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Protein Labeling Enhances Aptamer Selection by Methods of Kinetic Capillary Electrophoresis

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S *Supporting Information*

ABSTRACT: Methods of kinetic capillary electrophoresis (KCE) facilitate highly efficient selection of DNA aptamers for protein targets. The inability to detect native proteins at low concentrations in capillary electrophoresis creates, however, a significant obstacle for many important protein targets. Here we suggest that protein labeling with new Chromeo dyes can help to overcome this obstacle. By labeling a number of proteins with Chromeo P503, we show that the labeling procedure enables accurate detection of proteins in CE without significantly affecting their electrophoretic mobility or their ability to bind DNA. Moreover, Chromeo P503 does not appear to label the amino-groups of buffer components to a significant extent, making the labeling procedure compatible with a large number of selection and run buffers. Fluorescent labeling of protein targets with Chromeo dyes empowers selection of aptamers by KCE methods and promises to increase the rate at which aptamers for new targets are being developed and introduced in various applications.
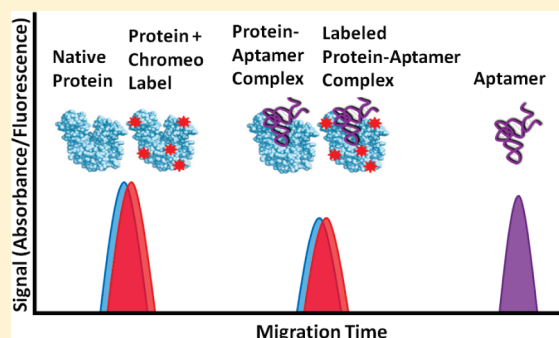
Aptamers are small ssDNA (RNA) oligonucleotides which can bind their targets with high selectivity and affinity.[1,2] Their molecular recognition capacity has been shown to parallel that of antibodies,[3] enabling the use of aptamers in several important applications as affinity probes, drug candidates, and drug delivery vehicles.[4−6] Aptamers are selected from large libraries of random DNA sequences using the following iterative process. First, the library is combined with the target and left to incubate, allowing for suitable DNA sequences to bind. Then the target-bound DNA is isolated from the unbound DNA in a partitioning step. Because of limited separation efficiency, the partitioning step typically needs to be repeated multiple times to obtain aptamers with the desired binding parameters. Moreover, partitioning leads to unavoidable aptamer-loss due to collection inefficiencies associated with the separation technique. As a result, PCR amplification is typically needed after each partitioning step in order to compensate for the DNA loss. This general procedure which involves multiple rounds of alternating aptamer partitioning and PCR amplification is referred to as systematic evolution of ligands by exponential enrichment (SELEX).[7] The development of highly efficient partitioning methods is critical, as this will increase the rate at which aptamers are selected for new targets.

Kinetic capillary electrophoresis (KCE) is a conceptual platform for homogeneous kinetic affinity methods which can, in particular, facilitate highly efficient partitioning of aptamers.[8] One to four rounds of KCE-based partitioning is generally sufficient to complete the selection process,[9] while other partitioning methods typically require more than 10 rounds. The high efficiency of KCE methods allows the intermediary PCR steps to be excluded during aptamer selection, by an approach entitled

Non-SELEX.[10] In addition, KCE methods uniquely facilitate selection of smart aptamers, aptamers with predefined binding parameters, which include the equilibrium and rate constants of interaction between the aptamer and target.[11] KCE-based aptamer selection can be optimized in three distinct steps: (i) finding conditions under which the protein does not interact with the capillary inner wall, (ii) maximizing the separation between the target protein and the DNA library, and (iii) determining the aptamer-collection window (Figure 1).

To perform these three steps of optimization, both the DNA library and protein target need to be detected. DNA can be easily tagged with a fluorescent label while maintaining its native electrophoretic properties; therefore, both UV absorption and laser induced fluorescence can be used for library detection. As for the protein, early works on fluorescent labeling demonstrated peak broadening and a significant shift in CE migration times, making the protein labeling procedure unsuitable for optimizing KCE-based aptamer selection.[12] Therefore, only a single means of protein detection, light absorption in UV, has been used to facilitate the selection process. This creates problems in all three steps of optimization. Light absorption has a poor detection limit in CE due to the short optical path-lengths of approximately 0.1 mm. Because of the multistep nature of protein purification, many proteins are unavailable at the detectable concentration of $\sim 10^{-5}$ M or higher. In these cases, optimization steps 1 and 2 cannot be carried out using detection by UV absorbance.
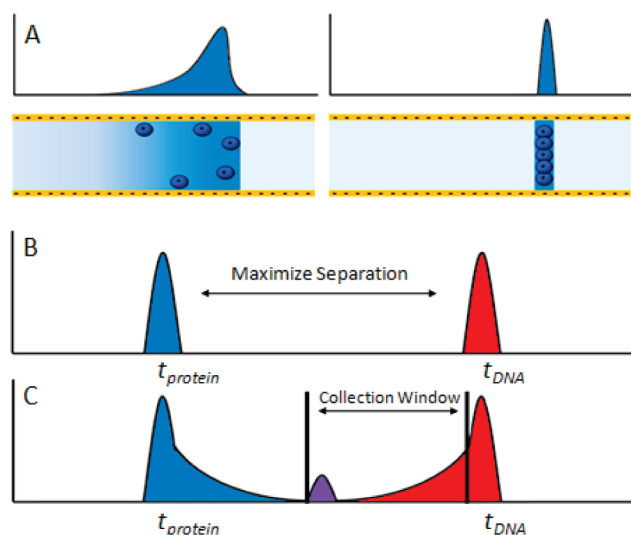
Figure 1. Schematic illustration of the three steps of optimization in KCE-based aptamer selection: prevention of protein adsorption to the walls (A), maximizing the protein−DNA separation window (B), and determination of the aptamer-collection window (C).

Table 1. Limits of Detection of Unlabeled and Chromeo-Labeled Proteins Detected with UV Absorbance and Fluorescence, Respectively

	limit of detection, nM	
protein	UV absorbance	fluorescence
AGP	2600	3
BSA	500	2
HSA	400	1
myoglobin	3100	120
α-lactalbumin	1500	60

The inability to detect the protein can also present a challenge in step 3, which typically relies solely on the fluorescently labeled DNA library. Under ideal circumstances, the labeled DNA is sufficient to detect both the complex and DNA, which will accordingly establish the aptamer collection window (see Figure 1C). However, if the protein has nuclease activity, the library may become partially degraded during the incubation period and these products of degradation can be confused with the complex (see the Supporting Information, Figures S1 and S2). It is also possible that the complex peak remains undetected during the bulk affinity assay even at the highest available target concentrations. In these situations, the mobility of the pure protein is used as a reference for boundary determination, which again relies on UV absorbance.

If not optimized, KCE-based aptamer selection has a much lower chance of success. The goal of this work was to find a means of fluorescently labeling the protein target so that it is compatible with KCE-based aptamer selection. Labeling reagents typically react with the positively charged ε-amine groups on the exposed lysine residues and alter the protein's charge upon conjugation. If the proteins charge-to-size ratio is affected by the labeling procedure, its corresponding electrophoretic velocity will be shifted making the migration time unreliable. A new series of
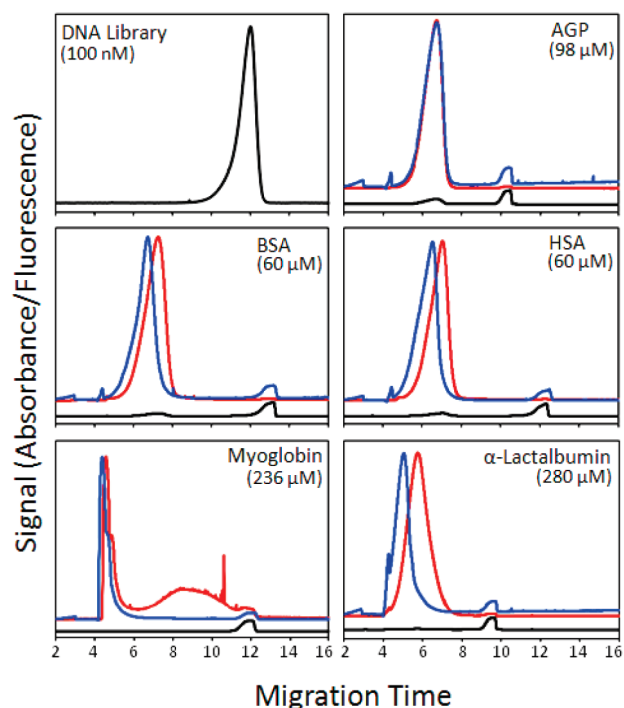

Figure 2. Effect of Chromeo labeling on protein mobility in CE. Unlabeled proteins were detected by UV absorbance at 214 nm using 50 μM fluorescein as a migration standard (blue traces). Chromeo-labeled proteins were prepared at the same concentrations which were used for UV absorbance detection. Fluorescence of labeled proteins was excited by a 488 nm solid-state laser and detected using a 610 nm filter (red traces). Fluorescence of the migration standard, 10 nM fluorescein, and the DNA library were excited using the same 488 nm laser and detected through a 520 nm filter (black traces).

fluorescent dyes, termed Chromeo, have recently become commercially available. These dyes can facilitate fluorogenic labeling without changing the protein's charge during the process. Dovichi and coauthors demonstrated that Chromeo dyes can be successfully used in capillary isoelectric focusing, as the protein's pI is maintained.[13,14] While not suggesting that protein mobility in CE would remain unaffected, this work had motivated us to explore this opportunity. Our results proved that Chromeo labeling of protein can facilitate the optimization of KCE-based aptamer selection for proteins.

RESULTS AND DISCUSSION

Ideally, the labeling would have three properties. First, it cannot significantly shift the protein's migration with respect to the time-window chosen for aptamer collection. Second, labeling should not prevent protein−DNA binding. Third, the labeling reaction should be compatible with commonly used amino-containing buffers such as Tris, glycine, etc. The goal of our work was to test these three properties of protein labeling using a dye from the Chromeo family. We chose Chromeo P503 to test this hypothesis given that its quantum yield increases by a factor of 50 when bound to the protein. This property effectively eliminates any background signal, allowing for sensitive detection. Chromeo P503 offers additional advantages over other Chromeo dyes, attributed to its unique spectral properties. The excitation wavelength at 503 nm is similar to both the FAM and Alexa488-labeled DNA libraries, while its emission is shifted to a
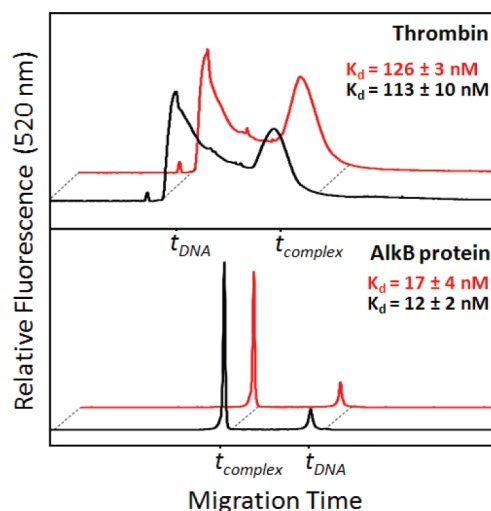
Figure 3. Influence of Chromeo-labeling on aptamer binding and affinity for thrombin (upper panel) and AlkB protein (lower panel). NECEEM binding assays were performed by using equilibrium mixtures containing labeled aptamer and either labeled (red lines) or unlabeled (black lines) protein. The concentrations of thrombin and its aptamer were 0.5 and 0.1 μM, respectively. The concentration of AlkB protein and its aptamer were 80 and 30 nM, respectively. Only DNA is detected when fluorescence is measured at 520 nm.

Figure 4. Peak identification of the thrombin—aptamer complex through the combined use of Chromeo-labeled thrombin and FAM-labeled aptamer. Black traces depict fluorescence at 520 nm while the red traces represent fluorescence at 610 nm. Upper panels show NECEEM electropherograms obtained with native thrombin; lower panels show NECEEM electropherograms obtained with Chromeo-labeled thrombin. The left panels show the measured signals, while the right panels show the same data with the red electropherograms rescaled to have the heights of the left peaks identical for black and red electropherograms. The concentrations of thrombin and aptamer were 0.5 and 0.1 μM, respectively.

much longer wavelength. This would allow both the protein and DNA to be excited simultaneously by the same laser source yet detected through different channels by using the appropriate filters.

We first confirmed that by fluorescently labeling proteins with Chromeo P503, detection in CE is significantly improved compared to light-absorbance of the native protein. Because of the significant variations that exist in the physical—chemical properties of the proteins, we decided to analyze a series of proteins as models; bovine serum albumin (BSA), human serum albumin (HSA), α_1-acid glycoprotein (AGP), myoglobin, and α-lactalbumin, which would allow us to make reliable conclusions with a reasonable power of prediction. Our results shown in Table 1 suggest that the lower limit of detection improves (for the five proteins studied) from an average value of 1620 to 35 nM when switching from UV light absorbance of unlabeled proteins to fluorescence detection of labeled proteins (see also the Supporting Information). This detection limit is suitable for KCE-based aptamer selection since proteins are typically available, and often used, at concentrations higher than 35 nM. Thus, for the majority of proteins, Chromeo labeling can facilitate the optimization of aptamer selection provided that the label does not influence the protein's electrophoretic mobility to a great extent.

In order to quantify the mobility shift attributed to the Chromeo label, we measured the difference in the migration times of native and Chromeo-labeled proteins with respect to the separation window between the protein and DNA library. These experiments were performed using the five previously listed proteins, as they were available at concentrations high enough to produce a reliable signal when using UV absorbance for detection. The top left panel in Figure 2 shows the mobility of the library; the remaining panels demonstrate the CE migration of the unlabeled and Chromeo-labeled proteins, detected using UV absorbance and laser induced fluorescence (LIF), respectively. For each protein tested, only a marginal
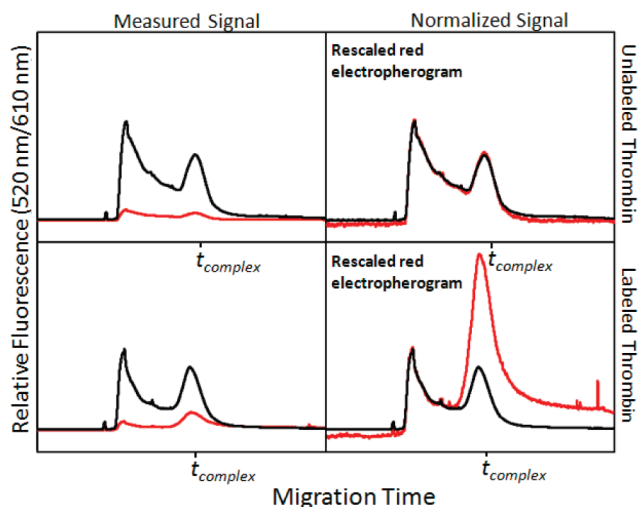
shift in electrophoretic mobility was observed following the labeling reaction. α-Lactalbumin expressed the greatest shift in mobility upon labeling, which was found to be approximately 10% of the protein-library separation window. Moreover, Chromeo P503 does not lead to labeling products with heterogeneous mobility since all labeled proteins migrate as individual peaks with widths similar to those of the native proteins, a result which could not be obtained by FQ-labeling.[12] From these results, we conclude that Chromeo-labeled proteins can be used in the first two steps of optimization for KCE-based aptamer selection.

For step 3 of the optimization process (determining the aptamer selection window), the labeling process should not significantly perturb protein—DNA binding. Figure 3 illustrates how protein labeling influences aptamer binding by using two proteins for which aptamers were readily available: thrombin[15] and AlkB.[16] These binding studies were performed by non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) which can be used to determine equilibrium (K_d) and rate (k_{off}) constants of the protein—aptamer complex dissociation.[17] With the use of a 520 nm filter, only the fluorescence emitted by the DNA library was detected and shown in each trace. We can easily see that the NECEEM electropherograms are similar for both the Chromeo-labeled and unlabeled proteins in both aptamer/protein models. This result was unexpected but is not counterintuitive. Indeed, while labeling can affect biomolecular interactions, the wide use of fluorescently labeled antibodies suggests that when it comes to binding other molecules, proteins have a relatively high tolerance toward the labeling of a limited number of residues. Our observation certainly does not prove that Chromeo labeling will be this inert to all protein—aptamer pairs. However, during step
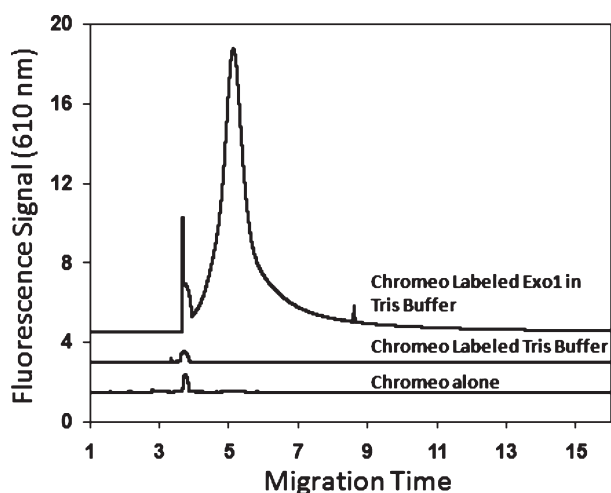
Figure 5. Negligible effect of amino-containing buffer components on fluorescence signal from Chromeo-labeled Exo 1 protein. The top trace shows the electropherogram obtained from Chromeo-labeled Exo1, which was stored in a 10 mM TrisHCl buffer. The middle trace is a control illustrating how this Tris buffer reacts with the Chromeo dye. The bottom trace is a second control which illustrates the peak obtained from the Chromeo dye alone.

3 of optimization, which relies on the interaction of the protein with the entire library (so-called bulk affinity assays), the influence of Chromeo labeling is expected to be negligible for the majority of proteins. The library provides an incredibly large number of DNA structures and even if labeling prevents the interaction of some structures, it may equally increase the protein's affinity to others. Since the bulk affinity assay is done prior to the collection and amplifications steps, the labeled protein can be used initially for optimization and predictive purposes while the unlabeled protein is subsequently used for aptamer selection. This approach would enhance the selection process and ensure that the aptamers will bind tightly to the native protein. Therefore, it is possible to use Chromeo-labeled proteins in step 3 of the optimization process.

We then showed that Chromeo labeling can help to correctly identify the protein—DNA complex from other unknown peaks such as library degradation products. This distinction can be made by simultaneously detecting the protein and DNA at two different wavelengths. Both the Chromeo-labeled protein and Alexa-labeled DNA can be excited using the same 488 nm laser line; however, their emission wavelengths are unique, 610 nm for the protein and 520 nm for the DNA. Thus, with the use of a two channel detection system, the fluorescence emitted from both the DNA and protein can be separated into two distinct electropherograms. The principle of complex distinction is based on the fact that the protein—DNA complex will fluoresce at both wavelengths, while any peaks attributed to DNA degradation or impurities would fluoresce only at a single wavelength (unless bound to the protein). To illustrate this approach, we conducted an experiment in which we used either Chromeo-labeled or unlabeled thrombin and its Alexa-labeled aptamer, using fluorescence detection at both 520 and 610 nm. NECEEM experiments were conducted in which we expect to observe two main peaks: unbound aptamer and protein-bound aptamer.

When the unlabeled protein is used in the equilibrium mixture (upper panels in Figure 4), only DNA fluoresces and ideally we

expect to see no signal in the 610 nm channel. However, the channel picks up the tail of Alexa fluorescence from the DNA and records a signal similar to that in the 520 nm channel but at a much lower intensity. The similarity can be confirmed by normalizing the signals from the two channels; the normalized signals are identical. The right-hand side peak is tentatively assigned to the protein—DNA complex but it may potentially be a product of DNA degradation that migrates differently from intact DNA.

To test whether or not the assignment is right, we use the Chromeo-labeled thrombin (lower panels in Figure 4). In this case, the signals at the two wavelengths are not similar; the right-hand side peak is higher relative to the left-hand side peak in the 610 nm channel. This becomes very obvious when the signals are normalized. Both the Chromeo label of the protein and the Alexa label of the DNA contribute to this peak while the left peak is solely the spectral tail of DNA fluorescence. This allows us to accurately conclude that the right-hand side peak is the complex and not any other product such as aptamer degradation. This simple model experiment proved that Chromeo labeling of the protein can facilitate the simple identification of the protein—aptamer complex from all other possible peaks. This distinction is crucial during the determination of the aptamer-selection window, step 3 in the optimization process, and throughout the course of selection.

Aptamer selection by KCE methods is often conducted in amino-group containing buffers, such as Tris and glycine. Since Chromeo dyes react with primary and terminal amino groups present on the proteins, they can potentially react with amino groups present in buffer components. The labeled buffer can, in turn, interfere with protein detection. To test this potential problem, we performed a labeling reaction using Exo1, a protein that was stored in a Tris-containing buffer. With comparison of the electropherogram of the labeled protein to that of the labeled storage buffer containing the Tris-component, the migration of the pure protein can be inferred. It is evident from Figure 5 that the Tris buffer, which is present at a much higher concentration than the Exo1, produced a much smaller peak relative to the protein. This suggests that Chromeo-labeling is compatible with amino-containing buffers.

■ CONCLUDING REMARKS

To conclude, we proved that labeling proteins with Chromeo can facilitate sensitive detection of proteins in CE and does not significantly affect protein mobility or DNA-binding properties. Along with low reactivity toward amino-groups present in buffer solutions, these findings make Chromeo labeling an important addition to the tool-box of KCE-based methods for aptamer selection. This new opportunity promises to significantly increase the rate of success in KCE-based aptamer selection and thus contribute to further development of aptamer-based diagnostic and therapeutic tools.

■ MATERIALS AND METHODS

Materials. Uncoated fused-silica capillaries with 75 μm inner diameter (375 μm outer diameter) were purchased from Polymicro (Phoenix, AZ). Chromeo P503 pyrylium dye was purchased from Active Motif (Burlington, ON, Canada). AlkB and Exo1, both native to *E. coli* were provided by Oxford University and Oxford Nanopores, respectively. Human-α-thrombin was

purchased from Haematologic Technologies Inc. (Essex Junction, VT). Bovine serum albumin (BSA), human serum albumin (HSA), α_1-acid glycoprotein (AGP), myoglobin, α-lactalbumin, and all other chemical reagents were purchased from Sigma Aldrich (Oakville, ON, Canada). The HPLC-purified AlkB aptamer (5′-fluorescein-TGC CTA GCG TTT CAT TGT CCC TTC TTA TTA GGT GAT AAT A-3′) and thrombin aptamer (5′-Alexa488-CGG TTG GTG TGG TTG GAA AAA AAA AAA AAA AAA AAA AAA A-3′) were purchased from Integrated DNA Technologies (Coralville, IA). The DNA was dissolved in 10 mM TrisHCl (pH 7.5) at a high stock concentration, which was then stored at −20 °C until use. All other solutions were prepared in deionized water and filtered through a 0.22 μm filter (Millipore, Nepean, ON, Canada).

Instrumentation. CE experiments were conducted using a P/ ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA) equipped with either a photodiode array (PDA) or laser induced fluorescence (LIF) detector. A 488 nm solid-state laser was used to excite Alexa-488/FAM labeled DNA and Chromeo labeled proteins. A two-channel detection system was implemented in order to detect both the protein and DNA simultaneously. A 520 nm filter, specific for the fluorescently labeled DNA, was placed in one channel, while a 610 nm filter, specific for the Chromeo-labeled proteins, was used in the second channel.

Electrophoresis Conditions. The separation buffer used in all CE experiments was 50 mM Tris-acetate (pH 8.2). Prior to each run, the uncoated capillary was conditioned by rinsing with 0.1 M HCl, 0.1 M NaOH, ddH$_2$O, followed by 50 mM Tris-acetate (pH 8.2) using a pressure of 137.9 kPa for 2 min. The polyvinyl alcohol (PVA) coated capillary was rinsed with ddH$_2$O and 50 mM Tris-acetate (pH 8.2) at 68.9 kPa for 4 min. A total of 50 nL of the sample was introduced into the capillary using a 3.4 kPa pressure pulse for 10 s. The cartridge temperature was controlled at 15 °C for each run.

Chromeo P503 Labeling. Proteins that were available in lyophilized powder form (BSA, HSA, AGP, myoglobin, and α-lactalbumin) were dissolved in 0.1 M NaHCO$_3$ (pH 8.3) to a concentration of 4 mg/mL. The protein solution was divided into two 200 μL aliquots, one of which was labeled with 2 μL of Chromeo P503 working solution. The protein solution was left to incubate at room temperature for 30 min to complete the conjugation reaction, which was observed by a color change from blue to red.

Thrombin, AlkB, and Exo1 were labeled by diluting the protein stock in a 1 to 100 mixture containing the Chromeo P503 working solution and 0.1 M NaHCO$_3$ (pH 8.3), respectively. The final concentration of labeled protein was 10 μM for thrombin and AlkB and 5.1 μM for Exo1. The solution was left to incubate overnight at 4 °C to maintain protein structure and activity. The Chromeo labeled-Tris buffer was prepared in a similar manner to Exo1; however, the storage buffer containing 10 mM TrisHCl (pH 8.0) was used in replace of the protein.

Migration Shift of Labeled Proteins. The unlabeled protein solution and 50 μM fluorescein, diluted in 0.1 M NaHCO$_3$ (pH 8.3), were coinjected into a 50 cm uncoated capillary (75 μm i.d.). The sample was then separated using 20 kV and detected with a PDA detector at 214 nm. The labeled protein and 10 nM fluorescein, diluted in 0.1 M NaHCO$_3$ (pH 8.3), were coinjected into the same 50 cm capillary, separated using 20 kV and detected by LIF through both the 520 and 610 nm channels.

NECEEM-Analysis of Protein−Aptamer Interaction. With the use of a thermal cycler (Eppendorf, Hamberg, Germany), 1 μM of the aptamer was heated to 95 °C for 1 min followed by a controlled decrease in temperature at a rate of 0.5 °C per second until the sample reached 25 °C. This temperature treatment, or "annealing", is necessary as it promotes the proper folding of the ssDNA aptamers.

A mixture containing 0.5 μM of labeled/unlabeled thrombin and 0.1 μM thrombin aptamer was prepared in a 20 mM Tris-acetate (pH 8.2) buffer supplemented with 5 mM KCl and 1 mM MgCl$_2$. The mixture was left to incubate for 15 min at room temperature prior to separation in a 50 cm PVA coated capillary, which was used reduce protein adsorption to the inner capillary surface. A voltage of 10 kV was used for the first 5 min of separation in order to avoid sample overheating in the uncooled region of the capillary. The voltage was then increased to 20 kV for the remainder of the run.

A mixture containing 80 nM labeled/unlabeled AlkB and 30 nM AlkB aptamer was prepared in a 50 mM HEPES (pH 7.5) buffer containing 50 mM NaCl. Following a 15 min incubation, the equilibrium mixture was injected into a 50 cm uncoated capillary. A voltage of 10 kV was applied for the first 5 min of the run, which was subsequently increased to 20 kV.

■ ASSOCIATED CONTENT

ⓈSupporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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Protein Labeling Enhances Aptamer Selection by Methods of Kinetic Capillary Electrophoresis

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Exonuclease I (Exo1) degradation of the ssDNA library during bulk affinity assay

An equilibrium mixture containing 3 μM Exo1 and 100 nM of an annealed DNA library were prepared in a 50 mM Tris-acetate (pH 8.2) incubation buffer containing 100 mM NaCl, 1 mM EDTA, and 0.5 mM MgCl$_2$. The mixture was left at room temperature for 15 minutes to ensure the protein and DNA had reached equilibrium. 50 nL of the mixture was injected into a 50 cm uncoated capillary and separated by applying 20 kV. Figure S1 depicts multiple peaks. Although these peaks may be mistakenly identified as Exo1-DNA complex, they are products of DNA degradation.
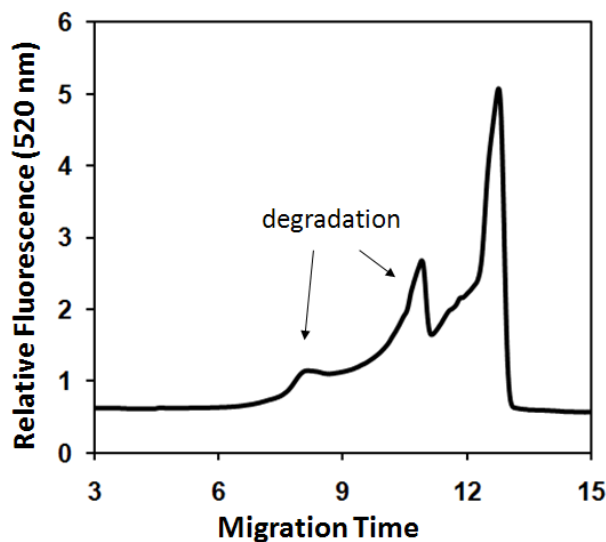

Figure S1. Equilibrium Mixture containing 3 μM Exo1 and 100 nM DNA library in a 50 mM Tris-acetate incubation buffer containing 100 mM NaCl, 1 mM EDTA, and 0.5 mM MgCl$_2$. The mixture was separated in a 50 mM Tris-acetate (pH 8.2) run buffer with an applied voltage of 20 kV. The DNA library is shown as the rightmost peak while the products of degradation are indicated with arrows.

The identity of these peaks was confirmed by performing capillary gel electrophoresis (CGE) using the same incubation buffer.   CGE can accurately discriminate between DNA that differs in length by only one nucleotide and, therefore, confirm if any DNA degradation has occurred. A DNA ladder containing 20 nucleotides (nt), 37 nt, 56 nt and 80 nt DNA markers was used to assess the length of the DNA present in the sample. By performing a co-injection of an equilibrium mixture and the DNA ladder, degradation can be accurately assessed. Figure S2 illustrates the DNA digestion following a 15 min incubation of 100 nM Exo1 and 5μM of the DNA library that was diluted in the same 50 mM Tris-acetate (pH 8.2), 100

mM NaCl, 1 mM EDTA, 0.5 mM MgCl$_2$ buffer. It is clear that degradation products are present, as ssDNA containing less than 20 nt are observed in the electropherogram.
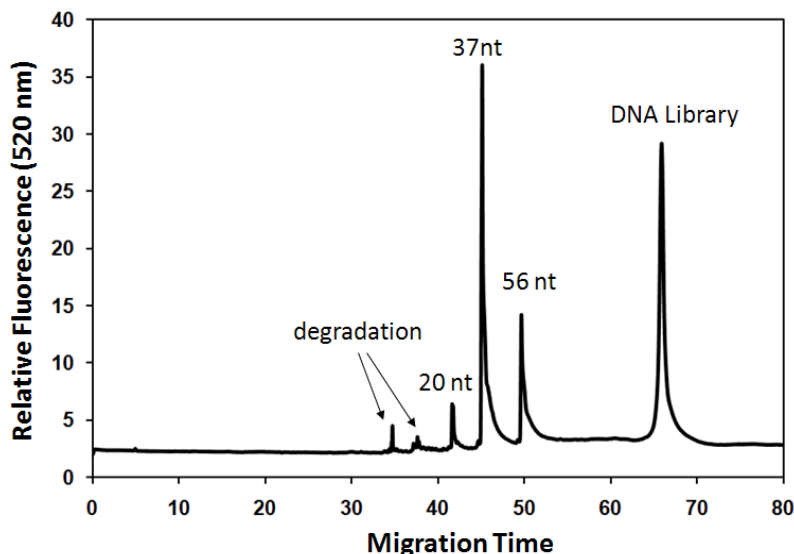

Figure S2. CGE results illustrating Exo1 activity in a 50 mM Tris-acetate (pH 8.2) buffer supplemented with 100 mM NaCl, 1 mM EDTA, and 0.5 mM MgCl$_2$. The DNA ladder and an equilibrium mixture containing 100 nM Exo1 and 5 μM of the DNA library were co-injected into the capillary. Products of DNA degradation having less than 20 nt in length are indicated with arrows.

This observation proves that DNA degradation occurs under these conditions, and the multiple peaks seen in Figure S1 are likely degraded ssDNA.

Establishing the Limit of Detection (LOD)

Solutions of labeled and unlabeled BSA, HSA, AGP, myoglobin and α-lactalbumin were prepared as serial dilutions using 100 mM NaHCO$_3$ (pH 8.3) dilution buffer. 50 nL of the sample was introduced into a 50 cm uncoated capillary (75 μm i.d.) and protein migration was induced by applying 20 kV. A PDA detector was used to detect the protein at 214 nm, while fluorescence emission at 610 nm was used to detect the labeled protein. The standard deviation of the background signal height (within the 1 to 2.5 minutes time frame) was determined for each sample. The standard deviation was then multiplied by 5 to determine the LOD in relative fluorescence units. This value was converted into units of concentration by normalizing it to the protein peak intensity at a known concentration. For each protein, detection limits obtained with UV absorbance and fluorescence were averaged and tabulated in Table 1 of the main text. LOD experiments are shown in figures S3, S4, S5, S6, and S7 with fluorescence detection shown in the top panel and UV detection shown in the lower panel.
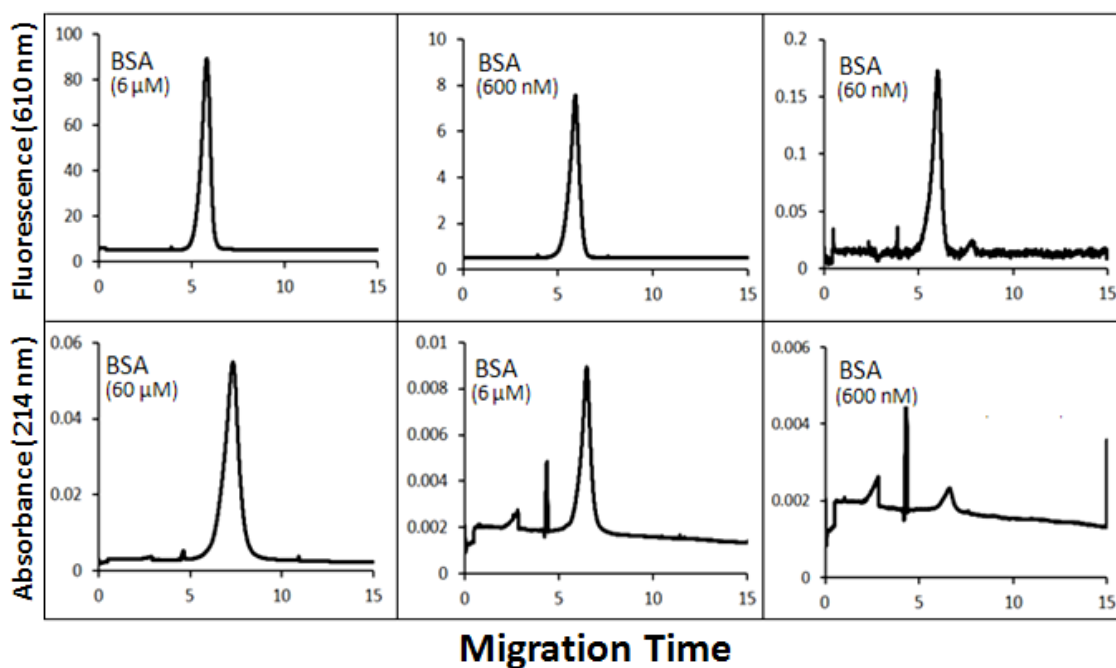
Figure S3. Electropherograms illustrating the improvement in CE detection of BSA by labeling protein with Chromeo P503. The top panel shows the data obtained using fluorescence detection while the lower panel illustrates the data obtained using UV absorbance detection. The respective concentrations are indicated in the right corners.
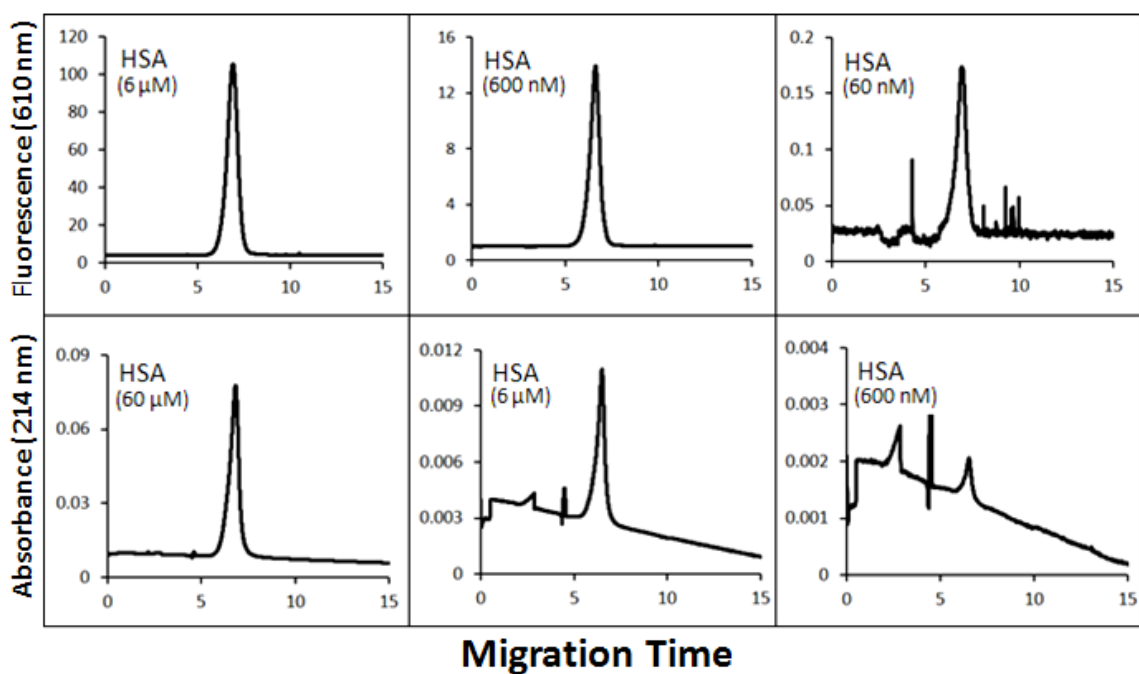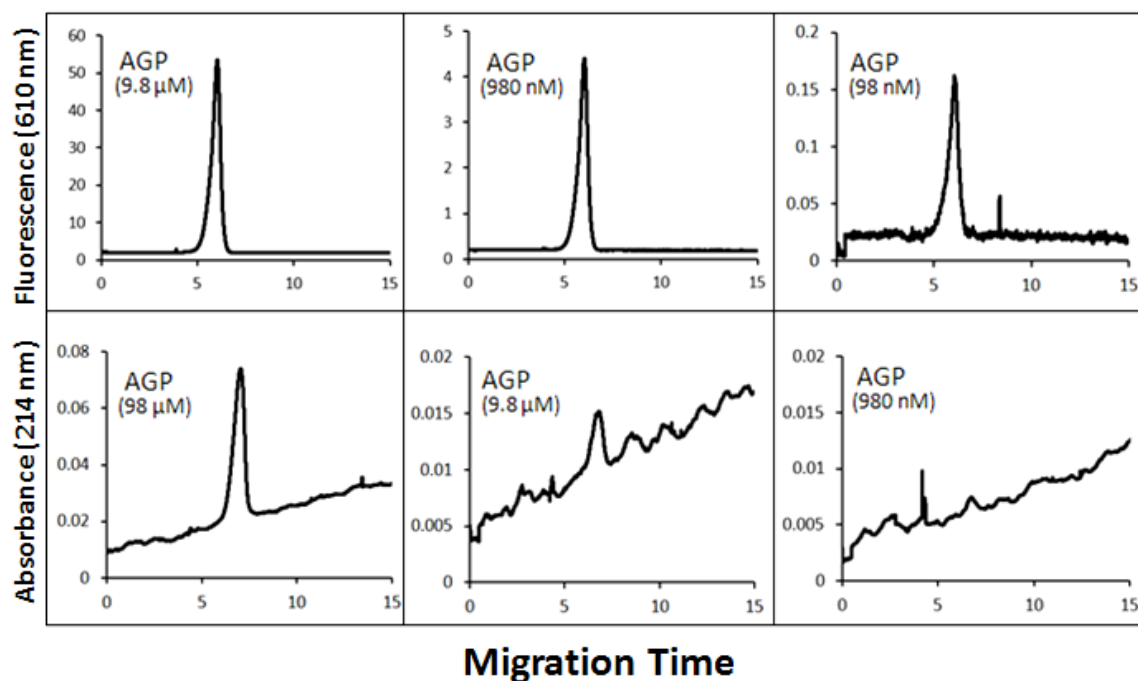

Figure S4. Electropherograms illustrating the improvement in CE detection of HSA by labeling protein with Chromeo P503. The top panel shows the data obtained using fluorescence detection while the lower panel illustrates the data obtained using UV absorbance detection. The respective concentrations are indicated in the right corners.

Figure S5. Electropherograms illustrating the improvement in CE detection of AGP by labeling protein with Chromeo P503. The top panel shows the data obtained using fluorescence detection while the lower panel illustrates the data obtained using UV absorbance detection. The respective concentrations are indicated in the right corners.
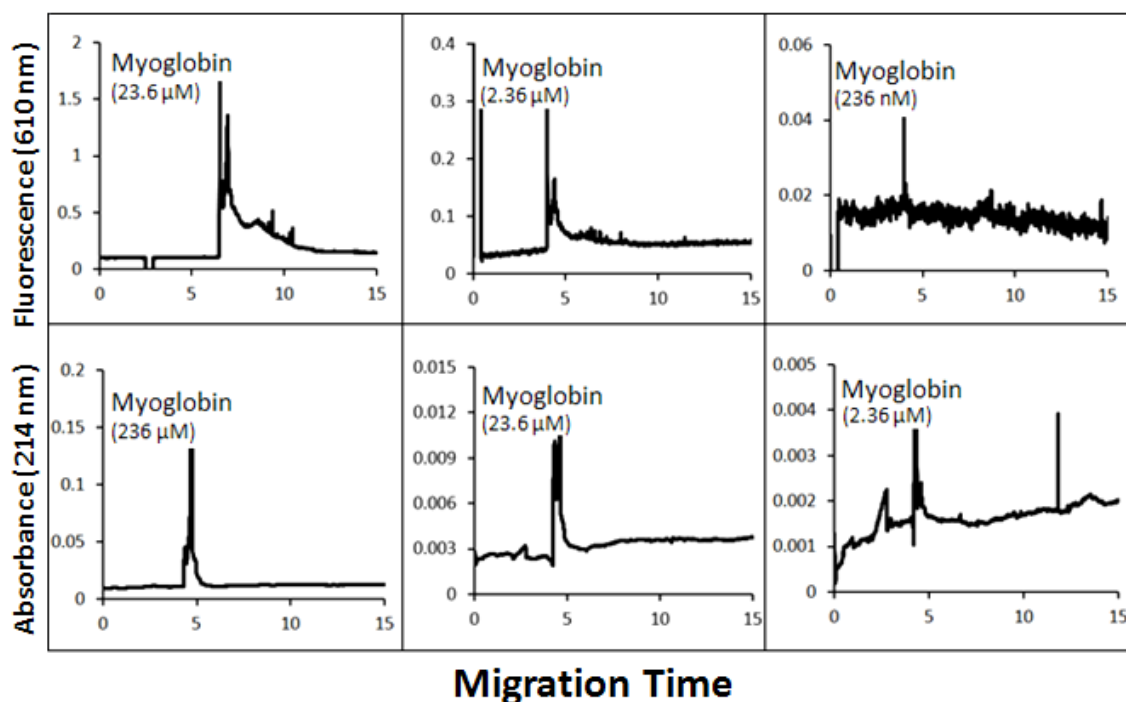

Figure S6. Electropherograms illustrating the improvement in CE detection of myoglobin by labeling protein with Chromeo P503. The top panel shows the data obtained using fluorescence detection while the lower panel illustrates the data obtained using UV absorbance detection. The respective concentrations are indicated in the right corners.
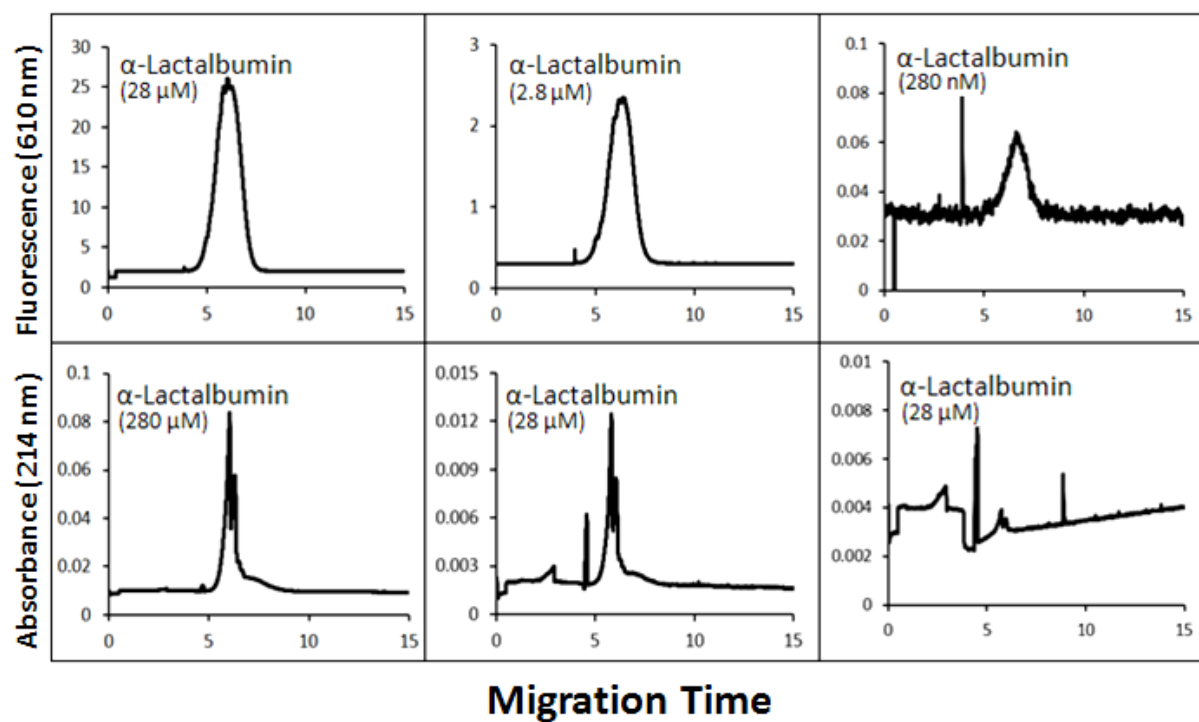
Figure S7. Electropherograms illustrating the improvement in CE detection of α-lactalbumin by labeling protein with Chromeo P503. The top panel shows the data obtained using fluorescence detection while the lower panel illustrates the data obtained using UV absorbance detection. The respective concentrations are indicated in the right corners.