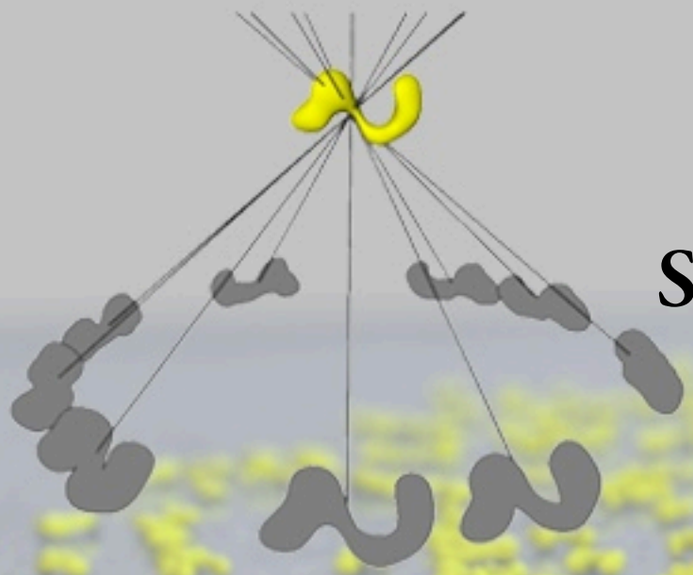


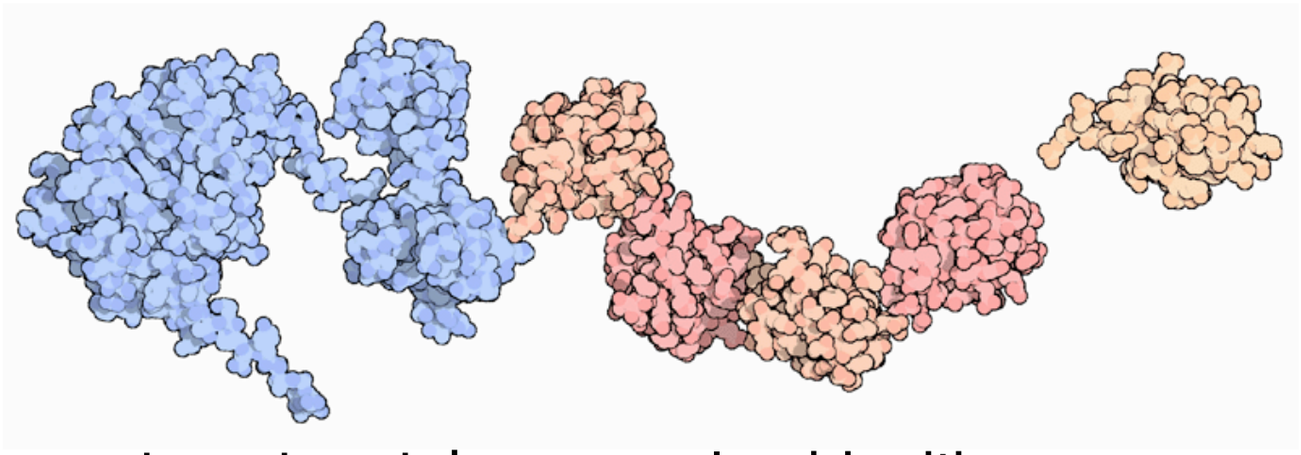
Dmitry Lyumkis

National Resource for Automated Molecular Microscopy

Single-Particle EM Reveals Large-Scale Conformational Variability of the Ltn1 E3 Ligase

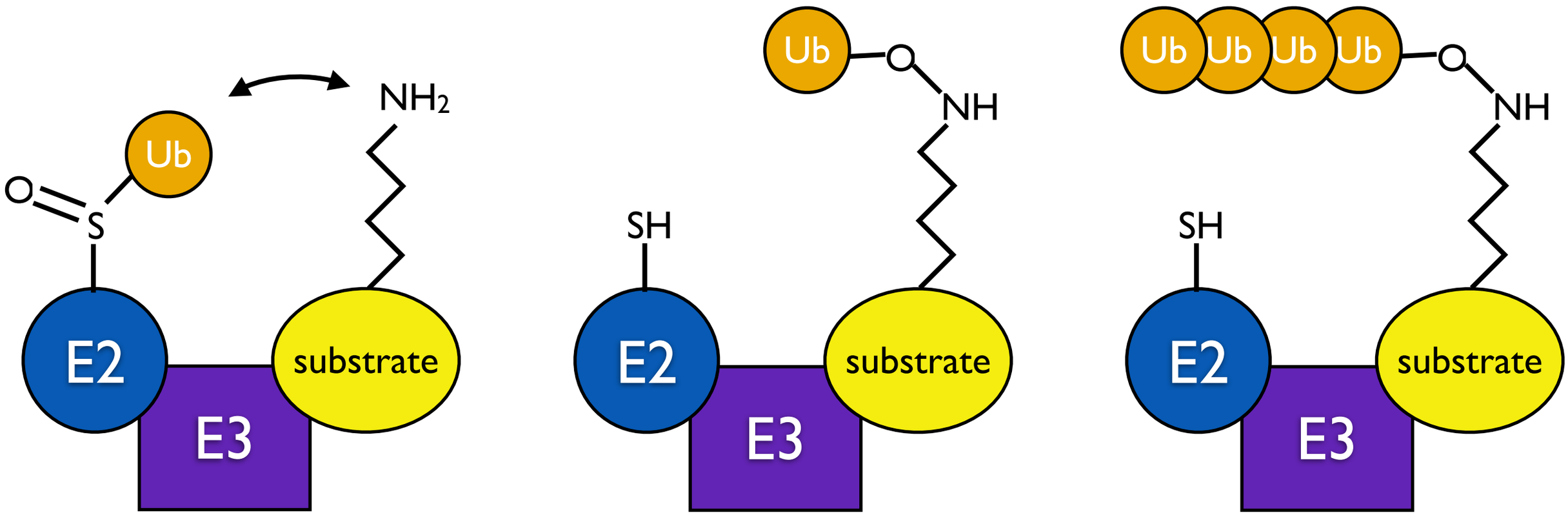
NRAMM cryo-EM workshop
November 14, 2012

E3 ubiquitin ligases



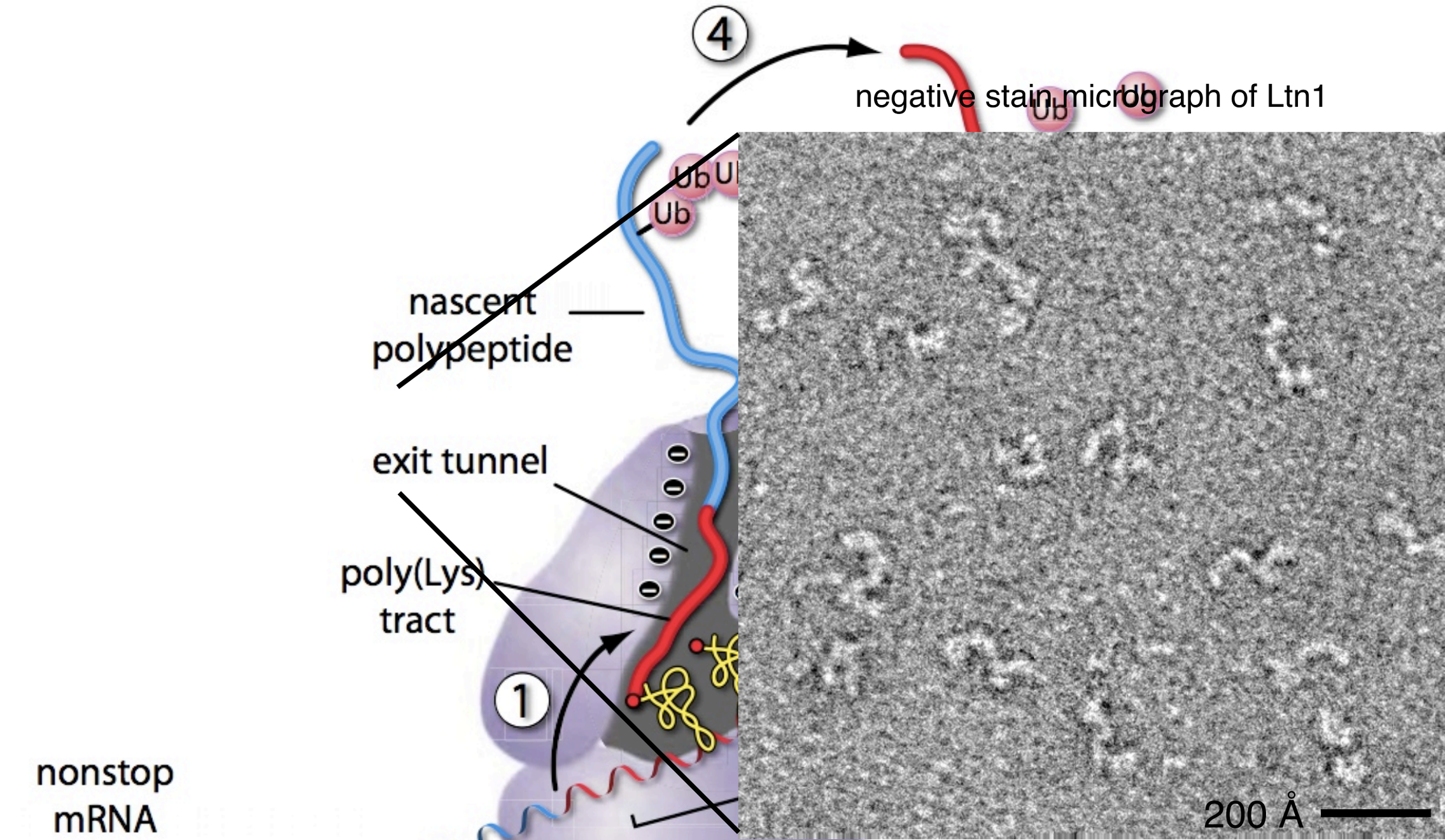
target protein poly-ubiquitin

1. proteasome-mediated protein degradation
2. progression of the cell cycle
3. apoptosis
4. DNA transcription and repair
5. ... and virtually every process in the cell



Komander, D. and M. Rape (2012). "The ubiquitin code." Annu Rev Biochem **81**: 203-229

Ltn1 is a RING-E3 ubiquitin ligase that is responsible for protein quality control in eukaryotes

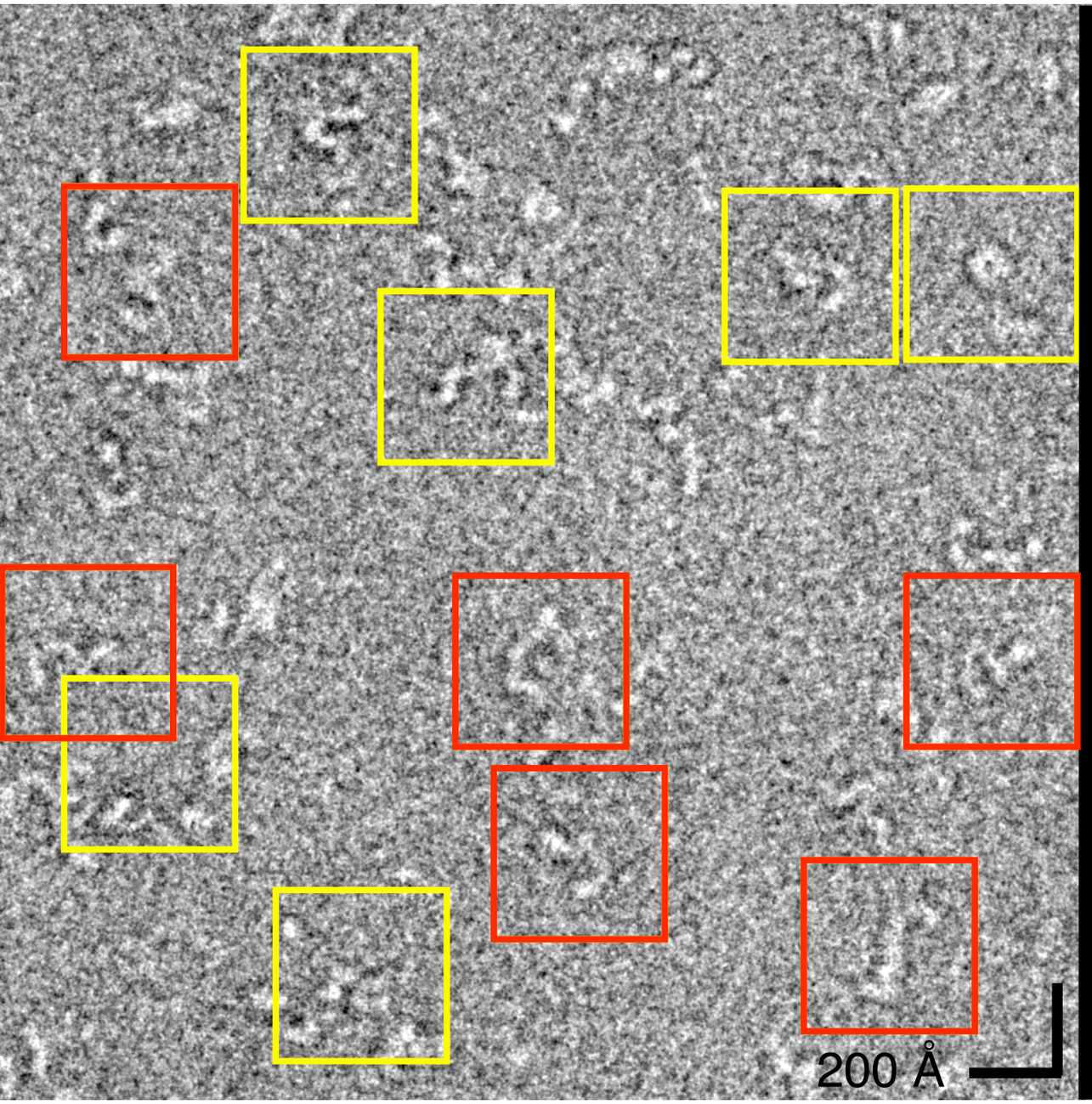


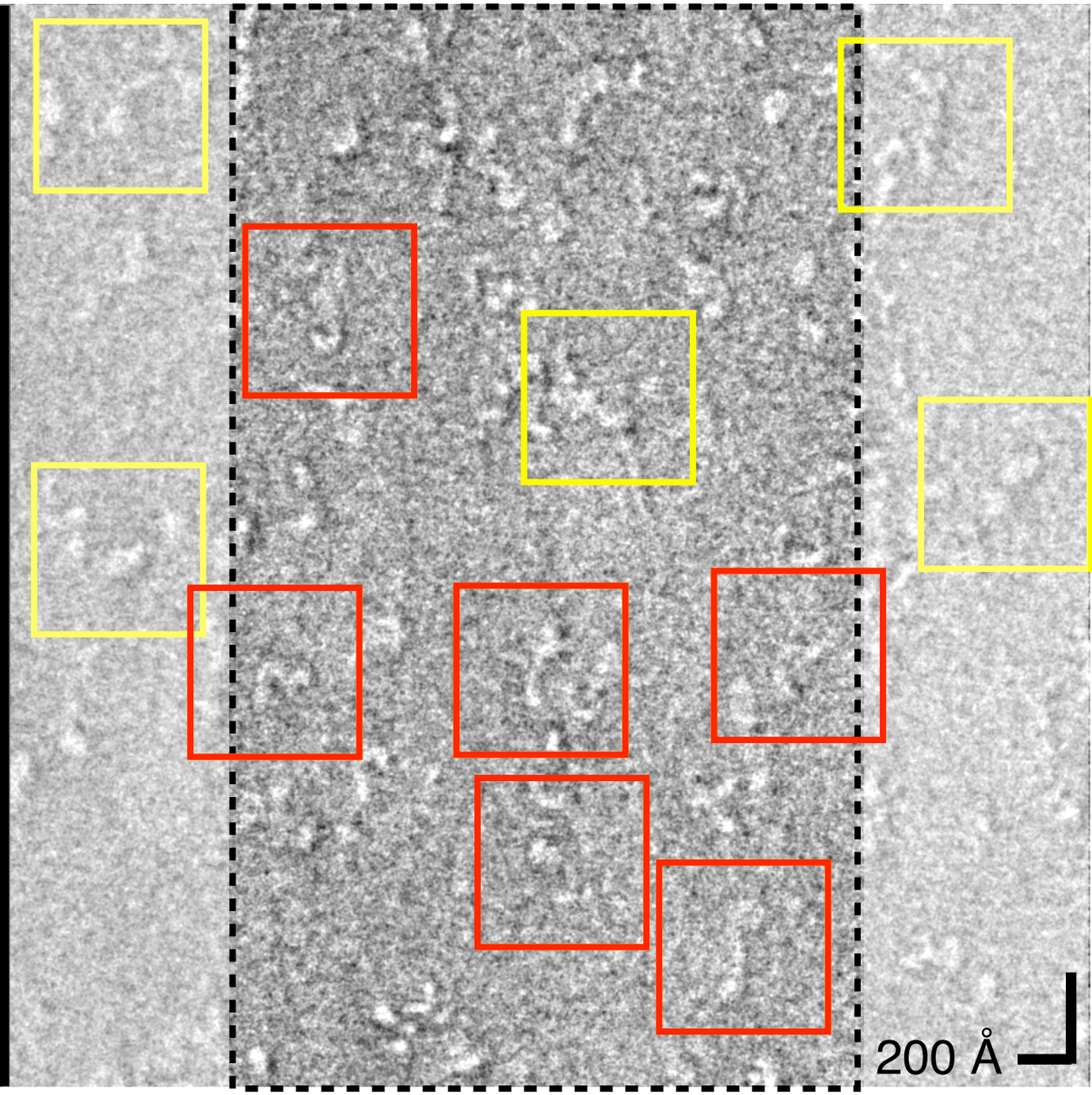
Bengtson, M. H. & Joazeiro, C. A. *Nature* **467**, 470-473 (2010)

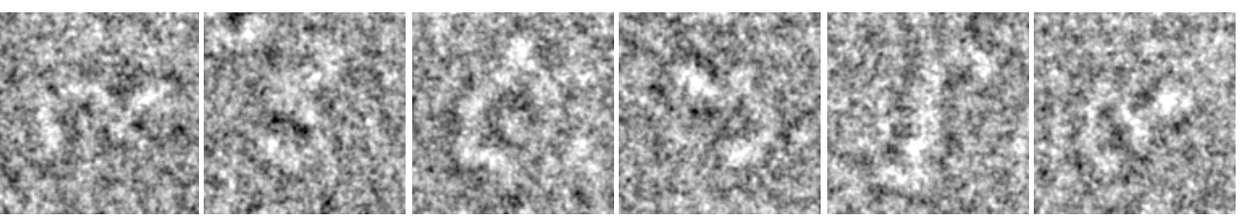
data collection and particle extraction

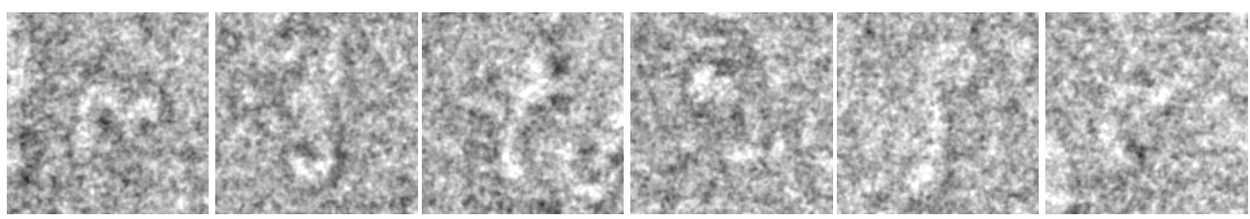
untilted micrograph region (0°, 2um def, 2% UF)

tilted micrograph region (55°, 2um def, 2% UF)









alignment, classification, & 3D reconstruction

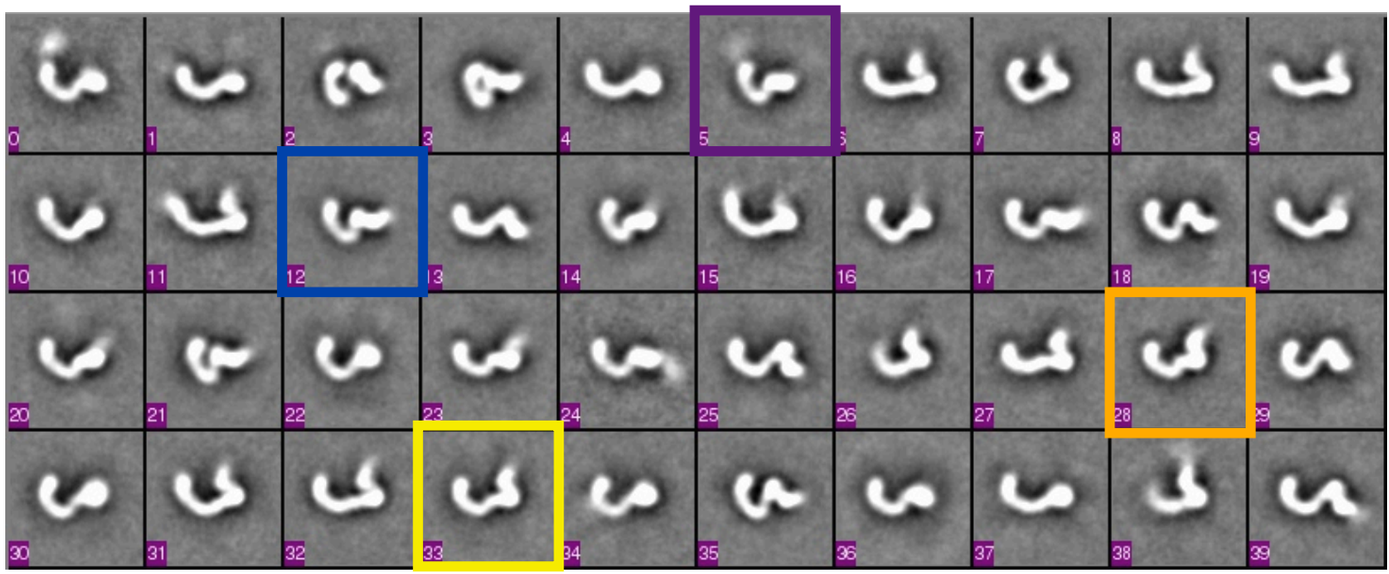
1. reference-free / reference-based alignment & classification

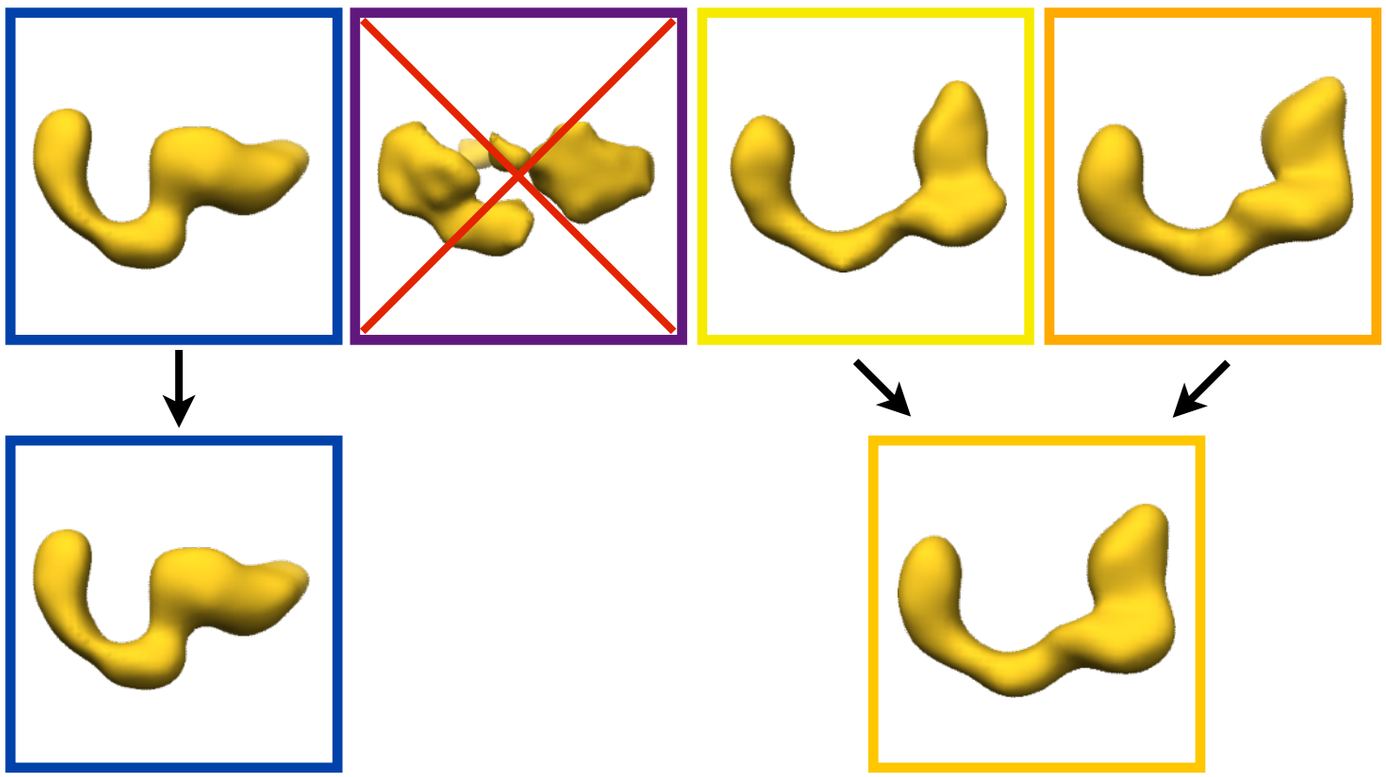
iterative

2. RCT reconstruction from tilted particles corresponding to **each** untilted class

3. combine reconstructions from similar volumes (different is >10° change in terminus rotation)

4. if RCT volume is adequate, remove reconstructed particles from data & repeat 1-3

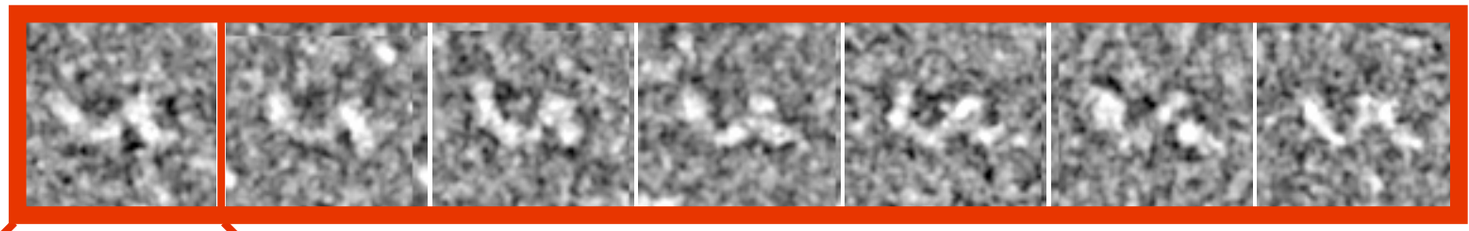




final volume: conformation 1

final volume: conformation 2

automated RCT infrastructure in Appion



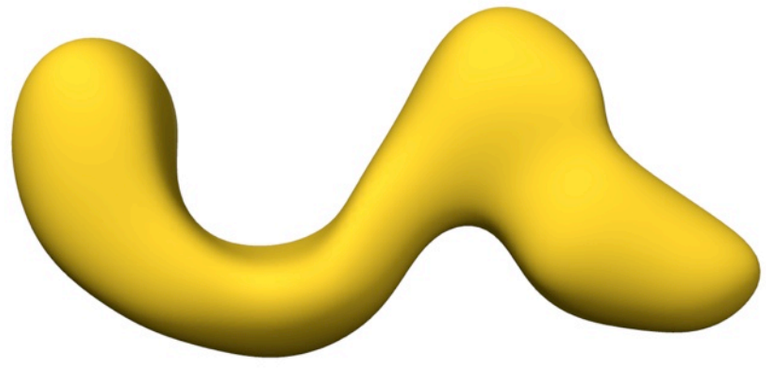
database record
class correspondence

database records

- original particle in stack
- alignment parameters (rot, shx, shy, mirror)
- class correspondence

1. tilted particle counterpart
2. tilted micrograph
3. tilt angle

iterative translational refinement using established Euler angles



RCT volume

automated RCT results

QuickTime Player File Edit View Share Window Help — Stop Recording — (84%) — Sat 5:50 PM — Administrator

RCT Volume Summary

image viewer | RCT Volu... | RCT Run Re... | makeDDRaw... | Particle Sele... | Hierarchical ... | Stack Report | Hierarchical ... | Hierarchical ... | http:/...10.hed

cronus3.scripps.edu/betamyamiweb/processing/rctsummary.php?expId=8589

Most Visited | Getting Started | Latest Headlines | database | Bookmarks

Appion

RCT Volume Summary Page

logged in as ***dlyumkis*** [Log Out]

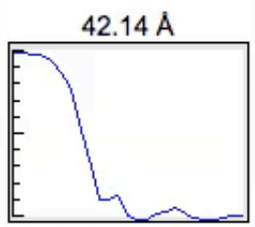
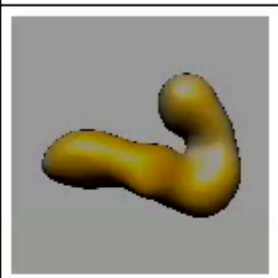
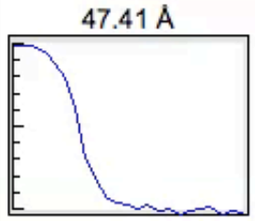
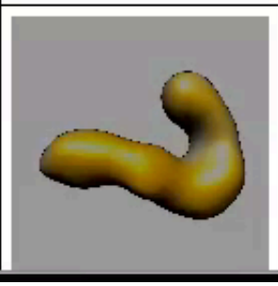
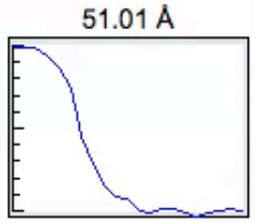
Project: Joazeiro - E3 ligase (224)
Session: **11mar23d** - data collection: free Listerin from Chris Lima, grid B1 from 11mar10c
Image Path: /ami/data15/leginon/11mar23d/rawdata

[Appion Stats]
[User Guide]

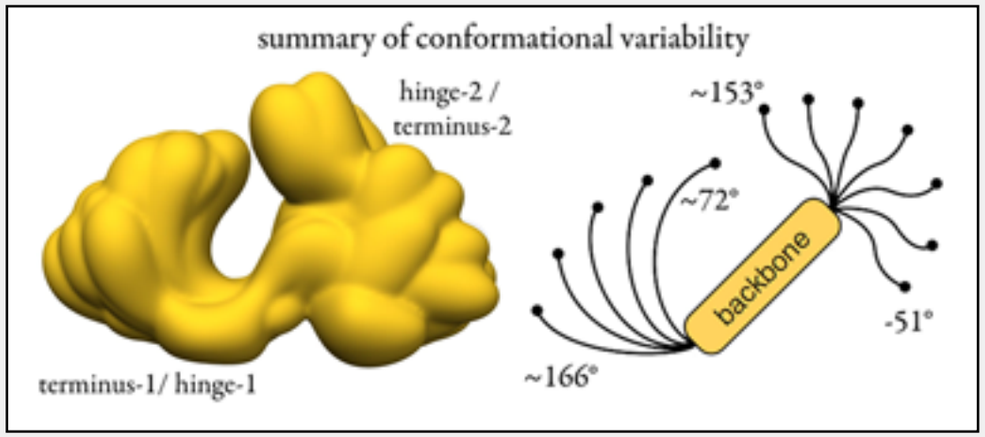
Hide | Expand | Contract

- **Object Selection : 8**
- Template Picking
  - 1 complete
- DoG Picking
  - 1 complete
- Signature
- Manual Picking
- Object Tracing
- Repeat from other session
- Align and Edit Tilt Pairs
- Auto Align Tilt Pairs
  - 4 complete
- **CTF Estimation**
- Estimate the CTF
  - 1 complete
- Repeat from other session
- **Stacks : 20**
- Stack creation
  - 20 complete
- more stack tools
- **Particle Alignment**
- Run Alignment
  - 32 complete
- Run Feature Analysis
  - 15 complete

[Show 196 hidden rct runs]

defid	name	image	num part	pixel size	box size	fsc res	rmeasure	description
648 hide	rct1clust309class192833		1,028 of 66,718	2.18 Å	160	 42.14 Å	18.59 Å	class refinement: combining classes 19 (rct264), 28 (rct273) & 33 (rct278) for increasing particle numbers in heatmap edit
505 hide	rct500clust463class245		828 of 66,718	2.18 Å	160	 47.41 Å	36.34 Å	sub-classification: tightly folded particles (refbased19), 10 averages, class 2, 4, & 5 edit
504 hide	rct499clust463class24		552 of 66,718	2.18 Å	160	 51.01 Å	16.45 Å	sub-classification: tightly folded particles (refbased19), 10 averages, class 2 & 4 edit

Ltn1 shows extensive conformational variability about two hinge regions



row merges

hinge 1: terminus-1 flexing

column merges

200 Å

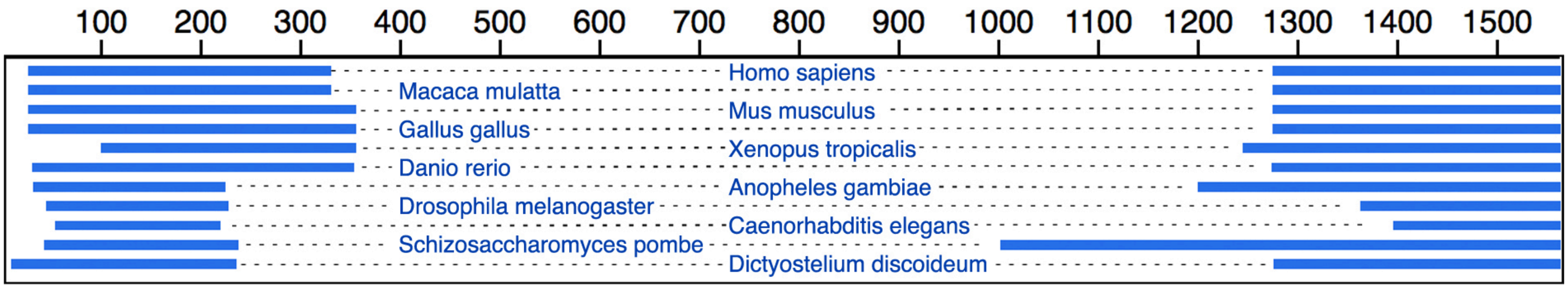
hinge 2: terminus-2 rotation

Ltn1 contains 2 conserved termini

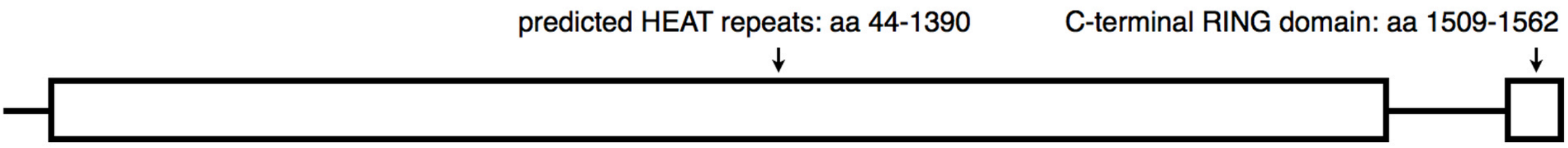
RING E3s are responsible for two functions:

(1) substrate recognition
(2) recruitment of E2-Ub conjugate

sequence conservation between orthologs

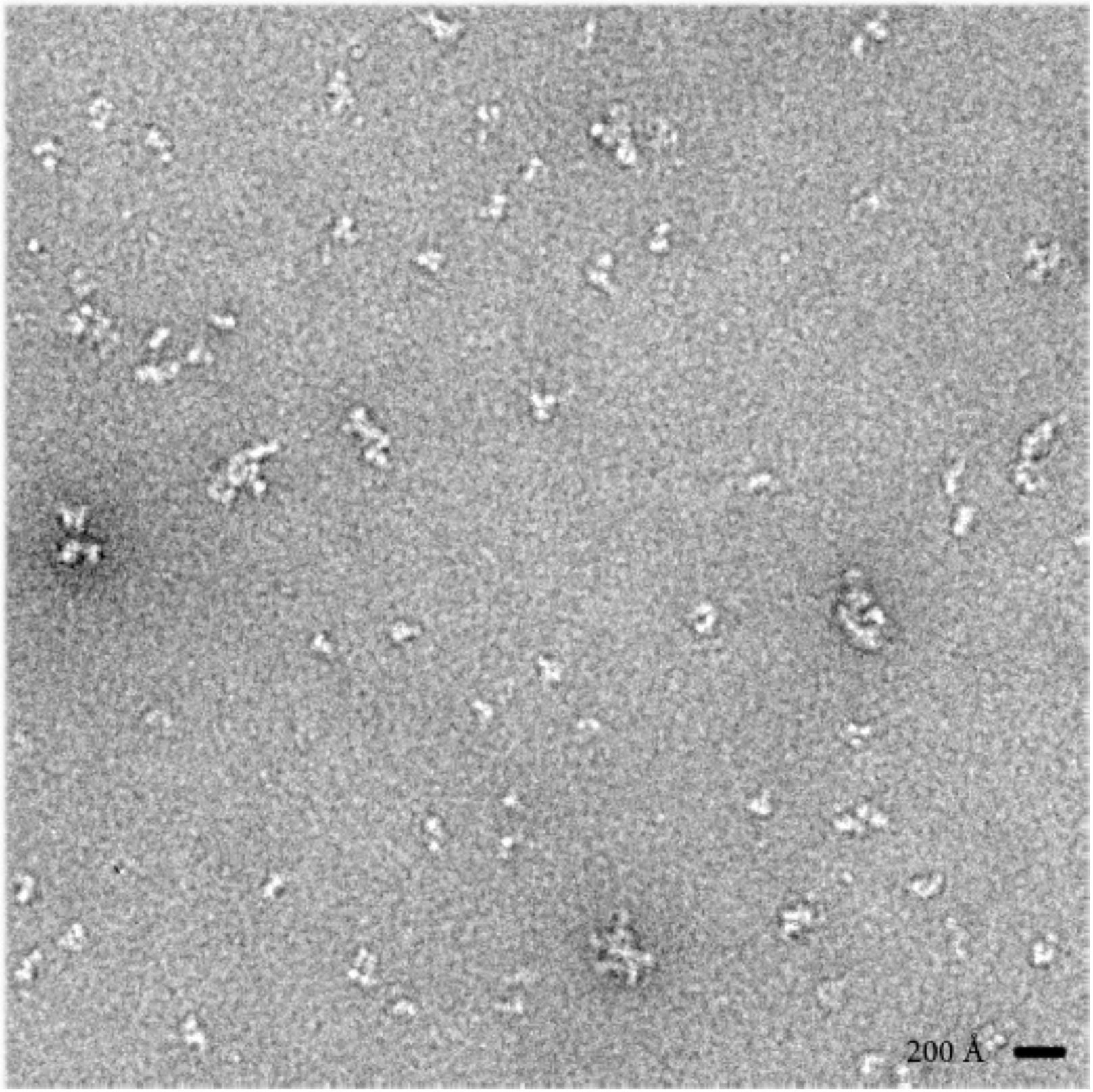


domain analysis



... so we made a truncated protein to orient the termini

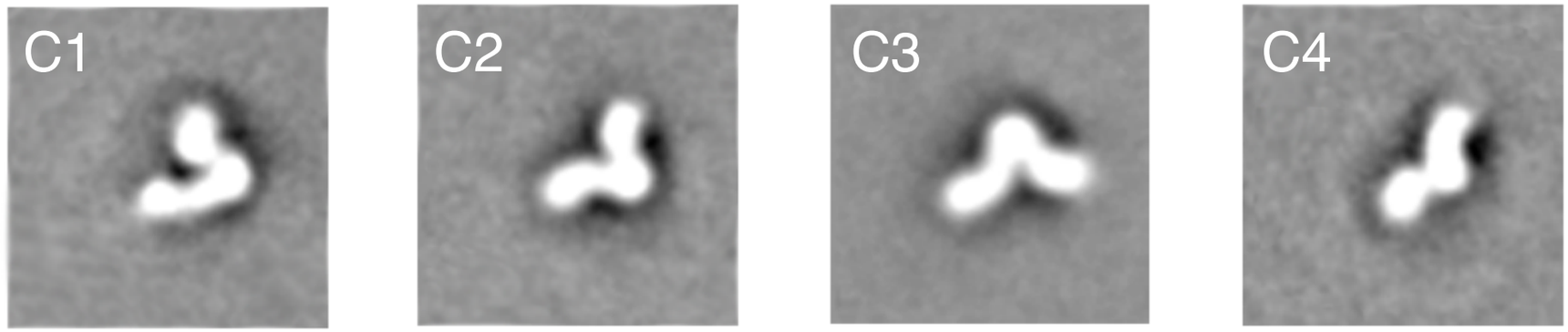
micrograph portion of ΔN-Ltn1 (amino acids 477-1562)



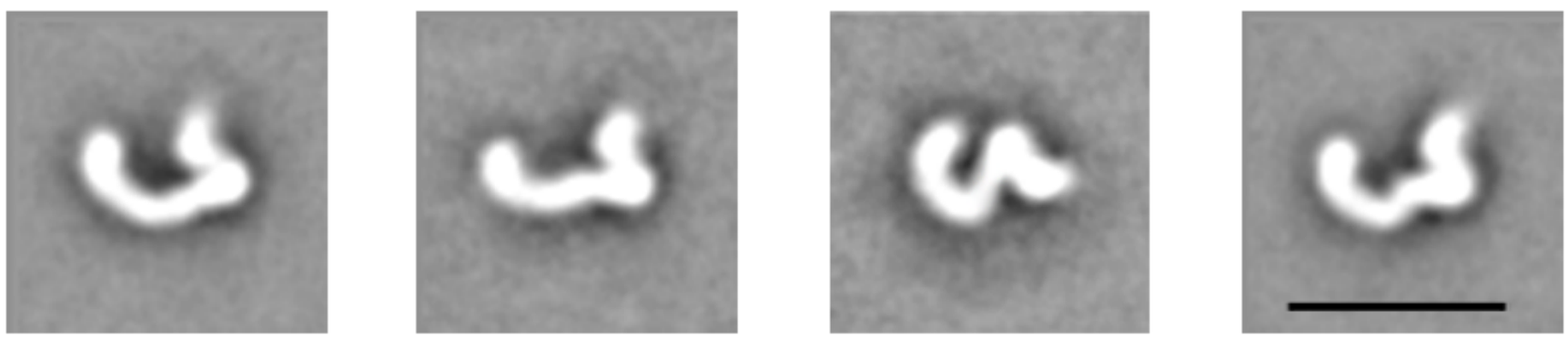
Orientation of Ltn1's N and C termini

RING E3s are responsible for two functions: (1) substrate recognition, (2) recruitment of E2-Ub conjugate

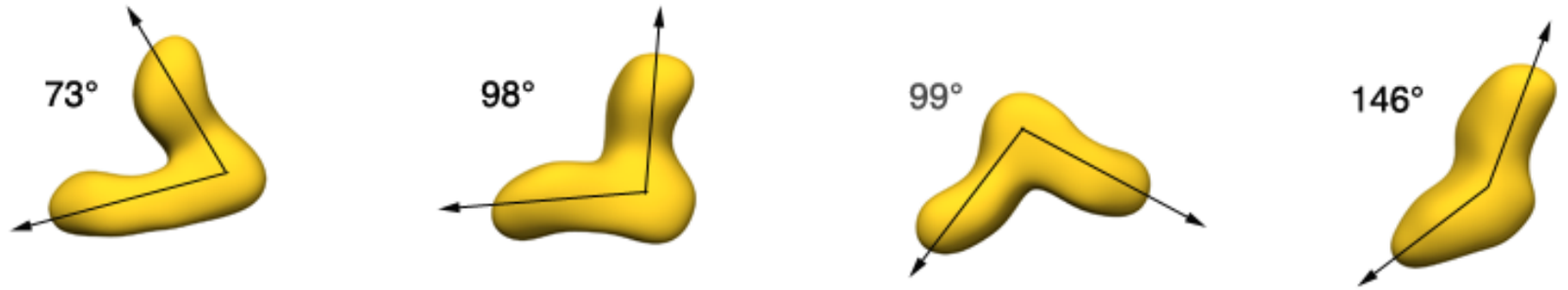
ΔN-Ltn1 Class Average
(amino acids 477-1562)



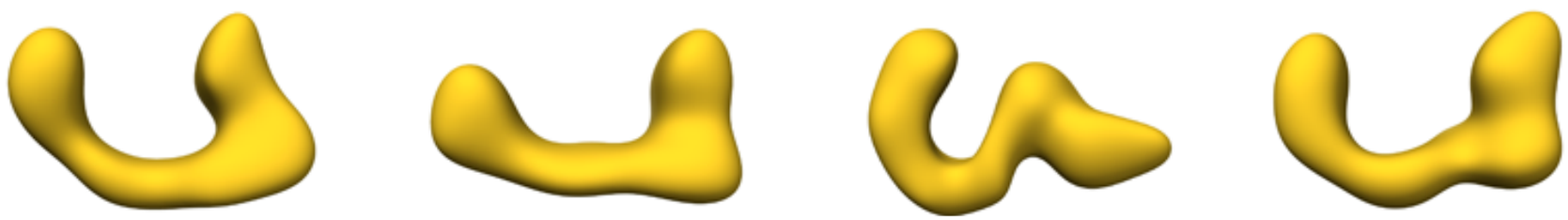
Ltn1 Class Average
(amino acids 1-1562)



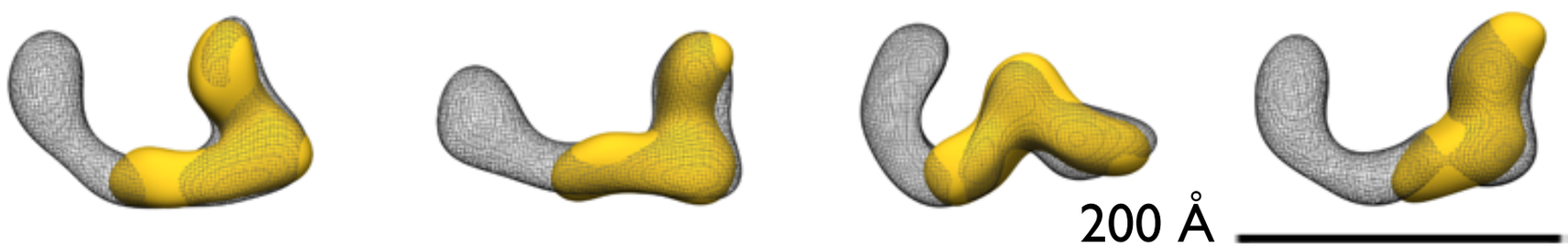
ΔN-Ltn1 3D reconstruction
(amino acids 477-1562)



Ltn1 3D reconstruction
(amino acids 477-1562)

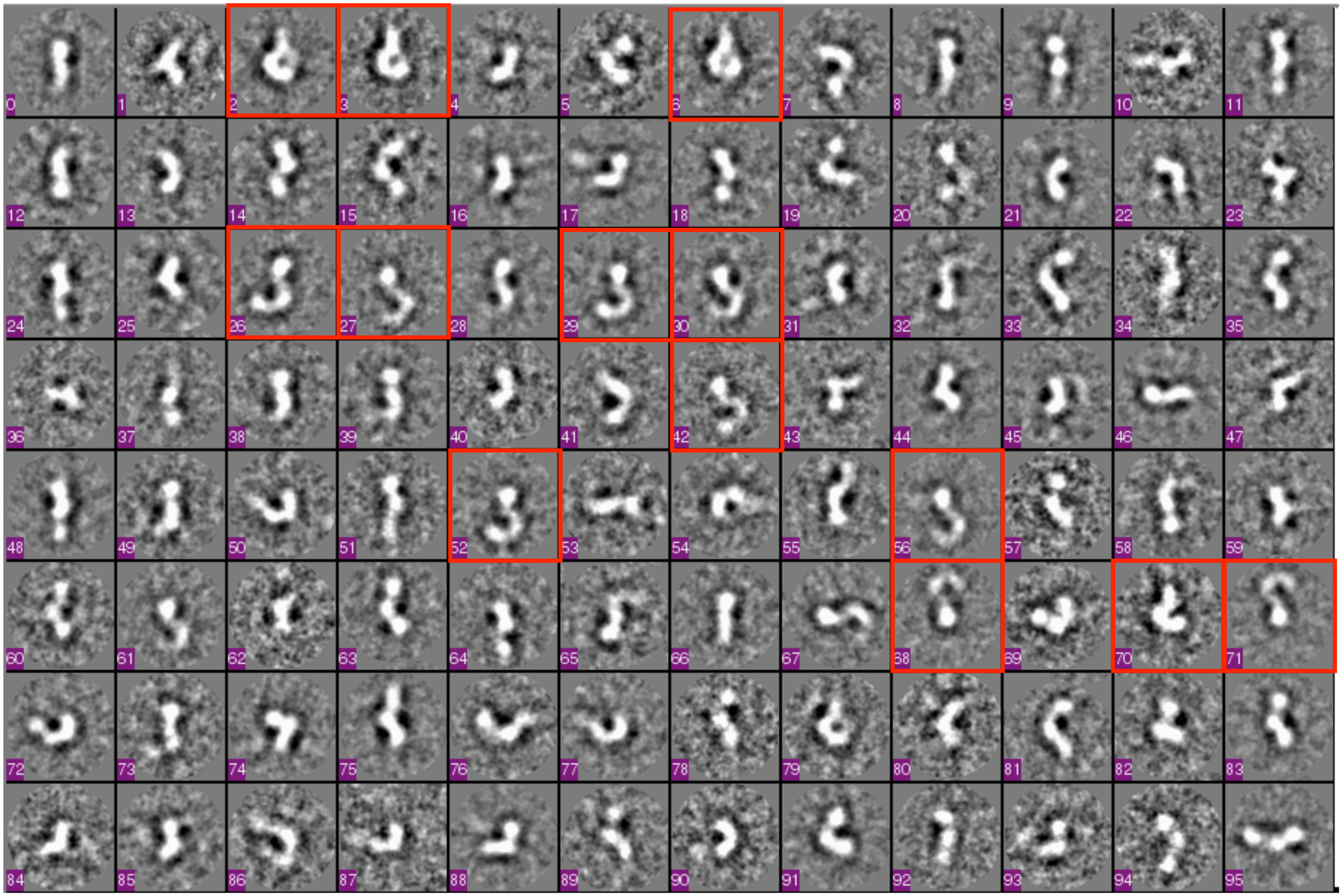


overlay

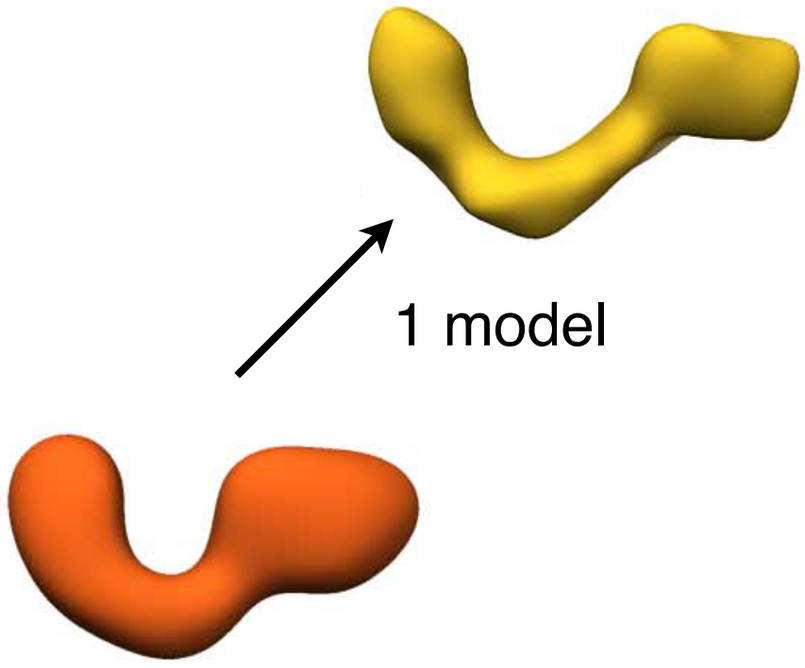


Ltn1 in vitreous ice

Does the same mobility occur in a more native environment?



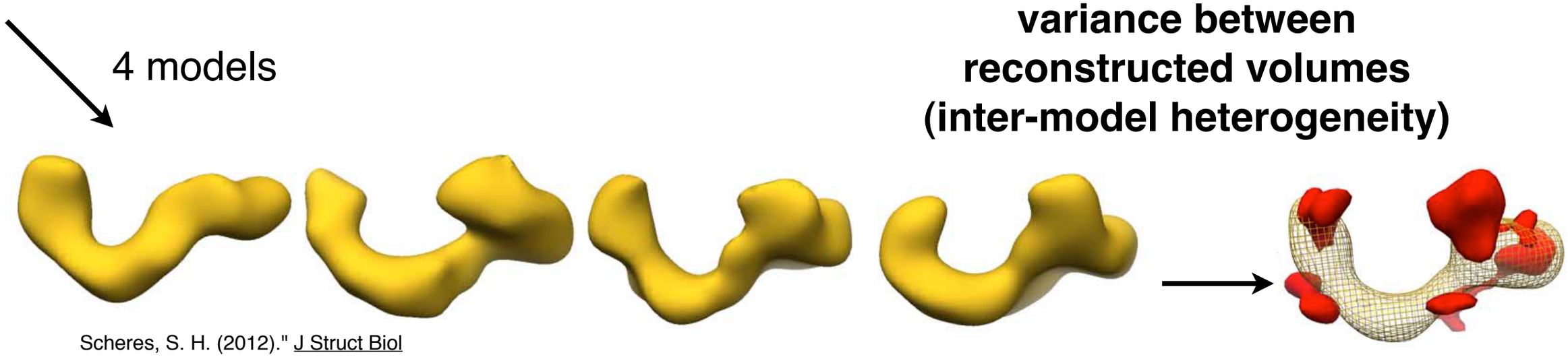
Mobility of Ltn1's termini is present in frozen hydrated preparations (cryo-EM)



primary variance within data (intra-model heterogeneity)

(1) recalculate 10,000 volumes by bootstrap resampling
(2) calculate variance

Penczek, P. A., M. Kimmel, et al. (2011). Structure **19**(11): 1582-1590

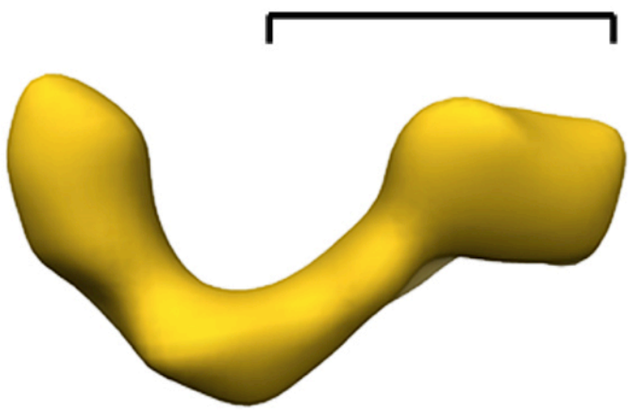


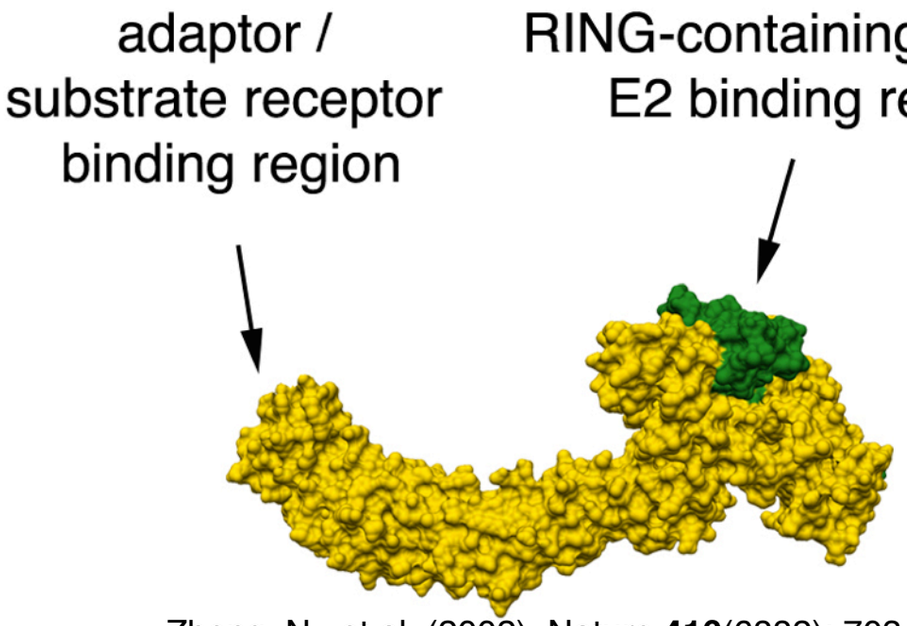
variance between reconstructed volumes (inter-model heterogeneity)

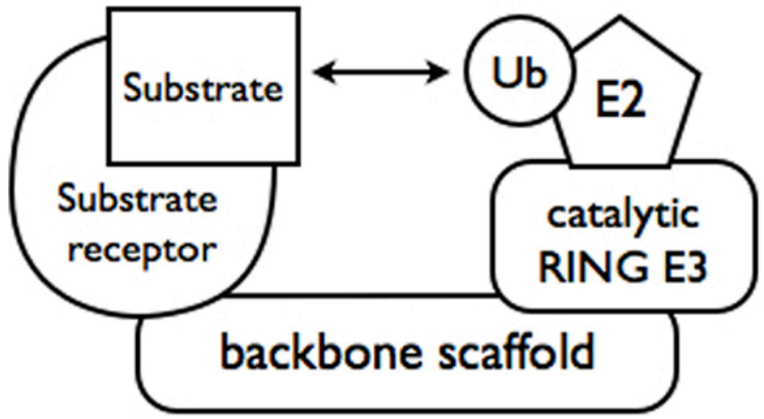
Scheres, S. H. (2012)." J Struct Biol

(1) Ltn1 exhibits similar heterogeneity in ice as it does in stain, and its basic architecture is preserved
(2) predominant heterogeneity within the structure accounts for the mobility of its C-terminus

Ltn1 is similar to CRL E3s in overall shape and size





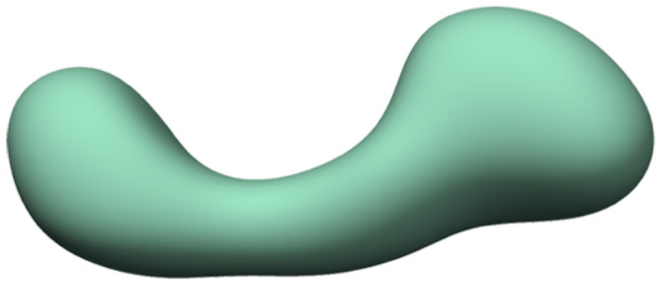


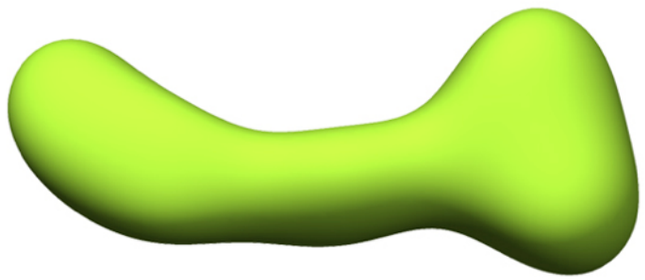
Zheng, N., et al. (2002). Nature **416**(6882): 703-709

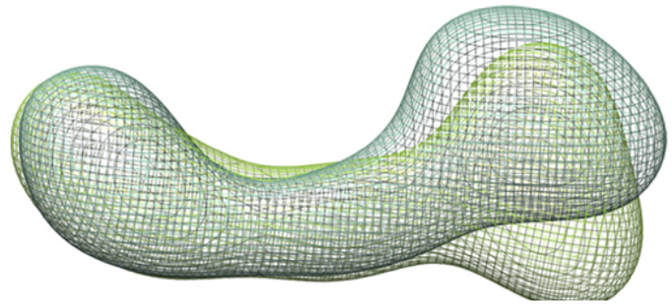
RCT reconstruction 1

RCT reconstruction 2

overlay



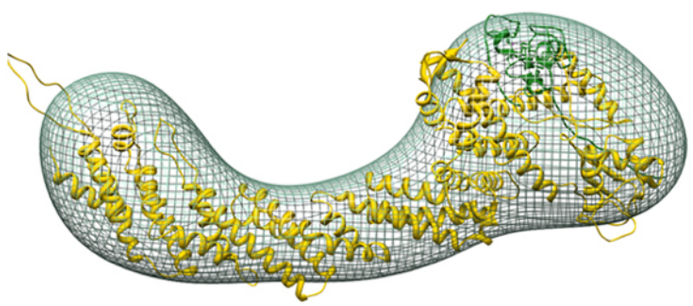


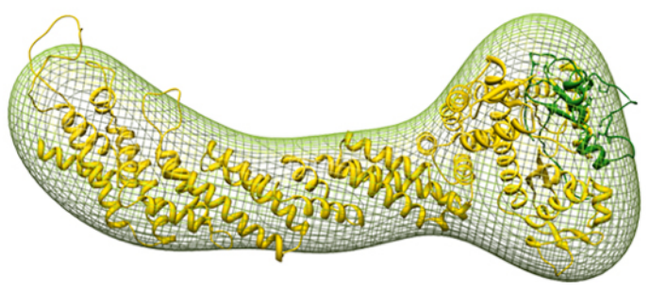


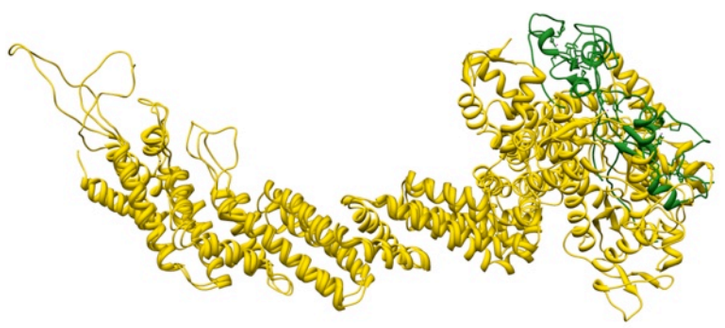
MD fit to 1

MD fit to 2

MD simulations





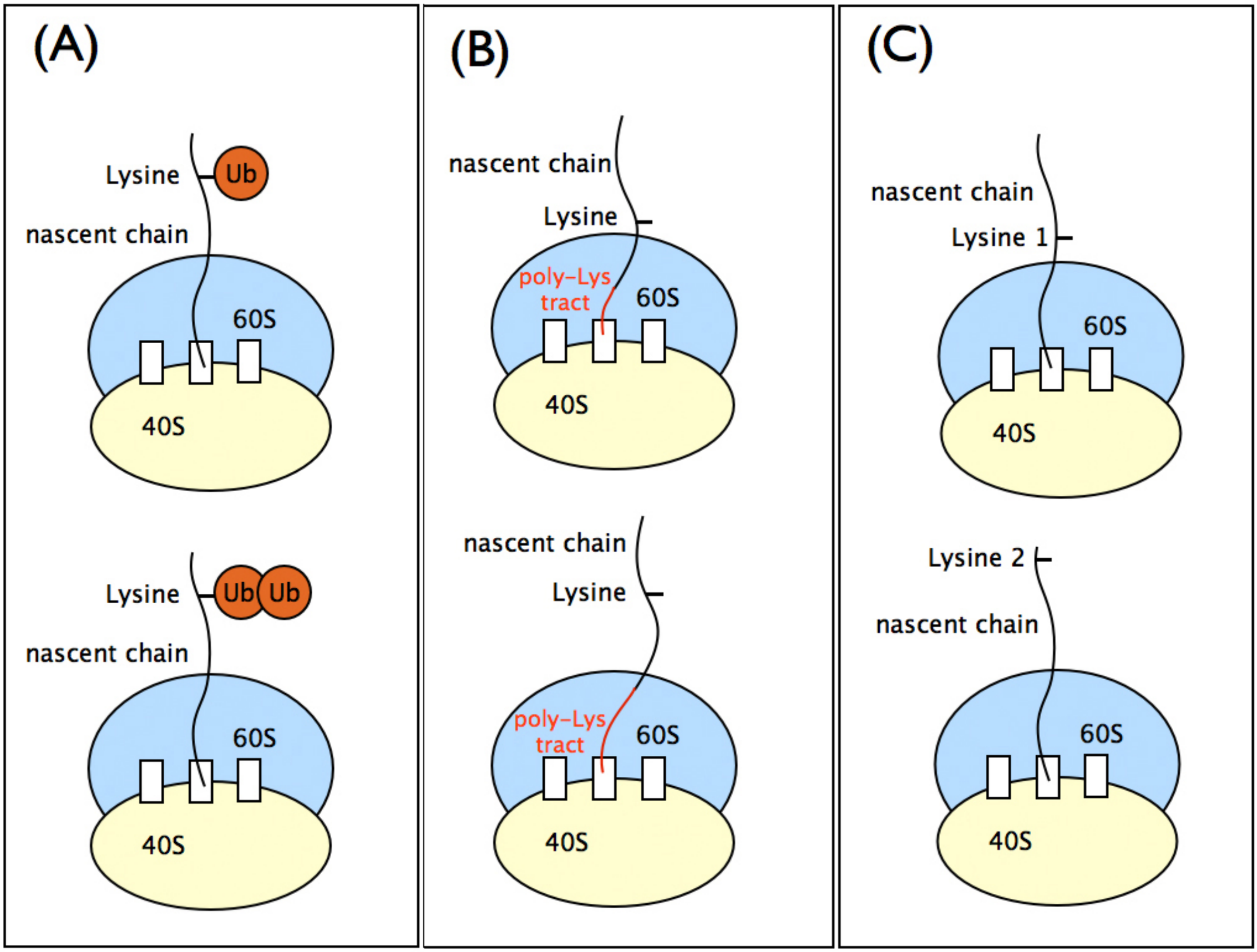


Liu, J. and R. Nussinov (2011). JBC **286**(47): 40934-40942

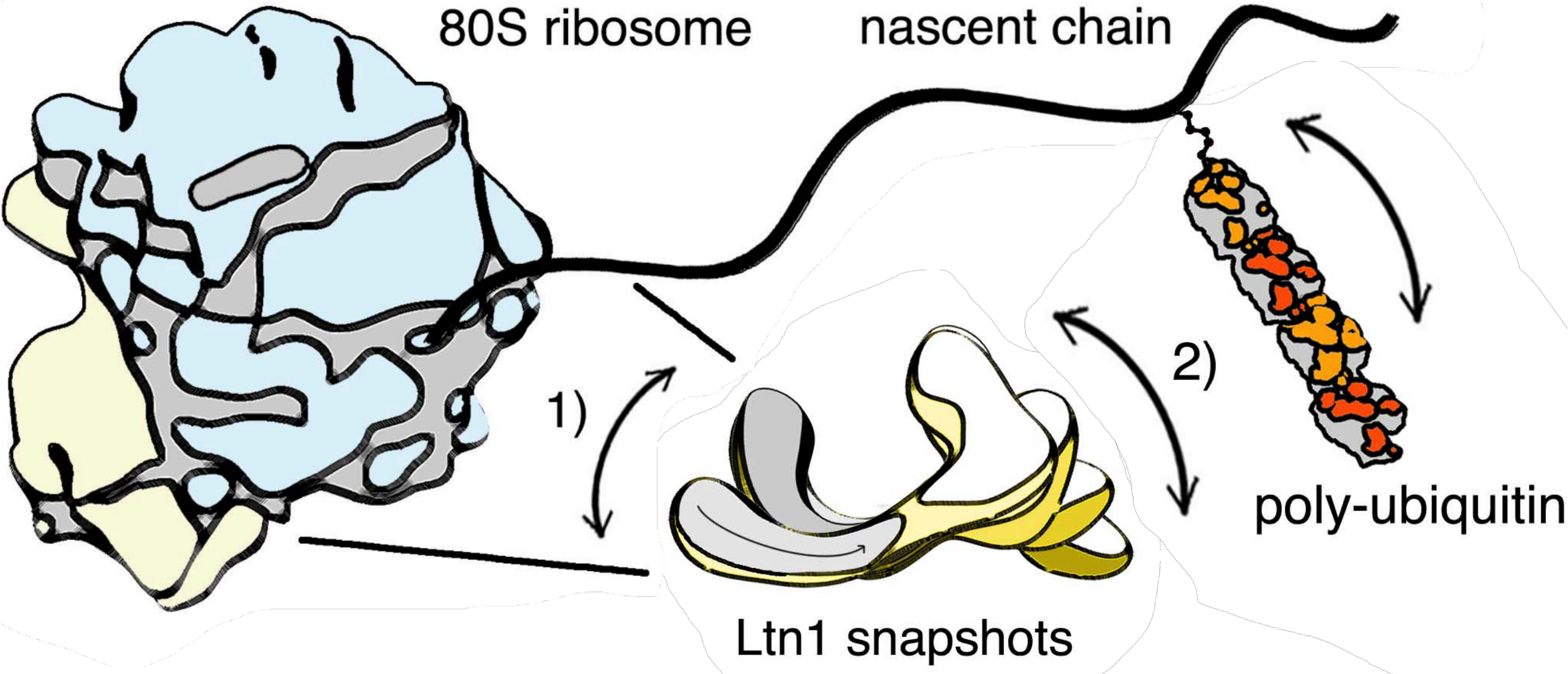
(1) The employed strategy using negative stain is identifying real mobility within the protein

(2) Ltn1's mobility cannot be simply explained by its large size and extended shape

relevance of flexibility within the ribosomal context of protein quality control



Model of Ltn1 E3 function within the context of protein quality control



protein structures drawn to scale

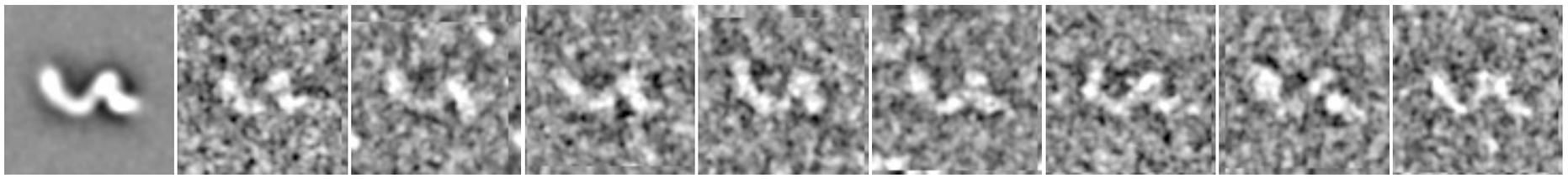
(1) mediate poly-ubiquitylation

(2) recognize **different** target lysines in a heterogeneous and dynamic environment specific to protein quality control

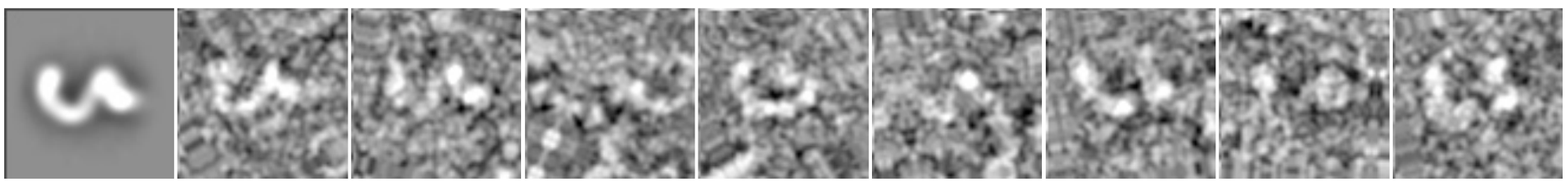
validation / optimization of RCT results

1. look at the particles within your class

homogeneous class

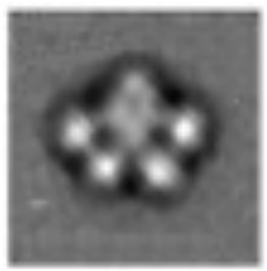


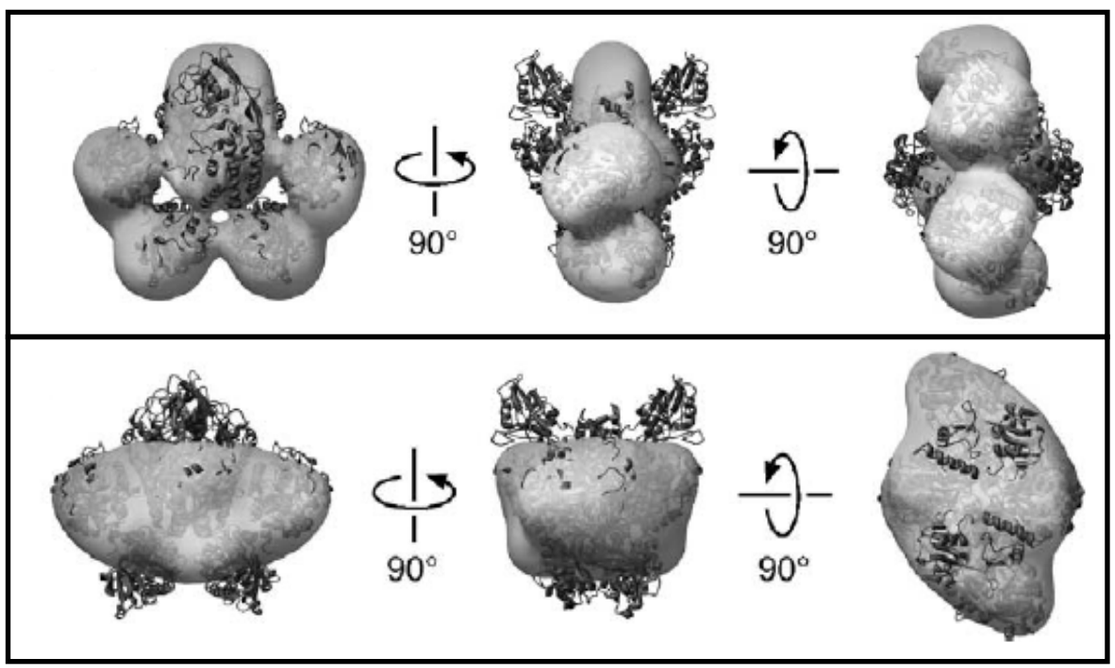
heterogeneous class

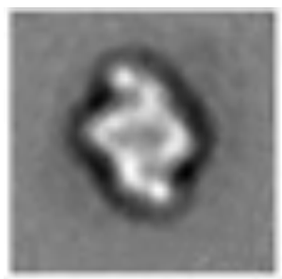


2. minor things - careful tilt-pair matching (especially when it is done automatically), FSC value, curve, etc.

3. effects of specimen flattening: different structure by RCT may not always mean different conformation and/or composition







Cheng, Y., E. Wolf, et al. (2006). "Single particle reconstructions of the transferrin-transferrin receptor complex obtained with different specimen preparation techniques." J Mol Biol **355**(5): 1048-1065

conclusions

1. Ltn1 is a highly dynamic protein and this may have functional implications for its role in protein quality control

2. Growing evidence that flexibility plays an important role in E3 ubiquitin ligases

3. RCT as a robust methodology for sorting heterogeneity in single-particle data
   - fair amount of automation (record keeping!)
   - data collection in stain ~= RCT

4. public availability of the negative stain dataset: **http://maskiton.scripps.edu**

Thanks to ...

Principal Investigators

Bridget Carragher
Clinton Potter
Ron Milligan
Claudio Joazeiro

Collaborators

Claudio Joazeiro
Mario Bengtson
Joong-Won Lee
Chris Lima
Selom Doamekpor
Matt Petroski
Tasha B. Toro

AMI / Joazeiro groups

see below