Supporting Information

Microglia membrane biomimetic platinum-based MOF-loaded quercetin nanodrug delivery system for the treatment of Alzheimer's disease

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1. Experimental section

1.1 Materials

$PtCl_2$ was purchased from Shanghai Bide Medical Technology Co., LTD. Trifluoroacetic acid was purchased from Jiangsu Aikang Biomedical Research and Development Co., LTD. Agarose, cholesterol, β-mercaptoethanol,5, 5-dimethyl-1-pyrroline-N-oxide (DMPO) were purchased from Zhenjiang Dewei Chemicals Co., LTD. Quercetin was purchased from Nanjing Herbal Source Biotechnology Co., LTD. Levamisole hydrochloride and 4 ', 6-diamidino-2-phenylindole (DAPI) were purchased from Zhenjiang Yibei Biotechnology Co., LTD. 2',7'-dichlorofluorescein diacetate (DCFH-DA) and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide (JC-1) was provided by MedChemExpress (MCE). 1,1' -dioctadecyl-3,3,3',3' -tetramethylindole dicarbanine perchlorate (DiD) and amyloid-beta peptide (1-42) were purchased from Beotemax Biotechnology Co., LTD. Human neuroblastoma cells (SH-SY5Y), mouse microglia cells (BV2), and mouse brain microvascular endothelial cells (bEnd.3) were purchased from the Cell Bank of the Chinese Academy of Sciences. Fetal bovine serum (FBS), MEM, GlutaPlus, HEPES and RPMI-1640 medium were purchased from Wuhan Saiwei Bio-Tech Co., LTD. All other reagents were of analytical grade.

1.2 Instruments

The morphological characteristics of the materials were observed by high-resolution transmission electron microscope (JEM-2100 HR, Japan). The dynamic light scattering (DLS) and zeta potential of the materials were measured by multi-angle particle size potential analyzer (nanobrok Omni, Brucellosis, USA), and the stability of the materials was determined by 7-day particle size results. Uv-vis and NIR spectra were determined with Shimadzu UV-2550. Elemental composition was analyzed using a multifunctional X-ray photoelectron spectrometer (XPS, ESCALAB QXi, Thermo Fisher, USA). The unabsorbed Cu^{2+} concentration was measured by atomic absorption spectrometer (AAS, tas-990f, PERSEE, China), and the adsorption efficiency of Cu^{2+} was calculated. The ability of the material to remove •OH was determined by electron spin resonance spectrometer (ESR, A300-10/12, Germany). Cellular uptake of the

material was observed by confocal laser scanning microscopy (CLSM, Leica TCS SP5, Germany). Fluorescence staining of cells and nematodes was photographed using an inverted fluorescence phase contrast biomicroscope (Ti2-U, Japan).

2. Methods

2.1 Drug loading

Pt-MOF was dispersed in ethanol solution containing Qu (mass ratio = 1:2). After stirring for 48 h at room temperature, the mixed solution was centrifuged (13000 r/min, 20 min), after which the unloaded Qu was washed off with ethanol, and the resulting product was dried for later use.

Based on the UV absorption standard curve of Qu in ethanol at 374 nm (Fig. S1), the drug loading of Pt-MOF was calculated to be 40% and the drug loading efficiency to be 34%. Drug loading (DLC) and drug loading efficiency (DLE) were calculated by the following formula:

$$\text{DLC}(\%) = \frac{Weight\ of\ loaded\ Qu}{total\ weight\ of\ Pt-MOF/Qu} \times 100 \quad (1)$$

$$\text{DLE}(\%) = \frac{Weight\ of\ loaded\ Qu}{initial\ weight\ of\ Qu} \times 100 \quad (2)$$

2.2 Microglial cell membrane extraction

Microglia membranes were extracted by repeated freeze-thaw. In this experiment, microglia were cultured in a cell incubator (37 ° C, 5% CO2). After the cells were overgrown, they were collected, centrifuged, resuspended in hypotonic lysate, placed in an ice bath for 30 min, and the cell suspension was frozen in liquid nitrogen, followed by thawing in a 37 ° C water bath. Subsequently, the cell suspension was centrifuged (800 g,10 min) to remove the cell content in the precipitate, and the supernatant was collected and centrifuged (18000 g,20 min) at 4 ° C to obtain the microglial cell membrane, which was resuspended in PBS (pH=7.4) and dispersed by sonication. The protein concentration of the membrane was determined by BCA protein kit. The resulting cell membrane suspension was also stored at − 80 ° C.

2.3 Drug release

Pt-MOF/Qu/BV2 (1 mg) was placed in a dialysis bag (3000 D) and then in 20 ml PBS (2 % SDS) buffer in a shaker (100 rpm), 1 ml of buffer containing Qu was removed

at specific time points, and buffer was replenished to 20 ml. The absorbance at λ=374 nm was measured using a UV spectrophotometer, and the cumulative drug release and drug release rate were calculated from the release standard curve (Fig. S2) using the following formula:

$$M_R = M_t + \frac{v}{V}\sum_{0}^{t-1} M_R \quad (3)$$

$$\text{drug release rate (\%)} = \frac{M_R}{M_L} \times 100 \quad (4)$$

In eq (3) and (4), M_R is the cumulative release of drug at t, M_t is the release of drug in the buffer at t, v is the volume of buffer solution removed, V is the total volume of buffer solution, and M_L is the loading of drug.

2.4 Antioxidant properties study

The ability to scavenge hydroxyl radicals (·OH) was evaluated by using 5,5-dimethyl-1-pyrrolidine-N-oxide (DMPO) to capture ·OH radicals. ·OH radicals were generated by irradiating hydrogen peroxide (H_2O_2) with light, then the material was added to scavenge the ·OH radicals. Subsequently, characteristic peaks were detected using an electron spin resonance (ESR) spectrometer.

Catalase activity (CAT) was measured indirectly using iodine titration. In this system, hydrogen peroxide will oxidize potassium iodide to elemental iodine in the presence of dilute sulfuric acid. We added Qu (4 μg/mL), Pt-MOF/BV2 (40 μg/mL) or Pt-MOF/Qu/BV2 (40 μg/mL) materials to this system beforehand and incubated for 6 h to decompose hydrogen peroxide. respectively, and then potassium iodide was added to generate elemental iodine, which showed significant UV absorption at 288 nm and 350 nm. The CAT activity of each group was measured by ultraviolet absorption of iodine.

In studies of superoxide dismutase (SOD) activity, superoxide anion (O_2^-) is generated by reacting xanthine (12 mM) with xanthine oxidase (1 U). Dihydroethidium (HE) can be oxidized by O2- to ethidium (Ex = 610 nm). Superoxide anion was removed from the reaction system by pre-adding Qu (4 μg/mL), Pt-MOF/BV2 (40 μg/mL) or Pt-MOF/Qu/BV2 (40 μg/mL) for 6 h, and then the fluorescence intensity of

ethidium was measured by a fluorescence spectrophotometer to evaluate SOD activity in each group.

2.5 Study on the chelating properties of Cu^{2+}

First, prepare Cu^{2+} standard solutions at concentrations of 1–5 μg/mL and establish a standard curve (Fig.S3). Subsequently, mix the material with Cu^{2+} at an equal mass ratio and incubate with shaking for 2 h. Next, transfer the mixture to an ultrafiltration tube and centrifuge (6000 r/min, 30 min) to separate unchelated Cu^{2+}. Finally, determine the concentration of free Cu^{2+} using atomic absorption spectroscopy (AAS).

2.6 Cellular uptake

SH-SY5Y cells were seeded in laser confocal culture dishes and allowed to grow to confluence before commencing the experiment. First, Pt-MOF/Qu/BV2 was mixed with the DiD fluorescent probe and incubated for 30 min (DiD binds to cell membrane lipid molecules to achieve material labeling). Subsequently, co-culture the DiD-labeled Pt-MOF/Qu/BV2 with SH-SY5Y cells for 3 hours. After incubation, fix cells with 4% paraformaldehyde at 4°C for 30 minutes, then stain cell nuclei with DAPI (1 μg/mL). Finally, wash with PBS to remove excess dye and observe cellular uptake of the material using a laser confocal microscope.

2.7 Intracellular ROS and mitochondrial membrane potential assessment

SH-SY5Y or BV2 cells were seeded in 6-well plates until confluent, followed by the addition of Aβ protofibrils (5 μM) to induce reactive oxygen species (ROS) production. The samples were then incubated for 24 h. Finally, cells were stained with the DCFH-DA fluorescent probe (1 μg/mL) for 30 min under dark conditions. After washing with PBS to remove excess dye, fluorescence images were captured using an inverted fluorescence microscope.

The assessment of mitochondrial membrane potential followed the same procedure as described above, with the final step involving the use of the JC-I fluorescent probe (10 μg/mL) to visualize changes in intracellular mitochondrial membrane potential.

2.8 Transmembrane transport experiment

bEnd.3 cells were seeded in the upper chamber of a Transwell plate, while SH-

SY5Y cells were seeded in the lower chamber to simulate the blood-brain barrier. Subsequently, DID-labeled Pt-MOF/Qu/BV2 (40 μg/mL) was added to the upper chamber and incubated for 6 hours. Finally, fluorescence images of material entering the lower chamber were captured using an inverted fluorescence microscope.

In addition, to investigate the permeability of Pt-MOF before and after membrane modification in Transwell model, the same upper chamber was spread with bEnd.3 cells. Pt-MOF and Pt-MOF/BV2 (40 μg/mL) were added to the upper chamber after the cells were full. The permeability of the material was assessed by measuring the Pt content in the solution using atomic absorption spectrometry.

2.9 Biocompatibility

After blood collection from mice, red blood cells were obtained by repeated centrifugation (3000 r/min, 15 min) and washing. The resulting red blood cell pellet was resuspended in 1× PBS to prepare a 4% red blood cell suspension. Subsequently, add different concentrations of the test material to the red blood cell suspension and incubate at 37°C for 2 hours. After incubation, collect the supernatant by centrifuging at 3000 r/min for 5 minutes. Finally, measure the absorbance of the supernatant at 570 nm using a microplate reader and calculate the hemolysis rate.

2.10 Distribution in vivo

ICR mice (9 weeks old, 20 g) used in the experiment were obtained from Jiangsu University and handled in accordance with the requirements of the Jiangsu University Animal Care and Use Committee (UJS-IACUC-2023091502). Pt-MOF/Qu/BV2 labeled with DID (0.2 mg/mL,200 μL,Calculated based on the weight of Pt-MOF) was administered to mice via tail vein injection, with PBS serving as the control. Six hours later, mice were euthanized, and brains and organs were dissected. Finally, the distribution of the material within the brain and organs was observed using an in vivo optical imaging system.

In addition, to investigate the accumulation of Pt in various organs and the plasma content of Pt, Pt-MOF /Qu/BV2 (0.2 mg/mL, 350 μL) was administered to mice (n=3) via the tail vein. Then the mice were euthanized at 1 h, 4 h, 8 h, 12 h and 24 h, and the organs were dissected and the blood was extracted. The Pt levels in the organs and

plasma were determined by atomic absorption spectrometry.

3. Supplementary Figures

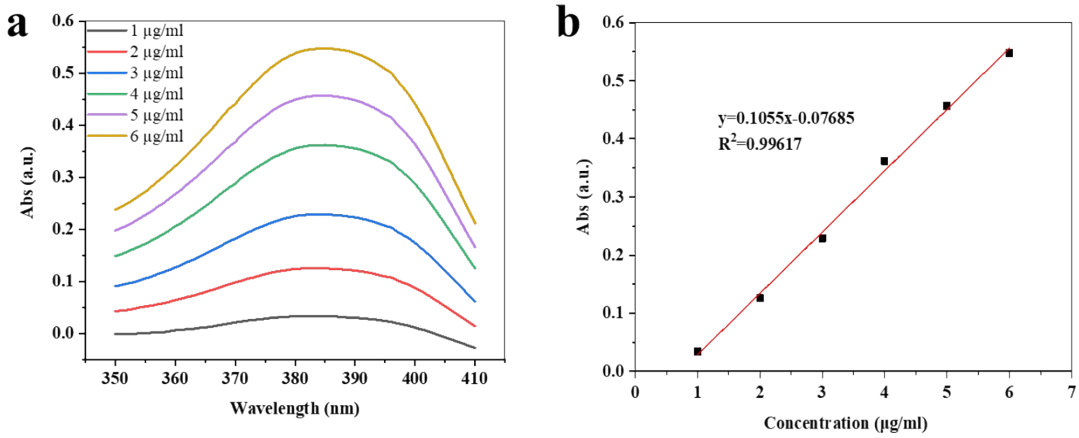

Fig.S1. (a) Uv absorption curves for different concentrations of quercetin in ethanol，（b）Drug loading standard curves for quercetin.

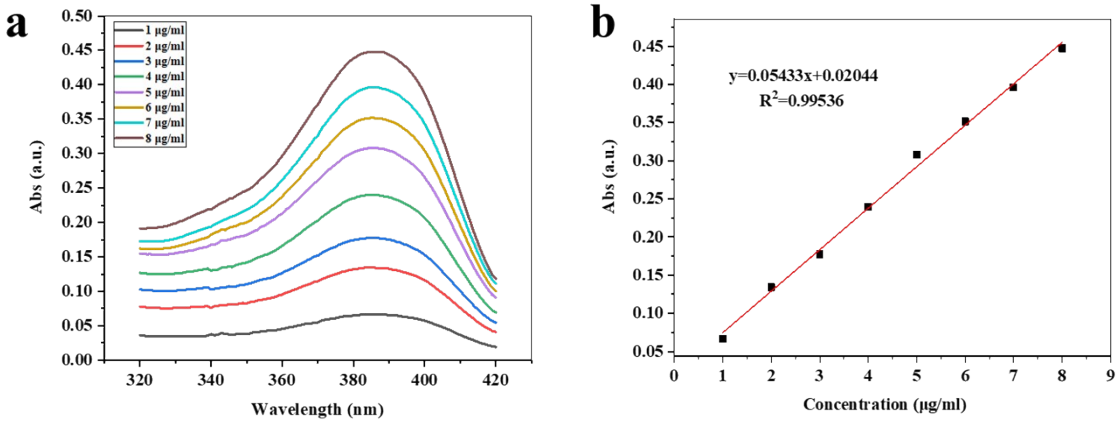

Fig.S2. (a) Uv absorption curves of different concentrations of quercetin in PBS (pH=7.4, 2% SDS)，（b）Standard curve for the release of quercetin.

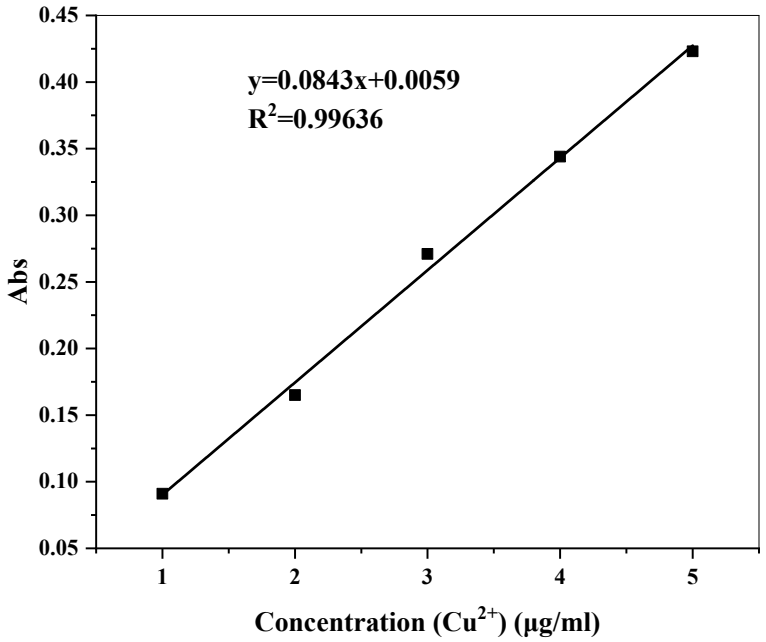

Fig.S3. The standard curve for Cu^{2+}.

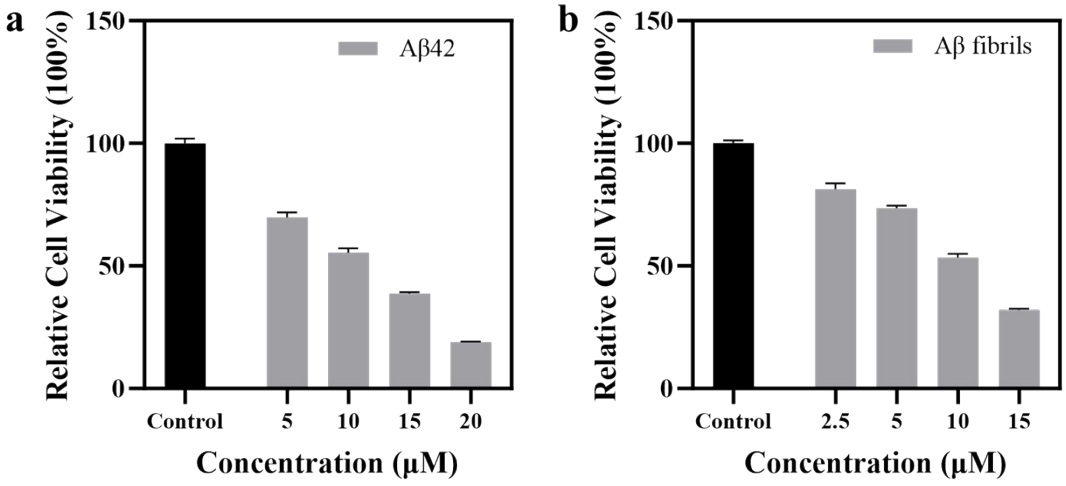

Fig.S4. Toxic effects of different concentrations of Aβ monomers (a) and Aβ fibrils (b) on SH-SY5Y cells. (mean ± SD, n = 3).

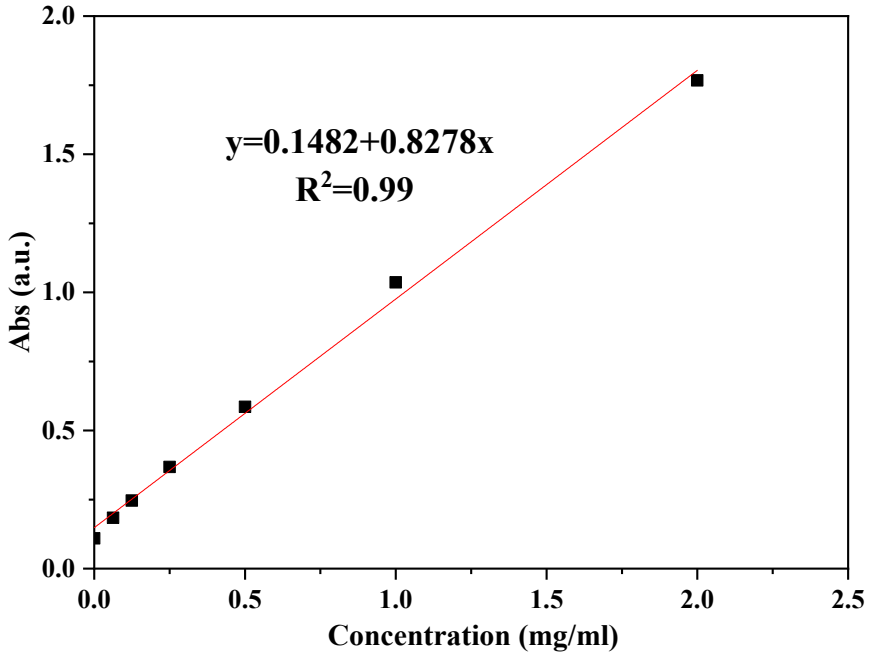

Fig.S5. The BCA protein kit was used to determine the standard curve.

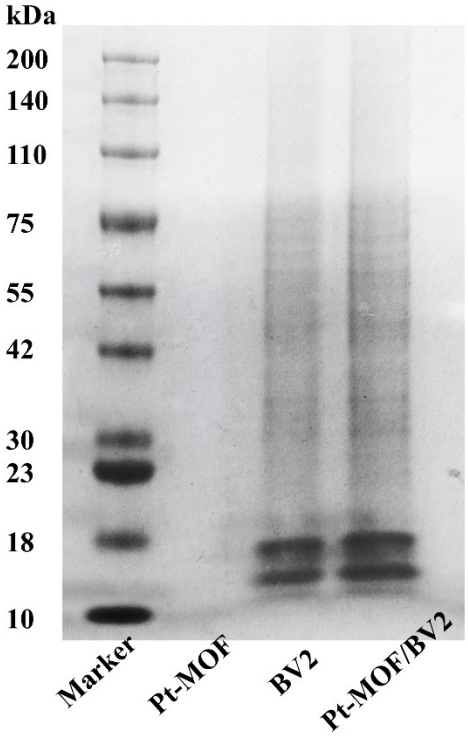

Fig.S6. SDS-PAGE protein analysis of Pt-MOF, BV2 and Pt-MOF/BV2.

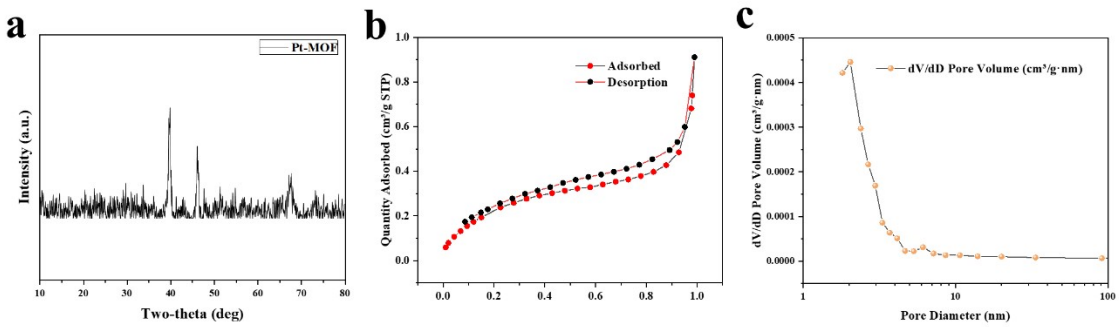

Fig.S7. (a) XRD analysis of Pt-MOF .(b) N_2 adsorption-desorption curves of Pt-MOF.(c) BJH Desorption dV/dD Pore Volume of Pt-MOF.

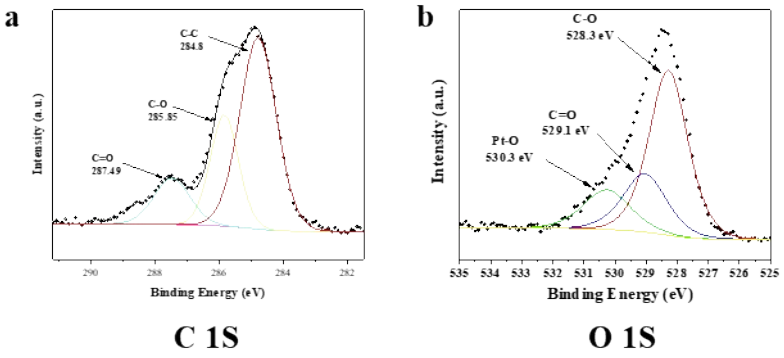

Fig.S8. Pt-MOF high-resolution spectra of (a) C1s, (b) O1s.

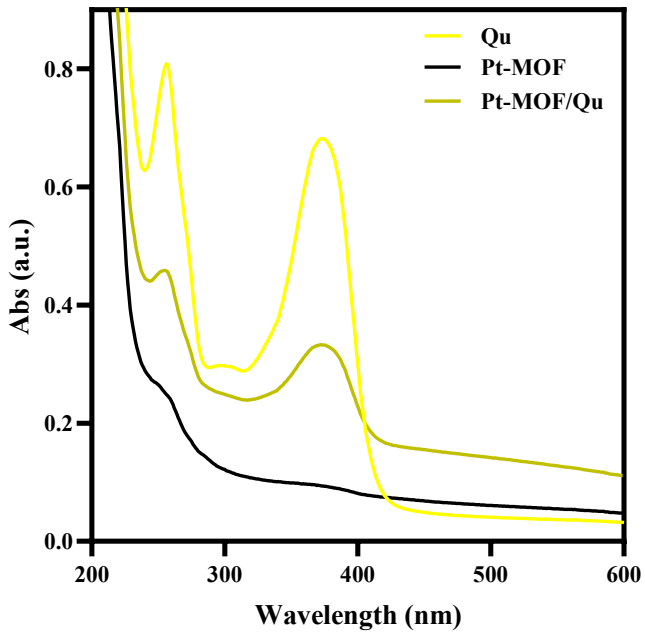

Fig.S9. UV absorption curves of Pt-MOF, Qu, and Pt-MOF/Qu.

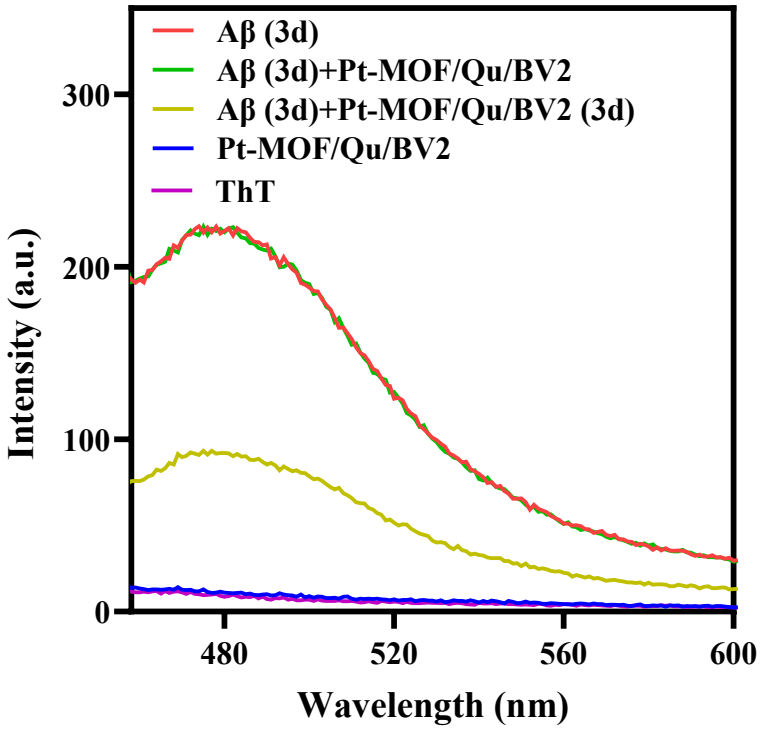

Fig.S10. Aβ monomer (incubation for 3 days), Aβ monomer (incubation for 3 days) +Pt-MOF/Qu/BV2, Aβ monomer +Pt-MOF/Qu/BV2 (co-incubation for 3 days), Pt-MOF/Qu/BV2, ThT Fluorescence intensity curves of the above five samples. Aβ (10 μM),Pt-MOF/Qu/BV2 (40 μg/mL), ThT (20 μM).

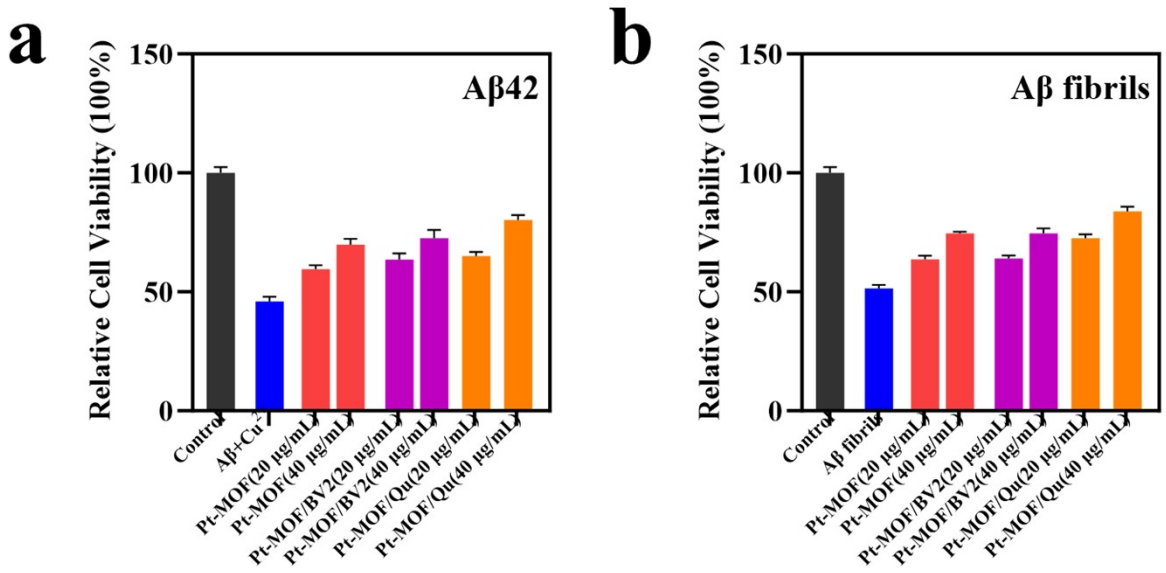

Fig.S11. (a) The ability of Pt-MOF, Pt-MOF/BV2, Pt-MOF/Qu to restore cell viability in the presence of Aβ monomer (15 μM)+Cu^{2+}.(b) The ability of Pt-MOF, Pt-MOF/BV2, Pt-MOF/Qu to restore cell viability in the presence of Aβ fibrils was investigated.

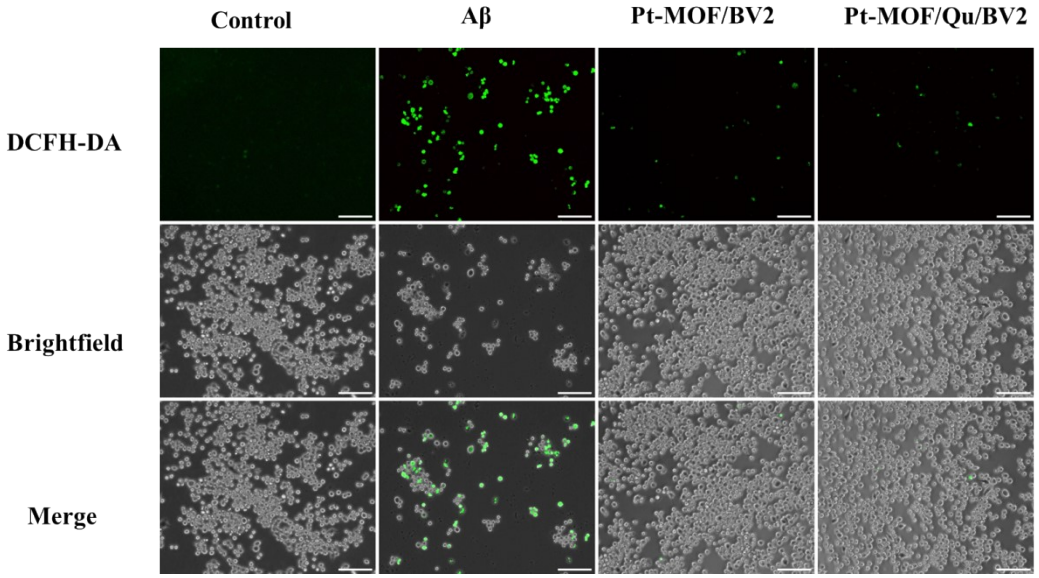

Fig.S12. Scavenging effect of materials on intracellular ROS in BV2(scale bar = 100 μm).

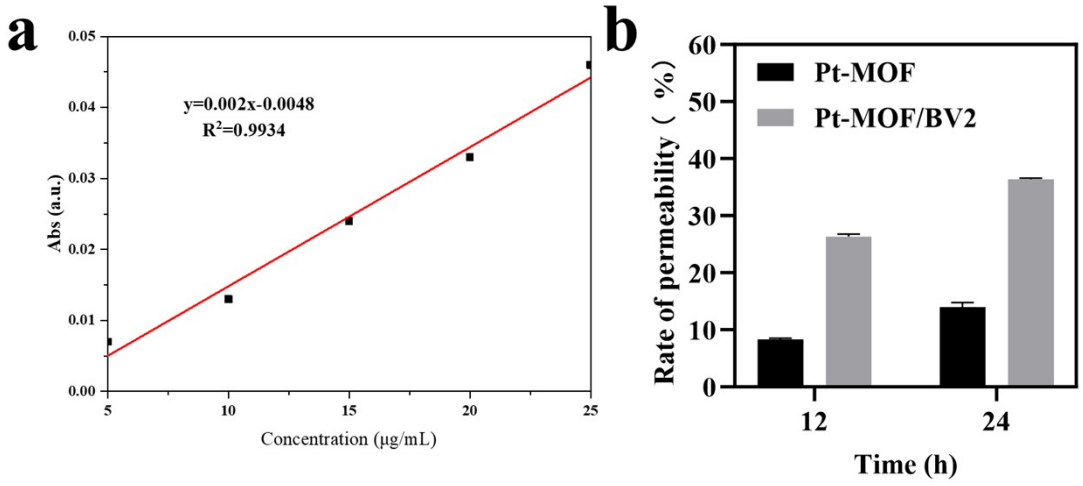

Fig.S13.(a) Standard curve of Pt measured by atomic absorption spectrometry.(b) Pt-MOF (40 μg/mL),Pt-MOF/BV2 (40 μg/mL)permeability in Transwell model (mean ± SD, n = 3).

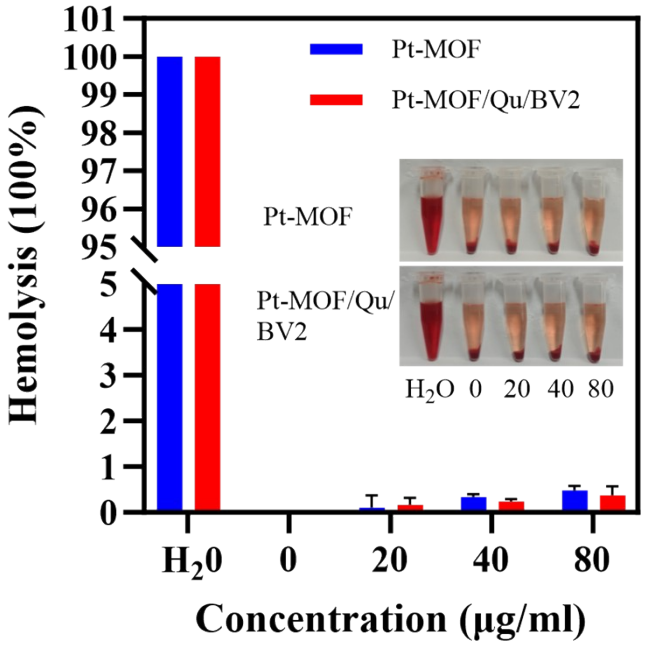

Fig.S14. Hemolysis rates of Pt-MOF and Pt-MOF/Qu/BV2(mean ± SD, n = 3).

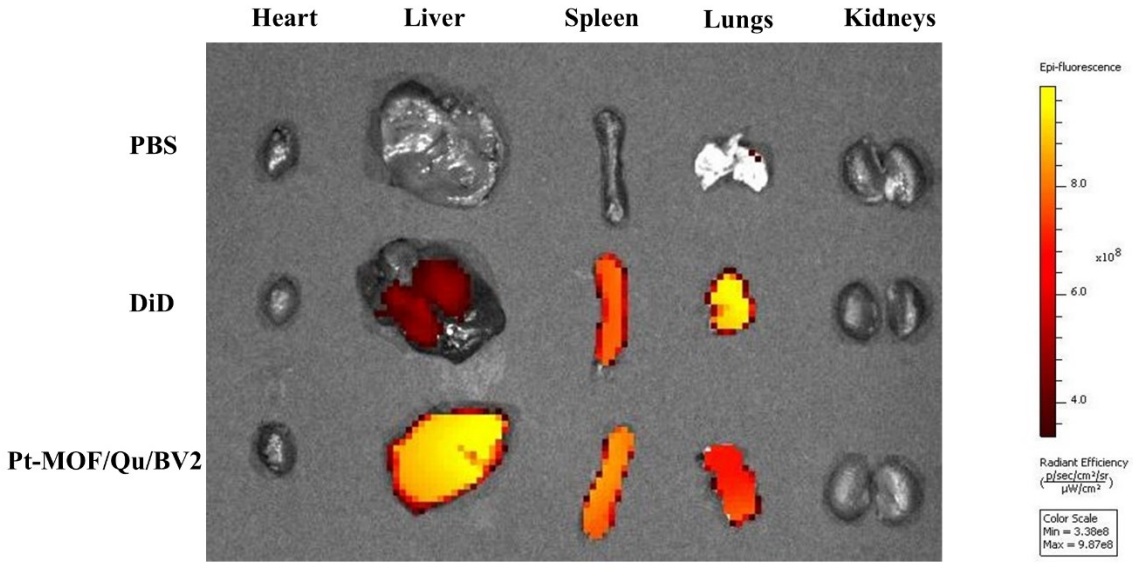

Fig.S15. Fluorescence images of mouse organs after tail vein injection of PBS, DiD, and Pt-MOF/Qu/BV2(mean ± SD, n = 3).

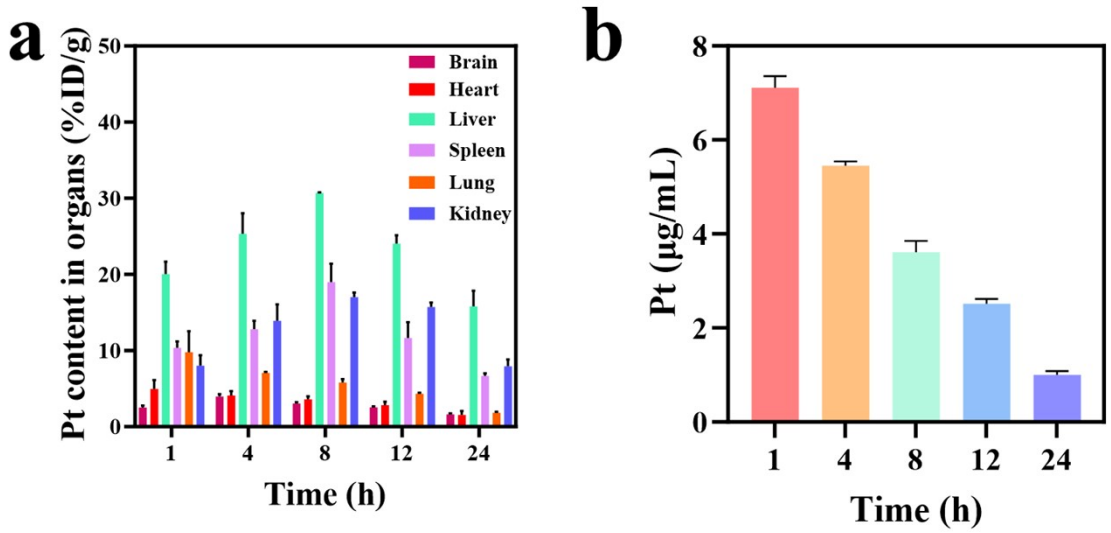

Fig.S16.(a) The content of Pt in various organs during 24 h in mice.(b) The content of Pt in the mouse blood over a 24-hour period(mean ± SD, n = 3).