Structural basis for the recognition of type-2 N-degron substrate by PRT1 E3 ubiquitin ligase

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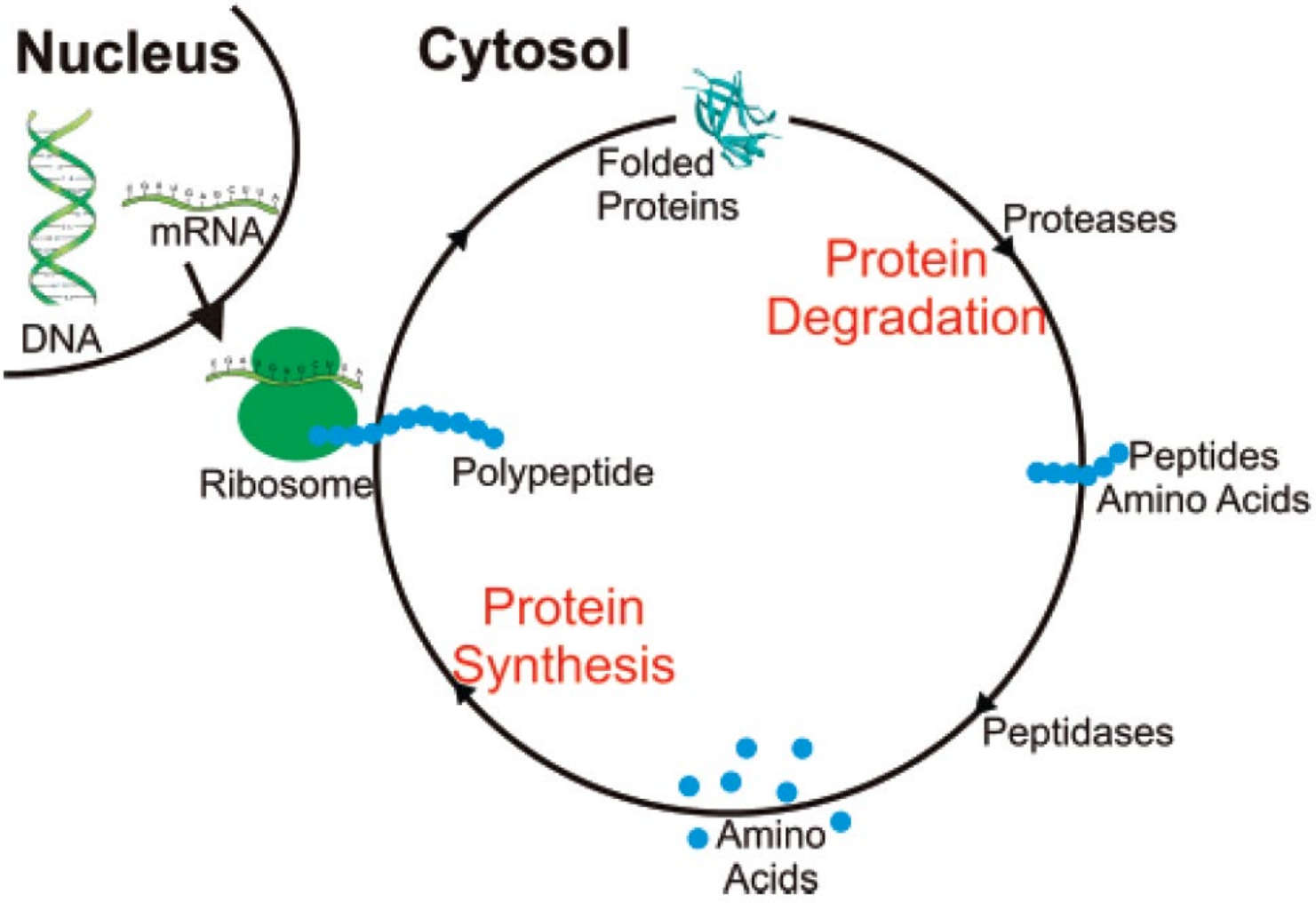
Human Life
http://kothemes.hiandroid.com/2954
Pitte Cocci

Life cycle of proteins



Voet *et al.*, Biochemistry textbook

N-terminal destabilizing residues (N-degron)

Bachmair *et al.* & Varshavsky, Science (1986)

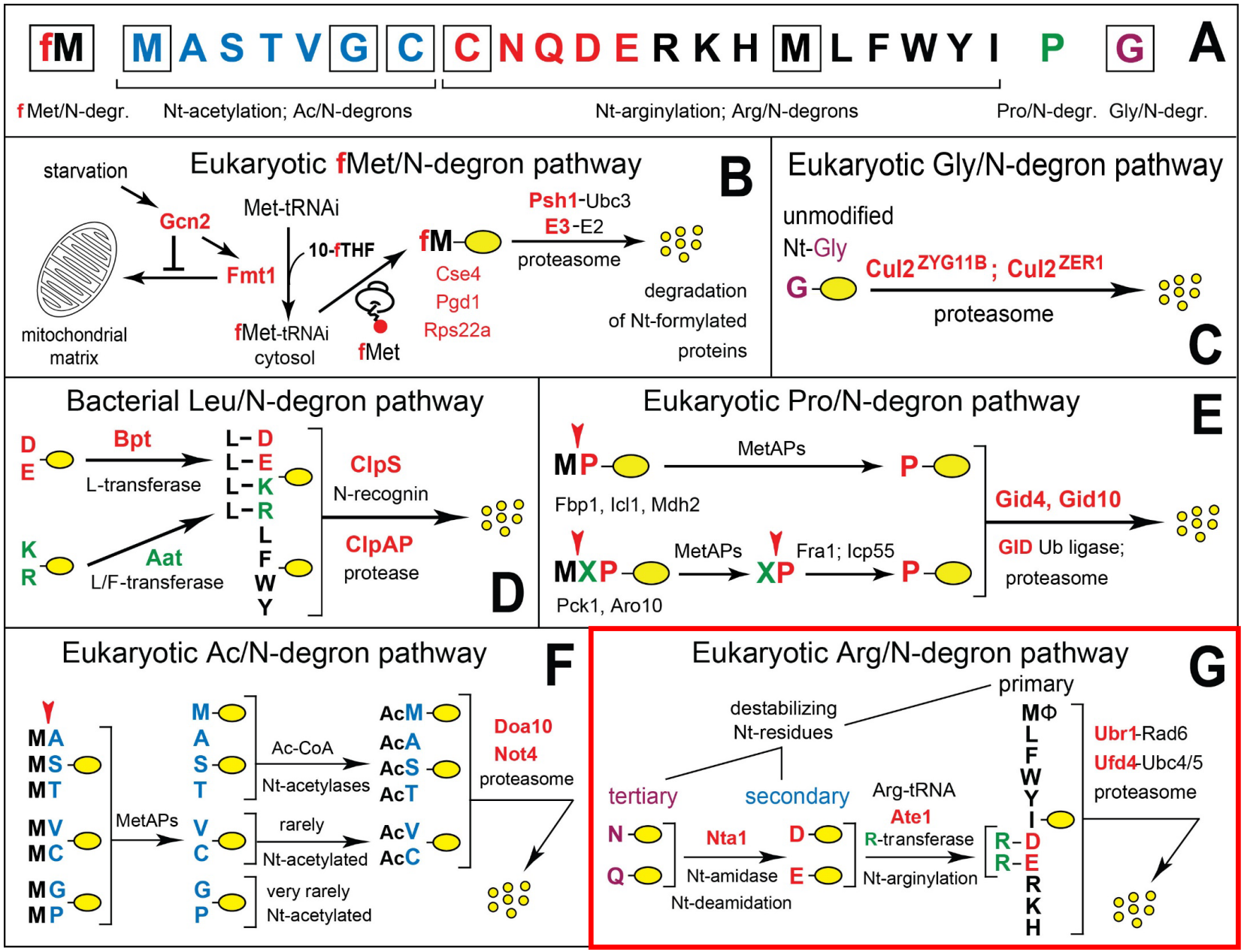
Residue X in ub-X-βgal	Radius of gyration of X (Å)	Deubiquitination of ub-X-βgal	$t_{1/2}$ of X-βgal
Met	1.80	+	>20 hours
Ser	1.08	+	
Ala	0.77	+	
Thr	1.24	+	
Val	1.29	+	
Gly	0	+	
Ile	1.56	+	~30 minutes
Glu	1.77	+	
Tyr	2.13	+	~10 minutes
Gln	1.75	+	
Phe	1.90	+	~3 minutes
Leu	1.54	+	
Asp	1.43	+	
Lys	2.08	+	
Arg	2.38	+	~2 minutes
Pro	1.25	–*	~7 minutes

Stabilizing

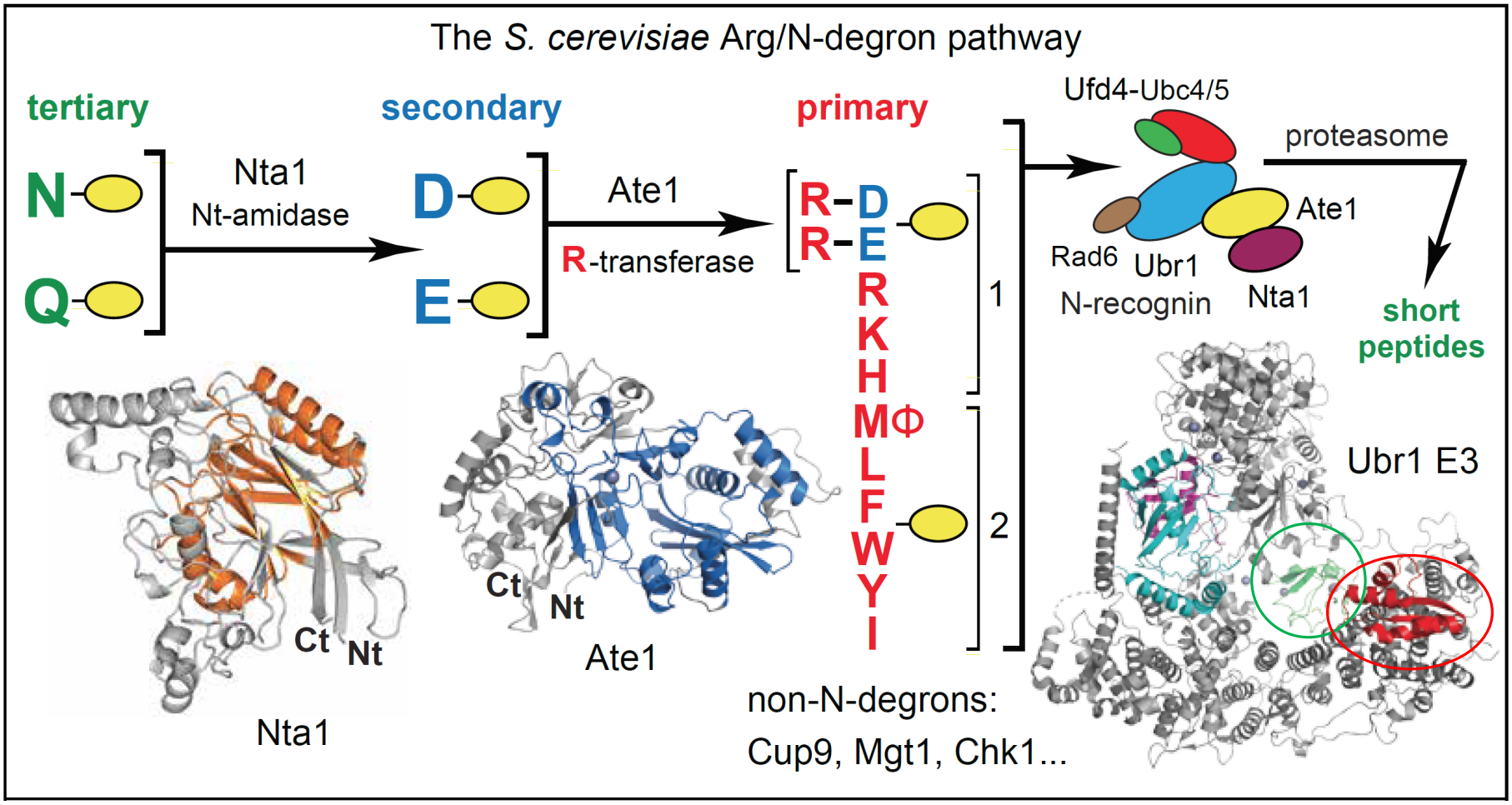
Destabilizing

The N-degron pathway

BH Kim *et al.*
PNAS (2022)



Structural biology of the Arg/N-degron pathway



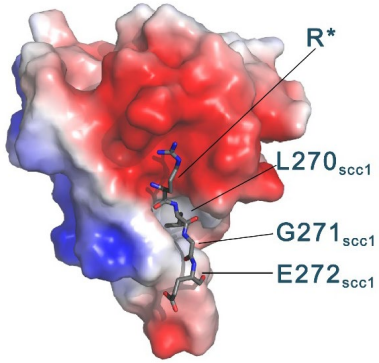
MK Kim *et al*. PNAS (2016)

BH Kim *et al*. PNAS (2022)
Van *et al*. JMB (2022)

WS Choi *et al*. Nat SMB (2010)
L Kim *et al*. Protein Sci (2021)
M Pan *et al*. Nature (2021)

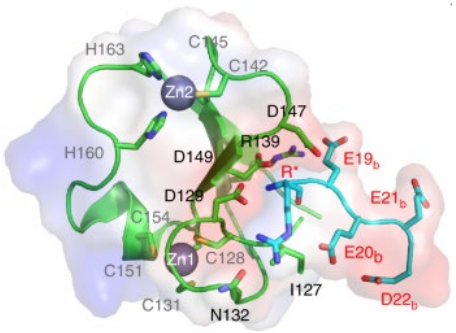
N-degron recognition by N-recognins

Ubr1-RLGS



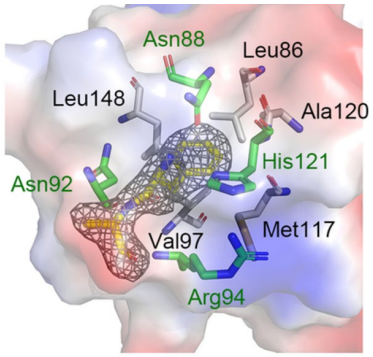
Choi *et al*. Nat SMB (’10)

p62/SQSTM1-REEED

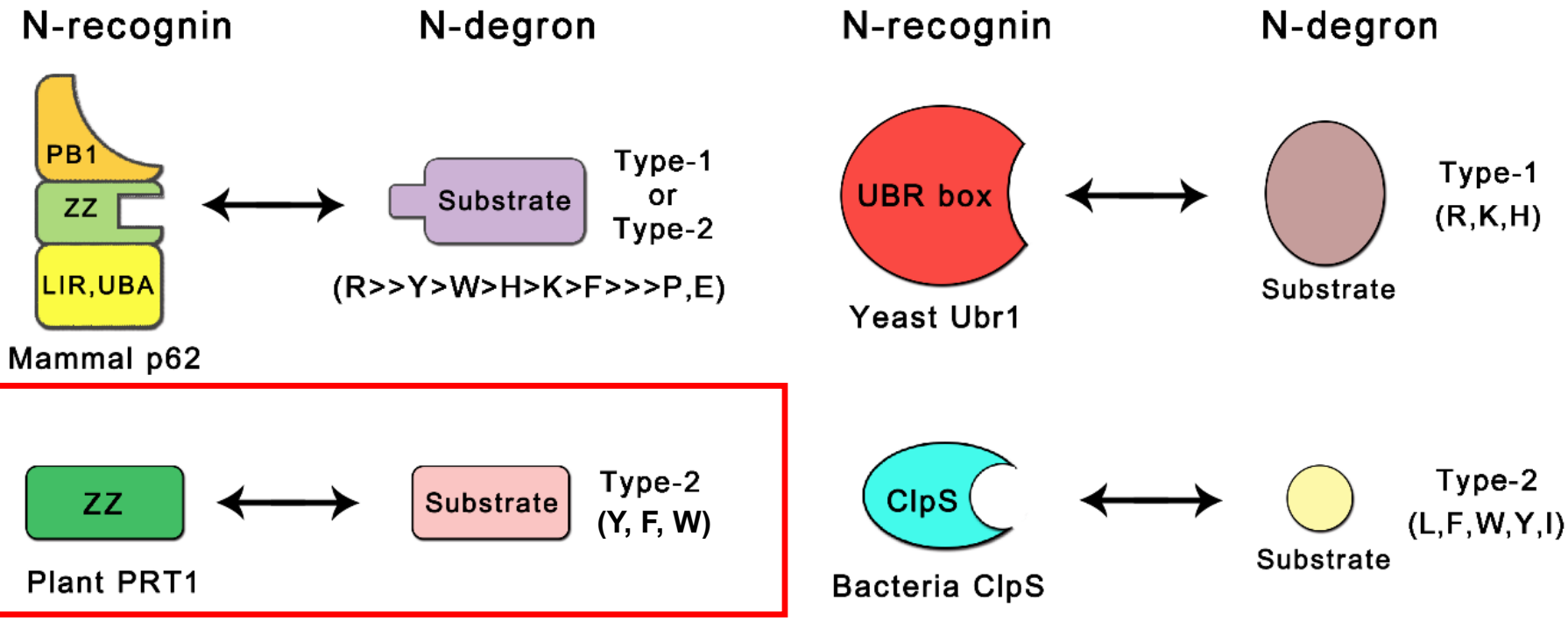


Kwon *et al*. Nat Comms (’18)

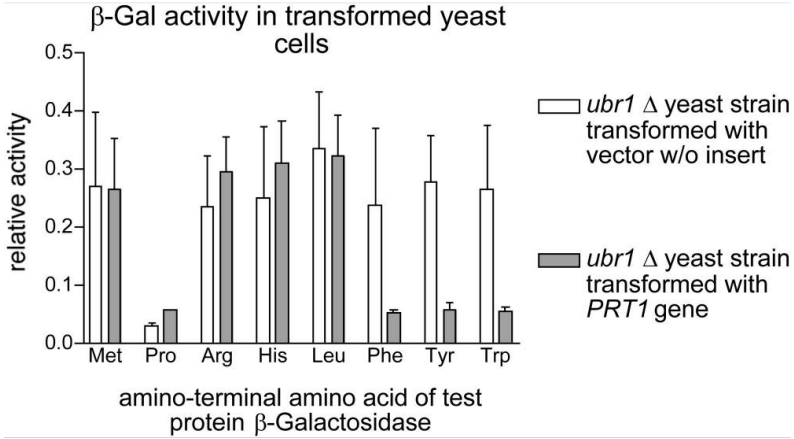
*At*ClpS1-FA



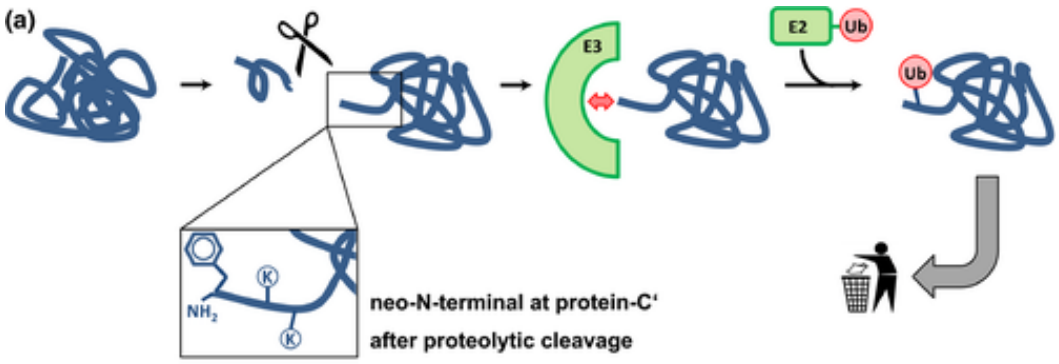
Kim *et al.* Protein Sci (’21)



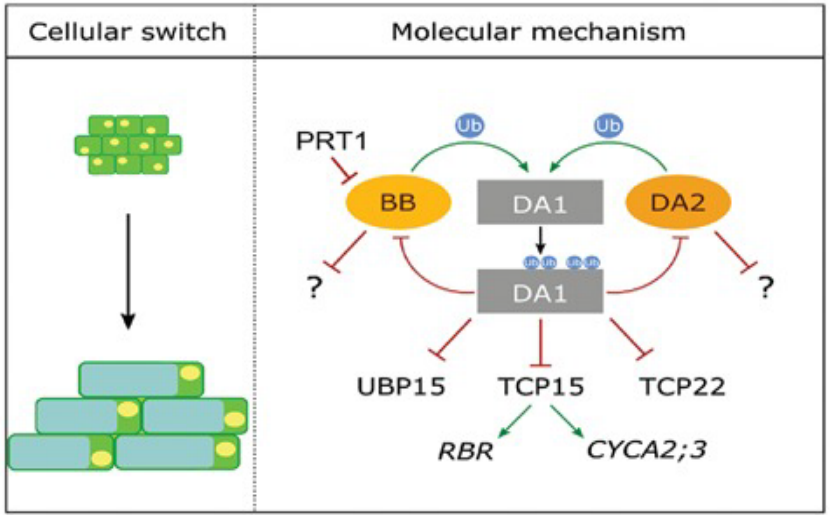
PRT1 (PROTEOLYSIS1) is a unique N-recognin E3 ubiquitin ligase



Stary *et al.* Plant Physiol (2003)

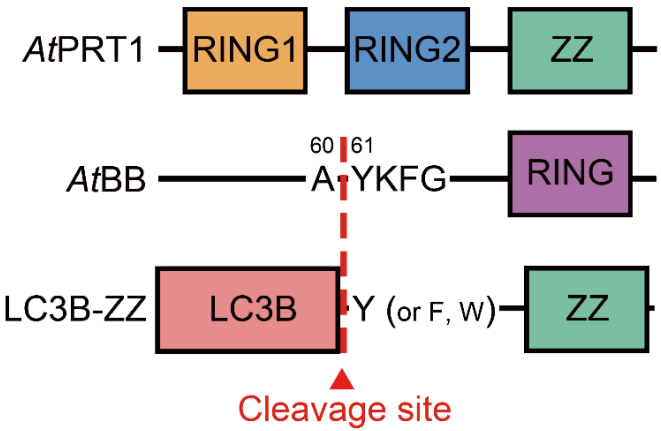


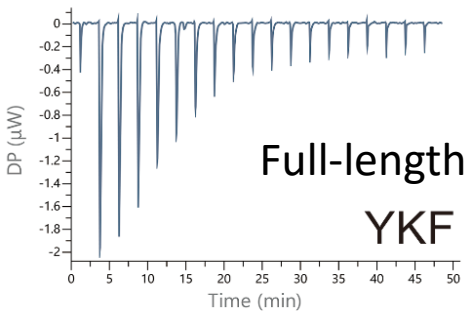
Mot *et al.* New Phytol (2018)

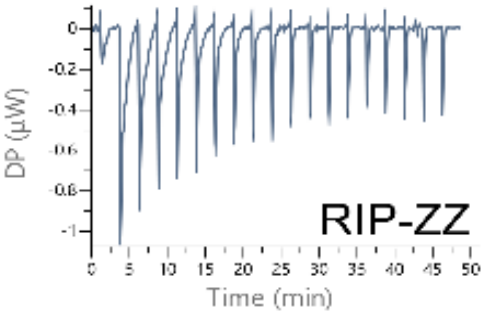


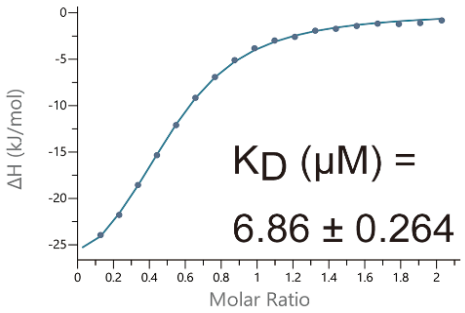
Dong *et al.* Genes Dev (2017)

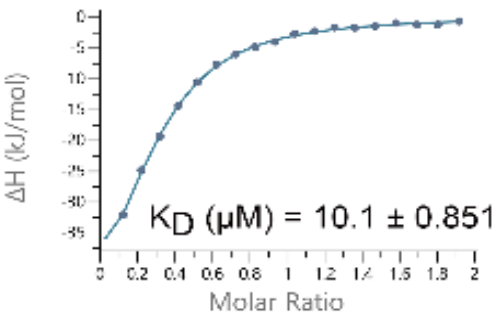
ZZ-domain is responsible for N-degron recognition

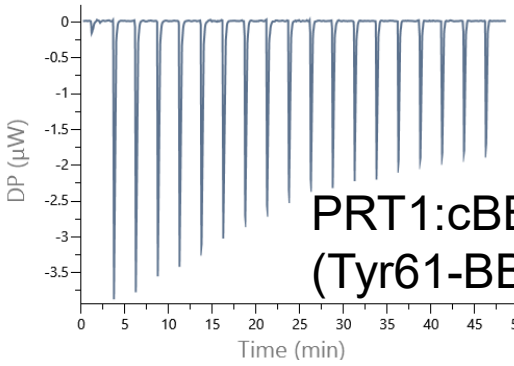


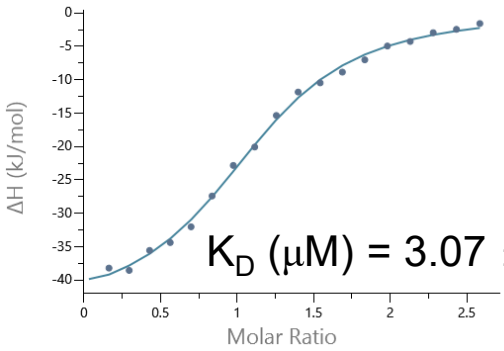


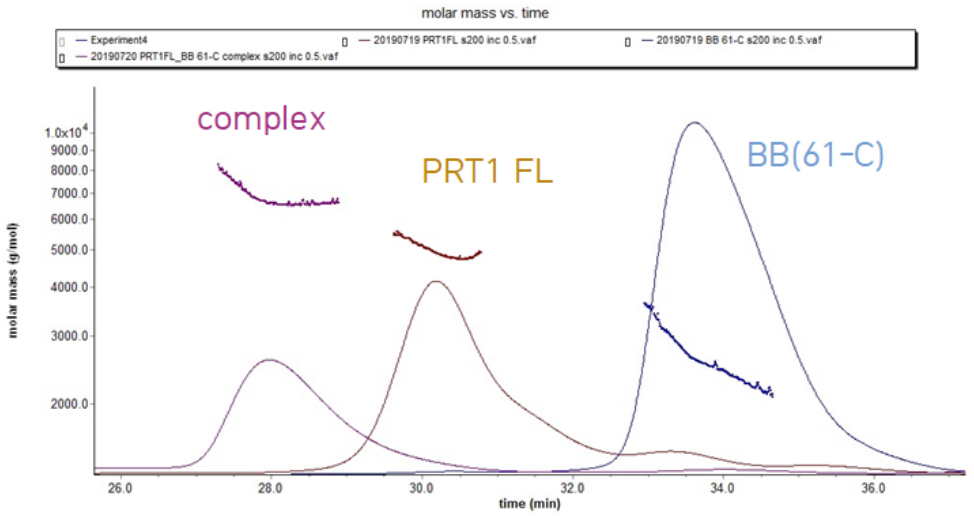












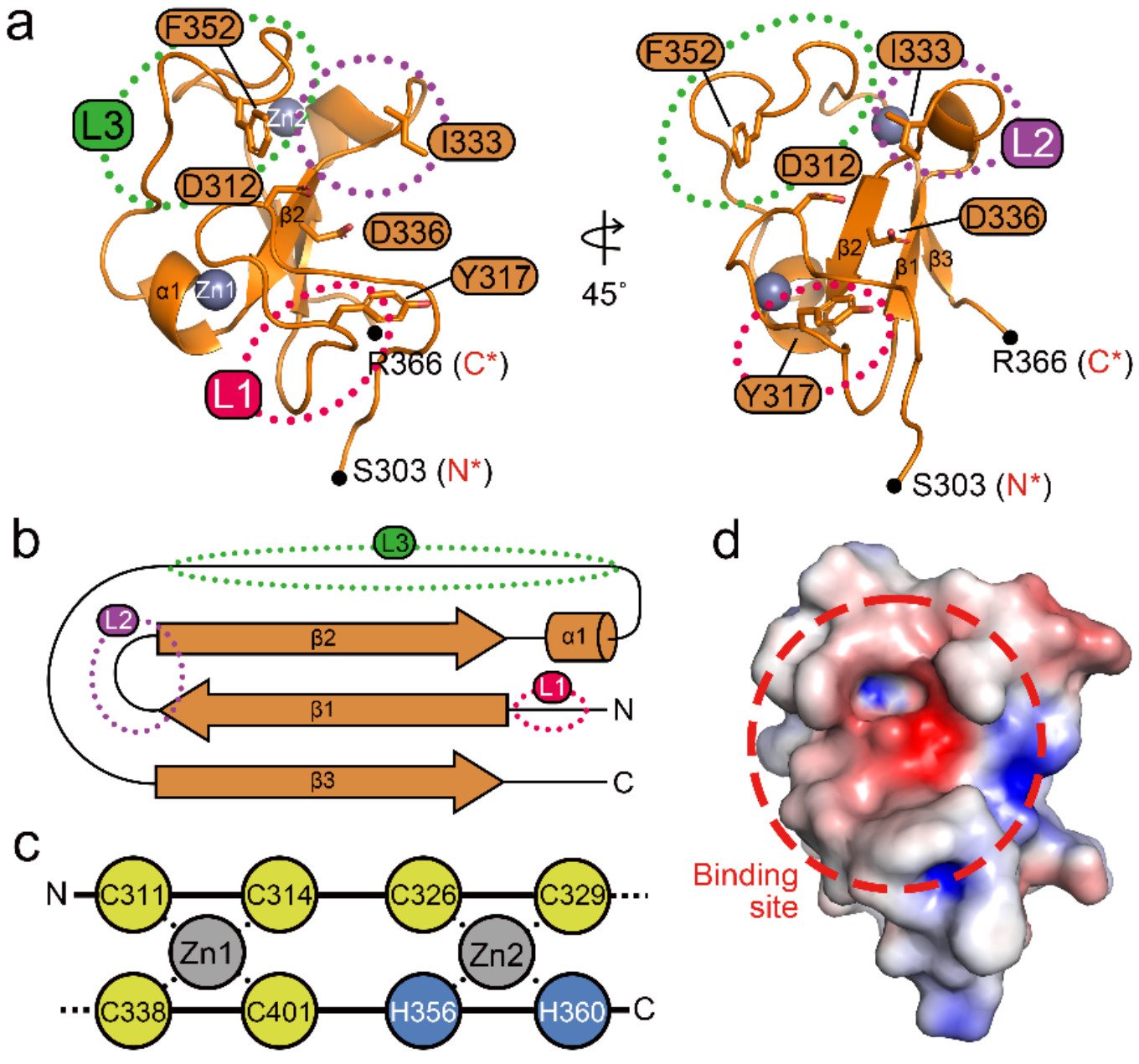
Data collection and phasing

	Apo PRT1^{ZZ}	YKFG-PRT1^{ZZ}	FKFG-PRT1^{ZZ}	WAAG-PRT1^{ZZ}
Data collection				
Space group	P 6_2 2 2	P 2_1 2_1 2_1	P 2_1 2_1 2_1	I 4_1 2 2
Cell dimensions				
a, b, c (Å)	78.0, 78.0, 70.5	48.3, 85.7, 86.0	48.7, 86.4, 89.8	106.3, 106.3, 97.1
α, β, γ (°)	90, 90, 120	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	1.28	1.28	1.28	1.28
Resolution (Å)	48.77–1.74	42.98–1.67	42.83–2.10	40.77–2.79
R_{merge}	0.084 (2.158)	0.160 (1.147)	0.174 (2.578)	0.126 (2.093)
I / σ (I)	32.13 (1.37)	20.09 (1.22)	11.57 (1.20)	18.48 (1.02)
Completeness (%)	97.24 (90.92)	98.62 (94.79)	99.67 (97.64)	99.56 (96.06)
Redundancy	21.4 (20.5)	6.8 (5.8)	14.0 (14.1)	28.8 (28.6)
Beamline	SP8 BL44XU	PAL 11C	SP8 BL44XU	SP8 BL44XU
SAD-Phasing				
No. of Zn atoms	2			
Initial figure of merit	0.434			

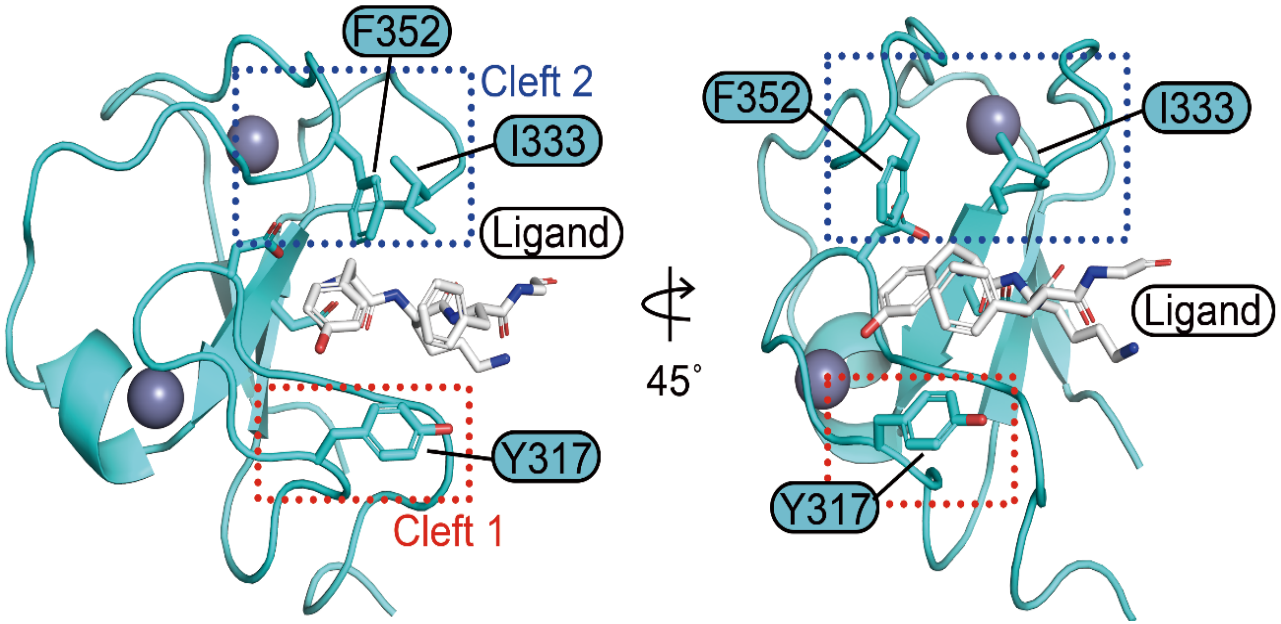
Refinement

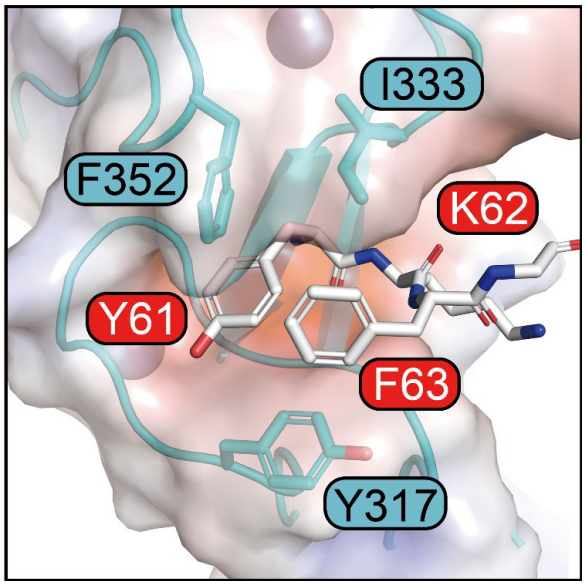
	Apo PRT1^{ZZ}	YKFG-PRT1^{ZZ}	FKFG-PRT1^{ZZ}	WAAG-PRT1^{ZZ}
Refinement				
Resolution (Å)	48.77–1.74	42.98–1.67	42.83–2.1	40.77–2.79
No. reflections	13,069 (1,191)	41,345 (3,911)	22,752 (2,193)	7,204 (683)
R_{work}	0.2350 (0.3869)	0.2107 (0.3427)	0.2572 (0.4646)	0.2345 (0.4684)
R_{free}	0.2787 (0.4442)	0.2368 (0.3780)	0.2945 (0.4967)	0.2613 (0.4440)
No. of atoms	557	3,455	3,255	1,059
Macromolecules	496	3,202	3,228	1,050
Hetero-atoms	2 Zn	12 Zn, 2 Mg	12 Zn, 2 Mg	4 Zn, 1 SO_4
Waters	33	239	13	-
Protein residues	59	405	408	136
B-factors (Å^2)	49.73	22.14	69.20	129.65
R.m.s. deviations				
Bond length (Å)	0.020	0.007	0.005	0.008
Bond angles (°)	1.73	1.01	0.66	0.95
Ramachandran stat.				
Favored (%)	100.00	98.71	98.47	98.48
Allowed (%)	0.00	1.29	1.53	1.52
Outliers (%)	0.00	0.00	0.00	0.00

Apo ZZ-domain structure of PRT1

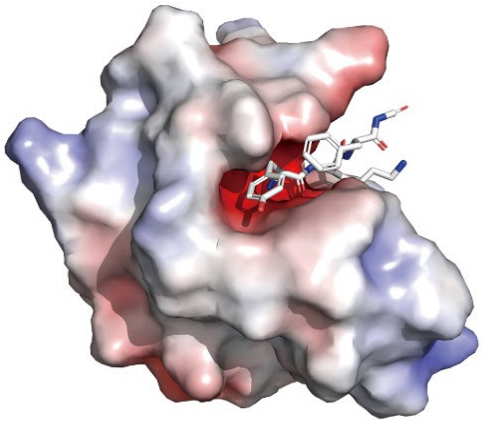


N-degron complex structure

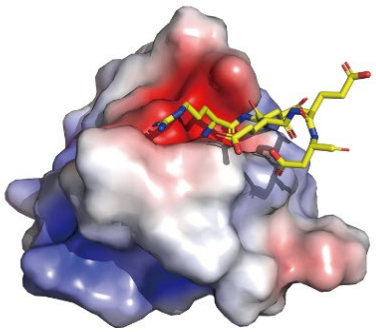




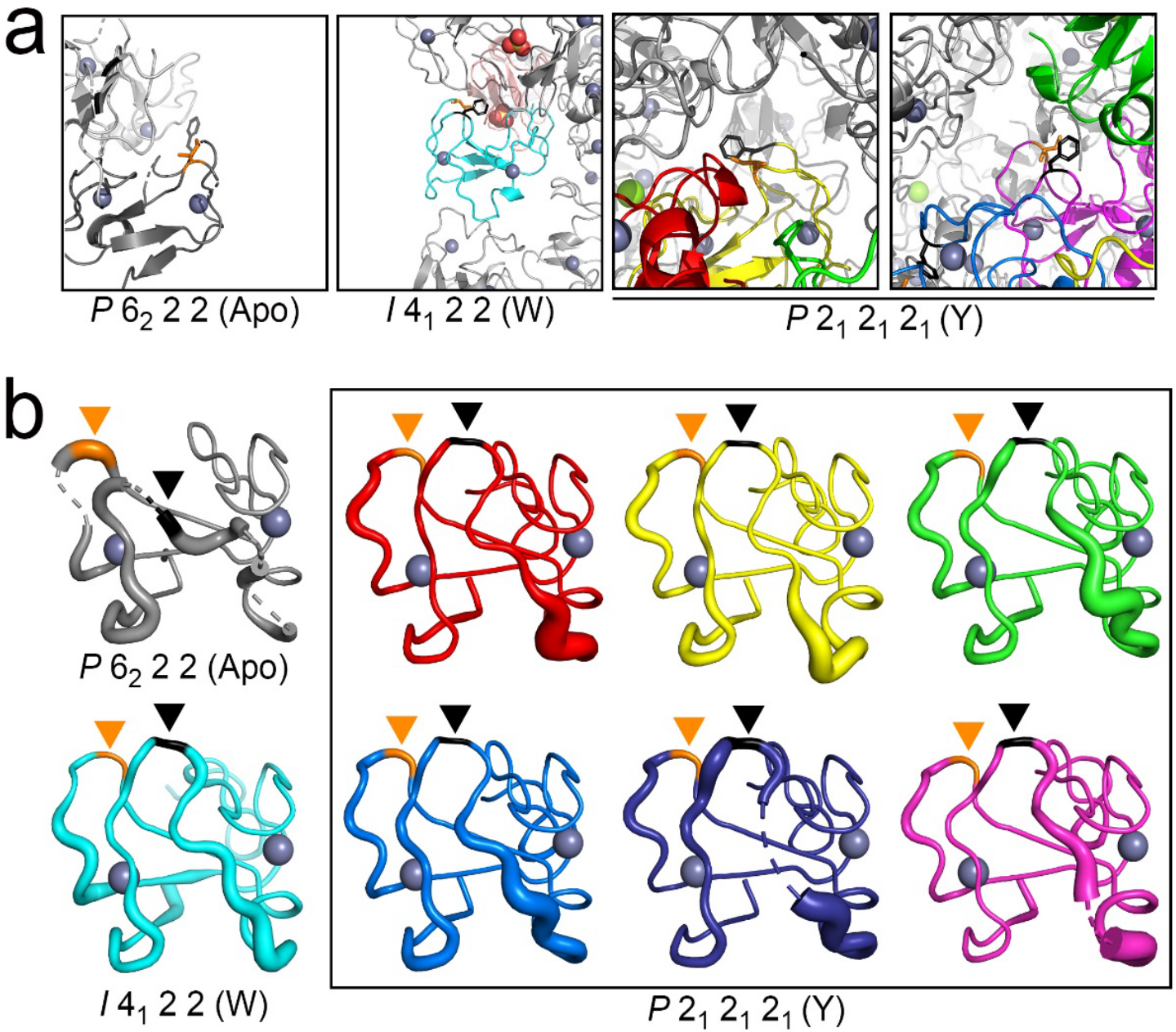
PRT1-ZZ



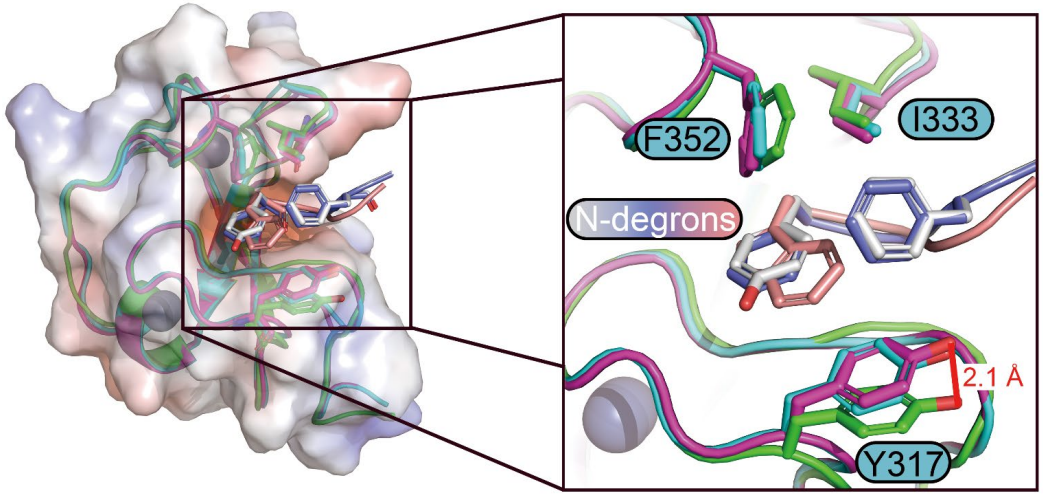
p62/SQSTM1-ZZ

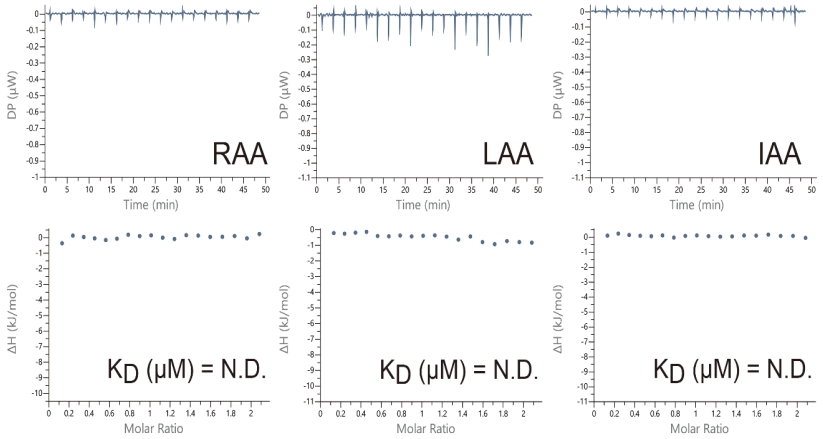


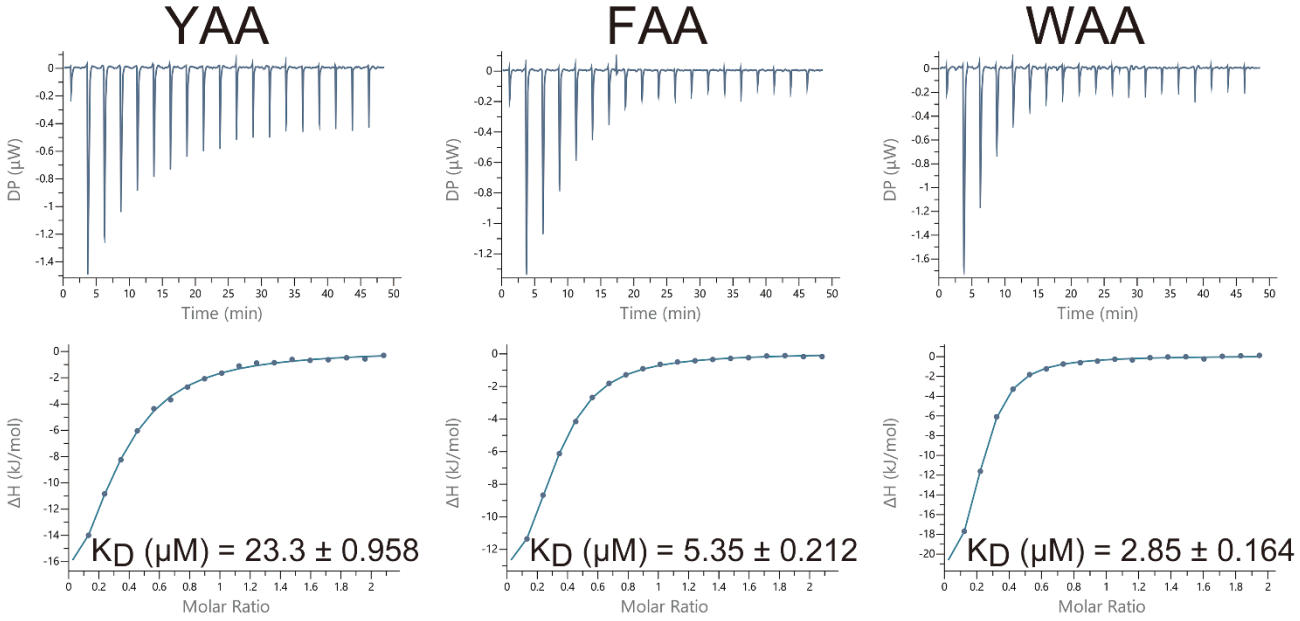
Crystal packing analysis and B-factors



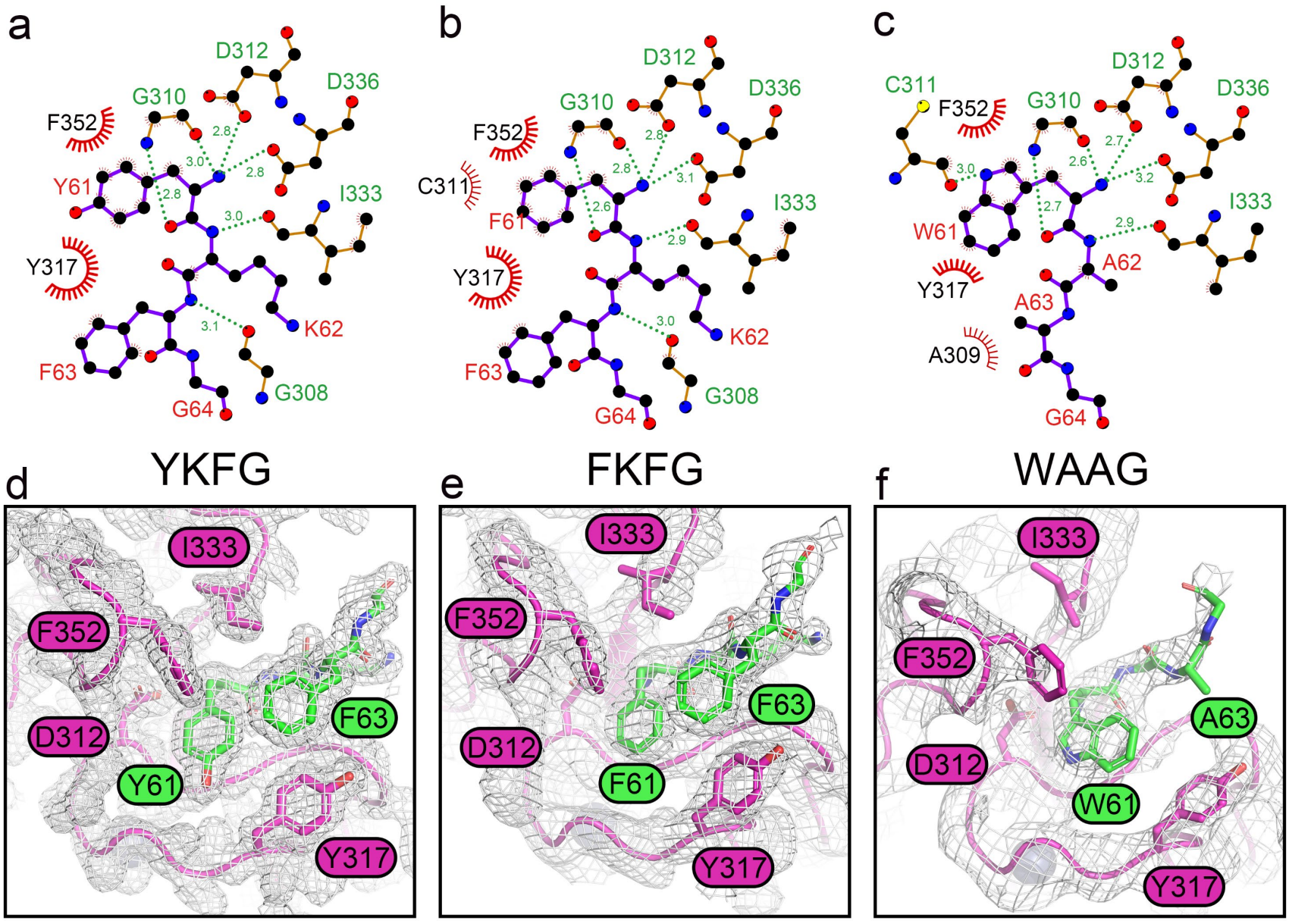
Specificity for the aromatic N-degrons



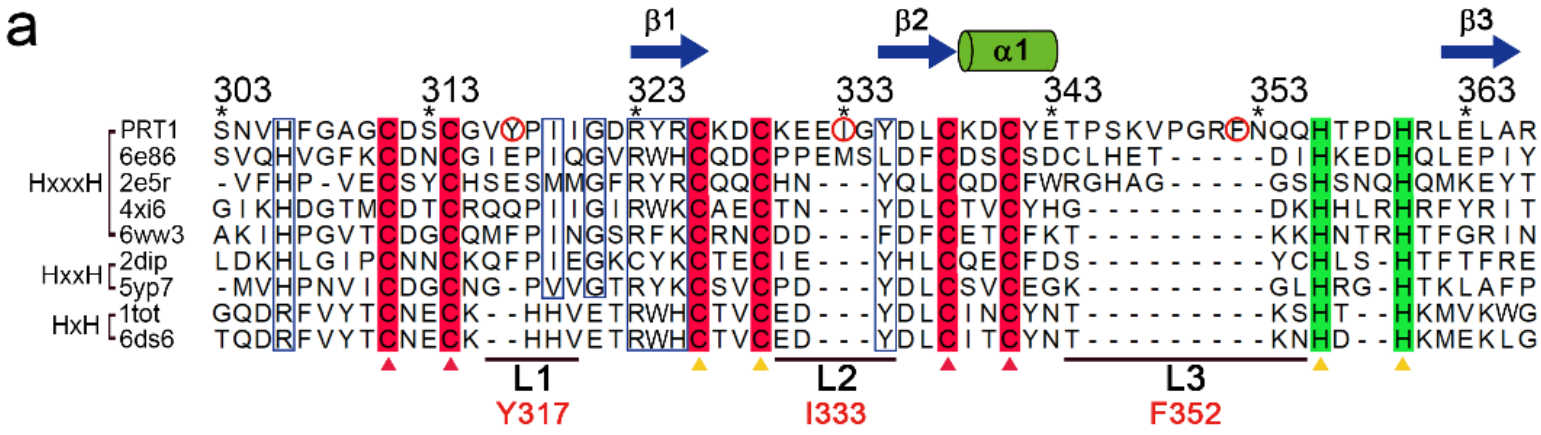


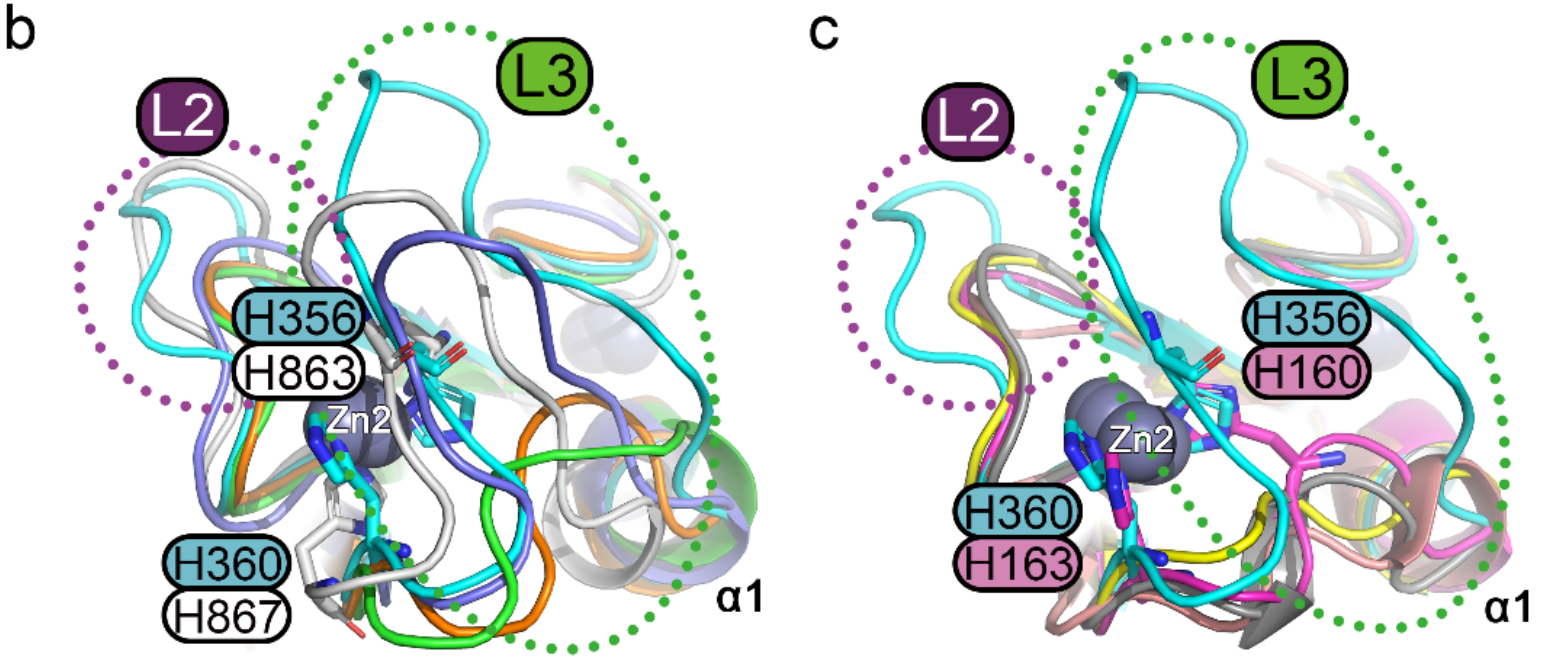


Hydrophobic interaction between PRT1 and BB



Unique conformational change in PRT1-ZZ

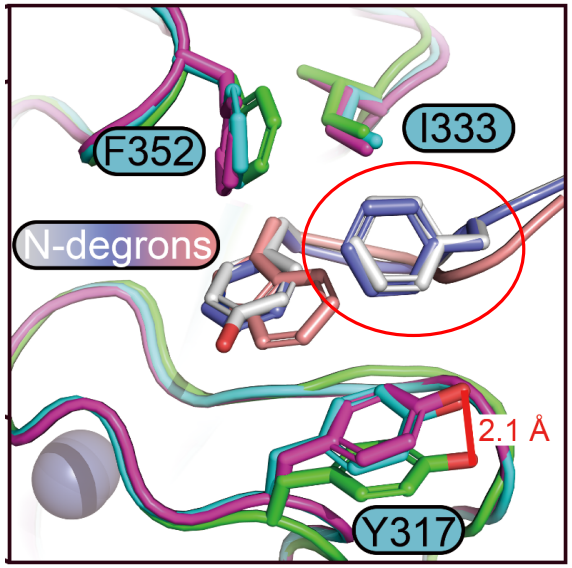


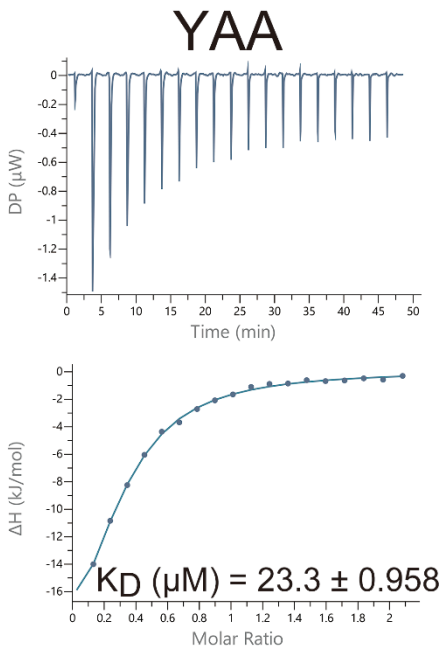


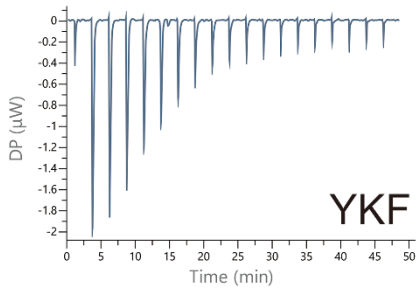
HxxxH *vs* HxxH

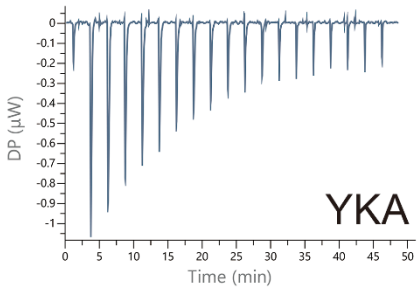
HxxxH *vs* HxH

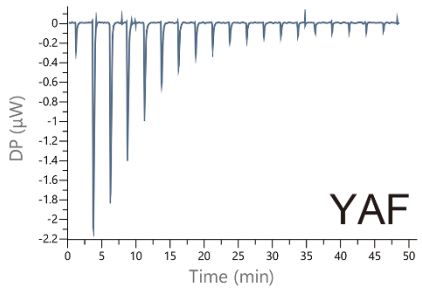
Role of the 3rd residue in N-degron

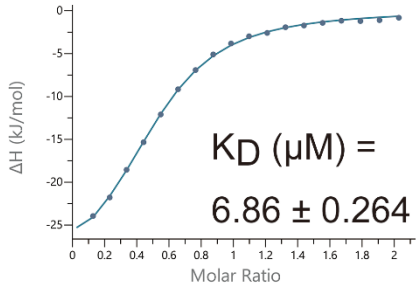


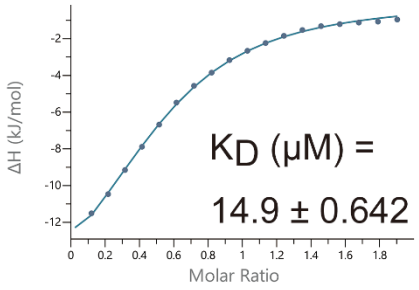


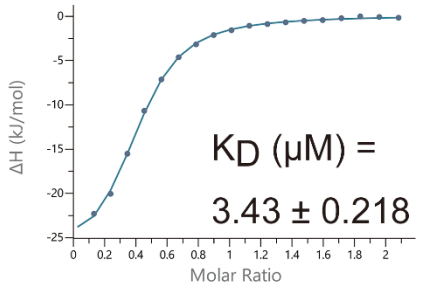




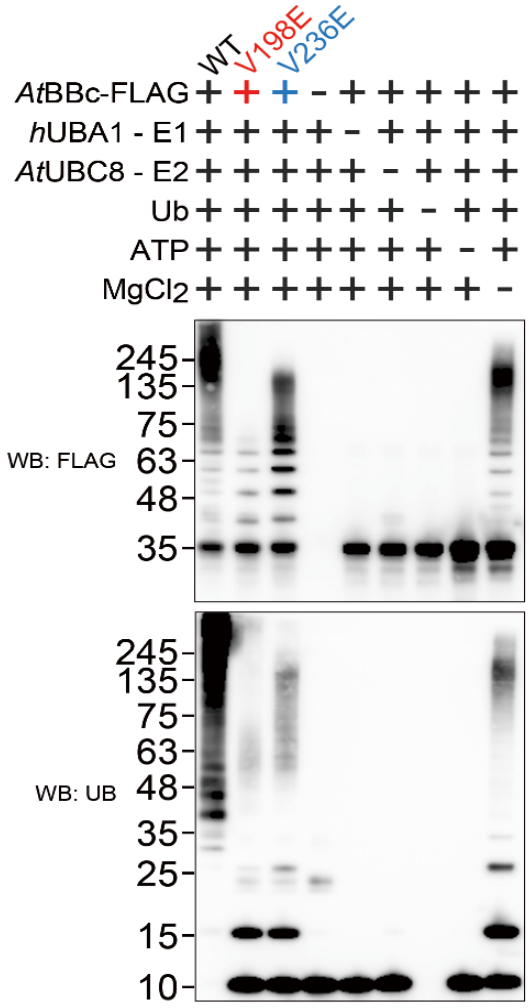


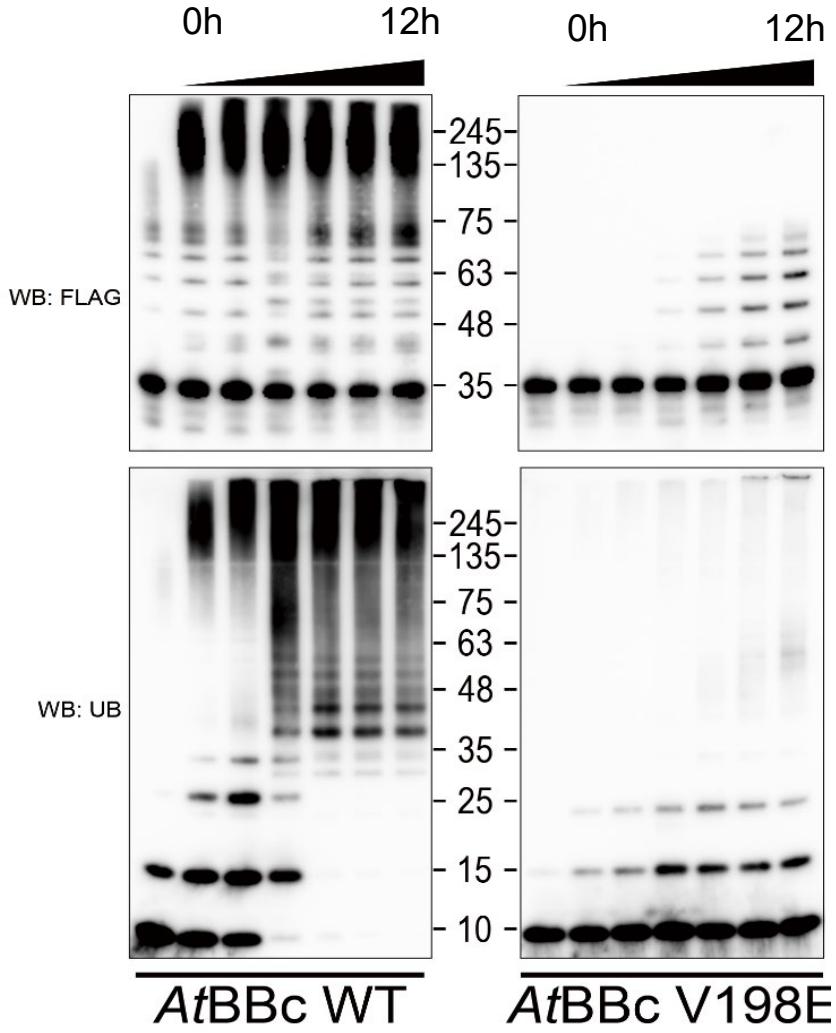




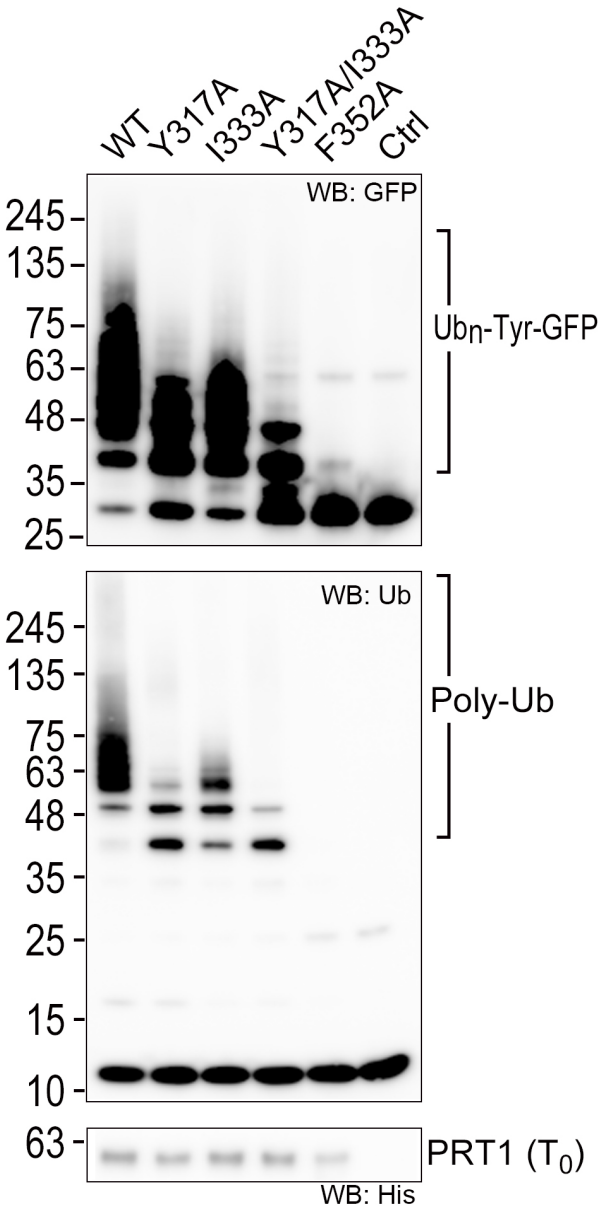


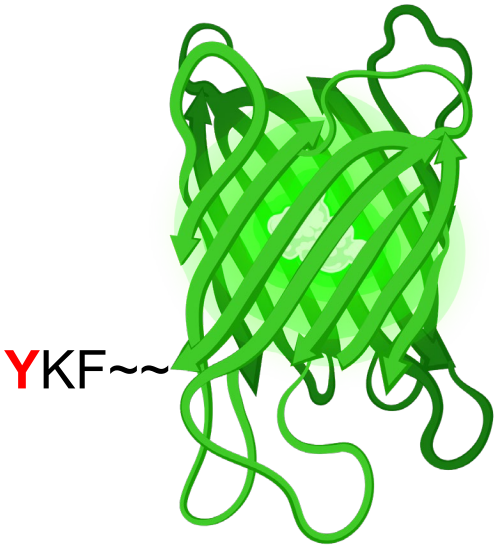
Modified BB substrate for ubiquitylation: V198E

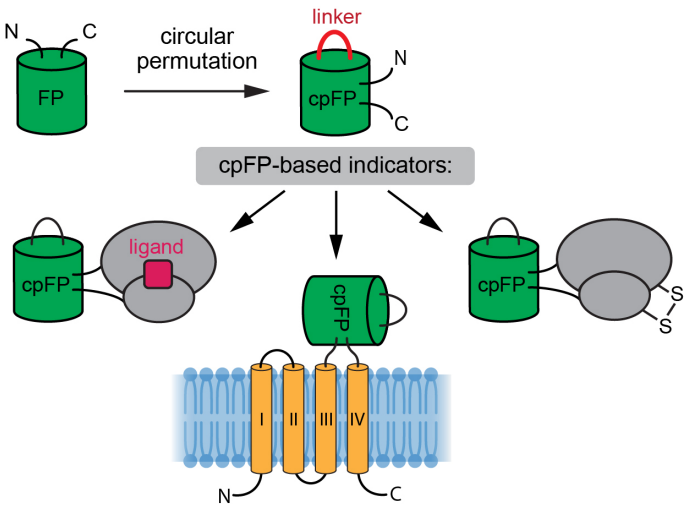




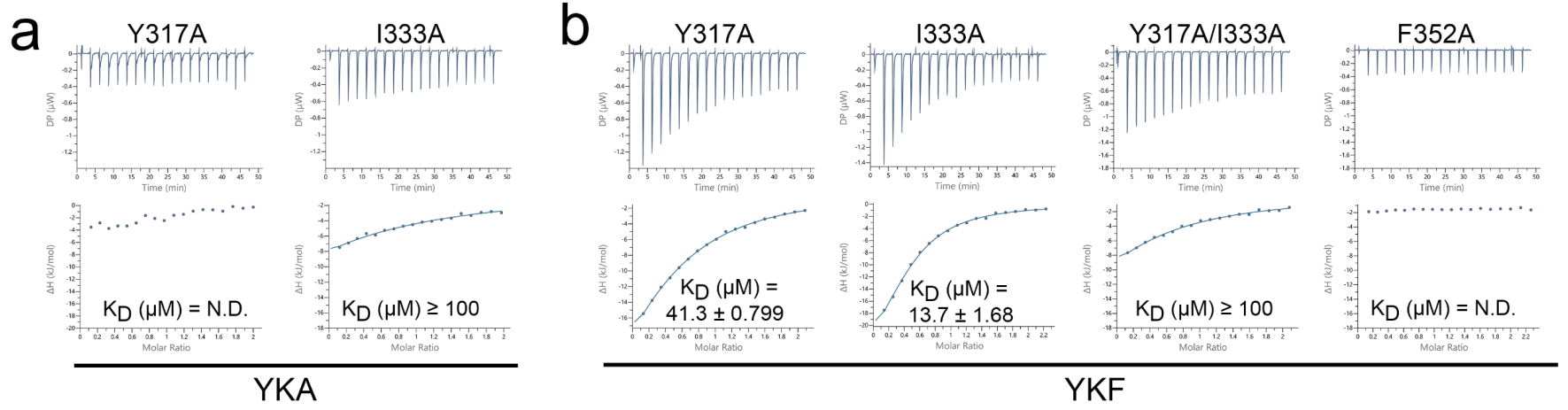
Model substrate for ubiquitylation: YKF-cpGFP

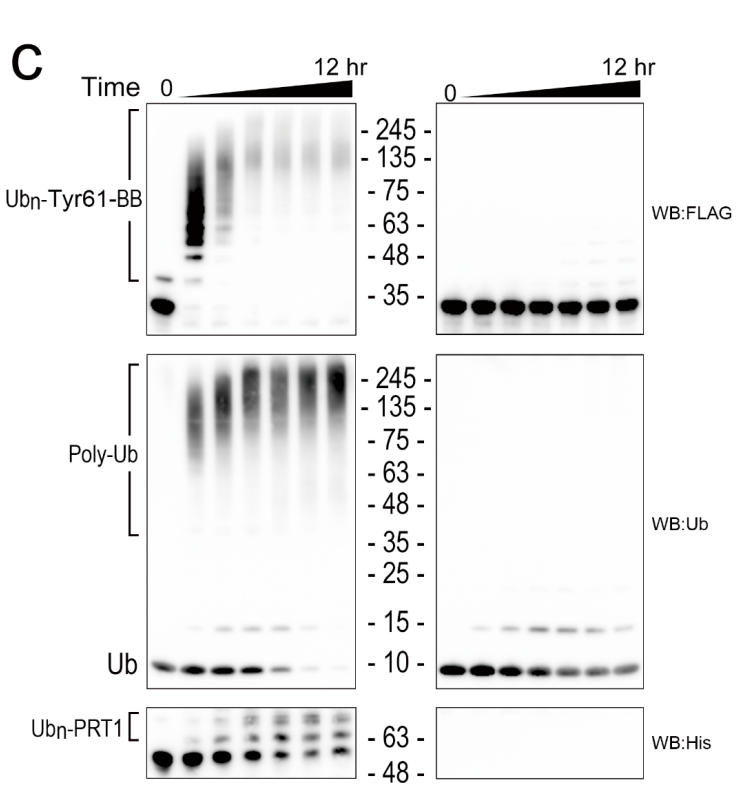


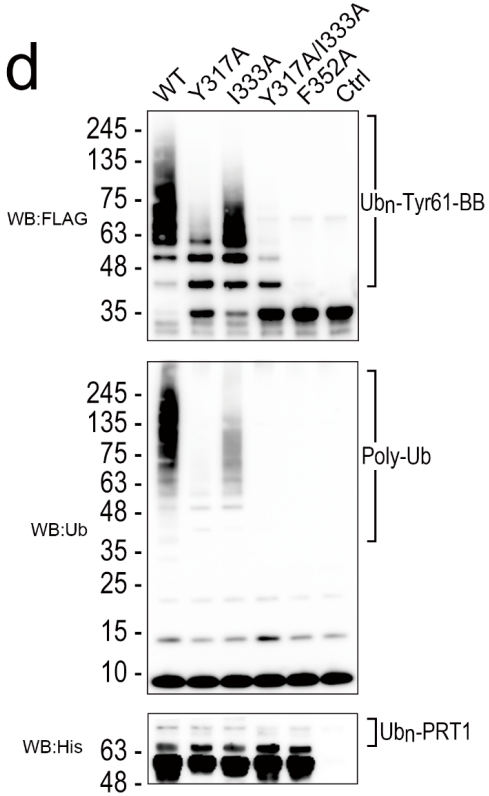


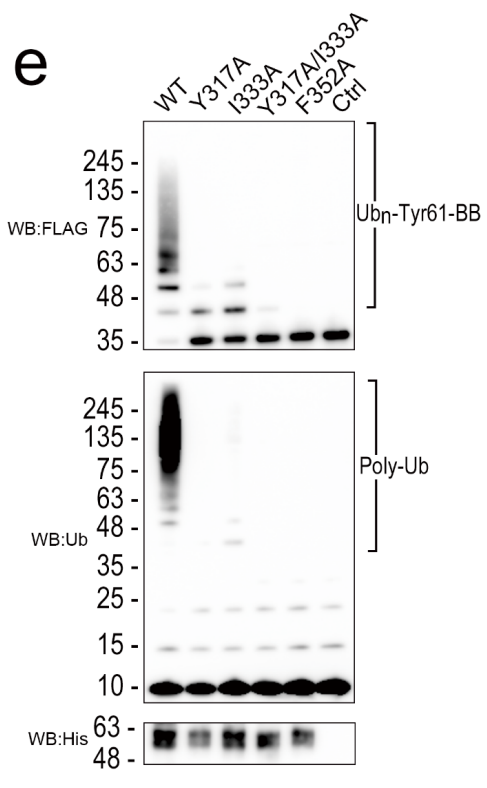


Ubiquitylation: binding and activity correlation

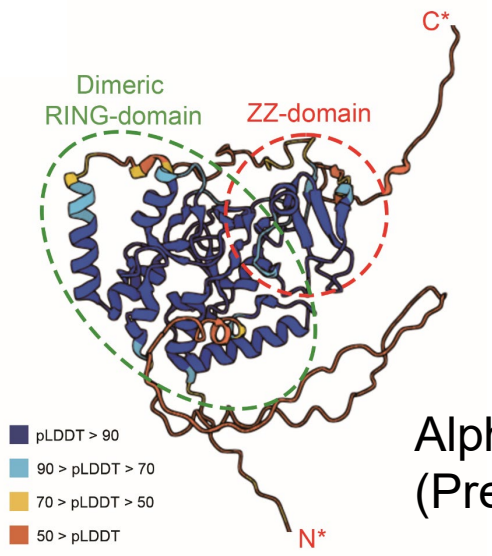




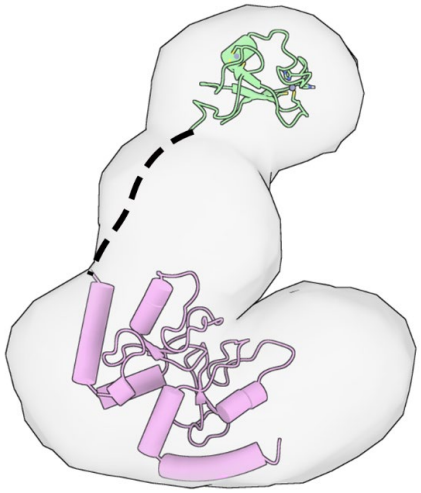




Overall structure of PRT1 (SAXS)

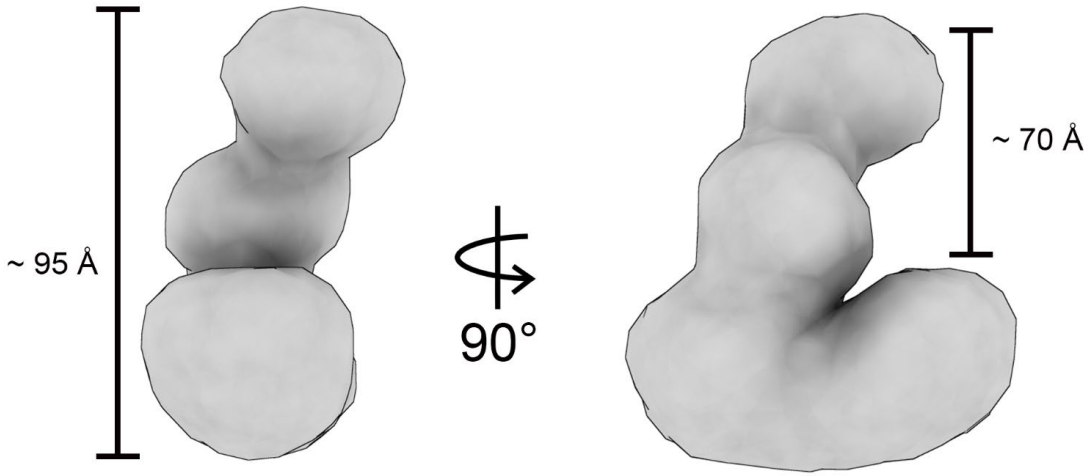


AlphaFold
(Predicted)

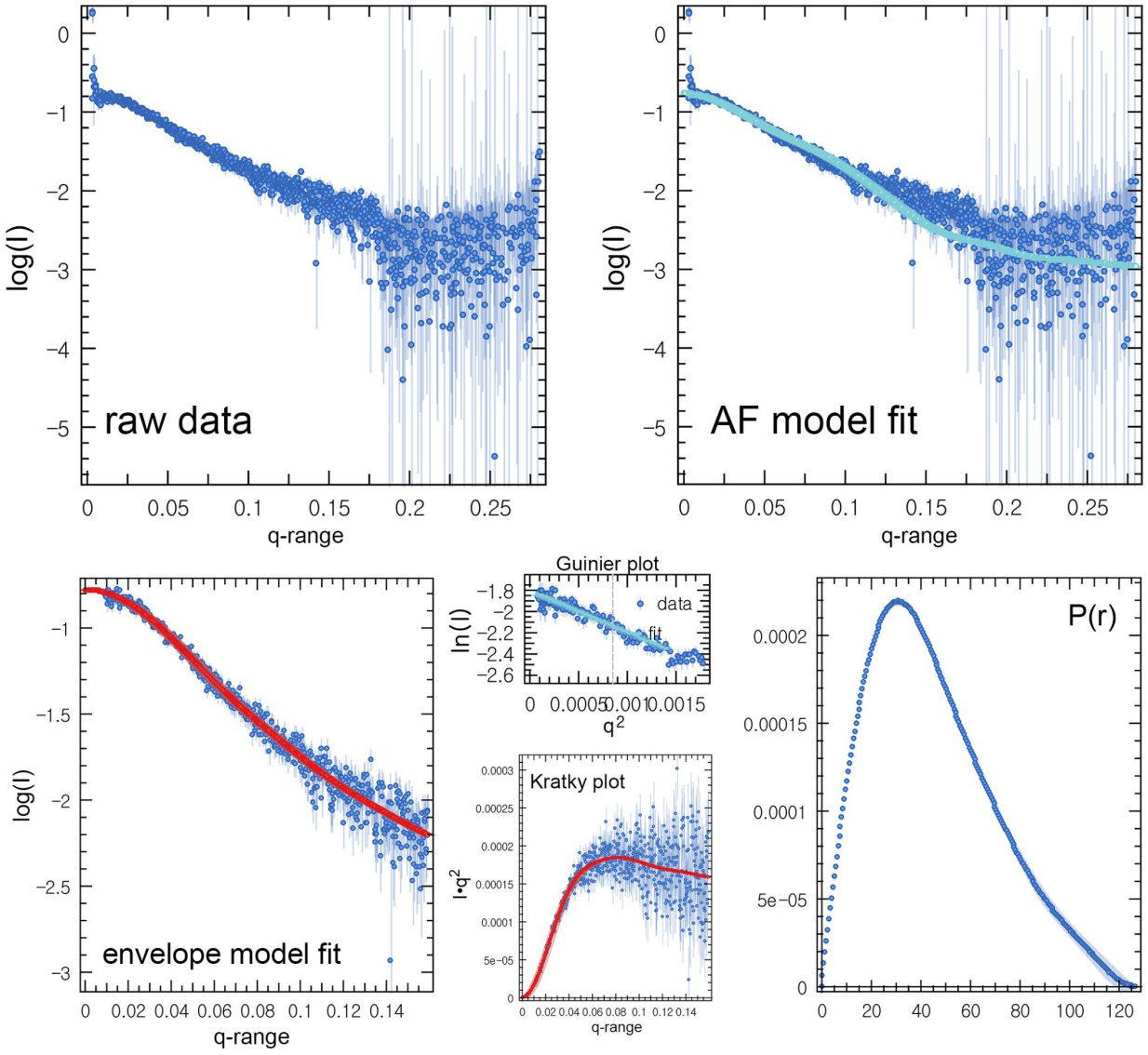


SAXS
(Experimental)

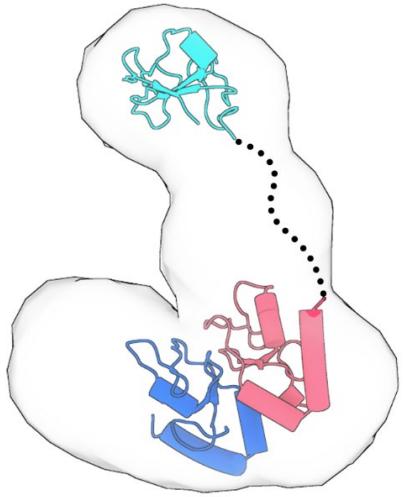
‘L’

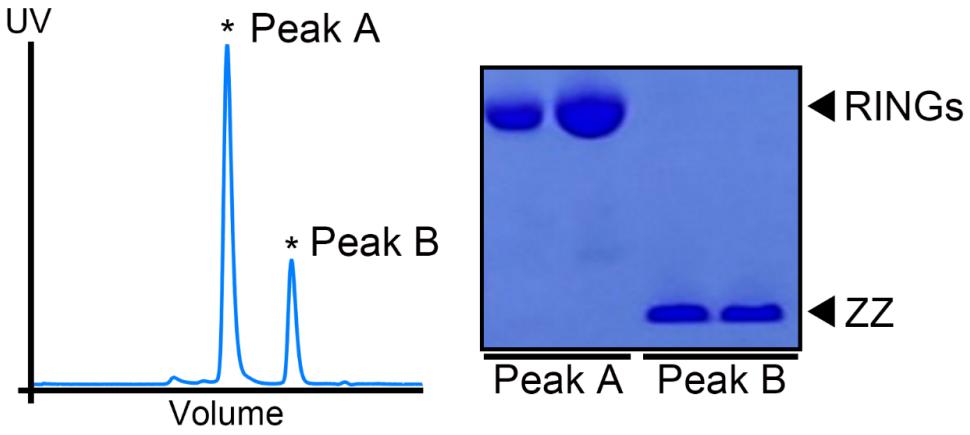


SAXS data analysis (protein sample vs AF model)



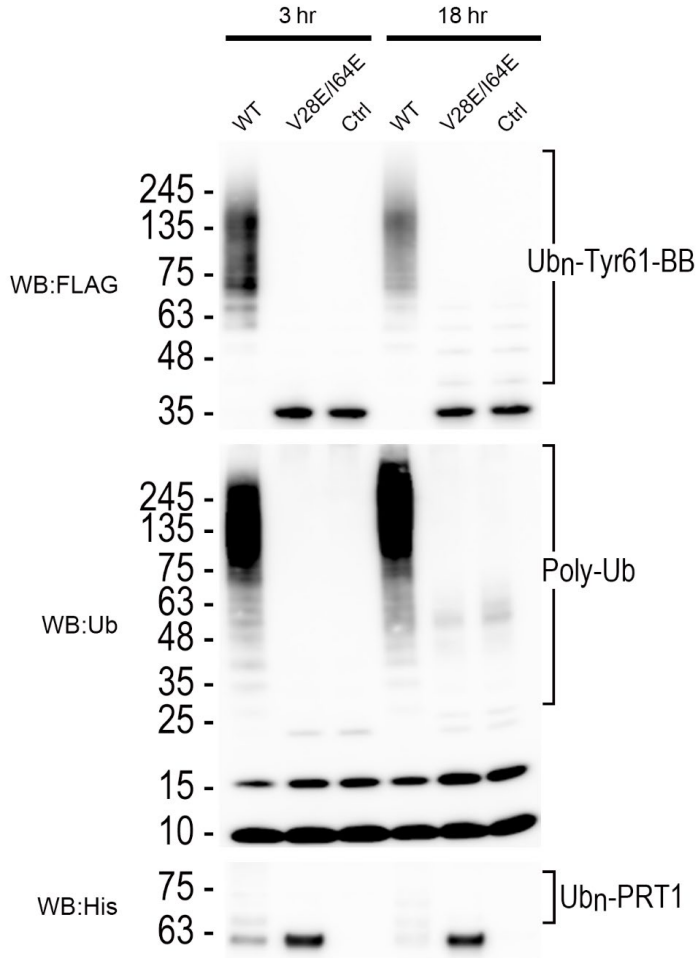
RING1 and ZZ-domain migrate separately



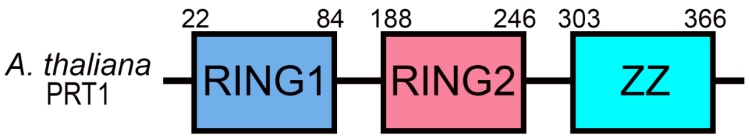


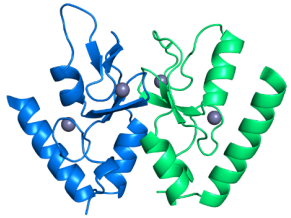
RING1 is critical for ubiquitylation

V28, I64 (putative E2 binding residues)

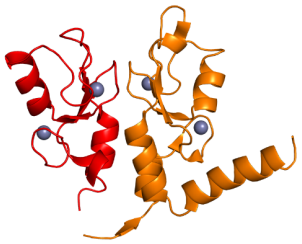


Intramolecular RING dimerization of PRT1

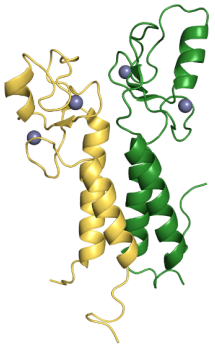




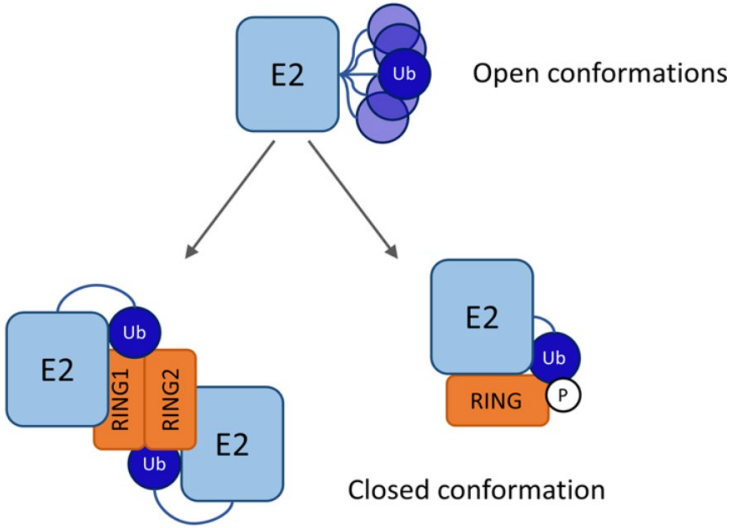
PRT1 AlphaFold
Intramolecular dimer

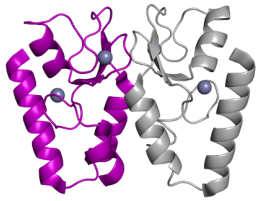


RING1B-BMI1 6wi8
Heterodimer
RMSD: 2.772

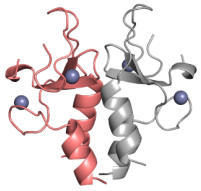


BRCA1-BARD1 1jm7
Heterodimer
RMSD: 1.760

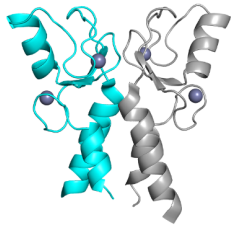




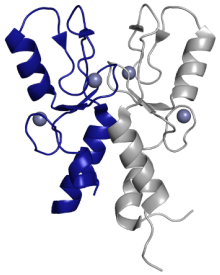
RNF8 4orh
Homodimer
RMSD: 2.991



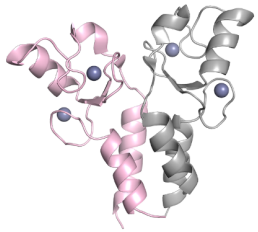
TRIM5α 4tkp
Homodimer
RMSD: 4.000



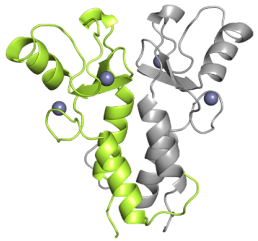
TRIM21 6fga
Homodimer
RMSD: 1.987



TRIM25 5eya
Homodimer
RMSD: 1.162



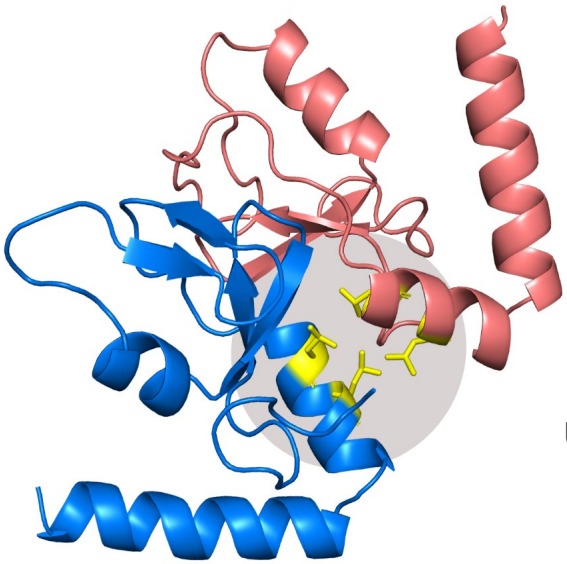
TRIM37 3lrq
Homodimer
RMSD: 1.764



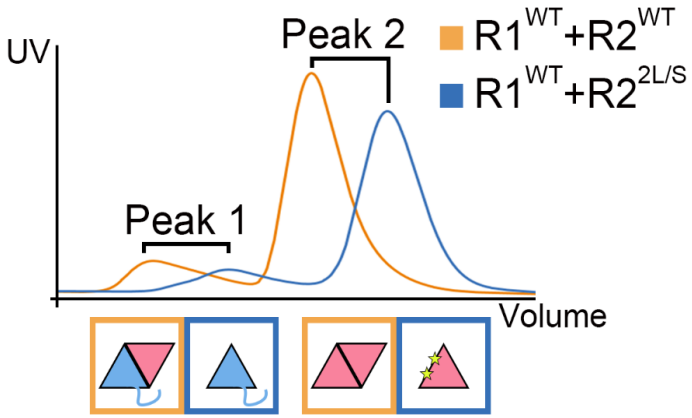
TRIM69 6yxe
Homodimer
RMSD: 3.318

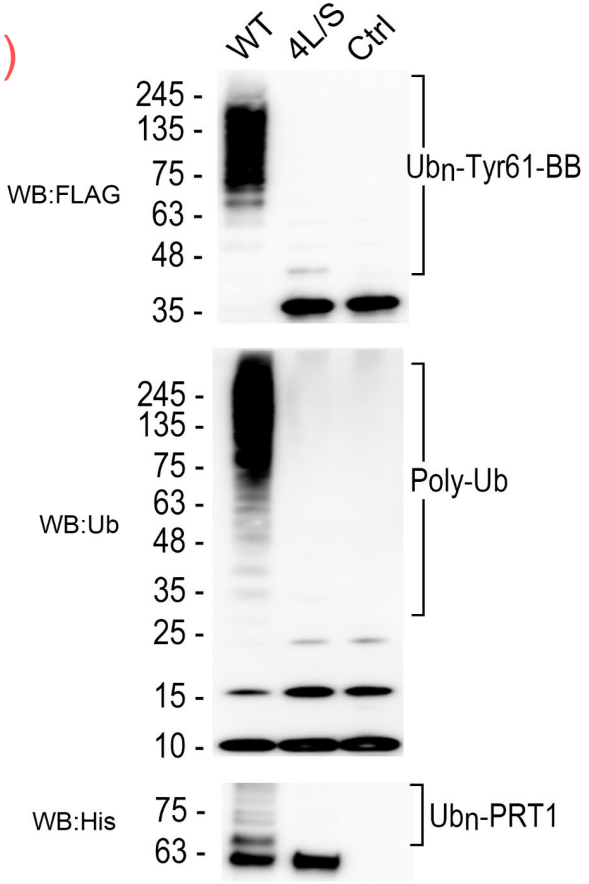
Filippo Fiorentini *et al.* & Katrin Rittinger (2020) *Biochemical Soc. Transaction*

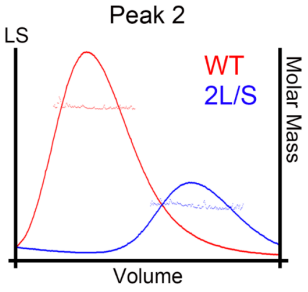
RING dimer is critical for ubiquitylation activity

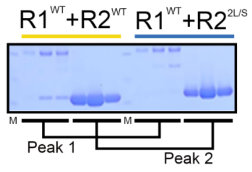


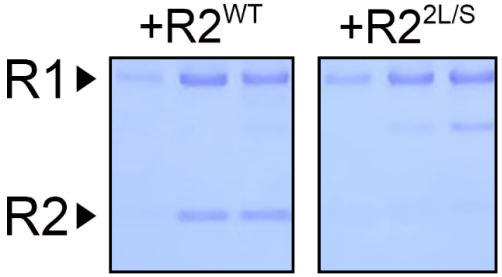
4L/S:
L79S/L82S in RING1 &
L246S/L249S in RING2: 2L/S)



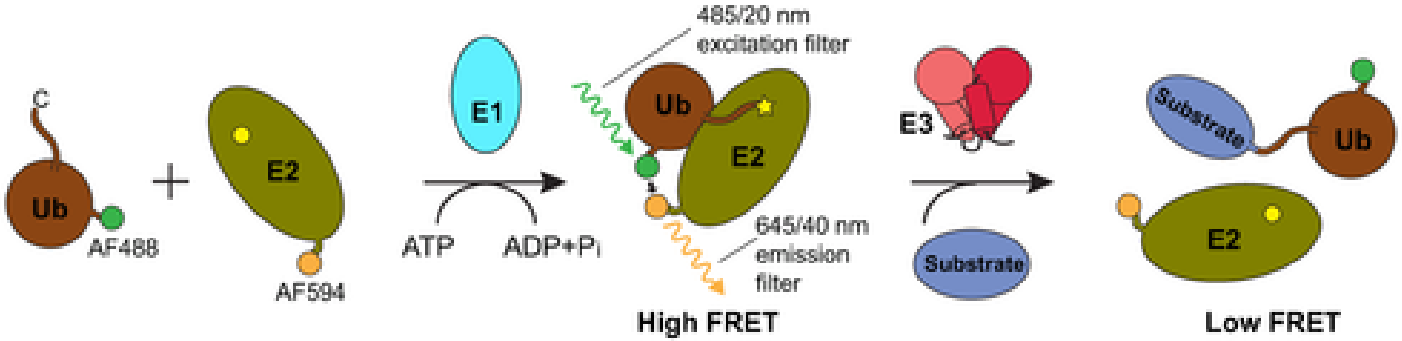


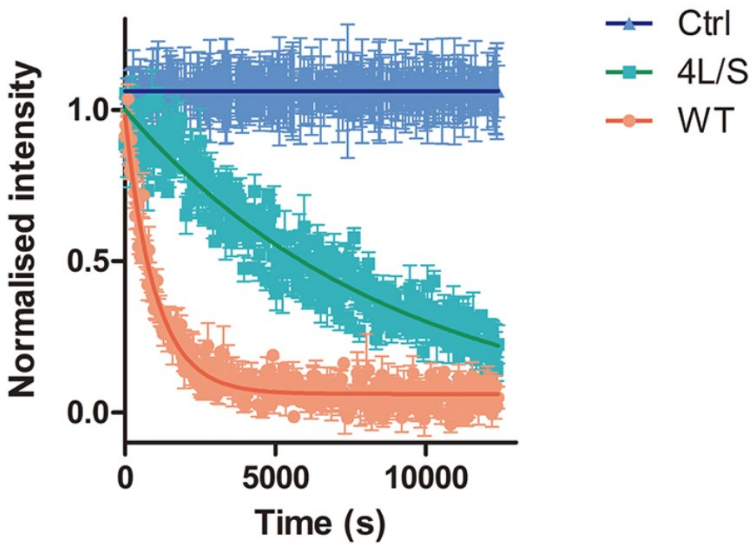






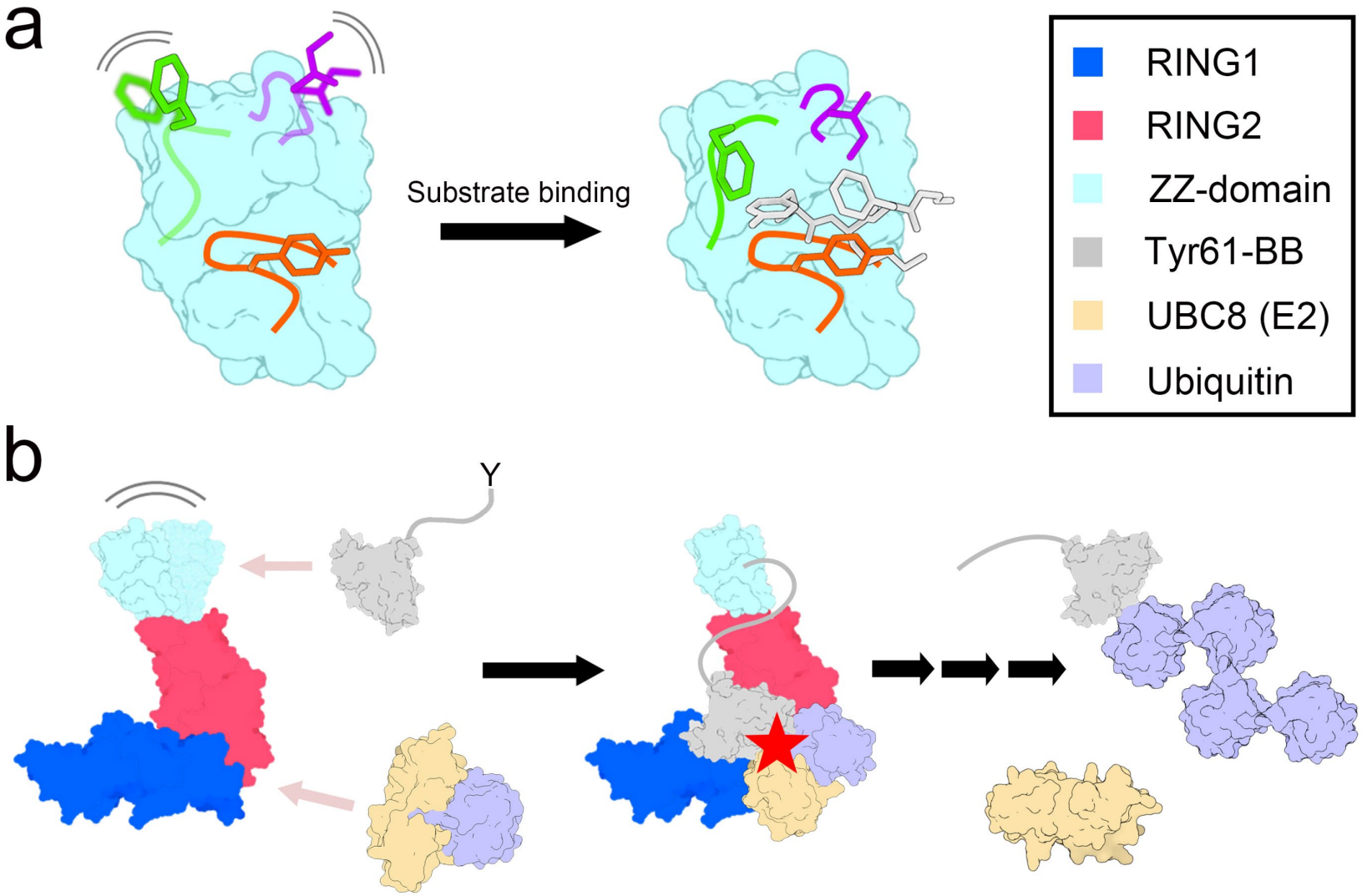
Ub discharge assay





	WT	4L/S
Half-life (sec)	696.6	6212
k (Decay rate)	$9.9951e^{-3}$	$1.1116e^{-3}$

Mode of action of PRT1



Summary

1. ZZ-domain recognizes the bulky hydrophobic type-2 N-degrons by the 3 key residues (Y317, I333, and F352).

2. The conformational change of long loops is the unique feature of PRT1.

3. The 3rd residue (Phe) of cleaved BB (Tyr61-BB substrate) is also participated in stabilizing the interaction.

4. The interaction between N-degron and PRT1 affects the ubiquitylation activity.

5. Intramolecular RING heterodimer is a key for the robust ubiquitylation of PRT1 N-recognin.

Acknowledgements

Thanks to Lab members

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Hejeong Shin
Minsang Kim
Ju Yeon Lee

Facilities

< X-ray >
PAL 11C
Spring-8 BL44XU

< SAXS >
PAL 4C

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