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Chemical Tools for Lipid Cell Biology

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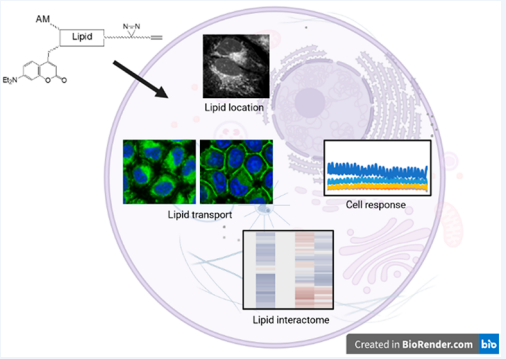
CONSPECTUS: Lipids are key components of all organisms. We are well educated in their use as fuel and their essential role to form membranes. We also know much about their biosynthesis and metabolism. We are also aware that most lipids have signaling character meaning that a change in their concentration or location constitutes a signal that helps a living cell to respond to changes in the environment or to fulfill its specific function ranging from secretion to cell division. What is much less understood is how lipids change location in cells over time and what other biomolecules they interact with at each stage of their lifetime. Due to the large number of often quite similar lipid species and the sometimes very short lifetime of signaling lipids, we need highly specific tools to manipulate and visualize lipids and lipid−protein interactions. If successfully applied, these tools provide fabulous opportunities for discovery.



In this Account, I summarize the development of synthetic tools from our lab that were designed to address crucial properties that allow them to function as tools in live cell experiments. Techniques to change the concentration of lipids by adding a small molecule or by light are described and complemented by examples of biological findings made when applying the tools. This ranges from chemical dimerizer-based systems to synthetic "caged" lipid derivatives. Furthermore, I discuss the problem of locating a lipid in an intact cell. Synthetic molecular probes are described that help to unravel the lipid location and to determine their binding proteins. These location studies require in-cell lipid tagging by click chemistry, photo-cross-linking to prevent further movement and the "caging" groups to avoid premature metabolism. The combination of these many technical features in a single tool allows for the analysis of not only lipid fluxes through metabolism but also lipid transport from one membrane to another as well as revealing the lipid interactome in a cell-dependent manner. This latter point is crucial because with these multifunctional tools in combination with lipidomics we can now address differences in healthy versus diseased cells and ultimately find the changes that are essential for disease development and new therapeutics that prevent these changes.

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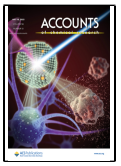

receptor internalization in the absence of stimuli and tyrosine phosphorylation. The receptor recycles back to the plasma membrane within 90 min.

I. INTRODUCTION

This Account begins with what cell biology demands from lipid tools rather than starting from a chemical, biochemical, or biophysical origin. For lipid structures and classes as well as key techniques to analyze lipids, I refer to the expert articles in this special issue and elsewhere.[5−8] Through extensive work over the past decades, we are quite well educated about the biosynthesis, metabolism, and typical location of lipids in cells. However, the cellular function of particular lipid species and their protein interactomes are largely elusive. For this reason, the community needs well designed lipid derivatives that can be used as discovery tools.

In an ideal experiment, we would be able to change the endogenous lipid composition noninvasively at will and at the same time monitor a large number of cellular events and molecules including the lipids themselves. This will be particularly useful if the target lipid has signaling function such that small changes of one lipid species have a significant cellular effect. For some studies, it might appear attractive to change the global lipid composition of the cell. In special biological conditions, this actually happens in a surprisingly dramatic fashion. For instance, after viral infections through COVID or flaviviruses, the host cell lipidome is massively altered to accommodate the viral replication centers.[9−11] However, manipulating the cells lipidome in a global way seems to be an overly drastic approach for a typical cell biology experiment, only rivalled by massively changing ATP or NADH levels. On the other hand, if a certain biophysical membrane property needs to be achieved, this might be the only possible way.

The visualization of lipids in cells is quite tricky. Unlike proteins, most lipids cannot be arrested by fixation. Therefore, additional techniques are required to prevent the lipid of interest from escaping this location. To make things worse, fluorophores often used to make molecules visible seem to drag lipids into the wrong membrane.[1,13,14] In addition, lipids seem to change location quite rapidly, and we therefore need fast and precise snapshots.

II. MANIPULATING CELLULAR LIPID LEVELS

Altering the concentration of a single lipid or the composition of a group of lipids in cells has been pursued to a limited extent by interfering with lipid biosynthesis and metabolism. A simple approach involves the use of specific enzyme inhibitors if they are available. Alternatively, biosynthetic enzymes can be switched by employing chemical dimerizers such as the well-known ternary FKBP−rapamycin−FRB system or one of the many alternatives.[15,16] Our own lab contributed a reversible chemical dimerizer system (rCD1) based on the interaction of a SNAP-tag with benzylguanine on one side and synthetic ligand of FKBP (SLF) with FKBP linked to the protein of interest on the other side (Figure 1).[12] After translocation of the lipid-modifying enzyme, the interaction could be reversed by adding FK506. With the help of rCD1, we were able to increase levels of the signaling lipid phosphatidylinositol 3,4,5-trisphosphate (PIP_3) at the plasma membrane for a limited amount of time and then study the dephosphorylation kinetics in intact cells once the kinase to make this lipid species was rapidly removed. This demonstrated that PTEN, the dominant PIP_3 phosphatase

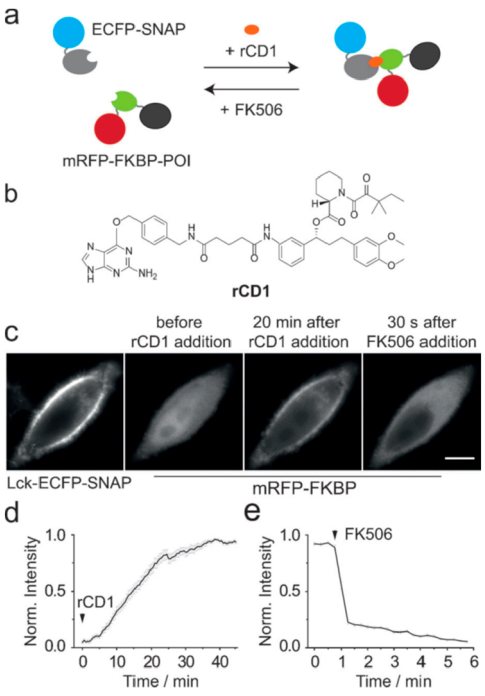

Figure 1. Mode of action of the reversible chemical dimerizer rCD1 (a). rCD1 (b) features benzylguanine to react with a SNAP tag and SLF which binds to an FKBP domain. The stronger binding of FK506 to FKBP outcompetes this interaction, rapidly altering the location of the protein of interest (POI). This works particularly well if the SNAP tag is anchored to the plasma membrane (c, d, e) or the nucleus. Data and images taken with permission from Feng et al.[12] Copyright 2014 Wiley-VCH.

in many cells, is a quite effective enzyme at the plasma membrane.[12] An even more elegant approach to change lipid concentrations can be achieved by using optogenetic tools such as the LOV2−ZDK1 system introduced by the Hahn group.[17] Although we know from preliminary experiments that this approach is useful for manipulating many lipid species, to date, optogenetics have only sparsely been applied to lipid cell biology. A prominent example from the De Camilli group uses optogenetics to switch phosphoinositide phosphatases and kinases on and off.[18]

A major requirement of lipid tools is that they need to provide a signal faster than the metabolism of the lipid of interest or the tool itself. For instance, if a lipid is added to cells and cell entry is slower than metabolism, there will be little effect observable unless the metabolite is active. This is also true for small molecules such as chemical dimerizers that are introduced to cells. In this respect, switches that are operated by light are much superior. It should be mentioned that the turnover rate of many lipids is much higher than expected. Concentrations of signaling lipids such as $PI(3,4,5)P_3$ or diacylglycerol can rise and fall within seconds rivalling signaling events such as calcium spikes. Even lipids that appear in bulk such as phosphatidylcholine species have a significant turnover rate that leads to a change in the fatty acid composition several times a day.[19] Tools that target single cells need to take these dynamic behaviors into account. In essence, one likes to be quick and subtle when changing intracellular lipid levels. Photoactivatable and photoswitchable tools are likely ideal in this respect, as we will see below.

An obvious way to change the lipid composition of a cell is to add the lipid. This works surprisingly poorly for signaling lipids.

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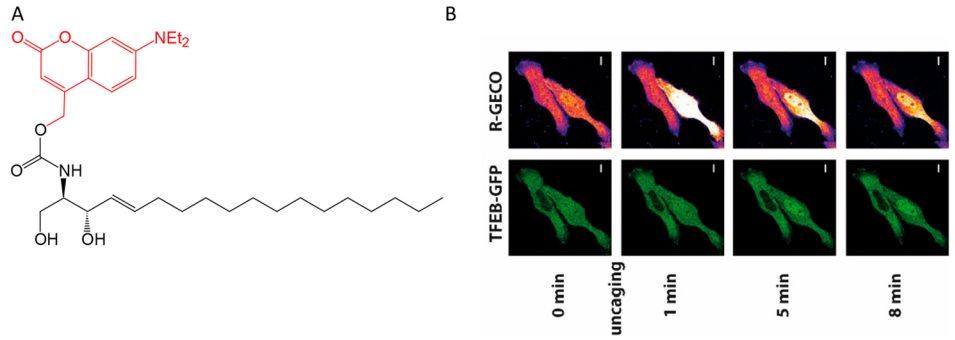

Figure 2. Structure of caged sphingosine with the photo-removable coumarin group in red (A). Intracellular calcium levels of HeLa cells expressing the red fluorescent calcium sensor R-GECO show a cell-specific calcium transient after uncaging of caged sphingosine in a small region of one (the right) cell for 3 s (B, upper panel). The lysosomal drop in calcium induced translocation of the transcription factor TFEB fused to GFP from the cytosol to the nucleus in the same cell (B, lower panel) while the other cell remained unchanged. Reproduced with permission from Höglinger et al.[3] Copyright 2015 eLife.

One reason is the high lipophilicity, which helps pass cell membranes but limits solubility in aqueous media. In some cases, nonionic detergents help to prevent precipitation; in others albumins were employed as carriers. Once the lipid enters the cell, the above-mentioned metabolism issue is prevalent for all lipids. A good example is sphingosine. In our hands, at low doses sphingosine was rapidly acylated to ceramide and no effect was observed. If the dose was increased, the ceramide levels became toxic. Therefore, a delivery system that ensures a lack of metabolism until the tool has entered the cell circumvents the above-mentioned problems. Our group used a caged version of sphingosine in which the caging group is a fluorescent diethylamino coumarin.[3] This coumarin is attached to the critical amino group of sphingosine via a carbamate group (Figure 2A) and not only prevents acylation to ceramide but also permits quantification of how much lipid derivative has entered the cell and in what membrane it ended up. By uncaging sphingosine in a subregion of the cell, we discovered that sphingosine is releasing calcium from the lysosome via a two-pore channel (Figure 2B). Surprisingly, a caged sphinganine derivative, which is just missing the double bond, was inactive. We further found that the drop in lysosomal calcium levels induced translocation of the transcription factor TFEB from the cytosol to the nucleus (Figure 2B), which subsequently led to the induction of autophagy.[3,20] This demonstrates that short lipid-based events can have lasting cellular effects. The use of caging groups will be detailed below after further tricks to deliver lipids to the cell's interior have been discussed.

Many lipids involved in signaling have charged groups. Negative charges of phosphate groups in particular prevent cell entrance. In this respect, phosphoinositides are the most notorious but phosphatidic acid and other phosphoglycerol lipid derivatives also share this feature. In the past, we developed a prodrug approach to mask the negative charges of phosphates by groups that are stable outside cells but subject to intracellular hydrolysis through endogenous hydrolases once the lipid derivative has entered cells.[21,22] We typically use acyloxymethyl esters because they do not add a large amount of lipophilicity and they keep the lipid derivatives quite soluble in the aqueous media of the cell dish. This technique also shifts the enzymatic hydrolysis step necessary to remove the protecting group away from the phosphate, as higher cells are not equipped with "phosphotriesterases". Alternatively, we also employed *S*-acetylthioethyl (SATE) groups.[14,23] SATE groups have the advantage that they are introduced together with the phosphate groups while acyloxymethyl esters are introduced through alkylation in the last synthetic step. The protected, uncharged molecules are usually soluble in toluene and extraction from the dried crude by this solvent provides a useful first cleaning step. AM esters are stable enough to be purified on reverse-phase HPLC columns but behave less favorably on silica. Our group synthesized many of the phosphoinositides that are involved in signaling events as AM ester derivatives including those of PI(3)P, PI(4,5)P_2, PI(3,4)P_2, and PI(3,4,5)P_3.[24−27] It should be mentioned that phosphates on the inositol ring might migrate during the enzymatic cleavage of AM esters.[28] For this reason, we added butyrates to mask the hydroxyl groups vicinal to the phosphates (Figure 3A). While this made the hydrolysis patterns even more complex, it prevented phosphate migration because the AM esters are hydrolyzed much more rapidly than the butyrates.[29] This being said, most of the butyrates need to be removed to restore biological activity. Cell entry of AM ester-covered lipids happens within a few minutes. However, the many enzymatic reaction steps plus the rapid metabolism of the biologically active lipid, i.e., PI(3)P or PI(3,4,5)P_3, still constitute a problem. A solution would be if we gave the cell more time to remove the bioactivatable protecting groups before metabolism sets in. For this reason, we turned to caged derivatives as caged lipids usually escape premature metabolism.

The synthesis of membrane-permeant caged phosphoinositides added major synthetic complexity compared to the noncaged derivatives as the caging should be located at a specific single phosphate. In addition, most caging groups are benzylic and hence reductive deprotection of protecting groups has to be avoided. As a result, we introduced fluorenylmethyl groups to the phosphates, and their removal under modestly basic conditions avoided the loss of caging groups from phosphates (see below for an example). Typical caging groups used for lipids are various nitrobenzyl groups or the above-mentioned 7-diethylamino-4-methylene-coumarin (DEAMC).[30] The advantages of the nitrobenzyl groups goes back to the pioneering experiments with caging groups by Trentham and colleagues and have been recently discussed in great detail.[31,32] Our group preferred the coumarin over the nitrobenzyl groups for various reasons. We wanted to reduce the impact of short wavelength light on cells, and DEAMC allowed us to use 405 nm blue light for uncaging. Most importantly, DEAMC is fluorescent and therefore cell entry and location of the lipid derivative can be followed by using very little excitation light. As a result, we were able to deliver the lipid derivative, give

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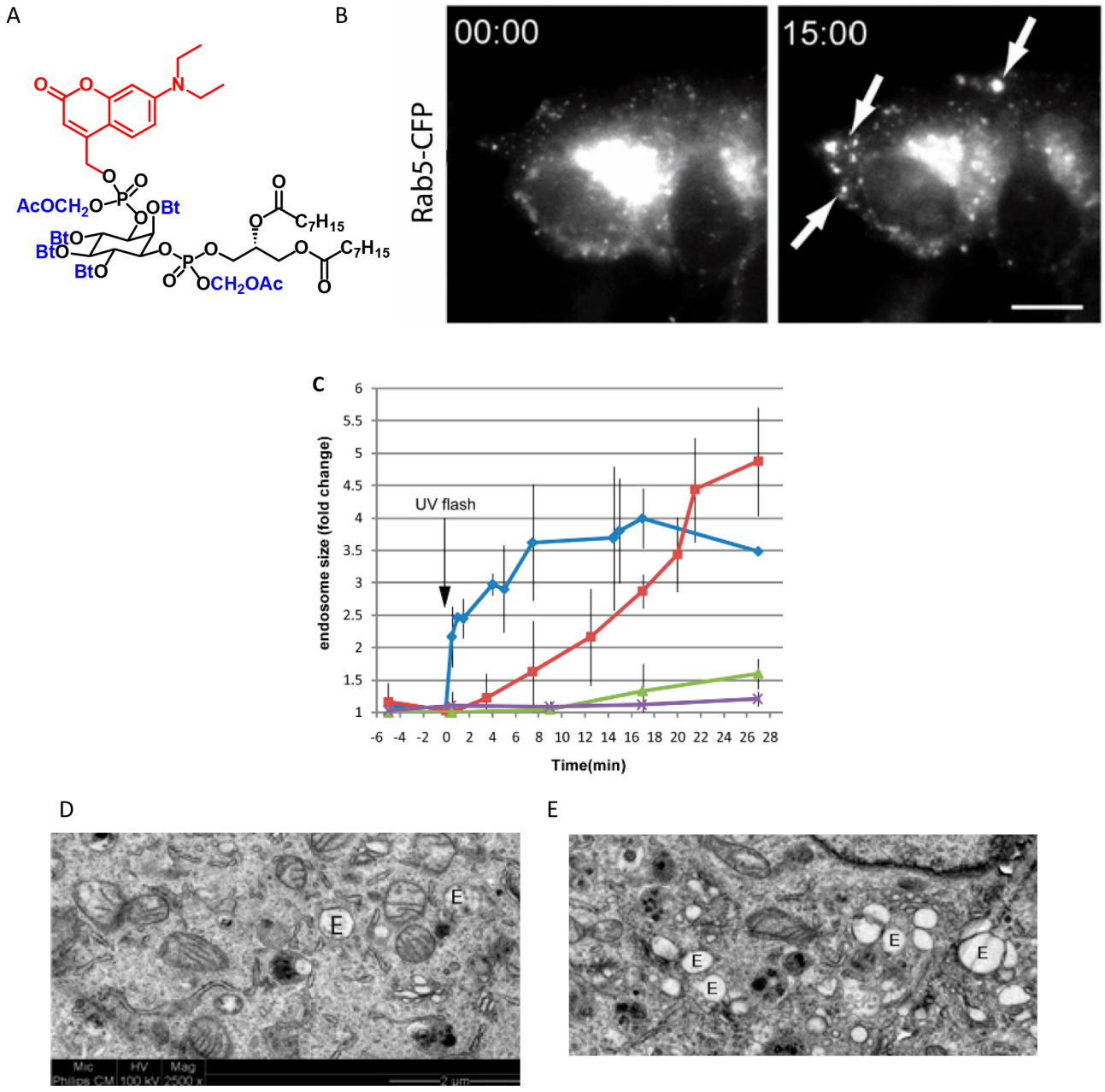

Figure 3. Structure of membrane-permeant, caged PI(3)P with the bioactivatable protecting groups in blue (A). Bioactivatable protecting groups (in blue) are removed by endogenous hydrolases. Incubation of HeLa cells with 100 μM membrane-permeant PI(3)P followed by uncaging led to rapid aggregation of Rab5-CFP-labeled endosomes within 15 min (B), much faster than through delivery of an uncaged PI(3)P derivative (C). Blue, caged PI(3)P; red, PI(3)P; green, PI(4)P; violet, UV only. EM micrographs confirmed endosome aggregation (D), E marks endosomes. Note that in panel E, there are many more endosomes than are marked. Endosomes are known to present PI(3)P-binding FYVE domains on their surface and the attached proteins are involved in endosomal fusion. Reproduced with permission from Subramanian et al.[26] Copyright 2010 Springer Nature.

the cell time to remove all protecting groups but the cage, and then start the experiment in the living cell with a short flash of light. The intrinsic fluorescence is not always beneficial, especially when fluorescent sensors are used that are also excited by blue light. Therefore, in some cases, we used nitroveratroyl cages to release lipids such as diacylglycerols.[33]

We usually preincubated cells with the caged lipids for 30 min to many hours. Even after long incubations, the caged lipids maintained their activity. A detailed metabolic study of a membrane-permeant and caged PIP_3 derivative showed that the AM esters were cleaved first, that butyrates needed up to 30 min to be hydrolyzed to a significant extent, and that the lipid lost some of its cage, potentially through phosphodiesterase activity.[29] In cell experiments, the latter does not seem to be a problem, likely because the sporadic release of an active lipid derivative is slower than its metabolism. This is, however, overcome by releasing the lipid by a flash of light. As is shown in the example of caged PI(3)P in Figure 3B, unleashing the lipid by light produces rapid aggregation of endosomes while the uncaged version needed more than 20 min to induce the same effect (Figure 3C).

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The introduction of caging groups not only complicates the synthesis, it also adds lipophilicity which sometimes requires the use of DMSO stock solutions and Pluronic F-127, especially when C_{16} or C_{18} fatty acids are included. It can be argued that these additives might compromise cell behavior and well-being. However, we always kept the final DMSO concentration at about 0.1% and used very little Pluronic. Then the cells were given quite some time, often overnight, to adjust to the new conditions before the flash of light started the experiment off. Not only did the long incubation times help the cells to adjust to the added chemicals, but the experiments also demonstrated that the caging groups successfully prevented the metabolism of a good portion of the exogenously added compounds.

In the meantime, we synthesized a large number of caged lipid derivatives other than phosphoinositides such as diacylglycerols,[33] sphinganine,[3] phosphatidic acid,[34] lyso-phosphatidic acid,[35] and quite recently the cannabinoid 2-arachidonyl glycerol (2-AG).[36] Other laboratories added many more caged lipid derivatives including anandamine,[37] caged ceramides,[38,39] and caged lipids that localized to particular cellular organelles and membranes via targeting groups.[40−43]

Design and synthesis of caged 2-AG was a tricky task because 2-AG is notorious for its migration of the acyl groups to the thermodynamically more stable *sn*1- or *sn*3-position. Further, a caging group on one of the hydroxyl groups would have generated a diacylglycerol derivative, which might have its own activity without uncaging. For this reason, we needed a group that covered both hydroxy groups but was removable in a single step. Success came by using an acetal that formed a 6-membered ring connected to the light-sensitive diethylamino coumarin. Interestingly, the removed coumarin was much less fluorescent. This way, the light impact and region of uncaging in a given cell could be easily followed.[36] With the help of caged 2-AG, we could demonstrate that cells use cannabinoids to stimulate sluggish β-cells and dampen the ones that are overly active.

III. DETECTING LIPID LOCATION

It is not trivial to monitor the effect of elevating or reducing the concentration of a lipid species in an intact cell. In some cases, there are dramatic outcomes as is shown above in the case of uncaging PI(3)P or sphingosine. Mostly, we are not really sure of what to look for. On the other hand, the ability to change one lipid concentration provides great opportunities for discovery, as will be discussed below. Generally, the most immediate readout is the translocation of a fluorescently labeled lipid binding domain from the cytosol to a given membrane. This imaging tool has been used many times to monitor appearance and disappearance of lipids through endogenous or induced enzymatic activity.[44−46] Our own group employed this technique several times. For instance, we employed the PH domain of Akt fused to EGFP (EGFP-PH_{akt}), to follow the activation of PI 3-kinase via the reversible chemical dimerizer rCD1, as mentioned above. Formation of PIP_3 led to a translocation of EGFP-PH_{akt} from the cytosol to the plasma membrane. Reversal of the interaction by FK506 immediately released the fluorescent domain.[12] Similarly, we used a C1 domain or protein kinase C fused to GFP (PKCα-GFP) to determine the effect of different fatty acid compositions on protein kinase translocation and its effect on opening calcium channels in the plasma membrane.[33] We found that local uncaging of the lipid induced a local increase of PKC but also a global calcium wave through the cell. However, the local combination of PKC activation and calcium influx at the uncaging site also generated a lasting local calcium signal.

The above examples demonstrate that the use of lipid tools can give otherwise difficult to achieve insight into cellular events. The thrill of using these tools comes from experiments that show an unusual outcome and lead to true discovery or new techniques in cell biology. The above-mentioned discovery of sphingosine-induced calcium release from lysosomes is one of these findings.

Another good example involved the PIP_3-induced internalization of growth factor receptors such as epidermal growth factor receptor (EGFR) or related receptor tyrosine kinases (RTKs) by following fluorescently tagged versions of the receptors. We found that when we uncaged PI(3,4,5)P_3 or PI(3,4)P_2 but not any other caged phosphoinositide, we generated a sufficient signal for receptor internalization in the absence of any kind of ligand, tyrosine phosphorylation, or receptor dimerization.[4] Interestingly, the internalized receptors recycled back to the plasma membrane within 60 to 90 min, likely because a lack of tyrosine phosphorylation prevented ubiquitination, a prerequisite to enter the endolysosomal path.[47] This phenomenon is restricted to RTKs and could be mimicked by inhibiting the lipid phosphatases PTEN and SHIP, which led to chronically increased PIP_3 levels. We believe that PI(3,4,5)P_3-induced RTK internalization is relevant in cell regions such as the growth cone of an axon or late stage cancer cells where receptor internalization and subsequent lysosomal destruction is not helping the cell to achieve its goal.

A third more technical example showed that uncaging a chemotactic lipid in the extracellular space made cells move. We synthesized caged lyso-phosphatidic acid (LPA), which is modestly soluble in an aqueous environment.[35] We uncaged by a train of 405 nm light flashes every 40 s for several hours at one small spot within a dish of migratory cancer cells known to express the LPA receptor. We then observed that these cells migrated to the spot where the illumination happened.

These discoveries and techniques strongly relied on the use of caged lipid derivatives. It should be mentioned that caged compounds are one of several groups of switching tools. A suitable alternative for studying lipids in cells and organisms are photoswitchable lipid derivatives.[48,49] These are mostly based on diazobenzene derivatives which transition from a *trans* to a *cis* conformation upon irradiation with long UV light and back to *trans* thermally or after illumination with visible light. Work with these lipid derivatives has been pioneered by the Trauner group. In collaborative efforts between our two laboratories, we contributed findings on how lipids regulate insulin secretion from pancreatic β-cells. Namely, we found that diacylglycerol limited the function of inward rectifying potassium channels, a major contributor to the calcium oscillations that ultimately drive insulin granule fusion with the plasma membrane.[50] Photoswitchable derivatives of fatty acids helped to study signaling and secretion via the β-cell fatty acid receptor GPR40.[51]

The value of caged and photoswitchable lipid derivatives is particularly high when lipid signaling is investigated in single cells. In addition, protection from premature metabolism by caging groups is also useful in other lipid tools. In the following, I am referring to molecules that allow determining the location and the interactome of a given lipid in cells, two notoriously difficult tasks. We have recently published numerous review and perspective articles on this topic.[52−54] In brief, lipid location is greatly influenced by artificially added aromatic moieties such as

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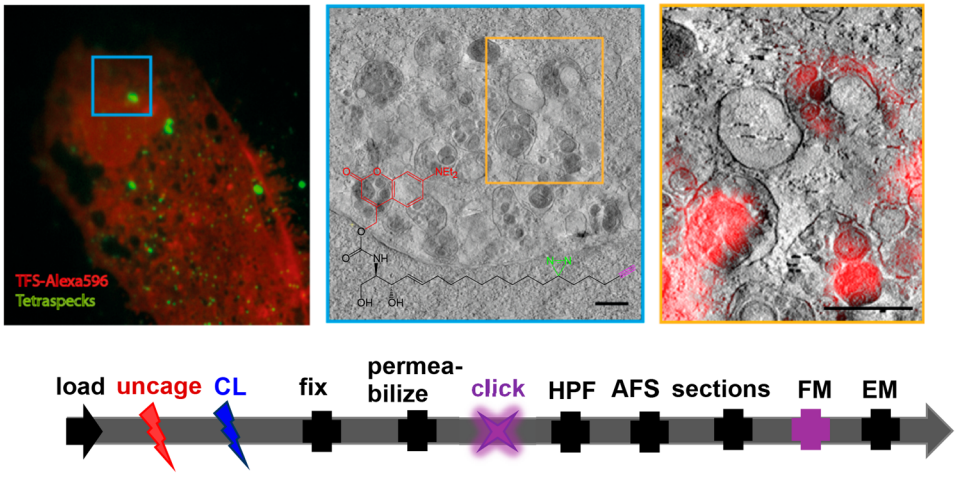

Figure 4. (top) Use of a multifunctional sphingosine (Sph) derivative (see middle panel, added functional groups color-coded) demonstrated the Sph location in late multivesicular endosomes of fibroblasts from a Niemann−Pick type C patient, as was determined by correlative light and electron microscopy. Photo-cross-linking preserved lipid localization; labeling was achieved with Alexa596 azide; thin sections were cut after high-pressure freezing and Lowricryl embedding. Tetraspecks are electron-dense fluorescent beads used to correlate locations in the light and electron micrographs. Scale bars are 500 nm. Partly reproduced with permission from Höglinger et al.[2] Copyright 2017 National Academy of Sciences. (bottom) Workflow for preparing CLEM samples with multifunctional lipids which involves orthogonal uncaging at 400 nm and photo-cross-linking of lipids (CL) at 350 nm, fluorescent labeling (click), high-pressure freezing (HPF), and vitrification (AFS) before the sample is sliced (300 nm) and investigated by fluorescence (FM) and finally electron microscopy (EM).

fluorophores. While fluorescently labeled lipid derivatives are very useful for *in vitro* applications,[55] we were seeking a method to label lipids at their respective cellular location. Based on initial work by Savic, Best, Thiele, and others, this was achieved by using click chemistry.[56−61] We included a variety with strained alkynes that did not require copper ion catalysis for successful labeling via click chemistry.[14] Because diffusion of lipids including labeled lipids can be very fast, a method was needed to fix the lipid location at a given point in time. Therefore, we and others synthesized lipid derivatives that featured an alkyne group as well as a diazirine, which is small and nonaromatic and can efficiently be photo-cross-linked with 350 nm light.[62] A good starting point were bifunctional lipid derivatives of fatty acids, cholesterol,[63] and later sphingosine.[3,64−66] The fatty acid tools were partly used to metabolically label major glycerolipids, especially phosphatidylcholine.[66] Bi- and trifunctional lipid derivatives are very useful for localizing the lipid derivative after fixation by tagging the molecule with a fluorophore via click chemistry. The method circumvents the cell localization problems that permanently fluorescently tagged lipid derivatives show. The largest advantage is that the lipid location can be determined with very high precision as is shown in Figure 4. The resolution limit here is the point-spread function of the light emitted from the fluorophore. In the shown case, a multifunctional sphingosine derivative was uncaged, photo-cross-linked, and labeled in fibroblasts from a Niemann−Pick type C patient.[2] Uncaging did not lead to photo-cross-linking. The fixed cells were then analyzed by correlative light and electron microscopy (CLEM) and the sphingosine location in late endosomes/early lysosomes, which is typical for this genetic lipid storage disease, was confirmed (Figure 4).

IV. DETECTING LIPID INTERACTOMES

The second application is the determination of lipid interactomes after photo-cross-linking (see below),[1,2,63] which often involves tagging with biotin by copper-catalyzed click chemistry and extraction via streptavidin beads. To avoid unspecific background, we frequently attach the photo-cross-linked lipid−protein conjugates directly to azide-bearing beads. After rigorous washing and proteolytic degradation, proteomic analysis reveals the lipid binding proteins.

Lipid photo-cross-linking was even successful in structural biology experiments. When nascent HIV particles were investigated by cryo-electron microscopy and particle averaging, the location of phosphatidylinositol 4,5-bisphosphate could be determined after photo-cross-linking a bifunctional lipid derivative.[67] As discussed above, lipids are rapidly metabolized in cells. Therefore, the addition of a third functionality, namely, a caging group, will prevent premature turnover and gives us full control over the metabolism.

We synthesized trifunctional derivatives bearing a cage, a diazirine, and an alkyne group of many lipids such as fatty acids, sphingosines, diacylglycerol, and several phosphoinositides.[1,2,13] While all derivatives are useful to determine their respective interactomes in a given cell type, the molecules also allow us to follow lipid transport in cells when rapid pulse-chase type experiments are performed. The principle is based on the fact that the aromatic caging group will inevitably draw the lipid derivative into endomembranes, mostly of the endoplasmic reticulum (ER) and the Golgi. This seems to be largely independent of the lipid headgroup. After uncaging, we observed a rapid transfer to the plasma membrane for phosphatidylinositol (PI) and higher phosphorylated PIs within less than a minute.[1,13] For phosphatidylinositol, proteomic analysis confirmed the well described PI transport proteins PITPA and PITPB.[13] When investigating the potential transport of PI(3,4,5)P_3 and PI(3,4)P_2, we employed the membrane-permeant trifunctional phosphoinositide derivatives to determine the lipid interactome in HeLa cells.

The synthesis of the trifunctional PI(3,4,5)P_3 derivative is shown in Figure 5A. Then we picked candidate proteins from the interactomes that were known to bind lipids and knocked them down by siRNA. Surprisingly, the knockdown of the lipid binding protein MPP6 and the plasma membrane flippase

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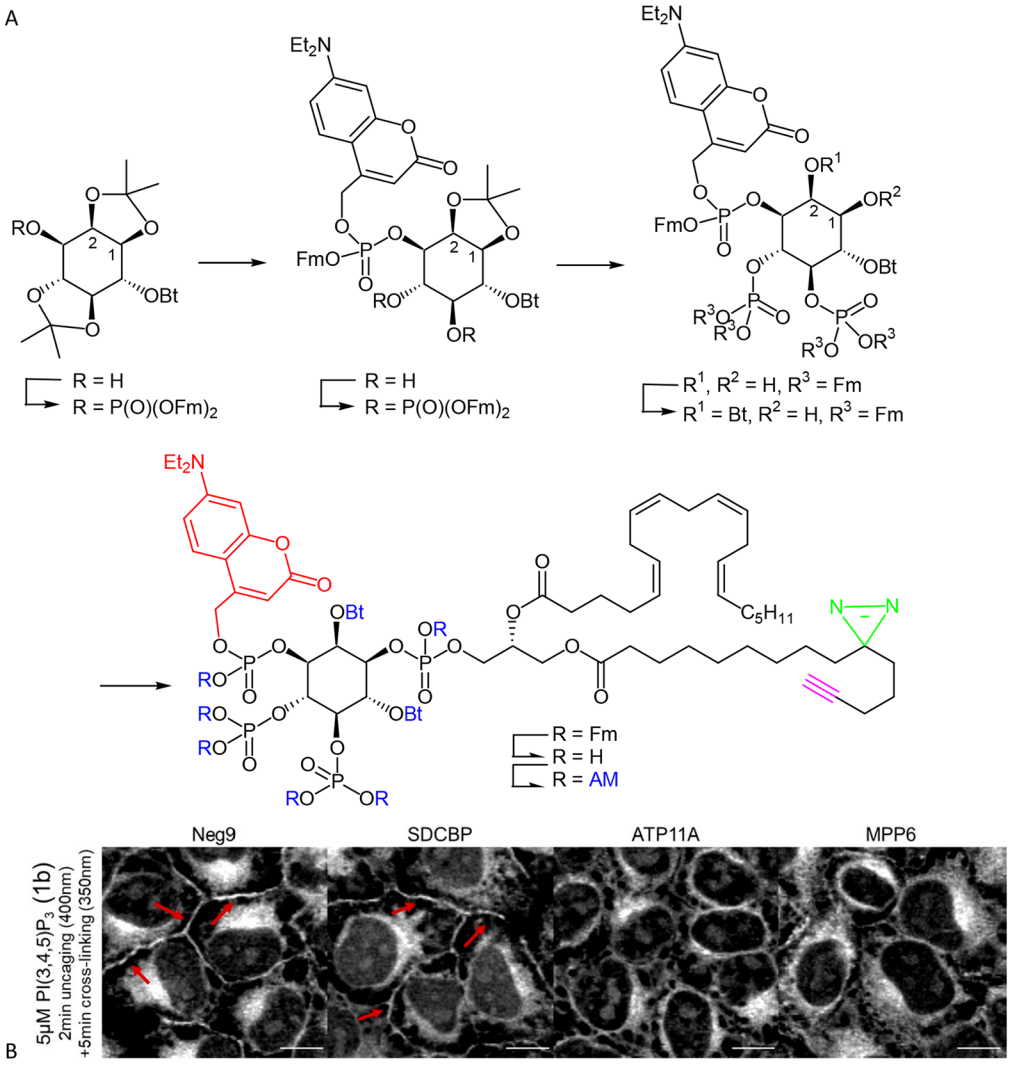

Figure 5. Synthesis of a membrane-permeant trifunctional $PI(3,4,5)P_3$ derivative. Functional additions are color-coded (A). Note that an enantiopure starting material is commercially available. Fm = fluorenylmethyl; Bt = butyryl; AM = acetoxymethyl. All synthetic details can be found in Mentel et al.[25] and Müller et al.[1] Location of uncaged, photo-cross-linked, and fluorescently tagged $PI(3,4,5)P_3$ in fixed cells previously transfected with an RNAi against putative lipid transport proteins (B). Reproduced with permission from Müller et al.[1] Copyright 2021 Wiley-VCH. Neg9 = random siRNA control. The knock-down of ATP11A and MPP6, but not SDCBP, led to persisting residence times of $PI(3,4,5)P_3$ in endomembranes, suggesting that these proteins are actively involved in phosphoinositide transport to the plasma membrane. Scale bar = 10 μm.

ATP11A blocked the transport of $PI(3,4,5)P_3$ from ER membranes to the plasma membrane (Figure 5B).[1] This observation suggested that higher phosphorylated lipids are frequently trafficked between the various cell membranes and might also explain why PIP_2 and $PI(3,4,5)P_3$ are rarely found as part of endomembranes. However, this finding raises many questions, namely, which lipids are transported, are they transported at membrane contact sites as is well described for PI(4)P and the ORP5/8 proteins[68] or through the cytosol, and are plasma membrane-bound lipid binding proteins such as ATP11A needed to incorporate lipids into the receiving membrane.

V. TRANSLATIONAL OUTLOOK

As shown above, multifunctionalized lipid derivatives are quite useful discovery tools. It might be speculated that they also have substantial translational value. Because they are able to unravel lipid interactomes, they provide means to compare interactomes of healthy versus diseased cells. For instance, it is well-known that virus infections, especially by RNA-based viruses such as Zika, Dengue, hepatitis C, and SARS-CoV2, require major membrane remodelling to provide sites for efficient virus replication and egress.[11,69,70] Our collaborators Fikadu Tafesse at OHSU and Jennifer Kyle from PNNL recently determined the lipidomes of SARS-CoV2 infected lung cells and found major differences in the lipid composition of host cells when compared to healthy cells.[9] Indeed, inhibition of key enzymes in lipid metabolism such as fatty acid biosynthesis largely reduced virus replication. While determining the lipid interactomes in virus-infected cells is work in progress,[54] it will be very interesting to perform similar experiments in different types of cells from diseases such as bacterial infections, cancer, or genetic diseases. More difficult will be applications in bacteria as the bacterial lipidomes are usually quite different from mammalian ones. This would require the synthesis of a very specialized toolset. From a translational point of view, the comparison of lipid interactomes in healthy and disease cells will provide new starting points for therapeutic campaigns.

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VI. CONCLUDING THOUGHTS

Chemical biology and its unique lipid tools will have a large impact on our future understanding of the lipid world in cell biology and disease. We will much better follow lipid locations in cells, especially when time-resolved light microscopy is applied or when optical imaging is combined with cryo-electron tomography. The latter technique will provide 3-dimensional images of lipid localization in a fairly undistorted fashion and with high organelle-specific information. By using cross-linking techniques, we will learn about the proteins that bind to lipids. The latter data sets will hopefully end up in databases making the information available to the entire community. These resources will bring the available knowledge base on lipids much closer to what we are used to at the protein level. However, for a more complete understanding of lipid biology, we need to address the high dynamics of lipid biochemistry in intact cells. This means that we will need more fluorescent sensors to help monitor lipid turnover rates and highly specific inhibitors and activators of key enzymes that drive lipid metabolism to manipulate the cellular equilibrium. Lipid chemical biology at the cellular level is starting to live up to the demands, and we will witness unravelling of lipid cell biology, a very complicated systems biology problem.

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Notes

The author declares no competing financial interest.

Biography

Carsten Schultz studied Chemistry at the University of Bremen, Germany. After his Ph.D. on inositol phosphates under the supervision of Bernd Jastorff, he moved to a postdoctoral position with Roger Y. Tsien at UC San Diego, USA, where he developed methods to deliver highly charged molecules to intact cells noninvasively. After his return to Germany, he worked independently towards his habilitation. After a short stay at the MPI for Molecular Physiology in Dortmund, Carsten was appointed as a group leader to the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, where he installed a Chemical Biology program focused on cell biology and signal transduction. He became a Senior Scientist with tenure in 2008. In the past 20 years, his research interests were majorly drawn toward the chemical biology of lipids, protein labeling techniques, and fluorescent sensors and reporters. In 2016, he was appointed the Helen Jo Whitsell Professor for Basic Science and Chair of the Department of Physiology & Pharmacology at Oregon Health and Science University in Portland, OR, USA. His engagement at EMBL ended in 2019. In 2019, the Departments of Physiology & Pharmacology and Biochemistry & Molecular Biology merged to form the Department of Chemical Physiology and Biochemistry in which Chemical Biology, Physiology, Biochemistry and Structural Biology are combined under one roof.

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