Supporting Information：

A triblock probe-polyA-probe electrochemical interfacial engineering for sensitive analysis of RNAi plant

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Contents:

1 Capillary electrophoresis result of the long target RNA prepared by in vitro transcription.

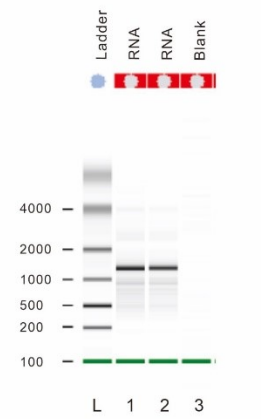

Fig S1, Capillary electrophoresis result of the long target RNA fragments prepared by in vitro transcription Lane L was the ladder; Lane 1 and lane 2 were our RNA molecule; Lane 3 was blank.

2 XPS study of our PAP interface

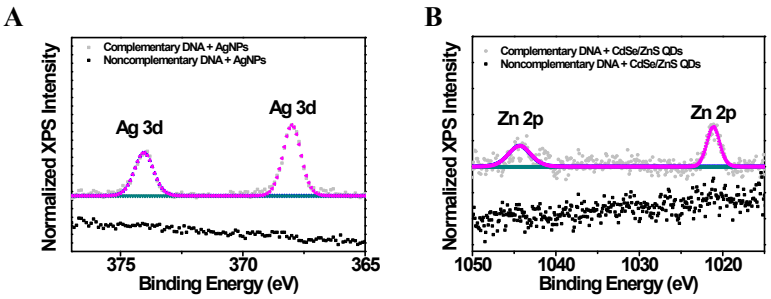

Fig S2, A) Zn 2p region XPS spectra of the PAP interface after hybridization with AgNPs labelled with target DNA and non-target DNA; B) Zn 2p region XPS spectra of the PAP interface after hybridization with CdSe/ZnS QDs labelled with target DNA and non-target DNA.

3 Optimization of the DMSO concentration

According to previous studies, DMSO can effectively weaken the hydrogen bond strength between bases and facilitate the opening of secondary structure. Therefore, we optimized the concentration of DMSO in the hybridization system to improve the efficiency of RNA hybridization. The experimental results are shown in Figure S1. When detecting 10 nM (Fig S1 A) target RNA, the highest S/N was achieved using 10 % DMSO. For 1 nM RNA, 8% DMSO produced the highest S/N (Fig S1 B). The highest S/N was obtained using 6% DMSO for the detection of 100 pM RNA (Fig S1 C). Too much DMSO suppressed the hybridization of low concentration target. Finally, we choose 6% DMSO for improved detection limit.

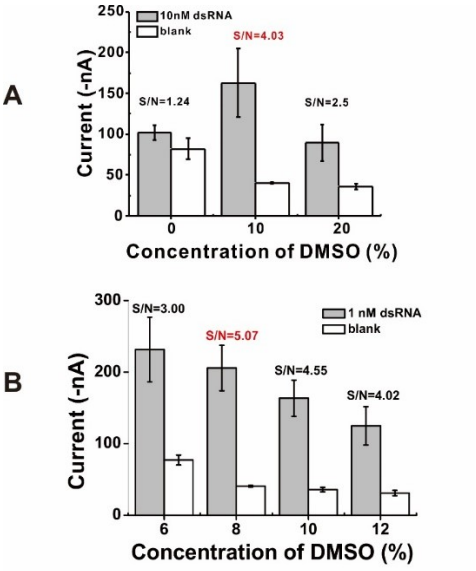

Figure S3. The analysis results of E-biosensors with different concentrations of DMSO in the pre-hybridization solution. The target RNA concentration was: A) 1 nM, B) 1 nM.

4 Optimization of the concentration of two main ions in the hybridization buffer (Na^+ and Mg^{2+})

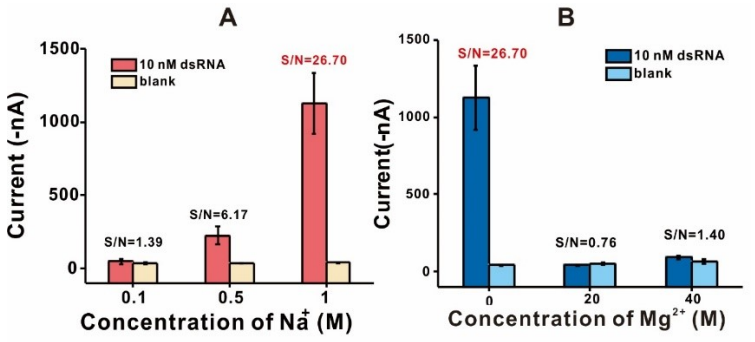

Figure S4. Optimization of Na^+(A) and Mg^{2+}(B) in hybridization buffer. The concentration of target RNA was 10 nM. The error bar is the standard deviation of three repeated experiments with three different electrodes.

5 Digital PCR quantification of the RNA samples extracted from the transgenic maize leaves

1) Preparation of samples:

To extract total plant RNA, frozen maize leaf sample was pulverized to a fine powder in a mortar chilled with liquid nitrogen. Then the powder was transferred to the lysis buffer and purified using a plant total RNA extraction kit. The quality and integrity of the extracted total RNA was evaluated by Agilent 2100 bioanalyzer, and the concentration was determined using a UV-visible spectrum (Nanodrop-2000) The purified RNA was then stored at -80 °C.

4 different RNA samples were extracted from different maize leaves: 11061.32, 11019.4, 11048.21 and a non-RNAi maize sample. Latter 3 samples were designed to produce non target RNA in this experiment.

Table S1: Concentration of the total RNA (UV quantification)

SAMPLE	Concentration（ng/μL）
11061.32	1060.5

11019.4	3268.5
11048.21	2149.3
Non-RNAi	1018.2

2) Digital quantification

RNA denaturation and total RNA reverse transcription was performed for cDNA (RNA extraction was directly applied in this step without further dilution)

Table S2: Reaction solutions for RNA denaturation and reverse transcription steps

Materials and reagents	Volume (μL)
RNA denaturation step	
Oligo dT primer	1
dNTP mixture	1
RNA template	11061.32:2.83
	11019.4: 0.92
	11048.21: 1.40
	Non-RNAi: 2.95
Total volume	10 (certain volume of water was needed)
Reverse transcription step	
Solution from denaturation step	10
5×PrimeScript II Buffer	4
RNase Inhibitor (40 U/μl)	0.5
PrimeScript II RTase	1
water	4.5
Total volume	20

Table S3: Digital PCR quantification (cDNA product from last step was directly applied without further dilution)

Materials and reagents	Volume (μL)
dPCR mix	10
F	0.4
R	0.4
Probe	0.2
cDNA	1
dd水	8
Total volume	20

3) Digital PCR results:

Table S4: Concentration of the foreign target RNA:

Samples	Concentration（copies/μL）	Average（copies/μL）
11061.32	273.02	279.0
	281.72	
	282.15	
Non-RNAi	0.679	0.6

	0.306	
	0.87	
11019.4	42.272	44.7
	48.206	
	43.484	
11048.21	175.2	177.0
	172	
	183.67	

Table S5: Concentration of an endogenous gene (hgmA)

Samples	Concentration（copies/μL）	Average（copies/μL）
11061.32	73.468	76.0
	79.771	
	74.815	
Non-RNAi	172.16	169.2
	169.48	
	166.09	