Split Fluorescent Tools to Unveil the Organelle Contactome and Beyond

Inter-organelle membrane contact sites have emerged as essential platforms coordinating calcium signaling, lipid metabolism, redox homeostasis, and cell fate decisions. Their dynamic organization is profoundly altered in aging, neurodegeneration, cancer, and other pathological conditions. However, the lack of suitable tools capable of monitoring these interactions with high spatial and temporal resolution has limited our understanding of their functional relevance. To overcome these limitations, we developed an expanding platform of split-GFP-based contact site reporters (SPLICS) to visualize, quantify, and functionally characterize organelle contact sites in living cells and in vivo. Using protein engineering, we generated a new generation of SPLICS reporters capable of detecting established and previously inaccessible organelle interfaces. These probes were validated in cultured mammalian cells and in vivo models using quantitative fluorescence microscopy, genetic perturbation, and biochemical approaches. In addition, functional reporters were developed to simultaneously monitor calcium, and potentially other signaling species, at membrane contact sites, together with engineered molecular rulers to investigate the relationship between intermembrane distance and signaling efficiency. The newly developed SPLICS reporters enable quantitative visualization of organelle contact sites with high specificity and spatiotemporal resolution, allowing the construction of dynamic maps of the cellular contactome. Functional reporters reveal localized calcium microdomains associated with specific contact sites, while rigid molecular linkers define the optimal intermembrane distances required for efficient signal exchange. Application of these technologies to disease models uncovers extensive remodeling of organelle communication networks and identifies contact-site alterations associated with cellular dysfunction. These next-generation SPLICS technologies therefore provide an integrated platform for investigating both the structural and functional organization of the organelle contactome. Beyond mapping intracellular connectivity, they enable direct interrogation of the biochemical communication occurring at membrane contact sites, offering powerful new tools to dissect organelle crosstalk and its contribution to cell death, stress adaptation, and human disease.