QUANTITATIVE SINGLE MOLECULE ANALYSIS OF ANCHORING PROTEIN OF THE INHIBITORY SYNAPSE

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Super-resolution techniques based on individual molecule localization (IML) provide the capability to study sub-cellular structures at the molecular scale and to access quantitative information about proteins distribution in biological systems [1]. In the specific case of inhibitory synapses, the post-synaptic area is enriched with the scaffold protein gephyrin that, by creating an highly tangled submembranous network, offers binding sites for the synaptic clustering of inhibitory receptors, like GABA\(_A\) and glycine receptors [2].

In this work we mapped the spatial distribution of gephyrin clusters along the 3D organization of neuronal dendrites. In particular, we focused on synaptic gephyrin clusters identified by their matching with specific marker of pre-synaptic terminals by means of a dual color STORM (Stochastic Optical Reconstruction Microscopy) acquisition scheme [3]. Then we exploited PALM (Photo-Activatable Localization Microscopy) in order to quantitatively monitor the rearrangement of the gephyrin clusters in response to a chemically induced form of long-term potentiation of inhibitory synapses (chem-iLTP) [2]. The choice of a photoactivatable fluorescent protein with a fully characterized photophysics (mEos) [4] combined with a quantitative approach based on clustering analysis coming from graph theory [5] gave us access to a more comprehensive set of parameters allowing the characterization of gephyrin distribution both at the post-synaptic and extra-synaptic areas. The data presented here suggest that, during the expression of chem-iLTP, gephyrin is redistributed from extra-synaptic to synaptic compartments. Interestingly, we observe that the increase of synaptic gephyrin is strictly linked to an increase of heterogeneity in the inner organization of the scaffold protein at the synapse that rearranges in nanodomains.