Impact of phosphorylation over the encapsulation of nucleosides analogues within porous iron(III) Metal Organic Frameworks MIL-100(Fe) nanoparticles.

Supplementary Information

Valentina Agostoni,a # Resmi Anand,b # Sandra Monti, b Shaun Hall, c
Guillaume Maurin, c Patricia Horcajada, d Christian Serre, d Kawthar Bouchemal, a
Ruxandra Gref a *

# equally contributing authors

* Corresponding author

a Faculté de Pharmacie, UMR 8612 CNRS, Université Paris-Sud, Châtenay-Malabry, France.
b CNR-Istituto per la Sintesi Organica e la Fotoreattività, Bologna, Italy.
c Institut Charles Gerhardt Montpellier, UMR 5253 CNRS, UM2, UM1, ENSCM, Montpellier, France.
d Institut Lavoisier, UMR 8180 CNRS Université de Versailles St Quentin en Yvelines, Versailles, France.
### 1. Kinetics of encapsulation of different AZT derivatives at different payloads

**Tab. S1:** Kinetics of Encapsulation of different AZT-TP payloads within MIL-100 nanoMOFs defined as encapsulation efficiency (EE, %) and drug payload (Payload, wt%).

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>TL =8 wt%</th>
<th></th>
<th>TL =19 wt%</th>
<th></th>
<th>TL =25.4 wt%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
</tr>
<tr>
<td>0.5</td>
<td>98.9 ±0.3</td>
<td>18.9 ±0.1</td>
<td>77.8 ±3.2</td>
<td>19.7 ±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>99.4 ±0.4</td>
<td>18.9 ±0.1</td>
<td>85.3 ±4.8</td>
<td>21.6 ±1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>97.1 ±2.9</td>
<td>18.5 ±0.5</td>
<td>89.1 ±2.8</td>
<td>22.6 ±0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>99.5 ±0.5</td>
<td>19 ± 0.1</td>
<td>93.4 ±2.4</td>
<td>23.7 ±0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tab. S2:** Kinetics of Encapsulation and AZT-MP payloads within MIL-100 nanoMOFs defined as encapsulation efficiency (EE, %) and drug payload (Payload, wt%).

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>TL =8 wt%</th>
<th></th>
<th>TL =19 wt%</th>
<th></th>
<th>TL =25.4 wt%</th>
<th></th>
<th>TL =40 wt%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
<td>EE (%)a</td>
<td>Payload (wt %)b</td>
</tr>
<tr>
<td>0.5</td>
<td>99.5 ±0.5</td>
<td>19 ±0.1</td>
<td>100 ±0.1</td>
<td>25.4 ±0.02</td>
<td>87.8 ±1.7</td>
<td>35.1 ±0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>99.4 ±0.7</td>
<td>19 ±0.1</td>
<td>99.9 ±0.1</td>
<td>25.4 ±0.1</td>
<td>94 ±8</td>
<td>37.6±3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>99.2 ±0.5</td>
<td>18.9 ±0.1</td>
<td>99.8 ±0.1</td>
<td>25.3 ±0.1</td>
<td>88.3 ±0.1</td>
<td>35.3±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>99.5 ±0.7</td>
<td>19 ±0.1</td>
<td>100 ±0.1</td>
<td>25.4 ±0.01</td>
<td>94.3 ±7.5</td>
<td>37.3±3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Tab. S3:** Kinetics of Encapsulation and AZT payloads within MIL-100 nanoMOFs defined as encapsulation efficiency (EE, %) and drug payload (Payload, wt%).

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>EE (%)*</th>
<th>Payload (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>8.7 ±6.2</td>
<td>1.2 ±0.8</td>
</tr>
<tr>
<td>4</td>
<td>8.5 ±3</td>
<td>1.1 ±0.4</td>
</tr>
<tr>
<td>8</td>
<td>9.1 ±2.1</td>
<td>9.1 ±2.1</td>
</tr>
<tr>
<td>24</td>
<td>9.1 ±3.7</td>
<td>1.2 ±0.5</td>
</tr>
</tbody>
</table>

* encapsulation efficiency = \( EE(\%) = \frac{\text{encapsulated Drug (mg)}}{\text{Drug Solution (mg)}} \times 100 \%

* payload = \( \text{Payload (wt\%) = } \frac{\text{encapsulated Drug (mg)}}{\text{MIL - 100 nanoMOFs (mg)}} \times 100 \%

**TL = 13.4 wt%**
2. NanoMOFs interaction with aqueous solutions of a phosphate salt evaluated by ITC

**Fig. S1:** AZT-MP (5 mM) dilution in water (red) or titration into a nanoMOFs aqueous solution 0.5 mM (black).

**Fig. S2:** MIL-100 nanoMOF (0.5 mM) binding isotherms of a K$_2$HPO$_4$ aqueous solution 5 mM (A) or 10 mM (B).
3. UV-visible absorption and circular dichroism titration of AZT-TP with nanoMOFs in PBS

**Fig. S3:** UV-visible absorption (A) and circular dichroism (B) titration of AZT-TP with MOF in PBS buffer (10^{-2} M, pH 6.5) at 22°C, cell pathlength 0.5cm, T=22 °C. Color code: drug alone, black solid; drug mixed with MOF, colored solid: 0.0 3 (red), 0.1 (green), 0.2mg/ml (blue). MOF alone (same color code, dotted lines).

4. UV-visible absorption and circular dichroism titration of AZT-TP with nanoMOFs in PBS

**Fig. S4:** Plot of reciprocal $\Delta \theta$ at $\lambda_{max}$ vs. reciprocal iron(III) trimer concentration after 24 h from preparation of the mixtures of AZT-TP (black) or AZT-MP (red) $1 \times 10^{-4}$ M and MIL-100 nanoMOFs from 0.02 to 0.20 mg/mL, in water, cell path 0.2 cm, T = 22°C.
Available Iron sites occupancy
Zeotype architecture MTN = 2[5^{12}] + [5^{12}.6^{4}]

[5^{12}] = small mesoporous cages (SC) delimited by 12 microporous pentagonal windows
[5^{12}.6^{4}] = large mesoporous cages (LC) delimited by 12 microporous pentagonal windows and 4 microporous hexagonal windows

20: Iron trimers/SC
28: Iron trimers/LC

\[ \text{Iron trimers}_{LC} \% = \frac{\text{Iron trimers}_{LC}}{\text{Iron trimers}_{MIL100}} \times 100 \% = \frac{28}{2[20] + 28} \times 100 \% = 41.18 \% \]

i) Iron sites occupancy by AZT-TP

Maximum experimental AZT-TP payload = 24.4 wt %
Molar weight (Mw) MIL-100 per iron trimer = 653 g mol\(^{-1}\)
Mw AZT-TP = 507.2 g mol\(^{-1}\)

The number of mol of AZT-TP per mol of trimer is deduced from:

\[ \frac{\text{AZTTP}_{mol}}{\text{MIL100 trimers}_{mol}} \text{ ratio} = 0.314 \]

This gives 31.4 AZT-TP molecules/100 trimers\(_{MIL100}\)

Considering that only the large cages are accessible to the drug, there are:

31.4 AZT-TP/41.18 trimers\(_{LC}\) = 0.76 AZT-TP molecules/ trimers\(_{BC}\)

This means that there is roughly one AZT-TP molecule interacting with each iron trimers within the large cages.
As each BC contains 28 trimers, there are about 21 AZT-TP molecules/LC

ii) Iron sites occupancy by AZT-MP
Maximum experimental AZT-MP payload = 36 wt %

Molar weight (Mw) MIL-100 per iron trimer = 653 g mol⁻¹

Mw AZT-TP = 381.28 g mol⁻¹

The number of mol of AZT-TP per mol of trimer is deduced from:

\[
\frac{AZT\text{MP}_{\text{mol}}}{\text{MIL100 trimers}_{\text{mol}}} = 0.617
\]

This gives 61.7 AZT-TP molecules/100 trimers MIL100

Considering that only the large cages are accessible to the drug, there are:

61.7 AZT-TP/41.18 trimers LC = 1.5 AZT-TP molecules/ trimers BC

Considering that two over three iron metal sites are accessible per trimer (the third site being occupied by oxide group) this means that there is roughly 1.5 AZT-TP molecule interacting with both the available metallic site per each iron trimer within the large cages.

As each BC contains 28 trimers, there are about 42 AZT-MP molecules/LC