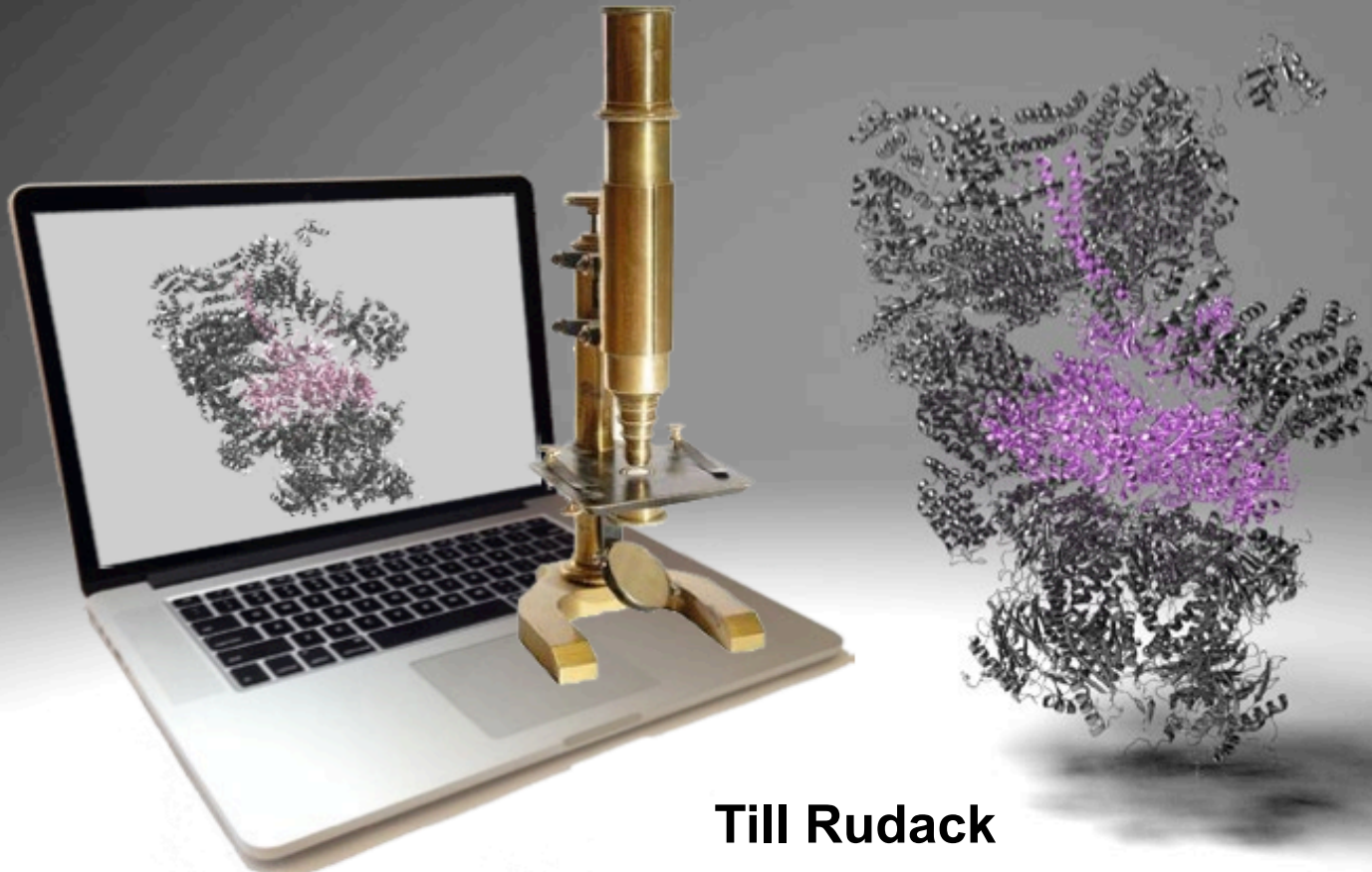


# Using MDFF

## Examples from Modern Research



**Till Rudack**

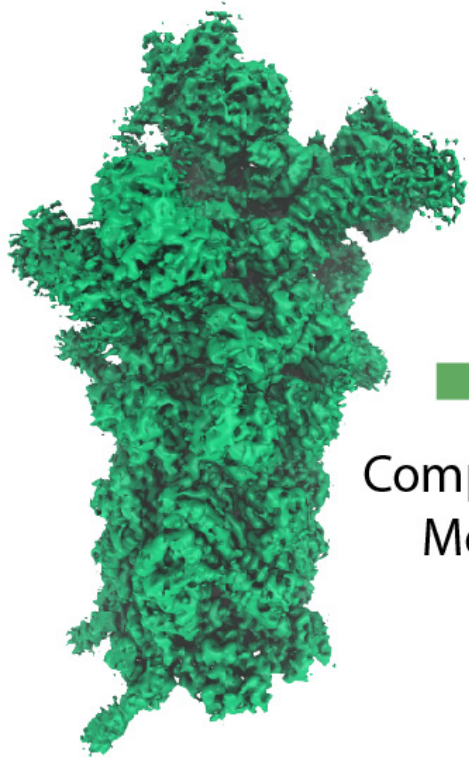
**Klaus Schulten Group - Theoretical and Computational Biophysics Group**

**NIH Center for Macromolecular Modeling and Bioinformatics**

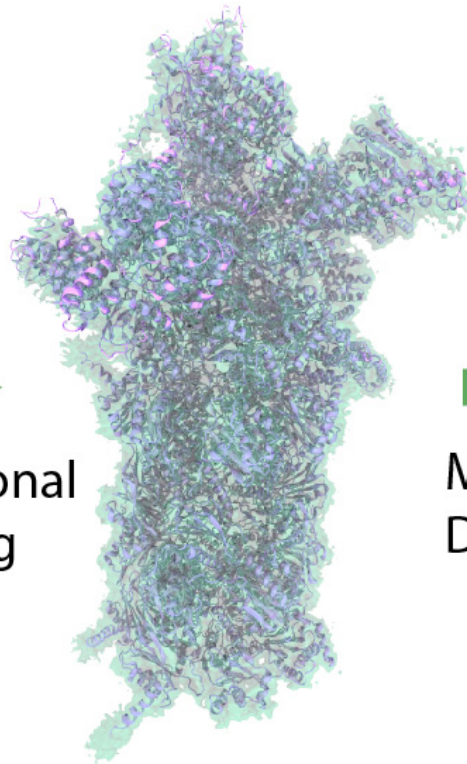
**University of Illinois at Urbana-Champaign**

06/15/16

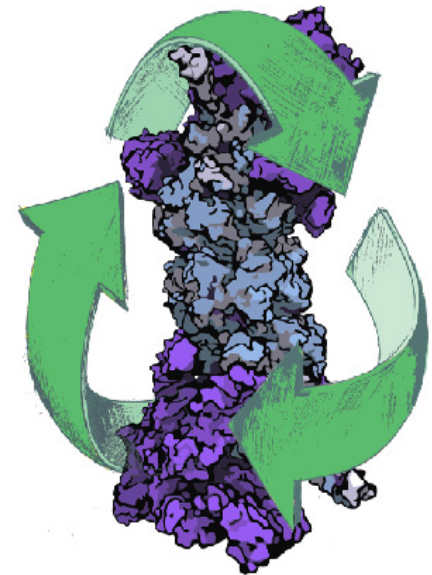
# Density



# Structure



# Function



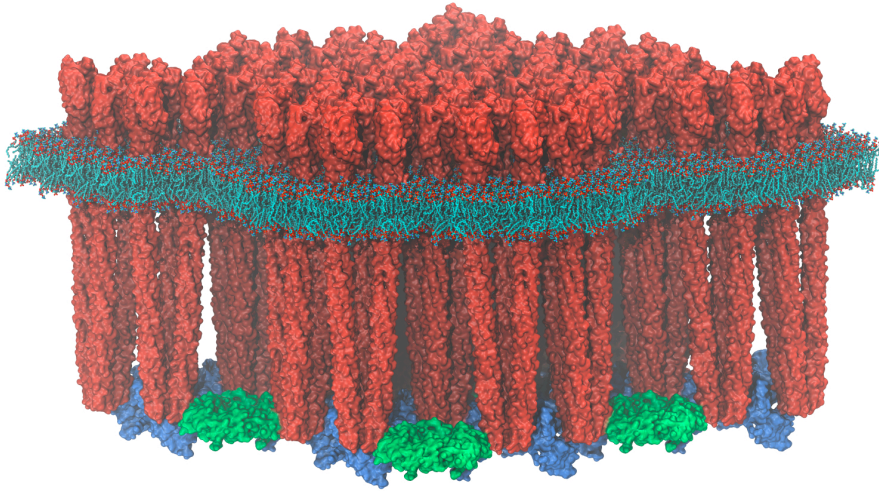
Computational  
Modeling

Molecular  
Dynamics

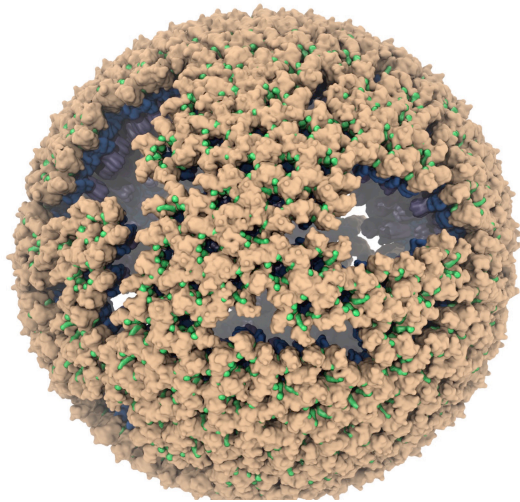
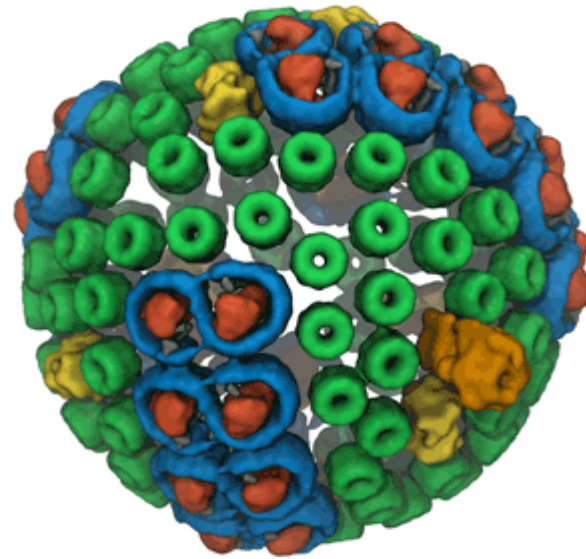


# A Sampling of TCBG's MDFF Projects

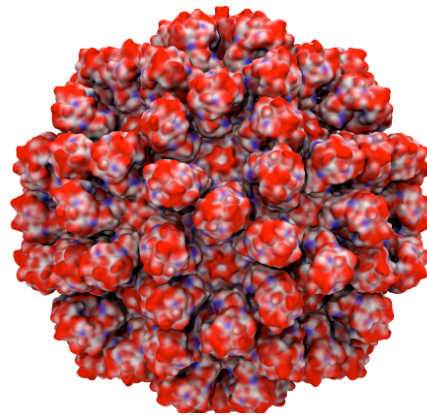
Chemosensory Array



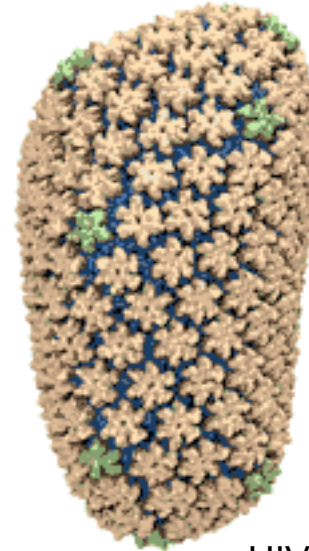
Chromatophore



Rous Sarcoma Virus



Theoretic  
Rabbit Hemorrhagic Disease

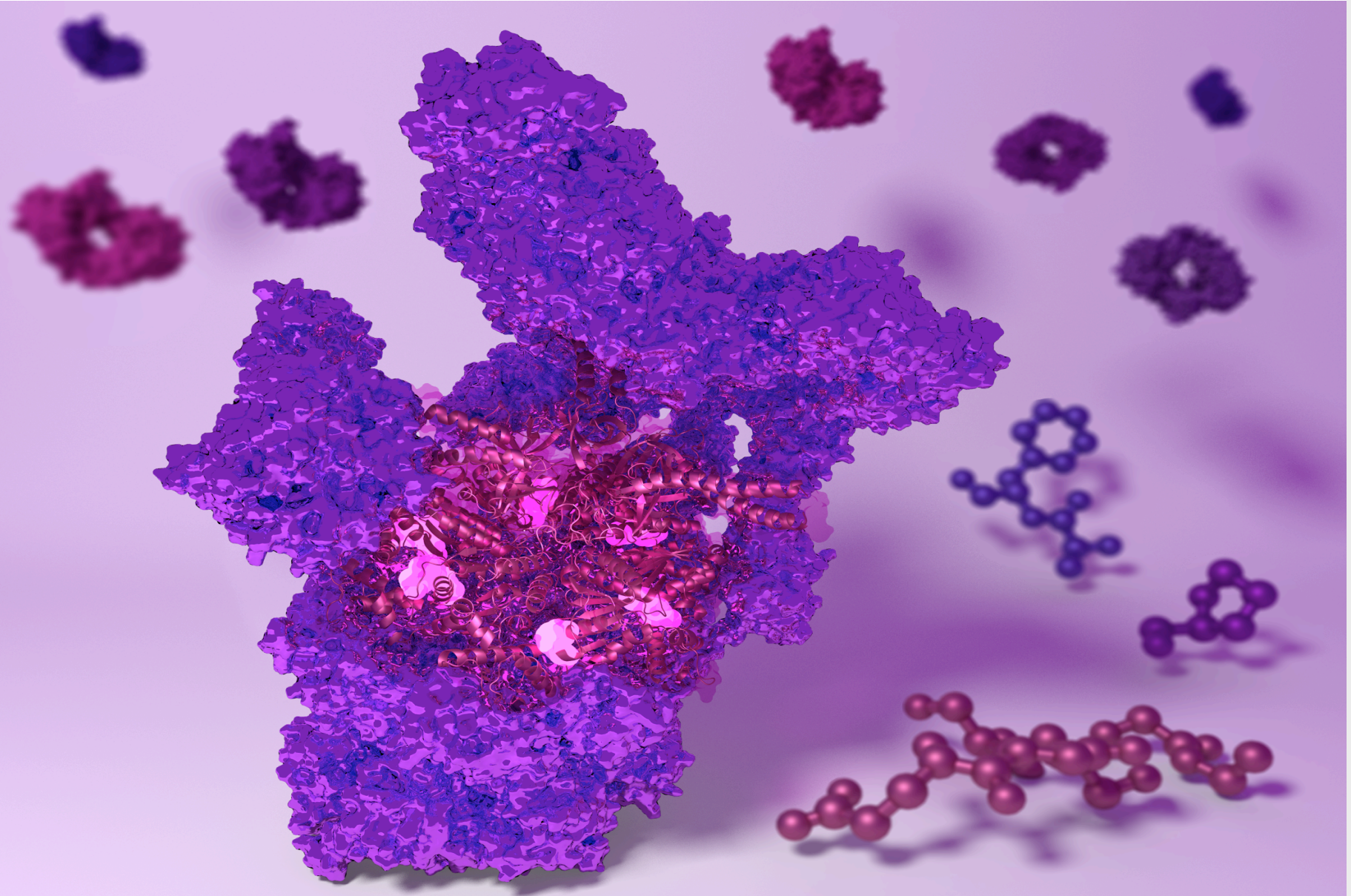


HIV



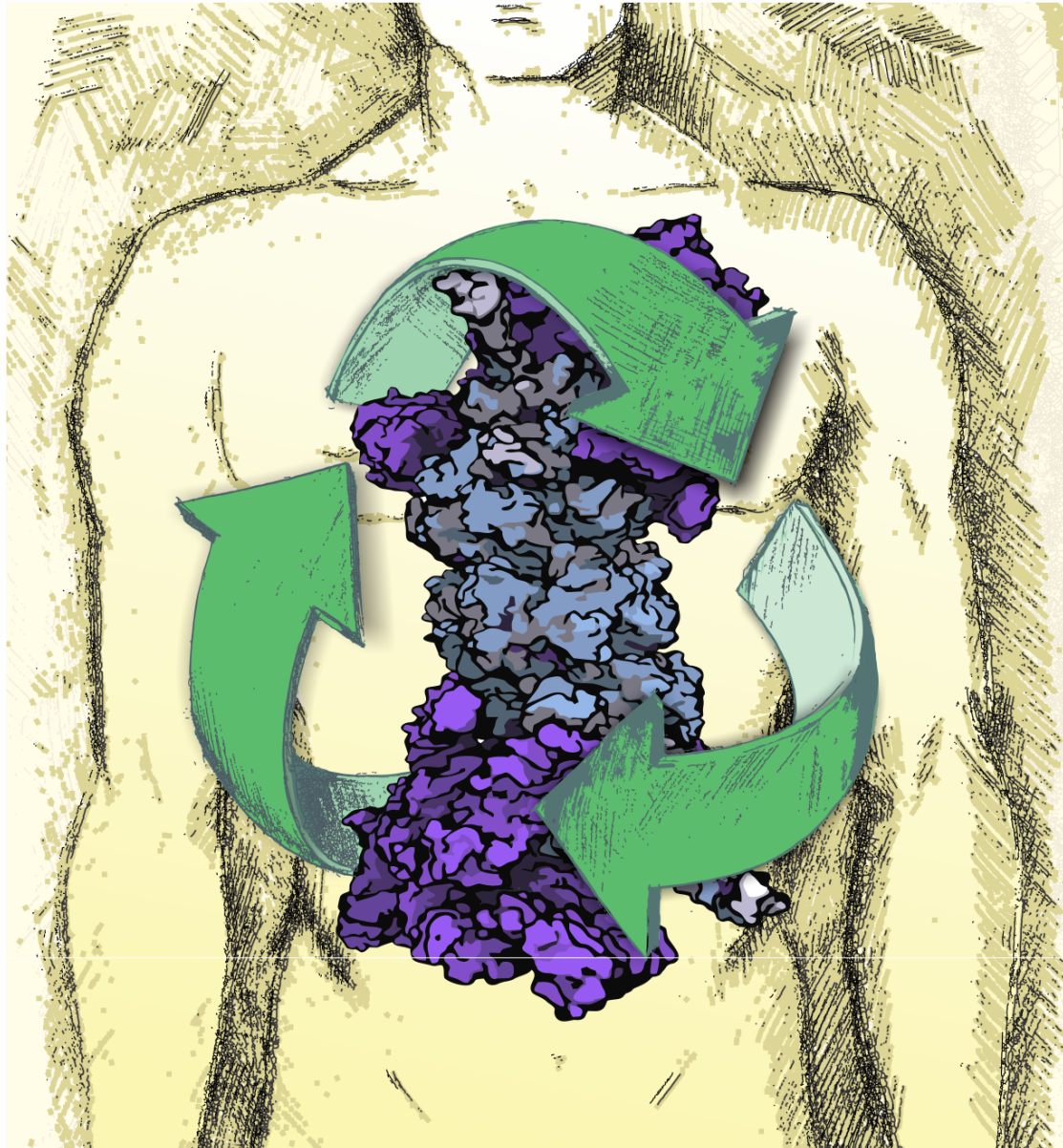
26S Proteasome

# Integrating experimental methods into computational modeling to obtain complete structural models

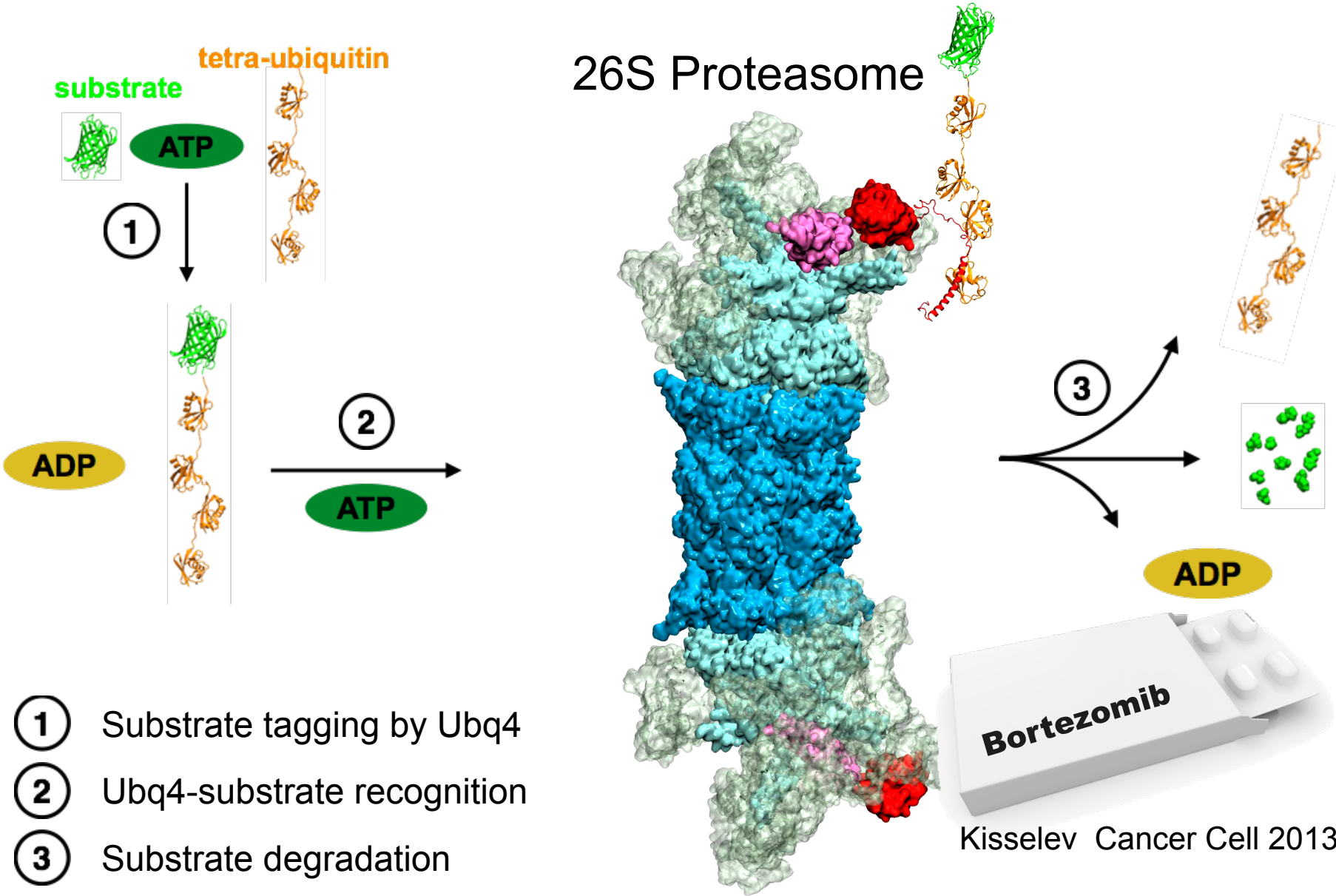




# The Recycling System of the Cell



# The ubiquitin proteasome proteolytic pathway



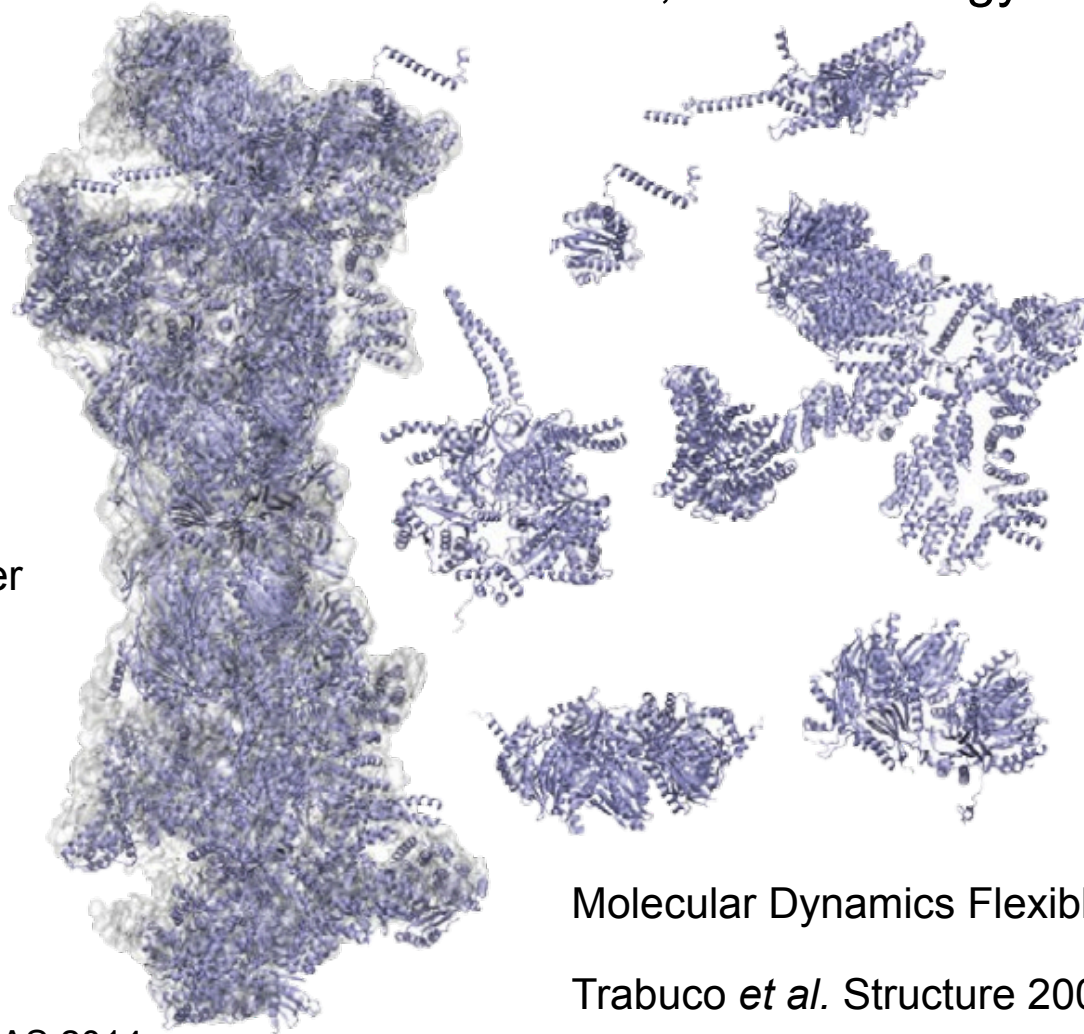
- 1 Substrate tagging by Ubq4
- 2 Ubq4-substrate recognition
- 3 Substrate degradation

Kisselev Cancer Cell 2013

# Near-atomic model of the 26S proteasome

Cryo-EM density

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



max planck institute  
of biochemistry



Wolfgang Baumeister  
Friedrich Foerster

PDB-ID 4CR2

EMDB-ID 2594

Resolution 7.7 Å

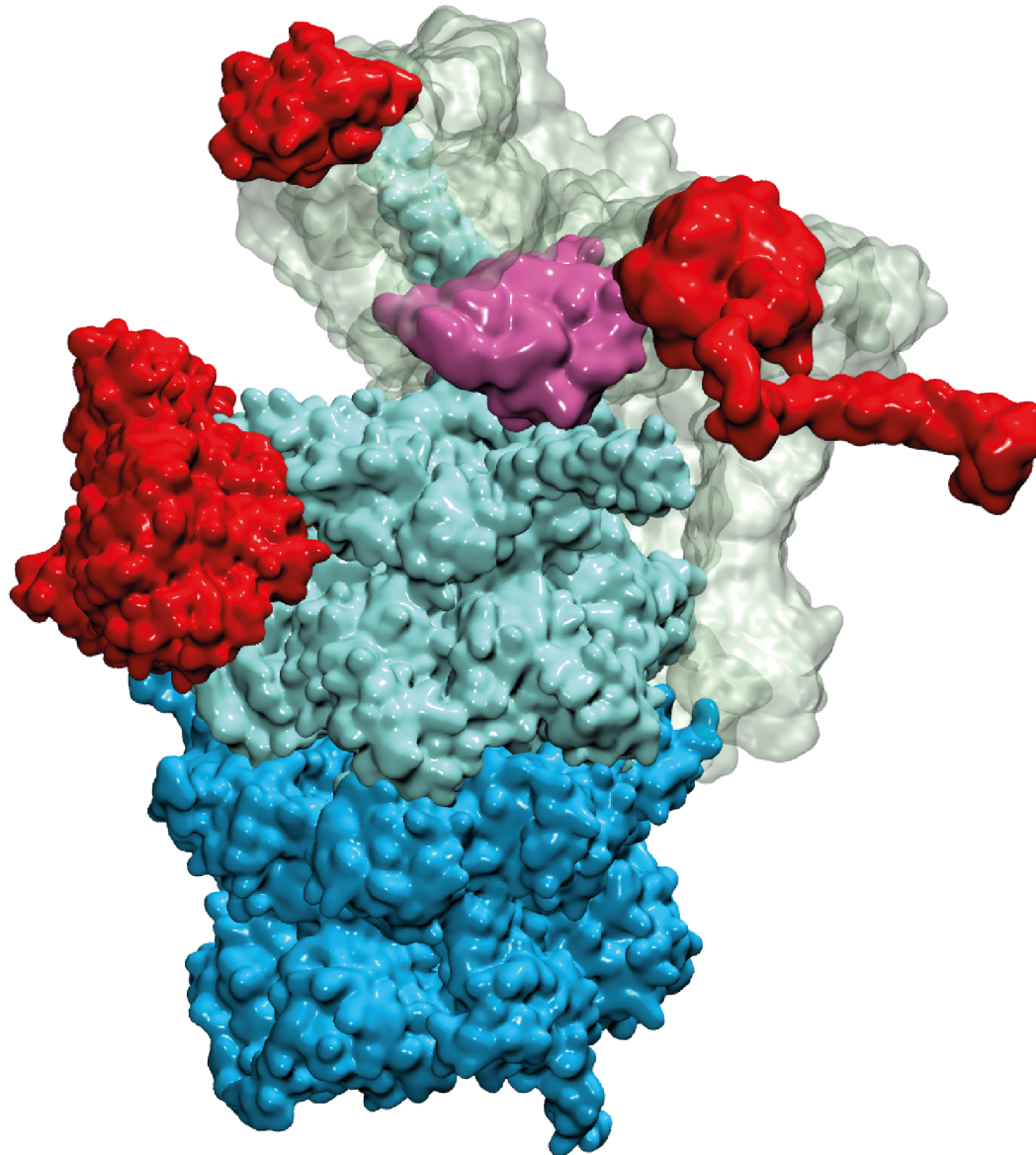
Unverdorben *et al.* PNAS 2014

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)

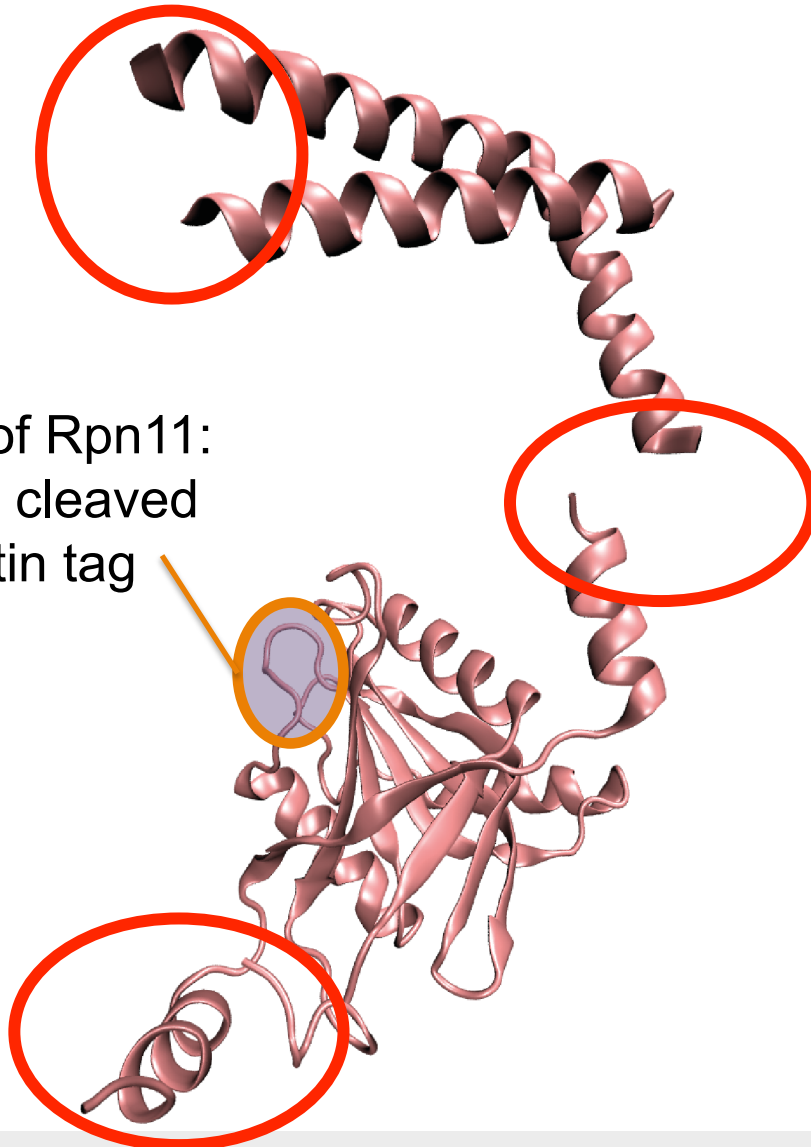
Substrate  
Degradation  
( $\alpha$ -ring,  $\beta$ -ring)





# Deubiquitylation subunit: Rpn11

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



Deubiquitylation  
(Rpn11)

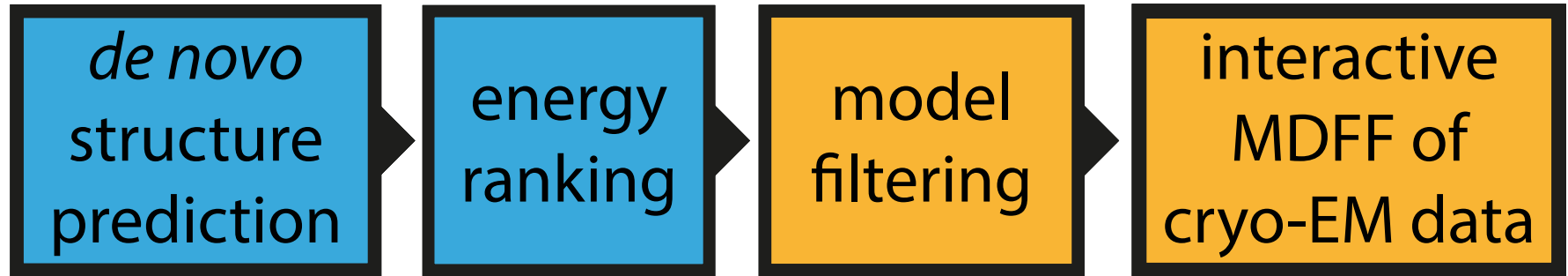
**Missing segments**  
**- highly flexible**  
**- ambiguous density**

Chain V of PDB-ID 4CR2



# Combining Rosetta and MDFF

*incomplete structural model deposited in the PDB*



*complete structural model that fits cryo-EM data*

**Rosetta**

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

**VMD/NAMD**

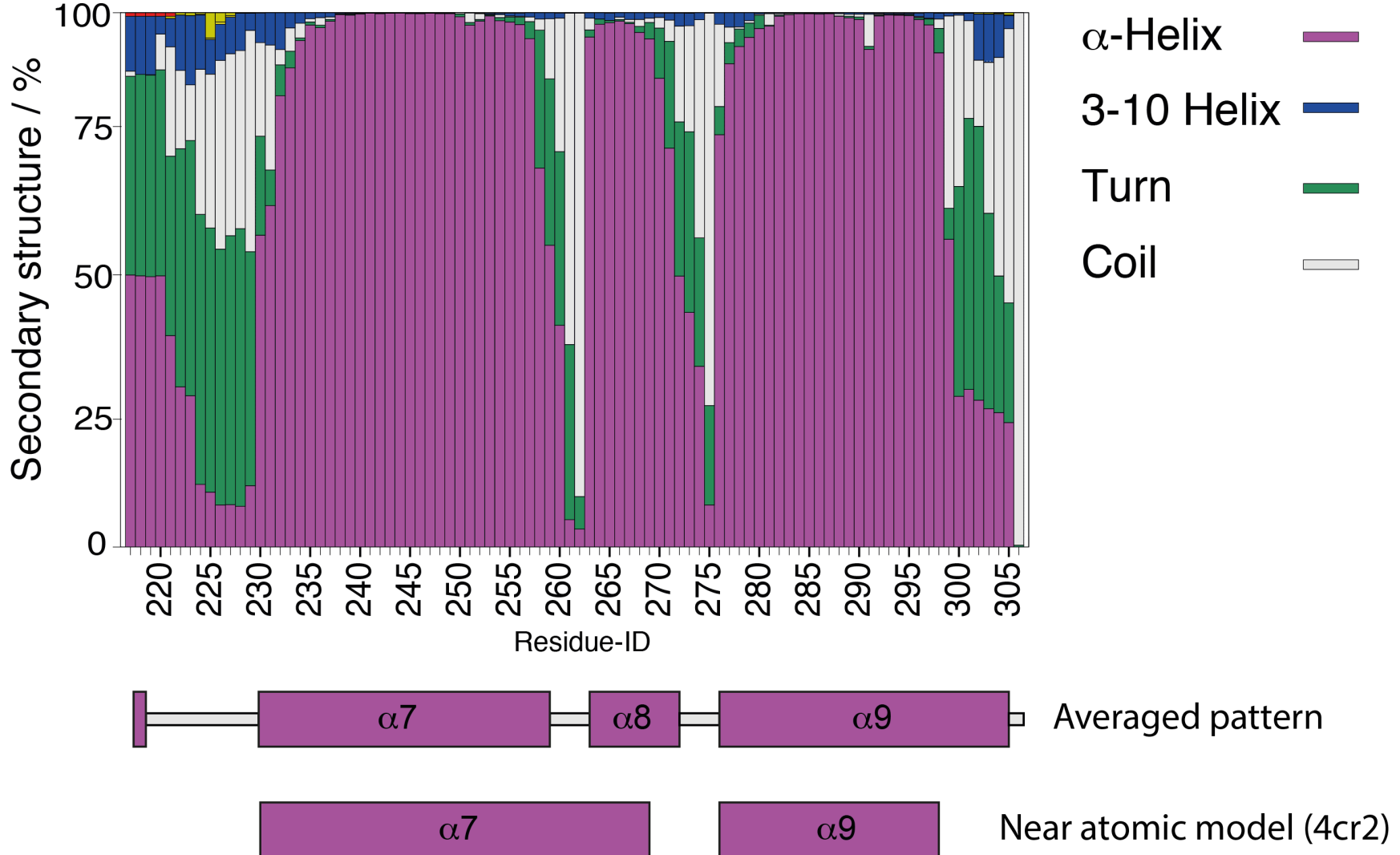
Humphrey *et al.* J. Mol. Graph. 1996  
Philips *et al.* J. Comput. Chem. 2005

**Integrating user expertise into *de novo* structure prediction**



# Model filtering by secondary structure

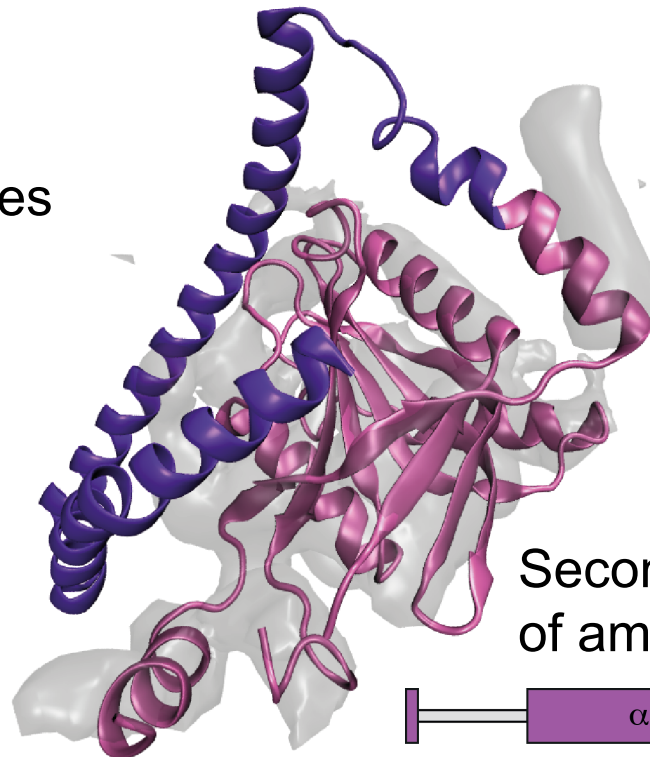
Secondary structure histogram of predicted ensembles of Rpn11's C-terminal tail



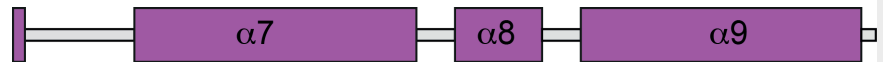
# Predicted model

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

Rosetta tends to build compact structures

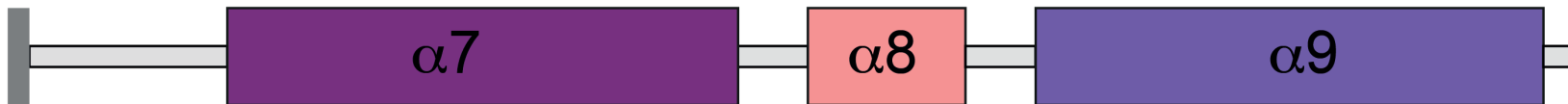
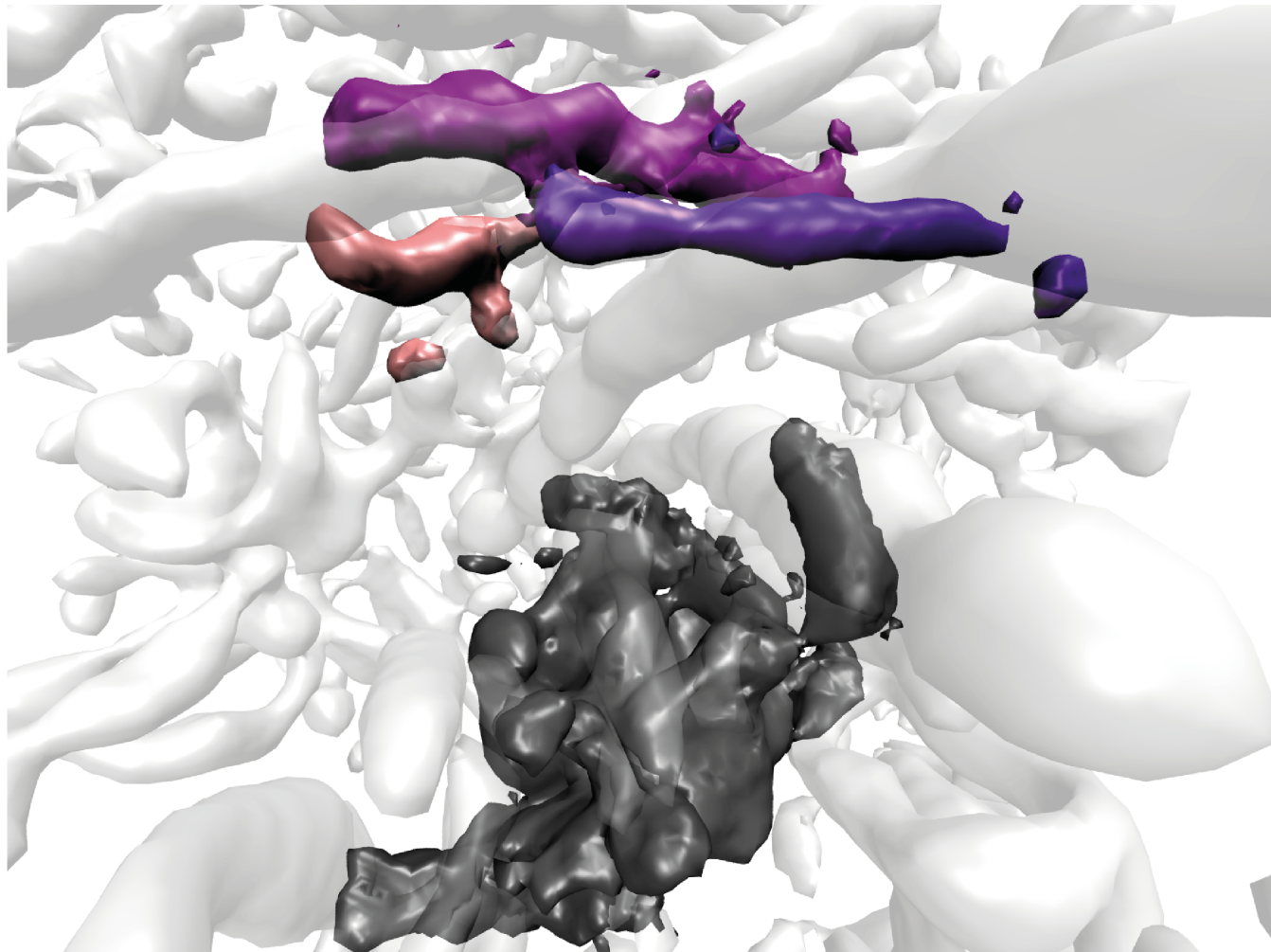


Secondary structure pattern of amino acids 217-306 (purple)

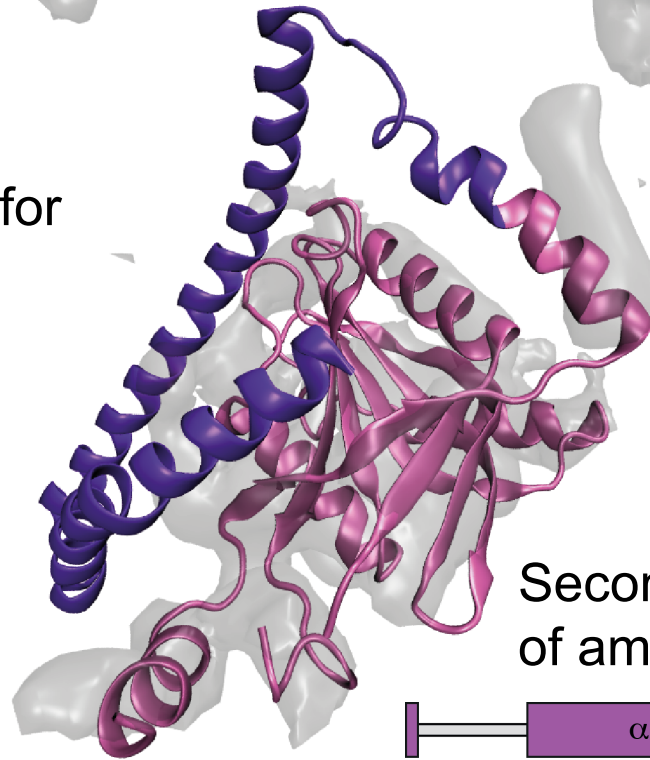
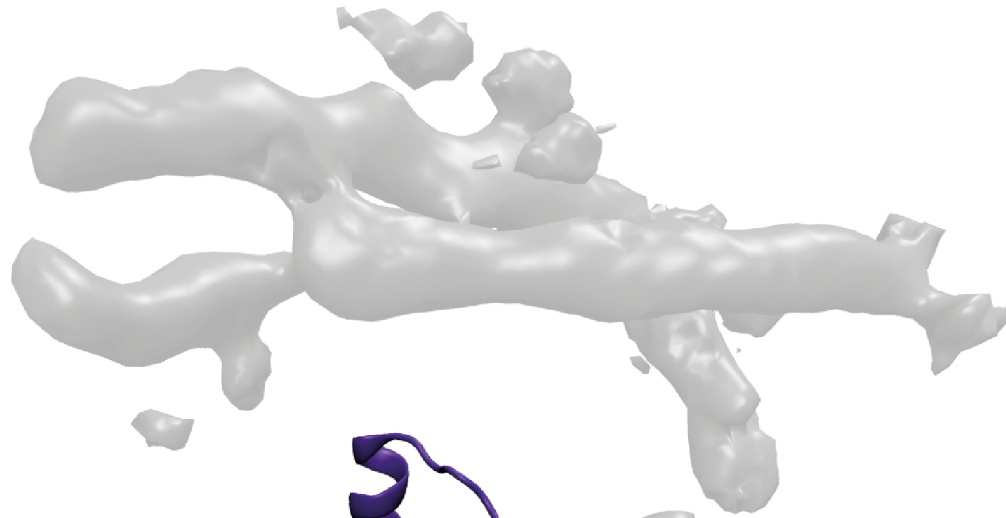




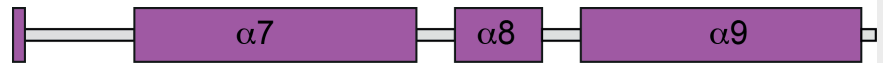
# Visual inspection of cryo-EM density



# Predicted model to initiate MDFD



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



Representative model  
of predicted ensemble for  
Rpn11's C-terminal tail



# Interactive Molecular Dynamics Flexible Fitting

The screenshot displays the VMD 1.9.3ab OpenGL Display window on the left, showing a protein structure with a purple ribbon and a pink ribbon, overlaid on a grey electron density map. The VMD Main window on the right shows the molecule list:

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
0	T	A	D	F	start_1.psf	4822	5	0
1	A	D	F		Rpn11_2_2594_density.0	0	0	1

The MDFF GUI window on the right shows the 'MDFF Connect' tab with buttons for 'Submit', 'Connect', 'Pause', and 'Finish'. The 'IMD Status' is 'Step 400'. The 'Cross Correlation Analysis' section is expanded, showing 'Calculate Real-Time Cross Correlation' with the following settings:

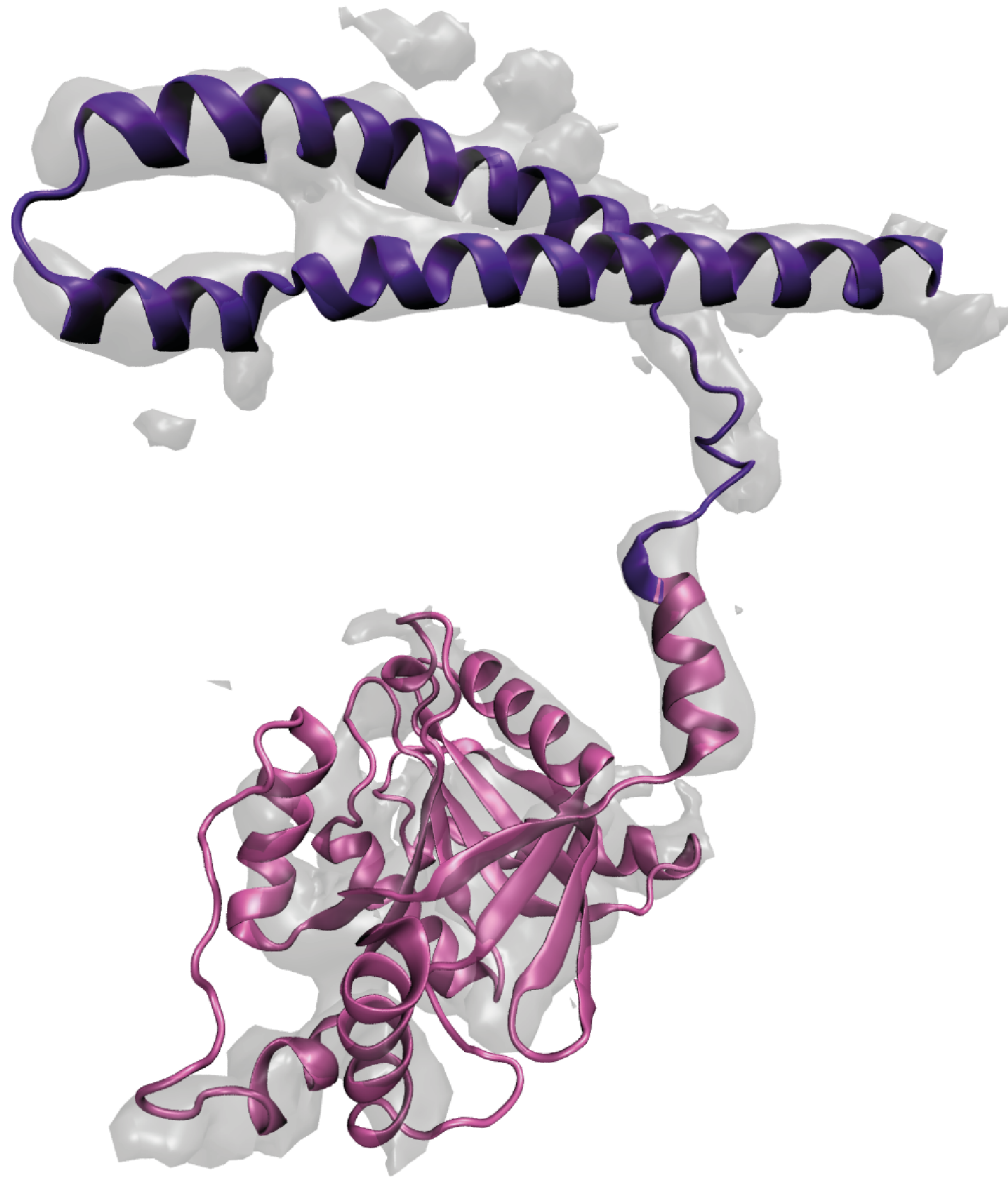
- Experimental Density (Mol ID): 1
- Selection: protein and noh
- Map Resolution: 7.7
- Use Threshold
- Threshold: [empty]

The 'Real-Time Cross Correlation' plot shows the cross correlation value on the y-axis (ranging from 0.696175 to 0.696575) versus the timestep on the x-axis (ranging from 150 to 400). The plot shows a noisy line that increases over time, reaching a value of approximately 0.696575 at timestep 400.

MDFF can be run on Cloud computing for low cost!



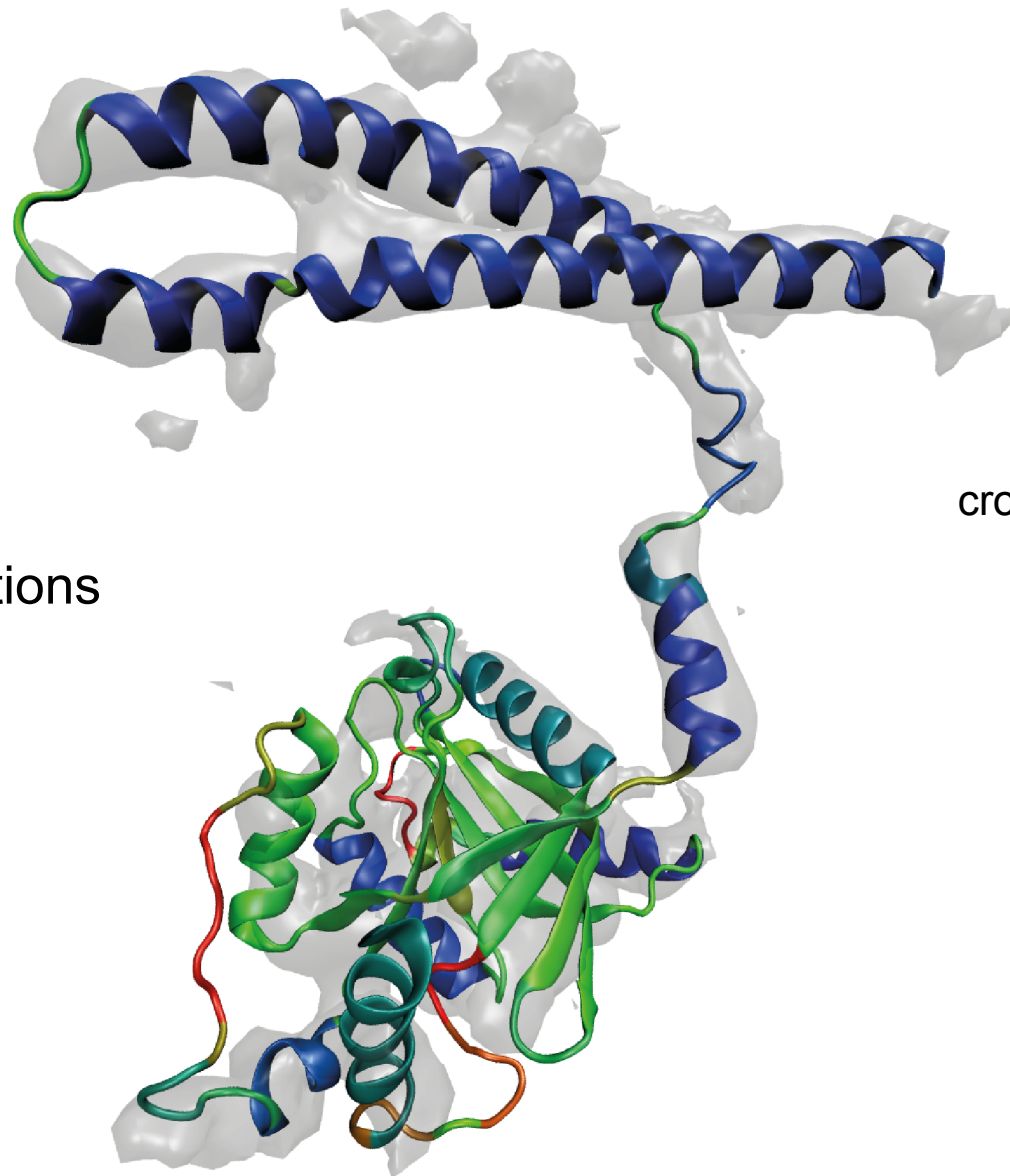
# Complete model of Rpn11 fitted to density







# Quality check by cross-correlations



cross correlation

0.65

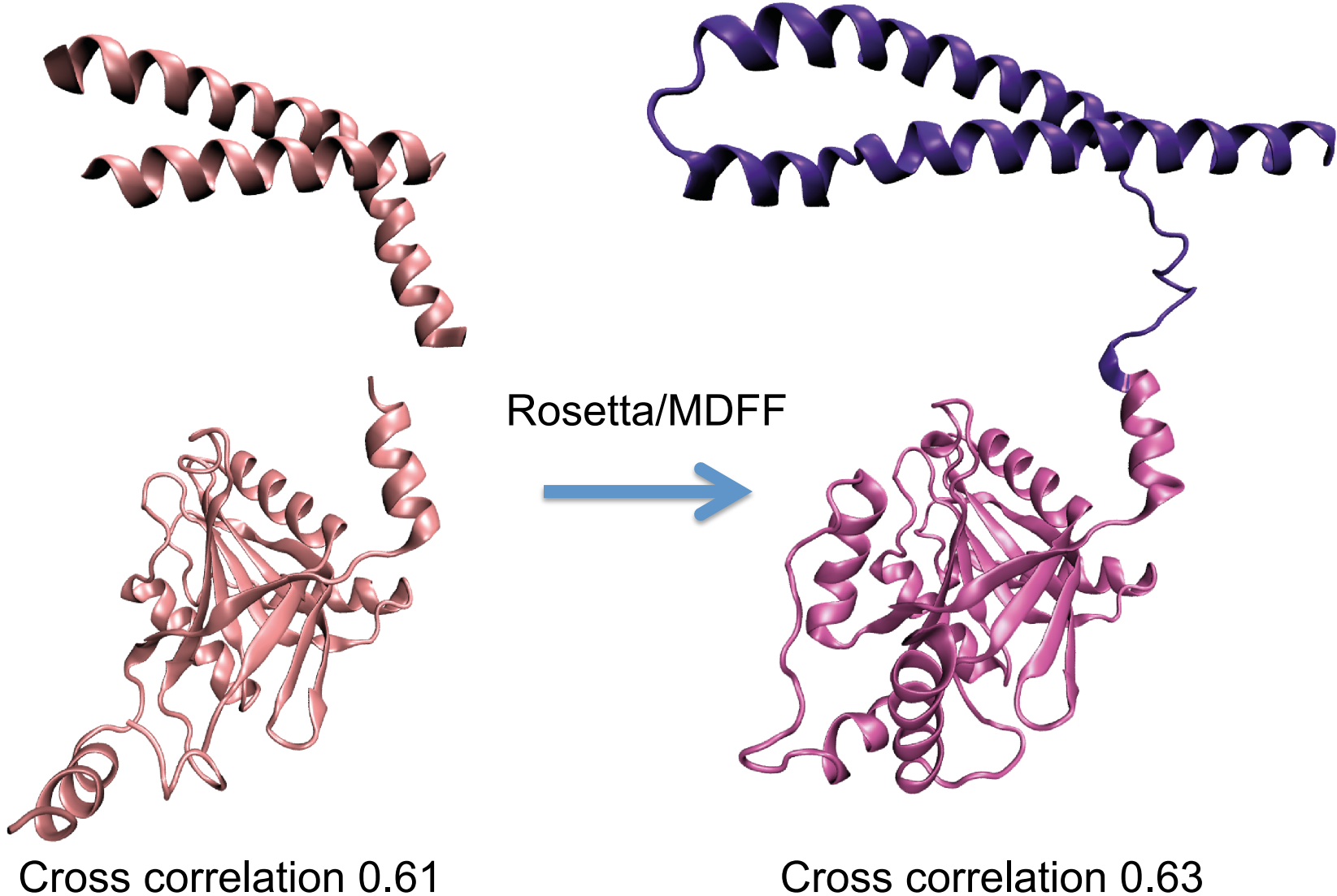
0

Rpn11 colored by  
local cross correlations

# Incomplete vs. complete model

Incomplete model

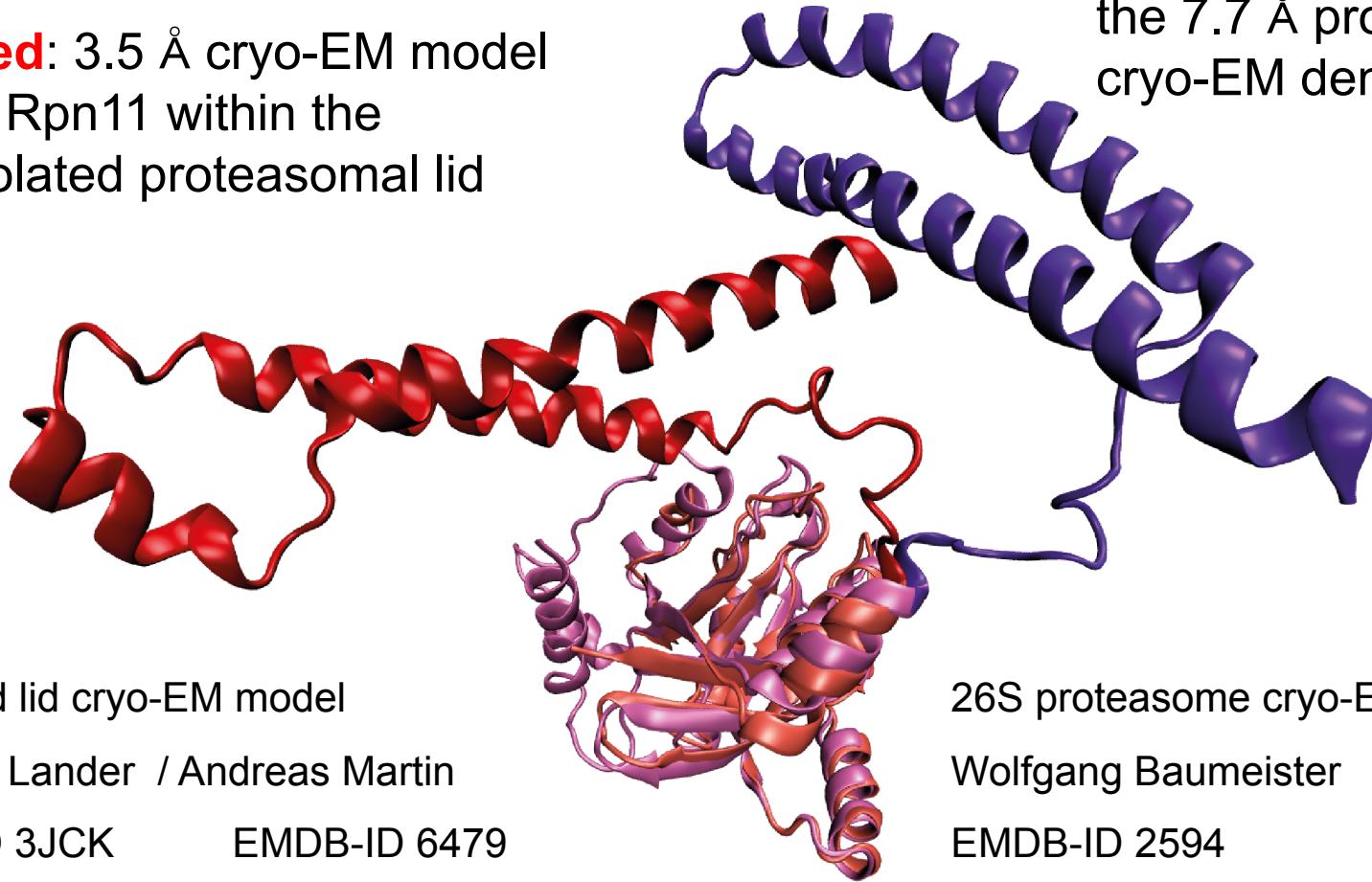
Complete model



# 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

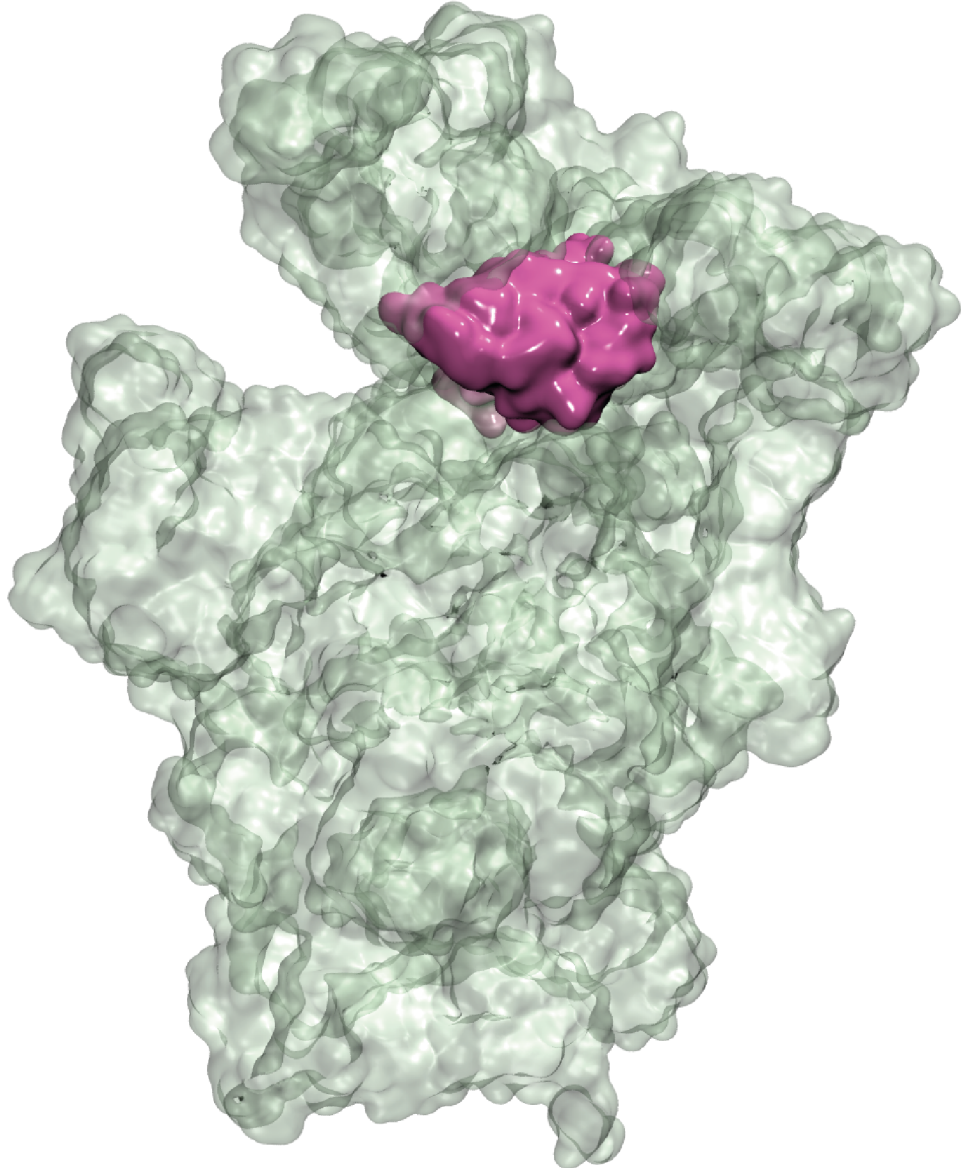
EMDB-ID 2594

Resolution 7.7 Å

Unverdorben *et al.* PNAS 2014



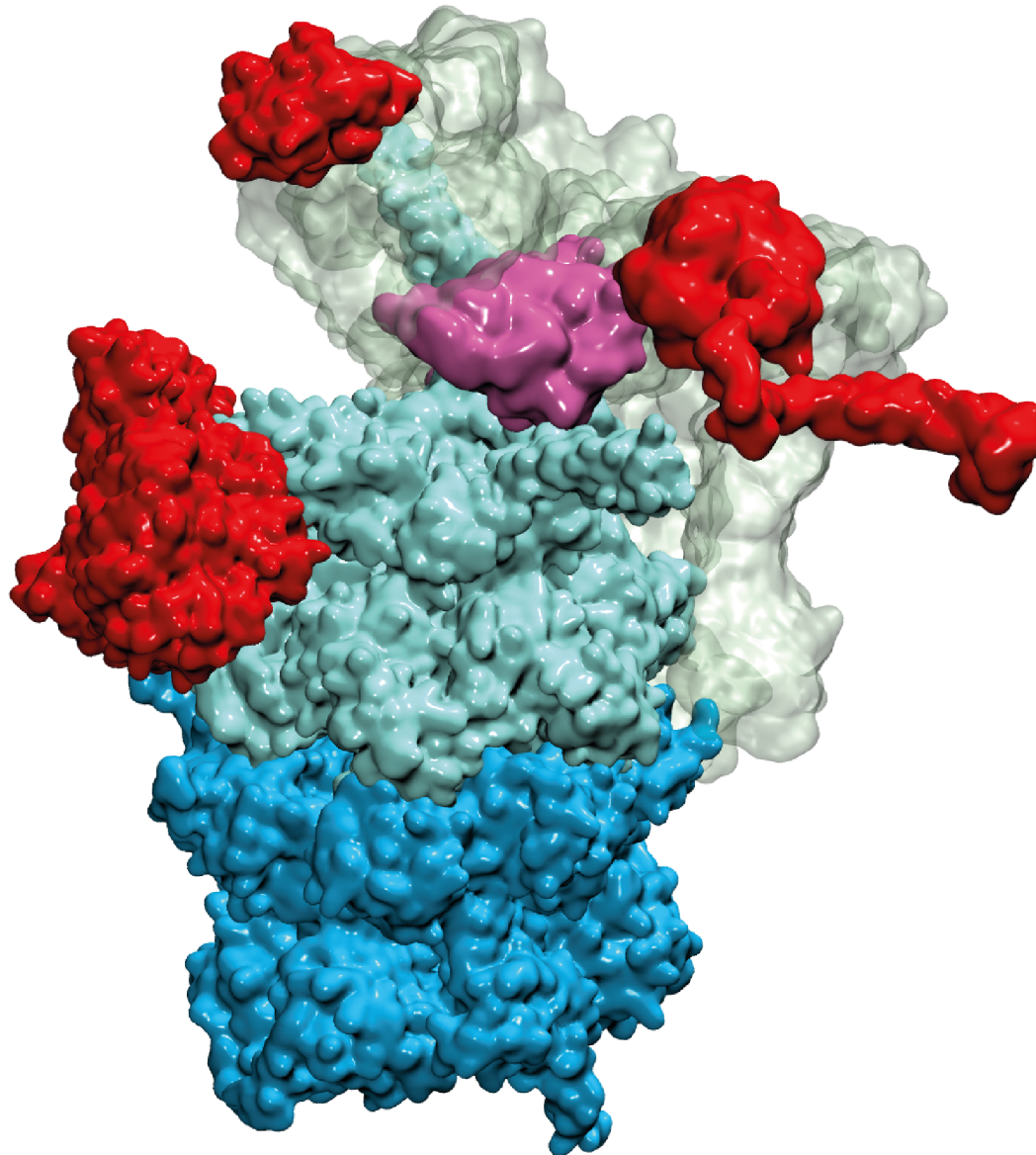
# Low vs. high resolution density model



Deubiquitylation  
(Rpn11)



# Functional subunits of the 26S proteasome



Ubiquitin  
Recognition  
(Rpn10, Rpn13, Rpn1)

Deubiquitylation  
(Rpn11)

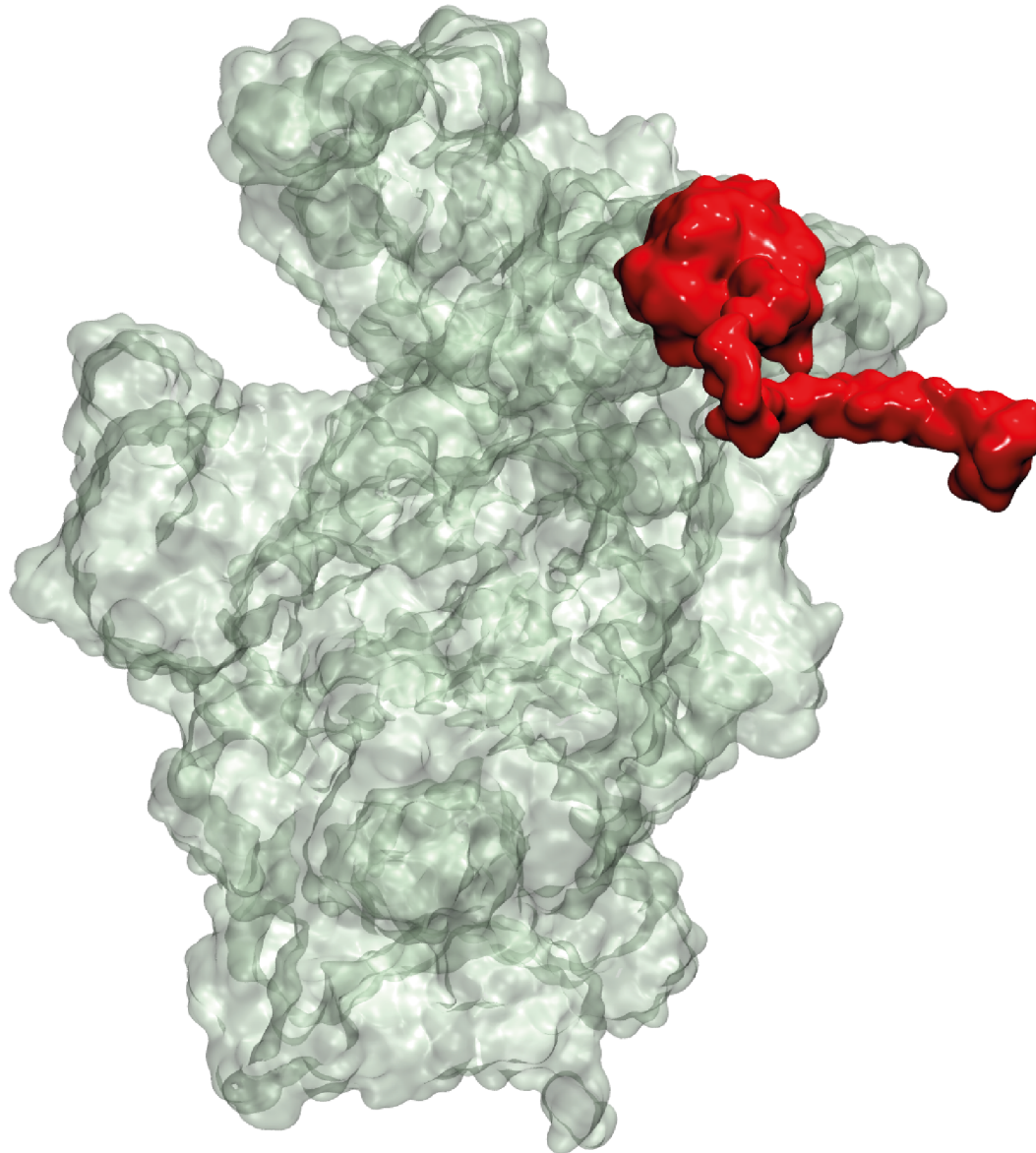
Substrate  
Unfolding  
(ATPase-ring)

Substrate  
Degradation  
( $\alpha$ -ring,  $\beta$ -ring)



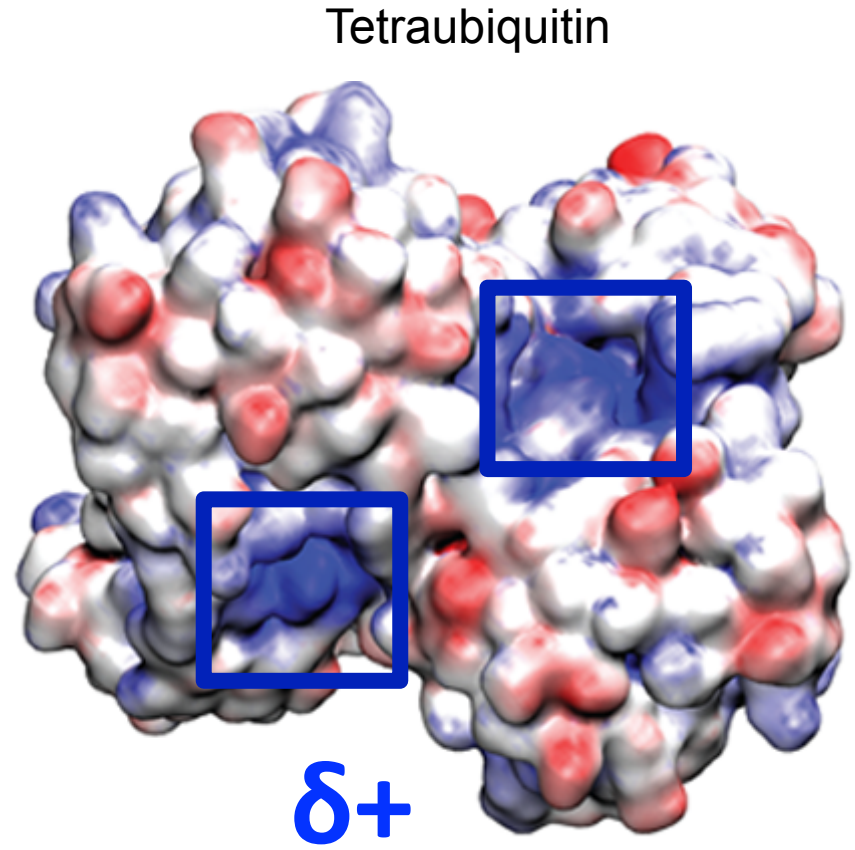
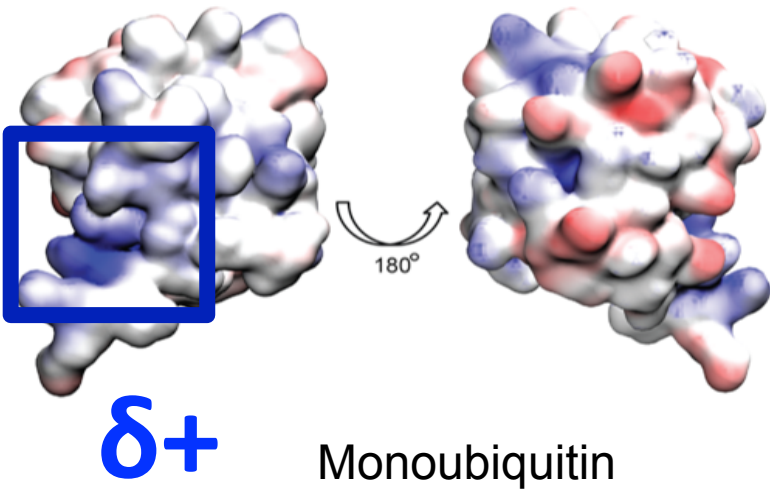
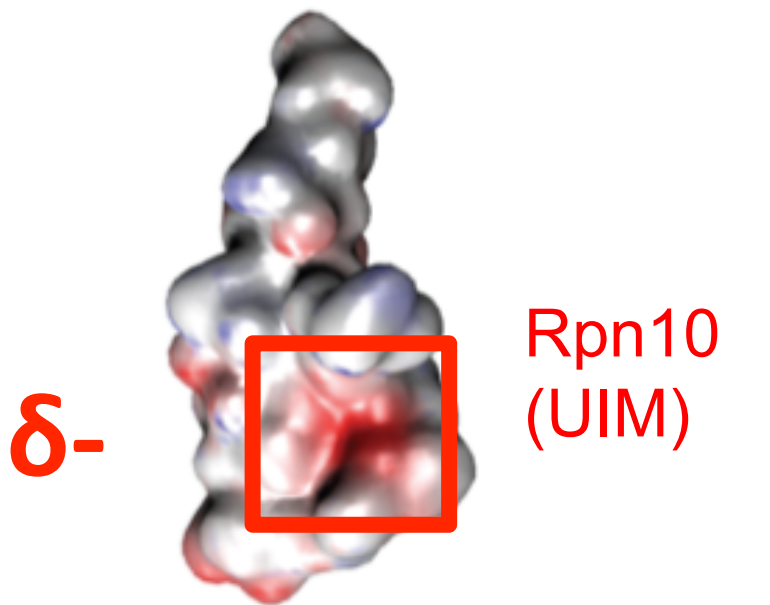
# Ubiquitin recognition by Rpn10

1



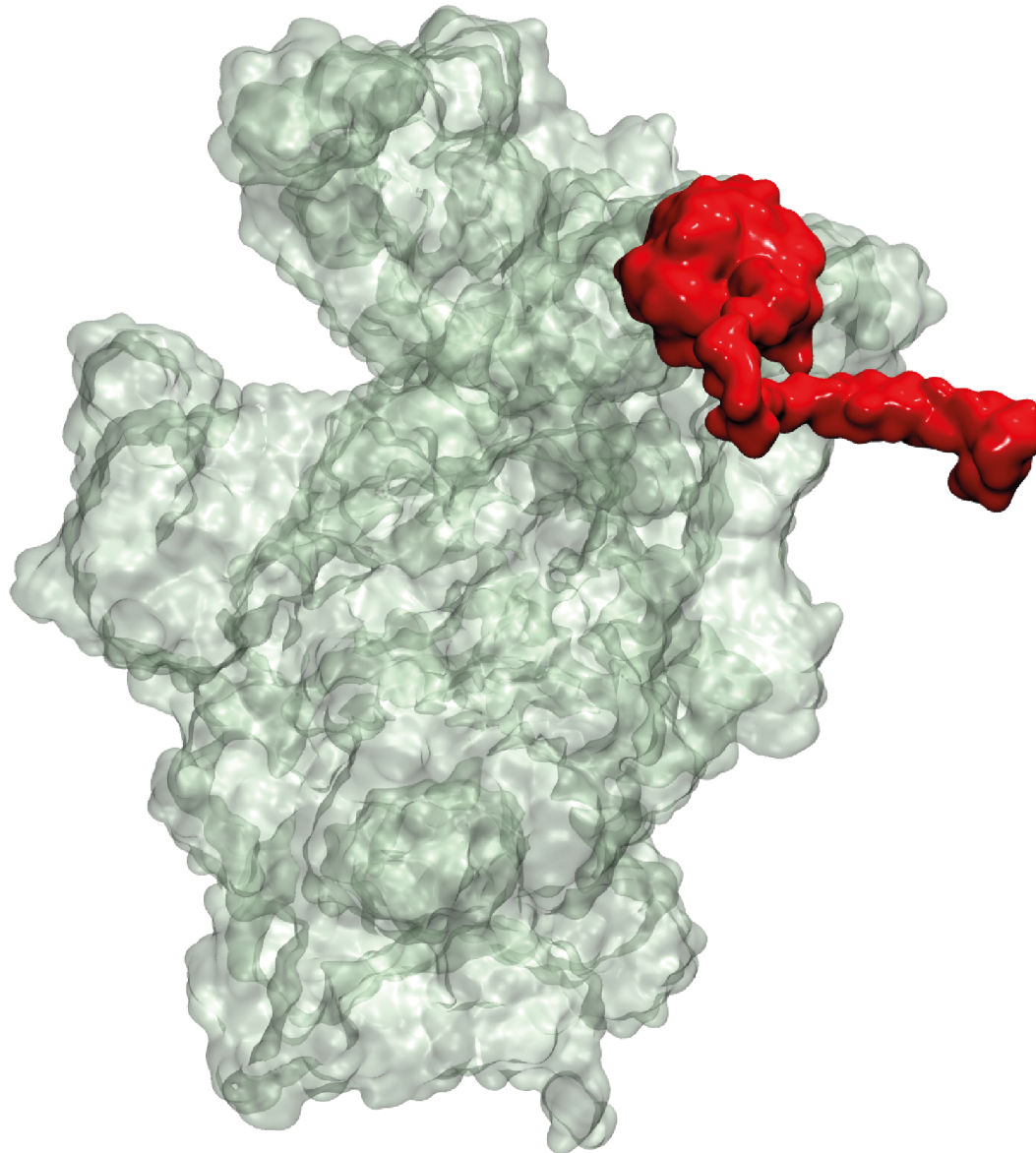
Ubiquitin  
Recognition  
(Rpn10)

# Ubiquitin Recognition





# Ubiquitin recognition by Rpn10

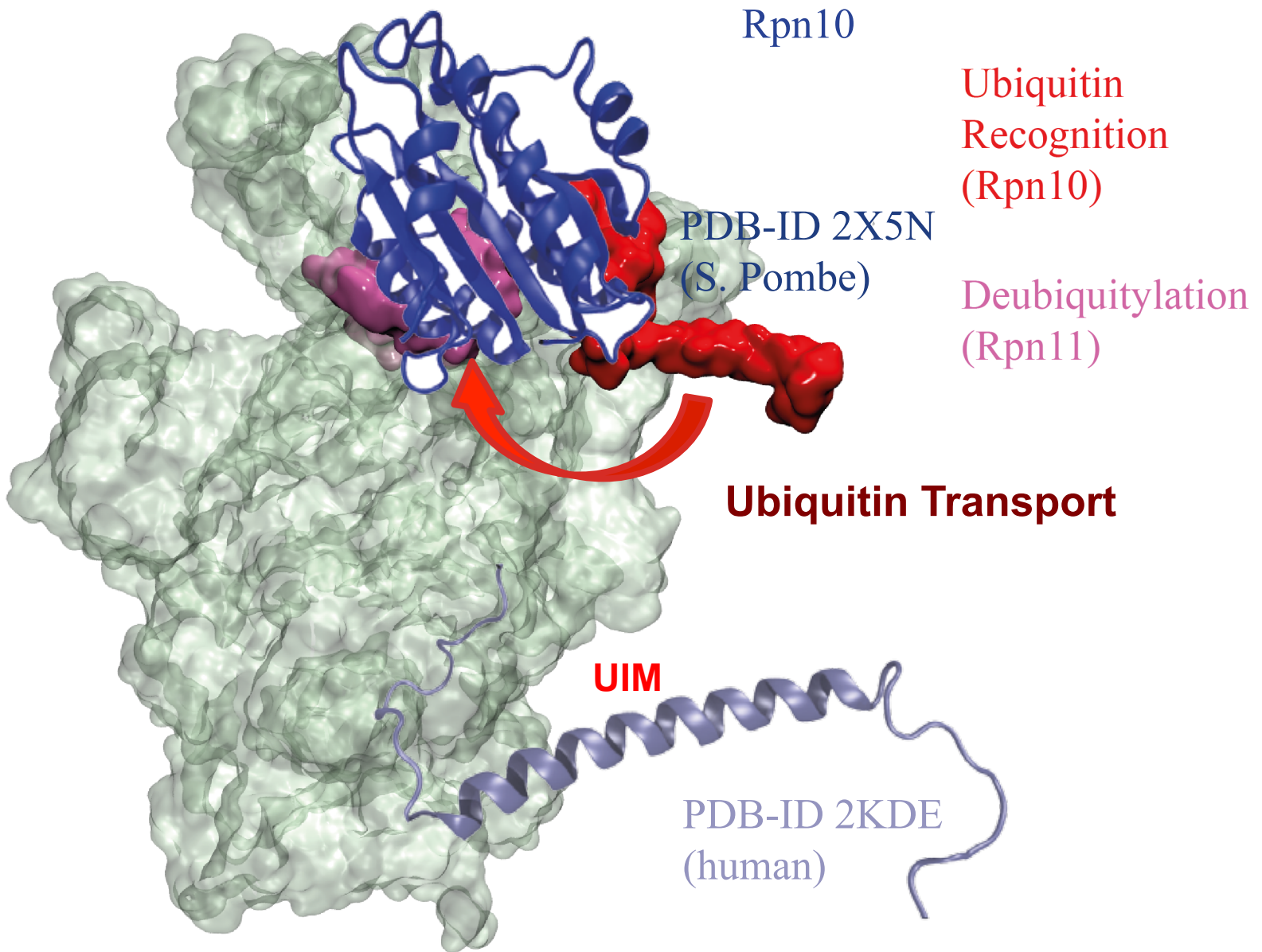


Ubiquitin  
Recognition  
(Rpn10)





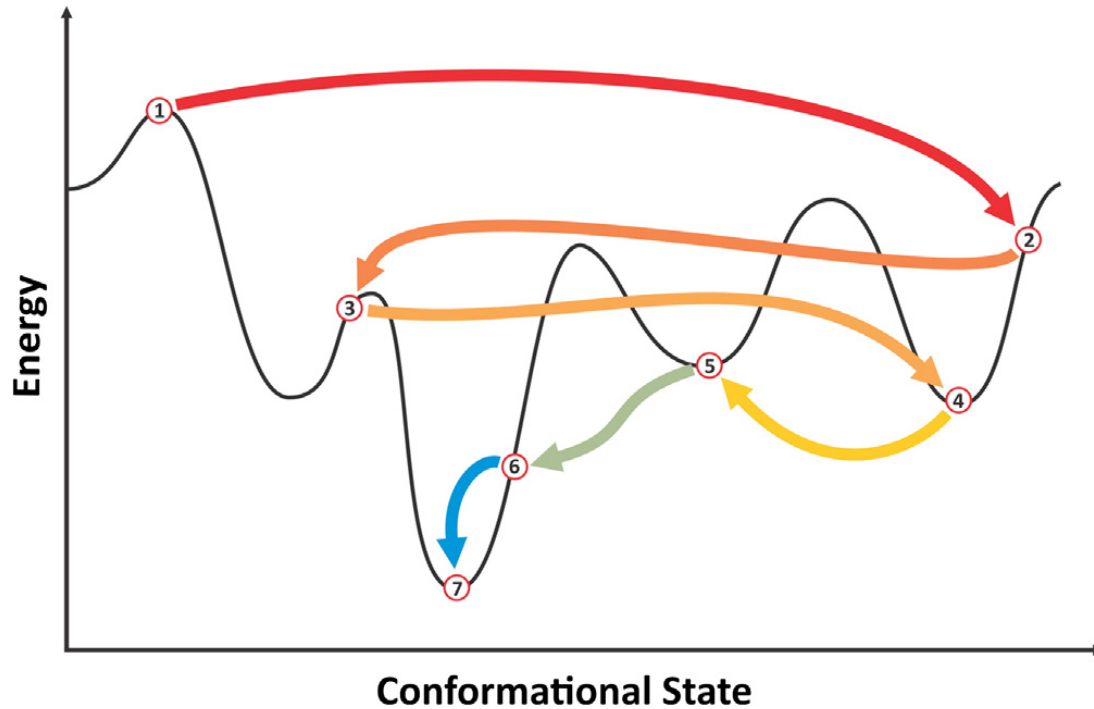
# Ubiquitin recognition and deubiquitylation



# 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.**



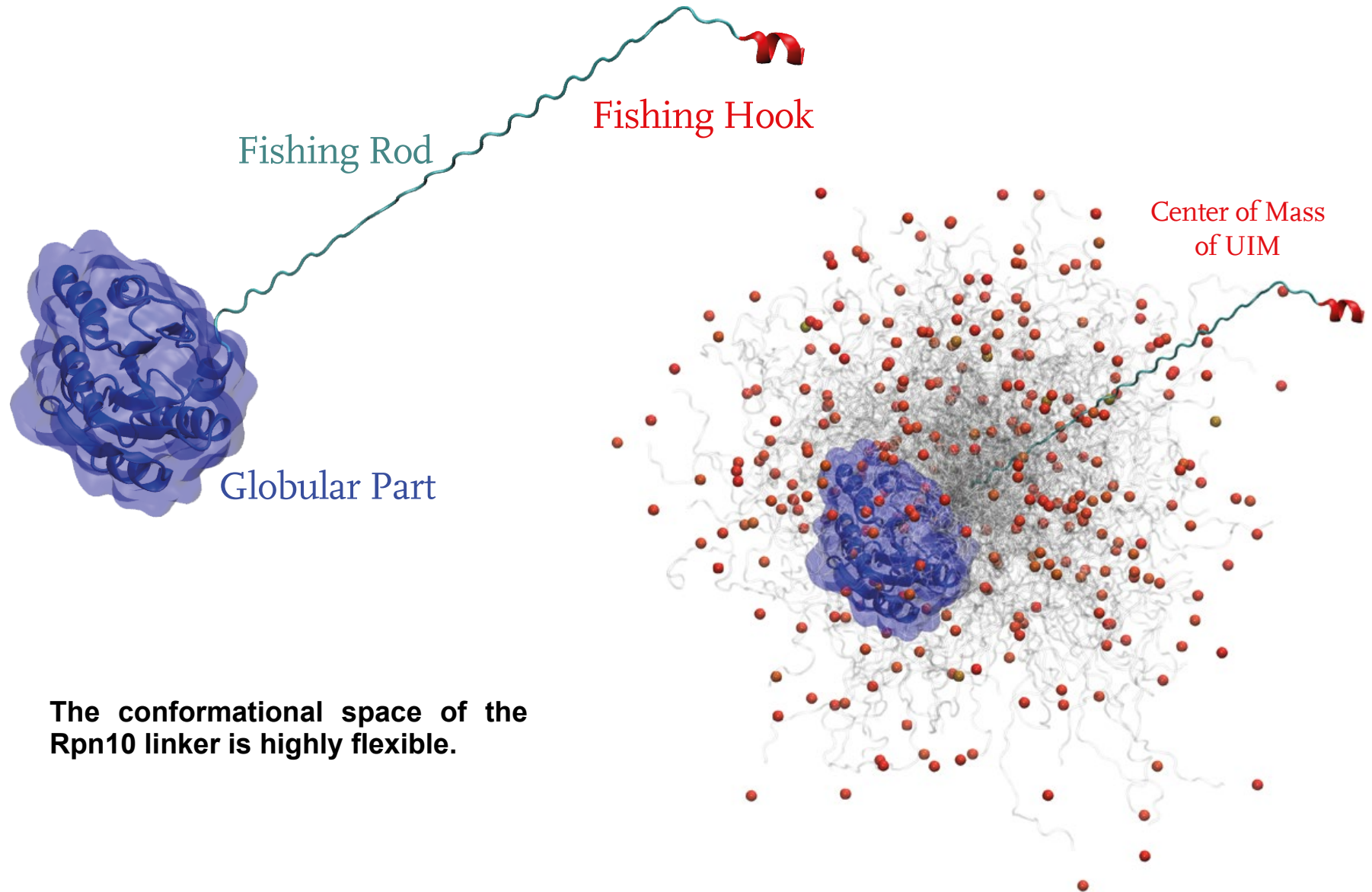
- Amino acid residues connecting Rpn10's UIM with the proteasome are likely to be disordered and stochastic searching algorithms such as GSA can be used to explore their conformational space

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



Rafael C. Bernardi Marcelo Melo

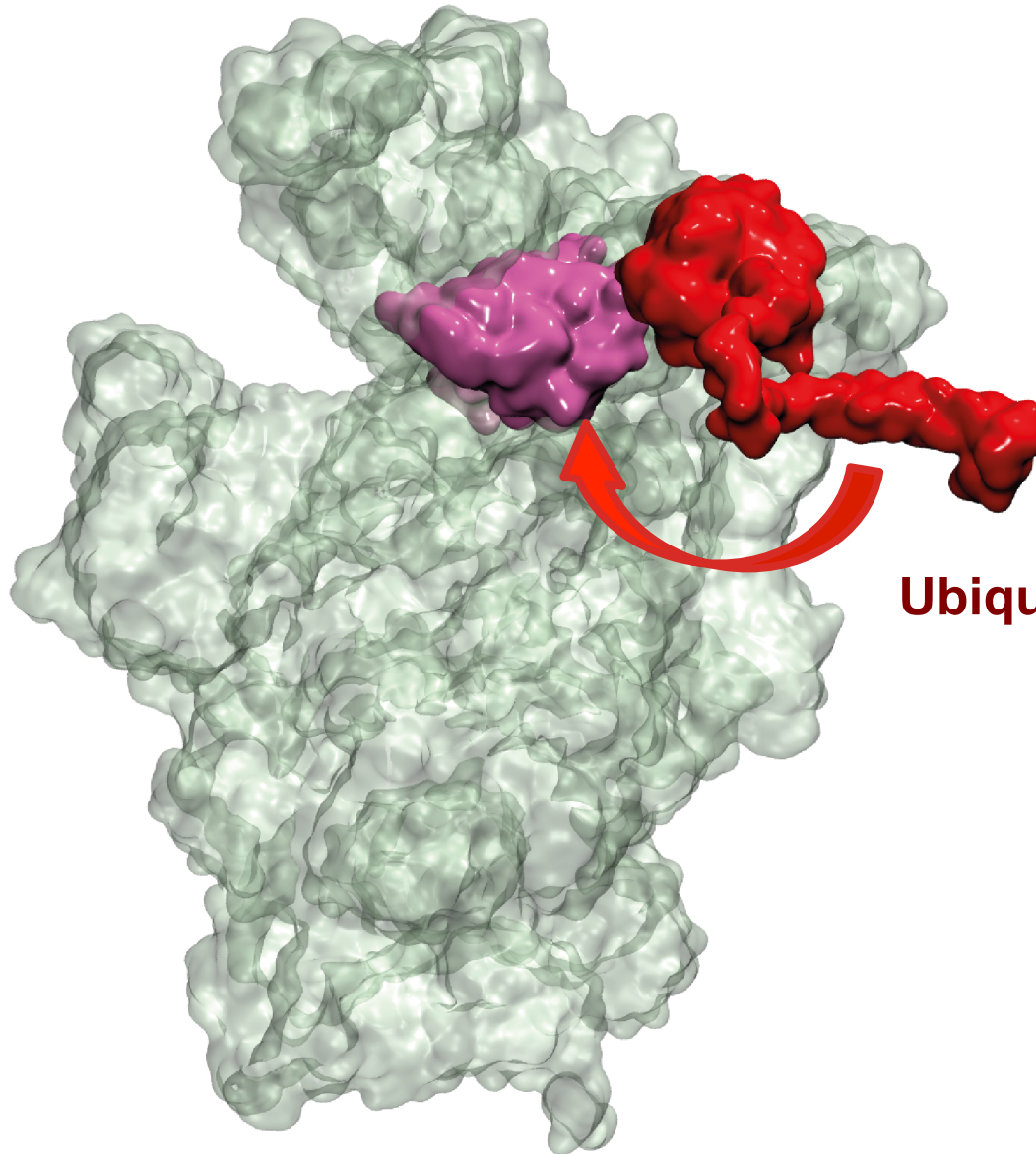
# Conformation Space of Rpn10 Anchor



The conformational space of the Rpn10 linker is highly flexible.



# Ubiquitin Transport to Deubiquitinase Rpn11



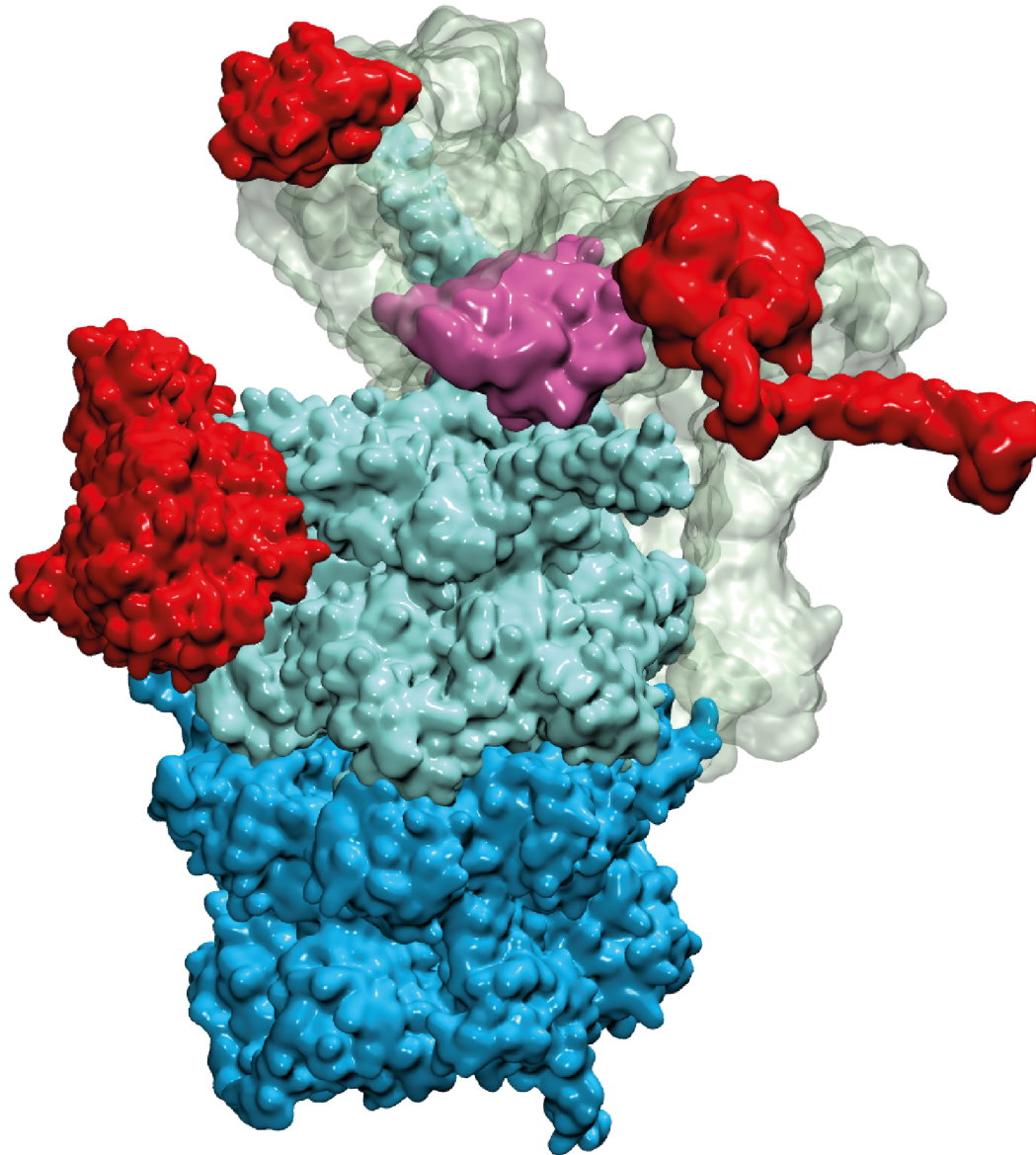
Ubiquitin  
Recognition  
(Rpn10)

Deubiquitylation  
(Rpn11)

**Ubiquitin Transport**



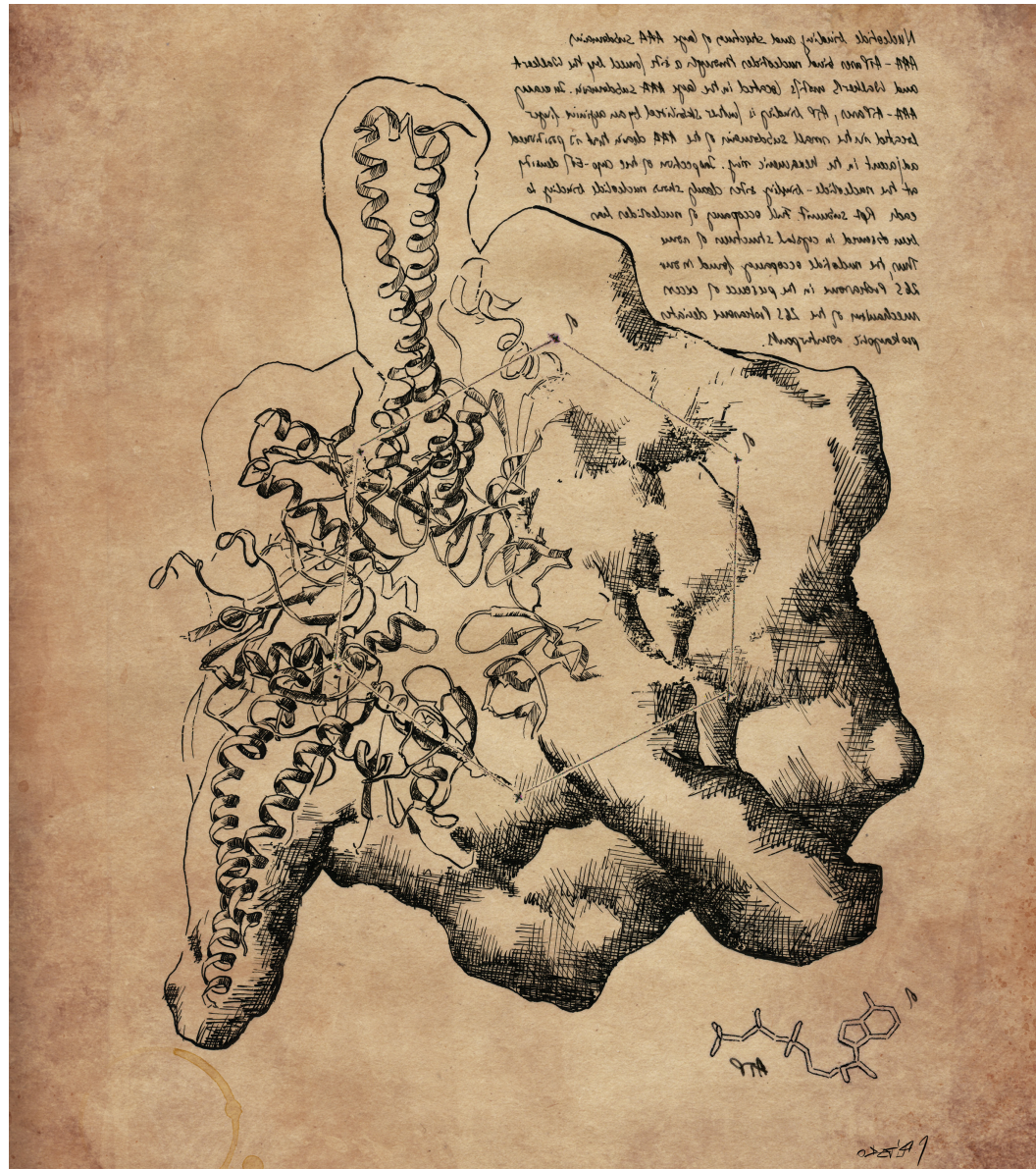
# Functional subunits of the 26S proteasome



Substrate  
Unfolding  
(ATPase-ring)

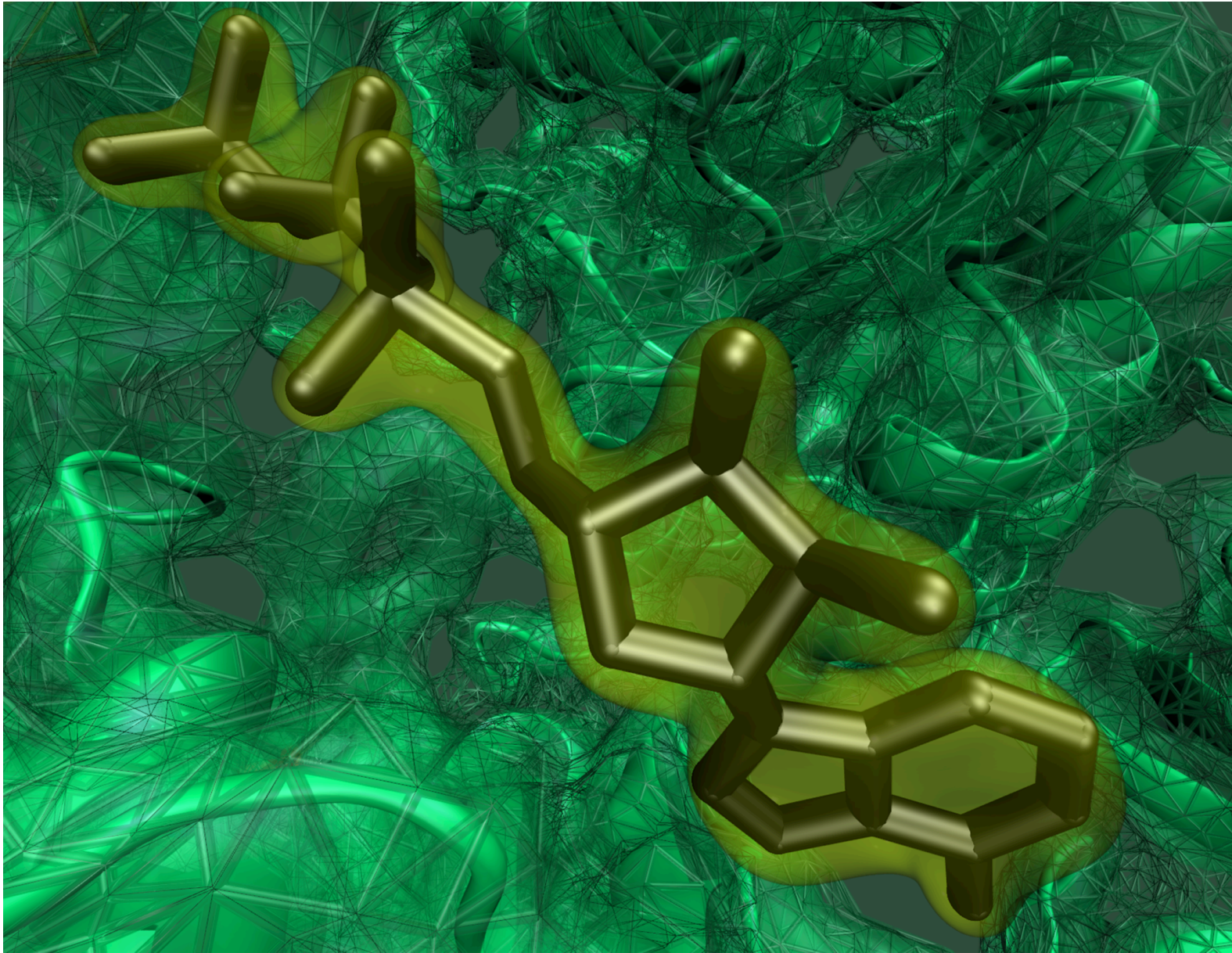


# The Motor of the Proteasome

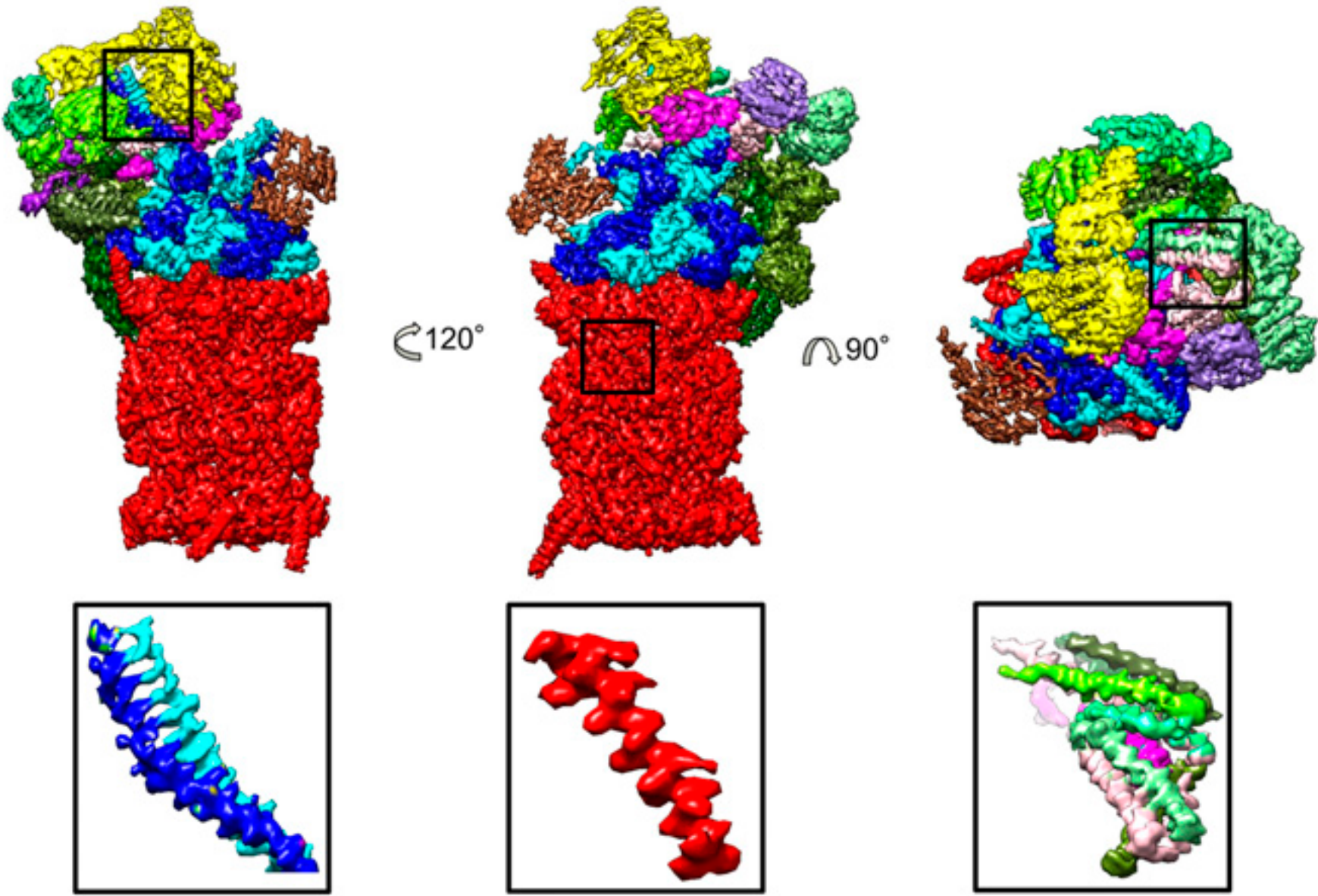




# Resolved nucleotides are needed

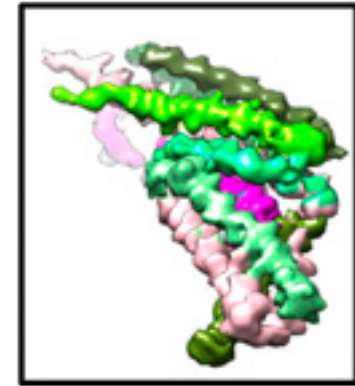
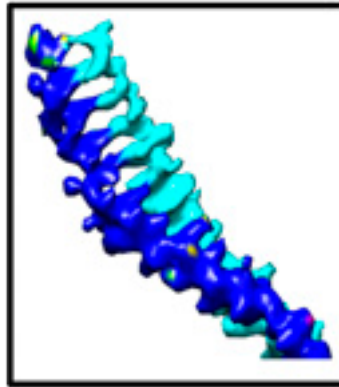
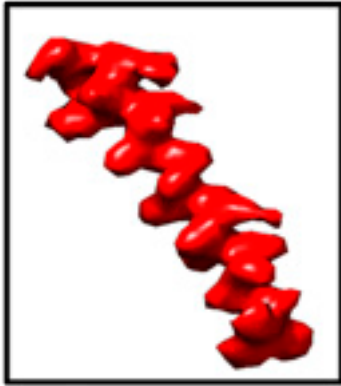


# 3.9 Å Resolution Density of the Human 26S Proteasome





# 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

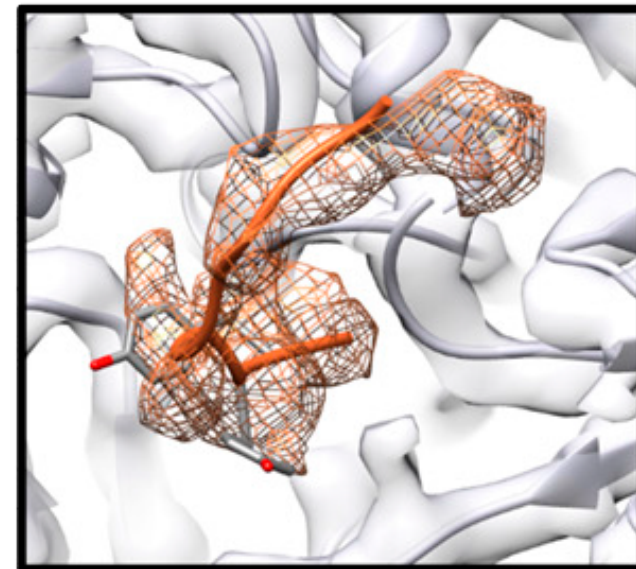
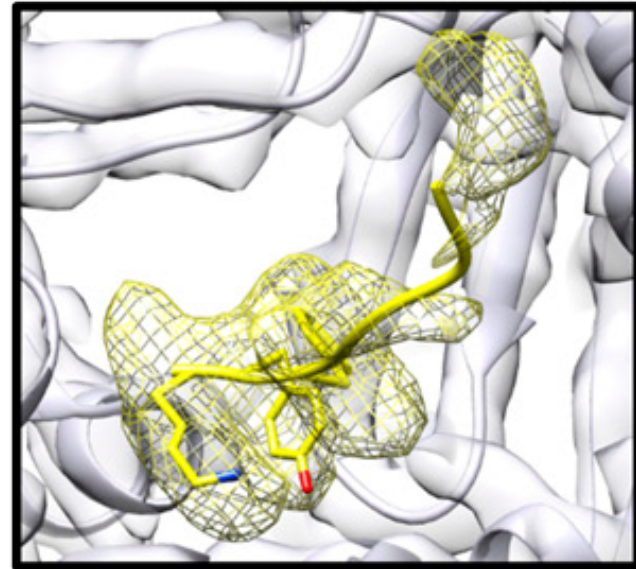
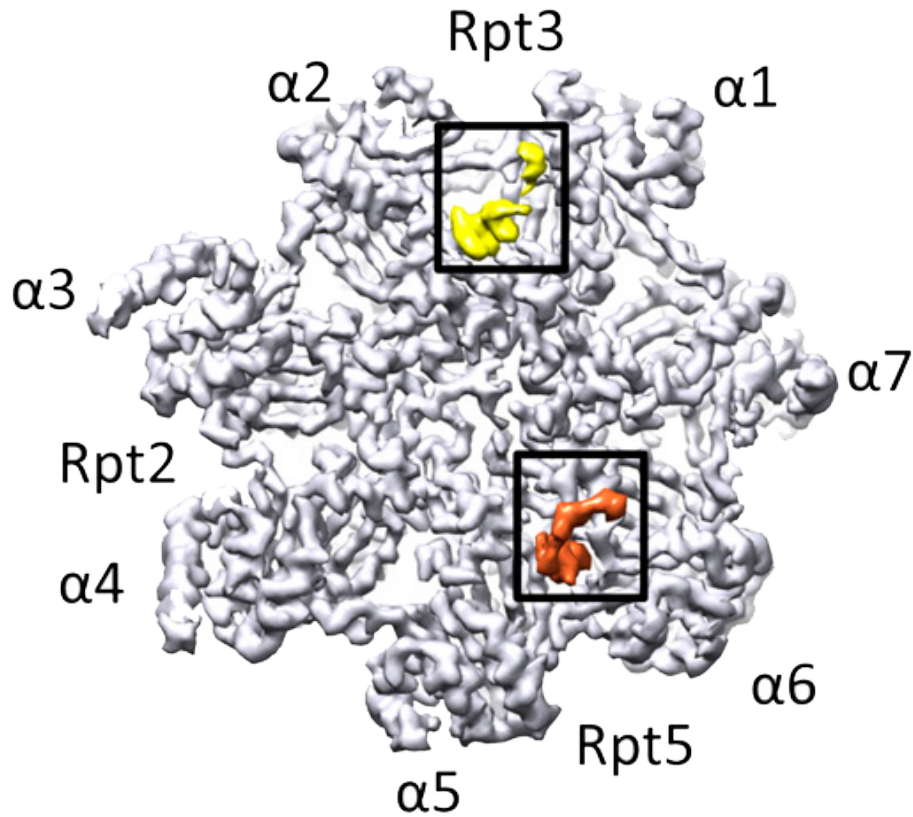
## Solution:

combining MDFF with

monte carlo based backbone and side chain rotamer search algorithms

in an iterative manner

# The ATPase Motor of the 26S Proteasome

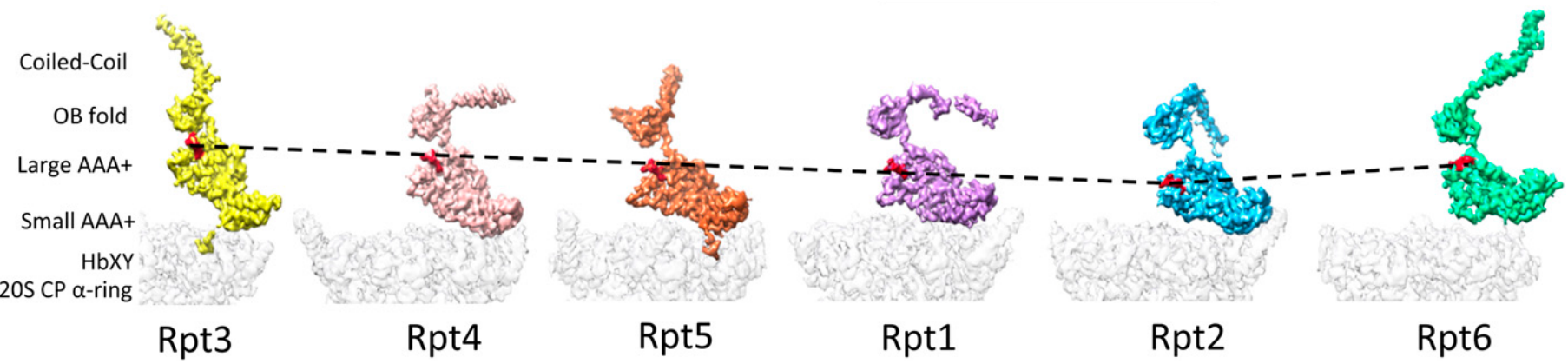
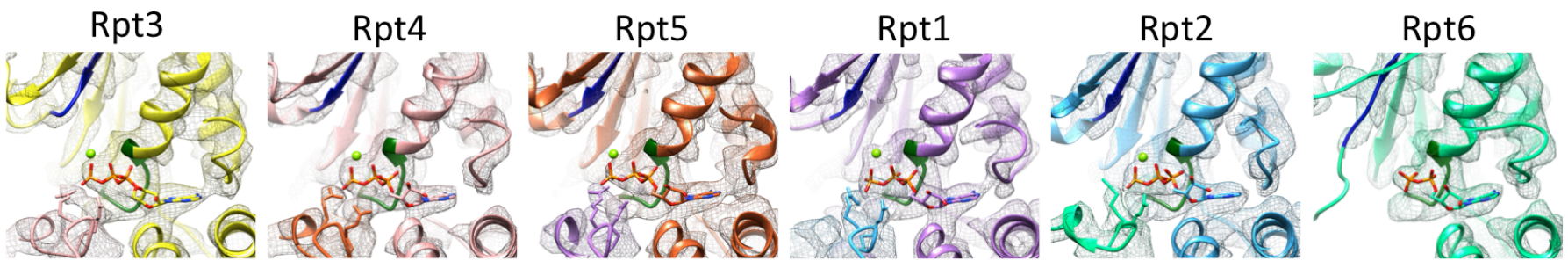
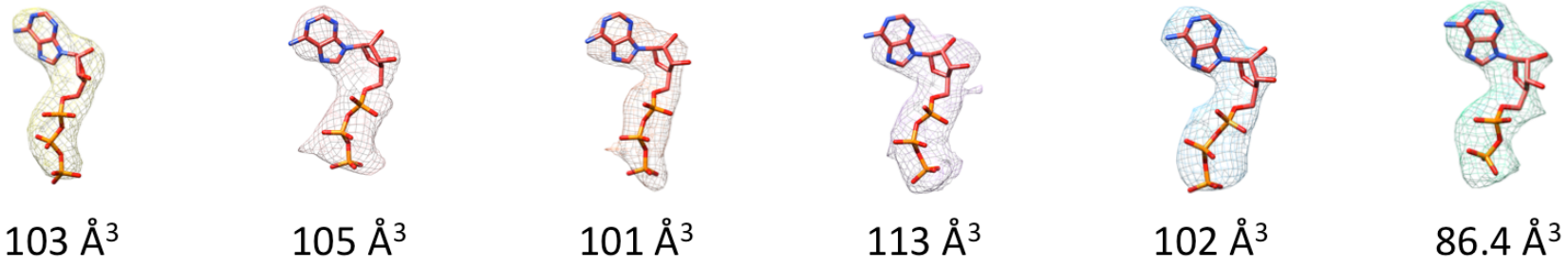


PDB-IDs: 5L4G, 5L4K

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 QM/MM simulations combined with path sampling techniques.

## NAMD QM/MM interface with MOPAC and ORCA will be released in the second semester of 2016

qwikMD - Easy and Fast Molecular Dynamics

Easy Run | Advanced Run | Basic Analysis | Advanced Analysis

Browser | Load

NMR State | Chain/type Selection | Structure Manipulation

Chain	Residue Range	Type	Representation	Color
-------	---------------	------	----------------	-------

Molecular Dynamics | SMD | MDFF | **QM/MM** | ABF

Solvent: Implicit | NaCl | Concentration: 0.15 mol/L

QM Software: MOPAC | Set Path | Number of QM Regions: 1

QM/MM Electrostatics: Cut-Off | PME

MD Protocol - Number of Steps

Classical	Minimization	Equilibration
5,000	500,000	1,000,000

Hybrid QM/MM: 100 | Minimization: 500 | Equilibration: 50,000

Classical: T= 27C | P= 1 atm | QM/MM: T= 27C | P= 1 atm

QM Calculation

QM ID	QM Region	Charge	Mult	QM Protocol	LiveSolvSel
1	157 atoms	0	1	PM7 XYZ T=2M 1SCF MOZYME ...	Center of Mass

Simulation Setup

Working Directory | Load | Save

Background: Black | White | Gradient | Color Scheme: VMD Classic

Prepare | Live Simulation | Reset | Restart from Last Step

Simulation Controls

Start MD Simulation

Pause | Detach | Finish

Progress: 45% | Completed 65ns of 150ns

Structure Manipulation

Res ID	Res NAME	Chain	Type
1	MET	A	protein
2	GLN	A	protein
3	ILE	A	protein
4	PHE	A	protein
5	VAL	A	protein
6	LYS	A	protein
7	THR	A	protein
8	LEU	A	protein
9	THR	A	protein
10	GLY	A	protein
11	LYS	A	protein
12	THR	A	protein
13	ILE	A	protein

Apply | Clear Selection

Structure Manipulation

Res ID	Res NAME	Chain	Type
1	MET	A	protein
2	GLN	A	protein
3	ILE	A	protein
4	PHE	A	protein
5	VAL	A	protein
6	LYS	A	protein
7	THR	A	protein
8	LEU	A	protein
9	THR	A	protein
10	GLY	A	protein
11	LYS	A	protein
12	THR	A	protein
13	ILE	A	protein

QM Region

atom selection

157 atoms selected

Point Charges

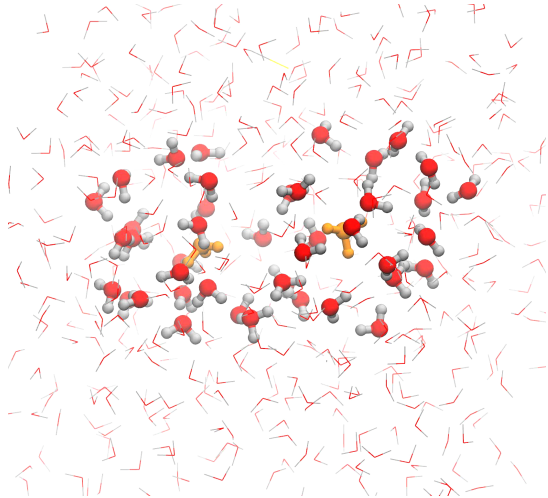
10 A from QM Region

2570 atoms selected

Add Solvent within 10 A

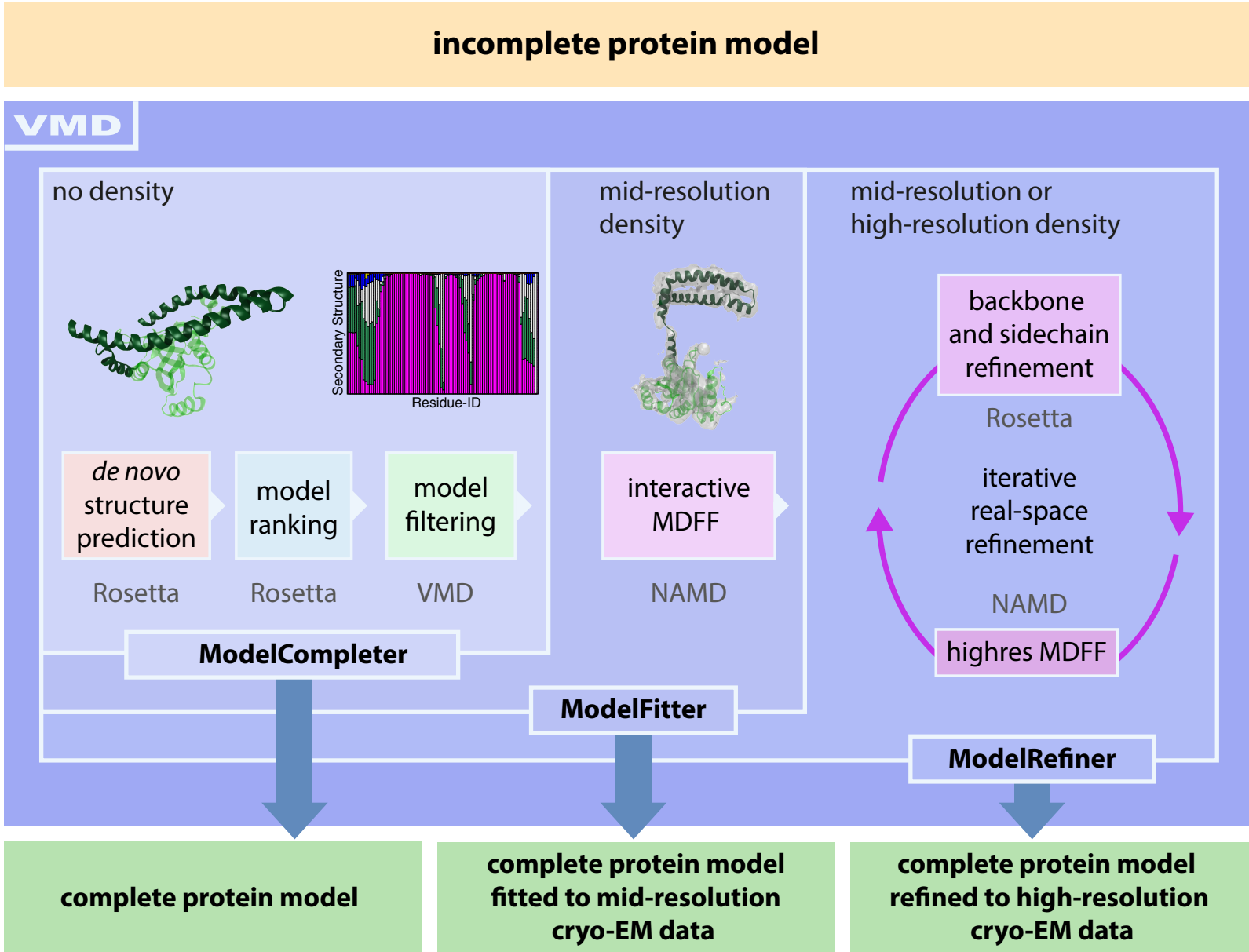
QM/MM Charge Zero

Apply | Clear Selection





# ModelMaker





# Conclusion

---

In order to obtain **complete** protein **structures** different **experimental** and **computational** methods need to be **integrated**

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



# Acknowledgments



Alexander von Humboldt  
Stiftung / Foundation



## Theory

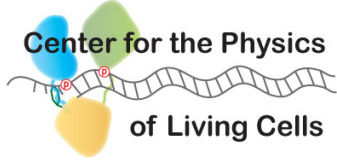
## Experiment



Klaus Schulten  
Ryan McGreevy



Wolfgang Baumeister  
Friedrich Förster  
Eri Sakata



## ModelMaker

Ryan McGreevy

## GSA

Rafael Bernardi  
Marcelo Mello



RUPRECHT-KARLS-  
UNIVERSITÄT  
HEIDELBERG



Technische Universität München

Maximilian Scheurer

Marc Siggel

Justin Porter