Live-cell Imaging of Phospholipase D Activity

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Technology description

The invention provides novel compounds that enable visualization and tracking of phosphatidic acid (PA) production by phospholipase D (PLD) at thecellularandsubcellularlevels using a simple two-step strategy called IMPACT –ImagingPhospholipase DActivity withClickableAlcohols viaTransphophatidylation.

Phospholipase D (PLD) enzymes impact cell signaling by synthesizing the pleiotropic lipid phosphatidic acid (PA). PA alone has been associated with diverse physiological changes, but how PA causes these effects remains unknown due to the lack of suitable tools for visualizing its production within cells. Cornell researchers have taken advantage of the enzymatic promiscuity of PLDs to develop a two-step strategy as shown in figure 1 involving novel, exogenous functionalized alcohols that enables the use of click chemistry to append imaging or other detection probes.

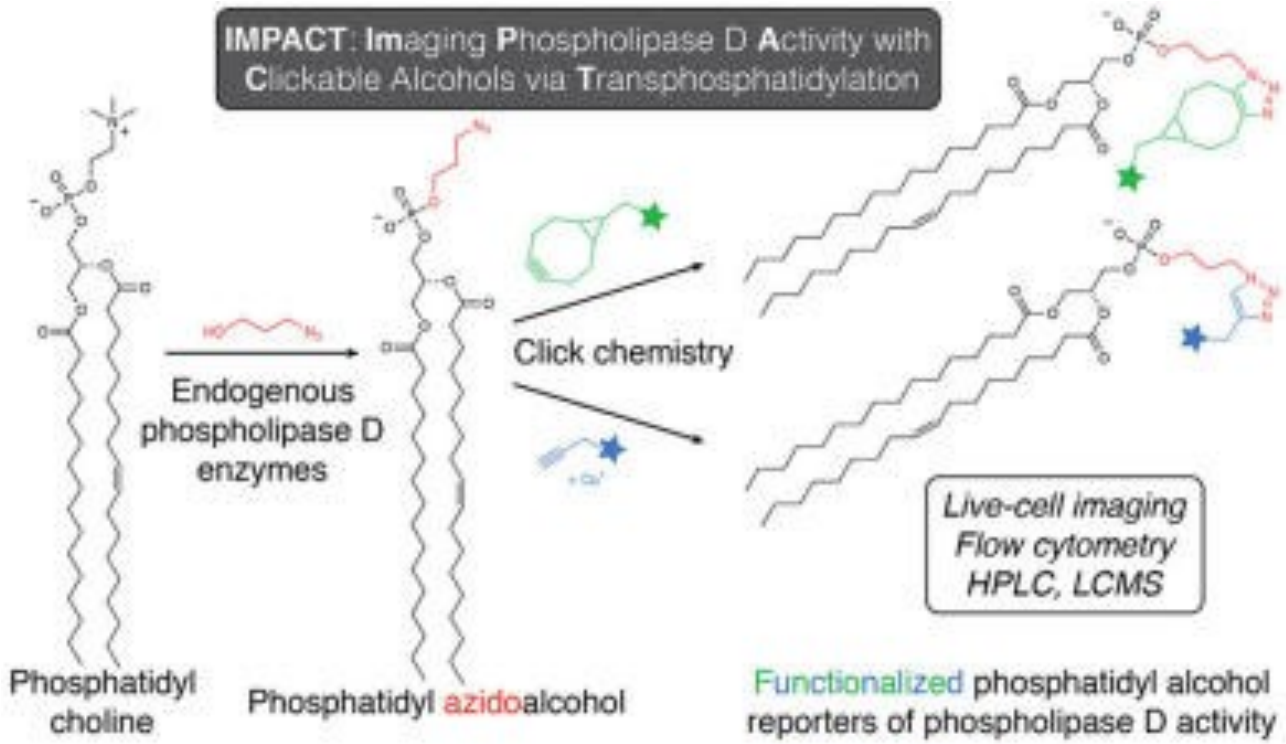

Figure 1: IMPACT

Proof of concept: Using various azidoalcohols as substrates, figure 2 shows live-cell imaging of PLD activity.

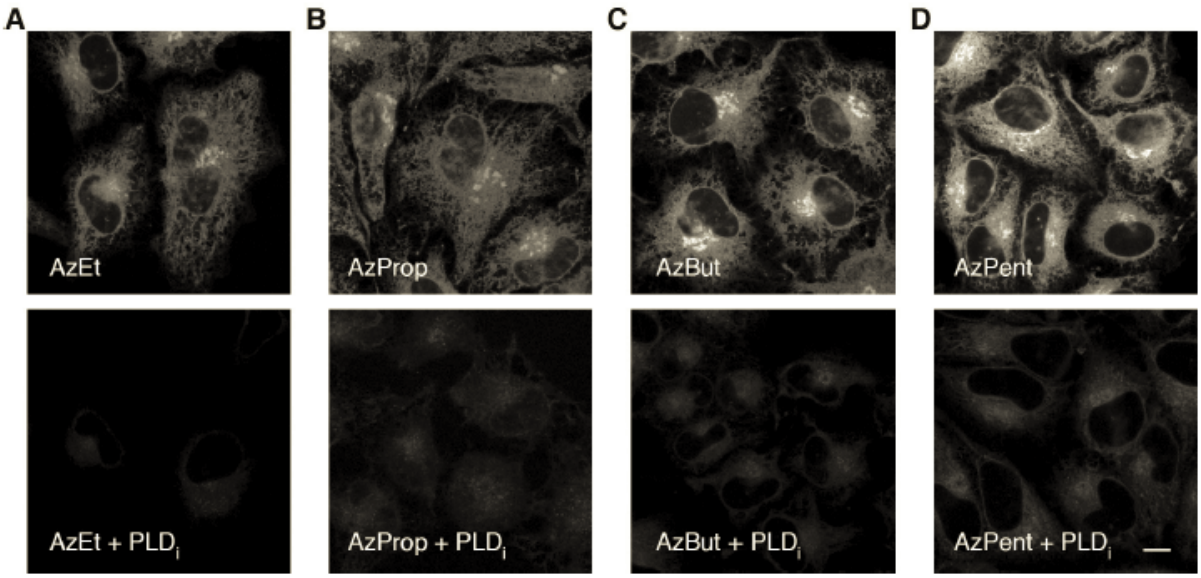

Figure 2: Evaluation of a panel of azidoalcohols for imaging PLD activity in HeLa cells. Cells were treated with PLDi (PLD inhibitor – bottom panel) or DMSO (top panel) followed by azidoalcohols AzET (A), AzProp (B), AzBut (C), AzPent (D) then incubated with BODIPY-cyclooctyne and imaged by confocal microscopy.

Application area

Tool for imaging PLD activity in live cells

Advantages

Does not perturb endogenous PA levels
Copper-free click chemistry means no copper toxicity to cells
High spatial and temporal resolution

Institution

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