Supporting Information: Instability of Cationic Gold Nanoparticle-Bioconjugates: The Role of Citrate Ions

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S1: TEM images and size distributions of different mean sizes of AuNPs: A) 6 nm, B) 8 nm and C) 16 nm. Scale bars are 100 nm.

S2: DLS measurements corresponding to different sizes of AuNPs and after the conjugation of AuNPs to peptidic biomolecules CIPGNVG-PEG_a **1**, CIPGNVG-PEG_b **2**, Ac-CIPGNVG-PEG_a **3** and Ac-CIPGNVG-PEG_b **4**.

Sample	Hydrodynamic Diameter nm (Pdl)
AuNP (6 nm)	9.7 (0.19)
AuNP (8 nm)	15.4 (0.39)
AuNP (16 nm)	23.0 (0.24)
AuNP (8 nm)–CIPGNVG-PEG _a	24.5 (0.36)
AuNP (6 nm)–CIPGNVG-PEG _b	50.9 (0.29)
AuNP (8 nm)–CIPGNVG-PEG _b	110.9 (0.14)
AuNP (16 nm)–CIPGNVG-PEG _b	398.6 (0.19)
AuNP (8 nm)–Ac-CIPGNVG-PEG _a	18.4 (0.35)
AuNP (8 nm)–Ac-CIPGNVG-PEG _b	87.8 (0.18)

S3: Z-Potential measurements corresponding to different sizes of AuNPs and after the conjugation of AuNPs to peptidic biomolecules CIPGNVG-PEG_a **1**, CIPGNVG-PEG_b **2**, Ac-CIPGNVG-PEG_a **3** and Ac-CIPGNVG-PEG_b **4**.

Sample	Z-potential mV (std. Dev. In mV)
AuNP (6 nm)	-33.9 (8.8)
AuNP (8 nm)	-43.0 (13.9)
AuNP (16 nm)	-43.2 (10.9)
AuNP (8 nm)–CIPGNVG-PEG _a	-54.4 (9.5)
AuNP (6 nm)–CIPGNVG-PEG _b	4.7 (5.0)
AuNP (8 nm)–CIPGNVG-PEG _b	11.8 (5.2)
AuNP (16 nm)–CIPGNVG-PEG _b	22.3 (5.9)
AuNP (8 nm)–Ac-CIPGNVG-PEG _a	-25.9 (8.4)
AuNP (8 nm)–Ac-CIPGNVG-PEG _b	-23.2 (7.1)

S4: UV-Vis absorption spectra of CIPGNVG-PEG_a–AuNP after the addition of an amount of ethanolamine (a, b, c), and after addition of ethanolamine followed by adjustment of the pH to 7.5 (d). Quantities refer to concentrations in the final solution.

S5: UV-Vis absorption spectra of the conjugation of peptidic biomolecules Ac-CIPGNVG-PEG_a **3** and Ac-CIPGNVG-PEG_b **4** to AuNPs of 8 nm after different reaction times.

S6: ^1H NMR spectrum in D_2O of the solution species present after exhaustive dialysis of CIPGNVG-PEG_b-AuNP (8 nm) conjugates.

Since the number of gold atoms in each NP is much larger than the number of biomolecules forming the SAM, the relaxation times are long and the NMR signals from the molecules are too broad to make meaningful assignments (Kalsin et al. *J. Am. Chem. Soc.* **2007**, 129, 6664). Despite this, the doublet signal at approximately 2.6 ppm could be assigned to citrate molecules which are close to a Au-surface, as this peak is in the region of the quartet at 2.5 ppm characteristic of free citrate molecules in a D_2O solution.

S7: UV-Vis absorption spectra of 8 nm AuNP (a), after the addition of AuNP to NaCl 0.05 M (b) and after the addition of AuNP to NaCl 0.05 M and CIPGNVG-PEG_a **1** 0.2 mM after 10 min. (c) and 1 hr

(d). Measurements taken 5 min. after the mixture of solutions. Quantities refer to concentrations in the final solution.

The immediate conjugation of thiolated molecules under the experimental conditions described in our work was demonstrated by a simple experiment. Citrate stabilized 8 nm AuNPs (5 ml) were added to: i) a 100 μ l solution 2.6 M of NaCl in H₂O, ii) a 100 μ l solution 2.6 M of NaCl and 10 mM of CIPGNVG-PEG_a **1**. An immediate (< 30 seconds) change of colour from red to blue could be observed in the first case where only NaCl was present. Under these circumstances, the high ionic strength of the salt screens the charges at the surface of the citrate stabilized AuNPs leading to rapid aggregation and precipitation. However when the addition was performed in the presence of an amount of the biomolecule **1**, it could not be observed any apparent aggregation after 10 min. (image inset). The resulting mixtures were also analyzed by UV-Vis spectroscopy and compared to the initial solution of AuNP, which further confirms the aggregation in the absence of the biomolecule **1**. By contrast, in the presence of biomolecule **1** the red-shift of the SPR band (from 517.5 to 520.5 nm) indicates the formation of a dense dielectric layer onto the AuNP surface and a satisfactory conjugation of this molecule to AuNPs after just few minutes. The SPR peak of this sample evolved from 520.5 to 521.5 nm after 1 hr due to the formation of a more ordered and dense packing of the molecules. However, after 1 day it could not be observed any further evolution in the UV-Vis absorption spectrum, which gives an idea of the completeness of the surface coverage at short times. In conclusion, the stability of the AuNP conjugates provides an interesting tool to give an idea of the rate of surfactants exchange, since the AuNPs are coated with thiolated molecules which present electrostatic repulsion faster than aggregation occurs.