

MemBright: a Family of Red to Near-Infrared Cyanine Fluorescent Membrane Probes for Mono and Two-Photon Cell and Tissue Imaging

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In addition to the basic function of cell barrier, the plasma membrane is the turntable for crucial processes in cell biology.¹ Therefore, its proper staining is critical in bio-imaging. Herein, we developed a family of six fluorescent membrane probes, called MemBright (MB), based on red to near-infrared dyes (cyanines 3, 3.5, 5, 5.5, 7 and 7.5) coupled to two amphiphilic zwitterionic anchor groups. These probes span their emission wavelengths from 560 to 830 nm and offer excellent contrast in imaging experiments due to their weak fluorescence in aqueous medium and their important turn-on effect when anchored to the plasma membrane. At low concentration (nanomolar range), MemBright probes display homogeneous and selective plasma membrane staining in live cancer cells, HeLa and KB, as well as primary hippocampal culture of neurones and astrocytes. They are also compatible with imaging of fixed cells and exhibit remarkably high two-photon absorption cross-section that allowed their application for two-photon imaging of live cells and liver tissue. Additionally, MemBright showed an enhanced labelling of neurons in brain slices providing good quality images of pyramidal neurons in the hippocampus by laser scanning confocal microscopy and by two-photon excitation imaging. Finally, super-resolution dSTORM imaging of the plasma membrane was performed using one of these probes. MemBright probes constitute a universal toolkit for biomembrane imaging with a variety of imaging techniques.

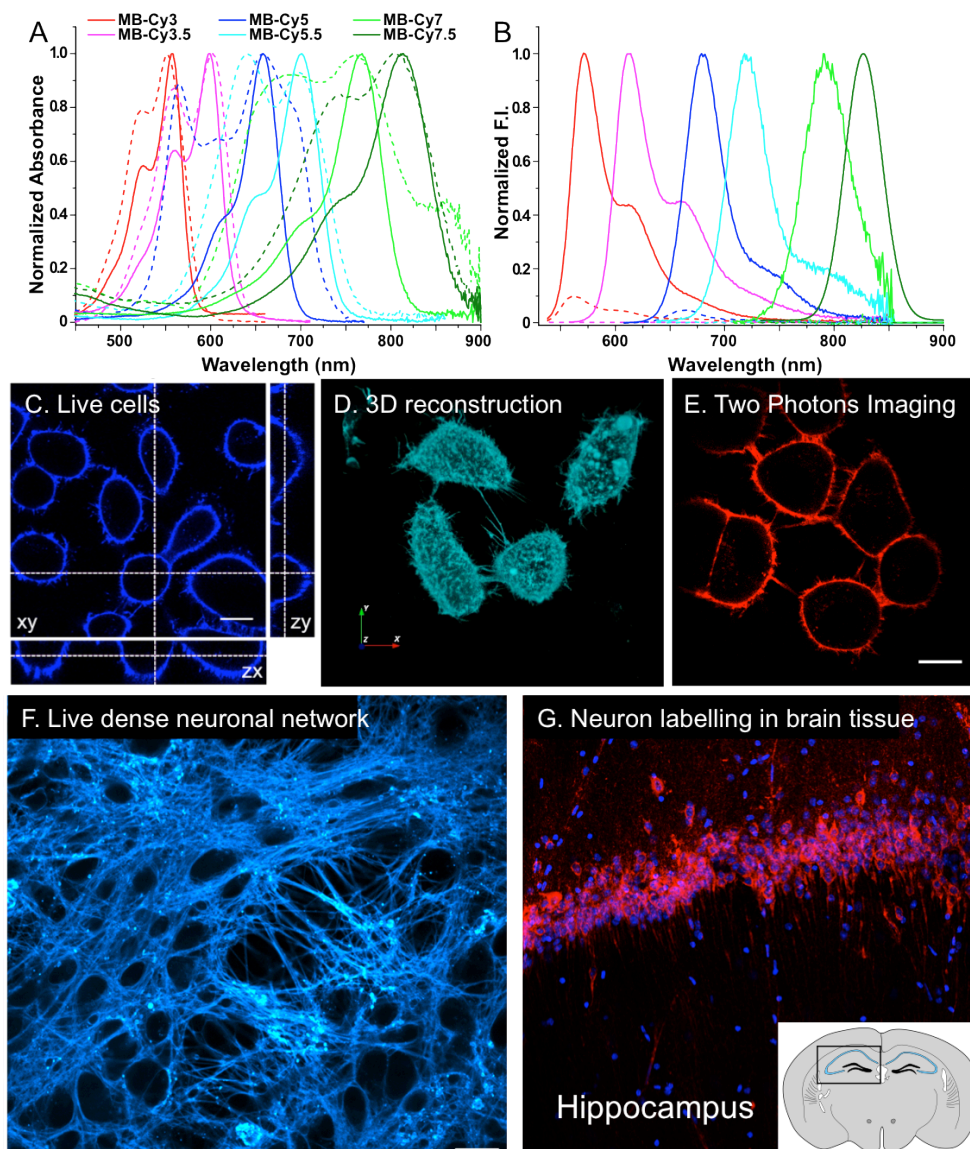


Figure 1. A) Normalized absorption and B) emission spectra of MemBright (200 nM) in water (dash lines) and the presence of DOPC vesicles (solid lines). C) Laser scanning confocal microscopy image of KB cells stained with MB-Cy5. D) 3D-image of live KB cells obtained by laser scanning confocal microscopy. E) Two-photon excitation imaging of KB cells stained with MB-Cy3.5. F) Spinning disc microscopy images of live dense neuronal network labeled with 20 nM MB-Cy5. G) Two-photon excitation imaging of a brain slice incubated with MB-Cy3.5 (5 μ M) providing the labeling of the pyramidal neurons in the hippocampus.

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References:

[1] Lingwood, D.; Simons, K. *Science* **2010**, *327*, 46–50.