Supporting Information

A power-free microfluidic chip for SNP genotyping using graphene oxide and a DNA intercalating dye

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Experimental Section

Materials and Instrumentation: SYBR Green I (SG) was obtained from Molecular Probes. The SG store solution was prepared with DMSO as 10,000×. GO was prepared according to our previous report.[24] Other chemicals were purchased from China National Pharmaceutical Group Corporation (Shanghai, China) as analytical grade and used without further purification. Fluorescence measurements were performed on an F-4500 fluorospectrameter (Hitachi, Japan). Fluorescence image was taken on a fluoscopy (Olympus BX43, USA). Oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. (China) and were purified by HPLC. All sequences were dissolved in MilliQ water and quantified as 100 μM by UV3010 (Hitachi, Japan) ready for use. The involved oligonucleotides are listed in Table 1.

Table 1 Sequences of oligonucleotides

Abbr / Type		Sequence
P1 / Probe		5'-TGCGAACCAGGAATT-3'
T1 / pcDNA[a]		5'-AATTCCTGGTTCGCA-3'
M	M1 / sm-T[b]	5'-AATTCCT<u>T</u>GTTCGCA-3'
	M2 / sm-A[b]	5'-AATTCCT<u>A</u>GTTCGCA-3'
	M3 / sm-C[b]	5'-AATTCCT<u>C</u>GTTCGCA-3'

a. perfectly complementary target; b. SNP target with single-base-mismatch (the mismatched base is underlined), and all SNP target were abbreviated as M, including M1, M2, and M3.

SG intercalation and GO quenching studies with diverse DNA structures. For DNA

hybridization, 2.5 μM of P1 and 2.5 μM of T1 (or M) were mixed in 4 μL of PBS (10 mM PB, 50 mM NaCl, pH 7.4) for 30 min at room temperature (RT). The control experiment was performed by employing equal volume of water instead of T1. For SG intercalation, 10 μL of SG (10×) was added and then Milli Q Water or PBS was used to make up 1 mL for fluorescence measurement (excitation at 497 nm, emission from 507 to 650 nm). For GO quenching analysis, 5 μL of GO (0.47 g/L) was added into above solutions and mixed vigorously, and the fluorescence spectra were recorded within 15s after addition of GO. For all the fluorescence data collection, a time gap from addition of GO to fluorescence spectra record was kept as 15s.

The optimization of salt concentration and temperature. 4 μL of hybridized DNA solution was added into 996 μL of PBS solutions with a series of NaCl concentrations (0.2, 0.5, 1.0, 2.0, 5.0, 10, 20, 50 mM) those were obtained by diluting with PBS (10 mM PB, 50 mM NaCl, pH 7.4). 10 μL of SG (10×) was added and mixed vigorously, and 5 μL of GO (0.47 g/L) was then added and incubated for 5 min for fluorescence detection. For GO quenching kinetics analysis, the fluorescence was recorded immediately after adding GO.

In order to gain the optimized temperature of SNP detection, 4 μL of hybridized solution, 996 μL of Milli Q water and 10 μL of SG (10 ×) were mixed and incubated at different temperatures (25, 30, 35, 40, 45, 50, and 55 ºC) for further fluorescence measurements. 5 μL of GO (0.47 g/L) was then added and incubated for 30s for fluorescence spectra record.

Sensitivity and Allele frequency analysis. For sensitivity analysis, P1 was hybridized with T1 (or M1) with a series of concentrations, to which 10 μL of SG (10 ×) were added and mixed vigorously, and fluorescence measurement was performed after addition of GO (5 μL, 0.47 g/L) for 5 min. The final concentration of P1 was 10 nM, and that of T1 (or M1) was 0.5 nM, 1 nM, 2 nM, 4 nM, 6 nM, 8 nM, and 10 nM, respectively. For allele frequency analysis, the mixture of M1 and T1 with different M1/(M1+T1) ratio (0, 5%, 10%, 20%, 40%, 60%, 80%, and 100%) was used and the total concentration of T1 and M1 is 20 nM.

Solid-phase assay on a PDMS-based microarray. The PDMS-based microarray was

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prepared as previous reported.[27] Briefly, a negative master was prepared on a silicon wafer with SU-8 photoresist. A plasma etcher was used to passivate the master, and then PDMS was cast on the surface of the master and followed by drying in oven. The PDMS was obtained through peeling off from the master as well as through-holes and waste reservoirs with appropriate size were punched. The PDMS chip was reversibly bonded to a flak PDMS slab (3 mm thick) and dimensions of the microchannels in the microfluidic chip are 100 μm (width) × 30 μm (height). The dimensions of whole chip are 3.5 cm (width) × 5 cm (length). For DNA detection, a mixture of DNA/SG in PBS was added to one inlet and equal volume of GO (0.47 g/L) to another inlet of the channel after the PDMS device was degassed in vacuum and sealed with a piece of adhesive tape. The fluorescence photo was taken on a fluoscopy (Olympus BX43, USA) and 490 nm exciting light source was employed.

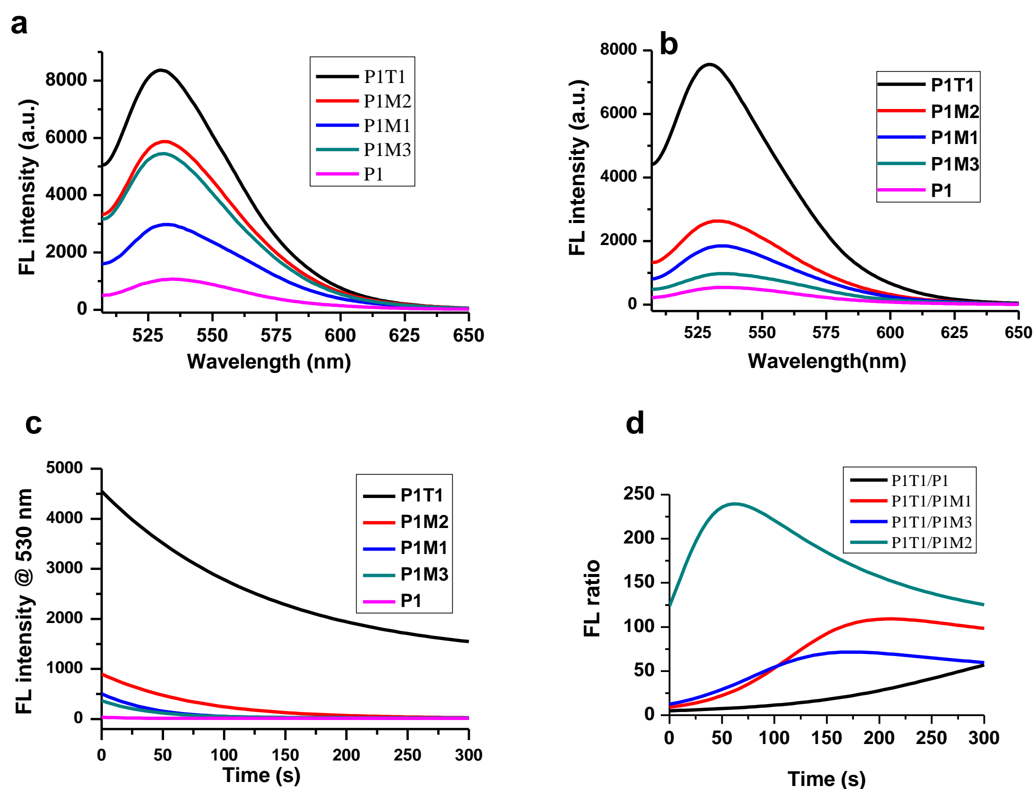
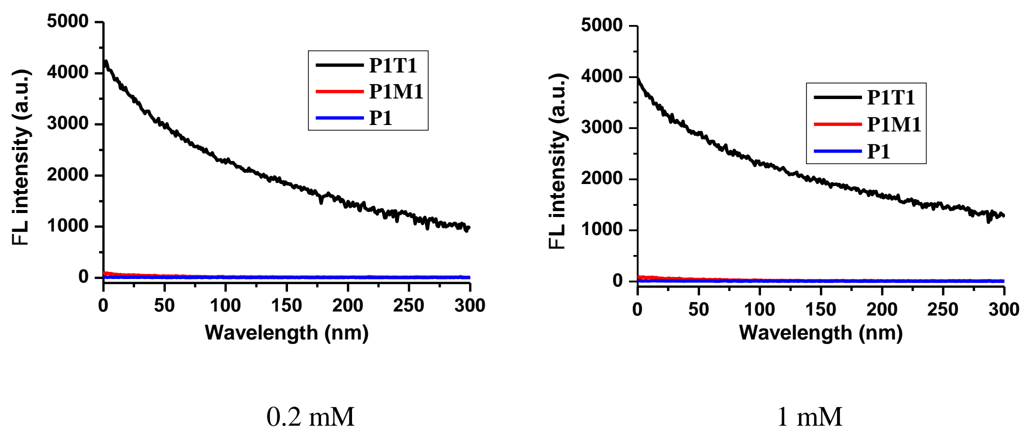


Figure S1 (a) Fluorescence spectra of SG with various DNA structures in 50 mM NaCl buffer. (b) Fluorescence spectra of SG/DNA complex in the absence of GO. (c) The quenching kinetics and (d) the FL ratio analysis for diverse DNA structures. The concentration of P1, T1, and M was 10 nM, respectively. 2.35 mg/L GO together with 0.01×SG was used. All procedures were performed in 0.2 mM NaCl buffer (except (a)) at RT. The fluorescence data was collected at 530 nm with Ex at 497 nm.



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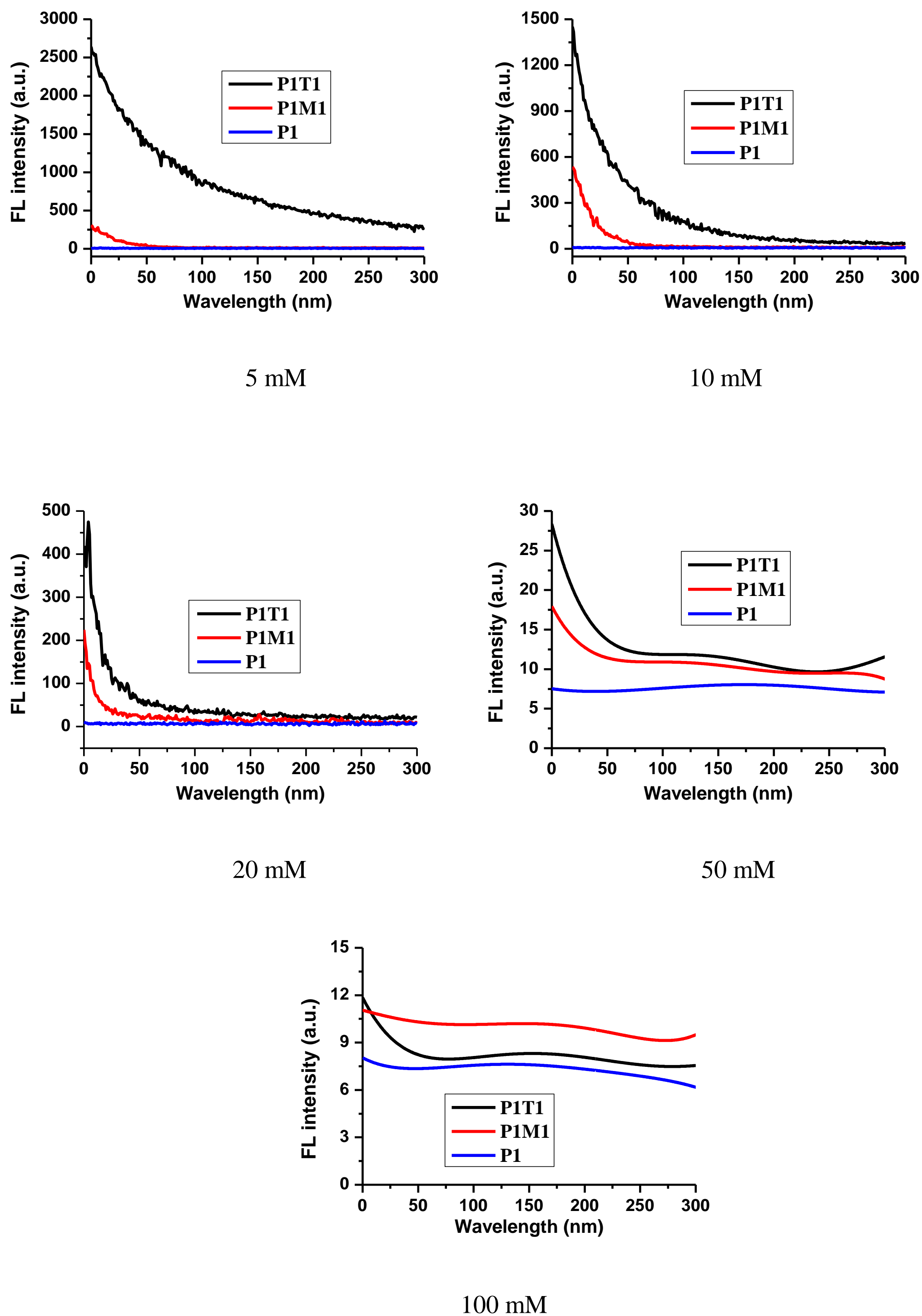

Figure S2 The fluorescence quenching kinetics of SG with various DNA structures by GO under different concentrations of PBS (0.2, 1, 5, 10, 20, 50, 100 mM NaCl). The concentration of probe DNA, pcDNA, SNP sequence was 10 nM. For ssDNA group, another 10 nM probe DNA was used instead of pcDNA. GO was 2.35 mg/L while SG was 0.01× in the FL cuvette, PBS containing 0.2 mM NaCl was used. All procedures were performed at RT. The FL data was collected at 525nm with Ex at 497 nm.

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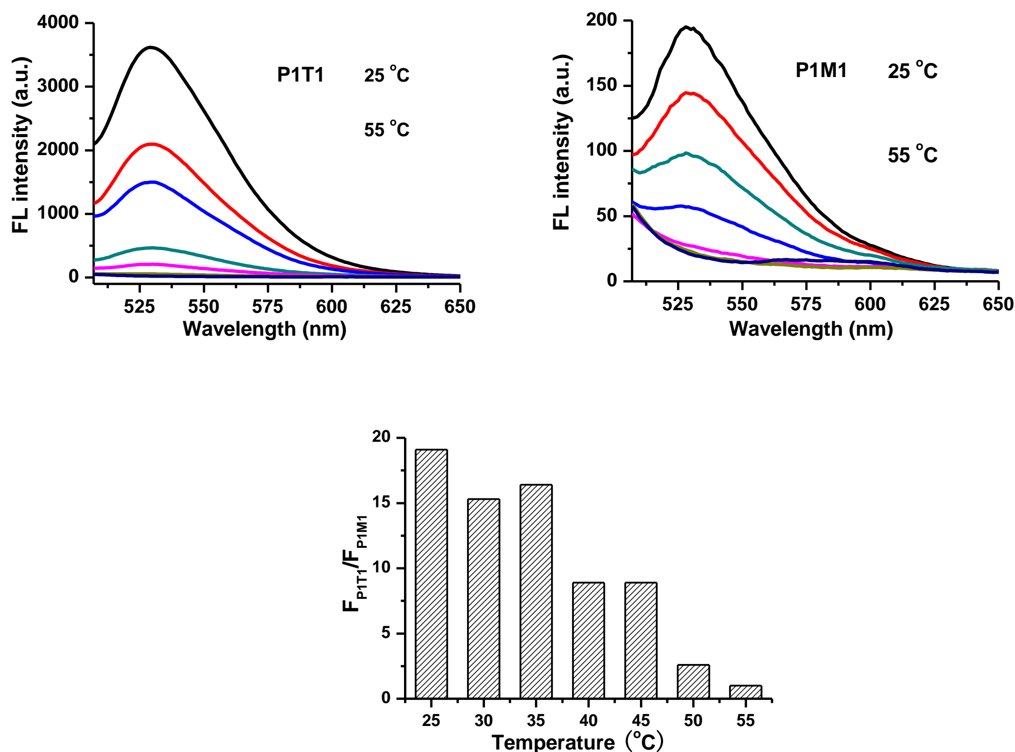

Figure S3 The fluorescence of P1T1 and P1M1, as well as F_{P1T1}/F_{P1M1} under different temperatures (25, 30, 35, 40, 45, 50, 55°C).

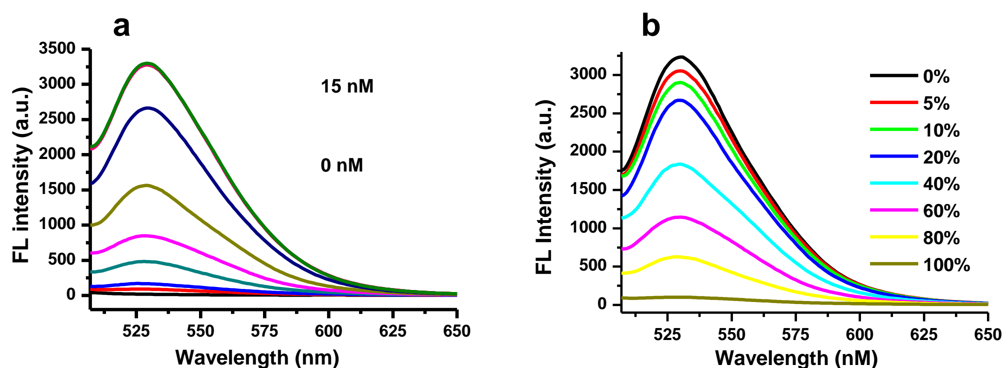

Figure S4. (a) Fluorescence spectra of SG/P1T1 in the presence of various concentrations of T1 (0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 15 nM). (b) Fluorescence spectra in the presence of different allele frequency (M1/(T1+M1) was 0, 5%, 10%, 20%, 40%, 60%, 80%, and 100%, and the total target DNA containing M1 and T1 was 20 nM.). Other conditions were same as Figure 1.

Table S1. FL ratio @530 nm of F_{P1T1}/F_{P1M1} and F_{P1T1}/F_{P1} in the presence of different concentrations of NaCl.

C_{NaCl} (mM)	0.2	1	5	10	20	50	100
F_{P1T1}/F_{P1M1}	82.2	123.6	27.1	3.55	2.45	1.41	1.18
F_{P1T1}/F_{P1}	273.9	191.2	53.3	5.69	3.27	1.53	1.46