Supporting Information for Analytical Chemistry

Pinpoint the Positions of Single Nucleotide Polymorphisms by a Nanocluster Dimer

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DNA-template AgNCs probes and target DNA hybridization experiments

For NCD-1, NC-probe-0 AgNCs solution (49 uL) was mixed with NC-probe-1 AgNCs (49 uL) solution, then DPBS (2.94 uL) was quickly added into the mixed probes solution to make sure the volume of solution same as that of NCD-4 after hybridization. NC-probe-0 and NC-probe-1 hybridized with each other. The solutions were kept in 37 °C water bath for 30 mins before the following fluorescence measurements. The experimental conditions of NCD-2 were same as NCD-1 except that NC-probe-1 AgNCs solution was replaced by NC-probe-2 AgNCs solution.

For NCD-4, NC-probe-0 AgNCs solution (49 uL) was mixed with 49 uL NC-probe-4 AgNCs solution (49 uL), then perfectly-matched (PM) DNA target (2.94 uL 250 uM, dissolved in DPBS) was quickly added into the mixed probes solution. The solutions were kept in 37 °C water bath for 30 mins before the following fluorescence measurements. The experimental conditions of NCD-3 were same as NCD-4 except that NC-probe-4 AgNCs solution was replaced by NC-probe-3 AgNCs solution.

The fluorescence excitation and emission spectral curves of Nanocluster Dimers (NCD-1, NCD-2, NCD-3 and NCD-4) after hybridization were shown in Figure S1-S4. The fluorescence emission spectral curves (excited at the maximum excitation wavelength) of Nanocluster Dimers (NCD-1, NCD-2, NCD-3 and NCD-4) before and after hybridization were also shown in Figure S1-S4.

The sensitivity of NCD-4 for target DNA detection

Under the optimal conditions, we investigated the sensitivity of the NCD-4 probe for target DNA detection. We obtained a linear response of the fluorescence intensity with respect to the concentration of PM DNA target over the range 50 nM–7.28 uM ($R^2 = 0.99$). Finally, we obtained the limit of detection (LOD) to be 27.4 nM. The linear equation is $y = 30.543x + 9.599$ (Figure S10).
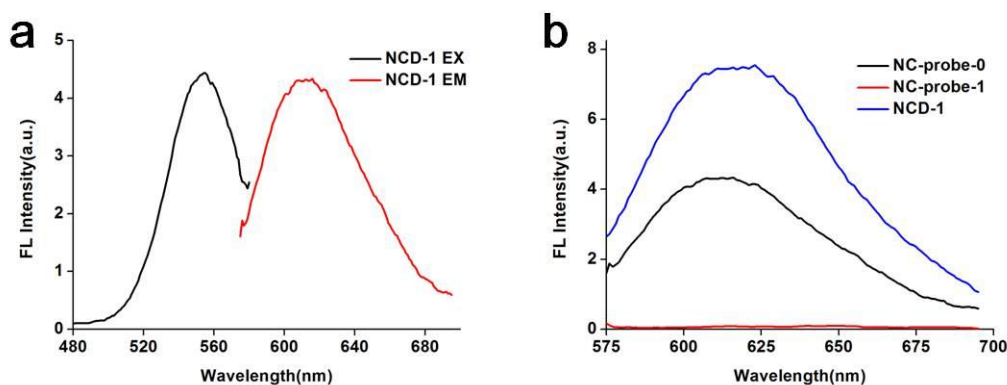
Figure S1. (a) Fluorescence excitation (in black) and emission (in red) spectra of NCD-1 after hybridization; (b) Fluorescence emission spectra of NC-probe-0 (in black), NC-probe-1 (in red), and NCD-1 (in blue) after hybridization excitated at 555 nm (Maximum emission of NCD-1 is at 613 nm).

Figure S2. (a) Fluorescence excitation (in black) and emission (in red) spectra of NCD-2 after hybridization; (b) Fluorescence emission spectra of NC-probe-0 (in black), NC-probe-2 (in red), and NCD-2 (in blue) after hybridization excitated at 565 nm (Maximum emission of NCD-2 is at 623 nm).

Figure S3. (a) Fluorescence excitation (in black) and emission (in red) spectra of NCD-3 after hybridization; (b) Fluorescence emission spectra of mixed solutions of NC-probe-0 and NC-probe-3 alone (in black), or hybridized with PM DNA target (in red) excitated at 560nm (Maximum emission of NCD-3 is at 625 nm).
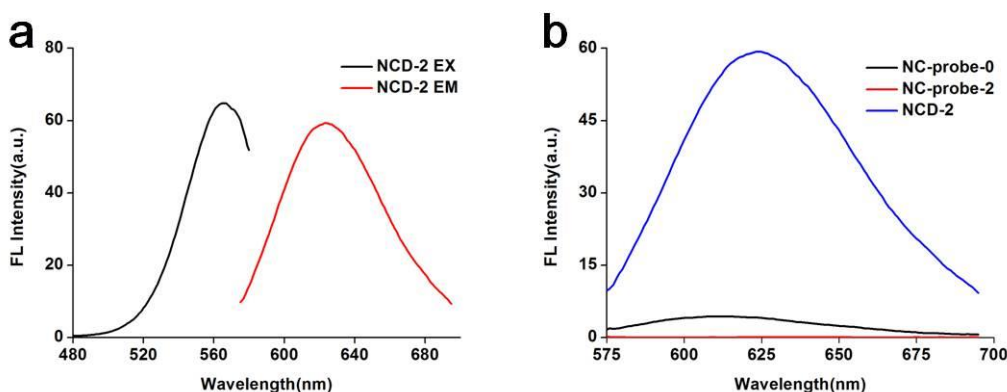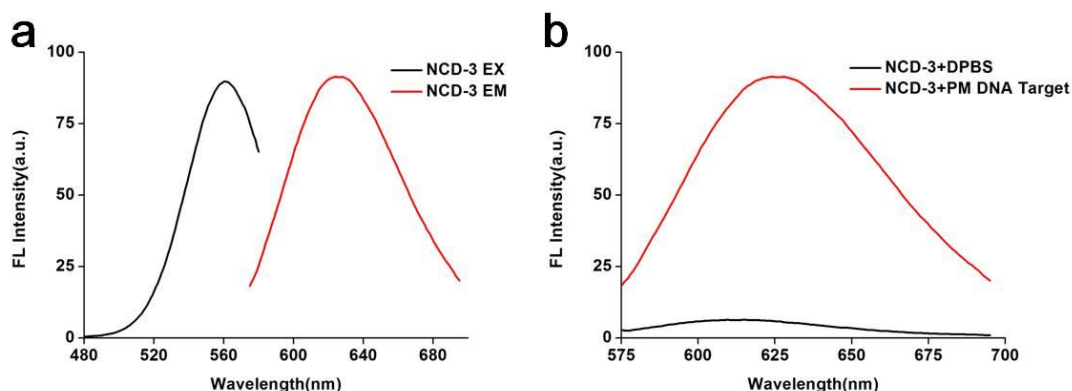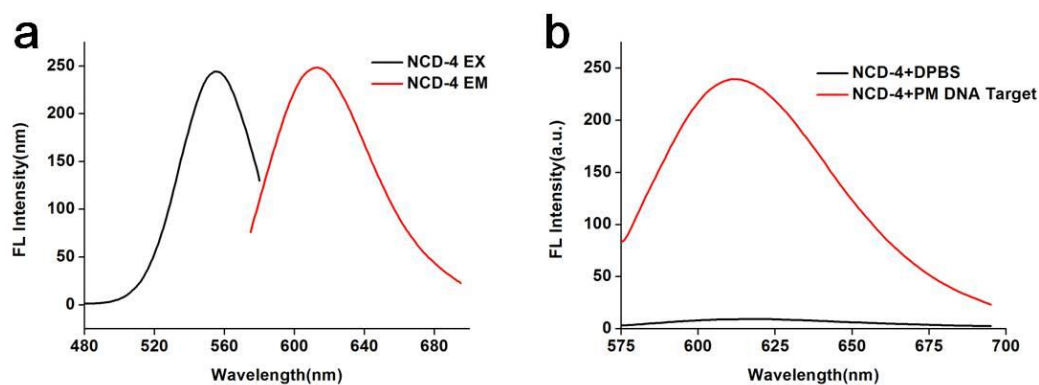
Figure S4. (a) Fluorescence excitation (in black) and emission (in red) spectra of NCD-4 after hybridization; (b) Fluorescence emission spectra of mixed solutions of NC-probe-0 and NC-probe-4 alone (in black), or hybridized with PM DNA target (in red) excited at 555nm (Maximum emission of NCD-4 is at 613 nm).

Figure S5. HRTEM micrographs of (a) AgNCs of NC probe-0 and NC probe-4 without target DNA, (b) The close proximity arrangement of two AgNCs of NC probe-0 and NC probe-4 with PM DNA. Scale bars = 20 nm.

Figure S6. The fluorescence decay of NC-probe-0 AgNCs (in green), NC-probe-4 AgNCs (in blue) and NCD-4 after hybridization (in red), and their fitting lines (in black, NC-probe-0 and NC-probe-4 with a mono-exponential function, NCD-4 with a dual-exponential function)

Figure S7. (a) Fluorescence intensities of NCD-4 for N2-P1, N2-P5 and N2-P9; (b) Fluorescence intensities of NCD-4 for N3-P1, N3-P5 and N3-P9; (c) Fluorescence intensities of NCD-4 for N4-P1, N4-P5 and N4-P9; (maximum emission 613nm, excited at 555nm, all the fluorescence intensities were measured at least three times)
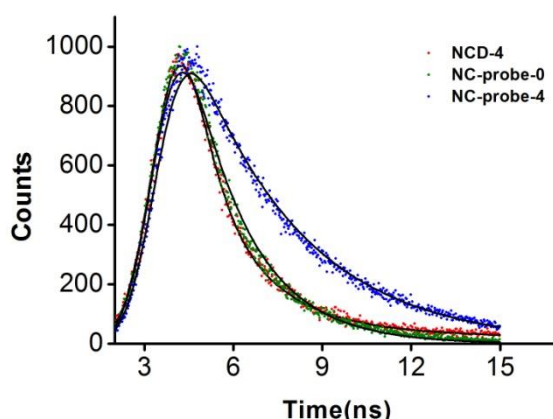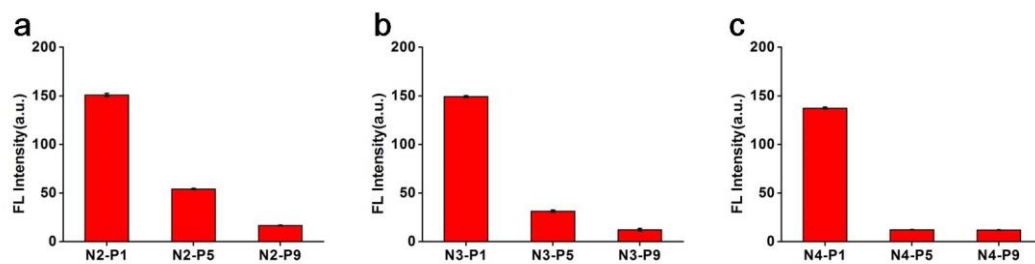

Figure S8. (a) Fluorescence intensities of NCD-4 for N1-P1, N2-P1, N3-P1 and N4-P1; (b) Fluorescence intensities of NCD-4 for N1-P9, N2-P9, N3-P9 and N4-P9; (maximum emission 613nm, excited at 555nm, all the fluorescence intensities were measured at least three times)

Figure S9. The kinetics of the mixed solutions of NC-probe-0 and NC-probe-4 alone, or hybridized with PM DNA target at 37 °C

Figure S10. (a) Fluorescence emission spectra (excited at 555nm) of the solution with various concentrations of PM DNA target: 0, 5.0×10^{-8}, 1.0×10^{-7}, 2.0×10^{-7}, 5.0×10^{-7}, 1.0×10^{-6}, 2.0×10^{-6}, 2.5×10^{-6}, 7.28×10^{-6} mol·L^{-1}; (b) Linear relationship between the fluorescence intensity (maximum emission 613nm) and the concentration of PM DNA (each error bar indicates the standard deviation).

Figure S11. Fluorescence emission spectra of mixed solutions of NC-probe-0-tsd and NC-probe-4-tsd hybridized with normal-C-target (in black), or TSD carrier-G-target (in red) excitated at 555nm (Maximum emission of NCD-4 is at 613 nm) in clean buffer (a); and in 15% diluted human serum (b); (c) Fluorescence intensities of emission spectra in Figure S11a-b at 613nm (all the fluorescence intensities were measured at least three times).

Table S1. All the Sequences of oligonucleotides used in this work. NC-nucleation sequences of NC-probes in italic and underlined. Hybridization sequences of NC-probes in boldface. The SNPs sites and the nucleotides of target DNA are shown in red and boldface. T nucleotides spacers of target DNA are shown in red and underlined. The SNP information of Tay-Sachs disease mutations is obtained from http://www.snpedia.com.

Name of Sequences	Sequences from 5' to 3'
NC probe-0	*CCCTAACTCCCC*AGATACAGCAAGAAGTT
NC probe-1	*CCTCCTTCCTCC*AACTTCTTGCTGTATCT
NC probe-2	AACTTCTTGCTGTATCT*CCTCCTTCCTCC*
NC probe-3	TTGAAGAACGACATAGA*CCTCCTTCCTCC*
NC-probe-4	TTGAAGAACGACATAGA*CCCTAACTCCCC*
PM DNA	AACTTCTTGCTGTATCTTCTATGTCGTTCTTCAA
N1-P1	AACTTCTTGCTGTATC**C**TCTATGTCGTTCTTCAA
N1-P5	AACTTCTTGCTG**A**ATCTTCTATGTCGTTCTTCAA
N1-P9	AACTTCTT**A**CTGTATCTTCTATGTCGTTCTTCAA
N2-P1	AACTTCTTGCTGTAT**GA**TCTATGTCGTTCTTCAA
N2-P5	AACTTCTTGCT**CA**ATCTTCTATGTCGTTCTTCAA
N2-P9	AACTTCT**AC**CTGTATCTTCTATGTCGTTCTTCAA
N3-P1	AACTTCTTGCTGTA**AGA**TCTATGTCGTTCTTCAA
N3-P5	AACTTCTTGC**ACA**ATCTTCTATGTCGTTCTTCAA
N3-P9	AACTTC**AAC**CTGTATCTTCTATGTCGTTCTTCAA
N4-P1	AACTTCTTGCTGT**TAGA**TCTATGTCGTTCTTCAA
N4-P5	AACTTCTTG**GACA**ATCTTCTATGTCGTTCTTCAA
N4-P9	AACTT**GAAC**CTGTATCTTCTATGTCGTTCTTCAA
P1	AACTTCTTGCTGTATC**C**TCTATGTCGTTCTTCAA
P2	AACTTCTTGCTGTAT**T**TTCTATGTCGTTCTTCAA
P3	AACTTCTTGCTGTA**G**CTTCTATGTCGTTCTTCAA
P4	AACTTCTTGCTGT**G**TCTTCTATGTCGTTCTTCAA
P5	AACTTCTTGCTG**A**ATCTTCTATGTCGTTCTTCAA
P6	AACTTCTTGCT**A**TATCTTCTATGTCGTTCTTCAA
P7	AACTTCTTGC**C**GTATCTTCTATGTCGTTCTTCAA
P8	AACTTCTTG**G**TGTATCTTCTATGTCGTTCTTCAA
P9	AACTTCTT**A**CTGTATCTTCTATGTCGTTCTTCAA
P10	AACTTCT**A**GCTGTATCTTCTATGTCGTTCTTCAA
P11	AACTTC**G**TGCTGTATCTTCTATGTCGTTCTTCAA
P12	AACTT**G**TTGCTGTATCTTCTATGTCGTTCTTCAA
P13	AACT**G**CTTGCTGTATCTTCTATGTCGTTCTTCAA
P14	AAC**A**TCTTGCTGTATCTTCTATGTCGTTCTTCAA
P15	AA**T**TTCTTGCTGTATCTTCTATGTCGTTCTTCAA
P16	A**C**CTTCTTGCTGTATCTTCTATGTCGTTCTTCAA
P17	**C**ACTTCTTGCTGTATCTTCTATGTCGTTCTTCAA
N1-P4 T-T	AACTTCTTGCTGT**T**TCTTCTATGTCGTTCTTCAA
N1-P4 T-C	AACTTCTTGCTGT**C**TCTTCTATGTCGTTCTTCAA

Name of Sequences	Sequences from 5' to 3'
N1-P4 T-G	AACTTCTTGCTGT**G**TCTTCTATGTCGTTCTTCAA
NC probe-0-tsd	*CCCTAACTCCCC*CCAATCACCAGAGCCTT
NC-probe-4-tsd	**ACATACAAGCCTCTCCA***CCCTAACTCCCC*
Normal-C-target	AAGGCT**C**TGGTGATTGGTGGAGAGGCTTGTATGT
TSD carrier-G-target	AAGGCT**G**TGGTGATTGGTGGAGAGGCTTGTATGT
PM DNA-spacer-1T	AACTTCTTGCTGTATCT**T**TCTATGTCGTTCTTCAA
PM DNA-spacer-5T	AACTTCTTGCTGTATCT-(T)$_5$-TCTATGTCGTTCTTCAA
PM DNA-spacer-10T	AACTTCTTGCTGTATCT-(T)$_{10}$-TCTATGTCGTTCTTCAA
PM DNA-spacer-15T	AACTTCTTGCTGTATCT-(T)$_{15}$-TCTATGTCGTTCTTCAA
PM DNA-spacer-20T	AACTTCTTGCTGTATCT-(T)$_{20}$-TCTATGTCGTTCTTCAA
PM DNA-spacer-25T	AACTTCTTGCTGTATCT-(T)$_{25}$-TCTATGTCGTTCTTCAA

Table S2. Gibbs binding free energy (ΔG) for the NC-Probe-0 and DNA target with single-base substitutions at different positions ("P0" means perfectly matched target). The values of ΔG shown in Table S2, are refered to the OligoAnalyzer 3.1 from the IDT Integrated DNA Technologies. http://sg.idtdna.com/calc/analyzer.

Mismatched position	P0	P1	P2	P3	P4	P5	P6	P7
ΔG/(kcal/mole)	-27.54	-25.95	-24.37	-22.9	-21.93	-20.59	-18.64	-17.04

P8	P9	P10	P11	P12	P13	P14	P15	P16	P17
-13.9	-11.95	-13.64	-15.6	-17.54	-19.14	-20.71	-22.66	-24.26	-25.6