Supporting Information

A Peptide-Based Conductive Hydrogel Capable of *in situ* Bioelectrocatalytic NADH Regeneration for Sustained Production of 1-Propanol

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Experimental section

Materials: Fmoc-FFKG were ordered from Jiangsu Ji tai Peptide Industry Science and Technology Co., Ltd. (Jiangsu, China). Alcohol dehydrogenase (ADH) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). *β*-Nicotinamide adenine dinucleotide (NAD^+) and reduced nicotinamide adenine dinucleotide (NADH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide, Bismuth (III) nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$), 4′,6-diamidino-2-phenylindole (DAPI), Rhodamine B (RhB), Sodium borohydride was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). All chemicals were used without further purification.

Characterization:

Scanning Electron Microscopy (SEM). The morphologies were conducted using a Zeiss Sigma 300 VP instrument. Images were obtained with secondary electron mode at 2 kV.

Transmission electron microscopy (TEM). The morphologies of Bi nanostucture, peptide and Peptide-Bi hydrogel was conducted using a Talos F200X G2 microscope from FEI Corporation, USA. The instrument was operated at a working voltage of 200 kV, and high-resolution transmission electron microscopy (HRTEM), high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), and energy-dispersive spectroscopy (EDS) elemental mapping were obtained. Bi nanoparticles doped into peptide hydrogel were conducted by EDS elemental mapping to ensure the accuracy and reliability of the elemental distribution maps.

The X-ray Diffraction (XRD). XRD analysis were measured on a Bruker D2 Phaser at room temperature with a count of 0.02°/step/0.06. The samples were frozen and lyophilized in polyethylene tubes.

Fluorescence Spectroscopy. Fluorescence emission spectra were measured by Fluorescence

Spectrophotometer F-4700 (Hitachi, Ltd., Japan). It was used to measure the encapsulated efficiency of the Rhodamine B-labeled ADH within Peptide-Bi hydrogel.

Zeta potential. The Zeta potential of assembled peptide based hydrogel were performed using Nano-ZS 90 Nanosizer (Malvern Instruments Ltd., Worcestershire, UK).

Circular Dichroism spectrum (CD). The CD spectra of the pure peptide hydrogel and Peptide-Bi hydrogel was obtained on BRIGHTTIME Chirascan.

X-Ray Photoelectron Spectroscopy (XPS). Measurements were performed on a Thermo Scientific K-Alpha+ spectrometer (Thermo Fisher Scientific, USA) using Al Kα radiation.

UV-Vis Spectroscopy. UV−vis absorbance spectra were detected the signal of NADH at the wavenumber of 340 nm on an UV-vis spectrophotometer (INESA, China).

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H NMR spectra were acquired on were performed on a 400 MHz Bruker superconducting-magnet NMR spectrometer. The amount of doping Bi element was analyzed by inductively coupled plasma optical emission spectrometry (Thermo Fisher iCAP PRO, USA). ^{1}H NMR spectra of final enzymatic production in the electrolyte were quantified by proton nuclear magnetic resonance (Bruker, Germen).

The Confocal Laser Scanning Microscopy (CLSM). CLSM was used to image the location of Rhodamine B-labeled ADH within Peptide-Bi hydrogel using a Nikon Ti2 confocal laser scanning microscope (Nikon, Japan).

Enzyme Catalytic Alcoholization of Propionaldehyde: Alcohol dehydrogenase (ADH) was applied to confirm the NADH generation. During the bioelectrocatalytic alcoholization of propionaldehyde, the Peptide-Bi hydrogel encapsulated enzyme (10 U) was as working electrode (ADH@Peptide-Bi Hydrogel), and the reactive electrolyte was PBS (18 mL, pH 6.5) containing

1.5 mM NAD^+ and 3.0 mM propionaldehyde. Prior to the measurements, all electrolyte solutions were thoroughly degassed by purging with N_2 (99.999%) for 30 min, and the control group was degassed N_2 into the electrolyte stirred at a speed of 200 rpm.

Product quantification: Proton nuclear magnetic resonance (^{1}H NMR) was used for product quantification. The samples were prepared according to a reported method.[1] The 460.0 μL of the reactive electrolyte was mixed with 100.0 μL of D_2O and 100.0 μL dimethylsulfone internal standard (100 ppm) and then added to the NMR tube. Relative concentrations were determined by calculating the integrated signal of methyl group of dimethylsulfone.

Enzyme Activity Assay: To carry out enzymatic activity, 1.0 mg free alcohol dehydrogenase was added into the electrochemical compartment as control group, alcohol dehydrogenase was encapsulated with Peptide-Bi hydrogel as electrocatalytic group. The free alcohol dehydrogenase out of the electrocatalytic system was used as blank group. The amount of NADH was monitored by UV-vis. Reaction aliquots were sampled every 0.5 h to monitor the alcohol dehydrogenase activity in each group. These samples were then 10 times diluted in the buffer solution (pH 7.2) and incubated for 2 min. The enzymatic alcoholization reaction was then initiated by the addition of propionaldehyde (3.0 mM) in the assay solution (pH 6.5, 1.5 mM NADH) and the conversion rate of each group was determined on the relative rate of the consumption of NADH using UV-vis absorption spectra at 340 nm for 2 h.

Cell culture

The biocompatibility of Peptide-Bi hydrogel was tested using mouse fibroblast cells (L929 cells, Sunncell, No: SNL-402). Meanwhile, peptides powder was sterilized by UV-ray before the gelation step for cells culture. According to the above preparation method for peptide-based hydrogel, they were transferred into 24-well plates to construct 3D microenvironment. During the

process of preparing Peptide-Bi hydrogel, the medium solution containing L929 cells was utilized to form the hydrogel after the 12 h incubation. The RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Cell Viability Assay

CCK-8 assay was utilized to assess the pure peptide and Peptide-Bi hydrogels fed Bi concentration with 1.0 mM, 2.0 mM. Briefly, L929 cells were seeded into 96-well plates with RPMI 1640 medium at 1.0×10^5 cells per well for 24 h incubation. Cell medium was replaced with the peptide and Peptide-Bi containing medium at designated concentrations (0, 25, 50, 150, 200, 250, 300 μg mL^{-1}) for another 24 h of incubation. The CCK-8 solution (20.0 μL) was then fed into each well according to manufacturer's protocol. The absorbance of each sample was measured by a microplate reader at 450 nm.

Statistical analysis

The data were presented as means ± standard deviations. SPSS 19.0 software (SPSS, Chicago, IL) was used to perform statistical analysis. The *T-test* was applied to verify the normal distribution of data. The center point of each error bar represents the mean of the triplicate measurements at least.

Supplementary Figures

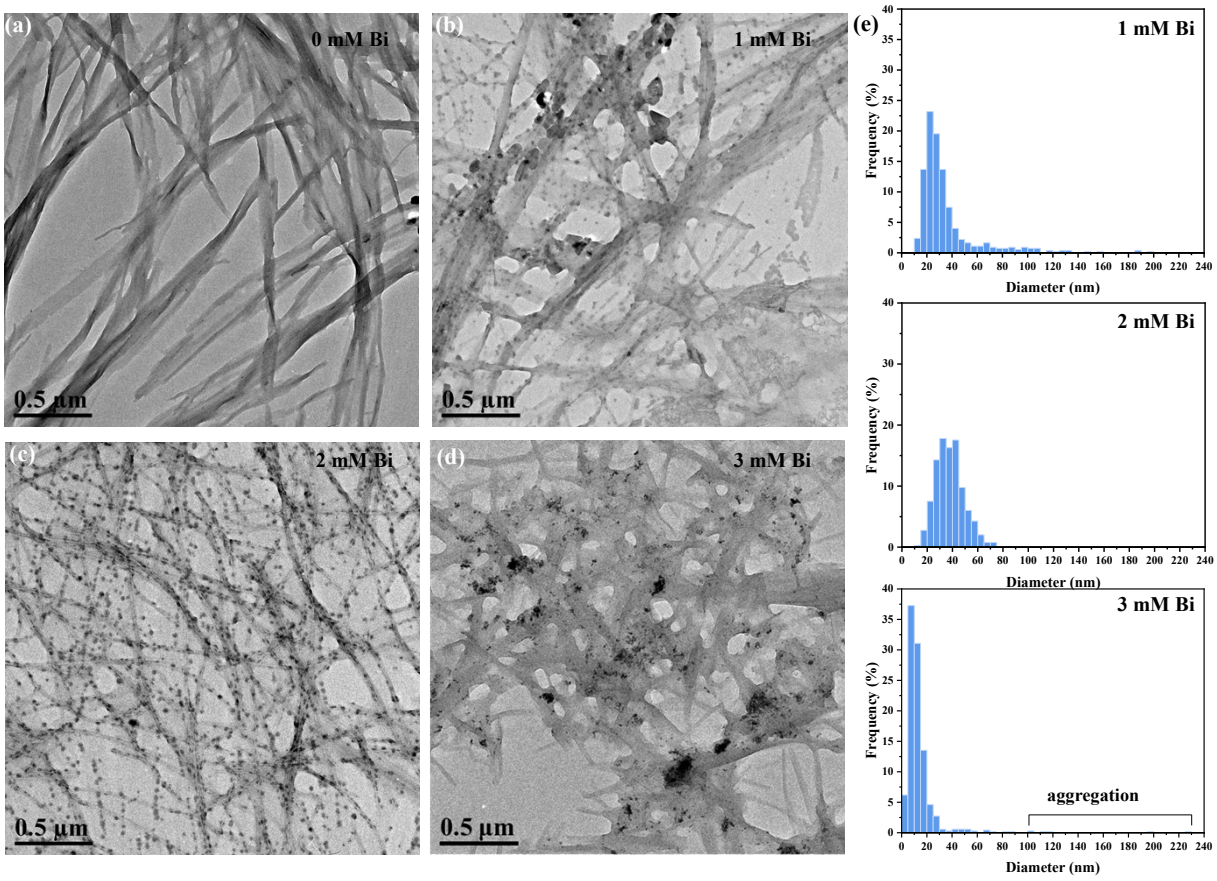

Figure S1. (a-d) The TEM images of peptide based hydrogel fed Bi ions with different concentration. (a) 0 mM, (b) 1.0 mM, (c) 2.0 mM, (d) 3.0 mM Bi concentration. (e) The Bi particle size distributions in the corresponding TEM image, respectively.

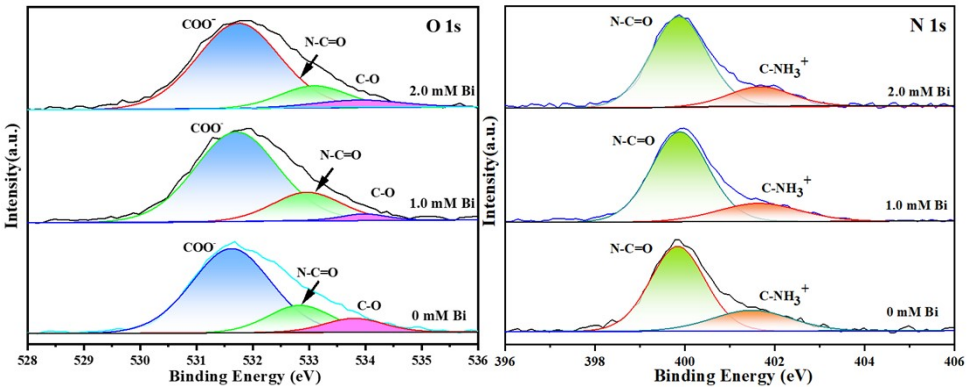

Figure S2. The O 1s and N 1s spectra of peptide hydrogel fed Bi ions with different concentration (1.0 mM, 2.0 mM) and peptide hydrogel.

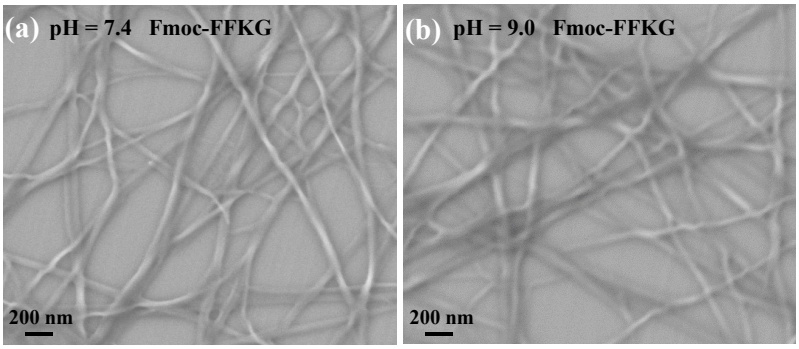

Figure S3. SEM images of assembly structure of Fmoc-FFKG at (a) pH 7.4 and (b) pH 9.0.

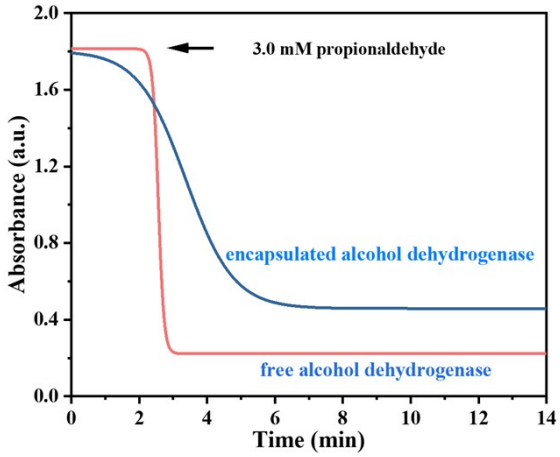

Figure S4. The catalysis activity of (a) free ADH and (b) encapsulated ADH in Peptide-Bi hydrogel.

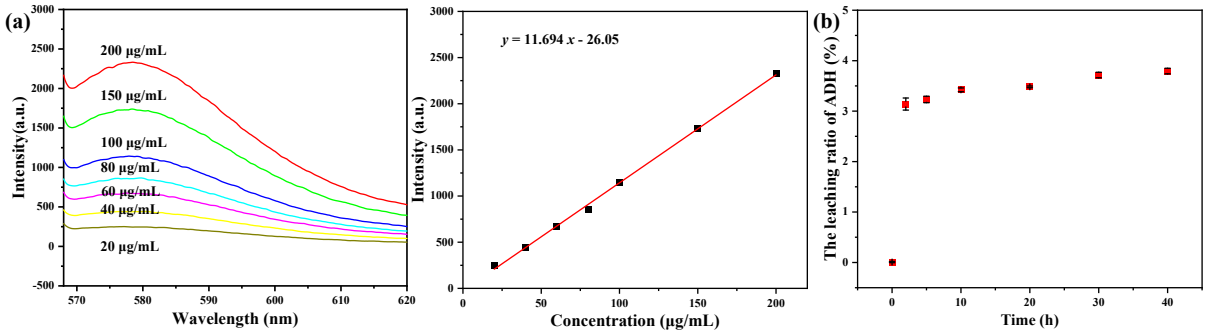

Figure S5. ADH leaching from the network of peptide-Bi hydrogel. (a-b) The standard curve of labbled ADH by RhB (λem = 578 nm). (c) The rate of ADH leaching at different times (0, 2, 5, 10, 20, 30, 40 h).

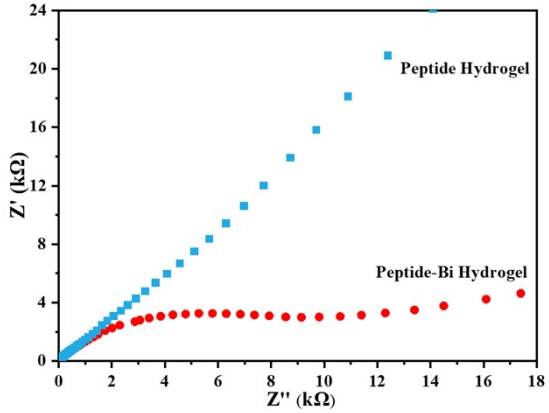

Figure S6. Charge transfer resistances result of Peptide-Bi hydrogel and peptide hydrogel in 1.5 mM NAD^+ electrocatalyte.

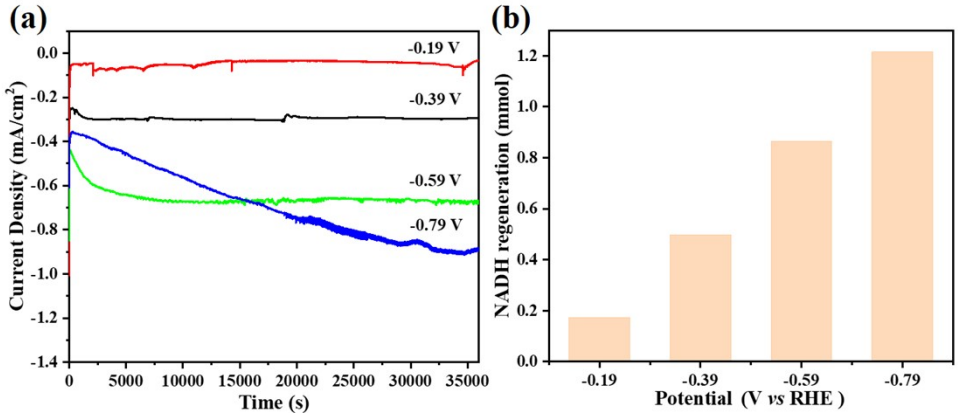

Figure S7. (a) I-t curve at different potentials and (b) and corresponding NADH regeneration.

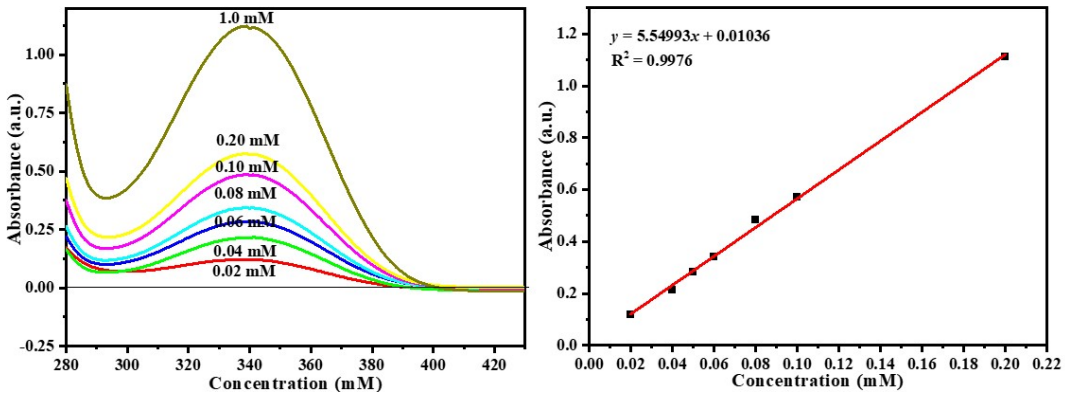

Figure S8. UV-vis absorption spectra of NADH at different concentrations of NADH solution and corresponding standard curve.

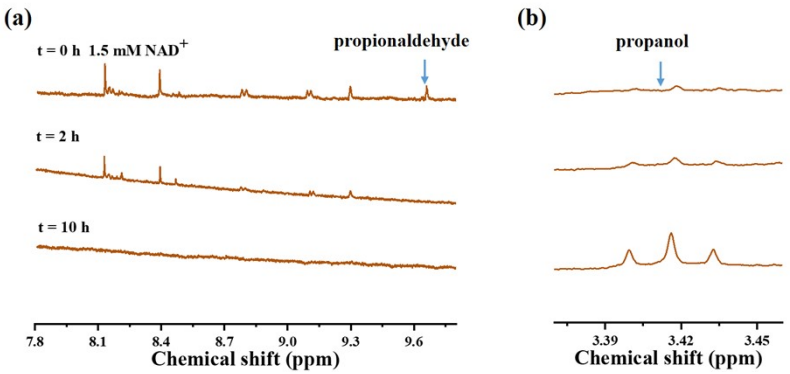

Figure S9. ^{1}H NMR spectra of NADH regeneration of 1.5 mM NAD^{+} in PBS at pH 6.5 with ADH@Peptide-Bi hydrogel. (a) The spectra exhibited the signal of propionaldehyde after different electrocatalytic time (0, 2, 10 h) in 3.0 mM propionaldehyde solution. (b) The spectrum exhibited the signal of propanol after different electrocatalytic time (0, 2, 10 h) in 3.0 mM propionaldehyde solution.

References

[1] A. Kurimoto; S. A. Nasseri; C. Hunt; M. Rooney; D. J. Dvorak; N. E. Lesage; R. P. Jansonius; S. G. Withers; C. P. Berlinguette, Bioelectrocatalysis with a palladium membrane reactor. *Nat. Commun.* **2023,** *14*, 1814.