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pH-Responsive Fluorescent Molecular Bottlebrushes Prepared by Atom Transfer Radical Polymerization

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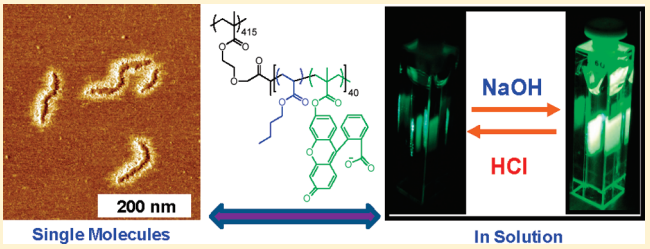
ABSTRACT: Fluorescein *O*-methacrylate was copolymerized with *n*-butyl acrylate by atom transfer radical polymerization in a "grafting from" reaction with a multifunctional linear macroinitiator to form pH responsive fluorescent bottlebrushes. The brush-like structure of the synthesized macromolecules was confirmed through molecular imaging by atomic force microscopy. NMR spectroscopy showed that the fluorescent units were successfully incorporated into the polymer. The synthesized bottlebrushes displayed highly fluorescent properties under basic conditions, yet showed no fluorescence under neutral or acidic conditions. The fluorescence could be turned on and off by changing the pH of the solution. These bottlebrush molecules could have potential applications in molecular imaging.



INTRODUCTION

Densely grafted copolymers are comb-shaped macromolecules that are also called bottlebrush copolymers due to their chain-extended brush-like shape.[1−8] This unique feature of the bottlebrush molecules has opened new pathways to several potential applications such as supersoft elastomers,[9] templates for nanoparticles and nanowires,[10−13] nanotubes,[14] photonic crystals,[15] nanoporous materials,[16] and molecular tensile machines.[17] Bottlebrushes can be prepared by using alkyne−azide click coupling reactions,[18] ionic polymerization,[19] ring-opening metathesis polymerization,[20] reversible addition−fragmentation chain transfer (RAFT) polymerization,[14,21−23] and atom transfer radical polymerization (ATRP).[6,24−33] Even though there are several successful reports on preparation of bottlebrushes via "grafting onto"[18] and "grafting through"[34−36] methods, so far most of the bottlebrushes were prepared using the "grafting from" method via ATRP.[1,25,37] Recent advances in the polymer synthesis has also allowed the preparation of a wide range of stimuli-responsive polymers that are sensitive to changes in temperature, light, pH, electrical field, and other stimuli.[7,38−71] pH-responsive polymers have been widely studied because pH is a convenient and effective stimuli in many applications.[72,73]

Fluorescent probes are used in a number of bioresponsive applications, ranging from drug delivery to genomics.[74,75] One of the most commonly used fluorescent dyes is fluorescein. In biomedical applications, fluorescein has several advantages over other dyes such as nontoxicity, high water solubility, and pH responsivity. Fluorescein shows highly fluorescent behavior under basic conditions and becomes nonfluorescent under neutral or acidic conditions.[76]

Herein we report a straightforward method for the preparation of pH-sensitive fluorescent molecular bottlebrushes by copolymerizing a fluorescein containing monomer, fluorescein *O*-methacrylate (FMA), with *n*-butyl acrylate (BA) in the side chains of the bottlebrush macromolecule. BA was chosen as the comonomer since it is easy to image the resulting material by using atomic force microscopy (AFM). This is due to its low glass transition temperature, $T_g \cong -50$ °C, and the tendency for poly(*n*-butyl acrylate) to adsorb onto certain surfaces. Although a very small amount of FMA was used during the copolymerization (0.25 mol %), the resulting bottlebrushes showed highly fluorescent properties. The fluorescence of the bottlebrushes can be switched on and off by adjusting the pH of the solution from basic to neutral. The bottlebrushes therefore have potential applications as molecular probes, for direct visualization of drug delivery capsules, and as molecular basicity indicators.

EXPERIMENTAL SECTION

Materials. All chemicals were purchased from Aldrich and used as received unless otherwise stated. 2-(Trimethylsilyloxy)ethyl methacrylate (HEMA-TMS) (99%) was purchased from Scientific Polymer Products and distilled under vacuum before use. BA was purchased from Acros and inhibitor/antioxidant was removed by passing through a basic alumina column prior to use. CuBr (98%) was purified by stirring with glacial acetic acid followed by filtering and washing the resulting solid with isopropanol.

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Equipment and Analysis. Molecular weights and M_w/M_n were determined by GPC, conducted with a Waters 515 pump and a Waters 2414 differential refractometer with PSS columns and THF as eluent. Linear poly(methyl methacrylate) standards were used for GPC calibration. Absorption and emission spectra of the $PBPEM_{415}$-*g*-$(PBA\text{-}co\text{-}PFMA)_{40}$ bottlebrushes were obtained using a Cary 50 UV−vis spectrophotometer and a FluoroMax-2 Jobin Yvon SPEX spectrofluorometer, respectively. Fluorescence emission spectra were recorded for neutral basic and acidic solutions at an excitation wavelength of 508 nm corresponding to the absorption maxima of the bottlebrushes. The samples were placed in a cuvette and continuously irradiated with 508 nm visible light using the spectrofluorometer. Digital pictures of the corresponding emission spectra were taken using a Canon SD600 digital camera in fluorescence mode. Confocal microscopy was carried out using a Carl Zeiss LSM 510 Meta NLO Confocor 3 inverted spectral confocal microscope using an excitation of 488 nm. Fluorescent brushes were drop-cast onto a glass coverslip, and the solvent was allowed to evaporate before imaging. Solution used for drop-cast was prepared as follows: Fluorescent brushes were mixed with nonfluorescent brushes with the ratio of 1:99 to dilute fluorescent brushes in solution to have clear images on surface. The concentration of the solution used was 0.0012 mg/mL.

Monomer conversion was determined by gas chromatography (GC) using a Shimadzu GC 14-A gas chromatograph equipped with a FID detector and ValcoBond 30 m VB WAX Megabore column. Dilute solutions (0.3 mg mL^{-1}) of the polymer sample were prepared using HPLC grade chloroform (Fisher Scientific). Monolayer samples were prepared using two different methods: (1) a Laurell Technologies Corp. (Model WS-400A-6NPP/Lite) spin-coater was used to prepare cast films, or (2) bottlebrushes were deposited onto grade V-4 mica substrates (SPI) using a KSV5000 Langmuir−Blodgett (LB) apparatus with Milli-Q double-distilled water as the subphase. Imaging of individual molecules within monolayers was carried out using a multimode atomic force microscope (AFM) (Veeco Metrology Group) in tapping mode. The AFM instrument was equipped with a Nanoscope IIIA control station and silicon cantilevers with resonance frequencies of ca.160 kHz. In-house-developed computer software was used for analysis of length distribution of the imaged macromolecules. Several images of ~300 molecules were analyzed to ensure a standard deviation of the mean below 5%.

Synthesis of the Bottlebrushes, Poly[BPEM-*g*-(PBA-*co*-PFMA)]. PBPEM (0.063 g, assuming 0.24 mmol of Br-initiating groups, was synthesized by following the previously reported procedure[1]), *n*-BA (12.3 g, 96 mmol), FMA (0.096 g, 0.24 mmol), anisole (2.0 mL), 4,4′-di(5-nonyl)-2,2′-bipyridine (dNbpy) (0.098 g, 0.24 mmol), and $CuBr_2$ (0.0013 g, 0.0060 mmol) were added to a 25 mL Schlenk flask, and the reaction mixture was degassed by three freeze−pump−thaw cycles. Then, CuBr (0.016 g, 0.114 mmol) was added under nitrogen. After stirring for 0.5 h at room temperature, to form the soluble catalyst complex, the flask was placed in a preheated oil bath at 80 °C. The polymerization was stopped after 25 h by cooling the flask to room temperature and opening the flask to air. The resulting polymer solution was purified by passing it through a column of neutral alumina, after which the polymer was precipitated by slowly adding this solution to cold methanol. The precipitate was separated, redissolved in THF, and reprecipitated into cold methanol three times. The resulting polymer was dried under vacuum at room temperature for 24 h; DP_{sc} of BA = 40, as determined by GC, $M_{n,GPC}$ = 585 000, M_w/M_n = 1.12.

RESULTS AND DISCUSSION

Synthesis of PBPEM-*b*-(PFMA-*co*-PBA). The synthetic procedure used for the preparation of fluorescent bottlebrushes is illustrated in Figure 1. ATRP was chosen for both the preparation of the backbone macroinitiator and for growing the side chains. Preparation of the macroinitiator has been reported previously.[1,36,37]

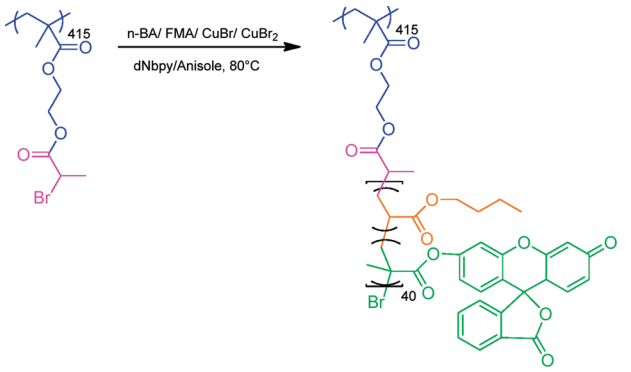

Figure 1. Preparation of Fluorescent Bottlebrushes by Copolymerization of Fluorescent FMA with BA in the Side Chains

Fluorescent moieties were introduced into a bottlebrush side chain by copolymerizing a pH-sensitive fluorescent monomer, FMA, with BA for the synthesis of brushes, using ATRP. The ratio of reagents employed was [BA]:[FMA]:[PBPEM]:[CuBr]:[$CuBr_2$]:[dNbpy] = 399:1:1:0.475:0.025:1 at 80 °C in 14% anisole. During the copolymerization the GPC traces shifted cleanly to the higher molecular weight region with increasing monomer conversion while preserving the narrow and symmetric molecular weight distribution (Figure 2).

The resulting bottlebrushes were precipitated by slow addition of the reaction mixture to cold methanol to remove excess monomer and the copper/ligand complex. After three precipitations, a slightly orange-colored polymer was obtained. Since FMA is completely soluble in methanol, this orange color is a sign of incorporation of FMA in the side chains.

^1H NMR spectroscopy was used to calculate the amount of FMA units incorporated into the side chains. In Figure 3, peak "b" represents the CH hydrogen of the phenyl of the FMA unit, while peak "a" corresponds to the CH_3 hydrogens from the BA unit. The integral ratio of "b" to ["b" + ("a"/3)] was calculated to be 0.009, which gives the mole percentage ratio of FMA unit in the side chains = 0.9%. Since 0.25% of FMA was present in the monomer feed, this calculation shows that FMA is 3.6 times more reactive than BA during the side-chain synthesis. This reactivity difference is consistent with the literature data on copolymerization of methyl methacrylate with methyl acrylate.[77,78] Since there are around 16 600 BA units per molecule (backbone DP × side-chain DP = 415 × 40), each bottlebrush molecule has around 150 FMA units.

AFM Analysis of the Bottlebrushes. The high content of BA units in the side chains of the bottlebrushes facilitated molecular imaging by AFM. The desorbed side chains aggregate on the top of the backbone whereas adsorbed chains act as spacers between the individual macromolecules. The overall effect is to create a distinctly delineated backbone, and this increases the topographic contrast between the surface and the molecule. In addition to separation and contrast enhancement, the strong repulsive steric interactions between the grafted side chains result in significant chain extension of the backbone.[1]

Films of PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes were prepared as described above and imaged using tapping-mode AFM.

Figure 4 shows height and phase images of bottlebrush polymers of PBA-*co*-PFMA on a mica substrate. While the height image (Figure 4a) demonstrates a densely packed monolayer of worm-like macromolecules, the phase image of isolated molecules (Figure 4b) enables imaging of the bottlebrush-like shell around the backbone. From the measured contour length L_n = 108 ± 6 nm and degree of polymerization of the backbone N = 415, one determines the backbone length per monomeric l_m unit as L_n/N = 0.26 ± 0.02 nm. This value corresponds to a fully stretched all trans C−C−C bond, l_{max} = 0.25 nm.[79] Also, the length polydispersity index PDI = 1.2 ± 0.1 is in agreement with that obtained by GPC. The distance D between bottlebrush molecules is D = 41 ± 3 nm. The number-average molecular weight M_n of the imaged macromolecules was determine using the AFM-LB technique.[80] As shown in Table 2, the AFM value M_n = (2.18 ± 0.27) × 10^6 agrees well with the calculated molecular weight M_n = 2.12 × 10^6 (Table 1).

Fluorescence Studies. The fluorescent properties of the bottlebrushes were examined by preparing 0.0078 wt % bottlebrush solutions using DMF:water 5:1 by volume ratio (colorless). The DMF was used to solubilize the BA units, and water was added to solubilize the FMA anionic units which were subsequently formed in basic media. The FMA units were ionized by adding one drop of 3 M NaOH aqueous solution. Since a very small amount of NaOH$_{(aq)}$ was added, it was assumed that the concentration of the species in the solution was not changed. The color of the solution instantly turned green. After ionization, FMA units were fully conjugated and show their fluorescent properties (Figure 5).

Images of irradiated samples of solutions of the bottlebrush copolymers are shown in Figure 6. Under neutral conditions, only the 508 nm excitation light is observed, indicating no

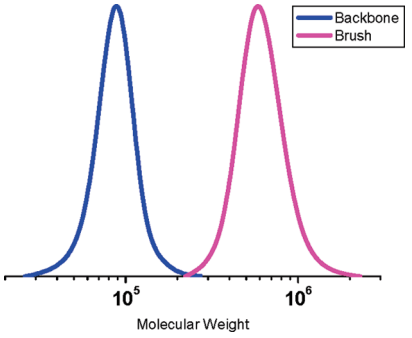

Figure 2. GPC traces of the PBPEM$_{415}$ (backbone) and [PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$] (bottlebrush). 10% monomer conversion was obtained in 25 h, resulting in [PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$] bottlebrushes.

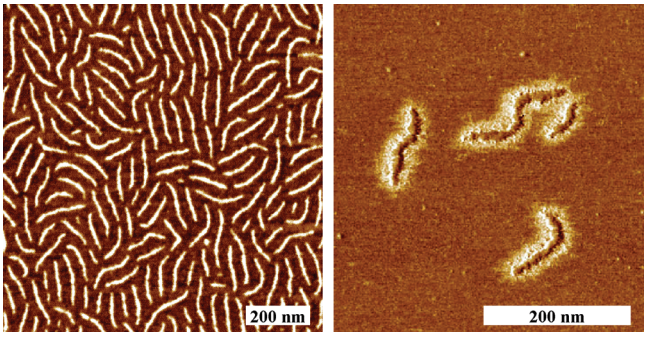

Figure 4. (a) Height image of PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes adsorbed on mica (LB sample) and (b) phase image of PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes adsorbed on mica (spin-casting).

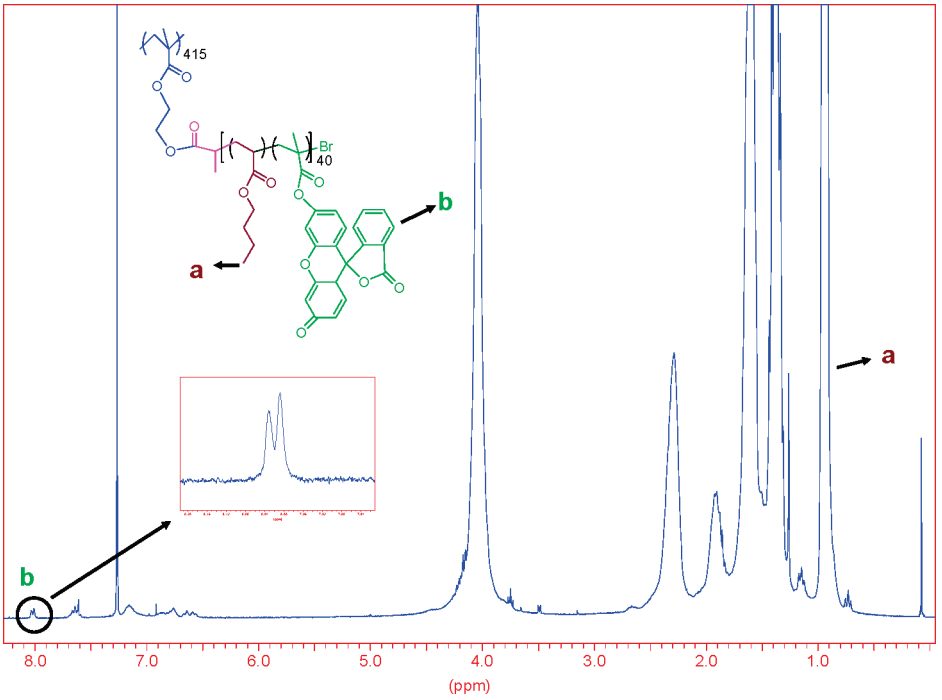

Figure 3. ^{1}H NMR spectrum of the [PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$] bottlebrushes.

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Table 1. Molecular Weight Data for Backbone and the Fluorescent Bottlebrushes

name	DP	$M_{n,theory}$	$M_{n,AFM}$	$M_{n,GPC}$	M_w/M_n
PHEMA-TMS$_{415}$	415[a]	81 000		82 800[a]	1.11[a]
PBPEM$_{415}$	415[a]	110 000		83 800[a]	1.09[a]
[PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$]	415−40[b]	2 230 000[b]	2 180 000	585 000[a]	1.12[a]

[a]Calculated from GPC-THF line by using PMMA standards. [b]Calculated by GC.

Table 2. Analysis of Length and Molecular Weight of PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ Polymers

name	L_n (nm)[a]	PDI[b]	D (nm)[c]	M_n (g/mol)[d]
PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$	108 ± 6	1.2 ± 0.1	41 ± 3	(2.18 ± 0.27) × 10^6

[a]Number-average contour length. [b]PDI (polydispersity index) = L_w/L_n. [c]Distance between molecules in a dense monolayer prepared by the LB technique. [d]Number-average molecular weight by AFM-LB technique [ref 69].

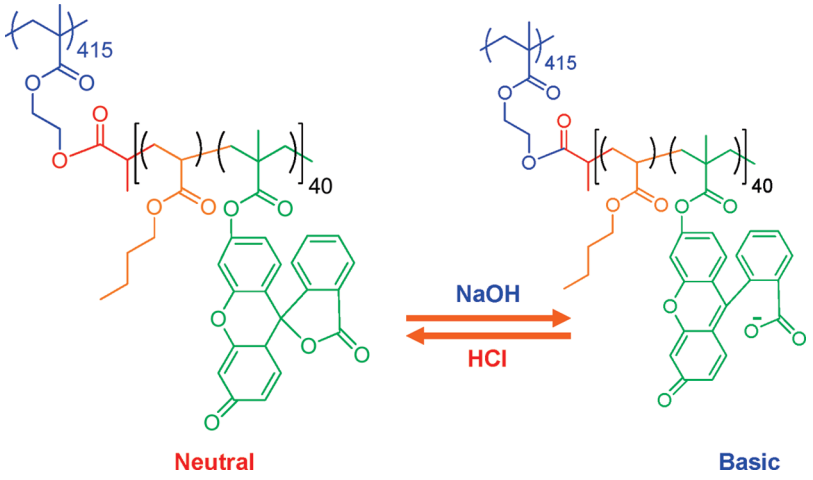

Figure 5. Schematic representation of the bottlebrushes under neutral and basic conditions.

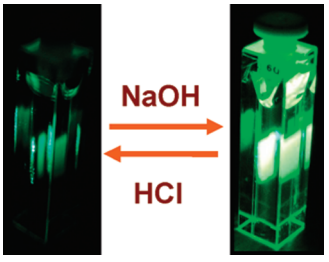

Figure 6. Solutions of bottlebrushes under neutral (left) and basic (right) conditions.

emission. However, under alkaline conditions, the solution becomes highly fluorescent and a green emission light is observed, corresponding to the 570 nm emission previously recorded on the spectrofluorometer (Figure 6).

The absorption and emission spectra of the PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes under different pH conditions are shown in Figure 7. The absorption and emission maxima are observed around 508 and 535 nm, respectively. Under acidic and neutral conditions (pH 6 and pH 7) the absorption and emission of the PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes are nonemissive, corresponding to the presence of the neutral

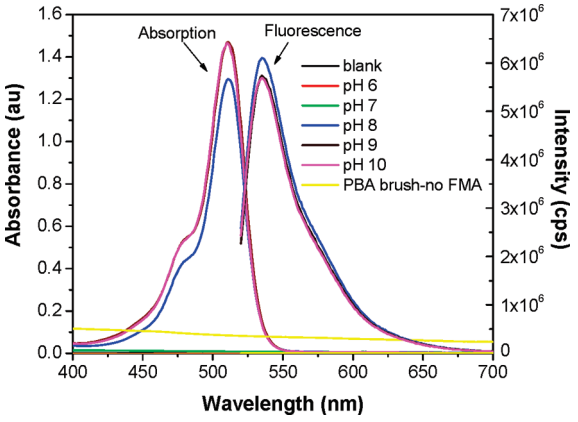

Figure 7. Absorption and emission spectra of PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes under different pH values.

form of fluorescein. However, under alkaline pH conditions (pH 8, pH 9, and pH 10) the PBPEM$_{415}$-*g*-(PBA-*co*-PFMA)$_{40}$ bottlebrushes "turn on" and are highly emissive, corresponding to the presence of the conjugated anionic form of the fluorescein molecule. No increase in the absorption intensity was observed for the brushes when the pH was changed from 9 to

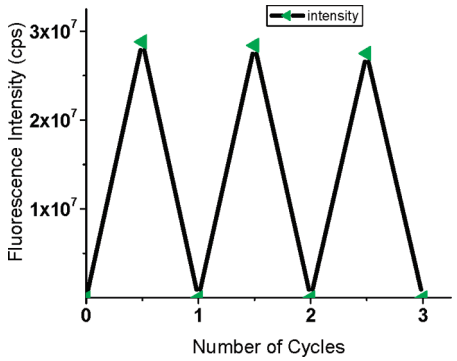

Figure 8. Turning the fluorescent properties of the bottlebrushes "on" and "off"; half numbers are basic conditions, and integral numbers are under neutral conditions.

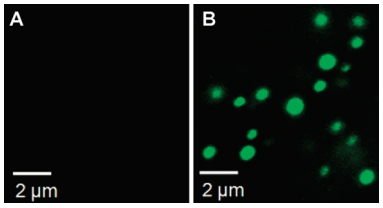

Figure 9. Confocal microscopy images of bottlebrush molecules: (A) fluorescent is off; (B) fluorescent is on.

10, showing that the highest possible value of ionized FMA % was reached when pH is around 9.

Experiments were repeated by conducting three neutral−basic cycles. Solutions of the bottlebrushes were made basic (pH 8) by adding one drop of 3.0 M $NaOH_{(aq)}$ and then made neutral by adding one drop of 3.0 M $HCl_{(aq)}$. Results for all cycles are given in Figure 8. Emission values were at the same level after each cycle, showing that the fluorescent property is quantitatively preserved.

The fluorescence quantum yield for the bottlebrushes was calculated to be 0.85 by using quinine sulfate as the standard. This value is very close to the value of FMA alone (0.91) which shows that even though around 150 FMA units were placed in one molecule, quenching of the fluorescence was considerably low. It is possible that the BA units provide a barrier between the FMA units, preventing quenching.

Fluorescence of individual bottlebrush molecules were imaged by using confocal microscopy (Figure 9). The bottlebrushes showed no fluorescence under neutral conditions (Figure 9A). Under basic conditions, strong fluorescence was observed (Figure 9B).

CONCLUSIONS

pH-responsive fluorescent bottlebrushes were prepared by introducing FMA units into regular PBA bottlebrushes. Incorporation of the fluorescent units was confirmed by NMR spectroscopy. Visualization of the bottlebrushes was provided by AFM experiments. The bottlebrushes displayed no fluorescence under neutral or acidic conditions, whereas the fluorescence was turned on by making the solution basic. The ability to turn the fluorescent property of the bottlebrushes on and off was demonstrated by repeatability experiments.

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