Title: Synthesis and cellular labeling of multifunctional phosphatidylinositol bis- and trisphosphate derivatives

Authors: Rainer Mueller, Ana Kojic, Mevlut Citir, and Carsten Schultz

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: Angew. Chem. Int. Ed. 10.1002/anie.202103599

Link to VoR: https://doi.org/10.1002/anie.202103599
Synthesis and cellular labeling of multifunctional phosphatidylinositol bis- and trisphosphate derivatives

Rainer Müller,1† Ana Kojic,1,2† Mevlut Citir,1,2 Carsten Schultz1,3*

1 European Molecular Biology Laboratory (EMBL), Cell Biology & Biophysics Unit, Meyerhofstr. 1, 69117 Heidelberg, Germany
2 Faculty of Biosciences, Collaboration for Joint Ph.D. Degree between EMBL and Heidelberg University, 69117 Heidelberg, Germany
3 Oregon Health & Science University, Department of Chemical Physiology and Biochemistry, 3181 SW Sam Jackson Park Rd, Portland, OR 97239-3098, USA

†These authors contributed equally. Corresponding author: C. Schultz, schulcar@ohsu.edu

Abstract – We synthesized the first multifunctionalized phosphoinositide polyphosphate derivatives featuring a photo-removable protecting group (“cage”), a photo-crosslinkable diazirine group and a terminal alkyne group useful for click chemistry. We demonstrate that the lipid derivatives readily enter cells. After photo-crosslinking, cell fixation and fluorescent tagging via click chemistry, we determined the intracellular location of the lipid derivatives before and after uncaging of the lipids. We find that there is rapid trafficking of PI(3,4)P₂ and PI(3,4,5)P₃ derivatives to the plasma membrane, opening the intriguing possibility that there is active transport of these lipids involved. We employed the photo-crosslinking and click chemistry functions to analyze the proteome of PI(3,4,5)P₃-binding proteins. From the latter, we validated by RNAi that the putative lipid binding proteins ATP11A and MPP6 are involved in the transport of PI(3,4,5)P₃ to the plasma membrane.

Introduction:

Phosphoinositides are important signaling lipids that contribute to the regulation of many cellular processes including survival, migration, secretion, and growth.1 The quite abundant phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] (ca. 0.1% of all lipids) was the first phosphoinositide to be recognized as a signaling lipid.2 The concomitant discovery of phosphatidylinositol 3-kinases (PI3Ks) and phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P₃] in the late 1980ies opened a new field in lipid signaling.3 Phosphatidylinositol 3,4-bisphosphate [PI(3,4)P₂] was discovered to be a metabolite of PI(3,4,5)P₃ that, however, is now known to trigger its own set of responses.4 Because it attracts pleckstrin homology (PH) domain-bearing lipid binding proteins to the plasma membrane similar to PI(3,4,5)P₃, the signaling pattern is difficult to distinguish. For instance, both lipids are a sufficient signal for internalization of growth factor receptors, although PI(3,4)P₂ was much less potent than PI(3,4,5)P₃.5 However, unlike PI(3,4,5)P₃, PI(3,4)P₂ was shown to be involved in the regulation of endosomal trafficking.4, 6

3-O-phosphorylated phosphoinositide concentrations are only about 0.1% of that of PI(4,5)P₂ at resting level and are recognized by specific protein domains. Due to the low abundance, it is difficult to determine concentrations of 3-O-phosphorylated PIPs such as PI(3,4,5)P₃, PI(3,4)P₂, and PI(3)P. Typically, fluorescent domain fusion proteins are used to estimate the phosphoinositide levels in intact cells.7 In cell batches, mass spectrometry is now sensitive enough to detect low levels of these highly charged lipids. However, these techniques come with caveats. For instance, pleckstrin homology (PH) domains do not distinguish well between PI(3,4,5)P₃ and PI(3,4)P₂.8 and mass spectrometry can distinguish between the isomers PI(4,5)P₂ and PI(3,4)P₂ only with difficulties.9 Even more challenging is the discovery of specific binding proteins of phosphoinositides. We therefore looked for tools that will help to...
locate the phosphoinositide of interest with high specificity in cells and 2) will at the same time permit finding binding proteins.

We decided to expand our panel of multi-functionalized lipid derivatives to the highly phosphorylated phosphoinositides. In the past, we and others equipped lipids such as cholesterol, sphingosine and diacylglycerol (DAG) with a photo-crosslinking diazirines as well as a terminal alkyne group for click chemistry.[10] Very recently, we equipped phosphatidylinositol with all of the named functional groups.[11] In a typical experiment, cells were incubated with the lipid and illuminated with 350 nm light for crosslinking.[12] Next, cells were fixed and subsequently a fluorophore was attached via click chemistry.[10a, 10e] Due to the protein-crosslinking, the lipid derivative stayed in place providing a precise location map at the time of crosslinking by fluorescence imaging. Alternatively, we attached biotin to the lipid and lipid-protein conjugates were isolated with streptavidine beads, washed and subjected to proteomic analysis.[10b]

As many lipids experience rapid metabolism inside cells, we equipped each lipid derivative with a photo-activatable protecting group, namely a 7-diethylamino-4-methylene coumarin, which is readily removable with 405 nm light.[13] This functional modification gives us excellent access to rapidly elevating the concentration of the lipid species of interest as was shown for many other lipids while preventing premature metabolism.[14] In the past, our group prepared numerous caged phosphoinositides.[11, 14b, 15] In this work, we will combine photoactivation with photo-crosslinking and fluorescent tagging. We previously demonstrated that 405 nm light is unable to induce significant crosslinking of a diazirine, making the two photochemical reactions subsequently applicable.[12] Unlike many other lipids, most phosphoinositides carry numerous phosphates with negative charges which make cell entry impossible. We therefore needed to apply bioactivatable protecting groups such as acetoxyethyl esters that are easily and rapidly removable by endogenous esterases inside cells.[16]

In this work, we present the synthesis of truly multifunctional phosphoinositide derivatives with all the above named features combined in one molecular tool (Figure 1). We show that the derivatives are biologically fully functional, for instance by inducing growth factor receptor endocytosis after photoactivation. We demonstrate not only spatial lipid distribution but also perform pulse-chase experiments by using rapid photoactivation.

Figure 1. Structures of multifunctionalized PI(3,4)P₂ (1a, 1c) and PI(3,4,5)P₃ (1b, 1d) derivatives featuring both a diazirine and an alkyne group on the sn1 fatty acid as well as a photo-activatable coumarin group (cage) on the 3-O-phosphate (1a, 1b). AM = acetoxyethyl, Bt = butyrate.
Results: The synthetic strategy for preparing multifunctionalized phosphoinositide derivatives was exemplified in our recent synthesis of trifunctional phosphatidylinositol.\(^{[11]}\) In brief, the photo-crosslinking diazirine and the alkyne group are both located in one of the two fatty acids. In order to be close to a natural fatty acid composition, we placed the functional groups on the saturated sn1 fatty acid and kept the arachidonic acid in the sn2 position unaltered. The resulting diacylglycerol (DAG) derivative is coupled to a n already caged head group. In the case of PI(3,4,5)P\(_3\), this head group featured a 7-diethylamino-4-methylene coumarin photoactivatable group at the functionally crucial 3-O-phosphate of the inositol moiety and was previously used for caging phosphoinositides.\(^{[11, 15]}\) In this work, we used a slightly improved synthetic procedure for the synthesis of the phosphoinositide head group that included the use of fluorenlylmethyl (Fm) groups for protecting the phosphates. Detailed synthetic procedures (Scheme S1) are described in the supplement. Head group and DAG moiety are coupled to form the fully protected lipid derivative by using an Fm-bearing phosphoramidite that was first reacted with the DAG and subsequently with the inositol head group. After oxidation to the phosphate triester, all phosphate protecting groups were gently removed and alkylation with acetoxyethyl bromide afforded the membrane-permeant bioactivatable lipid derivative.

For the synthesis of a trifunctional PI(3,4)P\(_2\) derivative, the head group synthesis (Scheme 1) required a different synthetic path than for previously non-caged PI(3,4)P\(_2\) derivatives,\(^{[17]}\) in order to regioselectively introducing the photoactivatable coumarin moiety to the 3-phosphate. We started from the commercially available enantiopure 2,3:5,6-diisopropylidene-myoinositol 2. Protection of the sterically less restricted 1-hydroxyl group by the bulky TBDPS group afforded alcohol 3. The single alcohol was protected to give the p-methoxybenzyl ether 4. The more labile ketal was removed and the two hydroxyl groups of 5 were esterified with butyric anhydride to give dibutyrate 6. The PMB group was removed and alcohol 7 was phosphorylated using the known di(fluorenlylmethyl)phosphoramidite 8 to afford 9. Removal of the ketal liberated the 2- and 3-hydroxyl groups (compound 10). The thermodynamically less stable 2-OH group was regioselectively esterified using a butyrate orthoester and selective acid-catalyzed ring-opening using Dowex in its protonated form. The resulting tributyrate 11 was subsequently phosphitylated with the known coumarin-bearing P(III) reagent 12 and oxidized to the bisphosphate triester 13. Finally, the TBDPS group was removed using 3HF\(\cdot\)NEt\(_3\). This latter reaction was tricky because the coumarin group on the phosphate is an excellent leaving group. Accordingly, we isolated the head group 14 in only 23% yield (total yield from 2: 8.3%). The synthesis of the corresponding head group for PIP\(_3\) derivatives was performed similar to our previously described path,\(^{[15a]}\) Improvements and additional details we describe in the supplement (Scheme S1).
Scheme 1. Synthesis of the caged PI(3,4)P₂ head group. Reagents and conditions: a) TBDPS-Cl, imidazole, pyridine, -10°C (25); b) PMB-O(C=NH)CCl₃, EtN/Pr₂, N,N-dimethylformamide, 22°C, 18 h; c) CH₃Cl:HCOOH 4:1, 22°C, 2 h, 70% (plus 30% recovery of starting material); d) BtCl₂, disopropylcarbodiimide, 4-dimethylaminopyridine, CH₂Cl₂, 22°C, 3 h, 87%; e) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; f) 1Pr₂NP(OFm)₂ (8), 1H-tetrazole, CH₂Cl₂, 22°C, 8 h, 2 AcO₂H/AcOH, -78-22°C, 1 h, 90% yield over two steps; g) CH₃Cl:HCOOH 5:95, 22 °C, 2 h, 100%; h) 1. n-C₃H₇:O(OMe)₂, poly(4-vinylpyridinium) trifluoroacetate, CH₂Cl₂, 22°C, 18 h; 2. DOWEX H⁺, MeCN, H₂O, 100% yield over two steps; i) 1Pr₂NP(OFm)(OCoum) (12), 1H-tetrazole, CH₂Cl₂, 22°C, 8 h, 2 AcO₂H/AcOH, -78-22°C, 1 h, 90% yield over two steps; j) 3HF*NEt₃, MeCN, 18 h, 55%. Bt=C₆H₅:CO₂⁻; PMB=pMeOC₆H₄CH₂⁺; TBDPS=SiPh₂Bu.

Scheme 2. Coupling of building blocks 14/15 and 17 by P(III) chemistry, followed by deprotection and alkylation of the phosphates with acetoxyethyl bromide. Reagents and conditions: a) for 1a and 1c: a) 14, 17, 1H-tetrazole, CH₂Cl₂, 23°C, 5 h; 2. AcO₂H/AcOH, -78-23°C, 1 h, 86% over two steps; b) MesNET, MeCN, 23°C, 0.5 h; c) AcOCH₂Br, iPr₂NET, MeCN, 23°C, 21 h, 50% for 1a and 24% for 1c over two steps. A for 1b: a) 15, 17, 1H-tetrazole, CH₂Cl₂, 23°C, 22 h; 2. AcO₂H/AcOH, -78-22°C, 1 h, 50% over two steps; b) MesNET, MeCN, 21°C, 10
Diaclyglycerol 16 was reacted to the Fm-protected phosphoramidite 17, as described previously (Scheme 2B). 11 Subsequently the inositol headgroups 14 and 15 were coupled to the lipid reagent 17, to give the fully protected PI(3,4)P₂ (18a) and PI(3,4,5)P₃ (18b) derivatives, respectively. Deprotection of the Fmoc and Fm groups in one step afforded the desired caged lipids 19a and 19b. Both were alkylated with acetoxymethyl bromide to give the bioactivatable caged lipid derivatives 1a and 1b, respectively. In the case of PI(3,4)P₂ deprotection, a fraction of the molecules lost the coumarin group. The following alkylation with acetoxymethyl bromide produced the PI(3,4)P₂ derivative 1c without caging group but membrane-permeant and with the diazirine and the alkyne in the sn1 fatty acid. The synthesis of the respective uncaged PI(3,4,5)P₃ derivative 1d is described in the supplementary material (Scheme S1).

The caged phosphoinositide derivatives were tested for cell entry and removal of the photoactivatable protecting group (“uncaging”). The latter was achieved by illuminating a 10 mM sample (1 nmol in total) of 1a or 1b (Figure 2), for 2 min in a test tube by a 1000W lamp (Newport) equipped with a 400nm longpass filter.

Figure 2. TLC of the non-illuminated and illuminated caged PI derivatives 1a and 1b on HPTLC silica 60, eluent chloroform:methanol:water:acetic acid (65:25:4:1) for 6 cm, then cyclohexane:ethyl acetate (1:1) for 9 cm. Prior to TLC lipids were labeled with fluorogenic 3-azido-7-diethylamino coumarin. Middle: 7-diethylamino 4-hydroxymethyl coumarin, the photolyzed “cage group”. The lower spots are likely the labeled phosphoinositides after uncaging.

Cell entry and fluorescence levels of the caged compounds 1a and 1b were quite similar. The compounds entered cells within a couple of minutes. Maximal fluorescence levels were already reached after about 1 min and stayed steady for at least 30 min (Figure 3). The optimal extracellular concentration for observing cell entry of 1a was at 2 µM, the one for the PI(3,4,5)P₃ derivative 1b was 5 µM. Lower concentrations led to slower entry while higher ones did not improve loading. Illumination with 375 nm laser light, as is commonly used for the uncaging reaction, lead to a strong but transient increase of the coumarin fluorescence, due to the known phenomenon that the released dye is brighter than its phosphate ester. 11 As was observed before for caged PI and is shown in Figure 3, the caged phosphoinositides accumulated predominantly in the ER and Golgi membrane network. We attribute this localization to an unexplained mechanism that draws lipids bearing aromatic groups into these endomembranes. 11, 18 For cell biology experiments, uncaging will provide the 3-phosphorylated phosphoinositides initially at endomembranes. However, the lipids transfer rapidly to the plasma membrane where most of the known biological binding partners and metabolic enzymes such as the phosphatases PTEN and SHIP have been identified.
Figure 3. Loading of trifunctional PI(3,4)P₂ (2 µM) and PI(3,4,5)P₃ (5 µM) derivatives into HeLa cells at 37°C, respectively (concentrations of 1, 2, 5 and 10 µM were tested, with lower concentrations showing slower compound loading and no further improvement of cell entry with higher concentrations). Illumination of cells with 405 nm laser light after 61 min led to a rapid increase of emission intensity due to the higher quantum yield of the released 7-diethylamino-4-hydroxymethyl-coumarin. Mean values of three replicates were normalized to the maximally observed fluorescence intensity. Error bars are standard deviation. B: selected images following the loading procedure as well as the release of the caging coumarin. Scale bar = 10 µm

Our group had previously shown that light-induced release of PIP₃ from membrane-permeant caged PIP₃ derivatives induced clathrin-coated pit-mediated endocytosis of receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR).⁵,¹⁵ We therefore incubated HeLa cells with the fluorescent EGFR-GFP fusion, incubated with the trifunctional PIP₃ derivative 1b and illuminated a subfraction of cells in the field of view with 375 nm blue light to uncage the PIP₃ derivative. Within 30 min, all illuminated cells showed the formation of dotted structures, likely endosomes, while in non-illuminated cells, the fluorescence remained at the plasma membrane (Figure 4). This control experiment demonstrated that the addition of the diazirine and the alkyne did not significantly alter the functionality of the caged PI(3,4,5)P₃.
Figure 4: PI(3,4,5)P$_3$ derivative activity after uncaging. The trifunctional PIP$_3$ derivative 1b fully induced EGFR-GFP internalization in HeLa cells after removal of the photoactivatable protecting group by a 6 sec 375 nm light flash shortly after the image on the left was taken. Only illuminated cells (white box) showed EGFR-GFP internalization. These images are representative of three independent experiments. Scale bar = 10 µm.

Due to the localization to endomembranes, it is important to show that the lipids are rapidly redistributed after uncaging. While redistribution is suspected because the derivatives are losing their only aromatic moiety, transport proteins are only known for phosphatidylinositol (PI) and PI(4)P but not for higher phosphorylated phosphoinositides.$^{[19]}$

Figure 5: PI(3,4)P$_2$ and PI(3,4,5)P$_3$ location. HeLa cells were incubated with 5 µM 1a or 1b, respectively, for 5 min and uncaged with >400 nm light for various time period. Subsequently, cells were photo-crosslinked with 350 nm UV light for 5 min and then fixed with 100% cold methanol. Non-crosslinked lipids and cleaved coumarin were extracted with methanol:chloroform:acetic acid (55:10:0.75). Lipid location was determined after click chemistry of Alexa 488 – picoly azide (green). A: Without exposure to 350 nm light, most of the lipid was washed away during fixation. B: Increased uncaging time led to more lipid detectable at the plasma membrane (arrows). The images are representative of two independent sets of experiments. Scale bar = 20 µm.

We therefore made use of the photo-crosslinking properties of the new tools and followed redistribution after uncaging. We first demonstrated that cross-linking led to a much higher retention of the phosphoinositide derivatives in HeLa cells compared to when crosslinking was omitted. In this case, most of the lipid was removed in the fixation process (Figure 5A). To look
at lipid transport, we uncaged the lipid derivatives prior to photo-crosslinking. We observed a quite rapid appearance of the fluorescence at the plasma membrane (Figure 5B, see the arrows, Figure S1). As this effect was observed after only 30 sec, we speculated that active transport might contribute to the change in location. We further showed that crosslinking was specific and can be out-competed by incubating the cells with a higher dose of a membrane-permeant caged PI(3,4)P₂ or PI(3,4,5)P₃ derivative, respectively, that lack the cross-linking diazirine (Figure S2). We therefore employed the possibility of attaching the cross-linked lipid-protein conjugates to azide beads for subsequent proteomic analysis.

All proteomic data were acquired in triplicate with or without exposure to UV light or without addition of a lipid derivative. Cells were fractionated into membrane and cytosolic fractions. Each fraction was investigated independently. Hits were selected if a protein showed an increase in spectral counts larger than 1.2-fold (log2 of 0.36) between UV and –UV conditions (–UV means no uncaging and cross-linking) or between +UV and control (no compound added but cells exposed to UV light). We chose spectral counts as a readout to permit the comparison to other proteomic analyses from our lab using multifunctional lipids.¹⁰°,¹² Hit proteins were only found in the membrane fraction. They had to appear in at least two of the three experimental repeats and had to feature a lipid-binding domain (Figure 6). Following these very strict criteria, we identified six proteins. Three of them (MPP6, SDCBP and ATP11A (Table 1, Table S1)) were known to be involved in lipid transport or were involved in protein trafficking or membrane organization,¹¹ respectively. They were selected as putative PI(3,4,5)P₃ derivate transporters and were investigated by RNAi knock down experiments. By testing the hypothesis that active transport is involved in trafficking of 3-phosphorylated phosphoinositides from endomembranes to the plasma membrane, we found that an RNAi against MPP6 and ATP11A but not SDCBP significantly delayed appearance of multifunctional PIP₃ at the plasma membrane (Figure 7, Figure S5).

Although the presented experiments are incomplete with respect to all hits from the proteomic screen, the results strongly support active transport of PIP₃ from endomembranes to the plasma membrane. This phenomenon might explain how cells are able to maintain a steep PIP₃ gradient over its quite closely connected membrane systems. The results also support the observation that PH domains predominantly recognize PI(3,4,5)P₃ at the plasma membrane.¹²² A broader study investigating other protein hits of the screen as well as the transport of other 3-phosphorylated phosphoinositides will be performed in the future.
Table 1. Putative lipid transport proteins detected by proteomic analysis after photo-crosslinking of TF-PI(3,4,5)P₃ and subsequent attachment to azide beads via click chemistry. Ratio increase and standard error were determined from three independent repeats.

| Protein                                      | Average spectral count control | Average spectral count -UV | Average spectral count +UV | Fold change +UV/control | Fold change +UV/-UV |
|----------------------------------------------|--------------------------------|----------------------------|----------------------------|-------------------------|---------------------|
| MAGUK p55 subfamily member 6 (MPP6)          | 4.333333                       | 2.333333                   | 6                          | 1.384615                | 2.571429            |
| Probable phospholipid-transporting ATPase IH (ATP11A) | 3                              | 5                          | 8.5                        | 2.833333                | 1.7                 |
| Syntenin-1 (SDCBP)                           | 3                              | 2.333333                   | 5                          | 1.666667                | 1.42857             |

![Figure 7](image_url) Location of uncaged, photo-crosslinked and fluorescently tagged PI(3,4,5)P₃ in fixed cells previously transfected with an RNAi against putative lipid transport proteins listed in Table 1. Neg9 = random siRNA control. The knock-down of ATP11A and MPP6, respectively, but not SDCBP, led to persisting residence times of PIP₃ in endomembranes. This suggests that these proteins are actively involved in phosphoinositide transport to the plasma membrane. The images are representative of three independent experiments. Red arrows points to plasma membrane staining through the fluorescently tagged lipid. Scale bar = 10 µm

In conclusion, we prepared multifunctional derivatives of PI(3,4)P₂ and PI(3,4,5)P₃. The functional modifications did not prevent biological activity with respect to receptor internalization, although this needs to be demonstrated again for a wider set of conditions. The multifunctional lipid derivatives will be exceptional discovery tools as for the first time their use will provide a comprehensive overview of 3-O-phosphorylated phosphoinositide binding proteins under various conditions. The current observation that the phosphoinositide derivatives are binding to putative lipid transport proteins opens new views on how phosphoinositides distribute in cells. Phosphoinositide transport by proteins is well established for phosphatidylinositol 4-phosphate. However, trafficking of 3-O-phosphorylated phosphoinositides represents a novel aspect in lipid signaling. The results demonstrate the discovery tool character of the multifunctional lipid derivatives.

Acknowledgement: We gratefully acknowledge funding from the NIH (R01GM127631) and Transregio 186, funded by the German Research Foundation (DFG). We thank the EMBL proteomics facility for the analysis of the proteomics data, especially Dr Per Haberkant and Dr Frank Stein. We also thank Dr Sergio Triana for his help with the creation of the heat map plots.

References:

[1] aT. Balla, *Physiological reviews* 2013, 93, 1019-1137; bR. R. Madsen, B. Vanhaesebroeck, *Sci Signal* 2020, 13.
[2] M. R. Hokin, L. E. Hokin, *J Biol Chem* 1953, 203, 967-977.
[3] aA. E. Traynor-Kaplan, A. L. Harris, B. L. Thompson, P. Taylor, L. A. Sklar, *Nature* 1988, 334, 353-356; bM. Whitman, C. P. Downes, M. Keeler, T. Keller, L. Cantley, *Nature* 1988, 332, 644-646.
[4] H. Wang, D. Loerke, C. Bruns, R. Muller, P. A. Koch, D. Puchkov, C. Schultz, V. Haucke, J Biol Chem 2020, 295, 1091-1104.
[5] V. Laketa, S. Zarbakhsh, A. Traynor-Kaplan, A. Macnamara, D. Subramanian, M. Putyrski, R. Mueller, A. Nadler, M. Mentel, J. Saez-Rodriguez, R. Pepperkok, C. Schultz, Science signaling 2014, 7, ra5.
[6] Y. Posor, M. Eichhorn-Gruenig, D. Puchkov, J. Schöneberg, A. Ullrich, A. Lampe, R. Müller, S. Zarbakhsh, F. Gulluni, E. Hirsch, M. Krauss, C. Schultz, J. Schmoranzer, F. Noé, V. Haucke, Nature 2013, 499, 233-237.
[7] P. Varnai, G. Gulyas, D. J. Toth, M. Sohn, N. Sengupta, T. Balla, Cell Calcium 2017, 64, 72-82.
[8] aM. A. Lemmon, Nat Rev Mol Cell Biol 2008, 9, 99-111; bl. G. Pemberton, T. Balla, Adv Exp Med Biol 2019, 1111, 77-137.
[9] C. Wang, J. P. Palavicini, M. Wang, L. Chen, K. Yang, P. A. Crawford, X. Han, Analytical chemistry 2016, 88, 12137-12144.
[10] aP. Haberkant, F. Stein, D. Höglinger, M. J. Gerl, B. Brügger, P. P. Van Veldhoven, J. Krijgsved, A. C. Gavin, C. Schultz, ACS chemical biology 2016, 11, 222-230; bD. Höglinger, A. Nadler, P. Haberkant, J. Kirkpatrick, M. Schifferer, F. Stein, S. Hauke, F. D. Porter, C. Schultz, Proceedings of the National Academy of Sciences of the United States of America 2017, 114, 1566-1571; ca. Nadler, G. Reither, S. Feng, F. Stein, S. Reither, R. Muller, C. Schultz, Angew Chem Int Ed Engl 2013, 52, 6330-6334; dm. Schuhmacher, A. T. Grasskamp, P. Barahtjan, N. Wagner, B. Lombardot, J. S. Schuhmacher, P. Sala, A. Lohmann, I. Henry, A. Shevchenko, U. Coskun, A. M. Walter, A. Nadler, Proc Natl Acad Sci U S A 2020, 117, 7729-7738; eM. J. Niphakis, K. M. Lum, A. B. Cognetta, 3rd, B. E. Correa, T. A. Ichu, J. Olucha, S. J. Brown, S. Kundu, F. Piscitelli, H. Rosen, B. F. Cravatt, Cell 2015, 161, 1668-1680.
[11] R. Müller, M. Citir, S. Hauke, C. Schultz, Chemistry (Weinheim an der Bergstrasse, Germany) 2020, 26, 384-389.
[12] P. Haberkant, R. Raijmakers, M. Wildwater, T. Sachsenheimer, B. Brügger, K. Maeda, M. Houweling, A. C. Gavin, C. Schultz, G. van Meer, A. J. Heck, J. C. Holthuis, Angewandte Chemie (International ed. in English) 2013, 52, 4033-4038.
[13] aD. Höglinger, A. Nadler, C. Schultz, Biochimica et biophysica acta 2014, 1841, 1085-1096; bA. Laguerre, C. Schultz, Curr Opin Cell Biol 2018, 53, 97-104.
[14] dF. Hovelmann, K. M. Kedziora, A. Nadler, R. Muller, K. Jalink, C. Schultz, Cell Chem Biol 2016, 23, 629-634; bC. Schultz, 2019, 141, 16544-16547.
[15] aM. Mentel, V. Laketa, D. Subramanian, H. Gillandt, C. Schultz, Angewandte Chemie (International ed. in English) 2011, 50, 3811-3814; bD. Subramanian, V. Laketa, R. Müller, C. Tischer, S. Zarbakhsh, R. Pepperkok, C. Schultz, Nature chemical biology 2010, 6, 324-326.
[16] C. Schultz, Bioorg Med Chem 2003, 11, 885-898.
[17] C. Dinkel, M. Moody, A. Traynor-Kaplan, C. Schultz, Angew Chem Int Ed Engl 2001, 40, 3004-3008.
[18] A. B. Neef, C. Schultz, Angew Chem Int Ed Engl 2009, 48, 1498-1500.
[19] A. Grabon, V. A. Bankaitis, M. I. McDermott, Journal of lipid research 2019, 60, 242-268.
[20] K. Segawa, S. Kurata, S. Nagata, Journal of Biological Chemistry 2016, 291, 762-772.
[21] aJ. M. Beekman, P. J. Coffer, J Cell Sci 2008, 121, 1349-1355; bF. Ye, M. Zeng, M. Zhang, Curr Opin Struct Biol 2018, 48, 6-15.
[22] W. S. Park, W. D. Heo, J. H. Whalen, N. A. O'Rourke, H. M. Bryan, T. Meyer, M. N. Teruel, Mol Cell 2008, 30, 381-392.
[23] J. Chung, F. Torta, K. Masai, L. Lucast, H. Czapla, L. B. Tanner, P. Narayanaswamy, M. R. Wenk, F. Nakatsu, P. De Camilli, Science 2015, 349, 428-432.