therefore useful landmarks for aligning the two structures.

Similarly, two contours of the Vta1p–Vps4p complex reconstruction were considered in detail (Fig. 7b), with one contour enclosing the full predicted molecular mass (824 kDa) and the other contour being extremely conservative, corresponding to the molecular mass of just the full-length Vps4p complex (578 kDa). Compared with full-length Vps4p, the Vta1p–Vps4p complex had significant extra densities around and over the nipple above the top ring and beneath the center of the bottom ring. These extra densities diminished but did not disappear even when the Vta1p–Vps4p complex was contoured at the exceedingly conservative level of 578 kDa.

Difference maps

Difference maps were also inspected. First, the ΔN-Vps4p, full-length Vps4p, and Vta1p–Vps4p maps were each normalized to have a mean of zero and a standard deviation of one. Three difference maps were then obtained by subtracting ΔN-Vps4p from the full-length Vps4p, full-length Vps4p from the Vta1p–Vps4p, and ΔN-Vps4p from the Vta1p–Vps4p (Fig. 8). As a control, the "internal" differences between the two half-maps of each reconstruction were also calculated (Fig. S8). Inspection of the difference map histograms (Fig. S9) suggested that the positive differences beyond approximately +3.5σ did in fact reflect real new protein densities. Strong positive differences between the full-length and ΔN-Vps4p maps were seen in the location of the nipple, the bottom-ring fins, above the top-ring shoulders, and high above the nipple disconnected from any other density (Fig. 8a). Of these, the appearance of the nipple is most likely to represent real new ordered protein, since it was large, continuous with the main body of the complex, and enclosed by all reasonable isosurfaces for the full-length complex and there was no comparable noise in that location in the "control" maps (Fig. S8). As a further internal control, there were a few voxels with negative differences at the −3.5σ significance level (not shown), but none was enclosed by any reasonable isosurface of either the ΔN- or the full-length Vps4p complex.

The differences between the Vta1p–Vps4p complex and the full-length Vps4p complex were simpler (Fig. 8b). Only two major densities appeared above +3.5σ, one above and around the nipple and the other beneath the bottom ring. Both were likely to be real and significant because they were connected to the main body of the complex, enclosed by all reasonable isosurfaces of the Vta1p–Vps4p complex, and there was no comparable noise in the control maps. The small extra densities on the fins and shoulders predicted by the isosurface comparisons (i.e., Fig. 7b) were missing in the difference maps at this significance level (+3.5σ). Indeed, they did not appear until the significance level was dropped to +1.5σ (data not shown), at which point so many other voxels were also enclosed that the map was judged to be noise. As the internal control, no density was seen below −3.5σ.

The differences between the Vta1p–Vps4p complex and the ΔN-Vps4p complex (Fig. 8c) resemble the differences between the Vta1p–Vps4p complex and the full-length Vps4p complex (Fig. 8b), with strong nipple-like density above the cup in the top ring and strong density underneath the center of the bottom ring. The size of the extra nipple density in Fig. 8c is like the sum of the extra nipple densities in Fig. 8a and b, as expected. Figure 8c also serves as an independent check on which differences in Fig. 8a are most likely to be meaningful: in principle, the differences between Fig. 8b and Fig. 8c should be the same as those shown in Fig. 8a. In both cases, the emergence of the nipple was consistent, but the smaller differences did not match and are therefore less reliable.

Comparison with other AAA ATPases

The oligomeric state of most type I (e.g., katanin, ClpX, and HslU) and type II (e.g., p97, NSF, ClpA,
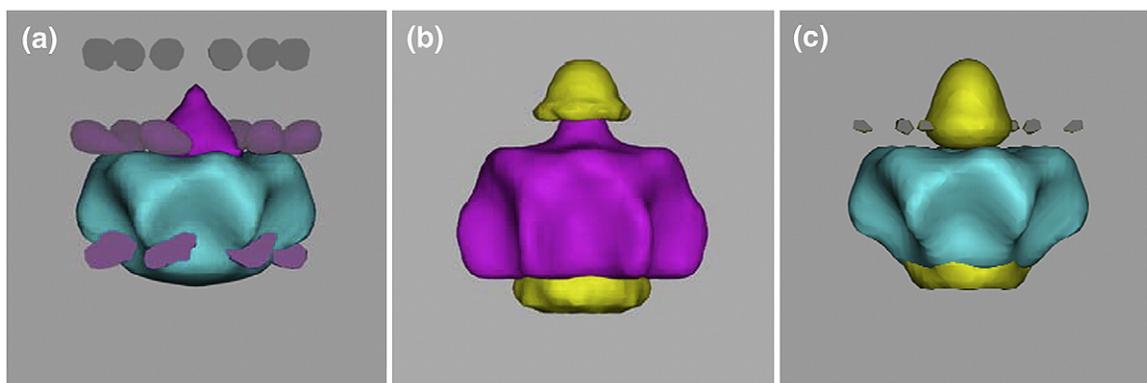

Fig. 8. Difference maps. (a) Full-length Vps4p minus ΔN-Vps4p, (b) Vta1p–Vps4p minus full-length Vps4p, and (c) Vta1p–Vps4p minus ΔN-Vps4p maps contoured at 3.5σ above the mean, superimposed on the three-dimensional reconstructions of the corresponding subcomplex. The largest difference densities in (a) are shown in purple, while those in (b) and (c) are shown in yellow. Additional smaller (shaded purple) or disconnected (gray) difference densities are also seen.