Exosome-coated Metal-Organic Framework Nanoparticles: An Efficient Drug Delivery Platform

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Methods and Characterization

Dynamic Light Scattering (DLS): DLS was performed on a *Zetasizer Nano Series* (Nano-ZS, *Malvern*) equipped with a laser with the wavelength λ = 633 nm. For sample preparation the freshly prepared nanoparticles (NPs) were dispersed in ethanol or phosphate-buffered saline (PBS) in the case of the capped NPs.

Scanning Electron Microscopy (SEM): All SEM micrographs were recorded with a *Helios NanoLab G3UC* (*FEI*) operating at 5 kV. During sample preparation an ethanolic NP dispersion was dried on a carbon film placed on an aluminum sample holder. The sample was stored overnight to evaporate the solvent followed by carbon sputtering prior to the measurement. For evaluation of the SEM micrographs the software *ImageJ v1.49.* was used.

Transmission Electron Microscopy (TEM): The TEM micrographs of the sample particles were taken on a *Titan Themis* (*Fei*) that was operated at an acceleration voltage of 300 kV. For sample preparation, an ethanolic NP solution was dried overnight on a carbon-coated copper grid.

Thermogravimetric Analysis (TGA): A dried sample of MIL-88A NPs (3.125 mg) was heated using a *TASC 414/4* (*Netzsch*). The sample was heated under synthetic air at 10 °C/min up to 900 °C. The resulting data was evaluated using the software *Proteus v4.3*.

Nitrogen sorption: Nitrogen sorption experiments were conducted with an *Autosorb-1* (*Quantachrome*). Prior to the measurement the sample (27.9 mg) was outgassed under high vacuum at 120 °C for 38 h. The resulting data was evaluated with the software *ASiQwin v3.0*. The linearized form of the BET equation was used to calculate BET surface areas. For the calculation of the pore size distribution a QSDFT equilibrium based model was used assuming slit and cylindrical pores.

X-Ray Diffraction: X-ray diffraction experiments were performed on the initial MIL-88A NPs. The samples were measured on a *STOE Transmissions-Diffraktometer System STADI P* operating in transmission mode. The setup is using $CuK_{\alpha 1}$-radiation with a wavelength λ = 015418 nm. The resulting diffraction pattern was evaluated using the software package *WinXPOW RawDat v3.0.2.5* and *WinXPOW PowDat_n v3.0.2.7.*

Fluorescence Microscopy: The fluorescence microscope images were recorded with a *Zeiss Observer SD* spinning disk confocal microscope using a Yokogawa CSU-X1 spinning disc unit and an oil objective with 63x magnification and BP 525/50 and LP 690/50 filters. The setup was heated to 37 °C and a CO_2 source was provided to keep the atmosphere at 5% CO_2. For both excitation of the calcein and the cell marker a laser with a wavelength λ = 488 nm was used. The images were processed with the *Zen* software by *Zeiss* to optimize contrast and provide the orthogonal views.

Fluorescence Spectroscopy: The fluorescence spectroscopy experiments were recorded with a MD-5020 setup from *PTI Photon Technology International*. The software *Felix32* was used for recording and evaluating the measured data.

UV/Vis Measurements: The UV/Vis measurements were performed on a Lambda 1050 UV/Vis/NIR spectrometer form *Perkin Elmer*. The software used to record the measured spectra was *Perkin Elmer UVWinLab*.

High-Content Quantification: The release quantification measurements were performed with a *ImageXpress Micro XLS* from *Molecular Devices* using an objective with 10x magnification with a GFP filter and the resulting images were evaluated with the *MetaXpress* software.

Cell culture: All cell experiments were prepared in a *Hera-Safe* cell culture unit from *Heraus*. The cells were incubated in *Hera Cell incubators* also from *Heraus*.

MTT Assays: The MTT-Assays were performed with a *Spectra Fluor Plus* from *Tecan* and were then evaluated with *Excel 2010*

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Synthesis of the uncoated and coated MIL-88A nanoparticles

Synthesis of MIL-88A NPs

MIL-88A NPs were synthesized in a microwave assisted approach based on the results of Chalati et al.[1] In this synthesis route an aqueous solution of $FeCl_3 \cdot 6\ H_2O$ (1.084 g, 4.01 mmol) and fumaric acid (485 mg, 4.18 mmol) are given to water (20 ml, Milli-Q). The reaction mixture was stirred until the metal salt was completely dissolved. The reaction mixture was then given into a Teflon tube (80 ml) and placed into a microwave oven (*Synthos 3000*, *Anton-Paar*) along with 3 additional vessels. Two of these vessels are filled with water (20 ml), the third vessel is filled with an aqueous $FeCl_3$ (20 ml, 1.084 g, 4.01 mmol) and is used to monitor the reaction progress. The vessels were heated under stirring with the sequence shown in Table S1:

Table S1: MW Heating program for the MIL-88(A) NPs synthesis

Heating	Dwelling	Cooling
30 s	5 min	45 min
To 80 °C	80 °C	To RT

To remove residual reactants the sample was subsequently washed via centrifugation (7840 rpm, 20 min) and redispersion of the pellet in ethanol (20 ml). This washing cycle was repeated 3 additional times. To remove also bulk material formed during the reaction, the dispersion was then centrifuged 3 times (3 min, 3000 rpm) and the pellet fraction of the product discarded.

Preparation of the exosome coating solution

Approximately 100000 HeLa cells were transferred to a 75 cm culture flask and incubated in 10 mL fetal bovine serum (FBS)-free Dulbecco's modified Eagle Medium (DMEM) for three days. The exosomes were then extracted from the medium using the *Exospin* kit from *Cell Guidance Systems* following the protocol from the kit.

Preparation of the loaded and coated particles

1 mg of MIL-88A NPs were solved in 1 mL of a 1 mM solution of calcein or suberohydroxamic acid (SBHA) and incubated overnight for loading. Next they were centrifuged for 5 min at 14000 rpm, to discard the supernatant and the pellet was dissolved in 0.2 mL of the exosome coating solution and 0.2 mL water and incubated for 2 h. The particles were then centrifuged (5 min at 14000 rpm) and redispersed in 1 mL PBS after washing several times.

Characterization of MIL-88A NPs

Scanning Electron microscopy

An overview picture of the MIL-88A NPs used in this work is presented in Figure S1.

The particles look uniform and feature a roundish morphology. For further characterization, the particles marked in the yellow box were used to determine a particle size distribution of the sample.[2]

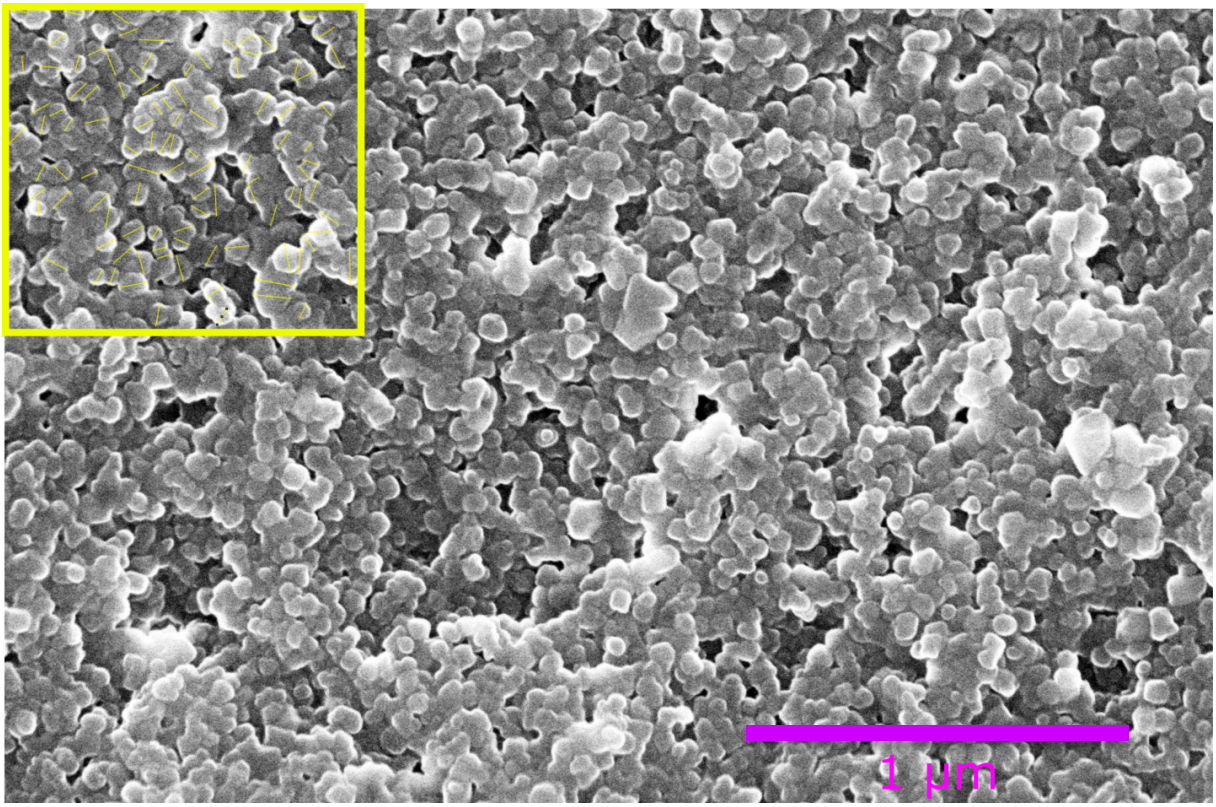

Figure S1: Scanning Electron micrograph of MIL-88A nanoparticles

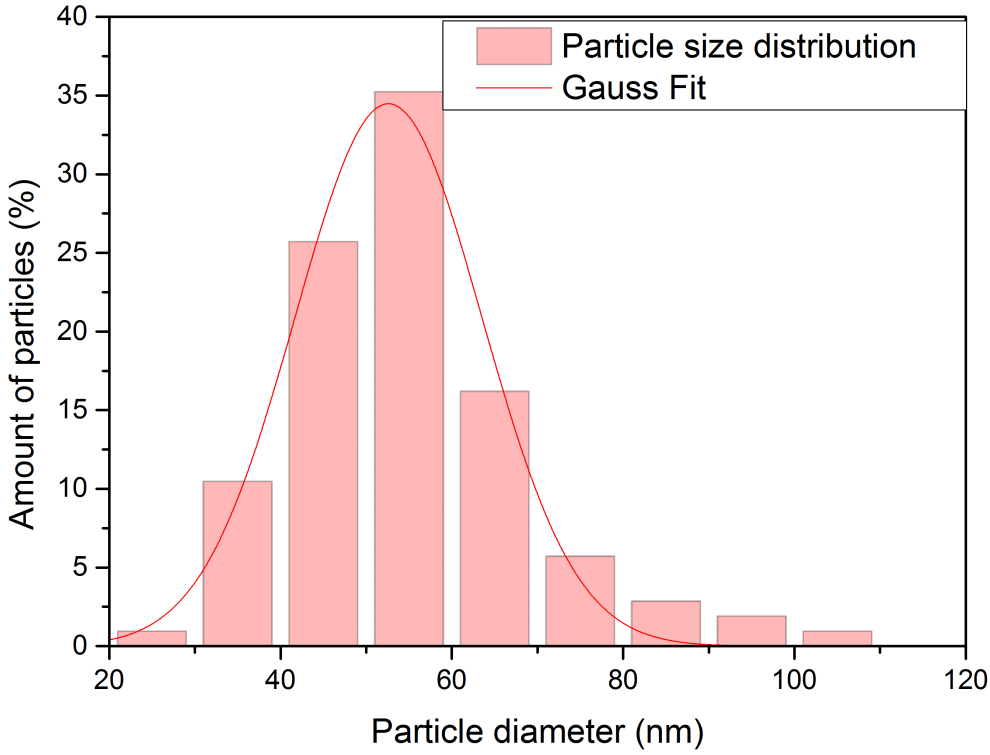

Figure S2: Particle size distribution determined from the SEM micrograph shown in Figure S1 (yellow box).

The particle size distribution was determined by manually measuring the diameter of ~ 150 particles (Figure S1 yellow box) and fitting the data with a Gaussian function using the software Origin v9.0.0.[2] This results in an average particle diameter of 52 nm with a standard deviation of 11 nm.

Transmission Electron microscopy

Figure S3 and S4 depict TEM micrographs of the MIL-88A NP sample. The particles feature a round morphology and are connected via thin necks. The MIL-88A sample is fairly homogenous, which is also shown in a particle size distribution given in Figure S5.

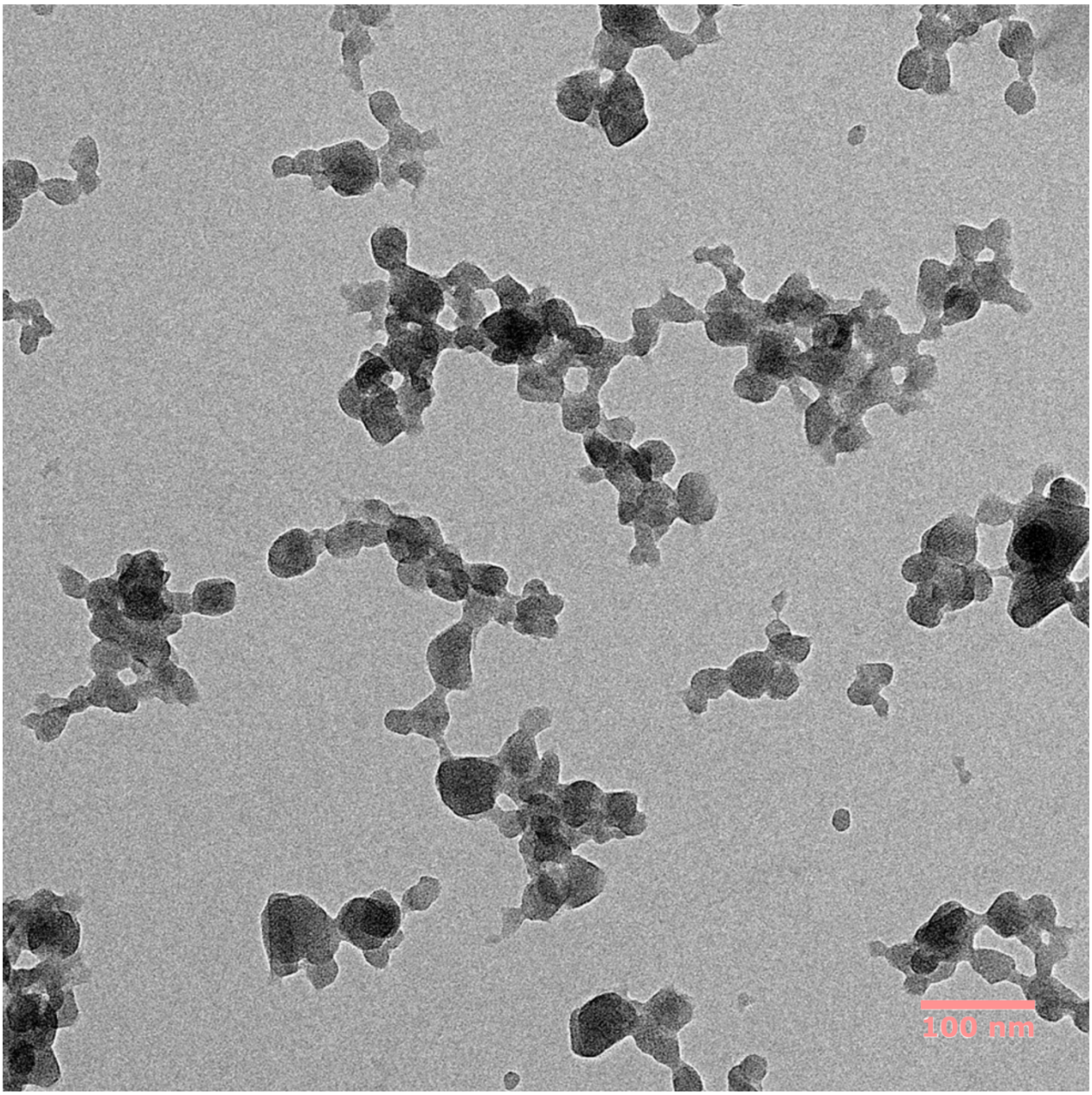

Figure S3: Transmission electron micrograph of MIL-88A NPs – detailed image.

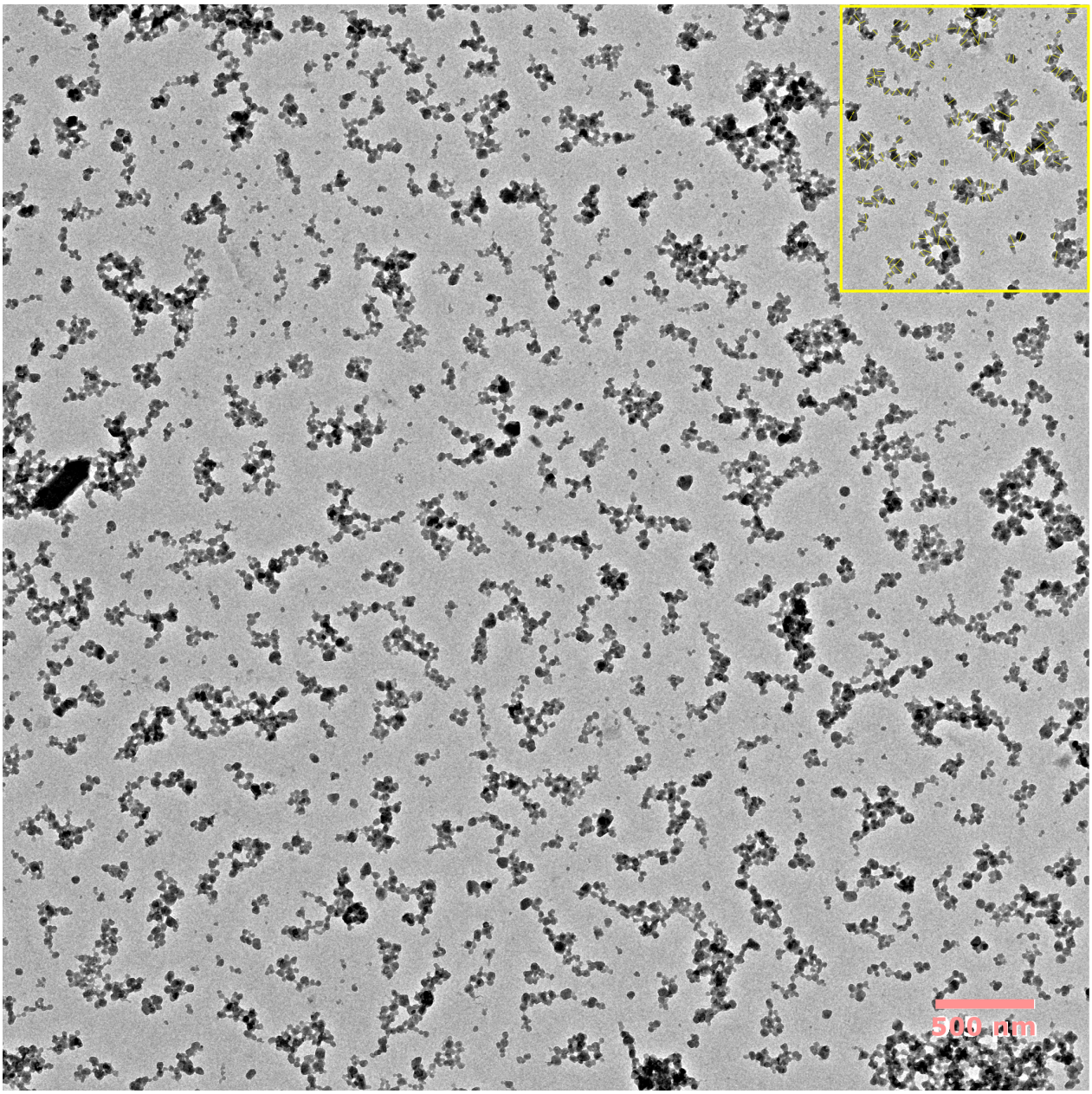

Figure S4: Transmission electron micrograph of MIL-88A NPs.

The particle size distribution was determined by manually measuring the diameter of ~ 200 particles and fitting the data with a Gaussian function using the software Origin v9.0.0.[2] The average diameter was determined at 36 nm with a standard deviation of 9 nm.

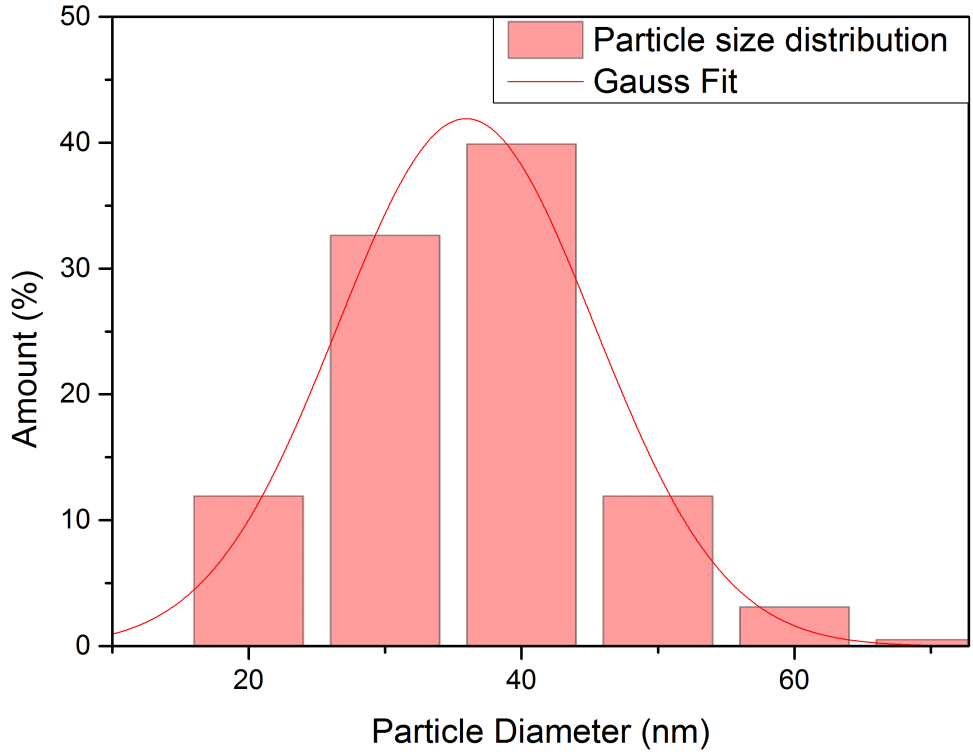

Figure S5: Particle Size distribution determined from the TEM micrograph shown in Figure S4 (yellow box).

This diameter differs from the results shown in the SEM measurements. This behavior however is not unusual in size determination of MOFs with SEM and TEM. Beam damage of a sample is a known problem in TEM mostly with high-energy electron beams (E > 100 keV).[2]

Dynamic Light Scattering

The results of the DLS measurements of the unfunctionalized MIL-88A NPs are shown in Figure S6. In water, the particles have an intensity based hydrodynamic average diameter of 99 nm with a polydispersity index (PDI) of 0.131, which translates to a good monodisperse particle size distribution.

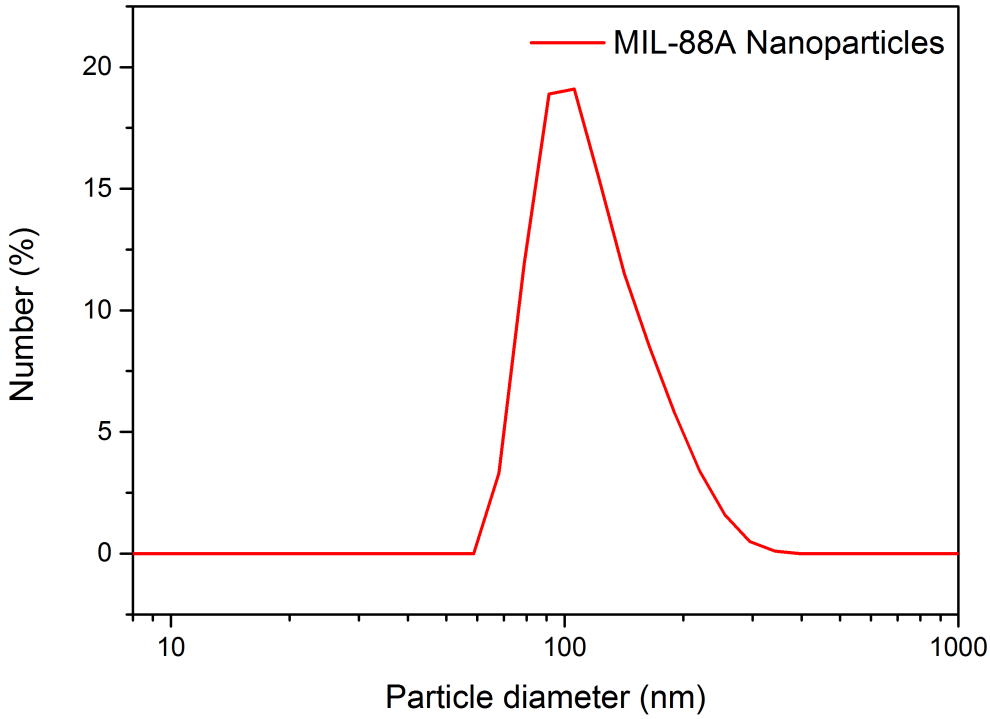

Figure S6: DLS size distribution of MIL-88A NPs showing the percentage of particles for each size.

DLS measurements of the exosome coated MIL-88A NPs can be seen in Figure S7. In PBS the exosome coated particles possess an average diameter of 101 nm (Figure S7). The measured PDI is 0.143 meaning that sample possesses a good monodispersity.

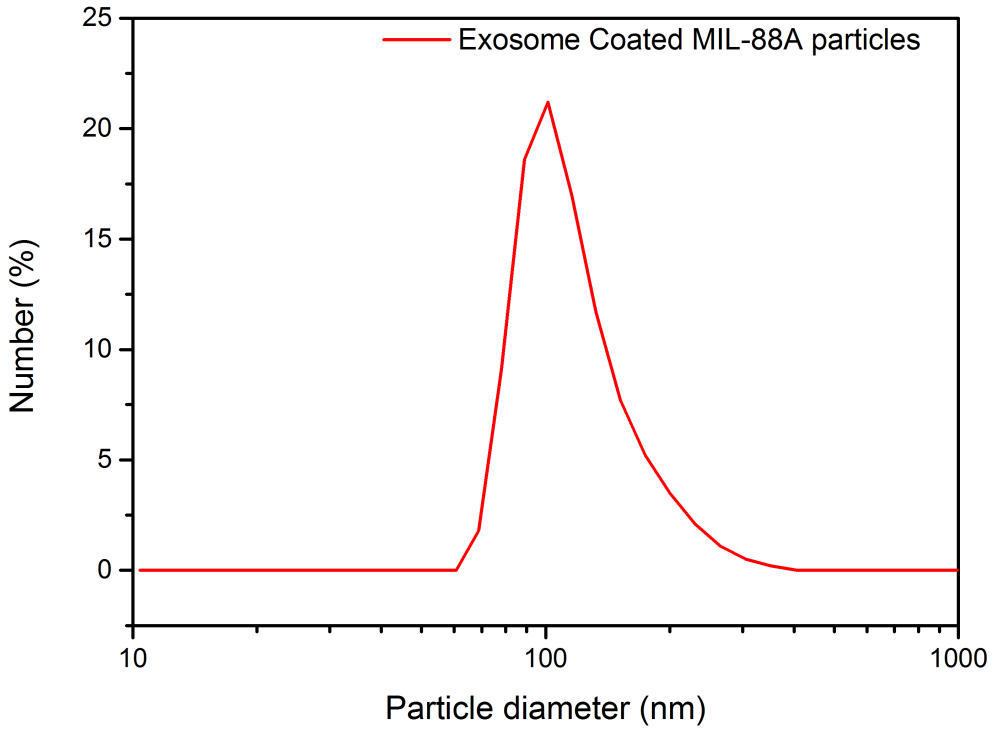

Figure S7: DLS size distribution of exosome coated MIL-88A NPs showing the percentage of particles for each size.

DLS measurements of the exosomes can be seen in Figure S8. In PBS the exosomes possess an average diameter of 100 nm. The measured PDI is 0.157 meaning that sample possesses a good monodispersity.

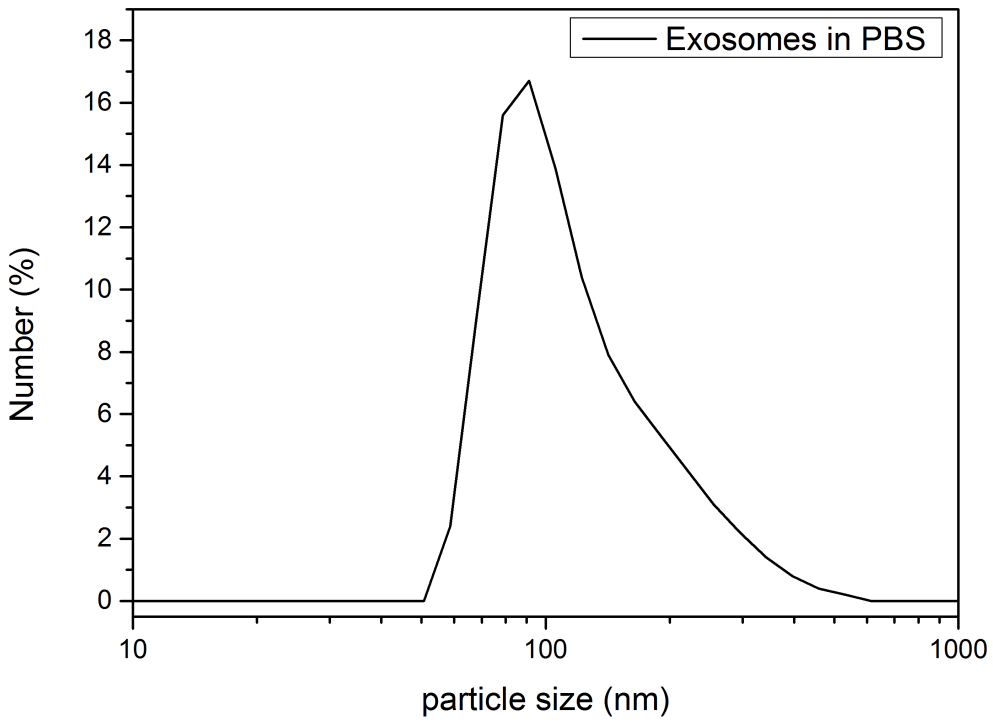

Figure S8: DLS size distribution of exosomes recovered with the Exospin kit showing the percentage of exosomes and extracellular vesicles for each size.

X-Ray Diffraction

Figure S9 shows the X-ray diffraction pattern of the initial MIL-88A NPs. The peak at 2θ = 10° is fairly weak suggesting an overall rather amorphous nature of the particles and less pronounced crystallinity than in other MOF structures. The diffractogram however is in good agreement with the data reported in literature for MIL-88A NPs.[1, 3]

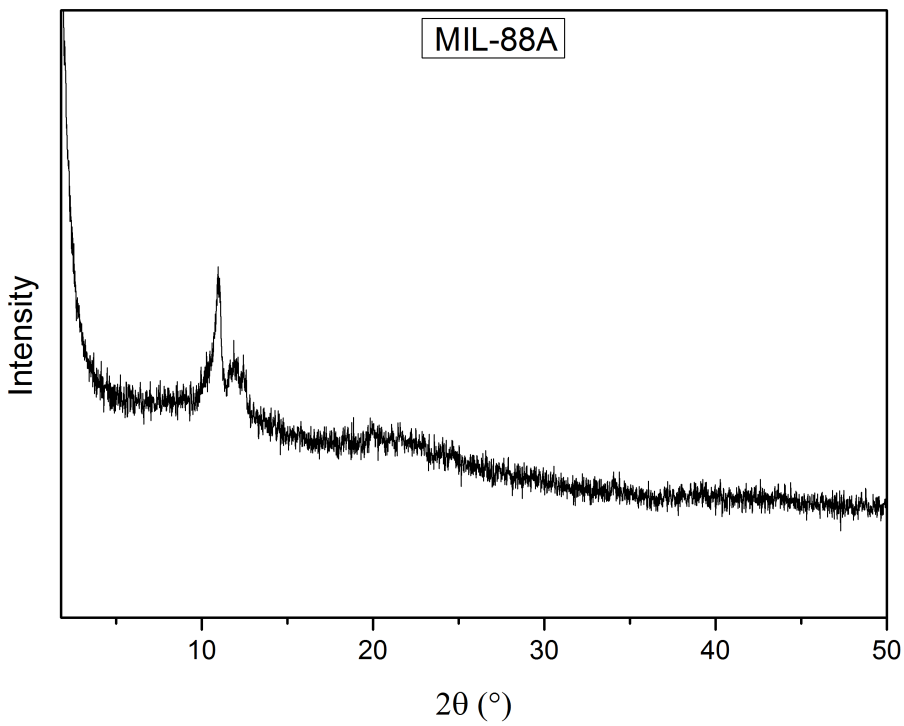

Figure S9: X-ray diffraction patter of dried MIL-88A NPs.

Thermogravimetric Analysis

The results of the thermogravimetric analysis of the initial MIL-88A NPs are shown in Figure S10. Up to 204 °C the residual solvent in the sample is desorbed resulting in a mass loss of 6 %. Subsequently, in a range between 204 – 433 °C the framework decomposes. The residual mass left of the sample remains at 42%. This data is in agreement with literature data for MIL-88A NPs and shows the successful synthesis of the MOF.[3]

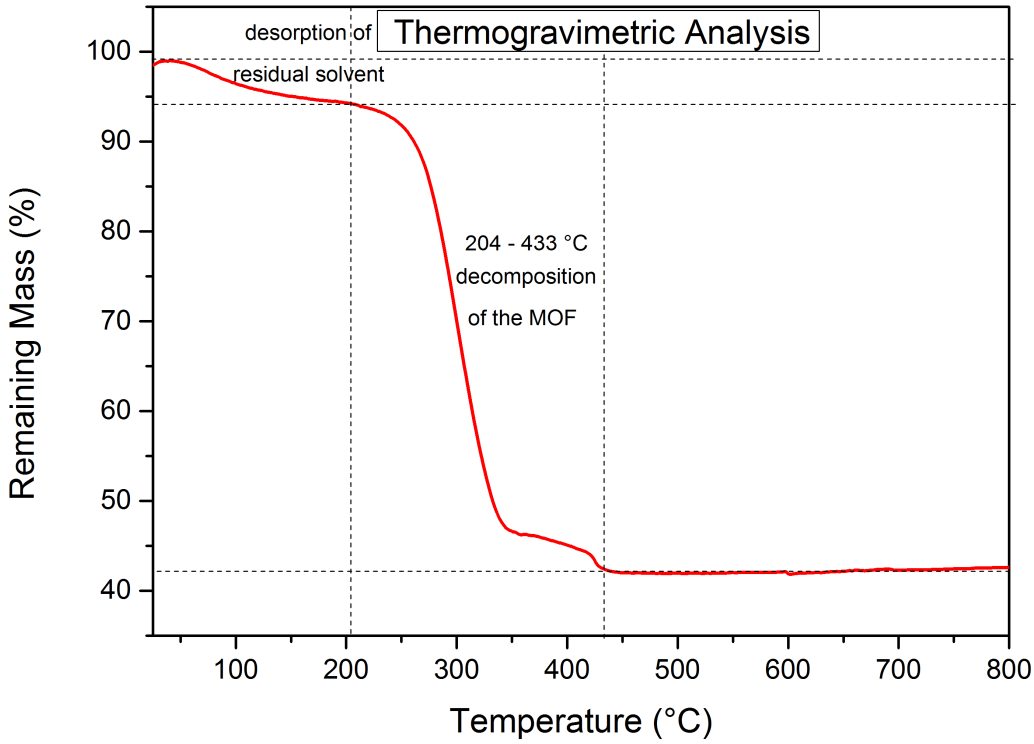

Figure S10: Thermogravimetric analysis of MIL-88A NPs.

Nitrogen Sorption

The results of the nitrogen sorption experiments are shown in Figure S11 which depicts the sorption isotherm and the corresponding pore size distribution. The results are summarized in Table S2 as well. The nitrogen uptake is in good agreement with reported data of MIL-88A NPs.[4]

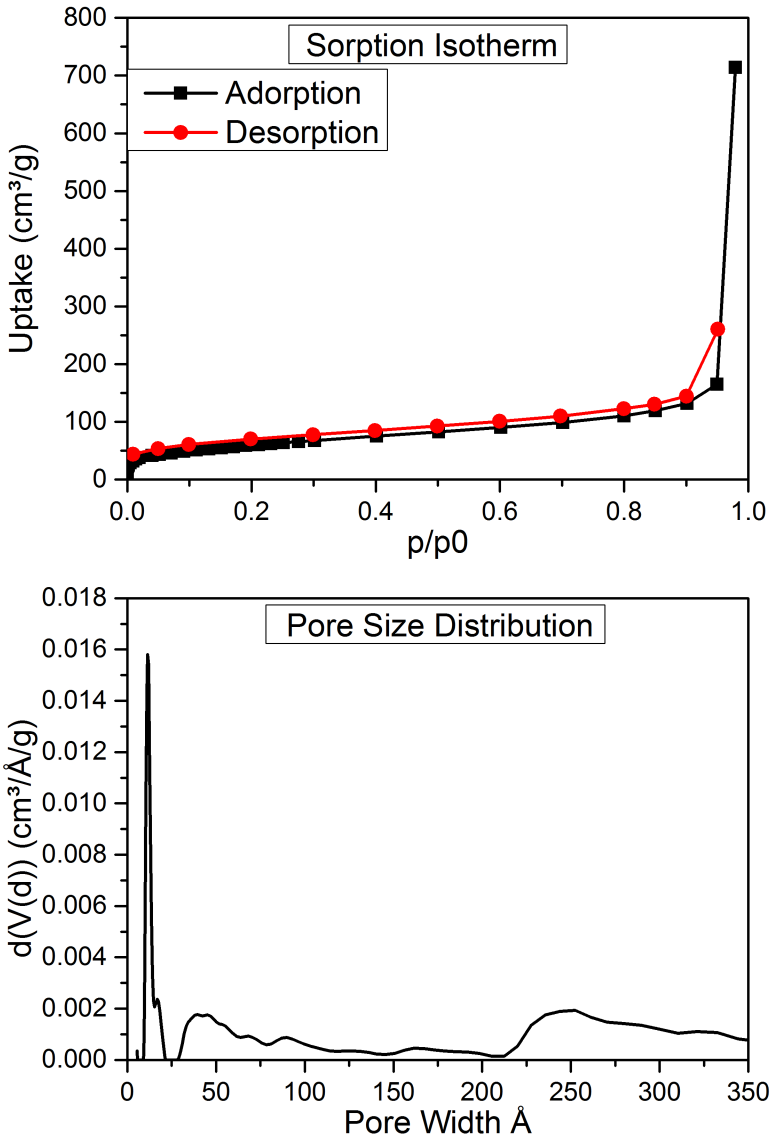

Figure S11: Nitrogen sorption isotherm and pore size distribution.

Table S2: Results of the BET analysis

Sample	**MIL-88A**
BET-surface area [m²/g]	218 m²/g
Relative pressure range used for calculation	0.11-0.23
Correlation coefficient	0.999
C-constant	Positive
Pore Size	11.44 Å

Stability Measurements

To investigate the stability of the particles in body relevant media, 500 µg of the coated and uncoated nanoparticles were dispersed in 1 mL ALF[5]. After a day of incubation the solutions were measured again. The solutions were clear and no particles could be recovered via centrifugation (Figure S12). The original particles seem to have been completely disassembled in the ALF media as it can be observed in the XRD and the SEM image (Figure S13, Figure S14).

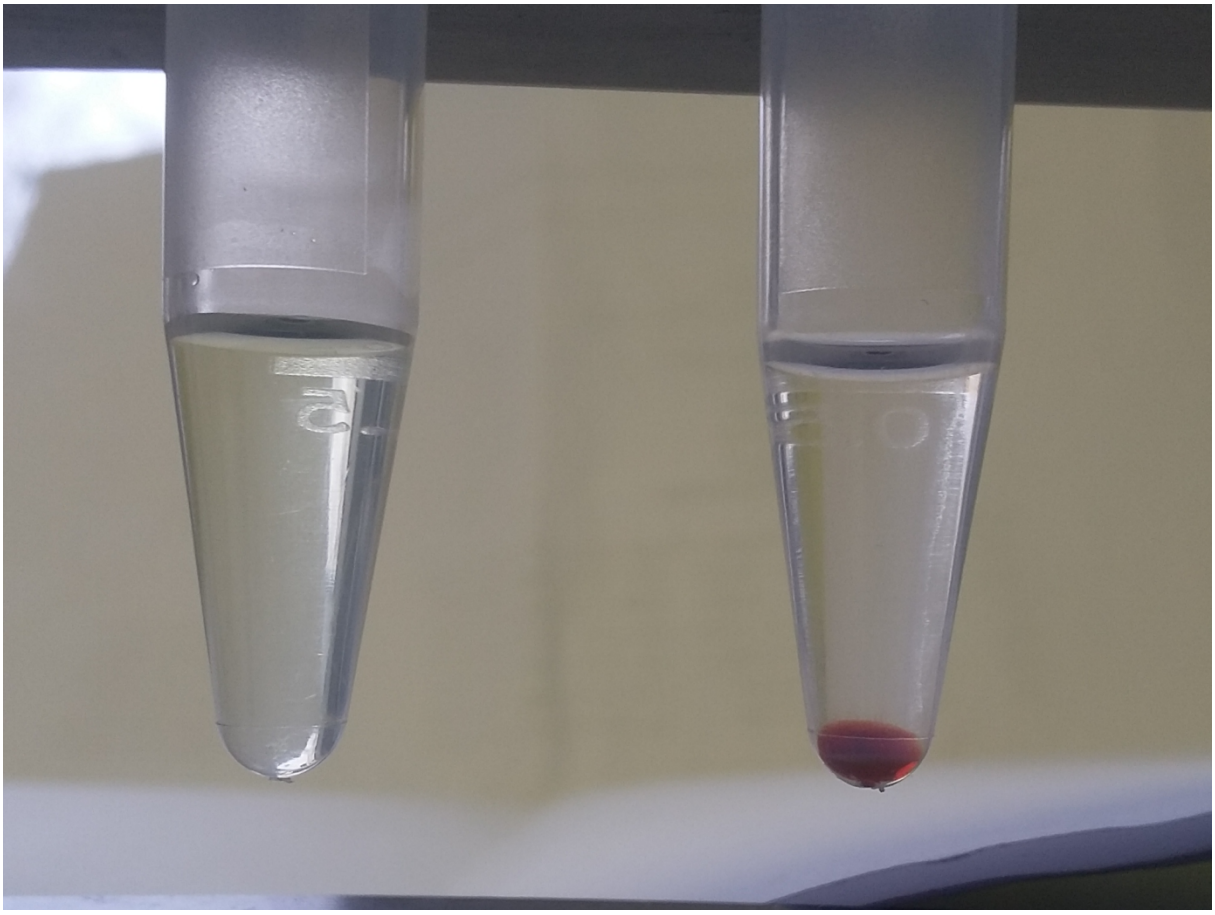

Figure S12: MIL-88A NPs before (right) and after (left) treatment with ALF. The particles in the untreated sample were recovered via centrifugation (14000 rpm, 5 min), while no particles could be recovered in the treated sample.

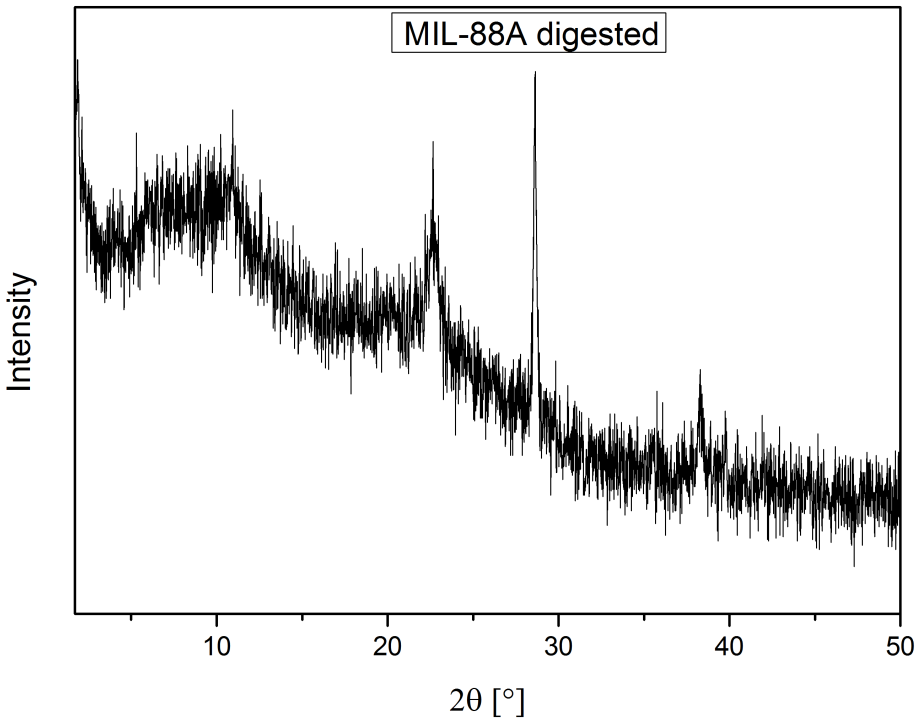

Figure S13: XRD of the residue after treatment of the MIL-88A nanoparticles with ALF, showing that the characteristic reflexes of MIL-88A at about 10° are no longer visible.

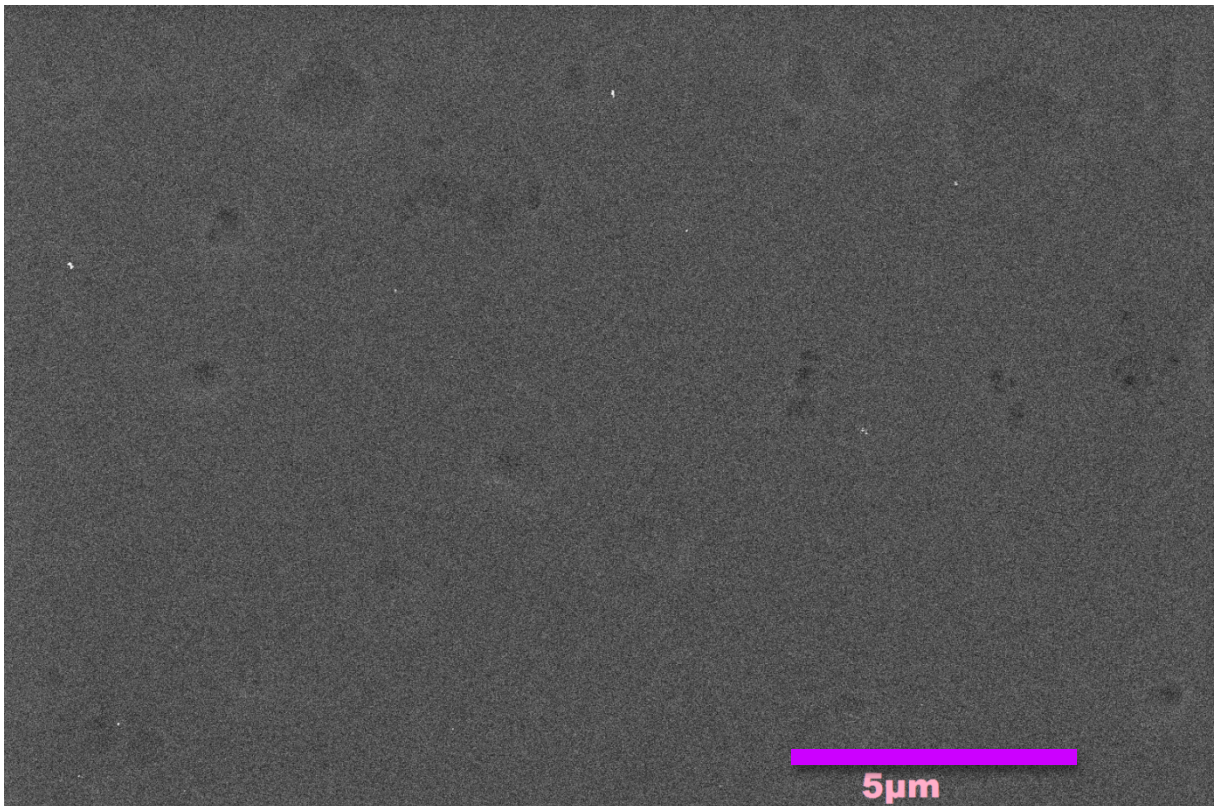

Figure S14: SEM images of the residue left after treating uncoated MIL-88A NPs with ALF, showing no discernible crystallites.

In addition to the ALF measurements the same procedures were carried out with an aqueous 10% FBS solution, showing no particle degradation after incubation overnight (Figure S15).

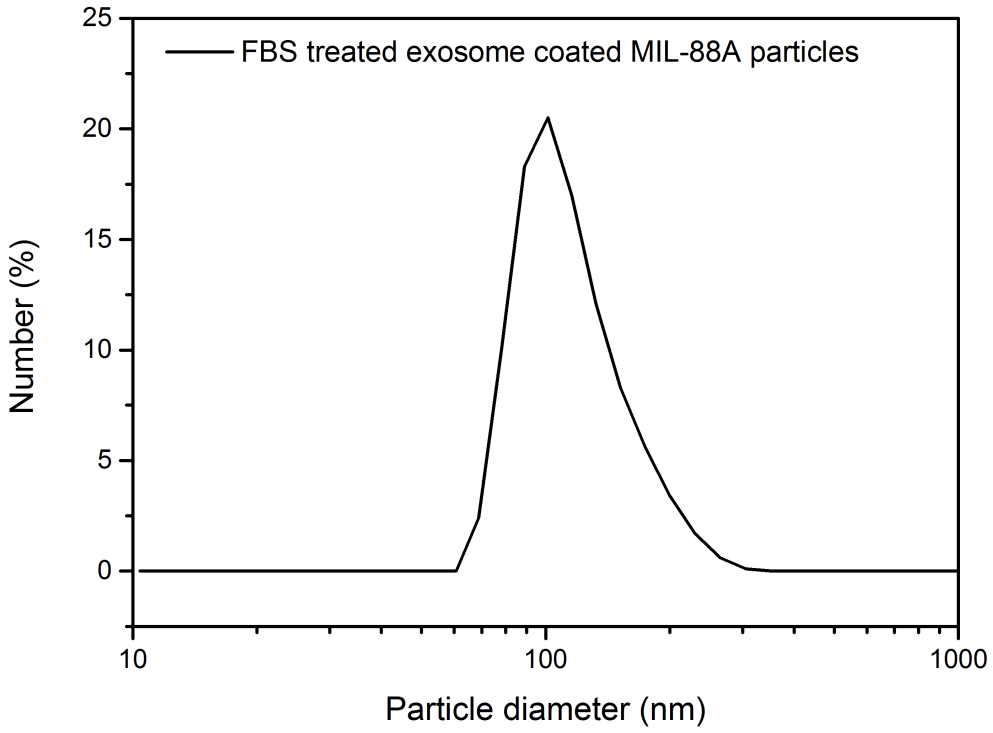

Figure S15: DLS size distribution of exosome coated MIL-88A NPs showing the percentage of particles for each size after overnight treatment with FBS.

Visualizing the Exosome coating

As proof of a successful exosome coating of the MOF nanoparticles we performed fluorescence colocalization experiments. We labeled the MOF nanoparticles (Figure S16, a) with Fluo-3, which stains the iron contained in the particles, and the exosomes with CellMaskOrange (Figure S16, b). Successful coating should result in a colocalization of both dyes, while they should be independently distributed in the case of failed coating. The merged image (Figure S16, c) of both channels shows that we indeed obtain colocalization of both dyes suggesting a successful coating. The slight offset of both channels can be explained by the time it took to switch between the different excitation wavelengths needed for the two different dyes: the particles slightly moved due to the Brownian motion preventing a complete overlap of both images in the merged channel. Careful control experiments excluded spectral overlap of the dyes and confirm that the colocalization of the signals is indeed due to successful coating.

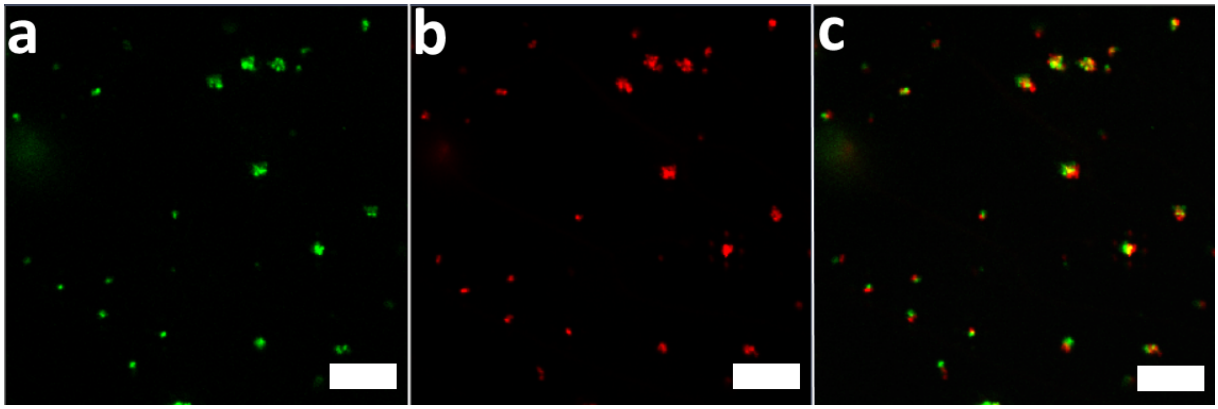

Figure S16: Fluorescence Colocalization Images of the exosome coated MIL-88A nanoparticles. The particles were marked with Fluo-3 (green, a) an iron marker, while the exosomes were marked with CellMask Orange (red, b). c shows the merged image of both channels.

Cell Release Experiments

For the cell release experiments 5000 HeLa cells were seeded in each well of ibidi 8-well plates and after one day 5, 10, 15 or 20 µg of the MIL-88A particles were added to each well. Incubation times of two, three and four days were investigated and can be seen in Figure S17, Figure S18 and Figure S19, respectively. The cells were marked with CellMask Orange, by adding 1 µL masking agent, incubating for 5 minutes at 37 °C/5 % CO_2 and washing three times with DMEM to remove the remaining masking agent. The microscope images were taken with a Zeiss spinning disk microscope with an oil objective with 63x magnification. In addition to normal images Z-Stacks were also recorded, allowing for an orthogonal view of the cells, proving that the particles have entered the cell after 2 days of incubation.

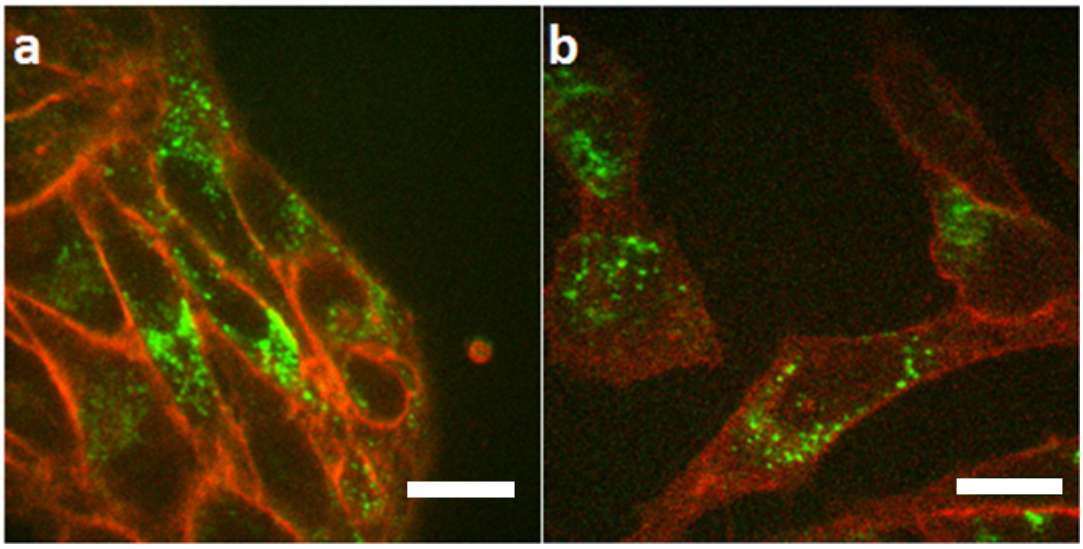

Figure S17: a, b) HeLa cells with exosome coated MIL-88A particles after two days of incubation. The scalebars (white) represent 20 µm.

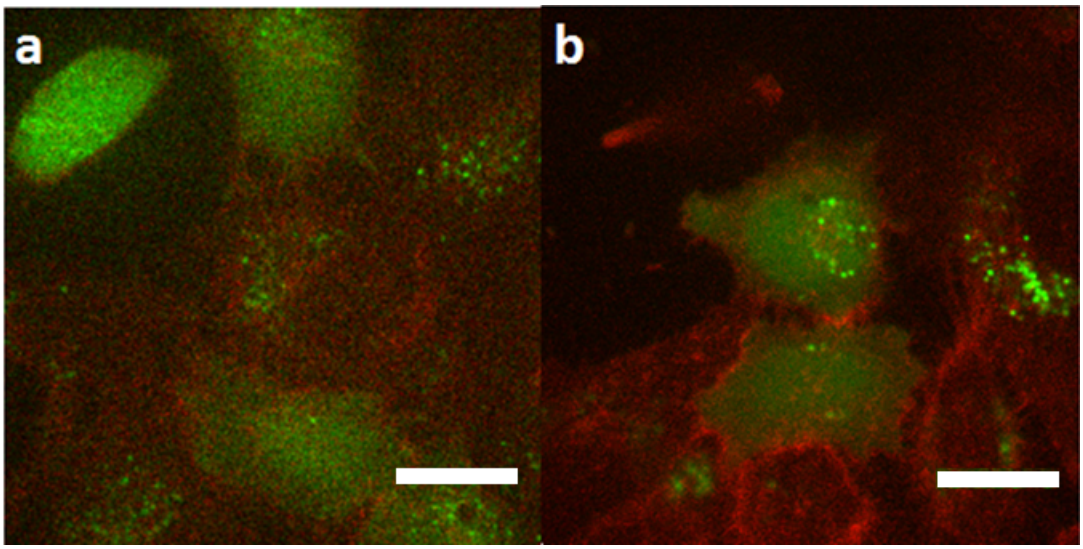

Figure S18: a, b) HeLa cells with exosome coated MIL-88A particles after three days of incubation. The scalebars (white) represent 20 µm.

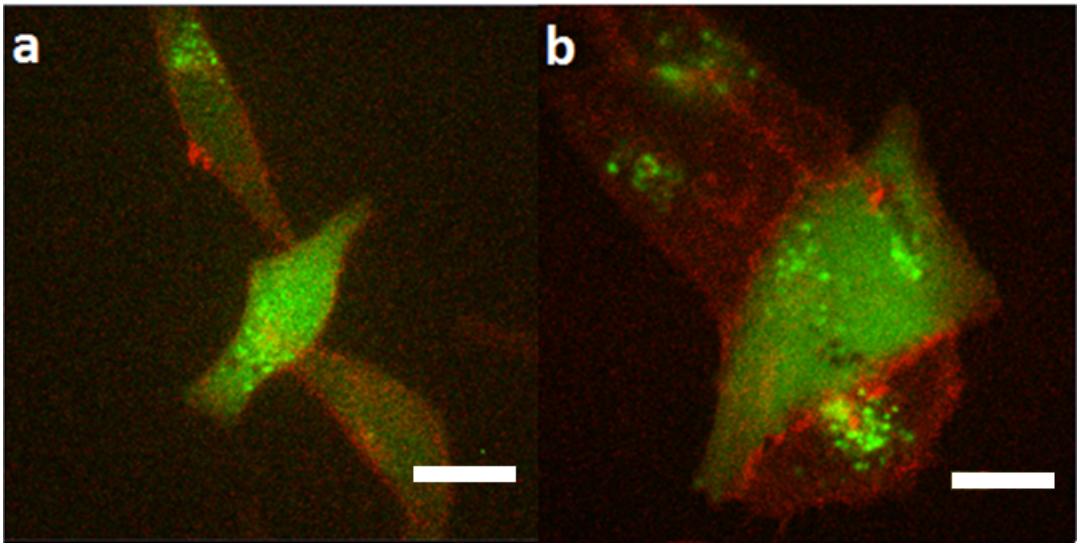

Figure S19: a, b) HeLa cells with exosome coated MIL-88A particles after four days of incubation. The scalebar (white) represents 20 µm.

Release Experiments

The release experiments were performed on a PTI fluorescence spectrometer. Hollow caps were filled with 50 µL of a 1 mg/mL particle stock solution for exosome coated and calcein loaded particles. Depending on the experiment either 150 µL water, 150 µL ALF or 90 µL water and 10 µL Triton 100-X were added. The caps were then sealed with a dialysis membrane and placed into cuvettes filled with either water or ALF and a stirring rod. The cuvettes were heated to 37 °C to simulate the cell environment. Each experiment lasted 14 h with one measurement per second. The excitation wavelength was 495 nm and the measured emission wavelength was 512 nm.

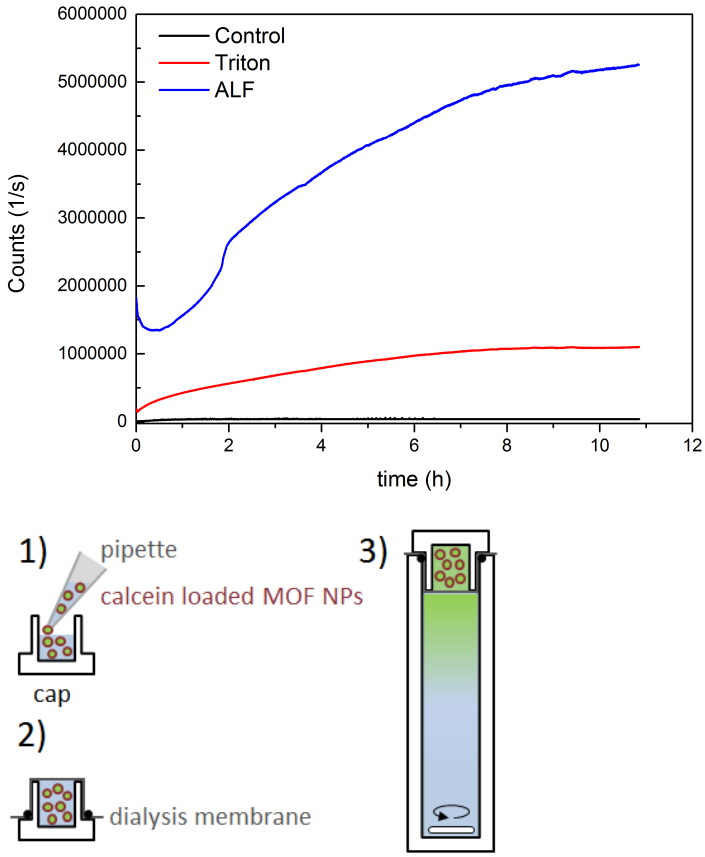

Figure S20: Fluorescence experiment of the exosome coated MIL-88A NPs showing that they are stable and do not release in water (black). When dissolved in ALF (blue) or Triton X-100 (red) containing media release from the particles can be observed that reaches saturation after about 10 hours. At the bottom a schematic of the measurement can be seen. 1) The cap is filled with loaded NPs. 2) The cap is sealed with a dialysis membrane. 3) The released calcein permeates through the membrane, while the particles stay in the cap.

To account for quenching 10 mL of a 1 mM solution of calcein were diluted with 990 mL PBS. Next 10 mL of this new solution were added to 4 mL water or ALF in cuvettes. In addition 10 mL triton X-100 was added to one sample. Then the fluorescence spectra of these solutions were measured to find a normalization factor between them (Figure S21). This factor was then used to calculate the offset for the individual fluorescence measurements. The normalization factor determined by comparing the measurements for water and triton X-100 to those for ALF is 2.17 and was implemented in the fluorescence experiments.

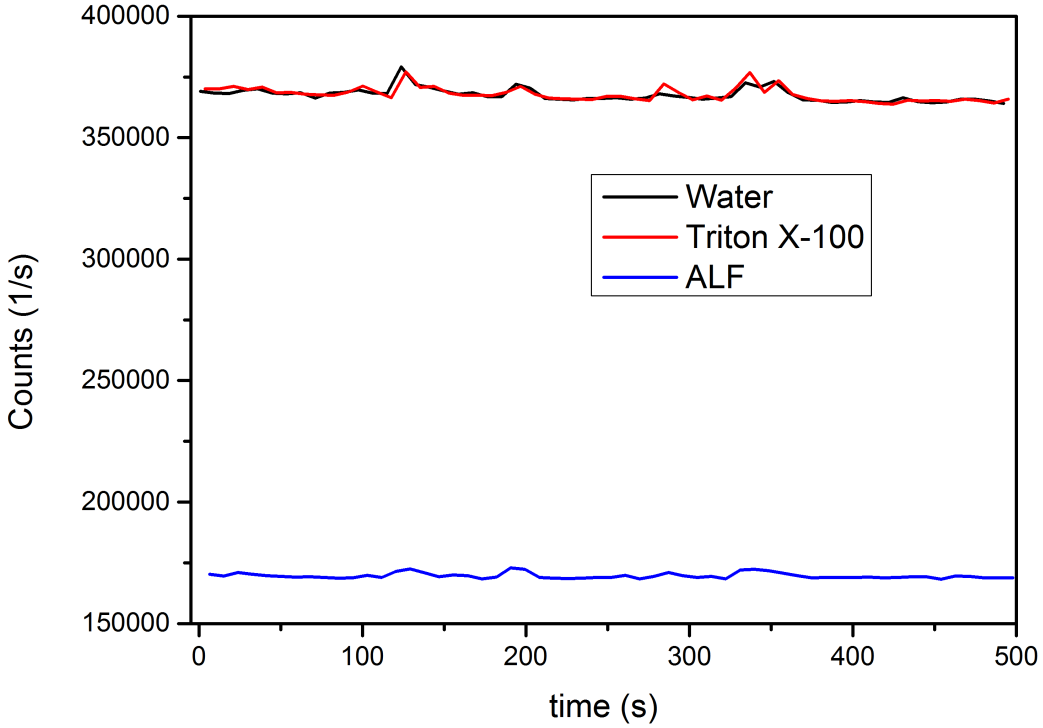

Figure S21: Fluorescence measurement to determine the quenching of the different media used in the fluorescence measurements.

UV/Vis measurements

To evaluate the loading capacity of the coated MIL-88A NPs, UV/Vis spectra of a 1 mM calcein solution, the remaining supernatant of the calcein solution after the particles were loaded, a 1 mg/mL solution of loaded particles dissolved by ALF, and a 1 mg/mL solution of coated, loaded particles were measured (Figure S22). In addition a solution containing the calculated amount of calcein loaded in the MOF was also measured. For each measurement 100 mL of the sample were diluted with 3 mL water.

The calcein loading capacity was calculated from the difference in absorbance of the measured solutions. The calcein stock solution used for loading the particles had a concentration of 1mM, i.e. 0.622 mg/mL. As 1 mL of the stock solution was used for loading, 1 mg of the particles have taken up 0.158 mg calcein or 15.8 wt%, using the ALF measurement as a basis. A quarter of the calcein was taken up by the MIL-88A particles and released. As the particles dissolve in ALF all of the loaded particles should be released. The difference in absorbance between the loaded particles and the particles dissolved in ALF can be attributed to quenching of the fluorescence as long as the calcein still resides in the MOF.

The spectrum of calcein in ALF and the sample containing the calculated amount of calcein match very well. This shows that the remains of the disintegrated MOF do not affect the UV/Vis measurements and this measurement can thus be used for determining the amount of encapsulated and released calcein.

In previous works, in which drugs like Doxorubicin and Paclitaxel were loaded into exosomes, loading efficiencies of 7.2 to 11.7% were reached.[6-8] These values are comparable but lower than those of the exosome coated MIL-88A NPs presented in this work which could reach loading capacities of up to 15.8 wt%.

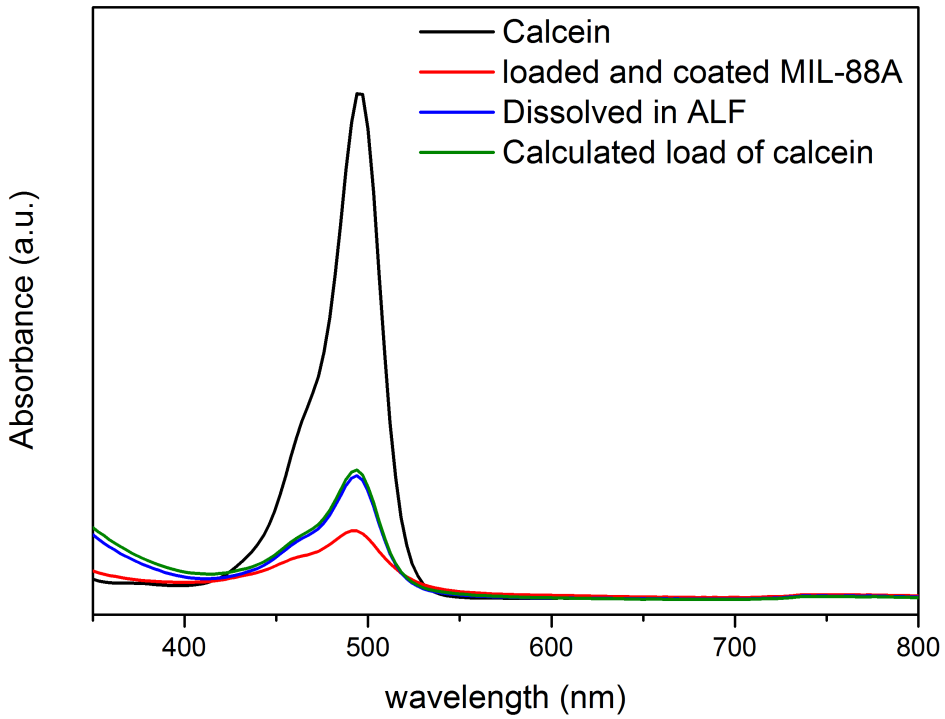

Figure S22: UV/Vis measurements of several different calcein and particle solutions

HDAC1 Assay to determine the SBHA loading

Due to SBHA being hardly detectable with standard analytical techniques, an HDAC1 activity assay was performed. SBHA is an inhibitor of HDAC1. The assay kit used was the HDAC1 Inhibitor Screening Assay Kit from Cayman Chemical. For the quantification different dilutions of SBHA were prepared and tested on their inhibition of HDAC1 and then compared to the inhibition of dissolved SBHA loaded particles to determine how much SBHA was loaded into the particles (Figure 4).

From that we calculated the theoretical dilution at 50% inhibition for SBHA (1:42.2) and the SBHA loaded MIL-88A NPs (1:5.40). From these dilutions and the concentrations of the stock solutions we could calculate how much SBHA (4.835 µg) and SBHA loaded particles (185 µg) were needed to inhibit 50% of the available HDAC1. By comparing these numbers we can then calculate the loading capacity: 13.1 % of the initially available 1 mM SBHA or 2.61 wt% in relation to the weight of the MOF NPs were loaded into the particles.

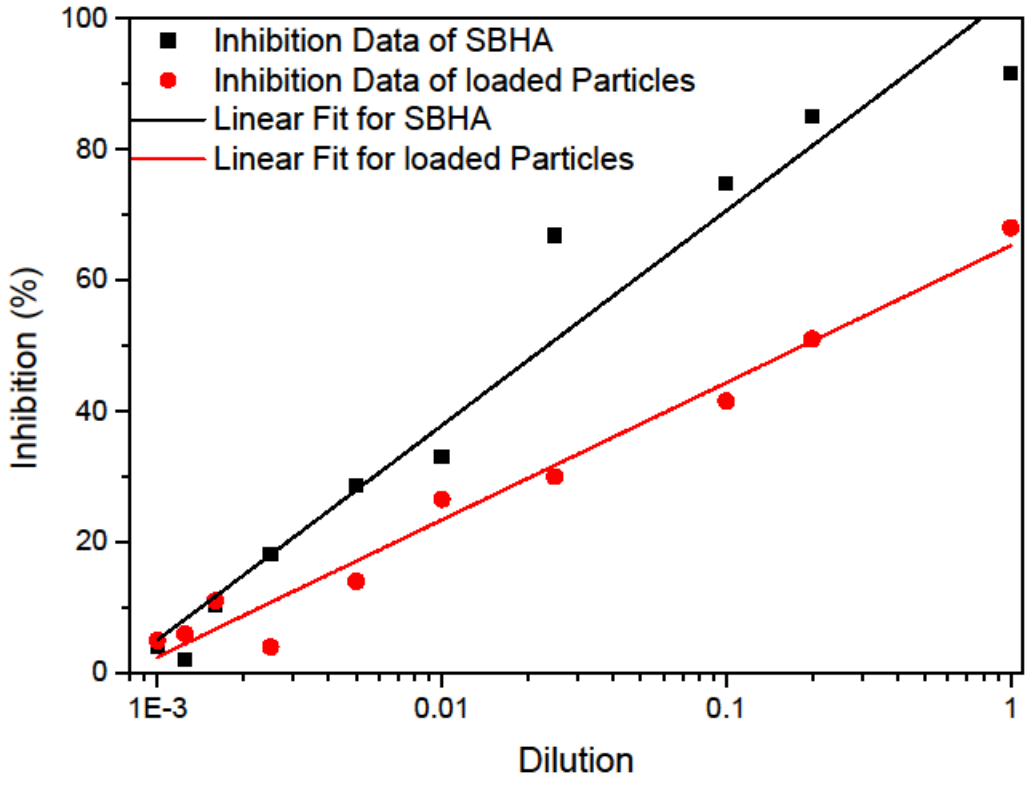

Figure S23: Graphical representation of the results of the HDAC1 Inhibition Assay.

High-Content Quantification Experiments

96-well plates from Costar were seeded with 5000 HeLa cells per well and a volume of 100 µL DMEM medium. After incubating for 1 day at 37 °C/5 % CO_2 the particles were added. Each concentration (0, 10, 20, …, 140, 150 µg/mL) was tested in three different wells. Before the measurement the plate was washed twice with 100 µL per well PBS to remove dead cells and excess calcein. Both transmission and GFP channel images were recorded. Four images were recorded per well. The images were evaluated with the MetaXpress software, by counting the number of cells showing release and the total number of cells and calculating the release percentage from these numbers.

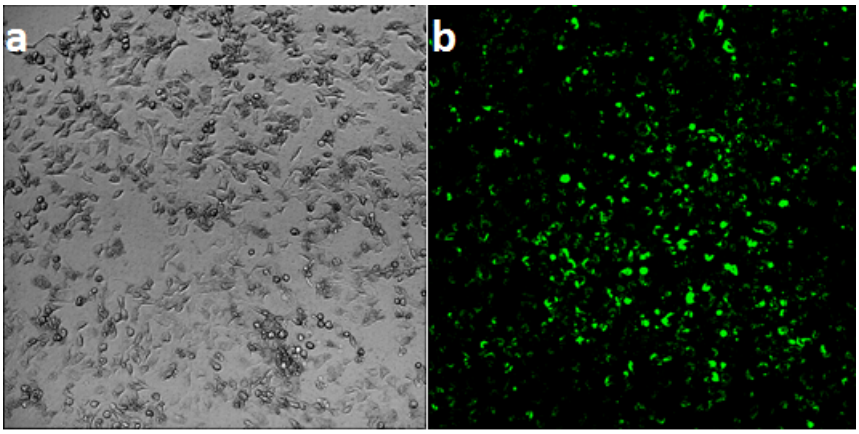

Figure S24: Sample images as used for the quantification measurements: a) Transmission image used to count the total number of cells; b) Fluorescence image used to count the number of cells showing release.

MTT-assays

5000 cells were seeded in each well of a 96-well plate and covered with 100 µL DMEM. After 1 day of incubation at 37 °C/5 % CO_2, the particles were added (0, 2, 4, 8, 20, 60, 100, 140 µg/mL). Each concentration was tested in triplicate. The supernatant was recovered via centrifugation (14000 rpm, 5 min) from a 1mg/mL loaded exosome coated MIL-88A NP stock solution after one day of incubation in PBS. The MTT-assays were performed after 3 and 4 days of incubation.

The MTT reagent concentration used was 0.5 mg reagent per 1 mL medium. The sample was washed three times with 100 µL HBSS buffer per well before 100 µL of the reagent solution was added in each well. After two hours of incubation at 37 °C/5 % CO_2 the wells were emptied and the whole plate was frozen at -80 °C for an hour. After defrosting 100 µL DMSO was added to each well and after dissolving any remaining crystals the MTT-assays were performed. MTT-assays were performed in triplicate and repeated on at least three different days.

In addition to the coated variants uncoated Mil-88A NPs were also investigated (Figure S25). They showed low toxicity with cell viabilities of over 70 %.

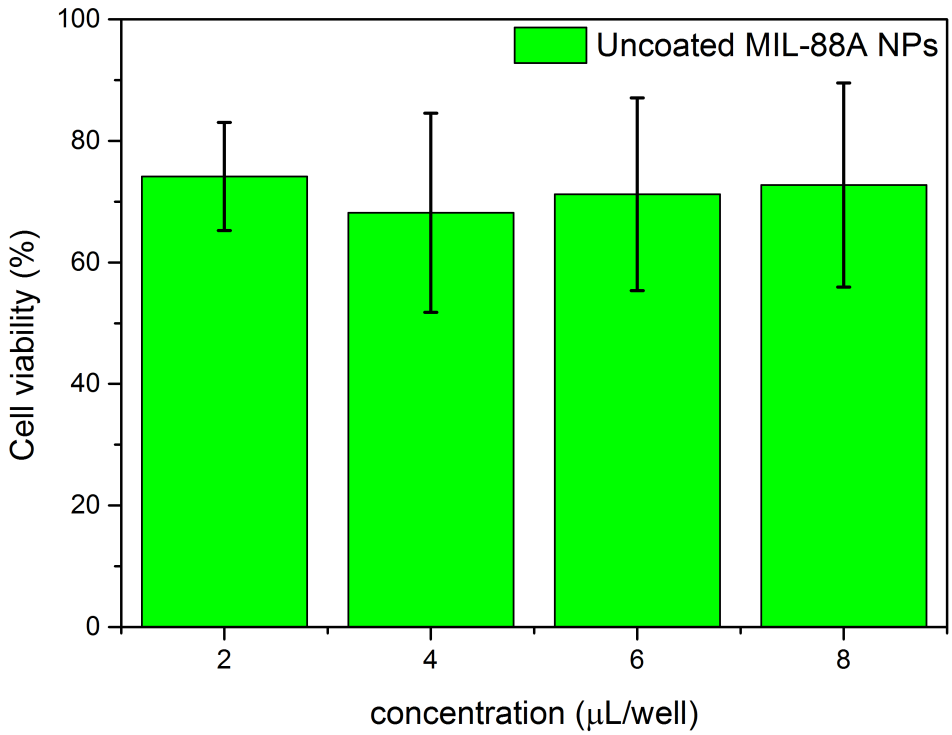

Figure S25: MTT Assay of HeLa cells after 3 days of incubation with uncoated MIL-88A NPs. The error bars signify the SD.

To investigate the efficacy of pure SBHA, a 1 mM solution of SBHA in PBS was added to HeLa cells in increasing amounts (Figure S26). Lower concentrations, below 20 µL/mL, proved to be nontoxic, while higher concentrations lead to very low cell viability of about 5%. Comparing the effect of free

SBHA with that of transported SBHA shows that the transported SBHA possesses a higher efficacy and leads to more cell deaths. 1 µL of SBHA in PBS corresponds to 0.204 mg of pure SBHA.

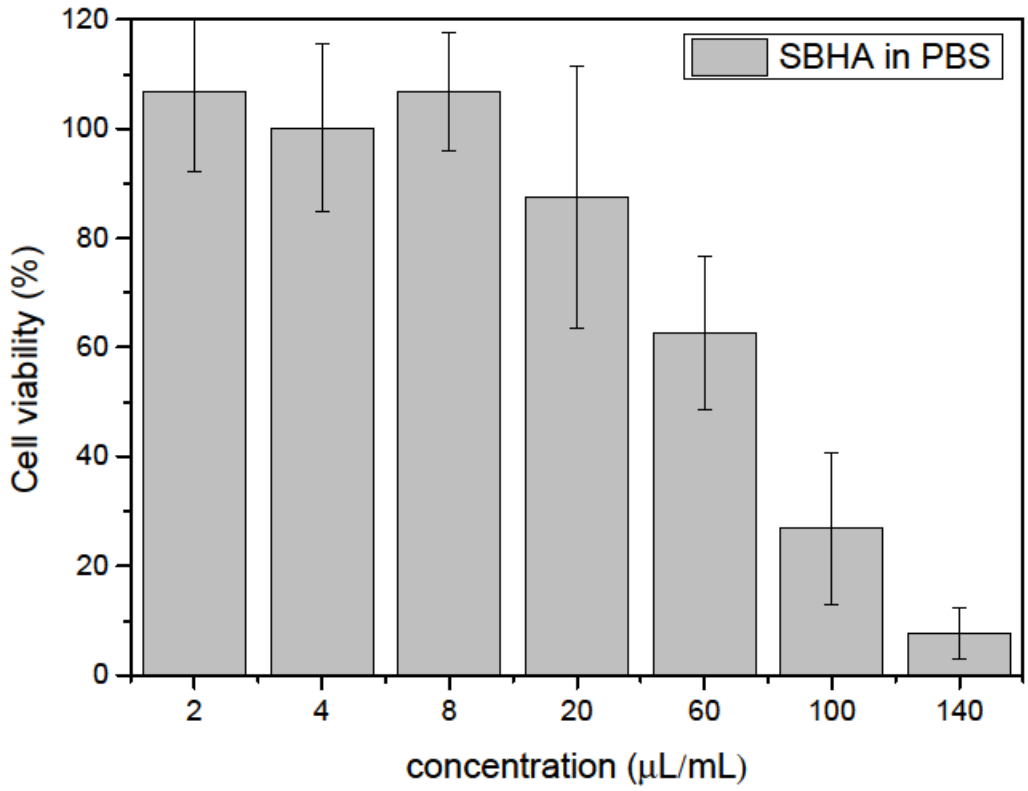

Figure S26: MTT Assay of HeLa cells after three days of incubation with a 1 mM solution of SBHA in PBS. The error bars signify the SD.

Cell targeting

Beyond potential inherent targeting through proteins naturally inserted into the exosomes, our exosome coated MOF NPs offer the option to additionally functionalize the exosome shell as has been shown earlier with liposomal coatings around NPs.[9-10] This can be done via spontaneous insertion of lipids functionalized with a targeting moiety such as folate. Folate receptors are known to be overexpressed on cancer cells and thus are a common receptor used for targeting. To show this optional targeting via inserted ligands, we added such folate functionalized lipids into the exosomal coating of the MOF NPs and performed targeting experiments

The MIL-88A NPs were loaded and coated as described previously, and then incubated overnight with 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(6-((folate)amino)hexanoyl) to replace some of the lipids in the exosome coating.[9] The modified particles are not taken up in media saturated with folate, but are efficiently taken up in HeLa cells incubated with folate-free DMEM (Figure S27). This shows their successful targeting.

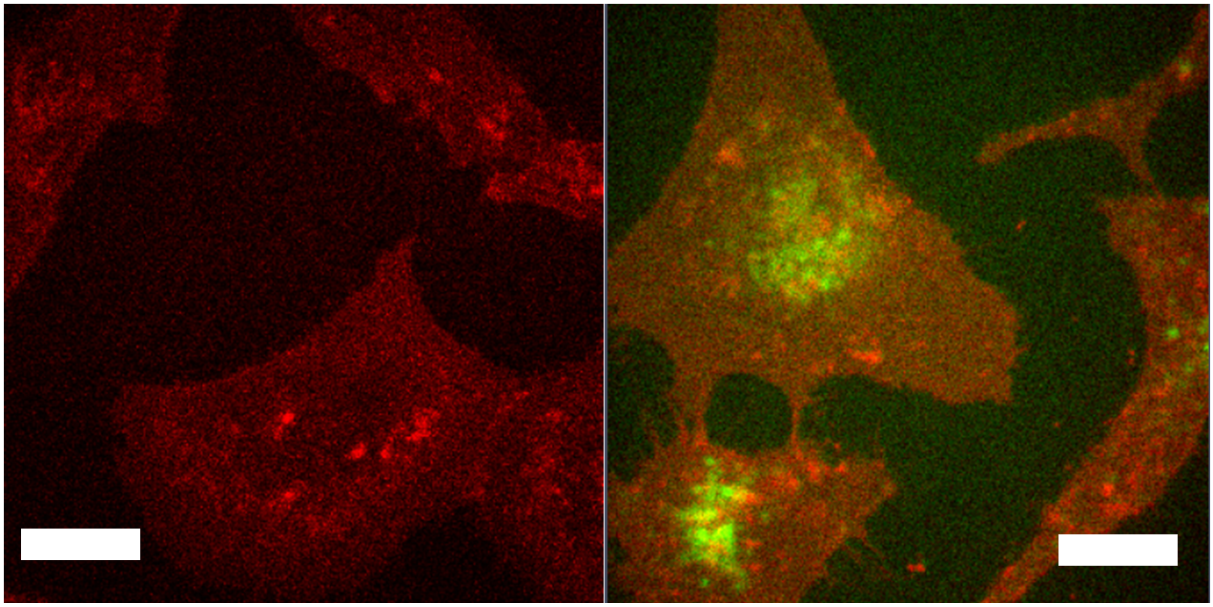

Figure S27: No uptake of folate modified exosome coated MIL particles in HeLa cells incubated in folate rich media (left). Uptake of folate modified exosome coated MIL particles in HeLa cells in normal folate free DMEM. The scale bars in both images represent 15 µm.

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