therefore in an equivalent position. In crystal form 2, the two ATPγS-bound Vps4 molecules are nearly equivalent, whereas the more open third molecule sits in a slightly different position within the spiral. Analogous 6-fold (or pseudo 6-fold) screw arrangements have been seen in the crystal structures of other AAA ATPases, which presumably reflects their propensity to form six membered rings[45; 46]. As discussed below, adjacent helices are connected by different types of two-fold symmetric packing interactions in the different Vps4 crystal forms.

Nucleotide-free Vps4 Dimerizes in Solution

The oligomeric state of the inactive, low molecular weight Vps4 complex has been controversial, and we therefore performed gel filtration and analytical ultracentrifugation analyses to examine the oligomerization of nucleotide-free Vps4 proteins. Pure recombinant Vps4 (MW=48,803 g/mol) and Vps4$_{\Delta MIT}$ (MW=37,700 g/mol) proteins eluted from a Superdex S200 gel filtration column as single species with apparent molecular weights of 127 kDa and 88 kDa, respectively. Thus, the Stokes radii of both constructs were most consistent with dimers, but could possibly be explained by extended monomers. Equilibrium sedimentation analyses were used to distinguish between these two possibilities because this approach provides shape-independent measures of protein solution mass.

As shown in Fig.4 and Supplemental Fig. 2, the radial distributions of full length Vps4 (panel 1) and Vps4$_{\Delta MIT}$ (panel 2) fit well to single-species dimer models, but not to monomer or trimer models. In each case, sedimentation data were collected at three different protein concentrations and two rotor speeds, yielding a total of six data