Supporting Information 1 Enzyme free and DNA-based multiplexer and demultiplexer 2 3 Changtong Wu,^{a,c} Kun Wang,^{a,c} Daoqing Fan,^a Chunyang Zhou,^{a,d} Yaqing Liu,^{*a,b} and 4 Erkang Wang*a ^a State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied 5 Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, P. R. China. E-mail: 6 7 yaqingliu@ciac.ac.cn, ekwang@ciac.ac.cn 8 ^b Key Laboratory of Food Nutrition and Safety (Tianjin University of Science and Technology), Ministry of Education, Tianjin, 300457, P.R.China. vaqingliu@tust.edu.cn. Tel: +86-22-60912484 9 ^c Department of Chemistry and Environmental Engineering, Changchun Universityof Science 10 and Technology, Changchun, China 11 ^d State Key Laboratory on Integrated Optoelectronics, College of Electronic Science and 12 Engineering, Jilin University, Changchun, China 13 14 15 **Experiment:** 1. Materials 16 17 All DNA used in this work were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). The DNA sequences were listed in Table S1. N-methyl mesoporphyrin IX (NMM) was 18 purchased from J&K Scientific Ltd. (Beijing, China). Other chemicals were of analytical grade 19 20 and were used without further purification. Graphene oxide (GO) was synthesized according to a modified Hummer's method.¹ The water used was purified by a Milli-Q system up to resistivity of 21 22 18.2 MΩ. 23 24 Table S1. DNA sequences used in the designed multiplexer and demultiplexer logic 25 operations. 26

	Strands	Sequences (5'-3')
DEMUX	F-DNA	FAM- GCT ACT CCG TCT CTT GTT TCG GTG
	IN1	GGG TCA CCA GAA CTG TAG ACG GAG TAG C

		$\mathbf{S}_{\text{DEMUX}}$	GCT ACT CCG TCT ACC CTT CTG GTG AGG GTG GGT GGG
MUX	P-DNA	GCA GTC CAT TAG TAG AGG GAT GCA GTG GGA AAA CAC	
		ATC GTC ACG AAT	
	INA	G GGC GGG ATG GGT CTA CTA ATG GAC TGC	
	INB	ATT CGT GAC GAT GTG TTT TGG G	
	S _{MUX}	GGG TTT TGG GTC TGC ATC CCT CTA CTA ATG GAC TGC	

2 To find the available DNA sequences for the developed higher-order logic gates, the designed 3 DNA sequences firstly mimicked website were on the http://mfold.rna.albany.edu/?q=DINAMelt/Twostate-melting and then modified according to the 4 5 experimental results. The above procedures were repeated until the available DNA sequences were 6 obtained.

7 Instruments:

8 Fluorescence measurements were carried out on a Fluoromax-4 spectrofluorometer (Horiba 9 JobinYvon, Inc., NJ, USA). The fluorescence spectra were recorded at room temperature by 10 irradiating FAM at 494 nm and NMM at 399 nm. Absorbance measurements were performed on a 11 Cary 500 Scan UV/Vis/NIR Spectrophotometer (Varian, USA). Circular dichroism (CD) spectra 12 of DNA were conducted on a JASCOJ-810 spectropolarimeter (Tokyo, Japan).

13 Logic Gate Operations:

The DNA were dissolved in water as stock solution and diluted with HEPES buffer (20mM HEPES, 150mM NaCl, 150mM KCl, pH 7.0,) and Tris-HCl buffer(10 mM Tris, 4mM MgCl₂, 16 15mM KCl, pH 7.40) for multiplexer and demultiplexer, respectively. All the diluted DNA strands 17 were heated at 88°C for 10 min and then cooled down slowly to room temperature before 18 experiments.

For multiplexer, the inputs DNA (final concentration: 200 nM INA, 150 nM INB, 300 nM S_{MUX})
were added in working buffer (including 100 nM P-DNA) one by one and incubated at room
temperature for 30 min. After that, NMM was added and further incubated for 30 min. Finally,
fluorescent emission spectra from 540 nm to 720 nm were recorded.

23 For demultiplexer, platform DNA strand (F-DNA) was firstly added into Tris-HCl buffer

containing 4 ug/mL GO and added inputs DNA 10 min later. After incubated for 30 min, the
 addition of 250 nM NMM and another 30 min incubating time was followed. Finally, the
 fluorescent analysis was performed. For FAM emission spectra, the excitation wavelength is 494
 nm, both of the excitation and emission slit width are 10 nm. The excitation wavelength is 399 nm
 for NMM. Both the excitation and emission slit width are 20 nm.

6 3. Native polyacrylamide gel electrophoresis (PAGE)

For both the logic gate, DNA solution with different inputs was firstly heated at 88°C for 10 8 min and then cooled down slowly to room temperature before experiment. Further, 10 uL DNA 9 solution mixed with gel red and 6×loading buffer was added for 15 % PAGE native 10 polyacrylamide gel electrophoresis analysis at a constant voltage of 120V for 90min. Finally, the 11 gels were scanned by a UV transilluminator.

12 **Results:**





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14 Figure. S1. AFM image of the synthesized GO (left) and the height profile (right).

15 The synthesized GO was characterized with atomic force microscopy (AFM) as shown in Figure 16 S1. The height of the GO was about 1.09 nm according to the detection of cross section, which is 17 consistent with previous report.²

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Fig.S2. Comparison of FAM fluorescence signal of F-DNA (100 nM) before (a) and after (b)
adding GO of 4ug/mL.

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Learned from Fig. S2, the fluorescence of FAM is quenched after the F-DNA anchors on GO
since GO can be used as a super nanoquencher to quench the fluorescence of various dyes via
long-range resonance energy transfer.³



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9 Fig.S3. FAM fluorescence response of F-DNA at the wavelength of 521 nm in the presence of10 various concentration of GO.

The optimal concentration of GO was explored by recording fluorescence response of FAM labled DNA, F-DNA. Learned from Fig. S3, the fluorescence intensity of FAM decreases along with increasing the concentration of GO and reaches a plateau at 4 µg/mL GO. Thus, 4 µg/mL of GO is selected for the experiments.

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2 Fig.S4. Fluorescence intensity of FAM at 521 nm as a function of the IN1 concentration for the3 developed demultiplexer.

As shown in Fig. S4, the FAM fluorescence intensity of GO/F-DNA increases with the increasing of the concentration of the IN1 and reaches a plateau up to 250 nM. Thus, 250 nM is selected for the following experiments.





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9 Figure S5 the quenching percentage of GO (A) and the restoring percentage with different pH.

As shown in Figure S5(A), the fluorescence of FAM on GO can be quenched about 98.5%, 98.1%, 93%, 85.3% in pH 4, 5, 6 and 7.4, respectively. Considering the logic operation of DEMUX, one has to consider the restore of the fluorescence of FAM. Learned from Figure S5(B), the fluorescence of FAM can be restore to the maximum in pH 7.4. In the case of pH 7.4, the ON/OFF ratio of FAM fluorescence reaches the maximum. Thus, pH 7.4 was selected for the experimental performance.

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2 Figure S6 the quenching percentage of GO via different concentration of MgCl₂.

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To reduce the background in the absence of any inputs, the influence of the concentration of Mg²⁺ on the fluorescence quench of FAM on GO was investigated as shown in Figure S6. According to the results, the background of the system reduces with increasing of the concentration of Mg²⁺ and reaches a plateau when the concentration of Mg²⁺ reaches 4 mM. Thus, 4 mM was selected as the concentration of Mg²⁺ in the experiments.

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11 Fig. S7. Circular dichlorism spectra for characterizing structure of DNAs in the demultiplexer12 logic operations.

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Learned from Fig. S7, the circular dichlorism (CD) spectra of F-DNA (curve a), IN1 (curve b) and selector DNA (S-DNA) (curve c) are of relatively low amplitude, indicating that the DNA strands possesses no obvious G4 structure. The amplitude of CD spectra are still low in the coexistence of F-DNA and IN1 (curve d), or coexistence of F-DNA and S-DNA (curve e). A significant change was monitored in the coexistence of F-DNA, IN1 and S-DNA (curve f). A 1 positive band near 265 nm and a negative band near 242 nm appear, indicating the formation of G-



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Fig. S8. Circular dichlorism spectra for characterizing structure of DNAs in the multiplexer
logic operations under the condition of switching off (A) and switching on (B) the selector.

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8 If the selector (S_{MUX}) is switched off, Fig. S8A, the CD spectra of P-DNA (curve a) and P-9 DNA/INB (curve b) are of relatively low amplitude, indicating that the DNA strands possesses no 10 obvious G4 structure. A significant change is monitored from the CD spectrum of P-DNA/INA 11 (curve c), A positive band near 265 nm and a negative band near 242 nm appear, indicating the 12 formation of G-quadruplex with a parallel structure. The addition of INB does not influence the G-13 quadruplex configuration of P-DNA/INA (curve d).

If the selector (S_{MUX}) is switched on, Fig. S8B, the CD spectra of P-DNA/S_{MUX} in the absence (curve a) and presence of INA (curve b) and are of relatively low amplitude. By combining the low fluorescence responses results shown in Fig. 3A in the main text, it is reasonable to draw a conclusion that the DNA strands possess no obvious G4 structure. Once INB is added, the amplitude of the CD spectra is significantly enhanced no matter adding (curve c) or free of (curve d) INA. By combining the low fluorescence responses results shown in Fig. 3A in the main text, it is reasonable to draw a conclusion that G-quadruplex configurations are formed in the cases.

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3 Fig. S9. PAGE analysis of DNA interactions for the 2:1 multiplexer logic operation.

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5 From Lane 1 to Lane 4, the belts indicate the ss-DNAs of P-DNA, INA, INB and S_{MUX} in 6 sequence. No obvious belts can be found from INA and INB. The possible reason is that the chain lengths of INA and INB are shorter than other ss-DNAs and duplexes. Belts appear at new 7 positions in the coexistence of P-DNA/INA (Lane 5), P-DNA/INB (Lane 6) and P-DNA/S_{MUX} 8 9 (Lane 7), indicating the formation of the corresponding duplex. In the coexistence of P-DNA, INA 10 and INB, a belt appears at position over than P-DNA/INA and P-DNA/INB (Lane 8), confirming that P-DNA can act as a linker to bind INA and INB together. In the coexistence of P-DNA, S_{MUX} 11 and INA (Lane 9), a belt appears at position where is similar as that of P-DNA/ S_{MUX} . The result 12 validates that the INA does not influence the interaction between P-DNA and S_{MUX} . In the 13 coexistence of P-DNA, S_{MUX} and INB (Lane 10), a belt appears at position where is over that of P-14 DNA/INB and P-DNA/S_{MUX}. The result validates that P-DNA can act as a linker to bind INB and 15 S_{MUX} together. In the coexistence of P-DNA, INA, INB and S_{MUX} (Lane 11), a belt appears at the 16 position where is similar as that in Lane 10, indicating that INA does not influence the interaction 17 18 among P-DNA, INB and S_{MUX}.

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