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Supporting Information

Water-triggered Self-assembly Polycondensation for the One-Pot Synthesis of Cyclomatrix Polyphosphazene Nanoparticles from Amino Acid Ester

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ToC:

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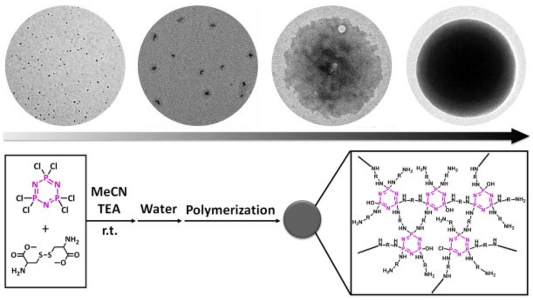

Experimental Section

Materials. HCCP (Adamas-beta) was recrystallized from dry hexane followed by sublimation (about −0.1 MPa) carried out twice. The melting point of the purified HCCP was 113-114°C. L-cystine methyl ester dihydrochloride (CysM·2HCl, GL Biochem Ltd.), triethylamine (TEA, Aldrich), acetonitrile (HPLC pure, Shanghai Chemical Reagents Corp.), and ethanol (Shanghai Chemical Reagents Corp.) were used without further purification. Hexane was distilled from P_2O_5. Water used in the experiments was purified to a resistivity higher than 18.2 MΩ·cm using a Hitech system.

Equipments. Liquid-state ^{31}P NMR spectra were recorded on a Varian Mercury Plus-400 nuclear magnetic resonance spectrometer (400 MHz) with 85% phosphoric acid as external reference. Solid-state ^{31}P NMR spectra were recorded on the same machine by an one-pulse sequence with the high-power DD technique. The proton $\pi/2$ pulse duration was 5 μs, and 2000 signal transients with a 100 s relaxation delay were accumulated. Magic-angle spinning was set at 10 kHz in order to ensure complete separation of side-band intensity from the central transition. Monopotassium phosphate was used as external reference (δ=0.01 ppm). Fourier-transform infrared (FTIR) spectra were recorded on a Paragon 1000 (Perkin-Elmer) spectrometer. Samples were dried overnight at 45 °C under vacuum and thoroughly mixed and crushed with KBr to fabricate KBr pellets. Molecular weight and polydispersity were estimated by using a Waters 1515-2414 (Waters, USA) gel permeation chromatograph (GPC) at 30 °C equipped with three linear mixed-B columns (Polymer Lab Corporation; pore size: 10 μm, column size: 300 × 7.5 mm) and a refractive index detector. DMF (0.01 mol/L LiBr) and polystyrene were used as the eluent (elution rate: 1.0 mL/min) and calibration standard, respectively. Scanning electron microscope (SEM) images were taken by using an FEI Nano 450 at an activation voltage of 800 V, and retarding field of 4000 V. Energy dispersive specrometer (EDS, Apollo X, EDAX Inc.) spectra were taken at the same machine with an operational voltage of 20 KeV. Transmission electron microscopy (TEM) images were taken by using an FEI-Tecnai G2 Spirit Biotwin operated at 120 kV accelerating voltage. Samples were prepared on the surface of 300-mesh Formvar-carbon film-coated copper grids. The size and distribution of all as-prepared particles were determined from SEM micro-graphs using Image J (V1.41, NIH, USA) for image analysis. Visual images were captured by a Canon IXUS 800IS digital camera (Canon, Japan).

DSC was obtained on a Perkin-Elmer DSC-7 (UK) instrument to observe the glass-transition temperature. Thermal degradation of the crosslinked microspheres was examined with a thermogravimetric analyzer (TGA) Perkin Elmer TGA-7 under nitrogen atmosphere. The mean size of nanoparticles was determined by DLS using a Malvern Nano_S instrument (Malvern, UK) at 25 °C. All of the measurements were repeated five times.

Preparation of PN-CysM oligomer solution. All processes were conducted in a dry nitrogen atmosphere at room temperature. HCCP (2.0 g, 5.75 mmol) and CysM·2HCl (6.37 g, 17.25 mmol) were added into a 150 mL round-bottom three-necked flask with 50 mL HPLC pure acetonitrile. After stirring for 30 min, TEA (10 mL) was added dropwise within 30 min. The reaction was then maintained for 2 d. The precipitate (salt triethylamine hydrochloride) was separated by centrifugation at a speed of 10000 rpm. The supernatant liquor was collected and stored in a 100 mL round-bottom flask in a dry nitrogen atmosphere.

Confirmation of the lower critical solubility parameter (LCSP) of the PN-CysM oligomer solution. A PN-CysM oligomer solution with an HCCP concentration of 20 $g \cdot L^{-1}$ was selected for the LCSP study. 3.0 mL of the as-prepared oligomer solution was injected into a 10 mL round-bottom flask. A certain amount of deionized water was then added dropwise (see Table S1). The white solid generated was judged by the naked eye, and the suspension was dropped onto a clean silicon wafer for the SEM study.

Table S1. The amount of deionized water added into the 3.0 mL PN-CysM oligomer solution and the corresponding Hildebrand solubility parameter.

V_{water} (mL)	1.5	2.1	2.7	3.0	3.3	3.6	3.9	4.2	5.1	6.0
δ_m [a]	15.7	16.6	17.2	17.5	17.8	18.1	18.3	18.5	19.0	19.4

[a] The δ_m was calculated according to Eq 1 in S5.

Preparation of regular PN-CysM spheres. A series of PN-CysM oligomer solutions with different concentrations of monomer HCCP (2 $g \cdot L^{-1}$, 5 $g \cdot L^{-1}$, 10 $g \cdot L^{-1}$, 20 $g \cdot L^{-1}$, 40 $g \cdot L^{-1}$) was prepared. 10 mL of each of the PN-CysM oligomer solution was injected into a 50 mL round-bottom flask. 12 mL deionized water was added dropwise to reach the LCSP over 5 min under stirring conditions. The white solid was collected by centrifugation and washed three times with deionized water and alcohol successively.

Particle formation mechanism study. The PN-CysM oligomer solution at a HCCP concentration of 2 g·L^{-1} was selected for the mechanism study. 10 mL of the oligomer solution was injected into a 50 mL round-bottom flask, to which 12 mL deionized water was added dropwise over 5 min. A small amount of the oligomer solution, and the solution triggered by water for 30 min, 1 h, and 2 h, was dropped onto the surface of 300-mesh Formvar-carbon film-coated copper grids for the TEM study, respectively.

Standard curve method to confirm the ratio of CysM section and cyclotriphosphazene section in the PN-CysM particles. 277 mg of CysM·2HCl was dissolved in 3 mL water (0.25 mmol/mL), and marked as Solution A. In addition, 174 mg of HCCP was dissolved in 2 mL alcohol (0.25 mmol/mL), and marked as Solution B. The two solutions were mixed together according to the amount shown in Table S2 and then dried before mixing with 2 g KBr to fabricate KBr pellets for the FT-IR study. The absorption intensity of the P=N stretching vibration peak (1240 cm^{-1}) in the obtained FT-IR spectra was normalized for intuitive description (Fig S1a). The molar ratio of CysM to cyclotriphosphazene was set as the horizontal axis, while the absorption intensity ratio of C=O bond (1746 cm^{-1}) to P=N bond (1240 cm^{-1}) was set as the vertical axis.

Table S2. The amount of Solution A and Solution B used, and the corresponding molar ratio of the CysM group and cyclotriphosphazene group in each mixture.

Solution A (μL)	100	120	133	143	150	156	160	164	167
Solution B (μL)	100	80	67	57	50	44	40	36	33
$\frac{n\,(CysM)}{n\,(cyclotriphosphazene)}$	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0

S1. FT-IR standard curve

For the purpose of understanding the precise ratio of CysM segments and cyclotriphosphazene segments inside the spheres, we established a standard curve (Fig. S1b). The estimated results indicate that there were 3.5 CysM segments on average attached to 1 cyclotriphosphazene segment.

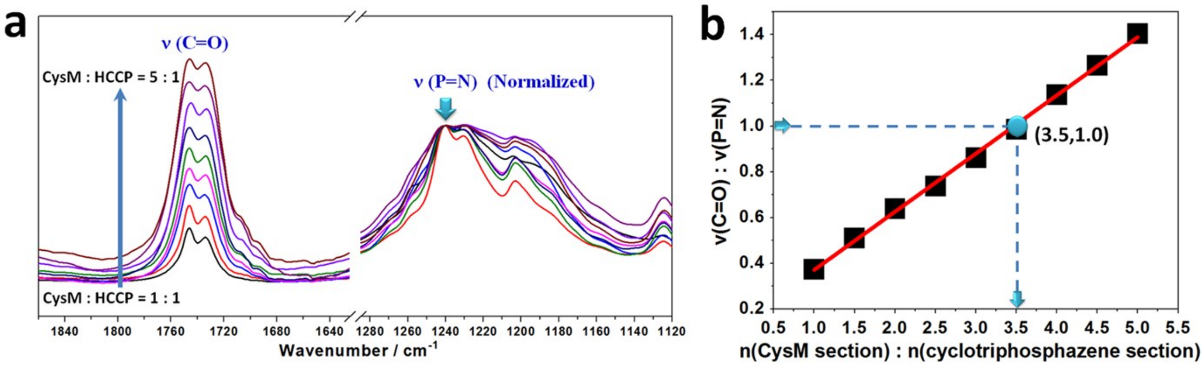

Figure S1. FT-IR standard curve. **(a)** The FT-IR spectra of the mixture with a mole ratio of CysM·2HCl to HCCP from 1.0 to 5.0, with an interval of 0.5. **(b)** The standard curve converted from the former FT-IR spectra.

S2. ^{31}P NMR of the oligomer

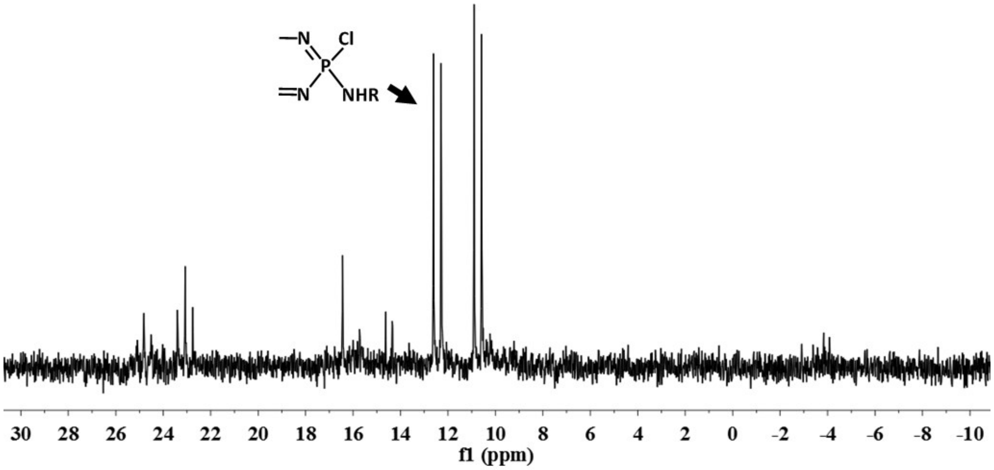

Figure S2. The liquid-state ^{31}P-{^{1}H} NMR of the oligomer ($CDCl_3$). Chemical shifts (δ/ppm) are with reference to external 85% H_3PO_4. This observation is in line with the results obtained for (primary amine)cyclotriphosphazenes.[1]

S3. The elastomer from PN-CysM oligomer and its anti-solvent ability

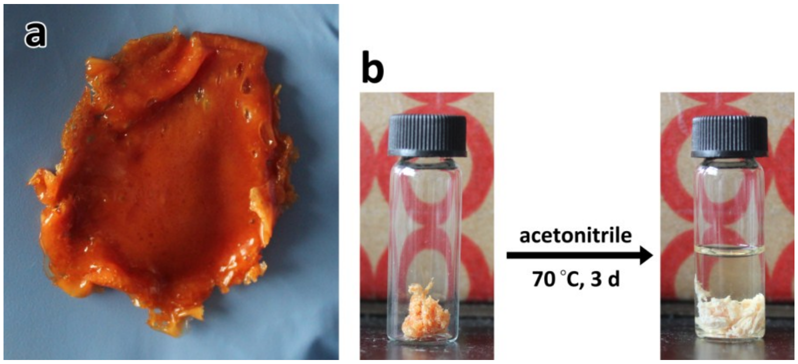

Figure S3. (a) PN-CysM polymer obtained by evaporating the solvent from the oligomer solution. **(b)** Change in the PN-CysM oligomer immersed in acetonitrile for 3 days at 70 ^{o}C.

S4. Solid-state ^{31}P NMR of PN-CysM particles

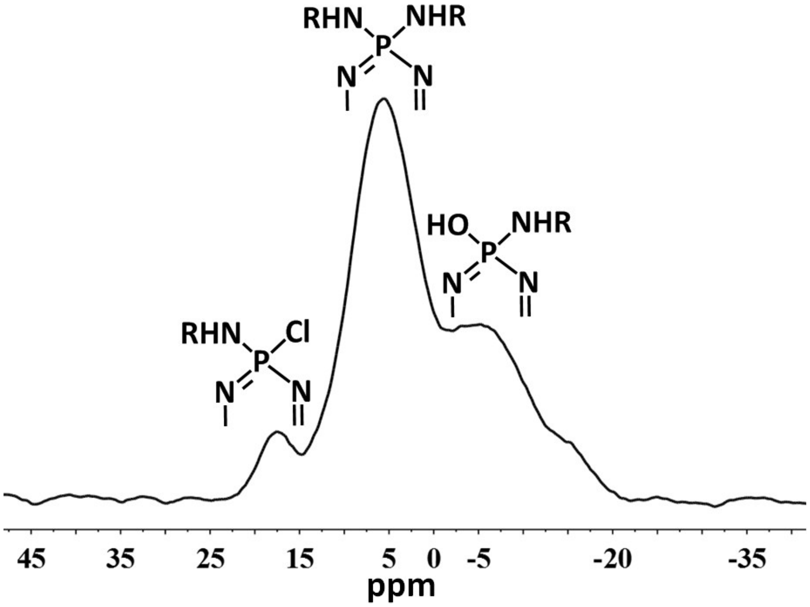

Figure S4. The solid-state ^{31}P-{^{1}H} NMR of PN-CysM particles. Chemical shifts (δ/ppm) are with reference to external KH_2PO_4 which was set to 0 ppm.

S5. Equation for calculating the Hildebrand solubility parameter (δ) of the mixed solution.

The Hildebrand solubility parameter of the mixed solution (δ_M) was calculated according to **Eq 1**, where the δ_1, δ_2 terms are the Hildebrand solubility parameter for acetonitrile (11.9) and water (23.5), respectively. The ϕ_1, ϕ_2 terms are the volume fraction of acetonitrile and water, respectively.[2]

$$\delta_M = \phi_1\delta_1 + \phi_2\delta_2 \qquad (1)$$

S6. DSC and TGA analysis of PN-CysM particles

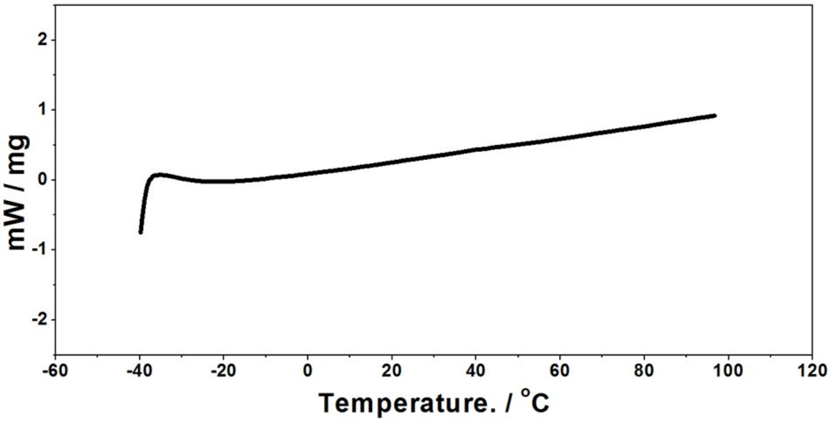

Figure S5. DSC curve of PN-CysM polyphosphazene particles.

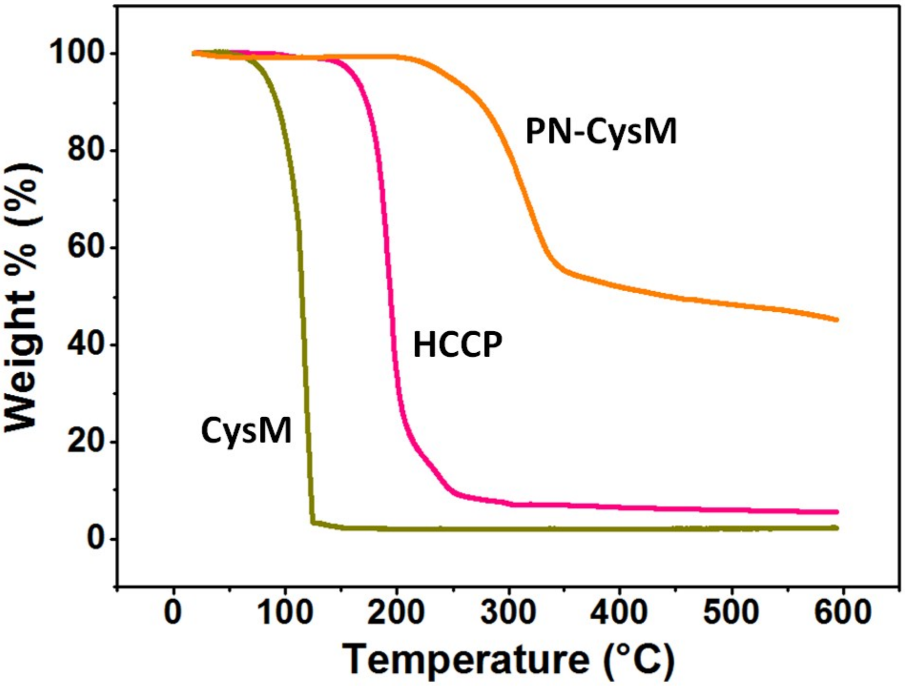

Figure S6. TGA curve of cystine, HCCP and PN-CysM polyphosphazene particles.

S7. DLS analysis of the particle growth process

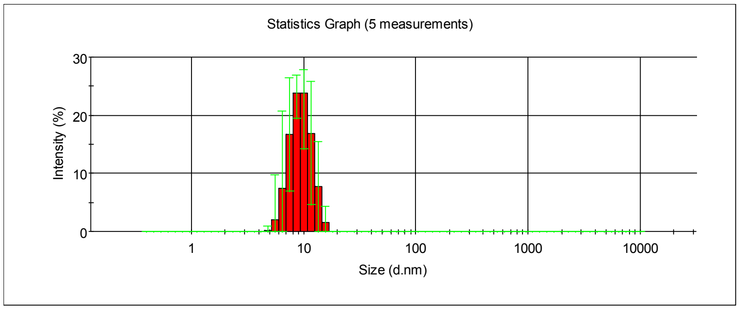

Figure S7a. DLS of oligomer solution.

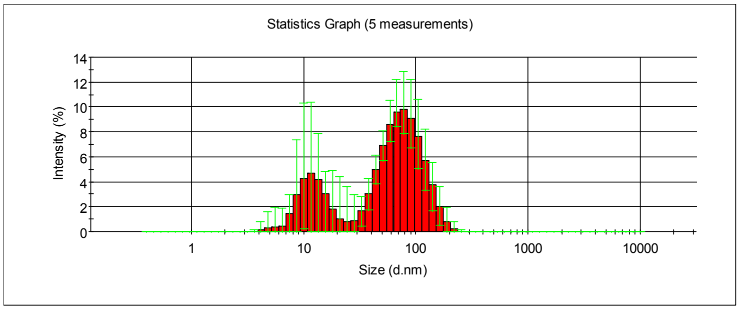

Figure S7b. DLS of 30 min growth of the oligomer at LCSP.

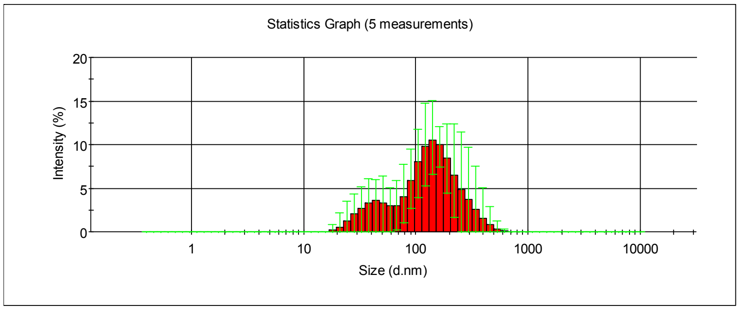

Figure S7c. DLS of 1 h growth of the oligomer at LCSP.

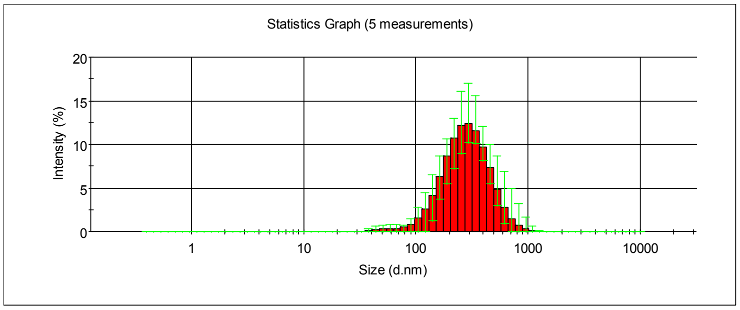

Figure S7d. DLS of 2 h growth of the oligomer at LCSP.

(1) Ganapathiappan, S.; Krishnamurthy, S. S. *J. Chem. Soc., Dalton Trans.* **1987**, 579.
(2) Hildebrand, J.; Scott, R. *Reinhold, New York*.