Electronic Supplementary Information for

Tethering-based chemogenetic approaches for modulation of protein function in live cells

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Chemogenetic strategies for modulating protein function

To date, several chemogenetic strategies have been developed for modulating protein function. The most prominent example is the "bump-and-hole" strategy (**Fig. S1a**).[1] This strategy has been well demonstrated for selective inhibition of kinases. A sensitized kinase can be generated by change of a conserved, bulky amino acid residue in the active site to glycine or alanine, creating a "hole". In parallel, a designer molecule containing a "bump" is generated by addition of a bulky group (e.g., *tert*-butyl) to a known kinase inhibitor. Due to the presence of the bump, the designer molecule can only inhibit the sensitized kinase and has no effect on any wild-type kinases. However, it is noteworthy that the introduction of a "hole" into the target enzyme sometimes abolishes the enzyme activity. Nevertheless, this strategy is powerful and has been extended to other proteins, including motor proteins, polymerases, and enzymes involved in post-translational modifications.[5]

The pairing of a sensitized protein and a designer small molecule can also be identified through random mutagenesis. Designer receptor exclusively activated by designer drugs (DREADD) is a representative example (**Fig. S1b**).[2] For instance, a G protein-coupled receptor (GPCR) was, in an experiment, mutated so that it did not respond to its cognate agonist (i.e., a small-molecule activator) but instead to a biologically inert ligand, namely clozapine-*N*-oxide (CNO). This and related approaches have been applied in multiple research fields.[2] However, this strategy is mostly limited to GPCRs and ligand-gated ion channels at present, likely due to the lack of appropriate high-throughput screening assays.

Another strategy followed for modulating protein function has been the use of a designer molecule to control the stability of the target protein fused with a protein-stabilizing or protein destabilizing domain (**Fig. S1c**).[3] Stability of the fusion protein is regulated by binding of the designer molecule to the protein stability domain. In this strategy, there is a pairing of a small molecule and a protein domain; binding of the small molecule either stabilizes the target protein or induces its degradation. Stabilization induced by the small molecule is analogous to gene overexpression, whereas destabilization induced by the small molecule is analogous to nucleic acid sequence disruption. Thus, this strategy is like a rapid version of genetic modulation, although the time-lag for protein synthesis or degradation still needs to be considered. It is also noteworthy that this strategy would not be suitable to dissect the individual contributions from catalytic and non-catalytic functions as the protein molecule is either present or absent.

Generation of a functional protein can also be regulated using inducible inteins or chemically induced dimerization. In the former case, the protein of interest is disrupted by an inducible intein; here, addition of the designer molecule triggers intein splicing, and formation of the full-length, functional target protein (**Fig. S1d**).[4] In the latter case, the protein of interest is split into two physically separated polypeptides fused to domains that can be chemically induced to dimerize; here, addition of the corresponding small molecule induces dimerization, yielding the target protein in the correct conformation for exerting its function (**Fig. S1e**).[5] In addition, chemically induced dimerization has been widely applied to control protein-protein interaction or subcellular localization of the target proteins.

Investigators have also modulated protein function using a small molecule to induce a conformational change in the protein (**Fig. S1f**). For example, the conformation of a circularly permutated bacterial dihydrofolate reductase (cpDHFR) has been shown to be affected by the presence of trimethoprim (TMP) and nicotinamide adenine dinucleotide phosphate (NADPH).[6] Here, cpDHFR was fused to a nanobody fusion protein, which was found to be functional only in the absence of the two small molecules. Addition of the two small molecules was observed to induce a conformational change of the cpDHFR, shielding the nanobody from interacting with its target. Note that TMP has a greater than 1,000-fold selectivity for bacterial DHFR relative to mammalian DHFR and can thus be employed as a biologically inert molecule in mammalian cells.

In addition to the strategies mentioned above, tethering is another powerful chemogenetic strategy for protein activation, inhibition, or reversible modulation (**Fig. S1g**). Here, the genetic modification involves introduction of a functional group with unique reactivity, so that the designer molecule can be covalently or non-covalently attached to the sensitized protein. Tethering-based chemogenetics can be used to modulate

either the catalytic function or biomolecular interaction of the target protein. The strategy provides flexibility often not available when using the bump-and-hole strategy, as the tethering point is not restricted to the small-molecule binding site. In addition, availability of a high-throughput screening assay is not required.
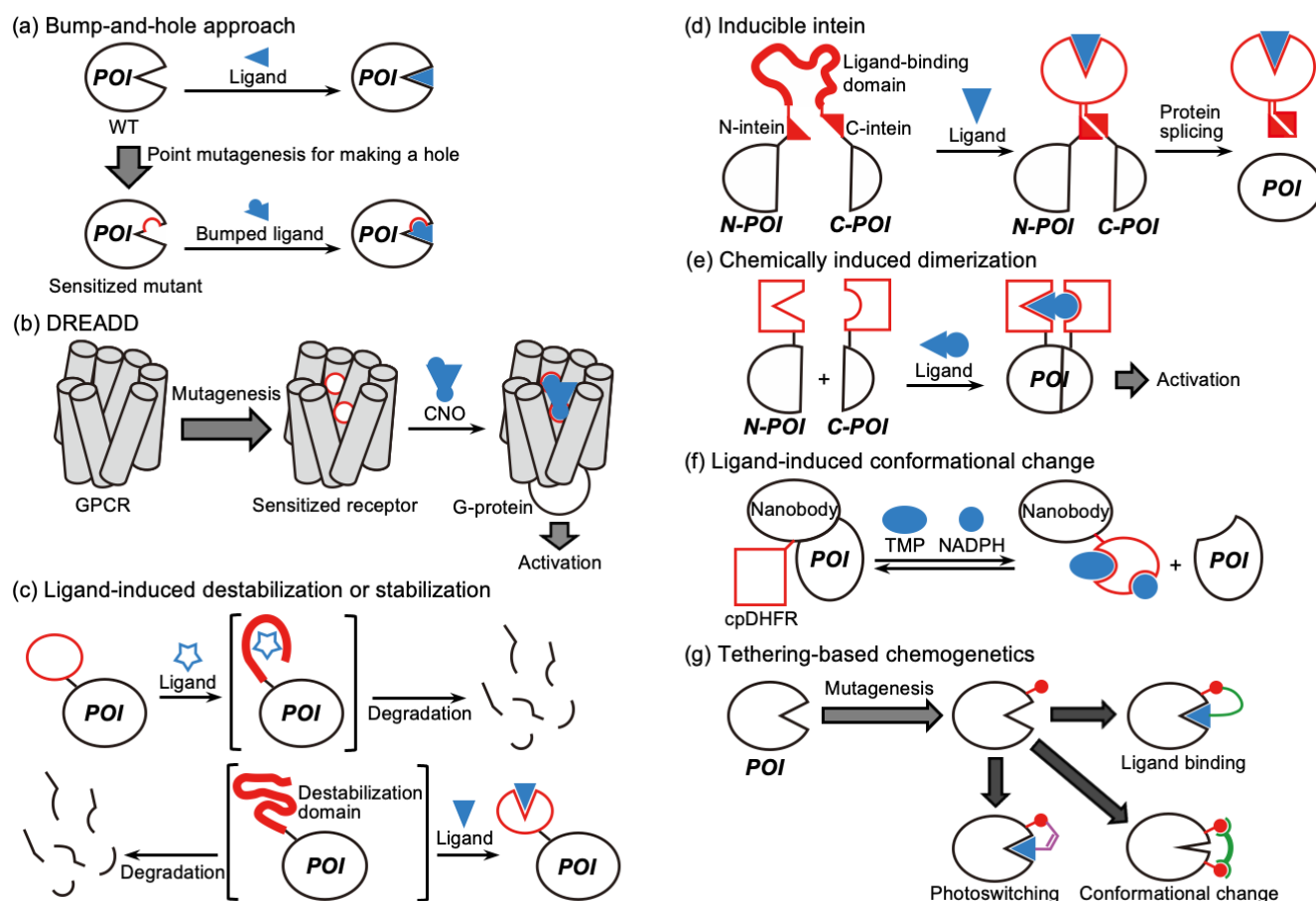
Fig. S1 Chemogenetic strategies for modulating protein function. The genetic modification for protein sensitization and the designer small molecule are highlighted in red and blue, respectively. CNO: clozapine-N-oxide, POI: protein of interest, cpDHFR: circularly permutated bacterial dihydrofolate reductase, TMP: trimethoprim, NADPH: nicotinamide adenine dinucleotide phosphate. (a) Bump-and-hole strategy for selective modulation of the sensitized protein. (b) DREADD, a typical example of generating a sensitized protein responsive to a designer small molecule (e.g., CNO) through random mutagenesis. (c) Fusion of a domain to control protein stability through either ligand-induced stabilization or degradation of the target protein. (d) Using an inducible intein for protein activation. (e) Chemically induced dimerization for correct folding of the target protein. (f) Ligand-induced conformational change for controlling selectivity and specificity of the nanobody. (g) Tethering-based chemogenetics for functional modulations of proteins.

References

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