The Human p97 Complex

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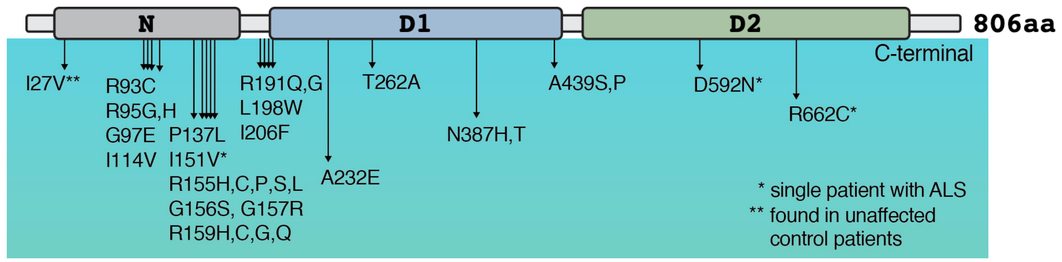
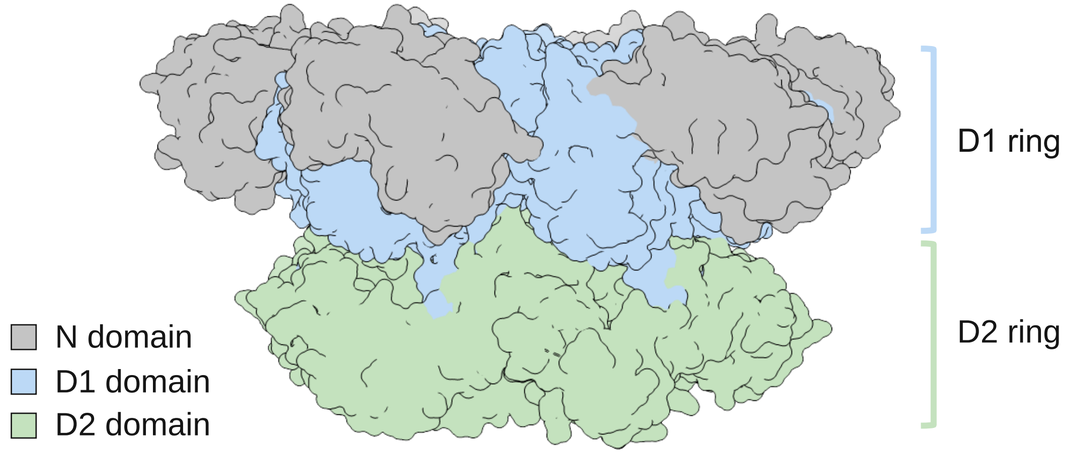
p97 (also known as VCP or Cdc48) is an abundant ATPase

- First discovered from yeast genetic screen
- Type-II AAA Family (ATPases Associated with various cellular Activity)
- One of the most abundant ATPases in cytoplasm
- Mutations associated with several neurodegenerative diseases
- Cancer/antiviral drug target

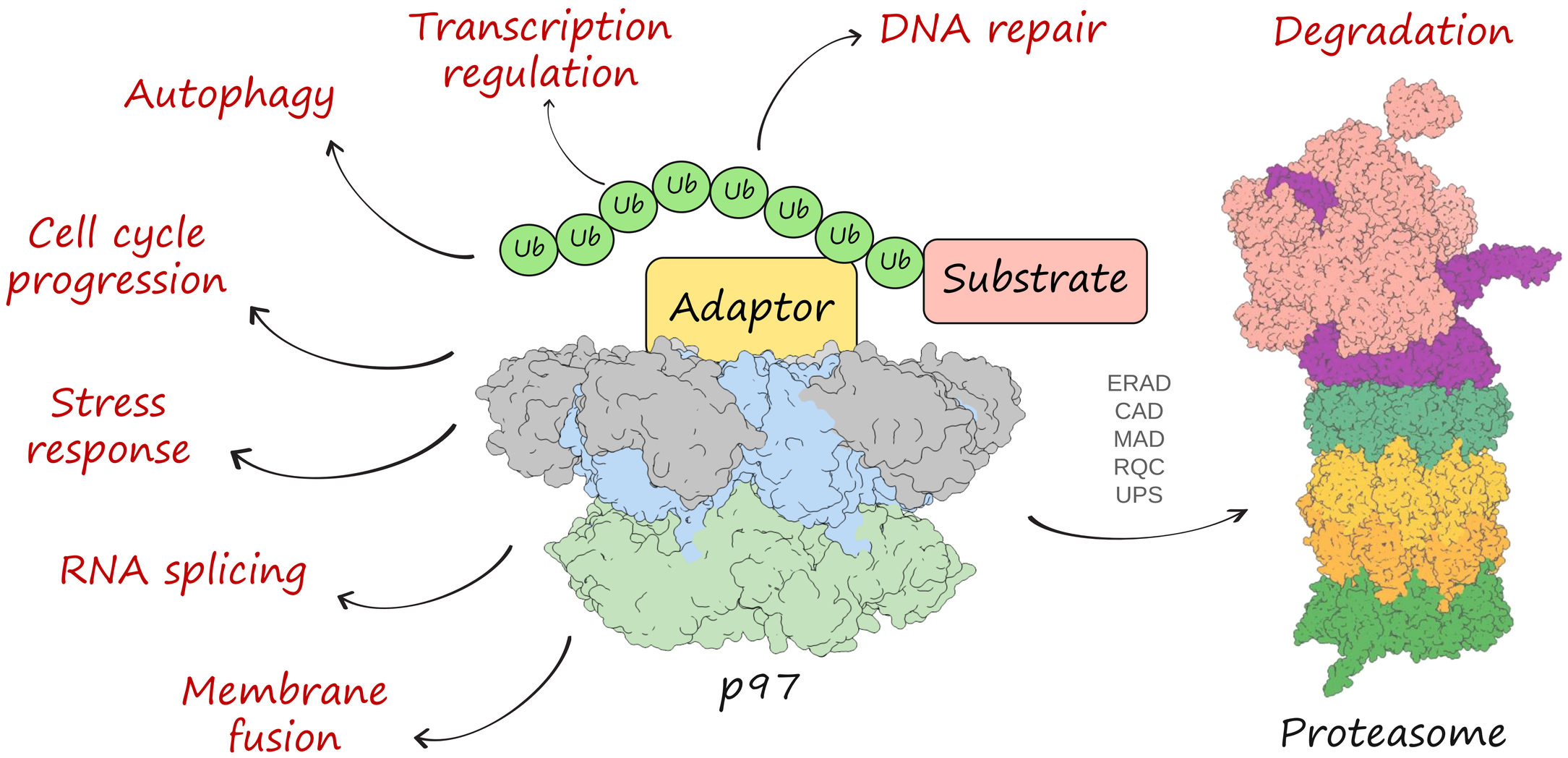


Meyer H and Weihl CC, *J. Cell Sci.*, 2014

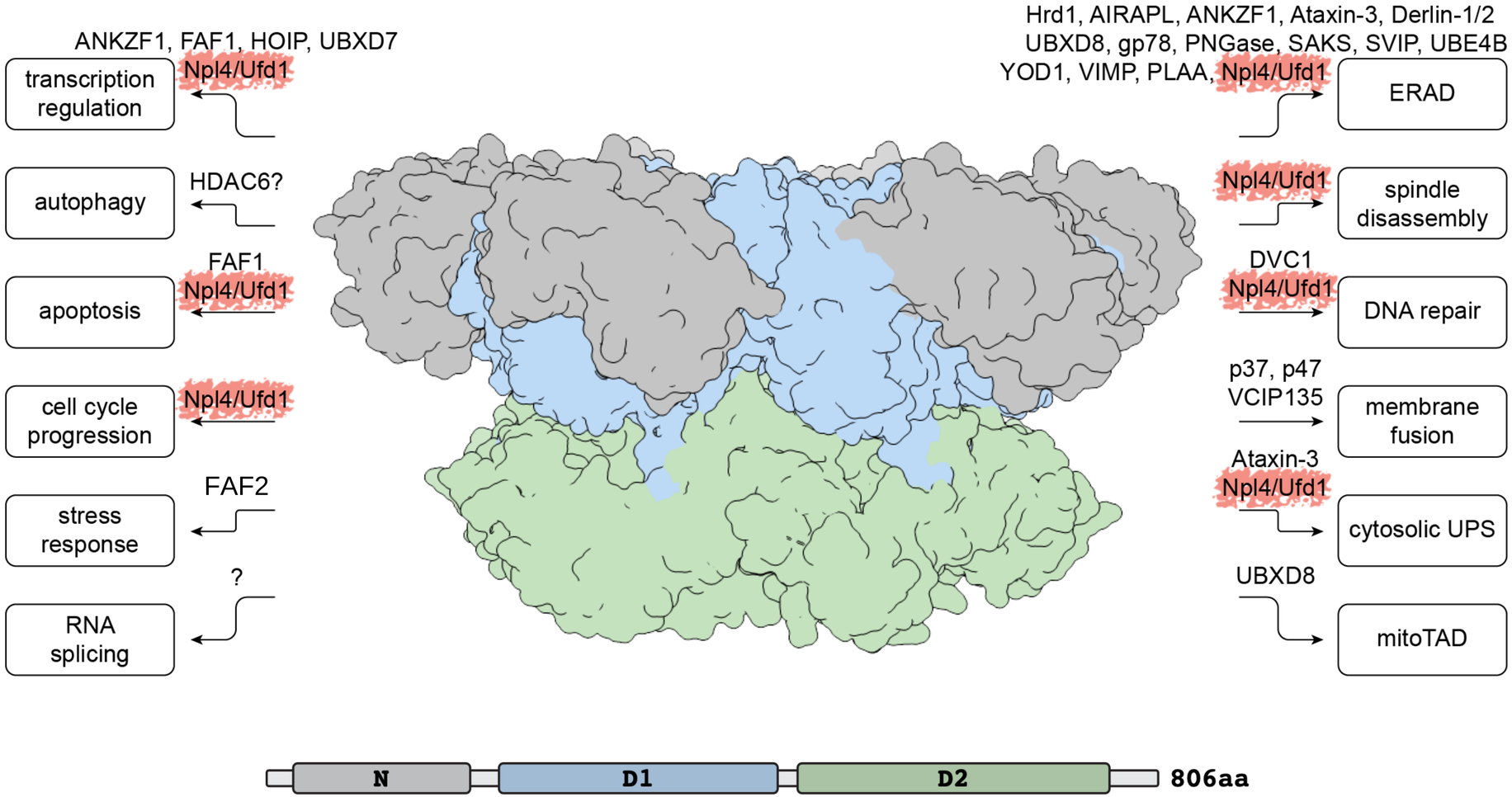


Davies JM, Brunger AT, Weis WI, *Structure,* 2008

p97 (VCP/Cdc48) is a central hub of cellular ubiquitin system



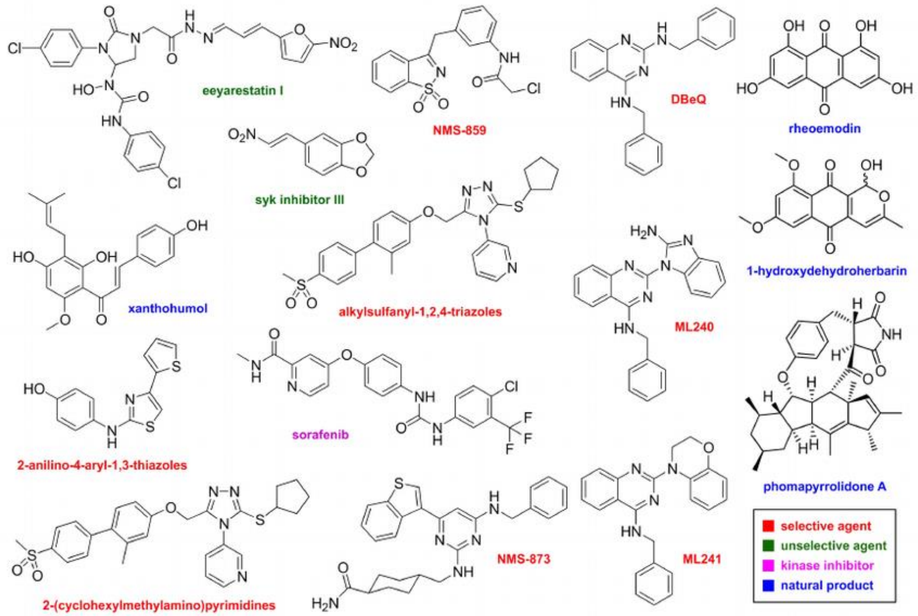
p97 participates in cellular processes via various adaptors



Adapted from Chapman E, *et al., Molecules,* 2015

Outstanding questions

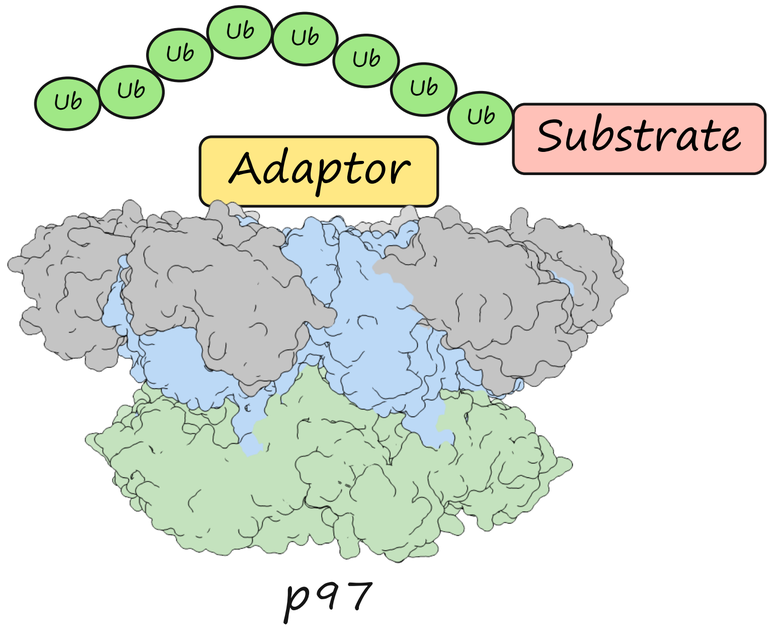
- How does p97 process ubiquitinated substrates via various cofactors?
- What are the physiological roles of disease mutations?
- What are the mechanisms of various inhibitors?

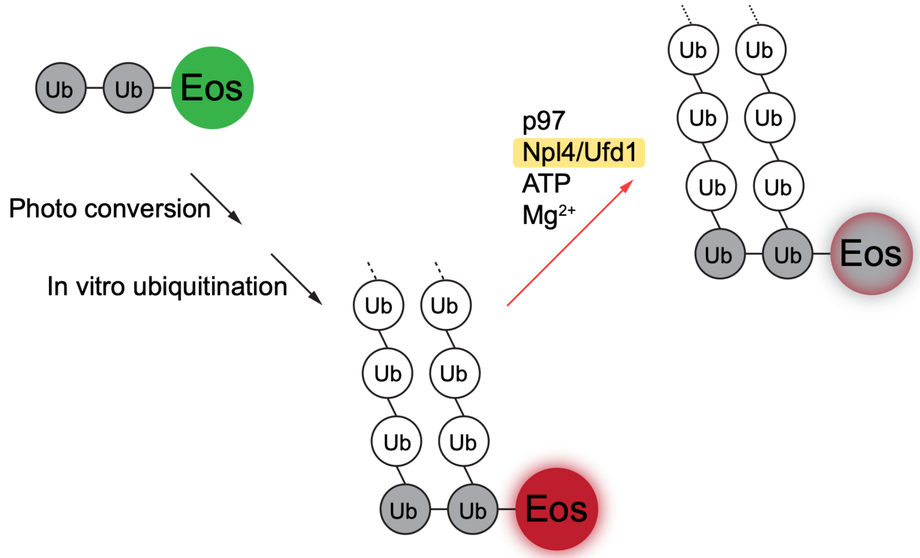


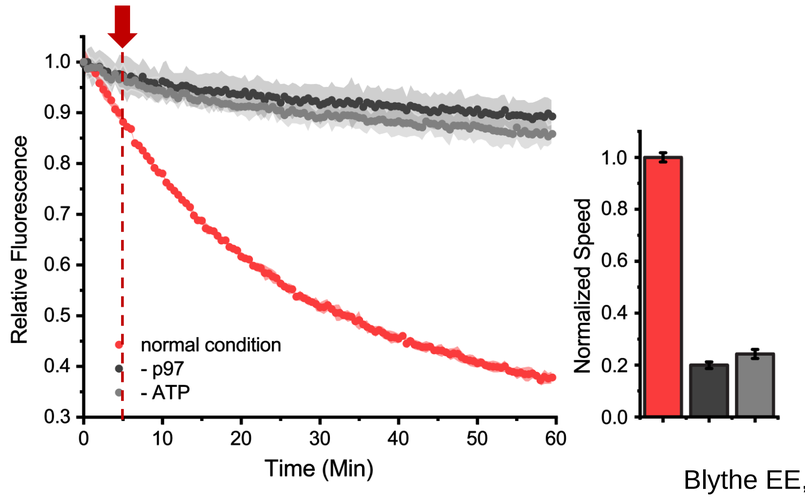
Chapman E, *et al., Molecules,* 2015

Capturing the p97 complex *in vitro*

Dr. Man Pan

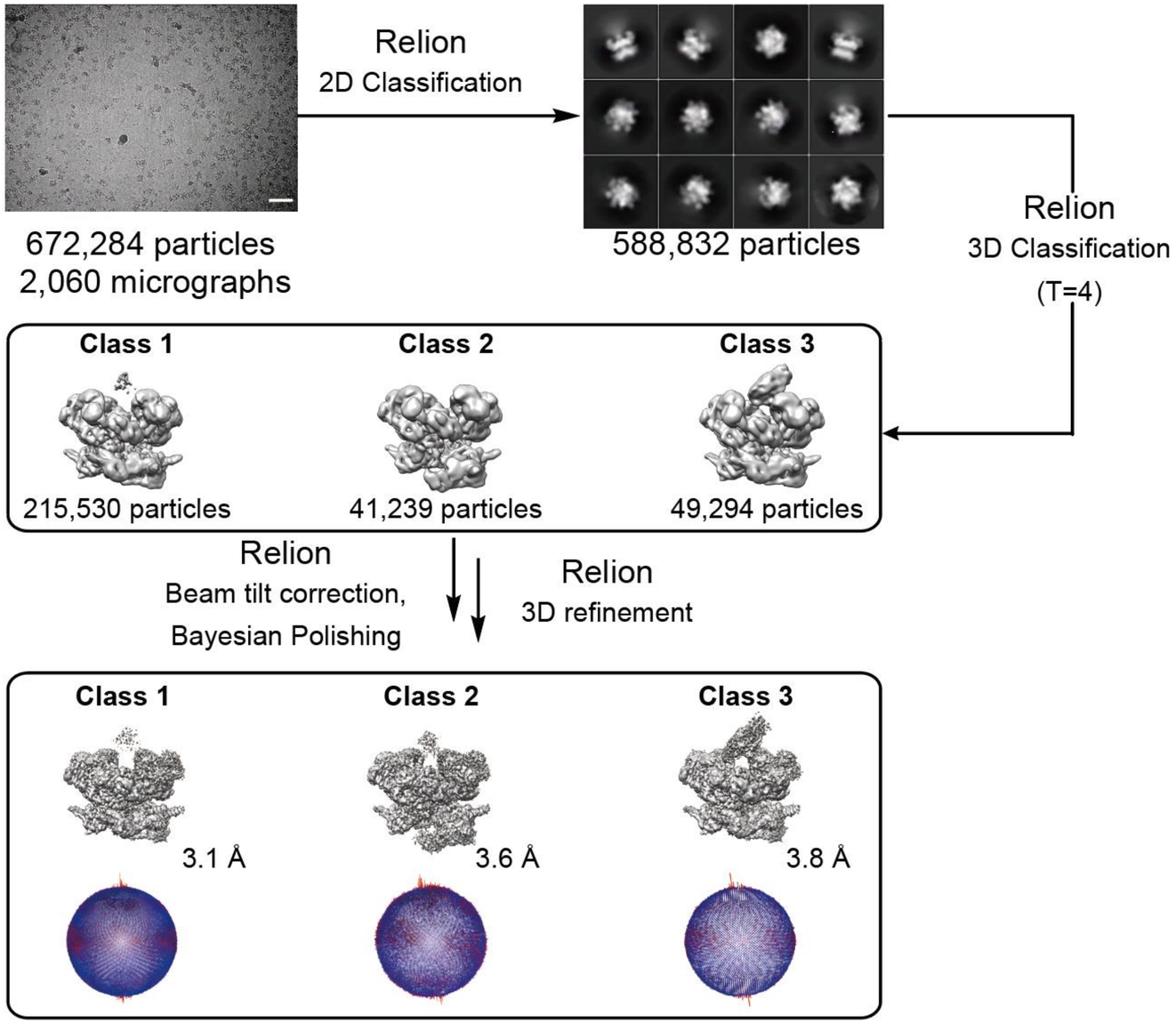


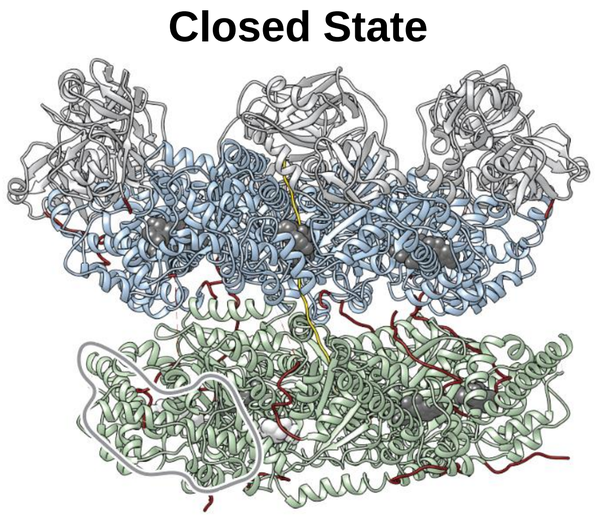


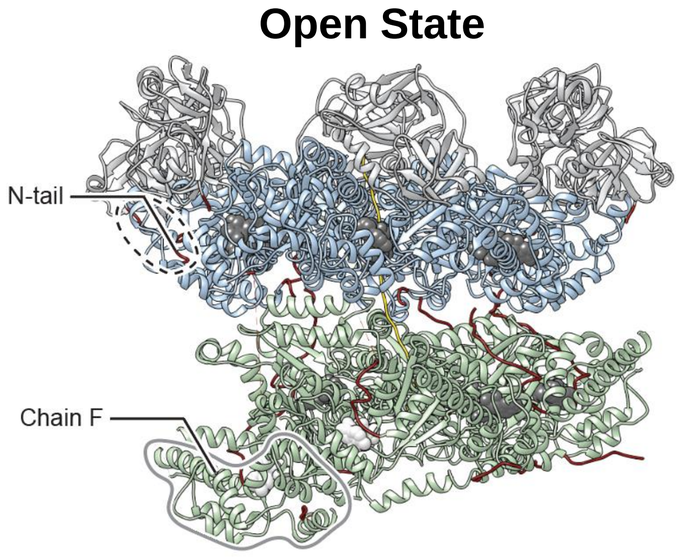


Blythe EE, et al., *PNAS*, 2017
Bodnar NO, Rapoport TA, *Cell*, 2017
Pan M, et al., *Nature Communications*, 2021

Single-particle cryo-EM analysis of the p97 complex



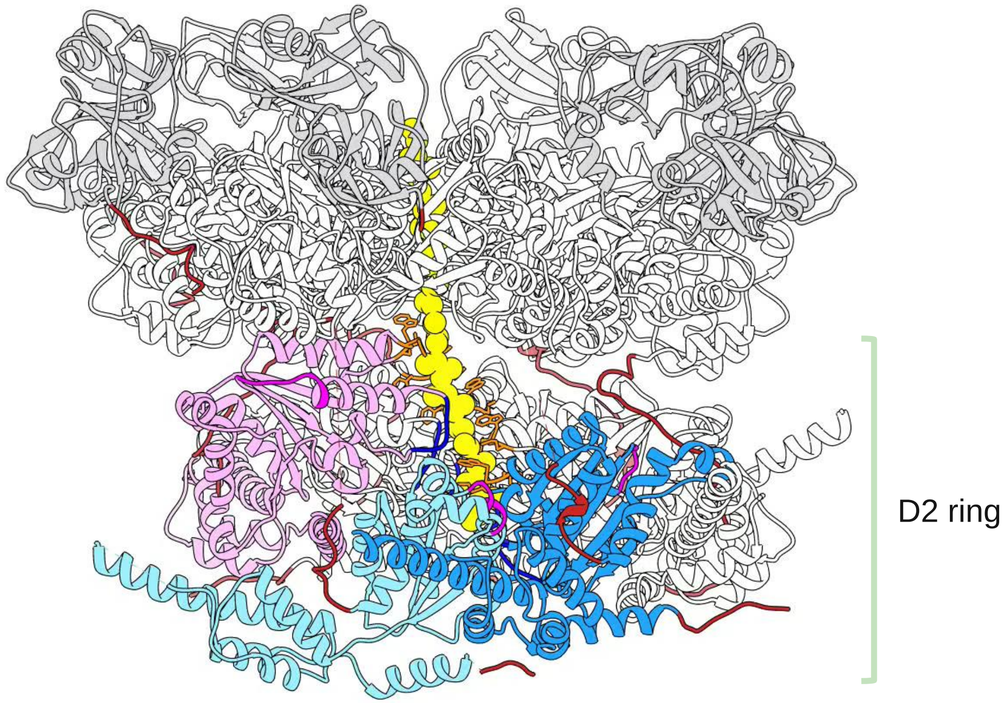




Pan M, et al., *NSMB*, 2021

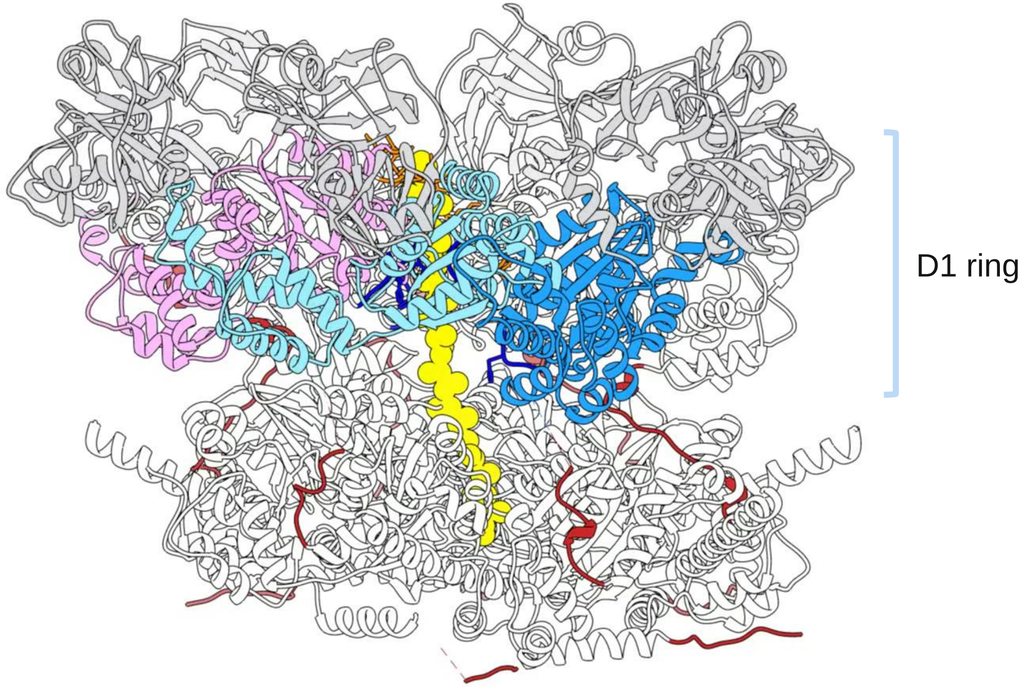
Staircase conformation and power stroke motion of D1 and D2 rings

Open State ↔ Closed State



CHAPSO dataset

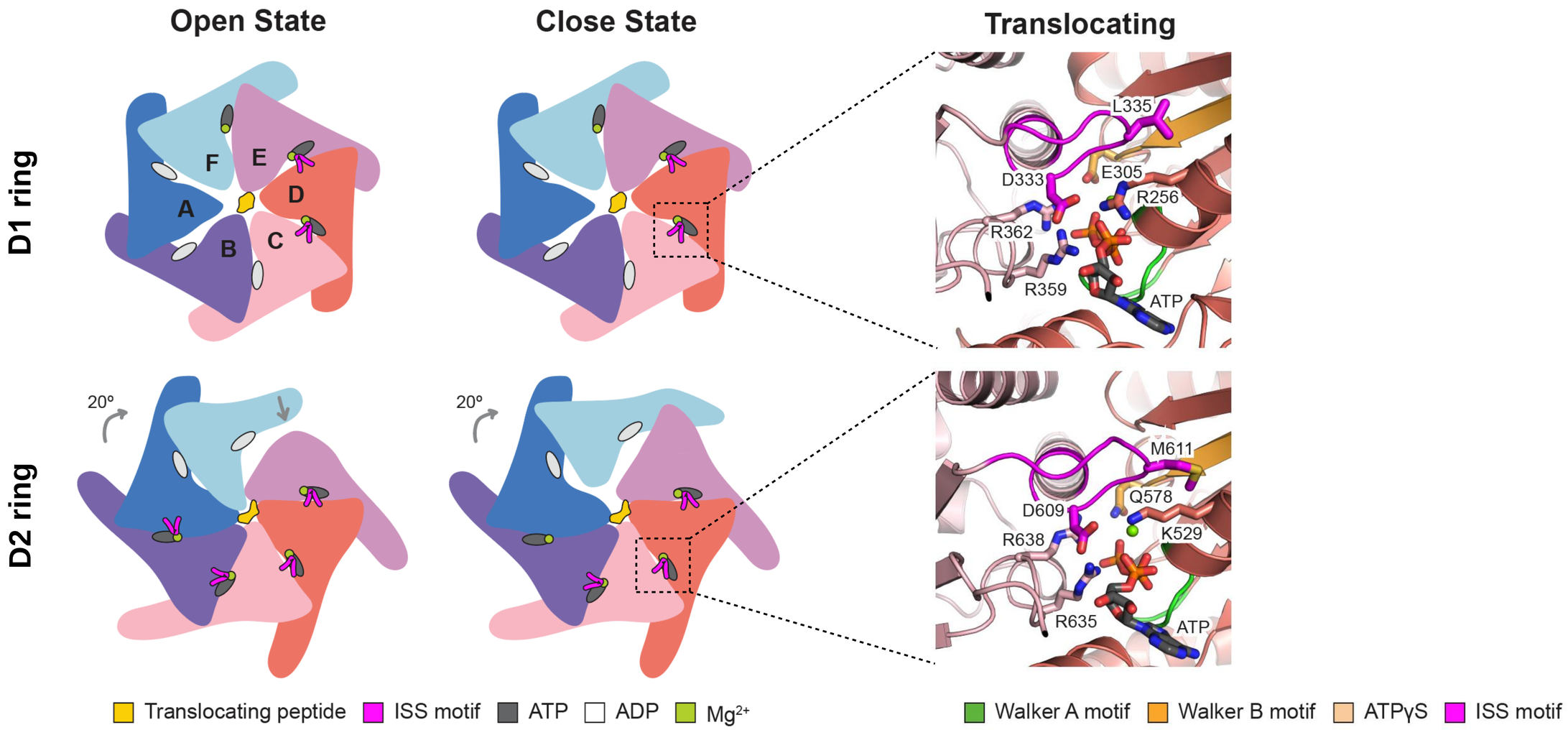
Three States



FOM dataset

Pan M, et al., *NSMB*, 2021

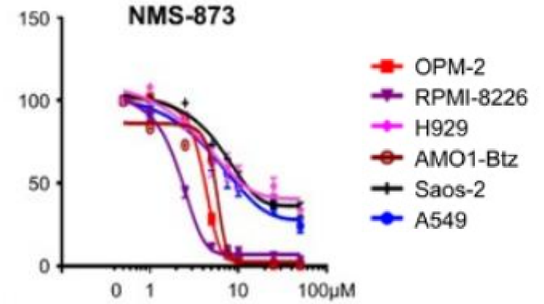
Inter-Subunit Sensing (ISS) motif is responsible for the staircase conformation



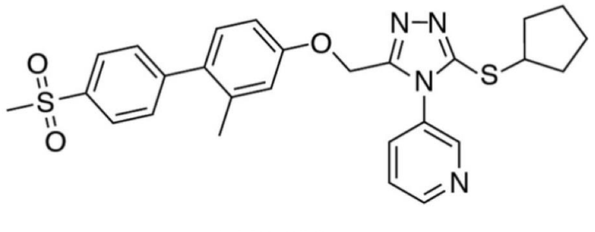
Pan M, et al., *NSMB*, 2021

NMS-873 is a non-competitive inhibitor of p97

Anticancer activity



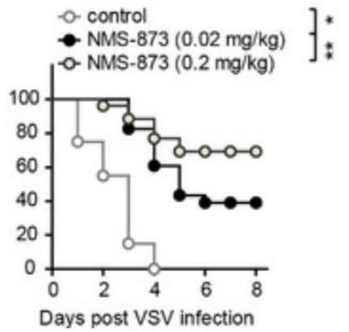
Parzych K, et al., *Cell Death & Disease*, 2015



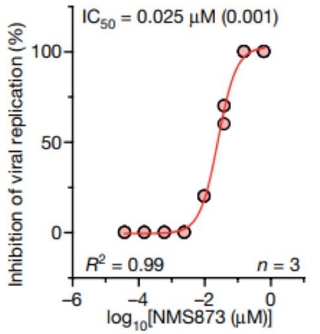
NMS-873

Magnaghi P, et al., *Nature Chem Biol*, 2013

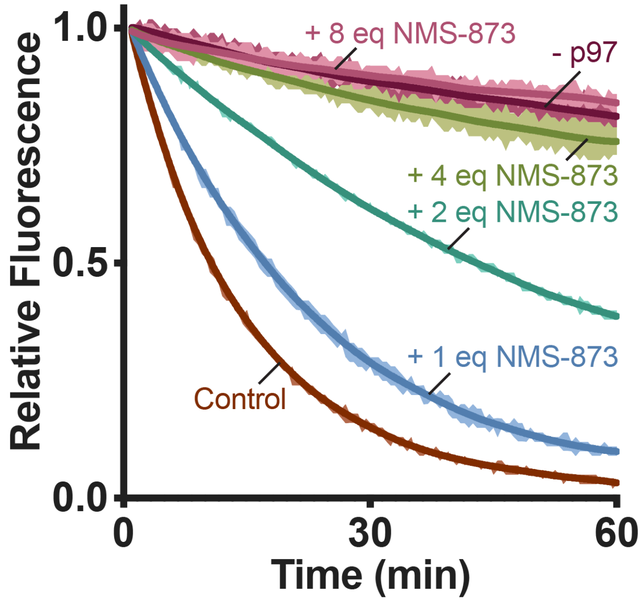
Antiviral activity



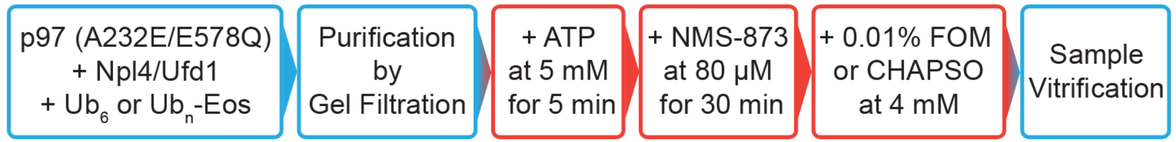
Hao Q, et al., *EMBO J*, 2015

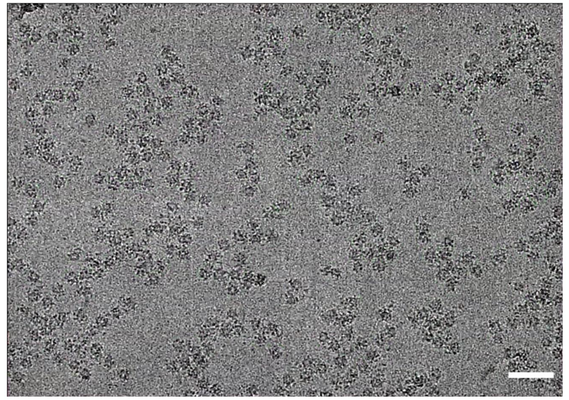


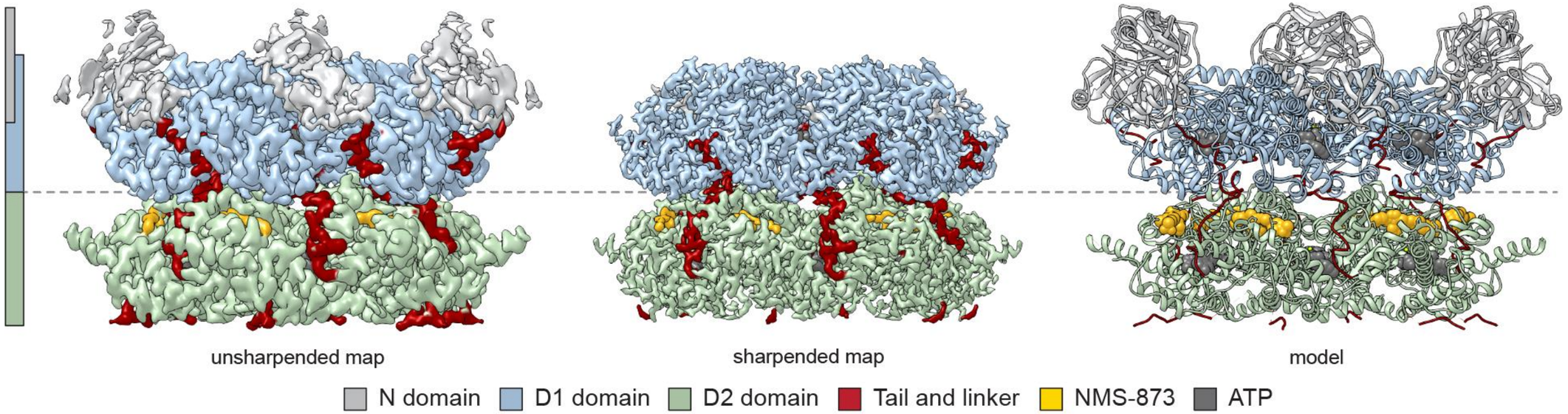
Bojkova D et al., *Nature*, 2020



Structure of human p97 in complex with NMS-873

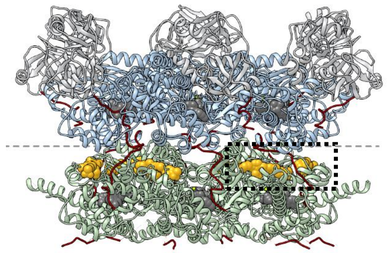


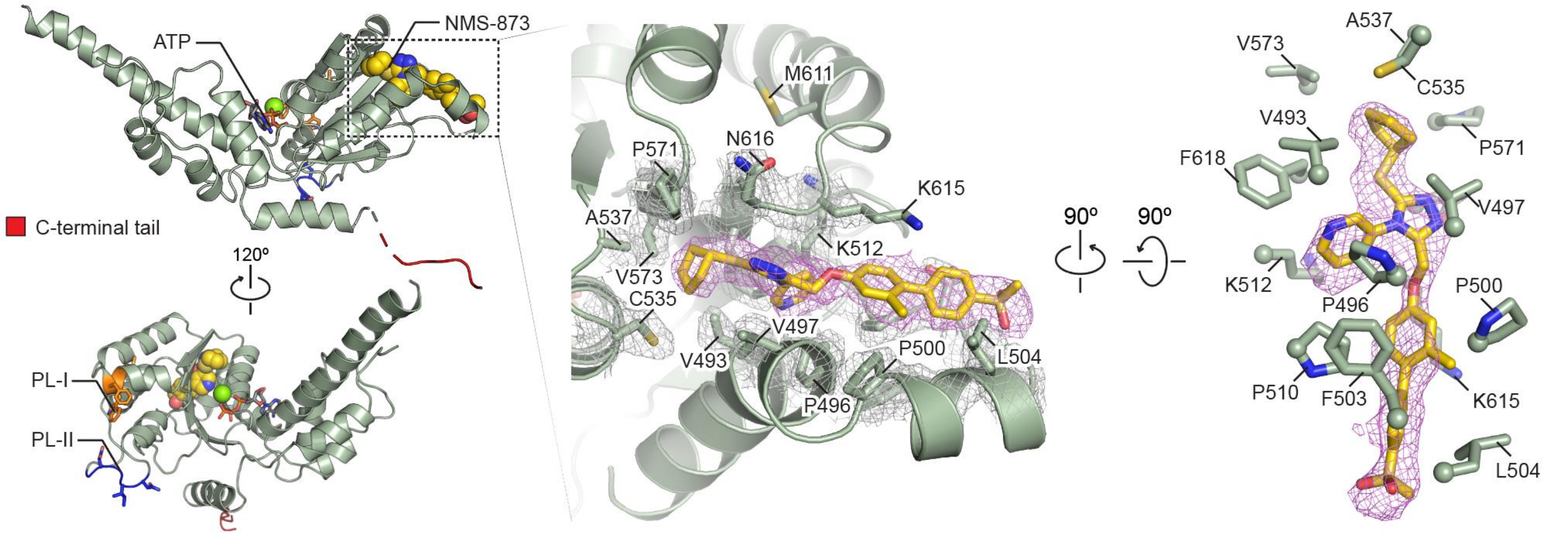


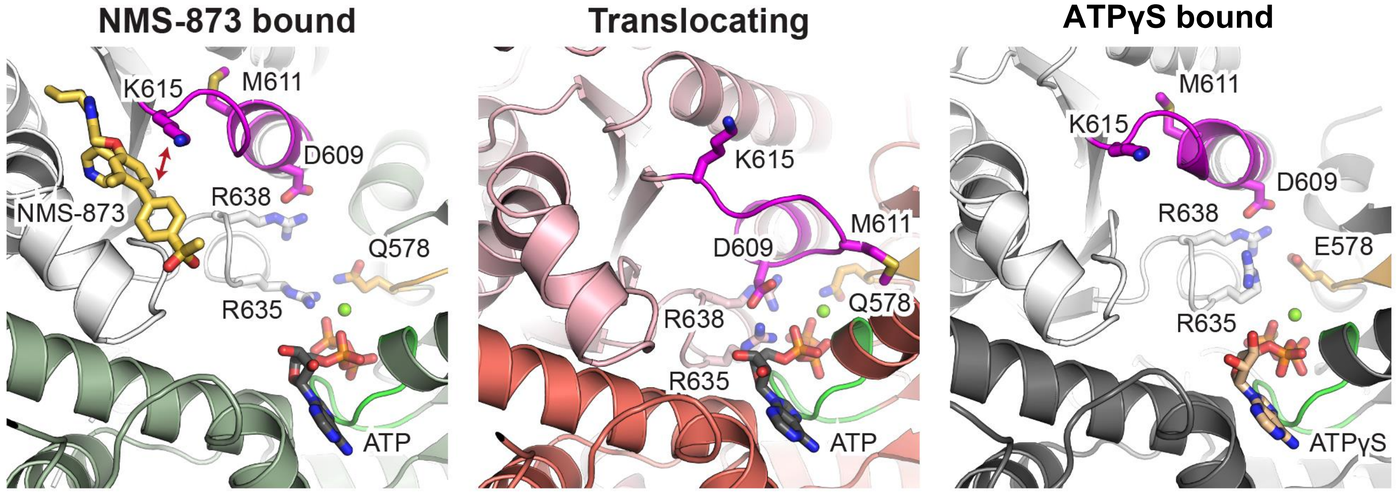


Pan M, et al., *NSMB*, 2021

NMS-873 binds at a cryptic groove in the D2 domain



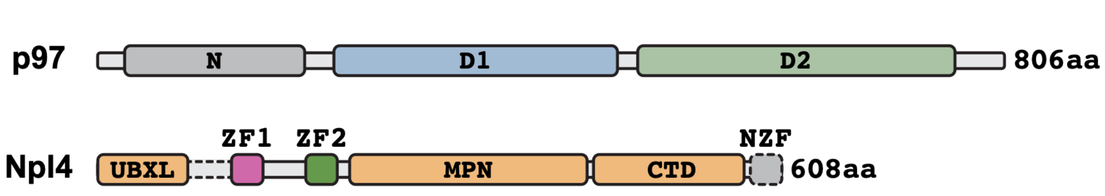


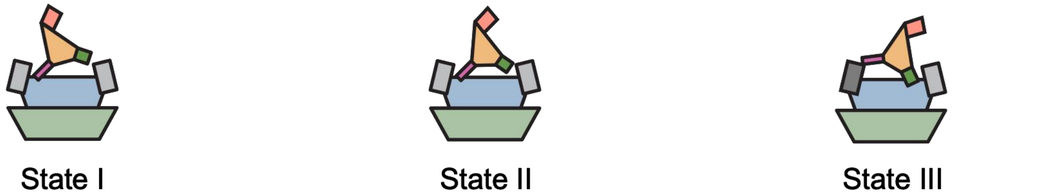


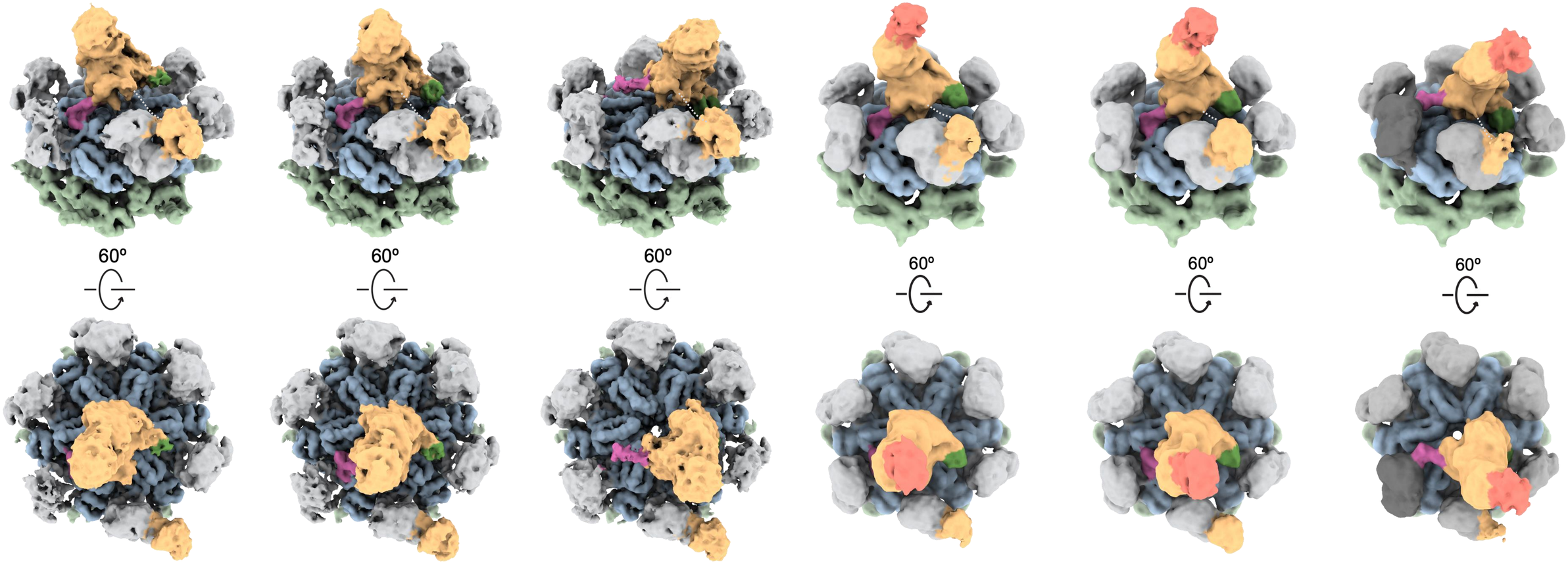
NMS-873 ATP Mg^{2+} Walker A motif Walker B motif ATPγS ISS motif

Pan M, et al., *NSMB*, 2021

Cryo-EM structures of p97 complex before translocation



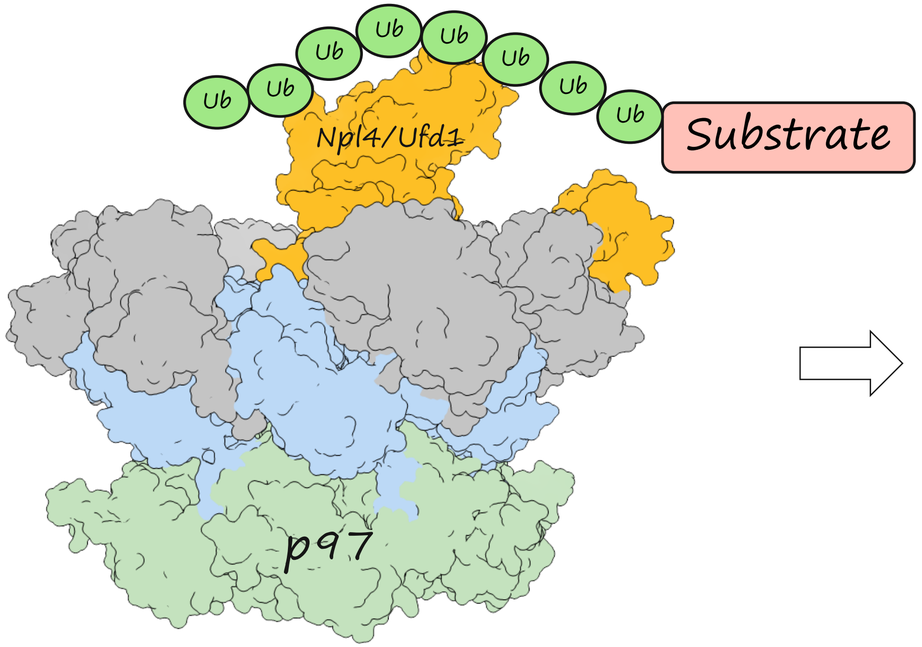




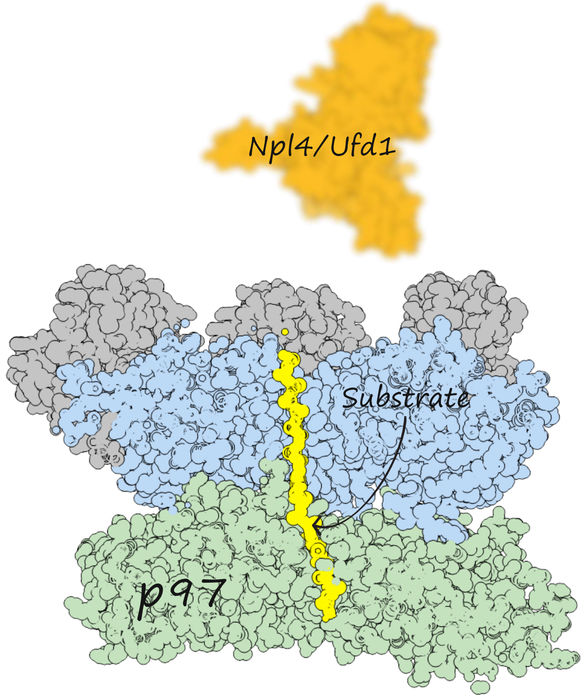
Pan M, et al., *Nature Communications*, 2021

Translocation mechanism of human p97

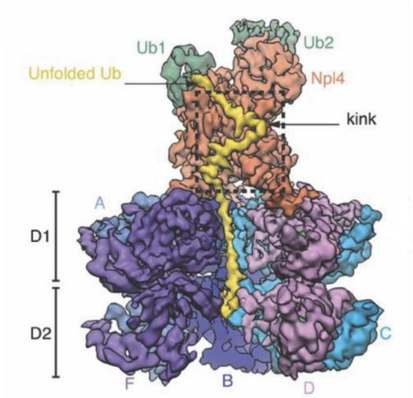
Substrate loading



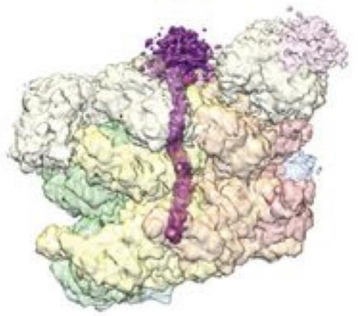
Translocating



Yeast Cdc48



Twomey EC, et al., *Science*. 2019



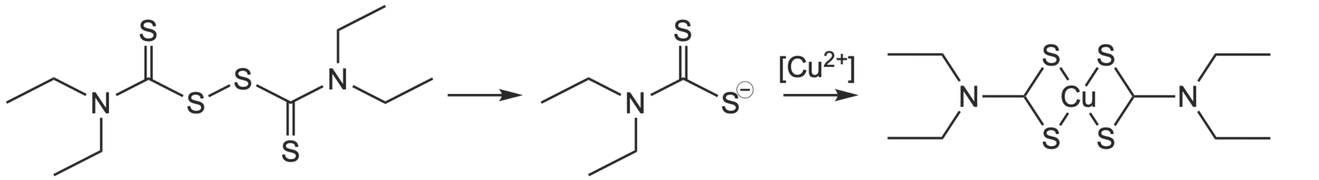
Cooney I, et al., *Science*. 2019

Disulfiram and Diethyldithiocarbamate-copper (CuET)

Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4

Zdenek Skrott, Martin Mistrik, [...] Jiri Bartek

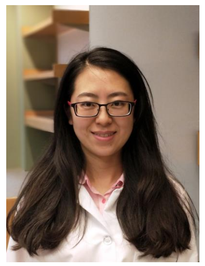
Nature **552**, 194–199(2017)



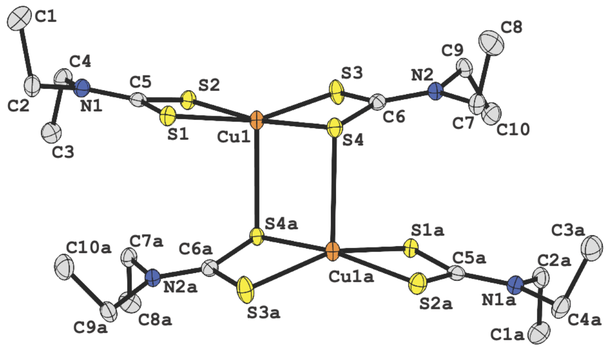
tetraethylthiuram disulfide
Disulfiram, DSF

diethyldithiocarbamate
DTC

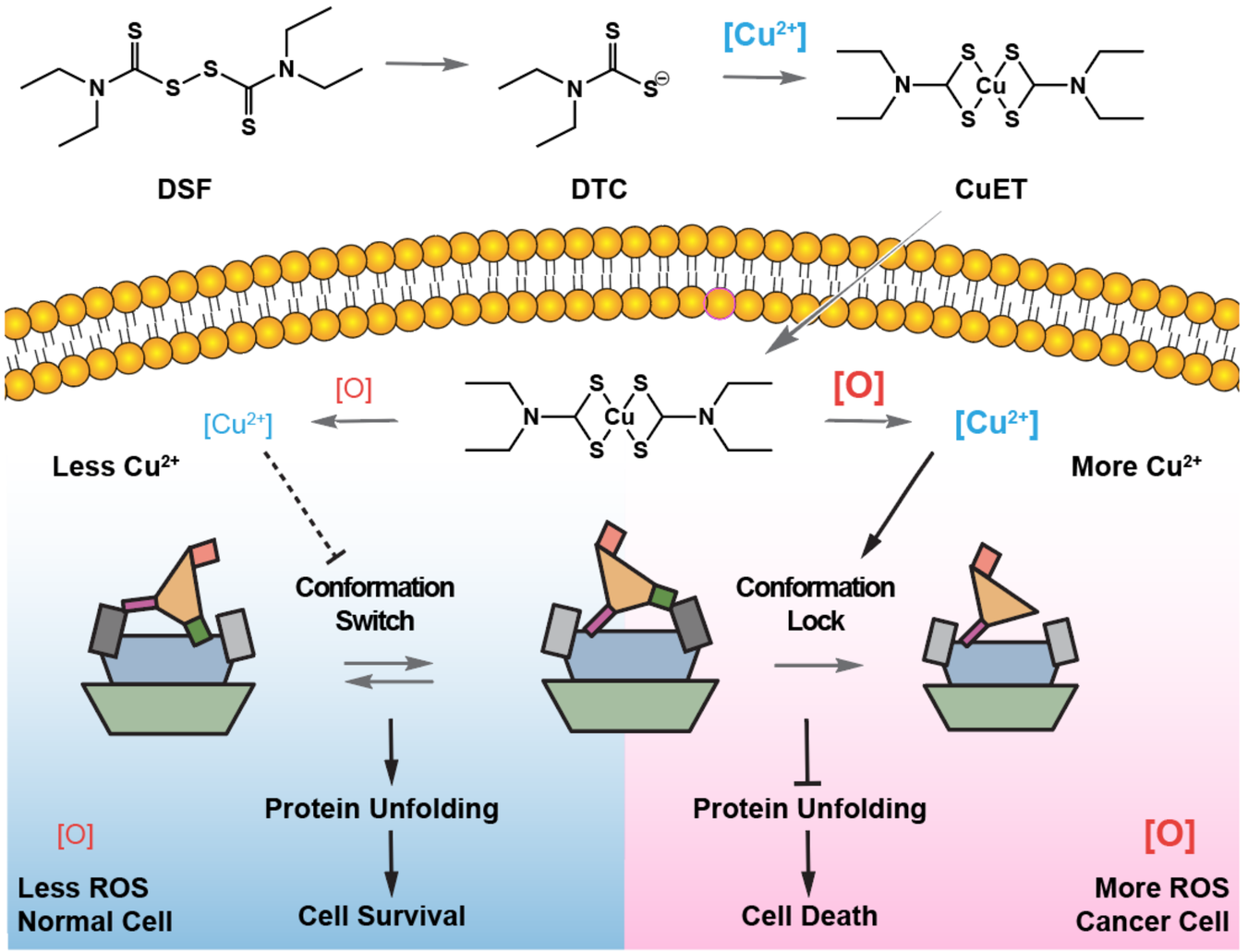
bis-(diethyldithiocarbamate)-copper
CuET



Dr. Yuanyuan Yu



CuET bypasses copper transporters



Pan M, et al., *Nature Communications*, 2021

Summary

- Structures of human p97 in working conditions revealed translocation mechanism
- ISS motif plays an important role in substrate translocation
- NMS-873 locks the ISS motif and allosterically inhibits the translocation of p97
- Disulfiram derivative CuET diffuses across plasma membrane and likely targets the zinc finger motifs of Npl4

Acknowledgement

Thank you for your attention!

Zhao Lab @ UChicago
Man Pan
Yuanyuan Yu
Yuan Xie
Jane Lodwick
Chang Liu

Liu Lab @ THU
Qingyun Zheng
Huasong Ai

EM Facility @ UChicago
Joe Austin
Tera Lavoie
James Fuller

Cryo-Electron Microscopy at the Frederick National Laboratory
Thomas Edwards
Adam Wier
Tara Fox

BMB Department @ UChicago