Soft Matter

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A soft supramolecular carrier with enhanced singlet oxygen photosensitizing properties†

Cite this: *Soft Matter*, 2013, **9**, 2453

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Herein we report the self-assembly of a supramolecular singlet oxygen photosensitizing system from an adamantane-functionalized, hexaanionic water-soluble zinc(II) phthalocyanine (PC) and β-cyclodextrin vesicles (CDV). Characterisation of the designed PC, which was synthesized by an asymmetric statistical condensation, was carried out by several analytical techniques such as MALDI-HRMS, NMR, IR, UV/vis as well as steady state and time resolved fluorescence spectroscopy. The influence of the docking of the PC to the CDVs on the PC photoluminescence as well as on the singlet oxygen photoproduction quantum yields was investigated. The results indicate that the host–guest interaction of the photosensitizer and the CDVs significantly prevents the formation of inactive aggregates, and enhances the photosensitizing ability of the PC. The supramolecular assembly constitutes a biocompatible photoactive platform for the design of phototherapeutic agents.

Received 11th October 2012
Accepted 17th December 2012

DOI: 10.1039/c2sm27353e

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Introduction

Photodynamic therapy (PDT) constitutes a non-invasive method for the inactivation of cancer cells or bacteria.[1] In this approach, irradiation of a photosensitizer (PS) with harmless red or near infrared light (λ > 650 nm) leads to the formation of cytotoxic singlet oxygen ($^1O_2$) by energy transfer from the lowest excited triplet state of the PS to molecular oxygen ($^3O_2$).[2] Since the introduction of phthalocyanines (PCs)[3,4] and porphyrins[5–7] as PS of $^1O_2$, several potential carrier systems for these rather hydrophobic molecules have been proposed.[8,9] PCs are known to be highly insoluble in aqueous environments, and consequently, only limitedly available *in vivo*. Several approaches to increase their water solubility have been investigated, which include among others the introduction of amine,[10,11] carboxylate[12] and hydroxyl[13] functions. Furthermore, it is well known that PCs tend to form inactive aggregates due to hydrophobic interactions. This behaviour decreases their ability to photosensitize $^1O_2$, as the stacked molecules release the absorbed energy mainly as heat.[14] In this sense, the introduction of charges on the periphery of the PC is expected to have a positive influence on the solubility while avoiding aggregation by electrostatic repulsion, thus enhancing their bioavailability and $^1O_2$ photoproduction.[11,12]

In the last few years, the first efforts were made to use organic and inorganic transporter systems for PCs, such as liposomes, surfactants and zeolites, to avoid aggregation and secondary interactions with tissues.[8,9] On the other hand, Mazzaglia *et al.* described the absorption of photosensitizers such as porphyrins in cyclodextrin (CD) nanoparticles, which consist of amphiphilic CDs bearing C_6 alkyl chains at the primary face and ethylene glycol units with amine functions at the secondary face. In this case, the negatively charged 5,10,15,20-tetrakis-(4-sulfonatophenyl)-21*H*,23*H*-porphyrin (TPPS) binds to the positively charged CD nanoparticles due to electrostatic interactions.[15–17] In 2000, Ravoo and Darcy reported another class of amphiphiles based on CDs.[18] By the introduction of alkyl chains (C_{12} or C_{16}) on the secondary face of the CD and oligoethylene glycol units on the primary face, amphiphiles were obtained that form stable

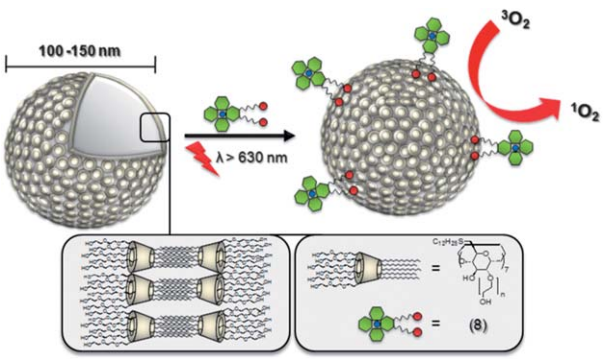

Fig. 1 Schematic representation of cyclodextrin vesicles decorated with phthalocyanine photosensitizer (**8**).

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† Electronic supplementary information (ESI) available: Details of reactions and measurements conducted. See DOI: 10.1039/c2sm27353e

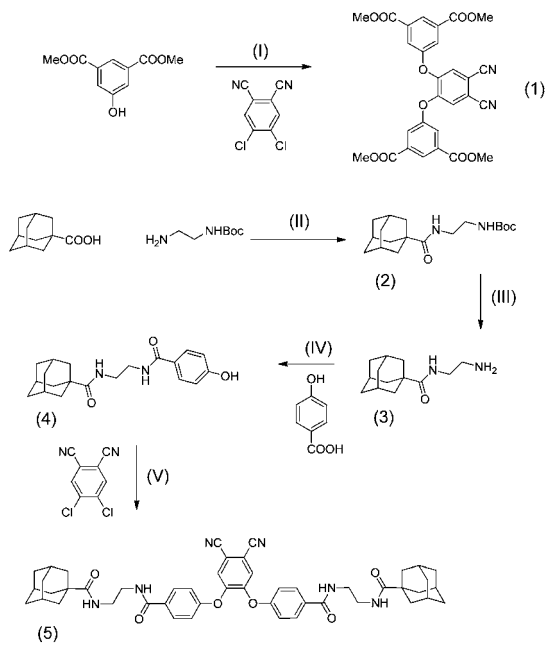
unilamellar cyclodextrin vesicles (CDV) in aqueous media. The cavities of the CDs embedded in the surface of these vesicles are available for complexation with hydrophobic guests such as adamantane,[19–23] *tert*-butylbenzene[24] and azobenzene[25,26] units so that the CDV can be decorated with functional guest molecules simply by mixing the host vesicles with the desired guests. Herein we describe the use of such supramolecular soft colloids for the immobilisation and transport of a tailored PS (Fig. 1). We synthesized a PC-adamantane derivative (**8**) in order to combine the photosensitizing capacity of the macrocycle with the binding ability to the CDV vesicles, which effectively prevents the formation of inactive aggregates.

Results and discussion

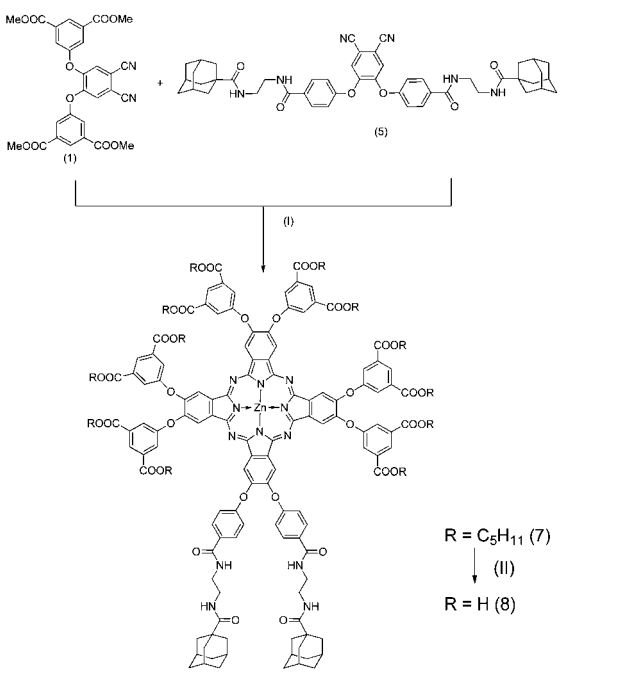
The synthesis of the PC derivative was carried out by using a statistical condensation of substituted phthalonitriles, starting from 1,2-dichlorophthalonitrile, which was converted into the tetraester (**1**) by aromatic nucleophilic substitution with an adequately substituted phenol derivative in the presence of K_2CO_3 (Scheme 1). The X-ray diffractogram of crystals of the resulting tetraester (**1**) was obtained. The crystal structure reveals the two nitrile groups as well as the ester moieties in the periphery, and corresponds to the space group $P\bar{1}$ (no. 2) with 2 molecules per unit cell (see ESI, Fig. S1†). A second dinitrile derivative bearing two adamantane moieties was also synthesized starting from adamantane carboxylate. This was coupled *via* an amide bond to mono Boc-protected ethylendiamine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), ethylcyano (hydroxyimino)acetate (Oxyma Pure®) and *N*-methylmorpholine (NMM) as coupling agents. Full details of the synthesis are provided in the ESI.†

After deprotection in acidic conditions with trifluoroacetic acid (TFA), the free amine was reacted with hydroxyl phenylcarboxylate as described above (*vide supra*). The deprotected hydroxyl group was then coupled to 1,2-dichlorophthalonitrile. The hydrophobic adamantane provides the ability to bind divalently to the surface of the CDV.[20] The phthalocyanine (**5**) and (**1**) were finally condensed statistically in a ratio of 3 : 1 employing DBU (1,8-diazabicyclo[5.4.0.]undec-7-ene) and anhydrous $Zn(OAc)_2$ in refluxing pentanol, yielding after purification by semipreparative HPLC the dark blue zinc(II) PC (**7**) (2.9% yield, Scheme 2). During the condensation, a transesterification of the methylesters to pentylesters was observed, which has been previously described by other authors.[12] The identity of the product was confirmed by high resolution mass-spectrometry, and ^{1}H-NMR and IR spectroscopy. In order to obtain the water soluble Zn(II) PC (**7**), the ester groups were removed by saponification with NaOH. After neutralization with HCl, a green precipitate was obtained which was found to be the desired asymmetrical, water soluble Zn(II) PC which was confirmed by MALDI-HR-MS. ^{1}H-NMR spectroscopy only showed broad, unstructured signals due to stacking of the PC (**8**), both in water and in DMSO (see ESI, Fig. S2†).

CDVs were prepared from an amphiphilic β-cyclodextrin substituted with 7 *n*-dodecylthiol units on the primary side and 7 oligo(ethylene glycol) units on the secondary side. These amphiphilic macrocycles form unilamellar bilayer vesicles upon dispersion and extrusion in aqueous solution. The synthesis of the amphiphilic cyclodextrin and the preparation and characterization of vesicles have been described in the literature.[19] In order to assess the influence of (**8**) on the structure of the CDVs, sizes and ζ-potentials were determined by dynamic light



Scheme 1 Synthesis of substituted phthalonitriles (I): K_2CO_3, DMF, 24 h, rt, (II): EDCI, Oxyma Pure®, NMM, DMF, 14 h, rt, (III): TFA, CH_2Cl_2, 4 h, rt, (IV): EDCI, Oxyma Pure®, NMM, DMF, 14 h, rt, (V): K_2CO_3, DMF, 24 h, rt.



Scheme 2 Statistical macrocyclisation of (**1**) and (**5**): (I) DBU, $Zn(OAc)_2 \cdot 5H_2O$, pentanol, 150 °C, 12 h, (II) THF, MeOH, H_2O, NaOH, rt, 6 h.

scattering (DLS). Bare CDVs have a characteristic diameter of around 100 nm after extrusion of a hydrated lipid film through a 100 nm polycarbonate membrane. The size distribution of the CDVs is provided in the ESI.† The CDVs have a slightly negative ζ-potential of around −11 mV, which is due to the preferential absorption of hydroxide ions by the ethylene glycol chains at the primary rim of the β-cyclodextrin (Fig. 2, entry 1).[19] Upon addition of (**8**) (added with 6 eq. of NaOMe), a significant drop of the ζ-potential to −25 mV was observed, which confirms the immobilization of the adamantane-functionalized PC on the CDV surface, since the large number of free carboxylate functions of the guest molecules provide a significant contribution to the negative surface potential of the CDVs. Furthermore, the repulsive forces between the functionalized vesicles play an important stabilizing role, and no increase in size was observed upon addition of (**8**) to the bare CDVs (Fig. 2, entry 2). To verify that the drop in the ζ-potential is due to the host–guest complexation between the hydrophobic adamantane residues of the PC and the free CD cavities of the vesicles, an excess (10 mM) of β-CD was added. It was observed that a displacement of the binding equilibrium occurs and that (**8**) is released from the CDVs. The ζ-potential of bare CDVs was recovered accordingly (Fig. 2, entry 3). The diameter and size distribution of the CDVs are not affected by the desorption of (**8**).

In Fig. 3 the normalized absorption of (**8**) is shown in comparison with (**8**) bound to the vesicles ((**8**)-CDV), both in aqueous solution. We note that in each experiment, 6 eq. of NaOMe were added with (**8**). The pH of the resulting solutions (with or without CDV) was slightly above 7. The photophysical properties of (**8**) and its derivatives are largely independent of pH, provided that the acidic or basic functional groups are decoupled from the photoactive macrocycle.[27] The absorption spectra display characteristic Soret-bands (360 nm) and Q-bands (685 nm), as previously reported for other zinc(II) phthalocyanines.[28,29] However, free (**8**) shows a pronounced shoulder around 620 nm, which diminishes upon attachment to vesicles, and constitutes distinctive evidence that aggregation of (**8**) is inhibited when it is immobilized on the CDVs.

In Fig. 4, the excitation and the emission spectra of (**8**) and (**8**)-CDV are depicted. The emission spectra can be attributed to the monomeric species and do not depend on the excitation wavelength. The absorption and the excitation spectra are coincident and in good agreement with previously reported spectroscopic studies of zinc(II) phthalocyanines.[30] Clearly, the peripheral water soluble carboxylic groups do not influence the photophysical properties of the macrocycle to any significant extent (Table 1).

Singlet oxygen quantum yields (Φ_Δ) were determined using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ADMADM) in aqueous solution as a fluorescent monitor (λ_{ex} = 380 nm). Irradiation of stirred, air saturated solutions of a reference compound (methylene blue, MB, Φ_Δ = 0.52) and either (**8**) or

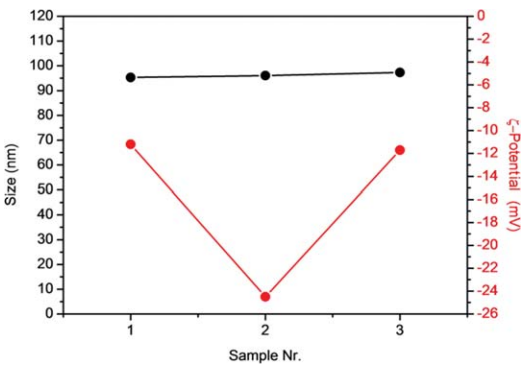


Fig. 2 Comparison of the size (nm) and the ζ-potential (mV) of (1) 0.1 mM CDV, (2) 0.1 mM CDV + 50 μM (**8**) and (3) 0.1 mM CDV + 50 μM (**8**) + 10 mM β-CD. The size distribution of CDVs is provided in the ESI.†

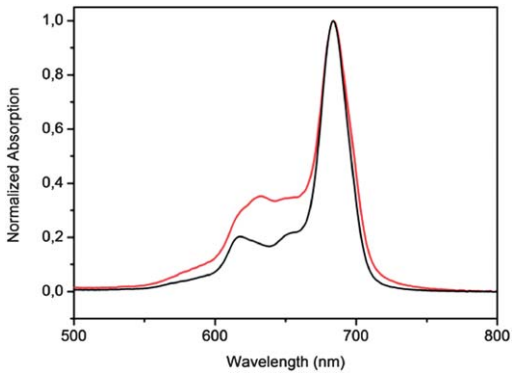


Fig. 3 Absorption spectra of (**8**) (red line) and (**8**)-CDV (black line), both recorded in distilled water.

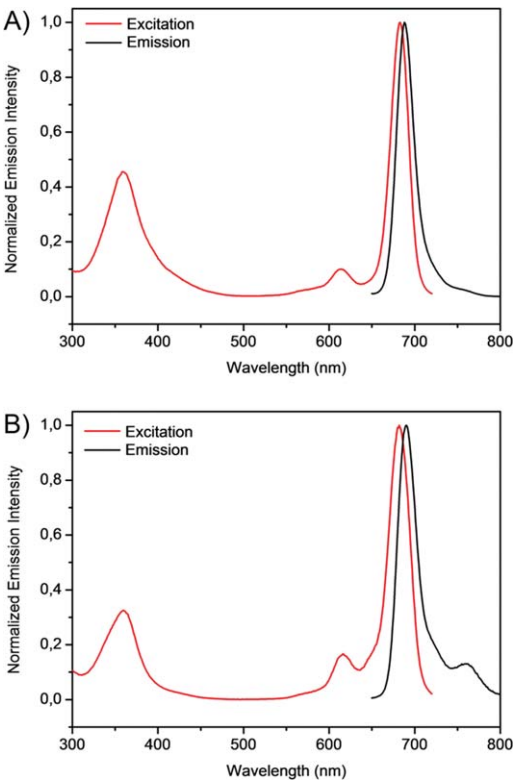


Fig. 4 Excitation (red) and emission (black) spectra of (**8**) (A) and (**8**)-CDV (B) in distilled water.

Table 1 Photophysical parameters of (**8**) in solution and bound to CDV in distilled water

	(**8**)	(**8**)-CDV
λ_{max}/nm (Soret-band)	358	360
λ_{max}/nm (Q-band)	682	682
λ_{max}/nm (emission)	690	690
τ_{fluo}/ns (±0.05)	3.24	0.63, 2.57
Φ_F (±0.02)	0.03	0.07
Φ_Δ (±0.02)	0.05	0.20

(**8**)-CDV in 10 × 10 mm quartz cuvettes led to a drop of the ABMDMA emission intensity over time (Fig. 5 and ESI, Fig. S4†). Comparison of the slopes of the fluorescence decays led to Φ_Δ (Fig. S4 and eqn (S1)†). This method was used in view of the fact that the phosphorescence of $^1O_2$ was not observable in the aqueous environment, even when D_2O was employed as a solvent. The hydroxyl groups of the CDV most likely quench the phosphorescence of $^1O_2$, thus preventing its quantitative determination in the near-infrared region of the electromagnetic spectrum (1275 nm). The Φ_F and the Φ_Δ of (**8**)-CDV are significantly higher (by a factor of roughly 3 within the experimental error) than for pure (**8**) in aqueous solution (Table 1). These results show the enhanced availability of monomeric chromophores in the (**8**)-CDV assembly for the photogeneration of $^1O_2$. By comparison with monomers of other octaoxosubstituted Zn(II) PCs ($\Phi_F = 0.30$ and $\Phi_\Delta = 0.70$), and assuming that a dimer absorbs like two monomers but does not show any fluorescence or $^1O_2$ photoproduction,[27] we conclude that about 25% of the photosensitizer molecules are active as monomeric species on the CDV. The host–guest interaction of two adamantane moieties with the cavities of the CDV compensates the free energy of aggregation. The driving force of the hydrophobic effect overcomes the stacking effect of PC, a process that is also driven by hydrophobic exclusion of the macrocycles. This approach constitutes a successful way to significantly avoid aggregation of PCs in aqueous media by immobilization on the surface of the vesicles.

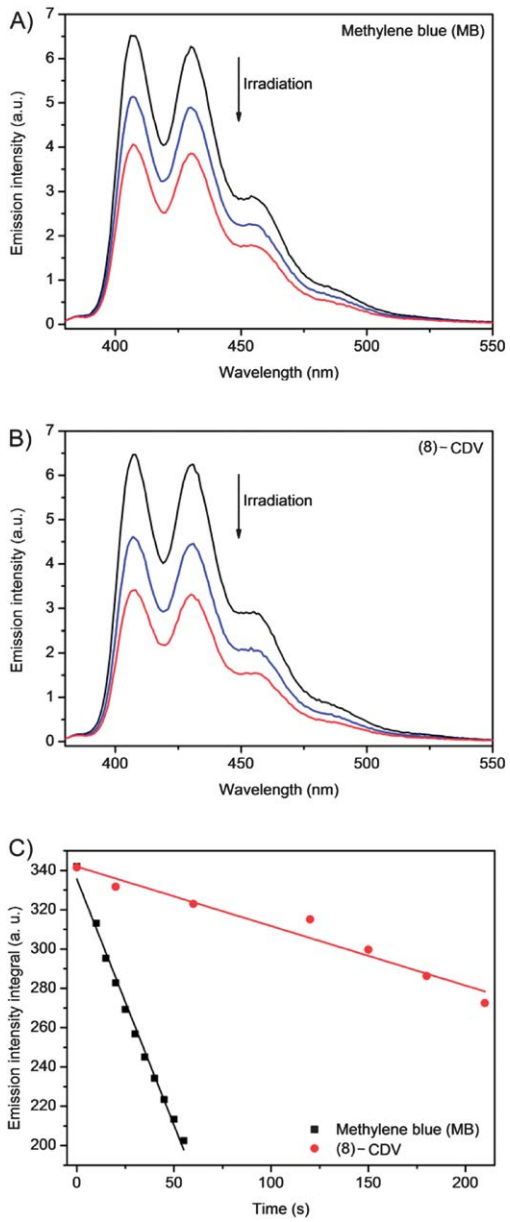


Fig. 5 Decaying emission spectra of ADMADM at different irradiation times: (A) MB (0 s, 25 s and 50 s) and (B) (**8**)-CDV (0 s, 240 s, 480 s). (C) Slopes of ADMADM decay for (**8**)-CDV (red) and for MB (black).

Conclusion

We have documented a synthetic route for a water soluble zinc(II) phthalocyanine bearing adamantane moieties. Vesicles formed by an amphiphilic β-cyclodextrin allow host–guest interactions and immobilization of the phthalocyanine in aqueous media, thus hindering the formation of inactive aggregates and enhancing the photosensitizing ability. This supramolecular assembly constitutes a biocompatible photoactive platform for the design of new phototherapeutic agents, and the possibility of combining this system with other adamantane-functionalized molecules for enhanced targeting or fluorescent properties opens the way for simultaneous detection and inactivation of cancer cells or bacteria.

Acknowledgements

The authors gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft (DFG grants Ra 1731/1-2 to BJR and Str1186/1-1 to CAS).

Notes and references

1 T. J. Dougherty, B. W. Henderson, C. J. Gomer, G. Jori, D. Kessel, M. Korbelik, J. Moan and Q. Peng, *J. Natl. Cancer Inst.*, 1998, **90**, 889–905.
2 C. Schweitzer and R. Schmidt, *Chem. Rev.*, 2003, **103**, 1685–1757.
3 M. Regehly, K. Greish, F. Rancan, H. Maeda, F. Böhm and B. Röder, *Bioconjugate Chem.*, 2007, **18**, 494–499.
4 K. K. Ishii, M. Shiine, Y. Shimizu, S. Hoshino, A. Hisaku, K. Sogawa and N. Kobayashi, *J. Phys. Chem. B*, 2008, **112**, 3138–3143.

5 R. K. Pandey, K. M. Smith and T. T. Dougherty, *J. Med. Chem.*, 1990, **33**, 2032–2038.
6 F. Schmitt, P. Govindaswamy, G. Süss-Fink, W. H. Ang, P. J. Dyson, L. Juillerat-Jeanneret and B. Therrien, *J. Med. Chem.*, 2008, **51**, 1811–1816.
7 N. Nishiyama, H. R. Stapert, G. Zhang, D. Takasu, D. Jiang, T. Nagano, T. Aida and K. Kataoka, *Bioconjugate Chem.*, 2003, **14**, 58–66.
8 C. A. Strassert, M. Otter, R. Q. Albuquerque, A. Höne, Y. Vida, B. Maier and L. De Cola, *Angew. Chem., Int. Ed.*, 2009, **48**, 7928–7931.
9 Y. N. Konan, R. Gurny and E. Allemann, *J. Photochem. Photobiol., B*, 2002, **66**, 89–106.
10 X. Jiang, S. Yeung, P. Lo, W. Fong and K. P. N. Dennis, *J. Med. Chem.*, 2011, **54**, 320–330.
11 H. Li, T. J. Jensen, F. R. Fronczek, H. Vicente and M. Graça, *J. Med. Chem.*, 2008, **51**, 502–511.
12 W. Liu, T. J. Jensen, F. R. Fronczek, R. P. Hammer, K. M. Smith, M. Graça and H. Vicente, *J. Med. Chem.*, 2005, **48**, 1033–1041.
13 M. Hu, N. Brasseur, S. Z. Yildiz, J. E. van Lier and C. C. Leznoff, *J. Med. Chem.*, 1998, **41**, 1789–1802.
14 D. A. Fernández, J. Awruch and L. E. Dicelio, *Photochem. Photobiol.*, 1996, **63**, 784–792.
15 A. Mazzaglia, A. Valerio, N. Micali, V. Villari, F. Quaglia, M. A. Castriciano, L. M. Scolaro, M. Giuffrè, G. Siracusano and M. T. Sciortino, *Chem. Commun.*, 2011, **47**, 9140–9142.
16 N. Kandoth, E. Vittorino, M. T. Sciortino, T. Parisi, I. Colao, A. Mazzaglia and S. Sortino, *Chem.–Eur. J.*, 2012, **6**, 1684–1690.
17 A. Mazzaglia, N. Angelini, R. Darcy, R. Donohue, D. Lombardo, N. Micali, M. T. Sciortino, V. Villari and L. M. Scolaro, *Chem.–Eur. J.*, 2003, **23**, 5762–5769.
18 B. J. Ravoo and R. Darcy, *Angew. Chem., Int. Ed.*, 2000, **39**, 4324–4326.
19 P. Falvey, C. W. Lim, R. Darcy, T. Revermann, U. Karst, M. Giesbers, A. T. M. Marcelis, A. Lazar, A. W. Coleman, D. N. Reinhoudt and B. J. Ravoo, *Chem.–Eur. J.*, 2005, **11**, 1171–1180.
20 C. W. Lim, B. J. Ravoo and D. N. Reinhoudt, *Chem. Commun.*, 2005, 5627–5629.
21 J. Voskuhl, M. C. A. Stuart and B. J. Ravoo, *Chem.–Eur. J.*, 2010, **16**, 2790–2796.
22 J. Voskuhl, T. Fenske, M. C. A. Stuart, B. Wibbeling, C. Schmuck and B. J. Ravoo, *Chem.–Eur. J.*, 2010, **16**, 8300–8306.
23 D. A. Uhlenheuer, D. Wasserberg, C. Haase, H. D. Nguyen, J. H. Schenkel, J. Huskens, B. J. Ravoo, P. Jonkheijm and L. Brunsveld, *Chem.–Eur. J.*, 2012, **18**, 6788–6794.
24 S. K. M. Nalluri, J. L. Bultema, E. J. Boekema and B. J. Ravoo, *Chem. Sci.*, 2011, **2**, 2383–2391.
25 S. K. M. Nalluri and B. J. Ravoo, *Angew. Chem., Int. Ed.*, 2010, **49**, 5371–5374.
26 S. K. M. Nalluri, J. Voskuhl, J. B. Bultema, E. J. Boekema and B. J. Ravoo, *Angew. Chem., Int. Ed.*, 2011, **50**, 9747–9751.
27 C. A. Strassert, G. M. Bilmes, J. Awruch and L. E. Dicelio, *Photochem. Photobiol. Sci.*, 2008, **7**, 738–747.
28 J. W. Owens, R. Smith, R. Robinson and M. Robins, *Inorg. Chim. Acta*, 1998, **279**, 226–231.
29 A. Ogunsipe, J. Chen and T. Nyokong, *New J. Chem.*, 2004, **28**, 822–827.
30 A. Ogunsipe, D. Maree and T. Nyokong, *J. Mol. Struct.*, 2003, **650**, 131–140.