SUPPORTING INFORMATION

Analysis of DNA in Phosphate Buffered Saline using Kinetic Capillary Electrophoresis

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1. Non-repeatability issues due to analyte interaction with sample vial

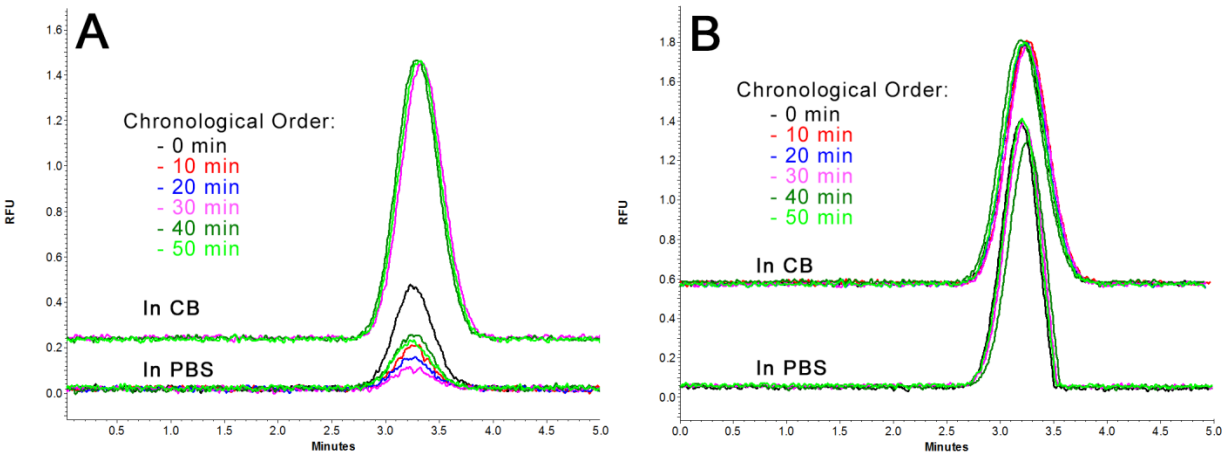

Figure S1. Signal repeatability of fluorescently-labelled DNA in CB and PBS buffers. A plug of 10 nM DNA was propagated through 10.1 cm of capillary length to detector (total length of capillary was 30 cm) by 0.30 psi (2.1 kPa) pressure. Six repetitions of each experiment were conducted in the chronological order signified by trace colors and presented in the legend. In each panel, top set of traces were obtained with CB as sample and run buffers, while bottom set was obtained with PBS. **Panel A:** signal repeatability with no additives in the sample; **Panel B:** signal repeatability with 1 mg/mL of BSA added to the sample for vial surface passivation. Addition of BSA significantly improves signal reproducibility. Quantum yield of fluorescently-labelled DNA in PBS is similar to one in CB.

2. Effects of pressure propagation on precision and accuracy of measurements of analyte mobility and peak broadening

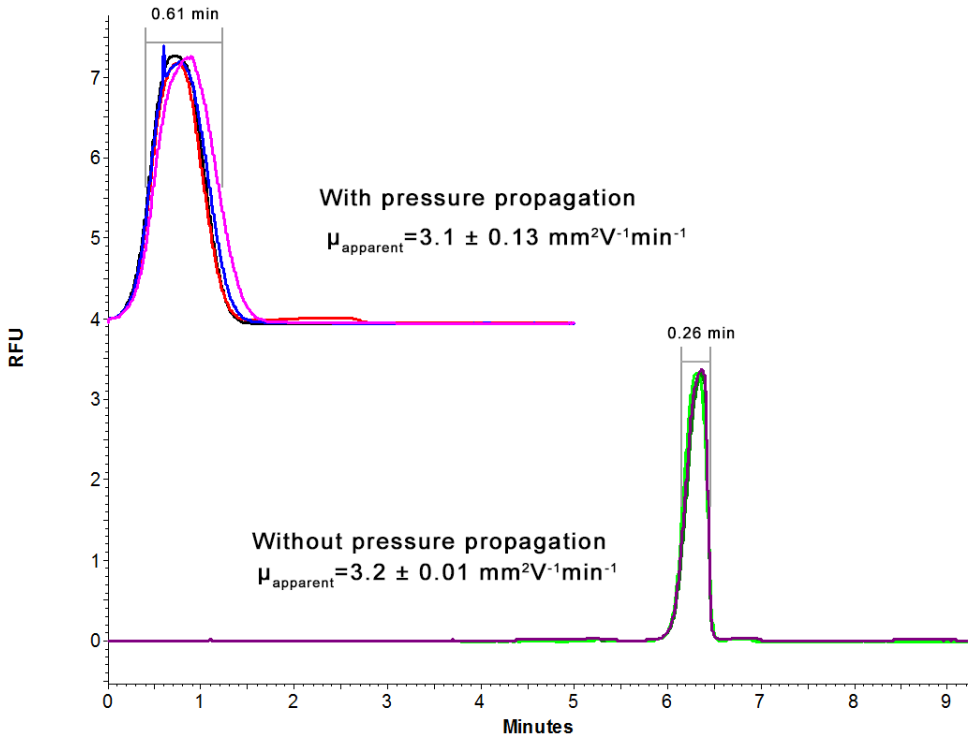

Figure S2. The influence of pressure propagation of DNA on accuracy and precision of electrophoretic mobility measurements in CB. All analyses were performed with the sample mixture of 50 nM DNA aptamer and 1 mg/mL BSA. The bottom set of traces shows migration of DNA in CB electrophoresis experiment without pressure propagation, and serves as a reference point for reproducibility, velocity, and peak widths. The top set of traces shows migration of the same DNA sample after it was pressure-propagated 9 cm closer to the detector. The length of capillary to detector was 10.1 cm (total length of capillary was 30 cm). The pressure propagation step was performed the same way as in experiments described in Figure 3 in the main text. The electrophoresis step was performed at a reduced strength of applied electric field (50 V/cm) to account for higher velocity of EOF in CB. Based on the velocity value from the bottom trace, the accuracy of the pressure propagation step is estimated to be within 0.03 mm, with a precision of 0.05 mm. Pressure propagation step increases the peak width 2.3-times. Thus, introduction of the pressure propagation step cannot account for the poor repeatability of migration times and peak broadening observed under similar conditions in PBS.

3. Dynamic DNA peak broadening in PBS-KCE

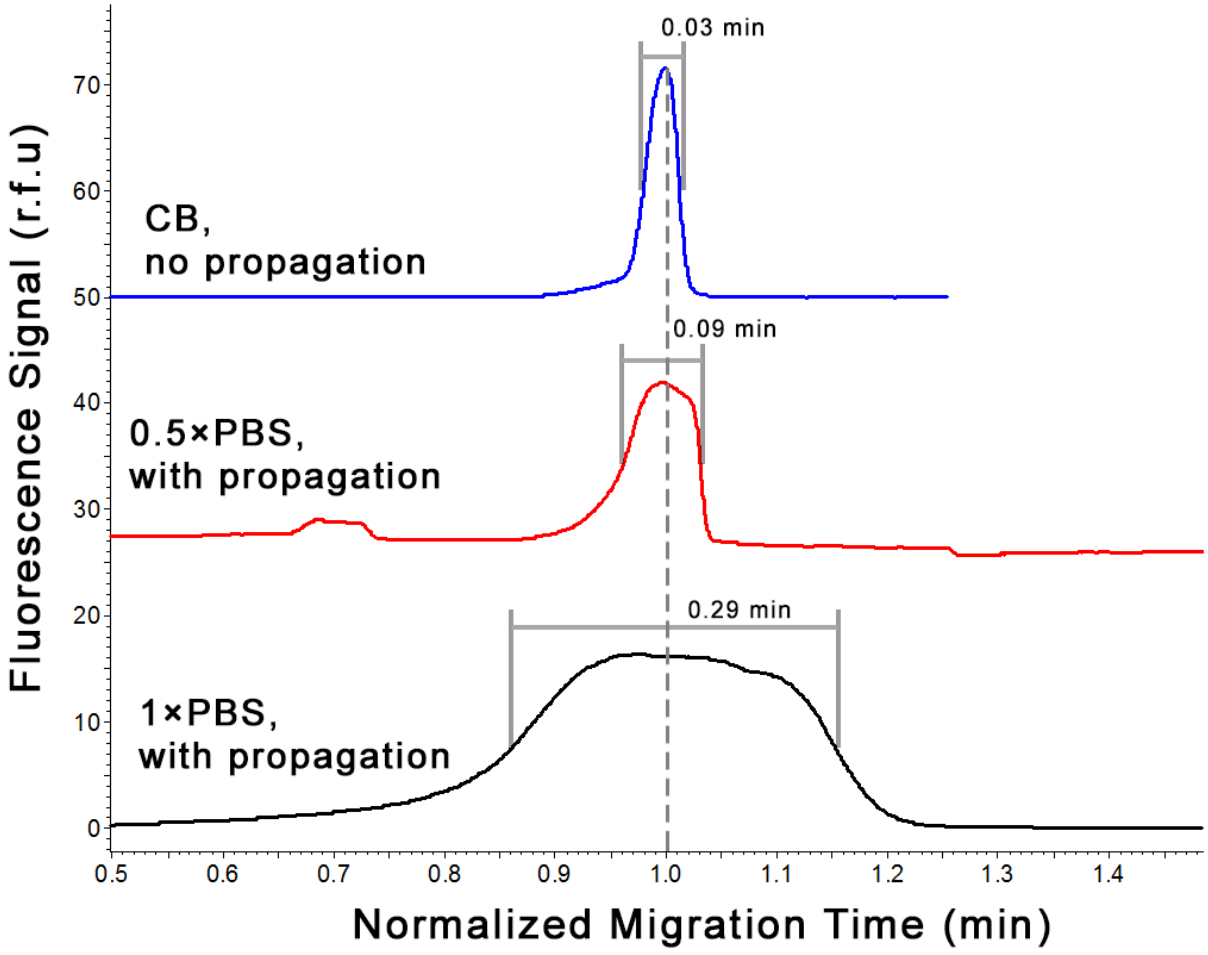

Figure S3: Comparison of widths of DNA peak in CB (blue trace), 2-times diluted PBS (0.5×PBS, red trace), and non-diluted PBS (1×PBS, black trace). The length of capillary to detector was 10.1 cm (total length of capillary was 30 cm). Electrophoresis was performed as in Figure 3 in the main text, with the exception that no sample propagation was applied in the CB experiments. For both PBS experiments, the post-injection pressure propagation step was 9 cm, making the distance between initial sample position and the detection window to 1.1 cm. Electrophoresis was carried at a 200 V/cm electric field. The capillary coolant temperature was set to 22 °C, with internal capillary temperature calculated to be 25 °C. Data shown is after normalization by residence time in the detector; the normalization was done by applying a multiplier to the *x*-axis equal to the reciprocal of the migration time of the peak. After normalization, the DNA peak width in non-diluted PBS is 3.2 times wider than in 2-times diluted PBS. This suggests that the ionic strength of the buffer plays a role in peak broadening. The 3-times increased peak width between CB and 2-times diluted PBS is not due to difference in ionic content (as the concentration of the major ionic species is similar), but due to the pressure propagation step.

4. Absence of DNA adsorption onto bare-silica capillary wall

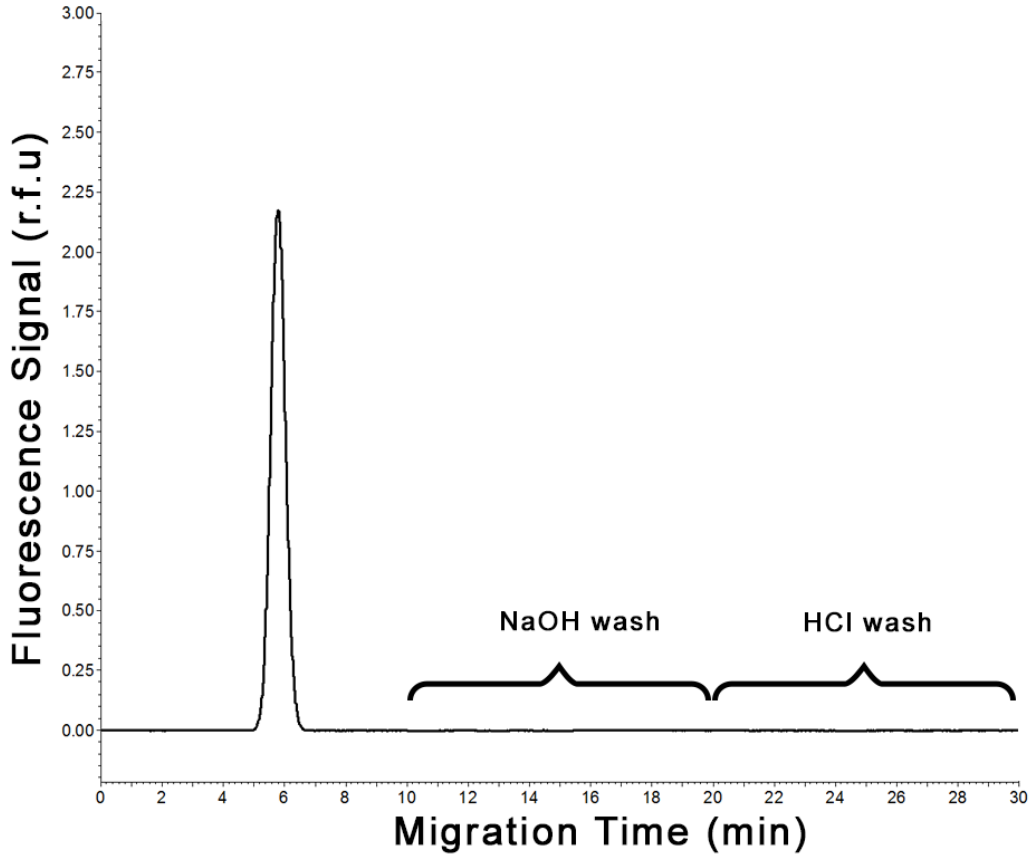

Figure S4: Analysis of DNA adsorption onto capillary walls. 50 nM DNA was driven through the capillary by 0.5 psi (3.4 kPa) pressure, with subsequent washes with 100 mM NaOH and 100 mM HCl, at the same pressure. The length of capillary to detector was 10.1 cm (total length of capillary was 30 cm). Lack of additional elution of DNA during the base and acid washes suggests that no adsorption of DNA on capillary walls occurs.

5. Effects of pressure-assisted electrophoresis on peak shape and width

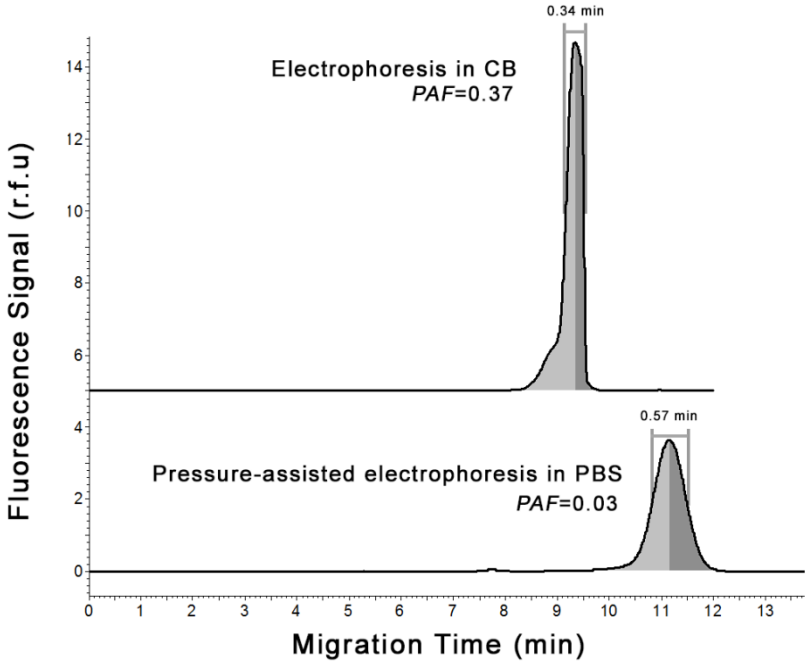

Figure S5: DNA peak shape and width as a result of electrophoresis in CB (top trace) and pressure-assisted electrophoresis in PBS (bottom trace). Both analyses were performed with a sample mixture of 50 nM DNA aptamer and 1 mg/mL of BSA. The length of capillary to detector was 39.9 cm (total length of capillary was 50 cm). Pressure-assisted electrophoresis increased peak width by 1.7 times, and decreased the peak asymmetry factor (*PAF*) by 10 times. PAF is calculated using the following formula:

$$PAF = (A_{front} - A_{tail})/(A_{front} + A_{tail})$$

where A_{front} and A_{tail} are front and tail areas of the peak, respectively, separated at the peak maximum. Values of *PAF* range between −1 and 1, with the value of 0 corresponding to a perfectly symmetric peak. The increased peak width is not expected to be detrimental to KCE data analysis, while the improvement in peak symmetry is expected to be beneficial, as described previously.[1]

Supporting References:

(1) Kanoatov, M.; Retif, C.; Cherney, L. T.; Krylov, S. N. *Anal. Chem.* **2012**, *84*, 149-154.