Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Electronic Supplementary Information

Towards optimisation of surface enhanced photodynamic therapy of breast cancer cells using gold nanoparticle-photosensitiser conjugates

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TEM analysis of the C11Pc/C3Pc-PEG-AuNPs

Analysis of the TEM for the C3Pc-PEG-AuNPs indicated that the nanoparticles have an average diameter of 3.83 ± 0.99 nm, following analysis of 562 nanoparticles, with a median value of 3.76 ± 0.99 nm. Analysis of the TEM for the C11Pc-PEG-AuNPs indicated that the nanoparticles have an average diameter of 3.41 ± 1.16 nm, following analysis of 282 nanoparticles, with a median value of 3.21 ± 1.35 nm. TEM images of the C3Pc-PEG-AuNPs and C11Pc-PEG-AuNPs, together with histograms showing their size distribution, are shown in Fig S1. The size distribution for C11Pc-PEG-AuNPs is somewhat skewed. Therefore, the median value of 3.76 ± 0.99 nm would be a better representation of the average size of the C11Pc-PEG-AuNPs.[1]

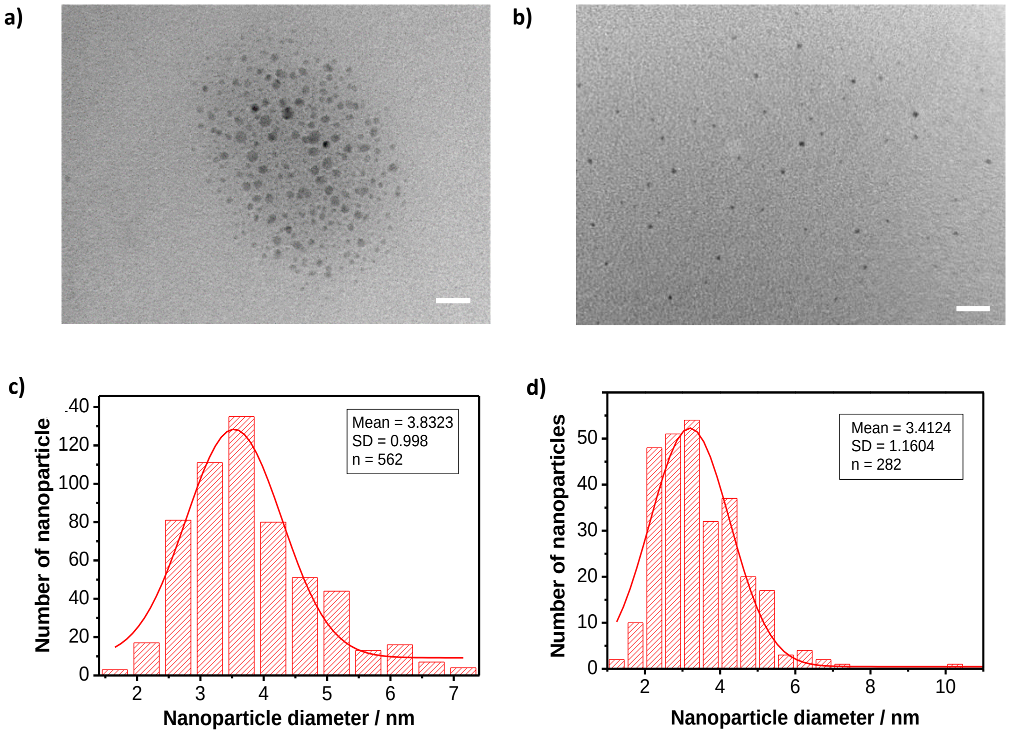

Fig. S1. Transmission electron micrographs of a sample of **(a)** C3Pc-PEG-AuNPs and **(b)** C11Pc-PEG-AuNPs, where the scale bars represent 20 nm. Size distributions showing the Gaussian fit of the **(c)** C3Pc-PEG-AuNPs with an average size of 3.83 ± 0.99 nm (n = 562) and **(d)** C11Pc-PEG-AuNPs with an average size of 3.41 ± 1.16 nm (n = 282).

Determination of the extinction coefficients of the phthalocyanines

C11Pc (2.27 mg) was dissolved in THF (2 mL) giving an initial concentration of 443 µM. C3Pc (24.5 mg) was dissolved in THF (5 mL) giving an initial concentration of 2,094 µM. These initial C11Pc and C3Pc solutions were then further diluted with THF to obtain concentrations ranging from 0-5 µM. These dilutions were prepared in volumetric flasks (2 mL). The dilutions were analysed by UV-vis spectroscopy between 300-800 nm starting with the lowest concentration. A calibration curve at 698 nm was created and a value for the extinction coefficient for each phthalocyanine was obtained. The values of the extinction coefficients obtained, through Fig S2, were 2.76×10^5 $M^{-1}\cdot cm^{-1}$ for C11Pc and 2.74×10^5 $M^{-1}\cdot cm^{-1}$ for C3Pc.

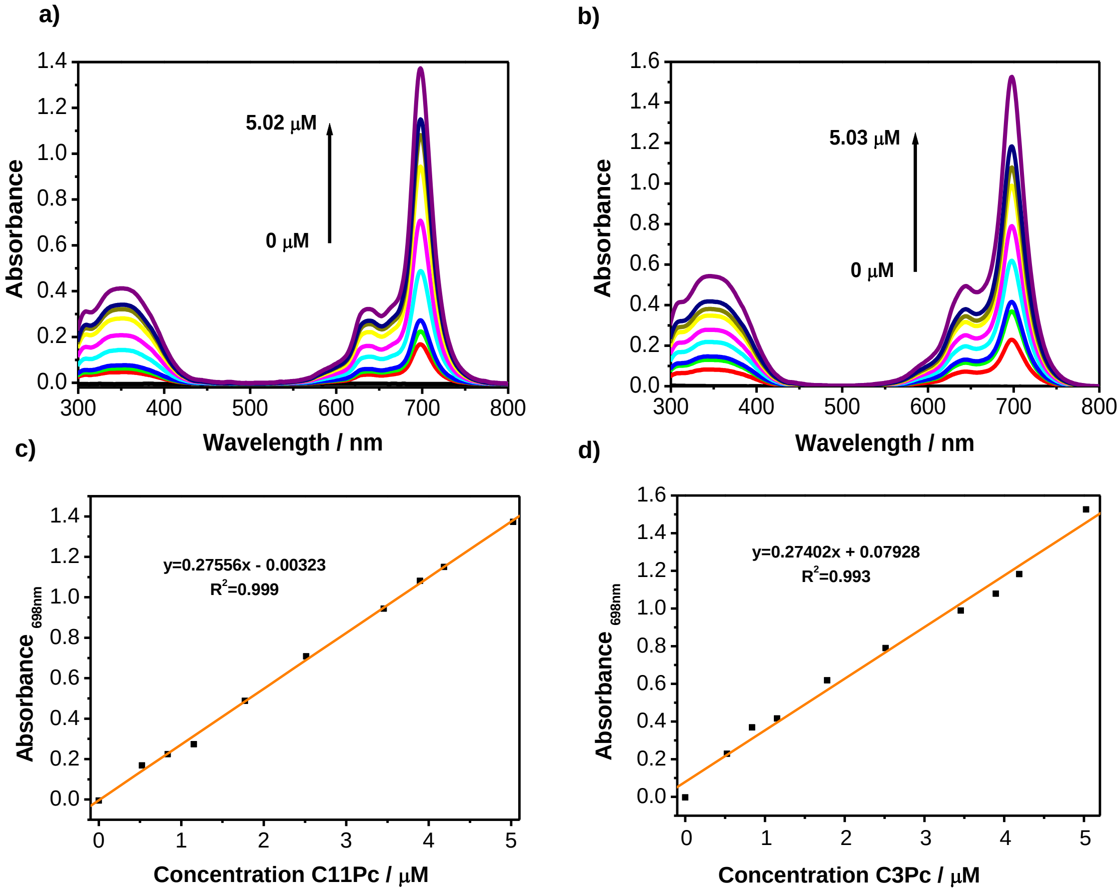

Fig. S2. UV-vis absorbance spectra of **(a)** C11Pc and **(b)** C3Pc in THF at various concentrations and their corresponding calibration curves giving the values for the extinction coefficients **(c)** C11Pc (2.76×10^5 $M^{-1}\cdot cm^{-1}$) and **(d)** C3Pc (2.74×10^5 $M^{-1}\cdot cm^{-1}$) measured using an absorbance intensity at 698 nm.

Estimation of the number of phthalocyanines per gold nanoparticle

The synthesised C11Pc-PEG-AuNPs and C3Pc-PEG-AuNPs were prepared following the same synthetic procedure with identical concentrations of Pc, PEG, gold chloride and sodium borohydride. However, the immobilisation of either of the phthalocyanines, C11Pc or C3Pc, on the surface of the AuNPs could be different due to the difference in the carbon chain length and the orientation of the macrocycle on the gold surface. In order to obtain a ratio of the concentration of Pc to the concentration of AuNPs in the sample, the concentrations of both the Pc and the AuNPs were calculated. For the phthalocyanines, the extinction coefficients reported above, together with the extinction intensity at 698 nm were used to obtain the concentration of the Pc in the sample *via* the Beer-Lambert law. The concentration of the AuNPs in the sample cannot be directly calculated due to the absence of a surface plasmon absorption band in the extinction spectrum of the AuNPs with diameters smaller than 10 nm. For this purpose, an estimation of the concentration of AuNPs in the sample was performed following the procedure described by Haiss *et al.*.[2] These authors experimentally determined the values for the extinction coefficients at 450 nm of AuNPs with a diameter of 2-100 nm. Additionally, the authors reported that it was possible to calculate the concentration of uncoated spherical AuNPs in water using the ε_{450} and the absorbance intensity at 450 nm, as shown in Equation S1. This method is more accurate for AuNPs larger than 5 nm in diameter, due to the surface effects taking place with smaller AuNPs. Considering that the AuNPs reported in this study are smaller than 5 nm and are coated, the method reported by Haiss *et al.* can only give an approximation of the concentration of AuNPs in the sample and thus only an estimation of the ratio of Pc to AuNPs can be calculated. The data required to calculate the concentrations of Pc and AuNPs, as well as the results of the ratio of Pc to AuNPs are shown in Table S1.

$$\mathrm{c} = \frac{\mathrm{A}_{450}}{\varepsilon_{450}}$$

Equation S1. Equation used to calculate the concentration of AuNPs in an aqueous solution, as reported by Haiss *et al.*.[2]

Table S1. Estimation of the ratio of Pc to AuNPs.

Sample	A_{450}	[AuNP] / M	A_{698}	[Pc] / M	Ratio Pc/Au
C3Pc-PEG-AuNPs	0.137	3.78×10^{-8}	1.026	3.75×10^{-6}	99
C11Pc-PEG-AuNPs	0.101	2.79×10^{-8}	0.886	3.22×10^{-6}	115

For 4 nm AuNPs, $\varepsilon_{450} = 3.62\times10^{6}\ \mathrm{M^{-1}\cdot cm^{-1}}$.[2]

The results in Table S1 suggest that the C3Pc-PEG-AuNPs have less phthalocyanine molecules immobilised per AuNP (*ca.* 99) as compared to C11Pc-PEG-AuNPs (*ca.* 115). As a result, at the same concentration of phthalocyanine, the C11Pc-PEG-AuNPs sample will contain less AuNPs than the C3Pc-PEG-AuNPs sample.

Estimation of the fluorescence quantum yields

The fluorescence quantum yields were obtained using a calibration curve of integrated fluorescence intensity (*i.e.,* area under the curve) *vs* absorbance at the excitation wavelength, 640 nm. The calibration curve produces a straight line with gradient m and intercept 0. The gradient is proportional to the fluorescence quantum yield of the sample. By comparing the gradient of the unknown samples to the gradient of the reference standard, the fluorescence quantum yields can be estimated. The gradients of each calibration curve were used to estimate the value for the fluorescence quantum yield, following Equation S2. The calibration curves obtained are shown in Fig S3, and the final fluorescence quantum yield values are shown in Table S2.

$$\emptyset_x = \emptyset_{ref}\left(\frac{\mathrm{Grad}_x}{\mathrm{Grad}_{ref}}\right)\left(\frac{n_x^2}{n_{ref}^2}\right)$$

Where:
Φ = Fluorescence quantum yield
Ref = Reference sample
x = Experimental samples
Grad = Gradient
n = Refractive index of solvent

Equation S2. Equation used to calculate the fluorescence quantum yields *via* the calibration method.

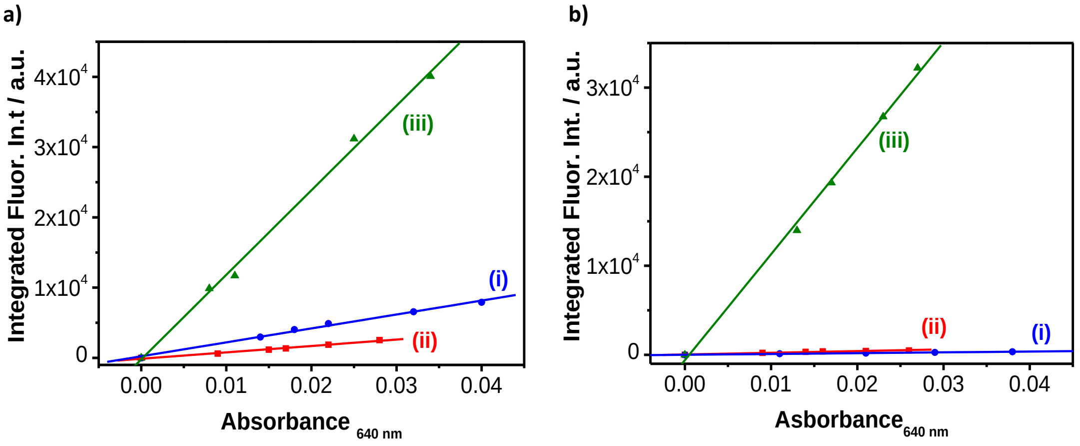

Fig. S3. (a) Calibration curves for the free (**i**) C11Pc and (**ii**) C3Pc in THF together with (**iii**) ZnPc-ref in toluene. **(b)** Calibration curves for (**i**) C11Pc-PEG-AuNPs and (**ii**) C3Pc-PEG-AUNPs in MES buffer, together with (**iii**) ZnPc-ref in toluene.

Table S2. Values obtained for the fluorescence quantum yields.

Figure	Sample	Gradient	R^2 value	Φ / %
S3 a)	ZnPc-ref	1.20×10^6	0.998	33.00
	Free C11Pc	1.89×10^5	0.997	4.80
	Free C3Pc	9.08×10^4	0.994	2.60
S3 b)	ZnPc-ref	1.19×10^6	0.999	33.00
	C11Pc-PEG-AuNPs	9.11×10^3	0.998	0.20
	C3Pc-PEG-AuNPs	1.91×10^4	0.980	0.42

$n_{THF}{}^{3}$ = 1.407; $n_{Toluene}{}^{4}$ = 1.496; $n_{H_2O}{}^{5}$ = 1.330

The fluorescence quantum yields for the free phthalocyanines, C11Pc and C3Pc, are rather low. A possible explanation for the low values obtained for the fluorescence quantum yields of the Pc in solution could be related to the dimeric structure of the phthalocyanines. Previous studies have reported that the strong fluorescence of monomeric phthalocyanines is usually quenched upon dimerisation and aggregation.[6-7] As an example, the Zefirov group reported the synthesis of a magnesium phthalocyanine (2-hydroxy-9(10), 16(17), 23(24)-tri-tert-butylphthalocyanine [Mg]) and its corresponding dimer.[8] The authors found that the fluorescence quantum yield of the dimer was 1.4 %, a value less than half that obtained for the monomeric form (4.8 %).[8]

The fluorescence quantum yields estimated for the Pc-PEG-AuNPs are also low, 0.42 % for C3Pc-PEG-AuNPs and 0.20 % for C11Pc-PEG-AuNPs. It is important to note that the refractive index of the MES buffer in which the nanoparticles were dissolved was taken to be the same as that of water (1.33), as reported in Table S2. However, MES buffer contains MES (50 nM) and Tween-20 (0.05 %). It has been reported that the presence of MES increases the refractive index of water, and this increase in refractive index is concomitant with an increase in the concentration of MES buffer in the sample.[9] As a result, the refractive index of MES buffer is expected to be higher than that of water, which would lead to higher values of the fluorescence quantum yields for the Pc-PEG-AuNPs than those reported here.

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