Manipulating single annealed polyelectrolyte under alternating current electric fields: Collapse versus accumulation

Shengqin Wang and Yingxi Zhu^{a)}

Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, Indiana 46556, USA

(Received 9 March 2012; accepted 17 April 2012; published online 1 May 2012)

Effective manipulation and understanding of the structural and dynamic behaviors of a single polyelectrolyte (PE) under alternating current (AC) electric fields are of great scientific and technological importance because of its intimate relevance to emerging bionanotechnology. In this work, we employ fluorescence correlation spectroscopy (FCS) to study the conformational and AC-electrokinetic behaviors of a model annealed PE, poly(2-vinyl pyridine) (P2VP) under both spatially uniform and non-uniform AC fields at a single molecule level. Under spatially uniform AC-fields, we observe a gradual and continuous coil-to-globule conformational transition (CGT) of single P2VP at varied AC-frequency when a critical AC-field strength is exceeded, in contrast to the pH-induced abrupt CGT in the absence of AC-fields. On the contrary, under spatially non-uniform AC-fields, we observe field-driven net flow and accumulation of P2VP near high AC-field regions due to combined AC electro-osmosis and dielectrophoresis but surprisingly no conformational change. Thus, distinct AC-electric polarization effect on single annealed PE subject to AC-field homogeneity is suggested. © 2012 American Institute of Physics.

[<http://dx.doi.org/10.1063/1.4710998>]

I. INTRODUCTION

Control and understanding of the molecular conformation and transport of polyelectrolytes (PEs), including biopolyelectrolytes such as DNAs and proteins, under external fields are of great importance in broad applications for drug delivery, biosensors, lab-on-chips, etc.^{1,2} Among many methods using various external stimuli or forcing fields, AC-electric fields combined with microfluidic devices have recently emerged as one of the efficient and versatile approaches; for instance, it has been exploited to control DNA conformation in hydrogels or concentrated polymer solutions,^{3,4} to ionize proteins or peptides for mass spectroscopy^{5,6} and to enhance protein crystallization.^{7,8} Essentially, AC-electric fields can be used to polarize and manipulate any type of colloids and macromolecules, neutral or charged, and its popularity and advantage over DC electric fields is centered on its effectiveness at high frequency and high voltage with minimal Faradaic reactions, which occur otherwise under DC-fields to limit applicable field voltage and current.⁹

Previous studies on the manipulation of PEs by AC electric fields mainly focus on DNA. Under non-uniform AC-fields, the dielectrophoresis (DEP) behaviors of fluorescence-labeled DNA are examined based on the detected change of DNA size or concentration.^{10–13} With growing interest in manipulating the conformation of DNAs for genomics, gene therapy, and biosensing, both uniform and non-uniform AC-fields have been applied to stretch a DNA chain up to its full chain length,^{14,15} which is often contributed to the AC-field induced torque, supplemented by a small bias force provided by AC-field induced flow; however, this common consensus of stretching DNAs under AC-

^{a)} Author to whom correspondence should be addressed. Electronic mail: yzhu3@nd.edu.

fields is challenged by recent observations of the collapse of DNA under uniform AC-fields of varied frequencies over the range of tens to several hundreds of Hz.^{16,17} However, such studies of PE structure and transport under AC-fields are mostly limited to a *quenched* PE (also designated as strong PE), including DNA, whose charges along its backbone are immobile and fixed, and the action of AC-electric polarization under AC-fields solely leads to the modification of the local counterion environment. Much additional complexity could arise when uniform AC-fields are applied to an *annealed* PE (also designated as weak PE), including the large groups of polyacids, polybases, and proteins, whose charges along its backbone are mobile, reversible, and show a strong dependence of local ionic environment; as such, the modification of local counterion concentrations by applied AC-fields can also cause considerable change in the charge fraction and imbalance of intramolecular interactions that stabilize its original conformation. Due to the complexity of the electric environment of an annealed PE, the study of conformational and transport response of an annealed PE chain to applied uniform and non-uniform AC-electric fields remains few. As the conformational transition of an annealed PE in principle resembles the compaction of DNA in cells or the folding of protein to its native state, the study of the conformational transition of an annealed PE subject to AC electric fields is important not only in the fundamental PE and biophysical science but also in the vast bionanotechnology.

In this work, we study the conformational and AC-electrokinetic behaviors of a model annealed PE in both spatially uniform and non-uniform AC electric fields at a single molecule level by using fluorescence correlation spectroscopy (FCS). Recently, we have employed the FCS techniques to enable the *in situ* DEP study of nanocolloids of diameter down to 10 nm and demonstrated it as an ultrafast and sensitive characterization method to examine the AC-electrokinetic response of nanocolloids and macromolecules under varied AC-fields, whose size is far smaller than the spatial resolution of microscopic imaging techniques.¹⁸ In this paper, we report our FCS study of poly(2-vinyl pyridine) (P2VP) under AC electric fields of varied electrode layouts, frequency, and strength. P2VP is a typical hydrophobic polybase and bears a weak base pyridine group on its repeating units, as its chemical structure and ionization equilibrium with proton are shown in Figure 1(a). As a polybase, P2VP is highly charged at low *pH* and adopts an expanded coil conformation due to predominant electrostatic repulsion, while it becomes weakly charged or uncharged at high *pH* and adopts a collapsed globule conformation due to predominant hydrophobic attraction. We have recently reported the realization of coil-to-globule conformational transition (CGT) of single P2VP chains in a salt-free aqueous solution under applied uniform AC-fields in a prior paper.¹⁹ Here, we present our comprehensive study of varied AC-field effects on P2VP structure and transport in microchannels. By comparing the measured profiles of hydrodynamic radius and concentration of single P2VP chains in response to varied AC-fields, we surprisingly observe the collapse of P2VP in the absence of flow under uniform AC-fields, whose collapse ratio strongly depends on salt concentration, *pH*, as well as AC-field frequency and strength, in sharp contrast to the accumulation of PVP molecules yet without apparent conformational change under non-uniform AC-fields, the latter of which exhibits the signature of AC electro-osmosis (AC-EO) and DEP; thus, distinct AC-electric polarization characteristics of an annealed PE subject to AC-field homogeneity is suggested.

II. EXPERIMENTAL SECTION

P2VP of $M_n = 135\,000$ g/mol and $M_w/M_n = 1.03$ (Polymer Source, Inc., Quebec, Canada) was labeled with a bright and stable fluorescence dye, Alexa Fluor 488 (Invitrogen, Inc.) on its amino-end functionalized group for single-molecule FCS experiments. Excessive free fluorescence dyes were thoroughly removed by size exclusion chromatographic column and verified by FCS. A stock solution of P2VP was first prepared by dissolving fluorescence-labeled P2VP in the HCl solution of *pH* = 2.0 and then diluted with deionized water (resistivity, $\Omega = 18.2$ M Ω /cm, Barnstead NanoPure II) or NaCl aqueous solutions of known ionic strength. Subsequently, different amounts of HCl solutions of *pH* = 2.0 were added to the P2VP aqueous solutions to reach the desired *pH* in a range of 3.0–4.7 as measured by a *pH* meter (Oakton *pH* 6)

FIG. 1. (a) Chemical structure of P2VP and its ionization equilibrium with proton. (b) Schematic diagram of experimental setup with spatially uniform AC-electric fields. The two parallel conducting surfaces are fabricated with a thin gold coating of 10 nm thick and subsequently coated with hydroxyl-terminated self-assembled monolayer to prevent P2VP from surface adsorption. (c) Schematic diagram of experimental setup with spatially non-uniform AC-electric fields with the blow-out of a pair of co-planar microelectrodes, in the middle of which a laser beam is focused near the coverslip surface. (d) Normalized autocorrelation functions, $G(\tau)$ by $G(0)$ for P2VP in salt-free aqueous solutions of $pH = 4.16$ in the absence of applied AC-field ($\blacktriangleleft$), and under applied spatially uniform AC-fields of $\omega = 10$ MHz ($\blacktriangleright$), 50 kHz ($\blacktriangledown$), and 5 kHz ($\blacktriangle$) at constant $E_{pp} = 400$ V/mm, and in 1 mM NaCl aqueous solution of $pH = 4.58$ in the absence of AC-field ($\bullet$) and under applied uniform AC-fields of $\omega = 10$ kHz ($\blacksquare$) at $E_{pp} = 400$ V/mm. (e) Measured R_h of P2VP as a function of pH in salt-free ($\blacksquare$) and 1 mM NaCl aqueous solution ($\bullet$).

for this work. To conduct the study of single P2VP chain conformation, the concentration of P2VP in dilute aqueous solutions was kept low and constant at ~ 2 nM.

Two microchannel layouts were fabricated to allow the application of spatially uniform and non-uniform AC electric fields to P2VP in aqueous solutions, as schematically illustrated in Figures 1(b) and 1(c), respectively. For the microchannel layout as illustrated in Figure 1(b), two parallel quartz coverslips were deposited with a thin gold layer of 10 nm thick and followed with a deposition of a hydroxyl-terminated self-assembled monolayer of 11-mercapto-1-undecanol (Aldrich) to prevent the adsorption of P2VP chains on gold electrode surfaces; these two conducting surfaces were assembled in a parallel geometry with a fixed gap separation of $L = 50 \mu m$ by using a double-side tape spacer (3 M) to produce spatially uniform AC-fields between two surfaces. For the microchannel layout to produce non-uniform AC-fields as illustrated in Figure 1(c), a pair of gold coplanar microelectrodes of each 200 nm thick and a fixed gap separation of 30 μm were fabricated on a glass coverslip by photolithography,^{18,20} and the microchannel was sealed with a transparent fluid cell injected with P2VP solution using optical UV glue (Nolan 80).

The diffusion coefficient, D , and concentration, $[c]$, of fluorescence-labeled P2VP were measured by FCS^{19,21,22} that was set up on an inverted microscope (Zeiss Axio A1) equipped with an oil-immersion objective lens (Plan Apochromat 100 $\times$, numerical aperture: 1.4). An argon laser of excitation wavelength, $\lambda_{ex} = 488$ nm (Melles Griot) is focused on the center

between two parallel conducting surfaces or in the middle spot between two microelectrode. The fluorescence intensity, $I(t)$, which fluctuates due to the transport of excited fluorescence P2VP chains in a confocal detection geometry, was detected separately by two single photon counting modules (Hamamatsu, Japan) after suppressing background noise by a combined series of optical filters and a dichroic mirror (Chroma Tech). The auto-correlation function, $G(\tau)$, of measured $I(t)$ was thereby obtained as $G(\tau) = \langle \delta I(t) \delta I(t + \tau) \rangle / \langle I(t) \rangle^2$ by using a multichannel FCS data acquisition system (ISS) via cross-correlation analysis, which removes the artifacts from detectors.

III. RESULTS AND DISCUSSION

The conformational dynamics of single P2VP chains in aqueous solutions in response to varied pH, added salt, and applied AC-electric fields is examined by FCS. Figure 1(d) exhibits representative autocorrelation function curves, $G(\tau)$ normalized by $G(0)$ for P2VP in dilute aqueous solution of selected pH with or without salts as well as under applied AC-fields of varied frequency, ω . The diffusion coefficient, D , of P2VP can be thereby obtained from measured $G(\tau)$ according to

$$G(\tau) = ([c]\pi^{1.5}z^2\bar{\omega})^{-1} \left(1 + \frac{4D\tau}{\bar{\omega}^2}\right)^{-1} \left(1 + \frac{4D\tau}{z^2}\right)^{-0.5}, \quad (1)$$

where $[c]$ is the concentration of fluorescence-labeled P2VP in the confocal volume of our FCS setup, which is calibrated at the room temperature ($\sim 25^\circ\text{C}$) by Rhodamine 6 G of known D ($= 280 \mu\text{m}^2/\text{s}$) in a dilute aqueous solution to be $\bar{\omega} = 0.26 \mu\text{m}$ in the lateral dimension and $z = 3 \mu\text{m}$ in the vertical dimension. With known water viscosity, η , the conformational structure of a P2VP chain in varied environmental conditions can be described by the measured hydrodynamic radius, R_h , based on the Stokes-Einstein equation,

$$R_h = \frac{k_B T}{6\pi\eta D}. \quad (2)$$

As shown in Figure 1(e), the measured R_h of P2VP decreases with increasing solution pH over a very narrow pH range, indicating the abrupt, first-order nature of the CGT for the annealed P2VP polyelectrolyte in dilute solution. The critical pH to induce the CGT of P2VP of $M_n = 135\,000 \text{ g/mol}$ is determined to be 4.20 and 4.60 in deionized water and 1 mM NaCl aqueous solution, respectively, in good agreement with the reported in the literature.²³

A. Collapse, without flow, of P2VP under uniform AC-fields

At a constant pH, we first examine the conformational response of single P2VP chain to applied uniform AC-fields of varied amplitude and frequency. Figure 1(d) exhibits the representative normalized $G(\tau)/G(0)$ curves for P2VP at pH = 4.16 in salt-free water and at pH = 4.58 in 1 mM NaCl salted water under varied peak-to-peak electric intensity, E_{pp} and ω , which are all well fitted with Eq. (1) to yield R_h , without any detectable AC-electrokinetic induced flow as another fitting equation (see Eq. (3) below) including flow velocity is also attempted. The extracted R_h of P2VP chains against increased E_{pp} at constant $\omega = 5, 50, \text{ and } 500 \text{ kHz}$ in deionized water and 1 mM NaCl aqueous solution is summarized in Figures 2(a) and 2(b), respectively. Apparently, the collapse of a P2VP coil into a globule of $R_h = 8 \text{ nm}$ occurs when a critical AC-field strength is exceeded. The critical AC electric intensity E_{cr} is defined as the approximately lowest E_{pp} value at which the onset of the P2VP CGT is observed. It is found that $E_{cr} \approx 320 \text{ V/mm}$ at $\omega = 5, 50, \text{ and } 500 \text{ kHz}$ in salt-free solutions and $\approx 280 \text{ V/mm}$ in 1 mM NaCl salt solution, exhibiting nearly ω -independence. As the CGT of hydrophobic polyacids or polybases can be induced by varying the local counterion gradient,²³ we postulate that at $E_{pp} > E_{cr}$, localized counterions are driven away from a P2VP chain, resulting in a decreased

FIG. 2. (a) Measured R_h of P2VP in salt-free solutions of $pH = 4.16$ against E_{pp} at $\omega = 500$ kHz (■), 50 kHz (●), and 5 kHz (▲). (b) Measured R_h of P2VP in 1 mM NaCl solution of $pH = 4.58$ against E_{pp} at $\omega = 500$ kHz (■), 50 kHz (●), and 5 kHz (▲). In both panels, the shade area indicates the region where P2VP remains in a coil conformational state, and the dashed line indicates the critical E_{cr} to induce the conformational transition of P2VP.

charge fraction and consequently an imbalance of intra-molecular electrostatic and hydrophobic interaction.

We then examine the AC-field frequency dependence of the conformational structure of P2VP chains in aqueous solution at constant $E_{pp} = 400$ V/mm and constant pH adjacent to its corresponding $pH_{CGT} = 4.20$ and 4.60 for P2VP in salt-free and 1 mM NaCl added aqueous solution, respectively. The lowest applied ω in this work was set at 5 kHz to avoid the polarization and corrosion of electrodes (as determined in Fig. S1 in supplementary material²⁴). As shown in Figure 3, the measured R_h of P2VP at $pH = 4.16$ gradually decreases with decreasing ω from 10 MHz to 5 kHz, indicating a continuous and gradual CGT under a uniform AC-field, in sharp contrast to the abrupt and first-order conformational transition by tuning solution pH .²³ AC-field induced CGT of P2VP in solutions appears similar to the recent observation of AC-field induced collapse of DNAs,¹⁶ which disagrees with the common consensus that PEs including DNAs can be usually stretched by DC or AC electric fields.^{3,4,25–27} However, the mechanism here associated with the AC-electric polarization of annealed hydrophobic PE bearing tunable and mobile charge sites is distinctly different from that with DNA or other quenched PEs bearing fixed charge sites along its backbone: for the latter case, the collapse of pre-stretched DNA is resulted from the anisotropic counterion polarization of DNA chains under micro- or nano-channel confinement; yet for our case, the collapse of P2VP takes places in a bulk solution without spatial confinement and thereby involves a possible mechanism with predominant hydrophobic attraction in comparison to the weakened electrostatic repulsion due to AC-field induced reduction of P2VP charge fraction as further discussed below. Similar behavior of AC-field induced CGT upon decreasing ω is also confirmed with P2VP in 1 mM NaCl solution at $pH = 4.58$ as shown in Fig. 3.

To further examine the generality of AC-field induced chain collapse, we repeat the experiments at varied constant $pH = 3.04–4.25$ across the $pH_{CGT} = 4.20$ for P2VP in salt-free solutions. It

FIG. 3. Normalized R_h of P2VP by its corresponding coil radius in salt-free solution of $pH=4.16$ (■) and 1 mM NaCl solution of $pH=4.58$ (●) against ω under applied uniform AC-fields of $E_{pp}=400$ V/mm.

is intriguing to observe in Figure 4(a) that the induced collapse of P2VP coils in uniform AC-fields only occurs over a narrow pH range of 4.15–4.18, in close vicinity to pH_{CGT} : the dimension of P2VP coil at $pH=4.16$ and 4.18 is gradually reduced as decreasing ω , yet the conformation of P2VP at $pH < 4.16$ or > 4.20 remains unchanged over the entirely varied ω from 20 MHz down to 5 kHz. Additionally, the effective frequency window to collapse a P2VP coil at $pH=4.18$ is smaller than that at $pH=4.16$ at which the CGT is progressed at a much slower pace and complete at $\omega=5$ kHz in contrast to $\omega=500$ kHz at $pH=4.18$. We exclude the effect of increased ionic strength at lower pH for the ineffectiveness of AC-electric polarization and collapse of P2VP coils, simply because a similar AC-field induced CGT is repeatedly observed at 1 mM NaCl solution of $pH=4.58$ whose effective ionic strength is obviously higher than that in salt-free solution of $pH=3.04$ and 4.02. Instead, we consider that the applied AC-polarization energy remains insufficient at our strongest applicable AC-field strength to considerably lower the energy barrier separating the coil and globule states at low pH to induce the CGT.²⁸

FIG. 4. (a) Measured R_h against ω for P2VP in salt-free solutions under applied *uniform* AC-fields of $E_{pp}=400$ V/mm at varied $pH=3.04$ (■), 4.02 (●), 4.16 (▲), 4.20 (▼), and 4.25 (◄). (b) Measured R_h against ω for P2VP in salt-free solutions under applied *non-uniform* AC-fields of peak-to-peak voltage, $V_{pp}=20$ V across two co-planar microelectrodes separated by $30\ \mu\text{m}$ at varied $pH=3.50$ (■), 4.16 (●), 4.21 (▲), and 4.25 (▼).

B. Flow and accumulation, without collapse, of P2VP under non-uniform AC-fields

To our surprise, no collapse of P2VP is observed under spatially non-uniform AC-fields of the similar range of E_{pp} and ω , as summarized in Figure 4(b). We have scrutinized the measured auto-correlation functions, $G(\tau)$ for P2VP at $pH = 4.16$ in non-uniform AC-field of $\omega = 10 \text{ kHz} - 20 \text{ MHz}$ and constant peak-to-peak voltage $V_{pp} = 20 \text{ V}$ across two co-planar electrodes of $L = 30 \mu\text{m}$, leading to an apparent maximal $E_{pp} = 670 \text{ V/mm}$ occurred in the horizontal plane of two microelectrodes. Considering the flow induced by inhomogeneous AC-fields, we have fitted $G(\tau)/G(0)$ using a model including both diffusion and lateral fluid net flow as

$$G(\tau) = ([c]\pi^{1.5}z^2\bar{\omega})^{-1} \left(1 + \frac{4D\tau}{\bar{\omega}^2}\right)^{-1} \left(1 + \frac{4D\tau}{z^2}\right)^{-0.5} \exp\left(-\frac{v\tau}{\bar{\omega}} \left(1 + \frac{4D\tau}{\bar{\omega}^2}\right)^{-1}\right), \quad (3)$$

where v is the net flow velocity of P2VP.²⁹ The curve fitting of obtained $G(\tau)$ under non-uniform AC-fields with Eq. (3) is demonstrated to be very good (see Figure S2 in supplementary material²⁴). The detected net flow at low frequency is clearly attributed to the AC-EO flow produced by the action of applied AC-field on its induced diffusive charge near the polarized electrode surface as further discussed below.^{30,31} Hence, in addition to extracted D and thereby hydrodynamic dimension, R_h , the time-averaged flow velocity, v and concentration, $[c]$ of P2VP in the confocal volume under inhomogeneous AC-fields are also obtained against ω as summarized in Figures 5(a) and 5(b), respectively.

For P2VP at varied pH , the measured $[c]$ is normalized by $[c]_0$ in the absence of applied AC-field that is $\sim 2.0 \text{ nM}$. With our co-planar electrode design, the high AC-field lines are pointed toward the electrodes^{18,20} and AC-polarized P2VP detected in the laser focal volume, which is centered between two microelectrodes and approximately $1.5 \mu\text{m}$ above the substrate, is expected to be driven toward the high AC-field region near the electrodes at ω below the positive DEP (pDEP)-to-negative DEP (nDEP) crossover frequency, ω_{cr} . We observe the accumulation of P2VP with $[c]/[c]_0 > 1$ at $\omega \leq 5 \text{ MHz}$ for all varied pH , suggesting pDEP characteristics of P2VP in response to applied non-uniform AC-fields, while $[c]/[c]_0$ approach the unity at $\omega > 10 \text{ MHz}$ for all varied pH except $pH = 3.50$, suggesting weak nDEP or zero DEP (oDEP); at $pH = 3.50$, it is rather intriguing to observe the increase of $[c]/[c]_0$ beyond our experimental uncertainty again as increasing ω from 10 MHz to 20 MHz . As we further examine the obtained ω -dependent P2VP concentration profile, we find that the concentration is peaked at $\omega = 20 - 50 \text{ kHz}$ for $pH = 4.16, 4.21$, and 4.25 and subsequently followed by a monotonic decrease to approach $[c]/[c]_0 = 1$ as increasing ω ; in contrast, at $pH = 3.50$, a peak plateau in the measured concentration is observed over a higher ω range from 200 kHz to 1 MHz and first followed by a decrease at $\omega = 1 - 5 \text{ MHz}$ and then by an apparent increase at $\omega = 10 - 20 \text{ MHz}$.

The low frequency region with the presence of a concentration peak appears to be manifested by the AC-EO flow of P2VP, in addition to the DEP effect.^{30,31} Recent AC-electrokinetic study of colloidal suspension has demonstrated that AC-EO flow becomes peaked at a characteristic relaxation frequency determined by the medium conductivity and the electrode double layer capacity near the electrodes that scales with $\omega_{eo} \sim \frac{D}{\lambda L} \sim 2 - 4 \text{ kHz}$ with $D_{ion} = 2.0 \times 10^{-9} \text{ m}^2/\text{s}$ for the Cl^- counterion, $L = 30 \mu\text{m}$ and the Debye screening length, $\lambda = 40 - 17 \text{ nm}$ at $pH = 3.50 - 4.25$, respectively and vanishes at lower or higher frequency limits.³² Experimentally, we observe that the obtained net flow velocity of P2VP monotonically decreases as increasing ω and eventually vanishes to zero at $\omega = 2 \text{ MHz}, 700 \text{ kHz}, 50 \text{ kHz}$, and 20 kHz at $pH = 3.5, 4.16, 4.21$, and 4.25 , respectively, which is considered at the upper frequency limit of AC-EO flow, $\omega_{eo,u}$. With decreasing pH , the resulting smaller λ leads to an increase in $\omega_{eo,u}$, which is clearly evidenced in Figure 5(c).

Thereafter, we believe that the accumulation of P2VP at $\omega > \omega_{eo,u}$ as observed in Figure 5(b) is resulted from the positive DEP force imposed on P2VP. At the steady state where the Brownian diffusive motion is canceled out by the normalization of $[c]_0$, the time-averaged concentration ratio, $[c]/[c]_0$ in the medium (m) of η and permittivity, ϵ_m , is expected to be controlled by the DEP mobility,

FIG. 5. (a) Measured flow rate, v and (b) normalized concentration, $[c]/[c]_0$ of P2VP against ω in salt-free aqueous solutions of varied $pH=3.50$ (■), 4.16 (●), 4.21 (▲), and 4.25 (▼) under applied non-uniform AC-fields of $V_{pp}=20$ V across two microelectrodes separated by $30\ \mu\text{m}$. (c) Upper frequency limit, $\omega_{eo,u}$ to induce AC-EO flow against solution pH .

$$\mu_{DEP} = \frac{R^2 \epsilon_m}{6\eta} \text{Re}[f_{CM}], \quad (4)$$

where $\text{Re}[f_{CM}]$ is the real part of ω -dependent dipolar Clausius-Mossotti (CM) factor of a P2VP molecule (p),

$$f_{CM} = (\tilde{\epsilon}_p - \tilde{\epsilon}_m) / (\tilde{\epsilon}_p + 2\tilde{\epsilon}_m) \quad (5)$$

and the complex permittivity, $\tilde{\epsilon}$, is related to the conductivity, σ and ϵ as $\tilde{\epsilon} = \epsilon - \frac{i\sigma}{\omega}$.³³ Because the surface conductance of P2VP molecule is higher than that of salt-free aqueous medium, P2VP is expected to experience pDEP to be driven towards high field regions near the electrodes at $\omega < \omega_{cr}$ at which $\text{Re}[f_{CM}] = 0$; conversely, at $\omega > \omega_{cr}$, P2VP is expected to experience nDEP or 0DEP to be driven to the bulk away from the electrodes that is the low field region in our electrode layout. Experimentally, we have observed the transition from pDEP to 0DEP (or weak nDEP) of P2VP with increasing ω across 10 MHz as exhibited by the shaded area in Figure 5(b); thus the ω_{cr} for P2VP at all varied pH is approximately 10 MHz, whose nearly independence of pH or medium conductivity is surprisingly consistent well with the classical DEP theory,^{33,34} yet sharply distinct from the abnormal DEP behavior of nanocolloidal and complex aggregates examined previously.^{18,20,35}

C. Discussion

It is clear in our experimental observation that the structural and dynamical responses of single P2VP chains to applied spatially uniform and non-uniform AC-fields are drastically different. Surprisingly, we have found that homogenous AC-fields can most effectively compress a P2VP chain, yet inhomogeneous AC-fields cause neither chain collapse nor elongation in contrast to the common consensus of hydrodynamic flow induced chain stretching, but lead to rapid and effective accumulation of P2VP instead.

We have observed in our previous work that the homogenous AC-field induced CGT is reversible upon sweeping ω when E_{pp} exceeds E_{cr} , yet a hysteresis in ω -dependent conformation is also observed.¹⁹ Here, we further reveal that both E_{cr} and effective ω -window to collapse a P2VP chain at constant pH is of salt concentration dependence. Our observation suggests that the continuous and reversible CGT under spatially uniform AC-fields is thermal noise-driven, when time-averaged induced dipole energy under AC-fields can considerably reduce the energy barrier that separates the distinct coil and globule conformations of a P2VP free chain in dilute solutions and is estimated to be 1.5–2 $k_B T$ by generalized ensemble Monte Carlo computer simulation.^{27,28}

For annealed PEs, such as P2VP, whose charge density is not constant but tunable by pH or applied electric fields, the local proton concentration in the vicinity of a PE chain is significantly different from that in the bulk due to counterion condensation. As salt concentration is increased, the gradient of local proton concentration normal to the annealed P2VP backbone becomes smaller, leading to the shift of pH_{CGT} to higher pH²³ as we confirm the shift of pH_{CGT} from 4.20 to 4.60 as deionized water is added with 1 mM NaCl. Under applied homogenous AC-fields, localized counterions can be driven away from a P2VP backbone to some distance extent at the half cycle of a given frequency, resulting in a greater counterion concentration gradient normal to a P2VP backbone so that the PE chain becomes polarized with an induced dipole, $p = 4\pi\epsilon R^3 \text{Re}[f_{CM}(\omega)]E$.^{34,36} Hence, the time-averaged induced dipole energy, $\langle pE \rangle = 4\pi\epsilon_m R^3 \text{Re}[K(\omega)]|E|^2$,³⁶ is expected to be positive at $\omega < \omega_{cr}$ corresponding to pDEP and lower the total free energy of a P2VP chain to be approximately $k_B T$ in the thermal fluctuation level, which allows the transition between a coil and a globule conformational state. The picture of AC-polarization modified energy landscape to dictate the CGT is supported by recent computer simulation predictions of the electrostatic potential change of annealed PE against charge fraction.²⁷ Moreover, the effective depletion of localized counterions to induce the collapse of a polarized P2VP chain is ω -dependent and the upper ω limit is determined by the pDEP-to-nDEP crossover frequency, ω_{cr} .

Alternatively, the effect of AC-electric polarization can be regarded equivalent to that from lowering salt concentration. As the localized proton concentration can be described by the Boltzmann distribution as

$$[H^+]_{local} = [H^+]_{bulk} \exp\left(-\frac{U}{kT}\right), \quad (6)$$

where U is the free energy of the local proton by taking the proton in the bulk solution as the ground energy state, the shift of local pH by an applied AC-field of root-mean-square strength, E_{rms} can be derived as

$$\Delta pH = 0.43\Delta U/k_B T = 0.43E_{rms}e\lambda/k_B T \quad (7)$$

Therefore, it is expected to achieve an increase of local pH , $\Delta pH = 0.04$ in a salt-free solution of $\lambda = 30$ nm so as to induce a CGT of P2VP at $pH = 4.16$ that otherwise occurs at $pH = 4.20$ in the absence of AC-fields; such AC-field resulting ΔpH thereby demands $E_{rms} = 80$ V/mm or equivalently $E_{pp} = 226$ V/mm, which semi-quantitatively agrees with our experimentally determined $E_{cr} = 320$ V/mm as indicated in Figure 2(a). Similarly, in 1 mM NaCl added solution of $\lambda = 10$ nm at $pH = 4.58$, a critical $E_{pp} = 339$ V/mm is estimated to cause an effective shift of $\Delta pH = 0.02$ in local pH to allow the collapse of a P2VP coil corresponding to its $pH_{CGT} = 4.60$, which is also approximate to our experimentally determined $E_{cr} = 280$ V/mm as indicated in Figure 2(b).

To our most surprise, we observe no conformational change of P2VP in response to spatially non-uniform AC-fields of similar E_{pp} and ω , but instead, net flow and accumulation of P2VP toward high field regions near two microelectrodes, which is in sharp contrast to the response of P2VP under uniform AC-fields that cause isotropic depletion of counterions and subsequent polarization normal to a P2VP chain. We postulate that due to the field-driven net motion of both P2VP chains and localized counterions, the depletion of localized counterions away from a P2VP chain is significantly weakened or suppressed, resulting in negligible counterion concentration-induced dipolar energy on a P2VP chain.^{37,38} In addition to the observed AC-EO and DEP effects on P2VP polarization under applied inhomogeneous AC-fields, other AC-electrokinetic effects such as AC-electrothermal and electrohydrodynamics could be also present and their combined effect could prevent a P2VP chain from either collapse or stretching, which could warrant a future study.

In the ω -window from ~ 100 kHz to 5–10 MHz, the net motion and accumulation of P2VP at varied $pH = 4.16$ – 4.25 are mainly manifested by pDEP: the existence of a plateau region of increased P2VP concentration at high $\omega < \omega_{cr}$ is in good agreement with the classical prediction of colloidal pDEP action on polyelectrolytes as well as experimental DEP study of DNAs.^{11,39} It should be noted that the observation of the “tail-up” in the measured P2VP concentration at $\omega > 10$ MHz at $pH = 3.50$ is unusual, which is similar to a recent report of an abnormal high- ω pDEP behavior of DNAs of 24 kbp or 48 kbp long,⁴⁰ but different from the commonly observed decrease in the DEP response of DNA at $\omega > 1$ MHz⁴¹ or 10 MHz;¹¹ however, we are unclear of the exact reason accountable for it. Additionally, we postulate that the slight increase in measured R_h of P2VP over $\omega = 200$ kHz–10 MHz at $pH = 3.50$ shown in Figure 4(b), where P2VP is fully charged and behaves similar to quenched PEs, is possibly resulted from AC-electrohydrodynamic stretching force, similar to stretching DNA under non-uniform AC-fields.

IV. CONCLUSION

We have employed FCS to study the conformational structure, net flow, and accumulation of a model annealed PE, P2VP, in response to spatially uniform and non-uniform AC electric fields at a single molecular level. The utilization of FCS is demonstrated as a sensitive and applicable method to *in situ* study the AC-electrokinetic dynamics of macromolecules in

nanometer sizes, which is far below the limit of conventional microscopic imaging techniques. Under uniform AC-fields, we observe a gradual collapse of a P2VP coil as decreasing AC-frequency when a critical AC-field strength is exceeded, in contrast to the *pH*-induced abrupt CGT; the critical AC-field E_{cr} and effective ω -window to achieve the fully conformational collapse both depend on solution *pH* and salt concentration. We conclude that such induced continuous and gradual CGT is resulted from AC-field induced migration and depletion of localized counterions in the diffusive layer near a P2VP chain, in a similar fashion as decreasing salt concentration. On the contrary, under spatially non-uniform AC-fields, we observe no conformational transition of P2VP and instead field-driven net flow and accumulation of P2VP near high AC-field regions. At low to intermediate ω regions, considerable net flow velocity in the order of tens $\mu\text{m/s}$ is detected and then vanishes with further increased ω , indicating the strong influence of AC-EO flow whose upper frequency window shows a strong dependence of ionic strength and agrees well with theoretical prediction; at higher ω , we observe the pDEP dominant effect to cause the concentration elevation of P2VP from the bulk concentration; as further increasing ω above 500 kHz–1 MHz, accumulated P2VP concentration decreases to approach zero, suggesting an apparent transition from pDEP to ODEP or weak nDEP when ω approaches to its characteristic dipolar relaxation frequency, ω_{cr} . The distinct conformational and transport behaviors of a single annealed PE chain under varied microelectrode layouts can be further exploited in lab-on-chip device design to effectively manipulate and transport PEs including DNA and protein under the application of varied AC-electric fields.

ACKNOWLEDGMENTS

We are grateful to the financial support from the US Department of Energy, Office of Basic Sciences, Division of Materials Science and Engineering under Grant No. DE-FG02-07ER46390 (polyelectrolyte conformational transition) and National Science Foundation under Grant No. CMMI-1129821 (molecular AC-electrokinetics). We are also indebted to the fruitful discussion with Professor H.-C. Chang at the University of Notre Dame.

- ¹F. Oosawa, *Polyelectrolytes* (Marcel Dekker, New York, 1971).
- ²S. Forster and M. Schmidt, *Physical Properties of Polymers* (Springer-Verlag, Berlin, 1995), Vol. 120, p. 51.
- ³M. Ueda, K. Yoshikawa, and M. Doi, *Polym. J.* **31**, 637 (1999).
- ⁴N. Kaji, M. Ueda, and Y. Baba, *Biophys. J.* **82**, 335 (2002).
- ⁵N. Chetwani, C. A. Cassou, D. B. Go, and H. C. Chang, *J. Am. Soc. Mass Spectrom.* **21**, 1852 (2010).
- ⁶N. Chetwani, C. A. Cassou, D. B. Go, and H. C. Chang, *Anal. Chem.* **83**, 3017 (2011).
- ⁷D. Hou and H. C. Chang, *Appl. Phys. Lett.* **92**, 223902 (2008).
- ⁸E. Servoli, D. Maniglio, A. Motta, and C. Migliaresi, *Macromol. Biosci.* **8**, 827 (2008).
- ⁹Z. Hammadi, J.-P. Astier, R. Morin, and S. Veessler, *Cryst. Growth Des.* **7**, 1472 (2007).
- ¹⁰C. L. Asbury, A. H. Diercks, and G. van den Engh, *Electrophoresis* **23**, 2658 (2002).
- ¹¹D. J. Bakewell and H. Morgan, *IEEE Trans. Nanobiosci.* **5**, 139 (2006).
- ¹²C. F. Chou, J. O. Tegenfeldt, O. Bakajin, S. S. Chan, E. C. Cox, N. Darnton, T. Duke, and R. H. Austin, *Biophys. J.* **83**, 2170 (2002).
- ¹³J. R. Du, Y. J. Juang, J. T. Wu, and H. H. Wei, *Biomicrofluidics* **2**, 044103 (2008).
- ¹⁴C. Walti, W. A. Germishuizen, P. Tosch, C. F. Kaminski, and A. G. Davies, *J. Phys. D: Appl. Phys.* **40**, 114 (2007).
- ¹⁵C. Walti, P. Tosch, A. G. Davies, W. A. Germishuizen, and C. F. Kaminski, *Appl. Phys. Lett.* **88**, 153901 (2006).
- ¹⁶C. Zhou, W. W. Reisner, R. J. Staunton, A. Ashan, R. H. Austin, and R. Riehn, *Phys. Rev. Lett.* **106**, 248103 (2011).
- ¹⁷J. Tang, N. Du, and P. S. Doyle, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 16153 (2011).
- ¹⁸V. E. Froude, J. I. Godfroy, S. Wang, H. Dombek, and Y. Zhu, *J. Phys. Chem. C* **114**, 18880 (2010).
- ¹⁹S. Wang, H.-C. Chang, and Y. Zhu, *Macromolecules* **43**, 7402 (2010).
- ²⁰P. D. Hoffman and Y. Zhu, *Appl. Phys. Lett.* **92**, 224103 (2008).
- ²¹D. Magde, E. Elson, and W. W. Webb, *Phys. Rev. Lett.* **29**, 705 (1972).
- ²²R. Rigler, U. Mets, J. Widengren, and P. Kask, *Eur. Biophys. J. Biophys. Lett.* **22**, 169 (1993).
- ²³S. Wang, S. Granick, and J. Zhao, *J. Chem. Phys.* **129**, 241102 (2008).
- ²⁴See supplementary material at <http://dx.doi.org/10.1063/1.4710998> for the detail of experimental data analysis.
- ²⁵A. E. Cohen, *Phys. Rev. Lett.* **91**, 235506 (2003).
- ²⁶P. Y. Hsiao and K. M. Wu, *J. Phys. Chem. B* **112**, 13177 (2008).
- ²⁷H. Liu, Y. Zhu, and E. Magnin, *Macromolecules* **43**, 4805 (2010).
- ²⁸T. Yamaguchi, T. Kiuchi, T. Matsuoka, and S. Koda, *Bull. Chem. Soc. Jpn.* **78**, 2098 (2005).
- ²⁹R. H. Kohler, P. Schwill, W. W. Webb, and M. R. Hanson, *J. Cell Sci.* **113**, 3921 (2000).
- ³⁰A. Ramos, H. Morgan, N. G. Green, and A. Castellanos, *J. Colloid Interface Sci.* **217**, 420 (1999).
- ³¹M. R. Bown and C. D. Meinhardt, *Microfluid. Nanofluid.* **2**, 513 (2006).
- ³²N. G. Green, A. Ramos, A. Gonzalez, H. Morgan, and A. Castellanos, *Phys. Rev. E* **61**, 4011 (2000).

- ³³H. A. Pohl, *Dielectrophoresis* (Cambridge University Press, Cambridge, 1978).
- ³⁴R. Pethig, [Biomicrofluidics](#) **4**, 022811 (2010).
- ³⁵L. Zhang and Y. Zhu, [Appl. Phys. Lett.](#) **96**, 141902 (2010).
- ³⁶S. Basuray and H.-C. Chang, [Phys. Rev. E](#) **75**, 060501 (2007).
- ³⁷G. S. Manning, [Biophys. Chem.](#) **7**, 95 (1977).
- ³⁸H. Isambert, A. Ajdari, J. L. Viovy, and J. Prost, [Phys. Rev. E](#) **56**, 5688 (1997).
- ³⁹S. Tuukkanen, A. Kuzyk, J. J. Toppari, H. Hakkinen, V. P. Hytonen, E. Niskanen, M. Rinkio, and P. Torma, [Nanotechnology](#) **18**, 295204 (2007).
- ⁴⁰A. Henning, F. F. Bier, and R. Holzel, [Biomicrofluidics](#) **4**, 022803 (2010).
- ⁴¹R. Yokokawa, Y. Manta, M. Namura, Y. Takizawa, N. C. H. Le, and S. Sugiyama, [Sens. Actuators B](#) **143**, 769 (2010).