Supporting Information

Methodology of reversible protein labeling for ratiometric fluorescent measurement

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1. Synthesis of dansyl-NTA-Ni\textsuperscript{2+} complex

Scheme

**Synthesis of dansyl-NTA**

\(N_{\alpha}, N_{\alpha}\text{-Bis(carboxymethyl)}\text{-L-lysine hydrate (182 mg, 695 \(\mu\)mol) and K}_2\text{CO}_3\ (317 mg, 2.30 mmol) were dissolved in acetonitrile/water (4.5 mL, acetonitrile/water, 7/2, v/v) and 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride) (300 mg, 1.11 mmol) was then added dropwise to the reaction mixture. Following a reaction for 32 h at room temperature in the dark under N\textsubscript{2} atmosphere, the solvent was removed from the reaction mixture by evaporation. After washing with acetonitrile and THF, the residue was purified using a LH-20 column chromatography (eluent: water) and was then re-purified by preparative TLC (eluent: acetonitrile/water, 2/1, v/v, \(R_f = 0.63\)) to give 266 mg (77 \%) dansyl-NTA as a yellow powder.**

\(^1\text{H-NMR (400 MHz, D}_2\text{O): }\delta \text{ (ppm) 0.81-1.32 (m, 6H), 2.24-2.31 (t, 1H), 2.80-2.85 (s, 6H), 2.85-3.10 (m, 6H), 7.31-7.36 (d, 1H), 7.61-7.67 (m, 2H), 8.21-8.29 (m, 2H), 8.42-8.47 (d, 1H).**

MALDI-TOF MS: Calcd. for C\(_{22}\)H\(_{29}\)N\(_3\)O\(_8\)S \([M-H]\)\(-\): \(m/z = 494.16\); Found 494.17.

**Synthesis of dansyl-NTA-Ni\textsuperscript{2+} complex**

NiCl\(_2\) (5.9 mg (46 \(\mu\)mol) in 6 \(\mu\)L 0.01 N HCl) was added to dansyl-NTA (10 mg (20 \(\mu\)mol) in 4 mL water) and the solution was brought to pH 8 by adding 10 mL 0.1 M NaHCO\(_3\). Following reaction for 1 h at room temperature in the dark, the product was purified using a Sep-Pak Plus cartridge (C18 long body) and dried to give 3.9 mg (35 \%) dansyl-NTA-Ni\textsuperscript{2+} complex.
\(^1\)H-NMR (400 MHz, D\(_2\)O): \(\delta\) (ppm) 2.00-2.44 (m, 7H), 2.46-3.00 (s, 6H), 3.20-3.55 (s, 6H), 3.55-3.66 (q, 2H), 7.20-7.46 (br., 1H), 7.54-7.98 (br., 2H), 8.30-8.68 (br., 3H). MALDI-TOF MS: Calcd. for C\(_{22}\)H\(_{29}\)N\(_3\)NiO\(_8\)S [M]\(^+\): \(m/z\) = 550.08; Found 550.18.

2. Synthesis of tags
All peptides (His\(_6\)Trp\(_3\), His\(_6\)Trp\(_2\), His\(_6\)Trp\(_1\), His\(_6\), Trp\(_3\)His\(_6\)-S-peptide, His\(_6\)-S-peptide) were synthesized with an automated peptide synthesizer by standard Fmoc chemistry using corresponding Fmoc amino acids. Crude peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) (column: YMC-pack ODS-A) eluting with acetonitrile/0.1\%TFA.

MALDI-TOF MS: His\(_6\)Trp\(_3\): Calcd. for C\(_{38}\)H\(_{47}\)N\(_{19}\)O\(_7\) [M+H]\(^+\): \(m/z\) = 882.40; Found 882.79. His\(_6\)Trp\(_2\): Calcd. for C\(_{60}\)H\(_{67}\)N\(_{23}\)O\(_9\) [M+H]\(^+\): \(m/z\) = 1254.56; Found 1254.20. His\(_6\)Trp\(_1\): Calcd. for C\(_{49}\)H\(_{57}\)N\(_{21}\)O\(_8\) [M+H]\(^+\): \(m/z\) = 1068.48; Found 1068.54. His\(_6\): Calcd. for C\(_{38}\)H\(_{47}\)N\(_{19}\)O\(_7\) [M+H]\(^+\): \(m/z\) = 882.40; Found 882.79. Trp\(_3\)His\(_6\)-S-peptide: Calcd. for C\(_{142}\)H\(_{191}\)N\(_{49}\)O\(_{32}\)S [M]\(^+\): \(m/z\) = 3126.46; Found 3124.84. His\(_6\)-S-peptide: Calcd. for C\(_{109}\)H\(_{161}\)N\(_{43}\)O\(_{29}\)S [M+H]\(^+\): \(m/z\) = 2569.23; Found 2569.76.

3. Fluorescence spectra
Fluorescence measurements were performed with a steady-state fluorescence instrument (Shimazu, RF-5300PC). To 100 mM phosphate buffer solution (pH 7.4) containing 5 \(\mu\)M dansyl-NTA-Ni\(^{2+}\) complex, 5 mM each peptide (His\(_6\)Trp\(_3\), His\(_6\)Trp\(_2\), His\(_6\)Trp\(_1\), His\(_6\)) was added at a final concentration of 30 \(\mu\)M, and fluorescence spectra were monitored (Figs. S1(a)-S4(a), Fig. 1 shows fluorescence spectra observed after 5 min from the addition of each peptide.). 50 mM ethylenediaminetetraacetic acid (EDTA) was subsequently added to the sample solution at a final concentration of 300 \(\mu\)M, and fluorescence spectra were monitored (Figs. S1(b)-S4(b)). 100 mM NiCl\(_2\) was subsequently added to the sample solution at a final concentration of 400 \(\mu\)M, and fluorescence spectra were monitored (Figs. S1(c)-S4(c)). The changes in fluorescent ratio \((F_{491}/F_{538})\) observed in a series of the above procedure \((F_{491}\) and \(F_{538}\) indicate fluorescence intensities at 491 nm and 538 nm, respectively) were calculated (Fig. 2 for His\(_6\)Trp\(_3\), Figs. S2(d)-S4(d) for His\(_6\)Trp\(_2\), His\(_6\)Trp\(_1\), His\(_6\)). For fluorescence measurements, an excitation wavelength of 330 nm was used.
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**Fig. S1** Fluorescence changes observed using His6Trp3. (a) Emission spectra of dansyl-NTA-Ni^{2+} complex (5 µM) upon the addition of His6Trp3 (30 µM). (b) Emission spectra of dansyl-NTA-Ni^{2+} complex observed by subsequent addition of EDTA (300 µM) to the sample solution in Fig. S1(a). (c) Emission spectra of dansyl-NTA-Ni^{2+} complex observed by subsequent addition of Ni^{2+} (400 µM) to the sample solution in Fig. S1(b). Fluorescent ratio ($F_{491}/F_{538}$) observed in a series of the above procedure is shown in Fig. 2. 100 mM phosphate buffer (pH 7.4) was used as solvent. Excitation wavelength was 330 nm.
Fig. S2  Fluorescence changes observed using His$_6$Trp$_2$.  (a) Emission spectra of dansyl-NTA-Ni$^{2+}$ complex (5 µM) upon the addition of His$_6$Trp$_2$ (30 µM).  (b) Emission spectra of dansyl-NTA-Ni$^{2+}$ complex observed by subsequent addition of EDTA (300 µM) to the sample solution in Fig. S2(a).  (c) Emission spectra of dansyl-NTA-Ni$^{2+}$ complex observed by subsequent addition of Ni$^{2+}$ (400 µM) to the sample solution in Fig. S2(b).  (d) Fluorescent ratio ($F_{491}/F_{538}$) observed in a series of the above procedure.  100 mM phosphate buffer (pH 7.4) was used as solvent.  Excitation wavelength was 330 nm.
Fig. S3  Fluorescence changes observed using His6Trp1.  (a) Emission spectra of dansyl-NTA-Ni$^{2+}$ complex (5 µM) upon the addition of His6Trp1 (30 µM).  (b) Emission spectra of dansyl-NTA-Ni$^{2+}$ complex observed by subsequent addition of EDTA (300 µM) to the sample solution in Fig. S3(a).  (c) Emission spectra of dansyl-NTA-Ni$^{2+}$ complex observed by subsequent addition of Ni$^{2+}$ (400 µM) to the sample solution in Fig. S3(b).  (d) Fluorescent ratio ($F_{491}/F_{538}$) observed in a series of the above procedure.  100 mM phosphate buffer (pH 7.4) was used as solvent.  Excitation wavelength was 330 nm.
Fig. S4  Fluorescence changes observed using His<sub>6</sub>.  (a) Emission spectra of dansyl-NTA-Ni<sup>2+</sup> complex (5 µM) upon the addition of His<sub>6</sub> (30 µM).  (b) Emission spectra of dansyl-NTA-Ni<sup>2+</sup> complex observed by subsequent addition of EDTA (300 µM) to the sample solution in Fig. S4(a).  (c) Emission spectra of dansyl-NTA-Ni<sup>2+</sup> complex observed by subsequent addition of Ni<sup>2+</sup> (400 µM) to the sample solution in Fig. S4(b).  (d) Fluorescent ratio ($F_{491}/F_{538}$) observed in a series of the above procedure.  100 mM phosphate buffer (pH 7.4) was used as solvent.  Excitation wavelength was 330 nm.
4. Experimental procedures for taking fluorescent images

Fluorescent images were obtained by irradiating samples using a mercury-xenon lamp (Moritex, MUV-202U). Band-pass filters, UV D-33S and Y-46 (Toshiba) were used for the lamp and a camera, respectively. RNase S’ was used as the model protein. RNase S’ is known as the reconstituted enzyme obtained by mixing equimolar amounts of S-protein and S-peptide (Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser) (J. -S., Kim, R. T. Raines, *Protein Science*, 1993, **2**, 348). We thus synthesized S-peptide linked with Trp$_3$His$_6$ (Trp$_3$His$_6$-S-peptide: Trp-Trp-Trp-His-His-His-His-His-His-Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser) and S-peptide linked with His$_6$ (His$_6$-S-peptide: His-His-His-His-His-His-His-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser), and prepared Trp$_3$His$_6$-tagged RNase S’ and His$_6$-tagged RNase S’ by mixing equimolar amounts of S-protein (purchased from Sigma) with Trp$_3$His$_6$-S-peptide and His$_6$-S-peptide, respectively, in 100 mM phosphate buffer solution (pH 7.4). After the addition of dansyl-NTA-Ni$^{2+}$ complex, fluorescent images were taken (Fig. 3 (left) for Trp$_3$His$_6$-tagged RNase S’, Fig. 3 (right) for His$_6$-tagged RNase S’).