# **FRET-Derived Ratiometric Fluorescent K<sup>+</sup> Sensors Fabricated from Thermoresponsive Poly**(*N***-isopropylacrylamide) Microgels Labeled with Crown Ether Moieties**

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We report on the fabrication of ratiometric fluorescent  $K^+$  sensors based on thermoresponsive poly(Nisopropylacrylamide) (PNIPAM) microgels covalently incorporated with K<sup>+</sup>-recognizing 4-acrylamidobenzo-18-crown-6 residues (B18C6Am), fluorescence resonance energy transfer (FRET) donor dyes, 4-(2acryloyloxyethylamino)-7-nitro-2,1,3-benzoxadiazole (NBDAE), and rhodamine-B-based FRET acceptors (RhBEA) by utilizing K<sup>+</sup>-induced changes in microgel volume phase transition (VPT) temperatures. P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels were synthesized via the free radical emulsion copolymerization technique. The spatial proximity between FRET pairs (NBDAE and RhBEA dyes) within microgels can be tuned via thermoinduced collapse and swelling of thermoresponsive microgels above and below VPT temperatures, leading to the facile modulation of FRET efficiencies. B18C6Am moieties within P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels can preferentially capture  $K^+$  via the formation of 1:1 molecular recognition complexes, resulting in the enhancement of microgel hydrophilicity and elevated VPT temperatures. Thus, the gradual addition of  $K^+$  into microgel dispersions at intermediate temperatures, i.e., between VPT temperatures of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels in the absence and presence of K<sup>+</sup> ions, respectively, can directly lead to the reswelling of initially collapsed microgels. This process can be monitored by changes in fluorescence intensity ratios, i.e., FRET efficiencies. The presence of FRET pairs within P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels allows for the facile in situ monitoring of thermoinduced and  $K^+$ -induced VPTs of dually responsive microgels. The response time for fluorescent  $K^+$ -sensing was further investigated via the stopped-flow technique, which reveals that the process completes within  $\sim 4$  s. This work represents the first report of thermoresponsive microgel-based ratiometric fluorescent sensors for both K<sup>+</sup> ions and temperatures.

#### Introduction

Metal-ion-responsive chemosensors have attracted increasing interest in the past decade due to their environmental and biological relevance.<sup>1–34</sup> Among them, the quantitative probing of K<sup>+</sup> ions is quite crucial due to their active roles of chemical signal transduction in biological systems. Compared to the design of typical colorimetric and fluorometric chemosensors of heavy metal ions (e.g.,  $Cu^{2+}$ ,  $Hg^{2+}$ , and  $Zn^{2+}$ , etc.), the optical detection of K<sup>+</sup> ions is quite tricky due to the fact that they generally exhibit weak affinity with conventional heavy-metalion-binding ligands and are quite inert in inducing chemical reactions of specially designed caged fluorophores.

To date, fluorescent  $K^+$  sensors can be categorized into two main types. The first one utilizes  $K^+$ -induced folding of biomolecules such as DNA. Takenaka et al.<sup>1</sup> constructed a  $K^+$ sensing oligonucleotide containing four GGG sequence sites and labeled with fluorescence resonance energy transfer (FRET) donor and acceptor moieties at both chain ends. The presence of  $K^+$  can induce the formation of a guanine quartet and enhance the FRET efficiency due to closer proximity between FRET pairs. Subsequently, Wang et al.<sup>2</sup> reported the preparation of selective  $K^+$ -sensing ensembles consisting of G-quadruplex DNA and cationic conjugated polymer (CCP). The specific binding of  $K^+$  ions to G-quadruplex DNA induces its folding into more condensed G-quartet DNA, accompanied with the enhanced FRET process from CCP to G-quartet DNA.

The second type of fluorescent  $K^+$  sensors is based on the molecular recognition of  $K^+$  ions by crown ethers or cryptands, which were covalently conjugated to small molecule fluorophores.<sup>3–5,35–37</sup> The detection mechanism is typically based on the  $K^+$ -modulated photoinduced electron transfer (PET) process. In a notable example, the covalent linkage of the PET-based small molecule ion-sensing motif based on  $K^+$ -cryptand recognition to natural polymers such as hydroxypropyl cellulose (HPC) enabled the construction of a portable commercialized blood/serum  $K^+$  analyzer in Roche OPTI CCA.<sup>37</sup> Fluorescent conjugated polymers can also be employed. Swager et al.<sup>7</sup> prepared selective fluorescent  $K^+$  sensors on the basis of  $K^+$ -induced aggregation of benzo-15-crown-5 (B15C5)-functionalized conjugated polymers, leading to the modulation of fluorescence emission intensities.

K<sup>+</sup>-binding crown ethers or cryptands can also be incorporated into thermoresponsive polymers,<sup>38–42</sup> microgels,<sup>43</sup> or gels<sup>44–46</sup> to construct nonfluorescent K<sup>+</sup> sensors by taking advantage of K<sup>+</sup>-crown ether molecular-recognition-induced changes in the hydrophilicity/hydrophobicity balance, which will affect the lower critical solution temperatures (LCSTs) or volume phase transition (VPT) temperatures of thermoresponsive polymers or gels. Typically, benzo-18-crown-6 (B18C6)

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SCHEME 1: (a) Synthetic Schemes Employed for the Preparation of Thermoresponsive and K<sup>+</sup>-Responsive P(NIPAM-B18C6Am-NBDAE-RhBEA) Microgels via Emulsion Polymerization; (b) Schematic Illustration for Tuning the Efficiency of FRET Processes within P(NIPAM-B18C6Am-NBDAE-RhBEA) Microgels by Temperature, K<sup>+</sup> Ions, and a Combination of Them



and benzo-15-crown-5 (B15C5) can form 1:1 and 2:1 molecular recognition complexes with  $K^{+,5,47,48}$  and induce increased hydrophilicity and hydrophobicity, respectively. Thermoresponsive poly(N-isopropylacrylamide) (PNIPAM), which is wellknown to possess a LCST at  $\sim$ 32 °C, has been typically employed as the polymer matrix to covalently attach crown ether functionalities. Dated back to 1993, Irie et al.<sup>38</sup> synthesized thermoresponsive PNIPAM functionalized with B18C6 moieties and reported that their LCSTs, determined by temperaturedependent optical transmittance measurements, linearly increase with KCl concentrations. This work can be regarded as a prototype of nonfluorescent K<sup>+</sup> sensors based on thermoresponsive and K<sup>+</sup>-responsive polymers. Later on, Yamaguchi et al.  $^{42,49-56}$  and Chu et al.  $^{40,41,43-45}$  systematically investigated the effects of K<sup>+</sup> ions on the thermal phase transitions of PNIPAMbased copolymer chains, microgels, hydrogels, membranes, and capsules functionalized with crown ether moieties, aiming at developing novel K<sup>+</sup> sensors and K<sup>+</sup>-responsive controlled release nanocarriers. It should be noted that, in the above examples, the quantitative probing of K<sup>+</sup> ions is mainly based on temperature-dependent changes in the turbidity of polymer solutions, size variations of microgel dispersions, or macroscopic dimensional changes of hydrogels. It would be highly desirable to integrate K<sup>+</sup>-induced thermal phase transitions of responsive polymers with fluorometric detection strategies, which is expected to allow more convenient, sensitive, and online monitoring of K<sup>+</sup> concentrations.

Previously, PNIPAM-based microgels or core-shell nanoparticles have been labeled with fluorescent donor and acceptor dyes for the detailed investigation of their VPT transition processes, as reported by the Lyon research group.<sup>57,58</sup> In one example, they simultaneously labeled the core region of core-shell PNIPAM microgels with FRET donors and acceptors, Cy5 and Cy5.5, and investigated the restriction exerted by the shell layer on the swelling/collapse of microgel cores. These systems can be considered as microgel-based fluorescent thermometers.

We recently reported two examples of thermoresponsive PNIPAM microgel-based  $Cu^{2+}$  sensors by covalently attaching  $Cu^{2+}$ -binding ligands and fluorophores into microgels.<sup>10,11</sup> The presence of  $Cu^{2+}$  can quench the fluorescence emission. Above the microgel VPTs,  $Cu^{2+}$  detection limits can be considerably enhanced due to cooperative binding of ligands to  $Cu^{2+}$  ions and closer proximity between  $Cu^{2+}$  and fluorophore reporter moieties.<sup>10,11</sup> In principle, the development of ratiometric fluorescent metal ion sensors would be more applicable as compared to single fluorophore reporter-based detection systems, as the former can effectively eliminate background interferences. We aim to develop a novel type of thermoresponsive microgel-based ratiometric fluorescent K<sup>+</sup> sensors by utilizing the molecular recognition complexation between K<sup>+</sup> ions and crown ether moieties.

Herein, we report on the fabrication of ratiometric fluorescent  $K^+$  sensors based on thermoresponsive PNIPAM microgels covalently incorporated with  $K^+$ -recognizing 4-acrylamidobenzo-18-crown-6 (B18C6Am), FRET donor dyes, 4-(2-acryloyloxy-ethylamino)-7-nitro-2,1,3-benzoxadiazole (NBDAE), and rhodamine-B-based FRET acceptors (RhBEA) by utilizing  $K^+$ -induced changes in microgel VPT temperatures (Scheme 1). Within P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels, the spatial proximity between FRET pairs (NBDAE and RhBEA dyes) can be tuned via thermoinduced collapse and swelling of thermoresponsive microgels, leading to the facile modulation of FRET efficiencies. The FRET process allows the comparison of relative fluorescence intensities of two different emission bands, and the "internal" calibration leads to ratiometric

detection of analytes, which can effectively exclude background interference. B18C6Am moieties within P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels can preferentially capture K<sup>+</sup> via the formation of 1:1 molecular recognition complexes, and this results in the enhancement of microgel hydrophilicity and elevated VPT temperatures. Thus, the gradual addition of K<sup>+</sup> ions into microgel dispersions at intermediate temperatures, i.e., between VPT temperatures of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels in the absence and presence of K<sup>+</sup> ions, respectively, can directly lead to the reswelling of initially collapsed microgels. This process can be monitored by changes in fluorescence intensity ratios, i.e., FRET efficiencies. The presence of FRET pairs within P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels allows for the in situ monitoring of thermoinduced and K<sup>+</sup>-induced VPTs of the reported dually responsive microgels.

### **Experimental Section**

Materials. N-Isopropylacrylamide (NIPAM, 97%, Tokyo Kasei Kagyo Co.) was purified by recrystallization from a mixture of benzene and n-hexane (1/3, v/v). 4-Acrylamidobenzo-18-crown-6 (B18C6Am, 98%, Acros) was used as received. Ammonium persulfate (APS) and N,N'-methylene-bis-acrylamide (BIS) were recrystallized from methanol and ethanol, respectively, and then stored at -20 °C prior to use. Nonionic surfactant, polyoxyethylene sorbitan monolaurate (Tween 20), was purchased from Amersco and used as received. Potassium chloride (KCl) was purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 MQ cm. 4-(2-Acryloyloxyethylamino)-7nitro-2,1,3-benzoxadiazole (NBDAE) and rhodamine-B-based FRET acceptor dye, RhBEA (Scheme 1b), were prepared according to literature procedures.<sup>59,60</sup>

Synthetic schemes employed for the preparation of thermoresponsive and K<sup>+</sup>-responsive P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels are shown in Scheme 1a. Thermoinduced modulation of FRET efficiencies within responsive P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels and K<sup>+</sup>-induced tuning of VPT temperatures and FRET efficiencies are shown in Scheme 1b.

Sample Preparation. Preparation of P(NIPAM-B18C6Am-**NBDAE-RhBEA**) Microgels. Typical procedures employed for the synthesis of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels with a B18C6Am feed content of 8.0 mol % (relative to the sum of NIPAM and B18C6Am) are as follows. Into a 100 mL three-necked round-bottom flask equipped with a mechanical Teflon stirrer, a reflux condenser, and a nitrogen gas inlet, NIPAM (0.40 g, 3.53 mmol), BIS (5.2 mg, 33.73  $\mu$ mol), B18C6Am monomer (0.12 g, 0.31 mmol), Tween 20 (25 mg, 0.02 mmol), and deionized water (50 mL) were charged. After degassing by bubbling with nitrogen for 30 min and heating to 70 °C, APS (25 mg, 110 µmol) dissolved in 1.0 mL of deionized water was injected under mechanical stirring at 400 rpm. NBDAE monomer (5.2 mg, 19.2 µmol) and RhBEA monomer (50.4 mg, 96  $\mu$ mol) in 2.0 mL of ethanol were then added dropwise over  $\sim 20$  min. The polymerization was conducted under stirring for 7 h. The dispersion was passed through glass wools in order to remove particulates and then dialyzed against deionized water for 5 days. Fresh deionized water was replaced approximately every 6 h.

**Characterization.** *Temperature-Dependent Turbidimetry.* The optical transmittance of aqueous solutions at a wavelength of 700 nm was acquired on a Unico UV/vis 2802PCS spectrophotometer. A thermostatically controlled cuvette was employed, and the heating rate was 0.2 °C min<sup>-1</sup>. The LCST was defined as the temperature corresponding to  $\sim 1\%$  decrease in optical transmittance.

Field-Emission Scanning Electron Microscope (FE-SEM). FE-SEM observations were conducted on a high resolution JEOL JSM-6700 field-emission scanning electron microscope. The samples for SEM observations were prepared by placing  $10 \,\mu$ L of microgel solutions on copper grids successively coated with thin films of Formvar and carbon.

*Laser Light Scattering (LLS).* A commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multitau digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He–Ne laser ( $\lambda_0 = 632$  nm) as the light source was employed for dynamic LLS measurements. Scattered light was collected at a fixed angle of 90° for a duration of ~5 min. Distribution averages and particle size distributions were computed using cumulant analysis and CONTIN routines. All data were averaged over three consecutive measurements.

*Fluorescence Measurements.* Fluorescence spectra were recorded using an RF-5301/PC (Shimadzu) spectrofluorometer. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath. The slit widths were set at 5 nm for excitation and 5 nm for emission. For all microgel-based FRET experiments, microgel concentrations are fixed at  $1.0 \times 10^{-5}$  g/mL.

**Stopped-Flow Fluorescence Measurements.**<sup>61</sup> Stopped-flow fluorescence measurements were carried out using a Bio-Logic SFM300/S stopped-flow instrument. The SFM-3/S is a three-syringe (10 mL) instrument in which all step-motor-driven syringes (S1, S2, S3) can be operated independently to carry out single- or double-mixing. The SFM-300/S stopped-flow device is attached to the MOS-250 spectrometer. For the fluorescence emission detection, the excitation wavelength was set at 470 nm and the emission wavelengths (20 nm slit widths in both cases) were set at 529 and 588 nm, respectively. The typical dead time is 2.6 ms using the FC-15 flow cell, and the kinetic data were fitted using the program Biokine (Bio-Logic).

## **Results and Discussion**

Synthesis of P(NIPAM-B18C6Am-NBDAE-RhBEA) Microgels. P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels were synthesized via free radical emulsion copolymerization of NIPAM, crown ether-containing monomer (B18C6Am), and FRET donor- and acceptor-based monomers (NBDAE and RhBEA) in the presence of BIS and Tween 20 at around neutral pH and 70 °C (Scheme 1a). The microgel samples possess a feed cross-linking density of 1.0 wt % (relative to the sum of NIPAM and B18C6Am), and the feed contents of B18C6Am monomer (relative to the sum of NIPAM and B18C6Am) is 8.0 mol %. As-synthesized microgels were characterized by SEM (see Figure 1), which revealed the presence of nearmonodisperse and spherical nanoparticles with an average diameter of ~100 nm for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels prepared with a B18C6Am feed content of 8.0 mol %.

Thermoregulated Volume Phase Transitions of P(NIPAM-B18C6Am-NBDAE-RhBEA) Microgels. Figure 2 shows the temperature-dependent intensity-average hydrodynamic radius,  $\langle R_h \rangle$ , recorded for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4, 1.0 × 10<sup>-5</sup> g/mL) in the absence of K<sup>+</sup> ions as determined by dynamic LLS. Upon heating,  $\langle R_h \rangle$ decreases from ~120 nm at 25 °C to 46 nm at 48 °C, i.e., ~17.8 times decrease in microgel hydrodynamic volumes. From Figure



Figure 1. Typical SEM images obtained by drying aqueous dispersions of thermoresponsive and  $K^+$ -responsive P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels prepared at a B18C6Am feed content of 8.0 mol %.



**Figure 2.** Temperature-dependent intensity-average hydrodynamic radius,  $\langle R_h \rangle$ , recorded for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4,  $1.0 \times 10^{-5}$  g/mL) in the absence of K<sup>+</sup> ions. The inset shows typical hydrodynamic radius distributions, *f*(*R*<sub>h</sub>), of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels at 25 and 48 °C, respectively.

2, we can tell that prominent microgel collapse occurs above ~30 °C, which should be ascribed to the LCST or the VPT temperature of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels. The inset in Figure 2 shows typical hydrodynamic radius distributions,  $f(R_h)$ , of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels at varying temperatures, yielding polydispersity indices ( $\mu_2/\Gamma^2$ , size distribution of microgel particles) of 0.07 and 0.04 at 25 and 48 °C, respectively. Microgel sizes determined by dynamic LLS measurements are in general agreement with those determined by SEM (Figure 1).

Lyon et al.<sup>57,58</sup> previously reported the use of FRET efficiencies to monitor the VPT process of thermoresponsive microgels simultaneously labeled with FRET donor and acceptor dyes. The thermoinduced microgel collapse or swelling leads to dramatic changes in microgel hydrodynamic volumes and concomitantly the considerable closer or farther proximity between labeled FRET donor and acceptors. Thus, thermoinduced changes in hydrodynamic volumes during VPTs can be quantitatively represented by changes in FRET efficiencies. Due to the sensitivity of the fluorometric technique and the detection principle (FRET process), the latter can probe more detailed information of the VPT process, as well as the internal chain dynamics within microgels.

P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels bear NB-DAE and RhBEA dyes as FRET donors and acceptors, respectively. The fluorescence emission spectrum of NBDAE well overlaps with the absorption spectrum of RhBEA dyes,



**Figure 3.** (a) Fluorescence emission spectra ( $\lambda_{ex} = 470$  nm; slit widths: Ex. 5 nm, Em. 5 nm) recorded at varying temperatures for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4, 1.0 × 10<sup>-5</sup> g/mL; microgels were prepared with a B18C6Am feed ratio of 8.0 mol %) in the absence of K<sup>+</sup>. (b) Temperature-dependent fluorescence intensity ratio changes,  $F_{588}/F_{529}$ , obtained for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions in the absence of K<sup>+</sup>.

and these two constitute an excellent FRET pair. Temperaturedependent fluorescence emission spectra during the VPT process of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels in the absence of  $K^+$  ions were then determined (Figure 3). From Figure 3a, we can apparently observe two emission peaks at around 529 and 588 nm, which are ascribed to the fluorescence emission of NBDAE and RhBEA dyes, respectively. In the temperature range 25-48 °C, the decrease of emission intensity at 529 nm and the increase of emission band at 588 nm are clearly evident. Figure 3b also plots temperature-dependent fluorescence intensity ratio changes,  $F_{588}/F_{529}$ , which increases from 1.1 at 25 °C to 4.1 at 48 °C. Thus, P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels can act as excellent fluorescent thermometers in the absence of K<sup>+</sup> ions. For the microgel dispersion prepared at 8.0 mol % B18C6Am feed content, we can discern an inflection point at  $\sim 30$  °C in the  $F_{588}/F_{529}$  vs temperature curve, and this correlates well with temperaturedependent dynamic LLS results (Figure 2). These results indicate that efficient energy transfer processes between NBDAE and RhBEA dyes can occur and heating above the VPT temperature of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels can considerably enhance FRET efficiencies.

K<sup>+</sup>-Ion-Regulated Volume Phase Transitions of P(NIPAM-B18C6Am-NBDAE-RhBEA) Microgels. PNIPAM is known to undergo coil-to-globule transition in aqueous solution upon heating above the LCST. The crown ether functionalities are expected to change the hydrophilicity/hydrophobicity balance when they capture metal ions via molecular recognition. Temperature-dependent optical transmittance measurements were employed to determine the changes of LCSTs of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels upon addition of varying amounts of K<sup>+</sup> ions (Figure 4). For microgel dispersions in the presence of varying concentrations of K<sup>+</sup> (0, 50, 100, 150, 200, 250, and 300  $\mu$ M), they possess different LCSTs, which were defined as the temperature corresponding to ~1% decrease in optical transmittance. In the absence of K<sup>+</sup>, crown ether moieties exhibit slight effects on the VPT temperature of



**Figure 4.** (a) Temperature-dependent optical transmittance at a wavelength of 700 nm obtained for aqueous dispersions of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels (pH 7.4,  $1.0 \times 10^{-4}$  g/mL; microgels were prepared with a B18C6Am feed ratio of 8.0 mol %) with varying amount of K<sup>+</sup> ions. (b) LCST of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions as a function of [K<sup>+</sup>]. LCSTs were defined as the temperature corresponding to ~1% decrease in optical transmittance.

P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels (~29 °C), as compared to conventional PNIPAM microgels. B18C6Am moieties can capture K<sup>+</sup> ions into their cavities via the formation of 1:1 molecular recognition complexes, and this will increase the hydrophilicity of microgels, accompanied with the increase of thermal phase transition temperatures. From Figure 4a, we can tell that the addition of 50 and 100  $\mu$ M K<sup>+</sup> ions does not exhibit appreciable effects on the LCSTs, which only slightly increase to 29.2 and 29.8 °C, respectively.

Considerable shift in LCST occurs in the presence of >150  $\mu$ M K<sup>+</sup> ions. In the K<sup>+</sup> concentration range of 150–300  $\mu$ M, LCSTs almost linearly increase from 32.2 to 38.2 °C, compared to the LCST value of 29 °C determined in the absence of K<sup>+</sup> ions (Figure 4b). On the other hand, PNIPAM microgels and homopolymers without covalently labeled crown ether functionalities typically exhibit a decrease of LCSTs in the presence of increasing concentrations of KCl. The considerable shift of LCSTs of crown-ether embedded thermoresponsive PNIPAM microgels in the presence of K<sup>+</sup> ions has previously been investigated by several research groups.43-46 The capture of K<sup>+</sup> ions by crown ether moieties increases the microgel hydrophilicity and introduces charge repulsion within microgels. These two aspects will contribute to the increase of LCSTs. The microgel dispersion at a concentration of  $1.0 \times 10^{-4}$  g/mL contains  $\sim 60 \,\mu\text{M}$  crown ether moieties. The fact that an excess of K<sup>+</sup> ions needs to be added to induce appreciable changes in LCSTs indicates that the molecular recognition between K<sup>+</sup> ions and B18C6Am is an equilibrium complexation process.

On the basis of the above analysis, we established that the addition of  $\sim$ 5-fold excess of K<sup>+</sup> ions into P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions leads to the increase of microgel LCSTs from 29 to 38.2 °C (Figure 4). If the temperature was maintained between these two critical values



**Figure 5.** Intensity-average hydrodynamic radius,  $\langle R_h \rangle$ , obtained for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4,  $1.0 \times 10^{-5}$  g/mL; microgels were prepared with a B18C6Am feed ratio of 8.0 mol %) with varying amount of K<sup>+</sup> at 38 °C.

(i.e., LCST<sub>1</sub> and LCST<sub>2</sub>, which represent thermal phase transition temperatures of microgels in the absence and presence of K<sup>+</sup> ions, respectively), microgels will be in the collapsed state at first. However, the addition of K<sup>+</sup> is expected to induce microgel reswelling due to the elevation of the LCST. We then employed dynamic LLS to investigate K<sup>+</sup>-induced microgel reswelling at 38 °C. Figure 5 shows the change of  $\langle R_h \rangle$  recorded for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions at 38 °C in the presence of increasing concentrations of K<sup>+</sup> ions (0–30  $\mu$ M).  $\langle R_h \rangle$  is ~68 nm in the absence of K<sup>+</sup> ions, whereas it dramatically increases to 125 nm at a K<sup>+</sup> concentration of 30  $\mu$ M, which is ~5 equiv relative to that of crown ether moieties. Compared to the  $\langle R_h \rangle$  value (120 nm) for microgels in the absence of K<sup>+</sup> ions at 25 °C, the slight increase of  $\langle R_{\rm h} \rangle$  (125 nm) for microgels at 38 °C in the presence of 5 equiv of  $K^+$ , which are in the swollen state, should be ascribed to charge repulsion and elevated hydrophilicity incurred by K<sup>+</sup>/ B18C6Am molecular recognition complexation.

In addition to the dynamic LLS technique for the characterization of the K+-ion-induced VPT process of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels, the covalent attachment of the FRET pair, NBDAE and RhBEA, also allows for convenient and more accurate monitoring of VPT processes, considering that changes in hydrodynamic volumes (~6.2 times in the range of  $0-30 \ \mu\text{M} \text{ K}^+$  ions at 38 °C, Figure 5) during VPT will considerably modulate the relative distances between FRET donors and acceptors. Figure 6 shows changes of fluorescence emission spectra and emission intensity ratios,  $F_{588}$ /  $F_{529}$ , for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels at 38 °C upon addition of varying amounts of KCl (0–30  $\mu$ M). Apparently, we can tell from Figure 6 the increase and decrease of emission intensities at 529 and 588 nm, which are characteristic of emissions of FRET donors (NBDAE) and acceptors (RhBEA), respectively. A comparison to the dynamic LLS results (Figure 5) clearly tells us that the dramatic decrease of fluorescence intensity ratios,  $F_{588}/F_{529}$ , is due to the VPT process (microgel reswelling), which leads to decrease of FRET efficiencies due to longer distances between the FRET pair. K<sup>+</sup> concentration-dependent changes of FRET efficiencies can also be employed as ratiometric  $K^+$  sensors. If we define the detection limit as the K<sup>+</sup> concentration at which a 10% change in fluorescence intensity ratio,  $F_{588}/F_{529}$ , can be measured by employing a  $1.0 \times 10^{-5}$  g/mL aqueous dispersion of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels, the detection limit of K<sup>+</sup> ions is  $\sim$ 3.6  $\mu$ M.

Fluorescent labeling of thermoresponsive PNIPAM microgels with FRET donors and acceptors also allows for the visual inspection of thermoinduced and K<sup>+</sup>-ion-induced VPTs. As



**Figure 6.** (a) Fluorescence emission spectra ( $\lambda_{ex} = 470$  nm; slit widths: Ex. 5 nm, Em. 5 nm) recorded at varying amount of K<sup>+</sup> for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4, 1.0 × 10<sup>-5</sup> g/mL; microgels were prepared with a B18C6Am feed ratio of 8.0 mol %) at 38 °C. (b) Fluorescence intensity ratio changes,  $F_{588}/F_{529}$ , obtained for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions as a function of [K<sup>+</sup>].



**Figure 7.** Photographs recorded under a 365 nm UV lamp for P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4,  $1.0 \times 10^{-4}$  g/mL; microgels were prepared with a B18C6Am feed ratio of 8.0 mol %) at varying conditions: (a) 25 °C; (b) 38 °C; (c) 38 °C upon addition of 300  $\mu$ M K<sup>+</sup>.

shown in Figure 7, upon heating the aqueous dispersion of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels at a concentration of  $1.0 \times 10^{-5}$  g/mL from 25 to 38 °C, the collapse of microgels leads to enhanced FRET efficiency due to closer proximity between NBDAE and RhBEA dyes. We can clearly discern the orange-to-red transition during the heating process. On the other hand, at a fixed temperature of 38 °C, addition of  $300 \,\mu\text{M}\,\text{K}^+$  ions (5 equiv relative to crown ether moieties) leads to the red-to-orange transition, which is associated with the K<sup>+</sup>triggered enhancement of the hydrophilicity of microgels and elevated LCST (Figures 4 and 6). Thus, we successfully demonstrated that FRET processes occurred within thermoresponsive microgels covalently labeled with crown ether moieties, and FRET donors and acceptors can be utilized for monitoring the thermotriggered and K<sup>+</sup>-ion-triggered volume phase transition processes. As FRET donors (NBDAE moieties) and acceptors (RhBEA residues) are expected to randomly distribute within PNIPAM-based microgels, there exists a distribution of FRET efficiencies between different donor-acceptor species with varying spatial distances. Thus, the obtained FRET



**Figure 8.** Selectivity of the aqueous dispersion (pH 7.4,  $1.0 \times 10^{-5}$  g/mL; microgels were prepared with a B18C6Am feed ratio of 8.0 mol %) of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels upon addition of 5.0 equiv of different metal ions (Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>) at 38 °C in the absence (light gray bars) and presence (gray bars) of 5.0 equiv of K<sup>+</sup>, respectively.

efficiencies can only be considered as an averaged value. Moreover, fluorescence emission at  $\sim$ 588 nm is a sum of contributions from the FRET process and the direct excitation of RhBEA residues; currently, it is quite difficult to quantify the relative contributions from both aspects.

A competition detection experiment was also conducted, in which we add 5.0 equiv of K<sup>+</sup> (relative to crown ether moieties) into the microgel dispersions in the presence of 5.0 equiv of Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> at 38 °C, respectively (Figure 8). From Figure 8, we can see that the addition of 5.0 equiv of K<sup>+</sup> ions exhibits prominent decrease of FRET efficiency even in the presence of other background metal ions. This indicates that there is no obvious interference from Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> ions, which is reasonable considering that the cavity size of B18C6Am can well fit K<sup>+</sup> ions.

Kinetic Process of K<sup>+</sup>-Induced Volume Phase Transitions of P(NIPAM-B18C6Am-NBDAE-RhBEA) Microgels. We are quite curious about the kinetics of K<sup>+</sup>-induced VPTs of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels, as the time scale is relevant to the performance of this novel type of fluorescent ratiometric K<sup>+</sup> sensors. The stopped-flow fluorescence technique was employed to characterize the kinetic process. Figure 9 shows the typical time dependence of fluorescence emission intensities obtained for the aqueous dispersion of P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels recorded at 529 and 588 nm, respectively, upon stopped-flow mixing microgel dispersions (pH 7.4) with varying amount of aqueous KCl at 38 °C. We can clearly observe the time-resolved increase of fluorescence emission intensity at 529 nm (emissions of NBDAE) and the decrease of emission intensity at 588 nm (emissions of RhBEA) upon stopped-flow mixing.

Figure 10a shows time-dependent fluorescence intensity ratio changes,  $F_{588}/F_{529}$ , and the decrease of intensity ratio with time is clearly evident. Moreover, at higher final KCl concentrations and a fixed final microgel concentration (1.0  $\times$  10<sup>-5</sup> g/mL), the final equilibrium intensity ratios are lower. Results obtained from kinetic studies (Figures 9 and 10) are in excellent agreement with equilibrium fluorescence results (Figure 6). From Figure 10, we can qualitatively tell that K<sup>+</sup>-induced microgel VPT completes within  $\sim 4$  s. This is quite fast, and the conventional spectrofluorometric technique cannot afford such high time resolution, whereas the stopped-flow technique with a typical dead time of  $\sim 2-3$  ms is an excellent technique. Kinetics traces shown in Figure 10a were further fitted with single exponential functions to obtain characteristic relaxation times ( $\tau$ ), and the results are shown in Figure 10b.  $\tau$  ranges from 0.74 to 1.56 s in the final  $K^+$  concentration range of 5-30



**Figure 9.** Time dependence of fluorescence emission intensities ( $\lambda_{ex}$  = 470 nm; slit widths: Ex. 20 nm, Em. 20 nm) recorded at (a) 529 nm and (b) 588 nm upon stopped-flow mixing P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4) with varying amount of aqueous KCl at 38 °C. The final concentration of microgels was fixed at 1.0 × 10<sup>-5</sup> g/mL, and the final KCl concentrations were varied.



**Figure 10.** (a) Time dependence of fluorescence intensity ratio changes  $(\lambda_{ex} = 470 \text{ nm}; \text{slit widths: Ex. 20 nm}, \text{Em. 20 nm}), F_{588}/F_{529}$ , recorded upon stopped-flow mixing P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions (pH 7.4) with varying amount of aqueous KCl at 38 °C. The final concentration of microgels was fixed at  $1.0 \times 10^{-5}$  g/mL, and the final KCl concentrations were varied. (b) Characteristic relaxation time,  $\tau$ , obtained from the single exponential fitting of dynamic traces upon stopped-flow mixing microgel dispersions with varying amounts of aqueous KCl solution.

 $\mu$ M. Higher K<sup>+</sup> concentrations provide larger driving forces for microgel reswelling. It should be noted that the characteristic relaxation times obtained here are much larger than those obtained for the purely pH-induced or thermoinduced VPT process of responsive microgels, suggesting that the diffusion of  $K^+$  ions into the microgel and subsequent formation of 1:1 molecular recognition complexes are the rate-determining step.

#### Conclusions

In summary, we synthesized thermoresponsive and K<sup>+</sup>responsive P(NIPAM-B18C6Am-NBDAE-RhBEA) microgels consisting of B18C6Am crown ether moieties, fluorescence resonance energy transfer (FRET) donors, NBDAE, and rhodamine-B-based FRET acceptors (RhBEA) via the free radical emulsion copolymerization technique. FRET efficiencies between NBDAE and RhBEA moieties can be employed to monitor the thermoinduced microgel collapse and swelling. Moreover, addition of K<sup>+</sup> into the P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel dispersions can considerably elevate the thermal phase transition temperatures due to the formation of crown ether- $K^+$  ion molecular recognition complexes, as evidenced from temperature-dependent optical transmittance, dynamic LLS, and spectrofluorometric characterization results. At temperatures located between the LCSTs of microgels in the absence and presence of  $K^+$  ions, the addition of proper amounts of K<sup>+</sup> ion can induce microgel reswelling, and this process can be facilely monitored by changes in FRET efficiencies. At a P(NIPAM-B18C6Am-NBDAE-RhBEA) microgel concentration of 1.0  $\times$  10<sup>-5</sup> g/mL, the detection limit of  $K^+$ ions is  $\sim$ 3.6  $\mu$ M at 38 °C with a response time of  $\sim$ 4 s, as evidenced from stopped-flow characterization results.

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#### **References and Notes**

- (1) Ueyama, H.; Takagi, M.; Takenaka, S. J. Am. Chem. Soc. 2002, 124, 14286–14287.
- (2) He, F.; Tang, Y. L.; Wang, S.; Li, Y. L.; Zhu, D. B. J. Am. Chem. Soc. 2005, 127, 12343–12346.

(3) Minta, A.; Tsien, R. Y. J. Biol. Chem. 1989, 264, 19449–19457.
(4) Crossley, R.; Goolamali, Z.; Sammes, P. G. J. Chem. Soc., Perkin Trans. 1994, 2, 1615–1623.

(5) Yamauchi, A.; Hayashita, T.; Nishizawa, S.; Watanabe, M.; Teramae, N. J. Am. Chem. Soc. **1999**, *121*, 2319–2320.

(6) Helgeson, R. C.; Czech, B. P.; Chapoteau, E.; Gebauer, C. R.; Kumar, A.; Cram, D. J. J. Am. Chem. Soc. **1989**, 111, 6339–6350.

(7) Kim, J.; McQuade, D. T.; McHugh, S. K.; Swager, T. M. Angew. Chem., Int. Ed. 2000, 39, 3868-+.

(8) Hsueh, S. Y.; Lai, C. C.; Liu, Y. H.; Peng, S. M.; Chiu, S. H. Angew. Chem., Int. Ed. 2007, 46, 2013–2017.

(9) Meier, M. A. R.; Schubert, U. S. Chem. Commun. 2005, 4610-4612.

(10) Yin, J.; Guan, X. F.; Wang, D.; Liu, S. Y. Langmuir 2009, 25, 11367–11374.

(11) Liu, T.; Hu, J. M.; Yin, J.; Zhang, Y. F.; Li, C. H.; Liu, S. Y. Chem. Mater. **2009**, *21*, 3439–3446.

(12) Kim, J. S.; Quang, D. T. Chem. Rev. 2007, 107, 3780-3799.

(13) Lourdes, B. D.; David, N. R.; Mercedes, C. C. Chem. Soc. Rev. 2007, 36, 993–1017.

(14) Royzen, M.; Durandin, A.; Young, V. G.; Geacintov, N. E.; Canary,
 J. W. J. Am. Chem. Soc. 2006, 128, 3854–3855.

(15) Brummer, O.; La Clair, J. J.; Janda, K. D. Org. Lett. **1999**, *1*, 415–418.

- (16) Wen, Z. C.; Yang, R.; He, H.; Jiang, Y. B. Chem. Commun. 2006, 106–108.
- (17) Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S. K. Org. Lett. 2006, 8, 371–374.
- (18) Gunnlaugsson, T.; Leonard, J. P.; Murray, N. S. Org. Lett. 2004, 6, 1557–1560.
- (19) Wu, Q. Y.; Anslyn, E. V. J. Am. Chem. Soc. 2004, 126, 14682-14683.
  - (20) Bag, B.; Bharadwaj, P. K. Org. Lett. 2005, 7, 1573-1576.
  - (21) Kramer, R. Angew. Chem., Int. Ed. 1998, 37, 772-773.

- (22) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. J. Am. Chem. Soc. 2000, 122, 968–969.
- (23) Inoue, M. B.; Medrano, F.; Inoue, M.; Raitsimring, A.; Fernando, Q. *Inorg. Chem.* **1997**, *36*, 2335–2340.
- (24) Ghosh, P.; Bharadwaj, P. K.; Mandal, S.; Ghosh, S. J. Am. Chem. Soc. 1996, 118, 1553–1554.
- (25) Martinez-Manez, R.; Sancenon, F. Chem. Rev. 2003, 103, 4419–4476.
- (26) deSilva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley,
- A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. Chem. Rev. 1997, 97, 1515–1566.
- (27) Czarnik, A. W. Acc. Chem. Res. 1994, 27, 302-308.
- (28) Zeng, H. H.; Thompson, R. B.; Maliwal, B. P.; Fones, G. R.; Moffett, J. W.; Fierke, C. A. Anal. Chem. 2003, 75, 6807–6812.
- (29) Beltramello, M.; Gatos, M.; Mancin, F.; Tecilla, P.; Tonellato, U. *Tetrahedron Lett.* **2001**, *42*, 9143–9146.
- (30) Yang, H.; Liu, Z. Q.; Zhou, Z. G.; Shi, E. X.; Li, F. Y.; Du, Y. K.; Yi, T.; Huang, C. H. *Tetrahedron Lett.* **2006**, *47*, 2911–2914.
- (31) Nouira, W.; Haddad, R.; Barhoumi, H.; Maaref, A.; Bausells, J.; Bessueille, F.; Leonard, D.; Jaffrezic-Renault, N.; Errachid, A. *Sens. Lett.* **2009**, *7*, 689–693.
- (32) Pawlowski, P.; Michalska, A.; Wojciechowski, M.; Golimowski, J.; Bulska, E.; Maksymiuk, K. *J. Solid State Electrochem.* **2009**, *13*, 107–113.
- (33) Xu, Y. P.; Ramu, Y.; Lu, Z. Nature 2008, 451, 826–U8.
- (34) Orvedahl, D. S.; Chan, A.; Degnan, S.; Murphy, C.; Fennell, S.; Krouwer, J. *Clin. Chem.* **1996**, *42*, 770–770.
- (35) Dietrich, B.; Lehn, J. M.; Sauvage, J. P.; Blanzat, J. *Tetrahedron* **1973**, *29*, 1629–1645.
- (36) Desilva, A. P.; Gunaratne, H. Q. N.; Sandanayake, K. R. A. S. *Tetrahedron Lett.* **1990**, *31*, 5193–5196.
- (37) He, H. R.; Mortellaro, M. A.; Leiner, M. J. P.; Fraatz, R. J.; Tusa, J. K. J. Am. Chem. Soc. 2003, 125, 1468–1469.
- (38) Irie, M.; Misumi, Y.; Tanaka, T. Polymer 1993, 34, 4531–4535.
  (39) Alexandratos, S. D.; Stine, C. L. React. Funct. Polym. 2004, 60, 3–16.
- (40) Ju, X. J.; Chu, L. Y.; Mi, P.; Song, H.; Lee, Y. M. Macromol. Rapid Commun. 2006, 27, 2072–2077.
- (41) Mi, P.; Chu, L. Y.; Ju, X. J.; Niu, C. H. Macromol. Rapid Commun. 2008, 29, 27–32.

- (42) Hara, N.; Ohashi, H.; Ito, T.; Yamaguchi, T. Macromolecules 2009, 42, 980–986.
- (43) Ju, X. J.; Liu, L.; Xie, R.; Niu, C. H.; Chu, L. Y. Polymer 2009, 50, 922–929.
- (44) Ju, X. J.; Chu, L. Y.; Liu, L.; Mi, P.; Lee, Y. M. J. Phys. Chem. B 2008, 112, 1112–1118.
- (45) Mi, P.; Ju, X. J.; Xie, R.; Wu, H. G.; Ma, J.; Chu, L. Y. Polymer **2010**, *51*, 1648–1653.
- (46) Zhang, X. Z.; Zhang, J. T.; Zhuo, R. X.; Chu, C. C. Polymer 2002, 43, 4823–4827.
- (47) Lin, S. Y.; Liu, S. W.; Lin, C. M.; Chen, C. H. Anal. Chem. 2002, 74, 330.
- (48) Xia, W. S.; Schmehl, R. H.; Li, C. J. J. Am. Chem. Soc. 1999, 121, 5599–5600.
- (49) Yamaguchi, T.; Ito, T.; Sato, T.; Shinbo, T.; Nakao, S. J. Am. Chem. Soc. **1999**, *121*, 4078–4079.
- (50) Ito, T.; Sato, Y.; Yamaguchi, T.; Nakao, S. *Macromolecules* **2004**, *37*, 3407–3414.
- (51) Okajima, S.; Sakai, Y.; Yamaguchi, T. Langmuir 2005, 21, 4043–4049.
- (52) Ito, T.; Yamaguchi, T. J. Am. Chem. Soc. 2004, 126, 6202-6203.
- (53) Ito, T.; Hioki, T.; Yamaguchi, T.; Shinbo, T.; Nakao, S.; Kimura,
- S. J. Am. Chem. Soc. 2002, 124, 7840-7846.
- (54) Ito, T.; Yamaguchi, T. Langmuir 2006, 22, 3945–3949.
- (55) Ito, T.; Yamaguchi, T. Angew. Chem., Int. Ed. 2006, 45, 5630–5633.
- (56) Chu, L. Y.; Yamaguchi, T.; Nakao, S. Adv. Mater. 2002, 14, 386–389.
- (57) Jones, C. D.; McGrath, J. G.; Lyon, L. A. J. Phys. Chem. B 2004, 108, 12652–12657.
- (58) Gan, D. J.; Lyon, L. A. J. Am. Chem. Soc. 2001, 123, 8203–8209.
  (59) Hansen, T. S.; Daugaard, A. E.; Hvilsted, S.; Larsen, N. B. Adv. Mater. 2009, 21, 4483-+.
- (60) Uchiyama, S.; Matsumura, Y.; de Silva, A. P.; Iwai, K. Anal. Chem. 2003, 75, 5926–5935.
- (61) Wang, D.; Yin, J.; Zhu, Z. Y.; Ge, Z. S.; Liu, H. W.; Armes, S. P.; Liu, S. Y. *Macromolecules* **2006**, *39*, 7378–7385.

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