5 Summary

For understanding the function of ligand-gated ion channels at a molecular level, the structure of such channels at a few Å-resolution has to be known. Since these receptors are complex membrane proteins, crystallization and follow-up x-ray structure analysis of these channels was not possible up to now. Therefore, a different approach to further clarify the structure of the nicotinic acetylcholine receptor is presented in this thesis. To examine the structure of these domains, it was intended to express some of the receptor's intracellular domains in different cellular systems.

Due to their likely participation in intracellular signal processing, studying these intracellular domains is of great value for neurochemical studies. For the rest of the receptor molecule, data and modellings of the structure are published but nothing is known about the intracellular loops. The loops contain for example phosphorylation sites in certain subunits, which are important for clustering of acetylcholine receptors at post-synaptic membranes, as mutation experiments had shown.

S*f*9-insect cells were used as eukaryotic expression system. Different DNA constructs were introduced into cells by baculovirus systems as well as by transfection with lipid reagents. The baculovirus expression however did not prove to be suitable for expressing the desired proteins. After infection the insect cells died before the expression of sufficient amounts of protein could be observed. In addition, the viral procedure is far too time consuming to test a large number of DNA constructs for optimizing the protein expression.

Finally, a stable S*f*9 cell line was established that expresses the intracellular loop of the δ-subunit. By using these cells, a procedure to purify the expressed δ-loop was developed. This procedure may be used to optimize the expression in order to obtain protein amounts sufficient for structural studies.

In *E. coli*, the expression of the intracellular loop in a prokaryotic context was tested for several DNA constructs encoding fusion proteins with soluble proteins like GST. Truncated versions of the intracellular loops of the δ- and the α7-subunits were introduced in different vectors. For some constructs a satisfying expression was achieved. However, the proteins could be purified only in small amounts applying high detergent concentrations, and with unpredictable yields. The GST-fusion protein of the α7-subunit was isolated from bacteria at a concentration of 30 µg/ml and a total yield of 45 µg.

As a third method, cell-free expression was tested using two *in vitro*-systems from the companies Roche and RiNA for the different intracellular loops. Only the intracellular δ-loop was once successfully expressed in a small amount and enriched by Ni^{2+}-affinity chromatography.

The expression in cell-free systems, in particular regarding unusual proteins, can therefore be considered to complement other expression methods. To obtain amounts of the nAChR intracellular loop, sufficient for solving its structure, the procedures here will have to be developed further.