**NMR study of the calmodulin/Munc13-1 complex reveals multiple simultaneous binding modes**

Mitcheell Maestre-Martinez[1], Fernando Rodriguez-Castaneda[1], Nicolas Coudevylle[1], Stefan Becker[1], Nils Brose[2], Teresa Carlomagno[1,3], Christian Griesinger[1]

[1] NMR-based Structural Biology, MPI for Biophysical Chemistry, Am Faßberg 11, 37077 Göttingen, Germany
[2] Molecular Neurobioly, MPI for Experimental Medicine, Hermann-Rein-Str. 3, 37075 Göttingen, Germany
[3] present address: European Molecular Biology Laboratory, Meyerhofstr.1, 69117 Heidelberg, Germany

The priming process is an essential and rate-limiting step for the post-docking/pre-fusion maturation of vesicles in regulated exocytosis [1]. The interactions between Munc13 and calmodulin (CaM) regulate the synaptic vesicle priming and synaptic efficacy in response to the residual $Ca^{2+}$ signal, thereby shaping the short-term plasticity characteristics during periods of synaptic activity [2]. Moreover, it has been recently shown that the Munc13s C1 domain lowers the energy barrier for synaptic vesicle fusion [3].

Munc13-1 features a CaM binding site (Munc13-$1^{458-492}$) located N-terminal to its C1 domain (Munc13-$1^{567-631}$), which binds CaM in a $Ca^{2+}$-dependent manner. The NMR solution structure of the CaM/Munc13-$1^{458-492}$ complex, presented here, features a novel (1-5-8-25-26) CaM binding motif, where the Munc13-1 residues W464, F468 and V471 anchor the protein to the C-terminal CaM domain, while L488 and W489 are attached to the N-terminal CaM domain. This structure features a flexible arrangement between both CaM domains, as no inter-domain NOEs were observed.

Additionally, we demonstrate the presence of an interaction between the C1 domain of Munc13-1 and CaM, which occurs in the NMR fast time scale. Chemical shifts perturbations (CSPs) and paramagnetic pseudo-contact shifts (PCSs) clearly identify the N-terminal CaM domain as the one binding the Munc13-1 C1 domain, while no interactions between Munc13-$1^{458-492}$ and the C1 domain were observed. Altogether, these results indicate that CaM binds simultaneously to the CaM-binding site and the C1 domain of Munc13-1. Thereby, the C-terminal CaM domain plays a leading role in the complex stabilization, through a high affinity interaction with the CaM-binding site of Munc13-1, while the N-terminal CaM domain seems to have a regulatory function by interacting simultaneously with the L488-W489 segment and the C1 domain.

[1] Brose, N.; Rosenmund, C. and Rettig, J., Curr Opin Neurobiol, **2000**, 10, 303-311.
[2] Junge, H.J.; Rhee, J-S.; Jahn, O.; Varoqueaux, F.; Spiess, J.; Waxham, M.N.; Rosenmund, C. and Brose, N., Cell, **2004**, 118, 389-401.
[3] Basu, J.; Betz, A.; Brose, N. and Rosenmund, C., J Neurosci, **2007**, 27, 1200-1210.