Electronic Supplementary Information

A Highly Sensitive Photoelectrochemical $VEGF_{165}$ Biosensor with dual signal amplification strategy by using $AgVO_3$ as photoactive material

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Experimental section

Chemicals and materials

Ammonium metavanadate (NH_4VO_3) was purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Silver nitrate ($AgNO_3$), Ascorbic acid (AA) and Poly acrylic acid (PAA) were supplied by Chengdu Kelong Chemical Inc. (Chengdu, China). 6-mercaptohexanol (MCH), Gold chloride ($HAuCl_4$), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) were acquired from Sigma-Aldrich (St. Louis, MO, USA). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were supplied by Beijing Chemical Reagent Co. (Beijing, China). Tris-hydroxymethylamino methane-hydrochloride (Tris) was supplied by Shanghai Roche Pharmaceutical Ltd. (Shanghai, China). Exonuclease III (Exo III) and $VEGF_{165}$ was supplied by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China).

1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl and 140 mM NaCl were used to prepare the solution 20 mM Tris-HCl solution (pH 7.4). potassium ferricyanide and potassium ferrocyanide were dissolved in PBS solution to prepare $[Fe(CN)_6]^{3-/4-}$ solution (pH 7.4, 5.0 mM) .

The oligonucleotides sequences were supplied by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences were as follows:

HP1: 5'-CCA CAC CAA CCT CTT CGT TTC TTC CTG GGG GAG TAT TGC GGA GGA AGG-3'

HP2: 5'-CAA CCT CTT CGT AGA GAG GTG TTT C CG AAG AGG TTG GTG TGG-3'

HP3: 5'-SH-CAT ACT CGA GCA GTC TAG AAA CAC CTC TCT ACG AAG AGG TTG TAG ACT GC -3'

S2 : 5'-GGG TAG GGC GGG TTG GGT TCA TGC AAC ATC TAG GAC TGC TCG AGT ATG-3'

S3: 5'-GGG TAG GGC GGG TTG GGT CTA GAT GTT GCA TGA CAT ACT CGA GCA GTC-3'

S4: 5'-ACC CAA CCC GCC CTA CCC-NH_2

Apparatus

The PEC tests were measured with a PEC workstation (Ivium, Netherlands). Electrochemical impedance spectroscopy (EIS) was measured with a CHI 660e electrochemistry workstation (Shanghai Chenhua Instrument, China). The morphologies of the prepared nanomaterials were characterized by scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and transmission electron microscope (TEM, JEM-2100, Japan). A three-electrode system consist of a platinum wire, an Saturated Calomel Electrode (SCE, saturated KCl) and a glassy carbon electrode (GCE, Φ = 4 mm), which is the reference electrodeas, the counter electrode and the working electrode successively.

Synthesis of $AgVO_3$

20 mL $AgNO_3$ aqueous solution (5 mmol/L) was mixed with 500 μL PAA (25 %), after stirring until a uniform suspension obtained, 20 mL NH_4VO_3 solution (5 mmol/L)

were then added dropwise into the as-prepared suspension under the condition of continuous stirring. The pH of the suspension was adjusted to 7.0 by ammonia solution. The suspension was ultrasonic for 40 min until the $AgVO_3$ crystallized.

Exonuclease III-aided target recycling

The mixture including 2 μL of 1 μM HP1, 2 μL of 1 μM HP2, 0.5 μL 20 units/L Exo III, and 20 μL $VEGF_{165}$ of different concentrations was incubated for 2 h at 37 ℃. All buffer was Tris-HCl solution (pH 7.4). Then the mixture was heated at 80 ℃ for 10 min to deactivate Exo III.[1] The obtained S1 solution was prepared for the following fabrication process of electrode.

Preparation of the PEC biosensor for $VEGF_{165}$ assay

Primarily, the bare GCE was polished with 0.3 μm alumina slurry and sonicated using ultrapure water to get mirror-like surface. Then were coated with Au NPs by electrotrolytic deposition under the potential of -0.2 V for 30 s. As show in Scheme 1, the electrodes were incubated with 10 μL of 1 μM HP3 at 4 °C for 12 h and blocked with 20 μL of 0.1 mM MCH at room temperature for 1 h. The solution of S1 coming from Exonuclease III-aided target recycling (20 μL) was then modified the electrodes at room temperature for 2 h to hybridize with HP3. Subsequently, the electrodes were incubated with 10 μL of 1 μM S2 and 10 μL of 1 μM S3 to trigger the HCR reaction at room temperature for 2 h. Subsequently, 10 μL of 0.1 M $AgVO_3$ was activated by 5 μL of 10 mM EDC and 20 mM NHS to crosslink with the amino group of S4 (10 μL, 1 μM) at room temperature for 1 h to obtain S4 modified $AgVO_3$ ($AgVO_3$-S4). Finally, 20 μL $AgVO_3$-S4 were immobilized onto the electrodes by hybridization of

S4 with the toehold strand at room temperature for 2 h. All buffer was Tris-HCl solution (pH 7.4).

PEC measurement

The PEC measurement was carried out under optimal experimental conditions of 5 mL 5.0 mM PBS solution (PH=7.0) containing 0.71 M electron donor AA, the light-emitting diode(LED) lights source acted as excitation light source with switching off-on-off for 10–20-10 s under the potential of 0.0 V. The excitation wavelength for PEC analysis was 460 nm.

Morphology characterizations of $AgVO_3$

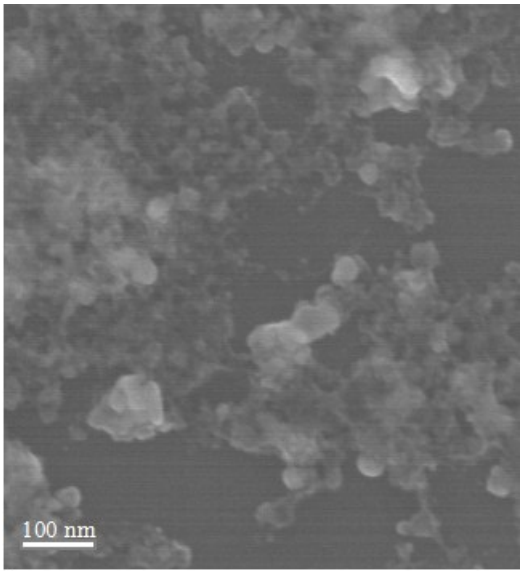

Fig. S1 SEM image of AgVO3 without PAA.

Characteristiction of the PEC biosensor

Photoelectrochemical (PEC) and electrochemical impedance spectroscopy (EIS) were used to survey stepwise electron transfer on the electrode. As shown in Fig. S2, the bare glassy carbon electrode (GCE) exhibited no photocurrent response (curve a). When Au NPs, HP3, MCH, S1, S2 and S3 were gradually modified on the electrode,

there was still no photocurrent signal. Finally, the PEC signal obtained after $AgVO_3$-S4 was immobilized on the surface of electrode by hybridization of S4 with the toehold strand (curve b). Fig. S2 shows the impedance spectrum of the electrode and its semicircle diameter equals electron transferresistance (R_{et}). When Au NPs was modified by electrodeposition on the electrode, the semicircle diameter was decreased due to the electrical conductivity of Au NPs (curve b). After the electrodes were modified with HP3, MCH, S1, S2 and S3, the semicircle diameter was successively increased because of the hindrance of electron transfer (curve c, d, e and f). Ultimately, when $AgVO_3$ was modified on the electrode, the decreased transfer resistance was observed because of the effectively promotion of electron transfer by $AgVO_3$ (curve g).

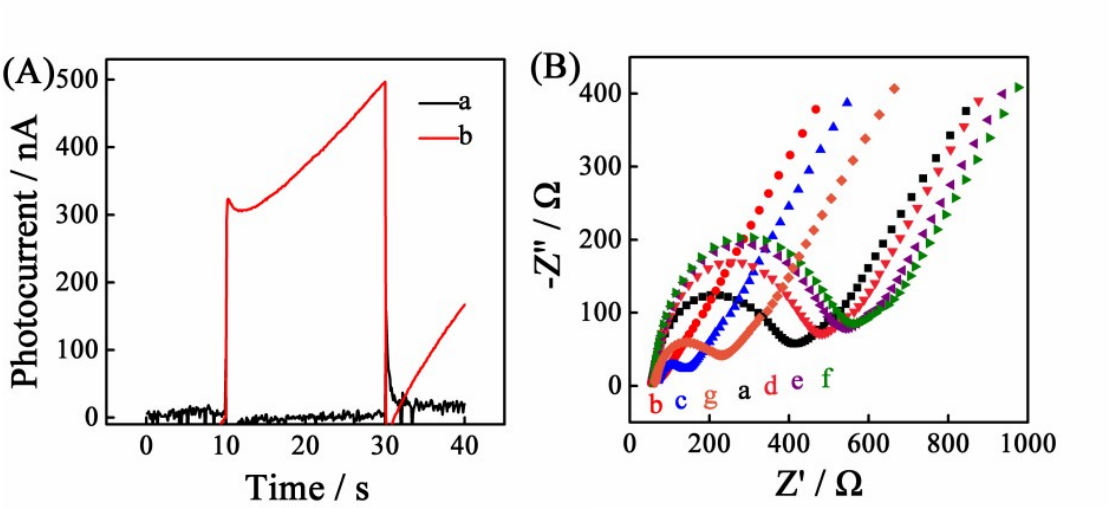

Fig. S2. PEC response (A) and EIS response (B) of (a) bare GCE, (b) Au NPs/GCE, (c)HP3/AuNPs/GCE, (d) MCH/HP3/AuNPs/GCE, (e) S1/MCH/HP3/AuNPs/GCE, (f) S2-S3/S1/MCH/HP3/AuNPs/GCE, (g) $AgVO_3$-S4/S2-S3/S1/MCH/HP3/AuNPs/GCE.

Optimization of AA concentration for the PEC biosensor

AA was used as an electron donor to improve the intensity of the photocurrent. Consequently, the effect of AA concentration was investigated. As shown in Figure S3, the photocurrent signal increased constantly with the increasing of AA concentration until 0.71 M. Therefore, 0.71 M was confirmed as the optimal concentration of AA.

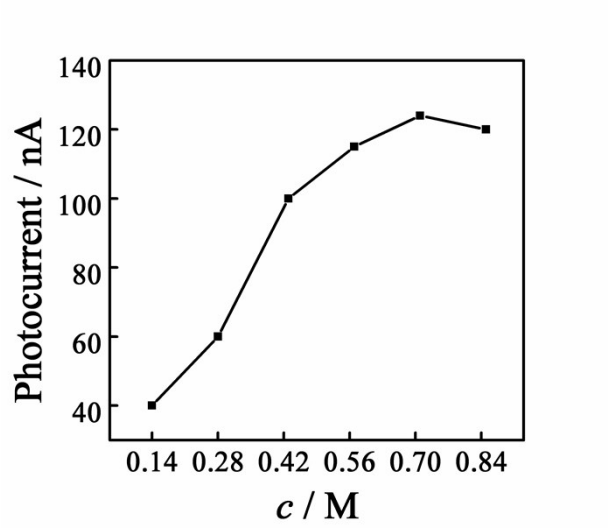

Fig. S3 Influences of AA concentration.

The selectivity of the biosensor for VEGF$_{165}$

The selectivity of this PEC aptasensor was evaluated by employing several interfering substances, which the concentration of interfering substances are 10 fold than the target VEGF$_{165}$. The experiments were performed by using 10 nM target VEGF$_{165}$ and 100 nM interferences of 20 μL for the preparation of the PEC biosensor, respectively. Despite the concentration of interfering substances are 10 fold than the target VEGF$_{165}$, obvious photocurrent increase was observed for 10 nM VEGF$_{165}$ (ΔI=320 nA). The corresponding photocurrent of 100 nM VEGF$_{121}$, PDGF-BB, PSA, β-Mg and lgG were negligible, indicating that the biosensor exhibits high specificity

for $VEGF_{165}$.

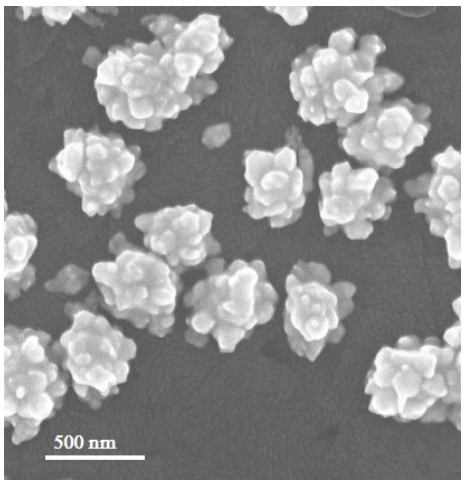

Fig. S4 The SEM image of Au NPs

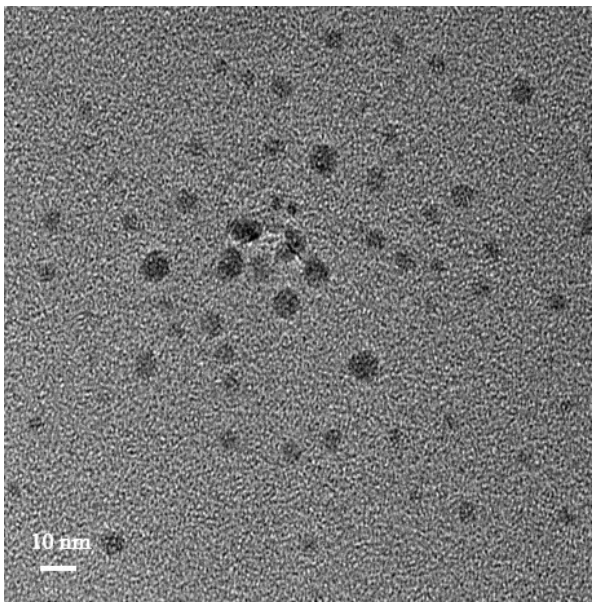

Fig. S5 The TEM image of $AgVO_3$-S4 particles

References

1 Y. W. Zhang, J. H. Liu, G. Wua, and W. Chen, *Nanoscale*, 2012, 4, 5300–5303.