Supporting Information:

Multimodal Enzyme Delivery and Therapy Enabled by Cell Membrane-Coated Metal–Organic Framework Nanoparticles

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Materials and Methods

Nanoparticle Preparation. To prepare the MOF nanoparticles, 0.2 mL solutions of zinc nitrate hexahydrate at 1 mg/mL (Sigma-Aldrich) and 2-methylimidazole at 20 mg/mL (Sigma-Aldrich) were added together. The mixture was vortexed for 30 s and left undisturbed for 3 h. Loading of the uricase (*Candida* sp., 5.3 U/mg; Sigma-Aldrich) was achieved by premixing an appropriate amount of the enzyme with the 2-methylimidazole solution to achieve uricase inputs ranging from 0.05 to 0.25 U. For nanoparticle coating, mouse RBC membrane or MΦ membrane was respectively derived from CD-1 mouse blood (BioreclamationIVT)[1] or murine J774 MΦs (TIB-67; American Type Culture Collection)[2] using previously reported protocols and suspended at 1 mg/mL in water. The membrane solution was then added to an equal volume of MOF or MOF-uricase nanoparticles for 30 min, followed by sequential extrusion through polycarbonate porous membranes (Whatman) using an Avanti mini extruder. The resulting nanoparticles were isolated by centrifugation at 10,000 *g* for 5 min and then resuspended in water for further use. Unless otherwise stated, studies were conducted with formulations made using uricase inputs of 0.1 U for RBC-MOF-uricase and 0.25 U for MΦ-MOF-uricase. RBC membrane vesicles and MΦ membrane vesicles were prepared by extruding purified cell membrane through the same set of porous membranes. All nanoparticle concentrations are expressed as membrane protein mass per unit volume.

Nanoparticle Characterization. Size and zeta potential were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS. To visualize nanoparticle morphology, the nanoparticle sample was adsorbed onto a carbon-coated 400-mesh copper grid (Electron Microscopy Sciences) and stained with 1 wt% uranyl acetate (Electron Microscopy Sciences), followed by imaging on a JEOL 1200 EX II transmission electron microscope. For the stability

study, nanoparticle samples were stored in PBS at room temperature, and size was measured periodically by DLS over the course of 8 days.

Quantification of Uricase Loading, Release, and Activity. For loading quantification, uricase was prelabeled with NHS-fluorescein (excitation/emission = 494/518 nm; Thermo Scientific), and the fluorescence of the supernatant after centrifugation of the extruded nanoparticles was measured using a BioTek Synergy Mx microplate reader. To quantify uricase release, at predetermined timepoints, aliquots of nanoparticles in PBS were centrifuged to pellet the nanoparticles, and the fluorescence of fluorescein in the supernatant was measured using a BioTek Synergy Mx microplate reader. The *in vitro* activity of RBC-MOF-uricase was quantified by an Amplex Red uric acid/uricase assay kit (Invitrogen), with uric acid as the substrate and free uricase as the standard. To study the *in vitro* activity of MΦ-MOF-uricase, monosodium urate was synthesized from uric acid (Sigma-Aldrich) according to literature[3] and used as the substrate for quantification using an Amplex Red uric acid/uricase assay kit.

Protein Characterization. Samples at 1 mg/mL protein content or an equivalent amount of MOF-uricase were prepared in NuPAGE Novex lithium dodecyl sulfate (LDS) sample loading buffer (Invitrogen) and run on 12-well Bolt 4%-12% Bis-Tris minigels (Invitrogen) in MOPS running buffer (Invitrogen). To visualize the overall protein profile, the gel was incubated in InstantBlue Protein Stain (Expedeon) for 1 h and imaged under a Bio-Rad Gel Doc XR system. To assess the presence of specific markers, proteins were transferred to 0.45 μm nitrocellulose membranes (Pierce) in Bolt transfer buffer (Novex) at 10 V for 60 min. The membranes were then blocked with 2% bovine serum albumin (Sigma-Aldrich) in PBS with 0.05% Tween 20 (National Scientific). Blots were probed with primary antibodies specific for mouse CD47 (miap301; Biolegend), IL1R1 (H-8, Santa Cruz Biotechnology), IL1R2 (3H4H4, Proteintech), TNFR1 (H-5,

Santa Cruz Biotechnology), TNFR2 (TR75-89, Santa Cruz Biotechnology), IL6Rα (D-8, Santa Cruz Biotechnology), or gp130 (E-8, Santa Cruz Biotechnology), followed by the appropriate horse radish peroxidase (HRP)-conjugated secondary antibodies (Biolegend). Development was done using ECL western blotting substrate (Pierce) in a Mini-Medical/90 developer (ImageWorks).

Uricase Degradation Study. All animal experiments were performed in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee of the University of California San Diego. Uricase-specific murine polyclonal antibodies were generated by immunizing a male CD-1 mouse (Envigo) with 0.1 mg uricase in Imject alum adjuvant (Thermo Scientific). Two boosters were given before collecting blood to obtain antibody-containing serum. For the study, 5 μL of 0.25% trypsin-EDTA (Gibco) was added into 100 μL aliquots containing 0.05 U of uricase in free form or encapsulated within RBC-MOF, and the mixtures were incubated at 37 ºC for increasing amounts of time. Uricase degradation profiling was achieved by western blot analysis as described above using the polyclonal anti-mouse uricase as the primary immunostain, followed by an HRP-conjugated anti-mouse IgG secondary antibody (Biolegend).

***In Vitro* Cytokine Binding.** To study the *in vitro* binding of proinflammatory cytokines, recombinant mouse IL1β (Biolegend), TNFα (Biolegend), or IL6 (Biolegend) at a final concentration of 8 ng/mL was mixed with nanoparticles at final concentrations ranging from 0 to 2 mg/mL. The mixtures were incubated for 2 h at 37 °C and then centrifuged at 16,100 *g* for 10 min to remove the nanoparticles. Cytokine concentrations in the supernatant were quantified by mouse IL1β, TNFα, or IL6 enzyme-linked immunosorbent assay (ELISA) kits (Biolegend) per the manufacturer's instructions.

***In Vivo* Biodistribution and Safety.** RBC-MOF-uricase was fluorescently labeled using 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine (excitation/emission = 644/665 nm; Invitrogen), and 400 μg of the nanoparticles was administered intravenously into adult C57BL/6 mice (Charles River Laboratories). Mice were euthanized at 24 h after nanoparticle administration for sample collection. For the biodistribution study, the major organs, including the liver, spleen, heart, lungs, kidneys, and blood, were collected. The organs were homogenized in 1 mL of PBS using a Biospec Mini-Beadbeater-16. Fluorescence was read using a Tecan Infinite M200 plate reader. To calculate the signal in the blood, total volume was estimated as 6% of mouse body weight. To obtain blood cell counts, whole blood was collected into potassium–EDTA collection tubes (Sarstedt), and analysis was performed by the UC San Diego Animal Care Program Diagnostic Services Laboratory. To perform the histological analysis, the major organs were sectioned and stained with H&E (Leica Biosystems), followed by imaging using a Hamamatsu Nanozoomer 2.0-HT slide scanning system.

***In Vivo* Treatment Efficacy Studies.** Hyperuricemia in mice was induced based on a previously reported procedure.[4] Food and water were withheld overnight prior to the study. Briefly, to increase the serum uric acid levels, adult C57BL/6 mice were injected intraperitoneally with 4 mg of allantoxanamide (BOC Sciences) suspended in 0.2 mL of 0.5% carboxymethylcellulose sodium (Grainger). After 2 h, the mice were intravenously administered with 0.2 mL of PBS, RBC-MOF-uricase at 2 mg/mL, or free uricase at an equivalent concentration. Blood samples were collected at predetermined timepoints and allowed to clot for 30 min at room temperature. After centrifugation to obtain the serum, the uric acid content was assayed using an Amplex Red uric acid/uricase assay kit.

Gout was induced according to a previously reported procedure.[5] Briefly, adult C57BL/6 mice were placed under anesthesia with a cocktail of ketamine (Pfizer) at 100 mg/kg and xylazine (Lloyd Laboratories) at 20 mg/kg. This was followed by intraarticular injection with 0.5 mg of monosodium urate in 25 μL of PBS into the ankle joint. After 24 h, the ankle joints were intraarticularly injected with 25 μL of PBS, MΦ-MOF at 2 mg/mL, RBC-MOF-uricase (inputted with 0.25 U of uricase) at 2 mg/mL, MΦ-MOF-uricase at 2 mg/mL, or free uricase at an equivalent concentration with the mice under anesthesia. Ankle joint swelling was measured with an electronic caliper at the indicated timepoints. To assess the remaining uric acid content and local proinflammatory cytokine (IL1β, TNFα, IL6) concentrations, mice were euthanized 48 h after treatment, and the periarticular tissues were collected. The tissues were homogenized in PBS containing a protease inhibitor cocktail (Sigma-Aldrich). After centrifugation, the supernatant was assayed for uric acid content using an Amplex Red uric acid/uricase assay kit and for cytokine concentrations by the appropriate ELISA kits per the manufacturer's instructions. To perform the histological analysis, the periarticular tissues were collected 48 h after treatment. The collected tissues were fixed with phosphate buffered formalin (Fisher Scientific), decalcified with 10% EDTA (Corning), and stained with H&E. Images were obtained using a Hamamatsu NanoZoomer 2.0-HT slide scanning system.

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