Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Ratiometric Fluorescence Detection of a Tag Fused Protein Using the Dual-Emission Artificial Molecular Probe

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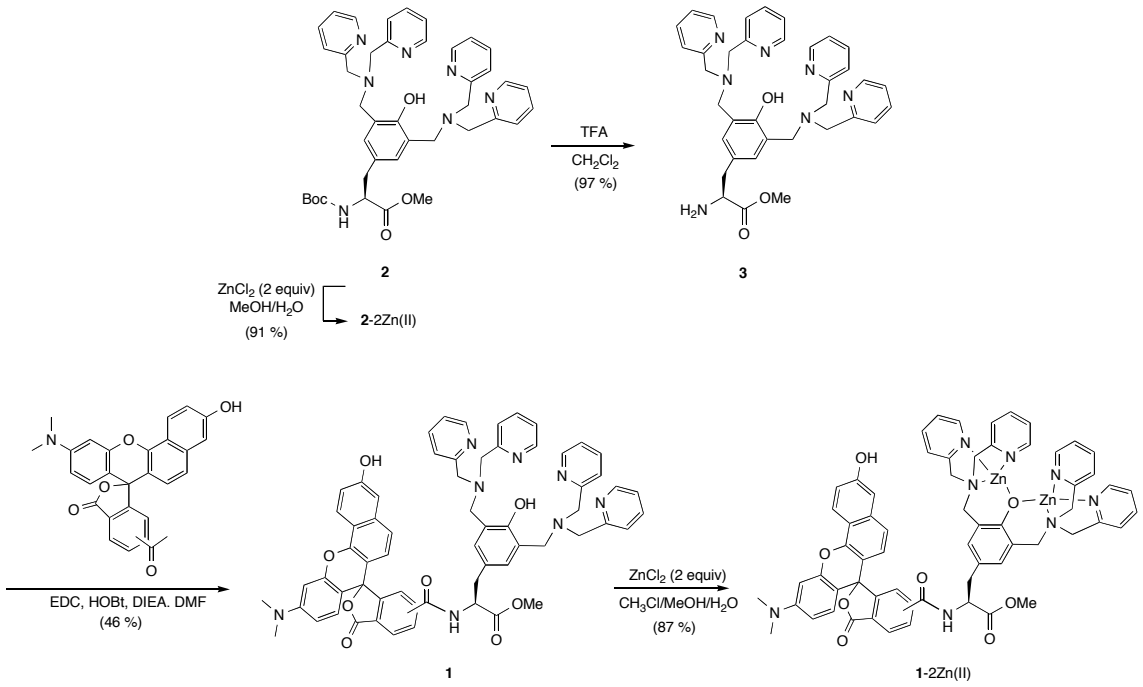
General methods

Unless otherwise noted, all chemical reagents were purchased from commercial suppliers and used without further purification. ^{1}H-NMR spectra were recorded using a JNM-EX400 (JEOL, 400 MHz) spectrometer and the chemical shifts (δppm) are referenced to the respective solvent. FAB mass spectra were recorded using a QP5050A (Shimadzu). Fluorescent spectra and Absorption spectra were recorded on a Perkin-Elmer LS55 spectrometer and U-2550 (Shimadzu), respectively. Reverse phase HPLC was conducted with a Lachrom (Hitachi) with C18 columns.

Synthesis and Compound Characterizations



Scheme S1 Synthesis of **1**-2Zn(II).

The details of syntesis of **2**, **2**-2Zn(II) and **3** were described previously.[8]

SNARF-DpaTyr (1) A mixture of **3** (20 mg, 0.033 mmol), carboxy SNARF (mixture of 5- and 6- carboxylate mixture) (15 mg, 0.033 mmol), HOBt・H_2O (7.5 mg, 0.050 mmol), EDC・HCl (9.4 mg, 0.050 mmol) and *N,N'*-diisopropylethylamine (30 mL, 0.17 mmol) in dry DMF (2 mL) was stirred at room temperature for 6 h. The mixture

was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was dissolved in CH_3Cl (containing a small amount of MeOH) and the solution was diluted with Hexane to form precipitate. The precipitate was filtered and washed with Hexane to give **1** (16 mg, 46%) as a purple powder. **1** was used in the next reaction without further purification. Due to assignment difficulty, ^{1}H NMR of **1** was measured after isolation of each isomer by reverse phase HPLC.

^{1}H NMR (400 MHz; CD_3OD; solvent)
isomer-1 : δ_H 3.00 – 3.06 (2H, m, CH_2), 3.43 (6H, s, NMe), 3.76 (3H, s, Me), 4.15 – 4.28 (12H, m, CH_2), 6.91 – 6.96 (1H, m, Ar-H), 7.24 – 7.29 (5H, m, Ar-H), 7.37 – 7.44 (12H, m, Ar-H), 7.56 – 7.61 (2H, m, Ar-H), 7.82 (4H, td, *J* = 8.0, 1.6 Hz, Ar-H), 8.07 - 8.12 (1H, m, Ar-H), 8.57 - 8.58 (5H, m, Ar-H), 8.83 (1H, d, *J* = 9.2 Hz, Ar-H).

isomer-2 : δ_H 2.82 – 2.97 (2H, m, CH_2), 3.42 (6H, s, NMe), 3.70 (3H, d, *J* = 4.0, Me), 4.00 – 4.20 (12H, m, CH_2), 6.58 (1H, dd, *J* = 7.2, 1.2 Hz, Ar-H), 6.87 – 7.00 (3H, m, Ar-H), 7.07 – 7.16 (5H, m, Ar-H), 7.22 – 7.31 (3H, m, Ar-H), 7.36 – 7.44 (10H, m, Ar-H), 7.55 (1H, t, *J* = 8.8 Hz, Ar-H), 7.66 (1H, dd, *J* = 14.2, 1.2 Hz, Ar-H), 7.82 (4H, qd, *J* = 7.8, 1.6 Hz, Ar-H), 7.98 – 8.05 (2H, m, Ar-H), 8.25 - 8.31 (1H, m, Ar-H), 8.53 - 8.63 (4H, m, Ar-H), 8.81 (1H, d, *J* = 8.8 Hz, Ar-H).

FAB-HRMS *m/e* calcd for $[M + H]^+$ 1053.4299, found 1053.4312.

SNARF-DpaTyr(Zn) (1-2Zn(II)) To a solution of **1** (mixture of the isomers 7.7 mg, 7.3 μmol) in distilled MeOH / $CHCl_3$ (1.5 mL / 0.5 mL) was added aqueous solution of $ZnCl_2$ (100 mM; 136 mL, 0.014 mmol), and the solution was stirred at room temperature for 1 h. The solution was concentrated by evaporation, and then lyophilized under vacuum. The obtained solid was suspended in ethyl acetate, filtered, and dried in vacuo to give **1**-2Zn(II) (7.9 mg, 87%) as a purple powder.

FAB-HRMS *m/e* calcd for $[SNARF\text{-}DpaTyr + 2Zn + 2Cl^-]^+$ 1249.2103, found 1249.2106.

Preparation of D4 peptide (Boc-DDDD-NH_2)

The Boc-DDDD-NH_2 was synthesized by the standard Boc chemistry in solution phase from Boc-Asp(Obzl)-OH and H-ASP(Obzl)-$CONH_2$. Detailed synthetic procedure and compound characterization was described previously.[8]

Preparations of the tag-fused RNases

The tagged RNases were constructed by the self-assembly of a S-protein (purchased from Aldrich) with a S-peptide[S1] tethering a D4-tag (DDDD) or His_6-tag (HHHHHH) at its N-terminus, which was synthesized by the automated peptide synthesizer and purified by reverse-phase HPLC. Detailed synthetic procedures and compound characterizations were described previously.[8]

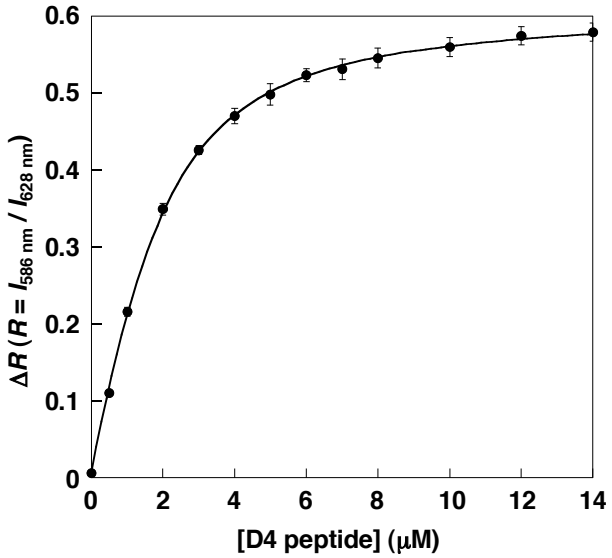


Fig. S1 Fluorescence titration curve of the emission intensity ratio R ($R = I_{586\ nm} / I_{628\ nm}$) of **1**-2Zn(II) (2 μM) upon addition of D4 peptide in 50 mM HEPES buffer, pH 7.2, at 25 ^{o}C.

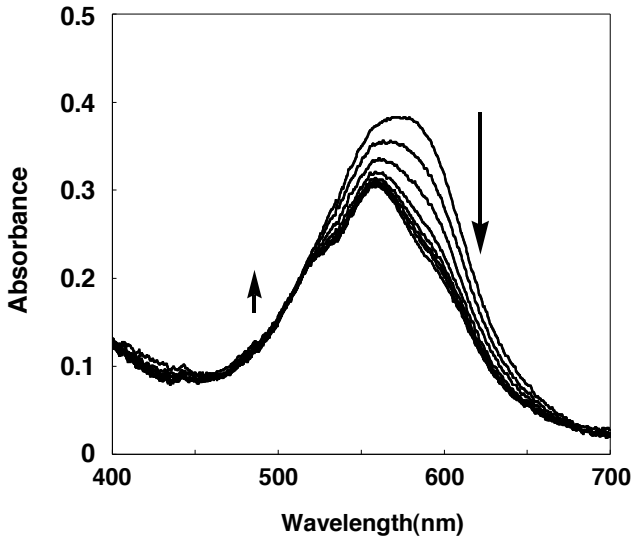


Fig. S2 Absorption spectral change of **1**-2Zn(II) (2 μM) upon the addition of D4 peptide : [D4 peptide] = 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 μM in 50 mM HEPES buffer, pH 7.2, at 25 ^{o}C.

References

S1. (a) P. R. Connelly, R. Varadarajan, J. M. Sturtevant and F. M. Richards, *Biochemistry*, 1990, **29**, 6108-6114. (b) I. Hamachi, R. Eboshi, J. Watanabe and S. Shinkai, *J. Am. Chem. Soc.*, 2000, **122**, 4530-4531.