Electronic Supplementary Information

Platinum(II) Non-Covalent Crosslinkers for Supramolecular DNA Hydrogels

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Experimental Section

Materials and reagents

Complexes **1**–**4** were synthesized according to the methods reported previously or by a slight modification of a literature procedure (ref. S1 and S2). Salmon testes DNA (smDNA, Sigma-Aldrich, D1626) and calf thymus DNA (ctDNA, Sigma-Aldrich, D1501) were dissolved in deionized water at 1.0 mg/mL by gentle magnetic stirring before use. Both DNAs are double-stranded molecules. The smDNA and ctDNA have average sizes of ~2 kilo base pairs and ~20 kilo base pairs, respectively. The smDNA possess a %G-C content of 41.2% according to the specification provided by Sigma-Aldrich. Sodium polyacrylates with a molecular weight of 10^7 (Sinopharm Chemical Reagent Co. Ltd.) were dissolved in deionized water at 1.0 mg/mL before use. Sodium polyacrylates with an average degree of polymerization of 50 were synthesized via atom transfer radical polymerization of *tert*-butyl acrylate (ref. S3), followed by hydrolysis using trifluoroacetic acid and neutralization using sodium hydroxide. L-Glutathione (AK Scientific, Inc., reduced 98%) was used as received.

Complex **2**. ^{1}H NMR (500 MHz, CD_3CN, 320 K, δ/ppm): δ = 0.96 (t, J = 6.8 Hz, 3H, alkyl), 1.42 (m, 4H, alkyl), 1.52 (m, 2H, alkyl), 1.86 (m, 2H, alkyl), 4.24 (t, J = 6.8 Hz, 2H, alkyl), 7.27-7.39 (m, 3H, $–C_6H_5$), 7.45 (d, 2H, J = 7.5 Hz, $–C_6H_5$), 7.66 (t, J = 6.3 Hz, 2H, trpy), 7.71 (s, 2H, trpy), 8.16-8.29 (m, 4H, trpy), 9.03 (d, J = 5.5 Hz, 2H, trpy). $^{13}C\{^1H\}$ NMR (125 MHz, CD_3CN, 320 K, δ/ppm): δ = 14.5, 23.4, 26.1, 29.4, 32.4, 72.0, 98.4, 102.8, 111.5, 126.3, 127.4, 127.9, 129.3, 130.1, 132.8, 142.3, 154.1,

155.8, 159.6, 170.3. HRMS (positive ESI) m/z found (calcd for $C_{29}H_{28}N_3OPt$): 629.1905 (629.1877).

$[Pt(bzimpy)(GS)]^+$ (purified product from **3a**–GSH reaction mixture). ^1H NMR (500 MHz, D_2O, 298 K, δ/ppm): δ = 1.52 (m, 1H, β-Glu), 1.63 (m, 1H, β-Glu), 1.73 (m, 1H, γ-Glu), 2.03 (m, 1H, γ-Glu), 2.61 (m, 1H, β-Cys), 2.82 (m, 1H, β-Cys), 3.31 (AB_q, $\Delta\nu$ = 36.3 Hz, J_{AB} = 18.0 Hz, 2H, α-Gly), 3.45 (t, J = 6.2 Hz, 1H, α-Glu), 4.09 (s, 6H, $–CH_3$), 4.47 (m, 1H, α-Cys), 7.31 (d, J = 7.2 Hz, 2H, bzimpy), 7.53 (m, 4H, bzimpy), 8.36 (d, J = 6.8 Hz, 2H, bzimpy), 8.49 (d, J = 7.5 Hz, 2H, bzimpy), 8.60 (t, J = 7.5 Hz, 1H, bzimpy). $^{13}C\{^1H\}$ NMR (125 MHz, D_2O, 298 K, δ/ppm): δ = 25.6 (β-Glu), 31.0 (γ-Glu), 32.3 ($–CH_3$), 33.6 (β-Cys), 41.0 (α-Gly), 53.5 (α-Glu), 57.8 (α-Cys), 112.1 (bzimpy), 116.9 (bzimpy), 124.4 (bzimpy), 126.4 (bzimpy), 126.7 (bzimpy), 134.7 (bzimpy), 139.1 (bzimpy), 143.0 (bzimpy), 146.9 (bzimpy), 153.5 (bzimpy), 172.4 (–COOH or –CONH–), 172.6 (–COOH or –CONH–), 173.0 (–COOH or –CONH–), 173.9 (–COOH or –CONH–). HRMS (positive ESI) m/z found (calcd for $C_{31}H_{33}N_8O_6PtS$): 840.1780 (840.1888).

Preparation of the platinum(II)–DNA hydrogels

Complex **1** in acetonitrile solution (1 mg/mL) was added into an aqueous solution of smDNA (1 mg/mL) at platinum(II)/base pair molar ratio of 1/1. Gel-like precipitates formed quickly upon the mixing of the platinum(II) complexes and smDNA. Unlike the transient presence of the gel-like precipitates in the mixture at complex **1**/base pair

molar ratio of 1 : 9, the gel-like precipitates formed at platinum(II)/base pair molar ratio of 1 : 1 were stable in the mixture. The gel-like precipitates were collected by centrifugation and washed with deionized water for three times. Hydrogels with desired shapes and tailorable sizes can be made by compressing the gel-like precipitates into silicone molds. The isolated yield and water content of the hydrogels can be readily calculated from the weight of the dried gels. Through the same procedures, when complex **2** in acetonitrile solution (1 mg/mL), complex **3a** in aqueous solution (1 mg/mL), complex **3b** in dimethylformamide solution (1 mg/mL) and complex **4** in dimethylformamide solution (1 mg/mL) were used in place of complex **1** in acetonitrile solution, **2**–DNA gel, **3a**–DNA gel, **3b**–DNA gel and **4**–DNA gel were obtained, respectively. The star-shaped **3a**–DNA gel was obtained by using the silicone mold **A** as shown in Fig. 2 in the main text. Agarose was available as a white powder which was dissolved in near-boiling water to give a gel on cooling. The platinum(II)–DNA gels in the present study were found to exhibit thermal stability even in boiling water. The agarose shell of the core-shell star-shaped hydrogels was formed by cooling a hot agarose solution in the silicone mold **D** with the presence of star-shaped **3a**–DNA gel.

Study of platinum release in intercalation-driven gel-to-sol transition

The freshly prepared hydrogels of alkynylplatinum(II) terpyridine complexes and smDNA were encapsulated by a piece of Kimwipes paper to avoid the suspension of hydrogel fragments during *in-situ* UV-vis measurements but were allowed to undergo

molecular diffusion freely through the relatively large meshes of the Kimwipes paper. The encapsulated hydrogels were immersed in smDNA solution (0.5 mg/mL) at room temperature to allow the platinum release driven by platinum(II)–DNA intercalation. The original colorless DNA solution turned yellow and *in-situ* UV-vis absorption spectra were recorded. Since the platinum(II) intercalation into DNA base pairs was much stronger than some non-specific interactions between the Kimwipes paper (cellulose, made from 100 % virgin wood fibers) and the platinum(II) complexes, the influence of Kimwipes paper on the platinum release kinetics should be insignificant.

Study of platinum release in GSH-triggered hydrogel dissociation

The freshly prepared hydrogels of $[Pt(bzimpy)Cl]^+$ and smDNA were incubated in aqueous GSH solution (10 mM, 25 mL) at room temperature to allow the platinum release. Periodically, a 1 mL aliquot of the release solution was taken out for ICP-MS measurement.

Physical measurements and instrumentation

^{1}H NMR spectra were recorded on a Bruker AVANCE 400, 500 or 600 (400, 500 and 600 MHz) Fourier-transform NMR spectrometer with chemical shifts reported relative to tetramethylsilane. High-resolution ESI mass spectra were collected on a Bruker maXis II high-resolution LC-QTOF mass spectrometer. 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. Transmission electron microscopy (TEM) experiments were performed on a Philips CM100 transmission electron microscope with an accelerating voltage of 100 kV. Selected area electron diffraction (SAED) experiments were carried out on FEI Tecnai G2 20 Scanning TEM with an accelerating voltage of 200 kV. Step-scanned powder X-ray diffraction (PXRD) data were collected on beamline 4B9A at the Beijing Synchrotron Radiation Facilities (BSRF) in the Institute of High Energy Physics (IHEP), Chinese Academy of Sciences (CAS). Zeta-potential analysis experiments were performed on a Malvern (UK) Zetasizer Nano ZS90 instrument. Circular dichroism (CD) measurements were recorded with a Jasco (Tokyo, Japan) J-815 CD spectropolarimeter. Inductively coupled plasma-mass spectrometer (ICP-MS) analysis was performed on a Agilent 7700x instrument.

Supporting Text and Figures

Text S1. Zeta potential analysis of the platinum(II)–DNA systems, which has been performed below the gelation concentration, shows the charge neutralization upon titration of the negatively charged DNA strands into the positively charged complex **1** (Fig. S1). Above the gelation concentration, charge neutralization leads to the destabilization of the platinum(II)–DNA systems and hence the formation of platinum(II)–DNA hydrogels (Fig. 1 in the main text). Besides smDNA with an average size of around 2 kilo base pairs, calf thymus DNA of approximately 20 kilo base pairs in size has shown hydrogel formation behaviors when mixed with the platinum(II) complexes as shown in Scheme 1 in the main text. Sodium polyacrylates with a molecular weight of 10^7 have been found to form hydrogels with platinum(II) complexes at a weight feed ratio of 1/1. Neither chemical reaction nor metal-ligand coordination can occur in the system of sodium polyacrylates and the platinum(II) complexes as shown in Scheme 1 in the main text. These, together with the observation that the hydrogels form immediately upon the mixing of the platinum(II) complexes and aqueous solutions of polyelectrolytes in these platinum(II)–polyelectrolyte systems, strongly suggest that the hydrogel formation in the present study is a physical process. The present study can serve as a modular approach for the fabrication of supramolecular hydrogels with diverse chemical compositions and functional properties by a flexible choice of polyelectrolytes and metal complexes. It is noteworthy that neither hydrogel formation occurs when sodium polyacrylates with an average degree of polymerization of 50 are mixed with

the platinum(II) complexes in the present study, nor hydrogel formation is observed when polyelectrolytes with low degrees of polymerization are used to interact with the platinum(II) complexes at low concentration in our previously reported studies of polyelectrolyte-induced aggregation (ref. 6 in the main text).

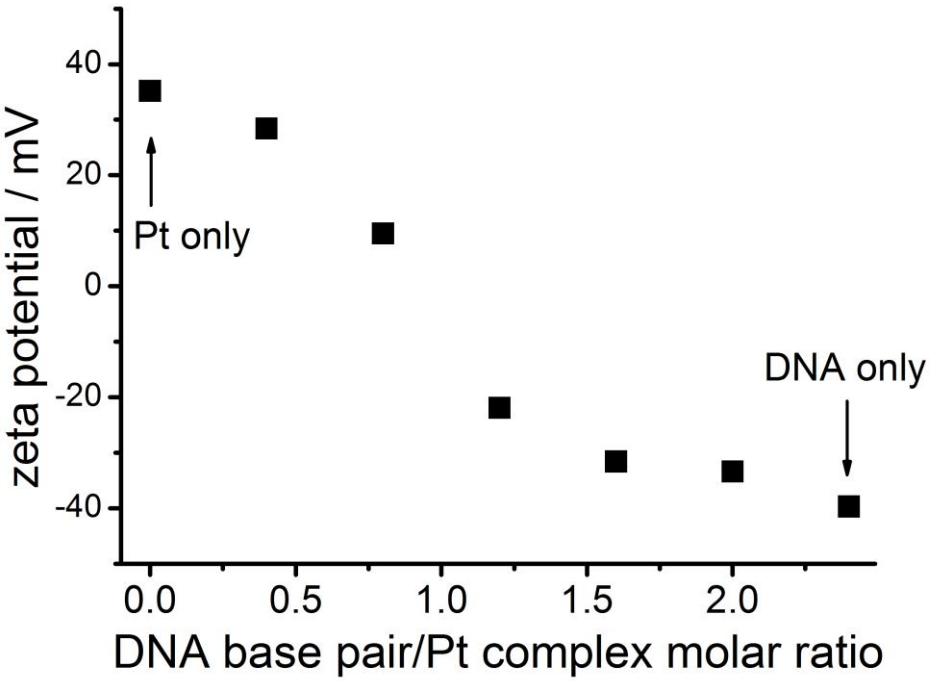

Fig. S1 Zeta potential analysis of the platinum(II)–DNA system of complex **1** and smDNA in an aqueous buffer solution (2 mM Tris–HCl, pH 7.4) at room temperature.

Text S2. Below the gelation concentration, the **3a**–DNA mixture at a platinum(II)/base pair molar ratio of 1/5 shows an increase of MMLCT absorption band in the region of 450 to 600 nm in its UV-vis spectrum (Fig. S2a), as well as a drastic intensity enhancement of the ^{3}MMLCT emission at 646 nm with respect to complex **3a** alone (Fig. S2b). These results strongly suggest the formation of $[Pt(bzimpy)Cl]^+$–DNA aggregates by metal–metal and π–π interactions and electrostatic attractions (ref. 15 in the main text). The CD spectral titration studies show insignificant changes in the region of 220 nm to 290 nm upon the addition of $[Pt(bzimpy)Cl]^+$ complexes into DNA in both cases of **3a**–DNA and **4**–DNA mixtures (Fig. S2c and S2d), indicative of the absence of platinum(II) intercalation in the $[Pt(bzimpy)Cl]^+$–DNA systems. In the case of **3a**–DNA mixture, the appearance of induced CD bands in the region of 300 to 400 nm that coincide with the region of intense intraligand absorption of the $[Pt(bzimpy)Cl]^+$–DNA aggregates can be tentatively attributed to the arrangement of $[Pt(bzimpy)Cl]^+$ complexes around DNA strands by electrostatic attractions (Fig. S2c). These spectroscopic studies indicate that, although the DNA is in excess, the strong tendency for the formation of the metal–metal and π–π interactions between $[Pt(bzimpy)Cl]^+$ complexes due to their large π-surface, as well as the additional hydrophobic interactions of C_{12} alkyl chains on complex **4**, can outcompete the platinum(II) intercalation into DNA base pairs. In the case of $[Pt(bzimpy)Cl]^+$ complex as non-covalent crosslinkers, the gel state is energetically more favorable than the sol state, and thus the intercalation-driven gel-to-sol transition pathway can be blocked. This can explain the enhanced stability

of the [Pt(bzimpy)Cl]$^{+}$–DNA hydrogels against DNA solution.

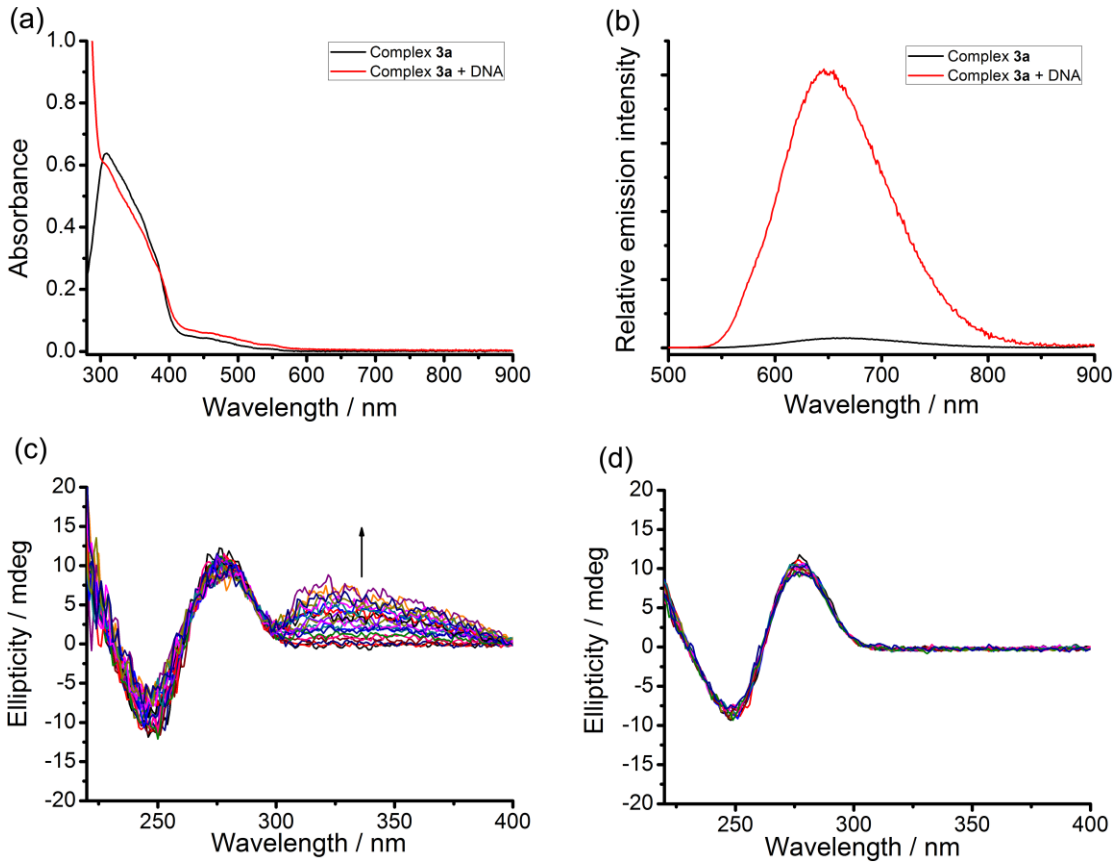

Fig. S2 (a) UV-Vis absorption spectra and (b) steady-state emission spectra (λ_{ex} = 387 nm) of the supramolecular system of complex **3a** (0.05 mM) and smDNA ([base pair] = 0.25 mM) in aqueous solution. (c, d) CD spectral changes of DNA with increasing platinum(II)/base pair molar ratio from 0 to 0.3 in (c) **3a**–DNA and (d) **4**–DNA supramolecular systems.

Text S3. Since GSH cannot react with smDNA in aqueous solution, studies of the chemical reaction between platinum(II) complexes and GSH in aqueous solution have been performed to investigate the mechanism of GSH-triggered dissociation of the $[Pt(bzimpy)Cl]^+$–DNA hydrogels. Complex **3a** was selected in the study since it is water-soluble, while complex **3b** with hexafluorophosphate counterions is insoluble in water. The ^1H NMR spectrum of complex **3a** in D_2O showed little signals due to the strong aggregation of complex **3a** in aqueous solution driven by metal–metal and π–π interactions (Fig. 8a in the main text). Upon the addition of GSH (40 mM) to the solution of complex **3a** (40 mM) in D_2O followed by incubation at room temperature for one week, the ^1H NMR spectrum of the reaction mixture showed significant changes (Fig.8a in the main text); similar ^1H NMR spectral changes have been observed in the reaction of complex **3a** in 10 mM GSH. The reaction product, a dark red solid, can be isolated by cooling the concentrated reaction mixture to 0 ℃ followed by vacuum filtration. ^1H NMR, $^{13}C\{^1H\}$ NMR, ^1H–^1H COSY and ^1H–^{13}C HSQC NMR experiments of the purified product were performed (Fig. 8 in the main text, Fig. S3 and Fig. S4). The new peaks at δ 2.61 ppm and 2.82 ppm in the ^1H NMR spectrum of the purified product that can be assigned to β-Cys protons (Fig. 8a in the main text), together with the large downfield shift of β-Cys carbon from δ 25.3 ppm to 33.6 ppm in the $^{13}C\{^1H\}$ NMR spectrum (Fig. 8b in the main text), indicated the involvement of the sulfur in the coordination of GSH to platinum(II). The $^{195}Pt\{^1H\}$ NMR spectrum with a peak at δ −3063 ppm (ref. 18 in the main text) and the HR ESI mass spectra of the reaction mixture further confirmed the platinum(II)–thiol

coordination (Fig. 8c in the main text and Fig. S5), that is, the ligand exchange reaction of chloro ligand in complex **3a** with glutathione. The ^{1}H NMR and ^{13}C{^{1}H} NMR spectra of the reaction mixture have also been given in Fig. 8 in the main text, indicating the purified product after cooling and filtration is the major product in the reaction mixture. The emergence of a set of signals in the region of aromatic protons in both ^{1}H NMR and ^{13}C{^{1}H} NMR spectra (Fig. 8a and 8b in the main text) indicated the deaggregation of the platinum(II) complexes due to the enhanced hydrophilicity provided by the GS moiety after platinum(II)–thiol coordination.

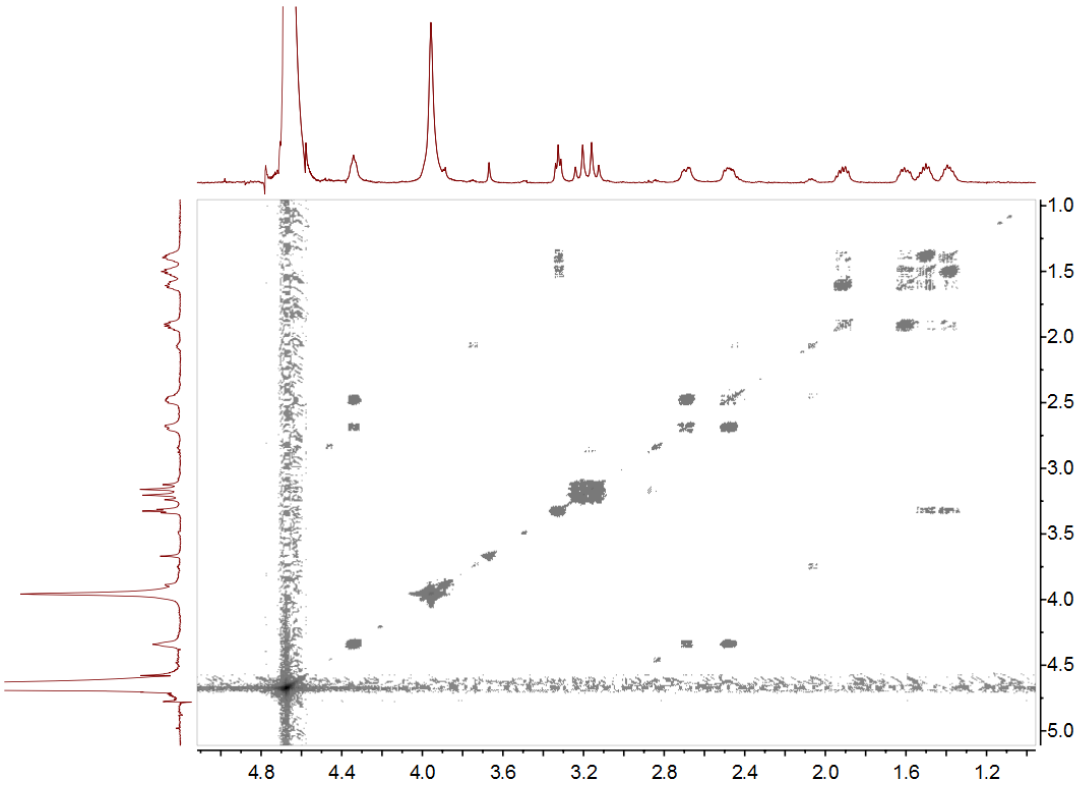

Fig. S3 ^{1}H–^{1}H COSY NMR spectrum (600 MHz) of the purified product from **3a**–DNA reaction mixture in D_2O at 298 K.

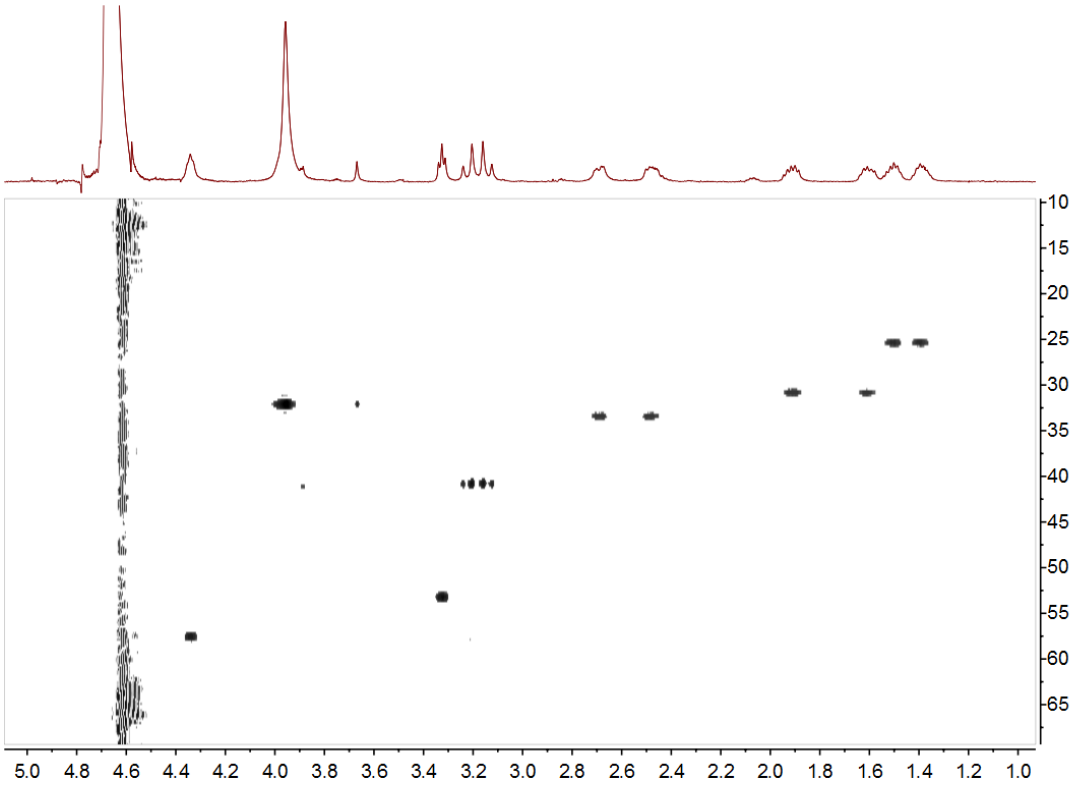

Fig. S4 $^{1}H–^{13}C$ HSQC NMR spectrum (600 MHz) of the purified product from **3a**–DNA reaction mixture in D_2O at 298 K.

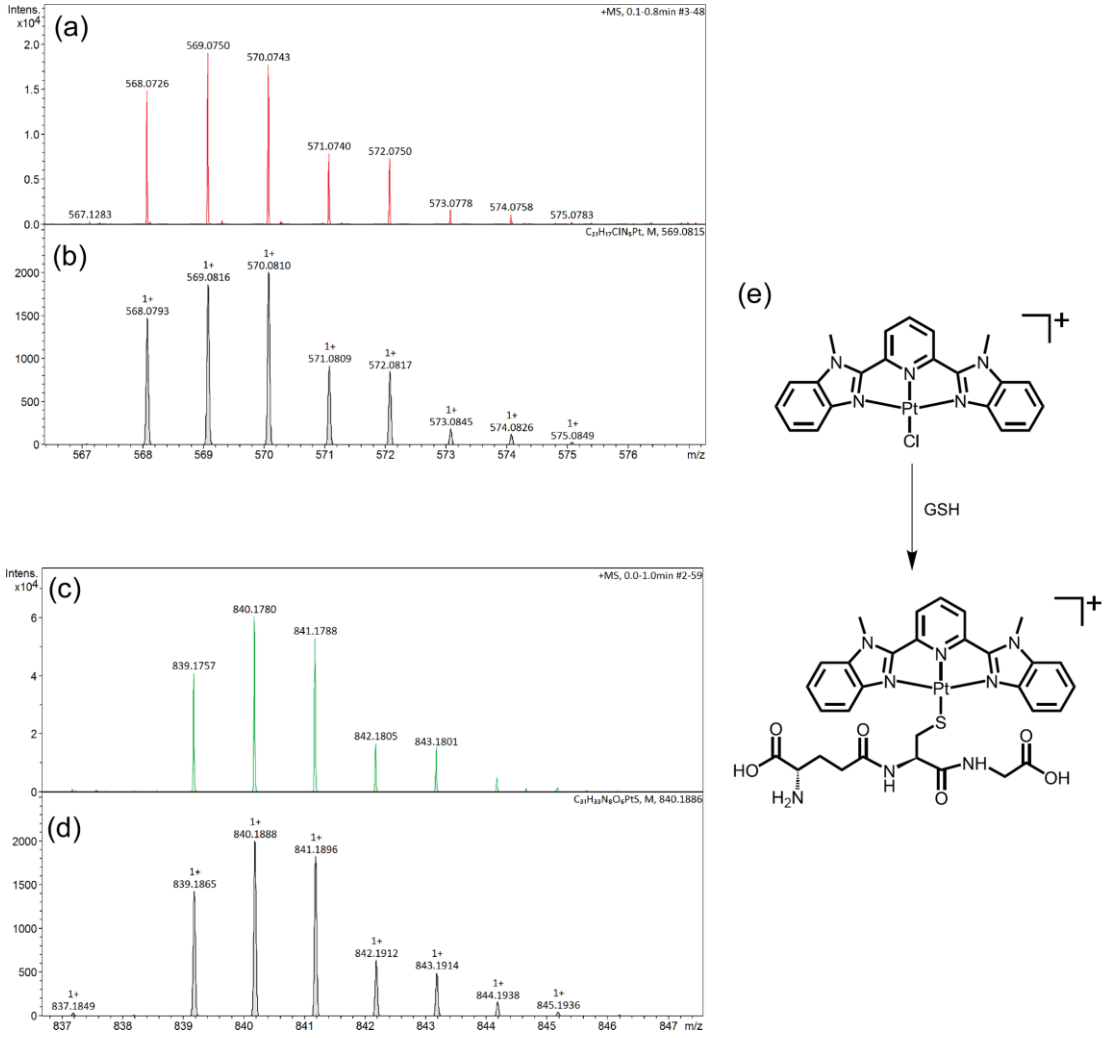

Fig. S5 (a, b) Expanded ion cluster at m/z 569 of the HR ESI mass spectrum of (a) complex **3a** and (b) its simulated isotope pattern. (c, d) Expanded ion cluster at m/z 840 of the HR ESI mass spectrum of (c) **3a**–DNA reaction mixture and (d) its simulated isotope pattern. (e) Ligand exchange reaction of chloro ligand in complex **3a** with GSH.

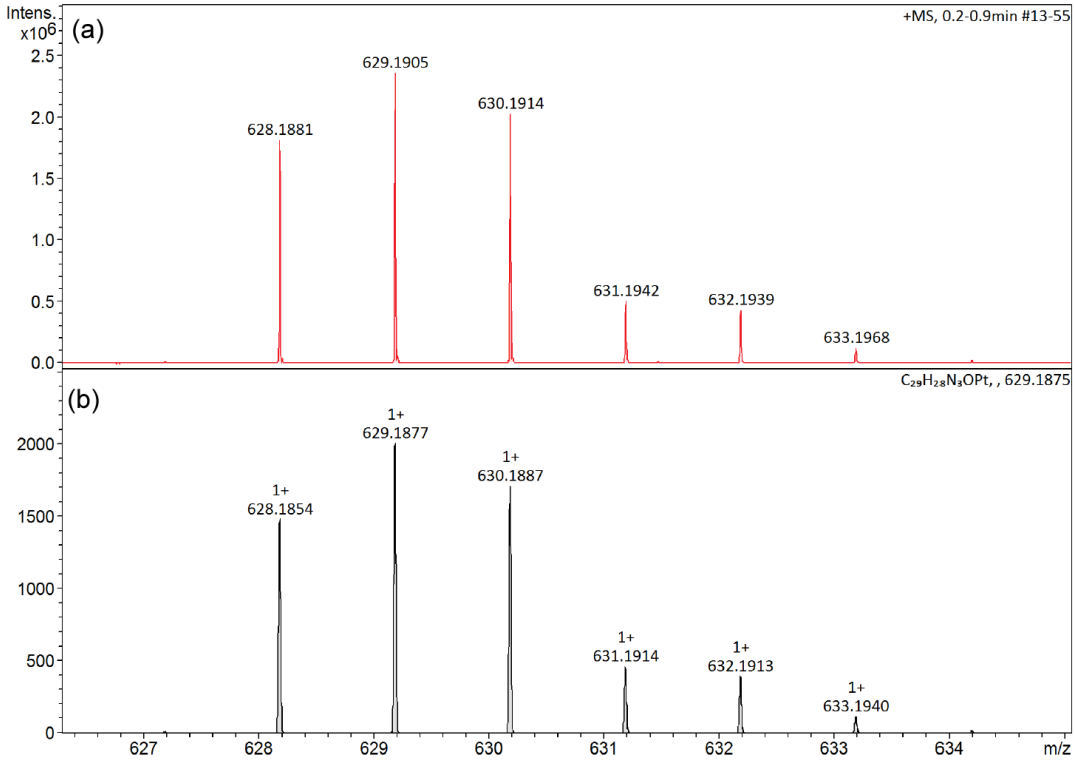

Fig. S6 Expanded ion cluster at m/z 629 of the HR ESI mass spectrum of (a) complex **2** and (b) its simulated isotope pattern.

References

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[S3] K. A. Davis and K. Matyjaszewski, *Macromolecules,* 2000, **33**, 4039.