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DNA Hybridization Assays Using Temperature Gradient Focusing and Peptide Nucleic Acids

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Abstract: Two types of DNA hybridization assays are demonstrated with temperature gradient focusing (TGF) and peptide nucleic acids (PNAs). In TGF, the application of a controlled temperature gradient along the length of a microchannel filled with an appropriate temperature-dependent buffer results in the formation of a gradient in both the electric field and electrophoretic velocity. Ionic species move in this gradient and concentrate at a unique point where the total velocity sums to zero. The first assay is a mixing assay in which PNA is allowed to flow through spatially focused DNA targets within a capillary. The second assay detects single base pair mutations (SBPM) by monitoring the fluorescence intensity of PNA/DNA duplexes as a function of temperature within the capillary. The SBPM analysis can be performed in less than 5 min with 100-fold more dilute analyte compared to conventional UV melting measurements.

Introduction

This report describes DNA hybridization assays in capillaries using temperature gradient focusing (TGF)[1] and peptide nucleic acids (PNAs).[2,3] TGF simultaneously concentrates and separates charged species based on the application of a temperature gradient to microfluidic or capillary channels. Here we demonstrate two different types of assays using TGF and PNA: (1) hybridization and detection of complementary PNA/DNA duplexes by stationary TGF and (2) single base pair mismatch (SBPM) detection of the R553X mutation in the cystic fibrosis gene by scanning TGF.

TGF is an equilibrium counterflow gradient focusing method similar to electric field gradient focusing. Unlike electric field gradient focusing, which requires electrical connections to the middle of a separation channel,[4−7] TGF requires only the application of a temperature gradient and the use of a buffer with a temperature-dependent ionic strength. As a result, TGF can be implemented in very simple microfluidic devices or even short capillaries as in the work described here. In TGF, charged analytes are focused by balancing their electrophoretic velocity against the bulk buffer velocity. The combination of the applied temperature gradient and the temperature-dependent ionic strength results in an electrophoretic velocity that is not constant everywhere, but varies along the temperature gradient. Consequently, the total velocity, which is given by the sum of the electrophoretic and bulk velocities, can be made equal to zero at a particular point along the temperature gradient. All of the analyte will then move toward that zero velocity point where it will be focused. Different analytes, with different electrophoretic mobilities, will be focused at different points along the gradient and thereby separated. Furthermore, in TGF, the position of the focused analyte bands can be easily controlled by adjusting the bulk velocity.

The hybridization of PNA with DNA was chosen for the TGF assays because the electrophoretic mobility of the PNA/DNA hybrid is significantly different from that of ssDNA targets. Consequently, the focused peaks due to the PNA/DNA hybrid and the ssDNA can be easily resolved. In addition, the fluorescently labeled PNA has nearly zero electrophoretic mobility, and thus it is only very weakly focused by TGF.[8]

The two types of TGF assays presented in this work are illustrated in Figure 1. We refer to the first assay, depicted in Figure 1a, as stationary TGF, because the position of the focused peaks is held roughly constant during the course of the experiment. The stationary assay involves three steps. First, negatively charged ssDNA targets are focused and concentrated at a predetermined point (and therefore temperature) in the capillary. Second, fluorescently labeled PNA is introduced into the capillary and is carried by bulk flow of the buffer *through* the stationary, focused band of ssDNA. Third, if PNA/DNA hybridization occurs, the hybrid focuses at a different spatial location because of its different eletrophoretic mobility, and a

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(8) The fluorescent label, TAMRA, attached to PNA, is zwitterionic and likely contributes a small net charge. However, no significant focusing of PNA was observed under the same experimental conditions.

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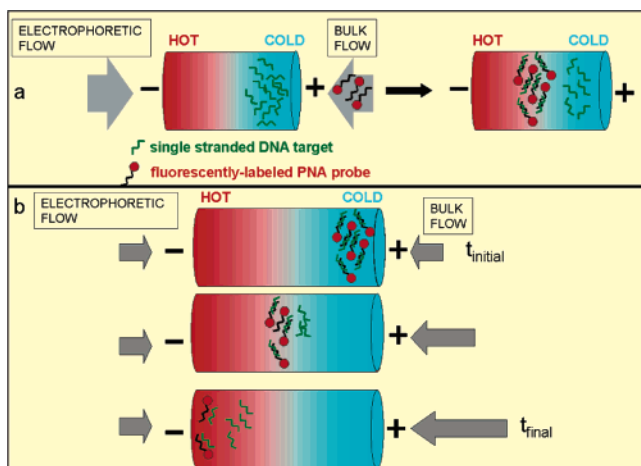

Figure 1. Schematic illustration of the two TGF assays in this report. The stationary assay (a) is performed by first focusing the ssDNA targets and then introducing the PNA probe into the bulk flow of the buffer. Scanning TGF (b) is performed by first focusing the DNA/PNA hybrid on the cold side of the temperature gradient and then changing the bulk flow to move the analyte spatially through the temperature gradient so that its melting temperature can be measured.

second fluorescent band is observed. If the PNA is not complementary to the focused DNA, it will not hybridize, and the neutral PNA will remain unfocused. Therefore, a second focused fluorescent band is not observed.

The second assay, depicted in Figure 1b, is referred to as scanning TGF, because the bulk flow of the buffer is changed in a controlled, stepwise fashion during the course of the experiment to move the focused peaks from the one end of the temperature gradient to the other. In this assay, fluorescently labeled PNA probes are hybridized with DNA targets before introduction in the capillary. TGF is then used to focus PNA/DNA hybrids at the low-temperature end of the gradient. The focused band of PNA/DNA hybrids is then gradually moved toward the high-temperature end of the gradient by adjusting the bulk flow rate. As the focused band moves to higher temperatures, the PNA/DNA hybrids thermally denature, and the melting point can be identified by a decrease in the intensity of the band. Scanning TGF can be used to detect single base pair mismatches by comparing the melting point of a perfect complementary PNA/DNA duplex to that of a PNA/DNA duplex containing a single base pair mismatch.

TGF provides two unique advantages for assays in capillary and microfluidic formats. First, TGF permits the spatial concentration of charged targets. In proteomic or genomic applications, protein or nucleic acid targets are typically present at nanomolar concentrations or less. These low concentrations combined with the low mass flow rates of microfluidic systems frequently result in unacceptably long analysis or equilibration times. Because TGF can be used to spatially focus biomolecular recognition probes as well as probe/target duplexes, the method can be used with very low concentration samples. Second, because of the unique focusing capabilities of TGF as described below, the problem of mixing target and probe molecules in microscale-confined geometries is greatly simplified. Effective mixing of reagents in microfluidic formats is difficult because of the low Reynolds number, laminar flow conditions where mixing of species is typically diffusion-limited. A variety of strategies for enhanced mixing within microfluidics have been reported, including special channel geometries[9,10] or modifications,[11−13] immobilization of reagents,[14,15] or external stimuli.[16,17] In contrast, the TGF approach does not require complex channel designs or reagent immobilization and thus represents an entirely new method for localizing a probe molecule and mixing it with target samples in microfluidic systems.

Table 1. DNA and PNA Sequences Used in This Study

Stationary TGF PNA/DNA Assay
PNA sequence N-terminus-C-terminus
Tam-OO-CAC-TGT-AGA-GCG-ATT-GCA
Complement DNA 5′-3′
Fl-TGC-AAT-CGC-TCT-ACA-GTG
Noncomplement DNA 5′-3′
Fl-GTC-AGT-CAG-TCA-GTC-AGT
Scanning TGF for SBPM Detection
PNA sequence R553 wild-type N-terminus-C-terminus
Tam-OO-CCT-GCT-CGT-TGA-CCT
DNA wild-type R553 5′-3′
Fl-AGG-TCA-ACG-AGC-AAG
DNA mutant R553 5′-3′
Fl-AGG-TCA-ATG-AGC-AAG
Fl = fluorescein, Tam = TAMRA

Experimental Section

Materials. Tris(hydroxymethyl)aminomethane base (Tris), phenol, and boric acid were purchased from Sigma[18] (St. Louis, MO) and used as received. Fluorescein and carboxytetramethylrhodamine (TAMRA) were purchased from Molecular Probes (Eugene, OR) and used as received. 2-[Methoxy(polyethyleneoxy)propyl]trimethoxysilane (PEG-silane) was purchased from Gelest, Inc. (Morrisville, PA) and used as received.[18] The fluorescently labeled ssDNA targets were purchased from Qiagen Operon (Valencia, CA) with a fluorescein label on the 5′ end.[18] Stock solutions were prepared in 0.1 M Tris, pH 8.2, buffer. TAMRA-labeled PNA probes were purchased from Applied Biosystems (Foster City, CA).[18] All solutions were prepared using 18.1 MΩ cm water. The DNA and PNA sequences used for the assays in this report are shown in Table 1.

The fused silica capillary (30 μm i.d.; 360 μm o.d.) was purchased from Polymicro Technologies, LLC (Phoenix, AZ).[18] An ∼5-mm optical window was prepared by burning a portion of the polyimide coating. The capillary was sandwiched between two polycarbonate sheets[18] (McMaster-Carr, Atlanta, GA), placed in a hydraulic press at 1000 lbs at 180 °C for 10 s, and cooled to 120 °C before releasing the pressure. The plastic served to provide mechanical stability to the bare capillary region and to enhance heat transfer from the copper blocks described in the TGF apparatus. The capillary was derivatized with PEG-silane (3 mM in toluene) by flowing the solution through the capillary for 4

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(18) Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

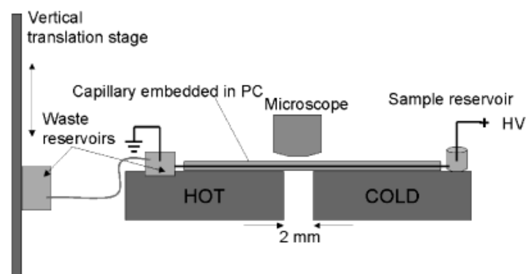

Figure 2. Schematic illustration of the TGF apparatus. Image is not to scale.

h. The reaction is preceded by a rinse in 1 M NaOH, water, ethanol, and toluene. The coating reduced the measured electroosmotic flow by a factor of 3 relative to the uncoated surface and minimized PNA adsorption to the walls of the capillary. For the stationary TGF assay, the capillary was 10-cm long, while the scanning TGF assay employed a 3-cm-long capillary. Each day a new capillary was derivatized.

Buffer Characterization and Temperature Measurements. The conductivity of 0.1 M Tris-phenol and 1 M Tris-borate was measured as a function of temperature with the use of a conductivity meter and a circulating water bath. The plot of ionic strength was constructed from the measured conductivities at temperatures ranging from 10 °C through 80 °C at 10 °C intervals and the known buffer viscosities.

The temperature gradient used for focusing was measured with a temperature-dependent dye (rhodamine B) by previously reported methods.[19] Briefly, the capillary was filled with a solution of rhodamine B, and fluorescence images were collected at each temperature gradient used and at a constant 20 °C, which served as a reference image. The temperature was calculated from the ratio of the intensity of the gradient image to the intensity of the reference image.

UV Melting Experiments. The melting transition curves of PNA/DNA duplexes were monitored from the absorbance at 260 nm with a Cary UV−visible spectrophotometer equipped with a Varian Peltier heater/cooler 6 × 6 multicell block.[18] The temperature was ramped from 25 °C to 95 °C to 25 °C at a rate of 1 °C min^{-1} with a 6-s hold time and data interval of 0.1 °C. A total of three cycles were performed. All PNA/DNA solutions were prepared in Tris buffer, pH 8.2, at a concentration of 1 μM.

TGF Apparatus. The prepared capillary device was connected to polypropylene sample and waste reservoirs via a hole drilled into the polypropylene and a Teflon-backed silicone septum, respectively; see Figure 2. The waste reservoir was connected to an additional reservoir attached to a computer-controlled vertical translation stage. Pressure-induced bulk flow was controlled by changing the height of the waste reservoir. The custom written Labview software program (National Instruments, Austin, TX) allowed step height and hold time to be manipulated.[18] The capillary device was anchored to a pair of copper blocks with a 2-mm gap between them. One side of the gap was temperature controlled by an electrical resistance heater employing a PID temperature controller while the other was maintained at 20 °C by a circulating water bath passing water through the copper block. The bulk velocity of the buffer was controlled by application of positive polarity voltage relative to the sample reservoir and pressure-induced flow. For reference, the bulk velocity moves from right to left in all line profiles and images shown, while negatively charged species move from left to right.

Fluorescence Microscopy. A fluorescence microscope equipped with a mercury arc lamp, 10× microscope objective, a dual-band filter set for TAMRA and fluorescein, and a three chip color CCD camera with frame-grabbing software were used to acquire all movies and images. The stationary TGF measurements were performed by first focusing a plug of the ssDNA target on the cold side of the temperature

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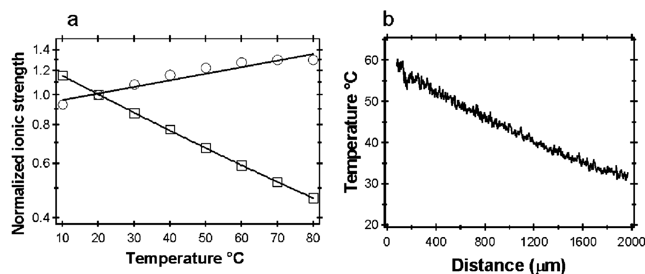

Figure 3. (a) Temperature-dependent ionic strength function of 1 M Tris-borate acid (○) and 0.1 M Tris-phenol (□). The solid lines are linear fits to the data. (b) The measured temperature profile along the 2-mm gap used for the experiments. The hot side of the gap was set to 65 °C, and the cold side was set to 20 °C.

gradient in a 10-cm-long capillary. The sample reservoir was then replaced with the same buffer containing no DNA, and focusing was performed until no further increase in DNA concentration was observed. The buffer was then replaced with buffer containing PNA. Images were then acquired at a rate of 1 frame every 20 s for 15 min. The scanning assays were performed by stepping the focused analyte through the temperature gradient at a step height of 0.5 mm and 4-s hold time in a 3-cm-long capillary. The images were triggered to the translation stage movements, and images were acquired at each step of the scan. The mutant and wild-type duplexes were prepared in 0.1 M Tris-phenol buffer at a concentration of 5 and 10 nM for the wild and mutant type, respectively, and incubated at room temperature for 10 min before performing the assay. The scanning TGF assay was repeated three times for each duplex.

Quantitative Fluorescence Measurements. The concentrations of ssDNA and PNA/DNA duplexes were calculated from a linear calibration curve obtained with stock solutions of fluorescein and TAMRA. The calibration was performed with five standard solutions of fluorescein and TAMRA in 0.1 M Tris-phenol under the same temperature conditions as the assays, 20 to 65 °C for the mixing assay and 20 to 80 °C for the SBPM assay.

Results and Discussion

Buffer Characterization and Temperature Measurements. Two important factors are critical for the success of TGF. First, the choice of an appropriate buffer determines the ability to perform the counterflow focusing method. Previously, two buffers were tested as TGF buffers. It was found that Tris-borate at both low and high concentrations was an appropriate TGF buffer system because the ionic strength is dependent on temperature. In this study, Tris-phenol was tested for use as a TGF buffer in an effort to find a buffer with a greater ionic strength change over the given temperature range. Figure 3a is a plot of the ionic strength vs temperature for both 1 M Tris-borate and 0.1 M Tris-phenol. As described previously, for Tris-borate 1 M was used because the conductivity of 0.01 M was too low and the ionic strength for 0.1 M Tris-borate was nonmonotonic.[1] We chose 0.1 M Tris-phenol because it had the appropriate conductivity and a steep ionic strength versus temperature curve. Two important observations can be drawn from this figure. First, the ionic strength change is in the opposite direction for these two buffer systems. Second, the Tris-phenol buffer has a steeper ionic strength change compared to Tris-borate. The higher ionic strength gradient results in a steeper velocity gradient for a given temperature gradient. The important consequence of this feature is that analytes with a wider range of electrophoretic mobilities can be simultaneously focused with the Tris-phenol buffer relative to the Tris-borate buffer. In fact,

a comparison of the focusing of PNA/DNA duplexes and free ssDNA in Tris-borate and Tris-phenol systems (data not shown) under similar focusing conditions (20−80 °C temperature gradient, 300 V cm^{-1}) revealed that with Tris-phenol, both bands could be easily focused (and resolved) at the same time within the 2-mm field of view of the microscope, whereas with the Tris-borate buffer they could not. For this reason, Tris-phenol was chosen as the buffer in these experiments.

A second factor important for TGF is the ability to form appropriate temperature gradients. The temperature gradient was verified inside the capillary with a dye whose quantum efficiency is temperature-dependent. Figure 3b is the measured temperature profile for a typical gradient employed in this study. As expected, the temperature is approximately linear across the 2-mm range.

Stationary TGF PNA/DNA Assay. The stationary TGF assay was demonstrated by hybridizing PNA probes with complementary ssDNA targets and comparing them to control experiments with mismatched targets. A temperature gradient of 20−65 °C across 2 mm and an applied voltage of 3000 V were used, and the assay was performed with a 10-cm-long capillary. The high-temperature end of the applied gradient was kept below the melting temperature of the PNA/DNA duplex (T_m > 65 °C) to ensure complete hybridization. First, fluorescently labeled 18-mer ssDNA targets were focused within the gradient from an initial concentration of 0.25 μM in the input reservoir. Labeling of ssDNA is not necessary for the assay, but was done to visualize the location of focused targets (labeling of the PNA is, of course, necessary). After sufficient focusing of ssDNA, the Tris-phenol buffer containing DNA in the input reservoir was replaced with the same buffer containing no DNA. The purpose of this step was to focus any remaining DNA in the capillary that had not yet reached its zero velocity point. By measuring the intensity of fluorescein emission, the final peak concentration of the focused ssDNA was estimated to be ~60 μM (corresponding to a total amount of ~7.5 fmol from the peak area) using the methods described in the Experimental Section. The buffer was then replaced with a buffer containing 1 μM of the TAMRA-labeled PNA probe. Figure 4a displays a series of line profiles monitoring the TAMRA fluorescence emission as a function of time after the introduction of PNA. The small peak visible at ~36 °C in traces i and ii is due to the green fluorescence interference of the focused, fluorescein-labeled ssDNA that is detected in the red channel of the CCD. After an initial lag period corresponding to the time required for the PNA to reach the focused DNA plug (~4 cm of capillary before the 2-mm gap), a new fluorescent band appears and grows with time between 45 and 55 °C that we assign to focused PNA/DNA hybrids. The observed peak asymmetry is due primarily to Taylor dispersion arising from internally generated and externally applied pressure gradients.[20] The line profiles in Figure 4b represent a control experiment where the same experiment was repeated with the noncomplementary 18-mer ssDNA target. Before introduction of the PNA, the noncomplementary ssDNA was focused to a final peak concentration of ~52 μM or a total amount of ~3.6 fmol from an initial concentration of 0.25 μM. As expected for a noncomplementary target, a band corresponding to TAMRA emission of focused PNA/DNA hybrids is not observed.

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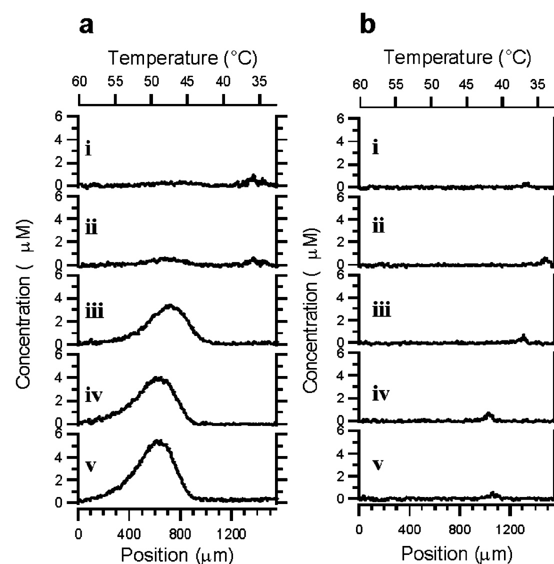

Figure 4. (a) Fluorescence line profiles of the PNA concentration collected as a function of time with perfectly complementary ssDNA (i) 0, (ii) 7.7, (iii) 10.7, (iv) 14.3, and (v) 15 min after introduction of PNA. (b) The results of the assay using noncomplementary ssDNA. The temperature measured inside the capillary is shown by the top horizontal axis, while the relative position along the capillary is shown by the bottom horizontal axis. Focusing conditions, 20 °C cold side (right), 65 °C hot side (left), 204 V cm^{-1} in 0.1 M Tris-phenol. Further details are provided in the text.

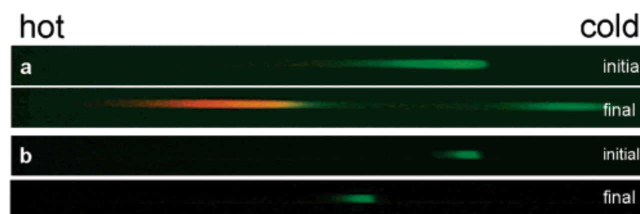

Figure 5. Fluorescence micrographs of the complement (a) and noncomplement (b) mixing assay. The final image was taken after 15 min of focusing time. The focusing conditions are given in Figure 4. For scale, each image is 2-mm long.

The fluorescence micrographs from which the line profiles of Figure 4 were obtained are shown in Figure 5. The PNA/DNA duplex and ssDNA focused bands are easily resolved from each other in the case of the perfect complement. Note the absence of an orange band in the noncomplement case. For illustration purposes only, a large amount of DNA was initially focused. The amount of ssDNA initially focused is also different for the two assays and is reflected by the differences in intensity and width of the green bands observed. Shifting of the spatial location of the focused ssDNA bands is also evident in the micrographs and line profiles for both experiments with the complement and noncomplement. We attribute the shifting behavior to changes in electroosmotic flow because of alteration of the surface condition of the capillary wall. In addition, changes in bulk flow also result from evaporation of buffer from the sample or waste reservoirs.

The calculated resolution between the PNA/DNA duplex and free ssDNA peaks was 1.7. The resolution is defined as $2(x_b - x_a)/(w_a + w_b)$ where x_a and x_b are the peak positions and w_a and w_b are the widths derived from a Gaussian peak fit. This resolution is more than sufficient to resolve the two peaks even with the observed tailing due to the peak overloading from the high input concentration of DNA. The resolution could easily be improved by reducing the steepness of the temperature

gradient or the concentration of the input sample. Although the apparent peak capacity from Figure 5a might seem low (calculated peak capacity = 6), for the hybridization assay described here, only a peak capacity of two is required. The peak capacity is defined as the length of the separation channel divided by 2σ, where σ is the standard deviation of the peak width. Again, the peak capacity can be improved by spanning a larger temperature gradient, application of a higher electric field, and not overloading the peaks (see, for example, the TGF scanning assay in the next section).

The distance and time required for the mixing and hybridization are related to the width of the focused band of the ssDNA target and the flow rate of the buffer, respectively. The width of the focused band depends on the identity of the TGF buffer as well as the amount that is focused. In the experiments illustrated here, the width varied from 50 to 200 μm, depending on the amount that was initially focused. Increasing the flow rate in microfluidic geometries typically increases the distance required for mixing. In contrast, increasing the flow rate in TGF mixing does not change the width of the focused band and actually improves the mixing rate.

Scanning TGF for SBPM Detection. In addition to the hybridization assay described above, TGF can also be used in a scanning mode to detect SBPMs, an important capability for the diagnosis of diseases such as cancer and cystic fibrosis. The example illustrated here is the interrogation of ssDNA targets that contain sequences relevant to a common SBPM, the R553X mutation found in the transmembrane conductance regulator (CFTR) gene, that results in cystic fibrosis.[21−23] In the scanning assay, a focused band of the PNA/DNA duplex is moved spatially across the temperature gradient by adjusting the bulk flow of the buffer. When the band reaches the melting temperature of the duplex, the fluorescence intensity decreases because the neutral, dissociated PNA strands are no longer focused and are therefore swept from the gradient region by bulk flow.

Figure 6 shows fluorescence micrographs of two different scanning TGF assays with the wild type, Figure 6a−c, and mutant, Figure 6d−f, DNA targets. In each case, as the focused PNA/DNA duplex (orange band) is moved from right ($T = 20$ °C) to left ($T = 80$ °C), the intensity decreases, and a new green band corresponding to ssDNA appears. The labeling of the ssDNA is not necessary but is done here to better visualize and understand the melting process. The wild type (Figure 6a−c) melts at a temperature higher (spatially to the left in the images) than the mutant sequence (Figure 6d−f). For example, at approximately the same spatial location, a green band appears in the mutant duplex (Figure 6e) but is absent in the wild type (Figure 6b).

Monitoring the fluorescence intensity as a function of position (and therefore temperature) reveals a plot analogous to that obtained with conventional UV melting measurements. A comparison of the two techniques is shown in Figure 7. The plot in Figure 7a shows the concentration of the PNA/DNA duplexes as a function of position (and temperature) for the TGF assay. The melting temperature from these measurements was assigned as the inflection point of the fit of the data to a sigmoid function. For comparison, conventional UV melts are shown in Figure 7b. The measured melting temperature, T_m, and the relative difference in T_m between the wild type and mutant PNA/DNA duplexes compared well to conventional UV measurements. The T_m of the wild and mutant duplexes measured using scanning TGF was 71.9 ± 1 °C and 62.9 ± 1 °C, respectively. In comparison, T_m values of 70.0 ± 1 °C and 63.2 ± 0.6 °C were determined from UV melt measurements for wild and mutant duplexes, respectively.

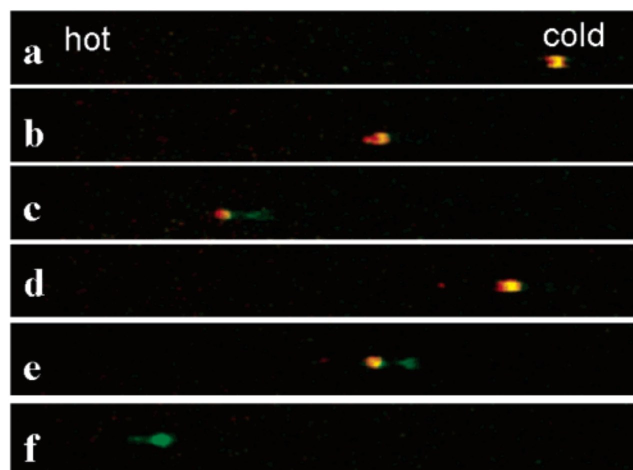

Figure 6. Fluorescence micrographs of wild-type R553 (a−c) and mutant R553X (d−f) PNA/DNA duplexes as a function of position in the capillary. Focusing conditions, 20 °C cold side, 80 °C hot side, 667 V cm^{-1} in 0.1 M Tris-phenol. The input concentration of PNA/DNA duplex was 5 nM (wild type) and 10 nM (mutant) in 0.1 M Tris-phenol. The orange band is the PNA/DNA duplex, while the green band is the melted ssDNA. For scale each image is ~1.5-mm long.

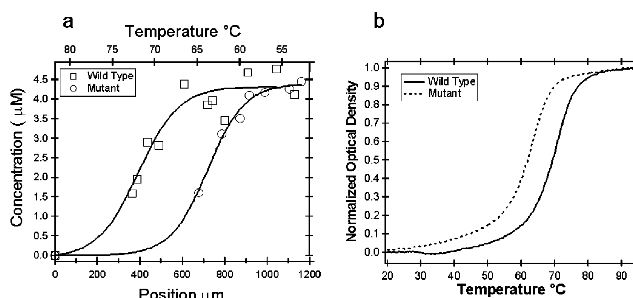

Figure 7. (a) Melting curves for the mutant (○) and wild type (□) PNA/DNA duplexes from the scanning TGF assay. The concentration is plotted as a function of position (bottom axis) and temperature (top axis). The solid and dashed lines are sigmoid fits to the data. For clarity, only one trial is shown for each sample. (b) A UV melting curve plotted as the normalized optical density versus temperature for the wild type (solid line) and mutant (dashed line) PNA/DNA duplexes. For clarity, only one temperature ramp is shown for each sample.

Linear temperature gradients formed within microchannel formats have been demonstrated previously by Cremer and coworkers and exploited for DNA/DNA mismatch studies.[24,25] In their work, a series of channels spanned a temperature gradient so that the particular reaction of interest could be monitored as function of temperature in one shot by filling the microchannels with the analyte of interest and simultaneously monitoring all

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microchannels. The key distinction between this method and TGF is the ability to concentrate analytes and therefore improve the limit of detection. When compared to conventional UV melting measurements, the scanning TGF assay can be performed with lower sample concentrations (10 nM compared with 1 μM) and much faster (<5 min for the TGF assay compared to greater than 60 min analysis for the UV measurement).

The concentration limit of detection (LOD) for the scanning assay can be estimated from the results shown in Figures 6 and 7. Under these experimental conditions, using a nonoptimal detector (mercury arc lamp and room temperature CCD camera), the peak height was 8.6 times greater than the background noise after 2 min of focusing time. Assuming a linear dependence of peak intensity with input concentration, this implies a concentration LOD of 2 nM. This LOD can easily be improved by using a better detection scheme and/or simply focusing for a longer time. To first order, the focused peak height is linearly proportional to time[1] so that an LOD in the picomolar range should be achievable with focusing times between 10 and 100 min. The sample well volume in the current apparatus was 150 μL. This implies a requirement of 300 fmol of DNA for detection, though again this is easily improved by using a smaller sample well. The ultimate mass limit of detection would be determined primarily by the detection system used. With state-of the art laser-induced fluorescence this could approach single molecule detection.[26]

Conclusions

TGF has been used to perform fast, simple nucleic acid assays that are easily incorporated in capillary or lab-on-a-chip formats. The significant advantages of TGF for microfluidic assays include the ability to concentrate targets prior to an assay, facile reaction of targets with probes because targets can be focused to a stationary point, the concentration and spatial separation of target-probe binding partners for sensitive detection, and the ability to thermally probe binding partners by operating TGF in a scanning mode. Finally, we note that the TGF approach described here is general and could also potentially be used to measure protein/protein, nucleic acid/protein, or drug/target binding.

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