

Synthesis and live-cell imaging of fluorescent sterols for analysis of intracellular cholesterol transport.

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Cellular cholesterol homeostasis relies on precise control of the sterol content of organelle membranes. Obtaining insight into cholesterol trafficking pathways and kinetics by live-cell imaging relies on two conditions. First, one needs to develop suitable analogs that resemble cholesterol as closely as possible with respect to their biophysical and biochemical properties. Second, the cholesterol analogs should have good fluorescence properties. This interferes, however, often with the first requirement, such that the imaging instrumentation must be optimized to collect photons from suboptimal fluorophores, but good cholesterol mimics, such as the intrinsically fluorescent sterols, cholestatrienol (CTL) or dehydroergosterol (DHE). CTL differs from cholesterol only in having two additional double bonds in the ring system, which is why it is slightly fluorescent in the ultraviolet (UV). In the first part of this protocol, we describe how to synthesize and image CTL in living cells relative to caveolin, a structural component of caveolae. In the second part, we explain in detail how to perform time-lapse experiments of commercially available BODIPY-tagged cholesterol (TopFluor-cholesterol®; TF-Chol) in comparison to DHE. Finally, using two-photon time-lapse imaging data of TF-Chol, we demonstrate how to use our imaging toolbox SpatTrack for tracking sterol rich vesicles in living cells over time.

Słowa kluczowe

Cholesterol trafficking, Fluorescent sterols, Life-cell imaging, SpatTrack

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