Fig. 3. Cryo-EM images of full-length Vps4p. Before cross-linking, most regions of the cryo-grid had clear ice devoid of protein, while other regions were filled with what appeared to be protein aggregates (top). After cross-linking (bottom), individual large protein complexes were routinely seen (examples of particles are encircled). The hexagon encloses one complex with visible hexagonal symmetry. The scale bar represents 100 nm.

(Fig. S1b and c). After cross-linking, individual, regular large protein complexes were routinely observed on the EM grids (Fig. 3, bottom).

The Vps4p oligomer displays 6-fold symmetry

The symmetry of the fully assembled Vps4p complex has not been unambiguously determined, and both double-ring pentamers (i.e., decamers)[18] and hexamers (i.e., dodecamers)[28] have been proposed. We therefore used three independent methods to look for rotational symmetries in the projection images of the three separate complexes. First, a two-dimensional classification procedure (the EMAN program *refine2d.py*) was used to separate each set of images into 50 classes such that related projection images were assigned to the same class.[69,70] Figure 4 shows the 18 resultant class averages most closely corresponding to "top" views for the three complexes. Six protruding densities can be recognized around the periphery in all 18 class averages of each complex, whereas none of the single class averages indicated 5- or 7-fold symmetry.

The second method to determine the symmetry of the complexes used a different EMAN program (*startcsym*), which deliberately searches for particles that exhibit a particular symmetry. The underlying algorithm simply rotates a particle by $360/n$ deg and
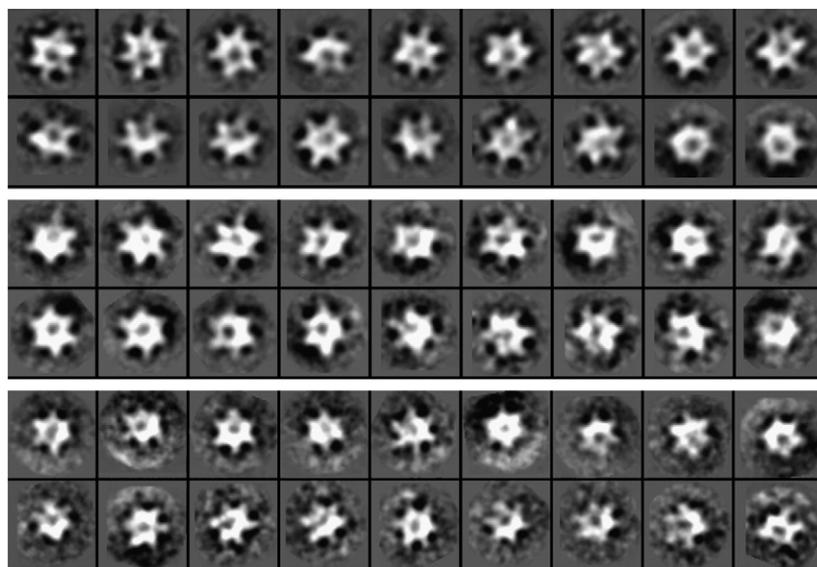

Fig. 4. Top views reveal 6-fold symmetry. Select class averages of (top) ΔN-Vps4p, (middle) full-length Vps4p, and (bottom) Vta1p–Vps4p complexes are shown. All the raw images were iteratively classified based on their similarity using singular value decomposition by *refine2d.py*. Particles within the same class were aligned and averaged to generate 50 class averages. The 18 class averages most closely corresponding to "top" views are shown, all of which suggest 6-fold symmetry.