Kinase-catalyzed Labeling: Chemical Approaches to Mapping Cell Signaling Pathways

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Kinases are key enzymes in cell signaling with clear roles in biological events and disease states. In fact, kinases are critical drug targets for a variety of clinical drugs, including anti-cancer agents. However, studies on cell signaling and development of new drug candidates are limited by the paucity of tools available to fully characterized kinase activity. Kinases mediate their influence on cell signaling by catalyzing phosphorylation of substrate proteins using adenosine 5’-triphosphate (ATP) as a cosubstrate. Our laboratory uncovered that kinases promiscuously tolerate as cosubstrates modified ATP analogs that contain a functional tag at the terminal phosphoryl (Figure 1). Exploiting this new property of kinases, we developed kinase-catalyzed labeling reactions to detect substrates in cell lysates. Kinase-catalyzed biotinylation using an ATP-biotin probe was employed to monitor and visualize phosphoproteins in cells (*J. Am. Chem. Soc.*, **2007,** *129,* 10-11; *Bioorg Med Chem,* **2016***, 24,* 12-19*; Chembiochem*, **2017,** *18,* 136-114; *J. Prot. Res*. **2021**, *20*, 4852-4861). In addition, kinase-catalyzed photocrosslinking using an ATP-arylazide probe facilitated kinase-substrate and kinase-associated protein identification (*Angewante Chemie*, **2010**, *49,* 1627-1630; *J. Am. Chem. Soc*, **2018**, *140*, 16299-16310; *Angewante Chemie*, **2021**, *60,* 9859-9862). In this talk, I will describe both published and unpublished technologies to study kinase activities in cells, along with our recent efforts to utilize ATP analogs for kinase-substrate identification and phosphoproteomics applications. By developing kinase-catalyzed labeling, we are pioneering new chemical tools to probe kinase activity and map the cell signaling events governed by kinases.



Figure 1- The mechanism of phosphoryl transfer from ATP (R= O-) to a substrate protein by kinases. The Pflum laboratory recently uncovered that -phosphate modified ATP analogs (R= biotin, fluorophore, or photocrosslinker) serve as kinase cosubstrates, allowing kinase-catalyzed phosphoprotein labeling.