Structure of the Rpn11–Rpn8 Dimer Reveals Mechanisms of Substrate Deubiquitination During Proteasomal Degradation

-Worden EJ, Padovani C, Martin A, Nat Struct Mol Biol. 2014 Mar;21(3):220-7

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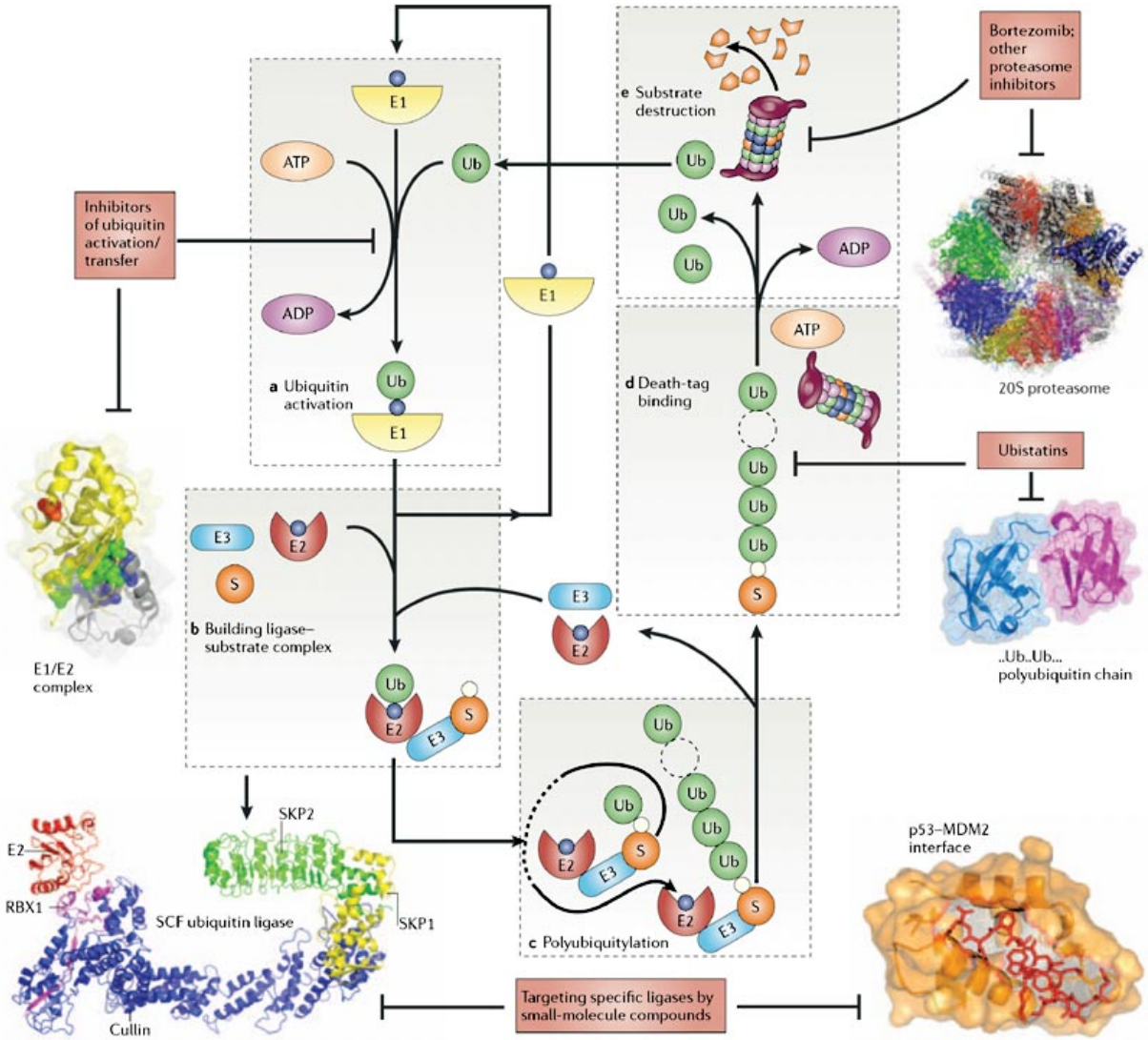
Why Me?

- Ubiquitin-Proteasome degradation system is so critical in many cellular processes that any proceedings in its structure studies is worthy a highlight
- Combination of X-ray structure of subunits and the EM structure of the full complex can dig out more information
- The strategy of protein expression employed in this paper might give some hints to us

Ubiquitin-Proteasome Degradation

- Strictly regulated ATP-Dependent protein degradation in all eukaryotic cells
  - Damaged
  - Misfolded
  - Obsolete
- Essential for many cellular processes
  - Cell Cycle Control
  - Regulation of Gene Expression
  - Apoptosis
  - Response to Cellular Stress
- Noble Prize in Chemistry to Araon Ciechnover, Avram Hershko and Irwin Rose, in 2004

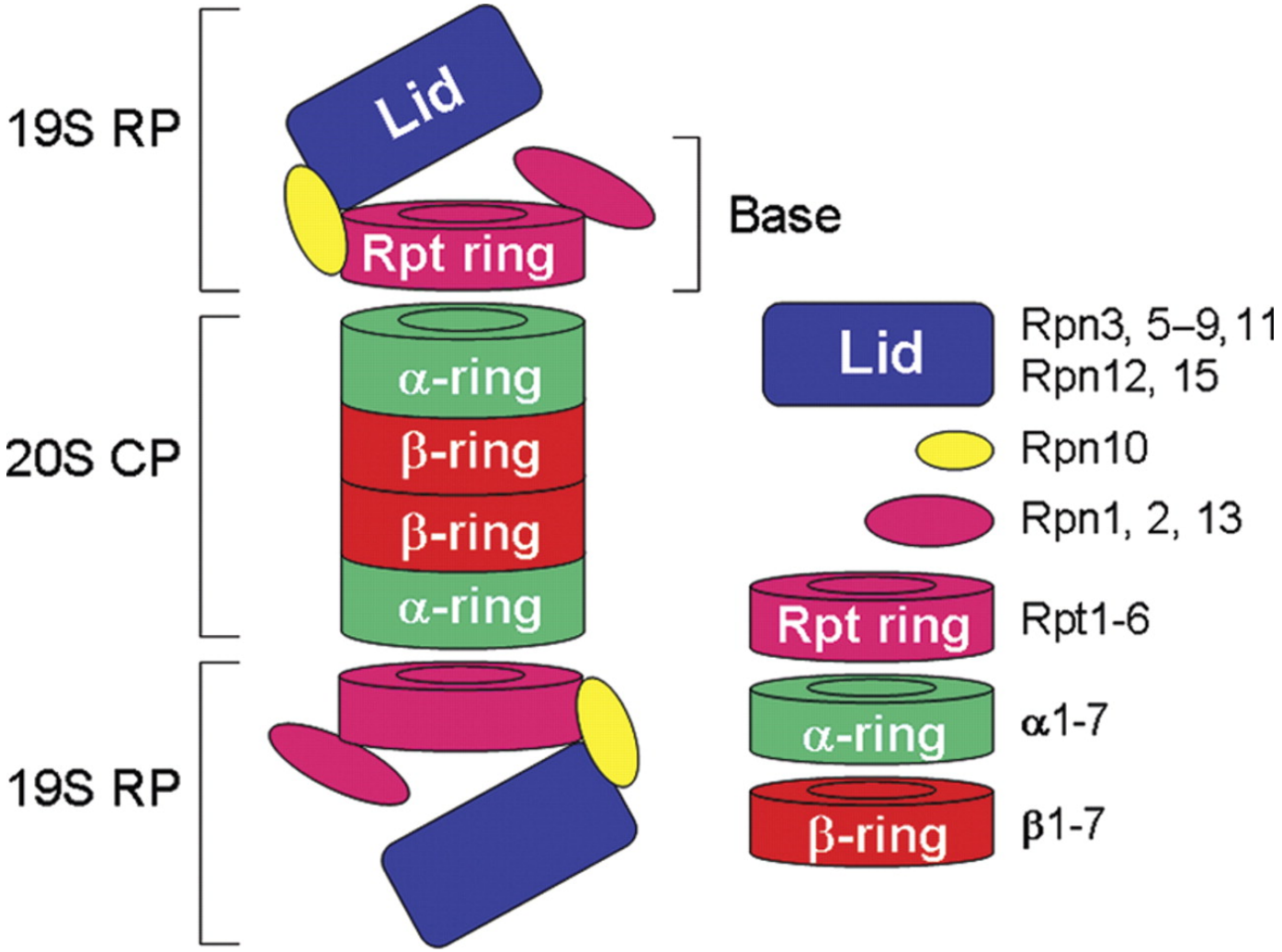
Ubiquitin-Proteasome Degradation Pathway



Nalepa *et al. Nature Reviews Drug Discovery* **5**, 596–613 (July 2006) | doi:10.1038/nrd2056

nature REVIEWS DRUG DISCOVERY

Schematic diagram of the 26S proteasome



Xie Y J Mol Cell Biol 2010;jmcb.mjq030

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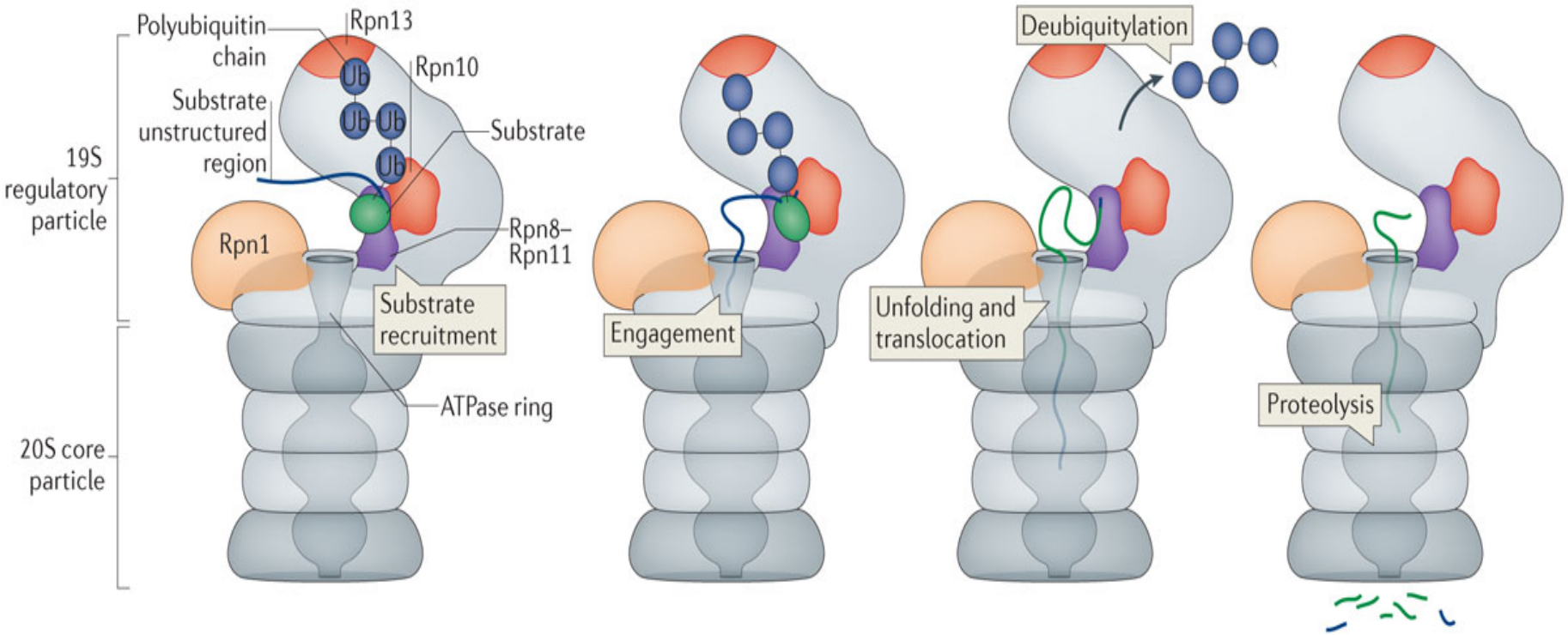
Steps of Proteasomal Degradation

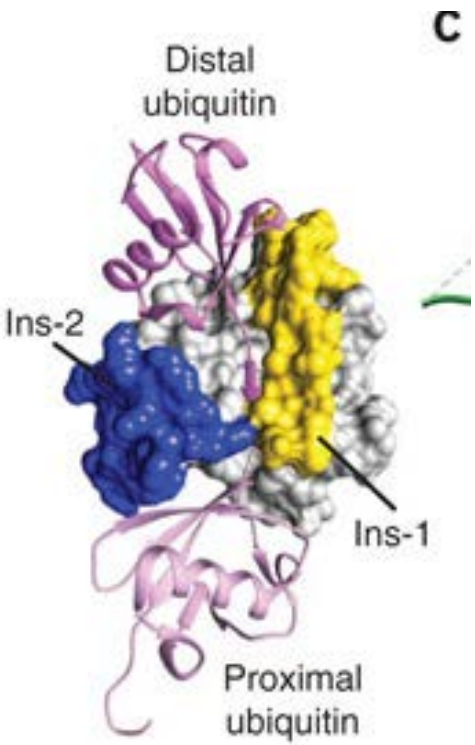


Nature Reviews | Molecular Cell Biology

Rpn11 and Rpn8

- Rpn11 is a Zn^{2+}-dependent Deubiquitinase(DUB) of the MPN-protein family, containing the sequence $EX_nHXHX_{10}D$ (where X is any residue, Insertion-1 & Insertion-2). It removes ubiquitin chain from condemned proteins before they get into ATPases.

- Rpn8 forms a dimer with Rpn11 in Cryo-EM structural image of the proteasome holoenzyme. It is the only other MPN-domain protein in proteasome, located right above the central pore of the base ATPases.

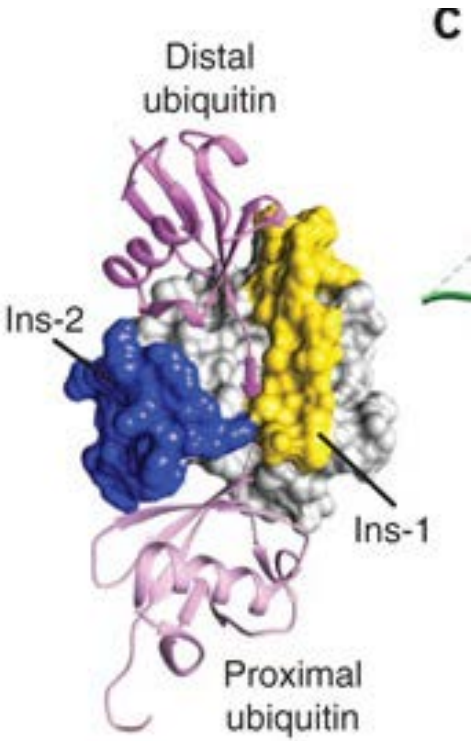


What is MPN domain

- MPN: Mpr1, Pad1 N-terminal domain, found first in Yeast
- Be part of large protein complexes
- Metalloenzyme
- Responsible for isopeptidase activity.
- Consisting of a canonical sequence (E-x[2]-H-S/T-H-x[7]-S-x[2]-D) and coordinating a zinc ion.
- Containing JAMM (JAB1-MPN-MOV34) metalloisopeptidase motif. The JAMM motif specifies a catalytic center essential for selective hydrolysis of linkages, contained between ubiquitin/ubiquitin-like proteins and target proteins or between ubiquitin monomers within a polymeric chain.

AMSH-LP

- Associated Molecule with the SH3 domain of STAM-like Protease
- Deubiquitinating enzymes (DUBs)
- The Zn(2+)-dependent DUBs AMSH and AMSH-LP regulate receptor trafficking by specifically cleaving Lys 63-linked polyubiquitin chains from internalized receptors.
- Consisting of a Zn(2+)-coordinating catalytic core and two characteristic insertions, Ins-1 and Ins-2.
- The distal ubiquitin interacts with Ins-1 and the core, whereas the proximal ubiquitin interacts with Ins-2 and the core. The core and Ins-1 form a catalytic groove that accommodates the Lys 63 side chain of the proximal ubiquitin and the isopeptide-linked carboxy-terminal tail of the distal ubiquitin. This is the first reported structure of a DUB in complex with an isopeptide-linked ubiquitin chain, which reveals the mechanism for Lys 63-linkage-specific deubiquitination by AMSH family members.



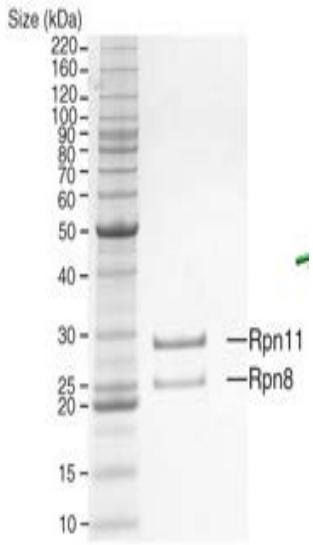
Questions addressed

- How to express and purify the complex of Rpn11 and Rpn8

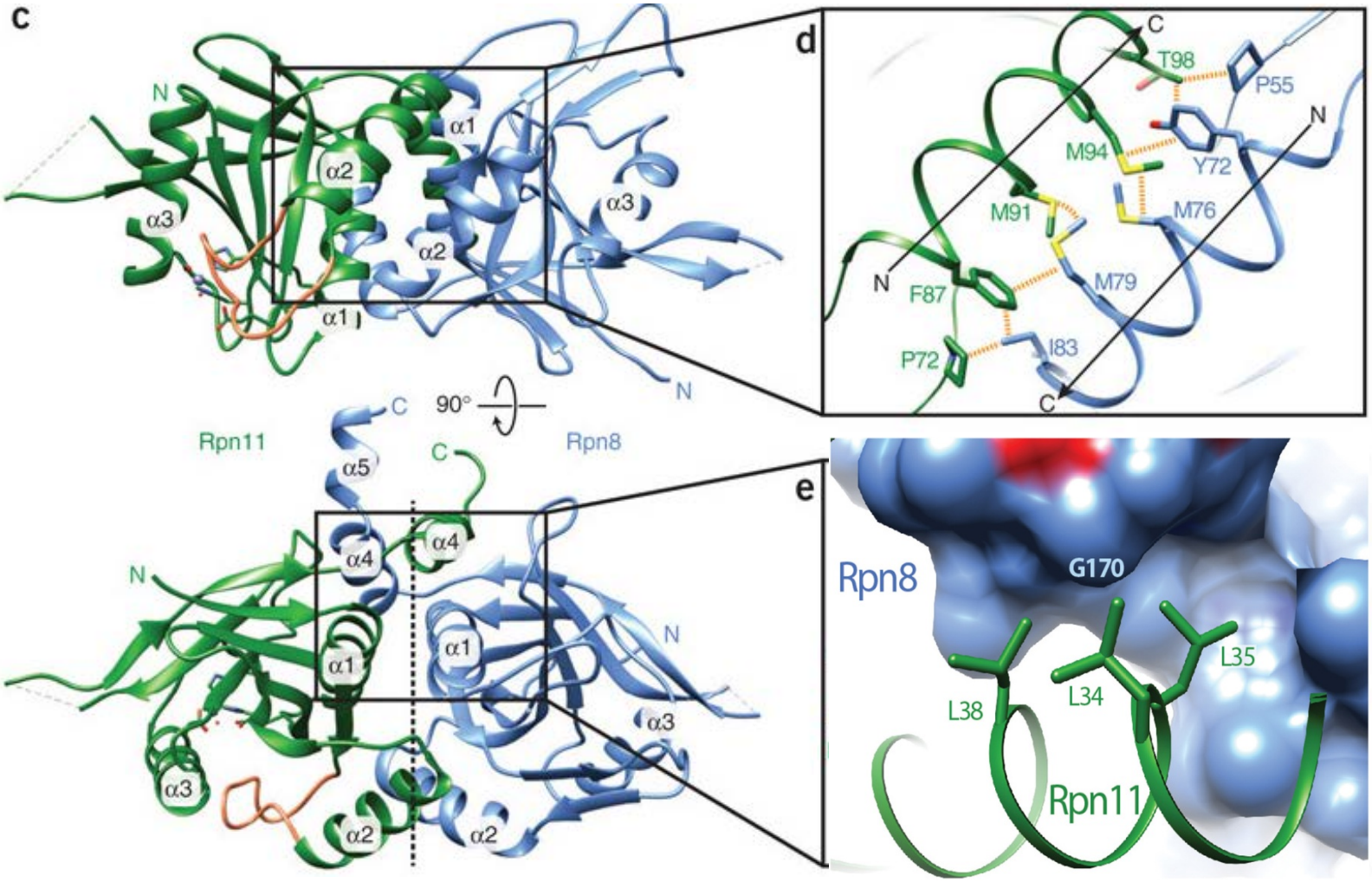
- What the atomic structure of the complex looks like and what we can learn from it.

Smart Strategy to Purify Rpn11/Rpn8

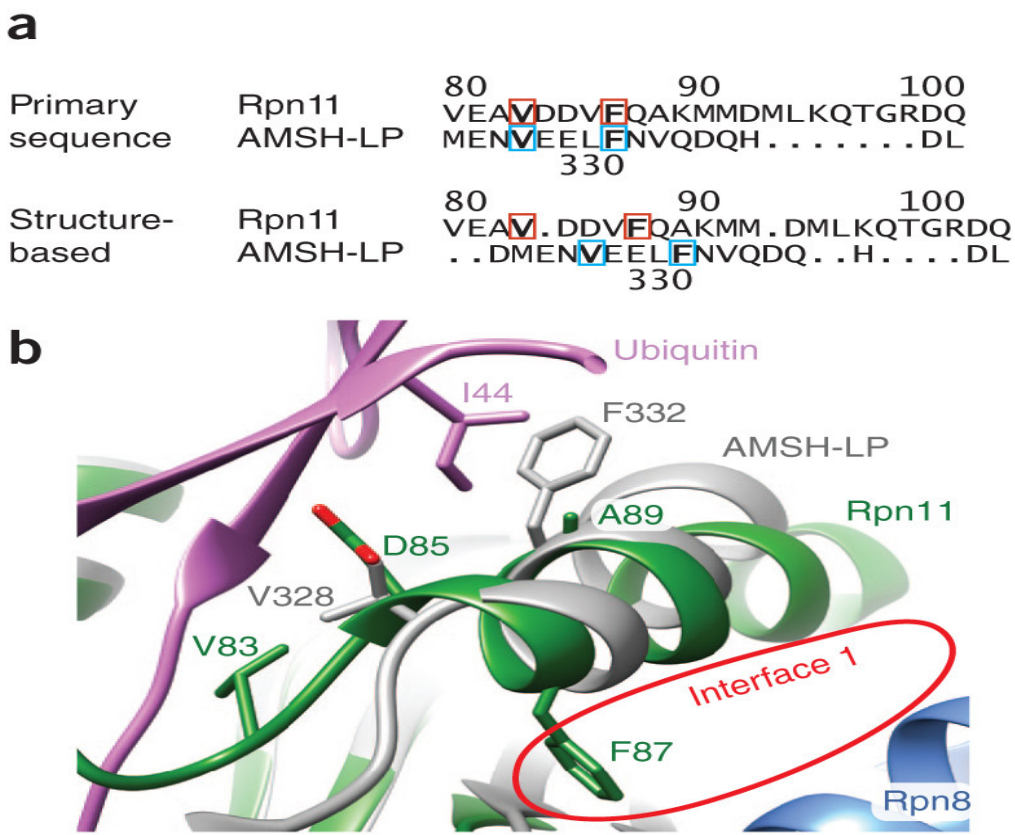
- Numerous previous attempts failed
- Why they successed:
  - Rpn11 and Rpn8 form a dimer in proteasome lid
  - Lid subcomplex is assembled through the formation of a large helical bundle consisting of the C-terminal helices of all subunits, including Rpn8 and Rpn11.
    - » “We thus reasoned that the numerous previous attempts to purify Rpn11 in isolation were unsuccessful because its MPN dimerization surface was unsaturated and its C-terminal helices were not paired with others in the helical bundle. “
  - Solution: Coexpress C-terminally truncated variants of Rnp11 and Rpn8.



Rpn11 and Rpn8 form a heterodimer through two distinct interfaces



Rpn11 Lacks Predicted Residues for Ubiquitin Binding



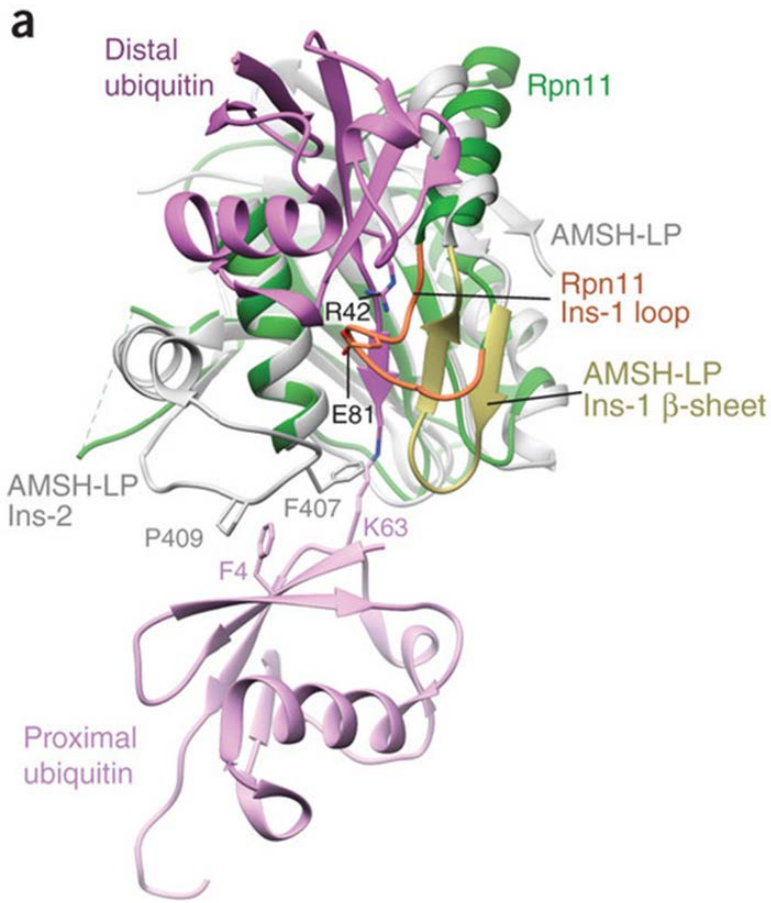
Rpn11 is missing a conserved binding site for the Ile44 patch of ubiquitin

Table 2: Kinetic parameters for the cleavage of Lys48-linked diubiquitin by wild-type or mutant Rpn11 in context of the Rpn11–Rpn8 heterodimer

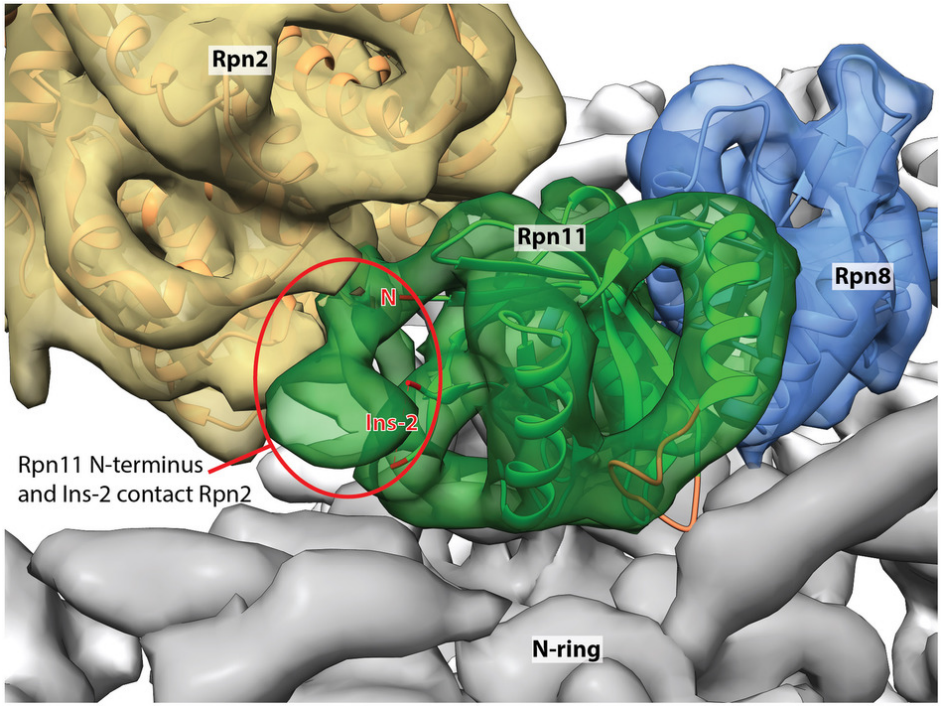
Rpn11	**Ub2 linkage type**	***Km (μM)***	***kcat (min^{-1})***
WT	48	120 ± 28	0.29 ± 0.11
A89F	48	36 ± 7	0.25 ± 0.06
D85V	48	205 ± 43	0.08 ± 0.01
L132A	48	320 ± 36	0.07 ± 0.01

Rpn11 and AMSH-LP bind ubiquitin in a similar orientation but differ considerably in their interaction with the Ile44 hydrophobic patch, but Rpn11 contains other conserved or highly similar residues to mediate ubiquitin binding in AMSH-LP

Ins-2 of Rpn11 Is Not Involved in Ubiquitin Binding

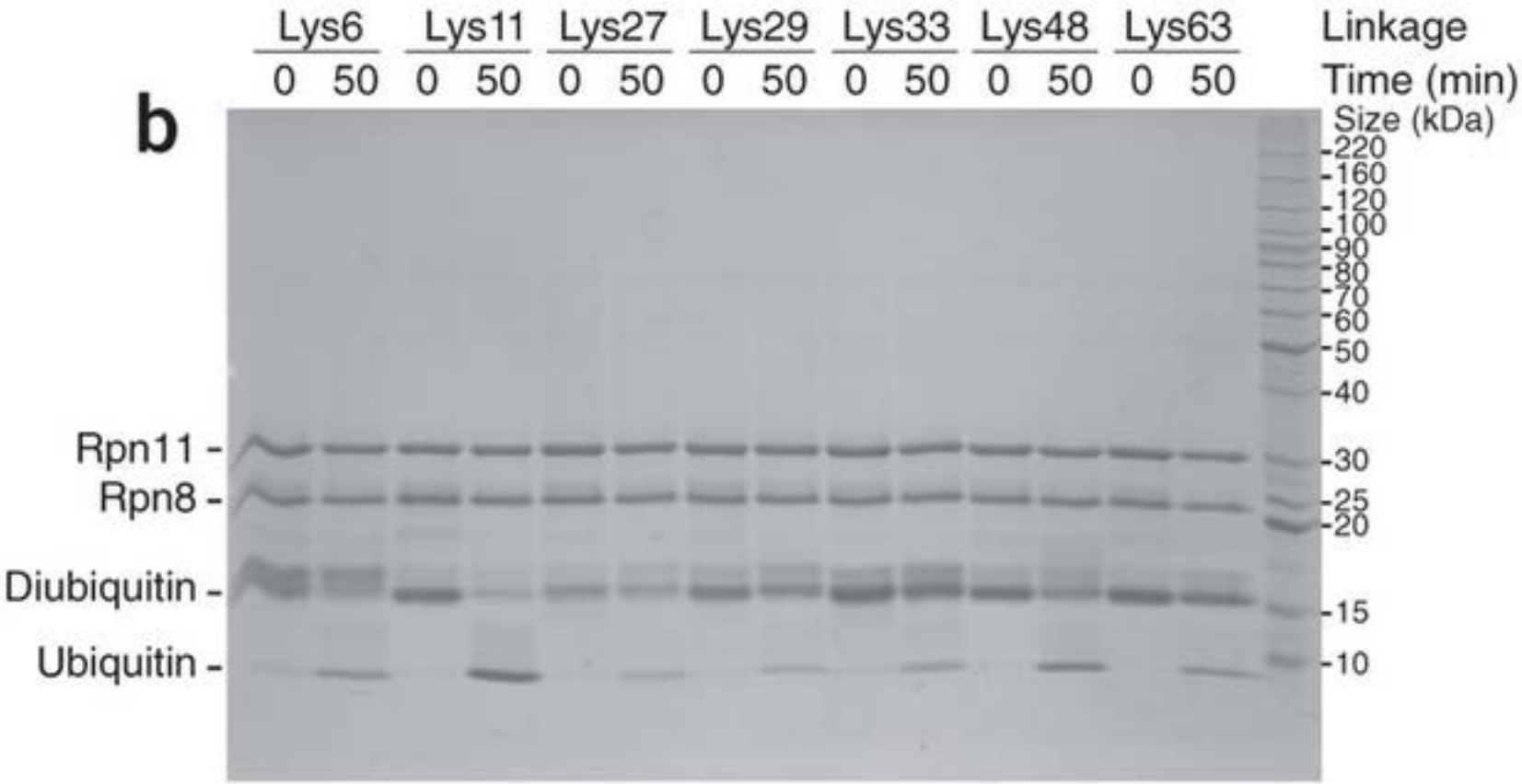


Superimposition of the crystal structures for ubiquitin-bound AMSH-LP and Rpn11



The N-terminus and Ins-2 region of Rpn11 contact Rpn2

Rpn11 Does Not exhibit Linkage Specificity



Missing Proximal Contacts Allow Rpn11 cleavage Promiscuity

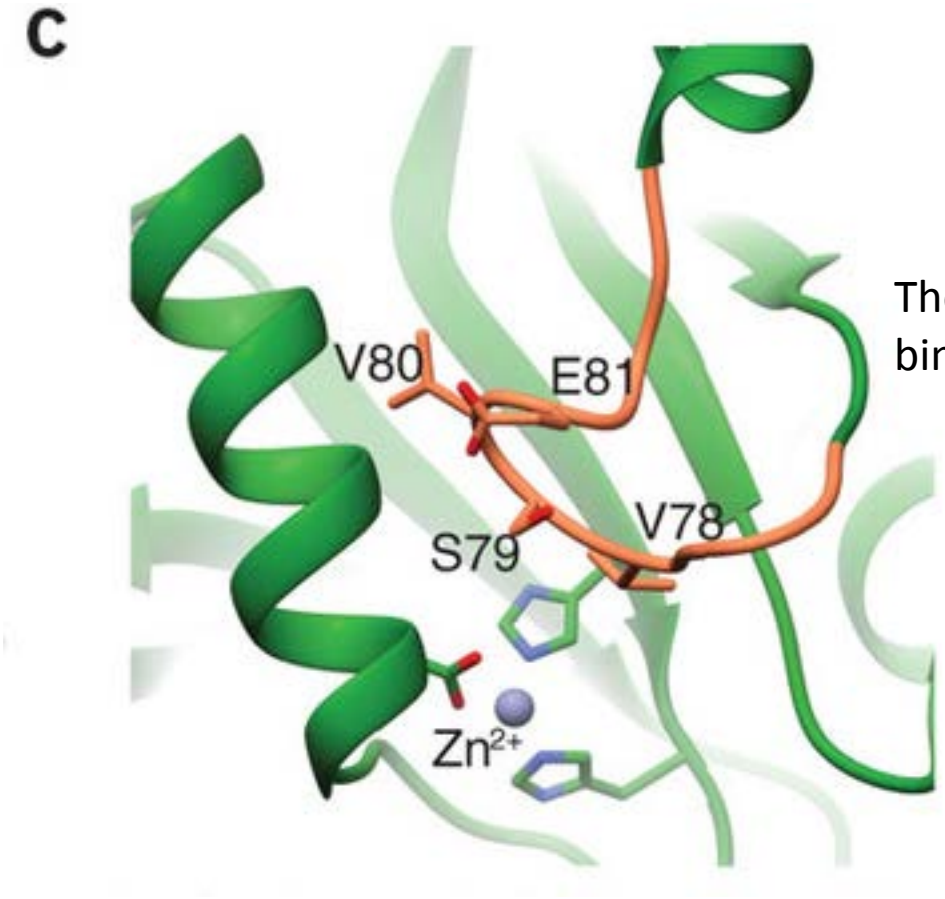
Rpn11 Does Not exhibit Linkage Specificity

Table 2: Kinetic parameters for the cleavage of Lys48- or Lys63-linked diubiquitin by wild-type or mutant Rpn11 in context of the Rpn11–Rpn8 heterodimer

Rpn11	**Ub2 linkage type**	***Km (μM)***	***kcat (min^{-1})***
WT	48	120 ± 28	0.29 ± 0.11
WT	63	299 ± 52	0.38 ± 0.05
WT	11	147 ± 43	0.74 ± 0.20
WT	Ubiquitin-lysine-TAMRA	20 ± 1	0.95 ± 0.02

A globular structure on the proximal side of the isopeptide bond leads to some steric hindrance in Rpn11 binding

Flexible Ins-1 Loop Positions Distal ubiquitin for Cleavage



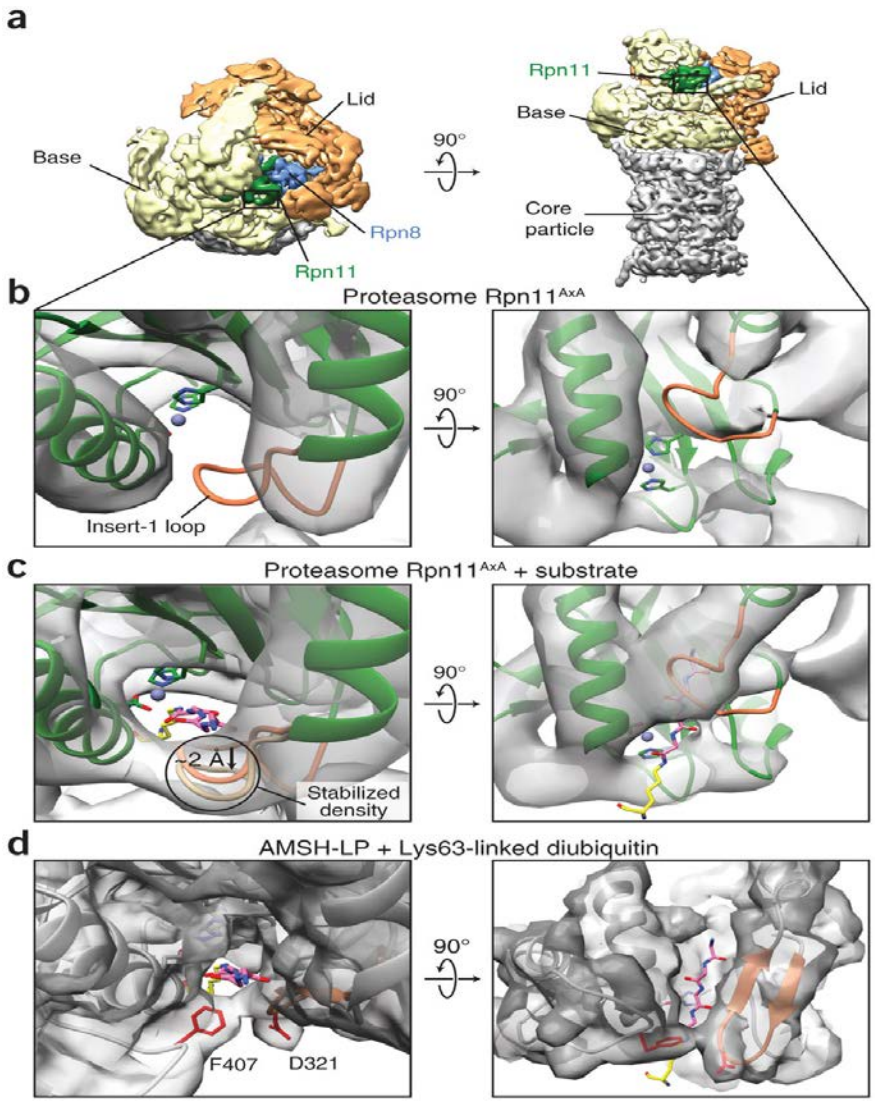
The Ins-1 loop covers the binding groove

Table : Kinetic parameters for the cleavage of Lys48-linked diubiquitin by wild-type or mutant Rpn11 in context of the Rpn11–Rpn8 heterodimer

Rpn11	**Ub2 linkage type**	***Km (μM)***	***kcat (min^{-1})***
WT	48	120 ± 28	0.29 ± 0.11
V78A	48	142 ± 41	0.20 ± 0.07
S79A	48	218 ± 50	0.13 ± 0.01
V80A	48	(Kd = 67 ± 35)	ND
E81A	48	515 ± 96	0.11 ± 0.01
Δ78–81	48	ND	ND
78–81 AAAA	48	ND	ND

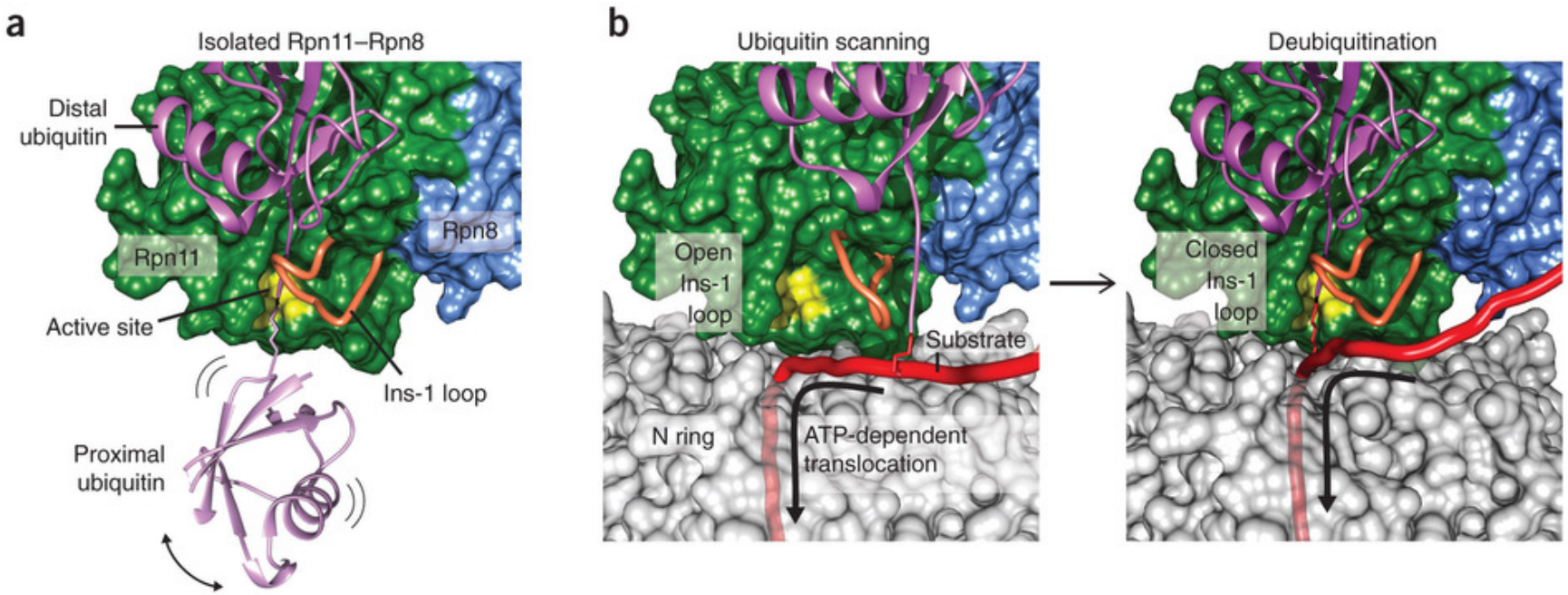
The Ins-1 loop may have an important role in positioning the C terminus of the distal ubiquitin for isopeptide-bond cleavage

Flexible Ins-1 Loop Positions Distal ubiquitin for Cleavage



The Ins-1 loop of Rpn11 Acts as a flap to fold over the ubiquitin C terminus

Model for Rpn11-mediated deubiquitination



Conclusion

- Atomic structure of Rpn11-Rpn8 dimer is solved.
  - Rpn11 lacks predicted residues for ubiquitin binding
  - Ins-2 of Rpn11 is not involved in ubiquitin binding
  - Flexible Ins-1 loop positions distal ubiquitin for cleavage
  - Rpn11 has low catalytic efficiency
  - Fewer substrate-stabilizing interactions makes Rpn11 less efficient but extremely promiscuous in cleavage

Take-Home Message

- Rpn11 is a DUB, but its structure determines its promiscuous in isopeptide cleavage.
- Think more if you are struggling for recombinant protein expression and purification

Thanks