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Electronic Supplementary Information

Hydrogel-polymersome composites as a sensing platform for monitoring food spoilage

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Size, morphology and stability of $PDMS_{25}$-*b*-$PMOXA_{10}$ polymersomes

Dynamic and static light scattering (DLS-SLS) were used to assess the size and architecture of the assemblies generated by the amphiphilic $PDMS_{25}$-*b*-$PMOXA_{10}$ diblock copolymers, both without and with encapsulated dye. The apparent values of the hydrodynamic radius (R_h) were determined by DLS measurements, while SLS data allowed for calculation of the radius of gyration (R_g). The structural parameter ($\rho = R_g/R_h$) was used to probe the vesicular morphology of the polymer self-assemblies. The obtained results are presented in **Figs. S1-S4**.

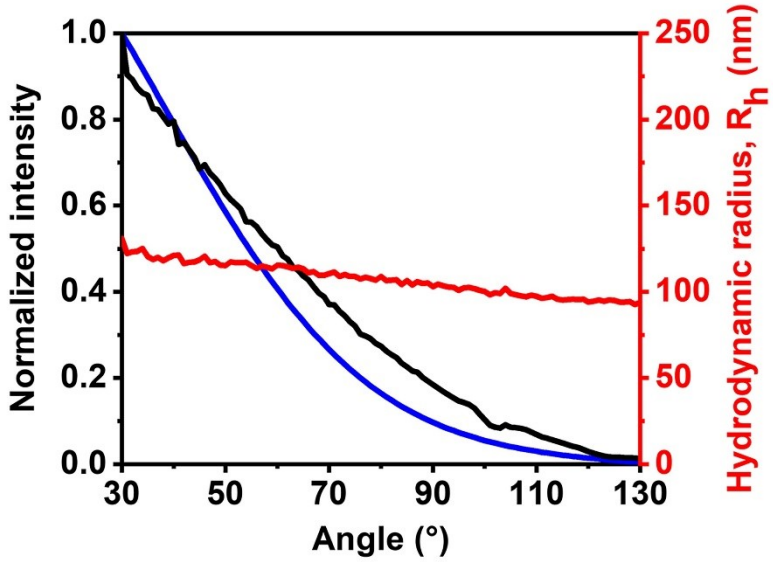

Fig. S1 Size determination of empty $PDMS_{25}$-*b*-$PMOXA_{10}$ polymersomes (Ps) via SLS-DLS. The scattering intensity at various angles (black curve) and the best Mie fit (blue curve) were used to determine the radius of gyration ($R_g = 120 \pm 6$ nm). The DLS profile provided the value of hydrodynamic radius ($R_h = 107 \pm 10$ nm) at different scattering angles (red curve). The R_g/R_h ratio of 1.12 confirms the hollow spherical morphology characteristic for polymersomes.

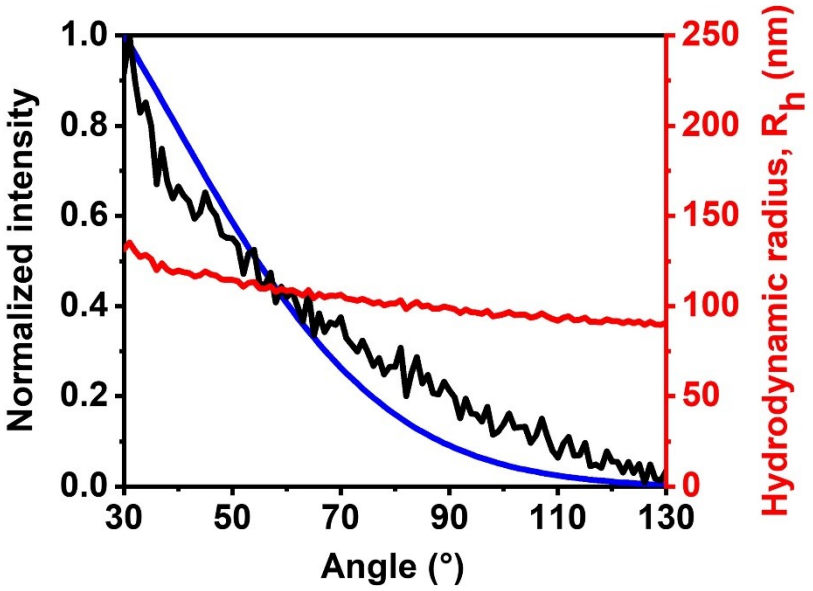

Fig. S2 SLS-DLS results for the CF-loaded polymersomes (CF-Ps). The scattering intensity at various angles (black curve) and the best Mie fit (blue curve) allow for determination of the radius of gyration ($R_g = 115 \pm 5$ nm), whereas the DLS profile indicates the hydrodynamic radius ($R_h = 103 \pm 11$ nm) at different scattering angles (red curve).

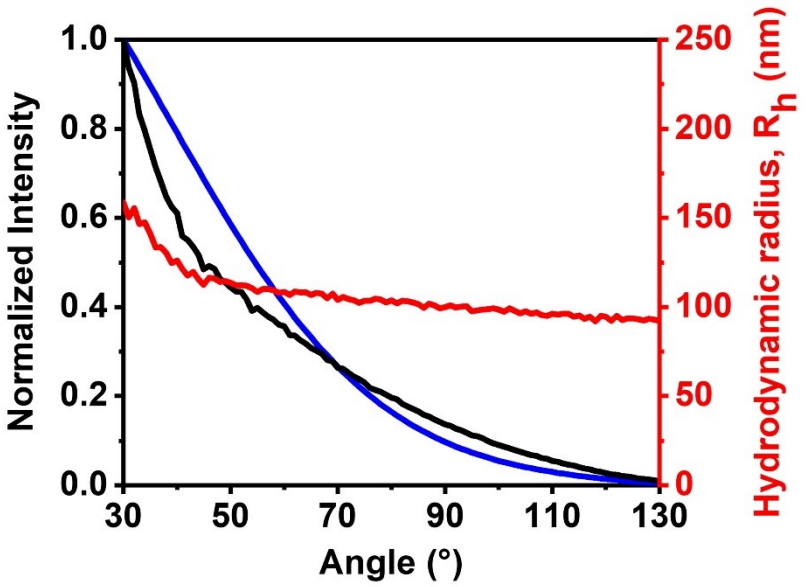

Fig. S3 Size determination of Py-loaded polymersomes (Py-Ps) via SLS-DLS. The scattering intensity at various angles (black curve) and the best Mie fit (blue curve) were used to determine the radius of gyration (R_g = 120 ± 6 nm), while the DLS profile provides hydrodynamic radius (R_h = 106 ± 14 nm) at different scattering angles (red curve).

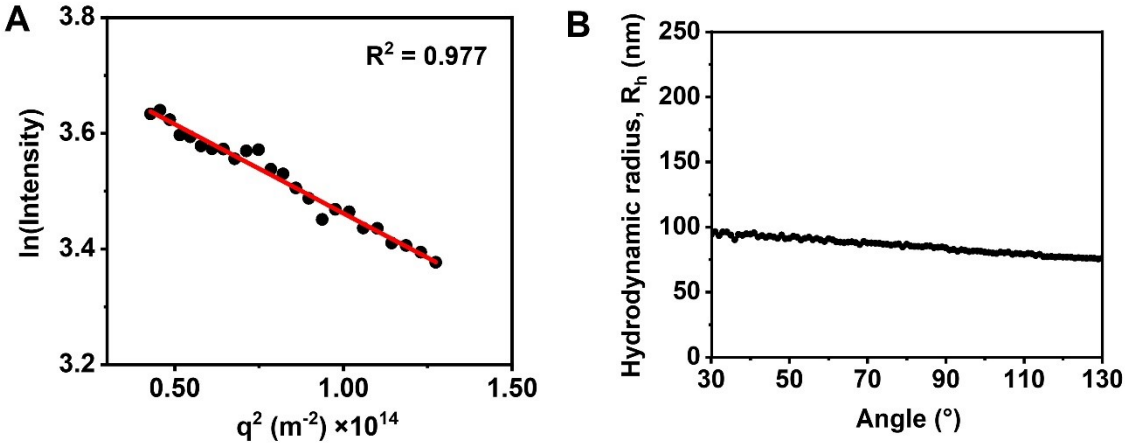

Fig. S4 SLS Guinier plot (A) and DLS profile (B) of BTB-loaded polymersomes (BTB-Ps). The Guinier plot shows the natural logarithm of the scattering intensity, ln(Intensity), versus the square of the scattering vector (q^2) in the low-angle range. The light scattering data (R_g = 96 ± 5 nm and R_h = 85 ± 6 nm indicate the presence of polymersomes.

As complementary analysis to assess the size distribution of the polymersomes, nanoparticle tracking analysis (NTA) was performed. The NTA measurements are represented in **Figs. S5** and **S8**.

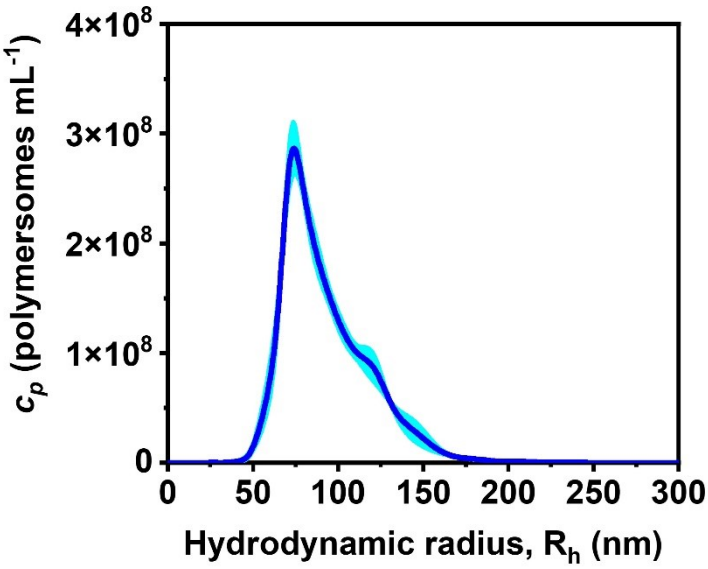

Fig. S5 Size distribution and concentration of empty polymersomes (Ps) determined by NTA. The initial dispersion was diluted 1:1000 with Milli-Q water (5 µg mL^{-1}). The cyan overlay refers to the standard deviation of the polymersomes count per mL. The R_h value is 93 ± 26 nm.

The typical spherical morphology of the polymersomes was confirmed by transmission electron microscopy (TEM). Empty polymersomes (Ps) are shown in **Fig. S6**, while micrographs of dye-loaded polymersomes are presented in **Fig. S7**.

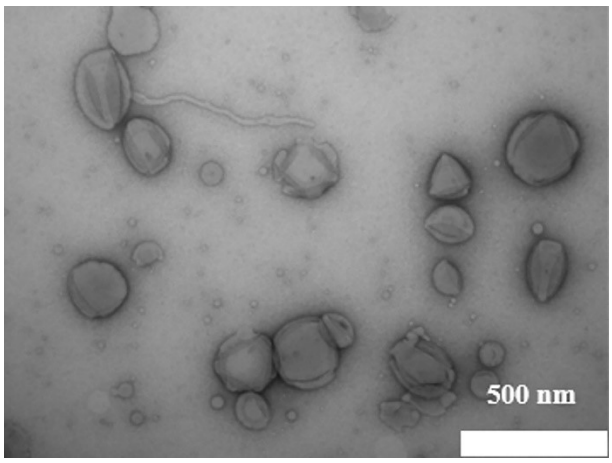

Fig. S6 TEM micrographs of empty polymersomes (Ps) revealing the deflated morphology typical for hollow nanoassemblies and showing their size polydispersity. Scale bar: 500 nm.

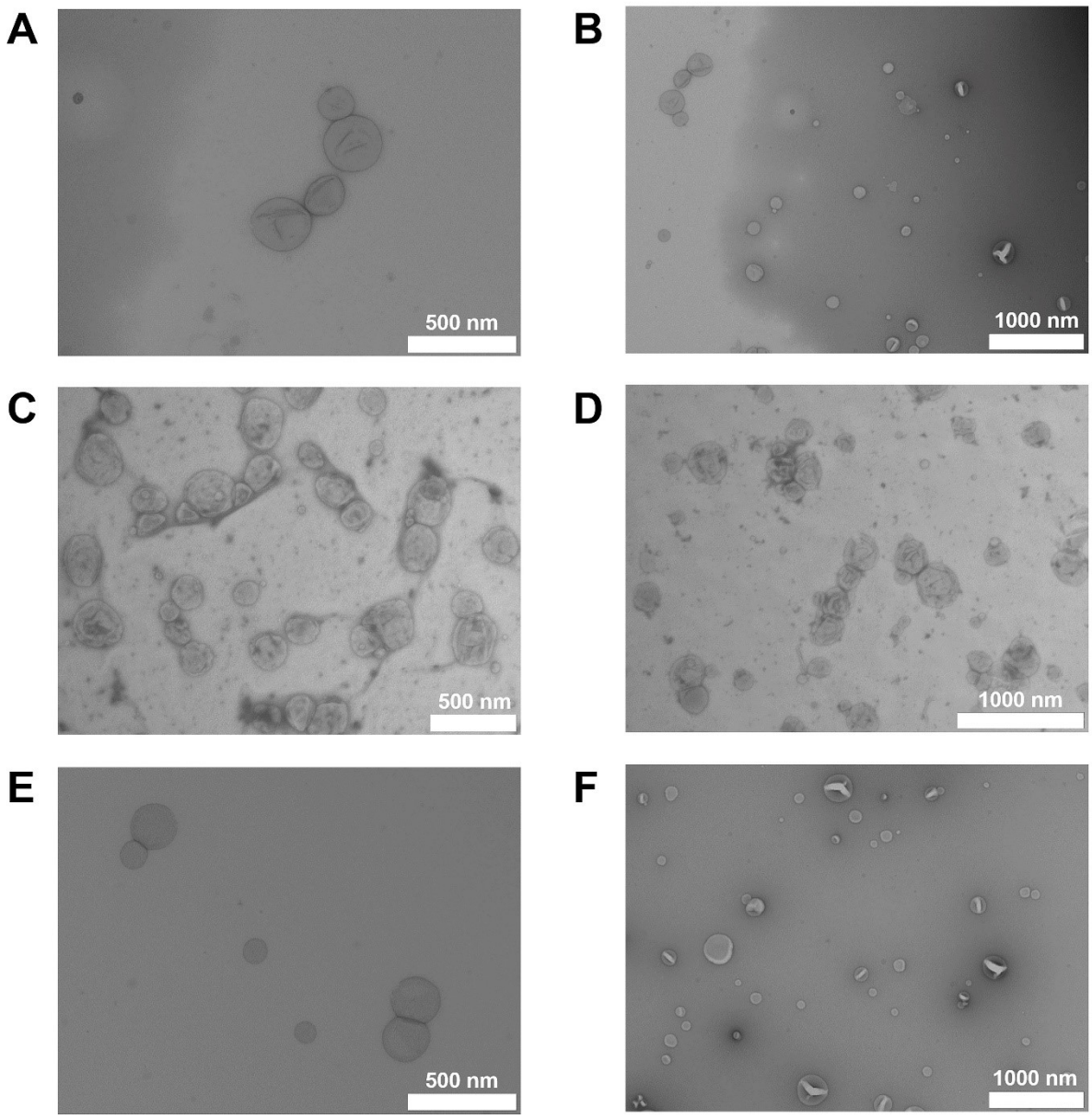

Fig. S7 TEM micrographs of dye-loaded polymersomes (dye–Ps): CF–Ps (A and B), Py–Ps (C and D) and BTB–Ps (E and F). Scale bars: 500 nm (left column) and 1000 nm (right column). The presence of encapsulated dyes does not affect the morphology of the self-assembled polymersomes.

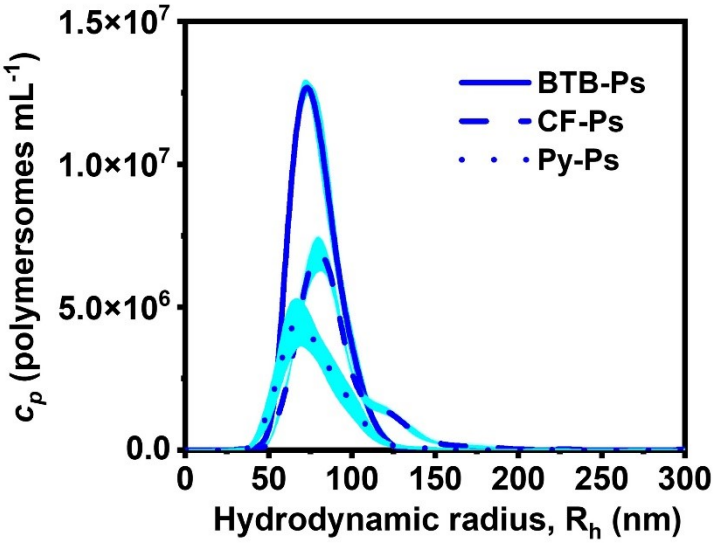

Fig. S8 Size distribution and concentration of BTB-Ps (solid line), CF-Ps (dashed line) and Py-Ps (dotted line) determined by NTA. The initial dispersions were diluted 1:1000 with Milli-Q water (5 µg mL^{-1}). The cyan overlay refers to the standard deviation of the polymersomes count per mL. The R_h values for CF-Ps, Py-Ps and BTB-Ps are 90 ± 24 nm, 77 ± 21 nm, and 79 ± 16 nm respectively.

To determine the optimum conditions for embedding polymersomes into the hydrogel, we analysed their stability in both the monomer mixture (**Fig. S9**) and the aqueous TEMED solution (**Fig. S10**).

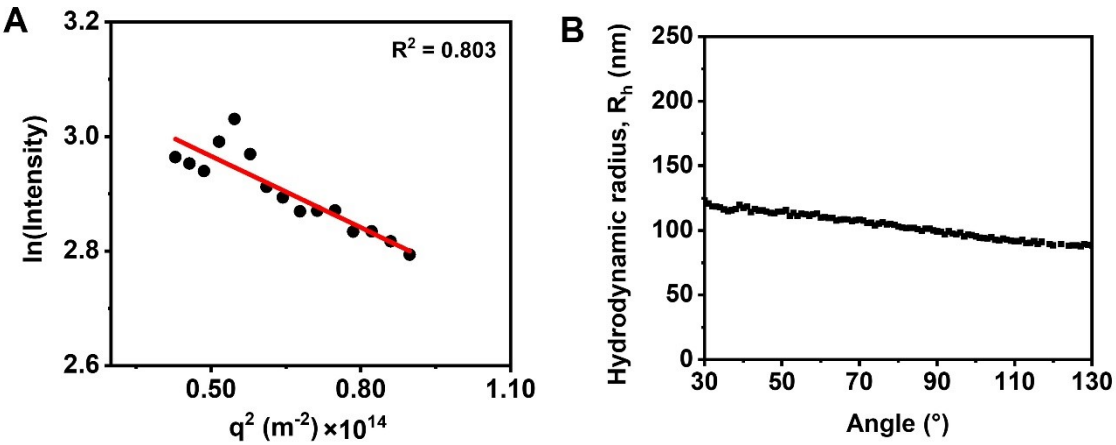

Fig. S9 SLS Guinier plot (A) and DLS profile (B) of empty polymersomes in the monomer mixture. Measurements resulted in R_g and R_h values of 112 ± 6 nm and 103 ± 10 nm respectively. The light scattering data indicate the presence of polymersomes and hence polymersome stability in the monomer mixture is confirmed.

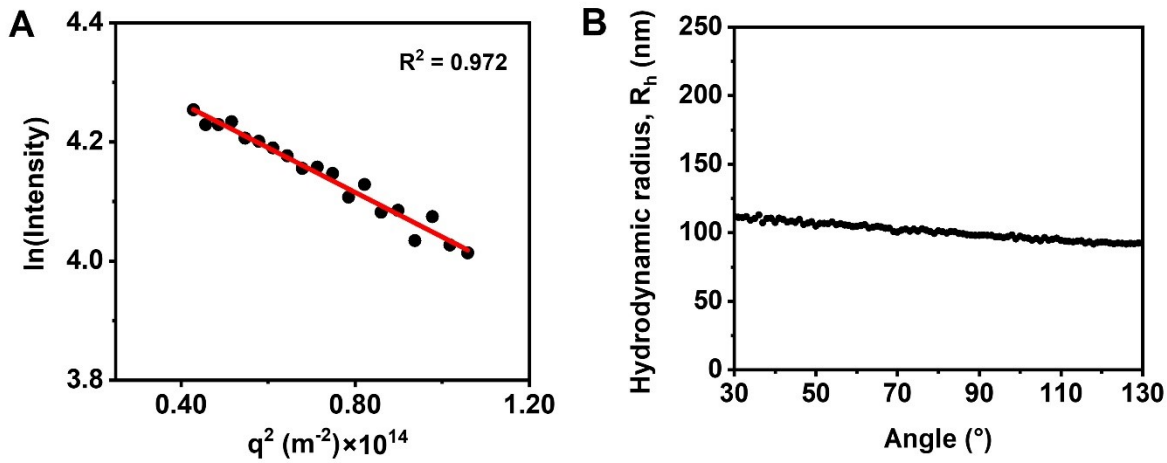

Fig. S10 SLS Guinier plot (A) and DLS profile (B) of empty polymersomes in TEMED solution. The R_g and R_h values are 105 ± 5 nm and 100 ± 6 nm respectively. The light scattering data point out polymersomes. An important requirement for this study, the stability of polymersomes in TEMED is also confirmed.

Behaviour of CF-Ps at different temperatures and in the presence of a surfactant

The effect of the surfactant Triton-X100 on polymersomes was evaluated using CF-Ps. In addition, a comparison of the fluorescence intensity for CF-Ps stored at 25 °C and 40 °C is shown in **Fig. S11**.

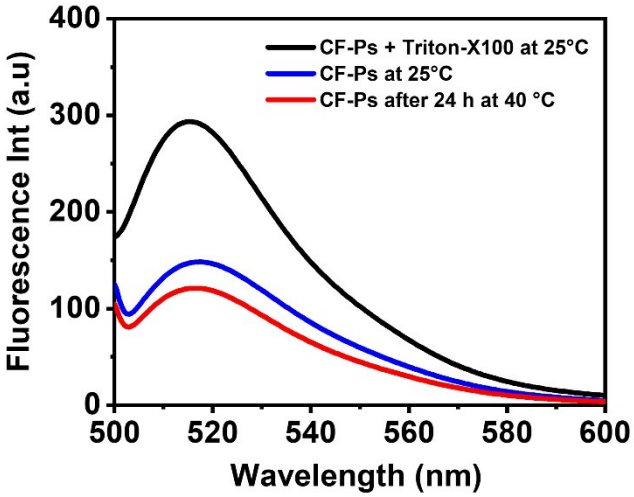

Fig. S11 Fluorescence emission spectra of CF-Ps at room temperature (blue curve), after heating to 40 °C for 24 h (red curve), and after the addition of the surfactant Triton-X100 (0.05%, v/v, in the final mixture; black curve). A sharp increase in fluorescence intensity is observed upon the addition of the surfactant due to the disruption of polymersome membrane. The minor difference in fluorescence between polymersomes measured at 25 °C and those measured after 24 h at 40 °C indicates that this temperature range does not affect polymersome stability.

Polymersome stability in HPCs after the addition of surfactant Triton X-100

A CLSM time-lapse series of a CF-HPC at 25 °C upon addition of surfactant is presented in **Fig. S12**. The gradual decrease in fluorescence intensity over time indicates the loss of dye due to the rupture of the polymersome membrane.

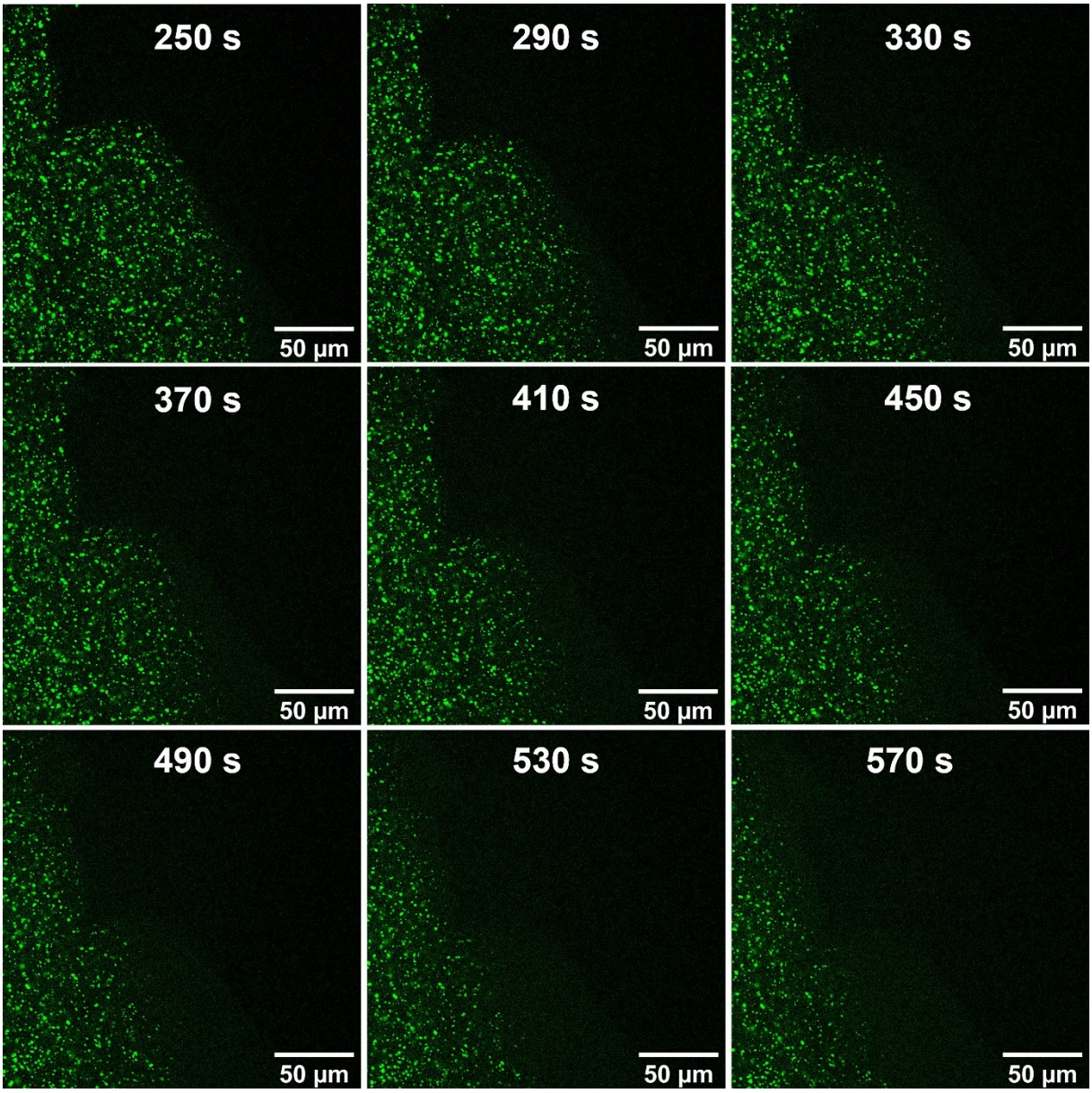

Fig. S12 Time-lapse series of a CF-HPC measured by CLSM after the addition of Triton-X100, indicating the disruption of polymersomes and the subsequent decrease in fluorescence due to the diffusion of CF out of the hydrogel and its removal by washing with Milli-Q water. The depicted spot was located at the edge of a CF-HPC. Within 500 s after the addition of surfactant, a significant decrease in fluorescence was observed.

BTB and BTB-loaded polymersomes at different concentrations of methylamine

The visual pH indication obtained by mixing 10 µL of free BTB (10 µM) with 90 µL of varying MA concentrations, as well as the effect of mixing 10 µL BTB-Ps dispersions with 90 µL of three different MA concentrations, is illustrated in **Fig S13**.

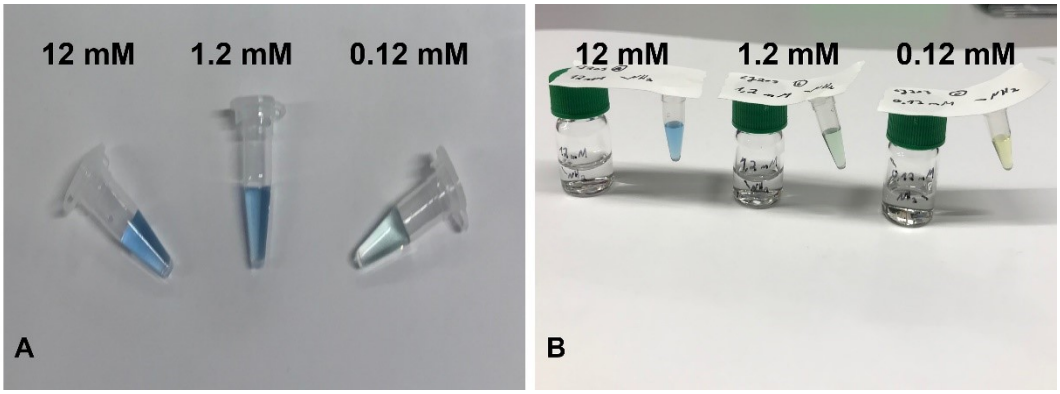

Fig. S13 Images of aliquots from aqueous solutions of free BTB (A) and purified BTB-Ps dispersions (B) in the presence of 12 mM, 1.2 mM and 0.12 mM methylamine (MA), respectively.

Amine-sensing by BTB-HPC after pre-heating at 40 °C

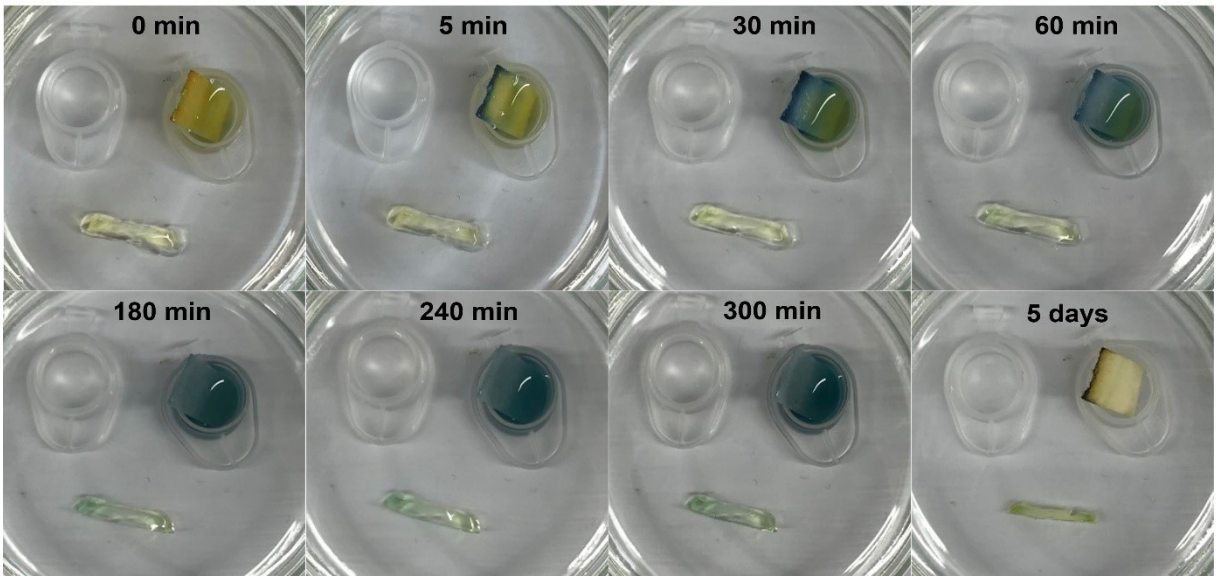

Fig. S14 The BTB-HPC response to the presence of an amine after pre-heating at 40 °C in Milli-Q water for 2 h. The top left reservoir contains 200 µL aqueous solution of 12 mM methylamine, while the top right reservoirs have small pieces of pH indicator paper in Milli-Q water. The optical images were taken at the following time points, from left to right: t = 0 min, 5 min, 30 min, 60 min, 180 min, 240 min, and 300 min. BTB-HPC responsiveness to the presence of amine after being stored at room temperature in Milli-Q water for 5 days.