Cryo-electron tomography on synapse in primary neurons

Pei Wang[1,2], Buyun Tian[1,2], Fengping Feng[1], Yanhong Xue[1*], Tao Xu[1,2*]
[1] National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[2] University of Chinese Academy of Sciences, Beijing 100049, China
***Correspondence: xueyanhong@ibp.ac.cn**

KEY WORDS Cryo-electron microscopy, neuron, active zone, vesicle

Synaptic transmission in neurons are multistep processes that are mediated by the fusion of vesicles and regulated by synaptic proteins. Vesicles are loaded with neurotransmitter, translocate to the plasma membrane, undergo tethering, docking and after several steps of priming, docked vesicles become fusion-competent and fuse with the PM in response to calcium influx. In neurons, the extraordinary temporal and spatial precision of neurotransmitter release relies on exact and specific interactions between presynaptic proteins, at specialized sites of presynaptic terminals called active zones (AZs). We culture primary neurons on EM golden grids and apply plunge freezing and cryo-electron tomography to characterize the organization of AZ proteins on different stages of vesicle docking in neurons, determine the role of specific AZ proteins in forming the structural organization, morphology and function of AZs, obtain their interactions with filaments, synaptic vesicles and plasma membranes and analyze the 3D structure of AZ proteins in situ. The aim is deciphering the functional nano-architecture of AZ in neuronal and neuroendocrine cells.

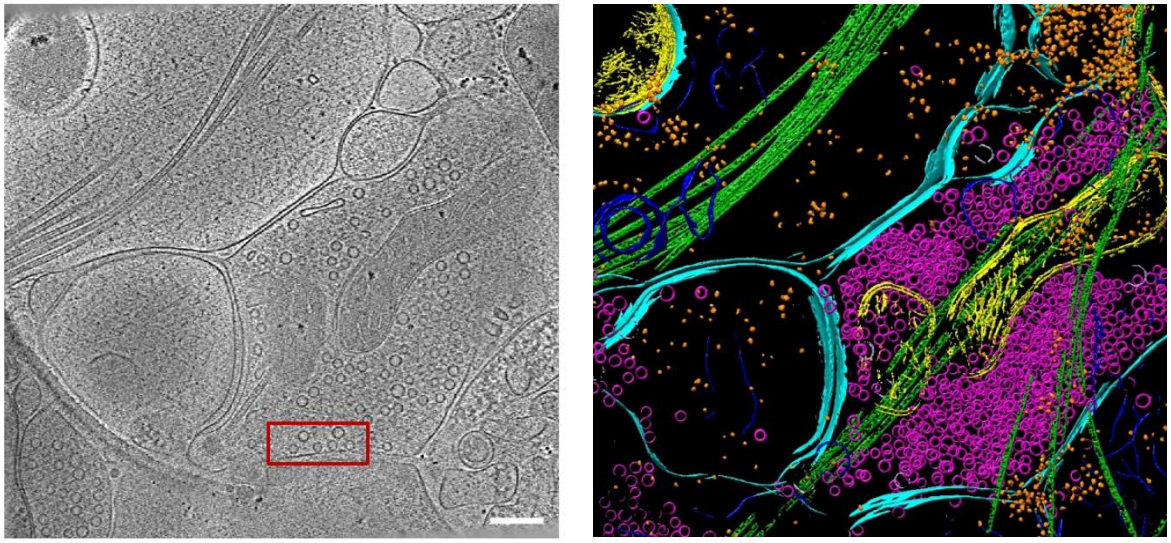

Figure 1: Slice and segmentation of tomogram shows SVs contact with PM in neuron synapse

[1] Sudhof, T. C. The presynaptic active zone, Neuron 75, 11-25 (2012)
[2] Gundelfinger, E. D., and Fejtova, A. Molecular organization and plasticity of the cytomatrix at the active zone, Current opinion in neurobiology 22, 423-430 (2012)
[3] Wilhelm, B. G., Mandad, S., Truckenbrodt, S., Haucke, V., Urlaub, H., and Rizzoli, S. O. Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins, Science 344, 1023-1028 (2014)
[4] Bei Liu, Yanhong Xue, Wei Zhao, Yan Chen, Chunyan Fan, Lusheng Gu, Yongdeng Zhang, Xiang Zhang, Lei Sun, Xiaojun Huang, Wei Ding, Fei Sun, Wei Ji & Tao Xu. Three-dimensional superresolution protein localization correlated with vitrified cellular context. SCIENTIFIC REPORTS. 5:13017 (2015)