and ClpB) AAA ATPases contains six subunits, with type I hexamers forming a single ring and type II hexamers adopting a double-ring structure in which each of the two AAA domains forms a separate ring. Figure 9 shows published cryo-EM reconstructions of ClpB,[47] NSF,[44] p97–p47,[39] and p97[35] together with our full-length Vps4p complex. (Note: We reversed the original assignment of rings in the Wilson-Kubalek p97 structure to make it consistent with the others.[35,36,39]) The five complexes were presumably all in the ATP or its analogue AMP–PNP-binding state, although it is unclear which, if any, of the ATPs have already been hydrolyzed in the stable hexameric complexes.[38] Note that the p97–p47 complex in Fig. 9 is composed of a p97 hexamer and a p47 trimer, and the p47 trimer is suggested to be the plug-like density located around the symmetry axis over the top ring.[39] Similarly, the NSF structure also contains the NSF adaptor protein αSNAP and substrate SNARE, which are all attributed to be the big bump-like density around the central axis over the top ring.[44] In the ClpB structure, the long spokes extending sideways out of the top ring are believed to be the coiled-coil M domain, unique to ClpB and absent in other AAA proteins.[45] The clear morphological similarities between Vps4p and the type II AAA ATPases constitute compelling evidence that the Vps4p complex is also a double ring.

As seen for Vps4p, the three type II AAA ATPases shown in Fig. 9 display very different conformations for their top and bottom rings. ClpB most closely resembles Vps4p, with the bottom ring closed and the top ring wide open with a large cavity. Because the top and bottom rings of type II AAA ATPases are formed by two AAA domains, some structural differences between the rings are to be expected. Indeed, all of the structures for type II AAA hexamers determined by cryo-EM, X-ray crystallography, or small-angle X-ray scattering[34–38,44,45,57,72,73] confirm that the two rings are structurally different, although the extent of this difference varies. In contrast, the two rings of the Vps4p complex are formed by exactly the same protein protomer, so the pronounced difference in the two Vps4p rings is noteworthy. It is possible, however, that the different conformations of the two rings reflect that the two rings are in different nucleotide conditions, despite the presence of a 1000-fold molar excess of ATP-γS during the cross-linking step.

N-terminal domain

The N-terminal domains that precede AAA domains are usually responsible for substrate recognition and binding.[74] The Vps4 N-terminus consists of a flexible linker and an MIT domain that is dispensable for ATP-dependent oligomerization and ATP hydrolysis *in vitro*. The MIT domain is required for recruitment of Vps4p to endosomal membranes,[18] and it forms a three-α-helix bundle that interacts directly with the C-terminal helices found on a subset of ESCRT-III proteins.[25–27] While the common bowl-like "core" structure was seen in both the ΔN- and full-length Vps4p reconstructions, the presence of the N-terminal domains in the full-length Vps4p structure caused a prominent nipple-like density to appear above the top ring on the symmetry axis. We interpret this new density to consist mainly of the MIT domains of the top ring, which would be positioned to bind ESCRT-III substrates directly above the cavity.

The six N-terminal domains of double-ring type II AAA ATPases are flexible and typically do not show up in averaged cryo-EM reconstructions. In those cases where the N-terminal domains have been immobilized, however, they were located above or around the D1 (top) ring. For example, the NSF N-terminal domain was suggested to lie above the

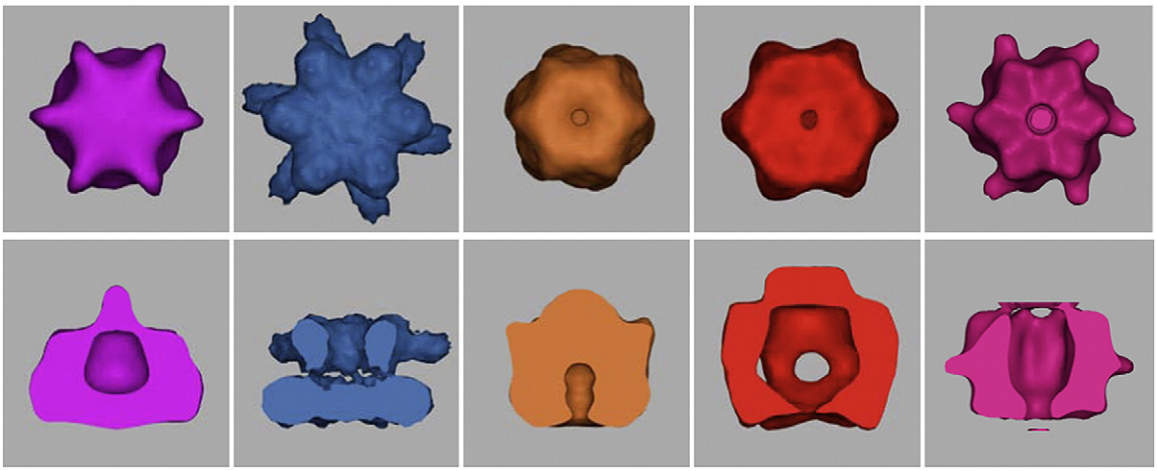

Fig. 9. Comparison of full-length Vps4p with cryo-EM reconstructions of type II AAA proteins. Full-length Vps4p, ClpB (Electron Microscopy Database ID 1243), NSF–αSNAP–SNARE (Electron Microscopy Database ID 1059), p97–p47 (Electron Microscopy Database ID 1191), and p97 (from E.M. Wilson-Kubalek) maps, all in presumably ATP-bound states, contoured at their full estimated molecular masses. The D2 rings of the type II AAA proteins face the viewer in the top row and comprise the lower rings in the bottom row.