# Supplementary material

# DNA Ultrasensitive Detection via SI-eRAFT and In Situ Metallization Dual-Signal Amplification

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**Table of contents:**



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**Apparatus.** All electrochemical measurements, including cyclic voltammogram (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out at room temperature on a CHI 760D electrochemical workstation (chenghua, Shanghai. China). Gold electrode (Φ= 2mm) was used as a working electrode. Reference electrode and the counter electrode were saturated calomel electrode (SCE) and platinum wire, respectively. The morphology of the electrode surface was observed by scanning electron microscope (EVO18, Carl Zeiss, German).

**Reagents.** All chemicals and reagents were analytical grade or higher, and used as received without further purification. Glycosyloxyethyl methacrylate (GEMA) and healthy normal human serum (NHS) were purchased from Sigma-Aldrich (St. Louis, MO) and Shanghai YiJi Industrial Co., Ltd. (Shanghai, China), respectively. Potassium chloride (KCl), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPAD), 6-mercapto-1-hexanol (MCH), Zirconium dichloride oxide octahydrate ($ZrOCl_2 \cdot 8H_2O$), tetrabutylammonium perchlorate (TBAP) and potassium hexafluorophosphate ($KPF_6$) were obtained from J&K Scientific Co., Ltd. (Shanghai, China). Sodium periodate ($NaIO_4$), N,N-Dimethylformamide (DMF), silver nitrate ($AgNO_3$), and other chemicals were ordered from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water used in all the assays was obtained from a Millipore Milli-Q water purification system (≥18.25 MΩ).

PNA probe with thiol groups at their 5'ends was synthesized by CP Biochem Co., Ltd. (Chengdu, China). All oligonucleotides were tailor-made by Sangon Biotechnology Co. Ltd. (Shanghai, China) with purity no less than 99%. The sequences are shown in Table S1.

**Table S1.** The detailed sequences of oligonucleotides

| Note | Sequence (5'-3') |
|---|---|
| Peptide nucleic acid (PNA) | HS-(CH2)11-AAC CAT ACA ACC TAC TAC CTC A |
| Complementary target ssDNA (T-DNA) | TGA GGT AGT AGG TTG TAT GGT T |
| Single -base mismatched ssDNA (1MT) | TGA GGT AGT AGG TTG TGT GGT T |
| Three-bases mismatched ssDNA (3MT) | TGA GGT ATT AGA TTG TGT GGT T |
| Non-complementary DNA (NC) | ACT TAC CTT TGC TCA TTG ACG A |

**Preparation of the Gold Electrode.** Prior to the gold electrode modification, it was polished to a mirror-like surface with an alumina slurry (1, 0.3 and 0.05 μm) on a microcloth pad for 5 min. The electrode was then treated with piranha solution (98% $H_2SO_4$ / 30% $H_2O_2$ = 3:1) (Caution: The solution is highly corrosive and oxidizing!) for 30 min. Afterward, the electrode was sonicated in ethanol and ultrapure water for a 2 min. Next, electrochemical cleaning was performed with 0.5 M $H_2SO_4$ to ensure the complete removal of impurities on the surface of the electrode. Subsequently, the electrode was rinsed thoroughly with ultrapure water and blown to dry with a stream of nitrogen.

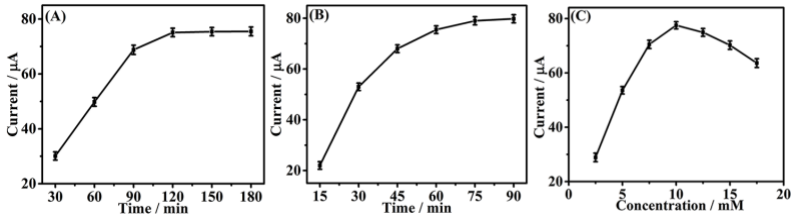

**Figure S1.** Optimization of the experimental conditions. (A) Effect of RAFT reaction time on the DPV response of the biosensor.; (B) Effect of in-situ metallization reaction time on the DPV response of the biosensor. (C) Effect of the concentration of $BrPhN_2^+$ on the DPV response of the biosensor. The error bars were acquired on the basis of six replicate experiments.

**Table S2.** Comparison of the detection limits and linear ranges of DNA biosensors

| Signal amplification strategy | linear range | LOD | ref. |
|---|---|---|---|
| **TdT** | 0.1 pM−1.0 nM | 20 fM | 1 |
| **RCA** | 10 aM−10 pM | 11 aM | 2 |
| **HCA** | 1.0 fM−1.0 pM | 0.4 fM | 3 |
| **SI-RAFT** | 1.0 fM to 1.0 μM | >1.0 fM | 4 |
| **AGET ATRP** | 0.1 nM−1.0 μM | 15 pM | 5 |
| **eRAFT** | 1 pM−10 aM | 5.4 aM | this work |

## REFERENCES

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