

# Chemical Science

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: F. Figueiredo, H. Madec, P. Mesdom, G. Salluce, Y. Tigherghar, K. Cariou, S. Roland, M. Sollogoub and G. Gasser, *Chem. Sci.*, 2025, DOI: 10.1039/D5SC04711K.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

# Encapsulating NHC-capped Copper(I) Complexes inside Cyclodextrin for Catalysis in Living Cells

Francisca Figueiredo<sup>a,#</sup>, Hugo Madec<sup>b,#</sup>, Pierre Mesdom<sup>a</sup>, Giulia Salluce<sup>a</sup>, Yanis Tigherghar<sup>b</sup>, Kevin Cariou<sup>a,\*</sup>, Sylvain Roland<sup>b,\*</sup>, Matthieu Sollogoub<sup>b,c,\*</sup>, Gilles Gasser<sup>a,\*</sup>

- a) Chimie ParisTech, PSL Université, CNRS, Institute of Chemistry for Life and Health Sciences, 75005 Paris, France.
- b) Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, IPCM, F-75005 Paris, France
- c) Institut Universitaire de France (IUF).

# These authors have contributed equally to the work and should be considered co-first authors.

Corresponding authors: Gilles Gasser, [gilles.gasser@chimieparistech.psl.eu](mailto:gilles.gasser@chimieparistech.psl.eu); Kevin Cariou, [kevin.cariou@chimieparistech.psl.eu](mailto:kevin.cariou@chimieparistech.psl.eu); WWW: [www.gassergroup.com](http://www.gassergroup.com); Matthieu Sollogoub, [matthieu.sollogoub@sorbonne-universite.fr](mailto:matthieu.sollogoub@sorbonne-universite.fr); Sylvain Roland, [sylvain.roland@sorbonne-universite.fr](mailto:sylvain.roland@sorbonne-universite.fr) WWW: <http://www.ipcm.fr/-Glycochimie-Organique>

ORCID Numbers:

Francisca Figueiredo: 0009-0003-1187-7684  
 Hugo Madec: 0009-0008-9509-0358  
 Pierre Mesdom: 0000-0003-1069-2138  
 Giulia Salluce: 0000-0002-1615-6822  
 Yanis Tigherghar: 0009-0000-5670-999X  
 Kevin Cariou: 0000-0002-5854-9632  
 Sylvain Roland: 0000-0002-2715-4283  
 Matthieu Sollogoub: 0000-0003-0500-5946  
 Gilles Gasser: 0000-0002-4244-5097

Keywords: carbene, copper complexes, cyclodextrins, in cell catalysis, inorganic chemical biology.

## Abstract

The development of "non-natural" chemical reactions inside living organisms is an expanding field of research. In this area, metal-based catalysis has been particularly scrutinised. However, most examples of catalysts developed so far are based on expensive and rare heavy metals such as ruthenium, iridium or palladium. For this reason, the development of catalysis in cells or *in vivo* with more accessible first-row metals is of great interest and could significantly increase the catalogue of reactions applicable in these complex environments. Herein, we demonstrate that encapsulating copper(I) *N*-heterocyclic carbene (NHC) catalysts inside the cavity of



modified cyclodextrins, renders these notoriously toxic complexes harmless towards CT26 cells at high concentrations.

Nevertheless, the catalytic activity of cyclodextrin(CD)-encapsulated NHC-copper complexes is preserved, allowing the deprotection of pinacol boronate ester groups outside and inside living cells to release phenol-based fluorophores. In cells, the production of fluorophore in the presence of CD-NHC-copper catalysts outperforms that induced by the cellular machinery alone and endogeneous ROS.

## Introduction

The development of metal-based catalysts has greatly expanded the toolbox available for synthesis in organic solvents and in water.<sup>1–4</sup> In the last decades, several research groups have investigated the applicability of well-established metal-catalysed reactions in more complex biorelevant environments, hoping to catalyse transformations that are yet to be seen in living organisms.<sup>5,6</sup> There are many challenges to overcome for the application of metal-based catalysts in living systems such as cells, the most common being deactivation by natural chelators,<sup>7</sup> unwanted toxicity,<sup>8</sup> and lack of direct quantitative methods to observe the incorporation of the compounds and to determine reaction conversions.<sup>9</sup> Consequently, although there are many examples of metal-based catalytic systems tested in biologically relevant conditions, involving nanoparticles,<sup>10,11</sup> or discrete metal complexes,<sup>12–15</sup> examples of application in living cells and organisms remain scarce.<sup>16–18</sup>

The most successful examples of catalysis in cells rely on ruthenium, iridium and palladium-based catalysts, which are rare and expensive metals from the second and third row. By comparison, the use of first-row metal complexes remains much less developed.<sup>19,20</sup> Here, a major additional challenge is the natural occurrence of some of these elements in cells and the existence of complex cellular mechanisms to regulate their levels.<sup>21,22</sup> In 2012, Meggers and co-workers reported an example of Fe<sup>III</sup>-based discrete catalyst, which operates an azide reduction in the presence of thiols and which was found to be active in HeLa cells.<sup>23</sup> Copper, another first-row metal, and its complexes, have also been studied. In this case, one of the strategies to avoid catalyst deactivation and reduce toxicity for the duration of in-cell experiments was to develop orthogonal reactions that are faster than unwanted competitive reactions of the metal with certain cell components.<sup>17</sup> Taran and co-workers



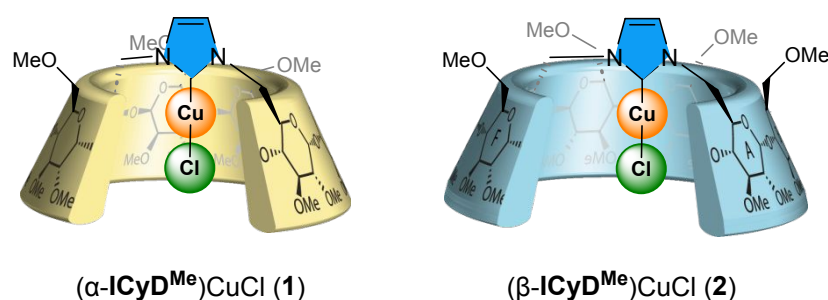
described in 2014 copper-catalysed azide-alkyne cycloadditions (CuAAC) using a copper-chelating azide based on tris(triazolylmethyl)amine scaffolds, which leads to very fast reactions and requires only one equivalent of  $\text{CuSO}_4$  per reaction, thus increasing biocompatibility. This system was successfully applied to a CuAAC reaction in HuH-7 human cancer cells.<sup>24</sup> Without using a chelating azide, the CuAAC reaction was first found to be much more sensitive to intracellular components, and in particular to the high concentrations of GSH.<sup>25</sup> Later on, however, Mascareñas and coworkers reported efficient CuAAC reactions in HeLa and A549 human cancer cells by using premade discrete tris(triazolylmethyl)amine copper complexes and abiotic small and freely diffusible alkynes and azides.<sup>26</sup> In 2021, another copper-catalysed life-compatible abiotic reaction was reported by the same group, who described N–H carbene insertion reactions in HeLa and MCF7 cells using  $\text{Cu}(\text{OAc})_2$  to generate benzoquinoxalines.<sup>13,27</sup> Interestingly,  $\text{Cu}(\text{OAc})_2$  showed only significant cytotoxicity at 100  $\mu\text{M}$  against HeLa cells after 24 h incubation.<sup>27</sup> These examples demonstrate that copper is a promising element for the design of biorthogonal metal-based catalysts, but has not yet been fully exploited in living organisms. It should be noted that the diversity of copper-catalysed reactions and copper ligands that have been used in cells remain very limited.

Another strategy to enhance the biocompatibility of metal complexes is based on the encapsulation within a host such as an enzyme, a cage, or a cavity.<sup>28–35</sup> By studying reactions in cell-like media and/or in the presence of GSH, it was found that encapsulation improved the tolerance of the metal (Cu, Au) to endogenous nucleophiles, and sometimes even increased the yield of the reactions.<sup>17,33–35</sup> However, this strategy has not been validated in cells. For these reasons, we decided to focus our attention on the use of "stealth" discrete copper(I) complexes, that could operate in cells without interfering with the intracellular copper machinery or with endogenous chelating agents. Our approach involves embedding of copper(I) complexes into cyclodextrins (CDs) as molecular containers reminiscent of enzyme active sites.

Some of us previously reported N-heterocyclic carbenes (NHC)-capped CD–metal complexes,<sup>36,37</sup> named  $[(\text{ICyD}^{\text{Me}})[\text{M}]]$ , based on permethylated CDs which makes them water soluble and in which the metal centre is encapsulated in the CD cavity



(M = Au<sup>I</sup>, Ag<sup>I</sup>, Cu<sup>I</sup>). Although deeply encapsulated, the metal centre is still catalytically active, and gold and silver complexes were shown to catalyse various transformations in pure water.<sup>38,39</sup> In particular, [( $\beta$ -**ICyD**<sup>Me</sup>)AuCl], derived from  $\beta$ -CD, was found to be an efficient catalyst for enantioselective cycloisomerization and hydroxylation-cyclization reactions.<sup>38</sup> In the present study, we hypothesized that (**ICyD**<sup>Me</sup>)-based copper(I) complexes **1** and **2** (**Figure 1**) should similarly be active catalysts for transformations in aqueous media, and thanks to metal encapsulation, could behave as biocompatible copper catalysts to allow catalysis in cells with low toxicity.



**Figure 1.** Water-soluble CD-NHC–copper(I) complexes [(α-**ICyD**<sup>Me</sup>)CuCl] (**1**) and [(β-**ICyD**<sup>Me</sup>)CuCl] (**2**) based on permethylated cyclodextrins, used in this study.

In the field of in cell catalysis, one of the most studied reaction is the deprotection of a fluorophore within cells through a cleavage reaction.<sup>16–18</sup> This approach enables the reactions to be monitored directly and in real-time by measuring fluorescence.<sup>9</sup> To the best of our knowledge, only one example with Cu<sup>I</sup>, using a tris(triazolylmethyl)amino copper(I) catalyst was reported by Chen and coworkers in 2019 for the cleavage of an internal propargyl carbamate linker.<sup>40</sup> However, the authors noted that the intracellular environment (i.e. high levels of GSH) could inhibit the reaction, and the study focused on targeted transformations on the surface of cancer cells. Interestingly, the methodology was applied to release the phenol-based drug Etoposide. Many fluorescent dyes and drugs contain phenol units,<sup>41,42</sup> but the release of such function through in cell metal-catalysis has been mainly performed with Pd and Ru, which trigger the cleavage of terminal allyl, propargyl, or allylcarbamates groups.<sup>16,17,43–45</sup> Another interesting masking group for the phenol function is the pinacol boronic ester (Bpin), which can regenerate the phenol via an *ipso*-hydroxylation deborylation reaction. This reaction can be triggered in cells by



reactive oxygen species (ROS) ( $O_2^{\cdot-}$ ,  $H_2O_2$ , peroxynitrite  $ONOO^-$ ) and various profluorophores for ROS detection in living organisms,<sup>46–48</sup> or prodrugs,<sup>49,50</sup> have been designed based on this transformation. Boronate esters are biocompatible and have been previously used for Suzuki-Miyaura couplings catalysed by Pd-containing NPs in mammalian cells.<sup>51</sup> On the other hand, several copper-based catalytic systems operating in water have been reported for the conversion of ArBpin compounds into phenol derivatives.<sup>52–54</sup>

In this study, we demonstrate the suitability of CD-encapsulated copper(I) complexes **1** and **2** as catalysts for deprotection of fluorescent dyes masked by pinacol boronic ester groups in complex cell culture media and in cell. We further show that the encapsulated  $Cu^I$  complexes can outperform the cellular machinery to convert boronic esters into phenol-based dyes, a reaction that can be competitively triggered by intracellular ROS. Overall, this work opens new perspectives for safe and precise catalysis in living cells with transition metals of the first row, for naturally occurring ones.

## Results

For our study, two water-soluble copper complexes  $[(\alpha\text{-ICyD}^{Me})CuCl]$  (**1**) and  $[(\beta\text{-ICyD}^{Me})CuCl]$  (**2**), with two different CD cavity sizes, were synthesized as previously reported by some of us.<sup>38</sup>

### Cytotoxicity of encapsulated $[(ICyD^{Me})CuCl]$ complexes

Complexes **1** and **2** are based on an NHC-Cu complex encapsulated in a CD. It has been widely demonstrated that "classical" non-encapsulated NHC copper(I) complexes can exhibit very high cellular cytotoxicity, superior to that of cisplatin, reaching tens of nanomolar levels on MCF-7 cancer cells.<sup>55–57</sup> To evaluate the level of cytotoxicity of CD-encapsulated NHC-copper complexes **1** and **2**, we determined the cell viability on murine colon cancer CT26 after incubation for a period of 48 h with increasing concentrations of copper complexes from 0.01 to 100  $\mu M$ . A non-encapsulated NHC copper(I) complex **NHC-CuCl** (see Figure S3 B), Cisplatin and  $CuSO_4$  were analysed under the same conditions for comparison. Cell viability was quantified by fluorescence using the resazurin assay. The results presented in Table 1 show that CD-encapsulated copper(I) complexes exhibit negligible cytotoxicity against CT26 cells after a relatively long incubation period (48 h), with  $IC_{50}$  values





above 100  $\mu\text{M}$  for both **1** and **2**. Under these conditions, cell viability is around 56-75% for **1** and 60-82% for **2** (see Figure S3 A). Compared to the non-encapsulated NHC copper(I) complex **NHC-CuCl**, which exhibits high cytotoxicity, these results clearly demonstrate the advantage of embedding the NHC-Cu(I) unit within a cavity. This protective encapsulation reduces toxicity and confirms that complexes **1** and **2** may serve as suitable copper catalysts for in-cell applications. It should be noted that various copper(II) salts such as  $\text{CuSO}_4$ ,  $\text{CuO}$  or  $\text{Cu}(\text{OAc})_2$  have been reported to have moderate to low cytotoxicity,<sup>27,58</sup> the latter depending on the cell line. Here, we observe that  $\text{CuSO}_4$  has a low toxicity on CT26 murine cells.

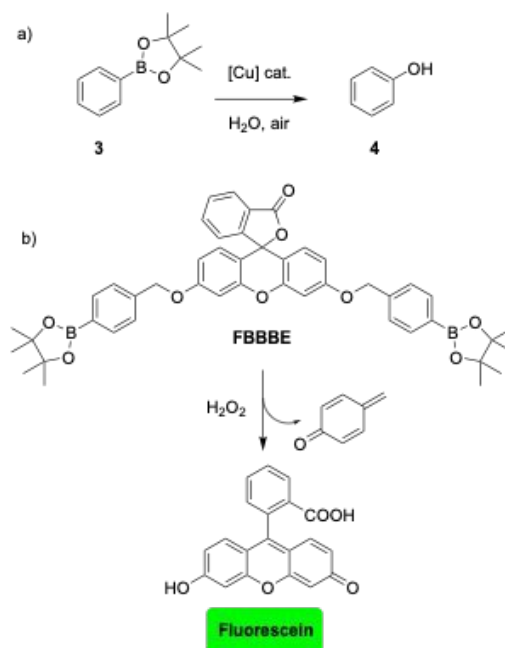
**Table 1.**  $\text{IC}_{50}$  values calculated for complex **1**, **2**, **NHC-CuCl**,  $\text{CuSO}_4$  and cisplatin in CT26 murine cells after an incubation period of 48 h in DMEM medium.

Compound	<b>1</b>	<b>2</b>	<b>NHC-CuCl</b>	<b>CuSO<sub>4</sub></b>	Cisplatin
$\text{IC}_{50}$ ( $\mu\text{M}$ )	>100	>100	$0.56 \pm 0.01$	>100	$4.14 \pm 0.45$

### *Ipso-hydroxylation deborylation reaction catalysed by [(ICyD<sup>Me</sup>)CuCl] in water*

Arylboronic acids  $\text{ArB}(\text{OH})_2$  and boronic esters can be converted into phenols using mild oxidants such as  $\text{H}_2\text{O}_2$ .<sup>59</sup> Metal-catalysed systems have also been developed, for which particular attention has been paid to the design of aerobic systems that operate with molecular oxygen, in order to use air as the oxidant, rather than  $\text{H}_2\text{O}_2$ .<sup>57,60</sup> Our attention was caught by a copper(II)-based catalytic system reported by Hu and coworkers, allowing oxidative hydroxylation of arylboronic acids under air and in water, which involves  $\text{CuSO}_4$  and 1,10-phenanthroline as a ligand.<sup>48</sup> Copper(I) species were also found to catalyse the reaction.<sup>46</sup> This system has been used to convert arylboronate esters, such as PhBpin (**3**), to phenol (**4**) (**Figure 2A**). Therefore, we wondered whether the same type of reaction could be applied by using encapsulated [(ICyD<sup>Me</sup>)CuCl] complexes **1** and **2** to the aerobic deprotection of relevant fluorescent dyes masked by pinacol boronic ester groups, such as **FBBBE**, which has been previously described.<sup>61</sup> This profluorophore includes the self-immolative linker BBE (Benzyl Boronate Ester), which is cleaved after oxidation of the C–B bond (phenol formation) to give fluorescein (**Figure 2B**).





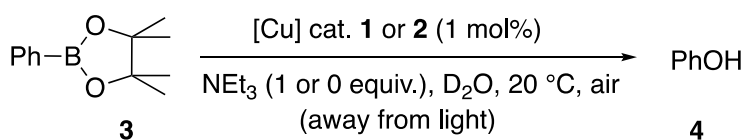
**Figure 2.** **A)** Copper-catalysed *ipso*-hydroxylation deborylation reaction; **B)** Principle of the self-immolative linker used in FBBBE profluorophore. FBBBE = Fluorescein bis(benzyl boronic ester).

The catalytic activity of encapsulated [(ICyD<sup>Me</sup>)CuCl] complexes **1** and **2** was first tested in the transformation of PhBpin (**3**) into phenol (**4**) as a model reaction with the aim of developing a system operating in water and under air atmosphere, and at a catalyst loading as low as possible, and compared to that of CuSO<sub>4</sub>. The generation of phenol **4** was monitored in D<sub>2</sub>O by <sup>1</sup>H NMR at 24, 48 and 72 h (see SI). The results, presented in **Table 2**, showed that both complexes **1**, **2**, and CuSO<sub>4</sub> catalysed the reaction in pure water with conversions reaching 81 to >95% after 72 h by using only 1 mol% of copper, thus demonstrating that copper(I) encapsulation was not detrimental to catalysis efficiency. By comparison with **1**, catalyst **2**, derived from the larger β-CD, is more active, leading to 91% conversion after 48 h and a complete conversion after 72 h. CuSO<sub>4</sub> has a similar efficiency as **2**. The reaction requires the presence of a base to achieve optimum conversion, which was obtained with 1 equiv. of NEt<sub>3</sub>. Hydroxide ions were found to be less favourable.<sup>59,62</sup> In the absence of NEt<sub>3</sub>, no formation of **4** was detected with complex **1** or CuSO<sub>4</sub>. Interestingly, we found that complex **2** could be active without a base, although the yield (13%) was lower than under optimum conditions.

**Table 2.** Oxidation of the C–B bond catalysed by [(ICyD<sup>Me</sup>)CuCl] complexes in water.







View Article Online  
DOI: 10.1039/D5SC04711K

[Cu]	NEt <sub>3</sub>	% of <b>4</b> at 24 h <sup>[a]</sup>	% of <b>4</b> at 48 h <sup>[a]</sup>	% of <b>4</b> at 72 h <sup>[a]</sup>
<b>1</b>	1 equiv.	25	70	81
<b>2</b>	1 equiv.	47	91	>95
CuSO <sub>4</sub>	1 equiv.	55	87	>95
<b>1</b>	-	<5	<5	<5
<b>2</b>	-	12	13	13
CuSO <sub>4</sub>	-	<5	<5	6

<sup>[a]</sup> % determined by <sup>1</sup>H NMR. For reactions with NEt<sub>3</sub> (fully homogeneous), the percentage represents the percentage of phenol (**4**) formed by comparison with the residual PhBpin (**3**). For reaction without NEt<sub>3</sub>, which are heterogeneous due to the low solubility of **3**, butadiene sulfone was used as an internal reference (see SI).

Having demonstrated that well-defined [(**ICyD<sup>Me</sup>**)CuCl] copper(I) complexes can be used for the aerobic *ipso*-hydroxylation deborylation reaction of **3** in water, we turned our attention to the activation of **FBBE** under the optimized catalytic conditions. However, the solubility of **FBBE** in pure water is very low, and we were not able to detect any conversion under these conditions. Consequently, as detailed later, the potential of copper complexes to activate **FBBE** was directly evaluated in cell cultures with DMEM as cell culture medium.

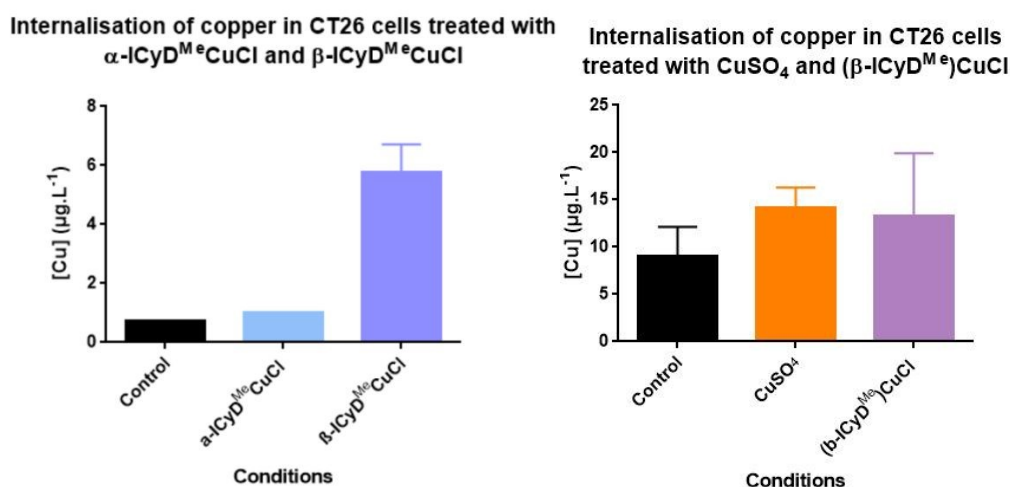
### Internalisation and stability studies

Prior to cellular tests, we assessed the internalisation of the CD-derived Cu<sup>I</sup> complexes and CuSO<sub>4</sub> in CT26 cells after incubation using cell fractionation followed by quantification using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), as previously performed by some of us with osmium, ruthenium and rhenium complexes.<sup>63–67</sup> Such studies have been performed on metal complexes of the second or third row. However, to perform such experiments with copper-based compounds poses an additional challenge. Copper is a metal that is naturally present in human cells, making necessary to ascertain that the copper concentration between non-treated cells and treated cells is significantly different to be able to conclude on the internalisation of the compounds. As a matter of fact, recent copper-



based cytotoxicity studies did not study the localisation of the complexes by ICP-MS,<sup>57,68,69</sup> except for Shad Gul *et al.* who successfully localised their complexes in the cytosol.<sup>70</sup> Their encouraging results motivated us to use this technique for our complexes.

Internalisation studies show that the copper concentration inside CT26 cells is up to six-fold higher with  $[(\beta\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**2**) compared to the control (non-treated cells) after 48 h of incubation. However, there is almost no detectable copper concentration difference between the control and  $[(\alpha\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**1**) (Figure 3). NMR over time of complex **2** in  $\text{D}_2\text{O}$  showed that it was stable at >94% after 4h. In the buffer solution (DMEM), UV-vis spectroscopy indicate that **2** remains stable for up to 4 hours of incubation (see SI, Fig. S6 and S7). Based on this, internalisation studies with  $[(\beta\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**2**) were repeated after 4 hours of incubation and compared to  $\text{CuSO}_4$  (Figure 3 B). The data show that copper is internalised in comparable amounts using either **2** or  $\text{CuSO}_4$ .



**Figure 3.** Internalisation of copper in cells treated **A)** (left) with  $[(\alpha\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**1**) and  $[(\beta\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**2**) compared to non-treated cells for 48 h; **B)** (right) with  $[(\beta\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**2**) or  $\text{CuSO}_4$  compared to non-treated cells for 4 h.

Given the catalytic, internalisation and stability results, we chose to proceed exclusively with catalyst **2**.

### Catalysis in the presence of living cells

Catalyst **2** was therefore used to operate catalysis in the presence of cells. To assess its efficiency, we studied the deprotection of **FBBBE** catalysed by copper by



monitoring the fluorescence intensity in cell cultures at different time points. We compared the efficiency of catalyst **2** vs. CuSO<sub>4</sub>, and vs. the cell innate oxidative ability. Control experiments included wells with (i) cells only, acting as a double negative control; (ii) catalyst **2** only; (iii) **FBBBE** only and (iv) CuSO<sub>4</sub> only. The experiments with **FBBBE** and catalyst and **FBBBE** and CuSO<sub>4</sub> were thus performed in regard of all these controls. To average the value, each condition included 36 samples in parallel. This set of experiments was repeated three times, to assess the reproducibility of the protocol and increase significance of small variations (Figures 4 and S8). The significance or non-significance of the data was determined by calculating the p-value between data sets (Tables S1, S2).

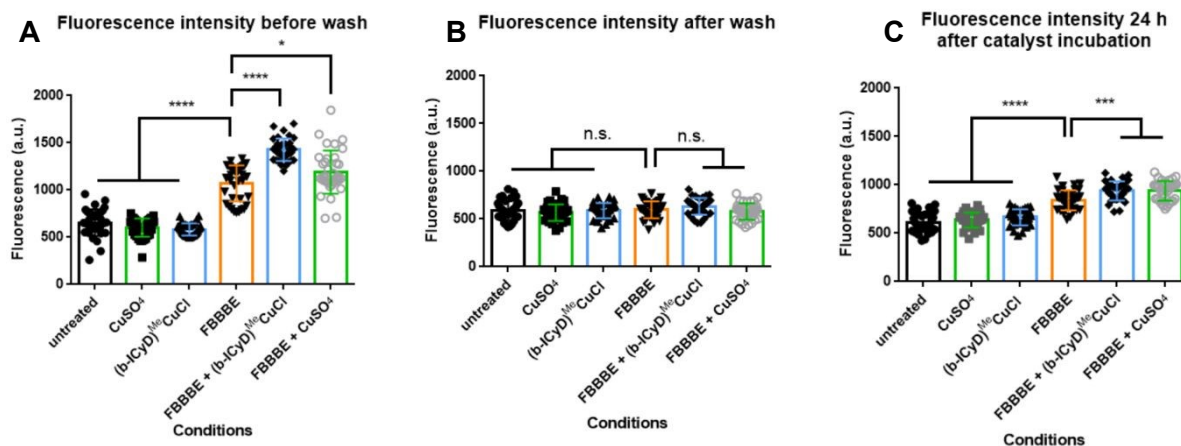
In all experiments, CT26 cells were incubated for 24 h, then treated with 50  $\mu$ M of **FBBBE**, if appropriate, and incubated overnight, and then treated with 100  $\mu$ M of catalyst **2** or CuSO<sub>4</sub> and then incubated for either 2 h or 4 h. After this incubation period, cells were washed and then incubated for 24 h and fluorescence measurement was performed (Figures 4 and S8).

Fluorescence intensity measurements, regardless of incubation time, consistently reveal a significant difference between **FBBBE**-treated cells and the three control groups lacking **FBBBE**. These results demonstrate that **FBBBE** undergoes deprotection in cells without the need for an external catalyst, likely via reaction with endogenous H<sub>2</sub>O<sub>2</sub>.<sup>1</sup> Notably, before washing the cell medium, the use of the CD-based catalyst **2** leads to a pronounced fluorescence enhancement ( $1426 \pm 118$  a.u.), and to a lesser extent when using CuSO<sub>4</sub> ( $1190 \pm 225$  a.u.), compared to the fluorescence without copper ( $1071 \pm 187$  a.u.), indicating that copper catalysts accelerate the deprotection reaction and thereby increase fluorescence intensity. These findings clearly show that encapsulated catalyst **2** effectively activates the **FBBBE** profluorophore through deprotection in the complex cellular environment. After washing the cells, this difference in fluorescence intensity is no longer present, and the overall fluorescence intensity drops from an average of  $1426 \pm 118$  a.u. (for dye + **2**, 2 h incubation) to  $633 \pm 86$  a.u. for all cells. This suggests that a good proportion of the fluorescence observed before the wash was coming from the extracellular media, that contained the dye and, when present, a copper catalyst. We then incubated the cells for an extra 24 h and witnessed an increase in the overall fluorescence of the cells that contained the dye (last 3 bars of **Figure 4** charts). This

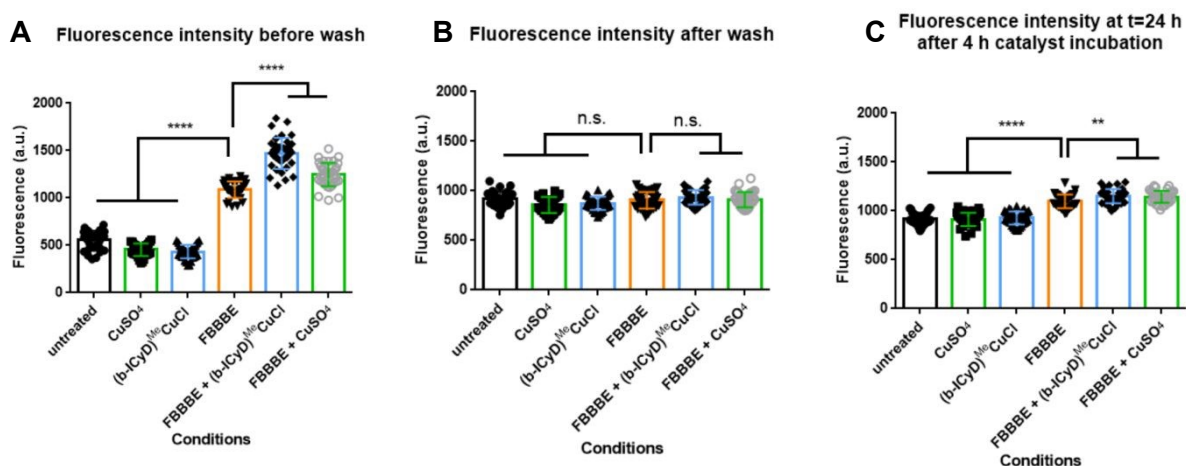


increase, up to  $936 \pm 98$  a.u., suggests that the fluorescence is now coming from the uncaging reaction of **FBBE** that is taking place *inside the cells*. This was later confirmed by confocal microscopy (**Figure 5**).

At  $t = 2$  h incubation of the catalyst



At  $t = 4$  h incubation of the catalyst



**Figure 4.** Bar charts detailing the fluorescence intensity of every tested condition for measurements after 2 h (Top) or 4 h (Bottom) incubation of the catalyst, before (A) and after (B) wash and at  $t = 24$  h after the wash (C).

At this stage, we focused mainly on the differences of fluorescence intensity measured at 24 h after cells wash and whether they were statistically significant. As stated above, we repeated the whole set of experiments three times (Figure S8) and examined the significance of the differences in intensity for each set (Table S2). The fluorescence intensity results shown in Table S2 confirm the reproducibility of the results obtained in Figure 4. First, the difference in fluorescence intensity between the cells that were not treated with the dye (controls 1, 2 & 3) and those that were



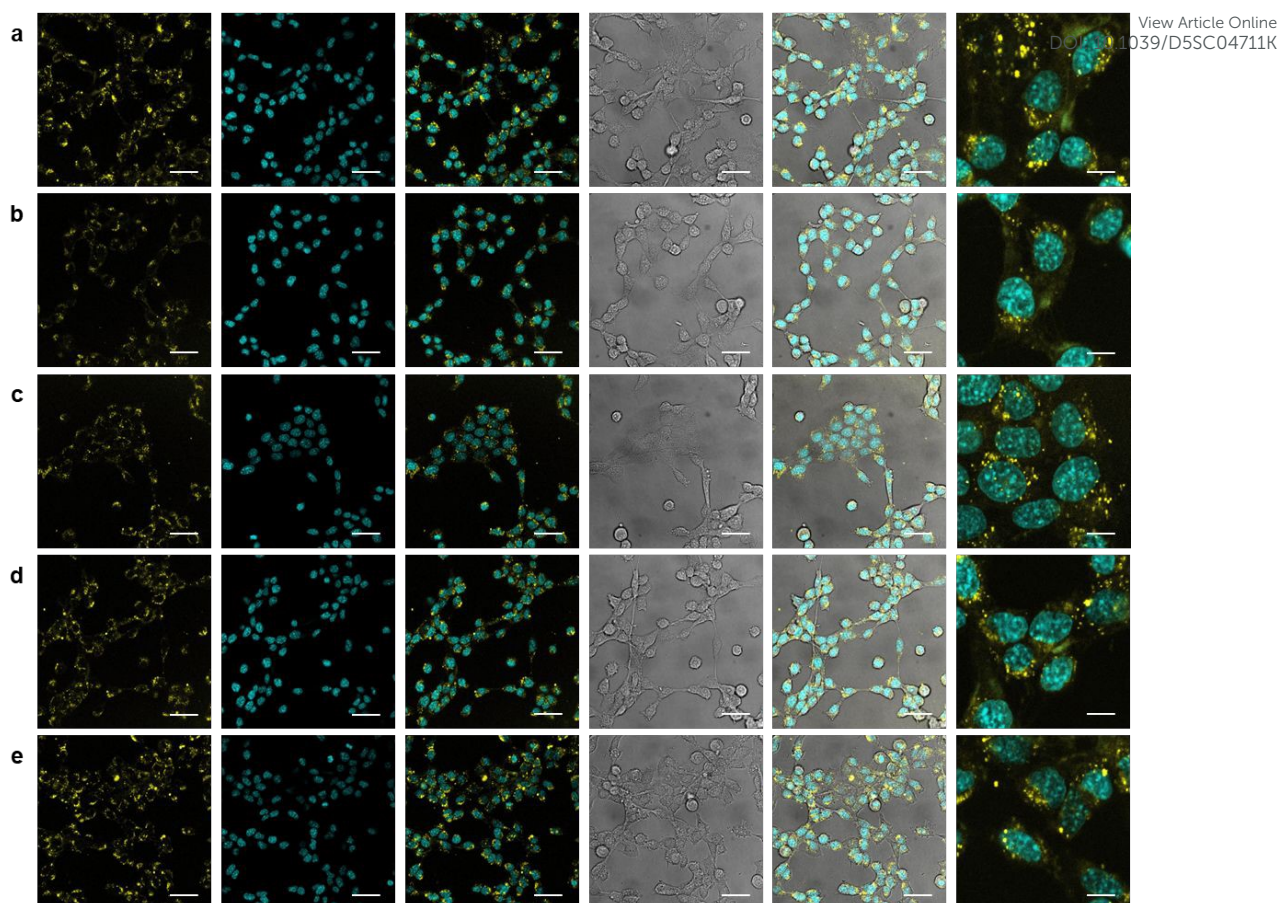
treated, is always significant (Table S2 column 1). A significant difference, although smaller, was also observed between cells treated with the dye-only control and those treated with the dye in combination with a copper catalyst, whether complex **2** or  $\text{CuSO}_4$ . These results show that adding copper to the cell is enhancing the cellular machinery for the deprotection of the dye. Furthermore, since complex **2** is as non-toxic, as easily internalised, and as efficient a catalyst as  $\text{CuSO}_4$ , we believe it is the actual catalyst responsible for the reaction inside cells. Consequently, we show that encapsulation of an NHC-Cu<sup>I</sup> complex in the cavity of a CD prevents its cytotoxicity yet allows reactivity inside cells.

Confocal microscopy experiments were conducted to observe the fluorescence inside the cells. In these experiments the measurements were performed after a 2 h or 4 h incubation of the catalyst, a cell wash, and then a 24 h incubation period. Fluorescence of the dye (yellow) is always visible from inside the cells in all experimental conditions (**Figure 5**). This confirms that the cell wash performed before a 24 h incubation period successfully removes all unwanted fluorescence coming from the uncaging reaction happening outside the cells (see **Figure 4** and **Table S2** significant). We can therefore confidently conclude that the fluorescence intensity measurements summarised in **Table 3** are from the uncaging reaction taking place inside the cells only.

View Article Online  
DOI: 10.1039/C5SC04711K







**Figure 5.** Intracellular fluorescence of fluorescein of CT26 cells treated only with FBBBE (a) or with FBBBE and **2** for 2 or 4 h (b and c, respectively), or with FBBBE and then with  $\text{CuSO}_4$  for 2 or 4 h (d and e, respectively) by confocal microscopy. Cells were imaged live following incubation with FBBBE (yellow), Cu(I) compounds (not fluorescent), and then with Hoechst 33342 (cyan). In each line, the first panel (from left to right) is the fluorescein signal (yellow), the second panel is the nuclei image (cyan), the third panel is the merge of fluorescent channels, the fourth is the bright field image, the fifth is the merge of all the channels, and the sixth is a zoom of the merge of fluorescent channels. Scale bars are 40  $\mu\text{m}$  for the non-zoomed images and 10  $\mu\text{m}$  for the zoomed images.

## Conclusion

In this study, we have successfully developed copper catalysis in complex cell culture medium and in cells with encapsulated NHC-copper(I) catalysts. To achieve this we used water-soluble  $[(\text{ICyD}^{\text{Me}})\text{CuCl}]$  complexes which are based on NHC-capped CD ligands, featuring a cavity reminiscent of enzyme active sites. The  $\text{IC}_{50}$  of these complexes was measured on CT26 cells and compared to a non-encapsulated





NHC-copper(I) complex. Contrary to the highly toxic standard NHC-Cu(I) complex, the encapsulated complexes  $[(\text{ICyD}^{\text{Me}})\text{CuCl}]$  showed no toxicity at all. This remarkable feature of the encapsulation makes them suitable for catalysis in cells. The catalytic properties of two complexes **1** and **2**, derived from  $\alpha$  and  $\beta$ -CD, respectively, were first studied in water, under different conditions. Their ability to transform arylboronic esters into phenol derivatives was demonstrated. Internalisation studies showed that **2** was more efficiently taken up by cells. In-cell uncaging tests on a fluorescein dye, protected by pinacol boronic ester groups (FBBBE) were performed inside CT26 cells with the CD-encapsulated copper(I) catalyst  $[(\beta\text{-ICyD}^{\text{Me}})\text{CuCl}]$  (**2**). A series of experiments with precise controls demonstrated that the fluorophore deprotection is accelerated in the presence of the catalyst in the complex cell culture medium as well as within the cells. Confocal microscopy clearly shows the fluorescence inside the cells that were incubated with the fluorophore and catalyst. Overall, we determined that: (i) The encapsulation within a CD cavity reduces the toxicity of the NHC-copper while keeping its reactivity; (ii) The size of the cavity influences catalytic activity, with  $\alpha$ -CD-derived catalysts inducing slower reactions compared to those derived from  $\beta$ -CD, which underscores the importance of the cavity in catalysis; (iii) Most importantly, we demonstrate for the first time that encapsulating a first-row metal within a CD enables catalysis inside a living organism.

This breakthrough paves the way for developing new first-row metal-based catalysts for applications in living organisms. These results are a strong incentive for the use of encapsulation strategies to protect the metal centres from natural chelators and reduce their innate toxicity while keeping a catalytical activity. Furthermore, the encapsulating structure can be used as a support to bind targeting moieties, allowing for the specific delivery of the active copper centre. This can have applications in cellular imaging or co-localisation studies and eventually could be considered for *in situ* pro-drug activation.

## Data Availability Statement

The datasets supporting this article have been uploaded as part of the supplementary information.

## Authors Contribution



F.F. and H.M. synthesized and characterized the compounds and wrote the first draft of this manuscript, which was then edited by K.C., S.R., M.S and G.G. H.M., Y.T. performed the catalysis experiments. F.F., P.M. and G.S. carried out biological experiments. K.C., S.R., M.S and G.G. designed and supervised the study.

View Article Online  
DOI: 10.1039/D3SC04711K

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgements

We are grateful for financial support from the ANR (ANR-20-CE07-0035), the ERC Consolidator Grant PhotoMedMet to G.G. (GA 681679), the program “Investissements d’Avenir” launched by the French Government and implemented by the ANR with the reference ANR-10-IDEX-0001-02 PSL (G.G.)

## References

- (1) Liu, Y.; Bai, Y. Design and Engineering of Metal Catalysts for Bio-Orthogonal Catalysis in Living Systems. *ACS Appl. Bio Mater.* **2020**, 3 (8), 4717–4746. <https://doi.org/10.1021/acsabm.0c00581>.
- (2) Chen, J.; Wang, J.; Li, K.; Wang, Y.; Gruebele, M.; Ferguson, A. L.; Zimmerman, S. C. Polymeric “Clickase” Accelerates the Copper Click Reaction of Small Molecules, Proteins, and Cells. *J. Am. Chem. Soc.* **2019**, 141 (24), 9693–9700. <https://doi.org/10.1021/jacs.9b04181>.
- (3) Luther, D. C.; Huang, R.; Jeon, T.; Zhang, X.; Lee, Y.-W.; Nagaraj, H.; Rotello, V. M. Delivery of Drugs, Proteins, and Nucleic Acids Using Inorganic Nanoparticles. *Advanced Drug Delivery Reviews* **2020**, 156, 188–213. <https://doi.org/10.1016/j.addr.2020.06.020>.
- (4) Thomas, S. R.; Casini, A. Gold Compounds for Catalysis and Metal-Mediated Transformations in Biological Systems. *Current Opinion in Chemical Biology* **2020**, 55, 103–110. <https://doi.org/10.1016/j.cbpa.2019.12.007>.
- (5) Hambley, T. W. Developing New Metal-Based Therapeutics: Challenges and Opportunities. *Dalton Trans.* **2007**, No. 43, 4929. <https://doi.org/10.1039/b706075k>.
- (6) Patra, M.; Gasser, G. Organometallic Compounds: An Opportunity for Chemical Biology? *ChemBioChem* **2012**, 13 (9), 1232–1252. <https://doi.org/10.1002/cbic.201200159>.
- (7) Corso, C. R.; Acco, A. Glutathione System in Animal Model of Solid Tumors: From Regulation to Therapeutic Target. *Critical Reviews in Oncology/Hematology* **2018**, 128, 43–57. <https://doi.org/10.1016/j.critrevonc.2018.05.014>.
- (8) Goyer, R. Nutrition and Metal Toxicity. *The American Journal of Clinical Nutrition* **1995**, 61 (3), 646S–650S. <https://doi.org/10.1093/ajcn/61.3.646S>.
- (9) Nguyen, D. P.; Nguyen, H. T. H.; Do, L. H. Tools and Methods for Investigating Synthetic Metal-Catalyzed Reactions in Living Cells. *ACS Catal.* **2021**, 11 (9), 5148–5165. <https://doi.org/10.1021/acscatal.1c00438>.
- (10) Soldevila-Barreda, J. J.; Metzler-Nolte, N. Intracellular Catalysis with Selected Metal Complexes and Metallic Nanoparticles: Advances toward the Development of Catalytic



- Metallodrugs. *Chem. Rev.* **2019**, *119* (2), 829–869. <https://doi.org/10.1021/acs.chemrev.8b00493>.
- (11) Rebelein, J. G.; Ward, T. R. In Vivo Catalyzed New-to-Nature Reactions. *Current Opinion in Biotechnology* **2018**, *53*, 106–114. <https://doi.org/10.1016/j.copbio.2017.12.008>.
- (12) Seoane, A.; Mascareñas, J. L. Exporting Homogeneous Transition Metal Catalysts to Biological Habitats. *European J Organic Chem* **2022**. <https://doi.org/10.1002/ejoc.202200118>.
- (13) Gutiérrez, S.; Tomás-Gamasa, M.; Mascareñas, J. L. Organometallic Catalysis in Aqueous and Biological Environments: Harnessing the Power of Metal Carbenes. *Chem. Sci.* **2022**, *13* (22), 6478–6495. <https://doi.org/10.1039/D2SC00721E>.
- (14) Martínez-Calvo, M.; Mascareñas, J. L. Organometallic Catalysis in Biological Media and Living Settings. *Coordination Chemistry Reviews* **2018**, *359*, 57–79. <https://doi.org/10.1016/j.ccr.2018.01.011>.
- (15) Destito, P.; Vidal, C.; López, F.; Mascareñas, J. L. Transition Metal-Promoted Reactions in Aqueous Media and Biological Settings. *Chem. Eur. J.* **2021**, *27* (15), 4789–4816. <https://doi.org/10.1002/chem.202003927>.
- (16) Madec, H.; Figueiredo, F.; Cariou, K.; Roland, S.; Sollogoub, M.; Gasser, G. Metal Complexes for Catalytic and Photocatalytic Reactions in Living Cells and Organisms. *Chem. Sci.* **2023**, *14* (3), 409–442. <https://doi.org/10.1039/D2SC05672K>.
- (17) James, C. C.; de Bruin, B.; Reek, J. N. H. Transition Metal Catalysis in Living Cells: Progress, Challenges, and Novel Supramolecular Solutions. *Angew Chem Int Ed* **2023**, *62* (41), e202306645. <https://doi.org/10.1002/anie.202306645>.
- (18) Chang, T.-C.; Tanaka, K. In Vivo Organic Synthesis by Metal Catalysts. *Bioorganic & Medicinal Chemistry* **2021**, *46*, 116353. <https://doi.org/10.1016/j.bmc.2021.116353>.
- (19) Casini, A.; Pöthig, A. Metals in Cancer Research: Beyond Platinum Metallodrugs. *ACS Cent. Sci.* **2024**, *10* (2), 242–250. <https://doi.org/10.1021/acscentsci.3c01340>.
- (20) Gourdon, L.; Cariou, K.; Gasser, G. Phototherapeutic Anticancer Strategies with First-Row Transition Metal Complexes: A Critical Review. *Chem. Soc. Rev.* **2022**, *51* (3), 1167–1195. <https://doi.org/10.1039/D1CS00609F>.
- (21) Chen, L.; Min, J.; Wang, F. Copper Homeostasis and Cuproptosis in Health and Disease. *Sig Transduct Target Ther* **2022**, *7* (1), 378. <https://doi.org/10.1038/s41392-022-01229-y>.
- (22) Kaplan, J. H.; Lutsenko, S. Copper Transport in Mammalian Cells: Special Care for a Metal with Special Needs. *Journal of Biological Chemistry* **2009**, *284* (38), 25461–25465. <https://doi.org/10.1074/jbc.R109.031286>.
- (23) Sasmal, P. K.; Carregal-Romero, S.; Han, A. A.; Streu, C. N.; Lin, Z.; Namikawa, K.; Elliott, S. L.; Köster, R. W.; Parak, W. J.; Meggers, E. Catalytic Azide Reduction in Biological Environments. *ChemBioChem* **2012**, *13* (8), 1116–1120. <https://doi.org/10.1002/cbic.201100719>.
- (24) Bevilacqua, V.; King, M.; Chaumontet, M.; Nothisen, M.; Gabillet, S.; Buisson, D.; Puente, C.; Wagner, A.; Taran, F. Copper-Chelating Azides for Efficient Click Conjugation Reactions in Complex Media. *Angewandte Chemie* **2014**, *126* (23), 5982–5986. <https://doi.org/10.1002/ange.201310671>.
- (25) Li, S.; Wang, L.; Yu, F.; Zhu, Z.; Shobaki, D.; Chen, H.; Wang, M.; Wang, J.; Qin, G.; Erasquin, U. J.; Ren, L.; Wang, Y.; Cai, C. Copper-Catalyzed Click Reaction on/in Live Cells. *Chem. Sci.* **2017**, *8* (3), 2107–2114. <https://doi.org/10.1039/C6SC02297A>.
- (26) Miguel-Ávila, J.; Tomás-Gamasa, M.; Olmos, A.; Pérez, P. J.; Mascareñas, J. L. Discrete Cu(I) Complexes for Azide–Alkyne Annulations of Small Molecules inside Mammalian Cells. *Chem. Sci.* **2018**, *9* (7), 1947–1952. <https://doi.org/10.1039/C7SC04643J>.
- (27) Gutiérrez, S.; Tomás-Gamasa, M.; Mascareñas, J. L. Exporting Metal-Carbene Chemistry to Live Mammalian Cells: Copper-Catalyzed Intracellular Synthesis of Quinoxalines Enabled by N–H Carbene Insertions. *Angew Chem Int Ed* **2021**, *60* (40), 22017–22025. <https://doi.org/10.1002/anie.202108899>.



- (28) Maity, B.; Taher, M.; Mazumdar, S.; Ueno, T. Artificial Metalloenzymes Based on Protein Assembly. *Coordination Chemistry Reviews* **2022**, *469*, 214593. <https://doi.org/10.1016/j.ccr.2022.214593>. View Article Online  
DOI: 10.1039/D5SC04711K
- (29) Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Lewis, J. C.; Ward, T. R. Artificial Metalloenzymes: Reaction Scope and Optimization Strategies. *Chem. Rev.* **2018**, *118* (1), 142–231. <https://doi.org/10.1021/acs.chemrev.7b00014>.
- (30) Moreno-Alcántar, G.; Casini, A. Bioinorganic Supramolecular Coordination Complexes and Their Biomedical Applications. *FEBS Letters* **2023**, *597* (1), 191–202. <https://doi.org/10.1002/1873-3468.14535>.
- (31) Diao, D.; Simaan, A. J.; Martinez, A.; Colombar, C. Bioinspired Complexes Confined in Well-Defined Capsules: Getting Closer to Metalloenzyme Functionalities. *Chem. Commun.* **2023**, *59* (29), 4288–4299. <https://doi.org/10.1039/D2CC06990C>.
- (32) Sathyan, A.; Deng, L.; Loman, T.; Palmans, A. R. A. Bioorthogonal Catalysis in Complex Media: Consequences of Using Polymeric Scaffold Materials on Catalyst Stability and Activity. *Catalysis Today* **2023**, *418*, 114116. <https://doi.org/10.1016/j.cattod.2023.114116>.
- (33) Nguyen, H. D.; Jana, R. D.; Campbell, D. T.; Tran, T. V.; Do, L. H. Lewis Acid-Driven Self-Assembly of Diiridium Macrocyclic Catalysts Imparts Substrate Selectivity and Glutathione Tolerance. *Chem. Sci.* **2023**, *14* (37), 10264–10272. <https://doi.org/10.1039/D3SC02836D>.
- (34) James, C. C.; Wu, D.; Bobylev, E. O.; Kros, A.; de Bruin, B.; Reek, J. N. H. Protection of a Gold Catalyst by a Supramolecular Cage Improves Bioorthogonality. *ChemCatChem* **2022**, *14* (23), e202200942. <https://doi.org/10.1002/cctc.202200942>.
- (35) Qiu, G.; Nava, P.; Martinez, A.; Colombar, C. A Tris(Benzyltriazolemethyl)Amine-Based Cage as a CuAAC Ligand Tolerant to Exogeneous Bulky Nucleophiles. *Chem. Commun.* **2021**, *57* (18), 2281–2284. <https://doi.org/10.1039/D0CC08005E>.
- (36) Zhang, P.; Tugny, C.; Meijide Suárez, J.; Guitet, M.; Derat, E.; Vanthuyne, N.; Zhang, Y.; Bistri, O.; Mouriès-Mansuy, V.; Ménand, M.; Roland, S.; Fensterbank, L.; Sollogoub, M. Artificial Chiral Metallo-Pockets Including a Single Metal Serving as Structural Probe and Catalytic Center. *Chem* **2017**, *3* (1), 174–191. <https://doi.org/10.1016/j.chempr.2017.05.009>.
- (37) Guitet, M.; Zhang, P.; Marcelo, F.; Tugny, C.; Jiménez-Barbero, J.; Buriez, O.; Amatore, C.; Mouriès-Mansuy, V.; Goddard, J.; Fensterbank, L.; Zhang, Y.; Roland, S.; Ménand, M.; Sollogoub, M. NHC-Capped Cyclodextrins (ICyDs): Insulated Metal Complexes, Commutable Multicoordination Sphere, and Cavity-Dependent Catalysis. *Angew Chem Int Ed* **2013**, *52* (28), 7213–7218. <https://doi.org/10.1002/anie.201301225>.
- (38) Zhu, X.; Xu, G.; Chamoireau, L.; Zhang, Y.; Mouriès-Mansuy, V.; Fensterbank, L.; Bistri-Aslanoff, O.; Roland, S.; Sollogoub, M. Permethylated NHC-Capped  $\alpha$ - and  $\beta$ -Cyclodextrins (ICyD<sup>Me</sup>) Regioselective and Enantioselective Gold-Catalysis in Pure Water. *Chemistry A European J* **2020**, *26* (68), 15901–15909. <https://doi.org/10.1002/chem.202001990>.
- (39) Roland, S.; Sollogoub, M. *N*-heterocyclic Carbene (NHC)-Capped Cyclodextrins for Cavity-Controlled Catalysis. In *Supramolecular Catalysis*; van Leeuwen, P. W. N. M., Raynal, M., Eds.; Wiley, 2022; pp 287–301. <https://doi.org/10.1002/9783527832033.ch20>.
- (40) Wang, X.; Liu, Y.; Fan, X.; Wang, J.; Ngai, W. S. C.; Zhang, H.; Li, J.; Zhang, G.; Lin, J.; Chen, P. R. Copper-Triggered Bioorthogonal Cleavage Reactions for Reversible Protein and Cell Surface Modifications. *J. Am. Chem. Soc.* **2019**, *141* (43), 17133–17141. <https://doi.org/10.1021/jacs.9b05833>.
- (41) Krämer, J.; Kang, R.; Grimm, L. M.; De Cola, L.; Picchetti, P.; Biedermann, F. Molecular Probes, Chemosensors, and Nanosensors for Optical Detection of Biorelevant Molecules and Ions in Aqueous Media and Biofluids. *Chem. Rev.* **2022**, *122* (3), 3459–3636. <https://doi.org/10.1021/acs.chemrev.1c00746>.





- (42) Scott, K. A.; Cox, P. B.; Njardarson, J. T. Phenols in Pharmaceuticals: Analysis of a Recurring Motif. *J. Med. Chem.* **2022**, *65* (10), 7044–7072. <https://doi.org/10.1021/acs.jmedchem.2c00223>.
- (43) Wang, J.; Zheng, S.; Liu, Y.; Zhang, Z.; Lin, Z.; Li, J.; Zhang, G.; Wang, X.; Li, J.; Chen, P. R. Palladium-Triggered Chemical Rescue of Intracellular Proteins via Genetically Encoded Allene-Caged Tyrosine. *J. Am. Chem. Soc.* **2016**, *138* (46), 15118–15121. <https://doi.org/10.1021/jacs.6b08933>.
- (44) Konč, J.; Sabatino, V.; Jiménez-Moreno, E.; Latocheski, E.; Pérez, L. R.; Day, J.; Domingos, J. B.; Bernardes, G. J. L. Controlled In-Cell Generation of Active Palladium(0) Species for Bioorthogonal Decaging. *Angew Chem Int Ed* **2022**, *61* (8), e202113519. <https://doi.org/10.1002/anie.202113519>.
- (45) Vidal, C.; Tomás-Gamasa, M.; Destito, P.; López, F.; Mascareñas, J. L. Concurrent and Orthogonal Gold(I) and Ruthenium(II) Catalysis inside Living Cells. *Nat Commun* **2018**, *9* (1), 1913. <https://doi.org/10.1038/s41467-018-04314-5>.
- (46) Lippert, A. R.; Van de Bittner, G. C.; Chang, C. J. Boronate Oxidation as a Bioorthogonal Reaction Approach for Studying the Chemistry of Hydrogen Peroxide in Living Systems. *Acc. Chem. Res.* **2011**, *44* (9), 793–804. <https://doi.org/10.1021/ar200126t>.
- (47) Fang, G.; Wang, H.; Bian, Z.; Sun, J.; Liu, A.; Fang, H.; Liu, B.; Yao, Q.; Wu, Z. Recent Development of Boronic Acid-Based Fluorescent Sensors. *RSC Adv.* **2018**, *8* (51), 29400–29427. <https://doi.org/10.1039/C8RA04503H>.
- (48) Wang, R.; Bian, Z.; Zhan, D.; Wu, Z.; Yao, Q.; Zhang, G. Boronic Acid-Based Sensors for Small-Molecule Reactive Species: A Review. *Dyes and Pigments* **2021**, *185*, 108885. <https://doi.org/10.1016/j.dyepig.2020.108885>.
- (49) Al-Omari, M. K.; Elaarag, M.; Al-Zoubi, R. M.; Al-Qudimat, A. R.; Zarour, A. A.; Al-Hurani, E. A.; Fares, Z. E.; Alkharraz, L. M.; Shkoor, M.; Bani-Yaseen, A. D.; Aboumarzouk, O. M.; Yassin, A.; Al-Ansari, A. A. Organoboronic Acids/Esters as Effective Drug and Prodrug Candidates in Cancer Treatments: Challenge and Hope. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2023**, *38* (1), 2220084. <https://doi.org/10.1080/14756366.2023.2220084>.
- (50) Peiró Cadahía, J.; Previtali, V.; Troelsen, N. S.; Clausen, M. H. Prodrug Strategies for Targeted Therapy Triggered by Reactive Oxygen Species. *Med. Chem. Commun.* **2019**, *10* (9), 1531–1549. <https://doi.org/10.1039/C9MD00169G>.
- (51) Yusop, R. M.; Unciti-Broceta, A.; Johansson, E. M. V.; Sánchez-Martín, R. M.; Bradley, M. Palladium-Mediated Intracellular Chemistry. *Nature Chem* **2011**, *3* (3), 239–243. <https://doi.org/10.1038/nchem.981>.
- (52) Joo, S.; Kwon, G.; Park, S.; Kim, S. Chemically Modified Chitosan as a Biopolymer Support in Copper-catalyzed *Ipso*-Hydroxylation of Arylboronic Acids in Water. *Bulletin Korean Chem Soc* **2019**, *40* (5), 465–468. <https://doi.org/10.1002/bkcs.11708>.
- (53) Bora, S. J.; Chetia, B. Novel CuCl<sub>2</sub>-Cryptand-[2.2.Benzo] Complex: A Base Free and Oxidant Free Catalyst for *Ipso*-Hydroxylation of Aryl/Heteroaryl-Boronic Acids in Water at Room Temperature. *Journal of Organometallic Chemistry* **2017**, *851*, 52–56. <https://doi.org/10.1016/j.jorganchem.2017.09.011>.
- (54) Xu, J.; Wang, X.; Shao, C.; Su, D.; Cheng, G.; Hu, Y. Highly Efficient Synthesis of Phenols by Copper-Catalyzed Oxidative Hydroxylation of Arylboronic Acids at Room Temperature in Water. *Org. Lett.* **2010**, *12* (9), 1964–1967. <https://doi.org/10.1021/ol1003884>.
- (55) Teyssot, M.-L.; Jarrousse, A.-S.; Manin, M.; Chevy, A.; Roche, S.; Norre, F.; Beaudoin, C.; Morel, L.; Boyer, D.; Mahiou, R.; Gautier, A. Metal-NHC Complexes: A Survey of Anti-Cancer Properties. *Dalton Trans.* **2009**, No. 35, 6894. <https://doi.org/10.1039/b906308k>.
- (56) Nayak, S.; Gaonkar, S. L. Coinage Metal *N*-Heterocyclic Carbene Complexes: Recent Synthetic Strategies and Medicinal Applications. *ChemMedChem* **2021**, *16* (9), 1360–1390. <https://doi.org/10.1002/cmdc.202000836>.



- (57) Elie, M.; Mahoro, G. U.; Duverger, E.; Renaud, J.-L.; Daniellou, R.; Gaillard, S. Cytotoxicity of Cationic NHC Copper(I) Complexes Coordinated to 2,2'-Bis-Pyridyl Ligands. *Journal of Organometallic Chemistry* **2019**, 893, 21–31. <https://doi.org/10.1016/j.jorganchem.2019.04.003>.
- (58) Shaligram, S.; Campbell, A. Toxicity of Copper Salts Is Dependent on Solubility Profile and Cell Type Tested. *Toxicology in Vitro* **2013**, 27 (2), 844–851. <https://doi.org/10.1016/j.tiv.2012.12.026>.
- (59) Hao, L.; Ding, G.; Deming, D. A.; Zhang, Q. Recent Advances in Green Synthesis of Functionalized Phenols from Aromatic Boronic Compounds. *Eur J Org Chem* **2019**, 2019 (44), 7307–7321. <https://doi.org/10.1002/efoc.201901303>.
- (60) Das, R.; Rohit, K. R.; Anilkumar, G. Recent Trends in Non-Noble Metal-Catalyzed Hydroxylation Reactions. *Journal of Organometallic Chemistry* **2022**, 977, 122456. <https://doi.org/10.1016/j.jorganchem.2022.122456>.
- (61) Daniel, K. B.; Agrawal, A.; Manchester, M.; Cohen, S. M. Readily Accessible Fluorescent Probes for Sensitive Biological Imaging of Hydrogen Peroxide. *ChemBioChem* **2013**, 14 (5), 593–598. <https://doi.org/10.1002/cbic.201200724>.
- (62) Sharma, M.; Adhikari, B.; Awoyemi, R. F.; Perkins, A. M.; Duckworth, A. K.; Donnadiou, B.; Wipf, D. O.; Stokes, S. L.; Emerson, J. P. Copper(II) NHC Catalyst for the Formation of Phenol from Arylboronic Acid. *Chemistry* **2022**, 4 (2), 560–575. <https://doi.org/10.3390/chemistry4020040>.
- (63) Vinck, R.; Dömötör, O.; Karges, J.; Jakubaszek, M.; Seguin, J.; Tharaud, M.; Guérineau, V.; Cariou, K.; Mignet, N.; Enyedy, É. A.; Gasser, G. *In Situ* Bioconjugation of a Maleimide-Functionalized Ruthenium-Based Photosensitizer to Albumin for Photodynamic Therapy. *Inorg. Chem.* **2023**, 62 (38), 15510–15526. <https://doi.org/10.1021/acs.inorgchem.3c01984>.
- (64) Wang, Y.; Mesdom, P.; Purkait, K.; Saubaméa, B.; Burckel, P.; Arnoux, P.; Frochot, C.; Cariou, K.; Rossel, T.; Gasser, G. Ru(II)/Os(II)-Based Carbonic Anhydrase Inhibitors as Photodynamic Therapy Photosensitizers for the Treatment of Hypoxic Tumours. *Chem. Sci.* **2023**, 14 (42), 11749–11760. <https://doi.org/10.1039/D3SC03932C>.
- (65) Gandioso, A.; Izquierdo-García, E.; Mesdom, P.; Arnoux, P.; Demeubayeva, N.; Burckel, P.; Saubaméa, B.; Bosch, M.; Frochot, C.; Marchán, V.; Gasser, G. Ru(II)-Cyanine Complexes as Promising Photodynamic Photosensitizers for the Treatment of Hypoxic Tumours with Highly Penetrating 770 Nm Near-Infrared Light. *Chemistry A European J* **2023**, 29 (61), e202301742. <https://doi.org/10.1002/chem.202301742>.
- (66) Enslin, L. E.; Purkait, K.; Pozza, M. D.; Saubamea, B.; Mesdom, P.; Visser, H. G.; Gasser, G.; Schutte-Smith, M. Rhenium(I) Tricarbonyl Complexes of 1,10-Phenanthroline Derivatives with Unexpectedly High Cytotoxicity. *Inorg. Chem.* **2023**, 62 (31), 12237–12251. <https://doi.org/10.1021/acs.inorgchem.3c00730>.
- (67) Ortega-Forte, E.; Rovira, A.; López-Corrales, M.; Hernández-García, A.; Ballester, F. J.; Izquierdo-García, E.; Jordà-Redondo, M.; Bosch, M.; Nonell, S.; Santana, M. D.; Ruiz, J.; Marchán, V.; Gasser, G. A Near-Infrared Light-Activatable Ru(II)-Coumarin Photosensitizer Active under Hypoxic Conditions. *Chem. Sci.* **2023**, 14 (26), 7170–7184. <https://doi.org/10.1039/D3SC01844J>.
- (68) Teyssot, M.; Jarrousse, A.; Chevy, A.; De Haze, A.; Beaudoin, C.; Manin, M.; Nolan, S. P.; Díez-González, S.; Morel, L.; Gautier, A. Toxicity of Copper(I)-NHC Complexes Against Human Tumor Cells: Induction of Cell Cycle Arrest, Apoptosis, and DNA Cleavage. *Chemistry A European J* **2009**, 15 (2), 314–318. <https://doi.org/10.1002/chem.200801992>.
- (69) Touj, N.; Nasr, I. S. A.; Koko, W. S.; Khan, T. A.; Özdemir, I.; Yasar, S.; Mansour, L.; Alresheedi, F.; Hamdi, N. Anticancer, Antimicrobial and Antiparasitical Activities of Copper(I) Complexes Based on *N*-Heterocyclic Carbene (NHC) Ligands Bearing Aryl Substituents. *Journal of Coordination Chemistry* **2020**, 73 (20–22), 2889–2905. <https://doi.org/10.1080/00958972.2020.1836359>.





- (70) Gul, N. S.; Khan, T.-M.; Chen, M.; Huang, K.-B.; Hou, C.; Choudhary, M. I.; Liang, H.; Chen, Z.-F. New Copper Complexes Inducing Bimodal Death through Apoptosis and Autophagy in A549 Cancer Cells. *Journal of Inorganic Biochemistry* **2020**, 213, 111260. <https://doi.org/10.1016/j.jinorgbio.2020.111260>. View Article Online  
DOI: 10.1039/D5SC04711K
- (71) Maiti, B. K.; Maia, L. B.; Moro, A. J.; Lima, J. C.; Cordas, C. M.; Moura, I.; Moura, J. J. G. Unusual Reduction Mechanism of Copper in Cysteine-Rich Environment. *Inorg. Chem.* **2018**, 57 (14), 8078–8088. <https://doi.org/10.1021/acs.inorgchem.8b00121>.



The datasets supporting this article have been uploaded as part of the supplementary information.

