*Electronic Supplementary Information*

# Enzyme-driven i-motif DNA folding for logic operations and fluorescent biosensing

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## Materials and Methods

Dual-labeled DNA was synthesized by Takara (Dalian, China). All other chemicals and enzymes used in this study were purchased from Aldrich-Sigma (St. Louis, MO,USA). The fluorescence spectra were recorded using Hitachi F-4500 spectrophotometer at room temperature.

**pH-dependent RG emission of dual-labeled DNA.** 20 nM of dual-labeled DNA solution was prepared by adding stocked DNA solution to 1.0 mM Tris buffer (containing 0.1 M NaCl) with pH ranging from 5.1 to 7.8. The RG emission spectra were measured with an excitation wavelength at 503 nm.

**"NOR" logic gate fabrication** To develop a logic gate with "NOR" function, to a mixed solution of DNA (20 nM in 1.0mM Tris buffer, pH = 6.8), acetylcholine (1.0mM), and glucose (25 mM) was added AChE (50 mU),or GOx (40 U), or AChE and GOx simultaneously. The RG emission spectra were measured after incubation for 30 min. at 37 $^{o}$C, the RG emission intensity was then compared and gated to the emission of solution without enzyme addition.

**"NAND" logic gate fabrication** The "NAND" logic gate was developed by adding INV (100 U) and GOx (40 U) to a mixed solution of DNA (20 nM in 1.0mM Tris buffer, pH = 6.8) and sucrose (25 mM). The RG emission intensity was measured and compared to that without enzyme addition or with only one enzyme.

**AChE activity assay and inhibitor identification** To demonstrate the use of the "NOR" DNA logic gate for enzyme activity assay, a mixture of DNA (20 nM) and acetylcholine (1.0 mM) in 1.0 mMTris buffer (pH = 6.8) was incubated with varied amounts of AChE (10 mU, 25 mU, 50 mU, and 100 mU) for 15 min. at room temperature, the RG emission intensity at 530 nm was monitored and compared to a solution without AChE addition. AChE inhibitor identification using the DNA logic gate was performed by incubating a mixed solution of DNA (20 nM), acetylcholine (1.0 mM), and AChE (50 mU) in the absence and presence of 10 μM neostigmine. The solution emission was then monitored and compared as described above. To determine the $IC_{50}$ of neostigmine against AChE, the solution of DNA (20 nM), acetylcholine (1.0 mM), and AChE (50 mU) was incubated with different concentration of neostigmine, AChE activity was assayed and compared based on the RG emissionchange in the presence of different concentration of neostigmine.

**Glucose sensing using "NOR" logic gate.** To develop a fluorometric glucose sensor, a mixed solution of DNA (20 nM) and GOx (40 U) was incubated with varied concentrations of glucosefor 30 min. RG fluorescence intensity at 530 nm (F) was recorded and normalized to that without glucose ($F_0$). To demonstrate a high selectivity of glucose sensing using the DNA logic gate, the sensing solution described above was incubated with 750 μM of galactose, mannose, fructose, or maltose.

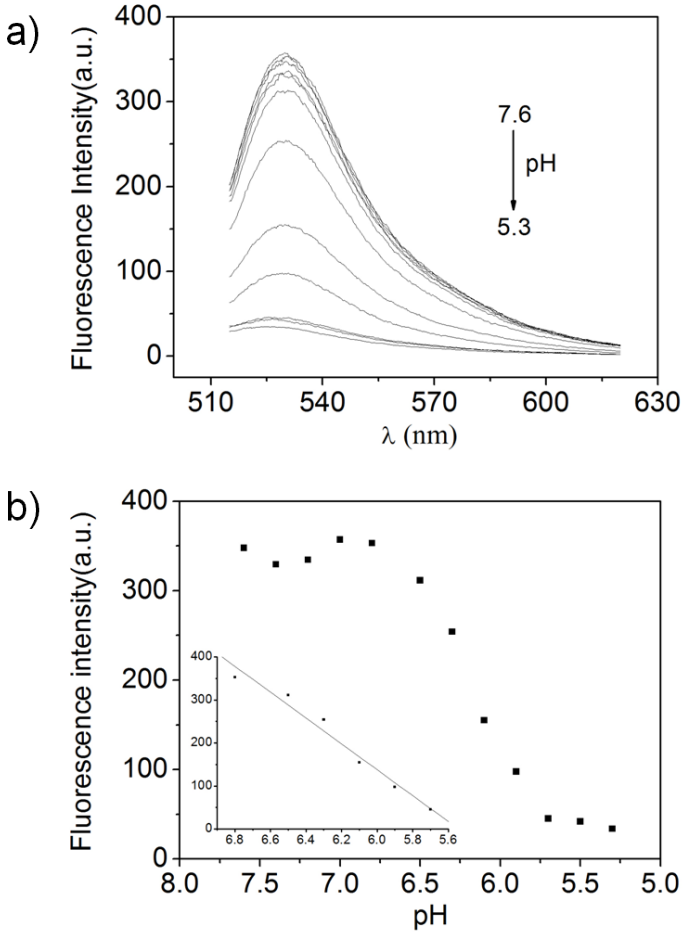


**Fig. S1** Fluorescence spectra (a) and emission intensity at 530 nm (b) of dual-labeled DNA (20 nM) in Tris buffered solutions (1.0 mM) containing 0.1 M NaCl with varied pH value. The fluorescence was measured with an excitation wavelength at 503 nm.

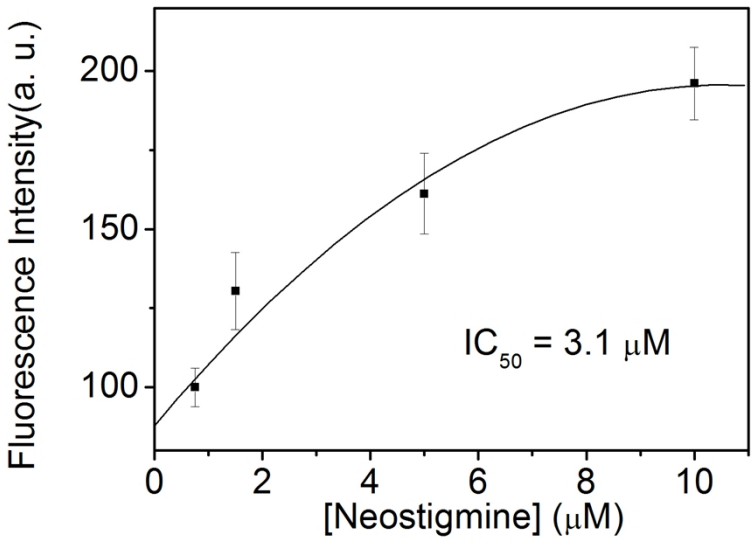


**Fig. S2** The effect of neostigmine concentration on AChE activity. A mixed solution of DNA (20 nM), acetylcholine (1.0 mM), and AChE (50 mU) was incubated with indicated concentration of neostigmine, RG emission intensity was monitored at 530 nm.

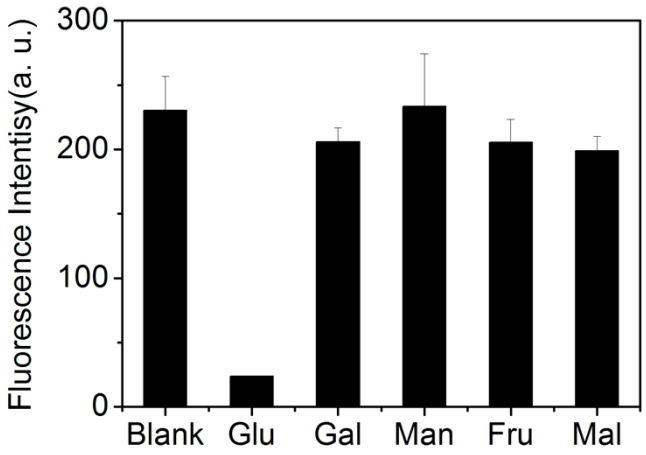


**Fig.S3** Selective glucose sensing using the "NOR" DNA logic gate. A mixed solution of DNA (20 nM) and GOx (40 U) was incubated with 750 μM of different saccharides as indicated, followed by RG emission measurement at 530 nm.