ELECTRONIC SUPPLEMENTARY INFORMATION

Organometallic Approach to sub-2 nm Thiolate-Protected Au Nanoclusters with Enhanced Catalytic and Therapeutic Properties

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Table of contents

1.	Experimental Section	.2
1	.1. Material and methods	.2
1	.2. Synthesis of pep9	.3
2.	Characterization	.4
2	.1. Colour	.4
2	.2. HAADF-STEM	.4
2	.3. XPS	.7
2	.4. FT-IR	.9
2	.5. NMR	11
3.	Catalysis: reduction of 4-nitrophenol to 4-aminophenol	12
4.	¹ O ₂ generation	13
5.	Cytotoxic study	15
6.	Supplementary references	16

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1. Experimental Section

1.1. Material and methods

The compound $[AuC_6F_5(tht)]$ was synthetized according to published procedure.¹ L-Cysteine (Cys) was purchased from Acros Organics, and L-Gluthathione reduced (GSH), triisopropilsilane (TIPS) and 1,3-diphenylisobenzofuran (DPBF) were purchased from Merk and used without further purification. Experiments have been conducted under air. All the synthetic approaches and properties measured described here have been repeated at least twice in order to ensure the reproducibility of the preparation methodology.

Temperature changes were monitored using a FLIR E6-XT thermographic camera.

UV-Vis absorption spectra of AuNCs samples (Cys (1), GSH (2) and pep9 (3)) and absorption spectra related to the reduction reactions of 4-nitrophenol to 4-aminophenol were recorded on Agilent 8453 Diode-array spectrophotometer. Spectra corresponding to the ${}^{1}O_{2}$ generation study were measured on a Perkin Elmer Lambda 265 spectrophotometer.

Samples for HAADF-STEM were directly drop-casted from reaction media (2–3 drops) over carboncoated Cu grids. HAADF-STEM images were obtained on a Titan instrument with Cs corrected probe (ThermoFisher Scientific, Waltham, MA, USA) at a working voltage of 300 kV, together with a HAADF detector (Fischione, Export, PA, USA).

For XPS samples, the AuNCs were synthesized following the synthetic procedure and were left stirring for 1 h. The solvent was then partially evaporated using a vacuum pump and then it was frozen using liquid nitrogen and left to lyophilize for two days, obtaining a brown solid. XPS experiments were performed in a Kratos AXIS Supra spectrometer, using a monochromatic Al K α 120W source operating at 15 kV or 8 mA. Wide scans were acquired at analyzer pass energy of 160 eV, whereas high-resolution narrow scans were performed at constant pass energy of 20 eV and steps of 0.1 eV. Basal pressure in the analysis chamber was less than 10⁻⁹ Torr. The spectra were obtained at room temperature. The binding energy (BE) scale was internally referenced to the C 1s peak (C-C = 284.9 eV).

¹H NMR spectra were measured with a 300 or 400 MHz spectrometer in D₂O. Chemical shifts are quoted relative to CFCl₃ (¹⁹F, external) and SiMe₄ (¹H, external).

Infrared spectra were recorded in the 4000-500 cm⁻¹ range using a PerkinElmer FT-IR Spectrum Two with an ATR accessory.

1.2. Synthesis of pep9.

The synthesis was performed automatically with Rink Amide MBHA resin (0.05 mmol) in a microwave assisted automated synthesizer. The fluorenylmethyloxycarbonyl (Fmoc) amino acids (5.0 equiv) were automatically coupled using oxyma pure/DIC (*N*,*N'*-diisopropylcarbodiimide). 20% (v/v) solution of piperidine in dimethylformamide (DMF) was used for Fmoc deprotection. Once synthesized, **pep9** was detached from the resin and all acid sensitive sidechain protecting groups were simultaneously removed using a 3 mL of a cleavage cocktail composed of [TFA 90%, thioanisole 5%, DODT (2,2 -(Ethylenedioxy)diethanethiol) 3%, and anisole 2%] under vigorous stirring for 2h at room temperature, followed by precipitation with cold diethyl ether. The solid precipitate was centrifuged the pallet was collected and the diethyl ether supernatant was discarded. The solid was dried under reduced pressure and the crude peptide was purified by semi-preparative HPLC on a Phenomenex Luna C18(2) column (10 μ , 250 mm × 21.2 mm) and a dual absorbance detector, with a flow rate of 20 mL/min and a linear gradient of H₂O containing 0.1%TFA/Acetonitrile 95/5 to 83/17 over 15 minutes. The retention time on the preparative scale HPLC was of 10.7 min.







Figure S2. HRMS MALDI calcd. for C₃₈H₆₄N₁₃O₁₂S [M+H]⁺: 926.4513 found: 926.4507.

2. Characterization

2.1. Colour



Figure S3. Colour of the reaction mixture: A) $Au(C_6F_5)(tht) + TIPS$ previous thiol addition B) AuNCs-Cys (1), C) AuNCs-GSH (2), D) AuNCs-pep9 (3).

The addition of TIPS to the Au(C_6F_5)(tht) solution in THF/H₂O results in an initial colour change from colourless to orange-brown, indicating the formation of AuNCs. The C_6F_5 ligand helps stabilize of these species, preventing aggregation during reduction. After one minute, the corresponding thiol is added, leading to a second colour change. Cys and GSH produce a darker brown solution, with GSH being slightly darker, while for pep9 results in a light brown colour. This second colour change may also suggest a greater reduction of the remaining Au(I), as thiolates can be oxidized, forming disulfide bonds.

2.2. HAADF-STEM



Figure S4. HAADF-STEM images of AuNCs-Cys (1).



Figure S5. HAADF-STEM images of AuNCs-GSH (2).



Figure S6. HAADF-STEM images of AuNCs-pep9 (3).





Figure S8. Diameter distribution for AuNCs-GSH (2).



Figure S9. Diameter distribution for AuNCs-pep9 (3).

2.3. XPS



Binding Energy (eV) Figure S10. Full XPS spectrum for AuNCs-SR (1, 2, 3).

Table S1. % atomic composition based on the XPS data for AuNCs.

AuNCs-	C 1s	O 1s	N 1s	Au 4f	S 2p	F 1s	Si 2s*
Cys (1)	55.54	15.26	6.23	3.70	6.15	13.12	-
GHS (2)	66.04	20.95	8.38	2.35	2.28	7.30	3.08
рер9 (3)	68.90	15.94	13.93	0.48	0.75	5.22	1.48

* Impurities from excess of reductant



Figure S11. High resolution XPS spectra for AuNCs-(1), (2), (3) for C 1s, O 1s, N 1s, S 2p and Au 4f.

The deconvolution of the C 1s line produces five chemical states: the first component (284.8 eV) can be attributed to C-C bonds, the second and third components (285.7-286.8 eV) to C-S and C-N bonds, while the fourth and fifth (287.5-289.2) can be associated to C=O and COOH carbon state. The N 1s signal is deconvoluted in two peaks, one at 399.8-400.2 eV attributed to the

chemical state of nitrogen in NH_2 and the other at 401.5-402.1 eV is compatible with nitrogen in the NH_3^+ state of the amino group.

The peak deconvolution in S2p spectra reveals the presence of different S chemical states: S1 component at 163.3-163.6 can be attributed to the chemisorbed sulfur species and the S2 one (164.5-164.8 eV) to a weakly bound to gold or unbound sulfur species. This interpretation has been previously proposed for the thiol/ gold system.² S3 and S4 in AuNCs **1** could be compatible with an oxidized species of S, the disulfide species.

The two oxidation states of gold observed in the AuNCs are absent in the precursor $[Au(C_6F_5)(tht)]$, and no signals match the Au(I) with this organometallic compound, indicating complete conversion and the presence of thiolate ligands on the AuNC surface.

2.4. FT-IR



Figure S12. FT-IR spectra of AuNCs-Cys (1), Cys is included as a comparison.



Figure S13. FT-IR spectra of AuNCs-GSH (2), GSH is included as a comparison.



Figure S14. FT-IR spectra of AuNCs-pep9 (3), pep9 is included as a comparison.



Figure S16. ¹⁹F NMR spectra of AuNCs-pep9 (3).

¹⁹F NMR of AuNCs-pep9(**3**) shows no fluorine signal corresponding to C_6F_5 , indicating the absence of gold precursor or other impurities. The signal observed at 75.6 ppm corresponds to the trifluoroacetic acid (TFA) employed during the peptide synthesis procedure.

3. Catalysis: reduction of 4-nitrophenol to 4-aminophenol



Scheme S1. Reduction reaction of 4-NP to 4-AP.



Figure S17. UV/Vis monitored reduction of 4-NP to 4-AP with AuNCs-GSH(2) as catalyst.



Figure S18. UV/Vis monitored reduction of 4-NP to 4-AP with AuNCs-pep9(3) as catalyst.



4. ¹O₂ generation



Figure S20. Absorbance decay spectra of DPBF measured at 410 nm for in the presence of AuNCs-pep9(3).



Figure S21. Appearance of the final solutions of the ¹O₂ generation experiment: (A) Control experiment. (B) AuNCs-Cys (1), (C) AuNCs-GSH (2) and (D) AuNCs-pep9 (3).

The decomposition of the catalysts AuNCs-Cys(1) and AuNCs-GSH(2) was observed during the experiment, which gave rise to aggregates and insoluble particles. This change in behaviour regarding ${}^{1}O_{2}$ generation can be attributed to the destabilization of the catalysts: for AuNCs-Cys(1), this occurred throughout the reaction, while for AuNCs-GSH(2), it happened primarily during the initial stages. In contrast, AuNCs-pep9(3) exhibited the same initial appearance, a transparent brownish solution.



Figure S22. Study of the catalase-like activity and laser effect with AuNCs-pep9(3) in the ¹O₂ generation reaction.

The same behaviour was observed in the experiment conducted with and without nanoclusters in the absence of H_2O_2 : a null decay was observed, indicating that no 1O_2 was generated. Therefore, in the presence of hydrogen peroxide, the nanoclusters generated singlet oxygen from the in situ

generated oxygen, proving their catalase-like activity. This study also indicated that the laser irradiation did not lead to the decomposition of the DPBF compound.

The effect of laser irradiation on the generation of singlet oxygen was also investigated. The difference between the measurements conducted in the absence of light and under laser irradiation is attributed to the photocatalytic effect of singlet oxygen generation from *in situ* generated oxygen. The observed decrease in absorbance in the experiment performed with H_2O_2 and AuNCs **3** in the absence of light could be explained by two mechanisms: the reaction of DPBF with triplet oxygen or the generation of singlet oxygen from the irradiation of the spectrophotometer used for absorbance measurements.





5. Cytotoxic study



Figure S23. Cytotoxicity curves for treated cells in the absence or presence of 855 nm light for 1h or 2h: (A) AuNCs-Cys(1), HDF. (B) AuNCs-pep9(3), HeLa. (C) AuNCs-Cys(1), HeLa.

6. Supplementary references

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