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Supporting Information

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Signal Amplifying Conjugated Polymer-DNA Hybrid Chips

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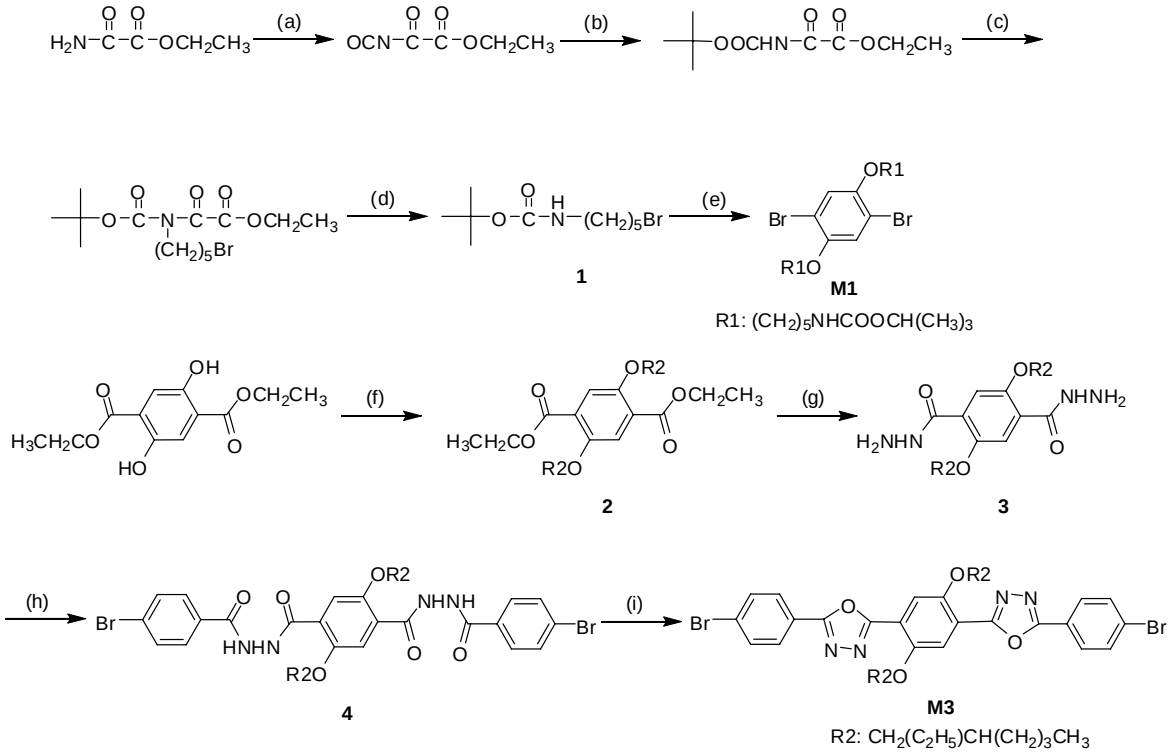
Section 1: Synthesis

Materials and Methods for monomer and P1 synthesis. All solvents and reagents were used without further purification as received from Sigma-Aldrich Chemical Co. UV/Vis absorption spectra were recorded with a Varian Cary50 UV/Vis spectrophotometer. Photoluminescence spectra and quantum yield in solution and solid state were obtained by using PTI QuantaMaster™ spectrofluorometers equipped with an integrating sphere. Gel permeation chromatography (GPC) was used to determine number and weight average molecular weights and molecular weight distributions, M_w/M_n, of the polymer samples with respect to polystyrene standards (Waters Corp.) in tetrahydrofuran as an eluent. ^{1}H NMR spectra (500 MHz) and ^{13}C NMR spectra (125 MHz) were obtained from Varian Inova 500 NMR instrumentation. High-resolution mass spectra were obtained from VG (Micromass) 70-250-S magnetic sector mass

spectrometer. Melting point was measured by PerkinElmer differential scanning calorimetry (DSC7).



Scheme 1. Monomer synthesis: (a) Oxalyl chloride, methylene chloride, 0 °C → 25 °C, 12 h. (b) *t*-butanol, toluene, 0 °C → 40 °C, 15 min. (c) 1, 5-dibromopentane, *t*-BuOK, DMF, 40 °C, 1 h. (d) LiOH, THF, water, r. t., 3 h. (e) 2, 5-dibromohydroquinone, K_2CO_3, DMF, 70 °C, 48 h. (f) 2-Ethylhexylbromide, K_2CO_3, DMF, 80 °C, 48 h. (g) H_2NNH_2, ethanol, 78 °C, 24 h. (h) 4-bromobenzoyl chloride, pyrindine, NMP, 12 h. (i) $POCl_3$, reflux, 12 h.

***N*-5-Bromopentyl *tert*-butyl carbamate (1).** The compound **1** was prepared by previous literature with slight modification of the length of alkyl side chain.[1] 25.6 mL Oxalyl chloride solution (50 mmol, 2 *M* in methylene chloride) was added to a 100 mL flask and it was cooled to 0 ^{o}C. Then, 5 g ethyl oxamate (43 mmol) was added to the reactor. The solution was refluxed overnight. After the removal of solvent and

unreacted oxalyl chloride, the product was purified by vacuum distillation at 65-70 ^{o}C. The obtained product was 2.38 g (yield 39 %). To 2.38 g ethyloxalyl isocyanate dissolved in 20 mL toluene was added dropwise 1.7 g *tert*-butanol dissolved in 4 mL toluene at 0 ^{o}C. The solution was heated to 40 ^{o}C for 15 min. After the removal of solvent and remaining *tert*-butanol, the crude product was dried in vacuum. The obtained product was 3.55 g (yield 96 %). To a 100 mL flask were added 35 mL DMF, 3.55 g *N*-*tert*-butoxycarbonyl ethyl oxamate, and 1.83 g potassium *tert*-butoxide (16.3 mmol). The solution was stirred at 60 ^{o}C for 1 h, and then the solution was added dropwise to the reactor containing 37.6 g 1,5-dibromopentane (10 equiv to ethyl oxamate). The solution was stirred at 60 ^{o}C for 1 h. After the removal of the unreacted 1,5-dibromopentane under vacuum, the product was extracted with methylene chloride. The solution was washed with water and dried with $MgSO_4$. The crude product was purified by column chromatography using ethyl acetate/hexanes (1/4, v/v). The obtained product was 3.9 g (yield 81 %). To a 100 mL flask were added 50 mL THF, 3.9 g *N*-*tert*-butyloxycarbonyl *N*-5-bromopentyl ethyl oxamate, and 1.66 g LiOH (39.7 mmol) dissolved in 20 mL water. The solution was stirred at room temperature for 3 h. The solution was diluted with water and extracted with methylene chloride. The organic layer was dried with $MgSO_4$ and concentrated to give product. The obtained product was 2.9 g (yield 83 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 4.55 (broad s, 1H, N-H), 3.40 (t, 2 H, CH_2), 3.13 (m, 2H, CH_2), 1.5-1.85 (m, 6H, CH_2), 1.41 (s, 9H).

Synthesis of *tert*-butyl 5.5'-(2,5-dibromo-1,4-phenylene)bis(oxy)bis(pentane-5,1-diyl)dicarbamate (M1). To a 250 mL 2-neck round-bottomed flask equipped with condenser were added compound **1** (5.00 g, 18.8 mmol), 2,5-dibromohydroquinone (1.69 g, 6.26 mmol), and potassium carbonate (3.46 g, 25.0 mmol) in 20 ml of

dimethylformamide (DMF). The flask was purged with extra pure Ar gas and placed in a 75 °C constant temperature oil bath. The reaction was carried out for 48 h with continuous stirring, and then cooled down. DMF was removed at reduced pressure by a rotary evaporator. The crude mixture was dissolved in chloroform and washed with water by extraction (4 times). The organic layer was dried by stirring with $MgSO_4$ and then filtered. The mixture was concentrated by the removal of chloroform. An additional purification was conducted by column chromatography. (ethyl acetate : hexane = 2 : 5 v/v). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 7.07 (s, 2H, aromatic), 4.58 (broad s, 2H, N-H), 3.95 (t, 4H, CH_2), 3.15 (m, 4H, CH_2), 1.5-1.85 (m, 12H, CH_2), 1.41 (s, 18H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ/ppm 160.0, 150.0, 118.5, 111,1, 79.1, 70.0, 40.4, 29.7, 28.7, 28.4, 23.2. HRMS (Voltage ES+) : calculated m/z of [M+Na]+ 659.1307; measured m/z 659.1315. Melting point: 98 °C.

Synthesis of 2,5-bis(2-ethylhexyloxy)terephthalohydrazide (**3**) 11.43 g of diethyl 2,5-bis(2-ethylhexyloxy)terephtalate (**2,** liquid at 25 °C, ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 7.34 (s, 2H, aromatic), 4.37 (q, 4H, -COOC<u>H</u>$_2$-), 3.89 (d, 4H, -OC<u>H</u>$_2$CH-), 1.73 (m, 2H, -C<u>H</u>-), 1.57-0.91 (m, 26H, alkyl), 0.90 (t, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ/ppm 166.4, 151.7, 124.5, 116.1, 71.8, 61.3, 39.5, 30.4, 29.1, 23.7, 23.0, 14.3, 14.1, 11.1. HRMS (Voltage ES+) : calculated m/z of [M+H]+ 479.3373; measured m/z 479.3361.), prepared with slight modification by previous literature[2], and hydrazine monohydrate (17 ml) were added into 100 ml anhydrous ethanol (99.5 %) and the mixture was stirred at 78 ^{o}C for 24 h. The mixture solution was cooled down and poured into 1800 ml water. Solids was collected by filtration and dried in vacuo. Additional recrystallization was done by ethanol to give a white cotton-like products of **3** (Yield: 46 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 9.18 (s, 2H, NH), 7.85 (s, 2H, aromatic),

4.17 (s, 4H, NH_2), 4.08 (d, 4H, CH_2), 1.83 (m, 2H, CH), 1.21-1.54 (m, 16H, CH_2), 0.97 (s, 12H, CH3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ/ppm 165.5, 151.0, 123.0, 115.7, 72.2, 39.4, 30.8, 29.0, 24.2, 23.0, 14.0, 11.1. HRMS (Voltage ES+) : calculated m/z of [M+H]+ 451.3284; measured m/z 451.3278. Melting point: 65 °C.

Synthesis of 5,5'-(2,5-bis(2-ethylhexyloxy)-1,4-phenylene)bis(2-(4-bromophenyl)-1,3,4-oxadiazole) (**M3**) To a 250 ml 2 neck round bottom flask were added compound **3** (4, 81 g, 10.7 mmol), 4-bromobenzoyl chloride (4,92 g, 22.47 mmol), pyridine (5 ml), and NMP 135 ml. After vigorous stirring for a while, the solution became a gel and the reaction continued overnight. The mixture was poured into 3000 ml of water and filtered to collect dihydrazide compound (**4**). Additional purification was done by recrystallization in chloroform. However, compound **4** showed a limited solubility in organic solvents, so reaction was proceeded without further characterization. 7.63 g of compound **4** was dissolved in 150 ml of phosphorus oxychloride and the solution was refluxed for 24 hr. The mixture was poured into 1500 ml water and the appearing solids were collected by filtration and drying in-vacuo. White powder product (**M3**) was obtained from recrystallization in benzene (Yield: 76 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 8.04, 7,69 (dd, J= 175, 11 Hz, 8H, aromatic), 7.86 (s, 2H, aromatic), 4.08 (d, 4H, CH_2), 1.84 (m, 2H, CH), 1.29-1.67 (m, 16H, CH_2), 0.96 (t, 6H, CH_3), 0.90 (t, 6H, CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ/ppm 164.7, 163.6, 151.0, 132.4, 128.4, 126.5, 122.9, 116.4, 114.4, 71.8, 39.7, 30.4, 29.1, 23.8, 23.0, 14.1, 11.2. HRMS (Voltage ES+) : calculated m/z of [M+H]+ 779.1808; measured m/z 779.1835. Melting point: 168 °C.

Polymer Synthesis (**P1**) To a 50 ml of Schlenk flask were added **M1** (132.8 mg, 0.208 mmol), **M2** (232.3 mg, 0.416 mmol), **M3** (162.4 mg, 0.208 mmol), THF (9ml) and 1M K_2CO_3 (5 ml). Degassed tetrakis(triphenylphosphine)palladium(0) (5 mol%) in THF (1

ml), prepared in a separate Schlenk, was transferred to the monomer mixture by cannula and the monomer solution was degassed by several cycles of vacuum and argon purging. Polymerization was carried out at 80 °C for 36 h. The solution of the reaction mixture was precipitated in 100 ml of methanol and filtered. Solid product was washed by water and acetone 3 times. Further purification was done by extraction with chloroform/water to give precursor polymer. 10 ml of trifluoroacetic acid (TFA) was carefully added to polymer in chloroform (10 ml) and the polymer solution was stirred at room temperature for 6 h to cleave *t*-BOC group. After evaporation of solvent and TFA, the polymer was re-dissolved in chloroform and washed with 1 M KOH solution, followed by NaCl, and deionized water to give yellow polymer (**P1**) (Yield: 120 mg). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 8.29 (d, 4H, aromatic), 7.93 (d, 4H, aromatic), 7.5-7.9 (m, 14H, aromatic), 7.10 (s, 2H, aromatic), 4.15 (d, 4H, CH_2), 3.95 (t, 4H, CH_2), 3.08 (m, 4H, CH_2), 2.09 (broad s, 4H, NH_2), 1.91 (m, 2H, CH), 1.12-1.83 (m, 84H, CH_2), 1.01 (t, 6H, CH_3), 0.93 (t, 6H, CH_3), 0.80 (t, 12H, CH_3). The number/weight average molecular weight was calculated with the polymer before cleavage of *t*-BOC due to the limited solubility of P1 in tetrahydrofuran as a GPC eluent, M_n = 51,000, PDI = 4.4.

Section 1 References

1. F. Berree, J.-P. Bazureau, G. Michelot, M. Le Corre, *Synth. Commun.* **1999**, *29*, 2685-2693.
2. T.-Y. Wu, R.-B. Sheu, Y. Chen, *Macromolecules* **2004**, *37*, 725-733

Section 2: DNA Chip Fabrication

Polymer immobilization onto a glass substrate. A glass slide (25 mm × 75 mm) was dipped in $NH_4OH/H_2O_2/H_2O$ (40 ml/40 ml/160 ml) at 80 °C for 1 h and rinsed with DI water (30 ml). After drying, the slide was soaked in pirahna solution (H_2SO_4 : H_2O_2 = 35 ml : 15 ml) for overnight, washed with DI water (30 ml) and dried with a stream of air. It was transferred into a solution of 97% aminopropyltrimethoxysilane (APTMS) (2 ml), DI water (2 ml), and methanol (48 ml) and sonicated for 30 min. It was rinsed with methanol (30 ml) and water (30 ml), and then dried with a stream of air. The slide was baked at 120 °C for 30 min. Amino-functionalized glass slide was reacted with 1,4-diphenylenediisothiocyanate (100 mg) in dimethylformamide (DMF) (54 ml) and pyridine (6 ml) for 2 h. It was washed with 30 ml of DMF, 30 ml of methylenechloride and dried. Only one side of the slide was reacted with **P1** (2 mg) in pyridine (0.5 ml) and chloroform (9 ml). The slide was subsequently washed with chloroform, methylene chlorides, and DI water. Further cleaning steps of the slide were achieved by sonication in chloroform for 5 min and drying in a vacuum oven. For comparison of FRET efficiency, an amine functionalized glass slide without **P1** (a glass after step (a) in Figure 3 (a)) was used as a control slide.

Light directed on-chip oligonucleotide synthesis.

The glass slide was enclosed in a holder connected to a DNA synthesizer. Oligonucleotide synthesis was performed using the standard phosphoramidite chemistry[3] except for the deprotection step, where a photogenerated acid (PGA) was used to deprotect the terminal dimethoxytrityl protecting group at selected reaction sites (Figure S1).[4] The synthesizer is coupled to an optical unit for digital photolithographic projection using a Digital Light Projector (Texas Instruments). At each deprotection

step, the slide holder was filled with the PGA precursor solution in CH_2Cl_2 and a predetermined light pattern was projected onto the device surface to trigger the formation of acid. The DNA synthesis reagents were obtained from Glen Research. A DNA patterned image after hybridization was obtained from a GenePix 4000B microarray scanner (Molecular Devices Corp.) with dual lasers (532 nm/17 mW, 635 nm/10mW).

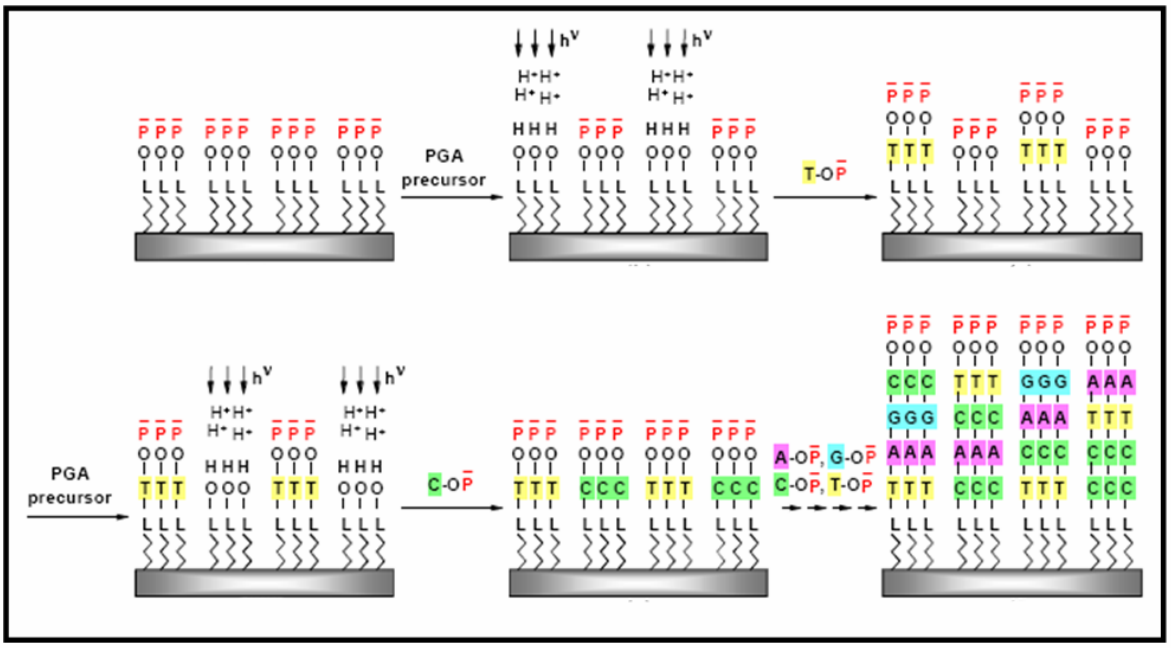


Figure S1. Schematic representation of light directed on-chip oligonucleotide synthesis.

Section 2 References

3. L. J. McBride, M. H. Caruthers, *Tetrahedron Lett.* **1983**, *24*, 245-248.
4. a) X. Gao, E. LeProust, H. Zhang, O. Srivannavit, E. Gulari, P. Yu, C. Nishiguchi, Q. Xiang, X. Zhou, *Nucleic Acids Res.* **2001**, *29*, 4744-4750; b) L. J. Hornbeck, *SPIE Eur. Proc.* **1996**, *2783*, 135-145.

Section 3: Hybridization Test

Hybridization Test and Analysis 1 ml of 6×SSPE (900 mM sodium chloride, 60 mM sodium hydrogen phosphate, 6 mM EDTA, pH 7.4) was added onto the slide and removed after 5 min. 150 μl (20×SSPE/Acetylated BSA/water = 3/1/6) of SSPE solution was dropped onto the slide and removed. Hybridization solution (20×SSPE: 15 μl, Acetylated BSA: 5 μl, water: 29 μl, HEX-labeled DNA, 5'-HEX-aca cat cac gga tgt-3': 0.5 μl, Cy5-labeledDNA, 5'-gaa ata atg ctt cgt cga tat tag act tct act gcg gat cat aca-Cy5-3': 0.5 μl) was heated to 65 °C to prevent loop formation of the target oligo. 2-3 drops of the hybridization solution of analyte DNA were applied onto the slide. The slide was kept in a humid chamber and incubated at 25 °C for 1 h. The slide was rinsed with 6×SSPE and iced water and dried with a stream of air. The slide was examined by using a fluorescence scanner and PL intensity before and after hybridization was investigated by using a fluorescent spectrophotometer (PTI QuantaMaster™ Spectrofluorometers with an integrating sphere). Hybridization tests with 1-mismatch (5'-HEX-aca cat ctc gga tgt-3') and a non-complementary DNA (5'-HEX-tgt gta gtg cct aca-3') were also conducted in the same condition as for the complementary DNA. The fluorescence images in Figure 4a inset were obtained by using BX41 Fluorescence microscope, DP71 digital camera (Olympus), and Microsuite 5 Biological Suite Software (Olympus) and are background (prehybridization) subtracted. Direct excitation of the dye and **P1** excitation for amplification were carried out at 500 nm and 405 nm, respectively.

Dimethyltrityl (DMT) quantification to measure the density of DNA on chips.

The amount of DMT, cleaved from the final cycle of oligo synthesis was measured by UV spectroscopy in order to compare the density of oligonucleotides synthesized on the P1-coated glass with the oligo density on the control (amine modified glass without **P1**). 0.1 M of *p*-toluenesulfonic acid monohydrate (TSA) (4 ml) was prepared in anhydrous acetonitrile and was treated to glass slides for 1 min. The DMT solution was measured by UV spectroscopy in order to quantify DMT concentration. This solution is easier to pipet than solutions containing methylene chloride and is acidic enough to neutralize any residual base. DMT absorption was determined by scanning from 400 nm to 600 nm by UV. A major peak corresponding to a DMT cation appears at 500 nm. There is a second peak at 410 nm with an extinction coefficient of 28,690.

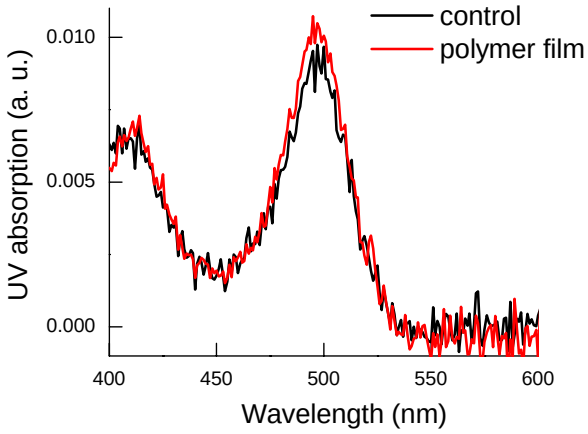


Figure S2. UV absorbance for DMT quantification

In Beer's law, the molar absorptivity (or extinction coefficient) is constant and the absorbance is proportional to concentration for a given DMT dissolved in a given solvent and measured at a given wavelength (410 nm).

$$A=\varepsilon \cdot b \cdot c$$

where A is the absorbance (no units, since $A=\log_{10}P_0/P$)
ε is the molar absorptivity with units of $L \cdot mol^{-1} \cdot cm^{-1}$

b is the path length of the sample (cm, 1 cm cuvette)

c is the concentration of the DMT in acetonitrile, expressed in $mol \cdot L^{-1}$.

The surface concentration of oligonucleotide in the slides (20 cm x 20 cm) measured using this equation is 2.44 $pmol \cdot cm^{-2}$. Both the polymer and control slide have similar numbers in oligo concentration.

Detection limit study.

Figure S3 showed the result of our detection limit study. In the picomolar concentration regime the fluorescence intensity from the target DNA slightly increases. From 10^{-10} molar concentration the signal intensity becomes significantly larger than the baseline. Therefore, the detection limit should be about 10^{-10} M or 20 picogram of the target DNA in 50 µl solution.

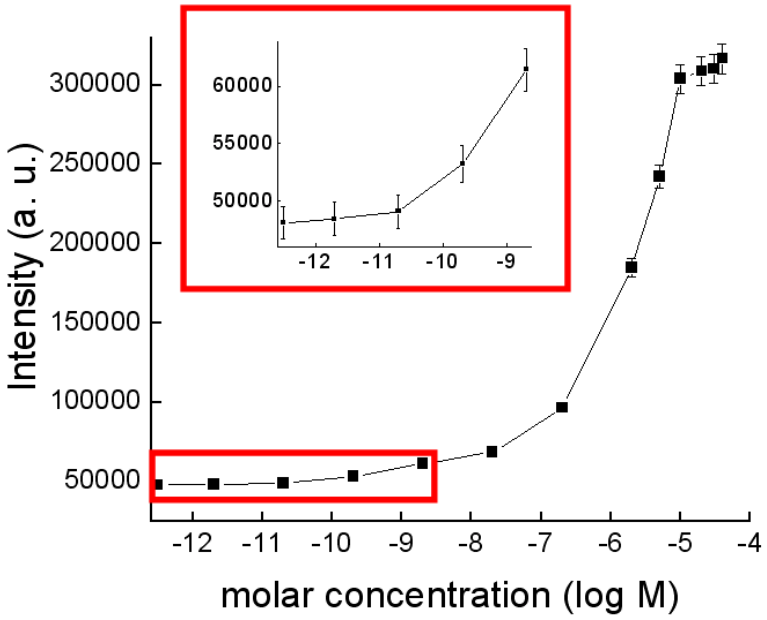


Figure S3. Profile of fluorescence intensity upon change of target DNA concentration