A New Family of Bright Dioxaborine-Based Merocyanine Fluorophore for Multicolour Imaging of Lipid Droplets in Live Cells

Mayeul Collot,[1] Pichandi Ashokkumar,[1] Lydia Danglot,[2] Andrey Klymchenko[1]

[1] Laboratoire de Biophotonique et Pharmacologie, UMR 7213 CNRS, Université de Strasbourg, 67401 ILLKIRCH, France.

[2] University Paris Diderot, Sorbonne Paris Cité, Institut Jacques Monod, CNRS UMR 7592, Membrane Traffic in Health and Disease, INSERM ERL U950, 75013 Paris, France.

E-mail: mayeul.collot@unistra.fr

Lipid droplets (LDs), also known as adiposomes or lipid bodies, are intracellular lipid-rich organelles that regulate the storage of neutral lipids including triglycerides and cholesterol esters.[1] In the recent years, LDs draw a considerable attention as it was found to be involved in many physiological processes including membrane synthesis and trafficking,[2, 3] protein degradation[4] but also inflammation[5] and pathologies like obesity, diabetes and atherosclerosis[6] as well as viral replication[7] and cancer.[5] Therefore it is of great importance to specifically image these vesicles in cells and tissues. Herein we present a family of new merocyanine fluorophores based on an indolenine moiety and a dioxaborine barbiturate derivative (fig. 1A). These so-called Stato-Merocyanine (SMCy) form soluble non-emissive aggregates in water (Fig. 1B) and span their fluorescence from yellow to the near infrared in oil (Fig. 1C) with an impressive fluorescence enhancement. The Stato-Merocyanine display high molar extinction coefficient (up to 390,000 $M^{-1}.cm^{-1}$) and high quantum yield values (up to 100%) with an enhanced photostability compared to merocyanines (MC-540) and carbocyanine (Cy5). All the members of this new family specifically stain the LDs in live cells with very low background noise. Unlike Nile red, a well-known lipid droplet marker, SMCy dyes possess narrow absorption and emission bands in the visible and thus allow multicolor imaging, up to 4 colours (Fig. 1D). SMCy proved to be compatible with fixation and led to high quality 3D images of lipid droplets in cells (Fig. 1E). Their high brightness and remarkably high two-photon absorption cross-section (SMCy5 and SMCy5.5) as well as their capacity to efficiently fluoresce in the near infrared region make them promising candidates for *ex vivo* and *in vivo* tissue imaging.

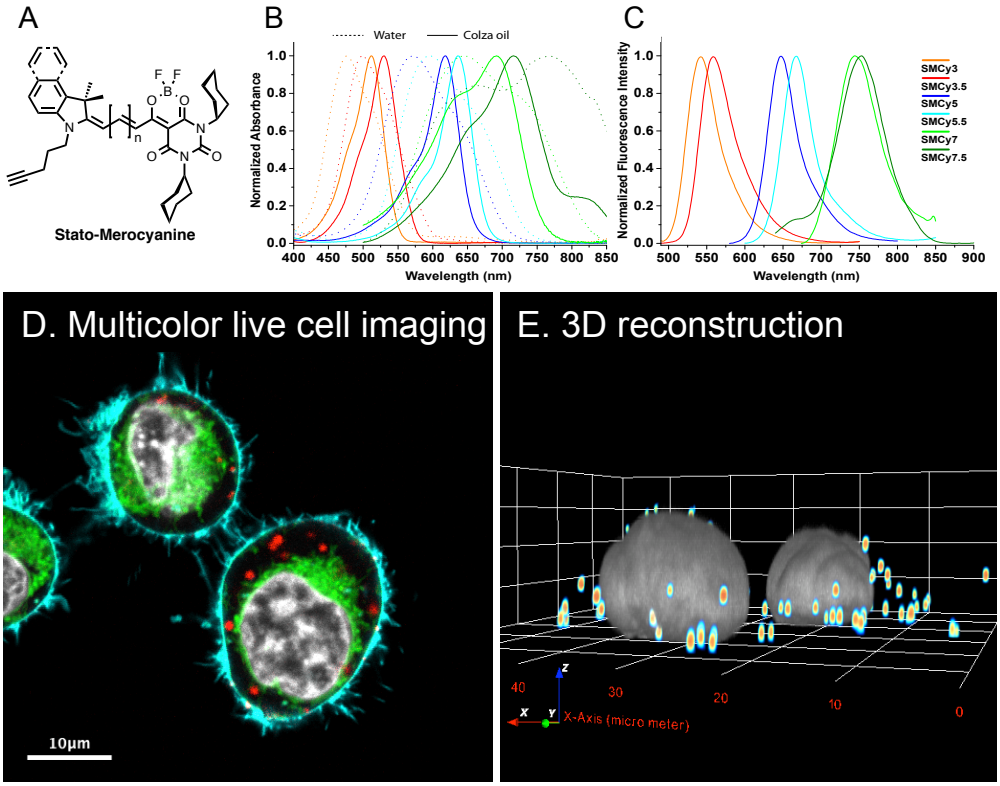

Figure 1. A) Structure of the SMCy dyes. B) Normalized absorption spectra of SMCy (1 µM) in water and oil. C) Emission spectra of SMCy dyes (1 µM) in oil. D) Laser scanning confocal microscopy image of KB cells, nuclei were stained with Hoechst (grey), plasma membranes with MemBright®-488 (cyan), mitochondria with Rhodamine C-12 (green) and the LDs with SMCy5 (red). E) 3D image of fixed KB cells (4% PFA) obtained by laser scanning confocal microscopy, the nuclei appear in grey (Hoechst) and the LDs in rainbow spots (SMCy5).

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