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Non-uniform Velocity of Homogeneous DNA in a Uniform Electric Field: Consequence of Electric-Field-Induced Slow Dissociation of Highly Stable DNA–Counterion Complexes

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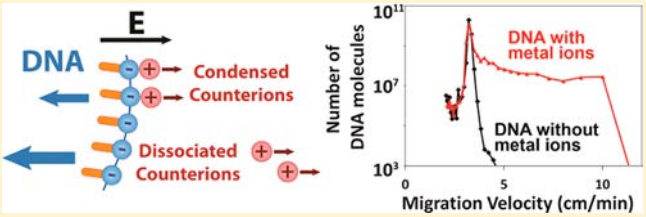
Supporting Information

ABSTRACT: Identical molecules move with identical velocities when placed in a uniform electric field within a uniform electrolyte. Here we report that homogeneous DNA does not obey this fundamental rule. While most DNA moves with similar velocities, a fraction of DNA moves with velocities that vary within a multiple-fold range. The size of this irregular fraction increases several orders of magnitude when exogenous counterions are added to DNA. The irregular fraction decreases several orders of magnitude when DNA counterions are removed by dialysis against deionized water in the presence of a strong electric field (0.6 kV/cm). Dialysis without the field is ineffective in decreasing the size of irregular fraction. These results suggest that (i) DNA can form very stable complexes with counterions, (ii) these complexes can be dissociated by an electric field, and (iii) the observed non-uniform velocity of DNA is caused by electric-field-induced slow dissociation of these stable complexes. Our findings help to better understand a fundamental property of DNA: its interaction with counterions. In addition, these findings suggest a practical way of making electromigration of DNA more uniform: removal of strongly bound DNA counterions by electro-dialysis against deionized water.



INTRODUCTION

When dissolved in water, DNA bears a high density of negative charge due to deprotonation of phosphate groups in its backbone.[1] The presence of this negative charge defines many properties of DNA, including its structure and ability to interact with other molecules.[2] When placed in an electric field, the negatively charged DNA migrates toward the positive electrode. The polymeric nature of DNA makes it simple to predict its molecular length and charge. As a result, electro-migration techniques, such as electrophoresis, are among the primary tools for studying DNA *in vitro*.[3,4]

A variety of electrophoretic platforms exists to facilitate different analytical goals. Each platform presents researchers with various degrees of separation power, sensitivity, limits of detection, and robustness. Due to efficient heat dissipation in small channels, techniques such as capillary or microchannel electrophoresis allow application of high-magnitude electric fields, and as a result make it possible to resolve DNA molecules in solution even without the use of sieving-matrixes.[5]

All electrophoretic techniques operate under the assumption that in a uniform electric field, within a uniform electrolyte, DNA migrates with a velocity that is defined by its length and conformation.[6] By extension, it is also assumed that identical DNA molecules migrate with a uniform velocity, when diffusion is accounted for. This is confirmed on a daily basis by thousands of researchers that observe uniform zones of DNA in properly performed electrophoresis experiments. Indeed, when accompanied by prevailing optical or radioactivity-based detection approaches, electrophoresis produces apparently uniform velocity profiles for homogeneous DNA samples. The assumption about uniform velocity of DNA migration in electrophoresis is, thus, widely accepted and not challenged.

Some applications, however, require that the products of DNA electrophoresis separation are subjected to procedures that are much more sensitive to presence of minute quantities of DNA. One example of such an application is DNA aptamer selection, during which DNA molecules are separated based on their interaction with a second "target" molecule, and are then subjected to PCR amplification.[7] It has been observed, that the efficiency of electrophoretic separation of DNA in such cases is lower than expected: a considerable amount of DNA appears in fractions that, theoretically, should be devoid of it.[8] Various explanations were proposed for this phenomenon, but its cause was never conclusively determined.

This work was partially motivated by our realization that typical electrophoresis experiments do not reveal detailed velocity profiles of DNA. They utilize optical or radioactivity-based detection approaches which are characterized by relatively poor limits of detection and dynamic ranges. Irregularities in DNA velocity cannot be noticed if the amount of DNA that moves with irregular velocities is below the limit of detection. We, hence, decided to study DNA velocity profiles in a larger dynamic range of DNA concentrations by using

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quantitative polymerase chain reaction (qPCR). This detection approach has a much lower limit of detection, when compared to the more commonly used alternatives, and can quantitate DNA in a range of concentrations of 10 orders of magnitude.[9] Here we report our findings that while the major fraction of DNA molecules does migrate with predictable velocities, there exists a fraction of DNA molecules that migrate with a wide range of irregular velocities. We have determined that the cause for this velocity heterogeneity, within a homogeneous DNA sample, lies in the ability of DNA to form stable complexes with its counterions. Irregular DNA migration profiles arise due to electric field-induced dissociation of such complexes.

RESULTS AND DISCUSSION

Capillary electrophoresis (CE) was used as a "one-dimensional" electro-migration technique with a perfect uniformity of the electric field parallel to the direction of migration.[10] Detection of DNA was performed with either commercially available fluorescence detection system or with qPCR, which is an off-line detection technique rarely used for DNA detection in CE. For qPCR detection, fractions were collected from the capillary output at 1-min intervals and then subjected to qPCR as described elsewhere.[8] CE of DNA can be carried out either in a capillary with coated inner walls, to suppress the electroosmotic flow (EOF) (Figure 1A), or in a bare-silica capillary with EOF (Figure 1B). In CE, the net velocity of DNA is, in general, a vector sum of the electrophoretic velocity and velocity of EOF, which are counter-directed between the two modes (Figure 1, top). Thus, for elution of DNA, the two modes require different polarities to be applied. We utilized both modes of CE for the first set of experiments.

A short plug of an 80-nt synthetic fluorescein-labeled single-stranded DNA (ssDNA) was injected into a capillary, a high voltage was applied, and migration time of DNA to a detection point was recorded both by measuring fluorescence of the label and by quantitating DNA in collected fractions by qPCR. As we expected, fluorescence detection exposed a typical Gaussian-shape peak (Figure 1, middle), which suggests uniform velocity of DNA normally dispersed due to peak-broadening phenomena such as diffusion.[11] The dynamic range of fluorescence detection was approximately 3 orders of magnitude. Presenting data in a log scale did not reveal any additional information. The results were different with qPCR detection, which allowed us to determine the quantity of DNA within a range of 10 orders of magnitude. Presenting the results of qPCR measurements in a log scale revealed a non-uniform velocity profile (Figure 1, bottom). Most DNA migrated with a uniform velocity, normally dispersed to yield the Gaussian distribution. However, there was also an irregular fraction of DNA, which migrated with velocities different from that of the main DNA zone, and varying within a several-fold range. The irregular fraction of DNA was below the limit of detection of fluorescence. The length of the DNA molecule did not significantly affect the observed velocity heterogeneity, as similar velocity profiles were observed by us for 71-nt and 120-nt DNA samples (Figure S1).

Various phenomena can potentially explain the observed irregular fraction of DNA. Strong distortion (fronting or tailing) of sample zones in CE can generally be caused by (i) sample diffusion, (ii) the anti-stacking effect, (iii) "friction" of DNA molecules along the walls of the capillary, (iv) DNA heterogeneity in size, (v) DNA heterogeneity in tertiary

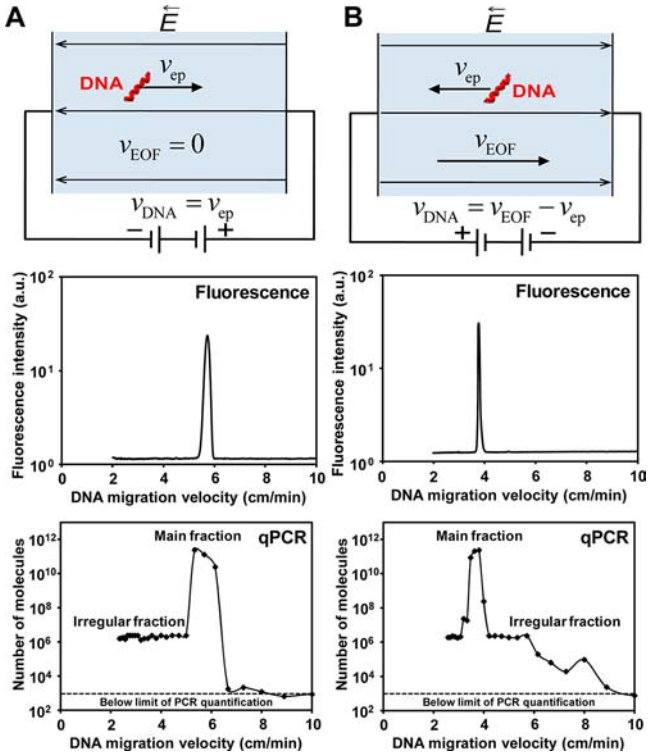

Figure 1. Migration of homogeneous DNA in a uniform electric field within a uniform electrolyte in the absence (A) and presence (B) of the electroosmotic flow. The top drawings show schematic representations of the DNA mobility experiments. The net velocity of DNA, v_{DNA}, depends on its electrophoretic velocity, v_{EP}, and the velocity of electroosmotic flow, v_{EOF}, in the electric field E. The middle and bottom graphs show electrophoretic migration profiles of fluorescein-labeled 80-nt ssDNA detected by fluorescence and quantitative PCR, respectively. DNA was dissolved in the run buffer, 50 mM Tris−acetate pH 8.3, to final concentrations of 10 μM and 250 nM for for qPCR-based and fluorescence-based detection, respectively. A 9-mm plug of DNA solution was injected by pressure into an 80-cm long capillary with inner diameter of 75 μm. Electro-migration was carried out with an electric field of 375 V/cm. The middle and bottom traces differ in shapes and slightly shifted along the abscissa. The shape difference is due to different frequency of signal acquisition: 4 Hz for fluorescence detection and 0.016 Hz for qPCR detection.

structure, and (vi) interaction of DNA with other species in solution.

Some of these can be refuted immediately as explanations of the observed phenomenon. DNA molecules are bulky and are generally characterized by slow diffusion coefficients. In our experiments, the front of the irregular fraction and the peak of the main fraction were separated by approximately 40 cm. Even with the greatest estimate for DNA diffusion coefficient, calculations show that it would take approximately 1500 days to establish the observed DNA distribution pattern by diffusion alone. This is in stark contrast to the 10-min elution time of the irregular fraction front. Thus, the irregular velocity of DNA cannot be explained by sample diffusion. Neither can it be explained by the anti-stacking effect, as in our experiments the sample electrolyte and the electrophoresis electrolyte were identical, and the DNA concentration was negligibly low when compared to the concentration of the buffer. Boundary phenomena, such as retardation of DNA molecules near the walls of the capillary due to "friction" also cannot explain the observed results. If DNA molecules were slowed down by

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interacting with the capillary surface, the irregular fraction of DNA would migrate slower than the major fraction regardless of presence of EOF. However, in the presence of EOF (and reversed direction of electric field), the irregular fraction migrated faster than the major fraction (Figure 1A, bottom). Finally, the observed irregular fraction cannot be explained by heterogeneity in DNA polymer lengths (e.g., as a result of sample degradation). Matrix-free electrophoresis methods, such as CE, separate molecules based on charge-to-size ratios. DNA molecules of various lengths are poorly resolved in matrix-free CE, as charge-to-size ratio of DNA is approximately constant across all polymer lengths. This is due to the fact that both the charge of DNA molecules and their friction coefficients are directly proportional to their polymer length. Experience shows that in CE, mobilities between DNA as long as 122 nt and as short as 15 nt differ by only ~10%. In current experiment, however, the mobility of the irregular fraction differed from that of the major fraction by a factor of 3. Furthermore, truncated DNA cannot be detected with the employed qPCR detection method, as shorter sequences would lack an adequate flanking regions required for PCR primer annealing. As a result, all qPCR detected sequences must be of the same length.

We noticed that the observed velocity profile was analogous to that reported for similar experiments with a highly heterogeneous DNA library containing ~10^{12} different sequences.[8] The nature of the velocity profile for the DNA library has never been deciphered, but it was suggested that the conformational heterogeneity of the library was the likely cause of the "heterogeneous" velocity. In our experiments, depicted in Figure 1, we studied a single DNA sequence that cannot provide a conformational diversity of the library, but can still result in multiple thermodynamically feasible conformations through DNA interaction with "itself" inter- or intramolecularly.[12] To examine whether or not the velocity non-uniformity could be explained by the conformational heterogeneity caused by DNA interaction with itself, we studied electro-migration of the double-stranded sample of the same DNA sequence (dsDNA). In contrast to ssDNA, dsDNA forms a much smaller variety of structures that primarily consist of the thermodynamically favorable double helix conformation. We found that the velocity profile of dsDNA was also non-uniform (Figure 2) and nearly identical to that of the ssDNA shown in Figure 1B, bottom. This result suggested that non-uniform velocity of DNA could not be explained by multiple conformations caused by intra- and intermolecular interactions of DNA.

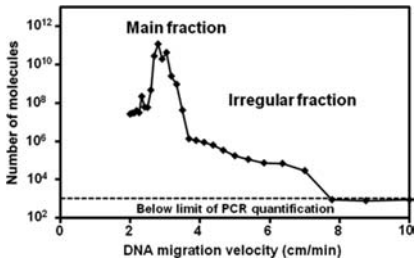

Figure 2. Migration profile of dsDNA in a uniform electric field measured with qPCR detection. Experimental conditions were similar to those described in legend to Figure 1.

After eliminating all identified alternative explanations for formation of the irregular fraction, we finally considered interaction of DNA with other species as the possible cause. DNA mobility was previously shown to be affected by interaction with cations.[13] Moreover, we noticed that the velocity pattern of DNA in Figure 1, bottom resembled those of DNA in analyses of DNA–protein binding by non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM).[14] The non-uniform velocity of DNA in NECCEM is caused by the slow dissociation of very stable affinity complexes between DNA and a protein, after the electric field disturbs equilibrium by removing free protein and free DNA from the surroundings of the DNA–protein complex. The electric field was shown to increase the rate of dissociation of protein–DNA complex.[15] While there was no protein present in experiments shown in Figures 1 and 2, the solution did contain a variety of positive counterions, which could bind to the negatively charged DNA and affect its electrophoretic velocity.[16−20] We thus hypothesized that the observed irregular velocity profile of DNA was caused by dissociation of DNA–counterion complexes.

To produce the observed NECEEM-like velocity profile, a portion of complexes between DNA and counterions must be very stable and slow-dissociating. DNA can form two general types of complexes with its counterions: diffusely bound complexes and condensation complexes.[17,21,22] Diffusely bound complexes are unstable, fast-dissociating and predominate in conditions of counterion abundance. In contrast, condensation complexes display higher stability, slower rates of dissociation and occur in conditions of counterion deficiency. The process of DNA counterion condensation was theoretically predicted by Manning in 1969, and since then has garnered strong experimental support.[22−24] Counterion condensation is driven by an excessively high native charge density of DNA molecules. Due to insufficient charge neutralization in conditions of counterion deficiency, the charge density of DNA may exceed a certain threshold value. This causes some counterions to become trapped, or condensed, in close vicinity of DNA, unable to escape through thermal energy alone. The stability of condensation complexes is inversely proportional to their concentration, with the complexes becoming more difficult to dissociate as their concentration decreases. At certain conditions of counterion deficiency, condensation complexes become so strong that their dissociation requires application of an external force, such as a strong electric field.[25]

Consideration of the counterion condensation theory makes dissociation of DNA–counterion complexes a plausible explanation for the observed heterogeneity of electrophoretic velocity of DNA. Tightly bound metal counterions are likely to be carried-over into sample solutions along with DNA from the time of its synthesis. Combined with the more bulky buffer counterions, these create conditions of counterion abundance, in which diffusely bound complexes comprise the vast majority of DNA–counterion interactions. Upon electrophoresis, diffusely bound fast-dissociating counterions separate from DNA in a matter of seconds, resulting in formation of a large fraction of DNA molecules with a uniform electrophoretic velocity. Furthermore, electrophoretic separation of diffusely bound counterions creates counterion deficiency around DNA, leading to formation of more stable condensation complexes. Electric-field-induced slow dissociation of such condensation complexes results in a small fraction of molecules with different mobilities. Importantly, the more condensation complexes dissociate from DNA, the more stable the remaining complexes become. As complex dissociation events are "probability-controlled", dissociation of more stable condensation com-

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plexes results in a wide-stretching irregular fraction of DNA. As such, this hypothesis is consistent with the shape and the small relative size of the irregular fraction.

We reasoned that if our hypothesis is true, then the extent of DNA velocity heterogeneity can be regulated by the concentration of DNA counterions in the solution. To increase the amount of counterions we simply added NaCl to the solution of DNA and incubated the mixture to reach equilibrium in interaction between DNA and Na^+. The experiment was similar to NECEEM in a sense that the run buffer did not contain NaCl and the injected DNA–Na^+ complexes were promoted to dissociate when the excess of Na^+ was separated from the complexes. These experiments clearly showed that the irregular fraction of fast-moving DNA increased with increasing concentration of NaCl in the equilibrium mixture. The increase was so significant that the irregular fraction was detectable by fluorescence and even had a peak of stable, fast-moving DNA–Na^+ complexes (Figure 3).

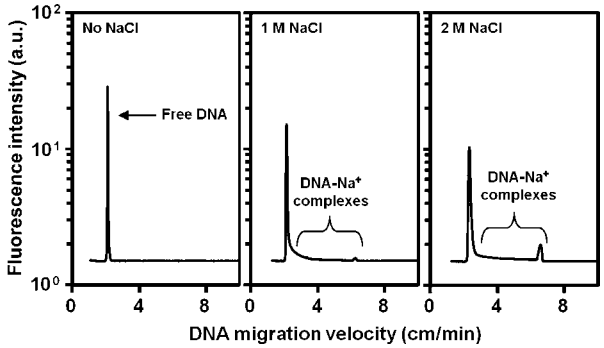

Figure 3. Dependence of ssDNA velocity in a uniform electric field on addition of Na^+ counterions. Experimental conditions were similar to those described in legend to Figure 1.

Similar results were obtained with other monovalent (Li^+ and K^+) and divalent (Mg^{2+} and Ca^{2+}) cations (Figure S2), confirming that DNA can form stable slow-equilibration complexes with metal counterions. It is difficult, however, to extract any meaningful kinetic information from these experiments due to a complex stoichiometry of binding and constantly changing rates of complex dissociation.

Next, we tested the effects of decreasing counterion concentration in solution on the size of the irregular fraction. First, we attempted to only decrease the concentration of the diffusely bound counterions by passive dialysis. We dissolved synthetic fluorescein-labeled 80-nt ssDNA in deionized water and subjected it to passive dialysis against deionized water for 8 h, with regular replacement of the diluent with fresh portion of deionized water. After dialysis, the content of the membrane bag was diluted with Tris–acetate run buffer and the resulting solution of DNA was run in CE to measure its velocity profile. The resulting velocity profile was similar to the one of the DNA sample prior to dialysis (Figure 4A, red and blue traces). This result suggested that the presence of fast-dissociating, diffusely bound counterions does not cause the observed DNA velocity heterogeneity.

Decreasing the amount of condensed counterions is not as trivial task as increasing it. Due to their stability, DNA counterion condensation complexes are difficult to dissociate by conventional deionization methods, such as filtration, precipitation, or passive dialysis. However, dissociation of condensed counterion complexes can be achieved more

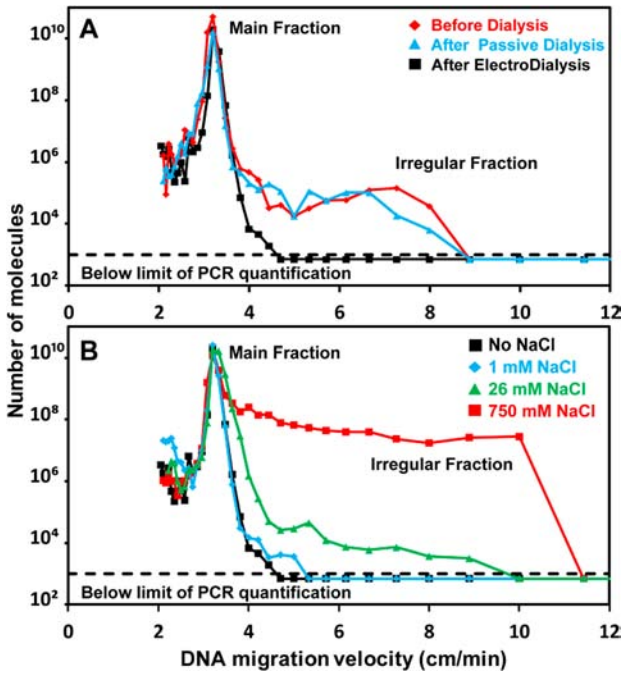

Figure 4. Influence of counterion depletion (A) and re-introduction (B) on migration of ssDNA in a uniform electric field measured with qPCR. In panel A, the red trace corresponds to DNA prior to dialysis (control experiment), the blue trace corresponds to DNA that was dialyzed against deionized water in the absence of electric field, and the black trace corresponds to DNA that was dialyzed against deionized water in the presence of electric field of 600 V/cm. In panel B, either 0, 1, 26, or 750 mM of NaCl was added to an electro-dialyzed sample of DNA before analysis by CE with qPCR detection (black, blue, green, and red traces, respectively). Experimental conditions for CE were similar to those described in the legend to Figure 1. Both the size of the DNA fraction with irregular migration velocities and the range of the irregular velocities showed strong dependence on Na^+ ion concentration.

efficiently by application of a strong electric field.[25] Thus, to test the effects of decreasing the concentration of condensed counterions on the size of the irregular fraction, we combined dialysis with the application of a strong electric field. Synthetic fluorescein-labeled 80-nt ssDNA was dissolved in deionized water and subjected to dialysis against deionized water in the presence of an electric field of 600 V/cm. The diluent was replaced with fresh deionized water with 1 min intervals. After 5 min of such electro-dialysis procedure, the content of the dialysis bag was diluted with Tris–acetate run buffer and the resulting solution of DNA was run in CE to determine DNA velocity profile. This time, the result was different: the fraction of irregularly moving DNA was drastically decreased (Figure 4A, black trace). The same experiment was repeated after incubating DNA at 4 °C for 96 h to ensure that the equilibrium of DNA with buffer ions is established. As expected the observed DNA velocity profile was not affected by incubation time (Figure S3). The effect of counterion depletion was reversible. Adding NaCl to the electro-dialyzed (i.e., counterion deficient) DNA increased the irregular fraction of DNA (Figure 4B) in a Na^+ concentration-dependent fashion. The obtained results allowed us to make two conclusions. First, an electric filed is required for efficient dissociation of the very stable condensed counterion–DNA complexes. Second, the size of the irregular fraction of fast-moving DNA decreases with

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decreasing the amount of metal counterions condensed on DNA.

CONCLUSIONS

In summary, our results strongly suggest that (i) DNA can form very stable complexes with at least a fraction of counterions, (ii) these complexes can be dissociated by an electric field, and (iii) the observed non-uniform velocity of DNA is caused by electric-field-induced slow dissociation of these stable complexes. The first two points are in good agreement with Manning's theory of counterion condensation on polyions.[25,26] Many aspects of this theory were previously difficult to examine due to the lack of suitable experimental techniques. Our results suggests that CE with qPCR detection, as well as electrodialysis against deonized water, can serve as powerful tools for testing many conclusions of Manning's theory.

The interactions of DNA with ions are of a great importance in nature.[20,26−28] The presence of electric fields in cells in close proximity to DNA have been observed.[29−31] Moreover, the shielding effect of both mono- and divalent cations was shown to modulate the strength of DNA−DNA or protein−DNA interactions occurring in live cells.[27,29] Our finding of electric field influence on DNA−counterion interactions has a potential to be biologically significant, since metal ions on the DNA are involved in DNA biochemistry in living cells.

Non-uniform velocity of homogeneous DNA in a uniform electric field may have many important practical implications. For example, this phenomenon is likely the sole cause of a previously reported DNA background of unknown nature in aptamer selection by electrophoresis-based partitioning.[8] It can also be a potential source of artifacts in aptamer-based ultrasensitive analyses of proteins in the presence of electric field, when interactions occur in near-physiological buffers, with relatively high ion content.[32] In pharmaceutics, our technique may help in both analysis and purification of nucleic acid based drug components, since aptamers are now becoming more widely used in medicine.[33,34]

EXPERIMENTAL SECTION

Materials. All chemicals were purchased from Sigma-Aldrich (Oakville, ON) unless otherwise stated. Fused-silica capillaries were purchased from Molex Polymicro (Phoenix, AZ). DNA sequences were custom synthesized by Integrated DNA Technologies (IDT, Coralville, IA). The 80-nt DNA samples had an additional HPLC purification cycle to remove the truncated oligomers. All synthetic DNA was subjected to a standard desalting by IDT, a procedure which is company claimed to almost completely remove all non-reacted protective groups and diffusely bound counterions from the DNA samples. All DNA was received as lyophilized pellet and resuspended in the sample/run buffer (50 mM Tris−acetate at pH 8.3) unless stated otherwise. All electromigration and NECEEM experiments were performed using 10 μM DNA samples for qPCR-based detection and 250 nM DNA samples for fluorescence-based detection. NanoDrop 1000 spectrometer (Thermo Scientific, Wilmington, DE) was used to verify DNA concentrations by measuring absorbance at 260 nm. Values were calculated based on manufacturer-provided extinction coefficients. For electrophoretic migration studies, a fluorescein-labeled ssDNA molecule was employed, with the following sequence: 5′-fluorescein-CTT CTG CCC GCC TCC TTC CTG GTA AAG TCA TTA ATA GGT GTG GGG TGC CGG GCA TTT CGG AGA CGA GAT AGG CGG ACA CT-3′. For dsDNA migration studies, an unlabeled complementary strand was separately synthesized, mixed in at 1:1 ratio, heated to 90 °C and then slowly cooled down to 4 °C. For the experiment with different cations we used a synthetic ssDNA library of the following sequence: 5′-fluorescein-CTT CTG CCC GCC TCC TTC CT -(N40)- AGA CGA GAT AGG CGG ACA CT-3′

DNA Dialysis. For all dialysis procedures, 50−200 μL of each DNA sample was prepared at 100 μM concentration, with deionized water as solvent. Roughly 3-cm-long portions of Spectra/Por 6 dialysis membrane bags (Spectrum Laboratories Inc., Rancho Dominguez, CA) with molecular weight cutoff value of 25 kDa, were used for all experiments. Prior to each procedure, dialysis membrane bags were soaked in deionized water for 30 min and thoroughly rinsed. The DNA sample was then transferred into the dialysis bag, and clamped off at each end, ensuring that no air bubbles were trapped inside the membrane bags. For passive dialysis, the membrane bags were placed into 500 mL of deionized water, and incubated for 8 h. The diluent water was exchanged every hour, for a total of eight times. The sample solution was then transferred from the membrane bag into a test tube. For electro-dialysis, the membrane bags with DNA samples were placed into a Minive Blotter chamber (Amersham-GE Healthcare, Baie d'Urfe, QC) containing 300 mL of deionized water. An electric field of 600 V/cm was then applied across the blotter chamber for one minute. At this point the diluent water was exchanged and the procedure repeated. The DNA sample was used for CE analysis after 5−7 repetitions of the procedure.

Capillary Electrophoresis. All CE procedures were performed using a P/ACE MDQ apparatus (Beckman Coulter, Mississauga, ON) equipped with a laser-induced fluorescence (LIF) detection system. All capillaries were 80 cm long (70 cm to the detector) and had an inner diameter of 75 μm and an outer diameter of 360 μm. The poly(vinyl alcohol)-coated capillary was prepared as described elsewhere.[35] Samples were injected into the capillary, prefilled with the run buffer (which was the same as the sample buffer, 50 mM Tris−acetate at pH 8.3), by a pressure pulse of 0.5 psi for 11 s, resulting in a plug with a length of 9 mm. Prior to every run, the coated capillaries were rinsed with a sequence of deionized water and the sample/run buffer. Uncoated capillaries were rinsed with a sequence of 6000 ppm sodium hypochlorite solution, 100 mM HCl, 100 mM NaOH, deionized water, and run buffer prior to every experiment. During electrophoresis, both inlet and outlet reservoirs contained the sample/run buffer solution. Separations were carried out by an electric field of 375 V/cm. For coated capillaries, electrophoresis was carried out with the negative electrode at the injection end of the capillary. For uncoated capillaries, electrophoresis was carried out with the positive electrode at the injection end of the capillary; the direction of the EOF was from the inlet to the outlet reservoir. The temperature in the cooled region of the capillary was maintained at 15 °C during separations. For experiments with fraction collection, uninterrupted electrophoresis was performed for the first 5 min of the run, after which the collection vial was switched every minute. Eluent was collected into vials containing 10 μL of run buffer. A total of 34 fractions were collected for each experiment. To prevent fraction cross-contamination, the outlet end of the capillary was momentarily dipped into a reservoir with a large volume of sample/run buffer in between every fraction collection step. Collected fractions were immediately analyzed through qPCR. All NECEEM experiments were conducted in uncoated capillaries, with the same procedures as described above, except for the fact that no fractions were collected, and electrophoresis was performed uninterrupted for 40 min. In NECEEM experiments, 250 nM ssDNA samples contained either 0, 1, or 2 M NaCl.

qPCR. qPCR reaction mixture was prepared by combining IQ SYBR Green Supermix from Bio-Rad (Mississauga, ON) with unlabeled DNA primers. The nucleotide sequence of the sense primer was 5′-CTT CTG CCC GCC TCC TTCC-3′ and the sequence of the anti-sense primer was 5′-AGT GTC CGC CTA TCT CGT CTC C-3′. Aliquots of 2 μL of each fraction were mixed with 18 μL of qPCR reaction mixture immediately before thermocycling. Besides the collected fractions, each qPCR experiment also included negative controls (no template control, fraction-collection buffer control and run buffer control), a set of standards (containing from 10^3 to 10^7 template molecules) and a set of 100× dilutions of the seven fractions with the highest expected DNA concentration. Each qPCR reaction was performed in duplicates. Thermocycling and real-time fluores-

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cence signal collection was performed with iCycler IQ system from Bio-Rad. Raw fluorescence signal data were background-subtracted and amplitude-normalized as described elsewhere.[36]

■ ASSOCIATED CONTENT

Supporting Information

Supporting results and figures. 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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