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Room-temperature phosphorescence logic gates developed from nucleic acid functionalized carbon dots and graphene oxide

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Part S1. Synthetic procedures of HOOC-CD

Carbon dots (CD) were prepared according to a modified version of the reported method (*Angew. Chem., Int. Ed.*, 2013, **52**, 3953). In a typical experiment, 1.2 g of citric acid and 0.75 mL of ethylenediamine were dissolved in 12 mL of double distilled water. After adequate mixing under agitation, the resultant mixture solution was transferred to a 50 mL poly(tetrafluoroethylene) (Teflon)-lined autoclave, and heated at 150 ^{o}C for 5 h. Then, the as-obtained product was subjected to dialysis against the double distilled water (retained molecular weight: 3.5 kDa) frequently (at least ten times in 24 hours) in order to achieve CD. After that, 25 mg of the as-prepared CD was dispersed in 25 mL of basic aqueous solution containing NaOH (1.25 g) and $ClCH_2COONa$ (1.25 g), followed by sonication treatment for 3 h in water bath, using the reported method (*Small*, 2010, **6**, 537). Undergoing these treatments above, the resultant NaOOC-CD was neutralized with HCl and dialysed to acquire HOOC-CD, which possessed a quantum yield of 7.3% (using quinine sulphate as the standard). In comparison with CD that obtained a high quantum yield of 77.1%, the quantum yield of HOOC-CD represented a dramatic decrease, which was attributed to the increase of electron-withdrawing carboxyl groups (*Biosens. Bioelectron.*, 2015, **63**, 506).

Part S2. Synthetic procedures of cDNA-CD

The conjugation of HOOC-CD and cDNA (*i.e.* oligonucleotide capture ssDNA: 5'-NH_2-TGC ATT ACT AAT CAG TGA GGC CTT-3') was conducted, based on a small modified version of the previous method (*Anal. Chem.*, 2011, **83**, 1307). Typically, 5 mg of the prepared HOOC-CD was dispersed in 7.5 mL of PBS (phosphate buffered saline, 0.1 M, pH 7.0) under ultrasonication, and a homogenous aqueous suspension was obtained. After that, 50 mg of succinic anhydride was added to the above mixture solution, which was then allowed to react for 2 h under stirring. After this reaction, the reaction was stopped and the as-obtained mixture solution was centrifuged and repeatedly washed with PBS. Then, 3 mg of EDC and 4.5 mg of NHS dispersed in 7.5 mL of Tris-HCl buffer (0.05 M, pH 7.2) containing 0.02 M of NaOH were added to the reaction mixture, and allowed the reaction to react for 30 min under stirring, followed by the addition of cDNA (50 μL). This reaction was incubated for 12 h. The excess of HOOC-CD was removed in a dialysis bag (retained molecular weight: 7 kDa) against water frequently. The resultant solution was centrifuged and washed with Tris-HCl buffer repeatedly. These obtained particles were resuspended in Tris-HCl buffer, and therefore HOOC-CD conjugated with cDNA (cDNA-CD) was obtained.

Part S3. Molecular structures of DNA oligonucleotides

DNA oligonucleotides with a concentration of 100 μM were purchased from Shanghai Reagent Corp. of China, and the sequences are listed as follows:

Capture ssDNA (cDNA): 5′-NH_2-TGCATTACTAATCAGTGAGGCCTT-3′

Target ssDNA (tDNA, representing a perfect match with cDNA):

3′-ACGTAATGATTAGTCACTCCGGAA-5′

Single-base mismatch ssDNA (mDNA): 3′-ACGTAATGATTAGACACTCCGGAA-5′

Materials and characterizations

DNA oligonucleotides were purchased from Shanghai Reagent Corp. of China. $ClCH_2COONa$, NHS and EDC were brought from Shanghai GL. Biochem Ltd. of China. GO used in this study was synthesized by using the well-established Hummers method (*J. Am. Chem. Soc.*, 1958, **80**, 1339). Other common chemicals and solvents were obtained from Shanghai Reagent Corp. of China (*i.e.*, from the commercial sources), and can be directly utilized as received without any further purification treatment.

The phosphorescence spectra were recorded on an LS-55 fluorescence spectrophotometer (PerkinElmer, USA) equipped with a quartz cell (1 cm × 1 cm) in the phosphorescence mode. Fourier transform infrared (FTIR) spectra were obtained under a transmission mode with a Nicolet 6700 FTIR spectrometer (Nicolet, USA). UV-visible absorption spectra were recorded with a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence emission spectra were measured on a FLSP 920 fluorescence spectrophotometer (Edinburgh Instrument, U.K.). Time-resolved fluorescence and phosphorescence decay by delay were performed on a FluoroMax-4 spectrofluorometer (Horiba, France). Transmission electron microscope (TEM) images were acquired by a JEOL JEM-1400 TEM operating at 120 kV of acceleration (JEOL, Japan).

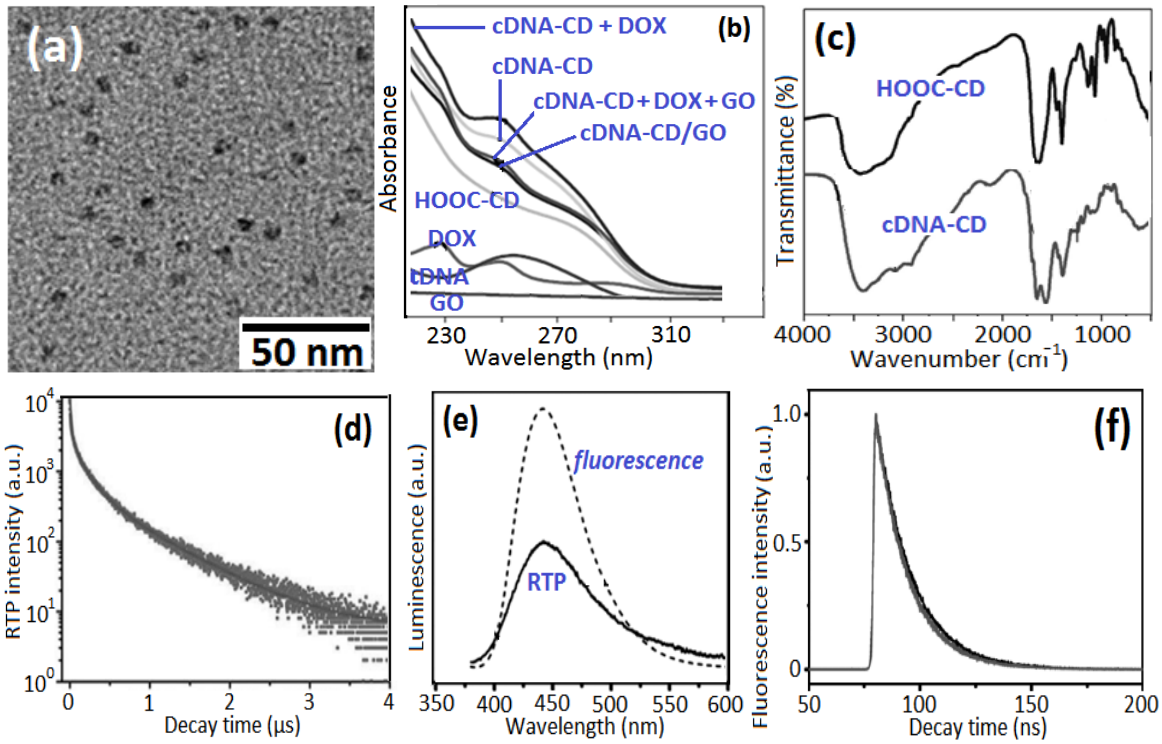

Fig. S1 (a) TEM images of HOOC-CD. (b) UV-vis absorption spectra and (c) FTIR spectra of HOOC-CD and cDNA-CD. (d) Time-resolved RTP spectrum of HOOC-CD. (e) RTP and fluorescence emission spectra of HOOC-CD. (f) Time-resolved fluorescence spectrum of HOOC-CD.

In the synthesis of CD, a high-temperature reaction of citric acid and ethlenediamine at 150 ^{o}C resulted in the formation of polymer-like nanoparticles (*i.e.* CD). TEM images of the as-prepared CD demonstrated homogeneous CD particles with the spherical shape and nearly uniform diameter of ~12 nm (Fig. S1a). In comparison with HOOC-CD, cDNA-CD represented a more intensive absorption below 300 nm (Fig. S1b). It might be attributed to the bonding of cDNA and CD. Similarly, cDNA-CD with the addition of DOX also exhibited an increasing absorption because of the bonding of CD and DOX. However, the addition of GO into the complexes of "cDNA-CD" and "cDNA-CD plus DOX" induced a weak decrease in the absorption spectrum, which should be due to the π-π stacking interactions between CD and GO. Fig. S1c showed FTIR spectra of HOOC-CD and cDNA-CD. The absorption bands at 1650 cm^{-1} and 3400 cm^{-1} were assigned to the C=O and O-H stretching vibration, respectively (*Angew. Chem. Int. Ed.*, 2013, **52**, 3953). Additionally, the characteristic absorption bands at 2920 cm^{-1}, 1390 cm^{-1}, 1240 cm^{-1}, 1135 cm^{-1} and 1090 cm^{-1} were assigned to the C-H stretching vibration, O-H deformation peak, C-OH stretching peak, and double C-O strength stretching, respectively. These results implied that the HOOC-CD had excellent water-solubility due to the oxygen-containing functional groups, enabling a facile functionalization with biomolecules (*e.g.* DNA). The absorptions of cDNA-CD conjugates were dramatically different from those of HOOC-CD located at 3100 cm^{-1}, 1560 cm^{-1} and 1240 cm^{-1}. These peaks were characteristic of –NH, –N–C=O and NHC=O stretching vibration, respectively, indicating the grafting of cDNA on cDNA-CD. The UV-vis and FTIR spectrum characterizations verified that cDNA was conjugated to HOOC-CD indeed. RTP decay spectrum of CD was shown in Fig. S1d. The curve could be fitted into a multiexponential function with four lifetimes. The average lifetime was calculated to be ~385 ms (excited at 325 nm, emitted at 440 nm), using the equation: $< \tau > = \Sigma\alpha_i\tau_i^2 / \Sigma\alpha_i\tau_i$. In comparison with the fluorescence characteristics of CD (as indicated in Fig. S1e), RTP emission profile of CD displayed only an increase in the longer wavelength of 500~600 nm, without the emission band shifted to a long wavelength. This result was similar to that of RTP arising from Mn doped ZnS QDs (*Anal. Chem.*, 2008, **80**, 3832). By contrast, the prepared CD exhibited a much stronger fluorescence emission than that of RTP, suggesting the fluorescence with a short lifetime of 15.3 ns (in Fig. S1f) and high quantum yield of 77.1%. As a reference, the corresponding characters of CD based on RTP were 385 ms and 7.3%, respectively.

In addition, to further exploit the RTP emission character of CD, we provided a detail explanation. In the UV-vis absorption spectrum of HOOC-CD, the peak centered at 245 nm is attributed to π-π* transition of C=O, and the peak at 340 nm is ascribed to the *n*-π* transition of C=O. The RTP excitation spectrum shows a broad band from 300 to 400 nm, and overlaps the band of C=O (*Nat. Chem.*, 2011, **3**, 205), implying that the RTP may be from C=O bonds on CD. Furthermore, the singlet and triplet states of aromatic carbonyl groups are close in energy, and the spin-orbit coupling is efficient, so that it is prone to intersystem crossing (*Chem. Commun.*, 2013, **49**, 5751). Consequently, it is reasonable to suppose that RTP originates from the aromatic carbonyl group on CD. Additionally, as well-established, polycyclic aromatic hydrocarbons are a family of compounds those can be directly determined by room temperature phosphorimetry (*Anal. Chim. Acta*, 2004, **516**, 213). The RTP of CD is also probably related to the graphitic structure that is similar to the polycyclic aromatic structure.

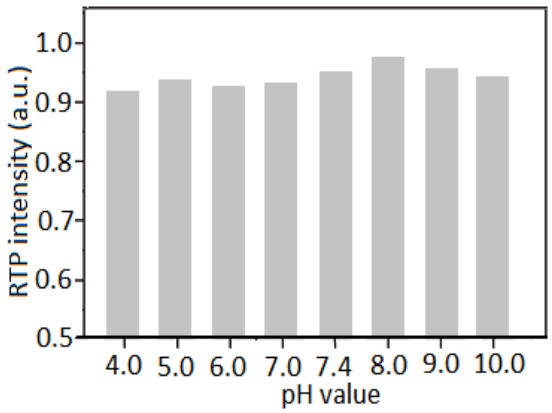

Fig. S2 RTP intensity responses of cDNA-CD/GO system in Tris-HCl buffer of different pH values.

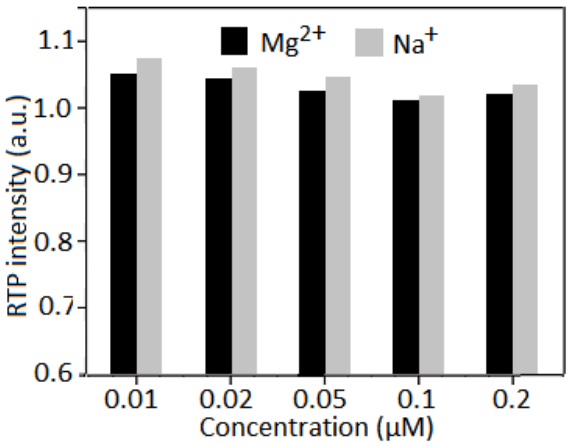

Fig. S3 RTP intensity responses of cDNA-CD/GO system in salt solutions of different concentrations.

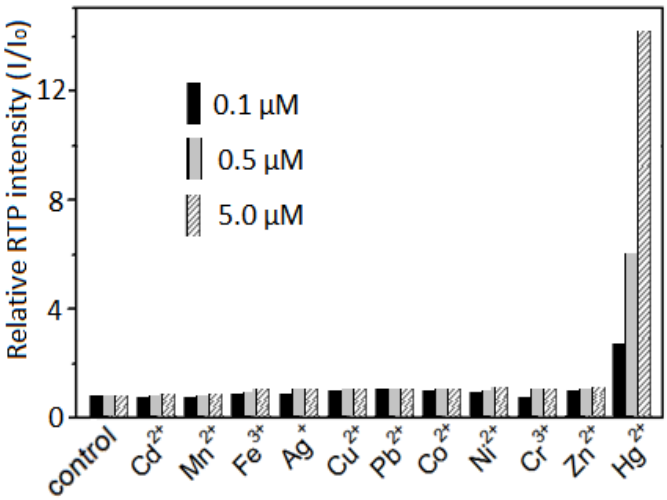

Fig. S4 RTP responses of CD to various metal ions of different concentrations (0.1, 0.5 and 5.0 μM).

Table S1

Detected concentrations of Hg^{2+} in real water samples using the cDNA-CD/GO system-based RTP "OR" logic gate.

Samples [a]	Added Hg^{2+} /μM	By logic gate /μM	RSD [b] (%)	Recovery (%)
Tap water 1	0.050	0.054 ± 0.002	3.704	108.0
Tap water 2	0.100	0.095 ± 0.004	4.210	95.0
Tap water 3	0.500	0.511 ± 0.009	1.761	102.2
Sea water 1	0.050	0.049 ± 0.002	4.081	98.0
Sea water 2	0.100	0.113 ± 0.005	4.425	113.0
Sea water 3	0.500	0.509 ± 0.015	2.947	101.8

[a] All concentrations were expressed as mean of six determinations ± standard deviation (SD).

[b] The relative standard deviation (RSD) was calculated as (SD/mean) × 100%.

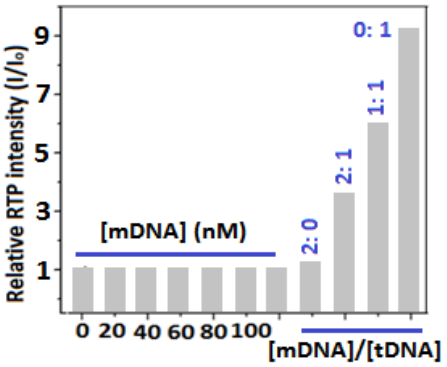

Fig. S5 Relative RTP intensities of cDNA-CD/GO system after incubating with mDNA of different concentrations, and with a mDNA and tDNA mixture (total 200 nM) with the [mDNA]/[tDNA] ratios at 2: 0, 2: 1, 1: 1 and 0: 1, respectively.

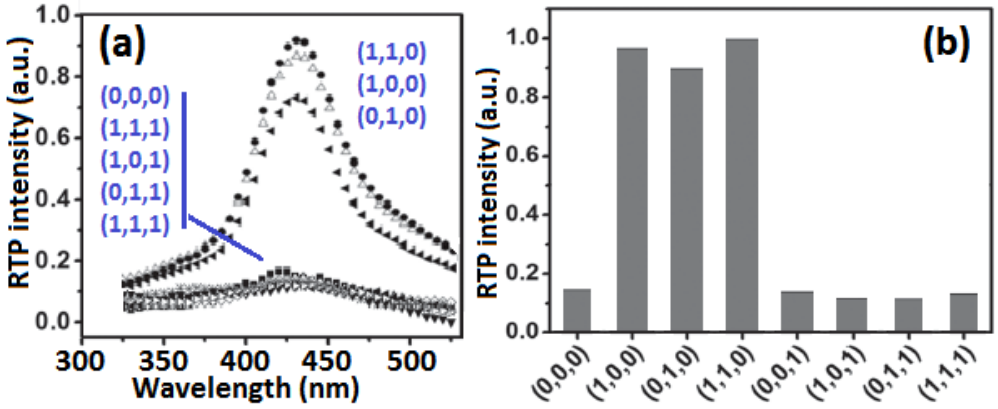

Fig. S6 The cDNA-CD/GO system-based "OR-INHIBIT" logic gate: (a) Normalized RTP spectra of the logic gate with different combinations of the inputs, and (b) normalized RTP intensities at 440 nm.