

Augmented interaction of multivalent arginine coated gold nanoclusters with lipid membranes and cells

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1. Synthesis of Au NCs

Au NCs were synthesized following the protocol described elsewhere[1].

Briefly for the SG/SG-3Arg (25/75), 12 mg of SG and 144.1 mg of SG-1Arg were dissolved in 3 mL of water, followed by the addition of 5 mL of H₂AuCl₄·3H₂O (20 mM) and 95 mL of distilled water. Therefore, the molar ratio Au:SG/SG-XArg was 1:1.5. The solution was stirred for 30 min and the pH maintained at 9. Then, the pH was decreased to 2 to stop the reduction process. To complete the reaction, the solution was stirred 4 more hours.

Afterwards, the solution was rotary evaporated, re-solubilized in 10 mL of water and ~12 mL of isopropanol (IPA) was added to precipitate the Au NCs. The Au NCs were collected by centrifugation and the supernatant was centrifuged twice to recover more Au NCs. The Au NCs were re-solubilized in water and the pH was adjusted to 7, before lyophilization.

The same procedure was applied to synthesize the others Au NCs by changing the ratio of ligands.

2. Au NCs characterization

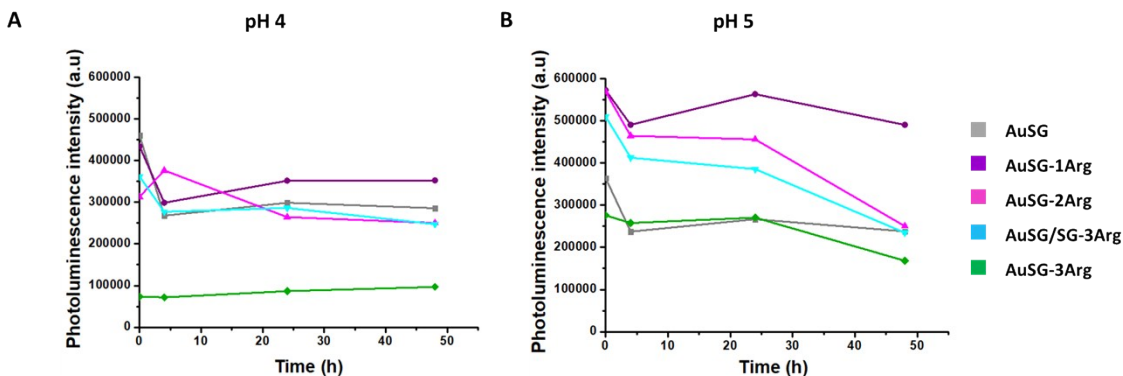


Figure S1. Evolution of the PL intensity ($\lambda_{max.}$) of the five different Au NCs diluted in PBS solution (0.04 mgAu/mL) as a function of the storage time (freshly prepared, 4, 24, 48 hours) at (A) pH 4 and (B) pH 5.

3. Au NCs' interaction with lipid bilayers

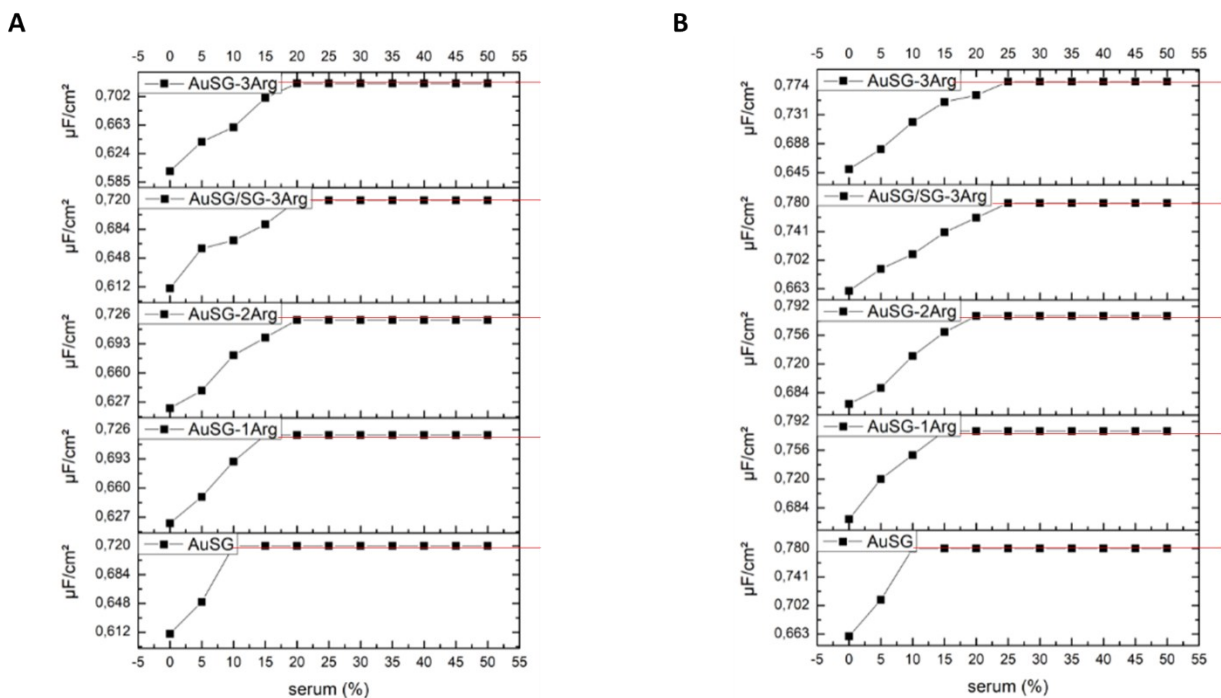


Figure S2. Capacitance measurements C_s of the five types of Au NCs as a function of the serum concentration with a (A) 98% DOPC: 2% DOPS and (B) 93% DOPC : 7% DOPS lipid membrane. The red line corresponds to the initial specific capacitance, *i.e.* the specific capacitance of a lipid bilayer without NCs.

4. *In vitro* measurements

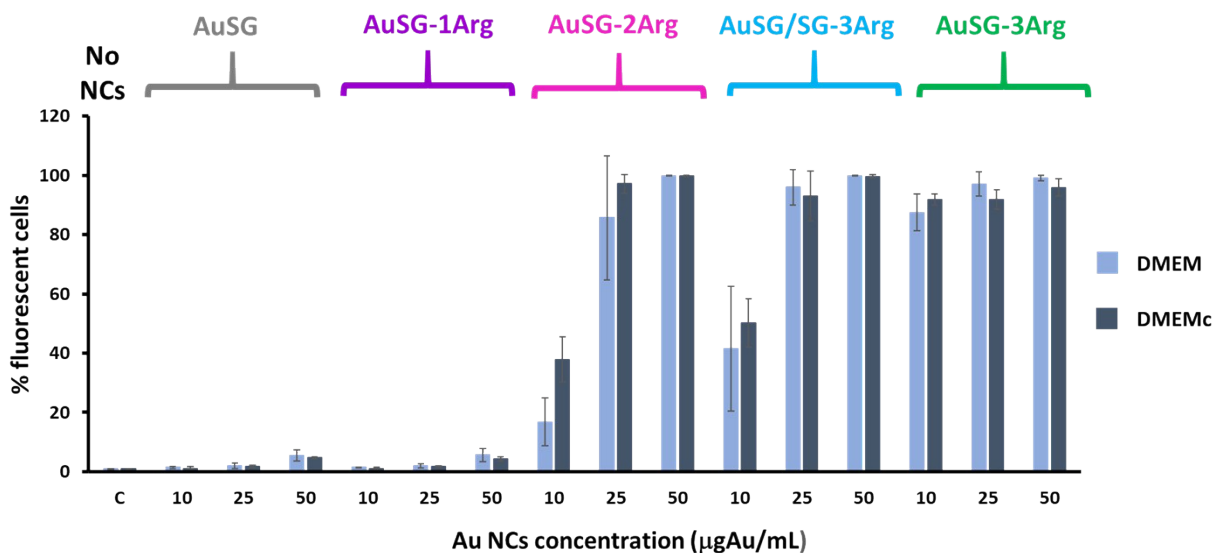


Figure S3. Fluorescence labelling of COLO 829 cells in suspension by the different Au NCs is Arginine and dose dependent. Flow cytometry measurements were performed with increasing concentrations of Au NCs (10, 25 and 50 µgAu/mL) in DMEM and DMEMc at 37°C for 30 min. Results are expressed as mean ± standard deviation (n = 3, number of cells per gate = 10 000 cells).

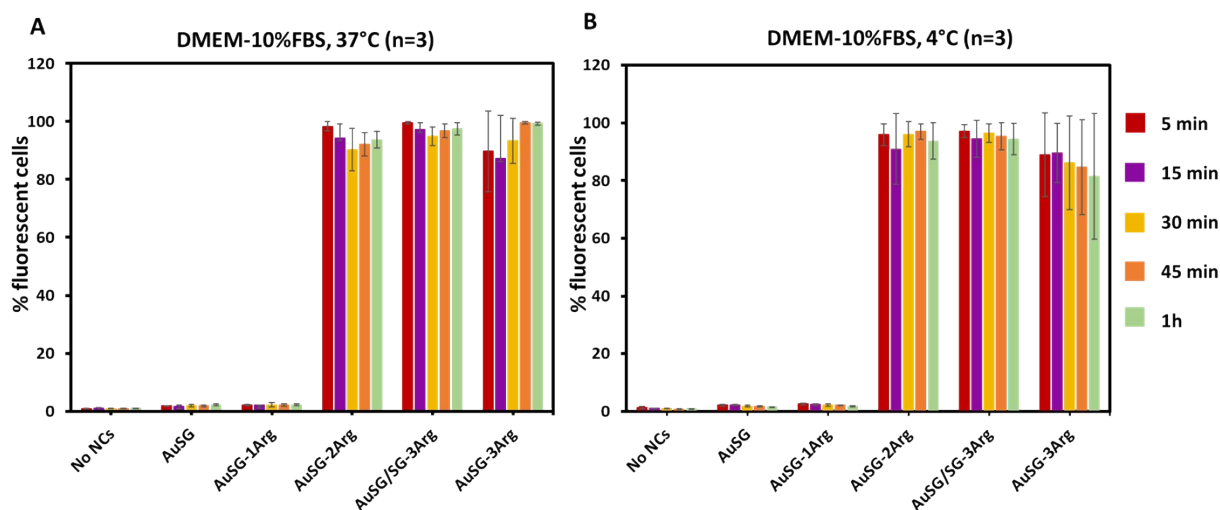


Figure S4. Flow cytometry measurements of COLO 828 cells in suspension with the five different Au NCs. Cells were incubated in DMEMc at 25 µg Au/mL for 5, 15, 30, 45 and 60 min to evaluate the kinetics of

particles interactions with the cells at (A) 37°C or (B) 4°C. Results are expressed as mean \pm standard deviation ($n = 3$, number of cells per gate = 10 000 cells).

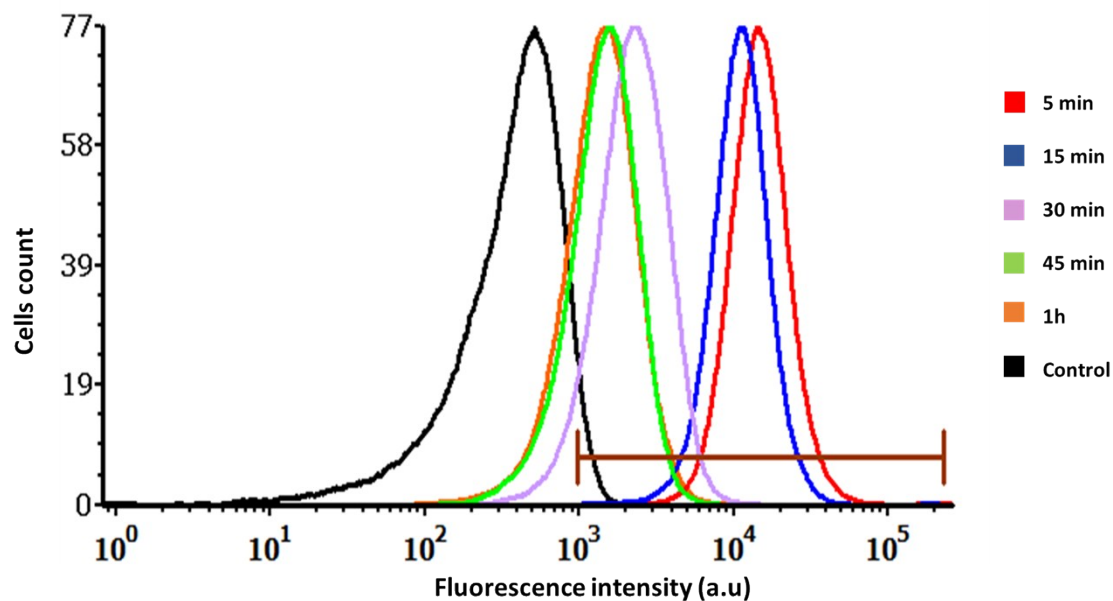


Figure S5. Representation of the variation of mean fluorescence intensity obtained by flow cytometry experiments of COLO 829 cells in suspension with the AuSG-2Arg. Experiments were performed at 37°C with 25 $\mu\text{gAu/mL}$ in DMEM and various incubation time (5, 15, 30, 45 or 60 min). The brown marker shows the percentage of positive cells regarding to the control experiments fixed at 1% (cells incubated without Au NCs).

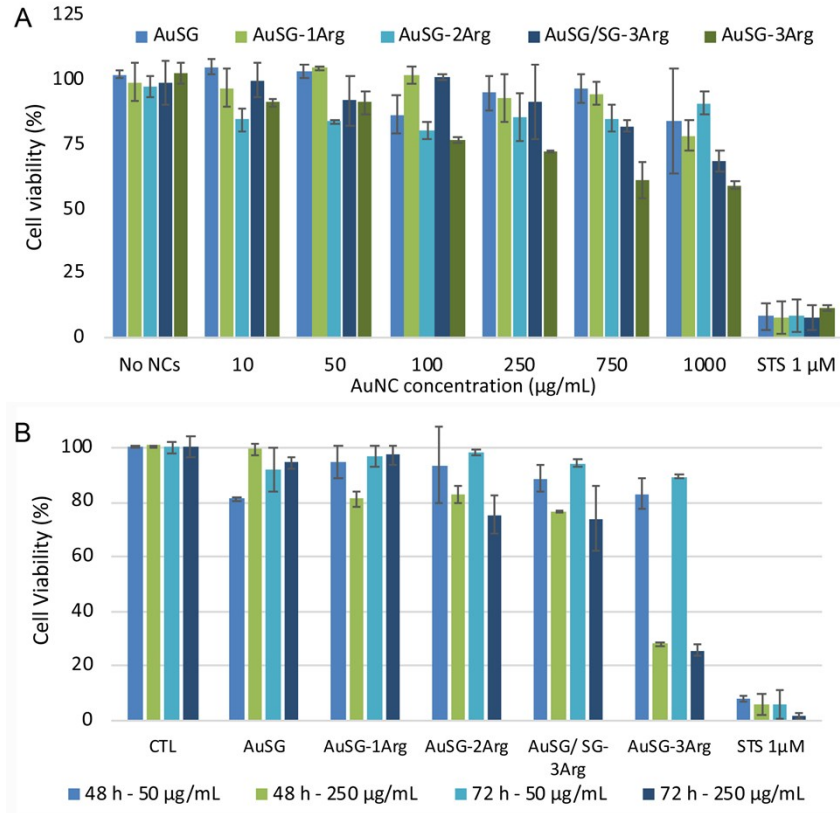


Figure S6. Evaluation of the cell viability in presence of increasing amounts of Au NCs. Human healthy MRC5 fibroblast cells were incubated with the five Au NCs either for 24 h (A) at 50, 100, 250, 500, 750 to 1000 µgAu/mL for 24 h in DMEMc, or for 48 and 72 h (B) at 50 and 250 µgAu/mL. Staurosporine was used as a cell death inducer. Cell viability was measured using a MTS assay. Results are expressed as mean \pm standard error (n = 3).

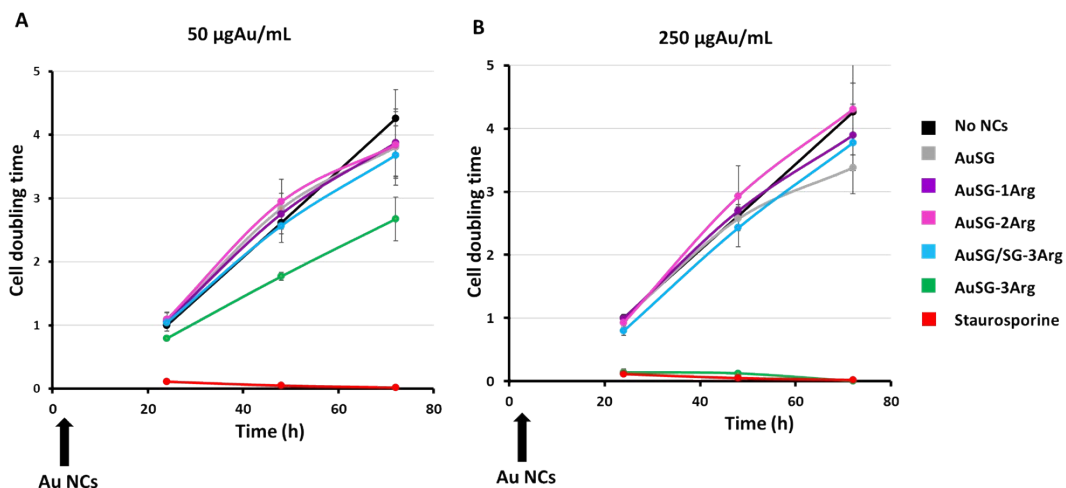


Figure S7. Evaluation of the cell viability and growth rate as a function of time. COLO 829 cells were incubated with the five Au NCs at (A) 50 and (B) 250 $\mu\text{gAu/mL}$ for 24 h, 48 h or 72 h in DMEMc. Staurosporine was used as a positive control of cell death. Cell viability was measured using a MTS assay. Results are expressed as mean \pm standard error ($n = 3$).

REFERENCES

1. Porret E, Jourdan M, Gennaro B *et al.* Investigation of the Spatial Conformation of Charged Ligands on the Optical Properties of Gold Nanoclusters *J. Phys.Chem. C* (2019) 123(43), 26705-26717.