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pH-Responsive Schizophrenic Diblock Copolymers Prepared by Polymerization-Induced Self-Assembly

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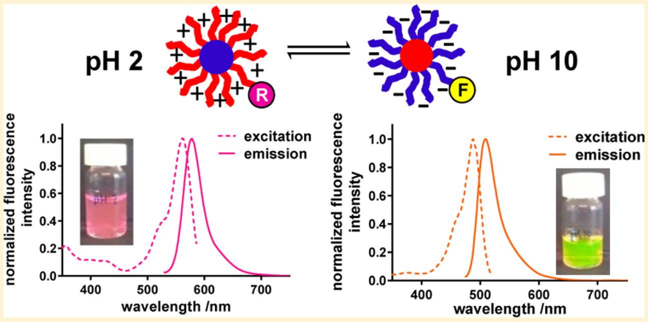
S *Supporting Information*

ABSTRACT: Polymerization-induced self-assembly (PISA) is used for the highly convenient and efficient preparation of ampholytic diblock copolymer nanoparticles directly in acidic aqueous solution. Cationic nanoparticles comprising a protonated polyamine stabilizer block and a hydrophobic polyacid core-forming block are formed at pH 2. Micelle inversion occurs at pH 10 to produce anionic nanoparticles with an ionized polyacid stabilizer block and a hydrophobic polyamine core-forming block. Macroscopic precipitation occurs at around pH 6−7, which lies close to the isoelectric point of this ampholytic diblock copolymer. Incorporation of fluorescein and rhodamine dye labels into the acid and amine blocks, respectively, leads to dual-color bifluorescent self-reporting pH-responsive nanoparticles.



INTRODUCTION

It is well-known that amphiphilic AB diblock copolymers self-assemble in aqueous solution to form spherical micelles, worm-like micelles, or vesicles.[1−4] Stimulus-responsive diblock copolymers have also been well-studied: amphiphilic character can be triggered by a change in pH[5−9] or temperature,[8−13] which leads to in situ self-assembly. In certain cases both blocks can be stimulus-responsive, which enables two types of micelles to be formed; such copolymers have been described as having "schizophrenic" character.[14−19] Literature examples include dual pH-responsive,[17,19−31] dual thermoresponsive,[32−35] and dual salt-responsive diblock copolymers[36] and also various copolymers exhibiting two types of stimulus-responsive behavior (e.g., pH plus temperature,[37−45] pH plus salt,[46] and CO_2 plus temperature[47]). In each case schizophrenic diblock copolymers were first prepared via solution polymerization and required post-polymerization processing in dilute solution to produce nanoparticles, with protecting group chemistry being required in some cases.[17,19,21,24,40] Such copolymers have been evaluated for biomedical applications, such as hemocompatibility,[48] protein purification,[49] and drug delivery.[6,44]

Polymerization-induced self-assembly (PISA) offers a robust strategy for the convenient synthesis of well-defined diblock copolymer nanoparticles directly in a wide range of solvents at high solids.[50−55] In most cases PISA offers an enhanced reaction rate compared to solution polymerization and hence relatively high final monomer conversions.[56,57] In particular, amphiphilic diblock copolymer nanoparticles can be prepared directly in water via reversible addition−fragmentation chain transfer (RAFT) aqueous dispersion polymerization[50,52,58−60] or RAFT aqueous emulsion polymerization.[52,61−63] RAFT-mediated PISA has been used to prepare thermosensitive diblock copolymer nano-objects.[64−66] For poly(glycerol monomethacrylate)−poly(2-hydroxypropyl methacrylate) (PGMA−PHPMA) block copolymer worms synthesized by RAFT aqueous dispersion polymerization, a worm-to-sphere transition can be induced by cooling from 20−25 °C to 5−10 °C.[67,68] The same morphological transition has been observed for poly(lauryl methacrylate)−poly(benzyl methacrylate) (PLMA−PBzMA) worms prepared by RAFT dispersion polymerization in *n*-dodecane, but in this case heating to 90 °C was required to induce the change in morphology.[69] Similarly, pH-responsive diblock copolymer nano-objects have been prepared using RAFT aqueous dispersion polymerization. For example, using either a carboxylic acid-functionalized chain transfer agent (CTA)[70] or a morpholine-functionalized CTA[71] leads to morphological transitions on adjusting the solution pH to either ionize or protonate end-groups located on the terminus of the stabilizer block for otherwise non-ionic diblock copolymers. However, as far as we are aware, ampholytic diblock copolymer nano-objects[72] have not yet been prepared by PISA. This omission is perhaps surprising because it is known that such copolymers have potential applications as pigment dispersants,[73−75] in addition to biomedical applications such as drug delivery.[76]

In the present work, PISA is utilized for the synthesis of new pH-responsive ampholytic schizophrenic diblock copolymers (Figure 1a). Unlike previous syntheses of schizophrenic diblock copolymers by solution polymerization, nanoparticles are produced directly in water at high solids. Thus, a poly(2-

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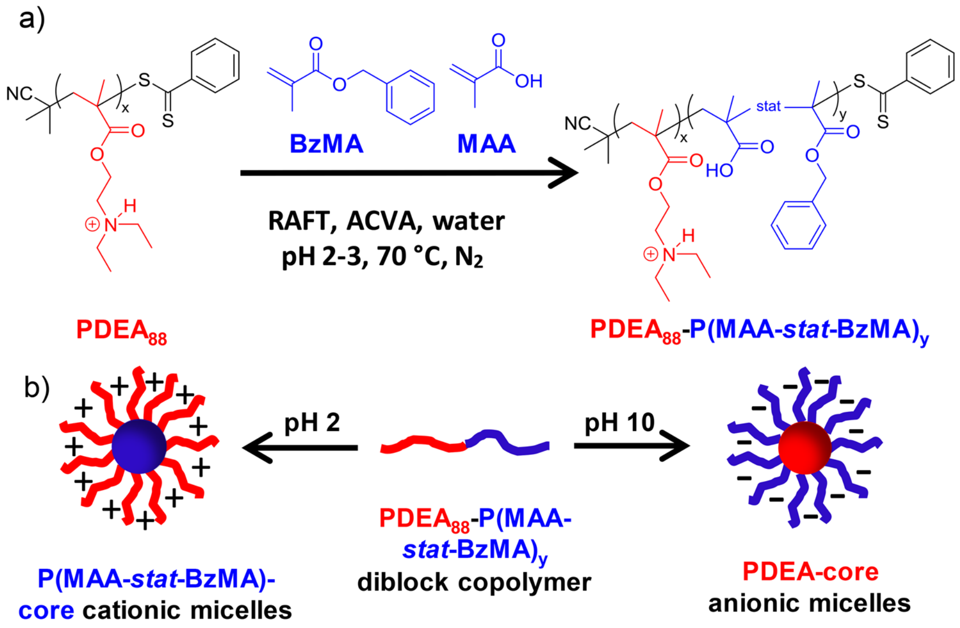

Figure 1. Schematic representation of (a) the RAFT aqueous emulsion copolymerization of methacrylic acid and benzyl methacrylate at 70 °C at 15% w/w using a $PDEA_{88}$ macro-CTA and (b) the schizophrenic micellization behavior of $PDEA_{88}$-P(MAA-*stat*-BzMA)$_y$ diblock copolymers in aqueous solution.

(diethylamino)ethyl methacrylate) ($PDEA_{88}$) CTA is chain-extended via RAFT aqueous emulsion copolymerization of benzyl methacrylate (BzMA) and methacrylic acid (MAA) at a pH of 2.5. PDEA is a weak polybase with a pK_a of approximately 7.3.[77] Therefore, the PDEA block is protonated and acts as a cationic steric stabilizer for the hydrophobic core-forming P(MAA-*stat*-BzMA) block. On switching the solution pH to pH 10, nanoparticle inversion occurs and the MAA residues become ionized. Thus the initial core-forming block becomes hydrophilic, while the PDEA stabilizer block becomes deprotonated and hence hydrophobic. These new dual pH-responsive sterically stabilized nanoparticles have been characterized using ^1H NMR spectroscopy, dynamic light scattering (DLS), aqueous electrophoresis, small-angle X-ray scattering (SAXS), and transmission electron microscopy (TEM). Moreover, appropriate fluorescent labeling of the copolymer chains leads to the formation of self-reporting nanoparticles that can act as both a colorimetric and a bifluorescent sensor.

■ EXPERIMENTAL SECTION

Materials. 2-(Diethylamino)ethyl methacrylate (DEA; 99%), methacrylic acid (MAA; 99%), and benzyl methacrylate (BzMA; 96%) were purchased from Sigma-Aldrich (UK) and used without further purification. 2,2′-Azobis(4-cyanovaleric acid) (ACVA; 98%) was purchased from Alfa Aesar (UK) and used as received. α,α′-Azobis(isobutyronitrile) (AIBN) was purchased from Molekula (UK) and used as received. 2-Cyano-2-propyl dithiobenzoate (CPDB; 97%) was purchased from Strem Chemicals (UK) and used without further purification. 2-(Methacryloyloxy)ethyl thiocarbamoyl rhodamine B (RhBMA) was purchased from Polysciences Europe GmbH (Germany) and was used as received. Fluorescein *O*-methacrylate (FMA, 97%) was purchased from Sigma-Aldrich (UK) and was used as received. Sodium hydroxide was purchased from VWR (UK). Dimethyl sulfoxide-d_6 and chloroform-*d* were purchased from Goss Scientific Instruments Ltd. (Cheshire, UK). All other solvents were purchased from Fisher Scientific (Loughborough, UK) and used as received. Deionized water was used for all experiments.

Preparation of $PDEA_x$ Macro-CTA. CPDB RAFT agent (0.4023 g, 1.817 mmol), DEA (40.000 g, 254.4 mmol), AIBN (0.0995 g, 0.6058 mmol; CPDB/ACVA molar ratio = 3.0), and toluene (27.0012 g, 60% w/w) were weighed into a round-bottom flask and degassed with nitrogen for 30 min. The reaction solution was then placed in an oil bath heated to 70 °C. The polymerization was allowed to proceed for 6.5 h, resulting in a monomer conversion of 63% as determined by ^1H NMR. The crude PDEA homopolymer was purified by dialysis against isopropanol (IPA) using a 1000 MWCO dialysis membrane (Spectrum Laboratories Inc.) to give a pure PDEA macro-CTA (<1% monomer remaining) with a mean degree of polymerization calculated to be 88 as determined by ^1H NMR in $CDCl_3$. THF GPC analysis indicated an M_n of 11 600 g mol^{-1} and an M_w/M_n of 1.17 (against a series of near-monodisperse poly(methyl methacrylate) (PMMA) calibration standards).

RAFT Aqueous Emulsion Polymerization of Methacrylic Acid and Benzyl Methacrylate. A typical protocol for the synthesis of $PDEA_{88}$−P($MAA_{0.7}$-*st*-$BzMA_{0.3}$)$_{100}$ diblock copolymer was as follows: $PDEA_{88}$ macro-CTA (0.1200 g), MAA monomer (0.0505 g, 0.5866 mmol), BzMA (0.0443 g, 0.2514 mmol), ACVA (0.5 mg, 1.676 μmol; CTA/initiator molar ratio = 5.0), and water (1.2198 g, 15% w/w) were weighed into a 25 mL round-bottom flask, and the pH of the reaction solution was adjusted to pH 2.5. The reaction solution was then purged with nitrogen for 30 min and subsequently placed in an oil bath heated to 70 °C. The resulting copolymer was analyzed by THF GPC following methylation with trimethylsilyldiazomethane (M_n = 9600 g mol^{-1}, M_w/M_n = 1.86 vs PMMA standards). ^1H NMR spectroscopy analysis of a sample dissolved in DMSO-d_6 indicated less than 1% residual monomer. DLS studies of a 0.10% w/w copolymer dispersion at pH 2 indicated an intensity-average particle diameter of 43 nm (DLS polydispersity, PDI = 0.09).

Preparation of $PDEA_x$ Macro-CTA Labeled with Rhodamine B Methacrylate. CPDB RAFT agent (0.0141 g, 0.064 mmol), DEA (1.200 g, 7.633 mmol), methacryloxyethyl thiocarbamoyl rhodamine B (0.0261 g, 0.038 16 mmol), AIBN (3.5 mg, 21.203 μmol; CPDB/ACVA molar ratio = 3.0), and toluene (0.8117 g, 60% w/w) were weighed into a round-bottom flask and degassed with nitrogen for 30 min. The reaction solution was then placed in an oil bath heated to 70 °C. The polymerization was allowed to proceed for 6.5 h, resulting in a DEA monomer conversion of 71% and a RhBMA conversion of 74% as determined by ^1H NMR. The crude P(DEA-RhBMA) polymer was purified by dialysis against isopropanol (IPA) using a 1000 MWCO dialysis membrane (Spectrum Laboratories Inc.) to give a pure P(DEA-*stat*-RhBMA) macro-CTA (<1% monomer remaining) with a mean degree of polymerization calculated to be 85 as determined by ^{1}H NMR spectroscopy in $CDCl_3$.

RAFT Aqueous Emulsion Polymerization of Methacrylic Acid and Benzyl Methacrylate with Fluorescein *O*-Methacrylate Fluorescent Label. P(DEA-*stat*-RhBMA)$_{85}$ macro-CTA (0.0800 g), MAA (0.0697 g, 0.8094 mmol), BzMA (0.0611 g, 0.3469 mol), fluorescein *O*-methacrylate (0.9 mg, 2.3124 μmol), ACVA (0.3 mg, 1.156 μmol; CTA/initiator molar ratio = 5.0), and water (1.1962 g, 15% w/w) were weighed into a 25 mL round-bottom flask, and the pH of the reaction solution was adjusted to pH 2.5. The reaction solution was then purged with nitrogen for 30 min and subsequently placed in an oil bath heated to 70 °C. DLS studies of a 0.10% w/w copolymer dispersion at pH 2 indicated an intensity-average particle diameter of 79 nm (DLS polydispersity, PDI = 0.10).

RAFT Solution Polymerization of Benzyl Methacrylate. A typical protocol for the synthesis of PDEA$_{88}$–PBzMA$_{300}$ diblock copolymer was as follows: PDEA$_{88}$ macro-CTA (0.1501 g), BzMA (0.4802 g, 2.7251 mmol), ACVA (0.5 mg, 1.676 μmol; CTA/initiator molar ratio = 5.0), and THF (0.9462 g, 40% w/w) were weighed into a 25 mL round-bottom flask. The reaction solution was then purged with nitrogen for 30 min and subsequently placed in an oil bath heated to 70 °C. The resulting copolymer was analyzed by THF GPC (M_n = 35 200 g mol^{-1}, M_w/M_n = 1.28 vs PMMA standards). ^{1}H NMR spectroscopy analysis of a sample dissolved in DMSO-d_6 indicated a monomer conversion of 74%.

RAFT Solution Polymerization of Methacrylic Acid and Benzyl Methacrylate. A protocol for the synthesis of P(MAA-*stat*-BzMA)$_{100}$ diblock copolymer was as follows: MAA (2.00 g, 23.24 mmol), BzMA (1.7550 g, 9.96 mmol), AIBN (10.9 mg, 66 μmol; CTA/initiator molar ratio = 5.0), and DMF (2.0844 g, 50% w/w) were weighed into a 25 mL round-bottom flask. The reaction solution was then purged with nitrogen for 30 min and subsequently placed in an oil bath heated to 60 °C. The resulting copolymer was analyzed by THF GPC (M_n = 12 200 g mol^{-1}, M_w/M_n = 1.19 vs PMMA standards) following exhaustive methylation with trimethylsilyldiazomethane. ^{1}H NMR spectroscopy analysis of a sample dissolved in DMF-d_7 indicated a MAA monomer conversion of 93% and a BzMA conversion of 80%.

Copolymer Characterization. *^{1}H NMR Spectroscopy.* All ^{1}H NMR spectra were recorded using a 400 MHz Bruker Avance-400 spectrometer using dimethyl sulfoxide-d_6, dimethylformamide-d_7, or deuterated chloroform.

Gel Permeation Chromatography (GPC). The molecular weights and polydispersities of the PDEA macro-CTA and PDEA–P(MAA-*stat*-BzMA) diblock copolymers were determined by GPC using THF eluent. The GPC setup consisted of two 5 μm (30 cm) Mixed C columns and a WellChrom K-2301 refractive index detector operating at 950 ± 30 nm. The mobile phase contained 2.0% v/v triethylamine and 0.05% w/v butylhydroxytoluene (BHT) with a toluene flow rate marker, and the flow rate was fixed at 1.0 mL min^{-1}. Copolymer solutions (1.0% w/v) were prepared in THF. A series of ten near-monodisperse poly(methyl methacrylate) standards (M_p values ranging from 1280 to 330 000 g mol^{-1}) were used for calibration. Data were analyzed using Agilent GPC/SEC software version 1.2.

Dynamic Light Scattering (DLS). The intensity-average hydrodynamic diameter of each batch of spherical diblock copolymer nanoparticles was determined using a Malvern Instruments Zetasizer NanoZS instrument. Aqueous dispersions (0.10% w/w) were analyzed, and data were averaged over three consecutive runs. Measurements were made at 25 °C.

Aqueous Electrophoresis. Zeta potentials were calculated from electrophoretic mobilities using the same Malvern Instruments Zetasizer NanoZS instrument equipped with an autotitrator (MPT-2 multipurpose titrator, Malvern Instruments). Copolymer dispersions were diluted using an aqueous solution of 1 mM KCl to a final concentration of 0.1% w/w solids, and the solution pH was adjusted from 2 to 10 by addition of either dilute NaOH or HCl, as required.

Transmission Electron Microscopy (TEM). Copper/palladium TEM grids (Agar Scientific) were coated in-house to yield a thin film of amorphous carbon. The grids were then subjected to a glow discharge for 30 s to create a hydrophilic surface. Individual samples (0.20% w/w aqueous dispersion, 10.0 μL) were adsorbed onto the freshly treated grids for 1 min and then blotted with filter paper to remove excess solution. To stain the colloidal aggregates, uranyl formate (9.0 μL of a 0.75% w/w solution) was absorbed onto the sample-loaded grid for 20 s and then carefully blotted to remove excess stain. The grids were then dried using a vacuum hose. Imaging was performed using a Philips CM100 instrument operating at 100 kV and equipped with a Gatan 1 k CCD camera.

Fluorescence Spectrophotometry. The fluorescence spectra were recorded with a 1.0 cm glass cuvette using a Horiba Scientific Fluoromax-4 spectrofluorometer and Fluoressence V3 software. Samples were diluted to a maximum visible light absorbance of no greater than 0.1 in order to avoid overloading the detector.

Small-Angle X-ray Scattering. SAXS data were collected using a laboratory SAXS insturment (Xeuss 2.0, Xenocs, France) equipped with a liquid gallium MetalJet X-ray source (Excillum, Sweden, wavelength λ = 0.134 nm), with motorized scatterless slits for beam collimation and a Dectris Pilatus 1M pixel detector (sample-to-detector distances 2.5 and 3.8 m). SAXS patterns were recorded over a q range of 0.004 Å^{-1} < q < 0.24 Å^{-1}, where $q = 4\pi \sin \theta/\lambda$ is the length of the scattering vector and θ is one-half of the scattering angle. A glass capillary of 2 mm diameter was used as a sample holder. Data were reduced using the Foxtrot software package developed by SOLEIL and further analyzed using Irena SAS macros[78] for Igor Pro.

■ RESULTS AND DISCUSSION

The PDEA macro-CTA was prepared at 60% w/w by RAFT solution polymerization in toluene at 70 °C (see Figure S1) using 2-cyanopropyl dithiobenzoate (CPDB) as a CTA. A degree of polymerization (DP) of 140 was targeted, and the DEA polymerization was terminated at 63% conversion to preserve RAFT chain-end functionality, yielding a PDEA macro-CTA with a mean DP of 88 as determined by ^{1}H NMR. Analysis by THF GPC (vs poly(methyl methacrylate) standards) indicated an apparent M_n of 11 600 g mol^{-1} and an M_w/M_n of 1.17, with the latter indicating a well-controlled RAFT polymerization.

This PDEA$_{88}$ macro-CTA was then chain-extended with a mixture of methacrylic acid (MAA) and benzyl methacrylate (BzMA) at pH 2.5 to form sterically stabilized diblock copolymer nanoparticles (Figure 1a). Unlike a previous study reported by Tam and co-workers,[79] 30 mol % BzMA comonomer was introduced to confer additional hydrophobic character to the polyacid block to ensure the formation of relatively compact micelles at low pH. Moreover, the additional pendant benzylic groups provided convenient ^{1}H NMR signals to aid subsequent copolymer characterization studies. [NB: The MAA/BzMA statistical (or possibly gradient) copolymer is expected to have a lower pK_a than that of PMAA homopolymer,[80] for which the pK_a is 5.4.[79,81,82] This is because ionization of the carboxylic acid groups should be easier for the statistical copolymer.[81]]

A series of PDEA$_{88}$–P(MAA-*stat*-BzMA)$_y$ diblock copolymers were synthesized at 15% w/w concentration (Table 1) while targeting core-forming block DPs ranging from 100 to 300, with all such blocks containing 30 mol % BzMA comonomer. Comonomer conversions of more than 97% were obtained within 6 h in all cases, even for the copolymer with the highest P(MAA-*stat*-BzMA) DP. In principle, methylation of the MAA residues in these diblock copolymers should enable THF GPC analysis. This approach is known to work well for other MAA-based copolymers.[83,84] However, in practice using excess trimethylsilyldiazomethane to methylate

Table 1. Summary of Monomer Conversions and GPC Molecular Weight Data Obtained for a Series of PDEA$_{88}$–P(MAA-*stat*-BzMA)$_y$ Diblock Copolymers Synthesized at pH 2.5 via RAFT Aqueous Emulsion Copolymerization of MAA and BzMA at 70 °C[a]

target core DP	MAA conv.[b] (%)	BzMA conv.[b] (%)	actual core DP[b]	M_n[c] (g mol^{-1})	M_w/M_n[c]
100	>99	99	100	9600	1.86
150	98	99	148	10700	1.78
200	>99	>99	199	13000	1.90
300	99	97	295	16000	1.76

[a] Conditions: [macro-CTA]:[initiator] molar ratio = 5.0; total solids concentration = 15% w/w. [b] Comonomer conversions determined by ^{1}H NMR studies in DMSO-d_6. Mean copolymer DP values were estimated from the overall conversion indicated from the residual vinyl comonomer signals in the ^{1}H NMR spectra because the presence of overlapping aromatic signals precluded end-group analysis. [c] Apparent molecular weight determined by THF GPC (refractive index detector, calibrated with poly(methyl methacrylate) standards) following methylation with excess trimethylsilyldiazomethane

PDEA$_{88}$–P(MAA-*stat*-BzMA)$_y$ in THF also caused some unwanted quaternization of the PDEA block.

After their methylation, the PDEA$_{88}$–P(MAA-*stat*-BzMA)$_y$ diblock copolymers exhibited relatively broad molecular weight distributions (MWD) when analyzed by GPC (see Figure S3a). However, control experiments confirmed that low degrees of quaternization were obtained when a near-monodisperse PDEA$_{88}$ homopolymer was subjected to the same methylation conditions, which led to an apparently broad MWD as a result of column adsorption problems (see Figure S3b,c). Thus, the GPC data obtained for such diblock copolymers are not a true reflection of their real MWDs, which are believed to be significantly narrower.

Unfortunately, attempts to use a stoichiometric quantity of trimethylsilyldiazomethane based on MAA residues only led to incomplete methylation (data not shown). However, chain extension of the PDEA$_{88}$ macro-CTA using just BzMA produced a relatively low dispersity (M_w/M_n = 1.28) and a relatively high blocking efficiency (Figure S3d). Moreover, THF GPC analysis of a fully methylated P(MAA-*stat*-BzMA)$_{89}$ statistical copolymer indicates a narrow MWD (M_w/M_n = 1.19). Thus, similar pseudoliving character is assumed for the PDEA$_{88}$–P(MAA-*stat*-BzMA)$_y$ diblock copolymers examined in this study.

Dynamic light scattering (DLS) was used to determine the intensity-average diameter of the nanoparticles after dilution to 0.10% w/w at pH 2 (Figure 2a). This technique indicated relatively narrow particle size distributions, with diameters ranging between 37 and 46 nm. A monotonic increase in particle size with increasing core DP was observed, which is typical for PISA formulations.[85−87] This can be fitted to a power law of the form $d = kN^{\alpha}$, where d is the core diameter, N is the mean DP of the core-forming block, and k depends on the Flory–Huggins parameter (see Figure S4).[86,88] An α exponent of 0.29 was calculated for this system. Comparison with our previous results for the RAFT aqueous emulsion polymerization of similar monomers[86,89] suggests that this size dependence is partly determined by the character of the polyelectrolytic stabilizer block (Figure S4). TEM studies show the presence of spherical nanoparticles (Figure 2a). This is confirmed by fitting the SAXS data to a spherical micelle model (see Figure 2c and Figure S5).

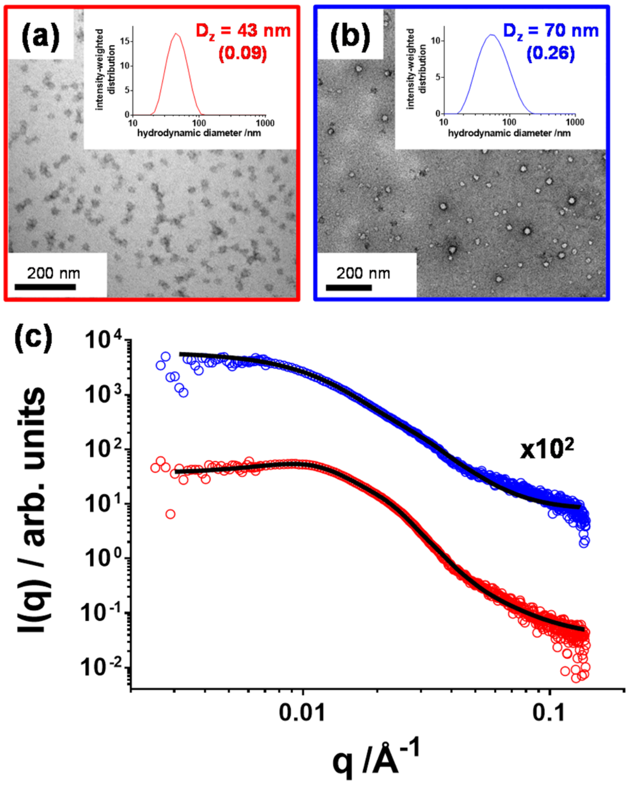

Figure 2. PDEA$_{88}$–P(MAA-*stat*-BzMA)$_{199}$ diblock copolymer prepared at 15% w/w by RAFT aqueous emulsion copolymerization at 70 °C: (a) TEM image obtained for PDEA-core micelles at pH 2 with inset DLS intensity-average size distributions recorded for 0.1% w/w aqueous dispersions at pH 2 and (b) P(MAA-*stat*-BzMA)-core micelles at pH 10 with inset DLS intensity-average size distributions recorded for 0.1% w/w aqueous dispersions at pH 10; (c) SAXS curves at pH 2 (red) and pH 10 (blue). Solid lines show fits to the data using a spherical micelle model.

When the pH of the dispersion is increased to pH 10, inverse micellization occurs as the amine group on the PDEA block is now deprotonated and hence uncharged, but the acidic PMAA residues become ionized. As a result, the hydrophobic PDEA block now forms the micelle core, stabilized by the anionic P(MAA-*stat*-BzMA) block. These micelles were also analyzed by DLS (Figure 2b) and found to be both somewhat larger and more polydisperse than those formed at pH 2. Spherical nanoparticles were observed by TEM (Figure 2b). SAXS studies verified that the nanoparticles were indeed spherical at pH 10, although somewhat polydisperse (Figure 2c and Figure S5).

Dilute aqueous dispersions of PDEA$_{88}$–P(MAA-*stat*-BzMA)$_y$ were analyzed by DLS (Figure 3a and Figure S6a) and aqueous electrophoresis as a function of pH (Figure 3b and Figure S6b). Relatively small cationic nanoparticles are observed at pH 2 with zeta potentials of approximately +40 mV. The dispersions became much more turbid between pH 6–8, indicating macroscopic flocculation at around the isoelectric point (IEP) (see Figure 3a). Above pH 8, weakly turbid micellar dispersions of highly anionic nanoparticles were obtained, with zeta potentials of approximately −40 mV. The IEP varied with copolymer composition, ranging from pH 7.57 for the shortest P(MAA-*stat*-BzMA) block (DP 100) to 6.32 for the longest block (DP 295) (Figure S6 and Table S1). Clearly, more

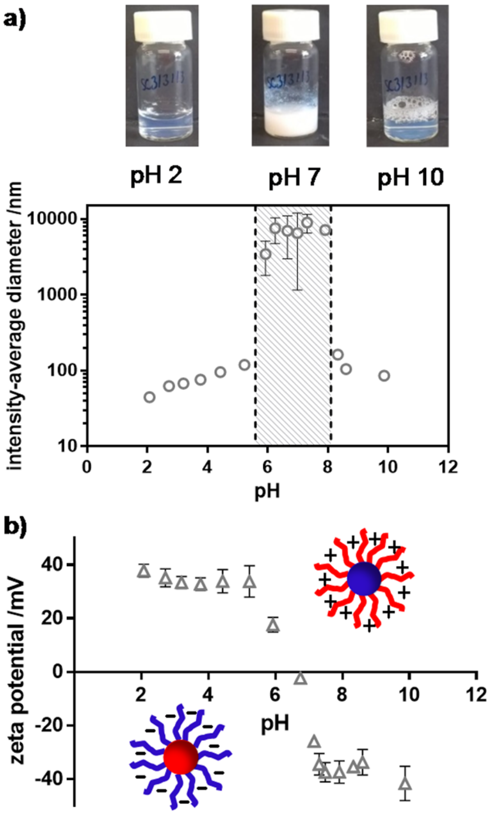

Figure 3. (a) Variation in hydrodynamic diameter as a function of solution pH for a 0.1% w/w aqueous dispersion of a $PDEA_{88}$–$P(MAA\text{-}stat\text{-}BzMA)_{199}$ copolymer (30 mol % BzMA content) at 25 °C, with digital photographs recorded at pH 2, 7, and 10. (b) Zeta potential vs pH curve recorded for a dilute aqueous dispersion of the same copolymer at 25 °C. All DLS data include standard deviations, but some lie within the size of the symbols used for the data points.

asymmetric copolymers exhibit lower IEPs as their PMAA content is increased.[90]

Schizophrenic micellization behavior was confirmed by studying the relative solvation of the polyacid and polybase blocks of these ampholytic copolymers using ^1H NMR spectroscopy. In DMSO-d_6, both the P(MAA-*stat*-BzMA) and PDEA blocks are well-solvated; hence all the expected signals are observed in the spectra (Figure S7a, peaks A–F correspond to PDEA, G and H are due to PMAA, and J–L are assigned to PBzMA). However, PDEA-core micelles are formed in D_2O/NaOD (adjusted to pH 10), so only those NMR signals corresponding to the MAA and BzMA residues are observed (Figure S7b). Conversely, only PDEA signals are visible in a DCl/D_2O mixture at pH 2 (Figure S7c), indicating that the polyacid block forms non-solvated micelle cores under these conditions.

We hypothesized that appropriate fluorescent labeling of such schizophrenic ampholytic diblock copolymers should enable their application as colorimetric and/or pH sensors. A rhodamine B label was chosen for the PDEA block. This chromophore, although pH-insensitive, is quenched by the deprotonated tertiary amine moieties in the PDEA block and thus only displays fluorescence below pH 6.[91,92] In contrast, fluorescein only displays fluorescence above pH 7 when its carboxylic group becomes ionized;[93] thus this chromophore was selected to label the P(MAA-*stat*-BzMA) block. Aggregation-induced quenching (AIQ)[94] most likely occurs for the rhodamine B and fluorescein labels when located within the nanoparticle cores at high and low pH, respectively. Both labels and their derivatives provide high molar extinction coefficients and fluorescence quantum yields and thus have been widely used as fluorescent probes. Dual-labeled copolymers have been developed previously to act as ratiometric pH probes by monitoring the fluorescence emission of two different species.[92,95] These two chromophores are also known to be a FRET pair, which can further enhance the efficacy of the pH sensor.[92,96]

Methacrylate-functional derivatives of these two fluorescent labels were incorporated at relatively low levels during the ampholytic copolymer PISA synthesis in each case (Figure S8). Thus, rhodamine B methacrylate (RhBMA) was statistically copolymerized with DEA to produce a rhodamine B-labeled $P(DEA_{85}\text{-}stat\text{-}RhBMA_{0.4})$ macro-CTA. Then fluorescein *O*-methacrylate (FMA) was added along with MAA and BzMA in the subsequent chain extension under PISA conditions to produce bifluorescent schizophrenic $P(DEA_{85}\text{-}stat\text{-}RhBMA_{0.4})$-$P(MAA_{139}\text{-}stat\text{-}BzMA_{49}\text{-}stat\text{-}FMA_{0.4})$ nanoparticles. Following exhaustive dialysis to remove any unreacted labels, these copolymers were characterized using both visible absorption and fluorescence spectroscopy.

PDEA-core nanoparticles are formed at pH 10, so fluorescence arising from the rhodamine label is largely quenched by the deprotonated tertiary amine groups obtained under these conditions, in addition to any AIQ effect. Since the fluorescein label is located on the P(MAA-*stat*-BzMA-*stat*-FMA) stabilizer chains that form the nanoparticle shell under these conditions, the latter chromophore becomes active, and so its fluorescence emission is observed at 509 nm when using an excitation wavelength of 470 nm (Figure 4b). As a result, a dilute aqueous dispersion of such nanoparticles appears yellow-green at pH 10 (see inset, Figure 4b).

However, the fluorescein label is no longer active when located within the micelle cores at pH 2, whereas the P(DEA-*stat*-RhBMA)-stabilizer chains become highly fluorescent under such conditions (peak at λ_{em} = 578 nm when using λ_{ex} = 525 nm; Figure 4a). In this case, a bright pink copolymer dispersion is observed (see inset, Figure 4a). The relative fluorescence intensity at the λ_{max} for each label can be calculated, which is denoted as I_{578}/I_{509} (Figure 4c). This I_{578}/I_{509} ratio is reduced dramatically from 52.4 to 0.138 as the pH is adjusted from 2 to 10. This corresponds to an 18-fold *reduction* in the rhodamine B signal and a 28-fold *enhancement* of the fluorescein signal. In principle, such behavior enables these schizophrenic micelles to act as self-reporting nanoparticles, providing information on their local pH via both colorimetry and fluorescence. This complementary fluorescence behavior also confirms the pH-driven location of each fluorophore within either the anionic (fluorescein) or cationic (rhodamine B) nanoparticle shell, thus providing compelling additional evidence for the schizophrenic nature of these nanoparticles.

■ CONCLUSIONS

In summary, doubly pH-responsive ampholytic diblock copolymer nanoparticles can be conveniently prepared via RAFT-mediated PISA in acidic aqueous media. Such formulations are a substantial improvement on earlier synthetic routes, which typically involve organic solvents and sometimes require protecting group chemistry. Aqueous electrophoresis, DLS and TEM studies confirmed that these diblock copolymers exhibit schizophrenic behavior: they exist in the form of

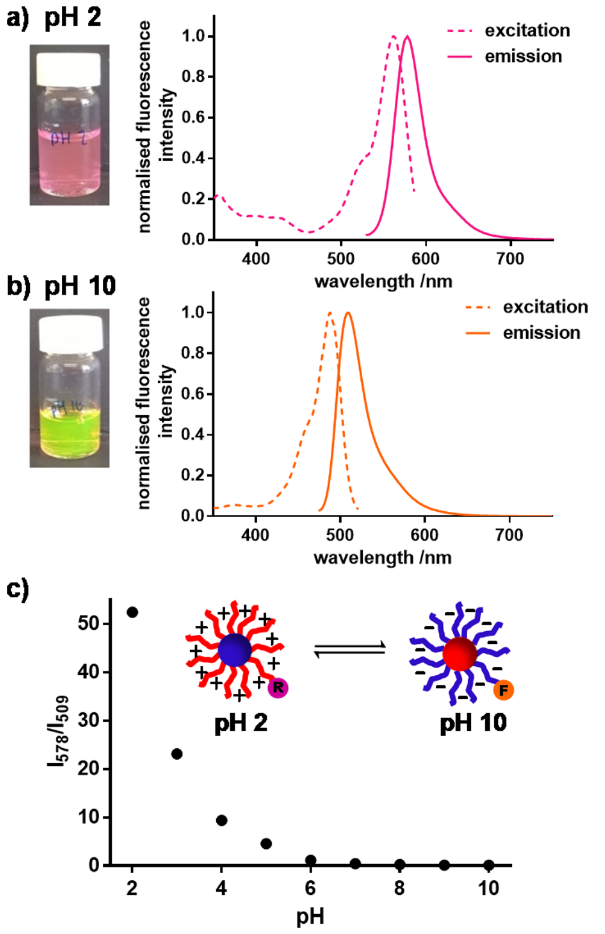

Figure 4. (a) Fluorescence spectra of schizophrenic P(DEA$_{85}$-*stat*-RhBMA$_{0.4}$)−P(MAA$_{139}$-*stat*-BzMA$_{49}$-*stat*-FMA$_{0.4}$) micelles recorded at pH 2 showing the excitation and emission of "active" rhodamine B (R) labels (excitation at λ_{em} = 595 nm, emission at λ_{ex} = 525 nm) with an inset digital photograph illustrating the resulting pink coloration; (b) fluorescence spectra recorded at pH 10 showing the excitation and emission of "active" fluorescein (F) labels (excitation at λ_{em} = 525 nm, emission at λ_{ex} = 470 nm) with an inset digital photograph illustrating the resulting yellow-green coloration; and (c) variation in fluorescence intensity ratio, I_{578}/I_{509}, between R and F labels with pH, with inset schematic cartoon showing the switch between anionic micelles at pH 10 containing "active" fluorescein labels and cationic micelles at pH 2 containing "active" rhodamine B labels.

cationic spheres below pH 6 and as anionic spheres above pH 8, with macroscopic flocculation occurring reversibly at intermediate pH. Moreover, inserting an appropriate fluorescent label into each block during the copolymer synthesis produced novel self-reporting nanoparticles that are both colorimetric and bifluorescent via aggregation-induced quenching.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.7b01005.

PDEA$_x$ reaction scheme, THF GPC curves, plot of mean particle diameter vs mean DP, further SAXS curves, table of IEP vs core DP, further pH titrations by DLS and aqueous electrophoresis, assigned ^{1}H NMR spectra showing changes in polymer solvation, P(DEA$_{85}$-*stat*-RhBMA$_{0.4}$)−P(MAA$_{139}$-*stat*-BzMA$_{49}$-*stat*-FMA$_{0.4}$) reaction scheme (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Antonietti, M.; Förster, S. Vesicles and Liposomes: A Self-Assembly Principle Beyond Lipids. *Adv. Mater.* **2003**, *15*, 1323−1333.

(2) Discher, D. E.; Eisenberg, A. Polymer Vesicles. *Science* **2002**, *297*, 967−973.

(3) Blanazs, A.; Armes, S. P.; Ryan, A. J. Self-Assembled Block Copolymer Aggregates: From Micelles to Vesicles and their Biological Applications. *Macromol. Rapid Commun.* **2009**, *30*, 267−277.

(4) Mai, Y. Y.; Eisenberg, A. Self-Assembly of Block Copolymers. *Chem. Soc. Rev.* **2012**, *41*, 5969−5985.

(5) Dai, S.; Ravi, P.; Tam, K. C. pH-Responsive Polymers: Synthesis, Properties and Applications. *Soft Matter* **2008**, *4*, 435−449.

(6) Bae, Y.; Fukushima, S.; Harada, A.; Kataoka, K. Design of Environment-Sensitive Supramolecular Assemblies for Intracellular Drug Delivery: Polymeric Micelles that are Responsive to Intracellular pH Change. *Angew. Chem., Int. Ed.* **2003**, *42*, 4640−4643.

(7) Kocak, G.; Tuncer, C.; Bütün, V. pH-Responsive Polymers. *Polym. Chem.* **2017**, *8*, 144−176.

(8) Wei, M.; Gao, Y.; Li, X.; Serpe, M. J. Stimuli-Responsive Polymers and their Applications. *Polym. Chem.* **2017**, *8*, 127−143.

(9) Moad, G. RAFT Polymerization to form Stimuli-Responsive Polymers. *Polym. Chem.* **2017**, *8*, 177−219.

(10) Pelton, R. Temperature-Sensitive Aqueous Microgels. *Adv. Colloid Interface Sci.* **2000**, *85*, 1−33.

(11) Dimitrov, I.; Trzebicka, B.; Müller, A. H. E.; Dworak, A.; Tsvetanov, C. B. Thermosensitive Water-Soluble Copolymers with Doubly Responsive Reversibly Interacting Entities. *Prog. Polym. Sci.* **2007**, *32*, 1275−1343.

(12) Strandman, S.; Zhu, X. X. Thermo-Responsive Block Copolymers with Multiple Phase Transition Temperatures in Aqueous Solutions. *Prog. Polym. Sci.* **2015**, *42*, 154−176.

(13) Roy, D.; Brooks, W. L. A.; Sumerlin, B. S. New Directions in Thermoresponsive Polymers. *Chem. Soc. Rev.* **2013**, *42*, 7214−7243.

(14) Bütün, V.; Billingham, N. C.; Armes, S. P. Unusual Aggregation Behavior of a Novel Tertiary Amine Methacrylate-Based Diblock Copolymer: Formation of Micelles and Reverse Micelles in Aqueous Solution. *J. Am. Chem. Soc.* **1998**, *120*, 11818−11819.

(15) Bütün, V.; Liu, S.; Weaver, J. V. M; Bories-Azeau, X.; Cai, Y.; Armes, S. P. A Brief Review of 'Schizophrenic' Block Copolymers. *React. Funct. Polym.* **2006**, *66* (66), 157−165.

(16) Liu, S.; Billingham, N. C.; Armes, S. P. A Schizophrenic Water-Soluble Diblock Copolymer. *Angew. Chem., Int. Ed.* **2001**, *40*, 2328−2331.

(17) Liu, S.; Armes, S. P. Polymeric Surfactants for the New Millennium: A pH-Responsive, Zwitterionic, Schizophrenic Diblock Copolymer. *Angew. Chem., Int. Ed.* **2002**, *41*, 1413−1416.

(18) Roy, D.; Cambre, J. N.; Sumerlin, B. S. Triply-Responsive Boronic Acid Block Copolymers: Solution Self-Assembly Induced by

Changes in Temperature, pH, or Sugar Concentration. *Chem. Commun.* **2009**, *7345*, 2106−8.

(19) Rodríguez-Hernández, J.; Lecommandoux, S. Reversible Inside-Out Micellization of pH-Responsive and Water-Soluble Vesicles based on Polypeptide Diblock Copolymers. *J. Am. Chem. Soc.* **2005**, *127*, 2026−2027.

(20) Vo, C.-D.; Armes, S. P.; Randall, D. P.; Sakai, K.; Biggs, S. Synthesis of Zwitterionic Diblock Copolymers without Protecting Group Chemistry. *Macromolecules* **2007**, *40*, 157−167.

(21) Wang, D.; Yin, J.; Zhu, Z.; Ge, Z.; Liu, H.; Armes, S. P.; Liu, S. Micelle Formation and Inversion Kinetics of a Schizophrenic Diblock Copolymer. *Macromolecules* **2006**, *39*, 7378−7385.

(22) Cai, Y.; Armes, S. P. Synthesis of Well-Defined Y-Shaped Zwitterionic Block Copolymers via Atom-Transfer Radical Polymerization. *Macromolecules* **2005**, *38*, 271−279.

(23) Bories-Azeau, X.; Armes, S. P.; Van Den Haak, H. J. W. Facile Synthesis of Zwitterionic Diblock Copolymers without Protecting Group Chemistry. *Macromolecules* **2004**, *37*, 2348−2352.

(24) Liu, S.; Armes, S. P. Synthesis and Aqueous Solution Behavior of a pH-Responsive Schizophrenic Diblock Copolymer. *Langmuir* **2003**, *19*, 4432−4438.

(25) Goloub, T.; de Keizer, A.; Cohen Stuart, M. A. Association Behavior of Ampholytic Diblock Copolymers. *Macromolecules* **1999**, *32*, 8441−8446.

(26) Gohy, J.-F.; Creutz, S.; Garcia, M.; Mahltig, B.; Stamm, M.; Jérôme, R. Aggregates Formed by Amphoteric Diblock Copolymers in Water. *Macromolecules* **2000**, *33*, 6378−6387.

(27) André, X.; Zhang, M.; Müller, A. H. E. Thermo- and pH-Responsive Micelles of Poly(acrylic acid)-*block*-Poly(N,N-diethylacrylamide). *Macromol. Rapid Commun.* **2005**, *26*, 558−563.

(28) Mao, B. W.; Gan, L. H.; Gan, Y. Y.; Tam, K. C.; Tan, O. K. Controlled One-Pot Synthesis of pH-Sensitive Self-Assembled Diblock Copolymers and their Aggregation Behavior. *Polymer* **2005**, *46*, 10045−10055.

(29) Agut, W.; Brûlet, A.; Schatz, C.; Taton, D.; Lecommandoux, S. pH and Temperature Responsive Polymeric Micelles and Polymersomes by Self-Assembly of Poly[2-(dimethylamino)ethyl methacrylate]-b-Poly(glutamic acid) Double Hydrophilic Block Copolymers. *Langmuir* **2010**, *26*, 10546−10554.

(30) Chen, L.; Chen, T.; Fang, W.; Wen, Y.; Lin, S.; Lin, J.; Cai, C. Synthesis and pH-Responsive "Schizophrenic" Aggregation of a Linear-Dendron-Like Polyampholyte Based on Oppositely Charged Polypeptides. *Biomacromolecules* **2013**, *14*, 4320−4330.

(31) Zhang, J.; Xiao, Y.; Luo, X.; Wen, L.; Heise, A.; Lang, M. Schizophrenic Poly(ε-caprolactone)s: Synthesis, Self-Assembly and Fluorescent Decoration. *Polym. Chem.* **2017**, *8*, 3261−3270.

(32) Weaver, J. V. M; Armes, S. P.; Bütün, V. Synthesis and Aqueous Solution Properties of a Well-Defined Thermo-Responsive Schizophrenic Diblock Copolymer. *Chem. Commun.* **2002**, 2122−2123.

(33) Arotçaréna, M.; Heise, B.; Ishaya, S.; Laschewsky, A. Switching the Inside and the Outside of Aggregates of Water-Soluble Block Copolymers with Double Thermoresponsivity. *J. Am. Chem. Soc.* **2002**, *124*, 3787−3793.

(34) Virtanen, J.; Arotçaréna, M.; Heise, B.; Ishaya, S.; Laschewsky, A.; Tenhu, H. Dissolution and Aggregation of a Poly(NIPA-*block*-sulfobetaine) Copolymer in Water and Saline Aqueous Solutions. *Langmuir* **2002**, *18*, 5360−5365.

(35) Vishnevetskaya, N. S.; Hildebrand, V.; Niebuur, B.-J.; Grillo, I.; Filippov, S. K.; Laschewsky, A.; Müller-Buschbaum, P.; Papadakis, C. M. Aggregation Behavior of Doubly Thermoresponsive Polysulfobetaine- b -poly(N -isopropylacrylamide) Diblock Copolymers. *Macromolecules* **2016**, *49*, 6655−6668.

(36) Wang, D.; Wu, T.; Wan, X.; Wang, X.; Liu, S. Purely Salt-Responsive Micelle Formation and Inversion Based on a Novel Schizophrenic Sulfobetaine Block Copolymer: Structure and Kinetics of Micellization. *Langmuir* **2007**, *23*, 11866−11874.

(37) Smith, A. E.; Xu, X.; Kirkland-York, S. E.; Savin, D. A.; McCormick, C. L. "Schizophrenic" Self-Assembly of Block Copolymers Synthesized via Aqueous RAFT Polymerization: From Micelles to Vesicles. *Macromolecules* **2010**, *43*, 1210−1217.

(38) Ge, Z.; Cai, Y.; Yin, J.; Zhu, Z.; Rao, J.; Liu, S. Synthesis and 'Schizophrenic' Micellization of Double Hydrophilic AB 4 Miktoarm Star and AB Diblock Copolymers: Structure and Kinetics of Micellization. *Langmuir* **2007**, *23*, 1114−1122.

(39) Jiang, X.; Ge, Z.; Xu, J.; Liu, H.; Liu, S. Fabrication of Multiresponsive Shell Cross-Linked Micelles Possessing pH-Controllable Core Swellability and Thermo-Tunable Corona Permeability. *Biomacromolecules* **2007**, *8*, 3184−3192.

(40) Rao, J.; Luo, Z.; Ge, Z.; Liu, H.; Liu, S. "Schizophrenic" Micellization Associated with Coil-to-Helix Transitions Based on Polypeptide Hybrid Double Hydrophilic Rod-Coil Diblock Copolymer. *Biomacromolecules* **2007**, *8*, 3871−3878.

(41) Zhang, Y.; Wu, T.; Liu, S. Micellization Kinetics of a Novel Multi-Responsive Double Hydrophilic Diblock Copolymer Studied by Stopped-Flow pH and Temperature Jump. *Macromol. Chem. Phys.* **2007**, *208*, 2492−2501.

(42) Jiang, X.; Zhang, G.; Narain, R.; Liu, S. Fabrication of Two Types of Shell-Cross-Linked Micelles with "Inverted" Structures in Aqueous Solution from Schizophrenic Water-Soluble ABC Triblock Copolymer via Click Chemistry. *Langmuir* **2009**, *25*, 2046−2054.

(43) Chang, C.; Wei, H.; Feng, J.; Wang, Z. C.; Wu, X. J.; Wu, D. Q.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. Temperature and pH Double Responsive Hybrid Cross-Linked Micelles Based on P(NIPAAm-co-MPMA)-b-P(DEA): RAFT Synthesis and "Schizophrenic" Micellization. *Macromolecules* **2009**, *42*, 4838−4844.

(44) Bastakoti, B. P.; Guragain, S.; Nakashima, K.; Yamauchi, Y. Stimuli-Induced Core − Corona Inversion of Micelle of Poly(acrylic acid)-*block*-Poly(N-isopropylacrylamide) and its Application in Drug Delivery. *Macromol. Chem. Phys.* **2015**, *216*, 287−291.

(45) Savoji, M. T.; Strandman, S.; Zhu, X. X. Invertible Vesicles and Micelles Formed by Dually-Responsive Diblock Random Copolymers in Aqueous Solutions. *Soft Matter* **2014**, *10*, 5886−5893.

(46) Bütün, V.; Top, R. B.; Ufuklar, S. Synthesis and Characterization of Novel "Schizophrenic" Water-Soluble Triblock Copolymers and Shell Cross-Linked Micelles. *Macromolecules* **2006**, *39*, 1216−1225.

(47) Feng, A.; Zhan, C.; Yan, Q.; Liu, B.; Yuan, J. A CO_2- and Temperature-Switchable "Schizophrenic" Block Copolymer: from Vesicles to Micelles. *Chem. Commun.* **2014**, *50*, 8958−8961.

(48) Shih, Y. J.; Chang, Y.; Deratani, A.; Quemener, D. "Schizophrenic" Hemocompatible Copolymers via Switchable Thermoresponsive Transition of Nonionic/Zwitterionic Block Self-Assembly in Human Blood. *Biomacromolecules* **2012**, *13*, 2849−2858.

(49) Patrickios, C. S.; Hertler, W. R.; Hatton, T. A. Protein Complexation with Acrylic Polyampholytes. *Biotechnol. Bioeng.* **1994**, *44*, 1031−1039.

(50) Warren, N. J.; Armes, S. P. Polymerization-Induced Self-Assembly of Block Copolymer Nano-Objects via RAFT Aqueous Dispersion Polymerization. *J. Am. Chem. Soc.* **2014**, *136*, 10174−10185.

(51) Derry, M. J.; Fielding, L. A.; Armes, S. P. Polymerization-Induced Self-Assembly of Block Copolymer Nanoparticles via RAFT Non-Aqueous Dispersion Polymerization. *Prog. Polym. Sci.* **2016**, *52*, 1−18.

(52) Canning, S. L.; Smith, G. N.; Armes, S. P. A Critical Appraisal of RAFT-Mediated Polymerization-Induced Self-Assembly. *Macromolecules* **2016**, *49*, 1985−2001.

(53) Sun, J. T.; Hong, C. Y.; Pan, C. Y. Formation of the Block Copolymer Aggregates via Polymerization-Induced Self-Assembly and Reorganization. *Soft Matter* **2012**, *8*, 7753−7767.

(54) Charleux, B.; Delaittre, G.; Rieger, J.; D'Agosto, F. Polymerization-Induced Self-Assembly: From Soluble Macromolecules to Block Copolymer Nano-Objects in One Step. *Macromolecules* **2012**, *45*, 6753−6765.

(55) Rieger, J. Guidelines for the Synthesis of Block Copolymer Particles of Various Morphologies by RAFT Dispersion Polymerization. *Macromol. Rapid Commun.* **2015**, *36*, 1458−1471.

DOI: 10.1021/acs.macromol.7b01005
Macromolecules 2017, 50, 6108−6116

(56) Blanazs, A.; Madsen, J.; Battaglia, G.; Ryan, A. J.; Armes, S. P. Mechanistic Insights for Block Copolymer Morphologies: How Do Worms Form Vesicles? *J. Am. Chem. Soc.* **2011**, *133*, 16581−16587.

(57) Chaduc, I.; Zhang, W.; Rieger, J.; Lansalot, M.; D'Agosto, F.; Charleux, B. Amphiphilic Block Copolymers from a Direct and One-Pot RAFT Synthesis in Water. *Macromol. Rapid Commun.* **2011**, *32*, 1270−1276.

(58) Boissé, S.; Rieger, J.; Belal, K.; Di-Cicco, A.; Beaunier, P.; Li, M. H.; Charleux, B. Amphiphilic Block Copolymer Nano-Fibers via RAFT-Mediated Polymerization in Aqueous Dispersed Systems. *Chem. Commun.* **2010**, *46*, 1950−1952.

(59) Zhou, W.; Qu, Q.; Xu, Y.; An, Z. Aqueous Polymerization-Induced Self-Assembly for the Synthesis of Ketone-Functionalized Nano-Objects with Low Polydispersity. *ACS Macro Lett.* **2015**, *4*, 495−499.

(60) Jiang, Y.; Xu, N.; Han, J.; Yu, Q.; Guo, L.; Gao, P.; Lu, X.; Cai, Y. The Direct Synthesis of Interface-Decorated Reactive Block Copolymer Nanoparticles via Polymerisation-Induced Self-Assembly. *Polym. Chem.* **2015**, *6*, 4955−4965.

(61) Zhang, X.; Boissé, S.; Zhang, W.; Beaunier, P.; D'Agosto, F.; Rieger, J.; Charleux, B. Well-Defined Amphiphilic Block Copolymers and Nano-objects Formed in Situ via RAFT-Mediated Aqueous Emulsion Polymerization. *Macromolecules* **2011**, *44*, 4149−4158.

(62) Ferguson, C. J.; Hughes, R. J.; Nguyen, D.; Pham, B. T. T.; Gilbert, R. G.; Serelis, A. K.; Such, C. H.; Hawkett, B. S. Ab Initio Emulsion Polymerization by RAFT-Controlled Self-Assembly. *Macromolecules* **2005**, *38*, 2191−2204.

(63) Ferguson, C. J.; Hughes, R. J.; Pham, B. T. T.; Hawkett, B. S.; Gilbert, R. G.; Serelis, A. K.; Such, C. H. Effective Ab Initio Emulsion Polymerization under RAFT Control. *Macromolecules* **2002**, *35*, 9243−9245.

(64) Pei, Y.; Lowe, A. B.; Roth, P. J. Stimulus-Responsive Nanoparticles and Associated (Reversible) Polymorphism via Polymerization Induced Self-assembly (PISA). *Macromol. Rapid Commun.* **2017**, *38*, 1600528.

(65) Pei, Y.; Thurairajah, L.; Sugita, O. R.; Lowe, A. B. RAFT Dispersion Polymerization in Nonpolar Media: Polymerization of 3-Phenylpropyl Methacrylate in n-Tetradecane with Poly(stearyl methacrylate) Homopolymers as Macro Chain Transfer Agents. *Macromolecules* **2015**, *48*, 236−244.

(66) Pei, Y.; Dharsana, N. C.; Van Hensbergen, J. A.; Burford, R. P.; Roth, P. J.; Lowe, A. B. RAFT Dispersion Polymerization of 3-Phenylpropyl Methacrylate with Poly[2-(dimethylamino)ethyl methacrylate] Macro-CTAs in Ethanol and Associated Thermoreversible Polymorphism. *Soft Matter* **2014**, *10*, 5787−5796.

(67) Blanazs, A.; Verber, R.; Mykhaylyk, O. O.; Ryan, A. J.; Heath, J. Z.; Douglas, C. W. I.; Armes, S. P. Sterilizable Gels from Thermoresponsive Block Copolymer Worms. *J. Am. Chem. Soc.* **2012**, *134*, 9741−9748.

(68) Verber, R.; Blanazs, A.; Armes, S. P. Rheological Studies of Thermo-Responsive Diblock Copolymer Worm Gels. *Soft Matter* **2012**, *8*, 9915−9922.

(69) Fielding, L. A.; Lane, J. A.; Derry, M. J.; Mykhaylyk, O. O.; Armes, S. P. Thermo-Responsive Diblock Copolymer Worm Gels in Non-Polar Solvents. *J. Am. Chem. Soc.* **2014**, *136*, 5790−5798.

(70) Lovett, J. R.; Warren, N. J.; Ratcliffe, L. P. D.; Kocik, M. K.; Armes, S. P. pH-Responsive Non-Ionic Diblock Copolymers: Ionization of Carboxylic Acid End-Groups Induces an Order-Order Morphological Transition. *Angew. Chem.* **2015**, *127*, 1295−1299.

(71) Penfold, N. J. W.; Lovett, J. R.; Warren, N. J.; Verstraete, P.; Smets, J.; Armes, S. P. pH-Responsive Non-Ionic Diblock Copolymers: Protonation of a Morpholine End-Group Induces an Order−Order Transition. *Polym. Chem.* **2016**, *7*, 79−88.

(72) Lowe, A. B.; Billingham, N. C.; Armes, S. P. Synthesis and Characterization of Zwitterionic Block Copolymers. *Macromolecules* **1998**, *31*, 5991−5998.

(73) Auschra, C.; Eckstein, E.; Mühlebach, A.; Zink, M. O.; Rime, F. Design of New Pigment Dispersants by Controlled Radical Polymerization. *Prog. Org. Coat.* **2002**, *45*, 83−93.

(74) Creutz, S.; Jerome, R.; Kaptijn, G. M. P.; Van Der Werf, A. W.; Akkerman, J. M. Design of Polymeric Dispersants for Waterborne Coatings. *J. Coat. Technol.* **1998**, *70*, 41−46.

(75) Creutz, S.; Jérôme, R. Effectiveness of Block Copolymers as Stabilizers for Aqueous Titanium Dioxide Dispersions of a High Solid Content. *Prog. Org. Coat.* **2000**, *40*, 21−29.

(76) Alarcon, C.; Pennadam, S.; Alexander, C. Stimuli Responsive Polymers for Biomedical Applications. *Chem. Soc. Rev.* **2005**, *34*, 276−285.

(77) Bütün, V.; Armes, S. P.; Billingham, N. C. Synthesis and Aqueous Solution Properties of Near-Monodisperse Tertiary Amine Methacrylate Homopolymers and Diblock Copolymers. *Polymer* **2001**, *42*, 5993−6008.

(78) Ilavsky, J.; Jemian, P. R. Irena: Tool Suite for Modeling and Analysis of Small-Angle Scattering. *J. Appl. Crystallogr.* **2009**, *42*, 347−353.

(79) Dai, S.; Ravi, P.; Tam, K. C.; Mao, B. W.; Gan, L. H. Novel pH-Responsive Amphiphilic Diblock Copolymers with Reversible Micellization Properties. *Langmuir* **2003**, *19*, 5175−5177.

(80) Yessine, M. A.; Lafleur, M.; Meier, C.; Petereit, H. U.; Leroux, J. C. Characterization of the Membrane-Destabilizing Properties of Different pH-Sensitive Methacrylic Acid Copolymers. *Biochim. Biophys. Acta, Biomembr.* **2003**, *1613*, 28−38.

(81) Merle, Y. Synthetic Polyampholytes. 5. Influence of Nearest-Neighbor Interactions on Potentiometric Curves. *J. Phys. Chem.* **1987**, *91*, 3092−3098.

(82) van Treslong, C. J. B. Evaluation of Potentiometric Data of Weak Polyelectrolytes Taking Account of Nearest-Neighbour Interaction. *Recl. des Trav. Chim. des Pays-Bas* **1978**, *97*, 13−21.

(83) Mykytiuk, J.; Armes, S. P.; Billingham, N. C. Group-Transfer Polymerization of Benzyl Methacrylate: A Convenient Method for Synthesis of Near-Monodisperse Poly(methacrylic acid)s. *Polym. Bull.* **1992**, *29*, 139−145.

(84) Katchalsky, A.; Eisenberg, H. Molecular Weight of Polyacrylic and Polymethacrylic Acid. *J. Polym. Sci.* **1951**, *6*, 145−154.

(85) Sugihara, S.; Blanazs, A.; Armes, S. P.; Ryan, A. J.; Lewis, A. L. Aqueous Dispersion Polymerization: A New Paradigm for In Situ Block Copolymer Self-Assembly in Concentrated Solution. *J. Am. Chem. Soc.* **2011**, *133*, 15707−15713.

(86) Cunningham, V. J.; Alswieleh, A. M.; Thompson, K. L.; Williams, M.; Leggett, G. J.; Armes, S. P.; Musa, O. M. Poly(glycerol monomethacrylate)−Poly(benzyl methacrylate) Diblock Copolymer Nanoparticles via RAFT Emulsion Polymerization: Synthesis, Characterization, and Interfacial Activity. *Macromolecules* **2014**, *47*, 5613−5623.

(87) Semsarilar, M.; Ladmiral, V.; Blanazs, A.; Armes, S. P. Poly(methacrylic acid)-Based AB and ABC Block Copolymer Nano-Objects Prepared via RAFT Alcoholic Dispersion Polymerization. *Polym. Chem.* **2014**, *5*, 3466−3475.

(88) Battaglia, G.; Ryan, A. Bilayers and Interdigitation in Block Copolymer Vesicles. *J. Am. Chem. Soc.* **2005**, *127*, 8757−8764.

(89) Cockram, A. A.; Neal, T. J.; Derry, M. J.; Mykhaylyk, O. O.; Williams, N. S. J.; Murray, M. W.; Emmett, S. N.; Armes, S. P. Effect of Monomer Solubility on the Evolution of Copolymer Morphology during Polymerization-Induced Self-Assembly in Aqueous Solution. *Macromolecules* **2017**, *50*, 796−802.

(90) Patrickios, C. S. Polypeptide Amino Acid Composition and Isoelectric Point 1. A Closed-Form Approximation. *J. Colloid Interface Sci.* **1995**, *175*, 256−260.

(91) Bakkialakshmi, S.; Selvarani, P.; Chenthamarai, S. Fluorescence Quenching of Rhodamine B Base by Two Amines. *Spectrochim. Acta, Part A* **2013**, *105*, 557−562.

(92) Hu, J.; Zhang, X.; Wang, D.; Hu, X.; Liu, T.; Zhang, G.; Liu, S. Ultrasensitive Ratiometric Fluorescent pH and Temperature Probes Constructed from Dye-labeled Thermoresponsive Double Hydrophilic Block Copolymers. *J. Mater. Chem.* **2011**, *21*, 19030−19038.

(93) Martin, M. M.; Lindqvist, L. The pH Dependence of Fluorescein Fluorescence. *J. Lumin.* **1975**, *10*, 381−390.

(94) Binks, J. B. *Photophysics of Aromatic Molecules*; Wiley: London, 1970.

(95) Sun, H.; Scharff-Poulsen, A. M.; Gu, H.; Almdal, K. Synthesis and Characterization of Ratiometric, pH Sensing Nanoparticles with Covalently Attached Fluorescent Dyes. *Chem. Mater.* **2006**, *18*, 3381−3384.

(96) Ma, B.; Xu, M.; Zeng, F.; Huang, L.; Wu, S. Micelle Nanoparticles for FRET-based Ratiometric Sensing of Mercury Ions in Water, Biological Fluids and Living Cells. *Nanotechnology* **2011**, *22*, 065501.

DOI: 10.1021/acs.macromol.7b01005
Macromolecules 2017, 50, 6108−6116