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Stephanie de Jong[1,2]
Nicolas Epelbaum[1,2]
Ruchi Liyanage[1,2]
Sergey N. Krylov[1,2]

[1]Department of Chemistry, York University, Toronto, Ontario, Canada
[2]Centre for Research on Biomolecular Interactions, York University, Toronto, Ontario, Canada

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Research Article

A semipermanent coating for preventing protein adsorption at physiological pH in kinetic capillary electrophoresis

Protein adsorption to the inner capillary wall hinders the use of kinetic capillary electrophoresis (KCE) when studying noncovalent protein–ligand interactions. Permanent and dynamic capillary coatings have been previously reported to alleviate much of the problems associated with protein adsorption. The characteristic limitations associated with permanent and dynamic coatings motivated us to look at a third type of coating – semipermanent. Here, we demonstrate that a semipermanent capillary coating, designed by Lucy and co-workers, comprised of dioctadecyldimethylammonium bromide (DODAB) and polyoxyethylene (POE) stearate, greatly reduces protein adsorption at physiological pH – a necessary requirement for KCE. The coating (i) does not inhibit protein–DNA complex formation, (ii) prevents the adsorption of the analytes, and (iii) supports an electoosmotic flow required for many applications of KCE. The coating was tested in three physiological buffers using a well-known DNA aptamer and four proteins that severely bind to bare silica capillaries as standards. For every protein, a condition was found under which the semipermanent coating effectively suppresses protein adhesion. While no coating can completely prevent the adsorption of all proteins, our findings suggest that the DODAB/POE stearate coating can have a broad impact on CE at large, as it prevents the absorption of several well studied, highly adhesive proteins at physiological pH.

Keywords:
Capillary electrophoresis / Kinetic capillary electrophoresis / Protein adhesion / Semipermanent coating
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1 Introduction

Kinetic capillary electrophoresis (KCE) refers to separation-based affinity methods that function as a platform for the kinetic analyses of noncovalent molecular interactions [1]. KCE methods have been implemented in several applications that range from defining both the kinetic and equilibrium constants of interaction (k_{off}, k_{on}, and K_d) between protein–ligand binding pairs [2–7] to the development of high-affinity DNA ligands (aptamers) to protein targets [8–14]. Although protein–ligand interactions are commonly studied by KCE, their kinetic analysis is often hindered by protein adsorption to the inner surface of bare silica capillaries. At pH values greater than 3, positively charged proteins tend to experience a significant ionic attraction to the negatively charged capillary surface. This protein–silica interaction usually leads to peak broadening, poor separation efficiency, and analyte depletion [15, 16]. A number of buffer and surface modifications have been introduced in order to alleviate protein adsorption along the capillary surface and, in turn, facilitate protein detection and separation. Some of the most common approaches include adjusting the run buffer pH [17, 18], implementing high salt concentrations [19, 20], and the use of dynamic surface modifiers or covalent capillary coatings [21–24]. Although many of these techniques have proven to be effective in reducing the surface adsorption of protein analytes, the majority are ill-suited for kinetic-based applications such as binding analysis and aptamer selection due to apparent incompatibilities with the fundamental KCE requirements. A buffer, together with any additive or coating, must satisfy three conditions in order to study biomolecular interactions by CE: (i) complex formation must not be inhibited, (ii) the adhesion of basic proteins and their respective ligand must be suppressed, and (iii) the EOF should be sufficiently strong to ensure that both

Correspondence: Professor Sergey N. Krylov, Department of Chemistry, York University, Toronto, Ontario M3J 1P3, Canada
E-mail: skrylov@yorku.ca
Fax: +1 416 736-5936

Abbreviations: DODAB, dioctadecyldimethylammonium bromide; **KCE**, kinetic capillary electrophoresis; **NECEEM**, nonequilibrium capillary electrophoresis of equilibrium mixtures; **NGAL**, neutrophil gelatinase associated lipocalin; **POE**, polyoxyethylene; **TES**, N-*Tris*(hydroxymethyl)methyl-2-aminoethanesulfonic acid

the ligand and complex are carried past the detector, while still maintaining an ideal separation distance.

To address the first condition, the run buffer of choice must preserve the integrity of both the protein and the protein–ligand complex. Although buffers of extreme alkaline or acidic pH promote the electrostatic repulsion between the protein and capillary, they can also denature protein analytes. Protein denaturation will certainly influence the formation of any complex and, consequently, prevent kinetic measurements as well as aptamer selection. As a result, for KCE-based analysis, the pH of the BGE should not deviate from the physiological range (i.e., 7–9). Alternatively, buffers of high ionic strength have also been implemented to reduce the irreversible protein adsorption along the capillary surface [19, 20]. The high number of salt ions present in the BGE serves as an ion-exchange mechanism, capable of displacing the protein from the anionic capillary surface. The main drawback of this approach is the excessive Joule heating associated with high-conductivity buffers. The increased capillary temperature will have a detrimental influence on complex stability, preventing their use in the KCE-analysis of binding pairs [25–27]. Moreover, the large number of surrounding ions will certainly interfere with the relatively weak interactions that constitute the protein–ligand complex and, as a result, reduce the apparent binding affinity [28].

To meet the second requirement, the capillary surface must be physically inert to both the protein and ligand of interest. Cationic coatings, which reverse the capillary surface charge, have proven to be successful in the separation of basic proteins [29, 30]. However, the resultant charge reversal tends to promote the adsorption of anionic molecules, restricting that coatings use in KCE methods involving negatively charged ligands, such as DNA (RNA) aptamers. Many permanent, polymer-based coatings eliminate the electrostatic adsorption of both positively and negatively charged molecules by completely neutralizing the surface charge. Unfortunately, nonionic coatings are often accompanied with a highly reduced or nonexistent EOF and are incapable of fully satisfying the third requirement for specific KCE applications. In the absence of an EOF, analytes are separated according to their overall size-to-charge ratio in the selected BGE. This creates a challenge in the kinetic analysis of proteins and ligands that differ in their overall charge polarity, a situation that is commonly encountered with cationic proteins and DNA aptamers. DNA molecules retain a uniform negative charge at physiological pH, and will migrate toward the anode with a relatively high velocity. However, predicting the electrophoretic migration of proteins is challenging as it depends, not only on their amino acid sequence, but also on their structural conformation and the pH value of the separation buffer. If the protein holds a net positive charge of considerable magnitude, it may effectively counterbalance the charged DNA and neutralize the complex appreciably. In the absence of a significant EOF, the separation time of such weakly charged analytes is rather lengthy and impractical for kinetic studies that demand high throughput, such as aptamer screening. Alternatively, if the free aptamer and protein–aptamer complex acquire a similar net charge and experience comparable migration velocities toward the anode, then a suppressed EOF may be suitable. To meet the third condition, it would be highly beneficial if the EOF could be tailored to meet the specific separation requirements of the ligand and complex of interest.

The number of coatings compatible with KCE-based binding analysis and aptamer selection is rather limited. While several dynamic additives, such as amine-containing molecules, polymers, and surfactants may satisfy the three requirements, they often lack the efficiency associated with more permanent coatings. Since their attachment is based on temporary interactions with the capillary, dynamic modifiers tend to compete with the protein for surface-binding sites and, thus, introduce an additional level of interaction. This will undoubtedly complicate the kinetic analysis of protein–ligand interaction and should be either avoided or compensated for. Furthermore, only a selected number of covalently bonded coatings are suitable for KCE, and often these coatings require complex synthesis and lengthy derivitization processes [31–33].

Lucy and co-workers have recently introduced a semipermanent double-layer coating consisting of a surfactant bilayer and diblock polymer that may potentially satisfy the three listed conditions [34]. The first layer consists of dioctadecyldimethylammonium bromide (DODAB), a cationic surfactant, which forms a stable bilayer along the inner surface of the capillary. DODAB effectively masks the negatively charged silanol groups and produces a highly anodic EOF. The second layer is formed using a diblock polymer, polyoxyethylene (POE) stearate, which consists of a hydrophobic stearate block that inserts into the lipid bilayer and a hydrophilic block that projects into the capillary lumen. The magnitude of the EOF can be reduced by increasing the length of the polymer's hydrophilic block, which allows for the optimized separation of either acidic or basic proteins [35]. Lucy's main focus was to suppress the EOF to facilitate the highly efficient separation of a multitude of proteins with similar p*I*. The prospective separation of samples containing both acidic and basic analytes in combination with a tunable EOF motivated us to explore this coating as a possible solution to protein adsorption in KCE.

2 Materials and methods

2.1 Materials

Untreated fused-silica capillaries with inner diameter of 75 μm and outer diameter of 375 μm were purchased from Polymicro (Phoenix, AZ, USA). The cationic DODAB surfactant, the diblock polymer, POE-8 stearate, and all other chemical reagents were used as received from Sigma-Aldrich (Oakville, ON, Canada) unless stated otherwise. Buffer solutions were prepared in Milli-Q quality deionized water and filtered through a 0.22-μm filter (Millipore, Nepean, ON, Canada). Run buffers containing

Tris, N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), sodium tetraborate, and sodium dihydrogen phosphate were prepared from the powdered compounds. Either glacial acetic acid, phosphoric acid (Caldeon Laboratories, Georgetown, ON, Canada) or NaOH was used adjust buffer pH to the values indicated. Benzyl alcohol was donated by Prof. Michael Organ (York University) and used as a neutral EOF marker. Chromeo P503 pyrylium dye was obtained from Active Motif (Burlington, ON, Canada). Lyophilized conalbumin (chicken egg white) and lysozyme (chicken egg white) were purchased from Sigma Aldrich, and human-α-thrombin was obtained from Haematologic Technologies (Essex Junction, VT, USA). Human neutrophil gelatinase associated lipocalin (NGAL), a protein that aids in iron regulation by sequestering siderophores, was provided by Dr. Jonathan Barasch at Columbia University. The thrombin specific aptamer (5′-Alexa488-CGG TTG GTG TGG TTG GAA AAA AAA AAA AAA AAA AAA AAA A-3′) was ordered from Integrated DNA Technologies (Coralville, IA, USA) and used as a generic DNA model.

2.2 Instrumentation

All experiments were performed using a P/ACE MDQ CE instrument (Beckman-Coulter, Fullerton, CA, USA). Pressure-based experiments were performed using LIF detection mode. A 488-nm laser line was used to excite fluorescence of the Chromeo-labeled proteins and Alexa488-labeled DNA. Band pass filters specific for 637 and 520 nm fluorescence emission were used to detect Chromeo-labeled proteins and Alexa488-labeled DNA, respectively. A photo diode array detector was setup to detect light absorbance of benzyl alcohol at 214 nm to establish the EOF in each buffer type.

2.3 DODAB/POE stearate preparation and coating application

Separate solutions of DODAB and POE-8 stearate were prepared in deionized water to obtain final concentrations of 0.1 mM DODAB and 0.01% POE-8 stearate. Due to the limited solubility in water, each reagent was subjected to an analogous series of heated sonicate/stir cycles. The sonicator (Aquasonic 50T, VWR Scientific Products, Radnor, PA, USA) was first heated in an incubator (C24 Incubator Shaker, New Brunswick Scientific, Enfield, CT, USA) overnight to reach a temperature of 60°C. The solution was then sonicated within the temperature-controlled incubator to ensure minimal thermal variation. After 30 min of sonication, the solution was then transferred to a magnetic stirrer/hot plate (CERAMAG Midi, IKA, Wilmington, NC, USA) and gently stirred with heating for 30 min at 60°C. The sonicate/stir process was performed for a minimum of two repetitions, until clear surfactant and polymer solutions were produced.

A 50 cm bare silica capillary (40 cm to detection window) was first preconditioned with methanol and 0.1 M NaOH, sequentially each at a pressure of 20 psi for 10 min. A total of 0.1 mM DODAB was flushed through the capillary (10 min at 20 psi) to generate stable surfactant layer along the surface. The polymer layer was then administered by rinsing with 0.01% (w/v) POE-8 stearate at 20 psi for 10 min. Excess coating was then removed by flushing the capillary with the selected run buffer for 5 min at 20 psi. The temperature was controlled at 25°C throughout the entire coating procedure.

2.4 Protein labeling

A 4 mg/mL solution of conalbumin and lysozyme was prepared by dissolving the lyophilized protein in 0.1 M $NaHCO_3$ (pH 8.3) solution. A 1 μL volume of Chromeo P503 working solution was added to 100 μL of each protein solution. For NGAL and thrombin labeling, a 1:100 working solution of Chromeo P503 was made in 0.1 M $NaHCO_3$ (pH 8.3). Equal parts of the diluted Chromeo working solution and NGAL (stock 92 μM) were mixed to prepare the conjugation reaction. Thrombin was labeled by diluting the protein stock (240 μM) in the 1:100 Chromeo mixture to obtain an intermediary concentration of 48 μM. All labeling reactions were left to incubate overnight at 4°C to complete the conjugation. Proteins were then further diluted in 0.1 M $NaHCO_3$ (pH 8.3) to the final concentrations prior to experimentation.

2.5 Experimental conditions

For all pressure-driven propagation experiments, 42 nL of each sample was introduced into the DODAB/POE-8 stearate-coated capillary by applying a pressure of 0.5 psi for 7 s. The analyte was then carried either 10 or 40 cm to the detector using a 0.5 psi pressure in the reverse and forward direction, respectively. For EOF measurements, 2 mM benzyl alcohol was injected into the capillary inlet and electro-migrated with +15 kV when using 100 mM TES (pH 7.5), 50 mM Tris acetate (pH 8.2), and 25 mM borax (pH 9.3) run buffers and −15 kV when with the 50 mM sodium phosphate (pH 3.0) BGE. The EOF mobility was calculated as:

$$\mu_{EOF} = \frac{L_t L_d}{U t_m} \quad (1)$$

where L_t is the capillary total length, L_d is the distance from the capillary inlet to the detection window, U is the separation voltage, and t_m the migration time of benzyl alcohol.

2.6 NECEEM (nonequilibrium capillary electrophoresis of equilibrium mixtures) analysis

The thrombin-binding aptamer, concentrated at 1 μM, was heated to 95°C for 1 min using a thermal cycler (Eppendorf, Hamberg, Germany). The sample was gradually cooled to a

final temperature of 25°C at a rate of 0.5°C/s. The temperature treatment was performed as an "annealing" step to generate the proper aptamer structure. A mixture consisting of 320 nM thrombin and 67 nM thrombin binding aptamer was prepared in 20 mM Tris acetate (pH 8.2) supplemented with 5 mM KCl and 1 mM $MgCl_2$. The mixture was left to incubate at room temperature for 15 min to establish chemical equilibrium. The sample was injected into the capillary outlet (10 cm to the detector) using a reverse pressure of 0.5 psi for 7 s and propagated 5 cm past the inefficiently cooled capillary outlet by applying a reversed pressure of 0.3 psi for 1 min. The mixture was then separated by applying an electric field of 400 V/cm with the cathode positioned at the capillary outlet. The equilibrium dissociation constant (K_d) for the thrombin-aptamer binding pair was determined using the following equation:

$$K_d = \frac{[T]_0 \{1 + A_1/(A_2 + A_3)\} - [Apt]_0}{1 + (A_2 + A_3)/A_1} \quad (2)$$

where T_0 and Apt_0 are the initial concentrations of thrombin and aptamer present in the equilibrium mixture, respectively. A_1, A_2, and A_3 refer to the integrated the peak area for unbound aptamer (A_1), thrombin–aptamer complex (A_2), and aptamer dissociated from complex (A_3). All area values were first adjusted for variations in the electrophoretic velocity of free and protein-bound aptamer.

3 Results and discussion

The DODAB/POE stearate system was initially introduced as a stable semipermanent coating capable of enhancing the separation of proteins with similar electrophoretic mobility. The coatings resolving power can be ascribed to both its suppressed EOF and ability to reduce analyte adsorption along the capillary surface. The purpose of this work was to assess whether the DODAB/POE stearate coating is practical for reducing protein adsorption in KCE-based studies. Generally, when sample denaturation is not a concern, BGE's of very low pH are employed to optimize peak separation between cationic proteins. The pH range suggested by Lucy's group (pH 3–4) is well below the p*I* of basic proteins and all analytes should achieve a full positive charge. When a low pH BGE is combined with a highly reduced anodic EOF and normal electric polarity, the separation between proteins is maximized. However, these conditions conflict with the coating requirements 1 and 3 (see Section 1), as low pH will denature protein samples and inhibit complex formation, while a highly suppressed EOF may complicate KCE analysis.

To resolve the issue of protein denaturation, we selected three buffers commonly used in KCE methods in replacement of the low pH BGE: (i) 50 mM Tris acetate (pH 8.2), (ii) 25 mM borax (pH 9.3), and (iii) 100 mM TES (pH 7.5). Although the choice of buffer is a trivial step, this immediately satisfies the first condition with respect to the BGE.

It was previously demonstrated by MacDonald et al. [35] that when DODAB and POE stearate solutions are introduced into the capillary bore sequentially, the magnitude of the reversed EOF can be controlled. By varying the length of the polymer chain used to generate the second layer, the EOF is fine-tuned, accordingly. Long-chain polymers (i.e., POE-40 and POE-100 stearate) have proven to be quite effective in reducing the EOF, while shorter polymers (i.e., POE-8 stearate) tend to retain a considerable anodic flow at low pH values. We opted to use POE-8 stearate for further investigation given that the reported EOF was significantly stronger, and initially perceived as simpler to implement in KCE analysis.

To evaluate the antiadhesive properties of the DODAB/POE-8 stearate coating when associated with the three chosen physiological buffers, we selected five representative analytes as models. The analytes consisted of a commonly used ligand (a DNA aptamer) and four proteins known to interact with the bare capillary surface; (i) conalbumin (MW = 75.8 kDa, p*I* = 6.7), (ii) thrombin (MW = 36.7 kDa, p*I* = 7.0-7.6), (iii) lysozyme (MW = 14.6 kDa, p*I* = 11), and (iv) NGAL (MW = 24.9 kDa, p*I* = 8.4). The degree of analyte adsorption along the coated capillary when using either 50 mM Tris acetate (pH 8.2), 25 mM borax (pH 9.3), 100 mM TES (pH 7.5), or 50 mM sodium phosphate (pH 3.0) as the selected BGE was monitored using a recently introduced pressure-based, pseudo dual-detection technique [36]. Briefly, a small sample plug is injected into the capillary outlet and propagated 10 cm to the detector by applying a low reversed pressure of 0.5 psi. The experiment is then repeated using the same sample and capillary, however, the injection is introduced at capillary inlet and carried a longer distance (40 cm) to the detector using a forward pressure of 0.5 psi. Protein recovery during the 30-cm propagation can then be estimated by integrating the peak area and comparing the values obtained from the two migration distances. To increase the sensitivity of this technique, all proteins were fluorescently labeled with Chromeo P503. This enabled the use of much lower sample concentrations than otherwise necessary for UV detection [37–39].

Analyte propagations of 10 and 40 cm were performed in triplicates attaining a standard deviation in integrated peak area of less than approximately 15% between repeated trials (see Supporting Information Tables S1–S5, a and b for area and SD values). For the four model proteins and DNA, sample recovery and peak shape were used as indicators of adsorption to the DODAB/POE-8 stearate-coated capillary in each of the three physiological buffers. A sodium phosphate (pH 3.0) buffer served as antiadhesive control to which all other experimental data was compared. We implemented a two-tier ranking system where the extent of analyte adsorption was first estimated qualitatively, and provided that the adsorption was minimal, a semiquantitative approach was then employed. Peak width, symmetry, and the amount of tailing observed for both the 10- and 40-cm propagation distances was used to visually assess the extent of adsorption. Significant peak deformation combined with a rise in the baseline generally indicates a high-affinity surface interactions attributed to irreversible sample adsorption. A representative example of such

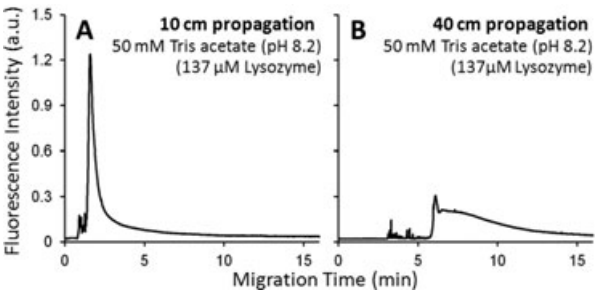

Figure 1. Representative electropherograms showing protein adsorption to the capillary wall during 10 cm (A) and 40 cm (B) pressure propagation trials. The diagram depicts 137 μM of Chromeo-labeled lysozyme in 50 mM Tris acetate buffer at pH 8.2.

Table 1. Antiadhesive ranking of DODAB/POE-8 stearate coating using selected background electrolytes of physiological pH and the sodium phosphate (pH 3.0) reference buffer by using percentage of sample recovered

Background electrolyte	Ranking				
	Conalbumin	Thrombin	NGAL	Lysozyme	Thrombin-binding aptamer
100 mM TES (pH 7.5)	1	3	2	1	1
50 mM Tris acetate (pH 8.2)	4	1	1	Adsorbed	1
25 mM Borax (pH 9.3)	3	2	1	2	1
50 mM sodium phosphate (pH 3.0)	2	1	1	1	1

a case is shown in Fig. 1 where 137 μM lysozyme was used as the protein sample and 50 mM Tris acetate (pH 8.2) as the physiological BGE. In such situations, the areas cannot be easily integrated due to excessive peak tailing (as indicated by exceedingly high SD in peak area, Supporting Information Table S5a–b) and are listed as "adsorbed" in the Table 1 ranking.

If the coating/electrolyte combination successfully suppressed the analyte–surface interaction, peaks appeared to be narrow, symmetrical, and well defined. In these cases, the extent of adsorption can be quantified through the percentage of analyte recovered during the 30 cm propagation by comparing peak area integration values at the 10 and 40 cm migration distances (detailed calculations are presented in Supporting Information Tables S1–S5, c). Our findings suggest that each analyte had demonstrated a unique response to the four buffers studied. Electrolytes were then ranked according to their antiadhesive capabilities toward each analyte, with the highest sample recovery classified as 1 (Table 1). Equal rankings were given to electrolytes whose efficiency and recovery capabilities were indistinguishable for the specified sample.

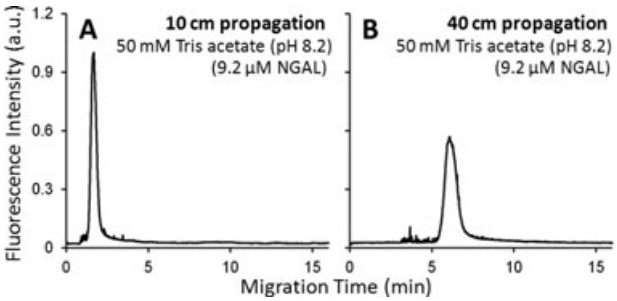

Figure 2. Representative electropherograms showing protein elution without adsorption to the capillary wall during 10 cm (A) and 40 cm (B) pressure propagation trials. The diagram depicts 9.2 μM of Chromeo-labeled NGAL in 50 mM Tris acetate buffer at pH 8.2.

It was found that the DODAB/POE-8 stearate combination is highly functional as an antiadhesive coating for the majority of proteins and electrolytes studied (see Supporting Information for pressure propagation figures). In all cases, protein adsorption was substantially reduced compared to similar runs performed in an untreated capillary [36]. Each of these model proteins had demonstrated severe adsorption to bare silica, which was conveyed either through excessive tailing, protein loss, or irreproducibility during the 10- and 40-cm propagation experiments (see Supporting Information Fig. S6 for NGAL and supporting information of Ref. [36] for conalbumin, lysozyme, and thrombin experiments). Figure 2 depicts the temporal pressure propagation profile of 9.2 μM NGAL at 10- and 40-cm distances using 50 mM Tris acetate (pH 8.2) in the DODAB/POE-8 stearate-coated capillary. In this particular set of experiments the percentage of sample recovery after 30 cm was found to be approximately 90% (Supporting Information Table S2c). This is a notable improvement from a similar experiment performed in an untreated fused-silica capillary, where NGAL was found to adsorb almost completely to the capillary surface (see Supporting Information Fig. S6).

For both NGAL and the thrombin specific DNA aptamer, the peak area and shapes were consistent during both the 40 and 10 cm propagations and each value was within the limits of the calculated SD (Supporting Information Figs. S3 and S5). Therefore, we can conclude that the amount of surface interaction is negligible for these two analytes regardless of the electrolyte identity or pH value. The other three proteins used in this study (conalbumin, thrombin, and lysozyme) had demonstrated an increased sensitivity to these two parameters, each imparting a distinct buffer preference. Lysozyme, in particular, had expressed a considerable degree of adsorption in all electrolytes, apart from 100 mM TES (pH 7.5) – which was shown to be quite effective (Supporting Information Fig. S4). Although the surface adhesion of both conalbumin and thrombin was found to be suppressed in each of the electrolytes when combined with the DODAB/POE-8 stearate coating, there are still notable distinctions that can be made in terms of peak area and percent recovery calculations for each tested buffer (Supporting Information

Figs. S1 and S2). These differences allowed us to accurately establish the rankings listed in Table 1 for these two proteins. It is evident that each analyzed protein had demonstrated a unique response to the three physiological electrolytes chosen in this study. As a result, it is not possible to generalize the coating performance according to the nature of the BGE, as the overall electrolyte efficiency depends largely on the proteins exposed amino acid residues and should be determined empirically. Factors such as electrolyte pH, the electromagnetic interactions with buffer ions, and hydrophobic effects are thought to collectively determine the extent of protein adsorption.

These results confirm that the DODAB/POE-8 stearate coating is capable of satisfying the second requirement when combined with a BGE of physiological pH. Moreover, since analyte interaction with the coated capillary surface was found to be negligible, we can further conclude that the coating itself does not interfere with the association and dissociation kinetics of noncovalent interactions studied with KCE. When performed with a BGE of physiological pH, our experimental results can determine whether the first coating requirement, with respect to both the coating and BGE, is satisfied.

To address the third criterion, we experimentally measured the EOF of the DODAB/POE-8 stearate-coated capillary in each described buffer using 2 mM benzyl alcohol as a migration standard. When using 50 mM sodium phosphate at pH 3.0, we obtained a fairly suppressed and anodic EOF consistent with the value previously reported by MacDonald et al. (-2.37×10^{-4} cm^2/Vs) [35] with 0.01% (w/v) POE-8 stearate. Our reversed EOF was moderately weaker (-1.82×10^{-4} cm^2/Vs), and can be attributed to either the slight variation in coating preparation, the solvent chosen for DODAB and POE-8 stearate dilution, or DODAB maturity [40]. In any case, the coated capillary appeared to be quite stable, and the minor standard deviation obtained between successive EOF measurements is indicative of a suitable capillary coating. When the pH of the electrolyte was increased to physiological values, the EOF reversed in its directionality (Table 2). This result was unexpected and suggests that the DODAB solution behaves differently as the pH is raised above 3.0 or when the identity of the buffer anion is changed [40].

In KCE, a typical EOF is in the range of 4×10^{-4} cm^2/Vs while the electrophoretic migration of a typical DNA molecule is greater than -2.5×10^{-4} cm^2/Vs. Under these standard conditions, both the complex and DNA ligand are able to reach the detector in less than 20 min. In the DODAB/POE-8 stearate coating at pH 8–9, DNA mobility is only slightly greater than that of the EOF, and the migration time to the detector would be in the order of hours. Although this may be beneficial for aptamer selection, it is generally unrealistic for simple kinetic studies. To reduce the separation time, one could potentially increase the polymer length, and replace POE-8 stearate with either POE-40 or POE-100 stearate when preparing the second layer. This would aid in masking the positively charged DODAB layer, and, thus, decrease the EOF. Alternatively, the separation method can be adapted in order to compensate for the prolonged migration times. To illustrate the simplicity of this modification, we performed a KCE method termed nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) [41] using a DODAB/POE-8 stearate-coated capillary with 50 mM Tris acetate (pH 8.2) employed as the separation buffer. An equilibrium mixture containing thrombin and its DNA aptamer was introduced into the coated capillary at the outlet end by applying a short pressure plug in the reversed direction. The mixture was then pressure propagated 5 cm past the inefficiently cooled region of the capillary to avoid sample overheating [42] leaving approximately a 5-cm migration distance to the detector. A voltage was then applied to induce sample separation with the anode placed at the capillary inlet. It can be clearly seen from Fig. 3 that both the aptamer and thrombin-aptamer complex reached the detector in less than 20 min, which is quite reasonable for KCE studies. In addition, the K_d obtained in the DODAB/POE-8 stearate-coated capillary is statistically equivalent to the value we had previously reported (95.7 ± 4.6 nM) for the thrombin-aptamer binding system using a PVA-coated capillary, which had also demonstrated negligible thrombin adsorption [36].

Table 2. EOF mobility of the DODAB/POE-8 stearate coating in each of the tested buffers

Background electrolyte	EOF × 10^4 (cm^2/Vs)
100 mM TES (pH 7.5)	0.84 ± 0.01
50 mM Tris acetate (pH 8.2)	1.69 ± 0.01
25 mM Borax (pH 9.3)	1.64 ± 0.01
50 mM Sodium phosphate (pH 3.0)	−1.82 ± 0.03

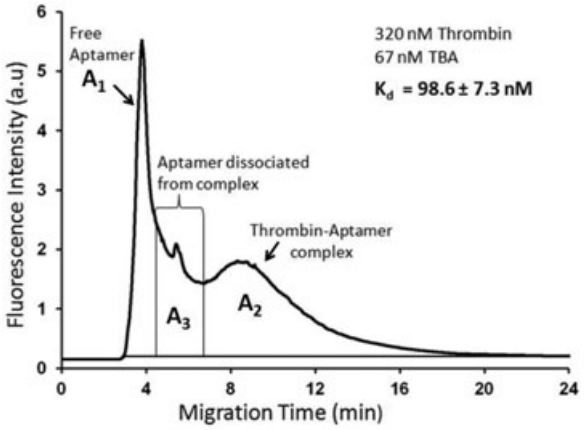

Figure 3. Electropherogram showing a NECEEM experiment utilizing the DODAB/POE-8 stearate to prevent protein adhesion. An equilibrium mixture containing 320 nM of thrombin and 67 nM thrombin binding aptamer was separated in a DODAB/POE-8 stearate-coated capillary with 50 mM Tris acetate (pH 8.2) employed as the run buffer. The sample was injected from the outlet end and pressure-propagated past the uncooled region so that the distance to the detection window was approximately 5 cm. The mixture was then separated using 20 kV and aptamer fluorescence was detected at 520 nm.

4 Concluding remarks

We describe, for the first time, a semipermanent capillary coating that is effective in preventing analyte adsorption for KCE methods. The DODAB/POE-8 stearate coating combined with a selected buffer of physiological pH is capable of satisfying all requirements needed for KCE compatibility. We proved that DODAB/POE-8 stearate suppresses adsorption of most analytes, does not inhibit complex formation, and maintains a reasonable EOF. The use of the DODAB/POE-8 stearate coating will help diversify the number of protein–ligand interactions studied by KCE. DODAB/POE-8 stearate-coated capillaries also have a potential use in KCE-based aptamer selection toward therapeutically important targets that would otherwise adsorb to untreated capillaries. Furthermore, this opportunity may assist in expanding the number of proteins used as targets for KCE-based aptamer selection.

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Supporting Information

A Semi-Permanent Coating for Preventing Protein Adsorption at Physiological pH in Kinetic Capilary Electrophoresis

Stephanie de Jong, Nicolas Epelbaum, Ruchi Liyanage and Sergey N. Krylov

Department of Chemistry and Centre for Research on Biomolecular Interactions, York University, Toronto, Ontario M3J 1P3, Canada

Influence of the background electrolyte and pH on analyte adsorption in the DODAB/POE-8 stearate coated capillary

In order to determine the extent of analyte-surface adsorption that occurred in the DODAB/POE-8 stearate coated capillary, we utilized a pressure-based sample propagation method with two virtual detection points that has been described previously (reference 13 in the main text). We chose 4 representative proteins which are known to experience strong affinity for the bare silica surface as well as a DNA aptamer given that it is a commonly used KCE ligand. The protein and DNA samples were first diluted in 0.1 M $NaHCO_3$ (pH 8.3) to obtain the following final sample concentrations: 5.3 μM conalbumin, 8.0 μM thrombin, 9.2 μM Neutrophil Gelatinase-Associated Lipocalin (NGAL), 137 μM Lysozyme and 100 nM thrombin specific aptamer. We evaluated the influence of background electrolyte pH on the amount of surface adhesion experienced by the sample in the DODAB/POE-8 stearate coated capillary. We selected four representative buffers, three of which are commonly used in KCE: (i) 25 mM borax (pH 9.3), (ii) 50 mM tris acetate (pH 8.2), and (iii) 100 mM TES (pH 7.5). The fourth run buffer, (iv) 50 mM sodium phosphate (pH 3.0), was of low pH and chosen as an anti-adhesive control. All previously reported research on DODAB/POE stearate coated capillaries had utilized electrolytes in this pH range, and 50 mM sodium phosphate (pH 3.0) is known to be quite effective in preventing adsorption. The analyte was injected into the DODAB/POE-8 stearate coated capillary by applying a low forward pressure of 0.5 psi for 7 s. The specified run buffer was then continuously introduced into the capillary with an applied pressure of 0.5 psi and functioned as a BGE. The sample was pressure-propagated until it reached the detector located at a 40 cm distance from the inlet. A second pressure-propagation experiment was then performed using the same capillary, sample and electrolyte; however, the analyte and buffer were each introduced at the capillary outlet using a reversed pressure of the same magnitude (0.5 psi) and carried 10 cm to the detection point. Each experiment was repeated in triplicates, achieving a standard deviation below 15% between repetitions, provided that the sample did not interact heavily with the capillary surface. Representative results illustrating the amount of protein adsorption in the DODAB/POE-8 stearate coated capillary are shown for each analyte and run buffer in Figs. S1, S2, S3, S4, and S5 below. Values obtained from the integrated peak areas at both 10 and 40 cm propagation distances along with the standard deviations are listed in Tables 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, and 5b below. The extent of analyte adsorption during the 30 cm migration was estimated by using the percentage recovery calculations presented in Tables 1c, 2c, 3c, 4c, and 5c.

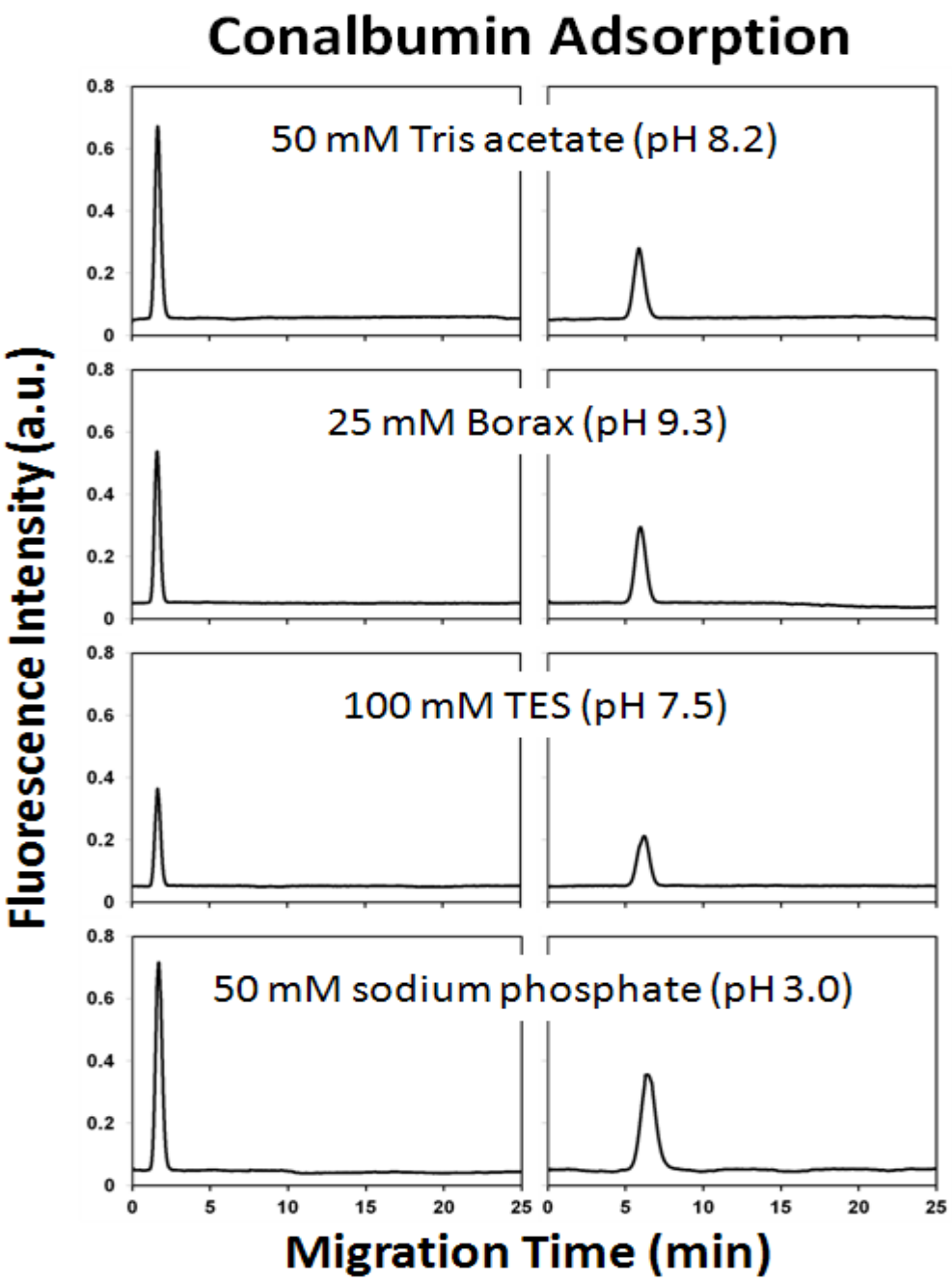


Figure S1. Temporal propagation patterns of 5.3 μM Chromeo-labeled conalbumin after 10-cm and 40-cm pressure-driven propagations using a DODAB/POE-8 stearate coated capillary using variable background electrolytes (BGE) (i) 50 mM tris-acetate (pH 8.2), (ii) 25 mM borax (pH 9.3), (iii) 100 mM TES (pH 7.5), and (iv) 50 mM sodium phosphate (pH 3.0).

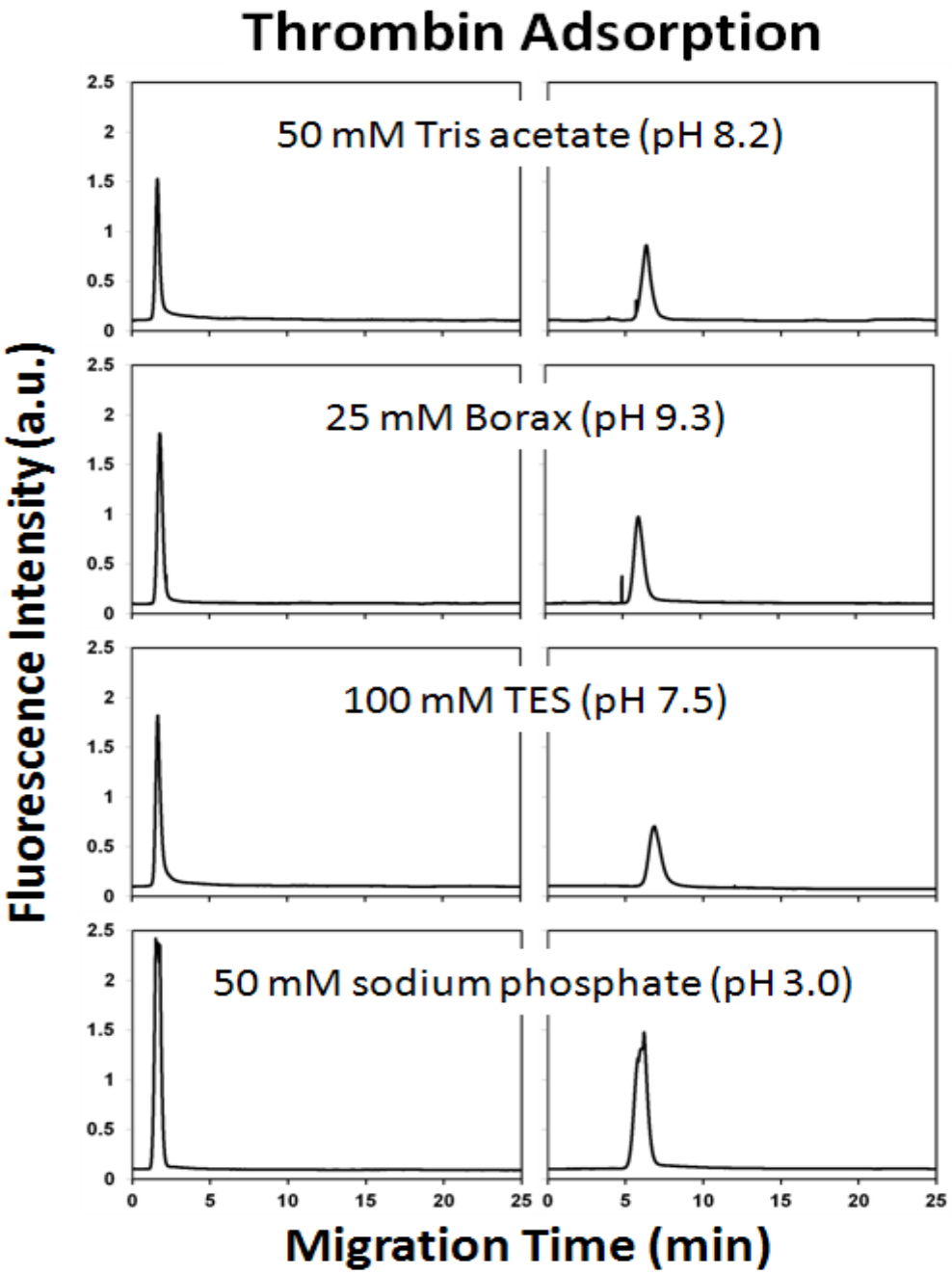


Figure S2. Temporal propagation patterns of 8.0 μM Chromeo-labeled thrombin after 10-cm and 40-cm pressure-driven propagations using a DODAB/POE-8 stearate coated capillary using variable background electrolytes (BGE) (i) 50 mM tris-acetate (pH 8.2), (ii) 25 mM borax (pH 9.3), (iii) 100 mM TES (pH 7.5), and (iv) 50 mM sodium phosphate (pH 3.0).

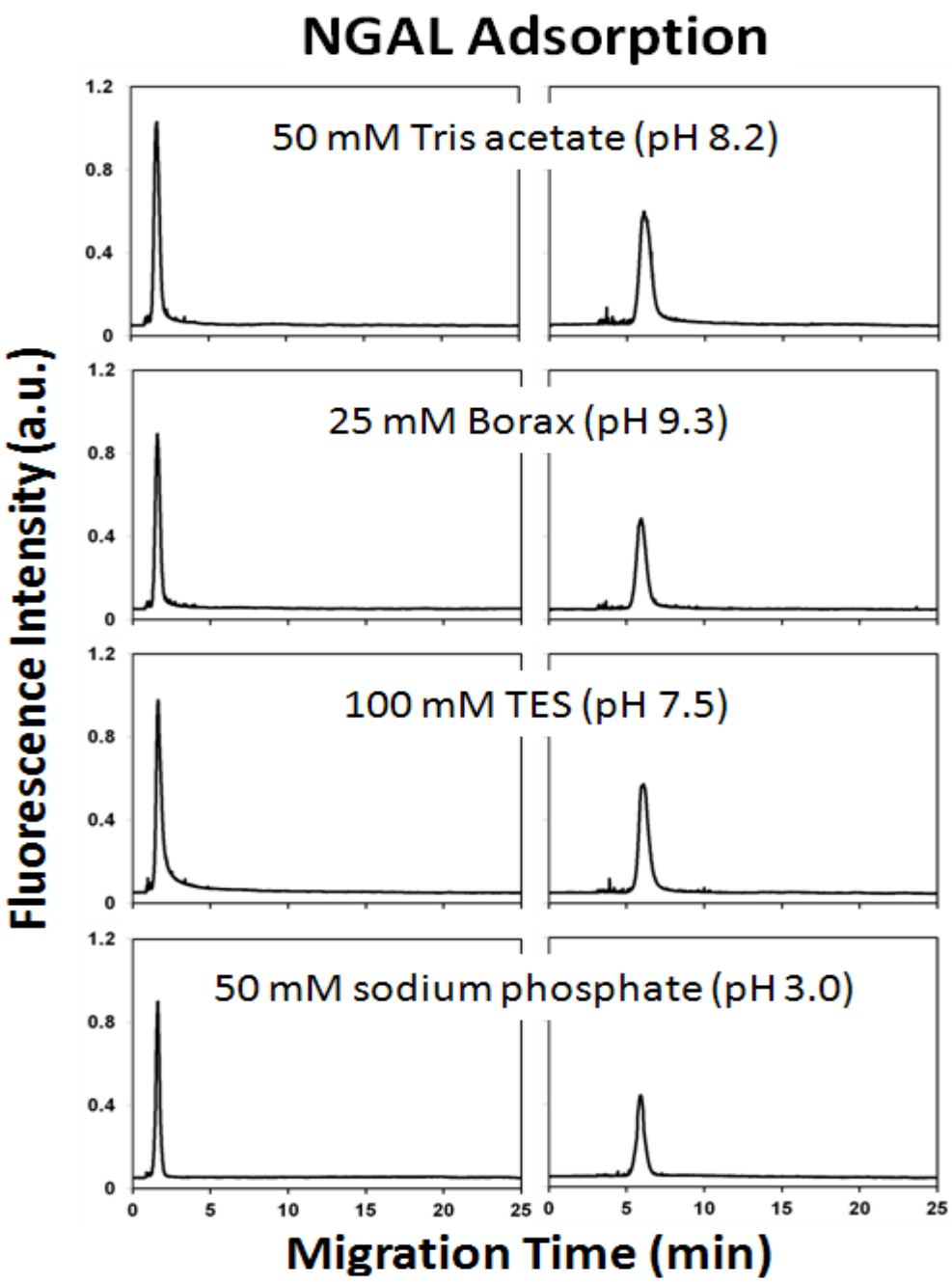


Figure S3. Temporal propagation patterns of 9.2 μM Chromeo-labeled NGAL after 10-cm and 40-cm pressure-driven propagations using a DODAB/POE-8 stearate coated capillary using variable background electrolytes (BGE) (i) 50 mM tris-acetate (pH 8.2), (ii) 25 mM borax (pH 9.3), (iii) 100 mM TES (pH 7.5), and (iv) 50 mM sodium phosphate (pH 3.0).

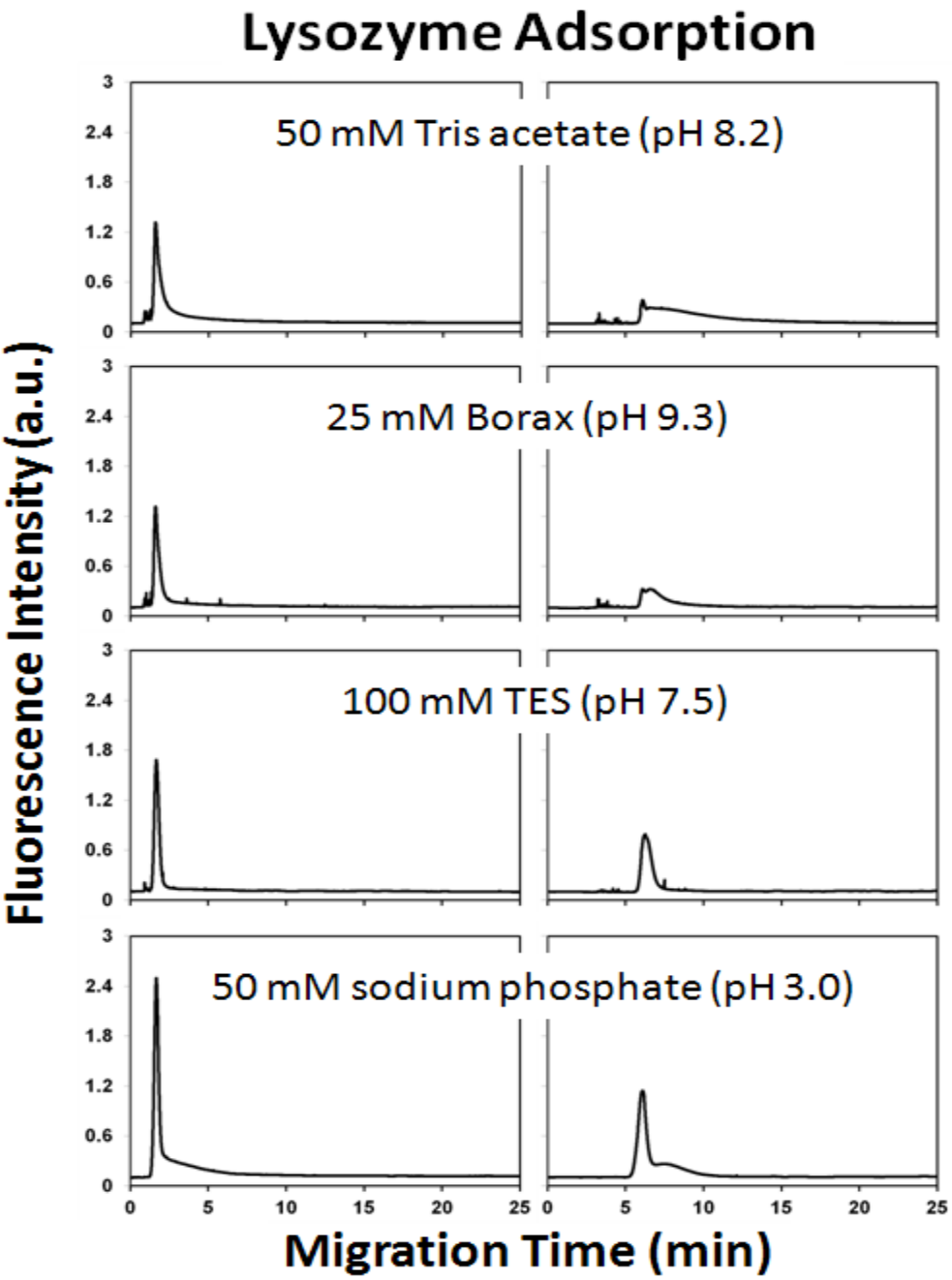


Figure S4. Temporal propagation patterns of 137 µM Chromeo-labeled lysozyme after 10-cm and 40-cm pressure-driven propagations using a DODAB/POE-8 stearate coated capillary using variable background electrolytes (BGE) (i) 50 mM tris-acetate (pH 8.2), (ii) 25 mM borax (pH 9.3), (iii) 100 mM TES (pH 7.5), and (iv) 50 mM sodium phosphate (pH 3.0).

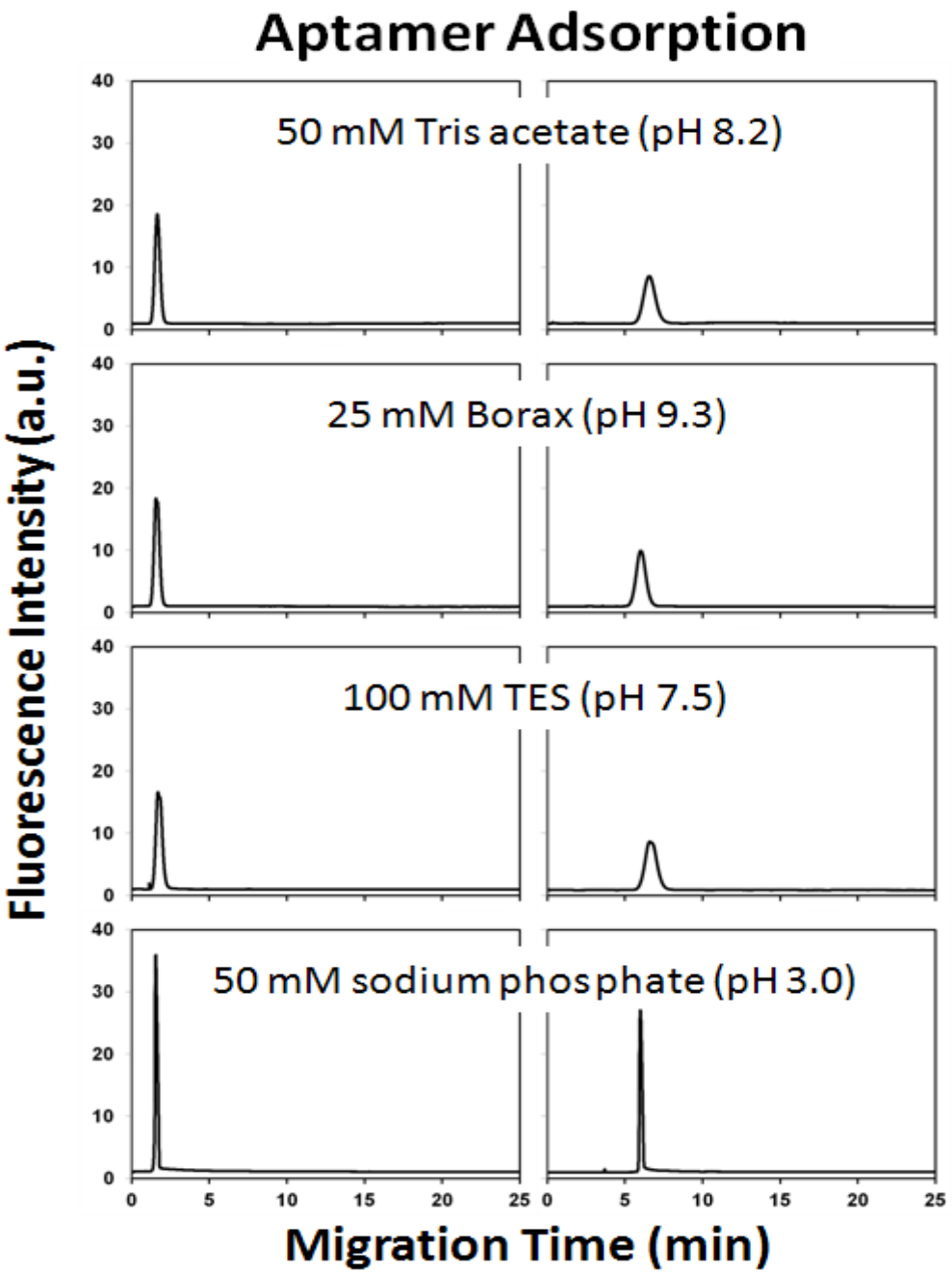


Figure S5. Temporal propagation patterns of 100 nM Alexa488-labeled thrombin binding aptamer after 10-cm and 40-cm pressure-driven propagations using a DODAB/POE-8 stearate coated capillary using variable background electrolytes (BGE) (i) 50 mM tris-acetate (pH 8.2), (ii) 25 mM borax (pH 9.3), (iii) 100 mM TES (pH 7.5), (iv) 50 mM sodium phosphate (pH 3.0).

[Table S1a] Area calculation for 5.3 μM conalbumin sample after 10 cm propagation

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	2006084	1957274	65497.43	3.35%
	2	1882838			
	3	1982900			
100 mM TES (pH 7.5)	1	817617	825909.3	7182.237	0.87%
	2	829944			
	3	830167			
50 mM tris acetate (pH 8.2)	1	1693299	1639693	49261.72	3.00%
	2	1629369			
	3	1596412			
25 mM borax (pH 9.3)	1	1474553	1320364	133786.9	10.13%
	2	1251525			
	3	1235013			

[Table S1b] Area calculation for 5.3 μM conalbumin sample after 40 cm propagation

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	1912995	1928248	135782.6	7.04%
	2	2071013			
	3	1800736			
100 mM TES (pH 7.5)	1	863056	838880	68625.83	8.18%
	2	761438			
	3	892146			
50 mM tris acetate (pH 8.2)	1	1156133	1134681	31079.48	2.74%
	2	1148870			
	3	1099039			
25 mM borax (pH 9.3)	1	1203553	1129007	66570.26	5.90%
	2	1107975			
	3	1075493			

[Table S1c] Percentage of 5.3 μM conalbumin recovered after a 30 cm propagation distance in the DODAB/POE-8 stearate coated capillary with the respective buffer

Buffer	Percent Recovery
50 mM sodium phosphate (pH 3.0)	99 ± 10 %
100 mM TES (pH 7.5)	102 ± 9 %
50 mM tris acetate (pH 8.2)	69 ± 6%
25 mM borax (pH 9.3)	86 ± 16 %

[Table S2a] Area calculation for 9.2 μM NGAL sample after 10 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	1567137	1571186	4778.657	0.30%
	2	1569964			
	3	1576457			
100 mM TES (pH 7.5)	1	2858390	2896888	187922.7	6.49%
	2	2731196			
	3	3101079			
50 mM tris acetate (pH 8.2)	1	3185467	2978667	180984.4	6.08%
	2	2901359			
	3	2849176			
25 mM borax (pH 9.3)	1	2180615	2108737	119518	5.67%
	2	1970770			
	3	2174826			

[Table S2b] Area calculation for 9.2 μM NGAL sample after 40 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	1523179	1645494	208571.5	12.68%
	2	1886322			
	3	1526982			
100 mM TES (pH 7.5)	1	2562506	2594344	28947.76	1.12%
	2	2619079			
	3	2601448			
50 mM tris acetate (pH 8.2)	1	2325852	2715882	395155	14.55%
	2	2705825			
	3	3115970			
25 mM borax (pH 9.3)	1	2094808	2142651	47723.95	2.23%
	2	2190255			
	3	2142889			

[Table S2c] Percentage of 9.2 μM NGAL recovered after a 30 cm propagation distance in the DODAB/POE-8 stearate coated capillary with the respective buffer

Buffer	Percent Recovery
50 mM sodium phosphate (pH 3.0)	105 ± 13%
100 mM TES (pH 7.5)	90 ± 8%
50 mM tris acetate (pH 8.2)	91 ± 21%
25 mM borax (pH 9.3)	102 ± 8%

[Table S3a] Area calculation for 8.0 μM thrombin sample after 10 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	6897542	6864694	138395.2	2.02%
	2	6712830			
	3	6983710			
100 mM TES (pH 7.5)	1	4423577	4665600	231106	4.95%
	2	4883970			
	3	4689252			
50 mM tris acetate (pH 8.2)	1	3898870	3600823	258954.3	7.19%
	2	3430983			
	3	3472616			
25 mM borax (pH 9.3)	1	4615710	4503915	101483.5	2.25%
	2	4417597			
	3	4478442			

[Table S3b] Area calculation for 8.0 μM thrombin sample after 40 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	6207593	6812831	524339.8	7.70%
	2	7129513			
	3	7101386			
100 mM TES (pH 7.5)	1	3316292	3331853	63349.05	1.90%
	2	3277735			
	3	3401533			
50 mM tris acetate (pH 8.2)	1	3470054	3687747	216626	5.87%
	2	3903290			
	3	3689897			
25 mM borax (pH 9.3)	1	6207593	6812831	524339.8	7.70%
	2	7129513			
	3	7101386			

[Table S3c] Percentage of 8.0 μM thrombin recovered after a 30 cm propagation distance in the DODAB/POE-8 stearate coated capillary with the respective buffer

Buffer	Percent Recovery
50 mM sodium phosphate (pH 3.0)	99 ± 8%
100 mM TES (pH 7.5)	71 ± 7%
50 mM tris acetate (pH 8.2)	102 ± 13%
25 mM borax (pH 9.3)	100 ± 6%

[Table S4a] Area calculation for 100 nM aptamer sample after 10 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	30480818	32816724	2870571	8.75%
	2	36021300			
	3	31948055			
100 mM TES (pH 7.5)	1	42737504	45008911	1967123	4.37%
	2	46134406			
	3	46154824			
50 mM tris acetate (pH 8.2)	1	42175283	42691702	1834902	4.30%
	2	44729476			
	3	41170347			
25 mM borax (pH 9.3)	1	41074688	42396311	1431427	3.38%
	2	43916755			
	3	42197490			

[Table S4b] Area calculation for 100 nM aptamer sample after 40 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	28687368	32035504	2908722	9.08%
	2	33940128			
	3	33479015			
100 mM TES (pH 7.5)	1	43159678	43301614	301193.7	0.70%
	2	43647552			
	3	43097613			
50 mM tris acetate (pH 8.2)	1	41788519	43478828	2959362	6.81%
	2	46895940			
	3	41752025			
25 mM borax (pH 9.3)	1	42706679	42660447	2225726	5.22%
	2	44862696			
	3	40411965			

[Table S4c] Percentage of 100 nM aptamer recovered after a 30 cm propagation distance in the DODAB/POE-8 stearate coated capillary with the respective buffer

Buffer	Percent Recovery
50 mM sodium phosphate (pH 3.0)	98 ± 18%
100 mM TES (pH 7.5)	96 ± 5%
50 mM tris acetate (pH 8.2)	102 ± 11%
25 mM borax (pH 9.3)	101 ± 9%

[Table S5a] Area calculation for 137 μM lysozyme sample after 10 cm propagation distance

Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	5985491	5950267	137629.2	2.31%
	2	5798449			
	3	6066861			
100 mM TES (pH 7.5)	1	4012065	3983023	37416.12	0.94%
	2	3940800			
	3	3996203			
50 mM tris acetate (pH 8.2)	1	3834332	4915203	984064	*20.02%*
	2	5152044			
	3	5759234			
25 mM borax (pH 9.3)	1	3974388	3930415	194789.8	4.96%
	2	3717397			
	3	4099459			

[Table S5b] Area calculation for 137 μM lysozyme sample after 40 cm propagation distance

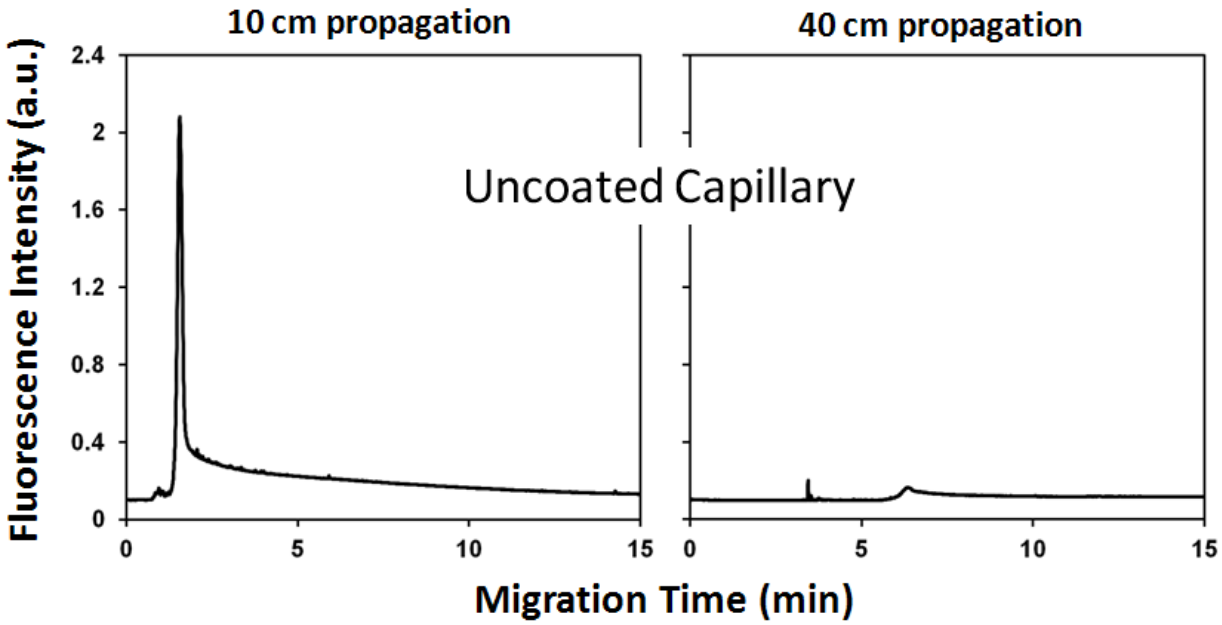
Buffer	Run #	Area	Average	Standard deviation (SD)	Average/SD
50 mM sodium phosphate (pH 3.0)	1	5670258	5739829	136140.2	2.37%
	2	5896697			
	3	5652533			
100 mM TES (pH 7.5)	1	3890077	3756399	221285.2	5.89%
	2	3878147			
	3	3500974			
50 mM tris acetate (pH 8.2)	1	4657537	5024353	3240621	*64.50%*
	2	8432774			
	3	1982747			
25 mM borax (pH 9.3)	1	3063922	2710647	321671.3	11.87%
	2	2633356			
	3	2434662			

[Table S5c] Percentage of 137 μM lysozyme recovered after a 30 cm propagation distance in the DODAB/POE-8 stearate coated capillary with the respective buffer

Buffer	Percent Recovery
50 mM sodium phosphate (pH 3.0)	96 ± 5%
100 mM TES (pH 7.5)	94 ± 7%
50 mM tris acetate (pH 8.2)	*102 ± 85%*
25 mM borax (pH 9.3)	69 ± 17%

Pressure-driven temporal propagation profile of NGAL using a bare silica capillary

The extent of NGAL adhesion onto an uncoated fused silica capillary was estimated by performing a set of pressure driven propagation experiments at two different detection distances, 10 cm and 40 cm. A sample containing 4.6 μM Chromeo-labeled NGAL was introduced into the capillary inlet by applying a pressure of 0.5 psi for 7 s, and then carried 40 cm to the detection point at a constant forward pressure of 0.5 psi in a 50 mM tris acetate (pH 8.2) run buffer. The experiment was then repeated by injecting the protein sample at the capillary outlet and propagated a 10 cm distance to the detector in a similar approach by applying a reversed pressure of 0.5 psi. By observing the results displayed in Figure S6, it is immediately evident that severe surface adsorption exists under these conditions.



[Figure S6] Pressure driven temporal propagation of 4.6 μM NGAL across a 10 cm and 40 cm distance in an uncoated fused silica capillary using 50 mM tris acetate (pH 8.2) as the background electrolyte. NGAL fluorescence was excited using a 488 nm solid state laser line and detected through a 610 nm filter.

NECEEM analysis of thrombin-aptamer binding system using DODAB/POE-8 stearate coated capillary

The NECEEM experiment was performed as indicated in the main text. However an unexplained peak appeared in the decay region, indicated in Figure S6. We attribute this peak to either DNA degradation or DNA impurity. When we performed the negative control experiment which consisted of 67 nM TBA diluted in the incubation buffer (20 mM Tris-acetate (pH 8.2); 5 mM KCl; 1 mM $MgCl_2$) without the addition of thrombin we also observed this a peak which reached the detector with similar migration time (shown in inset of Fig. S6).

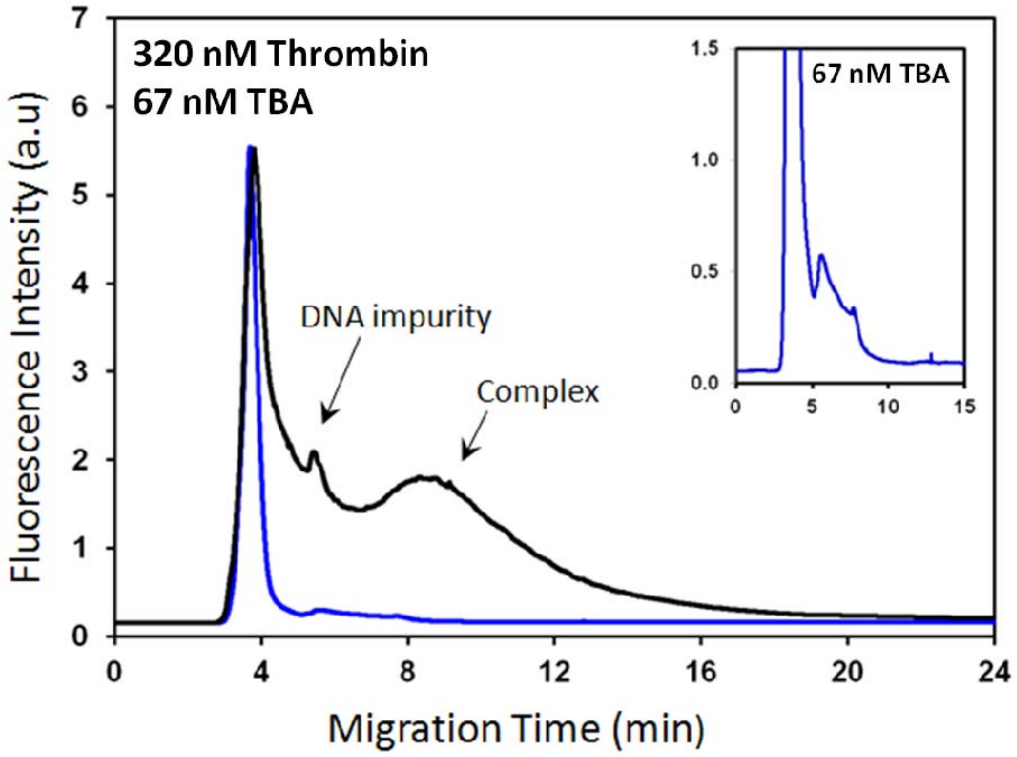


Figure S7. NECEEM electropherogram obtained in the DODAB/POE-8 stearate coated capillary with a 50 mM tris-acetate (pH 8.2) BGE. The equilibrium mixture consisted of 320 nM thrombin and 67 nM TBA, which was injected from the sample outlet using 0.5 psi reversed pressure and separated by applying an electric field of 400 V/cm under normal polarity (results shown in black trace). The control experiment (blue traces) was performed in the same manner with thrombin omitted from the equilibrium mixture.