

Atomically dispersed recognition unit for selective in vivo photoelectrochemical medicine detection

Received: 7 August 2023

Accepted: 1 October 2024

Published online: 12 October 2024

Xiankui Xu¹, Dawei Xu¹, Shen Lu¹, Xue Zhou¹, Shenbo Yang² & Zhonghai Zhang^{1,3,4,5} 

Continuous and long-term therapeutic monitoring of medicine molecules in biological systems will revolutionize healthcare by offering personalized pharmacokinetic reports. However, the extremely complex biological environment brings great challenges for in vivo molecule detection in living organisms. Here we introduce an in vivo photoelectrochemical biosensor following a reverse design strategy with single atoms as molecular recognition units. Atomic dispersion of Cu single atoms on TiO_{2-x} substrate create synergistic anchoring triple-site for efficiently and selectively capturing of dual-carbonyl group and neighboring dual-hydroxyl group of tetracycline molecules. The photoelectrode is encapsulated with antibiofouling layer and implanted into the vein of living mouse to enable long-term in vivo monitoring of tetracycline in real biological environments. It is important to note that our approach was exclusively tested in male mice, and therefore, the findings may not be generalizable to female mice or other species without further research. The rationally designed biological-components-free in vivo biosensor with excellent selectivity, robustness, and stability endows possibility for enabling personalized medicine guidance through real-time feedbacking information and providing direct and authentic medicine molecular analysis.

In vivo electrochemical biosensor is a minimally invasive sensor, which is designed to simply implant into a real biological system for continuous and long-term monitoring of target analytes¹. The in vivo electrochemical biosensor is a powerful tool to obtain real-time feedback information and provide direct and authentic chemical molecular detection and analysis in the biological systems. Therefore, it presents promising potential to revolutionize health care by enabling personalized medicine through continuous therapeutic drug monitoring, which can take the guesswork out of dosing by offering an individualized report on the pharmacokinetics of a medicine^{2,3}. However, the composition of actual biological system is extremely complex, which

makes the recognition elements are necessary components for in vivo biosensors and are essential to implement selective detection for their practical applications⁴. Most recognition units in biosensors are biological components, such as antibodies, enzymes, receptors, and artificial aptamers^{5–10}, whereas the inherent fragility, irreversible binding, non-selectivity, and possibility of degradation of these biological components in real in vivo environment severely limit their accuracy, robustness, drift over time, and lifetime of implantable biosensors. In addition, in vivo electrochemical sensor needs external electrical bias to induce oxidation/reduction reactions of biomolecules on the electrode surface¹¹, which results in undesirable

¹Shanghai Key Laboratory of Green Chemistry and Chemical Processes, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200241, China. ²Hongzhiwei Technology (Shanghai) Co., Ltd., 1599 Xinqin Road, Pudong, Shanghai, China. ³State Key Laboratory of Petroleum Molecular and Process Engineering, SKLPMPE, Sinopec Research Institute of Petroleum Processing Co., Ltd., 100083 Beijing, China. ⁴East China Normal University, Shanghai 200062, China. ⁵Institute of Eco-Chongming, East China Normal University, 20 Cuiniao Road, Chongming District, Shanghai 202162, China. ✉ e-mail: zhzhang@chem.ecnu.edu.cn

electrophysiological stimulation and thus brings out non-negligibly abnormal secretion of biomolecules from living cells¹². Therefore, a new strategy is urgently needed to avoid utilization of external electrical bias and implement selective in vivo biomolecule detection in a biological component free mode.

Photoelectrochemical (PEC) biosensor is an emerging and fast developing analytical device with incidence light as exciting resource and electrical current as readout signal^{13–15}. The formation of inner electric field on the interface of photoelectrode-electrolyte junctions effectively avoids utilization of external electrical bias¹⁶. Therefore, the PEC biosensor is a promising candidate to overcome electrical stimulation for in vivo detection, and near infrared (NIR) light driven or micro-optical fiber supported PEC biosensors have been proposed and implemented preliminary in vivo detection^{17–20}. However, the design and package of molecule recognition units on photoelectrode without using biological components are still highly desirable but challengeable to achieve high-selective and accurate in vivo detection.

Herein, we propose an innovative reverse design strategy to custom design and manufacture of photoelectrodes based on the unique molecular structure of target analytes, especially antibiotics. The atomic anchoring technology is employed to accurately and firmly “lock” antibiotic molecules on the surface of the photoelectrode for efficient capture and recognition of target molecules. In view of the wide applications of antibiotics in the medical field and their possible toxic side effects, especially the potential harm of excessive or long-term use of antibiotics on the human nervous system, liver system and immune system²¹, it is particularly important to monitor the process of antibiotic metabolism in vivo and in real time. This not only helps to guide clinical rational drug use, but also can effectively prevent the occurrence of adverse drug reactions. With the tetracycline (TET) as model, we have designed and prepared $\text{Cu}_1\text{-TiO}_{2-x}$ -based composite photoelectrode, where the dicarbonyl group and adjacent dihydroxyl group of TET can form stable coordination bonds with transition metals such as Cu²² and can interact with Ti site in titanium oxide (TiO_{2-x})^{23,24}, respectively. For traditional Cu nanoparticle (NPs)/ TiO_{2-x}

substrate system, unfortunately, only very limited TET molecules can be captured, which can be ascribed to the rare adjacent and synergistic anchoring sites. Single-atom catalysts are emerging and fast developing materials with wide utilization in many fields^{25–30}. The atomic dispersion of Cu atoms on TiO_{2-x} substrate ($\text{Cu}_1\text{-TiO}_{2-x}$) will be a good attempt to create synergistic anchoring sites for efficiently and selectively capturing of TET molecules. Based on these molecular interactions, the implantable PEC sensors with $\text{Cu}_1\text{-TiO}_{2-x}$ photoelectrode encapsulated antifouling layer (in-situ formation of metal-organic framework), implement selectively capture and identify TET molecules and long-term in vivo monitoring of TET metabolism in real biological environments.

Results

Photoelectrode design and molecular recognition mechanism

The design and manufacturing processes of $\text{Cu}_1\text{-TiO}_{2-x}$ nanocavity photonic crystal ($\text{Cu}_1\text{-TiO}_{2-x}$ NCPC) photoelectrodes are illustrated in Fig. 1a. The thin titanium wire with diameter of 0.1 mm was selected as electrode precursor and substrate. The detailed fabrication procedures can be found in Method in Supplementary information. The brief processes were described as follows. First, Ti wire was electrochemically anodized to form TiO_2 nanotubes (TiO_2 NTs) on its surface (SEM image in Supplementary Fig. 1). The TiO_2 NTs were totally removed in ultrasonic bath and left behind well-ordered nanocavity on Ti wire surface (SEM image in Supplementary Fig. 2). Expanding the optical absorption wavelength of photoelectrodes from the UV/vis light region into the NIR light region is a prerequisite for PEC in vivo detection³¹, therefore, the patterned Ti wire underwent the second anodization process to enlarge the size of nanocavity, and expanded the light absorption into NIR region. TiO_{2-x} NCPC was obtained after annealing treatment, and the test results of electron paramagnetic resonance spectroscopy (Supplementary Fig. 3) showed that there were many oxygen vacancies in the material. Then, a cryo-electrochemical deposition method was utilized to deposit Cu single atoms onto TiO_{2-x} . Finally, the TET molecules can be selectively captured by $\text{Cu}_1\text{-TiO}_{2-x}$.

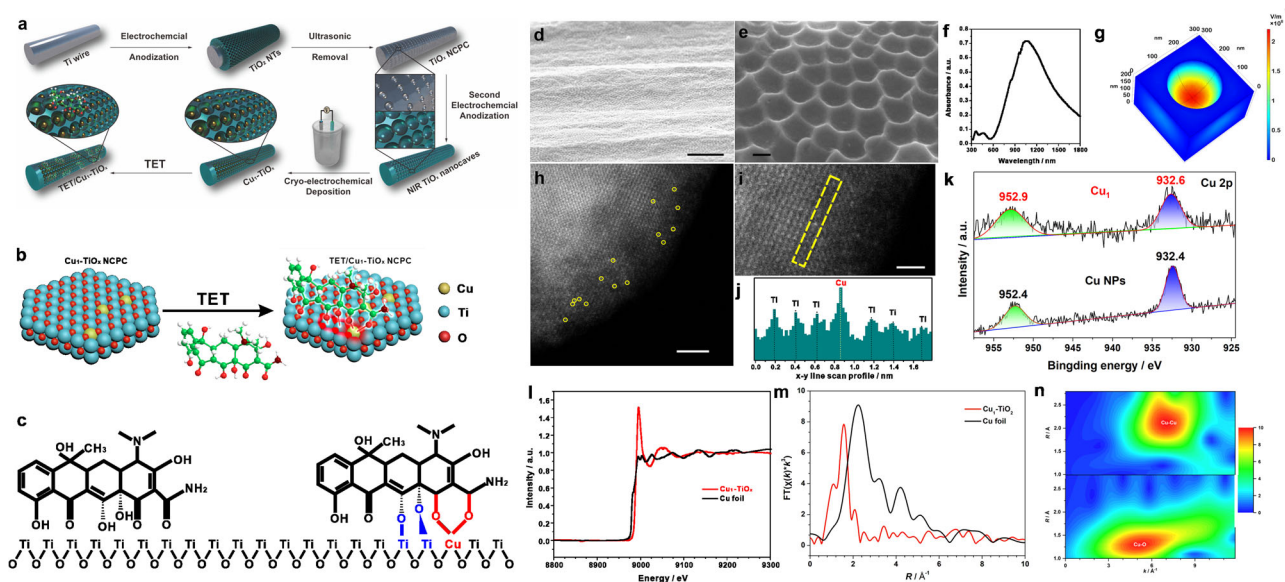


Fig. 1 | Material design and molecular recognition mechanism. **a** Schematic illustration of fabrication processes of $\text{Cu}_1\text{-TiO}_{2-x}$; **b** schematic diagram of triple-site recognition unit of $\text{Cu}_1\text{-TiO}_{2-x}$ for TET capture; **c** bonding dual-carbonyl group with Cu_1 and neighboring dual-hydroxyl group with Ti sites; **d** SEM image of $\text{Cu}_1\text{-TiO}_{2-x}$ the scale bar is 2 μm ; **e** enlarged SEM image of $\text{Cu}_1\text{-TiO}_{2-x}$ the scale bar is 100 nm; **f** DRS spectrum of $\text{Cu}_1\text{-TiO}_{2-x}$; **g** FDTD stimulation of hot-spot distribution on

the nanocavity, **h** AC HAADF STEM image of $\text{Cu}_1\text{-TiO}_{2-x}$ the scale bar is 2 nm; **i** enlarged AC HAADF STEM image of $\text{Cu}_1\text{-TiO}_{2-x}$ the scale bar is 1 nm; **j** x-y line scan profile measured from (i); **k** core-level XPS spectra of Cu 2p of $\text{Cu}_1\text{-TiO}_{2-x}$ and Cu NPs- TiO_{2-x} ; **l** Cu K-edge of near-edge XANES spectra for $\text{Cu}_1\text{-TiO}_{2-x}$ and Cu foil; **m** R-space of Cu K-edge XAFS spectra of $\text{Cu}_1\text{-TiO}_{2-x}$ and Cu foil; **n** wavelet transform of the k^2 -weighted EXAFS data of Cu L_{3-} edge of Cu_1 and Cu foil.

The molecule recognition mechanism of TET on $\text{Cu}_1\text{-TiO}_{2-x}$ was illustrated in Fig. 1b, where the Cu single atoms were distributed on the surface of TiO_{2-x} , and after introducing TET molecules, the dual-carbonyl group was coordinated with Cu single atom (red part in Fig. 1c) and the adjacent two Ti sites can bond with dual-hydroxyl group (blue part in Fig. 1c). Only the molecule with the same structure, dual-hydroxyl group and dual-carbonyl group in contiguous position can be selectively captured by the triple-site recognition unit with Cu single atoms and its neighboring two Ti sites in TiO_{2-x} , thus effectively avoided interference of false positive signals brought by single site recognition and greatly improved selectivity. In addition, all the elements of the recognition unit in $\text{Cu}_1\text{-TiO}_{2-x}$ were modified on the electrode surface through strong interaction (actually, components of the molecular recognition unit are the same elements of the electrode composition), which avoided utilization of biological components and guaranteed good stability and robustness in real organism environment for in vivo selective detection.

The morphology of $\text{Cu}_1\text{-TiO}_{2-x}$ was characterized from scanning electron microscope (SEM) images. As shown in Fig. 1d, the nanocavity with uniform size in large scale can be observed. Closer observation from enlarged SEM image (Fig. 1e) indicated that the size of nanocavity was about 220 nm with high periodicity, and induced photonic crystal property with optical absorption in NIR region (Fig. 1f). In addition, the size of nanocavity can be optimized through adjusting the second anodization duration and directly affected the optical absorption positions (Supplementary Fig. 4). The photonic property of TiO_{2-x} was further evaluated from finite-difference-time-domain (FDTD) simulation. As presented in Fig. 1g, the NIR photons were trapped into the bottom of the nanocavity with much enhanced electric field intensity. The NIR absorption activity of $\text{Cu}_1\text{-TiO}_{2-x}$ was essential for in vivo PEC detection as the NIR photons owned much deeper biological organism penetration depth than ultraviolet and visible light³².

To confirm the existence of Cu single atoms further directly on TiO_{2-x} , aberration-corrected high-angle annular dark field scanning transmission electron microscopy (AC HAADF-STEM) was employed and the image was presented in Fig. 1h. Clearly, the single Cu atoms, marked with yellow circles, were well dispersed on TiO_{2-x} . No Cu clusters or NPs can be detected, agreed well with the XRD patterns that no Cu crystals can be measured (Supplementary Fig. 5). The enlarged AC HAADF-STEM image (Fig. 1i) further distinguished single Cu atom and the line scan profile (Fig. 1j) indicated the Cu single atom possessed stronger signal intensity than Ti atoms. The HAADF-STEM image and the corresponding elemental mapping measurements were performed (Supplementary Fig. 6), in which the Ti, O, and Cu elements were detected and all of them were uniformly distributed. Furthermore, X-ray photo-electron spectroscopy (XPS) spectra of $\text{Cu}_1\text{-TiO}_{2-x}$ and Cu NPs- TiO_{2-x} were recorded (Supplementary Fig. 7). The core-level XPS of Cu 2p on $\text{Cu}_1\text{-TiO}_{2-x}$ and Cu NPs- TiO_{2-x} were examined and shown in Fig. 1k. The binding energy at 932.4 and 952.4 eV can be assigned to Cu 2p_{3/2} and Cu 2p_{1/2} respectively and corresponded to metallic Cu in Cu NPs- TiO_{2-x} sample³³. While, the binding energy positively shifted to 932.6 and 952.9 eV on $\text{Cu}_1\text{-TiO}_{2-x}$ sample, which implied the existence of higher valence state of Cu (Cu^{6+}) than valence state of metallic Cu³⁴.

X-ray absorption fine spectroscopy (XAFS), including X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS), can provide precise information about the local structure of the atoms and their surroundings, including oxidation state, types of neighboring atoms, bond distance, and coordination numbers³⁵. As shown in Fig. 1l, the XANES Cu k-edge spectra presented higher white line intensity on $\text{Cu}_1\text{-TiO}_{2-x}$ than that of Cu foil, agreed well with the XPS result that the Cu in $\text{Cu}_1\text{-TiO}_{2-x}$ owned higher chemical valent state than metallic Cu. The Fourier transformed (FT) k²-weighted EXAFS spectra (Fig. 1m) of $\text{Cu}_1\text{-TiO}_{2-x}$ displayed one prominent peak at 1.57 Å, which can be further fitted to be Cu-O

coordination (Supplementary Fig. 8 and Supplementary Table 1). In contrast, Cu foil showed a typical Cu-Cu pair at 2.23 Å. Corresponding to the coordination peak of Cu-O, which was quite different from those of the referenced Cu foil, implied no Cu clusters or NPs existence. The above results illustrated that the isolated Cu atoms were anchored at the atomic level in TiO_{2-x} and were coordinated to four O atoms (Supplementary Table 1). To verify correlative information more intuitively about coordination atoms, wavelet transform analysis was also displayed in Fig. 1n, in which the intensity maximum located at about 5.2 Å⁻¹ for Cu_1 and can be derived from Cu-O, which was considerably lower from the one at about 7 Å⁻¹ assigned to Cu-Cu in Cu foil. All of these about experimental results successfully confirmed the fabrication of Cu single atoms on TiO_{2-x} to assemble $\text{Cu}_1\text{-TiO}_{2-x}$ triple-site recognition unit photoelectrode.

Selective recognition of TET and PEC detection

The molecule recognition activity of TET adsorption on $\text{Cu}_1\text{-TiO}_{2-x}$ photoelectrode was investigated from Fourier transform infrared (FTIR) spectra and Raman spectra. As shown in Fig. 2a (Supplementary Fig. 9), the FTIR spectrum of TET presented a strong peak at 1640 cm⁻¹, which can be ascribed to stretching vibration of aromatic ring in TET^{36,37}. The TET/ TiO_{2-x} sample showed an identical IR spectrum to that of pristine TiO_{2-x} , indicating that no specific IR peaks from TET molecules were detected, thus suggesting that no TET molecules were adsorbed onto TiO_{2-x} . While, the TET/ $\text{Cu}_1\text{-TiO}_{2-x}$ presented a distinct FTIR peak at 1640 cm⁻¹, compared to the very weak peak at TET/Cu NPs- TiO_{2-x} , which clearly manifested effective and abundant TET capturing on $\text{Cu}_1\text{-TiO}_{2-x}$. The Raman spectra were also recorded and presented in Fig. 2b. The Raman active modes at 640 cm⁻¹ were correspond to presence of the anatase phase of TiO_{2-x} ³⁸. The TET showed two Raman peaks at 1450 cm⁻¹ and 1617 cm⁻¹, which can be attributed to $\nu(\text{CO})$ and $\delta(\text{NH})$ respectively³⁹. The Raman spectra revealed, as well, that TET has been efficiently adsorbed onto the surface of $\text{Cu}_1\text{-TiO}_{2-x}$. In addition, the adsorption behavior of $\text{Cu}_1\text{-TiO}_{2-x}$ towards various antibiotics on its surface was evaluated from Raman spectra (Supplementary Fig. 10), and indicated that $\text{Cu}_1\text{-TiO}_{2-x}$ exhibited a distinctive and selective adsorption effect solely towards TET. Both FTIR and Raman spectra uncovered selective adsorption of TET on $\text{Cu}_1\text{-TiO}_{2-x}$ and predicted success of triple-sites recognition strategy.

Except for selectively capturing, the photogenerated carrier dynamics between TET and $\text{Cu}_1\text{-TiO}_{2-x}$ were studied by femtosecond transient absorption spectroscopy (fs-TAS). As shown in Fig. 2c, the pure $\text{Cu}_1\text{-TiO}_{2-x}$ did not show obvious TA signal under laser pulse excitation, while, after adsorbed TET molecules, the TET/ $\text{Cu}_1\text{-TiO}_{2-x}$ exhibited ultra-fast and instantaneously increased absorption signals, which indicated that the photogenerated charge was instantaneously excited to the conduction band and transferred to TET. A faster decay time τ_1 (224 fs, 94%) was detected (Supplementary Fig. 11 and Supplementary Table 2) and attributed to charge capture by oxygen vacancy of TiO_{2-x} ⁴⁰. In addition, a slower decay time τ_2 (17 ps, 6%) was also measured and can be ascribed to the photogenerated charge recombination process⁴¹. After 50 ps, the observed TA intensity can be attributed to the electron transfer process between $\text{Cu}_1\text{-TiO}_{2-x}$ and TET, which promoted effective charge separation, further delayed self-trapping process of charge, and prolonged active charge lifetime⁴².

To further reveal the feasibility of $\text{Cu}_1\text{-TiO}_{2-x}$ based triple-site recognition unit for TET capturing, the density function theory (DFT) calculations were performed in DS-PAW software⁴³. The structural models of TiO_{2-x} and $\text{Cu}_1\text{-TiO}_{2-x}$ were confirmed from a series of characterization results (discussed in latter section) and the electronic structures for calculations were supplied in Supplementary Data 1. The DFT calculation results showed that the adsorption energy of TET on TiO_{2-x} was 0.88 eV, and the positive value indicated that the TET molecules were not thermodynamic spontaneous adsorption on the surface of TiO_{2-x} . While, the adsorption energy of TET on $\text{Cu}_1\text{-TiO}_{2-x}$

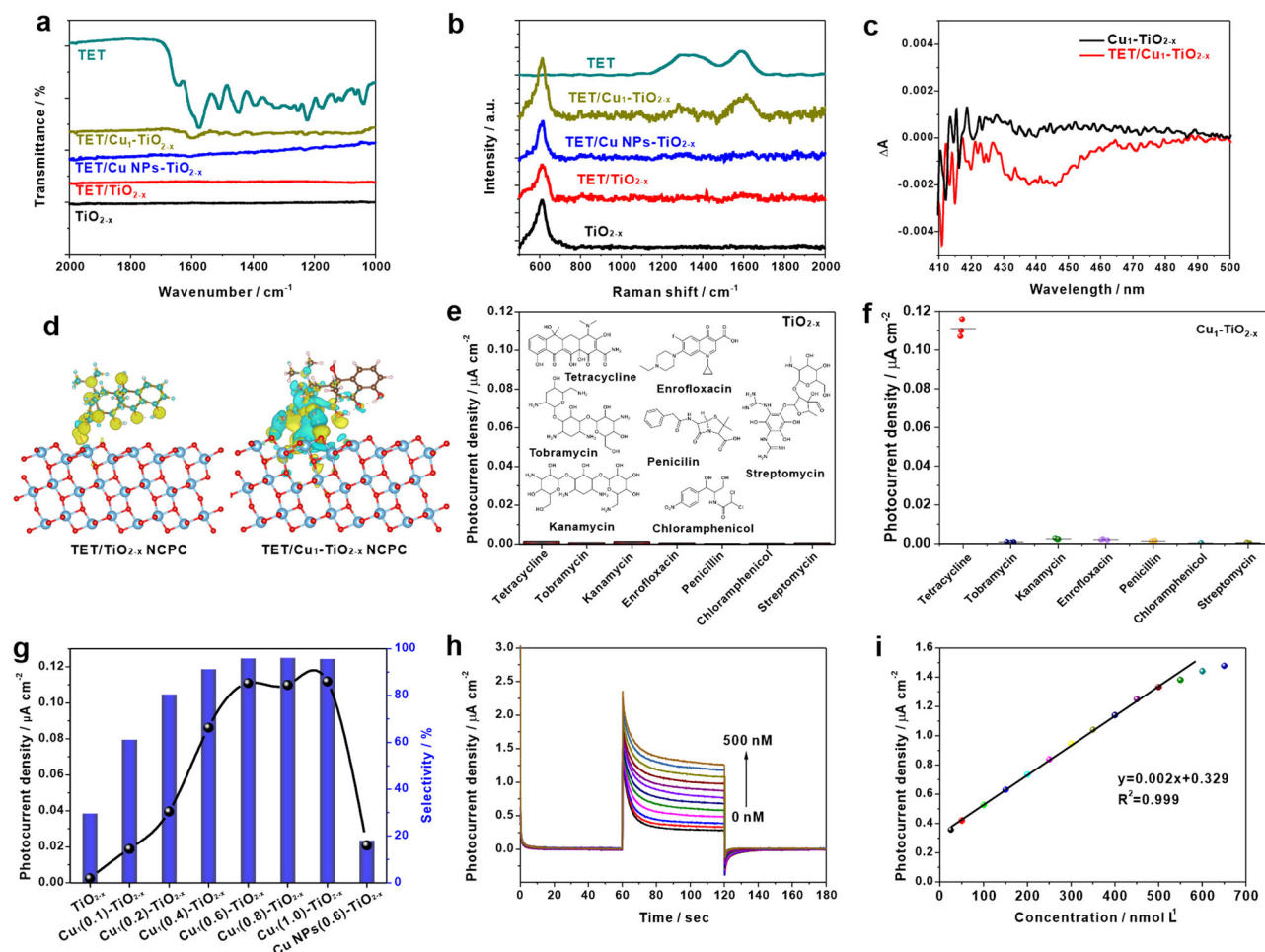


Fig. 2 | Selective recognition of TET and PEC detection on $\text{Cu}_1\text{-TiO}_{2-x}$. **a** FTIR spectra and **b** Raman spectra of TiO_{2-x} , TET/TiO_{2-x} , $\text{TET/Cu NPs-TiO}_{2-x}$, $\text{TET/Cu}_1\text{-TiO}_{2-x}$ and TET ; **c** fs-TAS decay kinetics of $\text{Cu}_1\text{-TiO}_{2-x}$ and $\text{TET/Cu}_1\text{-TiO}_{2-x}$; **d** DFT calculations of local charge density difference of TET/TiO_{2-x} and $\text{TET/Cu}_1\text{-TiO}_{2-x}$ NCPC, yellow part symbolizes local electron accumulation area and cyan part symbolizes local electron depletion area respectively; selectivity measurements of TET on **e** TiO_{2-x} and **f** $\text{Cu}_1\text{-TiO}_{2-x}$ with interferences of tobramycin, kanamycin,

enrofloxacin, penicillin, chloramphenicol, and streptomycin (all the chemical structures of TET and interferences were presented in the inset of **e**); **g** photocurrent response and selectivity of TET on TiO_{2-x} , $\text{Cu}_1\text{-TiO}_{2-x}$ with different Cu single atom contents, and Cu NPs- TiO_{2-x} ; **h** PEC detection of TET with different concentrations in range from 0 to 500 nM; **i** linear calibration curve for PEC TET detection.

reached -1.387 eV, so the negative and high value of absorption energy implied that the TET molecules were spontaneously and effectively adsorbed on the surface of $\text{Cu}_1\text{-TiO}_{2-x}$. To simulate potential electron transfer on the interface of TET and photoelectrodes (TiO_{2-x} and $\text{Cu}_1\text{-TiO}_{2-x}$), DFT calculations were performed and the spatial charge redistributions were analyzed and shown in Fig. 2d. The charge density difference increased by 0.068 eV on the interface of TET/TiO_{2-x} , which indicated slight charge transfer between TET/TiO_{2-x} . While, the charge density difference significantly increased to 0.124 eV for $\text{TET/Cu}_1\text{-TiO}_{2-x}$, which indicated efficient charge transfer between TET and $\text{Cu}_1\text{-TiO}_{2-x}$. More interestingly, the charge accumulation area is mostly located in the Cu_1 and its adjacent Ti sites, and the charge depletion area is in carbonyl and hydroxyl sides of TET (Supplementary Fig. 12), which suggested potential charge transfer from TET to $\text{Cu}_1\text{-TiO}_{2-x}$ through the “lock” bonds, and efficient and selective PEC response of TET on $\text{Cu}_1\text{-TiO}_{2-x}$ can be expected.

A series of PEC and electrochemical characterizations on TiO_{2-x} and $\text{Cu}_1\text{-TiO}_{2-x}$ have been performed and revealed significantly better PEC response on $\text{Cu}_1\text{-TiO}_{2-x}$ than that on TiO_{2-x} (Supplementary Fig. 13) due to high optical absorption in NIR region (Supplementary Fig. 14), low electronic resistance (Supplementary Fig. 15), and proper electronic structures (Supplementary Fig. 16). Under the irradiation of NIR

light, electron-hole pairs were generated on $\text{Cu}_1\text{-TiO}_x$ photoelectrode. These photogenerated electrons and holes migrated to the conduction and valence bands of the material, respectively. The photo-generated holes can participate in the oxidation process of TET molecules. In this process, TET molecules were oxidized and electrons were released. The photo-generated electrons were transmitted through an external circuit to form a detectable photocurrent signal. The selective PEC detection of TET on TiO_{2-x} and $\text{Cu}_1\text{-TiO}_{2-x}$ were evaluated and presented in Fig. 2e, f. The PEC response of TET and interferences, such as tobramycin, kanamycin, enrofloxacin, penicillin, chloramphenicol, and streptomycin, on TiO_{2-x} was recorded and showed insignificant photocurrent density, which indicated insufficient PEC detection activity on pristine TiO_{2-x} . While, on $\text{Cu}_1\text{-TiO}_{2-x}$ photoelectrodes (Fig. 2f), only TET was detected and contributed a high photocurrent response, other interference antibiotics just provided negligible photocurrent values, which revealed excellent selectivity of TET on $\text{Cu}_1\text{-TiO}_{2-x}$. In addition, the photocurrent responses of antibiotics on Cu NPs- TiO_{2-x} photoelectrodes were recorded, but also displayed a poor selectivity (Supplementary Fig. 17). To ascertain the exclusive selectivity of $\text{Cu}_1\text{-TiO}_{2-x}$ towards tetracycline structures, we performed selectivity assessments on three tetracycline-family antibiotics, doxycycline, minocycline, and tigecycline (Supplementary Fig. 18). The PEC test

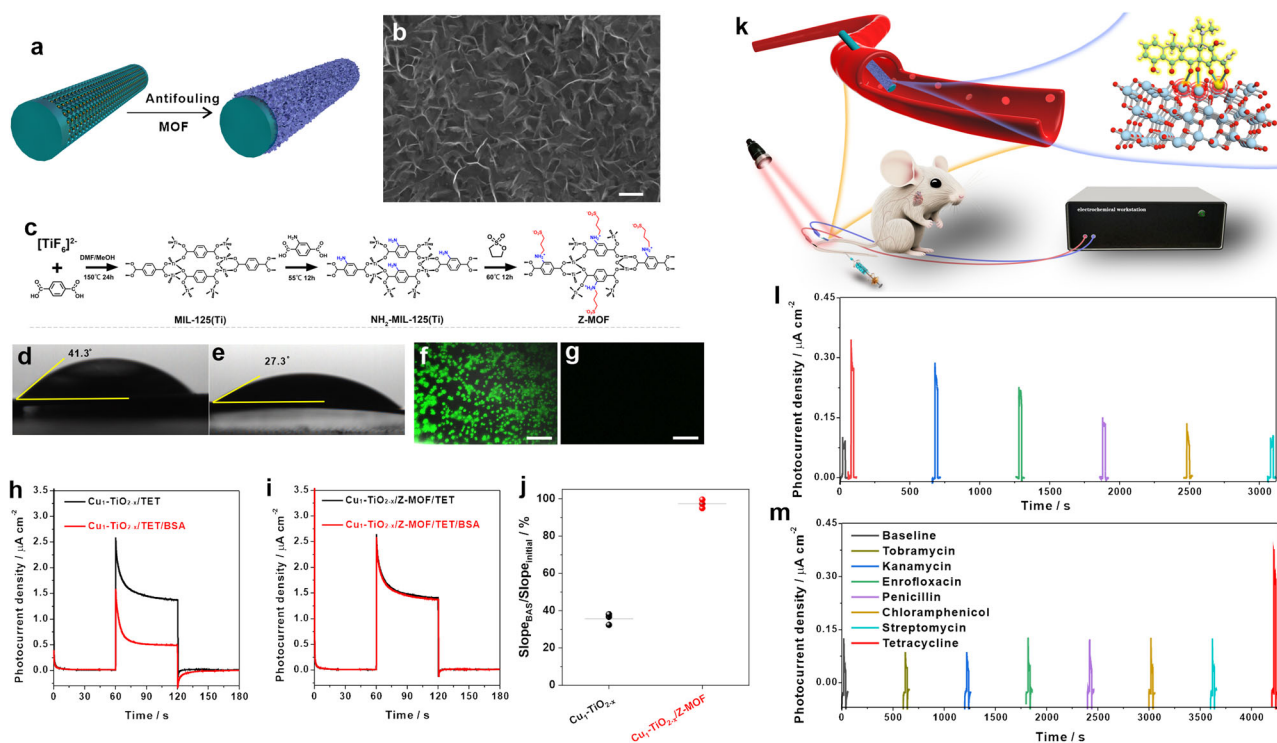


Fig. 3 | PEC in vivo detection of TET. **a** Schematic of encapsulation of antifouling layer on $\text{Cu}_1\text{-TiO}_{2-x}$; **b** SEM image of Z-MOF, the scale bar is 200 nm, and the SEM images of the three different regions show consistent morphologies; **c** chemical reaction process for formation of Z-MOF; static water contact angle on **d** $\text{Cu}_1\text{-TiO}_{2-x}$ and **e** $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$; confocal fluorescent images of **f** $\text{Cu}_1\text{-TiO}_{2-x}$ and **g** $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ after immersing in FITC-BSA solution, the scale bar is 200 μm , and the fluorescence images of the three different regions show consistent fluorescence distribution; photocurrent responses on **h** $\text{Cu}_1\text{-TiO}_{2-x}$ and **i** $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$

before and after incubating in BSA solution (20 mg mL^{-1}); **j** comparison of PEC detection performance with or without BSA as bio-contaminator on $\text{Cu}_1\text{-TiO}_{2-x}$ and $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$, error bars correspond to the Standard Deviation of five independent measurements ($n = 5$); **k** Schematic of PEC in vivo detection of TET on $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ in caudal vein of mouse; **l** PEC in vivo monitoring and tracking TET metabolism in living mouse; **m** in vivo selectivity measurements of TET on $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$.

results confirmed that tetracycline antibiotics, with their unique dicarbonyl and dihydroxyl structures, contributed photocurrent response, which further validated the molecular recognition manner of dihydroxyl and bicarbonyl groups of tetracycline antibiotics interacting with Cu single atom and two Ti sites respectively.

Furthermore, to ascertain the extensibility of our method to other transition metals, we investigated the adsorptive and selective capabilities of electrodes adorned with single Fe, Co, and Ni atoms towards TET molecules, leveraging their ability to coordinate with TET's dicarbonyl structure. According to the test results (Supplementary Fig. 19), all three single-atom/ TiO_{2-x} photoelectrodes showed the certain ability to adsorb tetracycline, although compared with the Cu single-atom electrode, their adsorption effect was inferior. This discovery further validated the potential of transition metal single atoms in the field of PEC biosensing, and revealed the differences in adsorption properties of different metal atoms.

The photocurrent density responses and selectivity of TiO_{2-x} , $\text{Cu}_1\text{-TiO}_{2-x}$ with different weight ratios of Cu single atom, and Cu NPs- TiO_{2-x} with controlled Cu amount were summarized in Fig. 2g. The $\text{Cu}_1(0.1)\text{-TiO}_{2-x}$ (refer to Cu single atom with weight ratio of 0.1% on TiO_{2-x}) showed increased photocurrent and selectivity than pristine TiO_{2-x} , and both factors increased with the increase of Cu single atom weight ratios. As the weight ratio of Cu single atoms reached 0.6%, both photocurrent and selectivity reached a plateau, and a further increase in weight ratio did not contribute to higher PEC detection performance. Therefore, in this paper the $\text{Cu}_1\text{-TiO}_{2-x}$ and Cu NPs- TiO_{2-x} referred to $\text{Cu}_1(0.6)\text{-TiO}_{2-x}$ and Cu NPs(0.6)- TiO_{2-x} (Supplementary Fig. 20). In addition, the reproducibility, repeatability, and stability of $\text{Cu}_1\text{-TiO}_{2-x}$ based photoelectrodes were also evaluated (Supplementary

Fig. 21) and presented good performances. Beneficial from excellent selectivity and high PEC response, the $\text{Cu}_1\text{-TiO}_{2-x}$ based photoelectrodes were used for PEC TET detection. As presented in Fig. 2h, PEC signal-on photocurrent responses with an increase in the concentrations of TET were recorded. In the range of 25 nM to 500 nM of TET, a linear relationship ($R^2 = 0.999$) between concentrations of TET and change of photocurrent density was achieved (Fig. 2i) with a low detection limit of 7.7 nM.

Antibiofouling and PEC in vivo detection of TET

The $\text{Cu}_1\text{-TiO}_{2-x}$ photoelectrodes were further modified with in-situ formed antibiofouling MOF (metal-organic framework) as antibiofouling layer (Fig. 3a) to implement reliable long-term in vivo detection in real biological media. The zwitterionic MOF (Z-MOF) with nanosheet structure (Fig. 3b) was prepared with a solvothermal method (Fig. 3c) to encapsulate on the surface of $\text{Cu}_1\text{-TiO}_{2-x}$. As the hydrophilic property of zwitterionic layer is the prerequisite for achieving outstanding antifouling performance, the static water contact angles on $\text{Cu}_1\text{-TiO}_{2-x}$ and $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ were measured. As shown in Fig. 3d, the water contact angle on bare $\text{Cu}_1\text{-TiO}_{2-x}$ was about 41.3° . After the deposition of Z-MOF, the water contact angle was reduced to 27.3° (Fig. 3e). The good hydrophilic property will weaken the hydrophobic interaction between electrode surface and proteins to improve the antifouling performance. In order to visual presentation the antibiofouling activity, the confocal fluorescent images of $\text{Cu}_1\text{-TiO}_{2-x}$ and $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ after incubating in fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) solution was recorded in Fig. 3f, g. Intensive bright green fluorescence can be observed on pristine $\text{Cu}_1\text{-TiO}_{2-x}$, while, no obvious fluorescence light

can be detected on $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$, and the test with FITC-HAS (human serum albumin) solution showed the same result (Supplementary Fig. 22), indicating its good anti-fouling activity.

The antibiofouling activity of $\text{Cu}_1\text{-TiO}_{2-x}$ and $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ was evaluated from their photocurrent responses before and after immersing in BSA solution. As shown in Fig. 3h, the photocurrent response of TET on $\text{Cu}_1\text{-TiO}_{2-x}$ was significantly reduced after BSA contamination due to nonspecific binding of proteins onto the electrode interface. While, the PEC response of TET on $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ still maintained high value after Z-MOF layer deposition (Fig. 3i). In addition, the antibiofouling performance was evaluated through comparison of the sensitivity slopes of linear relationship curve) with or without BSA as bio-contaminator. As presented in Fig. 3j, the $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ maintained more than 97% of sensitivity with existence of BSA (20 mg mL^{-1}), whereas, decreased to 37% on pristine $\text{Cu}_1\text{-TiO}_{2-x}$.

The rationally designed $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ possessed high sensitivity, good selectivity, and excellent antibiofouling activity, which would guarantee its successful practical in vivo bio-detection. All experimental procedures involving animals were conducted with the approval of the Animal Ethics Committee in East China Normal University, China. The male Wistar mice were anesthetized using chloral hydrate and wrapped in a heating pad to maintain the body temperature at 37°C . As illustrated in Fig. 3k, following the insertion of the micro-photoelectrode into the mouse's tail vein, a substantial photocurrent response was observed upon NIR light illumination. Subsequently, TET with a concentration of 100 nM was injected into caudal vein. As shown in Fig. 3l, the metabolism of TET in living mice was monitored and after 60 min, the photocurrent value was comparable to the blank value, indicating the complete metabolism of TET from the peripheral blood. The in vivo selectivity of TET on $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ was also investigated. As shown in Fig. 3m, after injection of 100 nM of interference antibiotics, no photocurrent change from its blank value was detected, while after injection of 100 nM TET, a significant increase of photocurrent was observed, which further confirmed excellent in vivo PEC selective detection of TET on $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$.

PEC in vivo monitoring of TET in wound infection model

Furthermore, to demonstrate potential practical applications of PEC in vivo monitoring of TET, the wound healing efficiency using a mouse model infected lesion with *E. coli* was evaluated. Wounds were introduced dorsally on anesthetized mice and inoculated with *E. coli* ($1 \times 10^7 \text{ CFU}$). In the treatment experiments, after inoculation for 12 h, four groups of mice were treated with PBS (phosphate buffered saline) as control group and TET in different delivery ways as experimental groups respectively (at least 3 replicates for each group). The control group was performed through injecting PBS solution into the tail vein of mice. The conventional TET (C-TET) treatment group was conducted through one-time injecting solution containing 11.25 mg/kg TET into the tail vein of mice every 24 h⁴⁴. Through in situ monitoring the concentrations of TET in blood of mice with $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$ photoelectrode, two therapeutic drug monitoring (TDM) TET administration groups were proposed, which were successively administered TET after completely metabolized (TDM-CM) or half metabolized (TDM-HM). In all TET treatments, the total injection amounts in 24 h were kept consistent with 11.25 mg/kg .

The wounds were monitored for 13 days, and the wound healing status with different treatments were shown in Fig. 4a (Supplementary Fig. 23). The wound area values were summarized in Fig. 4b. The control group presented the lowest healing rate, along with loss of body weight (Fig. 4c), which suggested bacteria interfering with the wound healing procedure. The C-TET group demonstrated an accelerated healing process, but was still much slower than TDM-CM and TDM-HM treatment groups. As shown in Fig. 4d, the TDM-CM group was first injected with 2.25 mg/kg TET, and after completely

metabolizing, that is, the concentration of TET in blood decreased to zero, the TET with the same concentration was injected again in the first 6 h of each 24 h. The TDM-HM group was administrated TET as half metabolized (Supplementary Fig. 24). The number of bacteria that survive in wound tissue was also determined using the colony forming unit (CFU) method in order to visually quantify the inhibition of wound infection. As shown in Fig. 4e, f (Supplementary Fig. 25), the number of bacterial colonies in the wound treated with TDM-CM and TDM-HM was significantly decreased when compared to other treated and untreated wounds.

In addition, after 13 days experiment, gross anatomical observation (Supplementary Fig. 26) showed insignificant difference in the overall ocular changes of all organs (heart, liver, spleen, lung, and kidney) of mice in PBS, C-TET and TDM-HM groups. While, the liver and lung of mice in TDM-HM group presented darker color, which can be speculated possible bleeding due to toxic injury of TET with high blood concentration. In order to further verify the toxic injury, histopathological examinations were conducted for closer observation of organ tissues. As shown in Fig. 4g (Supplementary Fig. 27), no obvious pathological changes can be found in PBS, TET, and TDM-CM treatment groups, whereas, in TDM-HM treatment group, liver injury hemorrhage, alveolar wall capillary dilatation and congestion, kidney tissue renal cortex and renal medulla hemorrhagic injury can be detected. These results indicated that overdosage of TET could lead to severely hemorrhagic damage in liver, lung, and kidney. All the above experiments demonstrated that real-time in vivo monitoring of medicine blood concentration is a highly effective strategy both for optimizing therapeutic effect and for reducing toxic side effects.

Discussion

In summary, a reverse design strategy was proposed to fabricate $\text{Cu}_1\text{-TiO}_{2-x}$ based photoelectrode with triple-site recognition unit for selective in vivo detection of TET molecules. With the adjacent and synergistic anchoring sites of Cu single atom on TiO_{2-x} , the dual-carbonyl group and neighboring dual-hydroxyl group of TET molecules were selectively captured by $\text{Cu}_1\text{-TiO}_{2-x}$. The $\text{Cu}_1\text{-TiO}_{2-x}$ based biological components free PEC in vivo sensors have implemented selective detection and continuous and long-term monitoring of TET metabolism in living mice with excellent selectivity and high sensitivity. We believed that the new reverse design strategy would open a new avenue of rationally designing biological components free recognition units for building a selective, robust, and stable in vivo detection platform, and facilitated reliable quantitative analysis of trace substances in complex systems.

Methods

Chemicals and materials

Titanium wire (0.1 mm 99.6%) was purchased from Jinjia Metal, China. Ethylene glycol (EG), ammonia fluoride (NH_4F), sodium sulfate (Na_2SO_4), copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), terephthalic acid, N,N-dimethylformamide, methanol, tetracycline (TET), kanamycin, tobramycin, enrofloxacin, chloramphenicol, penicillin and streptomycin were purchased from Macklin Chemical and used as received. Sulfuric acid (95–98%) (H_2SO_4) was supplied by Sinopharm Chemical Reagent Co., Ltd, China. Trichloroacetic aldehyde was supplied by Sangon Biotech, Shanghai, China. All aqueous solutions were prepared using deionized water (DI) with a resistivity of $18.2 \text{ M}\Omega \text{ cm}$ at 25°C .

Preparation of TiO_{2-x} NCPCs

The TiO_2 nanocavity photonic crystals (TiO_2 NCPCs) were synthesized using a two-step anodization process. Prior to the anodization process, the Ti wires underwent degreasing by sonicating in ethanol and DI water, followed by drying in a pure nitrogen stream. The anodization process employed a conventional two-electrode system with Ti wire

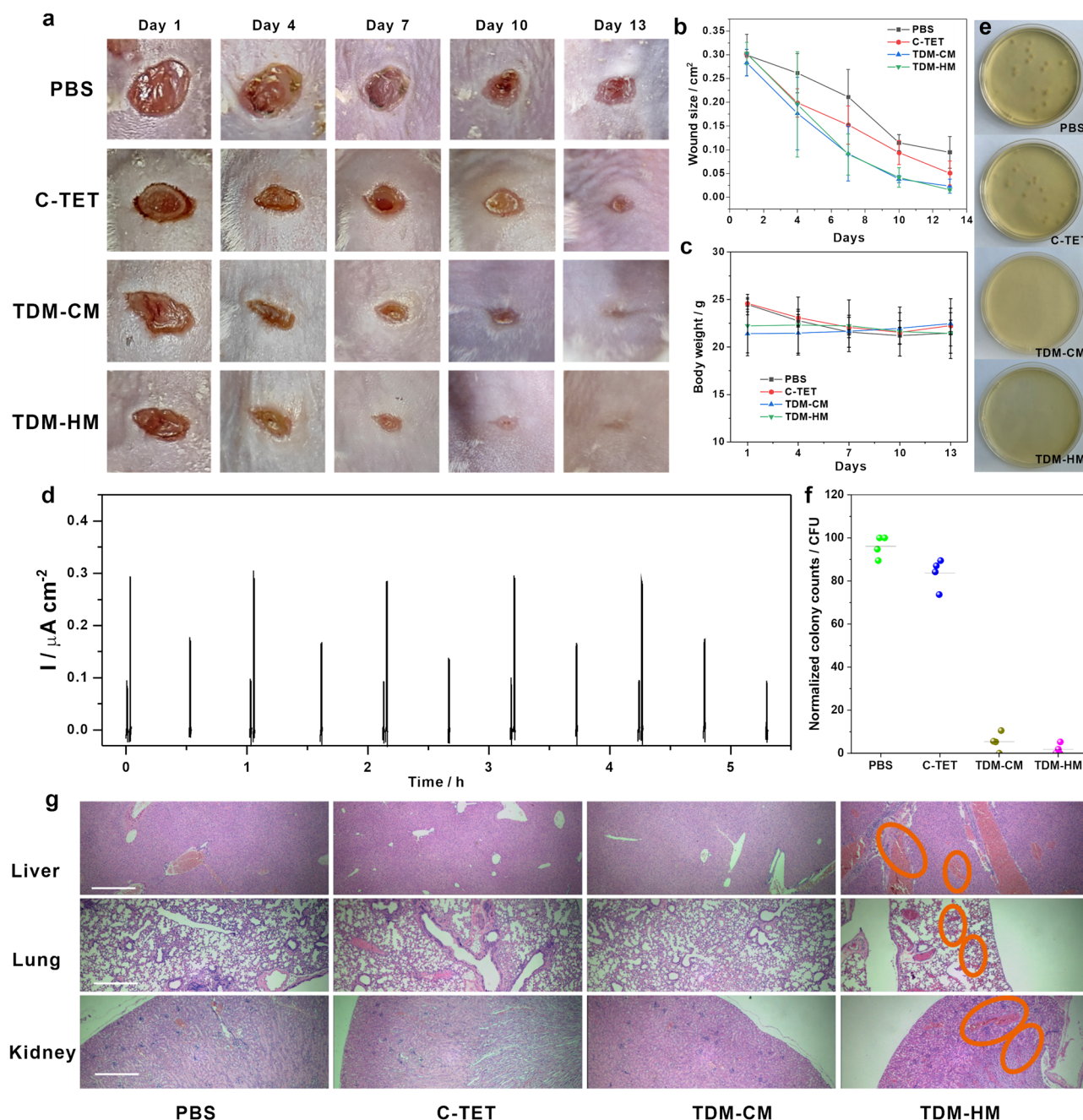


Fig. 4 | PEC in vivo monitoring of TET for practical application. **a** Photographs of *E. coli* infected wounds on days of 1, 4, 7, 10 and 13 with different treatments of PBS, C-TET, TDM-CM, and TDM-HM; **b** mean wound areas on mice with different treatments, error bars correspond to the standard deviation of five independent measurements ($n = 5$); **c** body weight changes of mice in *E. coli*-infected model after different treatments, error bars correspond to the standard deviation of five independent measurements ($n = 5$); **d** I-T curves of medicine concentration

monitoring in TDM-CM group; **e** photographs of agar plates of *E. coli* colony after PBS, C-TET, TDM-CM, and TDM-HM treatments; **f** survival rate of *E. coli* in the infected wound tissues using colony forming unit (CFU) method, error bars correspond to the standard deviation of five independent measurements ($n = 5$); **g** H and E staining images ($n = 3$) of liver, lung, and kidney tissue sections after PBS, TET, TDM-CM and TDM-HM treatments, the scale bar is 500 μm .

serving as the anode and a Pt foil serving as the cathode, respectively. The electrolytes used consisted of 0.32 wt% NH_4F in EG solution with 2.7 vol% water. All anodization steps were performed at an ice bath temperature. During the first anodization process, the Ti wires were anodized at 60 V for 0.5 h, and the as-grown nanotubes with a regular arrangement were subsequently removed from the Ti wire substrate by ultrasonication in DI water. The same Ti wire with patterned nanocavity then underwent the second anodization sequentially at 30 V for 0–20 s to adjust the size of the nanocavity. After the two-step anodization, the prepared TiO_{2-x} NCPC samples were cleaned with DI

water and dried with nitrogen gas. Finally, the prepared TiO_{2-x} NCPCs were annealed in air at 500 $^{\circ}\text{C}$ for 1 h with a heating rate of 5 $^{\circ}\text{C}/\text{min}$.

Fabrication of $\text{Cu}_1\text{-TiO}_{2-x}$

A dilute sulfuric acid solution (0.5 M) was used to prepare the Cu precursor, by adding 32.9 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The as-prepared TiO_2 NCPCs were immersed in 50 mL of the Cu precursor for 1 h. Next, a typical two-electrode system was set up with Pt foils as the working electrode and Ti wires as counter electrode. The Cu precursor and TiO_2 NCPCs were completely immersed in liquid nitrogen for about 5 min.

The frozen mixture was then slowly electrochemically reduced at -10 V potentials. After reduction, the frozen electrolyte was melted at room temperature, and the prepared $\text{Cu}_1\text{-TiO}_{2-x}$ was washed with DI water and dried with nitrogen gas.

Materials characterizations

The morphologies of samples were characterized by scanning electron microscopy (SEM, Gemini450, Zeiss). The transmission electron microscopy (TEM) images and energy dispersive spectroscopy (EDS) mapping were acquired using a JEM-2100F JEOL equipped with an X-ray energy dispersive spectrometer (EDS: X-Max 80T, Oxford, UK) for chemical composition analyses. For atomic resolution imaging, the measurements were performed on an aberration-corrected high-angle annular dark field scanning transmission electron microscopy (AC HAADF-STEM, JEOL Grand ARM300). The crystalline structure of the samples was analyzed by X-ray diffraction (XRD) [Bruker D8 Discover diffractometer, using $\text{Cu K}\alpha$ radiation ($1.540598 \text{ \AA}$)]. The diffuse reflectance UV-vis adsorption spectra were recorded on a spectrophotometer (Shimadzu, UV 3600) with fine BaSO_4 powder as reference. Photoelectron Spectroscopy (XPS) data were collected by an Axis Ultra instrument (Kratos, Analytical) under ultrahigh vacuum ($<10^{-8}$ torr) and using a monochromatic $\text{Al K}\alpha$ X-ray source operating at 150 W. The survey and high-resolution spectra were collected at fixed analyzer pass energies of 160 and 20 eV, respectively. Binding energies were referenced to the C $1s$ binding energy of adventitious carbon contamination which was set at 285.0 eV. The concentration of Cu atoms was directly measured by ICP-OES (Inductively coupled plasma optical emission spectroscopy, ThermoFisher ICAP7200HS). The femtosecond transient absorption spectroscopy (fs-TAS) experiments were performed based on a commercial fs-Ti/Sapphire regenerative amplifier laser system (Vitara, Astrella, Coherent Inc.) and an automated data acquisition transient absorption spectrometer (Helios Fire/EOS, Ultrafast Systems). The samples were photoexcited under 350 nm pump laser and probed at the wavelength range from 410 to 500 nm. The Fourier transform infrared (FTIR) spectroscopy characterization was carried out on Nicolet iS50, Thermo Fisher Scientific Inc. The Raman spectra were measured through DXR Raman Microscope (ThermoFisher). X-ray absorption spectroscopy (XAS) data from the Cu K side were collected at room temperature at beam 1WIB of the Beijing Synchrotron Radiation Facility (BSRF). The typical energy of the storage ring on the BSRF is 2.5 keV. The Si (111) twin monochromator was calibrated with a platinum sheet and then XAS data relative to the reference spectrum was collected. XAS analysis of the sample was performed using Lytle detector in fluorescence mode. Data analysis was performed by Athena and Artemis software included in IFFEFIT0.8.012. The theoretical scattering path for the extended X-ray absorption fine structure spectral curve fitting analysis is generated by the fast first shell theory. The electrochemical measurements were performed on CHI 660E electrochemical working station. The photocurrent was measured under the illumination of NIR light (infrared ray lamp bulb with wavelength range 760 – 1500 nm with intensity of 30 mW cm^{-2}). The electrochemical impedance spectra (EIS) were measured using a PGSTAT 302N Autolab Potentiostat/Galvanostat (Metrohm) equipped with a frequency analyzer module (FRA2) with an excitation signal of 10 mV amplitude. Afterward, the Mott–Schottky plots, which were impedance vs. potential measurement were performed at a fixed frequency of 5 kHz.

Fabrication of $\text{Cu}_1\text{-TiO}_{2-x}/\text{Z-MOF}$

Z-MOF on $\text{Cu}_1\text{-TiO}_{2-x}$ was synthesized using the method reported in the literature^{45,46}. Briefly, terephthalic acid (BDC, 16.6 mg, 0.1 mmol), and HCl ($100 \mu\text{L}$, 0.1 mol/L) were dissolved in 10 mL of a mixture of N, N-dimethylformamide (DMF, 9 mL) and methanol (MeOH, 1 mL) in a 50 mL Teflon-lined autoclave. Then, the solution was sonicated for 20 min. Then, the $\text{Cu}_1\text{-TiO}_{2-x}$ sample was added to the aforementioned

solution. The sealed vessel was maintained at 150°C for 24 h. The resulting MIL-125(Ti) was washed with DMF three times and dried in a vacuum oven at 80°C overnight. As-prepared MIL-125(Ti) was reacted with amino-p-dibenzoic acid at 55°C for 12 h to obtain $\text{NH}_2\text{-MIL-125(Ti)}$. The $\text{NH}_2\text{-MIL-125(Ti)}$ was then put into solution containing 2.5 g of propane sultone and 12.5 mL of acetone, after maintained at 60°C under stirring condition for 12 h, the zwitterionic MOF (Z-MOF) was prepared.

PEC detection of TET by $\text{Cu}_1\text{-TiO}_{2-x}$

The PEC characterization of $\text{Cu}_1\text{-TiO}_{2-x}$ were evaluated using a three-electrode configuration with the $\text{Cu}_1\text{-TiO}_{2-x}$, Ag/AgCl and Pt foil as working, reference, and counter electrode, respectively. The supporting electrolyte used was $0.1 \text{ M Na}_2\text{SO}_4$ ($\text{pH} = 7.4$) solution. The transient photocurrent response was evaluated under chopped light irradiation (light on/off cycles: 60 s) at a fixed electrode potential of 0.5 V vs. Ag/AgCl. The photocurrent was measured under an irradiation from an infrared light whose wavelength was ≥ 800 nm. The reproducibility and long-term stability of the $\text{Cu}_1\text{-TiO}_{2-x}$ were also tested by studying the current response intermittently in a period of 10 days.

In vivo PEC detection

All procedures involving animals were conducted with approval of the Animal Ethics Committee in East China Normal University, China. The male Wistar mice were anesthetized using chloral hydrate. Meanwhile, the Wistar mouse was wrapped in a heating pad to maintain the body temperature at 37°C . The transient photocurrent response was evaluated under chopped light irradiation (light on/off cycles: 20 s) at 0 V potential. The photocurrent was measured under an irradiation from an infrared light. The light source is an infrared lamp with a wavelength range between 760 – 1500 nm. At a position 15 cm from the light source, the optical power density is 30 mW cm^{-2} . The exposure time for each test cycle is 20 s. The irradiance dose per test cycle is 0.6 J cm^{-2} .

Construction and experimental grouping of *Escherichia coli* infected wound model mice

BALB/c mice (6 – 8 weeks old, purchased from the Laboratory Animal Center of East China Normal University) were shaved and skin wounds (8 mm in diameter) were established according to the standard procedures. After wound formation, $20 \mu\text{L}$ of *E. coli* MG1655 suspension (10^7 CFU/mL) was added to the wound area. *E. coli* wound model mice were successfully constructed after 12 h of inoculation. Subsequently, the mice were randomly placed in four cages ($n = 6/\text{group}$), labeled as PBS group (PBS treatment), conventional TET (C-TET) group (intravenous injection of 11.25 mg/kg TET, equivalent to 500 nmol, once daily), and TDM-CM group (therapeutic drug monitoring complete metabolism TET administration, intravenous injection of TET to maintain the blood concentration) and TDM-HM group (therapeutic drug monitoring half-metabolism TET administration, which refers to the reduction of TET concentration to half of its initial value and then administered, intravenous injection of TET to maintain high blood medicine concentration).

Measurement of the number of colonies in the wound

To evaluate the antibacterial effects of C-TET, TDM-CM, and TDM-HM treatments on bacteria at wound sites, mice were euthanized 13 days after drug treatment, and wound skin tissue was removed and weighed under sterile conditions. The equal-weight tissue was homogenized with ultrasonic crusher, and the tissue diluent was inoculated on LB AGAR plate and cultured at 37°C for 24 h for microbial count.

In vivo toxicity evaluation

After 13 days of treatment, all mice were euthanized, and gross anatomical observation was carried out to observe the changes of tissues

and organs (heart, liver, spleen, lung, kidney) of each group. Some other tissues and organs were fixed in formaldehyde solution, and paraffin slices were prepared for H&E (hematoxylin-eosin) staining for histopathological examination.

DFT calculation

All the calculations were carried out based on density functional theory (DFT) implemented in DS-PAW software. The electron-ion interactions were described by the projector augmented wave (PAW) method and the exchange correlation energy was described within Perdew-Burke-Ernzerhof (PBE) functional in the generalized gradient approximation (GGA). To avoid the interaction between two layers, a 15 Å vacuum layer was added at the top of surface. Only the top layer was relaxed during the optimization, and the bottom layer was fixed as the bulk layer. The surface optimization was done with an energy cutoff of 400 eV with a k-mesh of $3 \times 3 \times 1$, and an energy and force convergence of 1×10^{-5} eV and 0.02 eV/Å respectively. Van der Waals (vdW) interaction was considered at the DFT-D3 level as proposed by Grimme. The adsorption of molecules on the surfaces was computed under the same setting as mentioned before in the optimization of the bare surfaces. The adsorption energy of the molecules on the surfaces were calculated as:

$$E_{\text{abs}} = E_{\text{molecule@slab}} - E_{\text{slab}} - E_{\text{molecule}} \quad (1)$$

Atomic charges were calculated from electronic structure by Bader charge analysis. Differential charges were used to study the electron transfer.

Statistical analysis

All data analyses were performed in Origin 8.5 and statistical significance was defined as a two-tailed *p* value of less than 0.05 by *t*-test as description in comparison of two groups.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data that support the findings of the study are included in the main text and Supplementary Information files. Source data are provided with this paper.

References

- Li, J. et al. A tissue-like neurotransmitter sensor for the brain and gut. *Nature* **606**, 94–101 (2022).
- Ogata, G. et al. A microsensing system for the in vivo real-time detection of local drug kinetics. *Nat. Biomed. Eng.* **1**, 654–666 (2017).
- Lin, S. et al. Wearable microneedle-based electrochemical aptamer biosensing for precision dosing of drugs with narrow therapeutic windows. *Sci. Adv.* **8**, eabq4539 (2022).
- Zhang, J. F. et al. An ultrasensitive biosensor for high-resolution kinase activity imaging in awake mice. *Nat. Chem. Biol.* **17**, 39–46 (2021).
- Clifford, A. et al. Strategies for biomolecular analysis and continuous physiological monitoring. *J. Am. Soc. Chem.* **143**, 5281–5294 (2021).
- Zhang, Z. et al. Development of nucleic-acid-based electrochemical biosensors for clinical applications. *Angew. Chem. Int. Ed.* **61**, e202212496 (2022).
- Minopoli, A. et al. Ultrasensitive antibody-aptamer plasmonic biosensor for malaria biomarker detection in whole blood. *Nat. Commun.* **11**, 6134 (2020).
- DeLoache, W. C. et al. An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. *Nat. Chem. Biol.* **11**, 465–471 (2015).
- Wang, D. et al. An aptamer-based depot system for sustained release of small molecule therapeutics. *Nat. Commun.* **14**, 2444 (2023).
- Huang, P. J. & Liu, J. Simultaneous detection of L-lactate and D-glucose using DNA aptamers in human blood serum. *Angew. Chem. Int. Ed.* **62**, e202212879 (2023).
- Rong, G., Corrie, S. R. & Clark, H. A. In vivo biosensing: progress and perspectives. *ACS Sens.* **2**, 327–338 (2017).
- Wei, H. et al. Galvanic redox potentiometry based microelectrode array for synchronous ascorbate and single-unit recordings in rat brain. *Anal. Chem.* **92**, 10177–10182 (2020).
- Zhao, W. W., Xu, J. J. & Chen, H. Y. Photoelectrochemical DNA biosensors. *Chem. Rev.* **114**, 7421–7441 (2014).
- Shu, J. & Tang, D. Recent advances in photoelectrochemical sensing: from engineered photoactive materials to sensing devices and detection modes. *Anal. Chem.* **92**, 363–377 (2020).
- Liu, J., Fu, B. & Zhang, Z. Ionic current rectification triggered photoelectrochemical chiral sensing platform for recognition of amino acid enantiomers on self-standing nanochannel arrays. *Anal. Chem.* **92**, 8670–8674 (2020).
- Fu, B. & Zhang, Z. Rationally engineered photonic-plasmonic synergistic resonators in second near-infrared window for in vivo photoelectrochemical biodetection. *Nano. Lett.* **19**, 9069–9074 (2019).
- Wu, W. & Zhang, Z. Defect-engineered TiO₂ nanotube photonic crystals for the fabrication of near-infrared photoelectrochemical sensor. *J. Mater. Chem. B* **5**, 4883–4889 (2017).
- Lu, S., Fu, B. & Zhang, Z. Zwitterionic polymers coating anti-biofouling photoelectrochemical aptasensor for in vivo antibiotic metabolism monitoring and tracking. *Anal. Chem.* **94**, 14509–14516 (2022).
- Ye, X. et al. FRET modulated signaling: a versatile strategy to construct photoelectrochemical microsensors for in vivo analysis. *Angew. Chem. Int. Ed.* **60**, 11774–11778 (2021).
- Tao, L. et al. Implantable optical fiber microelectrode with anti-biofouling ability for in vivo photoelectrochemical analysis. *Chin. Chem. Lett.* **34**, 107481 (2023).
- Xu, J. et al. A smartphone-integrated method for visual detection of tetracycline. *Chem. Eng. J.* **416**, 127741 (2021).
- He, C. et al. Labile Cu(I) catalyst/spectator Cu(II) species in copper-catalyzed C–C coupling reaction: operando IR, in situ XANES/EXAFS evidence and kinetic investigations. *J. Am. Chem. Soc.* **135**, 488–493 (2013).
- Xin, Y. et al. Recognition unit-free and self-cleaning photoelectrochemical sensing platform on TiO₂ nanotube photonic crystals for sensitive and selective detection of dopamine release from mouse brain. *Biosens. Bioelectron.* **87**, 396–403 (2017).
- Zhao, Y., Wu, W., Fu, B., Gan, L. & Zhang, Z. Rationally design of near infrared light responsive micro-photoelectrodes for in vivo sensing of neurotransmitter molecules in mouse brain. *Chin. J. Chem.* **38**, 275–281 (2020).
- Qiao, B. et al. Single-atom catalysis of CO oxidation using Pt₁/FeO_x. *Nat. Chem.* **3**, 634–641 (2011).
- Zhang, Y. Heterogeneous catalysis: single atoms on a roll. *Nat. Rev. Chem.* **2**, 0151 (2018).
- Lee, B. H. et al. Reversible and cooperative photoactivation of single-atom Cu/TiO₂ photocatalysts. *Nat. Mater.* **18**, 620–626 (2019).
- Fang, Y. et al. Dual activation of molecular oxygen and surface lattice oxygen in single atom Cu₁/TiO₂ catalyst for CO oxidation. *Angew. Chem. Int. Ed.* **61**, e202212273 (2022).

29. Zhu, C., Fu, S., Shi, Q., Du, D. & Lin, Y. Single-atom electrocatalysts. *Angew. Chem. Int. Ed.* **56**, 13944–13960 (2017).
30. Tong, S., Fu, B., Gan, L. & Zhang, Z. Single atom catalysts for boosting electrocatalytic and photoelectrocatalytic performances. *J. Mater. Chem. A* **9**, 10731–10738 (2021).
31. Fu, B., Wu, W., Gan, L. & Zhang, Z. Bulk/surface defects engineered TiO₂ nanotube photonic crystals coupled with plasmonic gold nanoparticles for effective in vivo near-infrared light photoelectrochemical detection. *Anal. Chem.* **91**, 14611–14617 (2019).
32. Li, Z., Zhou, X., Yang, J., Fu, B. & Zhang, Z. Near-infrared-responsive photoelectrochemical aptasensing platform based on plasmonic nanoparticle-decorated two-dimensional photonic crystals. *ACS Appl. Mater. Interfaces* **11**, 21417–21423 (2019).
33. Fan, Q., Zhang, X., Ge, X., Bai, L. & He, D. Manipulating Cu nanoparticle surface oxidation states tunes catalytic selectivity toward CH₄ or C₂₊ products in CO₂ electroreduction. *Adv. Energy Mater.* **11**, 2101424 (2021).
34. Yu, Y. et al. Synergistic effect of Cu single atoms and Au–Cu alloy nanoparticles on TiO₂ for efficient CO₂ photoreduction. *ACS Nano* **15**, 14453–14464 (2021).
35. Li, P. et al. Supramolecular confinement of single Cu atoms in hydrogel frameworks for oxygen reduction electrocatalysis with high atom utilization. *Mater. Today* **35**, 78–86 (2020).
36. Janković, I., Šaponjić, Z., Čomor, M. & Nedeljković, J. Surface modification of colloidal TiO₂ nanoparticles with bidentate benzene derivatives. *J. Phys. Chem. C* **113**, 12645–12652 (2009).
37. Thelakkat, M. & Schmidt, H. Synthesis and properties of novel derivatives of 1,3,5-tris(diarylamino)benzenes for electroluminescent devices. *Adv. Mater.* **10**, 219–223 (1998).
38. Brognara, A., Bricchi, B., William, L., Brinza, O. & Konstantakopoulou, M. New mechanism for long photo-induced enhanced Raman spectroscopy in Au nanoparticles embedded in TiO₂. *Small* **18**, 2201088 (2022).
39. Jin, D., Bai, Y., Chen, H., Liu, S. & Chen, N. SERS detection of expired tetracycline hydrochloride with an optical fiber nano-probe. *Anal. Methods* **7**, 1307–1312 (2015).
40. Tang, J., Durrant, J. & Klug, D. Mechanism of photocatalytic water splitting in TiO₂. Reaction of water with photoholes, importance of charge carrier dynamics, and evidence for four-hole chemistry. *J. Am. Chem. Soc.* **130**, 13885–13891 (2008).
41. Jankulovska, M., Berger, T., Lana-Villarreal, T. & Gómez, R. A comparison of quantum-sized anatase and rutile nanowire thin films: devising differences in the electronic structure from photoelectrochemical measurements. *Electrochim. Acta* **62**, 172–180 (2012).
42. Li, S. et al. In situ construction of elemental phosphorus nanorod-modified TiO₂ photocatalysts for efficient visible-light-driven H₂ generation. *Appl. Catal. B Environ.* **297**, 120412 (2021).
43. Blöchl, P. E. Projector augmented-wave method. *Phys. Rev. B* **50**, 17953 (1994).
44. Zeng, Y. et al. Bacteriophage as an alternative to antibiotics promotes growth performance by regulating intestinal inflammation, intestinal barrier function and gut microbiota in weaned piglets. *Front. Vet. Sci.* **8**, 623899 (2021).
45. Dai, Z., Guo, J., Zhao, C., Gao, Z. & Song, Y. Y. Fabrication of homochiral metal–organic frameworks in TiO₂ nanochannels for in situ identification of 3,4-dihydroxyphenylalanine enantiomers. *Anal. Chem.* **93**, 11515–11524 (2021).
46. Wang, C. et al. Zwitterionic metal–organic frameworks modified polyamide membranes with enhanced water flux and antifouling capacity. *Chemosphere* **309**, 136684 (2022).

Acknowledgements

This research was supported by National Natural Science Foundation of China (No. 22174045).

Author contributions

X.X. and Z.Z. designed the experiments. X.X. and S.L. carried out the synthesis of photoelectrode and photoelectrochemical measurements. X.X. and X.Z. performed the morphological and elemental characterizations. D.X. and S.Y. performed the DFT calculations. All authors wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41467-024-53154-z>.

Correspondence and requests for materials should be addressed to Zhonghai Zhang.

Peer review information *Nature Communications* thanks the anonymous reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

Reprints and permissions information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2024