Zn\textsuperscript{2+}-Triggered Drug Release from Biocompatible Zirconium MOFs Equipped with Supramolecular Gates

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A new theranostic nanoparticle, comprising of monodisperse zirconium metal-organic frameworks (MOFs) as drug carriers and carboxylatopillar[5]arene-based supramolecular switches as gating entities, is constructed, and controlled drug release triggered by bio-friendly Zn\textsuperscript{2+} ions (abundant in synaptic vesicles) and auxiliary thermal stimulus is realized. This on-command drug delivery system exhibits large pore sizes for drug encapsulation, excellent biodegradability and biocompatibility, extremely low cytotoxicity and premature drug release, and superior dual-stimuli responsiveness, opening a new avenue in targeted drug delivery and controlled release of therapeutic agents, especially in the treatment of central nervous system diseases.

1. Introduction

Zinc ion, as the second most abundant trace element in human body, plays “ubiquitous biological roles” in maintaining the immune function, gene expression, apoptosis, enzyme regulation, sterilization, treatment of cancer, structure and function of myriad proteins involved in transcription, neurosecretory product or cofactor, protein trafficking, etc.\textsuperscript{[1]} The brain has the highest zinc content with respect to other organs: in brain extracellular fluids, free zinc is approximately 500 n\textsuperscript{M} in concentration; however, in the synaptic vesicles of some neurons called “zinc-containing” neurons, it probably exceeds 1 \times 10\textsuperscript{−3} M of concentration with only weak coordination with any endogenous ligand.\textsuperscript{[2]} Zinc has a powerful effect on the central nervous system (CNS), influencing the processes such as oxidative stress, apoptosis, immune defense, motor coordination, neurogenesis, memory, and synaptic plasticity.\textsuperscript{[3]} Zinc dyshomeostasis is a pathological feature of depression, Parkinson’s disease (PD), Alzheimer’s disease (AD), autism spectrum disorders, epilepsy (EL), amyotrophic lateral sclerosis (ALS), and schizophrenia.\textsuperscript{[4]} The elevated Zn\textsuperscript{2+} level in AD, PD, EL, diabetes, cancer, etc. provides a new perspective for targeted drug delivery\textsuperscript{[5–10]} and alleviates the limitation of traditional treatment for CNS diseases, such as untargeted treatment that may cause side effects and difficulty to be tuned according to patients.\textsuperscript{[11,12]}

On the other hand, metal-organic frameworks (MOFs)\textsuperscript{[13–16]} are gaining momentum recently, in particular from drug delivery and storage, attributing to their enormous porosity, high surface area, good biodegradability and biocompatibility, versatile functionality, and a wide array of potentials in cell imaging.\textsuperscript{[17–19]} UiO-66-NH\textsubscript{2}, a typical type of zirconium (Zr) based MOFs, contains linear 2-amino-1,4-benzenedicarboxylate (BDC-NH\textsubscript{2}) ligands and Zr\textsubscript{6}O\textsubscript{4}(OH)\textsubscript{4} clusters as 12-connected nodes, and has garnered increasing attention due to the excellent stable structure and successful post-synthetic modification (PSM).\textsuperscript{[20]} In the microporous framework (see the Supporting Information), each centric octahedral cage (free diameter: \textapprox 11 Å) connects with eight

DOI: 10.1002/smll.201500155
corner tetrahedral cages (free diameter: \(\approx 8\) Å) by means of trigonal windows (\(\approx 6\) Å).\(^{[21]}\) Zr, a silvery, lustrous, and strong transition metal, is widely distributed in nature and found in all biological systems and unique presence in the human brain, often in appreciable amounts.\(^{[22]}\) The promising mechanical, biocompatible characteristics, and extremely low toxicity of Zr compounds boost the burgeoning biomedical applications of Zr-containing agents\(^{[23]}\) and significantly Zr-based MOFs possess a wide array of potential applications in controlled drug delivery and storage.\(^{[24–26]}\)

The emergence of pillarenes (or pillararenes)\(^{[27–32]}\) has been one of the most significant achievements in supramolecular chemistry and materials science over the past 7 years. The superior structures, facile functionalization, and typical host–guest properties of pillarenes stimulated a tremendous upsurge of interest in artificial transmembrane channels,\(^{[33,34]}\) drug nanocarriers,\(^{[35–40]}\) gas sorption,\(^{[41,42]}\) MOFs,\(^{[43]}\) sensing and detection,\(^{[44]}\) stabilization of nanoparticles, biological applications,\(^{[45,46]}\) etc.\(^{[47–51]}\)

Herein, benign activation mechanisms have been applied to targeted drug release systems constructed from monodisperse nanoMOFs (NMOFs) and carboxylato-pillar[5]arene (CP5)-based stimuli-responsive supramolecular gates for CNS disease therapy for the first time (Figure 1). External heating as an important treatment of physical therapy in modern and traditional medicine has been introduced to regulate drug release from the gated nanocarriers. Meanwhile, Zn\(^{2+}\)-triggered drug release with large pore sizes for drug encapsulation, extremely low premature release, good biodegradability and biocompatibility, low cytotoxicity, and potential for imaging are especially advantageous for brain disease therapy. We envision that this new hybrid nanomaterial, which combines physical and natural biochemical treatment in one pot, will prove to be a novel drug delivery platform for CNS disease therapy in the near future.

### 2. Results and Discussion

#### 2.1. Preparation of CP5-Gatekeeper Mechanized NMOFs and Characterization

Figure 1 shows the operation of CP5-based mechanized UiO-66-NH\(_2\) nanocarrier systems. The synthesis of scaffold UiO-66-NH\(_2\) was conducted according to the literature report\(^{[20]}\) (Supporting Information). UiO-66-NH\(_2\) was first modified with positive charged quaternary ammonium salt (Q) stalks via PSM. Following the entrapment of 5-fluouracil (Fu) in MOF pores, the MOFs’ surfaces were capped with negatively charged CP5 rings through host–guest complexation with the Q stalks to form [2]pseudorotaxanes as the gating entities of the nanocarriers, thereby realizing the drug encapsulation and gate installation.

The peaks of Q and \(-\text{NH}\) in the \(^1\)H NMR spectra (Figures S5, S6, Supporting Information) and the peak of BDC-NH-Q in the electro-spray ionization mass spectrometry (ESI-MS, Figure S7, Supporting Information) certified that the Q stalks were successfully tethered to UiO-66-NH\(_2\), and 41% of NH\(_2\) groups of UiO-66-NH\(_2\) were reacted with Q stalks. Thermo-gravimetric analysis (TGA) indicated that UiO-66-NH-Q is thermally stable below 470 °C (Figure S9, Supporting Information). Meanwhile, powder X-ray diffraction (PXRD) patterns (Figure S8, Supporting Information) indicate that the porous scaffolds are still in the same crystal structure despite a slight decrease in intensity and a broadening of the (110) and (200) peaks caused by PSM. To further test the microcrystallinity of our newly synthesized materials, high-resolution transmission electron microscopy (HRTEM) images and electron-diffraction patterns were obtained (Figure 2), which confirmed that both UiO-66-NH\(_2\) and Fu-loaded, CP5-capped UiO-66-NH-Q had well-defined crystalline planes with interplanar \(d\) spacing of 0.298 nm, corresponding to the lattice spacing of (222) planes, and the
single-crystalline nature of these nanoparticles was vividly demonstrated by electron-diffraction patterns. Furthermore, the surface areas, pore volumes, and pore size distributions of UiO-66-NH₂ and UiO-66-NH-Q were analyzed from N₂ sorption isotherms for activated MOFs at 77 K (Figure 3). A Brunauer–Emmett–Teller (BET) surface area of 889 m² g⁻¹ and pore volume of 0.98 cm³ g⁻¹ were calculated by DFT for UiO-66-NH₂. The pore size distribution of UiO-66-NH₂ (Figure S11, Supporting Information) obtained by the non-localized DFT (NLDFT) method showed two main sharp peaks at about 0.6 nm and 1.0 nm. BET surface area and pore volume of UiO-66-NH-Q were calculated to be 686 m² g⁻¹ and 0.89 cm³ g⁻¹, pore size distribution of UiO-66-NH-Q (Figure S13, Supporting Information) showed two main sharp peaks at ≈0.6 nm and 1.1 nm. The little difference of these data of these two MOFs (Table 1) suggests that the pores and the structure of the nanoparticles were stable enough for grafting Q units onto the openings of the pores by covalent bonds; hence, UiO-66-NH-Q can be a promising candidate as drug carriers to construct mechanical nanovalves by further combining with supramolecular switches.

The morphology, size, dispersity, and surface texture of these nanoparticles were measured by scanning electron microscope (SEM), dynamic light scattering (DLS), and zeta potentials. UiO-66-NH₂, UiO-66-NH-Q, and Fu-loaded, CP5-capped UiO-66-NH-Q exhibited positive surface charges (Table 2), the zeta potential of Fu-loaded, CP5-capped UiO-66-NH-Q was measured to be ≈15.0 mV that also indicates that the newly constructed drug delivery system can not only maintain certain stability but also strong enough to transport drugs in biological media. The average diameter of the MOFs (0.1 mg mL⁻¹ in water) was calculated by DLS. The average particle size in solution became larger after surface modification and CP5 capping (Table 2). The average particle diameter of Fu-loaded, CP5-capped UiO-66-NH-Q was calculated to be ca. 330 nm, which was bigger than that by SEM because it was the hydration diameter instead of the exact real diameter. Interestingly, the nanoparticles are within the size range that can easily be taken up by cells (100–200 nm) as evidenced by SEM (Figure 2c), which makes the mechanical nanocarriers constructed from UiO-66-NH-Q and CP5 excellent candidates for targeted drug delivery.

2.2. Zn²⁺ and Thermal Dual Stimuli-Triggered Drug Release Profile

Over-expressed Zn²⁺ ions, which have a higher binding affinity (Kₐ = 391.9 m⁻¹, Figure S22, Supporting Information) toward CP5 in 2:1 stoichiometry, in synaptic vesicles can act as a competitive binding agent to regulate the supramolecular nanovalves to release Fu from the pores of NMOFs to diseased areas as a result of the Zn²⁺-induced dethreading of the CP5 rings from the Q stalks (Kₐ = 164.4 m⁻¹, Figure S18,

| Table 1. Surface areas, pore volumes, and pore size distributions of UiO-66-NH₂ and UiO-66-NH-Q. |
|---------------------------------|-----------------|-----------------|
|                                | UiO-66-NH₂      | UiO-66-NH-Q      |
| S_{BET} [m² g⁻¹]               | 889             | 686             |
| Vₚ [cm³ g⁻¹]                   | 0.98            | 0.89            |
| Pore size distribution [nm]    | 0.6, 1.0        | 0.6, 1.1        |
Supporting Information). The release curves of Fu-loaded, CP5-capped NMOFs are presented in Figure 4a. When the concentration of Zn$^{2+}$ (in vitro) is comparable to the concentration in the extracellular fluids, only 5% of Fu was released, indicating that Zn$^{2+}$ could trigger the systems with extremely low premature release. To investigate the influence of concentrations and kinetics on the cargo release systems, Zn$^{2+}$-triggered drug release was monitored under different concentrations of Zn$^{2+}$ ions, e.g., 500 nM, 1 $\times$ 10$^{-3}$ M, 5 $\times$ 10$^{-3}$ M, and 10 $\times$ 10$^{-3}$ M, as a function of time. It also shows that higher percentages of Fu were released from the NMOFs with an elevated Zn$^{2+}$ concentration, which means that the drug release rate will become faster and the release will be enhanced when the amount of Zn$^{2+}$ in the treated region is greater and indicates the important roles of the CP5 supramolecular switches. The new strategy offers important prospects for the potential treatment of CNS disease:

(1) Because of the high Zn$^{2+}$ concentration in the synaptic vesicles in the brain, this drug can be released from nanocontainers near the targeted synapse site.

(2) These nanocontainers can not only transport the desired medicine but also decrease adverse side effects.

(3) The concentration that induces release should be fine-tuned according to the varied Zn$^{2+}$ concentration in patients. Our mechanized NMOFs with supramolecular gates can achieve a better therapeutic effect by accounting for the different characteristics of patients.

On the other hand, external heating as an auxiliary biofriendly stimulus was employed to operate the CP5-based nanovalves on NMOFs. Upon increasing temperature, the

**Table 2. Zeta potential and average particle diameter obtained by DLS.**

<table>
<thead>
<tr>
<th>Zeta potential [mV]</th>
<th>Average Zeta potential [mV]</th>
<th>Particle diameter [nm]</th>
<th>Average Particle diameter [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>UIO-66-NH$_2$</td>
<td>23.1</td>
<td>223.0</td>
<td>223 ± 1</td>
</tr>
<tr>
<td></td>
<td>23.6</td>
<td>24.0 ± 1.1</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>25.2</td>
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<td>222.4</td>
</tr>
<tr>
<td>UIO-66-NH-Py</td>
<td>26.0</td>
<td>298.8</td>
<td>305 ± 9</td>
</tr>
<tr>
<td></td>
<td>21.6</td>
<td>22.5 ± 3.1</td>
<td>315.8</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td></td>
<td>302.1</td>
</tr>
<tr>
<td>Fu-loaded, CP5-capped UIO-66-NH-Py</td>
<td>13.1</td>
<td>346.8</td>
<td>326 ± 22</td>
</tr>
<tr>
<td></td>
<td>15.8</td>
<td>15.0 ± 1.7</td>
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<tr>
<td></td>
<td>16.2</td>
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<td>296.0</td>
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**Figure 4.** Release profiles of the Fu-loaded, CP5-capped UIO-66-NH-Q operated by (a) competitive binding with Zn$^{2+}$ and (b) thermal activation.
supramolecular interactions between the CP5 rings and the Q stalks are weakened.\textsuperscript{[53]} The amount of the released Fu gradually increased (Figure 4b). The premature release at either 25 °C or 37 °C is not obvious, but the system showed a relatively faster and higher premature release within the first 100 min at 37 °C. As an important treatment of physical therapy in the modern and traditional medicine, external heating acts synergically on the drug delivery with Zn\textsuperscript{2+} as another trigger.

Furthermore, a series of control experiments have also been done to prove the functionalization of CP5-based supramolecular gates in the UiO-66-NH\textsubscript{2} drug delivery system by comparing the difference of the release of Fu-loaded UiO-66-NH\textsubscript{2} without CP5 caps activated by Zn\textsuperscript{2+} (Figure S4, Supporting Information). Premature leakage without attaching the CP5 rings was significantly serious, almost all Fu molecules leak out and the encapsulation efficiency of NMOFs without attaching the CP5 rings (42 µmol g\textsuperscript{-1}) was obviously lower than that with the attachment of the CP5 rings (115 µmol g\textsuperscript{-1}). It indicated the significant role of CP5-based supramolecular switches in our system, which effectively prevented premature cargo leakage and showed a stimuli-responsive host–guest performance for controlled drug release.

2.3. Cytotoxicity

MTT cytotoxicity assay of 293 cells treated with UiO-66-NH\textsubscript{2} and CP5-capped UiO-66-NH\textsubscript{2} at various concentrations indicated that our novel nanocontainers had only negligible cytotoxicity to normal human cells, as deduced from the fact that the cell viabilities were higher than 96% even at a high concentration of 50 µg mL\textsuperscript{-1} (Figure 5). After capping, this system shows smaller cytotoxicity due to the biological friendly property of CP5 rings.\textsuperscript{[39]} Overall, the materials, before and after CP5 capping, possess negligible cytotoxicity at low concentrations, allowing them to be used as biocompatible nanocontainers for drug delivery and controlled release.

3. Conclusion

In conclusion, benign activation mechanisms have been applied to targeted drug release systems combining monodisperse NMOFs with CP5-based stimuli-responsive supramolecular switches as nanovalves for CNS disease therapy. UiO-66-NH\textsubscript{2} particles with microporous framework were selected as nanocontainers,\textsuperscript{1} H NMR, ESI-MS, PXRD, N\textsubscript{2} sorption isotherms, BET surface area and pore size distribution certified that, without affecting the porosity of the large channel, positive charged Q stalks were successfully installed on the surfaces via PSM. And then, Fu drug was loaded, negatively charged CP5 macrocycles were introduced to encircle the Q stalks via host–guest complexion to form [2]pseudorotaxanes as gates of the nanocarriers, thereby regulating the drug-controlled release. HRTEM, SEM, zeta potential, and DLS showed that this mechanical nanocontainer is mainly in monodisperse microparticle state with certain stability and within the size range that can easily be taken up by cells. MTT cytotoxicity assay of 293 cells indicated that the materials possess negligible cytotoxicity. Zn\textsuperscript{2+}-triggered drug release with extremely low premature release suggested an especially advantageous approach for brain disease therapy. Meanwhile, external heating as a treatment of physical therapy was introduced to regulate the drug release from NMOF nanocarriers. We envision that this new hybrid nanomaterial, which combines physical and natural biochemistry treatment in one pot and takes advantages of the properties of metal and organic ligands, will prove to be a new generation of drug delivery systems for CNS disease therapy in the near future.

4. Experimental Section

Materials and Methods: Starting materials and reagents were purchased from Aladdin, and used as received. All reagents were purchased from commercial sources and used without further purification, unless otherwise noted. Deionized water was used in all relevant experiments.\textsuperscript{1} H NMR spectra were recorded at 25 °C on a Bruker AVANCE III 300 MHz NMR and 500 MHz, TMS was used as an internal standard. Chemical shifts were reported in ppm relative to the signals corresponding to the residual nondeuterated solvent, and coupling constants were recorded in Hertz. ESI-MS was performed using Liquid Chromatography Mass Spectrometry Instrument (Agilent 1290-microOTOF Q II). TGA were carried out on a TGA/Q500 from room temperature to 900 °C at a heating rate of 10 °C min\textsuperscript{-1} in nitrogen flow. PXRD measurements were carried out using a Rigaku SmartLab III powder diffractometer. N\textsubscript{2} sorption isotherms at 77 K were measured on Quantachrome instruments ASIQMV/H002-S after pretreatment by heating the samples. The pore size distribution was estimated by the DFT method from a N\textsubscript{2} sorption experiment at 77 K. SEM images were collected on a JEOL JSM6700F. TEM images were collected on a JEM 2100F instrument at an accelerating voltage of 200 kV. UV–vis spectra were recorded on a Shimadzu UV-2550 instrument. DLS measurements were performed on a Zetasizer Nano ZS instrument. Zeta potential measurements were tested on a Zetasizer Nano 9300 instrument.

**PSM of UiO-66-NH\textsubscript{2}:** UiO-66-NH\textsubscript{2} was dried at 75 °C under vacuum for at least 12 h. The freshly dried UiO-66-NH\textsubscript{2} (28 mg,
-0.1 mmol equiv. of \(-\text{NH}_2\) was suspended in dry DMF (1 mL). To this suspension, dicyclohexylcarbodiimide (DCC, 0.08 g, 0.6 mmol), 4-dimethylaminopyridine (DMAP, 0.02 g, 0.2 mmol), and Q (0.04 g, 0.2 mmol) were added. The solution was stirred at 40 °C for 3 d in a glove box. The solid was isolated by centrifugation and washed three times with warm DMF, and then washed three times with warm water. Then, the product was dried under vacuum. \(\text{UIO}-66\)-NH-Q was characterized by NMR, TEM, SEM, PXRD, BET, DLS, TGA, and ESI-MS. Approximately, 5 mg of MOF (\(\text{UIO}-66\)-NH-Q or \(\text{UIO}-66\)-NH-H) was dried under vacuum at 100 °C overnight and digested with sonication in DMSO-\(d_6\) (500 μL) and \(\text{D}_2\text{SO}_4\) (90 μL) for the NMR test. For measuring ESI-MS, \(\text{UIO}-66\)-NH-Q (1 mg) was digested in DMSO (0.1 mL) and concentrated \(\text{H}_2\text{SO}_4\) (0.02 mL) with sonication, then \(\text{K}_2\text{CO}_3\) was added to the solution to remove the excess \(\text{H}_2\text{SO}_4\), and methanol (0.5 mL) was added to dilute the solution. The peak of BDC-NH-Q indicated that the generation of the \(\text{UIO}-66\)-NH-Q, as shown in Figure S7 (Supporting Information).

\textbf{Fu and CP5 Capping:} MOFs (3 mg) were suspended in an aqueous solution of \(\text{Fu}\) (1 mL, \(3.3 \times 10^{-3} \text{M}\)) for 12 h at room temperature. An excess amount of \(\text{CP5}\) (34 mg) was added to the above mixture. The resulting reaction mixture was stirred for 2 d at room temperature. The Fu-loaded, CP5-capped MOFs were washed with deionized \(\text{H}_2\text{O}\) by centrifugation and dried under vacuum.

\textbf{Controlled Release Experiments:} Fu-loaded, CP5-capped MOFs (1 mg) were suspended in deionized \(\text{H}_2\text{O}\) and then placed in a dialysis bag, followed by being immersed into deionized \(\text{H}_2\text{O}\) (3 mL) in a cuvette with gentle stirring. Activation of the mechanized NMOFs was accomplished by heating or addition of competitive binding agent. During this period of time, UV–vis absorption spectra of the solution were recorded at predetermined times. The amount of released cargo was quantified by plotting the absorption curve with cargo solutions of different concentrations as a function of time. Control experiments were carried out with the Fu-loaded NMOFs (1 mg). These Fu-loaded NMOFs were suspended in solutions and then stirred for 3 d to result in a complete drug release.

\textbf{Encapsulation Efficiency:} For the calculation of encapsulation efficiency, \(\text{Fu}\) release was triggered by adding \(\text{Zn(NO}_3\text{)}_2\cdot6\text{H}_2\text{O}\). MOF material (1 mg, the Fu-loaded, CP5-capped \(\text{UIO}-66\)-NH-Q or the Fu-loaded \(\text{UIO}-66\)-NH-Q) was put into a dialysis bag, which was immersed into the cuvette that was stirred gently with the \(\text{Zn(NO}_3\text{)}_2\cdot6\text{H}_2\text{O}\) aqueous solution (3 mL, \(10 \times 10^{-3} \text{M}\)). During this period of time, UV–vis absorption spectra of the solution were recorded at predetermined times. According to the Lambert–Beer Law, it can be calculated that for 1 mg of the Fu-loaded, CP5-capped \(\text{UIO}-66\)-NH-Q, 0.115 μmol/0.042 μmol of \(\text{Fu}\) molecules can be released. The results showed that the encapsulation efficiency of MOFs without attaching the CP5 rings was lower than that with attaching the CP5 rings. This revealed the important role of CP5-based supramolecular switches in our system.

\textbf{Supporting Information}

Supporting Information is available from the Wiley Online Library or from the author.

\textbf{Acknowledgements}

This research was supported by the National Natural Science Foundation of China (21272093 and 51473061), and the Fundamental Research Funds for the Central Universities (No. JCKY-QKJC05). We thank Prof. Yanyan Zhang in the Department of Psychology at Jilin University for insightful discussion on CNS disease therapy.

Received: January 18, 2015
Revised: March 18, 2015
Published online: April 28, 2015