Supporting information for: Recognition of Poly-ubiquitins by the Proteasome through Protein Re-folding Guided by Electrostatic and Hydrophobic Interactions

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Table S1: List of simulations performed.

System (residues)	force field	time (ns)	replicas	system size (atoms)
human S5a (196-306)	CHARMM22	200	1	75,000
human S5a (196-306)	CHARMM27	200	1	75,000
human S5a (196-306)	CHARMM36	200	1	75,000
yeast Rpn10 (208-268)	CHARMM22	200	3	54,000
S. pombe Rpn10 (190-243)	CHARMM22	200	1	39,000
human S5a (196-306):mono-ubiquitin	CHARMM22	200	3	93,000
yeast Rpn10 (208-268):mono-ubiquitin	CHARMM22	200	3	72,000
S. pombe Rpn10 (190-243):mono-ubiquitin	CHARMM22	200	3	70,000
human S5a (196-306):K48-Ubq4 (Ubq1,Ubq2)	CHARMM36	200	2	263,400
human S5a (196-306):K48-Ubq4 (Ubq1,Ubq3)	CHARMM36	200	1	200,700
human S5a (196-306):K48-Ubq4 (Ubq1,Ubq4)	CHARMM36	200	2	200,700
human S5a (196-306):K48-Ubq2	CHARMM36	200	1	126,500
human S5a (272-306):Ubq (Steered MD)	CHARMM36	40	2	73,800

Table S2: Comparison of simulated NOE pair distances with NMR data[S1,S2] for S5a/Rpn10 arm structures with and without ubiquitin bound.

Structures	$N_{\text{restraints}}$ (NMR)	N_{violated} (MD)	% agreement
S5a/Rpn10 arm (1YX4)	98	6	93.9%
S5a/Rpn10 arm : mono-ubiquitin (2KDE)	2,928	189	93.5%

Table S3: Experimentally determined K_d values for binding of 26S proteasome to different ubiquitin species and binding of human S5a to mono- and K48-linked diubiquitin.

	n_{ubq}	K_d (nM)	ΔG (kcal/mol)
K_{48}-linked Ubq_2 : 26S Proteasome[S3]	2	15,000	-6.62
K_{48}-linked Ubq_3 : 26S Proteasome[S3]	3	1,933	-7.84
K_{48}-linked Ubq_4 : 26S Proteasome[S3]	4	171	-9.29
K_{48}-linked Ubq_6 : 26S Proteasome[S3]	6	52	-10.00
Ubq_5 (linear) : 26S Proteasome[S3]	5	539	-8.60
Ubq_1 : (S5a UIM1)[S1]	1	350,000	-4.74
Ubq_1 : (S5a UIM1)[S1]	1	73,000	-5.68
K_{48}-linked Ubq_2 : (S5a)[S1]	2	8,900	-6.93

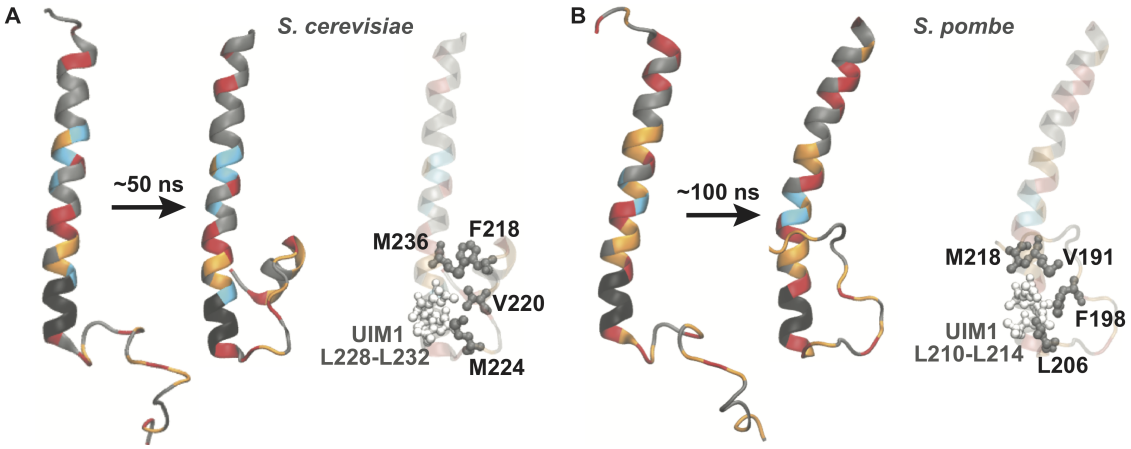

Figure S1: Properties of the flexible arm of the Rpn10 fragment in *S. cerevisiae* and *S. pombe* from MD simulations. A. The starting structure (left) and a representative structure (middle) of the Rpn10 fragment in *S. cerevisiae* from MD simulations, and the same structure highlighting interacting nonpolar residues/groups of the Rpn10 fragment on the right. Amino acids marked in red are negatively charged, blue are positively charged, orange are hydrophobic, black comprise UIM1, and grey cover the rest. B. The starting structure (left) and a representative structure (middle) of the Rpn10 fragment in *S. pombe* from MD simulations, and the same structure highlighting interacting nonpolar residues/groups of the Rpn10 fragment on the right. The color code is the same as in panel A.

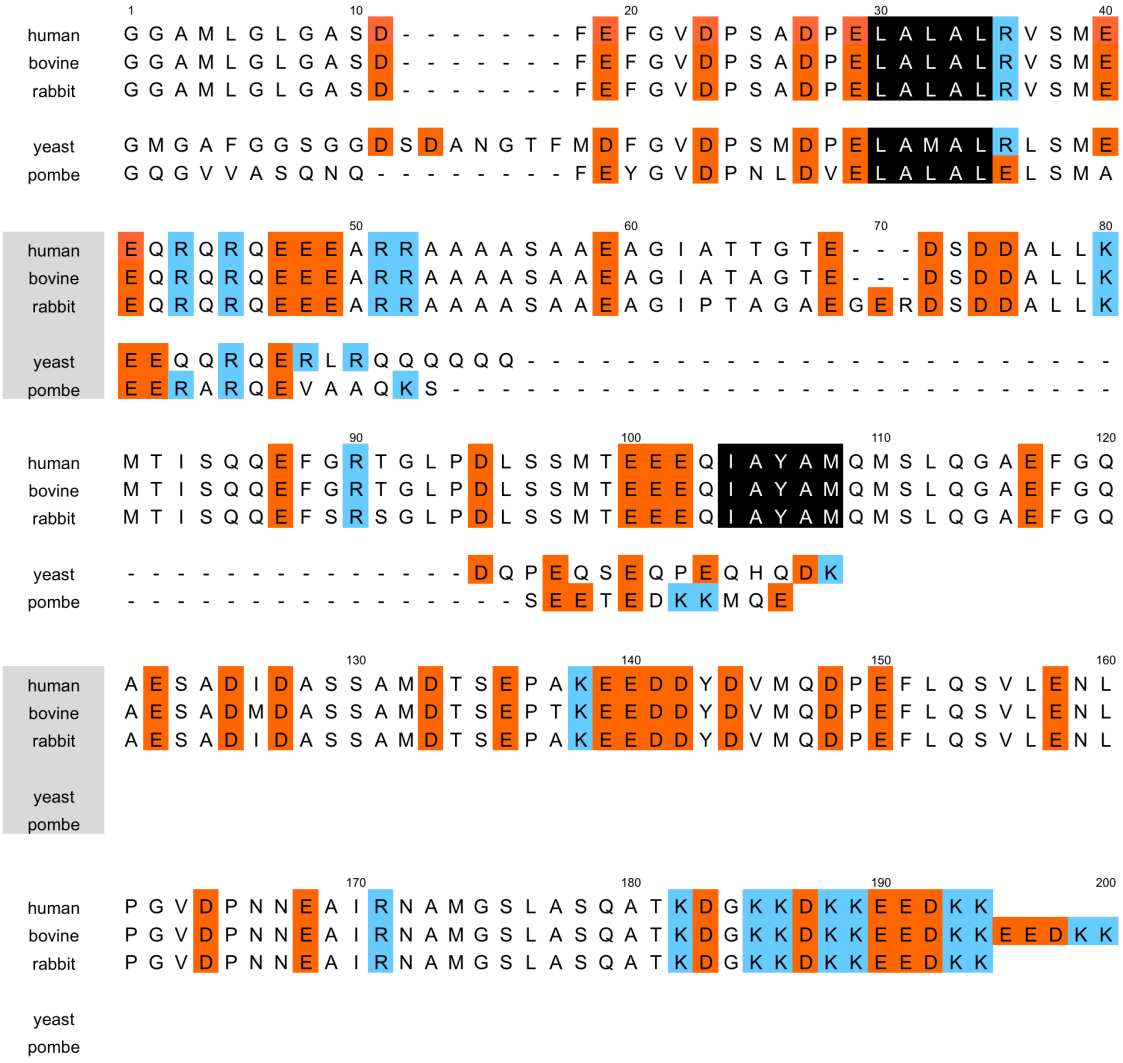

Figure S2: Aligned sequences of Rpn10 flexible arms of human, bovine, rabbit, *S. cerevisiae* and *S. pombe* species. The sequences of Rpn10 protein of human, bovine, rabbit, yeast, and *S. pombe* species correspond to UniProt[S4] codes P55036, Q58DA0, B7NZD2, P38886, and O94444, respectively.

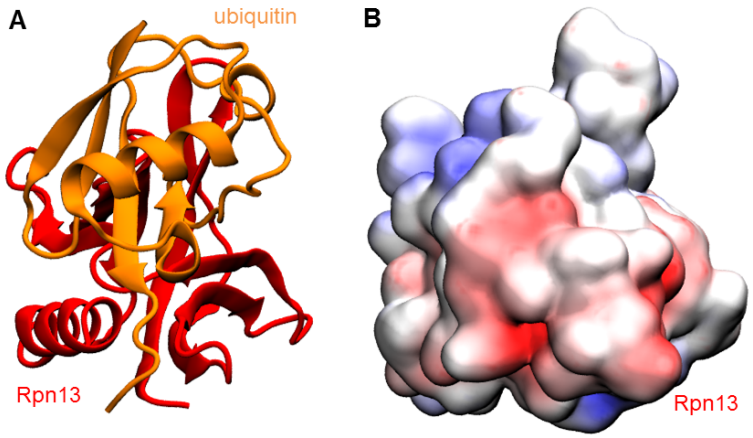

Figure S3: NMR structure of mouse Rpn13 bound to mono-ubiquitin (pdbID: 2Z59). A. Cartoon representation of the Rpn13-mono-ubiquitin complex. B. Surface of Rpn13 colored according to electrostatic potential, shown in the same orientation as in panel A; ubiquitin is removed to reveal the potential of the Rpn13-ubiquitin binding surface. The color scale of the electrostatic potential is the same as in Fig. 3 (in k_BT/e units).

References

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(S2) Wang, Q.; Young, P.; Walters, K. J. Structure of S5a Bound to Monoubiquitin Provides a Model for Polyubiquitin Recognition. *J. Mol. Biol.* **2005**, *348*, 727–739.

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(S4) Consortium, T. U. UniProt: A Hub for Protein Information. *Nucl. Acids Res.* **2015**, *43*, D204–D212.