Classifying and correlating structural heterogeneity *in situ* and *in vitro*

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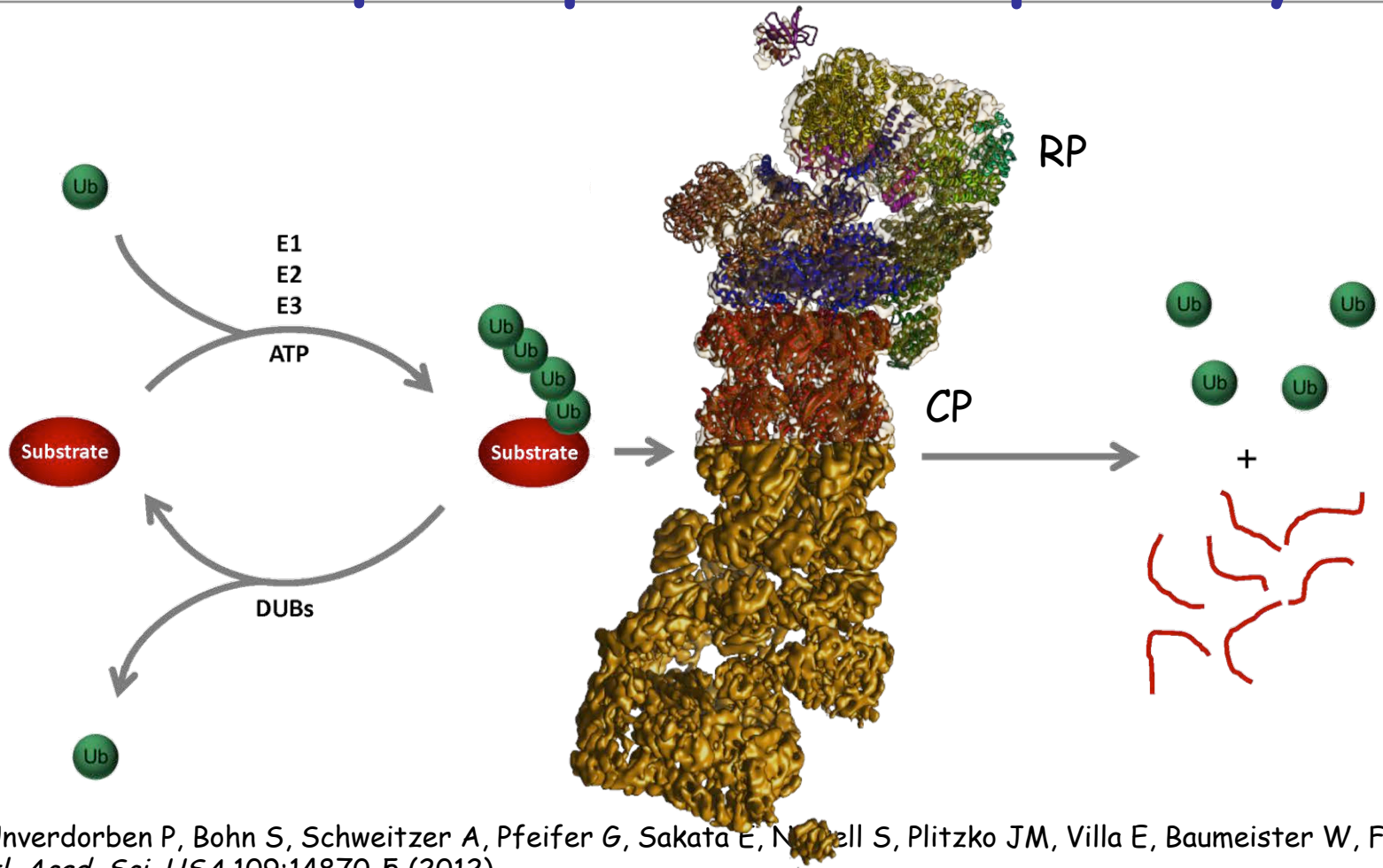
Granlibakken, March 31st 2016

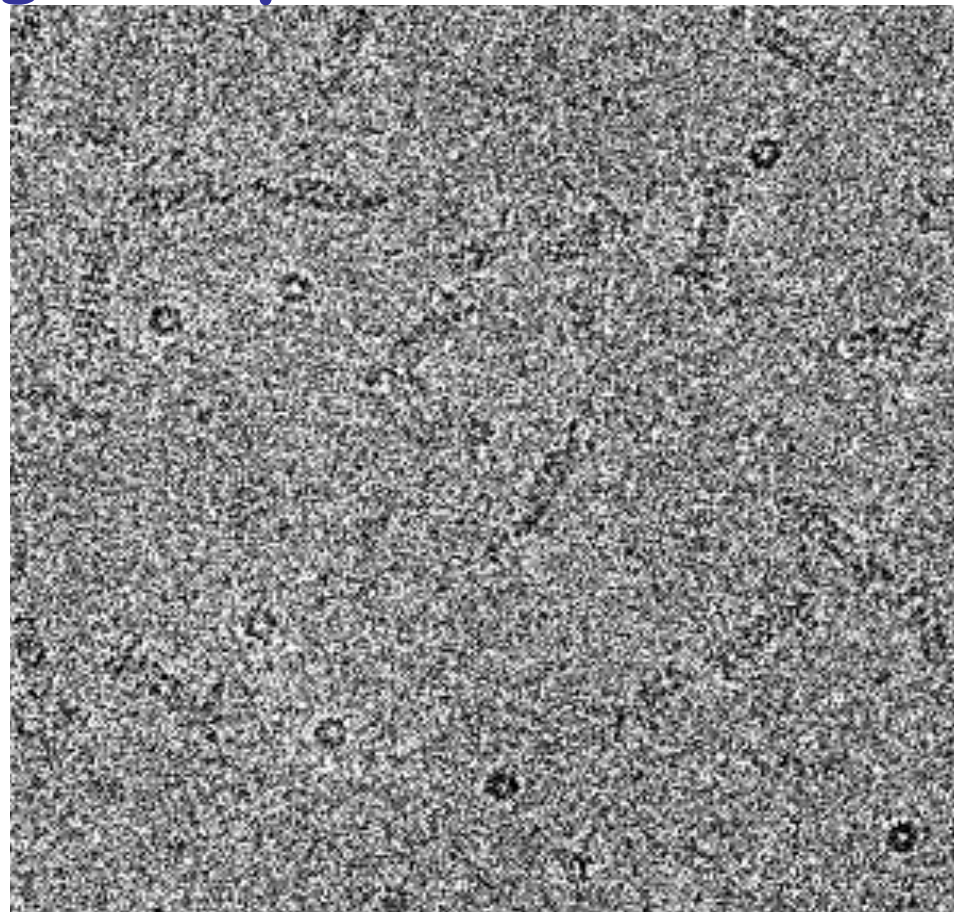
Max Planck Institute of Biochemistry

26S proteasome is most downstream element of ubiquitin proteasome pathway



Beck F, Unverdorben P, Bohn S, Schweitzer A, Pfeifer G, Sakata E, N[illegible]ell S, Plitzko JM, Villa E, Baumeister W, Förster F. *Proc. Natl. Acad. Sci. USA* 109:14870-5 (2012).

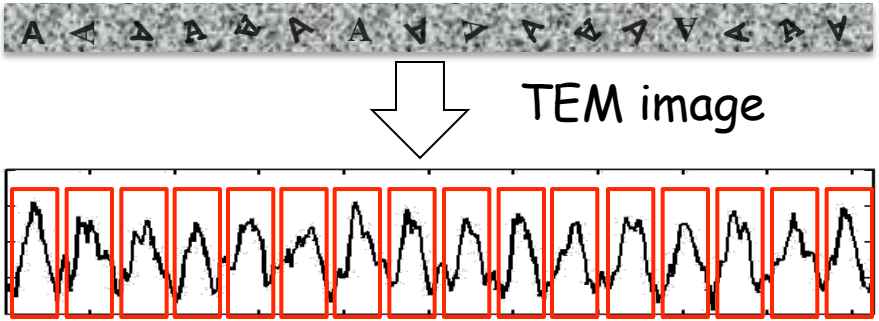
Variable composition and structural heterogeneity make 26S challenging



Bohn S, Beck F, Sakata E, Walzthoeni T, Beck M, Aebersold R, Förster F, Baumeister W & Nickell S.. Proc. Natl. Acad. Sci. USA 107:20992-7 (2010).

Single Particles can be imaged and statistically analyzed in 2D and 3D

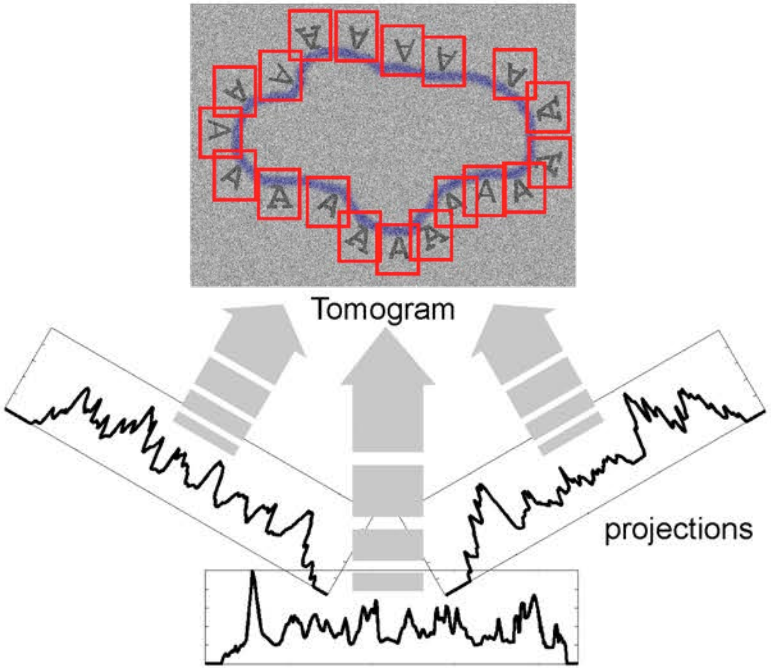
Purified complexes



Alignment &
3D reconstruction

3D classification

Complexes *in situ*



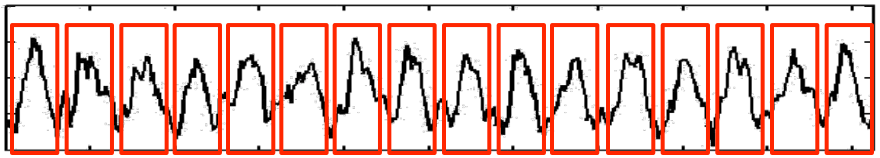
In vitro: isolated complexes

In vivo: whole cells

Single Particles can be imaged and statistically analyzed in 2D and 3D

Purified complexes

TEM image



Alignment &
3D reconstruction

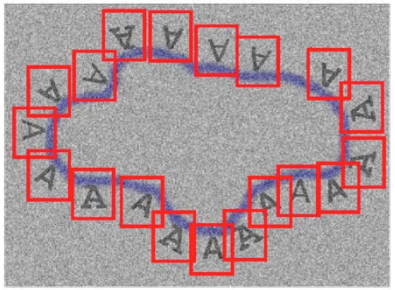
A

3D classification

A A A A

In vitro: isolated complexes

Complexes *in situ*



Alignment &
averaging

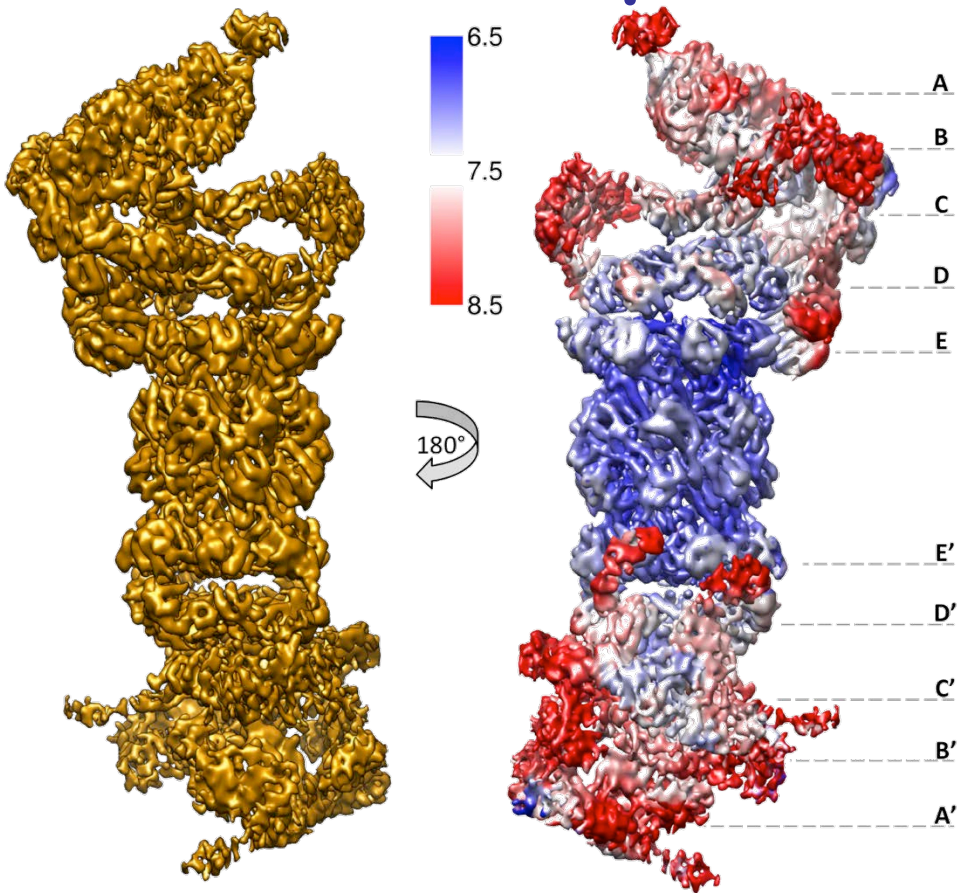
A

3D classification

A A A A

In vivo: whole cells

Single particle analysis suggests different conformers of 26S proteasome



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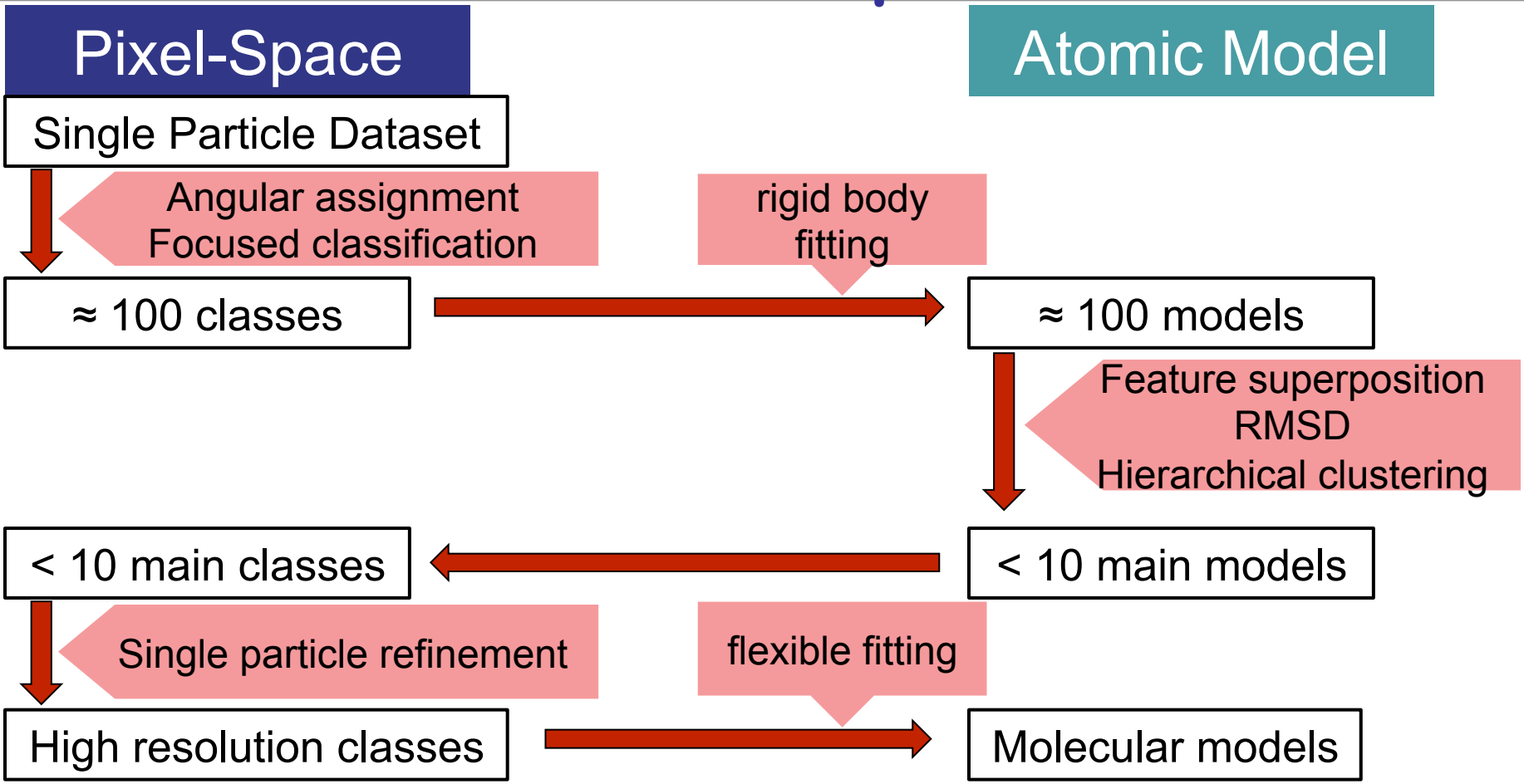
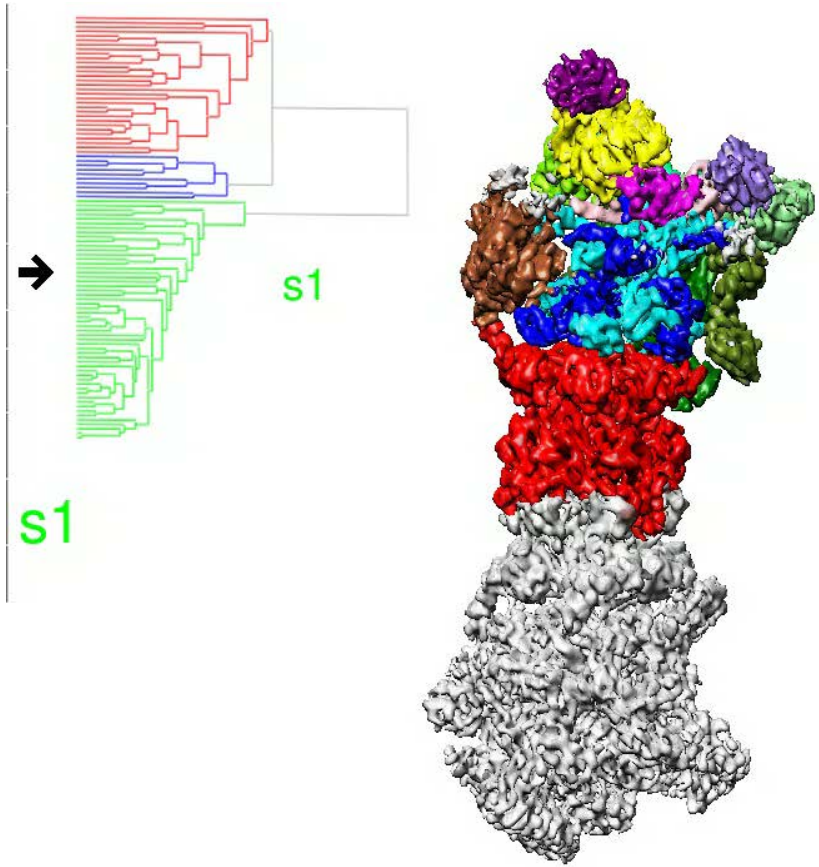
3D classification in voxel and atomic coordinate space

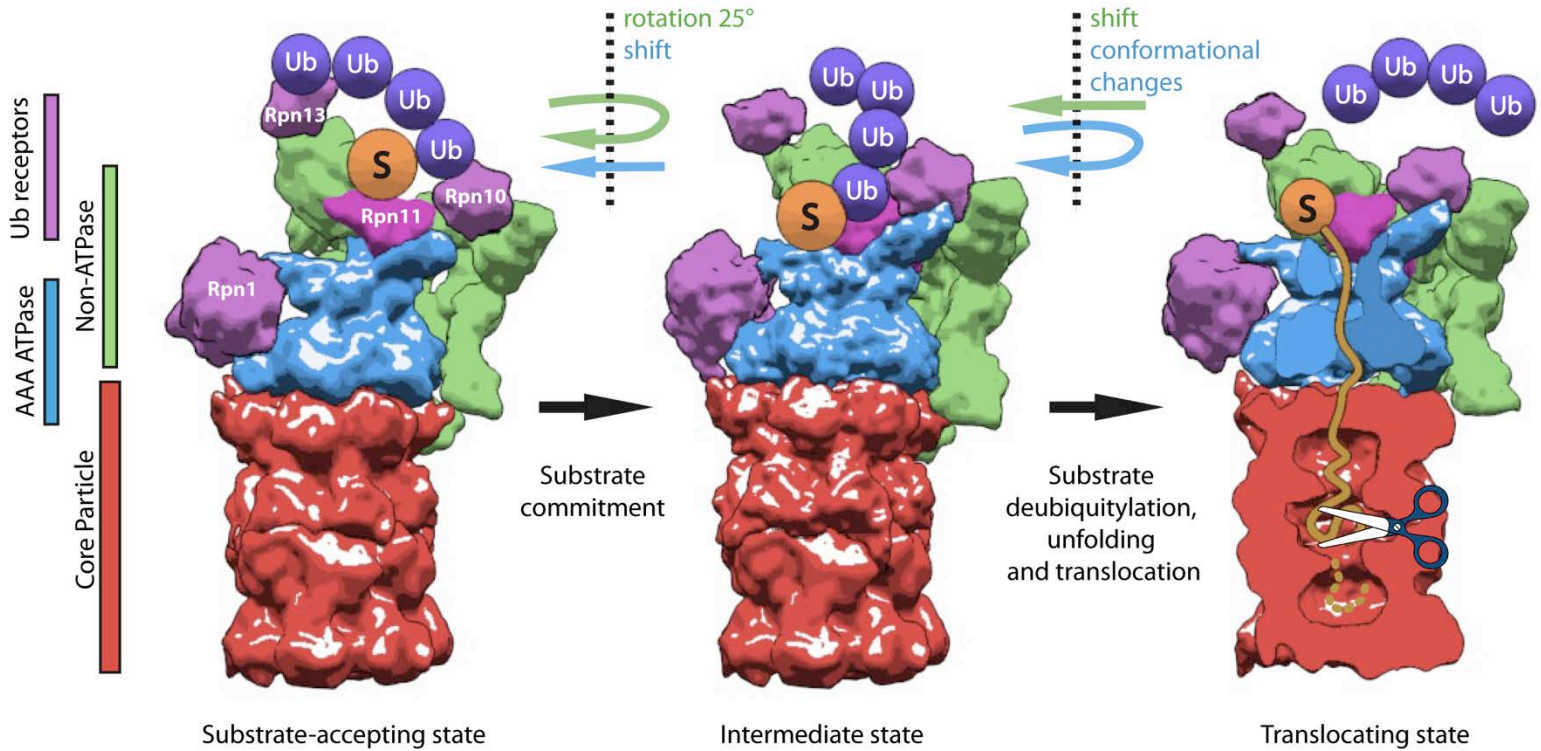


Deep classification of millions of particles reveals conformational changes



Unverdorben P, Beck F, Sledz P, Schweitzer A, Pfeifer G, Plitzko JM, Baumeister W & Förster F. *Proc. Natl. Acad. Sci. USA* 111:5544-9 (2014).

Model for mechanism of proteasomal degradation



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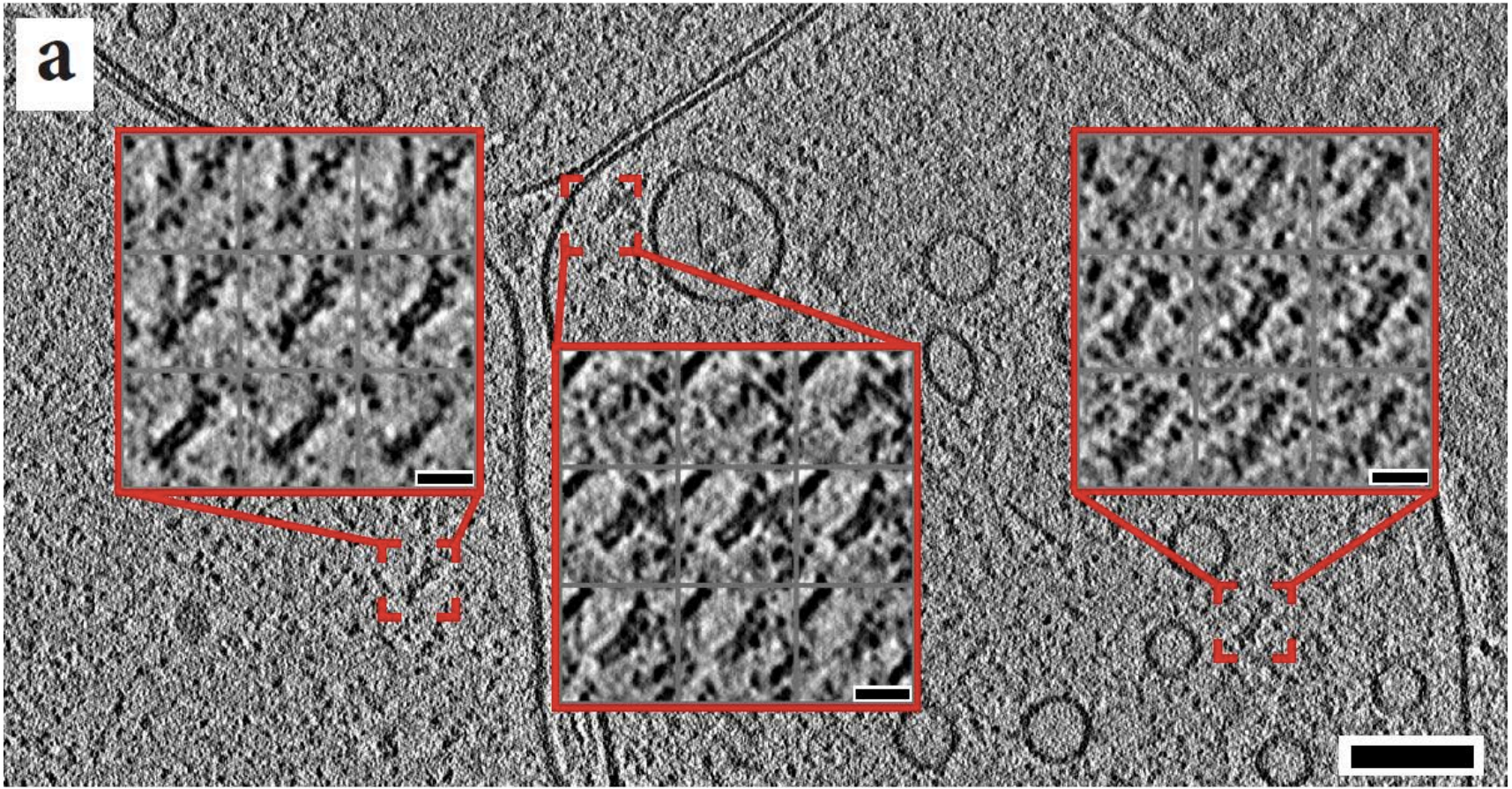
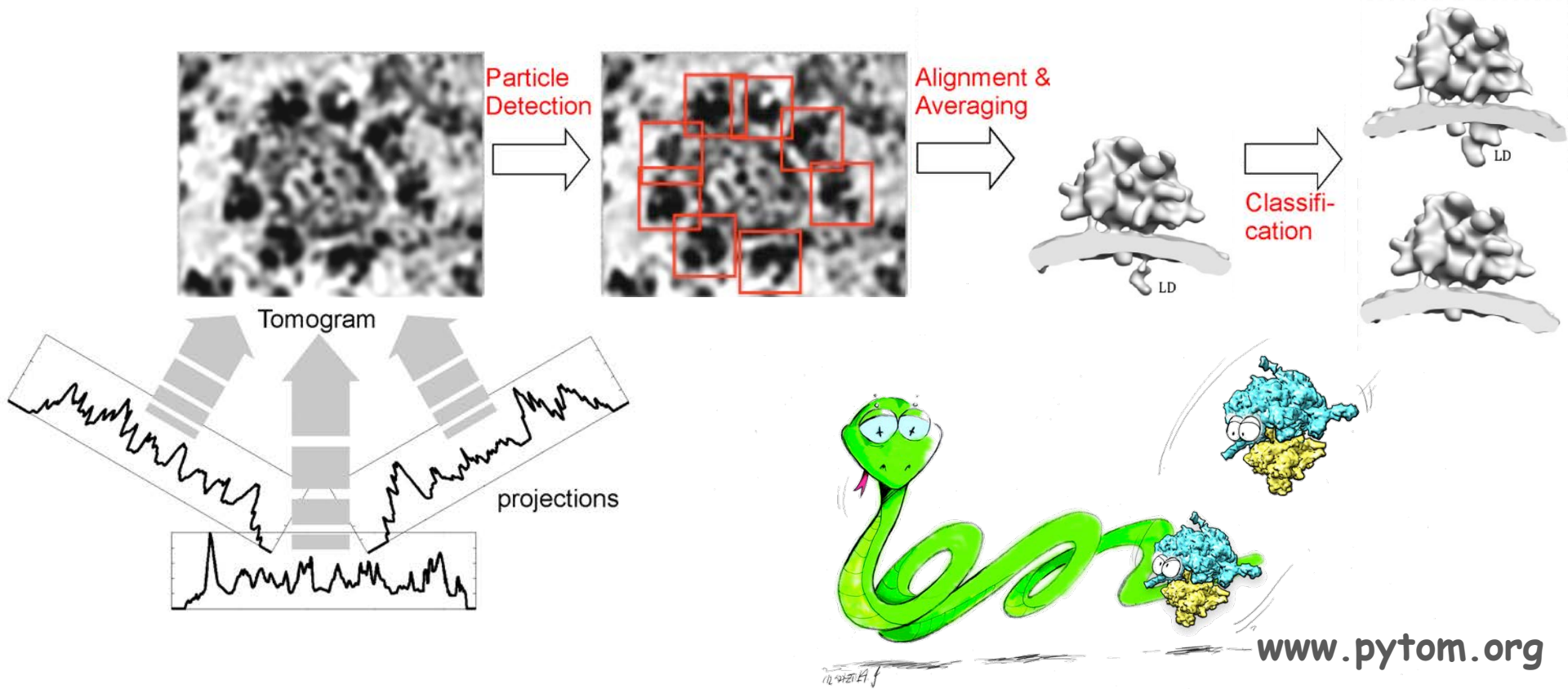
26S Proteasomes in intact neurons imaged by phase plate cryo-electron tomography



Asano S*, Fukuda Y*, Beck F, Aufderheide A, Förster F, Danev R, Baumeister W, *Science* 347:439-442 (2015)

Autofocused classification targets most variable regions of subtomograms



Hrabe T, Chen Y, Pfeffer S, Cuellar LK, Mangold AV & Förster F.. J. Struct. Biol. 178:177-88 (2012).

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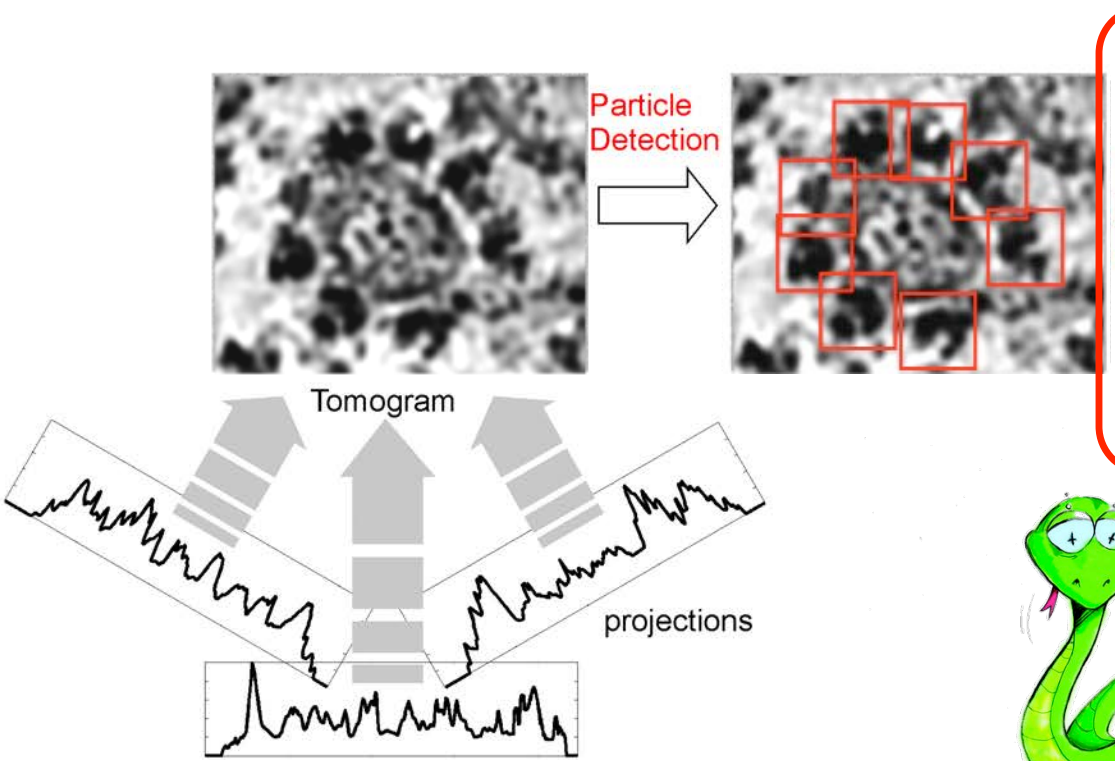
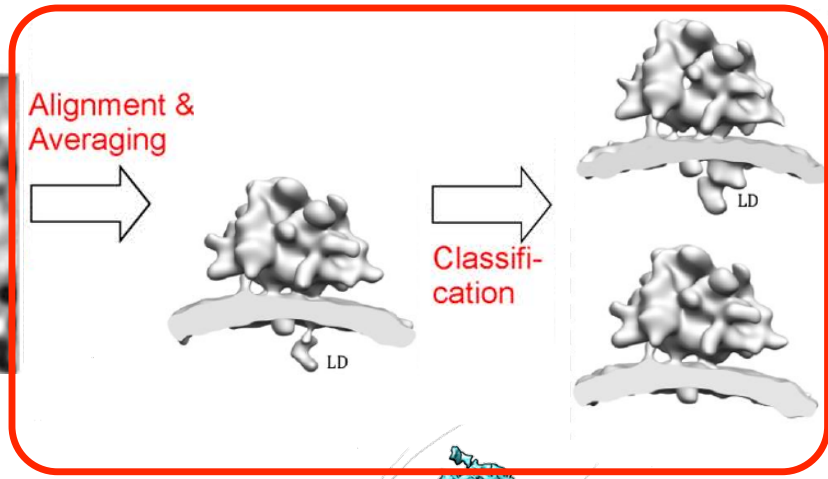
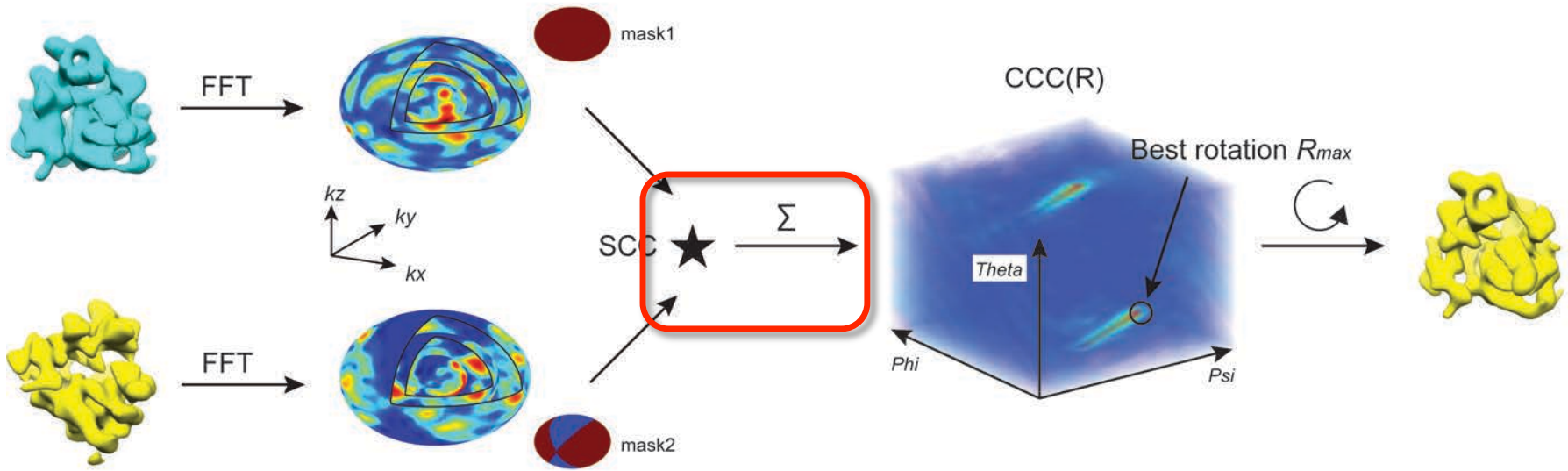




www.pytom.org

Chen Y, Pfeffer S, Fernandez JJ, Sorzano CO & Förster F, *Structure* 22:1528–37 (2014).

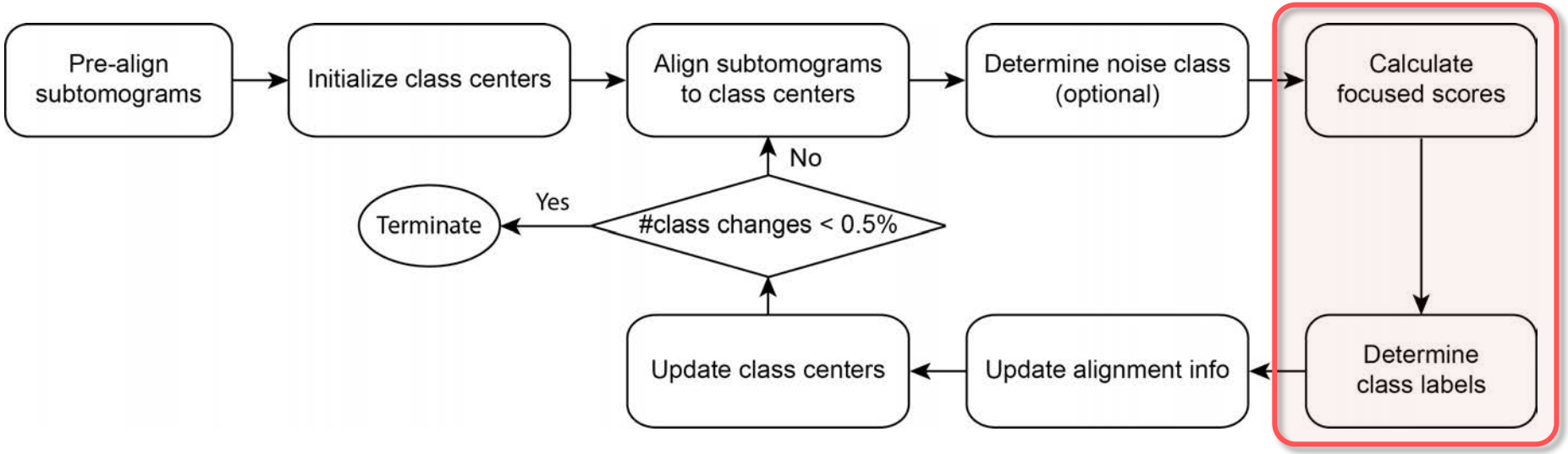
Fast Rotational Matching accelerates alignment by orders of magnitude



- Fourier transformed is expanded to spherical harmonics which allows fast rotational matching via generalized convolution theorem
- Common trick in X-ray crystallography (rotation function)

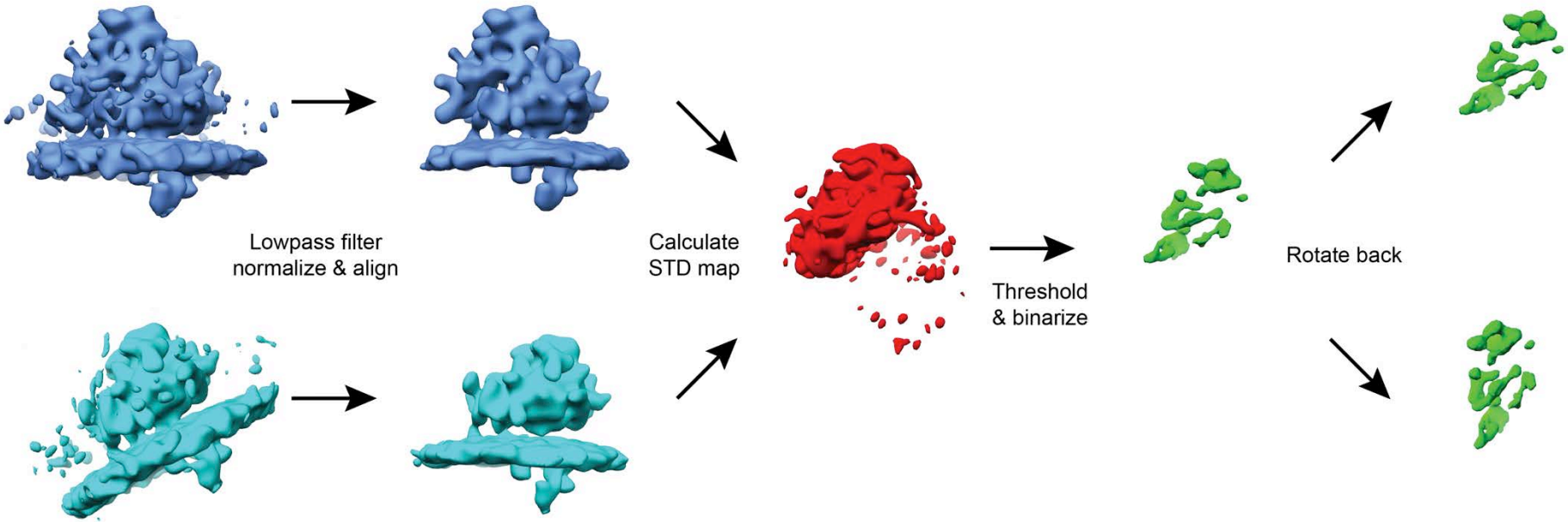
Chen Y, Pfeffer S, Hrabe T, Schuller JM, Förster F. *J. Struct. Biol.* 182:235-45 (2013).

Classification by iterative multi-reference alignment



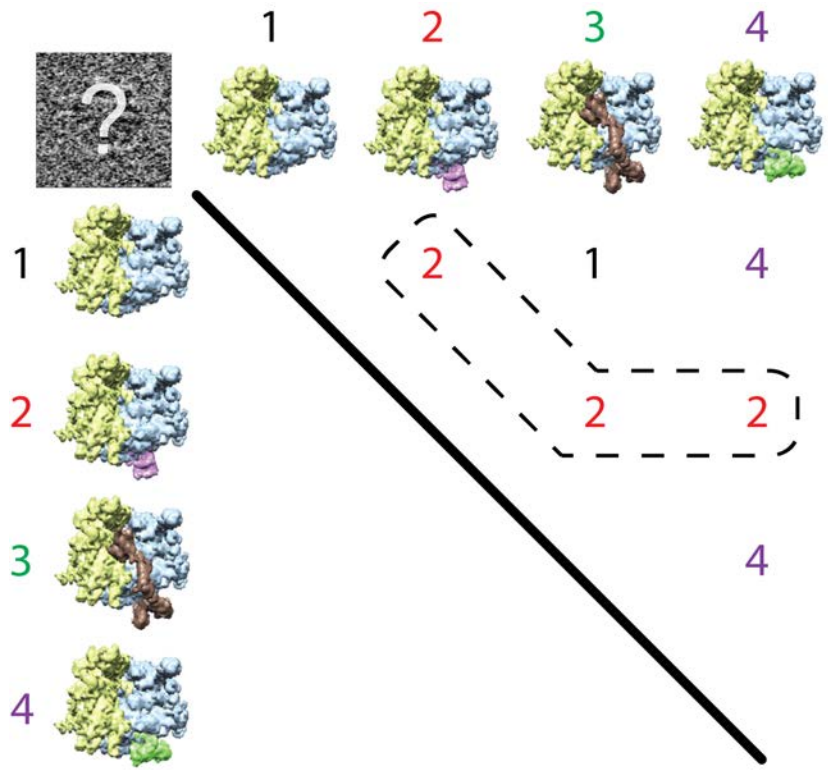
Chen Y, Pfeffer S, Fernandez JJ, Sorzano CO & Förster F, *Structure* 22:1528–37 (2014).

Determination of 3D mask for classification



Chen Y, Pfeffer S, Fernandez JJ, Sorzano CO & Förster F, *Structure* 22:1528–37 (2014).

Class assignment of particles by voting



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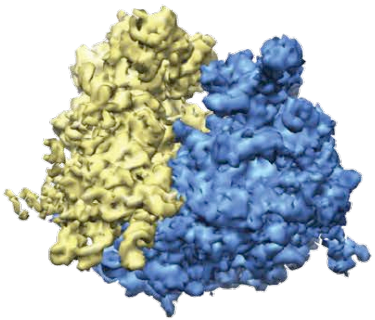
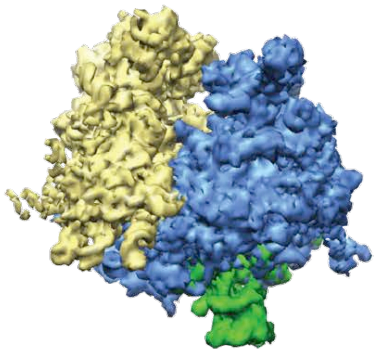
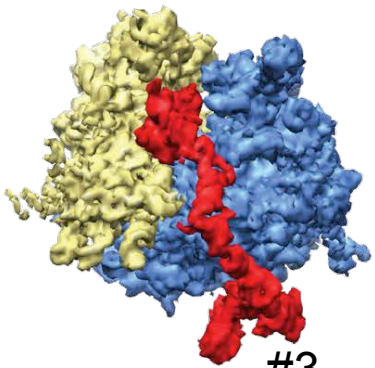
Application to simulated examples suggests superior classification performance



#1



#2



#3

noise

#0

CPCA			MLTOMO			AC3D		
	TPR	FPR		TPR	FPR		TPR	FPR
#0	100%	10%	#0	100%	1%	#0	93%	2.3%
#1	50.7%	22.4%	#1	70.7%	38.4%	#1	83.3%	2.8%
#2	36%	19.7%	#2	31%	19%	#2	98%	7%
#3	86%	0%	#3	14%	0%	#3	98%	0%

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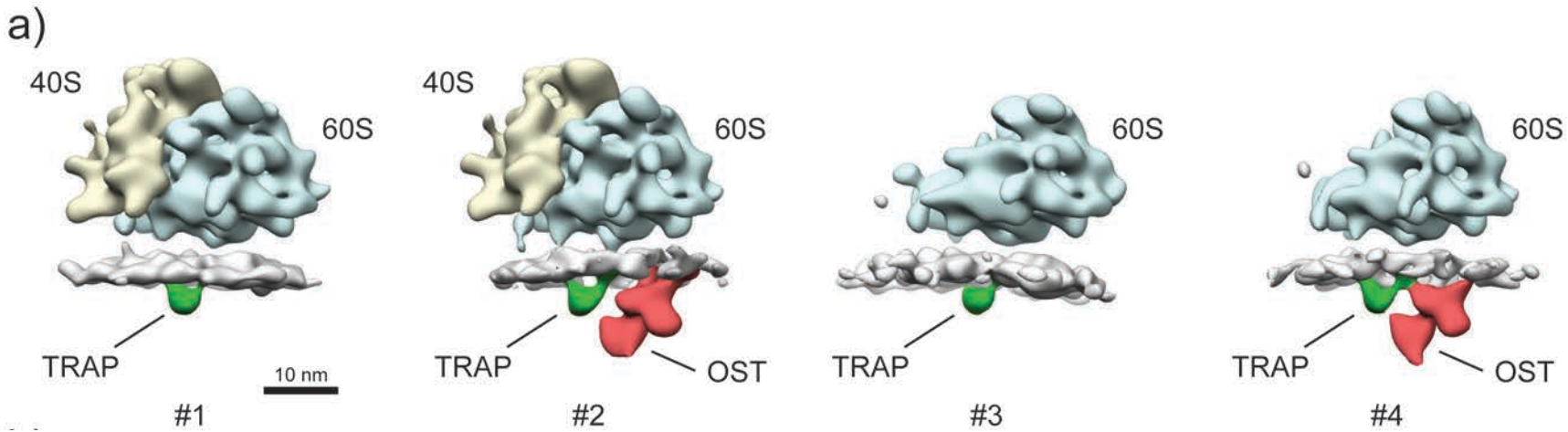
Autofocused classification reveals different Ribosome-Translocon States *in situ*



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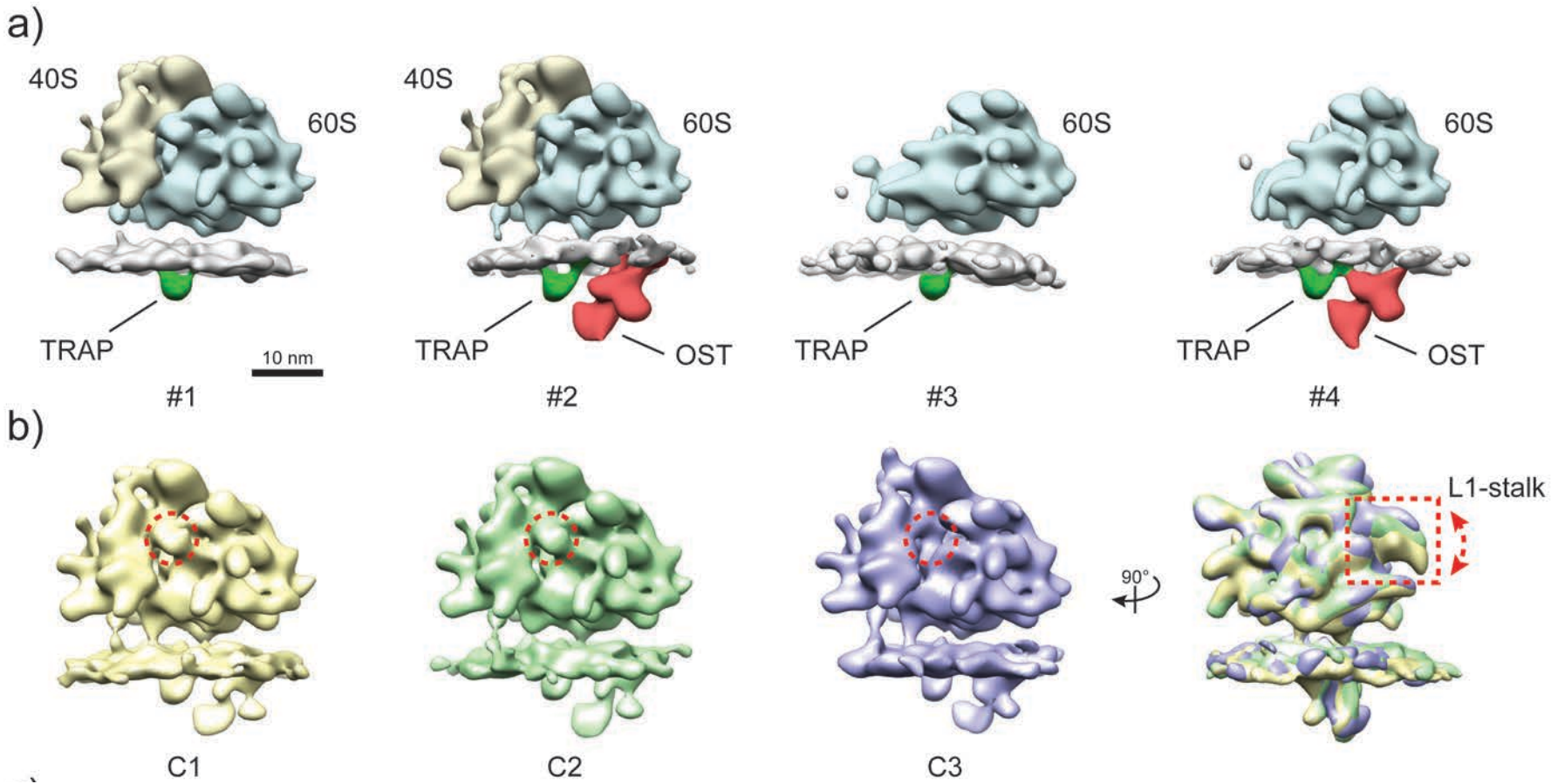

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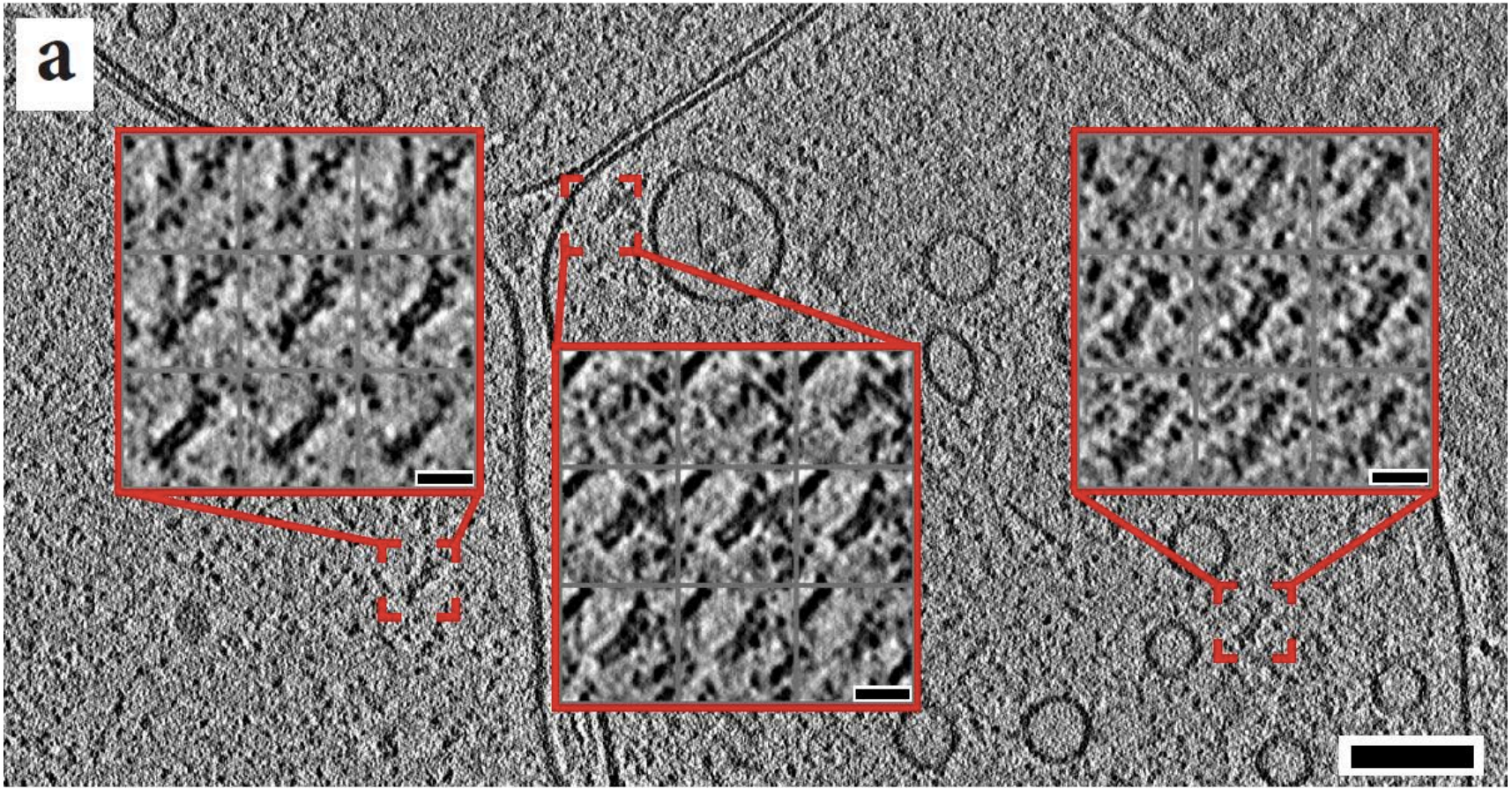
26S Proteasomes in intact neurons imaged by phase plate cryo-electron tomography



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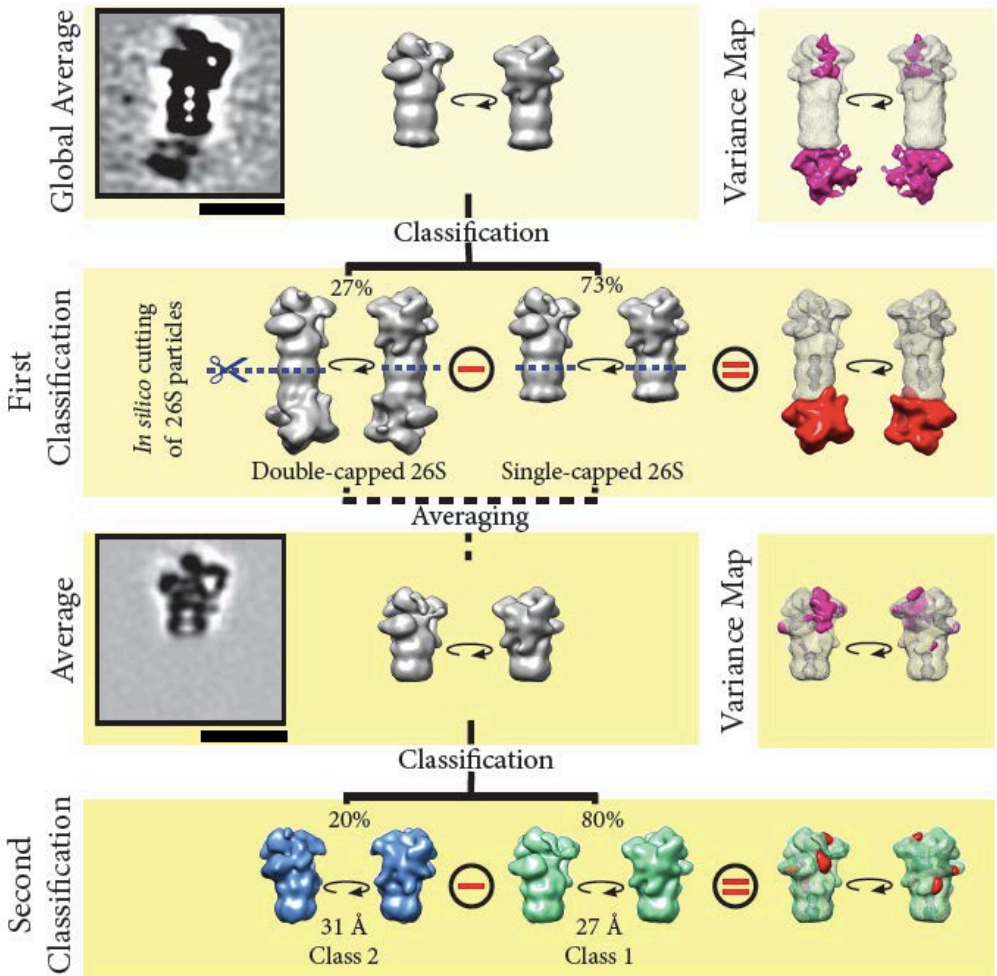
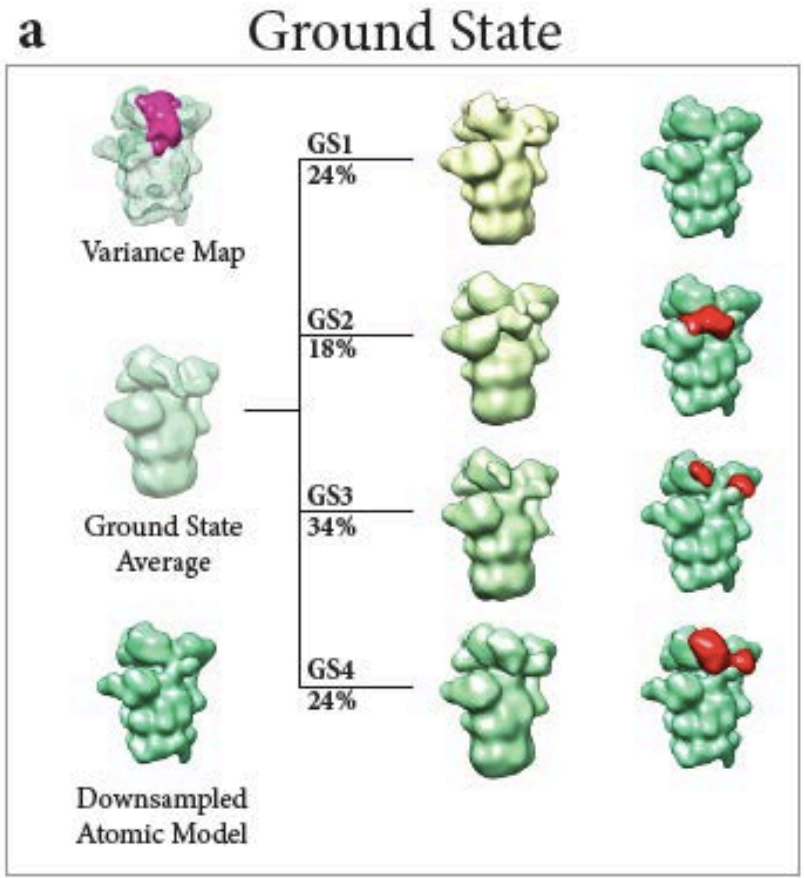
Classification of 26S proteasome conformation *in vivo* by AC3D





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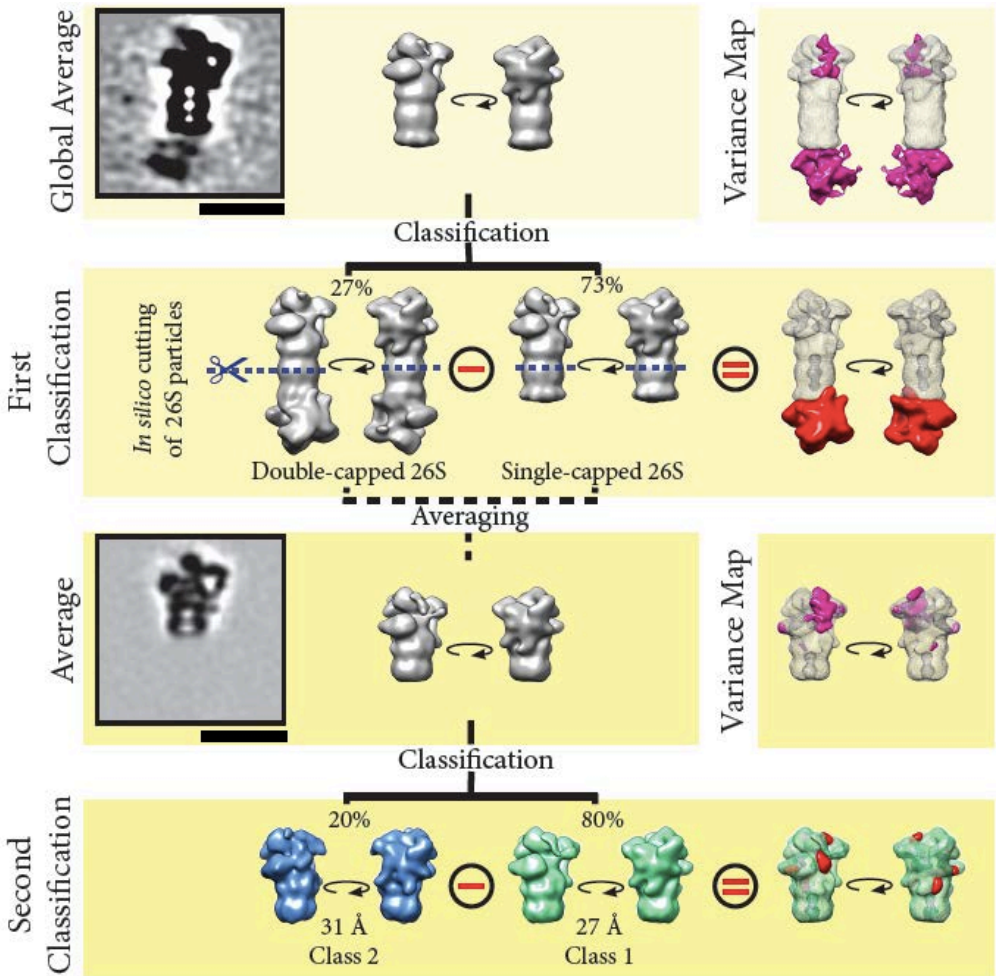
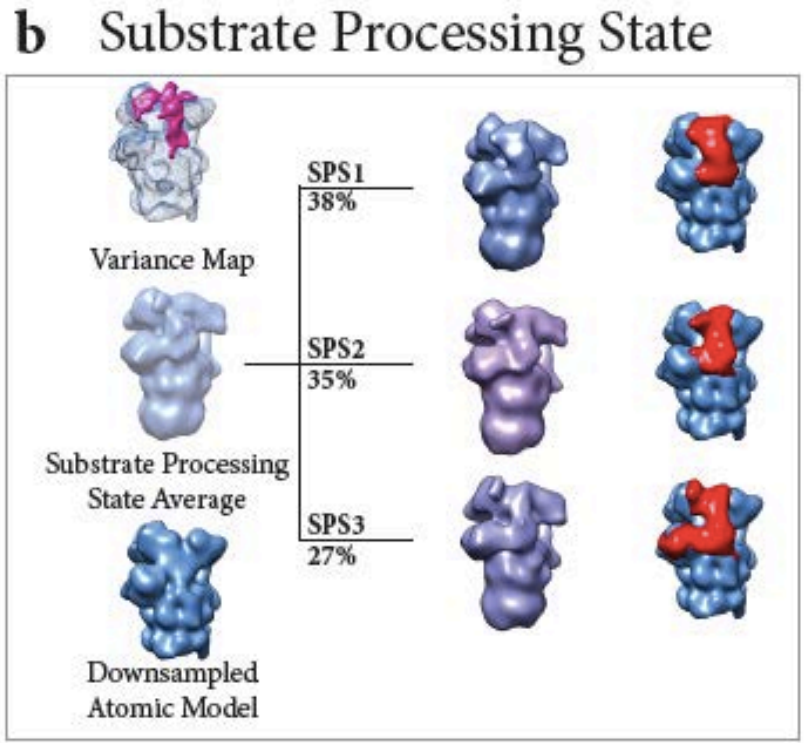
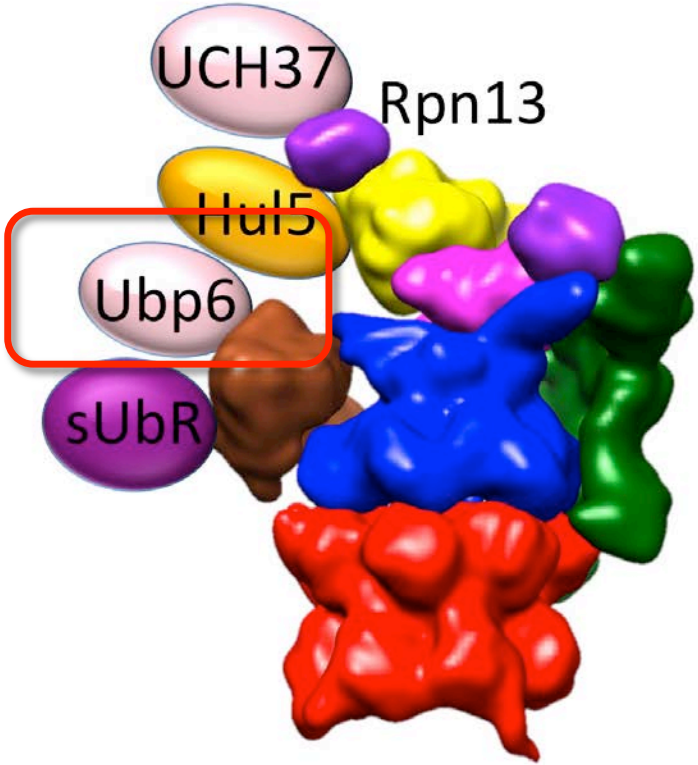


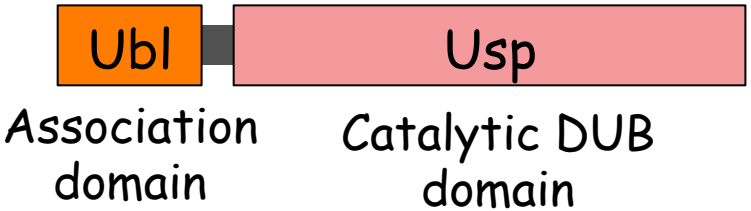


Asano S*, Fukuda Y*, Beck F, Aufderheide A, Förster F, Danev R, Baumeister W, *Science* 347:439-442 (2015)

26S proteasome associates with cofactors in the cell

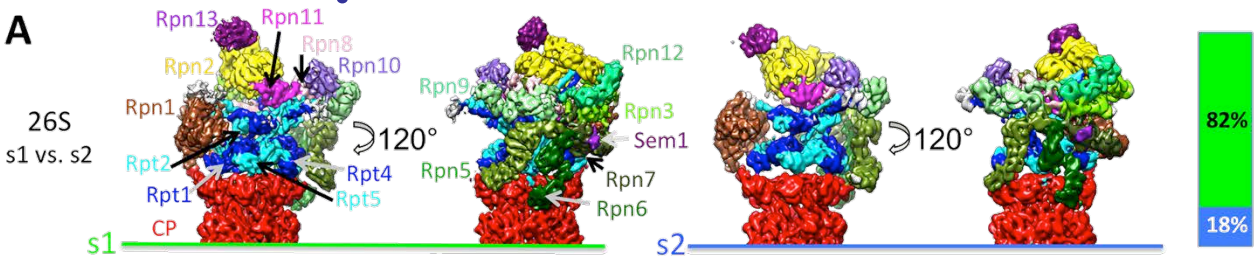


Deubiquitylating enzyme Ubp6



Förster F, Schuller JM, Unverdorben P & Aufderheide A. Biomolecules 4:774-794 (2014).

Ubp6-Ub alters conformation of 26S proteasomes



Aufderheide A, Beck F, Stengel F, Hartwig M, Schweitzer A, Pfeifer G, Goldberg AL, Sakata E, Baumeister W, Förster F. *Proc. Natl. Acad. Sci. USA* 112, 8626-8631 (2015).

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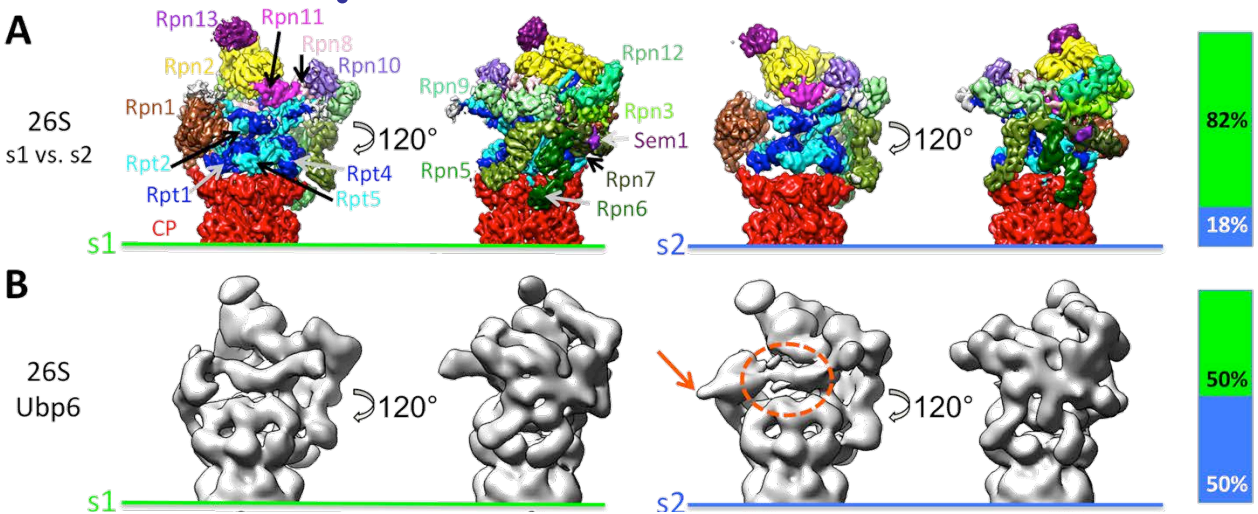

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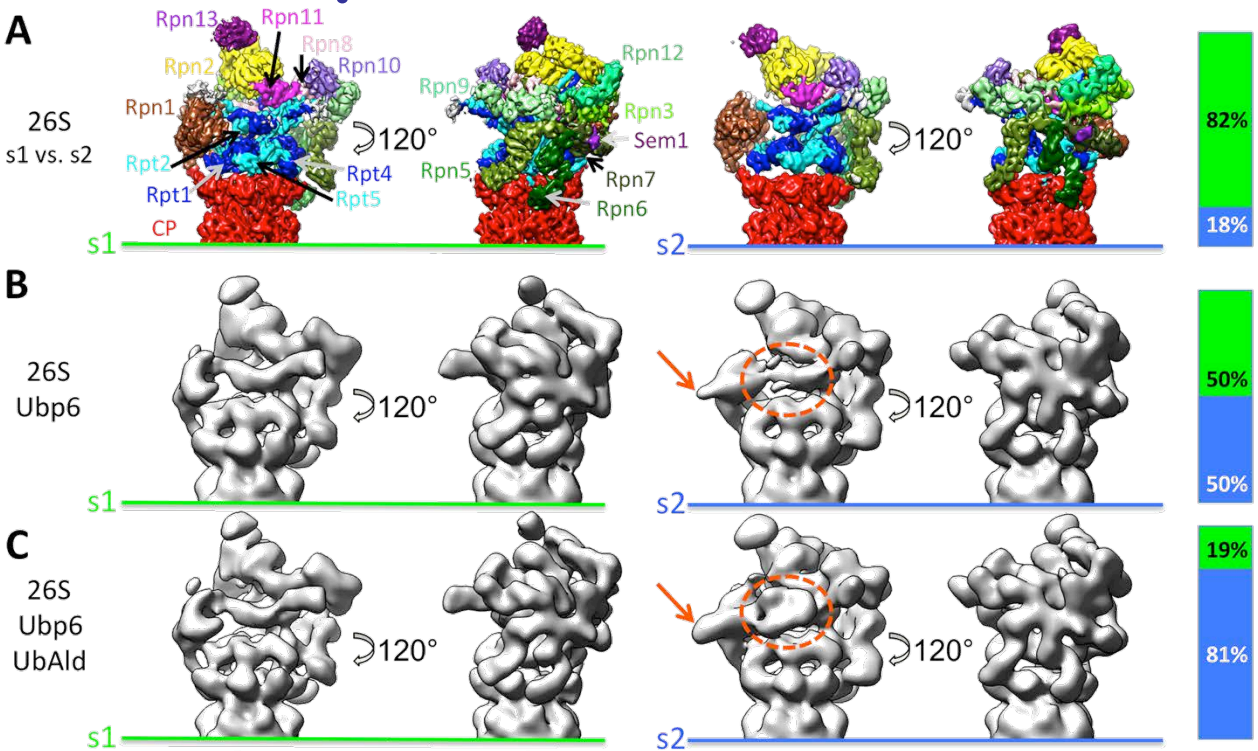

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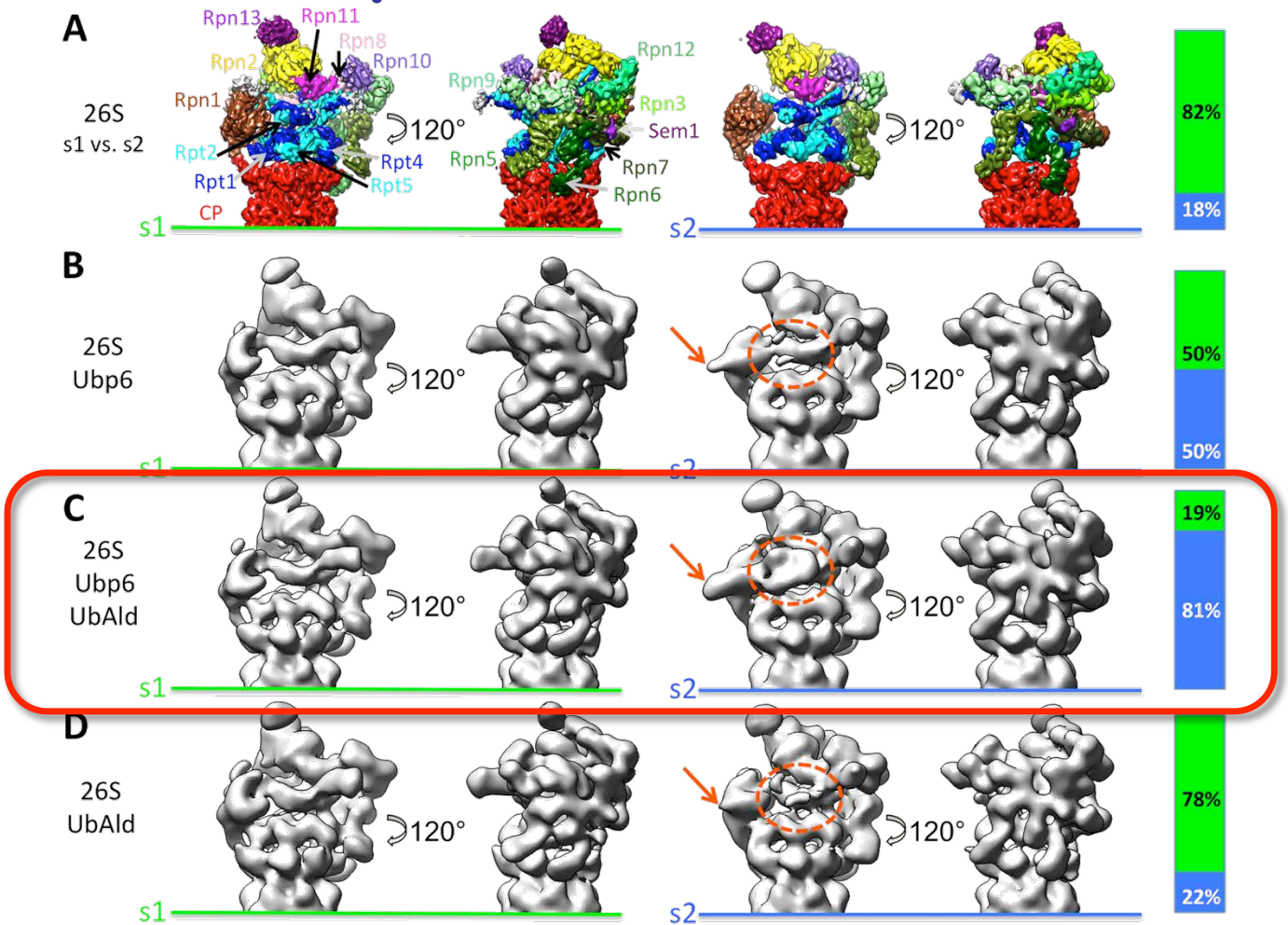
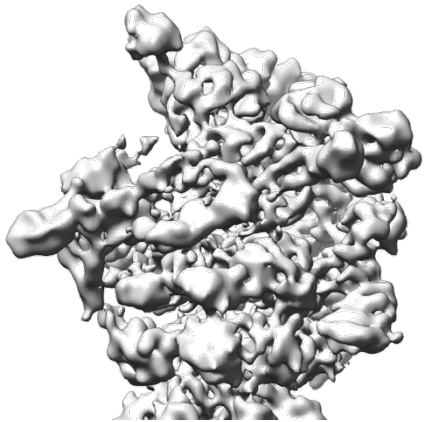
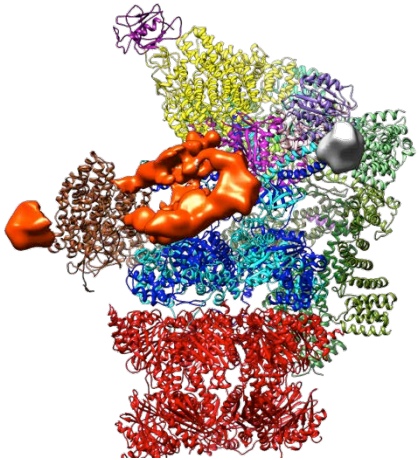
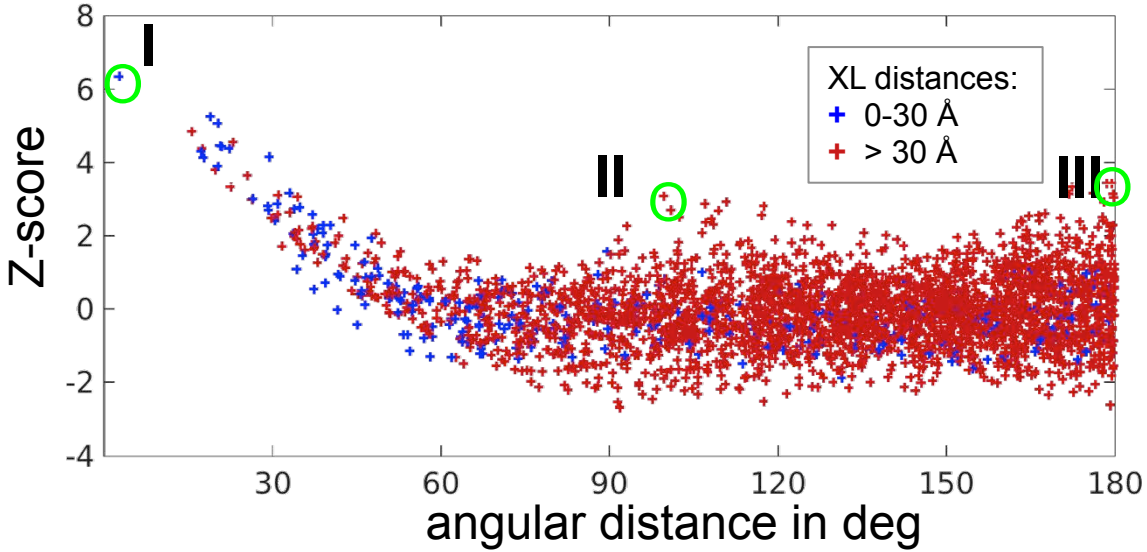
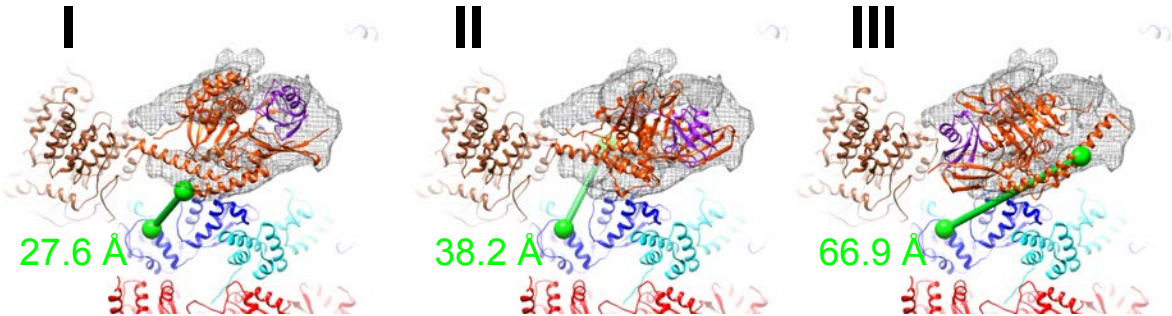


Usp domain can be fitted unambiguously into EM map









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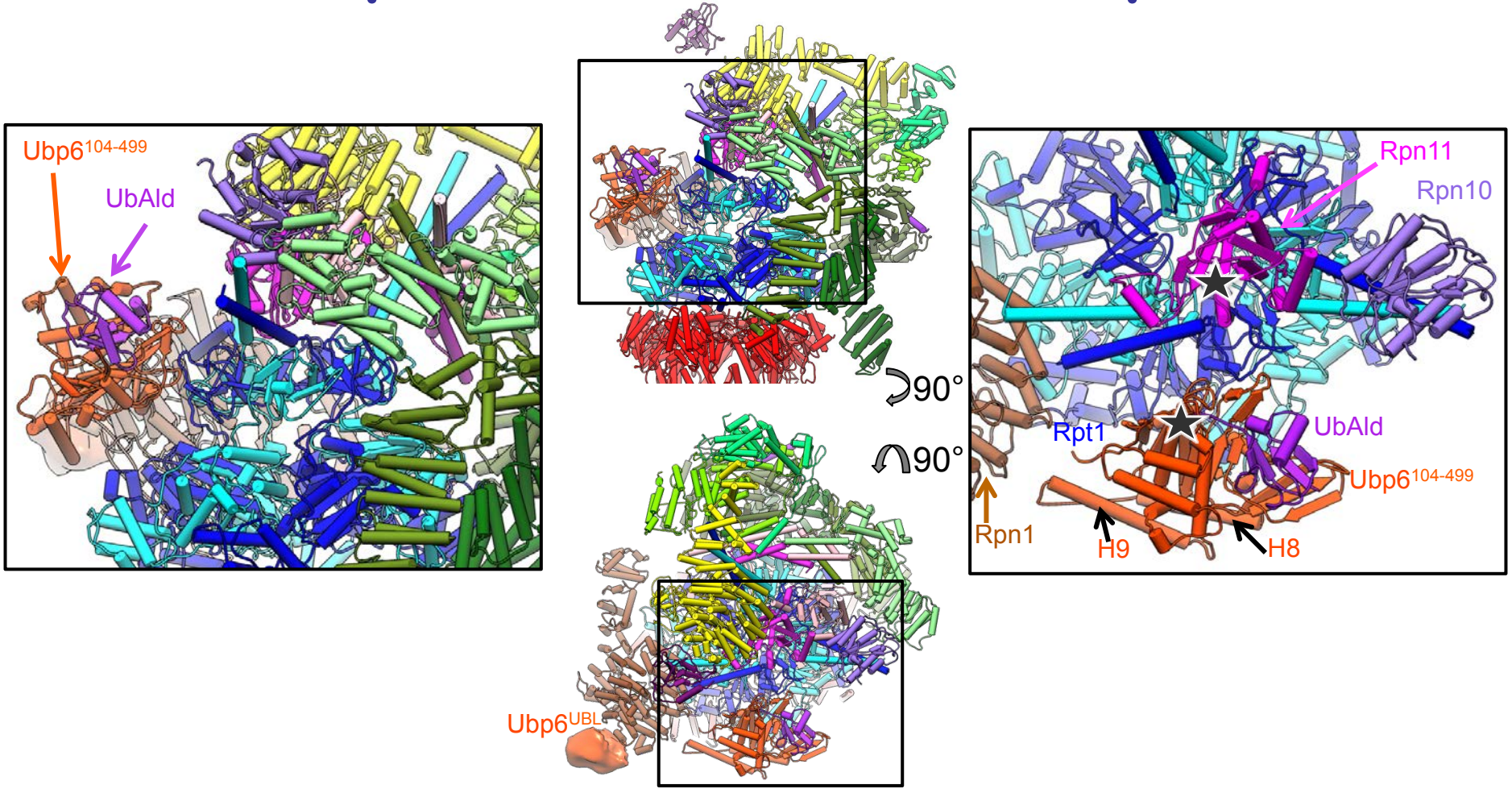
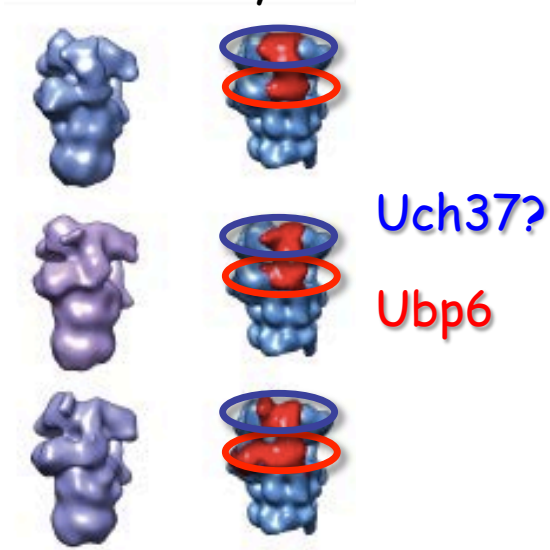
Ubp6 resides in proximity to Ub receptor Rpn10 and DUB Rpn11



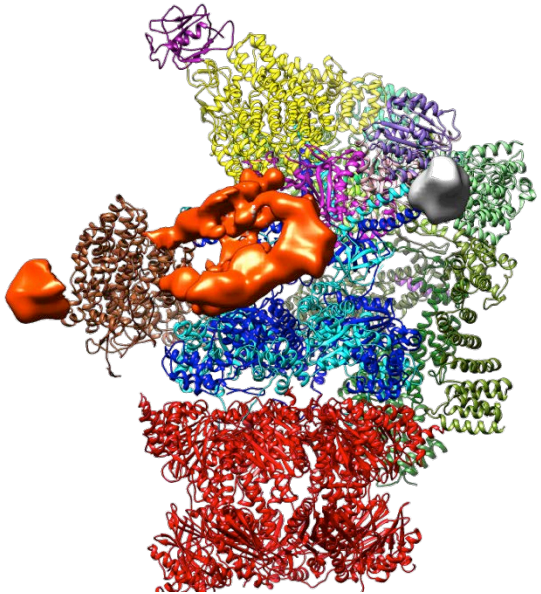
DUBs sandwich substrates and coordinate their degradation

In situ cryo-ET



Substrate-processing state

In vitro SPA

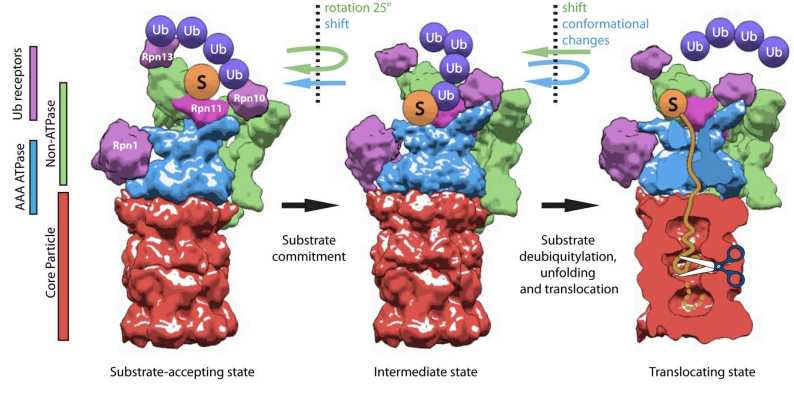


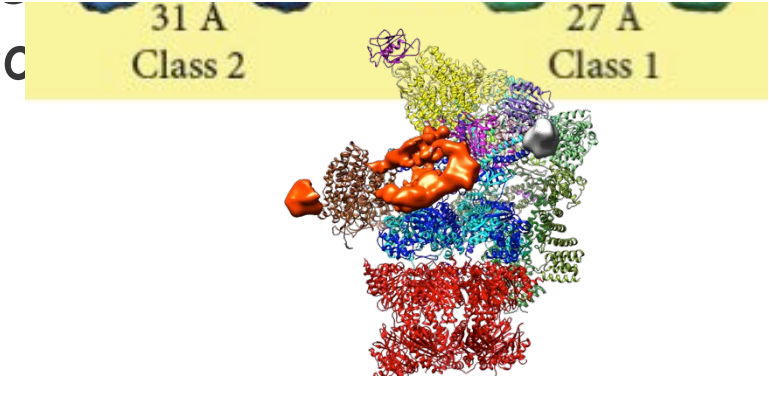
Summary

- Single particle classification reveals 3 major conformational states of the 26S proteasome *in vitro*

- AC3D reveals 2 distinct 26S c
its supramolecular interaction

- Single particle analysis identif
involved in activation of 26S pro





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Yuxiang Chen
Pia Unverdorben
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Florian Beck
Shoh Asano
Yoshiyuki Fukuda
Wolfgang Baumeister

LMU

Markus Pech
Stephan Wickles
Roland Beckmann

Universität des Saarlandes

Johanna Dudek
Richard Zimmermann

Technische Universität Kaiserslautern

Michael Wöllhaf
Johannes Herrmann

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Hudson Freeze

DFG Deutsche Forschungsgemeinschaft