Supplementary Information for the Manuscript Entitled

Microwave irradiation-assisted RAFT polymerization-induced self-assembly (MWI-PISA) of pH-responsive diblock copolymer nanoparticles

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**Instruments and Analyses:**

Microwave-assisted-PISA aqueous RAFT polymerizations were conducted on a Biotage Initiator Robot Sixty microwave system. The HPMA monomer, CTA, PHPMA-mCTA and PHPMA-\emph{b}-PDPA NPs were characterized by proton nuclear magnetic resonance (\emph{^1}H NMR) spectroscopy. \emph{^1}H NMR spectra were recorded on a Bruker Avance III 600 spectrometer operating at 600.272 MHz, using CDCl\textsubscript{3}, methanol-\emph{d}\textsubscript{4} or D\textsubscript{2}O (acidified with DCl; pH ~ 3.0) as the solvent at 295 K. For \emph{^1}H measurements single pulse experiments with a width of 90° pulse 18 μs, relaxation delay 10 s, 32 scans were applied.

The apparent average molecular weights (i.e., number-average molecular weight (\emph{M}_n) and weight-average molecular weight (\emph{M}_w) and the molecular weight distribution or dispersity (\emph{D}) of the PHPMA-mCTAs were determined by size exclusion chromatography (SEC) using an HPLC Ultimate 3000 system (Dionex, USA) equipped with an SEC column (TSKgel SuperAW3000 150 × 6 mm, 4 μm) and three detectors: ultraviolet/visible (UV/VIS), refractive index (RI, Optilab-rEX) and multiangle light scattering (MALS, DAWN EOS) (Wyatt Technology Co., USA). A mixture (80/20 vol %) of methanol and sodium acetate buffer (0.3 M, pH = 6.5) was used as the mobile phase.
The dynamic light scattering (DLS) measurements were performed using an ALV CGE laser goniometer consisting of a 22 mW HeNe linear polarized laser operating at a wavelength ($\lambda = 632.8$ nm), an ALV 6010 correlator, and a pair of avalanche photodiodes operating in pseudo-cross-correlation mode. The samples were loaded into 10 mm diameter glass cells and maintained at 37 ± 1 °C. The data were collected using the ALV Correlator Control software and the counting time was 45 s. The measured intensity correlation functions $g_2(t)$ were analyzed using the algorithm REPES (incorporated in the GENDIST program)$^{57}$, resulting in the distributions of relaxation times shown in equal area representation as $\tau A(\tau)$. The mean relaxation time or relaxation frequency ($\Gamma = \tau^{-1}$) is related to the diffusion coefficient ($D$) of the nanoparticles as $D = \frac{\Gamma}{q^2}$ where $q = \frac{4\pi n\sin \theta}{\lambda}$ is the scattering vector being $n$ the refractive index of the solvent and $\theta$ the scattering angle. The hydrodynamic radius ($R_H$) or the distributions of $R_H$ were calculated by using the Stokes-Einstein relation:

$$\quad R_H = \frac{k_B T q^2}{6 \pi \eta D \tau} \quad (1)$$

being $k_B$ the Boltzmann constant, $T$ the absolute temperature, and $\eta$ the viscosity of the solvent.

In the static light scattering (SLS), the scattering angle was varied from 30 to 150° with a 10° stepwise increase. The absolute light scattering is related to weight-average molar mass ($M_w(NPs)$) and to the radius of gyration ($R_G$) of the nanoparticles by the Zimm formalism represented as:

$$\quad K_c \frac{1}{R_G} = \frac{1}{M_w(NP)}(1 + \frac{R_G^2}{3q^2}) \quad (2)$$

Hence, measuring the light scattering intensity ($I_{sc}$) at a given angular range for one single diluted concentration, the value of $R_G$ can be estimated from the slope of the profiles and ($M_w(NPs)$) from the intercept.

The aggregation numbers were determined by the equation:

$$\quad N_{agg} = \frac{M_w(NPs)(SLS)}{M_w(SEC)} \quad (3)$$

The small-angle X-ray scattering (SAXS) experiments were recorded by using a pinhole camera (MolMet, Rigaku, Japan, modified by SAXSLAB/Xenocs) attached to a microfocused
X-ray beam generator (Rigaku MicroMax 003) operating at 50 kV and 0.6 mA (30 W). The samples were sealed into boro-silicate capillaries (∼2 mm diameter) and the scattering intensities were recorded by using a Pilatus 300 K detector with an exposure time of 480 min. The scattering profiles were fitted by using SASFit software. The form factors of spherical core–shell assemblies and bilayered vesicles were used during the fitting procedures. Correspondingly, the fitting parameters were the radius of the core ($R_c$) and thickness of the shell ($t_{shell}$), and the thickness of the hydrophilic PHPMA shell ($t_{hshell}$), the thickness of the hydrophobic PDPA layer ($t_{PDPA}$) and the radius of the aqueous lumen of the vesicles. The electron densities were determined based on the chemical structure of each polymer block and the mass densities, and the Q12 size dispersity of the samples was accounted for in the form factors by using a log-normal distribution.

The transmission electron microscopy (TEM) observations were performed on a Tecnai G2 Spirit Twin at 120 kV (FEI, Czech Republic), equipped with cryo-attachment (Gatan, cryo-specimen holder) using a bright field imaging mode at an accelerating voltage of 120 kV. The sample solution (4 µL) was deposited on an electron microscopy grid covered with lacey carbon supporting film (Agar Scientific) after hydrophilization by glow discharge. The solution excess was removed by blotting (Whatman no. 1 filter paper) for ∼1s and then the grid was immediately plunged into liquid ethane held at -181 °C. The frozen sample was transferred into the microscope and observed at -173 °C under the conditions described above (120 kV, bright field imaging).

**Synthesis of monomer**

1) **N-(2-hydroxypropyl) methacrylamide (HPMA)**

$\text{Na}_2\text{CO}_3$ (12.61 g, 119 mmol) was suspended in DCM (28 mL). The mixture was cooled to -10 °C and amino-2-propanol (8.35 mL, 108 mmol) was added. Freshly distilled methacryloyl chloride (10.56 mL, 108 mmol) in 11 mL of DCM was added dropwise with the solution kept between 25 and -10 °C (ethanol/dry ice mixture) and vigorously stirred. The mixture was stirred for 20 min at -5 °C and then left stirring to equilibrate to room temperature. Following filtration, the filtrate was dried twice with anhydrous Na$_2$SO$_4$ and concentrated until formation of crystallization seeds. The product was left to crystallize at -20 °C overnight, washed with cold DCM and recrystallized from acetone The crystals were dried in vacuo (16.1 g, 85%).
\begin{align*}
^1\text{H-NMR (600 MHz, DMSO, } \delta, \text{ ppm):} & \ 1.00 (d, 3H, CH(OH)-CH_3), 1.85 (q, 3H, C(CH_2)CH_3)), \\
& 3.05 (m, 2H, NH-CH_2), 3.68 (m, 1H, CH(OH)), 4.68 (d, 1H, CH(OH)), 5.31 (m, 1H, =CH_2), \\
& 5.65 (m, 1H, =CH_2), 7.80 (s, 1H, NH-CH_2) \text{ (see Figure S1 for assignments).}
\end{align*}

\begin{center}
Scheme S1. Synthesis of the N-(2-hydroxypropyl) methacrylamide (HPMA).
\end{center}

\begin{center}
Figure S1. \textsuperscript{1}H NMR spectrum of the N-(2-hydroxypropyl) methacrylamide (HPMA) in DMSO-d\textsubscript{6}.
\end{center}

2) 4-Cyano-4-(((ethylthio)carbonothioyl)thio)pentanoic acid (CTA)
Sodium hydride (60 % in mineral oil, 3.15 g, 0.079 mol) was added to diethyl ether (150 mL) in a 250 mL round bottom flask and was cooled to <10 °C. Ethanethiol (4.72 g, 0.076 mol) was added dropwise (over 20 minutes) to the suspension containing sodium hydride and the resultant mixture was allowed to stir for 10 minutes. To this solution, carbon disulfide (6 g, 0.079 mol) was then added dropwise. After a further 10 minutes, the resultant ethane trithiocarbonate sodium salt (7.0 g, 0.044 mol) was isolated by filtration by suction using a fritted glass funnel. The ethane trithiocarbonate sodium salt was resuspended in diethyl ether (100 mL) and iodine (5.6 g, 0.022 mol) was added and stirred for 45 minutes at room temperature. Subsequently, the brown suspension was washed two-fold with Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (200 mL of 5 wt. %) solution and once with brine (200 mL) to yield a yellow ether phase which was
collected, dried over MgSO$_4$, filtered and concentrated via rotary evaporation to yield a viscous yellow liquid (3.07 g, 0.011 mol). $^1$H-NMR (400 MHz, CDCl$_3$ $\delta$, ppm) 3.30 (q, 4H), 1.34 (t, 6H).

The bis(ethyl trithiocarbonate) was dissolved in ethyl acetate (100 mL) and 4,4'-azobis(4-cyanopentanoic acid) (V-501, 4.57 g, 0.0169 mol) was added. This solution was refluxed under nitrogen at 90-100 °C for 20 hours. The solution was concentrated and purified by flash silica chromatography (30.0 × 5.0 cm) using n-pentane/ethyl acetate/acetic acid (1/1/0.03 v/v/v). After rotary evaporation of the appropriate fractions (Rf = 0.65 in n-pentane/ethyl acetate/acetic acid 1/1/0.03 v/v/v), a viscous yellow liquid was obtained. The yellow liquid was placed at -20 °C, which yielded yellow crystals that were subsequently filtered, washed with ice-cold pentane and dried in vacuo. Yield: 5.76 g.

$^1$H-NMR (400 MHz, CDCl$_3$, $\delta$, ppm): 3.33 (q, 2H, CH$_2$CH$_3$), 2.67 (m, 2H, CH$_2$CH$_2$), 2.52 (m, 1H, CH$_2$CH$_2$), 2.39 (m, 1H, CH$_2$CH$_2$), 1.86 (s, 3H, CH$_3$), 1.34 (t, 3H, CH$_3$). Conversion by $^1$H-NMR spectroscopy 85% (See Figure S2 for assignments).

![Figure S2](image_url). $^1$H NMR spectrum of 4-cyano-4-(((ethylthio)carbonothioyl)thio)pentanoic acid in CDCl$_3$. 

Figure S2. $^1$H NMR spectrum of 4-cyano-4-(((ethylthio)carbonothioyl)thio)pentanoic acid in CDCl$_3$. 

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Figure S3. $^1$H NMR spectrum of the poly N-(2-hydroxypropyl) methacrylamide (PHPMA) in methanol-d$_4$.

Figure S4. SEC analysis in MeOH/acetate buffer of the PHPMA-mCTAs (in Table 1).
**Figure S5.** Dynamic light scattering particle size distributions obtained for PHPMA$_{23}$-$b$-PDPA$_n$ (n = 50, 75 and 100).

**Figure S6.** Cryo-TEM image of PHPMA$_{23}$-$b$-PDPA$_{75}$ nanoparticles formed in water at 10% w/w solids under MWI conditions.
Figure S7. Dynamic light scattering particle size distributions obtained for PHPMA$_{51}$-$b$-PDPA$_n$ (n = 50, 75 and 100).