Electronic supplementary information

Janus DNA Orthogonal Adsorption of Graphene Oxide and Metal Oxide Nanoparticles Enabling Sensing in Serum

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Materials and Methods

Chemicals. All of the DNA samples were from Integrated DNA Technologies (IDT, Coralville, IA, USA). Their sequences and modifications are shown in Table S1. Carboxyl graphene oxide (GO) was purchased from ACS Material (Medford, MA) and was used as received. Metal oxide nanoparticles (MONPs) were purchased from Sigma or US Research Nano. The detailed information of our MONPs is shown in Table S2. The MOs used were not modified with surfactants or other capping agents, and MOs dispersion was washed with Milli-Q water for three times before use. Sodium chloride, sodium phosphate dibasic heptahydrate, and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) were from Mandel Scientific (Guelph, ON, Canada). Bovine serum albumin (BSA) was form Sigma. Milli-Q water was used for all experiments.

Instrumentation. The ζ-potentials of these nanomaterials were measured using Zetasizer Nano 90 (Malvern) at 25 °C. Typically, 50 μg/mL of MONPs or GO were dispersed in HEPES buffer (10 mM, pH 7.6) for the measurement. The atomic force microscopy (AFM) image of GO was acquired using a Multimode IV instrument (Veeco, USA). A GO dispersion was deposited on a SiO_2 substrate pretreated with 3-amino-propyl trimethoxysilane (APTMS). The size and morphology of these nanomaterials were studied using transmission electron microscopy (TEM) (Philips CM10). The TEM samples were prepared by dropping the nanomaterial dispersion (25 μg/mL) on a copper grid followed by drying overnight at room temperature. The fluorescent images were acquired using a digital camera under a LED lamp (excitation at 470 nm). The fluorescent spectra were recorded using a Varian Eclipse fluorescence spectrophotometer.

Adsorption of DNA. Typically, a FAM-labeled DNA (100 nM, Table S1) was mixed with a nanomaterial in buffer A (HEPES 10 mM pH 7.6, NaCl 300 mM) for 1 h. The concentration of each material was: GO 20 μg/mL CeO_2 10 μg/mL, CoO 100 μg/mL, Cr_2O_3 500 μg/mL, Fe_2O_3 100 μg/mL, Fe_3O_4 125 μg/mL, NiO 50 μg/mL, and TiO_2 125 μg/mL. These concentrations were chosen (individually optimized) to achieve full DNA adsorption but not in great excess.

DNA displacement by phosphate or adenosine. To test the displacement of DNA by phosphate or adenosine, the prepared DNA/nanomaterial conjugates were incubated with phosphate (10 mM) or adenosine (5 mM) for 2 h, the samples were then centrifuged (15000 rpm, 10 min). Afterwards, the fluorescent images and the emission spectra were acquired. The reaction kinetics of displacement by phosphate were also monitored using a fluorescence microplate reader by exciting at 485 nm and monitoring emission at 535 nm. Typically, a final of 20 nM DNA (DNA1, or DNA2) was used, and phosphate solutions of various concentrations were then added to induce the DNA desorption (final phosphate concentration, 0, 0.01 mM, and 1 mM). The concentration of each was: GO 4 μg/mL CeO_2 2.5 μg/mL, CoO 20 μg/mL, Cr_2O_3 100 μg/mL, Fe_2O_3 20 μg/mL, Fe_3O_4 25 μg/mL, NiO 10 μg/mL, and TiO_2 25 μg/mL.

Desorption of probe DNA. To obtain cDNA-induced probe desorption, the DNA/NiO conjugate was first prepared by mixing DNA1 (20 nM) with NiO NPs (10 μg/mL) in buffer A for 2 h. Then cDNA of designed concentration was added to the conjugate, and the fluorescence was monitored for another 2 h. The selectivity test was carried out by adding 20 nM cDNA1, sDNA1, misDNA1 to the DNA/NiO conjugate.

To test the effect of BSA on the DNA desorption from GO, DNA/GO conjugate was first prepared by mixing DNA1 (10 nM) with GO (2 μg/mL) in buffer A for 2 h. Then BSA solutions of various concentrations were respectively introduced to induce DNA desorption, and the fluorescence was monitored for 1 h. The BSA-induced DNA recovery from MO platforms was performed similarly. The concentration of each material was: CeO_2 1 μg/mL, CoO 10 μg/mL, Cr_2O_3 50 μg/mL, Fe_2O_3 10 μg/mL, Fe_3O_4 12.5 μg/mL, NiO 5 μg/mL, and TiO_2 12.5 μg/mL. The DNA desorption in the presence of fetal bovine serum (FBS) was tested similar to the BSA-induced desorption.

Detection of cDNA. The DNA/NM conjugates were prepared by mixing DNA1 (10 nM) with each NM in buffer A for 2 h. The concentration of each material was: GO 2 μg/mL CeO_2 1 μg/mL, CoO 10 μg/mL, Cr_2O_3 50 μg/mL, Fe_2O_3 10 μg/mL, Fe_3O_4 12.5 μg/mL, NiO 5 μg/mL, and TiO_2 12.5 μg/mL. Then cDNA of various concentration was added to induce fluorescence recovery. The fluorescence intensities before and after adding cDNA for 2 h were recorded to calculate the fluorescence enhancement. The kinetics of cDNA-induced DNA1 desorption from GO and NiO in the presence of BSA was carried out by adding BSA (100 μg/mL) only or a mixture of BSA 100 μg/mL and cDNA (100 nM). To detect DNA in serum, cDNA of different concentrations was mixed with 10% FBS, and then 5 μL of the mixture was introduced to the DNA1/GO, DNA1/NiO or DNA1/CoO platform (95 μL).

Table S1. The sequences and modification of DNA oligonucleotides used in this work. FAM = carboxyfluorescein.

DNA names	Sequences (from 5′ to 3′) and modifications
DNA1	FAM-ACG CAT CTG TGA AGA GAA CCT GGG
DNA1-3′	ACG CAT CTG TGA AGA GAA CCT GGG-FAM
sDNA1	ACG CAT CTG TGA AGA GAA CCT GGG
cDNA1	CCC AGG TTC TCT TCA CAG ATG CGT
misDNA1	CCC AGG TTC TCT TCA CAC ATG CGT
cDNA1-3′	CCC AGG TTC TCT TCA CAG A
misDNA1-3′	CCC AGG TTC **C**CT TCA CAG A
DNA2	FAM-TCA CAG ATG CGT
DNA3	FAM-AAA AA
T_{30}	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
C_{30}	CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC
A_{30}	AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA

Table S2. Information of the MONPs used in this work.

Materials	Size*[a](d, nm)	Vendor	Catalog Number
CeO_2	5	Sigma	289744
CoO	50	US Research Nano.	US3051
Cr_2O_3	60	US Research Nano.	US3060
Fe_2O_3	50	US Research Nano.	US3200
Fe_3O_4	50	Sigma	637106
NiO	10-20	US Research Nano.	US3356
TiO_2	25	Sigma	637254

a: the information is provided the vectors.

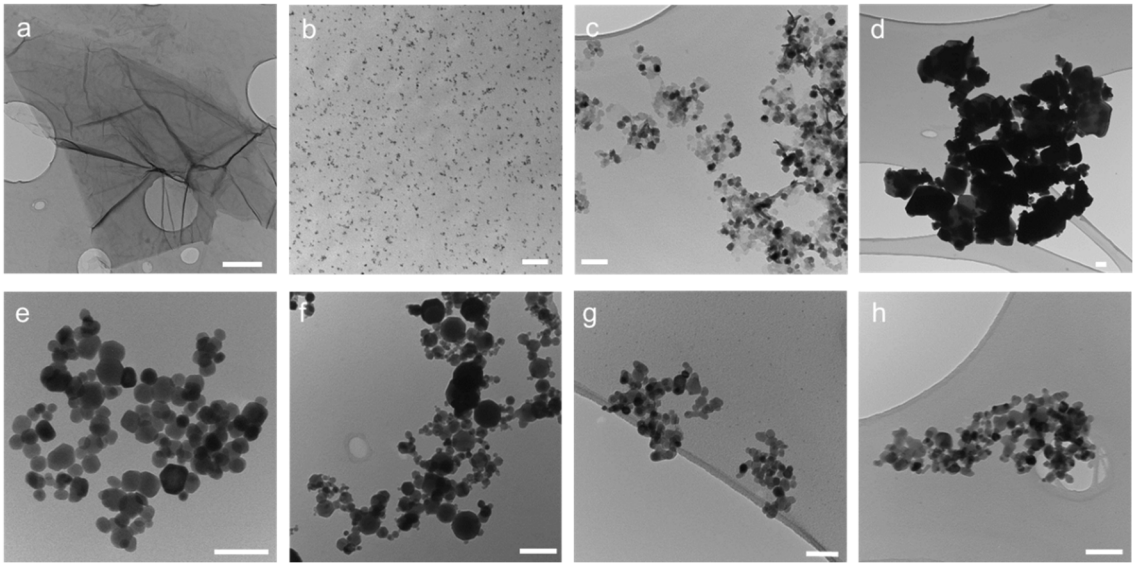


Figure S1. TEM micrographs of (a) GO, (b) CeO_2, (c) CoO, (d) Cr_2O_3, (e) Fe_3O_4 (f) Fe_2O_3, (g) NiO, and (h) TiO_2 NPs. The scale bar of (a) is 500 nm and the others 100 nm. It should be noted that aggregation of nanoparticles occurred due to the lack of strong surface capping ligands. To prepare the DNA/MONP conjugates, the MONPs were briefly sonicated before adding DNA to facilitate the dispersing.

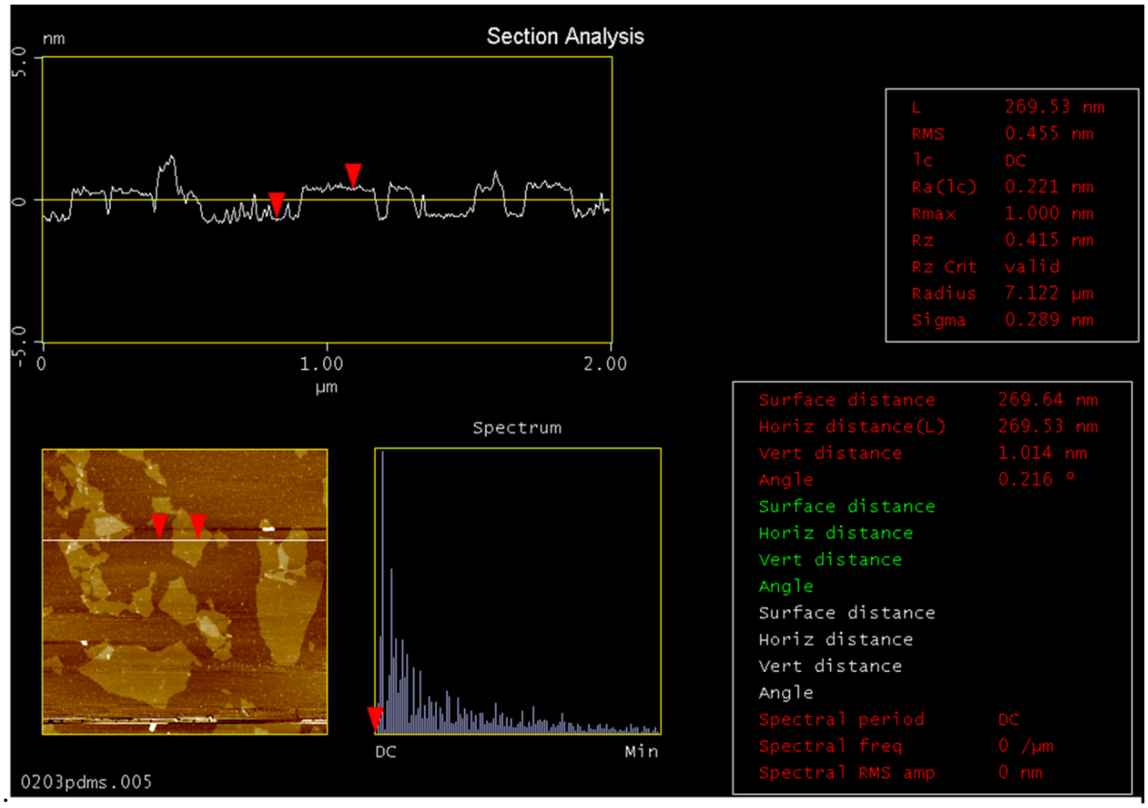
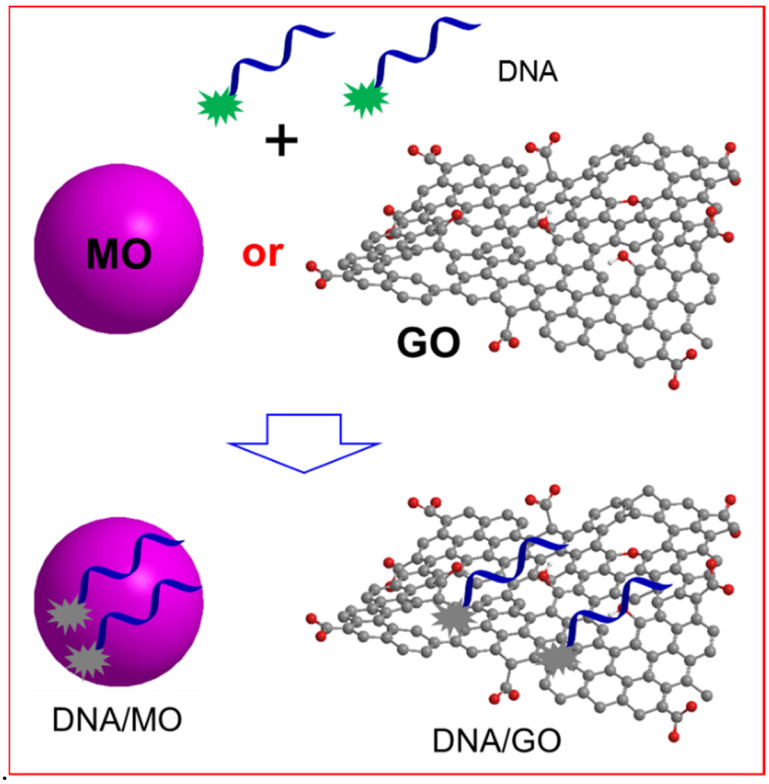


Figure S2. Atomic force microscopic (AFM) analysis of our graphene oxide (GO) used in this work.



Scheme S1. A scheme showing preparation of the sensors. FAM-labeled DNA oligonucleotides can be adsorbed by either MO or GO, resulting in fluorescence quenching. These adsorption complexes are used directly as sensors, which can show fluorescence enhancement in the presence of cDNA.

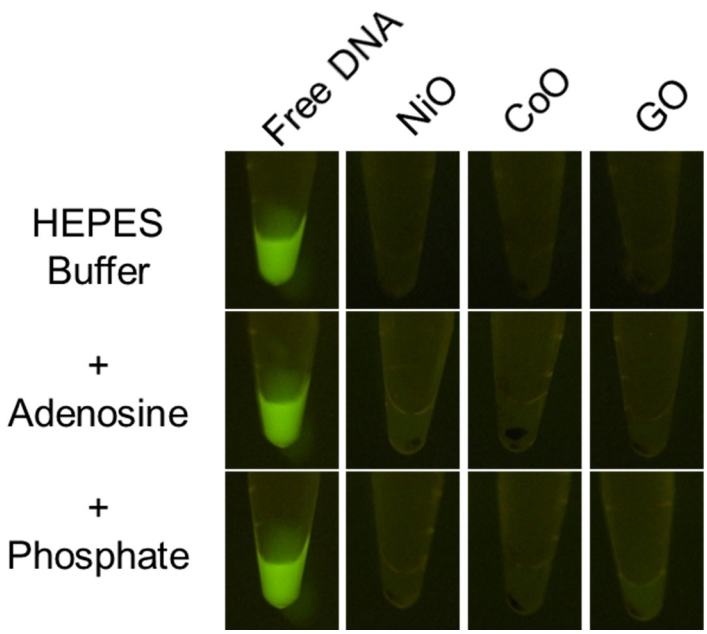


Figure S3. Images showing the DNA adsorption and fluorescence quenching of DNA1 (24-mer with FAM, 100 nM) by NiO (50 μg/mL), CoO (100 μg g/mL), and GO (20 μg /mL). Phosphate (final concentration = 10 mM, pH 7.6) and adenosine (final concentration = 5 mM) was added to induce the DNA desorption. No significant fluorescence recovery was observed, indicating the high affinity of DNA1 to these three materials.

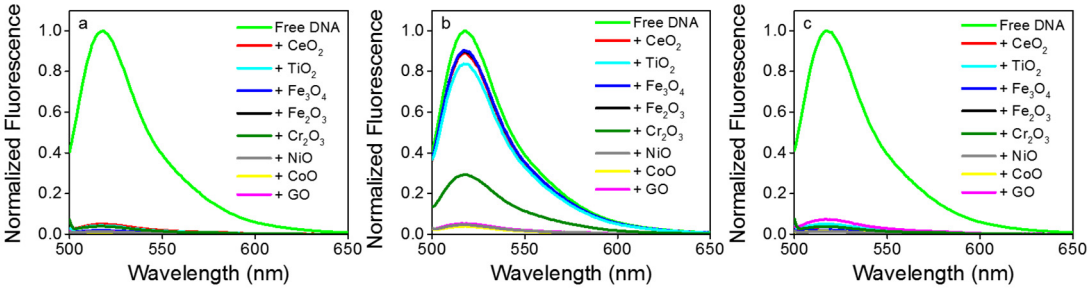
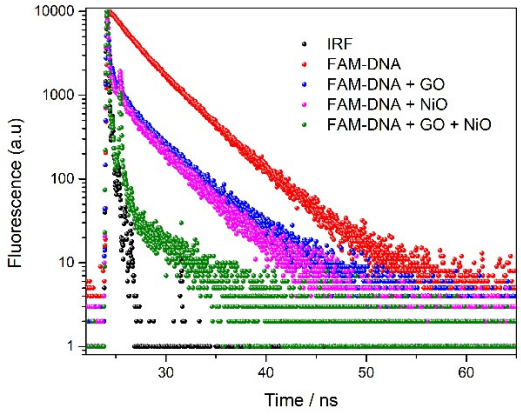


Figure S4. (a) Fluorescence spectra showing the DNA adsorption and desorption by CeO_2 (10 μg/mL), TiO_2 (125 μg/mL), Fe_3O_4 (125 μg/mL), Fe_2O_3 (100 μg/mL), Cr_2O_3 (500 μg/mL), NiO (50 μg/mL), CoO (100 μg/mL), and GO (20 μg/mL) using DNA1 (24-mer, 100 nM). The DNA desorption and fluorescence recovery was induced by (b) phosphate (pH 7.6 10 mM) or (c) adenosine (5 mM). Compared to the concentration of phosphate species (2.3 μM) or nucleosides (2.4 μM) in the probe DNA, the free phosphate ions or adenosine added was in great excess. For the phosphate displacement, among the seven MONPs, four materials (CeO_2, TiO_2, Fe_3O_4 and Fe_2O_3) show relatively weak interaction with DNA since nearly full fluorescence recovery was achieved, Cr_2O_3 shows moderate adsorption affinity, and NiO and CoO show high affinity. For adenosine displacement, only slightly fluorescence recovery was observed for GO but not for MONPs. The multiple interaction modes of DNA with GO may result in the strong binding affinity for the 24-mer DNA, which makes desorption less efficient by adenosine.



Sample	$\bar{\tau}$/ns	τ_i/ns	f_i[b]
FAM-DNA	2.79	1.26 ± 0.03	0.42
		3.88 ± 0.01	0.58
FAM-DNA + GO	0.54	0.02 ± 0.00	0.89
		3.37 ± 0.02	0.11
FAM-DNA + NiO	0.38	0.01 ± 0.00	0.91
		2.94 ± 0.03	0.09
FAM-DNA + GO + NiO	0.13	0.10 ± 0.00	0.98
		1.08 ± 0.01	0.02

$$\bar{\tau} = \sum f_i \tau_i = f_1 \tau_1 + f_2 \tau_2$$

Figure S5. Fluorescence lifetime analysis of FAM-labeled DNA1, and after its adsorption by GO and by NiO nanoparticles (left), and the quantitative fitted data (right). Fluorescence lifetime measurements were performed on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with a Delta-diode laser (405 nm, D-405, Horiba scientific) as the excitation source and a picosecond photon detection module (PPD-850, Horiba scientific) as the detector. The concentrations of FAM-DNA-1, GO, and NiO were 100 nM, 10 µg/mL, and 25 µg/mL, respectively. The free DNA has two roughly equal populations with lifetime averaged around 2.79 ns. After adding GO or NiO, the fluorescence was quenched by ~90%. For each case, the data were fitted to two lifetimes. The shorter component counted for ~90% of the DNA population with a lifetime of 0.02 ns for GO and 0.01 ns for NiO. Therefore, we assigned the mechanism to be dynamic quenching since the lifetime was significantly shortened, and the reason behind quenching for NiO is attributed to electron transfer of excited fluorophore.

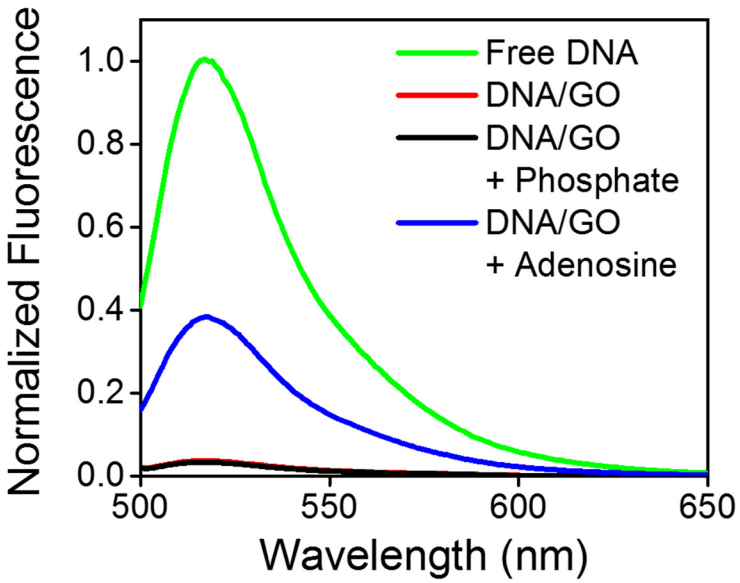


Figure S6. Fluorescence spectra showing the DNA desorption and fluorescence recovery of GO (20 μg/mL) using DNA3 (5-mer, 100 nM) induced by phosphate (pH 7.6, 10 mM) or adenosine (5 mM) in buffer A. Compared to the 24-mer DNA (DNA1), this short DNA (5-mer) has a much lower affinity to GO, resulting in ~ 40% fluorescence recovery. Howeer, adding phosphate has no effect on the DNA adsorption, confirming the base-binding modes of DNA on GO.

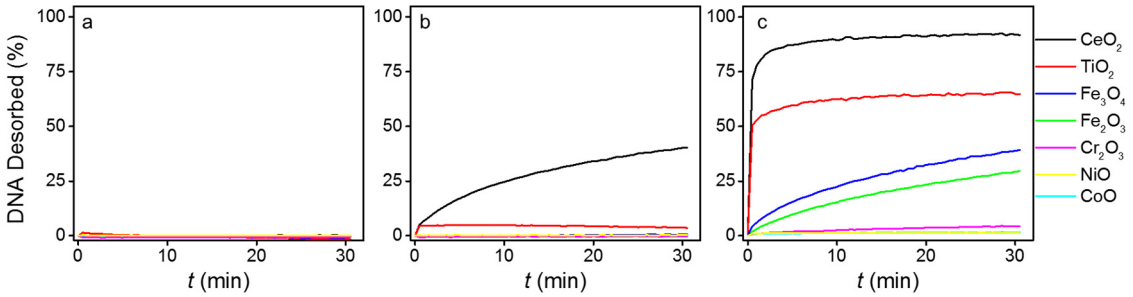


Figure S7. Desorption kinetics of DNA1 (24 mer, 20 nM) from MONP surface as a function of phosphate concentration: (a) no phosphate, (b) 0.01 mM, and (c) 1 mM. The reaction was carried out in buffer A. In the absence of phosphate ions, the fluorescence signal was stable within 30 min. When 0.01 mM phosphate was added, DNA on CeO_2 surface was gradually displaced. A minor fluorescence recovery was also observed for TiO_2. In the presence of 1 mM phosphate, DNA recovery was observed for all materials except for NiO and CoO. Based on the relative desorption kinetics, we can conclude that the order of DNA adsorption affinity with MONPs is (from low to high): CeO_2 < TiO_2 < Fe_3O_4 < Fe_2O_3 < Cr_2O_3 < NiO, CoO.

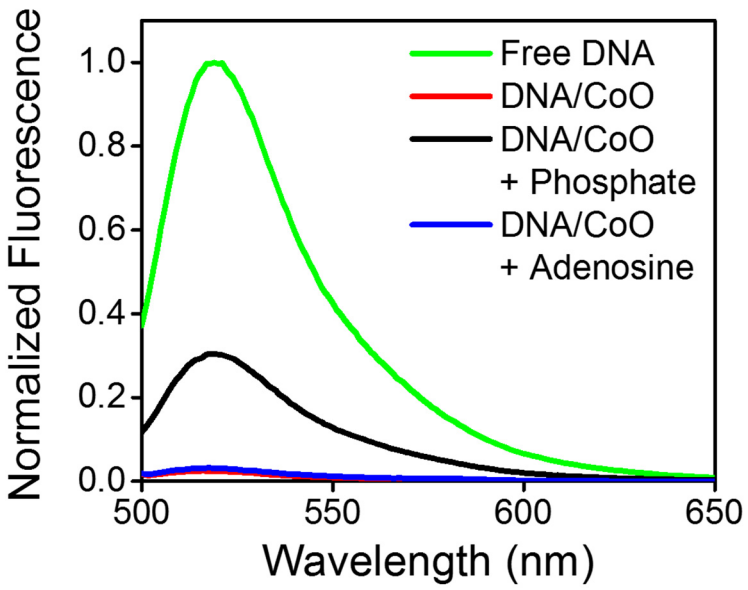


Figure S8. Fluorescence spectra showing the DNA desorption and fluorescence recovery of CoO (100 μg/mL) using DNA2 (12-mer, 100 nM) induced by phosphate (pH 7.6 10 mM) or adenosine (5 mM) in buffer A.

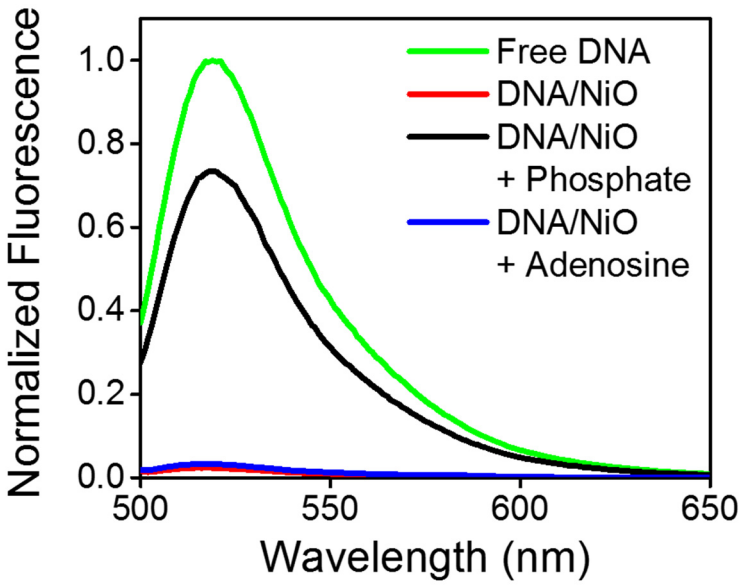


Figure S9. Fluorescence spectra showing the DNA desorption and fluorescence recovery of NiO (50 μg/mL) using DNA2 (12-mer, 100 nM) induced by phosphate (pH 7.6, 10 mM) or adenosine (5 mM) in buffer A.

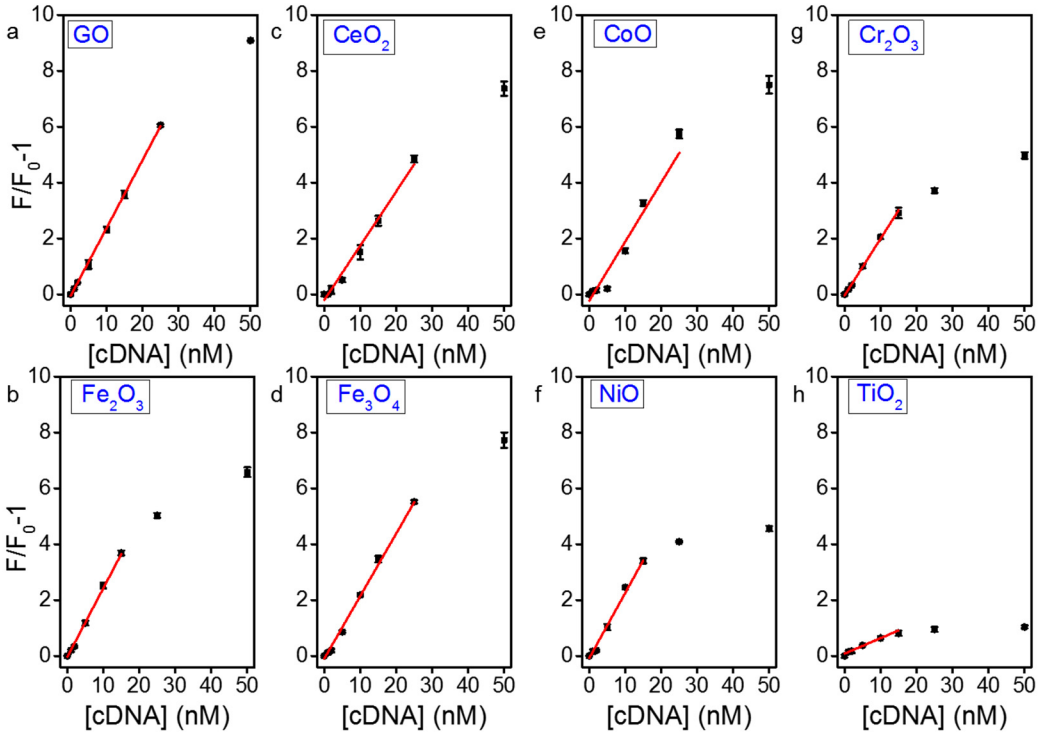


Figure S10. The fold of fluorescence enhancement over background with (a) GO, (b) CeO_2, (c) CoO, (d) Cr_2O_3, (e) Fe_2O_3, (f) Fe_3O_4, (g) NiO, and (h) TiO_2 NPs as a function of cDNA concentration. The fluorescence after adding cDNA for 2 h in buffer A. The red lines fit the linear range of signal responses. The probe concentration of all sensors were 10 nM.

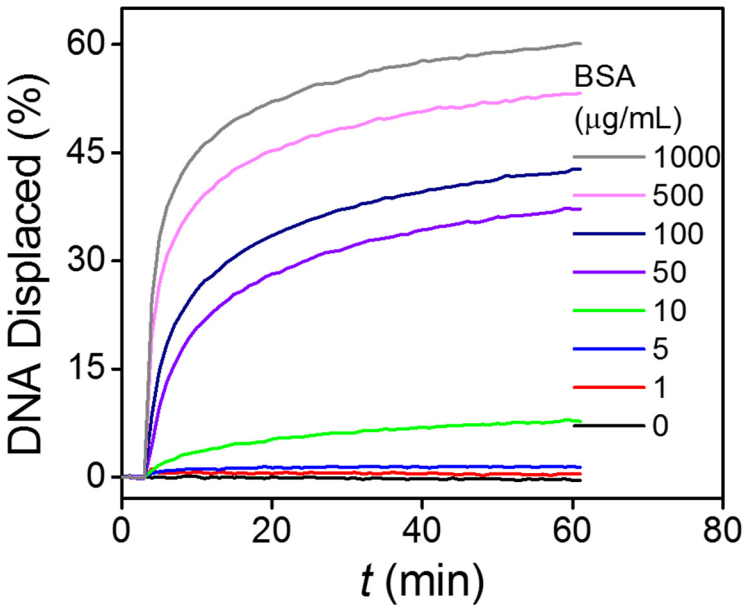


Figure S11. Kinetics of BSA-induced DNA desorption from GO in the presence of various concentrations of BSA. Then DNA/GO conjugate was prepared by mixing DNA1 (10 nM) with GO (2 μg/mL) in buffer A for 2 h. BSA was then added to induce fluorescence recovery. The DNA displaced was calculated by comparing the fluorescence recovery to the free probes without nanomaterials.

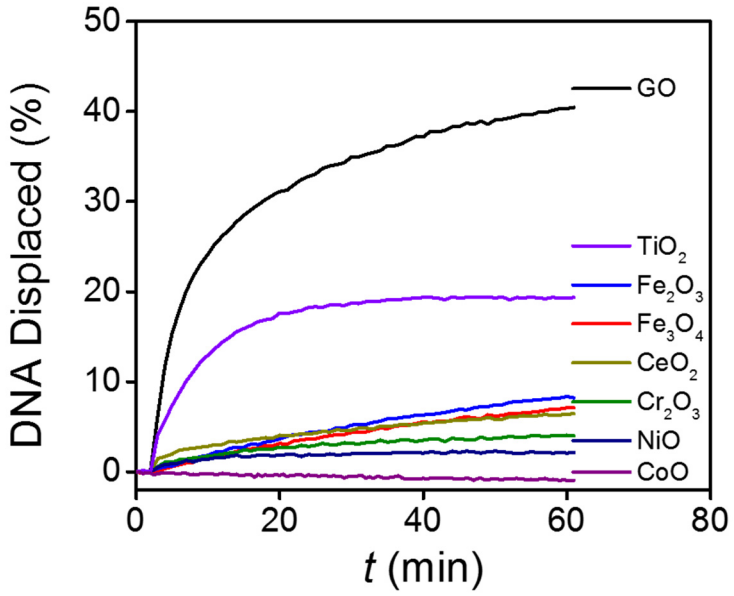


Figure S12. Kinetics of BSA-induced DNA desorption from GO or MONPs surface. The DNA/NPs conjugates were prepared by incubating DNA1 (10 nM) with different NMs in buffer A. BSA (final concentration = 100 μg/mL) was then added to induce fluorescence recovery. The DNA displaced was calculated by comparing the fluorescence recovery to the free probes without nanomaterials.

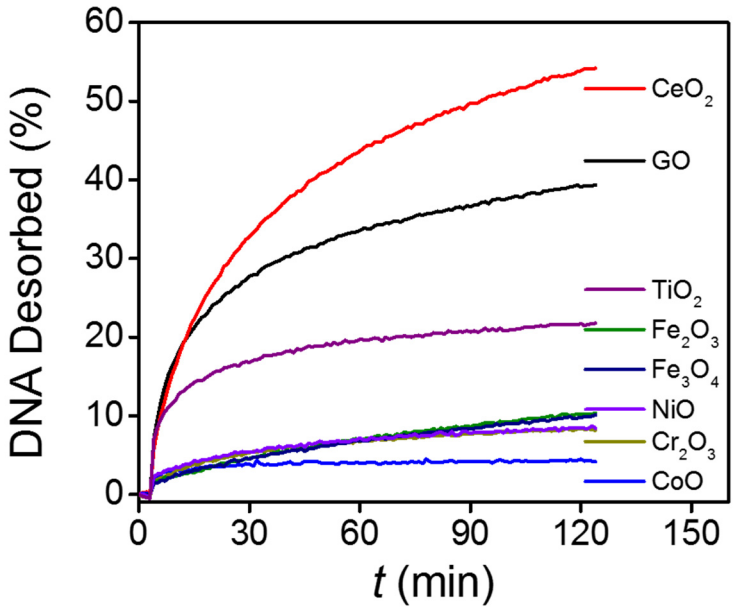


Figure S13. Kinetics of serum-induced DNA desorption from GO or MONPs surface. The DNA/NPs conjugates were prepared by incubating DNA1 (10 nM) with different nanomaterials in buffer A. FBS (final concentration = 0.5%) was then added to induce fluorescence recovery. The DNA displaced was calculated using the similar method to the BSA-induced desorption.