High-density single molecule mapping on live neurons give new insights into sub-diffraction organization of Postsynaptic molecules

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Abstract: The number and lateral organization of AMPA receptors in postsynapse is considered as a crucial factor in controlling synaptic plasticity and the fidelity of synaptic transmission [1]. The equilibrium between the number of synaptic and extrasynaptic AMPA receptors is decisive in controlling basal transmission and synaptic plasticity. This balance is regulated by the subunit composition of these receptors and their selective interaction with intracellular scaffold proteins. However, how the trafficking of these molecules is controlled remains unknown. Emergence of single molecule based super-resolution imaging techniques offers unique capabilities to monitor the live cell activity at the nanometric scale and various time scales. We combine high-density Single Particle Tracking (SPT) techniques like Photo Activation Localization Microscopy (PALM) [2] and Universal Point Accumulation In Nanoscale Topography (uPAINT) [3], on a multimodal microscope to map trajectories of individual molecules at an unprecedented spatial (~30nm) and temporal resolution (>50 Hz). We discuss the application of PALM in combination with Single Particle Tracking (SPT-PALM) to obtain high density single molecule trajectories on live hippocampal pyramidal neurons transfected with GluA1, GluA2, PSD95 and Homer molecules fused to photoconvertable EOS fluorescent protein. Complimentary to SPT-PALM we used uPAINT to compare the distribution of endogenous surface receptors using high-density stochastic labeling of surface receptors with antibodies tagged to fluorescent molecule ATTO647. Using a correlative approach to combine SPT-PALM and uPAINT on living neurons, we observed the spatio-temporal evolution of sub-diffraction sized nanodomains (<80nm) revealing a very dynamic short-range organization of molecules in the synapse. Additionally we determine and differentiate diffusional behavior from thousands of spatially discrete single molecule trajectories obtained by SPT-PALM and UPAINT from single cells. Finally we elucidate the importance of high-density mapping of single molecules from living neurons to comprehend finer details of molecular mechanisms important in the organization of an excitatory synapse.