# Charge on a weak polyelectrolyte

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Fluorescence measurements with single-molecule sensitivity are used to measure the hydrodynamic size and local $p$H of a weak polyelectrolyte, poly-2-vinyl pyridine end labeled with $p$H-sensitive dye, the polyelectrolyte having concentration so low (nanomolars) that molecular properties are resolvable only from fluorescence experiments and cannot be accessed by light scattering. We find that the local $p$H near the dye, inferred from its brightness, is consistently three orders of magnitude higher than the bulk $p$H. Upon varying the bulk $p$H, we measure the collapse point at which hydrophobic attraction overwhelms electrostatic repulsion between charged elements along the chain, and conclude that adding monovalent salt shifts this coil-to-globule collapse to higher $p$H than in the absence of salt. The influence of salt appears to shift the ionization equilibrium of this weak polyelectrolyte in the direction of the chain possessing enhanced electric charge at a given $p$H. Phenomenologically, this is opposite to the case for strong polyelectrolytes, although the mechanism differs. © *2008 American Institute of Physics.* [DOI: 10.1063/1.3055596]

That the electrostatic charge on a polymer chain adjusts to its surroundings (is not set solely by its chemical makeup) is fundamental to the functions of those polymers that dissolve in water—not only synthetic polyelectrolytes but also biopolymers. The large and sometimes contentious literature dedicated to understanding the impact of surrounding ions focuses on the case where permanent charges may be neutralized by the localization of counterions, known as "counterion condensation." [1–6] Here we are interested in polyelectrolytes that do not fit so cleanly into this paradigm—the large class of polyelectrolytes whose charge state is determined by the chemical equilibrium with protons that bind to ionizable groups along the chain. The former polyelectrolytes are known as "strong," the latter as "weak." The major difference between the strong and weak polyelectrolyte is that, for the former case, the charges are fixed (immobilized) on the chain, while the charges are mobile (dynamic) for the latter one. This may lead to a different nature of the interaction between the counterions and the charged chain, because the binding of counterions may in turn shift the ionization equilibrium, and this is the premise of the current study.

Testing this idea, we study a physical situation where the physical observable is exquisitely sensitive to small changes in the amount of charge—this is the coil-to-globule collapse transition, an instability at which solubility of the chain owing to its electrostatic charge is overwhelmed by hydrophobic attraction. [7–9] The few-molecule fluorescence methods employed in this study not only are responsive to the collapse transition itself but also allow us to gauge the local $p$H in the immediate vicinity of the polymer chains, at solution concentrations near the limit of infinite dilution, at least three orders of magnitude lower than studied previously by other methods. [10–13] The significance of seeking the limit of infinite dilution is that this is the regime where the localization of protons onto polymer chains, that they serve to ionize, carries the highest entropic cost.

The experiments were performed at the Chinese Academy of Sciences in Beijing using a homebuilt setup for fluorescence correlation spectroscopy (FCS) described elsewhere. [9] The solution concentration of the polymer, ~3.0 nM (moles of polymer/liter), corresponded to a mean distance >0.7 $\mu$m between chains. This much exceeds the electrostatic screening length; within the range of salt concentration studied, the Debye length varied from 5 to 30 nm—always comparable to or larger than the dimension of the polymer chain. At these nanomolar concentrations, on average, one dye resided within the confocal volume. Poly-2-vinylpyridine (P2VP) (number-average molecular weight $M_n$=109 800 g mol$^{-1}$ and ratio of weight-average to number-average molecular weight $M_w/M_n$=1.03) was purchased from Polymer Source (Quebec, Canada), and in our laboratory fluorescent dyes were attached to the amino group at one end. Different fluorescent dyes were employed according to the purpose at hand: Alexa Fluor 488, Bodipy FL, Oregon Green 488 (from Invitrogen Corporation), or FITC (from Aldrich). The labeled polymer samples were purified by both size exclusion chromatography and dialysis. During experiments, the $p$H of the solution could not be controlled by buffer, as the salt concentration in a buffer would have been undesirably high. Instead, it was controlled by adjusting the concentration of strong acids such as HCl, HBr, or HNO$_3$, whose low concentrations had a minimal effect on electrostatic screening. The experiments were conducted at

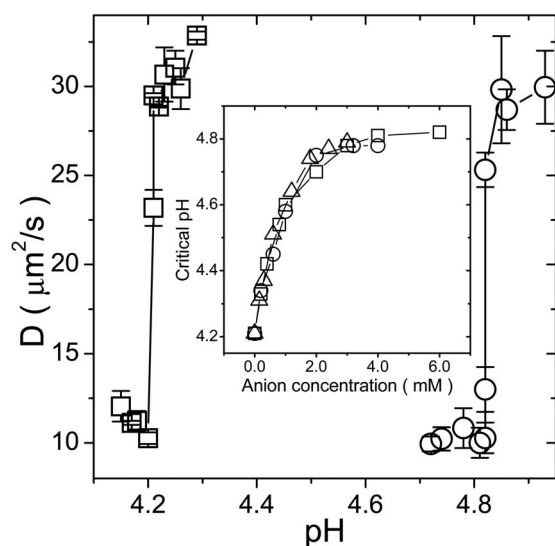a)Author to whom correspondence should be addressed. Electronic mail: jzhao@iccas.ac.cn.

FIG. 1. Diffusion coefficient of P2VP in aqueous solution plotted against pH of the solution without added salt (□) and with 6 mM NaCl (○), the experiments being conducted at ≈3.0 nM concentration with fluorophore Bodipy FL. Inset: critical pH at the collapse transition is plotted against chloride concentration for NaCl (□), MgCl$_2$ (○), and LaCl$_3$ (△). Other data (not shown) confirmed the same for different anions, Br$^-$ (KBr) and NO$_3^-$ (KNO$_3$), demonstrating the generality of this behavior.

room temperature, 22 °C. It is well known that each repeat unit along the P2VP polymer carries a pyridine that can associate with a proton to induce a positive electric charge.[14,15]

Figure 1 shows the first-order transition of these chains when its electrostatic charge falls below the level needed to overcome attraction between its hydrophobic segments. The translational diffusion coefficient measured by FCS ($D$), which is inversely proportional to its hydrodynamic size, is plotted against pH in salt-free solution and in the presence of monovalent salt.[9,16] Going beyond showing the existence of this first-order transition, these data show that the higher the salt concentration, the higher the critical pH at collapse, until the effect saturates. For a large number of additional experiments of this kind, the inset of Fig. 1 shows that regardless of the (monovalent) counterion chosen, the functional dependence was the same. It is understandable that ionic strength dependence saturates above 10 mM. This is because the entropy of the system scales as ln $c$, where $c$ denotes concentration, and reduction in free energy by increase in entropy scales as $\Delta c \cdot c^{-1}$, where $\Delta c$ denotes the change in concentration;[17] so naturally the latter term saturates at high concentration. The shift of transition point to higher pH with added salt implies that the local pH near the chain was reduced by salt. Experiments at even higher salt concentration proved to be not feasible because of the formation of aggregates due to electrostatic screening. The physics here differs from the well-documented field-assisted ionization of polyelectrolyte,[18] where the screening effect serves as the driving force and the ionic strength is at least two orders of magnitude higher than the current situation. Furthermore, we also discovered that not all salts have the same effect. For example, NaClO$_4$ did not show any critical pH upshift but a slight downshift (a full account of this issue will be described elsewhere). Provided screening effects had similarly
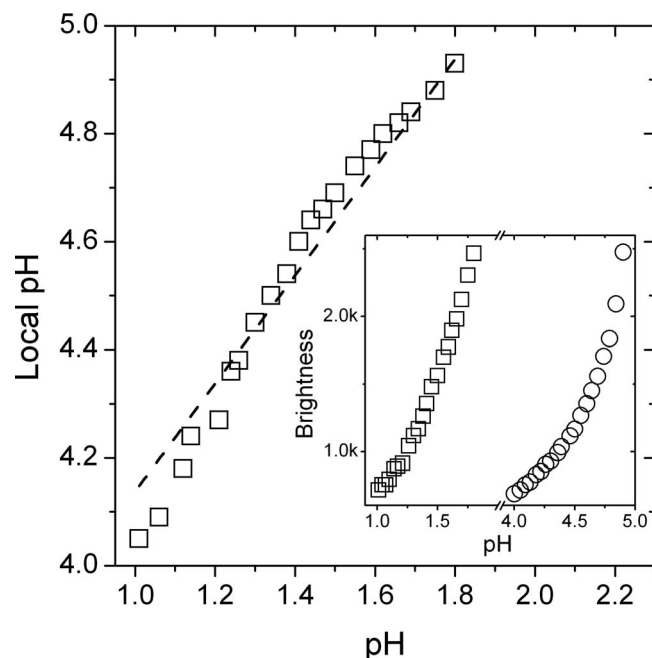


FIG. 2. Using a pH-sensitive dye to infer local pH. Inset: brightness of Oregon Green 488, measured from photon counting histograms compiled within the FCS confocal volume, plotted against solution pH for this fluorophore dissolved freely in solution (○) or attached to one end of the polymer chain (□). The dotted line emphasizes that these curves are shifted by 3 pH units on the horizontal axis. The unit for brightness is counts/second/molecule. Main figure: the local pH of polymer-attached fluorophore, defined as the pH at which the polymer-attached fluorophore displays the same brightness as when not attached, is plotted against bulk pH.

been the cause here, similar phenomenon should have been observed, and this was not the case.

A subtle point was the hypothetical possibility that not only protons, but also salt cations, might associate with the pyridine group on the polymer. However, the absence of such hypothetical complex formation was shown by NMR (nuclear magnetic resonance) characterization of aqueous solutions of pyridine and NaCl, for which no spectral difference in Na$^{23}$ was detected with and without the presence of pyridine.

In order to probe local pH near the polymer, pH-sensitive fluorophores were attached chemically to one end of the chains, and photon count histograms were constructed to quantify the brightness of fluorophores contained within the confocal volume.[19,20] The fluorophores were selected to have the property that their brightness increases with pH. The inset of Fig. 2 compares the brightness, as a function of pH, of Oregon Green 488 in free aqueous solutions and when attached to the polymer at otherwise the same solution conditions. The shift of the latter to lower pH than that of the free fluorophore is striking. For more quantification, we estimated the local pH of polymer-attached dye as the bulk pH at which the polymer-attached dye displayed that same brightness.[21] Results of this argument are summarized in the main part of Fig. 2, where local pH, near polymer-attached dye, is plotted against bulk pH. This huge difference in proton concentration is understood to result from electrostatic repulsion between protons and the positively charged polymer chain; that is why, for the polymer-
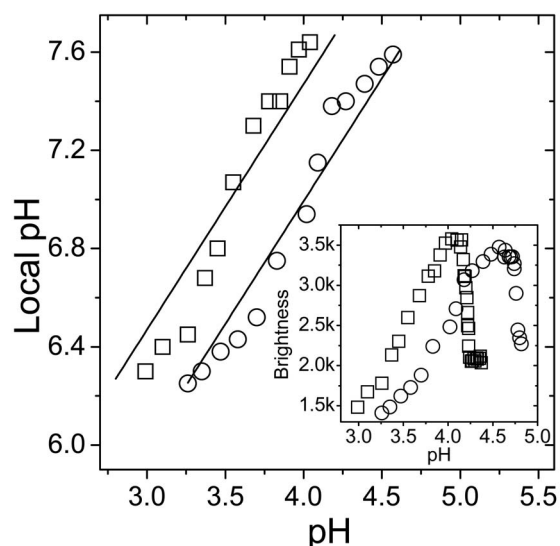
FIG. 3. Using a $p$H-sensitive dye to infer local $p$H in the presence of added salt. Inset: brightness of FITC (fluorescein isothiocyanate), measured from photon counting histograms compiled within the FCS confocal volume, plotted for this fluorophore dissolved freely in solution without added salt (□) and with 5 mM NaCl (○). The unit for brightness is counts/second/molecule. This calibrates data in the main figure. Main figure: local $p$H, defined as the $p$H at which the polymer-attached fluorophore displays the same brightness as when not attached, is plotted against $p$H without added salt (□) and with 5 mM NaCl (○). Dotted lines through the data are fits to the naive Boltzmann distribution discussed in the text.
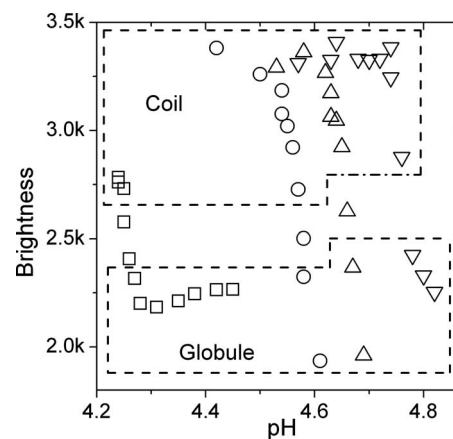


FIG. 4. Reversibility of the collapse transition when $p$H and ionic strength were varied separately in studies of FITC-attached polymer. Brightness is plotted against bulk $p$H. The unit for brightness is counts/second/molecule. First, polymer was dissolved without added salt, and collapse was induced by raising $p$H (□). Then the polymer was returned to the coil state by adding NaCl to reach a concentration of 1 mM, and collapse was induced by further raising the $p$H (○). This process was continued for 2 mM NaCl (△) and 5 mM NaCl (▽). The regimes of the coil and collapse conformations (fast and slower diffusions, respectively: $D \sim 33$ and 11 $\mu m^2/s$, respectively) are highlighted by the rectangles bordered with dotted lines. Note the sharp decrease in brightness even before the chain collapses into the globule conformation. The $p$H was raised by adding small amounts of strong base NaOH so that the increase in ionic strength can be ignored.

attached dye, three orders of magnitude higher *bulk* proton concentration is needed to achieve the same *local* concentration. Parenthetically, we note that these data loosely fit the linear relation expected from a naive Boltzmann distribution model based on local electric field, which we discuss further below (this is the dotted line through the data, which supposes an electric potential of $8.0 \times 10^{-2}$ V). Another contributing element is surely that the elevated counterion concentration at low $p$H screens the polymer charge such that the chain conformations change with $p$H, but no quantitative analysis is offered at this time.

Using a different $p$H-sensitive fluorophore (see caption of Fig. 3), we studied the regime of higher $p$H closer to the collapse transition and focused on the effect of added salt. First, experiments without added salt (not shown) confirmed the conclusion that the local $p$H was 3 $p$H units higher than that in the solution, just as for the different fluorophore in Fig. 2. In Fig. 3, local $p$H is plotted against bulk $p$H and one sees that the data in 5 mM salt are shifted 0.4–0.8 $p$H units upward. Note that regardless of whether salt is added, the fluorophore at first becomes brighter with increased $p$H, and then brightness passes through a maximum. The similarity of the curves regardless of added salt, especially the similar fluorophore brightness at the maxima, indicates nearly the same local critical $p$H.

By fitting both sets of data in Fig. 3 to the Boltzmann distribution function ($p$H$_{local} = p$H$_{bulk} + e\psi/kT$, in which $e$ is for unit charge, $\psi$ for electric potential, and $T$ for temperature), the shift of the data by salt addition shows a reduction of ~15% in electric potential with 5.0 mM salt, thus providing a rough quantification of the argument proposed at the

outset of this paper. However, it is interesting that the maximum brightness does not coincide with the collapse transition, as will be discussed in connection with Fig. 4. The drop of fluorophore brightness reflects higher local proton concentration and hence, by the argument introduced at the outset of this paper, reduced repulsion between the positively charged chain and the protons. A trivial hypothetical explanation would be that the brightness change reflects local hydrophobicity instead, but this was excluded by observing that no such phenomenon was observed with a different fluorophore attached to the same chain. This was Bodipy FL, an uncharged and hydrophobic dye known to be more fluorescent in a hydrophobic environment. When attached to these polymer chains, although a large brightness increase was observed after the chains' collapse because the dye became wrapped by neutral polymer segments, this fluorophore exhibited no change in brightness before the chains' collapse. Taken together, these considerations confirm that the chains continued to be partially charged right before collapse.

Finally, reversibility of the collapse transition was investigated by adjusting $p$H and salt concentration independently. We reasoned that as $p$H induces collapse but salt promotes solubility, it might be possible to switch the chains successively between collapsed and coil states while avoiding chain aggregation, owing to the infinite dilution conditions of this experiment. For this purpose, the chain conformation was monitored from diffusion coefficient using FCS, and local $p$H was monitored from fluorophore brightness based on photon counting histograms. Brightness was classified into three categories corresponding to these chain conformations: the extended coil state (slow diffusion: $D = \sim 11$ $\mu m^2/s$), collapsed globule state (fast diffusion: $D = \sim 33$ $\mu m^2/s$), and intermediate state 11 $\mu m^2/s < D < 33$ $\mu m^2/s$. No bright-

ness changes were observed when the chain was in the globule conformation.

The ideas of this paper are clearly confirmed by the sequence of data sets summarized in Fig. 4. The experiment began in the situation of no added salt. With adjustments in the direction of increasing solution $p$H, collapse to the globule conformation was observed. Then NaCl was added, causing the chain to regain the coil conformation, accompanied by increased fluorophore brightness. Continued successive elevation of $p$H and addition of more salt resulted in alternating collapse and re-expansion. This exhibits the interesting trend that charge on the polyelectrolyte *increases* when counterions bind to it.[22–25] Phenomenologically, this is opposite to the case for strong polyelectrolytes, although the mechanism differs. It is interesting to note that the chains appear to retain electric charge even after collapse. Perhaps this reflects charge on the surface of the resulting globule.

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