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Gold Nanoparticles Grafted by Reduced Glutathione With Thiol Function Preservation

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ABSTRACT

Gold nanoparticles (AuNP) are one of the most widely applied nanomaterials for biomedical purposes due to their ease of preparation, well-defined structure and simple surface functionalization. Herein, a method to anchor reduced glutathione (GSH, *i.e.* an important antioxidant tripeptide in all living organisms) onto AuNP surface with preservation of the GSH thiol function was reported. Using AuNP capped with dihydrolipoic acid (AuNP@DHLA) as starting material, GSH was anchored by an *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS)-mediated coupling between the carboxylate group of DHLA and the amine function of GSH to generate AuNP@DHLA-$(GSH)_n$. Yields of 7.5 ± 1.2 μM of thiol per nM of AuNP were obtained. These particles showed a markedly enhanced reductive capacity compared to AuNP@DHLA and free GSH evaluated by two assays commonly used in evaluation of antioxidant activities, *i.e.* 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and Ferric ion Reducing Antioxidant Power assays, and by direct potentiometry.

Among the different developed nanomaterials, gold nanoparticles (AuNP) are currently major tools developed for a wide range of applications such as therapy, diagnostic and biosensing [1–2]. Thus, some AuNP used as drug delivery platforms are evaluated for tumors treatment and others as sensors to detect various chemical or biological species [3]. Indeed, AuNP are characterized by interesting properties such as easy synthesis allowing the production of well-defined monodispersed particles [4] with high drug grafting capacities [1]. Various active compounds (such as synthetic drugs and endogenous compounds like nucleic acids, antibodies, peptides, proteins and enzymes) were already linked to AuNP [5]. Indeed, this inorganic support offers many advantages such as a high ratio of molecule binding and an increase of active pharmaceutical ingredient stability.

In this context, functionalization of AuNP is usually performed using compounds bearing a thiol function to provide particles being efficiently stabilized through the strong Au—S bond [4]. In cells, the release of the active component is triggered by intracellular reduced glutathione (GSH) through ligand exchange [1,6]. This compound is, indeed, the most abundant intracellular thiol (1 to 11 mM *versus* 0.002 mM in human plasma). In this medium, AuNP corona is composed of decreasing amounts of active pharmaceutical ingredient, in parallel to increasing amounts of GSH bound to the gold core. As previously shown, this can induce cytosolic as well as mitochondrial GSH depletion leading to further cell apoptosis [6–8]. Due to the high affinity of gold towards sulfur, binding thiol-containing peptides or proteins to AuNP with preservation of their thiol function still remains a challenge. Indeed, published studies always exploit the Au—S bond formation, which, in turn, may cancel the pharmacological effect of the compound. For instance, GSH has been directly linked to the core of AuNP [9–10] and even to gold or silver nanoclusters [11–13] for either further functionalization, or for reaction with toxic compounds annihilating its reductive properties.

In the present paper, GSH was anchored on AuNP with preservation of the thiol function and thus of its associated biochemical activity. The development of AuNP capped by dihydrolipoic acid (AuNP@DHLA), used as the starting platforms for the anchorage of GSH, has previously been optimized in order to limit the residual reactivity of the gold core [14]. These AuNP@DHLA are biocompatible [6] and resistant to ligand exchange even when high GSH concentrations corresponding to intracellular levels were used [6,8]. Herein, a physicochemical characterization (including their redox properties) of AuNP@DHLA grafted with GSH (AuNP@DHLA-$(GSH)_n$) is described.

Reduced glutathione was anchored at the surface of AuNP@DHLA through a classical coupling reaction using EDC and NHS [5], in order to create an amide bond between the carboxylate function of DHLA and the primary amine group of GSH. Due to the homogenous and dense DHLA capping of the particles (each particle bears *circa* 282 DHLA moieties) [6,14], this process allowed the preservation of GSH thiol function. The characterization of AuNP@DHLA-$(GSH)_n$ showed no significant differences compared to the native AuNP@DHLA, in

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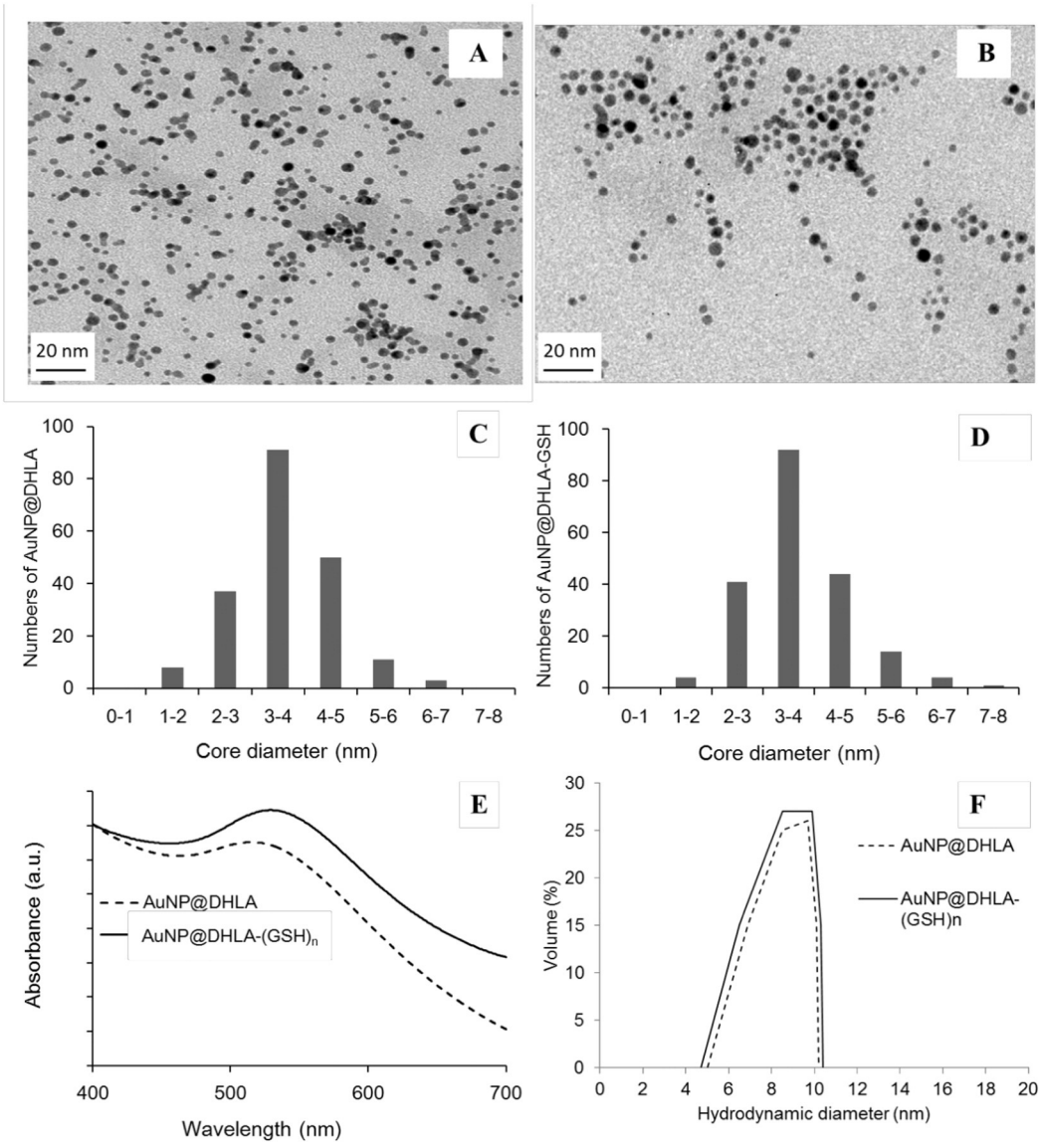

Fig. 1. Physicochemical characterization of AuNP@DHLA and AuNP@DHLA-$(GSH)_n$ ((A) and (B), respectively) using TEM, the Dc extracted from TEM pictures (C) and (D) (n = 200), their visible absorbance spectra (E), and Dh obtained by dynamic light scattering (F).

Table 1
Nanoparticle physicochemical characterization and results of the reductive capacity of AuNP@DHLA-$(GSH)_n$ particles evaluated by ABTS and FRAP assays.

	Parameter	AuNP@DHLA-$(GSH)_n$	AuNP@DHLA	GSH
Physicochemical characterization	Dc[a] (nm)	4.0 ± 0.9	3.5 ± 1.3	
	Dh[b] (nm)	7.6 ± 0.7	7.1 ± 1.4	
	ζ[c] (mV)	−23 ± 3	−46 ± 4	
	λ_{max}[d] (nm)	523 ± 2	517 ± 2	
	Gold content (μM)	105 ± 4	116 ± 7	
	ε ($\times 10^7\ M^{-1}\cdot cm^{-1}$)	0.75 ± 0.03	0.50 ± 0.02	
	Thiol content[e] (μM/nM of AuNP)	7.5 ± 1.2	<LOQ	
Redox tests	EC50[f] ($ABTS^{\bullet+}$) (nM)	2 ± 0[g]	35 ± 3[g]	19,300 ± 1,500
	FRAP value[h] (nM)	38 ± 7[g]	191 ± 31[g]	1,352,000 ± 122,000

Values are expressed as mean ± standard deviation of n = 3 independent measurements except for core diameter, Dc (n = 200).
[a] Dc: core diameter (TEM).
[b] Dh: hydrodynamic diameter (dynamic light scattering).
[c] ζ: zeta potential (electrophoretic mobility).
[d] λ_{max}: Surface Plasmon Band.
[e] Ellman's reaction (LOQ: limit of quantification of Ellman's assay is 3.25 μM).
[f] EC50: efficient concentration 50 corresponding to the half-maximum values of the concentration-response curves.
[g] Concentration expressed in AuNP.
[h] FRAP value corresponding to absorbance equivalent to 0.4 mM Fe^{2+}.