

DNA Assembled Gold Nanoparticles Polymeric Network Blocks Modular Highly Sensitive Electrochemical Biosensors for Protein Kinase Activity Analysis and Inhibition

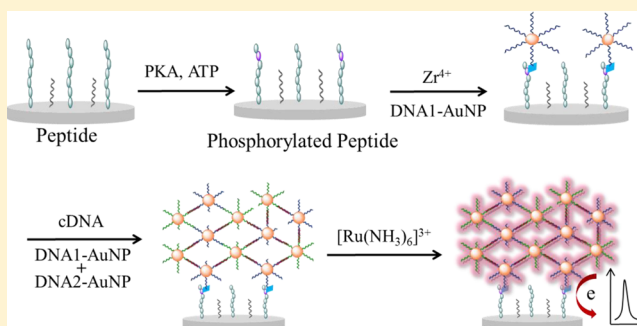
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S Supporting Information

ABSTRACT: A highly sensitive electrochemical biosensor was built for the detection of kinase activity based on the DNA induced gold nanoparticles (AuNPs) polymeric network block signal amplification. In this strategy, the DNA₁ conjugated AuNPs were integrated with the phosphorylated peptide by Zr⁴⁺ and assembled into DNA-AuNPs polymeric network block by the hybridization of cDNA with each side sequences of DNA₁ and joint DNA₂. The kinase activity was determined by the amperometric responses of [Ru(NH₃)₆]³⁺ absorbed on the network block by electrostatic interaction. Due to its excellent electroactivity and high accommodation of the DNA-AuNPs polymeric network block for [Ru(NH₃)₆]³⁺, the current signal was significantly amplified, affording a highly sensitive electrochemical analysis of kinase activity. The as-proposed biosensor presents a low detection limit of 0.03 U mL⁻¹ for protein kinase A (PKA) activity, wide linear range (from 0.03 to 40 U mL⁻¹), and excellent stability even in cell lysates and serum samples. This biosensor can also be applied for quantitative kinase inhibitor screening. Finally, the PKA activities from BE4S-2B, A549, and MCF-7 cell lysates were further analyzed, which provided a valuable strategy in developing a high-throughput assay of in vitro kinase activity and inhibitor screening for clinic diagnostics and therapeutics.



Over 30% of human proteins are modified by protein kinase, and they regulate the majority of cellular pathways including metabolism, cell growth, cellular signal communications, and survival differentiation.^{1–3} Over 500 protein kinase genes are contained in human genes, and they constitute about 2% of all human genes. The aberrant protein phosphorylation and kinase activity are linked with many common diseases, such as various forms of cancer^{4,5} and Alzheimer's disease.^{6,7} It is estimated that 25% of drug development efforts are now focused on protein kinase inhibitors discovery. As a result, accurate identification of protein kinases activity and their potential inhibitors is not only valuable to provide insights regarding the fundamental biochemical process of diseases but also essential to the protein kinase-targeted drug discovery and molecular-target therapies.

Recently, electrochemical kinase analysis has received much attention. Compared to other methods such as radioactive,⁸ fluorescence,^{9–12} and surface-plasma resonance^{13,14} analysis systems, electrochemical methods are simple, cost-effective, and sensitive. The measurements of phosphorylation reactions based on oxidation current of electroactive species such as tyrosine,¹⁵ ferrocene,¹⁶ etc. that conjugated on the substrate during the phosphorylation processes have been re-

ported.^{4,5,15–19} To simplify the detection procedure, a gold nanoparticle labeled phosphorylation process for the kinase assay was also designed in this group by measuring the redox currents of gold nanoparticles, yet a low sensitivity was achieved.²⁰ To avoid the modification of ATP substrate and amplify the signal, electroactive probes were also introduced by the coordination effect of phosphate group with some metallic ions such as Ti⁴⁺ and Zr⁴⁺.^{4,5} For example, a TiO₂-assisted silver enhanced electrochemical biosensor for kinase activity profiling was developed by the specific binding of phosphate groups with TiO₂ nanoparticles.⁴ A DNA-based strategy was also described by the chronocoulometric response of Ru(NH₃)₆³⁺ absorbed on the DNA-gold nanoparticles that linked with the phosphorylated peptide by Zr⁴⁺.⁵ Despite the improvement of these methods, it is still a challenge to develop sensitive, accurate, and rapid methods for the profiling of kinase activity and inhibition.

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DNA assembly nanotechnology has received explosive interest in biosensing as signal amplification probes owing to the specificity of DNA base-pairing hybridization and the specific enzymatic DNA polymeric manipulation as well as the DNA-nanoparticles assembly, which creates long concatamers containing multiple target molecules and signal probes.^{21–25} For example, “supersandwich” multiple DNA probes have been used for DNA biosensors by applying a signal DNA to hybridize with two target DNAs but in different complementary regions, leading to large signal amplification.^{23,26,27} In addition, long, linear, tandemly, and repetitive single strands of DNA can also be fabricated by the DNA amplification techniques such as rolling circle amplification (RCA) in the presence of DNA polymerase.^{28–34} The produced long DNA can be used as scaffolds that hybridize with the fluorescence molecules, enzymes, or nanoparticles conjugated to a short DNA sequence to form 1D polymeric nanoprobe or even a 3D DNA-nanoparticle array for sensitive biosensing.^{35–39} However, the intrinsic electron inert property of the polymeric DNA is an obstacle for their applications in electrochemical biosensors. Owing to their good biocompatibility, fascinating electrocatalytic activity, large surface area, excellent conductivity, and stability, gold nanoparticles have been widely used in electrochemistry, biosensing, imaging, and so on.^{40–42} The conductive DNA nanostructure was also reported by the conjugation of gold nanoparticles that showed great promise in bioelectronics applications.⁴³

In this work, a highly sensitive electrochemical biosensor for kinase activity analysis was built on the basis of the DNA assembly induced gold nanoparticles (AuNPs) polymeric network block signal amplification probes mediated by zirconium cation (Zr^{4+}). When the peptide on the electrode was phosphorylated by kinase, the multicoordinative interaction forms between Zr^{4+} ions and phosphate groups on the phosphorylated peptides and 5'-end of DNA_1 conjugated on gold nanoparticles (DNA_1 -AuNP) to capture the DNA_1 -AuNPs on the electrode. The outside sequence of DNA_1 and DNA_2 conjugated on gold nanoparticle can hybridize with the DNA sequence on each side of cDNA and assemble into DNA -AuNPs polymeric network block. As a result, the kinase activity can be analyzed by the amperometric responses of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ absorbed on the DNA -AuNPs network block through electrostatic interaction. Due to the excellent electroactivity and high accommodation of the DNA -AuNPs polymeric network block, the amperometric signal was significantly amplified, affording a highly sensitive electrochemical biosensor for kinase activity detection. The electrochemical biosensor can also be applied for quantitative kinase inhibitor screening. Moreover, detection of kinase activity in complicated biological samples such as serum and cell lysate was also conducted. This strategy shows great promise in the sensitive, selective, and high-throughput kinase activity assay and inhibitor screening.

■ EXPERIMENTAL SECTION

Reagents and Materials. Protein kinase A (PKA; catalytic subunit from bovine heart), $[\text{Ru}(\text{NH}_3)_6]^{3+}$, ellagic acid, and *N*-(3-chlorophenyl)-6,7-dimethoxy-4-quinazolinamine (Tyrphostin AG1478) were purchased from Sigma. Cysteine-terminated kemptide (CLRRASLG) was obtained from GL Biochem (Shanghai, China). 1-Hexanethiol was from J&K (Beijing, China). DNA_1 (5'-P-GCTTGTAGTAGTCTG-C6-SH-3'), DNA_2 (5'-SH-C6-CGTTTAGGATTTGTG-3'), and cDNA

(5'-CAGACTACTACAAGCTTTCACAAATCCTAAACG-3') were synthesized and purified by Sangon Inc. (Shanghai, China). Adenosine triphosphate (ATP), Dulbecco's Modified Eagle Medium (DMEM), and fetal bovine serum (FBS) were obtained from Dingguo Biological Products Company (China). Other reagents of analytical grade were provided from Beijing Chemical Company (China). $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (48% w/w) was obtained from Shanghai Reagent (Shanghai, China).

Preparation of DNA Conjugated Gold Nanoparticle Nanoprobes. The gold nanoparticles were prepared as reported previously.⁴⁴ In brief, 100 mL of 0.01% (w/v) HAuCl_4 solution was boiled with vigorous stirring, and 2.5 mL of 1% (w/v) trisodium citrate solution was quickly added to the boiling solution. Wine red solution was obtained, indicating the formation of AuNPs with diameter of 15 nm which were characterized by transmission electron microscopy (TEM) and UV-vis spectra (Figure S1 in the Supporting Information). The DNA conjugated gold nanoparticles were prepared by adding DNA_1 or DNA_2 into 1 mL of gold colloidal solution. After stirring for 24 h, 150 μL of 1 M NaCl was added dropwise. Finally, the mixture was centrifuged at 12 000 rpm for 10 min twice and dispersed in buffer containing 300 mM NaCl and 50 mM Tris-HCl, pH 7.4. The DNA conjugated AuNPs were characterized by UV-vis spectra (Figure S1B in the Supporting Information). The characteristic absorption peak of gold nanoparticles was red-shifted from 520 to 523 nm due to the decoration of DNA on Au nanoparticles. When the cDNA was incubated with DNA_2 -AuNPs, the characteristic absorption peak was further shifted to 528 nm, indicating the hybridization of cDNA with DNA_2 on the surface of AuNPs.

Assembly and Phosphorylation of Peptides on Gold Electrode. A gold electrode (diameter of 2 mm) was polished carefully first with 0.3 μM Al_2O_3 powder on fine abrasive paper and washed with water and then ultrasonically with ethanol and water. Prior to immobilization of kemptide, the gold electrode was activated in 0.5 M H_2SO_4 between 0.2 and 1.65 V (vs Ag/AgCl) until a reproducible cyclic voltammogram was obtained. After being cleaned with water and dried with N_2 , the electrode was immersed into a PBS (10 mM, pH 7.4) solution containing 500 μM cysteine terminated kemptide at room temperature for 12 h. After the incubation step, the electrode was washed thoroughly with PBS. Then, the electrode was immersed into 1 mM hexanethiol for 30 min to block the unmodified region of electrode and then washed thoroughly with blank PBS. A PKA-catalyzed phosphorylation reaction was performed by placing the modified electrode in an assay buffer solution (50 mM Tris-HCl and 20 mM MgCl_2 , pH 7.4) containing a desired amount of PKA and ATP at 37 °C for 1 h. For a PKA inhibitor assay, the procedures were similar as above, except for different concentrations of inhibitor were added in the PKA reaction mixture.

DNA-AuNPs Network Block Amplification Electrochemical Characterization of Peptide Phosphorylation. The phosphorylated peptide modified electrode was treated with 0.5 mM Zr^{4+} at room temperature for 1 h. Then, the electrode was rinsed absolutely with blank PBS and water. After being dried with N_2 , the electrode was successively coated with DNA_1 -AuNPs and followed DNA_2 -AuNPs and cDNA solution at 37 °C. The resulted electrode was thoroughly washed and nitrogen flow dried. Finally, the modified electrode was immersed in 50 μM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ solution for 10 min and washed for differential pulse voltammetry (DPV) characterization.

Preparation of Cell Lysates and FBS Samples. Cells were cultured in DMEM medium supplemented with 10% FBS at 37 °C in a humid atmosphere containing 5% CO₂. Subsequently, cells were treated with 1 mL of lysis buffer for 30 min at 4 °C. After the above step, the cell lysate was then centrifuged at 12 000 rpm for 20 min and the resulting supernatants were stored at –20 °C before use. The analyses of PKA activity in complex biological samples were performed by diluting the cell lysates into desired volumes of PKA assay buffer (50 mM Tris-HCl, 20 mM MgCl₂, 100 μM ATP, pH 7.4). The PKA activity in fetal calf serum samples was analyzed after a 10 times dilution by PBS.

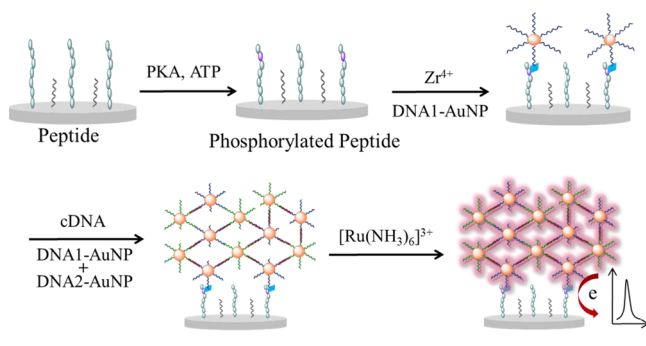
Electrochemical Measurements. All cyclic voltammetry and DPV experiments were carried on a conventional three-electrode system with a CHI802B electrochemical workstation (Chenhua Instrument Company of Shanghai, China) using the modified electrode as working electrode, and platinum wire and Ag/AgCl with saturated KCl solution were used as counter electrode and reference electrode, respectively. Electrochemical impedance spectroscopy (EIS) was conducted on SP-150 (Bio-Logic, France) in a solution containing 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] and 0.1 M KCl in the frequency range from 0.01 to 10⁵ Hz.

Apparatus and Characterization. UV–vis experiments were performed with a UV-3900 spectrophotometer (Hitachi, Japan). TEM images were obtained with a Hitachi model H-800 (Hitachi, Japan). Fourier transform infrared spectroscopy (FT-IR) spectra of the samples were recorded on a QUINX55 spectrometer (Brucher, Germany). Atomic force microscopy (AFM) with tapping mode characterization was conducted on a Nanoscope III (Digital Instrument) scanning probe microscope.

RESULTS AND DISCUSSION

DNA Assembled Gold Nanoparticles Polymeric Network Blocks Amplification Electrochemical Analysis of PKA Activity. Scheme 1 describes the configuration of

Scheme 1. Configuration of DNA-AuNPs Assembled Polymeric Network Amplified Electrochemical Biosensor for Kinase Activity Detection



electrochemical biosensor for kinase activity detection. The cysteine-terminated kemptides were assembled on the gold electrode through the Au–S bond. Then, hexanethiol was further assembled on the electrode to block blank binding sites. In the presence of PKA and ATP, the serine of substrate peptide was phosphorylated and linked with DNA₁-AuNPs by the multicoordinative interaction between Zr⁴⁺ and the intrinsic 5'-phosphate end of DNA₁.⁵ In the presence of cDNA, DNA₂-AuNPs and DNA₁-AuNPs were assembled to form a DNA-

AuNPs polymeric network block by the hybridization of cDNA with both DNA₁ and DNA₂ on each side sequence. Due to the excellent conductivity of gold nanoparticles, the network assembled electrode interface maintains good electron transfer ability compared with those polymeric DNA products. Moreover, such a conductive network block affords a large accommodation for [Ru(NH₃)₆]³⁺ ions adsorption by the electrostatic interaction because of the intrinsic negative charged properties of both the DNA backbone and AuNPs in the network, resulting in a significant signal amplification.

Characterization of the Biosensor. The surface coverage of kemptide on the electrode was calculated to be 3.2×10^{-11} mol cm⁻² by quantitatively characterizing the reduction desorption of sulfur atoms through cathodic stripping voltammetry. The phosphorylation of kemptide was confirmed by FT-IR spectra (Figure S2 in the Supporting Information). Besides the characteristic peaks of kemptide, two additional peaks at 995 and 1070 cm⁻¹ appeared and were attributed to the presence of phosphate groups catalyzed by PKA in the presence of ATP, indicating that phosphate groups were transferred to the serine residue of kemptide. Then, the Zr⁴⁺ ions were attached to the phosphorylated peptide. After careful washing with PBS solution, DNA₁-AuNPs was adsorbed onto the electrode by the stable interaction between Zr⁴⁺ and phosphate groups. Moreover, DNA-AuNPs network blocks were further formed on the electrode in the presence of cDNA by the hybridization of cDNA with both DNA₁ and DNA₂ on the gold nanoparticles. The formation of the DNA-AuNPs polymeric networks was also confirmed by AFM characterization (Figure S3 in the Supporting Information). It is clear that a few of the well-separated gold nanoparticles with a diameter of ca. 15 nm was dispersed on the phosphorylated surface of the electrode. As a comparison, a condensed aggregation of gold nanoparticle was observed in the presence of DNA₂-AuNPs, DNA₁-AuNPs, and cDNA. The gold nanoparticle polymeric network was formed by the hybridization of cDNA with both DNA₁ and DNA₂ on the gold nanoparticles, which provide a large accommodation for the adsorption of electroactive probes. The facts imply that the proposed strategy is feasible for the electrochemical signal amplification for kinase activity detection.

CV and EIS were also applied to characterize the assembly processes of the gold electrode using [Fe(CN)₆]^{4-/3-} as electroactive probes. As can be seen in Figure 1A, the bare gold electrode exhibited a couple of quasi-reversible redox peaks in curve a. After the modification of kemptide, the peak current decreased (curve b). This is ascribed to the electron inert feature of kemptide which partially blocks the electron transfer on the surface of the gold electrode. The peak current was further decreased after the phosphorylation by kinase (curve c), which was attributed to the electrostatic repulsion between the negative charged phosphate groups and electroactive probes. After successful treatment of Zr⁴⁺ and DNA₁-AuNPs, the electrode interface was further negatively charged due to the negatively charged DNA and citrate ligands on AuNPs. Thus, a decreased peak current was also observed (curve d) although there was excellent conductivity of the AuNPs. After the formation of gold polymeric network, the large negative charged DNA-AuNP network block appeared, which further inhibited the mass transfer of negative electroactive probes. As a result, a smaller peak current and wider redox peak gap arised (curve e). These results are well consistent with the phenomena in EIS. In the Niquist plots, the diameter of the

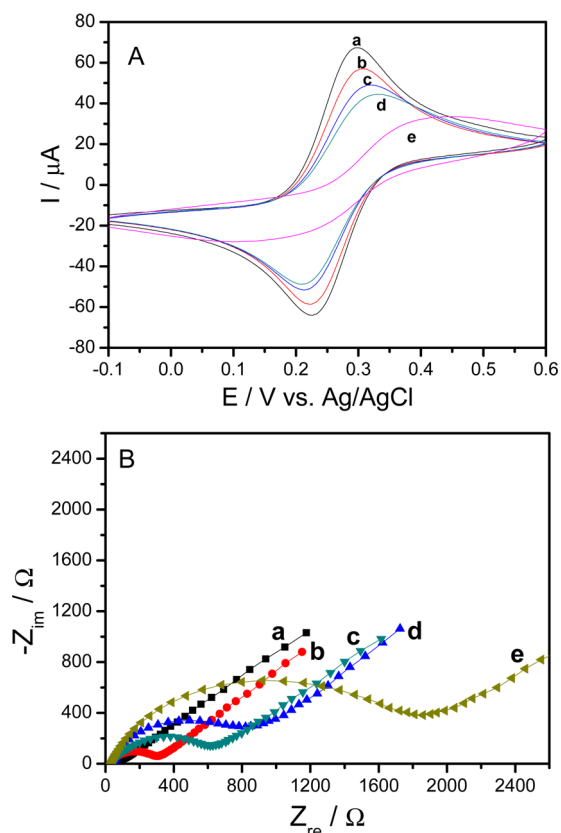


Figure 1. Cyclic voltammograms (A) and electrochemical impedance spectra (B) of bare gold electrode (a), kemptide modified gold electrode (b), phosphorylated kemptide modified electrode by PKA (c), Zr^{4+} mediated DNA₁-AuNPs assembled phosphorylated peptide modified electrode (d), and Zr^{4+} mediated DNA-AuNPs polymeric network assembled phosphorylated peptide modified electrode (e). These experiments were conducted in 0.1 M KCl solution with 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ electroactive probes. Scan rate is 100 mV/s. The frequency range is between 0.01 and 10^5 Hz.

semicircle is equal to the electron transfer resistance at the electrode interface. It was clear that the diameter of semicircles increased step by step with the assembly of kemptide, phosphorylation, and DNA₁-AuNPs, and it increased a lot after the assembly and formation of DNA-AuNPs network block. It indicates that the electron transfer resistance increased successively with sequential assembly of DNA-AuNP polymeric network probes.

DPV Behaviors of the Biosensor. Both DNA and gold nanoparticles are negative charged, and a large number of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ can be adsorbed by the DNA-AuNPs network block, so that the kinase activity can be detected on the basis of the redox response determined by $[\text{Ru}(\text{NH}_3)_6]^{3+}$. Figure 2 shows the DPV response of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ on the kemptide modified gold electrode before (curve a) and after (curve c) phosphorylation by PKA with the treatments of DNA-AuNPs network structure by Zr^{4+} . As it is shown, the peptide modified electrode before phosphorylation exhibits a very weak current signal. After the phosphorylation by kinase, a strong current signal at -0.2 V was observed due to the formation of DNA-AuNPs network block that adsorbs a large number of $[\text{Ru}(\text{NH}_3)_6]^{3+}$. To further confirm the signal amplification effect of the DNA-AuNPs polymeric network, the phosphorylated peptide modified electrode treated with DNA₁-AuNPs was also studied as shown in curve b. It is clear that the peak

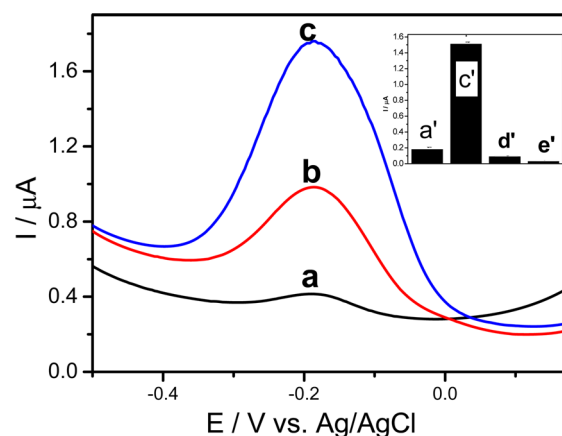


Figure 2. DPV responses of kemptide modified gold electrode before (a) and after (c) phosphorylation with the treatment of DNA-AuNPs network mediated by Zr^{4+} and the Zr^{4+} mediated DNA₁-AuNPs assembled phosphorylated peptide modified electrode (b). The inset shows the comparison of current intensities on kemptide modified electrode before (a') and after (c') phosphorylation with the treatment of DNA-AuNPs network mediated by Zr^{4+} ; the phosphorylated peptide modified electrode with DNA-AuNPs network block nanoprobe was measured in the absence of Zr^{4+} (d') and the peptide modified electrode without treatment (e'). DPV experiments were conducted in PBS solution after immersion in $50 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ for 10 min at a scan rate of 100 mV/s. The concentration of PKA is 25 U mL^{-1} .

current of the electrode just treated with DNA conjugated gold nanoparticle is much smaller than that of the DNA-AuNPs network block, which was similar to the AFM results, indicating that the DNA-AuNPs network block can significantly improve the current signal. In addition, a series of control experiments was also conducted, and the peak current responses were shown in the inset of Figure 2. Besides the peak current of column a' and c' that was obtained from curve a and curve c in Figure 2, respectively, peak current of the phosphorylated peptide modified electrode with DNA-AuNPs network block nanoprobe was measured in the absence of Zr^{4+} shown as column d'. A very small current signal was observed, indicating the important role of Zr^{4+} -mediated cross-linking in the sensor. Moreover, nearly no current signal was observed on the peptide modified electrode after the adsorption of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ions (column e' in the inset of Figure 2). The facts demonstrate that a such DNA-AuNPs network block signal amplification strategy can be used for sensitive electrochemical detection of kinase activity.

The temperature of enzyme catalyzed reaction and the concentration of ATP are critical parameters for the kinase-catalyzed reaction on the electrode surface and are optimized as shown in Figure S4 in the Supporting Information. It was observed that the largest current response was obtained at 37°C , so the temperature for the phosphorylation procedure by kinase was chosen as 37°C in these experiments. In addition, the current intensity of the peptide modified electrode increased with the increasing concentrations of ATP in the presence of PKA of 25 U mL^{-1} . The current signal reached a maximal value at the ATP concentration of $40 \mu\text{M}$ and maintained the intensity at higher concentration of ATP. Thus, the optimal concentration of ATP was $40 \mu\text{M}$.

Electrochemical Measurements of PKA Activity. The activity of protein kinase was evaluated under the same conditions with different concentrations of PKA. Figure 3

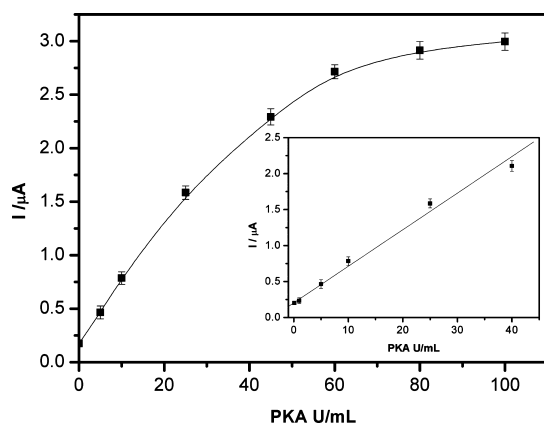


Figure 3. Current intensity as a function of PKA activities. The inset is the linear relationship between current intensity and the concentrations of PKA.

shows the current signal as a function of PKA activity in the range from 0 to 100 U mL⁻¹. The current signal increased accordingly with the increase of PKA concentration and reached a platform value after ca. 80 U mL⁻¹. A linear relationship between the current signal and the concentrations of PKA was obtained from 0.1 to 40 U mL⁻¹ and can be represented as $I = 0.2036 + 0.0508 \times c$ with the correlation coefficient of $R = 0.9959$, where I is the current intensity and c is the kinase activity. The detection limit of PKA was 0.03 U mL⁻¹ ($S/N = 3$), which was lower than that of previous reported electrochemical assays.^{4,4,5,45,46} The fact demonstrates that the proposed electrochemical method can be employed for highly sensitive kinase activity detection in a wide concentration range.

Repeatability of this biosensor assessed by assaying the same level of PKA activity of 25 U mL⁻¹ on five freshly prepared electrodes. The relative standard deviation is 4.96%. These results reveal that the as-designed ECL biosensor demonstrates acceptable reproducibility.

Kinase Activity Detection in Complex Biological Samples. To evaluate the as-designed biosensor in complex biological samples for clinic application, we applied this sensor in both FBS and cell lysates samples for kinase activity detection. The PKA in FBS samples was conducted by adding certain concentrations of PKA in ten times diluted FBS solutions, and the PKA activity was measured. Table S1, Supporting Information, shows the PKA activity in the FBS solution with three parallel measurements by using the as-designed electrochemical biosensor and comparing with reference values. The relative deviation between the electrochemical biosensor and reference value ranged from -2.17% to 1.6%, suggesting the excellent accuracy, and could be applied as a reliable technique for the kinase activity detection in serum samples.

Overexpression of protein kinase is generally associated with cell proliferation and neoplastic transformation. PKA is also served as a cancer biomarker for diagnostics and prognostics.^{47,48} In this work, three cell lysates from BE4S-2B normal cell line and two cancer cell lines of lung (A549) and breast (MCF-7) were measured as shown in Figure 4. It was observed that MCF-7 cell line expressed the highest level of PKA in the three cell lines, and the PKA expression levels of cancer cells are higher than normal cell (BE4S-2B). These experimental results confirm that the as-prepared electrochemical biosensor could

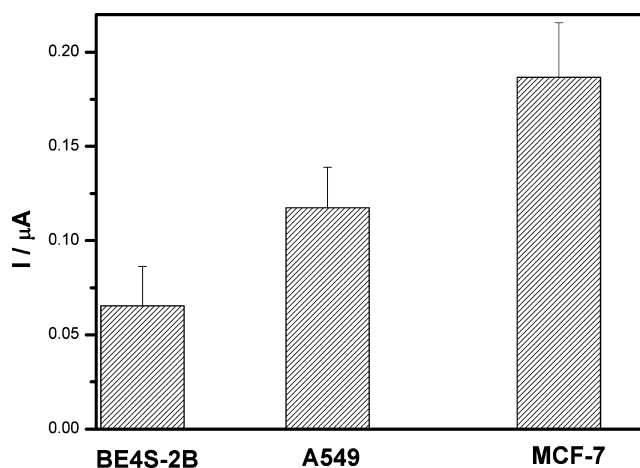


Figure 4. Detection of PKA activities from BE4S-2B, A549, and MCF-7 cell lysates. The signal intensities of cell lysates were subtracted by that of blank lysates.

be used to determine kinase activity in complex biological samples with high sensitivity.

Kinase Activity Inhibition Evaluation. The application and screening of kinase inhibitors for kinase activity regulation is important in disease diagnosis and clinical therapy. The DNA-AuNPs network block amplified electrochemical biosensor was also applied to quantitatively evaluate the inhibition of kinase in the presence of small molecule inhibitors. The kinase activities were measured with different concentrations of PKA inhibitors, and the half-maximal inhibition values (IC_{50}) were calculated. As shown in Figure 5a, the current responses

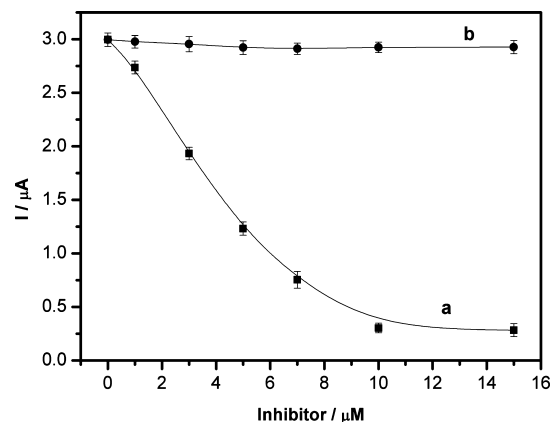


Figure 5. Relationship between DPV responses and concentrations of ellagic acid (a) and Tyrphostin AG 1478 (b). The concentrations of PKA and ATP are 100 U mL⁻¹ and 100 μM, respectively.

were recorded with different concentrations of ellagic acid which is a cell-permeable antioxidant with antimutagenic and anticarcinogenic characteristics. The current intensity decreased along with the increasing concentrations of ellagic acid and then reached a stable level when the concentrations of ellagic acid were over 10 μM. The IC_{50} was calculated to be 4.01 μM, which was compared with our previous result.⁴⁹ The PKA activity was also conducted in the presence of Tyrphostin AG 1478 which is a tyrosine kinase inhibitor but not a PKA inhibitor. As seen in Figure 4b, the current signal hardly had any changes even though the concentration of Tyrphostin AG 1478 was as high as 15 μM. These facts imply that the as-

designed DNA assembled DNA-AuNPs network amplified electrochemical biosensor has potential in quantitative kinase inhibitors screening.

CONCLUSION

In conclusion, a novel DNA assembled gold nanoparticles (AuNPs) polymeric network block signal amplification electrochemical biosensor has been developed for kinase activity and inhibition assay. The DNA-AuNPs polymeric network blocks present excellent electroactivity and high accommodation for $[\text{Ru}(\text{NH}_3)_6]^{3+}$ electrochemical oxidization and significantly amplified the amperometric signals, offering a highly sensitive electrochemical biosensor for kinase activity detection. This strategy provides a simple detection procedure and also avoids the environment sensitive DNA enzymic polymerization processes as well as the electron inert properties of DNA polymer. The as-designed electrochemical biosensor offers a highly sensitive strategy for PKA kinase activity monitoring even in the complex biological samples with a low detection limit of 0.03 U mL^{-1} , wide linear range, and good stability. Moreover, the biosensor also shows great potential in the application of an accurate and quantitative kinase inhibitor assay. The robust biosensor can also be ready for other kinase activities and inhibition assays, showing great promise in clinic diagnostics and drug discovery applications.

ASSOCIATED CONTENT

Supporting Information

Additional information (Table S1 and Figures S1–S4) as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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