All-optical interrogation of brain circuits with the Halotag technology

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The project I am working on and that will be soon subject of a publication, relies on the use of the HaloTag technology: a powerful tool mainly used for protein labeling and biological assays. When an animal or a tissue, genetically expressing the halotag construct, are incubated with a fluorescent JF-halotag ligand (ref #GA1121), the neurons of interest are stained in a rapid and irreversible manner. Here, we presented an innovative construct combining the expression of an opsin, fused with the halotag protein tag, with the aim of performing "all-optical" investigation of neuronal networks, *i.e* be able to photostimulate the opsin$^+$ neurons and simultaneously record their activity via a calcium indicator. In this context, one of the main issues the researchers undergo during current optogenetics experiments are the existence of different crosstalks related to the similar absorption and emission wavelengths of the different constructs. For instance, when we image neurons expressing commonly used opsins at a specific wavelenght, the latter also activates the neurons of interest. We here want to exploit the versatility of the JF-halotag ligand palette to tackle some of these crosstalk during all-optical investigation of neuronal networks: using an unique genetic tool (animal line expressing the construct), and the variety of fluorescent halotag ligand available (JF-dye, Promega), one can specifically choose the colors and then create the optimal environment for its experiment (non-overlap of excitation/emission spectra). We already showed that this new construct is powerful, allowing in mouse brain slices and zebrafish larvae, single-cell control under 2-photon excitation of large population of neurons. Moreover, the diversity of dyes allow specific combinations for multicolor imaging; we want to extend this technology to stain neurons at different time points of the developmental stages in order to follow the cells of interest and investigate their physiology and neuronal networks involving early and late born neurons. We believe that this innovative technology will open the way to novel possibilities of studies and will help the investigation of neuronal networks in living animals, facilitating the "all-optical" interrogation of brain circuits, in particular in the context of brain disease such as vision-related pathologies.