Novel tools for the Spatio-Temporal Photocontrol of protein-protein interactions

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Background incl. aims
The dynamic regulation of AMPA glutamate receptors (AMPARs) trafficking into and out of the synapse is a major mechanism underlying synaptic plasticity, a fundamental basis of learning and memory. However, the detailed molecular mechanisms governing the dynamics of these postsynaptic receptors are still not fully understood, stressing the need for new methods to modulate and monitor these processes. With the purpose of controlling locally, acutely and in a reversible manner the interaction between proteins involved in AMPAR trafficking we have developed original biomolecular tools based on optogenetics. These approaches will be effectively applied with spatial and temporal precision on selectively targeted individual cells and they will work based on two strategies: by reversibly immobilizing proteins by light, or light-controlling the binding state (association / dissociation) of proteins. The design and production of these tools have involved molecular/cell biology, biochemistry and protein engineering for later on, validating them in heterologous cells and neurons by Advanced Fluorescence Techniques. Our tools effectively address current technical limitations in neuroscience and could allow investigating questions from further biological disciplines.

Methods
The design and production of these optotools have involved molecular/cell biology, protein engineering and biophysical characterization, for later on, validated them in heterologous cells by Advanced Fluorescence Techniques. In order to characterize these approaches in transfected eukaryotic cells as a model of complex cellular context, a new red shifted FRET pair has been developed to monitor protein interactions. Besides heterologous cells, the approaches have been also validated by u-PAINT in neurons.

Results
On one hand, we have designed Photoactivatable competing ligands to destabilize AMPARs by disruption of the natural interaction between Stargazin and PSD-95. On the other hand, we have engineered photoswitchable crosslinkers to specifically recognize AMPAR subunits and control with spatio-temporal resolution the mobility and local number of AMPARs.

Conclusions
The approaches we have engineered should most importantly allow tackling fundamental questions related to AMPARs mobility but also be of high impact on a large scientific community as they could be transferable to many systems.

Keywords:

Optogenetics, super resolution, FLIM-FRET, PPI,

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