Supporting Information for

Site-Specific Covalent Labeling of His-tag Fused Proteins with a Reactive Ni(II)-NTA Probe

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Syntheses of the reactive probes

Scheme S1

**tert-Butyl 2,2'-{(1-tert-butoxy-6-(3-(chlorosulfonyl)benzamido)-1-oxohexan-2-ylazanediyl)diacetate (10)**

To an ice-cooled solution of 8 (107 μL, 0.67 mmol) in dry CH₂Cl₂ (2 mL) was added drowise a solution of 7 (144 mg, 0.33 mmol) and DIEA (150 μL, 0.84 mmol) in dry CH₂Cl₂ (1 mL). The reaction mixture was stirred for 0.5 h at 0 °C and further stirred for 1h at rt. After removal of the solvent by evaporation, the residue was purified by column chromatography on SiO₂ (hexane : ethyl acetate = 2 : 1) to give 10 (189 mg, 89%) as a brown oil. ¹H-NMR (400 MHz, CDCl₃) δ 8.59 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.44 (t, J = 6.0 Hz, 1H), 3.58-3.54 (m, 2H), 3.48 (d, J = 16.4 Hz, 2H), 3.42(d, J = 17.2 Hz, 2H), 3.31 (t, J = 7.6 Hz, 1H), 1.82-1.78 (m, 2H), 1.72-1.58 (m, 4H), 1.47 (s, 9H), 1.36 (s, 18H). FAB-MS m/e = 633 [M+H]+.

**tert-Butyl 2,2'-{(1-tert-butoxy-6-(3-(2-(2-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)ethoxy)ethoxysulfonyl)benzamido)-1-oxohexan-2-ylazanediyl)diacetate (13)**

To a stirred solution of 10 (37 mg, 0.11 mmol) in dry CH₂Cl₂ (3 mL) was added 12 (100 mg, 0.16 mmol), DMAP (4 mg, 0.036 mmol), and DIEA (52 μL, 0.32 mmol). The reaction mixture was stirred for 5 h at rt. After removal of the solvent by evaporation, the residue was purified by column chromatography on SiO₂ (CHCl₃ : MeOH = 100 : 1 → 70 : 1) to give 13 (26 mg, 27%) as a yellow amorphous powder. ¹H-NMR (400 MHz, CDCl₃) δ 8.67 (s, 1H), 8.42(s, 1H), 8.20 (d, J = 7.6 Hz, 2H), 8.02 (d, J = 8.0 Hz, 1H), 7.60 (t, J = 8.4 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.35 (t, J = 4.0 Hz, 1H), 6.64 (d, J = 8.8 Hz, 1H), 6.49 (s, 1H), 4.22 (t, J = 4.4 Hz, 2H), 3.68 (t, J = 4.8 Hz, 2H),...
3.58-3.54 (m, 2H), 3.51-3.39 (m, 8H), 3.30 (t, $J = 6.8$ Hz, 1H), 1.75-1.72 (m, 2H), 1.68-1.58 (m, 4H), 1.43 (s, 9H), 1.39 (s, 18H), 1.24 (t, $J = 7.2$ Hz, 6H). FAB-MS $m/e = 946$ [M+H]$^+$. 

**2,2'-(1-Carboxy-5-(3-(2-(2-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)ethoxy)ethoxysulfonyl)benzamido)pentylazanediyl)diacetic acid (15)**

To a solution of compound 13 (26 mg, 0.028 mmol) in dry CH$_2$Cl$_2$ (2 mL) was added TFA (1.5 ml), and the reaction mixture was stirred for 6 h at rt under N$_2$ atmosphere. After removal of the solvent in vacuo, the residue was treated with i-Pr$_2$O to give a precipitate, which was collected by filtration to give compound 15 (18 mg, 84%) as a yellow solid. $^1$H-NMR(400 MHz, CD$_3$OD) $\delta$ 8.60 (s, 1H), 8.12 (d, $J = 7.6$ Hz, 1H), 8.07 (d, $J = 8.0$ Hz, 1H), 7.70 (t, $J = 8.0$ Hz, 1H), 7.55 (d, $J = 9.2$ Hz, 1H), 6.82 (d, $J = 8.8$ Hz, 1H), 6.58 (s, 1H), 4.27 (t, $J = 4.4$ Hz, 2H), 3.74-3.62 (m, 6H), 3.56-3.47 (m, 9H), 3.38 (t, $J = 5.2$ Hz, 2H), 1.88-1.81 (m, 2H), 1.70-1.45 (m, 4H), 1.24 (t, $J = 6.8$ Hz, 6H). FAB-HRMS calcd for C$_{35}$H$_{45}$N$_4$O$_{14}$S [M+H]$^+$ $m/e = 777.2653$, found 777.2644.

**tert-Butyl 2,2'-(1-tert-butoxy-6-(2-chloro-5-(chlorosulfonyl)-4-fluorobenzamido)-1-oxohexan-2-ylazanediyl)diacetate (11)**

To a solution of 2-chloro-4-chlorosulfonyl-3-fluoro benzoic acid (200 mg, 0.73 mmol) in dry CH$_2$Cl$_2$ (3 mL) were added thionyl chloride (132 $\mu$L, 1.83 mmol) and dry DMF (2 dropos), and the solution was refluxed for 7 h under N$_2$ atmosphere, in which time formation of 9 was monitored by TLC (CHCl$_3$ : MeOH = 20 : 1). After removal of the solvent in vacuo, the crude of 9 was dissolved in dry CH$_2$Cl$_2$ (1 mL) and cooled in an ice-bath. A solution of 7 (210 mg, 0.23 mmol) and DIEA (213 $\mu$L, 1.22 mmol) in dry CH$_2$Cl$_2$ (2 mL) was added dropwise to the solution of 9. The reaction mixture was stirred for 30 min at 0°C and further stirred for 1h at rt. After removal of the solvent in vacuo, the residue was purified by column chromatography on SiO$_2$ (CHCl$_3$ → CHCl$_3$ : MeOH = 100 : 1) to give 11 (143 mg, 43%) as a brown oil. $^1$H-NMR(400 MHz, CDCl$_3$) $\delta$ 8.21 (d, $J = 6.8$ Hz, 1H), 7.39 (d, $J = 8.8$ Hz, 1H), 7.19 (t, $J = 4.0$ Hz, 1H), 3.62-3.56 (m, 2H), 3.43-3.35 (m, 4H), 3.30 (t, $J = 8.0$ Hz, 1H), 1.88-1.74 (m, 2H), 1.71-1.60 (m, 4H), 1.46 (s, 9H), 1.36 (s, 18H). FAB-MS $m/e = 685$ [M+H]$^+$. 

**tert-Butyl 2,2'-(1-tert-butoxy-6-(2-chloro-5-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)ethoxy)ethoxysulfonyl)-4-fluorobenzamido)-1-oxohexan-2-ylazanediyl)diacetate (14)**

Compound 11 (143 mg, 0.21 mmol) was used as a starting material. By the same
procedure described for the synthesis of 13, 14 (15 mg, 15%) was obtained as yellow amorphous powder. $^1$H-NMR(400 MHz, CDCl$_3$) δ 8.82 (t, $J$ = 4.8 Hz, 1H), 8.66 (s, 1H), 8.11 (d, $J$ = 7.2 Hz, 1H), 7.44 (d, $J$ = 9.6 Hz, 1H), 7.28 (d, $J$ = 9.6 Hz, 1H), 6.65 (d, $J$ = 9.6 Hz, 1H), 6.48 (s, 1H), 4.34 (t, $J$ = 4.4 Hz, 2H), 3.71 (t, $J$ = 4.8 Hz, 2H), 3.58-3.42 (m, 10H), 3.30 (t, $J$ = 8.0 Hz, 1H), 1.75-1.63 (m, 6H), 1.45 (s, 9H), 1.39 (s, 18H), 1.25 (t, $J$ = 6.8 Hz, 6H). FAB-MS $m/e$ = 997 [M+H]$^+$.  

2,2’-(1-Carboxy-5-(2-chloro-5-(2-(2-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)ethoxy)sulfonyl)-4-fluorobenzamido)pentylazanediyl)diacetic acid (16)

Compound 14 (15.2 mg, 0.015 mmol) was used as a starting material. By the same procedure described for the synthesis of 15, 16 (10.8 mg, 86%) was obtained as a yellow solid. $^1$H-NMR(400 MHz, CD$_3$OD) δ 8.63 (s, 1H), 8.00 (d, $J$ = 7.6 Hz, 1H), 7.60 (d, $J$ = 9.6 Hz, 1H), 7.57 (d, $J$ = 9.2 Hz, 1H), 6.83 (d, $J$ = 8.0 Hz, 1H), 6.59 (s, 1H), 4.37 (t, $J$ = 4.0 Hz, 2H), 3.76-3.64 (m, 6H), 3.59-3.48 (m, 9H), 3.38 (t, $J$ = 6.8 Hz, 2H), 1.92-1.81 (m, 2H), 1.79-1.55 (m, 4H), 1.25 (t, $J$ = 6.8 Hz, 6H). FAB-HRMS calcd for C$_{35}$H$_{42}$ClFN$_4$O$_{14}$S [M] $^+$ $m/e$ = 828.2091, found 828.2098.

Scheme S2

tert-Butyl 2,2’-(1-tert-butoxy-6-(2-chloro-4-fluoro-5-(pent-4-ynyloxysulfonyl)benzamido)-1-oxohexan-2-ylazanediyl)diacetate (17)

To a solution of 11 (161 mg 0.24 mmol) in dry CH$_2$Cl$_2$ (4 mL) was added 4-pentyl-1-ol (14.5 μL, 0.16 mmol), Et$_3$N (43 μL, 0.31 mmol) and Me$_3$N•HCl (3 mg, 0.031 mmol), and the reaction mixture was stirred for 7 h at rt. After dilution with Et$_2$O, organic layer was washed with water and brine followed by drying over Na$_2$SO$_4$. After removal of the solvent by evaporation, the residue was purified by column chromatography on SiO$_2$ (hexane : ethyl acetate = 3 : 1) to give 17.
(65 mg, 57%) as a colorless oil. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 8.13 (d, $J = 7.2$ Hz, 1H), 7.32 (d, $J = 8.8$ Hz, 1H), 7.01 (t, $J = 4.8$ Hz, 1H), 4.30 (t, $J = 6.4$ Hz, 2H), 3.58-3.53 (m, 2H), 3.41 (d, $J = 19.2$ Hz, 2H), 3.35 (d, $J = 21.2$ Hz, 2H), 2.33-2.29 (m, 2H), 1.95-1.88 (m, 2H), 1.75-1.54 (m, 6H), 1.46 (s, 9H), 1.37 (s, 18H). FAB-MS $m/e = 733$ [M+H]$^+$. 

2,2'-((1-Carboxy-5-(2-chloro-4-fluoro-5-(pent-4-nylosulfonyl)benzamido)pentylazanediyl)-diacetic acid (18)

Compound 17 (30 mg, 0.041 mmol) was used as a starting material. By the same procedure described for the synthesis of compound 15, 18 (18 mg, 79%) was obtained as a colorless solid. $^1$H-NMR (400 MHz, CD$_3$OD) $\delta$ 7.98 (d, $J = 6.8$ Hz, 1H), 7.66 (d, $J = 9.6$ Hz, 1H), 4.30 (t, $J = 5.6$ Hz, 2H), 3.74 (d, $J = 15.2$ Hz, 2H), 3.78 (d, $J = 24.8$ Hz, 2H), 3.54 (t, $J = 7.2$ Hz, 1H), 3.38 (t, $J = 6.8$ Hz, 2H), 2.30-2.26 (m, 2H), 2.21 (t, $J = 2.4$ Hz, 1H), 1.70-1.57 (m, 6H). FAB-MS $m/e = 565.1039$ [M]$^+$. FAB-HRMS calcd for C$_{22}$H$_{27}$ClFN$_2$O$_{10}$S [M+H]$^+$$m/e = 565.1059$ found 565.1039.
**tert-Butyl 2,2’-(6-(5-(2-(2-azidoethoxy)ethoxy)ethoxysulfonyl)-2-chloro-4-fluorobenzamido)-1-tert-butoxy-1-oxohexan-2-ylazanediyl)diacetate (19)**

Compound 11 (357 mg, 0.52 mmol) was used as a starting material. By the same procedure described for the synthesis of 13, 19 (175 mg, 63%) was obtained as a colorless oil. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 8.11 (d, $J = 7.2$ Hz, 1H), 7.30 (d, $J = 9.2$ Hz, 1H), 6.99 (m, 1H), 4.32 (t, $J = 4.8$ Hz, 2H), 3.74 (t, $J = 4.8$ Hz, 2H), 3.65 (t, $J = 4.8$ Hz, 2H), 3.62-3.58 (m, 4H), 3.54-3.52 (m, 2H), 3.46-3.36 (m, 6H), 3.29 (t, $J = 8.0$ Hz, 1H), 1.68-1.55 (m, 6H), 1.45 (s, 9H), 1.36 (s, 18H). FAB-MS $m/e = 824$ [M+H]$^+$.  

**tert-Butyl 2,2’-(1-tert-butoxy-6-(2-chloro-4-fluoro-5-(2-(2-(2-(5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethoxysulfonyl)benzamido)-1-oxohexan-2-ylazanediyl)diacetate (20)**

A suspension of 19 (78 mg, 0.094 mmol) and 10% Pd-C (8 mg) in dry AcOEt (3 mL) was vigorously stirred for 3h at rt under H$_2$ atmosphere. After filtration, the filtrate was concentrated in vacuo to give a crude product. To a solution of the crude product in dry DMF (2 mL) and added Biotin-OSu (16 mg, 0.047 mmol), and the mixture was stirred for 30 min at room temperature. After removal of the solvent in vacuo, the residue was purified by column chromatography on SiO$_2$ (CHCl$_3$ : MeOH = 7 : 1) to give 20 (20 mg). This material contained impurities, however was used for the next reaction without further purification. FAB-MS $m/e = 1024$ [M+H]$^+$.  

**2,2’-(1-Carboxy-5-(2-chloro-4-fluoro-5-(2-(2-(2-(5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethoxy)ethoxy)ethoxysulfonyl)benzamido)pentylazanediyl)diacetic acid (21)**

Compound 20 (20 mg, containing impurities) was used as a starting material. Deprotection by the same procedure described for the synthesis of 15 and the following HPLC purification to give 24 (1.2 mg, 2% from 19) was obtained as a white solid. FAB-HRMS calcd for C$_{33}$H$_{48}$ClFN$_5$O$_{14}$S [M+H]$^+$ $m/e = 856.2312$, found 856.2233.  

**Preparation of the reactive Ni(II)-complex probe (1, 2, 3, 5)**

A corresponding ligand solution (2 ~ 7mM of 15, 16, 18, or 21 in DMSO) was mixed with 1.1 equiv of NiCl$_2$ (2 mM in 50 mM HEPES, pH 7.2, 100 mM NaCl), and the solution was stand for 20 min at rt. This material was directly used for the labeling reaction.
Syntheses of His6 and His10 peptide

His6 (Ac-WAHHHHHH-NH$_2$) and His10 (Ac-WAHHHHHHHHHH-NH$_2$) peptides were manually synthesized by solid-phase peptide synthesis using the standard Fmoc-based coupling chemistry. The coupling reaction was performed with Rink Amide Resin (Novabiochem, 0.3 mmol) using 3 equiv of amino acid, 3 equiv of O-Benzotriazole-$N,N,N',N'$-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 3 equiv of 1-hydroxybenzotriazole (HOBT), and 6 equiv of DIEA. The completion of the coupling reaction (typically within 30 min) was confirmed by Kaiser test in each step. After acetylation of the N-terminus with Ac$_2$O in CH$_2$Cl$_2$, the peptide cleavage and side-chain deprotection were carried out by treatment with 5 mL of TFA containing triisopropylsilane (0.1 25 mL), and H$_2$O (0.125 mL) for 1.5 h at rt. Crude peptide was precipitated in tert-butyl methyl ether and then purified by reverse-phase HPLC (column; YMC-pack ODS-A, 250 x 10 mm, mobile phase; CH$_3$CN (containing 0.1% TFA) / H$_2$O (containing 0.1% TFA) = 5 / 95 → 40 / 60 (linear gradient over 40 min), flow rate; 3 mL/min, detection; UV (220 nm). Molecular weight of the peptide was confirmed by MALDI-TOF mass spectroscopy:

His6 peptide; Ac-WAHHHHHHH-NH$_2$ calcd for C$_{52}$H$_{63}$N$_{22}$O$_9$ [M + H]$^+$, m/e = 1139, found 1139.
His10 peptide; Ac-WAHHHHHHHHHH-NH$_2$ calcd for C$_{76}$H$_{91}$N$_{34}$O$_{13}$ [M + H]$^+$, m/e = 1687, found 1687.

Covalent labeling of His tag peptide

A solution of His6 or His10 peptide (5 μM), and reactive probe (1 or 2, 10 μM) in 50 mM HEPES, pH 7.2, 100 mM NaCl was incubated in a plastic tube at 37 °C for 7 hr, during which time the solution was sampled at the appropriate time (1, 2, 3, 5, 7 hr). The sampling solution were desalted with a Zip-Tip (C18 type, Millipore) and then subjected for MALDI-TOF mass analysis (Bruker, Autoflex III) using α-cyano-4-hydroxycinnamic acid as a matrix. The labeling yield (%) of the peptides was calculated based on the peak intensities (PI) of the labeled and unlabeled peptide, and defined as the following equation;

(\text{Sum of PIs of the labeled peptides}) / [(\text{Sum of PIs of the labeled peptides}) + (\text{Sum of PIs of the unlabeled peptide})]
Preparation of His10-EGFP and control EGFP lacking His10 tag

The DNA fragments coding His10 (MGSSHHHHHHHHHHSSGLVPRGS) was inserted into NcoI-NdeI site of pET-28a. The sequences of the 5’-phosphorylated DNA fragments were as follows: 5’- C ATG GGC AGC AGC CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT CAT AGC AGC GGC CTG GTG CCG CGC GGC AGC GG -3’ (forward) and 5’- T ACC GCT GCC GCG CGG CAC CAG GCC GCT GCT ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG GCT GCT GC -3’ (backward).

pET28a-His10-EGFP plasmid was transformed into *E. coli* BL21(DE3)pLysS. The cells were grown in 1 L of Terrific Broth (TB) at 37 °C until an optical density (OD) at 600 nm increased to 0.5 ~ 0.6, and further grown at 16 °C for 24 h with IPTG induction (0.3 mM). The cells were spun down for 40 min at 4000 rpm. The cells were re-suspended in 40 mL of Lysis buffer (50 mM HEPES, 100 mM NaCl, 10% glycerol) and lysed by sonication (10 shots x 20 sec, Branson Sonifier 450). Insoluble materials were removed by centrifugation for 10 min at 12,000 rpm (x2) to collect the soluble fraction, containing His10-EGFP. The EGFP purification was performed with TALON resin (Clontech). The soluble fraction (40 mL) was adsorbed on 1 mL of TALON resin pre-filled with washing buffer (50 mM HEPES, 100 mM NaCl, 30~50 mM imidazole, pH 7.2) in a plastic column. The resin was washed with washing buffer and then the resin-bound protein was eluted from the column with elution buffer (50mM HEPES, 100 mM NaCl, pH 7.2, 150 mM imidazole). The fractions containing the purified EGFP was collected and dialysed twice with HEPES buffer (50 mM HEPES, 100 mM NaCl, pH 7.2) to remove the excess imidazole. The concentration of His10-EGFP was determined by UV absorbance at 488 nm based on the reported extinction coefficient of EGFP (ε = 55,000 M⁻¹•cm⁻¹). The solution of the EGFP was stored at 4 °C.

Control EGFP lacking His10 tag used in Figure 3 was prepared from the purified His10-EGFP by the treatment with thrombin (1 unit per 10 mg of His10-EGFP in 50 mM HEPES, 100 mM NaCl, pH 7.2, 22 °C, 16 hr) to cleave the His10 site. The solution of the protein was suspended with Benzamidine Sepahrose 6B and incubated for 5 min at rt to remove thrombine. Dialysis with HEPES buffer (50 mM HEPES, 100 mM NaCl, pH 7.2) twice gave control EGFP, purity of which was confirmed by SDS-PAGE analysis.

Control EGFP lacking His10 tag used in Figure 4 was expressed in *E. coli* BL21(DE3)pLysS from pET28a-EGFP plasmid lacking His10 site DNA fragment.
Covalent modification of His10-EGFP with 2

A solution of His10-EGFP or control EGFP (5 µM), and 2 (15 µM) in 50 mM HEPES, pH 7.2, 100 mM NaCl was incubated in a plastic tube at 37 °C for 12 hr, during which time the solution was sampled at the appropriate time (1, 2, 3, 5, 7, 12 hr), mixed with loading buffer (containing 1.14 M of 2-mercaptoethanol) to quench the reaction. The collected samples were denatured by heating with loading buffer (95 °C, 3 min), and ran on SDS-polyacrylamide gel electrophoresis by the standard method. The in-gel fluorescence analysis was performed with LAS-4000 (FUJIFILM, 365 nm excitation and L41 band pass filter). The labeling yield (%) is defined as the labeled coumarine unit per total amount of protein, and calculated based on the fluorescent band intensity of the labeled protein, in which the authentic samples of human carbonic anhydrase quantitatively labeled with 3-caroxyl-7-diethylaminocoumarine was used as a fluorescence standard.

Introduction of an alkyne unit into His10-EGFP by the reaction with 3 and 4

A solution of His10-EGFP or control EGFP (5 µM), and 3 (20 µM) in 50 mM HEPES, pH 7.2, 100 mM NaCl was incubated in a plastic tube at 37 °C for 7 hr. The subsequent Huisgen reaction was conducted at rt for 30 min with coumarin azide 4 (500 µM, final conc.), CuCl2, ascorbic acid, and triazole ligand 11 (500 µM of each in final concentration), and incubated for 30 min at 20 °C. The mixtures were denatured by heating with loading buffer (95 °C, 3 min), and ran on SDS-polyacrylamide gel electrophoresis by the standard method. The in-gel fluorescence analysis was performed with LAS-4000 (FUJIFILM, 365 nm excitation and L41 band pass filter).

Introduction of a biotin unit into His10-EGFP by the reaction with 5

A solution of His10-EGFP or control EGFP (0.5 µM), and 5 (0.5 µM) in 50 mM HEPES, pH 7.2, 100 mM NaCl was incubated in a plastic tube at 25 °C for 30 min. The samples were ran on SDS-polyacrylamide gel electrophoresis and then transferred to a PVDF membrane. After treatment with 5% skim milk in TBS-T for 1hr at rt, the membrane was incubated with streptavidine-HRP conjugate (1/5000) for 1hr at rt, and then subjected for chemical luminescence analysis with LAS-4000 (FUJIFILM).

Selective Labeling of the His10-EGFP in the crude lysate of E. Coli cells

The cell lysate containing soluble protein mixture was obtained from E. coli BL21(DE3)pLysS expressing His10-EGFP or EGFP lacking His10 tag, total protein concentration of which was determined to be 3.0 or 1.2 mg/mL, respectively, using Protein Quantification
Kit-Rapid (Dojindo). The protein mixtures were incubated with 2 (15 μM) for 7 hr at 25 °C. The mixture was denatured by heating with loading buffer (95 °C, 3 min), and ran on SDS-polyacrylamide gel electrophoresis by the standard method. The in-gel fluorescence analysis was performed with LAS-4000 (FUJIFILM, 365 nm excitation and L41 band pass filter).

**One-pot orthogonal labeling of the tag fused proteins**

The protein mixture was prepared by mixing the purified His10-EGFP protein with the crude cell lysate of *E. coli* JM109 expressing MBP-CA6D4 protein, in which the concentration of His10-EGFP and MBP-CA6D4 was determined to be 5 μM and 13 μM, respectively. Probe 2 (15 μM) and 6S3 (20 μM) was added to this protein mixture, and the reaction mixture was incubated in a plastic tube at 4 °C for 2 hr. After addition of sodium pyrophosphate (3 mM in final concentration), the mixture was further incubated at 25 °C for 4 hr. The sample was ran on SDS-polyacrylamide gel electrophoresis. The in-gel fluorescence analysis was performed with ChemiDoc XRS (BIO-RAD, 365 nm excitation, and 480BP70 (coumarine) and 630BP30 (rhodamine) band pass filters).
**Figure S1.** In-gel fluorescence analysis of the cleaved EGFP unit. His10-EGFP (43 µg) was labeled with 2 and then treated with thrombin (2.15 unit in 50 mM HEPES, 100 mM NaCl, 22 °C, 16 hr) to cleave the His10-tag fragment.

**Figure S2.** MALDI-TOF mass analysis of the His10 tag fragment. His10-EGFP (43 µg) was labeled with 2 and then treated with thrombin (2.15 unit in 50 mM HEPES, 100 mM NaCl, 22 °C, 16 hr) to cleave the His10-tag fragment. The sequence of the cleaved His10-tag is as follows: GSSHHHHHHHHHHSSGLVPR (2316 Da).


References


