Multicolor Time-Resolved FRET microscopy reveals the impact of G protein-coupled receptor oligomerization on internalization processes.


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Introduction: Identifying the interacting partners and the dynamics of molecular networks constitutes the key point in understanding cellular processes. Methods based on resonance energy transfer strategies (RET) have often been developed to evidence the existence of such complexes. These methods however suffer from few drawbacks, such as only one complex at a time can be studied and most often its localization and evolution in time are not well-determined. Here we report an original multi-color time-resolved fluorescence RET microscopy method (i) allowing the detection of up to three different partners in a cellular context, (ii) compatible with kinetics experiments, and (iii) offering sub-cellular resolution.

Results: Multi-color time-resolved fluorescence RET microscopy method is based on the use of cryptate of lanthanide (Lumi4Tb) as donor. Thanks to the lanthanides long-lasting luminescence, the introduction of a time delay between the sample illumination and the image acquisition allows discriminating donor or FRET long-lasting fluorescence from short-lived fluorescence issued from auto-fluorescence or direct acceptor excitation. By using that method, we previously detected GPCR oligomers in native tissues [1]. Moreover, thanks to its multiple emission peaks and large Stokes shift, Lumi4Tb can be associated with various acceptors, ranging from fluorescein-like or dy647-like fluorophores to quantum dots (QD). These characteristics make the time-resolved FRET microscopy an easy technique to implement and to detect FRET signal. We used the approach to evidence the existence of G-protein coupled receptor oligomerization and to track their internalization after their stimulation with agonists. Finally, we evidenced cross-regulations between receptors during internalization processes when taking vasopressin V1a and V2 receptors as models.

Conclusion: We demonstrate the potential of the time-resolved FRET microscopy when studying G protein-coupled receptor oligomerization and internalization. The imaging capabilities of the method open a new window for studying the dynamics and structure of molecular complexes in cells.

Fig.1: Multicolor time-resolved FRET imaging. A mix of cells expressing either vasopressin CLIP-V2 or dopamine Halotag-D2 or SNAP-D2 receptors were plated and incubated in the presence of CLIP-Lumi4(Tb), CLIP-green, HALO-Lumi4(Tb), HALO-red and SNAP-Lumi4(Tb)-biotin and in a second step with quantum dots (QD605). Image 1 corresponds to the donor fluorescence, images 2 to 4 to the FRET signal. Bar size is 50 μm.

References: