Electronic Supporting Information for:

Amorphous metal-organic frameworks for drug delivery

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S1. Instruments

All PXRD data were collected in Bragg-Brentano geometry on a D8 Bruker diffractometer equipped with a primary Ge monochromator for Cu Kα1 and a Sol-X solid state detector. Collection conditions were: 2-70° in 2θ, 0.02° step size, 15 seconds/step, divergence slits 0.2 mm, receiving slit 0.2 mm. Samples for SEM were scattered onto spectroscopically-pure carbon tabs (TAAB Ltd UK) mounted on aluminium stubs. They were coated with 15 nm of gold in a Quorum Emitech K575X sputter coater to make them electrically conductive. They were imaged in an FEI XL30 FEGSEM, operated at 5 keV, using an Everhart Thornley secondary electron detector. Thermogravimetric analysis (TGA) was performed using a TA Instruments Q-500 series thermal gravimetric analyser, with the sample (0.7 - 5 mg) held on a platinum pan under a continuous flow of dry N_2 gas. TGA curves were obtained using a heating rate of 5 °C/min and up to 600 °C. N_2 adsorption isotherms were undertaken at 77 K using a Micromeritics TriStar instrument.

S2. Synthesis and Characterization

UiO-66 was obtained following the procedure described by Katz *et al.*[1] 0.125 g of $ZrCl_4$ was dissolved in 5 ml of DMF and 1 ml of HCl (37 %), while 0.123 g of terephthalic acid (BDC) was dissolved in 10 ml of DMF. The two solutions were mixed in a 25 ml teflon lined autoclave and heated at 80 °C for 16 hours. The resulting solid was collected by centrifugation at 5500 rpm for 10 minutes and then washed with DMF and ethanol three times. The white product was then dried at 90 °C in a vacuum oven in order to remove the solvents.

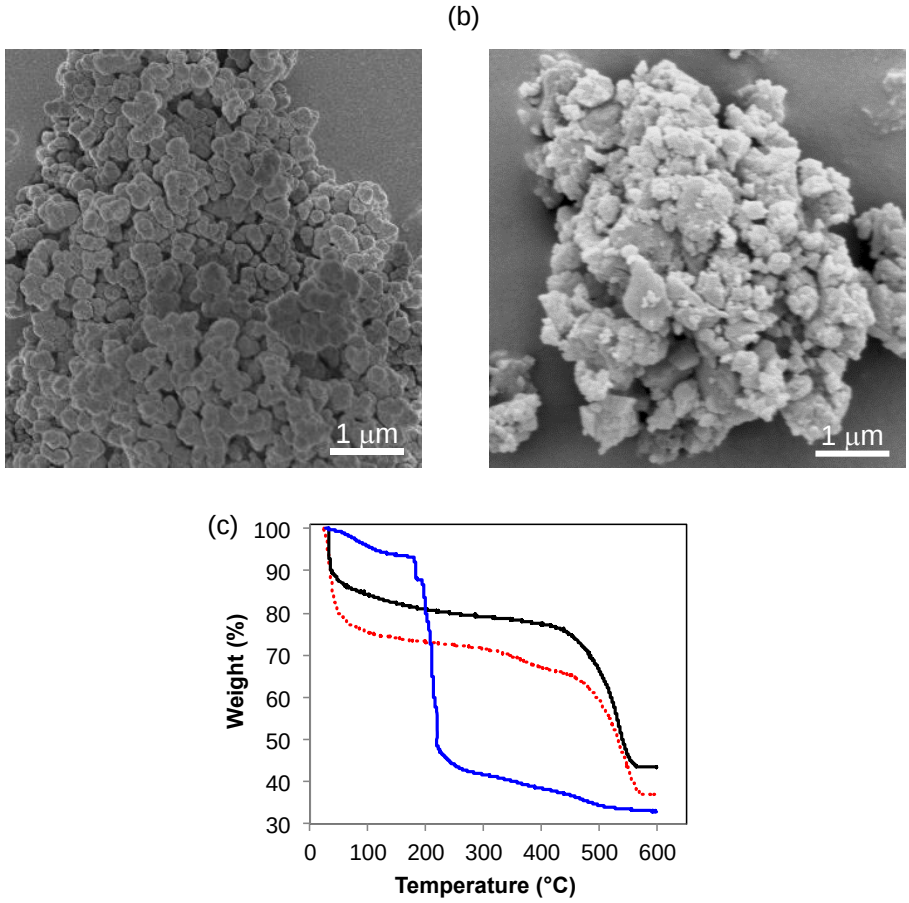

Figure S1. a) SEM images of UiO-66 (bar scale = 1 μm); **b)** SEM images of aUiO-66 (bar scale = 1 μm); **c)** TGA curves under dry N_2 gas of UiO-66, black solid line; cal@UiO-66, red dotted line; and pure calcein, blue solid line.

We analyzed the porosity of UiO-66 by using N_2 adsorption at 77 K (Figure S2). Prior to the analysis, 100 mg of the sample were evacuated for 24 h at 150 °C under vacuum. The BET area of synthesized UiO-66 is 1166 m^2/g, similar to the values previously reported in the literature.[2,3]

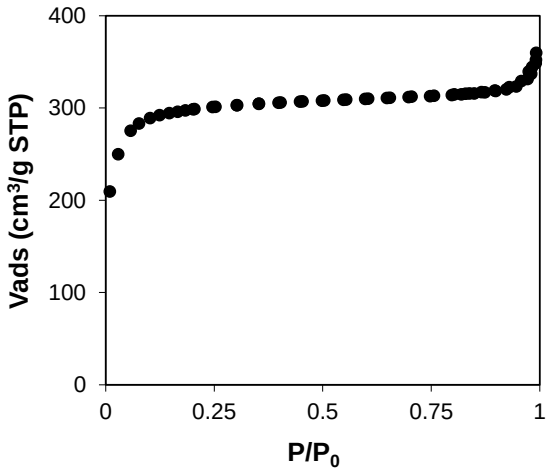

Figure S2. N_2 adsorption isotherms at 77 K on UiO-66.

S3. Drug Loading Experiments

The calcein adsorption was performed by soaking 20 mg of activated UiO66 into 5 mL of methanol calcein solution at 37°C under orbital agitation for 6 days. The loaded material was collected by centrifugation at 5500 rpm for 20 minutes, washed twice with methanol, centrifuged again for 10 minutes and dried overnight at 37°C to remove the solvent. The amount of drug adsorbed was quantified by using a UV-vis spectrophotometer at 498 nm measuring the amount of drug presents in the supernatant after the first centrifugation step. The loaded amount is given by the equation [1]:

$$\text{Loading (wt.\%)} = \frac{\text{calcein}_{\text{added}}\ (\text{mg})}{\text{calcein}_{\text{added}}\ (\text{mg}) + \text{material (mg)}} \qquad [1]$$

where $\text{calcein}_{\text{added}}$ is the amount of calcein at t=0, material is the amount of empty material added.

S4. MOF Amorphization

0.2 g of calcein loaded UiO-66 was placed in a stainless steel jar along with an 8 mm stainless steel ball. The jar was then oscillated at 20 Hz for 30 minutes using a Retsch MM200 mill resulting in amorphous loaded UiO-66 (*a*UiO-66).

S5. Delivery assays

Calcein release experiments were performed in an incubator at 37°C with orbital agitation and using phosphate buffered saline (PBS, 10mM) at pH 7.4 in order to simulate physiological conditions. 3 mg of drug-loaded particles were placed into a dialysis bag (MWCO 3500, molecular weight cut-off Da, Medicell International) with a total volume of 10 mL of PBS. At different times, 1 ml of PBS was taken and replaced with 1 ml of fresh PBS. The amount of drug released was measured by using a UV-vis spectrophotometer at 498 nm. The corrected concentration of calcein release is given by the equation [2]:

$$c_t = c'_t + \frac{v}{V}\sum_0^{t-1} c'_t \qquad [2]$$

where c_t is the corrected calcein concentration at time t, c'_t is the apparent calcein concentration, v is the sample taken and V is the total volume of the solution. Every experiment was performed by triplicate.

The kinetics of calcein delivery from crystalline and amorphous UiO-66 were adjusted using non-linear regressions in order to understand the release behaviour. For the crystalline material the delivery was adjusted to a simple hyperbola model [3]:

$$\text{N (wt. \%)} = \frac{N_{max}\, t}{(t_{1/2} + t)} \qquad [3]$$

where N is the amount released from the total drug-loaded amount in weight percent, N_{max} is the maximum amount released, t is time in days and $t_{1/2}$ is the time needed to get half of the maximum amount delivered. For cal@*a*UiO-66 it was not possible to adjust the delivery to a simple curve. In this case, we used a hyperbola model considering two different release stages [4]:

$$\text{N (wt. \%)} = \frac{N_{\max}(1)\, t}{(t_{1/2}(1) + t)} + \frac{N_{\max}(2)\, t}{(t_{1/2}(2) + t)} \qquad [4]$$

where N_{max} and $t_{1/2}$ are considered for two stages: *(1)* and *(2)*.

Figure S3 and Table S1 show the fitting of the experimental release and fitting parameters, respectively, for UiO-66 and *a*UiO-66. On one hand, the maximum calcein amount delivered by cal@UiO-66 is 97.07 wt% and half of that amount is reached at 1.86 h. On the other hand, the two-stage release pattern of *a*UiO-66 might be related to the existence of two different release phenomena. During the first one, release of calcein takes place presumably through desorption and diffusion along the amorphous pore texture of the material. About 58 % of the calcein is delivered this way, and half of this amount is reached at 14 h indicating the much slower diffusion compared with cal@UiO-66. The second release stage is a much slower process that might be associated to the partial dissolution of defects of the *a*UiO-66 to liberate the encapsulated calcein. Finally, as it was possible to detect the calcein delivered form the amorphous material it is possible to conclude that the ball-milling process is not provoking the degradation of the guest molecules.

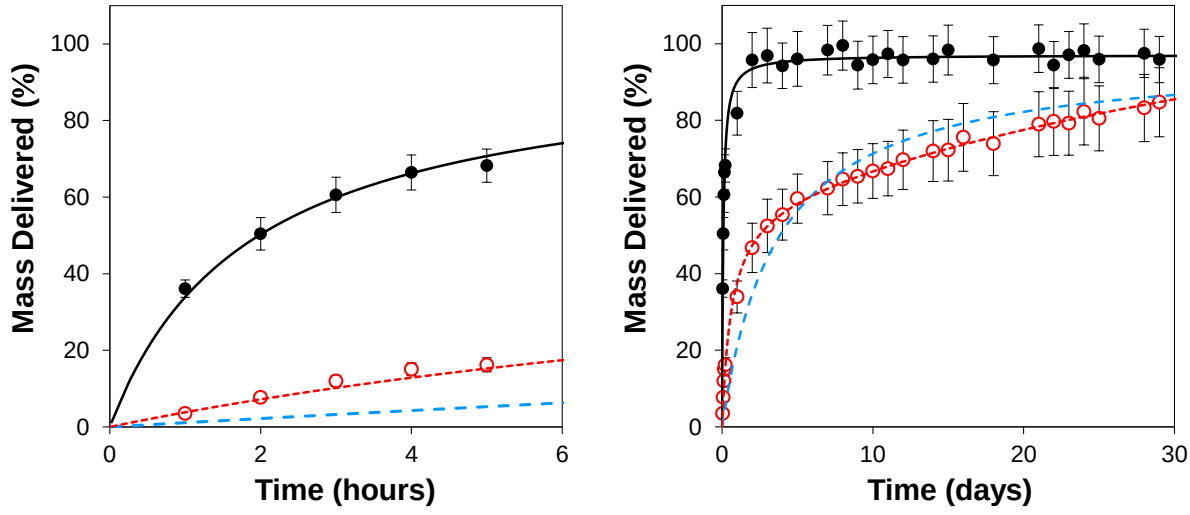

Figure S3. (left) Calcein release during the first 5 hours and **(right)** 30 days from UiO-66, black closed circles, and *a*UiO-66, red open circles. Black solid and red dotted lines represent the kinetic of delivery fitting using non-linear regression on UiO-66 and *a*UiO-66 respectively. Blue dashed line represents the fitting for *a*UiO-66 using Eq [3].

Table 1: Fit-curves for calcein release from crystalline and amorphous UiO-66.

Material	Equation	R^2
UiO-66	cal (wt%) = 97.07 t / (0.07762 + t)	0.9908
*a*UiO-66	cal (wt%) = 58.16 t / (0.6008 + t)+ 97.07 t / (72.24 + t)	0.9982

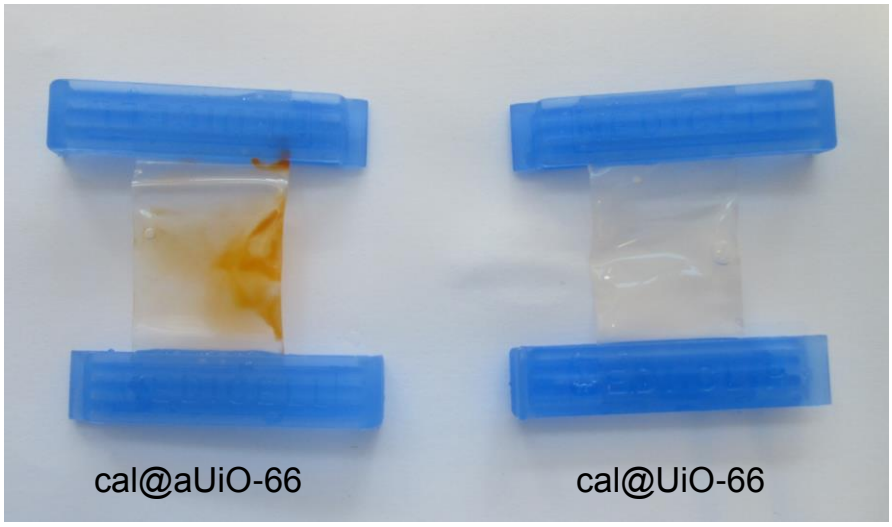

Figure S4. Pictures of the cal@aUiO-66 and cal@UiO-66 samples in the dialysis bags after 10 days release in PBS. Note the orange color from the presence of calcein in aUiO-66 but not in crystalline UiO-66.

S6. Cell culture

HeLa cells were maintained at 37ºC with 5% CO_2 in high rich glucose (4500 mg/L) Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 2mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were passaged three times a week (at 75-80% of confluence) at a density of 2.8 x 10^4 cell/cm^2.

S7. Cytotoxicity assays

The cytotoxicity activity of empty UiO-66 was investigated using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Promega, UK) reduction assay. The day before the experiment, the cells were seeded in to a 96 well plate at a density of 5 x 10^3 cells per well. Prior to the treatments, the cells were washed twice with PBS. The different MOF concentrations were dispersed in cell culture media containing 0.5 % of DMSO. Then they were added to the cells and incubated for 24 and 48 h at 37ºC with 5 % CO_2. To measure the toxicity, the cells were washed extensively to remove the solids, the media was replace with 100 µl of fresh culture media containing 20 µl of MTS/phenazine methosulfate (in a proportion 20:1) solution and the plate was incubated for 1h at 37ºC with 5 % CO_2. The plates were read at 490 nm. Finally, we confirmed that the DMSO concentration used was not toxic.

The IC50 values were measured at 24 and 48h. The values found were 1.503 ± 0.154 mg/mL and 1.357 ± 0.088 mg/mL for 24 and 48 h respectively. These values are similar to the reported for MIL Fe-based MOFs[4].

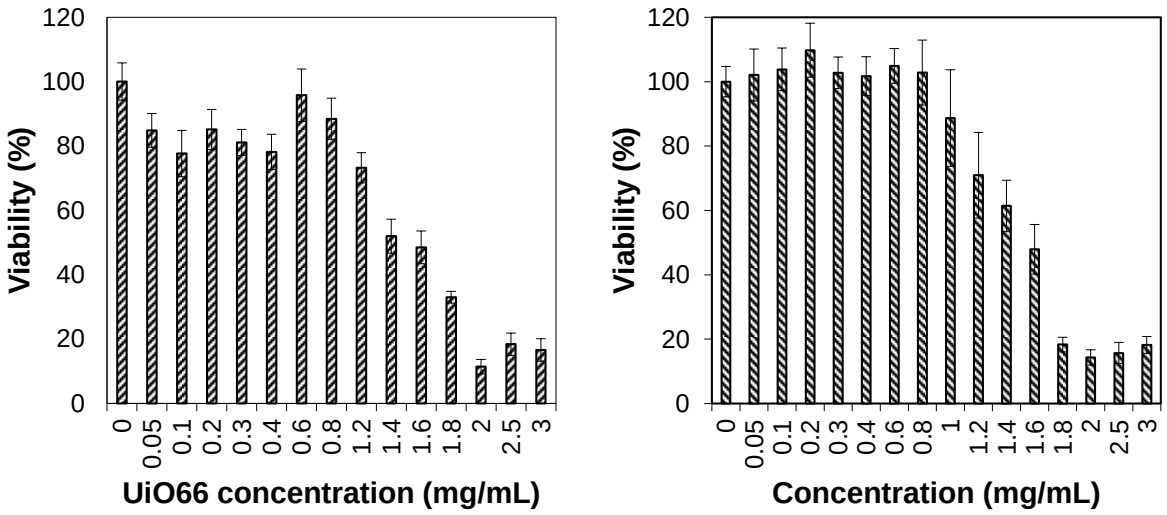

Figure S5. Metabolic activity of HeLa cells after exposure to UiO-66 particles at 24 h **(left)** and 48 h **(right)**.

S8. Confocal microscopy

For cell penetration assays, HeLa cells were seeded in a NUNC^{TM} imaging four-well plate at a density of 1.11 x 10^5 cell/mL and incubated for 24 h at 37ºC with 5 % CO_2 in DMEM supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 2mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were then washed twice with PBS and incubated with 0.25 mg/ml of either cal@UiO-66 or cal@*a*UiO-66 for 24 and 48 h. The loaded materials were well disperse in culture media before being added to the well plates containing the cells. Untreated cell and free calcein were included as controls (0.013 mg/ml). 5 µg/ml of both Hoechst 33342 (H33342) and propidium iodide (PI) were used for staining the nucleus and as a viability control respectively. After the incubation time, cell were washed several times to remove all the non-internalized particles. Untreated cells were fixed with 4 % p-formaldehyde (PFA) for 5 min and later all the samples were incubated with media containing the dyes H333421 and PI for 15 min. The cells were then washed extensively to remove the dyes and fresh media without phenol red was added to each sample. Finally, the four-well plate was placed on a Leica TCS SP5 confocal microscope to be imaged. The microscope was equipped with 405 diode, argon and HeNe lasers. Leica LAS AF software was used to analyse the images.

S9. Flow cytometry assays

Flow cytometry is a technique that allows measuring and analysing multiple properties of cells by passing a solution containing them through one or more lasers illumination intercepts. Then, a variety of light scattering from the sample is collected. There are three main characteristic determined by this methodology: 1) cell's size; 2) granularity that is related with the internal complexity of cells; and, 3) fluorescence intensity. The latter property permits to quantify and compare different cell samples. For example, cells incubated with free calcein, crystalline and amorphous MOFs.

HeLa cells were seed in a Cellstar 12-well plate at a density of 1 x 105 cell/ml and incubated for 48 h at 37ºC with 5 % CO_2 in DMEM supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin. Then, the each well was washed twice with PBS and incubated for 2 h with free calcein (37.625 mg/ml), cal@UiO-66 (0.5375 mg/ml) or cal@aUiO-66 (0.5375 mg/ml). After that, the media of each well was aspirated and the wells were washed extensively to remove

the solids. Then the cells were harvested by adding 0.2 mL of trypsin and incubating for 5 min at 37ºC with 5 % CO_2. The cells were recovered by centrifugation, 5 min at 1200 rpm and re-suspended in 250 μl of PBS. Then 1 μl of CellTrace calcein AM red/orange was added to each sample and incubated for 15 min at 37ºC with 5 % CO_2. Then the samples were centrifuged for 5 min at 1200 rpm and washed once with PBS. The final pellet was re-suspended in 200 μl in DMEM without phenol red supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 2mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. Finally the samples were measure in a Cytek DxP8 analyser cytometer within 30 min. All the analysis was done using FlowJo and Prism softwares.

In order to build the graph shown in Figures 4a and 4.b, we run control samples of unstrained or either stained with calcein or CellTrace calcein AM red/orange. These controls defined each section of the dot plot as follow: 1) unstained cells were located in the bottom-left section; 2) calcein AM red/orange negative but calcein positive were located in the bottom-right section; 3) calcein AM red/orange positive but calcein negative were located in the upper-left section; and, 4) calcein AM red/orange positive and calcein positive were located in the upper-right section of the graph. The color of the dots denotes the areas of high and low population density. Red and orange relate to the higher population density, blue and green to the lower one and yellow to the middle range.

S10. References

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