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Novel lipid tools and probes for biological investigations

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We present the latest advances in lipid tool development for studying cellular membrane trafficking and metabolism. We focus on chemical modifications that are introduced to natural lipid structures. The new functionalities are used to follow and interfere with lipid dynamics in intact cells.

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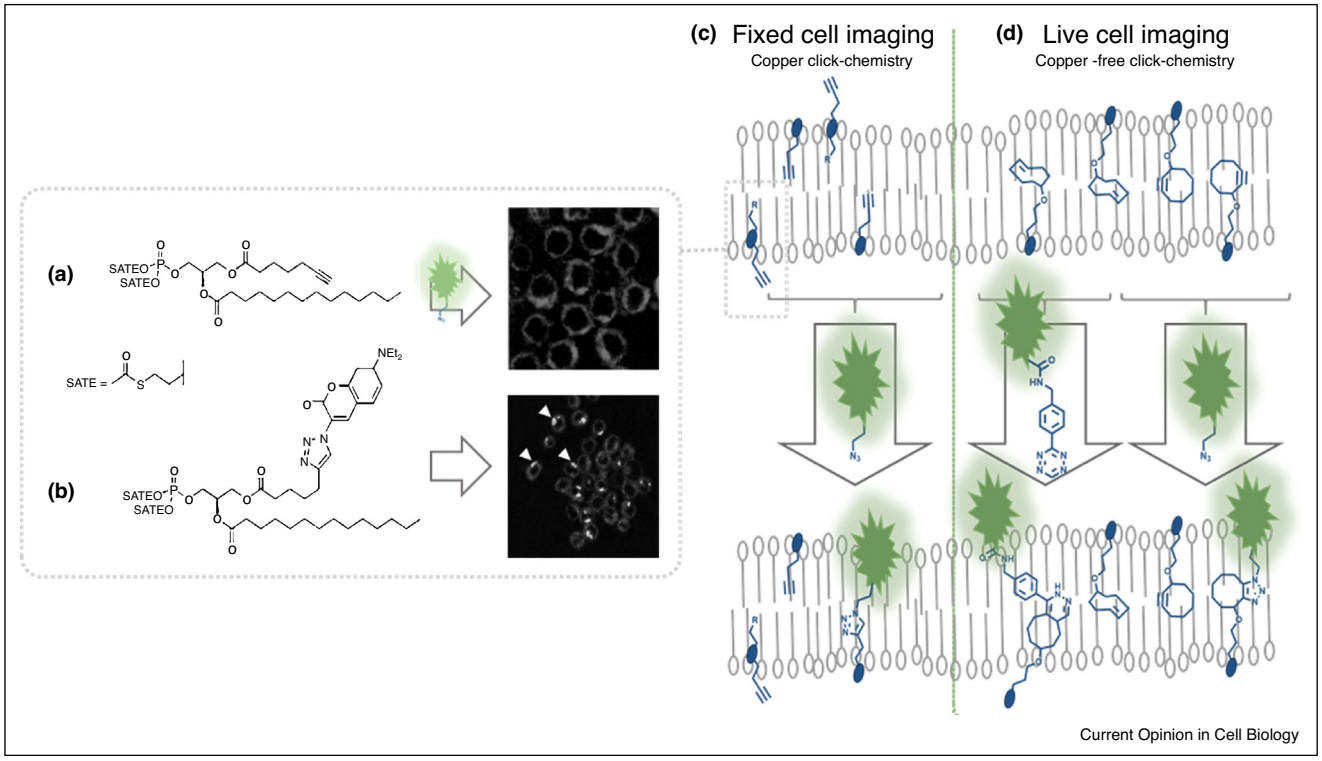
In a living cell hundreds of enzymes rigorously orchestrate the biosynthesis of thousands structurally different lipids [1]. The structural diversity of lipids is on a combination of head-groups and more or less unsaturated hydrocarbon moieties and carbon backbones. Interestingly, not only conjugated unsaturated double bonds but also aromatic groups are almost entirely excluded [2]. The high diversity of the lipid composition directly contributes to cell and organelles activities [3]. For instance, each organelle passing through the secretory pathway experiences major changes in their lipid composition. This affects bilayer thickness, lipid packing-density and surface charge [4,5] but also induces changes in signaling-relevant lipids such as phosphoinositides that determine the fate of the organelle [6]. Due to their intrinsic complexity, natural membrane systems are not easily manipulated without triggering important unnatural perturbations. Lipids and sterols play central roles in membrane plasticity and trafficking and the use of dynamic molecular tools is required to address lipid–lipid and lipid–protein interactions. Unlike nucleic acid structures or proteins, lipid derivatives cannot be genetically encoded. On the other hand, lipids are small molecules and when added to cells have a good chance of being incorporated into the natural membrane systems. Here, we discuss the recent development of novel lipid derivatives and probes for studying cellular membranes. From simple fluorescent lipids to complex tri-functional lipid probes, we report on how chemists and biologists have merged their efforts to provide a toolbox for better understanding lipid dynamics in cell biology.

Although manipulating a cellular membrane system with minimal invasion is a challenge, it is as difficult to study membranes in their natural constitution. For live cell experiments, fluorescence spectroscopy and imaging techniques allow for the least invasive observation of biological membranes, their manipulation, and their characteristic physical properties [7–9]. For this purpose, tagging at different positions of lipids (i.e. head-group labeled or acyl chain labeled) led to the preparation of numerous fluorescent lipid analogs [10–12]. A major limitation of this technique is caused by the volume occupied by the fluorophore compare to the lipid itself. The steric hindrance and the intrinsic properties of the label are responsible for a perturbation of the lipid properties that might influence its location, dynamics and molecular interactions. As mentioned above, lipids almost never have an aromatic group and fluorophores are intrinsically aromatic, thus leading to unpredictable and sometimes unusual location effects [13]. As an illustrative example, a recent comparative study described the biophysical properties of fluorescent cholesterol analogues whose structures featured a variety of tags located at different positions on the sterol scaffold [14]. The analogues demonstrated distinct and heterogeneous performances along different assays such as plasma membrane incorporation and in intracellular trafficking experiments. An aromatic and hence bulky fluorophore will likely induce a misconduct of the natural behavior of the lipid derivative (Figure 1a,b) and precautions need to be taken to avoid misinterpretation of experimental observations [15]. As discussed later, caged lipids constitute another striking example about how aromatic tags can dramatically affect the cellular location and the metabolism of the lipid (see below). This caveat of fluorescently labeled lipid probes for imaging requires alternative. One solution is provided by using functional groups that are not aromatic such as C–C triple bonds which may be used for attaching fluorophores (Figure 1c) in the intact cell via click chemistry [16–18]. Even better solutions seem to be methods that do not require any alteration of the lipid structures but detect endogenous lipid forms with high specificity. Indeed, there are antibodies available for some lipid species, for instance some of the phosphoinositides [19,20]. Another option is the detection of symmetric molecular vibrations of lipid double bonds by

Figure 1



Clickable lipid derivatives permit labeling and imaging of lipids and sterols in their original location after fixation and copper-catalyzed click chemistry **(a,c)**. If the pre-labeled lipid is added to cells, an entire different location is resumed suggesting that the aromatic fluorophore determines the location **(b)**. An advance is copper-free click chemistry, which is appropriate for live cell imaging of lipids **(d)**. *Trans*-cyclooctene-containing lipids spontaneously react with tetrazine-tagged dyes, while cytooctyne-bearing lipids will react with azido-fluorophores.

Raman spectroscopy [21–25]. Typically, hydrocarbon chain vibration of CH_2 and CH_3 groups and C–C and C–H stretching modes are observed. Thanks to the unique peak of the C–D bond (~2100 cm^{-1}) [26], the utilization of deuterium-labeled lipids contributed majorly to improve the specificity and the sensitivity of Raman spectroscopy for lipid identification [27]. However, while Raman imaging is an emerging discipline, it is still difficult to distinguish single lipid species unless a special molecular group (alkyne, azide) is introduced to the molecule of interest. Mass spectrometry imaging is another promising modality allowing for the detection and imaging of label-free lipids with high sensitivity, albeit with destruction of the sample. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) recently enabled measuring effects on the phospholipid composition by psychostimulants in the *Drosophila* brain [28] with a spatial resolution of 3 μM. Nanoscale SIMS (NanoSIMS) instruments provide even higher sensitivity and spatial sub-cellular resolution. Originally developed for material sciences, NanoSIMS recently allowed to image the distribution of stable isotope labels containing lipids with up to 50 nm spatial resolution [29]. The same group applied this modality to visualize cytosolic lipid droplets formed after administrating stable isotope-labeled fatty acid to mice [30]. However, this methodology necessitates complex sample preparations for biological materials. Indeed, NanoSIMS operates under ultra-high vacuum that requires the fixation and the removal of water of the samples before analysis.

Although Raman and mass-spectrometry imaging constitute two powerful methodologies to look at the intracellular distribution of lipids, other tools are needed to uncover lipid–protein interactions and lipid metabolism. This brings us back to chemically modified lipid derivatives. By using a sensible modification or indeed often a combination of modifications, it is possible to follow the dynamic of lipid metabolism and protein lipidation.

Clickable lipid derivatives

Alkyne-bearing derivatives can be efficiently used for the labeling and the imaging of lipids and sterols in a cellular environment. Synthetic alkyne-tagged PA analogs were one of the first tools that allowed imaging the dynamics of the lipid in both fixed and living cells. The alkyne tag can be a propargyl moiety that will react with an azido-fluorophore via copper-catalyzed click chemistry. A recent illustration of this technique was described by Jao *et al.* [31]. In this study, the authors reported the