

Organometallic Cages as Vehicles for Intracellular Release of Photosensitizers

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ABSTRACT: Water-soluble metalla-cages were used to deliver hydrophobic porphyrin molecules to cancer cells. After internalization, the photosensitizer was photoactivated, significantly increasing the cytotoxicity in cells. During the transport, the photosensitizer remains non-reactive to light, offering a new strategy to tackle overall photosensitization, a limitation often encountered in photodynamic therapy.

In recent years, the use of large vehicles to carry photosensitizers to cancer cells has attracted much interest.¹ Photosensitizers such as porphyrins and phthalocyanines are in general poorly water-soluble, unless highly substituted with hydrophilic groups.² Therefore, encapsulation of the photosensitizer within the hydrophobic cavity of water-soluble carriers provides an elegant strategy to transport photosensitizers in aqueous media, a necessity for biological applications. Moreover, most photosensitizers show poor selectivity to diseased cells and consequently generate an overall photosensitization of the entire body. Thus, spatial-controlled release of the photosensitizer remains one of the main challenges in photodynamic therapy.³

Recently, water-soluble arene ruthenium metalla-cages have been used to deliver hydrophobic molecules to cancer cells.⁴ In an extension to this work, we have now encapsulated porphyrin, a well-known lipophilic photosensitizer,⁵ in two cationic arene ruthenium metalla-cages (Figure 1). In the hexanuclear metalla-prism, $[\text{Ru}_6(\eta^6\text{-}p\text{-Pr}^i\text{C}_6\text{H}_4\text{Me})_6(\text{tpt})_2(\text{dobq})_3]^{6+}$ ($[\mathbf{1}]^{6+}$; $\text{tpt} = 2,4,6\text{-tris}(4\text{-pyridyl})\text{-}1,3,5\text{-triazine}$; $\text{dobq} = 2,5\text{-dioxido-}1,4\text{-benzoquinonato}$), porphyrin is trapped in the cavity of $[\mathbf{1}]^{6+}$, while in the larger octanuclear metalla-cube

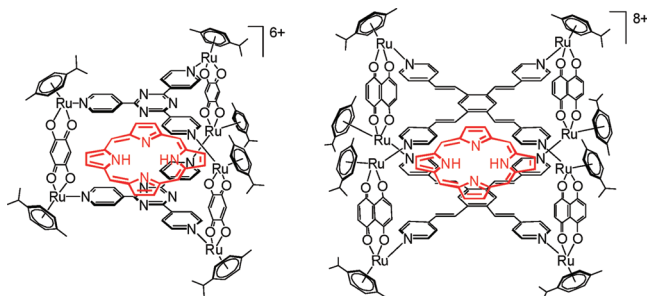


Figure 1. Molecular structures of $[\text{porphinC}\mathbf{1}]^{6+}$ and $[\text{porphinC}\mathbf{2}]^{8+}$.

$[\text{Ru}_8(\eta^6\text{-}p\text{-Pr}^i\text{C}_6\text{H}_4\text{Me})_8(\text{tpvb})_2(\text{donq})_4]^{8+}$ ($[\mathbf{2}]^{8+}$; $\text{tpvb} = 1,2,4,5\text{-tetrakis}\{2\text{-}(4\text{-pyridyl})\text{vinyl}\}\text{benzene}$; $\text{donq} = 5,8\text{-dioxido-}1,4\text{-naphthoquinonato}$), porphyrin is reversibly encapsulated and can be released without rupture of the cage compound. The antiproliferative activity and the phototoxicity of the empty cages and the porphyrinCage systems have been evaluated on human cancer cell lines from different phenotypes. Moreover, stability of the cages, uptake of the host–guest systems, and release of porphyrin after internalization in the cells have been studied by fluorescence spectroscopy.

Synthesis of the empty metalla-prism $[\mathbf{1}]^{6+}$ has been reported previously.⁴ However, synthesis of the carceplex $[\text{porphinC}\mathbf{1}]^{6+}$ is new and requires the addition of porphyrin during the formation of $[\mathbf{1}]^{6+}$ (see Supporting Information (SI)). The encapsulation of porphyrin in $[\mathbf{1}]^{6+}$ is easily monitored by ¹H NMR spectroscopy (Figure 2). Indeed, the signals associated with the protons of the porphyrin molecule are shifted upfield due to the encapsulation. Moreover, diffusion-ordered NMR spectroscopy (DOSY)⁶ clearly demonstrates that the porphyrin molecule is trapped in the hydrophobic cavity of $[\mathbf{1}]^{6+}$, as illustrated in Figure 2. The carceplex $[\text{porphinC}\mathbf{1}]^{6+}$ is isolated as the triflate salt.

Synthesis of the metalla-cube $[\mathbf{2}]^{8+}$ follows the same strategy using 4 equiv of $[\text{Ru}_2(\eta^6\text{-}p\text{-Pr}^i\text{C}_6\text{H}_4\text{Me})_2(\text{donq})\text{Cl}_2]^{7}$ and 2 equiv of tpvb^8 in methanol at reflux for 24 h (SI). Likewise, $[\text{porphinC}\mathbf{2}][\text{CF}_3\text{SO}_3]_8$ is prepared by adding 1 equiv of porphyrin during the formation of $[\mathbf{2}]^{8+}$. The empty cage and the host–guest system have been fully characterized by ¹H, ¹³C, and DOSY NMR spectroscopy, as well as by ESI-MS and elemental analysis. The encapsulation of porphyrin in the cavity of $[\mathbf{2}]^{8+}$ was confirmed by DOSY measurements (Figure 2). As compared to $[\text{porphinC}\mathbf{1}]^{6+}$, the proton signals of the encapsulated porphyrin molecule in $[\mathbf{2}]^{8+}$ are broad with a similar upfield shift, but as expected for a porphyrinCage system they are all diffusing with the proton signals of the cage. The broadness of the signals is due to the large cavity size of $[\mathbf{2}]^{8+}$, in which porphyrin is free to move. Indeed, Chem3D models of both⁹ $[\text{porphinC}\mathbf{1}]^{6+}$ and $[\text{porphinC}\mathbf{2}]^{8+}$ systems give clear pictures of the porphyrin environment in the cavities of $[\mathbf{1}]^{6+}$ and $[\mathbf{2}]^{8+}$ (Figure 3).

Spectroscopic measurements were realized on porphyrin, the empty cages as well as the porphyrinCage systems. UV–vis

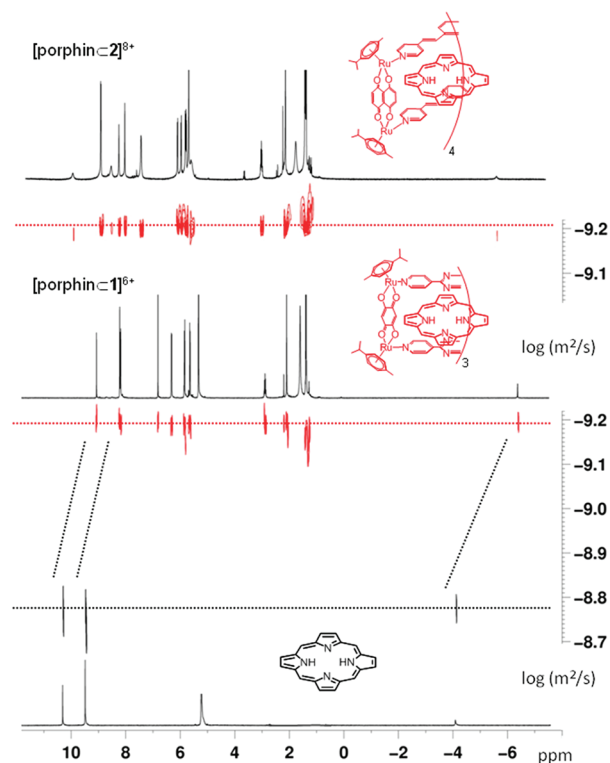


Figure 2. ^1H NMR (CD_2Cl_2 , 23 $^\circ\text{C}$) and DOSY spectra of porphyrin, $[\text{porphyrinC1}][\text{CF}_3\text{SO}_3]_6$, and $[\text{porphyrinC2}][\text{CF}_3\text{SO}_3]_8$.

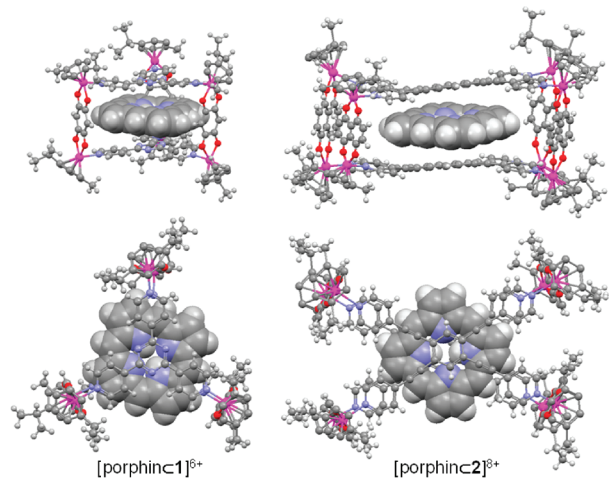


Figure 3. Chem3D models of the metalla-cages $[\text{porphyrinC1}]^{6+}$ and $[\text{porphyrinC2}]^{8+}$: side and top views.

absorption spectra reveal hypochromism of the characteristic porphyrin bands when trapped inside both cages (Figure S1), while the porphyrin fluorescence intensity almost vanishes upon encapsulation (see Figure 4). The strong hypochromism of the fluorescence is a useful phenomenon to study the uptake and stability of the systems as well as to follow the release of the guest by the cage compounds after internalization by the cells.¹⁰ In addition, the ability of porphyrin, the empty cages, and the porphyrinCage compounds to generate reactive oxygen species has been evaluated. All complexes $[\text{1}][\text{CF}_3\text{SO}_3]_6$, $[\text{2}][\text{CF}_3\text{SO}_3]_8$, $[\text{porphyrinC1}][\text{CF}_3\text{SO}_3]_6$, and $[\text{porphyrinC2}][\text{CF}_3\text{SO}_3]_8$ show no production of singlet oxygen in ethanol/DMSO as opposed to porphyrin, which possesses after excitation at 414 nm a singlet oxygen quantum yield of 97% (Table S1).

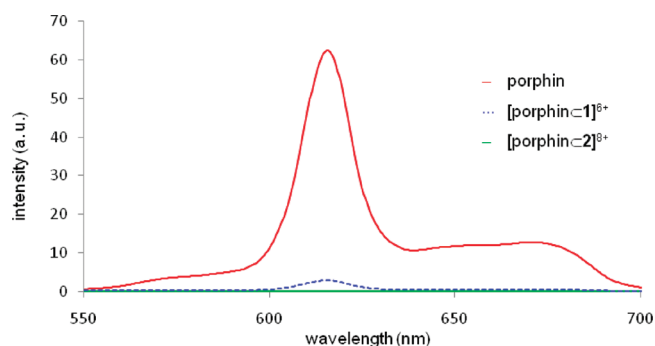


Figure 4. Fluorescence spectra of porphyrin and porphyrinCage systems (isopropanol/dmsol, 10^{-4} M, excitation 405 nm).

Consequently, empty and porphyrinCage systems can be considered harmless in term of phototoxicity; only after release of porphyrin is photoactivity regained.

The stability of the porphyrinCage systems has been evaluated under various biological conditions (see SI). All complexes are stable at physiological pH from 6 to 8 at 37 $^\circ\text{C}$. In the presence of oxidative (H_2O_2) or reductive (dithiothreitol) derivatives, no degradation of the host–guest systems is observed. Similarly, when exposed to complete culture medium, the porphyrinCage systems remain intact.

The uptake and release of porphyrin after internalization of the host–guest systems have been studied for various human cancer cells, Me300, A2780, A2780cisR, HeLa, and A549 (Table 1). The antiproliferative activity of the complexes in the

Table 1. Cytotoxicity of PorphyrinCage Systems for Various Human Cancer Cells after 72 h Incubation in the Dark

cells	IC_{50} (μM)	
	$[\text{porphyrinC1}]^{6+}$	$[\text{porphyrinC2}]^{8+}$
Me300	5.7 ± 0.9	5.0 ± 0.1
A2780	6.0 ± 0.8	12.0 ± 0.5
A2780cisR	5.2 ± 0.9	6.2 ± 1.7
HeLa	9.5 ± 1.3	12.5 ± 3.1
A549	8.5 ± 1.7	11.2 ± 5.0

dark was evaluated, demonstrating that the empty cages and porphyrinCage systems present moderate cytotoxicities with comparable values in the cell lines tested. All IC_{50} values are comprised in the range 5–12 μM (Table 1), and no significant differences were found between the empty and porphyrinCage systems. Moreover, despite the presence of eight ruthenium atoms per metalla-cage in $[\text{2}]^{8+}$, as opposed to only six in $[\text{1}]^{6+}$, $[\text{porphyrinC2}]^{8+}$ is slightly less cytotoxic than $[\text{porphyrinC1}]^{6+}$.

Interestingly, the porphyrin fluorescence could be detected intracellularly during the incubation of cells with the porphyrinCage systems. A stronger signal for $[\text{porphyrinC2}]^{8+}$ than $[\text{porphyrinC1}]^{6+}$ in all cell lines was observed, suggesting a higher porphyrin release inside the cells (Figure 5). The differences in fluorescence after 72 h incubation can be correlated to the nature of the porphyrinCage systems. Indeed, as emphasized in Figure 3, the release of porphyrin requires two different mechanisms depending on the cages: rupture of the cage in $[\text{porphyrinC1}]^{6+}$, while in $[\text{porphyrinC2}]^{8+}$ the porphyrin molecule can diffuse through an aperture without breakage of the cage.

The uptake of the porphyrinCage systems by cells was further visualized by fluorescence microscopy. Cells incubated with

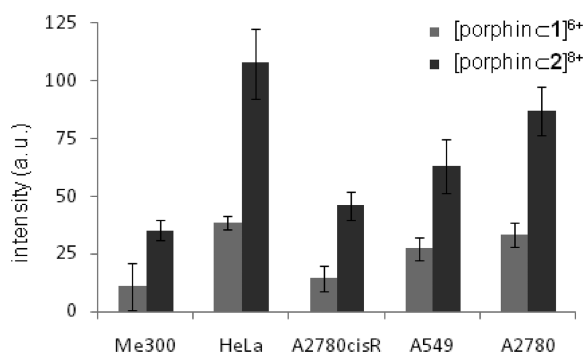


Figure 5. Fluorescence of porphyrin detected in cancer cells after 72 h incubation (5 μ M porphyrinCage systems).

[porphyrinC1]⁶⁺ did not present enough fluorescence to be detected, while incubation with [porphyrinC2]⁸⁺ revealed strong red and blue fluorescence spots corresponding to porphyrin molecules and empty cages, respectively (Figure 6). These

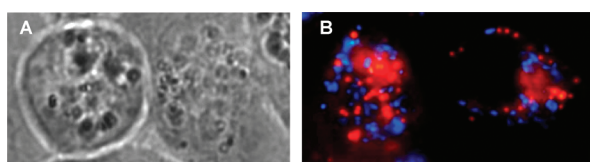


Figure 6. Fluorescence microscopy of HeLa cells incubated with [porphyrinC2]⁸⁺ (2 μ M, 20 h): (A) white light and (B) fluorescence.

findings confirm the intracellular release of porphyrin from the cage and also indicate that both the cage and porphyrin are located in different compartments of the cell and not in the nucleus.

The photodynamic efficiency of both porphyrinCage systems was evaluated in HeLa cells at 0.5 μ M concentration (\sim 20 times below the IC₅₀ concentration, 20 h incubation). Excellent phototoxicities were found for both cages, confirming the release of porphyrin from the cage (Figure 7). Moreover,

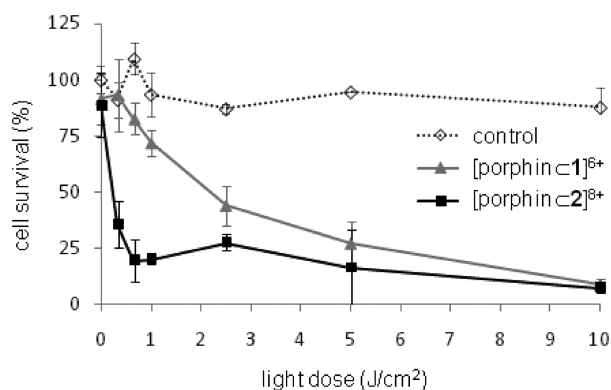


Figure 7. Photodynamic activity of porphyrinCage systems (0.5 μ M, 20 h, 488 nm irradiation) in HeLa cancer cells (control being cells irradiated without compound).

[porphyrinC2]⁸⁺ (0.2 J/cm²) was 10 times more photoactive than [porphyrinC1]⁶⁺ (2.1 J/cm²). This result is in complete agreement with intracellular measurements of porphyrin fluorescence and singlet oxygen quantum yield, linking the release of porphyrin with photoefficiency.

In conclusion, we have demonstrated that the metalla-cages were able to carry and deliver intracellularly photosensitizers

following uptake by cells. The release of porphyrin is higher for the larger cubic cage as compared to the smaller prismatic cage. These systems display hypochromism properties toward the photosensitizer loaded inside the cavity of the cage, resulting in the absence of phototoxic effect outside of cells. This ability defines our cages as very safe and powerful tools for new photodynamic strategies that may not induce overall photosensitization in patients and therefore allow better efficiency in photodynamic treatment.

■ ASSOCIATED CONTENT

📄 Supporting Information

Experimental details, UV-vis spectra, and singlet oxygen quantum yields. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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