Nanobodies reveal an extra-synaptic population of SNAP-25 and Syntaxin 1A in hippocampal neurons

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Synaptic vesicle fusion (exocytosis) is a precisely regulated process that entails the formation of SNARE complexes between the vesicle protein synaptobrevin 2 (VAMP2) and the plasma membrane proteins Syntaxin 1 and SNAP-25. The sub-cellular localization of the latter two molecules remains unclear, although they have been the subject of many recent investigations. To address this, we generated two novel camelid single domain antibodies (nanobodies) [1] specifically binding to SNAP-25 and Syntaxin 1A [2]. These probes penetrated more easily into samples and detected their targets more efficiently than conventional antibodies in crowded regions. When investigated by two-color Stimulated Emission Depletion (STED) microscopy [3], the nanobodies revealed substantial extra-synaptic populations for both SNAP-25 and Syntaxin 1A, which were poorly detected by conventional antibodies. Moreover, the extra-synaptic Syntaxin 1A molecules were recruited to synapses during stimulation, suggesting that these are physiologically-active molecules. We conclude that small probes as nanobodies are able to reveal qualitatively and quantitatively different organization patterns, when compared to conventional antibodies.

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