Supporting Information

Inhibition of cGAS-STING Pathway via an Endogenous Copper Ion-Responsive Covalent Organic Framework Nanozyme for Alzheimer's Disease Treatment

Haochen Zhang,[1,2] Junlin Ya,[1,2] Mengyu Sun,[1,2] Xiubo Du,[3] Jinsong Ren,[1,2] and Xiaogang Qu*[,1,2]

[1]Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, P. R. China

[2]School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, P. R. China

[3]College of Life Sciences and Oceanography, Shenzhen Key Laboratory of Microbial Genetic Engineering, Shenzhen University, Shenzhen 518060, China

*Corresponding author.
E-mail: xqu@ciac.ac.cn

Experimental Section

Materials

[2,2'-bipyridine]-5,5'-dicarbaldehyde, 4,4',4",4"'-(Pyrene-1,3,6,8-tetrayl) tetraaniline were purchased from Yanshen Technology Co., Ltd (Jilin, China). Anhydrous mesitylene, anhydrous dioxane and NADH were purchased from Aladdin (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), 1,4-dimethylpyridinium iodide, piperidine, and 3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Aβ42 peptides were purchased from NovoPep Limited (Shanghai, China). Octapeptide Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp (KLVFFAED) was customized from Sangon Biotechnology Co. (Shanghai, China). BCA protein assay kit, ROS assay kits, mitochondrial membrane potential assay kit, NAD+/NADH assay kit, beta tubulin rabbit monoclonal antibody, STING rabbit monoclonal antibody were purchased from Beyotime Biotechnology (Shanghai, China). cGAS rabbit polyclonal antibody was purchased from ABclonal Technology Co.,Ltd (Hubei, China). Other chemicals were purchased from Aladdin (Shanghai, China). Milli-Q water (18.2 MΩ; Millpore Co. USA) was used to prepare all buffers. Cell culture dishes and cell culture plates were bought from Guangzhou Jet Bio-Filtration Co., Ltd. All the chemical reagents were analytical grade and directly used without further purification unless otherwise specified.

Measurements and Characterizations

Fourier transform infrared (FT-IR) analysis was carried out on a Bruker Vertex 70 FT-IR spectrometer. The scanning electron microscope (SEM) images were taken by a Hitachi S-4800 FESEM at working voltage of 10 kV and working current of 10 A. Ultraviolet-Visible (UV-Vis) absorption spectra were recorded with a JASCO-V550 spectrometer. Powder crystal X-ray diffraction (PXRD) was performed Rigaku-Dmax 2500 diffractometer by using CuKα radiation. Adsorption isotherms of CO_2 were measured using the ASAP 2020 from Micromeritics Co. Ltd. Zeta potential and dynamic light scattering (DLS) measurements were performed on a Zetasizer 3000HS analyzer. Fluorescence spectrum was obtained with a JASCO FP-6500 spectrofluorometer. An Olympus BX-51 optical equipped with a CCD camera was used for capturing fluorescence images. X-ray photoelectron spectroscopy (XPS) data were acquired with a Perkin Elmer PHI 5600 instrument, while inductively coupled plasma mass spectrometry (ICP-MS) analyses were conducted using a TheroScientific Xseries instrument. The confocal laser scanning microscopy (CLSM) characterization was acquired by a (Nikon Eclipse Ni-E, Japan) top-of- the-line motorized upright. The flow cytometry data was obtained by BD LSRFortessa™ Cell Analyzer. Morris water maze (MWM) test was performed on WMT-100S Morris water maze (TECHMAN Co., LTD, Chengdu, China).

Synthesis of Py-BPy-COF: Py-TA (0.02 mmol, 11.3 mg) and 2,2'-BPy-DCA (0.04 mmol, 8.5 mg) were mixed in a Pyrex tube (10 mL) with a solution of mesitylene/dioxane/6M HOAc (5:5:1 by volume, 1.1 mL). The mixture was then degassed through three freeze-pump-thaw cycles. The reaction mixture was heated to 120 °C and allowed to react for 7 days. After the reaction, the product was collected by filtration and washed consecutively with deionized water (3 × 10 mL) and acetone (3 × 10 mL). It was then dried at 40 °C under vacuum for 48 hours, resulting in a yellow Py-BPy-COF.

Synthesis of PB-COF@NADH: 20 mg of Py-BPy-COF dry solid was dispersed in 80 mL of deionized water using ultrasonic treatment to obtain a uniformly dispersed aqueous suspension. Subsequently, 10 mL of NADH aqueous solution (1.0 mg·mL^{-1} in water) was added to the suspension. The mixture was stirred at room temperature for 24 hours. After centrifugation, the precipitates were washed three times with deionized water and then dried to obtain PB-COF@NADH. The NADH concentration was determined using an NAD+/NADH Assay Kit (Beyotime Biotechnology).

Synthesis of TP@PB-COF@NADH: For the modification of KLVFFAED (KD8), 6 mg COF was added to a 25 mL solution containing 6 mg KD8 in 1,4-dioxane. The mixture was then heated in an oil bath at 105 °C for 12 hours. The resulting precipitates were collected via centrifugation and washed with deionized water three times. Finally, the product TP@PB-COF was dried at room temperature. For the synthesis of TP@PB-COF@NADH, 10 mg TP@PB-COF was dispersed in 80 mL of deionized water using ultrasonic treatment. Subsequently, 10 mL of NADH (1.0 mg·mL^{-1}) was added. The

mixture was then stirred at for 24 hours. Next, the precipitates were collected via centrifugation and washed with deionized water 3 times. TP@PB-COF@NADH was obtained after being dried at room temperature.

Preparation of aggregate-free amyloid-β: The sample preparation followed the previously established protocol. Initially, 1.0 mg of lyophilized Aβ was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a concentration of 1.0 mg·mL^{-1} (240 μM). The solution was agitated in a 4 ℃ freezer for 4 hours to ensure complete dissolution, and subsequently stored at -20 °C as a stock solution. Before use, the HFIP solvent was evaporated using a gentle nitrogen stream, followed by dissolving the peptide in Milli-Q water.

Tyrosine fluorescence assay: The inherent tyrosine (Tyr10) fluorescence of Aβ42 was measured using fluorescence spectroscopy with an excitation wavelength (λ_{ex}) of 285 nm. Aβ42 (20 μM) was incubated in PBS (10 mM, pH 7.4) with Cu^{2+} (20 μM), Ca^{2+} (20 μM), Fe^{3+} (20 μM), and Zn^{2+} (20 μM). Subsequently, Py-BPy-COF was added to the mixture at a final concentration of 20 μg·mL^{-1}, and the fluorescence was monitored using fluorescence spectroscopy.

Ascorbate consumption assay and CCA experiments: To assess ascorbate (Asc) consumption, 1 μM Cu^{2+}, 100 μM Asc, and either 60 μg·mL^{-1} Py-BPy-COF or 12 μM Aβ were prepared in PBS (10 mM, pH 7.4) and analyzed using UV-Vis spectroscopy. The generation of hydroxyl radicals was evaluated by monitoring the oxidation of coumarin-3-carboxylic acid (3-CCA). The transformation of 3-CCA to 7-hydroxy-

coumarin-3-carboxylic acid (7-OH-CCA) was quantified by measuring the increase in fluorescence intensity at 450 ± 20 nm (λex = 395 ± 15 nm) [CCA] = 0.5 mM.

Measurement of photothermal performance: To assess the photothermal performance, an aqueous dispersion of Py-BPy-COF (80 μg·mL^{-1}) was illuminated with an 808 nm or 1064 nm near-infrared (NIR) laser (1.0 W·cm^{-2}). The temperature was precisely measured using an infrared camera with an accuracy of 0.1 °C. After irradiation, the laser was switched off, and the samples were cooled to its original temperature to generate heating and cooling curves. The stability of samples was then evaluated through five consecutive heating and cooling cycles. Under identical conditions, the photothermal performance of other samples was also evaluated.

Mimetic catalytic activity of PB-COF-Cu^{2+} for the oxidation of NADH to NAD+: The mimetic catalytic activity of PB-COF-Cu^{2+} in oxidizing NADH to NAD+ was evaluated using UV-vis spectrophotometry. Specifically, a reaction mixture containing PB-COF-Cu^{2+} (50 μg·mL^{-1} final concentration), NADH (0, 0.05 mM, 0.1 mM, 0.2mM, 0.3mM and 0.4 mM), H_2O_2 (10 mM final concentration) and HEPES buffer (50 mM pH 7.4) was prepared. The absorption changes at λ = 340 nm were monitored spectrophotometrically. The concentration of oxidized NADH was calculated using the Beer-Lambert law: A = εbc, where A is the absorbance, ε is the molar absorptivity (6200 M^{-1} cm^{-1}), b is the path length of the sample, and c is the concentration. Each experiment was replicated three times to ensure accuracy. The reaction rates were then analyzed using the Michaelis-Menten equation (V = Vmax×[S] / ([S] + Km)) to determine the Michaelis-Menten constants.

The influence of NIR-II irradiation on the NPX-like activity of PB-COF-Cu^{2+} was investigated. The reaction mixture of catalytic system was exposed to 1064 nm irradiation (1.0 W·cm^{-2}), and the absorption values at 340 nm were recorded every minute using a spectrophotometer. As a control, experiments were conducted on the reaction mixture of the catalytic system without irradiation.

Measurement of the NAD+/NADH Ratio: HT22 cells (rat pheochromocytoma) were cultured at a density of 1.0×10^5 cells per well in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% horse serum. The cells were maintained in a 5% CO_2 atmosphere at 37 °C. Subsequently, the culture medium was discarded, and the cells were washed with PBS. The cells were then treated with Aβ-Cu^{2+}, Aβ-Cu^{2+}+NADH, Aβ-Cu^{2+}+ PB-COF@NADH, or PBS ([Aβ42-Cu^{2+}] = 20 μM, [NADH] = 10 μg·mL^{-1}, [PB-COF@NADH] = 60 μg·mL^{-1}) in serum-free conditions for 12 hours. Specially, NIR-II laser irradiation (1064 nm, 0.7·W cm^{-2}) was employed for NIR-II group (10 min) during the incubation. After treatment, the cells were washed three times with PBS and lysed using 200 μl of lysis buffer. The NAD+/NADH ratio was determined using the NAD/NADH Assay Kit, following the manufacturer's instructions.

Detection of Reactive Oxygen Species (ROS) in HT22 Cells: HT22 cells were seeded at a density of 1×10^5 cells per well and cultured overnight to ensure adherence. Prior to various treatments, the cells were washed with phosphate-buffered saline (PBS) and then incubated in 1.0 mL of fresh DMEM medium (without serum) containing Aβ42-Cu^{2+} or Aβ42-Cu^{2+} + PB-COF@NADH ([Aβ42-Cu^{2+}] = 12 μM, [PB-COF@NADH] = 60 μg·mL^{-1}) for 6 hours. Specifically, NIR-II laser irradiation (1064 nm, 0.7·W cm^{-2})

was employed for NIR-II group (10 min) during the incubation. After that, the cells were washed with PBS and subsequently co-incubated with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The cells were then imaged using a fluorescence microscope and flow cytometry to assess ROS levels.

cGAS-STING signaling pathway assay: The HMC3 cells were incubated with various formulations: (1) control, (2) 12 μM Aβ42-Cu^{2+}, (3) 12 μM Aβ42-Cu^{2+} + 60 μg·mL^{-1} PB-COF@NADH + NIR-II laser (1064 nm, 0.7·W cm^{-2}). Then the protein samples were collected following cell lysis with RIPA lysis buffer. Then the concentrations of protein samples were quantified using a BCA protein detection kit (Beyotime Biotechnology) and adjusted to the same concentration. The proteins were then separated by SDS-PAGE gradient gel electrophoresis and transferred onto PVDF membranes. The membranes were incubated overnight at 4 °C with antibodies against cGAS (Rabbit Polyclonal Antibody), STING (Rabbit Polyclonal Antibody), and β-tubulin. Following this, the membranes were incubated with an HRP-conjugated goat anti-rabbit immunoglobulin G (IgG) secondary antibody. Protein bands were visualized using an enhanced chemiluminescence solution reaction.

Detection and Measurement of Microglial Cell Activation and Inflammatory Cytokine Levels: HMC3 cells were seeded at a density of 1×10^5 cells per well and cultured overnight to allow for adherence. After the initial culture period, the medium was discarded, and each well was washed with PBS. The cells were then treated with 12 μM Aβ42-Cu^{2+} or 60 μg·mL^{-1} PB-COF@NADH in DMEM without serum for 12 hours. Especially, NIR-II laser irradiation (1064 nm, 0.7·W cm^{-2}) was employed for

NIR-II group (10 min) during the incubation. After that, the supernatants were collected using centrifuge tubes. To quantify the levels of inflammatory cytokines TNF-α and IL-1β, enzyme-linked immunosorbent assay (ELISA) kits (Beijing Solarbio Science & Technology Co., Ltd.) were utilized.

Measurement of Mitochondrial Membrane Potential Following Treatment: HT22 cells were seeded at a density of 1.0×10^5 cells per well and cultured for 24 hours to allow for adherence. Subsequently, the cells were washed with PBS, and the medium was replaced with fresh DMEM without serum containing Aβ42-Cu^{2+}, Aβ42-Cu^{2+}+ PB-COF@NADH, or PBS ([Aβ42-Cu^{2+}] = 12 μM, [PB-COF@NADH] = 60 μg·mL^{-1}) for 6 hours. Particularly, NIR-II laser irradiation (1064 nm, 0.7·W cm^{-2}) was employed for NIR-II group (10 min) during the incubation. After being washed with PBS, the cells were stained with JC-1 according to the manufacturer's protocol and imaged using a confocal laser scanning microscope (CLSM).

Measurement of Intracellular ATP Levels: HT22 cells were seeded at a density of 1×10^5 cells per well and cultured overnight to allow for adherence. Subsequently, the cells were washed with PBS and treated with 1.0 mL of fresh DMEM without serum, containing Aβ42-Cu^{2+}, Aβ42-Cu^{2+} + NADH, Aβ42-Cu^{2+} + PB-COF@NADH, or PBS ([Aβ42-Cu^{2+}] = 12 μM, [NADH] = 10 μg·mL^{-1}, [PB-COF@NADH] = 60 μg·mL^{-1}) for 12 hours. Specially, NIR-II laser irradiation (1064 nm, 0.7·W cm^{-2}) was employed for NIR-II group (10 min) during the incubation. After that, the ATP levels in each group were measured using an ATP assay kit, following the manufacturer's instructions.

Cell viability assays: HT22 cells were seeded at a density of 5.0×10^4 cells per well and cultured overnight to allow for adherence. Subsequently, the culture medium was discarded, and the cells were washed with PBS. Various components, including Aβ42-Cu^{2+}, NADH, and PB-COF@NADH, were introduced into the DMEM without serum at the following concentrations for different experimental groups: [Aβ42-Cu^{2+}] = 12 μM, [NADH] = 10 μg·mL^{-1}, [PB-COF@NADH] = 60 μg·mL^{-1}. The cells were incubated with these components for 24 hours. Specifically, NIR-II laser irradiation (1064 nm, 0.7·W cm^{-2}) was employed to for NIR-II group (10 min) during the incubation. After that, the cell viability was assessed using the MTT method. The biocompatibility of Py-BPy-COF in HT22 cells was evaluated using the same methodology.

Annexin V/PI Assay: HT22 cells were randomly plated in 6-well plates cultured overnight to allow for adherence. Then the cells were incubated with Py-Bpy-COF for 24 h. After that, the cells were stained with 5 μL annexin V-FITC at room temperature for 15 min and followed by 10 μL PI for 5 min in the dark. The fluorescence intensity of cells was viewed by flow cytometry in green channel for annexin V-FITC and red channel for PI, respectively.

Animal Model: The APPswe/PS1M146V/tauP301L transgenic model mice (3×Tg-AD mice) were generously provided by the Xiubo Du group at Shenzhen University (Shenzhen, China). Control mice (C57BL/6 mice) were obtained from Jilin University. The Changchun Institute of Applied Chemistry Animal Care and Use Committee authorized all animal studies, which were conducted in compliance with the NIH

Publication No. 85-23 Rev. 1985 standards for the care and use of laboratory animals. Additionally, all animal care and handling practices followed the rules that Changchun Institute of Applied Chemistry's ethical committee had authorized.

***Ex Vivo* Fluorescence Imaging:** The 3×Tg-AD mice were administered an intraperitoneal injection of TP@PB-COF@NADH@Cy3 and PB-COF@NADH@Cy3 at a dosage of 12.5 mg/kg. After a 24-hour period, the mice were euthanized, and their brains, hearts, livers, spleens, lungs, and kidneys were excised for subsequent ex vivo fluorescence imaging analysis.

Morris water maze (MWM) test: Six-month-old male 3×Tg-AD mice and WT mice (n=5) were administered intraperitoneal injections of, NADH, TP@PB-COF, TP@PB-COF@NADH or saline (12.5 mg/kg) every 3 days and the NIR-II group were illuminated by 1064 nm laser every day (0.7 $W \cdot cm^{-2}$, 10 min) in a 15-day experimental period. After 2 weeks of continuous irradiation following the injections, the Morris water maze was employed to assess memory function in these mice. Prior to the maze testing, animals underwent a training phase consisting of five trials per day for six consecutive days, with the platform position remaining fixed throughout. A tracking system automatically recorded the experimental data. The swimming pool was divided into four equal quadrants, and each trial began with the mouse positioned at one of four predetermined starting points. Mice were allowed to swim for 90 seconds or until they found the platform. Trials were terminated if the mouse failed to locate the platform within the 90-second limit. The swimming speed, the percentage of time spent in the target quadrant relative to the total swimming time, the latency to find the platform, and

the number of times the mouse passed over the platform position were recorded for statistical analysis.

***In Vivo* Histological Analysis:** For hematoxylin and eosin (H&E) staining, the harvested tissues (heart, liver, spleen, lung, kidney, and brain) were initially fixed in 4% paraformaldehyde following saline treatment and then dehydrated. The dehydrated samples were embedded in paraffin, sectioned, and subjected to H&E staining. For Nissl staining, the brains were separately collected, preserved in 10% formalin, embedded in paraffin, and sectioned at a thickness of 3 μm. These brain sections were then subjected to Nissl staining to detect Nissl bodies in the cytoplasm of neurons within the hippocampus and cortex. Immunohistochemical analysis was performed to assess the brain Aβ burden with age-matched C57BL/6 mice serving as negative controls.

Assessment of Inflammatory Levels in the Brain: To investigate the levels of inflammation factors TNF-α and IL-1β in the brains of WT mice and 3×Tg-AD mouse models after treatments, the brain of mice was collected and homogenized for 4 min in the 0.2 mL tube with RIPA buffer. Then the solution was centrifuged at 100,000 × g for 30 min. Lastly, the inflammatory mediators of the supernatants was tested by the corresponding kits.

Hemolysis Test: 1 mL whole blood was collected in tubes containing Li-heparin from the Orbital venous of C57BL/6 mice. Then, 1 mL blood was mixed with the proper amount of 10 mM PBS (pH = 7.4), centrifuged 5 min at 2,500 rpm to remove the supernatant, and repeated 3-4 times until the supernatant becoming colorless

transparent. The precipitated erythrocytes were then dispersed in 10 mM PBS (pH = 7.4) to get erythrocyte suspension. The TP@PB-COF@NADH were added to the erythrocyte suspension to a final concentration of 25, 50, 100, 200, 400 μg mL^{-1}. Negative control was placed with erythrocyte suspension diluted with 10 mM PBS (pH = 7.4), and positive control was placed with erythrocyte suspension diluted with ultrapure water. The tubes were incubated for 8 h at room temperature, recording the hemolysis phenomenon. At the meanwhile, the specific 540 nm spectrophotometric absorptions of hemoglobin were analyzed. Calculation of the hemolysis rate (HR%) was by the following equation: $HR\% = (A_{TP@PB\text{-}COF@NADH} - A_{NC}) / (A_{PC} - A_{NC}) \times 100\%$. Where $A_{TP@PB\text{-}COF@NADH}$, A_{PC} and A_{NC} are the absorbance of the sample, the positive control and the negative control, respectively.

Statistical Analysis: All data were expressed as mean ± standard errors (n = 3 unless specified.). Statistical analysis was performed using Microsoft Excel, Origin 2020, and GraphPad Prism software. Statistical evaluation was performed using unpaired Student's two-sided t test analysis. Asterisks indicate significant differences (*P < 0.05, **P < 0.01, ***P < 0.001, ****p < 0.0001).

Supporting Figures and Tables

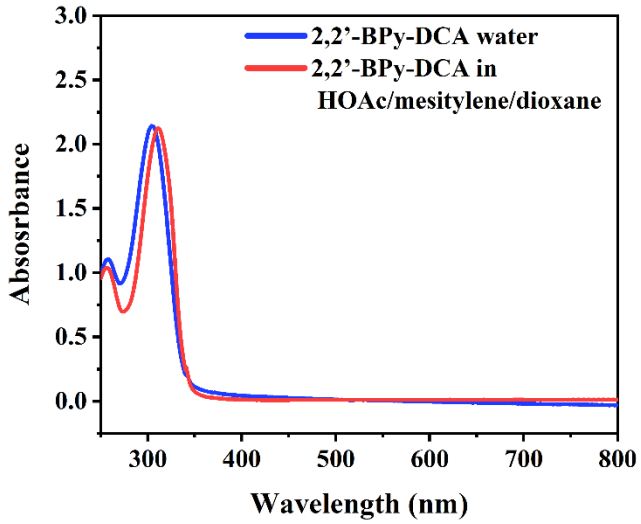

Figure S1. UV-vis spectra of 2,2'-BPy-DCA in water and a mixture of mesitylene/dioxane/6M HOAc (5/5/1, *v/v*), respectively. The pH values of 6M HOAc aqueous solution and a mixture of mesitylene/dioxane/6M HOAc are measured to be 1.53 and 3.15, respectively. Both are lower than the pKa value of 3.83, indicating the formation of mono-cation of 2,2'-BPyDCA. Owing to the presence of intramolecular H-bonding interaction, the *cis* form of 2,2'-BPy-DCA mono-cation is much more stable than the *trans* form. The two red-shifted peaks at 256 nm and 312 nm are attributed to the characteristic absorption of the mono-cationic species.

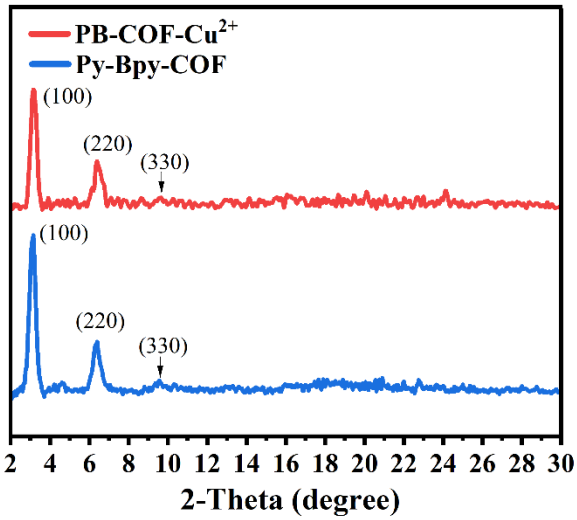

Figure S2. PXRD patterns of Py-Bpy-COF and PB-COF-Cu^{2+}.

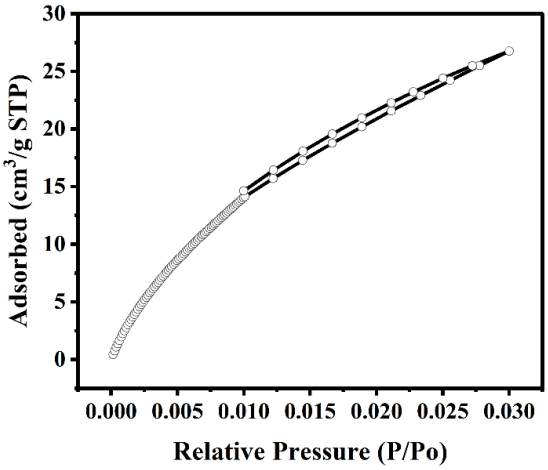

Figure S3. CO_2 adsorption isotherm of Py-Bpy-COF at 273 K.

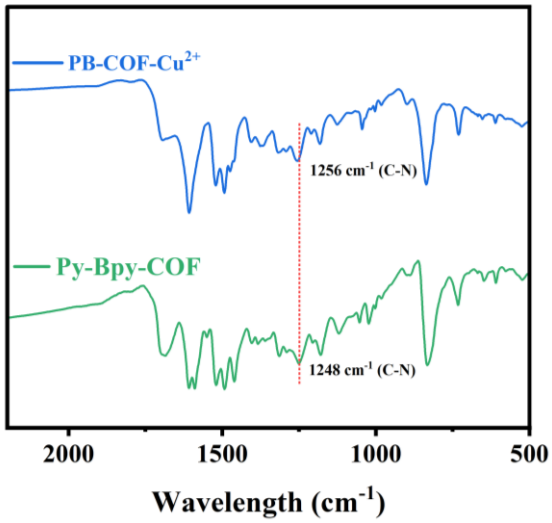

Figure S4. FT-IR spectra of Py-Bpy-COF and PB-COF-Cu^{2+}.

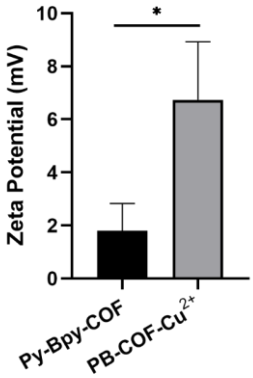

Figure S5. Zeta potential of Py-Bpy-COF and PB-COF-Cu^{2+}.

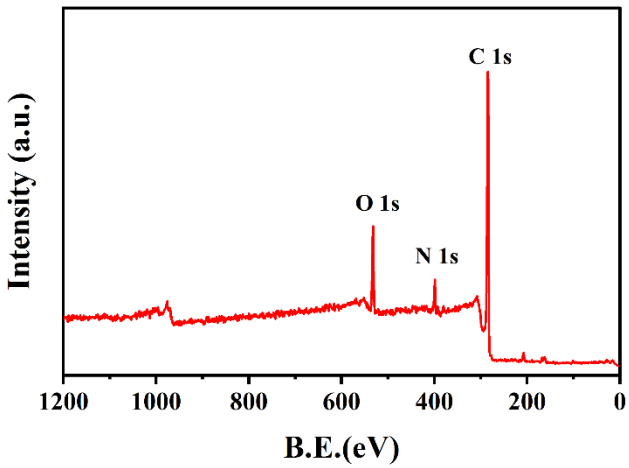

Figure S6. XPS spectrum of Py-Bpy-COF.

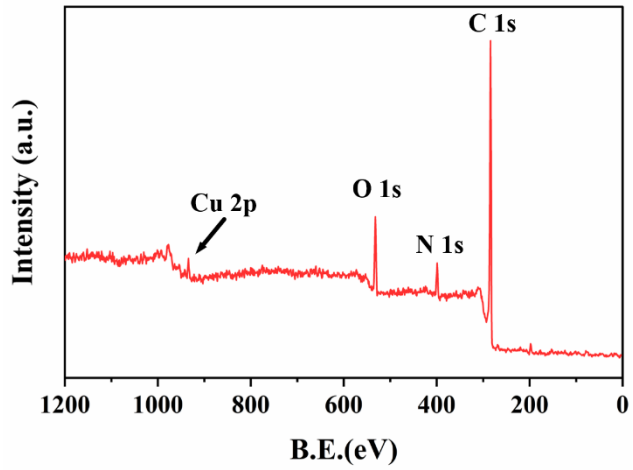

Figure S7. XPS spectrum of PB-COF-Cu^{2+}.

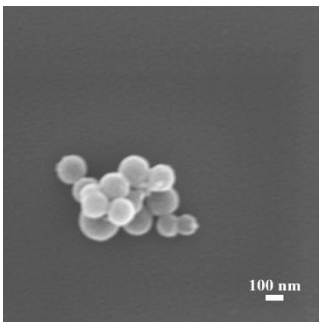

Figure S8. SEM image of PB-COF-Cu^{2+}.

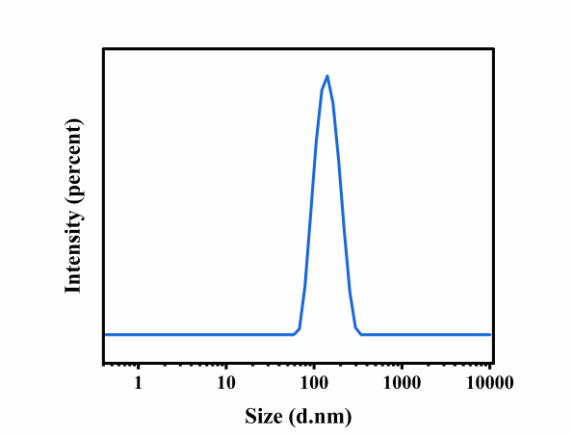

Figure S9. DLS profile of PB-COF-Cu^{2+}.

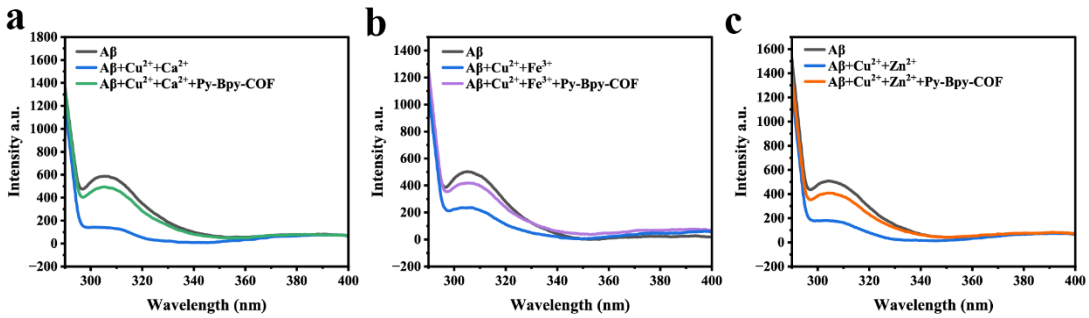

Figure S10. Extraction of Cu^{2+} from Aβ42 by Py-Bpy-COF in the presence of Ca^{2+}, Fe^{3+} and Zn^{2+}. [Aβ42] = 10 μM, [Cu^{2+}] = 10 μM, [Ca^{2+}] = 10 μM, [Fe^{3+}] = 10 μM, [Zn^{2+}] = 10 μM, [Py-Bpy-COF] = 50 μg·mL^{-1}, PBS (10 mM, pH 7.4)

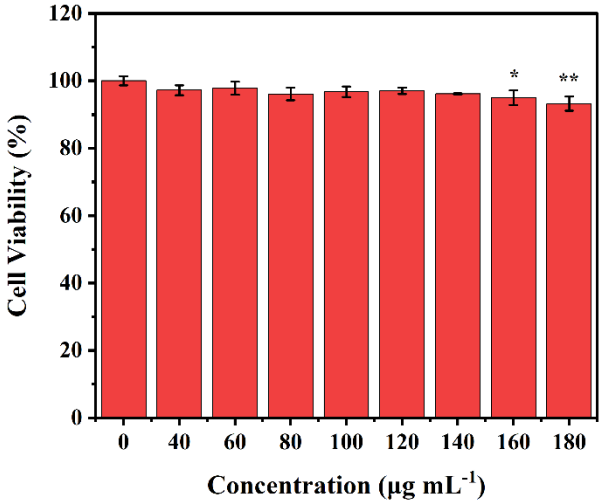

Figure S11. Cell viability of HT22 cells treated with Py-Bpy-COF at different concentration.

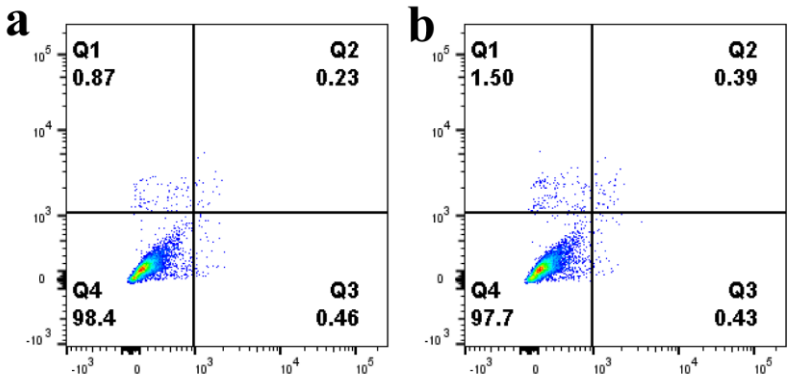

Figure S12. Flow cytometry of HT22 cells (a) without treatment and (b) treated with Py-Bpy-COF.

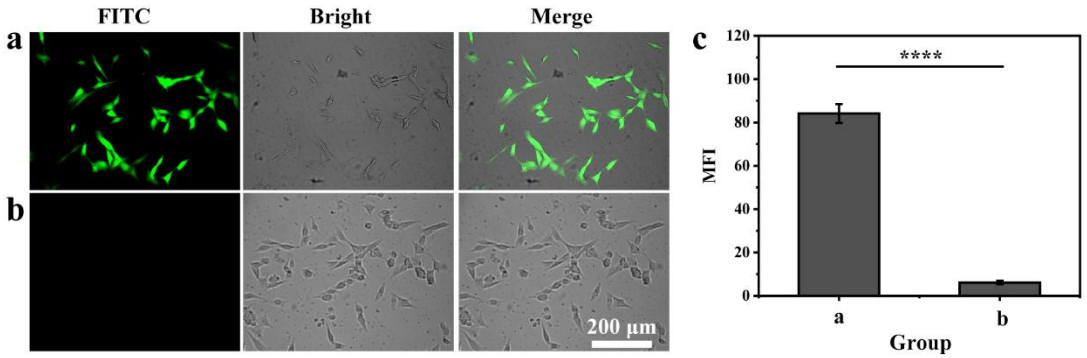

Figure S13. Fluorescence images of HT22 cells (a) treated with 12 μM Aβ42-Cu^{2+} alone and (a) in the presence of PB-COF@NADH (60 μg·mL^{-1}) with NIR-II irradiation (1064 nm, 0.7 mW·cm^{-2}), stained with the ROS probe DCFH-DA. (c) Quantitative analysis of the fluorescence intensity.

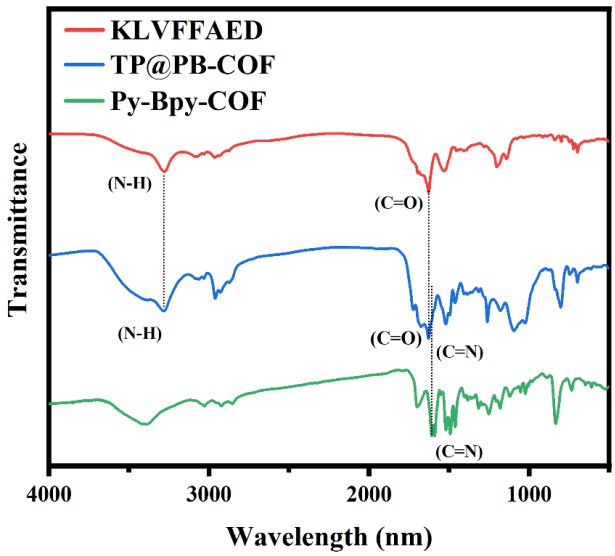

Figure S14. FTIR spectra of KLVFFAED (red), TP@PB-COF (blue), and Py-Bpy-COF (green).

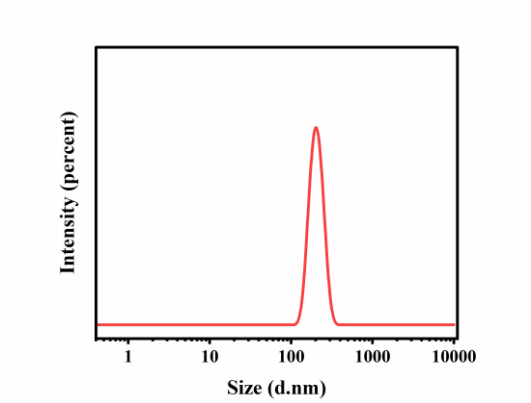

Figure S15. DLS result of TP@PB-COF@NADH.

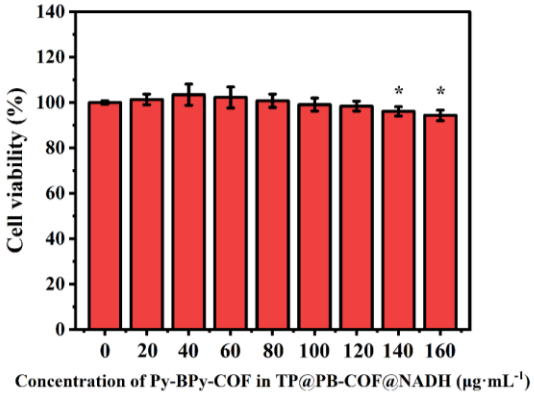

Figure S16. Cell viability of HT22 cells treated with TP@PB-COF@NADH at different concentrations.

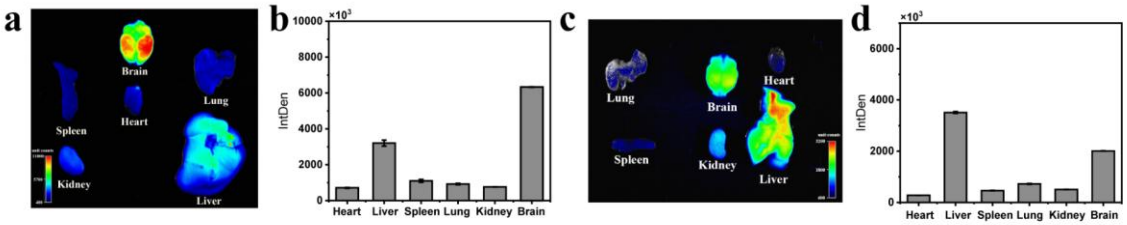

Figure S17. Fluorescence imaging of the brain and major organs from 3×Tg-AD mice 24 hours after intraperitoneal injection of TP@PB-COF@NADH@Cy3 (a) and PB-

COF@NADH@Cy3 (c), with corresponding quantitative analyses shown in (b) and (d), respectively.

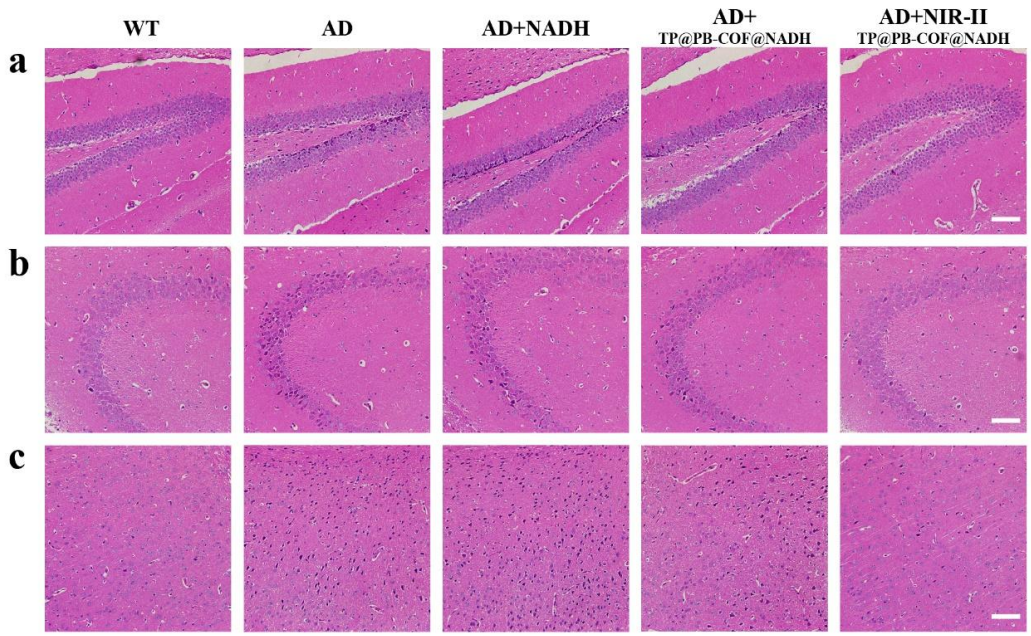

Figure S18. Images of H&E staining in the (a) DG, (b) hippocampus, and (c) cortex areas of brains in WT mice and 3×Tg-AD mice after different treatments. Scale bar = 100 μm.

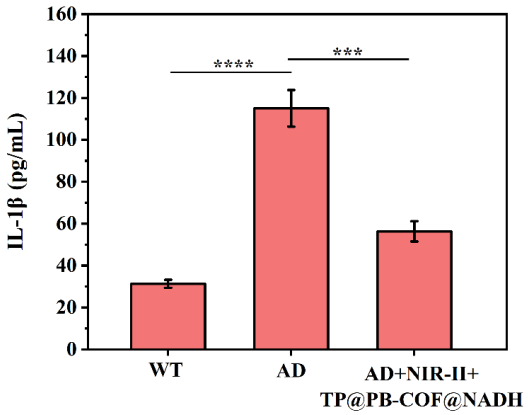

Figure S19. The levels of IL-1β in the brain of WT mice and 3×Tg-AD mice after treatment were measured by enzyme-linked immunosorbent assay (ELISA) kits.

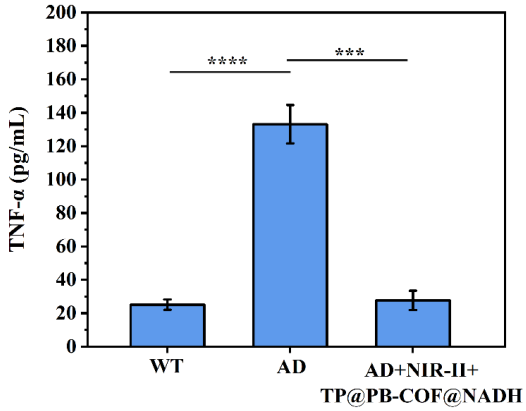

Figure S20. The levels of TNF-α in the brain of WT mice and 3×Tg-AD mice after treatment were measured by ELISA kits.

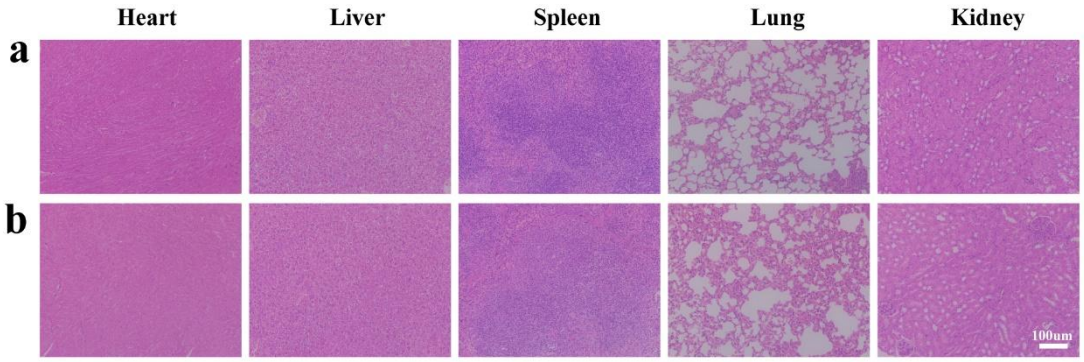

Figure S21. H&E staining of hearts, livers, spleens, lungs, and kidneys: (a) WT mice, (b) 3×Tg-AD mice treated with TP@PB-COF@NADH and NIR-II irradiation. Scale bar = 100 μm.

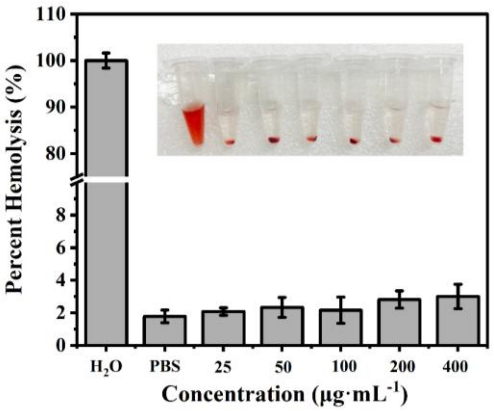

Figure S22. Hemolysis test results of TP@BP-COF@NADH.

References

[1] M. Ma, N. Gao, X. Li, Z. Liu, Z. Pi, X. Du, J. Ren, X. Qu, *ACS Nano* **2020**, *14*, 9894-9903.

[2] A. E. Behar, L. Sabater, M. Baskin, C. Hureau, G. Maayan, *Angew. Chem. Int. Ed.* **2021**, *60*, 24588-24597.

[3] Z. Mi, P. Yang, R. Wang, J. Unruangsri, W. Yang, C. Wang, J. Guo, *J. Am. Chem. Soc.* **2019**, *141*, 14433-14442.

[4] Z. Liu, Q. Deng, G. Qin, J. Yang, H. Zhang, J. Ren, X. Qu, *Chem* **2023**, *9*, 2016-2038.

[5] Q. Ren, H. Chen, Y. Chen, Z. Song, S. Ouyang, S. Lian, J. Tao, Y. Song, P. Zhao, *ACS Appl. Mater. Interfaces* **2023**, *15*, 4947-4958.

[6] P. Ling, Q. Zhang, T. Cao, F. Gao, *Angew. Chem. Int. Ed.* **2018**, *57*, 6819-6824.