pH-sensitive membrane probes for ratiometric imaging and monitoring of intracellular vesicle acidification

Sophie Walter*[1], Erwan Grandgirard[2], Marc Nothisen[1], and Mayeul Collot[1]

[1]Chémo-Biologie Synthétique et Thérapeutique – université de Strasbourg, Institut de Chimie - CNRS Chimie, Centre National de la Recherche Scientifique – France

[2]Institut de Génétique et de Biologie Moléculaire et Cellulaire – université de Strasbourg, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale : U964, Centre National de la Recherche Scientifique : UMR7104, université de Strasbourg : UMR7104, Institut National de la Santé et de la Recherche Médicale : U1258 – France

Résumé

Tracking pH variations within intracellular vesicles along the endocytic pathway is essential for a deeper understanding of cellular trafficking and metabolism. Although small-molecule fluorescent pH probes are valuable tools in bioimaging, they are typically not specifically targeted to intracellular vesicles or are directed primarily toward acidic lysosomes, thereby limiting the dynamic observation of vesicular acidification.
Here, we report the design of a novel ratiometric FRET-based pH probe that initially targets the plasma membrane (PM) and subsequently accumulates within intracellular vesicles via endocytosis. This probe combines two dyes: a spiroamide rhodamine acting as the FRET acceptor and a green BODIPY dye serving as the FRET donor. Spiroamide rhodamines featuring an intramolecular nucleophilic moiety are particularly well-suited due to their dynamic equilibrium between an emissive open form and a non-emissive spirocyclic form.

Upon exposure to the vesicular lumen, the probe enables real-time monitoring of vesicle acidification throughout the endocytic pathway. Importantly, this approach allows for statistical analysis of intracellular vesicle acidification under various biological conditions. Moreover, this ratiometric FRET probe provides the capability to track pH changes over time within individual vesicles, including highly dynamic and mobile populations.
Michelis et al. *Anal. Chem.* 2022, 94, 15, 5996–6003

Mots-Clés: pH, FRET, ratiometric, intracellular vesicle acidification

*Intervenant