Electronic Supplementary Information (ESI)

A SNAP-Tag Fluorogenic Probe Mimicking the Chromophore of the Red Fluorescent Protein Kaede

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Table of Contents

Supplementary Figures S1-S12
Tables S1

$^1$H NMR spectra of 3a – 3d, 4a – 4d

$^{13}$C NMR spectra of 3a – 3d, 4a – 4d
Figure S1 Fluorescence emission intensity of (a) 4a (5 μM), (b) 4b (5 μM), (c) 4c (5 μM), and (d) 4d (5 μM) in the presence of an increasing percentage of glycerol in methanol. Fluorescence measurement was carried out using a Tecan infinite M1000Pro fluorescence microplate reader at excitation wavelength of 530 nm and emission wavelengths of 630 nm.
Figure S2 Fluorescence emission spectra of (a) 4a (5 μM), (b) 4b (5 μM), (c) 4c (5 μM), and (d) 4d (5 μM) in various organic solvents, and their corresponding intensities of (e) 4a (5 μM), (f) 4b (5 μM), (g) 4c (5 μM), and (h) 4d (5 μM) at 630 nm; 1. Aqueous buffer, 2. DMSO, 3. Acetonitrile, 4. DMF, 5. Methanol, 6. Ethanol, 7. Ethyl acetate, 8. Tetrahydrofuran, 9. Benzene, 10. 1,4-Dioxane, 11. Glycerol. Fluorescence measurement was carried out using a Tecan infinite M1000Pro fluorescence microplate reader at excitation wavelength of 530 nm.
Figure S3 linear plots of the logarithm of the fluorescence emission intensity of (a) 4a (b) 4b (c) 4c (d) 4d at 630 nm as a function of the logarithm of viscosity. Method can be found in the Experimental Section.
Figure S4 Absorption spectra of (a) 4a (5 μM) (b) 4b (5 μM) (c) 4c (5 μM) (d) 4d (5 μM), fluorescence excitation spectra of (e) 4a (5 μM) (f) 4b (5 μM) (g) 4c (5 μM) (h) 4d (5 μM) and emission spectra of (i) 4a (5 μM) (j) 4b (5 μM) (k) 4c (5 μM) (l) 4c (5 μM) in three different viscous solvent systems. Fluorescence measurement was carried out using a Tecan infinite M1000Pro fluorescence microplate reader at excitation wavelength of 530 nm.
Figure S5 Fluorescence emission intensity of 4a, 4b, 4c, and 4d (5 μM) at 620 nm in the absence, or the presence HaloTag or SNAPf protein (10 μM). Fluorescence measurement was carried out using a Tecan infinite M1000Pro fluorescence microplate reader at excitation wavelength of 530 nm.
Figure S6 ESI-Mass spectra of (a) SNAPf after deconvolution (b) SNAPf before deconvolution (c) SNAPf upon conjugation with 4c after deconvolution (b) SNAPf upon conjugation with 4c before deconvolution.
Figure S7 (a) SDS-page gel images of cell lysates containing SNAPf WT or C145A in the absence or presence of 4c. (b) Fluorescence emission spectra of E. coli lysates expressing SNAPf C145A or WT upon conjugating with 4c. E. coli cells expressing SNAPf C145A or WT were harvested, resuspended in buffer (50 mM HEPES, 100 mM NaCl, and 1 mM DTT, pH 7.5), adjusted to OD$_{600}$ = 8.0, lysed using cell disruptor, and centrifuged at 21,000G for 30 min to acquire supernatant. Subsequently, 5 μM of 4c was added to half of the lysate for conjugation with SNAPf. Fluorescence measurement was carried out using a Tecan infinite M1000Pro fluorescence microplate reader at excitation wavelength of 530 nm.
Figure S8 Live-cell fluorescence imaging of HEK293T cells incubated with 4c. Images of cells expressing (a) SNAPf WT-EGFP (b) SNAPf C145A-EGFP. Blue: Hoechst 33342, Red: SNAPf•4c, Green: EGFP.
**Figure S9** Cytotoxicity of **4c** determined by LDH assay (n=3).
Figure S10  Fluorescence emission Intensity of (a) BG-CCVJ (5 μM) (b) 4c (5 μM) in buffer, BSA (2 mg/mL), or the presence of SNAPf (10 μM), and live-cell fluorescence imaging of non-washed or washed HEK293T cells with (c) BG-CCVJ (5 μM) (d) 4c (5 μM).
Figure S11 SDS-page analysis of HEK293T cells samples that were used in confocal imaging experiment. Cells were harvested, lysed in RIPA buffer, centrifuged to acquire supernatant, and adjusted to the same concentration using the Bradford assay kit.
Figure S12  Live-cell fluorescence imaging of HEK293T cells expressing SNAPf WT. HEK293T Cells were transiently transfected with the plasmid encoding SNAPf proteins. After protein expression for 24h, cells were incubated with 1 μM of 4c and Hoechst 33342 for 30 min, and images were taken via confocal microscopy.
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**Table S1** Photophysical properties of 4a, 4b, 4c, 4d and 4a•SNAPf conjugate. The values of 4a – 4d were determined in glycerol and the values of SNAPf•4c conjugate were determined in HEPES buffer (50 mM, pH 7.5) containing NaCl (100 mM) and dithiothreitol (1 mM), respectively.
$^1$H NMR Spectrum of 3a
$^1$H NMR Spectrum of $3b$
$^1$H NMR Spectrum of 3c
$^1$H NMR Spectrum of 3d
$^1$H NMR Spectrum of 4a
$^1$H NMR Spectrum of 4b
$^1$H NMR Spectrum of 4c
$^1$H NMR Spectrum of 4d
$^{13}$C NMR Spectrum of 3a

\[ 170.36 \quad 169.23 \quad 157.30 \quad 151.90 \quad 141.16 \quad 130.43 \quad 125.78 \quad 122.68 \quad 112.60 \quad 112.46 \quad 107.79 \]

3a
$^{13}$C NMR Spectrum of 3b

173.38 167.18 159.91 152.77 141.15 130.38 125.53 122.69 112.59 110.76

3b
$^{13}$C NMR Spectrum of 3c

3c
$^{13}$C NMR Spectrum of 3d
$^{13}$C NMR Spectrum of 4a

![Chemical Structure Image]
\textsuperscript{13}C NMR Spectrum of 4b
$^{13}$C NMR Spectrum of $4c$

$4c$
$^{13}$C NMR Spectrum of 4d