Electronic Supplementary Information for

Hot-spot-active magnetic graphene oxide substrate for microRNA detection based on cascaded chemiluminescence resonance energy transfer

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TableS1. Sequences of RNA and DNA used in this study

Name	Sequence (5′-3′)	Modification
miR-122	UGGAGUGUGACAAUGGUGUUUG	
miR-let-7a	UGAGGUAGUAGGUUGUAUAGUU	
miR-let-7b	UGAGGUAGUAGGUUGUGUGGUU	
I-1	CCGAGCCAAACACCATTGTCACACTCCAGCTCGGGGGTAGGGCGGGTTGGG	5′-amino group, 3′-fluorescein
I-1′	CCGAGCCAAACACCATTGTCACACTCCAGCTCGGGGGTAGGGCGGGTTGGG	
II-1	CCGAGCCTACAAACACCATGCTCGGGGGTAGGGCGGGTTGGG	5′-amino group, 3′-fluorescein
II-2	GTGACAATGGTGTTTGTAG	
II-3	CTACAAACACCATTGTCACACTCCA	
III-1	GGGTAGGGCGGGTTGGGCCTCCAGAGAGAGAGAGAGAGGGAGG	5′-fluorescein, 3′-amino group
III-2	TCTCTCTCGTGACAATGGTGTTTGTAGCTCTCTCT	
III-3	CTACAAACACCATTGTCACACTCCA	

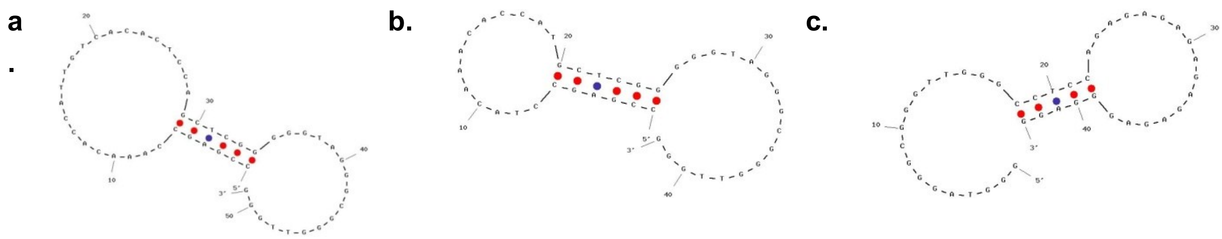

Fig. S1. The secondary structures of (a) I-1 (I-1′), (b) II-1 and (c) III-1 that were predicted using the OligoAnalyzer Tool of IDT (www.idtdna.com).

Relationship ofthe proposed three modes

In this assay, we proposed a C-CRET process from HRP-mimicking DNAzyme-catalyzed luminol-H_2O_2 to fluorescein and further to GO when HRP-mimicking DNAzyme/fluorescein was in a close proximity to GO surface. Accordingly, mode I was firstly fabricated by covalently immobilizing HRP-mimicking DNAzyme/fluorescein-labeled hairpin DNAs (hot-spot-generation probes) on magnetic GO (MGO), resulting in a signal "off" state due to the quenching of luminol/H_2O_2/HRP-mimicking DNAzyme/fluorescein CRET system by GO. Although mode I offered sensitiveand selective strategy for miRNA detection through MGO-based C-CRET hot-spot generation, its major disadvantage was the irreversibility and irreproducibility of the substrate for practical sensor applications, making it usable only once. Therefore, a new reversible and regenerable C-CRET hot-spot-active substrate for miRNA detection was fabricated as mode II through strand displacement reaction (SDR). While mode II offered a means of C-CRET hot-spot generation for miRNA detection with reproducibility and reversibility, it still suffered from an intrinsic limitation of sequence-dependence of II-1 on target miRNA. That is, to achieve detection of different target miRNAs, the sequence of II-1 had to be changed according to the target sequences, which would not qualify as a general approach for hot-spot creation. The problem of sequence-dependence also occurred in mode I, in which the sequence of I-1 also had to be changed with targets. To overcome all problems mentioned, mode III through forming a triple-helix molecular switch structure was proposed, which had the advantages of not only reproducibility and reversibility but also sequence-independence of hairpin probes on MGO.

Calculation of the surface coverage of hairpin probe I-1 on MGO. Firstly, the fluorescence calibration curve of hairpin probe I-1 (from 1.0×10^{-7} to 1.0×10^{-6} M) was prepared (Fig. S2). The surface coverage of hairpin probe I-1 on MGO was quantitatively evaluated from the differences of fluorescence intensities of DNA I-1 solution at ~518 nm before and after its attachment on MGO as follows.

The concentration of DNA I-1 before attachment (C_0) was $C_0 = 2.857\times10^{-6}$M

The concentration of DNA I-1 after attachment (C_1) was $C_1 = 8.875\times10^{-8}$ M

The volume of DNA I-1 solution (V) was V = 1050 μL

Thus, the moles of total DNA I-1 attached on MGO (N) were $N = (C_0 - C_1) \times V = (2.857\times10^{-6} - 8.875\times10^{-8}) \times 1050 \times 10^{-6} = 2.9 \times 10^{-9}$mol

The specific surface area of GO was 50 m^2/g

The volume and concentration of GO used to prepare the MGO were 0.6 mL and 1 mg/mL, respectively.

Thus, the surface coverage of hairpin probe I-1 on MGO was 2.9×10^{-9}mol/($0.6 \times 1 \times 10^{-3} \times 50$ m^2) = 9.6×10^{-8}mol/m^2 = 96 pmol/m^2.

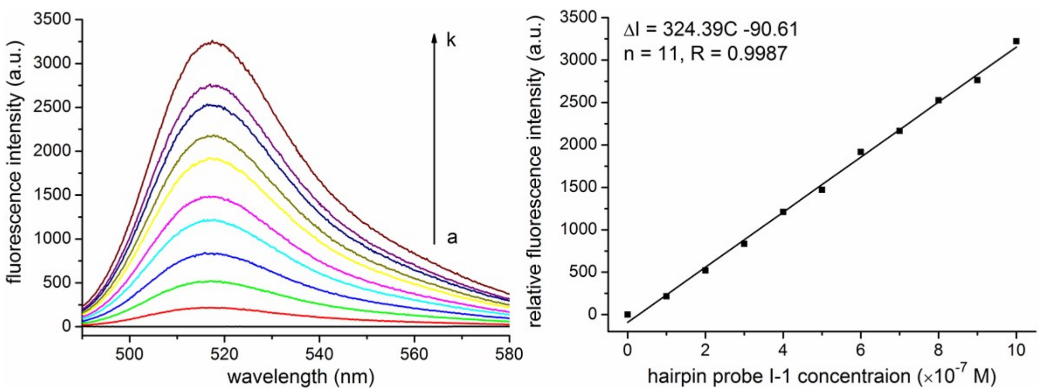

Fig. S2. Fluorescence intensities (A) and corresponding calibration curve (B) of standard hairpin probe I-1 solution with different. a→k: 0, 1.0×10^{-7}, 2.0×10^{-7}, 3.0×10^{-7}, 4.0×10^{-7}, 5.0×10^{-7}, 6.0×10^{-7}, 7.0×10^{-7}, 8.0×10^{-7}, 9.0×10^{-7}, and 1.0×10^{-6} M.

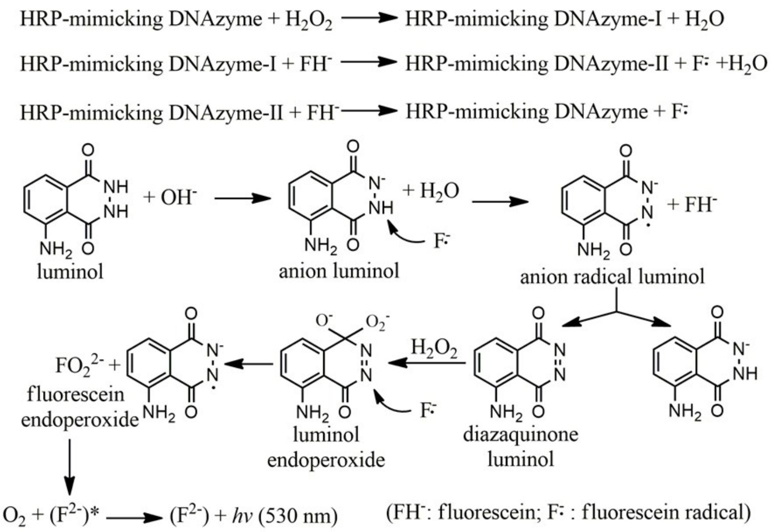

Fig. S3. CRET mechanism of luminol/H_2O_2/HRP-mimicking DNAzyme/fluorescein system.The fluorescein radical ($F\cdot^-$) increases the formation of the luminol radical ($L\cdot^-$). After the formation of luminolendoperoxide (LO_2^{2-}), an energy transfer from $LO_2^{2-}$to $F\cdot^-$forms a fluorescein endoperoxide (FO_2^{2-}), simultaneously liberating oxygen and emitting luminescence at ~530 nm.

mode I′

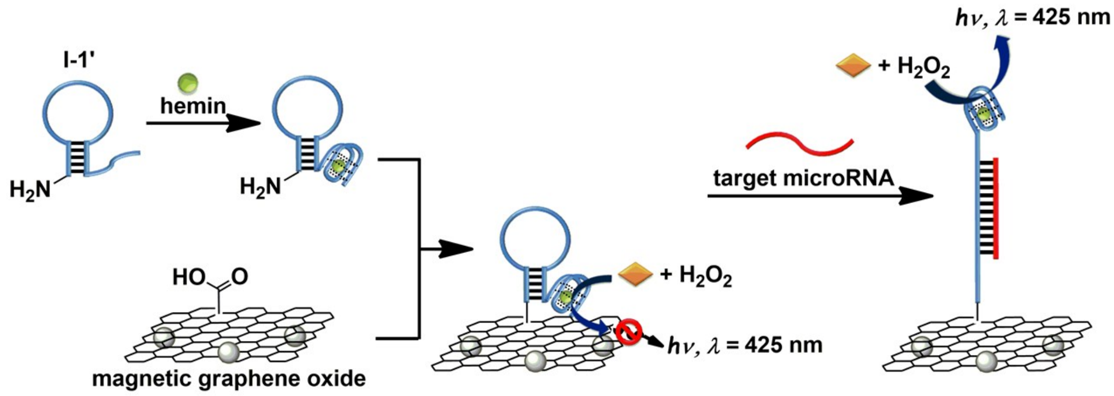

Fig. S4. Control experiment using hairpin probes I-1’ that is only modified with HRP mimicking DNAzyme (mode I) at 3′-end of I-1 to fabricate the hot-spot-substrate.

Table S2. Comparison between different modes for microRNA detection

strategy	advantage	disadvantage
mode I	Only onehairpin DNA (I-1) was required.	(i) The CRET hot-spot substrate was irreversible and not regenerable, making the sensor usable only once. (ii) The sequence of hairpin DNA (I-1) on substrate had to be changed according to target miRNA.
mode II	The fabricated CRET hot-spot substrate was reversible and regenerable.	The sequence of hairpin DNA (II-1) on substrate had to be changed according to target miRNA.
mode III	(i) The fabricated CRET hot-spot substrate was reversible and regenerable. (ii) Hairpin DNA (III-1) on substrate was sequence-independent.	All the disadvantages of modes I and II were successfully overcome.

Advantages of the proposed strategy

In comparison with earlier studies, the advantages of the proposed strategy can be summarized as follows. Firstly, the magnetic graphene oxide (MGO) was prepared via a carbodiimide-assisted covalent reaction using EDC as a coupling agent and NHS as an activator to active carboxyl groups on GO. The reaction was carried out at room temperature and easily operated. In contrast, most of the reported works for MGO preparation often involved with high temperature, nitrogen protection, complex chemical reactions, and complicated equipments.[1-4]

Secondly, due to the large specific surface and rich π electrons, GO can physically adsorb various biomolecules (such as DNA probes) with high affinity, while weak enough to allow quick desorption of probes in the presence of targets.[5] However, the physisorption system often suffered from no controllable organization,nonspecific displacement of probes by nontarget molecules and the false positive signal. To address this problem, herein we formed a C-CRET hot-spot-active substrate by covalently immobilizing hairpin DNA probes on GO via a carbodiimide-assisted covalent reaction. Upon target microRNA binding, the covalent hairpin probe only underwent a conformational change without leaving GO surface. As a result, the covalent substrate with good stability is more resistant to nonspecific probe release, which also supports the significance of not only controllable organization but also being reversible and regenerable,[6,7] especially through magnetic removal of reaction byproducts by the employment of MGO.

Thirdly, through theoretical calculations GO was a superquencher with long-range nanoscale energy transfer property.[8,9] In our work, instead of linear single-stranded DNA,[10,11] probes with hairpin structure covalently attached on MGO facilitated an efficient "on-off" signal switch for sensitive detection of microRNA target. In addition, unlike traditional CRET systems in which components were separatedfrom each other and large bioenzyme molecules (e.g. HRP) were used as catalysts, in this assay we used HRP-mimicking DNAzyme as catalyst and integrated it with fluorescent dye into single DNA hairpin probe involving nucleic acid functionalization instead of complicated synthesis and modification of biomolecules.

Last but not least, in the proposed luminol/H_2O_2/HRP-mimicking DNAzyme/fluorescein/GO C-CRET system, the energy transfer efficiency from luminol to fluorescein was calculated as high as 65.3%. In addition, in comparison with the luminol/H_2O_2/HRP-mimicking DNAzyme CL system, the total luminescence was enhanced ~2-fold for the luminol/H_2O_2/HRP-mimicking DNAzyme/fluorescein CRET system, which could be attributed to the enhancing role of fluorescein in the CRET system. That is, upon the addition of fluorescein into HRP-mimicking DNAzyme catalyzed luminol-H_2O_2 CL system, the total emission was enhanced. Thus, fluorescein served as both acceptor of luminol and enhancer of this chemiluminescence (CL) system. The mechanism was summarized as Fig. 1.[12] Additionally, 3′-end of hairpin probes were functionalized with fluorescein, while G-quadruplex that intercalated with hemin to yield an active HRP-mimicking DNAzyme was also incorporated at 3′-end that was immediately adjacent to FAM, which also make a contribution to a high CRET efficiency.

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