PhD Thesis Defense LARA LAPARRA 'Quantitative Nanoscale Imaging of Synaptic Protein Organization'

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March 28, 2018

Wednesday March 28, 15:00. ICFO Auditorium

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Single Molecule Biophotonics

ICFO-The Institute of Photonic Sciences

The arrival of super-resolution techniques has driven researchers to explore biological areas that were unreachable before. Such techniques not only allowed the improvement of spatial resolution in images but also the possibility to perform quantitative measurements at the single-molecule level. The interest in that particular field has been growing over the years and new and more sophisticated tools have been developed.

Neuroscience has been one of the first fields to adapt and benefit from super-resolution microscopy. These techniques opened a new window of opportunity to reveal spatial organization of the neuronal cytoskeleton and the molecular organization and dynamics of the synapse, structures below the spatial resolution limit of conventional light microscopy.

Protein organization and stoichiometry is central for synaptic transmission in neurons. Knowing the absolute numbers of proteins playing a key role in diseases can be of extreme interest in order to understand the mechanisms that lead to such neurological disorders.

In this thesis we exploited the super-resolution techniques to develop a pioneering method for quantitative single-molecule measurements and to unravel the organization of a synaptic protein complex that was never visualized before with nanoscale spatial resolution.

We developed a novel method in order to quantify the photoactivation efficiency of eight different photoswitchable fluorescent proteins commonly used in super-resolution experiments. We used the glycine receptor as a template because of its well-known stoichiometry and tagged eight different photoswitchable fluorescent proteins to the a- and Ss-subunits of this receptor and transiently transfected them to Xenopus oocytes. The fact that the fluorescent proteins are genetically encoded make them highly suitable for quantitative single-molecule counting. The photoactivation efficiency, which is the percentage of a fluorescent protein that photoactivates into a fluorescently detectable form, plays a critical role in properly interpreting quantitative measurements.

Moreover, we also focused our studies on super-resolution imaging of a synaptic protein complex, called LGI1 complex. This ensemble of proteins is one of the main key players involved in different neurological disorders. Leucine rich glioma activated 1 (LGI1) is a neuronal protein that forms a trans-synaptic bridge linking pre- and postsynaptic transmembrane proteins (ADAM22 and ADAM23) and helps to organize a multimeric complex at the synapse including AMPA receptors and voltage-gated potassium channels (VGKC). LGI1 autoimmune encephalitis is a severe neuropsychiatric disorder related to epilepsy where the patients produce autoantibodies against LGI1, which alter synaptic plasticity. However, the molecular mechanisms that lead to the observed problems in patients still remain largely unknown.

Using well-characterized synaptic markers as molecular standards, we determined the positioning of LGI1 and the other related proteins within the synaptic space at nanoscale resolution by means of multi-color STORM. Further, the comparison of this molecular architecture in healthy neurons versus neurons treated with antibodies from patients suffering from LGI1 autoimmune encephalitis showed that these antibodies impact the nanoscale organization of pre-synaptic proteins. These results suggested a loss of LGI1 interaction with pre-synaptic proteins upon antibody binding and gave further insight into early changes in pathology.

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