Electronic Supplementary Material (ESI) for ChemComm.

Supplementary material

Controllable signal molecule release from Au NPs-gated MSN for photocathodic detection of ultralow level AβO

Xuechen Zhang, Jinling Zhang, Yao Gao, Jianyue Yan, Wenbo Song *

College of Chemistry, Jilin University, Changchun 130012, PR China

*Corresponding Author: Email: wbsong@jlu.edu.cn

Tel：+86-431-85168352

Materials and Reagents

Indium tin oxide (ITO) glass was obtained from Wuhan Ge-Ao Ltd. TE buffer solution (10 mM, pH 7.4), tumor necrosis factor-alpha (TNF-α), lysozyme (Lys), insulin (Ins), α-synuclein (α-Syn) were purchased from Shanghai Sango Biotech Co., Ltd. Amyloid-beta peptide (Aβ) was purchased from Nanjing GenScript Biotech Co., Ltd. 3-aminopropyl triethoxysilane (APTES) were purchased from Aladdin Reagents (Shanghai) Co., Ltd. Other reagents were purchased from Sinopharm Chemical Reagents Co., Ltd. All of the reagents were used as received without further purification.

The oligonucleotides were synthesized by Sangon Biotech. Co., Ltd. with the following sequences: AβO-targeted aptamer, 5′-COOH-GCC TGT GTT GGG GCG GGT GCG-C_6-SH. Human serum purchased from Beijing Solarbio Science & Technology Co., Ltd was selected to demonstrate the possibility of practice application of the developed aptasensor. TE buffer solution (10 mM, pH 7.4) was employed to prepare the DNA solution. 10 mM PBS (pH 7.4) was used for the hybridization of DNA. And ultrapure water (18.2 MΩ) was used throughout the experiments for preparing all aqueous solutions. All solutions were immediately prepared before each experiment and stored at 4°C until use.

Apparatus

Morphology and microstructure were characterized by a field emission scanning electron microscope (FESEM; S-4800) and transmission electron microscope (TEM; JEM-2100F). The crystal structure of the samples was determined using a powder X-

ray diffractometer (XRD; shimadzu XRD-6000) with Cu Kα radiation. X-ray photoelectron spectroscopic (XPS) measurements were carried out on an ESCALAB 250 spectrometer with a mono X-Ray source Al Kα excitation (1486.6 eV) and C 1s peak (284. 6 eV) as the reference. UV-vis diffuse reflectance spectra (DRS) were obtained on an UV-3600 Shimadzu spectrophotometer equipped with an integrated sphere attachment. UV-vis absorption spectra were collected on the UV-1800 spectrophotometer (Shimadzu, Japan). Nitrogen adsorption-desorption isotherms were obtained on ASAP 2020 Plus HD88 (micromeritics instrument, United States). The zeta potential was measured using a zeta potential analyzer (zeta potential, Zetasizer Nano-ZS, Malvern, England). The Infrared spectra (IR) were recorded with a Nicolet Impact 410 FTIR infrared instrument using the KBr pellet technique. The PEC measurements were performed in a conventional three-electrode cell, with the as-prepared electrode as the working electrode, a Pt wire as the counter electrode and a silver chloride electrode as the reference electrode. The photocurrent measurements were conducted with a CHI 760E electrochemical workstation at a bias voltage of 0 V. A 500 W Xenon lamp with a 420 nm cutoff filter was used as a light source. The incident light intensity was 100 mW cm^{-2}. PEC detection was carried out in 10 mL Na_2SO_4 (0.5 M). Different concentrations of AβO were added into the 50-fold diluted human serum sample for the PEC detection to assess the practical application. Electrochemical impedance spectroscopic (EIS) analysis was performed in a 0.1 M KCl solution containing 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ mixture over the frequency range from 10 mHz to 100 KHz and applying perturbation amplitude of 5 mV.

Experimental section

Fabrication of MoS_2 QDs/Cu NWs photocathode. Typically, 5 mL 0.1M $Cu(NO_3)_2$ aqueous solution was mixed with 100 mL15 M NaOH aqueous solution in a 250 mL glass flask under sufficient stirring. 660 μL EDA and 100 μL 32 wt% $N_2H_4 \cdot H_2O$ were then added to the mixture. After stirring for 3 min, the flask was placed in an electronic oven at 80℃ for 40 min. The obtained Cu NWs were washed with 3 wt% $N_2H_4 \cdot H_2O$, ultrapure water and ethanol quickly, then vacuum-dried at 60℃ overnight. The N-acetyl-L-cysteine-capped MoS_2 QDs were prepared via a facile one-pot hydrothermal approach according to our previous report. The MoS_2 QDs/Cu NWs was obtained by a facile electrostatic self-assembly method. In brief, 40 mg Cu NWs was dispersed in 15 mL deaerated ultrapure water, followed by addition of 15 mg MoS_2 QDs (mass fraction of 27%) into the mixture. After sonication for 0.5 h and stirring for 2 h, the product was washed and vacuum-dried at 60°C for 12 h. After that, 20 μL MoS_2 QDs/Cu NWs suspension (2mg/mL) was deposited on the clean ITO surface (0.25 cm^2) and dried under 40°C.

Synthesis of Au NPs-Apt. In brief, 5 mL of 1 wt% sodium citrate was added into 90 mL ultrapure water and heated until reflux under vigorously stirring. Then 5 mL $HAuCl_4$ solution (0.2 wt%) was placed into the above solution and consistently stirred and heated for 1 h. This reaction was then cooled down to indoor temperature. After that, 100 μL PEG-2000 (100 μM) was dispersed in 2 mL Au NPs, followed by centrifuging at 10000 rpm to remove the residual PEG-2000. The resulting precipitate was dissolved into 1 mL aptamer (0.5 μM) for 12 h to obtain Au NPs-Apt.

Synthesis of Amino-Functionalized MSN. First, 0.28 g NaOH and 1.0 g CTAB were dispersed into 480 mL ultrapure water with persistent stirring for 20 min at room temperature. Following that, 5.0 g TEOS was injected into the above solution and heated to 80°C with consistent stirring for another 2 h. After cooling down, the white precipitate by centrifugation was washed with ultrapure water and methanol several times and dried under vacuum. To completely remove the surfactant template (CTAB), 1.0 g MSN was added into a mixture containing 1.0 mL HCl (37.2%) and 100 mL methanol, followed by heating under reflux for 6 h. The resulting substance was washed with water and methanol several times and then dried at 60°C. Then, 25 mg MSN was dispersed into 1 mL ethanol, then 0.8 mL APTES was mixed with the above suspension solution under vigorously stirring for 6 h. After that, the resulting substance was filtered and washed with ethanol and water for three times respectively and baked at 60°C.

Thionine loading and Au NPs Gated on MSN. 3 mg thionine (Th) and 10 mg NH_2-MSN were co-dropped into 1 mL ultrapure water and incubated for 12 h at indoor temperature. Subsequently, the precipitation was centrifuged and washed with ultrapure water. The resulting material was re-dispersed into 1 mL PBS (0.1 M) and then mixed with 50 μL Au NPs-Apt. After incubated at 4°C for 12 h, the mixture was rinsed with PBS several times. Ultimately, the resulting material was re-dispersed into 1mL PBS and stored at 4°C for further use.

Synthesis of water-soluble AβO. Briefly, Aβ lyophilized powder was dissolved in hexafluoroisopropanol (1mg/mL) and incubated overnight at room temperature. After

removing solvent by a gentle stream of nitrogen gas, Aβ monomer (AβM) was re-dispersed in dimethyl sulfoxide and stored at -20°C for further use. Aβ oligomer (AβO) was obtained by incubating AβM in darkness at 37°C for 12 h. The concentration of Aβ was calculated by measuring the absorbance at 276 nm with UV-vis spectroscopy[1], and the extinction coefficient was 1410 M^{-1} cm^{-1}. The calculated concentrations of AβO was consistent with that of AβM.

Determination of AβO. 12 μL AβO solution was added into 20 μL of Th-MSN@Au NPs-Apt. After incubation at 37°C for 1 h, the resulting solution was centrifuged at 8000 rpm and the supernatant was obtained. During this process, MSN and the released Au NPs were both precipitated and separated from Thionine. After that, 20 μL supernatant was incubated on the MoS_2 QDs/Cu NWs/ITO electrode at 37°C for 25 min. The resulting electrode (employed as working electrode) was subjected to PEC measurements at 0V in 0.5 M Na_2SO_4.

Determination of sample. Different concentration of AβO (0.5 pM and 5 pM) was added into 50-fold diluted human serum sample. 12 μL sample was then added into 20 μL of Th-MSN@Au NPs-Apt. The above solution was incubated at 37°C for 1 h and centrifuged at 8000 rpm. After that, 20 μL supernatant was incubated on the MoS_2 QDs/Cu NWs/ITO electrode at 37°C for 25 min. The resulting electrode was subjected to PEC measurements.

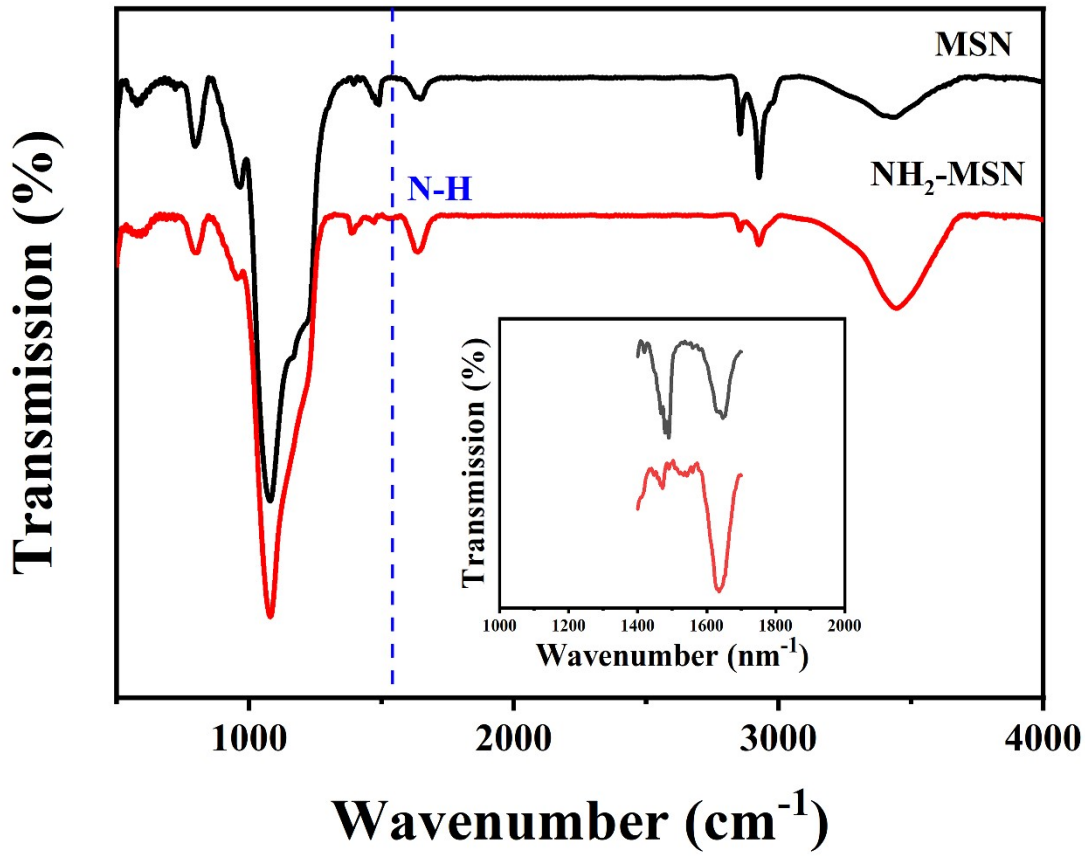

Fig. S1. FT-IR spectra of MSN and NH_2-MSN.

The FTIR spectra of amino-functionalized MSN exhibits a peak of N-H bending at 1530 cm^{-1}, demonstrating the successful preparation of amino functionalized MSN.

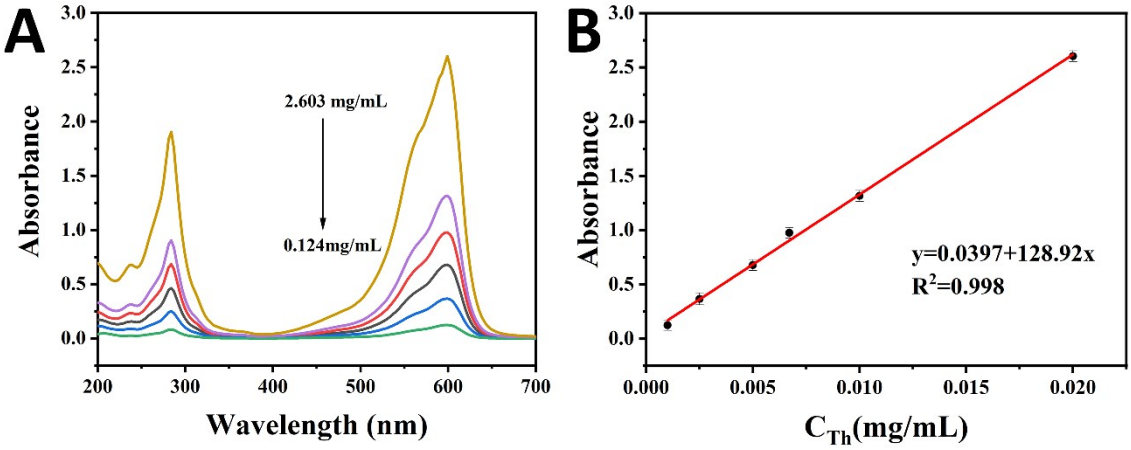

Fig. S2. (A) UV-vis absorption spectra to variable concentration of Th: 0.124mg/mL, 0.367 mg/mL, 0.679 mg/mL, 0.976 mg/mL, 1.316 mg/mL, 2.603 mg/mL and (B) calibration curve.

The mass of Th loaded in MSN was obtained by UV-vis absorption spectra. Initially, different concentrations of Th were configured and measured to obtain the corresponding UV-vis absorption response and calibration curve of concentration (the peak at 600nm was used for calculation). And the linear regression equation is y = 0.0397+128.92 C_{Th} (mg/mL) (R^2=0.998). Subsequently, Th released from 10 mg NH_2-MSN were dissolved in 40 mL water for UV-vis absorption spectra measurement. The corresponding UV-vis absorption response is 0.449. According to the linear regression equation, the mass of Th is 0.127 mg, and the mass fraction of Th in MSN is 1.27%.

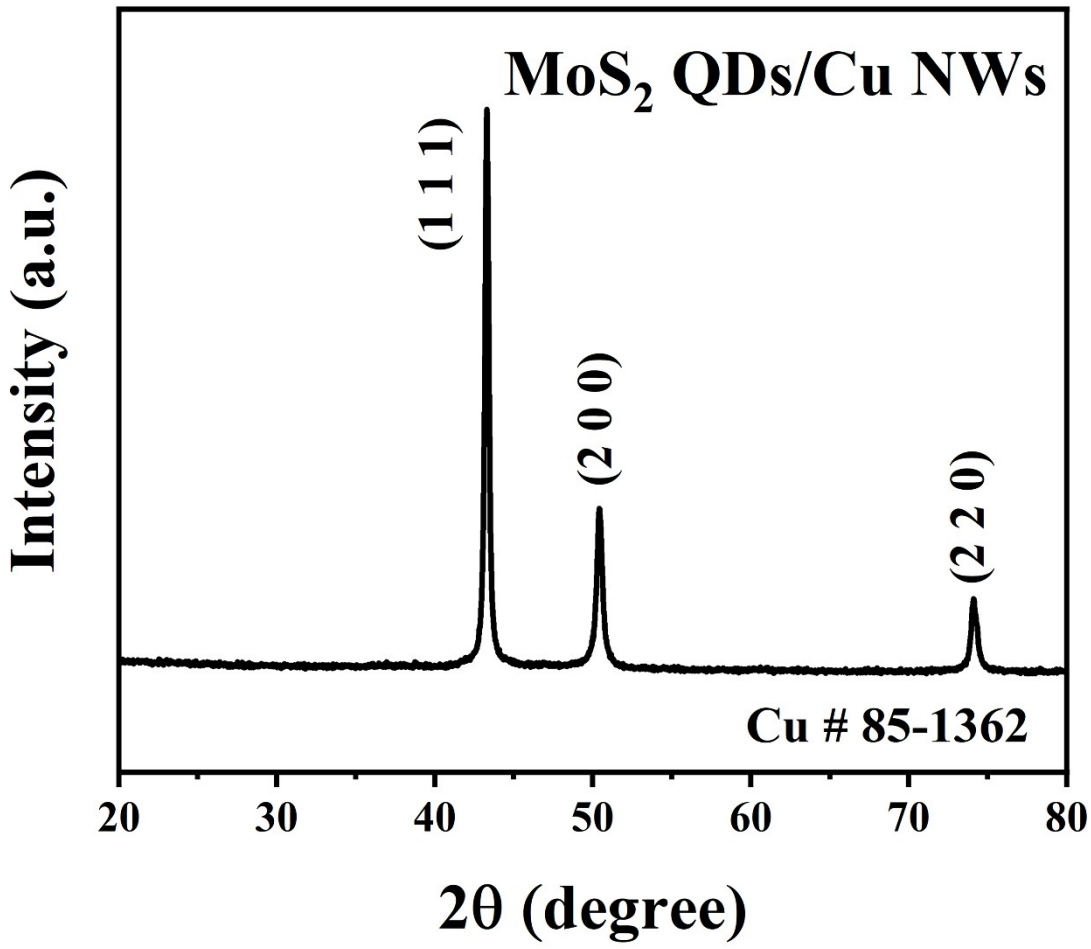

Fig. S3. XRD of MoS_2 QDs/Cu NWs.

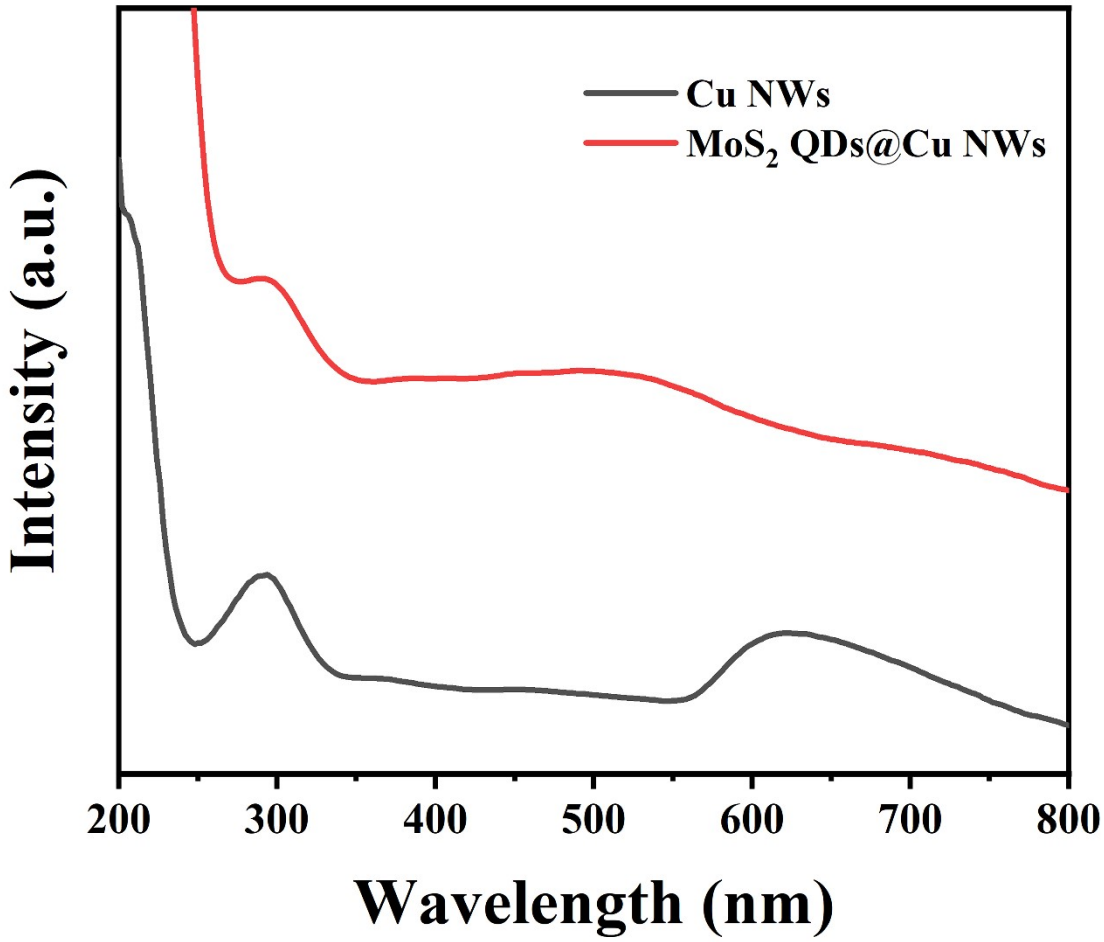

Fig. S4. UV-vis diffuse reflectance spectra of Cu NWs and MoS_2 QDs/Cu NWs.

The optical absorption property exhibited by UV-vis diffuse reflectance spectrum can validate the successful preparation of MoS_2 QDs/Cu NWs photoactive material. Two characteristic absorption peaks of Cu NWs at 292 nm and 623 nm can be observed (black curve in Fig. S4). After assembly with MoS_2 QDs, the two peaks have a blue-shift. Meanwhile, a substantially intensive absorption covering the range of 200 nm to 800 nm is exhibited, owing to strong light absorption property of the composite.

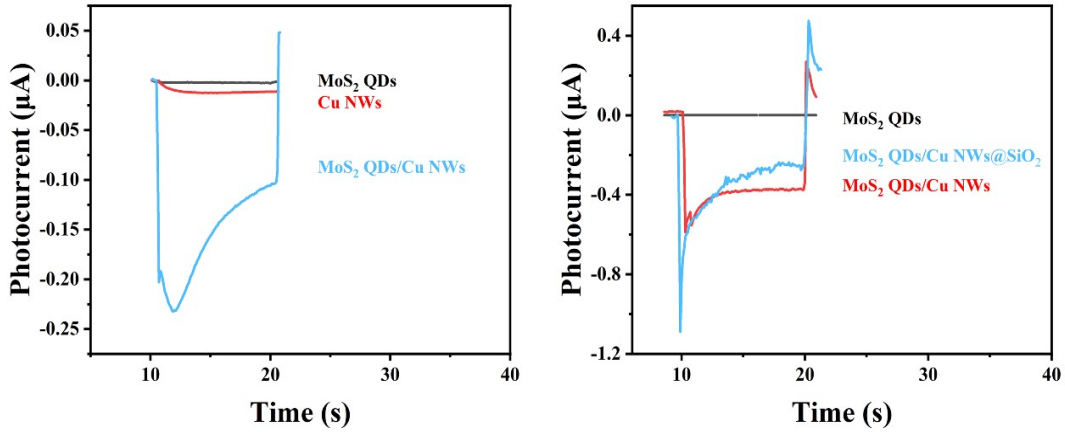

Fig. S5. (A) The photocurrent of MoS_2 QDs, Cu NWs and MoS_2 QDs/Cu NWs (under the illumination of 500 W Xenon lamp with a 550 nm cutoff filter). (B) The photocurrent of MoS_2 QDs, MoS_2 QDs/Cu NWs@SiO_2 and MoS_2 QDs/Cu NWs (under the illumination of 500 W Xenon lamp with a 420 nm cutoff filter).

Detailed calculation of LUMO/HOMO of Th

The LUMO and HOMO of Th are obtained according to the following equations:

(1) $E_g = 1240/\lambda$

(2) $LUMO = -e\ (\varphi_{red} + 4.71)$ (eV)

(3) $HOMO = LUMO - E_g$

(4) Evs NHE= −4.5 – Evs VACUUM

The absorption edge of Th is 629 nm (Fig. S6A), and the onset reduction potentials (φ_{red}) of Th is −0.07 V (Fig. S6B). Therefore, the LUMO and HOMO of Th are 0.14 V and 2.05 V.

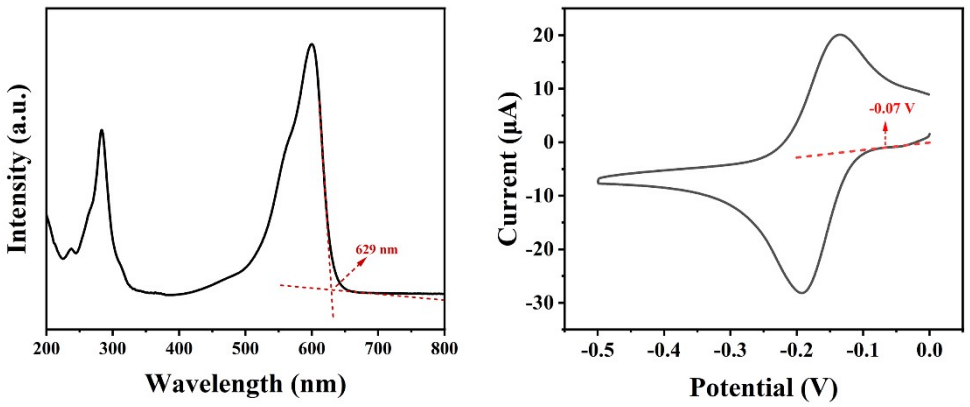

Fig. S6. (A) UV-vis absorption spectrum of Th. (B) Cyclic voltammogram during the process of Th electrochemical reduction.

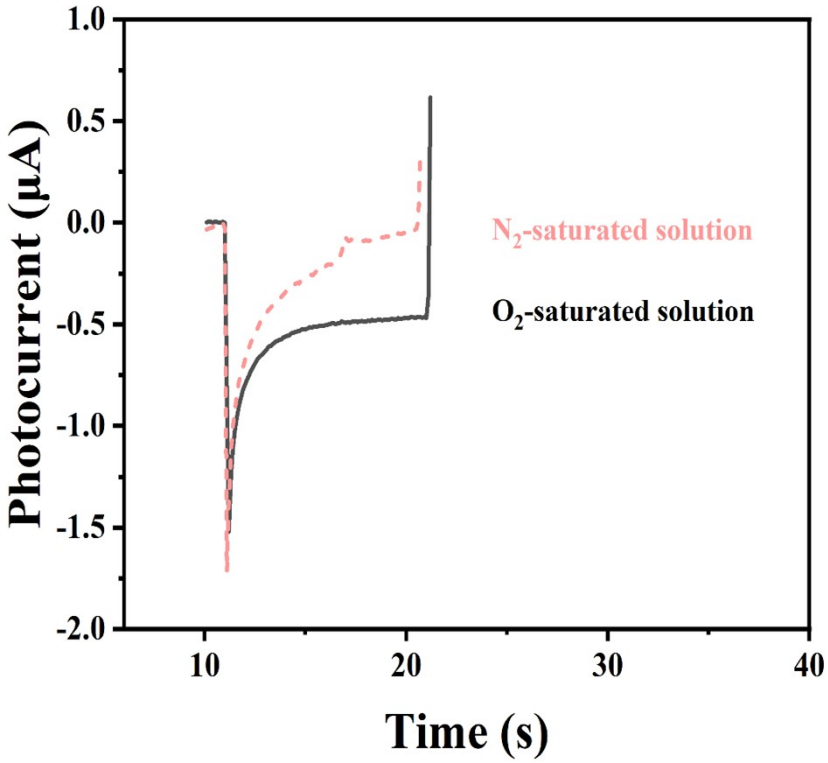

Fig. S7. Photocurrent response of Th/MoS_2 QDs/Cu NWs in nitrogen-saturated solution and oxygen-saturated solution.

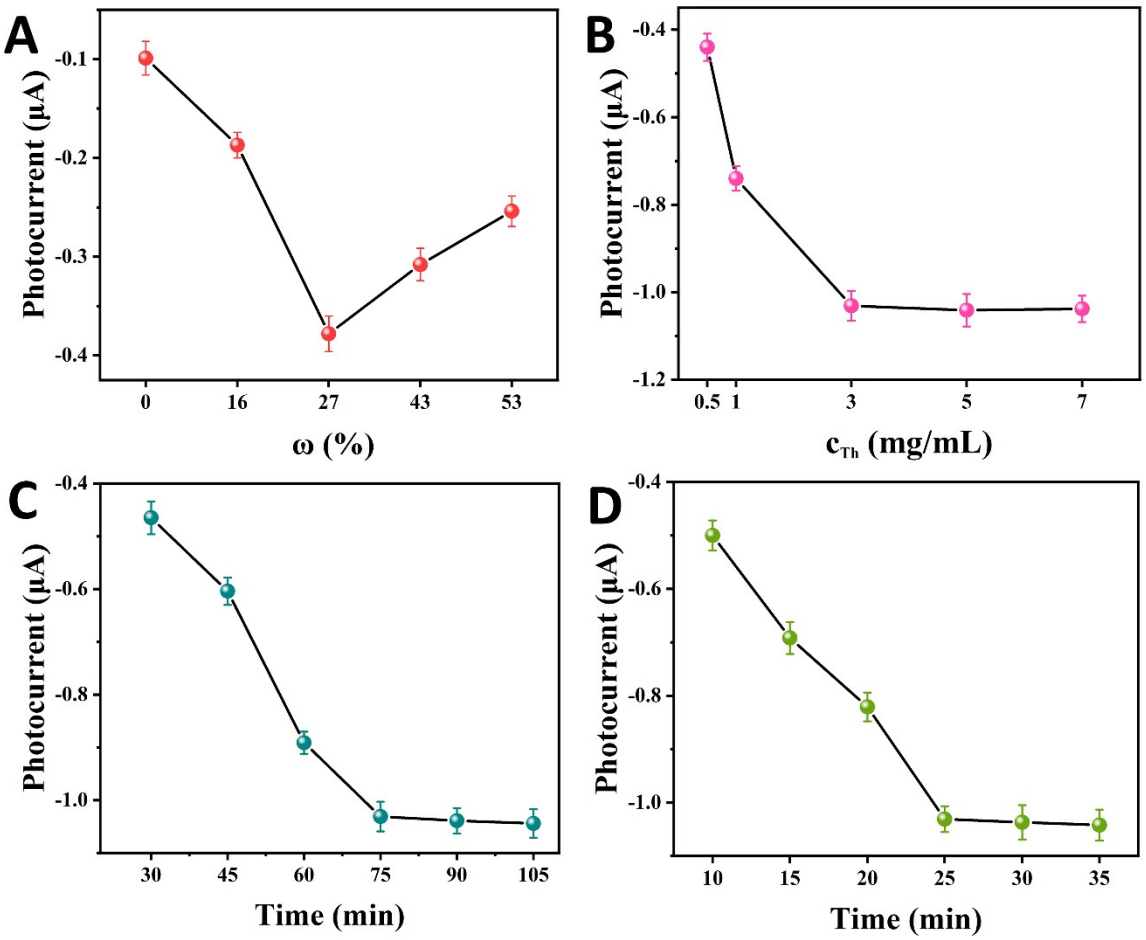

Fig. S8. Optimization of (A) the amount of MoS_2 QDs in the composite, (B) concentration of Th, (C) incubation time of AβO and (D) incubation time of Th.

For the purpose of achieving an optimal sensing performance of AβO detection, the experimental conditions are optimized. The ratio of MoS_2 QDs to Cu NWs in the photoelectric material may have a great impact on the photo-generated charge separation, transportation and recombination. The influence of the amount of MoS_2 QDs in the composite on the photocurrent response is studied. With the increase of the mass fraction of MoS_2 QDs from 0% to 27%, the photocurrent increases persistently and reaches the highest level. Then the photocurrent begins to decline (Fig. S8A). The amount of Th loading in the MSN can also affect the performance of PEC biosensor. A small amount of encapsulated Th signaling molecule is

disadvantageous to signal amplification, which induces low sensitivity of biosensor. Therefore, the Th saturation encapsulation in MSN is investigated by loading various concentration of Th and measuring the corresponding photocurrent. As shown in Fig. S8B, the photocurrent gradually enhances with the increase of Th concentration from 0.5 mg/mL to 3 mg/mL. After that, the photocurrent signal remains constant, indicating the saturated loading of Th in NH_2-MSN. The gatekeeper Au NPs-Apt is switched on via the specific recognition reaction between aptamer and AβO. The incubation time of AβO directly determines the efficiency of Th release, which affects the PEC response of the proposed sensor. Fig. S8C presents the optimization result of the incubation time of AβO. The photocurrent increases at prolonging the incubation time from 30 min to 75 min, which does not further increase after 75 min incubation. The incubation time of Th determines whether Th can closely bind on the photocathode surface. Fig. S8D displays the effect of incubation time of Th on the photocurrent. As one can see, with the increase of the incubation time from 10 min to 25 min, the value of the photocurrent increases significantly. When the incubation time surpasses 25 min, the photocurrent almost reaches a plateau. In conclusion, the optimal conditions for constructing PEC sensing platform, the amount of MoS_2 QDs in the composite, Th concentration in MSN, the incubation time of AβO and Th, are 27%, 3 mg/mL, 75 min and 25 min, respectively.

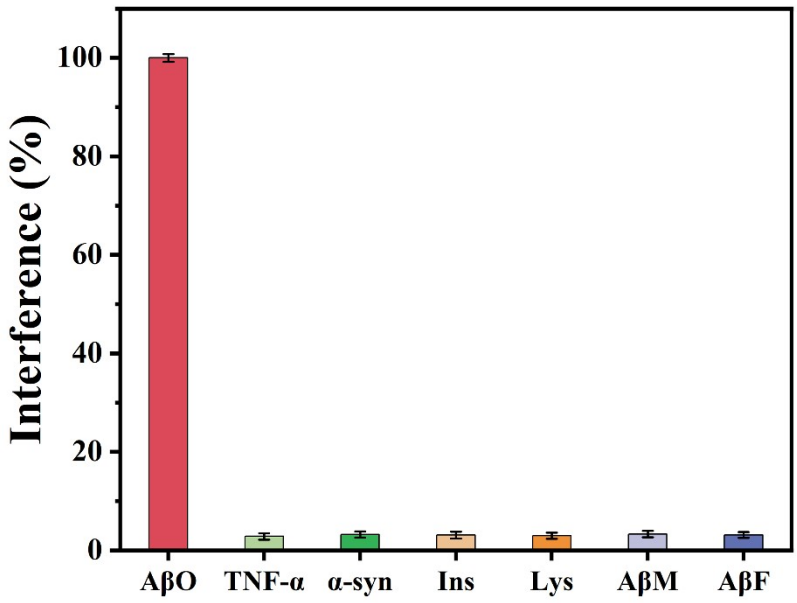

Fig. S9. Selectivity of the biosensor.

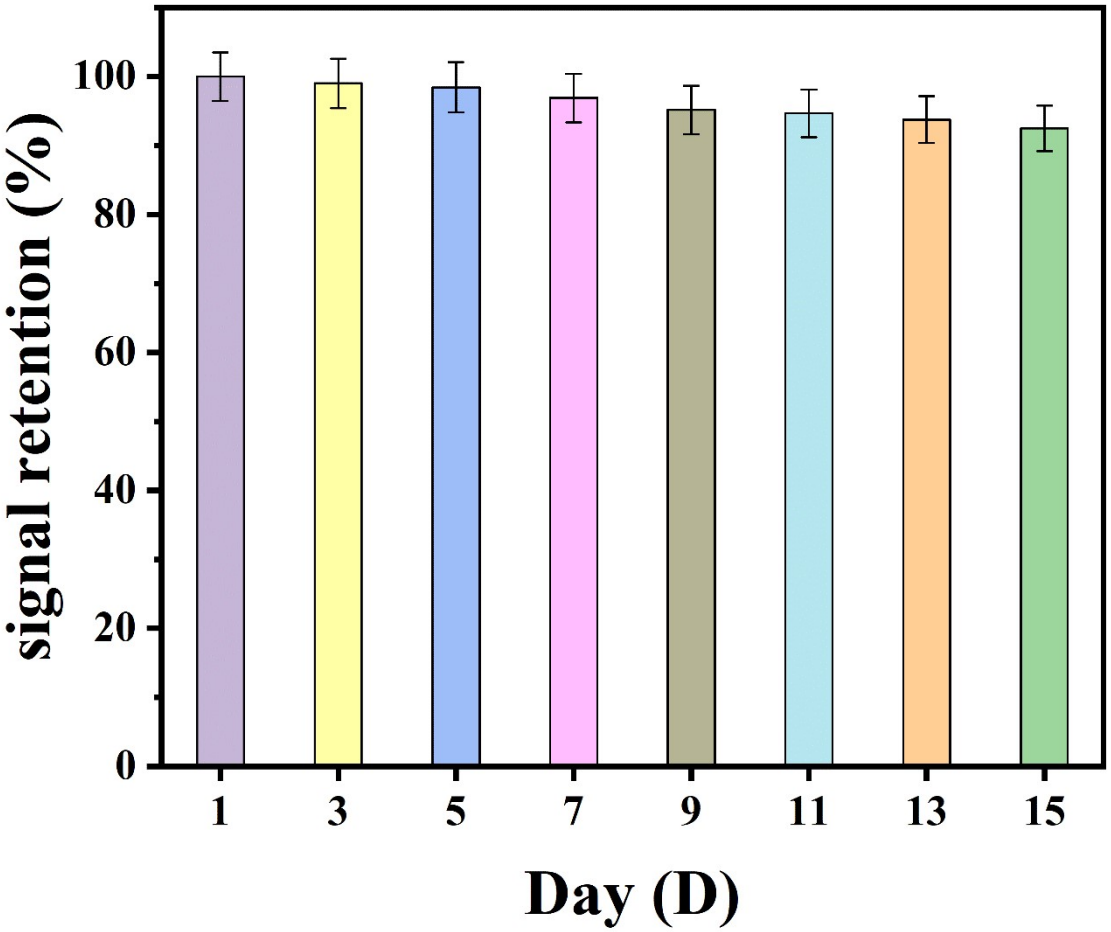

Fig. S10. Long-term storage stability of the proposed sensor.

Table S1. Performance comparison of the proposed PEC aptasensor with other reported methods.

No	Materials	method	Linear range	LOD	Ref.
1	3D DNA walker	FL	0.1 to 1 nM	22.3 pM	2
2	Au NP/MOF	ECL	0.1 pM to 10 pM,	71 fM	3
3	AptFc@SA-gold	EC	0.100 nM to 1.00 μM	93.0 pM	4
4	Au NPs	SPR	0.5 pM to 10 pM	0.2 pM	5
5	AuNPs	colorimetric	1 nM to 0.5 μM	0.5 nM	6
6	MoS_2 QDs/Cu NWs	PEC	5 fM to 0.5 μM	2.1 fM	**This work**

Table S2. Determination of AβO in human serum samples.

Sample	Spiked (pM)	Found (pM)	Recovery (%)	RSD (%)
serum 1	0.500	0.517	103.40	3.19
	5.000	4.906	98.12	3.04
serum 2	0.500	0.520	104.00	3.23
	5.000	5.198	103.96	3.31

References

1 J. Zhang, X. Zhang, Y. Gao, J. Yan and W. Song, *Biosens. Bioelectron.*, 2021, **176**, 112945.

2 Y. Yin, G. Chen, L. Gong, K. Ge, W. Pan, N. Li, J. O. achwa Machuki, Y. Yu, D. Geng, H. Dong and F. Gao, *Anal. Chem.*, 2020, **92**, 9247–9256.

3 L. Yin, Y. Wang, R. Tan, H. Li and Y. Tu, *Microchim. Acta*, **2021**,188:53.

4 C. Deng, H. Liu, S. Si, X. Zhu, Q. Tu, Y. Jin and J. Xiang, *Microchim. Acta*, 2020, **187**, 1–8.

5 Y. Zheng, X. Geng, X. Yang, S. Li, Y. Liu, X. Liu, Q. Wang, K. Wang, R. Jia and Y. Xu, *Anal. Chem.*, 2020, **92**, 2853–2858.

6 C. Deng, H. Liu, M. Zhang, H. Deng, C. Lei, L. Shen, B. Jiao, Q. Tu, Y. Jin, L. Xiang, W. Deng, Y. Xie and J. Xiang, *Anal. Chem.*, 2018, **90**, 1710–1717.