Supporting information

A novel microfluidic mixer based on dual-hydrodynamic focusing for interrogating the kinetics of DNA-protein interaction

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List of Supplementary Material

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Fluid control system. The fluid control system utilized compressed nitrogen gas as pressure source, one reservoir with buffer solution and the other two reservoirs with the sample solutions. The pressure was controlled by a pressure reducer and monitored by a pressure gauge (Fig. S1).

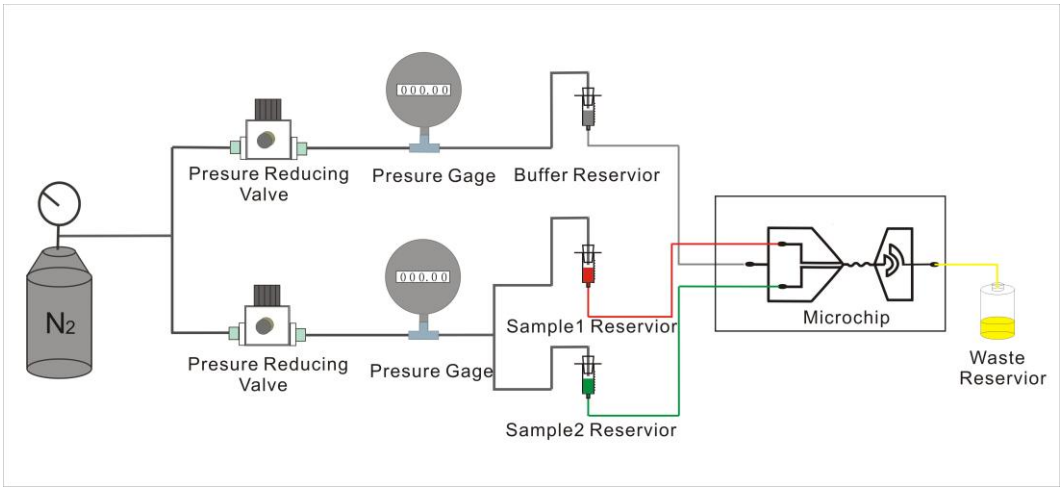

Fig. S1 Schematic of the fluidic control system.

Mixing result of the former structure. Fig. S2 showed the mixing result of our initial design. This design had a straight mixing channel (Fig.S2a). The mixing efficiency of fluorescein-labeled dextran and tetramethylrhodamine-labeled dextran at the observation channel was very poor (Fig. S2b).

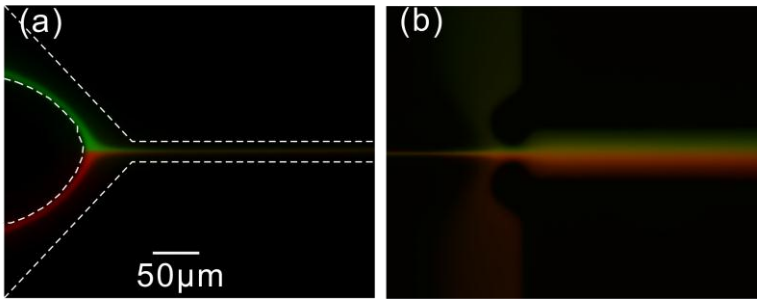

Fig. S2 Mixing result of the design with straight mixing channel.

Flow rate calibration. The volumetric flow rate through the inlet1 was measured by connecting the PTFE tubing to 100 μL Hamilton syringes, which were pressurized with compressed air.[1] When the fluorescence in the observation channel was homogeneous (Ps/Pc=1:1), the volumetric flow rate as a function of the driving pressure was shown in Fig. S3a. The volumetric flow rate showed very good linearity with pressure (Ps) between 12 kPa and 85 kPa.

To determine the flow rate in channels (the channels connected to the inlet 2/inlet 3 and the observation channel) where it was too low to measure with Hamilton syringes, we used fluorescent polystyrene beads (2.0 μm in diameter) as tracing particles to monitor the streamlines.[1] The microbeads were thoroughly mixed at a given ratio and suspended in buffer solution before use. Streamlines produced by the beads were recorded using a CCD camera. The longest streaklines corresponded to the beads traveling along the axis of symmetry (center line) of the channel, where the velocity was a maximum. The flow rate in the inlet 2 and inlet 3 was characterized as a function of Pc (Fig. S3b). For each P_c, the maximum velocity measured in the channel was converted to volumetric flow rate using the equations for developed laminar flow in a rectangular channel. When Pc was 35 kPa and 75 kPa, the volumetric flow rate through inlet 2 or inlet 3 was 0.27 μL/min and 0.52 μL/min, respectively. The flow rate in the observation channel was obtained for the calculation of the mixing dead time of the micromixer.

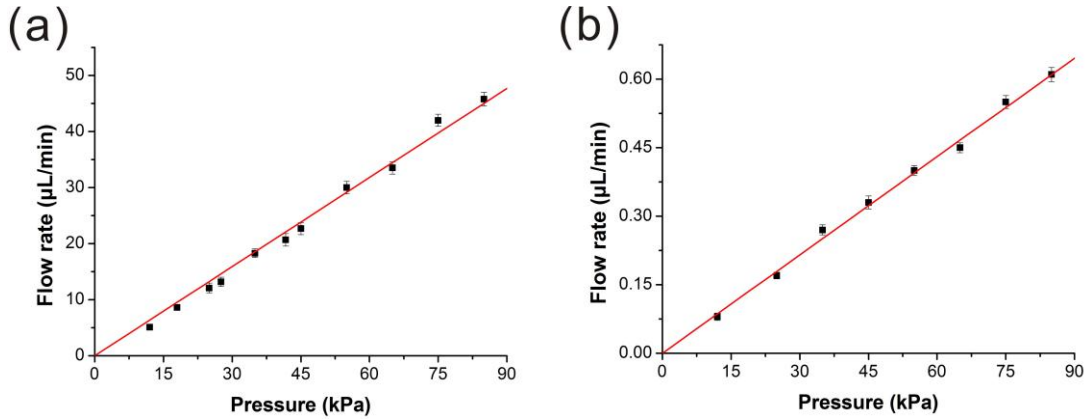

Fig. S3 Volumetric flow rates through the side channel (a) and the central channel (b) as a function of the pressure applied to inlet 1. The Ps/Pc (Ps, the pressure used at inlet 1; Pc, the pressure used at inlet 2 and inlet 3; pressure unit: kPa) was kept as 1:1, for the two solutions through the two central channels achieved complete mixing at this condition.

Fluorescence spectroscopy of the G-quardruplex. To verify the results obtained in the mixer, the FRET efficiency of the G-quadruplex under various conditions was further studied by fluorescence spectroscopy. Fluorescence measurements were carried out on a FP-6500 spectrofluorometer (Jasco, Japan) at 20 °C. The excitation and emission slits were both 10 nm. Excitation was set at 488 nm, and emission was collected from 500 to 650 nm. The fluorescence intensities were calculated by integrating the emission spectra from 500 to 530

nm for the donor and from 565 to 595 nm for the acceptor.[2] Solutions of 1 μM oligonucleotide (containing various concentrations of NaCl) and 0.5 μM SSBP were prepared and the mixtures were incubated at 20 °C for 24h.The FRET efficiency was calculated as shown in Table S1. The results showed that the binding of SSBP decreased the FRET efficiency of the G-quadruplex (except of the Control 1 with 0 mM NaCl), which fit well with the result obtained in the micromixer (Fig. 6) and the previous report.[3]

Table S1 FRET efficiency of various solutions.

Solution	FRET efficiency	Solution	FRET efficiency
DNA+Buffer	0.21	DNA+Buffer+SSBP	0.21
DNA+50 mM NaC	0.48	DNA+50 mM NaCl+SSBP	0.37
DNA+100 mM NaCl	0.65	DNA+100 mM NaCl+SSBP	0.47
DNA+150 mM NaCl	0.72	DNA+150 mM NaCl+SSBP	0.54

To confirm the result of Control 2 in the mixer, solutions of 1 μM oligonucleotide (containing 150 mM Na^+) and Tris-HCl buffer or 0.5 μM BSA were prepared. The mixtures were incubated at 20 °C for 24h and the fluorescence spectroscopy was shown as Fig. S4. The result displayed that the fluorescence of G-quadruplex/BSA was very similar to that of G-quadruplex/buffer, which revealed that no interaction occurred between G-quadruplex and BSA.

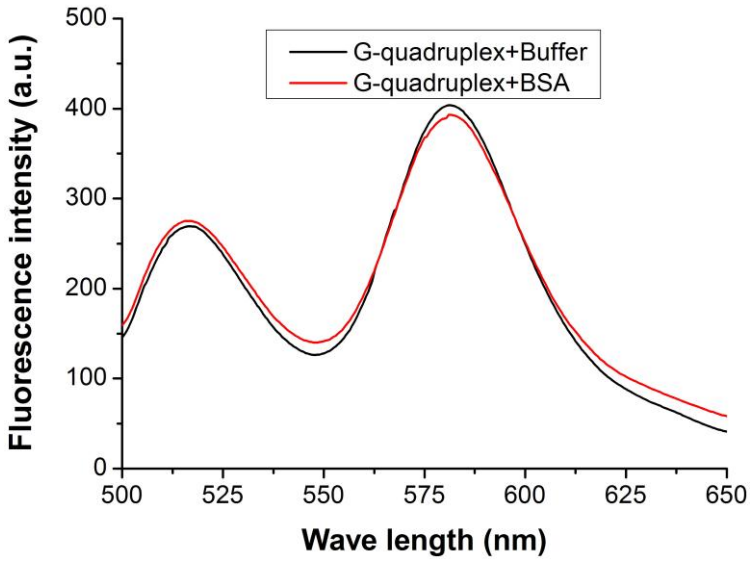

Fig. S4 Fluorescence spectroscopy of G-quadruplex/buffer and G-quadruplex/BSA.

Reference

(1) Gambin, Y.; VanDelinder, V.; Ferreon, A. C.; Lemke, E. A.; Groisman, A.; Deniz, A. A. *Nat. Methods* 2011, **8**, 239-241.

(2) Kan, Z. Y.; Yao, Y.; Wang, P.; Li, X. H.; Hao, Y. H.; Tan, Z. *Angew. Chem. Int. Edit.* 2006, **45**, 1629-1632.

(3) Zhuang, X. Y.; Tang, J.; Hao, Y. H.; Tan, Z. *J. Mol. Recognit.* 2007, **20**, 386-391.