Biological membranes are vital components of all living systems, forming the boundaries of cells and their organelles. They consist of a lipid bilayer and embedded proteins, which are nanomachines that fulfill key functions such as energy conversion, solute transport, secretion, and signal transduction. Some membrane proteins form domains, which can vary and adapt to the functional state of the cell. Intact membrane complexes or solubilized membrane-bound complexes can be studied by electron microscopy (EM) and single particle analysis. This technique allows obtaining structural information in a rather easy way up to 10-20 Å resolution without using crystals, which are obligatory in X-ray diffraction studies. The technique has gained in popularity at the cost of the crystallographic approach, which uses two-dimensional (2-D) crystals. Such 2-D crystals of purified membrane proteins, reconstituted in the presence of lipids, provide a close to native environment and allow the structure and function of membrane proteins to be assessed. It was demonstrated in the nineties that electron crystallography can solve high-resolution structures of membrane proteins and very recently single particle averaging established itself also as a high resolution technique. A cryo-EM reconstruction of viral protein 6 (VP6) of a rotavirus was similar in clarity to a 3.8-Å resolution map obtained from x-ray crystallography. At this resolution, most of the amino acid side chains produced recognizable density. For non-symmetric objects such as membrane proteins this resolution is not achievable, but single particle averaging at lower resolution is still attractive if applied to negatively stained specimens with a mass between about 200 kDa and 2000 kDa. A major reason is that thousands of projections can be processed very fast, yielding 2D projection maps of at least 20 Å resolution. The statistical analysis and classification procedures used in single particle analysis have been developed for sorting different projection views originating from different conformations or subunit compositions.

In this thesis we present here some examples which show that single particle electron microscopy can be wider applied than just performing a structure determination of a highly purified large protein complex. Obtaining two-dimensional projection maps is a fast process but assignment of these maps can be a tedious process. The method is very suitable for determination of transient complexes or complexes that are inherently heterogeneous or
simply small. The method works on the level of a specialized membrane, as we show here for the membrane complex of two pathogenic *Neisseria* species.

Chapter 1 gives a general introduction about the protein structure, with an emphasis on membrane proteins. It also includes general information about the examined organisms, some important cellular processes and protein complexes, relevant to the topics studied in this thesis. Theoretical aspects of electron microscopy (EM), specimen preparation and image analysis are also presented. This chapter further contains a compilation of the recent progress in the field of electron microscopy, with an emphasis on structural biology.

Chapter 2 presents a study on some membrane-associated peroxins (Pex proteins). These particular Pex proteins are involved in protein import over the membrane in peroxisomes. Studies were performed with a model organism, the yeast *Hansenula polymorpha*. Single particle electron microscopy was used to analyze the structure of purified Pex5p and its possible association with Pex20p. The results showed that Pex5p can physically interact with Pex20p. We observed that a multimeric Pex20p complex can bind to the periphery of the Pex5p tetramer. Moreover, in this Pex5p-Pex20p complex, the conformation of tetrameric Pex5p was changed from a closed conformation with a diameter of 115 Å into an open conformation of 134 Å. EM data also showed that the Pex5p-Pex20p complex was capable to bind native, folded catalase, a peroxisomal PTS1 protein. This suggests that the Pex5p-Pex20p complex may be functional as receptor complex.

After completing the practical work of Chapter 2, we realized that peroxisomal membrane protein complexes are good candidates for further EM investigation since virtually no direct structural information of these complexes is available. In chapter 3 a pilot study of the major peroxisomal membrane complexes in the yeast species *Saccharomyces cerevisiae* and *Hansenula polymorpha* is presented. We monitored the presence and purification of large, hypothetical pex complexes by western blotting with specific antibodies against Pex14p. The results showed that upon 1D and 2D/SDS Blue Native gel electrophoresis from peroxisomal fractions only a few protein bands could be attributed to peroxisomes. Moreover, no large Pex protein complexes were found, which we also proved by mass spectrometry. It was realized that this could be related to the relatively low protein content of the peroxisomal membranes compared to mitochondrial membranes. By using differential and sucrose density centrifugation to obtain a rather pure peroxisomal fraction, we faced the problem that
mitochondria were a major contaminant in the best purified peak fractions. We conclude that further purification of the initial membranes is crucial, which requires more advanced techniques and better separation protocols to separate peroxisomes from mitochondria.

Chapter 4 of this thesis presents three secondary transporter proteins: GltT of *Bacillus stearothermophilus*, CitS of *Klebsiella pneumoniae* and GltS of *Escherichia coli*, which were studied by single particle electron microscopy. Image processing showed that a GltT particle map with three-fold symmetry is in line with the trimer observed in the crystal structure of a homologous protein, Glt$_{Ph}$ of *Pyrococcus horikoshi*. The other secondary transporter: CitS showed two main views as a kidney-shaped particle and a biscuit-shaped particle, both with a long axis of 160 Å and a short axis of 84 Å. The last examined secondary transporter: GltS was similarly shaped but smaller with dimensions of 150 Å x 84 Å. By comparing the shapes and dimensions of the CitS and GltS particles with the GltT particle, we suggest that the former two were in the dimeric state. In addition, a 10 kDa protein tag (Biotin Acceptor Domain, BAD) was fused to the N-terminus of the CitS protein or inserted in the central cytoplasmic loop which connects the two putative domains of the protein. Results from single particle EM analysis of the two proteins revealed differently shaped particles relative to the untagged protein. N-terminally tagged CitS revealed a more globular shape relative to the wild type. The aspect ratios were 1.77 and 1.9, respectively. We observed, that the inserted BAD domain resulted in an extended particle with an aspect ratio of 2.14. A model is presented for the relative orientation of monomers in the CitS dimer.

In chapter 5 we studied the structure of the PilQ secretins from *Neisseria gonorrhoeae* and *Neisseria meningitidis*. The complex embedded in isolated outer membranes, was investigated by electron microscopy and single particle image analysis. Results showed that analyzed oligomers within the native membrane, revealed additional domains, which were not observed in studies using purified proteins. The structure of PilQ complexes of *N. gonorrhoeae* showed a double ring structure with a clear 14-fold symmetry of the outer ring, and a 14-15-fold symmetry in the inner ring. In addition, from the 14 protein densities of the outer ring 7 external spikes are protruding. Moreover, PilQ complexes of *N. meningitidis* also consisted of two rings, together with complexes partly or completely lacking the outer ring. Our results showed, that the outer ring of the *N. meningitidis* PilQ had a 19-fold symmetry and no external spikes were observed. The inner ring structures for both *N. gonorrhoeae* and *N. meningitidis* were of similar size as the structures previously observed from the isolated PilP/PilQ.
complexes, demonstrating that the second ring and the external spikes do not contain domains of the PilQ or PilP proteins.

Further investigation of the composition of the outer ring and the spikes of the PilQ complexes from several *N. gonorrhoeae* knock-out mutants showed some changes in the composition of the outer rings. A knock-out of PilP did not show presence of the spikes, which means that this protein might be directly or indirectly involved in the spike composition. Moreover, the PilP knock-out also showed a change in the symmetry of the outer ring from 14 to 19 fold. A PilF mutant also did not show spikes either, however, maintained the 14-fold symmetry of the outer membrane. We concluded, that PilC2 and PilW mutants yielded similar structures as observed for the wild-type, indicating that they are not involved in these structures. These results and our observations could indicate that our structure composed of two rings and spikes is a high rotational flexibility, especially the connection of the two rings is flexible. Therefore, we concluded that the second ring and spikes may have a role in stabilizing the anchoring of the PilP/PilQ complexes with the membranes.