Imaging approaches for analysis of cholesterol distribution and dynamics in the plasma membrane.

Autorzy

Daniel Wüstner

Maciej Modzel

Frederik Wendelboe Lund

Michael A. Lomholt

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Cholesterol is an important lipid component of the plasma membrane (PM) of mammalian cells, where it is involved in control of many physiological processes, such as endocytosis, cell migration, cell signalling and surface ruffling. In an attempt to explain these functions of cholesterol, several models have been put forward about cholesterol's lateral and transbilayer organization in the PM. In this article, we review imaging techniques developed over the last two decades for assessing the distribution and dynamics of cholesterol in the PM of mammalian cells. Particular focus is on fluorescence techniques to study the lateral and inter-leaflet distribution of suitable cholesterol analogues in the PM of living cells. We describe also several methods for determining lateral cholesterol dynamics in the PM including fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS), single particle tracking (SPT) and spot variation FCS coupled to stimulated emission depletion (STED) microscopy. For proper interpretation of such measurements, we provide some background in probe photophysics and diffusion phenomena occurring in cell membranes. In particular, we show the equivalence of the reaction-diffusion approach, as used in FRAP and FCS, and continuous time random walk (CTRW) models, as often invoked in SPT studies. We also discuss mass spectrometry (MS) based imaging of cholesterol in the PM of fixed cells and compare this method with fluorescence imaging of sterols. We conclude that evidence from many experimental techniques converges towards a model of a homogeneous distribution of cholesterol with largely free and unhindered diffusion in both leaflets of the PM.

Słowa kluczowe

Cholesterol, Fluorescence, Diffusion, Membrane, Fluorescence recovery after photobleaching, Fluorescence correlation spectroscopy, Super-resolution, Single particle tracking, Mass spectrometry imaging, Flip-flop, Endocytosis

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