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Resolving the Difference in Electric Potential within a Charged Macromolecule

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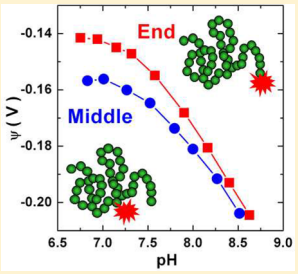
Supporting Information

ABSTRACT: The difference of the electric potential between the middle and end of polystyrenesulfonate (PSS^-) chain is discovered experimentally. Using a pH-responsive fluorophore attached to these two locations on the PSS^- chain, the local pH value was determined by single molecule fluorescence technique: photon counting histogram (PCH). By the observation of a very high accumulation of proton (2−3 orders of magnitude in concentration) at the vicinity of the PSS^- as a result of the electrostatic attraction between the charged chain and protons, the electric potential of the PSS^- chain is determined. A higher extent of counterion adsorption is discovered at the middle of the PSS^- chain than the chain end. The entropy effect of the counterion adsorption is also discovered—upon the dilution of protons, previously adsorbed counterions are detached from the chain.



INTRODUCTION

Polyelectrolyte is an important type of macromolecule carrying multiple permanent ions or ionizable groups on their main chain. The study of the physics and chemistry of polyelectrolytes has been attracting intensive research attention because polyelectrolytes have broad applications in numerous fields such as water treatment, daily life products, smart pharmaceutical products, bio- and medical materials, etc. More importantly, the physics of polyelectrolytes has been considered to be the key knowledge for the understanding of biological processes. Because of the long-range electrostatic interaction and the existence of multiple counterions, polyelectrolytes exhibit unique and complicated properties, which in turn have brought about difficulties to the understanding of their properties.[1−10]

The most important aspect of polyelectrolytes is the charge density or the electric potential of the chain as a whole or a part, which depends not only on the original chemical structure but also highly on the distribution of counterions.[5,6] Because of its chainlike molecular architecture, the inhomogeneity of the counterion distribution along the polyelectrolytes molecule has long been believed to exist. There have been studies by both theoretical analysis and computer simulation[7−9,11−19] that the charge density at the chain end is different from that of the middle portion of the molecular chain. Such a chain end effect of the charged macromolecules is a very important issue, considering the numerous processes in which the chain ends may play very important and essential roles, such as self-assembly of charged linear micelles,[20] polyelectrolyte chains adsorption on charged surfaces,[21] polyelectrolyte brushes and polyelectrolyte hydrogels,[22,23] and DNA translocation through protein channels,[24,25] etc.

However, up to now, there has been no direct experimental evidence reported about the chain end effect of the charge density or electric potential of the charged chain molecules. In this study, the difference of electric potential between the middle and the chain end of a model polyelectrolyte system, sodium polystyrenesulfonate (NaPSS), has been investigated successfully. By positioning a pH-sensitive fluorescent molecule with chemical linkage to the middle and the end of the NaPSS chain and by adopting a combination of fluorescence fluctuation spectroscopy−fluorescence correlation spectroscopy (FCS)[26−28] and photon counting histogram (PCH),[29,30] the difference in counterion distribution and the electric potential at these positions has been investigated at single molecular level.

EXPERIMENTAL SECTION

The spatial difference in electric potential within the NaPSS molecule is resolved by measuring the local pH value at the middle and end of the chain, based on that protons serve as the counterions of the PSS^- chain partially. By single molecule fluorescence methods, the local pH value was determined by measuring the fluorescence intensity of single pH-responsive fluorophore chemically positioned at different locations. The chemical structure of PSS^- polymer and the pH-responsive fluorescent molecule, Oregon Green 488 (OG488), are shown in Chart 1.

The preparation of NaPSS was conducted through sulfonation of polystyrene (PS). Two original PS samples were used: end-functionalized and middle-functionalized PS. Amino group-terminated PS (M_n = 120 × 10^3 g mol^{-1}, M_w/M_n = 1.05) was purchased from Polymer Source (Canada). Middle-functionalized PS (M_n = 117 × 10^3 g mol^{-1}, M_w/M_n = 1.16) was home-prepared by atom transfer radical polymerization.

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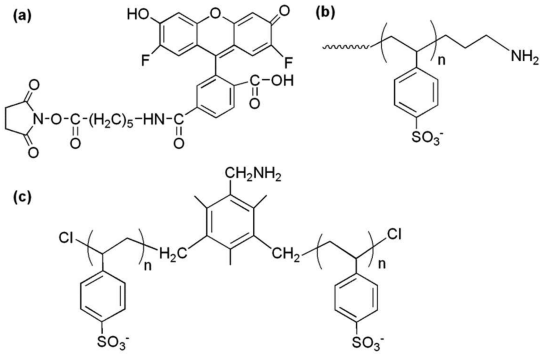
Chart 1. Chemical Structure of the pH-Responsive Oregon Green 488 (OG488), Succinimidyl Ester (a), Amino-Terminated Polystyrene Sulfonate (b), and Middle-Functionalized Polystyrene Sulfonate (c)



Synthesis of Polystyrene Middle-Functionalized by an Amino Group. The synthesis of the polystyrene functionalized at the middle by an amino group was conducted by atom transfer radical polymerization (ATRP), as shown in Scheme 1. The initiator (**1**) is first synthesized by the following protocol: 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene (2.0 g, 5.0 mmol) and sodium azide (0.26 g, 4.0 mmol) were dissolved in anhydrous dimethylformamide (30 mL). After stirring at 50 °C for 24 h, the solvent was evaporated and the residue was partitioned between dichloromethane (200 mL) and water (50 mL). The organic phase was washed with brine three times and dried over $MgSO_4$. The product was purified by flash column chromatography (silica gel, petroleum ether/dichloromethane 3:1).

Polystyrene (**2**) was synthesized via ATRP of styrene with **1**. A mixture of styrene (13.83 g, 0.133 mol), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (57.6 mg, 0.33 mmol), and **1** (20 mg, 0.055 mmol) was introduced into a polymerization tube with a magnetic stirrer and degassed by three freeze−pump−thaw cycles. The tube was then filled with nitrogen, after which CuCl (21.9 mg, 0.22 mmol) was quickly added into the frozen mixture. With the flask sealed, the mixture was evacuated and backfilled with nitrogen three times. The tube was heated to 80 °C in an oil bath. After stirring for 80 h, the polymerization was terminated by exposing the mixture to air and diluted with tetrahydrofuran (THF). The solution was passed through a column filled with basic alumina, and the polymer was precipitated in a large amount of methanol for three times and dried in a vacuum at 50 °C, producing the solid of **2**.

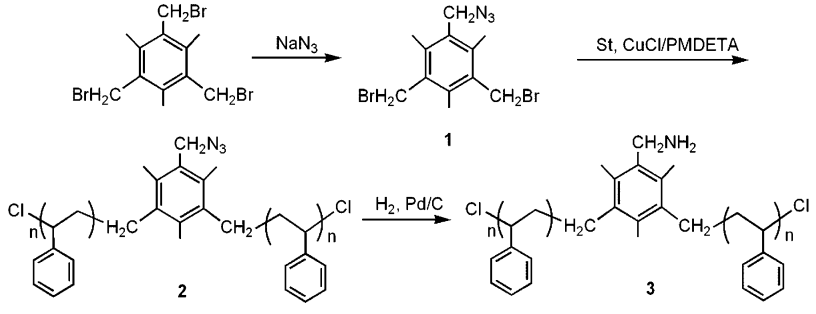
After three vacuum/H_2 cycles to remove air from the reaction tube, a mixture of **2** (200 mg) and 10% Pd/C (20 mg, 10% of the weight of the substrate) in THF (5 mL) was stirred under a hydrogen atmosphere at room temperature for 48 h. The reaction mixture was filtered using a sintered filter funnel. After the filtrate was concentrated, the polymer was precipitated in a large amount of methanol and dried in a vacuum at 50 °C to give **3** (Figure S2 in the Supporting Information).

Sulfonation and Labeling. The sulfonation of both polystyrene samples was conducted according to a published protocol,[31] and careful measurements have shown that the degree of sulfonation is more than 96% without any breakage of backbone under optimized experimental condition. The NaPSS polymers were later labeled with a pH-responsive fluorescent molecule, OG488 succinimidyl ester (chemical structure provided in Chart 1a). The labeled polymers were purified by both ultrafiltration and dialysis, and the sample purity was verified by careful control experiments. Control experiments also demonstrated no change in the fluorescence properties of OG488 due to the chemical linkage to the polymer chain (details in the Supporting Information).[32] It was also verified that OG488 itself showed no response to the presence of NaCl in the concentration range studied.

Measuring Local pH Value in the Vicinity of a Single PSS^- Chain. Single molecule fluorescence techniques—fluorescence correlation spectroscopy (FCS) and photon counting histogram (PCH)—were adopted to investigate the fluorescence emission intensity of single OG488 fluorophore chemically attached to PSS^- chain. These two techniques with single molecule sensitivity were combined together, monitoring the fluctuation of the fluorescence intensity inside the confocal excitation-detection volume. FCS analyzed the time-lag autocorrelation function of the fluctuation of the photon count and determined the diffusion coefficient (therefore the hydrodynamic radius), while PCH analyzed the deviation of the fluorescence photon counts from the standard Poisson distribution due to the fluctuation (the resulted super-Poisson distribution) and determined the fluorescence emission from single fluorophore within unit time, named the "brightness". The principles of these two well-established methods are detailed in a number of publications.[26−30] The experimental setup in this study was a home-built one based on the platform of an inverted microscopy (IX-71, Olympus, Japan) with a confocal geometry.[33−37] The 488 nm output of a solid state laser served as the excitation light source and was introduced into the sample solution through a water-immersion objective lens (Plan Apochromat 60×, numerical aperture = 1.2). The excited fluorescence was collected by the same objective lens. After passing a confocal pinhole with a diameter of 50 μm, the fluorescence was split into two parts with identical intensity and detected separately by two photomultiplier tube-based single photon counting module (H-7421, Hamamatsu, Japan). The single photon counting signal was recorded by a FCS-PCH data acquisition board (ISS, USA), and the data analysis was conducted by its software.

OG488-labeled PSS^- samples were dissolved in deionized water (18.2 $M\Omega\cdot cm^{-1}$) at a concentration of 5×10^{-9} M. The pH value of the solution (monitored by a pH meter) was controlled by adjusting the concentration of HCl or NaOH, instead of using a buffer solution in order to keep the level of added salt as low as possible. The resulting concentration of added sodium ions (Na^+) in the PSS^- solution is from 10^{-5} to 10^{-4} M. In order to suppress the possible contamination by dust and, more importantly, to avoid the fast uptake of carbon dioxide in the ambient condition, the sample solution was kept inside a sealed sample cell. The experiments were conducted at 25 °C.

Scheme 1. Chemical Protocol of the Synthesis of Polystyrene Functionalized at the Middle of the Chain by an Amino Group



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RESULTS AND DISCUSSION

Because of the electrostatic attraction between the PSS^- chain and protons (H^+), the local concentration of H^+ serves as the probe for the counterion distribution of PSS^- chain. The local proton concentration was determined by measuring the local pH value through the fluorescence brightness of the pH-responsive OG488 attached to the PSS^- chain. By taking the response curve of the brightness of free OG488 to the pH value of the solution as the master curve (inset of Figure 1), the local pH value of the PSS^- chain was measured.

Figure 1a shows the local pH value at the end and the middle of PSS^- chain as a function of pH value of the solution, in which the local pH value at the vicinity of the PSS^- chain (at the end and the middle) increases with the pH in the solution. Two features are immediately noticed: (1) Compared with the pH value in bulk solution, the local pH value at the vicinity of PSS^- chain is considerably lower by 2−3 pH units, corresponding to a difference in H^+ concentration by 2−3 orders of magnitude. This fact indicates the existence of the highly concentrated counterions near the charged chain because of the fact that H^+ constitutes partially the counterions due to its exchanging process with original counterion (Na^+). The dynamic exchange between counterions has been discovered by a previous study[37] and was also proved by a control experiment here, which, by spectral characterization, showed that Na^+ can be replaced by H^+ at prolonged dialysis of NaPSS in pure water. Therefore, the above result clearly shows a much denser distribution of counterions surrounding the PSS^- chain as a result of the electrostatic attraction between the charged chain and the counterions. This observation agrees well with the model of counterion adsorption[6] and also with a previous experimental study with a different system,[35] in which case the counterions form a cloudlike distribution around the charged chain. (2) The local pH value at the middle of PSS^- chain is always lower than that at the chain end—about 1.7 times (at pH 6.8, for example) difference in concentration (Figure 1b), exhibiting a higher counterion concentration in the middle of the PSS^- chain than at the end. Such a difference decreases continuously when the pH value is further raised, and the ratio dropped to 1.2 at pH 8.5.

The electric potential of the middle and the end of the PSS^- chain is determined by analyzing the distribution of protons using the universal Boltzmann distribution profile: $[H^+]_{local} = [H^+]_{bulk} \exp(-e\psi/k_BT)$, where $[H^+]_{local}$ and $[H^+]_{bulk}$ denote concentration of H^+ ions at the local vicinity of PSS^- chain and in the bulk solution, e the element charge, ψ the electric potential, k_B the Boltzmann constant, and T the absolute temperature. This relation leads to $pH_{local} = pH_{bulk} + 0.43(e\psi/k_BT)$, where pH_{local} and pH_{bulk} denote the local pH value at the PSS^- chain and that in the bulk solution. The data of the electric potential at the middle and the end of PSS^- chain as a function of pH value are presented in Figure 2, which provides a clear evidence of the chain-end effect of this charged macromolecule. The absolute value of the electric potential is higher for the middle of the PSS^- chain ($|\psi_{middle}|$) than that at

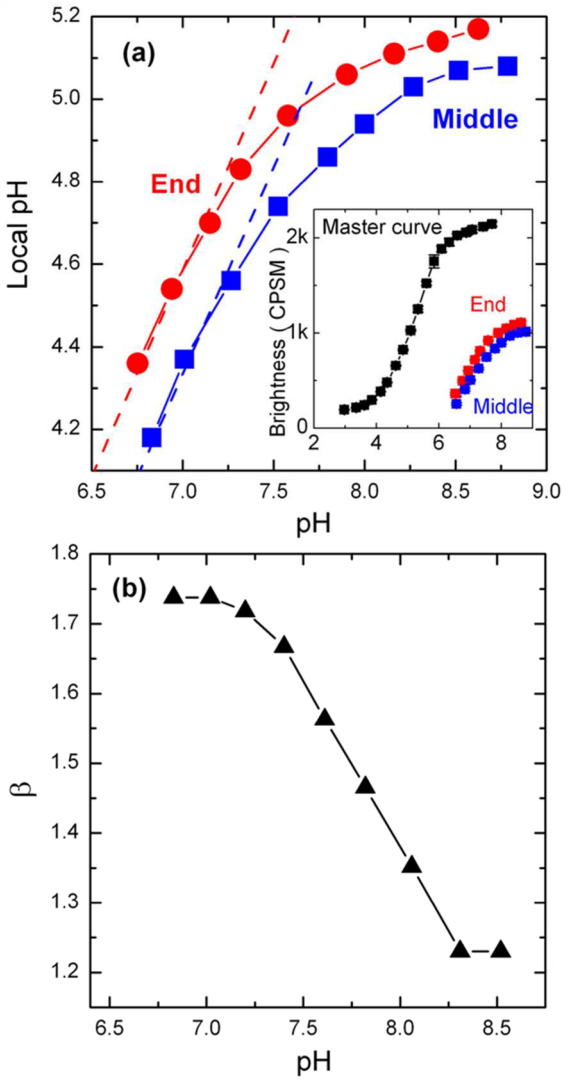

Figure 1. (a) Local pH value at the middle and the end of the PSS^- chain as a function of the pH value in the bulk solution, as determined by PCH measurements. The dashed lines denote the fitting by Boltzmann distribution function. Inset: pH dependence of the single molecule brightness of OG488 linked to the middle and the end of PSS^- chain. The response curve of free OG488 (black data point) is displayed as the master curve for local pH determination. (b) Ratio of proton concentration (β) the middle over that of the end of the PSS^- chain.

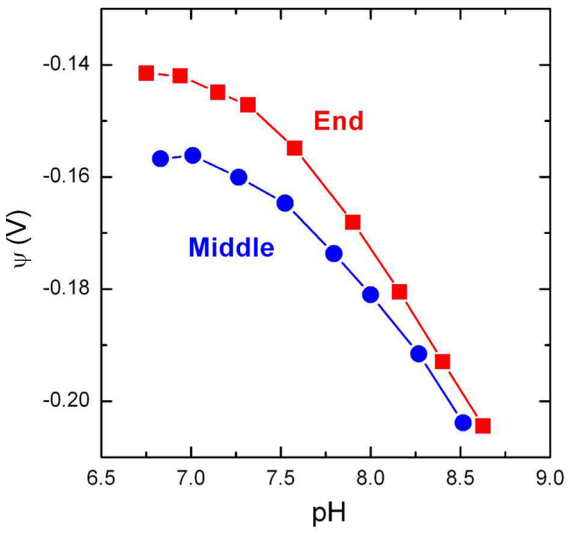

Figure 2. Electric potential (ψ) at the middle and the end of a single PSS^- chain as a function of pH value of the solution.

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the chain end ($|\psi_{end}|$). As an example, at pH 6.8, the value of $|\psi_{middle}|$ is slightly below 0.16 V and that of $|\psi_{end}|$ is 0.14 V.

The inhomogeneity of the counterion distribution of the PSS^- chain is clearly exposed—the counterion concentration is higher at the middle than that at the end of the chain. This difference originates from the original topological configuration, which generates the basic difference in the electric field at the chain—a counterion at the middle experiences the attraction from both sides of the chain along the dimension of the backbone while the one at the chain end only experiences attraction from one half. From another point of view, this is attributed to the probability for the chain end to be located with respect to the random coil of the polymer chain—the chain end has a higher probability to reside at the outer portion of the random coil of the PSS^- chain, where a lower concentration of counterions is found.[38]

The absolute values of electric potential of both locations remain constant below pH 7.0 but increase with the further elevation of pH value in the solution (Figure 2). Correspondingly, these results show that the charge density of the PSS^- chain is constant below pH 7.0 while the charge density increases beyond pH 7.0. This is a clear indication of the entropy effect to the counterion distribution. With the decrease of proton concentration (for pH < 7.0), the cloud of protons surrounding the PSS^- chain expands at first, resulting in the decrease of the local proton concentration without affecting the charge density of the PSS^- chain. This is further evidenced by the good fitting by Boltzmann distribution function (denoted by the dashed lines in Figure 1a). This observation also agrees well with a previous observation with a different system.[35] However, when the pH value is further increased, the dilution of protons makes the previously adsorbed (condensed) protons on the PSS^- chain be released and detached from the chain. The consequence of this process is the increase in the charge density of the PSS^- chain as demonstrated by the elevation of the amplitude of the electric potential. Meanwhile, with the further dilution of protons, more counterions are detached from the chain end than the middle of the chain, making the charge density or the electric potential increased more than the middle. This therefore reduces the difference in charge density between the middle and the end, evidenced by the reduction of the difference between $|\psi_{middle}|$ and $|\psi_{end}|$—it drops from 0.02 V at pH 6.8 to 0.004 V at pH 8.5.

The validity of the determination of the local pH values by PCH method is proved by a number of control experiments. The pH response of OG488 depends on its protonation process, and its spectrum changes continuously with the change of pH values.[39,40] As a consequence, the fluorescence intensity of the fluorophore increase continuously with the elevation of pH value around it. The advantage of the high sensitivity of the PCH method allows measurements of fluorescence intensity of the fluorophore (the brightness) at a single molecular level. Therefore, it is critical to prove that the chemical linkage to the polymer chain does not affect the properties of the pH-responsive fluorophore, for example, the pK_a value. This is done by the following control experiments which are all detailed in the Supporting Information. (1) The pH response of the OG488 with the chemical linkage to a monomer (styrenesulfonate) is identical to that of the free OG488, as measured by PCH. This demonstrates that local chemical linkage does not affect the fluorophore. This can be easily understood by the existence of the aliphatic spacer between the fluorophore and the polymer chain. This is further verified by the fact that the identical fluorescence emission spectra of the OG488 with and without chemical linkage to the PSS^- chain were recorded at identical pH local values, for both end- and middle-labeling. (2) The pH response of OG488 with linkage to a neutral polymer, poly(*N*-isopropylacrylamide), is identical to that of the free OG488, indicating the dominant role of the electrostatic interaction in the effect with PSS^- chain.

Separate measurements by using another pH-responsive fluorophore, Oregon Green 514 (OG514), also exhibit very similar results. (For clarity, all the details of the following measurements are provided in the Supporting Information.) By attaching OG514 to the middle and the end of the same PSS^- chain, the difference in electric potential between these two locations is clearly exposed by PCH measurements. Within about 10% offset in the electric potential value compared with what were determined by OG488, the difference between the end and the middle is obvious at a very similar level. Meanwhile, by taking the advantage of OG514 being a ratiometric pH indicator, its steady fluorescence excitation spectra with and without conjugation with the PSS^- chain were measured, and the results clearly exposed the huge difference in proton concentration near the PSS^- chain and the solution as well as the difference between the middle and the end of the PSS^- chain. Compared with what observed by the PCH method, an effect by the increased concentration of PSS^- chain showed up, as discussed later in the text. Besides, another set of separate measurements of quantum yield of the OG488 with and without the conjugation to PSS^- chain were conducted, and the results again show very similar results compared with those by the PCH method. Still, due to the lower sensitivity of the ordinary spectrometer, a concentration effect showed up compared with the single molecular PCH measurements. A discussion on concentration effect is provided later in the text.

Several other issues have been considered: (1) The concern of why the sodium ions do not fill the vacancies left by the detachment of the protons with the presence of 10^{-4}–10^{-5} M concentration of Na^+. This is attributed to the much weaker binding of Na^+ to the PSS^- chain than protons.[8] The weaker binding of Na^+ with PSS^- chain than H^+ is evidenced by a control experiment in which the local pH change of PSS^- chain is monitored under an increase of Na^+ concentration. Only a minor increase of the local pH value (~0.2) was observed by a change of NaCl concentration of 10^{-4} M. This is in a sharp contrast with the effect by proton dilution—a change of 10^{-6} M of protons induced a change of local pH value of 1.0. A detailed description is provided in the Supporting Information. (2) The possible effect of polymer concentration. The concentration of NaPSS is kept constant (5.0×10^{-9} M) during the PCH measurements, and no concentration effect is supposed to affect the results. It should be interesting to investigate the change of counterion distribution within one PSS^- chain at different polymer concentration because this difference may disappear when the counterion clouds of different molecules overlap. Our control experiments by addition of unlabeled NaPSS in the solution failed to provide sufficient information on this. The results showed that the effect by polymer concentration appeared at 0.1 mg mL^{-1}, but only the gradual replacement of H^+ by Na^+ was observed. However, a slight reduction of the difference of local pH at the middle and end of the chain was observed. Details are provided in the Supporting Information. (3) The difference of the dielectric constant of the medium in the center and at the outer rim of the random coil of the PSS^- chain.[41] With the presence of electrostatic interaction

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and the hydrophobic segments of the polymer, there has been predicted a inhomogeneity of the dielectric constant along the charged chain, which may affect the local pH value. However, it is rather difficult to address this issue by the methods in the current study.

CONCLUSIONS

In summary, the direct experimental evidence on the difference of counterion distribution and the electric potential between the middle and the end of PSS^- chain is discovered. Because of electrostatic attraction between the charged chain and counterions, the counterions are mostly distributed around the chain—a difference of 2−3 orders of magnitude in concentration compared to the bulk solution is exposed. More counterions are bound to the middle of the chain than at the chain end, demonstrating a clear end effect of this charged linear macromolecule. The entropy effect in counterion distribution is evidenced, in which previously adsorbed counterions are detached from the PSS^- chain, resulting in the electric potential increased upon dilution.

ASSOCIATED CONTENT

Supporting Information

Detailed information on the data analysis of single-molecule PCH experiments (Figure S1), additional figures about SEC (Figure S2), FCS (Figure S3), influence of labeling on the fluorescence properties of OG488 (Figures S4−S6), the measurements by steady fluorescence spectroscopy as well as the by using OG514 (Figures S7−S9), the effect of sodium ions (Figure S10), and effect of polyelectrolyte concentration (Figure S11). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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