Rationally Engineering Dual Missions in One Statistical Copolymer Nanocapsule: Bacterial Inhibition and Polycyclic Aromatic Hydrocarbon Capturing

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Experimental Section

Materials. Glycidyl methacrylate (GMA), 1-naphthylamine, bismuth(III) chloride, 2-(*tert*-butylamino)ethyl methacrylate (TA) and 2,2'-azobis(2-methylpropionitrile) (AIBN) were purchased from Aladdin Chemistry, Co. Anhydrous magnesium sulfate ($MgSO_4$), sodium bicarbonate ($NaHCO_3$), dichloromethane, dimethylformamide (DMF), and other solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China). The GMA monomer was passed through an alumina B column to remove the inhibitor before use. Dialysis

tube (8-14 kDa molecular weight cutoff) was supplied by Shanghai Genestar Bio-Technology Co., Ltd. $CDCl_3$ and DMSO-d_6 were purchased from J&K Scientific Ltd. Before use, AIBN was recrystallized from methanol and stored at 25 ^oC. DMF was dried using calcium hydride and distilled under reduced pressure. LB Agar and LB broth were purchase from Aladdin and used as received. Gram-negative bacterium *E. coli* (ATCC35218) and Gram-positive bacterium *S. areus* (ATCC29213) were purchased from Nanjing bianzhen biological technology Co., Ltd. The chain transfer agent (CTA), 4-cyanopentanoic acid dithiobenzoate (CPAD), was synthesized according to previous report.[1] Other chemicals were used without further purification unless otherwise specified.

Characterization. Waters Gel Permeation Chromatography with a refractive index signal detector (Waters 2414) was used to analyze the molecular weight and polydispersity of the copolymer with HPLC grade DMF as the eluent at a flow rate of 1.0 mL/min.

^1H NMR. ^1H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer at room temperature using $CDCl_3$ or DMSO-d_6 as the solvent.

DLS. The nanocapsule solutions were pulled into a standard cuvette and then tested in the ZETASIZER Nano series instrument (Malvern Instruments) to get the hydrodynamic diameters.

Zeta Potential. Zeta potential (ξ) studies were conducted at 25 ^oC using a ZETASIZER Nano series instrument (Malvern Instruments). It was measured at pH 7.0 (just after dialysis of nanocapsule without pH tuning).

Atomic Force Microscopy (AFM). AFM was employed to verify the hollow structure by height contrast. 10 μL of nanocapsule solution was dropped onto the silicon wafer, which was then

washed four times before sample preparation. The observation was conducted on a Seiko (SPA-300HV) instrument operating in a tapping mode at 200 – 400 kHz drive frequency.

Transmission Electron Microscopy (TEM). TEM images are obtained in a JEM-2100 electron microscope equipped with a Gatan 1k - 1k digital camera operating at an acceleration voltage of 200 kV. All nanocapsule solutions are diluted before dropped on the surface of amorphous carbon at ambient temperature. Then 10 μL of phosphotungstic acid solution (PTA; 2.0 w/w %, tuned to neutral pH using 1.0 M NaOH solution) was used to stain the samples for 1 min.

Scanning Electron Microscopy (SEM). SEM was utilized to observe the morphologies of the nanocapsules. To obtain SEM images, a drop of solution was spread on a silicon wafer and left until dryness. It was coated with platinum and viewed by a FEI Quanta 200 FEG electron microscopy operated at 15 kV. The images were recorded by a digital camera.

Fluorescence Spectroscopy. Fluorescent experiments were used in the fluorescence quenching test to evaluate the PAH-capturing capacity of the nanocapsules via a Lumina Fluorescence Spectrometer (ThermoFisher).

UV-Vis Spectroscopy. The UV-vis spectra were used to measure the optical density (OD) of the mixed solution in the antibacterial test via a UV759S UV-vis spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd.).

Synthetic Methods. *Synthesis of 2-Hydroxy-3-(naphthalen-1-ylamino)propyl Methacrylate (HNA) Monomer*. This monomer was synthesized and characterized according to our previous method.[2]

Synthesis of P(HNA_{23}-stat-TA_{20}) Statistical Copolymer by RAFT. A typical run was as following: AIBN radical initiator (2.6×10^{-3} g, 1.61×10^{-2} mmol), CPAD (3.0×10^{-2} g, 1.07×10^{-1} mmol), HNA (7.66×10^{-1} g, 2.7 mmol), TA (4.97×10^{-2} g, 2.7 mmol) and DMF (1.00 mL)

was added in a 25 mL flask with a stirrer bar. Then Ar was continuously purged for 30 min to remove air in the flask before immerged into the oil bath at 70 ^{o}C. After 48 h, the reaction was terminated by cooling down and exposure to air. After removing the DMF solvent by rotatory evaporation, the crude product was dissolved into dichloromethane and followed by precipitation in diethyl ether.

Self-Assembly of P(HNA$_{23}$-stat-TA$_{20}$) Statistical Copolymer into Nanocapsule. Typically, the P(HNA$_{23}$-*stat*-TA$_{20}$) statistical copolymer was dissolved in DMF at an initial concentration of 2.0 mg/mL. Water was then added dropwise to a volume ratio of 2:1 (water/DMF). The resultant nanocapsules were then purified by dialysis.

Antibacterial Test. The antibacterial test was performed according to our previously reported protocol.[3] Typically, the nanocapsule solution of different concentrations was placed into each cuvette with microorganism solution. Then optical density (OD) at 600 nm wavelength of visible light was measured at intervals using a UV-Vis spectrophotometer. To make the results clearer, the OD value of the standard sample was detracted for each solution. The MIC was recorded at the concentration at which less than half of the bacteria grew compared with the control sample after 23.5 h.

PAH Capturing. The aqueous nanocapsule solution (1.0 mL) of various concentrations was added in the quartz cuvette with 1.5 mL of deionized water. Then the fluorescence quenching process was recorded via fluorescence spectroscopy in the range of 350 ~ 500 nm (λ_{ex} = 335 nm). The intensity signals of the standard nanocapsule samples of different concentrations were detracted for each solution to minimize the disturbance. A calibration curve (Figure S8) was used to determine the final concentration.

Removability Test. *E. coli* (1.0 mL) in 0.7% Saline, aqueous solution of pyrene (C_{pyrene} = 137 ppb, 0.5 mL) and nanocapsule solution ($C_{nanocapsule}$ = 437 μg/mL, 0.5 mL) were added into a standard cuvette followed by the detection of UV-vis spectroscopy (in the range of 580 ~ 680 nm) and fluorescence spectroscopy (λ_{ex} = 335 nm, in the range of 350 ~ 500 nm) respectively before and after the filtration by a syringe filter membrane ($D_{pore\ ave} \approx$ 220 nm).

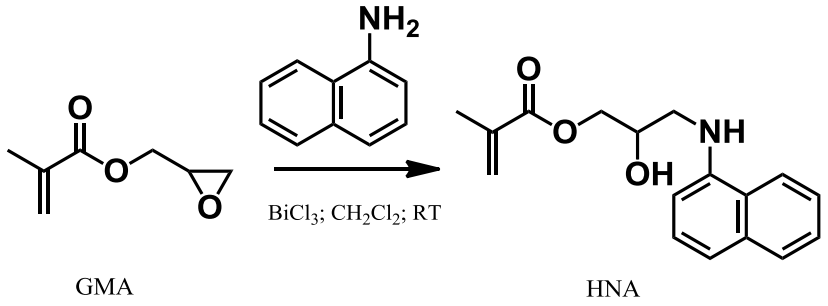

Figure S1. Synthesis of HNA monomer.

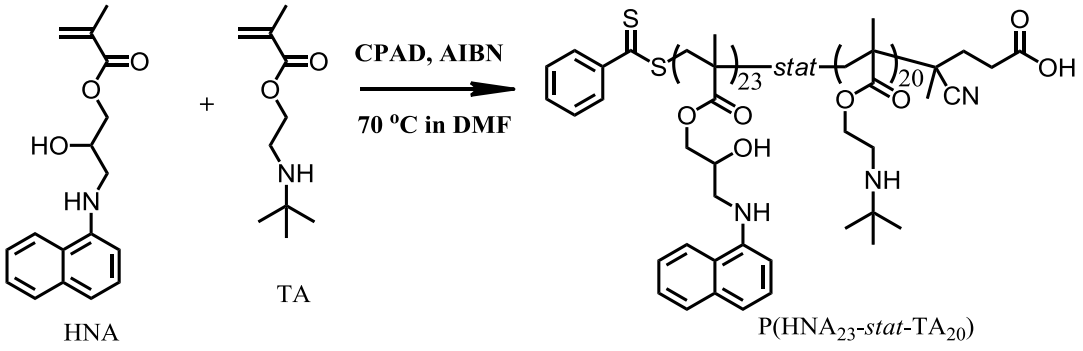

Figure S2. RAFT synthesis of P(HNA_{23}-*stat*-TA_{20}) statistical copolymer.

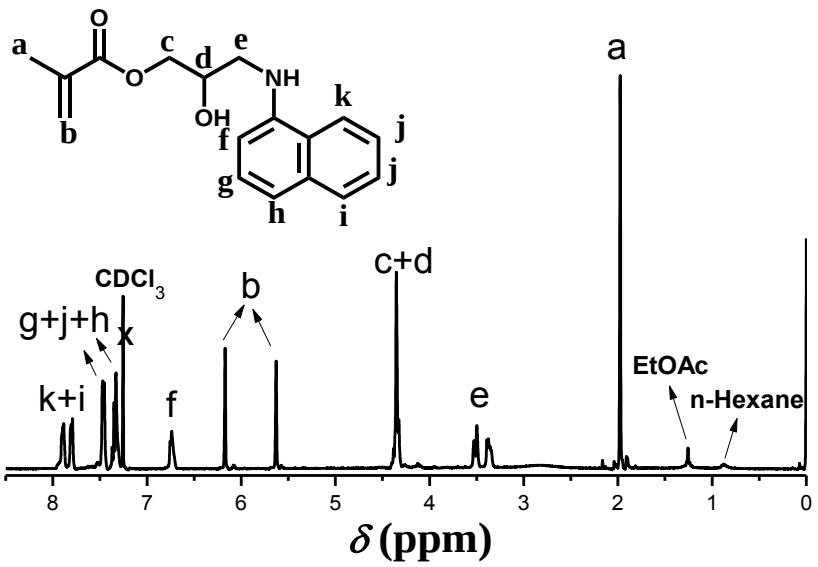

Figure S3. ^{1}H NMR spectrum of HNA in $CDCl_3$.

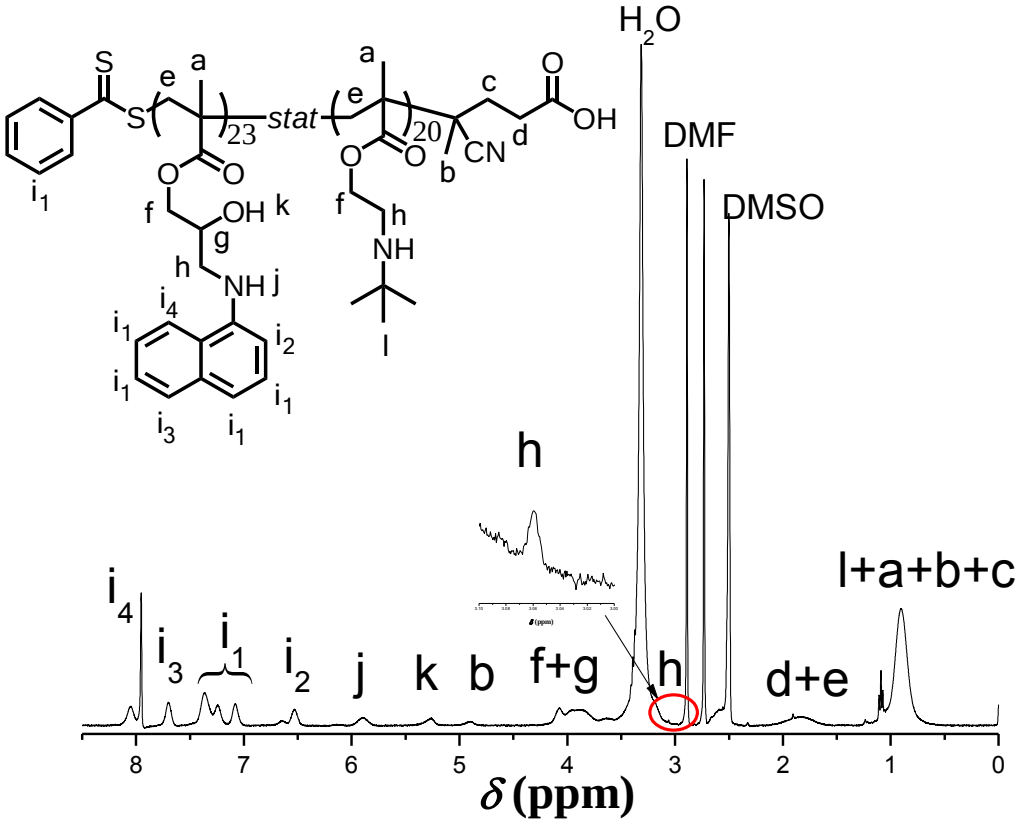

Figure S4. ^{1}H NMR spectrum of P(HNA$_{23}$-*stat*-TA$_{20}$) statistical copolymer in DMSO-d_6.

Calculation of the degree of polymerization of the statistical copolymer

Table S1. The integral areas of different peaks and the degree of polymerization (DP) of HNA (x) and TA (y) in the copolymer [†]

polymer	A_{i2}	A_{i1}	$A_{l+a+b+c}$	x	y
P(HNA$_x$-*stat*-TA$_y$)	S	4.22S	13.82S	23	20

[†] In Table S1, A_{i2} is the integral area of peak i_2 (PHNA, x); A_{i1} is the integral area of peak i_1 (PHNA and the benzene ring of the end group, $4x + 5$); $A_{l+a+b+c}$ is the integral area of peaks l, a, b and c [PHNA, PTA and the end group of CPAD, $9y + 3(x + y) + 2 + 3$] in Figure S4.

The integral area of peak i_2 was set to be S (*e.g.*, S = x for simplifying the calculation). The integral area of peak i is listed in Table S1. Since peak i_2 represents the HNA unit only and peak i_1 comes from both HNA unit and the end group of CPAD, the degree of polymerization of HNA (x) was determined as following:

$$A_{i_2} = 4x + 5 = 4.22\text{S}$$

$$A_{i_1} = x = \text{S}$$

Therefore, x is 22.73 ($\approx$ 23).

Since the integral area $A_{l+a+b+c}$ corresponds to the HNA unit, TA unit and the end group of CPAD, the degree of polymerization of TA (y) was determined as following:

$$A_{l+a+b+c} = 9y + 3(x + y) + 2 + 3 = 13.82\text{S}$$

Therefore, y is 20.08 ($\approx$ 20).

The molecular weight of P(HNA$_{23}$-*stat*-TA$_{20}$) is 10500 Da.

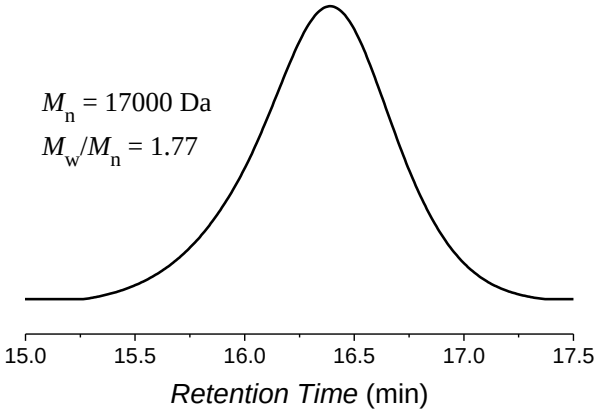

Figure S5. THF GPC trace of P(HNA_{23}-*stat*-TA_{20}) copolymer.

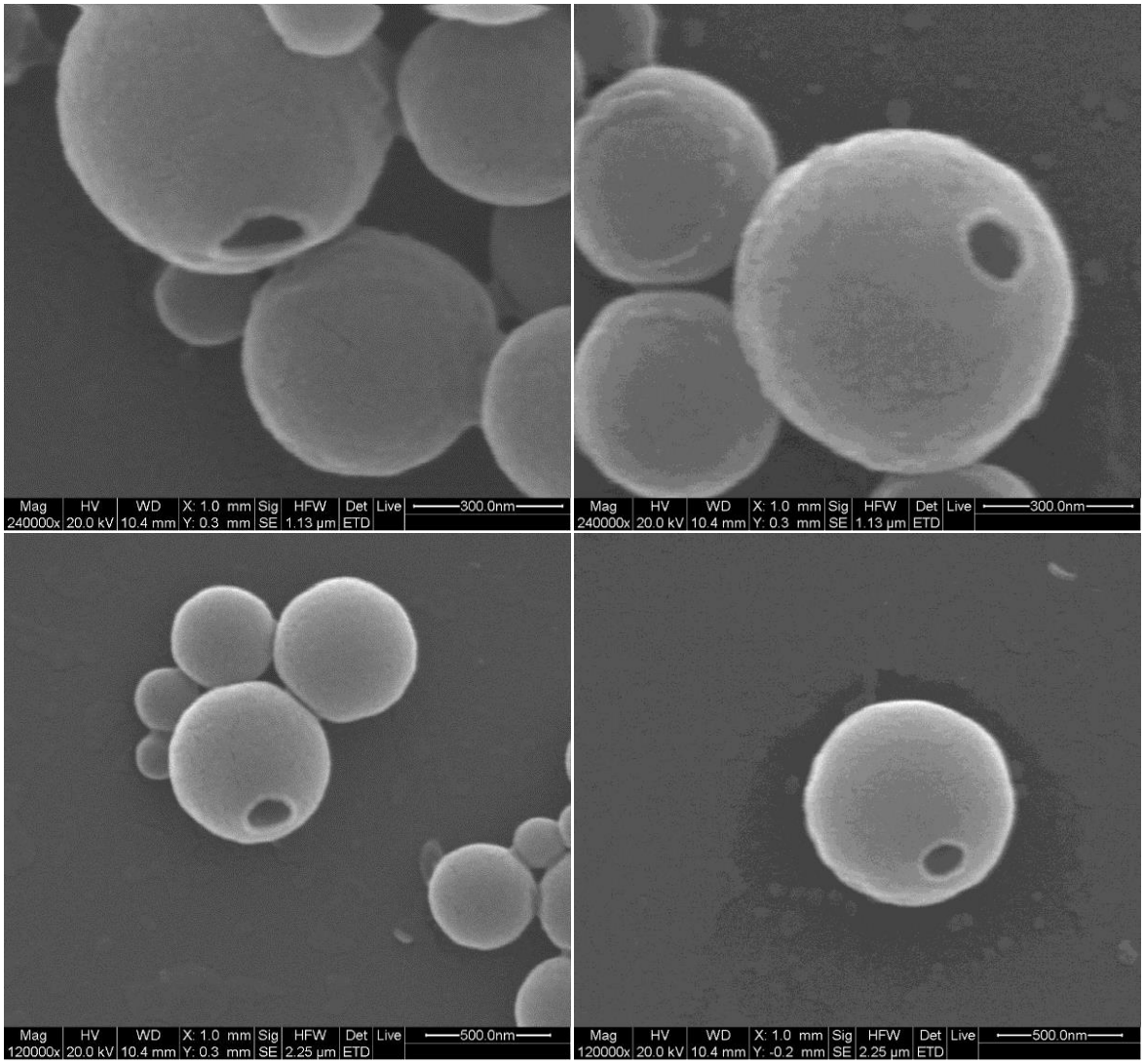

Figure S6. More SEM images of nanocapsules with open-mouth holes.

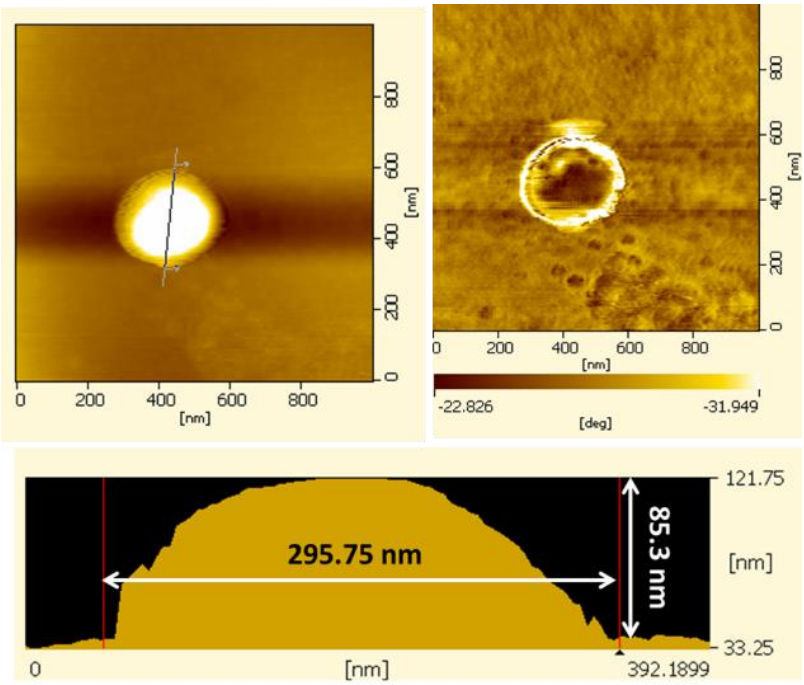

Figure S7. The typical AFM image of a nanocapsule. The height to diameter ratio is 1/3.5, indicating a hollow structure.

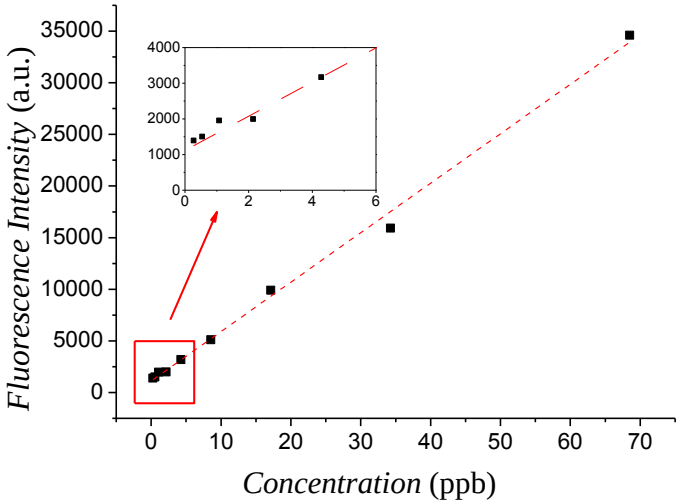

Figure S8. The correlated calibration curve of aqueous pyrene solution at 374 nm.

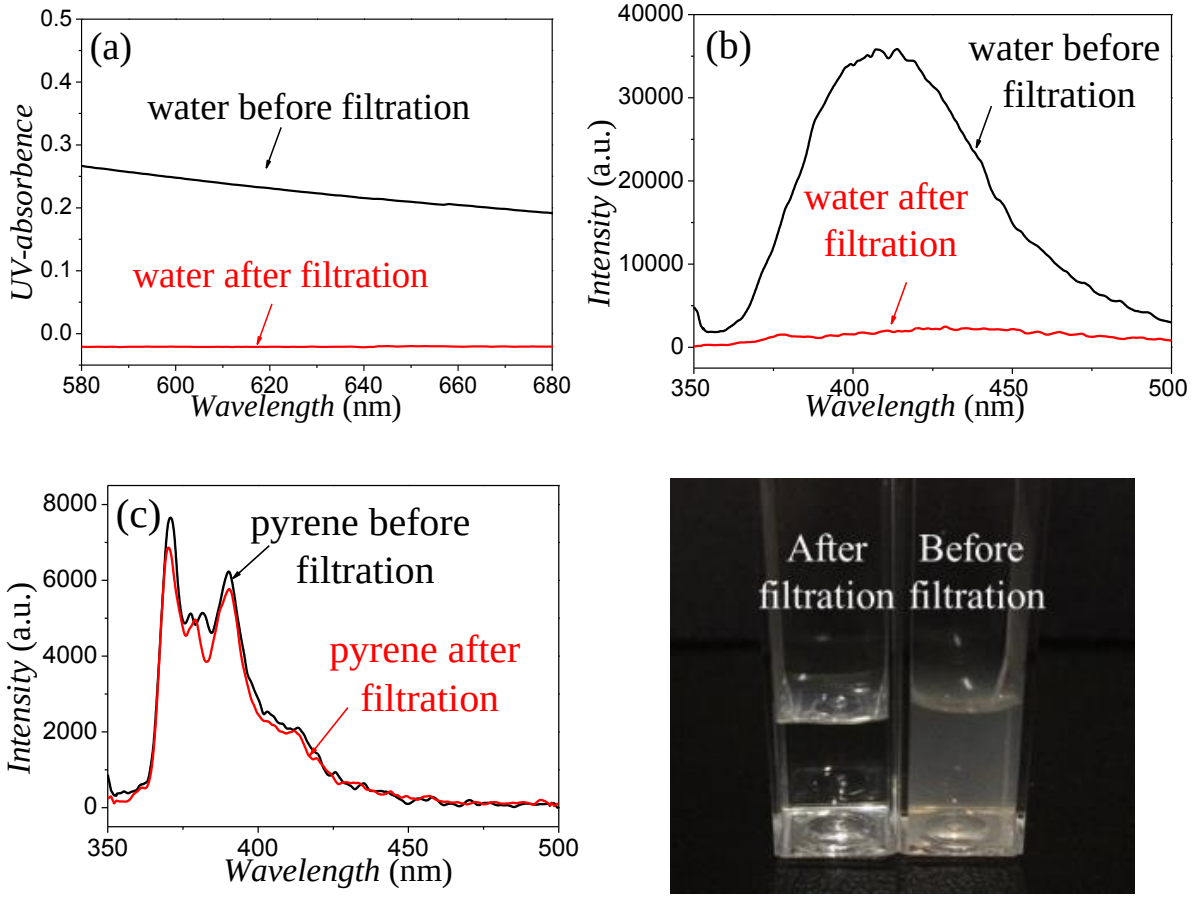

Figure S9. Separation of pyrene-adsorbed nanocapsules and bacteria from water. (a) UV-vis spectroscopy and (b-c) fluorescence spectroscopy (λ_{ex} = 335 nm) before and after the filtration by a filter membrane ($D_{pore} \approx$ 220 nm). (d) Digital photo of the mixture solution. Sample: (a), (b) and (d): 1.00 mL of *E. coli* in 0.7% saline, 0.5 mL of aqueous pyrene (137 ppb) and 0.5 mL of nanocapsules solution (437 μg/mL). (c) Control: aqueous pyrene solution (34.25 ppb).

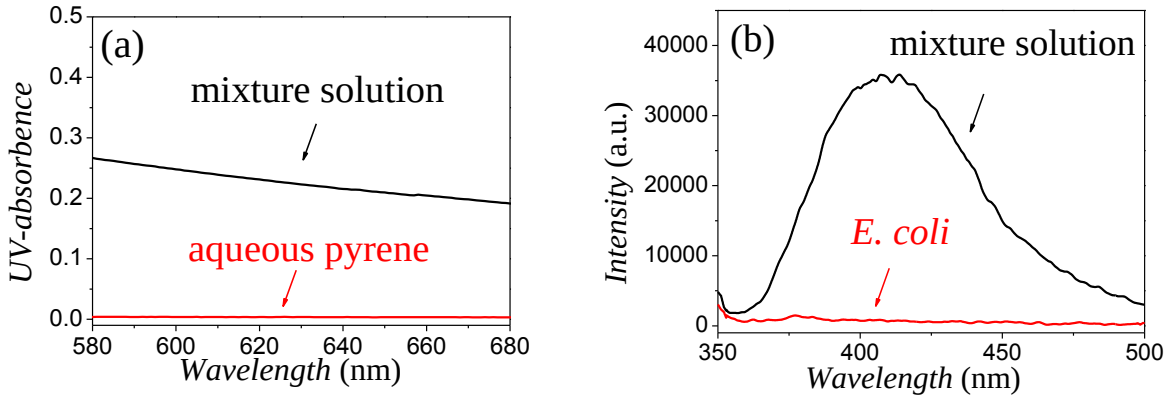

Figure S10. Comparison of the control samples with the mixture solution. (a) UV-vis spectroscopy of the mixture solution and the control sample of aqueous pyrene solution. The UV absorbance of aqueous pyrene is very little compared with the mixture solution. (b) Fluorescence spectroscopy of the mixture solution and the control sample of *E. coli*. The intensity of *E. coli* is very little compared with the mixture solution. Sample: mixture solution: 1.00 mL of *E. coli* in 0.7% saline, 0.5 mL of aqueous pyrene solution (137 ppb) and 0.5 mL of nanocapsules solution (437 μg/mL); aqueous pyrene: 2.00 mL of aqueous pyrene solution (34.25 ppb); *E. coli*: 1.00 mL of *E. coli* in 0.7% saline and 1.00 mL deionized water.

REFERENCES

(1) Oliveira, M. A. M.; Boyer, C.; Nele, M.; Pinto, J. C.; Zetterlund, P. B.; Davis, T. P. *Macromolecules* **2011**, *44*, 7167-7175.

(2) Zhu, Y. Q.; Fan, L.; Yang, B.; Du, J. Z. *ACS Nano* **2014**, *8*, 5022-5031.

(3) Zhou, C.; Wang, M.; Zou, K.; Chen, J.; Zhu, Y.; Du, J. Z. *ACS Macro Lett.* **2013**, *2*, 1021-1025.