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## PAPER

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# A mechanistic study of gold nanoparticles catalysis of O<sub>2</sub> reduction by ascorbate and hydroethidine, investigating reactive oxygen species reactivity†

Viacheslav Shcherbakov, \* Sergey A. Denisov  and Mehran Mostafavi \*

In this work, the mechanism of dioxygen reduction catalysed by gold nanoparticles (AuNPs) by two electron donors was investigated, *i.e.*, by sodium ascorbate and hydroethidine, focusing on potential ROS (reactive oxygen species) formation, such as O<sub>2</sub><sup>•−</sup> and H<sub>2</sub>O<sub>2</sub>. According to our results, when AuNPs catalyse the reduction of O<sub>2</sub>, ROS are formed only as intermediates on the surface of nanoparticles, and they are unavoidably reduced to water, catalysed by the AuNPs. Thus, the statement on ROS production in the presence of AuNPs often reported in the literature is excessive. The AuNPs can catalyze the oxidation of electron donors in the cell, *e.g.*, antioxidants causing oxidative stress. Therefore we propose that when explaining damage in the living cells observed in the presence of AuNP, the catalysis of redox reactions by AuNPs must be considered.

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## Introduction

Reactive oxygen species (ROS) are essential actors in cell biochemistry.<sup>1</sup> In biology, the reactivity of free radicals, including ROS, is a research domain under active investigation that has implications in, *e.g.*, aging processes, stress-activated diseases, and radiotherapy.<sup>2–7</sup>

Nanoparticles (NPs) are studied for different biomedical applications. One of the first examples was gold nanoparticles (AuNPs), which were considered an inert material and biocompatible. However, it has been known for a long time that materials' properties at the nanoscale are different from the bulk ones. Therefore, many NPs, including AuNPs possess catalytic activity in different reactions.<sup>8</sup> The idea that nanoparticles are not inert is gradually becoming more visible in biological research.

It is interesting to mention that the chemical activity of AuNPs is mainly raised in the context of ROS formation.<sup>9,10</sup> The idea that nanoparticles cause ROS formation probably drifted from the radiosensitization research field (known as ROS overproduction),<sup>11,12</sup> where nanoparticles are studied as radiosensitizing agents.<sup>9,10</sup> Briefly, using the fluorescence probes for ROS detection, it was shown that in the presence of AuNPs under ionizing radiation, the quantity of formed fluorescent product was significantly increased, which was interpreted as ROS overproduction, taking into account the linear dependence

of fluorescent product formation on ROS concentration. In our recent works, we showed that the increased formation of the fluorescent product could be explained not by additional ROS formation but by catalytic properties of AuNPs, which increase the yield of particular reaction products.<sup>13–15</sup> Thus, not considering the catalytic properties of nanoparticles in reactions involving organic radicals led to an invalid interpretation of the experimental data. At the same time, it is often mentioned that in the absence of ionizing radiation NP could produce ROS *via* catalysis,<sup>9,10</sup> *e.g.*, catalytic reduction of dioxygen to superoxide radical or hydrogen peroxide. Due to the high reactivity of ROS, their detection in biological systems is not readily achievable. Therefore, in biological works, the statement about catalytic ROS production is used to explain the observed biological effects, *e.g.*, oxidative stress.<sup>16</sup>

ROS formation and their reactivity in the presence of NPs are extensively discussed in the context of the enzymatic-like activity of nanomaterials. NPs, including gold, can catalyze reactions similar to natural enzymes.<sup>17–19</sup> They exhibit oxidase-like activity, which includes reactions involving oxygen,<sup>20,21</sup> hydrogen peroxide,<sup>22,23</sup> and superoxide radicals.<sup>23</sup> For example, ascorbate oxidation by dioxygen catalyzed by Au@Pt NPs was reported.<sup>24</sup> Unlike, L-ascorbate oxidase, in the presence of the NPs, it is stated that oxygen is reduced to hydrogen peroxide. However, other works show that AuNPs catalyze H<sub>2</sub>O<sub>2</sub> decomposition.<sup>22,23</sup>

The catalytic activity of NPs in biologically relevant reactions still does not receive proper attention, while it is crucial for further development of nanomedicine and understanding nanomaterials' toxicity. In our opinion, there is a demand for biologically orientated works in the chemistry of nanomaterials to provide a deeper and more convincing explanation of

Institut de Chimie Physique (ICP), CNRS/Université Paris-Saclay, 91405 Orsay, France.  
E-mail: [viacheslav.shcherbakov@universite-paris-saclay.fr](mailto:viacheslav.shcherbakov@universite-paris-saclay.fr); [mehran.mostafavi@universite-paris-saclay.fr](mailto:mehran.mostafavi@universite-paris-saclay.fr)

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biological effects observed in the presence of nanomaterials. For example, it was shown that AuNPs affect living cells' anti-oxidant status by inhibiting enzyme activity, *i.e.*, thioredoxin and glutathione reductases.<sup>25</sup> That shows clearly that the chemical activity of AuNPs cannot be limited to ROS formation.

We have recently shown that in many works when gold nanoparticles are used for both therapeutic and diagnostic purposes, the chemical effect of the nanoparticles is not taken into account, and this effect can have a powerful impact on the interpretation of the results.<sup>14,15</sup>

In this work, we have chosen two molecules: sodium ascorbate and hydroethidine, the first one as a model of an antioxidant that can be present in the biological medium and the second one as a model of a fluorescent probe used in ROS detection experiments. Here we aim to provide the mechanism of catalytic oxidation in the presence of AuNPs for both chosen molecules investigating potential ROS formation.

## Experimental

### Gold nanoparticles synthesis and supernatant preparation

We used 20 nm AuNPs prepared by reduction of potassium gold(III) chloride ( $\text{K}[\text{AuCl}_4]$ , 98%, Sigma Aldrich) with sodium borohydride ( $\text{NaBH}_4$ , 98%, Sigma Aldrich). Briefly, 3 ml of 100 mM  $\text{K}[\text{AuCl}_4]$  solution was mixed with 87.7 ml of deionized water (Milli-Q, 18 M $\Omega$  cm), and 9.3 ml of 100 mM  $\text{NaBH}_4$  was quickly added to the solution while actively stirring with a magnetic stirrer for 15 min. The final concentration of gold atoms was 3 mM.

AuNPs were characterized by several techniques: zeta potential of  $-20$  mV was measured by Zetasizer Nano ZS, Malvern; UV-vis absorption spectra were measured on Hewlett Packard 8453 spectrophotometer (Fig. S1 $\dagger$ ); the microphotography of the nanoparticles were taken by transmission electron microscopy (STEM 1400, JEOL, Fig. S2 $\dagger$ ).

The supernatant of AuNPs used as reference was prepared by adding NaCl to provoke particles aggregation with further sedimentation by centrifugation. Centrifugation was done in 2 ml Eppendorf microtubes utilizing an Eppendorf MiniSpin centrifuge with F-45-12-11 rotor at a speed of 13 400 rpm for 30 min. Then, the transparent liquid above the solid precipitate was gently taken.

The quality of the nanoparticle's removal from the solution was checked spectroscopically. The pH of AuNPs' suspension and its supernatant was *ca.* 7.

### Ascorbate oxidation

We studied the catalytic oxidation of sodium ascorbate by two oxidizers, oxygen, and hydrogen peroxide, in the presence of AuNPs. The concentration of sodium ascorbate (99%, Alfa Aesar) was determined by UV-vis absorption spectroscopy (Hewlett Packard 8453) using an extinction coefficient of  $14.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  at 265 nm measured in our laboratory. Studied solutions containing sodium ascorbate, oxygen or hydrogen peroxide, and AuNPs were placed into 1 mm quartz cuvette. The optical cuvette was covered by Parafilm M to prevent gas

exchange. The kinetics of ascorbate oxidation were measured by following a decrease of its absorption band at 265 nm.

**Oxidation by oxygen.** 3 ml of 4.5 mM sodium ascorbate solution and 3 ml of AuNPs suspension with 3 mM of gold atoms were bubbled with oxygen gas for 10 min. Then, 260  $\mu\text{l}$  of ascorbate solution and 130  $\mu\text{l}$  of AuNPs suspension were mixed directly in a 1 mm quartz cuvette. Thus, the final solution contained 3 mM of sodium ascorbate, AuNPs with 1 mM of gold atoms, and 1.22 mM of oxygen. The concentration of oxygen was calculated based on its solubility in water at ambient conditions.<sup>26</sup>

We also measured ascorbate stability at 1 mM concentration in oxygen-saturated solutions in the absence of AuNPs.

**Oxidation by hydrogen peroxide.** Before experiments with AuNPs, we studied ascorbate stability in the presence of 0.5 mM of  $\text{H}_2\text{O}_2$  (30% w/w, Sigma-Aldrich) in deoxygenated aqueous solution. Briefly, 3 ml of 1 mM sodium ascorbate solution containing 0.5 mM of  $\text{H}_2\text{O}_2$  was bubbled with Ar for 10 min. Ascorbate concentration was verified by absorption measurements every *ca.* 10 min.

For experiments with AuNPs, 3 ml of an aqueous solution containing 1.5 mM sodium ascorbate with 0.75 mM of  $\text{H}_2\text{O}_2$  and 3 ml of AuNPs suspension with 3 mM of gold atoms were deoxygenated by bubbling with Ar gas for 15 min. Then, 260  $\mu\text{l}$  of ascorbate solution and 130  $\mu\text{l}$  of AuNPs suspension were mixed directly in a 1 mm quartz cuvette. Thus, the final solution contained 1 mM of sodium ascorbate, 0.5 mM of  $\text{H}_2\text{O}_2$ , and AuNPs with 1 mM of gold atoms under an argon atmosphere.

### Hydrogen peroxide stability in the presence of AuNPs

The stability of hydrogen peroxide in the presence of AuNPs was measured by titanium(IV) oxysulfate solution (1.9–2.1%  $\text{TiOSO}_4$ , Sigma-Aldrich) titration method. Briefly, 0.5 ml of 20 mM  $\text{H}_2\text{O}_2$  was added to 5 ml of water or supernatant or AuNPs suspension with a gold atomic concentration of 3 mM. Then, the solution's volume was adjusted to 10 ml. 1.6 ml of each solution was taken every 10 min for 1 hour and mixed with 80  $\mu\text{l}$  of 10 wt% NaCl solution to cause AuNPs aggregation. Then, samples were centrifuged to precipitate AuNPs. Then, 1.5 ml of the samples were mixed with 1.5 ml of 1.9–2.1 wt%  $\text{TiOSO}_4$  solution and UV-vis absorption spectra were measured in 1 cm quartz optical cuvette.  $\text{H}_2\text{O}_2$  concentrations were calculated using an extinction coefficient of  $689 \text{ M}^{-1} \text{ cm}^{-1}$  at 407 nm of the yellow-colored complex of pertitanic acid ( $\text{H}_2\text{TiO}_4$ ).<sup>27</sup>

### Dihydroethidium oxidation by oxygen

Hydroethidine (HE,  $\leq 95\%$ , Sigma-Aldrich) was dissolved in DMSO to get 1 mM solution. The exact concentration was determined by absorption measurement at 345 nm using extinction coefficient of  $9750 \text{ M}^{-1} \text{ cm}^{-1}$ . Prior the measurement HE solution in DMSO was diluted 10 times by deionized water.

For the experiment on HE oxidation by oxygen in the presence of AuNPs, 1.5 ml of 110  $\mu\text{M}$  HE solution was mixed with 1.5 ml of AuNPs suspension ( $[\text{Au}] = 300 \mu\text{M}$ ) directly in 1 cm quartz cuvette. Then, the evolution of absorption spectra was recorded.

For the experiment on the reduction of the oxidized product obtained in the previous experiment, AuNPs were removed from

the solution by centrifugation. Then, 10  $\mu\text{l}$  of 30 mM  $\text{NaBH}_4$  solution was added to reduce the oxidized product. Then, the evolution of absorption spectra was recorded.

## Results

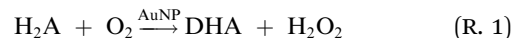
### Ascorbate oxidation by oxygen

In our work, we measured ascorbate ( $\text{H}_2\text{A}$ ) oxidation to dihydroascorbate (DHA) by following the disappearance of its absorption band at 265 nm (Fig. 1 insert). When ascorbate was added to AuNPs suspension, it caused a slight blue shift and a little increase in absorption of AuNPs plasmon band (Fig. 1 insert). A similar observation was reported in work utilizing surface plasmon spectroscopy of a single gold nanocrystals.<sup>28</sup> It has been suggested that the nanoparticle oxidizes the ascorbate ions, becoming negatively charged, which causes a blue shift in surface plasmon absorption. Then, when dioxygen molecules reach the AuNP surface, they uptake electrons, reducing the overall negative charge of AuNP, which causes a red shift of the plasmon band. However, we did not observe ascorbate oxidation by AuNPs in deoxygenated solution. Thus, we think that the reason for the observed blue shift is that ascorbate molecules work as surfactants interacting with AuNPs' surface, causing a change in nanoparticles absorption. For example, it was shown that the formation of  $\text{Cl}^-$  complexes with AuNPs affects the position of the surface plasmon resonance peak.<sup>29</sup>

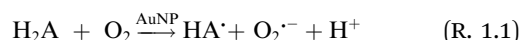
We observed that the absorption band of ascorbate ( $\text{H}_2\text{A}$ ) at 265 nm decreased within tens of minutes only when the solution contained both AuNPs and  $\text{O}_2$  (Fig. 1 insert). The experiment was designed in such a way as to observe the termination of the reaction when the oxidant (oxygen) is depleted.

In the oxygen-saturated solution,  $2.4 \pm 0.1$  mM of  $\text{H}_2\text{A}$  was consumed (Fig. 1), considering oxygen concentration of 1.22 mM, ascorbate :  $\text{O}_2$  ratio is 2 : 1. However, if we consider

oxygen reduction only to hydrogen peroxide (reaction (1)) as it was proposed elsewhere,<sup>24</sup> then the ascorbate :  $\text{O}_2$  ratio should be 1 : 1, which does not satisfy our experimental results; meaning that hydrogen peroxide is further reduced to water (reaction (2)), giving an overall reaction (3).

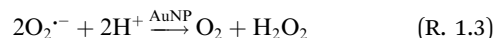


It was confirmed by ESR measurements at an ambient temperature that  $\text{H}_2\text{A}$  oxidation by oxygen catalysed by Pt@Au nanorods occurs through ascorbate radical formation ( $\text{HA}^\cdot$ ).<sup>24</sup> The same was observed for palladium concave nanocrystals.<sup>30</sup> Therefore, we can consider that reaction (1) includes two following steps:



Reaction (1.1), where ascorbate and superoxide radicals are formed, is catalysed by AuNPs because, in their absence, this reaction is very slow. Reaction (1.2) is not necessarily catalyzed by AuNPs since  $\text{O}_2^{\cdot-}$  is able to oxidize ascorbate radical. An important point is that reaction (1.1) is a limiting step. Therefore, the steady-state concentration of  $\text{O}_2^{\cdot-}$  is low, and it is localized at NPs' surface.

Reaction of superoxide radical with ascorbate radical is not specific and occurs by superoxide radical attachment to the ascorbate radical followed by cyclization and hydrolysis yielding DHA and  $\text{H}_2\text{O}_2$ .<sup>31</sup> Thus, simply by measuring the disappearance of ascorbate, we cannot understand whether the reaction between superoxide and the ascorbate radical occurs directly or whether it is catalyzed by nanoparticles. Moreover, it was reported that AuNPs possess superoxide dismutase-like activity.<sup>23</sup> Therefore, we can propose the disproportionation of two superoxide radicals into dioxygen and hydrogen peroxide catalyzed by gold (reaction (1.3)) as an alternative reaction mechanism.



Both proposed mechanisms, superoxide radical reduction (reaction (1.2)) and its disproportionation (reaction (1.3)), satisfy experimental observation that one  $\text{O}_2$  oxidizes two  $\text{H}_2\text{A}$  molecules in the presence of AuNPs. To examine the reactivity of  $\text{O}_2^{\cdot-}$  in the presence of AuNPs, we used another molecule probe, which is discussed in the next section.

### Superoxide radical fate in the presence of AuNPs

Superoxide radical formation *via* catalysis has a great interest for the biomedical application of NPs. The  $\text{O}_2^{\cdot-}$  formation in

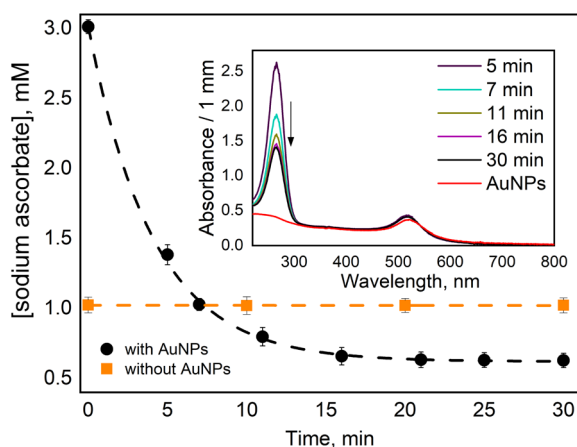


Fig. 1 Kinetic of ascorbate oxidation in oxygen-saturated solution ( $[\text{O}_2] = 1.2$  mM) in the absence (orange squares) and the presence of AuNPs (black dots,  $D_{\text{AuNP}} = 20$  nm,  $[\text{Au}] = 1$  mM). Inset: an example of UV-vis absorption spectra evolution of the solution containing AuNPs. The initial concentration was determined by diluting the stock solution (3 mM).

the presence of different NPs, including gold was even proposed as a chemical mechanism of AuNPs radiosensitizing effect.<sup>9,10</sup>

We used hydroethidine (HE) to study possible superoxide formation during the catalytic reduction of oxygen. HE is a fluorescent dye widely used in biological studies.<sup>32</sup> It has two oxidation products, ethidium ( $E^+$ ) and 2-hydroxyethidium cation ( $2-OH-E^+$ ) (Scheme 1).  $2-OH-E^+$  is considered a specific product of  $HE^{+}$  radical oxidation by  $O_2^{\cdot-}$ .

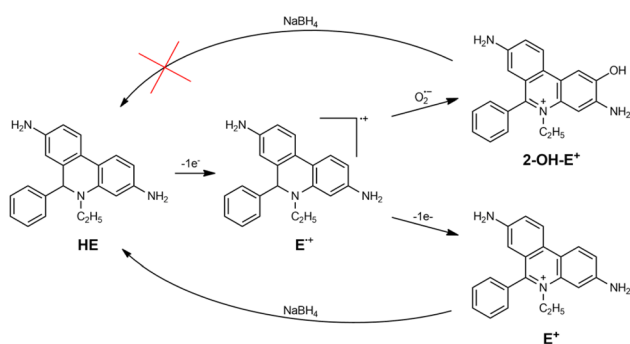
However, many studies showed that since fluorescent spectra of both oxidation products are similar, HE should not be considered a specific fluorescent probe for intercellular  $O_2^{\cdot-}$  detection.<sup>33</sup> Therefore, in our work, instead of fluorescence measurements, we used only absorption spectra which enabled us to differentiate between the two compounds. The experiments were based on the fact that  $E^+$  can be reduced to HE, but  $2-OH-E^+$  cannot (Scheme 1).

We observed a fast (minutes) transformation of the absorption spectrum of the sample containing HE and AuNPs (Fig. 2A). An increase at 290 nm and a decrease at 350 nm bear witness to HE oxidation. Similar to ascorbate experiments, HE oxidation does not occur in the absence of AuNPs or oxygen. This experiment was performed in an air atmosphere. Thus, the oxygen concentration in the solution is *ca.* 0.26 mM,<sup>26</sup> which is greater than the HE concentration allowing its total oxidation.

The broadening of the plasmon band of AuNPs around 500–600 nm is due to the aggregation of the particles in the presence of HE. This process was instantaneous and did not affect HE oxidation.

After the absorption spectrum stopped changing, AuNPs were removed from the solution by centrifugation. Then, we added 100  $\mu$ M of  $NaBH_4$  to the solution obtained after the particles removal and recorded the evolution of the absorption spectrum (Fig. 4B). We observed that the absorption maximum at 345 nm appeared back, and final absorption spectrum is similar to HE. Using  $\epsilon^{345nm} = 9750 \text{ M}^{-1} \text{ cm}^{-1}$ , we calculated HE concentration as 55  $\mu$ M, which equals to the initial HE concentration. Thus, we can conclude that the main product of HE oxidation by oxygen in the presence of AuNPs is  $E^+$ , and  $2-OH-E^+$  is not formed.

These experimental results mean that potentially formed  $O_2^{\cdot-}$  radicals do not react directly with the molecule or its



Scheme 1 Hydroethidine (HE) oxidation and reduction of ethidium ( $E^+$ ).

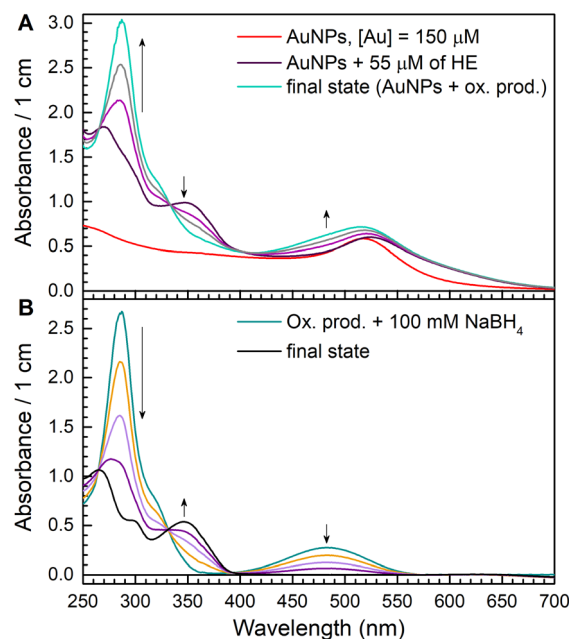


Fig. 2 Oxidation of HE (55  $\mu$ M) by oxygen (256  $\mu$ M, air atmosphere) in the presence of AuNPs ([Au] = 150  $\mu$ M) – (A) the resulting oxidation product was purified from AuNPs by centrifugation. Reduction of the oxidation product by  $NaBH_4$  (100  $\mu$ M) – (B).

radical. AuNPs catalyse reactions involving superoxide radicals. They either catalyse  $O_2^{\cdot-}$  reduction (like reaction (1.2)) or disproportionation (reaction (1.3)). We tend to think that nanoparticles work like an electron relay transferring an electron from an electron donor to electron acceptors. In this case, all transient species are formed and further reduced or oxidized depending on their RedOX potential on the nanoparticle's surface, being unable to interact with any other molecules. AuNPs could be considered as a microelectrode with adjustable RedOX potential depending on the potentials of reacting RedOX couples. This idea was proposed to explain corrosion and discussed elsewhere.<sup>34</sup>

### Hydrogen peroxide reactivity in the presence of AuNPs

To study  $H_2O_2$  reactivity in the presence of AuNPs, first, we studied its stability by  $TiOSO_4$  titration (Fig. 3 and S3<sup>†</sup>).

Partial  $H_2O_2$  decomposition was observed only in the presence of AuNPs within 60 min. Thus, we can conclude that AuNPs catalyse the disproportionation of  $H_2O_2$  into dioxygen and water (reaction (4)).



However, this reaction is slow to explain ascorbate oxidation by dioxygen, which is achieved within 10 min in our conditions (Fig. 1). Only 30% of  $H_2O_2$  was decomposed in 1 hour, which means that reaction (2) does not include  $H_2O_2$  disproportionation. Similar conclusion was made in work on glucose oxidation catalysed by AuNPs.<sup>22</sup>

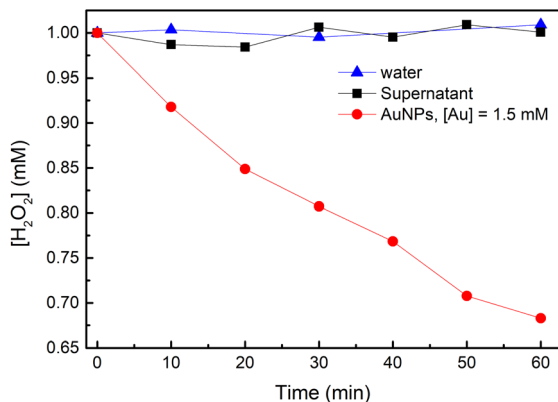


Fig. 3 Stability of  $\text{H}_2\text{O}_2$  in absence (blue – in water, black – in supernatant solution) and presence of AuNPs  $[\text{Au}] = 1.5 \text{ mM}$  (red).  $\text{H}_2\text{O}_2$  concentration was measured by  $\text{TiOSO}_4$  assay at pH 7. The concentration measurement is estimated to have an accuracy of ca. 6%.

It seems that AuNPs catalyse oxidation of electron donors like ascorbate by  $\text{H}_2\text{O}_2$ . To show that, we performed an experiment for ascorbate oxidation by  $\text{H}_2\text{O}_2$  in deoxygenated solution in the presence of AuNPs (Fig. 4). Similar to ascorbate oxidation by oxygen, the experiment was design to have ascorbate in excess.

0.5 mM of  $\text{H}_2\text{O}_2$  oxidized equal amount ( $0.53 \pm 0.03 \text{ mM}$ ) of ascorbate in the presence of AuNPs. Thus, the ratio of  $\text{H}_2\text{O}_2 : \text{O}_2$  is 1 : 1 (reaction (5)).

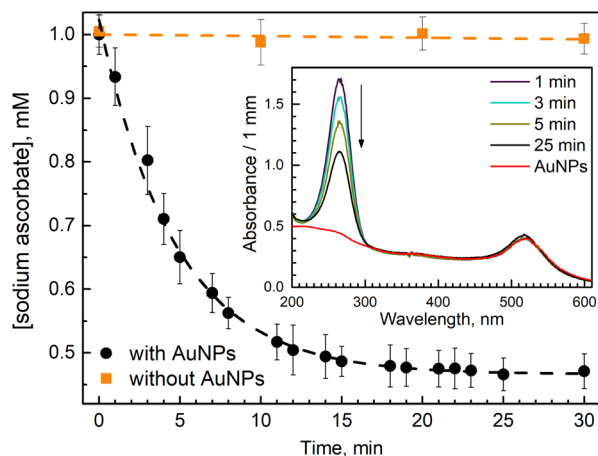
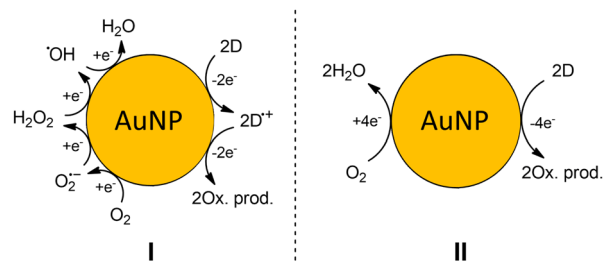


Fig. 4 Kinetic of ascorbate oxidation in the presence of 0.5 mM of  $\text{H}_2\text{O}_2$  without (orange squares) and with AuNPs (black dots,  $D_{\text{AuNP}} = 20 \text{ nm}$ ,  $[\text{Au}] = 1 \text{ mM}$ ) in deoxygenated aqueous solution (Ar atmosphere). Inset: an example of UV-vis absorption spectra evolution of the solution containing AuNPs.



Scheme 2 The generalized mechanism of oxygen reduction on the surface of AuNP.  $\text{H}^+$  is omitted for simplicity. (I) – step-by-step reduction of dioxygen (II) – overall reaction of dioxygen reduction.

Taking these results into account, we cannot consider  $\text{H}_2\text{O}_2$  as a stable product of oxygen reduction in the presence of AuNPs.

## Discussion

All reactions discussed in previous sections are generalized in Scheme 2 (I). It includes complete step-by-step reduction of dioxygen to water and ROS such as  $\text{O}_2^{\cdot-}$ ,  $\text{H}_2\text{O}_2$ , and  $\cdot\text{OH}$  that are formed as intermediates. In a living cell, the ROS cause non-specific damage (*i.e.*, oxidation), defend the organism against pathogens and are involved in the signaling pathways, where they specifically react with bio-molecules.

The lifetime of ROS in the cellular environment is very short (*e.g.*, for  $\cdot\text{OH}$  radical, it does not exceed a few nanoseconds). Therefore, one should consider the stationary concentration of ROS, which is dependent on the rate of its production and elimination. When the stationary concentration of ROS becomes higher, this is called “oxidative stress”. In other words, oxidative stress is an imbalance of ROS, antioxidants, and enzymatic systems responsible for ROS elimination. Thus if the concentration of antioxidants drops, oxidative stress is achieved.

Therefore, in our experiments with AuNPs we observe the catalysis of oxidation reactions by oxygen. We want to stress that ROS reactivity was not observed, even though they are formed as intermediates. It was clearly shown in the experiment on HE oxidation. Therefore, the mechanism could be simplified to 4 electron dioxygen reduction (Scheme 2 (II)). Now, if we translate our observation to the biological systems, we can state if one observes more oxidative damage in the presence of AuNP it does not necessarily mean ROS stationary concentration increases. The AuNP can catalyze the oxidation of electron donors, *e.g.*, antioxidants causing oxidative stress and detectable damage in the cell.

Despite the fact that ROS are formed during 4 electrons step-by-step reduction of dioxygen, these reactions occur locally on the surface of AuNP, and thus the release of ROS from the surface is unlikely to occur.

## Conclusions

In this work, we studied the mechanism of dioxygen reduction by two electron donors: sodium ascorbate and hydroethidine

catalysed by AuNPs, focusing on potential ROS formation such as  $O_2^{\cdot-}$  and  $H_2O_2$ . According to our results, when AuNPs catalyse the reduction of  $O_2$ , ROS are formed only as intermediates on the surface of NPs, and they are unavoidably reduced on-site to water catalysed by AuNPs. Thus, the statement on ROS production in the presence of AuNPs often reported in the literature is inaccurate. When AuNPs are absent, ROS can be generated and diffuse in the solution; in the presence of AuNPs, the catalytic effect of AuNPs causes the transformation of ROS into water. We also discussed the importance of catalytic activity of AuNPs in biological systems. Briefly, the main effect of AuNPs is catalysis of RedOx reactions, which includes  $O_2$  reduction, leading to the oxidation of electrons donors such as antioxidants. That eventually leads to oxidative stress. The RedOx reactions in biological systems are complex and not only include oxygen and  $H_2O_2$  as oxidants. Therefore, we suppose that biological observations of damage in the cells in the presence of AuNP should consider catalytic properties of AuNP in redox reactions.

## Author contributions

The contributions from V. Shcherbakov, according to 'CRediT – Contributor Roles Taxonomy', include conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing – original draft, writing – review & editing. The contributions from S. A. Denisov include conceptualization, formal analysis, validation, project administration, supervision, visualization, writing – original draft, writing – review & editing. The contributions from M. Mostafavi include conceptualization, formal analysis, validation, resources, project administration, supervision, visualization, writing – original draft, writing – review & editing.

## Conflicts of interest

There are no conflicts to declare.

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