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Supporting Information

A label-free silicon-based spherical nucleic acid enzyme (SNAzyme) for ultrasensitive chemiluminescence detection of acute myocardial infarction-related nucleic acids

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Supplementary experimental section

Preparation of SiO_2-CHO, SiO_2-SH, SiO_2-COC

5 mg SiO_2-NH_2 was resuspended in 3 mL of absolute ethanol, then 0.6 mL of glutaraldehyde aqueous solution (25%) was added, stirred at room temperature for 3 h, then washed with ethanol four times, and finally dried by a vacuum dryer to obtain SiO_2-CHO solid powder.

2 mg SiO_2 was resuspended in 0.48 mL of ethanol, then 20 μL of MPTMS was added, stirred at room temperature for 1 h, washed three times with ethanol, and finally dried by a vacuum dryer to obtain SiO_2-SH solid powder.

2 mg SiO_2 was resuspended in 2 mL of dry toluene, ultrasonically shake for 1 h to disperse, then add 20 μL of GPTMS, reflux toluene at 65°C for 1 h, then wash with dry toluene, acetone and ethanol in turn, and finally dry it in vacuum to obtain SiO_2-COC solid powder, and stored at 4°C for later use.

Preparation of SiO_2@ABEI

ABEI contains an amino group, as a result, it can be immobilized on the surface of SiO_2 by covalent bonding.[1] According to literature reports, -COOH, -CHO, -COC, -SH can all interact with -NH_2 through covalent bonding.[2-5] Next, ABEI will be further modified by SiO_2 containing the above four groups.

1 mg SiO_2-COOH was dispersed in 500 μL MES (pH 6.0, 25 mM) buffer containing 100 mM NHS, 120 mM EDC, and stirred for 1 h at room temperature, then washed sequentially with MES and PBS buffer (0.1 M, pH 7.4), and then nanoparticle was dispersed in 100 μL PBS containing 600 μM ABEI, stirred overnight at room temperature, then washed three times with water, and finally resuspended with water.

1 mg SiO_2-CHO was dispersed in 100 μL PBS containing 600 μM ABEI, stirred overnight at room temperature, then washed three times with water, and finally resuspended in water.

1 mg of SiO_2-COC was dispersed in 100 μL PBS containing 600 μM ABEI, stirred overnight at room temperature, then washed three times with water, and finally resuspended in water.

1 mg SiO_2-SH was dispersed in 250 μL GMBS solution (3.5 mM, DMSO), stirred at room temperature for 45 min, then washed once with DMSO and PBS, and resuspended in 100 μL PBS containing 600 μM ABEI, stirred overnight at room temperature, and then washed with water three times, finally resuspend in water.

Preparation of hemin solution

A certain amount of hemin solid was weighed and dissolved in dimethylSulfoxide (DMSO) solvent and diluted to 5 uM. For CL experiments or ABTS colorimetric experiments, the solution was diluted to the desired concentration with TE buffer.

Screening of the optimal preparation method of SiO_2@ABEI by chemiluminescence

50 μL of 2 mg/mL SiO_2@ABEI (modified by various methods) was mixed with 40 μL of 500 nM G4-DNAzyme (500 nM G4+500 nM hemin), and TE buffer containing 50 mM K^+ was added to 200 μL, and then injected into 96 wells Finally, 2 mM H_2O_2 was dissolved in BR buffer of pH 12, and the CL intensity was detected by a chemiluminescence microplate reader.

Recovery experiments

A volume of 180 μL 1% patients' serum containing 2 mg/mL SiO_2-capture@ABEI nanoprobe, 50 mM $MgAc_2$ was incubated with 2 μM caputure-9-G4 and five known concentrations of miDNA-499 at 37 °C for 2 h. After that, unreacted capture-9-G4 was removed by centrifugation. Then 180 μL TE buffer containing 50 mM KAc, 100 nM hemin and 2 mM $MgAc_2$ was added and incubated at 37 °C for 1 h, and then unbound hemin was removed by centrifugation. Finally, 50 μL of the reaction solution was injected into the 96-well plate, followed by 50 μL of BR Buffer containing 2 mM H_2O_2 (pH 12). The CL intensity of the sample can be collected by BMG LABTECH, which was substituted into the miDNA-499

correction curve equation to calculate the recovery concentration of the sample. Compared with the known standard concentration, the recovery rate was calculated.

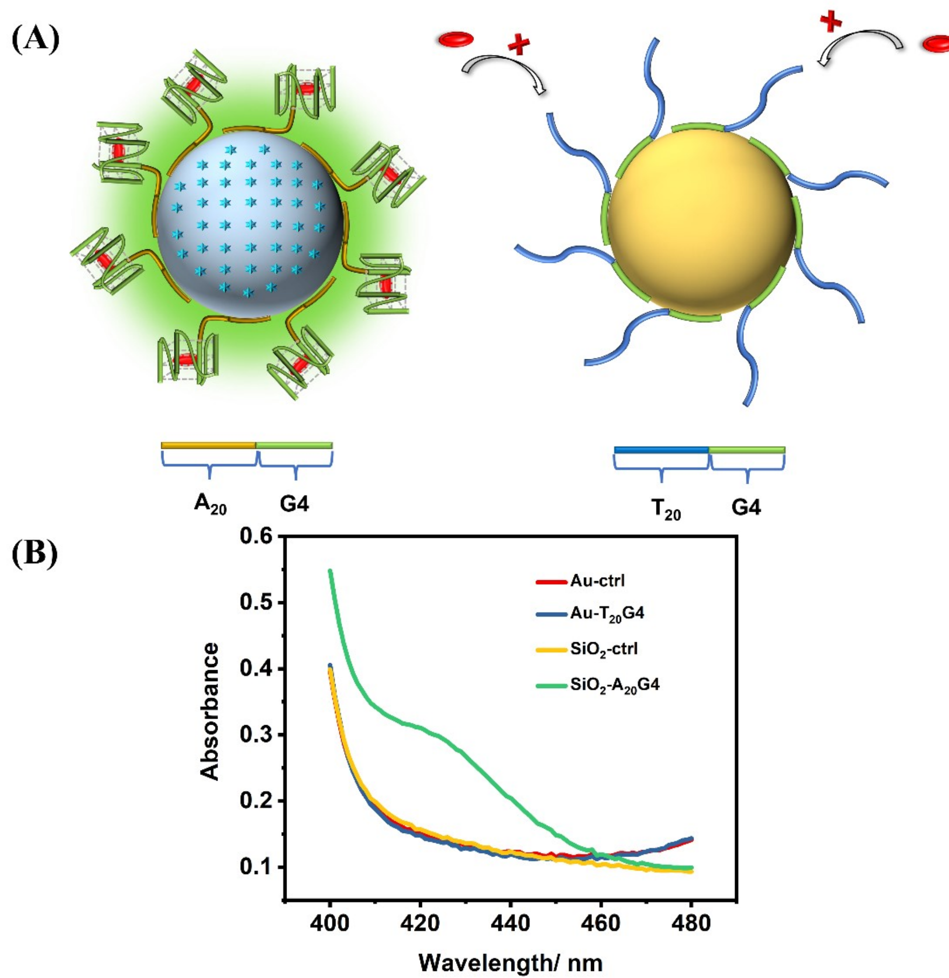

Fig. S1 (A) Schematic diagram of SiO_2 and AuNPs modifying label-free G4 and forming SNAzyme respectively. (B) The catalytic activities of AuNPs-DNA and SiO_2-DNA@ABEI were determined by colorimetry.

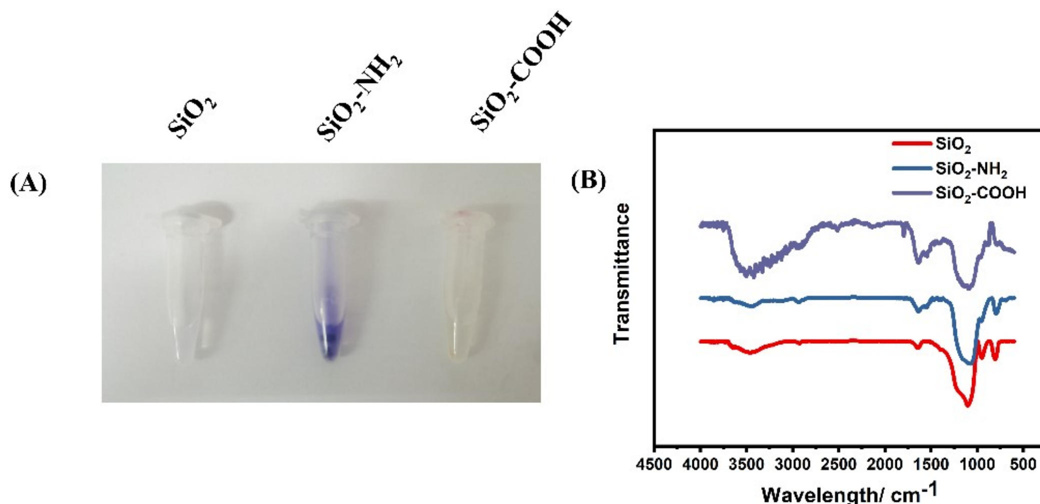

Fig. S2 (A) The color reaction of ninhydrin and (B) FTIR spectra characterized functional amino and carboxyl groups on SiO_2.

After SiO_2 is functionalized by APTES, there are a lot of amino groups on the surface, so under heating conditions, ninhydrin can react with SiO_2-NH_2 to form blue compounds[6]. When the carboxyl group is further modified, succinic anhydride consumes a large number of amino groups, resulting in almost fading of the color (Fig. S2A). Therefore, it is simple to judge whether the two functional groups are successfully functionalized by naked eyes.

Fig. S2A shows the infrared spectra of three SiO_2. Absorption peaks appear at 808 and 1103 cm^{-1} for all three types of SiO_2, and the absorption peaks are related to the symmetric and asymmetric stretching vibrations of the Si-O-Si band. The SiO_2-NH_2 sample has a characteristic peak at 1560, which is attributed to the N-H bending vibration generated by the amino functional group. Meanwhile, the characteristic peak of SiO_2-COOH at 1798 cm^{-1} is due to the stretching vibration of C=O easter. [7, 8]

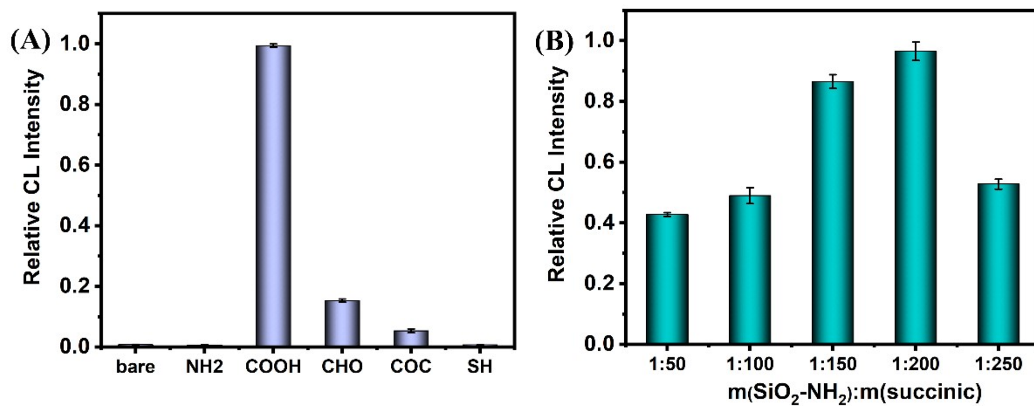

Fig. S3 (A) CL intensity of SiO_2 functionalized by different functional groups loaded with ABEI. (B) CL intensity of different carboxyl modification density on SiO_2 surface.

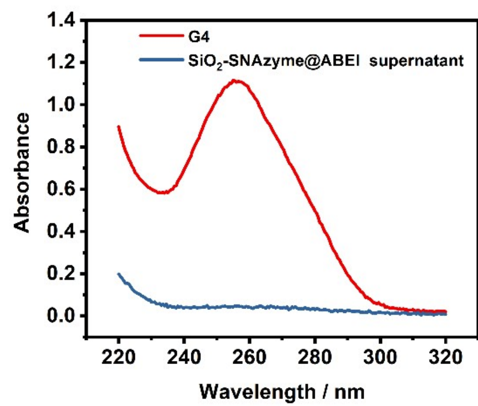

Fig. S4 UV−vis spectra of G4 in SiO_2-SNAzyme@ABEI supernatant.

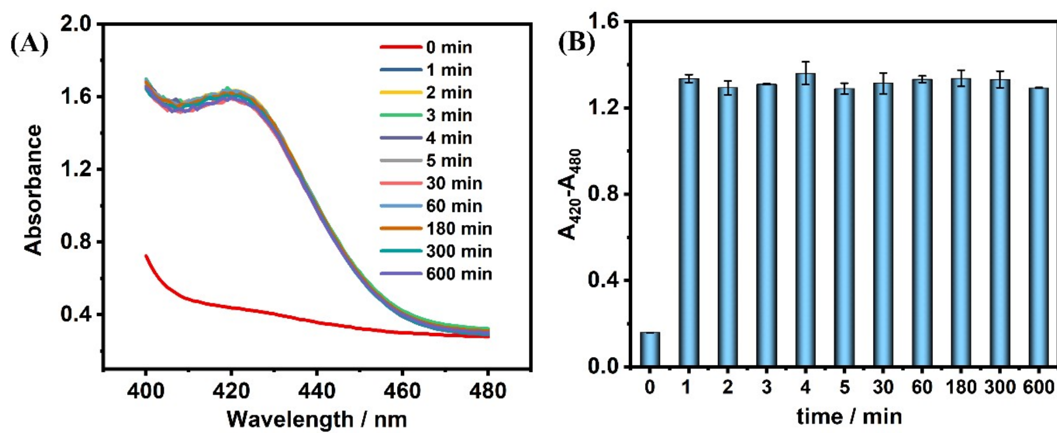

Fig. S5 Determination of the effect of reaction time of SiO_2@ABEI and G4 on DNA modification efficiency by colorimetry.(A) UV−vis spectra and (B) corresponding quantitative results for different reaction time of SiO_2@ABEI and G4.

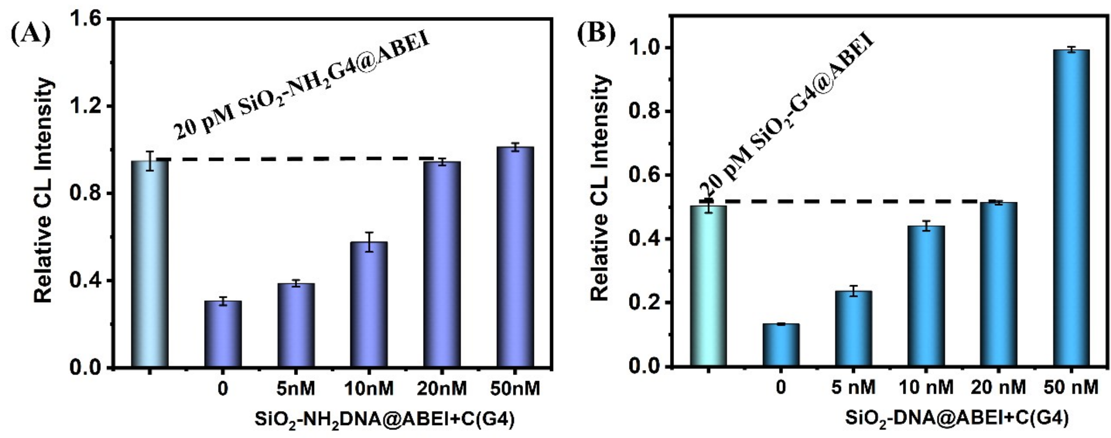

Fig. S6 Estimate the amount of G4 modification on SiO_2. (A) amino labeled G4. (B) label-free G4.

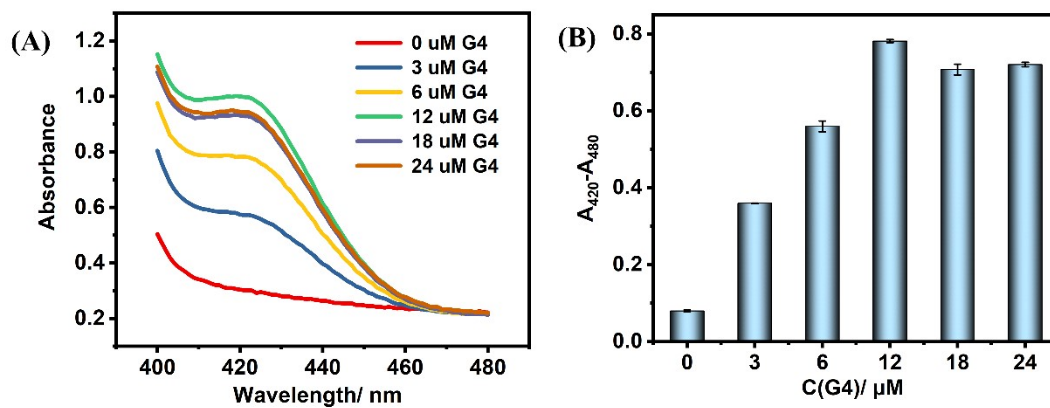

Fig. S7 The saturation concentration of SiO_2 modified G4 was determined by colorimetry, (A) UV−vis spectra and (B) corresponding quantitative results for different G4 initial modification concentration.

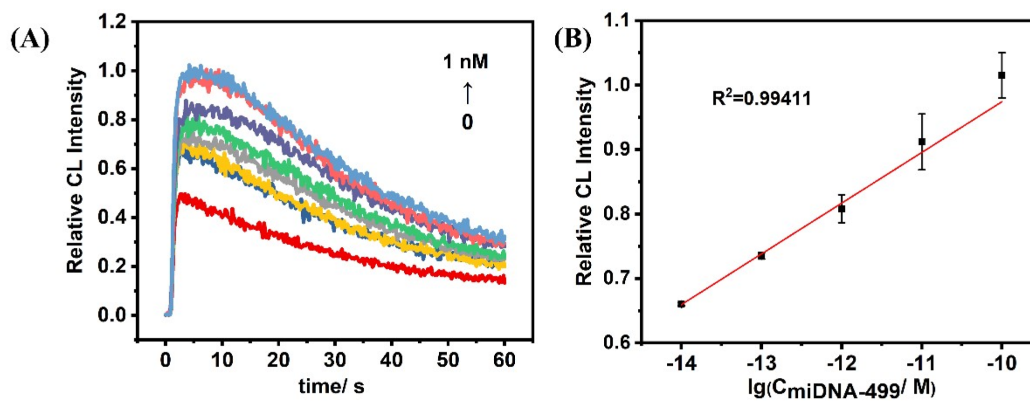

Fig. S8 Amino labeled DNA was used for linear detection of miDNA-499. (A) Representative kinetic curves of CL responses corresponding to different concentrations of miDNA-499. (B) Calibration curve of CL responses for miDNA-499 in range of 10 fM-100 pM.

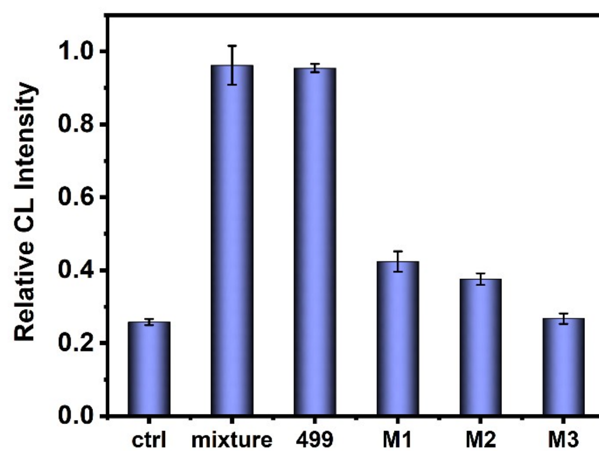

Fig. S9 Relative CL intensity of the biosensor in the absence of samples and in the presence of miDNA-499, miDNA-499, and strands with one (M1), two (M2), three (M3) mismatched bases of miDNA-499. The concentration of miDNA-499 and miDNA-499 are both 100 pM, while that of the base mutation strands are 1 nM.

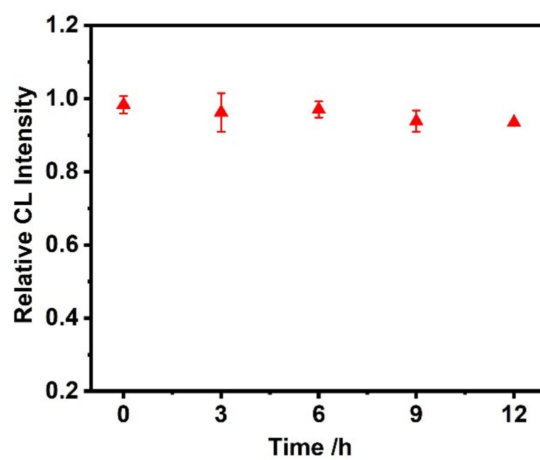

Fig. S10 Resistance to degradation of SiO_2-capture@ABEI tested in 1% human serum.

Table S1. Oligonucleotide sequences used in this work

Name	Sequence (5'-3')
NH_2-SiO_2-capture	AAACATCACTCAGTAACAGCGTTTTTT-NH_2
NH_2-PW17A	NH_2-TTTTTTTTTTGGGTAGGGCGGGTTGGGA
SiO_2-capture	AAACATCACTCAGTAACAGCGAAAAAAAAAAAAAAAAAAAA
A_{20}-ctrl	AAAAAAAAAAAAAAAAAAAAATTTTTCGTTTAGGATTTGTG
T_{20}-G4	TTTTTTTTTTTTTTTTTTTTTGGGTAGGGCGGGTTGGGA
C_{20}-G4	CCCCCCCCCCCCCCCCCCCCGGGTAGGGCGGGTTGGGA
A_{20}-G4	AAAAAAAAAAAAAAAAAAAAAGGGTAGGGCGGGTTGGGA
capture-6-G4	TTACTGGCAAGTCTTAATTTTTTGGGTAGGGCGGGTTGGGA
capture-7-G4	GTTACTGGCAAGTCTTAATTTTTTGGGTAGGGCGGGTTGGGA
capture-8-G4	TGTTACTGGCAAGTCTTAATTTTTTGGGTAGGGCGGGTTGGGA
capture-9-G4	CTGTTACTGGCAAGTCTTAATTTTTTGGGTAGGGCGGGTTGGGA
capture-10-G4	GCTGTTACTGGCAAGTCTTAATTTTTTGGGTAGGGCGGGTTGGGA
miDNA-499	TTAAGACTTGCAGTGATGTTT
miDNA-499-M1	TTAAGACTTGTAGTGATGTTT
miDNA-499-M2	TTAAGAGTTGCAGAGATGTTT
miDNA-499-M3	TTAATACTTGTAGTGACGTTT
miDNA-133a	AGCTGGTAAAATGGAACCAAAT
miDNA-208	AAGCTTTTTGCTCGAATTATGT
miDNA-328	CTGGCCCTCTCTGCCCTTCCGT

Table S2 Comparison of sensitivity for the detection of microRNAs

Method	Target	Linear Range	LOD	Ref.
chemiluminescence	miRNA-30b	8 pM-20 nM	4.5 pM	S[9]
chemiluminescence	miRNA-21	100 pM-1 nM	100 pM	S[10]
ECL	miRNA let-7a	10 fM-10 nM	1.49 fM	S[11]
qRT-PCR	miRNA-145	0.1 pg-1.0 mg	0.1 pg	S[12]
SERS	miRNA-141	100 fM-1 nM	100 fM	S[13]
chemiluminescence	miDNA-499	10 fM-100 pM	0.8 fM	This work

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