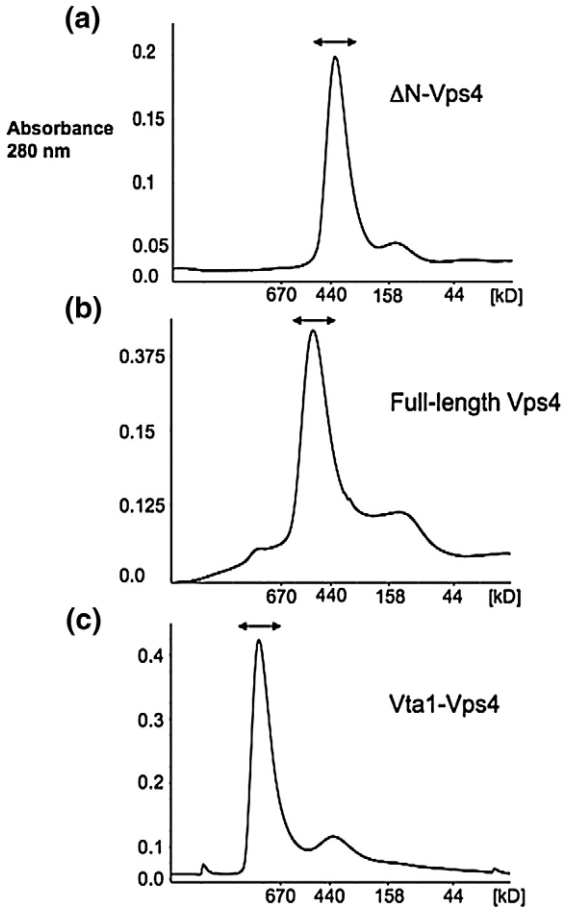

Fig. 1. Gel filtration analyses of multimeric Vps4p complexes. Multimeric ΔN-Vps4p, full-length Vps4p, and Vta1p–Vps4p complexes were isolated by size-exclusion chromatography (Superose 6) in buffer A (100 mM NaCl, 25 mM Tris–HCl, pH 7.5, 2 mM $MgCl_2$, 1 mM ATP, and 1 mM DTT). Elution positions of molecular mass standards are shown below each chromatogram.

densitometry (Fig. 2b). An equimolar mixture of Vta1p and Vps4p also produced the same 1:2 complex (Fig. 2a, red curve), but in this case, there was also a second peak composed of unbound Vta1p (Fig. 2a and c). Furthermore, the apparent size of the Vta1p–Vps4p complex did not shift to a higher mass, even when the two proteins were mixed in a 3:1 ratio. We therefore conclude that Vta1p and Vps4p form a complex of ~1:2 stoichiometry, even when Vta1p is present in excess.

In our initial attempts to visualize Vps4p complexes using cryo-EM, the samples were simply plunge-frozen and imaged. Unfortunately, the process of plunge-freezing the samples in thin films across EM grids destabilized the native protein complexes, presumably when they came in contact with the air–water interface. Consequently, most regions of the cryo-EM grids displayed clear ice devoid of protein, while a few were crowded with what appeared to be protein aggregates, and no intact, regular large protein complex was seen (Fig. 3, top). The complexes were chemically cross-linked with 0.02% glutaraldehyde and repurified by size-exclusion chromatography (Fig. S1a) to stabilize the protein assemblies for cryo-EM. The elution profiles were only slightly perturbed, indicating that, at least for the peak fractions used for imaging, the cross-linking procedure did not change the oligomeric state or essential structure of the complexes. SDS-PAGE analysis of the three protein complexes showed that the cross-linking was sufficient to covalently link nearly all the subunits of the complexes

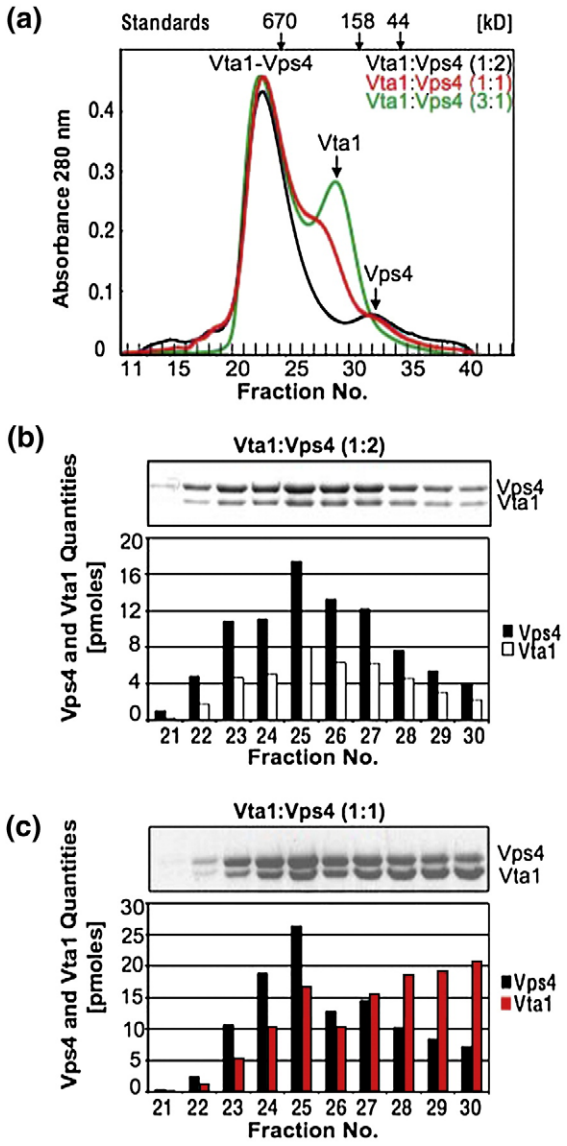

Fig. 2. The Vta1p–Vps4p complex exhibits 1:2 stoichiometry. (a) Size-exclusion chromatography of the complexes formed when Vta1p and Vps4p were mixed at molar ratios of 1:2 (black trace), 1:1 (red trace), and 3:1 (green trace). Samples were chromatographed on a Superdex-200HR column in the presence of 1 mM ATP. For reference, elution positions of size standards and of free Vps4p and Vta1p proteins are shown above (arrows). (b and c) SDS-PAGE analyses (top) and associated protein quantities (bottom) for fractions spanning the Vps4p–Vta1p complex peaks shown in (a) for the (b) 1:2 and (c) 1:1 mixtures.