Electronic Supplementary Information

Electrochemical sensing platform based on local repression of electrolyte diffusion for single-step, reagentless, sensitive detection of sequence-specific DNA-binding protein

Yun Zhang,* Fang Liu, Jinfang Nie,* Fuyang Jiang, Caibin Zhou, Jiani Yang, Jinlong Fan and Jianping Li

College of Chemistry and Bioengineering, Guilin University of Technology, Guilin 541004, China

* Corresponding author.

Tel.: +86 773 5896453; Fax: +86 773 5896839

E-mail address: zy_hnu@163.com; Niejinfang@glut.edu.cn.

The calculation of DNA surface coverage on the electrode

The surface coverage (Γ_{ap}) of the ferrocene (Fc)-labeled DNA probe modified on the gold electrode could be estimated from the amount of charge (Q) of the Fc's redox process in measuring cyclic voltammogram (CV) using the following formula: $\Gamma_{ap} = Q/nFA$, where n is number of electrons per reaction, F is Faraday constant and A is electrode's electroactive area. The total charge obtained from the anodic peak of CV curve a that is shown in Fig. 2A in the manuscript is ~9.60×10^{-8} C, while the real electroactive area of the gold electrode is calculated as ~52.6×10^{-3} cm^2 from the corresponding charge for the reduction of the oxide probe monolayer. Assuming that all of the Fc labels close to the electrode surface are electroactive, the surface coverage of the DNA probe is ~1.89×10^{-11} M/cm^2 (~1.14×10^{13} $molecules/cm^2$).

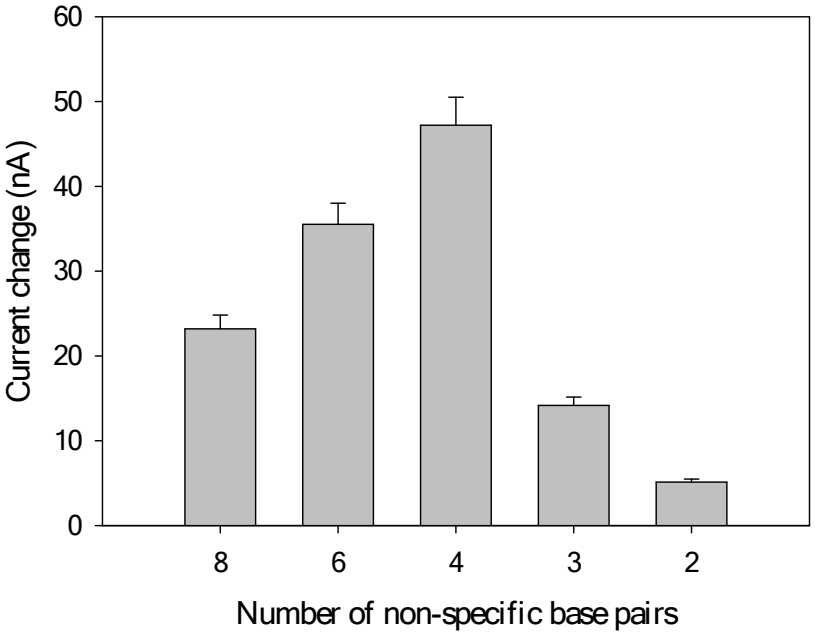

Fig. S1 The number optimization of the non-specific base pairs between the 5' end of the thiol-modified strand and the TATA box for biosensor fabrication. Each of the sensors fabricated with different number of non-specific base pairs and a fixed immobilization time of 2 h was used for the assay of a 5.04 nM TBP sample at 37 °C for 1 h. The number of non-specific base pairs that led to the greatest change of DPV peak current (i.e., 4) was chosen for biosensor fabrication in all following experiments. Each data point represents the average value of five repetitive experiments. Error bars reflect the standard deviations from the average values.

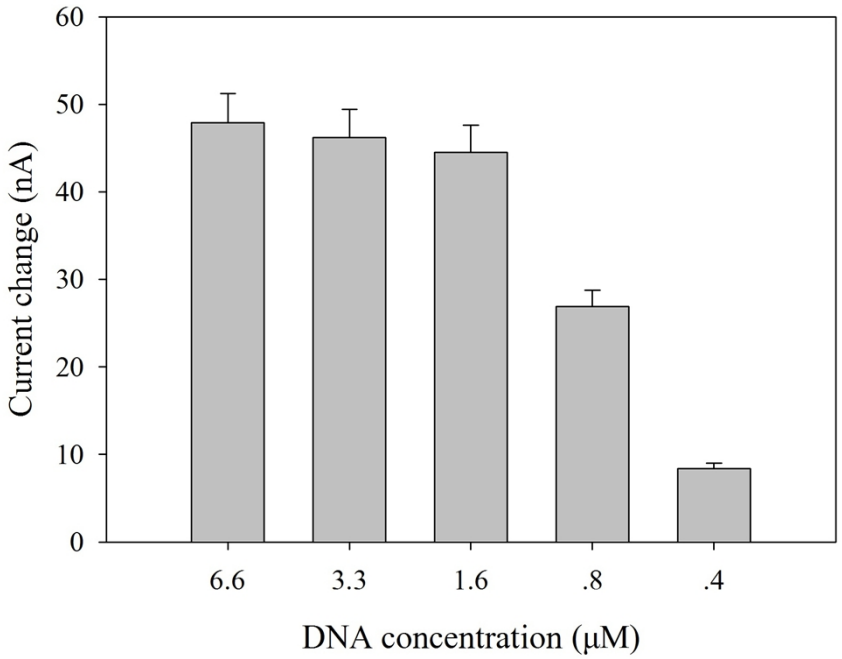

Fig. S2 The optimization of DNA concentration for biosensor fabrication. Each of the sensors fabricated at different DNA concentrations and a fixed immobilization time of 2 h was used for the assay of a 5.04 nM TBP sample at 37 °C for 1 h. The changes of DPV peak currents tend to slightly increase when using the DNA probe concentrations higher than 1.6 μM. Thus 1.6 μM was chosen as the optimized probe concentration for biosensor fabrication. Each data point represents the average value of five repetitive experiments. Error bars reflect the standard deviations from the average values.

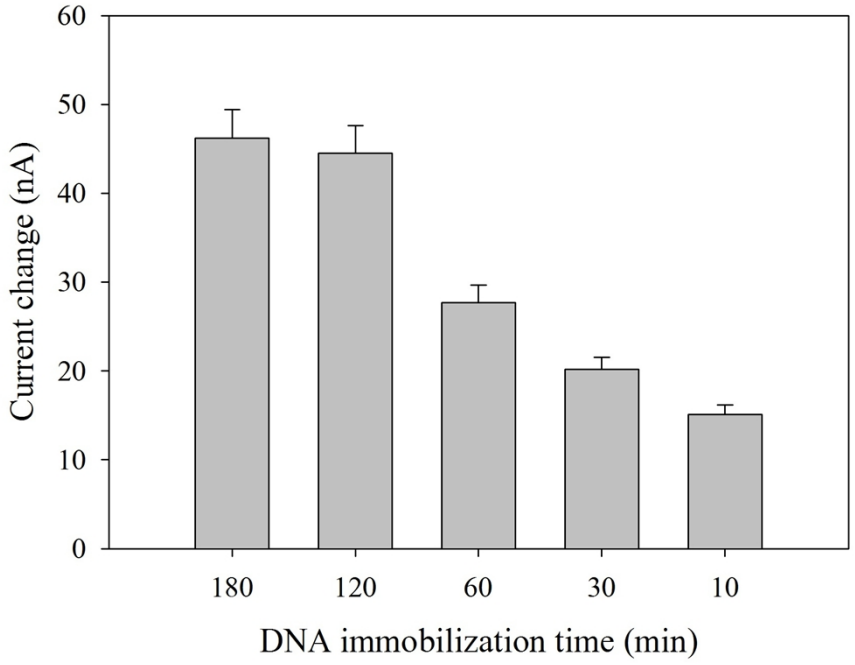

Fig. S3 The optimization of DNA immobilization time for biosensor fabrication. Each of the sensors fabricated at a fixed probe concentration of 1.6 μM and different immobilization time was used for the assay of a 5.04 nM TBP sample at 37 °C for 1 h. The changes of DPV peak currents tend to slightly increase when using the probe immobilization time higher than 120 min. Thus 120 min was chosen as the optimized immobilization time for biosensor fabrication. Each data point represents the average value of five repetitive experiments. Error bars reflect the standard deviations from the average values.

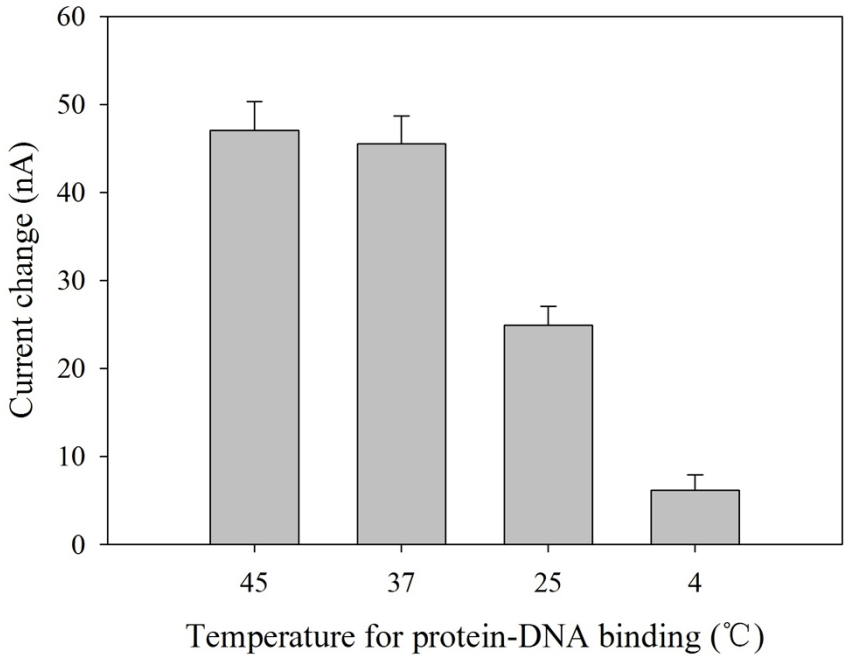

Fig. S4 The temperature optimization for protein-DNA binding. Each of the DNA-modified sensors fabricated at a fixed DNA concentration of 1.6 μM and a fixed immobilization time of 2 h was used for the assay of a 5.04 nM TBP sample at different temperatures for 1 h. The changes of DPV peak currents tend to slightly increase when using the reaction temperature higher than 37 °C. Thus 37 °C was chosen as the optimized temperature for sample assays. Each data point represents the average value of five repetitive experiments. Error bars reflect the standard deviations from the average values.

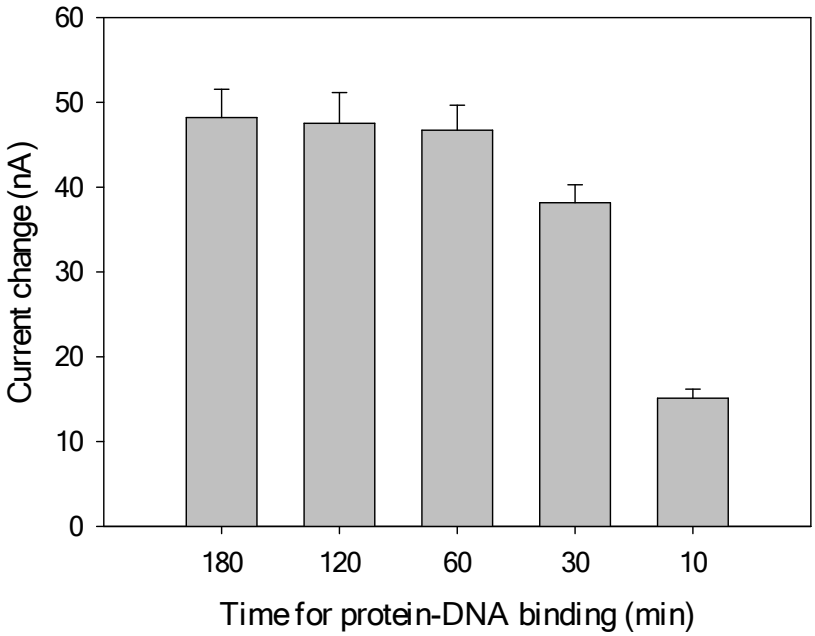

Fig. S5 The time optimization for protein-DNA binding. Each of the DNA-modified sensors fabricated at a fixed DNA concentration of 1.6 μM and a fixed immobilization time of 2 h was used for the assay of a 5.04 nM TBP sample at 37 °C for different periods of time. The changes of DPV peak currents tend to slightly increase after a period of reaction time longer than 60 min. Thus 60 min (1 h) was chosen as the optimized reaction time for sample assays. Each data point represents the average value of five repetitive experiments. Error bars reflect the standard deviations from the average values.

Table S1 Recovery of the Developed TBP Biosensor

Sample	Added TBP (nM)	Found TBP (nM)	Recovery (%)	RSD (%)
1	0.202	0.214	106	8.31
2	5.04	4.76	94.5	7.10
3	25.4	25.7	101	9.42