Phosphorylation-inducing chimeric small molecules

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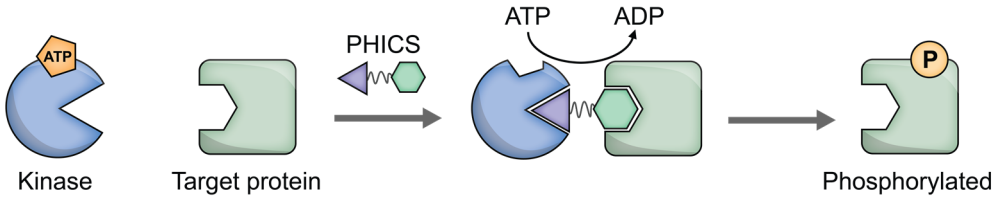
My laboratory focuses on applying chemistry-based approaches to propel the development of molecular and cellular modalities for various disorders. I will briefly introduce our efforts in cellular engineering using CRISPR-based technologies and the development of zinc-based prodrug systems, but my presentation will focus on Phosphorylation-Inducing Chimeric Small molecules (PHICS). Small molecules have been classically used to inhibit enzyme function (i.e., loss-of-function), but several new classes of small molecules are emerging that endow neo-functions to enzymes *via* proximity-mediated effects. I will describe the development and applications of PHICS, which can *induce* phosphorylation of a given target protein by forcing proximity between a kinase and the target protein (Scheme 1).

Scheme 1.



Platform development: The first-generation PHICS were formed by joining the target binder *via* a linker to a non-inhibitory kinase binder, which are scantily available. I will describe linkers based on addition-elimination reactions that enable the generation of PHICS using kinase inhibitors (abundantly available), allowing rapid development of PHICS for > 30 kinases (and their isoforms). Beyond PHICS, these linker chemistries may accelerate the development of chimeric molecules that induce/remove other post-translational modifications.

Novel bioactivities of PHICS: I will describe various activities of PHICS, including induction of phosphorylations that trigger signal transduction events and induction of neo-phosphorylations (unobserved endogenously and potentially immunogenic) that adversely affect the target protein's ability to interact with negatively charged biomolecules. For example, PHICS-mediated phosphorylation disrupted the ability of KRAS, a GTPase that translocates to the membrane, to interact with GTP/phospholipids. "Homo-PHICS" (i.e., a dimer of kinase binder) efficaciously inhibited the kinase by inducing ATP-binding pocket's phosphorylation that disrupts further ATP loading. This novel mechanism of action allowed PHICS to efficaciously kill cancer cells resistant to known drugs (e.g., Imatinib/Asciminib for CML, Ibrutinib for CLL).

We are currently evaluating the immunogenicity of neo-phosphorylations. Furthermore, we are determining if PHICS-mediated hyperphosphorylation can disrupt the activities of chemically-intractable targets (e.g., c-Myc:DNA interaction) or prevent pathologic protein aggregation. Overall, these studies lay the foundations for a novel therapeutic modality for cancer and demonstrate the power of synthetic chemistry to expand the chemical and functional diversity of proteins in cells using chimeric molecules.