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Supplementary Information:

# Sensitive and selective electrochemical DNA sensor for the analysis of cancer-related single nucleotide polymorphism

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**Table S1.** Oligonucleotides designed in the present study.

| Note | Sequence (5'-3') |
|---|---|
| Capture probe | $PO_3$-**TCTCAGCTGCTCGCTGTC** AATTCTCGTCTGTT HS |
| Biotin probe | Bio-tagcgcatcacagtcgcg |
| Stabilizing probe | HS-TCACA CTCATGACTGCTTCTTGT |
| Signaling probe 1 | $NH_2$-AGAGATCT ACAAGAAGCAGTCATGAG |
| Signaling probe 2 | $NH_2$-CGAGAATT ACAAGAAGCAGTCATGAG |
| Signaling probe 3 | $NH_2$-GACGAGAATT ACAAGAAGCAGTCATGAG |
| Signaling probe 4 | $NH_2$-CAGACGAGAATT ACAAGAAGCAGTCATGAG |
| Target DNA (T) | **GACAGCGAGCAGCTGAG A** cgcgactgtgatgcgcta |
| Mutant DNA1 (M1) | **GACAGCGAGCAGCTGAG** C cgcgactgtgatgcgcta |
| Mutant DNA2 (M2) | **GACAGCGAGCAGCTGAG** G cgcgactgtgatgcgcta |
| Mutant 3 DNA(M3) | **GACAGCGAGCAGCTGAG** T cgcgactgtgatgcgcta |
| Forward primer 1 | GTCAGAACCGGCTGGGGATG |
| Reverse primer 1 | CTCCTCCCAACTCATCCC GG |
| Capture probe 2 | $PO_3$- AGGACGCGGGTGCCGGGC AATTCTCGTCTGTT HS |
| Biotin probe 2 | Bio-tgtagatggccatggcg |
| Forward primer 2 | TACTCCCCTGCCCTCAACAA |
| Reverse primer 2 | CATCGCTATCTGAGCAGCGC |

Capture probe has a HS- moiety at the 3' terminus for self-assembly onto the gold electrode surface and a $PO_3$- at the 5' terminus as the substrate for DNA ligase. Biotin probe is modified with a biotin group at the 5' terminus. Stabilizing probe has a HS- moiety at the 5' terminus for self-assembly onto the surface of GNP. Signaling probe 1, 2, 3 or 4 has a $NH_2$- moiety at the 5' terminus for the modification of Fc. The regions in bold and in lowercase letter of the target DNA are complementary to the bold part of capture DNA and biotin probe, respectively. The underlined segments of signaling probes can hybridize with the underlined region of capture probe, while its gray segment is complementary to the gray region of stabilizing probe. The

boxed bases in mutant DNAs indicate alleles at the SNP site.

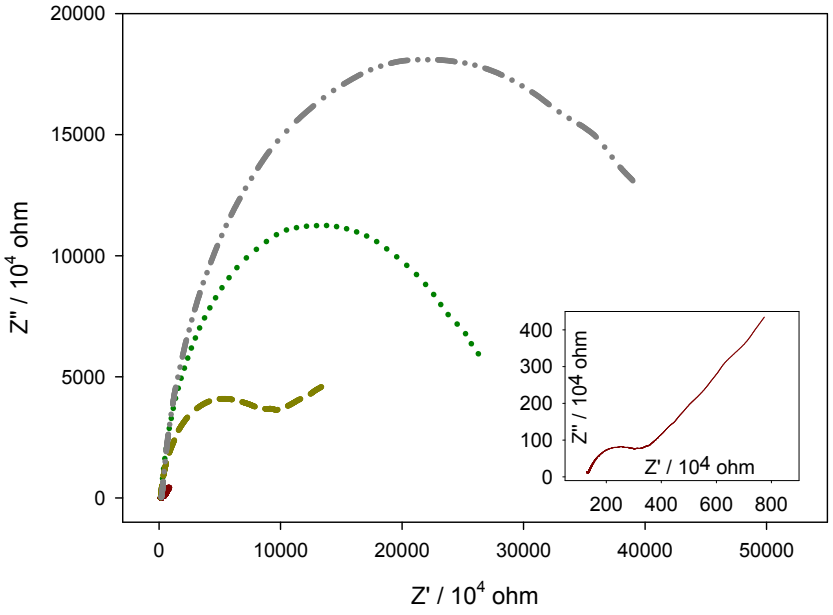


**Figure S1.** AC impedance of the present electrochemical DNA biosensor at different preparation stages: a) bare gold electrode (also shown in the Inset); b) the capture probe-modified electrode after ligation using target DNA (1 nM) as the template followed by heating dehybridization; c) the same as (b) but exposure to Fc-functioned GNPs; (d) the same as (c) but $NH_2$-functionalized GNPs were involved instead of Fc- functionalized GNPs.

As shown in Figure S1, an increase of impedance (curve b) is obtained in comparison with the bare electrode (curve a). This is directly associated with more surface-confined oligonucleotides, indicating the successful ligation of biotin probe to the electrode surface in the presence of the target DNA. When interacting with the Fc-functionalized GNPs, a decrease of Faraday impedance is observed (curve c). This should be attributed to the attachment of Fc tags which can facilitate the interfacial electron transfer. However, when Fc-functionalized GNPs were replaced with $NH_2$-functionalized GNPs, the electrochemical impedance increases significantly (curve d), further confirming that the modified GNPs are captured onto the sensing surface by

the target hybridization-induced biotin/streptavidin complexation and the Fc tag acts as an electrochemically active reporter (curve c).

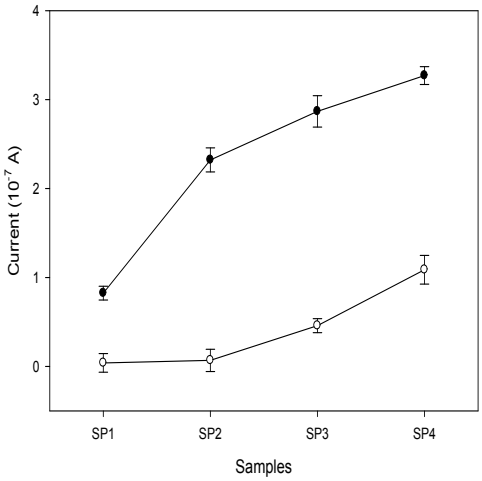


**Figure S2**. Optimization of the signaling probe. DPVs of this sensing system in the presence (black spots) and absence (circle spots) of 1 nM target DNA.

In this sensing system, the dragging strategy is dependent on the hybridization between the signalling probe and capture probe. To evaluate the influence of this strategy on target-responsive capability and to improve its performance, four signalling probes with different complementary sequences to the capture probe were designed. The performance of this sensing system with each of these four probes was individually tested under the same conditions. In the case of signalling probe 1 which is noncomplementary to the capture probe, the current signal induced by the target is only ~36 % of that with signalling probe 2 containing 8-mer nucleotides complementary to the capture probe. This demonstrates that the response signal can be improved by introducing a complementary sequence into signalling probe to hybridize with the capture probe. However, further lengthening the complementary sequence (e.g., signalling probe 3 and signalling probe 4, having 10-mer and 12-mer nucleotides complementary to the capture probe, respectively), the background signal increases substantially although the enhanced target response is detected, thus leading to a reduced signal-to-noise ratio by over 5-10 times compared to that with signalling probe 2. As demonstrated in our previous work,[1] this increased background comes from the ability of the signalling probe 3 (or the signalling probe 4) to hybridize the capture probe even with no target-induced biotin/streptavidin reaction. For the signalling probe 2, because of the relatively short complementary section, it cannot stably hybridize the capture probe. However, once the Fc-functionalized GNP is anchored on the electrode surface through the target-caused biotin/streptavidin complexation, hybridization

between the signalling probe 2 and the capture probe can be achieved due to the simultaneous multivalent effect.[1-3] With a maximum target-to-blank ratio, the signalling probe 2 was used in subsequent experiments.

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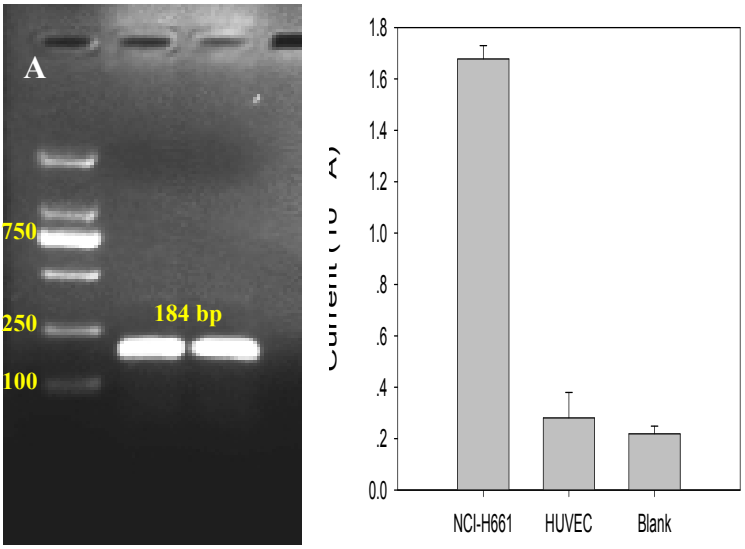


**Figure S3.** The practicality and generalization of this sensing system for PCR product detection (A) Image of 2% agarose gel electrophoresis for PCR amplicons. Lane M is the DNA marker (DL2,000 DNA Marker from Takara Biotechnology Co. Ltd. (Dalian, China), while lane 1 is the PCR product of NCI-H661 cells and lane 2 is the PCR products of HUVEC cells. (B) DPVs of the present biosensor for the detection of these PCR amplicons.

**References**

(1) L. Qiu, L. Qiu, Z. Wu, G..Shen, and R. Yu, *Anal. Chem.,* 2013, **85**, 8225-8231.

(2) C. Huang, Y. Huang, Z. Cao, W. Tan, and H. Chang, *Anal. Chem.,* 2005, **77**, 5735-5741.

(3) Huang, Y. Zhang, X. Xu, J. Jiang, G. Shen, and R. Yu, *J. Am. Chem. Soc.,* 2009, **131**, 2478-2480.