Supporting Information for:

Electrochemical Detection of Type 2 Diabetes Mellitus-Related SNP via DNA-Mediated Growth of Silver Nanoparticles on Single Walled Carbon Nanotubes

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EXPERIMENTAL SECTION

Materials and Apparatus. AgNO₃, NaBH₄ were obtained from Sigma-Aldrich Inc. SWCNTs were purchased from Carbon Nanotechnologies, Inc. Streptavidin-coated silica (SiO₂) microbeads (SiMBs, 2 μm) were purchased from Bangs Laboratories Inc. All chemicals were used as received, unless otherwise stated. β-cell lines were obtained from The Second Xiangya Hospital of Central South University (Hunan, China). RNase HII was obtained from New England Biolabs (NEB) Inc. DNA oligonucleotides were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). The sequences of these oligonucleotides are as follows ("rC" represents the RNA base “C” and the underlined letter represents the mismatched base at nucleotide position (np) 8344 in mtDNA). Transmission electron microscopic (TEM) images were conducted on a JEOL 1230 electron microscope operating at 200 kV. The UV absorption spectrum was measured using a U-4100 HITACHI spectrophotometer. Atomic force microscope (AFM) was conducted on a SPI3800-SPA400 microscope. Electrochemical measurements were performed with a CHI 660B Electrochemistry Workstation (CHI, Shanghai). A three-electrode system was used with a common Ag/AgCl reference and a Pt wire auxiliary electrodes placed in the central buffer solution containing 0.1 M NaCl. Differential pulse voltammetry (DPV) data were collected at 100 mV/s, 100 mM PBS (pH 7.5) unless otherwise indicated.

Preparation of ssDNA-Mediated SWCNT@AgNPs. Noncovalent functionalization of SWCNTs with ssDNA has been well established in earlier reports. The SWCNT/ssDNA suspension was then centrifuged at 14000 rpm for 1 h to remove aggregates. The SWCNT/ssDNA mixture solution was diluted to 0.5 mL with 20 mM Tris-HNO₃ buffer and aliquot of stock solution of silver nitrate (AgNO₃) was transferred into it. The solution was then incubated at 20 °C for 15 min to form the SWCNTs/ssDNA/Ag⁺ complex, following by centrifuged at 14000 rpm for 1 h and then washed with Tri-HNO₃ buffer for three times to remove free AgNO₃, and then stored at 4 °C for further usage. Then, 10 μL sodium borohydride (NaBH₄) was added to the solution with vigorously vortex. After incubation for 30 min at room temperature, the ssDNA-mediated SWCNT@AgNPs were formed.

Formation of DNA-Mediated AgNPs on the Surface of SWCNTs/GCE. The DNA-templated AgNPs on the surface of SWCNTs/GCE was prepared as follows. All
of following work solutions were prepared with 20 mM Tris-HNO₃ buffer solution (pH 7.2). First, the GCE was held upside down and 10 µL of SWCNTs solution (0.1 mg/mL, the concentration of SWCNT is excessive) was dropped onto the electrode surface. In order to make the solution evaporation, the electrode dropped with the solution was further covered under an infrared lamp. After cooling down, ssDNA was dropped onto the surface of SWCNTs/GCE and incubated for 2 h. Subsequently, the electrode was rinsed thoroughly with a copious amount of Mili-Q water. Then, 5 µL of stock solution of 20 µM AgNO₃ was transferred onto the surface of SWCNTs/GCE for 15 min to form DNA/Ag⁺ complex. The electrode was rinsed thoroughly with a copious amount of Mili-Q water again. The DNA-templated SWCNTs@AgNPs/GCE were formed by adding aliquot of NaBH₄ onto the surface of SWCNTs/GCE and incubated for 30 min. All DPV experiments were obtained from -0.2 V to 0.8 V with a scan rate of 0.1 V/s. Electrochemical impedance measurements in this work were obtained over a frequency range from 100 kHz to 1 Hz.

Quantitative Analysis of CP on the Surface of SiMBs. The biotin-labeled CP was first incubated with streptavidin-coated SiMBs. The mixtures were vortexed at room temperature for 1 h, followed by washing three times with PBS using centrifugation at 1 000 rpm to remove any CP that did not conjugate to the SiMBs. The absorption maximums (measured at 260 nm) of the supernatant, containing free CP removed from the SiMBs, were converted to molar concentrations of DNA by UV-Vis absorption using published sequence-dependent absorption coefficients. Finally, the average number of successfully conjugated oligonucleotides on the surface of SiMBs was obtained.

RNase HII-Assisted DPV Detection of 8344G Polymorphism. The biotin-labeled CP (50 nM) was first incubated with streptavidin-coated SiMBs (0.1 mg/mL). The mixtures were then vortexed and incubated at room temperature for 1 h, followed by washing three times with PBS and centrifugation at 1000 rpm to remove any DNA that did not conjugate to the SiMBs. The conjugates were dispersed in PBS and stored at 4 °C at a final concentration of 0.1 mg/mL. Then, target DNA was added into the solution containing CP-conjugated SiMBs, and the mixture was annealing to hybrid, followed by the addition of (0.1 U/µL) RNase HII. After reaction for 1 h, the solution was centrifuged to remove the CP-conjugated SiMBs. Next, the supernatant containing signal probe DNA was used to synthesize AgNPs and for the solid-state
DPV assay. For the real samples assay, mitochondria were extracted using Tissue Mitochondria Isolation Kit (Beyotime, shanghai) and their lysate was digested by Csp6I restriction enzyme (Thermo Scientific) before applied to our approach.

**Table S1.** The sequences of oligonucleotides used in this experiment.

<table>
<thead>
<tr>
<th>Capture probe (CP): 5’-biotin- AGGTGTGGGrCTCTCTTAATTCCCCC -3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1: 5’- TTAAGTTAAAGATTAAGAGAGCCAACACCTCTT -3’</td>
</tr>
<tr>
<td>T2: 5’- TTAAGTTAAAGATTAAGAGAACCACACCTCTT -3’</td>
</tr>
<tr>
<td>Signal probe (SP): 5’-TCTCTTAATTCCCCC-3’</td>
</tr>
</tbody>
</table>

**Table S2.** Detection of mutation target DNA in cell medium

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added T1 (nM)</th>
<th>Detection results (nM)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.095±0.004a</td>
<td>95.0 %</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1.031±0.051</td>
<td>103.1 %</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>8.965±0.601</td>
<td>89.7 %</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation of three determinations.

**Figure S1.** (A) TEM and (B) high resolution TEM images (HR-TEM) of ssDNA-mediated SWCNTs@AgNPs. [SWCNTs] = 0.1 mg/mL, [AgNO₃] = 20 µM, [NaBH₄] = 1 mM, [ssDNA] = 20 nM, respectively.
Figure S2. (A) AFM and (B) corresponding height profile of DNA-mediated SWCNTs@AgNPs. [SWCNTs] = 0.1 mg/mL, [AgNO$_3$] = 20 µM, [NaBH$_4$] = 1 mM, [ssDNA] = 20 nM, respectively.

Figure S3. The solid state transforming process of electron from Ag to the AgCl on the surface of SWCNT/GCE.

Figure S4. DPV scan of SWCNTs@AgNPs/GCE as function of different concentrations of ssDNA template (5 nM-50 nM). [SWCNTs] = 0.1 mg/mL, [AgNO$_3$] = 20 µM, [NaBH$_4$] = 1 mM.
Figure S5. DPV scan of SWCNTs@AgNPs/GCE by varying the concentration of AgNO$_3$ (from 0 to 25 μM). [SWCNTs] = 0.1 mg/mL, [ssDNA] = 50 nM, [NaBH$_4$] = 1 mM.

Figure S6. DPV scan of SWCNTs@AgNPs/GCE by varying the concentration of NaBH$_4$ (from 0.1 to 25 μM). [SWCNTs] = 0.5 mg/mL, [ssDNA] = 50 nM, [Ag$^+$] = 20 μM.

Figure S7. DPV scan of SWCNTs@AgNPs/GCE obtained by varying the silver growth time. [SWCNTs] = 0.1 mg/mL, [ssDNA] = 50 nM, [AgNO$_3$] = 20 μM, [NaBH$_4$] = 1 mM.
Figure S8. PAGE analysis of RNase HII-assisted cleavage of DNA stained by SYBR green II. Lane 1, 2, 3: capture probe, T2, T1, respectively; lane 4, 6: complex of capture probe with T2 and T1, respectively; lane 5, 7: complex of capture probe with T2 and T1 after cleavage by the RNase HII, respectively; lane 8: ssDNA. Concentration of DNA (CP, T1, T2 and SP) was 5 μM and the concentration of RNase HII was 0.1 U/μL, reaction time of hydrolysis by RNase HII was 0.5 h.

Figure S9. Reaction time and concentration of RNase HII assisted amplification-dependent peak current intensity responses obtained by monitoring the digestion of CP/T1 hybrid duplex by 0.002, 0.01, 0.02, 0.05 and 0.1 U/μL RNase HII for different time(from 10 to 60 min). [SWCNTs] = 0.5 mg/mL, [AgNO₃] = 20 μM, [NaBH₄] = 1 mM. [SiMBs] = 0.1 mg/mL, [CP] = 50 nM.
Figure S10. (A) Detection of mutation target DNA T1 in the presence of 10 nM wild-type DNA T2, and (B) The relationship between peak current intensity and T1 concentration (10 pM-10 nM) in the presence (black curve) and absence (red curve) of 10 nM T2. [SWCNTs]=0.1 mg/mL, [AgNO₃]=20 μM, [NaBH₄]=1 mM. [SiMBs]=0.1 mg/mL, [CP]=50 nM, [RNase HII]= 0.1 U/μL.

Figure S11. Application to samples with T1, T2 and crude extracts of carcinoma pancreatic β-cell lines from diabetes patients (1-10).
Figure S12. The representative electropherogram shows that these results are consistent with our proposed assay. For comparison, mtDNA from the same samples (1-10) were extracted using tissue mitochondria isolation kit and DNA extraction kit, and their PCR products were sent to Sangon Biotech for Sanger sequencing services.