solutions were applied to a Q Sepharose FF column (GE Healthcare) and washed with 25 mM Tris pH 8.0, 50 mM NaCl, 1 mM DTT before eluting with a gradient to 1 M NaCl. Monomeric ALIX was separated from dimer ($ALIX_{Bro1-V}$ and $ALIX_V$ samples) and aggregated species by size exclusion chromatography in 10 mM Tris pH 8.0, 100 mM NaCl, 1 mM DTT: $ALIX_{Bro1-V}$ (Superdex 200, GE Healthcare), $ALIX_{Bro1}$ and $ALIX_V$ (Superdex 75, GE Healthcare). In the case of $ALIX_{Bro1-V}$ and $ALIX_V$, monomeric ALIX was the predominant species, while dimeric ALIX was present in a much lower percentage. Furthermore, rigorous equilibrium analytical ultracentrifugation showed that monomeric $ALIX_V$ remained monomeric and that there was no appreciable equilibrium between monomer and dimer species.

Purification of Recombinant HIV-1 $p6^{Gag}$ and EIAV $p9^{Gag}$

GST-$p6^{Gag}$ and GST-$p9^{Gag}$ were expressed and lysed (50 mM Tris pH 8.0, 300 mM NaCl, 1 mM DTT) as for ALIX constructs. Clarified lysate was applied to a Glutathione GSTPrep column (GE Healthcare), washed, and eluted with lysis buffer supplemented with 20 mM glutathione. Protein fractions were combined, made to 2.5 mM $CaCl_2$, and incubated with thrombin (Novagen). Free GST and uncut GST fusion protein was separated from free HIV-1 p6 or EIAV p9 by size exclusion chromatography (Superdex 75, GE Healthcare) in 20 mM NaPhosphate pH 7.2, 150 mM NaCl. Residual GST was removed by an additional pass through the Glutathione GSTPrep column.