Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Diffusivity and Intercalation of Electroactive Dyes-Mediated Truly Ratiometric Homogeneous Electrochemical Strategy for Highly Sensitive Biosensing

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Materials and Apparatus

Chemicals and Reagents. All DNA and miRNAs used in this work were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) with their sequences listed in Table S1. Dithiothreitol (DTT), Gel Red, and alanine aminotransferase (ALT) were obtained from Solarbio (Beijing, China). Exonuclease III (Exo III) and Exonuclease I (Exo I) were purchased from Thermo Fisher Scientific (China) Co., Ltd. Tris(hydroxymethyl)aminomethane (Tris), L-serine, taurine, glucose, HCl, KCl, NaCl, methyl blue (MB), and $MgCl_2$ were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The Ag/AgCl electrodes (CHI111) and platinum wire electrodes (CHI115) were purchased from Shanghai CH instrument Co. LTD (Shanghai, China). The indium tin oxide (ITO) electrodes were purchased from Shenzhen Nanbo Display Technology Co. Ltd (Shenzhen, China). The diethylpyrocarbonate (DEPC) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Deionized water was obtained by a Milli-Q water purification system (Millipore Corp., Bedford, MA, U.S.A.). All reagents were analytical grade and all experiments used the DEPC-treated ultrapure water.

Apparatus. Electrochemical measurements were collected on an Autolab electrochemical workstation (Metrohm, Netherland) with a conventional three-electrode system: an ITO electrode as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire as the counter electrode. The images of gel electrophoresis were collected by the Gel Doc XR+ Imaging System (Bio-Rad Laboratories, Inc., U.S.A.).

Homogeneous Electrochemical Measurement. The ITO electrodes with the effective working area of 0.12 cm^2 were strictly treated using the previously reported method.[1] In brief, the ITO electrode was pretreated by being sequentially sonicated in an alconox solution (8 g of alconox per

liter of water), propan-2-ol, acetone, and ultrapure water for 15 min each. Then the ITO electrode was immersed in 1 mM NaOH solution for 5 h and sonicated in ultrapure water for 15 min. Finally, a negatively charged working electrode surface was obtained. The differential pulse voltammetric (DPV) measurement was carried out in the range from -0.4 to 0.5 V in 20 mM Tris-HCl buffer (100 mM NaCl, 10 mM $MgCl_2$, 20 mM KCl, 1 mM DTT, pH 7.5) with scanning rate of 10 $mV \cdot s^{-1}$.

RHE Detection of miRNA-182. All oligonucleotides were first treated through centrifugation for domiciliating in the tube's bottom and subsequently 20 mM Tris-HCl buffer (100 mM NaCl, 10 mM $MgCl_2$, 20 mM KCl, 1 mM DTT, pH 7.5) was added to obtain the stock solution of for experimental investigation. Prior to application, the solution containing Fc-H1/H2/H3 was heated to 95 ^oC for 5 min, respectively, and subsequently cooled down to 37 ^oC at a slow rate to form the desirable stem-loop configuration. The miRNA-182 assay was conducted as follows. 20 μL of Fc-H1 (5 μM), 20 μL of H2 (5 μM), 20 μL of H3 (5 μM), 10 μL of Exo III (2.0 U/μL), 10 μL of MB (30 μM), and 20 μL of target miRNA-182 with different concentrations were mixed together, and the mixed solution was permitted to react for 120 min at 37 ^oC. After the reaction, the DPV measurement of the system was performed in the range of -0.4~0.5 V with scanning rate of 10 $mV \cdot s^{-1}$.

Table S1. Sequences of the oligonucleotides used in the experiments[a]

Name	Sequence (5′-3′)
P1	TGAGGTAGTAGGTTGTAT
P2	ATACAACCTACTACCTCA
G1	GGGTTGGGCGGGATGGGT
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-96	UUUGGCACUAGCACAUUUUUGCU
miRNA-382	GAAGUUGUUCGUGGUGGAUUCG
miRNA-183	UAUGGCACUGGUAGAAUUCACU
miRNA-182	*UUUGGCAAUGGUAGAACU*CACACU
Fc-H1	TGGTAGAACTGGTTGTATAGATAGT*AGTTCTACCATTGCCAAA*-Fc
Fc-H1′	TGGTAGAACTGGTTGTATAGATAGTAGTTCTACCAATATATAT-Fc
Fc-H1″	ATATATATATGGTTGTATAGATAGTATATATATATTTGCCAAA-Fc
Fc-H1‴	ATATATATATGGTTGTATAGATAGTATATATATATATATATAT-Fc
H1	TGGTAGAACTGGTTGTATAGATAGT*AGTTCTACCATTGCCAAA*
H2	AGGGCGGGTGGGTTGTATAGTAGGCAAAGTAACTATACAACCAGTT CTACCATGGGTA
H3	TGGGTACTTTGCCTACTATACAATGGTAGAACTGGTTGTATAGTAG GGTAGGGCGGG

[a]In H1 and Fc-H1, the letters in italics represent the sequences complementary to the target miRNA-182. In Fc-H1, H1, H2, and H3, the underlined letters represent the sequences complementary to each other to form the stems of the hairpin probe, respectively.

Table S2. Biosensing performance of our strategy and other methods.

Analytical method	Target	Strategy	Linear Range	Detection Limit	Ref.
Ratiometric electrochemiluminescence	miRNA	electrode modification	0.02~120 pM 0.03~150 pM	6.3 fM 8.6 fM	2
Ratiometric electrochemiluminescence	miRNA	electrode modification	10 aM~100 pM	9.4 aM	3
Ratiometric photoelectrochemistry	miRNA	electrode modification	0.25~25000 fM	83.3 aM	4
Ratiometric electrochemistry	miRNA	electrode modification	0.1~100000 fM	11 aM	5
Ratiometric electrochemistry	miRNA	electrode modification	0.1~100.0 fM	67 aM	6
Ratiometric electrochemistry	DNA	electrode modification	0.01~0.8 pM	4.16 fM	7
Ratiometric electrochemistry	DNA	electrode modification	0.5~80 pM	0.12 pM	8
Ratiometric electrochemistry	miRNA	electrode modification	0.1~1500 fM	33 aM	9
Ratiometric electrochemistry	miRNA	material preparation	0.08~300 fM	25 aM	10
Ratiometric electrochemistry	miRNA	no electrode modification or material preparation	0.01~10 pM	10 fM	This work

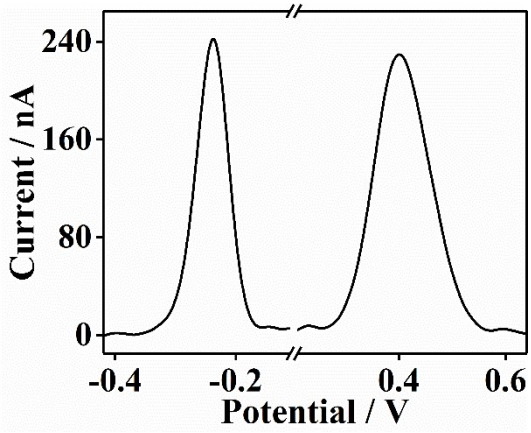

Fig. S1 The DPV curve of Fc-ssDNA and free MB molecules in the presence of Exo I.

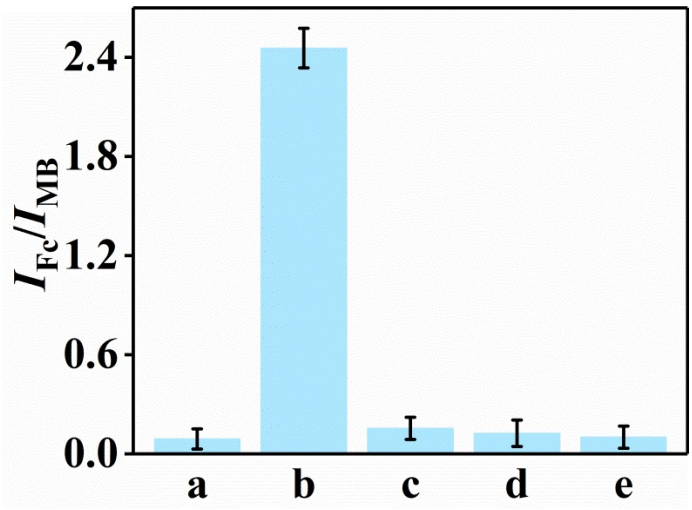

Fig. S2 The I_{Fc}/I_{MB} values of the ratiometric biosensor developed by different Fc-labeled DNA in the absence/presence of miRNA-182: (a) Fc-H1, in the absence of miRNA-182; (b) Fc-H1, in the presence of miRNA-182; (c) Fc-H1′, in the presence of miRNA-182; (d) Fc-H1″, in the presence of miRNA-182; (e) Fc-H1‴, in the presence of miRNA-182.

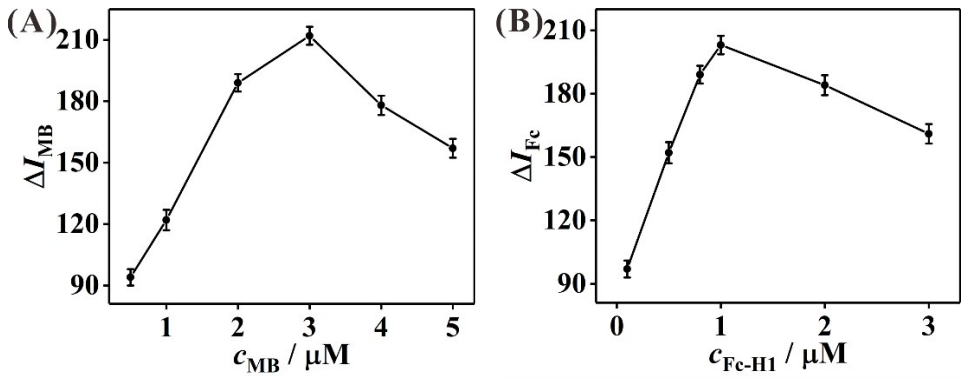

Fig. S3 (A) The DPV peak current change of MB (ΔI_{MB}) versus the MB concentration; (B)The DPV peak current change of Fc (ΔI_{Fc}) versus the Fc-H1 concentration.

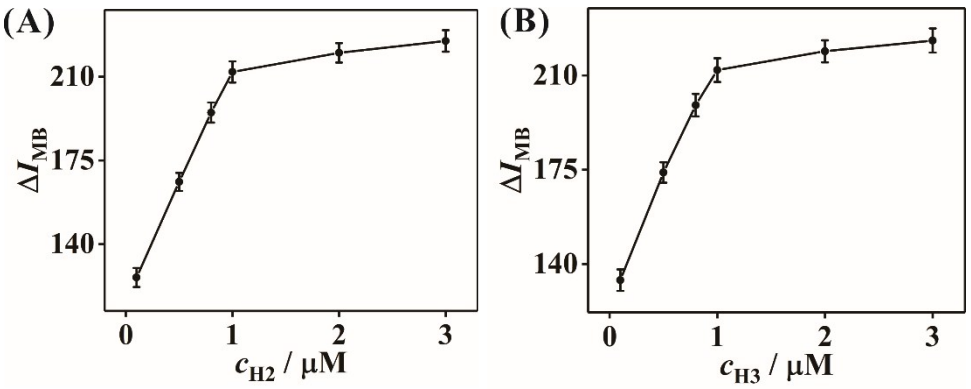

Fig. S4. (A) The DPV peak current change of MB (ΔI_{MB}) versus the H2 concentration; (B)The DPV peak current change of MB (ΔI_{MB}) versus the H3 concentration

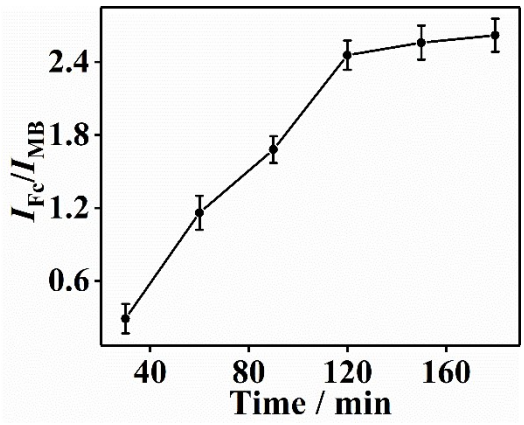

Fig. S5. The ratio of I_{Fc}/I_{MB} value versus the reaction time.

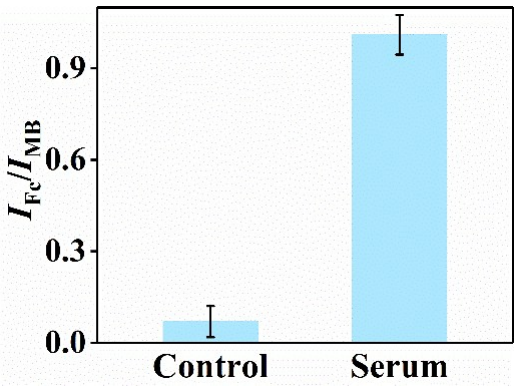

Fig. S6 The I_{Fc}/I_{MB} values versus different samples. Control sample: Fc-H1 + H2 + H3 + Exo III in Tris-HCl buffer; Serum sample: Fc-H1 + H2 + H3 + Exo III in diluted serum sample obtained from glioma patients.

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