Polymer capture by α -hemolysin pore upon salt concentration gradient

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We have measured the rate of capture of single molecules of sodium poly(styrene sulfonate) by α -hemolysin protein pore by varying applied voltage, pH, and the salt concentration asymmetry across the pore. We show that electrostatic interaction between the polyelectrolyte and the protein pore significantly affects the polymer capture rate in addition to the enhancement of drift arising from electrolyte concentration gradient. At higher pH values where the electrostatic interaction between the polymer and the α -hemolysin pore is repulsive, an antagonistic coupling with the drift induced by salt concentration gradient emerges. This antagonistic coupling results in a nonmonotonic dependence of the polymer capture rate on the salt concentration in the donor compartment. The coupling between the pore-polymer interaction and drift can be weakened by increasing the strength of the electric field that drives the polymer translocation. In contrast, at lower pH values where the polymer-pore interaction is attractive, a synergy with the additional drift from salt concentration asymmetry arises and the capture rate depends monotonically on the donor salt concentration. For higher pH, we identify two regimes for the enhancement of capture rate by salt concentration gradient: (a) drift-dominated regime, where the capture rate is roughly quadratic in the ratio of salt concentration in the receiver compartment to that in the donor compartment, and (b) antagonistic coupling regime at higher values of this ratio with a linear relation for the polymer capture rate. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4855075>]

I. INTRODUCTION

Electrophoretic capture of a single charged macromolecule in an electrolyte solution at the entrance of a nanopore and its subsequent threading through the pore have become an active area of research in the context of characterizing lengths and sequences of charged macromolecules such as polynucleotides, proteins, and synthetic polyelectrolytes.^{1–25} Based on experiments with a variety of pores and polymers, a rich phenomenology and theoretical formulations have emerged.²⁶ The basic experimental arrangement of the polymer translocation is the presence of a single nanopore embedded in a thin membrane partitioning an electrolyte solution into two compartments (*cis* and *trans*), across which an external electric field is applied. Under the external field, a charged polymer translocates from the *cis* to the *trans* through the nanopore (Fig. 1(a)). The translocation events are detected by measuring the ionic current which transiently drops when a polymer chain blocks the pore. The measured ionic current traces provide information on frequency and durations of blockade events, and blocked current values under various experimental conditions (Fig. 1(b)). These measures of translocation events depend on the various experimental conditions such as the sequence and concentration of the polymer, specificity of the nanopore, voltage gradient, temperature, identity and concentration of the electrolyte, and pH.

In addition, the effects of gradients in pH and salt concentration have been reported.^{20,27} The measured transloca-

tion time, blocked current, and capture rate are intrinsically stochastic and arise from the coupled conformational fluctuations of the polymer, the pore-polymer interaction, and the electrohydrodynamic flow fields in these systems. In order to fully understand the relative contributions of these factors, it is necessary to investigate the phenomenon under simpler conditions. We focus on the role of salt concentration gradient in the electrophoretic capture of flexible polyelectrolytes. We have chosen α -hemolysin pore as the nanopore, in view of its well-characterized nature.^{28,29}

The capture rate R_c of the polymer by the pore, independent of whether the captured polymer undergoes successful transport into the *trans* side or not, is typically taken as the reciprocal of the average time between two successive blockade events (Fig. 1(b)). In order for a blockade event to be detected, part of the chain must have entered the pore, which is associated with a free energy barrier. Before the encounter of the polymer with the pore, it needs to be transported to the pore entrance from the *cis* side, which is associated with a drift-diffusion mechanism in the presence of the external electric field at non-zero temperatures. The dependence of R_c on the polymer length, voltage gradient, and polymer concentration has been successfully addressed by considering the drift-diffusion and barrier penetration.^{30–32} In general terms, for weaker voltage gradients and shorter polymers, the free energy barrier dominates capture rate and for stronger voltage gradients and longer polymers, the drift dominates. In addition, the strength of the electric field in directing the polymer in the *cis* compartment towards the pore entrance can be augmented by creating a gradient in the electrolyte concentration across the pore. Specifically, as argued by Wanunu *et al.*,

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FIG. 1. (a) A schematic diagram of capture of a negatively charged polymer chain by α -hemolysin pore embedded in a lipid bilayer membrane driven by an externally applied electric field towards the positive electrode. (b) A typical ionic current trace of blockade events for 16 kg/mol sodium poly(styrene sulfonate) in 10 mM HEPES buffer with 1 M KCl (pH 7.5) at 140 mV. Ionic current through the protein pore is recorded in real time and a blockade is counted as a capture event whenever the current drops below the cut-off value of 75% of the open pore current. Times between two successive events, t_0 , are collected and the rate of polymer capture is obtained as the reciprocal of the average of t_0 , $1/\langle t_0 \rangle$.

lower salt concentration in the *cis* in comparison with that in *trans*, generates an additional pull of the polymer towards the pore.²⁷ This effect is due to the required continuity of current throughout the system while the access resistance is higher in the *cis* (due to lower electrolyte concentration) in comparison with the *trans* compartment.^{33,34}

In addition to contributing to the drift force on the polymer, a change in the salt concentration in the *cis* side can affect the electrostatic interaction between the polymer and the pore. In particular, such pore-polymer interactions can be very significant in the case of the α -hemolysin protein pore. It is already known that a charge-modification of even one amino acid residue per monomer in the heptameric α -hemolysin pore can significantly alter the kinetics of polymer transport.^{35,36} Also, a reduction in the net negative charge of the α -hemolysin protein pore by lowering the pH is shown to dramatically increase both the capture and threading of the polymer.²⁰

In this paper, we investigate the combined effects of additional drift on the polymer and the pore-polymer electrostatic interaction, both arising from an imposed salt concentration gradient across the pore. We have used α -hemolysin pore and sodium poly(styrene sulfonate) in our study. Although the α -hemolysin pore bears heterogeneous charge distribution,^{20,29,37} it is possible to determine the sign of the effective charge of α -hemolysin pore for capturing the polymer by changing salt concentration at a given pH. At high pH where the pore-polymer electrostatic interaction is repulsive, upon lowering the salt concentration both in *cis* and *trans* (leading to higher electrostatic repulsion between the polymer and pore), the capture rate should decrease. In addition, if we were to impose a salt concentration gradient ($c_{s,trans}/c_{s,cis} > 1$) (where $c_{s,trans}$ and $c_{s,cis}$ are, respectively, the salt concentrations in *trans* and *cis* compartments), then the enhanced drift part would mitigate the consequence of the polymer-pore repulsion. The relative importance of these two antagonistic contributions can be affected by the externally applied electric field and $c_{s,cis}$. On the other hand, if the pore-polymer interaction is attractive, we expect the polymer capture rate to increase as $c_{s,cis}$ is lowered (less screened pore-polymer attraction). The drift contribution is also in the direction of enhancing the capture rate upon lowering $c_{s,cis}$. As a result, we anticipate a synergistic effect of higher capture rate at low pH.

In efforts to explore the occurrence of the synergistic and antagonistic couplings between the drift and electrostatic interaction contributions and their consequences, we have devised a series of experiments along the following lines. First, we have measured the capture rate as a function of symmetric salt concentration at pH 7.5 and pH 4.5. These results offer proof for the inversion of polymer-pore repulsion into attraction as the pH is lowered. This is then followed by experiments under asymmetric salt concentrations ($c_{s,trans}/c_{s,cis} > 1$) at different pH values. We also have investigated the role of applied voltage difference on the polymer capture rate upon asymmetric salt concentrations. At higher applied voltage differences, the contribution from the drift part must overwhelm the contribution from the pore-polymer interaction. Our data presented below offer support to the above mechanisms of polymer capture.

II. MATERIALS AND METHODS

1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DphPC) and α -toxin from *Staphylococcus aureus* were purchased from Avanti Polar Lipid, Inc. (AL) and EMD Millipore (MA), respectively, and used to form an artificial lipid bilayer membrane and an α -hemolysin pore on it as described in previous reports.^{20,21} Sodium salt of poly(styrene sulfonate) (NaPSS) with weight average molecular weight of 16 kg/mol and polydispersity index of 1.13 was purchased from Scientific Polymer Products, Inc. (NY). Polydeoxythymidine (poly(dT)₈₀) was purchased from Integrated DNA Technologies, Inc. (IA). Potassium chloride (KCl) solutions with 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) in deionized water (18 M Ω cm) were used as buffer solutions for sample preparation and as experimental solutions. pH

values of the buffer solutions were adjusted by adding potassium hydroxide (KOH) or hydrochloric acid (HCl).

NaPSS and poly(dT)₈₀ samples were prepared in HEPES buffer with various KCl concentrations ranging 0.2–2 M with pH 4.5, 7.5, and 10. The concentrations of NaPSS and poly(dT)₈₀ used in this study are 0.5, 2, and 10 μ M for pH 4.5, 7.5, and 10, respectively. After forming an α -hemolysin pore in a buffer solution with desired pH and salt concentration, in asymmetric salt concentration experiments, the solution in *cis* side was exchanged by being flushed with buffer solutions with different KCl concentrations. Polymer concentration was controlled by flushing the *cis* side with excessive amount of polymer solution with specific polymer and salt concentrations.

Controlled electric potentials were applied between *cis* and *trans* side using silver/silver chloride (Ag/AgCl) electrodes connected to Axopatch 200B integrated patch clamp amplifier (Axon Instruments, CA). pClamp software (Molecular Devices, LLC., MA) recorded the ionic currents at 333.33 kHz which are collected by the Axopatch instrument and filtered at 10 kHz with a low-pass Bessel filter. MATLAB software (works, Inc., MA) was employed to detect the current blockade events.

Various characteristic current blockade levels are detected for polymers translocating through an α -hemolysin pore.^{1,2,20} Relatively weak and short blockades with spike-like current blockades represent collisions between polymer chains and the pore mouth. Once a chain gets into the pore mouth and occupies its vestibule region, 30%–70% of open-pore current is blocked. The current amplitude and duration of this state depend on identity of the polymer chain, polymer-pore interactions, *cis* and *trans* concentrations, and the applied voltage. When one chain end finds the narrowest region of the pore and enters into the β -barrel, the ionic current drops as much as 85%–95% of open-pore current with duration time proportional to the polymer chain length, which indicates that these deep blockades correspond to successful translocations through the pore. We set 25% of current drop as a cut-off level to detect polymer capture events, which means not only successful translocations but also unsuccessful events are considered as polymer captures. Polymer capture rate is calculated as $1/\langle t_0 \rangle$ where t_0 is an interval time between two successive events.

III. EXPERIMENTAL RESULTS

A. Symmetric salt concentrations

In order to establish the role of electrostatic interaction between the polymer and the pore on the capture rate of the polymer, we first conducted the measurements in symmetric *cis/trans* salt concentration environments. Under these conditions, the electric potential profiles near the pore mouth are expected to be the same on both sides. Hence, there is no additional drift contribution and the polymer capture is mainly due to the electrostatic interaction between the pore and the polymer, for a given electric field. The rate of capture for 16 kD NaPSS is plotted in Fig. 2 as a function of salt concentration for pH 4.5 and pH 7.5. The capture rates

FIG. 2. The capture rate of 16 kD NaPSS is plotted as a function of symmetric salt concentration in 10 mM HEPES buffer with (a) pH 4.5 and (b) pH 7.5. With increasing salt concentration, the capture rate decreases at pH 4.5 and increases at pH 7.5, indicating that the polymer-pore electrostatic interaction plays an important role in polymer capture by α -hemolysin pore.

in pH 4.5 are significantly higher than those in pH 7.5. This is consistent with our earlier result²⁰ and arises from attraction of the negatively charged polymer to the pore by the positive charges on the pore which are induced by lowering the pH. The more significant result is the opposite trends in the c_s -dependence of R_c for pH 7.5 and pH 4.5. The capture rate decreases with an increase in the salt concentration at pH 4.5, whereas it increases with an increase in c_s at pH 7.5. As already argued, at pH 7.5, the net interaction between the polymer and the pore is repulsive resulting in lower capture rates. As the salt concentration increases at this high pH, the electrostatic repulsion becomes weaker and consequently the capture rate increases. The increase in R_c with c_s at pH 7.5 saturates at about 1 M salt, as seen in Fig. 2(b). For pH 4.5, where the pore-polymer interaction is attractive, the opposite trend is observed: higher capture rate at lower salt concentrations.

FIG. 3. (a) Ionic current traces for capture events of 2 μM of 16 kD NaPSS by α -hemolysin pore at pH 7.5 under 160 mV for different *cis* and *trans* salt concentrations. For 0.5 and 1 M *trans* salt concentrations, the capture rate increases and then decreases with change in $c_{s,cis}$, while for 2 M of *trans* salt concentration, R_c increases as $c_{s,cis}$ decreases from 2 M to 0.2 M. (b)–(e) The rates of polymer capture of 16 kD NaPSS and poly(dT)₈₀ by α -hemolysin pore with asymmetric salt concentrations are measured in (b) and (c) pH 7.5, (d) pH 4.5, and (e) pH 10. Nonmonotonic dependence of R_c on *cis* salt concentration (at lower *trans* salt concentrations) is observed in pH 7.5 and pH 10 where there is antagonistic coupling between polymer-pore interaction and additional drift from salt concentration gradient. The shaded area indicates the region where the electrostatic repulsion between the polymer and the pore dominates R_c over the additional drift contribution. On the other hand, at pH 4.5, R_c monotonically increases with decreasing *cis* salt concentration because the electrostatic attraction between the polymer and the pore is synergistically coupled to the extra electric force from the salt concentration gradient.

B. Asymmetric salt concentrations

Having established that electrostatic interaction between the polymer and the α -hemolysin pore is significant in contributing to the polymer capture, we next investigated the role of salt concentration gradient. Under the asymmetric salt concentration conditions, both the additional drift and pore-polymer interaction contribute either synergistically or antagonistically as discussed above. The rates of capture of 16 kD NaPSS by α -hemolysin were measured for many combinations of *cis/trans* salt concentrations at a fixed electric field of 160 mV in 10 mM HEPES buffers with pH 7.5. Typical ionic current traces measured through α -hemolysin pore are shown in Fig. 3(a) and polymer capture rates are plotted in Fig. 3(b) for three different *trans* salt concentrations, namely, 0.5, 1.0, and 2 M KCl. For 2 M KCl, the capture rate continues to increase when the *cis* salt concentration is decreased. This feature is analogous to the observation by Wanunu *et al.* for the capture of dsDNA by solid-state nanopores.²⁷ On the other hand, for 0.5 M KCl, R_c first increases and then decreases upon lowering *cis* salt concentration. This nonmonotonicity is observed for 1 M KCl as well, except that the turning point is at lower *cis* salt concentration, as seen in Fig. 3(b). As already argued above, the nonmonotonic dependence of R_c on *cis* salt concentration at lower *trans* salt concentrations and higher pH is due to the antagonistic coupling between the pore-polymer interaction and the drift force originating from the salt concentration gradient. We attribute the trend of a decrease in R_c with lowering *cis* salt concentration to the electrostatic interaction between the polymer and the pore and the trend of an increase in R_c with lowering *cis* salt concentration to the extra drift arising from the salt concentration gradient. The regime of decrease in R_c with decreasing *cis* salt concentration is indicated by the shaded area in Fig. 3(b).

The occurrence of the nonmonotonic behavior in Fig. 3(b), which is not seen by Wanunu *et al.*, lies in the substantial electrostatic interaction between the polymer and the α -hemolysin pore. In order to further substantiate this difference, we have repeated the above experiments under asymmetric salt concentrations with the polynucleotide, poly(dT)₈₀. The results for 0.5 M KCl in *trans* are given in Fig. 3(c), where a similar nonmonotonicity is seen as in Fig. 3(b). Furthermore, at lower pH, the coupling between the drift force from salt concentration gradient and the pore-polymer interaction is expected to be synergistic. This expectation is confirmed by our results at pH 4.5, given in Fig. 3(d). For the same combinations of *trans* and *cis* salt concentrations as in Fig. 3(b), the dependence of R_c on *cis* salt concentration is monotonic in Fig. 3(d). As a further check, the measurements were repeated at pH 10. According to our proposed mechanism, the shaded area in Fig. 3(b) (for pH 7.5) representing polymer-pore repulsion should become larger at pH 10. The results are given in Fig. 3(e), fully consistent with our hypothesis.

The relative contribution of the pore-polymer interaction to the capture rate with respect to the drift force from the salt concentration gradient can be altered by the external electric field. At higher externally applied electric fields, the contribution from the pore-polymer interaction is expected to be

FIG. 4. The capture rate of 16 kD NaPSS in pH 7.5 with 0.5 M of *trans* salt concentration under different electric fields is plotted as a function of *cis* salt concentration. At higher voltage, drift dominates over the electrostatic repulsion between the polymer and the pore, resulting in monotonic behavior of R_c .

subdued in comparison with the induced drift. As a result, the nonmonotonic behavior reported in Fig. 3(b) is expected to be converted into a monotonic behavior expected for the regime of dominance by the induced drift. The results for the polymer capture rate as a function of voltage difference and *cis* salt concentration are given in Fig. 4 for *trans* 0.5 M KCl at pH 7.5. It is clear from Fig. 4 that for voltage differences of 220 mV and higher, the dependence of R_c on *cis* salt concentration is monotonic. For lower voltage differences, the behavior is nonmonotonic due to the antagonistic contribution from the polymer-pore interaction. There exists a crossover voltage difference of about 220 mV delineating the two regimes described in Fig. 3(b), for the conditions of the data given in Fig. 4.

IV. DISCUSSIONS AND CONCLUSIONS

The above data clearly demonstrate that the electrophoretic capture of single molecules of sodium poly(styrene sulfonate) by α -hemolysin protein pore under salt concentration gradients is controlled by a combination of pore-polymer electrostatic interaction and induced drift force emerging from the salt concentration asymmetry. At higher pH, the pore-polymer interaction is repulsive and as a result lowering the *cis* salt concentration makes this repulsion even stronger (due to less screening). This enhanced repulsion operates in the opposite direction, antagonistically, to the induced drift force facilitating polymer capture for $c_{s,trans}/c_{s,cis} > 1$. For higher pH values, the capture rate exhibits a nonmonotonic dependence on *cis* salt concentration (for a fixed higher *trans* salt concentration). This antagonistic coupling between the pore-polymer interaction and the drift can be suppressed by increasing the voltage difference. Since this effect is observed for the capture of both NaPSS and

FIG. 5. Log-log plot of the capture rate of 16 kD NaPSS vs. the salt concentration ratio, $c_{s,trans}/c_{s,cis}$. The solid lines are slopes of 1 and 2 to see the change in relative enhancement of the capture rate.

polydeoxythymidine (poly(dT)₈₀) through α -hemolysin pore, but not for dsDNA through a solid-state nanopore,²⁷ we attribute this effect to originate from the charge decoration of the α -hemolysin pore. At lower pH values, the pore-polymer interaction becomes attractive which becomes stronger at lower *cis* salt concentrations (due to less screening). Now, both the pore-polymer attractive interaction and the drift facilitate polymer capture and this synergistic effect leads to monotonic dependence of R_c on *cis* salt concentration (for a fixed higher *trans* salt concentration). All data presented above are consistent with this interpretation.

The capture rates of NaPSS by α -hemolysin for the various combinations of *cis* and *trans* salt concentrations for pH 7.5 are given in Fig. 5 as a double logarithmic plot of R_c versus $c_{s,trans}/c_{s,cis}$. The capture rate is enhanced by more than two orders of magnitude by changing $c_{s,trans}/c_{s,cis}$ from 1 to 10. Although this substantial enhancement of R_c under salt concentration gradients is similar to the observations by Wanunu *et al.*, quantitative details vary significantly. It is seen from Fig. 5 that there are two regimes. The slopes of 1 and 2 are indicated in the figure as a guide to the eye. In the first regime of lower values of $c_{s,trans}/c_{s,cis}$, the drift force dominates over the electrostatic interaction between the pore and polymer. In this regime, the dependence of R_c on $c_{s,trans}/c_{s,cis}$ is quadratic as indicated by a slope of about 2 in Fig. 5. In the second regime with a slope of about 1, the antagonistic coupling between the pore-polymer interaction and drift force is manifest. In this regime, as already discussed, the contribution from the pore-polymer repulsive interaction works against the drift force and lowers the enhancement of the capture rate. The crossover between these two regimes occurs at the *cis* salt concentration corresponding to the condition of almost complete screening of electrostatic interaction between the pore and polymer, which is 1 M for the experimental conditions of Fig. 5. This value is consistent with the saturation point for R_c as seen in Fig. 2(b). The emergence of these two regimes

is due to the nature of the α -hemolysin pore. Our results thus show that charge decoration on the pore is an additional handle in controlling the polymer capture in single molecule electrophoresis.

While the data presented in this paper clearly validate our proposed hypothesis of antagonistic/synergistic coupling between pore-polymer interaction and induced drift by salt concentration asymmetry, a theoretical description for such coupled forces under non-equilibrium conditions is presently lacking.

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- ¹J. J. Kasianowicz, E. Brandin, D. Branton, and D. W. Deamer, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13770 (1996).
- ²M. Akeson, D. Branton, J. J. Kasianowicz, E. Brandin, and D. W. Deamer, *Biophys. J.* **77**, 3227 (1999).
- ³A. J. Storm, J. H. Chen, X. S. Ling, H. W. Zandbergen, and C. Dekker, *J. Appl. Phys.* **98**, 014307 (2005).
- ⁴A. Meller, L. Nivon, and D. Branton, *Phys. Rev. Lett.* **86**, 3435 (2001).
- ⁵A. Meller, L. Nivon, E. Brandin, J. Golovchenko, and D. Branton, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1079 (2000).
- ⁶D. Branton, D. W. Deamer, A. Marziali, H. Bayley, S. A. Benner, T. Butler, M. Di Ventra, S. Garaj, A. Hibbs, X. H. Huang, S. B. Jovanovich, P. S. Krstic, S. Lindsay, X. S. S. Ling, C. H. Mastrangelo, A. Meller, J. S. Oliver, Y. V. Pershin, J. M. Ramsey, R. Riehn, G. V. Soni, V. Tabard-Cossa, M. Wanunu, M. Wiggin, and J. A. Schloss, *Nat. Biotechnol.* **26**, 1146 (2008).
- ⁷S. Benner, R. J. A. Chen, N. A. Wilson, R. Abu-Shumays, N. Hurt, K. R. Lieberman, D. W. Deamer, W. B. Dunbar, and M. Akeson, *Nat. Nanotechnol.* **2**, 718 (2007).
- ⁸K. R. Lieberman, G. M. Cherf, M. J. Doody, F. Olasagasti, Y. Kolodji, and M. Akeson, *J. Am. Chem. Soc.* **132**, 17961 (2010).
- ⁹Y. Jung, H. Bayley, and L. Movileanu, *J. Am. Chem. Soc.* **128**, 15332 (2006).
- ¹⁰S. E. Henrickson, E. A. DiMarzio, Q. Wang, V. M. Stanford, and J. J. Kasianowicz, *J. Chem. Phys.* **132**, 135101 (2010).
- ¹¹L. Movileanu, S. Howorka, O. Braha, and H. Bayley, *Nat. Biotechnol.* **18**, 1091 (2000).
- ¹²I. M. Derrington, T. Z. Butler, M. D. Collins, E. Manrao, M. Pavlenok, M. Niederweis, and J. H. Gundlach, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16060 (2010).
- ¹³T. Z. Butler, M. Pavlenok, I. M. Derrington, M. Niederweis, and J. H. Gundlach, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 20647 (2008).
- ¹⁴M. Wanunu, *Phys. Life Rev.* **9**, 125 (2012).
- ¹⁵M. Wanunu, D. Cohen-Karni, R. R. Johnson, L. Fields, J. Benner, N. Peterman, Y. Zheng, M. L. Klein, and M. Drndic, *J. Am. Chem. Soc.* **133**, 486 (2011).
- ¹⁶A. J. Storm, J. H. Chen, X. S. Ling, H. W. Zandbergen, and C. Dekker, *Nat. Mater.* **2**, 537 (2003).
- ¹⁷V. Tabard-Cossa, M. Wiggin, D. Trivedi, N. N. Jetha, J. R. Dwyer, and A. Marziali, *ACS Nano* **3**, 3009 (2009).
- ¹⁸M. Tsutsui, M. Taniguchi, K. Yokota, and T. Kawai, *Nat. Nanotechnol.* **5**, 286 (2010).
- ¹⁹E. Pennisi, *Science* **336**, 534 (2012).
- ²⁰C. T. A. Wong and M. Muthukumar, *J. Chem. Phys.* **133**, 045101 (2010).
- ²¹R. J. Murphy and M. Muthukumar, *J. Chem. Phys.* **126**, 051101 (2007).
- ²²G. Oukhaled, J. Mathé, A. L. Biance, L. Bacri, J. M. Betton, J. Pelta, and L. Auvray, *Phys. Rev. Lett.* **98**, 158101 (2007).
- ²³L. Brun, M. Pastoriza-Gallego, G. Oukhaled, J. Mathé, L. Bacri, L. Auvray, and J. Pelta, *Phys. Rev. Lett.* **100**, 158302 (2008).
- ²⁴A. Oukhaled, B. Cressiot, L. Bacri, M. Pastoriza-Gallego, J. M. Betton, E. Bourhis, R. Jede, J. Gierak, L. Auvray, and J. Pelta, *ACS Nano* **5**, 3628 (2011).

- ²⁵A. Fennouri, R. Daniel, M. Pastoriza-Gallego, L. Auvray, and J. Pelta, *Anal. Chem.* **85**, 8488 (2013).
- ²⁶M. Muthukumar, *Polymer Translocation* (Taylor & Francis, Boca Raton, 2011).
- ²⁷M. Wanunu, W. Morrison, Y. Rabin, A. Y. Grosberg, and A. Meller, *Nat. Nanotechnol.* **5**, 160 (2010).
- ²⁸J. E. Gouaux, O. Braha, M. R. Hobaugh, L. Song, S. Cheley, C. Shustak, and H. Bayley, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12828 (1994).
- ²⁹L. Song, M. R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, and J. E. Gouaux, *Science* **274**, 1859 (1996).
- ³⁰M. Muthukumar, *J. Chem. Phys.* **132**, 195101 (2010).
- ³¹S. E. Hendrickson, M. Misakian, B. Robertson, and J. J. Kasianowicz, *Phys. Rev. Lett.* **85**, 3057 (2000).
- ³²T. Ambjörnsson, S. P. Apell, Z. Konkoli, E. A. Di Marzo, and J. J. Kasianowicz, *J. Chem. Phys.* **117**, 4063 (2002).
- ³³J. E. Hall, *J. Gen. Physiol.* **66**, 531 (1975).
- ³⁴I. Vodyanoy and S. M. Bezrukov, *Biophys. J.* **62**, 10 (1992).
- ³⁵A. J. Wolfe, M. M. Mohammad, S. Cheley, H. Bayley, and L. Movileanu, *J. Am. Chem. Soc.* **129**, 14034 (2007).
- ³⁶G. Maglia, M. R. Restrepo, E. Mikhailova, and H. Bayley, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19720 (2008).
- ³⁷C. Y. Kong and M. Muthukumar, *J. Am. Chem. Soc.* **127**, 18252 (2005).