

TABLE S2. Features of recombinant proteins.

Protein	Molecular Mass	Oligomeric status ^(b)	Putative activity	Organism	Solubility ^(c)
CpxP	19.7	dimer	Inhibitor of the CpxA kinase	<i>E. coli</i>	soluble
ClpP1	22.5	tetradecamer	peptidase	<i>M. tuberculosis</i>	soluble
ClpP2	22.9	tetradecamer	peptidase	<i>M. tuberculosis</i>	partly soluble
GFP	27.4	monomer	Green Fluorescence Protein	<i>Aequorea victoria</i>	partly soluble
PA28 α	28	monomer and heptamer	Proteasome activator	<i>Rat</i>	soluble
PAN (Proteasome Activating Nucleotidase)	50	hexamer	ATPase	<i>Methanococcus jannaschii</i>	soluble
Nemo	51.8	dimer and trimer	ubiquitin binding	<i>Mouse</i>	-

(a) Molecular masses were calculated according to the amino acid composition of the recombinant protein and are given in kDa.

(b) The tetradecameric structure of ClpP1 and ClpP2 in solution is deduced from that of their ClpP homolog in *E. coli* (6). The hexameric structure of PAN was determined by size exclusion chromatography (7) and electron microscopy (5). The monomer–heptamer equilibrium self-association of PA28 α was determined by analytical ultracentrifugation (2) and crystallography analysis (3). Nemo was found to self-associate into a trimer by gel filtration and analytical ultracentrifugation (1) but its IKK binding domain is a dimer as seen by gel filtration, analytical ultracentrifugation, and X-ray crystallography (4).

(c) The solubility status was followed in the BL21(DE3) cells based on the presence of the protein in the soluble fraction after a centrifugation at 14,000 g.

1. **Agou, F., F. Ye, S. Goffinont, G. Courtois, S. Yamaoka, A. Israel, and M. Veron.** 2002. NEMO trimerizes through its coiled-coil C-terminal domain. *J Biol Chem* **277**:17464-75.
2. **Johnston, S. C., F. G. Whitby, C. Realini, M. Rechsteiner, and C. P. Hill.** 1997. The proteasome 11S regulator subunit REG alpha (PA28 alpha) is a heptamer. *Protein Sci* **6**:2469-73.
3. **Knowlton, J. R., S. C. Johnston, F. G. Whitby, C. Realini, Z. Zhang, M. Rechsteiner, and C. P. Hill.** 1997. Structure of the proteasome activator REGalpha (PA28alpha). *Nature* **390**:639-43.
4. **Rushe, M., L. Silvian, S. Bixler, L. L. Chen, A. Cheung, S. Bowes, H. Cuervo, S. Berkowitz, T. Zheng, K. Guckian, M. Pellegrini, and A. Lugovskoy.** 2008. Structure of a NEMO/IKK-Associating Domain Reveals Architecture of the Interaction Site. *Structure* **16**:798-808.
5. **Smith, D. M., G. Kafri, Y. Cheng, D. Ng, T. Walz, and A. L. Goldberg.** 2005. ATP binding to PAN or the 26S ATPases causes association with the 20S proteasome, gate opening, and translocation of unfolded proteins. *Mol Cell* **20**:687-98.
6. **Wang, J., J. A. Hartling, and J. M. Flanagan.** 1997. The structure of ClpP at 2.3 Å resolution suggests a model for ATP-dependent proteolysis. *Cell* **91**:447-56.
7. **Zwickl, P., D. Ng, K. M. Woo, H. P. Klenk, and A. L. Goldberg.** 1999. An archaeobacterial ATPase, homologous to ATPases in the eukaryotic 26 S proteasome, activates protein breakdown by 20 S proteasomes. *J Biol Chem* **274**:26008-14.