T^2 or H_{12}-GT-D^{51}. The polyhistidine tags were not removed prior to setting up crystallization trials. The full-length Blm10:proteasome crystals were poorly isomorphous and showed large variation in cell dimensions and even in space group.

Structure Determination

Diffraction data were collected at the National Synchrotron Light Source beamline X29 and processed using HKL (Otwinowski and Minor, 1997). Data were collected from the various crystals (Table 1) at 100K and at the wavelength indicated: c158 1.1 Å; c164 1.0 Å; c172 1.0688 Å; c280 1.0809 Å; c290 1.0 Å; c292 1.0642 Å. Many of the crystallographic calculations were performed using programs of the CCP4 suite (Collaborative Computational Project, 1994). The various crystal forms were phased by molecular replacement with PHASER (McCoy et al., 2007) using the unliganded structure of the *S. cerevisiae* proteasome (Groll et al., 1997) (pdb code 1ryp) as the search model. Map quality was greatly improved by non-crystallographic symmetry (NCS) averaging over the multiple copies of half proteasome:Blm10 complexes in the asymmetric unit and averaging between different crystal forms using DMMULTI (Cowtan, 1994). Map quality was further improved by application of a -50 $Å^2$ sharpening factor. Crystals belonging to space group $P2_1$ had four-fold NCS, and crystals belonging to space group $P2_12_12_1$ had two-fold NCS.

Model building with O (Jones et al., 1991) was aided by the identification of 20 methionine and 14 cysteine sites from crystals soaked in thimerosal, methyl mercury nitrate, or potassium platinum tetrachloride. Heavy atom derivates were prepared by adding aqueous stock solutions to the crystallization well solution to make the concentration indicated, followed by addition of 40 μL of this solution directly to the crystallization drop for the time indicated prior to mounting and plunging into liquid nitrogen: c164/FL-Thim, thimerosal, 6mM, 2 hours; c172/FL- $PtCl_4$, 6 mM, 2 hours; c290/Δ50-MeHg, $MeHgNO_2$, 1 mM, 10 minutes; c292/Δ50-$PtCl_4$, K2PtCl4 2mM, 24 hours. Due to non-isomorphism, the heavy atom derivative structures were determined individually by molecular replacement and their phases refined by NCS averaging. Anomalous difference Fourier maps were found to be more sensitive than isomorphous difference maps for the location of heavy atoms.

The best diffracting crystal structure was refined with REFMAC5 (Murshudov et al., 1997) and rebuilt with KiNG (Davis et al., 2007), with the final refinement calculations performed using Phenix (Adams