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 [1H, 15N] HSQC spectra were acquired in 256 transients over spectral widths of 6 kHz in the 1H dimension and 1.2 kHz in the 15N 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 1H chemical shift was referenced to 3-trimethylsilyl-propionate- 2, 2, 3, 3-d4 and the 15N chemical shift was referenced indirectly.


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/H₂O, 15 μL) was added into the CDDP saturated solution (ca 0.002 mol·L⁻¹, 0.15 mol·L⁻¹ NaCl/H₂O 300 μL), 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-[PtCl₄]²⁻ used for AFt-CDDP generation was formed in the ferritin solution (0.02 μmol, 0.15 mol·L⁻¹ NaClO₄/H₂O, 100 μL) with excess of K₂PtCl₄ (10 μmol, H₂O, 100 μL) at pH 8.5 after 2 h stirring. To this AFt-[PtCl₄]²⁻ solution, a NH₄⁺-NH₃ buffer ([NH₄⁺ + NH₃] = 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 NaClO₄/H₂O, 24 h) of the resulting solution, AFt-CDDP was obtained. The concentration and proportion of CDDP and TDDP was estimated by 2D [1H, 15N] HSQC NMR spectra shown in Figure S2. The concentration of the encapsulated Pt-NH₃ 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 $[^1H, ^{15}N]$ HSQC NMR spectrum of the solution ([Pt] = 20 mM) containing cisplatin (CDDP), transplatin (TDDP) and their aquated species in 95% H$_2$O/5% D$_2$O, pH

![Graph](image)

**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).