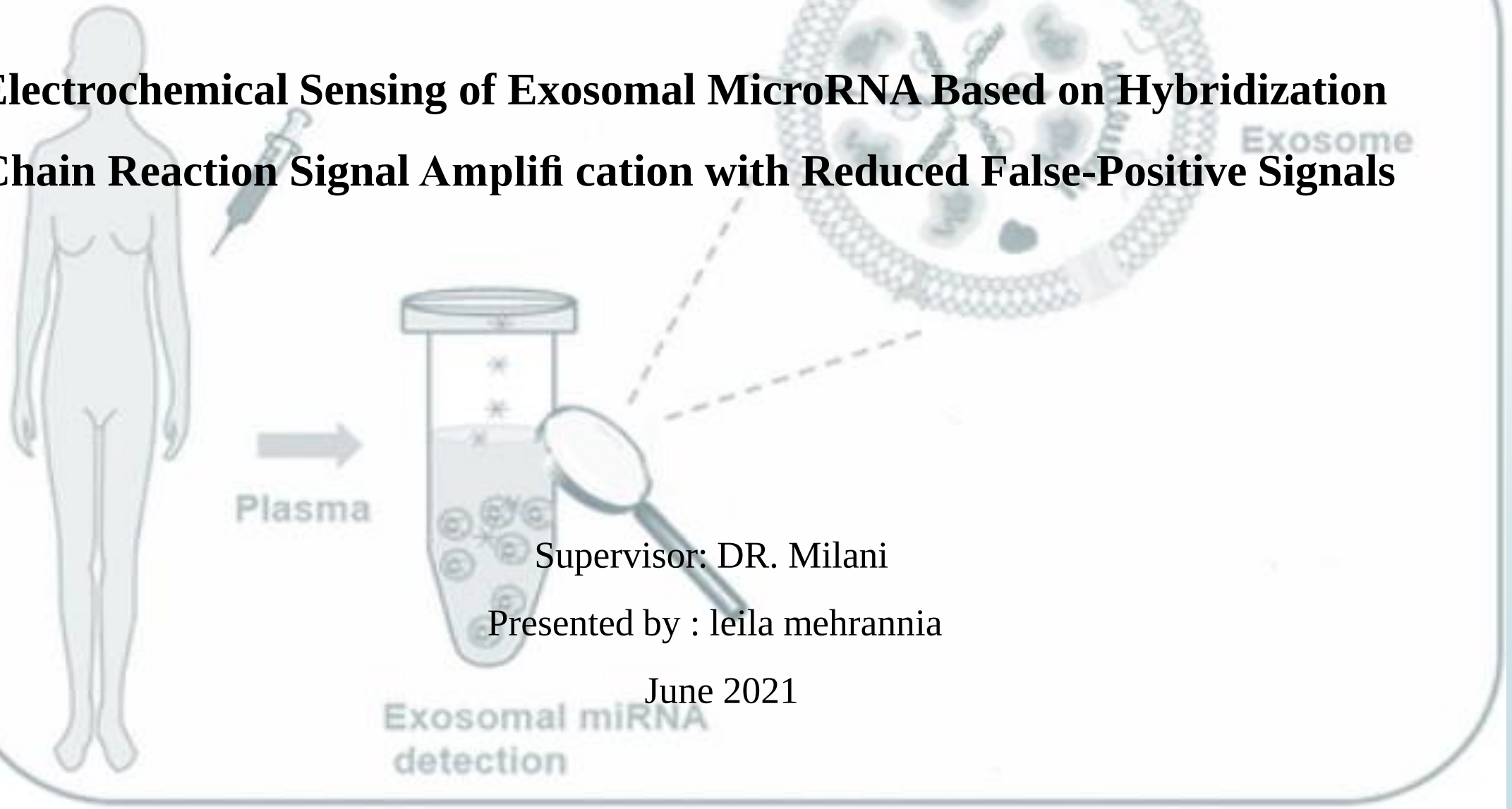
IN THE NAME OF GOD

Electrochemical Sensing of Exosomal MicroRNA Based on Hybridization Chain Reaction Signal Amplifi cation with Reduced False-Positive Signals



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Article

Electrochemical Sensing of Exosomal MicroRNA Based on Hybridization Chain Reaction Signal Amplification with Reduced False-Positive Signals

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Reasons for choosing this topic

- The abnormal expression of miRNAs can be found in exosomes secreted by different types of cancer cells, such as lung cancer, breast cancer, and prostate cancer. Therefore, the sensitive detection of exosomal miRNAs is highly desired in early cancer diagnosis and prognostic processes.

- Exosomes, especially cancer cell-derived exosomes and their transported molecules, are considered to be important noninvasive biomarkers for cancer diagnosis.

- Although The real-time (qRT-PCR) for detecting exosomal miRNAs has good sensitivity and specificity, it generally requires complicated and time-consuming processes and expensive and professional operation.

- In addition, the short length of microRNAs primers for use in the PCR assay decreases the sensitivity and increases false positive signals.

Introduction

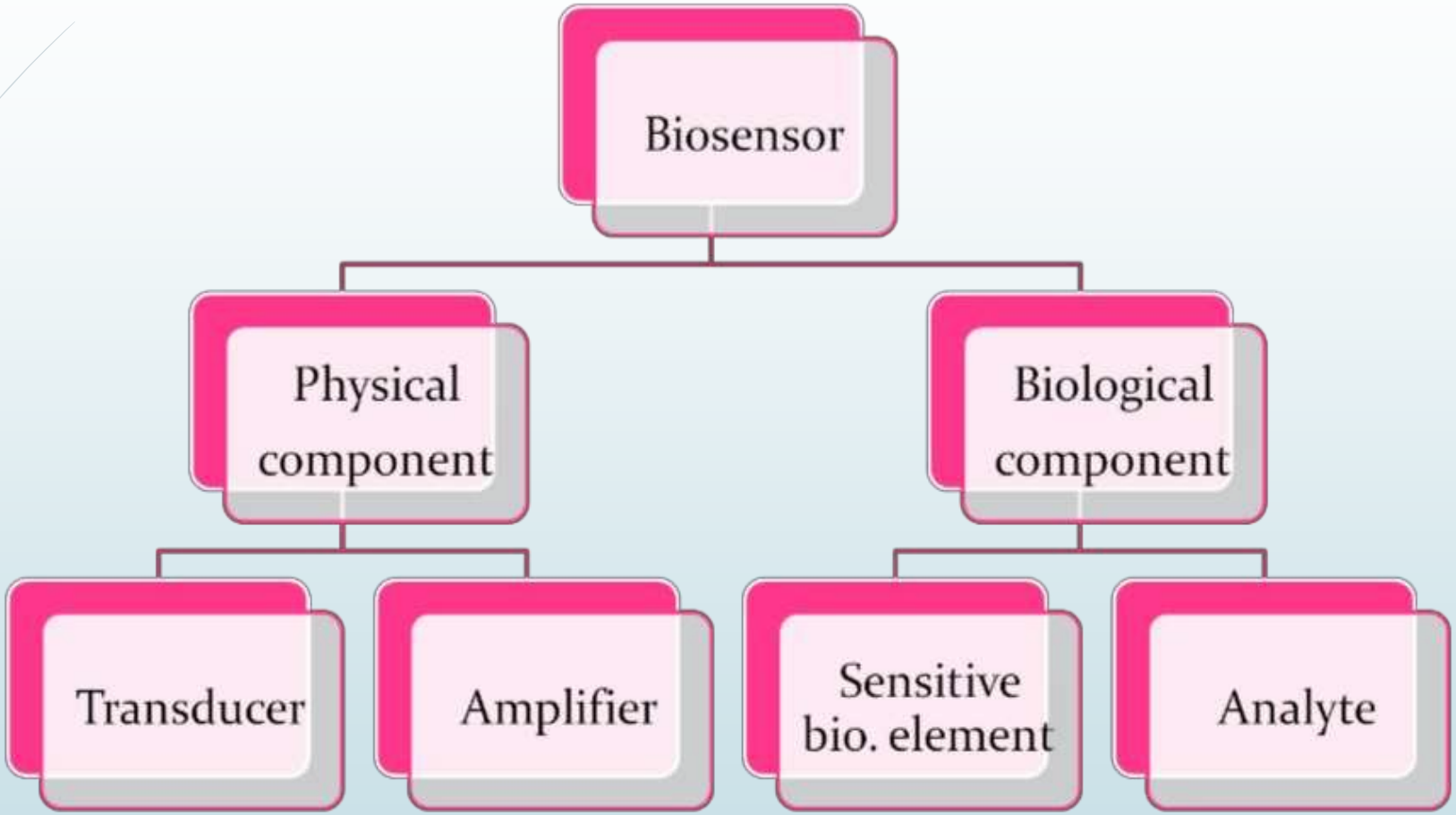
✓MicroRNAs (miRNAs) are a group of small noncoding RNA (approximately 22 nucleotides) molecules and play a critical role in the Posttranscriptional regulation of gene expression in many biological processes such as cell differentiation and cell apoptosis.

✓Exosomes are nanoscale extracellular vesicles secreted by almost all types of cells and are present in most body fluids.

✓Recent studies show that many miRNAs released into the blood circulation
are selectively concentrated in exosomes.

✓Compared with the free miRNAs in biological fluids, the exosomal miRNAs are extraordinarily stable and are related to the protection of the exosome lipid bilayer.

Introduction



fabrication an ultrasensitive electrochemical assay for exosomal miRNA detection

Electrochemical detection was performed at room temperature with A conventional three - electrode system:

- ✓a gold working electrode
- ✓a Pt wire counter electrode
- ✓a saturated calomel electrode (SCE) as a reference electrode).

Electrochemical analytical method: differential pulse voltammetry (DPV)

fabrication an ultrasensitive electrochemical assay for exosomal miRNA detection

1- The mixture of hpDNA probes and short 12 nucleotides single-stranded DNA (S-12) are first immobilized on a gold electrode via Au− S bonds with the − SH moiety at the 5 ′ -terminus of DNA.

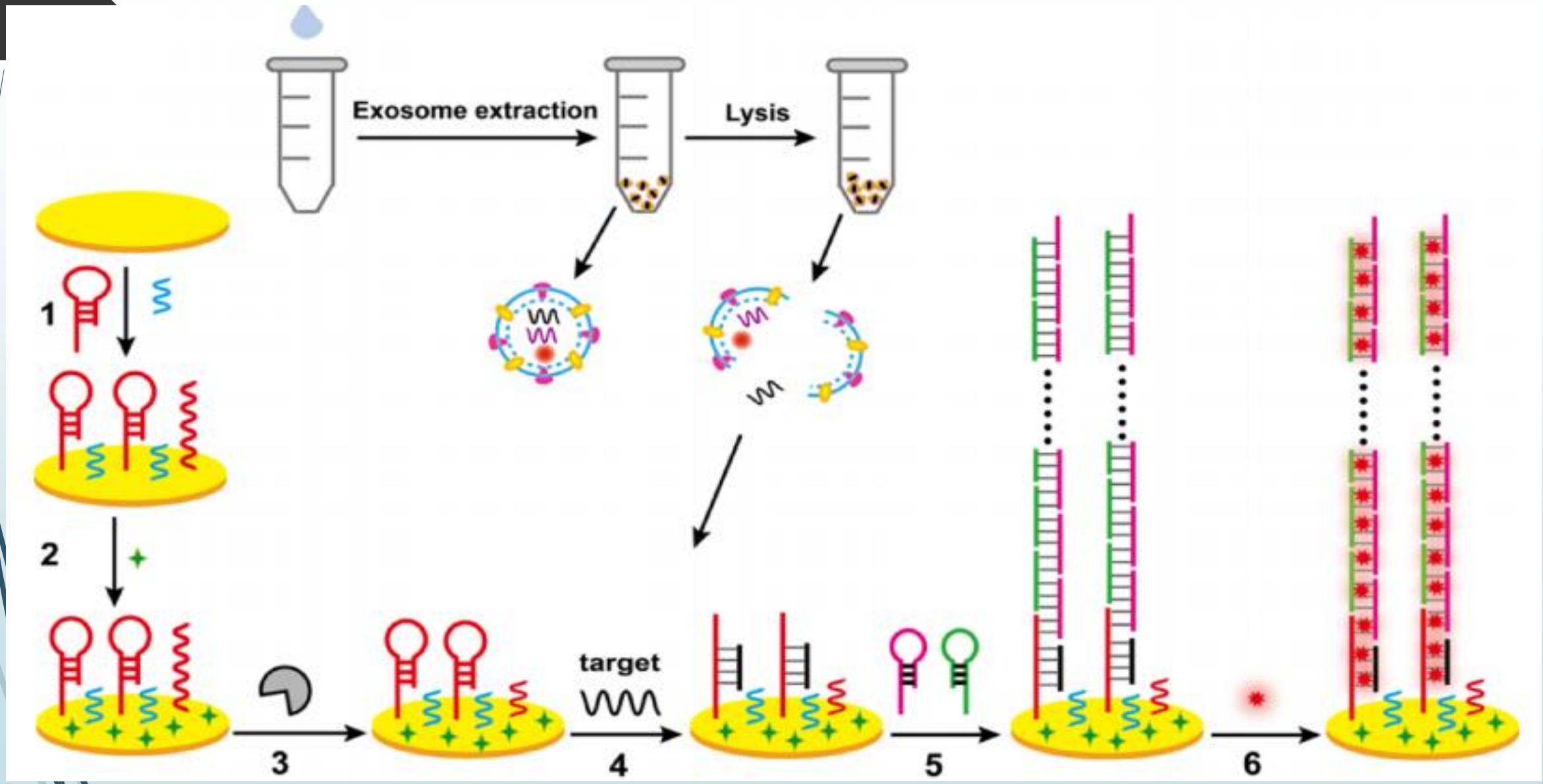
2- 6-mercapto-1-hexanol (MCH) is then added to block the unoccupied surface binding sites.

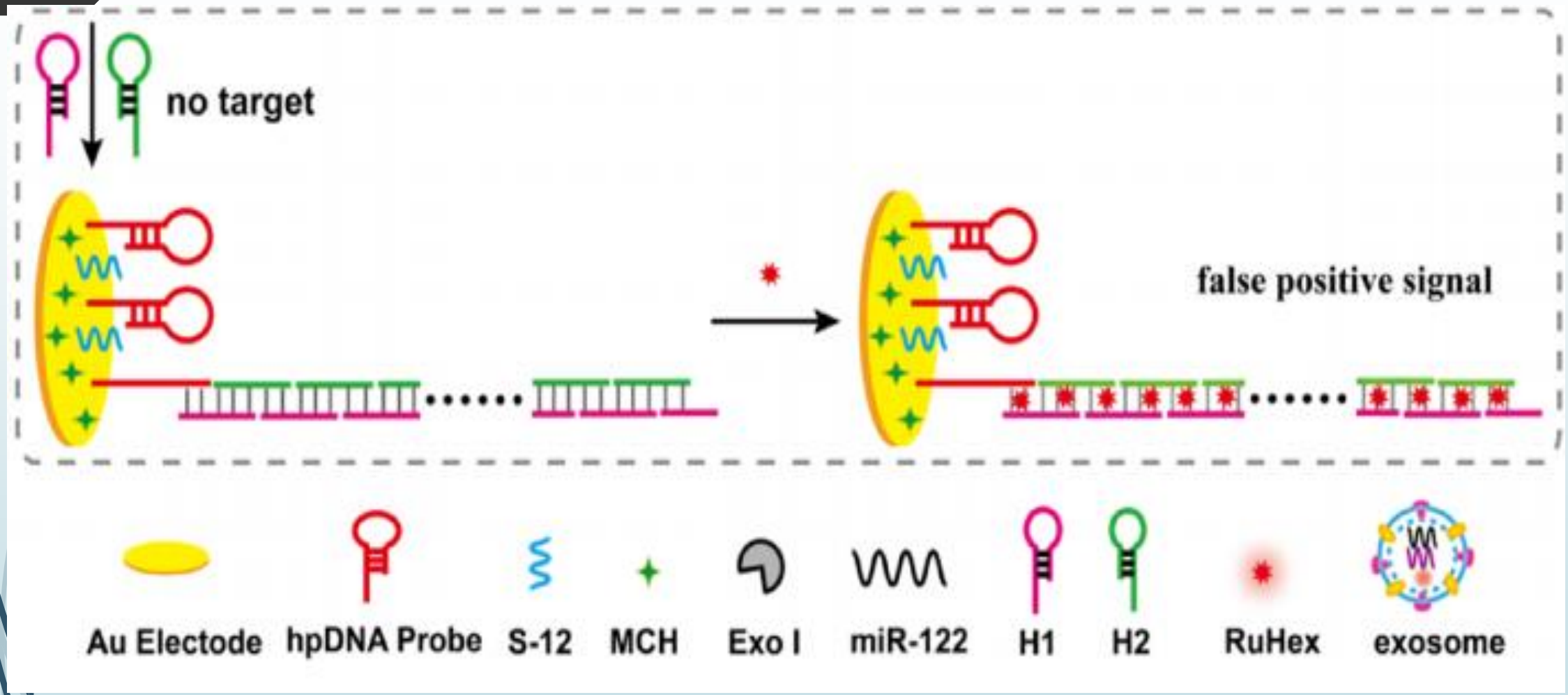
3- To reduce this false positive signal, The MCH/hpDNA/S-12-modified gold electrode is incubated with Exo I.

4- Exosomes of HepG2 (human hepatoma) and MCF-7 (human breast cancer) cells were isolated from the medium.

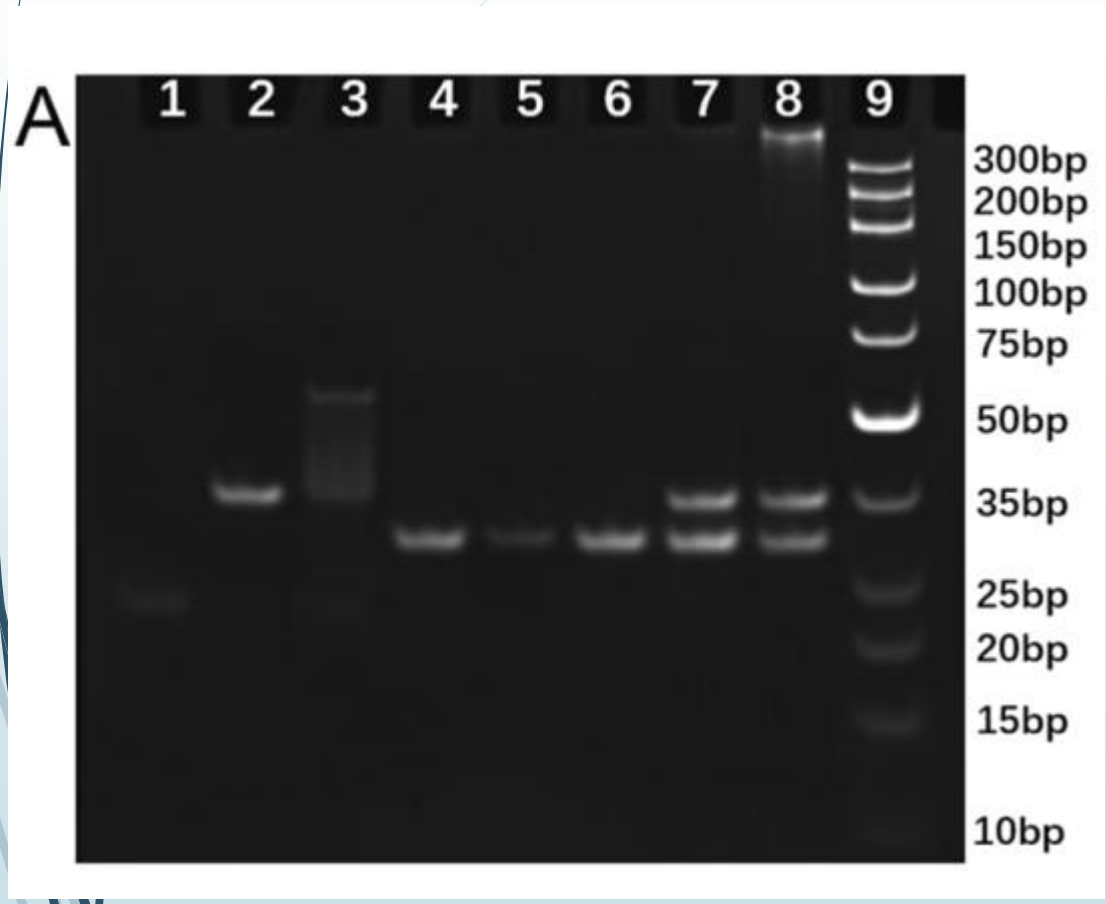
5- RNA was extracted from exosomes using the TRIzol total RNA isolation kit.

Detedction of Exosomal MicroRNA




no target
false positive signal
Au Electode
hpDNA Probe
S-12
MCH
Exo I
miR-122
H1
H2
RuHex
exosome

Feasibility of the Assay



Native PAGE gel analysis of different samples.

Lane 1: miR-122

lane 2 : hpDNA probe

lane 3 : mixture of miR-122 and hpDNA probe

lane 4 : H1

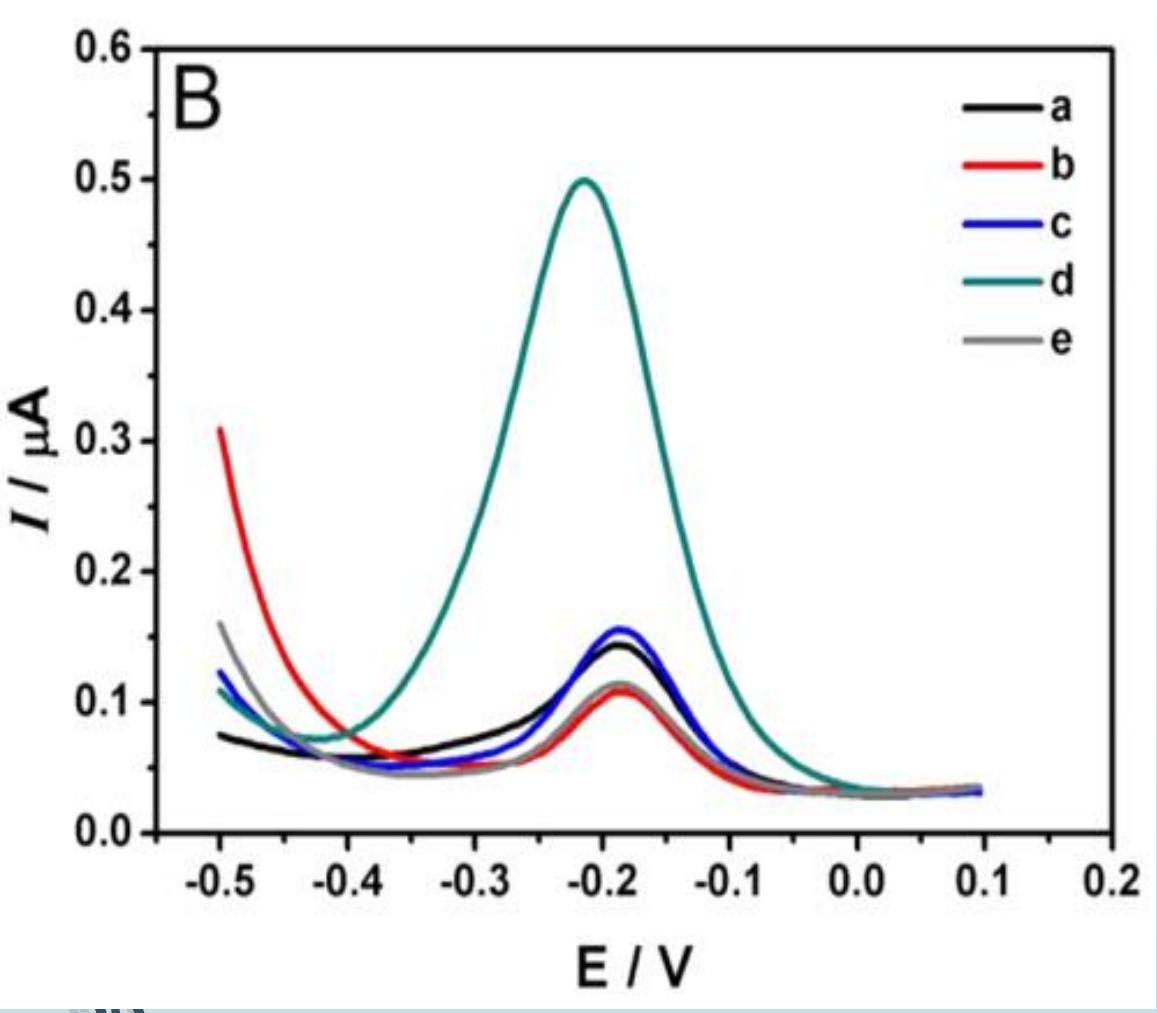
lane 5 : H2

lane 6 : mixture of H1 and H2

lane 7 : mixture of hpDNA probe, H1 and H2

lane 8 : mixture of hpDNA probe, H1,H2 and miR-122

Feasibility of the Assay



DPV response of the gold electrode at different stages:

(a) MCH/hpDNA/S-12-modified gold electrode

(b) after treatment with Exo I

(c) after capture of miR-122

(d) after the addition of H1 and H2

(e) after treatment with Exo I, H1, and H2, without the miR-122.

DPV parameters: − 0.5 to +0.2 V; amplitude, 50 mV; pulse width, 50 ms; pulse period, 0.2 s.

Optimization of Experimental Conditions.

1- lengths of ssDNA (S-12, S-24, and S-48)

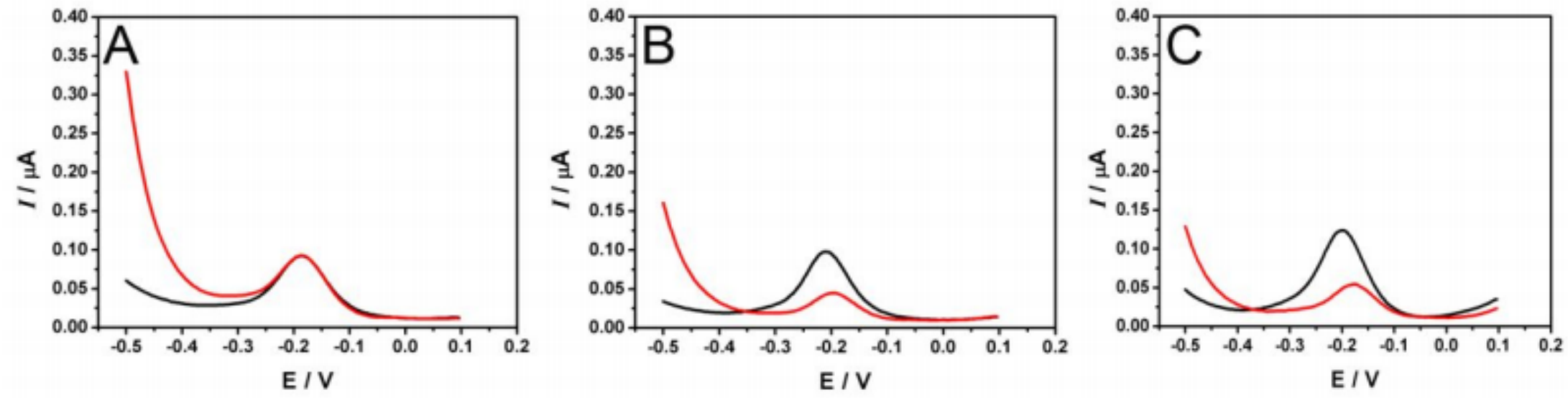


Figure S3. The DPV response of ssDNA before (black line) and after (red line) the addition of Exo I, respectively, (A) S-12; (B) S-24; (C) S-48.

Optimization of Experimental Conditions.

2 - the ratio of the hpDNA probes and S-12

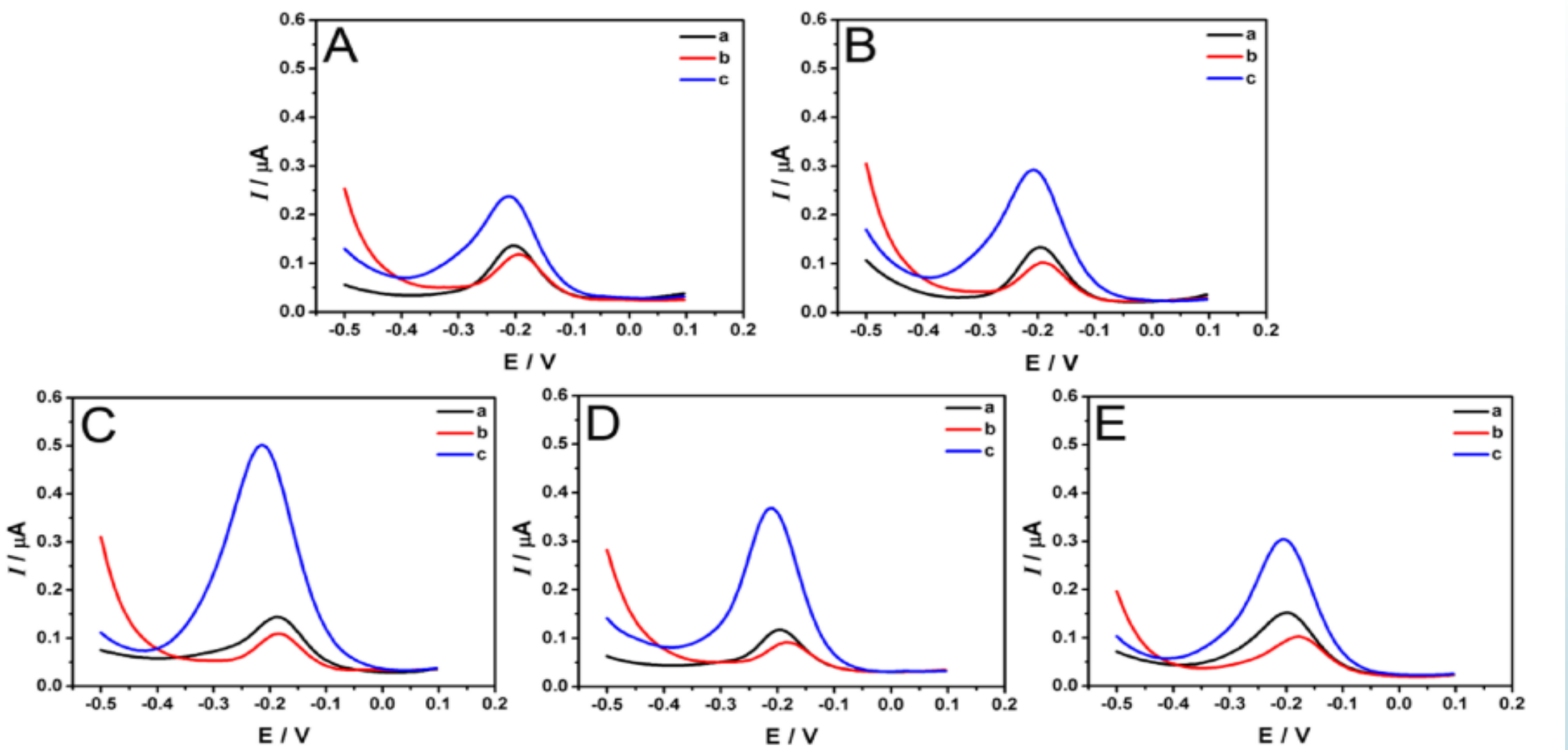


Figure 3. DPV response of the MCH/hpDNA/S-12modified gold electrode with different ratios of hpDNA and S-12. (A) 10:0; (B) 8:2; (C) 6:4; (D) 4:6; (E) 2:8. (a) MCH/hpDNA/S-12-modified gold electrode; (b) after digestion by Exo I (1.5 U/μL); (c) after the addition of miR-122, H1,

Optimization of Experimental Conditions.

3 - incubation time and concentration of Exo I.

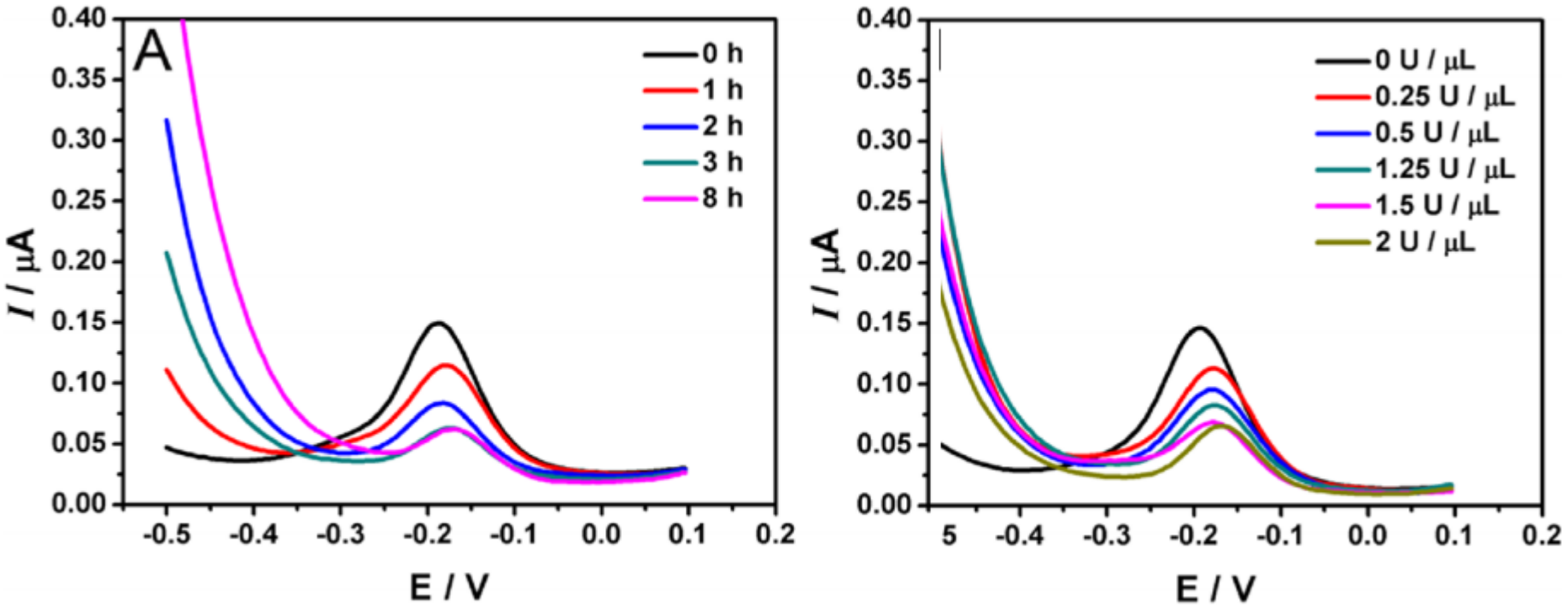


Figure 2. Optimization of (A) incubation time and (B) incubation concentrationof Exo I.

The false positive response

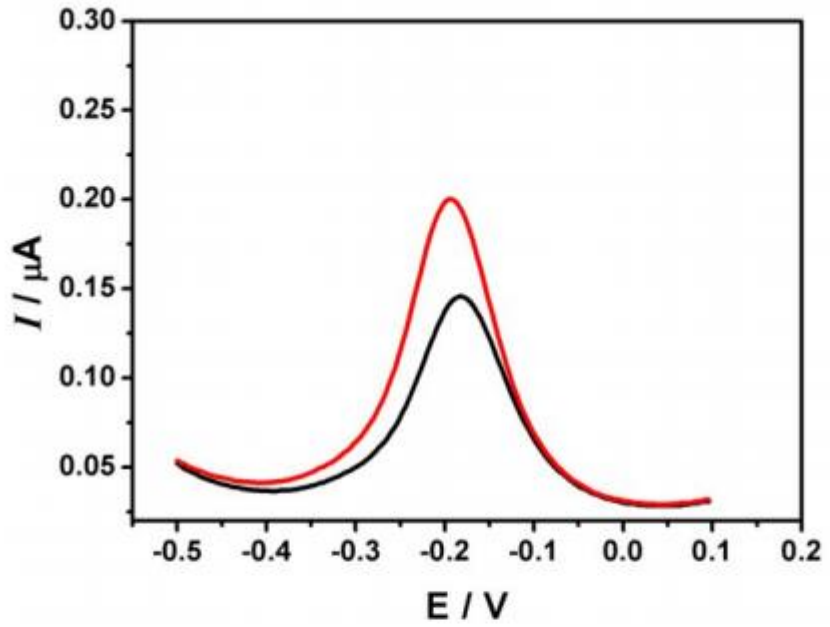


Figure S2. The DPV response of MCH/hpDNA modified gold electrode (black line) and H1/H2/MCH/hpDNA modified gold electrode (red line).

Exosomal MiRNA Detection

DPV response of different concentrations of miR-122

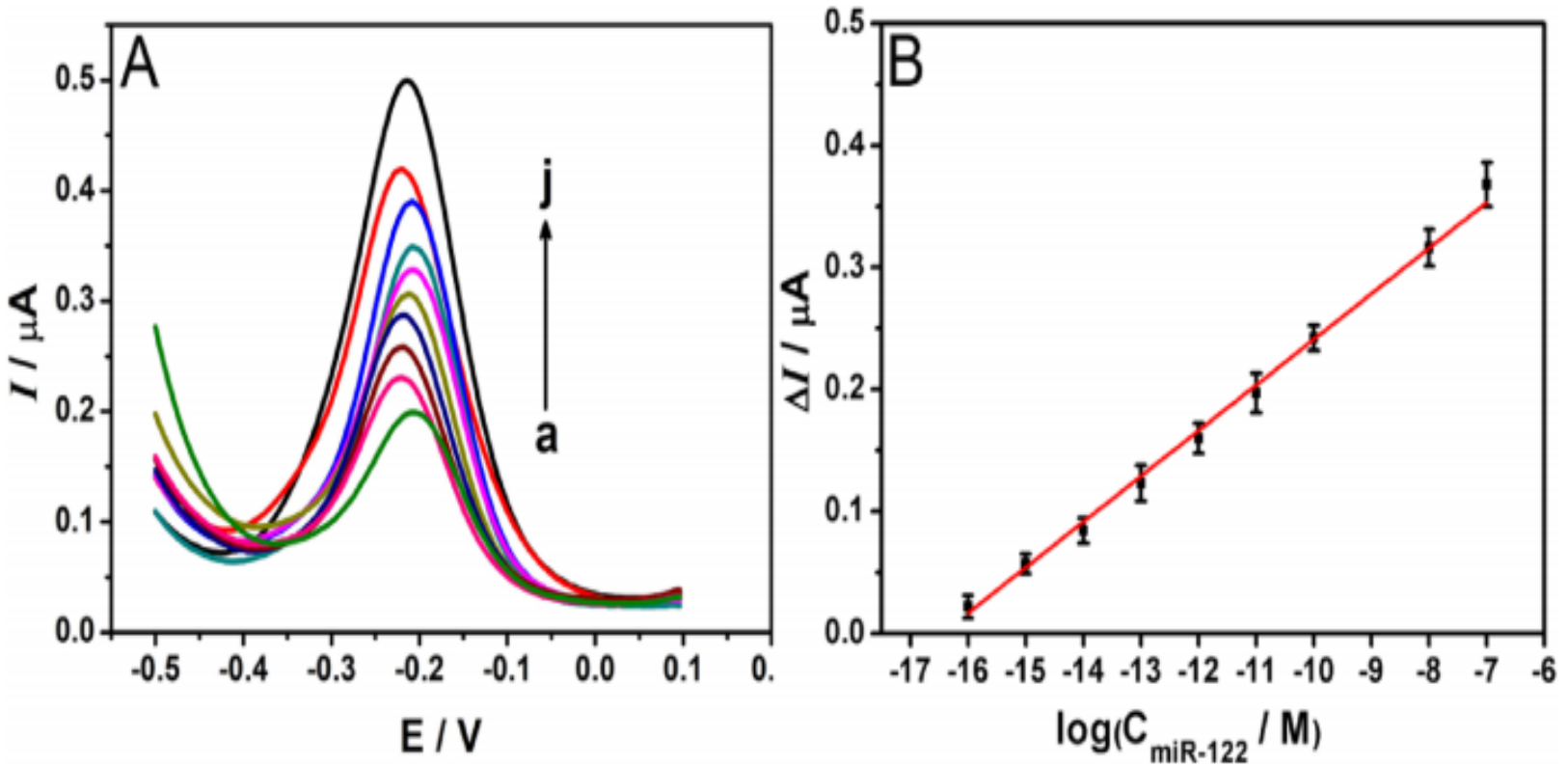
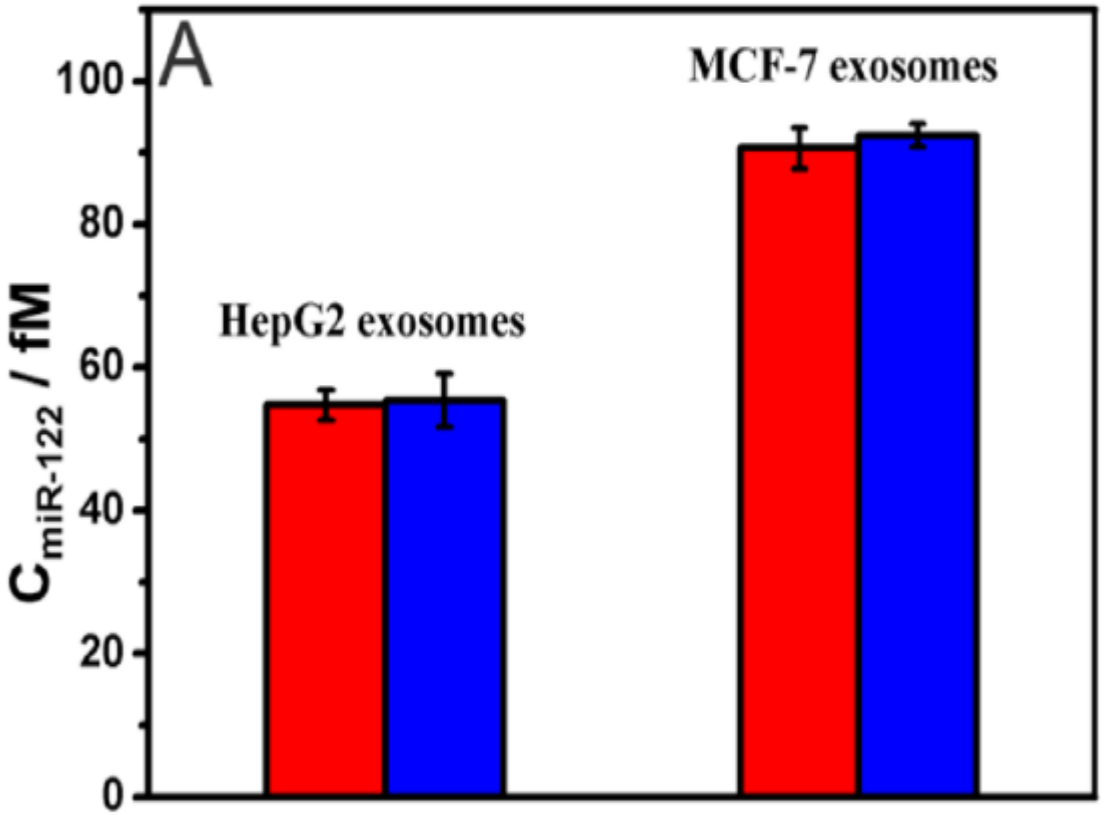
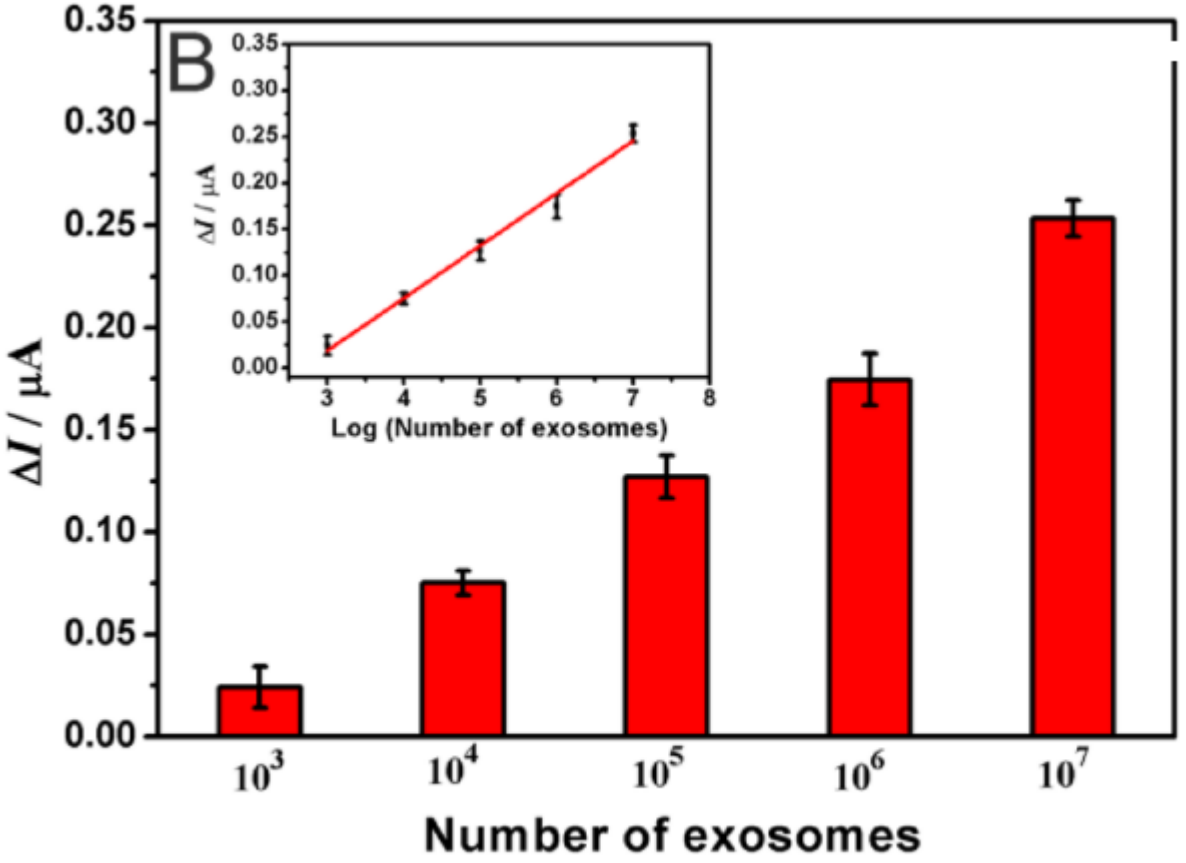


Figure 4. (A) DPV response of different concentrations of miR-122. The concentration of miR-122 is (a) 0.1 fM; (b) 1 fM; (c) 10 fM; (d) 100 fM; (e) 1 pM; (f) 10 pM; (g) 100 pM; (h) 1 nM; (i) 10 nM; (j) 100 nM; (B) Linear relationship between DPV signal changes and logarithm of miR-122 concentration.

Exosomal MiRNA Detection



Detection of miR-122 by the proposed assay (red bars) and qRT-PCR (blue bars).



Detection of miR-122 by the proposed assay in diferent amounts of MCF-7 exosomes.

Table 2. Comparison of Different Methods for Exosomal miRNA Detection

method	linear range (aM)	LOD (aM)	assay time (h)	ref
ratiometric fluorescence sensor	0–5 × 10^5	3 × 10^3	2	25
fluorescence sensor based on in situ detection	0–3 × 10^{10}	6.8 × 10^8	5.2	29
surface-enhanced Raman scattering	1.2 × 10^1–1.8 × 10^7	5 × 10^3	1	17
localized surface plasmon resonance	100–1 × 10^{10}	83	12	35
microfluidics chips	1 × 10^6–1 × 10^{11}	2 × 10^6	12.5	49
ratiometric electrochemical sensor based on bipedal DNA walkers	100–1 × 10^5	67	4.7	31
electrochemical assay based on exonuclease I and HCR amplification	100–1 × 10^{11}	53	3	this work

Selectivity and Reproducibility of the Assay

Selectivity

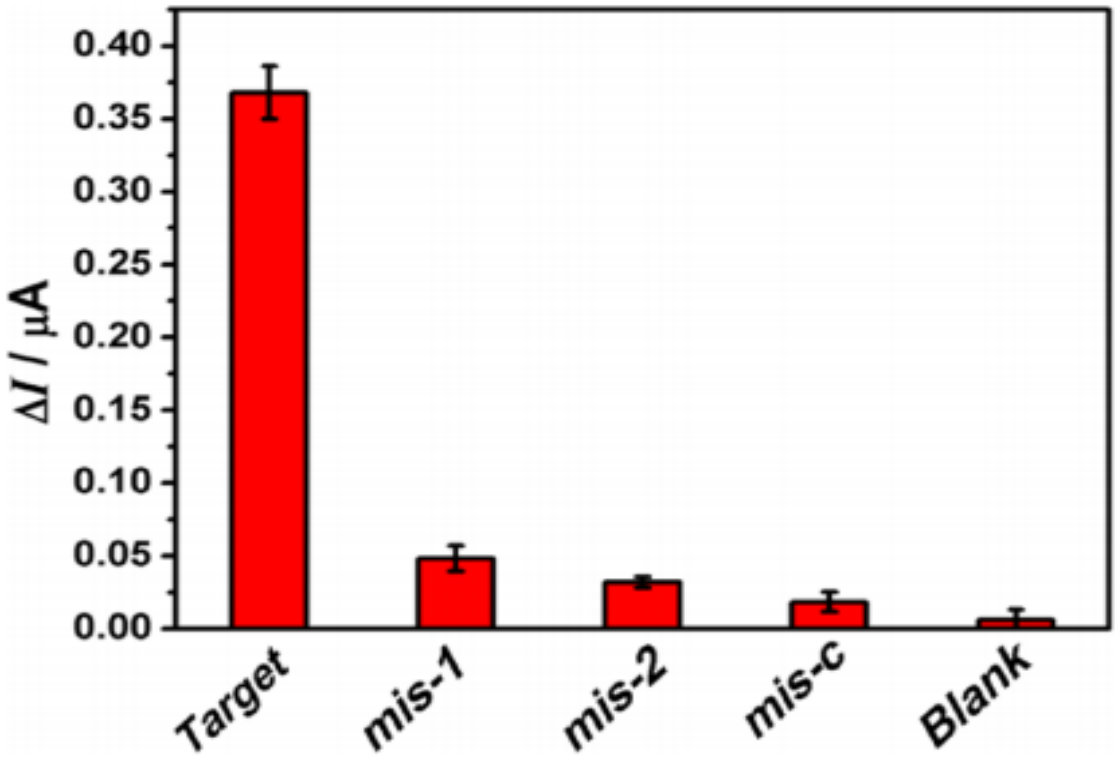


Figure 5. Selective investigation of the developed assay for detecting miR-122 against mis-1, mis-2, and mis-c at 100 nM.

Selectivity and Reproducibility of the Assay

Reproducibility

The reproducibility of the assay was also investigated. Six electrodes were used to detect target miRNAs of 100 pM and 1 fM, respectively. The relative standard deviations (RSDs) were approximately 3.46% and 2.69%, respectively, demonstrating the remarkable reproducibility of the assay.


Thank You!