bind between and contact adjacent subunits[18]. Vps4 is typical in this regard because ATP binding promotes enzyme assembly[16; 25], and Vps4 appears to have a canonical ATP binding site (although high resolution structures of ATP-bound Vps4 proteins have not previously been reported). Upon ATP binding, Type I ATPases generally assemble into single ring structures, whereas Type II ATPases generally form double ring structures in which the two ATPase cassettes form separate, stacked rings (e.g.,[21; 30]). The Type I Vps4 enzymes are atypical in this regard in that they appear to form double stacked rings[16; 25; 27; 31]. Crystal structures of double ring Vps4 complexes are not yet available, and current models for the stoichiometry and structures of this assembly have therefore been deduced from gel filtration [16; 25], protein crosslinking[16; 31], mutagenesis[25], single particle cryo-EM reconstructions[27; 31], and modeling studies[25; 27]. Vps4 proteins also bind an activator, Vta1/SBP1/LIP5, which contacts the β domain and promotes assembly and ATPase activity[25; 31; 32; 33; 34; 35; 36; 37; 38]. Recent biochemical and EM studies of the yeast Vps4-Vta1 supercomplex suggest that Vta1 may bind just one of the two rings in the double-ring assembly[31].

Although there is general agreement that Vps4 enzymes drive the ESCRT pathway by cycling between an inactive, low molecular weight state and an active, high molecular weight state, uncertainties surround the structures and stoichiometries of both the active and inactive conformations. For example, the inactive state of Vps4 has been described as both a monomer[28] and a dimer[16; 25; 27], and the nature of the putative dimer is not clear. Similarly, two different single particle reconstructions of the fully assembled core enzyme at moderate resolution have been interpreted as