Chemico-allosteric Control of Kinase Activity in Living Cells

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For many cellular behaviors, it is essential that signaling is precisely coordinated in space and time. Such spatiotemporal dynamics can only be fully understood using tools that examine and manipulate protein behavior in intact living cells. While it is very valuable to visualize signaling dynamics, testing hypotheses about spatio-temporal regulation requires that we actually control such activity by manipulating protein activity at precise times during live cell behavior. We developed an approach in which insertion of a ligand-controlled engineered domain (uniRapR) into the loops that are allosterically coupled to active sites. Such a domain renders kinases catalytically inactive; addition of a small molecule rapamycin rescues the catalytic activity by binding to uniRapR domain. We validated our tool by building switchable Src-family kinases in single cells and zebrafish. Activation of Src kinase leads to rapid induction of protrusion with polarized spreading in mamalian cells, and morphological changes with loss of cell–cell contacts in the epidermal tissue of zebrafish. Currently, we are extending this approach by controlling other protein families including guanine nucleotide exchange factors (GEFs). These switchable GEFs will allow us to investigate GEF-GTPase circuit dynamics in migrating cells.