Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Highly sensitive turn on detection of Ag^+ in aqueous solution and live cells with a symmetric fluorescent peptide

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1. Experimental Section

1.1. Reagents:

Fmoc protected amino acids and Rink Amide MBHA resin were from Novabiochem. 1-hydroxybenzotriazole(HOBt) and *N*,*N*''-diisopropylcarbodiimide (DIC) were from BeedTech. Other reagents for peptide synthesis including trifluoroacetic acid(TFA), *N*,*N*'-dimethylformamide (DMF), silver nanoparticles (10 nm particle size, 0.02 mg/mL in aqueous solution containing sodium citrate as stabilizer), and piperidine were purchased from Aldrich.

1.2. Solid phase synthesis and characterization of DPG1

The peptides were synthesized using Fmoc chemistry by solid phase peptide synthesis with Fmoc chemistry.[1] Fmoc protected amino acid was assembled on Rink Amide MBHA resin, as shown in Scheme 1. After deprotection of Fmoc group, the coupling of dansyl chloride was performed by the following procedure. To the resin bound amino acid (200 mg. 0.1 mmol), dansyl chloride (80 mg, 0.3 mmol, 3 equiv) in DMF (3 ml) and triethylamine (400 µl, 0.3 mmol, 3 equiv) were added. Cleavage of the peptide from the resin was achieved by treatment with a mixture of 3 ml TFA: TIS: H_2O (95:2.5:2.5, v/v/v) at room temperature for 4 h. After filtration and washing of the resin by TFA, a gentle stream of nitrogen was used to remove the excess TFA. The crude peptide was triturated with diethyl ether chilled at -20℃ and then centrifuged at 3,000 rpm for 10 min. The purity of crude peptide (>95%) were confirmed by analytical HPLC with a C18 column using a linear gradient of H_2O and acetonitrile (0–100% acetonitrile) containing 0.1% TFA. **DPG1** was synthesized by oxidation of crude peptide in the presence of 20 equiv of oxidized dithiothreitol (DTT). The oxidation reaction was monitored by HPLC and DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) assay by measuring the absorbance at 412 nm.[2] The resulting product was purified by prep-HPLC with a C18 column (TCI, Kaseisorb LC ODS 2000, 10.0 mm × 250 mm) using a water (0.1% TFA)-acetonitrile (0.1% TFA) gradient to give **DPG1**. The successful synthesis of **DPG1** was confirmed by ESI mass spectrometry (Platform II, micromass, Manchester, UK) and its purity (>98%) was confirmed by reverse phase analytical HPLC with a C18 column (TCI, Kaseisorb LC ODS 2000, 4.6 mm × 250 mm). ESI-MS: calcd

1287.42, obsd 1287.47 $[M + H^+]^+$. ^{1}H NMR (400 MHz, D_2O/CD_3CN): δ 1.86-1.84 (m, 2H), 2.12-2.08 (m, 2H), 2.58-2.62 (m, 1H), 2.95-2.97 (m, 1H), 2.99 (s, 6H), 3.20 (dd, *J* = 7.8, 1.6 Hz, 1 H), 3.29 (dd, *J* = 7.8, 1.6 Hz, 1H), 3.32-3.38 (m, 2H), 3.87 (dd, *J* =8.4, 1.8 Hz, 1H), 3.99 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.04-4.06 (m, 2H), 4.16-4.18 (m, 1H), 7.00 (s, 1H), 7.38 (d, *J* = 7.6, 1H), 7.56-7.59 (m, 1H), 7.61-7.63 (m, 1H), 7.74 (s, 1H), 8.14 (d, *J* = 8.4, 1H), 8.45 (d, *J* = 9.0 Hz, 1H), 8.63 (d, *J* = 9.0 Hz, 1H).

1.3. General fluorescence measurements

A stock solution of **DPG1** with the concentration of 1.0×10^{-3} M was prepared in 3rd distillation water. This stock solution was used for all spectrofluoremetric titrations after appropriate dilution. The fluorescence titration was carried out using the above referred solution after maintaining the pH of the solution to 7.4 using 10 mM HEPES buffer. Fluorescence emission spectrum of a sample in a 10 mm path length quartz cuvette was measured Perkin Elmer luminescence spectrophotometer (model LS 55). Emission spectra (400–700 nm) of **DPG1** in the presence of several metal ions (Na^+ and K^+ as nitrile anion and Ag^+, Ca^{2+},Cd^{2+}, Co^{2+}, Hg^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Al^{3+} and Zn^{2+} as perchlorate anion) were measured by excitation with 330 nm.

1.4. Determination of dissociation constant

The dissociation constant was calculated based on the titration curve of the sensor with metal ion. The fluorescence signal, F, is related to the equilibrium concentration of the complex (HL) between Host (H) and metal ion (L) by the following expression:

$$F = F_o + \Delta F * [HL]$$

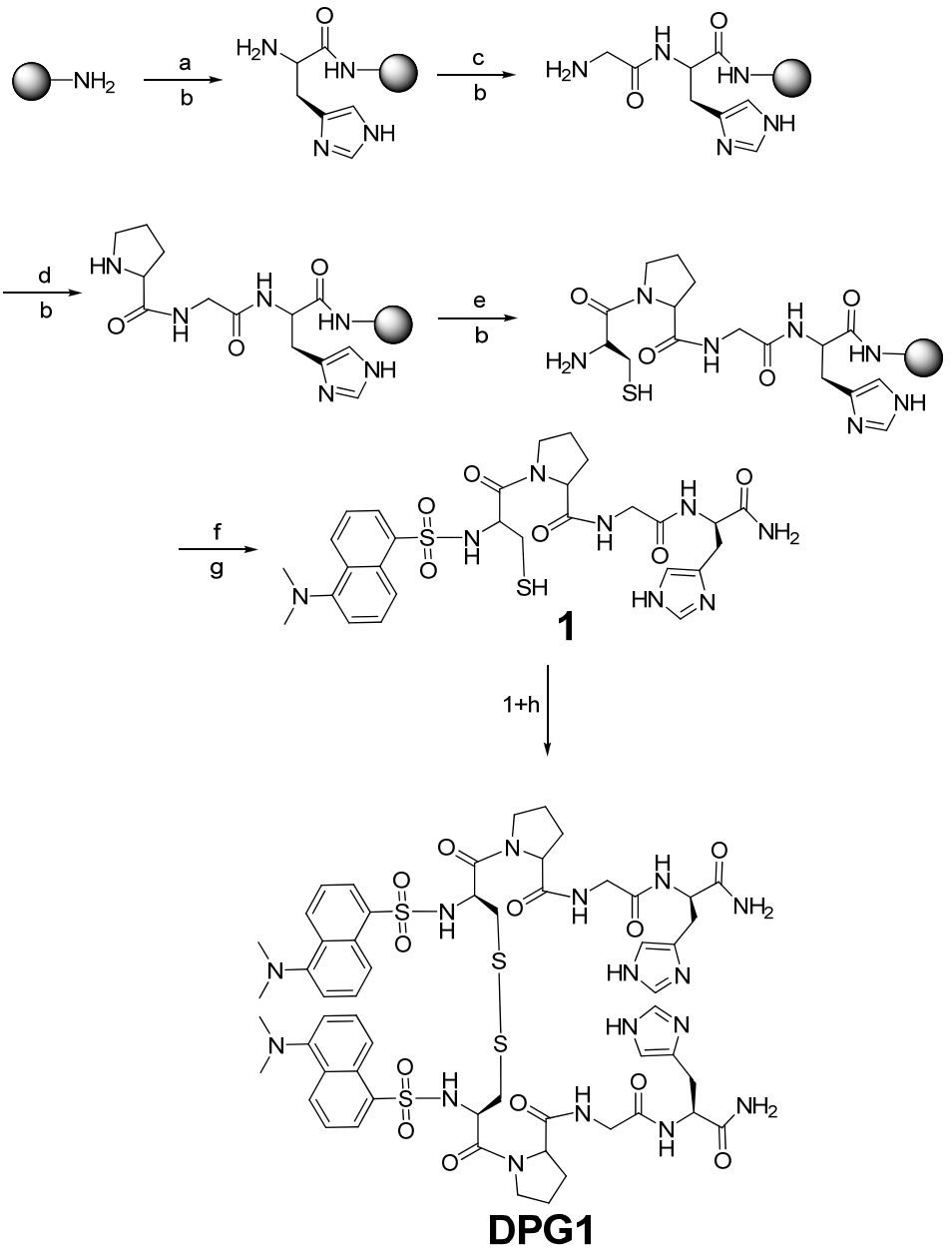
$$[HL] = 0.5\times [K_D + L_T + H_T - \{(- K_D - L_T - H_T)^2 - 4 L_T H_T\}^{1/2}]$$

Where F_o is the fluorescence of the probe only and ΔF is the change in fluorescence due to the formation of HL. Association constants were determined by a nonlinear least square fitting of the data with the equation.[3] The detection limit is calculated using the formula $3\sigma/m$, where σ is the standard deviation of the emission intensity of **DPG1** alone and, m is the slope between the intensity change at maximum intensity wavelength in relation to the

Ag^+ concentration.

1.5 Fluorescence measurements of silver nano particle(AgNP)

For the detection of AgNPs by **DPG1**, we utilized the oxidation reaction of AgNPs to Ag^+ by H_2O_2 under neutral conditions. A stock solution of AgNPs (100 μM) was prepared by dilution with water. A solution of H_2O_2 (5 equiv) was added into the stock solution of AgNPs (100 μM) and the resulting solution was stirred. Each solution (200 μl) containing AgNPs (100 μM) and H_2O_2 (5 equiv) was added into HEPES buffer solution at pH 7.4 containing **DPG1** at given time intervals (0, 2, 4, 6, 8, 10, 12, 14. 18 min). And then the fluorescence of the solution containing **DPG1** (5 μM), AgNPs (10 μM, 2 equiv), H_2O_2 (50 μM 5 equiv) was measured in 10 mM HEPES buffer solution at pH 7.4 (Figure S10). A solution of H_2O_2 (5 equiv) was added into the stock solution of AgNPs (100 μM) and the resulting solution was stirred for 4 min, to lead to the equilibration of AgNPs to Ag^+. Each solution with a different volume was added into HEPES buffer solution at pH 7.4 containing **DPG1** and the fluorescence spectrum of different concentration of AgNPs was measured in Figure S11.



a) Fmoc-His(Trt)-OH, HOBt, DIC; b) 20% Piperidine/DMF; c) Fmoc-Gly-OH, HOBt, DIC; d) Fmoc-Pro-OH, HOBt, DIC; e) Fmoc-Cys(Trt)-OH, HOBt, DIC; f) Dansyl chloride, TEA; g) TFA/TIS/H_2O h) Oxidized-DTT

Figure S1. Synthesis of **DPG1**.

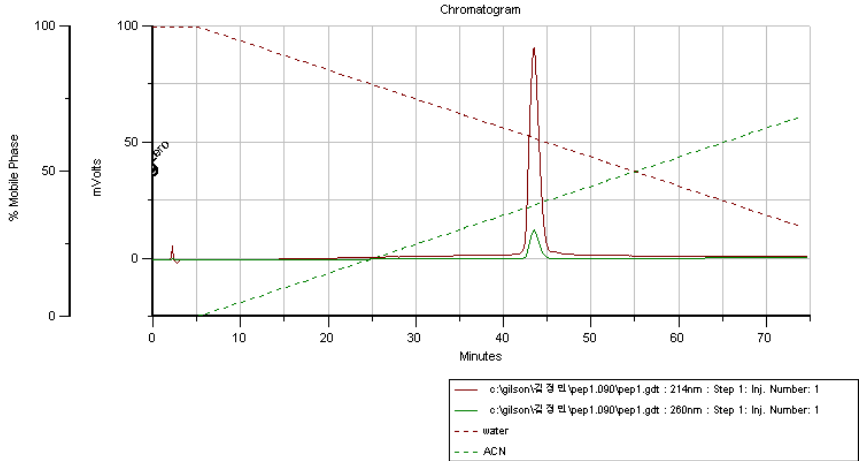


Figure S2. HPLC Chromatogram of **DPG1**.

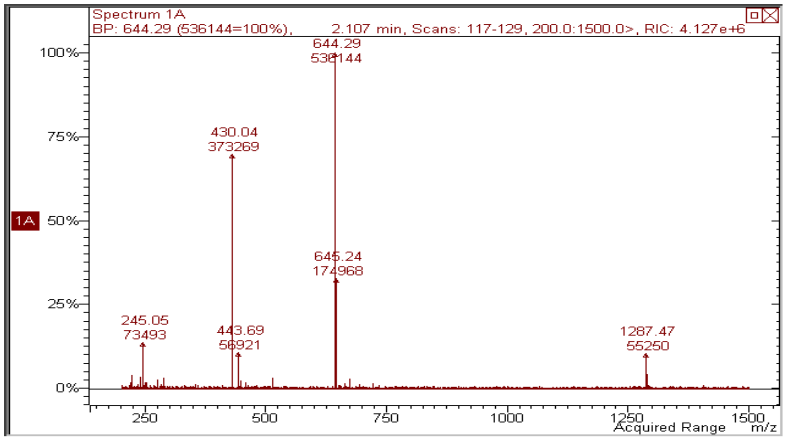


Figure S3. Mass spectrum of **DPG1** ($[M+H^+]^+$ Calcd. 1287.42; obsd. 1287.47).

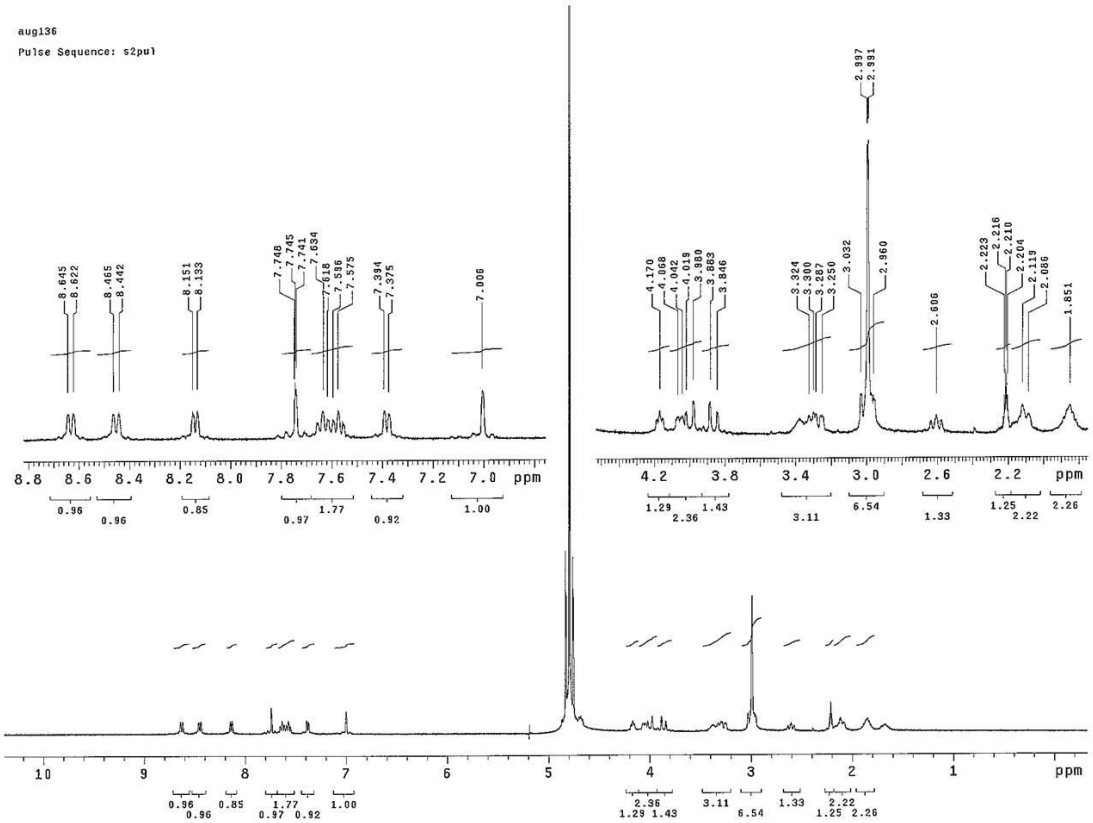


Figure S4. ^{1}H NMR of **DPG1** in D_2O/CD_3CN (80:20, v/v).

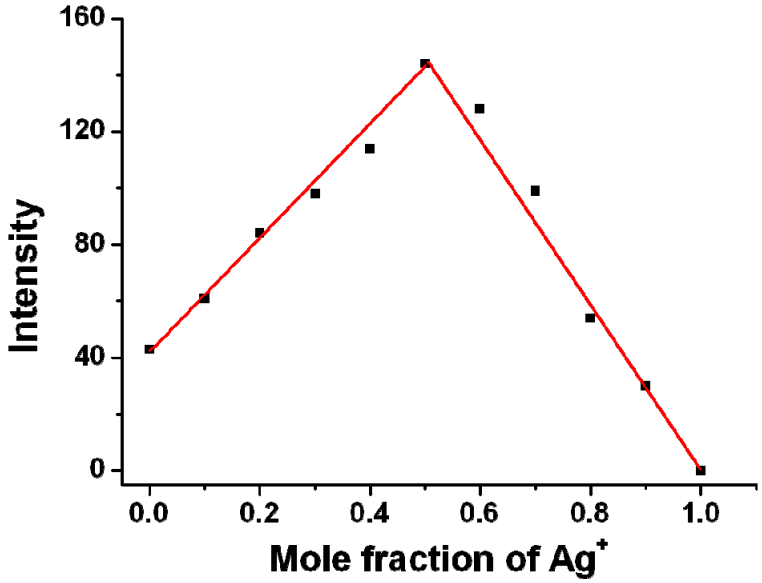


Figure S5. Job's plot for **DPG1** in 10 mM HEPES buffer solution at pH 7.4.

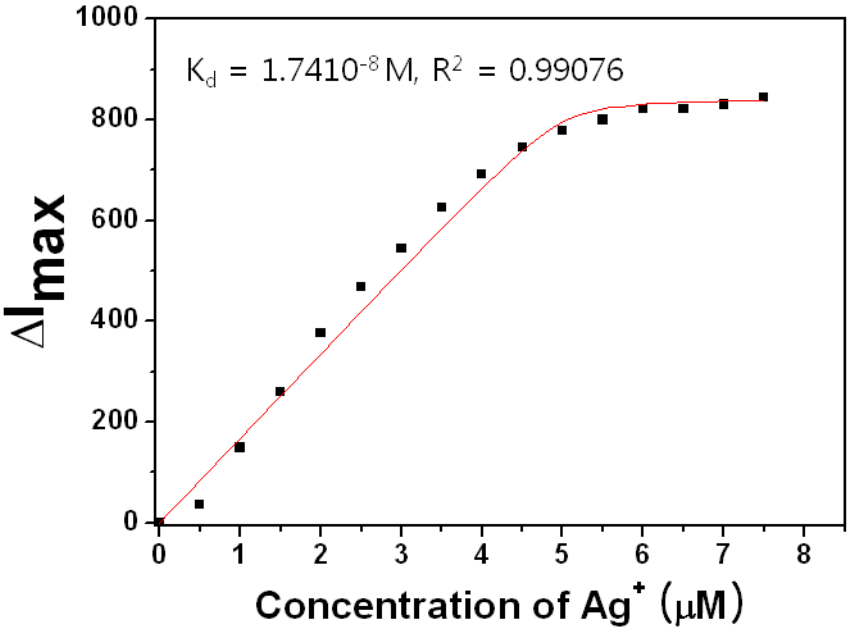


Figure S6. Titration of **DPG1** (5 μM) with Ag^+ in 10 mM HEPES buffer solution at pH 7.4.

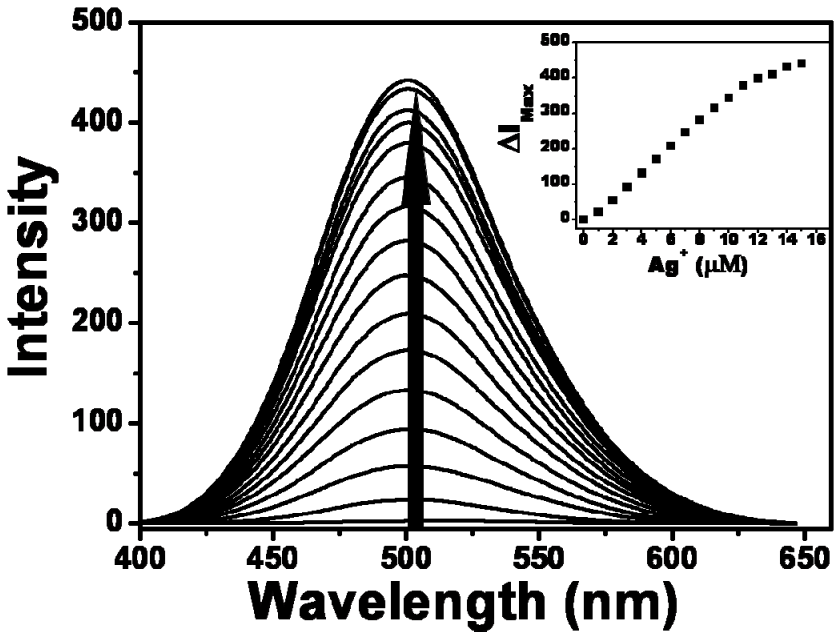


Figure S7. Titration of **DPG1** (10 μM) with Ag^+ in the presence of Cu^{2+} (2 equiv) in 10 mM HEPES buffer solution at pH 7.4.

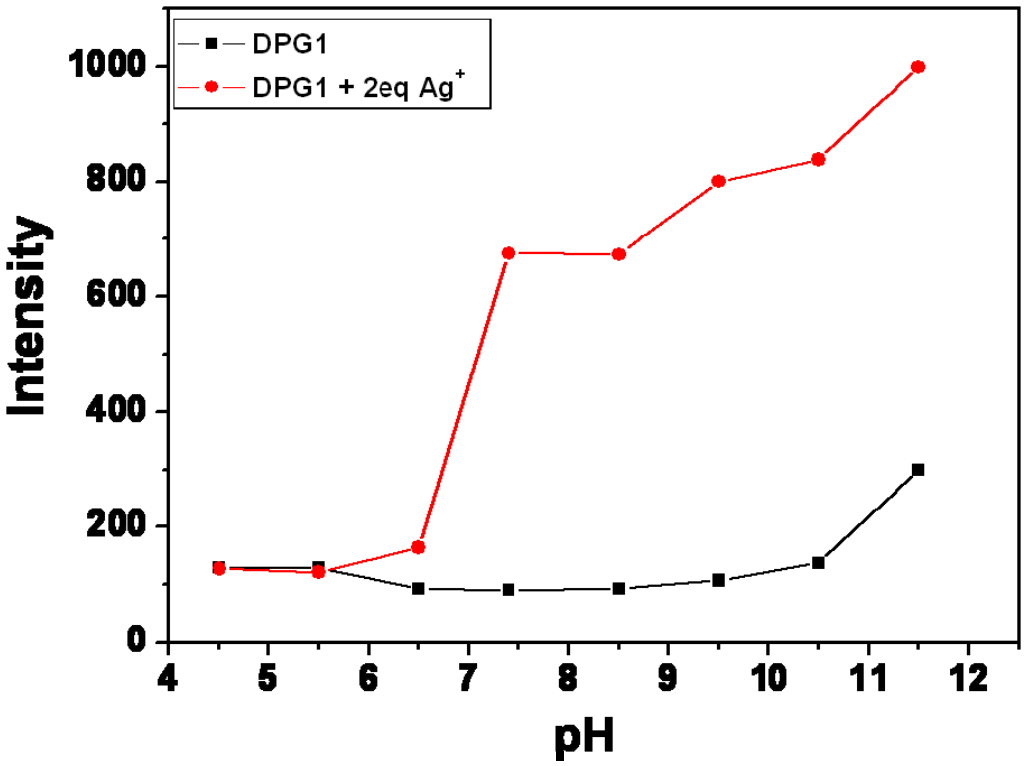


Figure S8. pH influence on the fluorescence intensity of **DPG1** in the absence and presence of Ag^{+} ions.

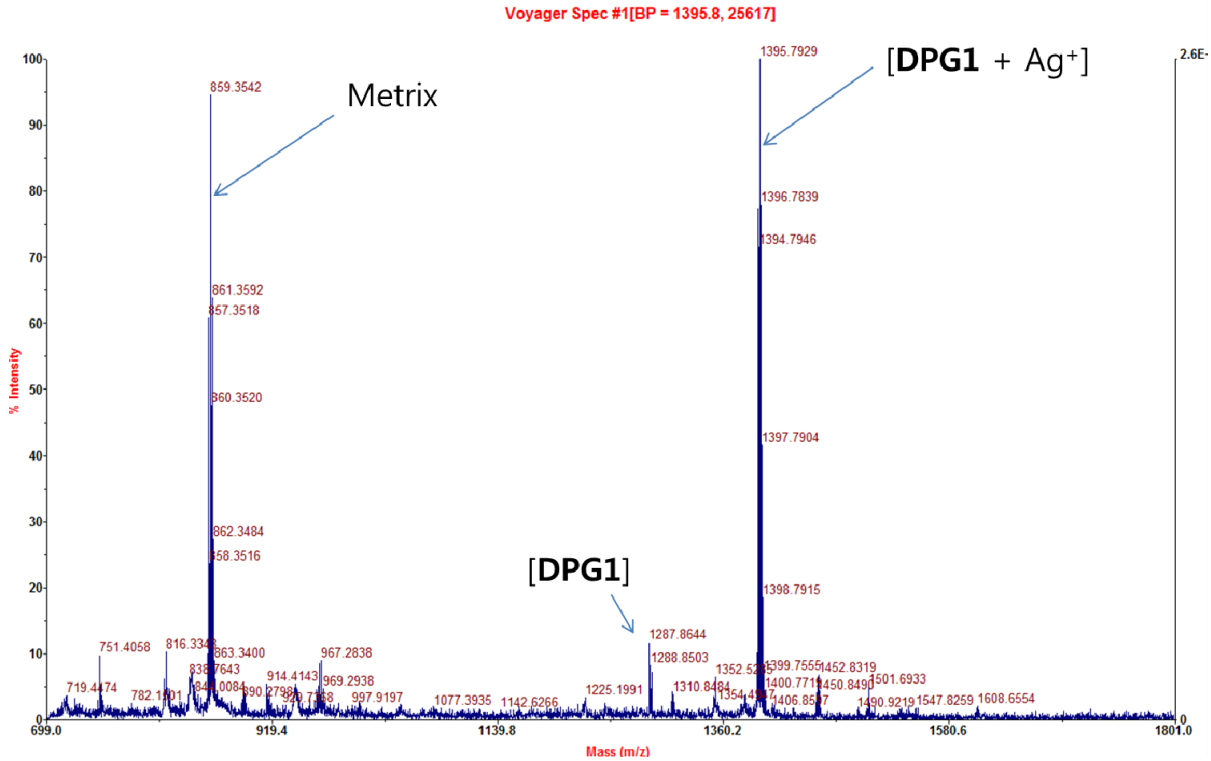


Figure S9. Mass spectrum of **DPG1** in the presence of Ag^{+} (2 equiv) ($[M + Ag^{+}]^{+}$ Calcd. 1393.31; obsd. 1394.79).

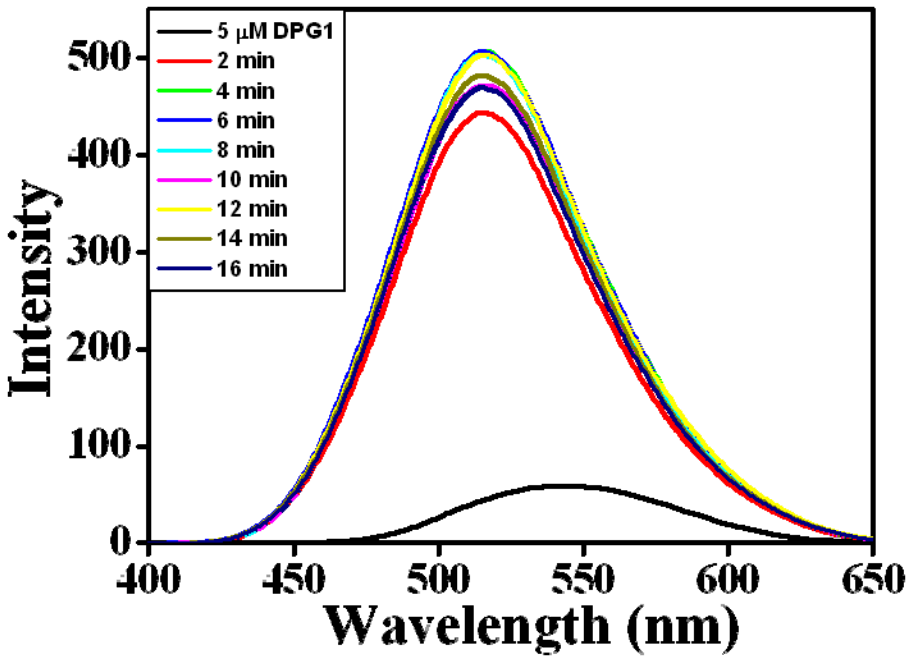


Figure S10. Changes in the fluorescence spectra of **DPG1** (5 μM) upon addition of AgNPs (10 μM) in the presence of 50 μM H_2O_2 (5 equiv) in 10 mM HEPES buffer solution at pH 7.4 (λ_{ex}=330 nm, slit=10/5).

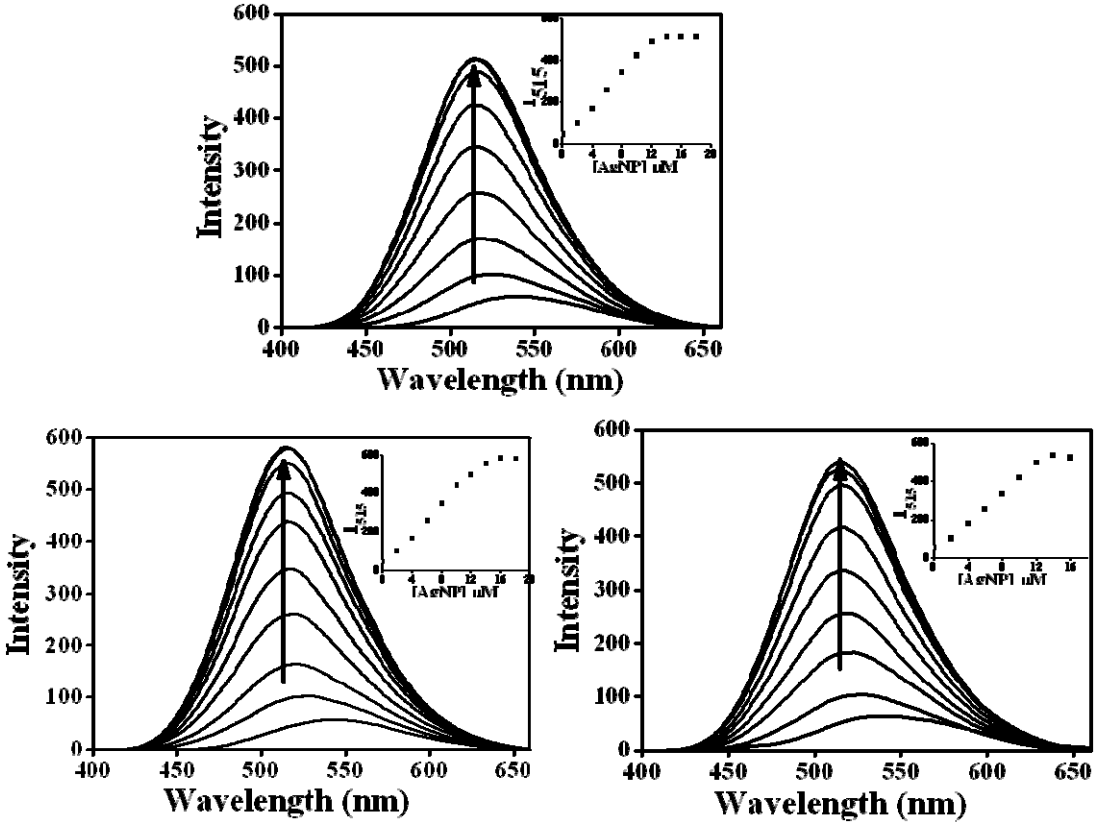


Figure S11. Changes in the fluorescence spectra of **DPG1** (5 μM) upon addition AgNPs (0, 2, 4, 6, 10, 12, 14 μM) in the presence of 50 μM H_2O_2 in 10 mM HEPES buffer solution at pH 7.4 (λ_{ex}=330 nm, slit = 10/5).

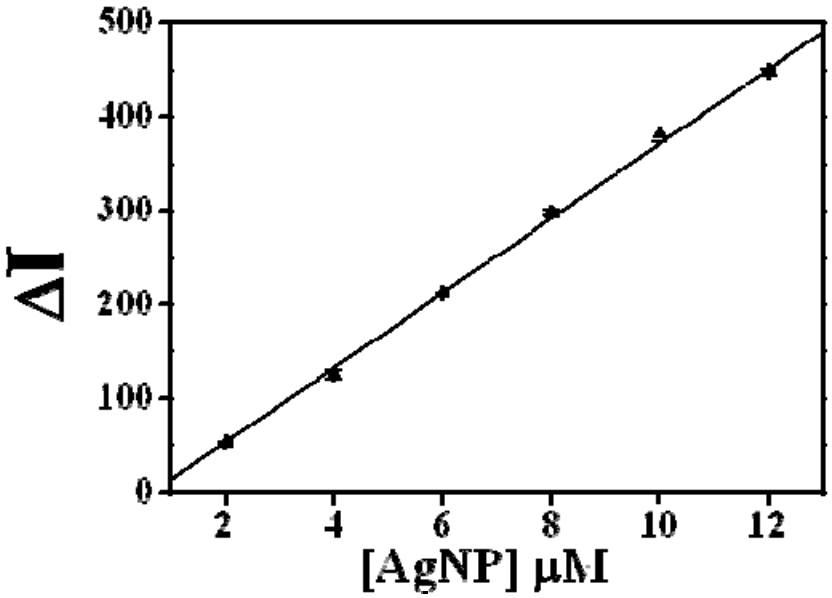


Figure S12. A standard plot that shows the linear relationship between the fluorescence intensity and the concentration of AgNPs.

References.

1. (a) S. S. Wang, *J. Org. Chem.,* 1975, **40**, 1235; (b) Wang, S. S., *J. Am. Chem. Soc.,* 1973, **95**, 1328; (c) G. B. Fields, *Methods in Enzymol*ogy, Academic Press, New York, 1997, **289**; (d) G. B. Fields, RL. Noble, *Int. J. Pept. Protein Res.,*1990, **35**, 161.

2. G. Bulaj, T. Kortemme, DP. Goldenberg, *Biochemistry*, 1998, **37**, 8965.

3. A. R. Reddi, T. R.Guzman, R. M. Breece, D. L. Tierney and B. R. Gibney, *J. Am. Chem. Soc.*, 2007, **129**, 12815.