Spatial distribution of the synaptic vesicle fusion events in the mammalian synapses correlated to the active zone and SNARE-protein clusters

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The synaptic vesicle (SV) fusion is an essential step of information transmission in the nervous system. This process requires temporally and spatially coordinated work of numerous different proteins. Meanwhile, studying this phenomenon with fluorescent techniques is challenging due to the confined space in the presynaptic bouton. In order to decipher the dynamics and nanoscale organization of the SV-fusion machinery by high-resolution microscopy, we grew hippocampal neurons on microstructured glass coverslips. This approach triggers the formation of purely presynaptic sites we call Xenapses.
Using pH-sensitive fluorescent probes in Xenapses allows marking single synaptic-vesicle fusion events with an unprecedented resolution. This approach called APALM (Active Potential-Activated Localization Microscopy) combined with the correlative TIRF-dSTORM procedure allows to overlay the synaptic-vesicle fusion sites with presynaptic nano-compartments and answer the question how form (internal architecture of the presynapse) fits function in the process of the synaptic vesicle fusion.
Spatial analysis shows that most of the SV-fusion events tend to localize close to Bassoon clusters (80-120 nm from the center of cluster). This tendency is even more prominent if we measure the distance between the centers of mass of the APALM clusters and centers of mass of the Bassoon clusters. In contrast, Syntaxin 1a and SNAP25B tend to coexist within separate clusters. Neither of these clusters overlaps with SV-fusion sites. That leads to a conclusion that hippocampal synapses SNARE-clusters do not serve as SV-docking sites.