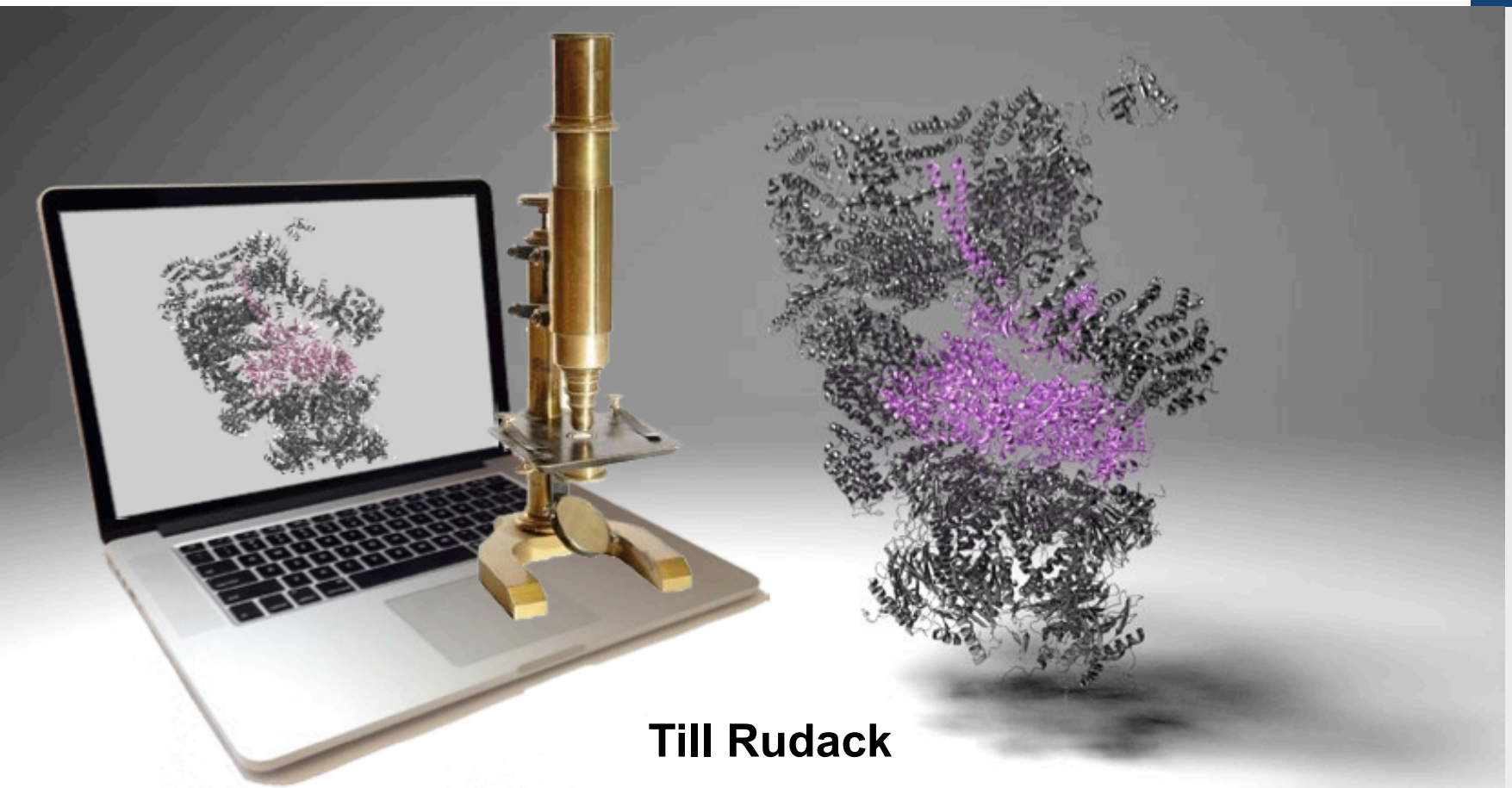
Integrative Modeling
Examples from Modern Research



Till Rudack

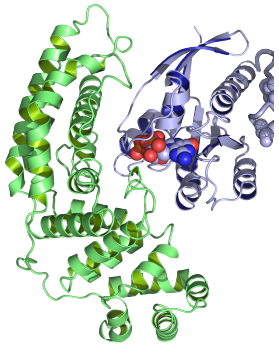
Klaus Schulten Group - Theoretical and Computational Biophysics Group

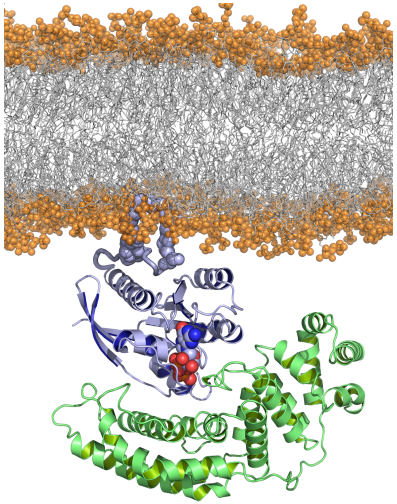
NIH Center for Macromolecular Modeling and Bioinformatics

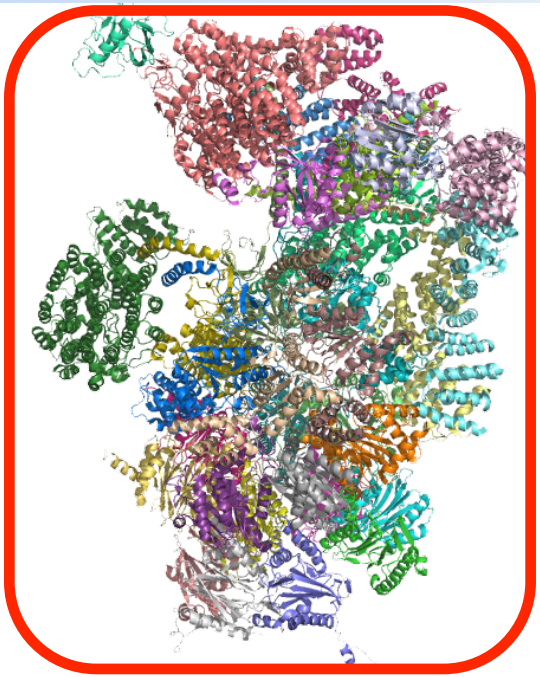
University of Illinois at Urbana-Champaign

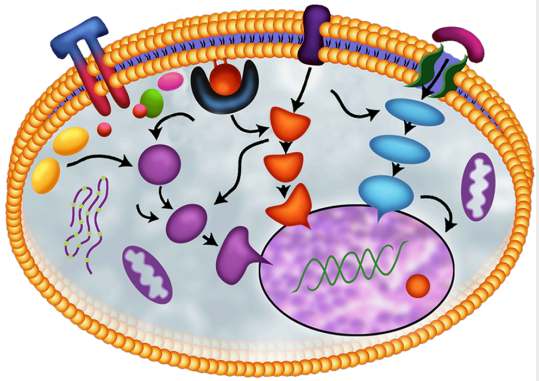
Application of MD simulations: Ras at Membrane

Protein In Solvent → **Protein + Membrane** → **Multi-Protein Complex** → **Cell**



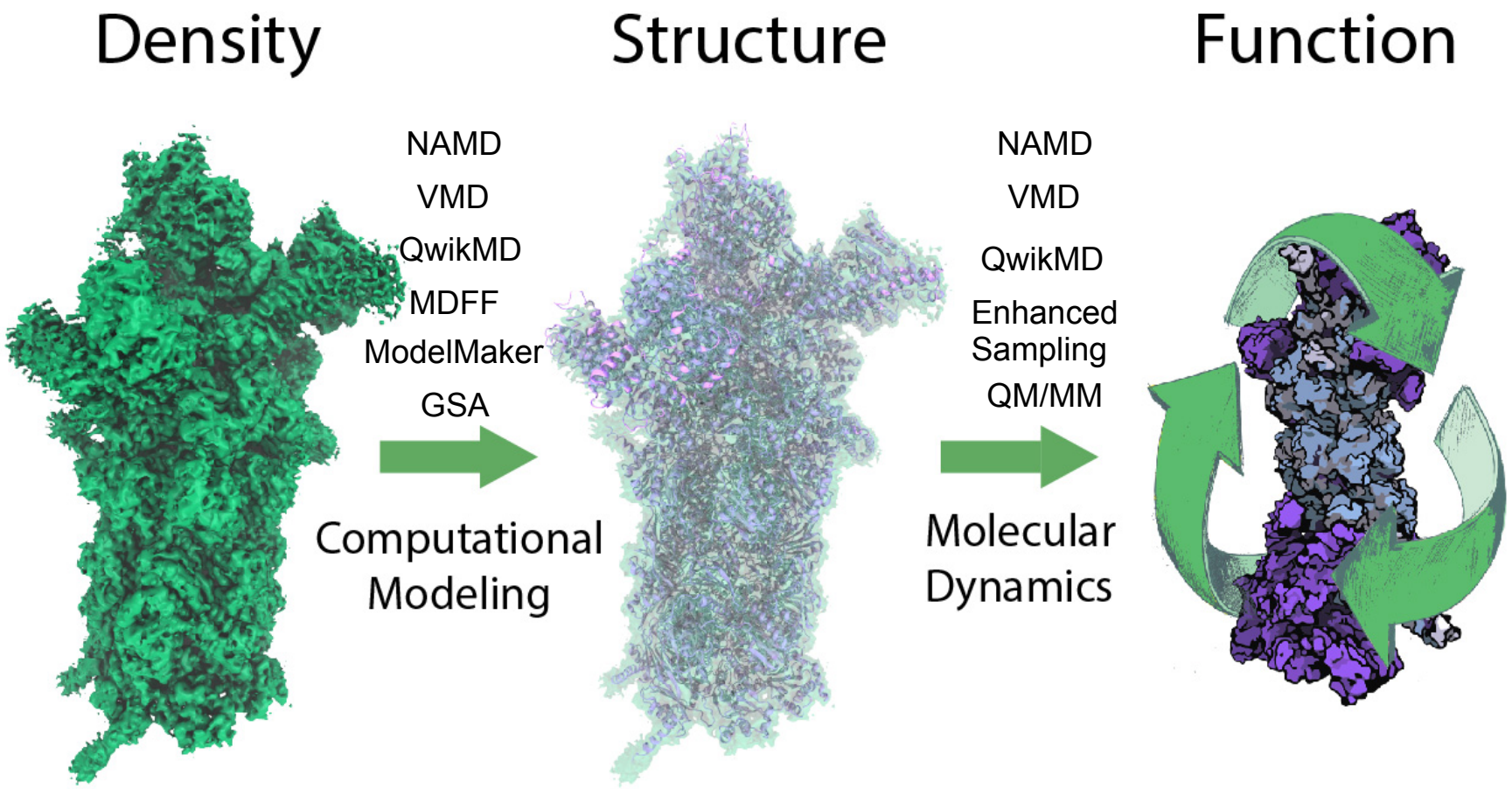






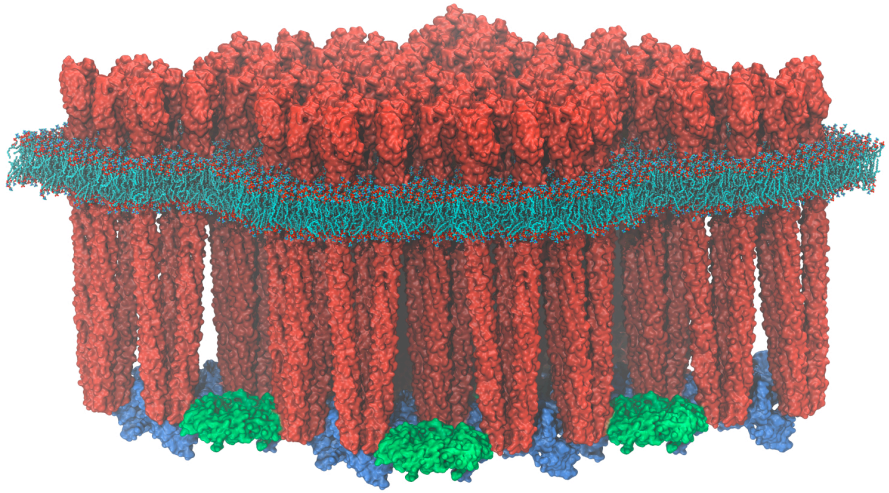
Molecular dynamics simulations connect **function** and **dynamics** to **structural data** from diverse **experimental sources** to investigate critical cellular processes occurring at the **sub-Ångstrom** level up to the **macromolecular** level.

The Key Strategy for Discoveries

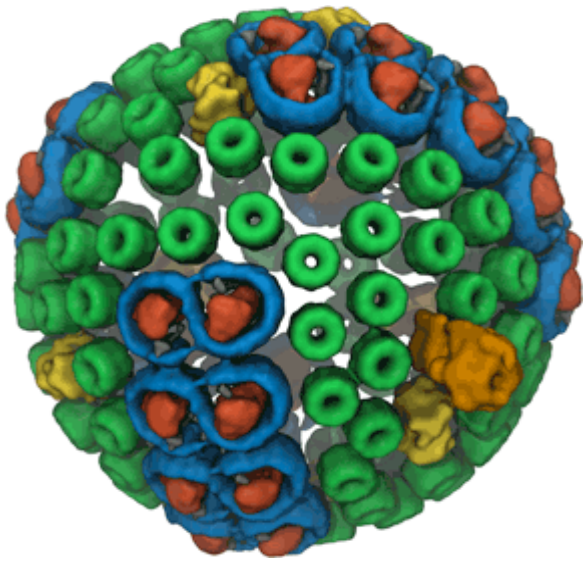


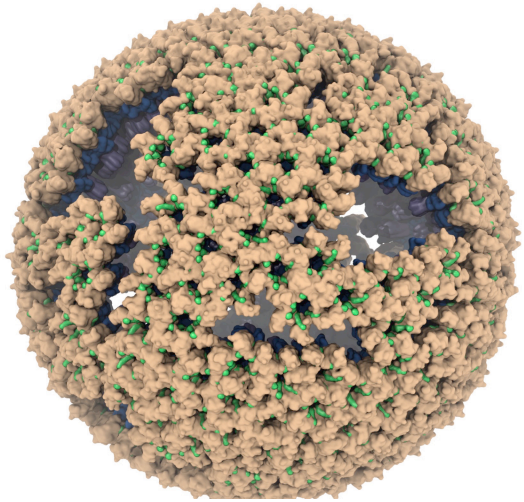
A Sampling of TCBG's MDFF Projects

Chemosensory Array

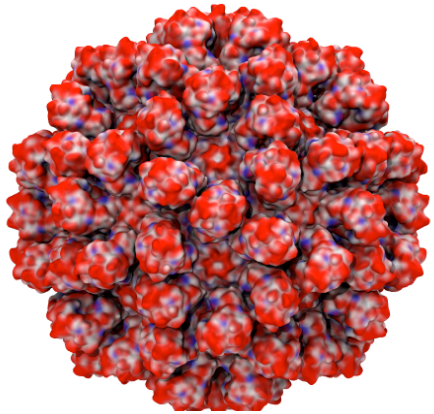


Chromatophore



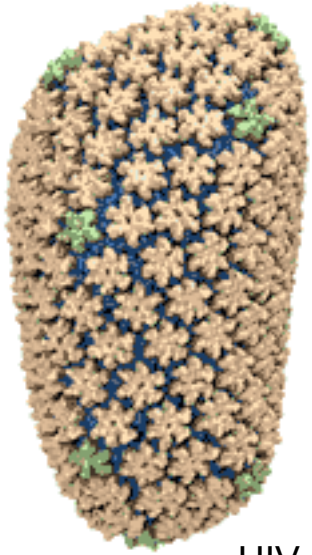


Rous Sarcoma Virus



Theoretic

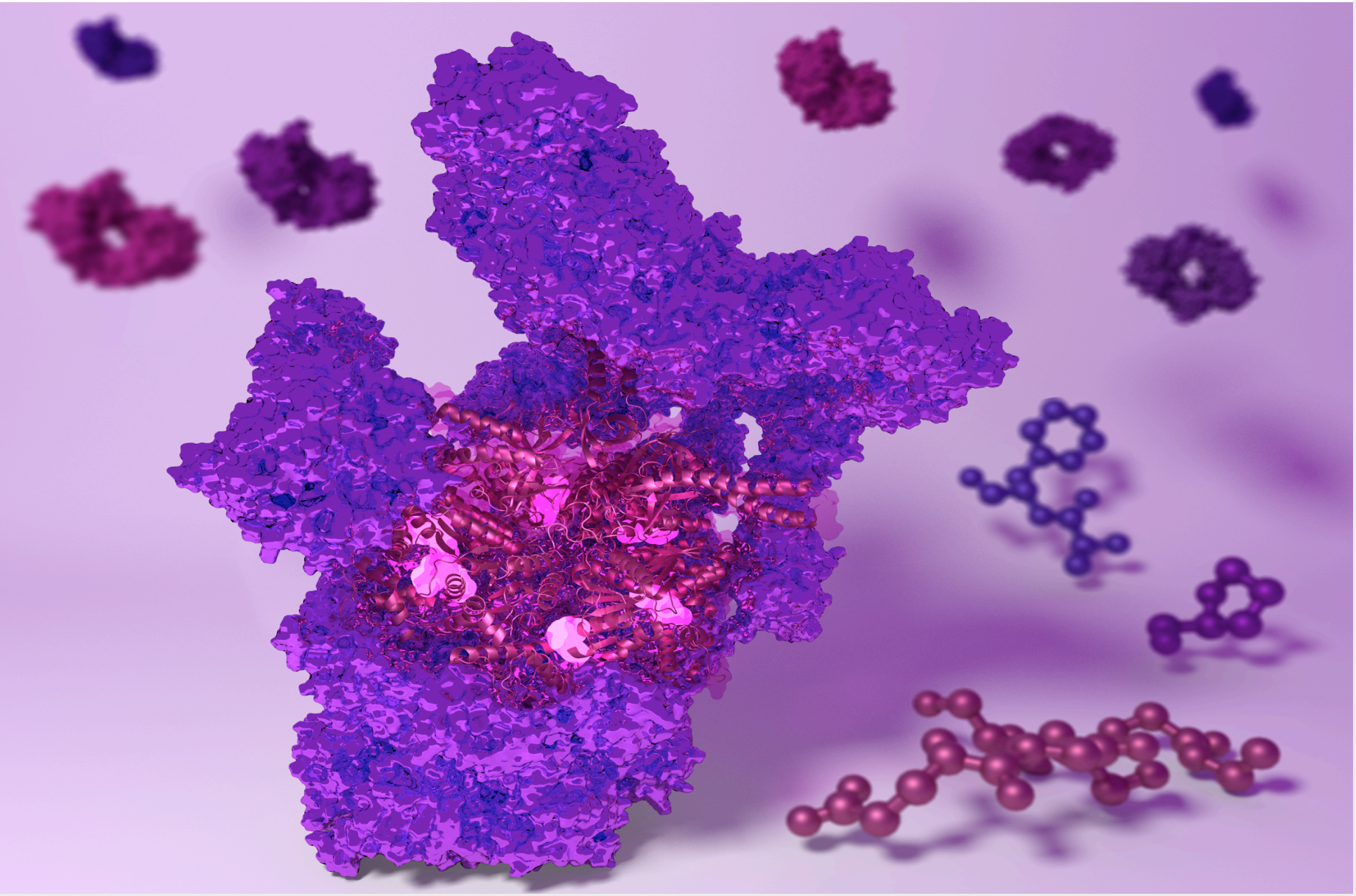
Rabbit Hemorrhagic Disease



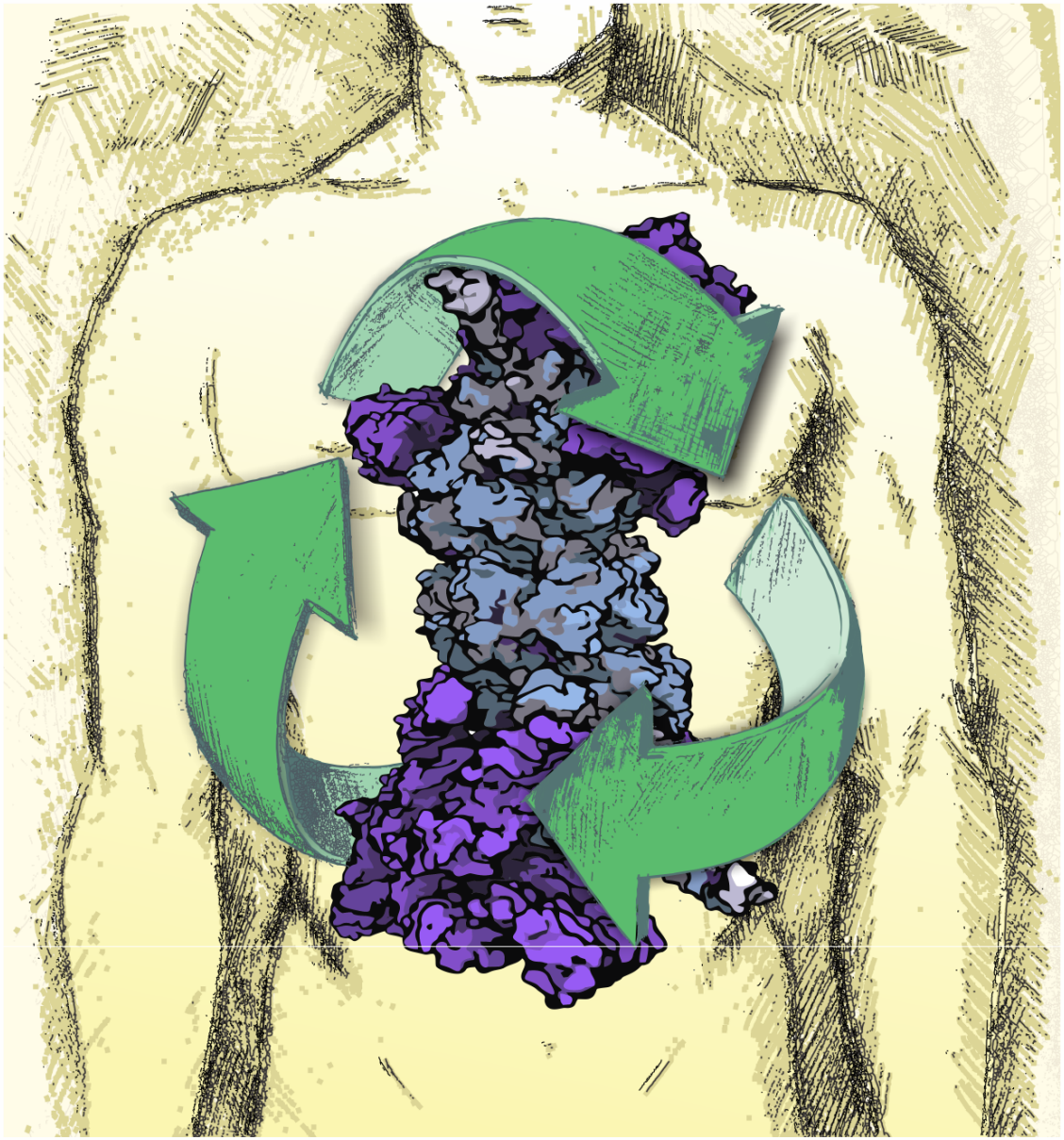
HIV

26S Proteasome

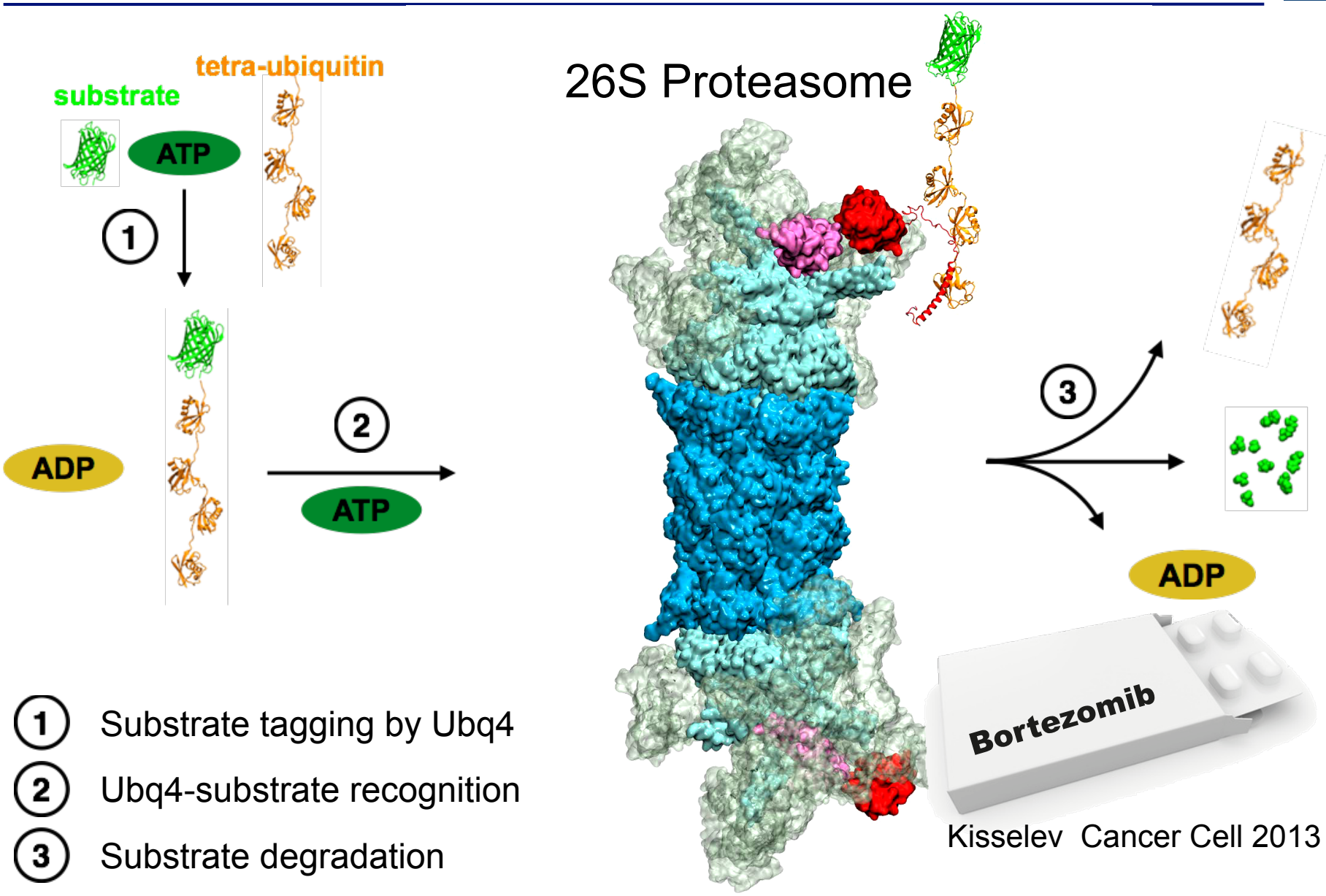
Integrating experimental methods into computational modeling



The Receycling System of the Cell



The ubiquitin proteasome proteolytic pathway



1. Substrate tagging by Ubq4
2. Ubq4-substrate recognition
3. Substrate degradation

Kisselev Cancer Cell 2013

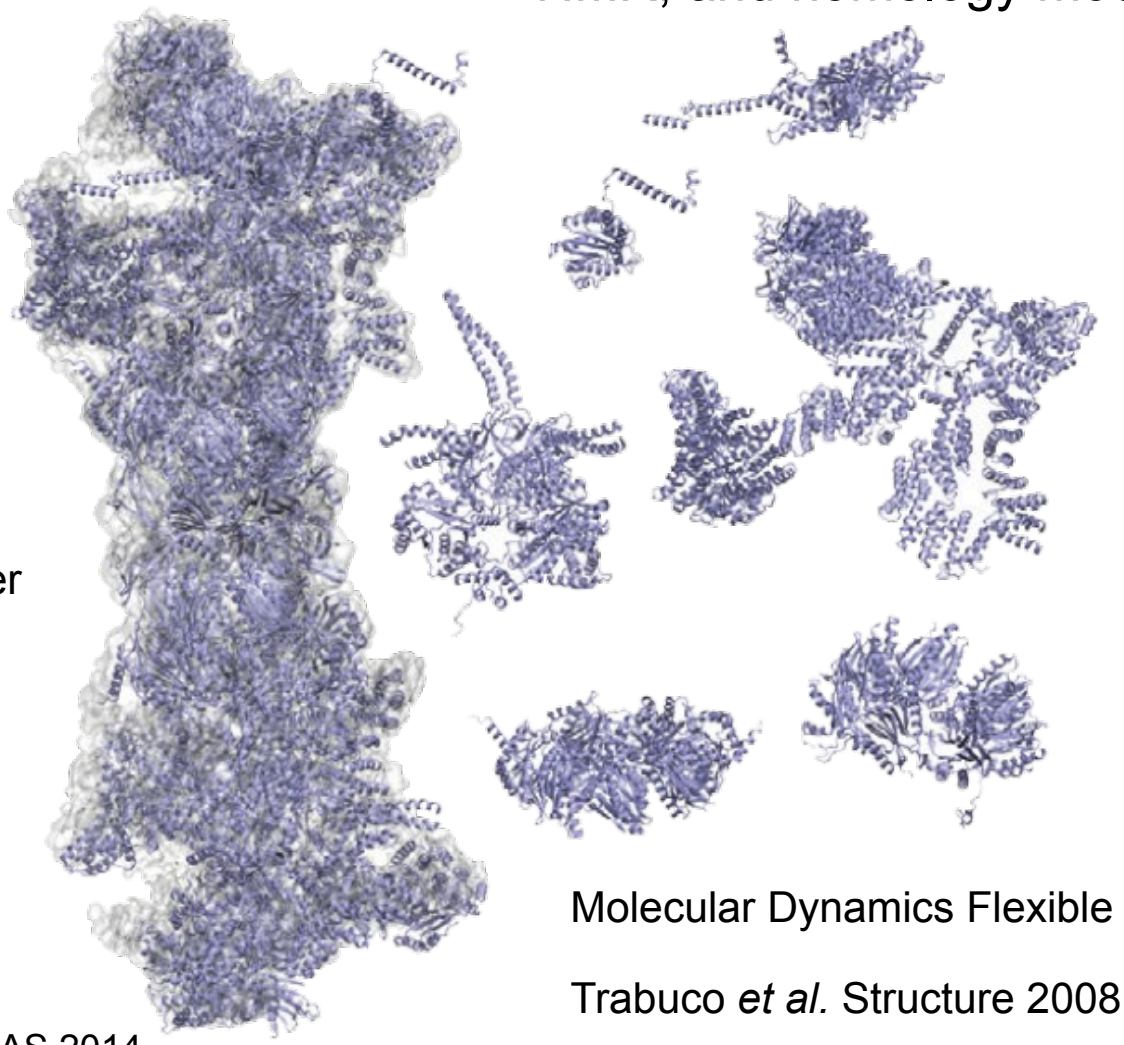
Near-atomic model of the 26S proteasome

Cryo-EM density

Subunits from X-ray crystalography, NMR, and homology modeling

max planck institute of biochemistry

Wolfgang Baumeister
Friedrich Foerster



PDB-ID 4CR2

EMDB-ID 2594

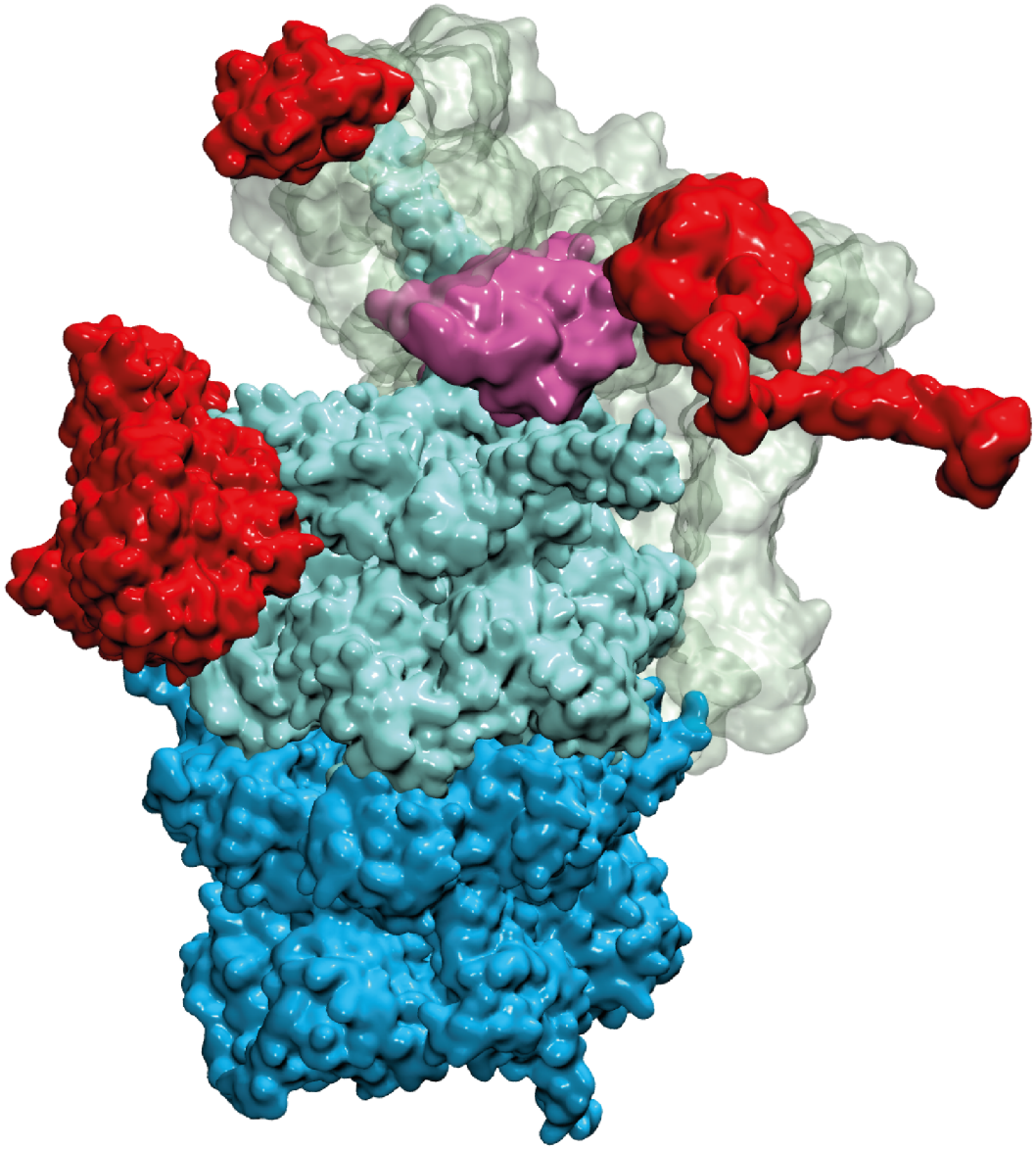
Resolution 7.7 Å

Unverdorben *et al.* PNAS 2014

Molecular Dynamics Flexible Fitting (MDFF):

Trabuco *et al.* Structure 2008

Functional subunits of the 26S proteasome



Ubiquitin
Recognition
(Rpn10, Rpn13, Rpn1)

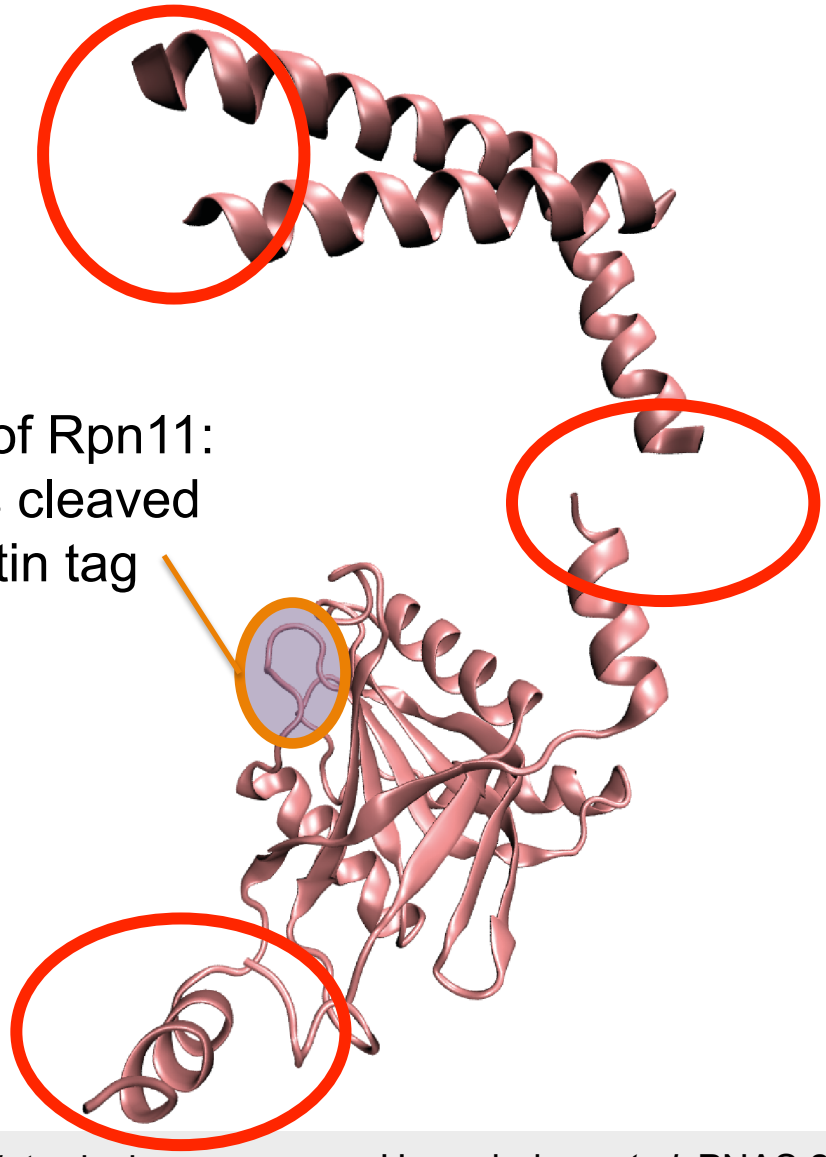
Deubiquitylation
(Rpn11)

Substrate
Unfolding
(ATPase-ring)

Substrate
Degradation
(α-ring, β-ring)

Deubiquitylation subunit: Rpn11

Complete models are a basic prerequisite to **perform MD simulations**



Deubiquitylation (Rpn11)

Active site of Rpn11: substrate is cleaved from ubiquitin tag

Missing segments

- highly flexible

- ambigous density

Chain V of PDB-ID 4CR2

Unverdorben *et al.* PNAS 2014

Combining Rosetta and MDFF through VMD

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

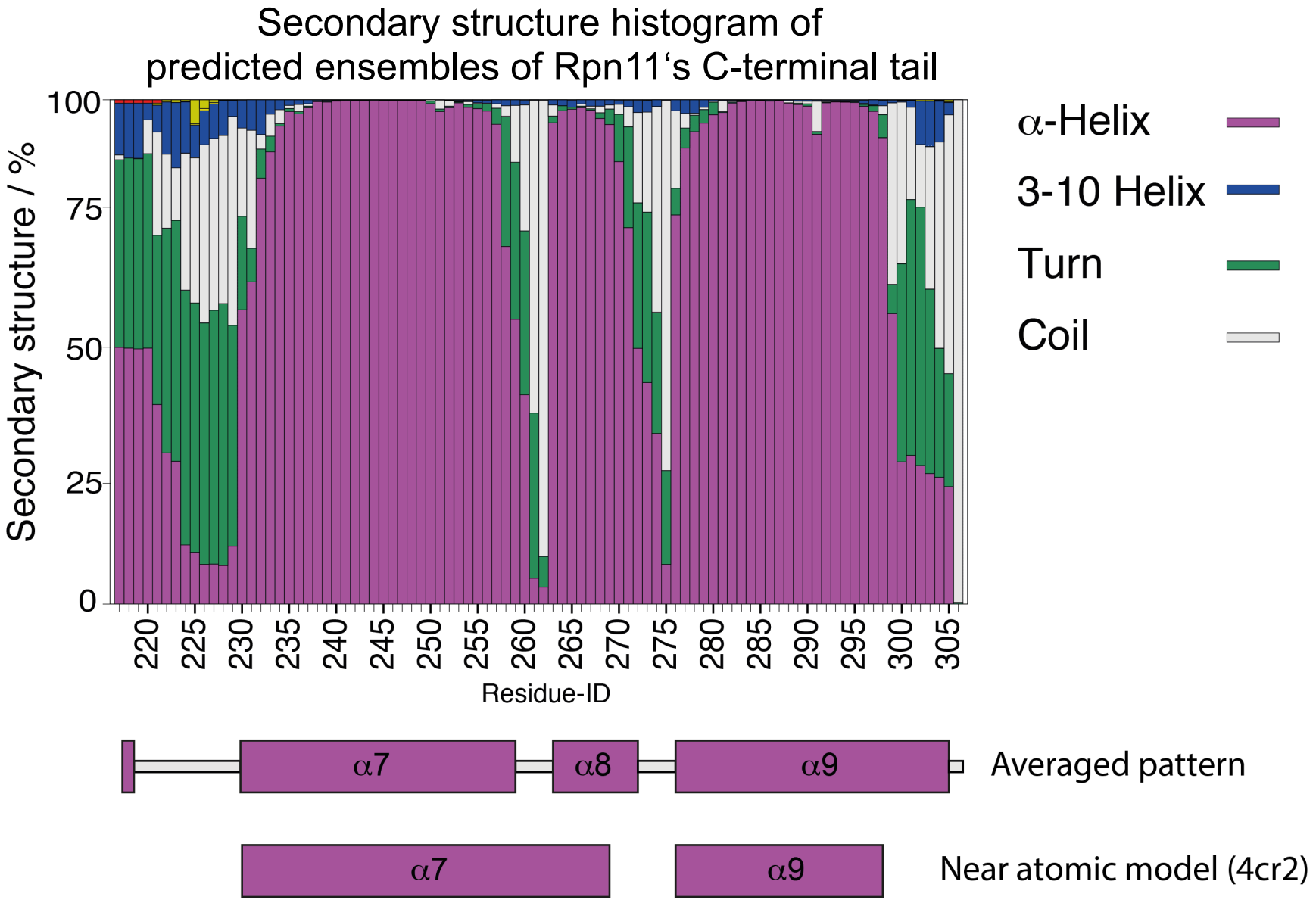
Leaver-Fay *et al.* Methods Enzymol. 2011
Porter *et al.* PLoS One 2015

VMD/NAMD

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

Integrating user expertise into *de novo* structure prediction

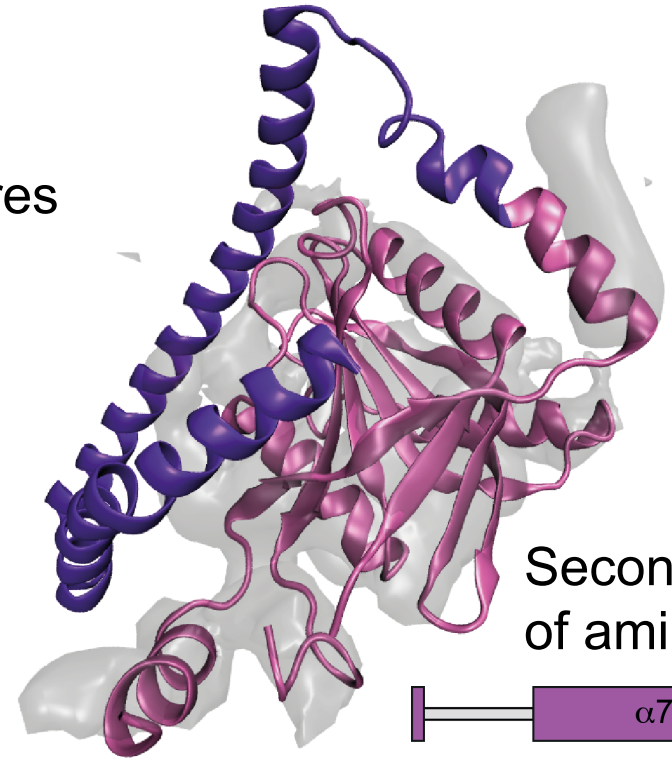
Model filtering by secondary structure



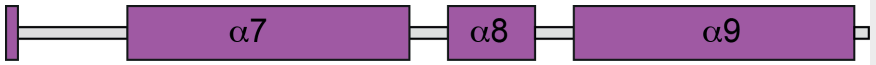
Predicted model

Represenative model of the
predicted averaged secondary structure pattern
for Rpn11‘s C-terminal tail (purple)

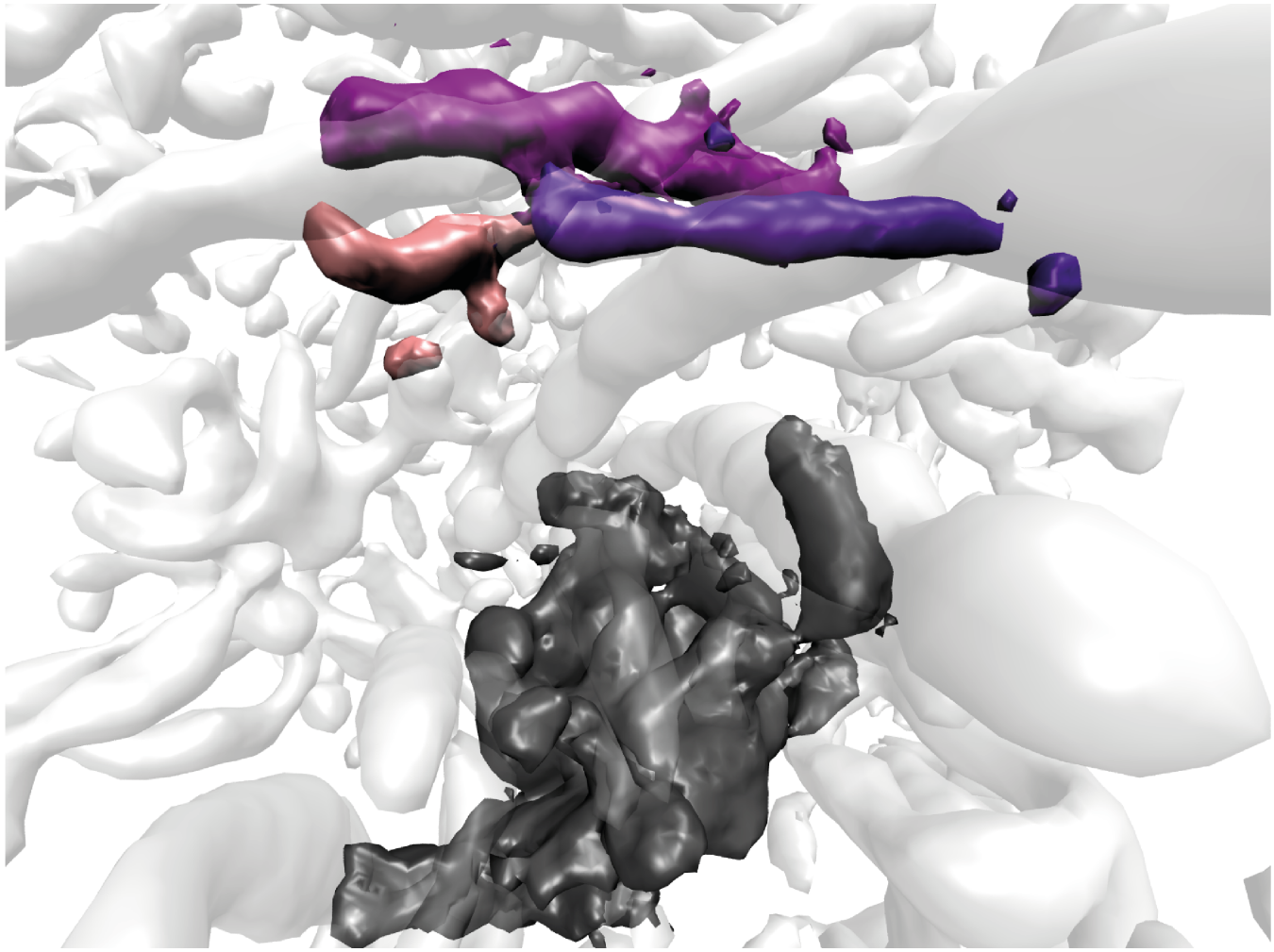
Rosetta tends to
build compact structures

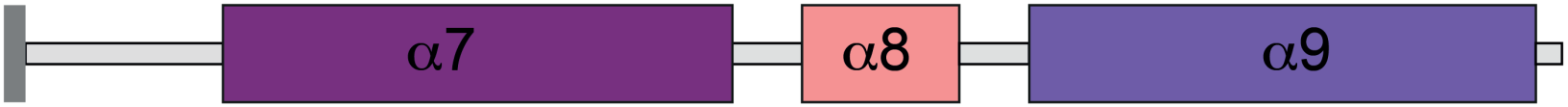


Secondary structure pattern
of amino acids 217-306 (purple)

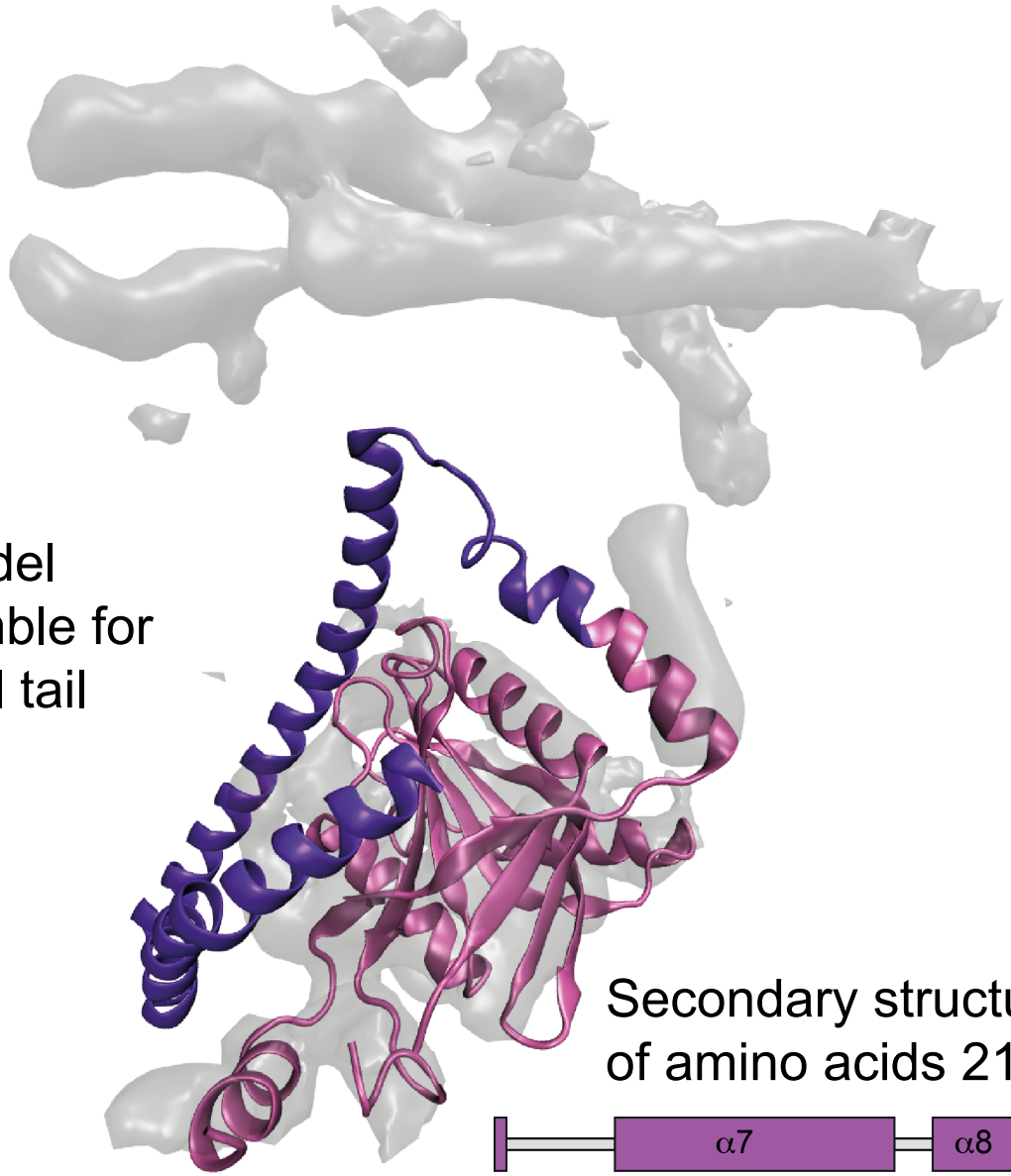


Visual inspection of cryo-EM density





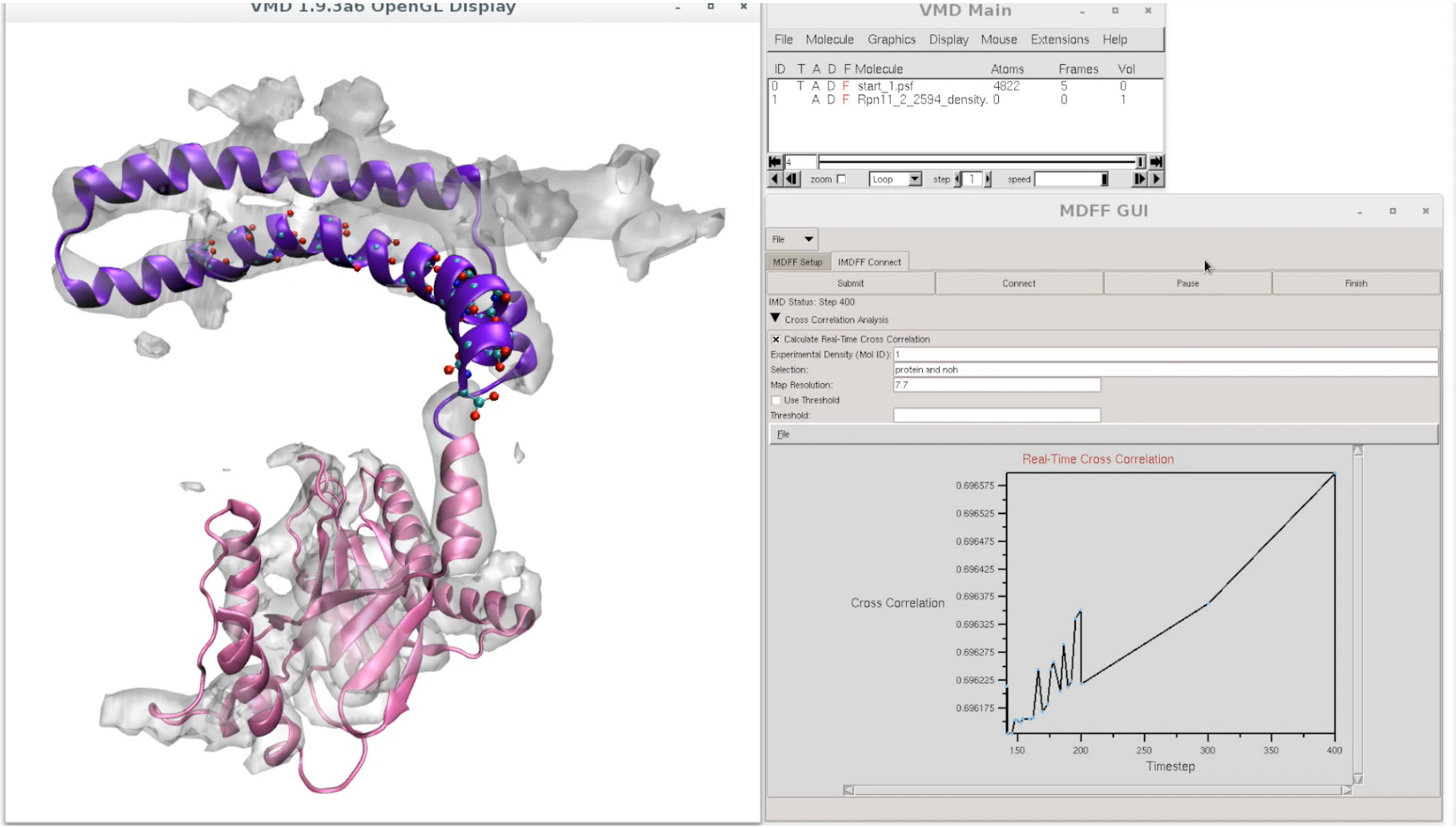
Predicted model to initiate MDFF



Represenative model
of predicted ensemble for
Rpn11‘s C-terminal tail

Secondary structure pattern
of amino acids 217-306 (purple)

Interactive Molecular Dynamics Flexible Fitting

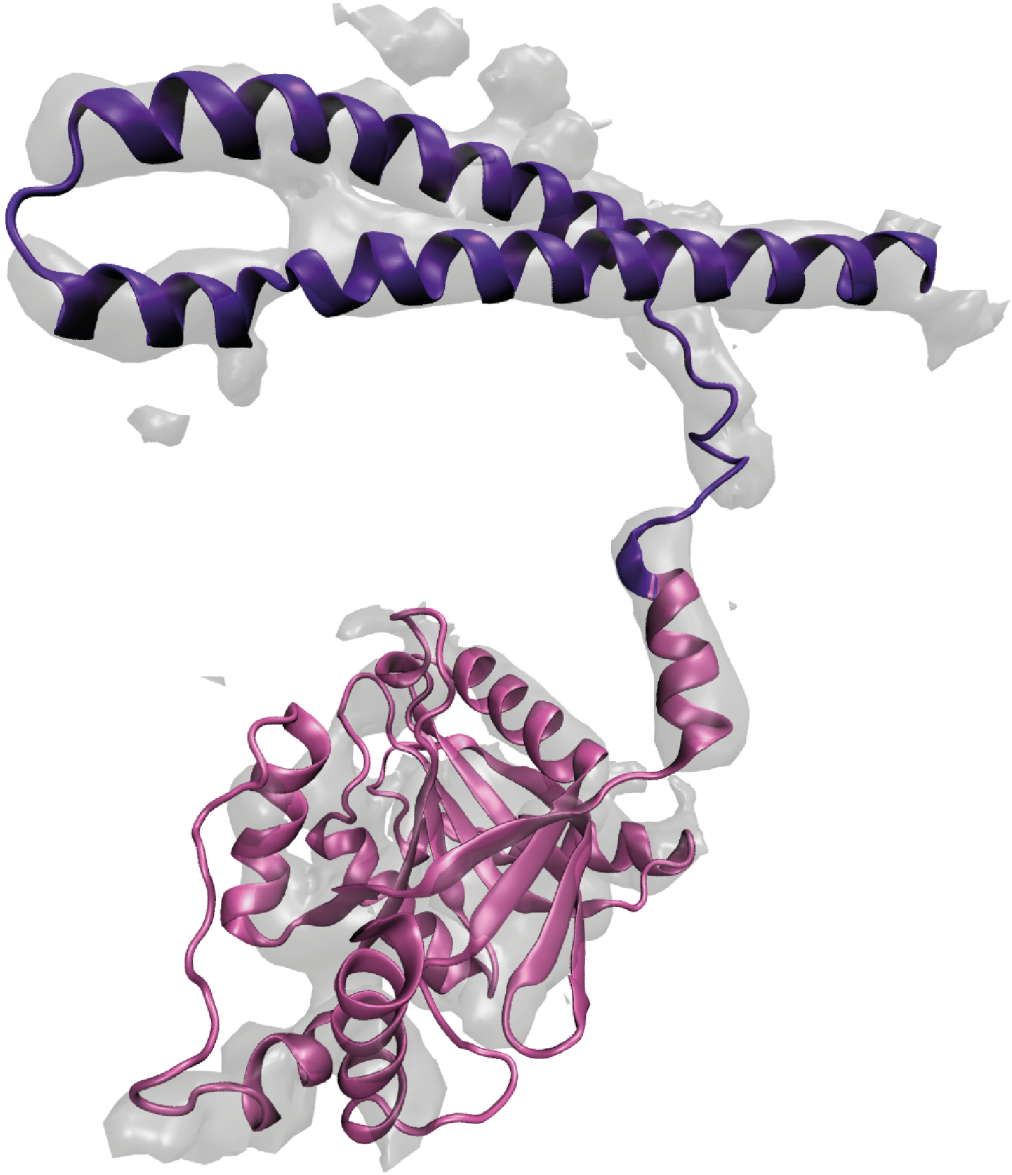


MDFF can be run on Cloud computing for a cup of a coffee!

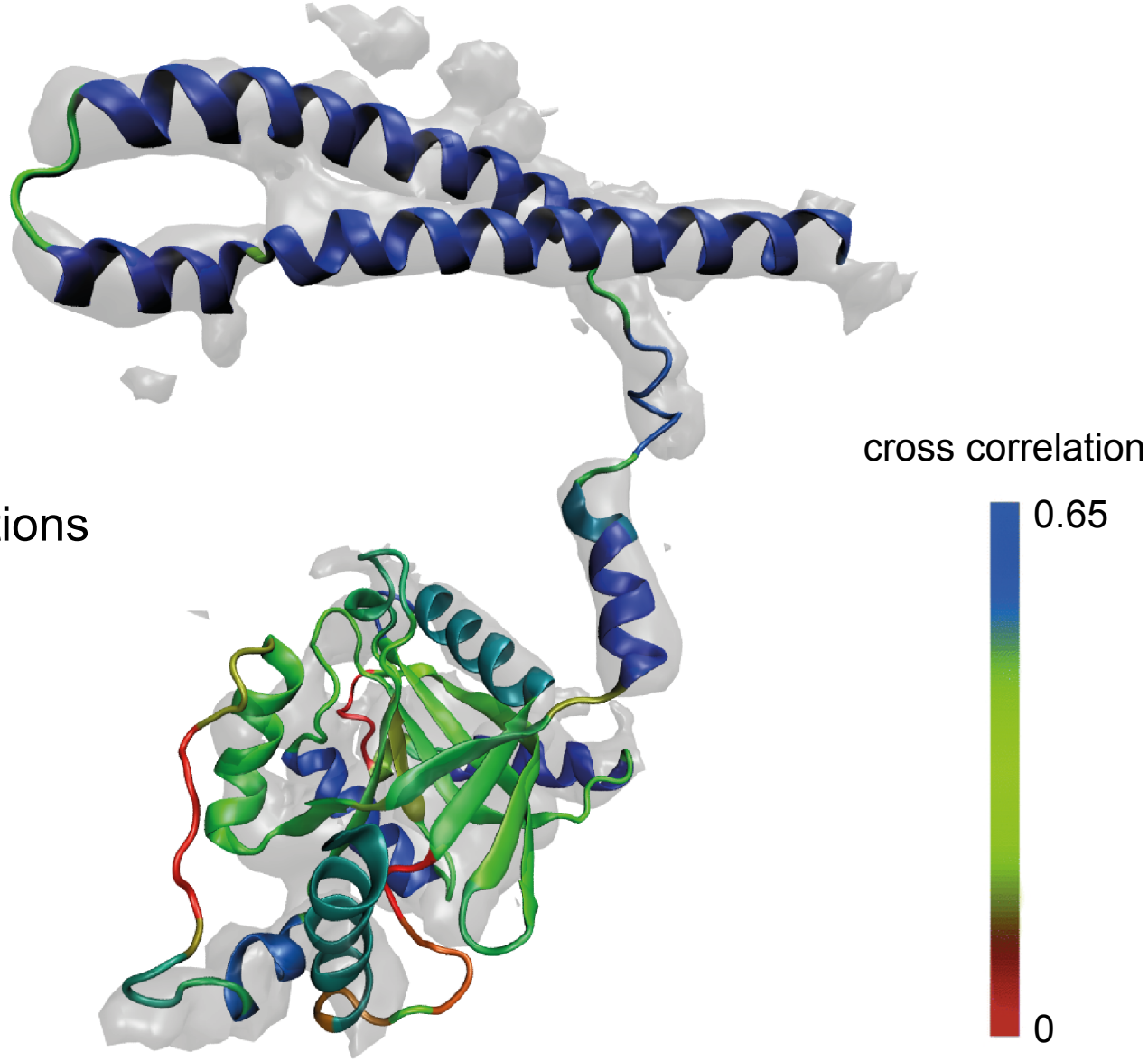
MDFF runs can be laucnhed through QwikMD!

MDFF Tutorial on You Tube and at http://www.ks.uiuc.edu/Research/mdff/

Complete model of Rpn11 fitted to density



Quality check by cross-correlations

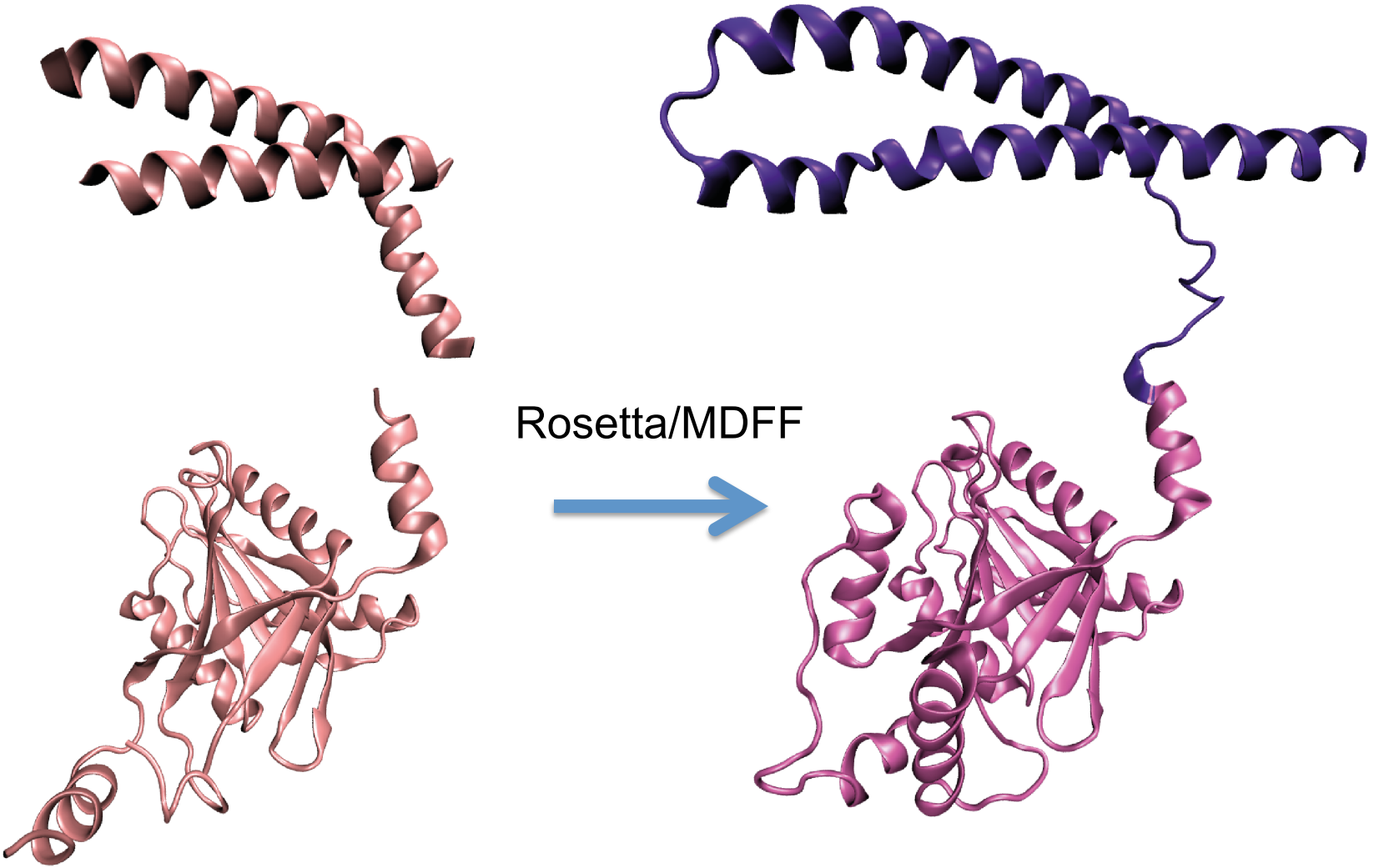


Rpn11 colored by
local cross correlations

Incomplete vs. complete model

Incomplete model

Complete model



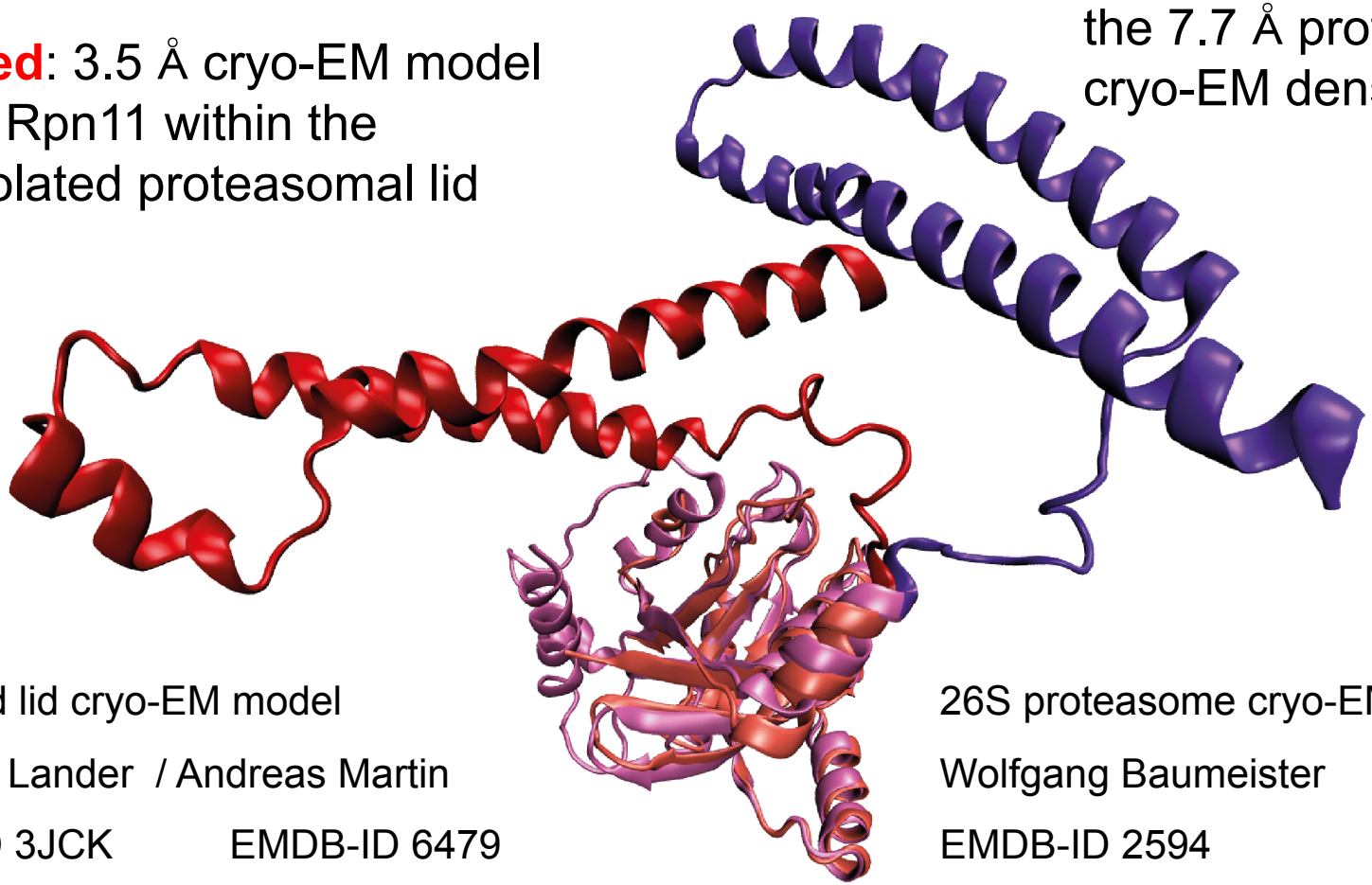
Cross correlation 0.61

Cross correlation 0.63

Low vs. high resolution density model

Red: 3.5 Å cryo-EM model of Rpn11 within the isolated proteasomal lid

Purple: completed Rpn11 model within the 7.7 Å proteasomal cryo-EM density



Isolated lid cryo-EM model

Gabriel Lander / Andreas Martin

PDB-ID 3JCK EMDB-ID 6479

Resolution 3.5 Å

Dambacher *et al.* eLife 2016

26S proteasome cryo-EM density

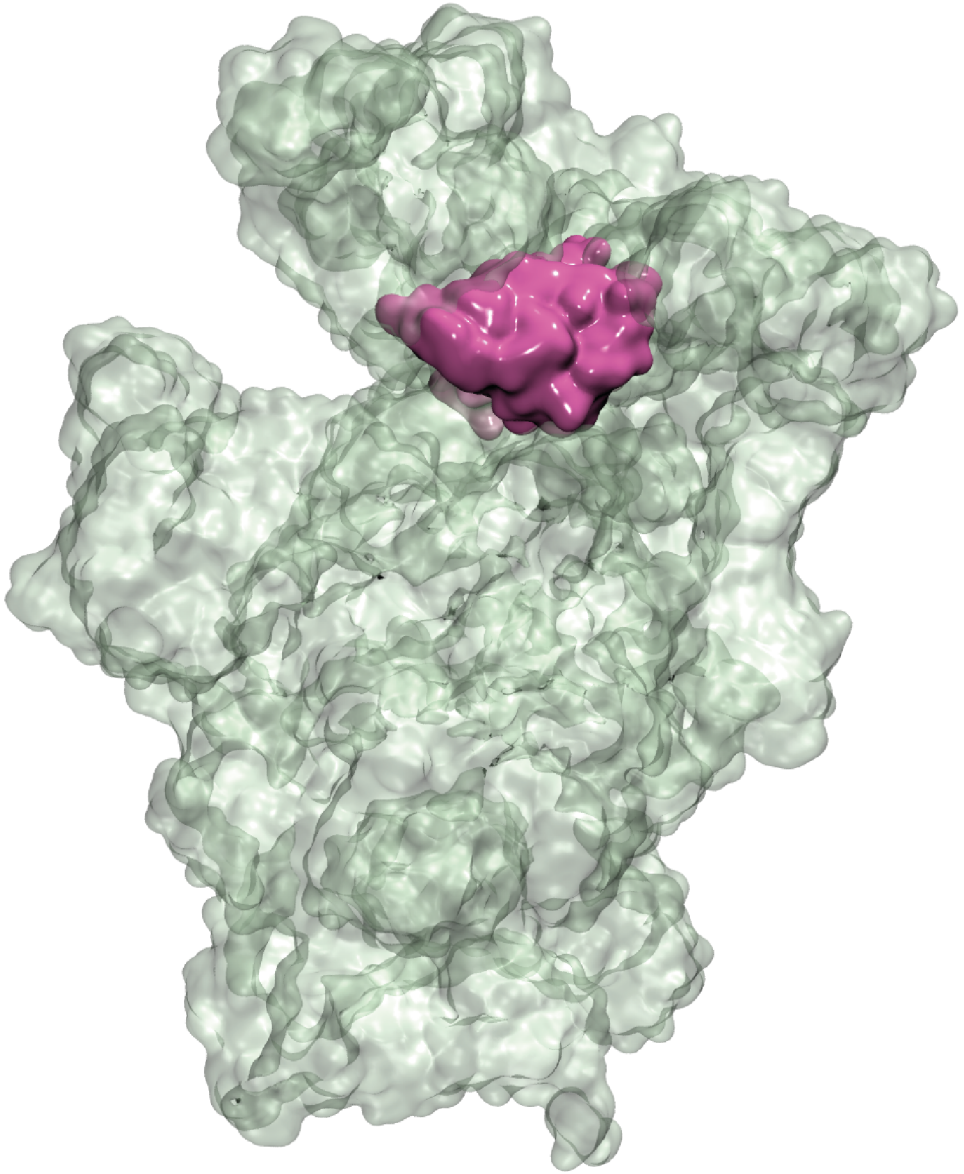
Wolfgang Baumeister

EMDB-ID 2594

Resolution 7.7 Å

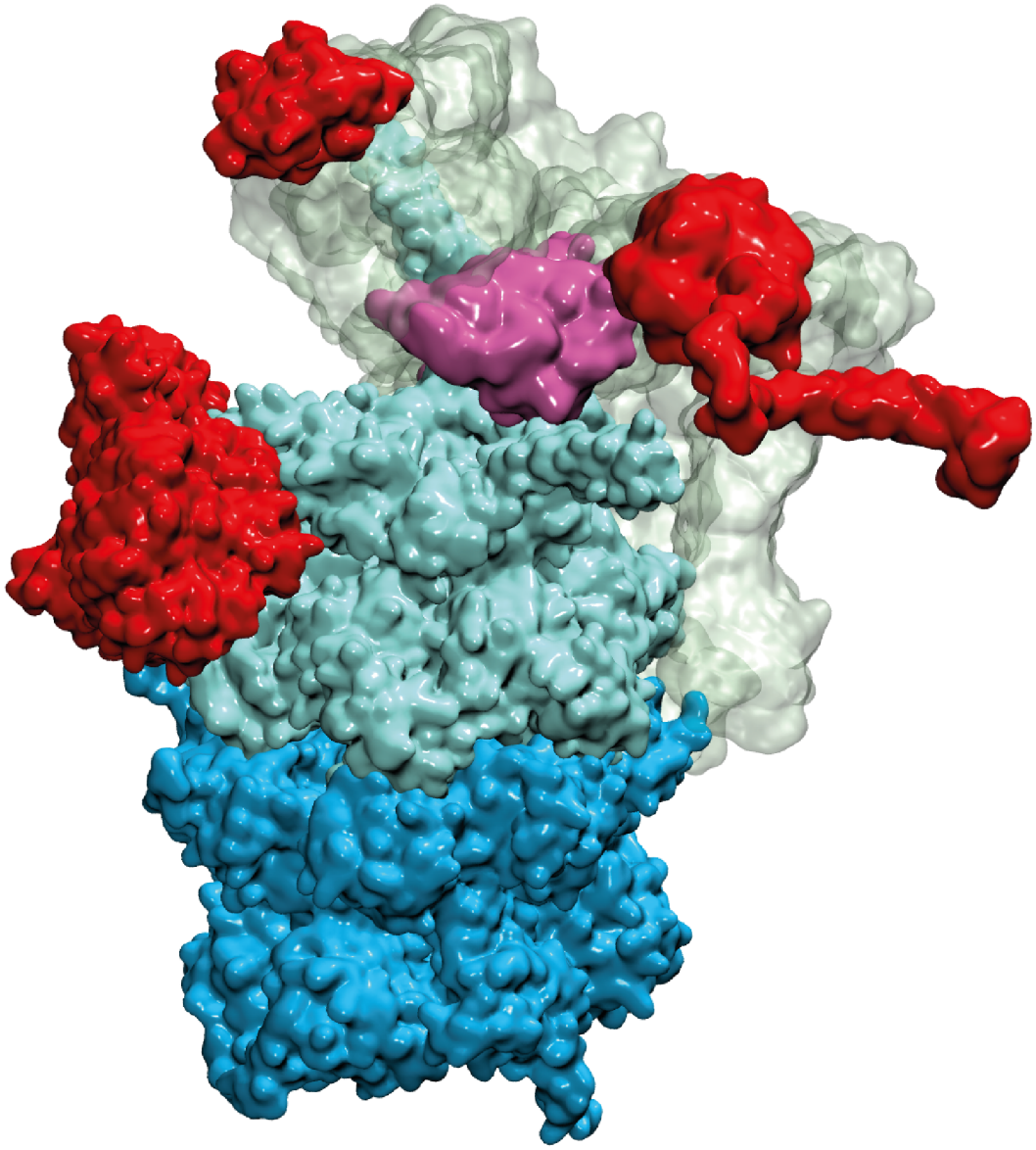
Unverdorben *et al.* PNAS 2014

Low vs. high resolution density model



Deubiquitylation
(Rpn11)

Functional subunits of the 26S proteasome



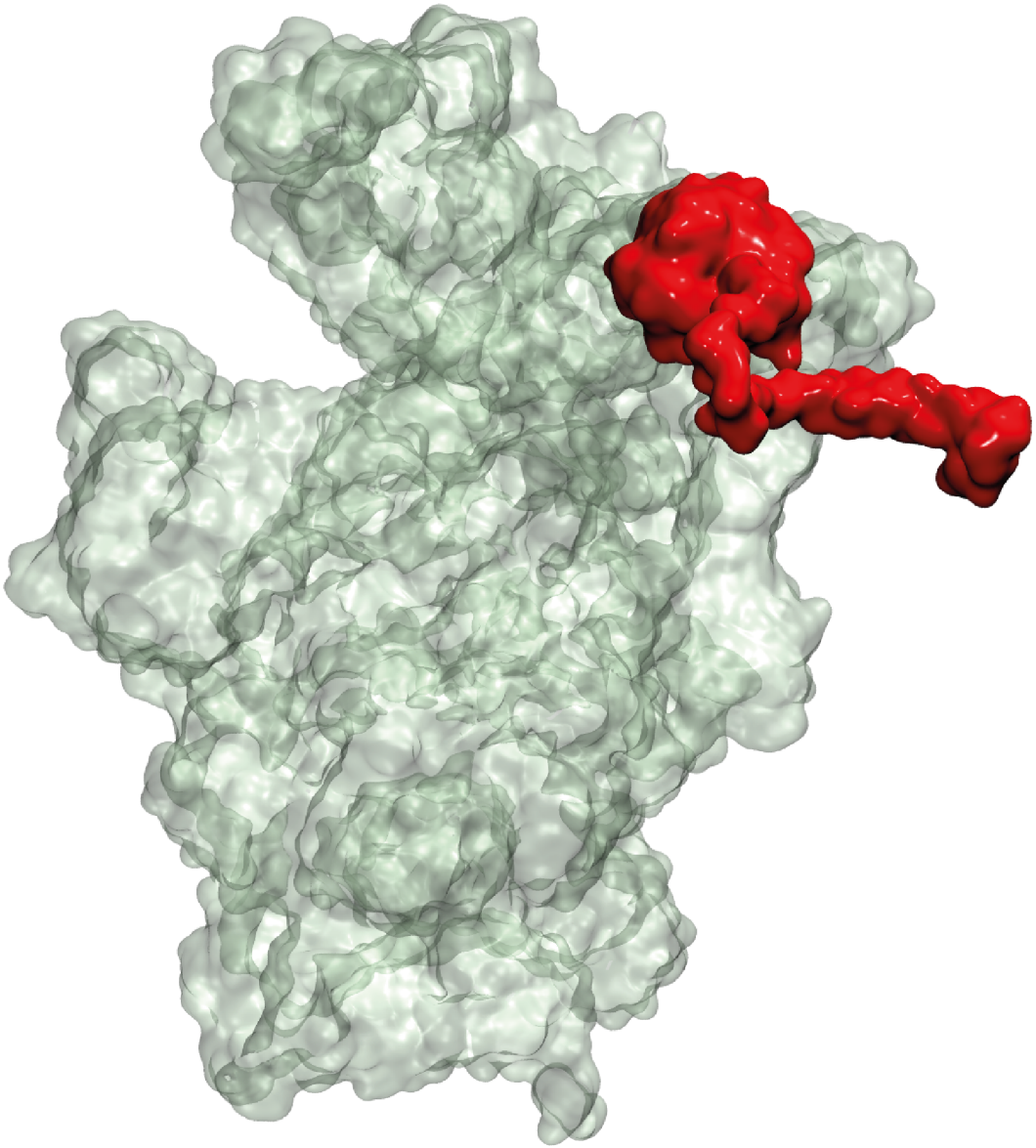
Ubiquitin
Recognition
(Rpn10, Rpn13, Rpn1)

Deubiquitylation
(Rpn11)

Substrate
Unfolding
(ATPase-ring)

Substrate
Degradation
(α-ring, β-ring)

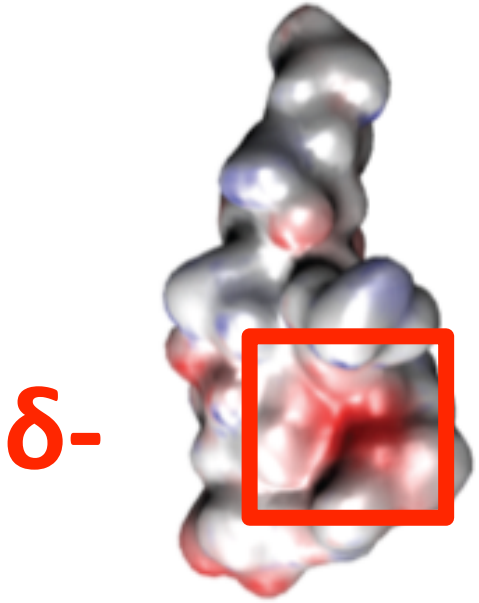
Ubiquitin recognition by Rpn10



Ubiquitin Recognition (Rpn10)

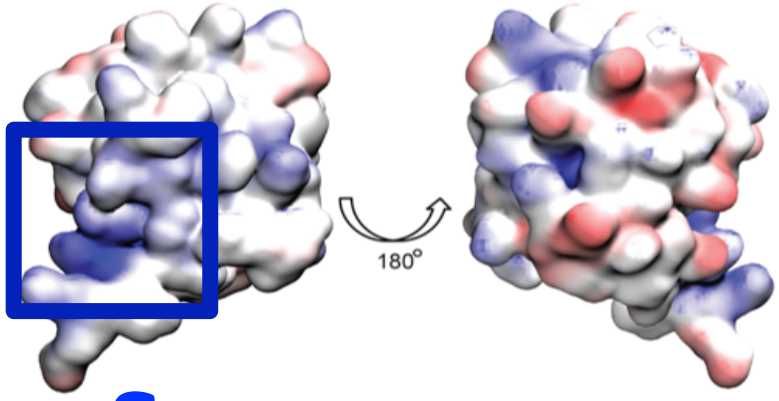
Zhang*, Vucovic*, Rudack*, Han, Schulten 2016 JPC B (in press)

Ubiquitin Recognition



δ-

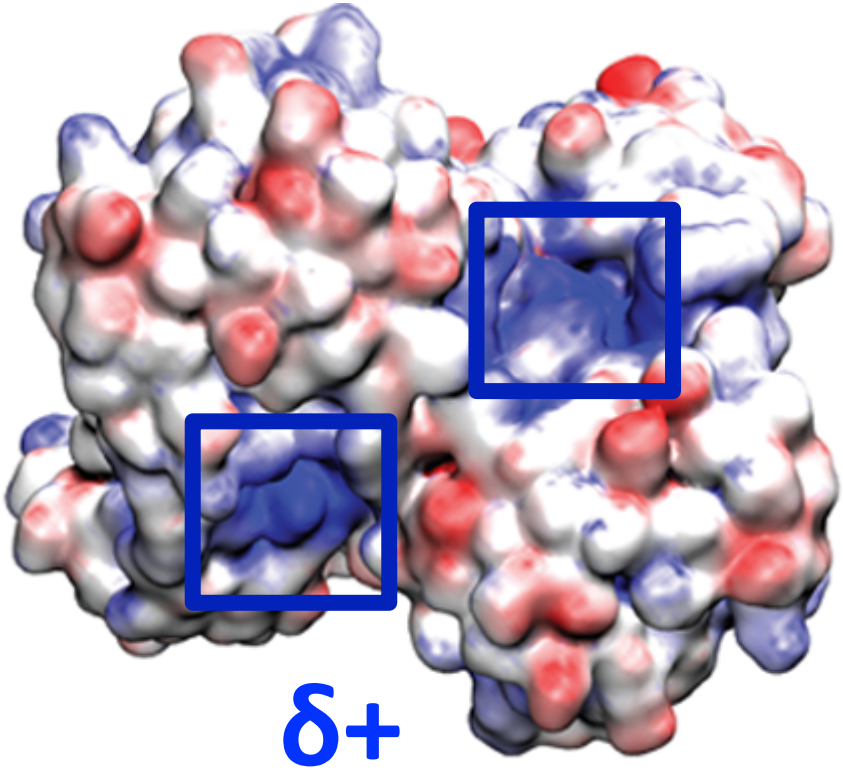
Rpn10
(UIM)



δ+

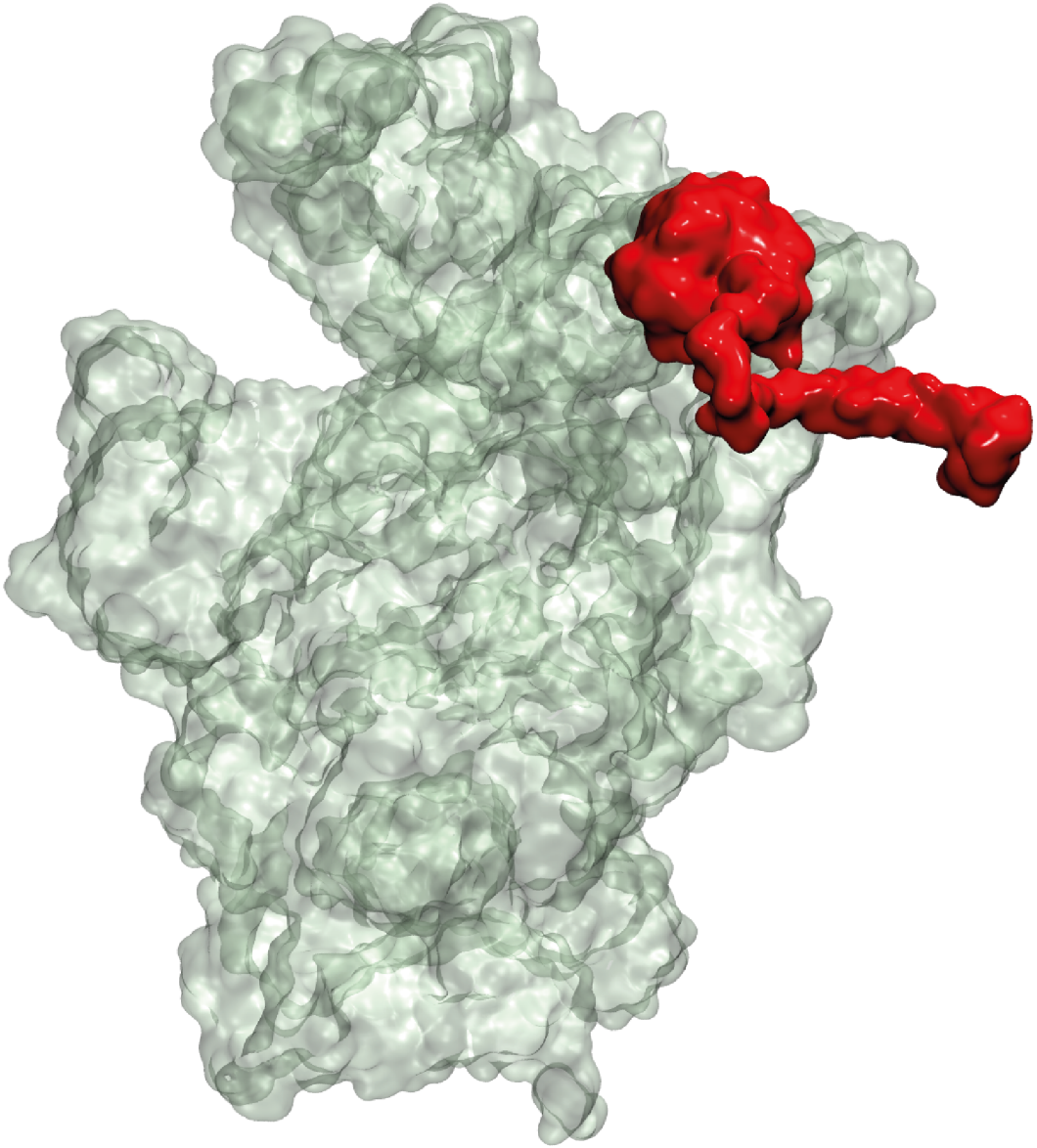
Monoubiquitin

Tetraubiquitin



δ+

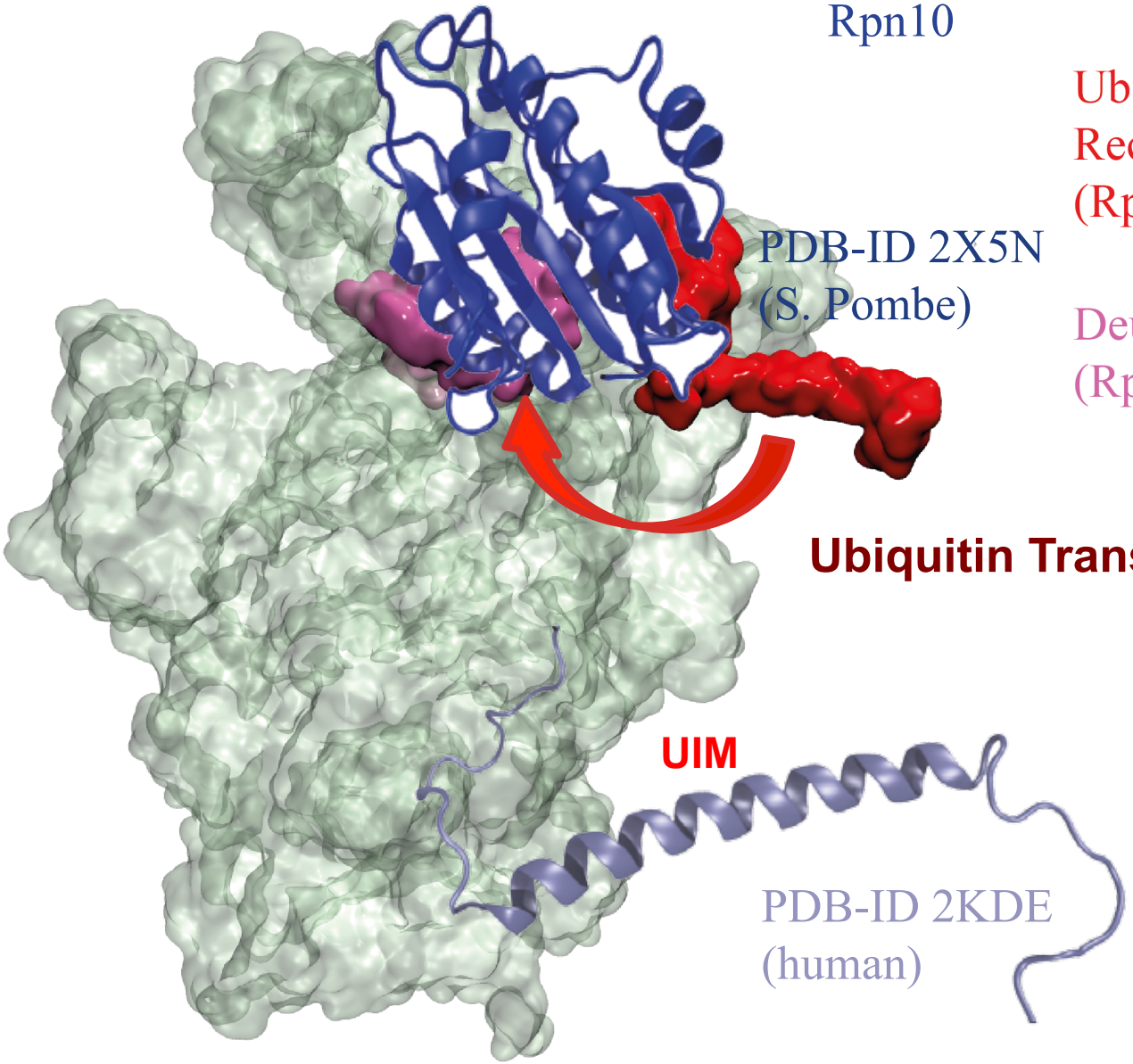
Ubiquitin recognition by Rpn10



Ubiquitin Recognition (Rpn10)

Zhang*, Vucovic*, **Rudack***, Han, Schulten 2016 JPC B (in press)

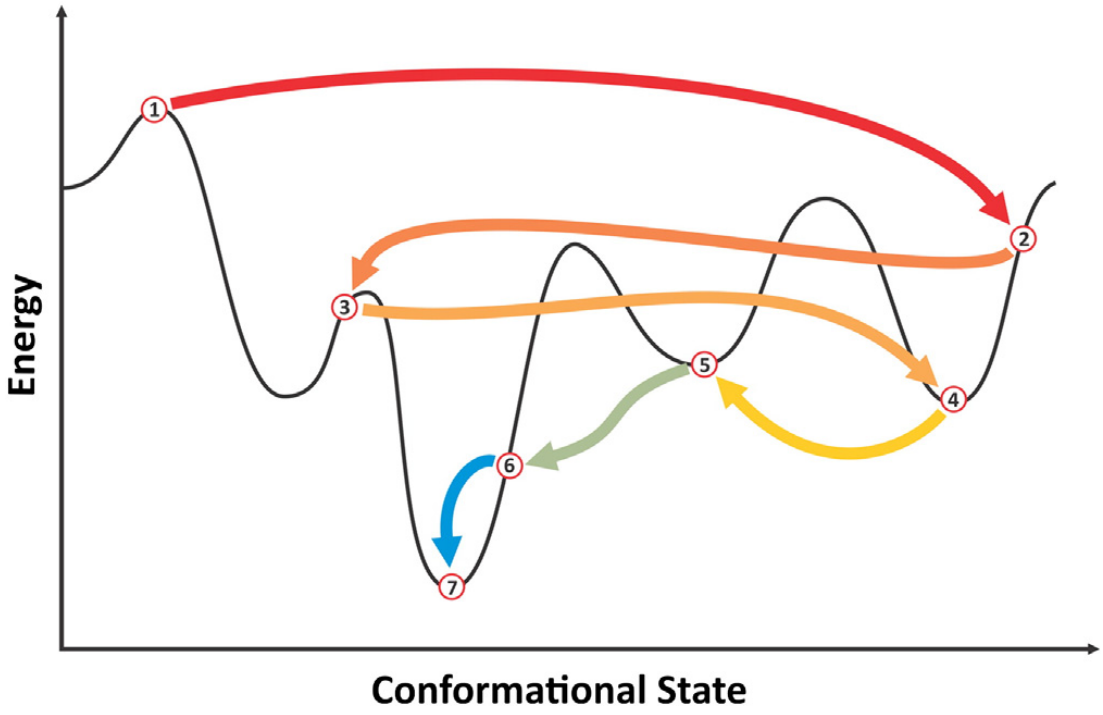
Ubiquitin recognition and deubiquitylation



Generalized Simulated Annealing – GSAFold

GSAFold NAMD Plugin – Allows *ab initio* structure prediction

New implementation of GSA on supercomputers allows the conformational search for large flexible regions.

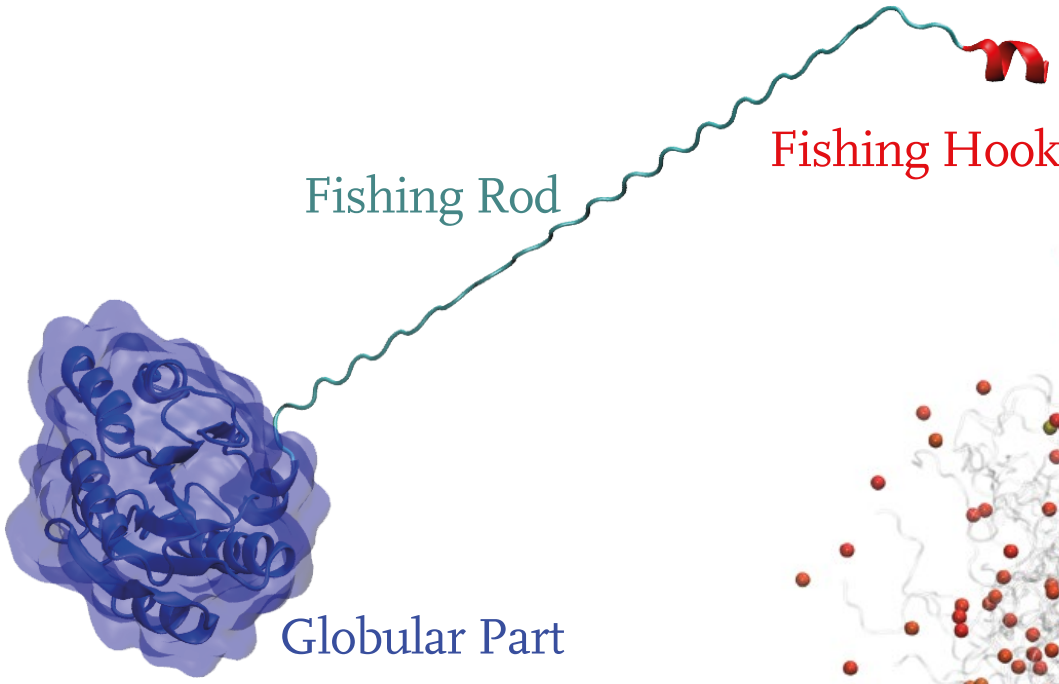


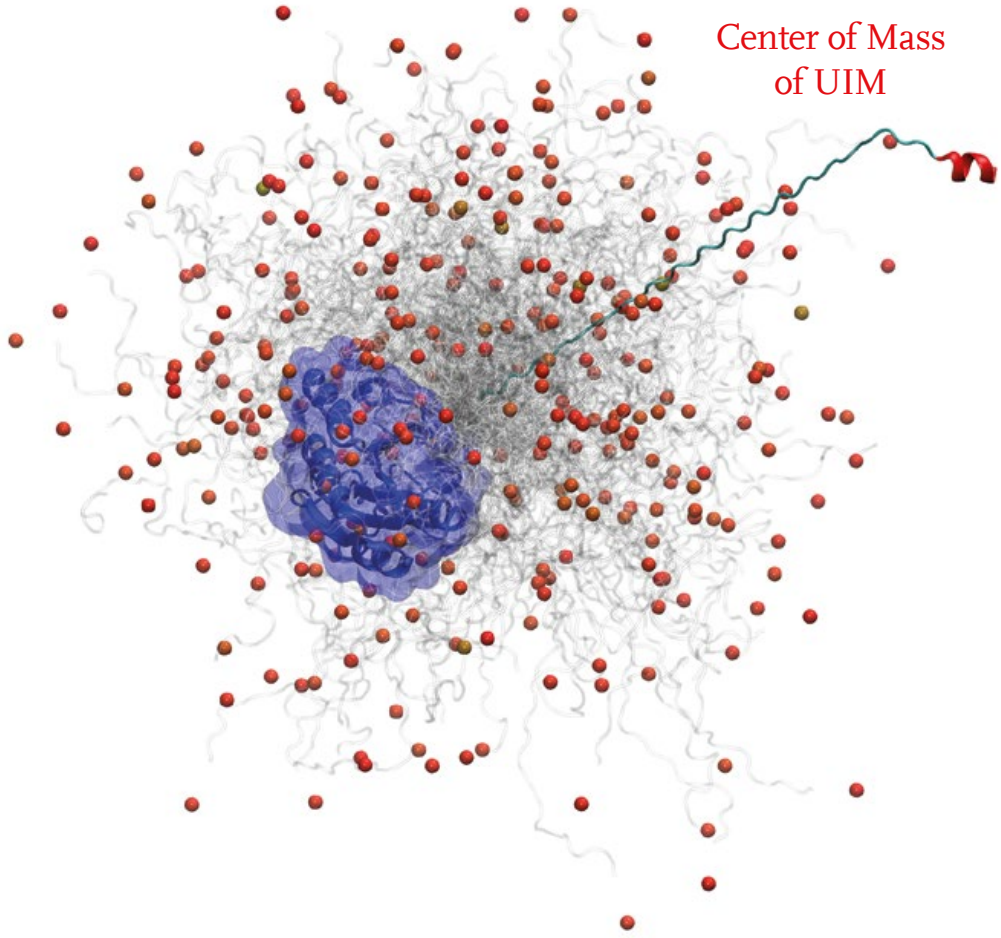
- GSAFold coupled to NAMD searches low-energy conformations to be used as starting points for the molecular dynamics studies.

- Amino acid residues connecting Rpn10's UIM with the proteasome are likely to be disordered and stochastic searching algorithms such as GSA can be used to explore their conformational space

Rafael C. Bernardi Marcelo Melo

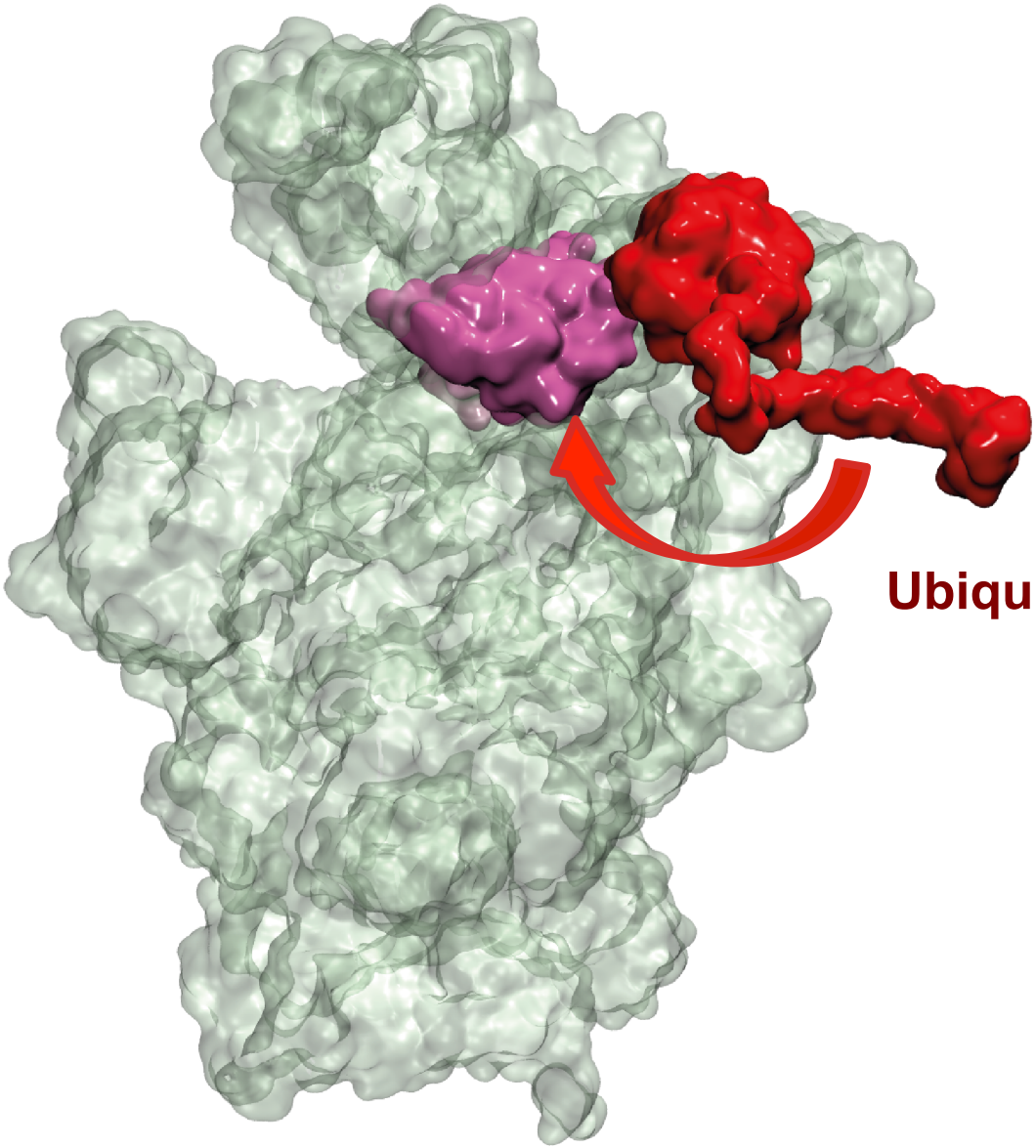
Conformation Space of Rpn10 Anchor





The conformational space of the Rpn10 linker is highly flexible.

Ubiquitin Transport to Deubiquitinase Rpn11

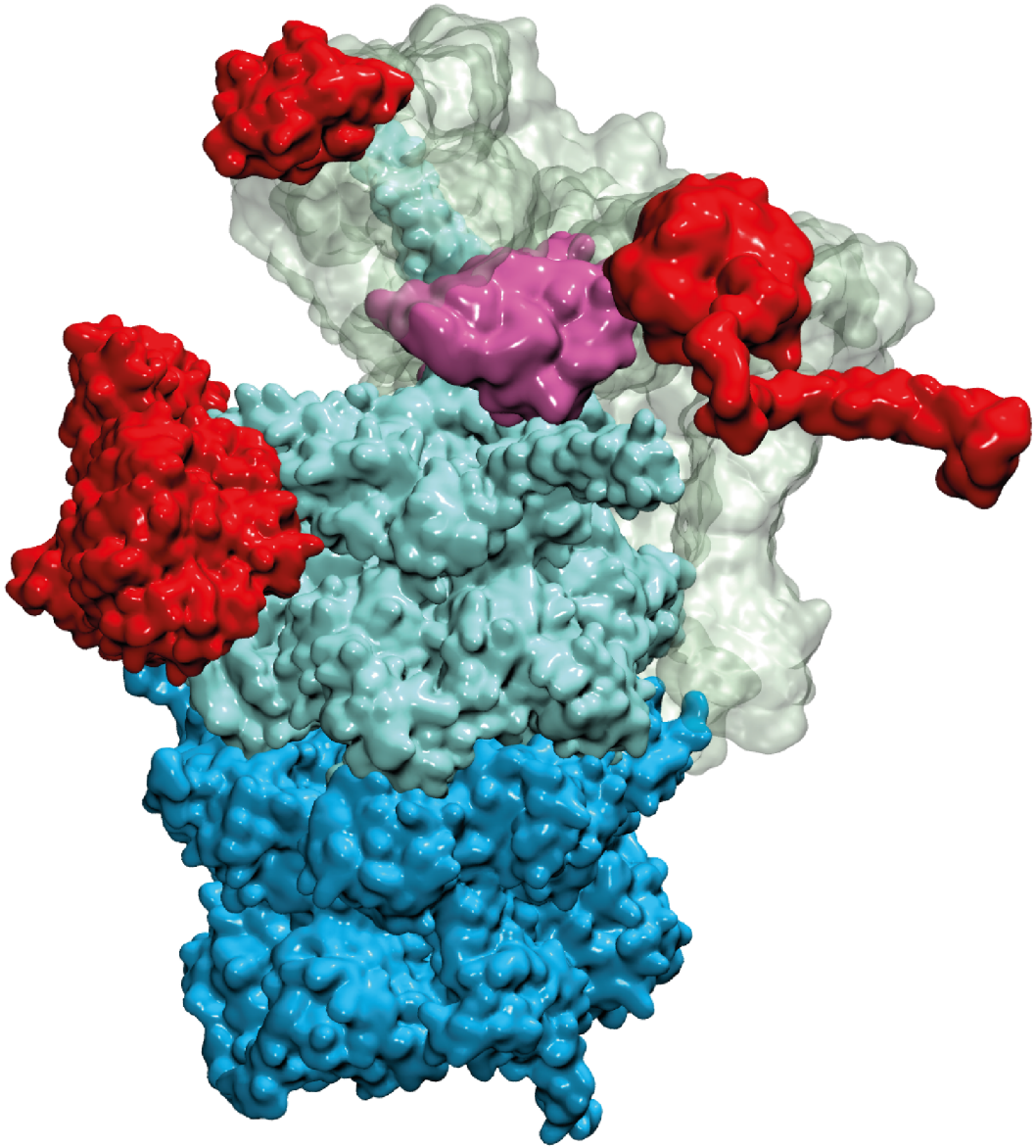


Ubiquitin Recognition (Rpn10)

Deubiquitylation (Rpn11)

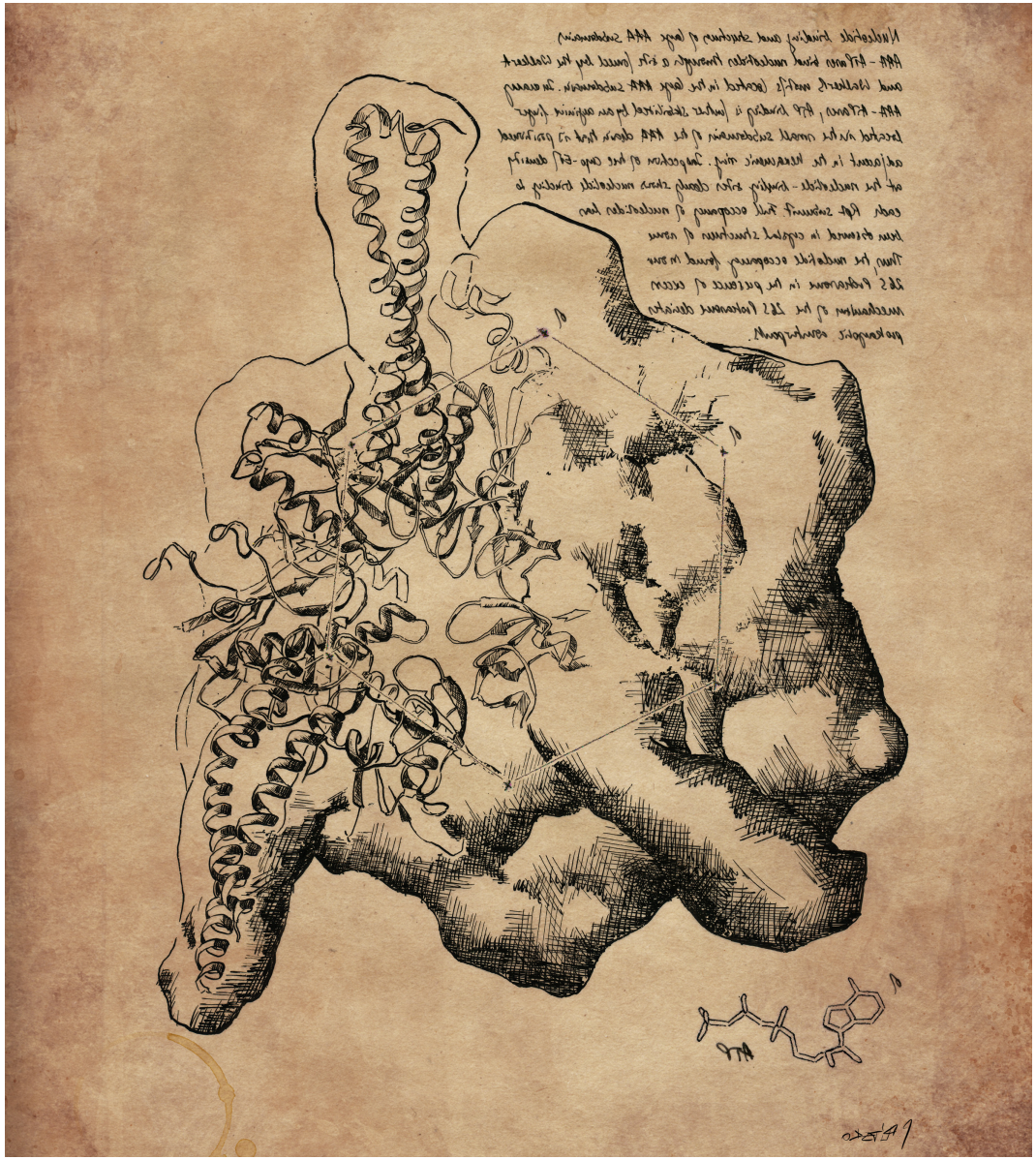
Ubiquitin Transport

Functional subunits of the 26S proteasome

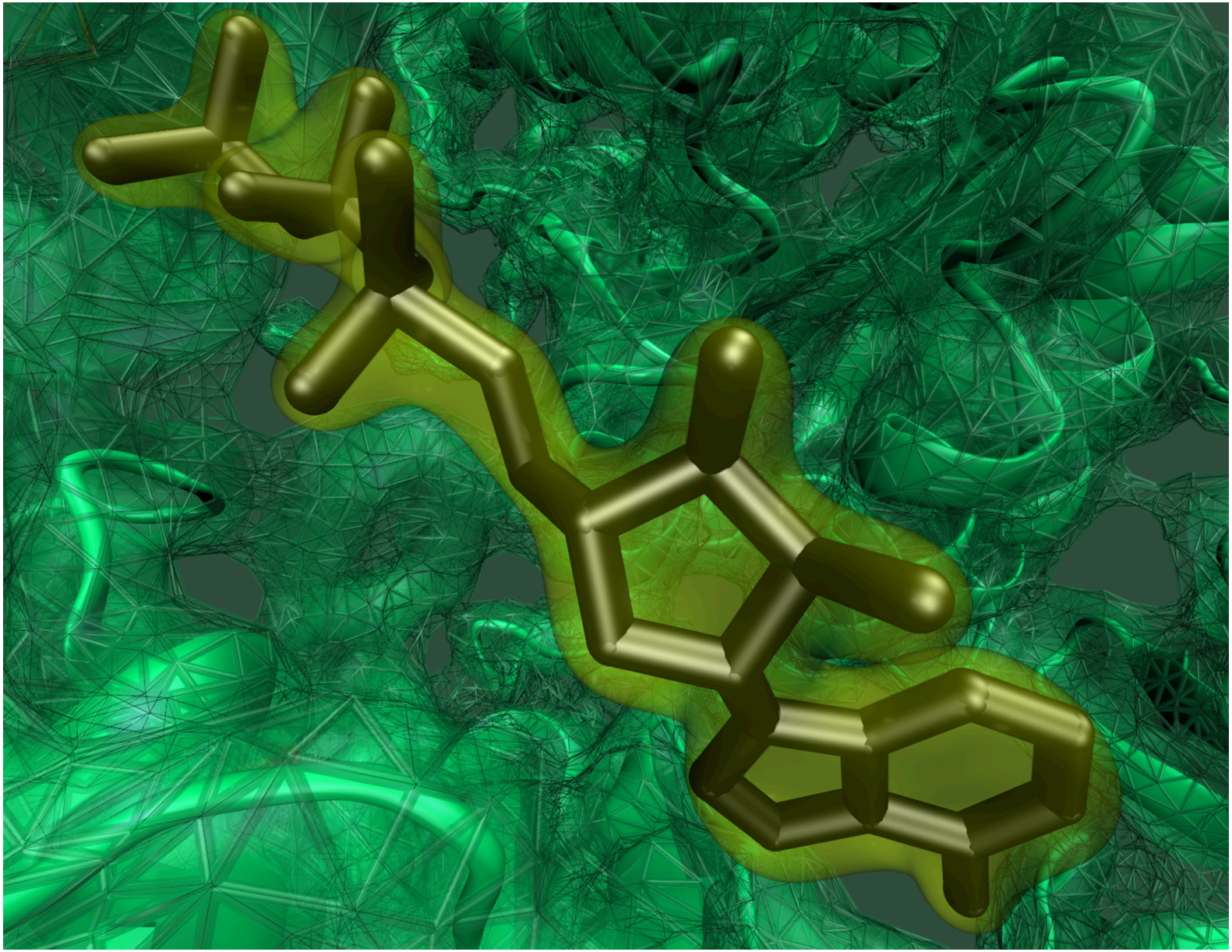


Substrate
Unfolding
(ATPase-ring)

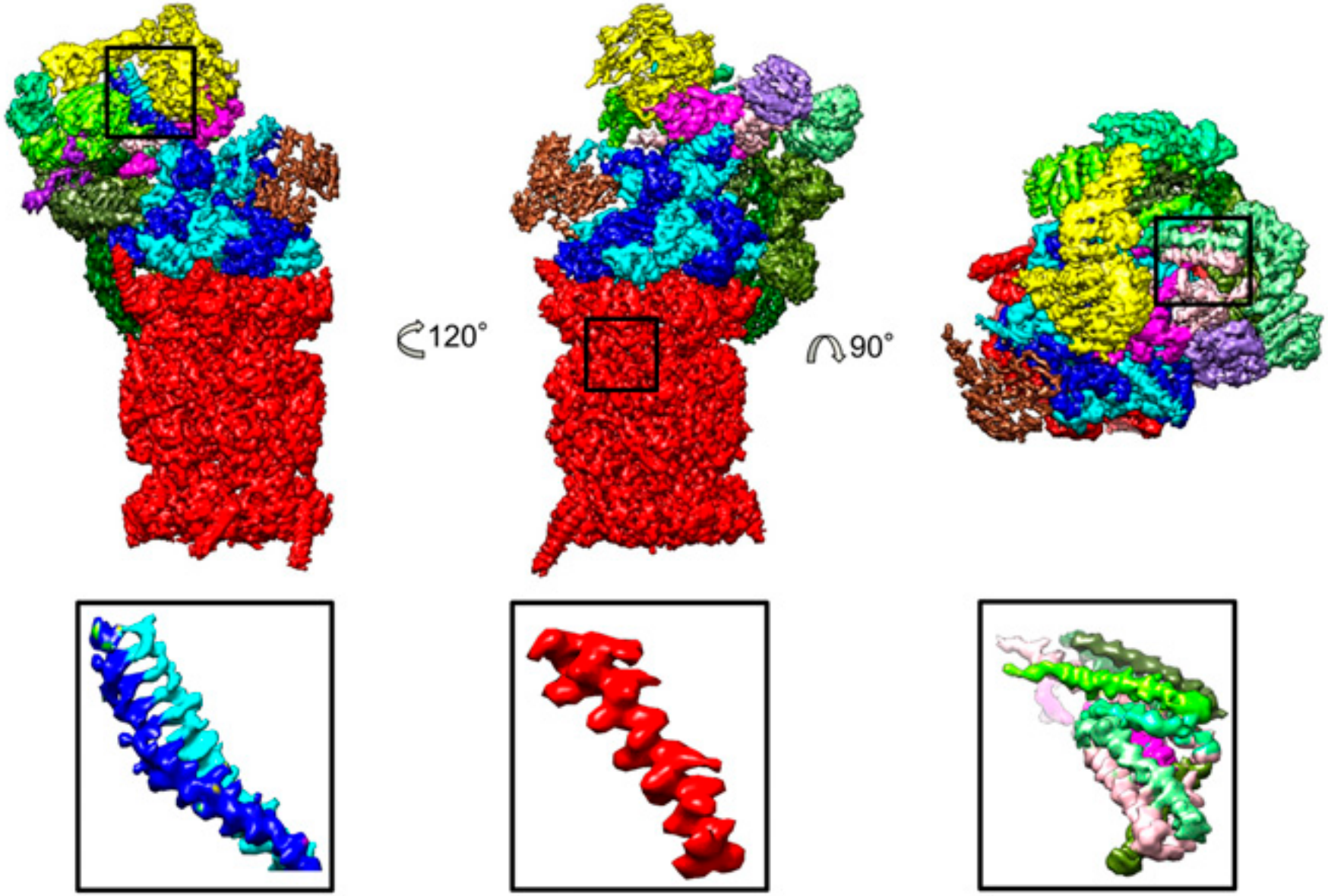
The Motor of the Proteasome



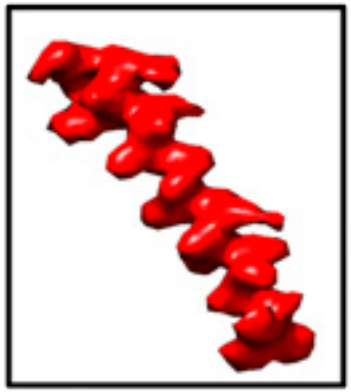
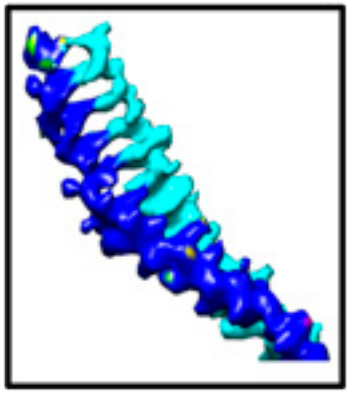
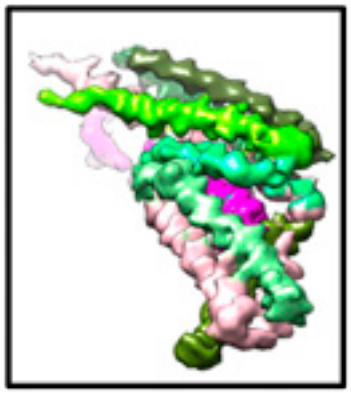
Resolved nucleotides are needed



3.9 Å Resolution Density of the Human 26S Proteasome



High-resolution Real Space Refinement with MDFF

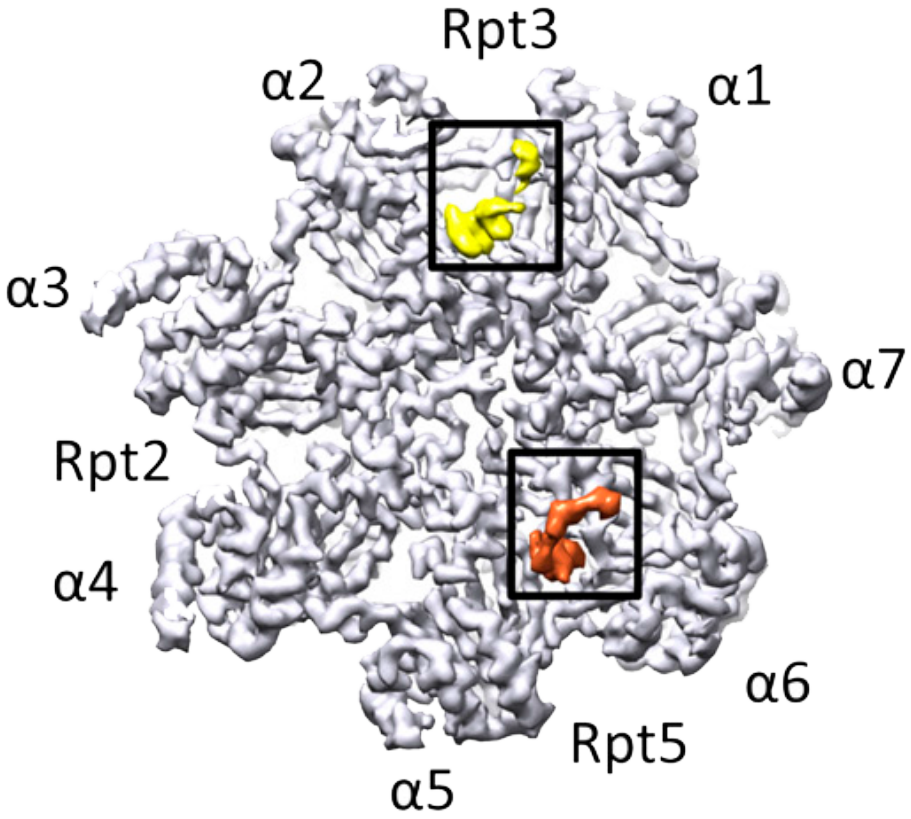
Advantage:
Positions of bulky side chains can be observed from density

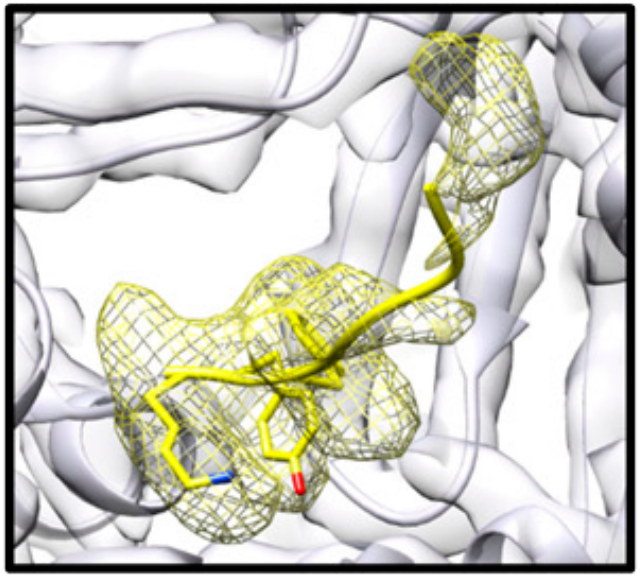
Challenge:
no detailed side chain orientation
X-ray structure refinement tools failed in the range of 4-5 Å resolution

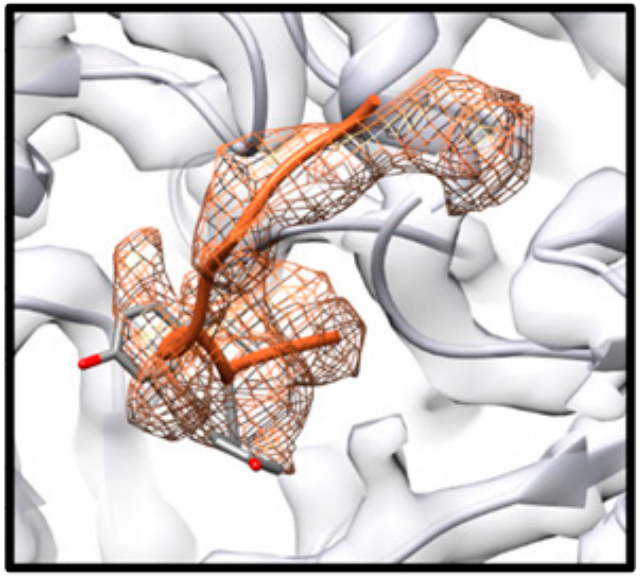
Solution:
combining MDFF with
monte carlo based backbone and side chain rotamer search algorithms
in an iterative manner

Goh, Hadden, Bernardi, Singharoy, McGreevy, Rudack, Cassidy, Schulten,
Annu. Rev. Biophys., 2016 45.1

The ATPase Motor of the 26S Proteasome



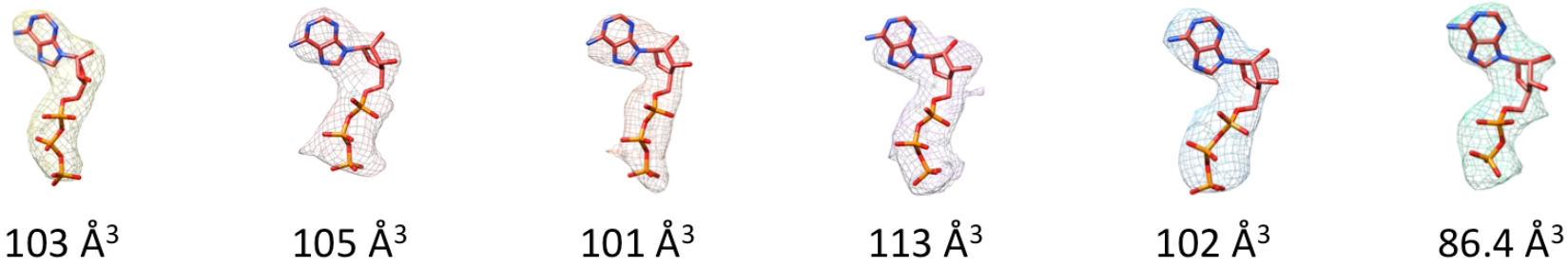


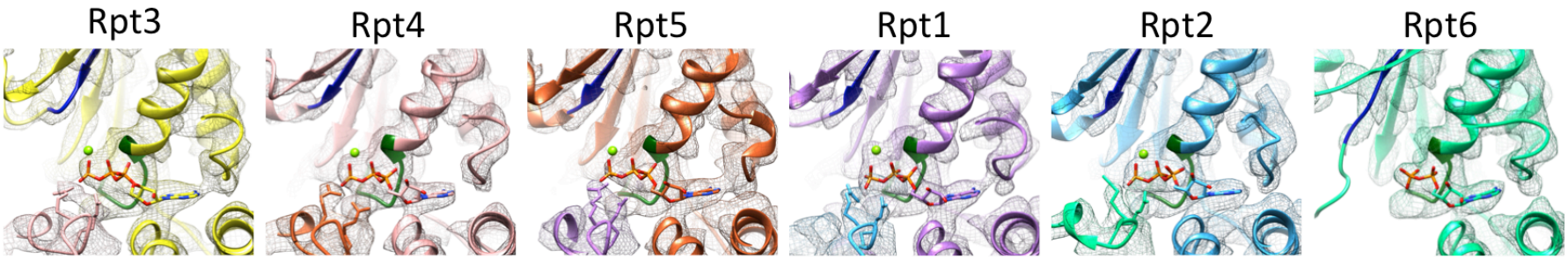


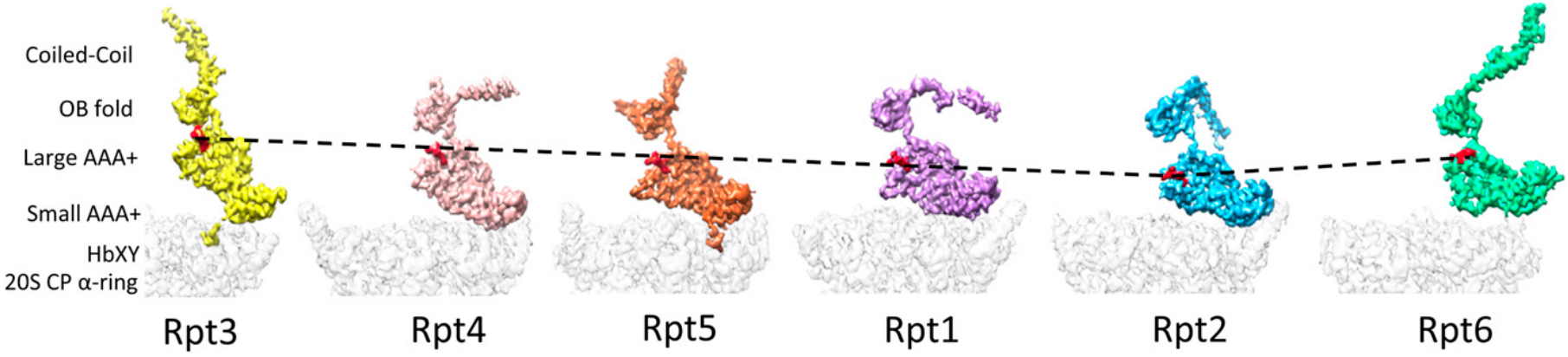
PDB-IDs: 5L4G, 5L4K
EMDB-ID: 4002

Schweitzer A, Aufderheide A, Rudack T, et al. "The structure of the 26S proteasome at a resolution of 3.9 Å." PNAS 2016 in press.

The Motor Action of Protein Unfolding

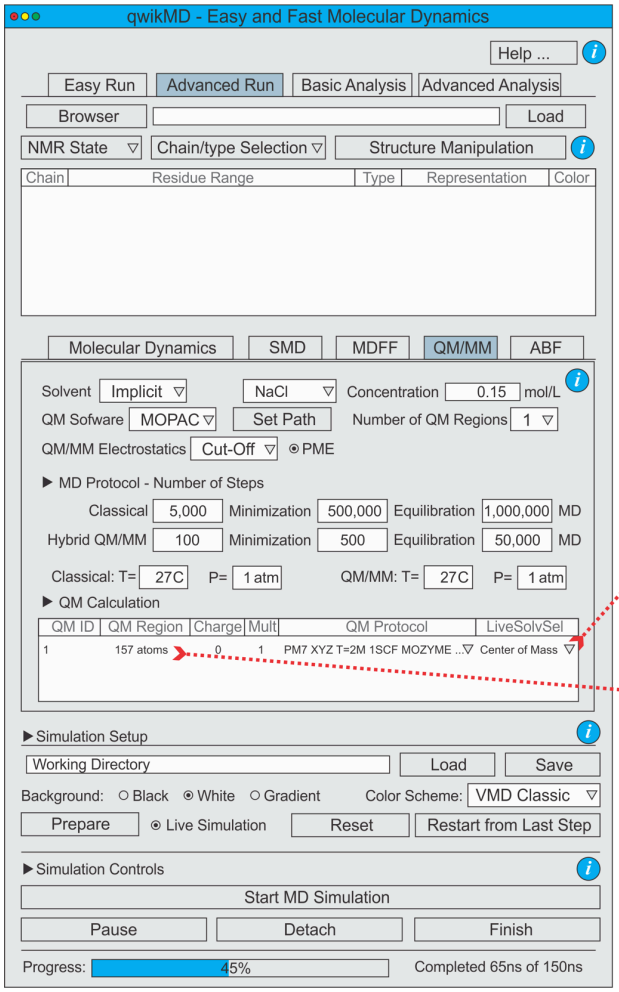




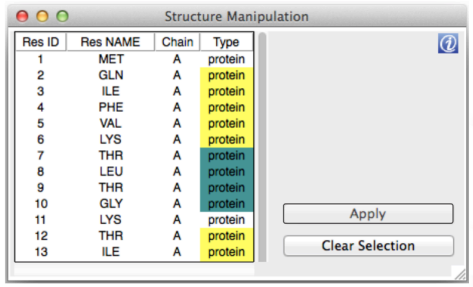


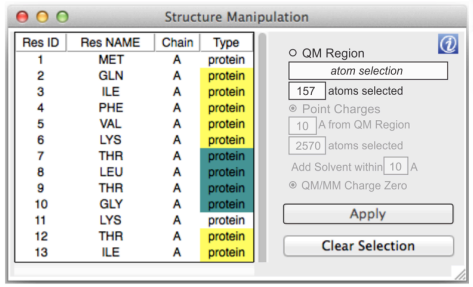
NAMD QM/MM Interface

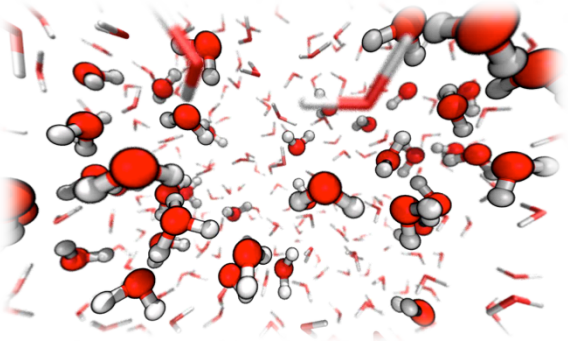
The atomic structure enable detailed investigations of the unfolding process by path sampling techniques. Chemical reaction in the active sites can be studied through QM/MM simulations.



NAMD QM/MM interface with MOPAC and ORCA is released in NAMD 2.12

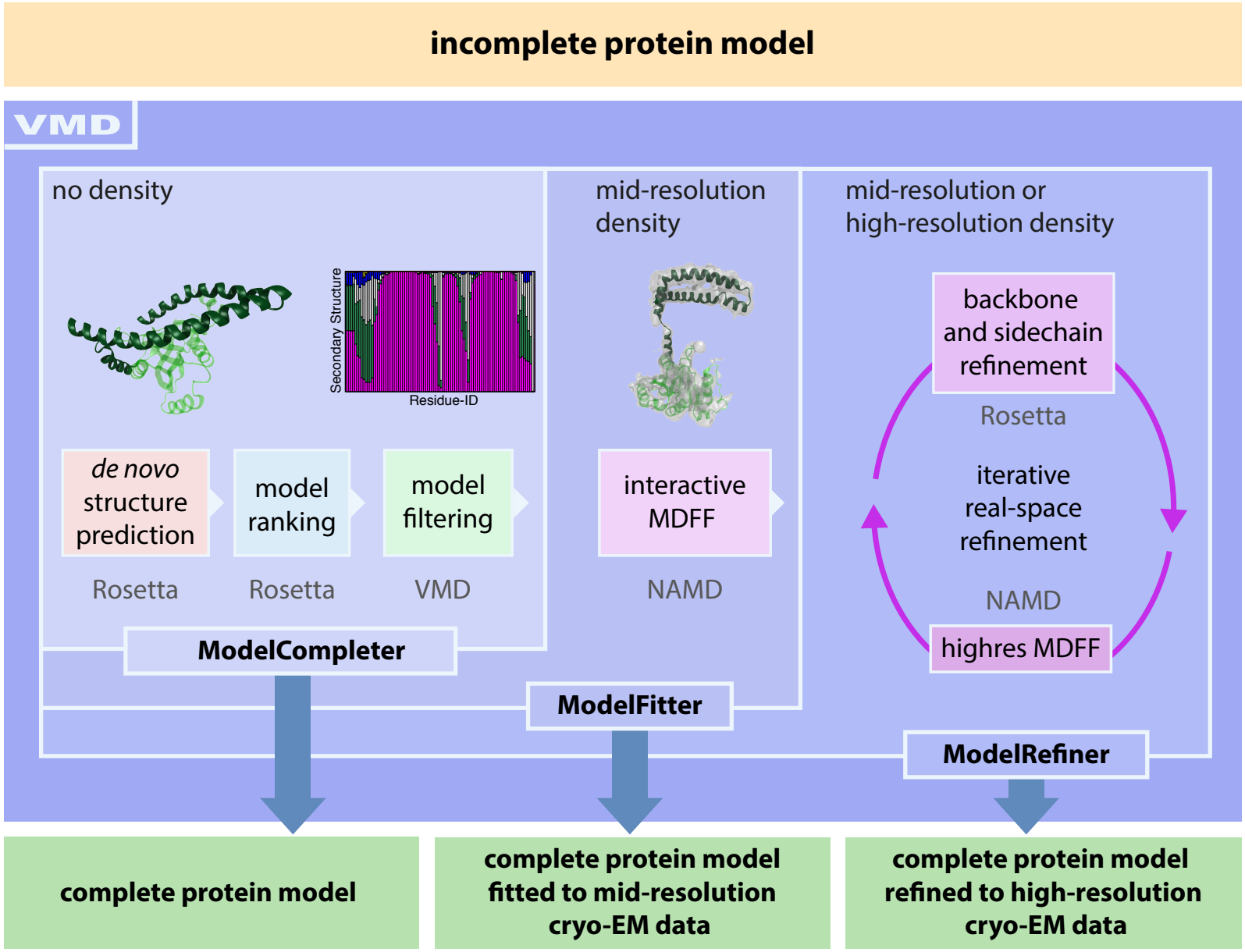






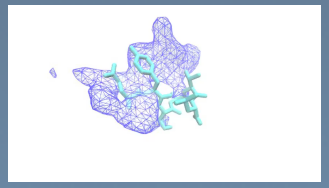
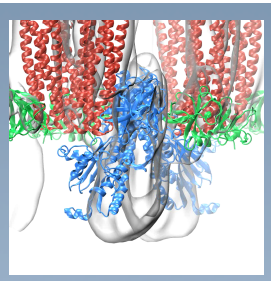
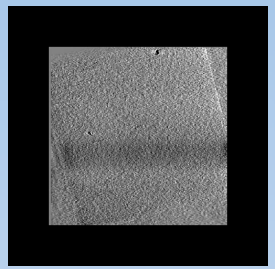
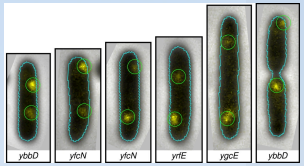
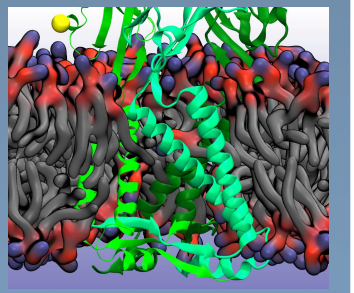
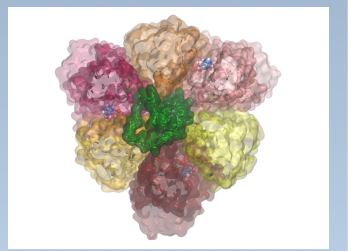
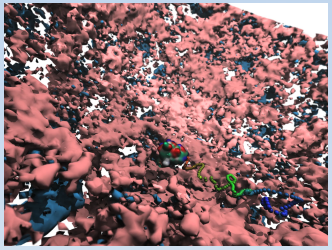
Next QwikMD release will support QM/MM

ModelMaker


incomplete protein model
VMD
no density
mid-resolution density
mid-resolution or high-resolution density
Secondary Structure
Residue-ID
de novo structure prediction
model ranking
model filtering
interactive MDFF
backbone and sidechain refinement
Rosetta
Rosetta
VMD
NAMD
iterative real-space refinement
NAMD
highres MDFF
ModelCompleter
ModelFitter
ModelRefiner
complete protein model
complete protein model fitted to mid-resolution cryo-EM data
complete protein model refined to high-resolution cryo-EM data

Bridging Computation and Experiment

Proteins → **Multi-Protein Complex** → **Organelles** → **Cell**

Experimental Method	  NMR X-ray		 cryo-EM	 FRET
Data Types	 X-ray Density	 Cryo-EM Density	 Cryo-EM Tomogram	 Brightfield Illumination
Function	 Membrane Permeation	 Motor Action	 Molecular Movement	 Cell Division

Take Home Message

In order to obtain **biomedical discoveries** different **experimental** and **computational** methods need to be **integrated.**

Automation is important but **user expertise** is equally important.

Acknowledgments

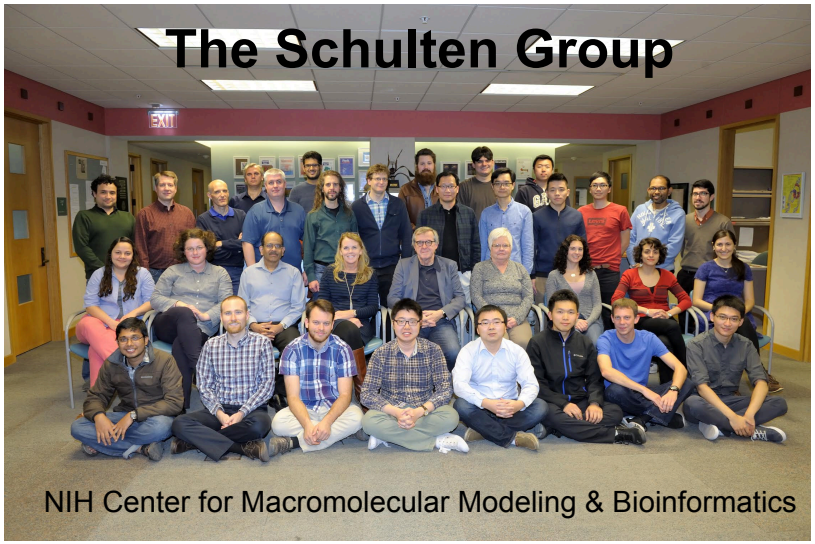
Alexander von Humboldt Stiftung/Foundation

National Institutes of Health

NSF

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

max planck institute of biochemistry



Center for the Physics of Living Cells

BLUE WATERS
SUSTAINED PETASCALE COMPUTING

Theory

Klaus Schulten
Ryan McGreevy

Experiment

Wolfgang Baumeister
Friedrich Förster
Antje Aufderheide

ModelMaker

Ryan McGreevy

GSA

Rafael Bernardi
Marcelo Mello

Workshop

Jodi Hadden

RUPRECHT-KARLS-
UNIVERSITÄT
HEIDELBERG

Technische Universität München

Maximilian Scheurer
Marc Siggel
Justin Porter