Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Encapsulation of Platinum Anticancer Drugs by Apoferritin

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Instruments and materials

The high resolution transmission electron micrograms were recorded using JEOL JEM-4000EX electron microscope at 400 kV. The platinum concentration was determined with ICP-AES using Jarrell-Ash J-A1100. The UV-vis spectra were recorded on a Shimazu UV-3100 spectrometer. The NMR spectra were recorded at 298 K on a Bruker Avance-600 spectrometer equipped with a TCI cryo-probe or a Bruker DRX 500 spectrometer. The 2D [^{1}H, ^{15}N] HSQC spectra were acquired in 256 transients over spectral widths of 6 kHz in the ^{1}H dimension and 1.2 kHz in the ^{15}N dimension using 2048 × 48 data points. Proton decoupling was achieved using GARP pulse sequence and water suppression was accomplished through pulse field gradients. The spectra were zero-filled and processed using a squared sine bell window function prior to Fourier transformation. The ^{1}H chemical shift was referenced to 3-trimethylsilyl-propionate- 2, 2, 3, 3-*d*4 and the ^{15}N chemical shift was referenced indirectly.

CDDP and CBDCA were purchased from Strem Chemicals. The ^{15}N-labelled CDDP and CBDCA were prepared by a modified literature method using $^{15}NH_4Cl$ as starting materials (a. Kerrison, S. J. S. Sadler, P. J. *J. Chem. Soc. Chem. Commun*. 1977, 861–865; b. Boreham, C. J. Broomhead, J. A. Fairlie, D. P. *Aust. J. Chem*. 1981, 34, 659–664).

Preparation of AFt-CDDP and AFt-CBDCA

Two methods were adopted to prepare the drug-loaded ferritin. In the unfolding-refolding method, an AFt solution (0.003 μmol, 0.15 mol·L^{-1} NaCl/H_2O, 15 μL) was added into the CDDP saturated solution (*ca* 0.002 mol·L^{-1}, 0.15 mol·L^{-1} NaCl/H_2O 300μL), and adjusted to pH 2.0 by HCl (0.1 mol·L^{-1}). The pH was maintained for about 15 min. When the dissociation of ferritin was completed, the pH value was adjusted to 7.5 using NaOH (0.1 mol·L^{-1}). The resulting solution was stirred at room temperature for 2 h. After eliminating the free CDDP molecules outside of ferritin by fully dialysis against NaCl (0.15 mol·L^{-1}) solution, the solution was centrifuged to eliminate precipitated protein that formed during the experimental process. AFt-CBDCA was prepared following the same procedure. In the *in situ* method, AFt-$[PtCl_4]^{2-}$ used for AFt-CDDP generation was formed in the ferritin solution (0.02 μmol, 0.15 mol·L^{-1} $NaClO_4/H_2O$, 100 μL) with excess of K_2PtCl_4 (10 μmol, H_2O, 100 μL) at pH 8.5 after 2 h stirring. To this AFt-$[PtCl_4]^{2-}$ solution, a NH_4^+-NH_3 buffer ([NH_4^+ + NH_3] = 0.3 mol·L^{-1}, pH 10) of equal volume was added and incubated at 8 ºC for 12 h. After an exhaustive dialysis (0.15 mol·L^{-1} $NaClO_4/H_2O$, 24 h) of the resulting solution, AFt-CDDP was obtained. The concentration and proportion of CDDP and TDDP was estimated by 2D [^{1}H, ^{15}N] HSQC NMR spectra shown in Figure S2. The concentration of the encapsulated Pt-NH_3 complexes was measured by ICP-AES. The concentration of the drug loaded protein

was determined by BCA protein assay (BCA Protein Assay Kit, Pierce), and the proportion of Pt atoms to protein was calculated accordingly.

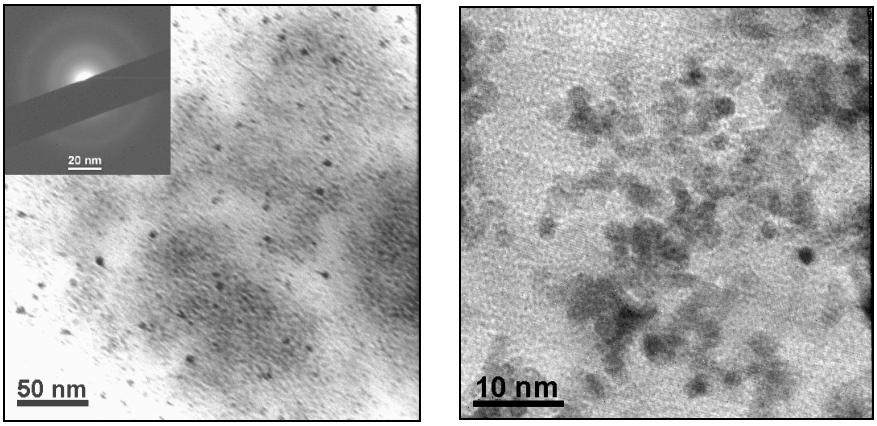

Fig. S1 TEM images of the AFt-Pt and the selected-area electron powder diffraction pattern of Pt nano-particles. The diffraction pattern has d-spacings of 2.26, 1.96 and 1.18 Å, consistent with the (111), (200), (311) reflections of Pt metal (face-center cube crystal, see 4-0802 in *Powder Diffraction File*, Joint Committee on Powder Diffraction Standards, USA, 1974). The results confirm that the metal core of ferritin is platinum.

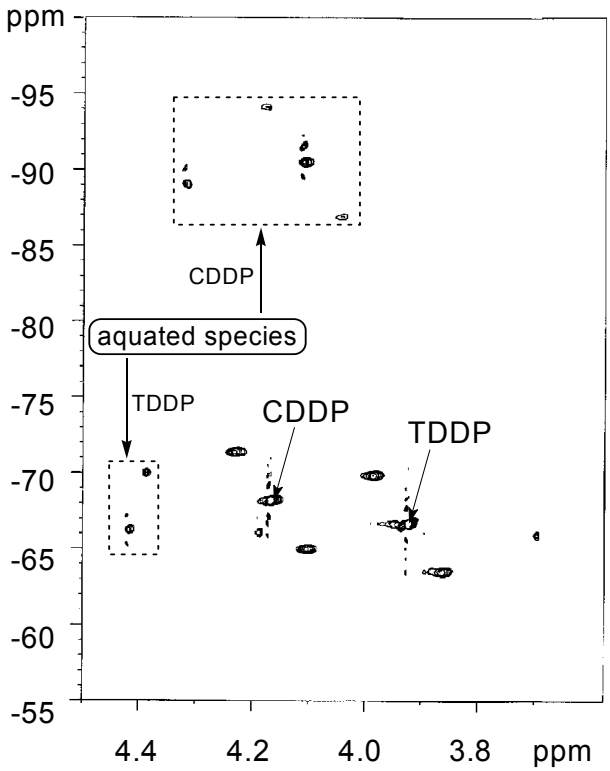

Fig. S2 The 2D [^{1}H, ^{15}N] HSQC NMR spectrum of the solution ([Pt] = 20 mM) containing cisplatin (CDDP), transplatin (TDDP) and their aquated species in 95% H_2O/ 5% D_2O, pH

5.8, 298K (for assignments, see S. J. Berners-Price, T. A. Frenkiel, U. Frey, J. D. Ranford, P. J. Sadler, *J. Chem. Soc., Chem. Commun.* 1992, 789–791; Berners-Price SJ, Ronconi L, Sadler PJ (2006) Prog Nucl Mag Res Sp 49: 65–98). The concentration and proportion of CDDP and TDDP were estimated using this spectrum.

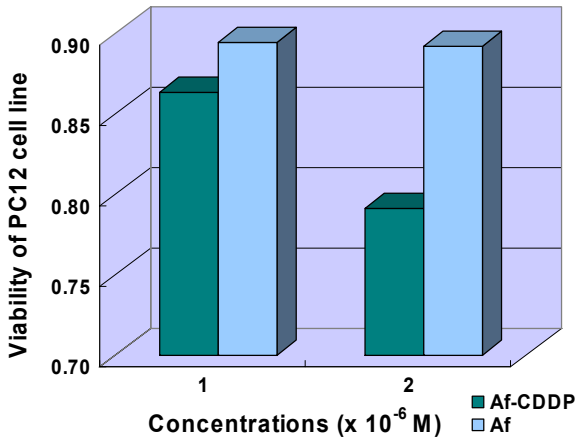

Fig. S3 The cytotoxic activity of AFt-CDDP against PC 12 cell line using AFt as a control (reference activity of CDDP is 0.88 and 0.74 at 1×10^{-6} and 2×10^{-6} M, respectively).