**INTERLACING BIOPHYSICAL METHODS TO UNVEIL MORPHOLOGY AND DYNAMICAL INTERACTIONS WITH MICROTUBULES OF Transient receptor potential vanilloid 1 (TRPV1) AT NANOSCALE IN LIVING CELLS**

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Transient Receptor Potential Vanilloid 1 (TRPV1) is a nonselective cation channel involved in the transmission and modulation of nociception, as well as in the integration of several painful stimuli.1 Recent evidences suggest a peculiar interaction between TRPV1 and microtubules for transmission of pain at the cellular level.2 By means of a high-sensitivity multiplex imaging approach, we monitored the nanoscale interactions of TRPV1 with microtubules. In more details, we first combined Temporal Image Correlation Spectroscopy (tICS) measurements with FRET, in order to unveil the dynamic properties of TRPV1-microtubule complex. Next, we used the combination of FRET with *i*MSD3 analysis based on Spatio-Temporal Image Correlation Spectroscpy (STICS) in order to demonstrate the presence of a directional diffusion of TRPV1 complexes with microtubules structures. Changes in TRPV1 aggregation upon receptor stimulation and/or microtubule depolymerization were investigated by fluorescence anisotropy associated to homo-FRET.4 The latter experiments highlighted that microtubule depolymerization induces the formation of large, non-functional aggregates. Finally, we applied single molecule localization techniques in order to improve the spatial resolution of TRPV1 detection. We demonstrated that TRPV1 is organized heterogeneously on plasma membrane in sub-micron membrane domains with different sizes by both super-resolution optical fluctuation imaging (SOFI) and photo-activated localization microscopy (PALM) by using the genetically-encoded photochromic reporter EYQ1.5 Our work clarified the membrane organization of TRPV1 at nanoscale in living cells, as well as the role of microtubule in preserving the receptor's functionality. In this context, future experiments will target specifically the nanoscale morphology of TRPV1-microtubules complexes on plasma membrane.

1. Latorre, R., Zaelzer, C. & Brauchi, S. *Q Rev Biophys* **42**, 201-246 (2009).

2. Goswami, C. et al. *J Neurochem* **91**, 1092-1103 (2004).

3. Di Rienzo, C., Gratton, E., Beltram, F. & Cardarelli, F. *Proc Nat Acad Sci USA* **110**, 12307-12312 (2013).

4. Tramier, M. & Coppey-Moisan, M. *Methods Cell Biol* **85**, 395-414 (2008).

5. Bizzarri, R. et al. *J Am Chem Soc* **132**, 85-95 (2010).