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Degradation Mechanism of Metal–Organic Framework Drug Nanocarriers Studied by Solid-State Nuclear Magnetic Resonance and X-ray Absorption Near-Edge Structure Spectroscopy

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1 Degradation mechanism of metal-organic framework
2 drug nanocarriers studied by solid-state NMR and
3 XANES spectroscopy

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15

16 ABSTRACT. Metal-organic framework nanoparticles (nanoMOFs) are novel porous drug delivery
17 systems whose features include high drug loading capacity, versatile functionalization,
18 biocompatibility, and biodegradability. However, little knowledge about the nature of nanoMOFs
19 degradation mechanism is one of many reasons that prevents their clinical use. MIL-100 (MIL
20 stands for Matériaux de l'Institut Lavoisier) is among the most studied nanoMOF for drug delivery.
21 Here, we investigate at the atomic scale the degradation mechanism of metal(III)-trimesate
22 nanoMIL-100 drug carrier in biological-mimicking phosphate medium. By using solid-state NMR
23 (ssNMR) spectroscopy, we found that the first step of nanoMIL-100(Al) degradation is the
24 substitution of labile water ligands, resulting in new coordination bonds between Al(III) and
25 phosphate ions, followed by the substitution of trimesate ligands leading to their release. The data
26 indicated that the reaction-limiting step most likely is the formation of an inorganic
27 aluminophosphate layer at the nanoparticle surface and that drug encapsulation and surface coating
28 affect the nanoMIL-100(Al) degradation. X-ray Absorption Near Edge Structure (XANES)
29 spectroscopy study of nanoMIL-100(Fe) degradation corroborates the hypothesized alteration
30 mechanism of nanoMIL-100(Al). From the ensemble of data, a stepwise degradation mechanism
31 representative for the nanoMIL-100 drug delivery system is proposed.

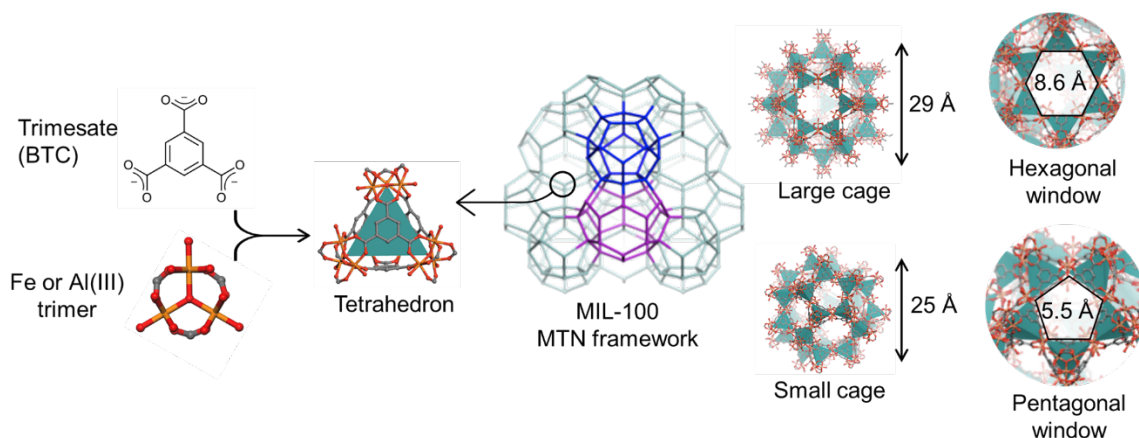
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33 1. Introduction

34 The concept of sustained drug release has been around since the 1960s¹; the quest has never
35 stopped to search for the most appropriate drug carriers for the delivery and controlled release of
36 therapeutic molecules to a targeted location in the patient. Since the first report in 2010²², metal-
37 organic framework nanoparticles (nanoMOFs) as drug carriers have gained increasing interest as
38 they combine the advantages of both their inorganic and organic constituents. Metal-organic

39 frameworks (MOFs) are porous materials made of metal-ligand coordination units assembled as
40 an ordered crystalline structure with high permanent porosity³⁻⁵. This high porosity enables MOFs
41 to capture a large amount of drug within a small amount of carrier, thus potentially reducing the
42 administered dose and related toxicity for the patients.

43 Among the most studied nanoMOFs for drug delivery to date is nanoMIL-100(Fe), belonging to
44 the MOF family of MIL-100. It consists of trivalent metal cations such as Al (III) or Fe (III), and
45 benzene-1,3,5-tricarboxylate ligands, also called trimesate or BTC^{6,7}. **Figure 1** illustrates the
46 structure of MIL-100. Its secondary building unit (SBU), the Fe or Al(III) metal trimer, consists
47 of three metal cations (III) coordinated with one central bridging oxygen and six carboxylate
48 groups. The 6-fold coordination sphere is completed with hydroxyl and water ligands. Two metal
49 cations coordinate with one water molecule per cation, and one cation coordinates with one
50 hydroxyl group. Every four SBUs connect through trimesate linkers, creating tetrahedra. These
51 tetrahedra build the MTN type MIL-100 framework, which possesses two kinds of pores: large
52 mesoporous cages of 29 Å diameter, accessible through hexagonal (8.6 Å) and pentagonal
53 windows (4.8-5.5 Å), and small mesoporous cages of 25 Å diameter, accessible exclusively
54 through pentagonal opening windows. The generally accepted formula is
55 $M_3O(H_2O)_2OH(C_6H_3(CO_2)_3)_2$ (M is Fe or Al)^{7,8}. In addition, the MIL-100 MOFs consist of
56 coordinative unsaturated metal sites (CUS) with strong Lewis acid properties produced upon
57 thermal activation and departure of labile ligands^{9,10}.



58
 59 **Figure 1.** Structure of the MOF family MIL-100. The Fe(III) or Al(III) trimer is 6-coordinate
 60 Fe or Al (III) coordinating with bridging oxygen, water, trimesate, and hydroxyl ligands.
 61 Tetrahedra, made of Fe or Al(III) trimers on their vertices and trimesates on their faces, are
 62 arranged following the MTN framework, creating two types of mesoporous cages: large cages with
 63 29 Å diameter, accessible through hexagonal (8.6 Å) and pentagonal (4.8-5.5 Å), and
 64 small cages with 25 Å diameter, accessible only through pentagonal windows. Adapted from
 65 reference¹¹.

66
 67 The high drug payload of nanoMIL-100(Fe) has been demonstrated for various drugs^{12,13,12,13},
 68 namely anticancer^{2,14,15}, antibiotic^{16,17}, antiretroviral¹⁸, and a combination of anti-inflammatory and
 69 antibiotics¹⁹. For example, the anticancer drug busulfan was loaded in nanoMIL-100(Fe) with a
 70 payload of 25 wt%, five and sixty times higher than its payload in polymer nanoparticles and in
 71 liposomes, respectively^{2,20,21}. Recently, the aluminum-trimesate nanoMIL-100(Al) was also
 72 explored for drug delivery. The MIL-100(Al) gel loaded with the anticancer drug doxorubicin
 73 (DOX) showed a remarkably high payload of 62 wt%²². More recently, DOX was efficiently
 74 encapsulated (74% encapsulation efficiency) in core-shell nanoMIL-100(Al) coated with γ -
 75 cyclodextrin (CD) citrate and was released progressively depending on the initial drug loading²³.

76 Both nanoMIL-100(Al) and nanoMIL-100(Fe) demonstrated high loading capacity for active
77 molecules bearing phosphate moieties because of the strong interaction between Al(III) or Fe(III)
78 CUS and phosphate group^{14,18,24}. Additionally, to improve the nanoMOF's colloidal stability and
79 to modulate their interaction with biological media and cells, the surface of nanoMIL-100(Fe) and
80 nanoMIL-100(Al) was coated covalently with polymers²⁵ or non-covalently with biopolymers,
81 lipids and carbohydrates^{15,26-31}. More importantly, for biomedical applications, nanoMIL-100(Fe)
82 has been shown non-toxic and biodegradable *in vitro*^{2,32,33} and *in vivo*^{2,21,34,35}. NanoMIL-100(Al)
83 has also been shown non-toxic *in vitro*³³. Still, many questions remain about the nanoMIL-100
84 carrier stability, for instance, toxicity might come from nanoMOF's degradation products³² or drug
85 encapsulation can modify nanoMOF's degradation kinetics²¹. To further progress the clinical
86 translation of nanoMOFs, a critical aspect that needs to be addressed is deciphering the degradation
87 mechanism of the nanoMIL-100 family.

88 However, there is little literature on the important topic of nanoMIL-100 degradation in
89 biological-relevant media and the effects of drug encapsulation and surface coating. A popular
90 hypothesis is that the degradation reaction of MIL-100(Fe) produces free iron(III) ions and trimesic
91 acid. Early studies found that the culprit of degradation is the phosphate component of the cell
92 medium^{18,36}. The higher the phosphate concentration, the higher the trimesate release, the more
93 degraded the nanoMOF¹⁶. Li *et al.* discovered that despite witnessing more than 30% trimesate
94 ligand loss, the MIL-100(Fe) nano- and micro-particles maintained their sizes and shapes upon
95 exposure to phosphate buffer³⁷. Using Raman and Mossbauer spectroscopy, the authors suspected
96 the formation of an amorphous shell of iron phosphate and/or iron oxide surrounding an intact
97 crystalline core. In addition, using *in situ* atomic force microscopy and high-resolution
98 transmission electron microscopy, Christodoulou *et al.* also found that the nanoMIL-100(Fe) and

99 microMIL-100(Fe) degraded without collapsing or changing their dimension¹¹¹¹. Degradation of
100 MIL-100(Fe) started at the particle surface, where the crystal planes at the surface disappeared
101 within 2 hours of incubation in phosphate buffer saline (PBS) at pH 7.4. However, none of these
102 techniques have given evidence at the atomic scale for the changes in iron coordination.

103 To study the MOF structure at the atomic scale, solid-state NMR (ssNMR) spectroscopy is a
104 non-destructive and valuable technique to get information about both the long-range crystal
105 structure (when combined with XRD and periodic first-principles calculations)³⁸ and short-range
106 local structure or interactions in MOFs. Deviations from periodic structures in MOFs can happen
107 after chemical modification or functionalization, which are often not detected by routine
108 characterization techniques such as X-ray diffraction and infrared spectroscopy. In this case,
109 ssNMR spectroscopy is a technique of choice for locally probing atoms, their neighboring
110 environments, and chemical bonds while keeping the MOF intact^{39,40}. It has been used extensively
111 to determine the structure of new MOFs^{7,41,42}, and the adsorption-desorption mechanism of liquid
112 and gases^{43,44}. For porous drug delivery systems, ssNMR spectroscopy has been mainly used to
113 study mesoporous silica⁴⁵⁻⁴⁷ and MOFs^{48,49}. For MIL-100(Fe), ssNMR spectroscopy gave indirect
114 proof about the interaction between the strong paramagnetic Fe(III) and the drug azidothymidine
115 triphosphate or the coating cyclodextrin phosphate (CD-P)^{18,26}. For diamagnetic MIL-100(Al),
116 ssNMR spectroscopy provided insights about host-guest interaction and location of the drugs
117 adenosine triphosphate (ATP) and doxorubicin, as well as the surface coating cyclodextrin-
118 phosphate (CD-P)^{23,24}.

119 In this work, we further use solid-state NMR spectroscopy to investigate the degradation
120 mechanism at the atomic scale of this nanoMIL-100(Al) in PBS, revealing a stepwise degradation
121 process. We also study the impact of ATP drug loading and CD-P surface modification on the

122 nanoMOF degradation. ATP and CD-P were chosen based on their well-known interaction
123 between molecules bearing phosphate moieties and the Al(III) cations of nanoMIL-100(Al)^{24,26}.
124 The ensemble of ssNMR data (²⁷Al, ³¹P, ³¹P-²⁷Al) reveals the evolution of the metal coordination
125 sphere in MIL-100 during degradation reaction, as a function of several parameters: MIL-100
126 structure, concentration of the degradation medium, interaction with surface coating and loaded
127 drug. To check whether our proposed degradation mechanism applies to the iron MIL-100 analog,
128 we studied the degradation of nanoMIL-100(Fe) in PBS by XANES spectroscopy, which was
129 selected because it can provide information about the local structure of iron(III) in MOFs^{50,51}. This
130 paper shows that using a combination of ssNMR and XANES spectroscopy allows better
131 understanding of the chemistry of nanoMIL-100 drug carriers and their degradation.

132

133 2. Experimental Section

134 Herein the most important experimental methods and techniques are summarized. Details are
135 given in the Supporting Information.

136 2.1. Synthesis of nanoMIL-100(Al) and nanoMIL-100(Fe)

137 NanoMIL-100(Al) and nanoMIL-100(Fe) were synthesized by microwave-assisted synthesis,
138 following a published procedure^{8,52}. All reagents and solvents were used without further
139 purification.

140 2.2. Drug encapsulation and surface coating of nanoMIL-100(Al)

141 NanoMOFs were loaded with ATP with the input loading 30 wt% of initial nanoMOF, following
142 a reported soaking procedure¹⁴. A 1.5 mg.mL⁻¹ aqueous solution of ATP disodium salt hydrate was
143 added to a determined milligram of nanoMOF (m_MOF). The final nanoMOF concentration was
144 5 mg.mL⁻¹. The weight of drug remaining in the supernatant after drug loading

145 (m_drug_remaining) was determined by HPLC based on a reported procedure⁵³. The actual drug
146 payload wt% was calculated as: $\frac{m_drug_input - m_drug_remaining}{m_MOF} \times 100$, where m_drug_input is the
147 weight of drug added to nanoMOF.

148 NanoMOFs were coated with phosphate- β -cyclodextrin (CD-P) at the theoretical coating 30
149 wt% of initial MOF, by adapting a published method²⁶. A 3 mg.mL⁻¹ aqueous solution of
150 phosphated β -cyclodextrin CD-P sodium salt (average 3-4 phosphate groups per cyclodextrin unit)
151 was added to a determined milligram of nanoMOF. The final nanoMOF concentration was 10
152 mg.mL⁻¹. The amount of coating remaining in the supernatant after surface coating
153 (m_coating_remaining) was determined by quantitative liquid-state NMR spectroscopy. The
154 actual surface coating wt% was calculated as: $\frac{m_coating_input - m_coating_remaining}{m_MOF} \times 100$, where
155 m_coating_input is the weight of drug added to nanoMOF.

156 2.3. Degradation of nanoMIL-100(Al) and nanoMIL-100(Fe)

157 NanoMOF was incubated in PBS pH 7.4 at 37°C for 2-5 days following two conditions: 10
158 mg.mL⁻¹ nanoMOF and PBS 10 mM, and 0.5 mg.mL⁻¹ nanoMOF and PBS 1 mM. PBS used is
159 Dulbecco's Phosphate-Buffered Saline 1X *i.e.*, 10 mM, pH 7.0-7.3, comprised of 8.1 mM
160 Na₂HPO₄, 1.5 mM KH₂PO₄, 138 mM NaCl, and 2.7 mM KCl.

161 2.4. Characterization of nanoMIL-100(Al)

162 Morphology and size of nanoMOF were observed by Transmission Electron Microscopy (TEM)
163 under a 120 kV microscope. Particle size of 0.5 mg.mL⁻¹ nanoMOF suspension in EtOH abs or
164 deionized (DI) water was measured by Dynamic Light Scattering (DLS). Specific surface area of
165 MOF was measured by the Brunauer–Emmett–Teller technique (BET) after degassing MOF
166 powder under vacuum at 100°C overnight. Crystallinity was evaluated by performing Powder X-
167 ray Diffraction (PXRD) at λ (Cu K α) of 1.5406 Å. Thermal stability was studied by

168 Thermogravimetric Analysis (TGA). The amount of trimesate and ATP in the supernatant
169 collected after nanoMOF degradation was determined by High-Performance Liquid
170 Chromatography (HPLC) following a published procedure³⁷. The amount of CD-P in the
171 supernatant was determined by NMR spectroscopy, performed at 9.4 T on a Bruker Avance
172 spectrometer equipped with a probe head of 5 mm. The chemical shifts were referenced to
173 tetramethylsilane for ¹H and ¹³C. Coordination structure of nanoMIL-100(Fe) was studied by
174 XANES spectroscopy recorded at the K-edge of Fe at 7112 eV at the SAMBA beamline in
175 Synchrotron SOLEIL, France. Experimental details are reported in the Supporting Information
176 (SI).

177 Coordination structure of nanoMIL-100(Al) was studied by Magic-Angle Spinning (MAS)
178 NMR spectroscopy performed at 11.7 T on a Bruker NEO NMR WB spectrometer equipped with
179 probe heads of 4 and 3.2 mm, except for the ³¹P-²⁷Al 2D NMR measurement, which was recorded
180 at 17.6 T with a 4 mm probe head. The chemical shifts were referenced to tetramethylsilane for ¹H
181 and ¹³C, to Al(NO₃)₃ 1 M solution for ²⁷Al, and to H₃PO₄ 85 wt% solution for ³¹P. **Table S1** in SI
182 reports details of NMR experiments.

183

184 3. Results and Discussion

185 3.1. Preparation and characterization of nanoMIL-100(Al)

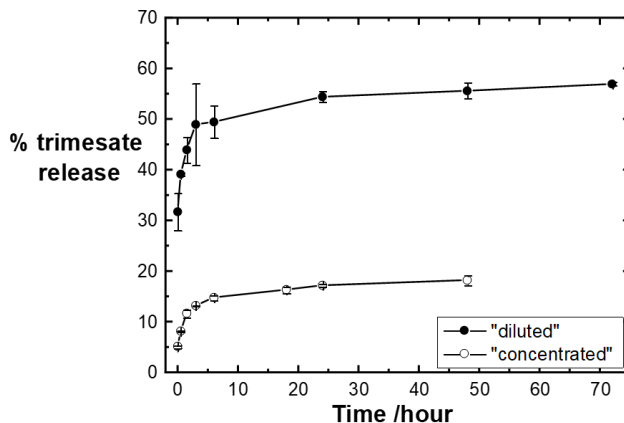
186 The first step of this study was the synthesis of nanoMIL-100(Al) suspensions adequate as drug
187 carriers, *i.e.* having a diameter lower than 200 nm and uniform size distribution. However,
188 following a published procedure⁵², the obtained MIL-100(Al) suspension had an inhomogeneous
189 size distribution. Therefore, we used an additional size separation step and successfully prepared
190 nanoMIL-100(Al) with hydrodynamic diameter Z-Average of 147 ± 14 nm and polydispersity

191 index PDI of 0.10 ± 0.02 (**Figure S1**). TEM images (**Figure 3a** and **Figure S2**) also displayed solid
192 nanoMIL-100(Al) particles with uniform shape and size (88 ± 25 nm). The nanoparticle size was
193 overestimated by DLS because in DLS, the average nanoparticle diameter is determined from
194 scattered light intensity and large objects scatter more light than small ones⁵⁴. The chemical
195 composition of the obtained nanoMIL-100(Al) was analyzed by TGA (**Figure S4**). It also showed
196 good crystallinity (XRD pattern in **Figure S5**) and a BET surface area of $1770 \text{ m}^2\cdot\text{g}^{-1}$ (N_2
197 adsorption isotherm in **Figure S3a**), which agree with published studies of MIL-100(Al)^{7,52}. ¹H
198 and ¹³C MAS NMR spectra in **Figure S6** and **Figure S7** show typical features for MIL-100(Al)
199 materials.

200 A recent study by our group detected an extra ²⁷Al peak in MIL-100(Al) nanoparticle, which
201 was not detected in the previous study of MIL-100(Al) microparticle⁷. This extra peak represents
202 the surface Al sites in MIL-100(Al) nanoparticle, indicated by high field NMR experiments ³¹P-
203 ²⁷Al *D*-HMQC and ²⁷Al 2D MQMAS²⁴. These surface species were reproduced in this study
204 (**Figure S8**) and are expected to play a crucial role in the degradation of nanoMIL-100(Al).

205 3.2. Degradation mechanism of nanoMIL-100(Al)

206 In the second step of this study, nanoMIL-100(Al) was incubated in PBS, composed of
207 phosphate monobasic and dibasic salts, at 37°C to mimic the physiological medium. All
208 measurements were performed *ex situ* by recovering the nanoparticles from suspension in PBS
209 after a given degradation period.



210 **Figure 2.** Kinetics of trimesate release, represented by the proportion of trimesate detected in the
 211 supernatant after degradation over the initial trimesate content in nanoMIL-100(Al). Two
 212 degradation conditions were named "diluted": 0.5 mg.mL⁻¹ MOF in PBS 1 mM, and
 213 "concentrated": 10 mg.mL⁻¹ MOF in PBS 10 mM, respectively.
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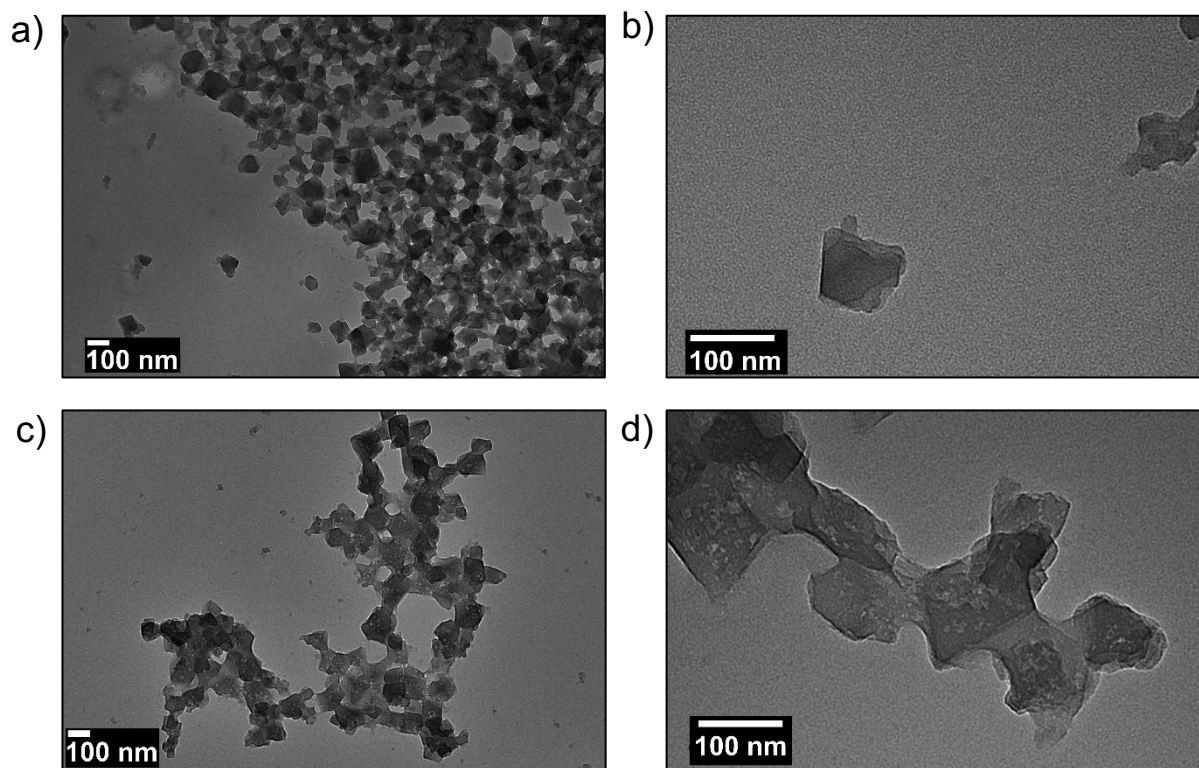
215 As a first step, to choose the suitable conditions for the NMR investigation, we determined the
 216 degradation kinetics of nanoMIL-100(Al) and the formation of trimesate by using HPLC. We
 217 chose two degradation conditions, "concentrated": 10 mg.mL⁻¹ nanoMIL-100(Al) in PBS 10 mM,
 218 2 days, and "diluted": 0.5 mg.mL⁻¹ nanoMIL-100(Al) in PBS 1 mM, 2 days. In the "concentrated"
 219 condition, the molar ratio of phosphate/Al₃ trimer (P/Al₃) is 0.54:1, meaning that nanoMIL-100(Al)
 220 is in excess, whereas in the "diluted" condition, the P/Al₃ is 11:1, and PBS is in excess compared
 221 to nanoMIL-100(Al). The percentage of released trimesate upon degradation in PBS is shown in
 222 **Figure 2.** As expected, the release profile of trimesate from nanoMIL-100(Al) had a similar pattern
 223 with MIL-100(Fe) and consisted of two stages. In the first three hours after incubation in PBS,
 224 there was a rapid generation of trimesic acid. Subsequently, the degradation reaction rate decreased
 225 gradually in the next 24 hours and reached equilibrium after 48-72 hours. This plateau of trimesate
 226 release after a given period was also observed in other works dealing with MIL-100(Fe)^{11,16,18}.

227 There could be two explanations for this degradation plateau: 1) insufficient amount of
228 phosphate to react with nanoMIL-100(Al), or 2) another mechanism preventing the reaction from
229 proceeding further. The first explanation was supported by the fact that the trimesate release was
230 higher in the “diluted” degradation, where the ratio P/Al₃ was higher than in the “concentrated”
231 degradation. To investigate further the limitation step of the nanoMIL-100 degradation reaction,
232 the morphology and structure of the degraded nanoMOF were characterized.

233 Modification of nanoMIL-100(Al) morphology after 48-hour degradation is shown in **Figure**
234 **3b** and **Figure S9**. The degraded nanoMIL-100(Al) retained their initial faceted shape and size
235 distribution 98 ± 24 nm, similar to the degraded nanoMIL-100(Fe) in Li *et al.*³⁷. However, the
236 degraded nanoMIL-100(Al) had holes on its surface, which is somewhat different from the
237 degraded nanoMIL-100(Fe), whose TEM images displayed particles with rounded edges and
238 without holes at the surface.

239

240



241 **Figure 3.** TEM images of intact nanoMIL-100(Al) at 10 k (a) and 30 k (b) magnification. TEM
 242 images of degraded nanoMIL-100(Al) at 10 k (c) and 30 k (d) magnification. Degradation was
 243 carried out with “diluted” condition: 0.5 mg.mL⁻¹ nanoMOF in PBS 10 mM.

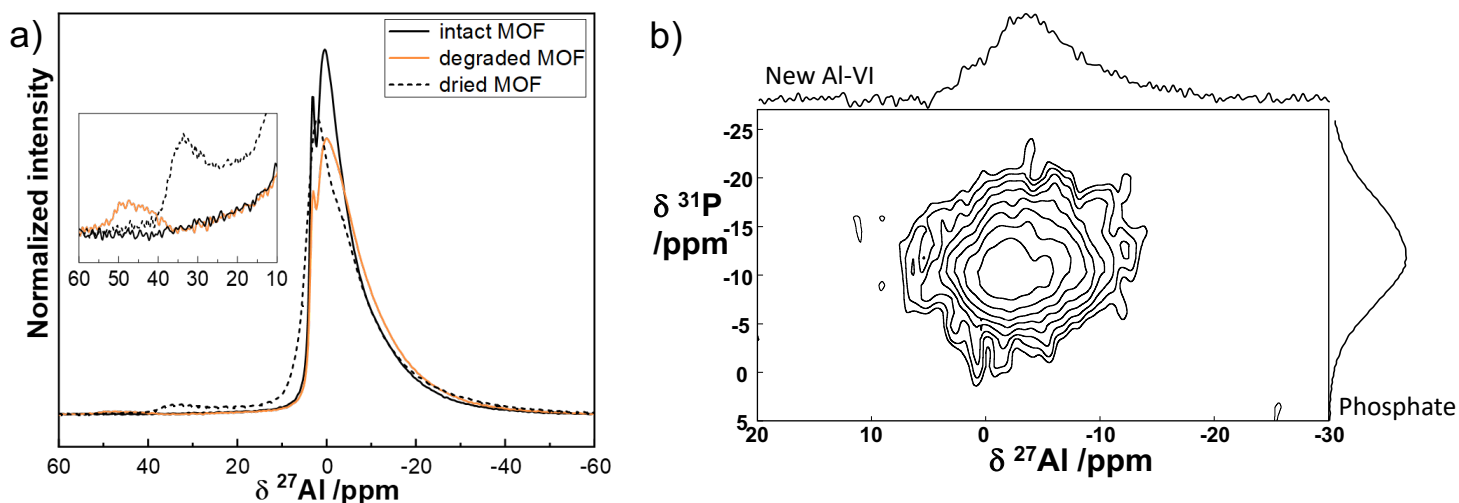
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245 More importantly, solid-state NMR spectroscopy demonstrates significant changes in the
 246 coordination sphere of nanoMIL-100(Al) after 48-hour degradation, indicated by the evolution of
 247 ²⁷Al MAS NMR spectra (**Figure 4a**). The first noticeable change is the growth of the ²⁷Al
 248 resonance around -2 ppm, a chemical shift characteristic of 6-coordinate Al cations coordinated
 249 to phosphate ions, coming from the MIL-100(Al) trimer and the degradation medium PBS,
 250 respectively. The presence of this new coordination mode for the 6-fold aluminum was confirmed
 251 in the ²⁷Al multiple-quantum MAS (MQMAS) NMR spectrum of the degraded nanoparticles
 252 (**Figure 5**). The formation of the Al-O-P bond was further evidenced by the ³¹P-²⁷Al 2D MAS
 253 NMR spectrum (**Figure 4b**), which showed a correlation peak between this ²⁷Al resonance at -2

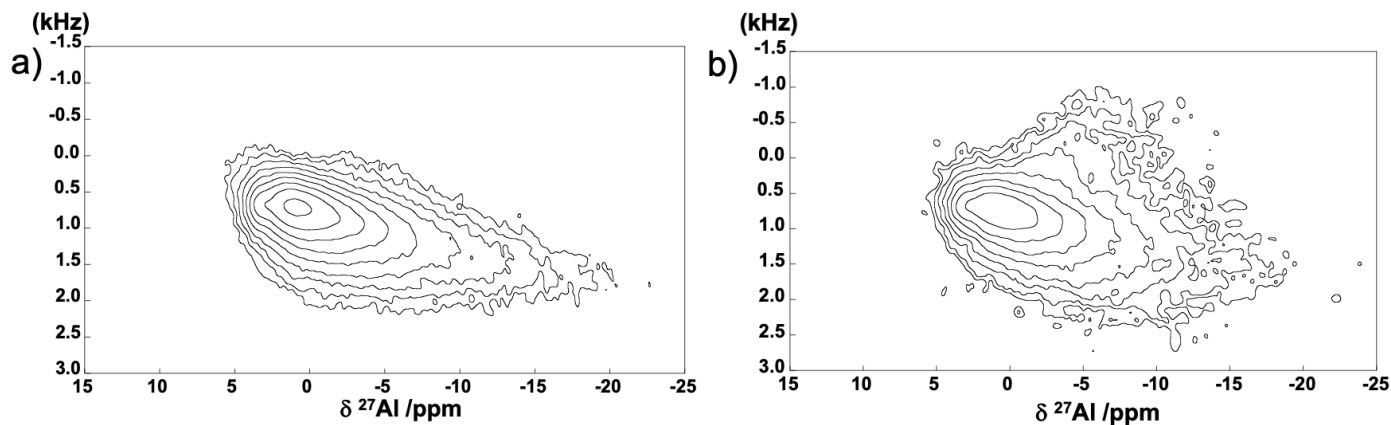
254 ppm and the ^{31}P resonances around -10 ppm. This ^{31}P - ^{27}Al correlation peak indicates close spatial
255 proximity within 5 \AA between these two nuclei, which fits the Al-O-P bond distance. It was also
256 confirmed by ^1H - ^{31}P and ^1H - ^{27}Al 2D NMR spectroscopy (**Figure S12** and **Figure S13**), showing,
257 in the degraded MIL-100(Al), the proximity between the phosphorus and the protons of the linker,
258 and between the new 6-coordinate Al(III) and the trimesate linker. Note that this degraded Al
259 species still remains in 6-coordination state as in the original MIL-100(Al) trimer, indicating that
260 substitution of an existing ligand such as water or trimesate occurred. Trimesate is a stronger Lewis
261 base than water, thus the metal-ligand bond Al-O-H between Al(III) and water is more easily
262 broken than the metal-ligand bond Al-O-C between Al(III) and trimesate. As a result, phosphate
263 ions from PBS should first replace the water ligand and form a strong Al-O-P coordination bond
264 with Al(III) center. Then, due to an increase in interaction between the Al metal center and the
265 newly bonded phosphate ligand, interaction of the Al center with the trimesate ligands decreases.
266 As the connection Al-O-C between carboxylic groups of the trimesate and Al weakens, phosphate
267 can also attack and replace the position of carboxylate. Free trimesic acid is liberated when all 6
268 Al-O-C bonds between a trimesate linker and Al trimers are disconnected.

269

270



271 **Figure 4.** a) ^{27}Al MAS NMR spectrum of intact (solid black line), degraded (orange), and
 272 dehydrated (black dash line) nanoMIL-100(Al). The inset graph shows the 40 ppm region
 273 corresponding to the 4 and 5-coordinate Al(III). b) 2D ^{31}P - ^{27}Al MAS NMR spectrum of degraded
 274 MOF in excess PBS.

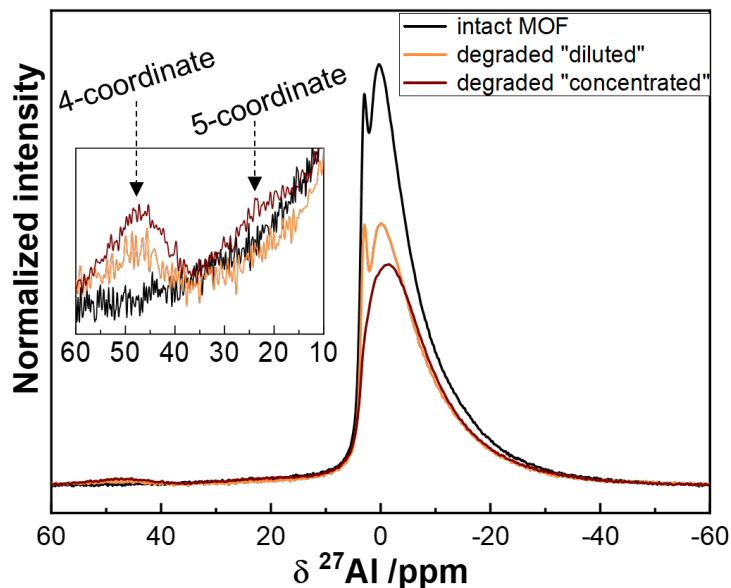


275
 276 **Figure 5.** ^{27}Al MQMAS spectrum of a) original and b) degraded nanoMIL-100(Al)

277
 278 Notably, the 1D ^{27}Al MAS NMR spectrum of the degraded nanoMIL-100(Al) (**Figure 4**) shows
 279 another new Al species at 48 ppm, whose chemical shift lies between the range of 4- and 5-
 280 coordinate aluminum cations. A decrease in the coordination number of aluminum cations in MIL-

281 100(Al) is a known phenomenon observed when heating the sample above 150°C. As in the study
282 of Haouas *et al.*⁵⁵, we also found that the coordinated water ligands were removed by heating, thus
283 creating 5-coordinate Al cations with a ²⁷Al chemical shift of 34 ppm (**Figure 4**). When the
284 dehydrated MIL-100(Al) re-adsorbed water from ambient air, this 5-coordinate Al cation returned
285 to 6-fold coordination without modifying the framework. However, in the degraded MIL-100(Al),
286 the degraded low-coordinate Al had a higher chemical shift of 48 ppm; and this chemical shift did
287 not change when the sample was exposed to air or heated above 150°C (**Figure S11**), implying
288 that this low-coordinate Al site no longer coordinates with water ligands. As Al(III) species bonded
289 with phosphate usually have lower ²⁷Al chemical shifts than the same coordination number Al(III)
290 species with no phosphate ligand due to a deshielding effect in NMR⁵⁶, we hypothesize that these
291 Al species at 48 ppm are 4-coordinate Al with at least one phosphate ligand. Such 4-coordinate-
292 Al is favorable in basic pH but not in neutral pH of PBS⁵⁶, which could explain why these species
293 have very low intensity in the ²⁷Al NMR spectrum. When the nanoMIL-100(Al) was degraded in
294 excess PBS (“diluted” condition), the intensity of the 4-coordinate ²⁷Al peak increased slightly,
295 and even a new 5-coordinate ²⁷Al peak was observed around 22-25 ppm (**Figure 6**). Therefore, we
296 ruled out the possibility of an intermediate product, and we assigned these 4- and 5-coordinate ²⁷Al
297 resonances to an inorganic aluminum phosphate AlPO₄^{58,59,57,58}. As the 4- and 5-coordinate ²⁷Al
298 peaks only account for 1% of the total Al amount, the amount of these degradation products is low,
299 and their formation may happen only at the surface. If these 4- and 5-coordinate aluminophosphate
300 form a non-porous layer, this layer can passivate the nanoMIL-100(Al) surface, prevent phosphate
301 from reacting with nanoMIL-100(Al), thus slowing down the degradation reaction. Blocked access
302 to the nanoMIL-100(Al) was supported by a 10-time decrease in BET surface area (**Figure S3**).

303 The existence of this passivation layer may explain how degradation and trimesate release reached
304 a plateau after 48 hours as determined by HPLC (**Figure 2**).



305
306 **Figure 6.** ^{27}Al MAS NMR of the degraded nanoMIL-100(Al) at the “diluted” and “concentrated”
307 conditions, demonstrating the effect of increasing P/Al_3 ratio on the nanoMOF degradation
308 reaction. The arrows indicate the position of 4- and 5-coordinate Aluminum species

309 The present ssNMR study about nanoMIL-100(Al) so far agrees with the hypothesis of Li *et al.*
310 about nanoMIL-100(Fe)³⁷ that aluminum phosphate/iron phosphate complex is the degradation
311 product of nanoMIL-100 reacting with phosphate buffer PBS. While Li *et al.* could only
312 hypothesize the formation of iron phosphate and iron oxide hematite and goethite³⁷, the present
313 study offers concrete evidence about the structure of the degraded nanoMIL-100(Al) by showing
314 the coordination states of Al and the likely formation of Al-O-P bond in the degradation product.

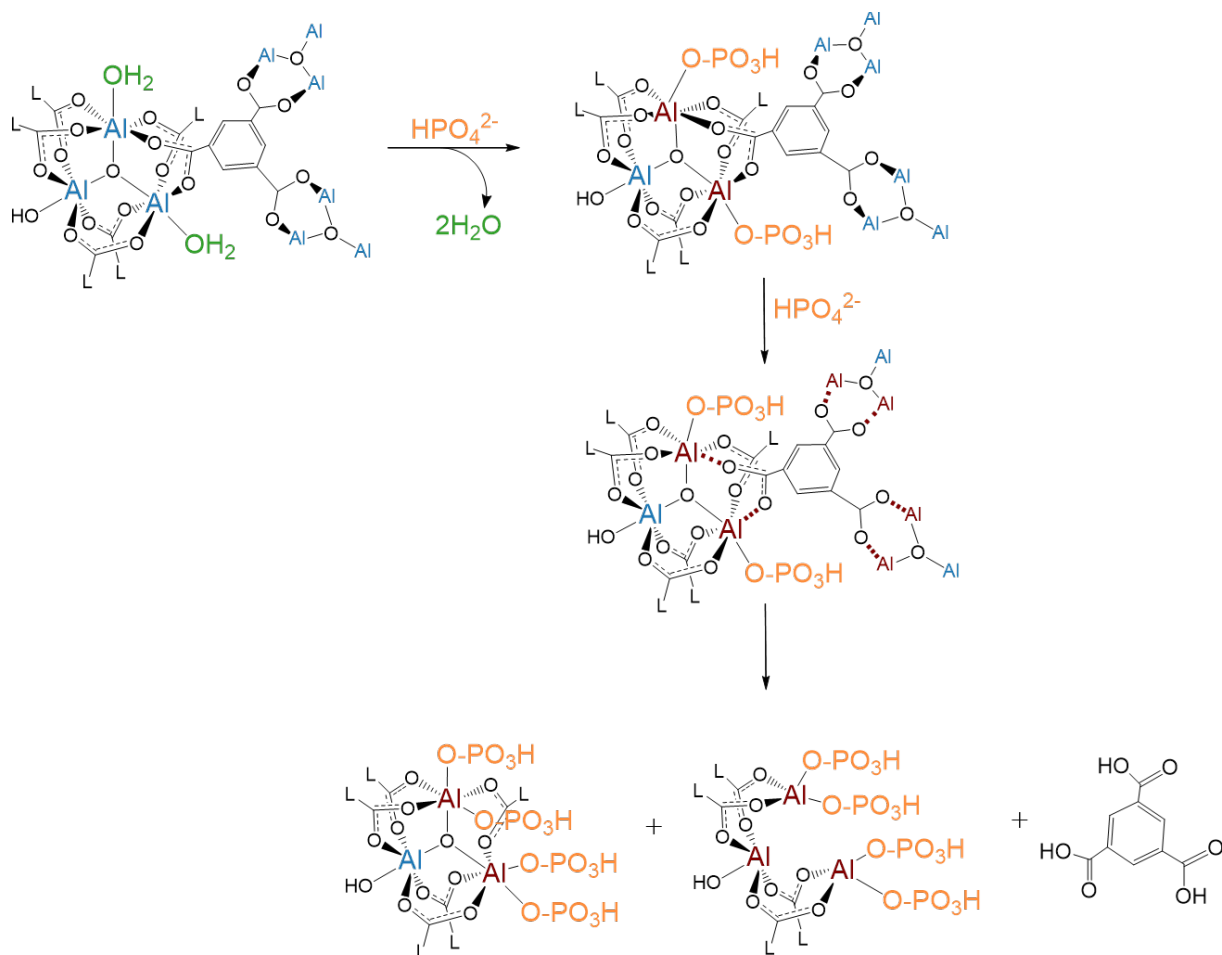
315 To summarize, we propose for the first time a molecular mechanism of nanoMIL-100(Al)
316 degradation in the mimicking biological medium PBS as follows (**Figure 7**):

317 1) Phosphate ions in PBS attack nanoMIL-100(Al) surface, substitute labile water ligands of
318 MIL-100(Al), and form coordination Al-O-P bond with MIL-100(Al). Consequently, the Al-O-C
319 coordination bonds between MIL-100(Al) trimer and trimesate linker are weakened.

320 2) Phosphate ions substitute carboxylate of trimesate linker. As the substitution of water and
321 trimesate continues, the formation of 6-fold aluminum atoms with a new coordination sphere (i.e.,
322 aluminum bonded with 1 to 2 phosphate groups) continues, producing the main degradation
323 products of nanoMIL-100(Al). The release of free trimesic acid starts when all six Al-O-C bonds
324 between a trimesate linker and Al trimers are broken.

325 3) New 4 and 5-coordinate Al species are produced, most likely as a layer of inorganic aluminum
326 phosphate AlPO_4 on the nanoMIL-100(Al) surface, due to cleavage of trimesate linker and the
327 aluminum-oxo center

328 4) After 6 hours, the insoluble aluminum phosphate species AlPO_4 passivate the MOF pore and
329 pore window. The intact trimers of nanoMIL-100(Al) are no longer exposed to a high amount of
330 phosphate. As a result, the degradation reaction reaches equilibrium, and the release of trimesate
331 reaches the maximum.



332

333 **Figure 7.** Proposed degradation mechanism of nanoMIL-100(Al) in phosphate buffer solution

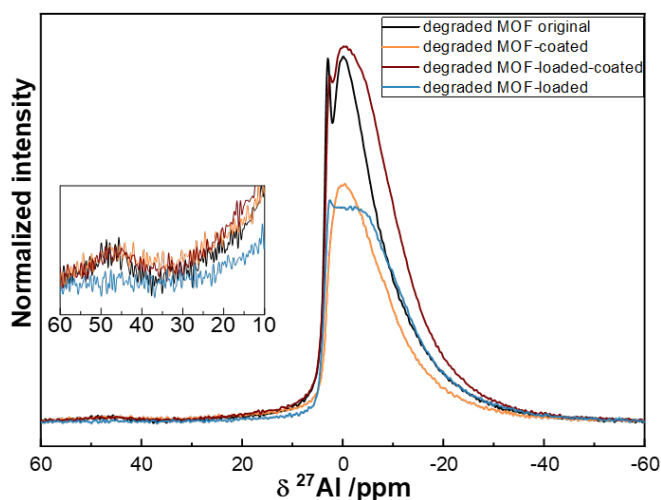
334

335 3.3. Degradation mechanism of drug-loaded and surface-coated nanoMIL-100(Al)

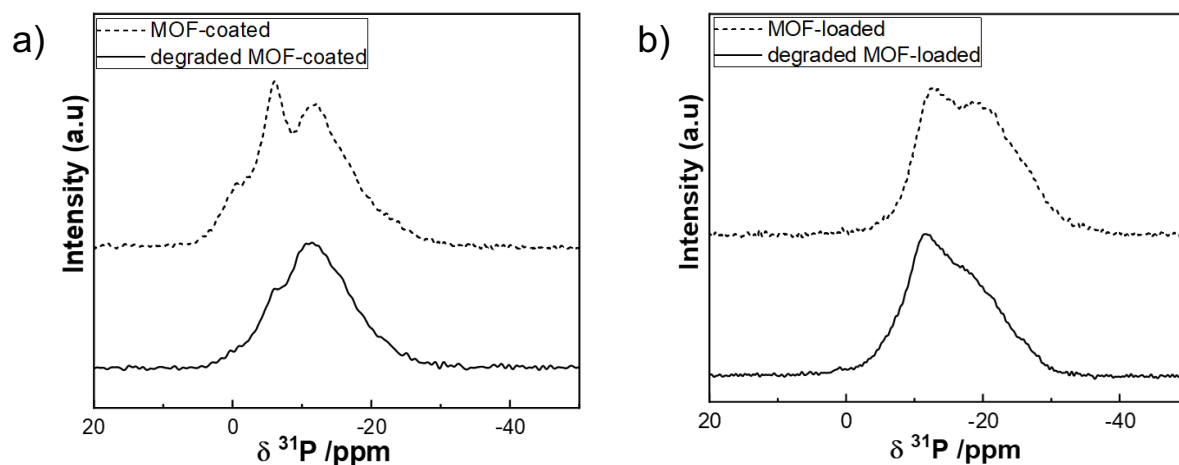
336 In the final step, we analyzed the effect of surface coating and drug loading on the nanoMIL-
 337 100(Al) degradation processes. As reported in the literature, the presence of loaded drug and
 338 surface coating could alter the physicochemical properties of nanoMOF^{21,26}, thus potentially
 339 affecting nanoMOF's degradation reaction.

340 NanoMIL-100(Al) was successfully loaded with adenosine triphosphate (ATP) at 18.6 ± 0.8
 341 wt%, and coated with β -cyclodextrin-phosphate (CD-P) at 28.3 ± 0.4 wt%. Before studying the
 342 degradation, the drug loading and surface coating quality were evaluated. Results of ²⁷Al and ³¹P

343 MAS NMR in **Figure S14a** and **Figure S14b** agree with our previous study²⁴. ³¹P MAS NMR
344 (**Figure S16b**) showed that the drug ATP is captured inside MIL-100(Al) by chemical adsorption
345 through Al-O-P coordination. ²⁷Al MAS NMR (**Figure S16a**) showed that the CD-P coating is
346 located at the surface and does not penetrate the MOF core. The integrity of the trimesate
347 framework was checked by TEM and ¹³C MAS NMR, see **Figure S15** and **Figure S17**,
348 respectively. From now on, we denote in the figures below the CD-P-surface-coated nanoMIL-
349 100(Al) as MOF-coated, the ATP-drug-loaded nanoMIL-100(Al) as MOF-loaded, the ATP-drug-
350 loaded-CDP-surface-coated nanoMIL-100(Al) as MOF-loaded-coated.



351
352 **Figure 8.** ²⁷Al MAS NMR spectra of degraded original and degraded modified nanoMIL-100(Al),
353 presenting the impact of drug encapsulation and surface coating on the nanoMOF degradation



354 **Figure 9.** ^{31}P MAS NMR of a) degraded CD-P-coated MIL-100(Al) compared to CD-P-coated
 355 MIL-100(Al), and b) degraded ATP-loaded-MIL-100(Al) compared to ATP-loaded-MIL-100(Al).
 356 CD-P and ATP are still present in the degraded nanoMOF after 4-5 days of degradation

357 Degradation of the CD-P coated nanoMIL-100(Al) is similar to degradation of the non-coated
 358 one. In **Figure 8**, the ^{27}Al MAS NMR spectrum of the degraded MOF-CDP shows both the hexa-
 359 coordinate Al-O-P around -2 ppm and the tetra-coordinate aluminophosphate at 48 ppm.
 360 Observing a similar degradation product, we speculate that, as CD-P covers approximately 30%
 361 of the nanoMIL-100(Al) surface²⁶, degradation reaction still occurs at the nanoMOF's surface not
 362 attached to CD-P, following the reaction mechanism proposed in the previous section.

363 Additionally, the ^{31}P MAS NMR spectrum of the degraded MOF-CDP in **Figure 9a** displays a
 364 shoulder around -10 ppm which belongs to CD-P, and the dominant signal of the phosphate
 365 coordinated with the MIL-100(Al) framework around -15 ppm. The presence of CD-P coating
 366 even after five days of degradation suggests that CD-P competes with inorganic phosphate ions to
 367 complex with Al(III). Consequently, PBS phosphates can only partially displace CD-P.

368 Meanwhile, the degradation reaction of the ATP-loaded nanoMIL-100(Al) exhibits features
 369 distinctive from that of the empty nanoMOF. No sign of low-coordinated Al was detected. ^{27}Al

370 NMR (**Figure 8**) still showed the 6-coordinate Al species around -2 ppm as the main degradation
371 product, while the Al species bonded with phosphate ATP was still present. Likewise, the ^{31}P MAS
372 NMR spectrum in **Figure 9b** shows both a peak of coordinated PBS phosphate and a shoulder
373 signal of ATP. Both ^{27}Al and ^{31}P NMR data consistently suggest that nanoMIL-100(Al) loaded
374 with ATP is less degraded than the empty one, and only a part of ATP is liberated from the
375 framework. Similar to the surface coating CD-P, the loaded drug ATP competes with inorganic
376 PBS phosphates to coordinate the Al(III) of nanoMIL-100(Al). The triphosphate ATP is a much
377 stronger complexing ligand towards Al(III) than the hydrogen phosphate and dihydrogen
378 phosphate ions⁶⁰, meaning that MIL-100(Al) forms a more stable coordination complex with ATP
379 than with phosphate ions. Therefore, it is difficult to break the coordination between ATP and
380 Al(III) by solely phosphate PBS, which explains the presence of ATP in the ^{31}P NMR spectrum,
381 even after days of degradation. However, the strong Al-O-P bond between Al(III) and ATP makes
382 the Al-O-C bond between Al(III) and trimesate linker more easily attacked by phosphate. We
383 hypothesize that phosphate attacks the Al-O-C bond with trimesate, forming hexa-coordinate Al-
384 O-P in the region -2 to -5 ppm observed in the ^{27}Al spectrum. When the Al center forms a new
385 coordination bond with inorganic phosphate, its coordination bond with ATP is weakened. As a
386 result, the probability is higher for phosphate to displace ATP from the framework. Another factor
387 to be considered is the steric effect caused by ATP. Bulky ATP possibly hinders phosphate
388 diffusion in the MIL-100(Al) trimer, thus preventing trimesate substitution.

389 In addition, the notable absence of the low-coordinate aluminophosphate can be explained by
390 the chelating capability of triphosphate ATP^{61,62}. ATP coordinates with MIL-100(Al) through the
391 terminal γ -phosphorus. During the breaking of the Al-O-C bond, the β -phosphorus of ATP can
392 compete with phosphate PBS ions and create a second coordination bond with Al. The bidentate

393 Al-ATP coordination not only halts the formation of low-coordinate Al but also prevents the 100%
394 release of ATP. Consistently, only 60% ATP was released in excessive PBS, while 100% of
395 trimesate ligand was found by HPLC (data not shown).

396 Finally, combining the effect of drug encapsulation and surface coating, **Figure 8** shows that
397 degradation of the ATP-loaded CD-P-surface-coated nanoMIL-100(Al) produces both 6-
398 coordinate aluminophosphate complex and 4-coordinate inorganic aluminophosphate, but with a
399 slightly lower proportion of 4-coordinate degraded complex (1.0%) than that in the surface-coated
400 empty nanoMIL-100(Al) (1.6%). Also, the ^{27}Al peak at 2.5 ppm belonging to the original
401 nanoMIL-100(Al) is more preserved in the degraded one in the presence of the drug ATP.

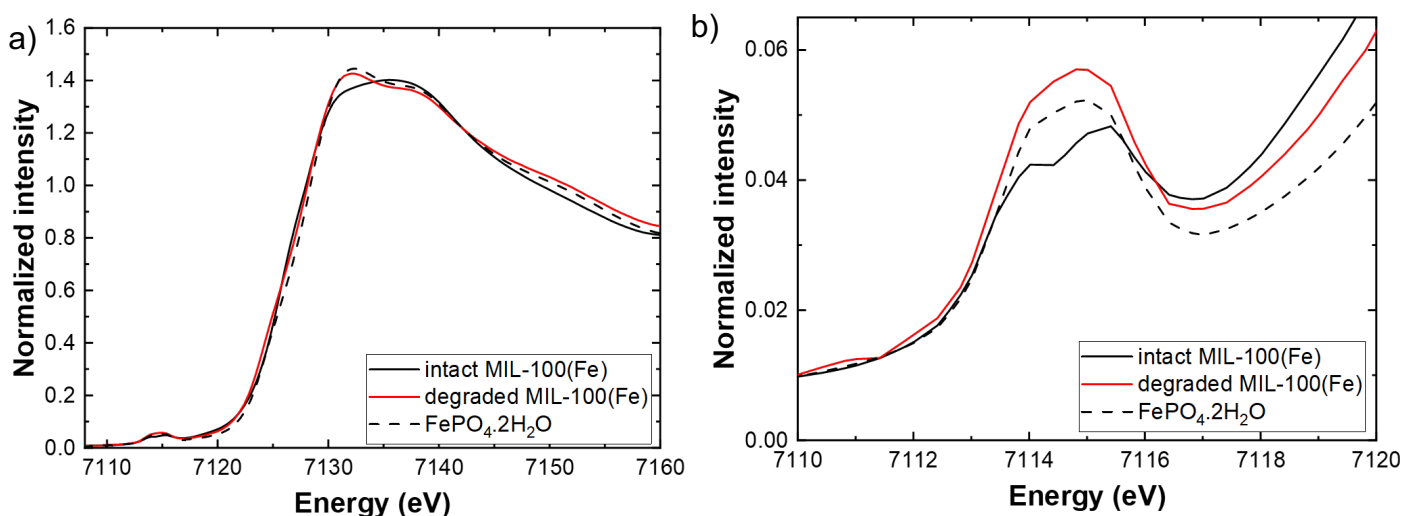
402 In summary, the degradation reaction of the modified MOF including the ATP-loaded and the
403 CD-P-surface coated nanoMIL100(Al) can be summarized as follows:

404 1) Surface coating with CD-P has a negligible effect on the degradation of nanoMIL-100(Al).
405 6-coordinate and 4-coordinate aluminum complexes with phosphate are formed as described in the
406 degradation mechanism of the original nanoMIL-100(Al)

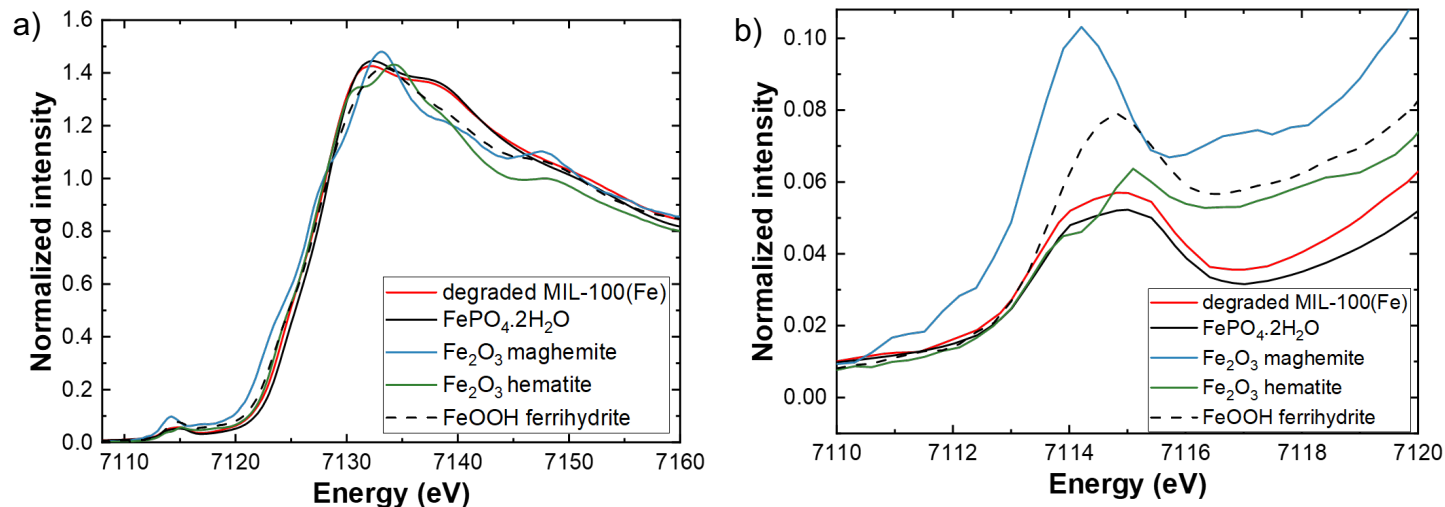
407 2) Encapsulation of ATP inside nanoMIL-100(Al) impacts significantly the nanoMOF
408 degradation. Degradation of ATP-loaded nanoMIL-100(Al) starts with the substitution of
409 trimesate Al-O-C bond, thus producing hexa-coordinate aluminophosphate and free trimesate.
410 However, the bulky ATP limits the amount of trimesate release. The coordination of PBS
411 phosphate with the Al trimer facilitates the dissociation of the Al-O-P bond with ATP. Then,
412 phosphate from PBS replaces the bonded ATP, leading to the release of free ATP. During the bond
413 dissociation process, the coordinated ATP can make the second coordination bond with the
414 degraded Al trimer. Consequently, no low-coordinated Al is created, and ATP is not entirely
415 released.

416 3.4. Degradation mechanism of nanoMIL-100(Fe), in comparison with nanoMIL-100(Al)

417 To evaluate whether our proposed degradation mechanism applies to the iron MIL-100 analog
418 nanoMIL-100(Fe), XANES spectroscopy was used to study the *ex situ* degradation of the original,
419 CD-P surface-coated, and ATP-loaded nanoMIL-100(Fe). Similar to nanoMIL-100(Al),
420 nanoMIL-100(Fe) was incubated in PBS at 37°C, then the degraded nanoparticles were recovered
421 after 48 hours. A “very diluted” condition: 0.5 mg.mL⁻¹ nanoMIL-100(Fe) in PBS 10 mM was used
422 to produce as much as possible degraded MIL-100(Fe) species.



423 **Figure 10.** a) Normalized Fe K-edge XANES spectra, and b) zoom on pre-edge region of degraded
424 nanoMIL-100(Fe) compared with original nanoMIL-100(Fe) and iron(III) phosphate dihydrate

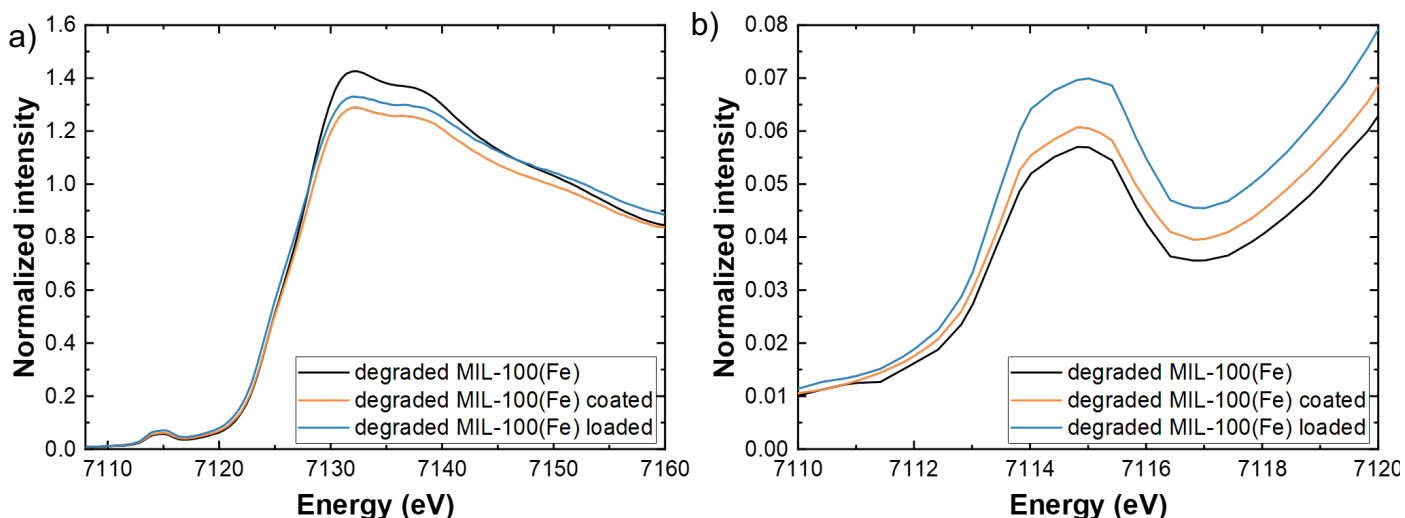


425 **Figure 11.** a) Normalized Fe K-edge XANES spectra, and b) zoom on the pre-edge region of
 426 degraded nanoMIL-100(Fe) compared with several Fe(III) possible degradation products, iron
 427 phosphate, iron oxides, and iron oxyhydroxide

428 The evolution of original nanoMIL-100(Fe) after degradation is shown in **Figure 10**. The
 429 XANES Fe K-edge spectrum of the degraded nanoMIL-100(Fe) is reminiscent of that of iron (III)
 430 phosphate dihydrate, a substance having 6-coordinate iron (III) bonds with phosphate through Fe-
 431 O-P bond⁶³. This similarity is also observed in the pre-edge region, where the intact MIL-100(Fe)
 432 shows two features at 7114 and 7115.4 eV, while the degraded MIL-100(Fe) and FePO₄·2H₂O
 433 only show a single, broad peak at 7114.9 eV. Moreover, the XANES spectrum of this degraded
 434 nanoMIL-100(Fe) is different from those of iron (III) oxide and iron (III) oxyhydroxide, excluding
 435 their presence in the degraded nanoMIL-100(Fe) (**Figure 11**). Therefore, we propose that the first
 436 step of nanoMIL-100(Fe) degradation also forms an iron-phosphate-6-coordinate species with Fe-
 437 O-P coordination bond, in the same way as illustrated in **Figure 7**, where phosphate from PBS
 438 replaces water ligand of nanoMIL-100(Fe) and coordinates with Fe(III). The 4-coordinate iron
 439 phosphate species were not detected due to its low amount, probably 1% of iron in the degraded
 440 nanoMIL-100(Fe). Note that nanoMIL-100(Fe) also became less porous after degradation (**Figure**

441 **S18**) with a smaller decrease (50% decrease) in the BET surface area than in nanoMIL-100(Al)
442 (90% decrease).

443 In addition, after degradation reaction, the oxidation number of Fe in nanoMIL-100(Fe) does not
444 change and remain as +III, as the degraded and original nanoMIL-100(Fe) have the same edge
445 position (**Figure 10a**), and the edge position of nanoMIL-100(Fe) is close to the values of Fe(III)
446 compounds (**Figure S19**). Previously observed by other authors using Mossbauer spectroscopy³⁷,
447 this maintenance of Fe oxidation number after nanoMIL-100(Fe) degradation is consistent with
448 our proposed degradation mechanism based on the model nanoMIL-100(Al) (**Figure 7**) which
449 describes degradation as an acid-base reaction.



450 **Figure 12.** a) Normalized Fe K-edge XANES spectra of degraded original and modified nanoMIL-
451 100(Fe), and b) zoom on the pre-edge region

452 The effect of ATP drug loading and CD-P surface coating on the nanoMIL-100(Fe) degradation
453 was also investigated. **Figure S20** shows that the ATP-drug-loaded and CD-P-surface-coated
454 nanoMIL-100(Fe) share the same XANES features as the original one, indicating that drug loading
455 and surface coating do not degrade nanoMIL-100(Fe). **Figure 12** shows that degradation of drug-
456 loaded, and surface-coated nanoMIL-100(Fe) produces similar species as obtained from

457 degradation of original one, as all three XANES and pre-edge spectra have similar shapes and
458 features. Once again, XANES spectroscopy shows that 6-coordinate iron-phosphate was formed
459 during the degradation reaction of nanoMIL-100(Fe).

460 This complementary study with XANES suggests that the first step in degradation of nanoMIL-
461 100(Fe) likely follows the degradation reaction of its aluminum analog, corroborating the ssNMR
462 study on nanoMIL-100(Al).

463

464 4. Conclusions

465 By using solid-state NMR spectroscopy, we obtained novel knowledge about the degradation
466 mechanism of nanoMIL-100(Al) and nanoMIL-100(Fe) in phosphate buffer. For the first time, we
467 find direct evidence about the change of the metal coordination sphere and the limiting reaction
468 step in the nanoMIL-100 degradation mechanism. First, upon substitution of labile water ligands
469 by phosphate, Al-O-P coordination bonds are formed between aluminum species at the surface of
470 nanoMOF and phosphate from the medium, creating 6-coordinate aluminophosphate. Second, the
471 substitution of trimesate by phosphate leads to the release of free trimesate and creates 4-coordinate
472 inorganic aluminum phosphate. This 4-coordinate aluminum, which has not been observed before,
473 can passivate the nanoMOF surface and slow down the degradation reaction. Additionally, our
474 study demonstrates that chemical modification such as drug encapsulation and surface coating can
475 impact the nanoMOF degradation, hence its drug release process, as a function of metal-ligand
476 bond strength.

477 To conclude, insights about the degradation mechanism of nanoMOF from this work contribute
478 to the understanding of MOF chemistry and help optimize the performance of nanoMOF drug
479 carriers. This study also highlights the useful role of ssNMR spectroscopy with the support of

480 XANES spectroscopy in investigating in depth the nanoMOF structure. Using ssNMR
481 spectroscopy, we were able to obtain enough evidence about the coordination sphere and the
482 reaction limiting step of nanoMIL-100(Al) degradation, whilst XANES spectroscopy provided
483 complementary proof on the reaction of nanoMIL-100(Fe). The obtained knowledge in this study
484 prompts new studies on understanding drug delivery chemistry and developing characterization
485 tools for drug nanocarrier. Comparative studies of MIL-100(Al) and MIL-100(Fe) are being
486 carried out to investigate the behaviors of MIL-100 analogs. Various types of drug compounds can
487 be explored when different host-guest interactions and degradation behaviors are expected. In the
488 future, we will develop a new NMR spectroscopy method to monitor *in situ* drug delivery
489 processes of nanoMOF.

490

491 ASSOCIATED CONTENT

492 **Supporting Information.** The supporting information contains: 1) experimental procedures of
493 nanoMOF preparation and characterization, 2) standard characterization of nanoMIL-100(Al):
494 DLS, TEM, XRD, BET, TGA, ¹H MAS NMR, ¹³C MAS NMR, ²⁷Al MAS and MQMAS NMR
495 spectra, 3) complementary NMR measurements on degraded nanoMIL-100(Al):, ³¹P, ¹H-³¹P, ¹H-
496 ²⁷Al. The file is available free of charge *via* the Internet at <http://pubs.acs.org>

497

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513 **Author Contributions**

514 C.M.C. and R.G. conceived the idea and designed the experiments. M.V. and I.C. synthesized

515 and characterized the materials. M.P. performed the high field MAS NMR spectroscopy

516 experiments. M.V., B.L-K., and S-T.D. performed and analyzed XANES spectroscopy

517 measurement. M.V. and C.M.C. analyzed data. All authors participated in the data discussion.

518 M.V. wrote the manuscript, and all authors revised the manuscript. All authors have approved

519 the final version of the manuscript.

520

521 **Notes**

522 The authors declare no competing financial interest.

523

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532

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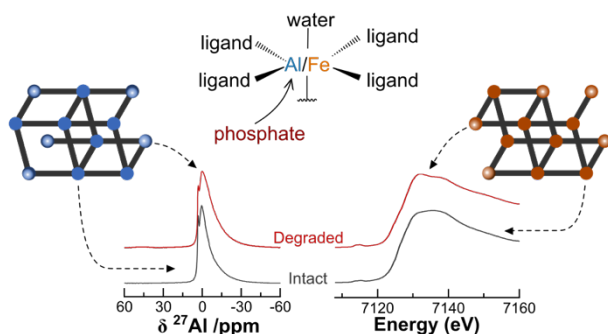
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