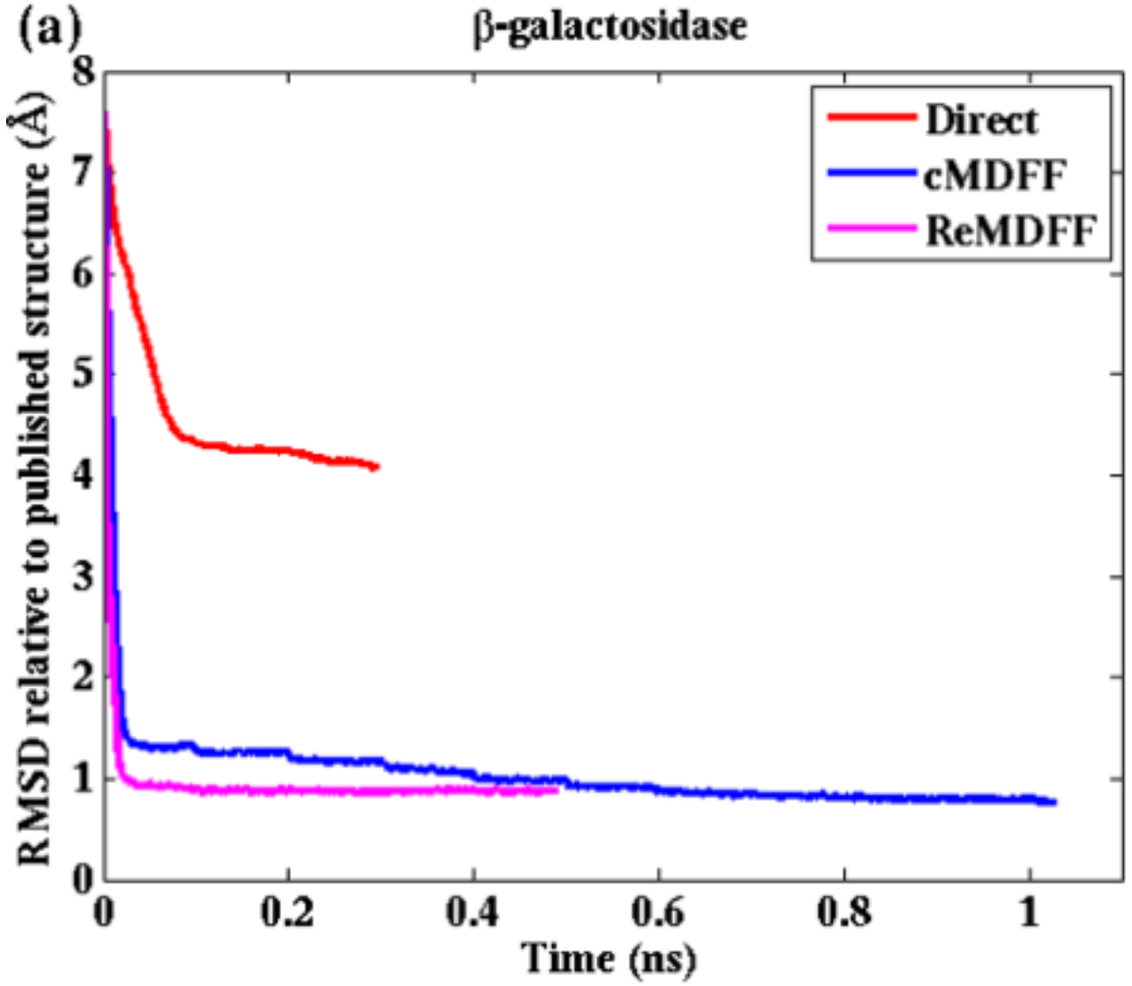


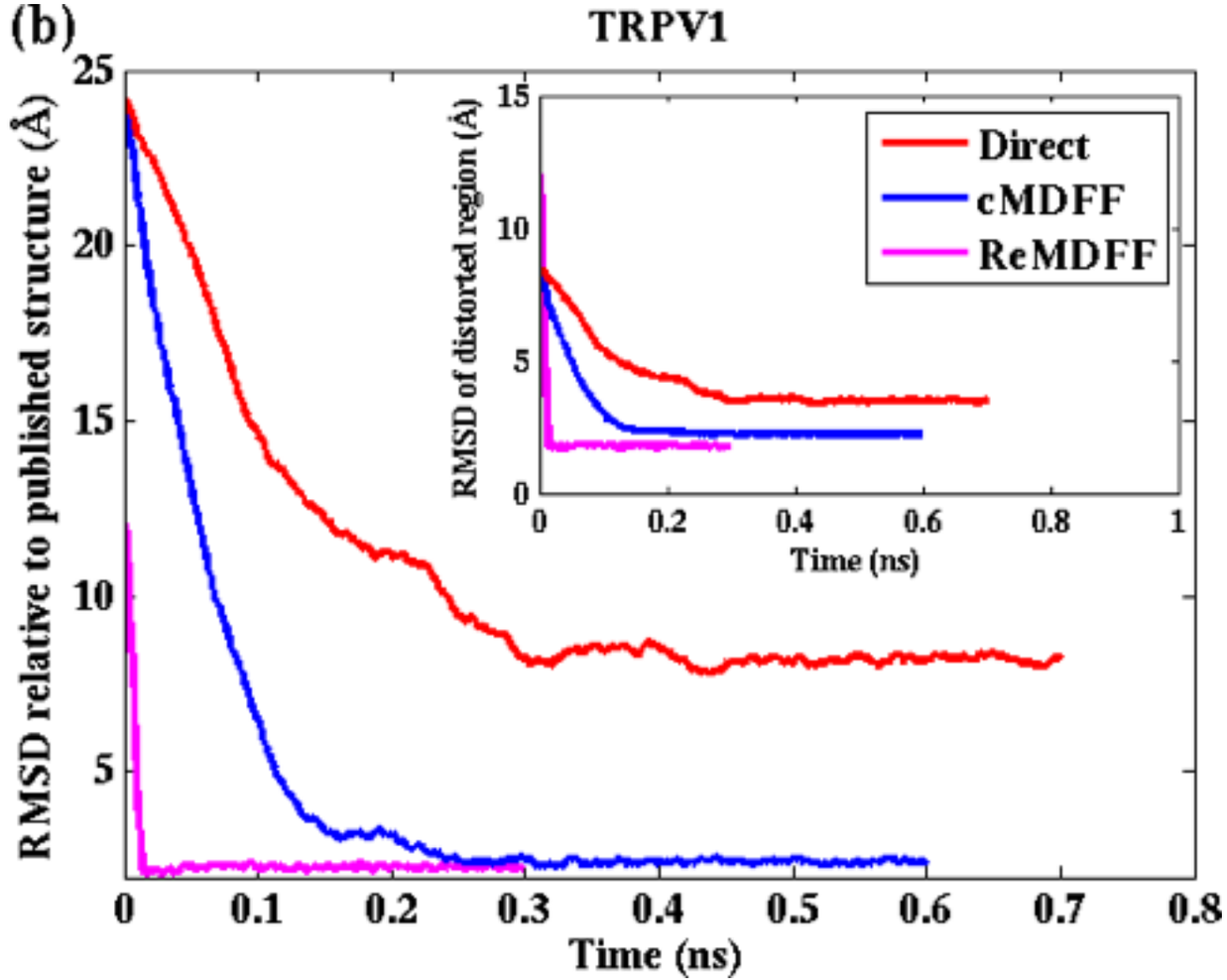
TRPV1 (3.4 Å)

M. Liao, E. Cao, D. Julius, Y. Cheng, Nature 504, 2013.

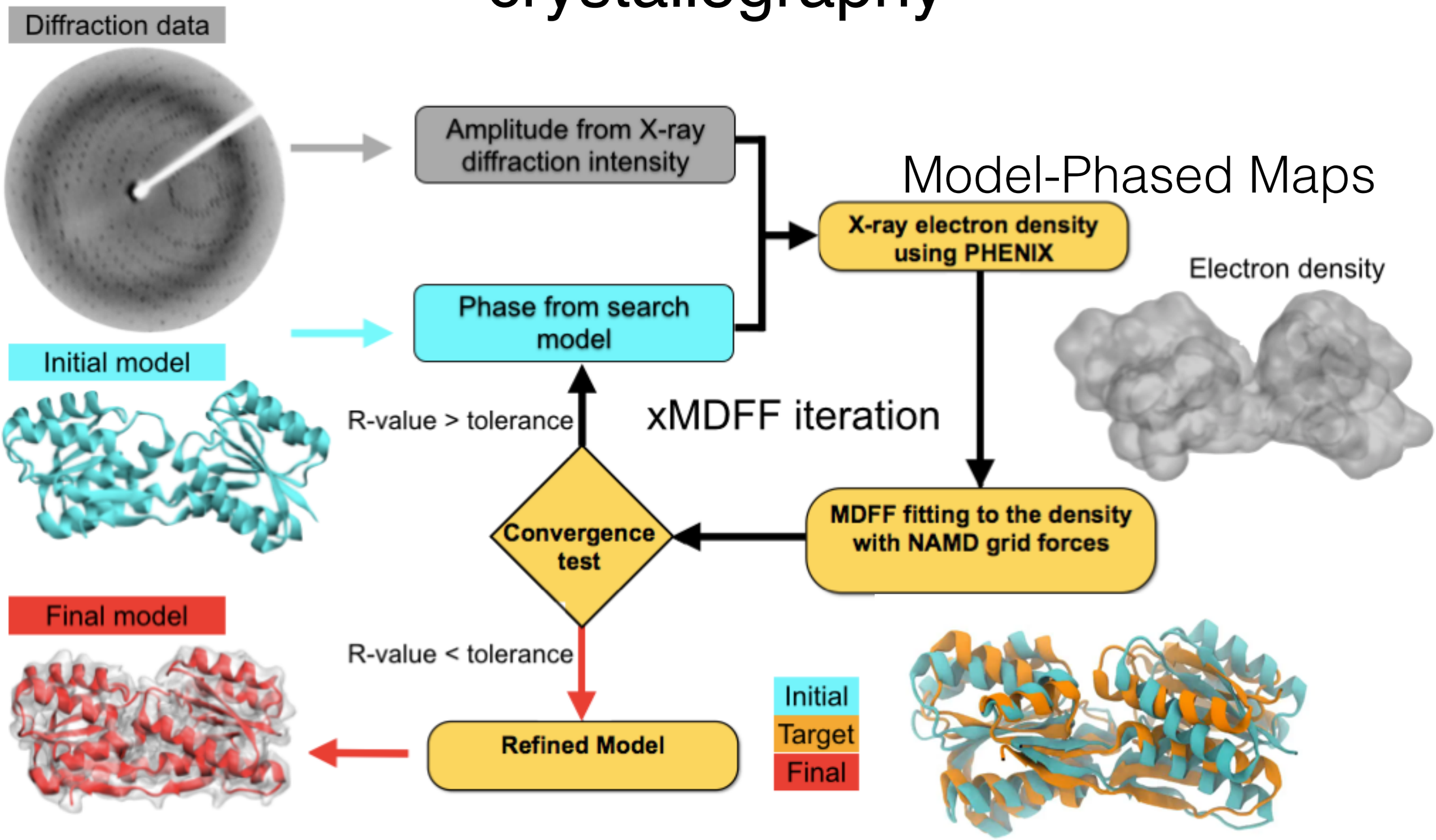
Resolution Exchange MDFF for **high-resolution** cryo-EM

- multiple maps blurred to varying resolution, like cMDFF
- independent parallel replicas (like Replica Exchange)
- each replica fits to a different map
- periodically exchange maps between replicas
- currently random exchange vs. parallel tempering which requires sufficient potential overlap of the energy distributions between neighboring replicas





xMDFF: MDFF for low-resolution x-ray crystallography



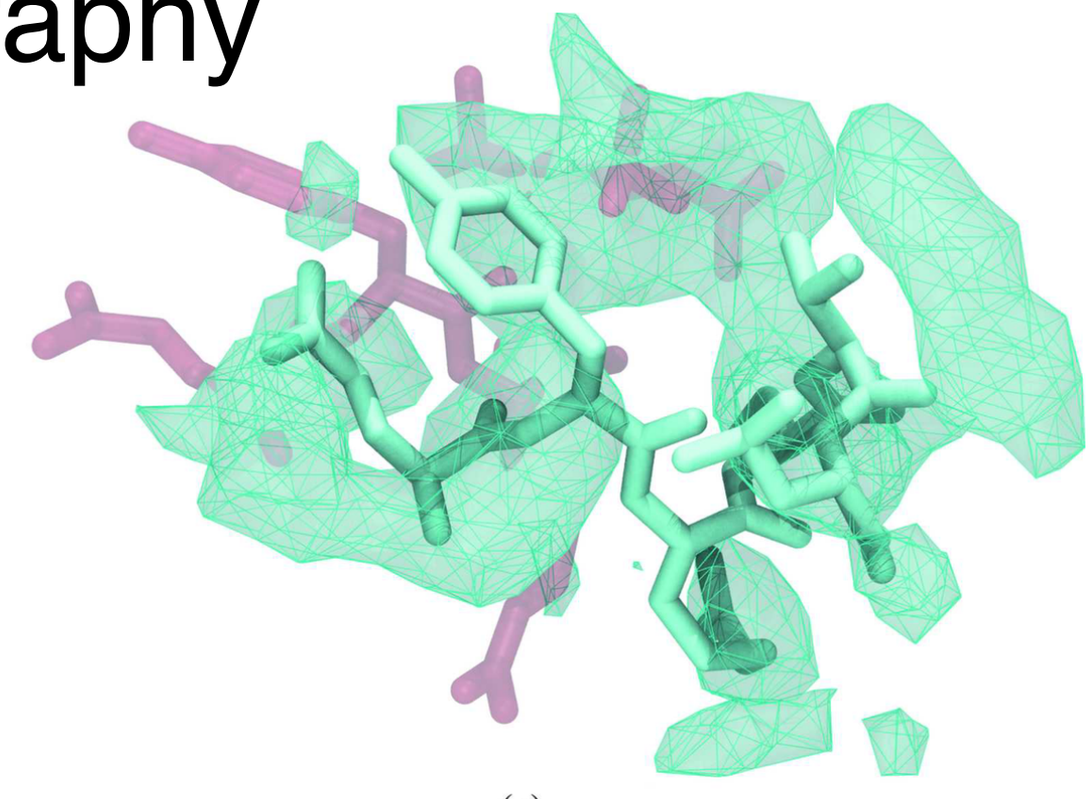
Ryan McGreevy*, Abhishek Singharoy*,et al. Acta Crystallographica D70, 2344-2355, 2014

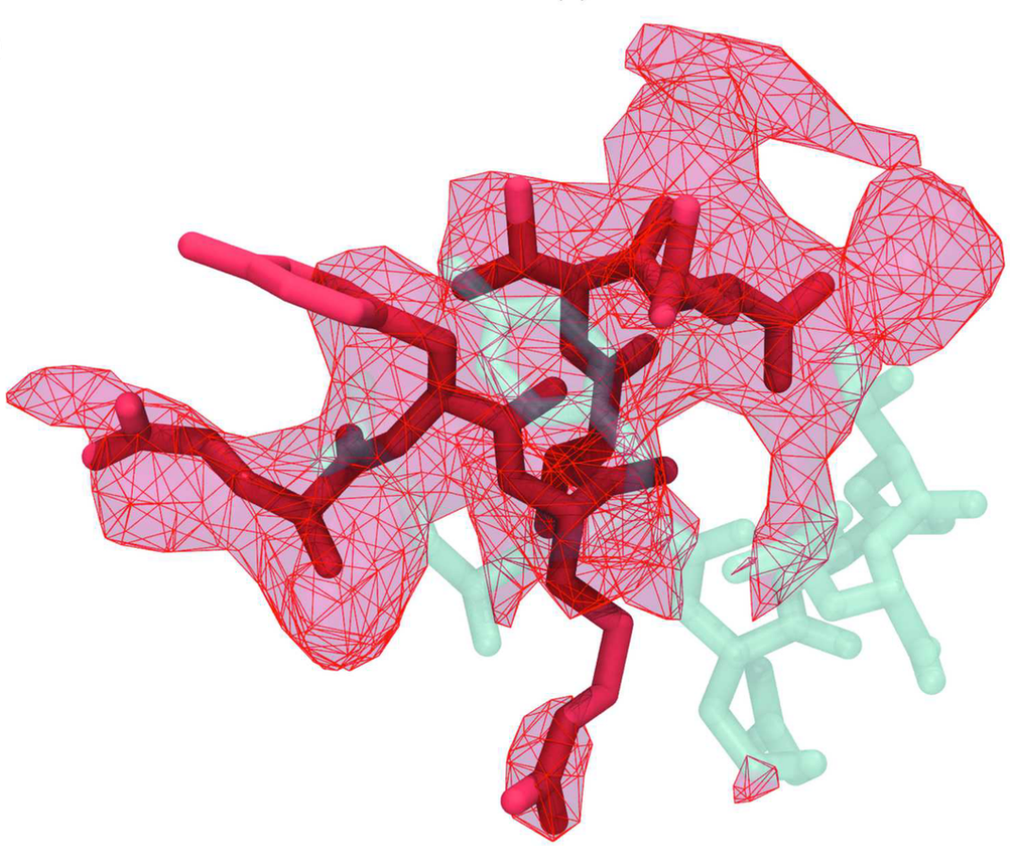
xMDFF: MDFF for low-resolution x-ray crystallography

- Periodically generate new 2mFo-DFc maps using phenix.maps

- "Difference" maps amplify the regions of the map in which portions of the true model are missing

- Can use any phenix.maps parameters, e.g., "Feature-enhanced maps" which reduce model bias and noise


(*a*)


(*b*)

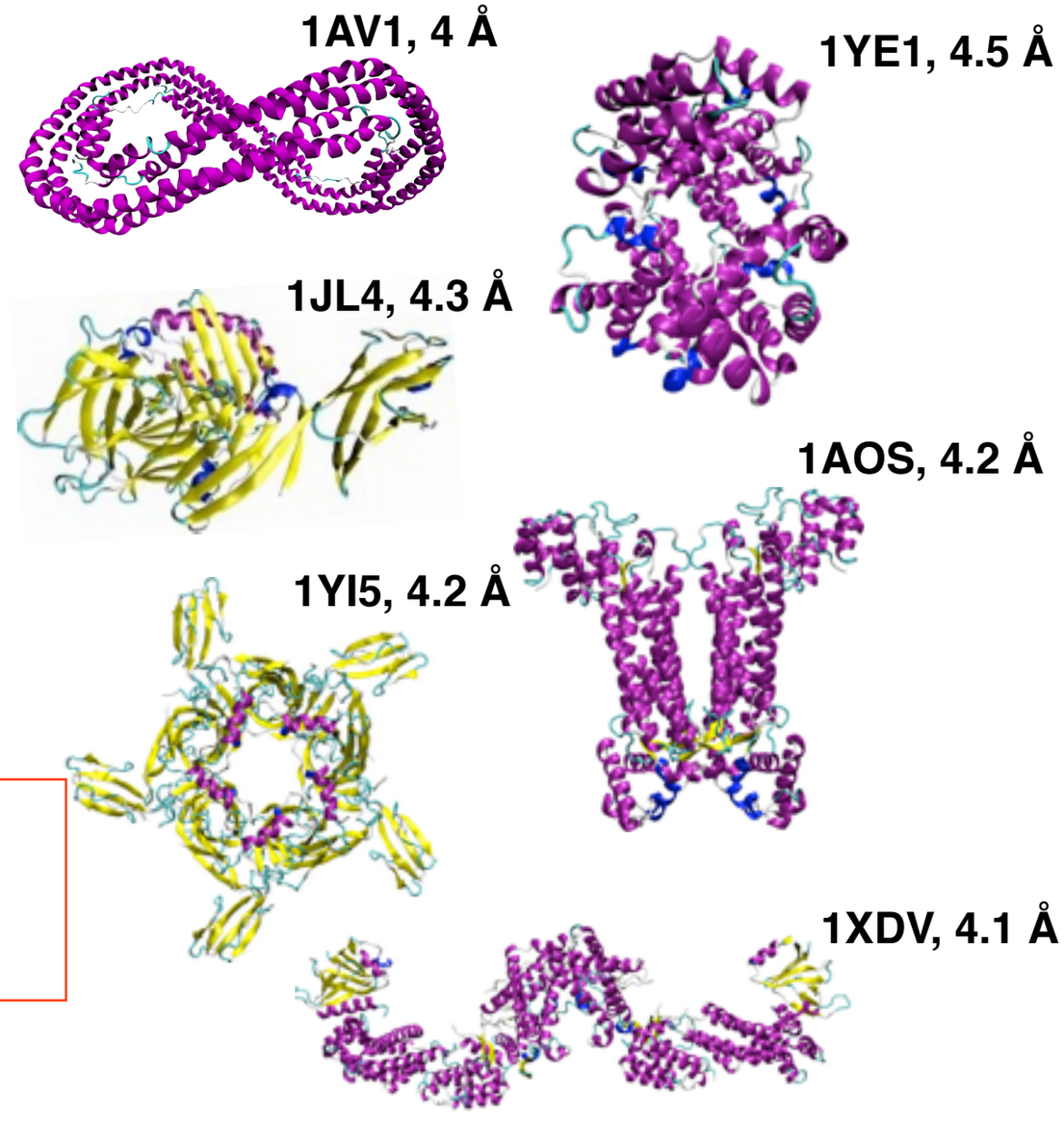
xMDFF Improves Structures Posted at the Protein Data Bank

Refinement statistics

PDB ID	Molprobity		R-work		R-free	
	initial	final	initial	final	initial	final
1AV1	3.72	1.94	0.38	0.33	0.42	0.34
1YE1	2.68	1.89	0.25	0.23	0.27	0.24
1JL4	3.24	1.47	0.36	0.33	0.42	0.38
1AOS	3.40	2.45	0.21	0.20	0.24	0.23
1XDV	2.87	2.01	0.39	0.29	0.41	0.33
1YI5	3.08	1.73	0.27	0.26	0.31	0.29

- *Better R-work and R-free values than published before.*
- *Close R-work and R-free implies less over-fitting.*
- *Improved geometry implied by low Molprobity score.*

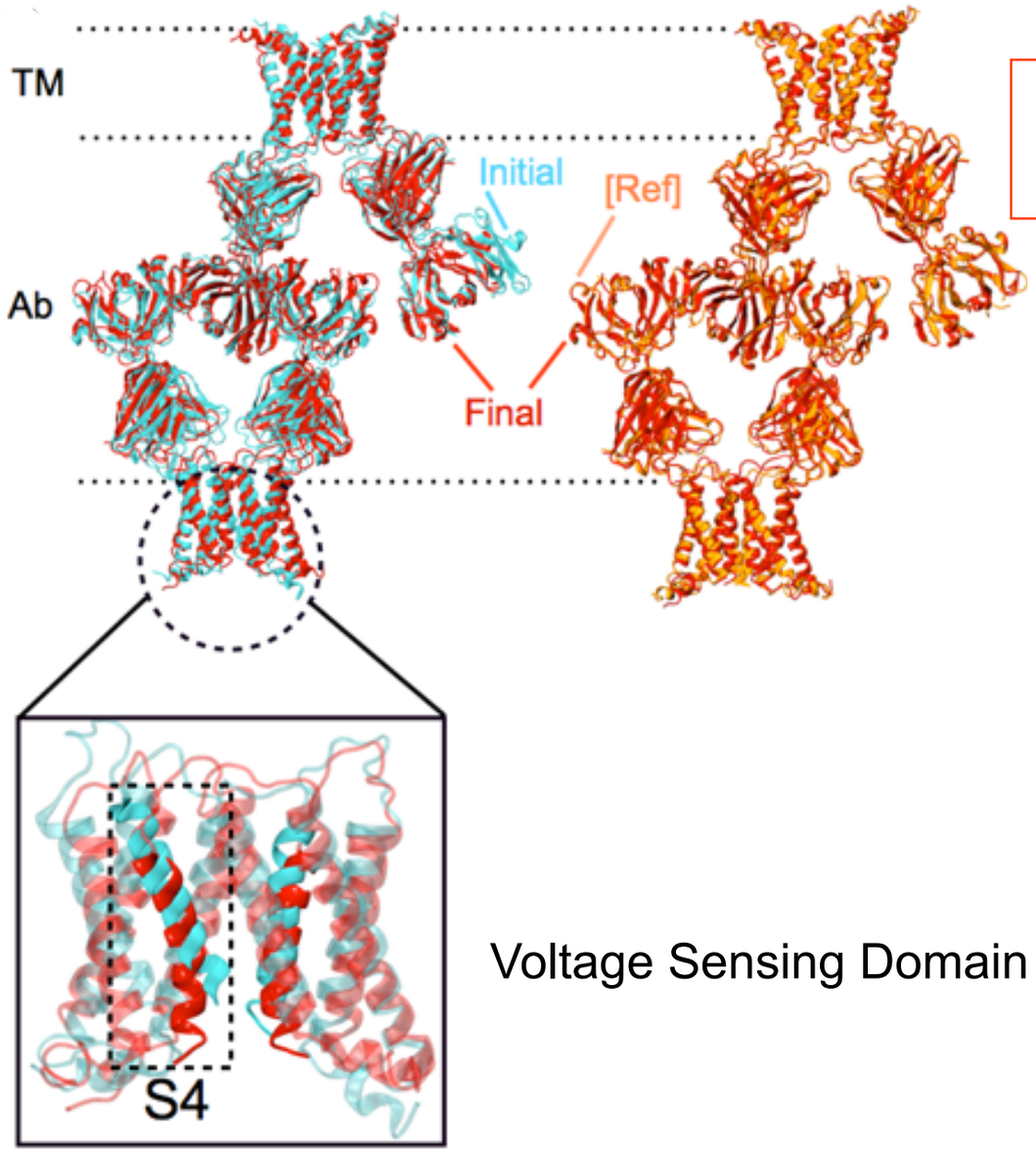
Structures



McGreevy, Singharoy, et. al. Acta Cryst. D70 2344-2355, 2014.

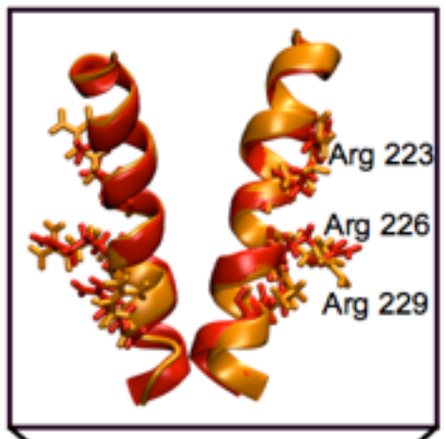
xMDFF Solves Voltage Sensor Protein Structure at 4 Å Resolution

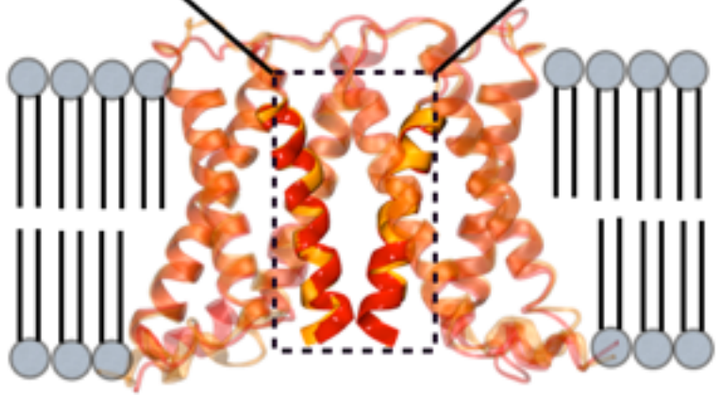
Collaboration with E. Perozo (U. Chicago)



Voltage Sensing Domain

- *xMDFF reproduces helix position and arginine alignment.*
- *Refined model confirms electrophysiological measurements.*





<u>Search model preparation</u>

Largest xMDFF structure has **2252 amino acids**

Search model used from **MUFOLD structure prediction** software (Dong Xu U. Missouri)

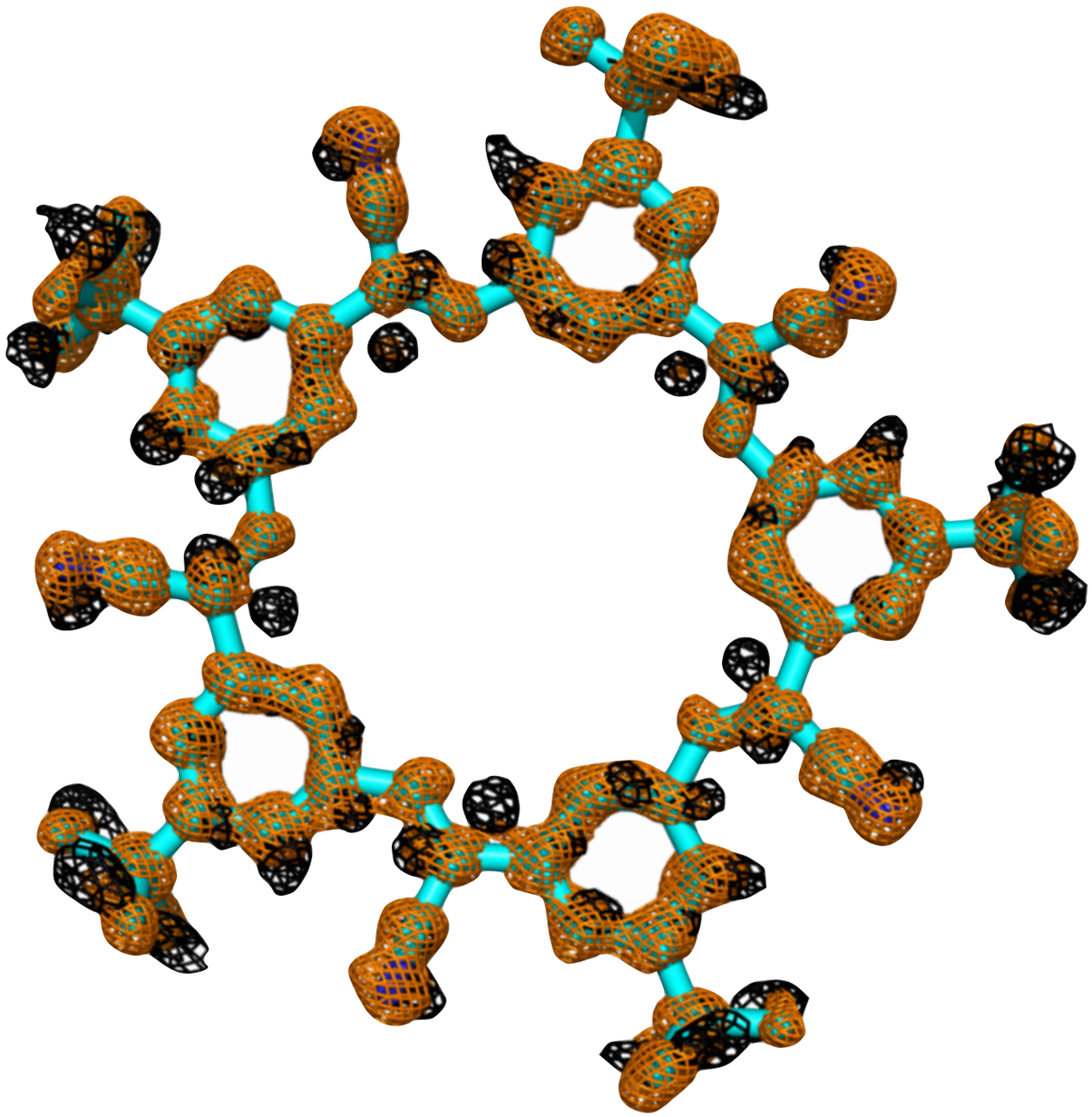
<u>Refinement statistics</u>

	initial	final
R:	0.45	0.26
R-free:	0.47	0.28
score:	3.07	2.19
helix RMSD:	4.65	1.34

Qufei Li, et. al. Nature Structural & Molecular Biology, 21:244-252, 2014

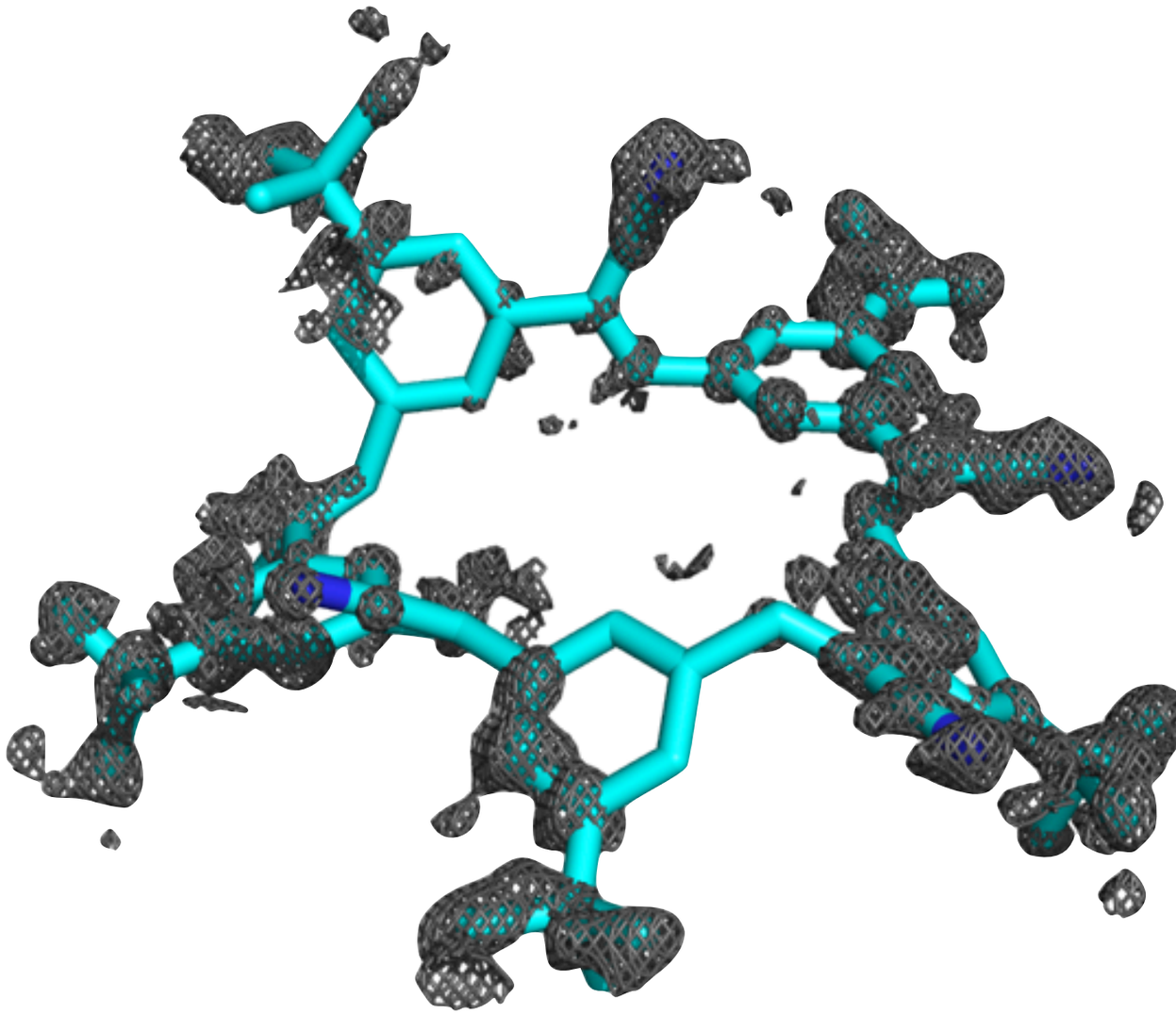
xMDFF for Abiological Materials

Cyanostar (2Å)



xMDFF-Phenix

(dual occupancy of CS shown in black and orange)



Phenix-only

Singharoy, et al. J. Am. Chem. Soc.137 (27), pp 8810–8818, 2015.