ALIX Crystallization and Data Collection

All crystals were grown in sitting drops using protein concentrated to 10 mg/ml in the size exclusion chromatography solution. SeMet $ALIX_V$ crystals grew at 4°C with a reservoir of 0.16-0.20 M Magnesium formate and 14% PEG-3350, and a drop of 1-2 μL reservoir and 2 μL protein solution. $ALIX_{Bro1}$ and $ALIX_{Bro1\text{-}V}$ both crystallized best at 13°C in drops with equal (0.5 – 2.0 μL) volumes of protein and reservoir solution: $ALIX_{Bro1}$ — 0.1 M NaMES pH 6.5, 10% PEG-20,000. $ALIX_{Bro1\text{-}V}$ – 8-9% PEG 4000, 0.10-0.25 M ammonium acetate, 0.10-0.15 M Magnesium acetate, 0.05 M Hepes pH 7.0.

$ALIX_{Bro1}$ and $ALIX_V$ crystals were cryoprotected in solutions of reservoir made up with 20% glycerol ($ALIX_V$; 5% and 10% intermediate steps). $ALIX_{Bro1\text{-}V}$ cryoprotection used reservoir made up with 30% MPD, achieved in 5% increments. Crystals were suspended in a nylon loop, plunged into liquid nitrogen, and maintained at 100 K during data collection. Crystallographic statistics are given in Supplemental Table S1.

ALIX Structure Solution and Refinement

The $ALIX_V$ structure was determined by the SAD method. The top 8 Se sites located with SHELX (Schneider and Pape, 2004; Sheldrick and Schneider, 1997) were used in SOLVE/RESOLVE (Terwilliger, 2002) to estimate phases at 3.1 Å. Phases were further improved in SIGMAA (Read, 1986) by averaging estimates obtained from two crystals, and used with amplitudes of crystal 1 for map calculation. Initial polyalanine models were built for the two molecules in the asymmetric unit and used to guide averaging of the map separately over the regions of arm1 and arm2 using MAMA