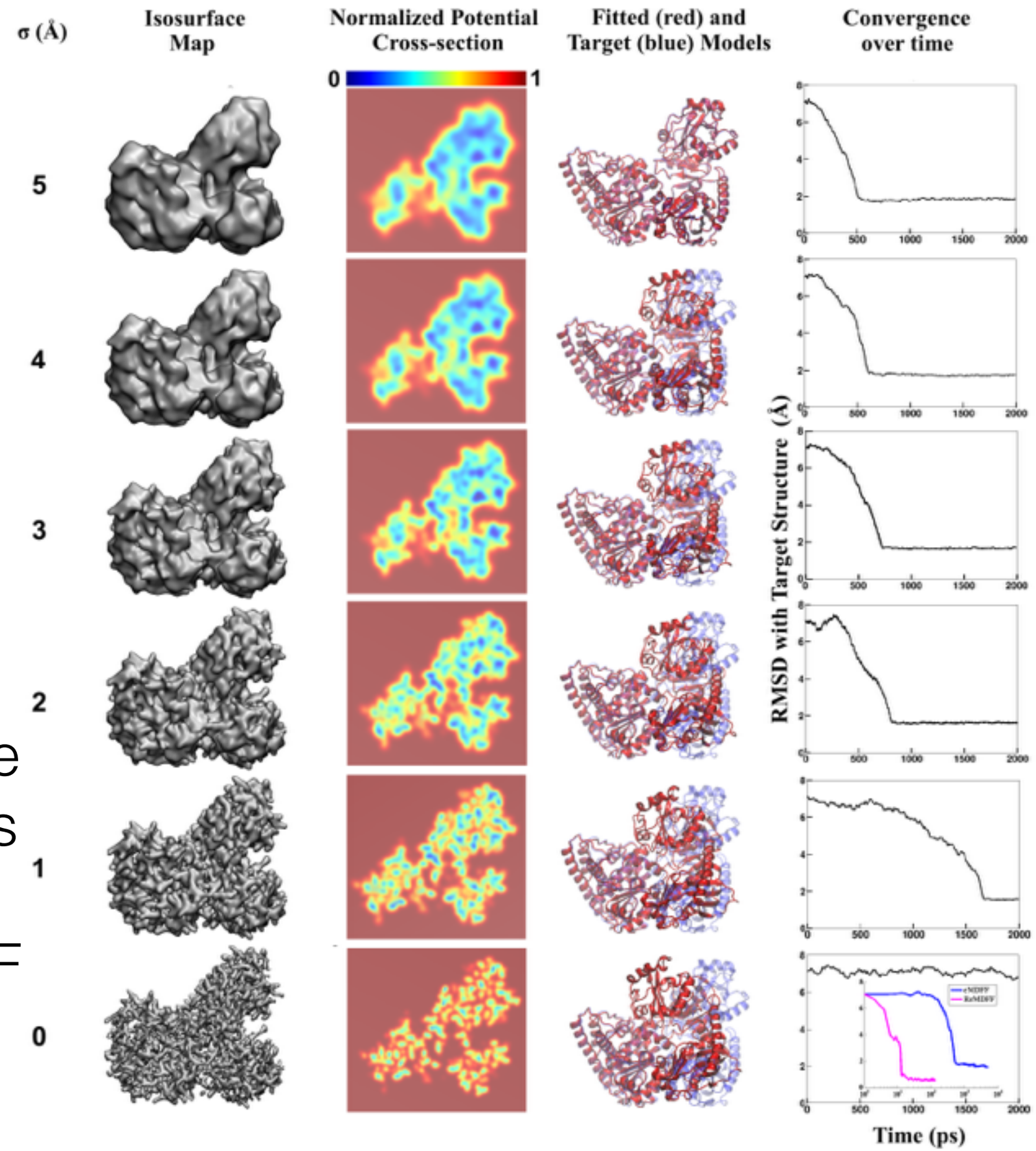
Molecular Dynamics Flexible Fitting
Advanced Techniques

Ryan McGreevy
Research Programmer

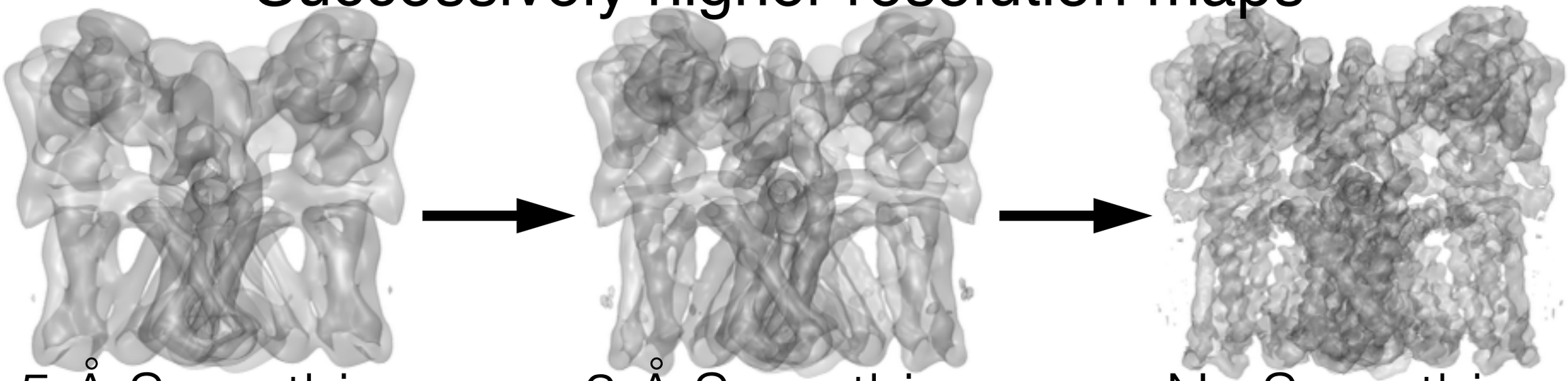
University of Illinois at Urbana-Champaign
NIH Resource for Macromolecular Modeling and Bioinformatics

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



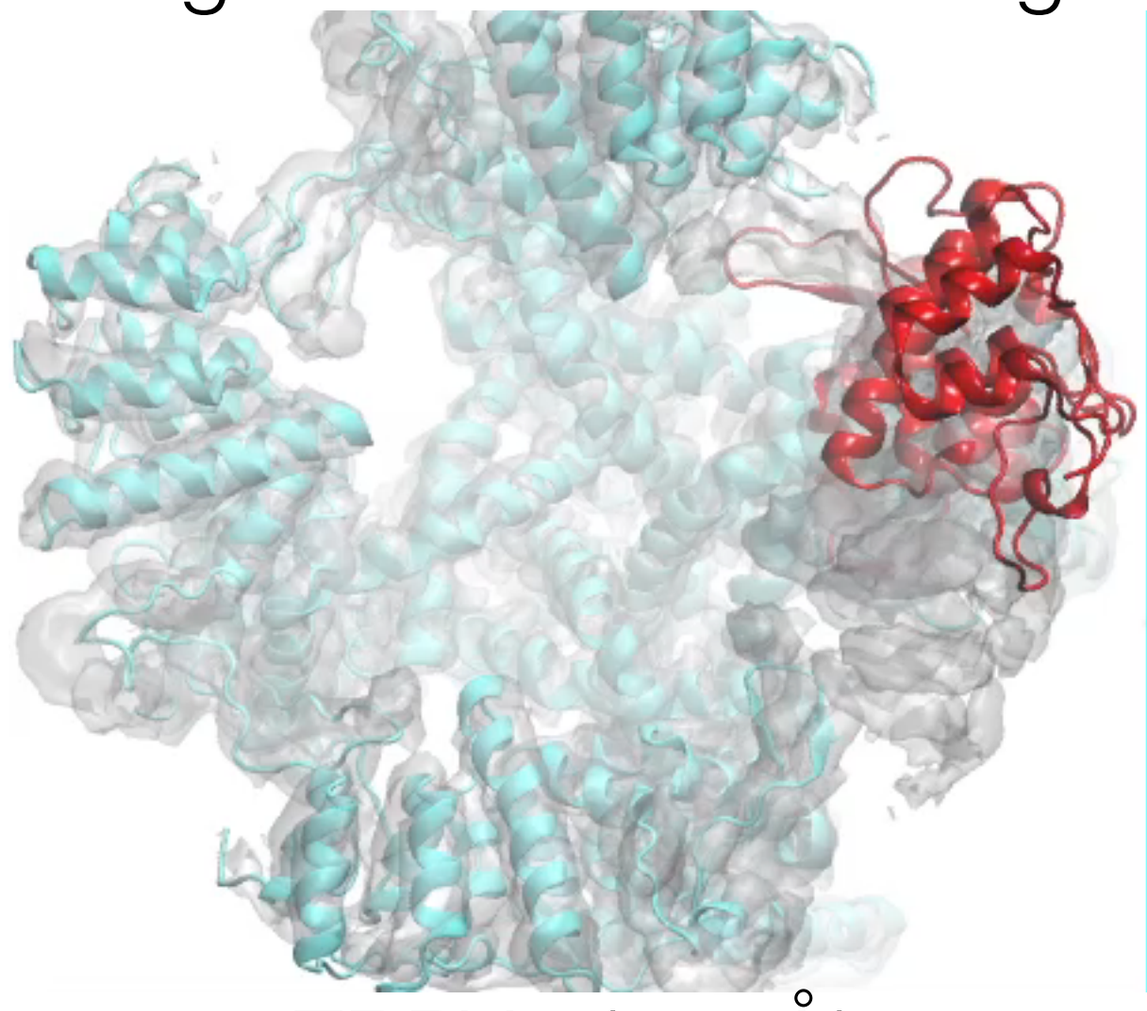
Cascade MDFF for **high-resolution** cryo-EM: Successively higher resolution maps



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

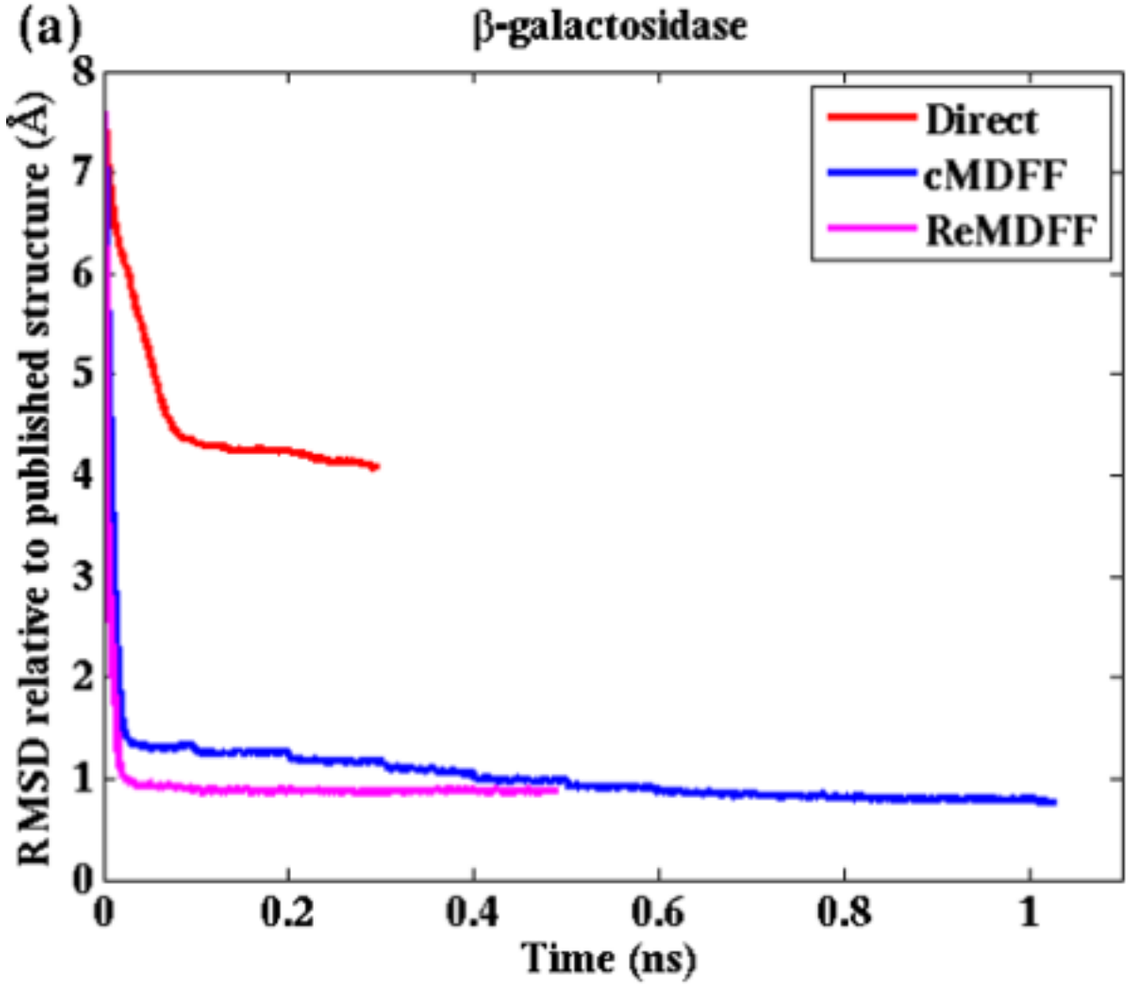


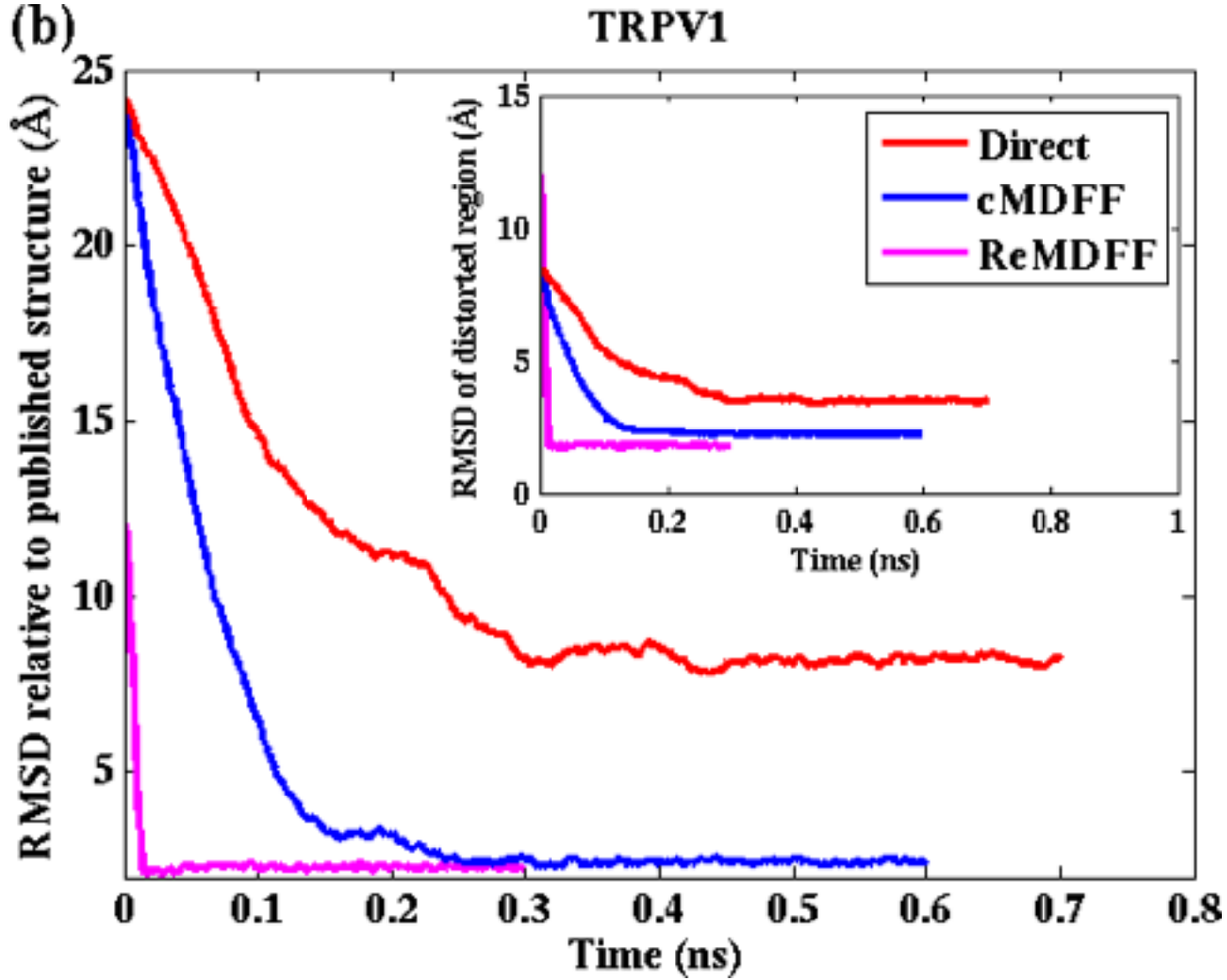
TRPV1 (3.4 Å)

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

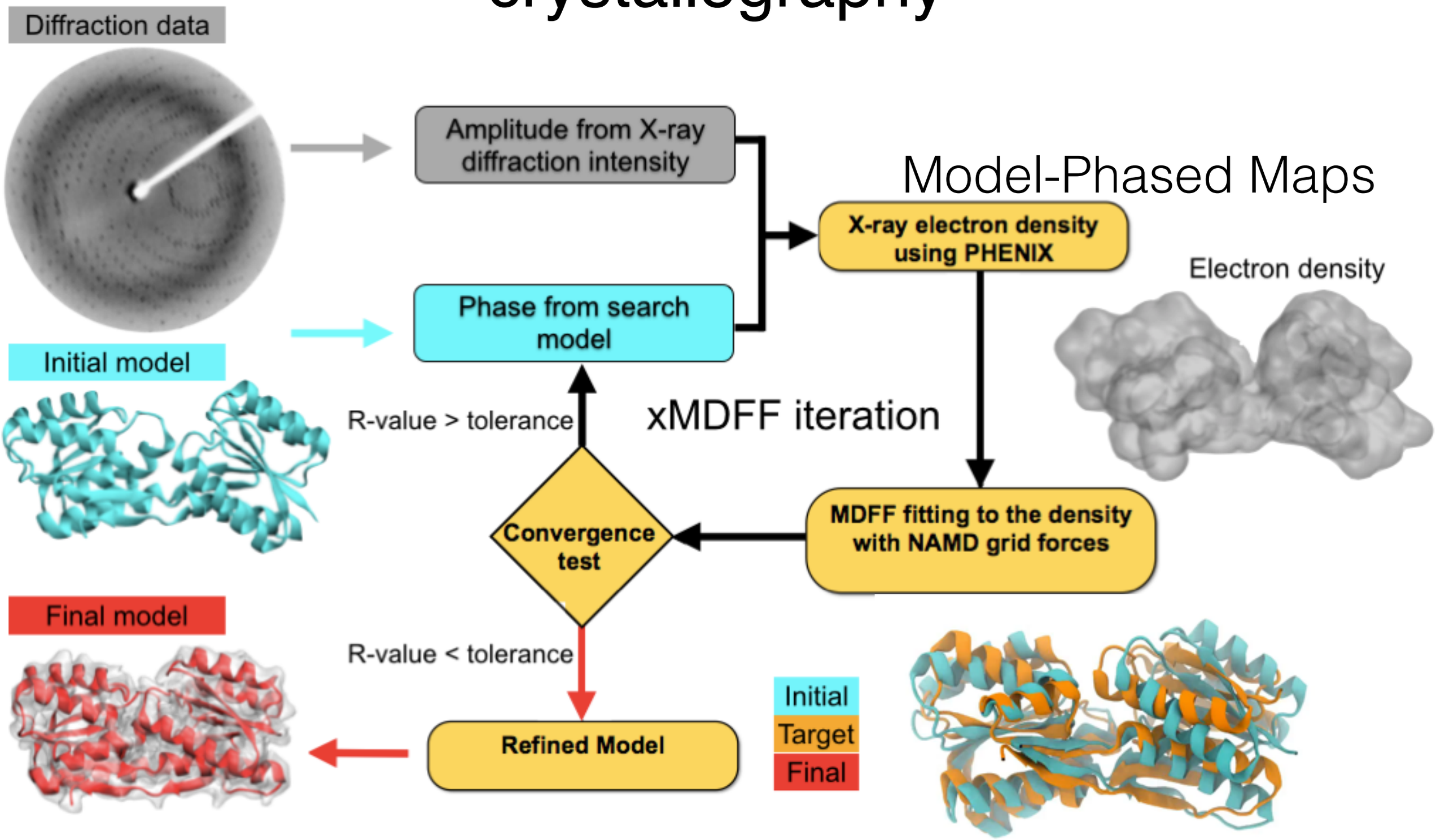
Resolution Exchange MDFF for **high-resolution** cryo-EM

- 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



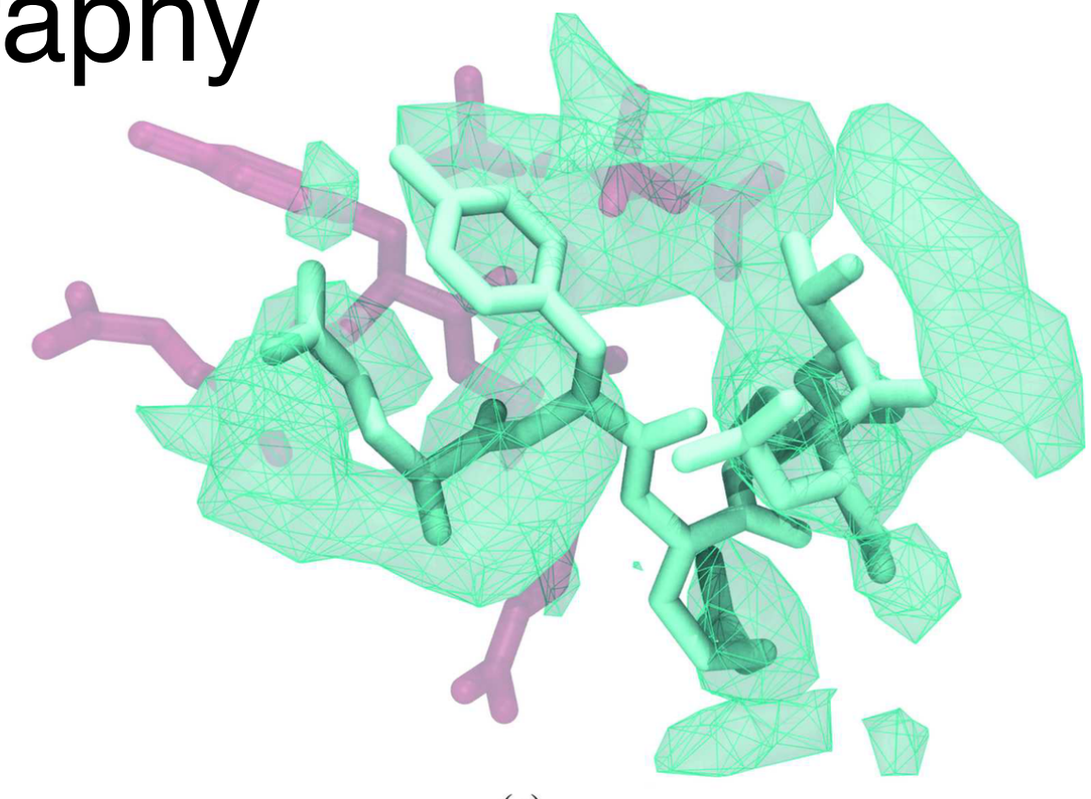
Ryan McGreevy*, Abhishek Singharoy*,et al. Acta Crystallographica D70, 2344-2355, 2014

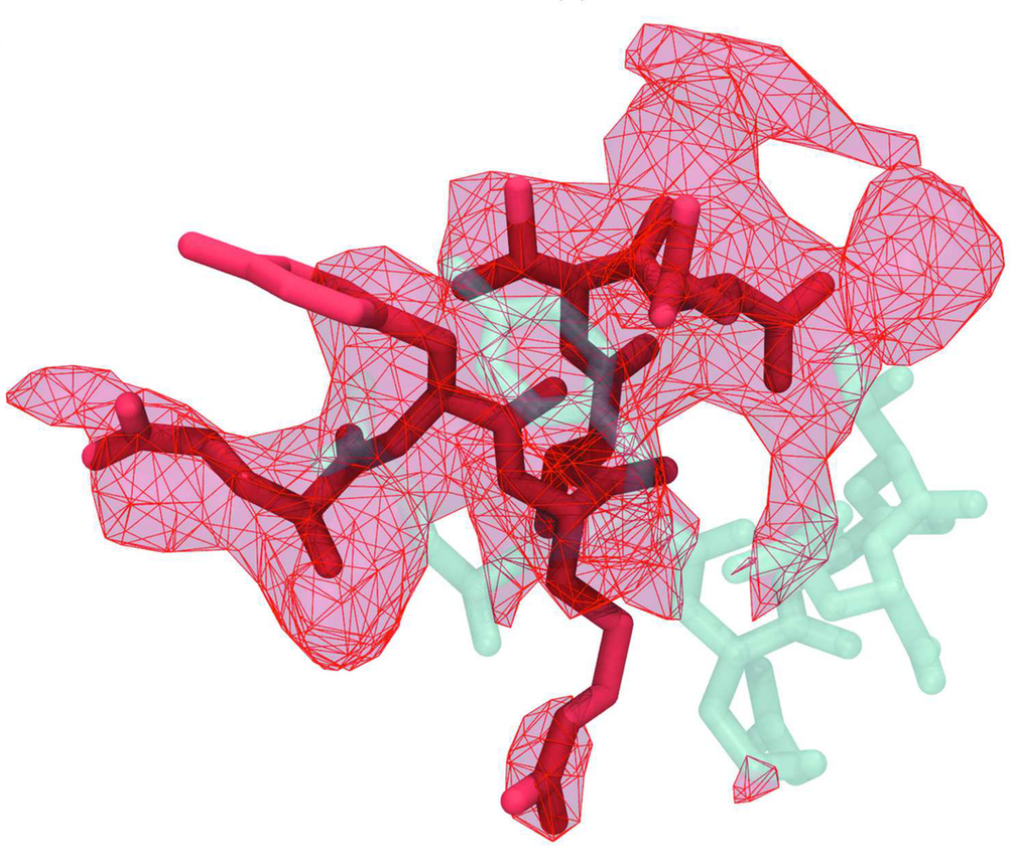
xMDFF: MDFF for low-resolution x-ray crystallography

- 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*)

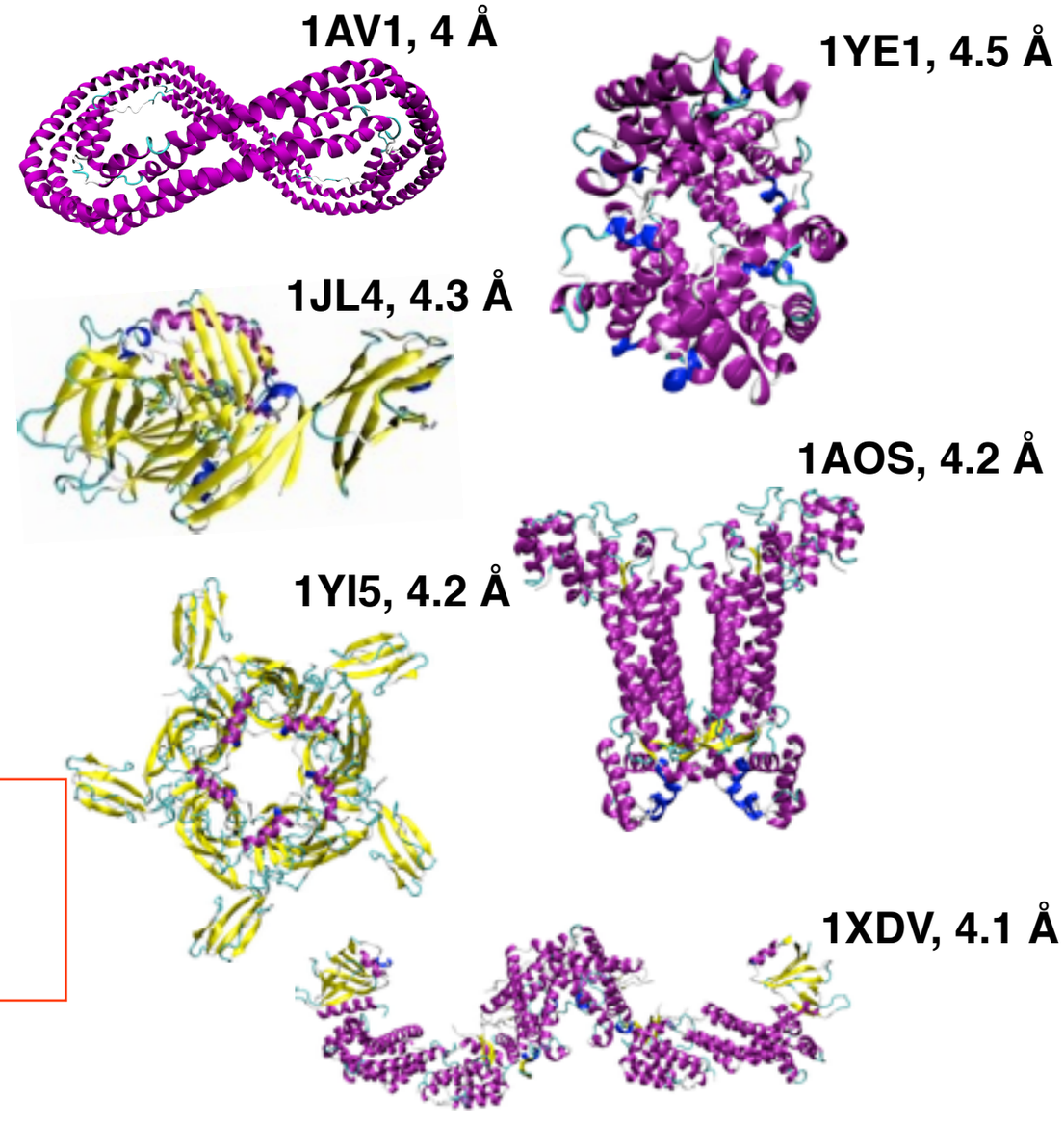
xMDFF Improves Structures Posted at the Protein Data Bank

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.*

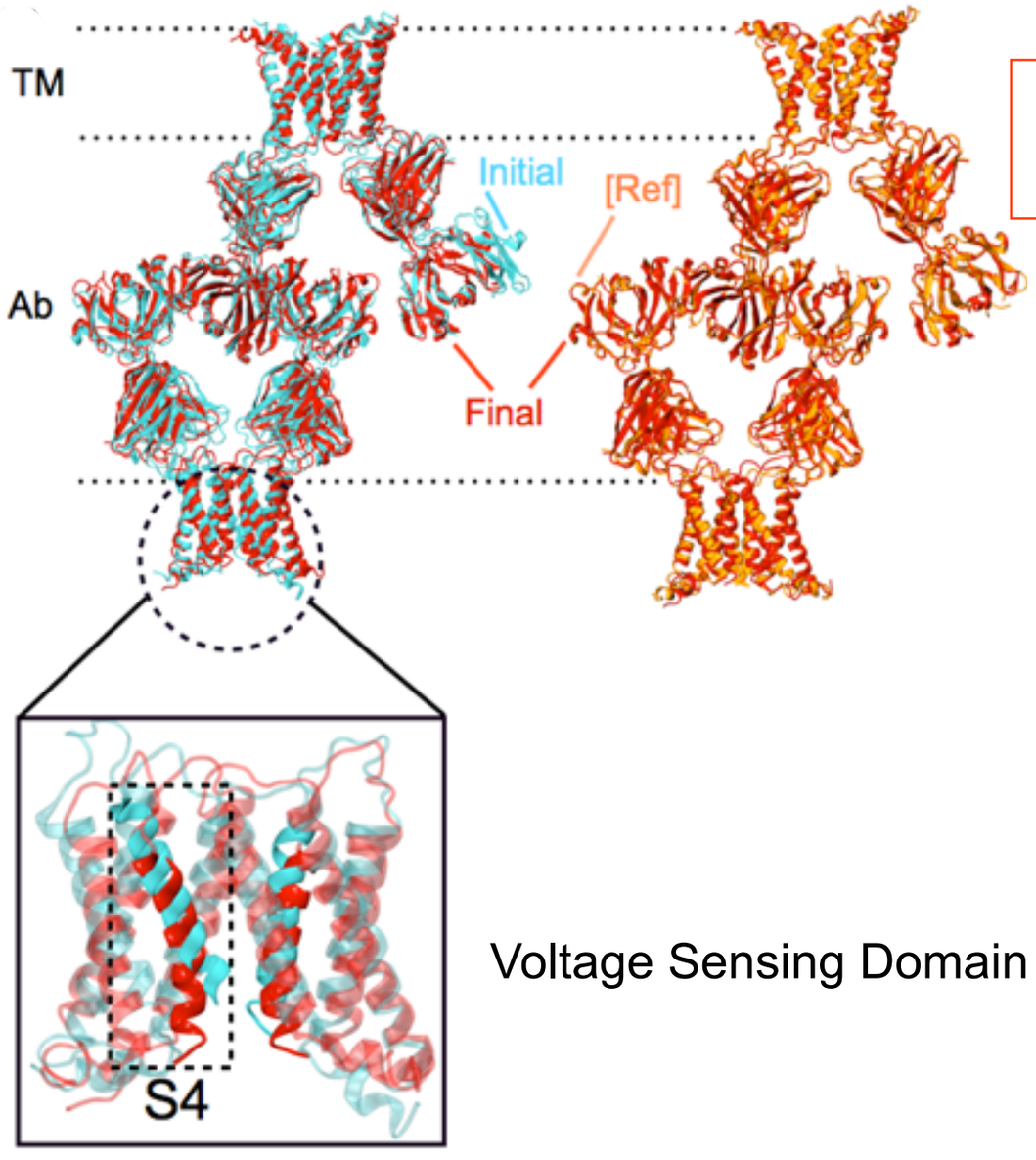
Structures



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

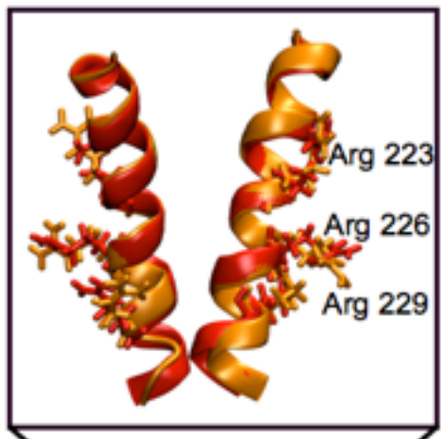
xMDFF Solves Voltage Sensor Protein Structure at 4 Å Resolution

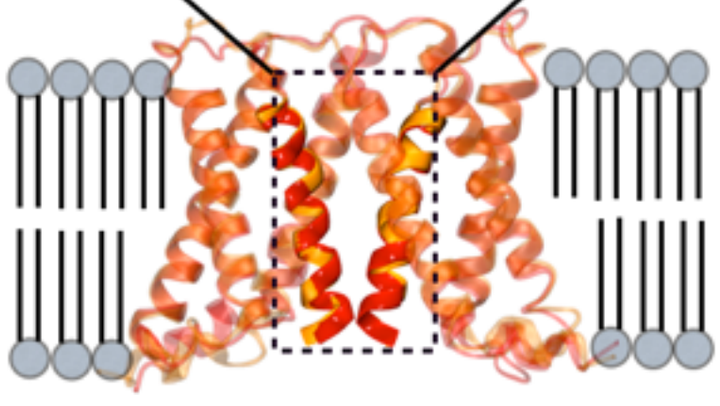
Collaboration with E. Perozo (U. Chicago)



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)

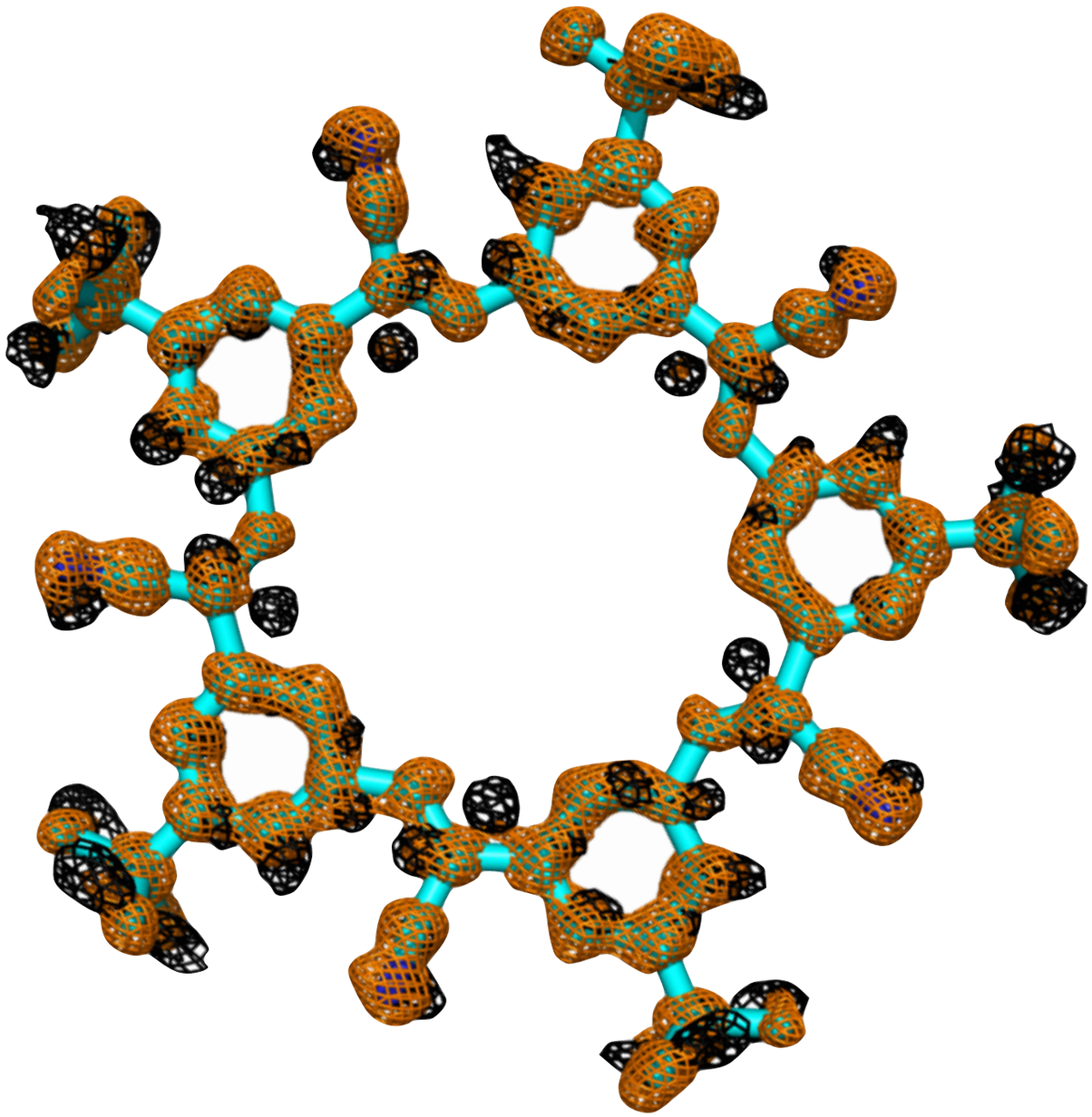
<u>Refinement statistics</u>

	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

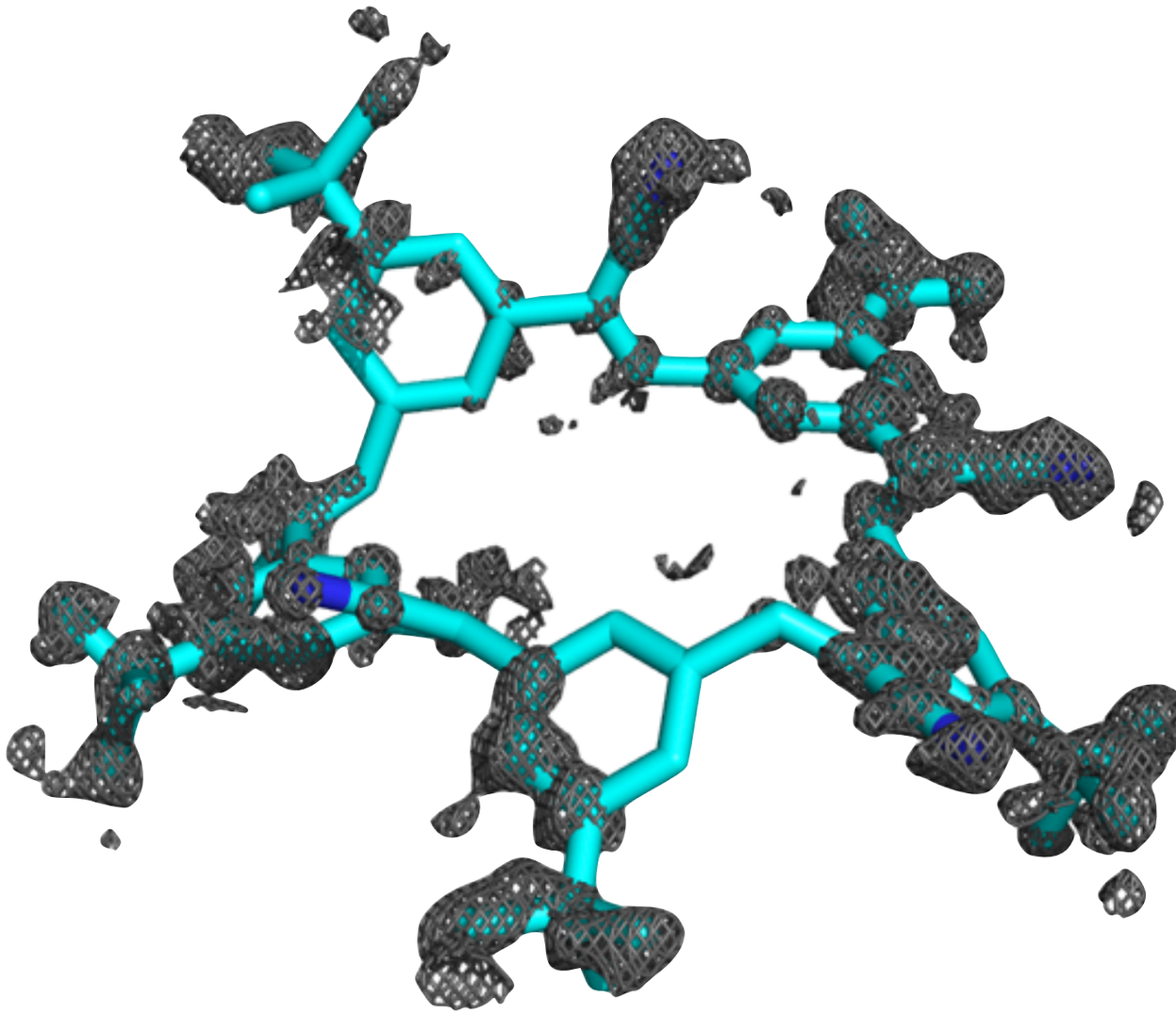
xMDFF for Abiological Materials

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.