Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

The preparation of the Electrochemiluminescent (ECL) active DNA nanoprobe for label-free and amplified ECL sensing microRNA

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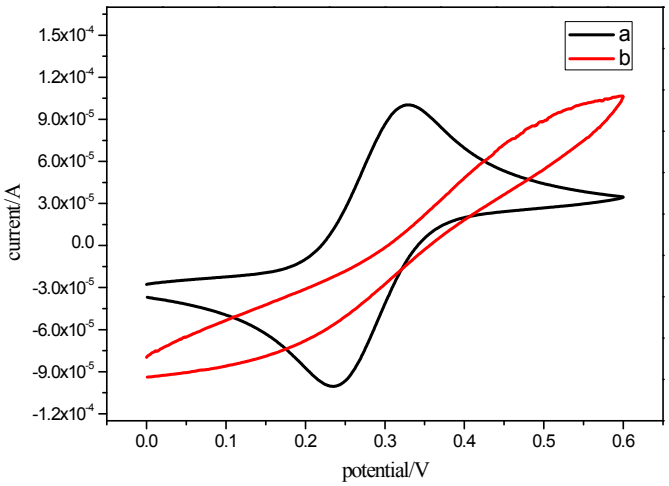

Figure S1. Cyclic voltammograms of bare GCE (a) and Nafion/MWNTS modified GCE (b) in $Fe(CN)_6^{3-/4-}$ solution (5.0 mM $Fe(CN)_6^{3-/4-}$, 0.1 M phosphate buffer solution, 0.1 M KCl, pH 7.4). Scan rate: 100 mV•s-1.

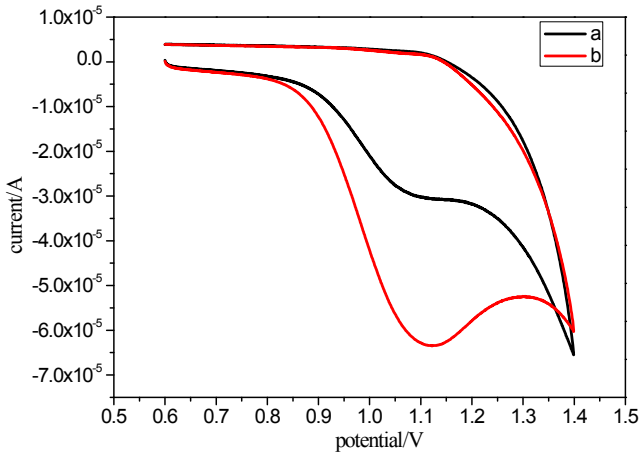

Figure S2. Cyclic voltammograms of DNA nanoprobes (a) and DNA nanoprobes hybridized with target miRNA (b) modified GCE in 50 mM Tris-HCl buffer (pH 7.4) solution. Scan rate: 100 mV•s-1.

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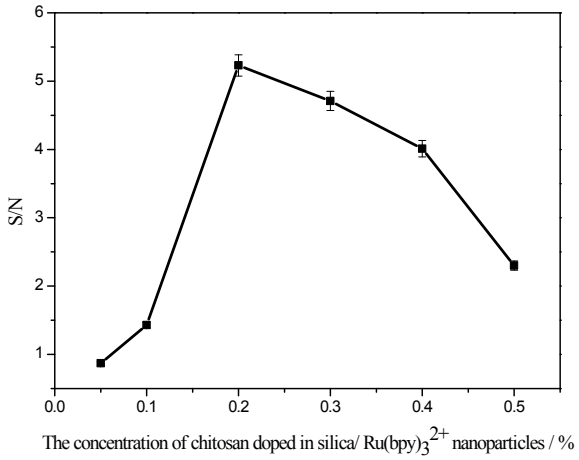


Figure S3. Effect of the concentration of chitosan doped in silica/ $Ru(bpy)_3^{2+}$ nanoparticles on the signal-to-noise. The signal-to-noise is the ECL intensity of DNA miRNA to DNA probe. The concentration of DNA nanoprobes is 1.1×10^{-9}M, concentration of let-7a miRNA is 10 pM. Other experimental conditions are same as described in the experimental section.

As can be seen in figure S3, ECL signal-to-noise ratio can reached the maximum when the concentration of chitosan is 0.2%, which is thus used for the NPs preparation in this work.

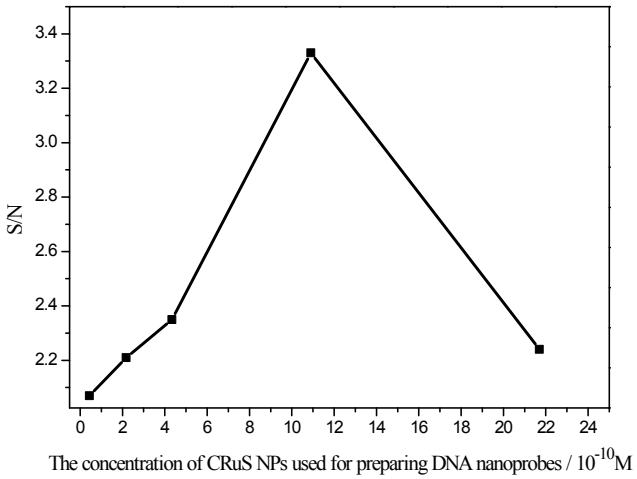


Figure S4. Effect of the concentration of CRuS NPs used for preparing DNA nanoprobes on the signal-to-noise. The signal-to-noise is the ECL intensity of DNA miRNA to DNA probe. The concentration of let-7a miRNA is 10 pM. Other experimental conditions are same as described in the experimental section.

Furthermore, the effect of concentration of CRuS NPs on the reaction system was studied. As shown in Figure S4, the ECL signal-to-noise increased with the concentration of CRuS NPs changing from 4.3×10^{-11} M to 2.2×10^{-9} M and reached a maximum at 1.1×10^{-9} M, then gradually decreased. These results indicated that only a small part of CRuS NPs were wrapped by the DNA probes and most DNA probes was free-state in supernatant when CRuS NPs of lower concentration was used. Thus, when target miRNA was added into the mentioned-above solution, most of them were consumed by free-state DNA probes instead of the interaction between target and DNA nanoprobes. However, while the concentration of CRuS NPs was higher than 1.1×10^{-9} M, CRuS NPs could not completely be wrapped by the insufficient amount of DNA probes. The uncompleted wrapping could not make DNA nanoprobes be electroneutral. Therefore, the introduction of target miRNA might not prevent DNA nanoprobes from being adsorbed at Nafion/MWNTS modified GCE.

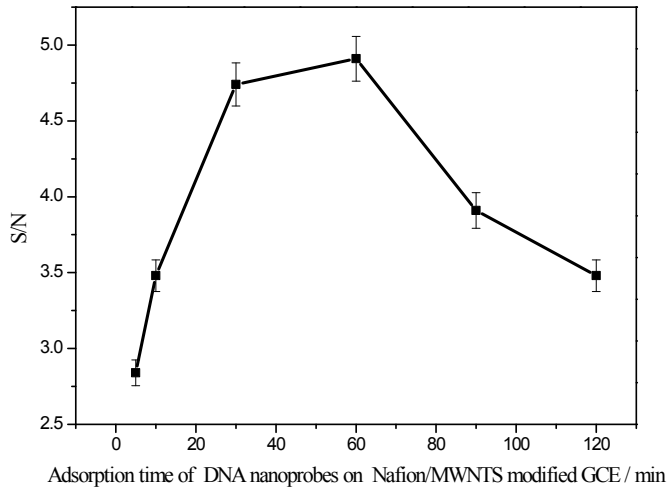


Figure S5. Effect of the adsorption time of DNA nanoprobes on Nafion/MWNTS modified GCE to the signal-to-noise. The signal-to-noise is the ECL intensity of DNA miRNA to DNA probe. The concentration of DNA nanoprobes is 1.1×10^{-9} M, concentration of let-7a miRNA is 10 pM. Other experimental conditions are same as described in the experimental section.

The adsorption time of CRuS NPs on modified GCE was examined. As shown in Figure S5, the ECL signal-to-noise could reach the peak when the adsorption time of NPs on modified GCE is 60 min. The possible reasons might be as described below: the NPs could not completely be adsorbed when the adsorb time was too short. However, when adsorbed time was too long, the part of hydrophobic groups on the DNA nanoprobes would had a certain interaction with hydrophobic Nafion film, leading to a weak assembly of CRuS NPs on the surface of the GCE. This weak assembly could result in the drop of signal-to-noise.

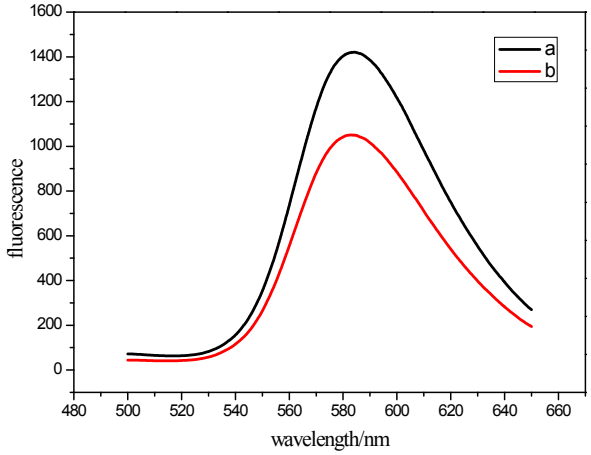


Figure S6. Fluorescence spectroscopy of CRuS NPs (a) and ferrocene-DNA nanoprobes (b); The concentration of ferrocene-DNA probes is 1.0 μM, the concentration of CRuS NPs is 2.2×10^{-9} M. Other experimental conditions are the same as described in the experimental section.

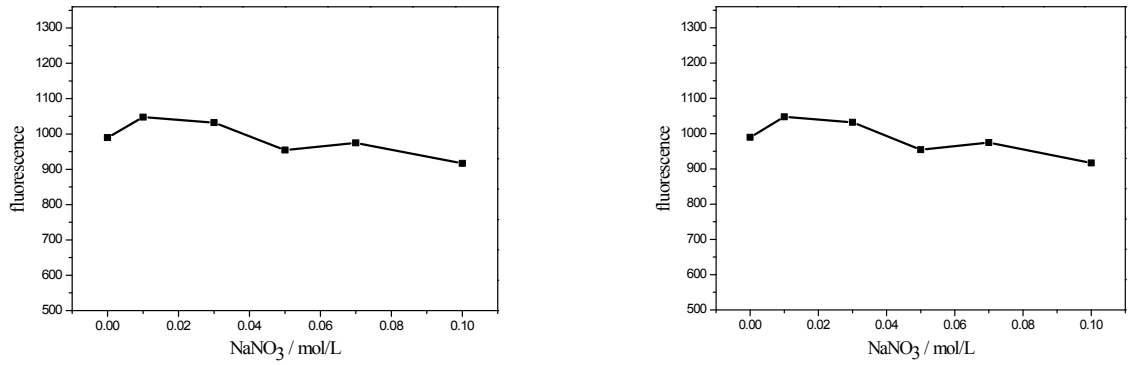
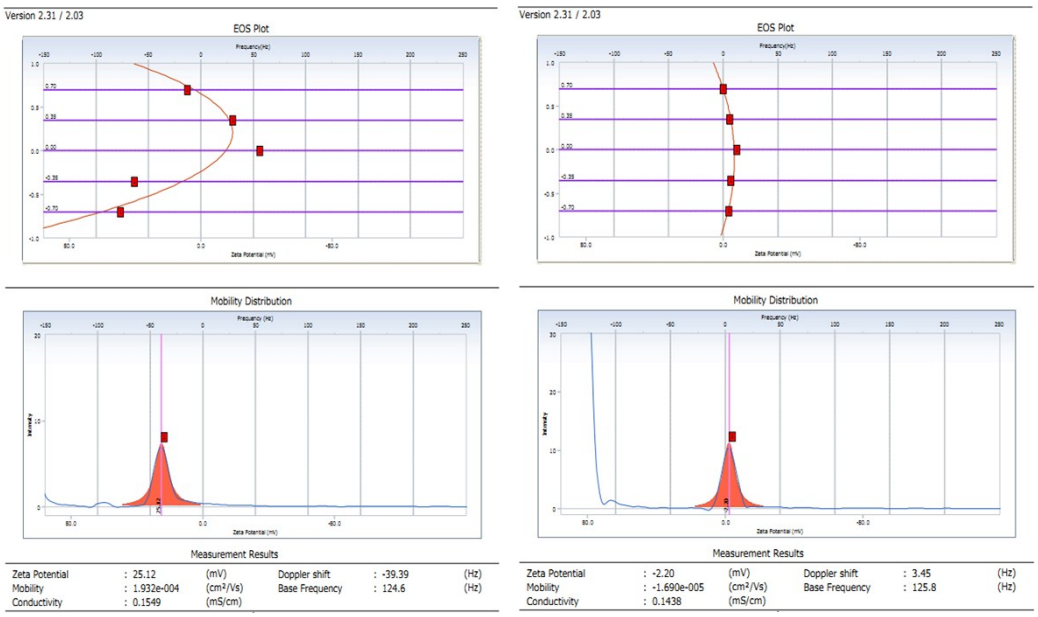


Figure S7. The time stability (A) and salt resistance (B) of the ferrocene-DNA nanoprobe; The concentration of ferrocene-DNA nanoprobe is 2.2×10^{-9} M. Other experimental conditions are the same as described in the experimental section.

The ferrocene labeling DNA probes were used to verify the stability of the DNA nanoprobe. Due to the quenching effect of ferrocene on $Ru(bpy)_3^{2+}$ fluorescence

signal (as can be seen in Figure S6), the fluorescence signal of CRuS NPs was partly quenched when ferrocene-DNA probes were adsorbed on CRuS NPs. Therefore, the fluorescence signal intensity of $Ru(bpy)_3^{2+}$ will remain low if the ferrocene-DNA probes were adsorbed on CRuS NPs steadily. Consequently, we can estimate the stability of DNA nanoprobes via the fluorescence signal of $Ru(bpy)_3^{2+}$.

We have explored the stability of the DNA nanoprobes from two aspects, time-stability and salt resistance. As shown in Figure S7, the fluorescence signal intensity of $Ru(bpy)_3^{2+}$ not rise significantly when the ferrocene-DNA nanoprobes were standing for a period of time (Figure S7 A) or add different concentrations of $NaNO_3$ into it (Figure S7 B). By this token, the DNA nanoprobe has good stability.



A B

Figure S8. The Zeta potential images of pure CRuS NPs (A) and DNA nanoprobe (B);

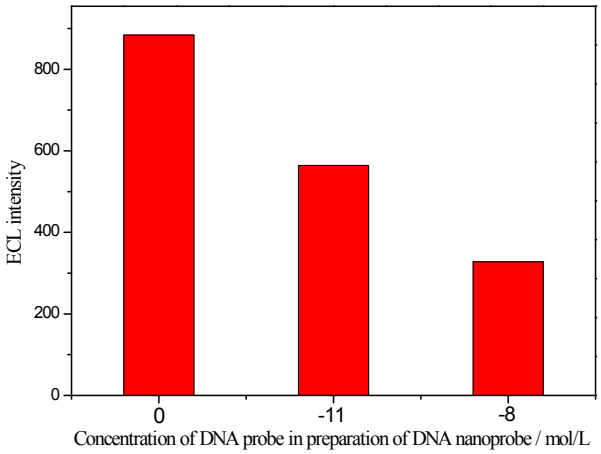


Figure S9. The ECL signal intensity of DNA nanoprobes prepared by different concentrations of DNA probe; The concentration of CRuS NPs is 1.1×10^{-9} M. Other experimental conditions are same as described in the experimental section.