Supporting Information

Biomimetic engineering of a neuroinflammation-targeted MOF nanozyme scaffolded with photo-trigger released CO for the treatment of Alzheimer's disease

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Experimental Section

Materials. Zirconium (IV) chloride ($ZrCl_4$), 1,3,5-Benzenetricarboxylic acid (H_3BTC), N, N-Dimethylformamide (DMF) and formic acid were purchased from Aldrich Chemical Co. All cell-culture used reagents were obtained from Gibco (Gibco Corporation, Grand Island, NY, USA). Aβ samples were customized from GL Biochem Ltd. (Shanghai, China). All ELISA kits and antibodies were purchased from Abcam (Shanghai, China). The anti-Ly6g antibody (FITC) (ab25024) was purchased from Abcam. PE anti-mouse CD16 antibody (S17014E) and FITC anti-mouse CD206 antibody (C068C2) were purchased from BioLegend. CO probe was purchased from GL Biochem (Shanghai) Ltd. All aqueous solutions were prepared using distilled water (18.2 MΩ, Milli-Q).

Apparatus and characterization. UV-vis absorbance measurement was carried out on a JASCO V-550 UV-vis spectrophotometer. Fluorescence spectra was detected by JASCO F-6000 fluorescence spectrometer with a Peltier temperature control accessory. FTIR spectra were carried out on a BRUKE Vertex 70 FT-IR spectrometer. Transmission electron microscopic (TEM) images were captured with a FEI TECNAI G2 20 high resolution transmission electron microscope operating at 200 kV. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The pore size was determined following the BJH method. The crystalline structures of the as prepared samples were evaluated by X-ray diffraction (XRD) analysis on a Rigaku-Dmax 2500 diffractometer by using CuKα radiation. The flow cytometry data was obtained by BD LSRFortessa™ Cell Analyzer.

Synthesis of MOF-808. The MOF-808 was synthesized according to a microwave method.[1] Briefly, H_3BTC (70 mg) and $ZrCl_4$ (233 mg) were dissolved in a solvent mixture of DMF/formic acid (15 mL/15 mL) and placed in a 100 mL boiling flask. The mixture was then transferred into a microwave oven. After microwave irradiation for 5 min, the resulting products were filtered and washed with DMF and then dried at 70 °C for 10 h.

Preparation of MOF/Fla. At the feeding weight ratio (Fla: MOF-808) of 3: 1, the MOF-808 (0.4 mg/mL) was mixed with Fla solution and the suspension was protected from light and stirred overnight. The as-prepared Fla loaded MOF-808 was collected by centrifugation. Then, the payload of Fla was calculated by measuring the absorbance of the difference between the supernatant after centrifugation and initial solution. The amount of adsorbed drug was determined by measuring the absorbance of the drug from the supernatant liquid, quantified from a standard curve. The loading capacity was calculated as follows: Loading capacity = $W/(W+W_{\text{MOF-808}}) \times 100\%$

W is the weight of adsorbed drug in the MOF-808, $W_{\text{MOF-808}}$ is the weight of total MOF-808.

Isolation of mature mouse neutrophil from bone marrow. Neutrophils were isolated from bone marrow of healthy mouse following the published protocols with minor modification.[2] Briefly, the bones were removed and freed of muscle and sinew. Then bone marrow was flushed from the bones with PBS. The suspension was added slowly into a three-layer Percoll gradient of 55 %, 65 %, and 78 % Percoll, respectively, diluted in PBS (v: v), and centrifuged (500 g, 30 min). As a result, bone marrow cells were layered and the mature neutrophils were recovered at the interface of the 65 % and 78 % fractions. The obtained neutrophils were identified with anti-Ly6g antibody (FITC). The use of antibodies strictly followed the product manual. Ly6g is selectively present at higher levels in neutrophils and is a specifc marker that separates neutrophils from other leukocytes.[3]

Preparation of cell membrane. Neutrophils membrane was derived following a previously published protocol.[4] Briefly, cells were washed with cold PBS and were then suspended in hypotonic lysing buffer containing protease inhibitor. Cells were then disrupted with a homogenizer. The suspension was centrifuged (20000 g, 20 min) at 4 °C. The precipitate was discarded and supernatant was centrifuged again (100000 g, 30 min). The RBC membrane was derived following our previous work.[5] Membrane

was stored at -80 °C for subsequent studies.

Membrane coating. Neutrophil membrane was added to MOF/Fla solution at a particle-to-membrane protein weight ratio of 2:1. Then, the solution was sonicated for 3 min at 4 °C to obtained Neu-MOF/Fla. RBC- MOF/Fla were obtained following the same procedure.

Measurements of CO release of Neu-MOF/Fla. The Neu-MOF/Fla solution (50 μg/mL) was illuminated (λ = 410 nm; power = 15 mW/cm^2) over the course of 6 min with an interval of 0.5 min for periodically measuring emission spectra of the solution mixture.

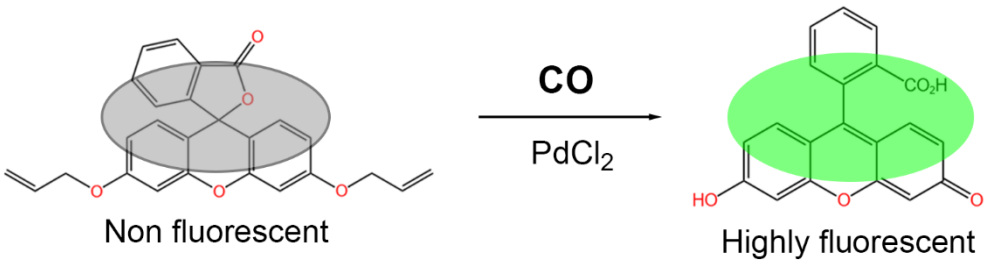
Hydrolysis of Aβ by MOF nanozymes. Aβ monomers (40 μM) were incubated with/without MOF nanozymes at 37 °C for 24 h. Then, the as-prepared samples were collected by centrifugation and utilized for MALDI-TOF-MS analysis.

Cell culture. BV2 cells were cultured in MEM medium (Gibco®, Life Technologies, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum and in a humidified 5 % CO_2 environment at 37 °C.

Two-photon confocal laser scanning microscopy (TP-CLSM) to trigger intracellular CO release. BV2 cells were seeded into 24-well plates at an initial density of 1.0 x 10^5 cells/cm^2 and allowed to adhere to the chamber slides for 24 hours. Neu-MOF/Fla dissolved in PBS buffer was added to a final concentration of 50 μg/mL per chamber. The cells were incubated for 6 h in media, protected from light. Then, the cells were washed three times with PBS buffer and irradiated with two-photon laser in a scanned manner (λ = 820 nm, using confocal laser source, 4100 mW/cm^2 at 4 % laser power) for different periods of time, followed by treated with DAPI (1 μg/mL) for 15 min and three washes with PBS buffer to remove all the residual dye. The cells were visualized under a confocal laser scanning microscope. Acquired images were

processed by universal adjustment to enhance contrast levels (same settings were applied for all acquired images in each detection channel).

Intracellular CO release of Neu-MOF/Fla detected with CO sensor. BV2 cells were seeded into 24-well plates at an initial density of 1.0 x 10^5 cells/cm^2 and allowed to adhere to the chamber slides for 24 hours. Neu-MOF/Fla dissolved in PBS buffer was added to a final concentration of 50 μg/mL in corresponding chamber. The cells were incubated for 6 h in media, protected from light. The culture chamber was then gently washed three times with plain culture media to remove residual nanoparticles. Next, a CO sensor (490 nm excitation, 527 nm emission) was added to the chambers for 30 min at a final concentration of 12.5 μM by diluting a DMSO solution of CO sensor (10 mM) with PBS (**Scheme S1**) [6]. The culture chamber was then gently washed twice with plain culture media to remove residual compound. Then, the cells of experimental group were washed three times with PBS buffer and irradiated with two-photon laser in a scanned manner (λ = 820 nm, using confocal laser source, 4100 mW/cm^2 at 4 % laser power) for 30 min, followed by treated with DAPI (1 μg/mL) for 15 min and three washes with PBS buffer to remove all the residual dye. The cells were visualized under a confocal laser scanning microscope. Acquired images were processed by universal adjustment to enhance contrast levels (same settings were applied for all acquired images in each detection channel).



Scheme S1. The CO sensing reaction for detecting the release of CO.

Immunofluorescent staining. The expression level of pro-inflammatory phenotypic marker CD16, anti-inflammatory phenotypic marker CD206 were assessed by immunofluorescent staining. BV2 cells (2 × 10^5/dish) seeded in a confocal dish were

inoculated with Neu-MOF/Fla (50 μg/mL) dispersed in completed MEM media for 4 h and irradiated with two-photon laser for 15 min, then treated with Aβ for 24 h. After fixation in 4 % paraformaldehyde for 20 min, the cells were blocked by 3 % fat-free milk in PBS for 1 h at room temperature. The cells were incubated with anti-CD16 antibody (PE) and anti-CD206 antibody (FITC) at 4 °C for 2 h. After staining the nuclear by DAPI, the cells were visualized under a confocal laser scanning microscope. The fluorescence intensity was quantified by Image J software (US National Institutes of Health).

Animal model. APPswe/PS1M146V/tauP301L transgenic AD model mice (3×Tg-AD mice), harbor a Psen1 PS1M146V mutation and the coinjected APPSwe and tauP301L transgenes. The control mice (C57BL/6 mice) were purchased from Jilin University. All animal experiments and maintenance protocols were complied with the Institutional Animal Care and Use Committee of Jilin University.

***Ex vivo* fluorescence imaging.** 3×Tg-AD mice were received intraperitoneal injection of Cy5-labelled Neu-MOF/Fla (2 mg/kg). After 6 h, all the mice were sacrificed. The brains, hearts, livers, spleens, lungs and kidneys were excised for *ex vivo* fluorescence imaging.

Morris water maze (MWM) test. The 3×Tg-AD mice (9-month-old, male) (n = 5) received intraperitoneal injection of saline or saline containing Neu-MOF/Fla (2 mg/kg) at the first day and the seventh day over a 15-day period, and irradiated with two-photon laser in a scanned manner (λ = 820 nm, using confocal laser source, 4100 mW/cm^2 at

4 % laser power) for 15 min. After the treatment, a water maze was used to test memory of 3×Tg-AD mice. Prior to water maze testing, animals were first tested with 5 trials per day for 5 consecutive days. The position of the platform remained unchanged throughout the experiment. Data were collected automatically using a tracking system. For data collection, the swimming pool was subdivided into four equal quadrants formed by imaging lines. At the start of each trial, a mouse was placed at one of the four fixed starting points, and allowed to swim for 90 s or until it found the target platform. If the animal was unable to locate the platform within 90 s, the trial was terminated. Time the mice spent swimming within the target quadrant, the time spent seeking for the target platform, and the latency frequency passing through the platform position were recorded for statistical analysis.

Supplemental figures

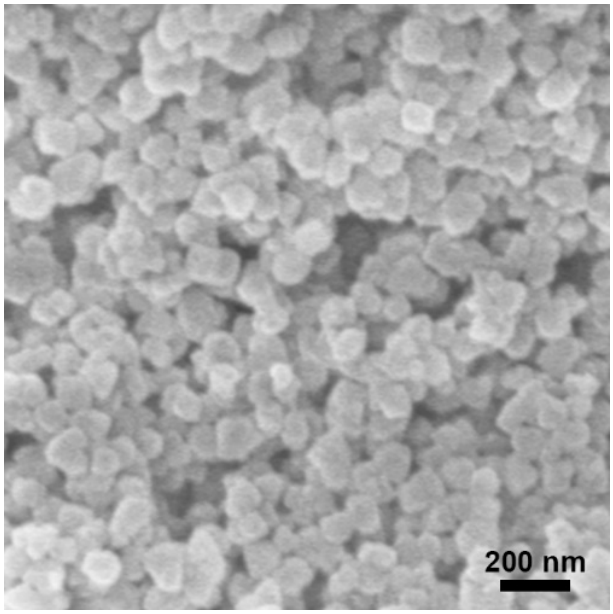


Figure S1. SEM images of MOF-808.

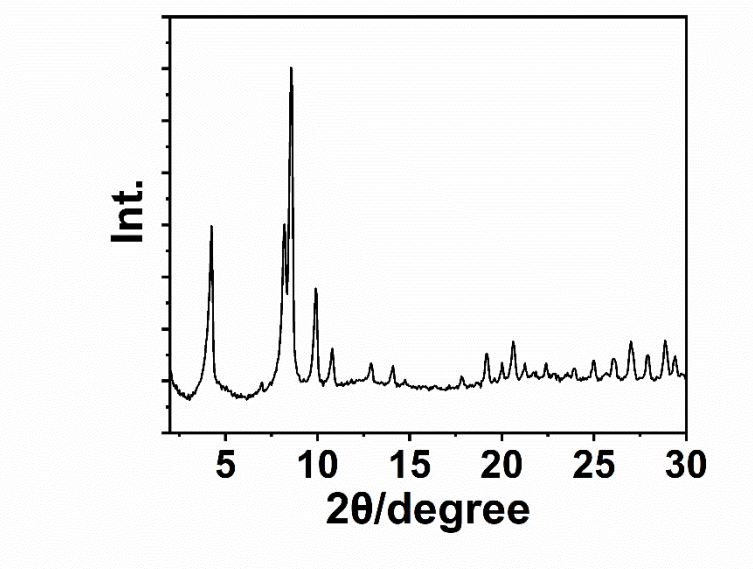


Figure S2. X-ray diffraction (XRD) patterns of MOF-808.

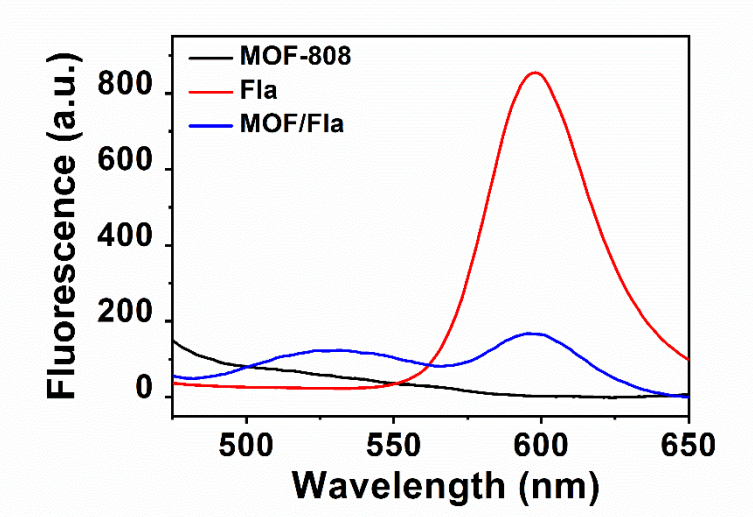


Figure S3. Fluorescence spectra of MOF-808, Fla and MOF/Fla.

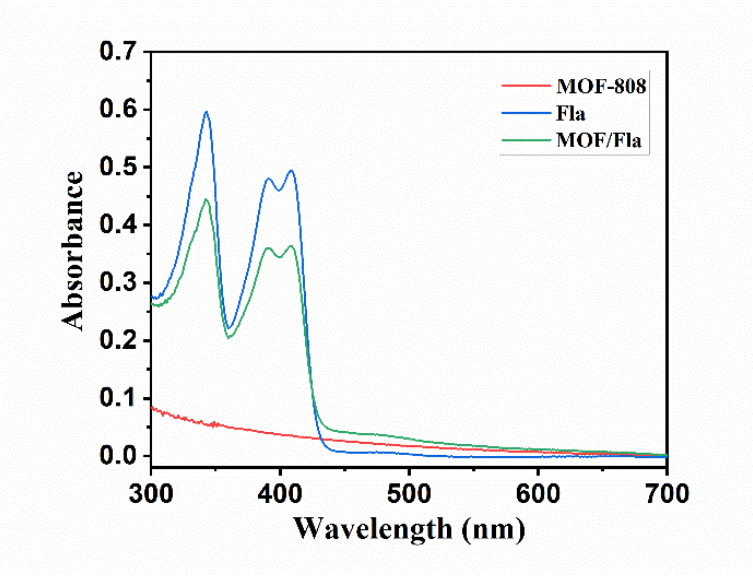


Figure S4. Absorption spectra of MOF-808, Fla and MOF/Fla.

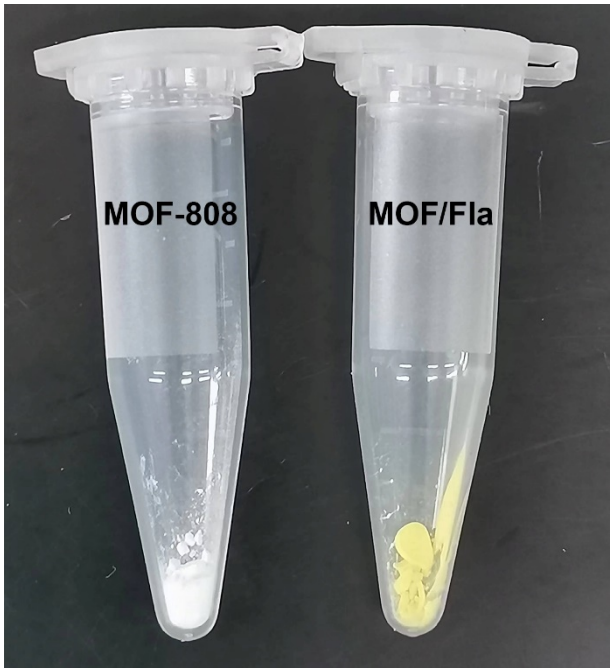


Figure S5. Typical pictures of MOF-808 and MOF/Fla.

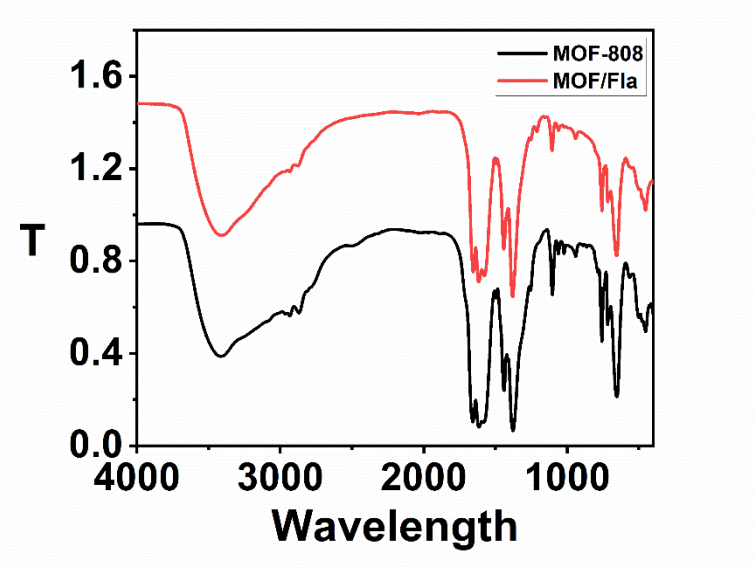


Figure S6. Fourier transform infrared spectroscopy (FTIR) of MOF-808 and MOF/Fla.

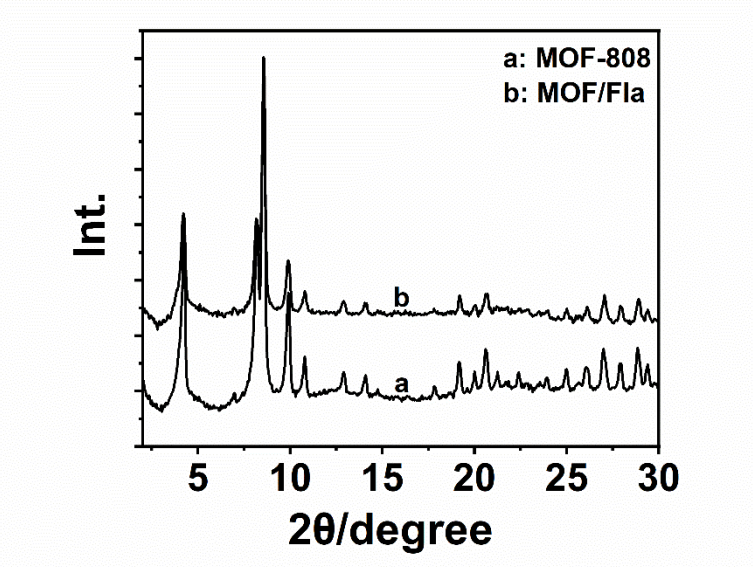


Figure S7. X-ray diffraction (XRD) patterns of MOF-808 and MOF/Fla.

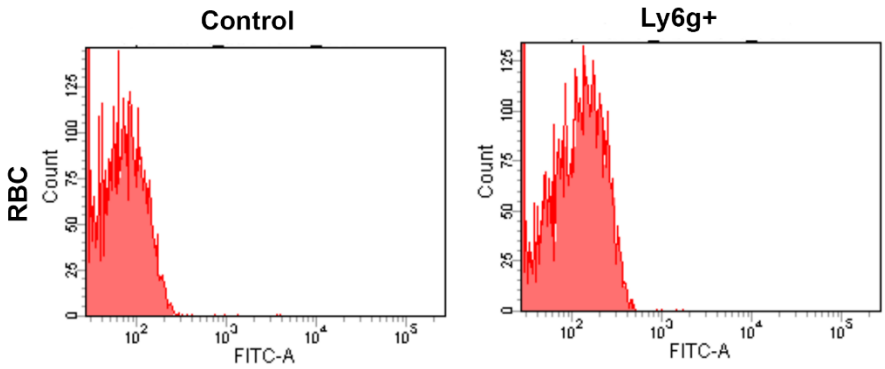


Figure S8. Flow cytometric analysis of the RBC stained with FITC-conjugated Ly6g antibodies.

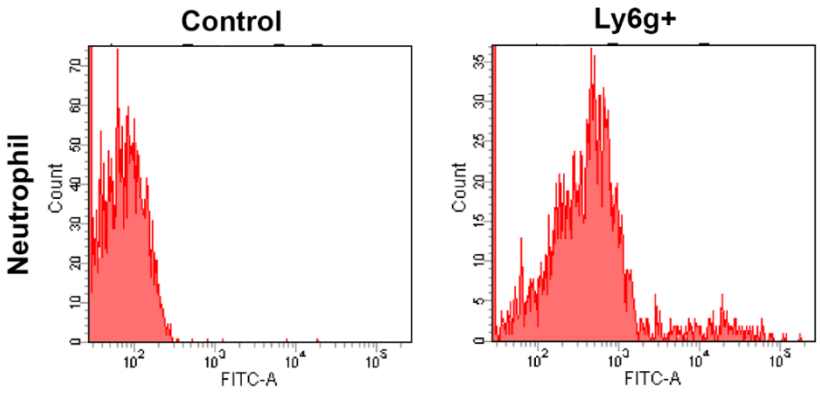


Figure S9. Flow cytometric analysis of the neutrophil stained with FITC-conjugated Ly6g antibodies.

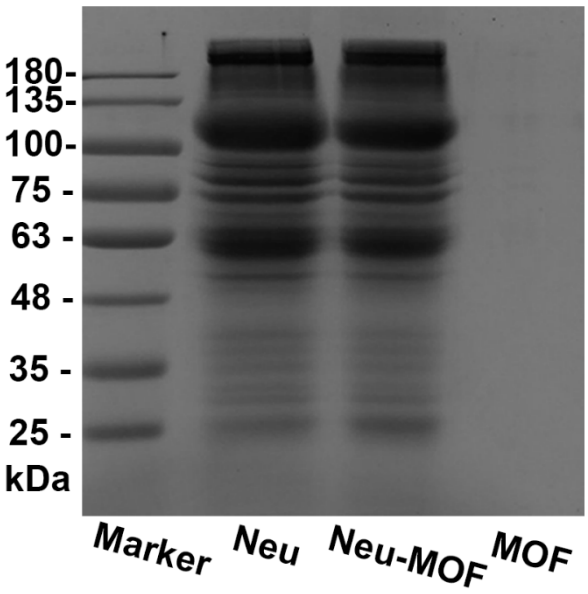


Figure S10. Protein analysis of neutrophil membrane, Neu-MOF, and MOF by using SDS-PAGE. Samples were stained with Coomassie Brilliant.

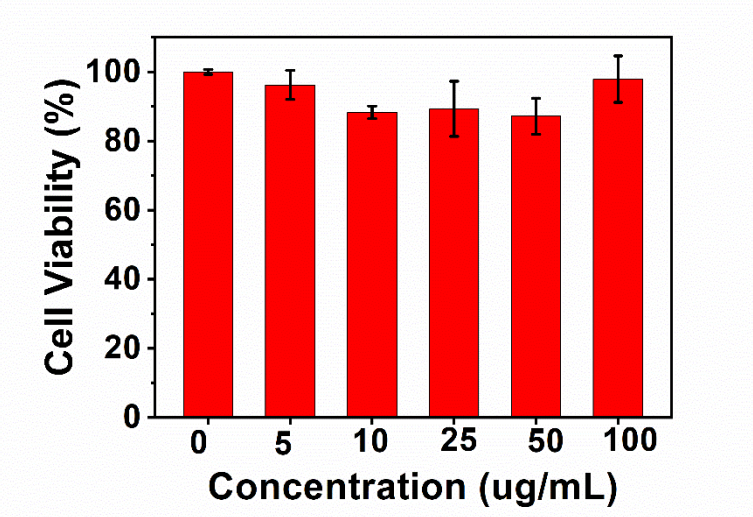


Figure S11. MTT assay of Neu-MOF/Fla at different concentrations co-incubated with BV2 cells for 24 h at our experimental concentration.

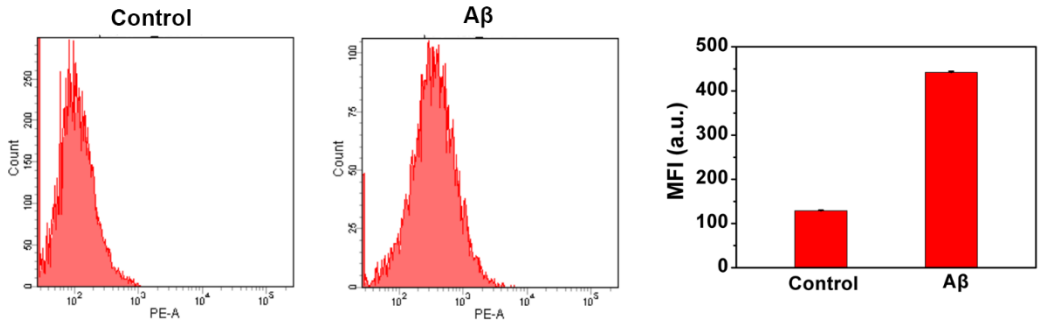


Figure S12. Flow cytometric analysis of the activation of BV2 cells by Aβ stained with PE-conjugated CD16 antibodies.

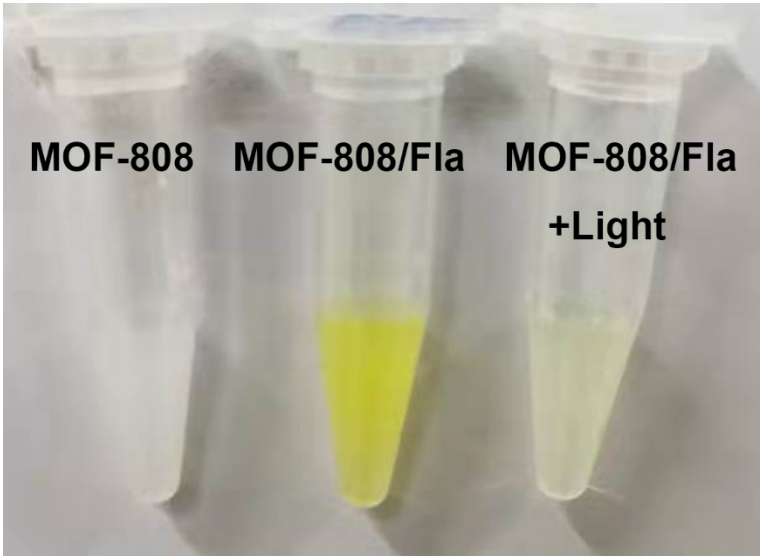


Figure S13. Typical pictures of Neu-MOF/Fla solution before and after irradiation.

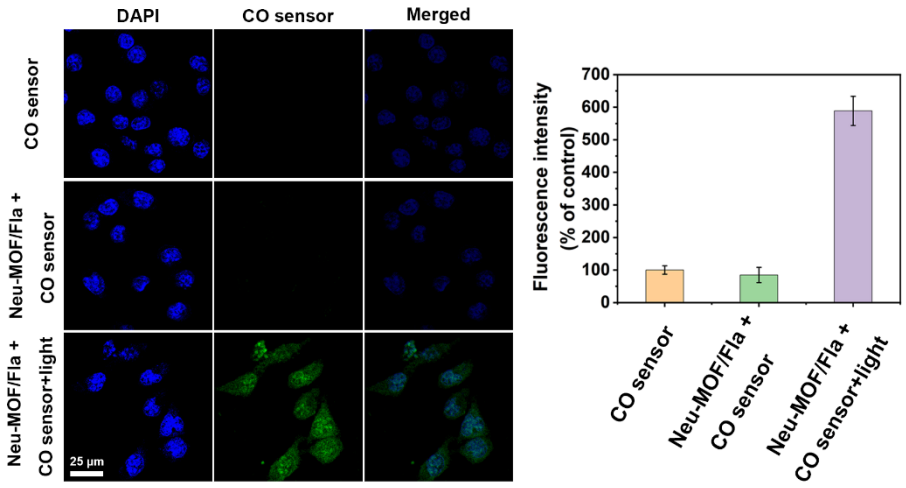


Figure S14. Intracellular CO release of Neu-MOF/Fla (50 μg/mL) under the illumination detected with CO sensor. Scale bar: 25 μm. Corresponding green fluorescence intensity of confocal microscopy images was measured by using Nikon Eclipse Analysis software.

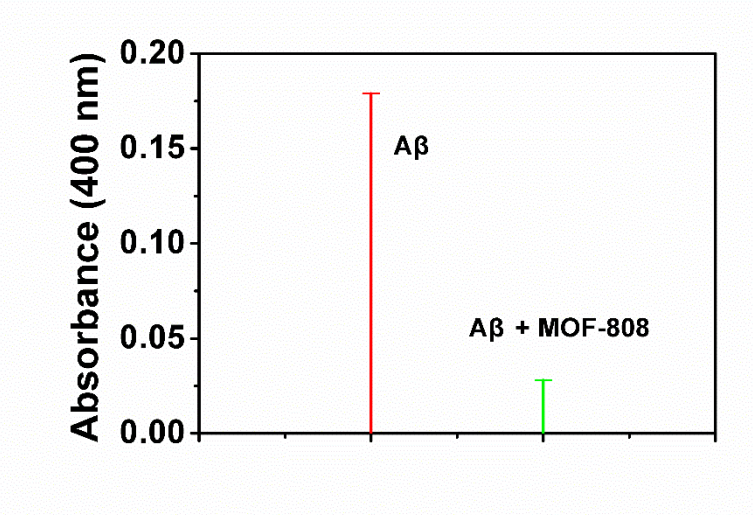


Figure S15. The turbidity value of Aβ samples after incubation without/with MOF-808.

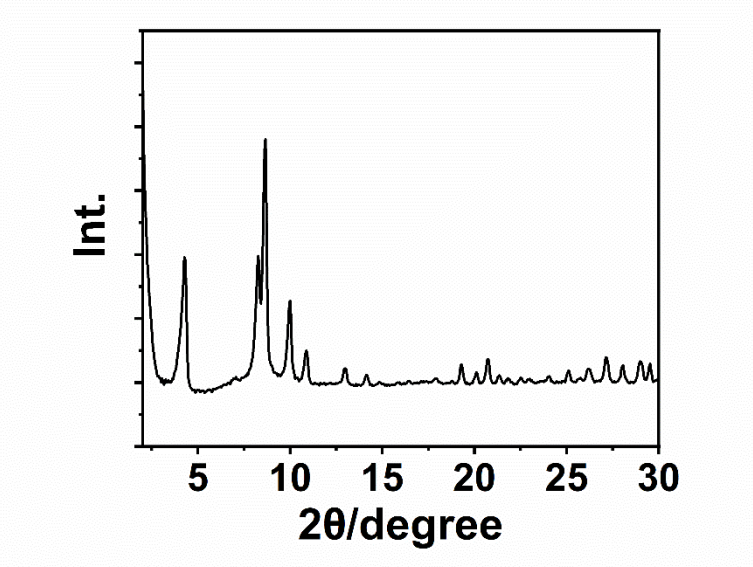


Figure S16. X-ray diffraction (XRD) patterns of MOF-808 after the reaction.

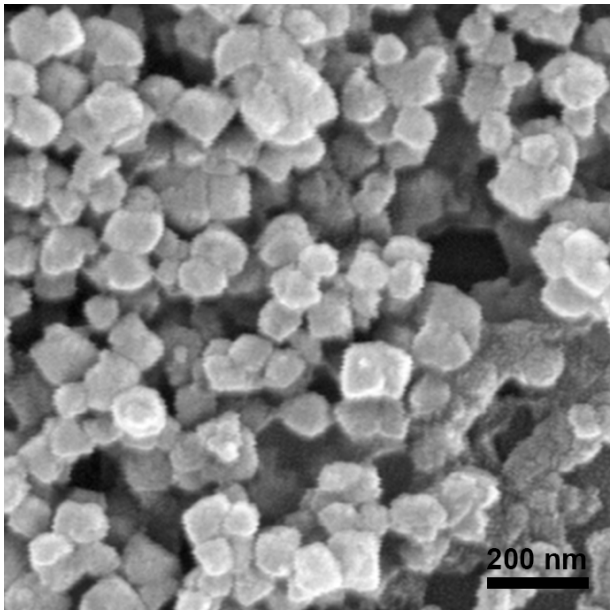


Figure S17. SEM images of MOF-808 after the reaction.

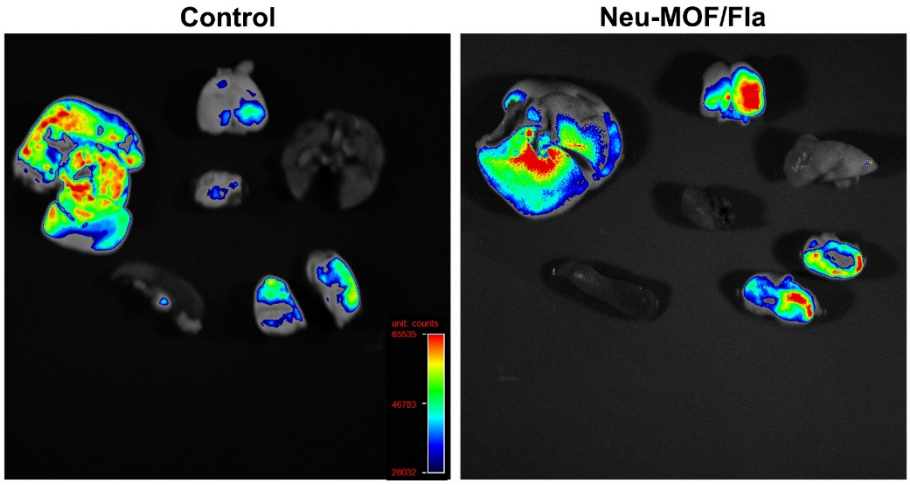


Figure S18. *Ex vivo* brains and organs fluorescence imaging of 3xTg-AD mice administrated with Neu-MOF/Fla.

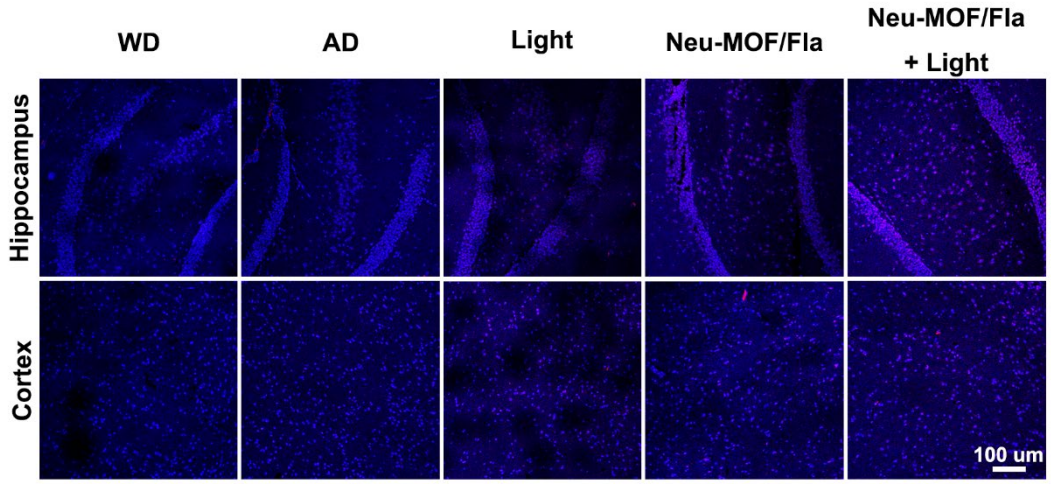


Figure S19. Immunofluorescence of CD206 in the hippocampus and cortex of 3×Tg-AD mice with different treatments.

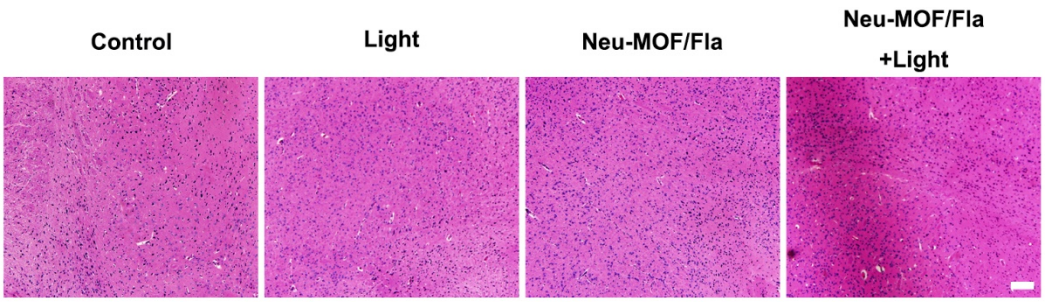


Figure S20. H&E staining of brain after 15 days of different treatment (scale bar = 100 μm).

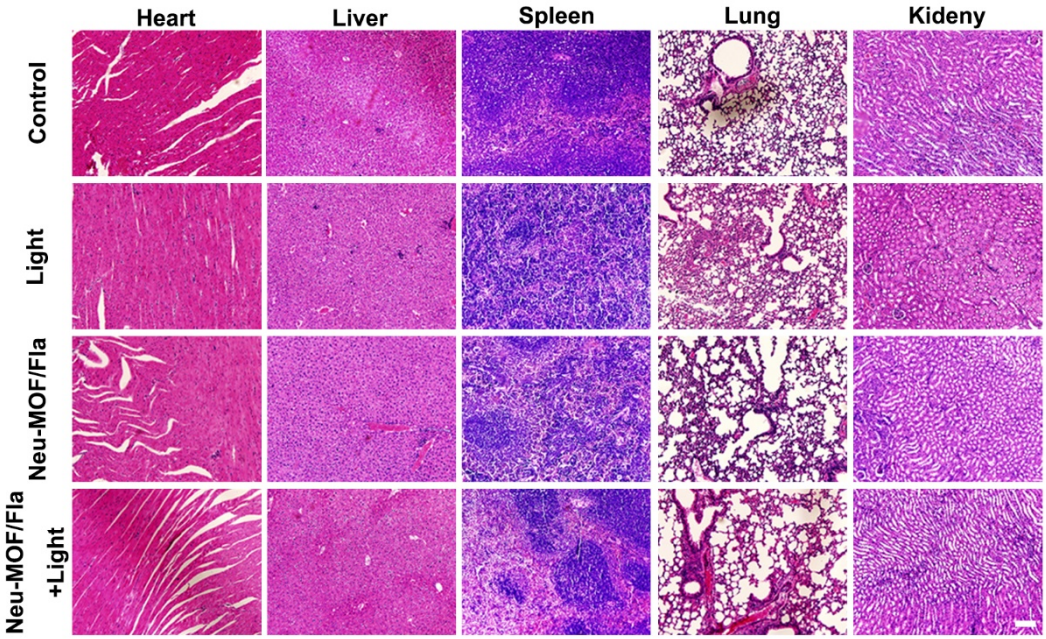


Figure S21. H&E staining of major organs after 15 days of different treatment (scale bar = 100 μm).

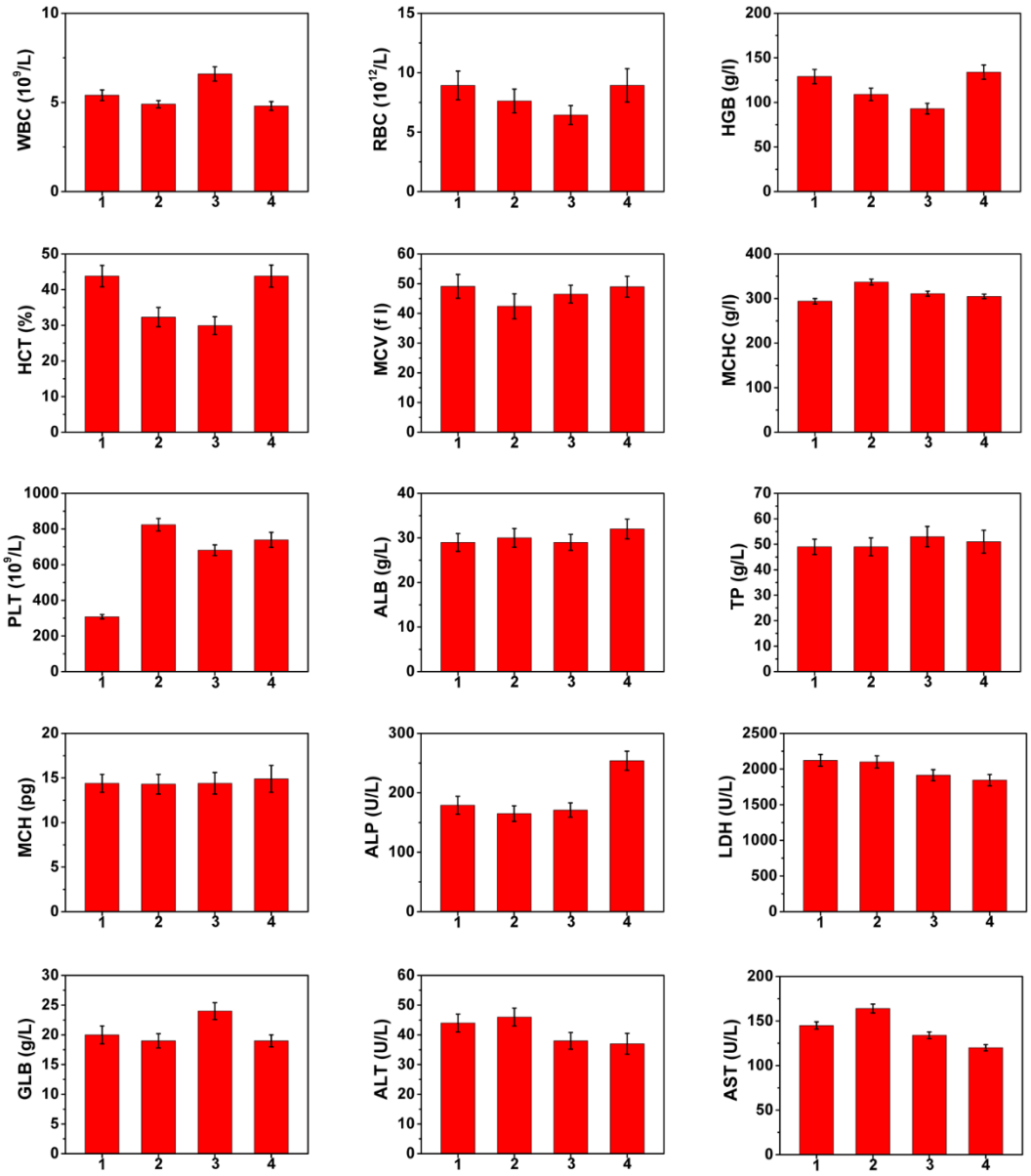


Figure S22. Blood biochemistry analysis of mice intravenously injected with different formulas: (1) saline (as a control), (2) Light, (3) Neu-MOF/Fla, (4) Neu-MOF/Fla + Light.

References

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6. Feng, S. M.; Liu, D. D.; Feng, W. Y.; Feng, G. Q., Allyl Fluorescein Ethers as Promising Fluorescent Probes for Carbon Monoxide Imaging in Living Cells. Anal. Chem. 2017, 89 (6), 3754-3760.