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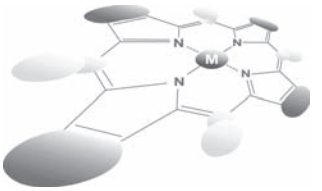

Porphyrin self-assembled monolayers and photodynamic oxidation of tryptophan

Isabelle Chambrier[a], David A. Russell[a], Derek E. Brundish[b], William G. Love[b], Giulio Jori*[c◊], M. Magaraggia[c] and Michael J. Cook*[a◊]

[a] *Wolfson Materials and Catalysis Center, School of Chemistry, University of East Anglia, Norwich NR4 7TJ, UK*
[b] *Destiny Pharma Ltd., Sussex Innovation Center, Science Park Square, Falmer, Brighton BN1 9SB, UK*
[c] *Department of Biology, University of Padova, via Trieste 75, 35121 Padova, Italy*

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ABSTRACT: The zinc and magnesium metalated derivatives of 5,5′-[12,12′-di(thiododecyloxy)-4,4′-phenyl)]-10,10′,15,15′,20,20′-hexakis(3,3′,4,4′,5,5′-hexakisdecyloxyphenyl)diporphyrin, **1b** and **1c**, have been synthesised and deposited to form self-assembled monolayer (SAM) films on the surface of gold-coated glass substrates. The SAM films have been characterized by RAIR spectroscopy and fluorescence spectroscopy. The potential for the porphyrin films to catalyze the oxidation of tryptophan within human serum albumin upon irradiation with white light has been demonstrated and attributed to the porphyrins acting as photosensitizers of oxygen to form oxidizing species.

KEYWORDS: porphyrin self-assembled monolayers, SAM films on gold, photodynamic surface.

INTRODUCTION

The anchoring of molecules onto the surface of a solid substrate to form self-assembled monolayers (SAMs) is now an established and well documented technology [1]. Molecules designed for particular applications contain a "functional core", *i.e*. a moiety that provides a desired effect or response to external stimuli, and a means for attaching it to a solid surface [2]. The latter frequently, but not invariably, exploits a tether chain that terminates with a functional group that reacts or interacts with the surface. A common combination for anchoring molecules to surfaces uses a thiol- or disulfide-terminated tether to form a thiolate linkage to gold; a second approach employs a silyl function to react with a silica/silicon surface [1, 2]. In previous work, one of our laboratories exploited both of these approaches to develop examples of phthalocyanine SAMs [3, 4]. SAMs deposited onto the surface of gold, supported on a glass slide, were investigated for optically based gas-sensing devices [5]. More recently these studies were extended to the surface coating of gold nanoparticles with a zinc phthalocyanine derivative in the presence of tetraoctylammonium bromide [6]. This construct has been exploited as a drug delivery system within a photodynamic therapy (PDT) protocol. Irradiation of these nanoparticle conjugates within HeLa cells induced substantial cell mortality through the photodynamic production of singlet oxygen [7].

We now report some parallel work using single-component porphyrin SAMs on the surface of gold and thus contribute to the burgeoning body of research into the characterization and applications of this type of SAM [8–11]. An interesting development has been the application of porphyrin SAMs for heterogeneous oxidation catalysis and this has exploited, for example, the redox behavior of Mn [9] and Co [10] contained within the macrocycle core. To our knowledge there has been no focus on exploiting porphyrin SAM surfaces for photodynamic oxidations. The present paper addresses this area, describing the synthesis of purpose-designed porphyrin derivatives and their deposition onto a gold surface supported on a glass slide. The role of these constructs in providing a "photodynamic surface" is demonstrated by the photooxidation of the tryptophyl residue in human

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*Correspondence to: Michael J. Cook, email: m.cook@uea.ac.uk, fax: +44 (0)1603-592015

serum albumin (HSA) when the films are illuminated with white light.

RESULTS AND DISCUSSION

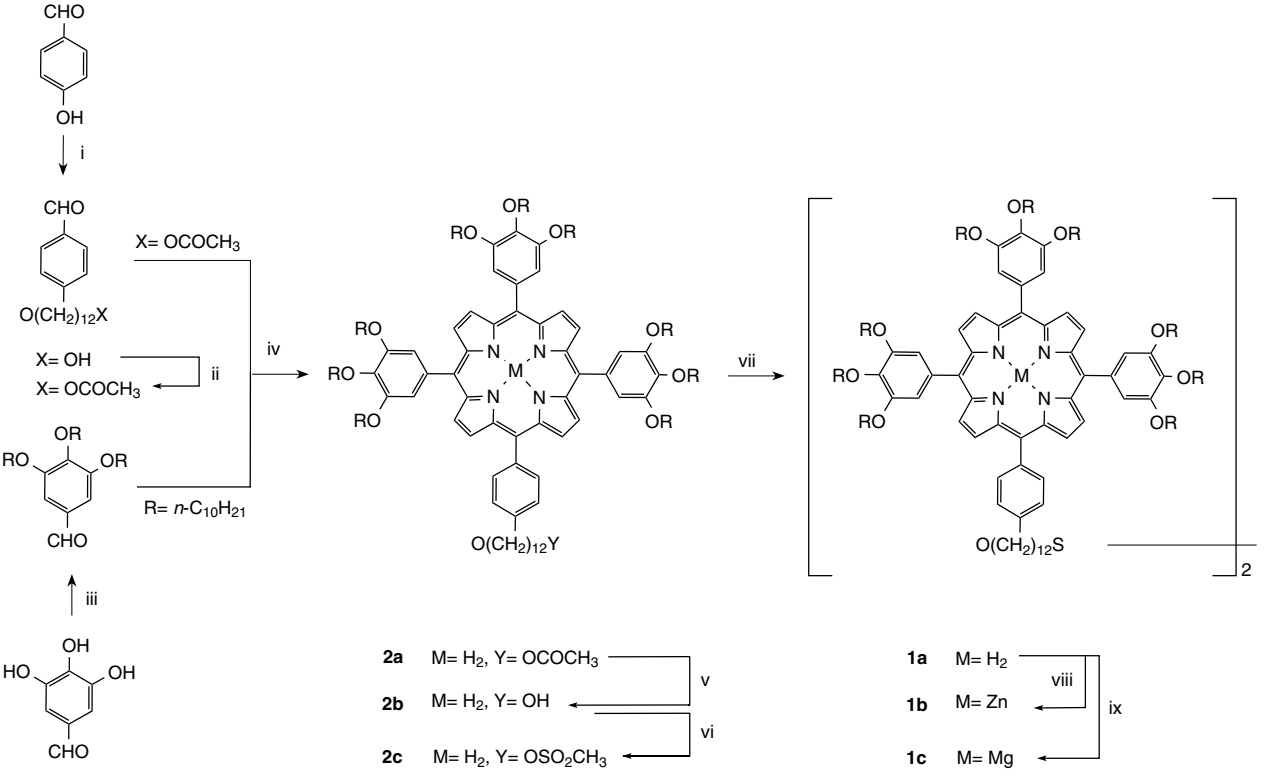
Design of compounds

The non-symmetrically tetra-aryl substituted porphyrin derivatives investigated for deposition as SAM films, **1b** and **1c** (Scheme 1), contain two porphyrin moieties linked through one of their aryl rings via a tether terminated with a disulfide unit. Three long-chain alkoxy groups on each of the remaining three aryl rings per porphyrin unit were incorporated to minimize self-association and the detrimental quenching effects this has on the electronically excited states of the porphyrin. Zinc and magnesium were chosen as central metals because of their limited adverse toxic properties so long as these ions are incorporated into the tetrapyrrolic macrocycle [12]. The route to prepare the compounds **1b** and **1c** proceeds *via* the synthesis of a non-symmetrically substitued metal-free porphyrin derivative with a tether terminated with a hydroxyl group **2b**. This functionality was deemed suitable for subsequent introduction of the disulfide moiety *via* the mesylate **2c**. Metalation of the ring with zinc and magnesium provided the final step of the synthetic work.

Synthesis

Aldehydes. The required aromatic aldehyde derivatives for the synthesis of the desired porphyrins were prepared from 4-hydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde. Yields were improved compared to those reported in the literature [13] by using a combination of both a phase-transfer catalyst, tetra-*n*-butylammonium iodide, to facilitate the substitution and a high boiling solvent.

Condensation. The mixed cyclotetramerization reaction (Step iv in Scheme 1) was first attempted using BF_3-etherate as the acid and p-chloranil as the oxidant. No porphyrins were obtained by this method. The reaction was therefore carried out using propionic acid under aerobic conditions. The efficiency of the reaction was further improved by using an oxidizing co-solvent, nitrobenzene [14]. Under these conditions there was no evidence of chlorin formation. However, on using 4-(12-hydroxydodecyloxy)benzaldehyde it was found that the product was partially esterified to form the propionate leading to mixtures and non-reproducible preparations. The acetate derivative of 4-(12-hydroxydodecyloxy)benzaldehyde (*i.e.* X = $OCOCH_3$ in Step iv) was therefore preferred as a precursor. Higher yields for the cyclotetramerization were obtained and purification of the product **2a** was straightforward. This compensated for the inclusion of the extra step required to deprotect **2a** to afford **2b**.



Scheme 1. Synthesis of compounds **1b** and **1c**. In all cases R = $C_{10}H_{21}$. *Reagents*: i. $Br(CH_2)_{12}OH$, MEK, K_2CO_3, KI, tetra-*n*-butylammonium iodide; ii. Ac_2O; iii. *n*-bromodecane, MEK, KI, K_2CO_3, tetra-*n*-butylammonium iodide; iv. pyrrole, propionic acid, nitrobenzene; v. NaOH; vi. Methanesulfonylchloride, Et_3N; vii. 1) Thiourea, 2) aq. NaOH; viii. $Zn(OAc)_2$; ix. $Mg(ClO_4)_2$, dry pyridine

***Metal-free porphyrin disulfide* 1a.** The free hydroxyl group of **2b** was converted into a mesylate, to afford **2c**, which was reacted with thiourea under aerobic conditions. The porphyrin disulfide derivative **1a** was obtained after hydrolysis using aqueous sodium hydroxide. The use of an aqueous solution of NaOH proved to be a satisfactory alternative to an NaOH solution in ethanol which led to a product whose ^{1}H NMR spectrum contained an unexplained extra set of signals.

***Metalated porphyrin disulfides* 1b *and* 1c.** Finally, the metalated derivatives **1b** and **1c** were obtained by refluxing the metal-free compound **1a** in refluxing THF and dry pyridine containing zinc acetate and magnesium perchlorate respectively. Expected changes in the UV-vis spectra were observed upon the introduction of the metal into the macrocycle. The four weak absorption bands at 516.0, 552.5, 592.5 and 650.5 nm present in the metal-free porphyrin **1a** are replaced by two weak bands at 557.0 and 598.0 nm, and 569.0 and 615.0 nm for the Zn and Mg derivatives, **1b** and **1c**, respectively. Similarly, the Soret band was shifted from 423.0 nm in the metal-free compound to 427.5 nm for the zinc derivative and 432.0 nm for the magnesium derivative.

Both products were characterized by ^{1}H NMR spectroscopy which showed the signal corresponding to the protons α to the sulfur atom as a triplet at 2.71 and 2.6 ppm for **1b** and **1c**, respectively. MALDI-TOF mass spectra showed isotopic clusters at 4601 [M^+] for **1b** and 4518 [M^+] for **1c**. Satisfactory C, H, N elemental analysis data confirmed the structures and attested to the purity of the compounds.

Formation and spectral characterization of self-assembled monolayer films of 1b and 1c

Conditions for the preparation of the gold-coated glass substrates are described in the Experimental section. The deposition of **1b** and **1c** to form SAM films on the substrates was undertaken by immersing substrates into a solution of each compound in cyclohexane at concentrations of *ca*. 2.5-5 $\times$ 10^{-7} M for 20 hours. The constructs were then rinsed with cyclohexane and allowed to dry in air. Reference (blank) substrates, *i.e*. without a SAM coating, were similarly immersed in neat cyclohexane for 20 hours and dried as above. Reflection-absorption infrared spectroscopy (RAIRS) was used to detect formation of SAM films on the gold surface.

Figure 1 shows the RAIR spectra (2600–3200 cm^{-1} region) of an example of a SAM film of **1b**. Comparable spectra were obtained for SAMs of **1c**. The absorption bands shown in Fig. 1 are assigned to the aliphatic C-H stretches associated with the peripheral alkyloxy chains *viz*. CH_3 (ν_{AS}) at 2959 cm^{-1}, CH_2 (ν_{AS}) at 2926 cm^{-1}, and CH_2 (ν_S) at 2857 cm^{-1}. Assignments of other bands in the spectra were not attempted. Intensities of the absorption bands showed good reproducibility over different regions of the film and from one film to another prepared in the same batch. The nature of the central metal ion does not seem to have any effect on SAM formation.

The thickness of the gold coating precluded acquisition of UV-vis absorption spectra. However, fluorescence emission spectra of the films and fluorescence excitation spectra were recorded. Examples of these are shown in Figs 2 and 3, respectively. The emission spectrum (Fig. 2) shows the fluorescence spectrum obtained upon irradiation at 415 nm. The emission is weak and gives rise to a broad band spectrum, not unexpected and comparable to that observed for phthalocyanine SAMs [5]. There is a narrower peak centered at *ca.* 620 nm which we tentatively assign to a Raman band. Evidence in support of this is that the band position varies with the irradiating wavelength. Its intensity can be ascribed to the effect of the gold surface, as exploited in surface enhanced resonance Raman scattering (SERRS) measurements. Estimation of the fluorescence quantum yields was attempted by comparing band intensities assigned to fluorescence

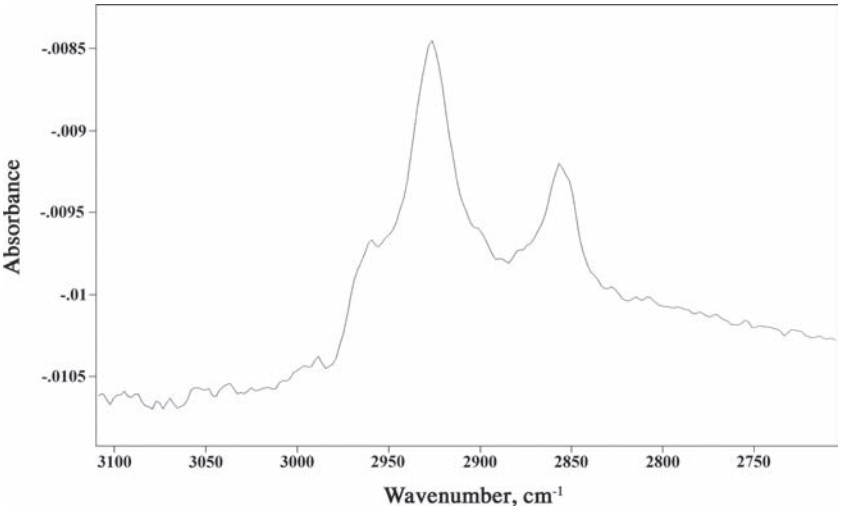


Fig. 1. RAIR spectrum of a SAM film of **1b** showing the 3100–2700 cm^{-1} region

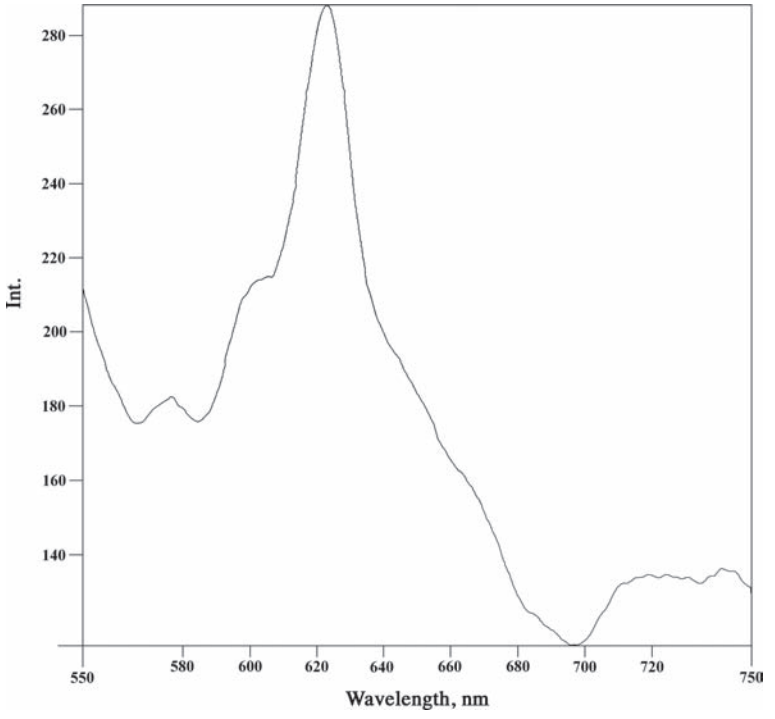


Fig. 2. Fluorescence emission spectrum from a SAM of **1b** upon irradiation at 415 nm

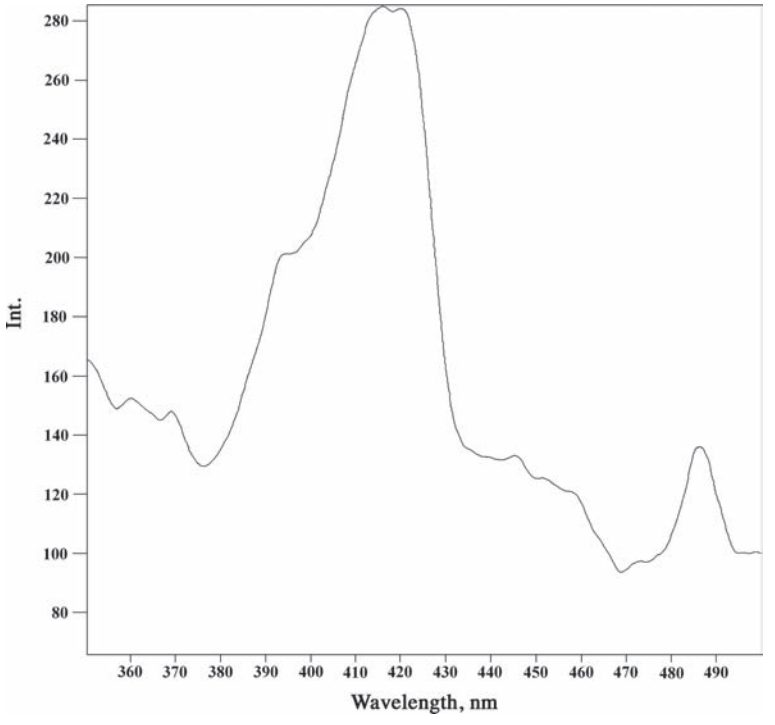


Fig. 3. Excitation spectrum of a film of **1b** (emission at 620 nm)

emission and band intensities in the excitation spectra with spectral data from solutions of haematoporphyrin IX used as a fluorescence standard (quantum yield 0.11 in a non-associated state in EtOH). This indirect approach, using data for seven films of **1b** and six films of **1c**, gave consistent data which indicated that for both types of SAM the quantum efficiency was *ca*. 0.02 with errors of the order of +/- 0.01. The low values may reflect a degree of quenching of the first excited singlet state of the molecules within the films through a variety of possible pathways, arising for example from their proximity to the gold surface or self-quenching through close association of the molecules despite the presence of the long alkoxy chains substituents [11, 15]. The occurrence of ground state interactions between porphyrin molecules in the SAMs is suggested by the broadness of the Soret band in the excitation spectrum and the presence of at least two maxima for such bands (Fig. 3); moreover, the poorly resolved emission bands above 700 nm (Fig. 2) is suggestive of the formation of weakly fluorescent side-by-side porphyrin dimers [12].

Photodynamic oxidation studies

The photosensitizing efficiency of the two types of porphyrin SAMs was tested against a tryptophan model, because the indole side chain of this amino acid is susceptible to photooxidative attack by both type I (radical-involving) and type II (singlet oxygen-involving) pathways [12, 16–18]. Thus, a kinetic study of its photosensitized modification provides reliable information on the photoefficiency of the porphyrin derivatives that is independent of the specific mechanism of the photoprocess. In particular, we selected a widely diffused protein, albumin, as a substrate since the three-dimensional structure of the polypeptide chain provides a "biological" microenvironment for the tryptophan (human serum albumin contains one tryptophyl residue per protein molecule). Most conveniently, the time-course of the photosensitized modification of tryptophyl residues can be readily followed by spectrophotofluorimetric analysis, namely by following the decrease in the intensity of the 290 nm-excited fluorescence emission in the 360 nm wavelength interval. Tryptophan is known to be largely converted into photooxidation products that are kynurenine or hydroxyindole based [18]. These do not interfere with the determination of tryptophan fluorescence, since their absorption and emission are shifted to the 400–500 nm spectral region [19]. For both SAMs, the photooxidation (white light irradiation) of the albumin-bound tryptophan sensitized by the porphyrin occurred according to first-order kinetics, as represented by semilogarithmic

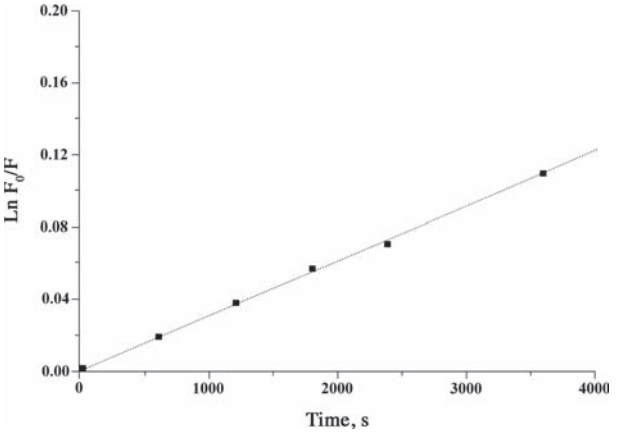


Fig. 4. Typical example of photokinetic plot describing the photosensitized oxidation of the tryptophyl residue in albumin for **1c**

plots (see Fig. 4). The slope of the plots allows the calculation of the rate constant k for the photoprocess; hence, k can be taken as a parameter defining the photosensitizing efficiency of the porphyrin SAMs. Under our experimental conditions, the rate constant for the photoprocesses is of the order of 2×10^5 s^{-1}; this value is about 20-fold smaller than those found for the photosensitized modification of tryptophan by free porphyrins [12, 20]. The decrease in the efficiency of a photosensitizer molecule, once it is immobilized on a solid matrix, is to be expected [17]. However, it is worth emphasizing that the photoefficiency of the immobilized porphyrins **1b** and **1c** is unusually large when compared with that observed for other matrix-bound photosensitizing agents [17].

EXPERIMENTAL

Equipment

^{1}H NMR spectra were measured in $CDCl_3$ at 270 MHz on a Jeol EX 270 or at 300 MHz on a Varian Gemini-300 using TMS as the internal reference. Mass spectra were obtained using the MALDI technique in Dithranol matrix or the FAB technique in Noba matrix. UV-vis spectra of solutions were measured in THF (unless otherwise specified) using a Hitachi U-3000 spectrophotometer. RAIRS experiments were performed using a Bio-Rad FTS40 Fourier transform infrared spectrometer. Fluorescence emission and fluorescence excitation spectra were obtained using a Perkin Elmer MPF4 spectrophotofluorimeter.

Materials

12-bromododecanol. 1,12-dodecanediol (50 g, 0.25 mol) in hydrogen bromide 48% (220 ml) was continuously extracted with petroleum ether (bp 80–100°C) (300 ml) for 18 h. The solvent was evaporated and the crude oil was filtered through silica gel (eluent: petroleum ether (bp 40–60°C)). A colorless fraction was obtained containing 1,12-dibromododecane (10.2 g, 13%). The silica gel was then eluted with acetone. A pale yellow oil was obtained after evaporation of the solvent which crystallized upon cooling. The title product was recrystallized from petroleum ether (bp 40–60°C). Yield 43.1 g (65%). ^{1}H NMR (60 MHz): δ, ppm 3.62 (t, 2H), 3.42 (t, 2H), 1.4 (brs, 21H).

4-[12-hydroxydodecyloxy]benzaldehyde. A mixture of 12-bromododecanol (15 g, 57 mmol), 4-hydroxybenzaldehyde (6.9 g, 57 mmol), potassium carbonate (excess), catalytic amounts of potassium iodide and tetra-*n*-butylammonium iodide was refluxed in methyl ethyl ketone (50 ml) for 16 h. After cooling, the solid was filtered off and washed with acetone. The combined organics were concentrated under reduced pressure. Diethyl ether was added to the oil and the flask placed in the fridge. The precipitate was filtered. Yield 12.5 g (72%). ^{1}H NMR (60 MHz): δ, ppm 9.9 (s, 1H), 7.9 (d, 2H), 7.0 (d, 2H), 4.1 (t, 2H), 3.62 (t, 2H), 1.3 (brs, 21H).

4-[12-acetyloxydodecyloxy]benzaldehyde. 4-[12-hydroxydodecyloxy]benzaldehyde (1.1 g, 3.6 mmol) was refluxed in acetic anhydride (15 ml) for 90 min. After cooling, the solution was poured into water with stirring. A precipitate formed which was filtered, washed with water and dried. Yield 1.1 g (88%). ^{1}H NMR (60 MHz): δ, ppm 9.82 (s, 1H), 7.8 (d, 2H), 7.0 (d, 2H), 4.0 (t, 4H), 2.02 (s, 3H), 1.3 (brs, 20H).

3,4,5-trisdecyloxybenzaldehyde. A mixture of 3,4,5-trihydroxybenzaldehyde (2 g, 13 mmol), 10-bromodecane (15 ml, excess), potassium carbonate (excess), catalytic amounts of potassium iodide and tetra-*n*-butylammonium iodide was refluxed in methyl ethyl ketone (25 ml) for 16 h. After cooling, the solution was filtered and the solid washed with acetone. The solvent was evaporated leaving an oil. This was filtered through silica gel (eluent: petroleum ether (bp 40–60°C)). Excess bromodecane was obtained after evaporation of the solvent. The silica gel was then eluted with acetone. The solvent was evaporated leaving a pale yellow oil which was used without further purification. Yield 7 g (93%). ^{1}H NMR (60 MHz): δ, ppm 9.8 (s, 1H), 7.1 (s, 2H), 4.1 (brt, 6H), 1.3 (brs, 48H), 0.9 (t, 9H).

5-(12-acetyloxydodecyloxyphenyl)-10,15,20-tri-(3,4,5-trisdecyloxyphenyl)porphyrin (2a). 3,4,5-trisdecyloxybenzaldehyde (3.51 g, 6 mmol) and 4-(12-acetyloxydodecyloxy)benzaldehyde (0.71 g, 2 mmol) were heated in propionic acid (40 ml) containing nitrobenzene (10 ml) to 130°C. Pyrrole (0.55 g, 8 mmol) was added at once and the temperature maintained for 3 h. The solution was then cooled and excess methanol added. The flask was placed in the fridge overnight. The dark oil at the bottom of the flask was separated and washed with methanol. The product was separated by column chromatography over silica gel (eluent: petroleum ether (bp 40–60°C)/THF 10:1) twice. The sample was used in the next stage without further purification. ^{1}H NMR (270 MHz): δ, ppm 8.86–8.94 (m, 8H), 8.1 (d, J = 8 Hz, 2H), 7.42 (s, 6H), 7.27 (d, J = 8 Hz, 2H), 4.29 (brt, 8H),

4.08 (t, 14H), 2.26 (s, 3H), 1.2–2.0 (m, 164H), 0.91 (t, 9H), 0.83 (t, 18H), -2.79 (s, 2H).

5-(12-hydroxydodecyloxyphenyl)-10,15,20-tri(3,4,5-trisdecyloxyphenyl)porphyrin (2b). 5-(12-acetyloxydodecyl-oxyphenyl)-10,15,20-tri(3,4,5-trisdecyloxyphenyl) porphyrin obtained above was refluxed in THF (10 ml). Ethanolic NaOH (excess) was added. The reaction was followed by tlc (eluent: petroleum ether (bp 40–60°C)/THF 10:1) until complete (rf starting material = 0.8, rf product = 0.2). The solvent was evaporated and the residue was chromatographed over silica gel (eluent: petroleum ether (bp 40–60°C)/THF 4:1). Yield 0.49 g (11% from benzaldehydes). ^{1}H NMR (270 MHz): δ, ppm 8.85–9.0 (m, 8H), 8.1 (d, J = 8 Hz, 2H), 7.43 (s, 6H), 7.3 (d, J = 8 Hz, 2H), 4.3 (t, 8H), 4.09 (t, 12H), 3.65 (brt, 2H), 1.2–2.05 (m, 164H), 0.91 (t, 9H), 0.84 (t, 18H), -2.77 (s, 2H).

5-(12-methanesulfonyloxydodecyloxyphenyl)-10,15,20-tri(3,4,5-trisdecyloxyphenyl)porphyrin (2c). 5-(12-hydroxydodecyloxyphenyl)-10,15,20-tri(3,4,5-trisdecyloxyphenyl)porphyrin (0.193 g, 86 μmol) was dissolved in dichloromethane (8 ml) and the solution cooled with a water bath. Et_3N (1 ml) was added, followed by methanesulfonylchloride (20 drops). The reaction was stirred at rt for 1 h. The organics were washed with a dilute HCl solution, brine, dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue was purified by column chromatography over silica gel (eluent: petroleum ether (bp 40–60°C)/THF 4:1). Yield 0.159 g (80%). ^{1}H NMR (270 MHz): δ, ppm 8.85–9.0 (m, 8H), 8.1 (d, J = 8 Hz, 2H), 7.43 (s, 6H), 7.28 (d, J = 8 Hz, 2H), 4.2–4.35 (m, 10H), 4.09 (t, 12H), 2.97 (s, 3H), 1.1–2.0 (m, 192H), 0.91 (t,9H), 0.84 (t, 18H), -2.77 (s, 2H).

5,5′-(12,12′-dithiodidodecyloxyphenyl)-10,10′,15,15′,20,20′-hexa(3,3′,4,4′,5,5′-hexakisdecyloxyphenyl) diporphyrin (1a). 5-(12-methanesulfonyloxydodecyloxyphenyl)-10,15,20-tri(3,4,5-trisdecyloxyphenyl)-porphyrin (159 mg, 0.07 mmol) and thiourea (excess) were refluxed in 1-pentanol (5 ml) until tlc (eluent: petroleum ether (bp 40–60°C)/THF 4:1) indicated no starting material was left (approx. 1 h) (rf product=0). Ethanol was added (2 ml) followed by aqueous NaOH (10%) (2 ml) and reflux continued for 5 min. The solution was left to cool and then added to dil. HCl (10%) and the organics were extracted with dichloromethane, washed with brine, dried ($MgSO_4$), filtered and the solvent evaporated. The residue was chromatographed over silica gel (eluent: petroleum ether (bp 40–60°C)/THF 4:1). Yield 114 mg (74%). ^{1}H NMR (270 MHz): δ, ppm 8.87–9.0 (m, 8H), 8.11 (d, J = 8 Hz, 2H), 7.44 (s, 6H), 7.3 (d, J = 8 Hz, 2H), 4.31 (t, 8H), 4.27 (t, 2H), 4.1 (t, 12H), 2.71 (t, 2H), 1.2–2.05 (m, 164H), 0.92 (t, 9H), 0.85 (t, 18H), -2.76 (s, 2H). UV-vis (THF): λ, nm 650.5, 592.5, 552.5, 516.0, 423.0.

5,5′-(12,12′-dithiodidodecyloxyphenyl)-10,10′,15,15′,20,20′-hexa(3,3′,4,4′,5,5′-hexakisdecyloxyphenyl)-diporphyrinatozinc (1b). 5,5′-(12,12′-dithiodidodecyloxyphenyl)-10,10′,15,15′,20,20′-hexa(3,3′,4,4′,5,5′-hexakisdecyloxyphenyl)diporphyrin (114 mg, 25 μmol) was refluxed in THF (5 ml). Zinc acetate dihydrate (20 mg, 90 μmol) was added and reflux continued for 30 min. The solvent was evaporated and the residue chromatographed through a short column over silica gel (eluent: petroleum ether (bp 40–60°C)/THF 4:1). Yield 116 mg (99%). ^{1}H NMR (270 MHz): δ, ppm 8.9–9.1 (m, 8H), 8.1 (d, J = 8 Hz, 2H), 7.42 (s, 6H), 7.27 (d, J = 8 Hz, 2H), 4.29 (t, 8H), 4.24 (t, 2H), 4.09 (t, 12H), 2.67 (t, 2H), 1.2–2.05 (m, 164H), 0.91 (t, 9H), 0.83 (t, 18H). UV-vis (THF): λ, nm (ε × 10^5 $M^{-1}.cm^{-1}$) 598.0 (0.12), 557.0 (0.38), 427.5 (12.6), 406.5 (0.86). MALDI-MS: isotopic clusters at m/z 4601 $[M]^+$ and 2300 ($[M]^{2+}$, 100%). Anal. calcd. for $C_{292}H_{462}N_8O_{20}S_2Zn_2$: C, 76.25; H, 10.12; N, 2.44%. Found: C, 76.39; H, 10.39; N, 2.12.

5,5′-(12,12′-dithiodidodecyloxyphenyl)-10,10′,15,15′,20,20′-hexa(3,3′,4,4′,5,5′-hexakisdecyloxyphenyl)diporphyrinatomagnesium (1c). 5,5′-(12,12′-dithiodidodecyloxyphenyl)-10,10′,15,15′,20,20′-hexa (3,3′,4,4′,5,5′-hexakisdecyloxyphenyl)diporphyrin (100 mg, 22 μmol) was refluxed in pyridine (10 ml, dried over KOH) under N_2 atmosphere. Magnesium perchlorate (10 mg, 80 μmol) was added and reflux continued for 4 h. After cooling, the mixture was poured onto water and extracted with diethyl ether. The organics were washed twice with dil. HCl (20%), brine, dried ($MgSO_4$), filtered and the solvent evaporated. The product was purified by column chromatography over silica gel (eluent: petroleum ether (bp 40–60°C)/THF 10:1). Yield 92 mg (93%). ^{1}H NMR (270 MHz): δ, ppm 8.8–9.1 (m, 8H), 8.1 (d, 2H), 7.25–7.5 (m, 8H), 3.9–4.4 (m, 22H), 2.7 (t, 2H), 1.1–2.1 (m, 164H), 0.8–1.0 (m, 27H). UV-vis (THF): λ_{max}, nm (ε × 10^5 $M^{-1}.cm^{-1}$) 615.0 (0.23), 569.0 (0.26), 432.0 (10.77). MALDI-MS: isotopic clusters at m/z 4518 ($[M]^+$, 100%) and 2260 $[M]^{2+}$. Anal. calcd. for $C_{292}H_{462}N_8O_{20}S_2Mg_2$: C, 77.63; H, 10.31; N, 2.48%. Found: C, 77.39; H, 10.28; N, 2.42.

Deposition of SAM films

Glass microscope slides (BDH Ltd.) were used as the substrates for the SAMs. The glass surface was wiped clean with a soft, detergent-free tissue and then washed with a stream of methanol in order to remove any bulk surface contamination. The glass substrates were then immersed into a solution of potassium hydroxide in aqueous methanol (100 g of potassium hydroxide was dissolved in 100 ml of Millipore water, then diluted to 250 ml with methanol) for 12 hours. The substrates were rinsed with fresh Millipore water and then dried in a stream of refluxing propan-2-ol. The resultant clean substrates were stored in sample jars, with air-tight lids.

The cleaned, dried glass substrates were then coated with a layer of chromium (99.999% purity, Johnson Matthey Ltd., 1 nm) followed by a layer of gold (99.999%

purity, Johnson Matthey Ltd., 45 nm). The chromium layer was deposited to ensure a good adhesion of the gold onto the glass surface. Both the chromium and gold layers were deposited by thermal evaporation under vacuum using an Edwards Auto 306 vacuum evaporator.

The SAMs were formed by immersing the freshly prepared gold-coated substrates into a solution of known concentration of porphyrin, typically between 2.5×10^{-7} and 5×10^{-7} M in cyclohexane at room temperature for 20 h.

Reference substrates for the SAMs were fabricated by immersing freshly prepared gold-coated substrates into cyclohexane for 20 h. The SAMs and the references were then washed in fresh cyclohexane, dried in air and subsequently stored in clean amber jars with air-tight lids.

Spectroscopic characterization of SAM films

The BioRad RAIR spectrometer housed a liquid nitrogen cooled MCT detector and was coupled with a Spectra-Tech FT85 reflectance unit. RAIR spectra of the films were acquired using p-polarized light at an incidence angle of 85°. For further details see reference 3c. The spectra were obtained from the co-addition of 1024 scans at a resolution of 4 cm^{-1}. Measurement of the fluorescence emission and fluorescence excitation spectra of the films was achieved using a Perkin Elmer MPF4 spectrophotofluorimeter equipped with accessories for front-surface fluorescence detection.

Photooxidation studies

Typically, a glass slide decorated with the SAM was placed in a Petri dish and covered by 2 ml of a phosphate-buffered (pH 7.4) aqueous solution of HSA; the initial protein concentration was 0.1 mM. The system thus obtained was irradiated, during gentle magnetic stirring, by full spectrum visible light emitted from a quartz/halogen lamp (Teclas, Lugano, Switzerland), which was operated at a fluence rate of 50 mW/cm^2. The light source was equipped with a heat-reflecting filter and a cut-off filter at 390 nm to eliminate any UV radiation. The light beam was piloted to the irradiation site by means of a bundle of optical fibers (external diameter = 0.8 cm). During irradiation, the temperature of the HSA solution was kept at 25°C by circulating thermostated water. At predetermined time intervals after the beginning of the irradiation, 0.1 ml aliquots of the HSA solution were taken and the tryptophan fluorescence emission was measured in a Perkin Elmer MPF4 spectrophotofluorimeter using 290 nm excitation and 360 nm emission; the excitation and emission slits were fixed at 7 nm.

CONCLUSION

The research has demonstrated that SAM films of derivatives of Zn and Mg metalated tetraphenylporphyrins (TPPs) substituted with three long alkoxy chains on each of three of the benzenoid rings, with a tether chain on the fourth, can serve as photodynamic surfaces. The choice of highly substituted TPP derivatives was made to disrupt or at least minimize close porphyrin-porphyrin ring interactions and this, together with a long tether to maintain a significant distance of the core from the gold surface, has ensured that the photoreactive electronically excited states of the molecules are not fully quenched. The latter has been demonstrated by the observation of fluorescence from the film and further by the photooxidation of the tryptophan moiety within human serum albumin. The efficiency of the porphyrin SAMs as heterogeneous catalysts for photooxidations has potential applications within chemical synthesis and also within medical therapies. Thus other porphyrin derivatives, deposited onto appropriate surfaces, could in principle be incorporated within medical devices or implants where a photodynamic effect could be advantageous.

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REFERENCES

1. a) Ulman A. *An Introduction to Ultrathin Organic Films from Langmuir-Blodgett to Self-Assembly* (1st edn.) Academic Press: California, 1991. b) Smith RK, Lewis PA and Weiss PS. *Prog. Surface Sci*. 2004; **75**: 1–68. c) Tao F and Bernasek SL. *Chem. Rev*. 2007; **107**: 1408–1453.
2. a) Kondo T and Uosaki K. *J. Photochem. Photobiol. C* 2007; **8**: 1–17. b) Bedioui F, Griveau S, Nyokong T, Appleby AJ, Caro CA, Gulppi M, Ochoa G and Zagal JH. *Phys. Chem. Chem. Phys.* 2007; **9**: 3383–3396. c) Love JC, Estroff LA, Kriebel JK, Nuzzo RG and Whitesides GM. *Chem. Rev*. 2005; **105**: 1103–1169. d) Schreiber F. *J. Phys-Condensed Matter* 2004; **16**: R881–R900. e) Witt D, Klajn R, Barski P and Grzybowski BA. *Curr. Org. Chem.* 2004; **8**: 1763–1797.
3. a) Chambrier I, Cook MJ and Russell DA. *Synthesis* 1996; 1283–1386. b) Simpson TRE, Revell DJ, Cook MJ and Russell DA. *Langmuir* 1997; **13**: 460–464. c) Revell DJ, Chambrier I, Cook MJ and Russell DA. *J. Mater. Chem*. 2000; **10**: 31–37.
4. Cook MJ, Hersans R, McMurdo J and Russell DA. *J. Mater. Chem*. 1996; **6**: 149–154.
5. a) Simpson TRE, Russell DA, Chambrier I, Cook MJ, Horn AB and Thorpe SC. *Sensors Actuators B* 1995; **29**: 353–357. b) Simpson TRE, Cook MJ, Petty MC, Thorpe SC and Russell DA. *Analyst* 1996; **121**: 1501–1505.
6. Hone DC, Walker PI, Evans-Gowing R, FitzGerald S, Beeby A, Chambrier I, Cook MJ and Russell DA. *Langmuir* 2002; **18**: 2985–2987.

7. Wieder ME, Hone DC, Cook MJ, Handsley MM, Gavrilovic J and Russell DA. *Photochem. Photobiol. Sci*. 2006; **5**: 727–734.
8. a) Watcharinyanon S, Nilsson D, Moons E, Shaporenko A, Zharnikov M, Albinsson B, Maartensson J and Johansson LSO. *Phys. Chem. Chem. Phys.* 2008; **10**: 5264–5275. b) Zuo G, Liu X, Yang J, Li X and Lu X. *J. Electroanal. Chem*. 2007; **605**: 81–88. c) Lu X, Zhang L, Li M, Wang X, Zhang Yan, Liu X and Zuo G. *Chem. Phys. Chem.* 2006; **7**: 854–862. d) Yasseri AA, Syomin D, Malinovskii LV, Loewe RS, Lindsey JS, Zaera F and Bocian DF. *J. Am. Chem. Soc*. 2004; **126**: 11944–11953. e) Rawlett AM, Hopson TJ, Amlani I, Zhang R, Tresek J, Nagahara LA, Tsui RK and Goronkin H. *Nanotechnology* 2003; **14**: 377–384. f) Bramblett AL, Boeckl MS, Hauch KD, Ratner BD, Sasaki T and Roger JW. *Surf. Interface Anal*. 2002; **33**: 506–515. g) Roth KM, Gryko DT, Clausen C, Li J, Lindsay JS, Kuhr WG and Bocian D. *J. Phys. Chem. B* 2002; **106**: 8639–8648. h) Imahori H, Hasobe T, Yamada H, Nishimaru Y, Yamazaki I and Fukuzumi S. *Langmuir* 2001; **17**: 4925–4931. i) Boeckl MS, Bramblett AL, Hauch KD, Sasaki T, Ratner BD and Roger JW. *Langmuir* 2000; **16**: 5644–5653. j) Imahori H, Norieda H, Nishimaru Y, Yanmazaki I, Higuchi K, Kato N, Motohiro T, Yamada H, Tamaki K, Arimura M and Sakata Y. *J. Phys. Chem. B* 2000; **104**: 1253–1260. k) Shimazu K, Takechi M, Fujii H, Suzuki M, Saiki H, Yoshimura T and Uosaki K. *Thin Solid Films* 1996; **273**: 250–253. l) Hutchison JE, Postlethwaite TA and Murray RW. *Langmuir* 1993; **9**: 3277–3283. m) Zak J, Yuan H, Ho M, Woo LK and Porter MD. *Langmuir* 1993; **9**: 2772–2774.
9. Benitez IO, Bujoli B, Camus LJ, Lee CM, Odobel F and Talham DR. *J. Am. Chem. Soc*. 2002; **124**: 4363–4370.
10. Berner S, Biela S, Ledung G, Gogoll A, Baeckvall J-E, Puglia C and Oscarsson S. *J. Catalysis* 2006; **244**: 86–91.
11. Zhang X-L, Chen L-G, Lv P, Gao H-Y, Wei S-J, Dong Z-C and Hou JG. *Appl. Phys. Lett*. 2008; **92**: 223118/1–223118/3.
12. Jori G and Spikes JD. In *Topics in Photomedicine*, Smith KC. (Ed.) Plenum Press: New York, 1983; pp 183–319.
13. Meier H, Prass E, Zerban G and Kosteyn K. *Z. Naturforsch. B* 1988; **43**: 889–896.
14. Johnstone RAW, Nunes MLPG, Pereira MM, Gonsalves AMDR and Serra AC. *Heterocycles* 1996; **43**: 1423–1437.
15. Imahori H, Kashiwagi Y, Endo Y, Hanada T, Nishimura Y, Yamazaki I, Araki Y, Ito O and Fukuzumi S. *Langmuir* 2004; **20**: 73–81.
16. Silva E, Ugarte R, Andrade A and Edwards AM. *J. Photochem. Photobiol. B* 1994; **23**: 43–48.
17. Jori G and Spikes JD. In *Oxygen and Oxy-radicals in Chemistry and Biology*, Rodgers MAJ and Powers EL. (Eds.) Academic Press: New York, 1981; pp 441–459.
18. Foote CS. In *Free Radicals in Biology*, Pryor WA. (Ed.) Academic Press: New York, 1976; pp 85–133.
19. Benassi CA, Scoffone E, Galiazzo G and Jori G. *Photochem. Photobiol.* 1987; **6**: 857–866.
20. Reddi E, Valduga G, Rodgers MAJ and Jori G. *Photochem. Photobiol*. 1991; **54**: 633–637.