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ABSTRACT

Proteins and their complex dynamic interactions regulate cellular mechanisms from sensing and transducing extracellular signals, to mediating genetic responses, regulating enzyme activity, and sustaining or changing cell morphology. To manipulate the molecular processes that govern the behavior and fate of cells, synthetically constructed, genetically encoded tools provide the means to precisely target proteins of interest (POIs), and control their subcellular localization and activity in vitro and in vivo. Ideal synthetic tools react to an orthogonal cue, i.e. a trigger that does not activate any other endogenous process, thereby allowing manipulation of the POI alone. In optogenetics, naturally occurring photosensory domain from plants, algae and bacteria are re-purposed and genetically fused to POIs. Chemogenetic tools consist of protein domains that recognize and bind small molecules. This work describes how the recently structurally and photochemically characterized green-light responsive cobalamin-binding domains (CBDs) from bacterial transcription factors were re-purposed to function as a green-light responsive optogenetic tool. In contrast to previously engineered optogenetic tools, CBDs do not induce protein-protein interaction (PPI), but rather confer a PPI already upon expression, which can be rapidly disrupted by illumination. This was employed to mimic inhibition of constitutive activity of a growth factor receptor, and successfully implement for cell signalling in mammalian cells and in vivo to rescue development in zebrafish. This work further describes the development and application of a chemically induced de-dimerizer (CDD) based on a recently identified and structurally described bacterial enzyme. CBDs and CDD expand the repertoire of synthetic tools by providing novel mechanisms of mediating PPIs, and by recognizing previously not utilized cues. In the future, they can readily be combined with existing synthetic tools to functionally manipulate PPIs in vitro and in vivo.