program SEDNTERP (Version 1.09, available on the World Wide Web)[58]. All data were analyzed using the program Heteroanalysis[59].

Crystallization

Crystals were grown by sitting drop vapor diffusion at 21℃. $Vps4_{104\text{-}437,E233Q}$ crystallized in drops comprising 2 μl of 13 mg/ml protein, 50 mM NaCl, 25 mM Tris pH 7.4, 1 mM DTT, 4 mM $MgCl_2$, and 2 mM ATPγS, mixed with 1 μl of reservoir solution (48% ethylene glycol (vol/vol), Bis-Tris pH 5.0). Crystals were cryo-cooled by plunging into liquid nitrogen directly from the crystallization solution. $Vps4_{122\text{-}437,E233Q}$ was crystallized in drops comprising 2 μl of 12 mg/ml protein, 100 mM NaCl, 25 mM Tris pH 7.4, 1 mM DTT, 4 mM $MgCl_2$, and 1 mM ATPγS, mixed with 2 μl reservoir solution (1.6 M ammonium sulfate, 0.1 M Bis-Tris pH 5.5). Crystals were briefly transferred to cryoprotectant (mother liquor made up with 20% glycerol), suspended in a nylon loop, and cryo-cooled by plunging into liquid nitrogen.

Structure Determination and Refinement

Diffraction data from $Vps4_{104\text{-}437}$ and $Vps4_{122\text{-}437}$ crystals were collected at Beamline X25 of the National Synchrotron Light Source, Brookhaven National Laboratory, and scaled and integrated by using the HKL2000 suite[60]. Crystal structures were determined by molecular replacement using PHASER[61]. A polyalanine/homology search based on the human VPS4B structure (pdb code 1XWI)[25] was used to determine $Vps4_{122\text{-}437,E233Q}$, and this refined structure was used to determine the structure of $Vps4_{104\text{-}437,E233Q}$. Model building was performed with COOT[62]. Structures