# Gold Octahedra nanoparticles ($Au_{0.03}$ and $Au_{0.045}$) : Synthesis and impact on marine clams *Ruditapes decussatus*

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**Abstract**

Increased use of gold nanoparticles (AuNPs) in several applications has led to a rise in concerns about their potential toxicity to aquatic organisms. In addition, toxicity of nanoparticles to aquatic organisms is related to their physical and chemical properties. In the present study, we synthesize two forms of gold octahedra nanoparticles ($Au\_{0.03}$ and $Au\_{0.045}$) in 1.3-propandiol with polyvinyl-pyrrolidone $K_{30}$ ($PVPK_{30}$) as capping agent using a polyol process. Shape, size and optical properties of the particles could be tuned by changing the molar ratio of PVP $K_{30}$ to metal salts. The anisotropy in nanoparticle shape showed strong localized surface plasmon resonance (SPR) in the near infrared region of the electromagnetic spectrum.

Environmental impact of Oct-AuNPs was determined in the marine bivalve, *Ruditapes decussatus* exposed to different concentrations of $Au\_{0.03}$ and $Au\_{0.045}$. The dynamic light scattering showed the stability and resistance of $Au\_{0.03}$ and $Au\_{0.045}$ in the natural seawater. No significant modification in vg-like proteins, MDA level and enzymatic activities were observed in treated clams with $Au\_{0.03}$ even at high concentration. In contrast, $Au\_{0.045}$ induced superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST) activities, in a concentration dependent manner indicating defense against oxidative stress. Enhanced lipid peroxidation represented by malondialdehyde content confirmed oxidative stress of $Au\_{0.045}$ at high concentration.

These results highlight the importance of the physical form of nanomaterials on their interactions with marine organisms and provide a useful guideline for future use of Oct-AuNPs. In addition, vitellogenin was shown not to be an appropriate biomarker for Oct-AuNPs contamination even at high concentration. We further show that Oct-AuNPs exhibited an important antioxidant response without inducing estrogenic disruption.

***Keywords:*** gold nano-octahedra; surface plasmon resonance; ecotoxicology, Biomarkers, Biomonitoring; Oxidative stress.

## 1. Introduction

Noble metal nanoparticles (NPs) have attracted increasing research attention in recent decades due to their interesting size-dependent optical, magnetic, electronic, and catalytic properties (Schmid, 2004; Astruc et al., 2005). The intrinsic properties of a metal nanostructure can be tailored by controlling its size, shape, composition and crystallinity. Shape-control has proven to be as effective as size-control in fine-tuning the properties and functions of metal nanostructures. Gold nanoparticles with their tunable Surface Plasmon Resonance (SPR) are popular for their wide range of practical applications such as catalysis, optics, biomedicine, and chemical sensing (Daniel and Astruc, 2004).

Development of simple and versatile synthesis methods for the preparation of AuNPs in a size- or shape-selected and controlled manner has been a challenging but intellectually satisfying task (Younan et al., 2012). Several published works have reported the synthesis of gold nanoparticles with interesting shapes using chemical, biological or physical methods (Matthew et al., 2008; Matthew et al., 2008; Vivek et al., 2009; Dreaden et al., 2012). A number of anisotropic gold nanostructures have been successfully synthesized on the basis of a polyol process in various polyol media (Poul et al., 2001; Sun and Xia, 2002; Guo et al., 2006 ; Seo et al., 2006; Li et al., 2007; Tang and Hamley, 2009). In addition, Li *et al*. (2008) have developed a low cost and straightforward PDDA (poly(diallyldimethylammonium) chloride)-mediated polyol route for the controllable synthesis of gold octahedral nanoparticles in ethylene glycol solution. The synthesis was conducted with a molar ratio of PDDA to $AuCl_4^-$ ions of 50 with addition of HCl. Li et al., 2007 synthesized octahedral Au particles of hundreds of nanometers in size by conducting the reaction in polyethylene glycol 600 (PEG 600) in the presence of PVP as surfactant and $NaBH_4$ as reducing agent. Triangular and polygonal gold micro-/nano-plates have been synthesized by Tang and Hamley, 2009 in 1,2-propanediol as both medium and reducing agent and PVP as a stabilizer (Tang and Hamley, 2009). Mezni et al. (2017) has prepared triangular gold nanoprisms of low dispersity and high crystallinity through a one-pot chemical process and using triethylene glycol (TREG) and polyvinylpyrrolidone (PVP) as solvent and capping agents, respectively. The triangular gold nanoprisms have been synthesized under conventional heating conditions, with the minimum amount of surfactant and without addition of any other reagent. Up to now, few have managed to obtain octahedral gold nanoparticles of low dispersion and high crystallinity by a simple chemical one-pot process without the addition of any other reagent. In this work we report, the synthesis of Gold octahedral nanoparticles ($Au\_{0.03}$ and $Au\_{0.045}$) in 1.3-propandiol medium as both solvent and reducing agent. The interest of this synthesis lies in the use of small quantity of surfactant in 1,3-propanediol medium ($R_{(PVP/Au)} << 1$).

Recently, the synthesis of nanoparticles of various forms has been explored. These are expected to interact differently with the marine organisms, affecting their biochemical status and biological responses (Canesi et al., 2012; Li et al., 2013; Katsumiti et al 2014; khazri et al., 2018). Therefore, it is important to understand how the forms of NPs affect their interactions with living organisms in the natural environment. Bivalves are candidates for uptake of pollutants during environmental contamination scenarios as they are filter-feeders known to bioconcentrate pollutants and contaminants very efficiently (Livingstone, 2001). The Mediterranean clam, *Ruditapes decussatus*, is already widely used as a sentinel species in aquatic toxicology due to its high tolerance for chemical contaminants (Dellali et al. 2001; Sellami et al. 2014). These organisms are abundant and farmed commercially around the Mediterranean Sea (Mohamed et al. 2003). They may represent a significant target for NPs in the aquatic environment (Canesi et al. 2012). AuNPs can induce reactive oxygen species (ROS) production in bivalves triggering oxidative stress and this is recognized as a common effect of NPs on marine organisms (Cid et al. 2015). ROS are normally detoxified by antioxidant defenses which include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione transferase (GST). Levels of antioxidant enzyme activity can provide valuable information on effects of NPs on a study organism (Cid et al. 2015). In addition, the yolk protein vitellogenin (Vtg) has long been used as a biomarker of feminization in marine organisms exposed to oestrogenic compounds (Sumpter and Jobling,1995) and is now used extensively as a reliable indicator of reproductive disruption (Matozzo et al., 2005). Despite links between NP exposure and adverse environmental effects in sentinel species such as clams, relatively little is known about how differing forms of these compounds could influence their interaction with bivalves. In addition, coating agents or surfactants are added to NP preparations in order to increase the stability of NPs in suspension media. These additives can influence significantly the toxicity of Oct-AuNPs, as already previously reported (Mano et al., 2012; Katsumit et al., 2014). To our knowledge, no exposure experiments of Oct-AuNPs with bivalves have previously been published. The present study aimed to characterize the effects of PVP coating Oct-AuNPs on modulation of antioxidant enzyme activities and reproduction in *R. decussatus*.

## 2. Experimental procedure

### 2.1. Synthesis of $Au\_{0.03}$ and $Au\_{0.045}$

Gold octahedral nanoparticles ($Au_{\_0.03}$ and $Au_{\_0.045}$) were produced by a modified polyol process involving a surface regulating polymer, polyvinyl-pyrrolidone (PVP $K_{30}$). Briefly, 25 ml of 1.3-propandiol (ACROS Organics, 98%) solution, containing 0.038 mmol of hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) (from Sigma-Aldrich), and a given amount of PVP (K30, Sigma-Aldrich) are mixed and heated to 100°C. The mixture was kept at this temperature for 30 min under continuous mechanical agitation. The molar ratio of PVP to $HAuCl_4$ ($R_{(PVP\ k30/Au)}$) was fixed at 0.03 and 0.045. Gold particles formed within minutes, and the final colloidal solution had a blue color. The product was separated by centrifugation, washed several times with ethanol/acetone solution and dispersed in ethanol.

### 2.2. Characterization

Morphological details of the synthesized gold particles were characterized by transmission electron microscopy (TEM)(JEOL-JFC 1600). Energy-dispersive X-ray spectrograph (EDX) attached to the TEM was used for elemental analysis. Selected area electron diffraction (SAED) was also conducted on the microscope, JEOL-JFC 1600. Optical absorption spectra of diluted AuNPs solution were acquired on a Perkin-Elmer Lambda 11 UV/VIS spectrophotometer. Raman experiments were performed using a Horiba-Jobin-Yvon XY spectrophotometer. The excitation laser beam was focused onto the sample through the 100X objective of a confocal microscope. The laser spot size was diffraction limited at the 633 nm excitation wavelength. The time evolution of the Raman spectra has been recorded with a time step of 0.5 s and an accumulation time of 0.5s. Dynamic light scattering (DLS) of gold octahedral nanoparticles ($Au_{\_0.03}$ and $Au_{\_0.045}$) in the seawater after 14 days of exposure was measured using an Amtec SM 200 Zetasizer operating with a He–Ne laser (632.8 nm).

### 2.3. Effects of $Au_{\_0.03}$ and $Au_{\_0.045}$ on *Ruditapes decussatus*

Clams *Ruditapes decussatus* were purchased from a site in Bizerte lagoon, Tunisia (37°1316.05′′N, 9°56′04.58′′E). Animals were distributed in 3L glass tanks and acclimated for a week on a 12 h light/dark cycle prior to exposure. After the acclimation, five experimental conditions were set up in triplicate of 10 individual clams per tank: Control, 0.1 and 1 mg/L $Au_{\_0.03}$ ($[Au_{\_0.03}]1$ = 0.1 mg/L and $[Au_{\_0.03}]2$ = 1 mg/L) and 0.1 and 1 mg/L $Au_{\_0.045}$ ($[Au_{\_0.045}]1$ = 0.1 mg/L and $[Au_{\_0.045}]2$ = 1 mg/L)). Control clams were not exposed to stressor ($Au_{\_0.03}$ and

$Au_{\_0.045}$). Exposed clams were subjected to daily concentrations of $Au_{\_0.03}$ and $Au_{\_0.045}$ set at 0.1 and 1 mg L−1 in seawater for a period of 14 days.

After 14 days of exposure, no evident mortality was observed and all animals were seen to be feeding normally. During the experimental period, salinity, temperature, dissolved oxygen and pH were measured daily with a thermo-salinity meter (LF 196; WTW, Weilheim, Germany), an oximeter (OXI 330/SET, WTW) and a pH meter (pH 330/SET-1, WTW), respectively. Temperature was maintained at 19 ± 2 °C, oxygen at 6.2 mg/L and the salinity was 32‰. Tanks were filled with natural sea water changed every 48 h and the environmental parameters were the same as those used for the acclimation period.

### 2.3.1. Determination of Vg-like proteins

Alkali-labile phosphates (ALP) levels were measured in cell-free haemolymph from clams (n = 10) exposed to $Au_{\_0.03}$ and $Au_{\_0.045}$ for 14 days. We selected this exposure period because this is an adequate time to induce variations in Vg levels in bivalves (Ricciardi et al., 2008. ALP levels were determined following the method of Blaise et al. [1999]. This approach, based on the determination of labile phosphates released by Vg after hydrolysis with alkali, was shown to be well correlated with the other direct assays. Five hundred mL of cell free haemolymph were mixed with 500 mL of t-butyl methyl ether (Sigma) for 30 min at room temperature. These emulsions were mixed by a Vortex agitator at least 3 times during the extraction period. A 400-mL sample of the ether phase was then mixed with 100 mL of 2 M Na OH for 60 min at 50 °C, to allow hydrolysis of bound phosphates. Levels of free phosphates were determined in the aqueous phase according to the phosphomolybdenum method of Stanton [1968]. A standard curve of known concentrations of inorganic phosphate was prepared. Results were expressed as mg ALP/mg proteins.

### 2.3.2. Determination of Superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST) activities and lipid peroxidation

Male and female clams selected from each treatment were homogenised by a polytron homogenizer in 10 mM Tris/HCl, pH 7.2, containing 500 mM sucrose, 1mM EDTA and 1 mM PMSF, supernatants were collected by centrifugation at 20.000 × g (4°C for 30 min). Antioxidant enzymatic activities were measured in the cytosolic fraction of 15 clams from controls and groups exposed to $Au_{\_0.03}$ and $Au_{\_0.045}$. Changes in optical density were quantified

using a Beckman DU500 spectrophotometer. Protein content was estimated by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. SOD activity was assessed by the ability of the enzyme to inhibit auto-oxidation of pyrogallol. We used 0.2 mM pyrogallol in air-equilibrated 50 mM Tris- buffer pH 8.20, containing 1 Mm EDTA (Marklund and Marklund, 1974) and is expressed in µmol/min/mg of total protein. CAT activity was measured by the decrease in absorbance at 240 nm due to $H_2O_2$ consumption (Aebi, 1979). The reaction volume and reaction time were 1 mL and 1min, respectively. The reaction solution contained 80 mM phosphate buffer, pH 6.5 and 50mM $H_2O_2$ and CAT activity was determined as nmol/min/mg protein. GST activity was measured by a modification of the method of Habig et al. (1974). There reaction mixture contained 200 µL supernatant, 2 mL phosphate buffer (0.125 M, pH 7.7, containing $Na_2$ EDTA, 0.05 M, 2–4 °C), $H_2O$ 400 µL, 200 µL 15mM 1-chloro-2, 4-dinitrobenzene (CDNB) dissolved in 95% ethanol and 200 µL 15 mM of reduced glutathione (GSH). GST activity was determined following the conjugation of GSH with CDNB at 340 nm. A unit of GST activity was defined as the amount of glutathione conjugate formed using 1nM GSH and CDNB/min per mg protein (nM 2, 4-dinitrophenyl glutathione/mg protein/min).

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using MDA as standard by the method of Buege and Aust (1978). One milliliter of the sample extract was mixed with 2 mL of the TCA-TBA-HCl reagent (15% (w/v) TCA, 0.375% (w/v) TBA and 0.25 N HCl). The contents were boiled for 15 min, cooled and centrifuged at 10.000 ×g to remove the precipitate. The absorbance was read at 535 nm and the MDA concentration of the sample was calculated using an extinction coefficient of $1.56 \times 10^5$ $M^{-1}$/cm. Lipid peroxidation was expressed as nmol of MDA/mg protein.

### 2.4. Statistical analyses

Statistical analysis was carried out using a statistical package (STATISTICA 8.0). Results of Vg like protein, MDA level and enzymatic activities were reported as mean ± standard deviation. The variation of each parameter among concentration was tested by one-way ANOVA ($p < 0.05$). Previously we tested the prerequisites for analysis of variance (normality and homogeneity of variances).When significant differences were found, Tukey's test was applied to determine which values differed significantly.

## 3. Results

### *3.1. $Au_{0.03}$ and $Au_{0.045}$ Characterization*

The UV-visible spectra of the $Au_{0.03}$ and $Au_{0.045}$ solutions are shown in Figure 1a. This two solutions were prepared to study the effect of $PVP_{K30}$ concentration on the shape of the resulting NPs and consequently on the variation of the plasmonic band as a function of each elaborated form. The two UV-visible spectra of the colloidal preparations show two plasmonic bands, which we attribute to the appearance of non-spherical particles.

Figure1.b shows Raman scattering spectra of the samples prepared in 1.3-propandiol obtained with the laser line of wavelength $\lambda = 633$ nm and acquisition time of 3 min. The two samples had a high Raman scattering intensity at this wavelength. This important enhancement of Raman scattering for an excitation close to the plasmon resonance of the samples is what is called exalted surface Raman (SERS). The vibration lines are related to the intermolecular vibrations of surfactant molecules on the surface Au-NPs. The line observed at 1480 $Cm^{-1}$ can be attributed to the C-N group of polyvinylpyrrolidone $K_{30}$. The presence of the $CH_2$ = CH group at 1473 $Cm^{-1}$ is noted. The appearance of a line at 1294 $cm^{-1}$ is the result of the group $CH_2$ attached to CN. The lines observed successively around 1065, 987.925 and 853$Cm^{-1}$ are due to the $CH_2$ alkyl groups of the ring. The line which appears towards 525 $Cm^{-1}$ is due to the group N-C = O.

Figure 2 (a and b) shows typical TEM images of $Au_{0.03}$ and $Au_{0.045}$ colloidal solutions at different molar ratio of PVP/Au. In this case, Au particles with different morphologies were obtained (rod-like, triangular nanoplates, cubiques nanoplates,). The size of the 2D gold objects was about 10 to 200 nm. When the molar ratio is 0.03, equilateral gold cubes nanoparticles with an average edge length of 25 nm were formed (Fig. 2a). This indicates that the appropriate molar ratio is vital for the formation of Oct-AuNPs. When the molar ratio was greater than 0.03 ($Au_{0.045}$), the AuNP became much thicker and agglomerated. We also observe a mixture of shape (triangular particles, octahedral and other).

Energy dispersive spectrum (EDX) analysis for such as-prepared sample confirms that the Oct-AuNPs consist of only gold (Fig. 2c, the copper element came from copper grid). The inset to Fig.2c, gives typical selected area electron diffraction (SAED) patterns obtained by directing the electron beam perpendicular to a single gold nanoplate deposited flat on the TEM grid.

### *3.2. Behaviour of gold Octahedra NPs in seawater and effects on clams Ruditapes decussatus*

#### *3.2.1. Physico-chemical evolution of Octahedra AuNPs in SW media*

*Dynamic light scattering analysis* (DLS) of $Au_{\_0.03}$ and $Au_{\_0.045}$ in natural seawater demonstrates a monomodal scattered intensity distribution with a major maximum at about 45 nm. According to the DLS data (Fig. 3), the Z-average particle diameter of $Au_{\_0.03}$ and $Au_{\_0.045}$ in natural seawater after 14 days of exposure is dav = 45 ± 1 nm and 50 ± 1 respectively. The position of the major peak of the scattered intensity distribution ((d ≈ 45 nm ($Au_{\_0.03}$) and 50 nm ($Au_{\_0.045}$) exceeds the size dispersion obtained from TEM images (Oct-AuNPs with an average size around 20 and 40 nm). From DLS we obtain the hydrodynamic diameter of the particle, defined as a sphere with the same translational diffusion coefficient as the particle being measured (assuming a hydration layer surrounding the particle or molecule). This small difference between the sizes of Oct-AuNPs is related to the hydrodynamic diameter measured and added by DLS. According to DLS and TEM data, $Au_{\_0.03}$ and $Au_{\_0.045}$ are stable in seawater and no agglomeration or aggregation was observed.

### *3.2.2. Hemolymph Vg-like protein levels response to Octahedra AuNPs exposure*

Difference of Vg-like protein means levels were recorded in females and males hemolymph. Thus, female controls exhibited approximately two-fold higher values than those of male controls (Figure 4). However, exposure to different Oct-AuNPs forms ($Au_{\_0.03}$ and $Au_{\_\mathbf{0.045}}$) and concentrations (0.1 and 1 mg/L) did not result in any significant alteration ($p > 0.05$) in Vg-like protein levels in the hemolymph of males and females compared to control.

### *3.2.3. Biomarker responses to Oct-AuNPs exposure*

SOD, CAT and GST activities of clams treated with $Au_{\_0.03}$ and $Au_{\_0.045}$ for 14 days, were determined (Fig. 5). $Au_{\_\mathbf{0.045}}$ induced concentration-dependent increase in antioxidant enzyme activity in both male and female. Indeed, SOD activity in female exposed to $[Au_{\_0.045}]1$= 0.1 mg/L and $[Au_{\_0.045}]2$ = 1 mg/L increased after 14 days of exposure by 26% and 28% respectively, compared to controls and by about 41% and 47% respectively in male (Fig. 5). In contrast, no effect in SOD activity ($p > 0.05$) was observed after 14 days exposure to $[Au_{\_\mathbf{0}.03}]1$= 0.1 mg/L and $[Au_{\_0.03}]2$ = 1 mg/L in females and males compared to control.

A similar pattern of variation in CAT activity was observed between males and females after 14 days exposure (Fig. 5). Exposure to $[Au_{\_0.045}]1$= 0.1 mg/L and $[Au_{\_0.045}]2$ = 1 mg/L caused a significant ($p < 0.05$) increase by approximately 29% and 43% in females and by about

30% and 50% in males respectively, compared to control group. No significant modification was observed in females and males CAT activities after exposure to $Au_{0.03}$.

Females GST activity increased from 32.2 ± 0.31 nmol/min/mg protein to 45.23 ± 0.18 nmol/min/mg protein in [$Au_{0.045}$]2-treated groups but no effects were evident on [$Au_{0.045}$]1-treatment ($p > 0.05$). Males GST activity increased also after exposure to [$Au_{0.045}$]2 by approximately 27%. Lipid peroxidation determined by measuring MDA content of clams exposed to [$Au_{0.03}$] were similar to the control after 14 days of exposure (Fig. 5). In contrast, [$Au_{0.045}$] = 1 mg/L increased MDA levels significantly ($p < 0.05$) for both sexes after 14 days of exposure.

## 4. Discussion

### *4.1. Oct-AuNPs stability*

PVP could act not only as a stabilizer layer to prevent the aggregation of the particles but also as a shape-controller to assist the formation of anisotropic metal nano-structures (Seo et al., 2006 ; Xiong et al., 2006 ; Li et al., 2007). At a lower molar ratio of PVP to gold, the nucleation and growth of gold nanoparticles were subjected to kinetic control. In this case, gold atoms would preferentially add to facets of the seeds with higher surface energy. We believe that PVP preferentially adsorbs on the {111} planes of Au nuclei and consequently the growth rate along the <111> direction is reduced while the growth rate along the <110> and <100> direction is enhanced, leading to the highly anisotropic growth of nuclei into nanostructure (Xiong et al., 2006; Xia et al., 2012). PVP can therefore play an important role in controlling the shape and monodispersity of gold nanoparticles but it cannot produce such shape-controlled uniform gold nanoparticles by itself without cooperation of polyol solvent. Indeed, the 1,3-propanediol can act not only as a solvent but also as a capping/stabilizing agent (Mezni et al., 2014). The 1,3-propanediol molecules are also adsorbed on the {111} oriented planes and thus contribute to slow down their growth, which explains the formation of gold nano-octahedral with a small PVP/Au molar ratio R=0.03 (Au_0.03).

The hexagonal symmetrical spots of the SAED pattern reveal clearly that these gold nanoplates are single crystals and the incident electron beam is perpendicular to {111} facet of the tested plate.

### *4.2. Effect of Oct-AuNPs contamination on Vitellogenins level and biochemical status*

Vitellogenins (Vg) are the major precursor of the egg yolk proteins in oviparous organisms (Wallace, 1985). They have been proposed as useful biomarkers in evaluating estrogenic effects of various chemicals including metals. In the present study, higher Vg-like protein levels in control females are attributed to the spawning phase of clams used in the investigation. Clams were collected during prespawning when the Vg levels are highest in females due to their natural sex hormones. These observations are in agreement with those reported for other bivalves (Gagné et al., 2005; Marin et al., 2003). No alterations were observed in both sexes after exposure to Oct-AuNPs. This could be related to the fact that Oct-AuNPs has not estrogenic effect, at least in clams. Additionally, the Oct-AuNPs may not interact with cellular estrogen receptors as observed for other chemicals (Matozzo et al., 2005). We can also hypothesize that Vitellogenin is not an appropriate biomarker of Oct-AuNPs contamination even at high concentration.

Induction of SOD, CAT and GST enzyme activities and MDA content are consistent with production of ROS in response to $Au\_{0.045}$ exposure since it is known that NPs are capable of crossing cell membranes, leading to cell damage (Li et al. 2013). However, lack of effects of $Au\_{0.03}$ form on oxidative parameters suggest that nanoparticles form represents an important variable in the interaction between NPs and living cells. Our results are dependent to the amounts of PVP $_{K30}$ adsorbed to the surface of the Oct-AuNPs and also with the shape of the particles obtained. Coating agents or surfactants are added to NP preparations in order to increase the stability in suspension media. These additives can influence significantly the toxicity of Oct-AuNPs, as already reported by other authors (Mano et al., 2012; Katsumit et al., 2014).Similar results were demonstrated in *Crassostrea virginica* exposed to PVP coated AgNPs (McCarthy, 2011).

The form-dependent uptake of Au observed in the present study may be related to effective mechanisms for particle sorting in bivalves (Dai et al., 2013). In addition, interactions and internalization of nanomaterials within cells is dependent to the shape and size (Nambara et al., 2016). In the present study, the form $Au\_{0.045}$ generates oxidative stress at high concentration and modulates the oxidative stress response in clams. This concentration dependent response is in agreement with previous studies related to environmental impact of nanomaterials on invertebrates **(**Canesi et al., 2012; Garcia-Negrete et al., 2013; Katsumit et al., 2014; Khazri et al., 2018).

## 5. Conclusion

In summary, single-crystalline Oct-AuNPs were successfully synthesized with well-defined shape and tunable size (~25 nm) by a modified polyol process in a 1.3-propanediol solution. This synthetic strategy provided an effective route for selective production of Oct-AuNPs. Under these conditions, functionalization of the Oct-AuNPs by other ligands of biological interest or by antibodies is directly possible and does not require any further purification. Besides our novel chemistry results, an environmental investigation using a multi-biomarker approach confirmed that Oct-AuNPs ecotoxicity to clams depends on NP form and concentration. No effect of the two considered nanosized materials on Vg-like proteins were found suggesting that Oct-AuNPs are not estrogenic disruptors.

## Acknowledgments

Anis Fkiri, gratefully acknowledges the support of the Ministry of Higher Education and Scientific Research of Tunisia.

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**Figures legend:**

**Figure 1:** (a) UV-visible spectra of $Au_{0.03}$ and $Au_{0.045}$ colloidal solutions prepared in the 1.3-propandiol at T = 100 °C, (b) Raman spectra of various samples prepared in 1.3-propandiol, excited with a laser source of wavelength $\lambda = 633$ nm for an acquisition time of 3 min.

**Figure 2:** TEM images of gold nanoparticles (a) $Au_{0.03}$, (b) $Au_{0.045}$ and (c) EDX spectrum of octahedra gold nanoparticles, the inset in fig. shows typical selected area electron diffraction (SAED) pattern from single nano-octahedra.

**Figure 3**: Dynamic light scattering (DLS) of $Au_{0.03}$ and $Au_{0.045}$ nanoparticles dispersed in natural seawater.

**Figure 4**: Vg-like protein levels expressed as µg ALP/mg proteins, in male haemolymph (A) and female (B) from control and treated clams with different form and different concentration of Oct-AuNPs. Values are means ± SD. Different letter: significant results: $p < 0.05$.

**Figure 5**: Superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST) activities and malondialdehyde content, in male and female from control and treated clams with different form and different concentration of Oct-AuNPs. Values are means ± SD. Different letters: significant results: $p < 0.05$.