Compartmentalized Supramolecular Hydrogels Based on Viral Nanocages towards Sophisticated Cargo Administration

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1. Materials & Methods

Materials were obtained from commercial suppliers and were used without further purification. $^1$H NMR spectra was recorded on a Bruker Ascend™ 400 MHz Nuclear Magnetic Resonance spectrometer. Dynamic Light Scattering (DLS) experiments were carried out by Microtrac Nanotrac Wave. UV-Vis spectra were recorded using a Perkin Elmer Lambda 850 UV spectrophotometer. Concentrations of CCMV and virus-like particles were determined using a Thermo Scientific NanoDrop 1000 Spectrophotometer. Transmission Electron Microscopy (TEM) analysis was performed using a Philips CM300ST-FEG Transmission Electron Microscope 300 kV, Scanning Electron Microscopy (SEM) analysis was performed using a ZEISS MERLIN HR-SEM. Rheological analysis was carried out on a Rheometer Physica UDS 200 (Anton-Paar) at room temperature. Experiments were performed on a 12.5 mm parallel-plate, 50 µL of hydrogels was added, the gap size was set to 0.2 mm. Rheological strain sweep was performed from 0.01% to 2000% with a fixed frequency of 1 Hz. Flow sweep was performed with a shear rate varying from 0.01 to 100 s$^{-1}$. Fast Protein Liquid Chromatography (FPLC) was carried out on ÄKTA Protein Purification Systems, equipped with a Superose™ 6, 10/300 GL column. Optical images were taken by Olympus Fluorescence Microscope with a TH4-200
halogen lamp. Statistics of cell viability was performed using TECAN i-control infinite 200 PRO microplate reader.

2. Experimental

2.1 Preparation of CCMV

CCMV was isolated and purified according to procedures in literature. The obtained virus was analyzed by UV/Vis spectroscopy, SDS-PAGE and FPLC and in agreement with literature.

2.2 Synthesis of CCMV-Vio

Scheme S1. Synthesis route of CCMV-Vio. Abbreviations see synthetic procedures.

Synthesis of compound 1 4,4′-dipyridine (3.12 g, 20 mmol) was dissolved in 50 mL DCM (dichloromethane), then CH$_3$I (2.8 g, 20 mmol) was added slowly. The solution was kept at 40 °C for 12 h. The precipitate was filtered off, and washed with DCM, dried in vacuum oven (60 °C) and finally obtained as a yellowish powder (4.3 g, yield 72%). $^1$H NMR (DMSO-$d_6$, ppm): δ = 9.15 (d, 2H), 8.88 (d, 2H), 8.62 (d, 2H), 8.05 (d, 2H), 4.39 (s, 3H).
Synthesis of **compound 2** Compound 1 (0.6 g, 2.0 mmol) and 2-bromoethanol (0.75 g, 6.0 mmol) were dissolved in 10 mL DMF (dimethylformamide). The solution was heated up to 80 °C, and kept for 5 h. The precipitate was filtered off, washed with DMF and acetonitrile, dried in vacuum oven (60 °C) and finally obtained as a yellow powder (0.42 g, yield 50%). ¹H NMR (DMSO-\textit{d}_6, ppm): δ = 9.27 (m, 4H), 8.75 (m, 4H), 4.73 (t, 2H), 4.39 (s, 3H), 3.88 (t, 2H).

Synthesis of **compound 4** Compound 2 (0.24 g, 0.6 mmol) was dissolved in 0.5 mL H₂O, then dropped into an aqueous solution (1 mL) of NH₄PF₆ (1.0 g, 6.1 mmol) while stirring. The precipitate was filtered off, washed with water, dried in vacuum oven (60 °C), and finally obtained compound 3 (0.25 g, yield 80%). Compound 3 (0.6 g, 1.2 mmol) was dissolved in 100 mL anhydrous acetonitrile. HMDI (4,4′-methylenedioxybenzyl isocyanate) (2.4 g, 14.4 mmol) and a drop of DTL (dibutyltin dilaurate) were added to the solution. The solution was stirred at room temperature for 24 h, and then concentrated to about 5 mL by rotation evaporation. The solution was precipitated using 100 mL anhydrous diethyl ether. The mixture was chilled at 4 °C for 1 h. The supernatant was decanted, and the residue was redissolved in a minimum amount of anhydrous acetonitrile, and precipitated using anhydrous diethyl ether again. The precipitate was dried under vacuum to obtain a yellowish solid (0.67 g, yield 82%). ¹H NMR (DMSO-\textit{d}_6, ppm): δ = 9.35 (d, 2H), 9.30 (d, 2H), 8.81 (d, 2H), 8.77 (d, 2H), 4.96 (t, 2H), 4.53 (t, 2H), 4.45 (s, 3H), 3.32 (m, 2H), 2.90 (m, 2H), 1.51 (m, 2H), 1.2-1.4 (m, 6H).

Synthesis of **CCMV-Vio** Compound 4 (67.4 mg, 0.1 mmol) was dissolved in 2 mL acetonitrile, and gradually added to an aqueous solution (2 mL) of TBAB (tetrabutylammonium bromide) (322 mg, 1.0 mmol) with rigorous stirring. A yellow precipitate was filtered off, washed with anhydrous acetonitrile (5 mL × 3) and diethylether, dried in vacuum oven (40 °C) and finally obtained as compound 5.

CCMV in virus buffer (22.4 mg/mL, 1 mL) was first dialyzed against phosphate buffer 7.2 buffer (0.1 M) at 4 °C (300 mL × 3), then diluted by the same buffer to a concentration of 5 mg/mL. Compound 5 (32.7 mg, 60 μmol, 60 eq. of capsid protein) was dissolved in 200 μL of precooling (4 °C) milliQ water. The solution of compound
was added into the CCMV solution in a 5 mL centrifugal tube every 30 min, in 4 batches, and mixed by roller mixer at 4 °C. The reaction was kept at 4 °C for 3 h, then dialyzed against PB 6.0 buffers with a gradually decreased salt concentration (from 100 mM to 50 mM, and finally 10 mM).

2.3 Synthesis of HPC-Np

![Scheme S2. Synthesis route of HPC-Np. Abbreviations see synthetic procedures.](image)

Hydroxylpropyl cellulose (HPC, 110 mg, M\textsubscript{n} ~ 1000 kDa) was dissolved in 10 mL anhydrous NMP (N-methyl-2-pyrrolidone) at 80 °C, then the solution was cooled down to 40 °C. 2-Naphthyl isocyanate (Np, 5 mg, 29.6 mmol) and a drop of DTL (dibutyltin dilaurate) were added into the above solution. The reaction was kept at 40 °C for 12 h. After that, the solution was first diluted by 20 mL diethylether, and then precipitated in 300 mL diethylether. The white precipitate was filtered off and redissolved in 20 mL H\textsubscript{2}O. The solution was dialyzed against pure water, and lyophilized to obtain white solid (100 mg, yield 91 %).

2.4 Determination of the Average Content of Viologen Group on Each Virus Nanoparticle

CCMV-Vio (4 mg/mL) 100 μL in PB 6.0 buffer and another 5 mL MilliQ water were degassed by using freeze-pump-thaw cycling. In a glove box under nitrogen atmosphere, 10 μL Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4} aqueous solution (10 mg/mL) was added into 40 μL of CCMV-Vio solution. The solution turned to be deep purple immediately, then it was diluted to 500 μL, and transferred into a cuvette (2 mm × 10 mm × 25 mm, W×L×H), sealed by a PTFE cap. UV-Vis spectrum was determined immediately after the cuvette was removed out of the glove box. The molar absorption coefficient of peak at 520 nm was calculated to be $\varepsilon_{520\text{nm}} = 0.76 \times 10^4$ L/(mol⋅cm), by taking compound 5 as a model compound. According to the UV-Vis spectrum, the concentration of viologen...
group in the CCMV-Vio solution was calculated to be $C_{\text{vio}} = 4.2 \times 10^4$ mol/L, and the concentration of capsid protein was about $C_{\text{cp}} = 2.0 \times 10^4$ mol/L, therefore, the degree of substitution was about 2.1 per capsid protein, and the average number of viologen group per virus nanoparticle was about 378.

### 2.5 Synthesis of CCMV-Np

Conjugation of CCMV particles with Np groups to obtain stable CCMV-Np particles was hard to achieve. The hydrophobic Np group not only hinders the reaction in aqueous solution, but also causes aggregation of virus particles. Therefore, a hydrophilic linker between CCMV and the Np group was necessary to stabilize the modified virus particles. In order to minimize steric effects during the supramolecular crosslinking, while having sufficient water solubility, tetraethylenepentamine (TEPA) was selected as a linker (Scheme S3). The Np group was then conjugated to the surface of virus particles through a CuAAC click reaction.

**Scheme S3. Synthesis route of CCMV-Np. Abbreviations see synthetic procedures.**

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**Synthesis of compound 6** Tetraethylenepentamine (3.8 g, 20 mmol) was dissolved in 100 mL of DCM. The solution was stirred and ethyl trifluoroacetate (5.68 g, 40 mmol) in 40 mL DCM was added dropwise at 0 °C. The reaction was kept at room
temperature for another 1 h. Then trimethylamine (TEA, 8.1 g, 80 mmol) and di-tert-
butyl pyrocarbonate ((Boc)$_2$O, 17.5 g, 80 mmol) in 20 mL DCM were added dropwise.
After incubation overnight, the solvent was evaporated to obtain a yellow oil. The
 crude product was recrystallized by adding 50 mL of n-hexane. The obtained white solid was then further purified by column chromatography (DCM as eluent) to get pure product (10.1 g, yield 74.1%). $^1$H NMR (CDCl$_3$, ppm): $\delta =$ 3.31(br, 12H), 2.79 (br, 4H), 1.44 (s, 27H).

Synthesis of compound 7

Compound 6 (5.0 g, 7.3 mmol) was suspended in ethanol (50 mL), NaOH aqueous solution (3 M, 50 mL) was slowly added under stirring. The reaction was monitored by TLC. After 3 h, the reaction was almost completed, the solution was then extracted by DCM (100 mL). The organic phase was washed by brine and water, dried by anhydrous Na$_2$SO$_4$. The solvent was then evaporated to obtain a yellowish oil, which was used directly for next step without further purification. $^1$H NMR (CDCl$_3$, ppm): $\delta =$ 3.33 (br, 12H), 3.04 (br, 4H of NH$_2$), 2.88 (br, 4H), 1.45 (s, 27H).

Synthesis of compound 8

2-Naphthoxyacetic acid (0.83 g, 4.1 mmol), ethylcarbodiimide hydrochloride (EDC-HCl) (0.94 g, 4.9 mmol) and N-hydroxysuccinimide (NHS) (0.56 g, 4.9 mmol) were dispersed in 20 mL anhydrous acetonitrile. The mixture was stirred at room temperature until the 2-naphthoxyacetic acid was completely dissolved and activated. The activated reactant was added dropwise into an acetonitrile solution of Compound 7 (2.0 g, 4.1 mmol, in 20 mL of acetonitrile). The reaction was monitored by TLC (CH$_2$Cl$_2$ : CH$_3$OH = 10 :1). After the reaction completed, the solvent was evaporated, and the residue was dissolved by DCM. The solution was then washed by brine and water, dried by anhydrous Na$_2$SO$_4$, and dried by rotation evaporation. The crude product was purified by column chromatography (CH$_2$Cl$_2$ : CH$_3$OH = 10 :1) to obtain a yellowish oil (1.4 g, yield 51%). $^1$H NMR (CDCl$_3$, ppm): $\delta =$ 7.77 (m, 3H), 7.46-7.37 (d, 2H), 7.23-7.14 (d, 2H), 4.60 (s, 2H), 3.70-3.30 (m, 16H), 1.43 (s, 27H).

Synthesis of compound 9

2-Azidoacetic acid (44.5 mg, 0.4 mmol), EDC-HCl (101.2 mg, 0.5mmol) and NHS (61.0 mg, 0.5 mmol) were dissolved in 5 mL
anhydrous acetonitrile. The solution was stirred at room temperature until 2-
azidoacetic acid was completely activated. Then compound 8 (269.5 mg, 0.4 mmol)
was added. The reaction was kept for 6 h, and monitored by TLC (CH₂Cl₂ : CH₃OH =
10 :1). When the reaction finished, the solvent was evaporated, and the residue was
dissolved by DCM. The solution was then washed by brine and water, dried by
anhydrous Na₂SO₄, and dried by rotation evaporation. The crude product was purified
by column chromatography (CH₂Cl₂ : CH₃OH = 25 :1) to obtain a colorless oil (272.5
mg, yield 90%). ¹H NMR (CDCl₃, ppm): δ = 7.81 (m, 3H), 7.61-7.47 (d, 2H), 7.24-
7.17 (d, 2H), 4.69 (s, 2H), 3.94 (s, 2H), 3.43-3.33 (m, 16H), 1.46 (s, 27H).

Synthesis of compound 10 Compound 9 (272.5 mg, 0.36 mmol) was dissolved in 3
mL of HCl /CH₃OH solution, the reaction was kept at 0 °C for 2 h. After the
evaporation of the solvent, white solid was obtained (200 mg, yield 98%). ¹H NMR
(D₂O, ppm): δ = 7.91-7.85 (m, 3H), 7.55-7.46 (d, 2H), 7.33 (m, 2H), 4.05 (s, 2H),
3.69-3.51 (m, 18H).

Synthesis of CCMV-Np. CCMV particles were firstly conjugated with alkyne
group. In general, 1 mL of CCMV solution (11.7 mg/mL) was dialyzed against PB pH
7.2 buffer (0.1 M) at 4 °C. Then 2-propynylamine (6.9 mg), EDC-HCl (24.2 mg) and
NHS (14.5 mg) were mixed with the CCMV solution. The mixture was incubated on a
roller mixer for 12 h at 4 °C. Then the solution was dialyzed against PB pH 7.2 buffer
to obtain a purified CCMV-alkyne solution. For the click reaction, in a typical
example, CCMV-alkyne (1 mg/mL, 2 mL) in PB 7.2 buffer (0.1 M), CuSO₄ (10
mg/mL, 16 µL), aminoguanidine hydrochloride (221 mg/mL, 16 µL), tris(3-
hydroxypropyltriazolylmethyl)amine (THPTA) ligand (27.2 mg/mL, 80 µL), aqueous
solution of compound 10 (2.4 mg in 200 µL water, 4.2 µmol) were mixed together in
a 2.5 mL Eppendorf centrifugal tube. Then freshly prepared sodium ascorbate solution
(62 mg/mL, 32 µL) was added into the tube. The tube was sealed immediately to
avoid the diffusion of oxygen from outside. The mixture was kept at 4 °C on a roller
mixer for 10 h. Then the solution was dialyzed against PB 6.0 buffer (0.1 M) to obtain
the target product.

2.6 Synthesis of HPC-Vio
Scheme S4. Synthesis route of HPC-Vio.

Hydroxylpropyl cellulose (102 mg, $M_n \sim 1000$ kDa) was dissolved in 10 mL anhydrous NMP at 80 °C, then the solution was cooled down to 40 °C. Compound 4 (20 mg, 0.03 mmol) and a drop of dibutyltin dilaurate (DTL) were added into the above solution. The reaction was kept at 40 °C for 12 h. After that, the solution was first diluted by 20 mL diethylether, and then precipitated in 300 mL diethylether. The light yellow precipitate was filtered off and redissolved in 20 mL $H_2O$. The solution was dialyzed against pure water, and lyophilized to obtain yellow solid (90 mg, yield 88%).

2.7 Preparation of Compartmentalized and Conventional Supramolecular Hydrogels

In a typical experiment, CCMV-Vio (4 mg/mL, 4 mL) in PB 6.0 buffer (10 mM), 30 mg of HPC-Np, and 2.6 mg of CB[8] were mixed together. The mixture was stirred at room temperature for 10 h to obtain a homogeneous mixture. The sticky solution was lyophilized to obtain a white, fluffy solid. Finally, 750 μL of water was added to the solid, a homogeneous supramolecular hydrogel with 4 wt% of polymer formed in 12 hours.

For the preparation of compartmentalization supramolecular hydrogel with ZnPc loaded, HPC-Np (13 mg), CB[8] (1.6 mg) were mixed in 2 mL of PB 6.0 buffer (10 mM), and CCMV-Vio/ZnPc ($C_{vio} = 0.35$ mM, 5.4 mg, 13 wt% of ZnPc) in 2.8 mL PB 6.0 buffer was added. The mixture was stirred at room temperature for 10 h to obtain a homogeneous mixture. The solution was lyophilized to obtain a white solid. Finally, 325 μL of water was added to the solid, a homogeneous supramolecular hydrogel with 4 wt% of polymer formed in 12 hours. The loading content of ZnPc was 3.5 wt%.

For the preparation of conventional supramolecular hydrogel, HPC-Np (6 mg), HPC-Vio (6 mg), CB[8] (0.4 mg) and ZnPc (0.45 mg) were mixed in 2 mL PB 6.0
buffer (10 mM). The mixture was stirred at room temperature for 10 h to obtain a homogeneous mixture. The solution was lyophilized to obtain a white solid. Finally, 300 μL of water was added to the solid, a homogeneous supramolecular hydrogel with 4 wt% of polymer formed in 12 hours. The loading content of ZnPc was 3.5 wt%.

2.8 Cargo Release Study

A 250 μL hydrogel sample was prepared in a 2 mL vial, then 1 mL of PBS buffer (pH 7.4, 0.1 M) release solution was carefully added. The sample vial was then incubated in a water bath equilibrated at 25 °C. The buffer solution was replaced with fresh one at a certain time interval. The ZnPc content of each collected buffer solution was determined by UV-Vis spectrophotometer.

2.9 Photodynamic Effect Study

RAW macrophages were grown in Dulbecco’s modified Eagle’s medium (DMEM) + 10% fetal bovine serum (FBS) + 1% penicillin, streptomycin solution (PS) at 37 °C. Approximately \(10^{14}\) ZnPc-loaded VLPs and released VLPs in their corresponding buffer or approximately \(10^{14}\) ZnPc molecules in pH 7.5 PBS buffer were mixed with \(10^4\) seeded cells in TC-coated 96 well plates with 100 μL growth medium and incubated at 37 °C for about 18 h. Experiments for each sample were done in triplicate. Afterwards the cells were washed three times with the LIFE cell imaging solution/HEPES (Thermo Fisher Scientific) to remove the excess of material. The washed cells were irradiated using a lamp fitted with a filter for 60 min in the HEPES buffer. After incubation at 37 °C for 1 h, propidium iodide (PI) was added to the cell culture, and images were taken with a fluorescence microscope immediately. To evaluate the cell viability, Calcein violet AM (Thermo Fisher Scientific) was added to the cell culture to 1 μM. After 15 min, the samples were characterized by a microplate reader. Calculation of cell viability was carried out by taking a positive control sample and blank sample as references.

3. Results and Discussion

3.1 Characterization of CCMV-Vio
Dynamic light scattering (DLS) analysis showed a narrow size distribution with an average diameter around 22 nm, indicating no aggregation of the particles after modification. The size of CCMV-Vio determined by DLS was a bit smaller than native CCMV, which may be attributed to the change of surface charge.

### 3.2 Characterization of CCMV-Np

![Figure S2. DLS spectrum of CCMV-Np. The number average size of the particles was around 18 nm.](image-url)
In the $^1$H NMR spectrum of HPC-Np, besides a single peak at 1.0 ppm and multiple peaks around 3.0 – 4.0 ppm that belong to the cellulose backbone, two peaks at 7.5 and 7.9 ppm were ascribed to the naphthyl group. Meanwhile, two absorption peaks at 280 nm and 325 nm in the UV-Vis spectrum also indicate the existence of naphthalene group. The degree of substitution could hardly be calculated from the NMR spectrum, owing to the relatively poor signal-noise-ratio. However, the UV-Vis spectrum is sensitive enough, and the content of naphthalene group was estimated to be 75 μmol per gram of polymer according to the Lambert-Beer law.

3.4 Characterization of HPC-Vio
The obtained HPC-Vio showed a characteristic absorption at $\lambda = 260$ nm in the UV-Vis spectrum, which belongs to viologen group (SI Figure S4a). In the $^1$H NMR spectrum, two doublets at 8.5 and 9.1 ppm are the characteristic signals for viologen. The number of viologen groups was estimated to be 68 $\mu$mol/g from the $^1$H NMR spectrum.

3.5 Characterization of Supramolecular Hydrogels
Figure S5. (a) 4 wt% Supramolecular hydrogel with light yellow color formed by CCMV-Vio, HPC-Np and CB[8]. (b) In a control experiment, a turbid solution instead of hydrogel was obtained by mixing CCMV-Vio, HPC-Np and CB[7] together. (c) UV-Vis spectra of the mixture of HPC-Np, CCMV-Vio and CB[8], and control experiments.

For the mixture of HPC-Np and CCMV-Vio, no significant absorption peak was observed in the wavelength region of 400 – 500 nm. However, when the CB[8] was added, weak and broad peaks appeared in this region. As a result, the supramolecular hydrogel appeared yellow, while all of the three individual components are colorless. A similar phenomenon was also observed in a model system, which contained HPC-Np, CB[8] and unmodified methyl viologen.

Figure S6. A 2 wt% supramolecular hydrogel with yellow color prepared through pathway B.

3.6 Preparation and Characterization of CCMV/ZnPc

ZnPc was synthesized according to a literature procedure. ZnPc (2 mg, 2.0 μmol) was dissolved in 10 mL of Tris buffer (pH 7.2, 50 mM tris-base, 300 mM NaCl) under the assistance of ultra-sonication and heating at 60 °C, then the solution was
cooled down to room temperature. Capsid protein (8 mg, 0.4 μM) in the same Tris buffer was then added into the above solution. The mixture was incubated on a roller mixer at 4 °C for at least 1 h (Scheme S5). According to the literature, VLPs with $T = 1$ particles will form in this way. The sample was then purified by FPLC, and characterized by DLS, UV-Vis spectrometer, and TEM.

Scheme S5. Synthesis of CCMV/ZnPc.

Figure S7. (a) FPLC chromatogram of CCMV/ZnPc; (b) DLS result showed that the particle size was around 18 nm; (c) UV-Vis spectra of ZnPc and CCMV/ZnPc. (d) TEM image of CCMV/ZnPc.

Elution volume of the main peak in FPLC chromatogram was 11.5 mL, suggesting the formation of $T = 1$ protein cages. A strong signal at 600 nm monitored by UV-Vis spectrometer suggested a successful encapsulation of ZnPc. The DLS measurement showed that the size of the particles was around 18 nm, which further confirmed the
structure of protein cages. In UV-Vis spectrum, CCMV/ZnPc showed three main
peaks at 280 nm, 322 nm and 595 nm. The peak at 280 nm belongs to capsid protein,
while the other two peaks should derive from ZnPc. In contrast to non-encapsulated
ZnPc, the absorption peaks of ZnPc after encapsulation shifted to the left, owing to a
higher degree of packing inside protein cages. TEM analysis showed spherical
nanoparticles with size mostly around 18 nm, which was in consistent to DLS result.

![CCMV/ZnPc](image1)  ![ZnPc](image2)  ![CCMV/ZnPc](image3)  ![ZnPc](image4)

**Figure S8. Solution stability of CCMV/ZnPc (with 0.1 mM of ZnPc) and ZnPc (0.1 mM) in PBS
buffer.**

3.7 Preparation of CCMV-Vio/ZnPc

In a typical experiment, CCMV/ZnPc (10 mg, 0.1 μM of CP) in 5 mL of Tris pH
7.2 buffer was dialyzed against a PB pH 7.2 buffer (0.1 M) to remove the tris-base,
then the solution was cooled down to 4 °C. MV-NCO (16.5 mg, 30 μmol) was
dissolved in 200 μL of the same PB pH 7.2 buffer at 4 °C, 50 μL of the solution was
added into CCMV/ZnPc solution under rigorous stirring (by turbo mixer) every 20
min. The mixture was kept at 4 °C on a roller mixer for another 3 h. Then the solution
was dialyzed against PB pH 6.0 buffer (0.1 M) to remove the excess reactant. After
dialysis, the volume of the solution was maintained at 5 mL, to keep the concentration
of CP to be constant.

3.8 Rheology Study of ZnPc Loaded Compartmentalized and Conventional
Supramolecular Hydrogels
Figure S9. Rheology study of compartmentalized and conventional supramolecular hydrogels. (a) Rheological strain sweep was performed from 0.01% to 2000% with a fixed frequency of 1 Hz at room temperature; (b) Flow sweep was performed with a shear rate varying from 0.01 to 100 $s^{-1}$ at room temperature.

### 3.9 Study of Cargo Release Mechanism

The released solutions were concentrated via centrifugation, and characterized by FPLC. As shown in Figure S10a, only a sharp peak with elution volume corresponding to the void volume of column was observed, suggesting large particles existed in the released solution. The strong absorption at 600 nm of this fraction indicated the containing of ZnPc in these particles. TEM analysis (Figure S10b) of the collected fraction revealed the large particles are VLPs covered and crosslinked by polymers.

Figure S10. (a) FPLC chromatogram of released samples; (b) TEM image of released VLPs.

### 3.10 Statistical Analysis of Cell Experiment Data
The statistical analysis of emission raw data was carried out using SPSS 22.0 software. Independent samples tests clearly show that the difference of cell viability in each set of samples with/without irradiation has statistical significance (p < 0.01), which further confirms the activity of ZnPc in all the three sets of samples. The reductions of cell viability for all the three sets of samples were comparable with differences having relatively low practical significance, analysis of variance also shows that the differences between ZnPc/VLP and between ZnPc/rel VLP have low statistical significance (p > 0.05), suggesting the retention of activity of ZnPc after encapsulation and releasing from the hydrogel.

Table S1. Independent samples test for each set of samples (sample size: 12)

| sample | irradiation | mean       | std. deviation | std. error mean | p   |
|--------|-------------|------------|----------------|-----------------|-----|
| ZnPc   | no          | 16058.833  | 4306.651       | 1243.223        | 0.000 |
|        | yes         | 9367.583   | 2545.586       | 734.847         |     |
| VLP    | no          | 19469.667  | 6178.940       | 1783.706        | 0.002 |
|        | yes         | 11996.000  | 2887.860       | 833.653         |     |
| rel VLP| no          | 14093.750  | 2746.543       | 792.859         | 0.000 |
|        | yes         | 8180.833   | 3981.072       | 1149.237        |     |

Table S2. Analysis of variance (ANOVA) of three sets of samples after irradiation

| (I) sample | (J) sample | mean difference (I-J) | std. error | p   |
|------------|------------|-----------------------|------------|-----|
| ZnPc       | VLP        | -2628.417              | 1305.303   | 0.148 |
|            | rel VLP    | 1186.750               | 1305.303   | 0.665 |
| VLP        | ZnPc       | 2628.417               | 1305.303   | 0.148 |
|            | rel VLP    | 3815.167               | 1305.303   | 0.022 |
| rel VLP    | ZnPc       | -1186.750              | 1305.303   | 0.665 |
|            | VLP        | -3815.167              | 1305.303   | 0.022 |

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