sets for each protein. The six data sets were globally fit to single species models in which the molecular weight was allowed to float, and also to monomer, dimer, or trimer models with fixed molecular weights. The distribution of Vps4 fit a protein with a predicted mass of 96,210 g/mol, which matched the mass expected for a Vps4 dimer ($M_{obs}/M_{monomer}$ = 1.97). Data for three initial concentrations of Vps4 centrifuged at 12,000 RPM are shown together with the global fits (Fig. 4, panel 1), and the small, random residuals indicate that the data were satisfactorily fit by a simple single species model (lower panels). Global fits to the three data sets collected at 16,000 RPM were also satisfactory (data not shown). Similarly, when fixed monomer, dimer, or trimer molecular weights were used during the global fitting procedure, the dimer model was clearly the best fit to the data (Supplemental Fig. 2). Likewise, sedimentation data for the $Vps4_{\Delta MIT}$ protein estimated a solution mass of 82,435 g/mol ($M_{obs}/M_{monomer}$ = 2.19, Fig. 4, panel 2), and the dimer model was also the best fit of the different possible fixed molecular weight models (Supplemental Fig. 2).

As in most of our other experiments, these analyses utilized Vps4 proteins with E233Q mutations because the constructs were also used to examine the effects of ATP binding in the absence of hydrolysis. To ensure that the E233Q mutation did not alter the oligomerization state, gel filtration analyses were also performed on wild type $Vps4_{\Delta MIT}$ proteins (both $Vps4_{101-437}$ and $Vps4_{124-437}$). In both cases, these proteins eluted with the same retention times as their E233Q analogues, indicating that the E233Q mutation did not affect the oligomerization state (not shown). We therefore conclude that in the absence of nucleotide, Vps4 and $Vps4_{\Delta MIT}$ proteins form stable dimers in solution.