BRINGING PROTEINS TOGETHER INSIDE LIVING CELLS USING LIGHT-ACTIVATED CHEMICAL DIMERIZERS

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Fundamental biological processes including cell division, migration, and death, are driven by protein interactions. Regulation of protein localization is one of the mechanisms cells utilize to control cellular events with high spatial and temporal precision. Therefore, several techniques have been developed to provide control of protein interactions and localization. A number of elegant approaches employ naturally light-responsive proteins, also known as optogenetics, to reversibly induce protein–protein binding interactions with subcellular precision. However, the application of these light-inducible protein systems to various intracellular locations beyond the plasma membrane has been limited. Moreover, to achieve sustained interactions in some applications, most of these optogenetic systems require continuous illumination, increasing the risk of phototoxicity. Another robust and widely utilized technique to control protein interactions via small molecules is the chemically-induced dimerization (CID) of proteins; the most classic example of this technique being rapamycin-induced dimerization. However, the lack of spatiotemporal control and reversibility in this system has necessitated the development of new dimerizers in the past two decades. By combining light-inducible features with the CID technique, we have created a novel platform to rapidly and reversibly induce protein dimerization using light with high specificity in living cells. This is accomplished with subcellular spatiotemporal resolution using a series of novel, cell-permeable, photoactivatable, and photocleavable chemical dimerizers. The modular design of our system has allowed us to tailor the properties of our molecules for studying various protein functions and biological pathways inside living cells. Furthermore, we demonstrate the utility of our system by applying it to manipulate dynamic biological events including organelle transport and spindle assembly checkpoint. This work establishes a foundation for optogenetic control over protein function and highlights the advantages of a hybrid chemical and genetic approach. We envision our tools to be readily adapted to experimentally probe complex signaling networks and other cellular processes that depend upon spatiotemporal regulation of protein localization on biologically-relevant timescales.