Supplementary Information *for*

Evaluating Effectiveness of Electron Shuttles in Environments with a WO_3 Nanoprobe

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This SI contains a 14-page document, including a 4-page description of the WO_3 synthesis, test paper characterization, membrane fabrication and bacterial culture, 1 table, 7 Fig.s, and this cover page.

Synthesis of WO_3 Nanorods

The reagents in this study were purchased from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). All solutions were prepared using the ultrapure water from a Milli-Q system (Millipore, Nillerica, USA) with a resistivity of 18.2 MΩ/cm. The tungsten trioxide (WO_3) was synthesized by a hydrothermal process according to the protocol of Wang et al. Briefly, 0.825 g of $Na_2WO_4 \cdot 2H_2O$ and 0.290 g of NaCl were dissolved in 20 mL of deionized water (Milli-Q, Millipore Inc., USA), followed by slowly adding 3 M HCl under continuous magnetic stirring. The solution was then transferred into a 45-mL Teflon-lined autoclave after adjusting pH to 2.0. After 24-h hydrothermal reaction at 180 °C, the system was naturally cooled to room temperature (25 ± 2 °C). The obtained product was repeatedly washed with deionized water for three times and then dried at 70 °C.

The WO_3 suspension was prepared by adding 1.25 g of WO_3 nanorods into 50-mL deionized water, followed by 20-min ultrasonic dispersing.

Characterizations of WO_3 Nanorods

X-ray powder diffraction (XRD) patterns were obtained using Smartlab diffractometer (Rigaku Co., Japan) between 10°-70° (2θ) with a scan step of 10° min^{-1} to identify the purity and phase of the prepared crystalline WO_3. The tests were conducted at 40 kV and 200 mA using Cu Kα radiation (λ = 1.5418 Å). The as-synthesized WO_3 nanorods were imaged by scanning electron microscopy (SEM, JSM-6700F, JEOL Co., Japan) using an accelerating voltage of 20 kV. Before imaging, the

samples were coated with Pt by a sputtering machine at a beam current of 20 mA for 45 s. The morphology of WO_3 nanorods was observed by transmission electron microscopy (TEM, JEM 2011, JEOL Co., Japan) with an accelerating voltage of 100 kV.

Spectroelectrochemical Analysis of WO_3

The Nafion/WO_3 hybrid membrane was prepared by a solution casting process. First, WO_3 was dispersed in a 5 wt% Nafion solution, and then the suspension was applied on a piece of glass. The solvent was then removed by heating at 80 °C. The thin film would be used in water contact angle measurements as the control group.

A platinum wire was used as the counter electrode, while an Ag/AgCl electrode was selected as the reference electrode. The cyclic voltammetry (CV) curves were obtained on an electrochemical workstation (CHI760D, CHI Instruments Co., China). *In situ* electrochemical absorption at 400 nm was recorded on a UV-visible spectrophotometer (UV-2450, Shimadzu Co., Japan), and the electrolysis with a constant potential was conducted in a solution containing 0.1 M Na_2SO_4.

Bacterial Strains and Cultures

Bacteria were cultivated aerobically in Luria-Bertani broth. The culture was grown at 30 °C and agitated at a rate of 150 rpm until the late stationary phase. The culture supernatants of Δbfe and Oef were collected by centrifugation at 5000 rpm for 5 min and the residual cells were filtered by filter membrane (0.22 μm) to remove the residual

cells.

A sterilized mineral salt medium without any electron acceptor or donor was used for dilution. The microbial electrochromic experiments were operated in minerals solution while supplemented with 20 mM acetate as an electron donor. Mineral solution contained: NH_4Cl 1.5 g/L, KCl 0.1 g/L, $CaCl_2$ 0.05 g/L, K_2HPO_4 0.225 g/L, KH_2PO_4 0.225 g/L, $MgSO_4·7H_2O$ 0.117 g/L, $NaHCO_3$ 2.5 g/L, HEPES 11.867 g/L and 10 mL trace element solution, which was buffered to pH 7.0 by adding KOH solution (5 mol/L). The trace element stock solution contained (per liter): nitrilotriacetic 1.5 g, $MnCl_2·4H_2O$ 0.1 g, $FeSO_4·7H_2O$ 0.3 g, $CoCl_2·6H_2O$ 0.17 g, $ZnCl_2$ 0.1 g, $CuSO_4·5H_2O$ 0.04 g, $KAl(SO_4)_2·12H_2O$ 0.005 g, $NaMoO_4$ 0.009 g, H_3BO_3 0.005 g, $NiCl_2$ 0.12 g, $NaWO_4·2H_2O$ 0.02 g, Na_2SeO_4 0.1 g. All the mediums were purged with high purity N_2 for 20 min and autoclaved for 20 min at 121 °C.

Obtained bacterial cultures were centrifuged at 5,000 rpm for 6 min to collect the cells. After washed three times with sterilized mineral salt medium and re-suspended in sterilized mineral salt medium., the cells were centrifuged, resuspended in sterilized mineral salt medium supplemented with electron acceptor. The bacteria concentration was determined by their optical density at 600 nm wavelength (OD_{600}). The suspension of *S. oneidensis* MR-1 was adjusted to an OD_{600} value of 20.

Preparation of Actual Samples

Escherichia coli, *Tetrahymena pyriformis*, and *Canis lupus familiaris* were selected as the representative species of prokaryotes, single-celled eukaryotes, and mammals, respectively. The supernatants of *E. coli* and *Tetrahymena pyriformis* were

collected by centrifugation (6000 rpm, 5 min) and then filtrated through a 0.22 μm filter membrane. The feces extracts of *Canis lupus familiaris* were obtained using ethyl acetate extraction followed by extraction through solid-phase micro-extraction for gas chromatography-mass spectrometer (GC-MS) analysis. The ESs were determined by GC-MS (7980B GC System, Agilent Co., USA) using a non-polar J&M DB 5MS 0.25 μm, 30 m × 0.25 mm column with a temperature ramp of 50 ˚C for 3 min, 2 °C/min up to 280 °C holding for 5 min.

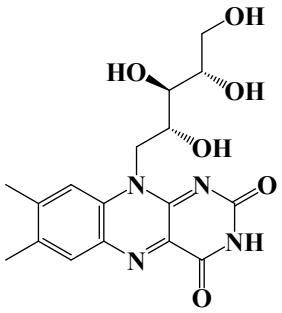
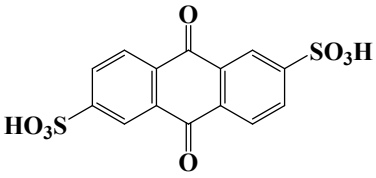
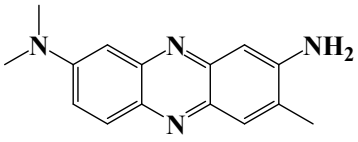
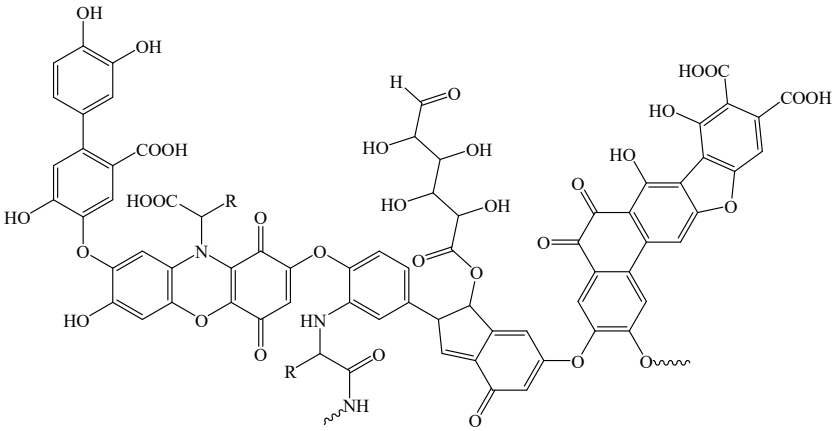
Preparation and Characterizations of the Test Paper

Sodium alginate was oven-dried at 70 °C for 24 h, and then dissolved in deionized water (0.25 wt. %), which was heated in a water bath at 80 °C for 20 min until the feculent suspension turned clear. Next, the WO_3 nanorods were suspended in deionized water (2.5 wt. %) under continuous ultrasonic dispersion for 20 min. Thereafter, sodium alginate solution was added into the WO_3 suspension (volume ratio = 4:1) under continuous magnetic stirring. As shown in **Fig. S1**, the mixture was poured onto a clean flat indium tin oxide (ITO) glass and evenly spread by gravity within 30 s, followed by immersing into a $CaCl_2$ solution (2 wt. %). After soaking for 2 h, the ITO glass was dried at 65 °C for 5 min. Later, the prepared test paper was peeled from the glass and stuck onto a clean glass wall until dry. Finally, the test paper was cut into 8 mm × 30 mm pieces for the subsequent experiments.

The wettability of the test paper was measured by a water contact angle system (JC 2000G, Zhongchen Co., China). The test paper was imaged using SEM (JSM-6700F, JEOL Co., Japan) and its elemental configuration was analyzed by SEM with energy

dispersive spectroscopy (Phenom ProX, Thermo Fisher Scientific Inc., USA). To examine whether the WO_3 nanorods were embedded successfully, the surface roughness profile was measured by atomic force microscopy (SmartSPM, Horiba Co., Japan). The diffusion rate in the test paper was measured in a permeation test cell with RF as the model ES. The RF concentration was measured every 3 min by recording the fluorescence spectra of the solution on both sides of the test paper using an Aqualog spectrometer (Horiba Co., Japan). The mechanical strength of the fabricated test paper was measured (DMA Q800, TA Instruments Co., USA).

Table S1. Structural formula of mentioned ES.

ES	structure formula
RF	
AQDS	
neutral Red	
humic acid	

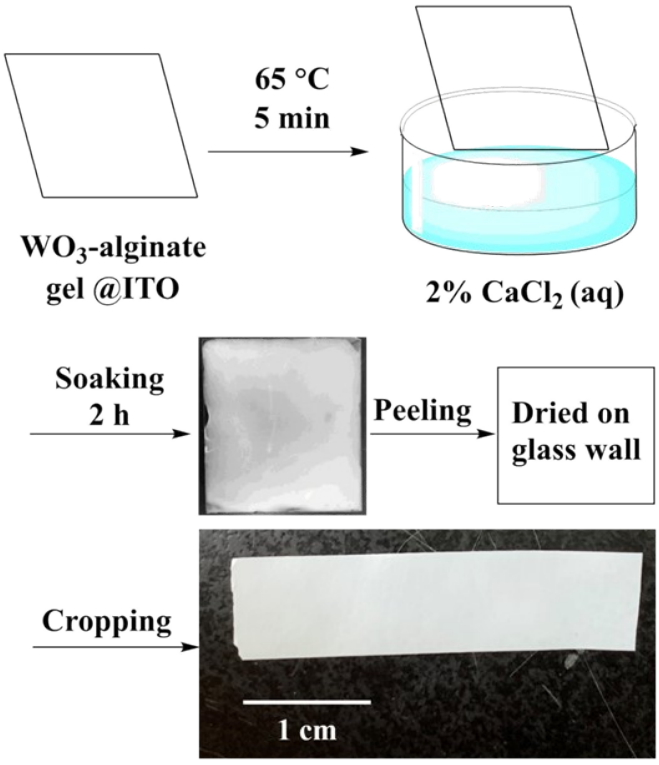


Fig. S1. Workflow of the WO_3 test paper preparation.

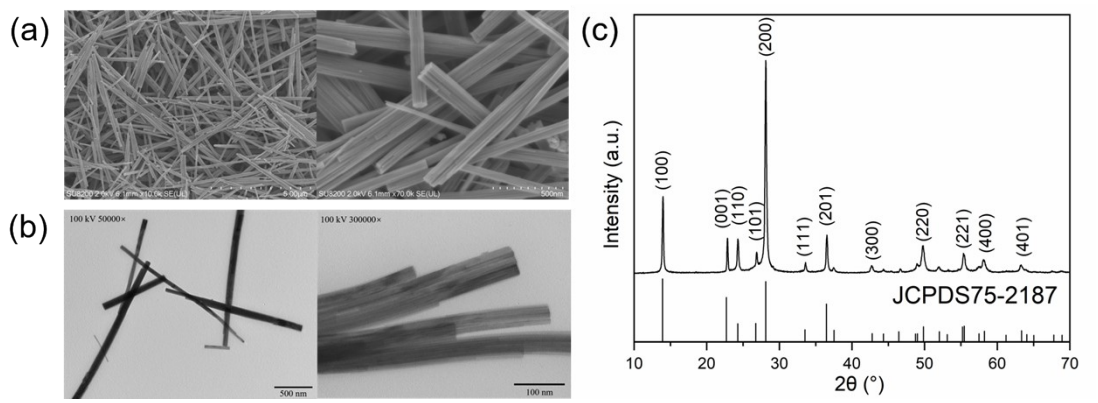


Fig. S2. Characteristics of WO_3 nanorods: (a) SEM image; (b) TEM image; and (c) representative XRD pattern.

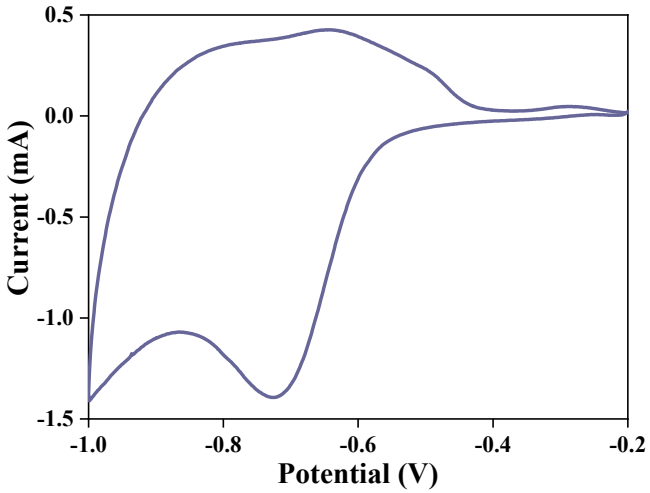


Fig. S3. The CV curve of WO_3 redox reaction.

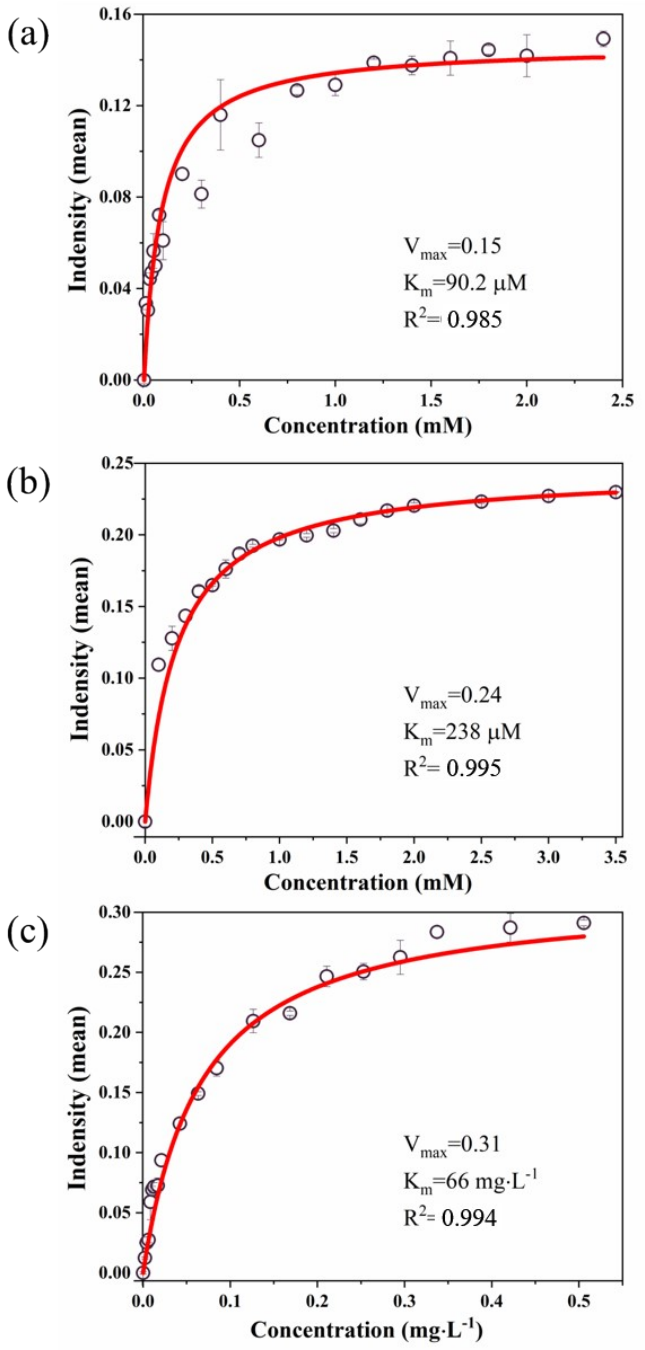


Fig. S4. The effect of concentration on the shuttling rate of ESs in the bioelectrochromism.

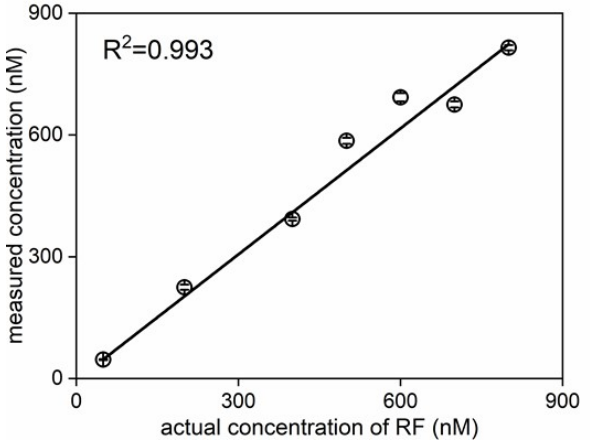


Fig. S5. RF concentration in the AQDS solutions with the amplification of the calculated $K_{shuttle}$.

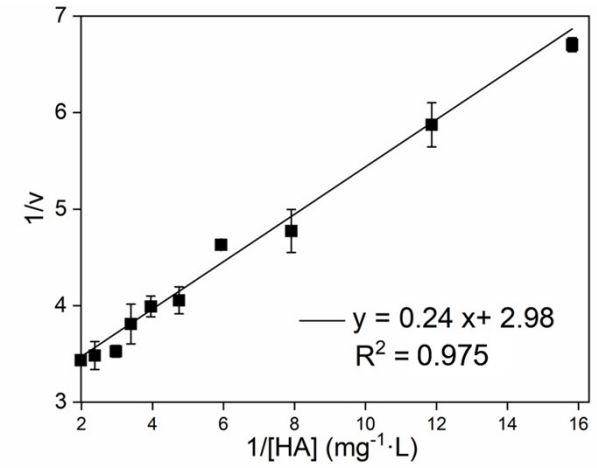


Fig. S6. The double-reciprocal plot of the HA concentration and color change.

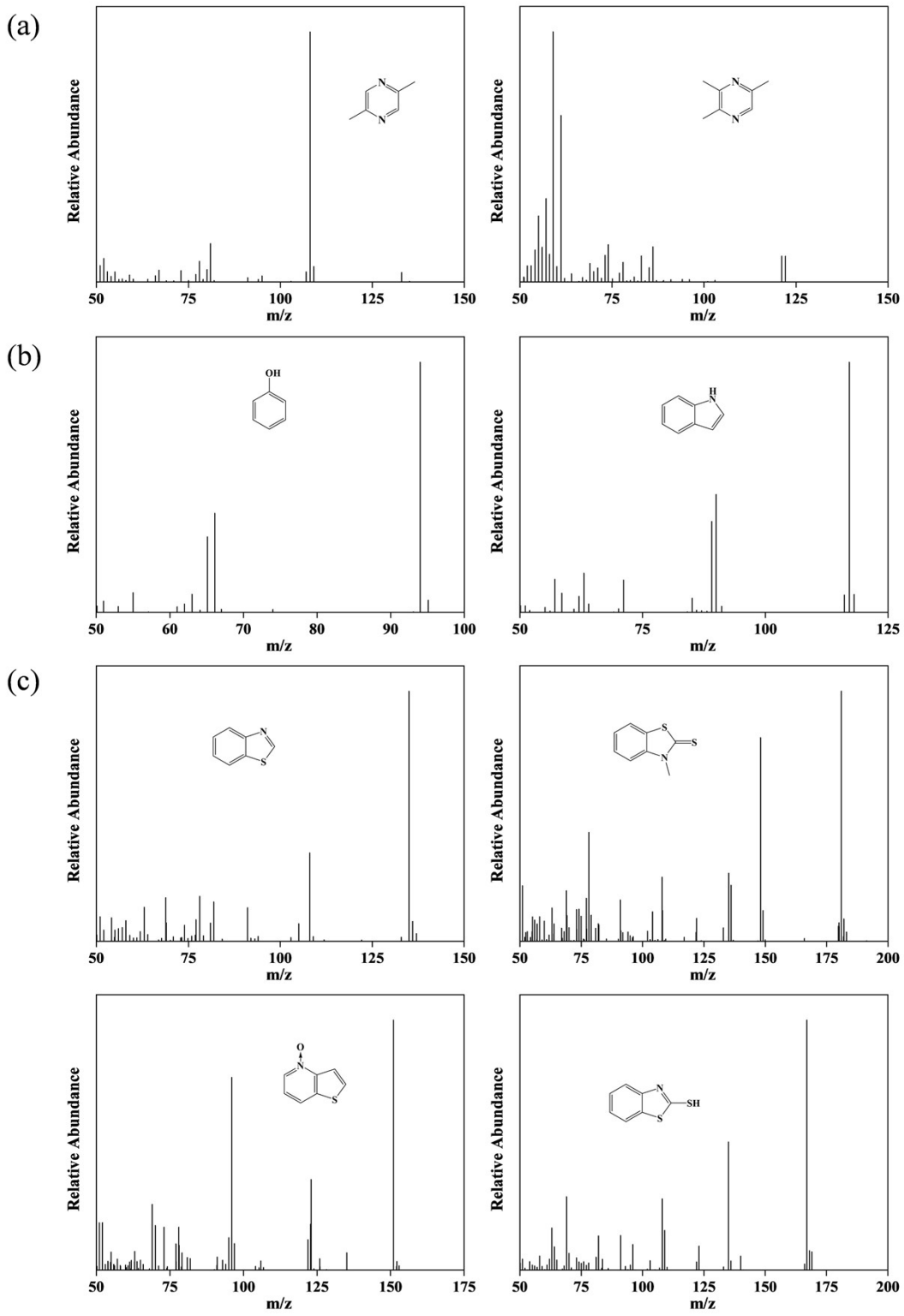


Fig. S7. The results of identification of electron shuttles existing in different solution using GC-MS. (a) Culture supernatant of *Tetrahymena pyriformis*. (b) Feces extract of *Canis lupus familiaris*. (c) Culture supernatant of *E. coli*.