Electronic Supplementary Information(ESI)

Fluorous Interaction Induced Self-Assembly of Tobacco Mosaic Virus Coat Protein for Cisplatin Delivery

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EXPERIMENTAL SECTION

Chemicals and Materials

TMV was extracted from infected Nicotiana benthamiana plants following a literature protocol.[24] 2,2,3,3,4,4,4-heptafluoro-butylamin ($NH_2CH_2CF_2CF_2CF_3$, J&K Scientific Ltd), 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC, Aladdin Industrial Corporation), hydroxybenzotriazole (HOBt, Aladdin Industrial Corporation), dimethylsulfoxide (DMSO, Aladdin Industrial Corporation) and ultra-pure water produced by Milli Direct-Q5 were used for interior surface modification of TMV. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium hydrate (NaOH), potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$), glacial acetic acid (CH_3COOH), sodium acetate (CH_3COONa), hydrochloric acid (HCl) supplied from Beijing Chemical Works and tris (hydroxymethyl) aminomethane (Tris) supplied from Aladdin Industrial Corporation were used for preparing different buffer solutions. Rhodamineisothiocyanate (RB) supplied from Aladdin was used for labeled F-TMVCP NPs. Lowry Protein Assay Kit purchased from Beijing Solarbio Science & Technology Co. Ltd was used for protein concentration measurement.

Carbon support films purchased from Beijing Xinxing Braim Technology Co.Ltd and uranyl acetate dye purchased from the Beijing Zhongjingkeyi Technology Co.Ltd were used for preparing samples for TEM observation.

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin (P/S) were all from Life Technologies and used for cell culture. Cell Counting Kit-8 (CCK-8, DOjinDO Molecular Technologies) were used for cytotoxicity measurement. Lyso Tracker Deep Red, Mito Tracker Deep Red and Hoechst purchased from Life Technologies were used for the staining of lysosomes, mitochondria and nuclei to explore the intracellular distribution of CDDP@F-TMVCP NPs.

All reagents were of analytical grade and used without further purification unless otherwise stated.

Fluorous ponytail Conjugation on the TMV Interior Surface

92 μL of 10 mg/mL EDC aqueous solution was added into 8.9 mL of 100 mM pH=7.4 HEPES-NaOH buffer containing 10 mg of TMV and mix. After 30 min, 193 μL of 20 mg/mL HOBt in DMSO (50 eq per coat protein) and 190 μL of $NH_2CH_2CF_2CF_2CF_3$ in DMSO (100 eq per coat protein) were added into the mixture. Then another two times 92 μL of 10 mg/mL EDC (totally 25 eq per coat protein for the three times) in H_2O were added into the mixture at 6 and 18 h. This reaction is performed at 4 °C for 72 h. The products were purified by dialysis and gel chromatographic separation.

Dissociating TMV or F-TMV with acetic acid

Two additional volume of acetic acid was added into TMV or F-TMV aqueous solution, stored at 4 °C for 20 min. The resulting suspension was centrifuged at 10000 rpm for 10 min to pellet the precipitated RNA. The resulting supernatant was passed through a Sephadex G-25 column pre-equilibrated with H_2O. The protein containing eluent was dialyzed against deionized water. After about a period of 24 h, the coat protein resulted in precipitation. The precipitate was pelleted via centrifugation at 10,000 rpm, 4 °C for 10 min and the supernatant was discarded. The pellet was then dissolved with 100mM pH=8.0 Tris-HCl buffer and dialyzed against 100mM pH=8.0 Tris-HCl buffer at 4 °C overnight with dialysis cartridge (MW cutoff = 3.5~5 kD).

Reassembling of TMVCP and F-TMVCP

The resulting coat protein solutions were further dialyzed against different condition, which includes different ionic strength buffer solutions and different temperatures. For example, 20 mM/100 mM pH=5.5 HAc-NaAc buffer, 20 mM/100 mM pH=7.4 K_2HPO_4-KH_2PO_4 buffer and 20mM/100mM pH=8.0 Tris-HCl buffer at 4 °C or 25 °C, respectively.

Prepare CDDP@F-TMVCP NPs

Add 80 eq CDDP aqueous solution into F-TMVCP NPs (0.5mg/ml in aqueous solution) at 4 °C for 24h. The uncoordinated cisplatin was removed by dialyze. Then CDDP@F-TMVCP NPs was obtained. Protein concentration was 0.16 mg/ml determined by Lowry Protein Assay Kit and CDDP concentration was 17.98 μg/ml

determined by inductively coupled plasma atomic emission spectrometry (ICP-AMS). The coordination number (CN), which is used to indicate the number of loaded CDDP per coat protein, is calculated as 6.55 following the equation (1):

$$CN = \frac{C_{CDDP}/M_{CDDP}}{C_{protein}/M_{protein}} \quad (1)$$

where C_{CDDP} and $C_{protein}$ represent the concentration of CDDP and protein, respectively. And M_{CDDP} and $M_{protein}$ represent the molecular weight of CDDP and protein, respectively.

Characterization

Bio-Rad SDS-PAGE system and MALDI-TOF MS were applied to confirm the success of chemical conjugation. MALDI-TOF MS was acquired on an AB SCIEX MALDI TOF-TOF 5800 Analyzer (AB SCIEX USA) equipped with a neodymium: yttrium-aluminum-garnet laser (laser wavelength was 349 nm), in reflection positive-ion mode. The mass spectrometer was externally calibrated using peptide calibration mix (AB SCIEX, USA).

The morphology of F-TMVCP NPs in dry state was observed with a transmission electron microscopy (TEM, HT-7700 HITACHI, Japan) with an accelerating voltage of 100 kV and the samples were stained by 0.2% uranium acetate before observation. The morphology of F-TMVCP NPs in aqueous solution was observed with a transmission electron microscopy (TEM, FEI Tecnai, USA) with an accelerating voltage of 200kV. FEI Vitrobot was used for preparing the samples for Cyro-TEM.

The height of F-TMVCP NPs in dry state was performed with an atomic force microscope (AFM, Bruker Multimode 8). A Tapping mode image is shown in Fig. 2.

Zeta potentials were measured by Zetasizer nano ZSP (Malvern) with protein concentration of 0.1 mg/ml. Zeta potentials at pH 3.0, pH 4.0 and pH 5.0 were measured in HAc-NaAc buffer solution; zeta potentials at pH 6.0, pH 7.0 and pH 8.0 were measured in KH_2PO_4-K_2HPO_4 buffer solution; zeta potential at pH 9.0 was measured in H_3BO_3-$Na_2B_4O_7$ buffer solution. The ionic strength of buffer solutions was 10 mM. The hydrodynamic diameter was determined by Dynamic Light Scattering (Dybapro NanoStar). Circular dichroism spectra were measured by Jasco

Circular dichroism J-815. Absorption spectra were determined through a U-3900 UV−vis spectrophotometer. Inductively coupled plasma atomic emission spectrometry (ICP-AMS, Varian 710-OES) was used to confirm the concentration of CDDP.

Confocal laser scanning microscopy (CLSM, Nikon Corporation A1R MP) was used to observe the distribution of CDDP@F-TMVCP NPs in cells.

Cell Culture

HeLa (human cervical cancer epithelial cell) were incubated in DMEM medium with 1% antibiotics (penicillin-streptomycin) and 10% FBS at 37 °C in a humidified atmosphere containing 5% CO_2.

Cytotoxicity Assay

HeLa cells were seeded in a 96-well plate at a density of 8000 cells per well and cultured in 5% CO_2 at 37 °C overnight. Then the culture medium was replaced by different concentration of samples (CDDP@F-TMVCP NPs, free CDDP and F-TMVCP NPs) in fresh growth medium. After 24h of incubation, the growth medium was replaced by 100 μL fresh growth medium containing CCK-8.

Then the plates were incubated for 2-3 h before recording the absorbance at 450 nm using EnSpire Multimode Plate Reader (PerkinElmer). The cell viability was determined as followed equation:

$$Cell\ Viability\ (\%) = \frac{I_{sample}}{I_{control}} \times 100 \quad (2)$$

where I_{sample} and $I_{control}$ represent the intensity determined for cells treated with samples and for control cells (untreated), respectively.

Intracellular Distribution and Colocalization

Rhodamineisothiocyanate (RB) was labeled on F-TMVCP NPs in 100 mM pH 9 Na_2CO_3-$NaHCO_3$ buffer solution. The resulting sample was purified by dialysis against aqueous solution. Then F-TMVCP NPs labeled with RB was blended with 80 eq CDDP at 4 °C for 24 hours and purified by dialysis against aqueous solution. This resulted in the CDDP@F-TMVCP NPs labeled RB.

2×10^5 HeLa cells were cultured overnight in glass bottom cell dishes. CDDP@F-

TMVCP NPs labeled with RB were added to cells with final concentration of 2 μg/mL CDDP and incubated for 24 h. Medium was changed to a new one containing 50 nM Lyso Tracker Deep Red, 50 nM Mito Tracker Deep Red and 10 μM Hoechst 33342 and incubated for 30 minutes. The resulting cells were observed by CLSM.

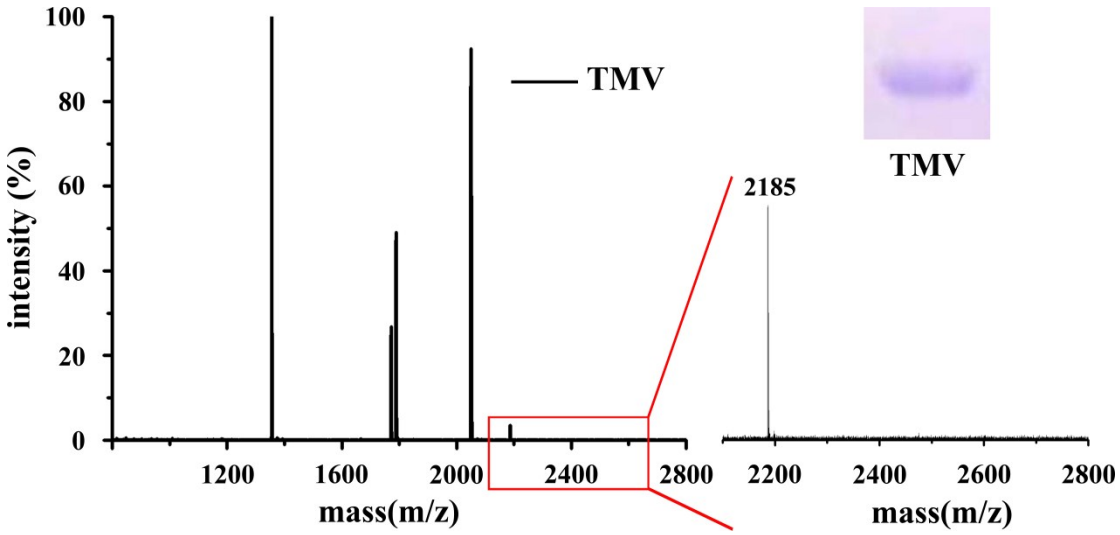

Fig. S1 MALDI-TOF MS result of peptides mixture obtained after trypsin digestion of TMV. The enlarged figure shows the MS result at 2100-2800 m/z value and the inset figure shows SDS-PAGE analysis of TMV visualized by coomassie staining.

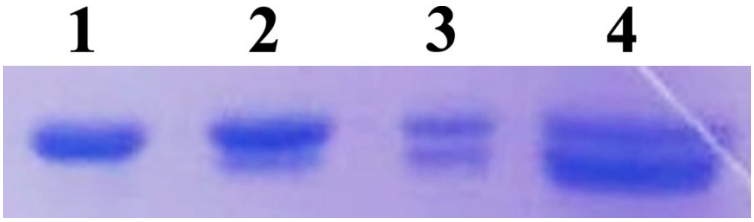

Fig. S2 SDS-PAGE visualized by coomassie brilliant blue staining. Lane 1 represents TMV, lanes 2~4 represent F-TMV prepared with different reaction time 14h, 24h and 72h, respectively. The fluorous ponytail equivalent to TMVCP was 100 eq. The proportions of the following bands that increase with the reaction time are 23.7%, 41.4% and 68.8%, respectively.

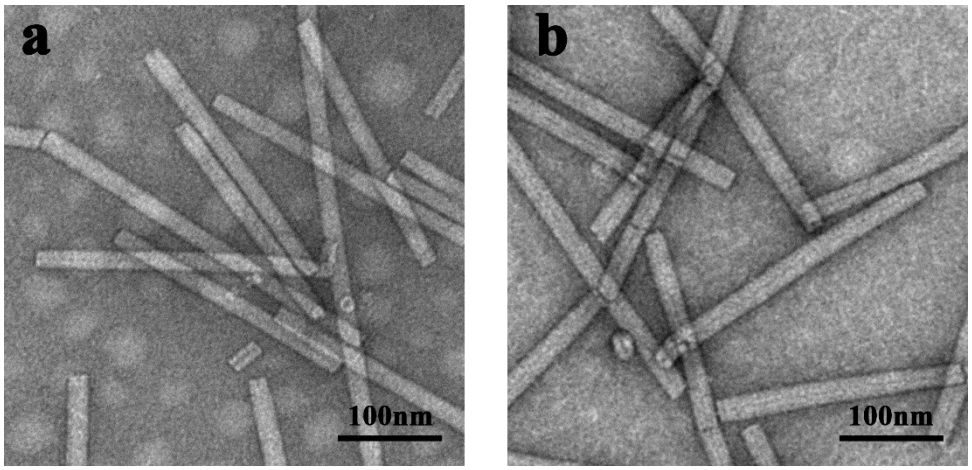

Fig. S3 TEM images of TMV (a) and F-TMV (b) in aqueous solution.

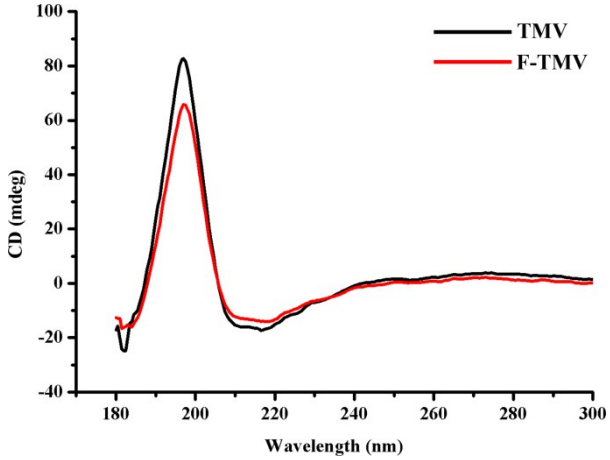

Fig. S4 CD curves of the TMV and F-TMV in aqueous solution.

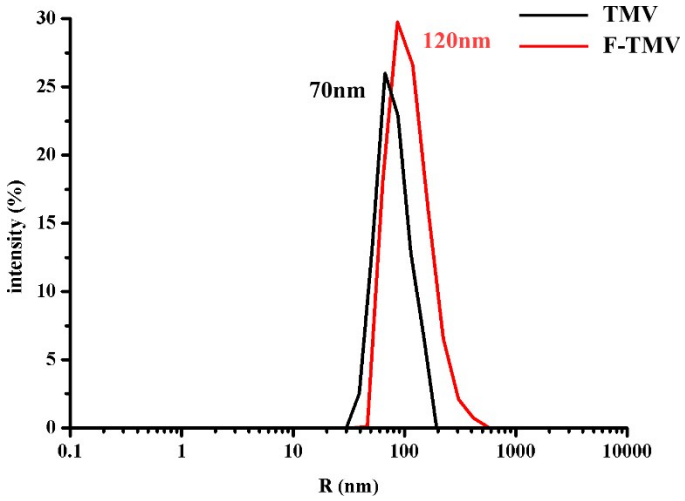

Fig. S5 DLS curves of the TMV and F-TMV in aqueous solution.

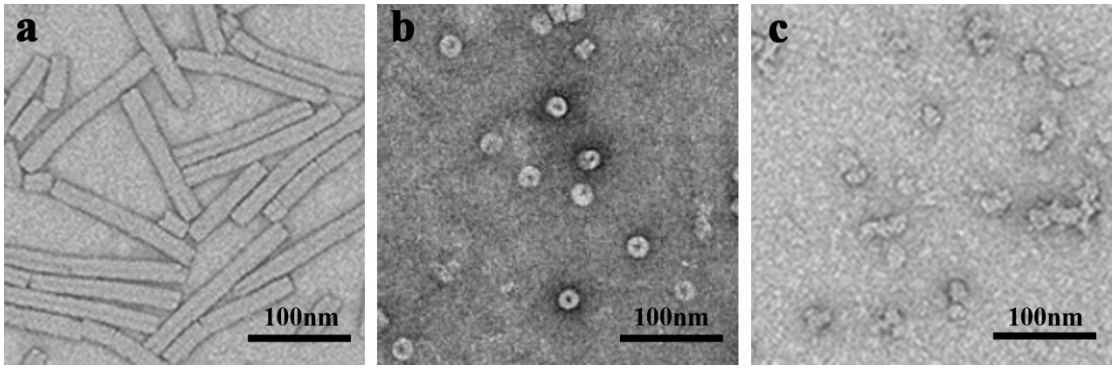

Fig. S6 TEM images of self-assembly of C-TMVCP in 20 mM pH 5.5 HAc-NaAc buffer (a), in 20 mM pH 7.4 K_2HPO_4-KH_2PO_4 buffer (b) and in 20 mM pH 8.0 Tris-HCl buffer (c), respectively.

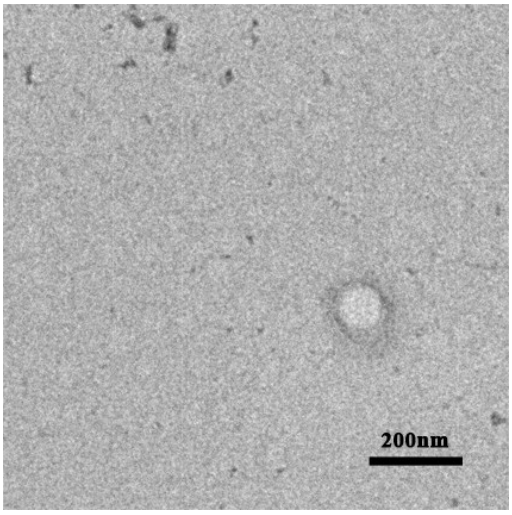

Fig. S7 TEM image of the F-TMVCP NPs by adding 5% v/v 1,1,1,3,3-hexafluoro-2-propanol.

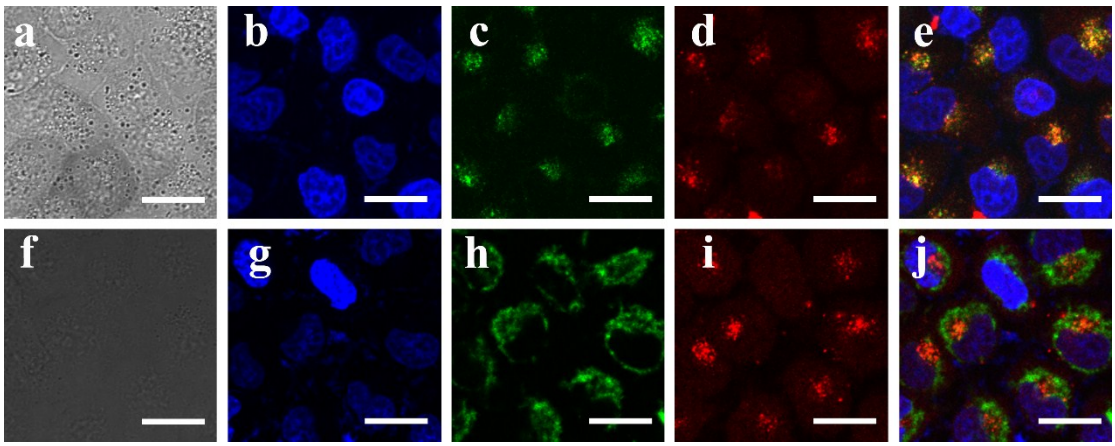

Fig. S8 Intracellular distribution of CDDP@F-TMVCP NPs after incubation in HeLa Cells for 24 hours. (a) and (f) are the DIC pictures of the cells. The blue color in (b), (e), (g) and (j) shows the nuclei stained with Hoechst 33342. Red color in (d), (e), (i) and (j) indicates CDDP@F-TMVCP NP labeled with rhodamine B. Green color in (c) and (e) shows endo/lysosome distribution stained by LysoTracker Deep Red. Green color in (h) and (j) shows mitochondria distribution stained by MitoTracker Deep Red. Scale bars: 20 μm.