A versatile inducible protein translocation tool for controlling plasma membrane signaling

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The inner leaflet of the plasma membrane (iPM) serves as a platform for intracellular signaling networks. Therefore, the ability to rapidly recruit signaling proteins to the iPM is a powerful approach for cell signaling research. For this purpose, several methods based on chemically induced dimerization (CID) have been developed. However, most of the CID tools require the expression of two protein components to control a single target protein and are ill-suited for controlling proteins reversibly and repeatedly.

The SLIPT (self-localizing ligand-induced protein translocation) system developed by us is a methodology to control protein translocation.[1,2] In SLIPT, a small-molecule hybrid ligand with an organelle/membrane localization ability is used to recruit its binding protein from the cytoplasm to the target site. Here, we present a versatile chemogenetic SLIPT system for iPM-specific protein recruitment. We created an engineered SLIPT tag, termed ^{iK6}DHFR (**Fig. a**), by inserting a hexalysine (K6) sequence into the loop region of *Escherichia coli* dihydrofolate reductase (eDHFR).[3] The ^{iK6}DHFR-fused proteins can be recruited specifically from the cytoplasm to the iPM within a few minutes with the myristoyl-D-Cys-tethered trimethoprim ligand (m^DcTMP).[2] The ^{iK6}DHFR/m^DcTMP system is applicable to control various signal processes, such as Raf/ERK activation, Ca^{2+} signaling, and cAMP generation. We also succeeded in manipulating the localization and activity of signaling proteins repeatedly by using two chemicals, m^DcTMP and TMP (**Fig. b**). In this presentation, we will report the details and applications of the ^{iK6}DHFR/m^DcTMP platform.

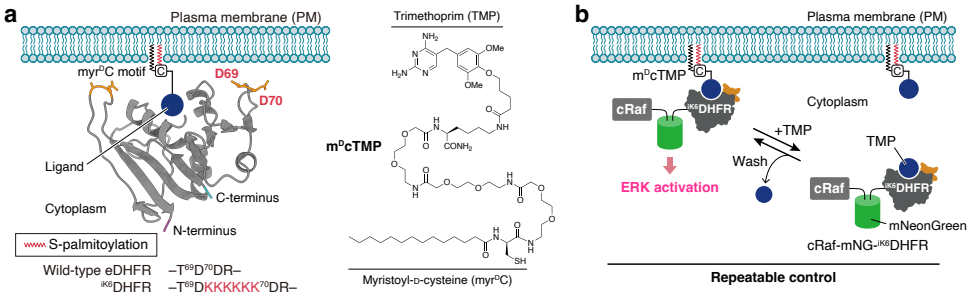

Fig. (**a**) Design of ^{iK6}DHFR and structure of m^DcTMP. (**b**) Synthetic chemical oscillation of ERK.

1) M. Ishida *et al.*, *J. Am. Chem. Soc.* **2013**, *135*, 12684. 2) A. Nakamura *et al.*, *ACS Chem. Biol.* **2020**, *15*, 1004. 3) Y. Hatano *et al.*, *bioRxiv* DOI: 10.1101/2021.03.16.435568.