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Assembling a Mixed Phthalocyanine–Porphyrin Array in Aqueous Media through Host–Guest Interactions†

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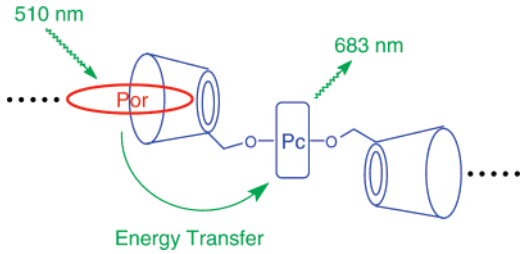
ABSTRACT



A stable 1:1 host–guest complex is formed between a silicon(IV) phthalocyanine conjugated axially with two permethylated β-cyclodextrin units and a tetrasulfonated porphyrin. The complex exhibits a light-harvesting property and works as an efficient photosensitizing system, killing HT29 human colon adenocarcinoma cells with an IC_{50} value of 0.09 μM.

Supramolecular chemistry provides a powerful tool to construct complex chemical systems from small building blocks by self-assembly and recognition processes based on noncovalent interactions.[1] This approach has been widely used to build sophisticated porphyrin scaffolds for the collection, conduction, and conversion of light and redox energy.[2] Phthalocyanines are another important class of functional dyes which exhibit intriguing electronic and optical characteristics. Self-assembled systems involving these macrocycles are relatively rare.[3] On the basis that phthalocyanines and porphyrins have complementary absorptions in the visible region and exhibit distinct redox and photophysical properties, hetero-arrays of these macrocycles would be of particular interest. Apart from the potential use as novel optoelectronic materials, these systems can also serve as models for the study of energy and electron-transfer processes in artificial photosynthetic systems. In addition, both classes of these tetrapyrrole derivatives are efficient photosensitizers for photodynamic therapy.[4] They may work in a cooperative manner in the hybrid systems. A substantial number of these hetero-arrays have been reported, in many of which the phthalocyanine and porphyrin units are linked either co-

† Dedicated to Professor Tien-Yau Luh on the occasion of his 60th birthday.

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valently[5] or through a metal center forming sandwich-type complexes.[6] A supramolecular approach has not been extensively used to construct these mixed tetrapyrrole systems. To our knowledge, only a few face-to-face aggregates held by electrostatic[7] or other[8] interactions and several axially bound coordination complexes[9] have been reported. Supramolecular hetero-arrays of these tetrapyrrole derivatives held by host−guest interactions have not been reported so far. We describe herein a novel β-cyclodextrin-conjugated phthalocyanine and its complexation with a tetrasulfonated porphyrin in aqueous media. The resulting 1:1 host−guest complex exhibits a light-harvesting property and works as an efficient photosensitizing system for photodynamic therapy.

It was reported that heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMe-β-CD) forms very stable 2:1 inclusion complexes with water-soluble *meso*-tetrasubstituted porphyrins in aqueous media.[10] This moiety was therefore selected to conjugate with a phthalocyanine core. Treatment of the readily available silicon(IV) phthalocyanine dichloride with mono-6-hydroxy permethylated β-cyclodextrin[11] and NaH in toluene gave the expected disubstituted product **1** in 42% yield (Figure 1) (see the Supporting Information). This

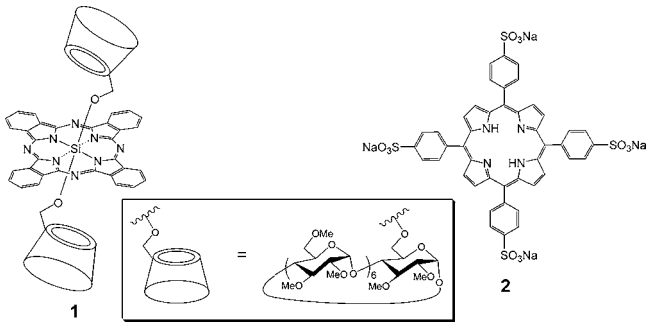

Figure 1. Structures of the cyclodextrin-conjugated phthalocyanine **1** and the tetrasulfonated porphyrin **2**.

compound, which represents a very rare covalently linked cyclodextrin−phthalocyanine conjugate,[12] had good solubility in many organic solvents and could be purified readily by silica-gel column chromatography. However, the solubility of this compound in water was rather limited. Only in the presence of a small amount of organic solvents such as methanol could a dilute aqueous solution of **1** be prepared.

Compound **1** was characterized with various spectroscopic methods. The high-resolution ESI mass spectrum showed two major envelopes peaking at *m*/*z* 3391.5 and 1705.8 for the ions $[M + Na]^+$ and $[M + 2Na]^{2+}$, respectively, with the expected isotopic pattern. The ^{1}H NMR spectrum in $CDCl_3$ showed two downfield AA′BB′ multiplets at δ 9.57−9.60 and 8.30−8.33 assignable to the phthalocyanine α and β ring protons, respectively. The OCH_2 protons above and below the phthalocyanine ring resonated as two upfield-shifted doublets at δ −0.49 and −2.49 as a result of the phthalocyanine ring current. The signals for the permethylated β-cyclodextrin moiety spread in a relatively wide region (from δ 0.04 to 5.01), again due to the ring current effect. The $^{13}C\{^1H\}$ NMR spectrum of **1** in $CDCl_3$ clearly showed four aromatic signals for the phthalocyanine ring. Due to the loss of C_7 symmetry of the cyclodextrin unit, the signals for C_1 and one of the methyl groups were each split into seven signals (at δ 94.4−99.6 and 61.1−62.3, respectively), while the other signals were partially overlapped.

The complexation of **1** with the tetrasulfonated porphyrin **2** (Figure 1) was first studied by NMR spectroscopy. The ^{1}H NMR spectrum of an equimolar mixture of **1** and **2** in D_2O was very complicated, which precluded a complete assignment. Some of the very upfield signals (from δ −2.2 to 1.0), however, could be unambiguously assigned to the glucopyranoside unit directly linked to the silicon phthalocyanine by 2D experiments including COSY, TOCSY, and ROESY (see the Supporting Information). The signals for **2** were split extensively in the region δ 6.9−9.5. As shown in the ROESY spectrum, these protons were correlated with the cyclodextrin moiety, suggesting that **2** is encapsulated in the cyclodextrin cavity.

Figure 2 shows the change in absorption spectrum of **1** upon addition of **2** in water. The Q-band of **1** at 679 nm is gradually decreased in intensity, giving two bands at 675 and 690 nm with an isobestic point at 687 nm. As shown in the inset, the change in absorbance for the bands at 679 and 690 nm becomes steady when 1 equiv of **2** is added. This strongly suggests that a 1:1 inclusion complex is formed between **1** and **2**. As β-cyclodextrin derivatives usually bind to charged *meso*-tetrasubstituted porphyrins in a 2:1 manner,[10] compound **1** bearing two TMe-β-CD units logically can bind to one equiv. of **2** forming a head-to-tail polymeric complex, although a 2:2 cyclic supramolecular complex cannot be precluded at this stage. The negative-ion mode ESI mass spectrum of a 1:1 mixture of **1** and **2** showed the

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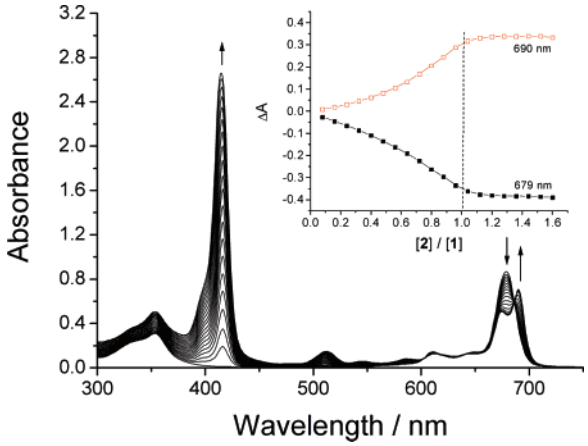

Figure 2. Change in absorption spectrum of **1** (5.0 μM) upon addition of **2** in water. The inset plots the change in absorbance of the Q bands at 679 and 690 nm versus the concentration ratio [**2**]/[**1**].

base peak at *m*/*z* 1074.89. The value as well as the isotopic pattern were in good agreement with the ion [**1** + **2** − 4Na]$^{4-}$. The signals for the higher aggregates of **1** + **2** were not found.

Upon excitation at 610 nm, phthalocyanine **1** in water gave a strong fluorescence at 683 nm. The signal was quenched gradually upon titration with porphyrin **2** and the change in fluorescence intensity again became steady when 1 equiv of **2** was added (Figure 3). The transient absorption spectrum

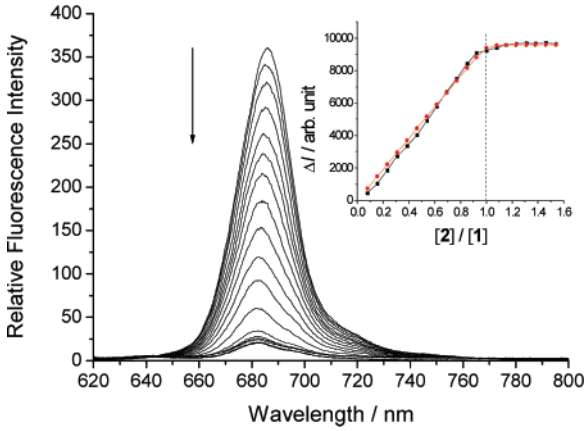

Figure 3. Change in fluorescence spectrum of **1** (6.5 μM) upon addition of **2** in water. The inset plots the observed (■, black) and calculated (●, red) changes in fluorescence intensity (area under 620−800 nm) versus the concentration ratio [**2**]/[**1**].

of a 1:1 mixture of **1** and **2** in water showed a weak band at ca. 580 nm (see the Supporting Information), which can be attributed to the transient absorption of the radical anion of **1**.[13] This observation supported that the fluorescence quenching was due to a photoinduced electron-transfer process. By applying eq 1 derived by Inoue et al.,[14] where ΔI is the change in fluorescence intensity of **1** upon addition of **2**, $[H]_0$ and $[G]_0$ are the initial concentrations of host and guest (i.e., **1** and **2** in this case), α is a proportionality coefficient, and K is the complex stability constant, the K value for this system was determined to be $(2.1 \pm 1.3) \times 10^8$ M^{-1} by a nonlinear least-squares method (inset of Figure 3). Kano et al. studied the complexation of TMe-β-CD with a series of charged porphyrins including compound **2**.[10b] It was found that the K values in water were too large to be determined. The value for the complex TMe-β-CD/**2**, however, could be determined to be $(2.0 \pm 1.3) \times 10^4$ M^{-1} in ethylene glycol/water (3:1). The very large binding constant found in this study is in line with these results.

$$\Delta I = \{\alpha([H]_0 + [G]_0 + 1/K) \pm \sqrt{\alpha^2([H]_0 + [G]_0 + 1/K)^2 - 4\alpha^2[H]_0[G]_0}\}/2 \quad (1)$$

Upon excitation at 510 nm, where only porphyrin **2** has a weak absorption, the 1:1 supramolecular complex of **1** and **2** in water emitted strongly at 683 nm, which could be ascribed to the fluorescence of the phthalocyanine unit. A weak emission at 639 nm due to **2** was also observed. The fluorescence quantum yield of **2** decreased from 0.20 to 0.01 upon complexation with **1** [with reference to *meso*-tetraphenylporphyrin in benzene ($\Phi_f = 0.11$)].[15] Excitation of **1** in water at this position did not give the emission band at 683 nm. These observations indicated the presence of an efficient singlet−singlet energy transfer process, from the excited porphyrin to the phthalocyanine component in the complex. This was confirmed by the excitation spectrum monitored at 683 nm, in which apart from the signals corresponding to the absorptions of **1** (at 353, 611, 649, and 679 nm), a strong band at 417 nm and a weak band at 510 nm, corresponding to the Soret and Q bands of **2**, respectively, also appeared. The transient absorption at ca. 580 nm, attributable to the radical anion of **1**, was also seen upon excitation at 510 nm (see the Supporting Information). Hence electron transfer is a way to depopulate the first excited-state of **1**, no matter if it is generated by direct excitation of **1** or through an energy transfer from the excited **2**.

The light-harvesting efficiency quantified as energy transfer quantum yield (Φ_{ENT}) was estimated by comparing the normalized (at 610 nm, where only the phthalocyanine component absorbs) absorption and excitation spectra at the porphyrin's Soret band region (see the Supporting Information).[16] The value was calculated to be 47%, showing that this is a rather efficient process. Since the fluorescence of **2** was greatly reduced in the presence of **1**, this value suggested that apart from the energy transfer pathway, an electron-transfer route might also be involved in the depopulation of

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the excited state of **2**. It is worth noting that although energy transfer is common and often highly efficient in covalently linked phthalocyanine-porphyrin hetero-arrays,[5] this has not been observed in the supramolecular counterparts reported so far.[7−9] The light-harvesting property of the host−guest complex of **1** and **2** can be attributed to the exceptionally strong binding of the two components. In fact, host−guest light-harvesting antenna systems based on cyclodextrins have been documented.[17]

As both phthalocyanines and porphyrins can serve as photosensitizers for photodynamic therapy,[4] we briefly examined the in vitro photodynamic activity of this host−guest system. Figure 4 shows the effects of phthalocyanine

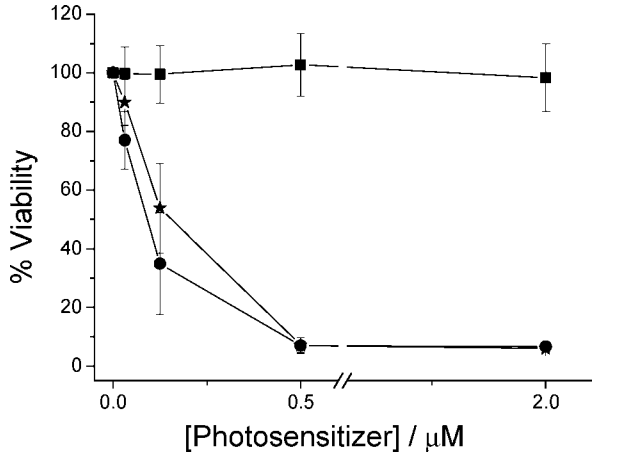

Figure 4. Photocytotoxicities of **1** (in Cremophor EL) (★), **2** (■), and the 1:1 host−guest complex of **1** and **2** (●) against HT29 cells (λ > 610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± SEM of three independent experiments, each performed in quadruplicate.

1, porphyrin **2**, and their 1:1 host−guest complex on HT29 human colon adenocarcinoma cells upon illumination with a red light (λ > 610 nm, total fluence = 48 J cm^{-2}). The cell viabilities were determined by MTT assay as described previously.[18] Due to the limited solubility of **1** in water, this compound was formulated with Cremophor EL as an emulsion, while the other two systems could simply be formulated with water. In the absence of light, all these systems were not cytotoxic. As shown in Figure 4, porphyrin **2** has virtually no effect on the cell viability because of its negligible absorption at λ > 610 nm. By contrast, both phthalocyanine **1** and the host−guest complex are highly photocytotoxic with a 50% growth-inhibitory ratio (IC_{50}) of 0.15 and 0.09 μM, respectively. Their dose−response curves are very similar. As revealed by fluorescence microscopy, phthalocyanine **1** in both systems could enter the cells causing intracellular fluorescence (excited at 630 nm and monitored at >660 nm). However, while the fluorescence was rather uniform in the cytoplasm of the cells for **1** in Cremophor EL emulsion, it appeared as bright and granular spots for the inclusion complex (see the Supporting Information). The different appearance suggested that the two systems may have different cellular uptake and localization properties. Nevertheless, the results showed that the 1:1 host−guest complex in water is as effective as **1** in Cremophor EL as a photosensitizing system. Porphyrin **2** enhances the water solubility and facilitates the formulation of phthalocyanine **1** through complex formation, preventing the use of Cremophor EL, which may cause serious hypersensitivity reactions.[19] It is envisaged that a careful selection of other photosensitizing guest species which have appropriate absorption and photophysical properties may lead to synergistic and improved photosensitizing systems. This work is being pursued.

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Supporting Information Available: Experimental procedures and characterization data for **1**; COSY, TOCSY, and ROESY spectra of **1** in $CHCl_3$ and the 1:1 inclusion complex of **1** and **2** in D_2O; transient absorption spectra and normalized absorption and excitation spectra of the 1:1 inclusion complex of **1** and **2** in water; microscopic images of HT29 cells after incubation with **1** in Cremophor EL or the 1:1 inclusion complex of **1** and **2** in water. This material is available free of charge via the Internet at http://pubs.acs.org.

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