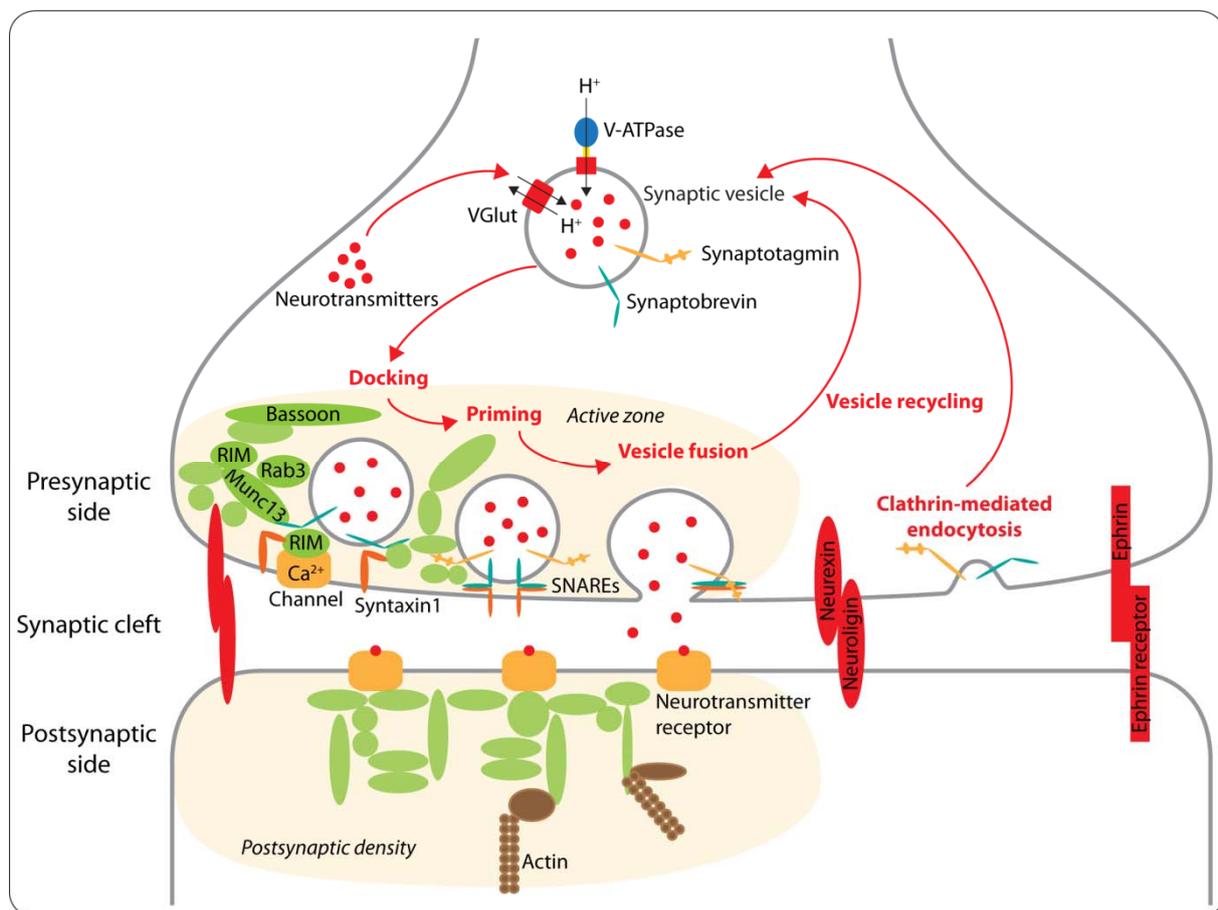




**PROTEIN-LIPID-INTERACTIONS IN SYNAPTIC VESICLES AND THE NEURONAL SYNAPSE**

Synapses are intercellular junctions between neurons in the nervous system. They are organised into a presynaptic terminal, the synaptic cleft and the postsynaptic density. Information that arrives in form of an action potential at the presynaptic terminal is transmitted to the postsynaptic neuron via chemical neurotransmitters, which are stored in synaptic vesicles (SVs) of the presynaptic cytoplasm.



**Schematic of a synapse.** SVs are loaded with neurotransmitters in the cytoplasm of the presynaptic terminal. Docking, priming and fusion of SVs takes place in the active zone whereupon they are recycled and reloaded. Neurotransmitters are released into the synaptic cleft to forward the signal to the postsynaptic density via neurotransmitter receptors in the postsynaptic membrane.

SVs are small storage organelles located at the presynaptic cytoplasm of every neuron. They contain neurotransmitters such as glutamate or  $\gamma$ -aminobutyric acid and pass through a trafficking cycle in the nerve terminal. The SV cycle includes import of neurotransmitters into the vesicles, docking, priming and fusion with the presynaptic

membrane to release neurotransmitters as well as endocytosis and recycling of the vesicles.

Whereas the essential proteins governing the SV cycle have been identified in the last decades, we still have only little knowledge about their exact operation and sequence in which they interact to carry out the individual steps of the SV cycle. Like other trafficking mechanisms, the vesicle cycle appears to be governed by non-covalent protein-protein and protein-lipid interactions that are assembled on demand and dissociate again when the task is completed. The resulting supra-molecular complexes involve interactions between soluble proteins and membrane components with as yet undefined stoichiometries, making it very difficult to identify their protein-protein interactions. In particular, membrane fusion involves not only interactions between membrane proteins, but also relies on formation of microdomains in which proteins and certain lipids are clustered and whose composition is largely unclear. Indeed, the inability to define these membrane protein assemblies and to study their properties constitutes a major problem preventing a complete molecular understanding of exocytosis and recycling.

So far, knowledge about protein complexes operating on the synaptic membranes is based on the study of isolated proteins (usually binary or ternary interactions) and pull-downs or immunoprecipitations in detergents that disrupt the native membrane environment. We develop new mass spectrometric techniques to address these protein assemblies. The analysis of SVs will serve as a starting point for a more comprehensive analysis of synaptic protein complexes at the presynaptic side. This will include for instance the still enigmatic architecture of docked synaptic vesicles; to date it is not known where exactly SVs bind the presynaptic membrane, which proteins interact or what protein stoichiometries the involved complexes have.

**Medical relevance for neurodegenerative disease.** SVs are unique in that they must be faithfully regenerated during each exo-/endocytotic cycle, requiring control mechanisms ensuring their complete and correct assembly which could be mediated by loose but specific “superclusters”. The investigation of these clusters and their interactions in the synapse will prove highly important to fully understand the mechanism of signal transduction in neurons. Only this knowledge can pave the way for new therapeutic interventions addressing neurodegenerative disorders and diseases. We hope to shed light on important protein-protein and protein-lipid interactions in neuronal synapses and thus extend our knowledge about the mechanisms of signal transduction in nerve cells allowing generation of functional models to provide therapeutic targets for clinical research.

The relevance of the identified protein-lipid interactions for neurodegenerative disease will therefore be investigated. For this, synaptic vesicles and synapses purified from healthy and diseased tissue will be compared yielding differences in their protein-protein and protein-lipid interactions. Another important aspect is the study of proteins that cause neurodegeneration such as  $\alpha$ -synuclein. Misfolding and subsequent aggregation often play an important role in neurodegenerative disease.

The interactions in misfolded and aggregated states will therefore be studied in detail to provide novel insights and contribute to the understanding of their interactions in the synapse which in most cases causes degeneration.

**Structural mass spectrometry.** We combine various cross-linking strategies with proteomics, lipidomics and mass spectrometry of intact protein complexes (“native” MS) to study protein-lipid assemblies in SVs and neuronal synapses. While proteomics and lipidomics are employed to identify the components of the protein-lipid-assemblies, structural mass spectrometric techniques deliver novel insights into protein-lipid-interactions. Native MS is the analysis of intact proteins and protein complexes in the gas phase of a mass spectrometer and reveals their composition, stoichiometry, topology, heterogeneity, subunit interactions as well as ligand binding. It covers a wide  $m/z$  range and allows the analysis of small protein subunits up to large protein assemblies in the same measurement. This is beneficial for heterogeneous protein assemblies such as large protein assemblies in SVs. Native MS will be complemented with various (chemical) cross-linking strategies to gain further insights into direct contact sites in the assemblies.