



Structural Studies of an AAA+ ATPase N-ethylmaleimide Sensitive Factor

Minglei Zhao

**Axel Brunger Lab
Stanford University**

**Yifan Cheng Lab
University of California, San Francisco**

NRAMM Workshop, November 10th, 2014

Outline

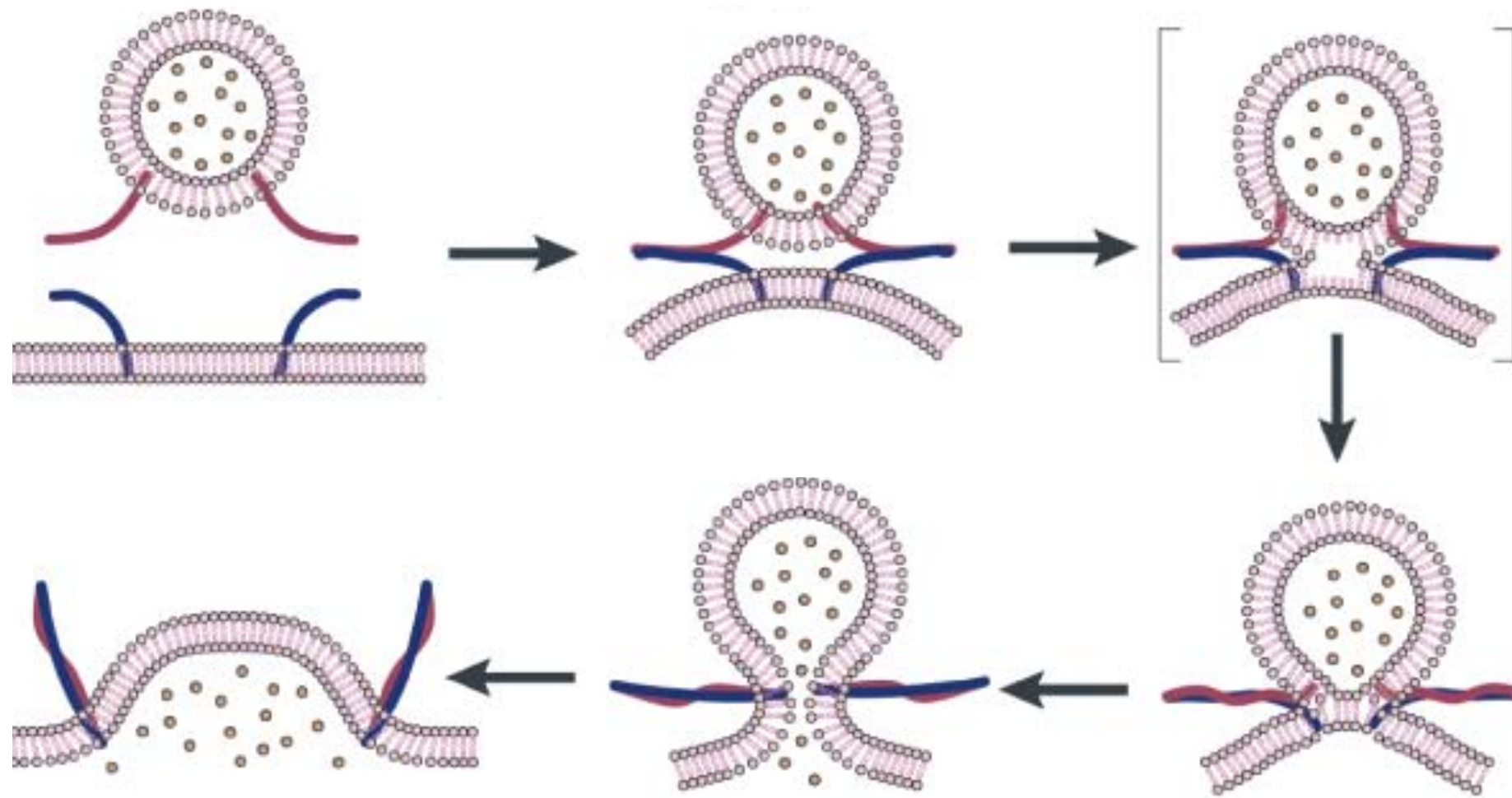
[This talk will highlight the biology while also drawing attention to the technical advances that made it possible.]

The Nobel Prize in Physiology and Medicine (2013)



"for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"

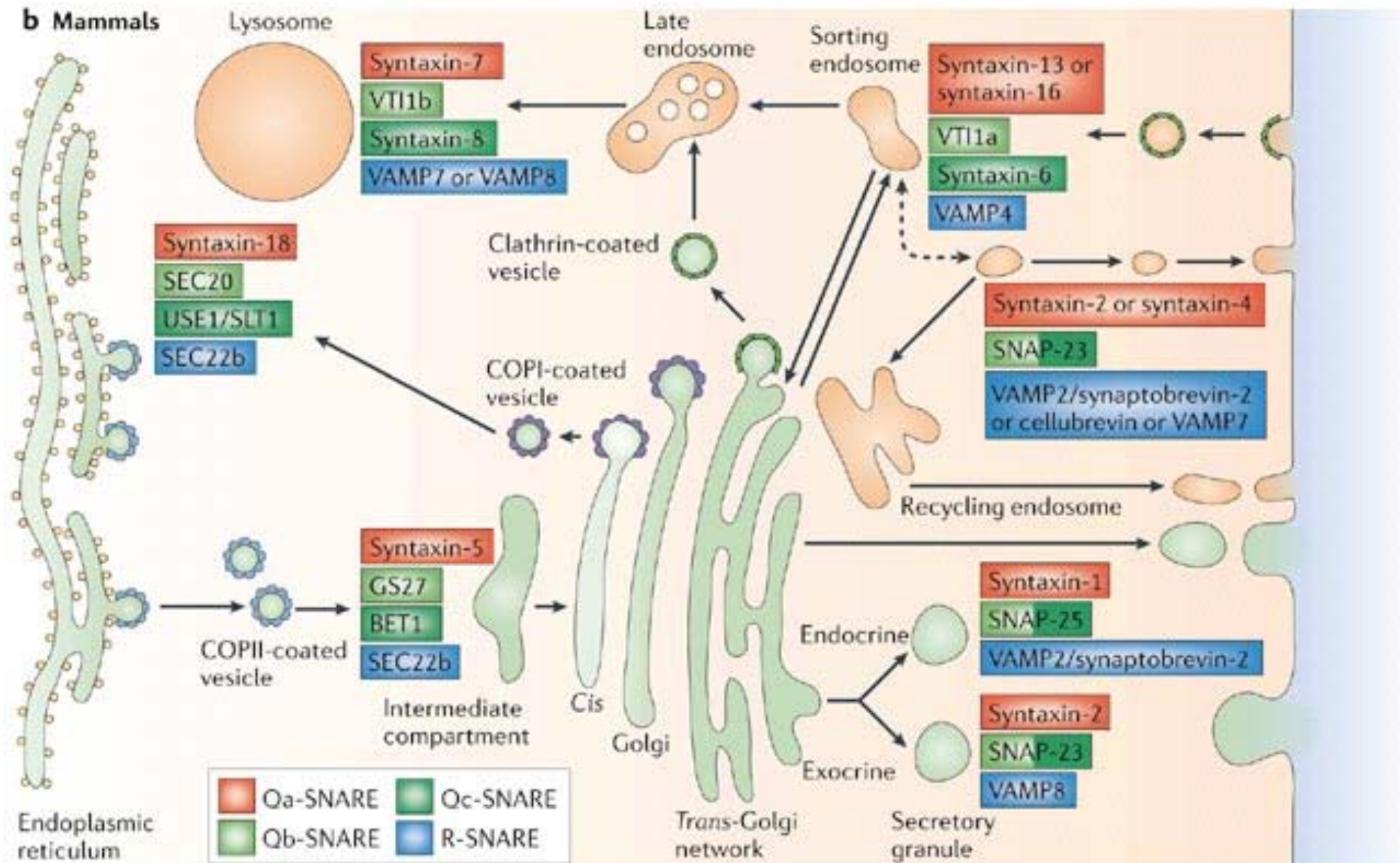
SNARE mediated vesicle/membrane fusion



Nature Reviews | Molecular Cell Biology

Chen *et al.*, 2001

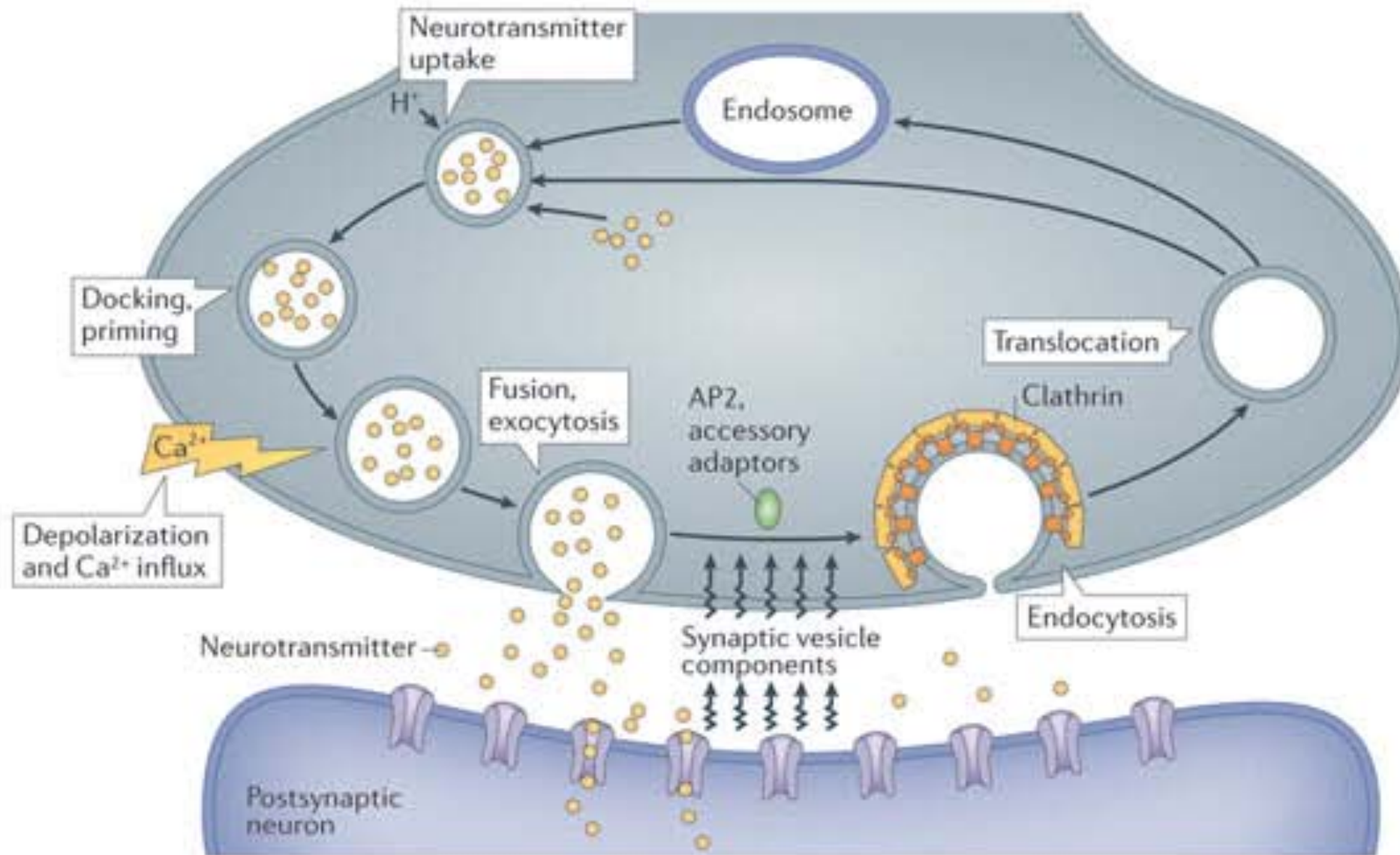
SNAREs involved many fusion systems



Copyright © 2006 Nature Publishing Group
Nature Reviews | Molecular Cell Biology

Jahn R, *et al.*, 2006

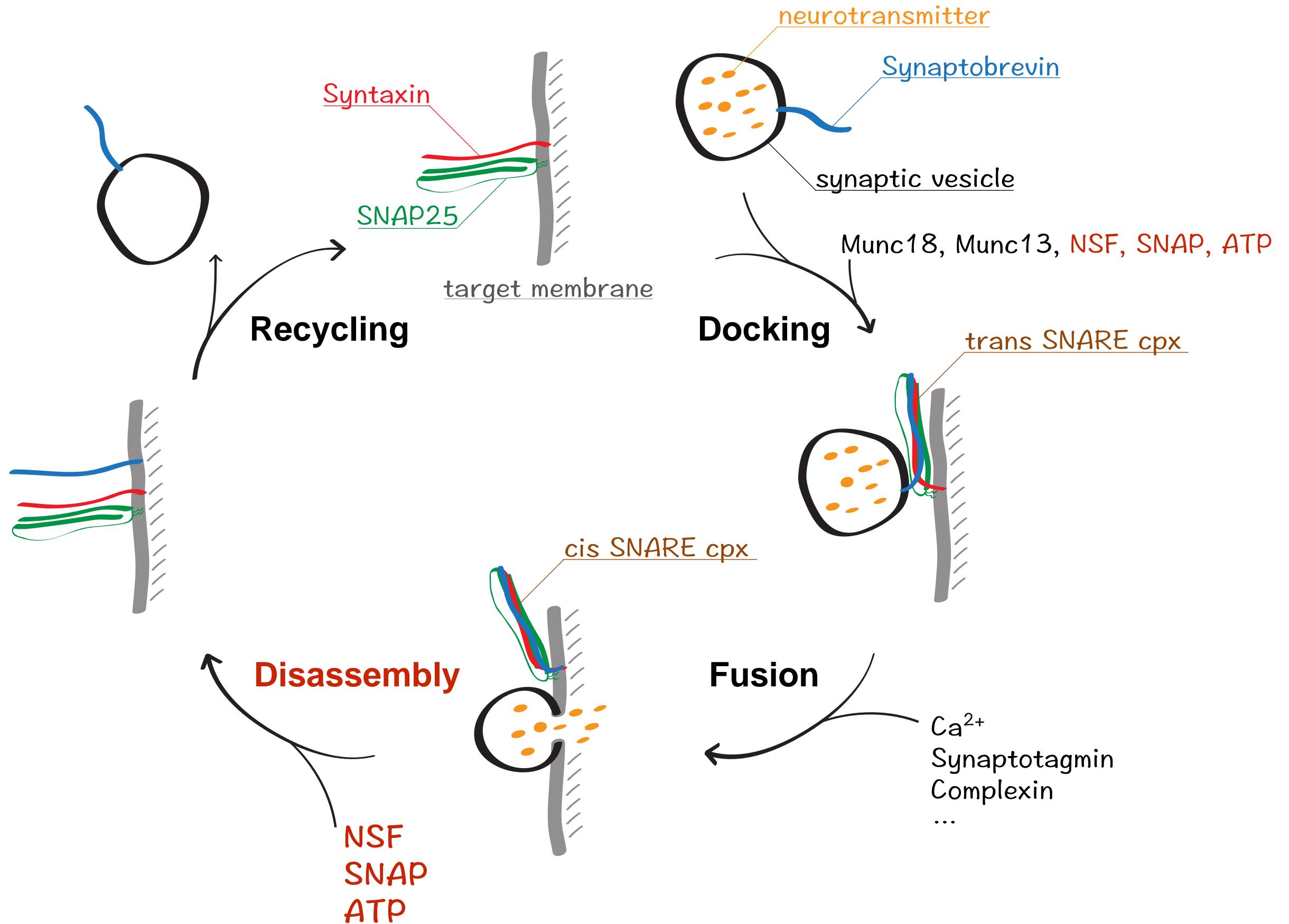
SNARE mediated neurotransmitter release



Nature Reviews | Molecular Cell Biology

McMahon HT, *et al.*, 2006

Synaptic vesicle fusion cycle

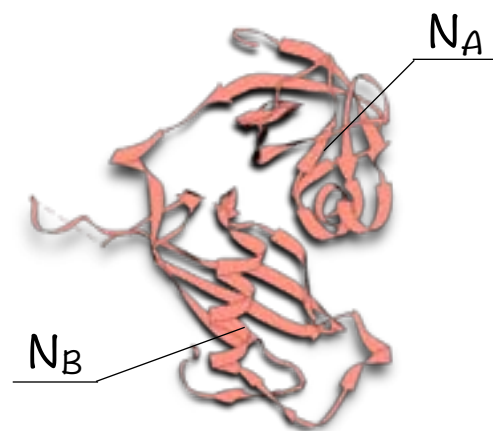


N-ethylmaleimide Sensitive Factor (NSF)

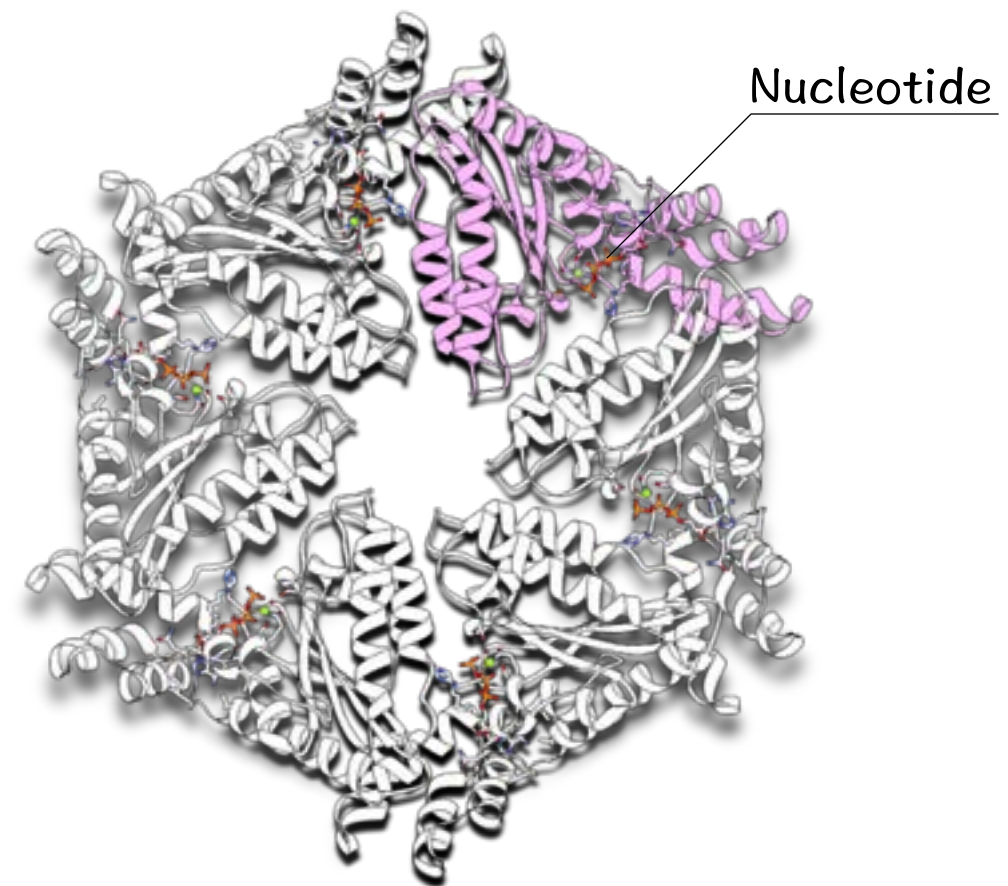
- First purified in 1988 by [James Rothman's group](#) from CHO cells (Block *et al.*, *PNAS*, 1988).
- One of the first identified machinery involved in vesicle traffic.
- [AAA+](#) superfamily member, homomeric hexamer, ~500 kDa.
- Very conserved in eukaryotes:

Organism	Identity to Human (%)
Baker's yeast	46
Arabidopsis	45
Worm	54
Fruit fly	63
Mammal	99

N-ethylmaleimide Sensitive Factor (NSF)



Yu *et al.*, *NSMB*, 1999
May *et al.*, *Nat. Cell. Bio.*, 1999

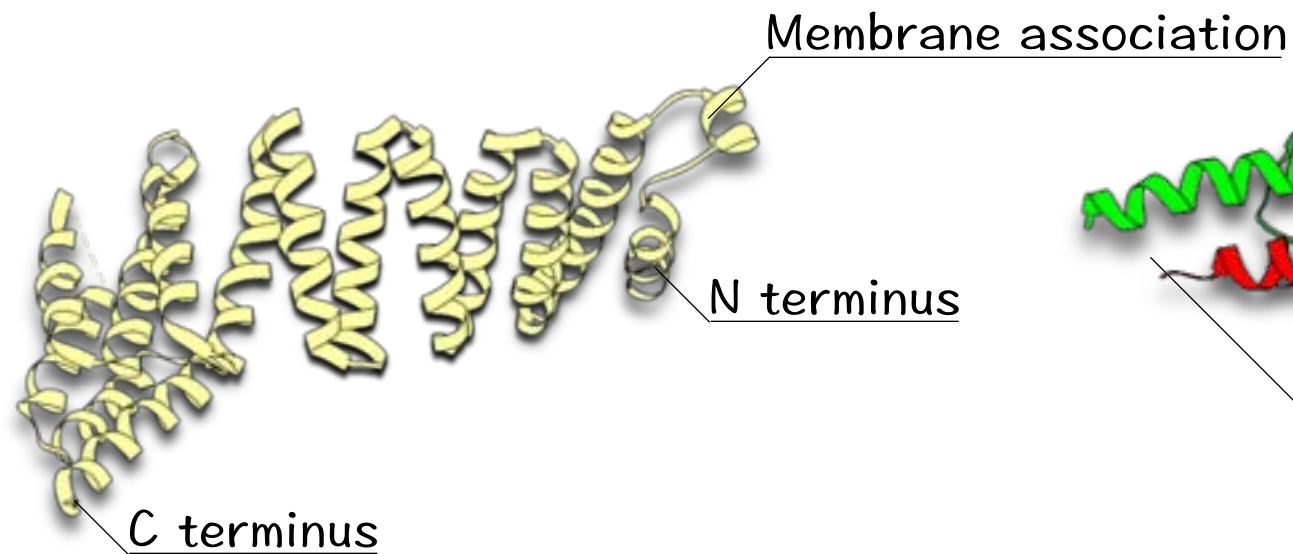


Yu *et al.*, *NSMB*, 1998
Lenzen *et al.*, *Cell*, 1998

NSF interacts with SNAREs via SNAPs

SNAP

(Soluble NSF Attachment Protein)

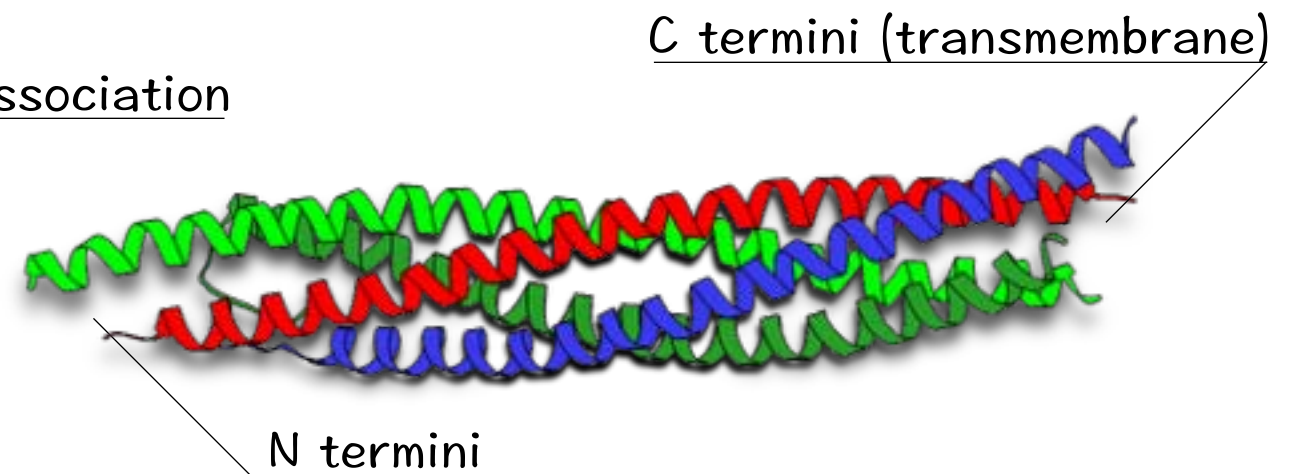


yeast homolog **Sec17p**

Rice et al., *Mol. Cell*, 1999

SNARE

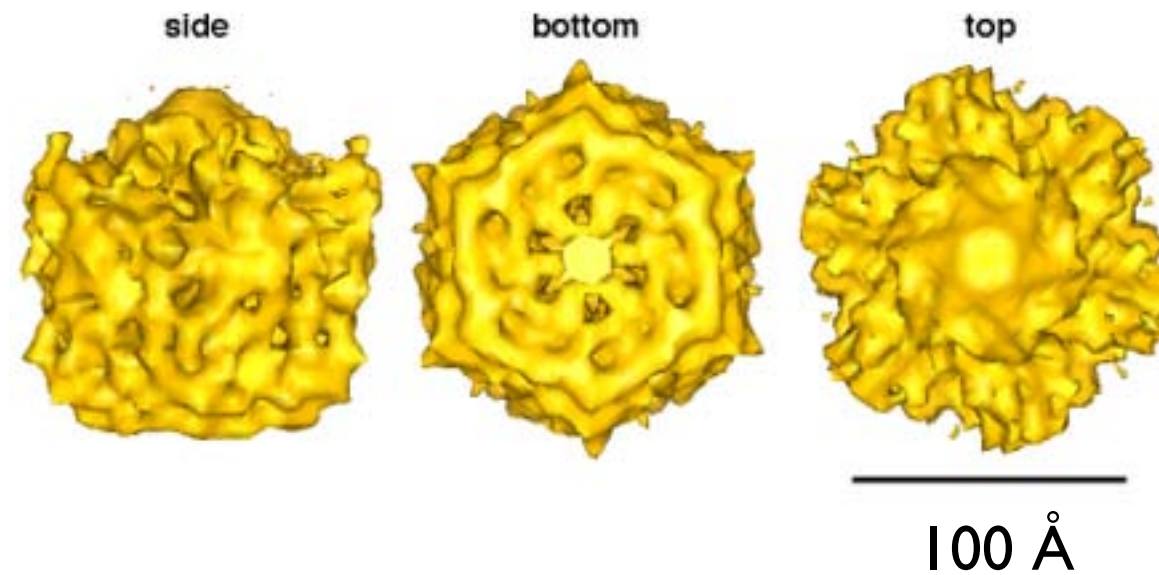
(SNAP Receptors)



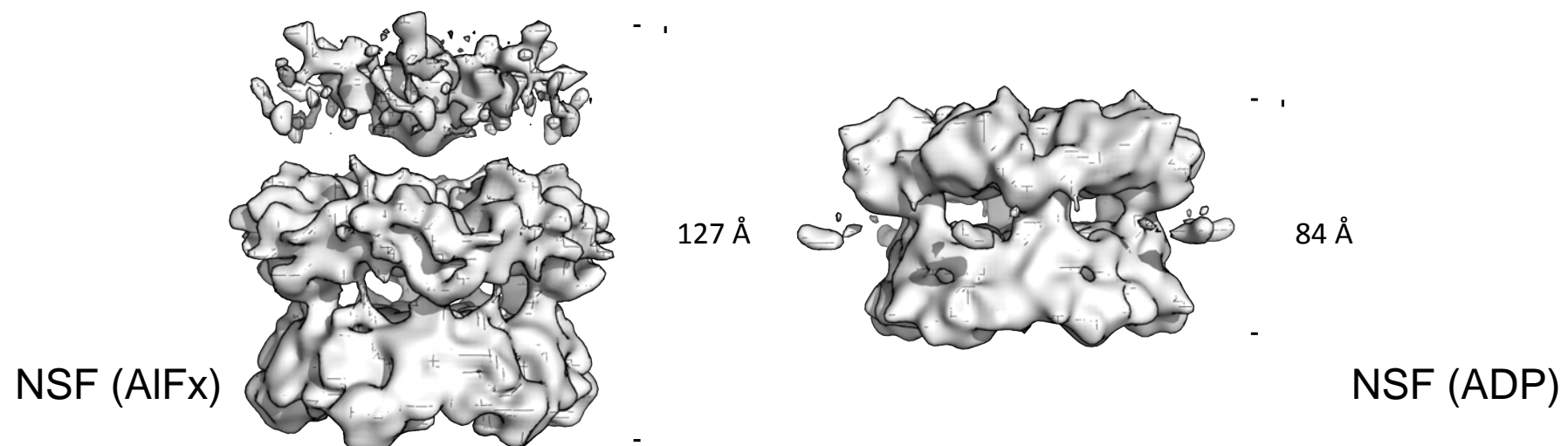
Core of the neuronal SNARE complex
(**Synaptobrevin2**-**Syntaxin1**-**SNAP25**)

Sutton et al., *Nature*, 1998

Previous EM Reconstructions of NSF

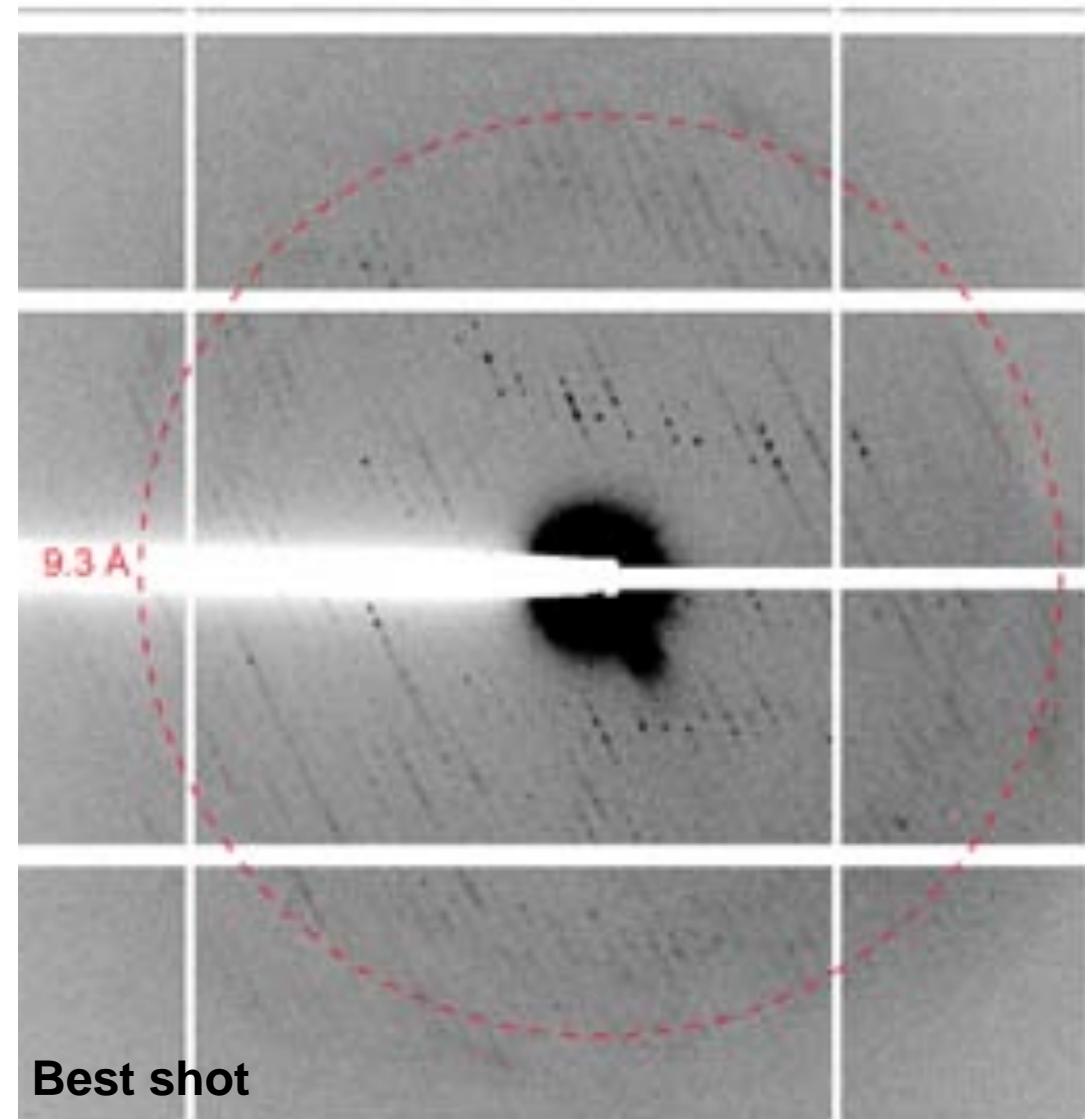
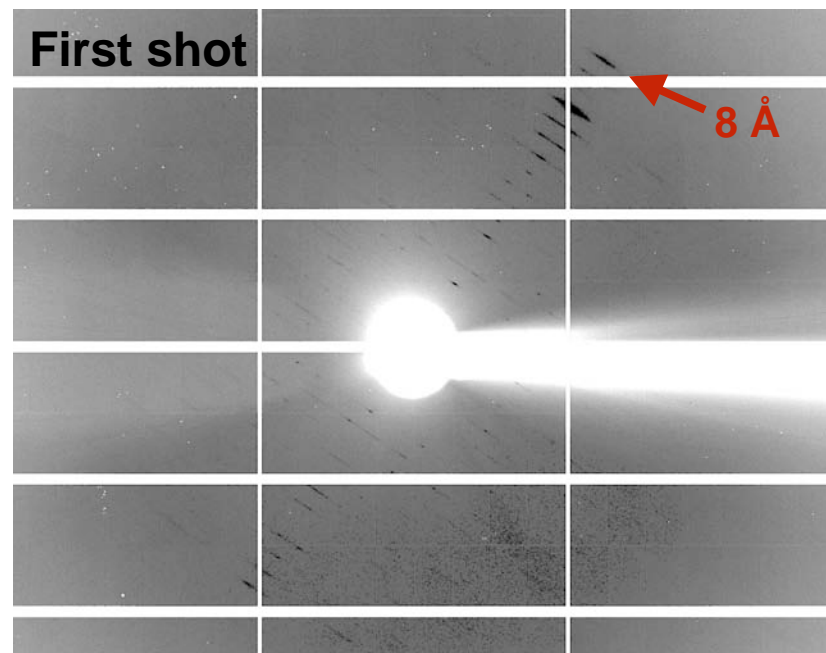
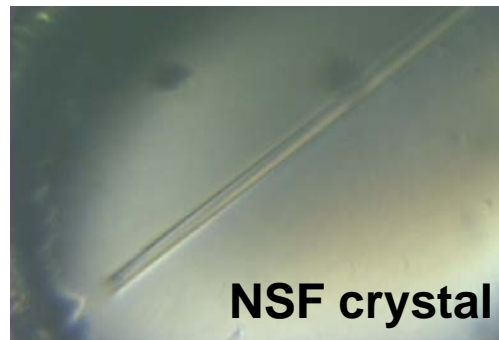


Cryo-EM reconstruction of 20S at ~ 12 Å (Furst et al., *EMBO J*, 2003)

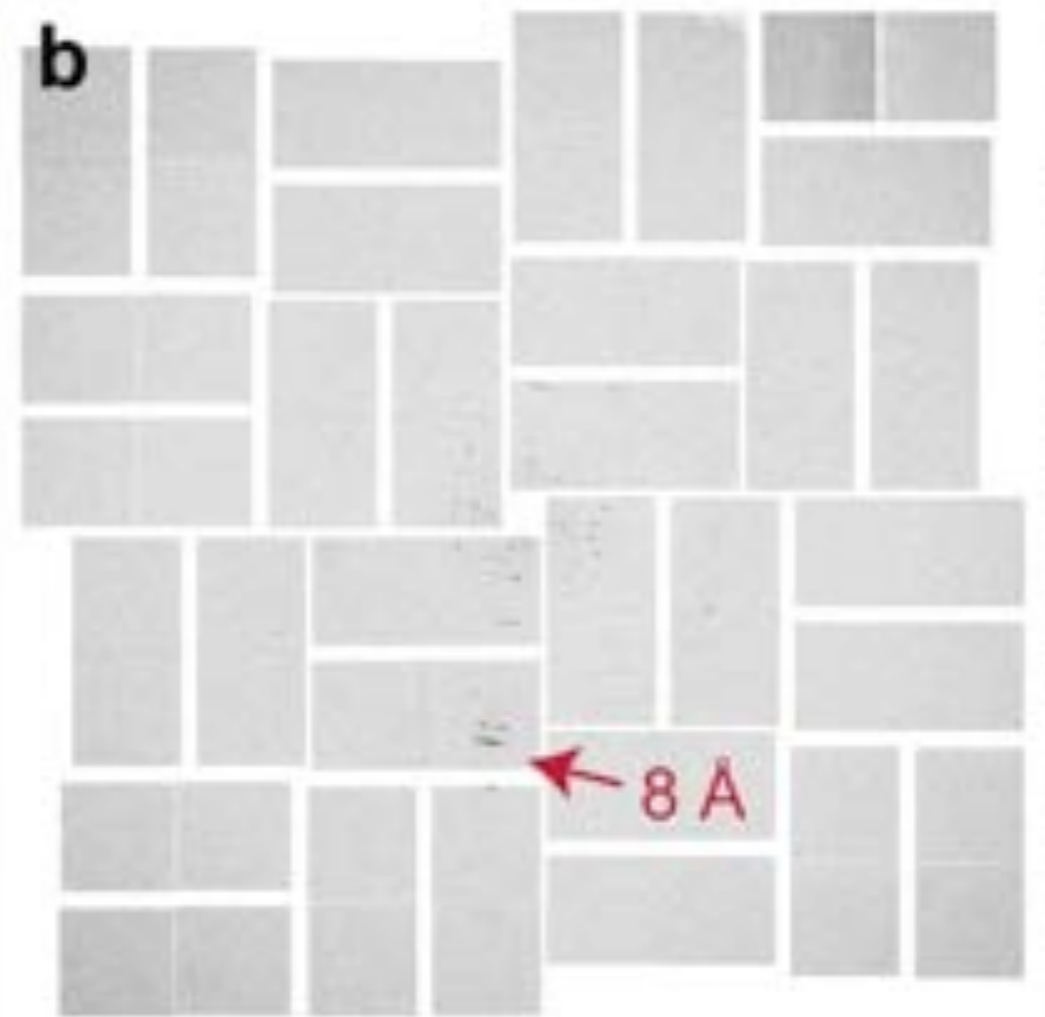
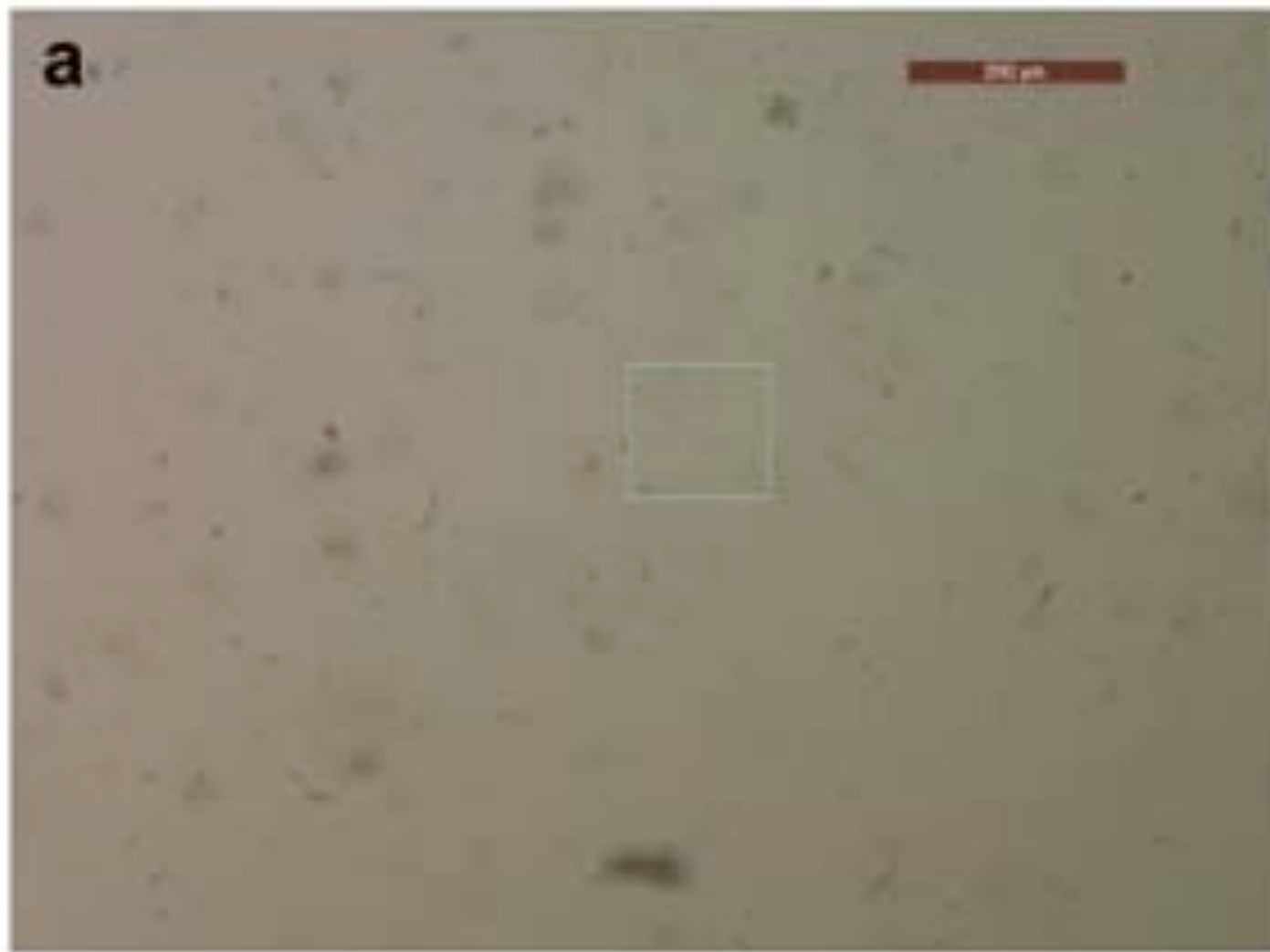


Cryo-EM reconstruction of NSF at lower resolution (Chang et al., *NSMB*, 2012)

NSF crystals diffract to $\sim 8\text{\AA}$

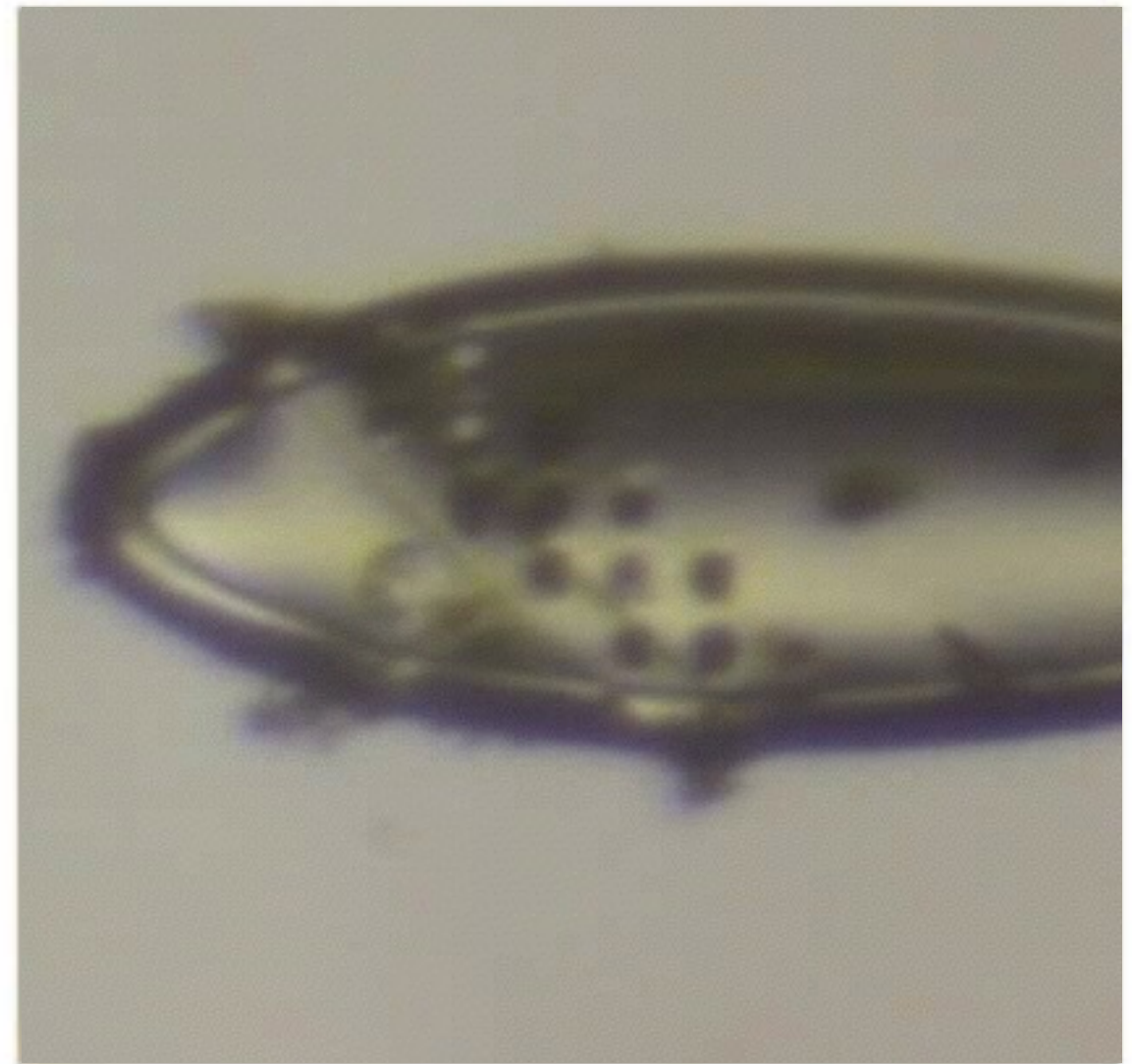
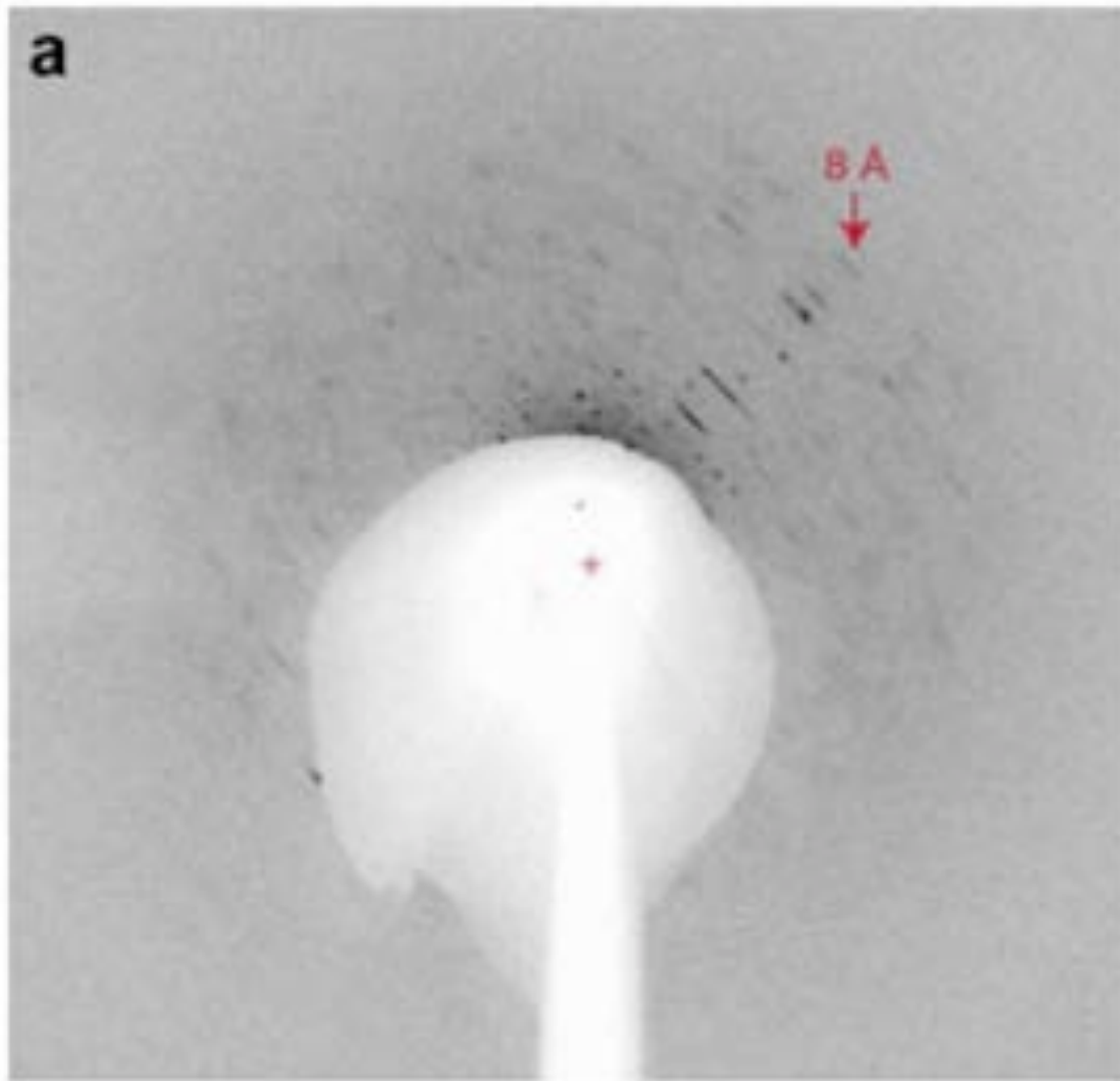


NSF crystal diffraction using X-ray free electron laser (XFEL)



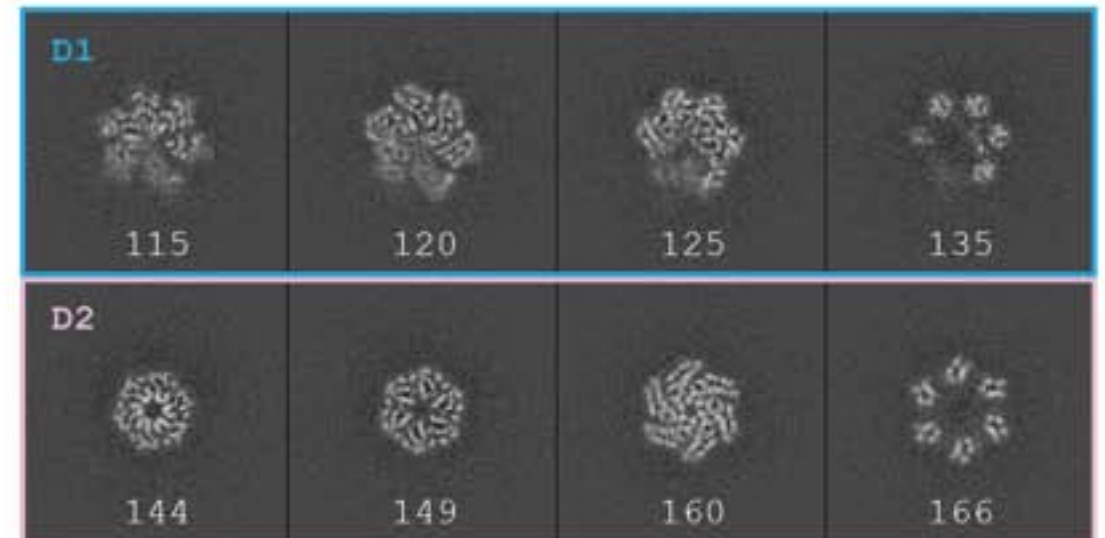
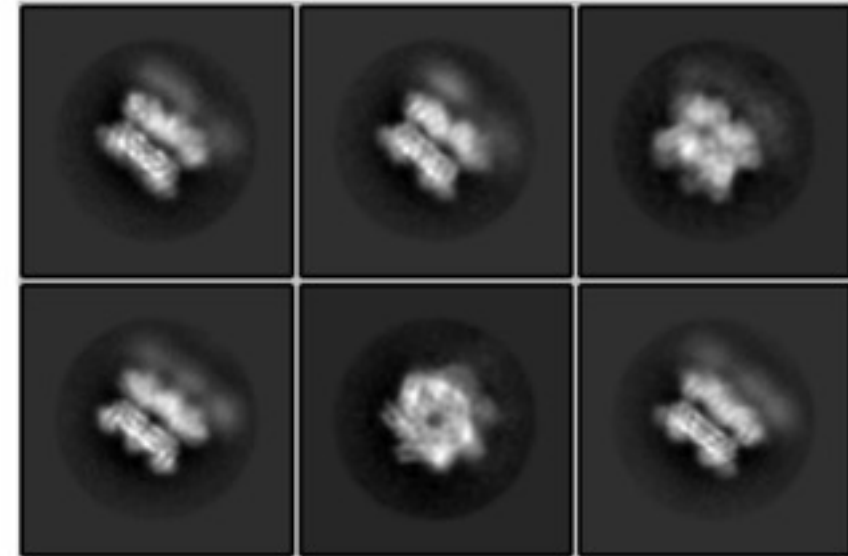
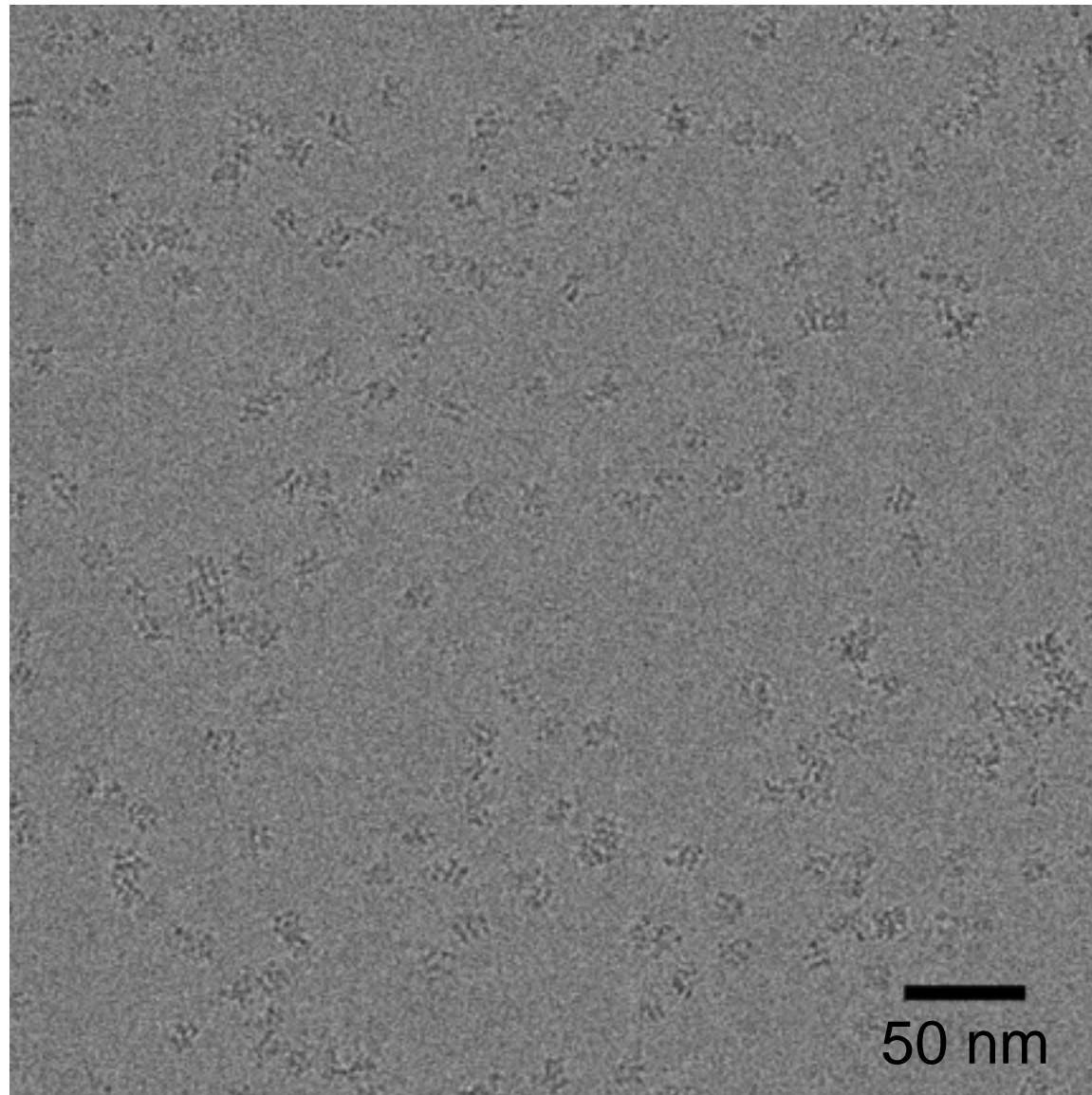
CXI station, LCLS

NSF crystal diffraction using X-ray free electron laser (XFEL)

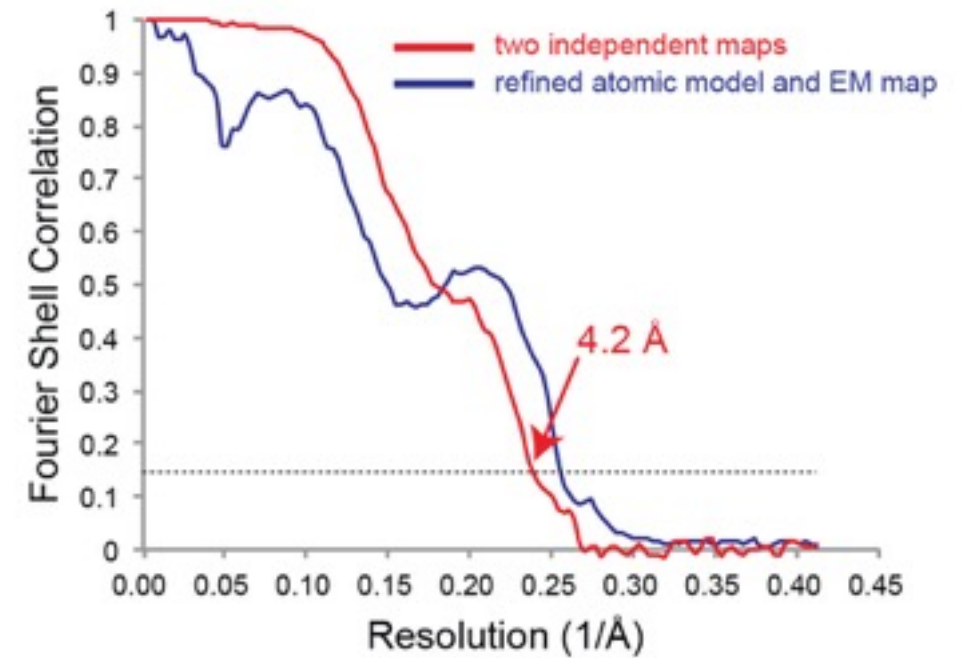
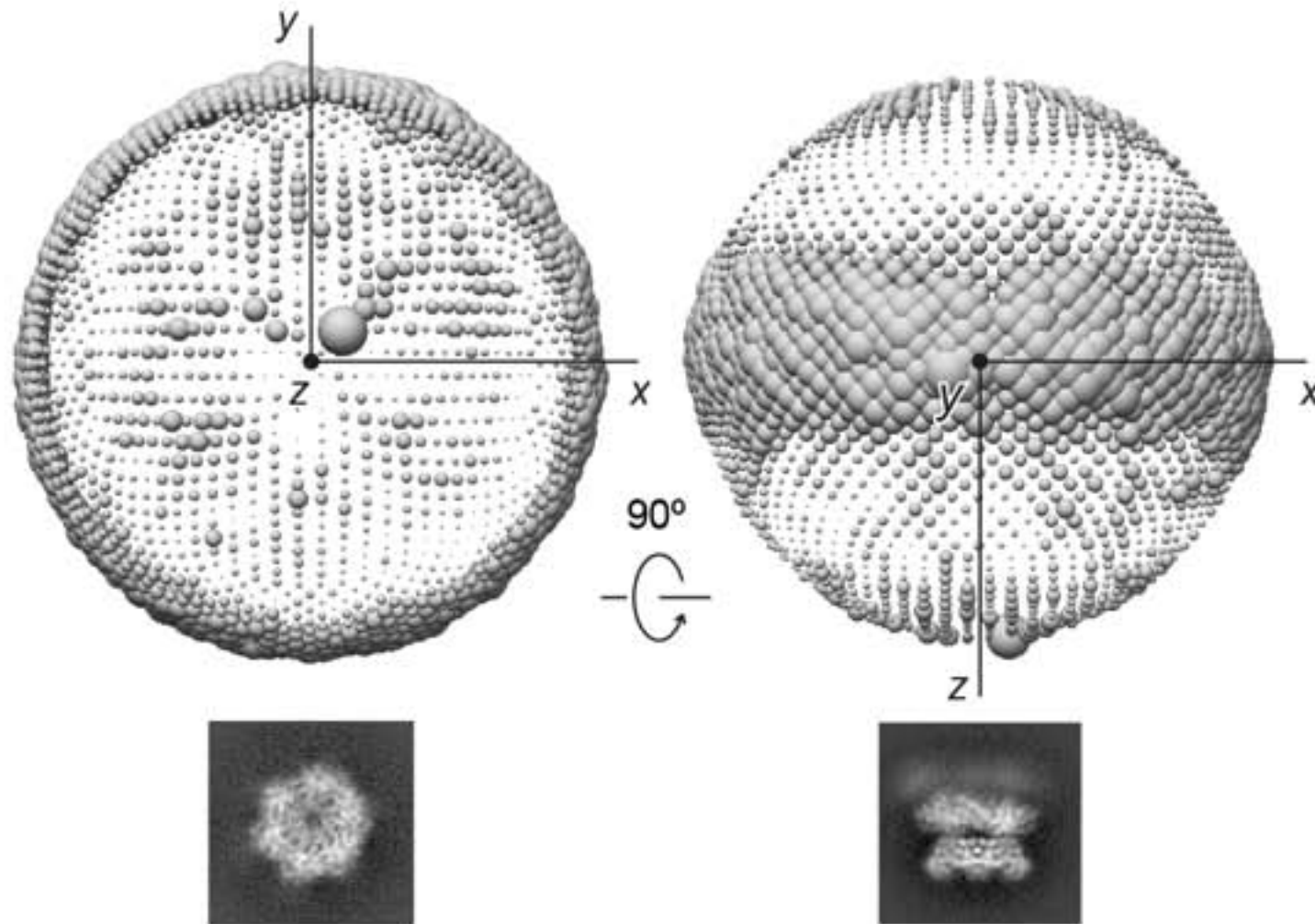


XPP station, LCLS

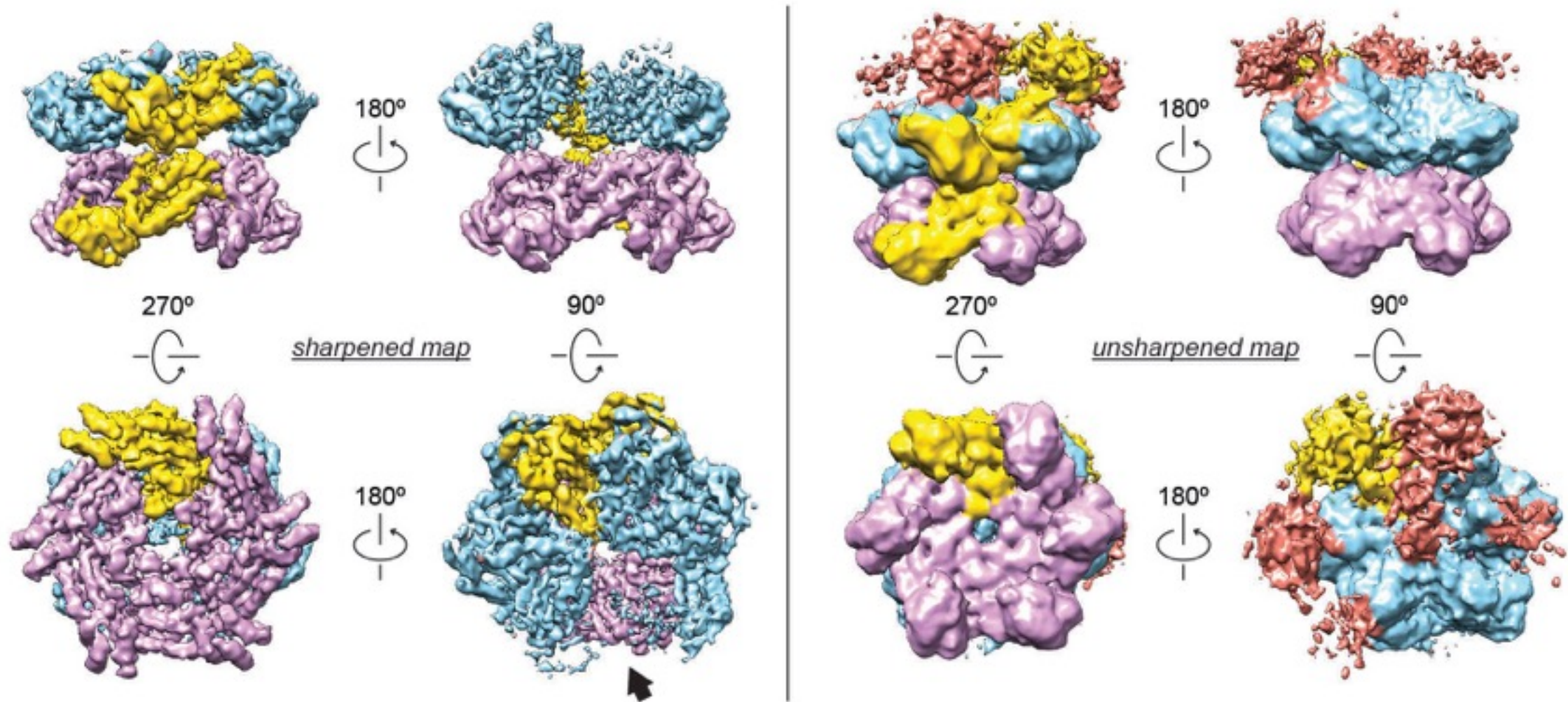
3D reconstruction of ATP-bound NSF by single-particle cryo-EM



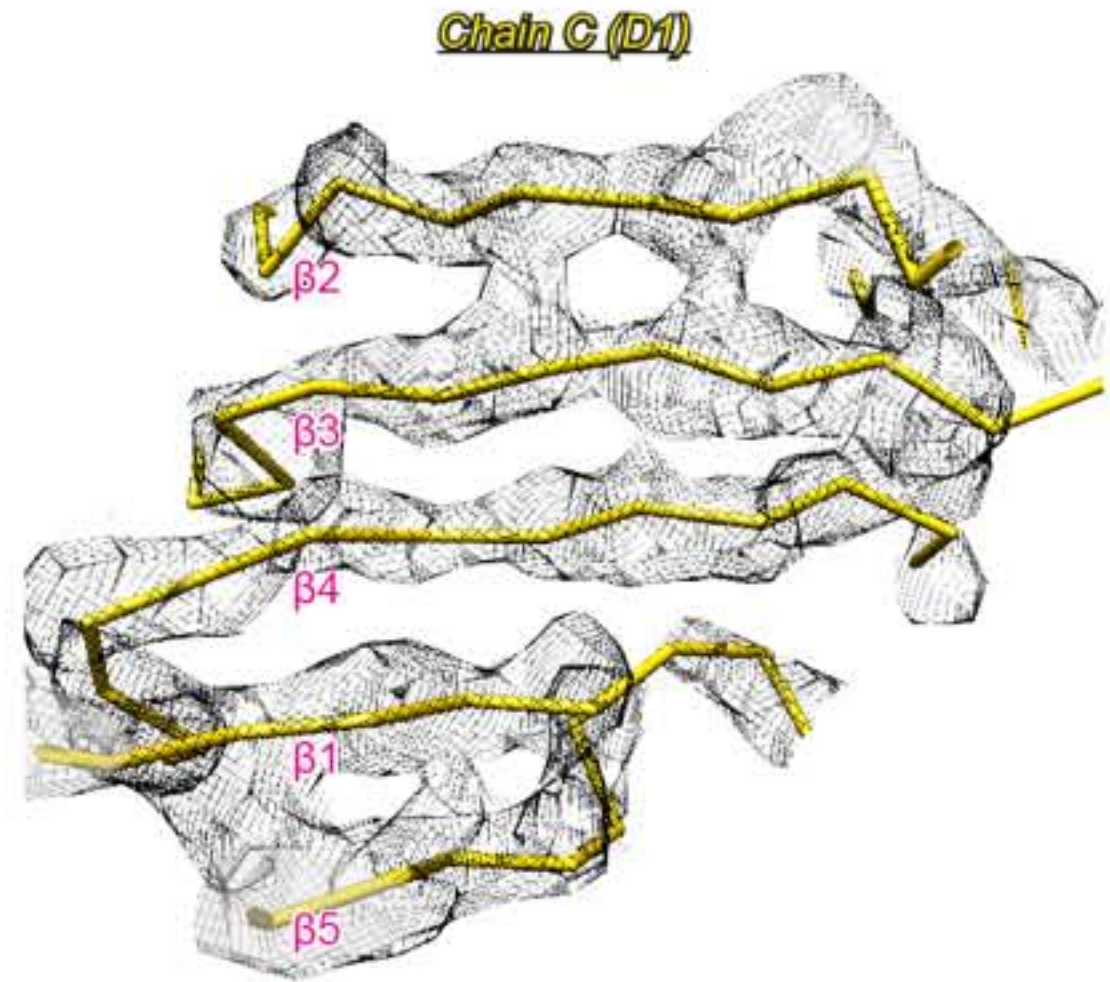
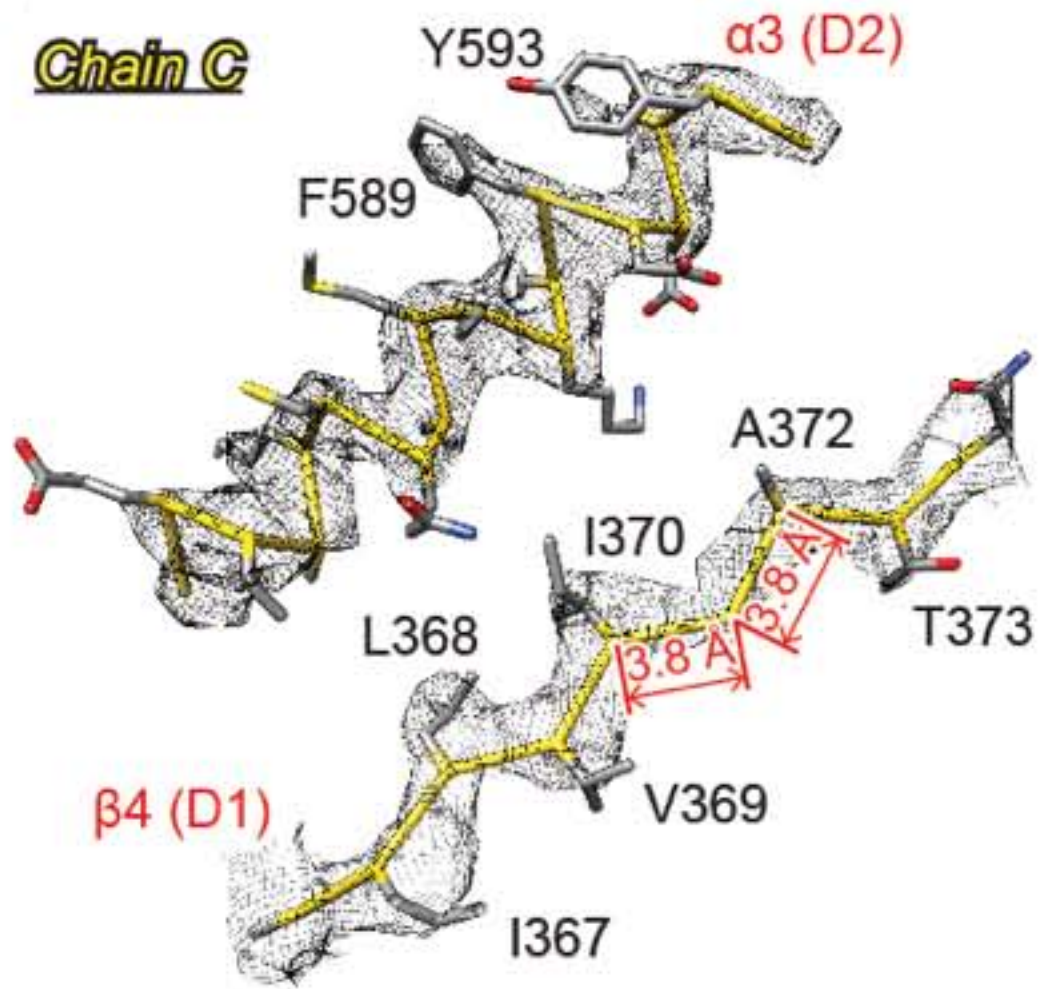
3D reconstruction of ATP-bound NSF by single-particle cryo-EM



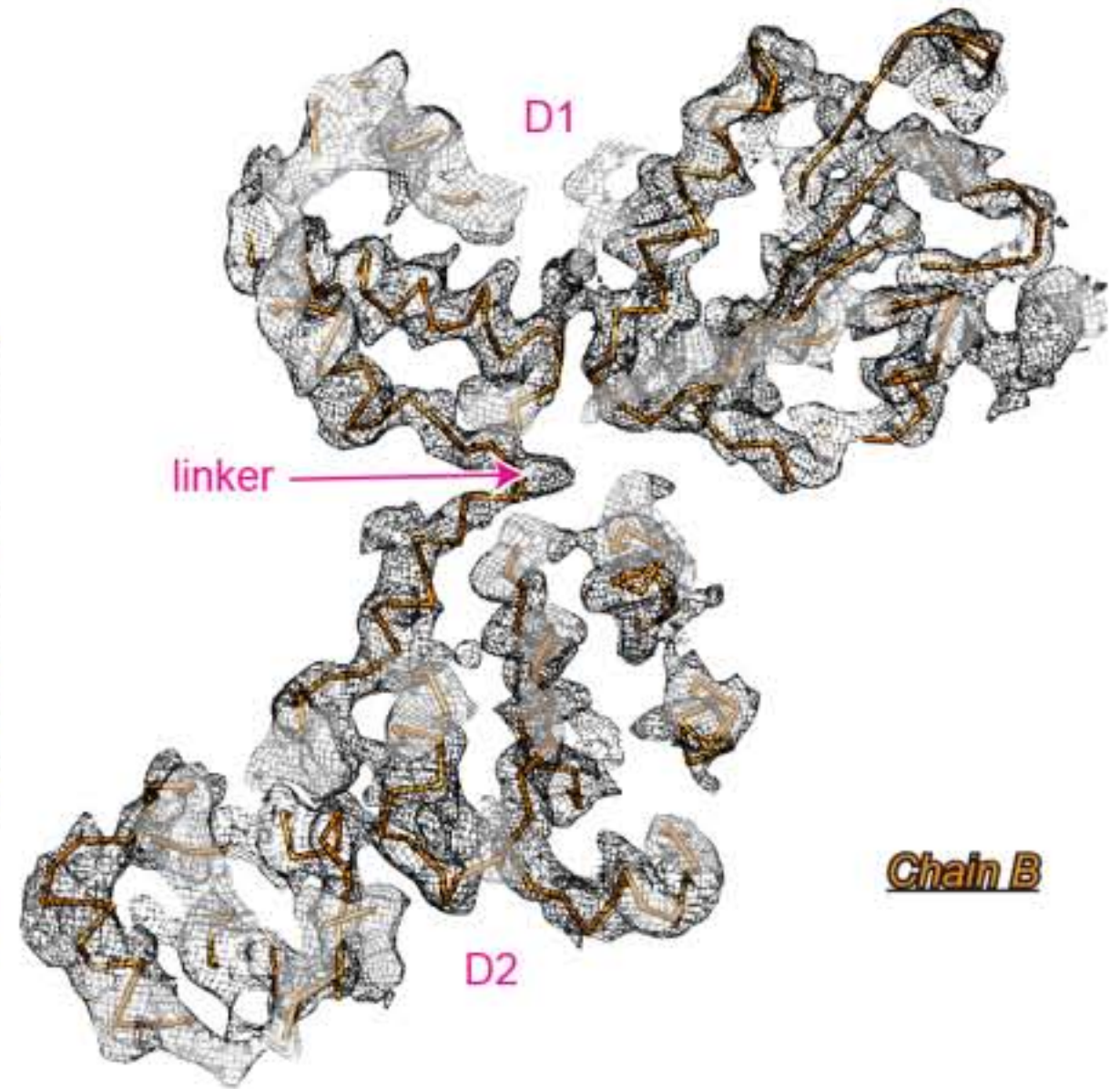
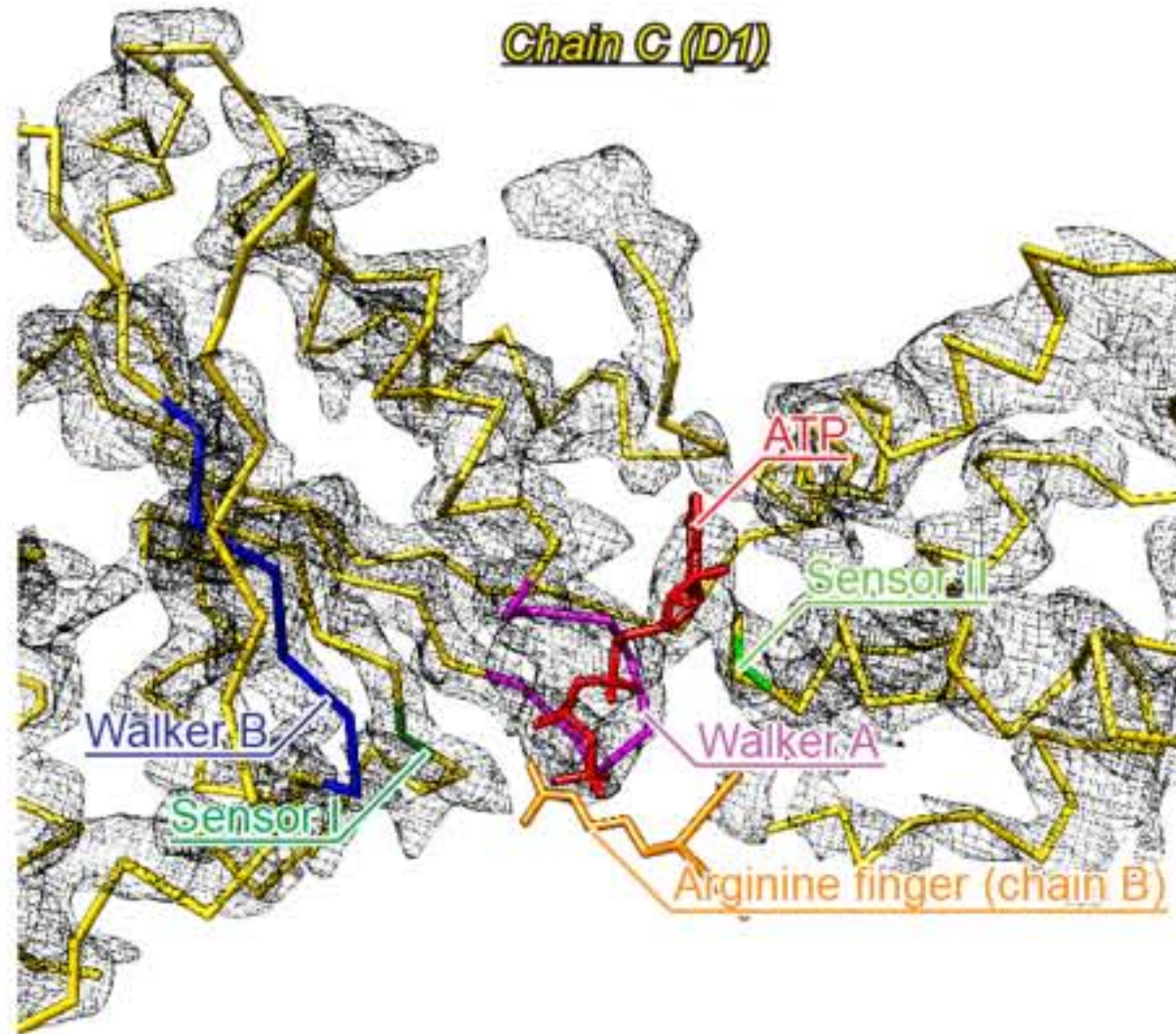
Maps of ATP-bound NSF



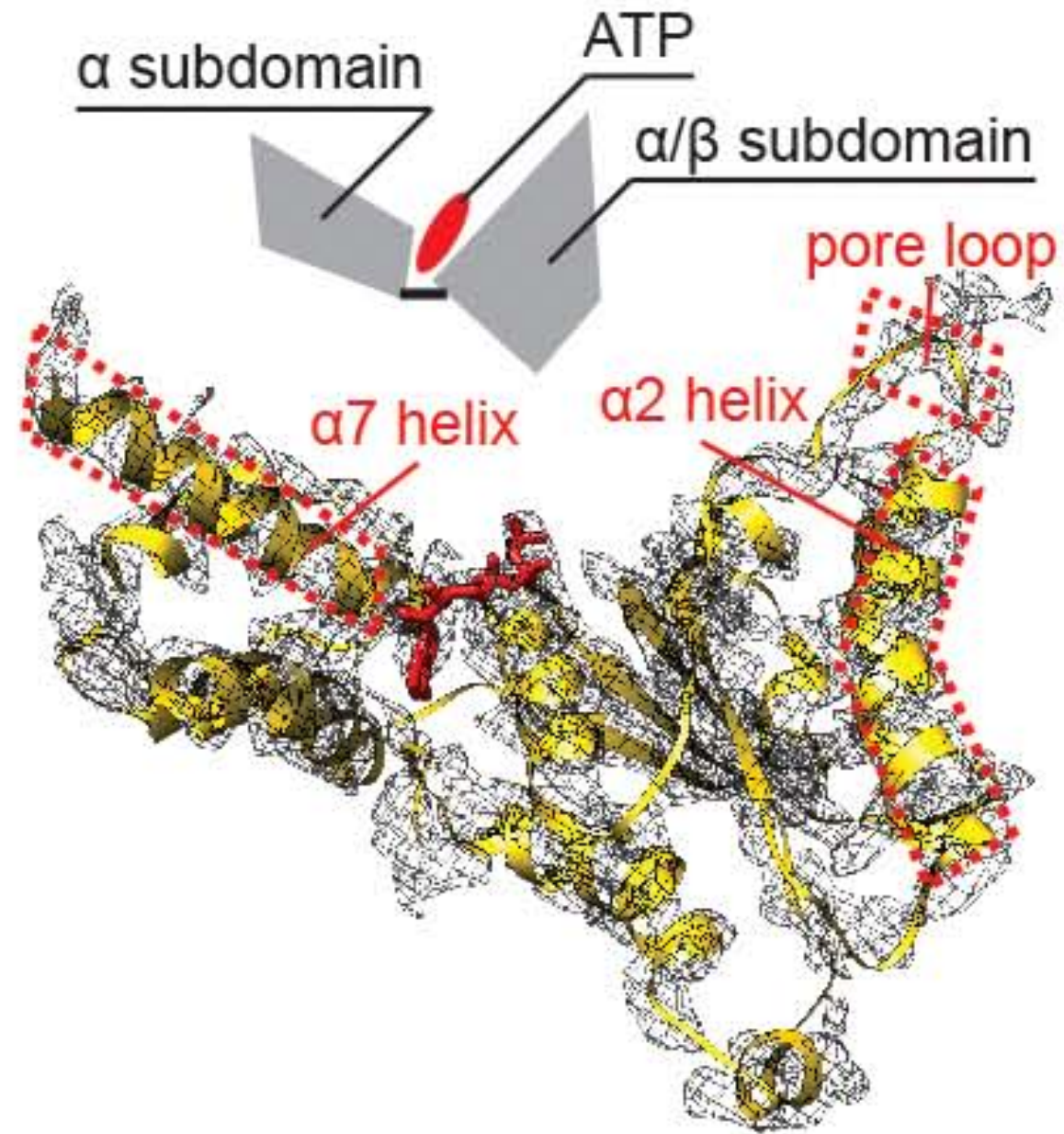
Structural features of ATP-bound NSF



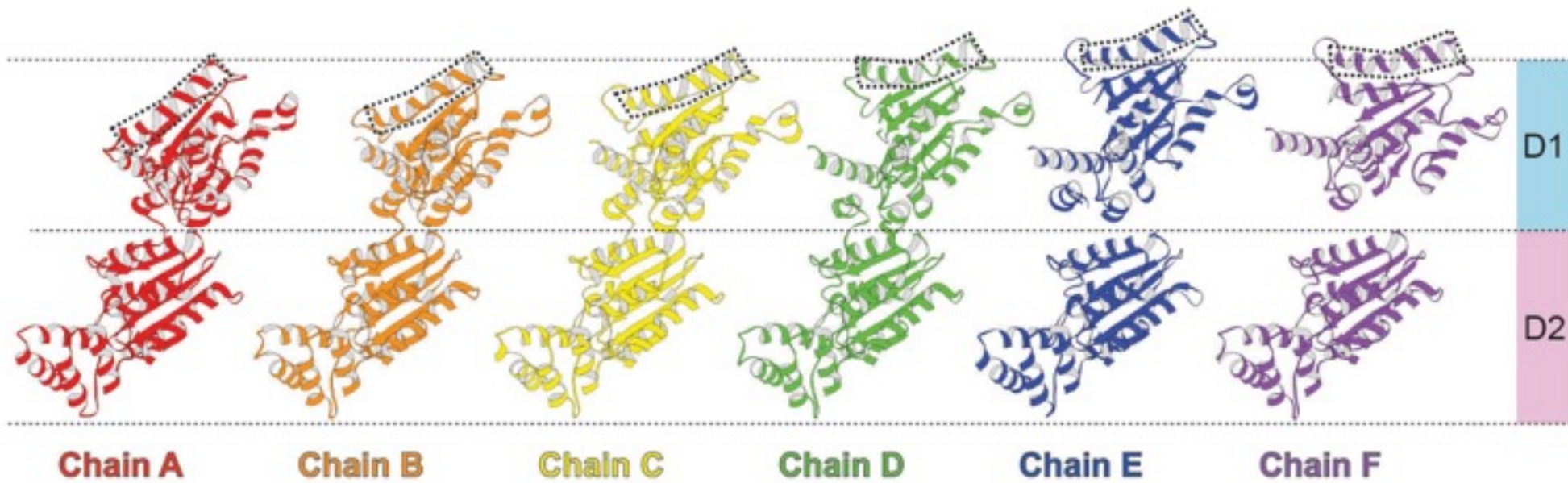
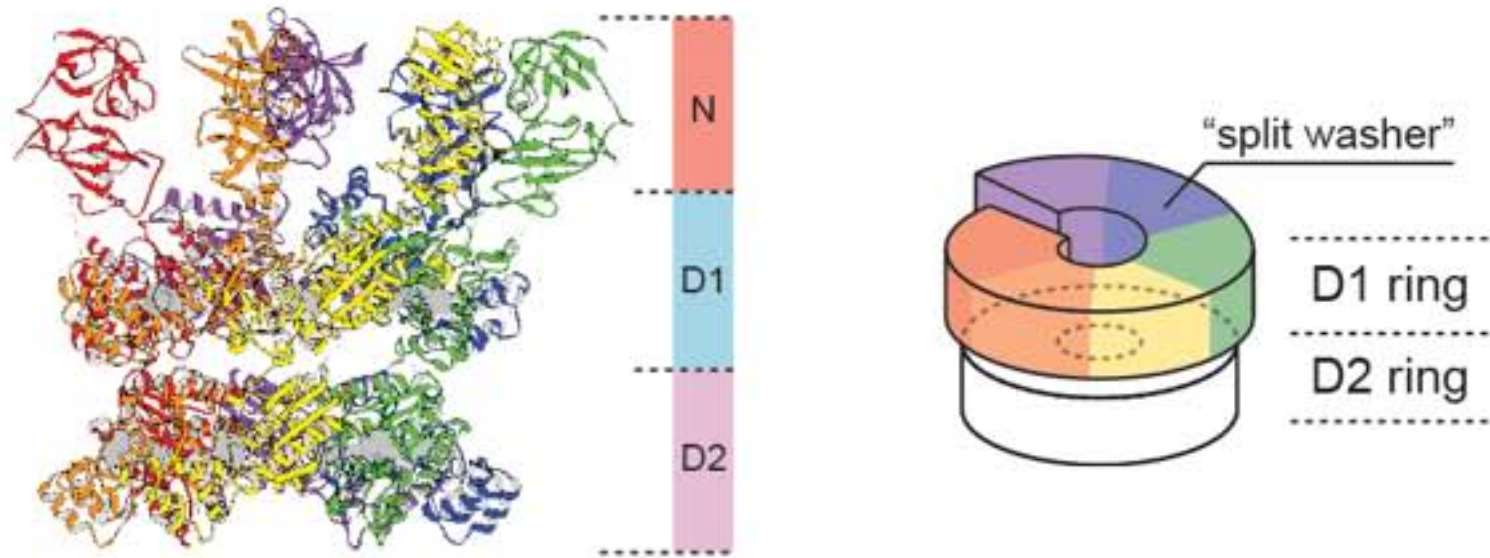
Structural features of ATP-bound NSF



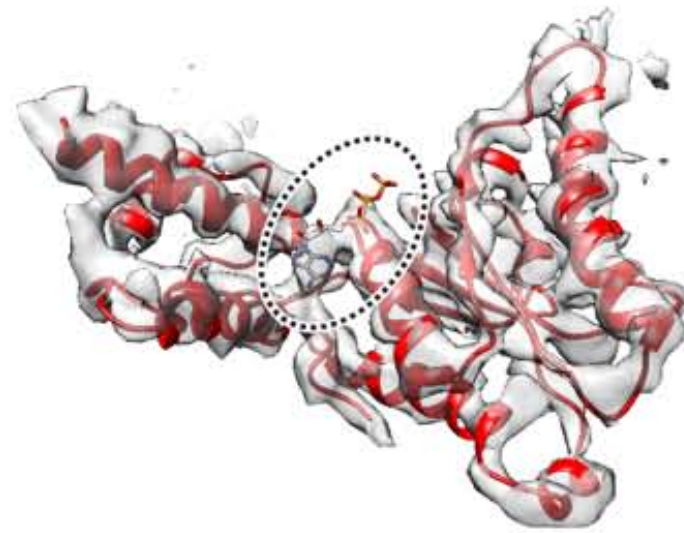
Model of the D1 domain



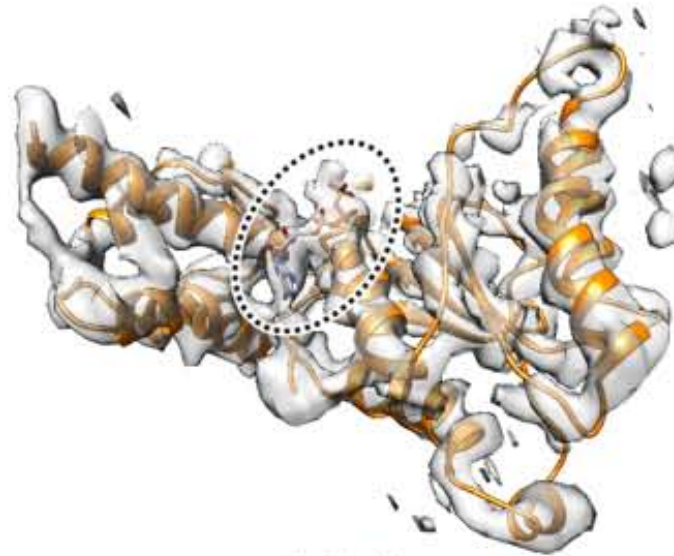
D1 ring of ATP-bound NSF is like a “split washer”



Nucleotide-binding pockets of the D1 domains



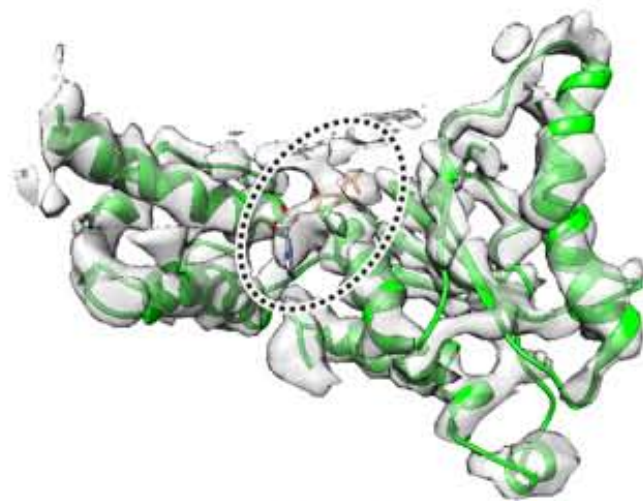
chain A



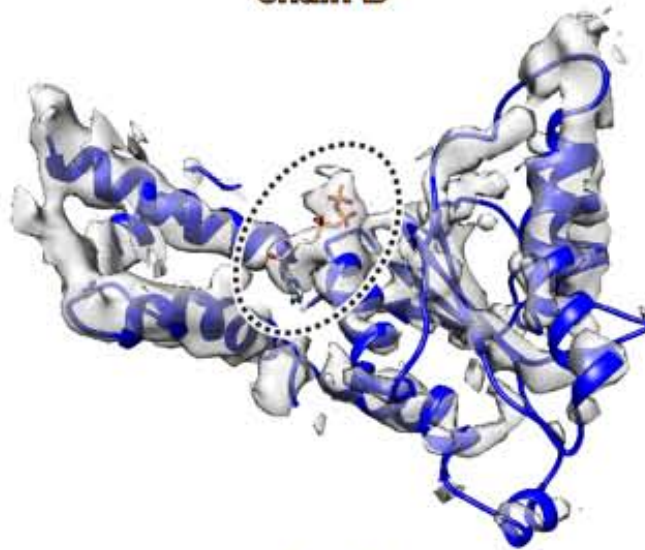
chain B



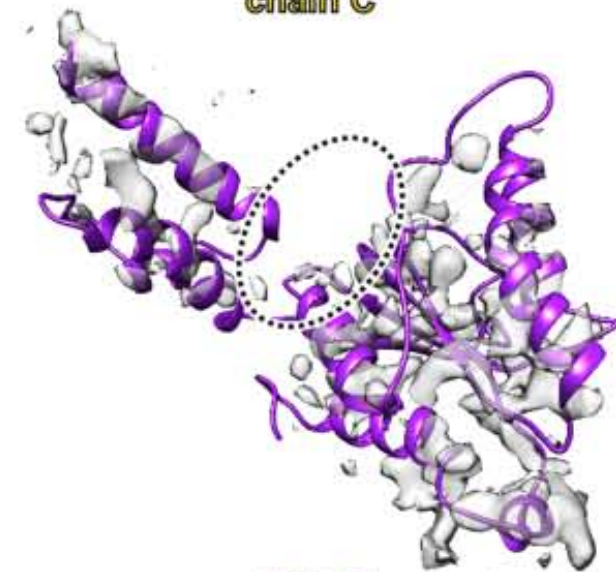
chain C



chain D

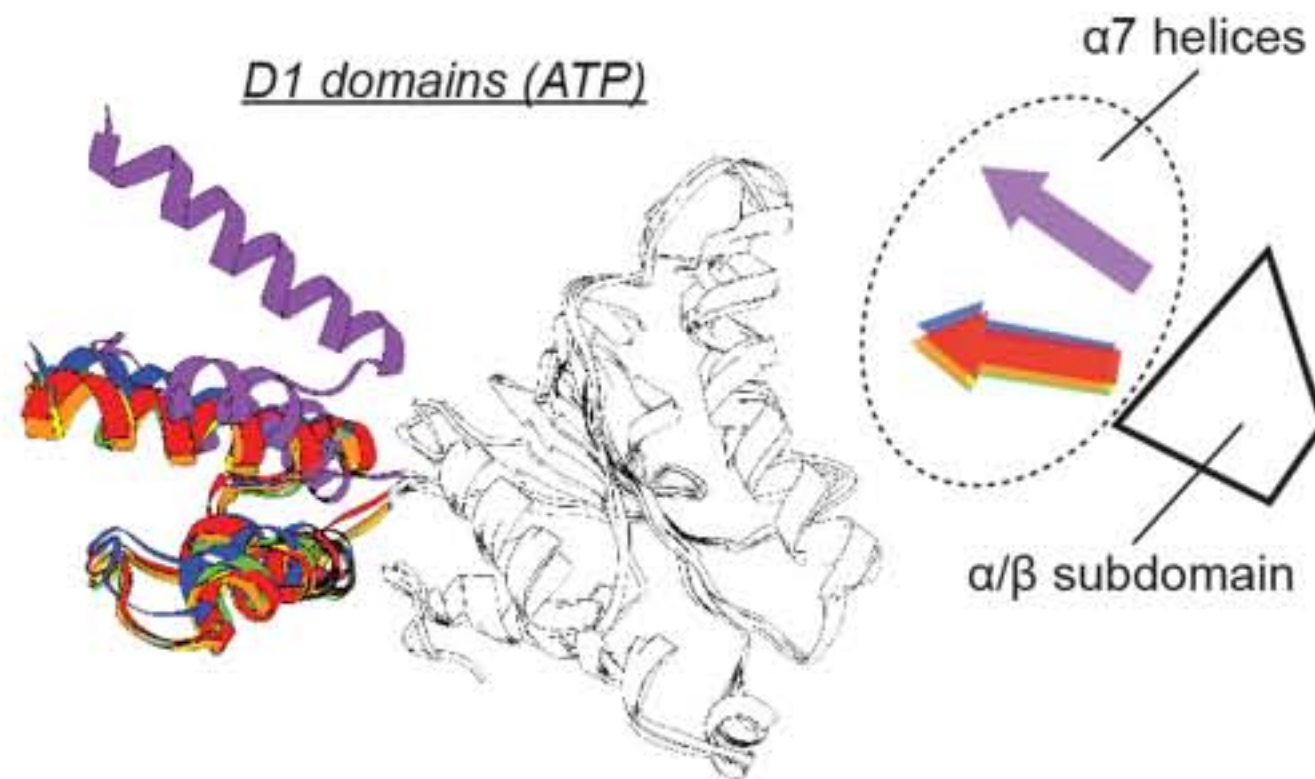


chain E

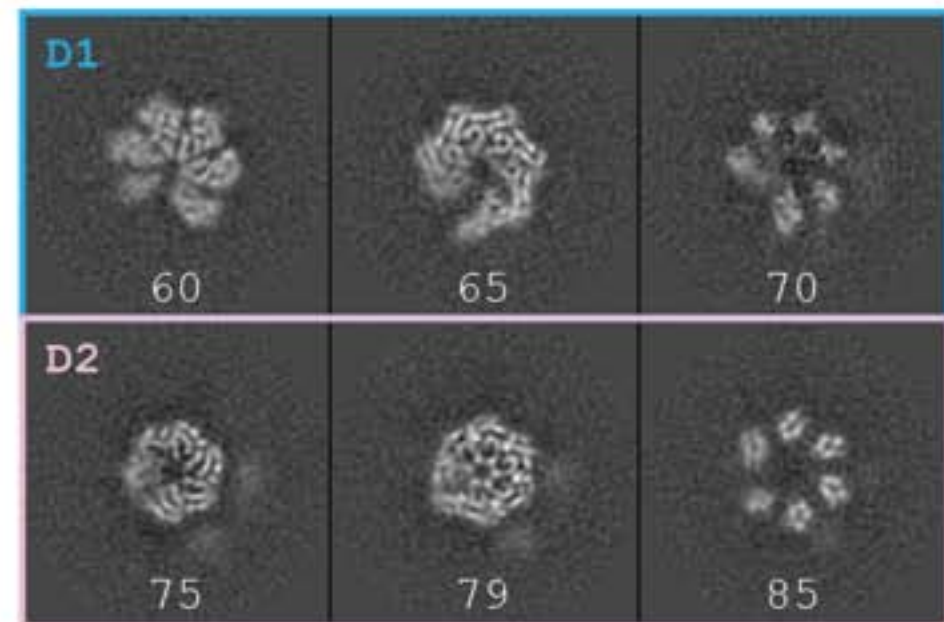
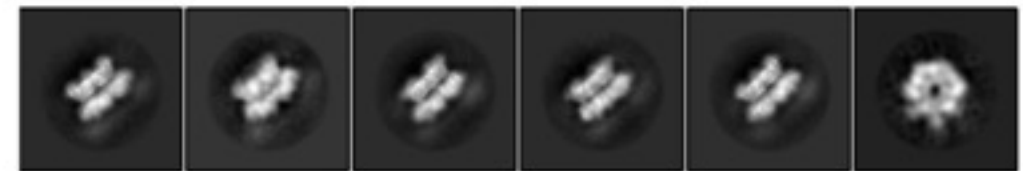
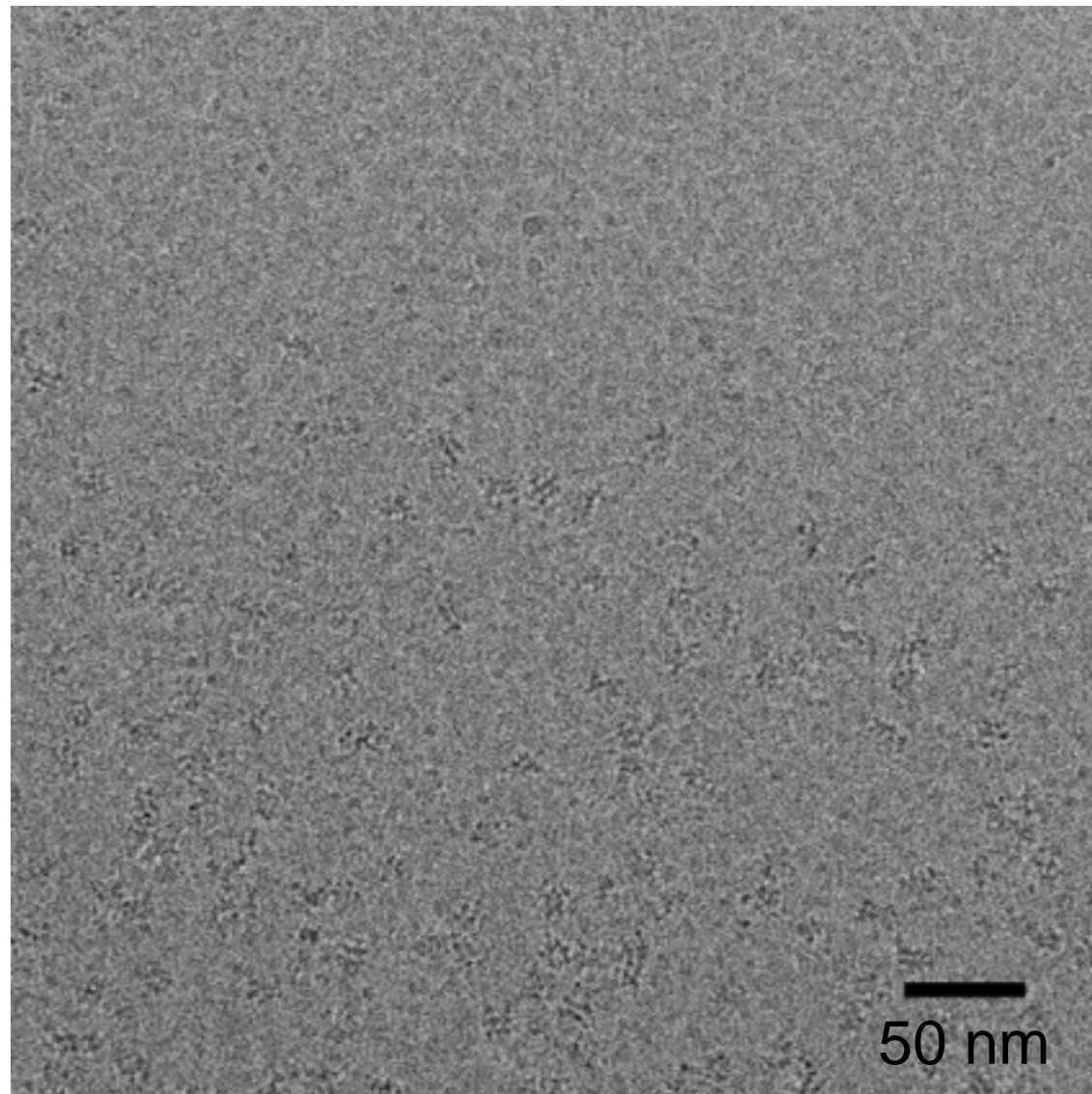


chain F

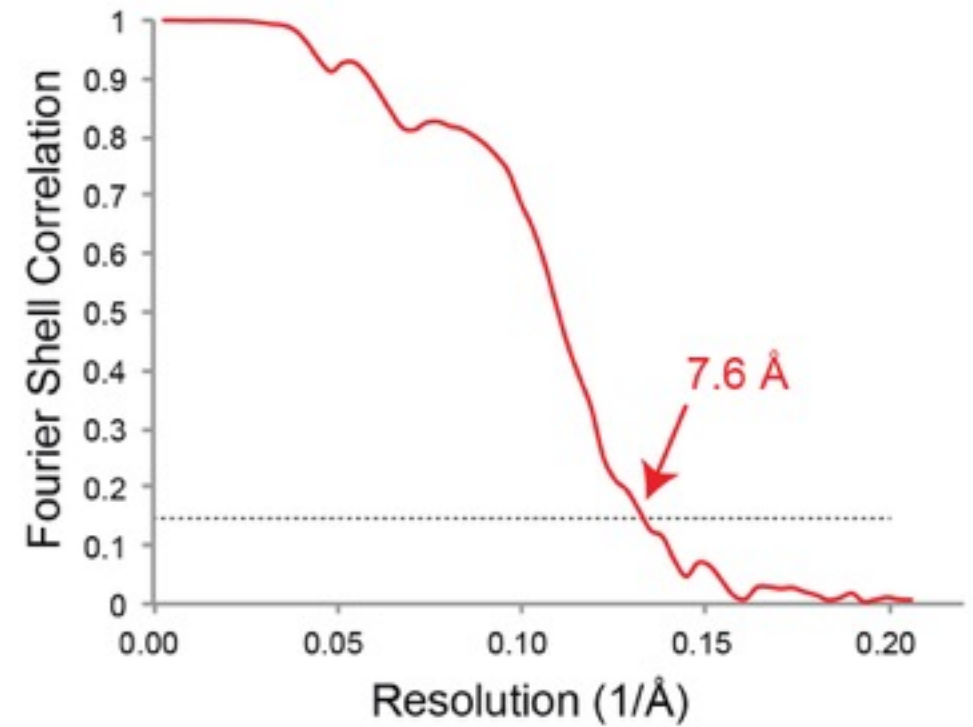
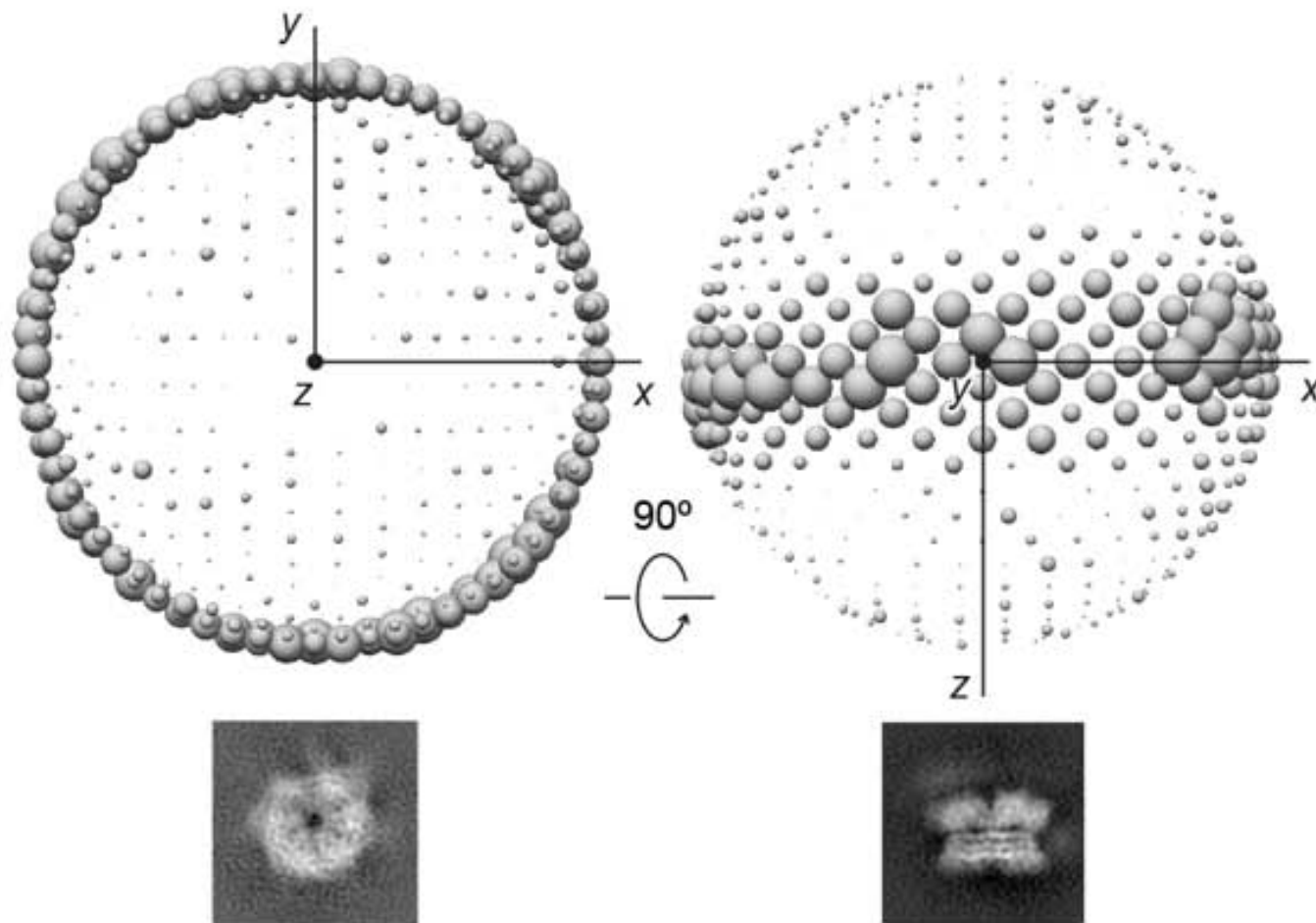
Superposition of the D1 domains



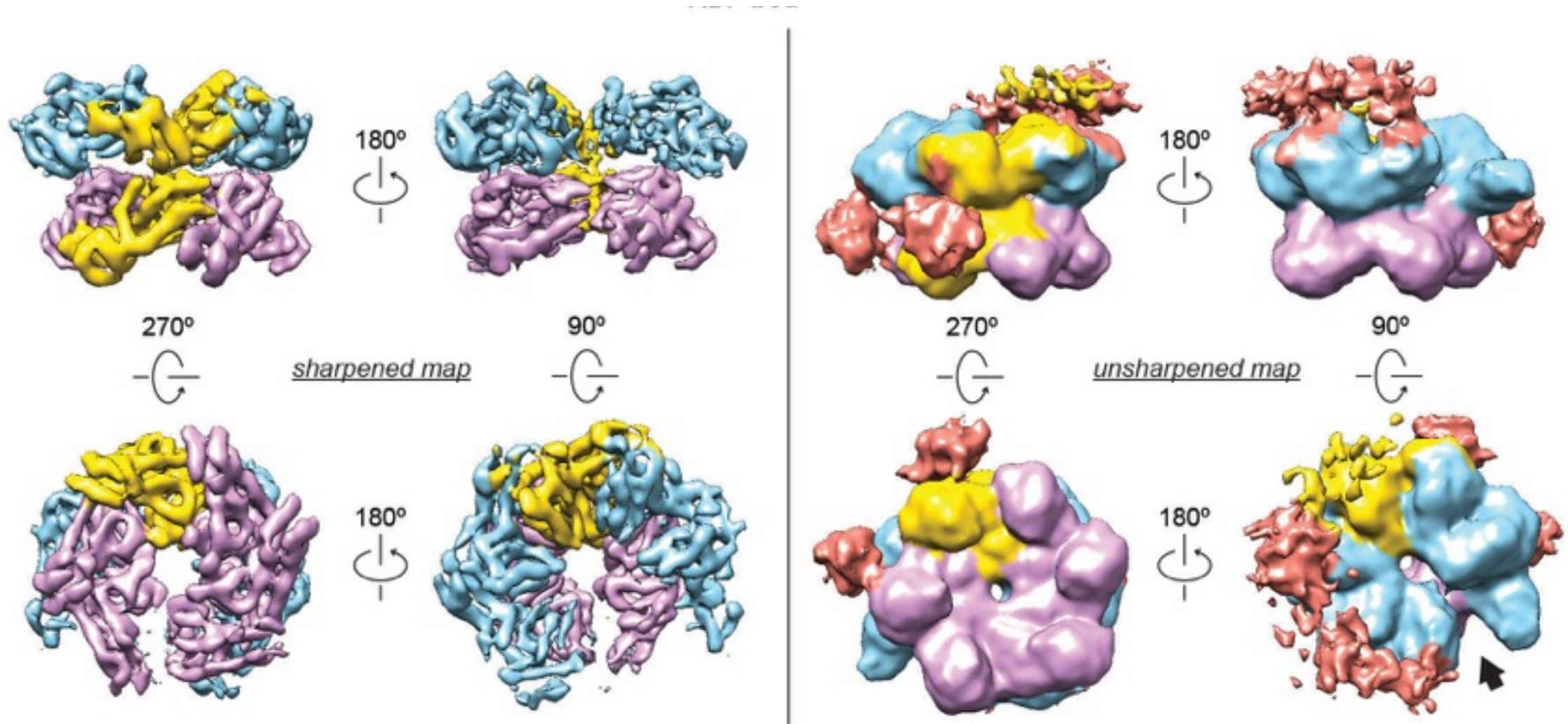
3D reconstruction of ADP-bound NSF by single-particle cryo-EM



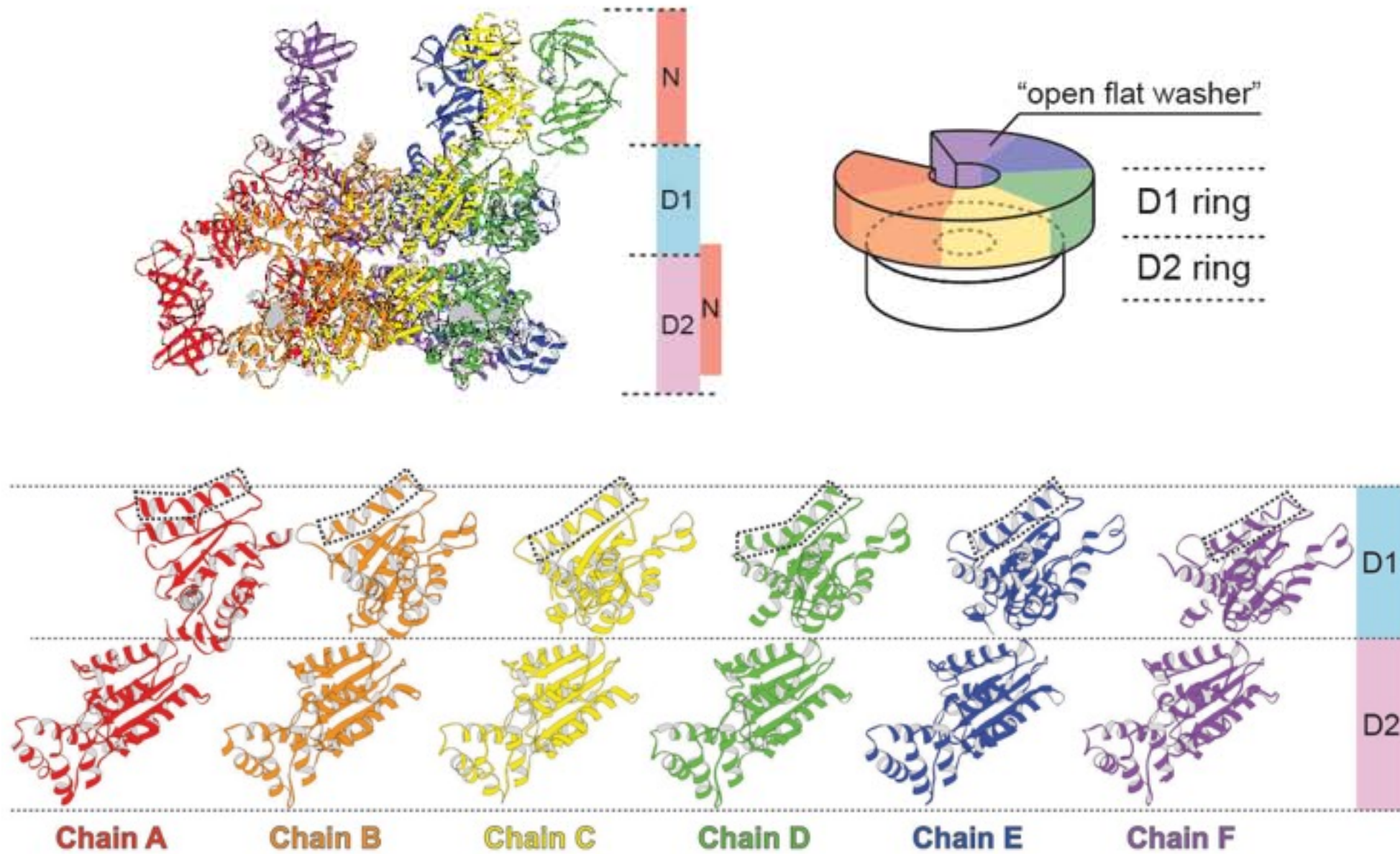
3D reconstruction of ADP-bound NSF by single-particle cryo-EM



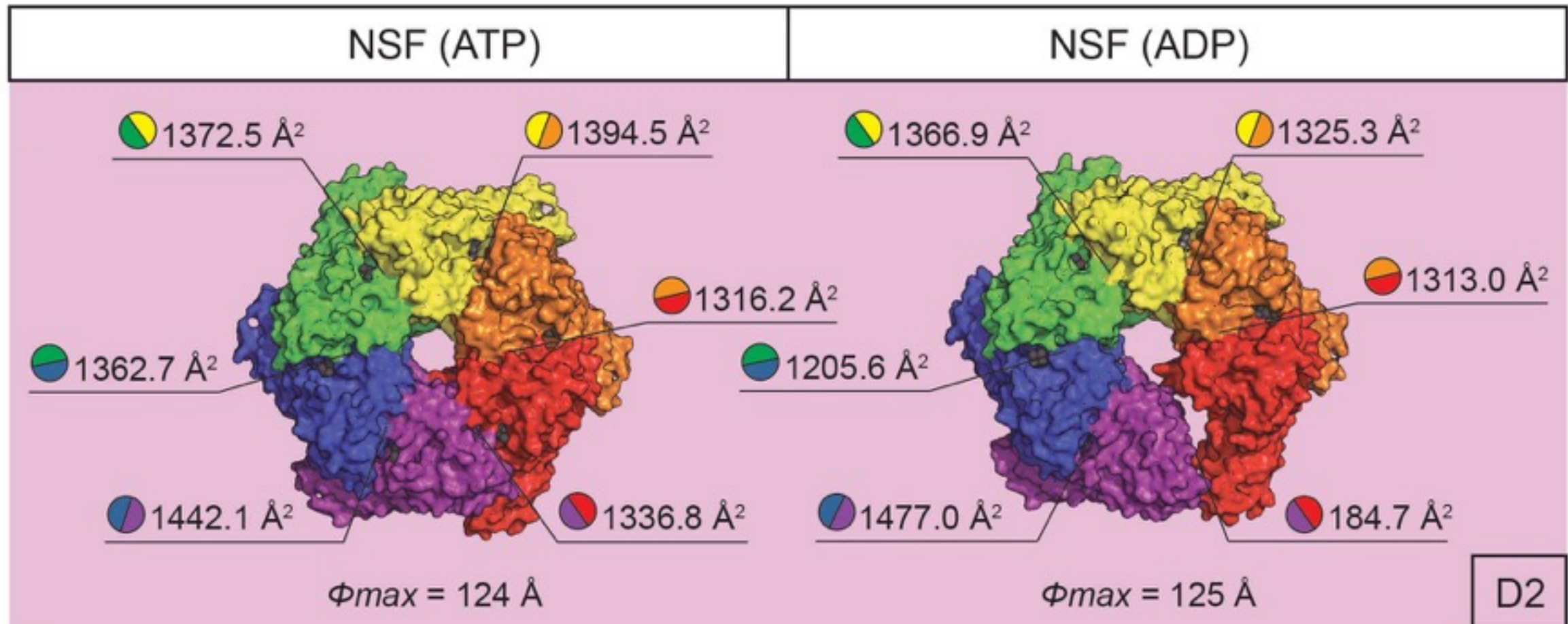
Maps of ADP-bound NSF



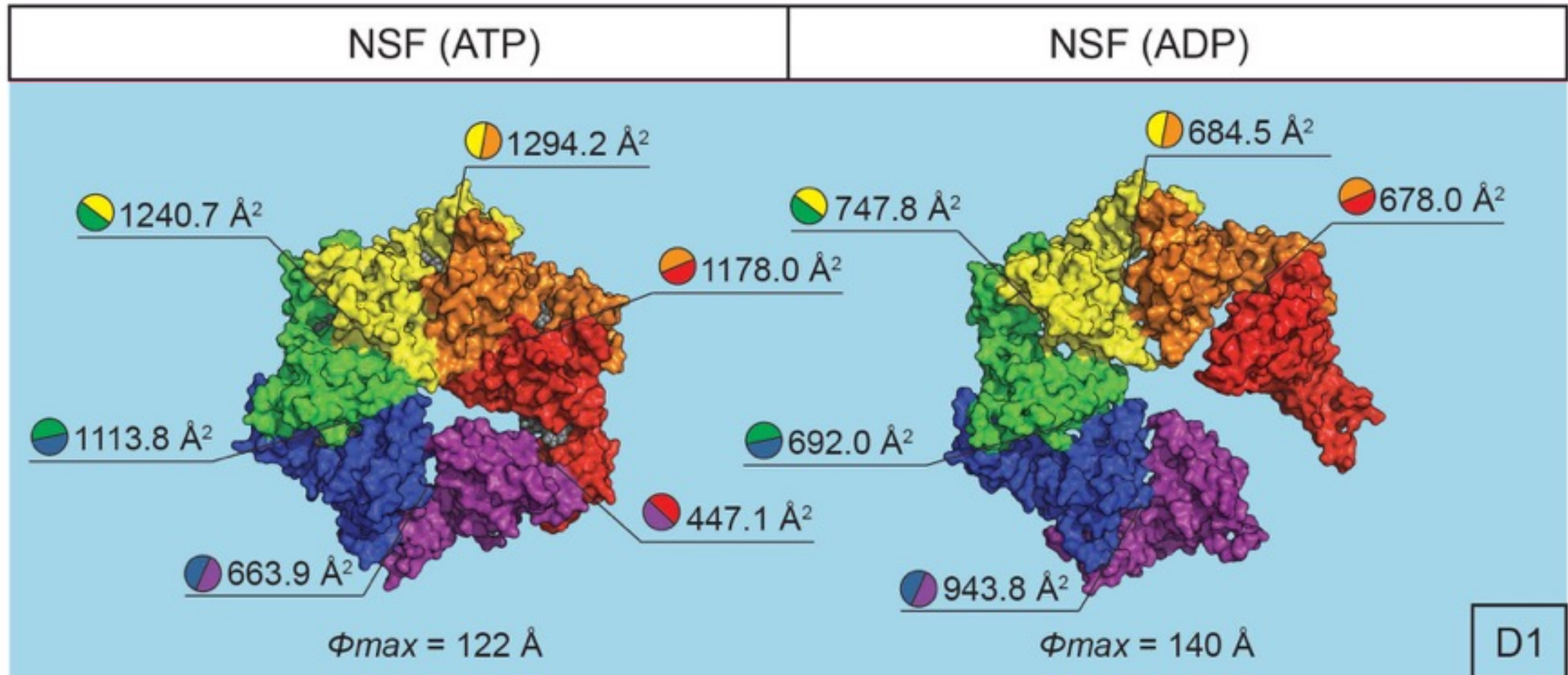
D1 ring of ADP-bound NSF is an “open flat washer”



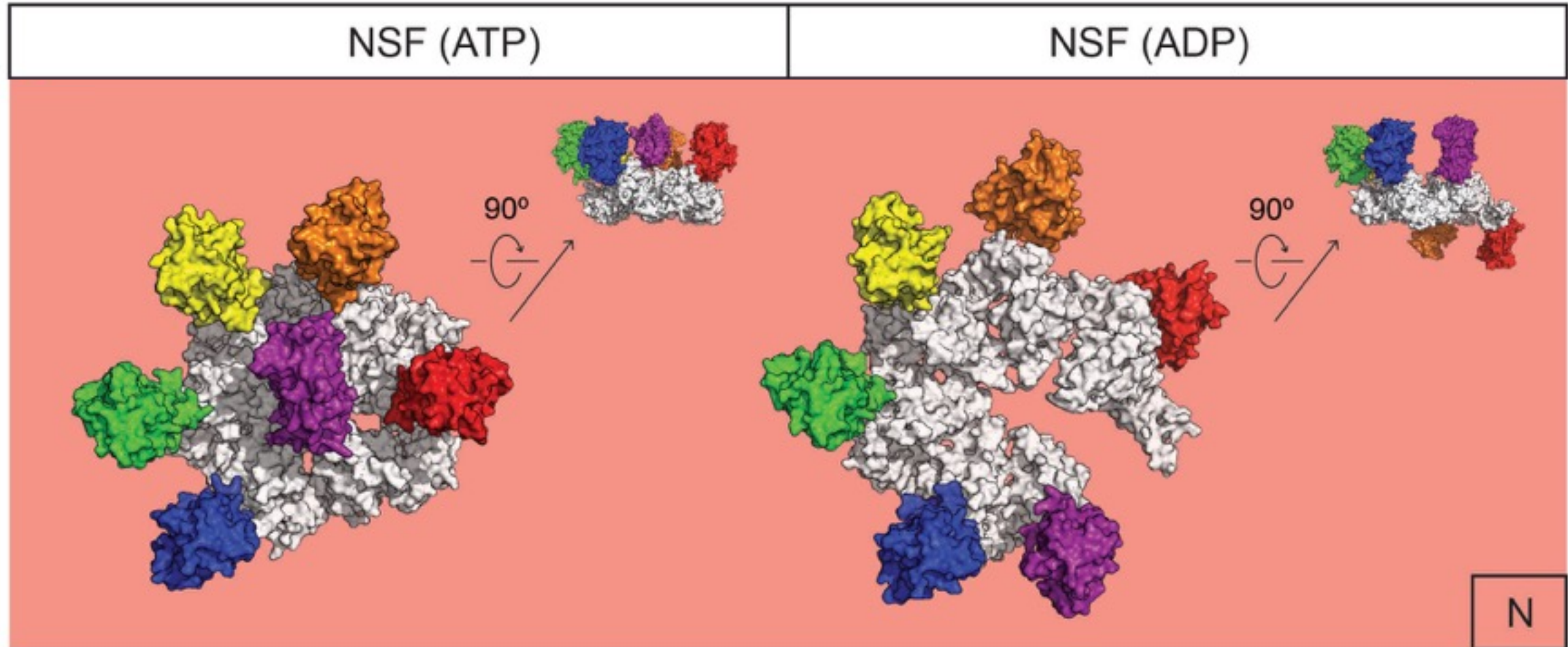
ATP-bound NSF vs. ADP-bound NSF



ATP-bound NSF vs. ADP-bound NSF



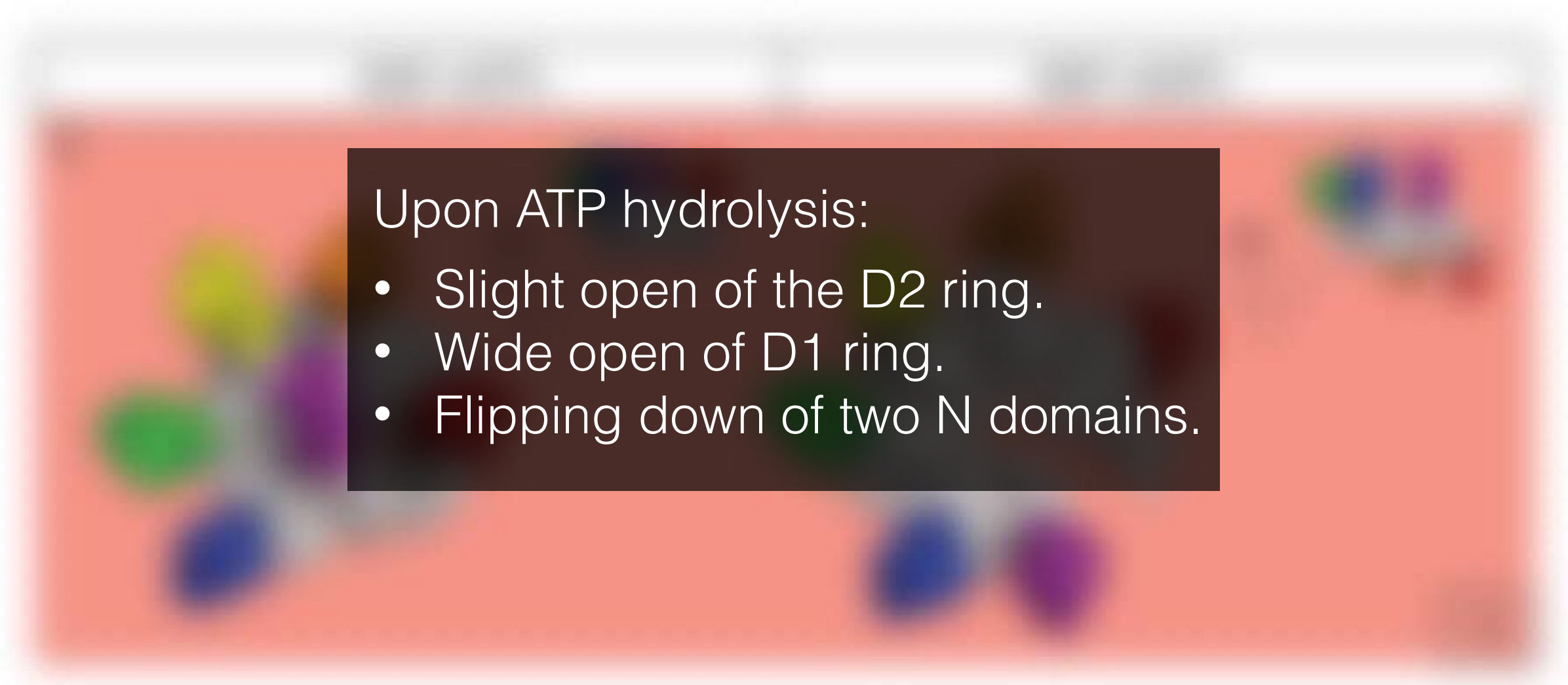
ATP-bound NSF vs. ADP-bound NSF



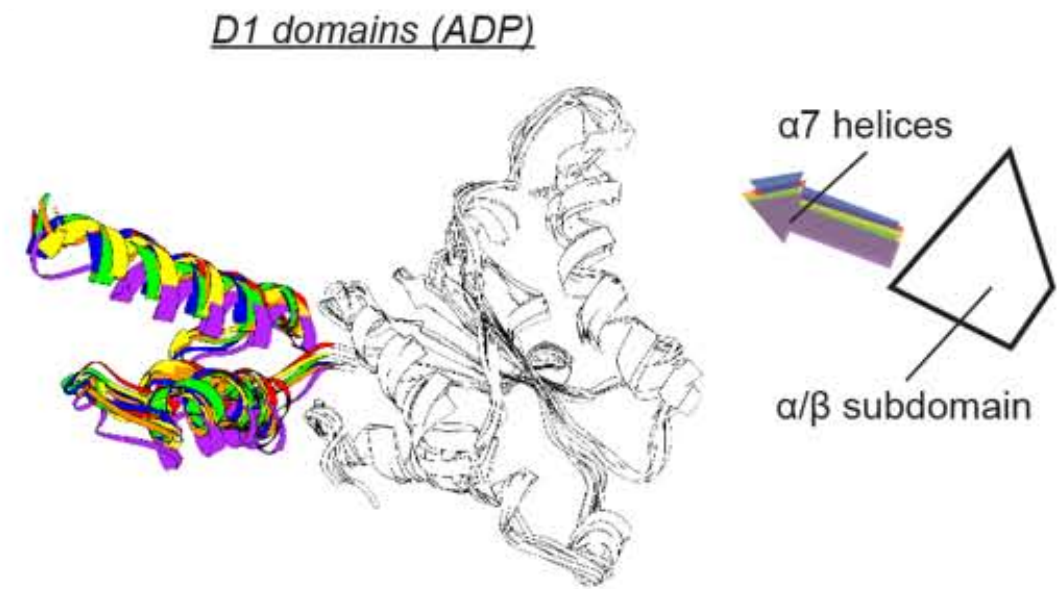
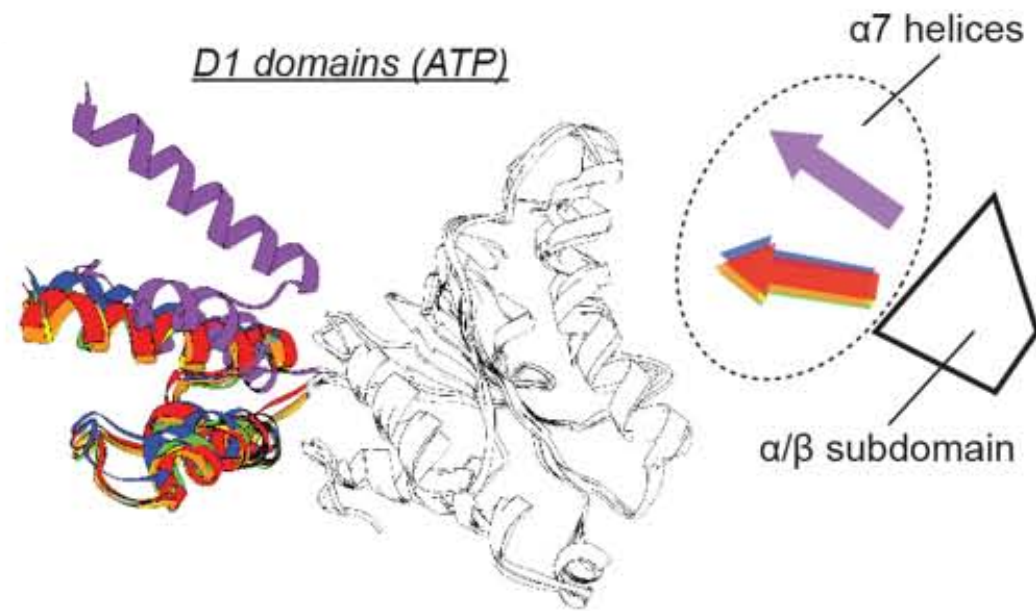
ATP-bound NSF vs. ADP-bound NSF

Upon ATP hydrolysis:

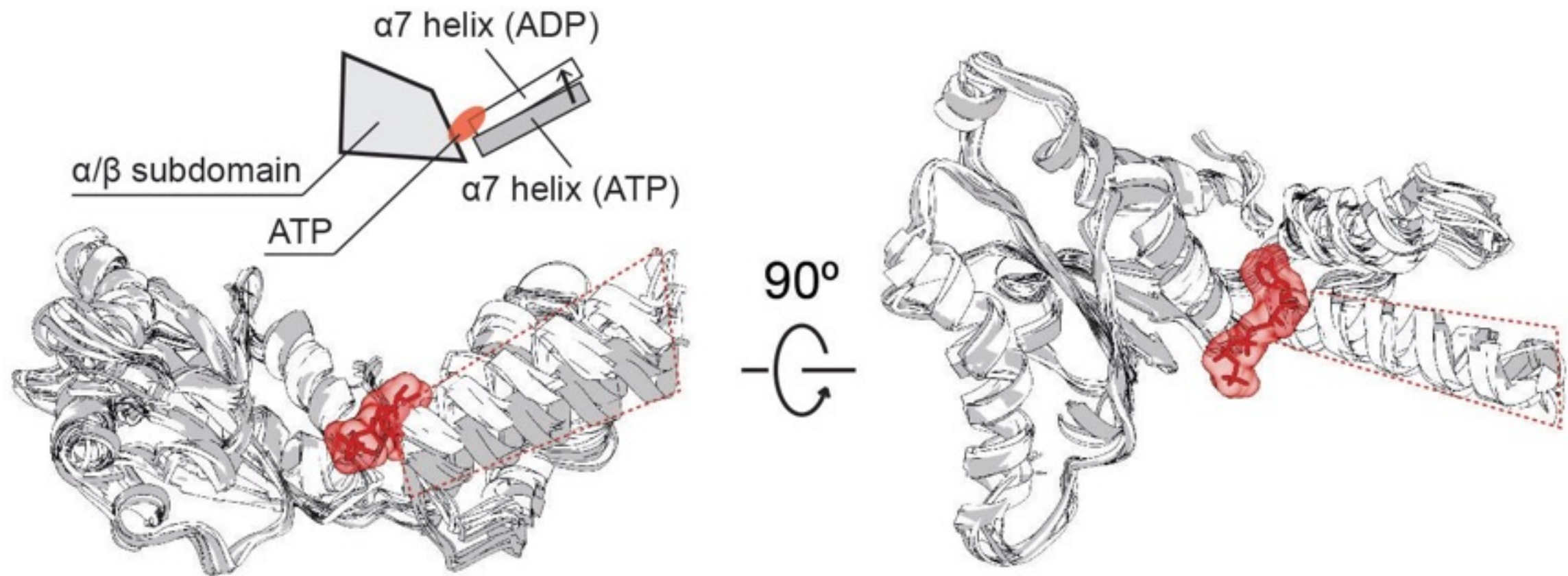
- Slight open of the D2 ring.
- Wide open of D1 ring.
- Flipping down of two N domains.



Superposition of the D1 domains

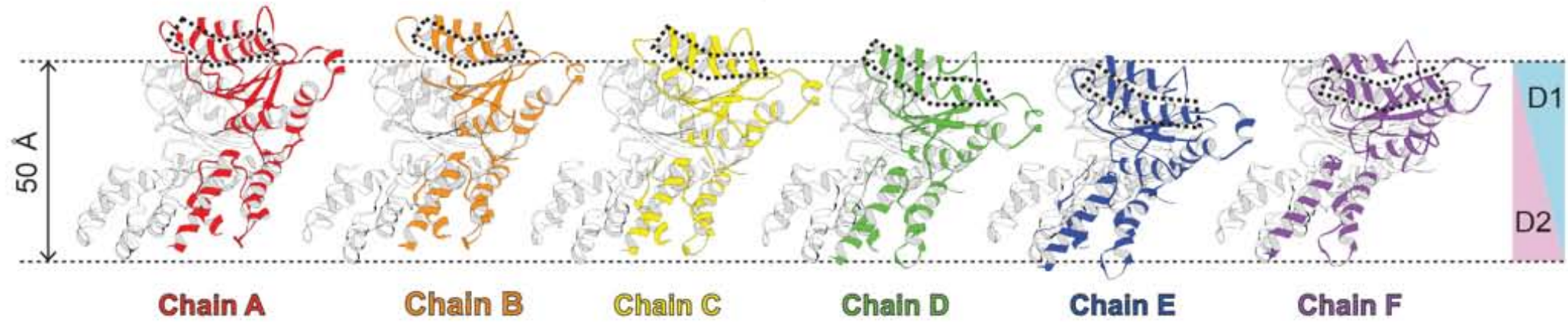


Conformational change of D1 domains upon ATP hydrolysis

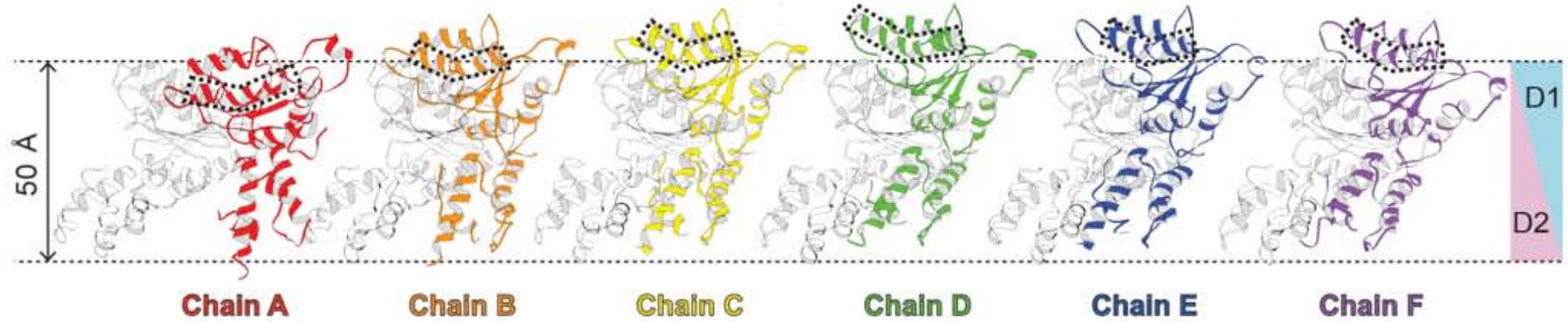


Outward movement of the D1 ring upon ATP hydrolysis

ATP-bound NSF



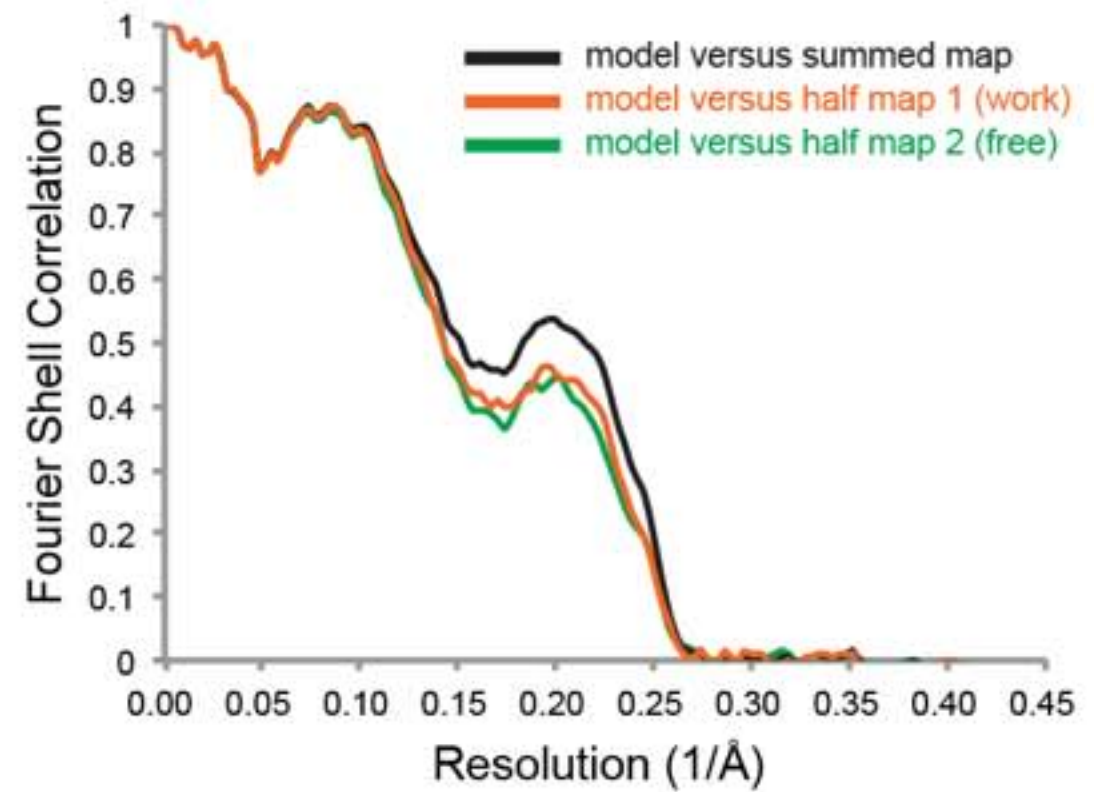
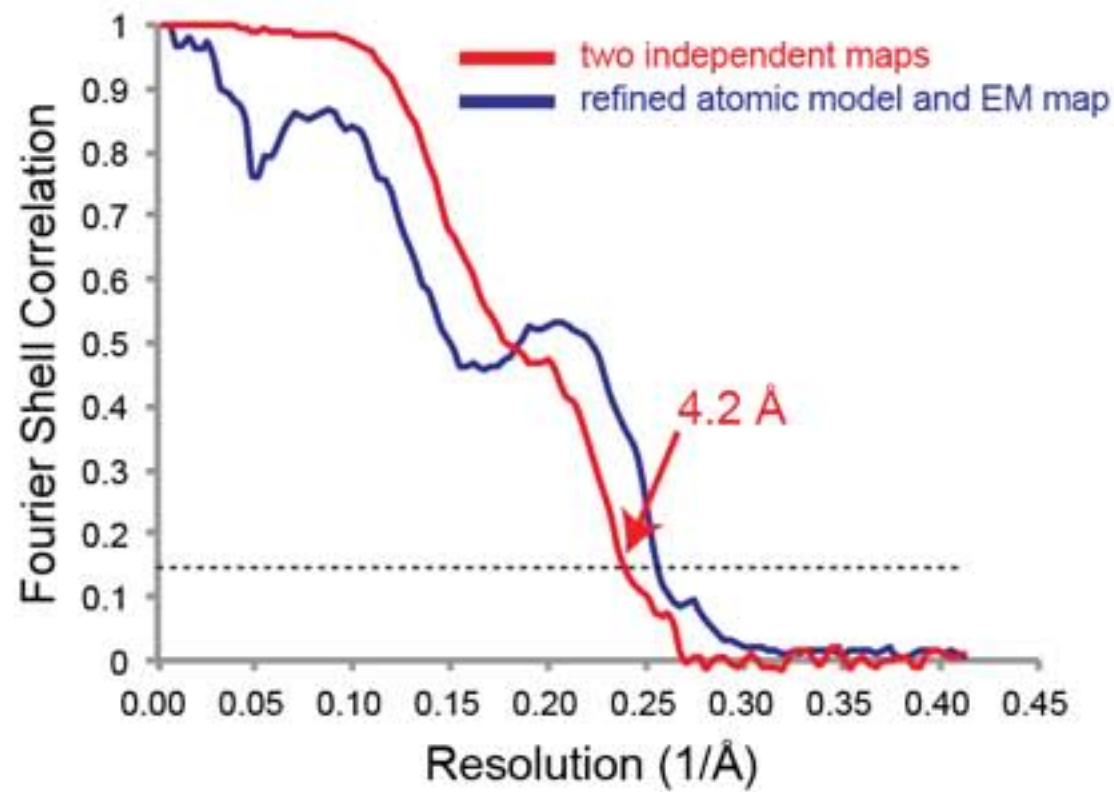
ADP-bound NSF



Single-particle cryo-EM vs. X-ray crystallography (personal experience)

	X-ray	cryo-EM
Sample preparation	Crystals!	Crystals?
Data collection	Mostly remote 10 min/dataset 360 degree	Remote? 1~2 days/dataset How much is enough?
Data processing	Concurrently to Several hours	1 week?
Model building	COOT Methods for low resolution model building	COOT? More tools needed!
Cross-validation	Rwork/Rfree	Better methods?

Model validation of ATP-bound NSF





STANFORD
SCHOOL OF MEDICINE

UCSF

University of California
San Francisco



Axel Brunger



Qiangjun Zhou



Yifan Cheng



Shenping Wu



Daniel Cipriano



Sandro Vivona

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

