A multifunctional nanoassembly of mesogen-bearing amphiphiles and porphyrins for the simultaneous photodelivery of nitric oxide and singlet oxygen†

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We report a molecular nanoassembly able to supply simultaneously, in the same region of space and under the exclusive control of visible light, nitric oxide and singlet oxygen, two species playing a key role in the therapy of cancer; the considerable fluorescence of this nanoaggregate and its reduced size (*ca.* 40 nm) represent additional advantages that make this photoactive vehicle an appealing candidate to be tested in biological systems.

In recent years, there has been an explosion of interest in nitric oxide (NO),[1] a simple diatomic free radical, for the central role it occupies in the maintenance and regulation of numerous physiological processes, encompassing neurotransmission, vasodilation and hormone secretion.[2] Besides its involvement in these vital functions, one of the most exciting properties of NO is its anticancer activity.[3] In this regard, NO releasing systems exclusively controlled by light inputs are particularly appealing due to the accurate dosage control of NO they offer with respect to those based on spontaneous thermolysis. This is a crucial point for the use of NO in anticancer therapy. In their excellent review on NO donors with anticancer activity,[4] Wang and coworkers have outlined how NO can act as a *double-edged sword* either inhibiting or encouraging tumor proliferation depending on the dose. They also stressed that for the effective use of NO in anticancer therapy its combination with common antitumor drugs is highly desirable.[4]

Porphyrins have extensively proven their activity as anticancer drugs in photodynamic cancer therapy (PDT).[5] In this well-known non-invasive treatment, porphyrins excited with visible light transfer the energy of their lowest triplet state to nearby oxygen molecules to generate reactive singlet oxygen, $^1O_2$ ($^1\Delta_g$), the actual photodynamically active species inducing cellular death.[6] However, incorporation of porphyrins within appropriate biovectors is often necessary in PDT and the preservation of the photophysical properties of the drug is a key pre-requisite for such delivery systems.[7]

On these grounds the achievement of photoactive carriers able to photogenerate NO themselves and, at the same time, to entrap porphyrins without alteration of their photodynamic activity, represents an intriguing objective to pursue. In fact, such systems can, in principle, produce synergistic effects due to the simultaneous photogeneration of NO and $^1O_2$ ($^1\Delta_g$) in the same region of space, maximizing the efficiency of the phototreatment.

With this in mind, and stimulated by our recent results on NO photoreleasing nanosystems,[8a–c] we report herein a multifunctional micellar nanoassembly formed by the mesogen-bearing cationic amphiphilic **1** and the anionic 5,10,15,20-tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphyrin (TPPS), as a fluorescent delivery vehicle able to supply NO and $^1O_2$ ($^1\Delta_g$) under the control of visible light (Fig. 1).

Compound **1** was easily synthesized in two steps (see ESI†) and incorporates a commercial nitroaniline derivative that we have recently discovered to be a suitable NO releasing unit under visible light stimulation.[8b,c] In particular, the photochemical mechanism involves a nitro-to-nitrite rearrangement followed by the rupture of the O–N bond to generate a phenoxyl radical and NO.[8a–c]

The aggregation behavior of **1**, which readily dissolves in water at room temperature, was investigated by conductivity and dynamic light scattering (DLS) measurements (Fig. 2). The plots of the specific conductivity and DLS intensity *vs.* the surfactant concentration are in excellent agreement with each other. From the break points it can be established that the critical aggregation concentration (cac) of **1** is *ca.* 0.7 mM. It is noteworthy that such a value is more than one order of magnitude smaller than that expected for a cationic surfactant containing both the same alkyl chain and the polar head and, according to the literature, this can be explained as a result of a drastic decrease of ΔG°_{agg} due to the terminal mesogenic unit.[9] The size distribution obtained by DLS measurements performed above the cac (inset, Fig. 2) is relatively

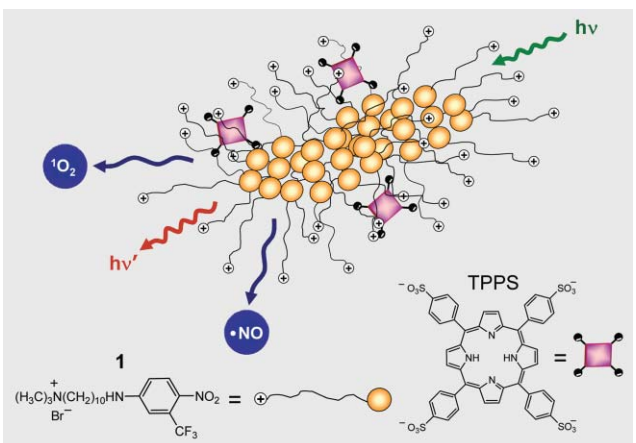

Fig. 1 Idealized view of the **1**–TPPS multifunctional nanoassembly.

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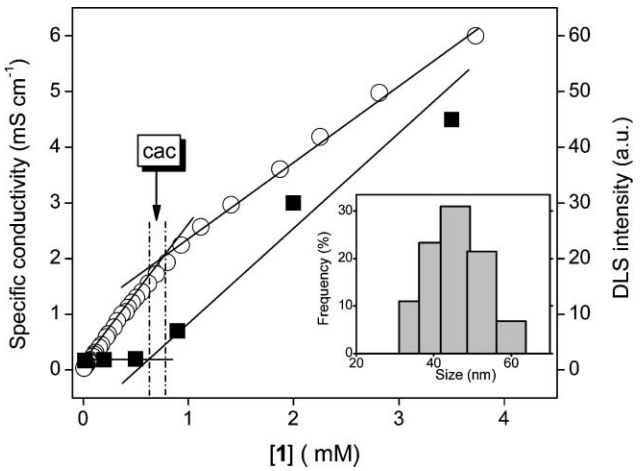

Fig. 2 Specific conductivity (○) and DLS intensity (■) of aqueous solutions of **1** as a function of surfactant concentration. The inset shows the size distribution of **1** micellar aggregates.

narrow with a mean diameter = 41.5 nm and a σ = 14.1 nm.[10] This value is larger than that expected for regular spherical micelles of **1** but is perfectly in line with the results reported for similar amphiphiles of the type *cationic group–decyl spacer–aromatic tail*, which are shown to form elongated micellar aggregates in aqueous solution.[9]

The self-assembly of TPPS and micelles of **1** is strongly encouraged by favorable electrostatic interactions in view of their opposite charges, and was demonstrated by UV–Vis absorption and emission spectroscopy. As shown in Fig. 3A, the absorption spectrum of TPPS in the presence of micelles (spectrum c) does not reproduce the sum of the single components (spectrum d), accounting for an interaction of the porphyrin with the micellar system in the ground state. Clearer evidence for such an interaction is provided by a comparison of the spectra (b) and (e) where it can be readily noted that TPPS undergoes hypochromism and red-shift of the Soret absorption upon binding with micelles. Besides, a red-shift was also observed in the $Q_{(0,0)}$-band peaks (see inset) as well as in the typical double band emission of TPPS (Fig. 3B). The fluorescence quantum yield was only slightly affected by the micelles, going from 0.16 (for the free TPPS) to 0.11, and did not exhibit any dependence on the excitation wavelength, suggesting the presence of a single population of fluorophores within the micelles. This spectroscopic scenario excellently reflects that reported for the same porphyrin in the presence of optically transparent cationic micelles[11] and is also very similar to that recently found in the case of cationic amphiphilic drug-carriers.[12] Accordingly, our data are consistent with the entangling of TPPS mainly as a monomer within the micellar network. DLS measurements were also performed in the presence of TPPS and showed only negligible changes in the size of the micellar aggregates (see ESI†).

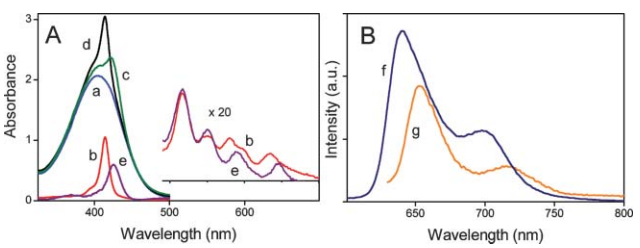

Fig. 3 A: absorption spectra in the Soret band, for (a) **1** (2 mM), (b) TPPS (20 μM) and (c) their mixture in aqueous solution. Cell path: 1 mm. The sum (d) = (a) + (b) and the difference (e) = (c) − (a) spectra are also shown, for the sake of comparison. The magnified (b) and (e) spectra in the Q-bands region are also shown in the inset. B: Fluorescence spectra (λ_{exc} = 580 nm) of TPPS (f) in the absence and (g) in the presence of **1** (2 mM).

The most convenient method for testing the suitability of the **1**–TPPS nanoassembly to photogenerate NO and $^1O_2$ ($^1\Delta_g$) is the direct and real-time monitoring of these transient species. To this end, we used an ultrasensitive NO electrode, which directly detects NO concentration by an amperometric technique and a time-resolved luminescence apparatus to monitor the typical infrared luminescence of $^1O_2$ ($^1\Delta_g$) at 1270 nm[13] (ESI†). Fig. 4 shows unambiguous evidence for the light-controlled generation of NO and $^1O_2$ ($^1\Delta_g$) from the nanoassembly. In fact, we observed linear photogeneration of NO which promptly stopped when the light was turned off, besides the characteristic infrared luminescence of $^1O_2$ ($^1\Delta_g$) decaying with first-order kinetics in *ca.* 4 μs.

A further, remarkable point of interest of this work is the photochemical independence of the two photoactive units of the nanoaggregate. Comparative photochemical experiments demonstrated that the photogeneration efficiency of NO ($\Phi_{NO} \sim 0.01$) and $^1O_2$ ($^1\Delta_g$) ($\Phi_\Delta \sim 0.6$) from the nanoassembly is virtually the same as that of the single components (Fig. 5), ruling out any

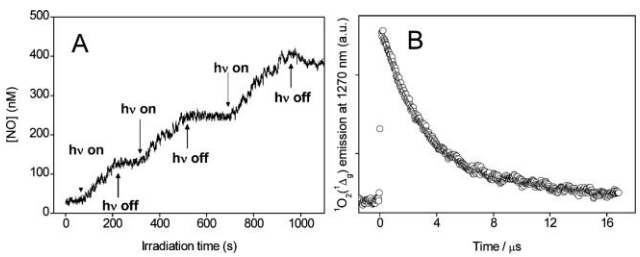

Fig. 4 A: NO released upon 450 nm light irradiation of **1**–TPPS nanoassembly in aqueous solution. (B) Representative decay kinetics of $^1O_2$ ($^1\Delta_g$) and related first-order fitting observed upon 532 nm laser excitation of **1**–TPPS nanoassembly in aqueous solution. [**1**] = 2 mM, [TPPS] = 20 μM.

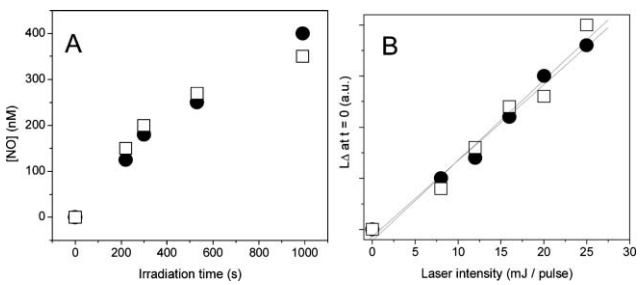

Fig. 5 A: photochemical NO release from micellar solution of **1** (2 mM) in the absence (□) and in the presence (●) of TPPS (20 μM). λ_{exc} = 450 nm. B: Luminescence intensity of $^1O_2$ ($^1\Delta_g$) at initial time (LΔ at t = 0) as a function of the laser intensity in the case of aqueous solution of TPPS (20 μM) in the absence (□) and in the presence (●) of **1** (2 mM). λ_{exc} = 532 nm. The data are corrected for the slight difference of photons absorbed by the different samples at the excitation wavelengths.

quenching effect due to potential photoinduced energy and/or electron transfer involving the two chromophores.

In summary, we report a photoactivable micellar nanoassembly that, to our knowledge, represents the *first molecular system* able to deliver simultaneously, in the same region of space, and under the exclusive control of visible light, NO and $^1O_2$ ($^1\Delta_g$), two species playing a key role in the therapy of cancer. The considerable fluorescence of the nanoaggregate, a useful tool for mapping its localization in cells, and its reduced sizes, represent additional advantages that make this photoactive vehicle an appealing candidate to be tested in biological research. Studies concerning this are currently in progress. Finally, we believe that the extension of the present approach to a variety of *ad-hoc* chosen NO photodonors and porphyrin derivatives may pave the way for the development of novel classes of nanoscaled, photoactivable systems in the emerging field of nanomedicine, for multimodal therapy.

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