Supplemental Experimental Procedures

Protein Preparation

Double capped *S. cerevisiae* proteasome:Blm10 and proteasome:Δ50Blm10 complexes were prepared largely as described (Iwanczyk et al., 2006). Briefly, *S. cerevisiae* strain SDL135 expressing proteasome subunit Pre1/β4 tagged with protein A at the C-terminus (Leggett et al., 2002) (kind gift of Daniel Finley and David Leggett) was grown in a 36 L fermentor in YPD+glucose at 30°C for 2 days to saturation, and harvested by centrifugation. Polyhistidine-tagged Blm10 was expressed from pTF155/pCPH1327 (full length) or pCPH1328 (Δ50) in a 36L fermentor or shaker flasks in synthetic medium with raffinose to an OD^{600} of 0.7 at 30°C, whereupon expression was induced by the addition of galactose to 1.1% and the culture grown overnight and harvested by centrifugation. Cell lysis was performed under liquid nitrogen using a freezer mill 6850 pulverizor (SPEX CentriPrep Group). Subsequent steps were performed at 4°C. Typical preparations started with 80g of cell paste expressing tagged proteasome and 80g of cell paste expressing Blm10, and followed the published protocol (Iwanczyk et al., 2006) to give a typical yield of 2-4 mg of complex. Protein was concentrated to 20-25 mg/ml in 50mM Tris pH 7.5, 50mM NaCl, 1mM EDTA, and 0.5mM dithiothreitol (DTT) using a spin filtration device. The concentrated protein was buffer exchanged in the same solution with fresh DTT using a G50-sephadex spin column.

Crystallization

Immediately prior to setting up crystallization trials, the protein sample was centrifuged at 16,000 *g* at 4°C for 10 minutes. Blm10:proteasome complex crystals were grown by vapor diffusion in drops comprising 0.5μL protein and 0.5μL reservoir against a reservoir of 5-6% PEG 8k, 0.1M Na/K phosphate pH 6.2, 0.2M NaCl, and 18-30% of ethylene glycol. Crystals were harvested by addition of ~50 μL of well solution to the drop immediately prior to suspending the crystal in a nylon loop and plunging into liquid nitrogen. Crystals with full-length Blm10 and Blm10 missing the first 50 amino acid residues (Blm10Δ50) grew under the same conditions and generally had similar morphologies, although the Blm10Δ50 complex crystals grew more reproducibly in about 2-3 weeks and diffracted more strongly. Growth of full-length Blm10 complex crystals took from weeks to months and was highly non-reproducible, with the large majority of preparations not yielding usable crystals. Both of the constructs had N-terminal extensions of 12 histidine residues, and started with the sequence H_{12}-G-