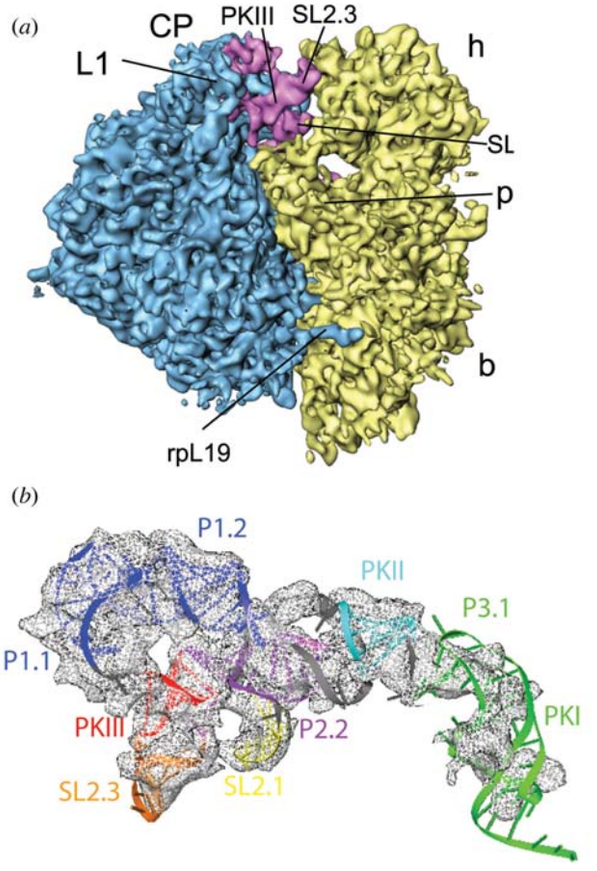

Fig. 2.5.7.6. A high-resolution cryo-EM map allows backbone tracing. (*a*) Cryo-EM map of the cricket paralysis virus (CrPV) IRES RNA in complex with the yeast 80S ribosome determined at 7.3 Å resolution. The map is shown from the L1 protuberance side with the ribosomal 40S subunit in yellow, the 60S subunit in blue and the CrPV IRES in magenta. Landmarks for the 40S subunit: b, body; h, head; p, platform. Landmarks for the 60S subunit: CP, central protuberance; L1, L1 protuberance. PKIII denotes helix PKIII of the CrPV IRES RNA and SL the two stem loops present in the secondary structure of the RNA. (*b*) Structure of the CrPV IRES RNA. Based on the cryo-EM map and additional biochemical knowledge, the complete chain of the RNA is found (189 nucleotides could be traced). The IRES molecular model is shown as a coloured ribbon docked into the cryo-EM density (grey mesh). PK and P denote the individual helical elements of the IRES and SL denotes the stem loops (Schüler *et al.*, 2006).

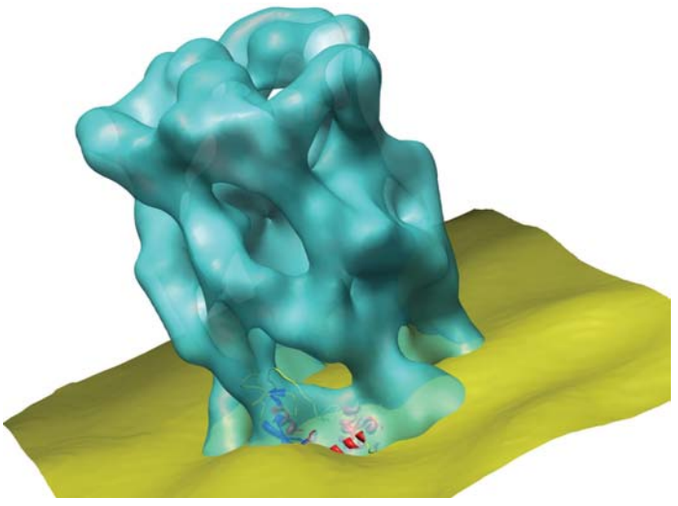

Fig. 2.5.7.7. Hrs (blue) embedded into the membrane (yellow) of an early endosome. Two functional domains have been docked into the cryo-EM density map of hexameric Hrs, determined to 16 Å resolution, and are shown as ribbons coloured by secondary structure (Pullan *et al.*, 2006). The structures of the VHS and FYVE domains have been determined crystallographically (Mao *et al.*, 2000) and are docked into the EM density map of the Hrs. The knowledge of the location of the FYVE and VHS domains, which are reported to bind to PI(3)P molecules found within the endosomal membrane (Kutateladze *et al.*, 1999), has guided the hypothetical placement of Hrs within the endosomal membrane. The immersion of the end caps of Hrs into the endosomal membrane demonstrates an 'end-on' binding model of the Hrs particles with the membrane. According to this model, either end cap can embed into the membrane, allowing the other end cap to carry out other essential protein trafficking functions, or to embed into another membrane, thus preventing fusion of membranes during early endosomal fusion.

macromolecules that comprise only protein and generally it is very difficult to delineate at intermediate resolution subunits of large macromolecular assemblies, automatically or not, in the absence of independent knowledge about their shape. The reason is that both the density and shape of the subunits are affected by the limited resolution differently depending on their spatial context. In general, subunits that are isolated and located on the surface or protruding from the structure will have relatively lower density while at the same time their overall shape will be better preserved and easier to discern. Subunits located inside the structure and surrounded by other structural elements, while having higher density, are more difficult to recognize, as they fuse with the surrounding mass densities. Therefore, it is difficult to provide a general method that could cope with the problem of automated mass-density analysis.

As most cryo-EM structures are determined at intermediate resolution, the most common mode of analysis is either to compare the map with the available X-ray crystallographic structures of its domains or to consider the result in the context of larger, subcellular structures obtained by electron tomography. In both cases correlation techniques are used extensively to obtain objective results or to validate the results obtained by manual fitting.

In docking of X-ray crystallographic structures into EM maps, the first step is the conversion of atomic coordinates from X-ray molecular models, as given in Protein Data Bank (PDB) files, into an electron-density map in a way that would mimic the physical image formation process. Although sophisticated methods of computational emulation of the image-formation process in the electron microscope are available, very simple approaches to conversion yield quite satisfactory results at the resolution of the EM results. The most common one is to assume that the Coulomb potential of an atom is proportional to its atomic number and add these atomic numbers within a Euclidean grid with a cell size equal to the EM pixel size in Å. The atomic coordinates of atoms are interpolated within the grid using trilinear interpolation. After such conversion, the X-ray map can be handled using the general image-processing tools of a single-particle software package. Initial orientation (or orientations, if the general placement is not immediately visually apparent) of the X-ray map can be easily performed manually within any number of graphical packages, for example *Chimera* (Pettersen *et al.*, 2004). The initial six orientation parameters (three translations and three Eulerian angles) are next transferred to the EM package (for details see Baldwin & Penczek, 2007) and the manual docking is refined using correlation techniques (Fig. 2.5.7.7). Similarly, the handedness of the EM map can be established or confirmed by performing fitting of the X-ray determined structure to two EM maps that differ by their hand.

Docking of EM maps into the broader cellular context of structures determined by electron tomography can provide information about the distribution of complexes and their interactions within the cell. Conceptually, the approach is very similar to that of particle picking, *i.e.*, template matching, with the main difference being that calculations are performed in three instead of two dimensions. Given a 3D structure of a single-particle EM complex, a set of 3D templates is prepared by rotating the