**Chemical Tools that IMPACT Lipid Signaling**

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The fidelity of intracellular signaling pathways requires that cells control the production of signaling agents in space and in time. Phosphatidic acid (PA) is both a central phospholipid biosynthetic intermediate and a multifunctional lipid second messenger produced at several discrete subcellular locations. The modes of action of PA can differ based on upstream stimulus, biosynthetic source, and site of production. How cells regulate the local production of PA to direct diverse signaling outcomes remains elusive. To begin to unravel these questions, we have focused our efforts on improving and expanding the toolkit for both visualizing and perturbing cellular PA production, with spatiotemporal precision. Toward the first goal, we have harnessed the exquisite selectivity of chemoenzymatic labeling and click chemistry tagging to develop a method for directly visualizing PA production by phospholipase D (PLD) enzymes. This method, termed IMPACT for Imaging PLD Activity with Clickable Alcohols via Transphosphatidylation, has revealed sites of PLD-mediated PA signaling elicited by diverse physiological stimuli and features subcellular, organelle-level resolution. To complement these tools for visualizing PA production, we have also generated a suite of light-controllable, optogenetic PLDs (optoPLDs) to precisely generate tunable amounts of PA, on demand, at specific organelle membranes. We will also describe select applications of IMPACT and optoPLD to, respectively, visualize and control the locations and dynamics of important cellular pathways that intersect with PLD signaling, including GPCR activation and the Hippo pathway. Collectively, these new approaches represent powerful and precise approaches for revealing spatiotemporally defined functions of PA in response to physiological and pathological stimuli. More broadly, our work highlights the power of combining bioorthogonal chemistries, chemoenzymatic tagging, directed evolution, and optogenetics to shed light on cell signaling pathways.