# Molecular Dynamics Flexible Fitting

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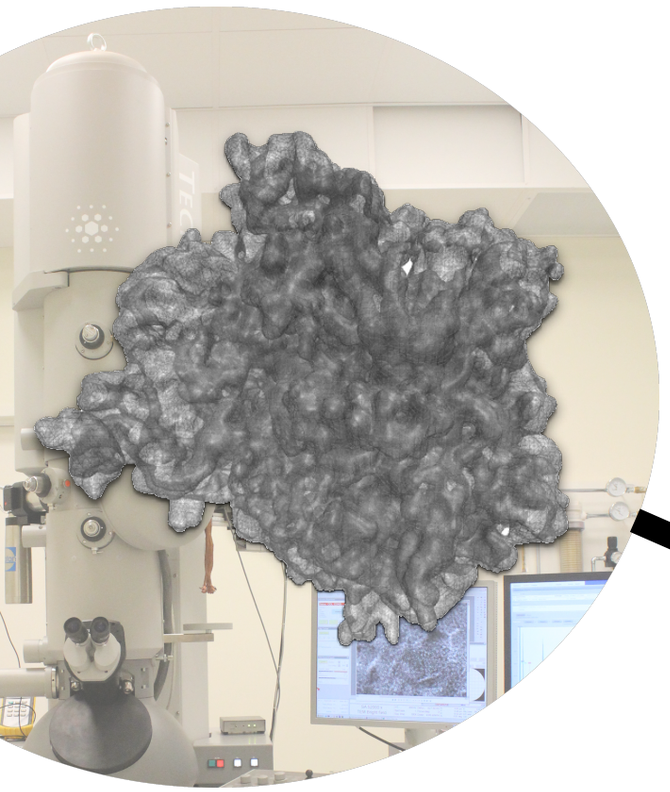
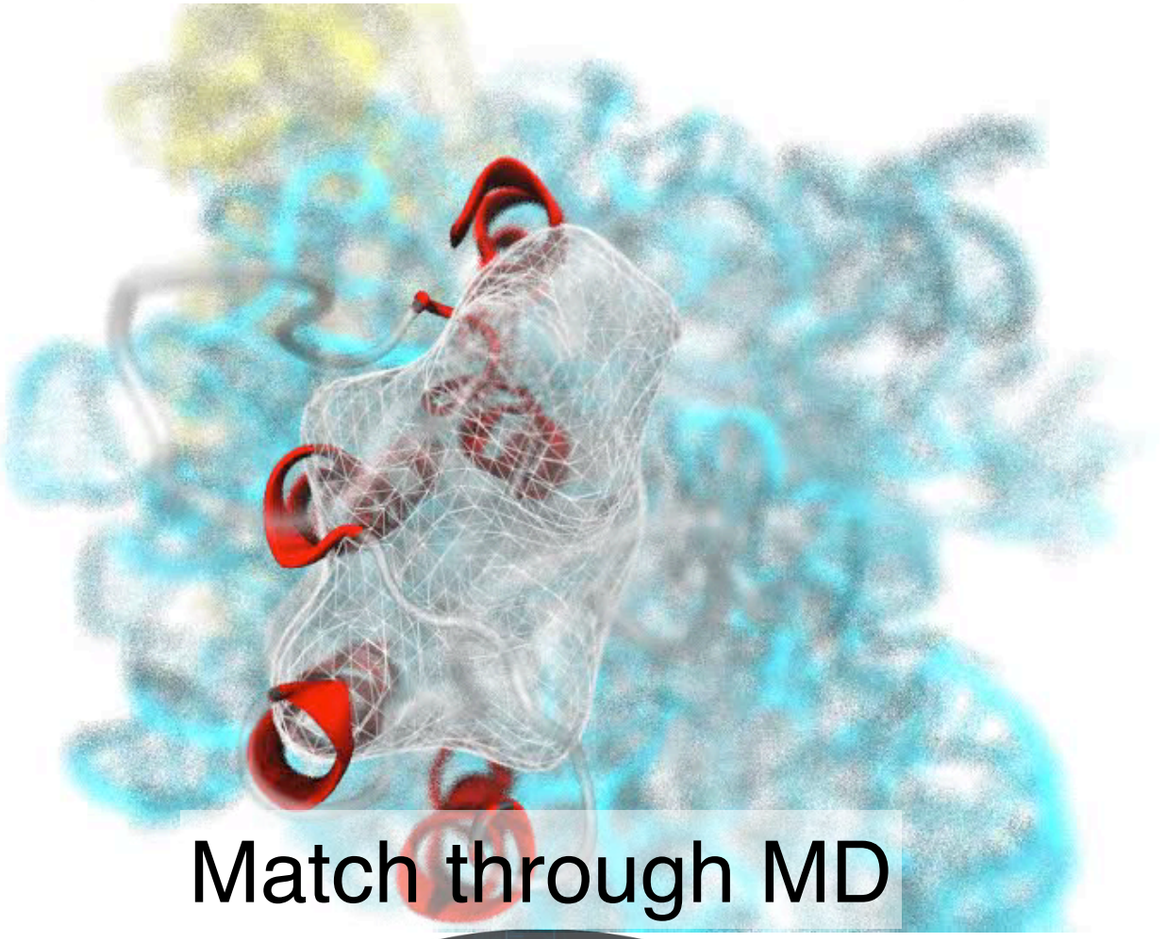
# **M**olecular **D**ynamics **F**lexible **F**itting

(Ribosome-bound YidC)

Electron Microscope

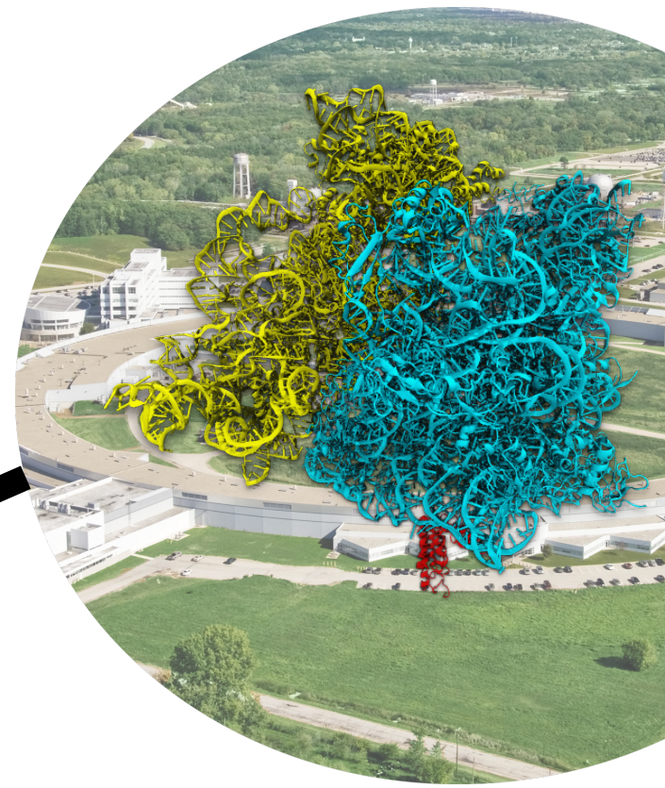


EM density map

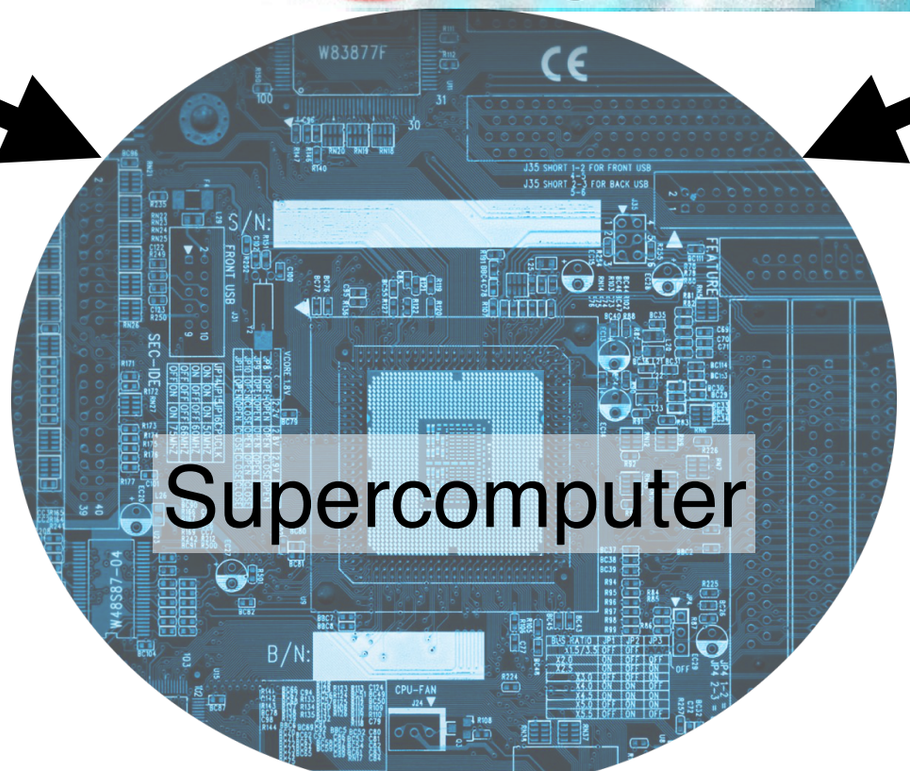


Match through MD

APS Synchrotron



crystallographic structure



Supercomputer

# Molecular Dynamics Flexible Fitting - Theory

Two terms are added to the MD potential

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

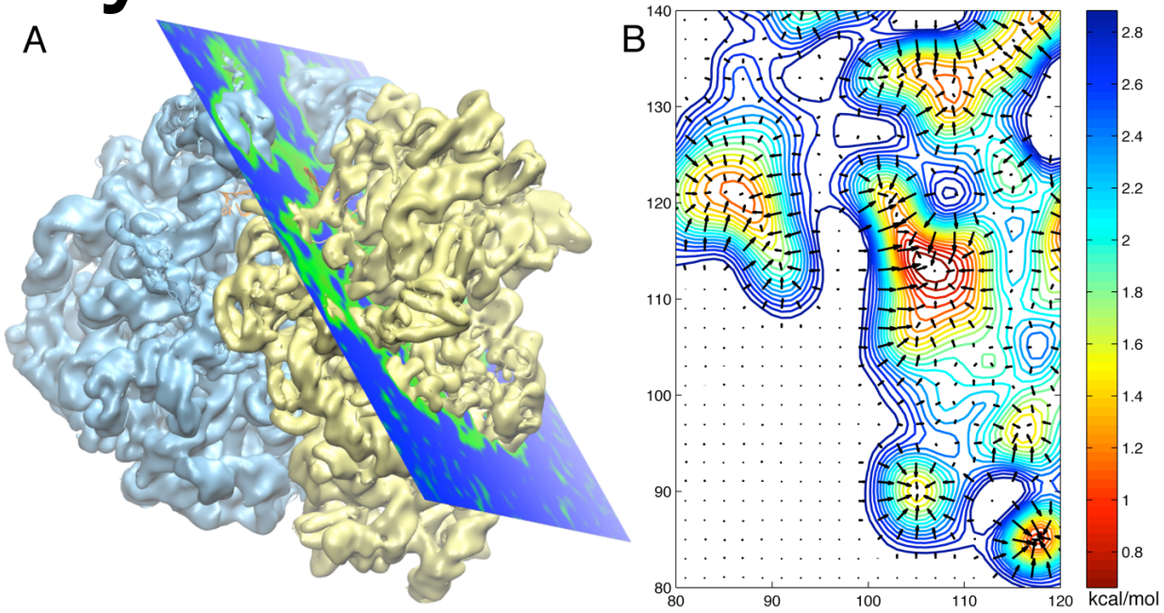
An external potential derived from the EM map is defined on a grid as

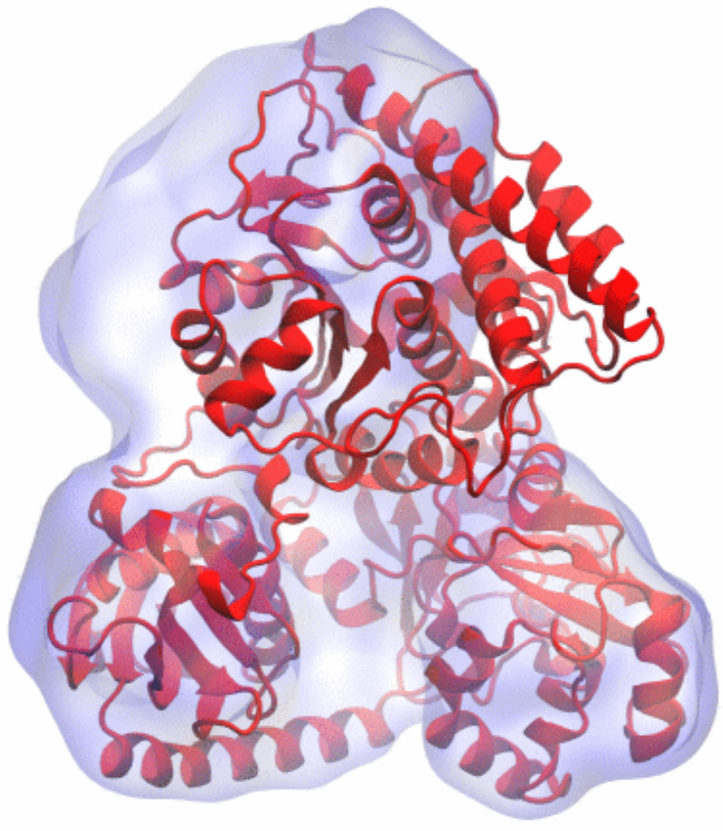
$$U_{EM}(\mathbf{R}) = \sum_j w_j V_{EM}(\mathbf{r}_j)$$

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

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.**

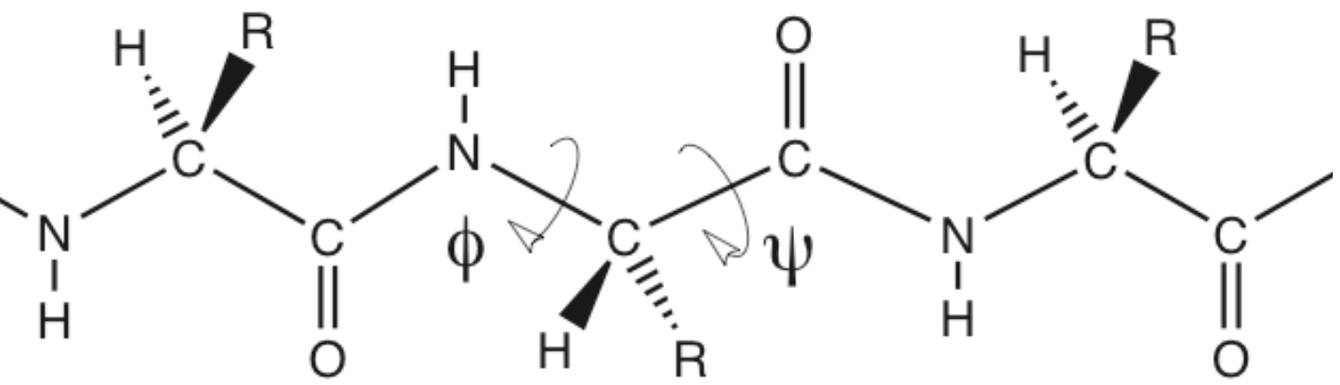
**[2] Trabuco et al. *Methods* (2009) 49:174-180.**

# Secondary structure restraints

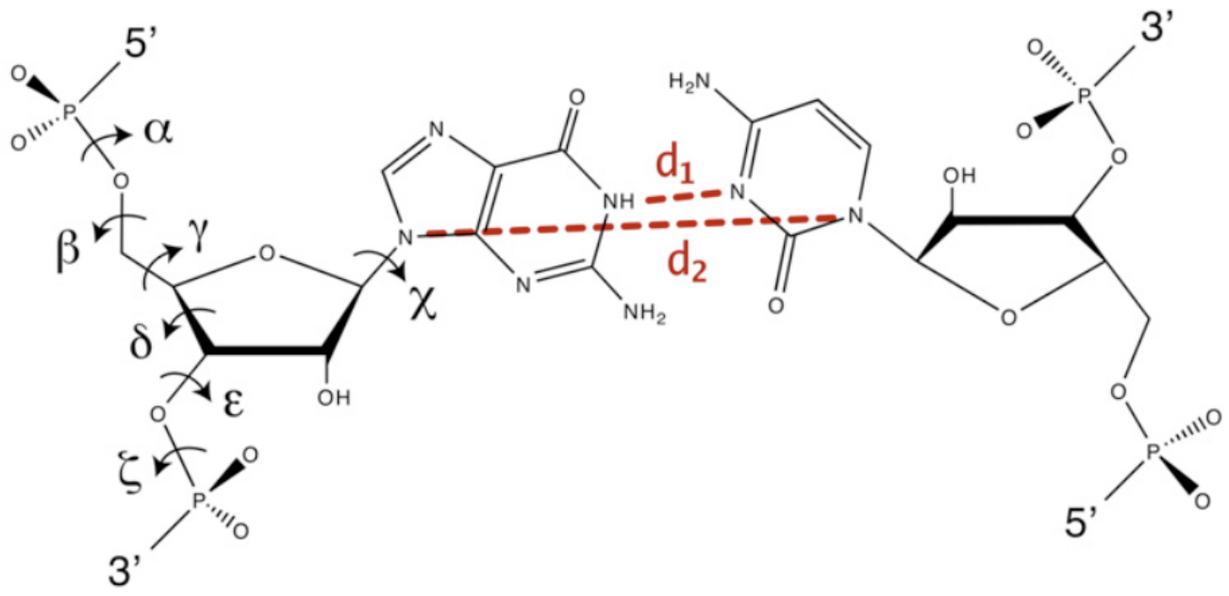
Harmonic restraints are applied to preserve secondary structure of proteins and nucleic acids, avoiding “overfitting.”

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

For proteins, $\phi$ and $\psi$ dihedral angles of residues within helices or beta strands are restrained.



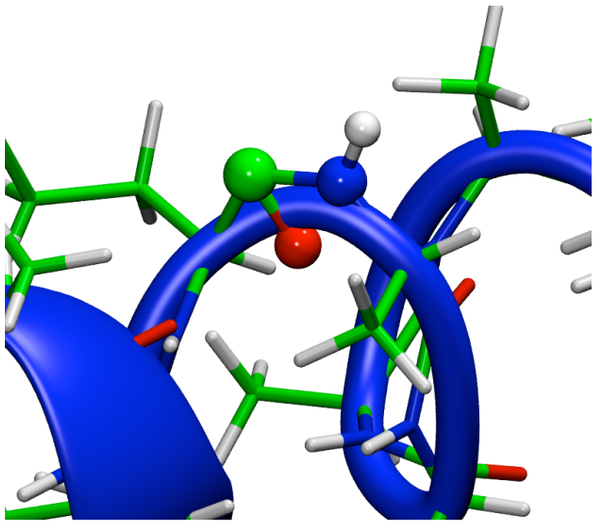

For nucleic acids, distance and dihedral restraints are applied to a selected set of base pairs.



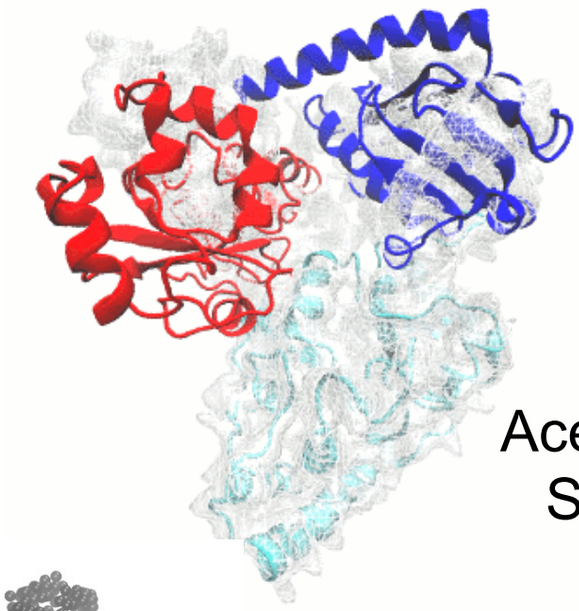
# Additional Restraints

## Cis-peptide and Chirality

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

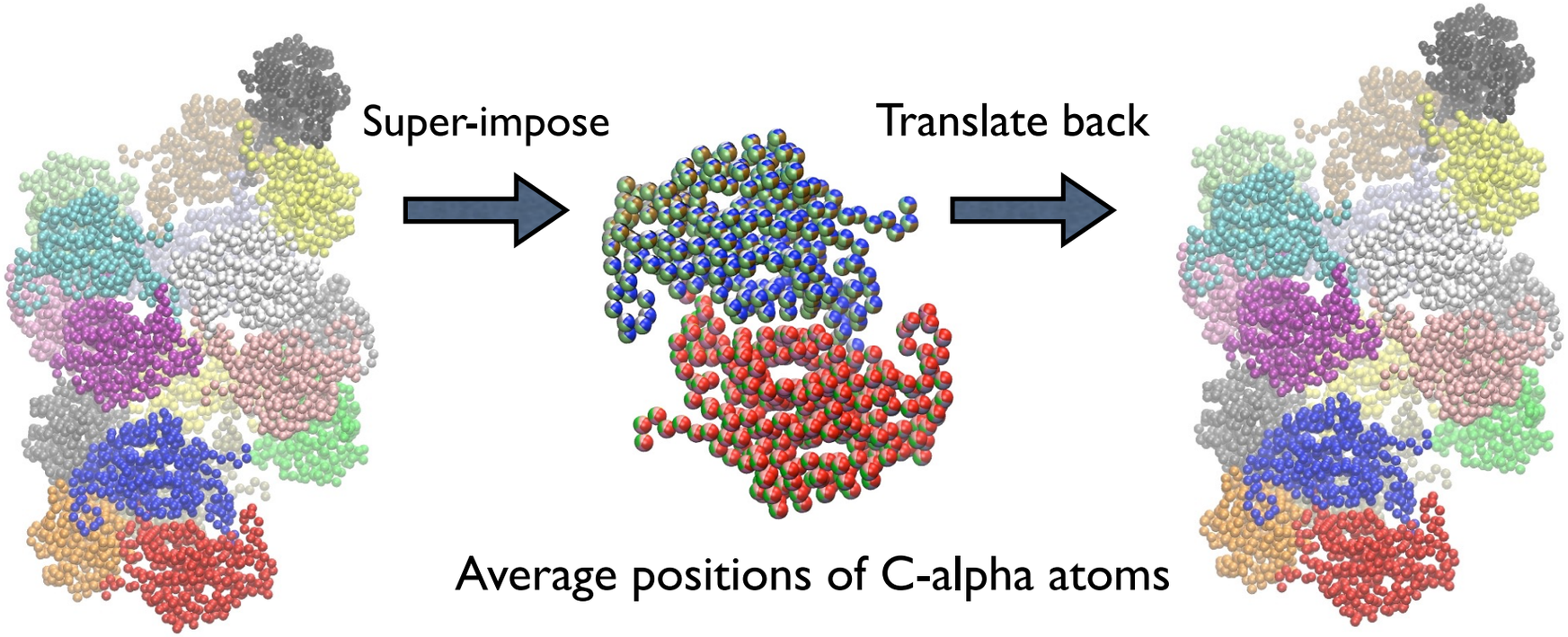


## Domain-wise

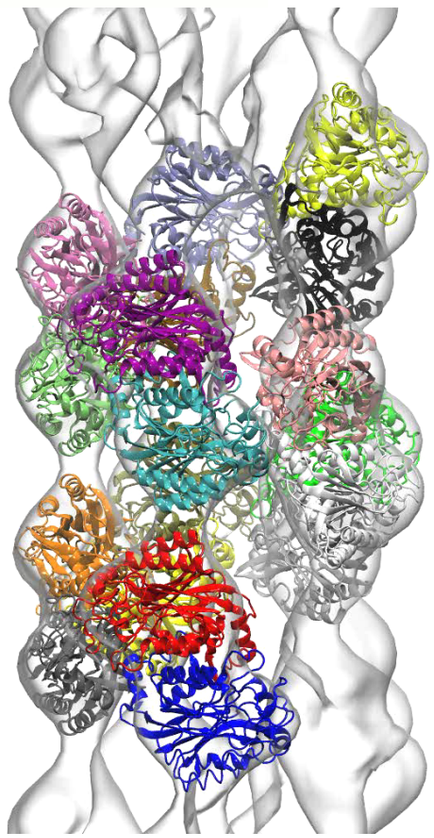


Acetyl – CoA Synthase

## Symmetry




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

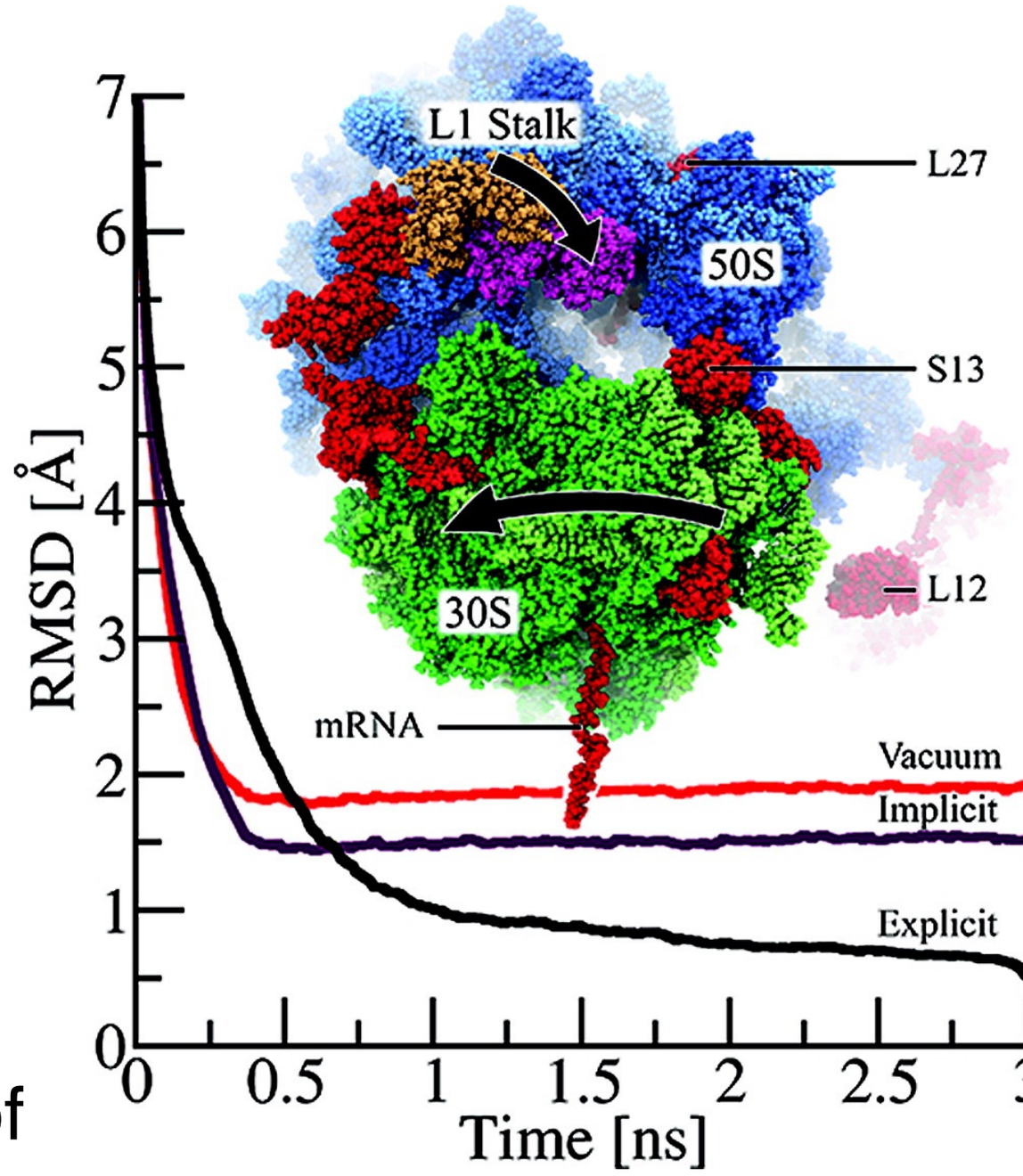


B. pumilus cyanide dihydratase

# Simulation Environment

MDFF can be run in different environments:

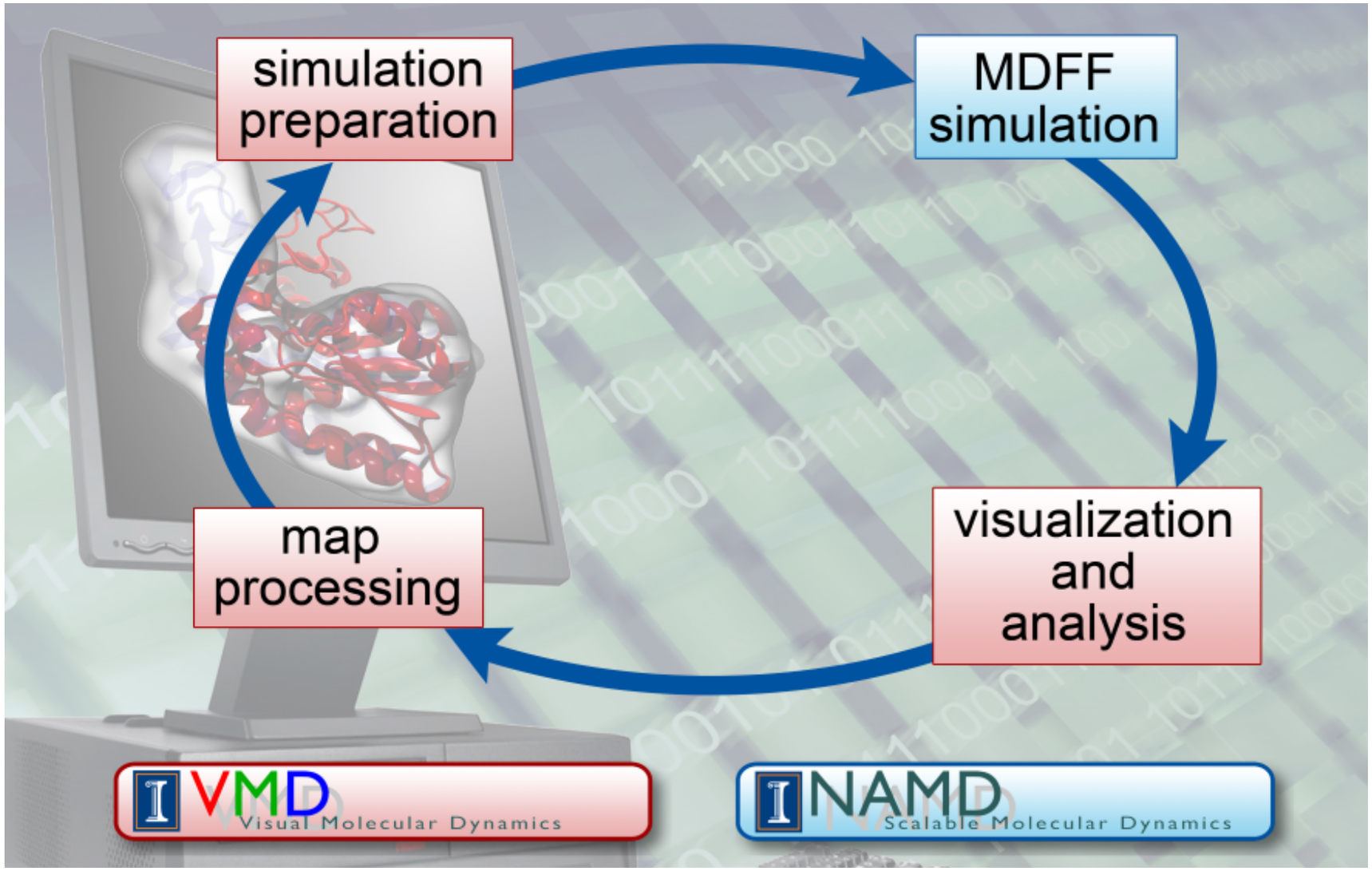
1. <u>Vacuum</u>
   - No water molecules
   - Fastest but potentially inaccurate
2. <u>Explicit Solvent</u>
   - Explicit atomic detail water molecules
   - Computationally slow and introduces effects of viscous drag
3. <u>Implicit Solvent</u>
   - generalized Born approximation of electrostatics
  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

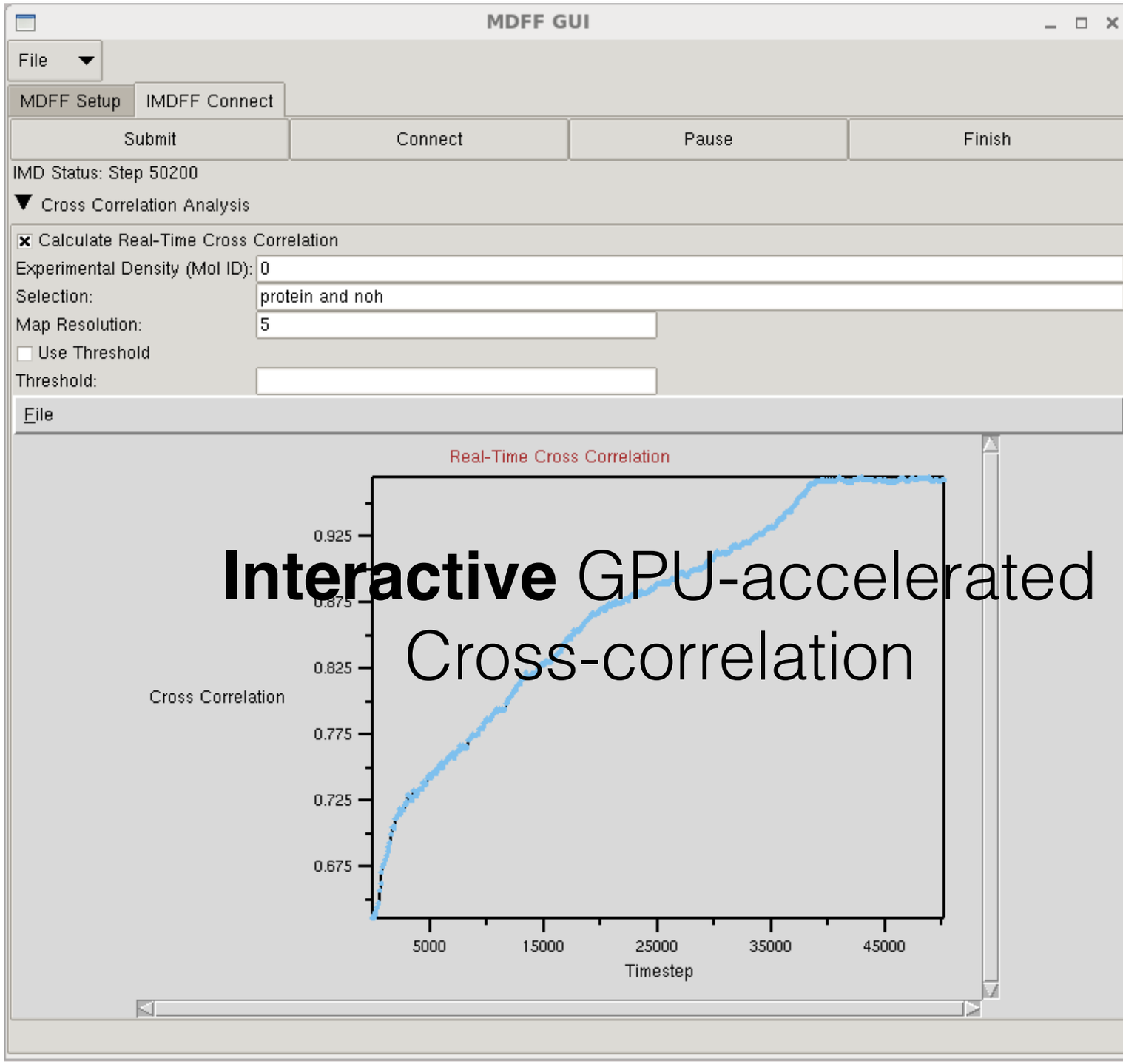
**Output:** produces simulation trajectory from which an ensemble of structures can be extracted

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




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

# Obtaining Initial Structures

1. X-ray crystallography or NMR structures

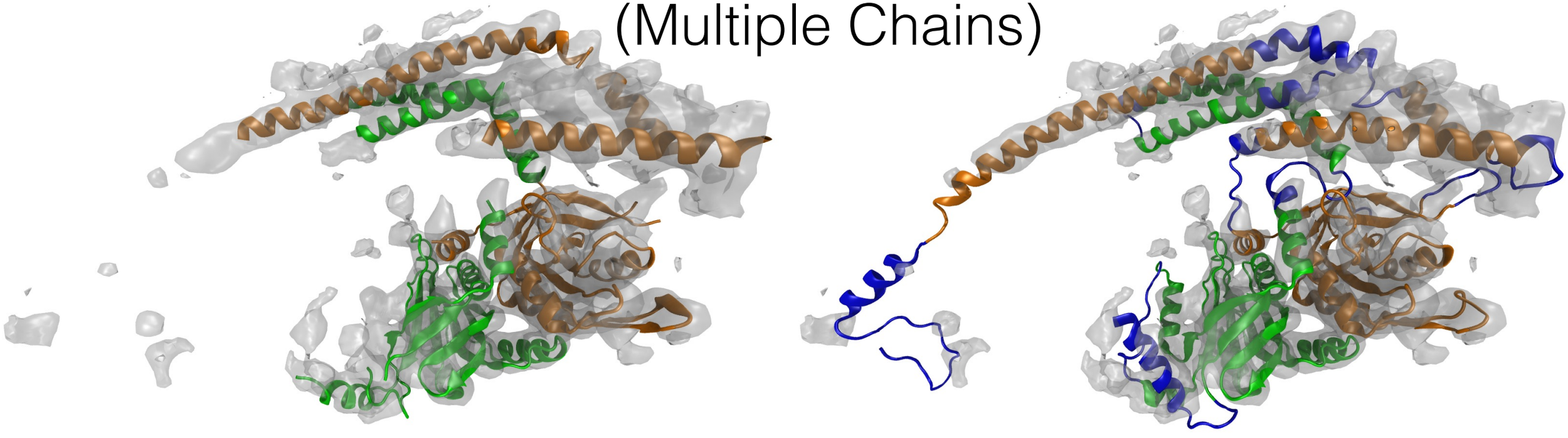
2. Refine structures from low-res X-ray data with xMDFF

   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)

ModelMaker (Multiple Chains)

Rpn8/Rpn11



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**

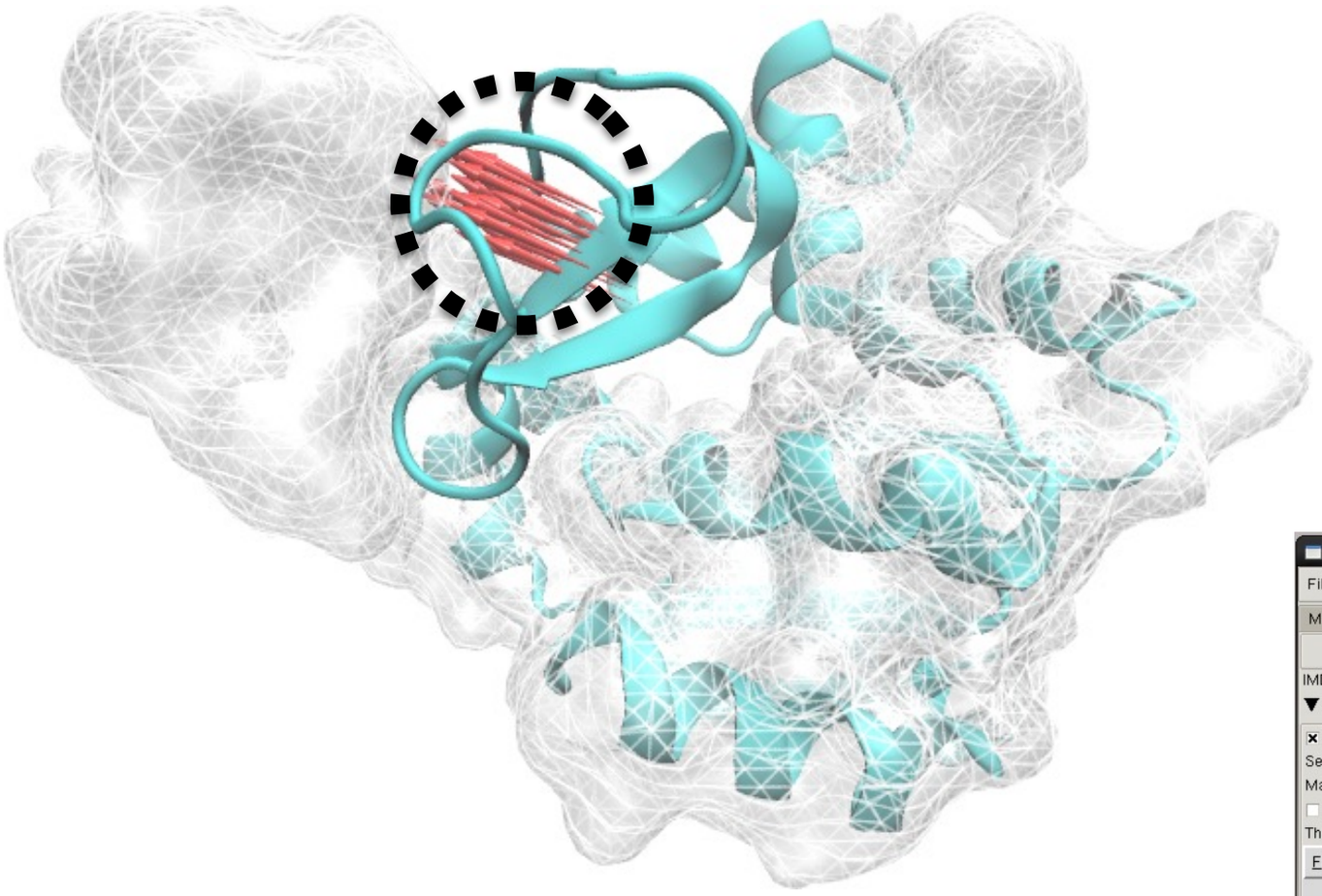
Leaver-Fay *et al.* Methods Enzymol. 2011
Porter *et al.* PLoS One 2015

**VMD/NAMD**

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

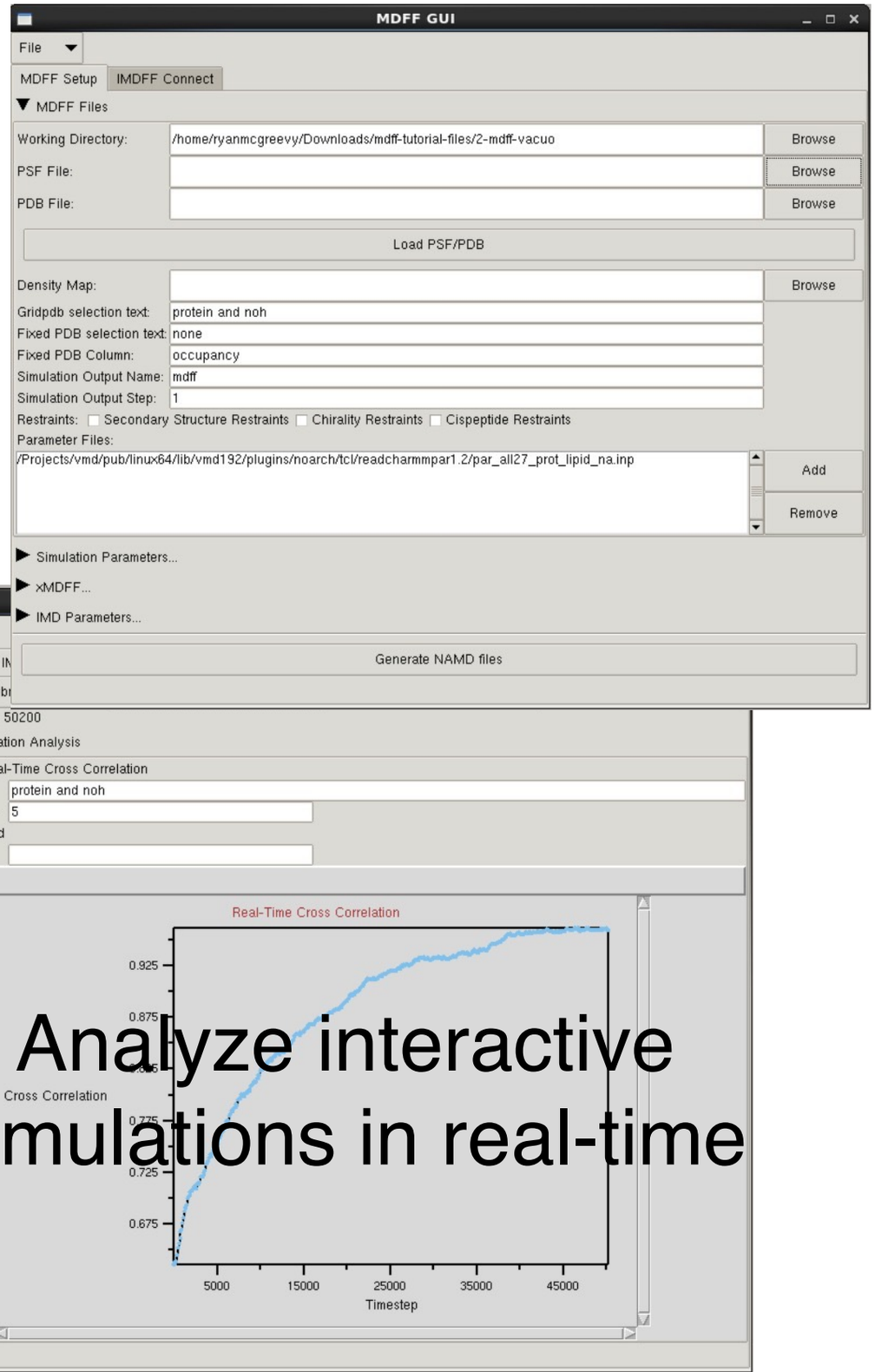
# Interactive Modeling with MDFF GUI

- 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

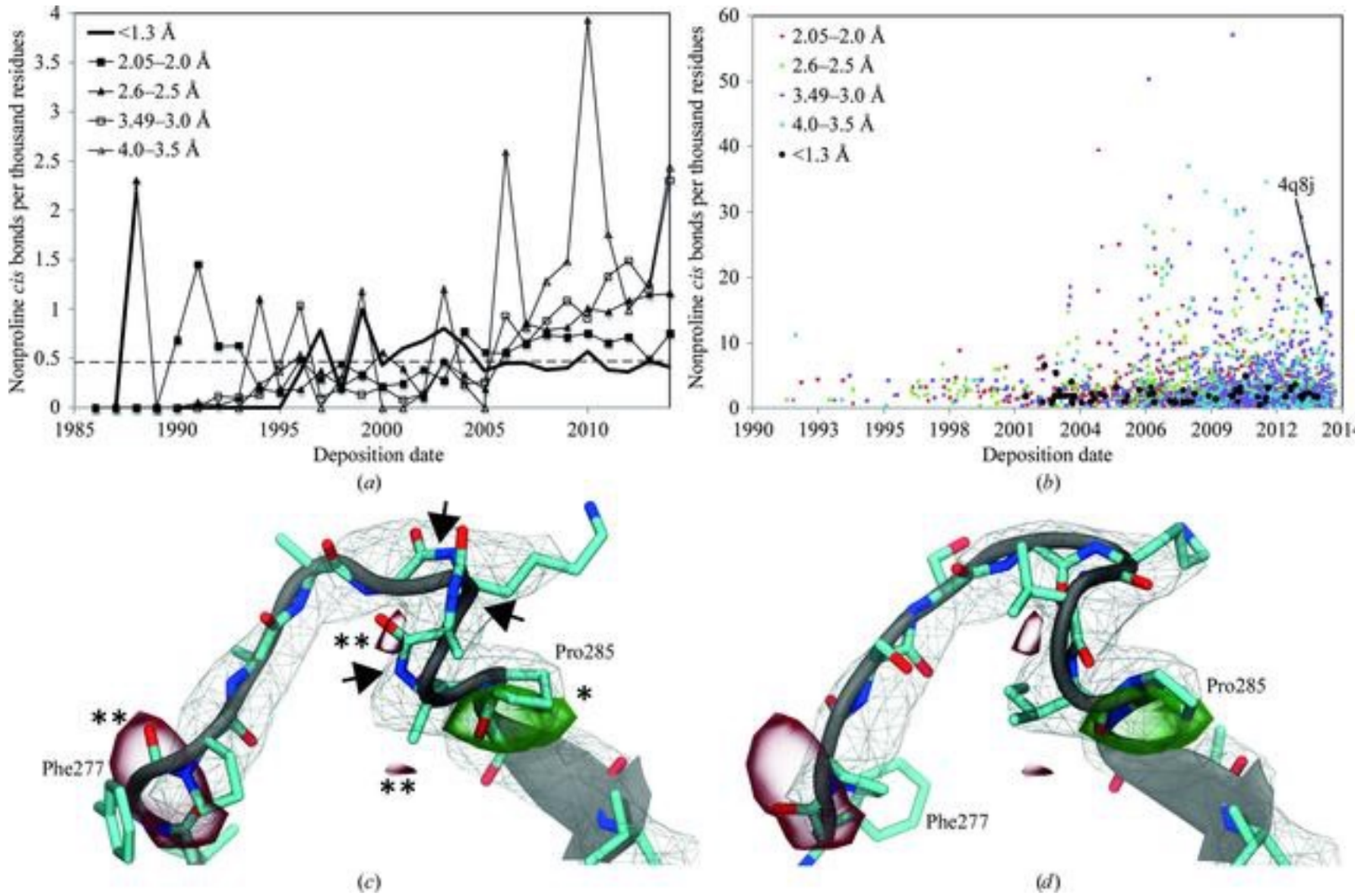



Analyze interactive simulations in real-time

# Importance of Checking Initial Structure

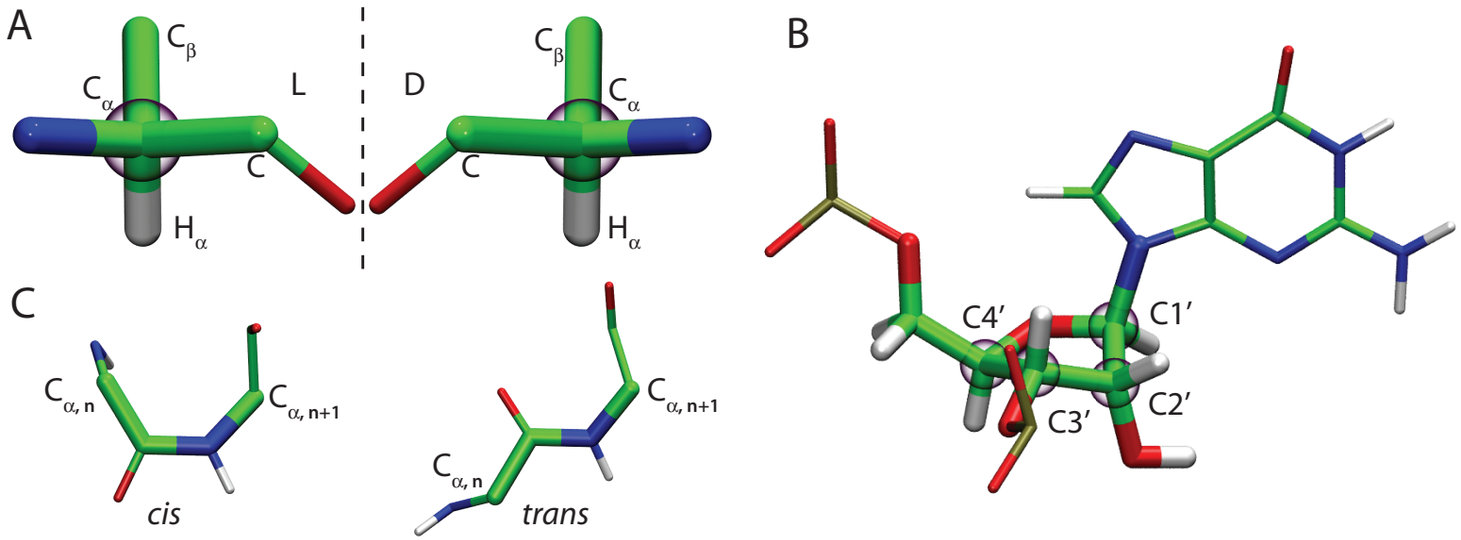
<0.05% non-proline bonds found in the cis conformation natively, however:

- 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

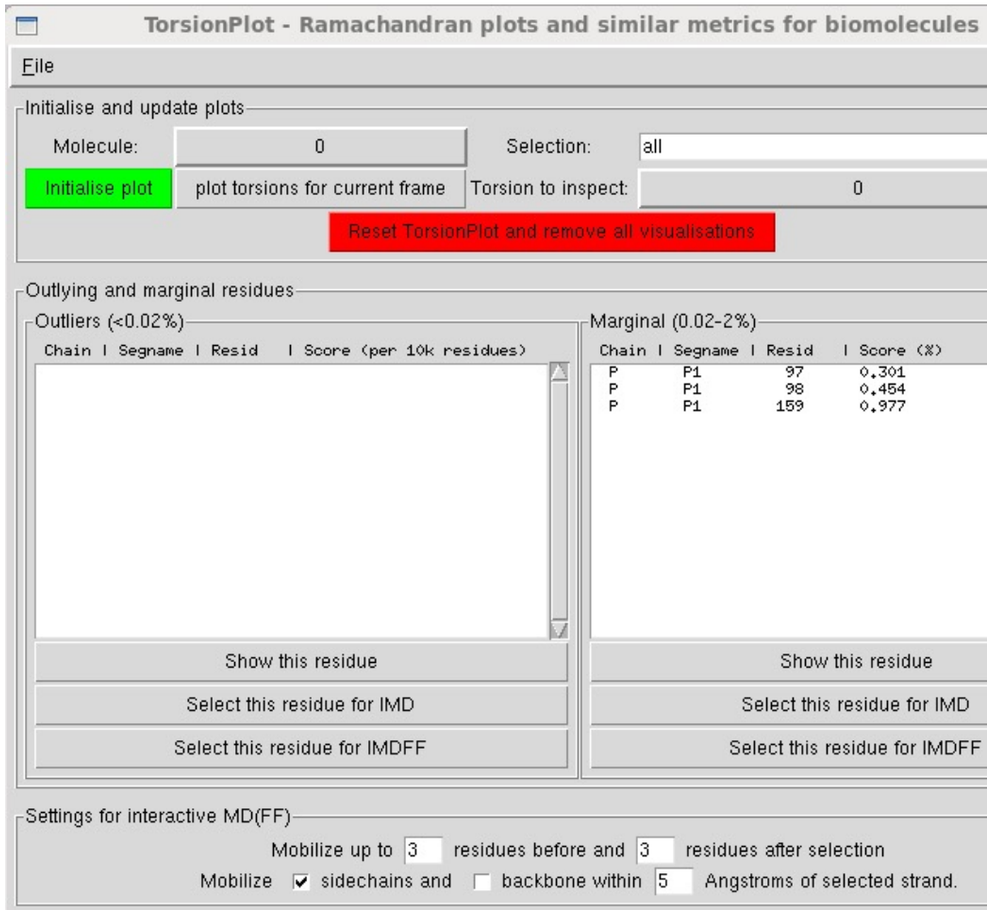


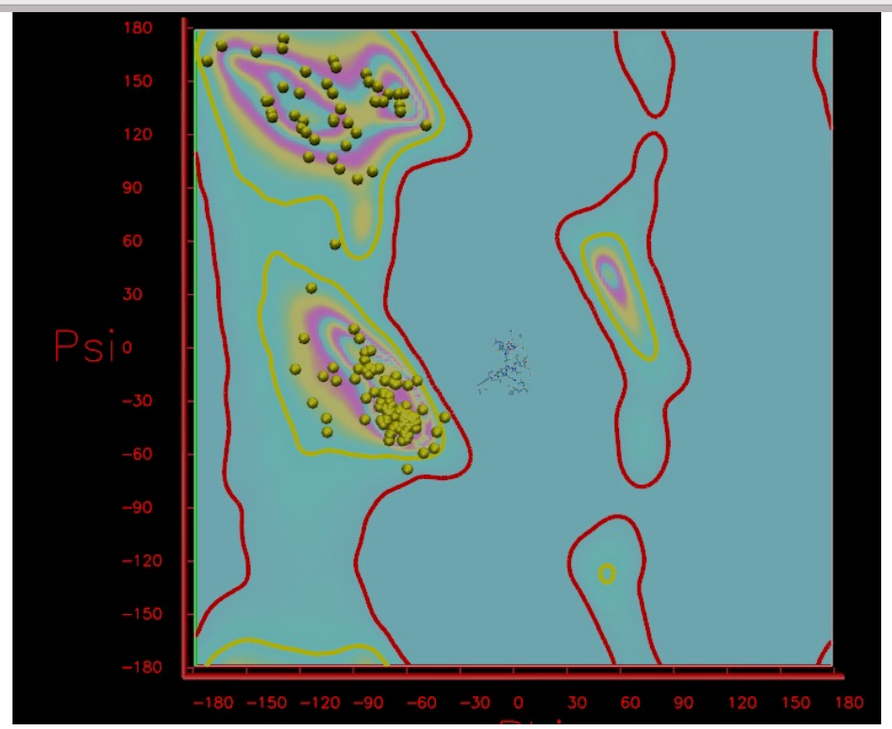

**Eduard Schreiner, et al. BMC Bioinformatics, 12, 190, 2011**

- 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

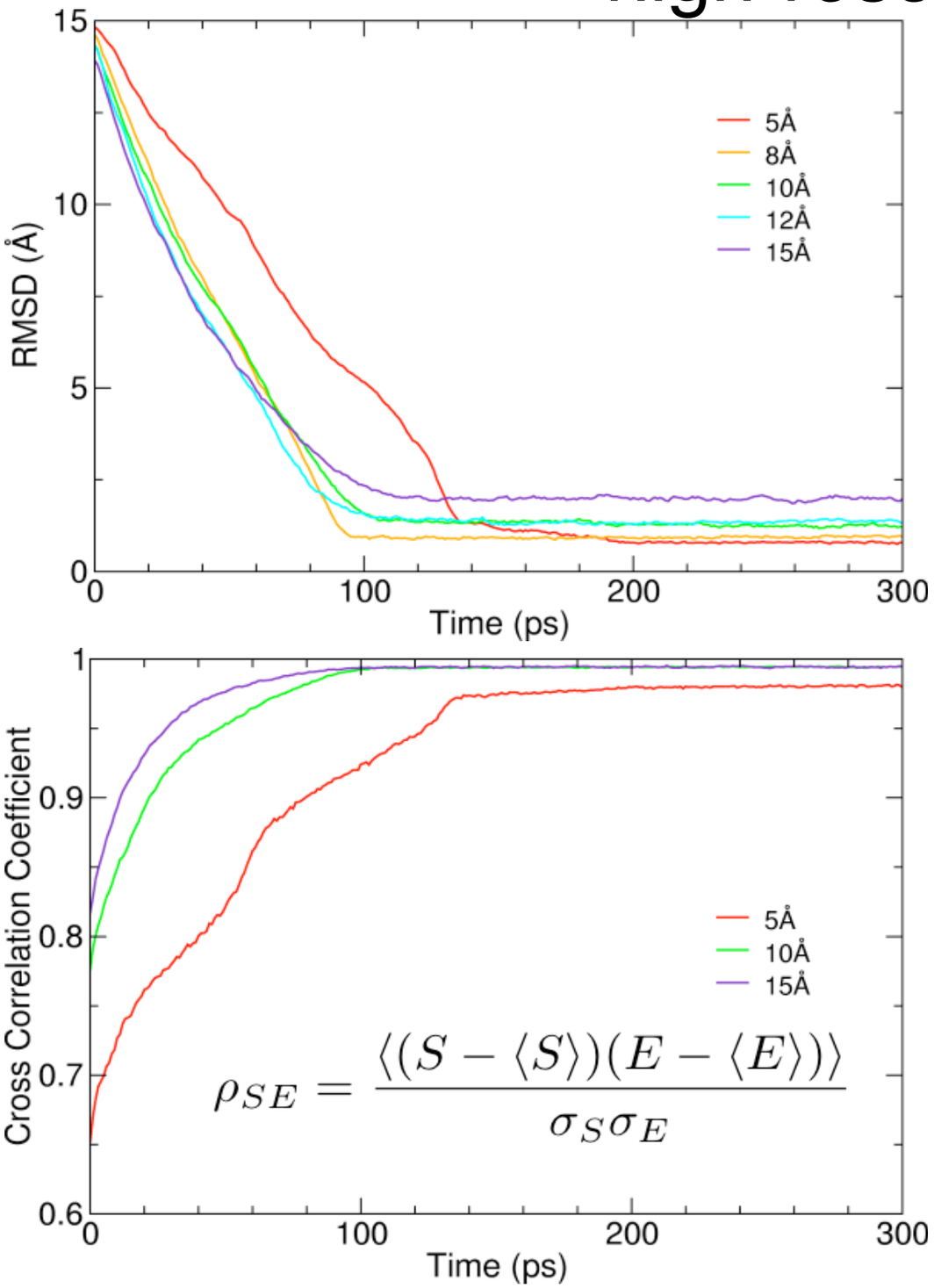


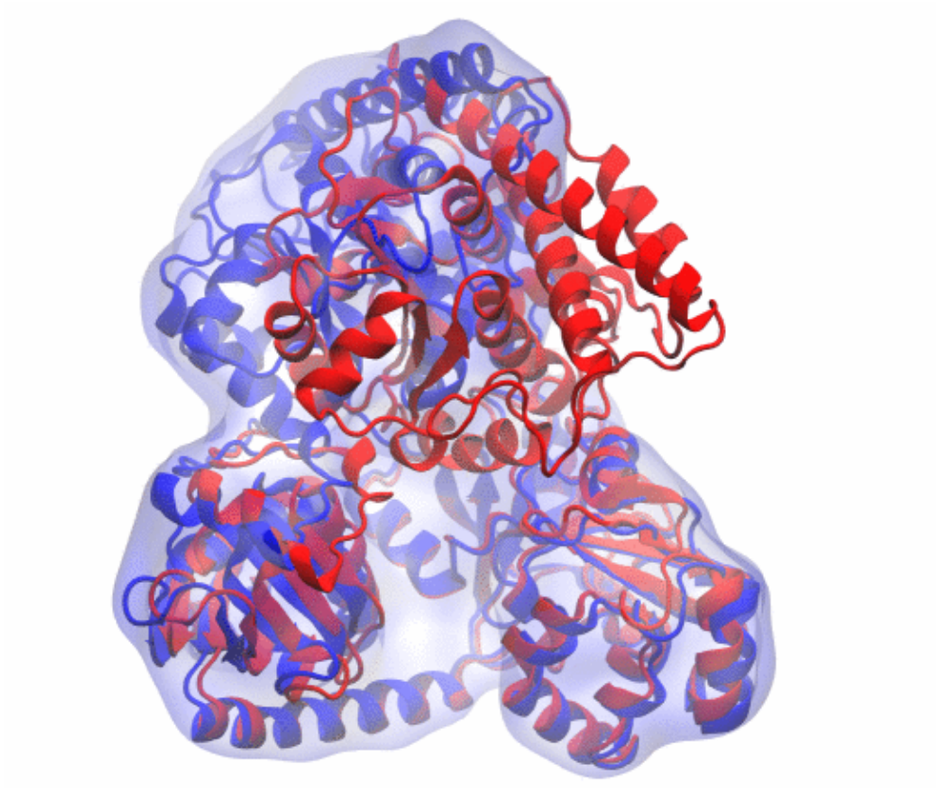




TorsionPlot Plugin new in VMD 1.9.3

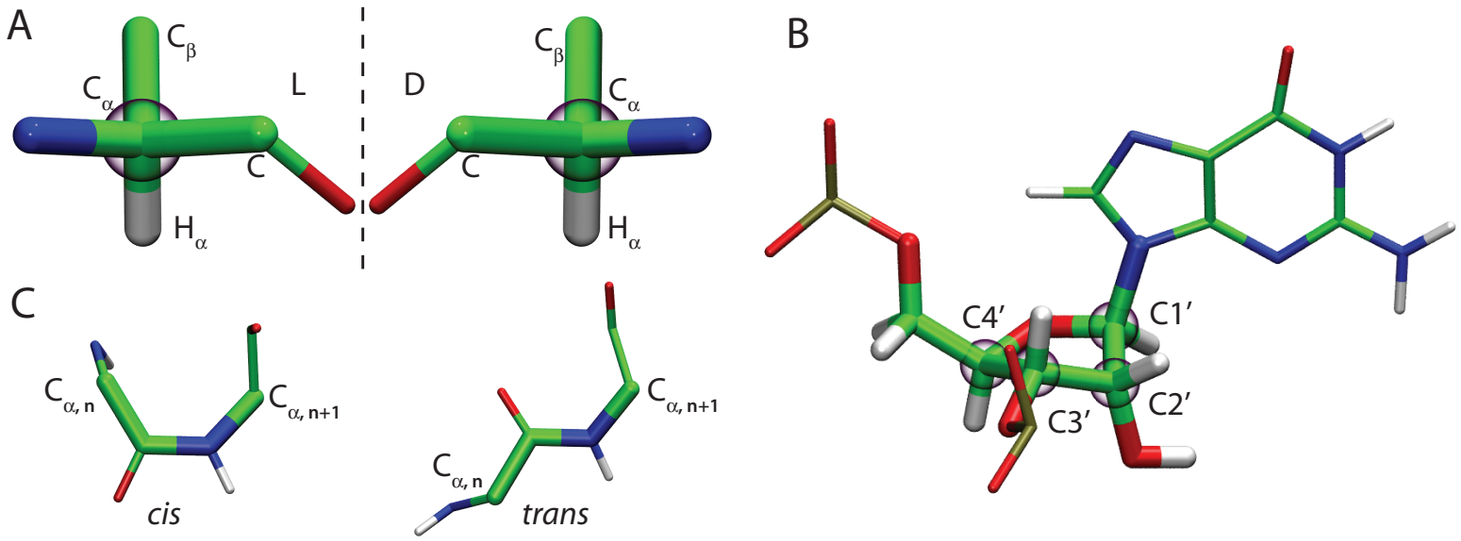
# Analyzing MDFF Model Quality 0: **Known Structures**

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
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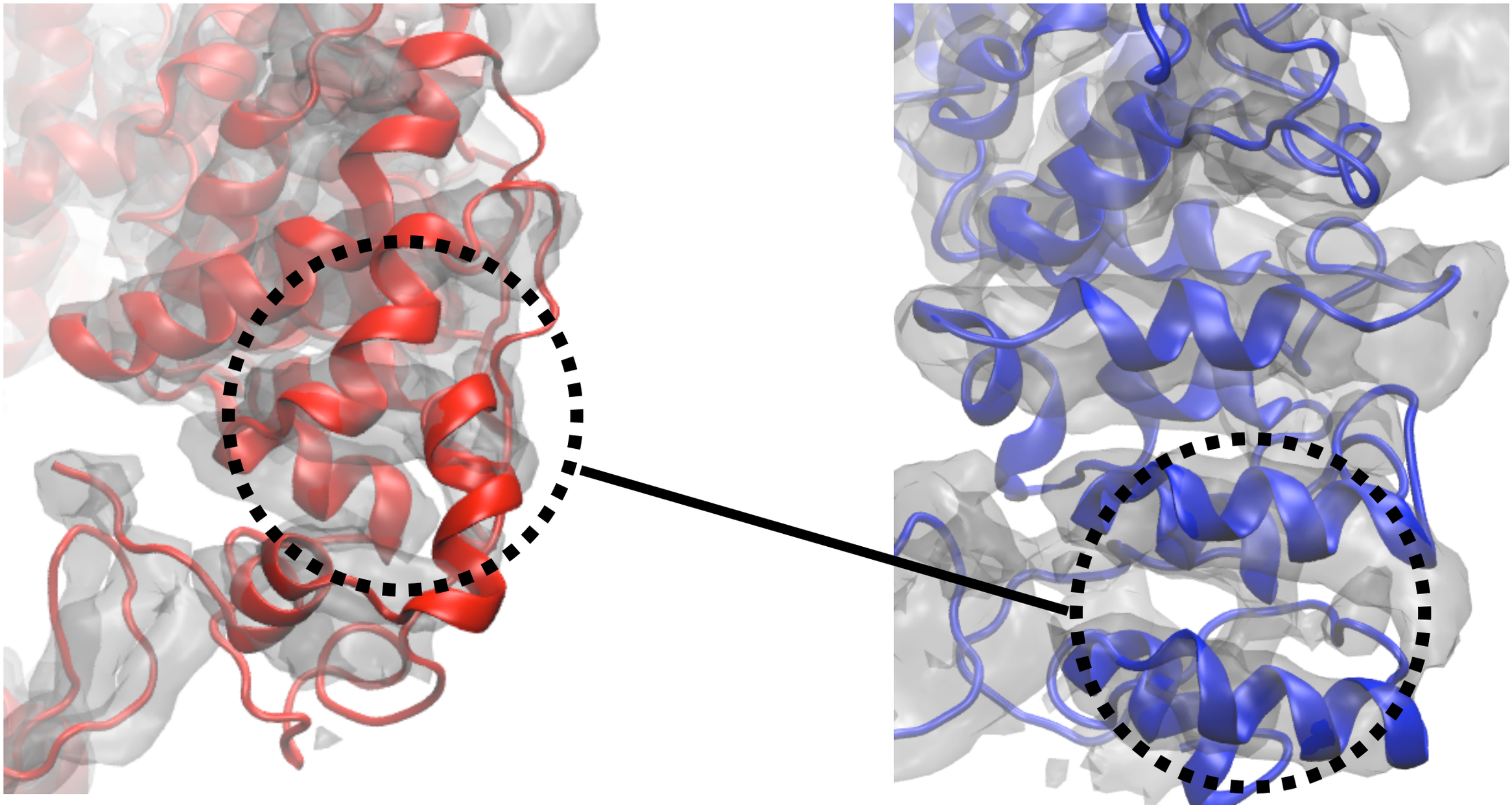
## xMDFF refined structures

| 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

(a)

Good Fit

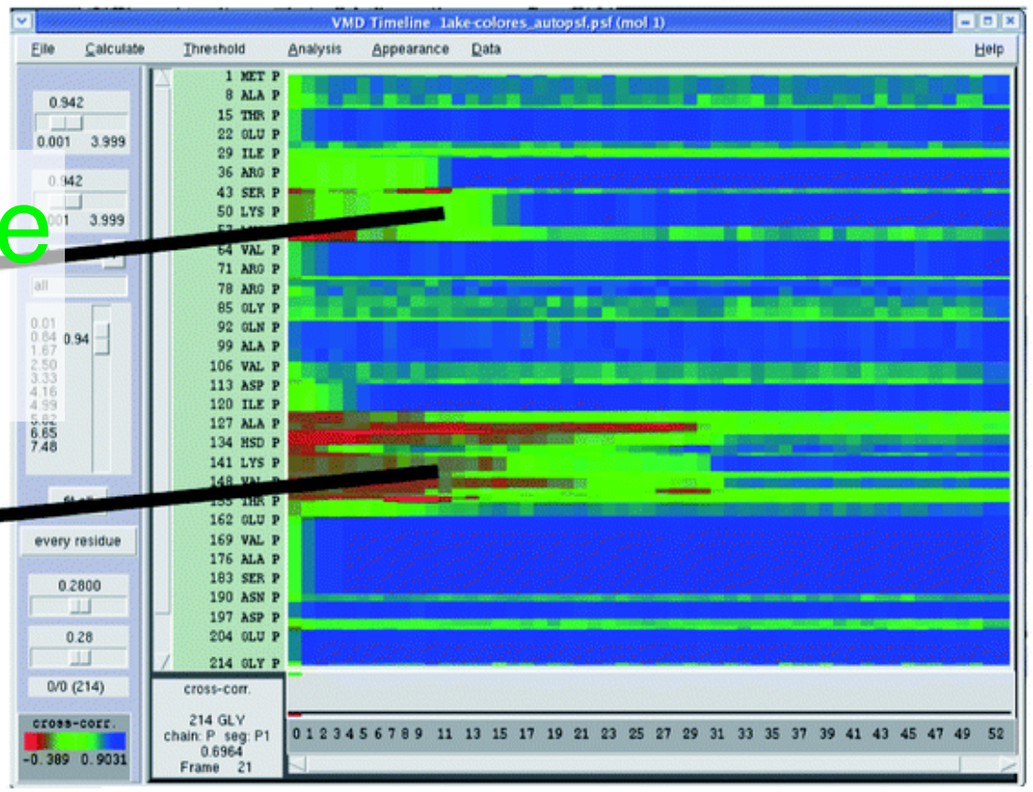
Intermediate Fit

(b)

Bad Fit

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

(c)



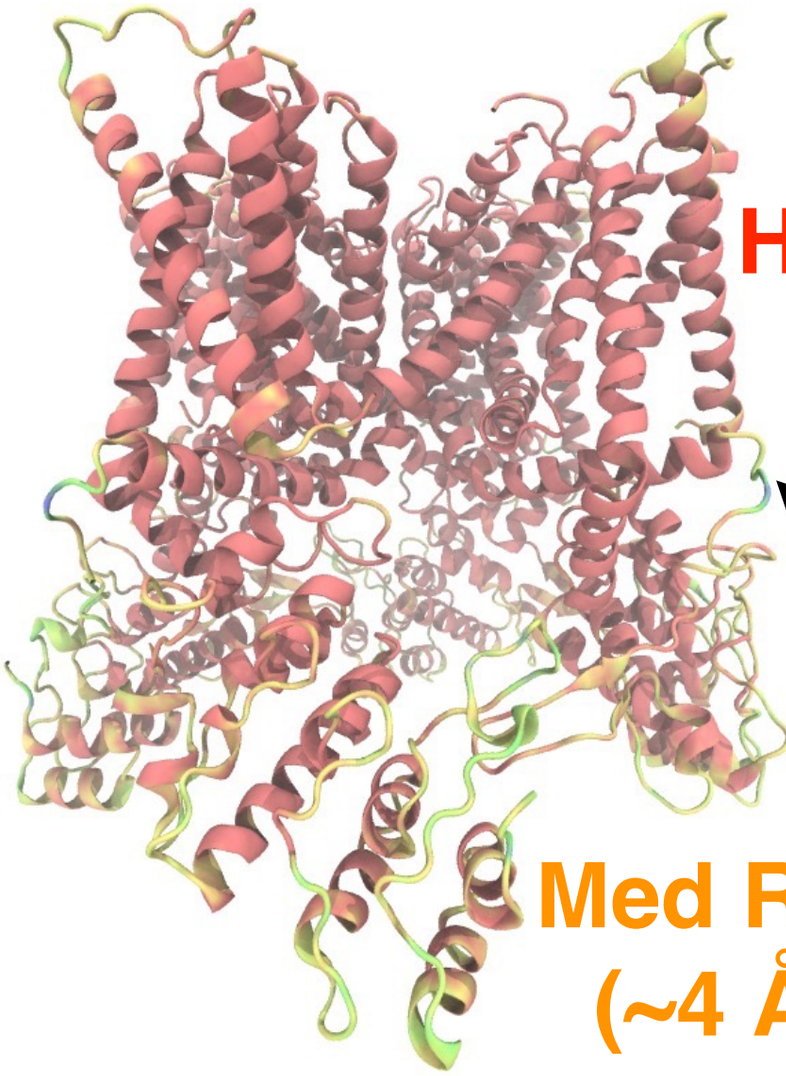

-.309 .9031

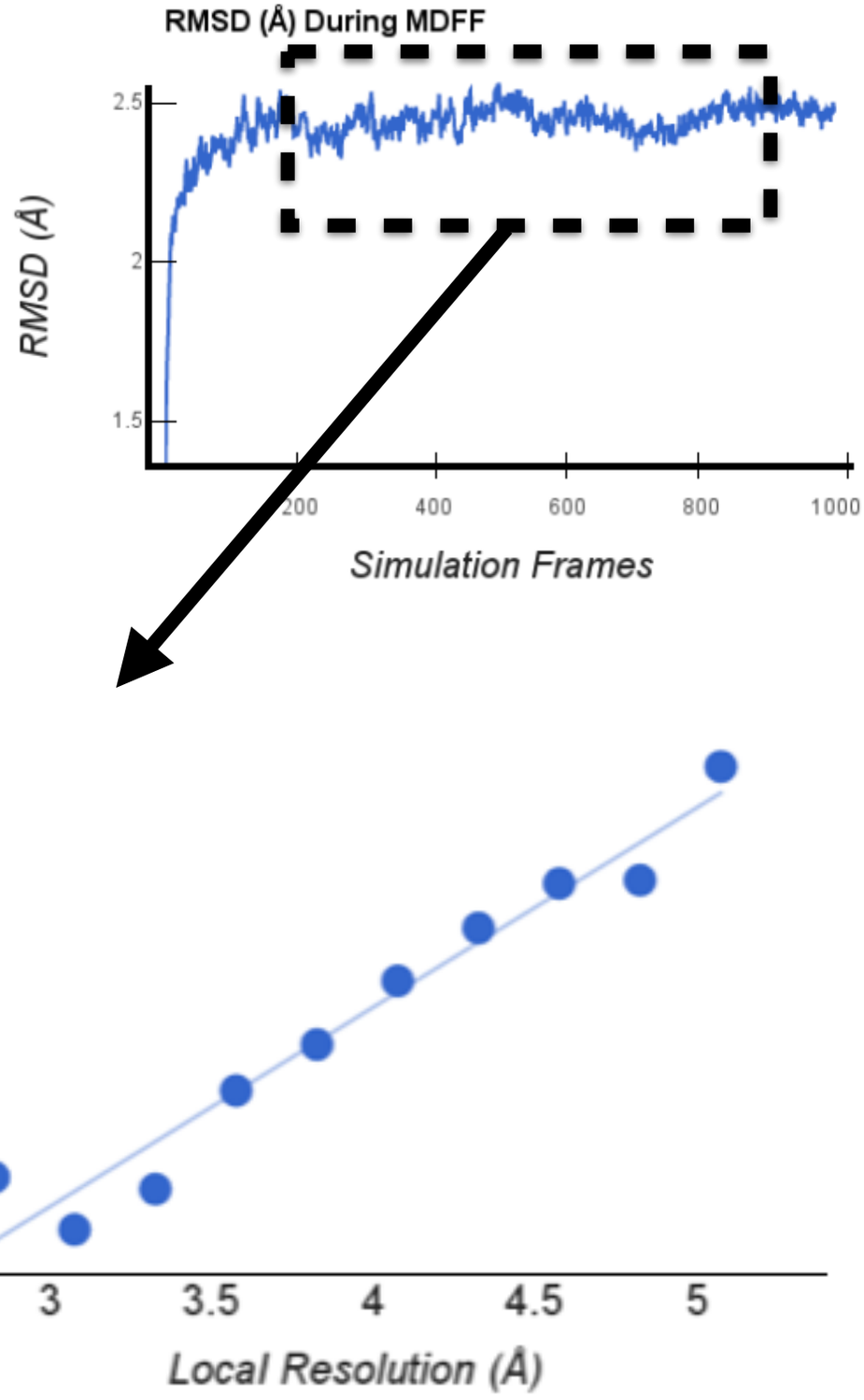
**John E. Stone, et al. Faraday Discussion, 169:265-283, 2014**

# 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




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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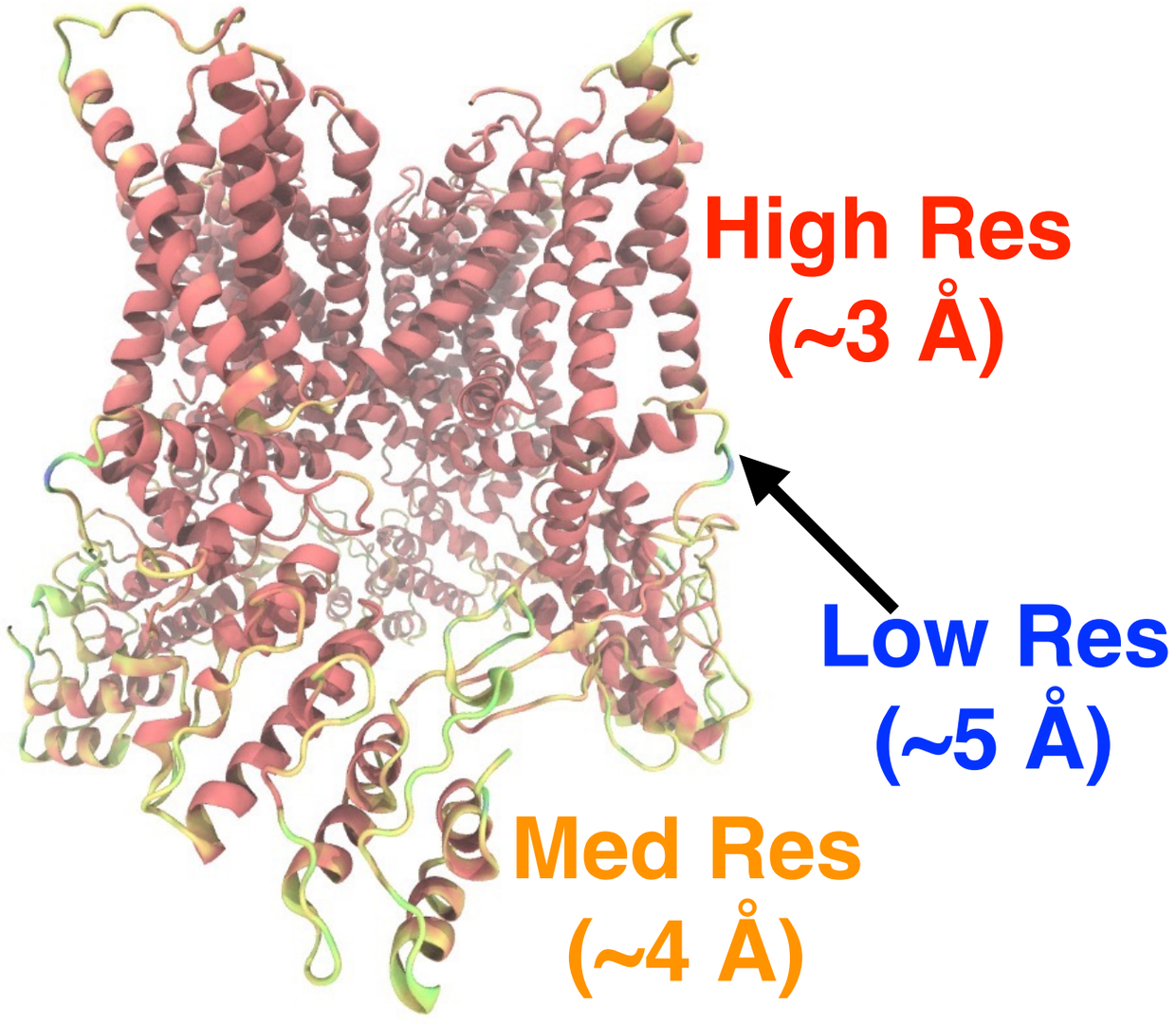
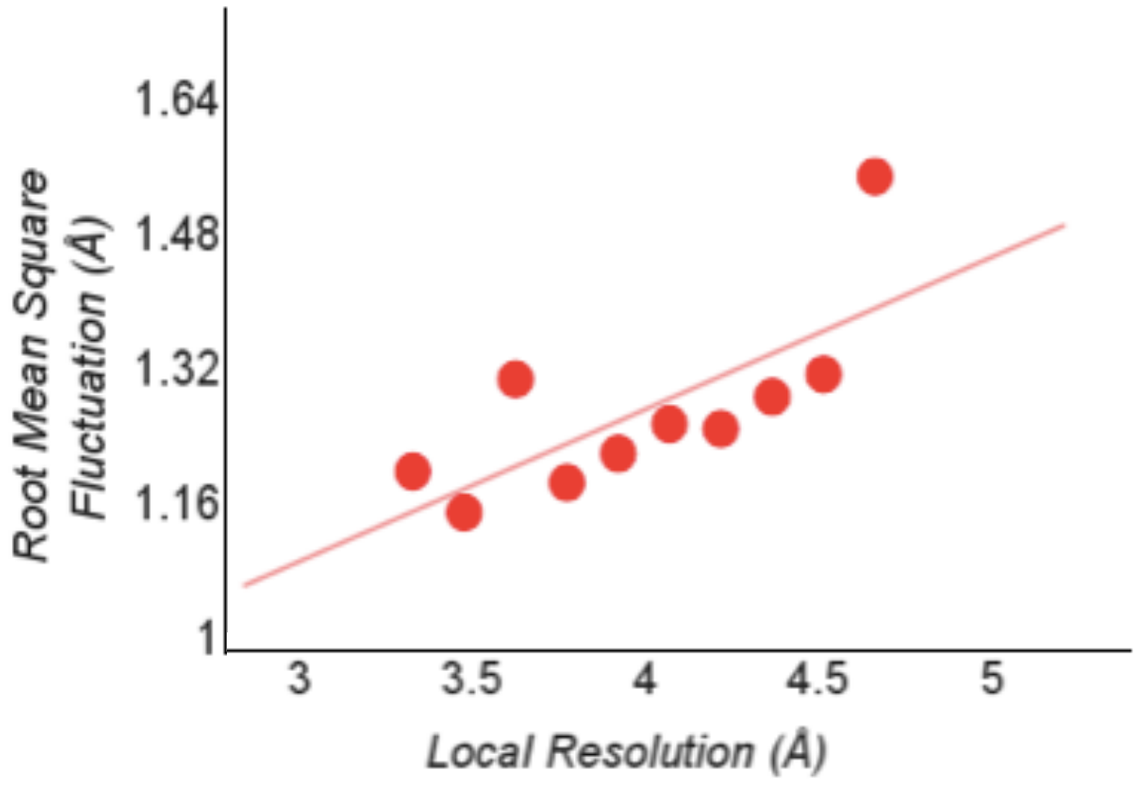



RMSF During Equilibration




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) |

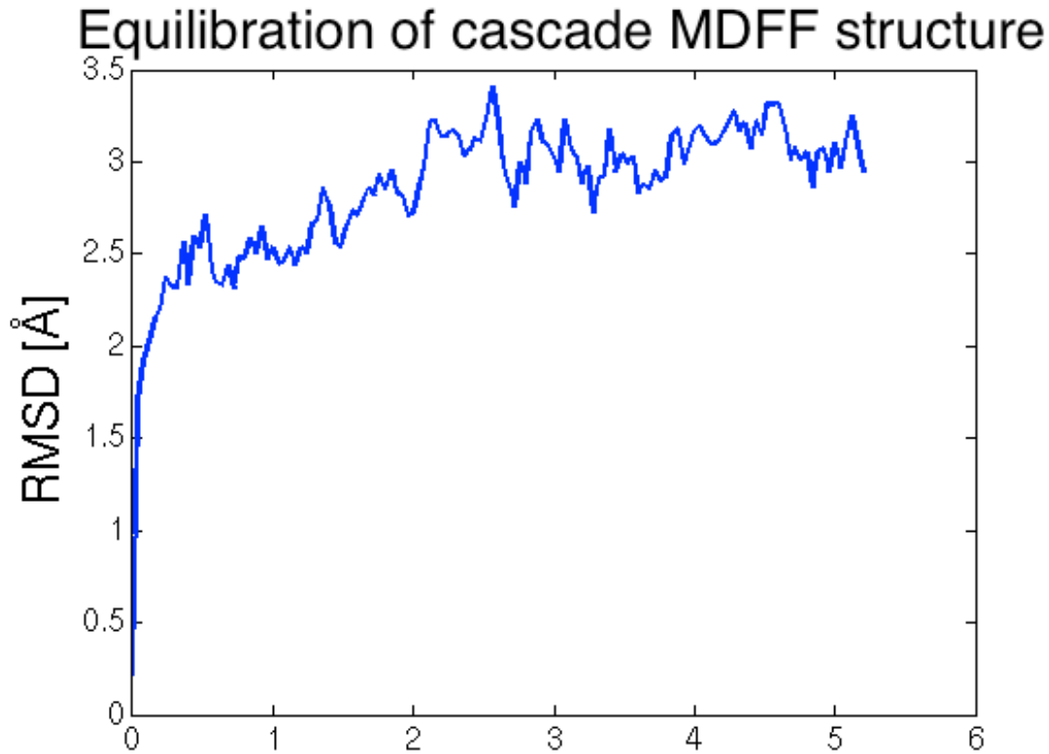
CC of reference structure w.r.t. each half map was 0.719

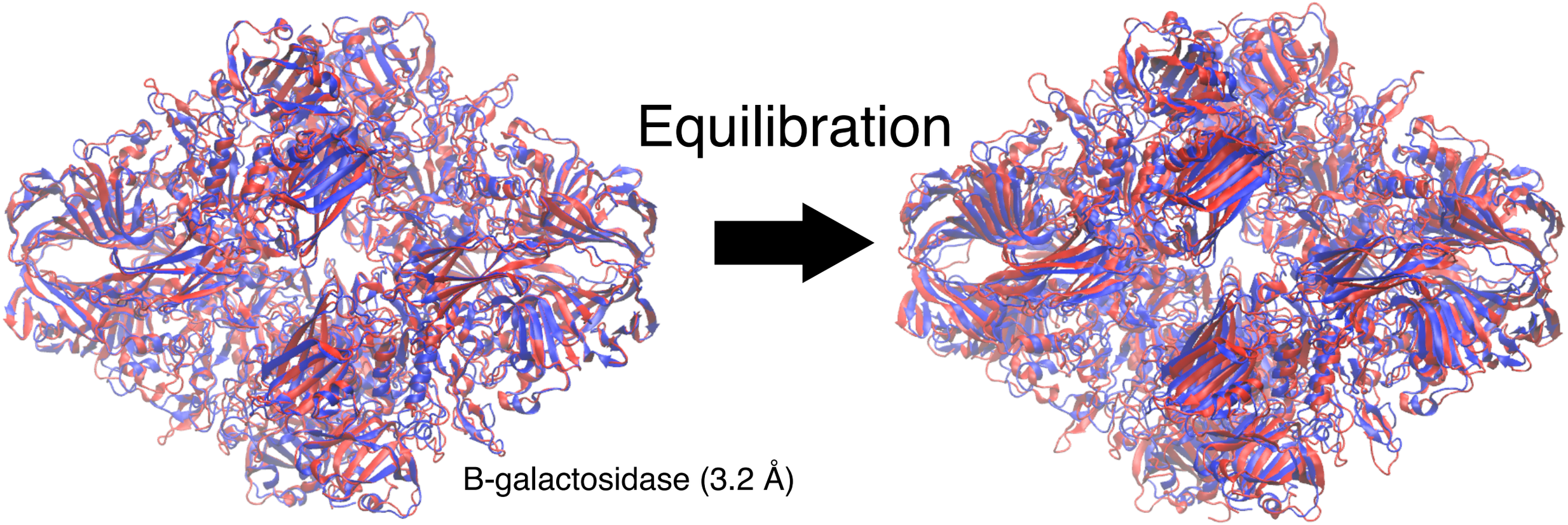
TRPV1 EM map and structure from M. Liao, E. Cao, D. Julius, Y. Cheng, Nature 504, 2013.

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

## Stability of structure during equilibration

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

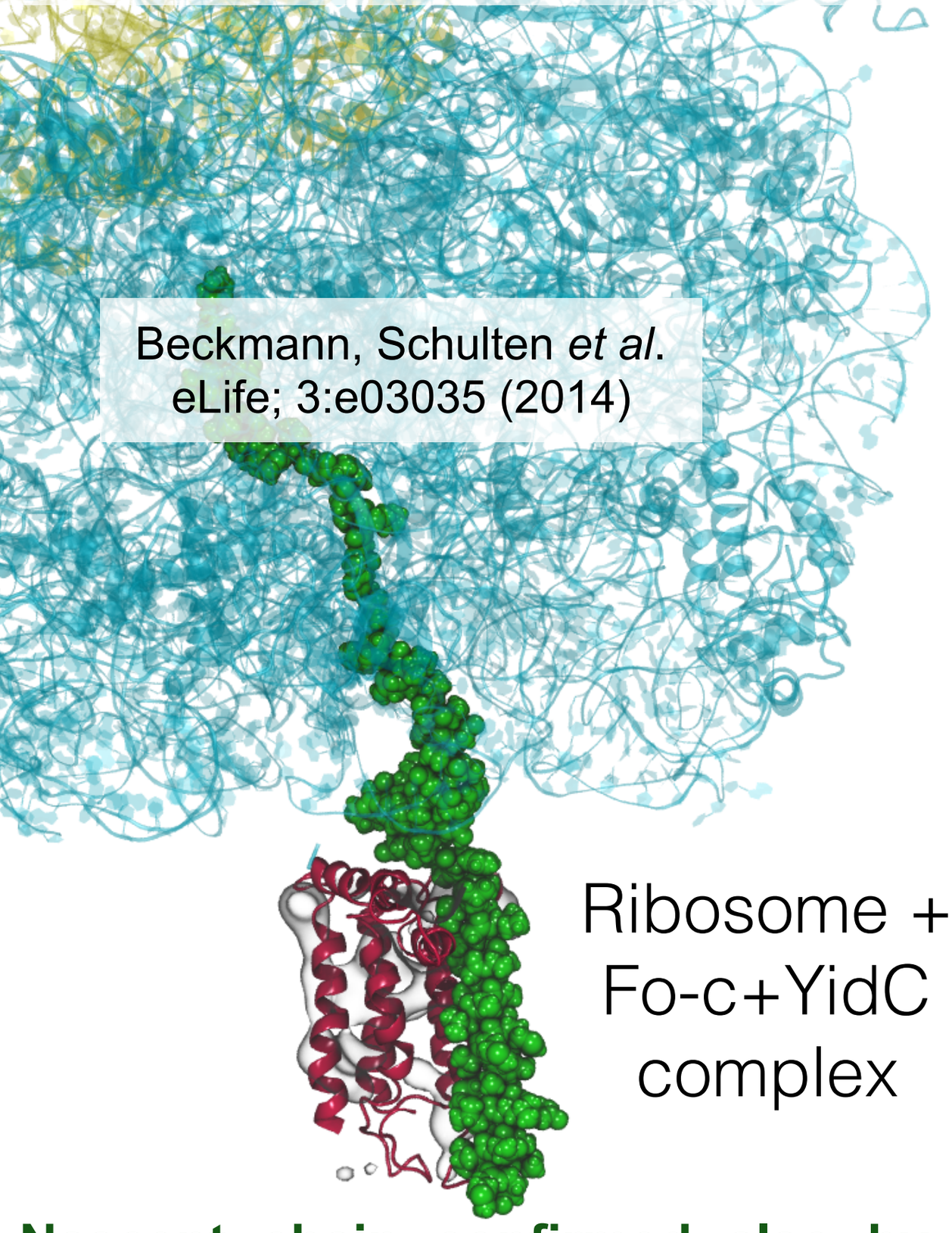



B-galactosidase (3.2 Å)

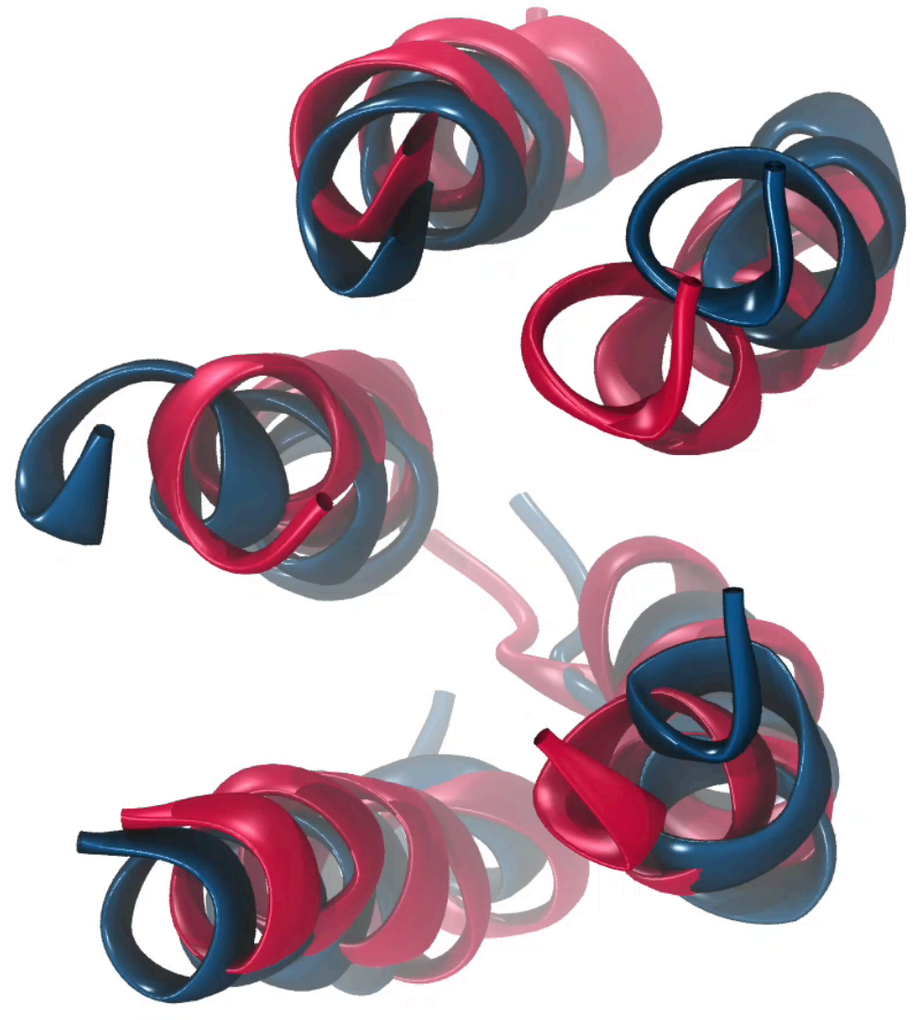
A. Bartesaghi, D. Matthies, S. Banerjee, A. Merk, S. Subramaniam, Proc. Natl. Acad. Sci. 111, 2014.

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




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.**

# Molecular Dynamics Flexible Fitting
# Advanced Techniques

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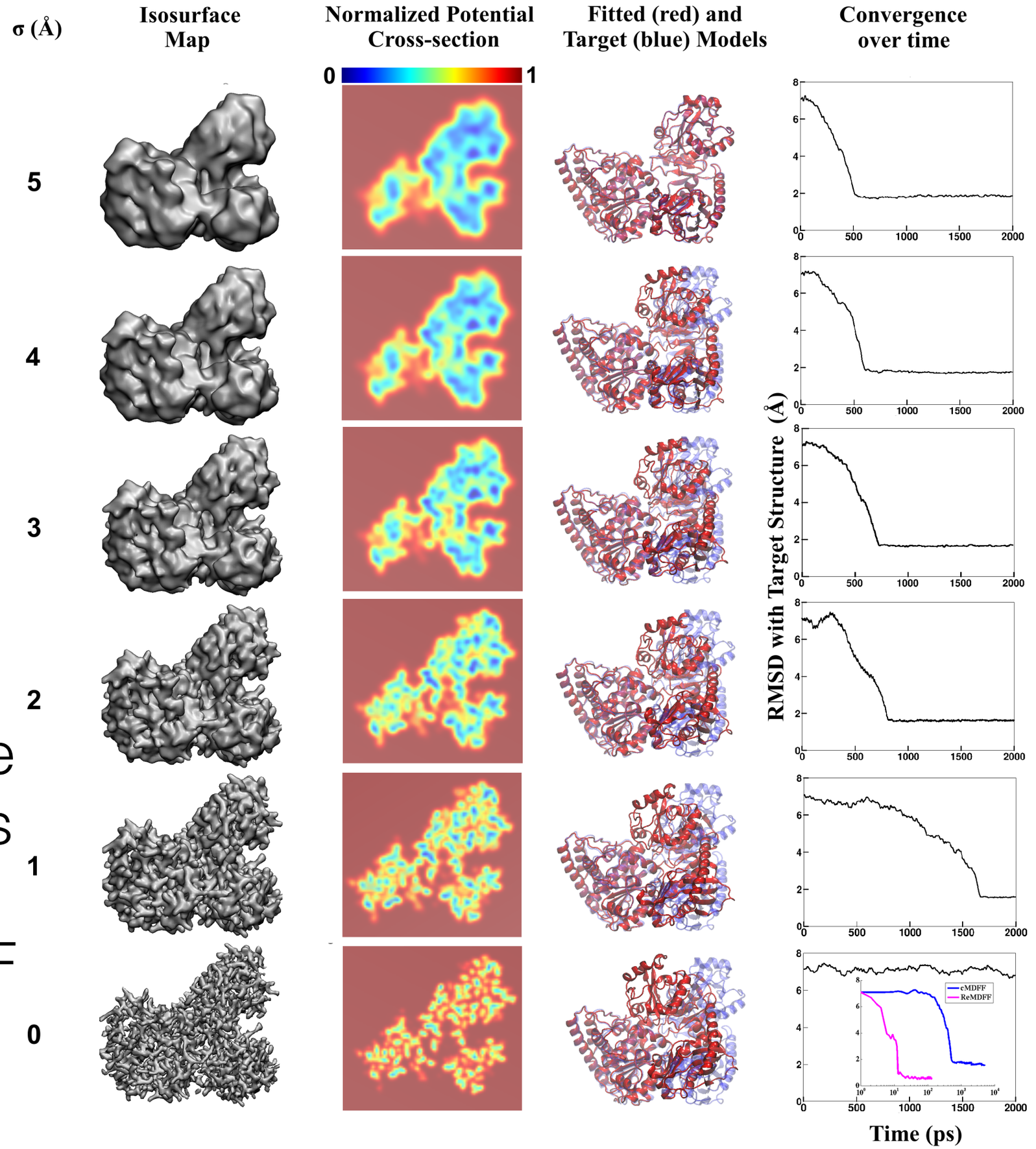
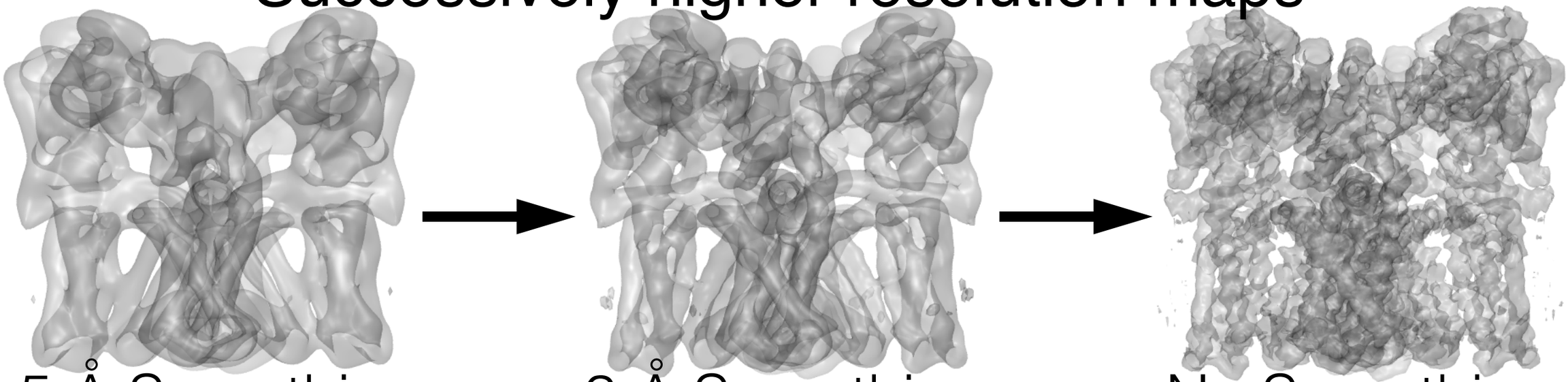
# MDFF for **high-resolution** cryo-EM

- 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



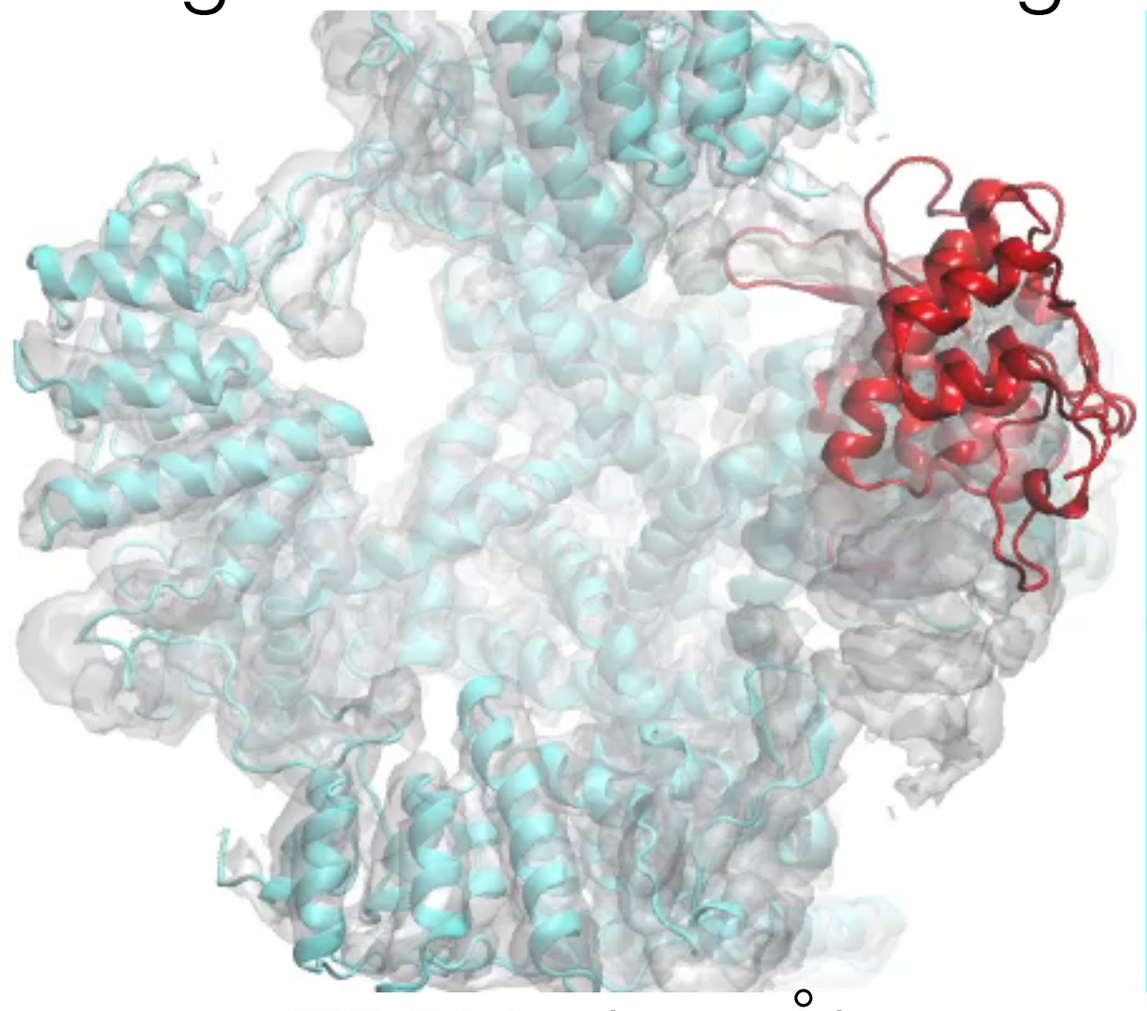
# Cascade MDFF for **high-resolution** cryo-EM:
## Successively higher resolution maps



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

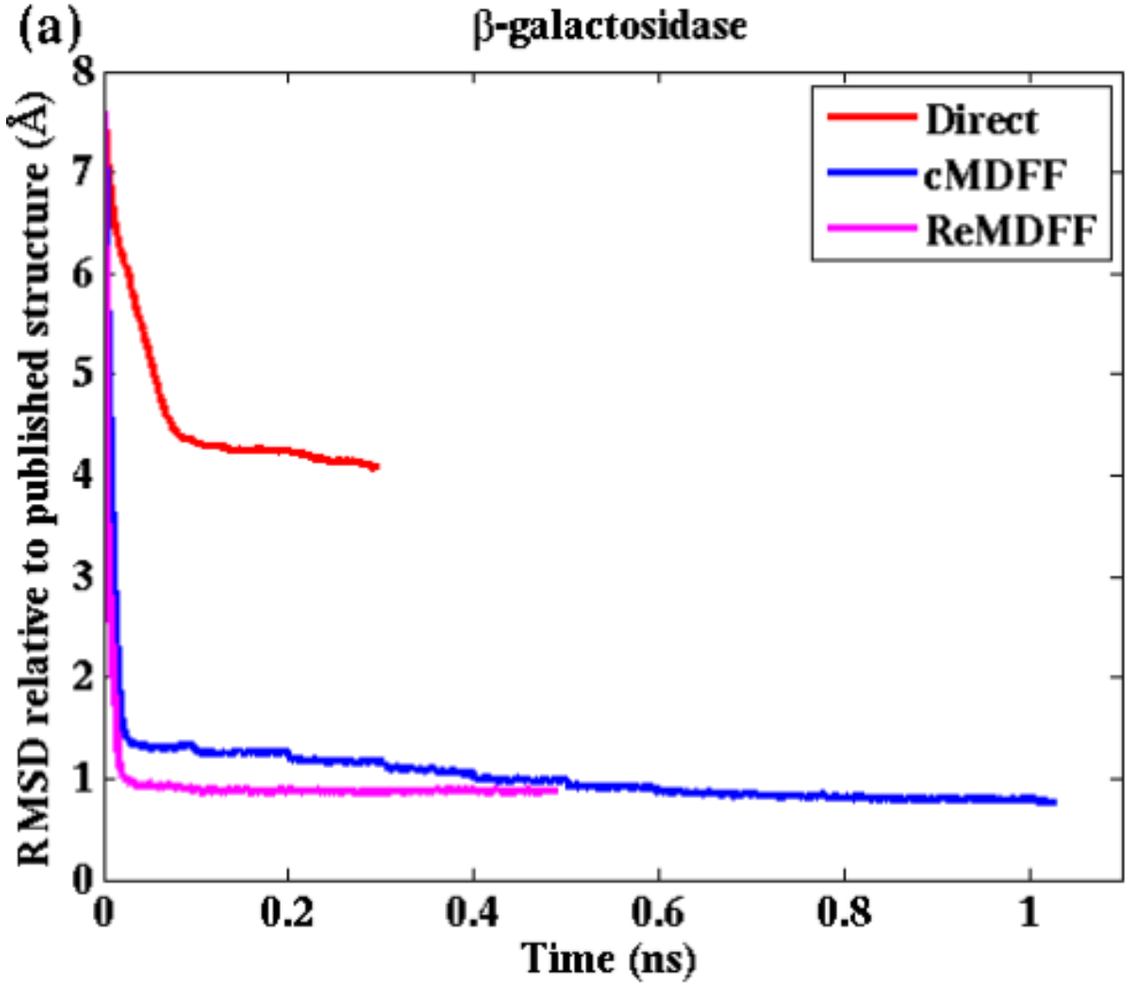


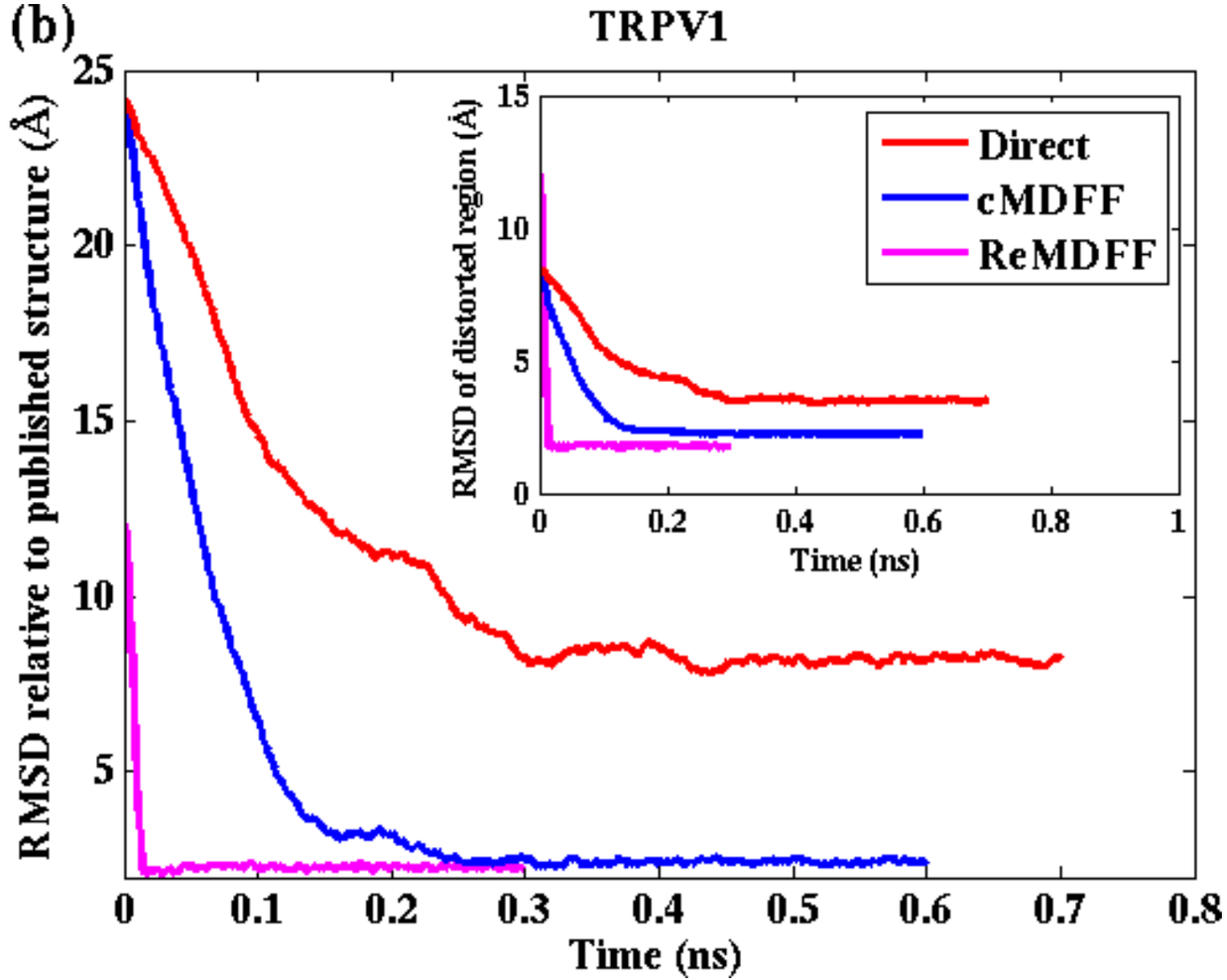
TRPV1 (3.4 Å)

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

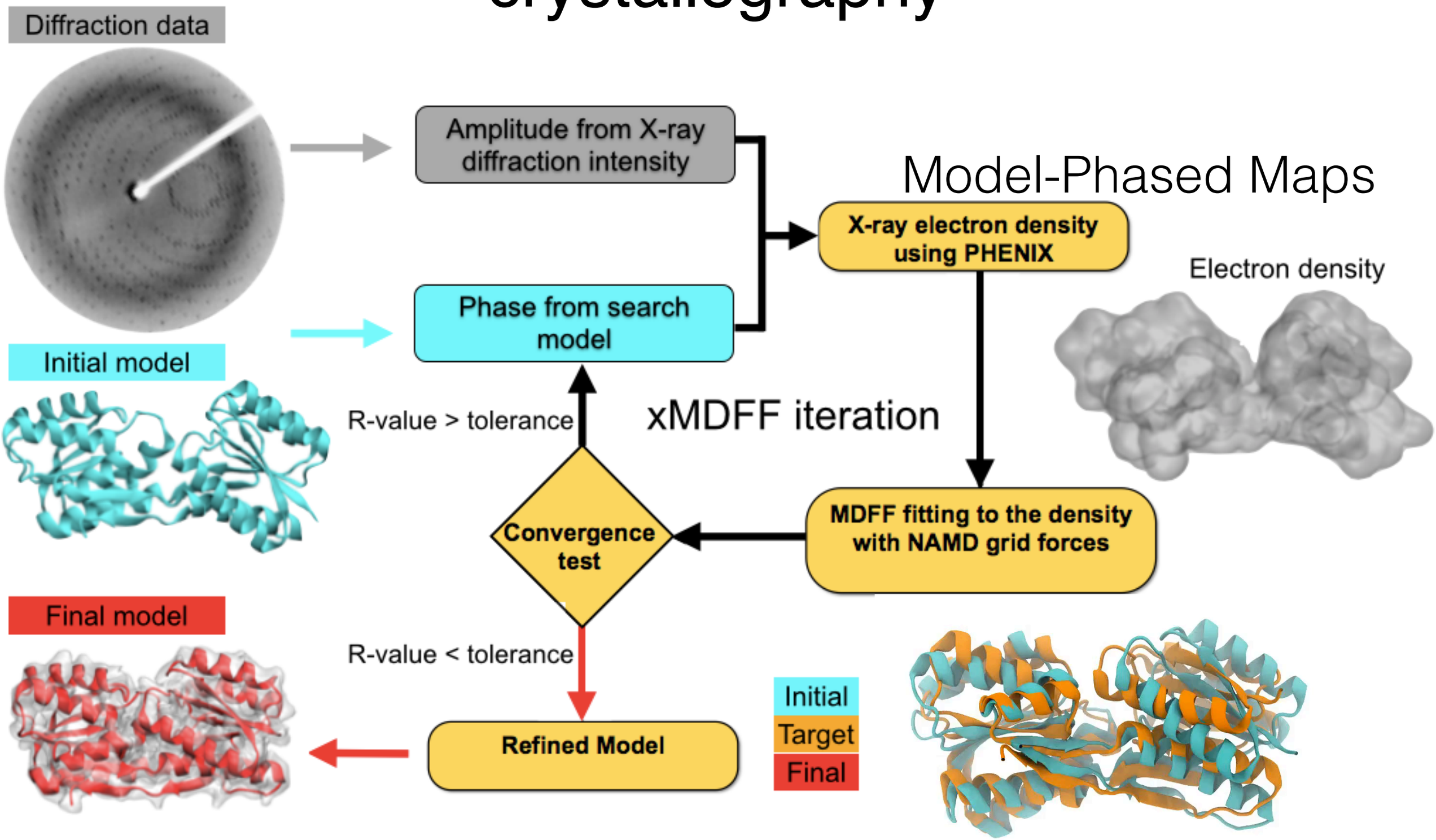
# Resolution Exchange MDFF for **high-resolution** cryo-EM

- 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



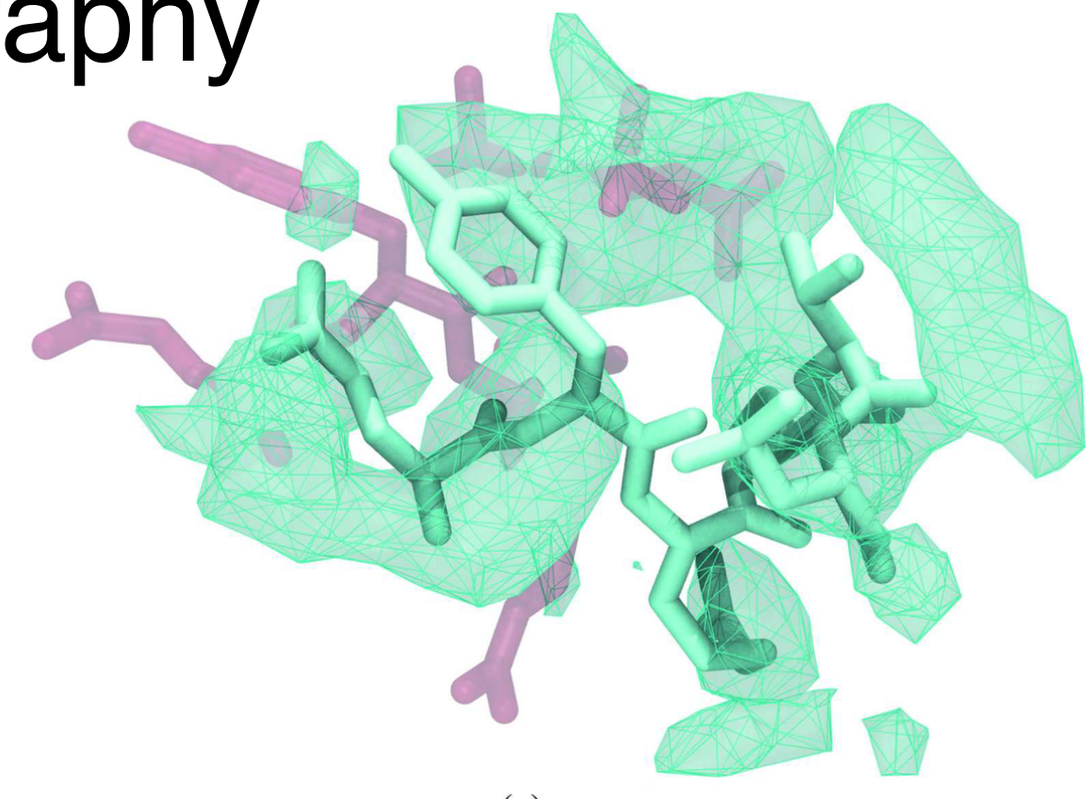

**Ryan McGreevy*, Abhishek Singharoy*,et al. Acta Crystallographica D70, 2344-2355, 2014**

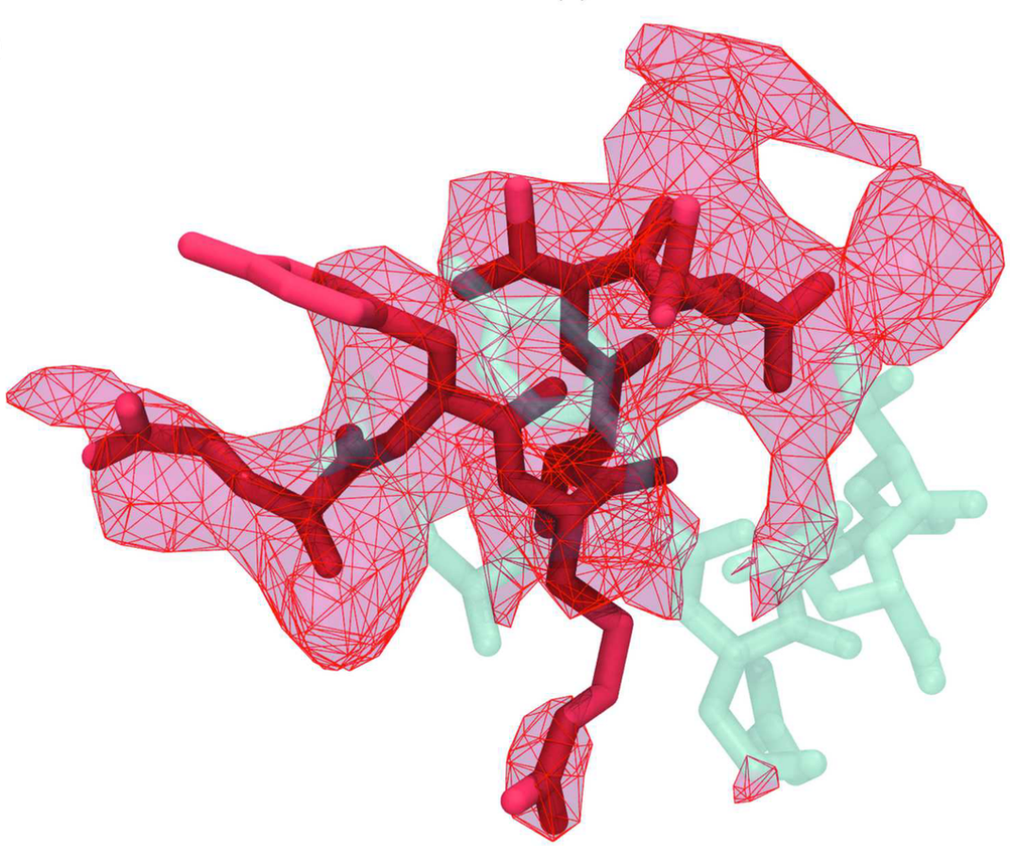
# xMDFF: MDFF for low-resolution x-ray crystallography

- 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*)

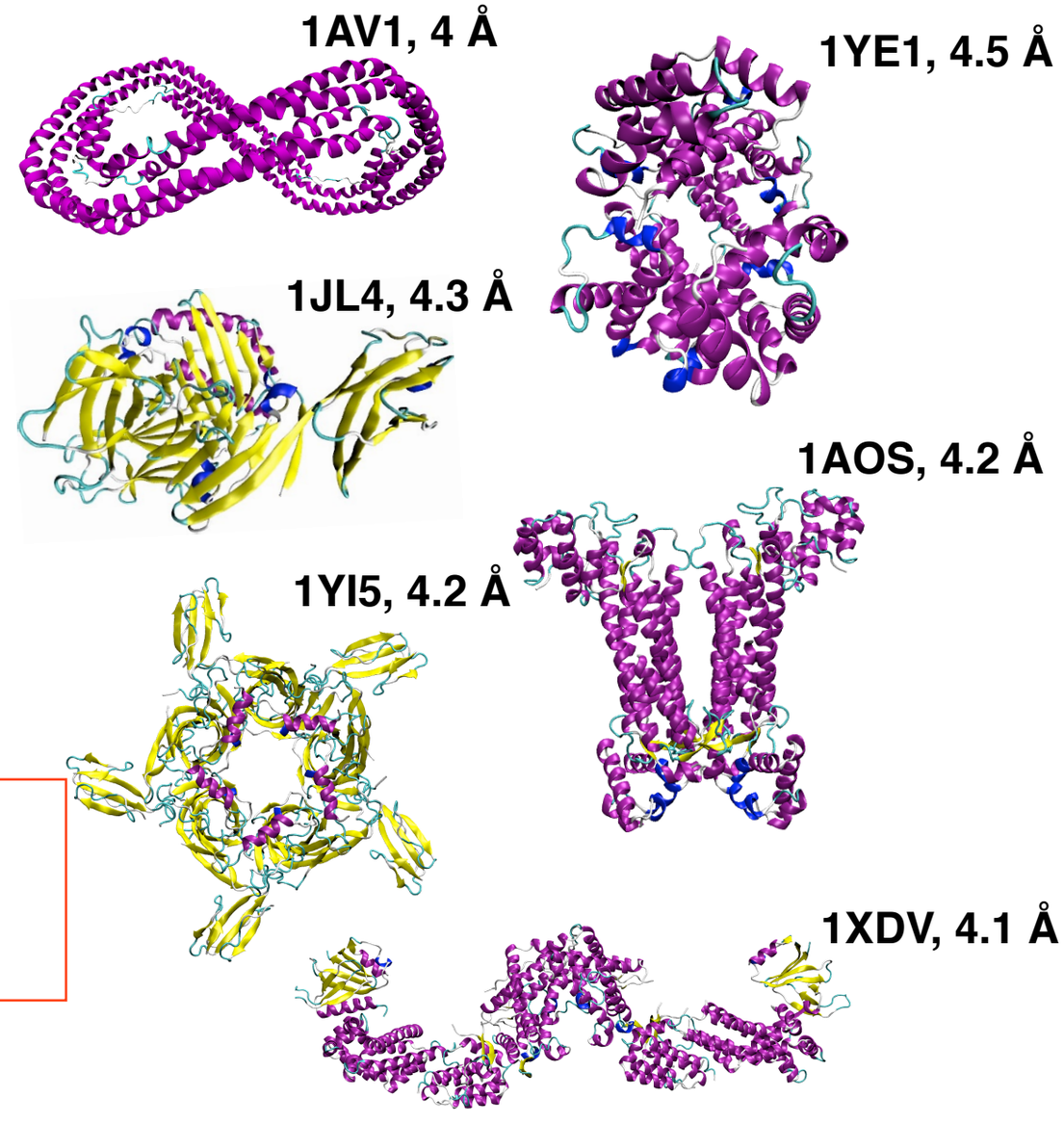
# xMDFF Improves Structures Posted at the Protein Data Bank

## Refinement statistics

| PDB ID | Molprobity initial | Molprobity final | R-work initial | R-work final | R-free initial | R-free 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.*

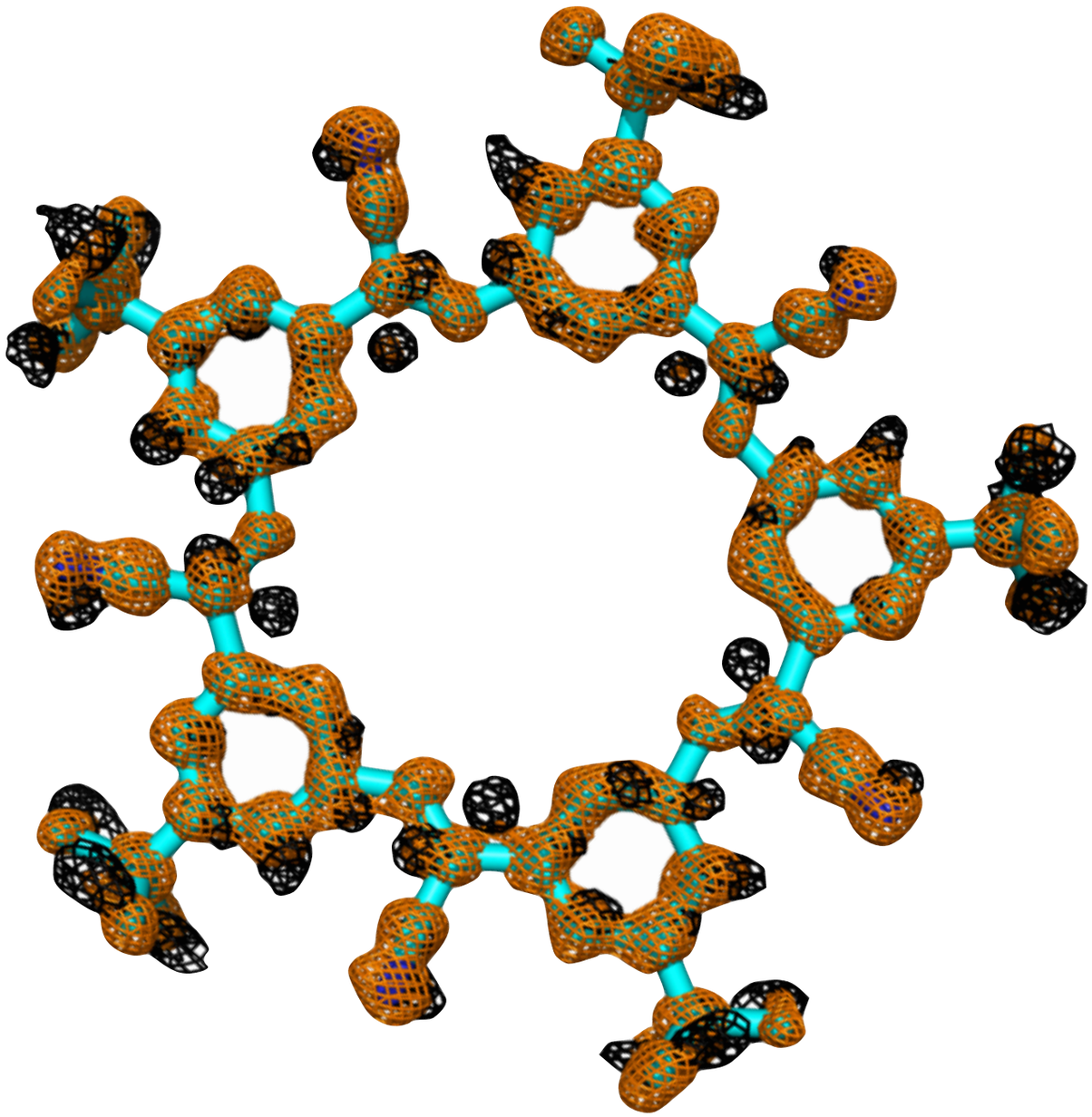
## Structures




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

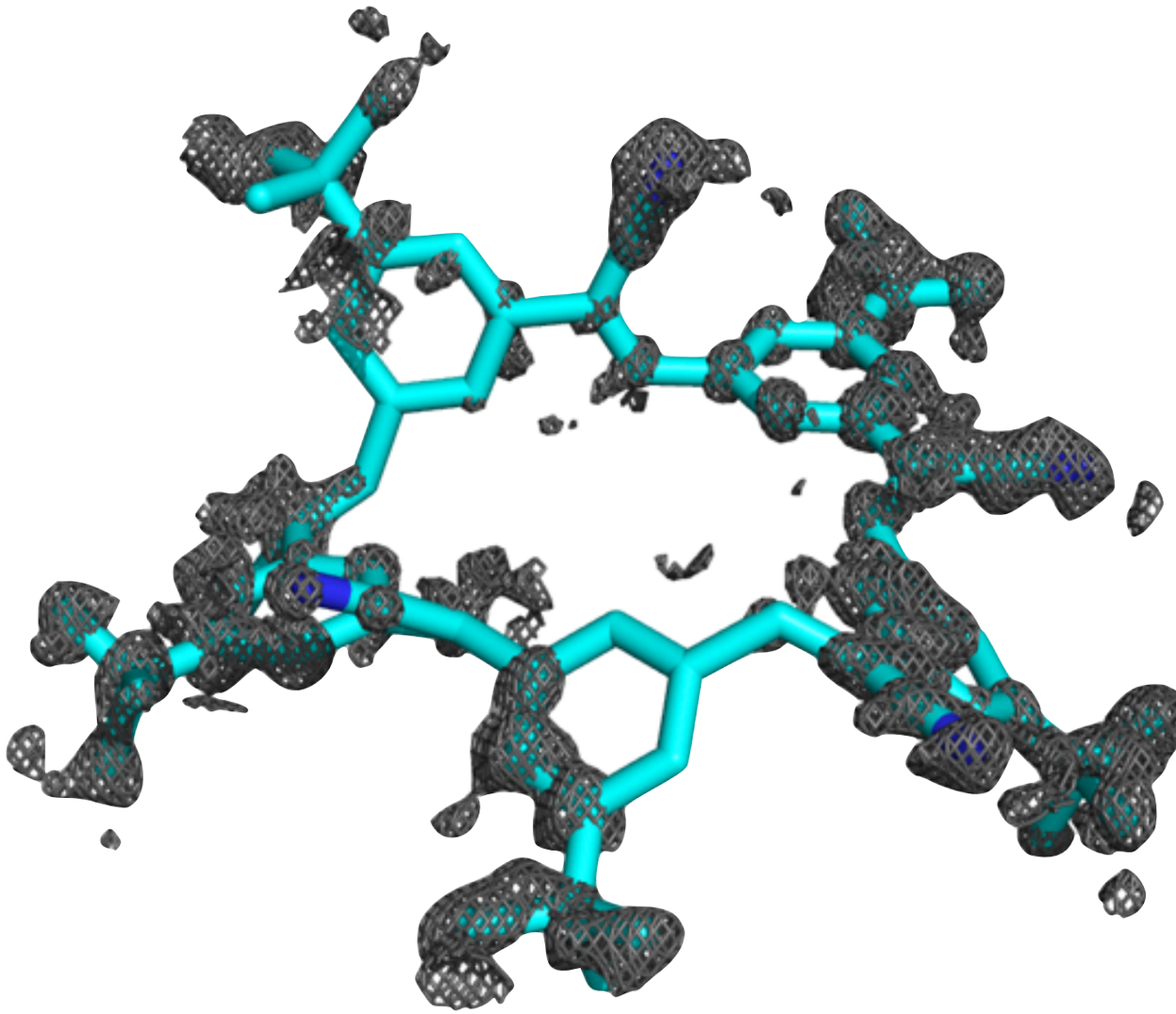
# xMDFF for Abiological Materials

## 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.

# 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

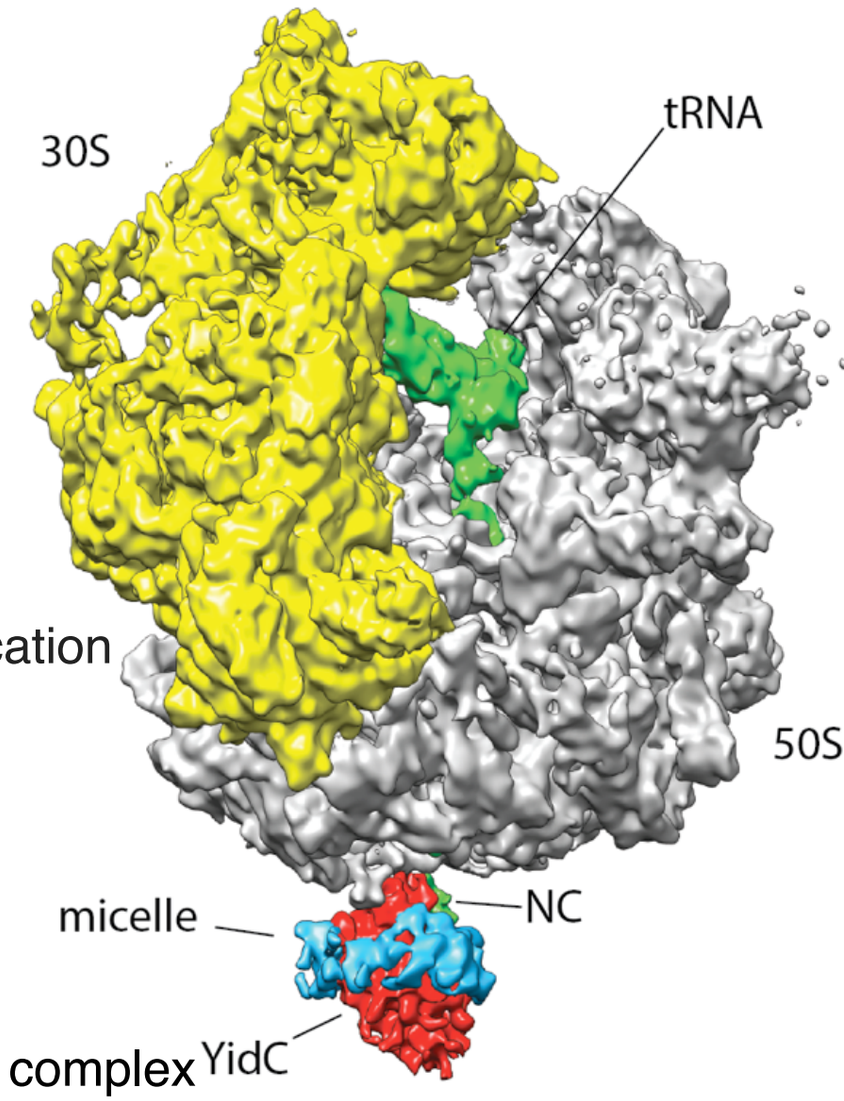
Lasker et al. ***PNAS*** (2012): Proteasome

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/

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