Supporting Information

Design and Synthesis of Fluorescent Probe Based on “On-Off” Behavior of Dansyl Group through Controlling Quenching Efficiency of Cyanopyranyl Group by Interaction with Proteins

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Materials and methods
Reagents

Broad-range molecular mass standards for SDS-PAGE were obtained from Merck and contained approximately equal-weight mixtures of ovotransferrin (78 KDa), bovine serum albumin (BSA) (66 kDa), ovalbumin (43 kDa), and carboanhydrase (30 kDa).

The other chemicals used were of analytical reagent grade and were purchased from TCI (Tokyo, Japan), Wako (Osaka, Japan), Aldrich, Bio-Rad, Invitrogen, and GE-Healthcare.

Synthesis of fluorometric reagent compound 1

The synthetic approach for compound 1 is shown in Scheme S1.

\[ \text{Scheme S1. Synthetic approach for compound 1.} \]

Detailed preparation methods and characterizations for each compound are as follows:

\[ \text{N-(2-Bromoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (3). To a} \]
solution of 2-bromoethylamine hydrobromide (1.07 g, 4.88 mmol) and triethylamine (0.98 g, 9.76 mmol) in 50 mL THF, dansyl chloride (1.44 g, 5.37 mmol) was added through a dropping funnel with ice bath cooling, stirred for 30 min, and then warmed to room temperature for 12 hours. After addition of water, the solvent was evaporated in vacuo. The residue was dissolved in ethyl acetate, washed with water, and dried over Na$_2$SO$_4$. After removal of the solvent, the product was purified by column chromatography (SiO$_2$; n-hexane/ethyl acetate = 2:1 v/v).

Yield: 89 %.

$^1$H NMR (500 MHz, CDCl$_3$, r.t., TMS, $\delta$): 2.89 (6H, s), 3.30–3.32 (4H, m), 5.19 (1H, t), 7.19 (1H, d), 7.52 (1H, t), 7.57 (1H, t), 8.24 (2H, t), 8.55 (1H, d).

$^{13}$C NMR (125 MHz, CDCl$_3$, r.t., TMS, $\delta$): 31.7, 44.6, 45.4, 115.4, 118.6, 123.1, 128.6, 129.5, 129.6, 129.9, 130.8, 134.5, 152.1.

ESI-MS(+) m/z [M + H]$^+$ = 357.17, calcd for C$_{14}$H$_{17}$BrN$_2$O$_2$S 356.02

Anal. Calcd for C$_{14}$H$_{17}$BrN$_2$O$_2$S: C, 47.07; H, 4.80; N, 7.84. Found: C, 47.01; H, 4.76; N, 7.83.

5-(Dimethylamino)-N-(2-(4-formylphenoxy)ethyl)naphthalene-1-sulfonamide (4).

To a solution of N-(2-bromoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (1.55 g, 4.19 mmol) in 100 mL acetone, 4-hydroxybenzaldehyde (0.56 g, 4.61 mmol) and potassium carbonate (3.18 g, 23.05 mmol) were added and then refluxed for 24 hours under argon atmosphere. After removal of the solvent, the residue was dissolved in ethyl acetate, washed with brine, and dried over Na$_2$SO$_4$. After removal of the solvent, the product was purified by column chromatography (SiO$_2$; n-hexane/ethylacetate = 1:1 v/v).

Yield: 74 %.

$^1$H NMR (CDCl$_3$, 500MHz, r.t., TMS, $\delta$): 2.85 (6H, s), 3.36–3.39 (2H, m), 3.87-3.89 (2H, m), 5.26 (1H, t), 6.69 (2H, d), 2.70 (1H, d), 7.49–7.53 (2H, m), 7.72 (2H, d), 8.24-8.28 (2H, m), 8.52 (1H, d), 9.86 (1H, s).

$^{13}$C NMR (125 MHz, CDCl$_3$, r.t., TMS, $\delta$): 42.5, 45.4, 66.4, 114.5, 115.2, 118.4, 123.1, 128.6, 129.5, 129.9, 130.3, 130.7, 131.8, 134.8, 152.1, 162.8, 190.7.

ESI-MS(+) m/z [M + H]$^+$ = 399.26, calcd for C$_{21}$H$_{22}$N$_2$O$_4$S 398.13

Anal. Calcd for C$_{21}$H$_{22}$N$_2$O$_4$S: C, 63.30; H, 5.56; N, 7.03. Found: C, 63.38; H, 5.52; N, 7.07.

(E)-N-(2-(4-(2-(4-(Dicyanomethylene)-6-methyl-4H-pyran-2-yl)vinyl)phenoxy)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (compound 1). To a solution of
5-(dimethylamino)-N-(2-(4-formylphenoxy)ethyl)naphthalene-1-sulfonamide (0.72 g, 1.74 mmol) in 70 mL ethanol, 4-(dicyanomethylene)-2,6-dimethyl-4H-pyran (0.30 g, 1.74 mmol) and piperidine (0.18 g, 2.09 mmol) were added and then refluxed for 12 hours under argon atmosphere. After removal of the solvent, the residue was washed with water and dried over Na$_2$SO$_4$. After removal of the solvent, the product was purified by column chromatography (SiO$_2$; chloroform/methanol = 10:1 v/v).

Yield: 67 %.

$^1$H-NMR (CDCl$_3$, 500MHz, r.t., TMS, $\delta$): 2.40 (3H, s), 2.87 (6H, s), 3.33-3.36 (2H, m), 3.89-3.91 (2H, m), 5.16 (1H, t), 6.53-6.56 (2H, m), 6.65-6.69 (3H, m), 7.15 (1H, d), 7.34-7.40 (3H, m), 7.51-7.57 (2H, m), 8.26-8.28 (m, 2H), 8.54 (d, 1H).

$^{13}$C NMR (125 MHz, CDCl$_3$, r.t., TMS, $\delta$): 22.6, 29.7, 31.6, 42.6, 45.4, 66.4, 106.4, 106.7, 114.9, 115.1, 115.3, 116.1, 118.5, 123.2, 127.8, 128.1, 128.6, 129.4, 129.6, 130.0, 130.7, 134.7, 137.5, 152.1, 156.4, 159.4, 159.8, 161.9.

ESI-MS(+) m/z [M + H]$^+$ = 553.25, calcld for C$_{31}$H$_{28}$N$_4$O$_4$S 552.18


Extinction coefficient of compound 1 itself was 18000 at 340 nm ($\lambda_{max}$). Extinction coefficient of compound 1 in the presence of 10 $\mu$g/mL of BSA was 19000 at 340 nm ($\lambda_{max}$).

**Synthesis of fluorometric reagent Reference 2**

The synthetic approach for reference 2 is shown in Scheme S2.

Detailed preparation methods and characterizations for each compound are as follows:

5-(dimethylamino)-N-(2-phenoxyethyl)naphthalene-1-sulfonamide (Reference 2). To a solution of N-(2-Bromoethyl)-5-(dimethylamino)naphthalene-1-sulfonamide
(0.43 g, 1.21 mmol) in 50 mL acetone, phenol (0.14 g, 1.44 mmol) and K₂CO₃ (0.99 g, 7.20 mmol) were added and then refluxed for 12 hours under nitrogen atmosphere. After removal of the solvent, the residue was dissolved in ethyl acetate, washed with water and dried over Na₂SO₄. After removal of the solvent, the product was purified by column chromatography (SiO₂; n-hexane : ethyl acetate = 1.5 : 1 v/v).

Yield: 72%.

¹H-NMR (CDCl₃, 500MHz, r.t., TMS, δ) 2.87 (6H, s), 3.30-3.34 (2H, m), 3.82-3.84 (2H, m), 5.17 (1H, t), 6.62 (2H, d), 6.92 (1H, t), 7.14-7.18 (3H, m), 7.49-7.62 (2H, m), 8.25 (2H, d), 8.53 (1H, d).

¹³C NMR (500 MHz, CDCl₃, r.t., TMS, δ): 43.1, 45.8, 66.4, 114.7, 115.7, 119.0, 121.7, 123.5, 128.9, 129.8, 129.9, 130.0, 130.3, 131.0, 135.2, 152.4, 158.3.

ESI-MS(+) m/z [M + H]⁺ = 371.53, calcd for C₂₀H₂₂N₂O₃S 370.14


Synthesis of fluorometric reagent Reference 3

The synthetic approach for reference 3 is shown in Scheme S3.

![Scheme S3](image)

Reference 3

**Scheme S3.** Synthetic approach for reference 3.

Detailed preparation methods and characterizations for each compound are as follows:

4-methoxybenzaldehyde (6). To a solution of 4-hydroxybenzaldehyde (1.00 g, 8.18 mmol) in 100 mL acetone, methyl iodide (11.62 g, 81.88 mmol) and K₂CO₃ (5.65 g,
40.90 mmol) were added and then refluxed for 12 hours under nitrogen atmosphere. After removal of the solvent, the residue was dissolved in ethyl acetate, washed with water and dried over Na$_2$SO$_4$. After removal of the solvent, the product was purified by column chromatography (SiO$_2$; $n$-hexane : ethyl acetate = 1 : 1 v/v).

Yield: 92 %.

$^1$H-NMR (CDCl$_3$, 500MHz, r.t., TMS, $\delta$) 3.89 (3H, s), 7.00 (2H, d), 7.84 (2H, d), 9.88 (1H, s).

$^{13}$C NMR (500 MHz, CDCl$_3$, r.t., TMS, $\delta$): 55.6, 114.3, 130.0, 132.0, 164.6, 190.8.

ESI-MS(+) m/z [M + Na]$^+$ = 159.2, calcd for C$_8$H$_8$O$_2$ 136.0

Anal. Calcd for C$_8$H$_8$O$_2$: C, 70.57; H, 5.92. Found: C, 70.54; H, 5.95.

(E)-2-2-(4-dicyanomethylene)-6-methyl-4H-pyran-4-ylidene (Reference 3). To a solution of 4-methoxybenzaldehyde (0.20 g, 1.47 mmol) in 20 mL EtOH, 4-(dicyanomethylene)-2,6-dimethyl-4H-pyran (0.25 g, 1.47 mmol) and piperidine (0.15 g, 1.76 mmol) were added and then refluxed for 12 hours under argon atmosphere. After removal of the solvent, the residue was washed with water and dried over Na$_2$SO$_4$. After removal of the solvent, the product was purified by column chromatography (SiO$_2$; $n$-hexane : ethyl acetate = 1:1 v/v).

Yield: 67 %.

$^1$H-NMR (CDCl$_3$, 500MHz, r.t., TMS, $\delta$) 2.39 (3H, s), 3.86 (3H, s), 6.52-6.53 (1H, m), 6.58 (d, 1H), 6.64-6.65 (1H, m), 6.94 (2H, d), 7.39 (1H, d), 7.48 (2H, d).

$^{13}$C NMR (500 MHz, CDCl$_3$, r.t., TMS, $\delta$): 19.6, 55.1, 106.0, 106.1, 114.3, 114.8, 115.4, 126.9, 129.1, 137.4, 156.0, 159.2, 161.2, 161.5.

ESI-MS(+) m/z [M + Na]$^+$ = 313.3, calcd for C$_{18}$H$_{14}$N$_2$O$_2$ 290.1

Anal. Calcd for C$_{18}$H$_{14}$N$_2$O$_2$: C, 74.47; H, 4.86. Found: C, 74.49; H, 4.83.

**SDS-PAGE and 2-DE staining protocols for compound 1 and gel imaging procedure.** Compound 1 was dissolved in DMSO and then diluted 1000 times with a mixture of 25 mM phosphate buffer solution (pH 3.0), H$_2$O, and MeOH (in the ratio 45:45:10 (v/v), respectively). After SDS-PAGE, the gel was exposed to the solution that contained compound 1, for 60 min, and then washed with water for 15 min. Fluorescent gel imaging was carried out using ChemiDoc MP imaging system (Bio-rad, USA). The excitation wavelength used was 365 nm. The obtained gel images were analyzed using Image Lab software. The resulting fluorescent gel images were black/white inverted.
**Miscellaneous.** The $^1$H NMR spectra and $^{13}$C NMR spectra were recorded on a Bruker AV500M spectrometer. The chemical shifts are reported in parts per million relative to tetramethylsilane, which is used as the internal reference. The mass spectrometer for the measurement of molecular weights of compound 1 and its intermediates was a Micromass ZQ2000 (Waters; USA). The absorption spectra were recorded at 25°C on a JASCO V-670 UV/visible spectrophotometer. The fluorescence spectra were recorded at 25°C on a JASCO FP-6500 spectrofluorophotometer. The separation of the proteins by SDS-PAGE was carried out using the XV PANTERA System (DRC Co., Ltd., Tokyo, Japan). 2-DE was performed by Auto2D (Sharp Manufacturing Systems Corporation, Osaka, Japan).
Fig. S1 Plot of the fluorescence intensity at 480 nm as a function of the BSA concentration. [compound 1] = 18.1 μM. Solvent: HEPES buffer solution (pH 7.0). Excitation wavelength: 340 nm. Temperature: room temperature.
**Fig. S2** Excitation spectrum (red line) and fluorescence spectrum (blue line) of reference 2 in the presence of 50 µg/mL BSA (a); fluorescence spectra of reference 2 before (blue line) and after (red line) the addition of 50 µg/mL BSA (b); excitation spectrum (red line) and fluorescence spectrum (blue line) of reference 3 in the presence of 50 µg/mL BSA (c); and fluorescence spectra of reference 3 before (blue line) and after (red line) the addition of 50 µg/mL BSA (d). [reference 2] = [reference 3] = 18.1 µM. Solvent: HEPES buffer solution (pH 7.0). Excitation wavelength: 345 nm for reference 2, and 450 nm for reference 3. Temperature: room temperature.
**Fig. S3** Fluorescence spectra of compound 1 (red line), reference 2 (blue line), and reference 3 (green line) in the absence of BSA (a). [compound 1] = [reference 2] = [reference 3] = 18.1 µM. Solvent: HEPES buffer solution (pH 7.0). Excitation wavelength: 340 nm for compound 1, 345 nm for reference 2, and 450 nm for reference 3. Temperature: room temperature.