

in water (0.1 M Tris-phenol buffer). A voltage of +3000 V was applied to the 10° C. end of the capillary and the 80° C. end was electrically grounded. FIG. 8 is the line profile monitoring the fluorescence emission from the PNA/DNA duplex that resulted from the mixing and the unreacted ssDNA. In FIG. 8, the top panel is a plot depicting a line profile before the introduction of PNA into the bulk flow and the bottom panel is a plot depicting a line profile 20 minutes after the introduction of PNA into the bulk flow. The single peak in the top panel is the ssDNA after 10 minutes of focusing from an initial concentration of 0.4 μm. The bottom panel shows two peaks corresponding to the PNA/DNA duplex and free ssDNA.

Additional experiments were performed with different ssDNA sequences that included perfect matches and perfect mismatches to a PNA probe sequence. The mismatched sequences did not hybridize and a new fluorescent band was not observed.

In an alternative measurement using temperature gradient focusing, the interactions between two materials are observable as a function of spatial position within a temperature gradient. For example, this method can be used to study duplex matching by first mixing together the two materials and allowing the two to hybridize to form a duplex. Subsequently, the hybridized sample is then focused along the cold side of the microchannel 710. The bulk flow is then adjusted so that the duplex moves towards the hot side of the fluid conduit. The duplex can be detected along the temperature gradient and the melting temperature of the duplex can be measured by detecting the change in intensity of the duplex peak as it is moved towards the hot end of the gradient. When the duplex reaches a position along the gradient where the local temperature is equal to the melting temperature of the duplex, the two materials forming the duplex will come apart and move away from the focused duplex band, and the duplex band will consequently decrease in intensity.

The following non-limiting example is provided to give a better understanding of how the interaction between two materials can be observed using temperature gradient focusing. In this example, a fluorescence melting profile of a PNA/DNA duplex that contained a single base pair mismatch (SBPM) was studied. The temperature gradient focusing separation was performed in a 30 μm I.D. fused silica capillary, 3 cm long. The capillary was mechanically and thermally anchored to two copper blocks at different temperatures to create a temperature gradient along a 2 mm section approximately midway along the length of the capillary. A temperature gradient from 20° C. to 80° C. was applied. The fluid used was 0.1 mol/L Tris(hydroxymethyl)aminomethane, 0.1 mol/L phenol in water (0.1 M Tris-phenol buffer). A solution of equal amounts of the PNA and DNA was prepared in the 0.1 M Tris-phenol buffer and introduced into the capillary via a fluid reservoir on the 20° C. end of the capillary. A voltage of +2000 V was applied to the 20° C. end of the capillary and the 80° C. end was electrically grounded. The pressure applied to the 80° C. end of the capillary was adjusted to control the bulk flow rate so that the PNA/DNA duplex was first focused on the 20° C. end of the 2 mm gradient section. The bulk flow was then progressively adjusted so that focused duplex band was moved spatially from the 20° C. end to the 80° C. end of the 2 mm gradient section. A plot of the measured fluorescence intensity as a function of distance along the gradient section is shown in FIG. 9 with the 20° C. on the right side of the graph and the 80° C. end on the left side of the graph. The green channel emission (circles) corresponds to free ssDNA while the red channel emission (squares) corresponds to the PNA/DNA

duplex that contains a single base pair mismatch. The melting temperature can be identified as the temperature at the position where the red channel curve has decreased to about half its initial intensity (approximately 800 μm in FIG. 9). Additional experiments compared the results obtained with a single base pair mismatch with those obtained when the DNA and PNA sequences were perfectly matched. After measuring the temperature inside the capillary to determine the temperature at each position along the capillary, a 7° C. difference was observed in the melting temperature between the perfect match and the single base pair mismatch. This result is comparable to results obtained with conventional UV absorbance-based melting curve experiments.

The two examples described above can be combined in an assay to first determine if there is a hybridization or binding interaction between two materials, and then if a hybridization event occurs, further testing of the melting temperature of the duplex can be performed by scanning the focused duplex through the temperature gradient and monitoring the signal as a function of position. The assay involves focusing ssDNA on the cold side of the gradient, and then flowing the PNA through the sample. If a hybridization event occurs, the resulting focused duplex peak can be concentrated on the cold side of the gradient to a desired concentration before sequentially scanning through the temperature gradient by changing the bulk flow so the duplex will move from cold to hot side of the gradient. The melting temperature can be determined from data such as the melting curve shown in FIG. 9. Comparisons of different melting curves and melting temperatures can then be used to determine the degree of mismatch (perfect match, single base pair mismatch, etc.) of the DNA and PNA sequences.

In another form of a mixing reaction/interaction using temperature gradient focusing, the PNA material can be replaced with a second single stranded DNA material that has been covalently or otherwise bound to a 'drag tag' or a molecule with an electrophoretic mobility that is less than that of the single stranded DNA by modifying the teaching of Vreeland, W. N., Meagher, R. J. & Barron, A. E. (2002) *Analytical Chemistry* 74, 4328-4333. Normally, there is only a very small difference in the electrophoretic mobility of single stranded and double stranded DNA. Consequently, it would be difficult to resolve the focused bands corresponding to the free single stranded DNA and the DNA/DNA duplex. Drag tags would therefore be used to reduce the electrophoretic mobility of the second single stranded DNA so that the drag-tag-DNA/DNA duplex would focus at a different position than the first single stranded DNA, so that both could be independently detected.

The present mixing reactions/interactions temperature gradient focusing can also be used to study enzyme/substrate interactions. For example, an enzyme could be initially focused within the separation conduit. The substrate could then be added to the bulk flow. The appearance of a focused product would signify that the enzyme/substrate reaction had occurred. Alternatively, the substrate could be focused first and subsequently introduce the enzyme into the bulk flow.

The present temperature gradient focusing methods for mixing reactions/interactions can be combined by selecting various aspects of the previously described methods. For example, nucleic acid-protein interactions or those that involve drug-target binding events can be analyzed using the present method. The only requirement is that at least one of the two input materials can be focused using temperature gradient focusing.

It will now be apparent to one of ordinary skill in the art that the present mixing reaction using temperature gradient focus-