A dual-inhibition aptamer gated OPECT biosensor based on MOFs-derived CAU-17/Bi_2S_3 Z-scheme heterojunction for rapid detection of bacterial quorum sensing signal molecules

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1. Materials and reagents

Methanol (CH_3OH), anhydrous ethanol (CH_3CH_2OH), N, N-dimethylformamide (DMF), sodium sulfide (Na_2S) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bismuth nitrate ($Bi(NO_3)_3 \cdot 5H_2O$), mercaptohexanol (MCH), sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), Phenol, were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4$), thioglycolic acid (TGA), trimesic acid (H_3BTC), ascorbic acid-2-phosphate (AAP), and perfluorinated resin (Nafion) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Phosphate buffered saline (PBS), ascorbic acid (AA), 1-ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Alkaline phosphatase (ALP) was purchased from Beijing Coolaber Science & Technology Co., Ltd. (Beijing, China). FTO was purchased from Zhuhai Kaivo Optoelectronic Technology Co., Ltd. (Zhuhai, China). N-(3-Oxodecanoyl)-L-homoserine lactone (3-O-C10-HL), N-hexanoyl-L-homoserine lactone (C6-HL) and N-octanoyl-L-homoserine lactone (C8-HL) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). Aptamers and single-stranded DNA (ssDNA) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All solutions were prepared with Milli-Q water (Millipore, USA). Natural seawater was obtained from Shilaoren beach, Qingdao, China.

The sequences of aptamers and ssDNA were listed as follows.

A1:

5'-NH_2-$(CH_2)_6$

TGGACCTTGCGATTGACAGCAGACATGAGTCTCAGGACATGGACCTTGCGATTGACAGCAGACATGAGTCTCAGGAC-3'.

A2:

5'-NH_2-$(CH_2)_6$

TGGACCTTGCGATTGACAGCGGAAAGCGCATAGGTGCACTAGGTTCTGAAGACCA TAGACGCAGACATGAGTCTCAGGAC-3'.

A3:

5'-NH_2-$(CH_2)_6$

TGGACCTTGCGATTGACAGCATGGCATGAGAGCATAAGGTACATGGGTTGAGACT ATCTCGCAGACATGAGTCTCAGGAC-3'.

A4:

5'-NH_2-(CH_2)6

TGGACCTTGCGATTGACAGCATGGAAACCGGGAGGTACATAGATAGTCCGAGTTC AGGCCGCAGACATGAGTCTCAGGAC-3'.

The DNA sequence of ssDNA was listed as follows.

5'-SH-$(CH_2)_6$

GTCCTGAGACTCATGTCTGCTGTCAATCGCAATTCATGCAGAAGTAGACTCATGTC TGCTGTCAATCGCAAGGTCCA-3'.

2. Characterization

The field-emission scanning electron microscopy (FESEM) images of the samples were obtained using a Hitachi Regulus 8100 microscope (Japan), and energy dispersive X-ray photoelectron spectroscopy (EDX) analysis was performed to understand the elemental composition distribution of the samples. High-resolution transmission electron microscopy (HRTEM) images were observed by JEM-2100 microscope (Japan) to further characterize the microstructure of the samples. The compositions and chemical valence of the elements on the surface of the material were analyzed using ESCALAB 250 (USA) X-ray photoelectron spectroscopy (XPS) instrument. The UV-visible diffuse reflectance spectra (UV-DRS) were examined in the range of 200-800 nm by UV-visible spectrophotometer (Hitachi U-3900H, Japan) with $BaSO_4$ as reference. Electron spin resonance spectroscopy (ESR) was conducted on the Endor spectrometer (JEOL JES-FA300, Japan) at room temperature. 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as the spin-trapping agents for the

detection of ·OH and $\cdot O_2^-$, while 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) was served as the scavengers of h^+. The signals were collected at dark and under light irradiation with a 500 W Xe lamp as light source for 10 min. The specific recognition ability of the self-screening aptamers of 3-O-C10-HL was evaluated by circular dichroism spectrometer (JASCOJ-1500, Japan). Magnetron sputtering was performed using PVD75 Proline SP equipped with Cr and Au as the sputtering source. The PEDOT: PSS film was spin-coated using a KW-4A spin-coating machine. PEC and OPECT measurements are carried out with PEC/OPECT detectors (Nandaguang, Nanjing, China). The light intensity is 3.5 $W \cdot cm^{-2}$.

3. Preparation of OECT devices

The OECT devices were prepared by the following method. Before sputtering, the soda-lime glass was ultrasonically cleaned with acetone, ethanol and ultrapure water, and then dried and plasma cleaned. Next, magnetron sputtering technology was used to sputter 10 nm of chromium layer and 100 nm of gold layer on the surface of soda-lime glass to form a pattern. The length of the pattern was 0.2 mm and the width was 6.0 mm. Then the patterned soda-lime glass was cleaned by plasma again. The next step was to cover the surface of the soda-lime glass with a PEDOT: PSS solution containing 5 % volume ratio of dimethyl sulfoxide and spin it at a speed of 3500 $rpm \cdot min^{-1}$ to form a film. Finally, the samples were annealed at 180 °C in argon atmosphere for 60 min.

The underlying principle was predicated on the electrochemical doping and dedoping processes intrinsic to OECT. The detailed process of doping and dedoping can be described as $n(PEDOT^+: PSS^-) + M^{n+} + n^{e-} \rightleftarrows nPEDOT^0 + M^{n+}: nPSS^-$, where n represents the charge of the cation and M^{n+} denotes the cation in the electrolyte. Under the illumination of light, the grating electrode would generate a positive voltage, thereby creating a forward electric field towards the channel direction, the cations in the electrolyte would be introduced into the channel's $PEDOT^+: PSS^-$ film. The cation implantation would result in the oxidation of $PEDOT^+$ in its high conductivity state transforming into the reduced $PEDOT^0$ in its low conductivity state.

4. Aptamer selection by SELEX

The aptamer of 3-O-C10-HL was self-screened by the SELEX method. An 80 nt oligonucleotide ssDNA library consisting of a central 40 nt randomized region flanked by two 20 nt primer binding sites was used as the initial library (5'-TGGACCTTGCGATTGACAGC-N40-GCAGACATGAGTCTCAGGAC-3'). The ssDNA library and subsequent aptamer pools were amplified by symmetric PCR with a forward primer (5'-CAGCTCAGAAGCTTGATCCT-3') and biotinylated reverse primer (5'-biotin-GTCCTGAGACTAATGTCTGC-3').

Before each round of screening, the ssDNA library was denatured: heated at 95 ℃ for 5 min, then rapidly cooled in ice bath for 10 min, and placed at 25 ℃ for 10 min. The purpose was to fold into a complex structural conformation. In the first round of screening, 400 mL of denatured random ssDNA library (2 nmol for initial round; 200 pmol for subsequent rounds [1]) was incubated with 200 pmol N-(3-Oxodecanoyl)-L-homoserine lactone (3-O-C10-HL) at 25 ℃ for 2 h with slight shaking. Subsequently, 2 $mg \cdot mL^{-1}$ graphene oxide (GO) solution (mass ratio of GO/ssDNA was 200:1 [2]) was added and incubated at 25 ℃ for another 1 h. After 1 h incubation, this mixture containing GO was centrifuged at 13000 rpm for 10 min at 4 ℃, and the supernatant was collected.

The collected supernatant was amplified by Polymerase Chain Reaction (PCR). The PCR mixture was consisted of 1 mL ssDNA template, 1 mL forward primer (10 mM), 1 mL biotinylated reverse primer (10 mM), 25 mL Taq PCR master mix, and 22 mL sterilized water. PCR was carried out as follows: pre-denaturation at 95 ℃ for 3 min, followed by 20 cycles of denaturation at 95 ℃ for 30 s, annealing at 50.7 ℃ for 30 s, extension at 72 ℃ for 35 s, then a final extension at 72 ℃ for 5 min and cooled at 4 ℃. PCR products were purified by Monarch DNA Gel Extraction Kit. Subsequently, 3% agarose gel electrophoresis and imaging were used to identify PCR products. To prepare ssDNA, biotinylated double stranded DNA was isolated by streptavidin modified magnetic beads [3]. Non-biotinylated ssDNA was collected and quantified using the Nano Drop 2000 spectrophotometer, which was then used as the nascent ssDNA pool for the next round of SELEX.

In order to improve the affinity and specificity of the selected aptamer, structural analogues of 3-O-C10-HL were used for counter selection in the third, sixth, and ninth rounds of screening. To

perform counter selection, denatured ssDNA was incubated with the counter targets. Then GO solution was added and incubated for a period of time, and then centrifuged to remove the supernatant. Subsequently, 3-O-C10-HL was added to the GO precipitate, which resulted in the competitive separation of ssDNA with high affinity for 3-O-C10-HL from the GO surface. In addition, as the number of screening rounds increased, the experimental conditions were continued to change to improve the rigor of screening. The screening steps for subsequent rounds were as same as the above steps. After twelve rounds of screening, the enriched library obtained was subjected to high-throughput sequencing by GENEWIZ Co., Ltd. (Suzhou, China) to obtain the nucleotide sequence and sequence abundance of each ssDNA in the library.

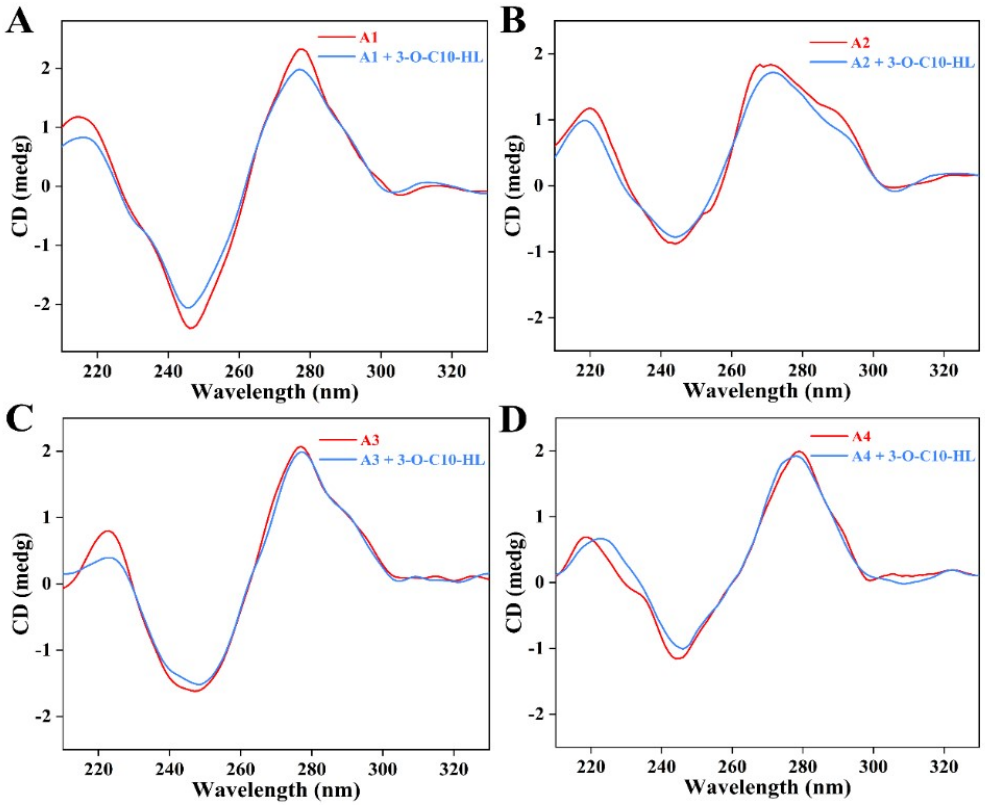

Fig. S1. CD spectra of A1 (A), A2 (B), A3 (C), and A4 (D) aptamer at 1 μM concentration before (red) and after (blue) incubation with 1 μM 3-O-C10-HL.

5. Characterization of materials

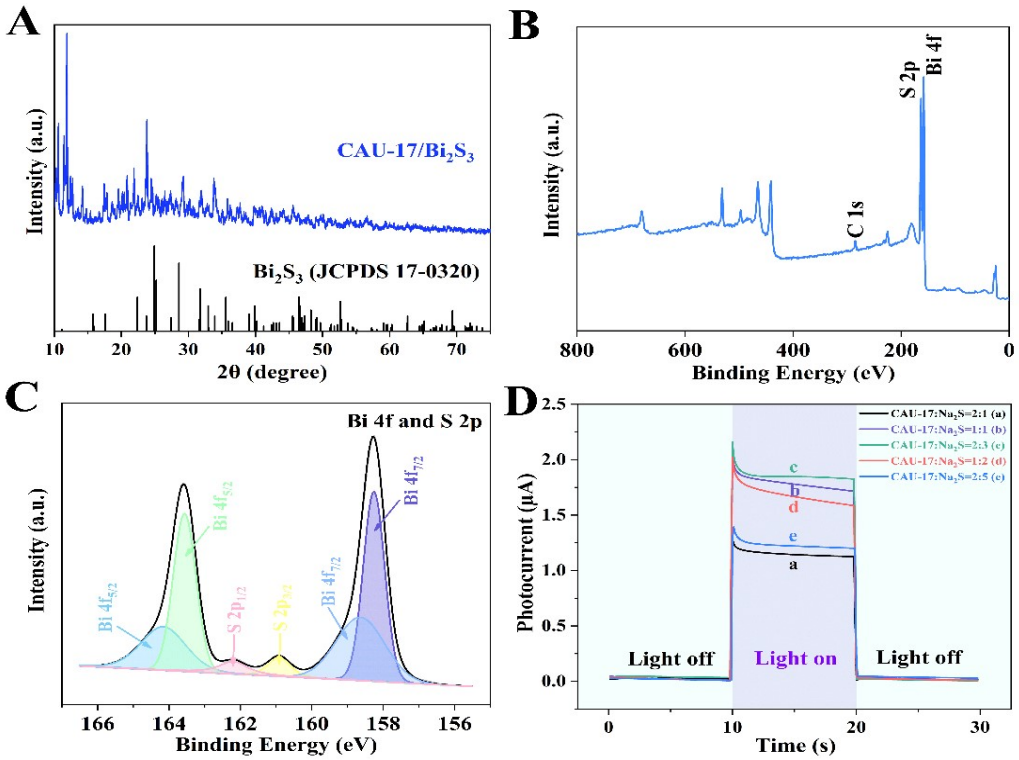

Fig. S2. XRD pattern of CAU-17/Bi_2S_3 (A); The survey XPS spectrum of CAU-17/Bi_2S_3 (B), High-resolution XPS spectra of Bi 4f and S 2p for CAU-17/Bi_2S_3 (C); The photocurrent responses of CAU-17/Bi_2S_3 with different molar ratios (D).

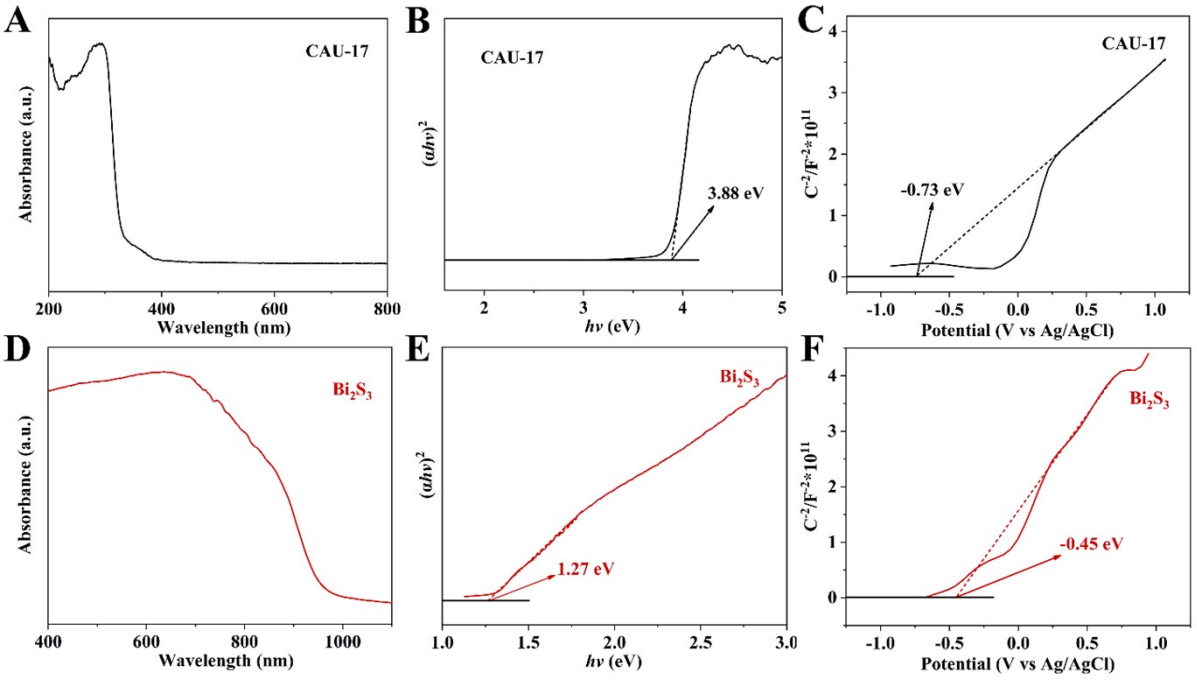

Fig. S3. UV-DRS of CAU-17 (A) and Bi_2S_3 (D); The corresponding Tauc diagrams (B and E) and Mott-Schottky (C and F) curves for CAU-17 and Bi_2S_3.

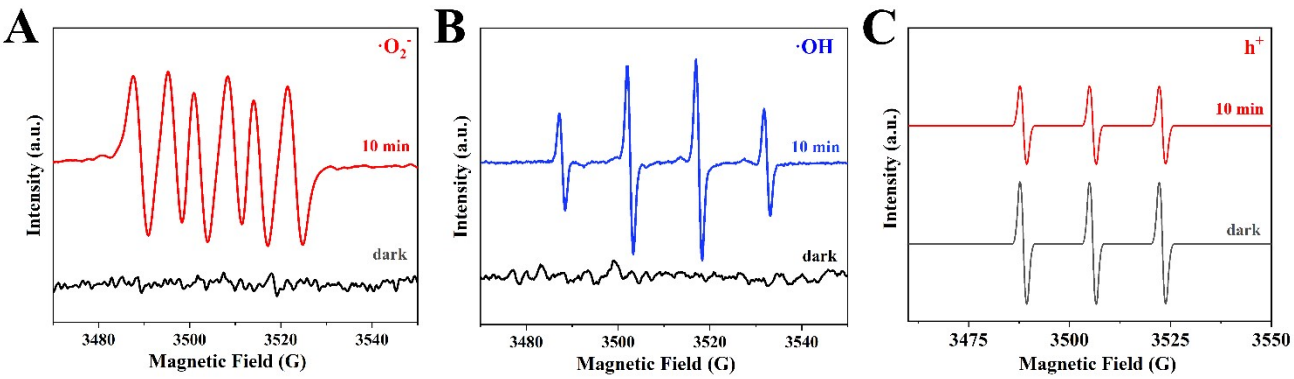

Fig. S4. ESR signals of DMPO-·O_2^- (A), DMPO-·OH (B) and TEMPO-h^+ (C) for CAU-17/Bi_2S_3.

5. OPECT detection of 3-O-C10-HL

Table S1. Comparisons of this work with recent published methods for the determination of quorum sensing signal molecules.

Method	Sample	LOD (M)	Reference
MALDI-MS	3-oxo AHL	5.0×10^{-16}	[4]
TLC-Biosensor	N-3-oxo-C6-HSL	2.0×10^{-14}	[5]
MMIES	AHL	8.0×10^{-10}	[6]
Network of bacteria-based delivery systems (BacteriaBots)	AHL	1.0×10^{-9}	[7]
LC-MS/MS	AHL	1.6×10^{-9}	[8]
TLC-Biosensor	C4-HSL	5.0×10^{-9}	[9, 10]
Paper strip whole cell biosensor	AHL	1.0×10^{-8}	[11]
GC-EI-MS	AHL	$3.2\text{-}6.2\times10^{-6}$	[12]
OPECT	3-O-C10-HL	4.41×10^{-13}	This work

Table S2. The recovery rates of standard addition method in seawater.

Sample	Added (M)	Found (M)	Recovery	RSD
1	1×10^{-11}	1.13×10^{-11}	108.33%	8.27%
2	5×10^{-11}	4.79×10^{-11}	97.80%	5.80%

3	10×10^{-11}	10.56×10^{-11}	103.03%	5.89%
4	50×10^{-11}	50.96×10^{-11}	100.67%	2.25%

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