

Electronic Supporting Information for the Article

A DNA-based Electrochemical Strategy for Label-free Monitoring the Activity and Inhibition of Protein Kinase

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Experimental section

1. Materials and reagents.

Peptide, LRRASLGGGGC and IYAAPKKGGGC were synthesized by GL Biochem Ltd (Shanghai, China). cAMP-dependent protein kinase (PKA, catalytic subunit) was obtained from New England Biolabs, Inc. (Ipswich, MA, USA). Thiolated 15-bp DNA1 (5'-HPO₄⁻-TATCCTGATTTTTT-C₆-SH-3') and DNA2 (5'-TATCCTGATTTTTT-C₆-SH-3') was synthesized and purified by Sangon Inc. (Shanghai, China). H-89 was obtained from EMD Biosciences (Calbiochem, La Jolla, CA). Other reagents, such as ATP, ZrOCl₂, [Ru(NH₃)₆]³⁺ (RuHex) were purchased from Sigma. All solutions were prepared and diluted using ultrapure water (18.3 MΩ·cm) from the Millipore Milli-Q system. The synthesis of 13-nm gold nanoparticles (AuNPs) and their functionalization with DNA were following the published protocol.¹

2. Peptide self-assembly on gold electrodes.

Gold electrode (2 mm in diameter, CH Instruments Inc.) was first polished on microcloth with Gamma alumina suspension (0.05 μm) for 5 min. Residual alumina powder was removed by sonicating electrode in ethanol and water for 5 min, respectively. Then the electrode was electrochemically cleaned to remove any remaining impurities following the reported protocol.² After being dried with nitrogen, the electrode was immediately used for peptide immobilization.

The gold working electrode was coated with 4 μL of 0.2 mM peptide solution and kept overnight at 4 $^{\circ}\text{C}$. After the incubation step, the electrode was washed with blank Tris-HCl buffer solution (10mM, pH 7.5, 25 $^{\circ}\text{C}$, TBS). Then unmodified region of the electrode was blocked by immersing the electrode into 0.1 mM hexanethiol ethanol solution for 30 min and then rinsing the surface with blank TBS.

The surface coverage of the peptides on the Au electrode was evaluated by the quartz crystal microbalance measurement. The relation between the frequency change (Δf) of the quartz crystal and the mass change on the electrode surface of quartz crystal (ΔM) obeys the Sauerbrey equation:

$$\Delta f = -2.26 \times 10^6 F_0^2 \Delta M/A$$

Where F_0 is the resonant frequency of the quartz crystal (9 MHz in the present case); A is the mass sensitive area of the electrode (cm^2). The frequency of the quartz crystal in air was measured and labeled as f_1 . After peptides were immobilized on the electrode surface of the quartz crystal and then the electrode was washed and dried, the related frequency of the quartz crystal in air was measured again and labeled as f_2 . From the frequency change ($\Delta f = f_2 - f_1$, -20 Hz in this paper), the surface coverage of the peptides on the gold electrode is calculated to be 4.8×10^{-10} mol/ cm^2 .

3. Peptide phosphorylation or inhibition at gold electrodes.

PKA was supplied in the buffer containing 20 mM Tris-HCl (pH 7.5 at 25 $^{\circ}\text{C}$), 50 mM NaCl, 1 mM Na₂EDTA, 2 mM 1,4-Dithiothreitol (DTT) and 50% glycerol. PKA reaction mixture (200 μL) contained a certain concentration of PKA, 0.2 mM ATP, and PKA-reaction-buffer (20 mM Tris-HCl (pH 7.5 at 25 $^{\circ}\text{C}$), 10 mM MgCl₂). The

peptide-immobilized electrode was placed in the reaction mixture and incubated at 30 °C for 2 h. After incubation, the electrodes were washed with blank TBS to remove the excess reagents. For inhibitor assay, the same experiments were performed, except for involvement of different concentration of H-89 in the reaction mixture. For Scanning electron microscopy (SEM) experiment, the peptide phosphorylation proceeded under the standard reaction condition with 100 U mL⁻¹ PKA.

4. Recognition of phosphorylation sites and linkage between phosphorylated peptide and DNA functionalized Au nanoparticles (DNA-AuNPs) by Zr⁴⁺.

The abovementioned-phosphorylated peptide electrodes were treated with 4 μL Zr⁴⁺ solution (0.2 mM in TBS) at room temperature for 1h. Then the electrodes were carefully washed with (1) TBS containing 0.5% Tween 20 three times, every time for 2 min; (2) blank TBS for 2 min; (3) water for 2 min, respectively. After being dried with nitrogen, the electrodes were coated with 4 μL DNA-AuNPs at room temperature for 1h. Then the electrodes were washed following the abovementioned process and dried by nitrogen as before.

5. Electrochemical chronocoulometric measurements.

30 μL RuHex (10 mM) was added to 6 mL TBS, and the solution was purged thoroughly with nitrogen for 10 min. All electrochemical measurements were performed with a CHI660a electrochemical workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode configuration was employed for all electrochemical experiments, which involved a gold working electrode, a platinum auxiliary electrode, and a saturated calomel reference electrode (SCE). Electrochemical detecting was performed by chronocoulometry (CC) with the following parameters:² initial potential: 0.2 V; final potential: -0.5 V; number of steps: 2; pulse width: 0.25 s; sample interval: 0.002; sensitivity (C or A/V): 5e⁻⁵ A/V.

6. Electrochemical impedance measurements.

Electrochemical impedance spectroscopy (EIS) was used to monitor the

modification process of the electrode and the corresponding results are shown in Fig. S1. For comparison, the EIS data of the bare Au electrode are shown in the inset of Fig. S1. In EIS, the semicircle diameter equals the electron-transfer resistance, R_{et} . This resistance controls the electron-transfer (eT) kinetics of the redox probe at the electrode interface. According to the EIS results shown in Fig. S1, the modification process of the electrode was achieved successfully.

References

- (1) Jin, R. C.; Wu, G. S.; Li, Z.; Mirkin, C. A.; Schatz, G. C. J. Am. Chem. Soc. 2003, 125, 1643 – 1654.
- (2) Zhang, J.; Song, S.; Wang, L.; Pan, D.; Fan, C. Nat. Protoc. 2007, 2, 2888 – 2895.
- (3) Zhang, J.; Song, S.; Zhang, L.; Wang, L.; Wu, H.; Pan, D.; Fan, C. J. Am. Chem. Soc. 2006, 128, 8575–8580.

Supporting Figures

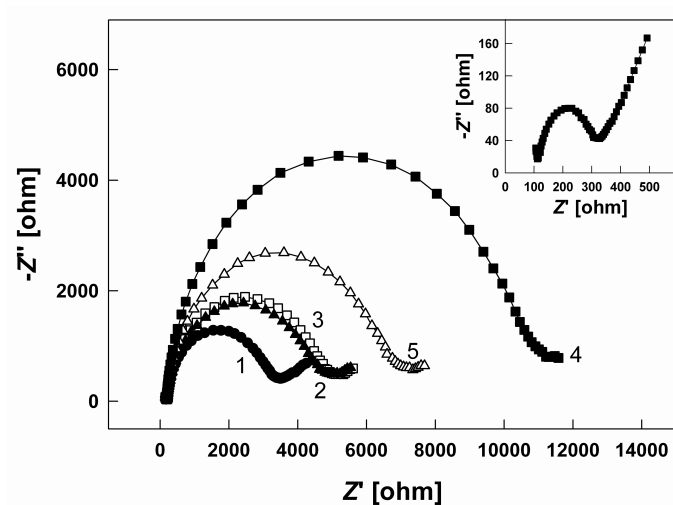


Fig. S1. Nyquist plots obtained with peptide-modified electrode (1), peptide-modified electrode blocked by hexanethiol (2), and electrodes with phosphorylation by PKA (3), phosphorylated peptide bound with DNA–AuNPs by Zr^{4+} (4), peptide incubated in the presence of well-known inhibitor H-89, 0.05 μ M, and then bound with DNA–AuNPs by Zr^{4+} (5). The inset is the nyquist plot of bare Au electrode. The Nyquist plots obtained with the electrodes in Tris–HCl buffer containing 0.1 M NaCl and 5 mM $[Fe(CN)_6]^{3/4-}$. EIS conditions: 100 kHz–0.05 Hz, 10 mV rms, 0.18 V vs SCE.

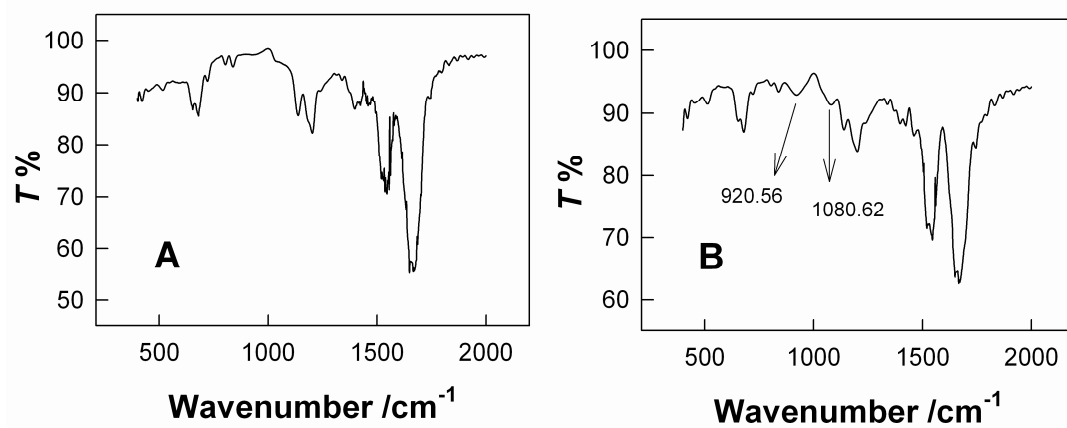


Fig. S2. FT-IR spectra of the peptide-functionalized electrode with (B) and without (A) PKA treatment. Note that the bare electrode without peptide modification was used for the blank FT-IR spectrum.

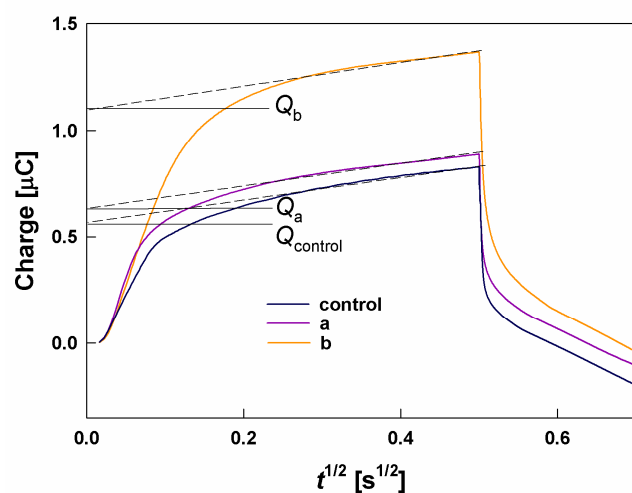


Fig. S3. Representative chronocoulometric curves for gold electrodes modified with peptide and MCH (Q_{control}), peptide phosphorylated by PKA (Q_a), phosphorylated peptide bound with DNA–AuNPs by Zr^{4+} (Q_b).

Redox charges of RuHex bound to DNA are obtained from chronocoulometric intercepts at $t = 0$.³ The signal is defined as the increment of the redox charge, i.e., signal = $Q_a - Q_{\text{control}}$; or signal = $Q_b - Q_{\text{control}}$.

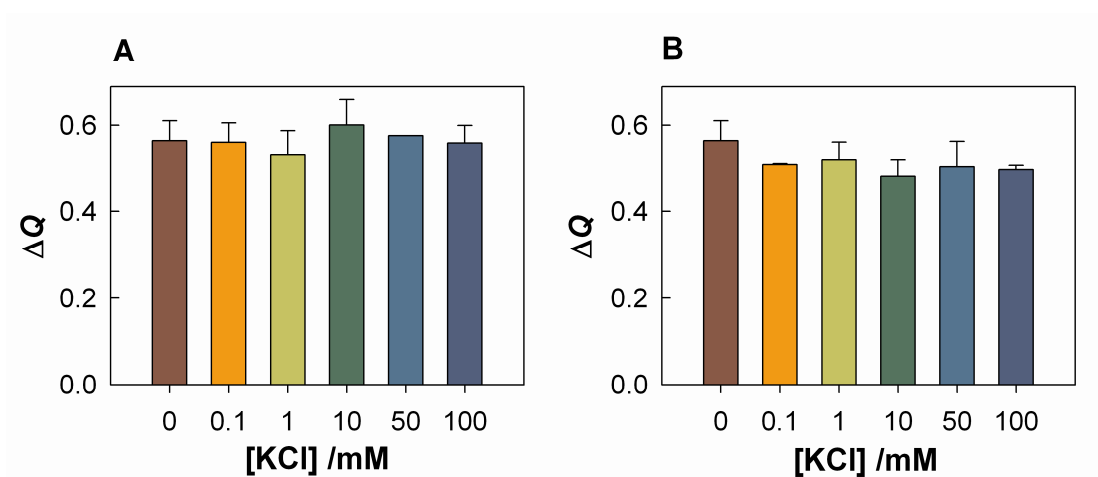


Fig. S4. Effect of the concentration of KCl in the solution of Zr^{4+} (A) or DNA-AuNPs (B) on the redox charge. No obvious disparity of the redox charge was observed after increasing the salt concentration, being probably due to the strong coordination interaction between Zr^{4+} ions and phosphates rather than electrostatic adherence that is sensitive to the ionic strength.

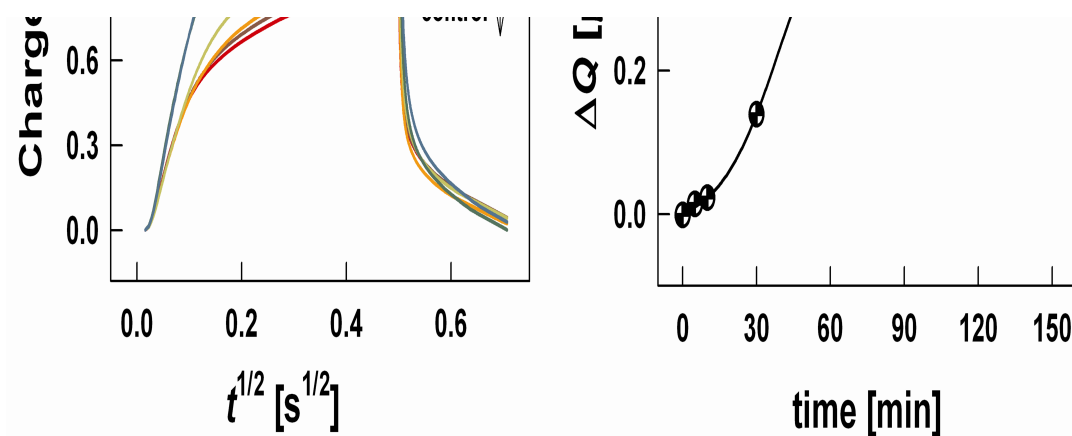


Fig. S5. (A) Chronocoulometric curves of the electrodes with different incubation time for phosphorylation at 30 °C, 100 U mL⁻¹ PKA, 0.2 mM ATP. (B) The dependence of charge response on incubation time.

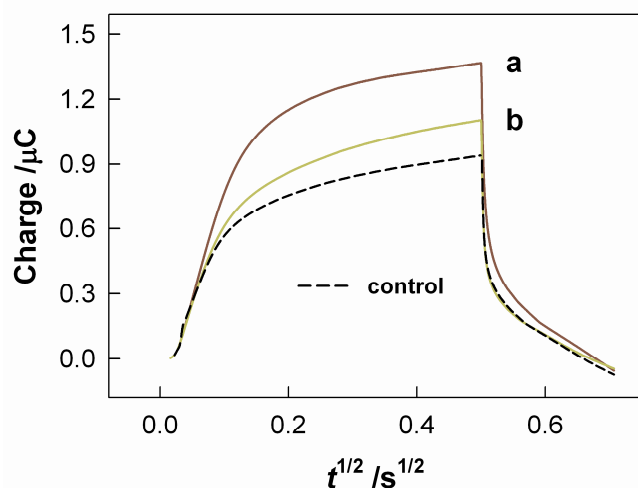


Fig. S6. Chronocoulometric curves of the phosphorylated electrodes for kinase assay using DNA-AuNPs with (a) or without (b) the 5'-phosphate modification. The control curve was obtained from the phosphorylated electrode only treated by Zr^{4+} ions. From the results, we found that when using DNA-AuNPs without the 5'-phosphate modification instead of analogue with the 5'-phosphate modification, the corresponding sensitivity for PKA assay was largely suppressed, and the change of the redox charge to 100 U mL^{-1} PKA using DNA-AuNPs without the 5'-phosphate modification decreased to 33% of that using terminal phosphate modified DNA-AuNPs. This is because the binding strength between Zr^{4+} and the phosphodiester groups of the DNA backbone is much weaker than that between Zr^{4+} and the phosphate group. (Similar phenomenon has also been reported by the references: *J. Am. Chem. Soc.*, 2008, **130**, 6243; *J. Am. Chem. Soc.*, 2004, **126**, 1497).