Title: Chemogenetic manipulation of endogenous proteins in fission yeast using a self-localizing ligand-induced protein translocation (SLIPT) system

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Supplementary Figures

Figure S1

Figure S1. Microfluidic devices used in this study.

(A and B) Schematic representation of the microfluidic devices used in this study.

(A) In the microfluidic device (Y04C-02), fission yeast cells are loaded from the cell inlet and sandwiched between the chamber and the glass bottom in the middle region. Culture medium is supplied from inlets 1 to 6.

(B) In the microfluidic device (Y04T-04), fission yeast cells are loaded from the cell inlet, and trapped in the glass-bottomed culture chamber. Culture medium is supplied from inlets 1 to 6.
Figure S2

Confocal fluorescence images of fission yeast cells expressing eDHFR-mNG before (left) and after 30 min (right) of treatment with m^D)cTMP (10 μM). Scale bars, 10 μm.
Figure S3. Nuclear translocation of the DHFR fusion protein by hoeTMP.

(A) Chemical structure of hoeTMP.

(B) Confocal fluorescence images (left) and DIC image (right) of fission yeast cells expressing iK6DHFR-mNG after 60 min of treatment with hoeTMP (10 μM). Scale bar, 10 μm.
Figure S4

**Figure S4. Time-lapse imaging of fission yeast using an agar pad.**

(A) The procedure for time-lapse imaging of fission yeast using an agar pad is shown.

(B) Confocal fluorescence images of fission yeast cells expressing $^{iK6}$DHFR-mNG were taken every 5 min. The representative images are shown at the indicated time points after the addition of 10 μM $m^DcTMP_{4Me}$. Scale bar, 10 μm.

(C) Time course of $m^DcTMP_{4Me}$-induced $^{iK6}$DHFR-mNG translocation to the PM. Normalized fluorescence intensity in the cytoplasm was plotted as a function of time. Data are presented as mean ± SD (n = 6 cells).
Figure S5. Endogenous tagging of iK6DHFR to the cdc2 gene.

(A) Schematic representation of endogenous tagging of iK6DHFR to the cdc2 gene.

(B) Multiple septa formation in fission yeast cells expressing Cdc2-iK6DHFR-mNG after 100 min of treatment with mPcTMP4Me (10 μM). White arrowheads indicate the septum positions. Scale bar, 10 μm.
(C) Confocal fluorescence images (top) and DIC images (bottom) of fission yeast cells expressing \(iK_6\)DHFR-mNG were taken every 5 min. The representative images are shown at the indicated time after the addition of 10 μM \(m^Dc\text{TMP}_{4\text{Me}}\). Scale bar, 10 μm.

(D) A heat map showing the temporal profile of the cell length of fission yeast cells expressing \(iK_6\)DHFR-mNG in the presence of \(m^Dc\text{TMP}_{4\text{Me}}\) (n = 10 cells).
Supplementary Movies

Movie S1. m^DcTMP-induced transient membrane translocation of iK6DHFR-mNG in fission yeast. Fission yeast cells expressing iK6DHFR-mNG were added to 10 μM m^DcTMP, and time-lapse imaged. Scale bar, 10 μm.

Movie S2. m^DcTMP_4Me-induced sustained membrane translocation of iK6DHFR-mNG in fission yeast. Fission yeast cells expressing iK6DHFR-mNG were added to 10 μM m^DcTMP_4Me, and time-lapse imaged. Scale bar, 10 μm.

Movie S3. m^DcTMP_4Me-induced translocation of endogenous Cdc2 for the plasma membrane. Fission yeast cells expressing Cdc2-iK6DHFR-mNG were added to 10 μM m^DcTMP_4Me, and time-lapse imaged. Scale bar, 10 μm.

Movie S4. m^DcTMP_4Me-induced translocation of endogenous Spg1 for the plasma membrane. Fission yeast cells expressing Spg1-iK6DHFR-mNG were added to 10 μM m^DcTMP_4Me, and time-lapse imaged. Scale bar, 10 μm.
# Supplementary Tables

## Table S1. Plasmids used in this study.

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*kan: kanamycin resistance gene (kanMX6).

*mNeonGreen: the codon-optimized mNeonGreen for *Schizosaccharomyces pombe*.

*eDHFR(69K6): ^K6DHFR.*
Table S2. Fission yeast strains used in this study.

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</table>

*nat: nourseothricin resistance gene (natMX6).
*kan: kanamycin resistance gene (kanMX6).
*mNeonGreen: the codon-optimized mNeonGreen for Schizosaccharomyces pombe.
*eDHFR(69K6): iK6DHFR.
Supplementary Methods

General materials and methods.

All chemical reagents and solvents were purchased from commercial suppliers (Watanabe Chemical Industries, Tokyo Chemical Industry, FUJIFILM Wako Pure Chemical Corp., and Kanto Chemical) and used without further purification. Reversed-phase HPLC was performed on a Hitachi LaChrom Elite system with UV detection at 220 nm using a YMC-Pack C4 column (10 × 250 mm or 20 × 250 mm). $^1$H NMR spectra were recorded on a Bruker AVANCE III HD400SJ (400 MHz) spectrometer. $^1$H NMR chemical shifts were referenced to tetramethylsilane (0 ppm). High-resolution mass spectra were measured on a Thermo Scientific Exactive Plus Orbitrap mass spectrometer.

Reagent abbreviations

DBU: 1,8-diazabicyclo[5.4.0]7-undecene
DIPEA: N,N-diisopropylethylamine
DMF: N,N-dimethylformamide
Fmoc-Adox-OH: Fmoc-8-amino-3,6-dioxaocanoic acid
HATU: 1-((dimethylamino)(dimethyliminio)methyl)-1H-[1,2,3]triazolo[4,5-b]pyridine
3-oxide hexafluorophosphate
HBTU: 1-((dimethylamino)(dimethyliminio)methyl)-1H-benzo[d][1,2,3]triazole
3-oxide hexafluorophosphate
HOAt: 3H-[1,2,3]triazolo[4,5-b]pyridin-3-ol
HOBt: 1H-benzo[d][1,2,3]triazol-1-ol monohydrate
NMP: N-methyl-2-pyrrolidone
o-Ns-Cl: 2-nitrobenzenesulfonyl chloride
TFA: trifluoroacetic acid
TIPS: triisopropylsilane
**Synthesis of compound 1 (m^PcTMP_4Me).**

Compound 1 was synthesized manually on Sieber amide resin (0.79 mmol/g) (38 mg, 30 μmol) using standard Fmoc-based solid-phase peptide synthesis protocols, as shown in Scheme S1. Fmoc deprotection was performed with 20% piperidine in DMF at room temperature for 15 min. After washing the resin with DMF, Fmoc-Lys(Aloc)-OH was coupled to the resin at room temperature with a mixture of Fmoc-Lys(Aloc)-OH (3.1 eq.), HBTU (3.0 eq.), HOBt (3.0 eq.), and DIPEA (6.0 eq.) in DMF. The coupling reaction was monitored by the Kaiser test. After Fmoc deprotection and washing,
N-methylation and subsequent amino acid coupling reactions were performed using the procedure previously reported \(^2\). The Fmoc-deprotected resin was treated with a mixture of \(\alpha\)-Ns-Cl (4.0 eq.) and 2,4,6-collidine (10.0 eq.) in NMP for 15 min. After washing the resin with NMP, the resin was treated twice with dimethyl sulfate (10.0 eq.) in NMP for 2 min each time. After washing the resin with NMP, the resin was treated twice with a mixture of 2-mercaptoethanol (10.0 eq.) and DBU (5.0 eq.) in NMP for 5 min each time, yielding the N-methylated N-terminus. Fmoc-Adox-OH was coupled to the resin using Fmoc-Adox-OH (3.1 eq.), HATU (3.0 eq.), HOAt (3.0 eq.), and DIPEA (6.0 eq.) in NMP. The coupling reaction was monitored by the chloranil test \(^3\). Subsequent Fmoc deprotection, N-methylation, and coupling reactions of Fmoc-Adox-OH and Fmoc-D-Cys(Mmt)-OH were performed as described above. After Fmoc deprotection, the N-terminus was myristoylated with a mixture of myristic acid (4.1 eq.), HBTU (4.0 eq.), HOBt (4.0 eq.), and DIPEA (8.0 eq.) in DMF/CH\(_2\)Cl\(_2\) (1/1). After washing the resin with DMF, MeOH, and CH\(_2\)Cl\(_2\), the resin was dried \textit{in vacuo}. The Aloc group of the lysine residue was deprotected by treatment with CHCl\(_3\) containing Pd(PPh\(_3\))\(_4\) (3.0 eq.), 5.0% acetic acid, and 2.5% 4-methylmorpholine under an Ar atmosphere. The resin was washed with DMF containing 0.5% DIPEA (×5), DMF containing 0.5% sodium diethyldithiocarbamate (×5), and DMF only (×5). The deprotected side chain of the lysine residue was then N-methylated as described above, and compound \(2\) \(^4\) was coupled to the lysine side chain with a mixture of \(2\) (4.1 eq.), HATU (4.0 eq.), HOAt (4.0 eq.), and DIPEA (8.0 eq.) in NMP/DMF (1/1). After washing the resin with NMP, DMF, MeOH, and CH\(_2\)Cl\(_2\), the resin was dried \textit{in vacuo}. Deprotection and cleavage from the resin were performed with CH\(_2\)Cl\(_2\) containing 5% TFA and 2.5% TIPS. The crude product was purified by reversed-phase HPLC using a semi-preparative C4 column (a linear gradient of MeCN containing 0.1% TFA and 0.1% aqueous TFA) to afford \(1\) as a white solid [8.4 mg, 22% (as a mono-TFA salt)].

\(^{1}\)H NMR (400 MHz, CD\(_3\)OD): mixture of rotamers \(\delta\) 7.21 (1H, s), 6.56 (2H, s), 5.09–4.87 (2H, m), 4.36–4.18 (4H, m), 3.93 (2H, t, \(J = 6.0\) Hz), 3.80 (6H, s), 3.76–3.49 (14H, m), 3.49–3.33 (4H, m), 3.21 (2H, m), 3.04 (3H, m), 3.00–2.75 (9H, m), 2.67 (2H, m), 2.47 (2H, m), 2.23 (2H, t, \(J = 4.0\) Hz), 2.05–1.48 (10H, m), 1.38–1.15 (22H, m), 0.89 (3H, t, \(J = 6.0\) Hz).

HRMS (ESI): calculated for [M+H]\(^+\), 1163.7108; found, 1163.7061.

\textbf{Synthesis of m\textsuperscript{D}cTMP and hoeTMP}

m\textsuperscript{D}cTMP and hoeTMP were synthesized as described previously \(^5,6\).
References


