

Electronic Supplementary Information

Surface Functional DNA Density Control by Programmable Molecular Defects

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Experimental Section

Materials. 2-Mercaptoethanol (MCE), 11-mercaptoundecanol (MCU), hexaammineruthenium(III) $\{[\text{Ru}(\text{NH}_3)_6]^{3+}\}$ chloride, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), ethylenediaminetetraacetic acid disodium salt (Na_2 -EDTA), adenosine, and mercury(II) chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris(hydroxymethyl)-aminomethane (TRIS) and tris(2-carboxyethyl)phosphine (TCEP) were obtained from Acros Organics (Geel, Belgium). 10x phosphate buffered saline (PBS) containing 1.37 M NaCl, 0.027 M KCl, 0.10 M Na_2HPO_4 , and 0.018 M KH_2PO_4 was purchased from Bioman Scientific Co., Ltd (Taipei, Taiwan). Cocaine was provided by Department and Graduate Institute of Forensic Medicine, National Taiwan University (Taipei, Taiwan). Deionized water (>18 M Ω ·cm) was obtained from the ELGA PURELAB classic system (Taipei, Taiwan).

DNA sequences. DNA strands purified by HPLC were purchased from PURIGO Biotechnology Co., Ltd. (Taipei, Taiwan). The DNA sequences are listed as following:

(1) DNA#1 (30 bp, high affinity to mercury ion):

5' HS-(CH₂)₆-ACT CAT GAT TCT TTC TTC CCC TTG TTT GTT-FAM-3'

(FAM: carboxyfluorescein)

(2) DNA#2 (42 bp, high affinity to human immunodeficiency virus type 1, HIV):

5' HS-(CH₂)₆-GCG ACT GGG ATT AAA TAA AAT AGT AAG AAT GTA TAG CCC
AGT-FAM-3'

(3) DNA#3 (35 bp, high affinity to adenosine):

5' HS-(CH₂)₆-ACT CAT GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-FAM-3'

(4) DNA#4 (47 bp, high affinity to cocaine):

5' HS-(CH₂)₆-ACT CAT GAG GGA GAC AAG GAA AAT CCT TCA ATG AAG TGG GTC
TCC C -FAM-3'

(5) HIV (33 bp)

5'-GCT ATA CAT TCT TAC TAT TTT ATT TAA TCC CAG-3'

(6) Random 1 (33 bp)

5'-ATC CCA TTC TGC AGC TTC CTC ATT GAT GGT CTC-3'

(7) Random 2 (35 bp)

5'-ACC TTC CTC CGC AAT ACT CCC CCA GGT TCA TGA GT-3'

(8) Random 3 (47 bp)

5'-ACC CGA ACC TGG GCT ACC ACC CTT AAT CCC CGA GAC CAT CAA TGA GG-3'

(9) Random 4 (48 bp)

5'-AAG CTG CAG AAT GGG ATA CCC GAA CCT GGG CTA CCA CCC TTA ATC CCC-3'

Instruments. The stamp surface activation was carried out by an oxygen plasma cleaner (Harrick Plasma PDC-32G, Ithaca, NY, USA). Fluorescence images were obtained by a Zeiss epifluorescence microscope (Axio Imager. M2, Carl Zeiss microscopy, Jena, Germany) equipped with an X-Cite® 120LED (Lumen Dynamics Group Inc., Mississauga, Canada) lamp and a fluorescence filter set with excitation and emission wavelengths of 480±15 nm and 535±20 nm, respectively. CV measurements were performed using the CH Instruments 627A electrochemical analyzer (Austin, TX, USA).

Post-Chemical Lift-Off Substrates Preparation. Silicon substrates (Mustec Corp., Hsinchu, Taiwan) with 100 nm-thick Au and 5 nm chromium adhesive layer were prepared by thermal

evaporation. The Au substrates were immersed in 0.5 mM MCU ethanolic solution for over 6 h to form self-assembled monolayers. After the MCU SAM formation, the substrates were washed by ethanol to remove excess MCU molecules, and blown dry with nitrogen gas. PDMS stamps (with square pattern, smiley or crying face shapes) were fabricated by standard photolithography-created masters. A 10:1 mass ratio of SYLGARD 184 silicone elastomer base and curing agent (Dow Corning, Midland, MI, USA) was thoroughly mixed, degassed under vacuum, cast onto master molds, and cured on an aluminum-top hot plate at 100°C overnight. The PDMS stamps were separated from the master molds, sequentially rinsed by acetone and isopropanol, and then blown dry by nitrogen gas. The prepared stamps were activated by 40 s exposure to oxygen plasma at a power of 18 W with 0.5 mbar oxygen flow. Thereafter, the stamps were conformal sealed onto the MCU SAM-modified substrates to initiate contact-induced reaction for a various amount of duration. The contact-sealed stamps were finally separated from the Au substrates, creating post-chemical lift-off surfaces.

For multiple chemical lift-off operation, an oxygen plasma-activated PDMS stamp with 10 μm line features was first conformal sealed onto the MCU SAM-modified substrate horizontally. After separating the contact sealed stamp from the Au substrate, identical treatments were sequentially employed on the same surface by different orientations as described in the main text.

Tethering DNA onto Post-Chemical Lift-Off Substrates. Before tethering DNA onto CLL-treated substrates, 5 μL of 10 μM thiolated DNA solution was first mixed with 5 μL of 20 μM reducing agent TCEP in 25 mM TRIS buffer (150 mM NaCl, pH 8.2) for 30 min. The DNA solution was then diluted to a final concentration of 0.5 μM in 25 mM TRIS buffer (150 mM NaCl, pH 8.2), and quickly dropped onto the substrates to anchor DNA molecules into the post-chemical

lift-off regions. After typically 1 h of incubation, the substrates were gently rinsed by deionized water, immersed in buffer solution, and stored at 4°C in dark.

For multiplexed DNA device fabrication, a featureless PDMS stamp after 40 s of oxygen plasma treatment was conformal sealed onto a MCU SAM-modified Au substrate for 1 h. After stamp removal, the Au substrate was conformal sealed to another plasma-treated stamp carrying 20 μm -wide microchannels. 5 μM DNA#1, DNA#3, and DNA#4 solutions (in 25 mM TRIS buffer, 150 mM NaCl, 1 μM TCEP, pH 8.2) were introduced into separated channels for 1 h molecule insertion. The microchannel stamp was then quickly separated from the Au substrate in a DI water bath. Finally, the Au surface was gently rinsed with DI water and 25 mM TRIS buffer (pH 7.4) before further use.

Cyclic Voltammetry (CV) Measurements. Electrochemical experiments were performed on the CH Instruments 627A electrochemical analyzer in a three-electrode system consisting of the prepared substrate (with an exposed area of 0.28 cm^2), an Ag/AgCl (3 M KCl) reference electrode, and a Pt wire counter electrode. CV measurements were carried out with 1 mM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in 25 mM TRIS buffer (pH 7.4) at a scan rate of 100 mV/s. The fourth cycle (the fourth forward scan and reverse scan) in a total of five cycles is recorded for each experimental condition.

Surface DNA Density Quantification. A literature-reported fluorescence-based determining method was used to quantify the FAM-labeled thiolated DNA modified on surfaces.¹ Briefly, the substrates were incubated with 50 μL of 20 mM MCE (in 1xPBS, pH 7.4) for 12 h to disengage all DNA from the surface. (Note: A fixed surface area with a 0.3 cm radius circle was employed to all surfaces.) The solutions were then collected and diluted 4 fold by 1x PBS (pH 7.4) before

fluorescence measurements. Fluorescence intensities at 520 nm were converted to molar concentrations of DNA by interpolation from a standard linear calibration curve. The calibration curve was prepared with known concentrations of FAM-labeled DNA in 5 mM MCE (in 1x PBS, pH 7.4). It should be noted that same pH, salt content, and MCE concentration were used in both samples and standard solutions to minimize the optical property fluctuation of FAM. Finally, the surface DNA density was obtained via dividing the calculated DNA amount by the fixed surface area.

Capturing Targets. Before target introduction, the sensing substrates were rinsed with 25 mM HEPES buffer (pH 7.4), 25 mM TRIS buffer (150 mM NaCl, pH 7.4), or 25 mM TRIS buffer (pH 7.4) in advance. Target solutions, i.e. Hg²⁺ in 25 mM HEPES buffer (pH 7.4), HIV in 25 mM TRIS buffer (150 mM NaCl, pH 7.4), adenosine or cocaine in 25 mM TRIS buffer (pH 7.4), was thereafter dropped onto the substrates for 5 min incubation. The target capturing ability of substrate-anchored DNA on this platform was determined by a series of fluorescence images. The relative fluorescence intensity was processed with the rectangle function in ZEN 2012 (blue edition) Service Pack 2 software (Carl Zeiss microscopy, Jena, Germany). All the target capturing experiments were repeated three times and the relative errors are demonstrated in the corresponding figures.

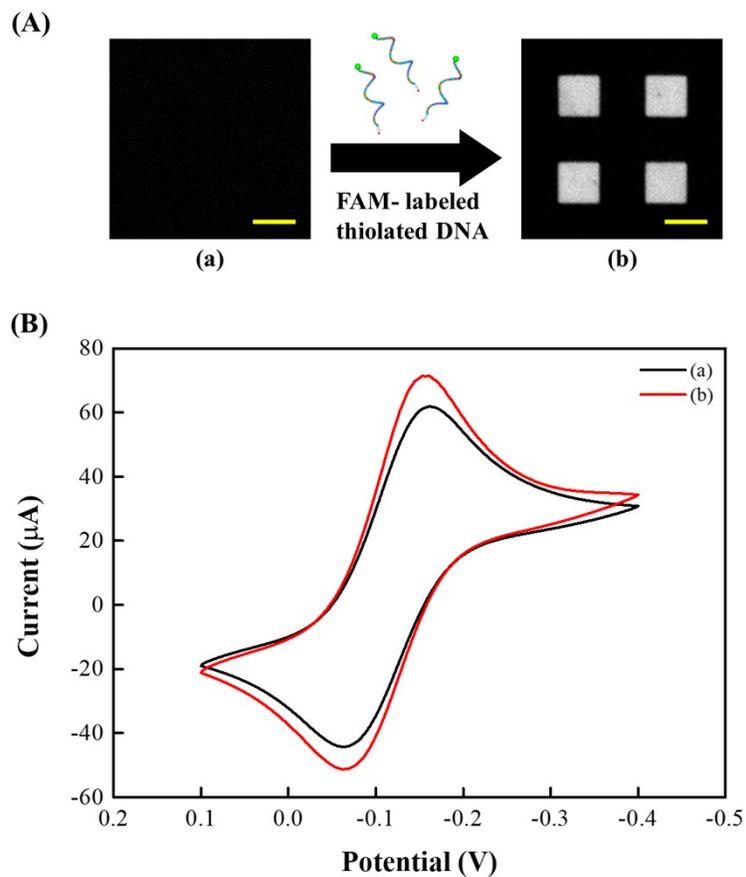


Fig. S1. (A) Fluorescence images (square pattern) and (B) cyclic voltammograms (featureless) of 1 mM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ on different substrates: (a) MCU modified Au surfaces after the CLL treatment and (b) post-CLL substrates after FAM-labeled DNA tethering. The scale bars are 20 μm . (Cyclic voltammetry experiments of $\text{Fe}(\text{CN})_6^-$ were also performed and showed similar results.)

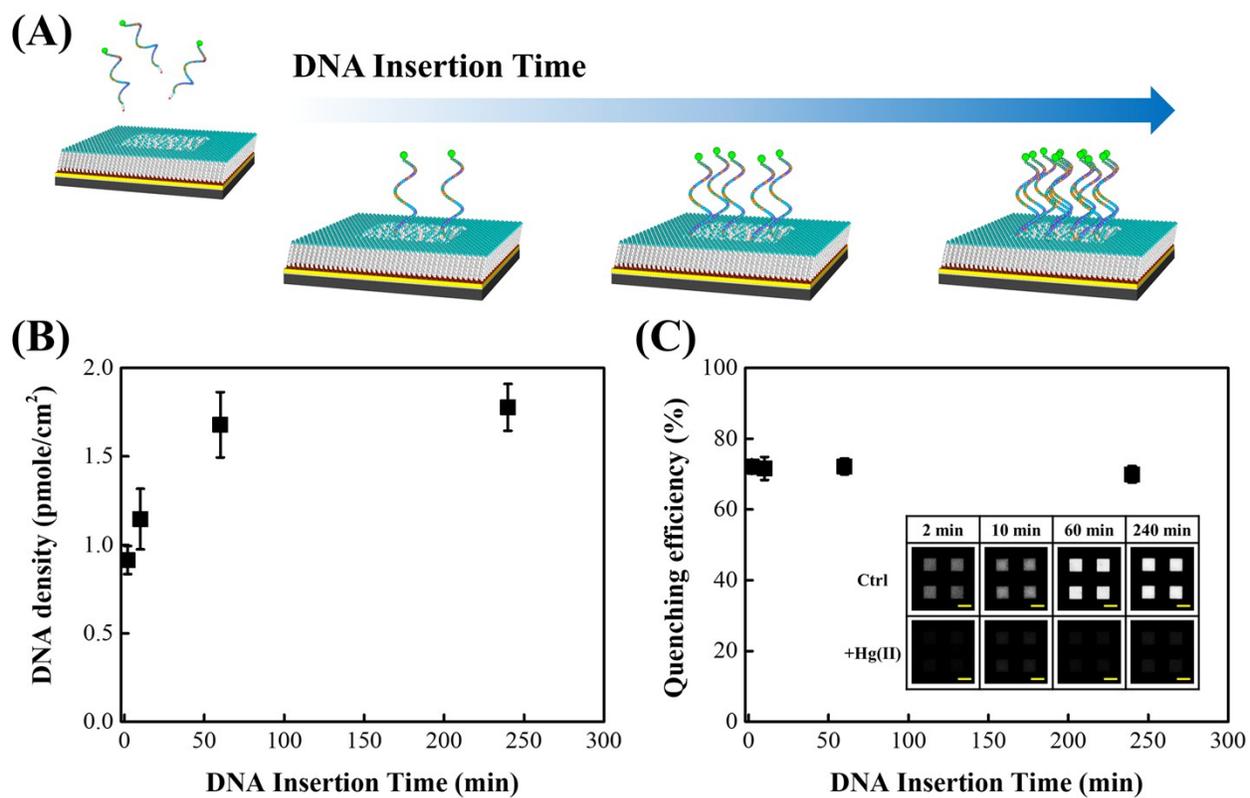


Fig. S2. (A) Schematic illustration of the DNA insertion time effects. (B) The obtained surface DNA density and (C) surface probe fluorescence signal quenching efficiency upon target introduction. DNA#1 and Hg(II) were used as the testing pair. (N = 3) Inset: The corresponding fluorescence images of DNA#1-tethered substrates upon Hg(II) introduction. The scale bars are 20 μ m.

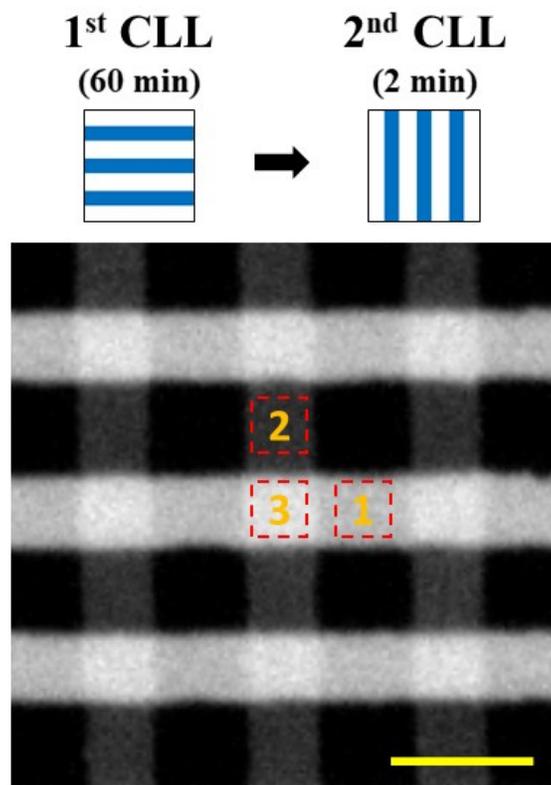


Fig. S3. Region indications in the fluorescence image rendering a lattice pattern fabricated by double CLL operation and subsequent DNA insertion, where 60 min (horizontally) and 2 min (vertically) of stamp seal time was applied sequentially. Region 1: 60 min, region 2: 2 min, region 3: 60+2 min. The scale bar is 20 μm .

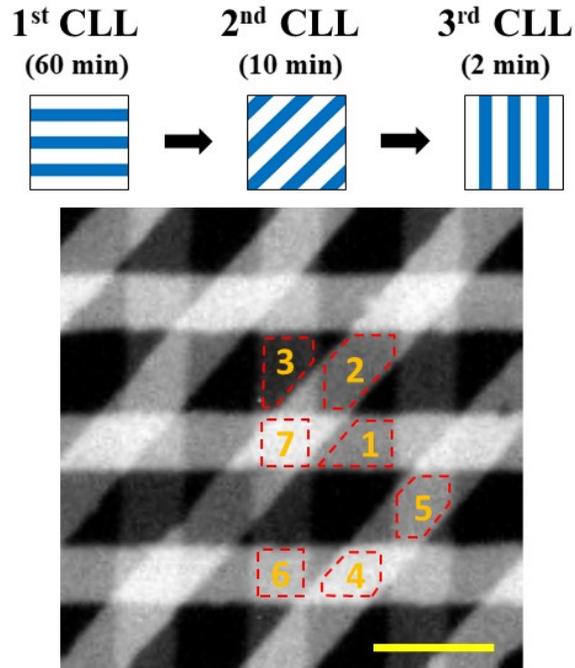


Fig. S4. Region indications in the fluorescence image rendering a complex mosaic pattern created by triple CLL operation and subsequent DNA insertion, where 60 min (horizontally), 10 min (45 degree tilted angle), and 2 min (vertically) of stamp seal time was applied sequentially. Region 1: 60 min, region 2: 10 min, region 3: 2 min, region 4: 60+10 min, region 5: 10+2 min, region 6: 60+2, region 7: 60+10+2 min. The scale bars are 20 μm .

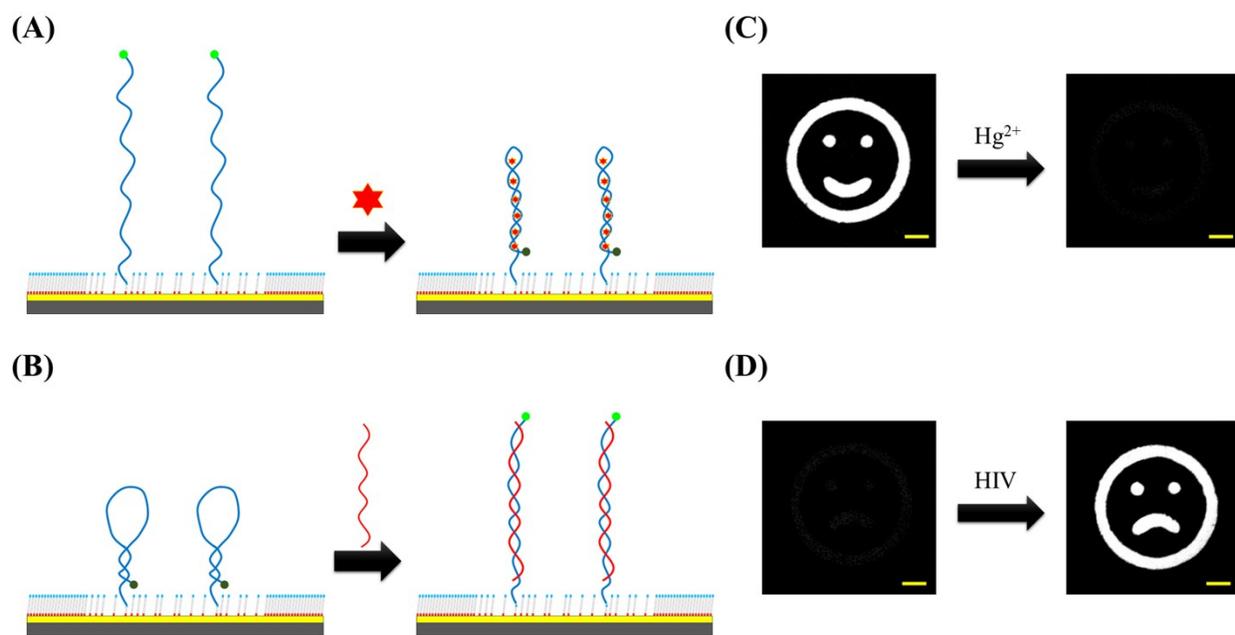


Fig. S5. Schematic illustration of fluorescence-based (A) “turn-off” and (B) “turn-on” assays. (C) and (D): Fluorescence images of DNA#1 and DNA#2 tethered surfaces before and after the addition of Hg(II) and HIV, respectively. The scale bars are 20 μm .

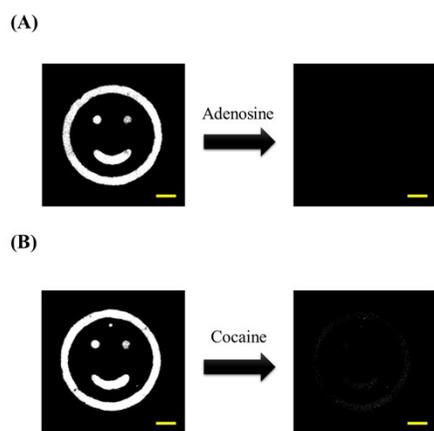


Fig. S6. Fluorescence images of (A) DNA#3 and (B) DNA#4 tethered surfaces before and after the addition of adenosine and cocaine, respectively. The scale bars are 20 μm .

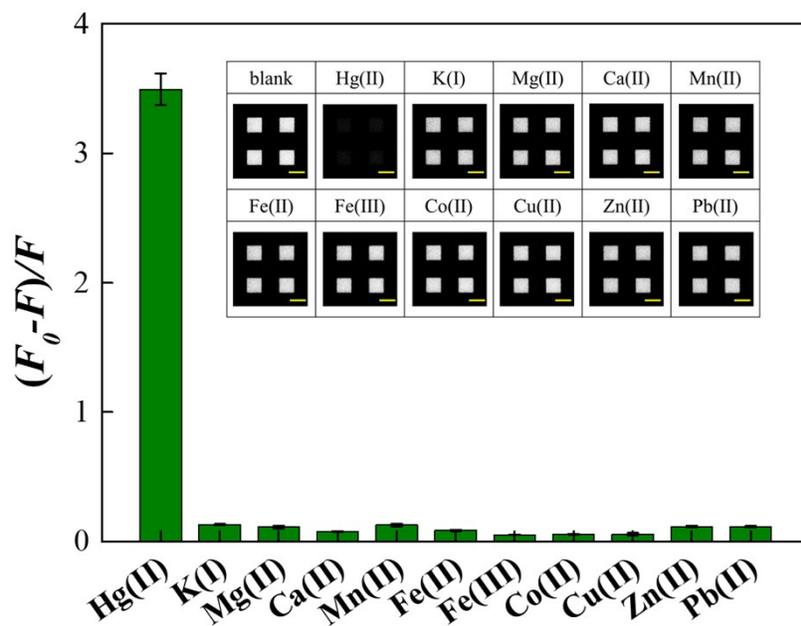


Fig. S7. Selectivity examination of the DNA#1 tethered substrate toward Hg(II). All substrates were independently treated by 100 nM solutes. F_0 and F represent corresponding relative fluorescence intensities without and with solutes, respectively. Each data point is obtained from the average fluorescence signal of individual squares ($N = 4$). The error bars indicate the standard deviation. The scale bars are 20 μm .

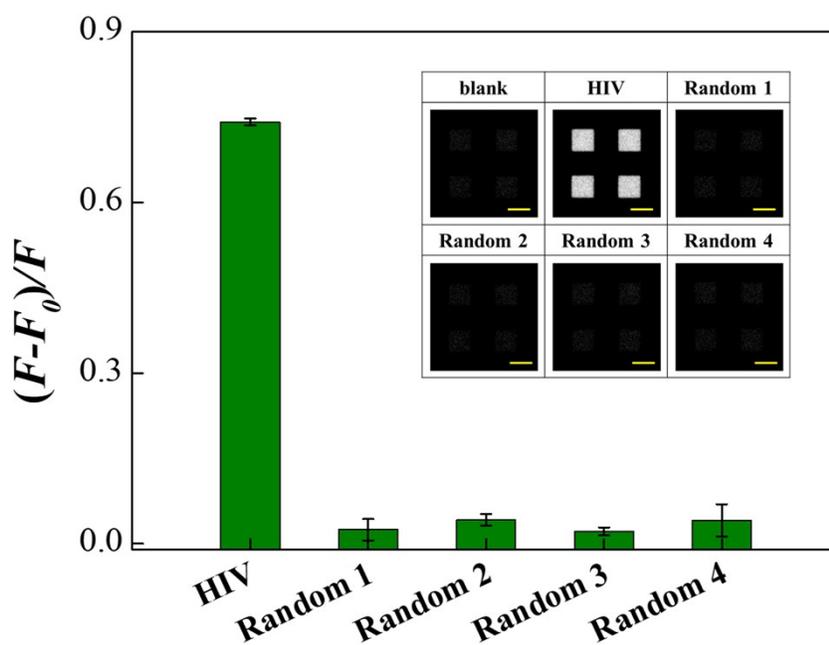


Fig. S8. Selectivity examination of the DNA#2 tethered substrate toward HIV. All substrates were independently treated by 100 nM solutes. F_0 and F represent corresponding relative fluorescence intensities without and with solutes, respectively. Each data point is obtained from the average fluorescence signal of individual squares ($N = 4$). The error bars indicate the standard deviation. The scale bars are 20 μm .

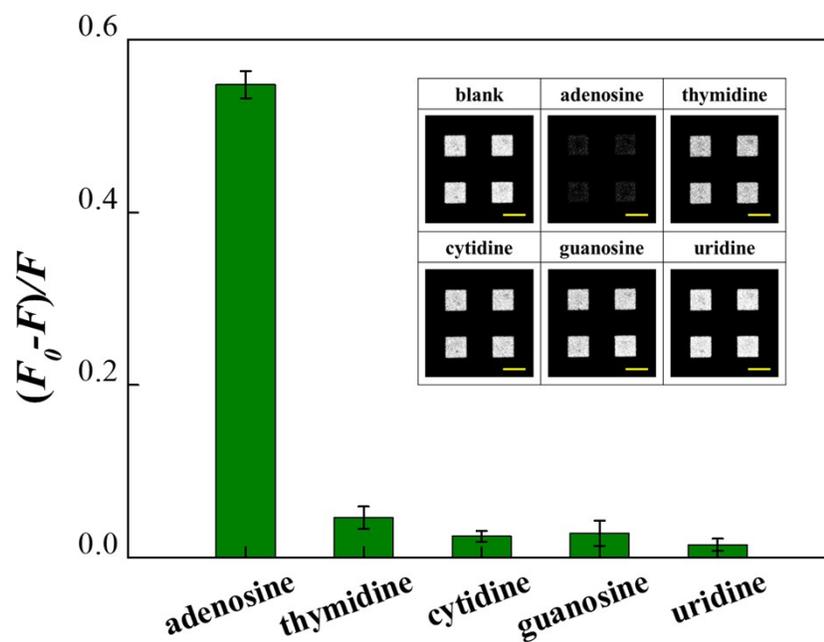


Fig. S9. Selectivity examination of the DNA#3 tethered substrate toward adenosine. All substrates were independently treated by 1 mM solutes. F_0 and F represent corresponding relative fluorescence intensities without and with solutes, respectively. Each data point is obtained from the average fluorescence signal of individual squares ($N = 4$). The error bars indicate the standard deviation. The scale bars are 20 μm .

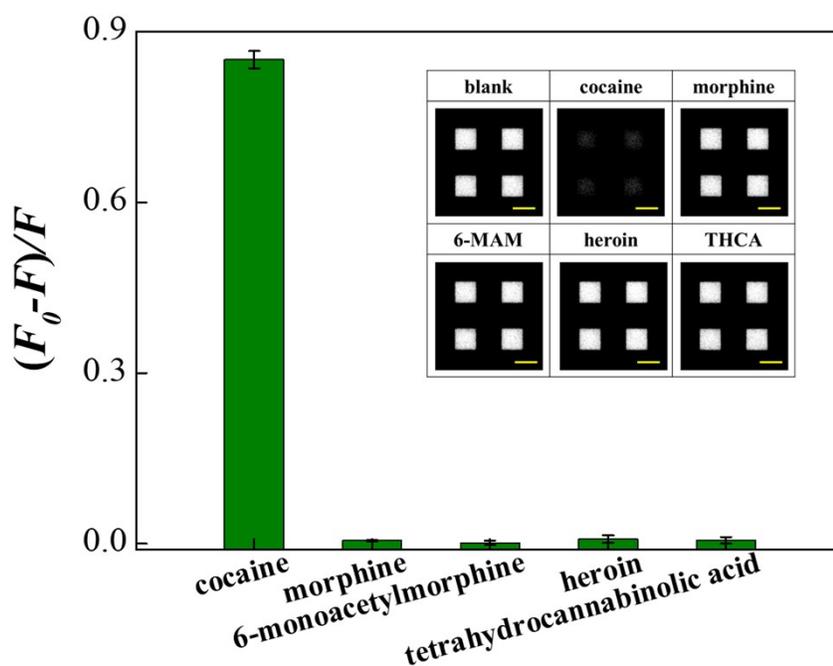


Fig. S10. Selectivity examination of the DNA#4 tethered substrate toward cocaine. All substrates were independently treated by 1 mM solutes. F_0 and F represent corresponding relative fluorescence intensities without and with solutes, respectively. Each data point is obtained from the average fluorescence signal of individual squares ($N = 4$). The error bars indicate the standard deviation. The scale bars are 20 μm .

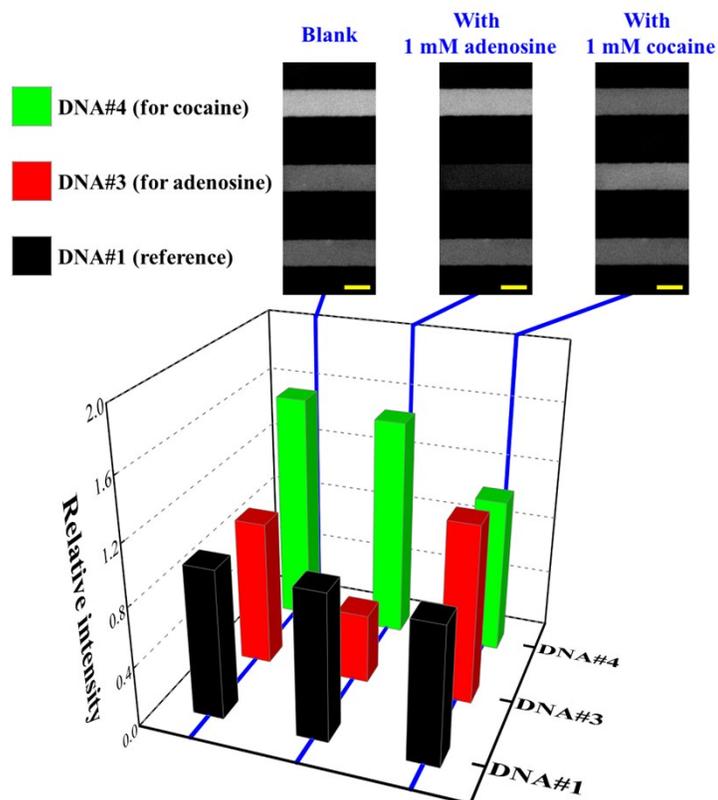


Fig. S11. The multiplexed surface tethered with three different types of DNA probes. Bottom position: anchored with DNA#1, as reference. Middle position: anchored with DNA#3, for adenosine recognition. Top position: anchored with DNA#4, for cocaine recognition. The scale bars are 20 μm .

REFERENCES

1. L. M. Demers; C. A. Mirkin; R. C. Mucic; R. A. Reynolds; R. L. Letsinger; R. Elghanian; G. Viswanadham, *Anal. Chem.* **2000**, 72, 5535-5541.