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Dynamic chemogenetic reporters to investigate organelle coupling

The close proximity between intracellular organelles is critical to regulate fundamental cell pathways, such as Ca2+ signaling, lipid metabolism, cell death and ER stress. Notably, alterations of organelle contacts have been reported in several pathologies. Therefore, defining the regulation of organelle juxtaposition and its contribution to different cellular functions is critical. However, efforts aimed at visualizing organelle contacts have been hindered by the lack of dynamic reporters allowing to follow membrane contact sites with sufficient spatiotemporal resolution.

We hence developed and characterized a set of reversible fluorescent probes to detect the juxtaposition between endoplasmic reticulum (ER), mitochondria and the plasma membrane . These sensors are based on splitFAST, a chemogenetic reporter originally developed to detect dynamic protein-protein interactions in the green, red or far-red spectrum.

By virtue of the intrinsic reversibility of splitFAST, these probes allowed to detect the dynamics of contact sites after different cellular treatments at high spatiotemporal resolution. Using confocal airyscan or lattice light-sheet microscopy, we followed ER-mitochondria contact sites in HeLa and Cos7 cells. We observed that the probe correctly marks and follows the transient docking of the two organelles. Moreover, we found that contacts are extremely dynamic, moving along the mitochondrial and ER networks and undergoing themselves continuous remodeling based on several fusion and fission events. By taking advantage of the dynamicity of the splitFAST-based probes, our future investigations will focus on the study of the mechanisms governing the plasticity of organelle contacts.

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