Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

MOF nanoparticles coated by lipid bilayers and their uptake by cancer cells

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Methods and Characterization

Powder X-ray diffraction (PXRD) measurements were performed using a Bruker D8 diffractometer (Cu-$K_{\alpha1}$ = 1.5406 Å; Cu-$K_{\alpha2}$ = 1.5444 Å) in *theta-theta* geometry equipped with a Lynx-Eye detector. The powder samples were measured between 2° and 45° *two theta*, with a step-size of 0.05° *two theta*.

Scanning electron microscopy (SEM) images were recorded with a JEOL JSM-6500F microscope equipped with a field emission gun, operated at an acceleration voltage of 5 kV and a working distance of 10 mm. Prior to measurements a thin gold layer (purity: 99.95%) was deposited on the samples using an Oerlikon Leybold Vacuum UNIVEX 350 sputter coater system operated at a base pressure of $1x10^{-6}$ mbar, an Argon pressure of $1x10^{-2}$ mbar, a power of 25 W and a sputtering time of 5 min.

Transmission electron microscopy (TEM). All samples were investigated with a FEI Titan 80-300 operating at 80 kV with a high-angle annular dark field detector. A droplet of the diluted nanoparticle solution in absolute ethanol was dried on a carboncoated copper grid.

Nitrogen sorption measurements were performed on a Quantachrome Instruments Autosorb at 77 K. Sample outgassing was performed for 12 hours at 393 K. Pore size and pore volume were calculated by a NLDFT equilibrium model of N_2 on silica, based on the adsorption branch of the isotherms. BET surface area was calculated over the range of partial pressure between 0.05 – 0.20 p/p_0. The pore volume was calculated based on the uptake (cm^3/g) at a relative pressure of 0.30 p/p_0.

Thermogravimetric (TG) analyses of the bulk samples were performed on a Netzsch STA 440 C TG/DSC with a heating rate of 1 K min^{-1} in a stream of synthetic air at about 25 mL min^{-1}.

Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer-Nano instrument equipped with a 4 mW He-Ne laser (633 nm) and an avalanche photodiode. The hydrodynamic radius of the particles was determined by dynamic light scattering in a diluted aqueous suspension.

Fluorescence Correlation Spectroscopy (FCS) is a powerful single-molecule detection technique to characterize interactions and dynamics of fluorescent particles or molecules by correlating their fluorescence fluctuations, in a confocal detection volume ($\sim$1fL $= 10^{-15}$L) in time.[1] Brownian motion or active transport lets particles diffuse though the volume causing spontaneous intensity fluctuations similar to photodynamic processes and chemical reactions. The temporal autocorrelation function is defined by $G(\tau) = \frac{\langle F(t)F(t+\tau)\rangle}{\langle F\rangle^2}$ and provides information about the dynamic properties of the measured sample such as diffusion times and average numbers of particles within the focal volume. From these values the hydrodynamic radius and the particle concentration can be determined.

An extension of FCS is dual-color fluorescence cross-correlation (FCCS), which provides access to binding properties of two differently labeled species of particles in the sample.[2] Lasers with two different wavelengths focused to the same spot excite both of the two fluorophore types with different emission spectra. The two fluorescence signals get separated by a dichroic mirror and are recorded individually. By correlating the fluctuations $F_1(t)$ and $F_2(t)$ not only with themselves (autocorrelation) but also crosswise, the ensuing cross-correlation curve $G(\tau) = \frac{\langle F_1(t)F_2(t+\tau)\rangle}{\langle F_1(t)\rangle\langle F_2(t)\rangle}$ yields the amount of particles that show coincidence of both fluorescence colors. These results are accessible by fitting the correlation curves according to $G(\tau) = G(0)\frac{1}{1+\frac{\tau}{\tau_D}}\frac{1}{\sqrt{1+\frac{\tau}{S^2\tau_D}}}$, where S is the structure parameter, the ratio between the lateral and the axial confocal volume radius while τ_D is the mean time a particle needs to cross the focal volume.[3] The amplitude G(0) contains the mean particle count $N = (G(0))^{-1}$ within the focal volume of the autocorrelations.

The FCCS measurements were conducted with a ConfoCor2 (Zeiss, Jena) setup with a 40x NA1.2 water immersion objective employing a red 633nm HeNe- and a blue 488nm Ar-laser for excitation of the two fluorophores Atto633 (bound to MOF nanoparticles) and BODIPY FL (DHPE-Lipid embedded in the DOPC bilayer)

Due to physical and technical reasons, the alignment of the two exciting laser beams leads to slightly displaced laser foci. In addition to other effects, this causes non-overlapping correlation curves even if a perfect coincidence of both labels is obtained.

In order to subsequently correct these deviations, a DNA double-strand assumed to be perfectly double-labeled with both green and red fluorescent dye (similar to the dyes of the MOF-lipid sample) was measured with the same configuration as the MOF sample.

The data were fitted using OriginPro9 (Fig. S-1). By adjusting the fitted curves of the correlation of the MOF particles (red, ch1) and the cross correlation (black) to the one of the lipids (blue, ch2) we obtained factors to correct the MOF-lipid measurements. The blue-green (ch2) data-set was chosen to overlay the other data-sets because the alignment of the confocal beam path and detection path was optimized to the blue laser (488nm) and therefore the ch2 data provides the most reliable results.

The correction factors obtained are listed in Table 1. To correct the raw data, the formula $G(\tau)_{corrected} = \left((G_{raw}(\tau \cdot \alpha) - 1) \cdot \beta\right) + 1$ was applied. The subtraction and addition of 1 is necessary because the minimum value of the correlation curves is 1.

	α	β
Ch1 (red)	0,3513	0,7298
Cross-correlation (black)	0,2920	3,0922

Table S-1: Correction factors obtained from FCCS Data of a double labeled DNA double strand.

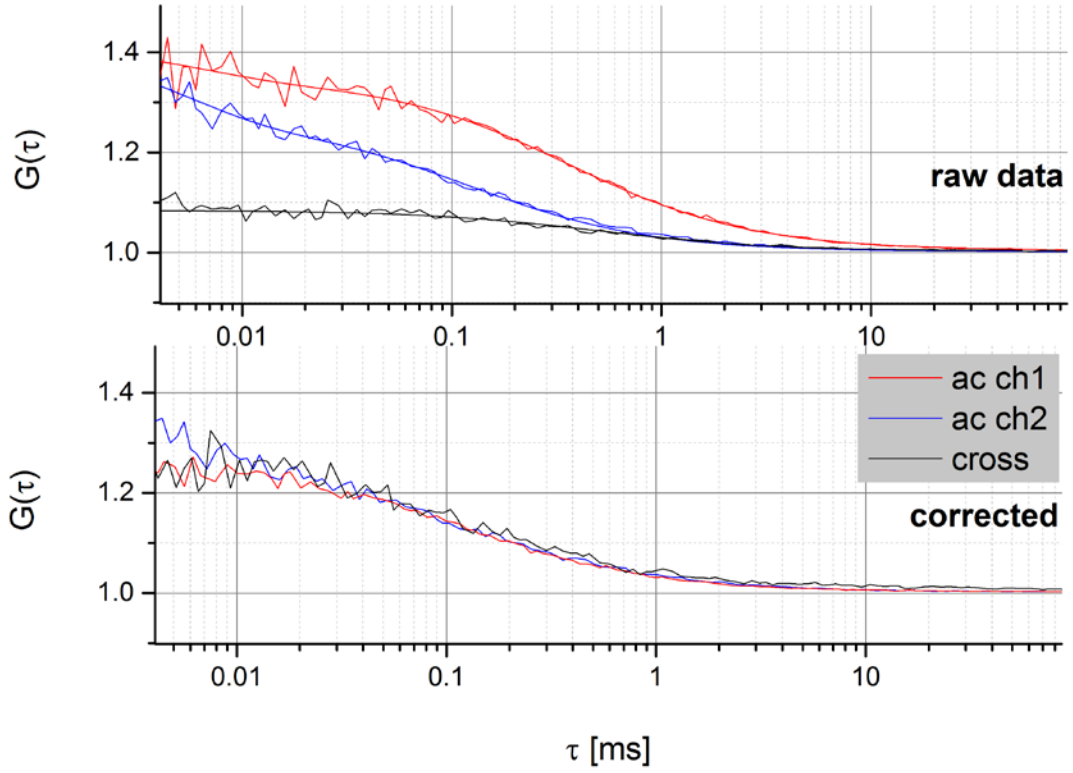

Fig. S-1 FCCS Data of a doubly labeled DNA double strand. The raw data (top) shows that the autocorrelations (red and blue) and cross-correlation (black) are not overlapping due to inevitable minor setup misalignments. To account for this, the fitting results were used to obtain correction factors to align all three curves (bottom). The same procedure was applied to the lipid-MOF results.

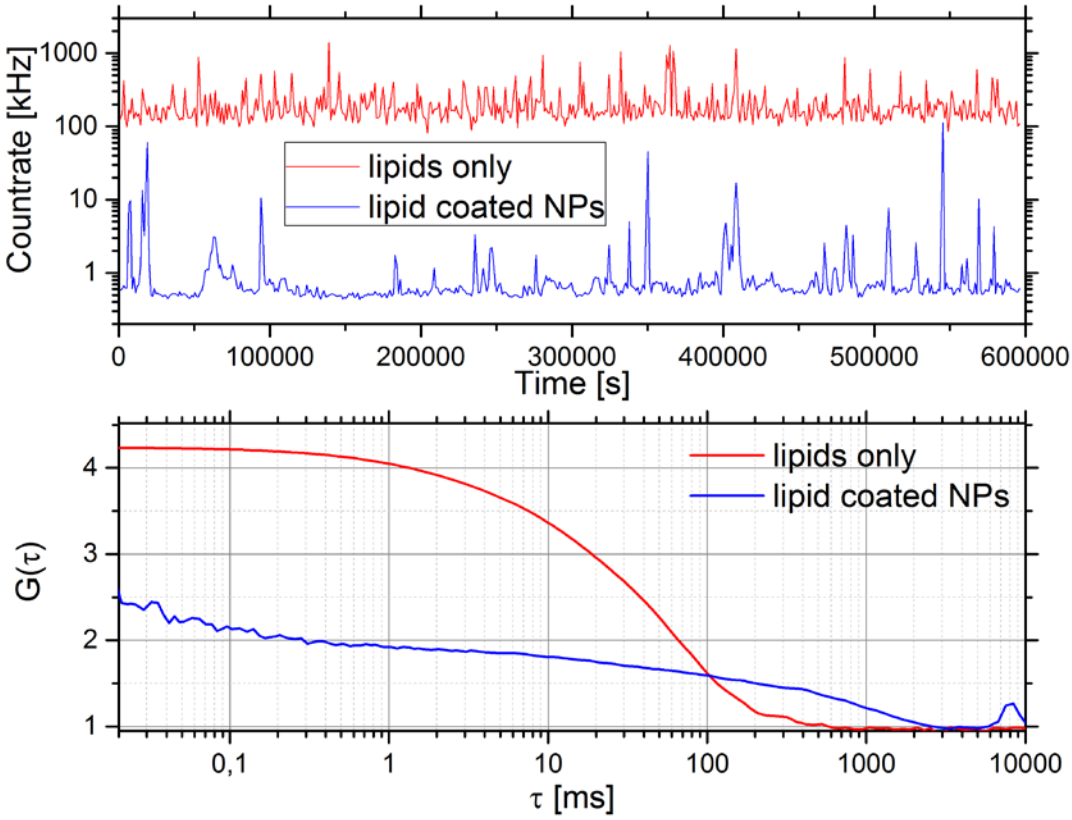

Fig. S-2 FCS Data of labeled lipids with and without MIL-100(Fe) NPs.

For the **fluorescence release experiments** an amount of 200 µL of the aqueous suspension containing MIL-101(Cr)@DOPC or MIL-100(Fe)@DOPC loaded with fluorescein (for preparation see experimental section) was transferred into the cap of a quartz cuvette (Fig. S-2, 1). The cap was sealed with a dialysis membrane (Fig. S-2, 2) and put on top of a cuvette that was filled with 3 ml H_2O. Only dye molecules can pass the membrane, but no nanoparticles. Consequently, dye molecules that were released from the pores of the particles are responsible for the measured fluorescence intensity. During fluorescence measurement, the water inside the cuvette was stirred (Fig. S-2, 3) and was heated to 37 °C. For the fluorescence measurement with a PTI spectrofluorometer (model 810/814, Photon Technology International), the monochromator slit was set to 1.25 mm, all other slits to 1.00 mm. The excitation wavelength of fluorescein (sodium salt) is 490 nm, the emission wavelength 512 nm. The measurement was run for 1 h with 1 point/min. After the addition of 20 µL of absolute Triton X-100 into the cap-system, the lysis of the lipid bilayer on the MOF nanoparticles allows the diffusion of the dye molecules from the pores and their detection in the cuvette (Fig. S-2, 3).

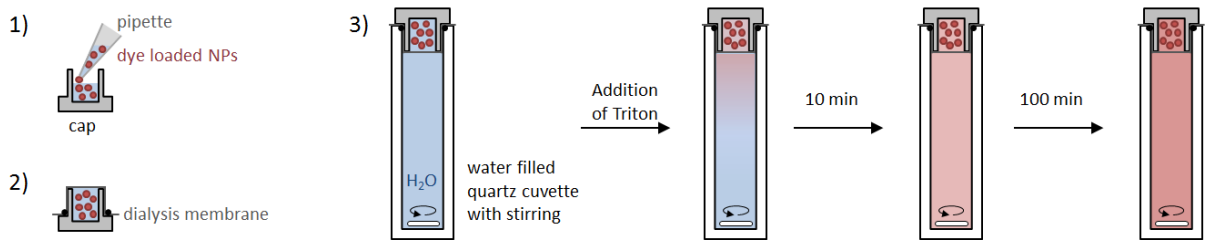

Fig. S-3 Scheme of the fluorescence release experiment. The sample was filled into a cap system that is closed by a dialysis membrane; the volume of the fluorescence cuvette was filled with water.

Confocal laser scanning microscopy and *in vitro* uptake of the nanoparticles. Membranes of bladder carcinoma cells were stained with the red fluorescence dye PKH26 (Sigma-Aldrich, St. Louis, MO, USA) according to manufacturer's instructions. In brief, adhered cells were detached, washed and incubated for 2 min with PKH26 dye solution. After further washing steps, cells were seeded on ibidi µ-slides (Ibidi, Munich, Germany) The next day, cells were treated with 20µl Atto-633 labelled MOF nanoparticles for indicated time points and fluorescence intensities were assessed using a Zeiss LSM 510 Meta microscope.

Impedance-based real-time cell monitoring. Cellular behaviour of MOF treated cells was analysed by utilizing the xCELLigence System (ACEA Biosciences, San Diego, CA, USA), which monitors cellular growth in real-time by measuring the electrical impedance across interdigitated microelectrodes covering the bottom of E-plates. Impedance is displayed as cell index values. T24 bladder carcinoma cells were seeded at a density of 5000 cells per well in E-plates and different charges of MOF nanoparticles (MOF#1 and #2) and amounts (4µl MOF/100µl medium and 8µl MOF/100µl medium) were added directly to the wells after about 18 h. Cell index tracings were normalized shortly after addition of the particles.

Experimental section

Chemicals

Chromium(III) nitrate nonahydrate (99%, *Aldrich*), terephthalic acid (98%, *Aldrich*), ethanol (99%, *Aldrich*) 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, *Avanti Polar Lipids*), fluorescein sodium salt suitable for fluorescence (*Fluka*), triton X-100 (*Aldrich*), N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (BODIPY® FL DHPE, *Invitrogen*).

Synthesis of MIL-101(Cr) nanoparticles

The microwave synthesis of MIL-101(Cr) nanoparticles was based on a modified procedure reported in the literature.[4, 5] An amount of 20 mL (1.11 mol) of H_2O was added to 615 mg (3.70 mmol) terephthalic acid and 1.48 g $Cr(NO_3)_3 \cdot 9\,H_2O$ (3.70 mmol). This mixture was put into a Teflon tube, sealed and placed in the microwave reactor (Microwave, Synthos, *Anton Paar*). Four tubes were filled and inserted into the reactor: one tube contained the reaction mixtures described above; the remaining tubes including the reference tube with the pressure/temperature sensor (PT sensor) were filled with 20 mL H_2O. For the synthesis, a temperature programme was applied with a ramp of 4 min to 180 °C and a holding time of 2 min at 180 °C. After the sample had cooled down to room temperature, it was filtrated and washed with 50 ml EtOH to remove residual e.g. terephthalic acid. For purification, the filtrate was centrifuged and redispersed in 50 ml EtOH three times. The sample was centrifuged at 20000 rpm (47808 rcf) for 60 min. Afterwards the sample was characterized by DLS, XRD, IR, TGA, BET, REM and TEM measurements.

Synthesis of MIL-100(Fe) nanoparticles

For the microwave synthesis of MIL-100 (Fe) nanoparticles, iron(III) chloride hexahydrate (2.43 g, 9.00 mmol) and trimesic acid (0.84 g, 4.00 mmol) in 30 ml H_2O were put into a Teflon tube, sealed and placed in the microwave reactor (Microwave, Synthos, *Anton Paar*).[6] The mixture was heated to 130 °C under solvothermal conditions (p = 2.5 bar) within 30 seconds, kept at 130 °C for 4 minutes and 30 seconds and the tube was cooled down to room temperature. For the purification of the solid, the reaction mixture was centrifuged

(20000 rpm = 47808 rcf, 20 min), the solvent was removed and the pellet was redispersed in 50 ml EtOH. This cycle was repeated two times and the dispersed solid was allowed to sediment overnight. The supernatant of the sedimented suspension was filtrated (filter discs grade: 391, *Sartorius Stedim Biotech*) three times, yielding MIL-100(Fe) nanoparticles. Afterwards the sample was characterized by DLS, XRD, IR, TGA, BET, REM and TEM measurements.

Synthesis of MIL-101(Cr)@DOPC and MIL-100(Fe)@DOPC nanoparticles with encapsulated dyes for fluorescence release and for in vitro experiments

The amount of 1 mg MIL-101(Cr) or MIL-100(Fe) nanoparticles was dispersed in 1 mL of a 1 mM aqueous solution of fluorescein (sodium salt). 24 h later the samples were centrifuged for 5 min at 14000 rpm (16873 rcf). For the application of the lipid layer, the sample was redispersed in 100 µL of a 3.6 mM DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) solution in a 60/40 (v/v) H_2O/EtOH mixture. 900 µL H_2O was added and mixed as quickly as possible. By increasing the water concentration, the lipid molecules precipitate and are expected to cover the nanoparticle surface with a lipid layer. For purification, the suspension was centrifuged (5 min, 14000 rpm = 16873 rcf), redispersed in 1 mL H_2O and again centrifuged. Finally the nanoparticles were redispersed in 200 µL H_2O.

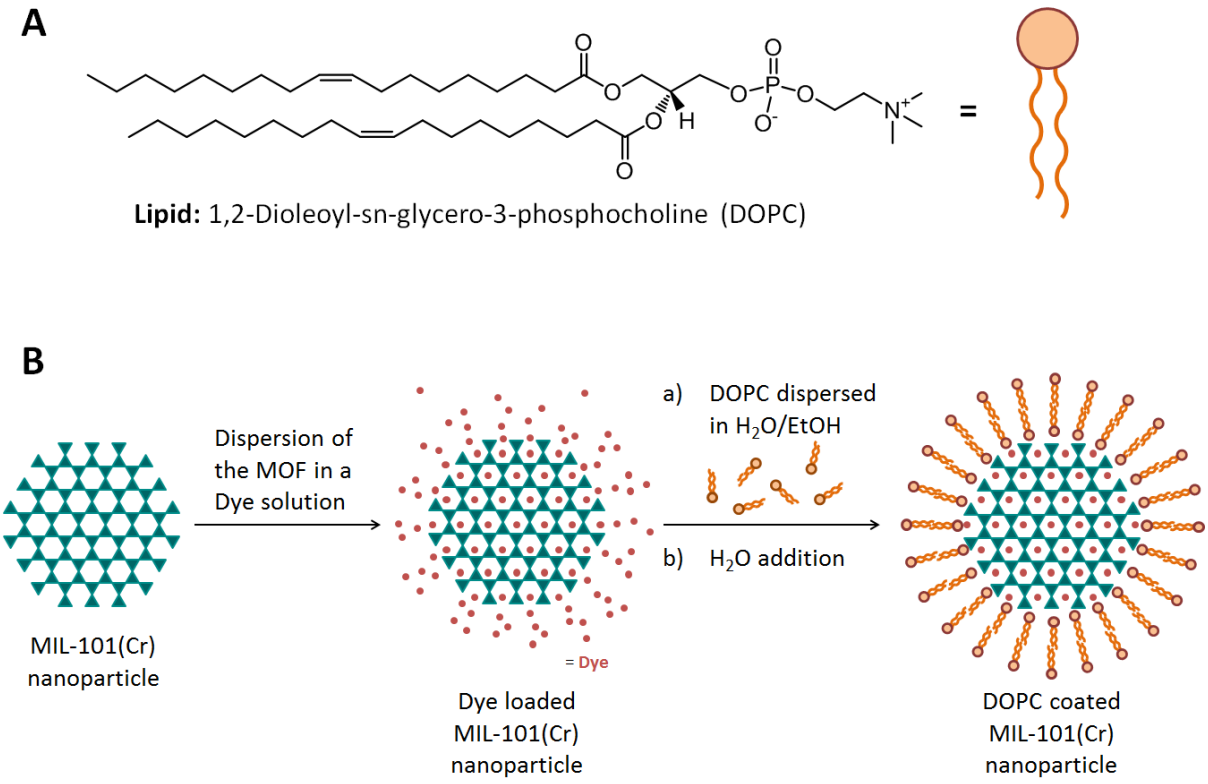

Fig. S-4 (A) Illustration of the lipid DOPC. **(B)** Schematic depiction of MIL-101(Cr) nanoparticles which are loaded with a dye in the first step, and coated with a lipid bilayer on the MOF nanoparticle surface in the second step.

Synthesis of labeled MIL-101(Cr)@DOPC nanoparticles for FCCS measurements

Loading of MOFs with dye. The amount of 1 µL ATTO 633 NHS (ATTOTec) stock solution (c = 1 mg/ml) was mixed with 100 µL MilliQ water (bi-distilled water from a Millipore system (Milli-Q Academic A10)) just before adding 25µL of this solution to 250 µL of a 10 mg/mL aqueous MOF suspension. This labeling solution was then stirred at room temperature for 48 hours. The nanoparticles were separated from free ATTO 633 molecules by centrifugation (19.000rpm = 20138 rcf, 45min) and resuspending with 1mL MilliQ water, and repeating this cycle 5 times.

Lipid preparation. The amount of 2.5 mg DOPC lipid (1,2-dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids) was mixed with 0.2 µg BODIPY FL DOPE lipid (N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, Invitrogen) in chloroform (99.995 mol% DOPC and 0.005 mol% BODIPY FL DHPE). After evaporating the chloroform with nitrogen gas, the

lipids were further dried in a vacuum overnight. The lipids were then dissolved in 1 mL of a 40 % ethanol/60 % water (v/v) solution to a final concentration of 2.5 mg/mL.

Lipid coating of the MOFs. The amount of 2.5mg labeled MOFs (labeling solution) were centrifuged (19.000 rpm = 20138 rcf, 45min). Afterwards 100 µL of the DOPC/BODIPY FL DHPE lipid in ethanol/water mixture was added. To induce the formation of lipid bilayer on the MOF surface, we quickly added 900 µL of MilliQ water. Afterwards the sample was ready to use for the FCCS measurements.

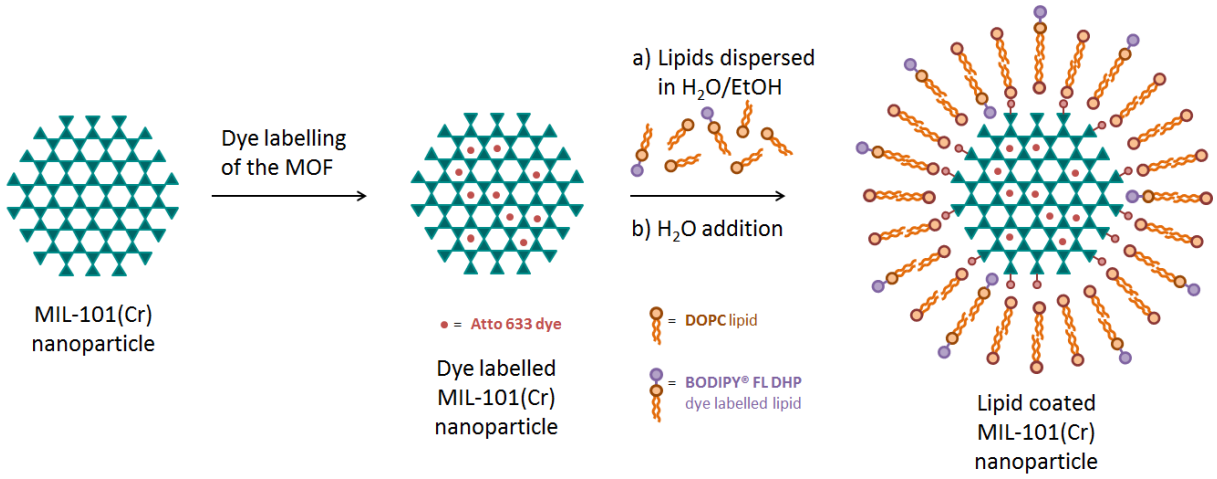

Fig. S-5 Schematic illustration of the dye labelling of MIL-101(Cr) nanoparticles in the first step and the formation of a labeld lipid bilayer on the MOF surface in the second step.

Supplementary Figures

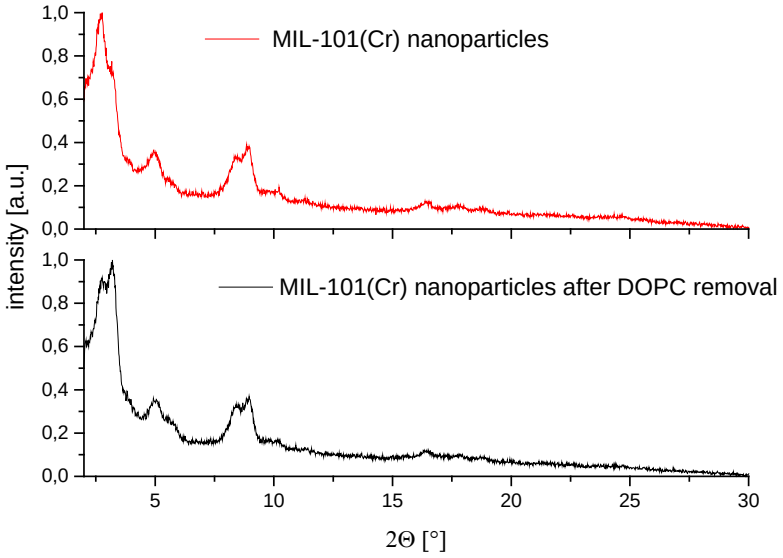

Fig. S-6 X-ray powder diffraction patterns of uncoated MIL-101(Cr) nanoparticles (top) and DOPC coated MIL-101(Cr) nanoparticles after removal of the lipid (bottom).

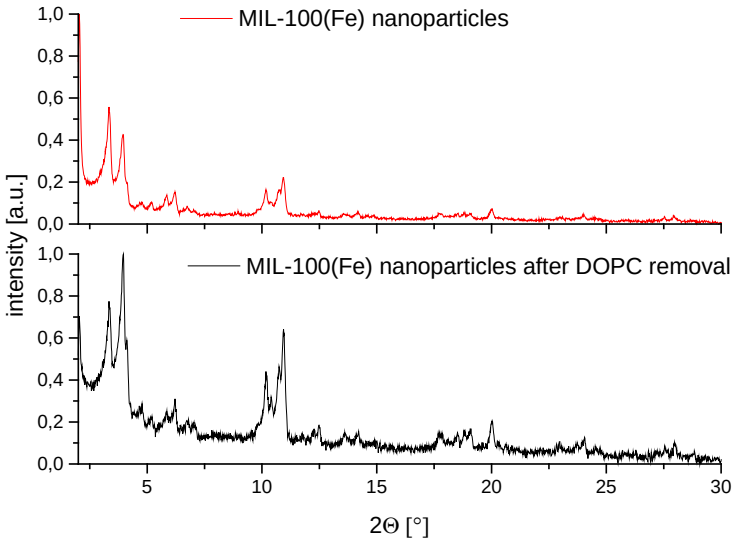

Fig. S-7 X-ray powder diffraction patterns of uncoated MIL-100(Fe) nanoparticles (top) and DOPC coated MIL-100(Fe) nanoparticles after removal of the lipid (bottom).

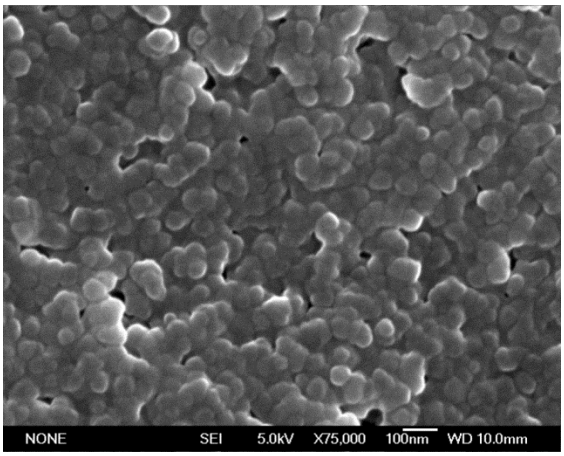

Fig. S-8 Scanning electron micrograph of MIL-101(Cr) nanoparticles.

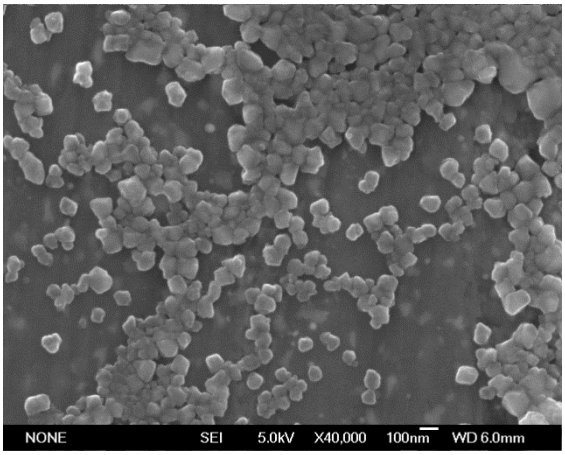

Fig. S-9 Scanning electron micrograph of MIL-100(Fe) nanoparticles.

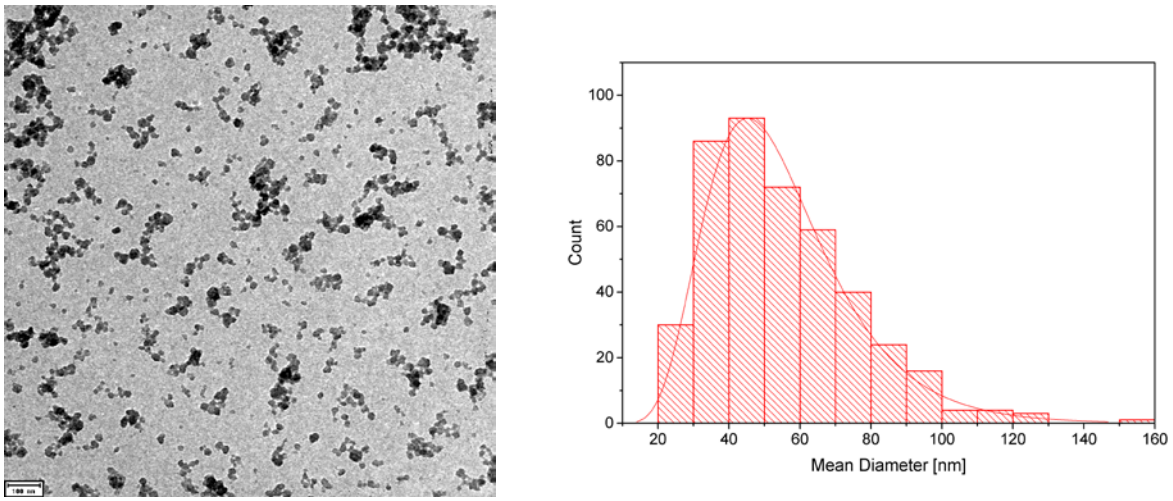

Fig. S-10 Transmission electron micrograph of MIL-101(Cr) nanoparticles (left). Size distribution of MIL-101(Cr) nanoparticles from the TEM picture (right).

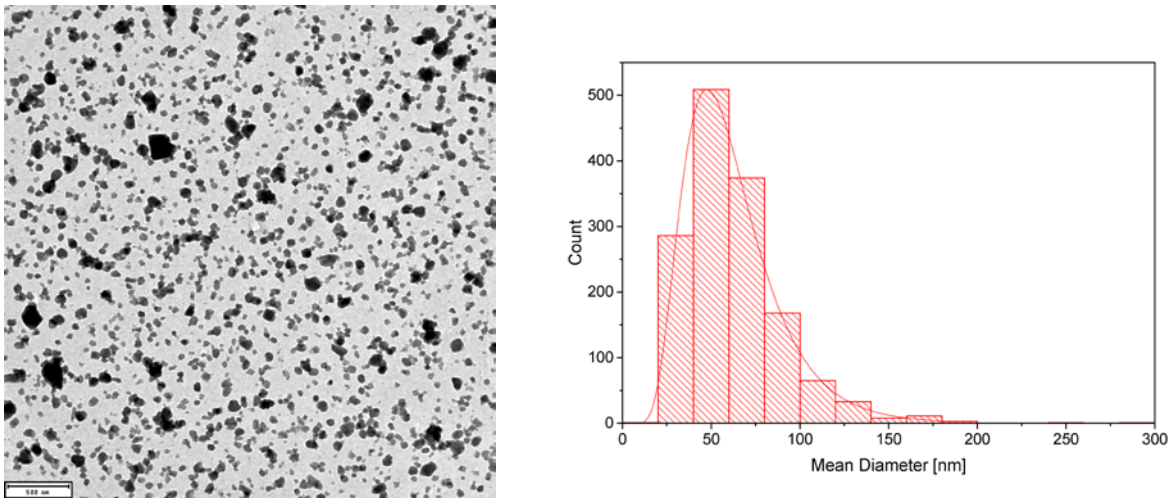

Fig. S-11 Transmission electron micrograph of MIL-100(Fe) nanoparticles (left). Size distribution of MIL-100(Fe) nanoparticles from the TEM picture (right).

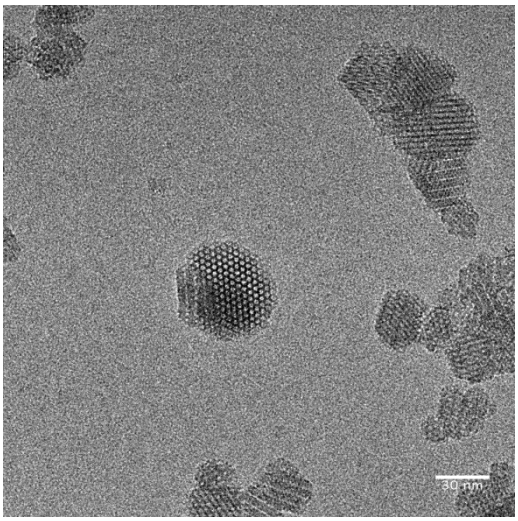

Fig. S-12 Transmission electron micrograph of MIL-101(Cr) nanoparticles – detailed image.

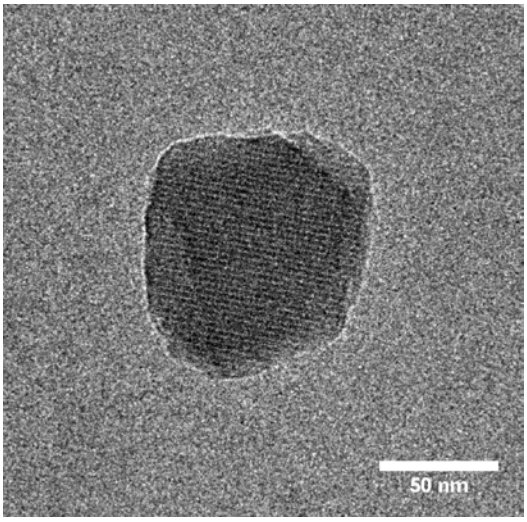

Fig. S-13 Transmission electron micrograph of MIL-100(Fe) nanoparticle – detailed image.

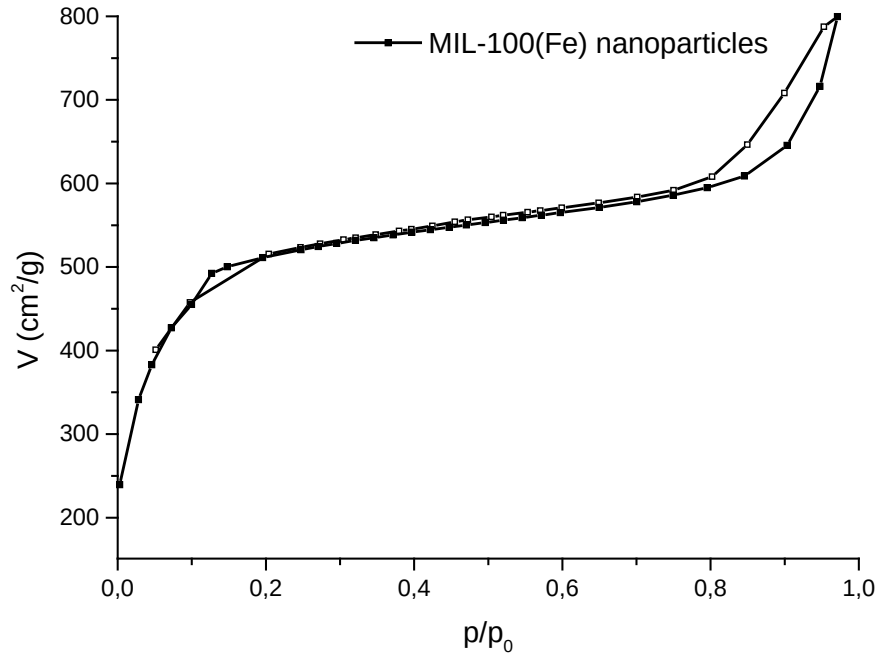

Fig. S-14 Nitrogen sorption isotherm of MIL-100(Fe) nanoparticles. Calculated BET surface: 2004 m^2/g.

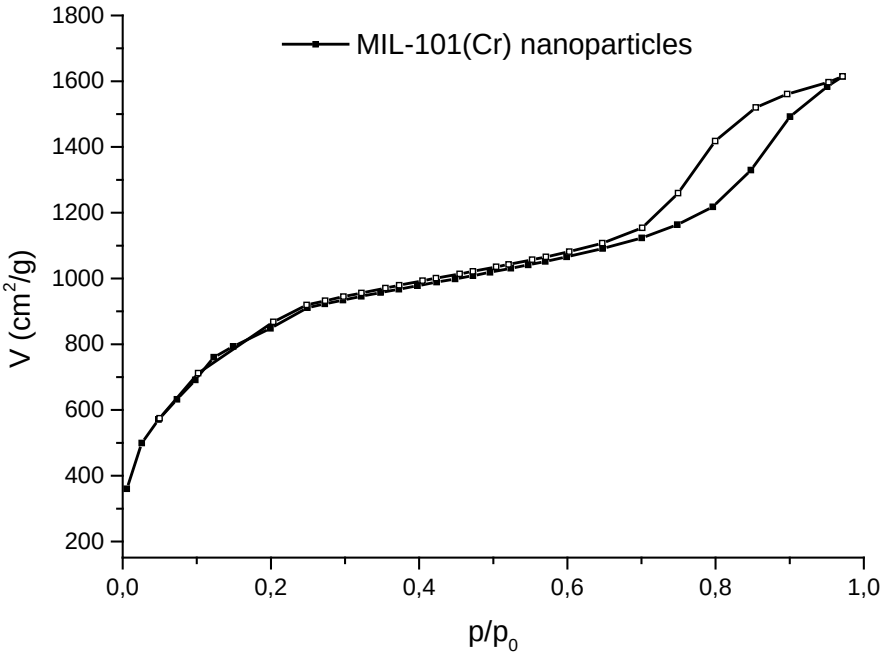

Fig. S-15 Nitrogen sorption isotherm of MIL-101(Cr) nanoparticles. Calculated BET surface: 3205 m^2/g.

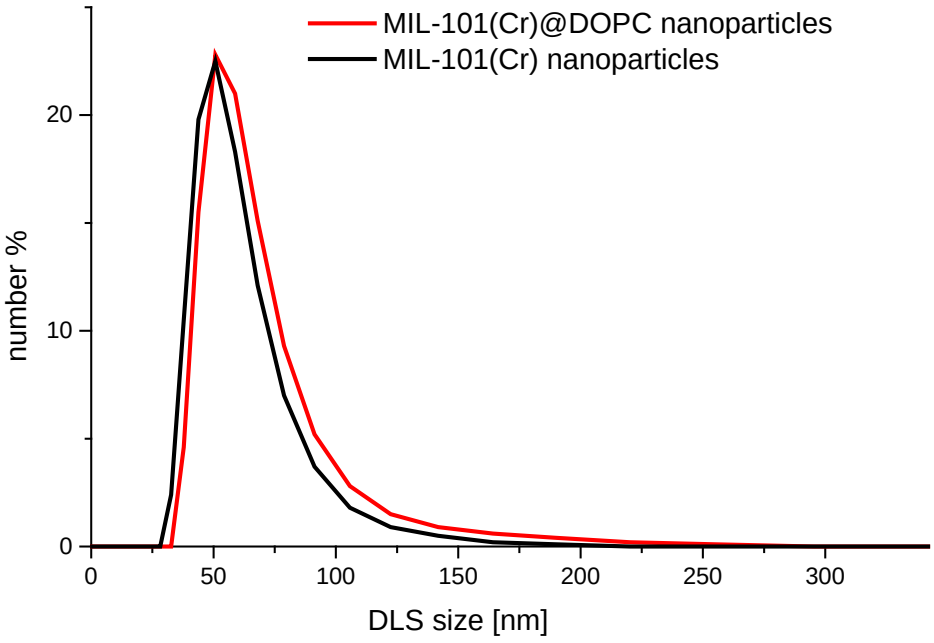

Fig. S-16 DLS size distribution (measured in water) by number comparing uncoated and DOPC-coated MIL-101(Cr) nanoparticles.

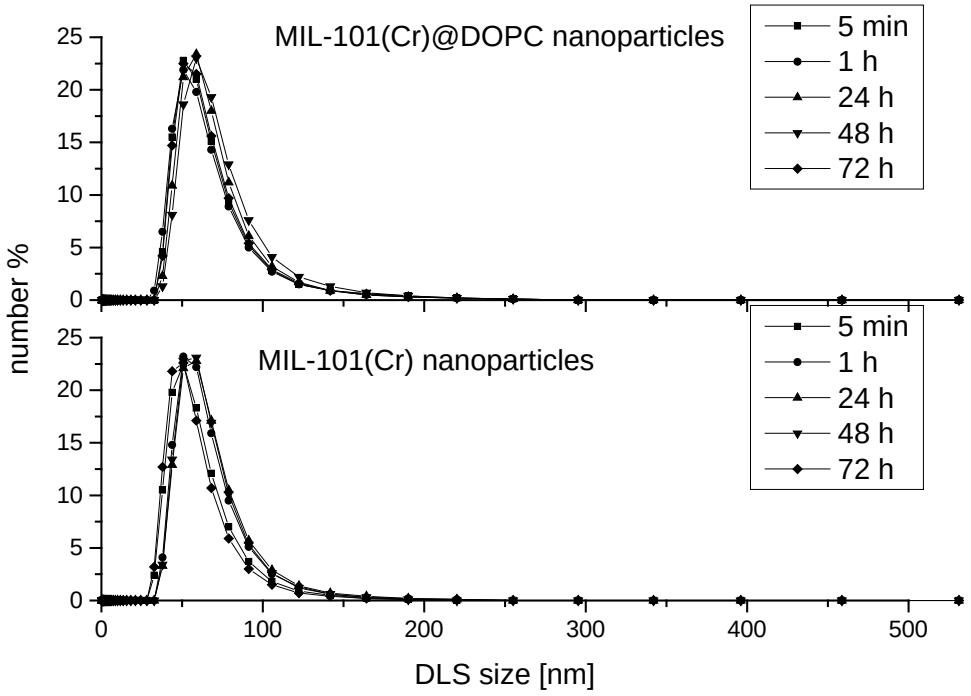

Fig. S-17 DLS size distributions by number comparing uncoated and DOPC-coated MIL-101(Cr) nanoparticles over a time period of 72 h.

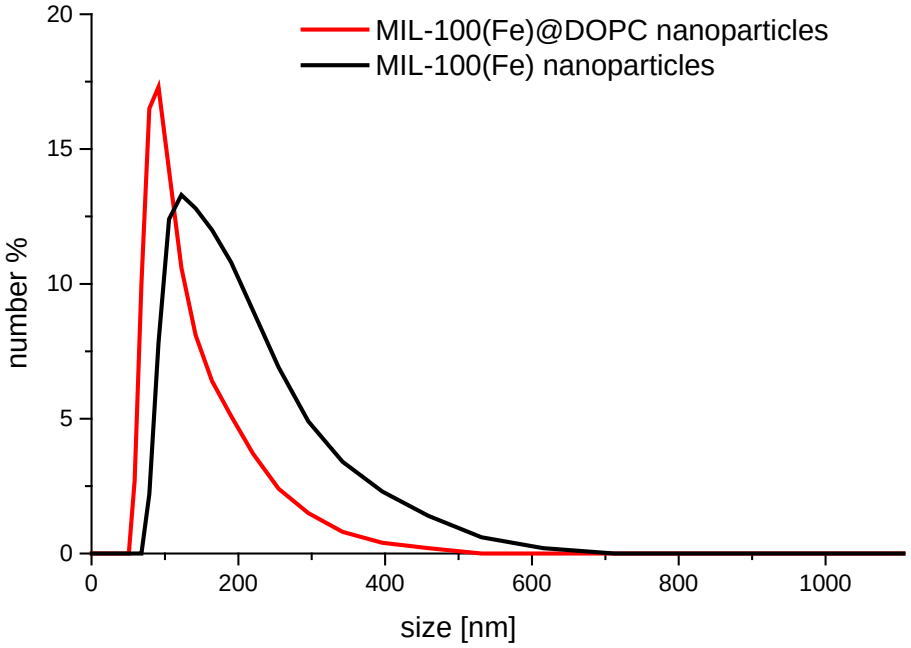

Fig. S-18 DLS size distribution by number (measured in water) comparing uncoated and DOPC-coated MIL-100(Fe) nanoparticles.

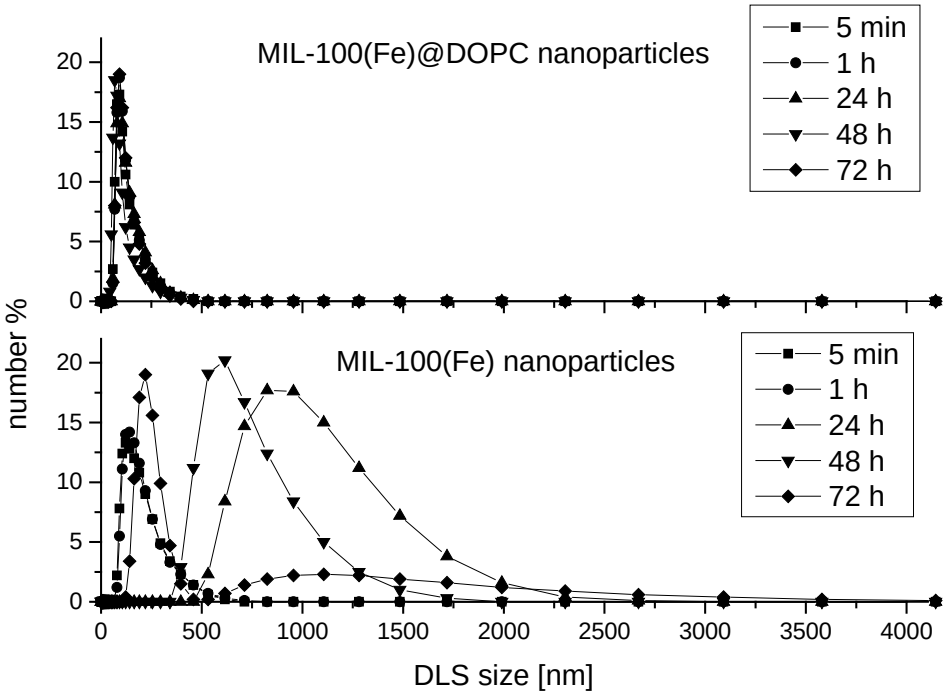

Fig. S-19 DLS size distribution by number, comparing uncoated and DOPC-coated MIL-100(Fe) nanoparticles over a time period of 72 h.

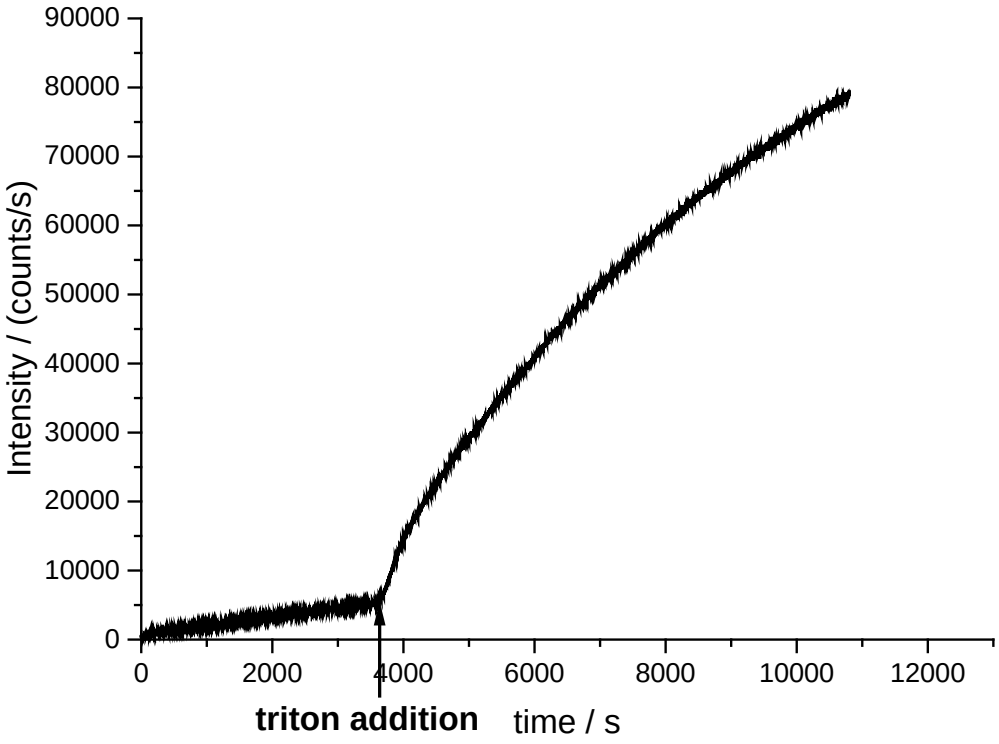

Fig. S-20 Fluorescein release from DOPC-coated MIL-100(Fe) nanoparticles before and after addition of Triton X-100.

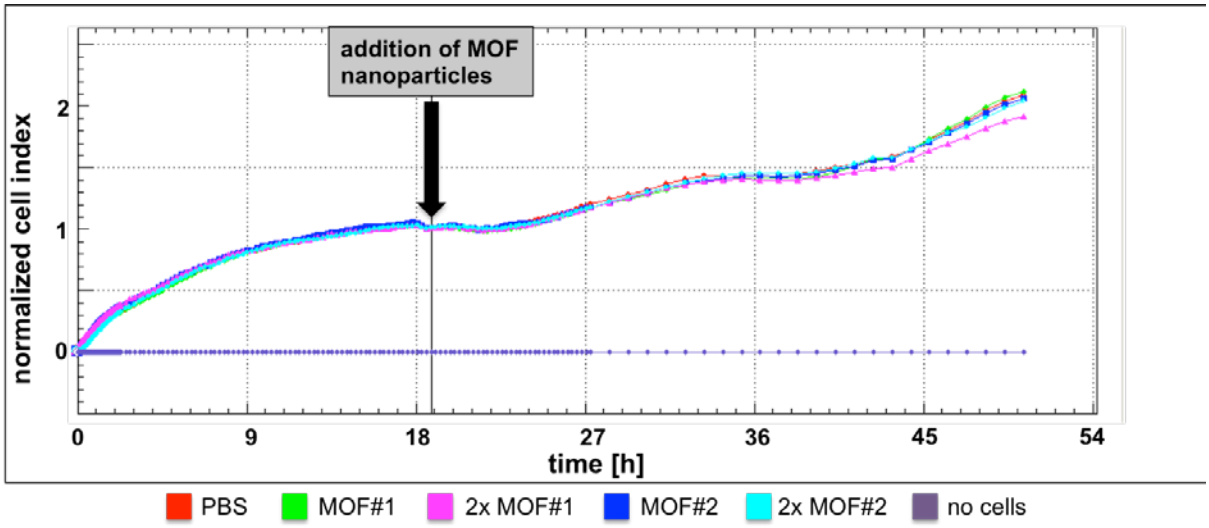

Fig. S-21 Impedance measurements of cell cultures. Bladder carcinoma cells were seeded on xCELLigence E-plates and treated at indicated time points with different charges (MOF#1 and MOF#2) and amounts of 6,4 µl

and 12,8 µl of MIL-101(Cr)@DOPC nanoparticles (c = 1 mg/ml) per 200 µl medium. Similar cell index values indicate that cells incubated with MOF nanoparticles show a behaviour very similar to PBS-treated control cells.

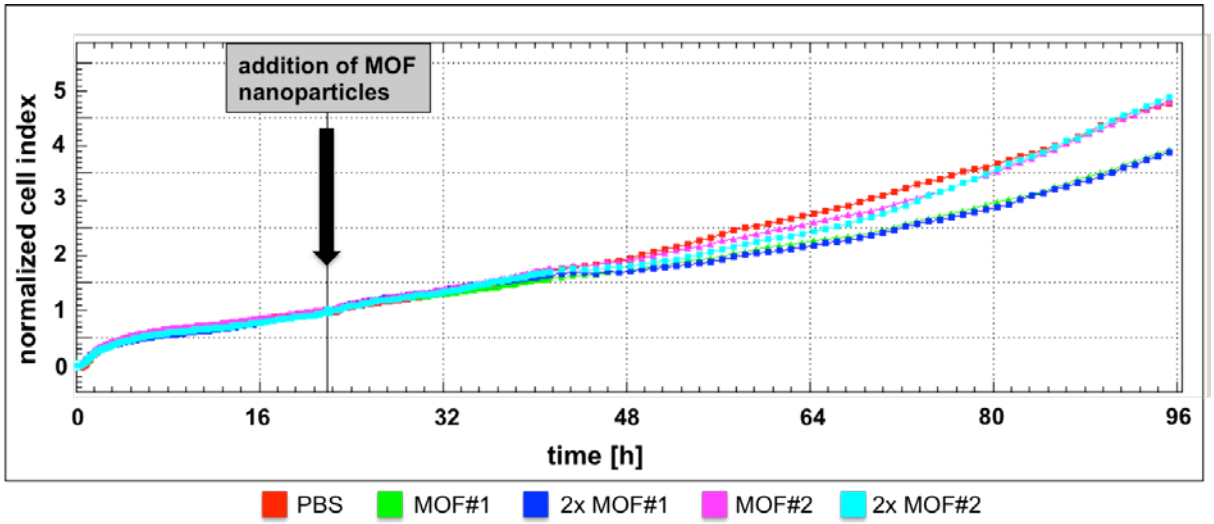

Fig. S-22 Impedance measurements of cell cultures. Bladder carcinoma cells were seeded on xCELLigence E-plates and treated at indicated time points with different charges (MOF#1 and MOF#2) and amounts of 6,4 µl and 12,8 µl of MIL-100(Fe)@DOPC nanoparticles (c = 1 mg/ml) per 200 µl medium. Similar cell index values indicate that cells incubated with MOF nanoparticles show a behaviour similar to PBS-treated control cells.

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