RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

RSC Advances

RSCPublishing

COMMUNICATION

Difunctional biogenic Au nanoparticles for colorimetric detection and removal of Hg^{2+}

Cite this: DOI: 10.1039/x0xx00000x

Hao Zhou,[ab] Yuanyuan Qu,*[a] Duanxing Li,[a] Xuwang Zhang,[a] Qiao Ma,[a] Wenli Shen[a] and Jiti Zhou[a]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/journal

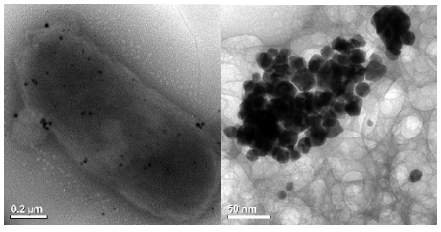
Biogenic Au nanoparticles (AuNPs) produced by *Cupriavidus metallidurans* SHE could act as colorimetric sensor and scavenger of Hg^{2+} based on biological reduction mediated formation of amalgam.

Mercury poses significant human health hazards even in trace amounts. Among those different mercury species, Hg^{2+} is the predominant form in aqueous phase and hydrosphere.[1] Therefore, developing high sensitive sensors for detecting Hg^{2+} quickly and precisely is indisputably important. Au nanoparticles (AuNPs) have been made as excellent sensor for Hg^{2+} due to the sensitivity arising from the high extinction coefficients of surface plasmon resonance (SPR) adsorptions or quenching effects.[2] Recently, there are more and more concerns on the biological synthesis of AuNPs (termed biogenic AuNPs) due to its cost effective, biocompatible and eco-friendly merits.[3] Nevertheless, reports about biogenic AuNPs based Hg^{2+} sensor are limited.

Cupriavidus metallidurans are well-established bacteria which harbor numerous metal resistant genes involving Mn^{2+}, Cu^{2+}, Zn^{2+}, and Hg^{2+} detoxification.[4] The model bacterium, *Cupriavidus metallidurans* CH34, attracted a striking concern for its unique capacity to take up highly toxic Au^{3+} complex and spit out pure bulky gold. The purple AuNPs were observed in the cultures of strain CH34.[5] However, neither the characterization nor the application of this biogenic AuNPs has been investigated.

Recently we isolated a strain termed SHE, which was identified as *Cupriavidus metallidurans* based on 16S rRNA gene sequence analysis.[6] Firstly, the AuNPs synthesis capacity of strain SHE was confirmed. The resting cells of *Cupriavidus* sp. SHE (OD_{660nm} = 2.0) were incubated with different concentrations of $HAuCl_4$ (0.5, 1, 2.5, 5 and 10 mM) for 48 h. After incubation, there were no observable color changes with 0.5, 1 and 2.5 mM $HAuCl_4$, while the cells incubated with 5 and 10 mM $HAuCl_4$ (termed $AuNPs_5$ and $AuNPs_{10}$) turned wine-red and dark green after 72 h, respectively (Fig. S1). After centrifugation at 3000 × *g* for 5 min, the supernatant became colorless. The pellet was washed twice using Milli-Q water, and further treated with 5 min water bath sonication. There was no absorbance of supernatant over 200-800 nm, which meant that most of the AuNPs were intracellular or membrane-associated[7]. These results showed a concentration-dependent AuNPs precipitation capacity of strain SHE. According to previous report, Au^{3+} was firstly transformed and accumulated as Au^{+}-S complex, then as a cellular defence mechanism, methylation of the Au^{+}-C complexes happened and the corresponded speciation was observed[5]. Therefore, the unobserved AuNPs in lower $HAuCl_4$ concentrations might be due to the excessive accumulation of Au^{+}-S and Au^{+}-C complexes.

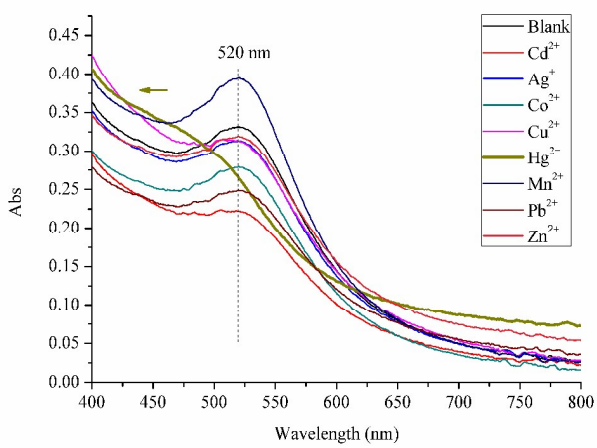
Next, the as-synthesized AuNPs were separated from bacterial cells through heating at 121°C for 20min , and characterized by UV-visible, dynamic light scattering (DLS) and transmission electron microscopy (TEM) analyses. The $AuNPs_5$ and $AuNPs_{10}$ showed the absorption peaks at 520 and 600 nm, respectively. DLS measurements of the AuNPs showed that the size distributions of $AuNPs_5$ and $AuNPs_{10}$ were 13.67±1.46 nm and 60.46±24.81 nm. The higher concentration of $HAuCl_4$, i.e. 10 mM, contained more reducing moieties, which increased the excess growth of AuNPs through secondary reduction process. Therefore, a larger diameter of AuNPs formed as a result. Given that a smaller size of AuNPs usually had better catalytic and sensing performance, $AuNPs_5$ was further characterized by TEM.[8] TEM images showed both microbe associated (Fig. 1a) and intracellular AuNPs (Fig. 1b) released after heating at 121 °C.



(a) (b)

Fig. 1 TEM images of biogenic AuNPs produced by *Cupriavidus metallidurans* SHE (5 mM $HAuCl_4$). (a) AuNPs formed around the cell wall of strain SHE. (b) Intracellular AuNPs.

Then, the application of AuNPs in colorimetric detection of various metal ions was assayed. 1 mM of Pb^{2+}, Hg^{2+}, Au^{3+}, Ag^{+}, Cu^{2+}, Co^{2+}, Mn^{2+} and Ni^{2+} were added into colloid AuNPs, respectively. The metal ions except Hg^{2+} showed no significant shift in the SPR band of AuNPs, and the colors of the dispersions remained wine-red for at least 24 h (Fig. 2a). Unlike the L-tyrosine mediated AuNPs, there was no significant response (red-shift) for Pb^{2+} in this case, which exhibited a better selectivity on Hg^{2+}.[9] In the case of Hg^{2+}, a significant shoulder peak of the SPR absorption spectra was observed, and the color of dispersions became slightly orange within 1 h. Meanwhile, gray precipitates could be observed in the bottom of tube after 12 h (Fig. 2b). This phenomenon was different from most of the AuNPs-based Hg^{2+} determination methods.[10] According to the previous report, a blue shift of SPR band usually meant the formation of core-shell Hg-Au amalgam, and the decrease in intensity depended on the thickness of Hg shell.[11] As the elemental mercury Hg^0 could be easily separated from the liquid, the biogenic AuNPs may act as both sensor and scavenger of Hg^{2+}.

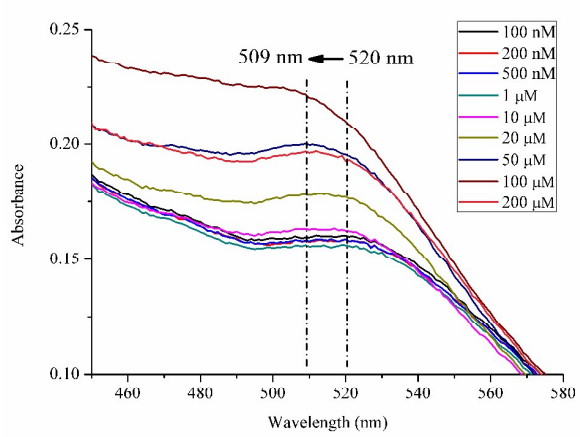


(a)

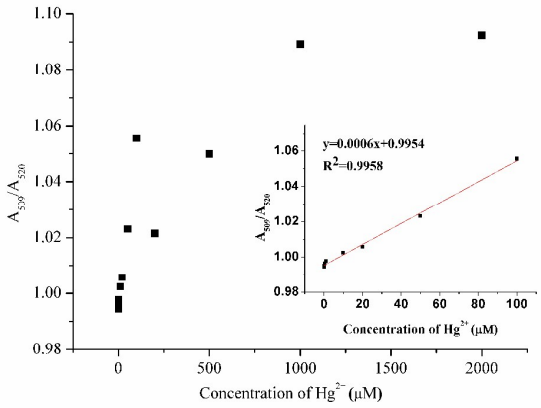
(b)

Fig. 2 (a) UV-visible absorption spectra of the biogenic AuNPs in the presence of 1 mM different metal ions. (b) AuNPs with (right) and without (left) 1 mM Hg^{2+}.

Due to the specificity on Hg^{2+}, the colorimetric detection of Hg^{2+} by biogenic AuNPs was investigated in details. In the assays with different Hg^{2+} concentrations (ranging from 100 nM to 2 mM), it was shown that the λ_{max} of SPR band firstly exhibited a gradually blue shift from 520 nm to 509 nm. Then, a shoulder peak appeared around 500 nm when Hg^{2+} concentration increased to 200 μM, and the original peak disappeared (Fig. 3a). The change of SPR band caused the color of the system changed from wine-red to orange. Fig. 3b showed the calibration curve for the detection of Hg^{2+} by AuNPs. When Hg^{2+} concentrations were in the range of 100 nM to 100 μM, a linear correlation between the absorbance ratio (A_{509}/A_{520}) and the Hg^{2+} concentration was observed (R^2=0.9958) with the lowest detection limit of 13.2 nM, which is comparable to the other AuNPs or AgNPs based methods (Table. S1).[9, 12-14] Therefore, the biogenic AuNPs could be used for the detection of Hg^{2+} in relatively wide concentrations (100 nM to 100 μM). When the concentration of Hg^{2+} was higher than 100 μM, it exhibited a non-linear change. The colorimetric sensor was also tested in a real drinking water sample. There was no Hg^{2+} could be detected using both AuNPs and ICP-MS analyses, thus standard solutions containing Hg^{2+} were spiked into the drinking water samples. The results showed that no matter Hg^{2+} existed alone, or coexisted with other metal ions, the concentrations detected by AuNPs was in agreement with the result of ICP analysis (Table. S2). It indicated this colorimetric sensor could be a simple and expedient method for Hg^{2+} determination in drinking water.



(a)



(b)

Fig.3 (a) UV-visible spectra of biogenic AuNPs with different concentrations (100 nM to 200 μM) of Hg^{2+}. (b) The calibration curve for the detection of Hg^{2+} by AuNPs.

Besides the colorimetric performance of biogenic AuNPs, the capacity of biogenic AuNPs for removing mercury was also assayed by ICP-MS. Five different initial concentrations of Hg^{2+} were added into the colloid AuNPs and incubated for 48 h. As shown in Fig. 4, at all tested concentrations, the Hg^{2+} in solution could be eliminated in different degree. The elimination process of mercury could be divided into two regimes: a quick drop of Hg^0 elimination rates from 60% to 23% (25 μM to 100 μM), and a much slower decrease from 23% to 13% (100 μM to 1000 μM). It could be explained by chemical adsorption of Hg^0 (quick stage) and formation of amalgam (slow stage). In other reports, the amalgamation of Au-Hg at higher

Hg^{2+} concentration and long exposure would always lead to the aggregation of particles, thus resulting in a significant red-shift in UV-visible spectrum.[1] However, in our case, the red-shift was not observed neither with even 5 mM Hg^{2+}, nor incubating the mixture for 144 h. The possible explanation should be that the coated biological molecules were charged, which could avoid the aggregation of larger amalgam particles by coulomb repulsion. This result showed that the biogenic AuNPs could remove Hg^{2+} efficiently.

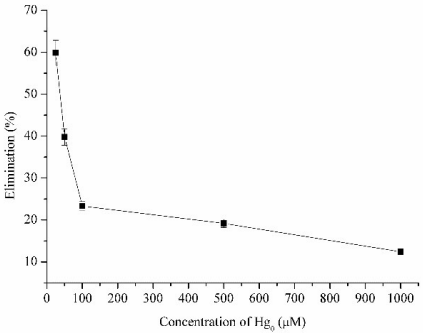
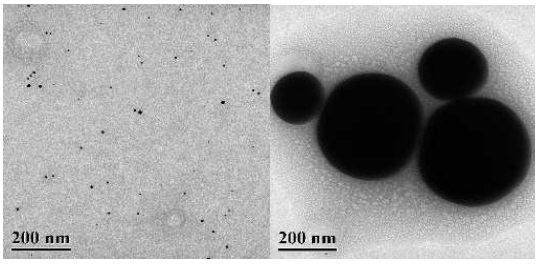
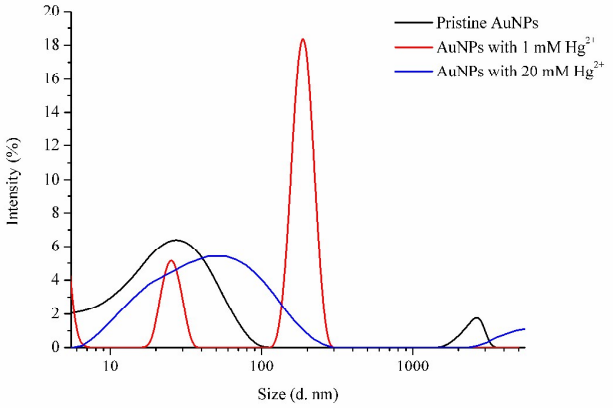


Fig.4 Remove of Hg_0 by biogenic AuNPs. The concentration of Au is 5.6 mg/L.

As we thought the blue shift of SPR band was caused by the formation of Au-Hg amalgam, TEM and DLS were further employed to compare the morphology and size distribution of AuNPs with different Hg^{2+} concentrations. This microscopic morphology change of AuNPs was shown in Fig. 5. When the Hg^{2+} content was 20 μM, the size of AuNPs did not have significant difference with the sole AuNPs (Fig. 5a and 5b). However, after adding 1 mM Hg^{2+}, the particles were still spheres but partly aggregated. In addition, there were some biological molecules adsorbed on the surface of nanoparticles. EDX analysis showed the local molar ratios of Hg/Au increased from 58.6/41.4 to 93.3/6.7 (atom %), which indicated AuNPs were embedded by thicker Hg shell at higher Hg concentrations (Fig. S3). The size distributions of amalgam particles were analysed by DLS (Fig. 5c). The original AuNPs and AuNPs with 20 μM Hg^{2+} had similar polydispersity, and the particle size of the latter had a slightly increase, which corresponded to the coated Hg_0 atoms. When 1 mM Hg^{2+} was incubated with AuNPs, the size distribution became narrower, and the average hydrodynamic diameter further increased to 203±27 nm. The larger particles should be a fusion of AuNPs embedded into the mercury-rich matrix. Thick Hg shell dramatically affected the SPR band of AuNPs, leading to the appearance of shoulder peak in UV-visible spectrum.



(a) (b)



(c)

Fig.5 TEM images and DLS analysis of AuNPs and Au-Hg amalgams. (a) AuNPs with 20 μM Hg^{2+}, (b) AuNPs with 1mM Hg^{2+}, (c) Z-average diameter of AuNPs and Au-Hg amalgams.

Since no extra reduction agents, such as $NaBH_4$ and sodium citrate, were added into the colloid AuNPs, it is interesting to know how Hg^{2+} was reduced. After AuNPs released from bacterial cells after sterilization, there would be many biological molecules, such as peptides, DNA and oligosaccharides, adsorbed on the surface of AuNPs due to their highly biological affinity.[15] Comparative experiment was performed using the sterilization product of the cells without AuNPs, and gray precipitates also appeared. Since the previous report showed free thiols in amino acid sidechain (such as cysteine) could convert Hg^{2+} to Hg_0, We determined whether the colloid AuNPs contained proteins or peptides by Bradford method.[16] This result showed protein/peptide existed in the system with a concentration about 11.4 μg/ml, which might mediate the Hg^{2+} reduction. The existence of proteins/peptides capping in the surface of AuNPs was also confirmed by FTIR studies (Fig. S4). The peaks centered at 1653 cm^{-1} and 1517 cm^{-1} corresponded to the amide I and amide II regions of proteins/peptides. In addition, the peaks centered at 1046 cm^{-1} in the crude extracts without AuNPs became two peaks centered at 1063 cm^{-1} and 1037 cm^{-1} when interacted with AuNPs, indicating the AuNPs may interacted with C-O group[17]. Meanwhile, although mercury reductases existed in many *Curpriavidus* spp. strains, they should not be the reducers of Hg^{2+} in this case due to the high temperature treatment.[18]Overall, the biogenic AuNPs generation from strain SHE and working mechanism was proposed in Fig. 6. The membrane associated Au^{3+} related reductase firstly reduced Au^{3+} to intracellular AuNPs, then AuNPs coated with biological molecule released into the solution after sterilization. Those peptides or amino acids with free thiols could reduce Hg^{2+} to Hg_0, and make Hg_0 easily adsorb on the surface of AuNPs. When the concentration of Hg^{2+} was relatively low (< 100 μM), AuNPs could be used as a colorimetric sensor for Hg^{2+} due to the chemical adsorption of Hg^0. Meanwhile, the biogenic AuNPs could remove biological reduced Hg through forming amalgam.

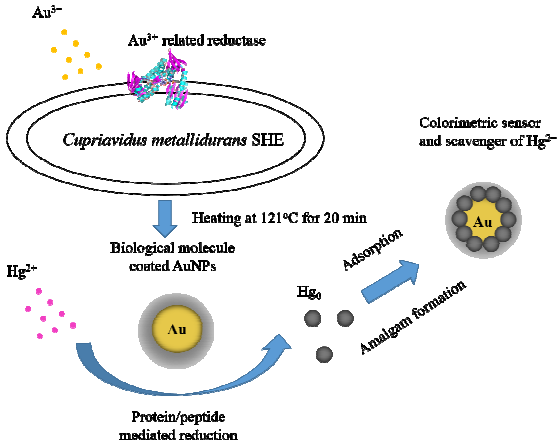


Fig.6 Schematic illustration of difunctional AuNPs generation and working mechanism.

In conclusion, we demonstrated the biogenic AuNPs from *Curpriavidus* sp. SHE had two roles when meeting Hg^{2+}: (1) an unusual blue-shift response for determining the concentrations of Hg^{2+} in a wide range, and (2) an effective mercury remover based on formation of Au-Hg amalgam. As many metabolic products of bacteria contain free thiols, this difunctional biogenic AuNPs should be easily synthesized by various other microbes. To the best of our knowledge, this is the first report about AuNPs without any extra modification could be used as an effective colorimetric sensor and scavenger of Hg^{2+}.

The authors gratefully acknowledge the financial supports from the National Natural Science Foundation of China (No. 21176040), the Program for New Century Excellent Talents in University (No. NCET-13-0077) and the Fundamental Research Funds for the Central Universities (No. DUT14YQ107). We also acknowledge Dr. Jun Ke of Dalian University of Technology for useful discussions.

Notes and references

[a] Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China. E-mail: qyy@dlut.edu.cn

[b] Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Food and Environmental, Dalian University of Technology, Panjin Campus, Panjin 124221, China.

† Electronic Supplementary Information (ESI) available: Experimental details, digital images of as-synthesized AuNPs and EDX spectra of AuNPs with different concentrations of Hg^{2+}. See DOI: 10.1039/c000000x/

1 I. Ojea-Jimenez, X. Lopez, J. Arbiol and V.puntes, *ACS Nano*, 2012, **6**, 2253.

2 C. W. Liu, Y. T. Hsieh, C. C. Huang, Z. H. Lin and H. T. Chang, *Chem. Commum.*, 2008, **19**, 2242.

3 (a) A. Mishra, M. Kumari, S. Pandey, V. Chaudhry, K. C. Gupta and C. S. Nautiyal, *Bioresour. Technol.*, 2014, **166**, 235; (b) A. Gangula, R. Podila, L. Karanam, C. Janardhana and A. M. Rao, *Langmuir*, 2011, **27**, 15288.

4 (a) T. von Rozycki and D. H. Nies, *Antonie van Leeuwenhoek*, 2009, **96,** 115; (b) P. Monsieurs, H.Moors, R. van Houdt, P. J. Janssen, A. Janssen, I. Coninx, M. Mergeay and N. Leys, *Biometals*, 2011, **24**, 1133-1151.

5. F. Reith, B. Estchmann, C. Grosse, H. Moors, M. A. Benotmane, P. Monsieurs, G. Grass, C. Doonan, S. Vogt, B. Lai, G. Martinez-Criado, G. N. George, D. H. Nies, M. Mergeay, A. Pring, G. Southam and J. Brugger, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 17757.

6. Y. Y. Qu, E. Shen, Q. Ma, Z. J. Zhang, Z. Y. Liu, W. L. Shen, J. W. Wang, D. X. Li, H. J. Li and J. T. Zhou. *J. Environ. Sci.*, Accepted.

7. P. S. Vijayakumar and B. L. V. Prasad, *Langmuir*, 2009, **25**, 11741.

8. K. Saha, S. S. Agasti, C. Kim, X. Li and V. M. Rotello, *Chem. Rev.*, 2012, **112**, 2739.

9. M. Annadhasan, T. Muthukumarasamyvel, V. Sankar Babu and N. Rajendiran, *ACS Sustainable Chem. Eng.*, 2014, **2**, 887.

10. (a) D. B. Liu, W. Qu, W. W. Chen, W. Zhang, Z. Wang and X. Y. Jiang, *Anal. Chem.*, 2010, **82**, 9606. (b) T. T. Lou, Z. P. Chen, Y. Q. Wang and L. X. Chen, *ACS Appl. Mater. Interfaces*, 2011, **3**, 1568.

11. T. Morris, H. Copeland, E. McLinden, S. Wilson and G. Szulczewski, *Langmuir*, 2002, **18**, 7261.

12. K. Farhadi, M. Forough, R. Molaei, S. Hajizadeh and A. Rafipour, *Sens. Actuators, B*, 2012, 161, 880.

13. R. M. Tripathi, R. K. Gupta, P. Singh, A. S. Bhadwal, A. Shrivastav, N. Kumar and B. R. Shrivastav, *Sens. Actuators, B*, 2014, 204, 637.

14. Y. Wang, F. Yang and X. R. Yang, *Biosens. Bioelectron.*, 2010, 25, 1994.

15. N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. Lytton-Jean, M. S. Han and C. A. Mirkin, *Science*, 2006, **312**, 1027.

16. A. G. Thawari, V.K. Hinge, M. Temgire and C. P. Rao, *RSC Adv.*, 2014, **4**, 53429.

17. J. Y. Xin, K. Lin, Y. Wang and C. G. Xia, *J. Korean. Soc. Appl. Bi*, 2015, **58**, 387.

18. N. Wiesemann, J. Mohr, G. Grosse, M. Herzberg, G. Hause, F. Reith and D. H. Nies, *J. Bacteriol*, 2013, **195**, 2298.

