

A supramolecular strategy to assemble multifunctional viral nanoparticles

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1. Materials

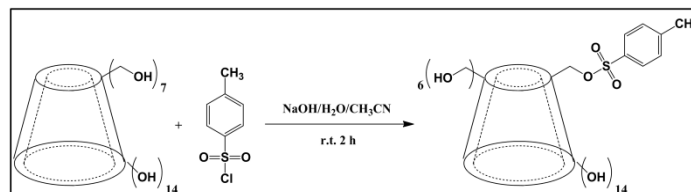
β -cyclodextrin (β -CD), p-toluenesulfonyl chloride, sodium azide (NaN_3), 3-aminophenylacetylene, copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), sodium ascorbate (NaAsc), rhodamine B (RhB), folic acid (FA), doxorubicin hydrochloride ($\text{Dox} \cdot \text{HCl}$), PEG (MW 2000 Da), 1-adamantanemethanol, 1-adamantanecarboxylic acid, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC \cdot HCl), *N*-hydroxysuccinimide (NHS), *N,N'*-carbonyldiimidazole (CDI), 4-dimethylaminopyridine (DMAP), Dubelcco's Modified Eagle's Medium (DMEM), 4',6-diamidino-2-phenylindole (DAPI), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and other reagents were local commercial products and used as received. All organic solvents were dried and distilled before used.

2. Measurements

^1H NMR spectra were characterized on a Bruker AVANCE DRX 400 M spectrometer. FT-IR spectra were acquired on a Nicolet 6700 spectrometer. UV-Vis spectra were acquired on a TU-1901 spectrometer (Beijing Purkinje General Instrument Co., Ltd.). MALDI-TOF MS analysis was performed using a Bruker Ultra-Flex I TOF/TOF mass spectrometer. Transmission electron microscopy (TEM) measurements were carried out on a JEOL JEM-1011 microscope operating at an accelerating voltage of 100 kV. Fast Protein Liquid Chromatography (FPLC) analysis was performed on an AKTA explorer (GE Biotech) instrument using Sephadex G-25 column and Superose-6 size-exclusion column.

3. Synthesis of β -CD derivatives and Ada derivatives

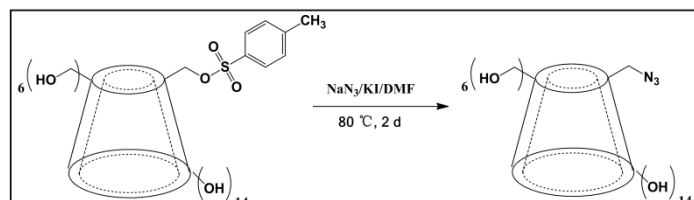
3.1 Mono-6-(p-toluenesulfonyl)-6-deoxyl- β -cyclodextrin (Ts- β -CD)



Ts- β -CD was synthesized using the reported protocol.¹ β -CD (60 g, 52.9 mmol) was suspended in 500 mL of pure water, and NaOH (6.57 g, 164.0 mmol) in 20 mL of water was added dropwise over 10 min. The mixture became homogenous. Then p-toluenesulfonyl chloride (10.08 g, 52.9 mmol) in 30 mL of acetonitrile (CH_3CN) was added dropwise over 10 min. After 2 h of stirring at room temperature, the unreacted p-toluenesulfonyl chloride was removed by suction filtration and the filtrate was cooled at 4

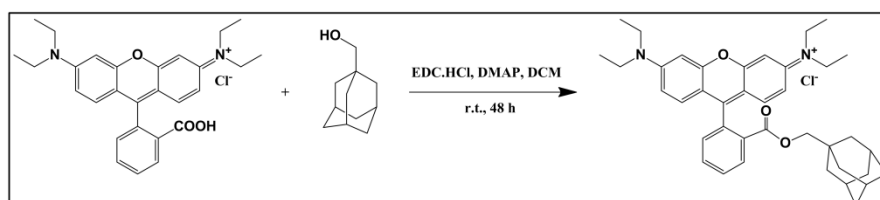
°C for 12 h. The resulting white precipitate was collected by suction filtration, washed twice with ether and dried under vacuum to afford a white solid (8.2 g, 12 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm): 7.75 (d, $J=7.6$ Hz, 2H), 7.43 (d, $J=7.6$ Hz, 2H), 5.71 (m, 14H), 4.84 (s, 4H), 4.77 (s, 3H), 4.49-4.17 (m, 6H), 3.65-3.43 (m, 28H), 3.37-3.22 (m, overlaps with HDO), 2.43 (s, 3H).

3.2 Mono-6-azide-6-deoxyl- β -cyclodextrin (β -CD-azide)



β -CD-azide was synthesized using the reported protocol.¹ Ts- β -CD (2.50 g, 1.94 mmol) was dissolved in 15 mL of DMF, then NaN_3 (1.26 g, 19.40 mmol) and KI (0.161 g, 0.97 mmol) were added. The reaction was allowed to proceed for 48 h at 80 °C. The resulting mixture was cooled to room temperature before 500 mL of acetone was added to produce a white precipitate. The precipitate was collected by suction filtration, recrystallized from 10 mL of pure water twice and dried under vacuum to yield a pure white solid (1.68 g, 75 %). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm): 5.71 (m, 14H), 4.87 (shoulder, 1H), 4.82 (s, 6H), 4.45 (m, 6H), 3.65-3.43 (m, 28H), 3.37-3.22 (br s, overlaps with HOD). FTIR (KBr): 2105 cm^{-1} (N_3).

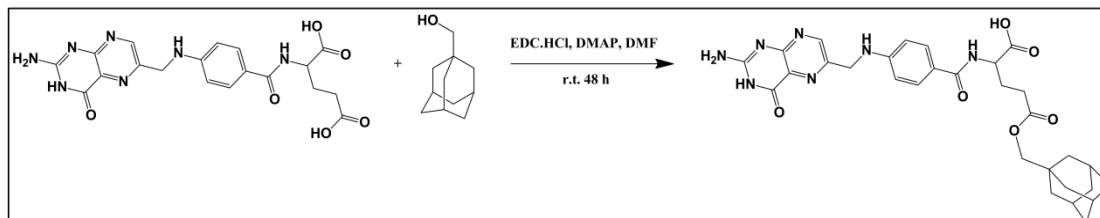
3.3 Adamantyl-rhodamine B (Ada-RhB)



RhB (0.958 g, 2.0 mmol) was dissolved in 30 mL of dichloromethane (DCM), then 1-adamantanemethanol (0.366 g, 2.2 mmol), EDC·HCl (0.767 g, 4.0 mmol) and DMAP (0.098 g, 0.8 mmol) were added. The reaction was allowed to proceed for 48 h at room temperature under argon in the dark. The resulting mixture was washed with 20 mL of pure water twice. Then the aqueous layer was extracted three times by DCM and all organic layers were combined, dried over anhydrous Na_2SO_4 , filtrated, concentrated and purified via silica gel chromatography ($\text{CH}_3\text{OH}/\text{CHCl}_3$, v/v, 1/10) to afford a purple solid (0.853 g, 68 %). ^1H NMR (400 MHz, CDCl_3) δ (ppm): 8.28 (dd, $J_1=8.0$ Hz, $J_2=0.8$ Hz, 1H), 7.81 (td, $J_1=7.2$ Hz, $J_2=1.2$ Hz, 1H), 7.74 (td, $J_1=7.2$ Hz, $J_2=1.2$ Hz, 1H), 7.32 (dd, $J_1=6.4$ Hz, $J_2=0.8$ Hz, 1H), 7.11 (d, $J=9.6$ Hz, 2H), 6.94 (dd, $J_1=7.2$ Hz, $J_2=2.4$ Hz, 2H), 6.86 (d, $J=$

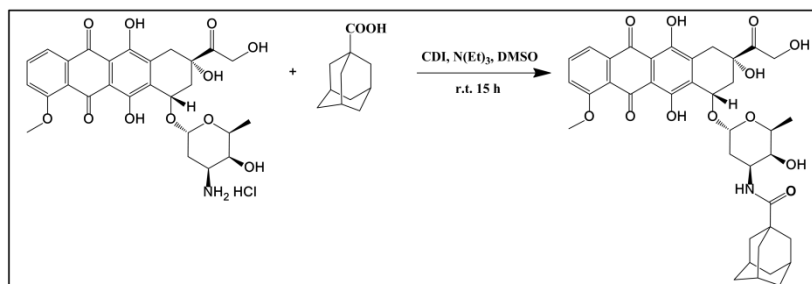
2.4 Hz, 2H), 3.66 (q, $J = 7.2$ Hz, 8H), 3.60 (s, 2H), 1.85 (s, 3H), 1.65 (d, $J = 12.4$ Hz, 6H), 1.48 (d, $J = 11.2$ Hz, 6H), 1.33 (t, $J = 7.2$ Hz, 12H), 1.24 (d, $J = 2.0$ Hz, 6H).

3.4 Adamantyl-folic acid (Ada-FA)



FA (1.500 g, 3.398 mmol) was dissolved in 50 mL of DMF, then 1-adamantanemethanol (0.565 g, 3.398 mmol), EDC·HCl (1.303 g, 6.796 mmol) and DMAP (0.166 g, 1.359 mmol) were added. The reaction was allowed to proceed for 48 h at room temperature under argon in the dark. The resulting mixture was poured into 200 mL of acetone and stirred for 30 min to form an orange-yellow precipitate. The precipitate was collected by suction filtration, washed with acetone and dried under vacuum to yield an orange-yellow solid (1.260 g, 63%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm): 11.42 (s, 1H), 8.65 (s, 1H), 8.12 (m, 1H), 7.72 (m, 1H), 7.64 (m, 2H), 6.91 (m, 2H), 6.64 (m, 2H), 4.49 (m, 2H), 4.34 (m, 1H), 3.61 (s, 2H), 2.33 (m, 2H), 1.89-1.85 (m, 4H), 1.63 (d, $J = 12.8$ Hz, 6H), 1.56 (d, $J = 11.2$ Hz, 6H), 1.45 (d, $J = 2.0$ Hz, 6H).

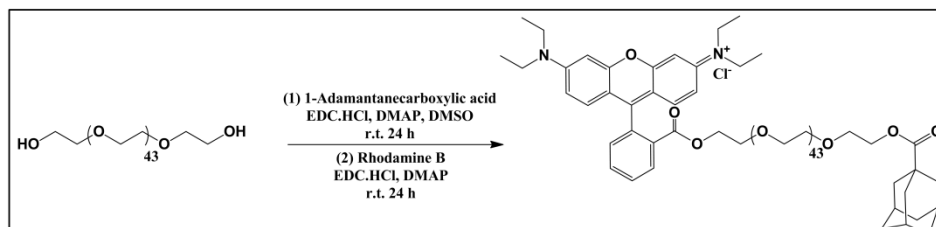
3.5 Adamantyl-doxorubicin (Ada-Dox)



Ada-Dox was synthesized using the reported protocol.² 1-adamantanecarboxylic acid (35.2 mg, 0.19 mmol) was dissolved in 2 mL of DMSO, then CDI (45.4 mg, 0.28 mmol) and triethylamine (200 μL) were added under the protection of argon. The reaction was allowed to proceed for 3 h at room temperature before Dox·HCl (93.3 mg, 0.17 mmol) dissolved in 1 mL of DMSO was added into above mixture and further stirred for 12 h at room temperature under argon. Ada-Dox was purified via silica gel chromatography ($\text{CH}_3\text{OH}/\text{CHCl}_3$, v/v, 1/5) to get a purple-red solid (77.2 mg, 68%). ^1H NMR (400 MHz, CD_3OD) δ (ppm): 8.04 (d, $J = 8.0$ Hz, 1H), 7.90 (t, $J = 8.0$ Hz, 1H), 7.64 (d, $J = 8.0$ Hz, 1H), 5.50 (d, $J = 2.8$ Hz, 1H), 5.17 (d, $J = 2.0$ Hz, 1H), 4.74 (d, $J = 1.6$ Hz, 2H), 4.32 (q, $J = 6.8$ Hz), 4.09 (s, 3H),

3.69 (s, 1H), 3.63-3.59 (m, 1H), 3.38 (s, 1H), 3.12 (q, $J=18.8$ Hz, 2H), 2.4 (d, $J=14.4$ Hz, 1H), 2.23 (dd, $J_1=10.0$ Hz, $J_2=4.8$ Hz, 1H), 2.23 (dd, $J_1=10.0$ Hz, $J_2=4.8$ Hz, 1H), 2.10 (dd, $J_1=9.2$ Hz, $J_2=4.0$ Hz, 1H), 2.03 (s, 3H), 1.94 (d, $J=1.6$ Hz, 6H), 1.82 (d, $J=12.4$ Hz, 3H), 1.77 (d, $J=12.0$ Hz, 3H), 1.32 (d, $J=6.8$ Hz, 3H).

3.6 Adamantyl-PEG2000-rhodamine B (Ada-PEG2000-RhB)



PEG (MW 2000 Da) (500 mg, 0.250 mmol) was dissolved in 2 mL of DMSO, then a fresh mixture of 1-adamantanecarboxylic acid (45 mg, 0.250 mmol), EDC·HCl (240 mg, 1.250 mmol), DMAP (15 mg, 0.125 mmol) and DMSO (1 mL) was added dropwise under vigorously stirring. The reaction was allowed to proceed for 24 h at room temperature under argon. Then a fresh mixture of RhB (120 mg, 0.250 mmol), EDC·HCl (240 mg, 1.250 mol) and DMSO (1 mL) was added. The reaction was carried out for further 24 h at room temperature under argon in the dark. The resulting mixture was transferred into a dialysis bag (MWCO= 1000 Da) and dialyzed in pure water for 7 d. The resulting solution was freeze-dried to yield a purple-red solid (512 mg, 78%). ¹H NMR (400 MHz, CDCl₃) δ (ppm): PEG {3.57}; RhB {8.39-8.28, 8.02, 7.81, 7.35, 7.00, 6.88, 6.77, 3.66 (overlap with PEG), 1.18-1.09}; Ada {1.94, 1.82, 1.64}.

4. Preparation of TMV, TMV- β -CD and TMV- β -CD conjugates

4.1 TMV

TMV was obtained by the reported protocol.³ Tobacco plants approximately 1 month old were inoculated with TMV solution (0.025 mg/mL). The infected leaves were then harvested and frozen. For virus purification, the leaves were crushed and added to 100 mM potassium phosphate buffer at pH 7.8 with 0.2% β -mercaptoethanol. The mixture was centrifuged for 15 min before the supernatant was treated with a mixture of CHCl₃ and 1-butanol (v/v=1:1). The aqueous portion was separated and TMV was precipitated by the addition of polyethylene glycol (MW 8000 Da) and NaCl. The resultant pellets were resuspended in 10 mM potassium phosphate buffer at pH 7.8. After a final round of ultracentrifugation at 45,000 rpm for 2.5 h with a Beckman 50.2 Ti rotor, pure TMV obtained as a clear

pellet were resuspended overnight in 10 mM potassium-phosphate buffer at pH 7.8.

4.2 TMV- β -CD

a) Diazonium-coupling reaction

80 μ L of 0.3 M of p-toluenesulfonic acid in water and 150 μ L of 78 mg/mL of 3-aminophenylacetylene in acetonitrile were mixed together at 4 $^{\circ}$ C. Then ice-cold 50 μ L of 207 mg/mL of NaNO₂ in water was added. The reaction was allowed to proceed for 1 h at 4 $^{\circ}$ C. Then 15 mg of TMV in 4.8 mL of 0.1 M pH 9.0 borate buffer was added into above diazonium salt solution. The diazonium-coupling reaction was allowed to proceed for 2 h at 4 $^{\circ}$ C. TMV-Alkyne was centrifuged at 9,500 rpm for 15 min to remove precipitate, and recovered by ultracentrifugation at 45,000 rpm/50.2 Ti for 2.5 h at 4 $^{\circ}$ C. Then the resulting TMV-Alkyne was dialyzed in 10 mM pH 7.8 potassium phosphate buffer for 48 h at 4 $^{\circ}$ C to remove remaining small molecules. TMV-Alkyne concentration was measured using a Modified Lowry Protein Assay Kit.

b) CuAAC reaction

15 mg of TMV-Alkyne was dissolved in 3.5 mL of 10 mM pH 7.8 potassium phosphate buffer, then β -CD-azide (93 mg, 100-fold molar excess) in 1 mL of DMSO and a pre-mixed solution of CuSO₄ (100 mM, 125 μ L) and NaAsc (200 mM, 125 μ L) were added. The reaction was allowed to proceed for 48 h at 4 $^{\circ}$ C. TMV- β -CD was dialyzed in 10 mM pH 7.8 potassium phosphate buffer for 48 h at 4 $^{\circ}$ C, and centrifuged at 9,500 rpm for 15 min to remove precipitate, and recovered by ultracentrifugation at 45,000 rpm/50.2 Ti for 2.5 h at 4 $^{\circ}$ C. TMV- β -CD concentration was measured using a Modified Lowry Protein Assay Kit.

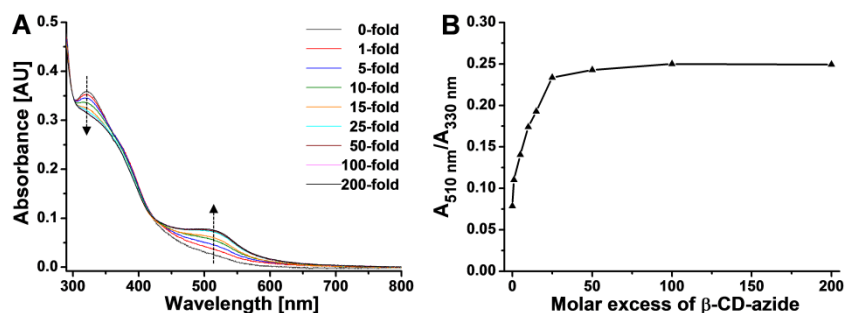


Fig. S1 (A) UV-Vis spectra of the resulting TMV- β -CD prepared by the CuAAC reaction while varying molar excess of β -CD-azide relative to TMV-Alkyne subunit proteins were used. (B) The relationship between absorbance ratio ($A_{510 \text{ nm}}/A_{330 \text{ nm}}$) of TMV- β -CD and molar excess of β -CD-azide.

4.3 TMV- β -CD/Ada-RhB (or Ada-FA, Ada-Dox, Ada-PEG2000-RhB)

General protocol was described as follows: Ada-RhB (or Ada-FA, Ada-Dox, Ada-PEG2000-RhB) (10-fold molar excess relative to the subunit proteins) dissolved in DMSO was added dropwise into TMV- β -CD in 10 mM pH 7.8 potassium phosphate buffer (using unmodified TMV as a control). The final virus concentration kept at 1 mg/mL in DMSO/buffer (v/v, 1/4). The mixture was incubated 30 min at 4 °C. The resulting solution was dialyzed in 10 mM pH 7.8 potassium phosphate buffer for 24 h at 4 °C, and recovered by ultracentrifugation at 45,000 rpm/50.2 Ti for 2.5 h at 4 °C, and monitored in situ by SEC using Sephadex G-25 column or Superose-6 size-exclusion column. All samples were analyzed in 10 mM pH 7.8 potassium phosphate buffer at a flow rate of 1 mL/min. The eluted fractions were collected and used in the next experiments.

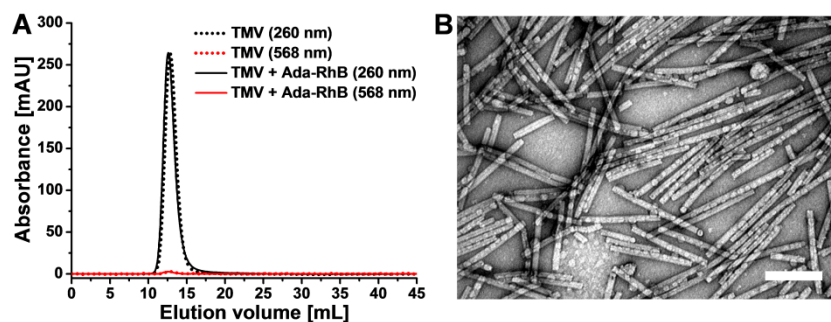


Fig. S2 Characterization of TMV- β -CD/Ada-RhB. (A) SEC diagram of TMV + Ada-RhB (solid) and TMV (dot) monitored at 260 nm (black) and 568 nm (red). (B) TEM image of uranyl acetate-stained TMV- β -CD/Ada-RhB. The scale bar is 200 nm.

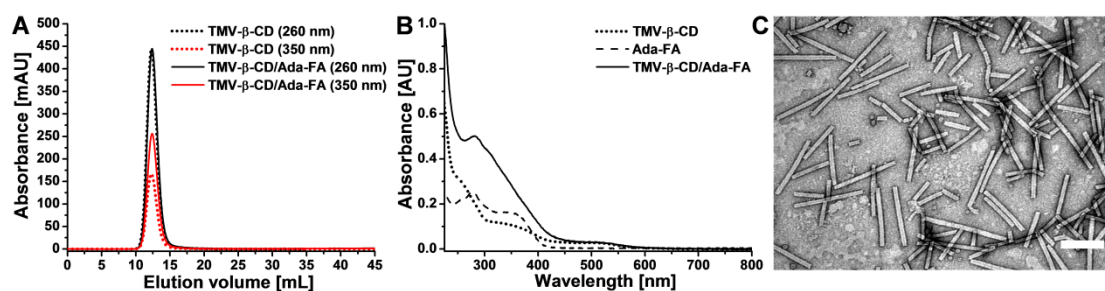


Fig. S3 Characterization of TMV- β -CD/Ada-FA. (A) SEC diagram of TMV- β -CD/Ada-FA (solid) and TMV- β -CD (dot) monitored at 260 nm (black) and 350 nm (red). The eluted fraction was collected and further analyzed. (B) UV-Vis spectra of TMV- β -CD, Ada-FA and TMV- β -CD/Ada-FA. (C) TEM image of uranyl acetate-stained TMV- β -CD/Ada-FA. The scale bar is 200 nm.

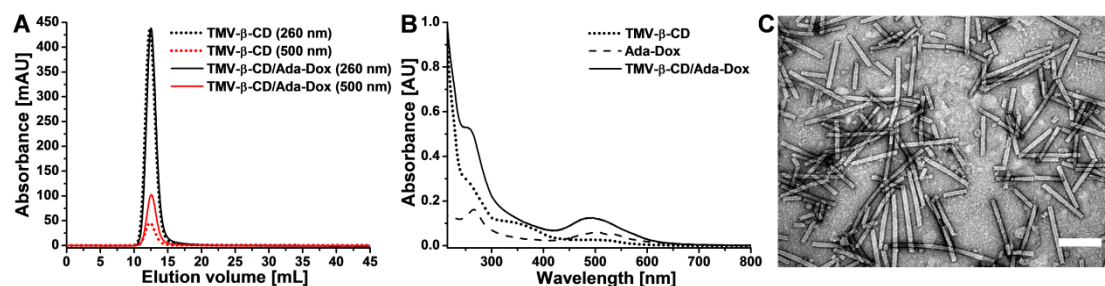


Fig. S4 Characterization of TMV-β-CD/Ada-Dox. (A) SEC diagram of TMV-β-CD/Ada-Dox (solid) and TMV-β-CD (dot) monitored at 260 nm (black) and 500 nm (red). The eluted fraction was collected and further analyzed. (B) UV-Vis spectra of TMV-β-CD, Ada-Dox and TMV-β-CD/Ada-Dox. (C) TEM image of uranyl acetate-stained TMV-β-CD/Ada-Dox. The scale bar is 200 nm.

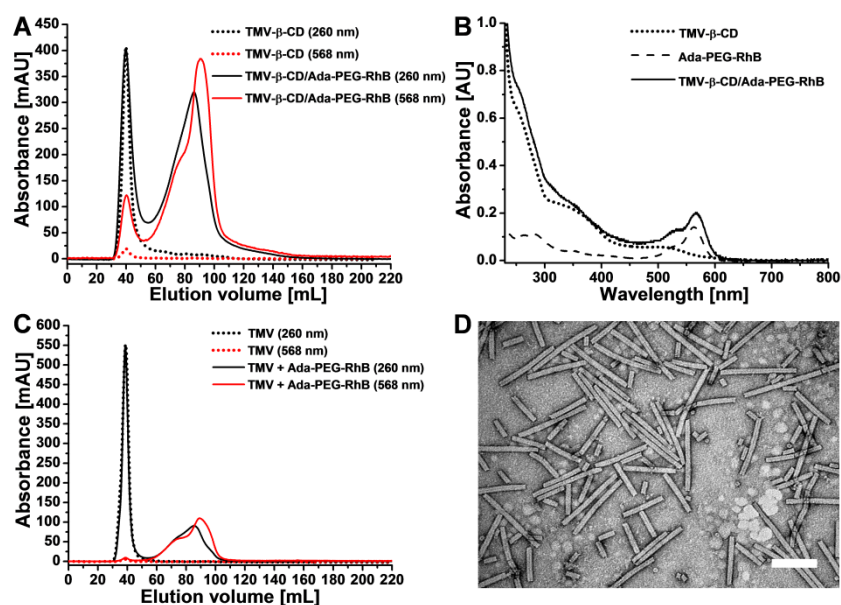


Fig. S5 Characterization of TMV-β-CD/Ada-PEG2000-RhB. (A) SEC diagram of TMV-β-CD/Ada-PEG2000-RhB (solid) and TMV-β-CD (dot) monitored at 260 nm (black) and 568 nm (red). The eluted fraction was collected and further analyzed. (B) UV-Vis spectra of TMV-β-CD, Ada-PEG2000-RhB and TMV-β-CD/Ada-PEG2000-RhB. (C) SEC diagram of TMV + Ada-PEG2000-RhB (solid) and TMV (dot) monitored at 260 nm (black) and 568 nm (red). (D) TEM image of uranyl acetate-stained TMV-β-CD/Ada-PEG2000-RhB. The scale bar is 200 nm.

4.4 TMV-β-CD/Ada-FA/Ada-RhB (or Ada-Dox)

General protocol was described as follows: various molar ratios of Ada-FA and Ada-RhB (or Ada-Dox) co-dissolved in DMSO was added dropwise into TMV-β-CD in 10 mM pH 7.8 potassium phosphate buffer with 10 μL transferpettor under continuously stirring. The final virus concentration kept at 1 mg/mL in DMSO/buffer (v/v, 1/4). The mixture was stirred for 30 min and incubated overnight at 4 °C. The resulting solution was dialyzed in 10 mM pH 7.8 potassium phosphate buffer for 24 h at 4 °C, and recovered by ultracentrifugation at 45,000 rpm/50.2 Ti for 2.5 h at 4 °C, and treated by SEC. The eluted fractions were collected and used in the next experiments.

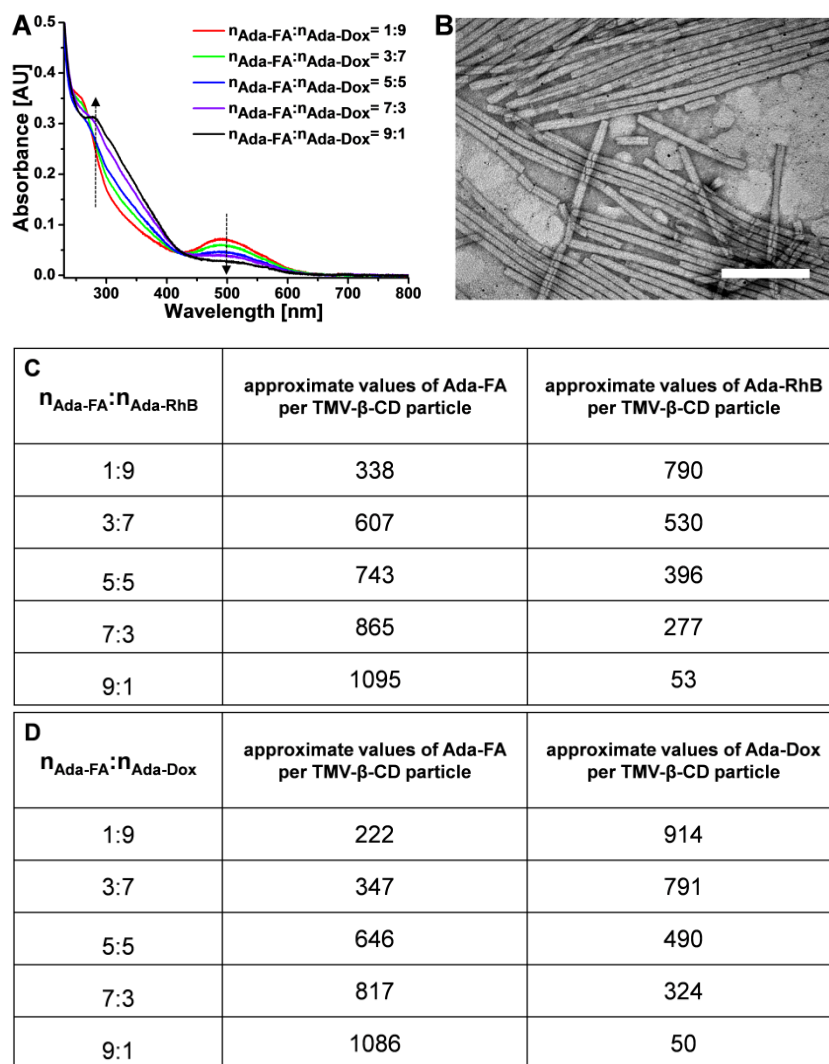
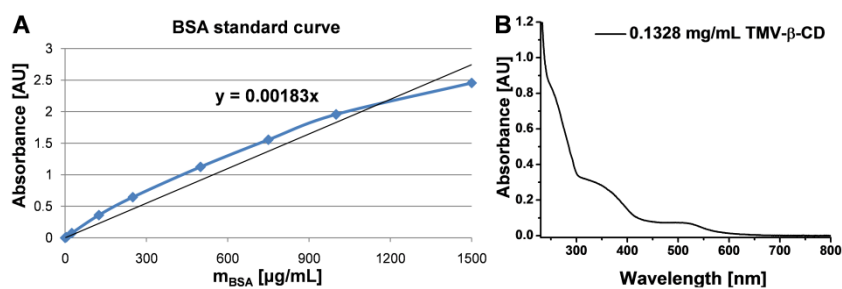


Fig. S6 (A) UV-Vis spectra of TMV- β -CD/Ada-FA/Ada-Dox with varying molar ratios of Ada-FA and Ada-Dox ($n_{\text{Ada-FA}}:n_{\text{Ada-Dox}}$) used. (B) TEM image of uranyl acetate-stained TMV- β -CD/Ada-FA/Ada-Dox ($n_{\text{Ada-FA}}:n_{\text{Ada-Dox}}=1:9$). The scale bar is 200 nm. (C) The approximate number of Ada-FA and Ada-RhB simultaneously displaying on per TMV- β -CD particle; (D) The approximate number of Ada-FA and Ada-Dox simultaneously displaying on per TMV- β -CD particle.

5. Quantitative analysis

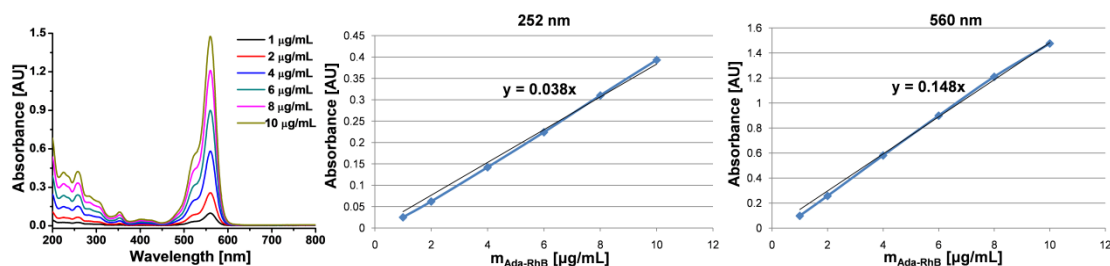
a) BSA standard curve and TMV- β -CD concentration



The absorbance ($\lambda_{750 \text{ nm}}$) of TMV- β -CD mother solution obtained by Modified Lowry Protein Assay

was 1.215 AU. According to the BSA standard curve ($\lambda_{750 \text{ nm}}$) obtained by Modified Lowry Protein Assay (A), the concentration of TMV- β -CD mother solution was calculated to be 1.328 mg/mL {i.e., $C = (1.215/0.00183)*2 \mu\text{g/mL} = 1.328 \text{ mg/mL}$ }. The 10-fold diluted TMV- β -CD solution (i.e., $C' = 0.1328 \text{ mg/mL}$) was subjected to UV-Vis spectroscopy assay (B).

b) Ada-RhB standard curves



c) Typical calculation protocol

We hypothesize that the absorbance of TMV- β -CD/Ada-RhB is the linear superposition of the absorbance of Ada-RhB and TMV- β -CD (i.e., $A_{\text{TMV-}\beta\text{-CD/Ada-RhB}} = A_{\text{TMV-}\beta\text{-CD}} + A_{\text{Ada-RhB}}$). For TMV- β -CD/Ada-FA/Ada-RhB, its absorbance is the linear superposition of the absorbance of Ada-FA, Ada-RhB and TMV- β -CD (i.e., $A_{\text{TMV-}\beta\text{-CD/Ada-FA/Ada-RhB}} = A_{\text{TMV-}\beta\text{-CD}} + A_{\text{Ada-FA}} + A_{\text{Ada-RhB}}$). It can be found that the UV-Vis spectrum of Ada-RhB moiety on the TMV- β -CD shows 8 nm shifts toward longer wavelength compared to that of free Ada-RhB. Using TMV- β -CD/Ada-RhB as an example, the typical calculation protocol was described below:

$$A_{\text{TMV-}\beta\text{-CD}} + A_{\text{Ada-RhB}} = A_{\text{(TMV-}\beta\text{-CD/Ada-RhB)}}$$

	$A_{\text{TMV-}\beta\text{-CD}}$ (0.1328 mg/mL)	$A_{\text{Ada-RhB}}$ (1 $\mu\text{g/mL}$)	$A_{\text{TMV-}\beta\text{-CD / Ada-RhB}}$
568 nm	0.027	0.148	0.300
260 nm	0.764	0.038	0.686

$$\begin{cases} 0.027*a + 0.148*b = 0.300 \text{ (568 nm)} \\ 0.764*a + 0.038*b = 0.686 \text{ (260 nm)} \end{cases} \implies \begin{cases} a = 0.806 \\ b = 1.880 \end{cases}$$

The actual mass of Ada-RhB moieties attached on 1 mg of TMV- β -CD: $m_{\text{Ada-RhB}}/m_{\text{TMV-}\beta\text{-CD}} = (1 \mu\text{g/mL} * b) / (0.1328 \text{ mg/mL} * a) = 17.6 \mu\text{g/mg}$.

The maximum mass of Ada-RhB moieties attached on 1 mg of TMV (i.e., every TMV particle has 2130 Ada-RhB moieties): $m_{\text{Ada-RhB}}/m_{\text{TMV}} = (m_{\text{TMV}}/M_{\text{TMV}}) * 2130 * M_{\text{Ada-RhB}} = 33.4 \mu\text{g/mg}$.

So the Ada-RhB labeling efficiency = $17.6/33.4 = 52.7\%$, i.e., the number of Ada-RhB moieties per

TMV- β -CD particle = $2130 \times 52.7\% = 1123$, which is consistent with the number of β -CD units per TMV particle.

6. Drug release experiment

TMV- β -CD/Ada-Dox in phosphate buffered saline (PBS, pH 5.0 and 7.4) was transferred to a dialysis bag (MWCO = 3500 Da) and immersed in a tube containing phosphate buffered saline (PBS, pH 5.0 and 7.4) while shaking on an orbital shaker at 100 rpm at 37 °C. At specific time intervals, aliquots of buffer solution outside the dialysis bag was withdrawn and subjected to photometrical assay at 500 nm. The same volume of fresh medium was replaced after every measurement. The experiment was performed in triplicate.

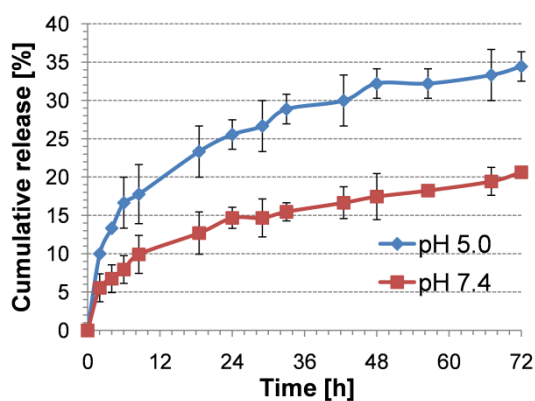


Fig. S7 The sustained release of Ada-Dox from TMV- β -CD/Ada-Dox at pH 5.0 and 7.4.

7. Cellular uptake study

HepG2 cells and NIH-3T3 cells were seeded into 24-well microplates at a density of 1.5×10^4 cell/mL in 500 μ L of DMEM containing 10 % FBS. After incubated for 24 h, the cultural medium was replaced by 2 % FBS-medium containing TMV- β -CD/Ada-Dox and TMV- β -CD/Ada-FA/Ada-Dox while maintaining a constant drug molar concentration of 1.84 μ M at 37 °C. After 24 h, the cultural medium was removed, and cells were washed three times with PBS. After fixation in 4 % paraformaldehyde in PBS for 30 min at room temperature, and cells were washed three times with PBS again. Later, cell nucleus was stained with DAPI for 30 min followed by several washes with PBS. Finally, cells were observed by a LSM 700 confocal laser scanning microscope imaging system (Carl Zeiss). Excitation wavelength was 350 nm and 488 nm for DAPI and Ada-Dox, respectively.

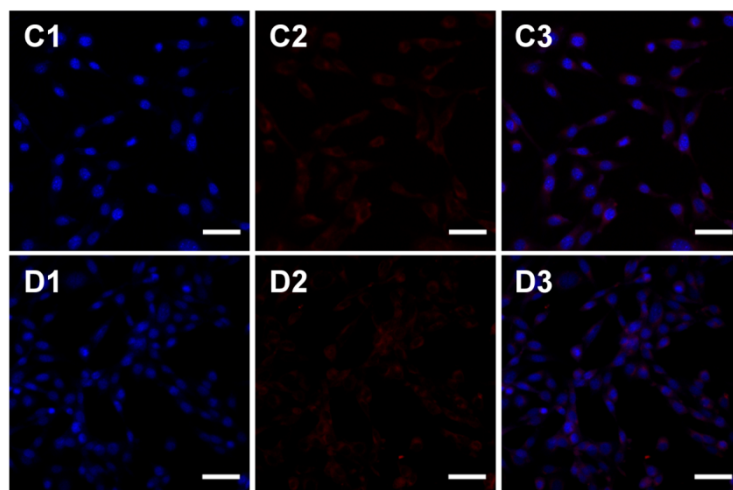


Fig. S8 Fluorescent microscopy images of NIH-3T3 cells. (C, D) were NIH-3T3 cells incubated with TMV-β-CD/Ada-Dox and TMV-β-CD/Ada-FA/Ada-Dox for 24 h, respectively. **1, 2, 3** indicated the DAPI, Ada-Dox and merged channels, respectively. The scale bar is 50 μm.

8. Cell viability study

HepG2 cells and NIH-3T3 cells were seeded into 96-well microplates at a density of 1.5×10^4 cell/mL in 200 μL of 10 % FBS-medium. After incubated for 24 h, the cultural medium was replaced by 5 % FBS-medium containing different concentration of (a) Dox, (b) Ada-Dox, (c) TMV-β-CD/Ada-Dox, (d) TMV-β-CD/Ada-FA/Ada-Dox ($n_{\text{Ada-FA}}:n_{\text{Ada-Dox}} = 1:9$). After further incubation for 72 h, 100 μL of 10 % sterilized MTT (5 mg/mL) stock solution in serum-free medium was added to each well. After 4 h, unreacted dye was aspiration, and the formazan crystals were dissolved in DMSO (100 μL) per well and measured in a microplate reader at the wavelength of 570 nm.

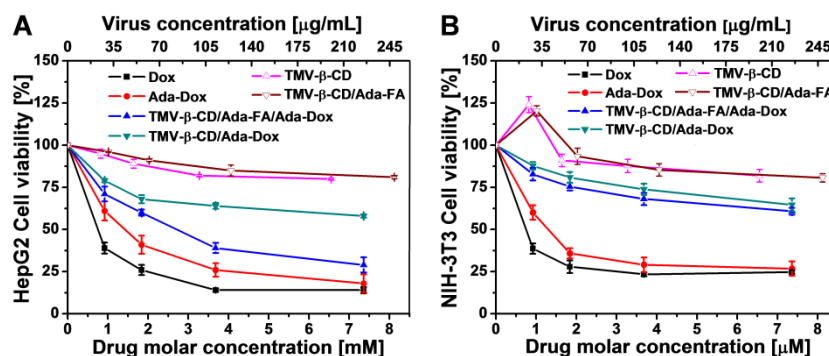


Fig. S9 Cytotoxicities of TMV-β-CD, TMV-β-CD/Ada-FA, Dox, Ada-Dox, TMV-β-CD/Ada-Dox and TMV-β-CD/Ada-FA/Ada-Dox against HepG2 cells (A) and NIH-3T3 cells (B) after 72 h of incubation measured by MTT assays.

9. References

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