SUPPORTING INFORMATION for

Efficient nanostructured platforms for Thiram formulation

Tatiana A. Huertas Navarro[a,b], Jazmín Torres[c,d], Martina Foresi[a,b], Mariana A. Fernández[a,b], Mónica C. García[c,d], Alejandro M. Granados[a,b] *, Alejandro M. Fracaroli[a,b] *

[a] Universidad Nacional de Córdoba (UNC), Facultad de Ciencias Químicas, Departamento de Química Orgánica, X5000HUA, Córdoba, Argentina.

[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC), X5000HUA, Córdoba, Argentina.

[c] Universidad Nacional de Córdoba (UNC), Facultad de Ciencias Químicas, Departamento de Ciencias Farmacéuticas, X5000HUA, Córdoba, Argentina.

[d] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA-CONICET), X5000HUA, Córdoba, Argentina.

a.fracaroli@unc.edu.ar y ale.granados@unc.edu.ar

MATERIALS AND METHODS

All chemicals, unless specified, were obtained from Sintorgan and Anedra. N,N−Dimethylformamide (DMF), formic acid, methanol, acetone, acetic acid, isopropanol, absolute ethanol, sodium acetate were used as received, and without further purification. The non-ionic surfactants Tween 80 (Tw80) and Span 80 (Sp80) were provided by Riedel-de Haën and Fluka, respectively. Thiram (≥ 98 %) was acquired in Sigma Aldrich. Water was purified in Millipore equipment (Milli Q quality).

MOFs synthesis

MOF-808 (octahedral crystals of 500 nm side): was obtained by solvothermal synthesis in N,N−dimethylformamide (DMF) using a method adapted from the literature[1] . Briefly, our process, for each batch, consisted of mixing N,N-dimethylformamide (10 mL), $ZrOCl_2 \cdot 8H_2O$ (0.16 g, 0.50 mmol) and 10 mL of 1,3,5-benzenetricarboxylic acid (**BTC**, 0.11 g, 0.52 mmol), formic acid (20 mL) in 100 mL vials. Once the reagents mixture was homogenized (by mixing and sonication for 5 min.), the vials were sealed and placed in an oven at 130°C for 3 days. After this period was allowed to reach room temperature, the white solid obtained was separated from the reaction medium by centrifugation at 8000 rpm for 3 minutes, discarding the supernatant. The solid obtained was washed with N,N-dimethylformamide three times a day for 3 days. This washing was repeated for three more days with methanol. Finally, the solid was collected by filtration and dried under vacuum. 0.16 g (0.12 mmol) of a white crystalline power was recovered by low-pressure filtration, corresponding to a 23.5 % reaction yield (based on the Zr precursor added and the molecular formula of MOF-808, $C_{24}H_{16}O_{32}Zr_6$).

nMOF-808 (octahedral crystals of 50-60 nm side)[2]

Zr_6 Oxo cluster synthesis

$ZrCl_4$ (2.0 g, 8.58 mmol) was added to a mixture of 3 mL of acetic acid and 5 mL of isopropanol under stirring at 500 rpm and heated at 120 °C for 60 min. After this period, the reaction mixture was allowed to reach room temperature. The white solid crystalized was collected for centrifugation at 9000 rpm for 5 min and subsequently washed with acetone twice, before drying it under vacuum at room temperature. The dry powder mass was 1.45 g (1.40 mmol), corresponding to an almost quantitative reaction yield (>99 %), assuming the oxo cluster molecular formula $Zr_6(\mu3{-}O)_4(\mu3\text{-}OH)_4$ $(CH_3COO^-)_6$ and considering the Zr salt as the limiting reagent of the reaction.

nMOF-808 synthesis

The Zr_6 oxo clusters obtained above (0.6 g; 0.59 mmol), were added under constant stirring (600 rpm) to a mixture of formic acid (0.75 mL), and H_2O (1.25 mL). This mixture was then stirred at room temperature until it was completely colorless. Then, 1,3,5-benzenetricarboxylic acid (150 mg, 0.7 mmol) was added, and the reaction was stirred overnight. The solid formed was collected by centrifugation at 9000 rpm for 5 min washed with mixtures of H_2O/acetone (25 mL/30 mL) and EtOH/acetone (25 mL/30 mL) twice, respectively. The collected solid was dried under vacuum at room temperature for 3 hours, affording 0.150 g (0.11 mmol) of a white crystalline powder, equivalent to 18.6 % reaction yield, with respect to added Zr_6 oxo clusters.

Characterization of MOF-808 and nMOF-808

The crystallinity and phase purity of the synthesized MOF-808 and nMOF-808 were sought to be confirmed by comparing their powder X-ray diffraction (PXRD) patterns with the reported material[1]. The measurements are carried out in a Malvern Panalytical X'Pert 1D. MOF-808 and nMOF-808 activation, were found to be highly crystalline, and to have the same MOF-808 structure reported previously, by the coincidence of 2θ angles and relative intensity, to previously published topology.

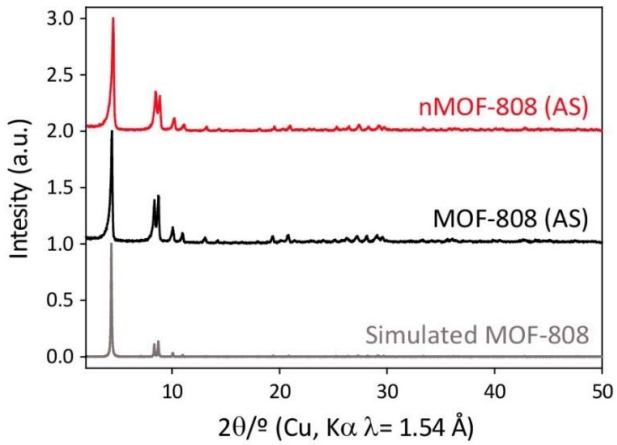

Figure S1. Powder X-ray diffraction (PXRD) pattern's comparison. MOF-808 simulated PXRD from reported structure (grey), materials prepared and activated in this work: MOF-808 (black) and nMOF-808 (red).

Their crystal morphology and size were evaluated by scanning electron microscopy (SEM, Figure 2) using a Carl Zeiss Σigma FE-SEM instrument. In both cases, it is possible to observe clearly the crystal's octahedral morphology and the order of magnitude in their size difference.

Additionally, we measured MOF-808 and nMOF-808 porosity by using a Micromeritics ASAP2020 instrument, where the N_2 isotherms were determined at 77 K. We also employed the BET model to estimate apparent surface area (Figure S2). Besides having the same topology and structure, we found nMOF-808 to have a considerably smaller surface area compared to MOF-808 ($SA_{nMOF-808}$ = 848 $m^2.g^{-1}$ and $SA_{MOF-808}$ = 1570 $m^2.g^{-1}$).

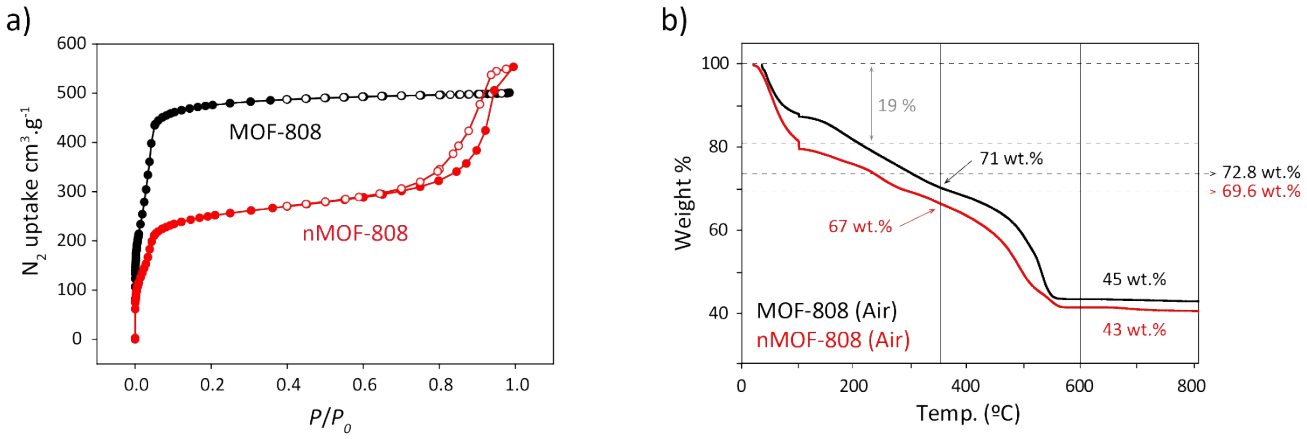

Figure S2. **a)** Comparison of N_2 isotherms obtained for MOF-808 (black curve), and nMOF-808 (red). **b)** Comparison of thermogravimetric analysis (TGA) results for MOF-808 (black curve), and nMOF-808 (red).

The activation of MOF-808 and nMOF-808 involved the removal of adsorbed molecules, such as solvents or ambient moisture, through a controlled thermal treatment under vacuum. During this process, both materials were subjected to a programmed temperature and pressure sequence in the Micromeritics VacPrep 061 equipment, achieving the desorption of impurities and effective pore opening. The activated samples were used for both their characterization (PRXD, surface area, SEM etc) and for Thiram adsorption experiments.

Finally, the colloidal stability of nMOF-808 and Thiram@nMOF-808 was studied by photon correlation spectroscopy. Samples were dispersed in water at 2.5 mg/mL and key stability parameters, including hydrodynamic diameter (d_H), polydispersity index (PDI), and electrokinetic potential (ζ) were studied by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively (Malvern Zetasizer® Nano-ZS 90). Samples were diluted 1/10 and sonicated for 20 min to ensure adequate dispersion. Measurements of d_H and size distribution (indicated by PDI) were conducted in a 1 mL polystyrene cuvette at room temperature, while ζ values were determined using a U-shaped capillary cuvette. All parameters were measured in triplicate, with data processed using Zetasizer® software version 7.12 to ensure accuracy and reproducibility. All measurements were performed in automatic mode, and as

stated in the manuscript, MOF-808 did not show any stability. In fact, not even the first measurement displayed stable values. Hydrodynamic diameter (d_H/nm, purple), electrokinetic potential (ζ/mV, blue) determined by DLS for MOF-808. The polydispersity index (PDI) was found to be in the range of 1.23 to 1.70 in the first measurement.

MOFs-Thiram Interaction

Thiram Photoprotection by MOF-808

The UV-vis spectra comparison for Thiram solutions in chloroform and Thiram@nMOF-808 before and after 3 h of continuous irradiation are summarized in Figure S3, below.

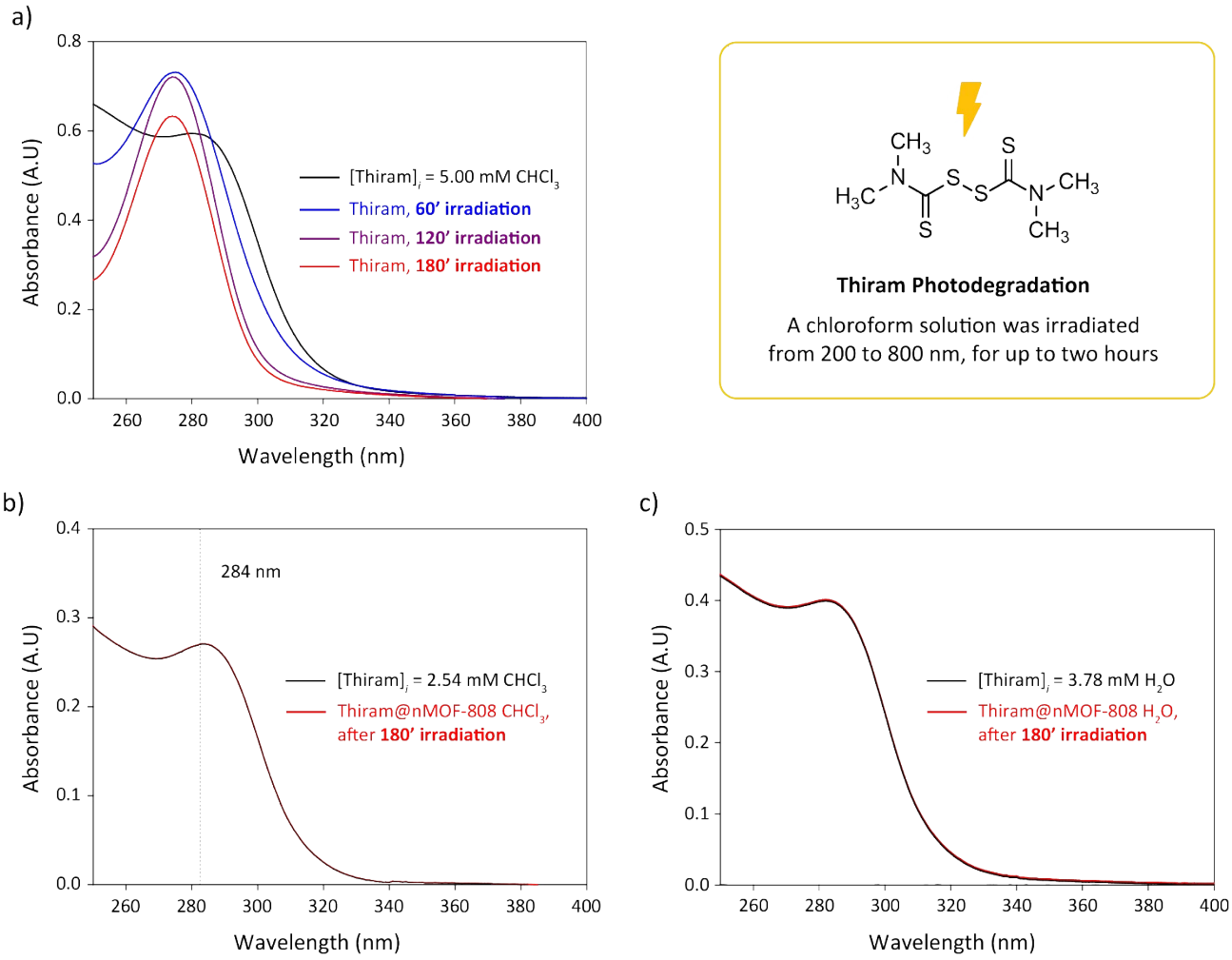

Figure S3. a) Comparison of the UV-Vis spectra of [Thiram] = 5.00 mM chloroform solution freshly prepared (black curve), after 60 min. irradiation (blue), 120' irradiation (purple), and 180' irr. (red). b) Photostability achieved after 180' irradiation of Thiram included in nMOF-808, and release in $CHCl_3$. c) Photostability achieved after 180' irradiation of Thiram@nMOF-808, and release in H_2O.

Thiram UV-vis absorption coefficient (ε) determination

As usual, after exploring both the best solvent for the inclusion-release of Thiram and its photostability, we prepared a series of solutions to determine the linear range of concentrations and calculate the UV-

vis absorptive coefficient (ε_{284nm} = 10.5 10^3 $M^{-1}.cm^{-1}$). As can be seen in the figure below (Figure S4), once protected from light, the calibration curve showed an acceptable error range.

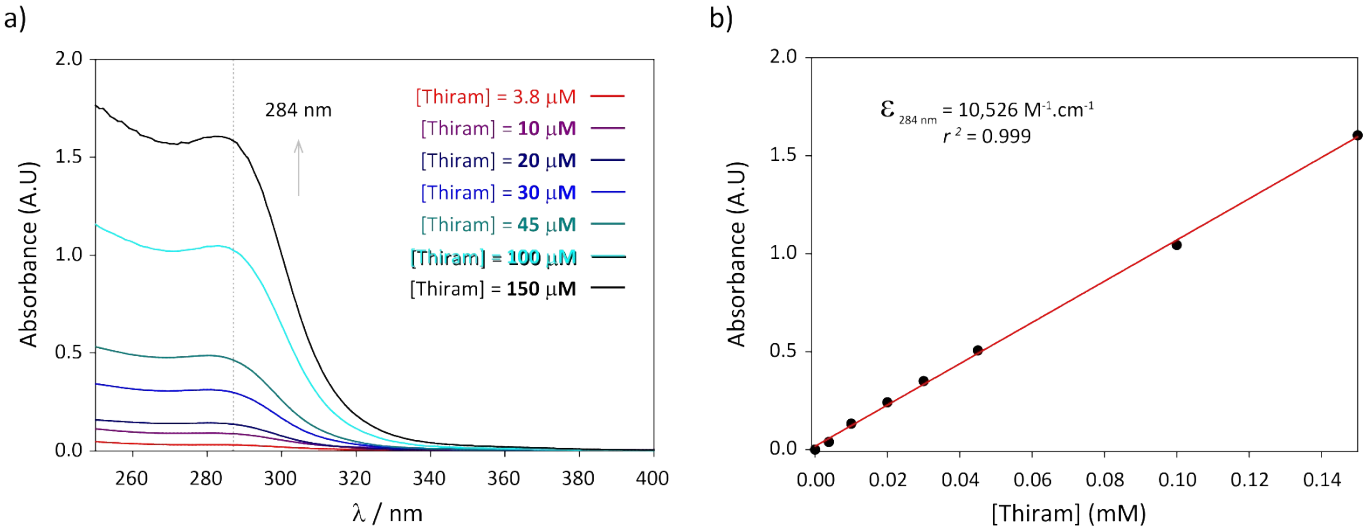

Figure S4. Calibration curve obtained for Thiram in $CHCl_3$, at 25 ºC.

Thiram inclusion in MOF-808

The equilibrium time for Thiram inclusion into the MOF pores was determined by measuring the UV-Vis absorption spectra throughout the whole inclusion period. Different samples were taken from the supernatant at different times (from 0 min to t = 300 min). As can be noted in the following Figure S5, the equilibrium for Thiram inclusion into nMOF-808 pores is reached at 180 min.

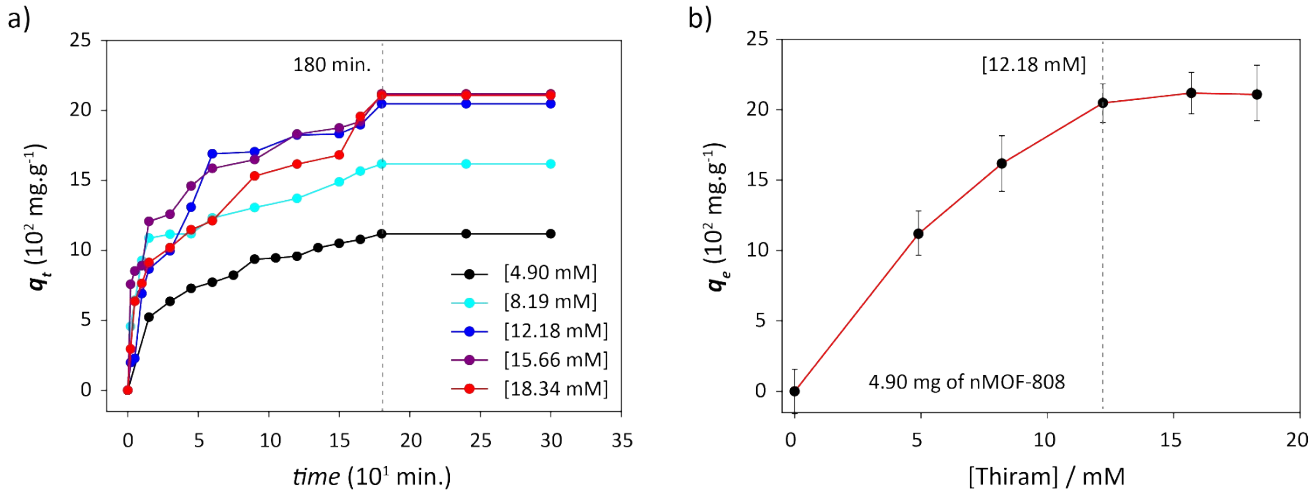

Figure S5. a) Calculated mass of included Thiram in nMOF-808 at a given time ($\boldsymbol{q_t}$); and b) mass of Thiram at the equilibrium point ($\boldsymbol{q_e}$) for different $[Thiram]_i$.

Different spectra were taken at different initial concentrations of Thiram ($[Thiram]_i$), to conclude about the maximum uptake of this fungicide for a given mass of absorbent material (4.90 mg of nMOF-808). We found that this amount of adsorbent is saturated at 180 min when $[Thiram]_i$ = 12.18 mM.

Once we have determined the equilibrium time and maximum uptake of fungicide into the MOF pores, a series of UV-vis adsorption experiments was performed by adding MOF-808 or nMOF-808 into 20 mL vials containing 5 mL of Thiram solutions in chloroform, according to the following table (Table S1).

Table S1. Different absorbent materials and fungicide amounts studied to find the right adsorption model and details on the mechanism that takes place

MOFs / mg	$[Thiram]_i^a$	$[Thiram]_i^b$	$[Thiram]_i^c$	$[Thiram]_i^d$	$[Thiram]_i^e$	$[Thiram]_i^f$
MOF-808 / 25 mg	4.90	8.00	13.09	15.00	19.90	25.16
nMOF-808 / 25 mg						25.16
nMOF-808 / 5 mg	4.90	8.19	12.18	15.66	18.34	

In the particular case of nMOF-808, when using 5 mg of the adsorbent, 5 different concentrations of Thiram in $CHCl_3$ were explored as can be seen in the Table above: 4.90; 8.19; 12.18; 15.66; 18.34 mM.

In a typical procedure, the suspensions were stirred at room temperature, protected from light, and after a given time and the re-precipitation of the MOFs, an aliquot of the supernatant was taken and diluted as follows:

- $0 < [Thiram]_i \geq 15.66$ mM: 50 μL of supernatant solution were diluted to a final volume of 5 mL with $CHCl_3$ (dilution factor of 100).
- $[Thiram]_i = 18.34$ mM: 5 μL of supernatant solution were diluted to a final volume of 5 mL with $CHCl_3$ (dilution factor of 1000).

such that the resulting concentrations were within the Beer-Lambert linear range obtained in the calibration curve (see Figure S4). The concentration of Thiram in the supernatant was determined based on the dilution carried out (*ut supra*), and the absorbance was measured at $\lambda_{max} = 284$ nm to calculate the concentration using the molar extinction coefficient of Thiram ($\varepsilon_{280nm} = 1.05\ 10^4\ M^{-1}\ cm^{-1}$).

Second loading cycle of Thiram into Thiram@nMOF-808

The batch of nMOF-808 loaded with thiram using an initial concentration of 23 mM was subjected to a second loading (2nd cycle). For this, once the first absorption equilibrium was reached, the supernatant was separated by centrifugation at 9000 rpm, and the solid was washed with chloroform to eliminate the thiram fraction that may have remained on the surface of the material. After this, it was dried under vacuum for 5 hours. The dry thiram@nMOF-808 was again dispersed in a solution of 23 mM thiram in chloroform, repeating the UV-Vis absorption measurements detailed for the 1st cycle to determine if there was an additional uptake of thiram that could be observed (Figure S6).

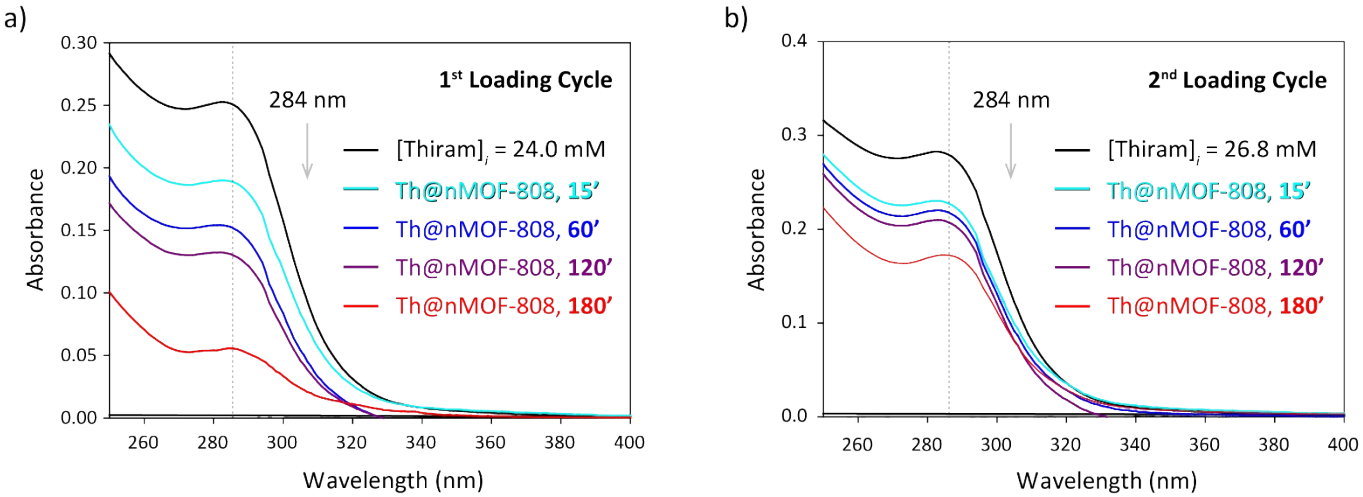

Figure S6. Thiram inclusion cycles in nMOF-808. Results obtained from (a) the 1st loading cycle, and (b) the 2nd loading of Thiram.

Langmuir and Freundlich models fitting for the experimental data

The results of fitting the experimental data to both Langmuir and Freundlich models, and to their corresponding linearized versions (Eq. 4 and 5, manuscript), are found below (Figure S7).

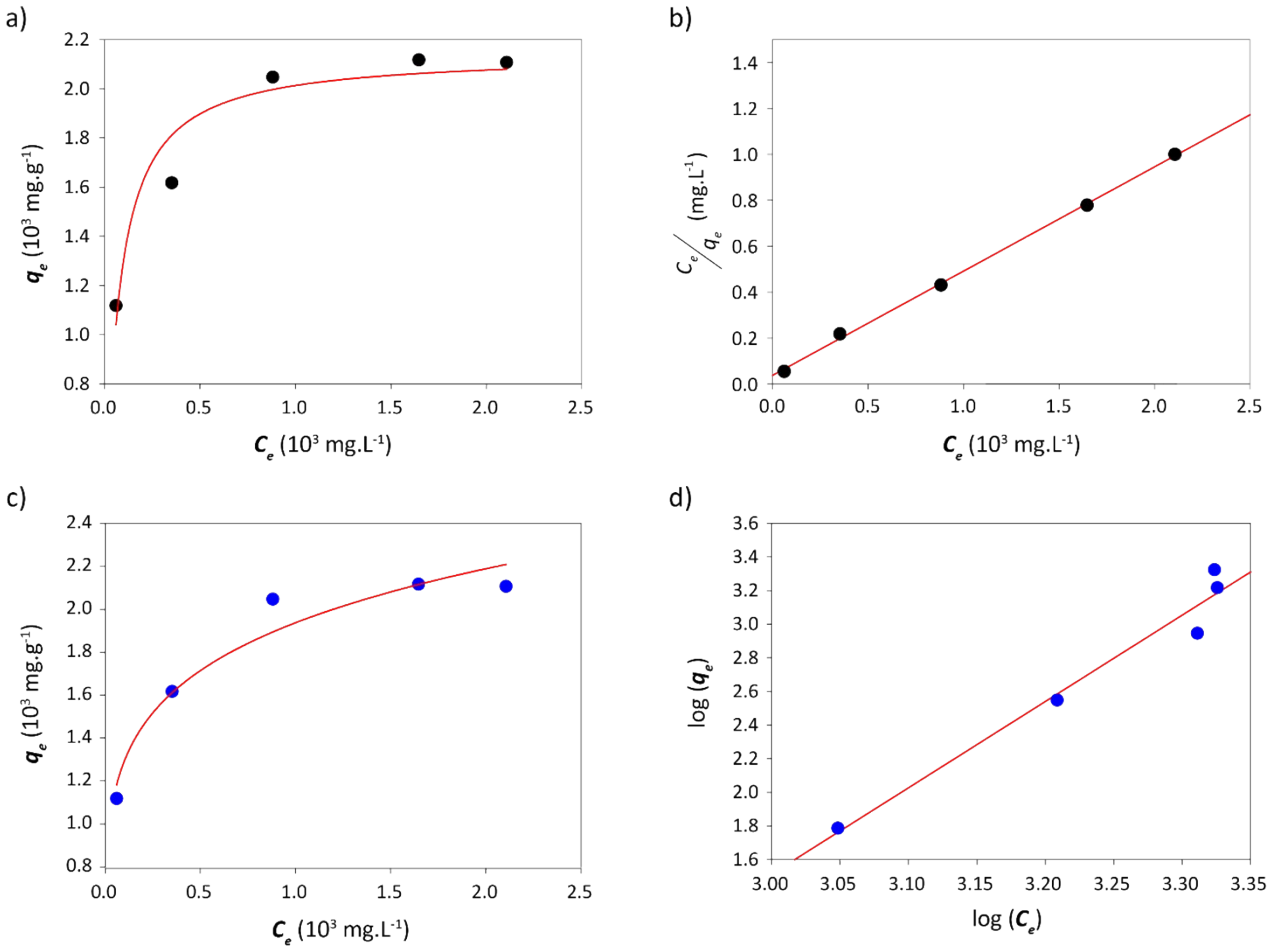

Figure S7. Non-linear and linear fittings for the experimental data to the (a, b) Langmuir and (c, d) Freundlich models.

Kinetic model fitting for the data obtained at [Thiram] = 12.2 mM, in contact with 5 mg of nMOF-808

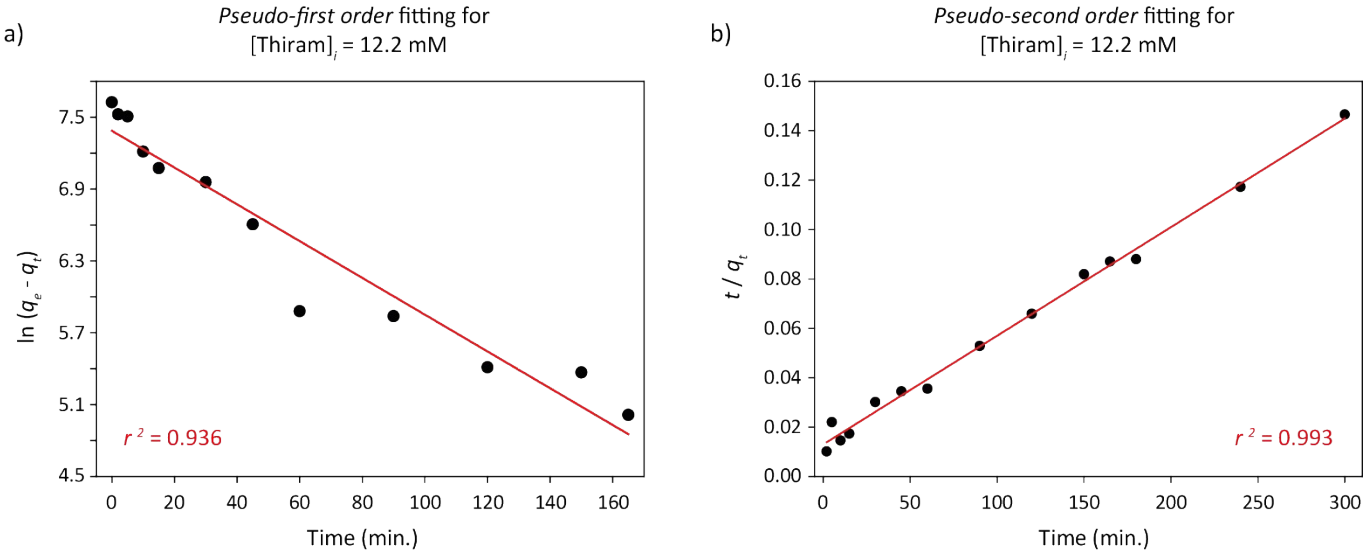

Figure S8. a) pseudo first-order model fitting and b) pseudo-second order model fitting for the data obtained using [Thiram]$_i$ = 12.2 mM, in contact with 5 mg of nMOF-808.

Adsorption efficiency of the Thiram inclusion process into nMOF-808 (Hall *et. al.,*)[3]

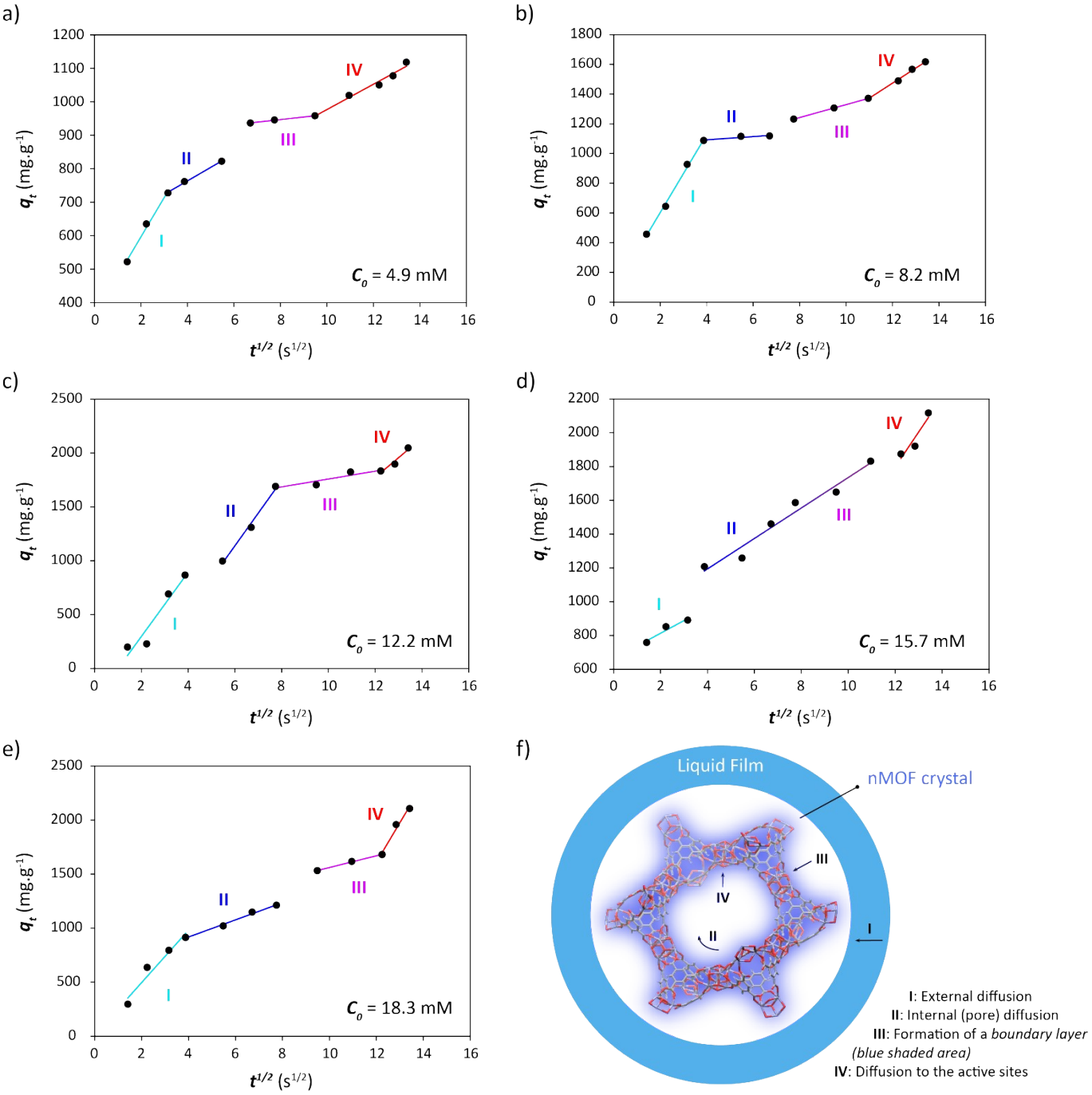

Figure S9. Graphs of q_t vs. $t^{1/2}$ for different C_0 of Thiram: (a) 4.9 , (b) 8.2 , (c) 12.2 , (d) 15.7 , and (e) 18.3 mM. (f) Schematic representation of the inter- and intraparticle diffusion model. In all cases, the formation of the boundary layer is, at least, partially responsible for the adsorption process rate, while the intraparticle diffusion does not seem to be the rate-determining step in any case, being almost the steepest portion in the graphs (IV).

Adsorption efficiency of the Thiram inclusion process into nMOF-808 (Hall *et. al.*) [see ref. 3]

Table S2. List of calculated values corresponding to k_i and Θ obtained from the correlation of the different linear steps, I, II, III, and IV, from q_t vs. $t^{1/2}$ graphs, for different C_0 values.

		Time stages			
C_0, mM	Parameters	I	II	III	IV
4.9	k_i, mg.(g.min$^{1/2}$)$^{-1}$	117	40	7.68	39
	Θ, mg.g^{-1}	361	601	885	598
	r^2	0.991	0.996	0.996	0.975
8.2	k_i, mg.(g.min$^{1/2}$)$^{-1}$	263	11	44	101
	Θ, mg.g^{-1}	77	1047	893	260
	r^2	0.996	0.876	0.999	0.995
12.2	k_i, mg.(g.min$^{1/2}$)$^{-1}$	298	303	36	182
	Θ, mg.g^{-1}	-300	-684	1394	-419
	r^2	0.918	0.989	0.861	0.942
15.7	k_i, mg.(g.min$^{1/2}$)$^{-1}$	75	90		207
	Θ, mg.g^{-1}	662	832		-686
	r^2	0.928	0.962		0.878
18.3	k_i, mg.(g.min$^{1/2}$)$^{-1}$	242	79	54	364
	Ord., mg.g^{-1}	10	600	1019	-2768
	r^2	0.944	0.990	0.998	0.975

Photodegradation test

A certain amount of Thiram-nMOF was placed in a quartz cell. A spectrophotometer with a 20 W halogen lamp and deuterium lamps was used as a light source. The irradiance was 20 W cm^{-2} and the distance between the quartz tube and the light source was 5 cm. The sample was irradiated for 4 hours. It was then dried under vacuum for 3 hours. Finally, it was resuspended in 5 mL of chloroform, shaken, and decanted for 30 min. The Thiram concentration in the supernatant was measured by UV-Vis spectrophotometry.

Incorporation of Thiram in aggregates formed by amphiphilic molecules

Preparation of niosomes

Niosomes were prepared using the film hydration method. Appropriate amounts of stock solutions of surfactants in absolute ethanol were placed in a round flask. After evaporation of the solvent (Buchi Labortechnik AG), the film obtained was hydrated with Milli Q water and left in a stirring water bath (Grant Instruments) at 60 °C and 80 rpm for 30 minutes. Finally, the temperature was set at 25 °C throughout the night.

Formulations with different proportions of surfactants were tested (Tw80:Sp80 at 50:50 and 40:60 ratios). Also, different concentrations of surfactants were assayed.

In Thiram-containing niosomes, the fungicide was added to the organic solvent at the film formation stage. Niosomes loaded with Thiram were purified using a Sephadex G-25 column (GE Healthcare) or by dialysis (through a benzoylated cellulose membrane, Sigma-Aldrich). The membrane containing 2 mL of loaded niosomes was immersed in 200 mL of Milli Q water for 18 h with stirring.

Four niosomes formulations were prepared for different assays:

Niosomes 1: Tw80:Sp80/1:1, $[Surfactant]_{total}$ = 10 mM; $[Thiram]_i$ = 0.05 mM.
Niosomes 2: Tw80:Sp80/1:1, $[Surfactant]_{total}$ = 10 mM; $[Thiram]_i$ = 0.1 mM.
Niosomes 3: Tw80:Sp80/1:1, $[Surfactant]_{total}$ = 30 mM; $[Thiram]_i$ = 0.1 mM.
Niosomes 4: Tw80:Sp80/1:1.5, $[Surfactant]_{total}$ = 10 mM; $[Thiram]_i$ = 0.1 mM.

Once the formulations, a part of them was purified and the concentration of Thiram in the purified and unpurified loaded niosomes was determined.

Characterization of niosomes

The diameter and PDI (polydispersity index) of the vesicles were determined by DLS (Delsa™ Nano Submicron Beckman Coulter).

Niosomes morphology was obtained by transmission electron microscopy (Fig. 2c, TEM, Jeol 1200 EX II). 2 % uranyl acetate was used to stain the samples.

Encapsulation efficiency (EE)

The EE was calculated by Eq. S1.

$$\%EE = \frac{[Thiram]_{nios.}}{[Thiram]_i} \times 100 \qquad \text{Eq. S1}$$

The concentration of Thiram in niosomes ($[Thiram]_{nios.}$), corresponds to the concentration loaded in the vesicles, after purification, whereas the concentration of Thiram initial means the concentration of fungicide before purification of niosomes. These concentrations were quantified by HPLC (HP series 1100 chromatograph, Hewlett Packard, Agilent Technologies). A Zorbax Eclipse Plus C18 column (4.6 mm x 250 mm, 5 µm, Agilent Technologies) was used. The mobile phase was methanol/sodium acetate-acetic acid pH=6, 7 mM (60:40), flow rate of 1 mL min^{-1}, detection at 230, 254 and 276 nm. The retention time of Thiram in these conditions was 6 minutes (Figure S10a). A calibration plot was performed from the measured areas (Figure S10b).

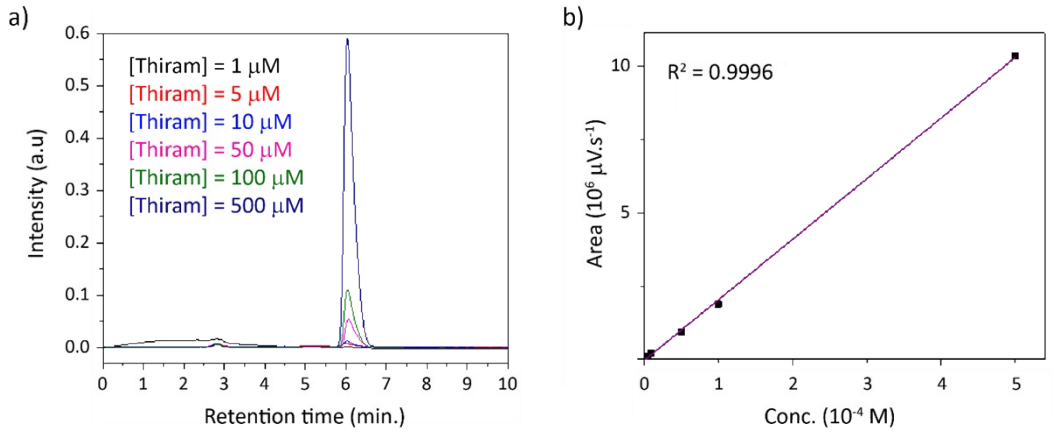

Figure S10. a) HPLC chromatograms of Thiram at different concentrations. b) Calibration plot.

***In vitro* release experiments**

The release studies were performed using a vertical Franz cell. 2 mL of Thiram-loaded niosomes were placed in the donor compartment of the cell. The receptor compartment was filled with 21 mL of Milli Q water. Release was followed for 3.5 hours at 25 °C. Quantification of Thiram was performed by HPLC (Figure S11).

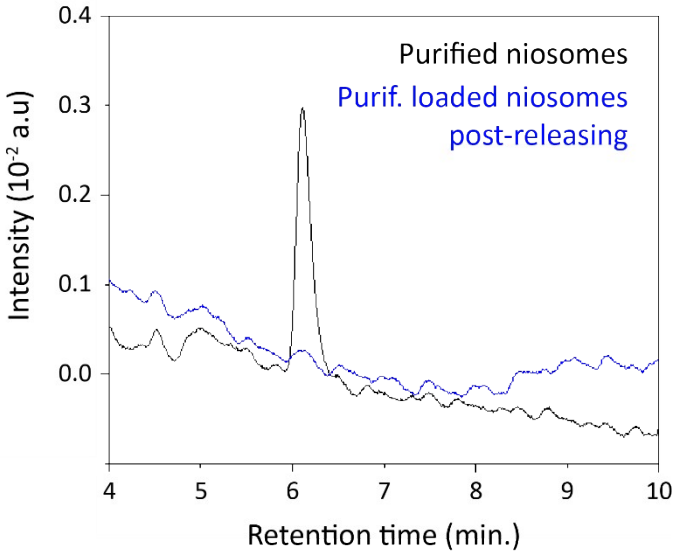

Figure S11. Chromatograms of loaded niosomes with Thiram before and after release.

Solubility tests

Experiments measuring apparent Thiram solubility were carried out in different media, using only aqueous solution, 10 mM niosomes, or Tween 80 solutions at different concentrations. Films of Thiram (from a concentrated solution in chloroform to assure an excess of fungicide) were hydrated with water, Tween 80 micelles 5 or 10 mM, or Tween 80:Span 80 niosomes, $[Surfactant]_{total}$ = 10 Mm, respectively, and left for 48 hours at 25 °C under stirring. The quantification of the fungicide was done by HPLC as was previously described.

Effect of the temperature on the stability of Thiram

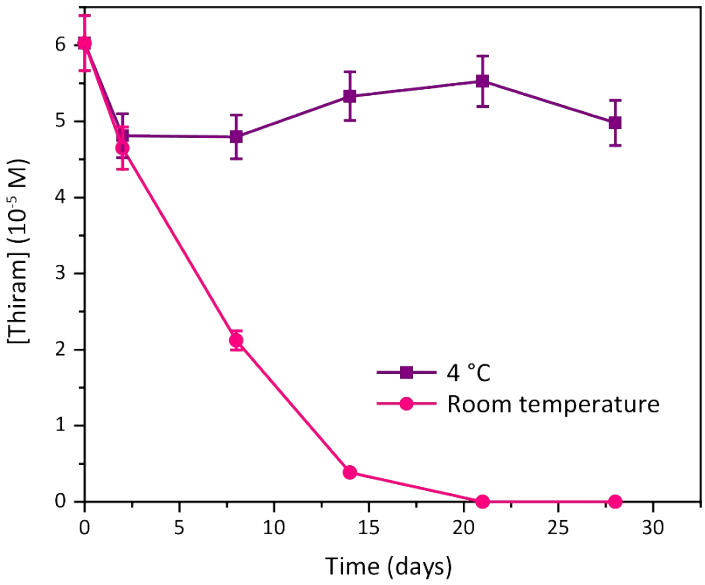

Figure S12. Effect of the temperature on the stability of Thiram in niosomes, stored at 4°C (purple) and at room temperature (pink). $[Thiram]_i$ = 0.05 mM.

Biological evaluation

Hemolytic potential and hemocompatibility

To evaluate the hemolytic potential, we conducted two *in vitro* hemolysis assays to assess the effect of Thiram on erythrocyte membrane integrity. In one of them, whole blood was used, while in the other, red blood cells (RBC) were used. Human blood samples were collected with ethical consent and donated in anonymity according to the regulations of the Blood Bank, National University of Cordoba. Healthy volunteers were fully informed regarding the purposes of the study, and informed written consent was obtained. The experimental protocol was approved by the Institutional Health Research Ethics Committee (CIEIS) of the Faculty of Medicine, National University of Cordoba (Res. 107-07042025).

Dispersions of Thiram@nMOF-808 (1 mL) were prepared with Thiram concentrations of 500, 250, 125, 62.5, and 31.25 µM in isosmotic phosphate buffer solution (PBS) at pH 7.4. Free Thiram at the same concentration was used as a reference. Additionally, unloaded nMOF-808 platforms were studied to evaluate their safety, using equivalent proportions to those used in the Thiram@nMOF-808.

When using whole blood, each sample (1 mL) was mixed with human blood to achieve a final concentration of 5 % v/v. The samples were incubated at 37 °C for 2 h under gentle mechanical stirring. After incubation, each sample was centrifuged at 25 °C and 1000 rpm for 5 min (Eppendorf Centrifuge 5804). Milli-Q water and isosmotic PBS at pH 7.4 were used as positive and negative hemolysis controls, respectively, and processed under the same conditions as the samples.

When using the RBC, firstly the whole blood (5 mL) was washed with 10 mL of isosmotic PBS pH 7.4 and centrifuged at 1500 rpm for 10 min to remove plasma and other cellular components. The erythrocyte pellet was washed twice more with isosmotic PBS, centrifugated each time at 1500 rpm for 10 min and the supernatant was discarded. After the final wash, erythrocytes were resuspended to a final volume of

10 mL in PBS pH 7.4. For each test, 200 µL of this diluted erythrocyte suspension was added to 200 µL of each treatment, using the concentrations described above. The samples were incubated at 37 °C for 2 h under gentle mechanical stirring. After incubation, each sample was centrifuged at 25 °C at 4000 rpm for 10 min. The same positive and negative controls were used.

The hemolysis percentage (H%) was determined by measuring the absorbance of the supernatant at λ=540 nm, using UV-vis spectrophotometry (Shimadzu MultiSpec - 1501) and calculated with Eq. S2:

$$H\% = \left(\frac{Abs._{\text{sample}} - Abs._{\text{control (-)}}}{Abs._{\text{control (+)}} - Abs._{\text{control (-)}}} \right) \times 100 \qquad \text{Eq. S2}$$

The experiments were performed in triplicate, and the results are presented as mean ± σ. These consistent approaches ensured comparability of hemolytic potential across treatments and conc.

Cytotoxicity assay

The murine fibroblast cell line (NIH/3T3) was acquired from American Type Culture Collection (ATCC). These cells were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM, HyClone™) supplemented with 10 % fetal bovine serum (HyClone™), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco™). The cell cultures were maintained in a humidified incubator at 37 °C with 5 % CO_2.

To assess *in vitro* cytotoxicity, cell experiments were performed against NIH-3T3 murine fibroblast cells. Thiram@nMOF-808 was tested on the cells at different concentrations (250, 125, 62.5, and 31.25 µM) and compared with the cytotoxicity of free Thiram. The cytotoxicity of nMOF-808 platforms without Thiram was also evaluated at the same proportions as those used in the Thiram@nMOF-808 samples.

Cell viability was measured using the resazurin-based alamarBlue® assay, a fluorometric method in which viable cells reduce the blue (resazurin), nonfluorescent compound, to the pink (resorufin), fluorescent product. The amount of resorufin produced indicates cell growth, as it is proportional to the number of viable cells through mitochondrial respiration[4]. Briefly, 5,000 cells/ well were seeded in 96-well plates and incubated in a culture medium for 24 h to allow full cell attachment. Subsequently, the cell medium was replaced with the treatments: free Thiram, Thiram@nMOF-808, and nMOF-808. Before the treatments, the three solid samples were reconstituted in sterile DMSO (ATCC®) and diluted in a culture medium containing 10 % v/v alamarBlue®, with the final DMSO concentration kept below 0.8 %. Treated cells were incubated for an additional 24 h, and fluorescence intensity was measured using a microplate reader (Biotek®, excitation/emission = 535 nm/590 nm), according to the manufacturer's recommendations. Cell viability was expressed as a percentage relative to the viability of untreated control cells. Results were presented as mean ± standard error of the mean (SEM), and data were derived from two independent experiments.

Statistical analysis

GraphPad Prism® software was used to perform statistical analyses and mean values of different experimental conditions were compared. Analysis of variance (ANOVA) was employed, followed by

Bonferroni post hoc test. Differences between the mean values were considered significant at a *p*-value of < 0.05.

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