"Proof-of-principle" concept for label-free detection of glucose and α-glucosidase activity through the electrostatic assembly of alkynylplatinum(II) terpyridyl complexes

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Supporting Information

Experiment Section:

Materials: α-Glucosidase was purchased from Sigma-Aldrich. All other reagents were of analytical grade and were used without further purification. The reactions were performed under an inert atmosphere of nitrogen unless specified otherwise.

Instrumentation: ^{1}H NMR spectra were recorded with a Bruker AVANCE 400 (400 MHz) Fourier transform NMR spectrometer at ambient temperature with tetramethylsilane (Me_4Si) as an internal reference. 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. Unless specified otherwise, the emission spectra were corrected for PMT response.

Synthesis of [Pt(tpy)(C≡C$C_6H_4CH_2NMe_3$-4)]$(OTf)_2$, (1): The ligand H-C≡C$C_6H_4CH_2NMe_3$-4 and complex **1** were synthesized as reported previously.[1]

Synthesis of PAAPBA: According to the literature methods,[2] AAPBA monomer (100 mg) was mixed with acrylamide (148.9 mg) and radical initiator AIBN (4.3 mg) in absolute ethanol (8 mL). The solution mixture was degassed by freeze-pump-thaw cycles. Polymerization was carried out by UV light illumination at 365 nm for 15 hours. After polymerization, a milky white solution was obtained. The polymer was isolated by centrifugation (6000 rpm, 20 min) after adding 40 mL acetone, and further purified by washing with same volume of acetone several times. The polymer was then dried under vacuum. ^{1}H NMR analysis confirmed the formation of the copolymer and the composition of AAPBA unit of the polymer was calculated to be

20 % (Fig. S4). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.60-2.20 (br, -C***H***$_2$-), 2.20-2.60 (br, O=CC***H***-), 7.20-8.00 (br, Ar***H***).

α-Glucosidase assay: In order to determine the activity of the enzyme at physiological pH and temperature, the enzyme and maltose were first incubated separately in aqueous buffer solution (50 mM potassium phosphate, pH 6.8) at 37 ^oC for 20 min. Then they were mixed and the assay was immediately started, i.e. t =0. At desired time interval t, 40 μL solution mixture was withdrawn and transferred to 460 μL of the solution containing complex **1** and PAAPBA in a basic buffer-CH_3CN solution (30 mM Tris-HCl, 30 mM NaCl, 20 % CH_3CN, pH 9.0). The enzyme was inactivated in this solution mixture (Fig. S5) by the presence of organic solvent and the basic environment, and the emission spectra were recorded by excitation at 300 nm to determine I_t, which was the relative emission intensity at 800 nm at time t.

Correlation of I_t with [Maltose]$_r$: To correlate I_t with the concentration of maltose reacted at time t, [Maltose]$_r$, a calibration curve of emission at 800 nm against glucose concentration (Fig. S2) was obtained by a titration of glucose in 50 mM potassium phosphate buffer solution at pH 6.8 into the solution containing complex **1** and PAAPBA in the buffer-CH_3CN solution (30 mM Tris-HCl, 30 mM NaCl, 20 % CH_3CN, pH 9.0). From this curve and considerations on the dilution of glucose by the sensing solution which is equal to a factor of 12.5, I_t can be correlated with the concentration of glucose formed at time t, [Glucose]$_t$, directly. As 1 mole of maltose gives 2 moles of glucose after catalytic cleavage of the glucosidic linkage, [Maltose]$_r$ is equal to half of [Glucose]$_t$ and a graph of [Maltose]$_r$ against time with different initial maltose concentration can be obtained (Fig. S3). From the slope of the linear region of the graph of [Maltose]$_r$ against time at t = 0, the initial rate of catalytic

reaction by α-glucosidase, V_0, at a particular initial maltose concentration can be determined.

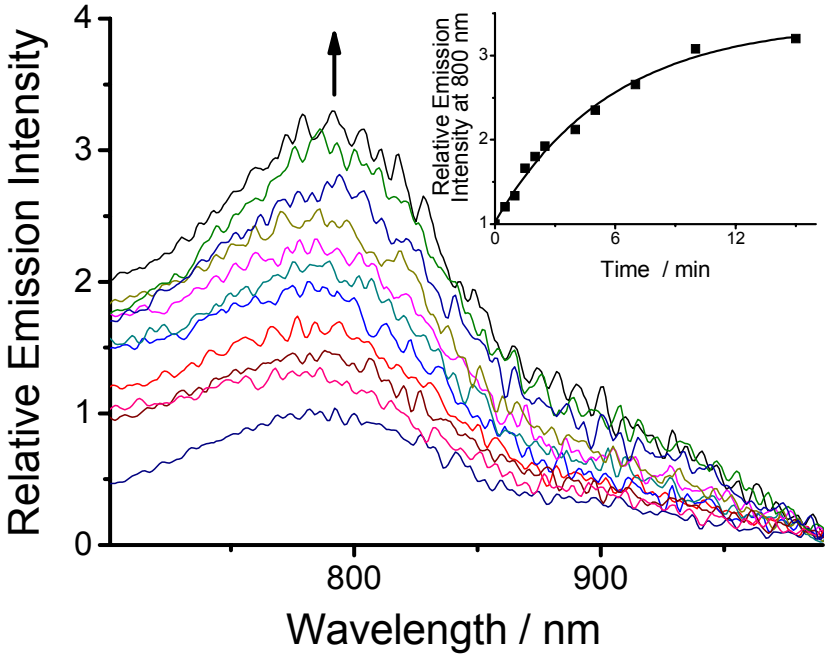

Fig. S1 Emission spectral changes of **1** and PAAPBA in 460 μL buffer-CH_3CN solution (30 mM Tris-HCl, 30 mM NaCl, 20% CH_3CN, pH 9.0) upon addition of 40 μL aqueous buffer solution (50 mM potassium phosphate, pH 6.8) which contained an incubated mixture of 0.4 mg/mL α-glucosidase and 64 mM maltose at different time intervals *t*. Concentrations of **1** and boronic acid moieties of the polymer in the final 500 μL solution mixture are both 83.3 μM. Inset: Plot of the relative emission intensity at 800 nm versus time *t*.

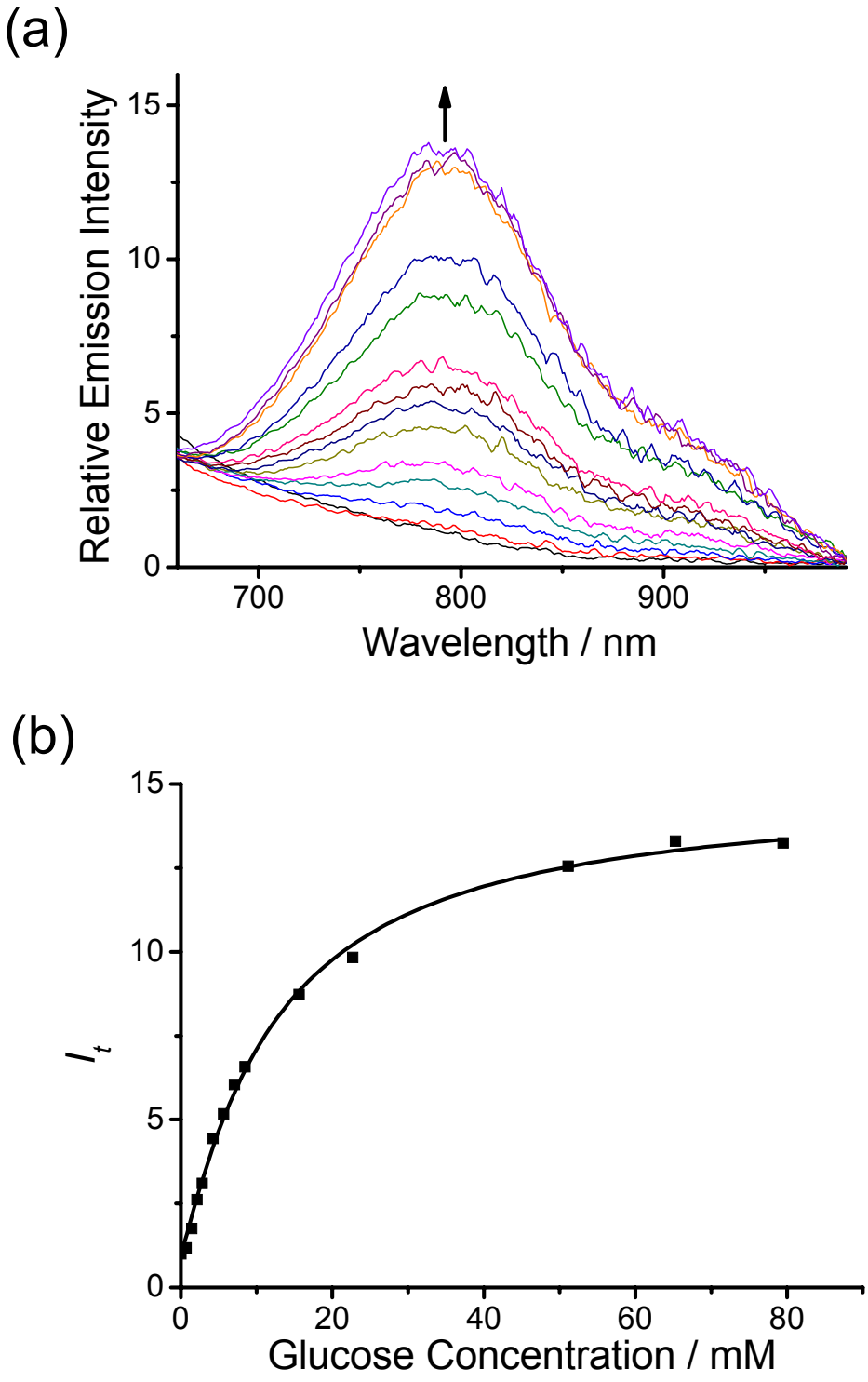

Fig. S2 (a) Emission spectral changes of complex **1** and PAAPBA in buffer-CH_3CN solution (30 mM Tris-HCl, 30 mM NaCl, 20 % CH_3CN, pH 9.0) upon mixing with different concentration of glucose in aqueous buffer solution (50 mM potassium phosphate, pH 6.8). Concentrations of **1** and boronic acid moieties of the polymer in the final solution mixture are both 83.3 μM. (b) Calibration curve of I_t versus glucose concentration.

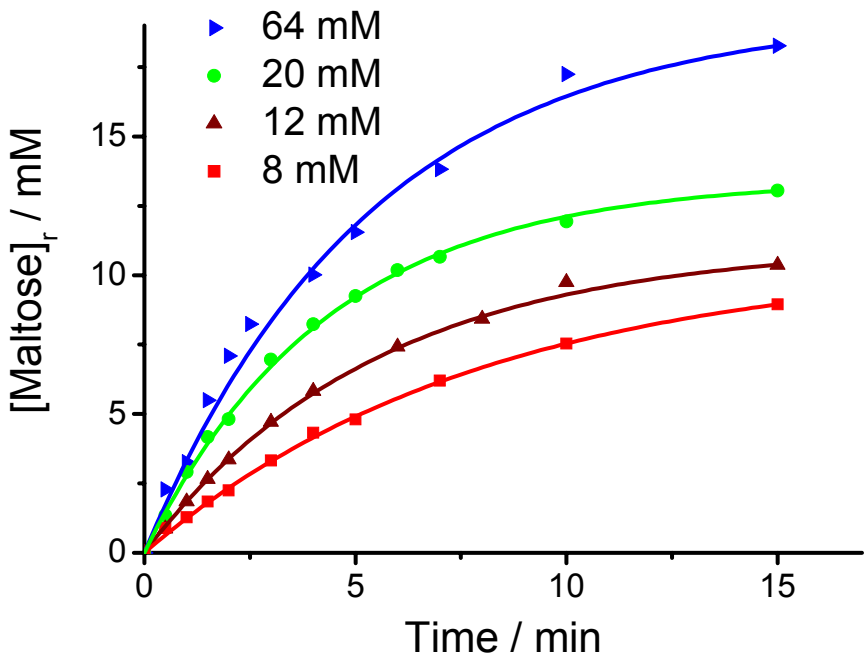

Fig. S3 Plot of $[Maltose]_r$ against time with 8, 12, 20 and 64 mM initial maltose concentration. Solution condition: 500 μL solution mixture composed of 460 μL buffer-CH_3CN solution (30 mM Tris-HCl, 30 mM NaCl, 20% CH_3CN, pH 9.0) and 40 μL aqueous buffer solution (50 mM potassium phosphate, pH 6.8) with 0.4 mg/mL α-glucosidase. Concentrations of **1** and boronic acid moieties of the polymer in the final 500 μL solution mixture are both 83.3 μM.

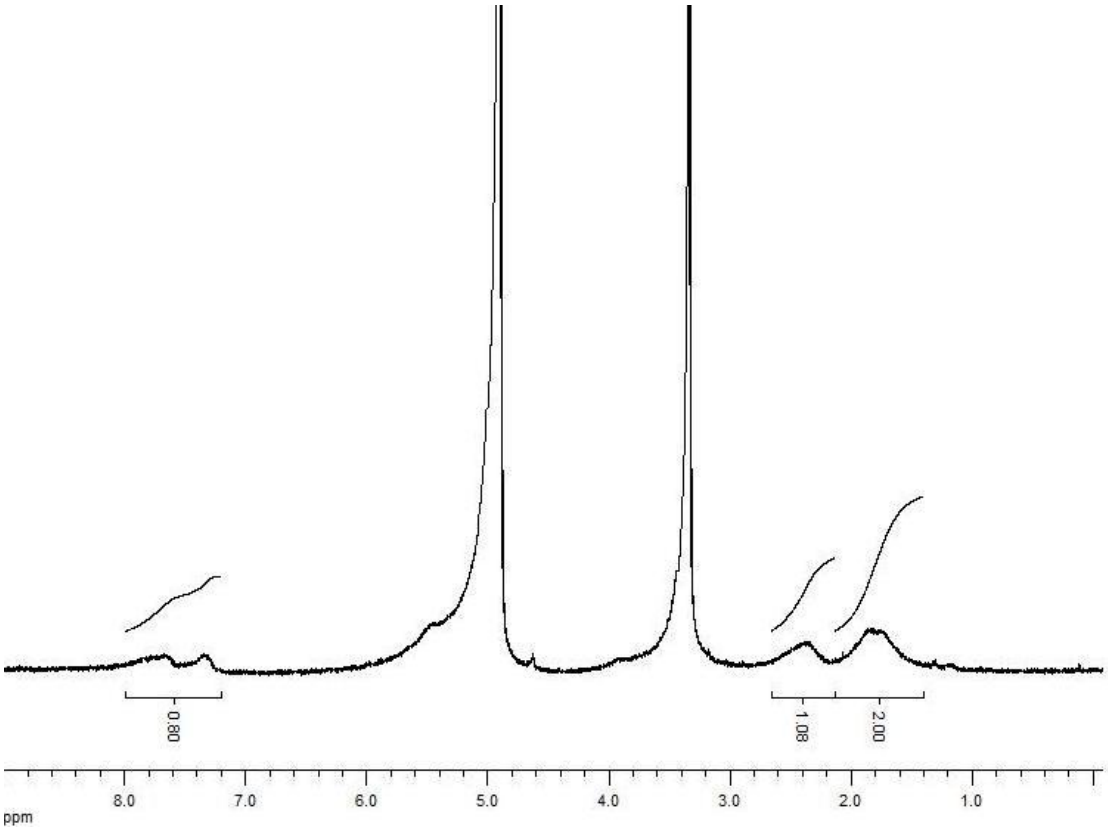

Fig. S4 ^{1}H NMR spectrum of boronic acid-containing polymer in CD_3OD.

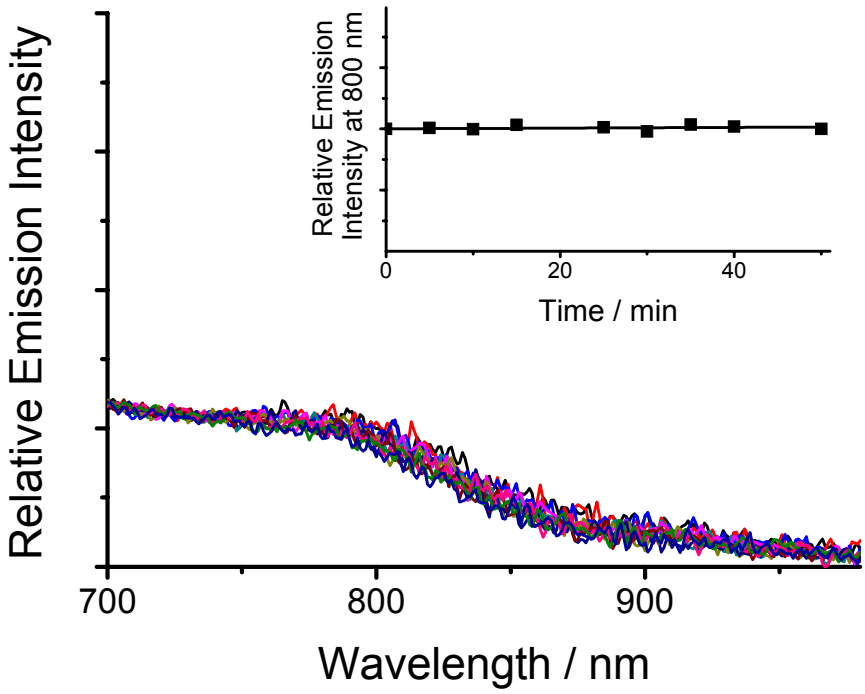

Fig. S5 Inactivation of α-glucosidase by basic buffer-CH_3CN solution: Emission spectral changes of complex **1** and PAAPBA in 460 μL buffer-CH_3CN solution (30 mM Tris-HCl, 30 mM NaCl, 20 % CH_3CN, pH 9.0) with time upon mixing with 40 μL aqueous buffer solution (50 mM potassium phosphate, pH 6.8) which contained an incubated mixture of 0.4 mg/mL α-glucosidase and 64 mM maltose. Concentrations of **1** and boronic acid moieties of the polymer in the final 500 μL solution mixture are both 83.3 μM. Inset: Plot of the relative emission intensity at 800 nm versus time.

References:

1. C. Yu, K. H. Y. Chan, K. M. C. Wong and V. W. W. Yam, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, 103, 19652; (e) C. Yu, K. H. Y. Chan, K. M. C. Wong and V. W. W. Yam, *Chem.–Eur. J.* **2008**, 14, 4577
2. (a) S. Kitano, Y. Koyama, K. Kataoka, T. Okano and Y. Sakurai, *J. Controlled Release*, **1992**, 19, 162-170. (b) K. Kataoka, H. Miyazaki, M. Bunya, T. Okano and Y. Sakurai, *J. Am. Chem. Soc.* **1998**, 120, 12694-12695.