Supporting Information for

DNAzyme Walker-Driven, Electrode-Surface Label/Wash-Free Ultrasensitive ECL miRNA Detection via ssDNA-Enhanced Peroxidase Activity of g-C_3N_4 Nanosheets

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Table S1. Oligonucleotide sequences used throughout the experiment.

Oligo Name	Sequence (from 5′ to 3′)
Substrate DNA	HS-$(T)_{14}$ CACTATrAGGAAGAGATTATATATACC
DNAzyme DNA	HS- $(T)_{42}$AATCGTGATAGGGGTTCTCTTCTCCGAGCCGGTCGAAATAGT
Locking DNA	AAGAGAACCCCTATCACGATTAGCATTAA
MiRNA-155	UUAAUGCUAAUCGUGAUAGGGGU
MiRNA-21	UAGCUUAUCAGACUGAUGUUGA
MiRNA-144	UACAGUAUAGAUGAUGUACU
let-7a	UGAGGUAGUAGGUUGUAUAGUU
DNA b	TTTGCAACACTC
DNA c	GGAAGAGATTATATATACC
DNA d	GCAGCCCCTAACCCTAACCCTAAAATCCGTCGAGCAGAGTT
DNA e	AAGAGAACCCCTATCACGATTAGCATTAA
DNA f	CAGTCGCAACACTCAAGGCACGACTGTTTTTT
DNA g	CGTGCCTTGAGTGTTGCTGAGGAGGCACG
DNA h	GTTAGGGGCTGCGTCACACTCAA

Table S2. Standard addition recovery experiments of miRNA-155.

Number	Detected(fM)	spiked (fM)	Detected (fM)	Recovery (%)	RSD (%)
1	Not detected		5.09	101.80	
2	Not detected	5	5.22	104.41	1.67
3	Not detected		5.24	104.89	
4	Not detected		52.00	104.01	
5	Not detected	50	47.97	95.94	4.77
6	Not detected		47.78	95.57	
7	Not detected		516.44	103.29	
8	Not detected	500	482.82	96.56	3.63
9	Not detected		487.86	97.57	

Optimization of Experimental Conditions

Fig. S1A shows the ECL intensity generated in the presence of different concentrations of luminol (fixed concentrations of H_2O_2 with 2×10^{-3} M, and ssDNA/g-C_3N_4 NS with 100 μg·mL^{-1}), and Fig. S1B depicts the ECL intensity in the presence of different concentrations of H_2O_2 (fixed concentrations of luminol with 10^{-10} M, and ssDNA/g-C_3N_4 NS with 100 μg·mL^{-1}). As the concentration of luminol or H_2O_2 increases, the ECL signal is intensified, and the ssDNA/g-C_3N_4 NS can be used as a catalytic label for the detection of H_2O_2.

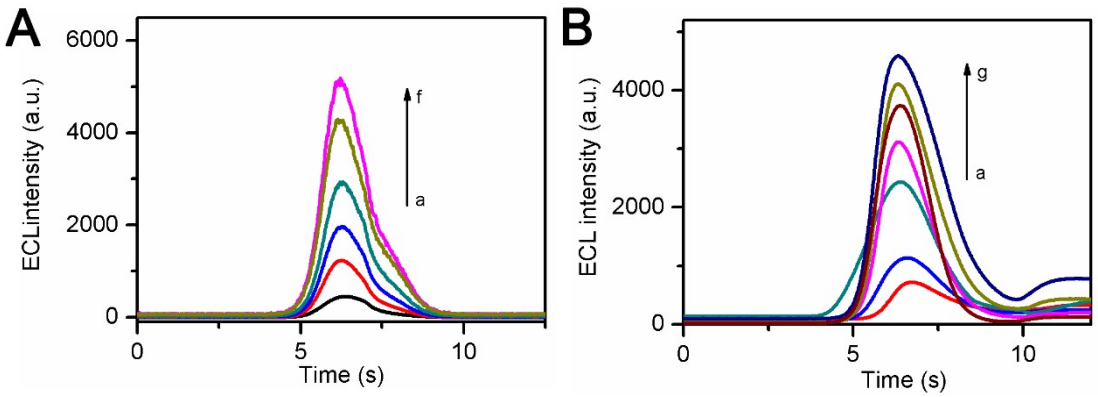

Fig. S1. (A) ECL spectra generated upon the oxidation of variable concentrations of luminol in the presence of 2×10^{-3} M H_2O_2 and 100 μg·mL^{-1} ssDNA/g-C_3N_4 NS (a to f: 10^{-12} to 10^{-7} M). (B) ECL spectra generated upon the oxidation of luminol by variable concentrations of H_2O_2 in the presence of 10^{-10} M luminol and 100 μg·mL^{-1} ssDNA/g-C_3N_4 NS (a to g: 2×10^{-7}, 2×10^{-6}, 2×10^{-5}, 2×10^{-4}, 2×10^{-3}, 2×10^{-2}, 2×10^{-1} M).

To determine the conditions for optimal assay performance, the optimization conditions should be studied systematically for the following relevant variables: (1) concentration of Mn^{2+}; (2) cleavage time.

Further effort is required to understand and better control the concentration of Mn^{2+} to dominate the DNAzyme walker. Fig. S2 shows the effect of the concentration of Mn^{2+} on the ECL intensity. The ECL signal reached a plateau when the concentration of Mn^{2+} was beyond 8 μM.

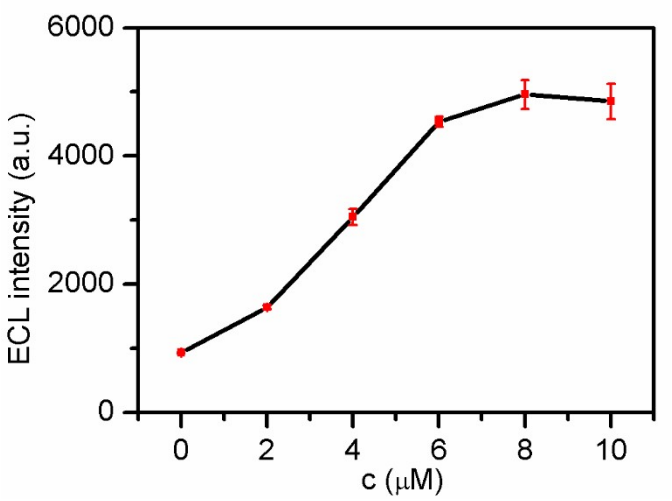

Fig. S2. The optimization of Mn^{2+} concentrations on the ECL intensity by catalytic oxidation of luminol in the presence of H_2O_2 and g-C_3N_4 NS.

The target-triggered recycling amplification releases ssDNA by switching the DNAzyme walker from a locked (inactive) state to an unlocked (active) state via Mn^{2+}-dependent cleavage. So Mn^{2+}-dependent DNAzyme cleavage time had a great effect on the efficiency of enzyme cascade amplification, and the optimum cleavage time of Mn^{2+}-dependent DNAzyme was investigated. The results show, the ECL increased and then remained stable, almost constant after 2 h, until the time exceeded 3 h, as seen in Fig. S3. Therefore, the cleavage time was fixed at 2 h for further exploration.

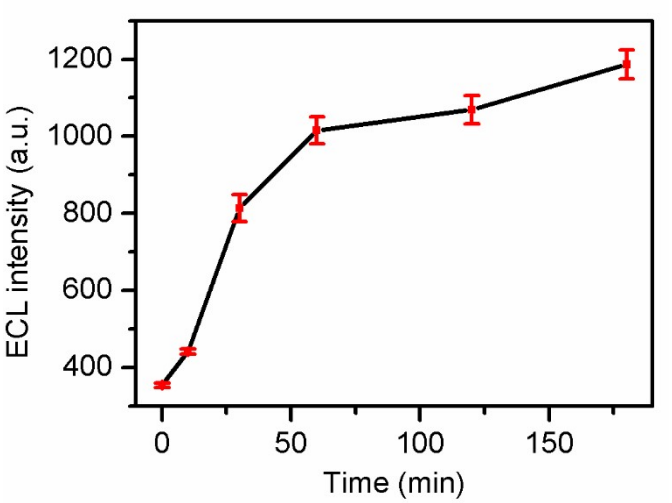

Fig. S3. The study of the effect of DNAzyme cleavage time on the ECL intensity by catalytic oxidation of luminol in the presence of H_2O_2 and g-C_3N_4 NS.

The ECL behaviors of various lengths of the ssDNA and hairpin DNA were exploited while keeping the total concentration of nucleosides constant. In detail, the number of bases of DNA was as follows (a: none; b:12; c:19; d:41; e:29; f:32; g: hairpin 29; h: hairpin 23), and the results were exhibited in Fig. S4. For ssDNA-modified g-C_3N_4 NS, the catalytic activity increased with the length of the ssDNA, indicating that the enhancement originates from the surface-bound DNA. The activity was enhanced significantly when the number of bases reached 19, while the enhancement of the hairpin DNA was weaker, due to the g-C_3N_4 NS's stronger affinity to ssDNA than dsDNA.

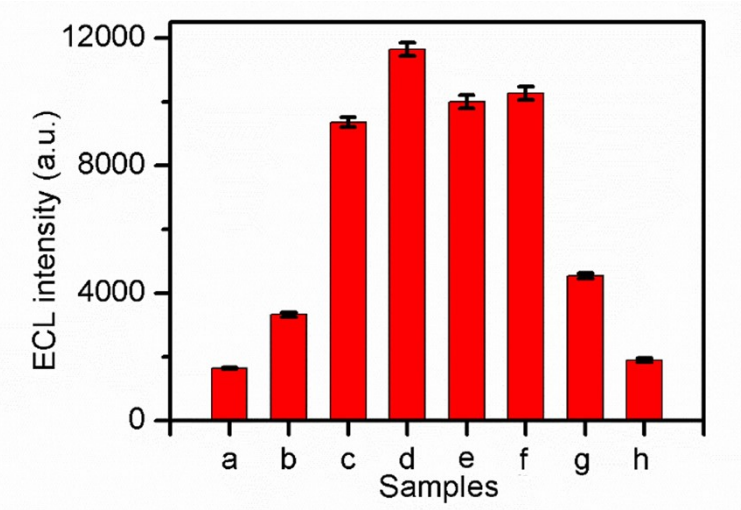

Fig. S4. The study of the effect of DNA length on the ECL intensity by catalytic oxidation of luminol in the presence of H_2O_2 and g-C_3N_4 NS.