Aqueous-only, pH-induced nanoassembly of dual pK_a-driven contraphilic block copolymers†

Nam S. Lee, Yali Li, C. Marcus Ruda and Karen L. Wooley*

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pH-Responsive block copolymers, having two segments with functionalities of differing pK_a, were prepared by NMP, providing a "green" route to the assembly of core–shell functionalizable nanostructures.

Stimuli-responsive block copolymers are of significant interest due to their ability to impose morphological, charge, or structural changes in their self-assembled nanostructures in response to a specific stimulus. One of the most intriguing benefits that some of these systems exhibit is a reversible nanoassembly process. External stimuli such as pH,[1] temperature,[2] light,[3] or a combination of these[4] have been shown to afford chemically-driven structural reorganization events. For instance, Kataoka and co-workers[5] reported spontaneous formation of polyion complex micelles from a series of oppositely-charged block ionomers in aqueous media, which allow for the incorporation of charged macromolecules, such as proteins and nucleic acids. Armes and co-workers, employed several techniques[6] to probe the pH-controlled assembly–disassembly of diblock and triblock copolymers into supramolecular micelles[7] and vesicles,[8] comprised of block segments having amino groups. The assembly process was triggered by deprotonation of the amines upon elevation of the pH. Dual functionalities have been employed in a few cases to achieve protonation–deprotonation of selective domains within the nanoscale assemblies,[9] including also an elegant example that employed an oligomer of poly(L-glutamic acid) and poly(L-lysine), PGA_{15}-*b*-$PLys_{15}$, to afford reversible inside-out vesicle reorganizations, for which vesicles existed at low (<4) and high (>10) pH values.[10]

Our aim is to utilize block copolymers having dual functional blocks, whose pK_a values are significantly different from each other, thereby providing a wide range of solution pH values, including physiological pH, over which distinct micelles can exist. Moreover, we are interested in the pH-responsive functionalities also possessing orthogonal chemical reactivities. The examples of pH-responsive materials based upon tertiary amines do not offer rich functionalization chemistries, and the system based upon glutamic acid and lysine could experience complications from complementary reactivities for the two block segments. Herein, we report aqueous-only, pH-induced reversible assembly of micelles from poly(acrylic acid)-*b*-poly(*p*-hydroxystyrene) (PAA-*b*-P*p*HS), which reveals its amphiphilicity over a pH range of 10 to 4. At higher pH values (>10), the entire polymer is water soluble, and at lower pH values (<4), it is water insoluble. Although the core constituting segment, poly(*p*-hydroxystyrene), has found prolific application in photoresist technologies,[11] its employment as a pH-responsive material for nanoassembly–disassembly is unique.

The diblock copolymer was prepared by controlled radical polymerization, which allowed for the convenient incorporation of protected functionalities into the block segments that would lead ultimately to the core and the shell regions of the micellar assemblies (Fig. 1). Poly(*tert*-butyl acrylate)$_{131}$-*b*-poly(4-acetoxystyrene)$_{76}$ (**3**), P*t*BA_{131}-*b*-$PAcS_{76}$, was synthesized *via* sequential nitroxide mediated radical polymerization (NMP) reactions.[12] P*t*BA_{131} was prepared by NMP of *tert*-butyl acrylate, and then was followed by chain extension with 4-acetoxystyrene to yield **3**. Base-promoted cleavage of the acetoxy groups and then acid-catalyzed deprotection of the *tert*-butyl residues afforded PAA_{131}-*b*-P*p*HS_{76}, **1** (Fig. 1). The pH-responsive diblock copolymer (**1**) was purified by dialysis against nanopure water for 3 days to remove residual trifluoroacetic acid and was then lyophilized.

With pK_a values of *ca.* 4 and 10 for the PAA and P*p*HS block segments, this block copolymer was expected to assemble into micelles at pH values below 10 and to aggregate and precipitate at pH values below 4. The purified polymer was dissolved in deuterium oxide at room temperature, which immediately resulted in a solution of pH 4. At this point, the P*p*HS segment was protonated, while a sufficient number of the acrylic acid residues remained deprotonated to provide for solubility, effecting amphiphilicity of the diblock copolymer. Thus, the diblock copolymers had spontaneously assembled into micelles, **2**. D_2O-Suppressed ^1H NMR spectra (Fig. 2) of the diblock copolymer were then obtained as a function of increasing pH (controlled by adding NaOD solution drop-wise to achieve the desired pH) to determine the diblock copolymer's critical pH values. A slight upfield shift was observed for the methine resonance from 2.4 to 2.2 ppm, which occurred upon raising the pH to 6. This resonance continued to move to higher field as the pH was increased to 9, and is attributed to deprotonation of acrylic acid groups. From pH 9 to 11, as the P*p*HS core underwent deprotonation, the micelle disassembled into diblock copolymer chains due to conversion to entirely hydrophilic polymer chains. This process, revealed the P*p*HS core and caused its disassembly, which was shown by the

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Department of Chemistry, Department of Radiology, Washington University in Saint Louis, Saint Louis, MO 63130, USA.
E-mail: klwooley@artsci.wustl.edu; Fax: +1 314 935 9844;
Tel: +1 314 935 7136

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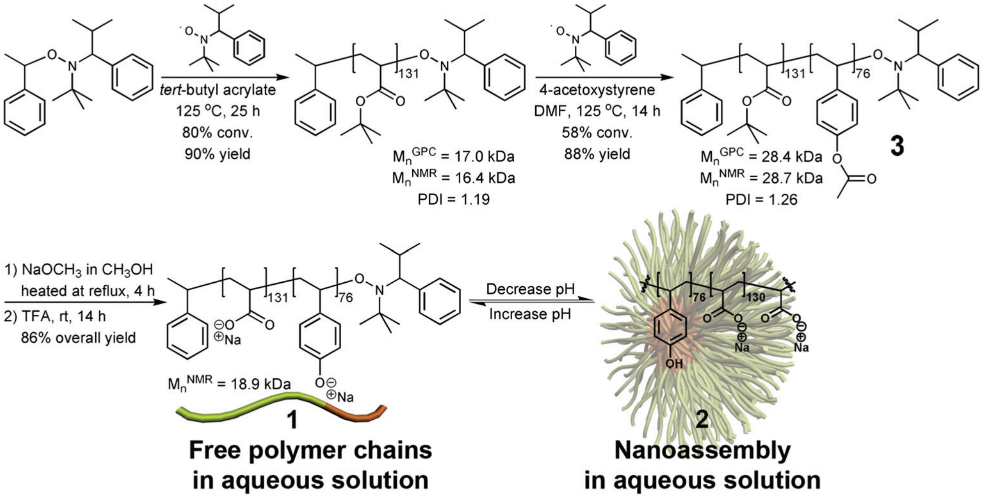

Fig. 1 Preparation of PAA$_{131}$-*b*-P*p*HS$_{76}$ and its pH-induced micelle assembly in aqueous solution.

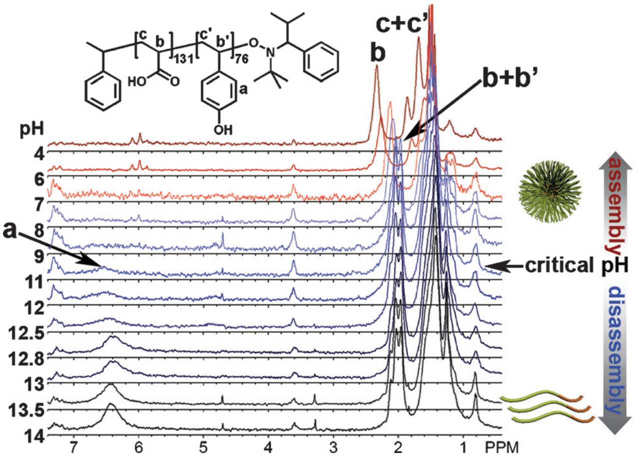

Fig. 2 A composite of ^{1}H NMR spectra (500 MHz) of PAA$_{131}$-*b*-P*p*HS$_{76}$, as amphiphilic micellar assemblies, **2**, at low pH and disassembly into solvated hydrophilic polymer chains, **1**, as the pH is elevated.

appearance of the aromatic protons of the P*p*HS block (resonating at 6.4 ppm) and by their gradual approach toward full integration as the pH was raised to 14. Furthermore, the appearance of benzylic protons (b′) and their growth from pH 9 to 14 also confirmed the solubilization of the P*p*HS. Below a solution pH of 3, the diblock copolymer precipitated from the aqueous solution. These results (large-scale precipitated aggregates below pH 4; micellar assemblies between pH 4 and 10) were in agreement with the pK_a values of AA (*ca.* 4) and *p*HS (*ca.* 10).

The hydrodynamic diameters (D_h) of the micelles were measured by dynamic light scattering (DLS) for individual samples prepared at six pH values (Fig. 3, left panel). The number-average D_h of the micelles were *ca.* 25 nm at pH 7.7 and increased to *ca.* 65 nm at pH 3. No correlation could be fit to the DLS data collected for samples at pH values above 8, due to a lack of micellar nanoassemblies and insufficient light scattering. The increase in average particle size at low pH was attributed to aggregation of the micelles, caused by reduction in electrostatic repulsions among particles as the PAA shell became protonated. Furthermore, data from ^{1}H NMR diffusion (D) experiments gave hydrodynamic radius (R_{hyd}) values as a function of pH that were in good agreement with the DLS data: D_{pH6} of 1.60×10^{-6} cm^2 s^{-1}, which correlated to $R_{\mathrm{hyd(pH6)}}$ of 29 nm, and D_{pH4} of 7.64×10^{-7} cm^2 s^{-1}, which corresponded to $R_{\mathrm{hyd(pH4)}}$ of 62 nm.

The integrity of the micellar assemblies upon deposition onto substrates and drying was also probed by atomic force microscopy (AFM) and transmission electron microscopy (TEM). A typical micelle at pH 7 had an approximate height of *ca.* 5 nm, as measured by AFM (Fig. 3, right panel). The size of the core of the micelle remained *ca.* 16 nm in diameter from pH 3.2 to 7.1, as measured by TEM (Fig. 4), suggesting that the P*p*HS core retained its stability, with the increasing apparent particle size at low pH being due to particle–particle aggregation events.

We have demonstrated a pH-induced nanoassembly of PAA-*b*-P*p*HS, which avoids the need for organic solvents and contains functionalities that provide opportunities for further physical and chemical manipulations of the core and shell domains. Although the two components for this diblock are quite common, their combination provides a unique opportunity to tune the aqueous-based assembly–disassembly processes over a wide pH range. In addition, the phenolic groups of the core may lead to iodination or other chemistries for labeling

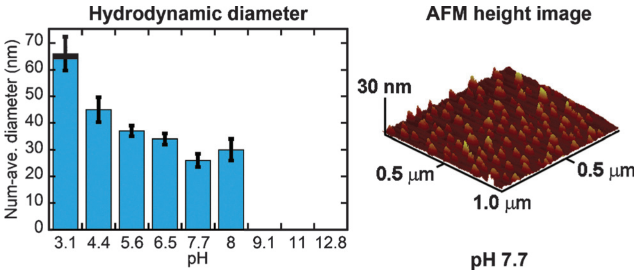

Fig. 3 Number-average D_h data for micelle **2** as a function of pH (left), as measured by DLS. Tapping-mode AFM image of micelle **2** (right), collected after deposition of the sample at solution pH 7.7 onto freshly-cleaved mica and allowing to dry under ambient conditions.

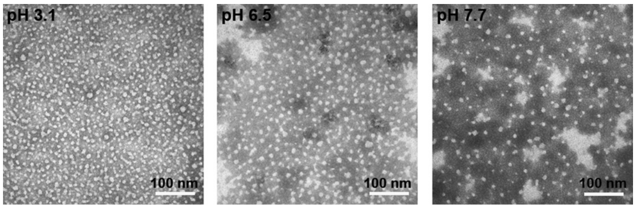

Fig. 4 TEM images of micelle **2**, collected on samples deposited from solutions at pH values of 3.1, 6.5 and 7.7, onto carbon-coated copper grids, negatively-stained with 1% phosphotungstic acid.

within the nanostructure, while the acrylic acid groups of the shell can be used for attachment of various units to mediate biological interactions. Ongoing work is transforming this aqueous-based assembly–disassembly system into functional nanodevices, and also into covalent entities that will undergo reversible, pH-driven swelling–deswelling and physical reorganization.

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