

# OBSERVING DYNAMIC INTERACTIONS OF STORE-OPERATED CALCIUM CHANNEL PROTEINS BY FLUORESCENCE LIFETIME IMAGING MICROSCOPY

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In electrically excitable cells such as rat neuroendocrine pheochromocytoma PC12 cells, the Ca<sup>2+</sup> influx essential for regulating several vital processes is major from voltage-gated Ca<sup>2+</sup> channels. The unique Ca<sup>2+</sup> entry pathway, namely store-operated Ca<sup>2+</sup> channels (SOCCs) constructed by Orai1 with STIM1 were recently proved to functional existed in this cell model. However, it is still unrevealed to define detail mechanism about the interactions between Orai1 and STIM1 during store depletion *in vivo*. Herein we further investigated the dynamic protein-protein interactions *in situ* through co-expressing the green fluorescence protein (EGFP)-Orai1 (donor) and mOrange-STIM1 (acceptor) as a fluorescence resonance energy transfer (FRET) pair under one-photon excitation fluorescence lifetime imaging microscopy (FLIM). The activity of SOCs (store-operated Ca<sup>2+</sup> entry, SOCE) was measured using fura-2 imaging method. With the strategy of time-correlated single photon counting, the lifetime values of each single pixel occupied within any region of the cell to form the lifetime map of every single cells. In summary, we further show the dynamic interactions between these two SOCC proteins comparing the FLIM maps of various conditions.

