

# Electronic Supplementary Material

## “Cracking the Immune Fingerprint of Metal-Organic Frameworks”

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### Index

#### Table of Content

##### Experimental Section

1. Materials
2. Synthesis of MIL-100(Fe) nanoparticles
3. Synthesis of MIL-100(Al) nanoparticles
4. Synthesis of ZIF-8(Zn) nanoparticles
5. Physicochemical characterization
6. *In vitro* cell studies
  - 6.1. Cells and culture
  - 6.2. Cytotoxicity assays
  - 6.3. ROS production
  - 6.4. Complement activation
  - 6.5. Cytokines profile evaluation
7. Statistics

##### Figures

- S1.** TEM images.
- S2.** XRPD patterns.
- S3.** Cytotoxicity assays. Cell viability of J774.A1 cell line after 24 h.

##### References

## **Experimental Section**

### **1. Materials**

Iron(III) chloride hexahydrate (97%), 1,3,5-benzene tricarboxylic acid (trimesic acid, BTC; 95%), aluminium(III) nitrate nonahydrate (99.9%), zinc(II) nitrate hexahydrate (98%), phosphate buffered saline (PBS) solution (0.01 M, pH=7.4), trypsin-Ethylenediaminetetraacetic acid (EDTA), thiazolyl blue tetrazolium bromide (MTT), Zymosan A from *Saccharomyces cerevisiae*, lectin (red kidney bean), phytohemagglutinin (PHA) were purchased from Sigma-Aldrich. Ethanol (96 %), 2-methylimidazole (99%), methanol (99.5%) and nitric acid were obtained from VWR. RPMI (Roswell Park Memorial Institute medium) 1640 medium supplemented with glutamax-1 from Gibco-Life Technologies (see ref<sup>[1]</sup> for composition). Similarly, 2',7'-dichlorofluorescein diacetate (2.5  $\mu$ M; DCFH-DA), L-glutamine (2 mM), Tris/EDTA (10 mM, pH 7.4) were purchased from Life Technologies. Heat-inactivated fetal bovine serum (FBS), dimethylsulfoxide (DMSO;  $\geq$  99.7 %), and penicillin/streptomycin (100 U.mL<sup>-1</sup>), were provided by Fischer. Polyvinylidene difluoride membrane (PVDF, Immun-Blot, Bio-Rad; Hercules, CA), Tris Buffered Saline with Tween 20, 1x (TBS-T), and 5-bromo 4-chloro 3'-indolylphosphate *p*-toluidine salt (BCIP), were purchased by Bio-Rad Laboratories, Hercules, CA. Ficoll-Paque PLUS<sup>TM</sup> (GE Healthcare), lipopolysaccharide (LPS; InvivoGen, San Diego, CA), phorbol 12-myristate-13-acetate (PMA; Abcam, Biochemicals), Monoclonal mouse antibody anti-human C3/C3b (IgG2b mouse anti- C3/C3b, Abcam-ab1187, Biochemicals), human Th1/Th2 11plex Ready-to-Use FlowCytomix Multiplex kit , (eBioscience, Affimetrix) and goat secondary antibodies anti-Ig mouse linked to alkaline phosphatase (AP) (DO486, Dako) were also used. All materials were used as received, without further purification.

### **2. Synthesis of MIL-100(Fe) nanoparticles**

MIL-100(Fe) NPs were prepared by microwave-assisted hydrothermal synthesis according to a previously reported procedure.<sup>[21]</sup> Briefly, 845 mg of trimesic acid together with 2.43 g of FeCl<sub>3</sub>·6H<sub>2</sub>O in 20 mL of distilled water were added into a Teflon lined autoclave at 130°C for 4 min in a microwave oven (1600 W) with a heating ramp of 2 min. The NPs were recovered by centrifugation (10500 rpm, 15 min). The activation of MIL-100(Fe) NPs, previously described elsewhere,<sup>[26]</sup> consisted in the re-dispersion and centrifugation (10500 rpm, 15 min) of 2.5 g of the NPs in 20 mL water for 15 min. This procedure was repeated twice in water and five successive times in fresh ethanol (20 mL). Further activation was carried out by re-dispersing the solid in 20 mL of a 0.1 M KF solution. The mixture was kept under magnetic stirring for 1 h 40 min under ambient conditions. Immediately after, NPs were collected by centrifugation (10500 rpm, 20 min) and washed twice with 20 mL of Milli-Q water and once with 20 mL of ethanol following the process described above. Activated MIL-100(Fe) NPs were isolated by centrifugation (10500 rpm, 15 min) and stored wet with a few droplets of fresh ethanol.

### **3. Synthesis of MIL-100(Al) nanoparticles**

MIL-100(Al) NPs were hydrothermally synthesized by a microwave-assisted route.<sup>[21]</sup> Briefly, a solution of 1.43 g of aluminum nitrate nonahydrate, 1.21 g of trimethyl-1,3,5- trimesate and 4 mL of nitric acid (4 M) dissolved in distilled water (20 mL) was heated at 210°C for 30 min using a hydrothermal microwave-assisted method (1600 W). The resulting mixture was cooled down with an ice bath and recovered by centrifugation (10500 rpm, 20 min). MIL-100(Al) NPs were then activated by dispersing the recovered solid into 50 mL of methanol overnight under vigorous stirring. The solid was recovered by centrifugation (10500 rpm, 20 min), exchanged twice with ethanol and kept wet for storage.

### **4. Synthesis of ZIF-8(Zn) nanoparticles**

ZIF-8(Zn) NPs were synthesized by as previously reported.<sup>[23]</sup> Briefly, a solution of 1.47 g of Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O in 100 mL of methanol was rapidly poured when it be dissolved into a solution of 3.24 g of 2-methylimidazole in 100 mL of methanol under vigorous stirring at room temperature (RT). The mixture slowly turned turbid and after 45 min the ZIF-8(Zn) NPs were recovered by centrifugation (10500 rpm, 20 min). The activation was performed readily redispersing the material in 20 mL absolute ethanol and recovering by centrifugation (10500 rpm, 20 min).

### **5. Physicochemical characterization**

X-ray powder diffraction (XRPD) patterns were collected in a D8 Advance Bruker diffractometer with Cu K $\alpha$ 1 radiation ( $\lambda$  = 1.54056 Å). Profiles were generally collected in the 3-20° 2 $\theta$  range with a typical step size of 0.02° in continuous mode. Transmission electron microscopy (TEM) images were collected with a Hitachi H-8100

microscope operating at 200 kV and equipped with an LaB6 filament. Fourier transform infrared (FTIR) spectra were collected using a Nicolet 6700 instrument (Thermo scientific, USA) from 4000 to 400  $\text{cm}^{-1}$ .  $\text{N}_2$  sorption isotherms were obtained at 77K using a BELsorp Mini (Bel, Japan). Samples were previously outgassed (140°C) under vacuum for 3 h. Thermogravimetric analyses (TGA; 5-10 mg) were collected on a Perkin Elmer Diamond TGA/DTA STA 6000 (25–600°C at 3°C·min<sup>-1</sup> with an  $\text{O}_2$  flow of 20 mL·min<sup>-1</sup>). Particle size was monitored by Dynamic Light Scattering (DLS) on a Zetasizer Nano (Malvern Instruments). Samples were prepared by dispersing the NPs at 0.1 mg·mL<sup>-1</sup> at RT in the desired media by the use of an ultrasound tip (10% amplitude for 1 min; Digital Sonifer 450, Branson). Similarly, for biocompatibility assays, the NP solutions were prepared a 10-fold higher concentration (due to a 1/10 direct dilution in each), yielding diverse concentrations range (more details in 2.6.2. section).

## 6. *In vitro* cell studies

### 6.1. Cells and culture

The J774.A1 (ATCC<sup>®</sup>TIB-67<sup>™</sup>) and HL-60 (ATCC<sup>®</sup>CCL-240<sup>™</sup>) cell lines were cultured in RPMI 1640 medium supplemented with 10% of heat-inactivated FBS, L-glutamine and 1% penicillin/streptomycin.<sup>[19,33]</sup> Both cell lines were grown at 37°C in a humidified 5%  $\text{CO}_2$  atmosphere and passaged twice a week (at 80% of confluence) at a density of  $5 \times 10^4$  cells *per*  $\text{cm}^2$ , being harvested by trypsinization (1% trypsin-EDTA solution).

### 6.2. Cytotoxicity assays

The cytotoxic activity of MIL-100(Fe), MIL-100(Al) and ZIF-8(Zn) was analyzed by the colorimetric MTT assay.<sup>[26,2]</sup> Adherent J774.A1 cells were seeded 24 h prior to the assay in 96-well plates at a density of  $1 \times 10^5$  cells *per* well in RPMI supplemented with 10% FBS. The MIL-100(Fe & Al) and ZIF-8(Zn) colloidal solutions were prepared at a 10-fold higher concentration (due to a 1/10 direct dilution: 20  $\mu\text{L}$  of the NP solutions were added to a final volume of 200  $\mu\text{L}$  *per* well), yielding different concentrations (from 200 to 1200  $\mu\text{g} \cdot \text{mL}^{-1}$ ). Subsequently, all these treatments were added into the cells for 24 h, keeping at 37°C with a 5%  $\text{CO}_2$  atmosphere. NPs in culture medium and culture medium alone were also tested to discard any interference in the assay. The treatments were removed and replaced with 100  $\mu\text{L}$  of fresh medium and the cytotoxicity was determined by adding the MTT reactant (10  $\mu\text{L}$  of 5 mg·mL<sup>-1</sup> in phosphate buffered saline (PBS), incubation at 37°C during 2 h) followed by a PBS washing with 200  $\mu\text{L}$ , ending with 200  $\mu\text{L}$  of Dimethyl Sulfoxide (DMSO) added to each well. Absorbance was determined at  $\lambda = 539$  nm in a plate reader after shaking. The percentage of cell viability was calculated by the absorbance measurements of control growth and test growth in the presence of the formulations at various concentrations.

### 6.3. ROS production

The cells were seeded 24 h prior to the assay in 96-well plates (U bottom) at a density of  $1 \times 10^4$  cells *per* well in 200  $\mu\text{L}$  of cell culture medium (RPMI supplemented with 10% FBS). The stimulus-containing solutions of 100  $\mu\text{L}$  were added to the cells at the final concentrations of 25 or 250  $\mu\text{g} \cdot \text{mL}^{-1}$  of each MOF prototype. Basal and negative controls were considered as the cells in the absence of stimulus or the cells in the presence of a ROS sensitive probe (2',7'-dichlorofluorescein diacetate-DCFH-DA; Sigma Aldrich Co.), respectively. As positive control, the cells were incubated with a ROS inductor, phorbol PMA (10  $\mu\text{M}$ ) at 37°C. After diverse incubation times (1, 4, 8 & 24 h), cells were centrifuged (900 rpm, 5 min) and kept in contact with the ROS probe (1  $\mu\text{L}$  *per* 200  $\mu\text{L}$  of cells) at 37 °C, under dark conditions. After 30 min incubation, two PBS-washes were performed, followed with the quantification of the 2',7'-dichlorofluorescein (DCF) fluorescence by flow cytometry (FC500, Beckman-Coulter; Miami, FL) on the total living population region.<sup>[3,4]</sup> Propidium iodide (PI) was also added to the samples to label the dead cell population.

### 6.4. Complement activation

The influence of the NPs on the activation of the complement cascade was evaluated by Western blot with a monoclonal anti-C3/C3b antibody (mAb), as previously described.<sup>[19,20]</sup> Thanks to the Institutional ethics approval, issued by the Ethics Committee for Clinical Research (Xunta de Galicia, Spain, 2013/272), a pool of human sera from healthy donors was included in the study under their written informed consent. All methods were performed in accordance with relevant guidelines and regulation. These human serum samples were incubated with two different concentrations of MIL-100(Fe), MIL-100(Al) and ZIF-8(Zn) NPs (25 and 250  $\mu\text{g} \cdot \text{mL}^{-1}$ ). Zymosan and PBS were used as positive and negative controls, respectively. Equal volumes of plasma, PBS buffer and NPs (50  $\mu\text{L}$  each) were mixed together and incubated at 37°C for 1 h. The mixture was centrifuged (13200 rpm, 30 min) in order to separate the NPs. Supernatants containing complement proteins were loaded

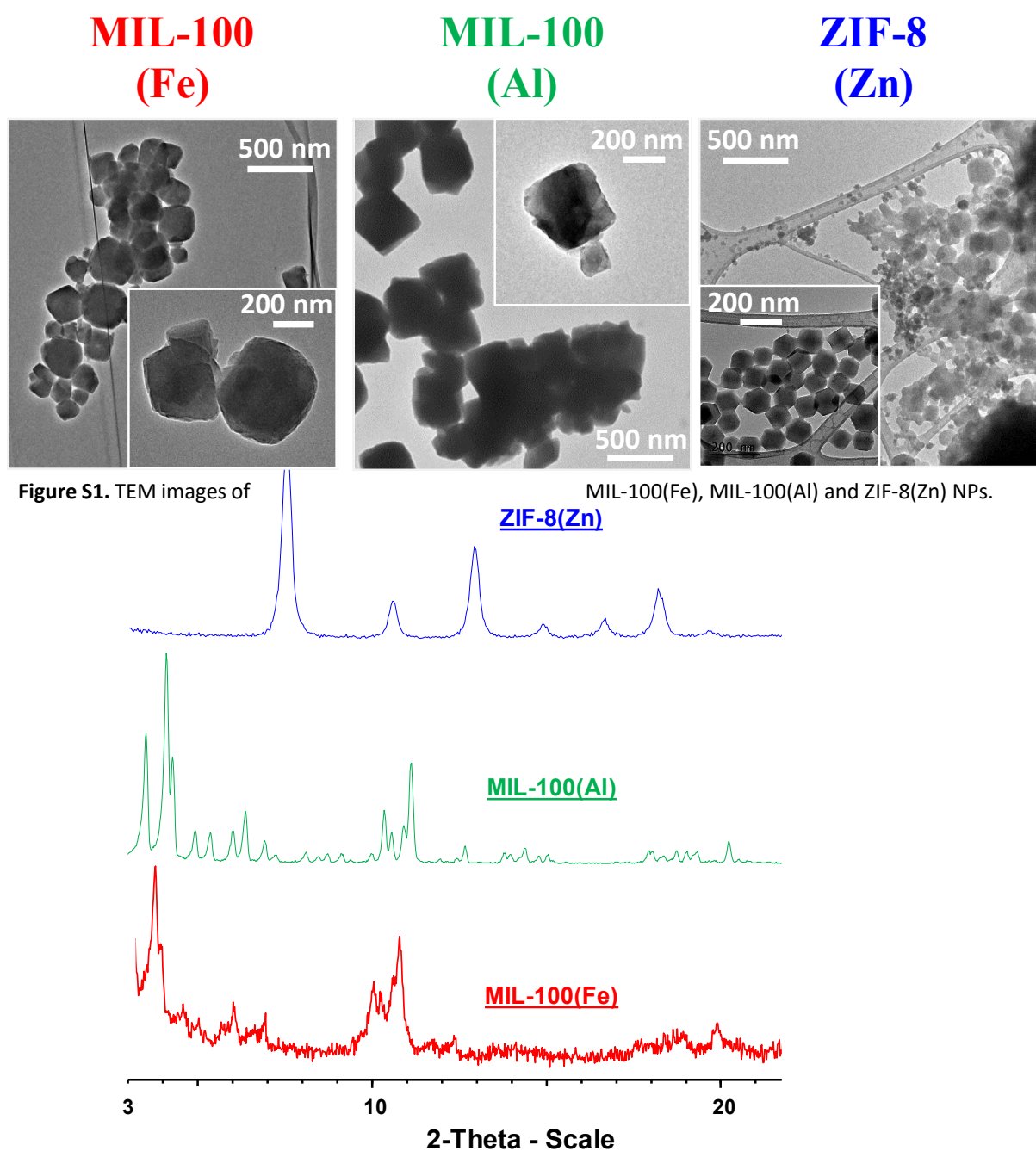
(2  $\mu\text{L}$ ) onto a 10% SDS-PAGE gel and then transferred to a polyvinylidene difluoride (PVDF) membrane using the Transblot Semidry Transfer Equipment (Bio-Rad; Hercules, CA).

#### 6.5. Cytokines profile evaluation

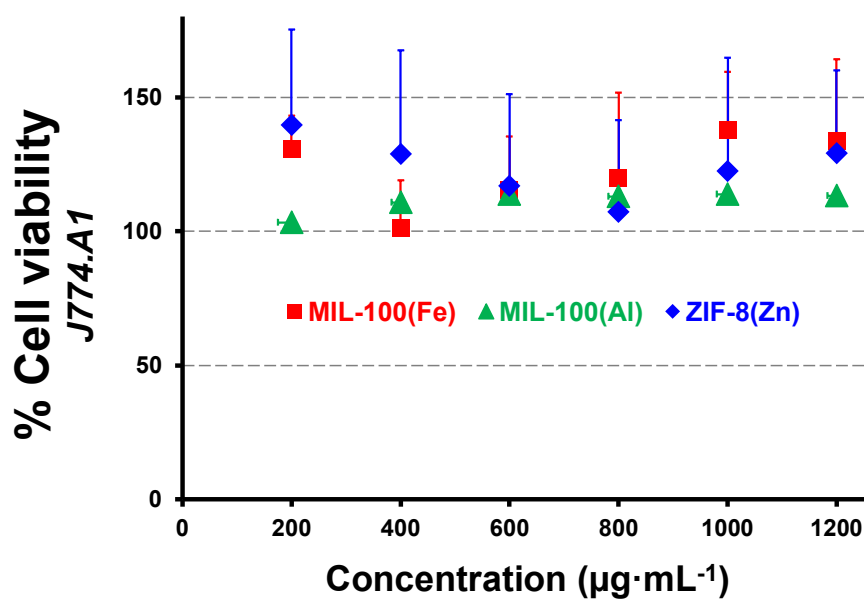
Cytokine production of human peripheral blood mononuclear cells (PBMCs) was evaluated in cell supernatants after 24 h in contact with two different concentrations of MIL-100(Fe), MIL-100(Al) and ZIF-8(Zn) NPs (25 and 250  $\mu\text{g}\cdot\text{mL}^{-1}$ ) by using the FlowCytomix™ kit, as previously reported.<sup>[19,20]</sup> The samples were analyzed with the flow cytometer (FC500, Beckman-Coulter; Miami, FL) and positive (lipopolysaccharide «LPS» at 1  $\text{mg}\cdot\text{mL}^{-1}$  and PHA at 10  $\text{mg}\cdot\text{mL}^{-1}$ ) and negative (PBS alone) controls were included for comparison.

#### 7. Statistics

The results of the different assays are represented as mean  $\pm$  standard deviation (SD). Ordinary Two-way ANOVA analysis of variance followed by a Tukey's multiple comparison test were carried out to determine significant differences using GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA, USA). Each experiment was performed at least three times ( $n \geq 3$ ). In the graphs, the results are indicated as:  $P > 0.05$ ,  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$  and  $****P \leq 0.0001$ .



**Figure S2.** XRPD patterns of MIL-100(Fe), MIL-100(Al) and ZIF-8(Zn) NPs.



**Figure S3.** Cell viability of murine macrophages J774.A1 after 24 h of incubation with MIL-100(Fe), MIL-100(Al) or ZIF-8(Zn) NPs.

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