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Supporting information

A novel fluoro-chromogenic click reaction for the labelling of proteins and nanoparticles with near-IR theranostic agents

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1. Instrumentation and materials.

1. 1 Techniques for the identification of compounds

¹H and ¹³C-NMR spectra were recorded on a Varian 400-MR spectrometer working at 400 MHz for ¹H or 100.6 MHz for ¹³C. All NMR data were obtained in CDCl₃. Chemical shifts are reported in parts per million (ppm, δ) and are referenced to the residual signal of the solvent. Coupling constants are reported in Hertz (Hz). Spectral splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (complex multiplet).

HPLC-MS analysis was carried out in an Agilent 1260 infinity Series chromatograph equipped with photodiode array detector (200–600 nm) and coupled to an Agilent 6130 quadrupole LC/MS. Separation was achieved using a Discovery® C18 (15m x 4.6 mm, 5µm particle size) column and an ACN:MeOH 85:15 mixture at 1.0 mL/min flow rate. The DAD detector was set at 400 nm matching the Soret band of porphycenes. API-ES positive ionization mode at 80V cone voltage was used. Fragmentations were scanned from 500 to 2500 m/z. Peaks were identified by their characteristic MS fragmentations.

1.2 Techniques for the characterisation of nanostructures

The size and morphology of tetraethylenglycol alkyl-thiol stabilized gold nanoclusters were determined by means of transmission electron microscopy (TEM). For TEM examination a single drop (15 μ L) of the EtOH solution of the nanostructures was placed onto a copper grid coated with a carbon film. The grid was left to dry in air at room temperature. TEM analysis were carried out in a JEOL 2010F microscope, working at 200 kV. The particle size distribution was evaluated from several micrographs by means of an automatic image analyser software.

1.3 Spectroscopic techniques

The photophysical properties of porphycene derivatives were measured in spectroscopic grade acetone.

Absorption spectra were recorded on a Varian Cary 6000i dual-beam UV/Vis spectrophotometer. Fluorescence emission spectra were recorded on a Jobin Yvon-Spex Fluoromax-4 spectrofluorometer. Fluorescence quantum yields (Φ_F) were determined by comparison of the area under the emission curves for solutions of 9-ITPPo and 9-amide porphycene derivatives in acetone with TPP in toluene ($\Phi_F=0,11$)¹ or ATAZPos and TAZPos in acetone with ICG in DMSO ($\Phi_F=0,12$)² respectively. The absorbance of both sample and reference solutions were below 0.05 in order to prevent inner filter effects and matched at the excitation wavelength. Emission intensities were corrected for the refractive index of the solvent.

Fluorescence decays were recorded with a time-correlated single photon counting system (Fluotime200, PicoQuant GmbH, Berlin, Germany) equipped with a red sensitive photomultiplier. Excitation was achieved by means of a 375 nm picosecond diode laser working at 10 MHz repetition rate and the photon counting frequency was kept below 1%. Fluorescence lifetimes were determined using PicoQuant *FluoFit 4.6* data analysis software.

The specific ${}^{1}O_{2}$ near-infrared phosphorescence kinetics were detected by means of a customized PicoQuant Fluotime 200 system described in detail elsewhere.³ The time-resolved emission signals were fitted by Eq. (1):

$$\mathbf{S}_{t} = \mathbf{S}_{0} \times \frac{\tau_{\Delta}}{\tau_{\Delta} - \tau_{T}} \left(\mathbf{e}^{-t/\tau_{\Delta}} - \mathbf{e}^{-t/\tau_{T}} \right)$$
 Eq. (1)

where S_0 is proportional to Φ_{Δ} , τ_{Δ} is the ¹O₂ lifetime and τ_{T} is the lifetime of the photosensitizer's triplet state. The Φ_{Δ} values were determined by comparing S_0 for 9-ITPPo, ATAZPos and TAZPos and a reference photosensitize, TPP ($\Phi_{\Delta}=0,63$).⁴

Triplet lifetimes of the argon fluxed samples were monitored with a home-built nanosecond flash photolysis setup using a Q-switched Nd:YAG laser (Surelite I-10, Continuum) for excitation at 355 nm and a Xe lamp (PTI, 75 W) for probing the transient absorption with right-angle geometry. The analysing beam passed through a dual grating monochromator (mod. 101, PTI) and was detected by a Hamamatsu R928 photomultiplier. The signal was fed to a Lecroy WaveSurfer 454 oscilloscope for digitizing and averaging (typically 10 shots) and finally transferred to a PC computer for data storage and analysis. Kinetic analysis of the individual transients decays afforded the values of $\tau_{\rm T}$.

1.4 Computational methods

Geometry optimizations of ground states were performed using the DFT method with B3LYP functional at 6-31G(d) level of theory. Gaussian 09 software was used for all calculations⁵.

2. Experimental section

9-amino-2,7,12,17-tetraphenylporphycene (9-ATPPo) was prepared in agreement with previously described synthetic procedures.⁶ This compound was used as starting reagent for the synthesis of the novel porphycene derivatives described below (Scheme S1).



Scheme S1: General synthetic scheme for the preparation of thiazolo[4,5-c]porphycenes

2.1. Synthesis of 9-isothiocyanate-2,7,12,17-tetraphenylporphycene



Scheme S2: Synthesis of 9-isothiocyanate-2,7,12,17-tetraphenylporphycene

To a solution of 9-ATPPo (50 mg, 7.5.10⁻² mmol) in CH₂Cl₂ (10 mL) was added 1,1'thiocarbonyldi-2(1H)-pyridone (53 mg, 3 eq, 0.23 mmol). The mixture was stirred 16h at room temperature. The solvent was evaporated and the mixture was purified by chromatography with silica column eluting with CH₂Cl₂. The product was then crystallized, by dissolving it in toluene (5 mL) and pouring it in acetonitrile (50 mL), to provide a green product (38 mg, 5.6.10⁻² mmol, 75 %). ¹H NMR (400 MHz, CDCl₃) δ 9.70 – 9.61 (ABq, 2H, *J*_{AB} = 11.1 Hz), 9.55 (s, 1H), 9.43 (s, 1H), 9.39 (s, 2H), 9.30 (s, 1H), 8.29 – 8.19 (m, 6H), 8.08 – 8.00 (m, 2H), 7.88 – 7.59 (m, 12H), 3.77 (s, 1H), 3.29 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 145.47, 145.15, 144.79, 143.64, 143.08, 142.96, 139.93, 138.03, 137.42, 136.14, 135.75, 135.18, 134.83, 134.03, 133.52, 131.54, 131.51, 131.48, 130.91, 129.47, 129.29, 129.26, 128.73, 128.41, 128.22, 127.94, 127.30, 124.14, 124.07, 118.27, 115.57, 115.16, 114.83. MS (API-ES) Calculated for C₄₅H₃₀N₅S [(M+H)⁺]: 672.22. Found: *m/z*=672.2. Retention time: 21.49 min.



UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 399 (8.8 $\cdot 10^4$), 540 (4.25 $\cdot 10^3$), 584 (1.95 $\cdot 10^4$), 643 (3.0 $\cdot 10^4$), 656 (2.85 $\cdot 10^4$), 667 (2.1 $\cdot 10^4$).

Figure S1: ¹H-NMR spectra of 9-ITPPo



Figure S2: ¹³C-NMR spectra of 9-ITPPo



Figure S3: Chromatogram of 9-ITPPo using UV/Vis detection at 400 nm.

2. 2. Synthesis of 2-aminothiazolo[4,5-c]tetraphenylporphycenes



Scheme S3: Synthesis of 2-aminothiazole-fused tetraphenylporphycene derivatives

Synthesis of ATAZPo1

To a solution of 9-isothiocyanate-2,7,12,17-tetraphenylporphycene (9-ITPPo, 5 mg, 7.10⁻³ mmol) in THF (0.5 mL) was added a large excess of ammonium hydroxide 30% (1 mL, 7 mmol). The mixture was stirred 3h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated to provide a green product (5mg, 97%). ¹H NMR (400 MHz, CDCl₃) δ 9.61 (d, *J* = 11.1 Hz, 1H), 9.49 (d, *J* = 11.1 Hz, 1H), 9.41 (s, 1H), 9.39 (s, 1H), 9.36 (s, 2H), 8.30 – 8.23 (m, 4H), 8.04 (m, 2H), 7.98 – 7.92 (m, 2H), 7.81 – 7.59 (m, 11H), 7.54 – 7.47 (m, 1H), 6.08 (s, 1H), 5.48 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 165.17, 145.25, 144.74, 143.73, 143.17, 143.10, 143.08, 142.64, 142.28, 141.10, 140.26, 137.78, 136.98, 136.65, 136.33, 135.19, 134.39, 134.11, 131.48, 131.42, 131.38, 131.36, 130.98, 130.95, 130.63, 129.98, 129.40, 129.23, 129.21, 129.14, 129.09, 129.01, 128.70, 128.04, 127.88, 127.82, 127.71, 127.63, 126.37, 125.23, 123.64, 123.59, 123.16, 115.93, 112.2. MS (API-ES) Calculated for C₄₅H₃₀N₆S [(M+H)⁺]: 687.23. Found: *m/z*=687.2 Retention time: 7.15 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 390 (1.01 $\cdot 10^5$), 412 (8.28 $\cdot 10^4$), 583 (6.1 $\cdot 10^3$), 627 (2.9 $\cdot 10^4$), 685 (6.5 $\cdot 10^4$), 745 (9.3 $\cdot 10^3$).



Figure S5: ¹³C-NMR spectra of ATAZPo1



Figure S6: Chromatogram of ATAZPo1 using UV/Vis detection at 400 nm.

To a solution of 9-ITPPo (3 mg, 4.10^{-3} mmol) in CH₂Cl₂ (2 mL) was added *n*-butylamine (50 µL, 0.6 mmol, 150 eq). The mixture was stirred 3h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated to provide a green product (3 mg, 95%). ¹H NMR (400 MHz, CDCl₃) δ 9.63 (d, J = 11.1 Hz, 1H), 9.54 (d, J = 11.1 Hz, 1H), 9.43 (s, 2H), 9.41 (s, 1H), 9.38 (s, 1H), 8.27 (m, 4H), 8.08 (m, 2H), 7.97 – 7.92 (m, 2H), 7.81 – 7.69 (m, 7H), 7.67 – 7.60 (m, 4H), 7.51 (m, 1H), 5.92 (s, 1H), 5.31 (s, 1H), 5.11 (s, 1H), 3.23 (m, 2H), 1.42 – 1.32 (m, 4H), 0.95 (t, J = 7.3 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 164.05, 145.04, 144.09, 143.89, 143.42, 142.76, 142.65, 142.58, 140.65, 139.62, 137.75, 136.84, 136.56, 136.52, 135.72, 134.42, 133.87, 131.42, 131.37, 131.12, 130.90, 129.31, 129.10, 129.04, 128.74, 128.04, 127.94, 127.80, 127.75, 126.60, 125.58, 123.64, 123.47, 123.22, 115.74, 112.80, 77.48, 77.16, 76.84, 44.51, 31.87, 20.14, 14.01. MS (API-ES) Calculated for C₄₅H₃₀N₆S [(M+H)⁺]: 743.29. Found: *m/z*=743.2. Retention time: 14.50 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 394 (1.06 $\cdot 10^{5}$), 414 (8.6 $\cdot 10^{4}$), 584 (1.0 $\cdot 10^{4}$), 632 (3.4 $\cdot 10^{4}$), 699 (6.8 $\cdot 10^{4}$), 743 (2.0 $\cdot 10^{4}$).



Figure S7: ¹H-NMR spectra of ATAZPo2



Figure S8: ¹³C-NMR spectra of ATAZPo2



Figure S9: Chromatogram of ATAZPo2 using UV/Vis detection at 400 nm.

To a solution of 9-ITPPo (3 mg, 4.10^{-3} mmol) in CH₂Cl₂ (2 mL) was added diethylamine (4 µL, 0.04 mmol, 10 eq). The mixture was stirred 5h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated to provide a green product (3 mg, 95%). ¹H NMR (400 MHz, CDCl₃) δ 9.61 (d, J = 11.1 Hz, 1H), 9.49 (d, J = 11.1 Hz, 1H), 9.41 (d, J = 1.0 Hz, 1H), 9.39 (s, 1H), 9.37-9.36 (s, 2H), 8.28-8.24 (m, 4H), 8.04-8.02 (m, 2H), 7.98 – 7.92 (m, 2H), 7.81 – 7.59 (m, 12H), 7.56 – 7.48 (m, 2H), 6.09 (s, 1H), 5.49 (s, 1H), 3.36 (q, J = 7.2 Hz, 4H), 1.13 (t, J = 7.1 Hz, 6H). ¹³C NMR (100 MHz, CDCl₃) 165.01, 145.10, 144.59, 143.59, 143.59, 142.95, 142.93, 140.92, 140.92, 137.61, 136.82, 136.48, 136.18, 135.03, 134.25, 133.96, 131.25, 131.19, 130.77, 130.48, 129.04, 128.92, 128.84, 128.54, 127.88, 127.71, 127.65, 127.54, 126.20, 125.06, 123.44, 115.77, 112.11, 52.78, 29.69. MS (API-ES) Calculated for C₄₅H₃₀N₆S [(M+H)⁺]: 743.29. Found: *m/z*=743.2. Retention time: 20.03 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 387 (9.6 $\cdot 10^4$), 405 (8.7 $\cdot 10^4$), 424 (8.7 $\cdot 10^4$), 583 (8.3 $\cdot 10^3$), 632 (1.2 $\cdot 10^4$), 702 (6.4 $\cdot 10^4$), 763 (1.4 $\cdot 10^4$).



Figure S10: ¹H-NMR spectra of ATAZPo3



Figure S11: ¹³C-NMR spectra of ATAZPo3



Figure S12: Chromatogram of ATAZPo3 using UV/Vis detection at 400 nm.

To a solution of 9-ITPPo (5 mg, 7.5.10⁻³ mmol) in CH₂Cl₂ (2 mL) was added piperazine (20 mg, 0.2 mmol, 30 eq). The mixture was stirred 3h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated, the product was purified by chromatography with silica column eluting with cyclohexane:toluene gradient starting from v:v=3:1 to v:v=0:1 to provide a green product (4 mg, 90%). The NMR spectrum shows two conformers at room temperature. ¹H NMR (400 MHz, CDCl₃) δ 9.61 (d, J = 11.2, 1H), 9.51 (d, J = 11.1, 1H), 9.43 – 9.36 (m, 4H), 8.26 (m, 4H), 8.04 (m, 2H), 7.96 (m, 2H), 7.84 – 7.60 (m, 11H), 7.49 (m, 1H), 5.94 (s, 1H), 5.33 (s, 1H), 3.47 – 3.29 (m, 4H), 2.95 – 2.89 (m, 1H), 2.78 (m, 2H), 2.62 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 165.67, 165.46, 144.86, 144.13, 143.00, 142.89, 142.41, 142.23, 139.41, 136.64, 136.38, 135.91, 135.83, 134.56, 133.63, 133.60, 131.24, 131.20, 131.16, 130.84, 130.77, 129.14, 128.93, 128.88, 128.63, 127.81, 127.69, 126.15, 123.61, 123.13, 112.67, 50.86, 48.88, 45.79, 45.55. MS (API-ES) Calculated for C₄₉H₃₈N₇S [(M+H)⁺]: 755.94, [(M+3H₂O+2H)⁺]:783.96. Found: *m/z*=784.2. Retention time: 5.74 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 392 (1.05 $\cdot 10^{5}$), 398 (1.07 $\cdot 10^{5}$), 420 (8.9 $\cdot 10^{4}$), 583 (7.0 $\cdot 10^{3}$), 625 (3.6 $\cdot 10^{4}$), 688 (6.8 $\cdot 10^{4}$), 744 (1.8 $\cdot 10^{4}$).



Figure S13: ¹H-NMR spectra of ATAZPo4



Figure S14: ¹³C-NMR spectra of ATAZPo4



Figure S15: Chromatogram of ATAZPo4 using UV/Vis detection at 400 nm.

To a solution of 9-ITPPo (5 mg, 7.5.10⁻³ mmol) in CH₂Cl₂ (2 mL) was added morpholine (20 mg, 0.2 mmol, 30 eq). The mixture was stirred 3h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated, the product was purified by chromatography with silica column eluting with cyclohexane:toluene gradient starting from v:v=3:1 to v:v=0:1 to provide a green product (4 mg, 90%). ¹H NMR (400 MHz, CDCl₃) 9.62 (d, *J* = 11.1 Hz, 1H), 9.53 (d, *J* = 11.1 Hz, 1H), 9.44 – 9.40 (m, 2H), 9.40 (s, 1H), 9.38 (s, 1H), 8.32 – 8.17 (m, 4H), 8.09 – 7.99 (m, 2H), 7.99 – 7.92 (m, 2H), 7.86 – 7.57 (m, 11H), 7.49 (m, 1H), 5.90 (s, 1H), 5.28 (s, 1H), 3.76 (t, *J* = 4.9 Hz, 4H), 3.36 (t, *J* = 4.9 Hz, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 165.51, 145.02, 144.33, 143.59, 143.12, 142.97, 142.26, 140.92, 139.25, 137.39, 136.57, 136.33, 136.09, 134.78, 133.43, 131.24, 131.20, 131.17, 130.78, 129.17, 128.94, 128.89, 128.68, 127.83, 127.77, 127.70, 126.19, 125.19, 123.67, 123.23, 115.60, 112.86, 66.33, 47.90. MS (API-ES) Calculated for C₄₉H₃₇N₆OS [(M+H)⁺]: 757.27, Found: *m/z*=757.2. Retention time: 14.74 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 386 (5.7 $\cdot 10^4$), 398 (5.8 $\cdot 10^4$), 414 (5.2 $\cdot 10^4$), 583 (4.7 $\cdot 10^3$), 624 (2.0 $\cdot 10^4$), 688 (3.7 $\cdot 10^4$), 740 (1.2 $\cdot 10^4$).



Figure S16: ¹H-NMR spectra of ATAZPo5



Figure S17: ¹³C-NMR spectra of ATAZPo5



Figure S18: Chromatogram of ATAZPo5 using UV/Vis detection at 400 nm.

To a solution of 9-ITPPo (1 mg, $1.5 \cdot 10^{-3}$ mmol) in CH₂Cl₂ (2 mL) was added aniline (5µl, 0.04 mmol, 30 eq). The mixture was stirred 24h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated, the product was purified by chromatography with silica column eluting with cyclohexane:toluene gradient starting from v:v=3:1 to v:v=0:1 to provide a green product (0.8 mg, 80%).¹H NMR (400 MHz, CDCl₃) δ 9.62 (d, *J* = 11.0 Hz, 1H), 9.54 (d, *J* = 11.1 Hz, 1H), 9.42 (m, 2H), 9.41 (m, 1H), 9.39 (m, 1H), 8.29 – 8.24 (m, 4H), 8.15 (m, 2H), 7.99 (m, 2H), 7.81 – 7.69 (m, 11H), 7.66 (m, 2H), 7.59 (m, 1H), 7.53 (m, 3H), 5.99 (s, 1H), 5.32 (m, 2H). MS (API-ES) Calculated for C₅₁H₃₄N₆S [(M+H)⁺]: 762.92, Found: *m*/*z*=762.9. Retention time: 14.42 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 386 (7.6 · 10⁴), 418 (7.4 · 10⁴), 586 (9.0 · 10³), 633 (2.7 · 10⁴), 692 (5.5 · 10⁴), 750 (1.2 · 10⁴).



To a solution of 9-ITPPo (5 mg, $7.5.10^{-3}$ mmol) in dichloromethane (2 mL) was added O-(2aminoethyl)-O'-(2-azidoethyl)nonaethylene glycol (NH₂-PEG-N₃, 9 mg, $1.5.10^{-2}$ mmol, 2 eq). The mixture was stirred 3h at room temperature. Then, water was added and the product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated, the product was purified by chromatography with silica column eluting with cyclohexane:toluene gradient starting from v:v=3:1 to v:v=0:1 to provide a green product (6 mg, 85%). ¹H NMR (400 MHz, CDCl₃) δ 9.63 (d, *J* = 11.1 Hz, 1H), 9.53 (d, *J* = 11.1 Hz, 1H), 9.42 (d, *J* = 0.8 Hz, 2H), 9.40 (d, *J* = 1.2 Hz, 1H), 9.37 (s, 1H), 8.26 (m, 4H), 8.07 – 8.00 (m, 2H), 7.96 – 7.91 (m, 2H), 7.81 - 7.57 (m, 11H), 7.52 - 7.46 (m, 1H), 6.34 (s, 1H), 5.90 (s, 1H), 5.32 (s, 1H), 4.11 (s, 2H), 3.66 - 3.51 (m, 44H), 3.41 - 3.31 (m, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 173.26, 163.85, 143.93, 143.74, 143.06, 142.59, 142.56, 140.84, 139.28, 137.64, 136.68, 136.41, 136.17, 135.75, 134.23, 133.66, 132.13, 132.03, 131.94, 131.92, 131.25, 131.20, 130.86, 129.13, 128.94, 128.88, 128.53, 127.92, 127.77, 127.63, 127.58, 126.26, 125.38, 123.51, 123.20, 115.48, 70.48, 70.46, 70.44, 70.43, 62.13, 50.62. MS (API-ES) Calculated for C₆₉H₇₇N₉O₁₁S [(M+H)⁺]: 1240.55, C₆₉H₇₇N₉O₁₁SNa [(M+Na)⁺]: 1262.54, Found: *m/z*=1240.4, 1262.4. Retention time: 8.15 min.

UV/Vis λ_{max}/nm ($\epsilon/M^{-1} \cdot cm^{-1}$): 388 (1.0·10⁵), 415 (8.4·10⁴), 586 (7.8·10³), 628 (3.2·10⁴), 693 (6.6·10⁴), 748 (1.7·10⁴).



Figure S21: ¹H-NMR spectra of ATAZPo7



Figure S22: ¹³C-NMR spectra of ATAZPo7



Figure S23: Chromatogram of ATAZPo7 using UV/Vis detection at 400 nm.

2. 3. Synthesis of 9-benzamido-2,7,12,17-tetraphenylporphycene



Scheme S4: Synthesis of 9-benzamido-2,7,12,17-tetraphenylporphycene (9-AmPo)

To a solution of 9-ATPPo (3 mg, $4.8 \cdot 10^{-3}$ mmol) in 5 mL of THF were added 2 µl of benzoyl chloride ($1.2 \cdot 10^{-2}$ mmol, 2.5 eq) and $4.8 \cdot 10^{-3}$ mmol of pyridine (1 eq). The mixture was stirred for 2 h. Then, water was added and the pH was adjusted to 12 with NaOH. The product was extracted with CH₂Cl₂, the organic layer was dried with anhydrous Na₂SO₄ and evaporated. Finally the product was purified by thin layer silica chromatography eluting with CH₂Cl₂ to provide a blue-greenish product (2.5 mg, 80%). ¹H NMR (400 MHz, CDCl₃) δ 10.52 (s, 1H), 9.85 (d, *J* = 11.2 Hz, 1H), 9.78 (d, *J* = 11.2 Hz, 1H), 9.65 (s, 1H), 9.61 (s, 1H), 9.55 (s, 1H), 9.52 (s, 1H), 9.45 (s, 1H), 8.48 (d, *J* = 7.5 Hz, 2H), 8.30 (ddd, *J* = 10.5, 8.1, 1.4 Hz, 4H), 8.19 – 8.12 (m, 1H), 8.00 – 7.92 (m, 2H), 7.81 (ddd, *J* = 12.7, 7.6, 5.4 Hz, 6H), 7.71 – 7.64 (m, 3H), 7.60 – 7.50 (m, 5H), 7.42 (dt, *J* = 16.8, 7.7 Hz, 5H), 4.76 (s, 1H), 4.39 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) 166.07, 145.59, 145.17, 144.54, 143.77, 142.23, 141.02, 140.85, 136.41, 136.31, 135.84, 134.77, 134.65, 134.11, 133.84, 131.62, 131.39, 131.33, 130.11, 129.17, 129.02, 128.99, 128.38, 127.91, 127.84, 127.77, 127.11, 127.00, 126.07, 124.06, 123.82, 123.51, 115.50, 114.09, 113.24. MS (API-ES) Calculated for C₅₁H₃₅N₅O [(M+H)⁺]: 734.87, C₁₀₂H₇₄N₁₀O₂ [(2M+H)⁺]: 1468.7, Found: *m/z*=734.2, 1468.4. Retention time: 8.15 min.



Figure S24: ¹H-NMR spectra of 9-AmPo



2.4 Synthesis of 2-phenylthiazolo[4,5-c]tetraphenylporphycene



Scheme S5: Synthesis of of 2-phenylthiazolo[4,5-c]tetraphenylporphycene (TAZPo)

To a solution of 9-AmPo (3 mg, $4.0 \cdot 10^{-3}$ mmol) in 0.4 mL of toluene were added 4 mg of the Lawesson's reagent ($1.0 \cdot 10^{-2}$ mmol, 2.5 eq). The mixture was stirred and heated at 120 °C using microwaves for 1.5 h. Then, the solvent was reduced under reduced pressure and the crude was purified by thin layer silica chromatography eluting with toluene to provide a blue-greenish

product (2.5 mg, 81%). ¹H NMR (400 MHz, CDCl₃) δ 9.51 (s, 2H), 9.43 (d, J = 1.0 Hz, 1H), 9.38 (s, 1H), 9.36 (d, J = 1.0 Hz, 1H), 9.35 (s, 1H), 8.21 – 8.17 (m, 4H), 8.09 – 8.04 (m, 2H), 7.94 (m, 3H), 7.76 – 7.69 (m, 8H), 7.68 – 7.64 (m, 4H), 7.62 – 7.57 (m, 4H), 5.99 (s, 1H), 5.32 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) 174.32, 144.81, 144.71, 142.56, 141.83, 140.54, 138.98, 137.42, 137.39, 136.89, 136.06, 135.98, 133.81, 132.14, 131.18, 131.07, 130.50, 129.45, 129.00, 128.77, 128.07, 127.87, 127.68, 127.09, 126.50, 126.24, 124.29, 123.78, 115.04, 114.72, 114.66. MS (API-ES) Calculated for C₅₁H₃₄N₅S [(M+H)⁺]: 748.9, C₁₀₂H₆₇N₁₀S₂ [(2M+H)⁺]: 1496.8, Found: *m/z*=748.2, 1496.4. Retention time: 3.37 min.



Figure S27: ¹H-NMR spectra of TAZPo.



Figure S29: Chromatogram of TAZPo using UV/Vis detection at 400 nm.

2.5 Synthesis of tetraethylenglycol alkyl-thiol stabilized gold nanoclusters

This synthesis was adapted from previously described synthesis of tri- and hexaethylenglycol alky-thiol stabilized gold nanoclusters with some modifications.⁷ Briefly, 30 mg of 23-mercapto-3,6,9,12-tetraoxatricosan-1-ol (90 μ mol) and 3.3 mg of 1-amino-3,6,9,12-tetraoxatricosane-23-thiol (10 μ mol) were mixed with 10 mg of tetracloroauric acid trihydrate (30 μ mol) in 7.0 mL of MeOH. 0.6 mL of a freshly prepared solution of NaBH₄ (1.3 M) was added at an input speed of 4.8 mL/h under stirring. The mixture was stirred at room temperature during 2.5 h and then the solvent was removed under reduced pressure. Next, the remaining crude was dispersed in 15 mL of a 2:1 water:ethanol mixture and purified by dialysis twice (10h, 900 mL). The aqueous solution was basified using a 0.1 M solution of KOH and extracted

several times with $CHCl_3$. The organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. Finally, the brownish solid powder was suspended in 20 mL of absolute EtOH.

2.6 Labelling procedure of gold nanoclusters

100 μ L of 9-ITPPo (100 μ M) in toluene were gently added to 2 mL of AuNCs (2.9 μ M) in ethanol. The mixture was stirred at room temperature for 24 h and several absorption spectra were recorded at different times (Figure S30). After the reaction completion, the solvent removed under reduced pressure. Next, the greenish powder was redispersed in water and purified by successive water dialysis.



Figure S30: Time evolution of the absorption spectra of 9-ITPPo upon binding to AuNCs.

2.7 Labelling procedure of bovine serum albumin

20 μ L of 9-ITPPo (16 mM) were added to a stirred solution of bovine serum albumin (BSA, 5 mg, 0.08 μ mol) in a sodium bicarbonate/sodium carbonate buffer (250 mL, pH 9.4). The reaction vessel was protected from light and stirred for 36 h at room temperature. The bioconjugate was purified using the acetone precipitation method previously reported by Hamblin *et al.*⁸. Briefly, the crude bioconjugate preparations were diluted with 1.5 mL of bicarbonate/carbonate buffer and 20 mL of acetone at 4 °C were slowly added. After 5 h, the green suspension was centrifuged at 4000g and washed several times with acetone until colourless supernatant. Then, the pellet was redissolved in 20 mL of PBS and exhaustively dialyzed against 1 L of PBS for 3 days in order to remove all traces of acetone.

3. Additional results

3.1 TEM examination of AuNCs

For size determination, a single drop (15 μ L) of the EtOH solution of the gold nanoclusters was placed onto a copper grid coated with a carbon film. The grid was left to dry in air at room temperature. TEM analysis was carried out in a JEOL 2010F microscope working at 200 kV. The particle size distribution of the gold nanoclusters was evaluated from several micrographs by means of an automatic image analyser software (Figure S31).



Figure S31: a) Schematic representation of tetraethylenglycol alkyl-thiol stabilized gold nanoclusters (TEG-AuNCs). b) TEG-AuNCs micrography at 120000X. c) TEG-AuNCs micrography at 500000X and d) particle size distribution.

3.2 Time evolution of the absorption spectra of 9-ITPPo upon reaction with *n*-butylamine



Figure S32: Time evolution of the absorption spectra of a mixture of 9-ITPPo (0.015 mM) and n-butylamine (3 mM) in toluene. The blue line corresponds to the absorption spectra at time 0 (previous to the addition of *N*-butylamine), the green line, the absorption of the intermediate (namely, 9-TUPo) and the red line, the absorption spectra of the sample 60 h later.

3.3 ¹H-NMR evolution of 9-ITPPo upon reaction with *n*-butylamine

The evolution of the reaction between 9-ITPPo and *n*-butylamine was characterised by means on ¹H-NMR spectroscopy. ¹H-NMR spectra were recorded at different steps: **A**) ¹H-NMR of 9-ITPPo in CDCl₃ prior to the addition of *n*-butylamine, **B**) 10 minutes after the addition of *n*-butylamine and **C**) 24 h after the addition (Figure S33).

A) ¹H NMR (400 MHz, CDCl₃) δ 9.70 – 9.61 (ABq, 2H, J_{AB} = 11.1 Hz), 9.55 (s, 1H), 9.43 (s, 1H), 9.39 (s, 2H), 9.30 (s, 1H), 8.29 – 8.19 (m, 6H), 8.08 – 8.00 (m, 2H), 7.88 – 7.59 (m, 12H).

B) ¹H NMR (400 MHz, CDCl₃) δ 9.81 (s, 1H), 9.69 (d, *J* = 11 Hz, 1H), 9.61 (d, *J* = 11 Hz, 1H), 9.41 (m, 4H), 8.25 (t, *J* = 7.3 Hz, 4H), 8.06 (d, *J* = 7.5 Hz, 2H), 7.91 (m, 1H), 7.85 – 7.63 (m, 11H).

C) ¹H NMR (400 MHz, CDCl₃) δ 9.63 (d, J = 11.1 Hz, 1H), 9.54 (d, J = 11.1 Hz, 1H), 9.43 (s, 2H), 9.41 (s, 1H), 9.38 (s, 1H), 8.27 (m, 4H), 8.08 (m, 2H), 7.97 - 7.92 (m, 2H), 7.81 - 7.69 (m, 7H), 7.67 - 7.60 (m, 4H), 7.51 (m, 1H).

¹H chemical shifts and integrals of **B**) are consistent with the formation of the 9-thiourea derivative.



Figure S33: ¹H-NMR of a mixture of 9-ITPPo and *n*-butylamine in CDCl₃. Spectra were recorded at different time intervals: A) 1H-NMR of 9-ITPPo in CDCl₃ prior to the addition of *n*-butylamine, B) 10 minutes after the addition of *n*-butylamine and C) 24 h after the addition.

3.3 Absorption spectrum of ATAZPo derivatives



Figure S34: Absorption spectrum of ATAZPo derivatives in acetone

3.3 Phosphorescence decays at 1275 nm for 9-ITPPo and ATAZPo2



Figure S35: Phosphorescence decays at 1275 nm upon excitation at 355 nm of 9-ITPPo and ATAZPo2 in acetone. The decays of an optically matched solution of TPP are shown for comparison.

3.4 Optical properties of 9-benzamido porphycene and 2-phenylthiazolo[4,5-c] 2,7,12,17-tetraphenylporphycene.

Similarly to ATAZPos, both the absorption and emission spectra of TAZPo is red shifted with respect to its corresponding 9-AmPo precursor (Figure S36 and Table S1). The most relevant property of this new porphycene derivative is an unprecedented 2-fold enhancement of its photosensitising properties which makes it an even better candidate for photodynamic therapy.



Figure S36: Absorption and fluorescence spectra of 9-AmPo (blue line) and its TAZPo derivative (red line).

| Compound | λm ^[a] | $\lambda_{F}^{[b]}$ | $arPhi_{F}^{[c]}$ | $	au_{s}^{[d]}$ | $	au_{	ext{T}}^{[e]}$ | $arPhi_{\Delta}^{[\mathrm{f}]}$ |
|----------|-------------------|---------------------|-------------------|-----------------|-----------------------|---------------------------------|
| 9-AmPo | 696 | 708 | 0.05 | 1 | 60 | 0.14 |
| TAZPo | 700 | 720 | 0.03 | <0.2 | 20 | 0.31 |

Table S1. Optical properties of 9-AmPo and its TAZPo derivative in acetone.

[a] Wavelength of the lowest-energy absorption band (nm); [b] Wavelength of fluorescence (nm); [c]
Fluorescence quantum yield; [d] Singlet state lifetime in air (ns); [e] Triplet state lifetime in argon (μs);
[f] Singlet oxygen quantum yield.

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