Aptamer-induced self-assembly of a NIR-emissive platinum(II) terpyridyl complex for label- and immobilization-free detection of lysozyme and thrombin

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Supporting Information

Instrumentation: ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz) Fourier transform NMR spectrometer at ambient temperature with tetramethylsilane (Me_4Si) as an internal reference. Positive-ion fast atom bombardment (FAB) mass spectra were recorded on a Thermo Scientific DFS High Resolution Magnetic Sector mass spectrometer. Infrared (IR) spectra were recorded on a Bio-Rad FTS-7 Fourier transform infrared spectrophotometer (4000 – 400 cm^{-1}). Elemental analyses were performed on a Flash EA 1112 elemental analyzer at the Institute of Chemistry of the Chinese Academy of Sciences, Beijing. UV-Vis absorption spectra were recorded on a Cary 50 (Varian) spectrophotometer equipped with a Xenon flash lamp. Steady state emission spectra were recorded using a Spex Fluorolog-3 Model FL3-211 fluorescence spectrofluorometer equipped with a R2658P PMT detector. CD measurements were recorded using a Jasco (Tokyo, Japan) J-815 CD spectropolarimeter. Microquartz cuvettes with 10-mm path length and 2-mm window width were used for UV-vis, emission and CD measurements.

Chemicals and Materials: Lysozyme aptamer (LA) (5'-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3')[1] and 15-mer thrombin-binding aptamer (TBA) (5'-GGT TGG TGT GGT TGG-3')[2] were synthesized from Tech Dragon Ltd., Hong Kong, China. Hemin was purchased from Alfa Aesar. Fetal bovine serum (FBS) was purchased from GIBCO. Lysozyme (molecular weight MW = 14.4; isoelectric point pI = 11), thrombin (MW = 37.4; pI = 6.35 – 7.6), human serum albumin (MW = 66; pI = 4.7), bovine serum albumin (MW = 69; pI = 4.7), myoglobin (MW = 17.6; pI = 6.8), trypsin (MW = 24; pI = 10.1 – 10.5) and other chemicals used were purchased from Sigma-Aldrich. Deionized water (18.2 MΩ) used for measurements was purified by ELGA PURELAB UHQ system. 1-(Propargyloxy)benzene-3,5-dimethanol was

synthesized by modification of literature reported procedures.[3]

Synthesis of 1: 1 was synthesized by the reaction of [Pt(tpy)Cl]OTf (200 mg, 0.33 mmol) with 1-(propargyloxy)benzene-3,5-dimethanol (192 mg, 1 mmol) in degassed DMF (10 ml) in the presence of triethylamine (1 ml) and a catalytic amount of CuI. The reaction mixture was stirred overnight at room temperature. After addition of diethyl ether (150 mL), the mixture was stirred for further 15 min. The precipitate formed was filtered and washed with methanol (10 ml). Subsequent recrystallization by diffusion of diethyl ether vapour into the acetonitrile-methanol solution of **1** gave the pure product as an orange solid. Yield: 110 mg (44%). ^{1}H NMR (400 MHz, d^6-DMSO, 298 K, δ/ppm): δ 8.86-8.85 (d, *J* = 5.5 Hz, 2H; terpyridyl H), 8.64-8.56 (m, 5H; terpyridyl H), 8.48-8.44 (dt, *J* = 1.4, 7.9 Hz, 2H; terpyridyl H), 7.78-7.74 (dt, *J* =1.3, 7.4 Hz, 2H; terpyridyl H), 6.95 (s, 2H, –C_6H_3–), 6.89 (s, 1H, –C_6H_3–), 5.24-5.21 (t, *J* = 5.5 Hz, 2H; –CH_2O***H***), 5.05 (s, 2H, –CH_2O–), 4.48-4.47 (d, *J* = 5.2 Hz, 4H; –C***H***$_2$OH); IR (Nujol, *v*/cm-1): 2135 (w) *v*(C≡C), 1155 (s) *v*(S=O); positive-ion FAB-MS, *m/z*: 619 [M – OTf]$^+$; elemental analysis calcd (%) for $C_{27}H_{22}N_3F_3SO_6Pt\cdot H_2O$: C 41.22, H 3.08, N 5.34; found: C 41.13, H 3.33, N 5.54.

Assay procedure for lysozyme measurement: Mixture of 10 µl of LA (100 µM), 50 µl of buffer solution (0.2 M Tris, 1 M NaCl, 50 mM $MgCl_2$, pH 7.5) and 300 µl of water was incubated at 80^{o}C for 5 min, allowed slowly cool down to room temperature.[1,4] 17.5 µl of 1.2 mM of complex **1** and 25 µl of acetonitrile were added and the resultant mixture was incubated at ambient temperature for 30 min. Lysozyme and water of appropriate amount were added to reach the final volume as 500 µl, further incubated at ambient temperature for 15 min before

measurement. All concentrations in the final assay solution mixture were calculated with buffer solution of 20 mM Tris, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.5 in 5% (v/v) acetonitrile, 2 μM LA and 42 μM of **1**. Both UV-Vis and emission spectra were then recorded at ambient temperature. The emission spectra were recorded at an excitation wavelength of 300 nm and corrected for PMT response.

Assay procedure for TBA-complex 1 measurement without the presence of hemin: Mixture of 30 μl of TBA (100 μM), 50 μl of buffer solution (20 mM Tris, 140 mM NaCl, 5 mM KCl, pH 7.5),[5] 300 μl of water and 10 μl of DMSO were incubated at ambient temperature for 1 hour. 17.5 μl of 1.2 mM of complex **1** and water of appropriate amount were added to reach the final volume as 500 μl and the resultant mixture was further incubated at ambient temperature for 30 min before measurement. All concentrations in the final assay solution mixture were calculated with buffer solution of 2 mM Tris, 14 mM NaCl, 0.5 mM KCl, pH 7.5 in 2% (v/v) DMSO, 6 μM TBA, 42 μM of **1**. Both UV-Vis and emission spectra were then recorded at ambient temperature. The emission spectra were recorded at an excitation wavelength of 297 nm and corrected for PMT response.

Assay procedure for TBA-hemin-complex 1 measurement and thrombin measurement: Stock solution of hemin (0.5 mM) was prepared in DMSO and stored in dark at -20^{o}C prior to use.[6] It was diluted to the required concentration with water when used. The assay procedure was similar to that for the measurement without the presence of hemin except that the mixture of TBA, buffer solution, water and 4 μl DMSO were incubated at ambient temperature for 30 min, followed by addition of 50 μl of hemin (60 μM) to the mixture and incubation for another 30 min

prior to the addition of **1**.[7] After incubation with **1**, thrombin of appropriate amount were added and further incubated at room temperature for 30 min before measurement. All concentrations in the final assay solution mixture were calculated with buffer solution of 2 mM Tris, 14 mM NaCl, 0.5 mM KCl, pH 7.5 in 2% (v/v) DMSO, 6 μM TBA, 6 μM hemin, 42 μM of **1**. Both UV-Vis and emission spectra were then recorded at ambient temperature. The emission spectra were recorded at an excitation wavelength of 297 nm and corrected for PMT response.

Assay procedure for measurement of control experiments: For the measurement with the presence of interfering protein, the assay procedure was similar to that for the measurement of the target protein except the corresponding target protein was replaced by same concentration of the interfering protein. For the measurement with the presence of both target and interfering proteins, the assay procedure was similar to that for the measurement of the target protein alone except the interfering protein of same concentration was added with the target protein before final incubation prior to the measurement.

Assay procedure for measurements in biological media: FBS and human saliva were selected for investigation. FBS was used without further treatment. Human saliva was freshly collected from human and was centrifuged to get supernatant for use.[1] The assay procedure was similar to that for the measurement of the target protein in original buffer except that 5 μl of the medium was added along with aptamer-containing mixture at the very beginning for the first incubation. The concentration of medium in the final assay solution mixture (500 μl) was 1 % (v/v) (diluted in 100 times).

References:

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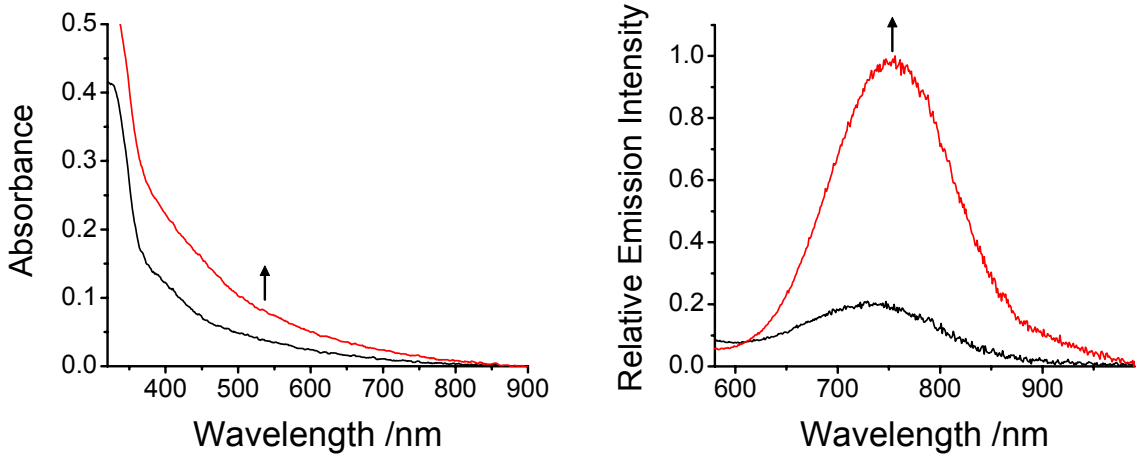

Fig. S1 (Left) UV-Vis absorption and (right) emission spectral changes of 42 μM of complex **1** (—) and in the presence of 2 μM of LA (—). Medium: 20 mM Tris, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.5, 5% (v/v) acetonitrile.

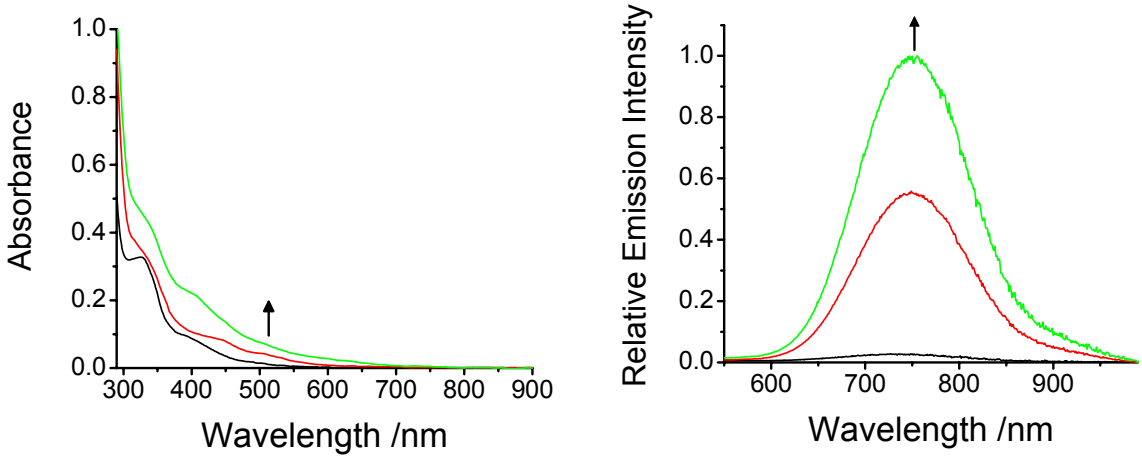

Fig. S2 (Left) UV-Vis absorption and (right) emission spectral changes of 42 μM of complex **1** (—), in the presence of 6 μM TBA (—), and in the presence of 6 μM TBA and 6 μM hemin (—). Medium: 2 mM Tris, 14 mM NaCl, 0.5 mM KCl, pH 7.5, 2% (v/v) DMSO.

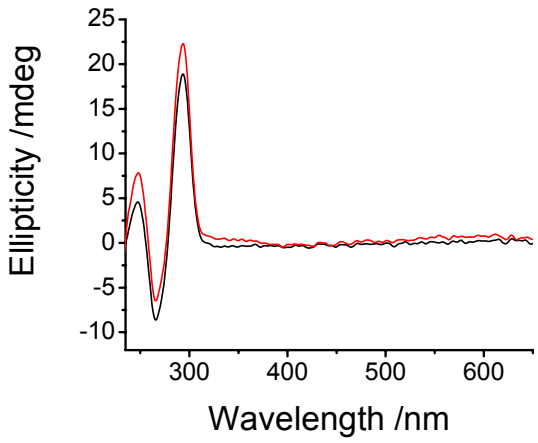

Fig. S3 CD spectra of 6 μM TBA (—), 6 μM TBA and 6 μM hemin (—). Medium: 2 mM Tris, 14 mM NaCl, 0.5 mM KCl, pH 7.5, 2% (v/v) DMSO.

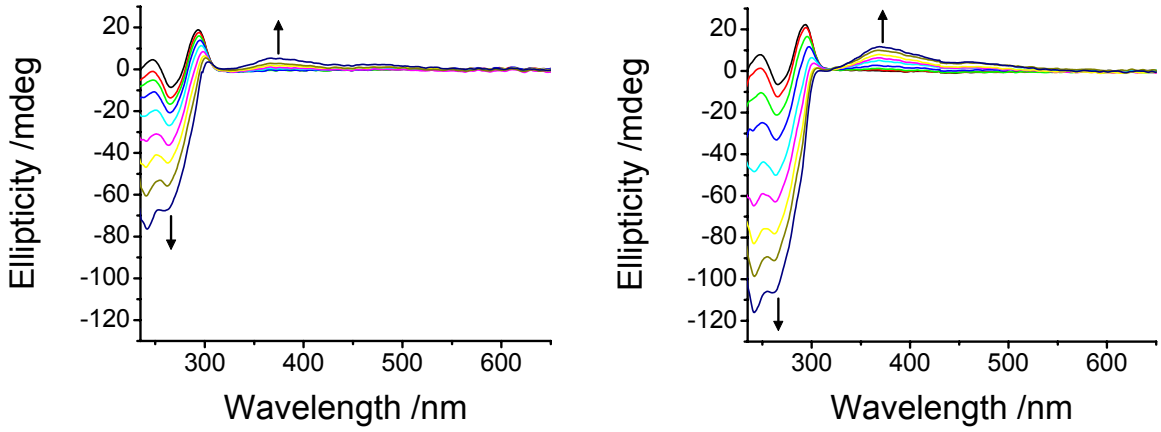

Fig. S4 CD spectra of 6 μM TBA (left), and 6 μM TBA and 6 μM hemin (right) upon addition of various concentration of **1** up to 42 μM. Medium: 2 mM Tris, 14 mM NaCl, 0.5 mM KCl, pH 7.5, 2% (v/v) DMSO.

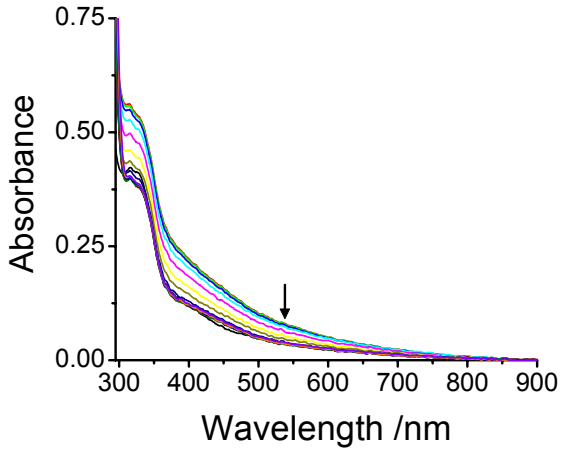

Fig. S5 UV-Vis absorption spectral changes of complex **1** upon addition of various concentrations of lysozyme to LA–complex mixture. Final concentration: 2 μM of LA and 42 μM of **1**. Medium: 20 mM Tris, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.5, 5% (v/v) acetonitrile.

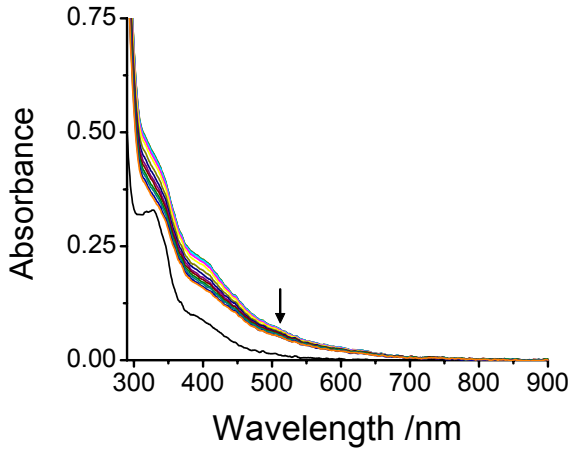

Fig. S6 UV-Vis absorption spectral changes of complex **1** upon addition of various concentrations of thrombin to TBA–hemin–complex mixture. Final concentration: 6 μM of TBA, 6 μM of hemin and 42 μM of **1**. Medium: 2 mM Tris, 14 mM NaCl, 0.5 mM KCl, pH 7.5, 2% (v/v) DMSO.

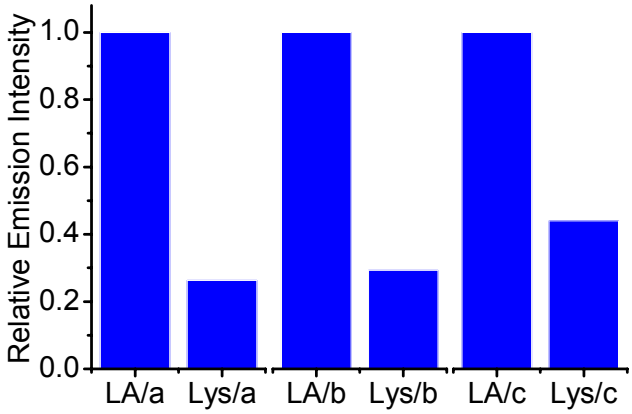

Fig. S7 Analysis for potential application of LA–complex mixture. Lysozyme (Lys) detection in (a) buffer, (b) diluted fetal bovine serum (FBS) and (c) diluted human saliva, with emission intensity relative to that in the absence of lysozyme in their corresponding media. Final concentration: 2 μM of LA, 42 μM of **1** and 24 μM of Lys.

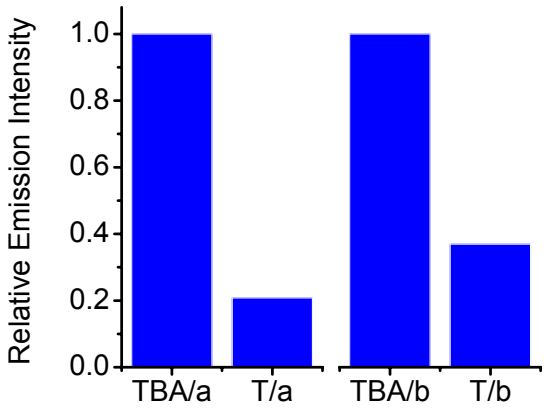

Fig. S8 Analysis for potential application of TBA–hemin–complex mixture. Thrombin (T) detection in (a) buffer and (b) diluted fetal bovine serum (FBS), with emission intensity relative to that in the absence of thrombin in their corresponding media. Final concentration: 6 μM of TBA, 6 μM of hemin, 42 μM of **1** and 6 μM of T.