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A fluorometric graphene oxide-based assay for determination of *agrB* gene transcription in methicillin-resistant *Staphylococcus aureus* by coupling exonuclease III-assisted target recycling and hybridization chain reaction

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Table. S1 Oligonucleotide sequences used in this work.

Nucleotide sequences	Sequence (5'-3')
Target	TGGTGCTCATGCAAAGTCTTCGATA
Hairpin 1	TCGGCGTGCAGTCGCCGAGAAGACTTTGCATGAGCACCA
Hairpin 2	TGCAGTCGTACGACTGCACGCCGACAGT-FAM
Hairpin 3	FAM-CGTACGACTGCATCGGCGTGCAGTGTACGTCGT
One mismatch	TG**C**TGCTCATGCAAAGTCTTCGATA
Two mismatches	TG**C**TGCTCATG**G**AAAGTCTTCGATA
Five mismatches	TG**C**T**C**CTCATG**G**AAAGT**G**TTC**C**ATA

Table S2 Repeatability and reproducibility of this sensor for mRNA detection

	Concentration (pM)	Group 1 (FL)	Group 2 (FL)	Group 3 (FL)	Group 4 (FL)	Group 5 (FL)	Average (FL)	RSD (%)
Repeatability	0.1	135.42	131.99	129.38	132.83	137.14	133.35	2.27
	10	251.37	246.59	248.53	253.27	252.47	250.45	1.12
	1000	362.16	358.24	367.58	360.26	365.19	362.69	1.03
Reproducibility	0.1	141.36	137.94	136.82	143.84	141.14	140.22	2.02
	10	263.97	258.93	256.23	260.1	263.03	260.45	1.20
	1000	374.92	367.36	365.12	372.07	370.21	369.94	1.04

Table. S3 The expression level of target RNA at different culture times (n=3)

Culture times (h)	Detected by this method	Detected by qRT-PCR
0	1	1
8	1.744	1.852
16	3.120	3.192
24	4.760	4.794
32	6.377	6.285
40	8.139	8.199
48	9.512	9.368

Table. S4 The expression level of target RNA after treatment with different concentrations of berberine (n=3)

Concentrations of berberine (μg□mL^{-1})	Detected by this method	Detected by qRT-PCR
0	1	1
20	0.877	0.902
40	0.794	0.783
60	0.647	0.639
80	0.501	0.516
100	0.325	0.336
120	0.145	0.165

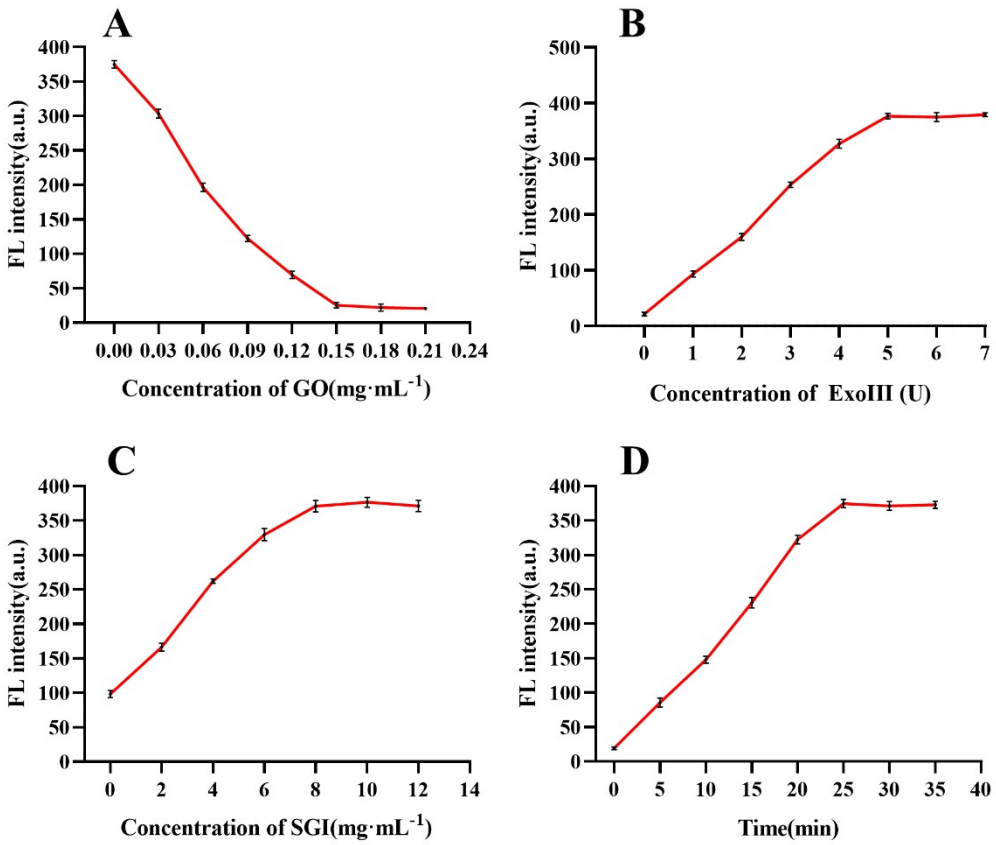

Figure. S1 Optimization of the experimental conditions. (a) Optimization of the concentration of GO. (HP1: 10 nM. HP2: 50 nM. HP3: 50 nM. SG: 8 μL. Exo III: 5 U. GO concentration: 0-0.21 mg□mL^{-1}); (b) Optimization of the volume of SG (HP1: 10 nM. HP2: 50 nM. HP3: 50nM. Exo III: 5 U. GO concentration: 0.15 mg□mL^{-1}. Target: 1 nM. Reaction time: 25 min. SG volumes: 0-12 μL); (c) Optimization of the concentration of Exo III (HP1: 10 nM. HP2: 50 nM. HP3: 50nM. GO concentration: 0.15 mg□mL^{-1}. SG volume: 8 μL. Target: 1nM. Reaction time: 25 min. Exo III: 0-7 U); (d) Optimization of the reaction time (HP1: 10 nM. HP2: 50 nM. HP3: 50nM. GO concentration: 0.15 mg□mL^{-1}. SG volume: 8 μL. Exo III: 5 U. Target: 1 nM. Reaction time: 0-35 min). λem = 514 nm and λex = 480 nm. Error bar SD (n = 3)

Optimization of the experimental conditions (Experimental steps)

First, the optimum concentration of GO was studied. 1 μL of 1 μM HP1 (final concentration: 10 nM), 5 μL each of HP2 and HP3 (final concentration: 50 nM), 0.5 μL of Exo III (10 U μL^{-1}), 8 μL of a working solution of SGI (diluted 100-fold), and different concentrations of GO ranging from 0 to 0.21 mg□mL^{-1} were mixed and incubated for 25 min at 37 °C. Second, the optimum volume of SGI was investigated. 1 μL of 1 μM HP1 (final concentration: 10 nM), 5 μL each of HP2 and HP3 (final concentration: 50 nM), 0.5 μL of Exo III (10 U μL^{-1}), 0.1 μL of 1 μM target RNA (final concentration 1 nM), 15 μL of GO (1 $mg \cdot mL^{-1}$), and seven different volumes of SGI ranging from 0 to 12 μL were mixed and incubated for 25 min at 37°C. Third, the optimum concentration of Exo III was surveyed. 1 μL of 1 μM HP1 (final concentration: 10 nM), 5 μL each of HP2 and HP3 (final concentration: 50 nM), 12 μL of SGI, 0.1 μL of 1 μM target RNA (final concentration 1 nM), 15 μL of GO (1 $mg \cdot mL^{-1}$), and eight different concentrations of Exo III ranging from 0 to 7 U were mixed and incubated for 25 min at 37°C. Lastly, the optimum reaction time was also discussed. 1 μL of 1 μM HP1 (final concentration: 10 nM), 5 μL each of HP2 and HP3 (final concentration: 50 nM), 12 μL of SGI, 0.1 μL of 1 μM target RNA (final concentration 1 nM), 15 μL of GO (1 $mg \cdot mL^{-1}$), and 0.5 μL of Exo III (10 U μL^{-1}) were mixed at 37°C, then the fluorescence was monitored every 5 minutes. The volume of the reaction were all 100 μL. All the concentration were the final concentration. The fluorescence emission spectra were recorded at 514 nm with excitation at 480 nm.

Optimization of the assay conditions (Results and Discussion)

To achieve the best result, the optimal concentration of GO was investigated. Fig. S1a displayed the fluorescence of this sensing platform upon the addition of different concentrations of GO. With the concentrations of GO increased, the fluorescence intensity gradually decreased. The fluorescence would not change until the concentration of GO peaked 0.15 mg · mL^{-1}. The result indicated that all of the FAM-labeled oligonucleotides have been adsorbed onto the surface of GO, resulting in more than 94.5% of the fluorescence being quenched. It also showed that this bioassay had low background value. Therefore, 0.15 mg · mL^{-1} was selected for the subsequent experiments. In addition, the optimum volume of SGI was surveyed. As shown in Fig. S1b, the fluorescence intensity was improved gradually with the increase of SGI, and the it became constant when the volume of SGI reached 8 μL. This result revealed that dsDNAs obtained from HCR had been intercalated by SGI completely, generating dual fluorescence through the synergistic effect of dsDNA-SGI. Hence, 8 μL of SG is chosen as the optimal volume for the next experiments. Furthermore, the optimal concentration of Exo III is studied. As shown in Fig. S1c, with the the increase of Exo III, the fluorescence intensity increased gradually. The fluorescence intensity displayed no obvious change when concentration of Exo III reached 5 U. Fig. S1d showed the fluorescence was nearly constant when the reaction time of this bioassay attained 25 min. These demonstrated that high fluorescence yield could be obtained, revealing the approach had a high S/N ratio and good sensitivity. Therefore, the optimal concentration of Exo III and optimal reaction time are 5 U and 25 min, respectively.