Supporting Information for

A Dual-Gated Polymersome Nanoreactor

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1. Materials

Polyethylene oxide monomethylether (M_n = 1900), 2-(diisopropylamino)ethyl methacrylate (DPA), *N*-isopropylacrylamide (NIPAM), 2,2'-azobis(2-methylpropionitrile) (AIBN), 6,8-difluoro-4-methylumbelliferyl octanoate (DiFMU), 7-hydroxy-4-methylcoumarin, 2-bromoethanol, tricaprylylmethylammonium chloride (R = C8-C10), dicyclohexylcarbodiimide (DCC, ≥ 99%), 4-dimethylaminopyridine (DMAP, ≥ 95%) and phosphotungstic acid were purchased from Aladdin Chemistry, Co., Ltd. (Shanghai, China). Potassium carbonate (K_2CO_3), sodium hydroxide (NaOH), magnesium sulfate ($MgSO_4$), triethylamine (TEA, ≥ 99.0%), acetonitrile (ACN, ≥ 99.5%), acetone (≥ 99.5%), carbon disulfide, dichloromethane (DCM, ≥ 99.9%), trichloromethane (99.0%), tetrahydrofuran (THF, ≥ 99.9%), *N,N*-dimethylformamide (DMF, ≥ 99.9%) and other solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China) and used as received. *Candida Antarctica* Lipase B (CALB, 32kDa) and p-nitrophenyl acetate were purchased from Sigma-Aldrich. $CDCl_3$ was purchased from J&K Scientific Ltd (Shanghai, China).

2. Characterization

2.1 Proton Nuclear Magnetic Resonance (^{1}H NMR)

^{1}H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer at room temperature. Samples (5.0 mg) were dissolved in 0.5 mL of $CDCl_3$ prior to measurements.

2.2 Dynamic Light Scattering (DLS)

The apparent hydrodynamic diameters (D_h) and polydispersities (*PD*s) of the polymersomes were determined by DLS using a ZETASIZER Nano series instrument (Malvern Instruments ZS 90) at a fixed scattering angle of 90°. Data processing was carried out using cumulant analysis of the

experimental correlation function and calculated from the computed diffusion coefficients using the Stokes-Einstein equation. Each reported measurement was conducted for three runs.

2.3 Transmission Electron Microscopy (TEM)

The polymersomes aqueous solution (8.0 μL, 0.1 mg/mL) was dropped onto a copper grid covered by carbon layer and dried at 40 °C or 25 °C and stained by neutral (pH 7.0) or acidic (pH 4.0) 1.0% phosphotungstic acid solution for 1 min. In particular, to prepare the TEM samples at 40 °C, the pre-heated copper grids loaded with the cross-linked polymersomes were dried out in an oven at 40 °C to minimize the influence of temperature change on the morphology of thermally responsive polymersomes. TEM Images were recorded on a JEOL JEM-2100F instrument at 200 kV equipped with a Gatan 894 Ultrascan 1k CCD camera.

2.4 Fluorescence Spectroscopy

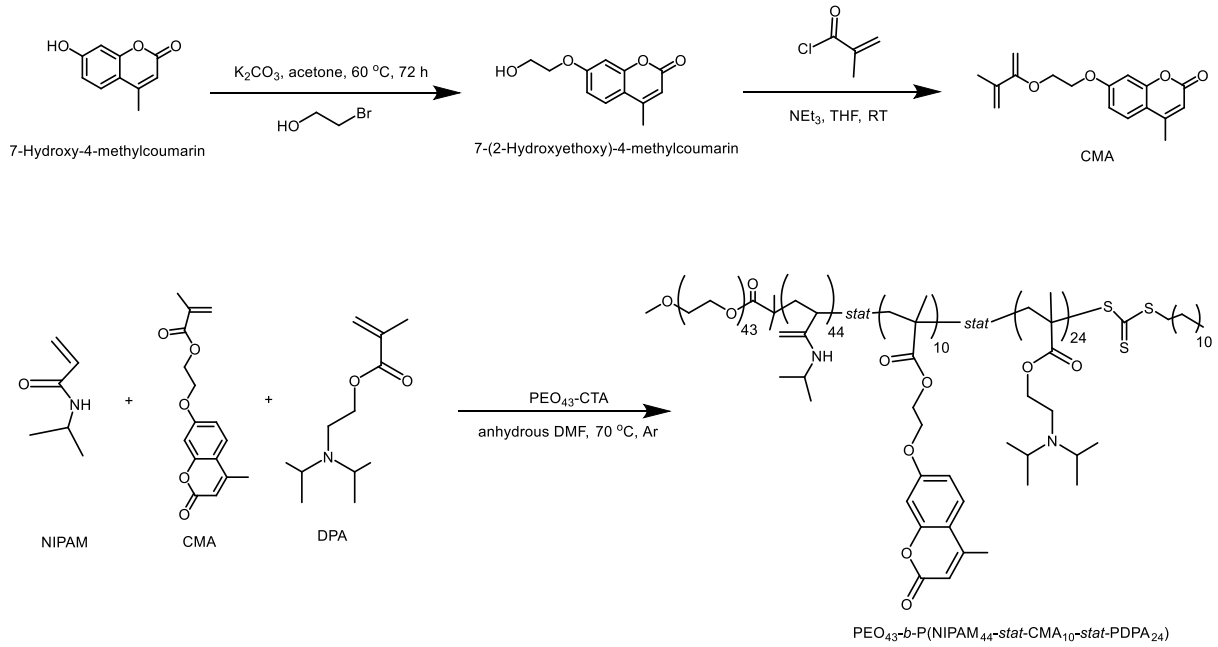
Fluorescence experiments were carried out *via* a Lumina fluorescence spectrometer (Thermo Fisher).

2.5 UV-vis Spectroscopy

The UV-vis spectra were acquired using a UV759S UV-vis spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd.). All the samples were analyzed using quartz cuvettes.

3. Scheme and Figures

Scheme S1. Synthesis route of PEO_{43}-P(NIPAM$_{44}$-*stat*-CMA$_{10}$-*stat*-DPA$_{24}$) block copolymer.



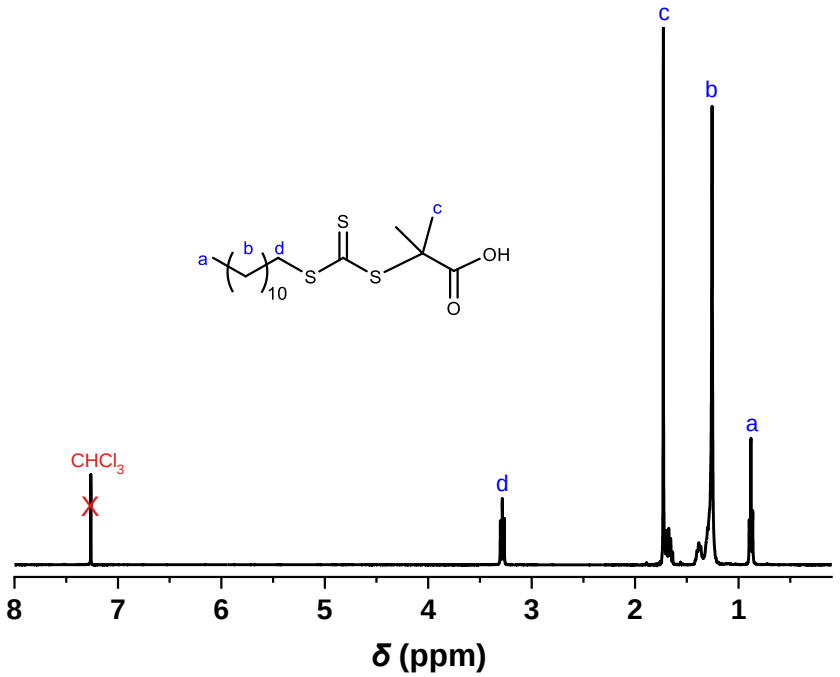


Fig. S1. ^{1}H NMR spectrum of DDMAT in $CDCl_3$.

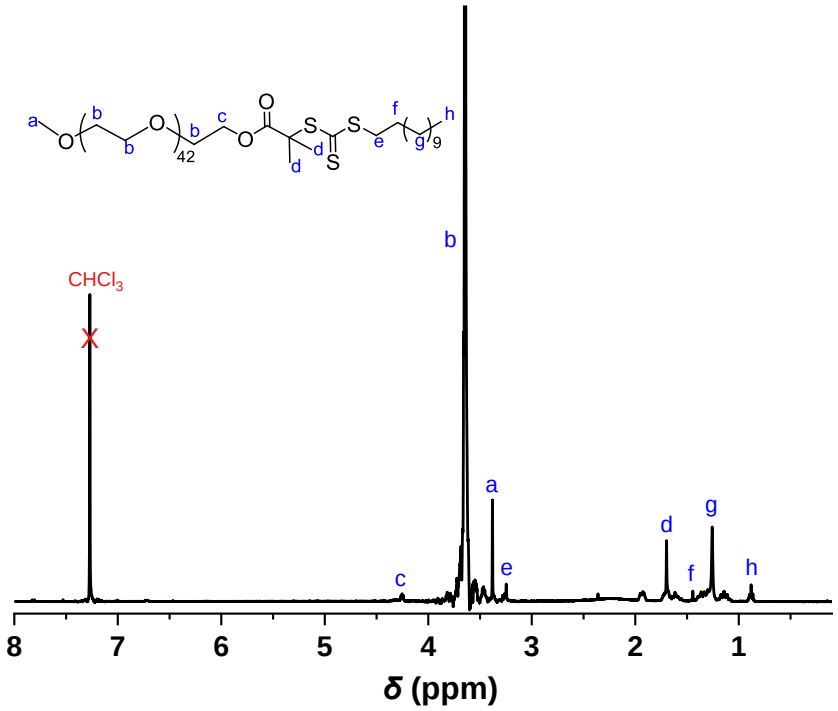


Fig. S2. ^{1}H NMR spectrum of PEO-DDMAT in $CDCl_3$.

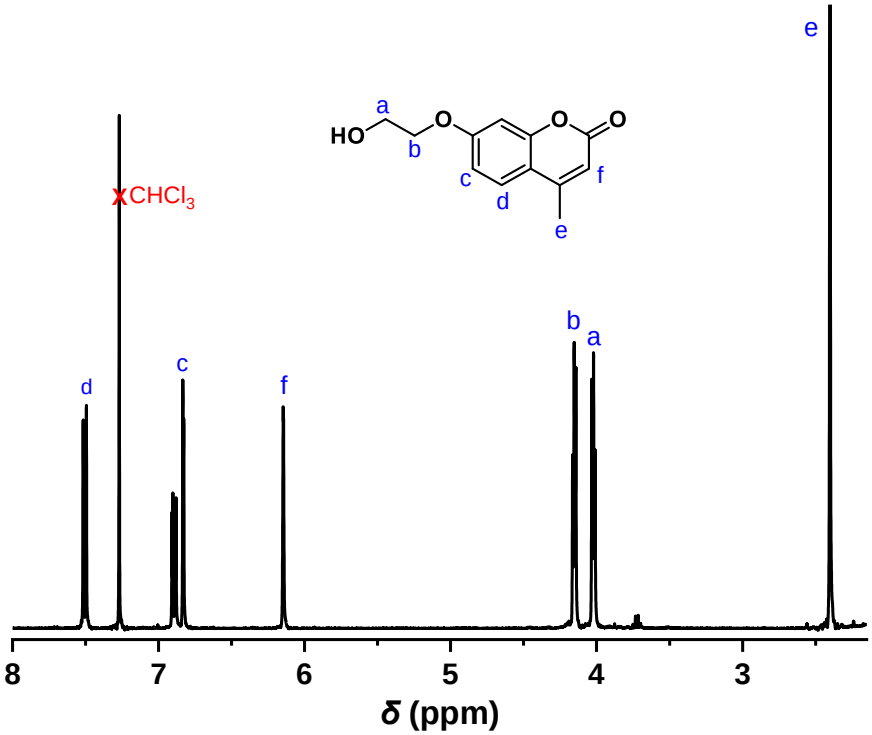


Fig. S3. ^{1}H NMR spectrum of HEMC in $CDCl_3$.

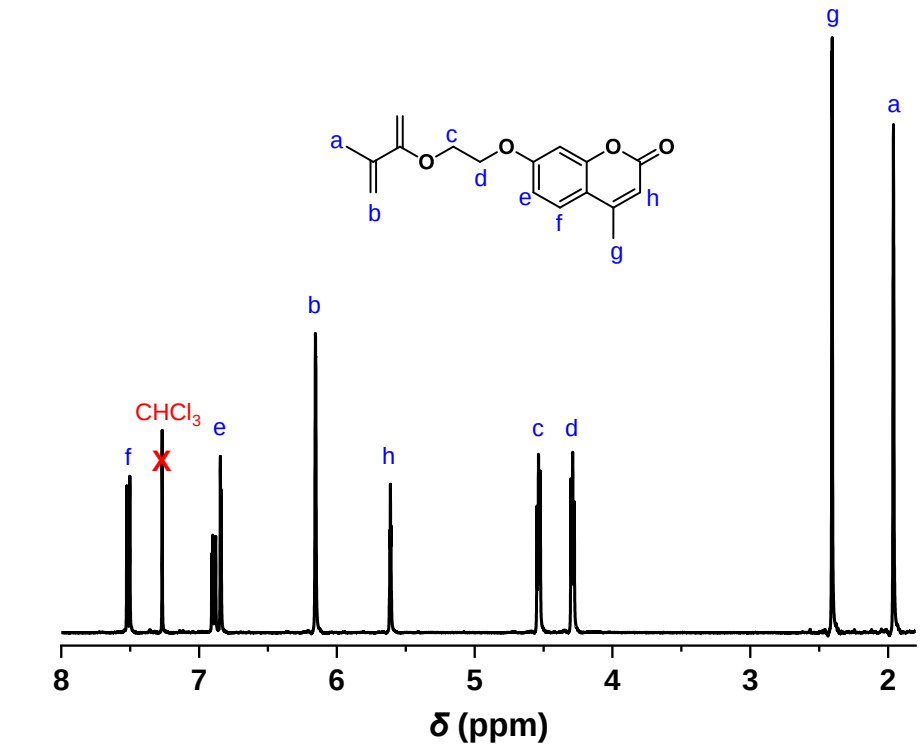


Fig. S4. ^{1}H NMR spectrum of CMA in $CDCl_3$.

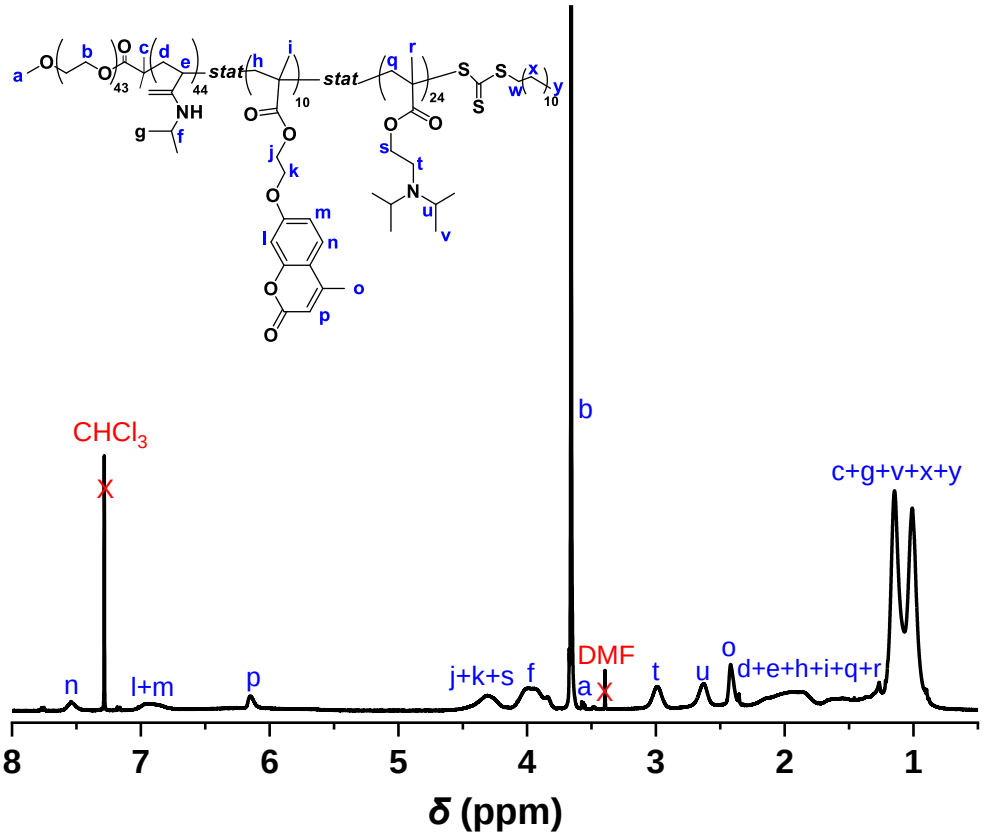


Fig. S5. ^{1}H NMR spectrum of PEO_{43}-P(NIPAM$_{44}$-*stat*-CMA$_{10}$-*stat*-DPA$_{24}$) in $CDCl_3$.

To calculate the DP (degree of polymerization) of PNIPAM, PCMA and PDPA block, the total integral area of peaks *a, b* is defined as A_{ab} and set as 170.00. The integral area of peak *f*, defined as A_f, can be therefore obtained as 43.94. The integral area of peak *p*, defined as A_p, can be therefore obtained as 10.08. The integral area of peak *t*, defined as A_t, can be therefore obtained as 48.31. Then the DP of the PNIPAM, PCMA and PDPA block should satisfy the following equation:

According to peak *f*: $DP_{PNIPAM} = A_d \approx 44$

According to peak *p*: $DP_{PCMA} = A_d \approx 10$

According to peak *t*: $DP_{PDPA} = \frac{A_t}{2} = \frac{48.31}{2} \approx 24$

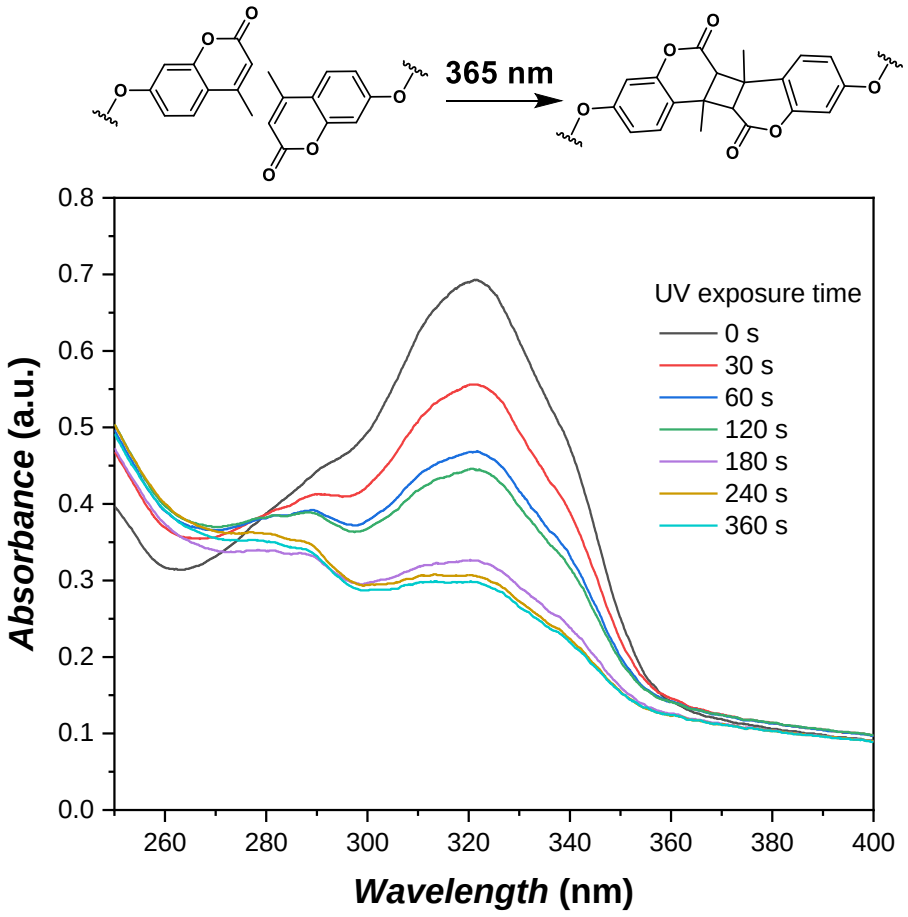


Fig. S6. UV-vis spectra of polymersomes under different UV exposure time (from 0 to 360 s). $C_{polymersomes}$ = 0.1 mg/mL.

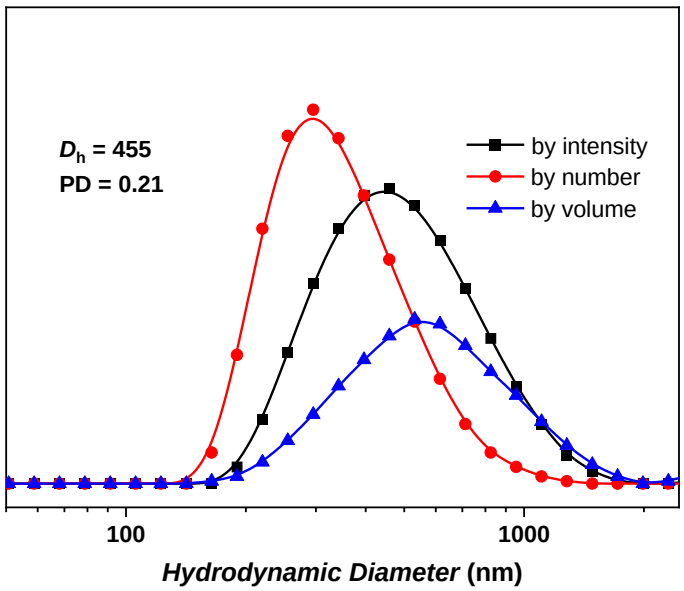


Fig. S7. DLS study of polymersomes self-assembled at 40 °C (C_{ini} = 2.0 mg/mL).

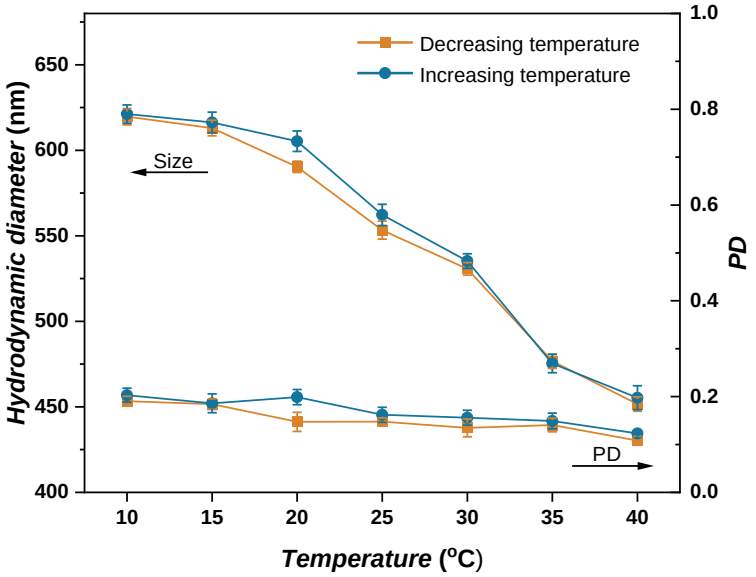


Fig. S8. Temperature-dependent size and *PD*s changes during the heating−cooling cycles.

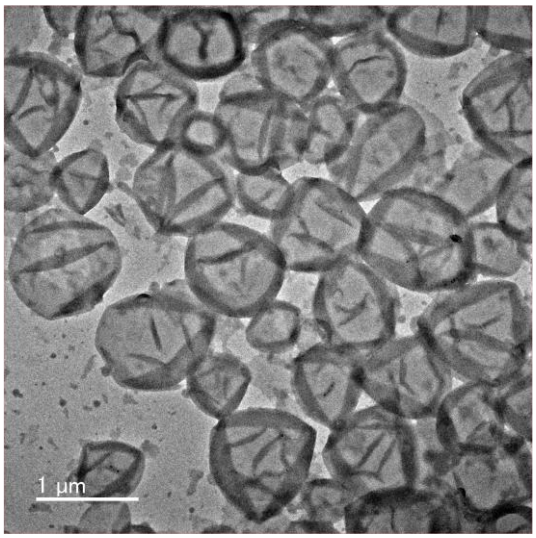


Fig. S9. TEM image of the swollen polymersomes at 25 °C.

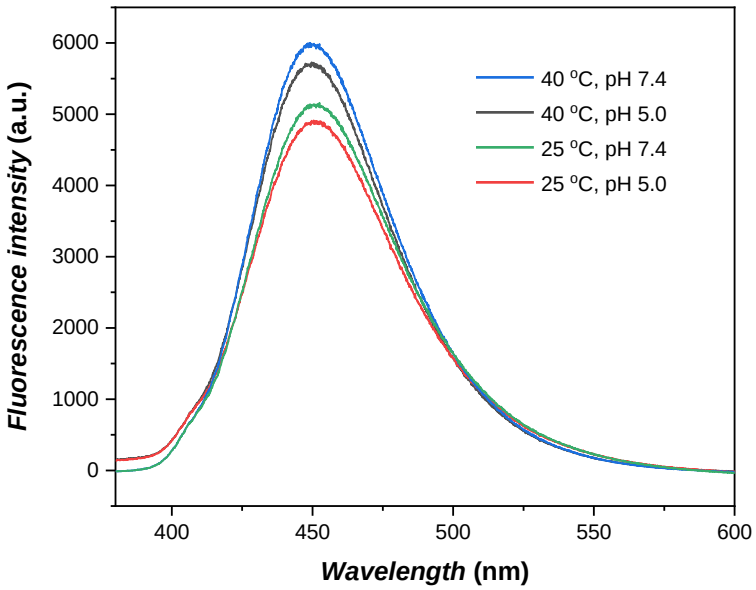


Fig. S10. Activity assay of free CALB under different temperature and pH conditions (pH 7.4 or 5.0, 40 °C or 25 °C).