mutated to prevent nucleotide hydrolysis (or both). These higher order assemblies appear to be dodecamers composed of two non-equivalent hexameric rings. The conclusion that VPS4 likely forms hexameric rings is based upon: 1) six-fold symmetry observed in cryo-EM reconstructions of assembled $Vps4_{\Delta MIT}$ and Vps4 complexes[31], 2) the finding that equilibrium sedimentation data for the Vps4 assembly is best fit by a dodecamer model[25], and 3) strong homologies between Vps4 and the related p97 D1 and spastin proteins, both of which form hexameric rings[52; 54; 55]. The Vps4 dimer/oligomer assembly cycle is therefore reminiscent of NtrC, another AAA ATPase that also forms catalytically inactive dimers, and then assembles into active hexameric rings upon ATP binding[56; 57]. In both cases, the dimers are “off pathway” with respect to hexameric ring assembly, and therefore appear to represent autoinhibited states that negatively regulate enzymatic activity. There is no indication, that the inhibitory dimers of NtrC and Vps4 proteins are structurally related, however, because the NtrC dimer subunits associate in a two-fold symmetric, head to tail orientation that is completely distinct from the orientations of subunits in the hexamer.

Models for Vps4 Assemblies

All $Vps4_{\Delta MIT}$ crystal forms reported to date show a similar packing arrangement in which the subunits form a continuous helix with a six-fold screw axis. These helices, in turn exhibit different types of two-fold symmetric packing interactions in different crystal forms, but our experiments indicate that none of these symmetric crystallographic interfaces is likely to mediate Vps4 dimerization in solution. In