nature materials

SUPPLEMENTARY INFORMATION

DOI: 10.1038/NMAT2608

1. Synthesis conditions and characterization (XRD, IR, TGA, SEM, Nanosizer)

MIL-88A_nano

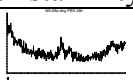
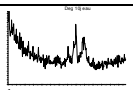
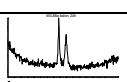
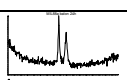
General synthesis type : a solution of $FeCl_3.6H_2O$ (1 mmol; Alfa Aesar, 98%) and fumaric acid (1 mmol; Acros, 99%) in 5 mL of solvent (dimethylformamide (DMF; Fluka, 98%), absolute ethanol (Aldrich, 99%), methanol (Aldrich, 99%) or distilled water) in presence or not of an additive (1mL of acetic acid (Aldrich, 99%) or 0.4 mL of sodium hydroxide 2M (Alfa Aesar, 98%)) was placed into a Teflon-liner steel autoclave at a temperature of 65, 100 or 150°C during different times (from 30 min to 3 days). Then, the obtained precipitated was recovered by centrifugation at 5000 rpm (1400 x g) for 10 min.

To remove the solvent, 200mg of solids were suspended into a 100 mL of deionised water overnight and after centrifuged.

Synthesis in water

The optimised synthesis of MIL-88A_nano was performed from a solution of $FeCl_3.6H_2O$ (1 mmol; Alfa Aesar, 98%) and fumaric acid (1 mmol; Acros, 99%) in 5 mL of distilled water using a Paar bomb at 65 or 100 or150°C for 0.5-74 h, recovering the orange precipitate by centrifugation at 5000 rpm (1398 x g) for 10 min.

Table S1.

Temperature °C	Time(h)	Size (nm)	Cristallinity	Yield
65	2	150 ± 35		1% ± 0,2
	6	250 ± 55		3,5% ± 1,1
	16	410 ± 90		40% ± 8
	72	630 ± 125		65% ± 12

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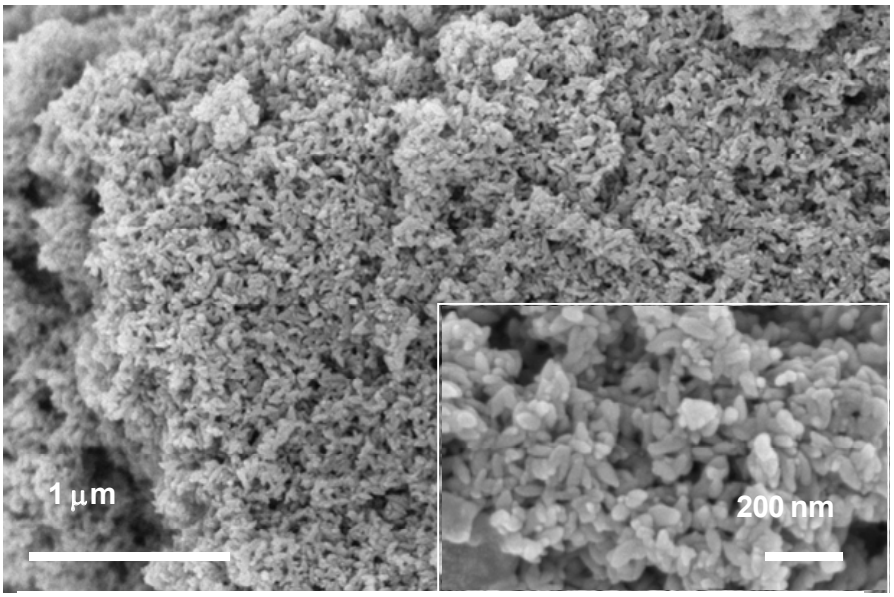
MIL-89_nano

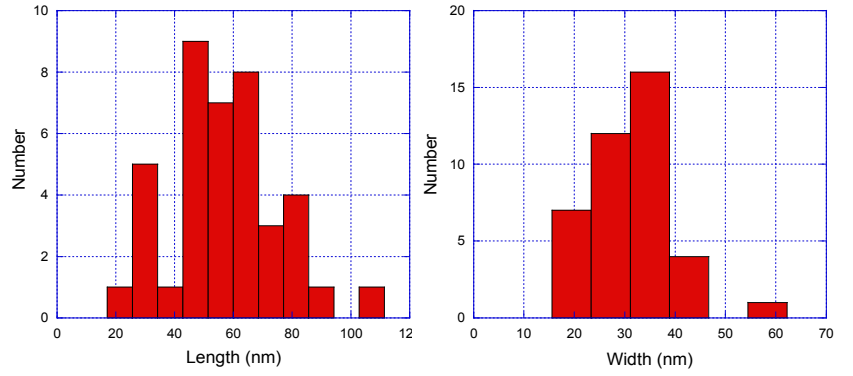
1) Synthesis in methanol

MIL-89_nano solid is obtained as nanoparticles of a 50-100 nm from a solution of iron acetate (1 mmol, synthesis reported in ref) and *trans,trans*-muconic acid (1 mmol; Fluka, 97%) in 5 mL of methanol (Riedel-de Haën, 99.8%) with 0.25 mL of sodium hydroxide 2M (Alfa Aesar, 98%) placed into a Teflon-liner steel autoclave at a temperature of 100°C during 6 h. After that, the obtained precipitated was recovered by centrifugation at 5000 rpm (1400 x g) for 10 min.

With the purpose of removing the solvent, 200mg of solids were suspended into a 100 mL of deionised water overnight and after centrifuged.

The estimated gyration diameter by light scattering methods is 205±6 nm (PDI 0.86). However, spherical particles slightly elongated with a homogeneous distribution of 56±19 in length and 32±9 nm in width are observed by SEM (Figure S1).





Minimum	18,181818	Minimum	18,181818
Maximum	109,09091	Maximum	54,545456
Sum	2240,9091	Sum	1277,2727
Points	40	Points	40
Mean	56,022728	Mean	31,931818
Median	54,545456	Median	36,363636
RMS	59,234965	RMS	33,099212
Std Deviation	19,486617	Std Deviation	8,8240252
Variance	379,72824	Variance	77,86342
Std Error	3,0811047	Std Error	1,3952009
Skewness	0,31928392	Skewness	0,1986866
Kurtosis	0,11416981	Kurtosis	-0,27270579

Figure S1. (top) SEM images of MIL-89_nano solid; (bottom) Statistics of particle size from SEM data

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2) Synthesis in ethanol

$FeCl_3.6H_2O$ (1 mmol; Alfa Aesar, 98%) and *trans,trans*-muconic acid (1 mmol; Fluka, 97%) in 10 mL of absolute ethanol (Aldrich, 99%) are placed in a Paar bomb at 100°C for 15h, recovering the brown precipitate by centrifugation at 10500 rpm for 15 min.

To remove the solvent and free acid, 200mg of solids were suspended into a 100 mL of deionised water overnight and after centrifuged.

The estimated gyration diameter by light scattering methods is 263±25 nm.

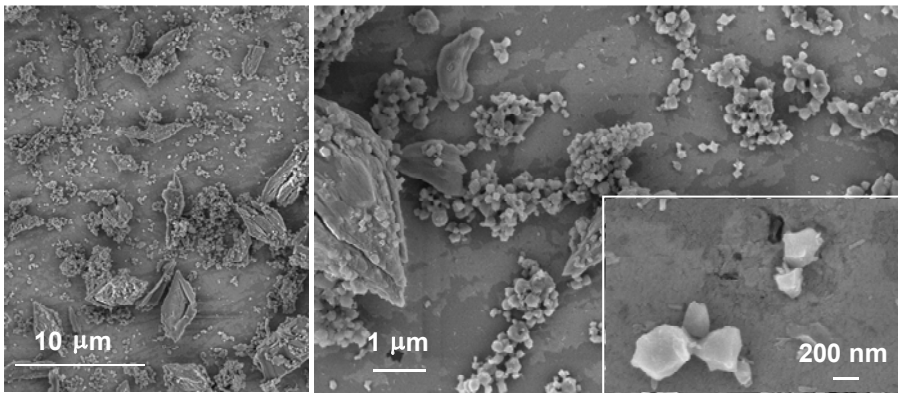
MIL-53_nano

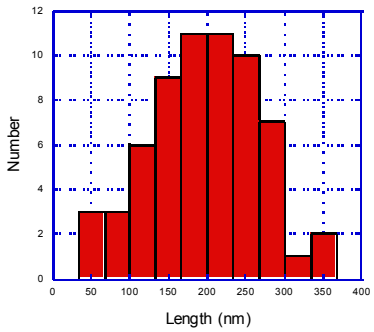
1) Synthesis in DMF

The synthesis of MIL-53_nano phase was performed from a solution of $FeCl_3.6H_2O$ (1 mmol; Alfa Aesar, 98%) and terephthalic acid (1 mmol; 1,4-BDC; Aldrich, 98%) in 5 mL dimethylformamide (DMF; Fluka, 98%) using a Paar bomb at 150°C for 2h, recovering the yellow precipitate by centrifugation at 5000 rpm (1400 x g) for 10 min.

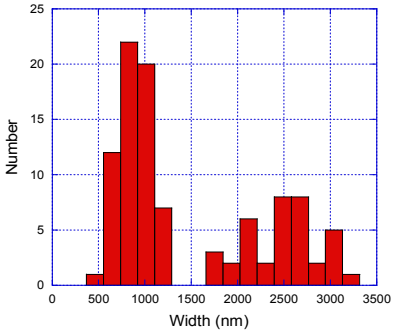
To remove the solvent, 200mg of solids were suspended into a 100 mL of deionised water overnight and after centrifuged.

SEM images show two populations of particles, one bigger rombohedrical crystals (1.2±2 in length and 1.5±0.8 μm in width) and other smaller pseudo spherical particles (~350 nm), which are quite aggregated (Figure S2).





Minimum	40,909092
Maximum	6727,2729
Sum	97509,091
Points	81
Mean	1203,8159
Median	218,18182
RMS	2287,5751
Std Deviation	1957,3259
Variance	3831124,8
Std Error	217,48066
Skewness	1,5142911
Kurtosis	0,54430874



Minimum	545,45453
Maximum	3272,7273
Sum	154000
Points	99
Mean	1555,5555
Median	1090,9091
RMS	1753,391
Std Deviation	813,20783
Variance	661306,98
Std Error	81,730462
Skewness	0.67797308
Kurtosis	-1,1301273

Figure S2. (top) SEM images of MIL-53nano obtained by microwave synthesis (0.18 HNO_3); (bottom) Statistics of particle size from SEM data

2) Synthesis in water

Iron terephthalate MIL-53(Fe) has been synthesized at 220°C for 30 min under microwave irradiation at 600 W. The molar composition of reactant mixture was 1.0 Fe : 1.0 H_2BDC (1,4-benzene dicarboxylate) : 0.18 HNO_3 or 0.36 HNO_3: 54 H_2O. The reactant mixture was loaded in a Teflon autoclave, sealed and placed in a microwave oven (Mars-5, CEM). The autoclave was heated up to 220°C within 2 min and kept at the same temperature for 30 min. The resulting solid was cooled down and the crystalline MIL-53(Fe) product in the solution was filtered off using a filter paper (ADVAMTEC 5C) and then sequentially stirred and

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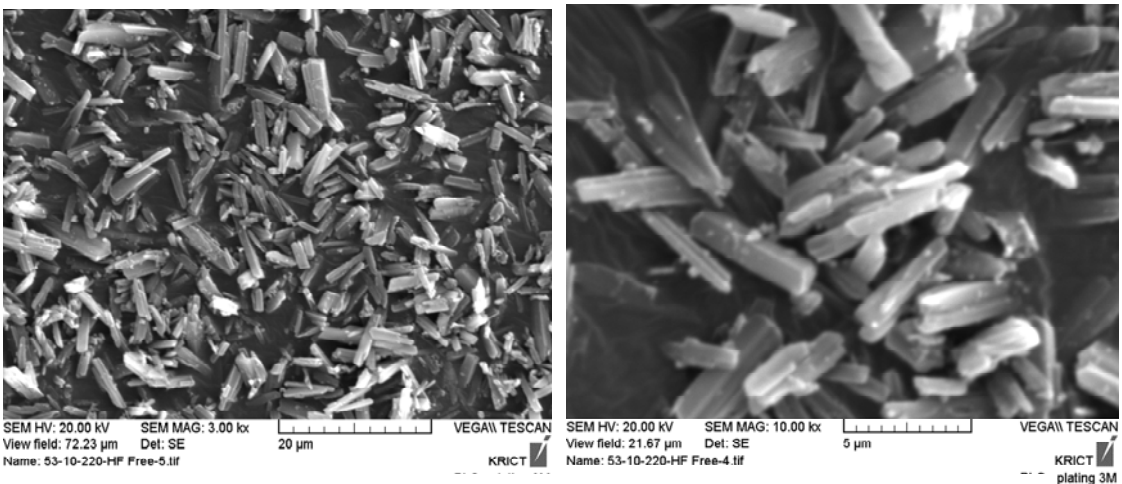
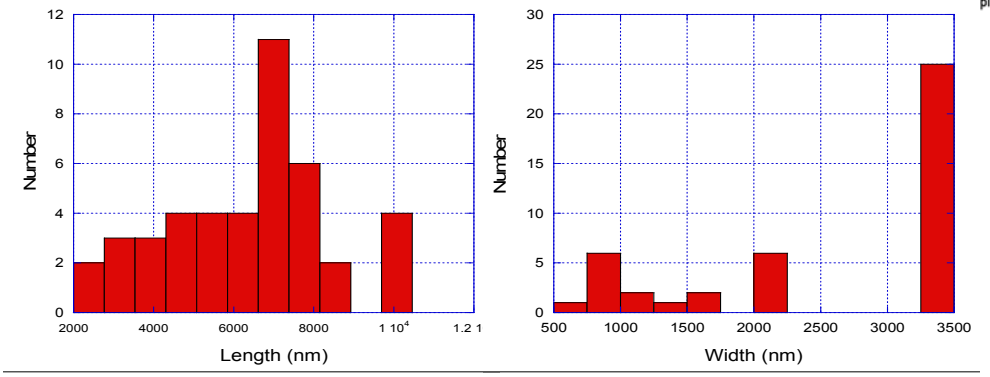
washed with ethanol at room temperature for 3 h to remove the free acid. The solid was finally dried overnight at 100°C (Figure S3).

Rod particles of 6,3±2 in length and 2.5±1 μm in width are observed by SEM (Figure S4).





Minimum	2068,9656
Maximum	10000
Sum	269068,97
Points	43
Mean	6257,4178
Median	6666,6665
RMS	6579,3644
Std Deviation	2056,9776
Variance	4231156,8
Std Error	313,68614
Skewness	0,03011283
Kurtosis	-0,62551202

Minimum	517,24139
Maximum	3333,3333
Sum	107919,54
Points	43
Mean	2509,7567
Median	3333,3333
RMS	2712,2205
Std Deviation	1040,4011
Variance	1082434,4
Std Error	158,65968
Skewness	-0,63771993
Kurtosis	-1,2863722

Figure S3. (top) SEM images of MIL-53(Fe) obtained by microwave synthesis (0.18 HNO_3); (bottom) Statistics of particle size from SEM data

MIL-100_nano

MIL-100_nano has been obtained from a solution of Fe powder (8 mmol; WaKo, 99%), 1,3,5-benzenetricarboxylic acid (1,3,5-BTC; 5.3 mmol; Aldrich, 95%), HF (4 mmol; Aldrich 48% in water) in 40ml of water at 200°C for 30 min under microwave irradiation at 600 W. The reactant mixture was loaded in a Teflon autoclave, sealed and placed in a microwave oven (Mars-5, CEM). The autoclave was heated up to reaction temperature within 2 min and kept at the same temperature for 30 min. The resulting solid was cooled down, filtered and washed with ethanol. The solid was finally dried overnight at 150°C under air atmosphere.

The estimated gyration diameter by light scattering methods is 315±9 nm (PDI=0.378). However, this is due to the very important particle aggregation since smaller spherical particles of 59±46 nm are observed by SEM (Figure S4).

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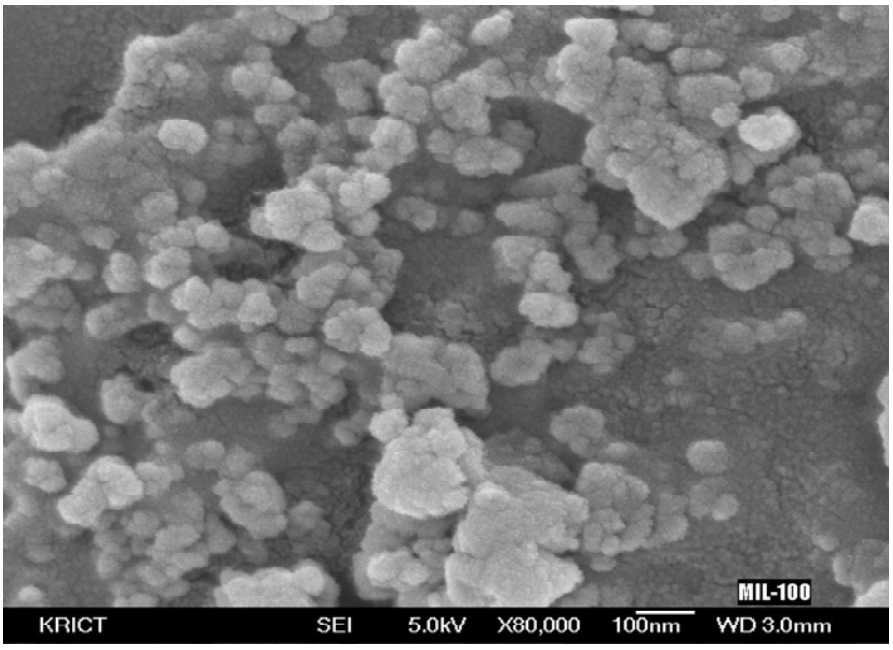

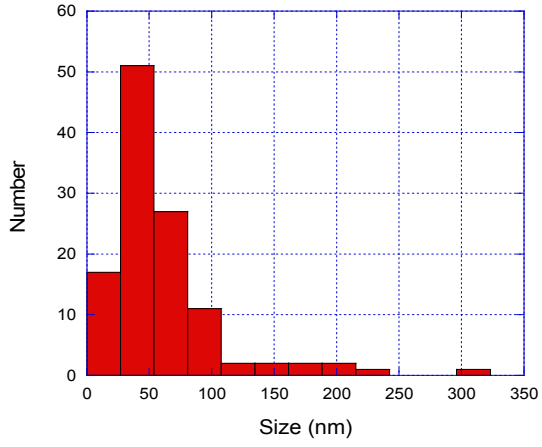

Minimum	8,5699997
Maximum	305
Sum	6868,32
Points	116
Mean	59,209655
Median	43,75
RMS	74,977373
Std Deviation	46,197638
Variance	2134,2217
Std Error	4,289343
Skewness	2,5281789
Kurtosis	8,1107799

Figure S4. (top) SEM of MIL-100nano microwave synthesis; (bottom) Statistics of particle size from SEM data

MIL-101_NH2_nano

MIL-101_NH2_nano was obtained from a solution of 90.5 mg of aminoterephthalic acid (0.5 mmol; Aldrich, 99%) and 135 mg of $FeCl_3.6H_2O$ (0.5 mmol; Alfa Aesar, 98%) in 25 mL of water placed into a Teflon-liner at 60°C for 5 min under microwave irradiation at 400W. The obtained precipitated was recovered by centrifugation at 10500 rpm for 10 min.

With the purpose of removing the free acid, the solid was washed with absolute ethanol for 5 min, centrifuged and kept wet.

The estimated gyration diameter by light scattering methods is 231±9 nm (PDI=0.136). Octahedral particles of 173±60 nm nm are observed by SEM (Figure S5).

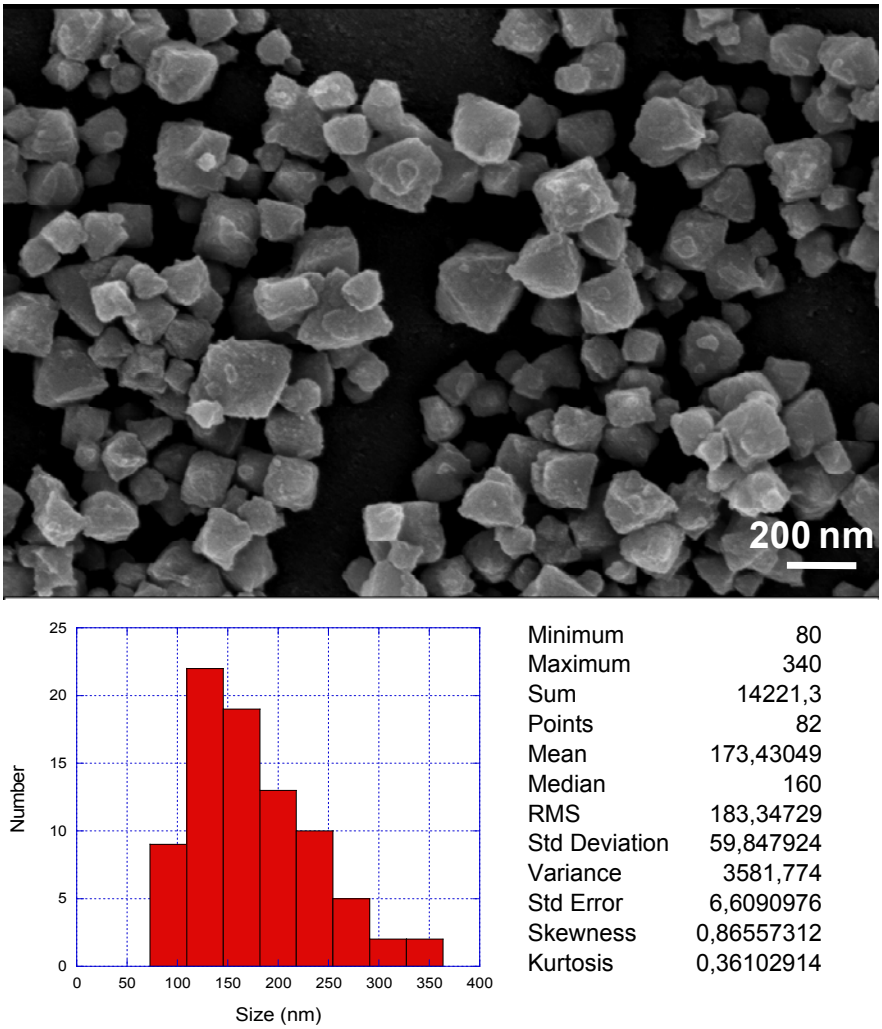

Figure S5. (top) SEM of MIL-101_NH2_nano microwave synthesis; (bottom) Statistics of particle size from SEM data

2. PEGylation during synthesis: MIL-88A-PEG_nano and MIL-89-PEG_nano

MIL-PEG-88A_nano and MIL-PEG-89_nano were synthesized using the same procedure as for MIL-88A_nano (see section 6) and MIL-89_nano (see section 1), by adding 100 mg of alpha monomethoxy -omega-amino poly(ethylenglycol) (CH3-O-PEG-NH2, 5000 MW, Iris Biotech) to the mentioned solution of $FeCl_3.6H_2O$ and dicarboxylic acid, using a Paar bomb at

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150°C/2h and 100°C/6h, respectively. The precipitates were recovered by centrifugation at 5000 rpm (1400 x g) for 10 min.

To remove the solvent, 200mg of solids were suspended into a 100 mL of deionised water overnight and after centrifuged.

The estimated particle size using the light scattering method was 250±58 nm for MIL-88A-PEG_nano. However, SEM micrographs show longer particles of 231±98 in length and 52±36 nm in width (Figure 2 in the text)

The estimated gyration diameter by light scattering methods is 92±4 nm (PDI=0.573) for MIL-89-PEG_nano. However, spherical particles slightly elongated with a homogeneous distribution of 72±22 nm are observed by SEM (Figure S6).

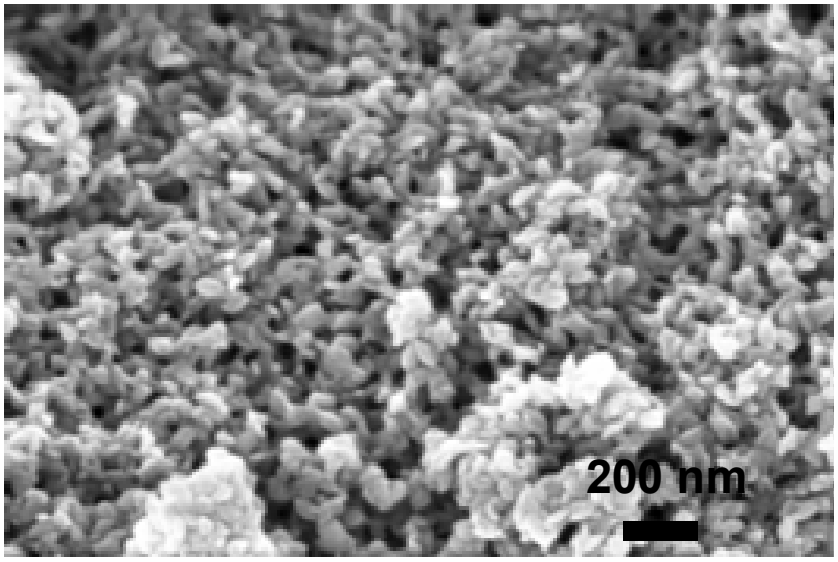

Figure S6. SEM of MIL-89-PEG_nano

3. PEGylation post-synthesis: MIL-88A-PEG_nano and MIL-100-PEG_nano

MIL 100 and MIL 88A nanoparticles were prepared as previously described (section 1).

30 mg of these dehydrated nanoparticles (100°C/12h) were suspended in 2 mL of CH3-O-PEG-NH2 solution (5 mg/mL of water). The reaction was carried on at 30°C for 3h. The ratio of solid: PEG was 10:100.

The coated nanoparticles were recovered by centrifugation 10000 rpm (5600 x g) for 10 min and washed two times by water to remove non grafted CH3-O-PEG-NH2. PEG amount in the supernatants was determined by a colorimetric method.(*2*)

Nanoparticles were dried for 16 h in an oven at 50 °C, under vacuum

10mg of dried nanoparticles were degraded in 2 mL HCl 5M during 16 h at 80 °C. After neutralisation with KOH 5M, the amount of PEG was determined by the same colorimetric method.

4. Dextran-fluorescein-biotin surface modification

In this case, micrometric particles of around 1-2 µm were chosen in order to distinguish the location of the Dextran-fluorescein-biotin detection in the material by confocal laser scanning microscopy (CLSM),

Thus, 5 mg of MIL-100, synthesized as previously reported (~1-2 µm) (*3*), were suspended in 0.5 mL of distilled water. Then, 0.5 mL of an aquose solution of dextran-fluorescein-biotin (5 mg/mL; 10000 MW, lysine fixable, Molecular Probes) were added, incubating at room temperature under stirring for 24 h. The particles were collected by centrifugation at 5000 rpm (1400 x g) for 15 min, and washed with distilled water.

Fluorescein was detected by a confocal laser scanning microscopy (CLSM), using a *microscope* CLSM *Zeiss Axiovert 200M* (excitation 488 nm, emission 515 nm) (Figure S7).

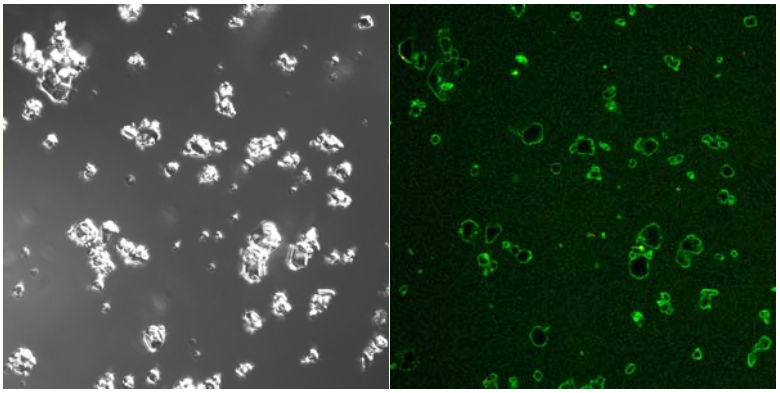

Figure S7. CLSM images of dextrane-fluoresceine-biotine-modified MIL-100(Fe) (~1-2 µm).

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5. Chitosan surface modification

For this study we have used chitosan grafted with alkyl (C12, lauryl) side chains. The degrees of modification were 2%wt (sample named Q25) and 7%wt (sample named Q100).

7 mg of chitosan were added to a solution of FeCl3.6H2O (1 mmol, 270 mg; Alfa Aesar, 98%) and fumaric acid (1 mmol, 116 mg; Acros, 99%) in 5 mL of distilled water, placed into a Teflon-liner steel autoclave and stirred for 45 min. The solution was heated at a temperature of 80°C for 12 h. After that, the obtained precipitated was recovered by centrifugation at 14000 x g) for 10 min and washed with acetone.

The formation of the MIL-88A phase was observed by XRD patterns (figure S8). These data also show the

maintenance of the typical flexibility of the porosity of Mil-88A in the presence of water.

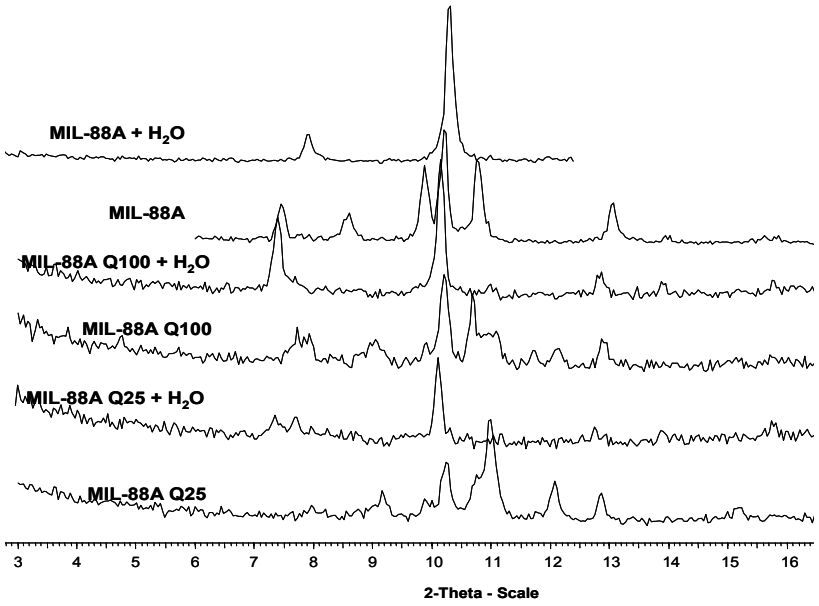

Figure S8. XRD patterns ofnanoMOFs modified with chitosan (MIL-88A_Q25 and MIL-88A_Q100) and non-modified nanoMIL-88A, in the presence or not of water.

MIL88A-Q25 and MIL-88A-Q100 showed a chitosan surface modification, estimated by TGA, of 16% and 22% (wt) in relation to the dehydrated solid, respectively (figure S9).

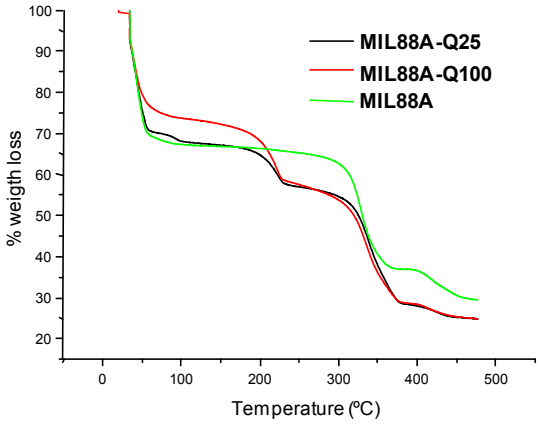

Figure S9. TGA of nanoMIL-88A modified or not with chitosan

6. *In vitro* degradation of MIL-100 and MIL-88A

The degradation of MIL-100 and MIL-88A s_nano was studied by suspending 10 mg of the nanoMOF in 5mLof a phosphate buffer solution (PBS, pH 7.4) at 37°C. These suspensions were kept under bidimensional stirring for different incubation times. At each time point, an aliquot of 2.5 mL of supernatant was recovered by centrifugation (10000 rpm (5600 x g)/30 min) and replaced with the same volume of fresh PBS (at 37°C). Released organic linker, fumaric or trimesic acid, was quantified in the supernatant by HPLC. Experiment was done in triplicate.

The concentration of the delivered trimesic acid and fumaric acid was determined using a RP-HPLC system (Reversed phase liquid chromatography) Alliance Waters equipped with a PDA spectrophotometer detector at λ= 215 nm and 210nm, respectively. Sunfire® C18 reverse-phase column (5µm, 4.6 X 150 mm), supplied for Waters, were employed. The mobile phase consisted on methanol (90 vol%) (Aldrich, HPLC grade) and buffer phosphate 0.02M pH 2.5 (10 vol%) (Aldrich, HPLC grade). The flow rate was 0.8 $mL \cdot min^{-1}$ and the column

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temperature was 25ºC. The effluent was monitored at 215 nm and the injection volume was 50 µL.

Several fumaric and trimesic acid solutions with concentrations of 0-0.1-0.25-0.5-1-5-10-50 and 0-0.-0.25-0.5-1-5-10 $\mu g \cdot mL^{-1}$ in PBS, repectively, were used as standards.

Retention time of fumaric and trimesic acid was about 5.5 and 5.6 minutes, respectively.

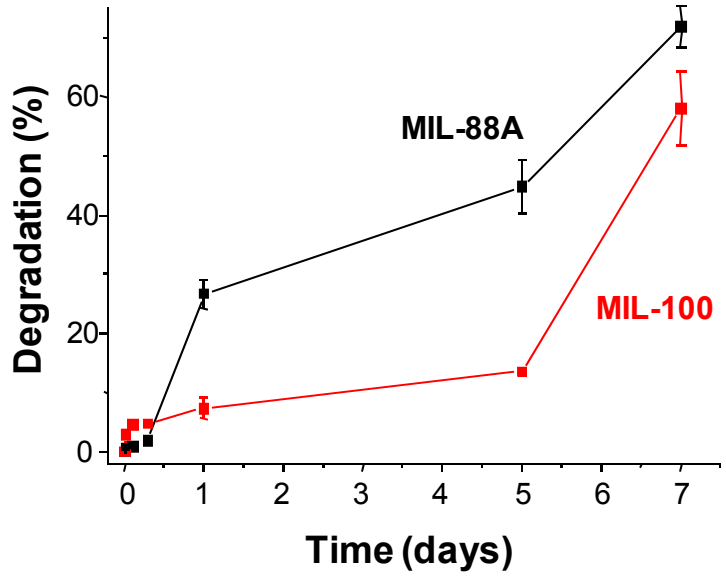

Figure S10. Release of organic acid from MIL 88A_nano and MIL-100_nano under simulated physiological conditions.

44% and 14% of the total amount of the organic linker in the hybrid nanoparticles was released from MIL-88A and MIL-100, respectively, after 5 days of incubation. MIL-100 shows a higher *in vitro* stability. More than 50% of degradation (72 and 58% for MIL-88A and MIL-100, respectively) was achieved after 7 days (Fig. S10).

7. *In vivo* toxicity tests

Materials

The synthesis of MIL-88A_nano was performed from a solution of $FeCl_3.6H_2O$ (1 mmol; Alfa Aesar, 98%) and fumaric acid (1 mmol; Acros, 99%) in 5 mL dimethylformamide (DMF; Fluka, 98%) with 0.4 mL of sodium hydroxide 2M (Alfa Aesar, 98%) using a Paar bomb at 150°C for 2h, recovering the orange precipitate by centrifugation at 5000 rpm (1400 x g) for 10 min.

To remove the solvent, 200mg of solids were suspended into a 100 mL of deionised water overnight and after centrifuged.

The estimated particle size using the light scattering method was 834 ±11 nm (PDI=328). However, SEM micrographs show longer particles with a polydisperse diameter of 327±118 x 135±56 nm and (Figure S11)

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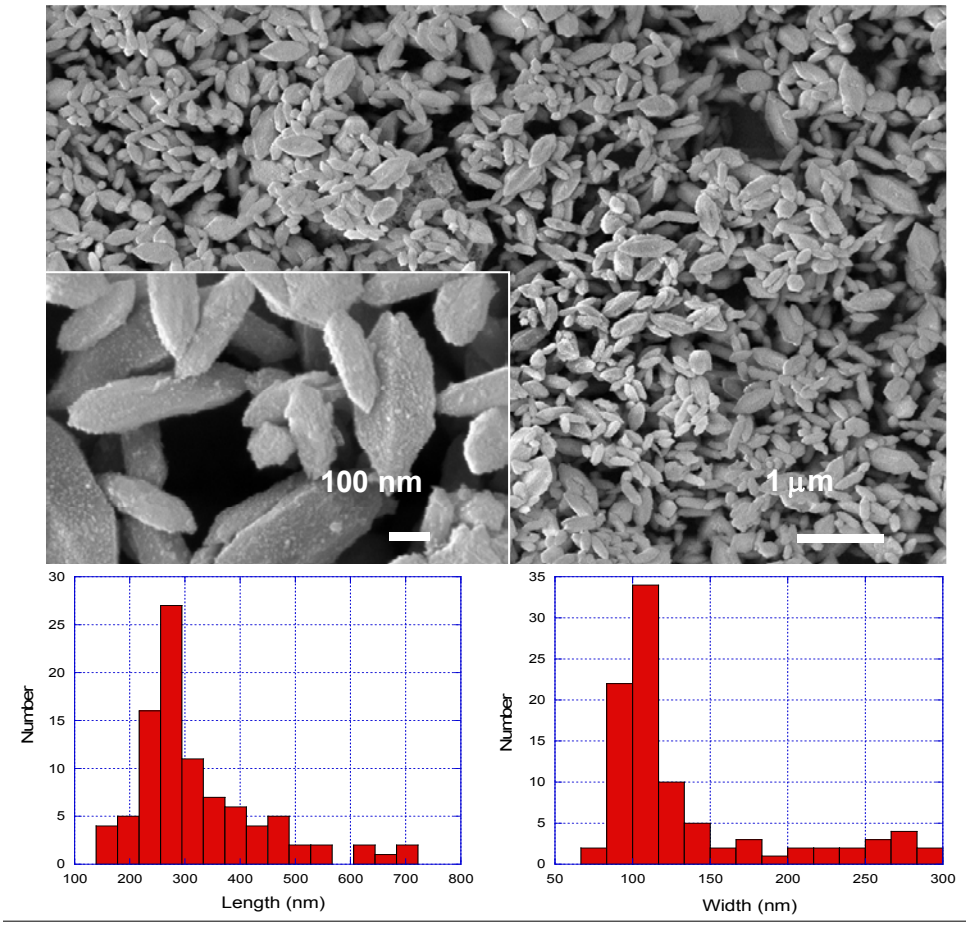

Minimum	145,45454
Maximum	709,09088
Sum	30772,727
Points	94
Mean	327,36944
Median	290,90909
RMS	347,66358
Std Deviation	117,67124
Variance	13846,52
Std Error	12,136861
Skewness	1,3625312
Kurtosis	1,9083736

Minimum	72,727272
Maximum	290,90909
Sum	12654,545
Points	94
Mean	134,62282
Median	109,09091
RMS	145,86951
Std Deviation	56,467072
Variance	3188,5302
Std Error	5,8241338
Skewness	1,4949045
Kurtosis	1,0304666

Figure S11. (top) SEM images of MIL-88A_nano solid; (bottom) Statistics of particle size from SEM data

Moreover, both fumaric acid and iron are endogenous. Thus, the normal levels of iron in human are: 80-180 μg/dL in adult males, 60-160 μg/dL in adult females, 50 a 120 μg/dl in children and 100 a 250 μg/dL in babies less than 1 year old. In normal conditions, the iron content should be around 50 mg of iron per kg of corporal weight (3.8 g for men and 2.3 g for women).*(4)*

The normal levels for the fumaric acid, an endogenous acid implicated in the Krebs cycle, are: in blood 3 mg/L, in brain tissue 150 mg/kg, in kidney tissue 95 mg/kg, in liver 78 mg/kg and in muscle 23 mg/kg (in rats).

MIL-88Bt_nano has been synthesis from a solution of $FeCl_3.6H_2O$ (1 mmol; Alfa Aesar, 98%) and 1,4-benzenetetramethyldicarboxylic acid (1 mmol; Chem Service) in 10 mL of dimethylformamide (Fluka, 98%) with 0.4 mL of NaOH 2M. This solution is places in a Paar bomb type and heated at 100°C for 2 hours. After cooling the Paar bomb under water, the solid was recovered by centrifugation at 5000 rpm (1400 x g) for 10 minutes.

To remove the solvent inside the pores, 200 mg of this product are suspended in 100mL of distilled water under stirring for 15h. Then, the solid is recovered by centrifugation at 5000 rpm (1400 x g) for 10 minutes.

The estimated gyration diameter by light scattering methods shows two distributions of spherical nanoparticles, one of them majority of 52±13 and the other around 139±14 nm. As observed by the SEM (37±13 nm; Figure S12), some aggregates are formed.

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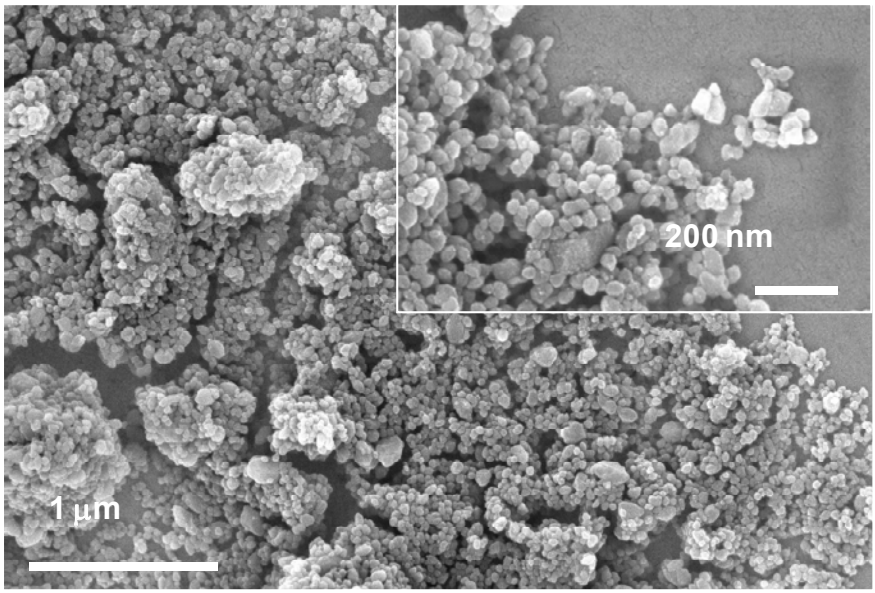

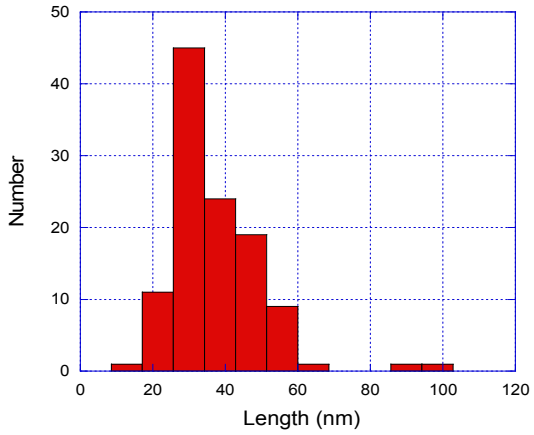

Minimum	13,636364
Maximum	100
Sum	4182,7273
Points	112
Mean	37,345779
Median	31,818182
RMS	39,393929
Std Deviation	12,593268
Variance	158,5904
Std Error	1,189952
Skewness	1,7967831
Kurtosis	5,8282088

Figure S12. (top) SEM images of MIL-88Bt_nano solid; (bottom) Statistics of particle size from SEM data.

MIL-100_nano was obtained by microwave synthesis as described above (section 1 of supporting information)

Animals

Wistar females rats (4-weeks-old, 161.36 ± 16.1 g) were obtained from the central animal care facilities, Janvier R Centre d'Elevage, France.

Acute in vivo toxicity tests

Toxicity, assessed by animal behavior, evolution of the animal and organs weights, enzymatic activity (cyp-450) as well as serum tests evaluating liver function and the inflammatory reaction were studied in female rats.

220 mg/kg of MIL-88A (150 et 500 nm; called MIL-88A group), 110 mg/kg of MIL-88Bt (50 et 140 nm, named MIL-88Bt group) suspended in a 0.5 mL aqueous solution of glucose 5% and 0.5 mL of glucose solution 5% (control group, called Glu group) were intravenously injected in the jugular vein under isofluorane anesthesia to 4 groups (1 day, 1 week, 1 and 3 months) of 8 rats.

Iron carboxylates suspensions were dispersed by ultrasounds and then, slightly settled in order to remove the bigger particles. The concentration of the injected suspension was determined by weight variation and UV-Vis spectroscopy (quantification of the colored complex Fe^{2+} - bipyridine at 520 nm). The administered concentration is limited by the suspension stability. Thus, the stability of the suspension 220 mg/kg of MIL-88Bt did not allowed the correct i.v. administration, choosing 110 mg/kg as higher dose.

Organs (spleen, kidneys, liver and heart) were extracted under isofluorane anesthesia at 1day, 1 week, 1 and 3 months, washed with a NaCl 0.9% solution at 4°C and conserved at -80°C after immersion in liquid nitrogen. 4/8 livers were kept at 4°C for the determination of the cytochrome P-450 (Cyp-450) activity.

Animal behavior and weight evolution of the animals was totally normal in comparison to the control group (Figure S13). Heart and kidneys did not show a significant variation, except a slightly increase in the kidneys weight after one day of injection. Spleen and liver weight increased after 1 week of the administration, coming back to normal values after 1 and 3 months, respectively. The weight increase of liver is a consequence of the iron accumulation,

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as we have verified by tissue observation (Figure S13). MIL-88Bt showed a slower kinetics for returning to normal values of liver weight.

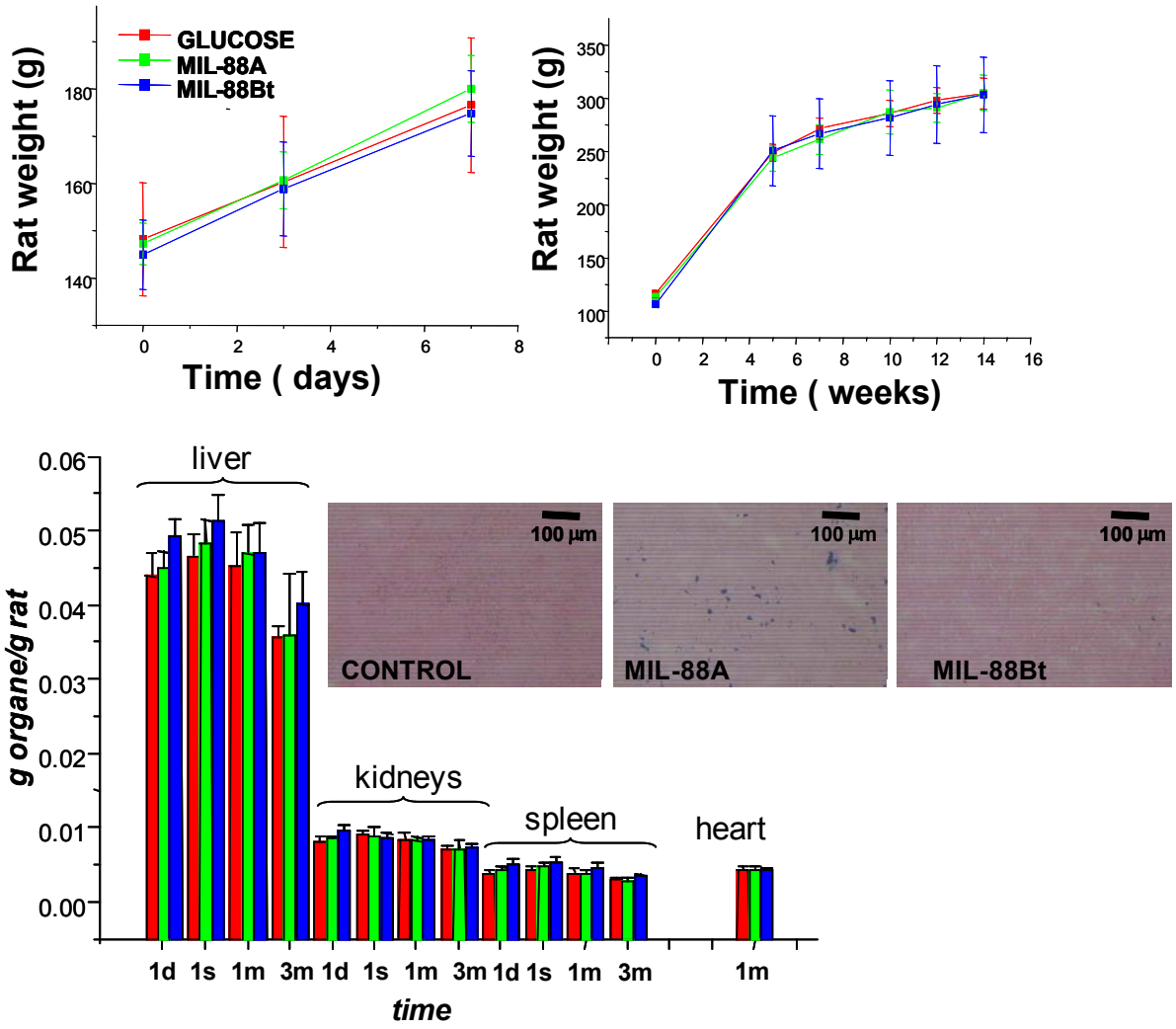

Figure S13. (Up) Rat weight evolution; (Down) Organs weight evolution and hepatic histology (5 µm) after 1 week of MIL88A and MIL-88Bt administration in comparison with the control group (Hematoxylin-eoxin coloration + Prusse coloration).

Iron accumulation (in blue) is more important when MIL-88A is administered. This can be due to the administration of a higher concentration of MIL-88A, but also to a faster degradation of this material since its higher hydrophilic character.

Activity of Cyp-450 was quantified by the reported method (*5*), by spectroscopic determination (microsomes + sodium dithionite + CO at 450 nm) of the Cyp-450/total protein ratio (in the microsome extract (total protein quantification using BCA test PIERCE (lot #HI106096)). The Glucose group (control) and Phenobarbital injected rats were used as negative and positive controls, respectively. Thus, we can conclude that these porous iron carboxylates are not metabolized by Cyp-450. Organic linkers could be directly removed since the high polarity of the organic linkers (diacids). Moreover, fumaric acid is an endogenous acid, which can reuse in the Krebs cycle.

One milliliter of blood was collected from each animal by jugular puncture on days 1, 3, 7 and 14 days, and 1, 2 and 3, and was centrifuged at 5600 g for 4 min. Serum samples were stored at -18°C until analysis. Serum levels of interleukin-6 (IL-6) were measured using a Quantikine ELISA kit (R&D Systems, France). Variables evaluating liver function, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), total bilirubin, cholesterol and albumin as well as serum iron were measured on an Integra 800 analyser (Roche Diagnostics, Meylan , France). The inter-assay coefficients of variation were below 5% for all parameters. Results were expressed as median (10^{th}, 90^{th} centile). Non parametric tests (Kruskal-Wallis test, Mann-Whitney test) were used for comparison among the different groups and between days. A p value equal or less than 0.05 was considered to represent a significant difference.

The quantification of interkeukine-6 (IL-6; ELISA kit Quantikine, Rat IL-6 R&D Systems, France), involved in the immunitary and inflammatory reaction, allows to determinate an inflammatory reaction after 1 day of the nanoparticles i.v. administration with normal values after 3 days. This could be explained for the local inflammation due to the i.v. injection. Thus, we can conclude that no inflammatory process is due to the nanoparticles presence in the body.

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No change in any liver function tests was observed over the study period, except a slight increase in AST (216[178, 265] vs 174[147, 227] U/l, + 19%) and ALP (249[196, 304] vs 186[134, 236] U/l, + 25%) on the day following MIL-88A injection as compared to the control group (p<0.01 for both parameters), with return to similar values as those in the latter group by day 3. On the other hand, albumin level was slightly lower (33.5[29.0, 35.4] vs 37.5[34.6, 39.7] g/l, -11 %, p<0.01) in MIL-88Bt-treated group on day 3 and was normalized by day 7. Those results indicate transitory abnormalities (cytolysis, inflammatory reaction) related to nanoparticles injection but at last, liver function is preserved.

An intriguing finding was the dramatic fall in iron level (18.3[8.9, 25.9] vs 41.2[22.9, 63.7], -56 %, p<0.001) on the day following MIL-88A injection as compared to the control group. Iron concentrations started to increase on day 3 (37.7[19.8, 51.1] vs 47.7[27.0, 64.9], p<0.03) and became comparable to those in control group one week later.

220 mg/kg of MIL-100_nano (100-200 nm; called MIL-100 group) and 78 mg/kg trimesic acid (called 1,3,5-BTC group) suspended in a 0.5 mL aqueous solution of glucose 10% and 0.5 mL of glucose solution 10% (control group, called Glu group) were intravenously injected in the jugular vein under isofluorane anesthesia to 2 groups (1 day and 1 week) of 6 rats.

Iron carboxylates suspensions were dispersed by ultrasounds and then, slightly settled in order to remove the bigger particles. The concentration of the injected suspension was determined by weight variation and UV-Vis spectroscopy (quantification of the colored complex Fe^{2+} - bipyridine at 520 nm). The administered concentration is limited by the suspension stability. Thus, the stability of the suspension of 220 mg/kg of MIL-100_nano was higher in 10% glucose than in 5% glucose.

Organs (spleen, kidneys, liver, lungs, brain, ovaries, thymus and heart) were extracted under isofluorane anesthesia at 1and 7 days, washed with a NaCl 0.9% solution at 4°C and conserved at -80°C after immersion in liquid nitrogen..

Animal behavior and weight evolution of the animals was totally normal in comparison to the control group (Figure S14). Brain, heart, thymus and kidneys did not show a significant variation. Lungs, spleen and liver weight increased after 1 day and 1 week of the administration of MIL-100 material (Figure S14).

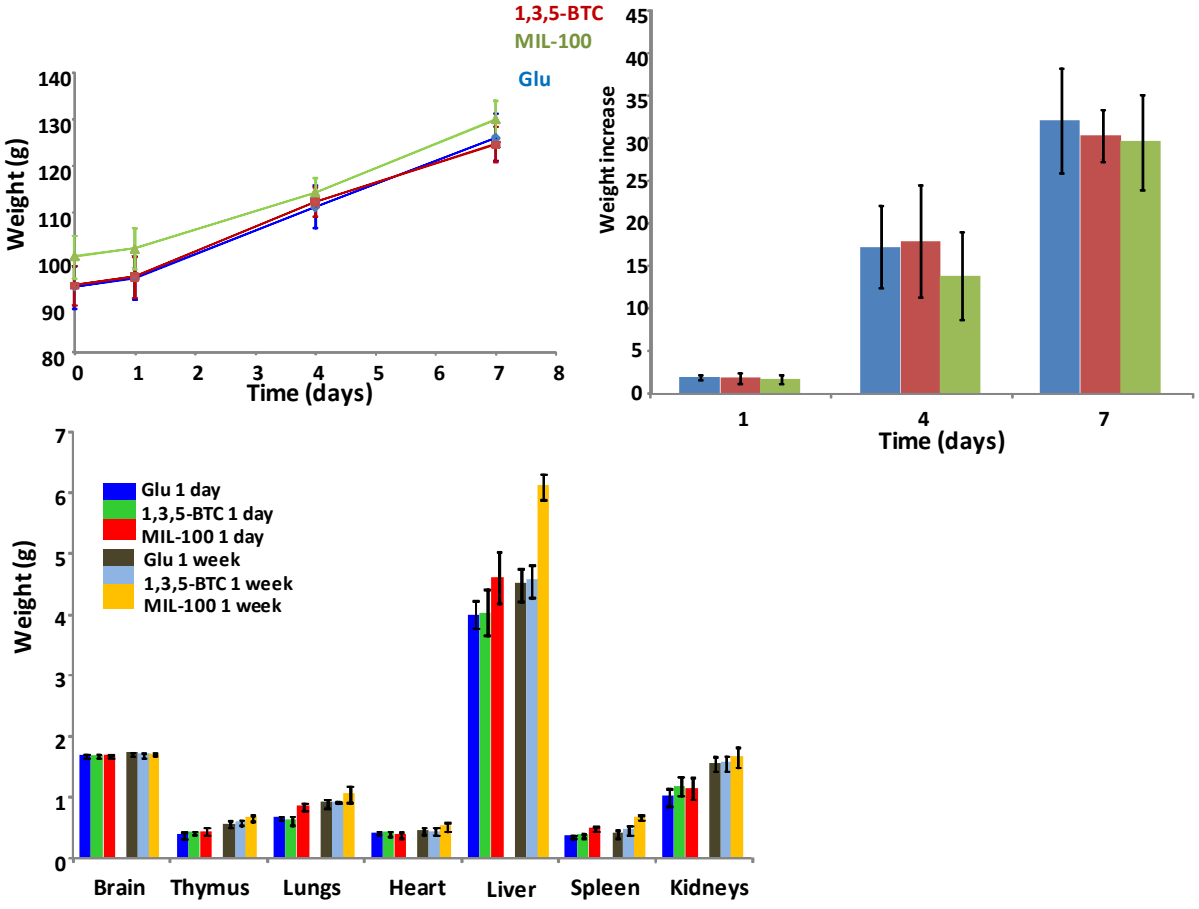

Figure S14. (Top) Rat weight evolution; (bottom left) Organs weight evolution

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Subacute in vivo toxicity tests

25 or 150 mg/kg of MIL-88A (150 et 500 nm; called 25 and 150 mg/kg, respectively) suspended in a 0.5 mL aqueous solution of glucose 5%, 0.5 mL of a solution 25 mg/kg of fumaric acid (called Fumaric group) and 0.5 mL of glucose solution 5% (control group, called Glu group) were daily intravenously injected during 4 days in the jugular vein under isofluorane anesthesia to 2 groups (5 and 10 days) of 4 rats.

Figure S15 shows that the evolution of the rats' weight is similar for all the groups. However, when the weight increase is calculated, some differences are observed. Thus, the weight increase was 20.7, 17.5, 12.3 and 26.1% for the Glu, 25 mg/kg, 150 mg/kg and fumaric group, respectively. Considering the increase weight of Glu group as normal value, the growth for 25 and 150 mg/kg was 0.84 and 0.59, respectively. Thus, the administration of 4 consecutive doses of MIL-88A leads to a decrease of the weight, being directly proportional to the administered dose.

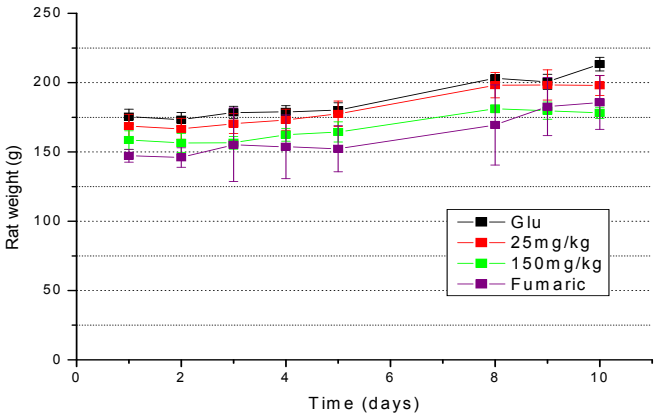

Figure S15. Evolution of the rat weight.

There is no significant difference of the weight of the spleen, kidneys and heart between the control group and the other groups (Figure S16). The lungs weight seems to increase after the

administration of the MIL-88A material. It could be due to the retention of the bigger particles in the smaller capillaries of this organ. Although the weight of the liver seems to decrease when the nanoMIL88A is administered, the difference is not enough significant.

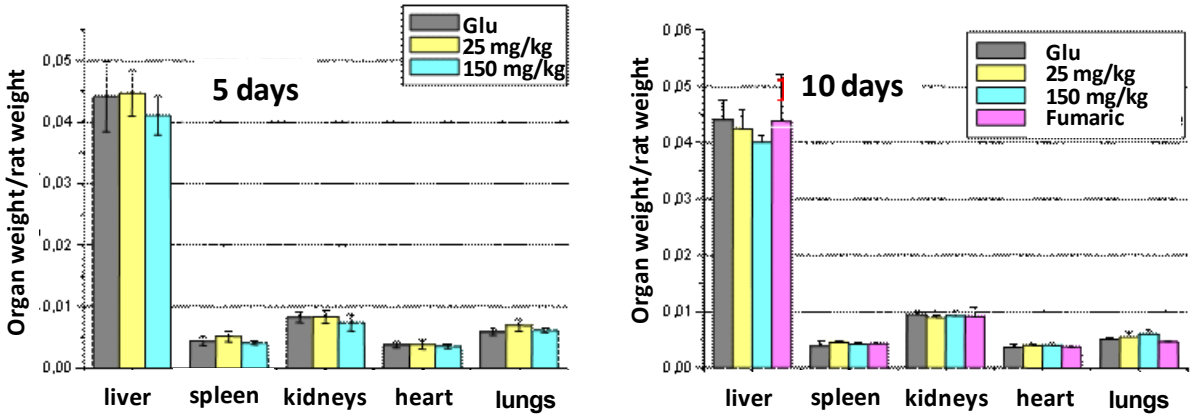

Figure S16. Organ weight

Water and food consummation is lower when 25 and 150 mg/kg of MIL-88A were administered, in total agreement with the decreasing of the gain weight (Figure S17).

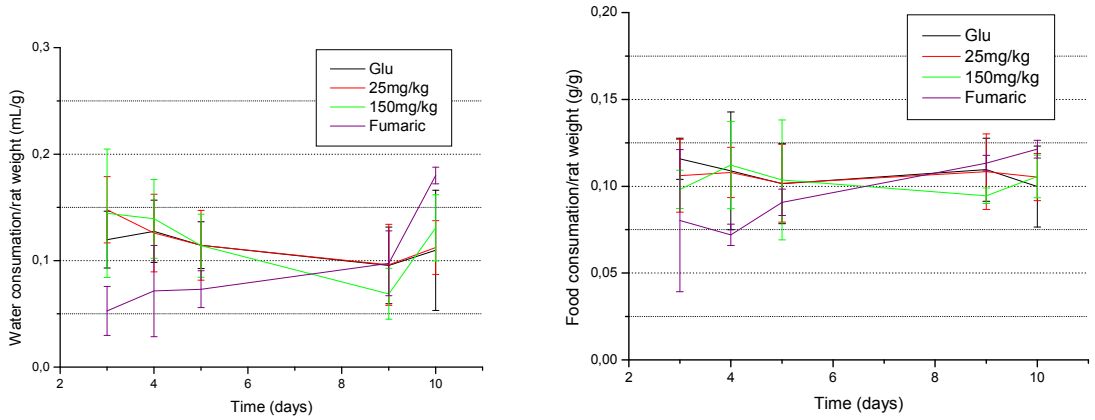

Figure S17. Food and water consummation

There is no significant evidence of the IL-6 variation after the administration of the 25 and 150 mg/kg of MIL-88A in comparison with the control group (Glu), so no immune and inflammatory reaction apart from the inflammatory reaction caused by the injection.

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Analysis of biological variables did not show any significant change in the course of the study, therefore indicating a good hepatic tolerance.

8. MTT tests

J774.A1 cells (mouse macrophages, ECACC No. 91051511) were grown in RMPI 1640 GlutaMAX™ I supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin (100 UI/mL) and streptomycin (100 µg/mL). Cells were maintained in a humidified incubator with 95% air/5% CO2 at 37 °C. The cytotoxicity of nanoparticles towards J774.A1 cells was determined using MTT assays (*6*) in 96-well plates (3 · 104 cells per well) after incubation (24 h) with nanoparticles. The protein content was assessed using a Bio-Rad DC Protein Assay after cell lysis with 0.1% Triton X-100.

The IC50 of the MIL 88A nanoparticles was 57 ± 11 µg/mL

9. Busulfan encapsulation

Busulfan (Bu; 1,4-bis(methylsulfonyloxy)butane; Fluka), was adsorbed into the material pores (MIL-89, MIL-88A, MIL-53 and MIL-100) by the suspension of 25 mg of dehydrated solid into 2.5 mL of a Bu solution at 80% of saturation, using different solvents, at room temperature and under stirring for 16 h. The Bu-loading particles were collected by centrifugation at 5000 rpm (1400 x g) for 15 min and dried under vacuum for 3 days. The solvents were selected according to a high busulfan solubility (Table S2) and a lower boiling point for an easier removal, using acetone, acetonitrile, chloroforme and dichloromethane. Experiments were done in quadruplicate

Bu-loading materials are characterized by XRD and the drug content is estimated by elemental analysis.

The maximum loading was achieved using dichloromethane as solvent for MIL-100 and MIL-53 materials, and acetonitrile for MIL-89 and MIL-88A.

Table S2. Busulfan solubility in different organic solvents.

	Busulfan solubility (mg/mL)
Acetonitrile	30
Acetone	20
Dichloromethane	10
Chloroforme	8

The structure maintaining of the rigid solid MIL-100 after the drug insertion was confirmed by XRD (Figure S18). Moreover, XRD of flexible phases show a variation on the XRD pattern and the cell parameters. Thus, we can estimate that the drug content, according to the pore opening of the MIL-53 solid, is approximately 15%, which is in total agreement with the elemental analysis data.

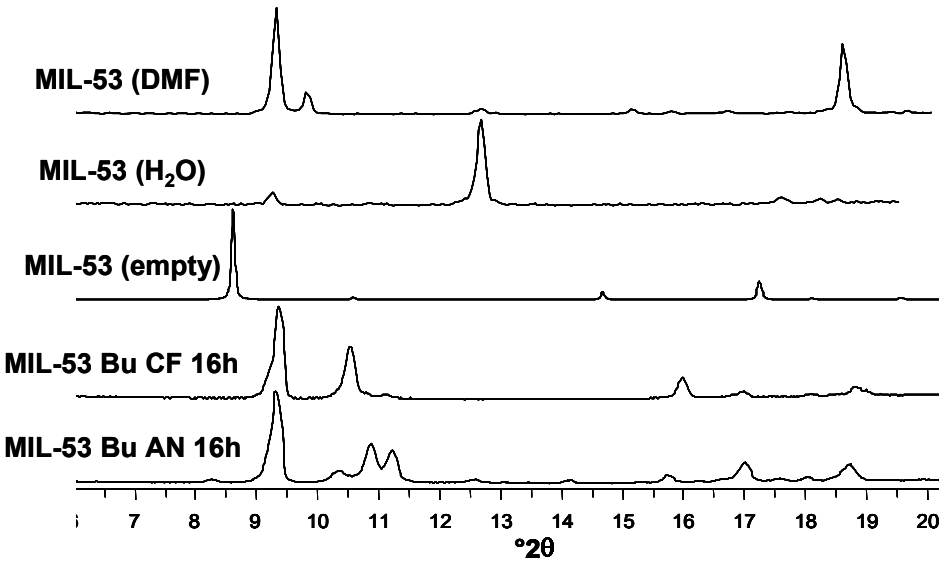

Figure S18. XRD patterns of MIL-53 solids containing : Dimethylformamide (DMF), H_2O, and Busulfan (adsorbed from chloroforme or acetonitrile solutions). XRD pattern of the dehydrated form is also represented for a better understanding.

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Busulfan activity test

The cytotoxicity of Busulfan, NP of MIL100 loaded with Busulfan and empty NP was studied in human leukemia (CCRF-CEM) and human multiple myeloma (RPMI-8226) cell lines. All cell lines were maintained in RPMI-1640 medium (Lonza) supplemented with 10% heat –inactivated fetal calf serum and, 10^5 U/L Penicillin G, 100 mg/L streptomycine in a humidified atmosphere of 5% carbon dioxide at 37°C.

Cells were plated on 96-well flat-bottom microtiter plates (TPP). Seven Busulfan concentrations (range 0.001mM to 10 mM) were made by serial dilutions of buslufan dissolved in dimethylsulfoxide DMSO (BIO BASIC INC). 2µL of each Busulfan solution were added to 200µL of the complete cell culture medium.

In the same way, seven concentrations of empty NP suspensions in RPMI-1640 were prepared (range 0.001mM to 10mM).

Seven suspensions of NP loaded with busulfan 25% w/w% (Busulfan/NP) in RPMI-1640 were made in order to have a range of concentration of Busulfan (0,001mM to 10mM). 20µL of these suspensions (empty NP and Busulfan-loaded NP) were added to 200µL of the complete cell culture medium and the cells were exposed to 2µL of DMSO, corresponding to the quantity used in Busulfan solutions.

After 48 hours of incubation the chemosensitivity was evaluated by Thiazolyl blue tetrazolium bromide (98%, Sigma) in PBS (Lonza) at a concentration of 5mg/mL. 25µL of this MTT reagent was added to each well. Within the next 3 hours, mitochondrial aldehyde dehydrogenase of viable cells reduced the yellow soluble MTT reagent to water-insoluble blue formazane crystals.

The plates of CCRF-CEM and RPMI-8226 cells were centrifuged (500 g) for 5 minutes and then the cell culture medium was evacuated. The formazane crystals were dissolved by adding 200µL of DMSO to each well in the case of RPMI-8226 cells and 75µL to each well in the

case of CCRF-CEM cells. The absorbance of the dissolved formazane blue dye was measured at 570 nm using an automated Mutiscan Ascent 7000 miroplate reader (Thermolab Electron Co.)

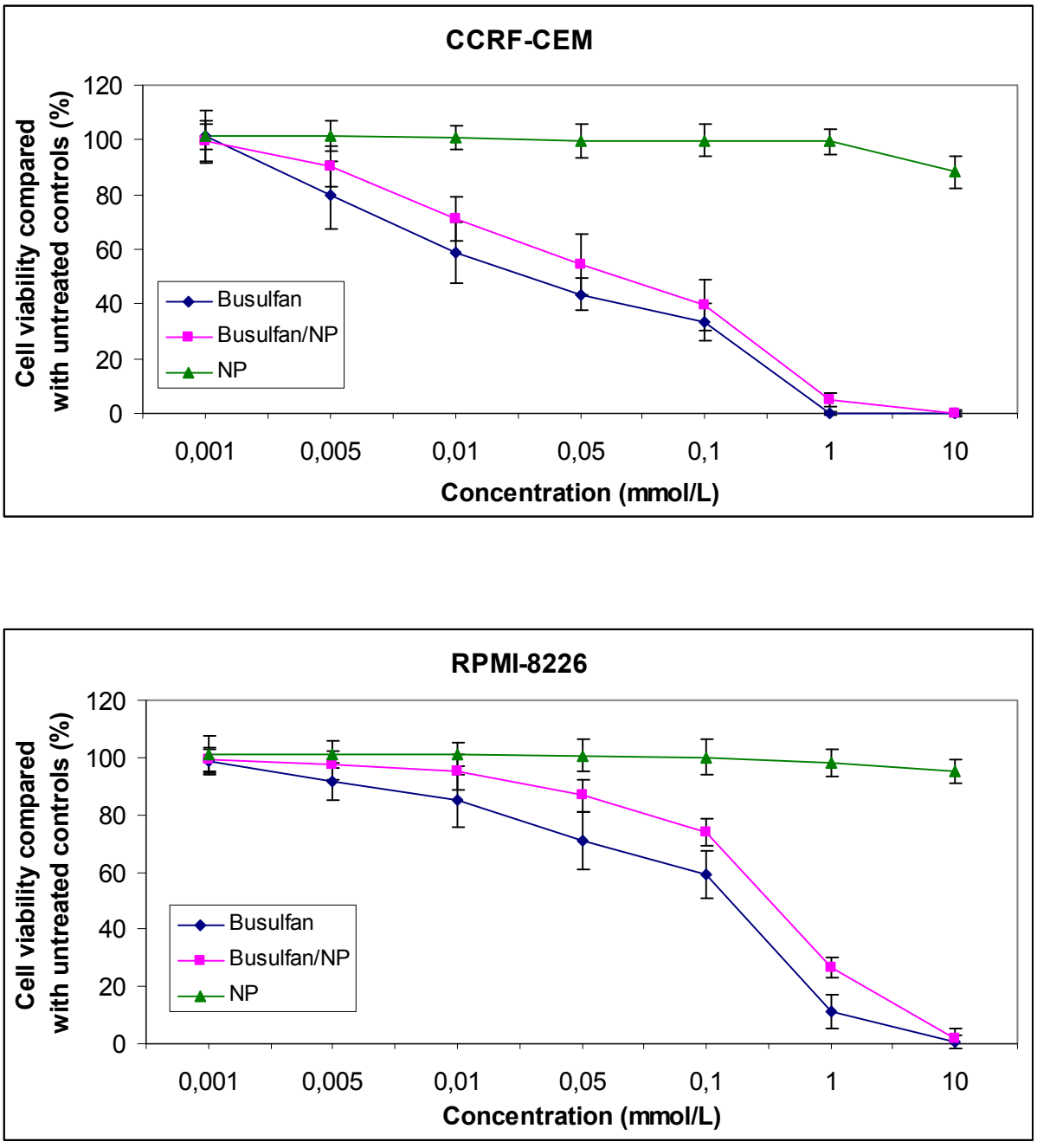

Figure S19. Cytotoxicity of Busulfan, MIL100_nano loaded with Busulfan and empty MIL 100_nano on human leukemia (CCRF-CEM) and human multiple myeloma (RPMI-8226) cell lines.

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10. AZT-TP Entrapment

We have studied the capacity of MIL-53, MIL-88A, MIL-101_NH2 and MIL 100 nanoparticles, PEGylated or not, to load AZT-TP (Azidothymidine triphosphate (AZT-TP; 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl) tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1H,3H)-dione, Moravek) using tritium-labelled AZT-TP (^{3}H-AZTP ; Moravek; 3,8Ci/mmol, 1mCi/ml, 133,4µg/ml, 250µl)

Experiments were done in quadruplicate, suspending about 2,5 mg of dehydrated MIL-100 (150°C/night) in 500 µL of a 1mg/mL AZT-TP aqueous solution(50µl of 3H AZT-TP+ 3ml of AZT-TP). The impregnation was performed under stirring for 16 hours.

The AZT-TP-containing nanoparticles were recovered by centrifugation (10000 rpm (5600 x g) /10 min, room temperature) and dried under vacuum during 3 days. The radioactivity in the supernatant was determined by radioactivity counting.

The nanoparticle pellet was degraded under acidic conditions (HCl 5M at 50°C for overnight) and the radioactivity was counted using a Beckman Coulter apparatus(LS 6500 multi purpose scintillation counter) and the AZT-TP adsorbed in the materials calculated by the difference with the mother solution radioactivity.

The loadings of AZT-TP are shown in Table S3.

Table S3. Encapsulation of AZT-TP in MIL-101_NH2 and MIL-100 solid PEGylated or not.

Sample	Loading (wt%)	Efficiency (%)
MIL100	10.3	97.7
MIL100 2 incubations	21.2	85.5
MIL100-PEG	5	75
MIL100-PEG 2 incubations	8	60
MIL101_NH2	25.4	97
MIL101_NH2 2 incubations	42.0	90.4

AZT- TP delivery:

The release of AZT-TP was studied by suspending 2.5 mg of MIL-100 nanoparticles containing 21 wt% AZT-TP, respectively, in 1mL PBS (pH 7.4, Aldrich). These suspensions were kept under bidimensional agitation for different incubation times (from 30 min to 10 days). At each time point, an aliquot of 0.5 mL of supernatant was recovered by centrifugation (1000 rpm (5600 x g)/10 min) and replaced with the same volume of fresh PBS. Released AZT-TP was quantified by radioactivity measurement. At the end of the release, the nanoparticle pellet was degraded under acidic conditions (1 mL HCl 5M at 50°C overnight) and the radioactivity was counted, confirming the total release of AZT-TP. Experiments were done in quadruplicate.

It is important to point out that a significant "burst effect" has been observed in the case of MIL 53 nanoMOFs which possess a pore size smaller than the drug AZT-TP. Thus, these additional experiments have shown that the drug could only be adsorbed at the external surface of the nanoparticles. Moreover, as the drug was located only at the surface, the loading was very poor (< 1%) and most of the adsorbed drug was released in the first 30 min of incubation (Figure S20).

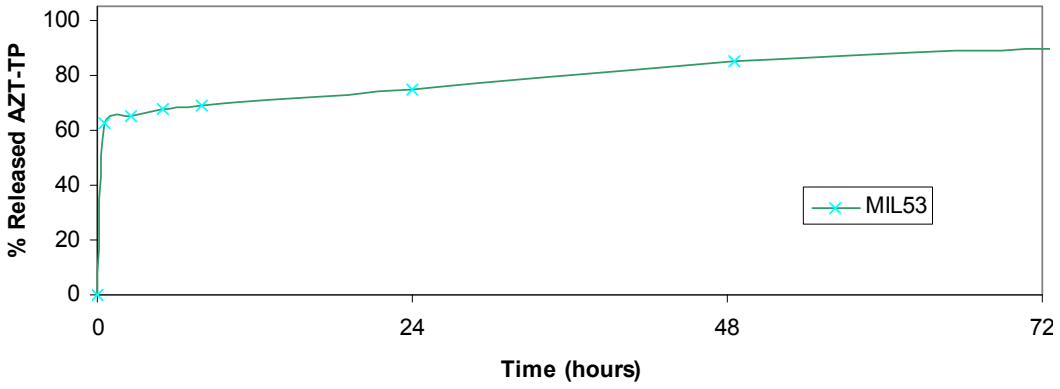

Figure S20. AZT-TP delivery from MIL-53

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HIV-Activity test

Peripheral blood mononuclear cells (PBMC) were isolated from the blood of healthy donors and submitted to anti-HIV assay as previously described (*7,8*). Briefly, phytohemaglutinin-P (PHA-P)-activated were pre-treated for 24 hours by six concentrations of each drug (1:5 dilutions between 1 µM and 0.32 nM of equivalent AZT concentration) and infected with one hundred 50% tissue culture infectious doses (TCID50) of the X4-tropic HIV-1-LAI strain (*9*). Five compounds were tested in our experiments, empty MIL100, AZTTP-charged MIL100, empty MIL100-PEG, AZTTP-charged MIL100-PEG and azidothymidine (AZT) as reference. This virus was amplified *in vitro* on PHA-P-activated PBMC. Viral stock was titrated using PHA-P-activated PBMC, and 50% TCID50 were calculated using Kärber's formula (*10*). Cell supernatants were collected at day 7 post-infection and stored at -20°C. Viral replication was measured by quantifying reverse transcriptase (RT) activity in cell culture supernatants using the RetroSys HIV RT kit (Innovagen). Experiments were performed in triplicate and on three independent blood donors.

11. CDV encapsulation

Cidofovir (L (S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine; Moravek, aqueous solution 400 µg /mL) was loaded in 2mg of MIL-101_NH2, MIL-100, MIL-88A and MIL-89 nanoparticles by soaking in a mixture of 1 mL of CDV and 30 µL of ^{14}C- CDV (14C-CDV; Moravek; 56 Ci/mmol, 0.1mCi/ml, 498.6µg/ml, 250µl). The impregnation time was 16 hours. The nanoparticles were recovered by centrifugation (5600 g, 15 min, room temperature) and dried under vacuum during 3 days. The radioactivity in the supernatant was determined by radioactivity counting (Beckman Coulter apparatus (LS 6500 multi purpose scintillation counter). Experiments were done in quadruplicate

The nanoparticle pellet was degraded under acidic conditions (1 mL HCl 5M at 50°C overnight) and the radioactivity was counted.

Table S4. Encapsulation of CDV in MIL-101_NH2 and MIL-100 solid.

Sample	Loading (wt%)	Efficiency (%)
MIL100	10.6	61.0
MIL100 2 incubations	16.1	46.2
MIL101_NH2	22.1	76.0
MIL101_NH2 2 incubations	41.9	68.1

CDV delivery:

The release of CDV was studied by suspending 2.5 mg of MIL-100 nanoparticles containing 16.1 wt% CDV in 1mL PBS (pH 7.4, Aldrich). These suspensions were kept under bidimensional agitation for different incubation times (from 30 min to 10 days). At each time point, an aliquot of 0.5 mL of supernatant was recovered by centrifugation (1000 rpm (5600 x g)/10 min) and replaced with the same volume of fresh PBS. Released CDV was quantified by radioactivity measurement. At the end of the release, the nanoparticle pellet was degraded under acidic conditions (1 mL HCl 5M at 50°C overnight) and the radioactivity was counted, confirming the total release of CDV. Experiments were done in quadruplicate.

12. Doxorubicin encapsulation

Doxorubicin hydrochloride (8S,10S)-10-(4-amino-5-hydroxy-6-methyl-tetrahydro-2H-pyran-2-yloxy)-,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione; Aldrich 98%) was loaded in 5mg of MIL-100 nanoparticles by soaking in 1.5 mL of a solution of Doxorubicin in water (10 mg/mL) for 24 hours. The impregnation was repeated two times.

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The nanoparticles were recovered by centrifugation (5600 g, 15 min, room temperature) and dried under vacuum during 3 days. Doxorubicin was quantified by fluorescence spectroscopy (emission maximum at 551 nm when excited at 472 nm).

Table S5. Encapsulation of Doxo in MIL-100 solid.

Sample	Loading (wt%)	Efficiency (%)
MIL100	8.5	20.8
MIL100 2 incubations	9.1	11.2

Doxorubicin delivery:

The release of Doxorubicin was studied by suspending 5 mg of MIL-100 nanoparticles containing 9.1wt% Doxorubicin in 2mL PBS (pH 7.4, Aldrich). These suspensions were kept under bidimensional agitation for different incubation times (from 30 min to 10 days). At each time point, an aliquot of 0.5 mL of supernatant was recovered by centrifugation (1000 rpm (5600 x g)/10 min) and replaced with the same volume of fresh PBS. Released Doxorubicin was quantified by fluorescence spectroscopy (emission maximum at 583 nm when excited at 472 nm).

13. Encapsulation of molecules of interest for cosmetics

Benzophenone-3 (2-Hydroxy-4-Methoxy Benzophenone, UVA and UVB solar filter, BZ3), benzophenone-4 (2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid, UVA and UVB solar filter, BZ4), caffeine (liporegulator, C) and urea (hydrating agent, U) were chosen as cosmetic agents for their encapsulation into the flexible and rigid iron carboxylates MIL-53 and MOF, MIL-100, respectively.

150 mg of solid, previously dehydrated (100°C for MIL-100 and 150°C for MIL-53 for 12h), were suspended into 10 mL of an aqueous solution of the cosmetic agent (C, U and BZ4; see concentration in Table S5) under stirring. After 3 days, the cosmetic containing solids were recovered by centrifugation at 5000 rpm for 15 min and dry under air.

The molecules of interest for cosmetics content into the materials were quantified by elemental analysis and TGA.

5 mg of MIL-53 were suspended into 10 mL of a benzophenone-3 solution under stirring for 12 h. The BZ3 solution was prepared by the dilution of 1mL of a 1g/L solution in dimethylsulfoxide (DMSO) in 100 mL of MiliQ water (final concentration 10 µg/mL). The BZ3 containing particles were recovered by centrifugation at 30000 rpm for 30 min.

The BZ3 content in the material was estimated by quantification of the BZ3 in the supernatant using UV-Vis spectroscopy (at 298 nm).

The crystalline structure was kept after the molecule of interest for cosmetics encapsulation for all the materials, as evidenced by XRD.

Table S6: Quantification of the molecules of interest for cosmetics in MIL-53 and MIL-100 solids

Solid	Cosmetic	Cosmetic solubility (mg/mL)	Concentration (mg/mL)	Cosmetic content (%wt)
MIL-100	**C**	22	22	24.2
	U	1080	500	69.2
	BZ4	100	10	15.2
MIL-53	**C**	22	22	23.1
	U	1080	500	63.5
	BZ4	100	10	5.0
	BZ3	3.7	0.01	1.5

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14. Magnetic resonance imaging (MRI)

In vitro Magnetic Resonance Imaging

Samples

For each type of nanoparticles, 6 samples containing different concentration of were prepared by suspending them in a water-glucose (5%) solution (C: 1mg/ml,0.5mg/ml, 0.2 mg/ml, 0.1mg/ml, 0.05mg/ml, 0 mg/ml).

MRI experiments were performed on a 9.4T vertical wide-bore magnet (Oxford, UK) driven by PARAVISION and equipped with a micro-imaging kit (Bruker®, Germany) and a 15 mm inner diameter bird-cage coil. Spin-echo imaging experiments were used for the determination of T1 and T2 relaxation times (FOV 15x15mm; slice thickness 1mm; acquisition matrix 32x32). T1 were determined using the saturation recovery method (Spin-echo sequence; TE=10 ms TR=4000, 2000, 1000, 500, 200, 100 ms) and T2 using the Carr-Purcell-Meiboom-Gill method (RARE sequence; rare factor 8 and 8 echo-images; TR/TE=15000/8 ms). For a given contrast agent all the samples were examined together. Before measuring relaxation times, the field homogeneity was optimized using a 1.5 diameter tube filled with the solution of dilution. Then the contrast agent samples were introduced in th tube. A scout image and a PRESS spectrum localized in the 5% glucose-water sample were recorded to verify the position of the samples and the field homogeneity. Then the T1 and T2 measurement experiments were carried out, resulting in a total acquisition time less than 6 min. The relaxivity of each type of nanoparticles at a given magnetic field was given by the slope of the straight line representing the relaxation rates versus the concentration of the product

***In vivo* MRI examination**

Animal preparation:

Wistar females (4-weeks-old, 161.36 ± 16.1 g) were obtained from the central animal care facilities, Janvier R Centre d'Elevage, France.

22, 44 and 220 mg/kg of MIL-88A (150 et 500 nm; called group 88A) suspended in a 0.5 mL aqueous solution of glucose 5% were intravenously injected in the jugular vein under isofluorane anesthesia to two rats. Rats without treatment were used as control. Rats were sacrificed by an overdose of isofluorane and immediately observed by MRI.

MRI experiments:

Imaging was performed at 300MHz on a 7T horizontal-bore magnet (Oxford, UK) driven by Paravision (Bruker®, Germany) and equipped with a 360 mT/m actively shielded gradient device (I.D.= 90 mm). For MRI examination, rat**s** were killed (isoflurane over dosage), introduced in 60 mm bird-cage coil and positioned using MRI scouting gradient-echo images in the three orthogonal directions. After the shimming process, RARE [TR/TE=1781.21/8.8ms; Rare Factor = 4 ; 4 averages; 39 1mm contiguous slices; FOV 70*70mm; acquisition matrix 512*384; reconstruction matrix 512*512; in plane resolution 136m*136m; time experiment 8m32s] and FLASH [TR/TE=564.4/6.7ms; 2 averages; 39 1mm contiguous slices; FOV 70*70mm; acquisition matrix 512*384; reconstruction matrix 512*512; in plane resolution 136m*136m; time experiment 7m13s] experiments were recorded

MRI observation protocol:

Four slices were analyzed in each zone of interest [liver (a) and spleen region (b)]. On each slide, region of interest (ROI) were define as seen on Figure S21 (a) [4 ROI in dorsal muscle, 12 ROI in liver] and (b) [4 ROI in dorsal muscle and the whole area for spleen]

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Then, in each zone of interest, global mean for each organ was calculated, and normalized to dorsal muscle's one. [(*dm*): dorsal muscle; (*k*) kidney, (*li*) liver, (*s*) spleen, (*st*) stomach]

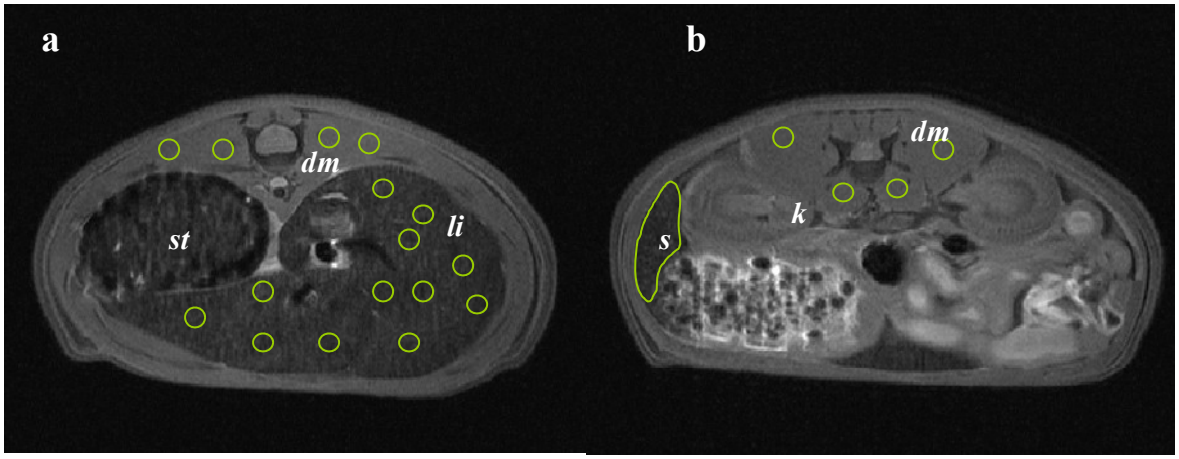

Figure S21. Region of interest choice, in dorsal muscle (*dm*), liver (*li*) and spleen (*s*)

For each sequence type (Spin echo or Gradient Echo), Relative normalized Intensity (*RIn*) was calculated for liver and spleen (Figure S22), showing a significant decrease in the *RIn* after treatment.

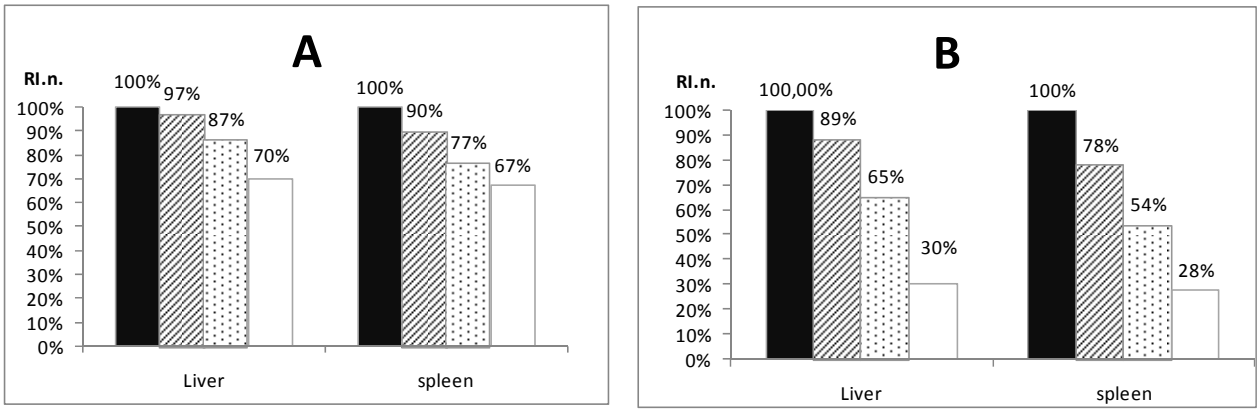

Figure S22. Relative Normalized intensity (*RIn.*), calculated for liver and spleen [(A) Spin echo sequences (RARE), (B) Gradient echo sequence (FLASH)] [*RIn.*= (normalized organ intensity /control normalized intensity)*100)] (Black =control, Hatched = treated with 22mg/kg, points = treated with 44mg/kg and white = treated with 220mg/kg)

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