Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Supramolecular polymer nanocapsules by enzymatic covalent condensation: biocompatible and biodegradable drug-delivery systems for chemo-photothermal anticancer therapy

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Contents

General Information

Materials

2-Chlorotrityl chloride resin, Fmoc-protected amino acids (Fmoc-Phe-OH, Fmoc-Gly-OH and Fmoc-Tyr(tBu)-OH), benzotriazole- 1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), N-methylmorpholine (NMM), N-hydroxybenzotriazole (HOBt), triisopropylsilane (TIS) and trifluoroacetic acid (TFA) were purchased from GL Biochem (Shanghai) Ltd. Dimethyl formamide (DMF) and dichloromethane (DCM) were purchased from Tiantai Industrial Corporation (Tianjin) and strictly dried with sodium. Cucurbit[8]uril (CB[8]) was purchased in Sigma-Aldrich. Horseradish peroxidase (HRP) was purchased from Aladdin Industrial Corporation (Shanghai). Rhodamine B (RhB), doxorubicin hydrochloride (DOX) and indocyanine green (ICG) were purchased in Energy Chemical Ltd (Shanghai). Other chemical reagents were purchased from Sinopharm Group Ltd (Shanghai).

Instruments and measurements

^{1}H NMR spectra and ^{13}C NMR spectra were recorded with a Bruker AVANCE III 500 instrument using a tetramethylsilane (TMS) proton signal as the internal standard. ESI-MS spectrometric analyses were performed at the Thermo Finnigan-LCQ Advantage Mass Spectrometer. HPLC experiments were performed on a Shimadzu HPLC system equipped with a C18 column and a SPD-20A UV-Vis detector. Dynamic light scattering (DLS) measurements were performed at a Malvern Instrument Zetasizer Nano ZS instrument with the temperature of 25 °C. The measurements of UV-vis absorption spectra were recorded on a Shimadzu 3100 UV spectrophotometer. The measurements of fluorescence spectra were performed on a Shimadzu RF-5301 PC spectrofluorimeter. The measurements of atomic force microscopy (AFM) were performed on Nanoscope III a controller, Veeco Metrology, Santa Barbara, CA using tapping modetin with a SiN_4 tip. Scanning electron microscopy (SEM) images were recorded with a JEOL JSM-6700F instrument. Transmission electron microscopy (TEM) images were recorded with a JEM-2100F instrument with an accelerating voltage of 200 kV. Confocal laser scanning microscopy (CLSM) was performed on an Olympus FV1000 measurement.

Experimental Section

Peptide synthesis

Peptide FG_3Y_2 (NH2-Phe-Gly-Gly-Gly-Tyr-Tyr-COOH) was synthesized by a common method of solid phase peptide synthesis (SPPS).[1] ^{1}H NMR (500 MHz, D2O, 25℃) δ(ppm): 7.32 (m, 3H), 7.21 (d, 2H), 7.02 (d, 2H), 6.95 (d, 2H), 6.74 (dd, 4H), 4.40 (m, 2H), 4.21 (t, 1H), 3.86 (m, 6H), 3.13 (m, 2H), 3.02-2.83 (m, 4H); ^{13}C NMR (500 MHz, D2O, 25 ℃) δ(ppm): 174.26, 172.38, 171.65, 171.32, 170.61, 169.78, 154.42, 133.72, 130.65, 130.48, 129.39, 129.18, 128.25, 128.03, 127.97, 115.37, 55.0, 54.49, 54.08, 42.51, 42.35, 42.25, 36.78, 36.30, 35.91.$C_{33}H_{38}N_6O_9$, ESI-MS: calc. $(M+H)^+$ = 663.69, obsvd. $(M+H)^+$ = 663.7.

Preparation of supramonomers

CB[8] (1.0 mM) was added to a protonated FG_3Y_2 solution (2.0 mM). The mixture was subjected to intense sonication for 1 minute and the transparent solution was obtained, indicating that FG_3Y_2 and CB[8] had combined and the supermonomers were obtained. Supermonomers of other concentrations were prepared by diluting the original solution directly in pure water.

Preparation of supramolecular polymer

HRP (0.5×10^{-4} mM) and H_2O_2 (2.0 mM) were added to the supermonomer solution (0.5 mM). The supermonomers were cross-linked and the supramolecular nanocapsules were generated in 40 minutes.

Preparation of RhB-NCs

Supramonomers (0.5 mM) and RhB (1 mg/ mL) were induced to produce RhB-NCs by enzyme-catalysis in 40 minutes. The unloaded RhB was removed by dialysis (Molecular Weight Cut-off: 1000).

Preparation of DOX-NCs

Supramonomers (0.5 mM) and DOX (1 mg/ mL) were mixed and they were induced to produce DOX-NCs by enzyme-catalysis in 40 minutes. The unloaded DOX was removed by dialysis (Molecular Weight Cut-off: 1000).

Preparation of ICG-NCs

Supramonomers (0.5 mM) and ICG (1 mg/ mL) were induced to construct ICG-NCs by enzyme-catalysis in 40 minutes. The unloaded ICG was removed by dialysis (Molecular Weight Cut-off: 1000).

Preparation of DOX/ICG-NCs

Supramonomers (0.5 mM) in the presence of DOX (1 mg/ mL) and ICG (1 mg/ mL) were induced by enzyme-catalysis to produce DOX/ICG-NCs in 40 minutes. The unloaded DOX and ICG were removed by dialysis (Molecular Weight Cut-off: 1000).

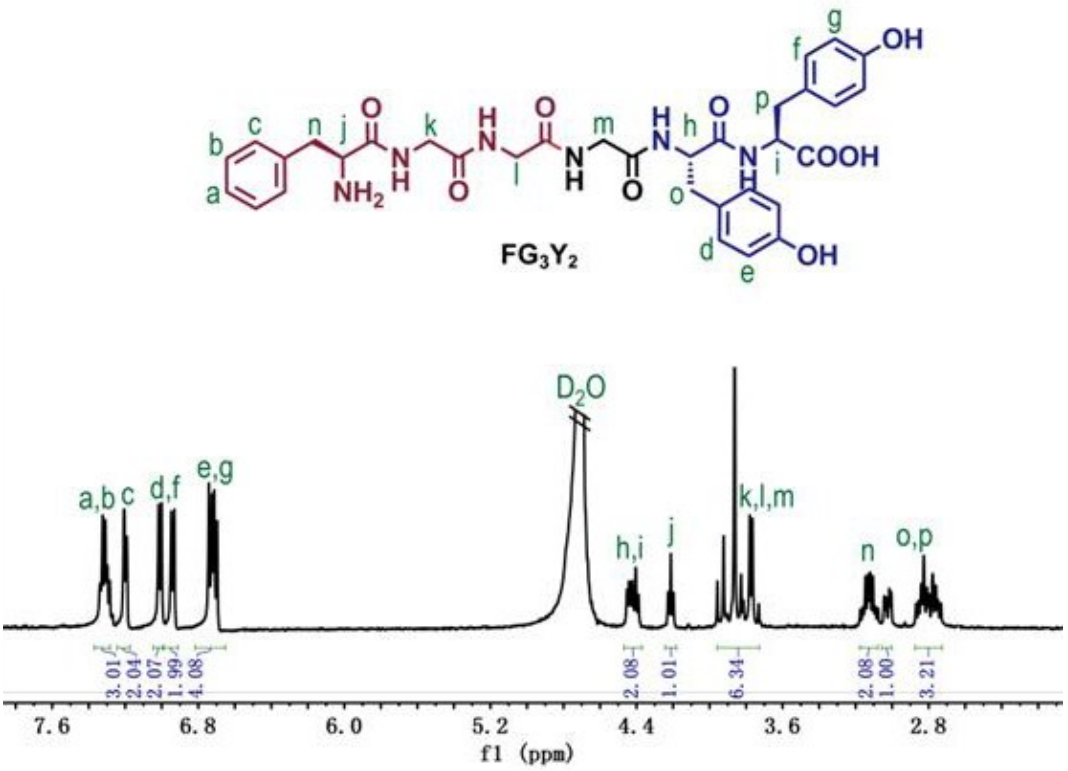

Fig. S1 ^{1}H NMR spectrum recorded for FG_3Y_2 in D2O.

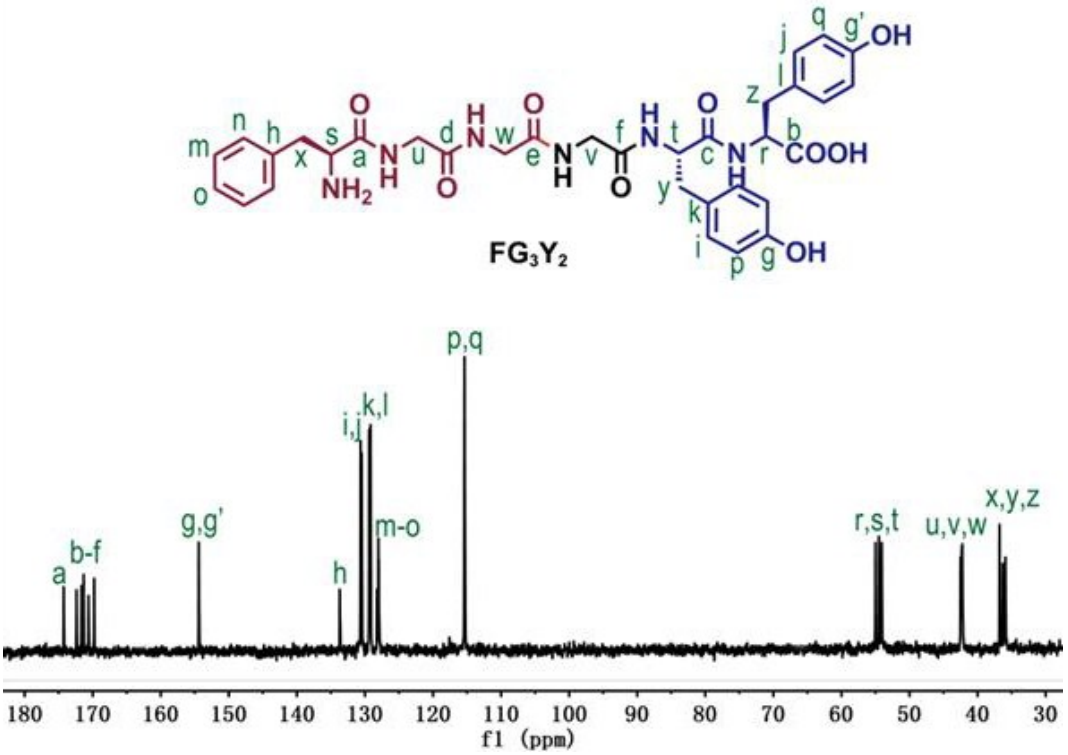

Fig. S2 ^{13}C NMR spectrum recorded for FG_3Y_2 in D_2O.

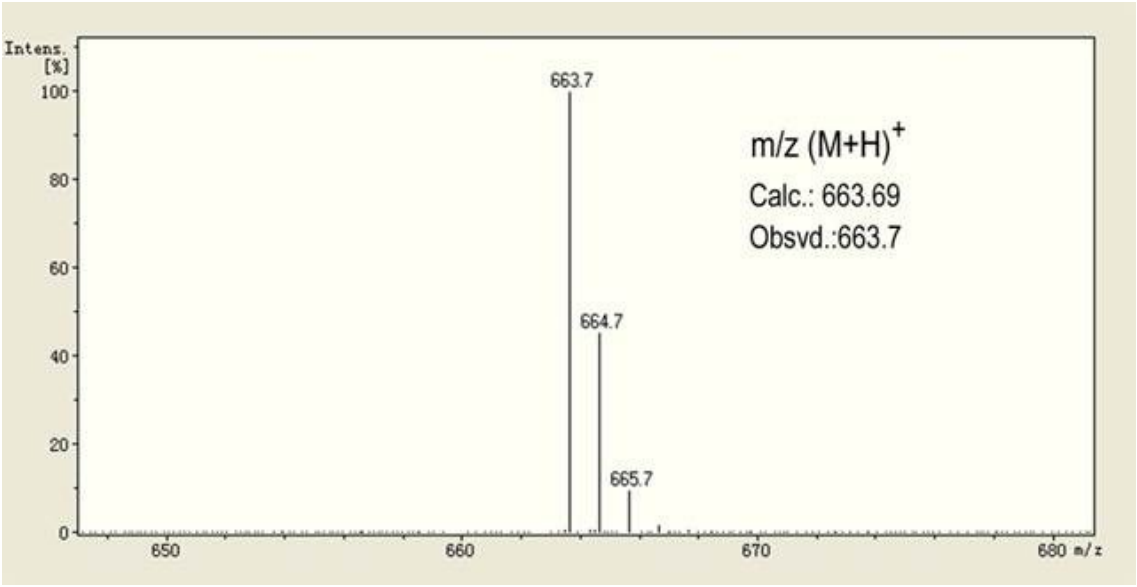

Fig. S3 ESI-MS spectrum recorded for FG_3Y_2.

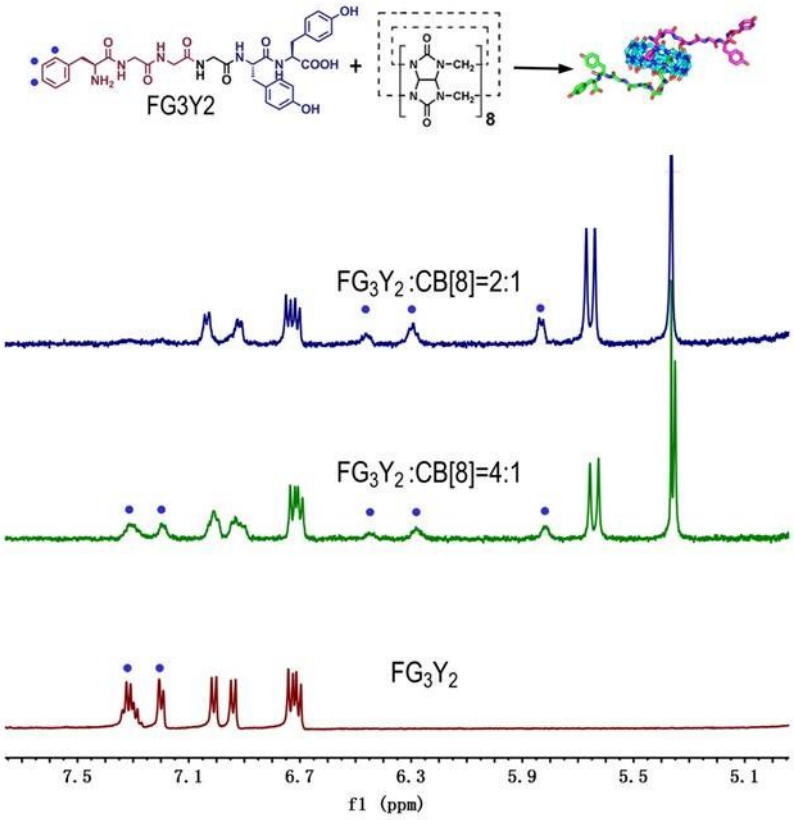

Fig. S4 Partial ^{1}H NMR spectra of FG_3Y_2 and the mixture of FG_3Y_2 with CB[8] at different molar ratio.

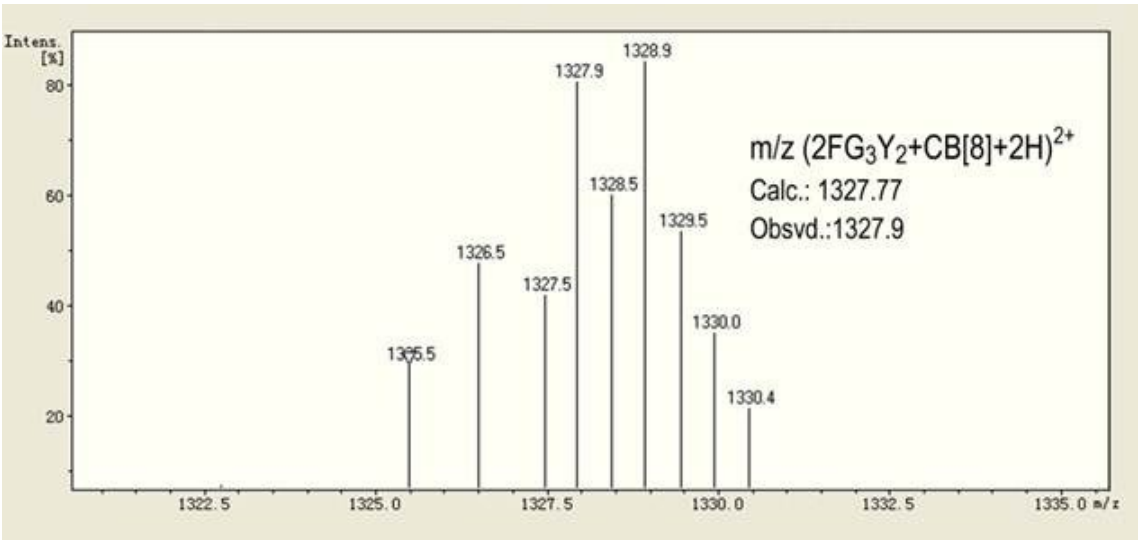

Fig. S5 ESI-MS spectrum of the supramonomer prepared by the complexation of FG_3Y_2 and CB[8] in a molar ratio of 2:1 in aqueous solution.

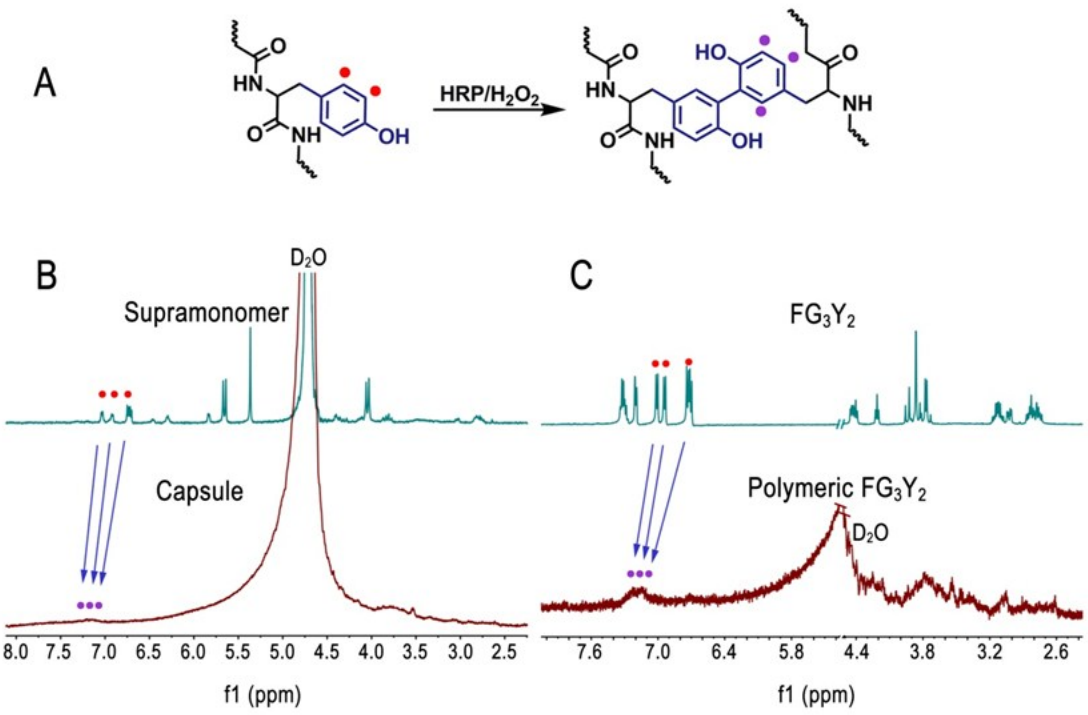

Fig. S6 (A) HRP-catalyzed tyrosing-tyrosing crosslinking through the C-C bond formation in the presence of H_2O_2, (B) Partial ^{1}H NMR spectra of the supramonomer and the formed capsule under the catalysis of HRP, (C) Partial ^{1}H NMR spectra of FG_3Y_2 and the polymeric product under the catalysis of HRP.

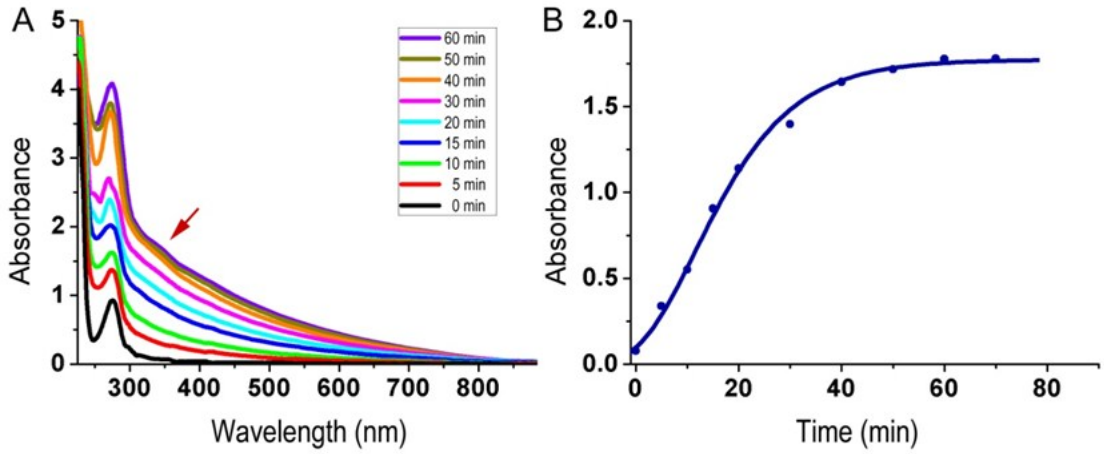

Fig. S7 (A) UV-vis absorption spectra of the supramonomers before and after the addition of HRP and H_2O_2 to trigger the polymerization, (B) Plot of the UV-vis absorbance versus reaction time during the polymerization process. The curve was drawn according to panel A.

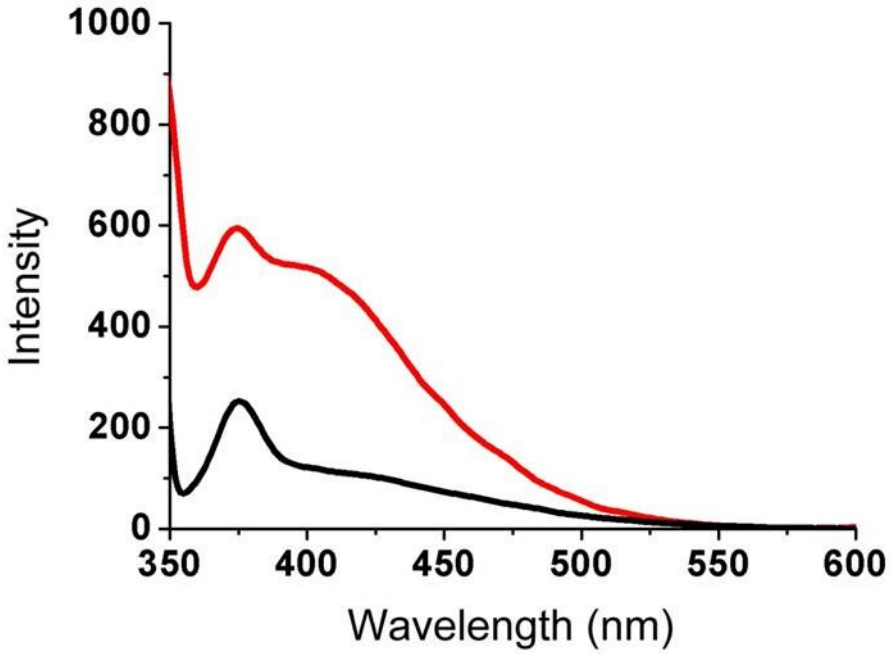

Fig. S8 Fluorescence emission spectra of the supramonomers and the formed capsules from them.

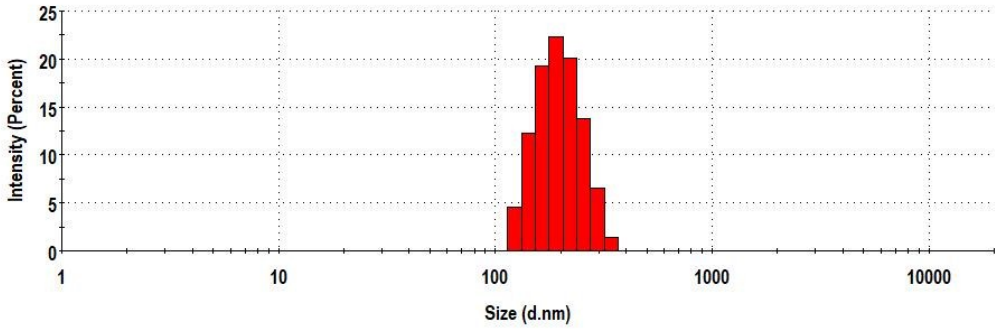

Fig. S9 Hydrodynamic size of the supramolecular capsules measured by DLS.

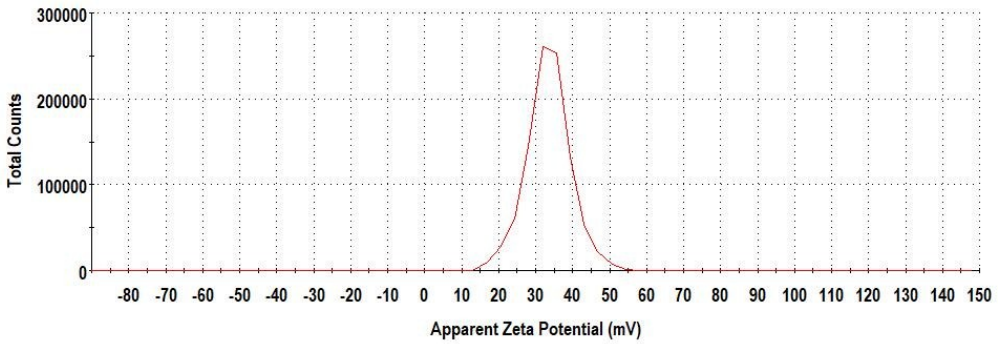

Fig. S10 Apparent zeta potential of the supramolecular capsules measured by DLS.

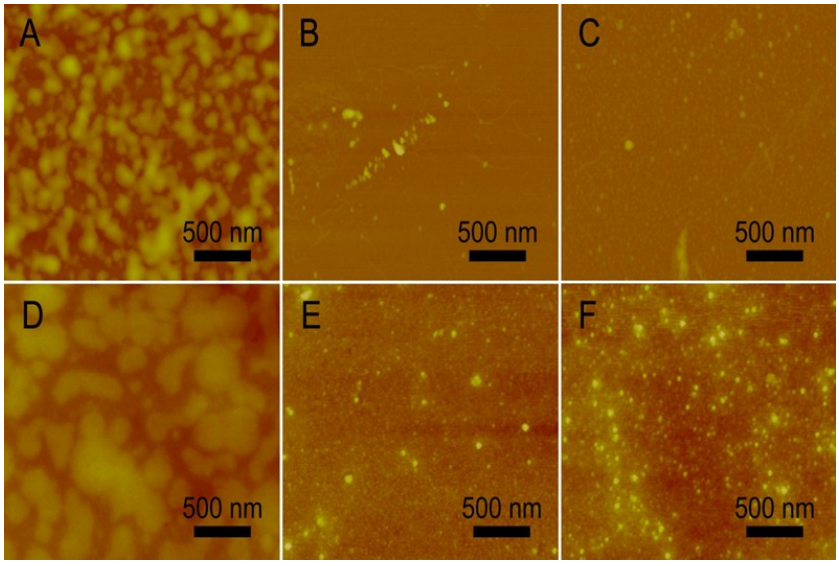

Fig. S11 AFM images of the control samples. (A) supramonomer, (B) supramonomer and HRP, (C) supramonomer and H_2O_2, (D) FG_3Y_2, HRP and H_2O_2, (E) HRP, (F) HRP and H_2O_2.

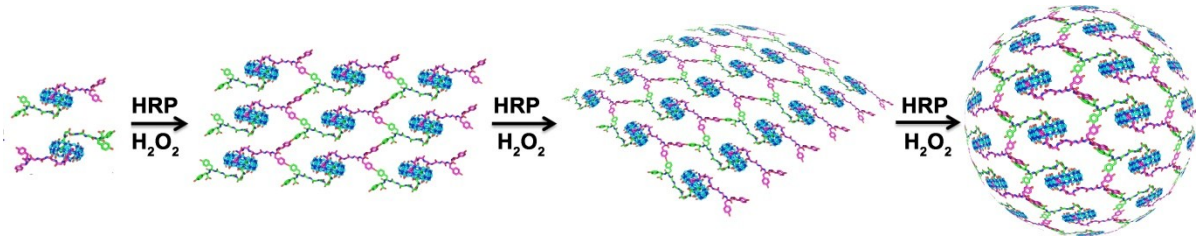

Fig. S12 Schematic illustration of the formation of nanocapsules.

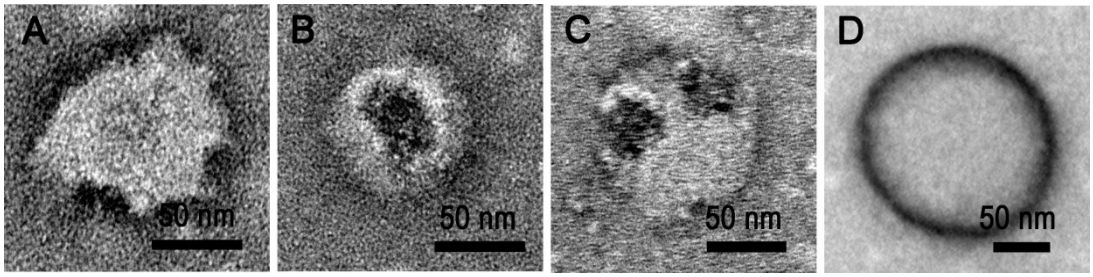

Fig. S13 TEM images at each stage of the polymerization of the nanocapsules. (A), (B), (C) and (D) at 5min, 10min, 20min and 40min. The samples were negatively stained with phosphotungstic acid.

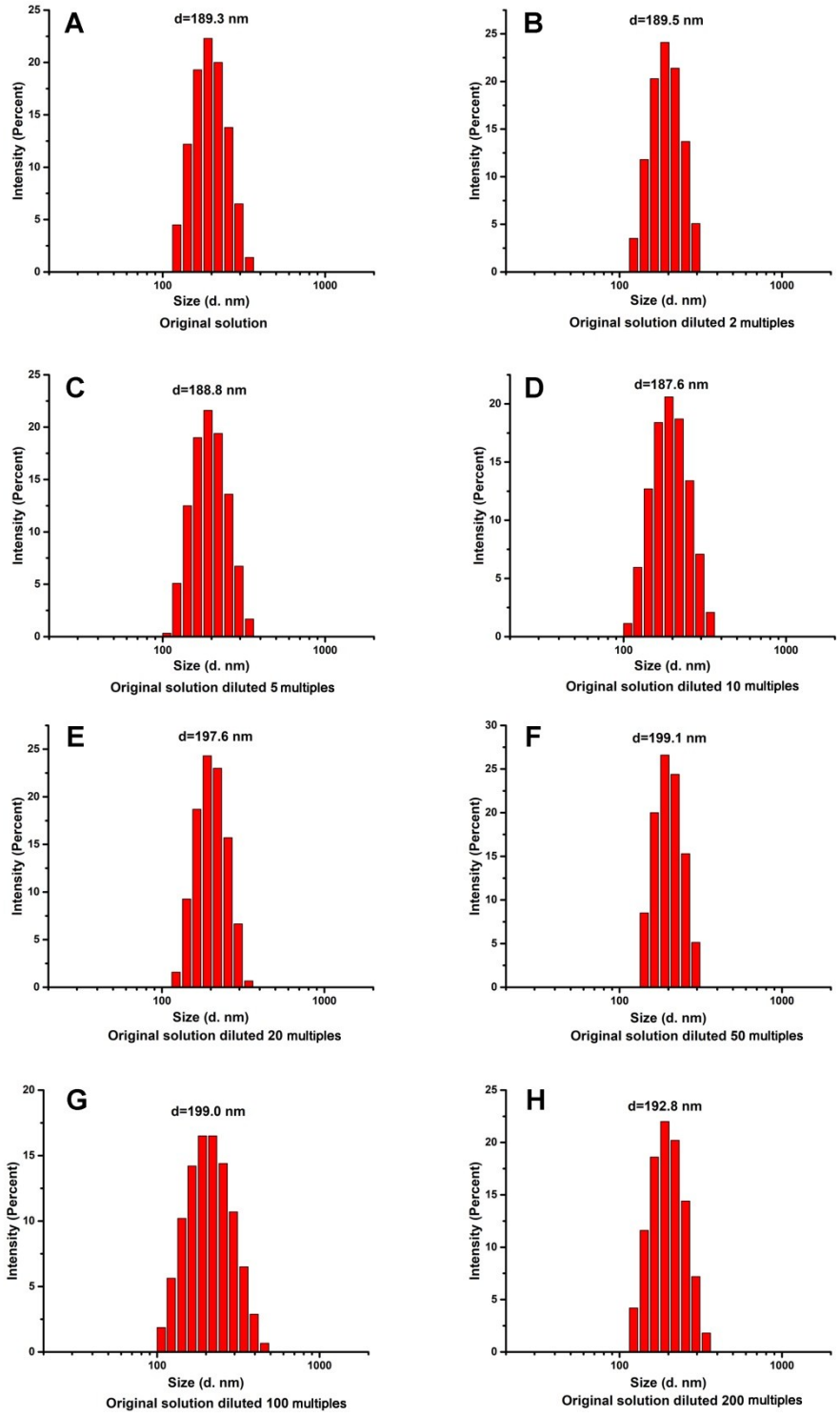

Fig. S14 Hydrodynamic sizes of the supramolecular capsules measured by DLS. (A) original solution, (B), (C), (D), (E), (F), (G) and (H), original solution diluted 2 multiples, 5 multiples, 10 multiples, 20 multiples, 50 multiples, 100 multiples and 200 multiples.

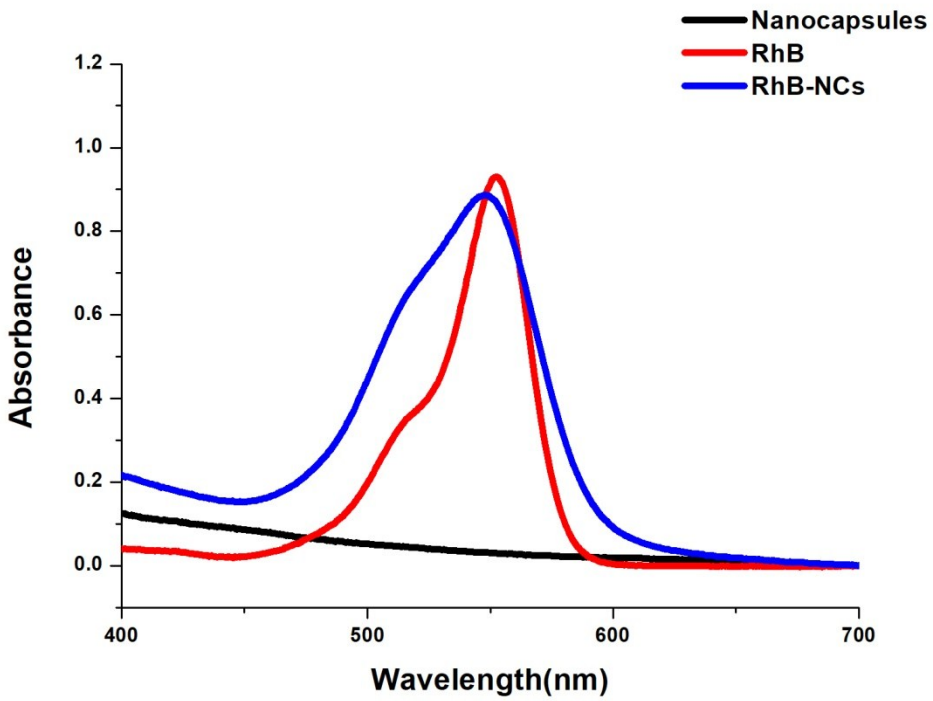

Fig. S15 UV-vis absorption spectra of nanocapsules, RhB and RhB-NCs.

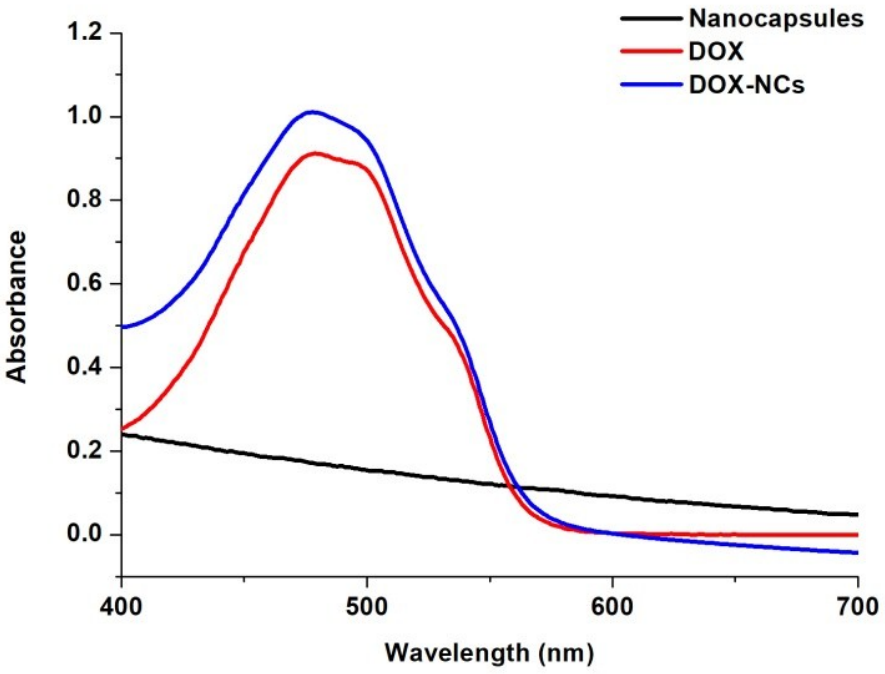

Fig. S16 UV-vis absorption spectra of nanocapsules, DOX and DOX-NCs.

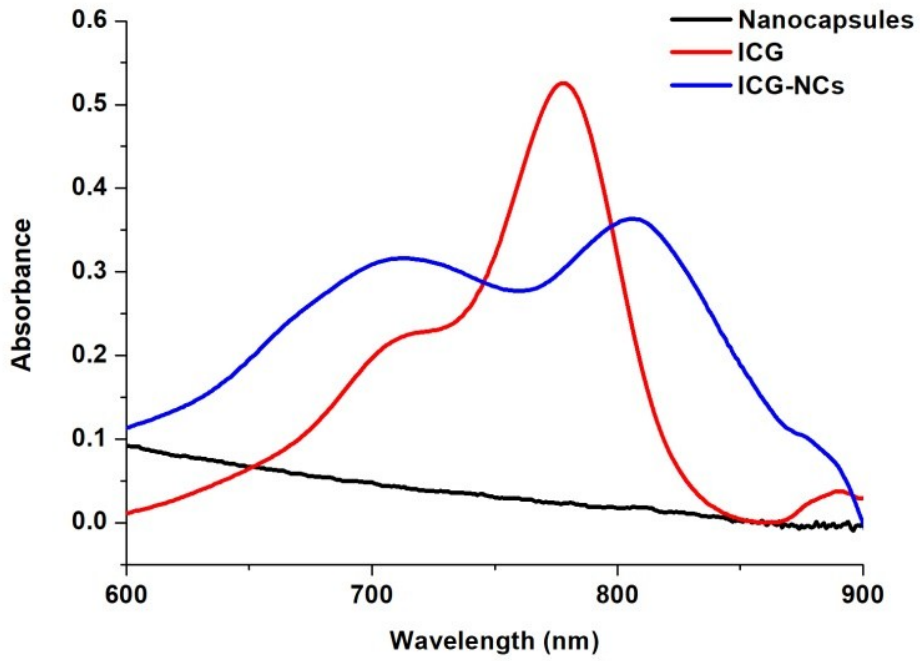

Fig. S17 UV-vis absorption spectra of nanocapsules, ICG and ICG-NCs.

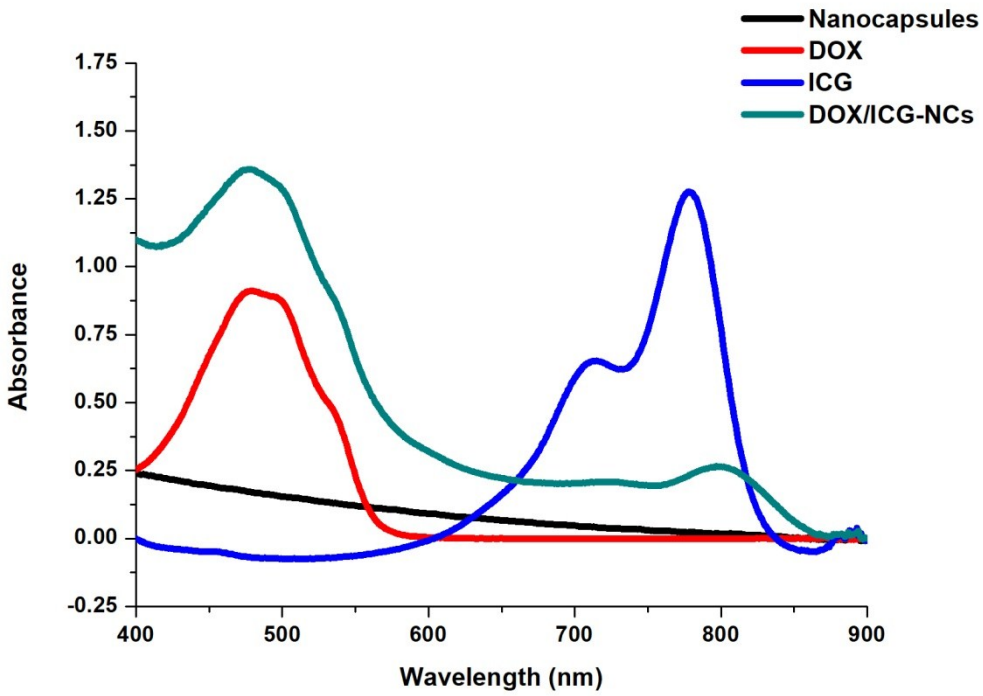

Fig. S18 UV-vis absorption spectra of nanocapsules, DOX, ICG and DOX/ICG-NCs.

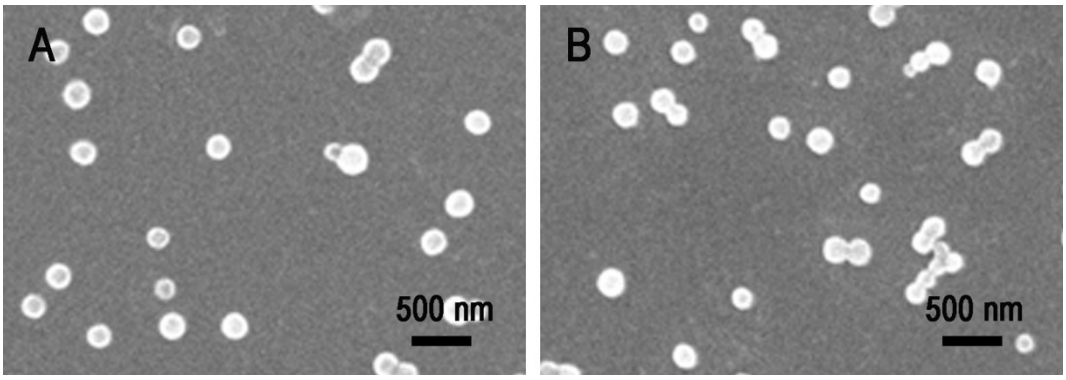

Fig. S19 SEM images of (A) RhB-NCs and (B) ICG/DOX-NCs.

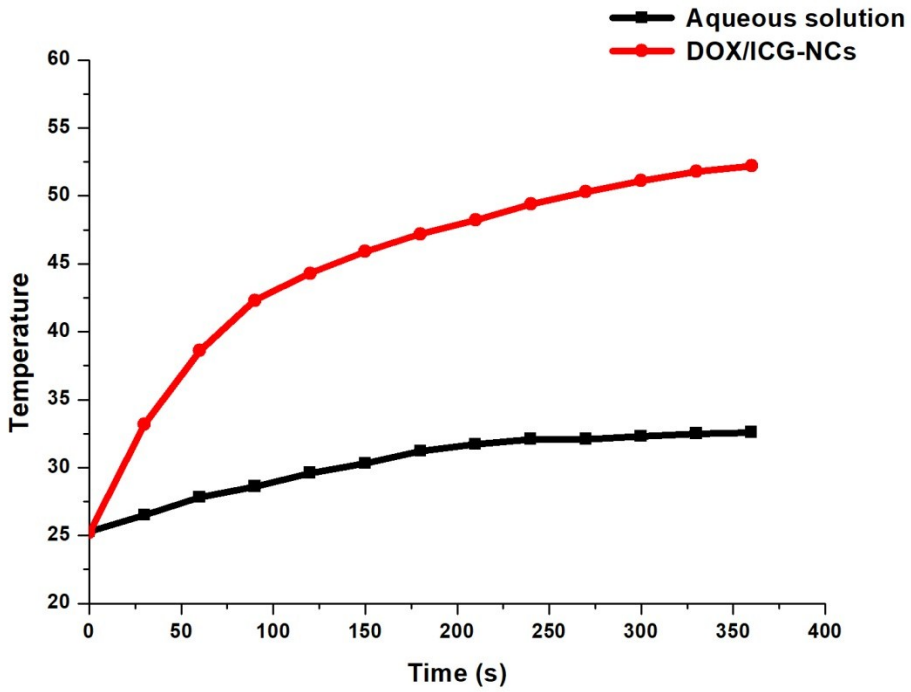

Fig. S20 Photothermal effect of free aqueous solution and ICG/DOX-NCs under near-infrared irradiation of 808 nm laser (1 W/ cm^2).

References

1. Z. H., Y. Fang, Q. Luo, S. Liu, G. An, C. Hou, C. Lang, J. Xu, Z. Dong and J. Liu, *Chem. Commun.*, 2016, **52**, 2083—2086.