

Supplementary Information
for
**Selective labeling of tag-fused protein by
tryptophan-sensitized luminescence of a terbium complex**

**Tasuku Hirayama,^a Masayasu Taki,^{*a,b} Atsushi Kodan,^c Hiroaki Kato^d and
Yukio Yamamoto^a**

^a *Graduate School of Human and Environmental Studies*

Kyoto University, Yoshida-nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan

^b *Graduate School of Global Environmental Studies*

Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan

^c *Institute for Integrated Cell-Material Sciences, Kyoto University, 69 Konoe-cho, Yoshida,
Sakyo-ku, Kyoto 606-8501, Japan*

^d *Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Kyoto
University, 46-29 Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan*

* To whom correspondence should be addressed

Tel: +81-75-753-7871; Fax: +81-75-753-6833

Email address: taki@chem.mbox.media.kyoto-u.ac.jp

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1. Synthesis

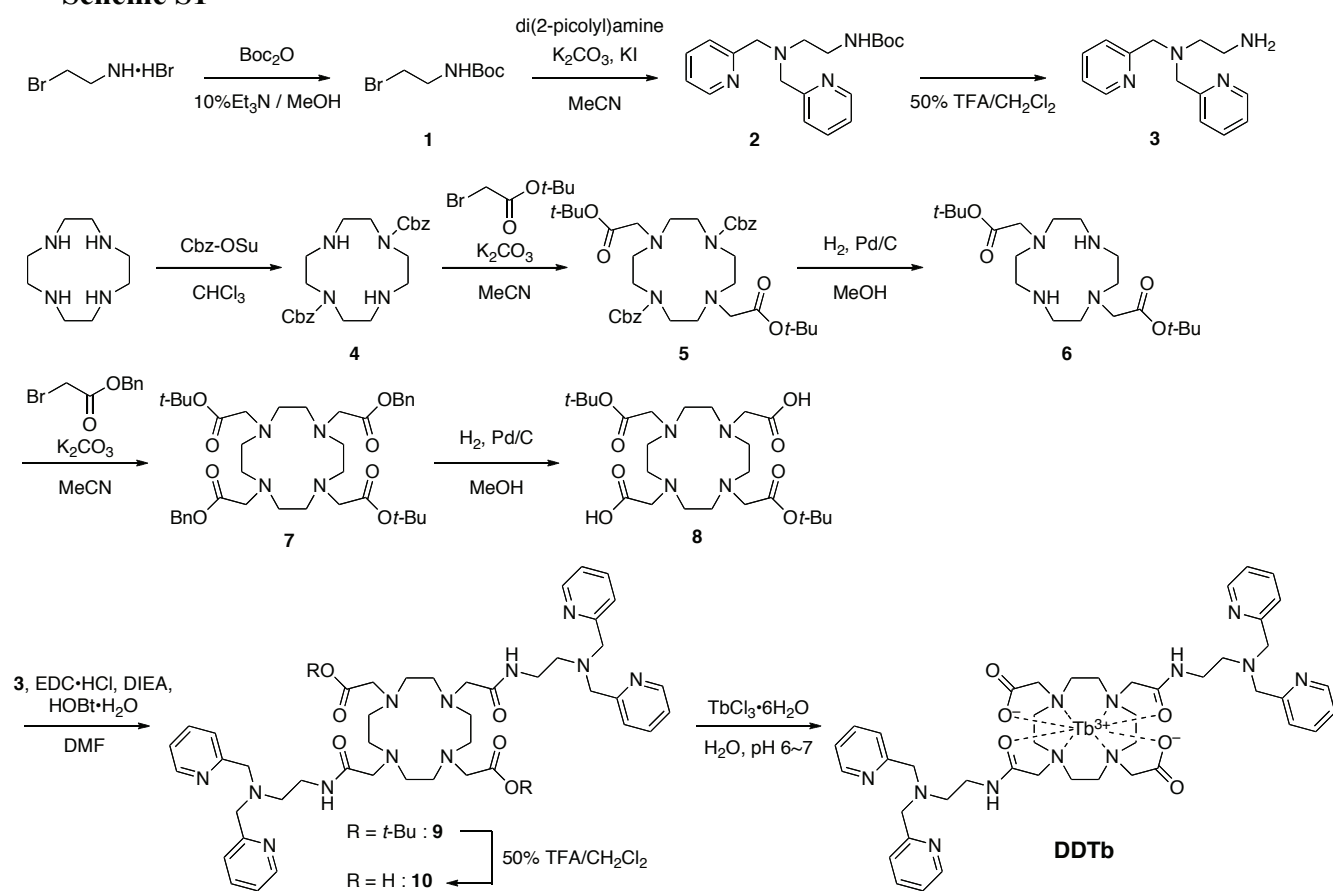
General. All chemicals used in this study were commercial products of the highest available purity and were further purified by the standard methods, if necessary. NMR spectra were recorded on a JEOL JNM-EX-270 (at 270 MHz to ^1H , 68 MHz to ^{13}C) or a JEOL alpha-500 (at 500 MHz to ^1H , 126 MHz to ^{13}C). Chemical shifts are given in ppm relative to tetramethylsilane (TMS) and J values are given in Hz. HRMS were done with a JEOL JMS-700. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) was performed on a Voyager-DE STR system (Applied Biosystems). Elemental analysis was done with a Yanaco CHN Corder MT-5. TLC analyses were performed on Silica gel 60-F₂₅₄ (Merck). Flash chromatography was performed on silica gel (Merck Silica Gel 60). Reverse phase HPLC was performed on Waters Delta 600 system (4.6 × 150 mm, XBridge™C₁₈ for analytical HPLC, and 10 × 250 mm for preparative HPLC).

Abbreviation

DMF	: <i>N,N</i> -dimethylformamide
TFA	: trifluoroacetic acid
HOBt•H ₂ O	: 1-hydroxybenzotriazole monohydrate
EDC•HCl	: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HBTU	: <i>O</i> -benzotriazole- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
IPTG	: isopropyl β - <i>D</i> -thiogalactopyranoside
PMSF	: phenylmethylsulfonyl fluoride

Synthesis of DDTb

Scheme S1



2-(Di(2-picolyl)amino)ethylamine (**3**) was synthesized as reported procedure with modification.^[i]

tert-Butyl (2-bromoethyl)carbamate (**1**)

To a cooled solution of 2-bromoethylamine hydrobromide (1.0 g, 4.9 mmol) in 10 % triethylamine/MeOH was added di-*t*-butyl dicarbonate (1.1 g, 4.9 mmol). After refluxing for 8 h, the solvent was evaporated. The residue was dissolved in AcOEt (50 mL). The solution was washed successively with 0.1 M HCl, sat. NaHCO_3 , water, and brine, and then dried over potassium carbonate. Evaporation of the solvent yielded the title compound (0.63 g, 58 %). $^1\text{H-NMR}$ (CDCl_3 , 270 MHz): δ 1.46 (9 H, s), 3.46 (2 H, q, $J = 5.1$ Hz), 3.53 (4 H, t, $J = 5.1$ Hz).

tert-Butyl [2-(di(2-picolyl)amino)ethyl]carbamate (**2**)

To a mixture of **1** (633 mg, 2.83 mmol), potassium carbonate (390 mg, 2.83 mmol), and potassium iodide (95 mg, 0.57 mmol) in MeCN (15 mL) was added di(2-picolyl)amine (558 mg, 2.83 mmol). After refluxing overnight, the mixture was filtered through a pad of celite and the filtrate was evaporated. The residue was dissolved in AcOEt (50 mL), and the mixture was washed with water (30 mL \times 3). The organic layer was dried over potassium carbonate before evaporation. The residue was purified by flash chromatography (AcOEt-MeOH, 10:1) to give the title compound (388 mg,

40 %). ¹H-NMR (CDCl₃, 270 MHz): δ 1.45 (9 H, s), 2.70 (2 H, t, *J* = 6.3 Hz), 3.23 (2 H, q, *J* = 6.3 Hz), 3.87 (4 H, s), 7.15 (2 H, t, *J* = 4.7 Hz), 7.42 (2 H, d, *J* = 7.9 Hz), 7.64 (2 H, td, *J* = 7.9, 2.0 Hz), 8.55 (2 H, dd, *J* = 4.7, 0.7 Hz).

2-(Di(2-picolyl)amino)ethylamine (3)

A solution of **2** (388 mg, 1.13 mmol) in 50% TFA/CH₂Cl₂ was stirred at room temperature overnight. After evaporation, water was added to the residue, and pH of the mixture was adjusted to 12 by addition of 2 M NaOH. The aqueous layer was extracted by CH₂Cl₂ (30 mL × 3), and then the combined organic layer was dried over potassium carbonate before evaporation to yield the title compound (237 mg, 87 %). ¹H-NMR (CDCl₃, 270 MHz): 2.70 (2 H, t, *J* = 5.6 Hz), 2.80 (2 H, q, *J* = 5.6 Hz), 3.85 (4 H, s), 7.15 (2 H, t, *J* = 6.0 Hz), 7.50 (2 H, d, *J* = 7.9 Hz), 7.66 (2 H, td, *J* = 7.9, 2.0 Hz), 8.54 (2 H, d, *J* = 4.7 Hz).

1,7-Bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (4)

Symmetrical di-protection of 1,4,7,11-tetraazacyclododecane (cyclen) was performed by the reported procedure.⁽ⁱⁱ⁾ In brief, 1.0 g (5.8 mmol) of cyclen (purchased from Aldrich Chemical Company Inc.) and 2.9 g (11.6 mmol) of *N*-(benzyloxycarbonyloxy)succinimide (Cbz-OSu) was dissolved in CHCl₃ (20 mL), and the solution was stirred at room temperature for 2 days. After evaporation, the residue was suspended in 1 M NaOH, and the aqueous suspension was extracted by CH₂Cl₂ (40 mL × 5). The organic layer was washed with brine, dried over K₂CO₃, and then evaporated to yield di-protected cyclen **4** (2.56 g, 100 %). This compound was used without further purification. ¹H-NMR (CDCl₃, 270 MHz): δ 2.75-2.94 (8 H, m_{br}), 3.43 (8 H, m_{br}), 5.15 (4 H, s), 7.27-7.35 (10 H, m).

1,7-Bis(benzyloxycarbonyl)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (5)

To a mixture of **4** (2.56 g, 5.8 mmol) and potassium carbonate (2.0 g, 14.5 mmol) in MeCN (30 mL) was added *tert*-butyl bromoacetate (2.26 g, 11.6 mmol). After refluxing overnight, the mixture was filtered through a pad of celite, and then evaporated. The residue was purified by flash chromatography (CH₂Cl₂-MeOH, 98:2) to give **5** (2.52 g, 65 %) as a colorless gum. ¹H-NMR (CDCl₃, 270 MHz): δ 1.43 (18 H, s), 2.87 (8 H, s_{br}), 3.42-3.49 (12 H, m_{br}), 5.12 (4 H, s), 7.27-7.35 (10 H, m).

1,7-Bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (6)

A mixture of **5** (2.52 g, 3.7 mmol) and 10% Pd/C (100 mg) in MeOH (40 mL) was stirred under hydrogen atmosphere (2.0 kg/cm²) at room temperature overnight. After filtration through a pad of celite, evaporation of the solvent gave **6** (1.5 g, 100%) as a white solid. ¹H-NMR (CDCl₃, 270 MHz): δ 1.46 (18 H, s), 2.62 (8 H, s_{br}), 2.80 (8 H, s_{br}), 3.31 (4 H, s).

1,7-Bis(benzyloxycarbonylmethyl)-4,10-bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (7)

To a mixture of **6** (330 mg, 0.75 mmol) and potassium carbonate (228 mg, 1.7 mmol) in MeCN (15 mL) was added benzyl bromoacetate (344 mg, 1.5 mmol). After refluxing overnight, the mixture was filtered through a pad of celite, and then evaporated. The residue was purified by flash chromatography (CH₂Cl₂-MeOH, 97:3) to give **7** (246 mg, 47%) as a colorless gum. ¹H-NMR (CDCl₃, 270 MHz): δ 1.42 (18 H, s), 2.30-3.50 (24 H, m_{br}), 5.15 (4 H, s), 7.30-7.38 (10 H, m).

1,7-Bis(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (8)

A mixture of **7** (487 mg, 0.70 mmol) and 10% Pd/C (50 mg) in MeOH (20 mL) was stirred under hydrogen atmosphere (2.0 kg/cm²) at room temperature overnight. After filtration through a pad of celite, evaporation of the solvent gave **8** (361 mg, 100%) as a white solid. ¹H-NMR (CDCl₃, 270 MHz): δ 1.48 (18 H, s), 2.30-3.50 (24 H, m_{br}).

1,7-Bis(*tert*-butoxycarbonylmethyl)-4,10-bis{[*N*-(di(2-picolyl)aminoethyl)carbamoyl]methyl}-1,4,7,10-tetraazacyclododecane (9)

A mixture of **3** (98 mg, 0.29 mmol), **8** (75 mg, 0.15 mmol), EDC•HCl (55 mg, 0.29 mmol), ethyl-diisopropylamine (49 μL, 0.29 mmol), and HOBt•H₂O (39 mg, 0.29 mmol) in DMF (5 mL) was stirred at room temperature for 24 h, and then the solvent was removed under reduced pressure. CH₂Cl₂ (30 mL) was added to the residue, and then the mixture was washed successively with 0.5 M NaOH (30 mL × 2), 10% NaHCO₃ (30 mL), and brine (30 mL). The organic layer was dried over potassium carbonate before evaporation. The residue was purified by flash column chromatography (CH₂Cl₂-MeOH-28% NH₃aq, 100:10:1) to give **9** (45 mg, 31%) as a beige solid. ¹H-NMR (CDCl₃, 500 MHz): δ 1.32 (18 H, s), 2.35-2.60 (16 H, m_{br}), 2.76 (4 H, t, *J* = 5.6 Hz), 3.25 (8 H, s_{br}), 3.39 (4 H, q, *J* = 5.6 Hz), 3.85 (8 H, s), 7.15 (4 H, t, *J* = 4.8 Hz), 7.47 (4 H, d, *J* = 7.6 Hz), 7.66 (4 H, td, *J* = 7.6, 1.8 Hz), 8.47 (2H, s_{br}), 8.54 (4 H, d, *J* = 4.8 Hz); ¹³C-NMR (CDCl₃, 126 MHz) 28.1, 37.6, 50.6, 50.8, 53.2, 55.7, 56.6, 60.0, 81.5, 122.1, 123.4, 136.7, 148.9, 159.3, 171.2, 171.5; HRMS (FAB): calcd for C₅₂H₇₆ N₁₂O₆Na (M⁺) 987.5903, found 987.5901.

1,7-Bis{[N-(di(2-picolyl)aminoethyl)carbamoyl]methyl}-1,4,7,10-tetraazacyclododecane-4,10-diacetic acid (10)

A solution of **9** (45 mg, 0.047 mmol) in 50% TFA/CH₂Cl₂ (10 mL) was stirred at room temperature overnight. The solvent was evaporated, and then diethyl ether was added. A precipitate was collected by filtration and dried in vacuo to yield **10** (40 mg, 100%). ¹H-NMR (CD₃OD, 500 MHz): δ 2.90-3.45 (32 H, m_{br}), 4.26 (8 H, s), 7.54 (4 H, t, *J* = 2.8 Hz), 7.47 (4 H, d, *J* = 4.3 Hz), 7.66 (4 H, t, *J* = 4.3 Hz), 8.60 (4 H, d, *J* = 2.8 Hz); ¹³C-NMR (CD₃OD, 126 MHz): δ 37.1, 50.7, 51.8, 55.2, 56.2, 58.0, 126.0, 126.9, 142.8, 147.4, 154.4, 162.6, 162.9; HRMS (FAB): calcd for C₄₄H₆₁N₁₂O₆ (MH⁺) 853.4832, found 853.4841.

DDTb

A solution of **10** (40 mg, 0.047 mmol) and TbCl₃•6H₂O (17.5 mg, 0.047 mmol) in water (5 mL) was stirred at 80 °C overnight with pH of the solution adjusted to ~7 by addition of 2 M NaOH, and then pH was brought up to 11 by 2 M NaOH before filtration. After readjustment of pH to ~6 with 6 M HCl, ethanol (10 mL) was added. The mixture was filtered through a pad of celite and the filtrate was evaporated. The residue was dissolved into a minimum amount of ethanol, and diethyl ether (10 mL) was added. A pale yellow precipitate was collected and dried in vacuo to afford DDTb as a pale yellow powder. The crude DDTb was purified by reverse-phase HPLC (XBridgeTMC₁₈ 10 × 250 mm) with a linear gradient 100% A (0.1% TFA in water) and 0% B (0.1% TFA in acetonitrile) to 65% A and 35% B at 4 mL/min over 15 min. The fraction was lyophilized to give pure DDTb (40 mg, 45%) as a pale yellow hygroscopic solid. HRMS (FAB): calcd for C₄₄H₅₈N₁₂O₆Tb (M⁺) 1009.3839, found 1009.3848; Anal. Calcd for C₅₈H₇₃F₂₁N₁₂O₂₅Tb (DDTb+6H⁺+7CF₃COO⁻+6H₂O): C, 36.74; H, 3.88; N, 8.86. Found: C, 36.69; H, 3.50; N, 8.67.

DDTbZn₂

To a solution of 10 μmol DDTb in Milli-Q water (1.8 mL) was added 100 mM ZnCl₂ (0.2 mL). The 5 mM stock solution of DDTbZn₂ thus prepared was stored in refrigerator at -10 °C.

DDEuZn₂

DDEu was synthesized with the same procedure as for DDTb except that Eu(OTf)₃ was used instead of TbCl₃•6H₂O. DDEuZn₂ was prepared as described for DDTbZn₂.

Peptides Synthesis and Purification

The model peptides were synthesized by solid phase synthesis (Fmoc-method) using Fmoc-His(Trt)-OH, Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, and Fmoc-Asp(*t*-Bu)-OH. In brief, Fmoc-NH-SAL-resin (0.1 mmol, purchased from Watanabe Chemical Industries, Ltd.) pre-swollen in DMF was deprotected by shaking in 20% piperidine/DMF. After washing with DMF (3 mL \times 5), the Fmoc-protected amino acid (0.3 mmol), HBTU (0.3 mmol), HOBt \cdot H₂O (0.3 mmol), and DIEA (0.3 mmol) was added to a suspension of the resin in DMF (3 mL). The coupling and deprotecting processes were confirmed by the ninhydrin reaction. The suspension was shaken until the reaction was completed. Then, the resin was washed with DMF (3 mL \times 5), and Fmoc group was removed by shaking in 20% piperidine/DMF followed by washing with DMF. This procedure was repeated to obtain a desired peptide. After cleavage from the resin and deprotection of the side-chain by cleavage cocktail (95% TFA, 2.5% water, 2.5% triisopropylsilane), the crude peptide was purified by HPLC (XBridgeTMC₁₈ 10 \times 250 mm) with a linear gradient from 100% solvent A (0.1% TFA in water) and 0% B (0.1% TFA in acetonitrile) to 60% A and 40% B at 4 mL/min over 15 min and the collected fraction was lyophilized to obtain the pure peptide. Each peptide was identified by MALDI-TOF-mass (matrix: CHCA)

H6A2W1: calcd for [M+H]⁺ 1169.23, found 1169.28

D8W1: calcd for [M+H]⁺ 1124.32, found 1124.40

D8A2W1: calcd for [M+H]⁺ 1266.40, found 1266.23

D8A4W1: calcd for [M-H]⁻ 1407.47, found 1407.55

2. Preparation of Tag-fused Glutathione S-Transferase (GST)

The D8A4W1 (DDDDAAWAADDDD) was expressed as the fusion protein with GST at the N terminus. The D8A4W1 tag was introduced into the pGEX-6P-1 vector (Amersham Biosciences, Uppsala, Sweden) via insertion of an oligonucleotide cassette (forward sequence, 5'- GATCC GAT GAC GAT GAC GCC GCA TGG GCC GCA GAT GAC GAT GAC TGA G; reverse sequence, 5'- AATTC TCA GTC ATC GTC ATC TGC GGC CCA TGC GGC GTC ATC GTC ATC G) at the restriction sites of *Bam*HI and *Eco*RI. The resulting construct was termed pGEX-6P-1-D8A4W1 encoding GST-D8A4W1. The DNA sequence of the insert was checked in both orientations by using a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA). The sequence analysis was carried out by an ABI PRISM 3100 sequencer (Applied Biosystems). The pGEX-6P-1-D8A4W1 was transformed into *E. coli* strain BL21(DE3). The resultant *E. coli* transformant was cultured in Luria-Bertani medium (2 L) containing 50 μ g/ml ampicillin at 37 °C on a shaker at 200 rpm. When the OD₆₀₀ reached 0.5~0.6, 1 mM IPTG was added to induce protein expression, and the culture was held at 37 °C on a shaker at 200 rpm for 3 h.

Cells were harvested by centrifugation, and suspended in 12 mL of sonication buffer (50 mM Tris•HCl, 120 mM NaCl, 1 mM EDTA, 5 mM DTT, 1 mM PMSF, pH 7.4). The suspended cells were disrupted using a Branson Sonifier Model W-250 (Branson Ultrasonics Corp., Danbury, CT, USA) equipped with a horn-tip (70% line voltage, 100% duty cycle, 10 s × 10). To improve solubility of the fusion protein, the given supernatant was solubilized with 1% Triton X-100. After the mixture was incubated for 30 min at 4 °C, the insoluble materials were sedimented by centrifugation at 12,000 × g for 10 min. The supernatant was then incubated with 1 mL of Glutathione Sepharose 4B (Amersham Biosciences) for 1 h. The column was washed with 10 bed volumes of wash buffer (50 mM Tris•HCl, 120 mM NaCl, 1 mM EDTA, pH 7.4), and the GST-D8A4W1 was then eluted with elution buffer (50 mM Tris•HCl, 120 mM NaCl, 16 mM glutathione, pH 8.0). The buffer of the fractions containing GST-D8A4W1 was exchanged to binding buffer (50 mM HEPES, 150 mM NaCl) through a Sephadex G-25 PD-10 column (Amersham Biosciences). Protein concentration was measured using a bicinchronic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) and bovine serum albumin as the standard. The purity of the GST-D8A4W1 preparation was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). GST without the tag was obtained by the same procedure as described above, except that pGEX-6P-1 encoding GST was transformed into BL21(DE3).

3. Steady-state Absorption and Fluorescence Spectroscopy

The UV absorption spectra were recorded on a Hewlett-Packard 8453 spectrometer. Fluorescence spectra were recorded using a Hitachi F-2500 spectrometer with the photomultiplier voltage of 700 V. To reduce fluctuations of the excitation intensity during measurement, the lamp was kept on for 1 h prior to the experiment. The path length was 1 cm with a cell volume of 3.0 mL. For UV-vis titration of DDTb with Zn²⁺, the initial absorbance of 100 μM DDTb in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃) was measured, and Zn²⁺ aliquots (10 μM each) were titrated into the solution. The absorption bands at 275 nm were plotted against the molar ratio of [Zn²⁺]/[DDTb]. The stoichiometries for the reaction between DDTbZn₂ and each model peptide were determined by fluorescence titration (λ_{ex} = 280 nm, slit width 2.5 nm). DDTbZn₂ aliquots (10 μM × 15) were titrated into a peptide solution (70 μM) in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃), and the emission intensities at 545 nm were plotted against the molar ratio of [DDTbZn₂]/[peptide]. For the determination of the dissociation constants (K_d) by fluorescence spectroscopy (λ_{ex} = 290 nm, slit width 5.0 nm), DDTbZn₂ aliquots (0.1 μM × 20) were titrated into a peptide solution (1 μM) in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃). The subtracted fluorescent intensities (ΔF = F - F₀, where F₀ means the initial fluorescence intensity, and ΔF_∞ means saturated fluorescent intensity) at

545 nm were plotted against [DDTbZn₂] added, and the experimental data were analyzed by non-linear least square curve fitting using the following equation:

$$\Delta F = \frac{\Delta F_{\infty} \{ [\text{pep}]_0 + [\text{L}] + K_d - \sqrt{([\text{pep}]_0 + [\text{L}] + K_d)^2 - 4[\text{pep}]_0 \times [\text{L}]} \}}{2[\text{pep}]_0}$$

in which [pep]₀ represents the total concentration of the peptide (1.0×10^{-6} or 0.5×10^{-6} M), and [L] does the total concentration of DDTbZn₂ added.

4. Isothermal Titration Calorimetry (ITC)

All experiments were performed in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃) at 25 °C using a VP-ITC MicroCalorimeter (MicroCal, Northampton, USA). The cell volume was 1.453 mL, and the syringe volume was 250 μL. DDTbZn₂ (100 μM) was titrated into the cell (a 10 μL volume per injection) containing the peptide (10 μM). In each experiment, 25 injections were made with a 180 s interval between injections. The stirring rate was 307 rpm throughout the experiment. The ITC binding curves were analyzed using the single-site binding equation in the MicroCal ORIGIN software package provided by the manufacturer, and the K_d , n , ΔH , and ΔS values were calculated.

5. Time-resolved Luminescence and Luminescence Lifetime Measurements

The time resolved luminescence spectra of DDTbZn₂ (100 μM) were measured in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃) containing each peptide by using Perkin-Elmer LS-55 (Beaconsfield, Buckinghamshire, England). The slit width was 10 nm for both of excitation and emission. All spectra were measured by using delay time of 0.05 ms, excitation wavelength of 280 nm, and gate time of 1.00 ms. Luminescence lifetimes of DDTbZn₂ (100 μM) were measured in aqueous buffered solution (50 mM HEPES, 100 mM KNO₃) of H₂O (pH 7.4) or D₂O (pD 7.4, based on the equation pD = pH + 0.40^[iii]) containing 100 μM of the peptide. The data ($\lambda_{\text{ex}} = 280$ nm, $\lambda_{\text{em}} = 545$ nm) were fitted to a single exponential curve obeying the below equation, where I_0 and I are the luminescence intensities at the time $t = 0$ and time t respectively, and τ is the luminescence emission lifetime.

$$I = I_0 \exp(-t/\tau)$$

Calculation of q values was done by the below equation.^[iv]

$$q = 5 \left(\frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} - 0.06 \right)$$

Time Resolved Luminescence Measurements with GST-D8A4W1 and Native GST

Time resolved luminescence spectra of DDTbZn₂ with purified GST-D8A4W1 and native GST were measured under the same experimental conditions described above except for the concentrations of the samples ([DDTbZn₂] = 50 μM, [GST] = 0.5 mg/mL). The lysate solutions were prepared by sonication of GST-D8A4W1 or native GST expressed *E. coli* in a buffer (50 mM Tris•HCl, 120 mM NaCl, 1 mM PMSF, pH 7.4) before removal of insoluble material by centrifugation (12,000 × g, 10 min). The obtained solutions were then diluted with 50 mM HEPES (pH 7.4) until the total concentrations of proteins were reached to ca. 1 mg/mL, which were determined by BCA assay.

6. NMR spectroscopy

¹H NMR spectra of D8A4W1 in the presence and absence of DDEuZn₂ were measured by using JEOL alpha-500 at 25 °C. The samples were prepared as follows. A solution of D8A4W1 (1 mM) in 50 mM HEPES buffer (pH 7.0, 1 mL) was lyophilized, and the dried residue was re-dissolved in 1 mL of D₂O. This process was repeated 3 times. Finally, the sample was dissolved in 1 mL of D₂O to afford a pD-buffered solution (pD = 7.4) of D8A4W1. A pD-buffered solution of DDEuZn₂ (1 mM) was prepared with the same procedure as described for D8A4W1. The DDEuZn₂ solution (0.5 mL) was added to the solution of D8A4W1 (0.5 mL), and the resulting complex (1 mM in 50 mM HEPES, pD = 7.4) was measured.

6. References

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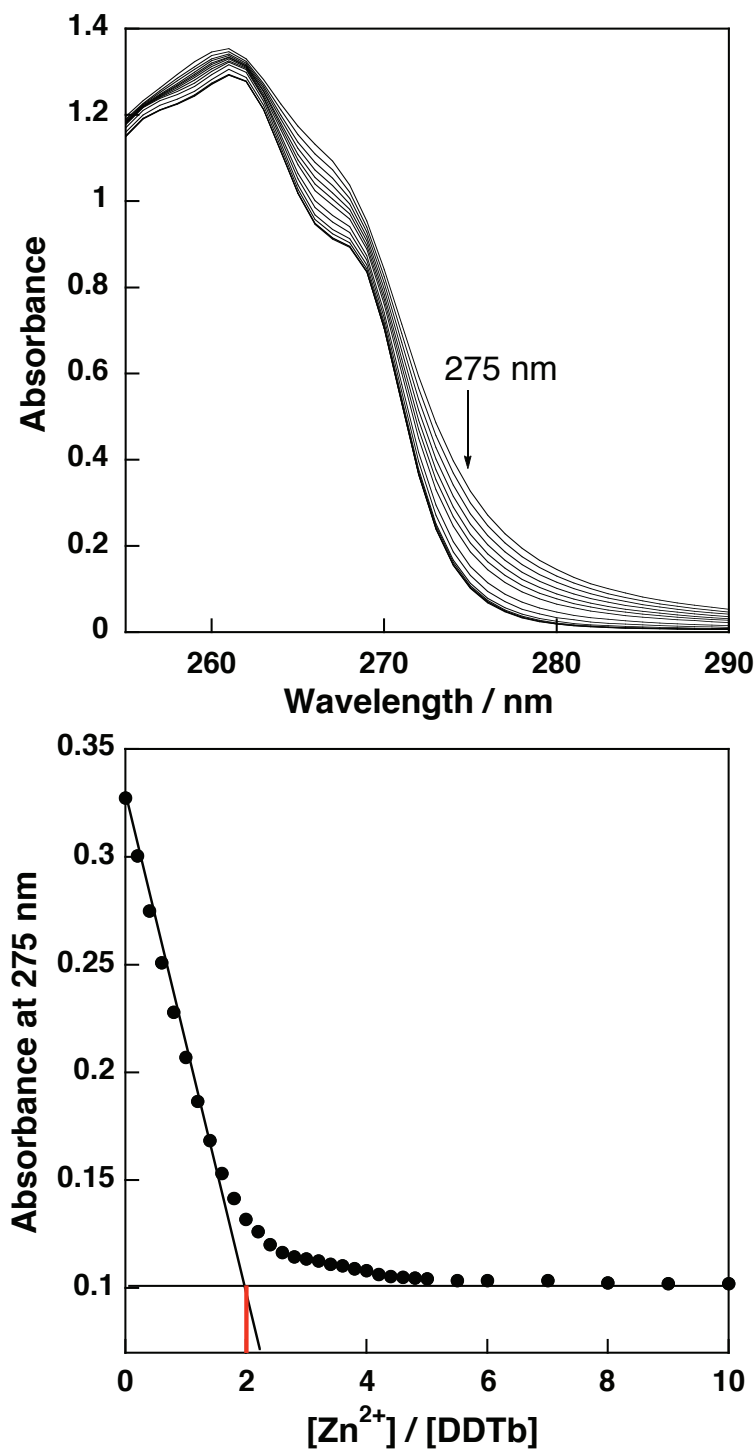


Fig. S1 UV-vis spectral change upon addition of Zn²⁺ ion (0–1 mM) to 100 μM DDTb in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃) (top). Molar ratio plot of absorbance at 275 nm (bottom).

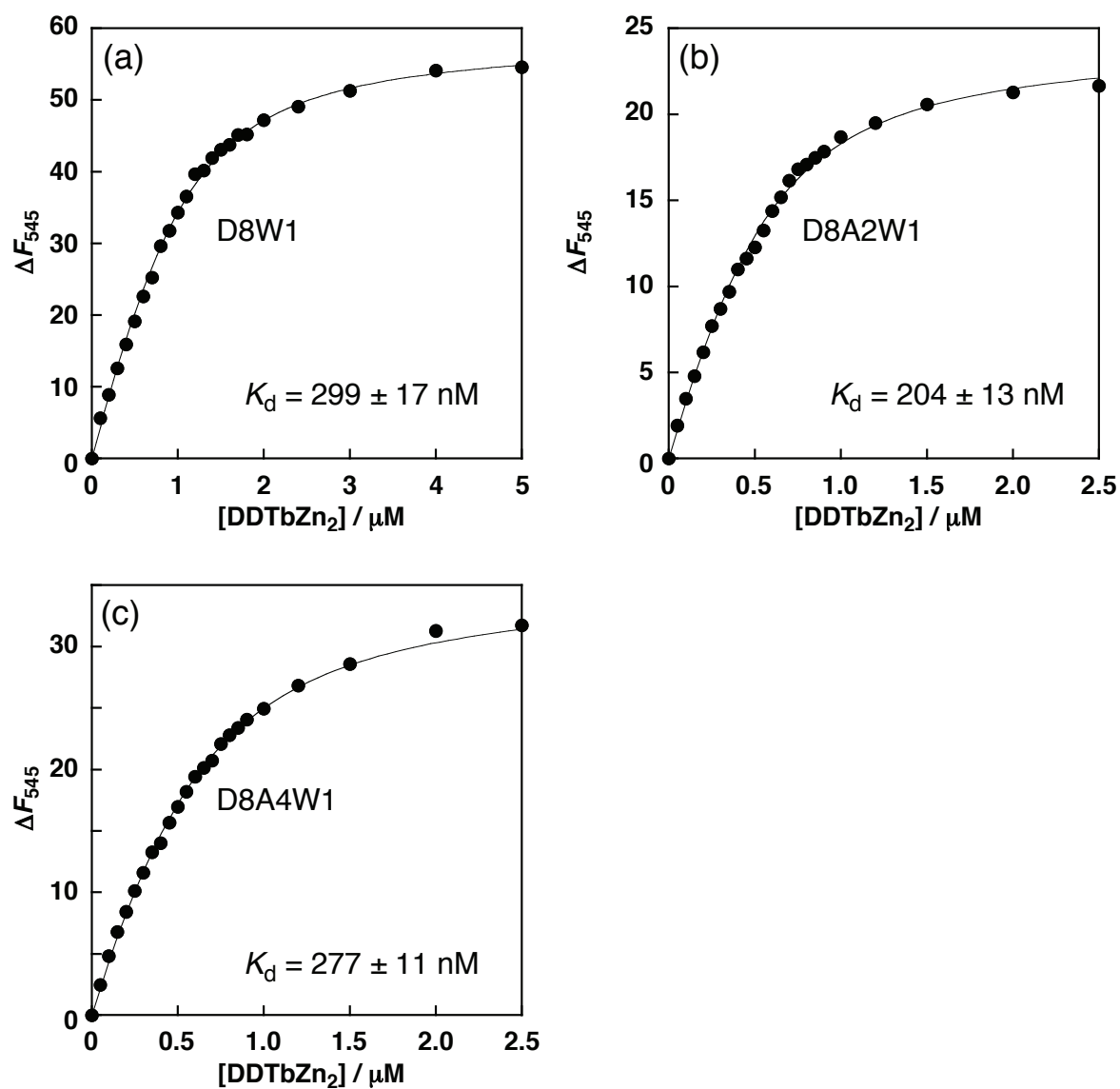


Fig. S2 Plots of $\Delta F (= F - F_0)$ at 545 nm with the best-fitting binding curves. Each plot was obtained by adding DDTbZn_2 to the solution of (a) $1.0 \mu\text{M}$ D8W1, (b) $0.5 \mu\text{M}$ D8A2W1, and (c) $0.5 \mu\text{M}$ D8A4W1 in 50 mM HEPES buffer (pH 7.4, 100 mM KNO_3) at 25 °C.

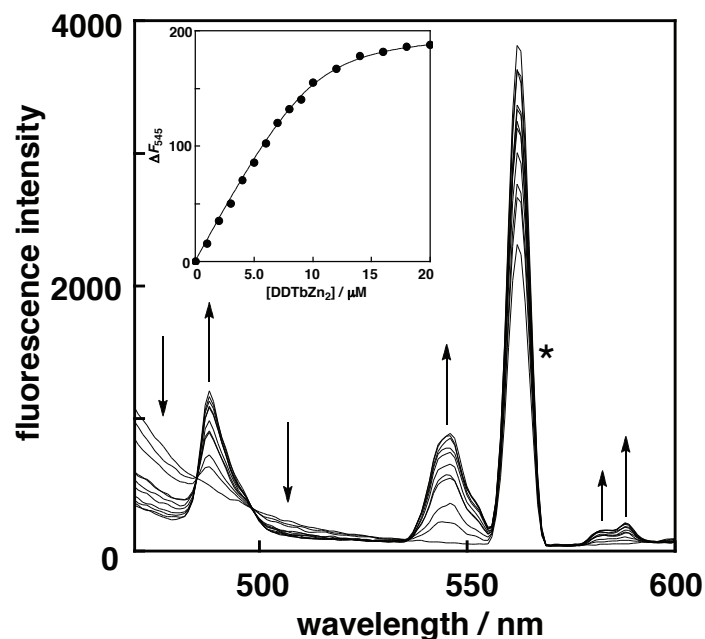


Fig. S3 Fluorescent spectral changes ($\lambda_{\text{ex}} = 280 \text{ nm}$) upon addition of DDTbZn₂ (0–100 μM) to a 70 μM solution of E8A4W1 in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃). The asterisks indicate scattered excitation light. Inset: Plots of ΔF ($= F - F_0$) at 545 nm with the best-fitting binding curves. Each plot was obtained by adding DDTbZn₂ to the solution of 10 μM E8A4W1 in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃) at 25 °C. Dissociation constant (K_d) between the peptide and DDTbZn₂ was calculated to be 700 nM.

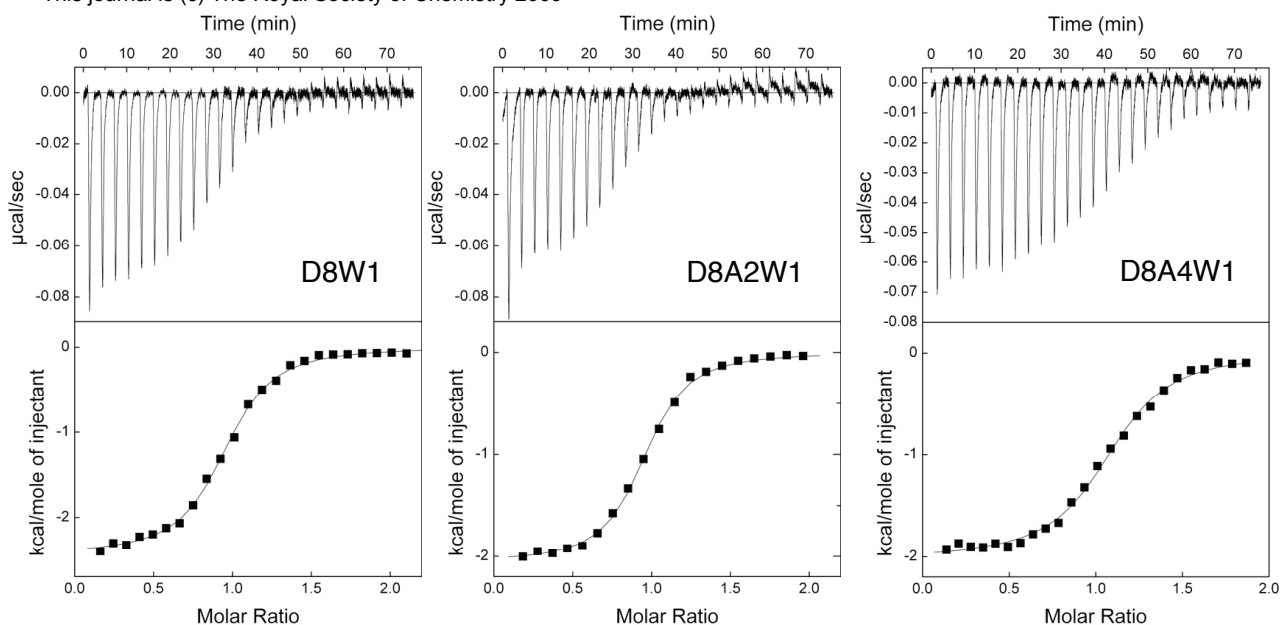


Fig. S4 ITC thermograms (top) and titration curves (bottom) of the model peptides (left: D8W1, middle: D8A2W1, right: D8A4W1) titrated against DDTbZn₂. Fitting lines were calculated using the single-site binding equation in the MicroCal ORIGIN software. Measurement conditions: [peptide] = 10 μM , [DDTbZn₂] = 100 μM (10 μL \times 25 injections), 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃), 25 $^{\circ}\text{C}$.

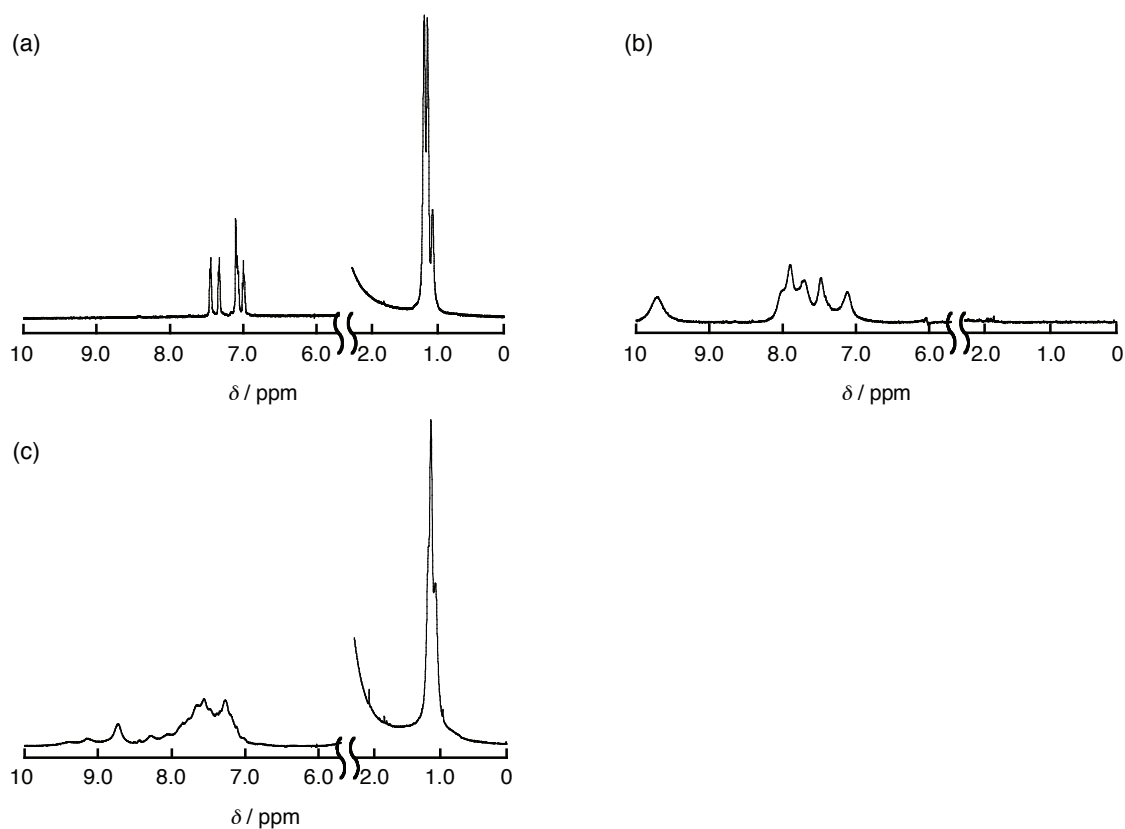


Fig. S5 $^1\text{H-NMR}$ spectra of (a) 1 mM D8A4W1, (b) 1 mM DDEuZn₂, and (c) 1 mM D8A4W1 in the presence of 1 eq of DDEuZn₂. All spectra were measured in 50 mM HEPES buffer (pD = 7.4) at 25 °C. Peaks at 1.2 ppm are assigned to alanine side chains, and peaks at 7.0~7.5 ppm are assigned to an indole ring in the tryptophan and pyridine rings in DDEuZn₂. (A line-broadening was also found in the pyridine rings in DDEuZn₂ as shown in (b))

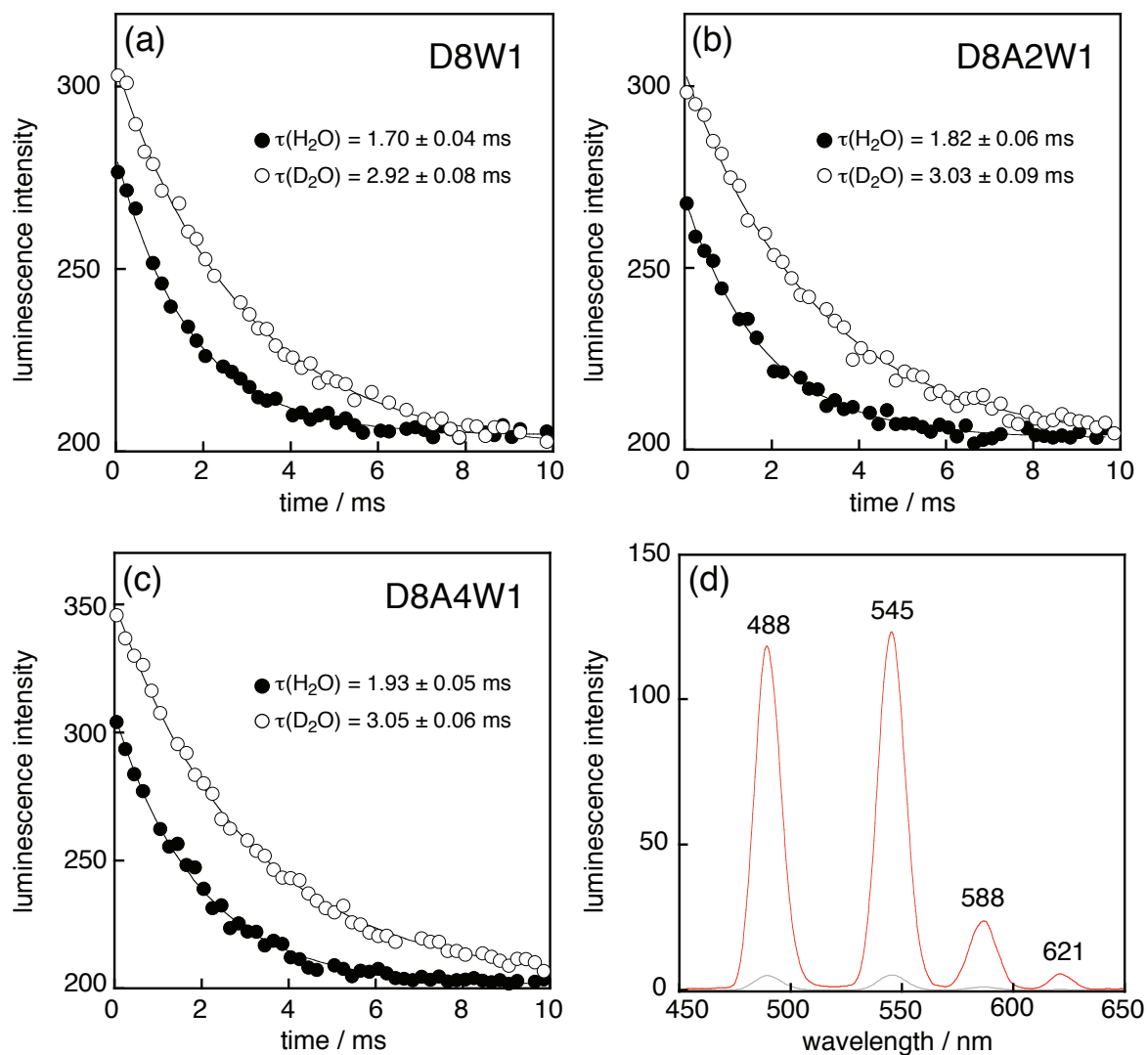


Fig. S6 Luminescent decays of the Tb^{3+} ion in the resultant structures of $DDTbZn_2$ and (a) D8W1, (b) D8A2W1, and (c) D8A4W1. The concentrations of $DDTbZn_2$ and the peptide were $100 \mu M$ in 50 mM HEPES buffer (pH 7.4, 100 mM KNO_3). The excitation wavelength was 280 nm, and Tb^{3+} -sensitized emission was observed at 545 nm. The data were fitted to a single exponential curve. (d) Luminescence spectra of $DDTbZn_2$ in the presence (red line) and absence (gray line) of model peptide.

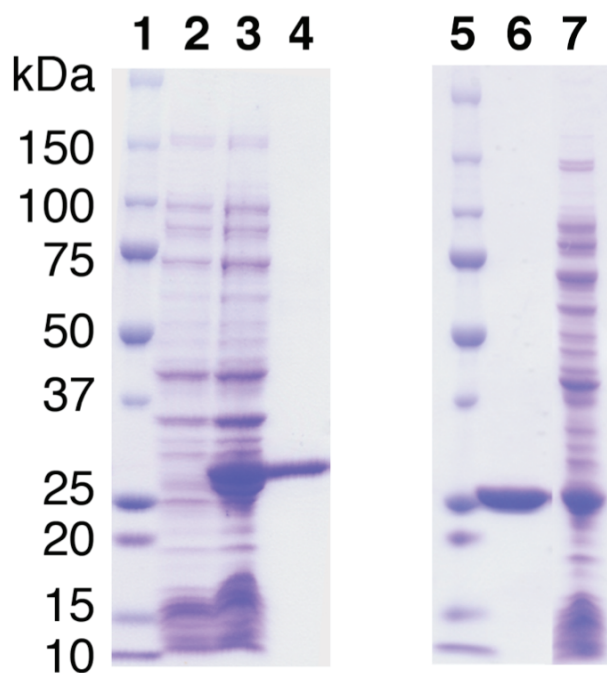


Fig. S7 SDS-PAGE analysis for expression and purification of GST-D8A4W1 and GST. Comassie Brilliant Blue staining of a 5-20% gradient polyacrylamide gel. The lanes are as follows: *lane 1*, precision plus protein standards (Bio-Rad Laboratories, Hercules, CA, USA); *lane 2*, uninduced cell harboring pGEX-6P-1-D8A4W1; *lane 3*, induced cell harboring pGEX-6P-1-D8A4W1; *lane 4*, purified GST-D8A4W1; *lane 5*, precision plus protein standards; *lane 6*, purified GST; *lane 7*, induced cell harboring pGEX-6P-1, respectively.

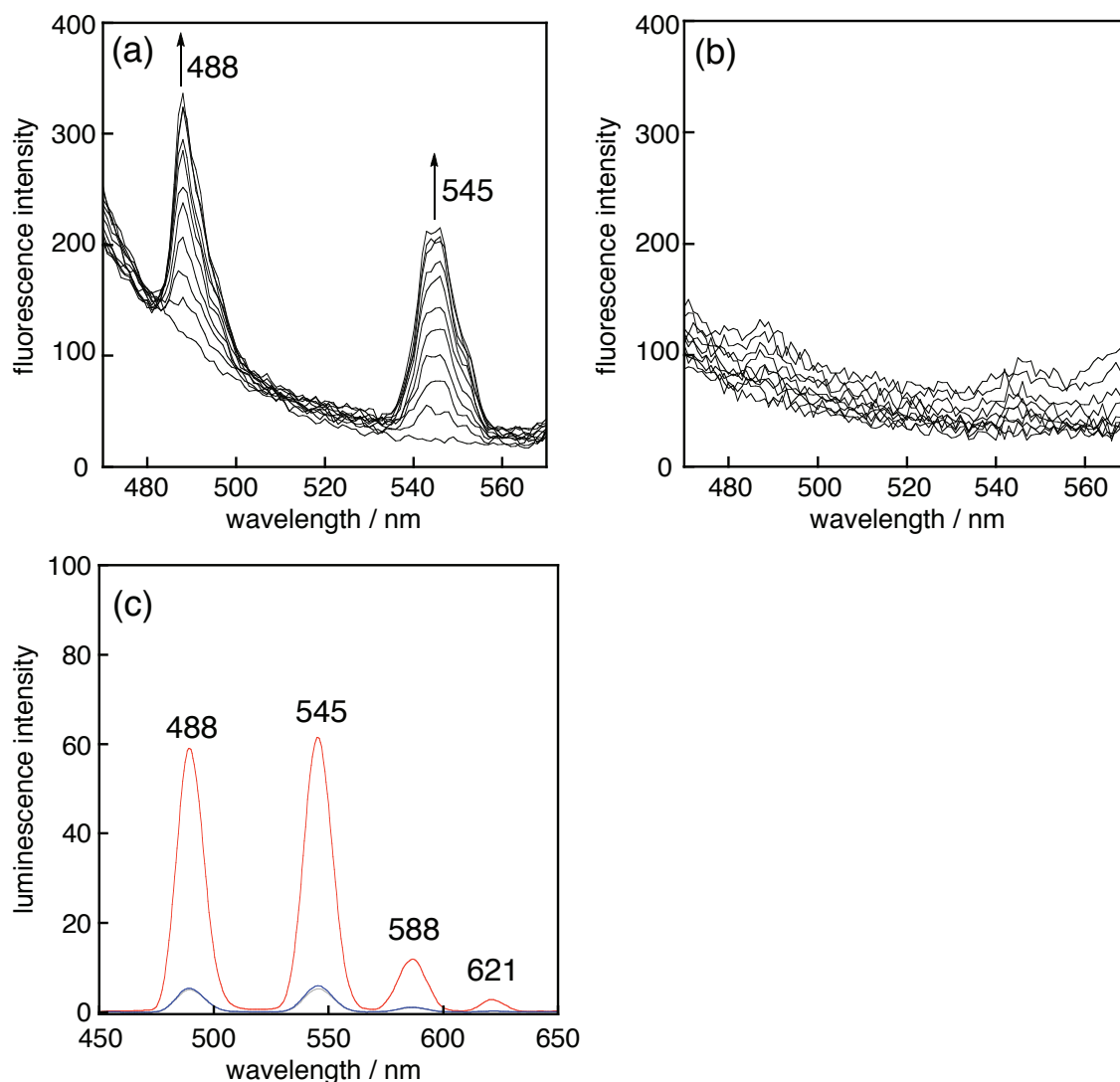


Fig. S8 Fluorescent spectral changes ($\lambda_{\text{ex}} = 290 \text{ nm}$) upon addition of DDTbZn_2 ($0\text{--}40 \mu\text{M}$) to a solution of (a) GST-D8A4W1 and (b) native GST in 50 mM HEPES buffer (pH 7.4, 100 mM KNO_3). The concentration of the protein is 0.5 mg/mL. (c) Time-resolved luminescence spectra of DDTbZn_2 in 50 mM HEPES (pH 7.4) in the presence of GST-D8A4W1 (red line) and native GST (blue line). The gray line (almost overlapped with blue line) indicates the luminescence spectrum of DDTbZn_2 .

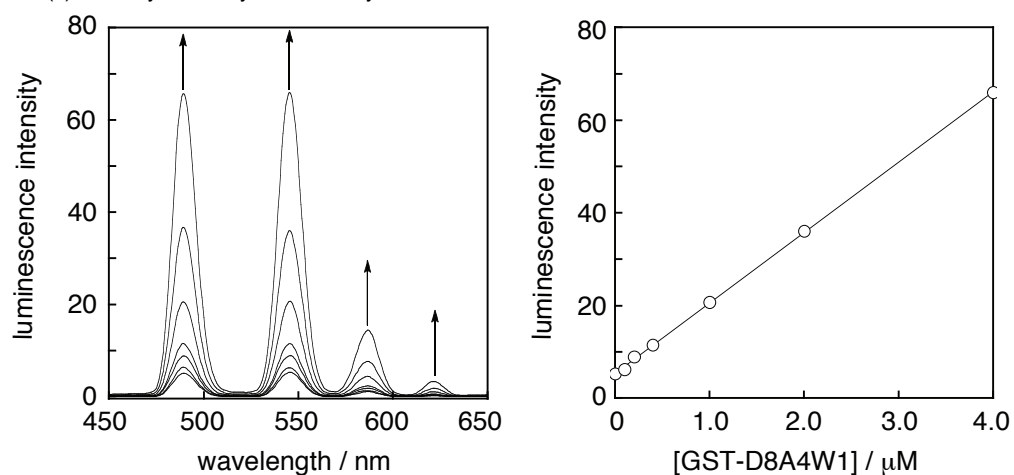


Fig. S9 Right: Luminescence spectral changes in time-resolved measurement ($\lambda_{\text{ex}} = 280 \text{ nm}$) upon addition of GST-D8A4W1 (0–4 μM) to a solution of 40 μM DDTbZn₂ in 50 mM HEPES buffer (pH 7.4, 100 mM KNO₃). Left: Plots of luminescence intensity at 545 nm in each concentration.