A modular ligand-directed approach to label endogenous aminergic GPCRs in live cells

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In the last two decades, new technologies based on luminescence have been developed to monitor the organization, signalling or ligand binding of G Protein-Coupled Receptors. These technologies rely on the overexpression of genetically modified (and/or fluorescently tagged) receptors of interest. However, there is an increasing interest in developping approaches to conjugate chemical labels to specific residues of native GPCRs, despite of the low reactivity and the high abundance of such residues.

Ligand directed approaches, may offer a solution to this problem. Such approaches consist in molecular probes that include a selective ligand moiety and a reactive moiety. Upon ligand binding, the labelling is directed to a nucleophilic amino acid in the vicinity of the binding pocket. However, this approach requires the use of non-nucleophilic ligand moieties, which is particularly difficult since many GPCR ligands contain amines or other nucleophilic functional groups.

In the present work, we developed an innovative ligand-directed toolbox based on a novel approach. The later uses molecular modules to build fluorescent ligand-directed probes to label an archetypical aminergic GPCR (D1R). Our molecular probes can be readily prepared before the labelling reaction from two molecular modules: an activated electrophilic linker which includes a fluorescent dye and a GPCR ligand that may include nucleophilic groups. Thanks to a fast and specific chemical reaction, the nucleophilic ligand can barely react with the activated linker before it is bound to the native target GPCR and the labelling reaction occurs. Subsequently, the ligand will unbind the GPCR pocket, leaving the receptor fluorescently labelled and fully functional.

This novel labelling approach allowed us to label endogenous D1 receptor both in transfected cells and primary cultures of neurons and will pave the way to develop new reagents and assays to monitor endogenous GPCRs distribution and activity in their native environment.