Electronic Supplementary Material (ESI) for Nanoscale

Electronic Supporting Information

Synthesis, Purification and Mass Spectrometric Characterisation of a Fluorescent Au_9@BSA Nanocluster and its Enzymatic Digestion by Trypsin

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Experimental details (Tables S1-S4) and Figures S1 - S6

Table S1 Instrumental parameters of the HPLC-UV/Vis system

Instrument	Agilent 1100 Series Liquid Chromatograph
Column	Superdex-200 10/300 GL, 13 µm particle size, 10 × 300 mm
Flow rate	0.6 mL min^{-1} (room temperature)
Injection volume	30 µL
Pressure	12 bar
Mobile phase	Ammonium acetate 50 mM (pH 6.8)
Detection wavelength	280 nm

Table S2 Instrumental parameters of the HPLC-ICP-MS system

Instrument	Thermo Fisher Scientific X-Series^{II} ICP-MS
RF power	1400 W
Auxiliary gas flow	0.80 L min^{-1}
Coolant gas flow	14.5 L min^{-1}
Nebulizer gas flow	0.90 L min^{-1}
Sampler cone	Nickel
Skimmer cone	Nickel
Dwell time	100 ms
Spray chamber	Conical spray chamber
Nebulizer	Concentric nebulizer, Meinhard type
Isotope monitored	$^{197}Au^{+}$
Column	Superdex-200 10/300 GL, 13 µm particle size, 10 × 300 mm
Flow rate	0.6 mL min^{-1} (room temperature)
Injection volume	50 µL

Table S3 Operational parameters of the MALDI-ToF-MS system

Instrument	Voyager-DE STR (Applied Biosystems)
Mode of operation	Linear
Extraction mode	Delayed
Polarity	Positive
N_2 laser	337 nm, 3 ns pulse width, 1600 mJ
Acquisition control	Manual
Accelerating voltage	25 kV
Grid voltage	92 %
Delay time	400 ns
Number of laser shots	50 / spectrum
Matrix	2-Nitrophloroglucinol (NPG)

Sample preparation for MALDI-MS measurements was performed on a stainless steel hydrophobic target (Voyager 96-2 Sample Plate P/N V700813) using the dried-droplet technique. For that, an aliquot (5 µL) of the sample solution and an equal aliquot of the matrix solution were mixed on the target in the given order and dried at room temperature. The matrix solution for MALDI-MS was prepared by dissolving 5 mg of 2-nitrophloroglucinol (NPG) in 1 mL of 50% acetonitrile and the proper amount of diammonium hydrogencitrate. In order to confirm that the presence of acetonitrile did not influence the stability of the studied nanoclusters, the MALDI sample preparation was carried out with methanol as organic modifier. Similar results as shown in figure 2b were obtained under these conditions as well.

Table S4 Operational parameters of the ESI-MS system

Instrument	ESI-Q-TOF QStar XL model (Applied Biosystems)
Ion spray voltage	4 kV
Nebulizer gas	Nitrogen
Calibration standard solution	Reserpine solution

The ESI-Q-TOF instrument used for this study was a QStar XL model (Applied Biosystems) equipped with the ion spray source (voltage 4 kV) and using nitrogen as nebulizer gas. Mass calibration was performed daily using a standard solution of reserpine (1 pmol/μL). All samples were reconstituted in 70% acetonitrile / 0.1% formic acid to an appropriate concentration and injected at a flow of 5 μL min^{-1}. The scanned mass range was chosen from m/z 500 to 4000. A Bayesian deconvolution algorithm available in the Analyst software (Applied Biosystems) was applied.

Further experimental details

TEM: Microscopic images were obtained using JEOL JEM-2100F (Jeol Ltd. Tokyo, Japan).

XPS: XPS was performed on a SPECS spectrometer with a Phoibos 100 hemispherical analyser at a pressure below 10^{-7} Pa. The X-ray source was Mg K_{α} (1253.6 eV) operated at 11.81 kV and 150 W. The photo-excited electrons were analysed in constant pass energy mode, using pass energy of 20 eV, energy step of 0.1 eV and dwell time of 0.5 s. The spectra are the result of 60 scans.

FTIR: FT-IR measurements were carried out at room temperature on a Varian FT-IR spectrometer (Varian 620-IR and Varian 670-IR). All the spectra were taken using 16 scans in the range of 600-4000 cm^{-1}.

Figure S1 Size distribution (a) of the raw product and (b) after fraction collection.

(a)

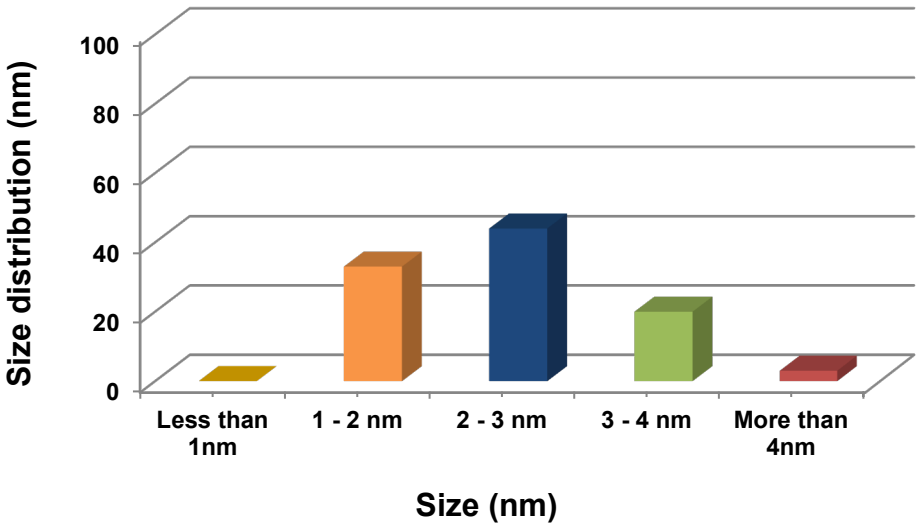


Figure S1 Size distribution (a) of the raw product and (b) after fraction collection.

(b)

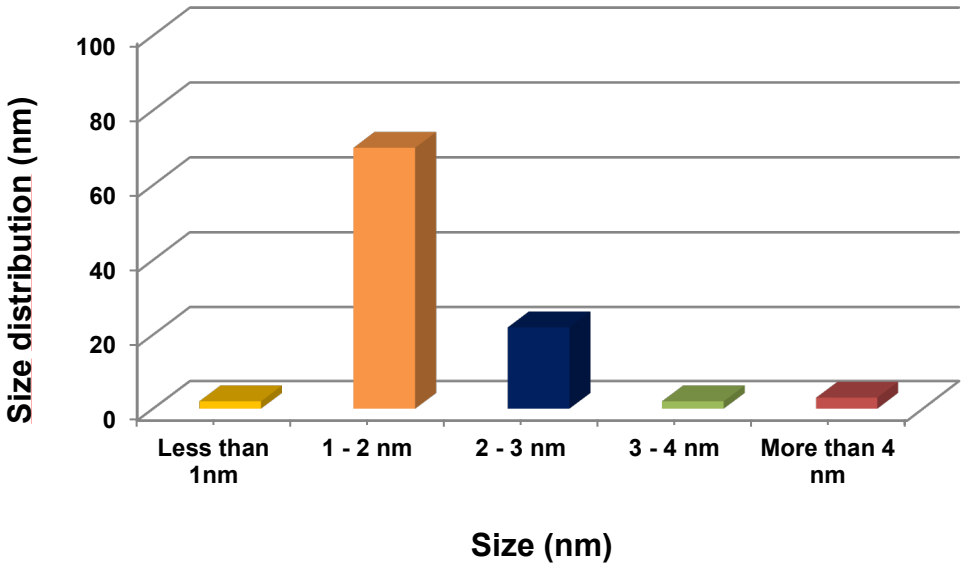


Figure S2 FTIR spectra of BSA and the Au_9@BSA nanocluster

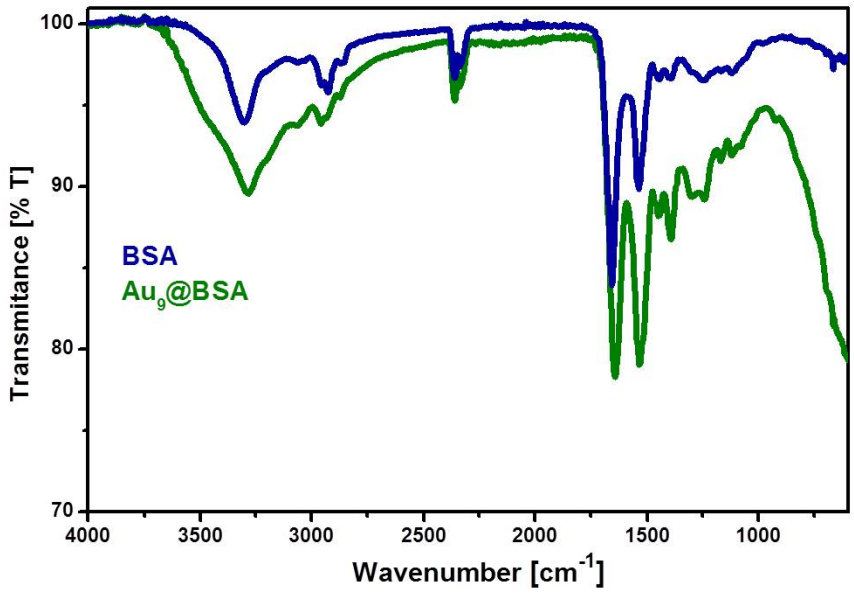


Figure S3 XPS spectrum of the Au_9@BSA nanocluster

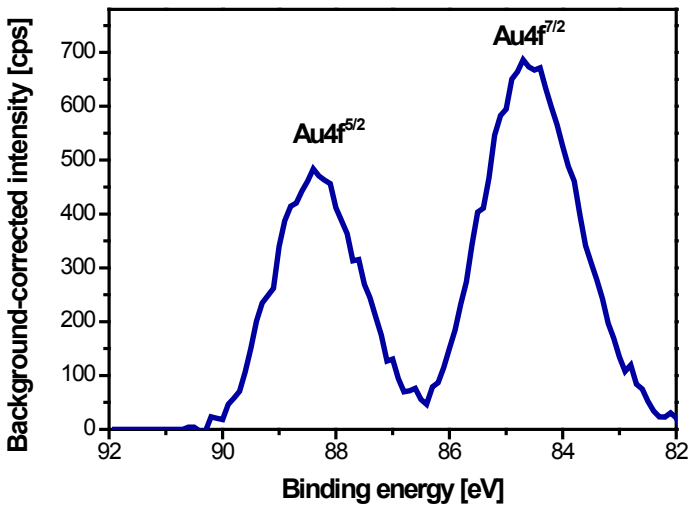
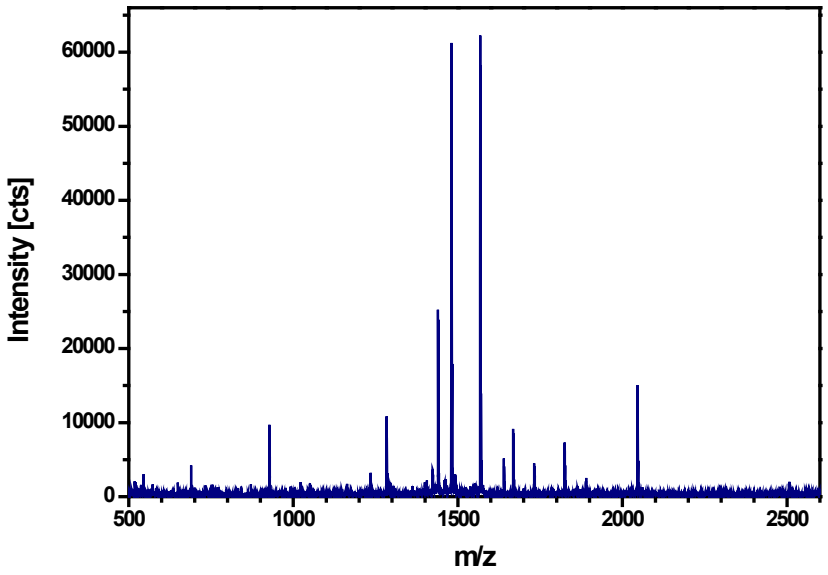


Figure S4 Mass spectra of the resulting peptides after tryptic digestion (a) of pure BSA and (b) of the Au_9@BSA nanocluster.

(a)



(b)

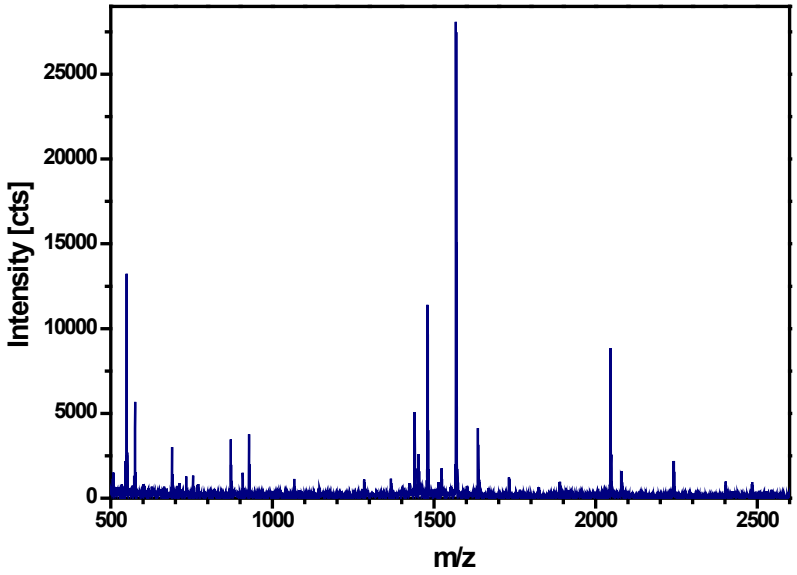


Figure S5 Size-exclusion chromatograms of Au_9@BSA and Au_9@$BSA_{trypsinated}$.

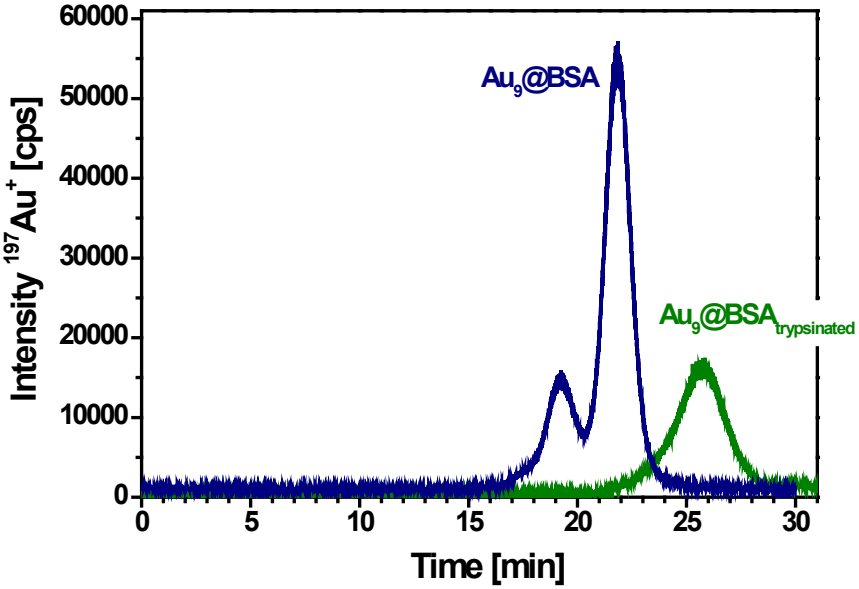
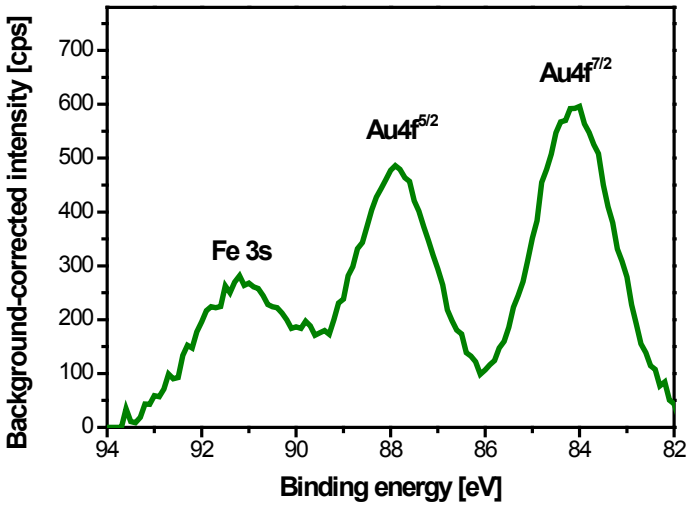


Figure S6 XPS spectrum of the Au_9@BSA after trypsination



Acknowledgements

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