Supporting Information

Enzymatic Biofuel Cell-Based Self-Powered Biosensing of Protein Kinase Activity and Inhibition via Thiophosphorylation-Mediated Interface Engineering

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Experimental section

Materials and reagents.

Cysteine-terminated peptide (CLRRASLG) was purchased from GenScript Biotechnology Co. Ltd. (Nanjing, China). Protein kinase A (PKA, catalytic subunit from bovine heart), adenosine 5′-[γ-thio] triphosphate tetra-lithium salt (ATP-s), nicotinamide adenine dinucleotide (NAD^+/NADH), PKA inhibitor H-89 dihydrochloride hydrate and 4, 4', 5, 5', 6, 6'-hexahydroxydiphenic acid 2, 6, 2', 6'-dilactone (ellagic acid), tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 6-mercapto-1-hexanol (MCH), were purchased from Sigma-Aldrich (St. Louis, MO.U.S.A.). Glucose dehydrogenase (GDH) was obtained by cell surface display according to our previous work.[1] The activity of the obtained GDH was 1.23 ± 0.03 U/mg cells. Carbon nanotube (CNT, purity > 99.9%, Electrical conductivity >100 S cm^{-1}) was purchased from Nanjing Ji Cang Nano Tech Co., LTD (Nanjing, China). β-D-Glucose was commercially available from Tokyo Chemical Industry Co. Ltd. (Japan). AuNPs were prepared according to the literature by adding a sodium citrate solution to a boiling $HAuCl_4$ solution.[2] Other chemicals were of analytical grade and were used as received without further purification. Ultrapure water (resistivity >18.2 MΩ cm at 25°C) obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, U.S.A.) was used throughout the experiments.

Apparatus and instrumentation.

Transmission electron microscopy (TEM) images were recorded on a HT7700 microscope (Hitachi, Japan) operated at 100 kV. Cyclic voltammetric (CV), and differential pulse

voltammetric (DPV) measurements were carried out on a CHI 660E electrochemical workstation (Shanghai, China) employing a three-electrode system: a fabricated bioanode or biocathode, an Ag/AgCl electrode and a platinum wire as the working electrode, the reference electrode and the counter electrode, respectively. Electrochemical impedance spectroscopy (EIS) was carried on an Autolab PGSTAT 302N electrochemical analyzer (Metrohm Autolab, The Netherlands) within a frequency range of 0.1 Hz to 100 kHz and with 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ used as the probe. The E^{OCV} of EBFC was measured on a CHI 660E electrochemical workstation by connecting the bioanode and the biocathode placed in the electrolytic cell.

Synthesis of SiO_2@AuNPs.

The preparation of SiO_2@AuNPs was according to our previous work[3], with slight modifications. Briefly, 1.8 mL of TEOS was mixed with 15 mL ethanol and stirred evenly, and then the above solution was added to the mixture of 1.0 mL of $NH_3 \cdot H_2O$ (28%), 10 mL of ethanol, and 5 mL of water. Subsequently, the mixture was stirred at room temperature for 2 h to obtain SiO_2 spheres. Excess TEOS and ammonia were removed by centrifugation (10 000 rpm, 15 min), and the obtained precipitate was washed with ultrapure water and ethanol. Subsequently, 0.4 mL of APTES was added dropwise to the obtained SiO_2 sphere ethanol solution and stirred at room temperature for 4 h. Then the solution was centrifuged and washed at least four times with ethanol to remove excess reactants. Subsequently, 500 μL of 10 nM carboxy group modified AuNPs and 1 mg mL^{-1} EDC/NHS were incubated with 100 μL of 1 mg mL^{-1} amino-functionalized SiO_2 sphere suspension overnight at room temperature. Surplus AuNPs were separated by centrifugation (7000 rpm, 10 min), and the precipitate was SiO_2@AuNPs.

Preparation of the Biocathode.

30 μL of the as-prepared AuNPs (50 nM) was dropped on the surface of the indium tin oxide (ITO) electrode (0.5 × 0.5 cm) and dried at 37°C. Then the substrate electrode was immediately coated with 30 μL of 11.4 μM peptide solution and kept at room temperature for 12 h to achieve the self-assembly of peptide on the AuNPs-modified electrode through the Au-S bond. Afterwards, the peptide-modified electrode was thoroughly rinsed with phosphate buffer (PB) (10 mM, pH 7.4) to remove excess peptide, and then 20 μL of 1 mM MCH for 30 min was coated to block the nonspecific binding sites on the electrode, followed by rinsing with PB. Subsequently, PKA-catalyzed peptide thiophosphorylation was performed by incubating the peptide/AuNPs/ITO into 30 μL of assay buffer pH 7.4 50 mM Tris-HCl containing 20 mM $MgCl_2$, a desired amount of PKA, and ATP-s at 37°C for 1 h. In the inhibitor assay, different desired concentrations of inhibitors were also introduced in the assay buffer, and the procedures were similar as the above process. Finally, 30 μL of the obtained SiO_2@AuNPs was coated on the modified electrode at room temperature for 30 min, followed by rinsing with PB. The SiO_2@AuNPs-thiophosphorylated peptide/AuNPs-modified biocathode was obtained and stored at 4°C when not in use.

Preparation of the GDH/CNT Bioanode.

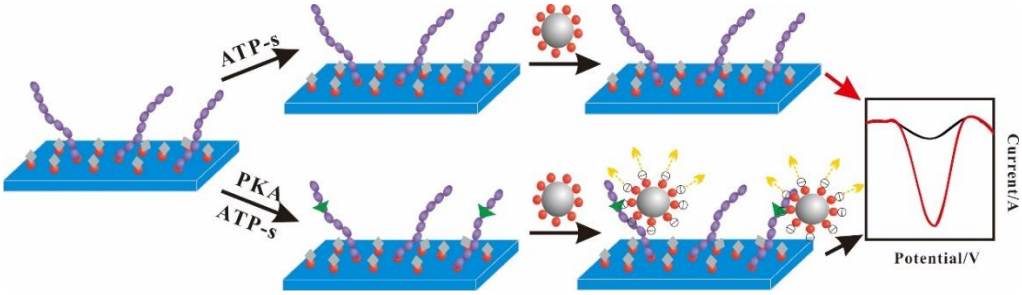
50 μL of 1 mg mL^{-1} CNT was coated on the surface of carbon paper (CP) electrode (0.5 × 0.5 cm) and dried at 37°C. Subsequently, the CNTs/CP electrode was immersed into a solution containing 1 mg mL^{-1} EDC and 1 mg mL^{-1} NHS for 30 min to activate the carboxyl group of CNT. After being rinsed with ultrapure water to eliminate excess EDC and NHS, the activated electrode was incubated with 50 μL of GDH solution at 37°C for 30 min to obtain the GDH/CNT bioanode.

Construction of EBFC-Based Self-Powered Biosensing Platform.

The GDH/CNT bioanode for the glucose oxidation occurred in anodic chamber, in which, the anolyte was 15 mL of 10 mM PB (pH 7.4) containing 8 mM glucose and 2 mM NAD^+. Simultaneously, the SiO_2@AuNPs-phosphorylated peptide/AuNPs/ITO biocathode for the $[Fe(CN)_6]^{3-}$ reduction occurred in cathodic chamber. 15 mL of 10 mM $K_3[Fe(CN)_6]$ dissolved in pH 7.4 10 mM PB was selected as the catholyte. The two chambers were separated with a Nafion membrane (DuPont™ Nafion® PFSA NRE-211). The open circuit voltage of EBFC was tested by connecting the bioanode and biocathode placed in the electrolytic cell. Polarization curves of the EBFC were measured by linear sweep voltammetry starting from the open-circuit value at a scan rate of 10 mV s^{-1}. The relationship of power output and current was calculated based on the polarization curve by the formula of $P=UI$. All measurements were carried out at 25 °C.

Cell Culture and Cell Lysis

Human breast adenocarcinoma cell line (MCF-7) and human lung carcinoma cells (A549) were seeded in DMEM (Dulbecco's Modified Eagle Medium, Gibco, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 μg mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin in 5% CO_2 37°C incubator. The cells were collected in the exponential phase of growth and washed three times with phosphate buffered saline. Then the cells were lysed referring to the manufacturer's recommended protocol, and the extracted supernatants were stored in freezing tubes at -20 °C and used in the following experiments.



Scheme S1. Schematic illustration of the assembly of the biocathode of EBFC.

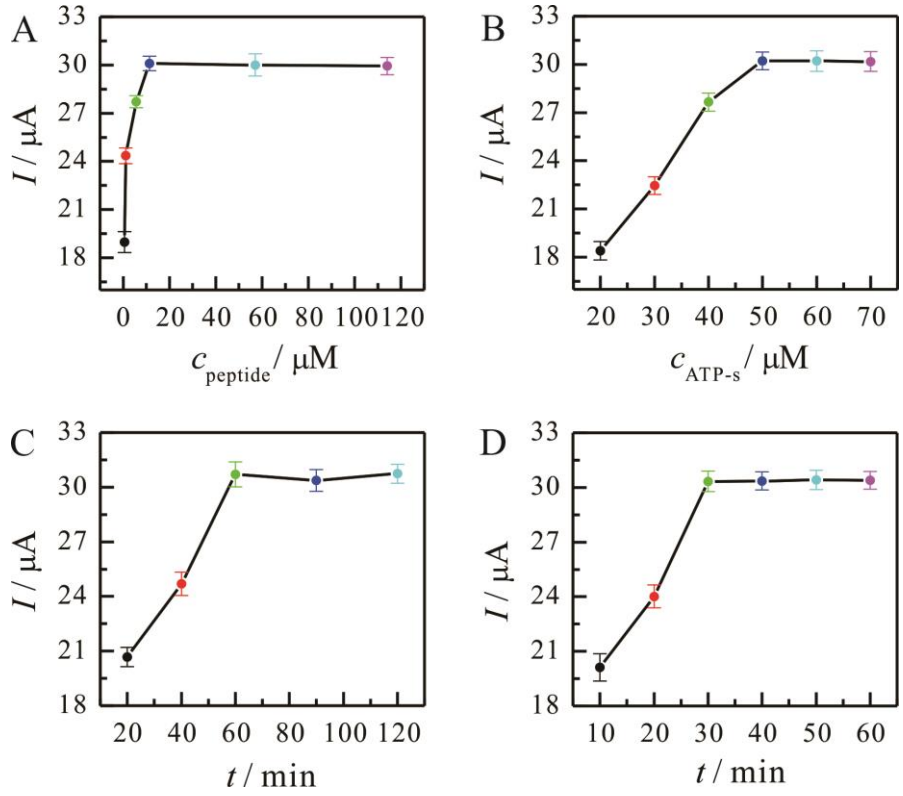


Fig. S1. Effect of (A) peptide concentration, (B) ATP-s concentration, (C) the thiophosphorylation time, and (D) the incubation time between SiO_2@AuNPs and modified electrode. All experiments were carried out in the presence of 20 U mL^{-1} PKA in PB buffer (10 mM, pH 7.4) containing 10 mM $[Fe(CN)_6]^{3-}$.

To achieve the best performance of EBFC-based self-powered biosensing platform, the concentration of peptide and ATP-s, the thiophosphorylation time, and the incubation time of SiO_2@AuNPs composite at the interface of the electrode were optimized. From Fig. S1A, with the peptide concentrations increased, the reduction current of $[Fe(CN)_6]^{3-}$ continually enhanced until reached a plateau when the concentration was higher than 11.4 μM, suggesting excessive peptide would be washed off once sufficient peptide were completely bound to AuNPs on the electrode surface. Therefore, 11.4 μM of peptide was used. In addition, ATP-s could provide the thiophosphate groups during the thiophosphorylation process., the variation trend was similar to that of peptide concentration, and with the increase of ATP-s concentration in the presence of 20 U mL^{-1} PKA, the current intensity also increased until the appearance of plateau (Fig. S1B). Hence, the optimal concentration of ATP-s could be regarded as 50 μM. Afterwards, the thiophosphorylation time is also a critical parameter for the PKA-catalyzed reaction on the surface

of electrode. As shown in Fig. S1C, the current intensity almost kept unchanged after 1 h, indicating the thiophosphorylation process of peptide completed. Hence, 1 h was preferable in the reaction system. Likewise, 30 min was adequate for the incubation time between SiO_2@AuNPs and the modified electrode (Fig. S1D).

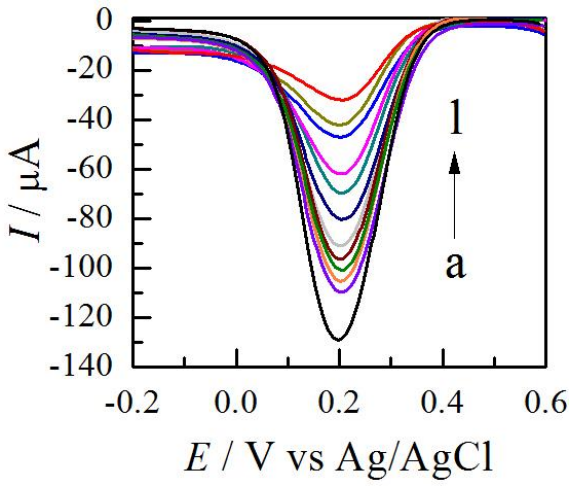


Fig. S2. DPV signal of the biocathode with different PKA concentrations (a-l: 0, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 20 U mL^{-1}).

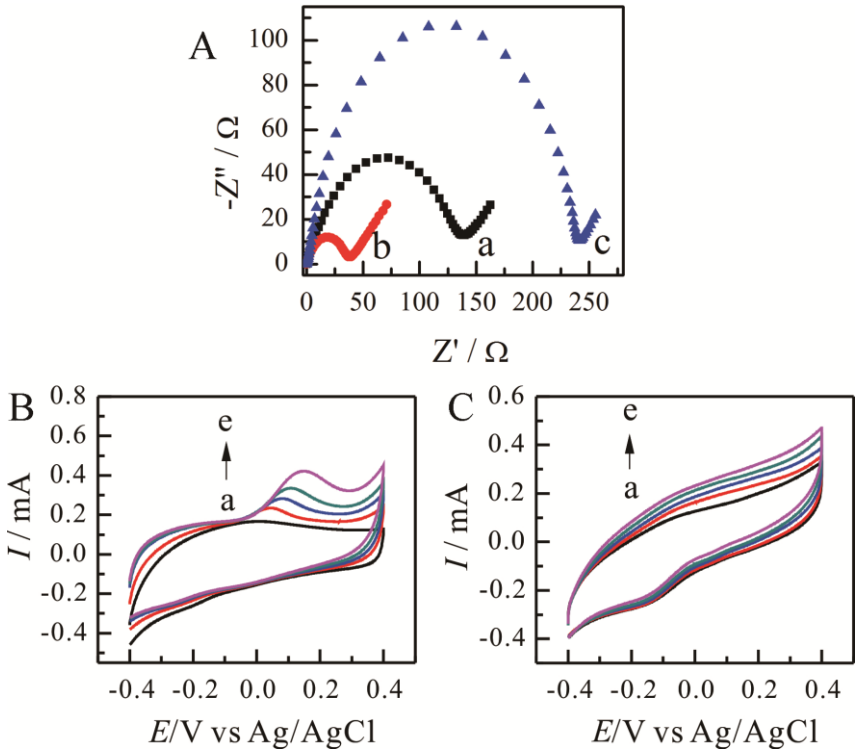


Fig. S3. (A) EIS of the bare CP electrode (a), the CNT/CP electrode (b), and the GDH/CNT/CP bioanode (c). (B) CVs of GDH/CNT bioanode in the absence (a) and the presence of 2 mM (b), 4 mM (c), 6 mM (d), and 8 mM (e) NADH in PB (10 mM, pH 7.4). (C) CVs of the GDH/CNT bioanode in PB (10 mM, pH 7.4) containing 2 mM NAD^+, in the absence (a) and presence of 2 mM (b), 4 mM (c), 6 mM (d), and 8 mM (e) glucose. υ = 50 mV s^{-1}.

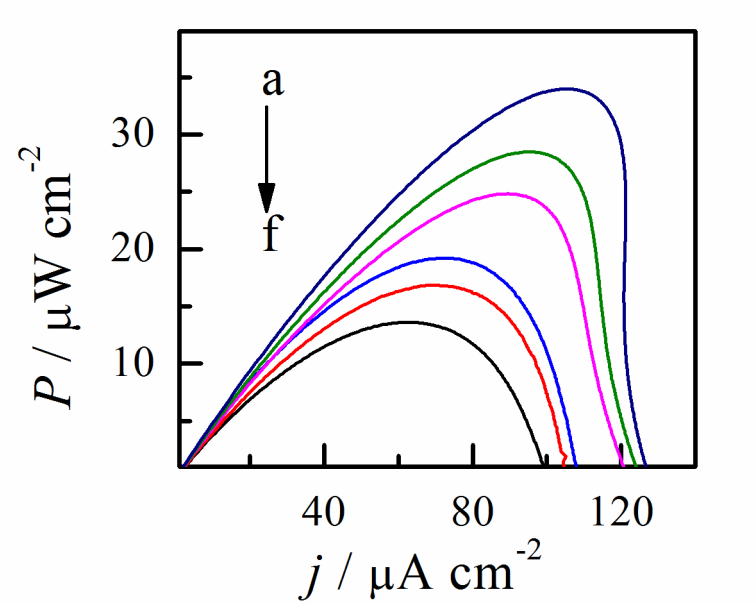


Fig. S4. Power output curves of the self-powered biosensor in the presence of different PKA concentrations (a→f: 0.1, 0.5, 1, 5, 10, 20 U mL^{-1}).

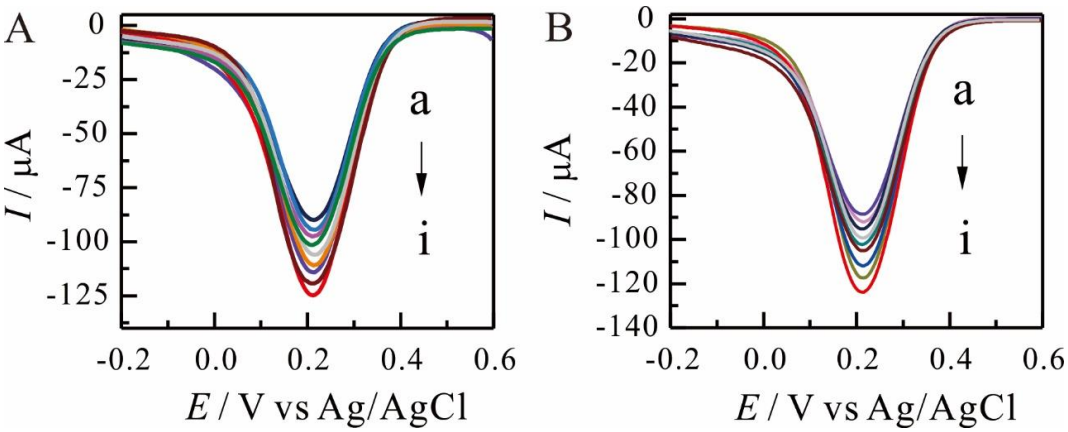


Fig. S5. DPV of the biocathode in the presence of different concentrations of ellagic acid (A) and H-89 (B) (a-i: 0.01, 0.05, 0.1, 0.5, 1, 3, 5, 7, 10 μM). The concentrations of PKA and ATP-s are 20 U mL^{-1} and 50 μM, respectively.

Table S1. Comparison of analytical performance for PKA assay by our method and those reported in literature

Method	Strategy	LOD ($U\ mL^{-1}$)	Dynamic range ($U\ mL^{-1}$)	Ref.
Self-powered Biosensor	Phosphorylation-mediated interface engineering	0.00022	0.0005-20	This work
PEC[a]	Signal amplification strategy triggered by PAMAM dendrimer and alkaline phosphatase	0.048	0.100-100	4
PEC	Localized surface plasmon resonance enhancement and dye sensitization	0.005	0.008-1	5
PEC	Phosphorylated graphite-like carbon nitride	0.077	0.050-50	6
ECL[b]	Graphene quantum dots and luminol	0.005	0.010-10	7
Electrochemistry	Gold nanoparticles-carbon nanospheres, phos-tag-biotin and β-galactosidase	0.014	0.050-100	8
Electrochemistry	Phos-tag and enzymatic signal amplification	0.150	0.500-25	9
Electrochemistry	DNA induced AuNPs polymeric network block signal amplification	0.030	0.030-40	10
Electrochemistry	Homogenous	0.100	5-50	11
Electrochemistry	Ferric ions coordinated to phosphorylated sites as electrocatalysts	0.030	0.100-25	12
Fluorescence	Metal Nanoclusters	0.100	0.400-3	13
Fluorescence	Green synthesis of peptide-templated gold Nanoclusters	0.004	0.010-40	14

[a] Photoelectrochemistry; [b] electrochemiluminescence

Table S2 Measurement of PKA in cell lysates used the as-proposed self-powered biosensing method

Cell lysates	PKA Concentration (U/mL)			Recovery (%)
	Detected	Added	Found	
MCF-7	0.019	0.02	0.042	107.7
		0.04	0.061	103.4
A549	0.013	0.02	0.031	93.9
		0.04	0.052	98.1

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