DISCUSSION

Vps4 Oligomerization

At steady state, Vps4 is distributed throughout the cytoplasm in an inactive state, but can be recruited to membrane sites of action where it binds ATP and associates into enzymatically active higher order complexes[16]. Our studies imply that Vps4 is likely a dimer in its inactive state. Specifically, we found that Vps4 forms stable dimers in solution, as evidenced by the unambiguous fit of equilibrium sedimentation data to a single species dimer model and by the identification of a series of different point mutations that block $Vps4_{\Delta MIT}$ dimerization. As is true for any dimeric protein, however, Vps4 will dissociate into monomeric species at sufficiently low protein concentrations. Indeed, we observed both monomeric and dimeric species for the Human VPS4B protein[25], presumably because the human VPS4B homodimer is less stable than the yeast Vps4 homodimer. Moreover, we observed that even the yeast Vps4 protein exhibited concentration-dependent mobility in gel filtration experiments performed at low micromolar concentrations, indicating that monomeric yeast Vps4 species can also be detected at low protein concentrations under non-equilibrium conditions (not shown). These observations raise the possibility that monomeric Vps4 species may accumulate in vivo if the protein concentration is sufficiently low. Nevertheless, the dissociation constant for the yeast Vps4 dimer must be less than ~5 μM in order to allow an adequate fit by a single species dimer model over the concentration ranges tested (Fig. 4 and data not shown).

ATP binding promotes formation of higher order Vps4 assemblies, provided that non-hydrolyzable ATP analogs such as ATPγS are used, or that the Vps4 protein is