Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Highly Selective Colorimetric Bacteria Sensing Based on Protein-Capped Nanoparticles

Suyan Qiu[a,b], Zhenyu Lin*[b], Yaomin Zhou[a], Donggen Wang[a], Lijuan Yuan[a], Yihua Wei[a], Tingcan Dai[a], Linguang Luo*[a], Guonan Chen[b]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A rapid and cost-effective colorimetric sensor has been developed for the determination of bacteria (bacillus subtilis was selected as an example). The sensor was designed relying on the lysozyme-capped AuNPs with advantages of effective amplification and high specificity. In the sensing system, lysozyme had a strong binding ability with bacillus subtilis, and induced a color change of the solution from light purple to purplish red effectively. The lowest detectable concentration for bacillus subtilis was 4.5×10^3 CFU/mL by the naked eye. And similar results were discernable via the UV-Vis absorption measurement. Particularly, a good specificity was perceived through the statistical analysis method using the SPSS software (version 17.0). This simple colorimetric sensor may therefore be a rapid and specific method for bacterial assay in complex samples.

Introduction

Diverse of bacteria are widespread in drinking water, food and food processing, which are closely related to our daily lives. The bacterial infection has been drawn increasing attention because a large number of bacteria can cause severe illness.[1] For the clinical diagnosis of infectious diseases, rapid determination and identification of bacteria is very important to time-consuming culturing. Therefore, it is essential to develop reliable methods for bacteria detection assay.[2] Several techniques are available including bacterial culture technologies,[3] polymerase chain reaction methodologies,[4] immunoassays,[5] quartz crystal microbalance analyses,[6] and electrochemical methods.[7,8] These techniques own the advantages of high sensitivity or good specificity, but most of them required trained operators, expensive reagents, and considerable sample processing prior to analysis.

Recently, optical sensors have been attracted much concern in various fields,[9,10] such as biomedical, industrial, automotive and electronics. Optical absorption and luminescence are widely used as optical transduction techniques. Among them, the colorimetric sensors[11] have become growing interesting in biological and chemical analysis by their various advantages, such as easy operation and miniaturization, without expensive and sophisticated instrumentation, and depending on the human eyes conveniently.[12-16] Up to now, various colorimetric sensors for diverse of analytes have been developed based on gold nanoparticles (AuNPs) with their numerous advantages including unique surface plasmon resonance, high surface area, excellent biocompatibility and distance-dependent optical properties.[17-20] What is more, the AuNPs capped with proteins can produce highly specific or multiple functions in the sensing system. The reason may lie in that those proteins may conduct the arrangement of enzymatically active composites and produce materials that identify substrates specifically.[21]

Herein, a novel colorimetric sensor for monitoring the level of bacteria was designed based on lysozyme-capped AuNPs. Bacillus species, gram-positive bacteria with low-pI, allow the characteristics of omnipresence in soil and high thermal tolerance, and can rapidly grow in liquid culture and form resistant spores readily. Several bacillus species are well known to cause diseases in humans.[22] For example, bacillus anthracis[23] can cause the acute infectious anthrax disease by eating or inhaling the foods contaminated with anthrax spores.[24] Moreover, the bacillus anthrax is considered to be a potential biological weapon in view of its high toxicity and fast infection. It is of great importance for human health to develop a simple and rapid method for the bacillus anthracis detection.[25,26] Taking into account its toxicity and infection, bacillus subtilis that considered to be a safe biological agent has been often used as a simulant for bacillus anthracis because of their closely related genetic.[27] So in this study, bacillus subtilis was selected as a model to demonstrate the feasibility of this colorimetric assay. Our early report showed[28] that the AuNPs solution could be induced to aggregate together by lysozyme, and accompanied with the solution color changing from red to purple. In this study, it was noteworthy that the AuNPs was dispersed again in the solution when bacillus subtilis had been added into the lysozyme-capped AuNPs solution. Meanwhile, the color of the solution changed from purple to purplish red. The reason may lie in that spore coats of bacillus subtilis contain a large number of low-pI proteins which are sensitive to lysozyme.[29] Based on these, a sensitive and specific colorimetric sensor was established for the bacillus subtilis determination.

Materials and Methods

Chemicals

Lysozyme, $HAuCl_4$, trisodium citrate, ployvinylpyrrolidone (PVP, Mw = 40000) and other reagents were purchased from Alfa Aesar China (Tianjin) Co. Ltd. and used as received. Without special indication, all reagents and solvents were of analytical grade or better and used directly without further purification. Bacillus subtilis, escherichia coli and staphylococcus aureus were cultivated by Fuzhou University.

The pH value of 3-(N-morpholino)-propane sulfonic acid (MOPS, 10 mM) buffer solution was adjusted to be 7.5 with NaOH solution.

Instruments

Zeta potential (ζ) and dynamic light scattering (DLS) were performed on a Brookhaven Instrument with He/Ne laser at 632.8 nm at scattering angles of 90 at 25 ^{o}C (Brookhaven Instrument Corporation, USA). Transmission electron microscopy (Tecnai 20, FEI-Philips) were used to obtain high-resolution images with a field emission gun operating at 200 kV. UV-Visible (UV-Vis) absorption spectra were measured by a Lambda 750 UV-Vis spectrophotometer (PerkinElmer, American) at room temperature. The photographs were taken with a Panasonic DMC-FX35 digital camera (Panasonic, Japan). Raman spectra were acquired on a Renishaw inVia laser Raman spectrometer (Renishaw PLC, Gloucestershire, UK) with an excitation laser at 532 nm.

Preparation of AuNPs solution

AuNPs with an average diameter of 13 nm were prepared according to the literature.[30] Briefly, all glassware used in the preparation was cleaned in aqua regia (1 part HNO_3, 3 parts HCl). A solution of $HAuCl_4$ (0.206 mg/mL) in 25 mL of deionized water was stirred and heated to reflux, and then added 1.0 mL 1.3% trisodium citrate solution into the above hot water. The above solution was heated under reflux with vigorous stirring for another 15 min, and accompanied by the variation of color from pale yellow to deep red. Finally, the solution was cooled to room temperature with a slow and continuous stirring, and then was stored in 4.0 ^{o}C for later experiment. The morphology of AuNPs solution was measured by TEM (The image was displayed in Fig. S2 in the Supplementary Information).

Preparation of lysozyme-capped AuNPs solution

100 μL AuNPs and ployvinylpyrrolidone (PVP, 0.25 mM) were added into MOPS buffer solution, incubated for 2 min to form PVP-capped AuNPs solution, which can help to remain the high stability of AuNPs for a long time. Then lysozyme was added into the above PVP-capped AuNPs solution, incubated for 5 min to form lysozyme-capped AuNPs solution. Finally, the above solution was subjected to UV-Vis spectrophotometer measurement.

Detection of bacillus subtilis

The quantitation of bacillus subtilis was measured by plating serial dilutions of the bacterial and counting the number of colonies before use. Subsequently, bacillus subtilis were separated from culture medium by centrifuging at 4000 rpm for 5.0 min and washed with deionized water for three times. The supernatant was discarded and the remaining bacillus subtilis was re-dispersed in 1.0 mL MOPS buffer solution, and stored at 4.0 ^{o}C. For bacterial detection, various amounts of bacillus subtilis were added into the lysozyme-capped AuNPs solution, shaken for 20 s. And then the above mixture was subjected to UV-Vis spectrophotometer measurement. To reduce the interference from the background for the response signal of the sensing system, the ratio of the absorption intensity at 521 and 602 nm was used to analysis. All measurements were repeated three times and the standard deviation was calculated as the error analysis.

It is difficult to recognize the differences among the similar responses by depending on the UV-Vis absorption spectra directly. While the subtle differences in UV-Vis absorption response patterns can be discriminated in the canonical score plot by the statistical analysis, in which discriminant functions were deduced by maximizing the separation of the variation within classes. Therefore, the statistical analysis method was used to investigate the specificity of the sensing system by the SPSS software, and the data was summarized in the canonical score plot through transforming the raw data to canonical factors.

Results and discussion

Mechanism of the colorimetric sensor

The AuNPs capped with proteins can not only promote the specificity of the sensing system because of the selective interaction between proteins and targets, but also amplify the sensing signal due to their excellent interaction between AuNPs and proteins. Since lysozyme was very sensitive to the bacteria, the lysozyme-capped AuNPs was used to develop a sensitive and selective sensing system for bacteria detection in this study, in which bacillus subtilis was selected as an example. Upon the addition of bacillus subtilis into the lysozyme-capped AuNPs solution, the electrostatic interaction and hydrogen bond formed easily between lysozyme and bacillus subtilis in a short time.[31-35] The lysozyme was removed from the surfaces of AuNPs, and accompanied by the solution color changed from purple to purplish red which may be attributed to the fact that a part of aggregated AuNPs was re-dispersed well in the solution (see Fig. 1). Based on which, a colorimetric sensor with high specificity and sensitivity was established to detect the bacillus subtilis.

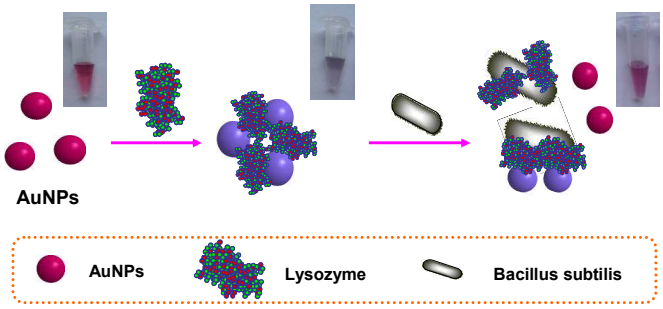

Fig. 1 Schematic of the colorimetric sensor for bacillus subtilis detection

Characteristics of the colorimetric sensor

The UV-Vis absorption lysozyme-capped AuNPs solution in

the presence and absence of bacillus subtilis were investigated to confirm our concept. As shown in Fig. 2, the color of lysozyme-capped AuNPs solution was light purple (Inset in Fig. 2Ba) and the maximum absorption peak was at around 630 nm (Fig. 2Aa). However, the color of the solution changed from light purple to purplish red with the addition of bacillus subtilis into the lysozyme-capped AuNPs solution (Inset in Fig. 2Bb). We concluded that the color of purplish red may contain both red and purple color. This deduction was further verified through the corresponding UV-Vis absorption. As can be seen in Fig. 2Ab, it was well known that the absorption intensity at about 521 nm increased, while the intensity at the other absorption band shifted from 630 nm to 600 nm increased as well. The reason may lie in that bacillus subtilis with negative charge could couple with lysozyme through electrostatic interaction and hydrogen bond, which was confirmed by Raman measurement (See Fig.S1 in the Supplementary Information). However, owing to the large size of bacillus subtilis, a part of bacillus subtilis was induced to combine directly with lysozyme that remained on the surfaces of the aggregated AuNPs. While the other part of bacillus subtilis was attached onto lysozyme which was desorbed from the surfaces of AuNPs, causing the AuNPs re-dispersed well in the solution. As a consequence, a color of purplish red including both red and purple was recognized obviously in the solution.

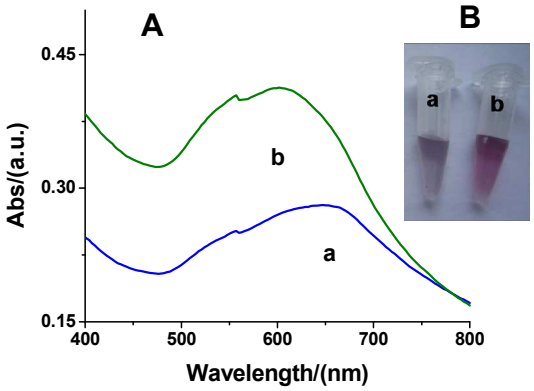

Fig. 2 (A) UV-Vis absorption spectra and the corresponding solution color (B) of the lysozyme-capped AuNPs solution in the absence (a) and presence of 6.0×10^4 CFU/mL bacillus subtilis (b).

To further certify the proposed mechanism, zeta potential (ζ) measurements and the average hydrodynamic diameters of a series of AuNPs solution were performed. ζ potential was measured for 40 times for each sample, and the average value was calculated for analysis and summarized in Table S1 (See Supplementary Information). The charge on lysozyme-capped AuNPs solution was -11.47 mV, which attributed to the fact that the AuNPs solution was induced from well disperse to aggregate together by lysozyme.[28] While the charge (-21.95 mV) was close to that of the well-dispersed AuNPs solution (-23.39 mV) with the addition of bacillus subtilis into the lysozyme-capped AuNPs solution. It was because that the aggregated AuNPs was dispersed again in the presence of bacillus subtilis. Similar results were exhibited in the dynamic light scattering data as well (See Fig. 3). The average hydrodynamic diameter of lysozyme-capped AuNPs solution that distributed mainly in the range of 966.7 nm to 2783.3 nm (Fig. 3b) was much larger than that of the PVP-capped AuNPs with the main range from 46.9 nm to 97.1 nm (Fig. 3a). This result may be caused by the fact that the PVP-capped AuNPs was induced to aggregate together by lysozyme. While it was perceived apparently that the hydrodynamic diameter intensity at the range of 966.7 nm to 2783.3 nm sharply decreased when bacillus subtilis (1.5×10^5 CFU/mL) was added into the lysozyme-capped AuNPs solution (Fig. 3c). Besides, it was noteworthy that both new obvious hydrodynamic diameter distributions were discovered. One was over the range from 58.1 nm to 96.2 nm which was well overlap with the range of PVP-capped AuNPs. We concluded that a part of AuNPs was well dispersed again in the solution. Another part that distributed in the range from 4501.8 nm to 6709.5 nm which maybe attributed to the both complexes of bacillus subtilis with lysozyme that was free in the solution and remained on the surface of AuNPs.

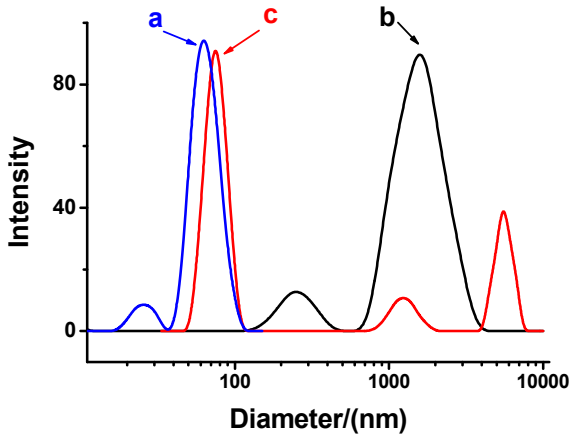

Fig. 3 DLS meaurements of AuNPs solution in different conditions: (a) PVP-capped AuNPs solution; (b) lysozyme-capped AuNPs solution; (c) lysozyme-capped AuNPs solution with the addition of bacillus subtilis (1.5×10^5 CFU/mL).

The effect of lysozyme concentration on the sensor

Since the UV-Vis absorption intensity was influenced by lysozyme concentration, the effect of lysozyme concentration was explored. Fig. S3 exhibited the change of the value of A_{521}/A_{602} ratio (where A_{521} and A_{602} indicated the UV/Vis absorption intensities at 521 nm and 602 nm, respectively.) upon adding different concentrations of lysozyme into the sensing system. The ratio decreased sharply upon increasing the concentration of lysozyme, and saturated at over 1.0 μg/mL. Therefore, 1.0 μg/mL of lysozyme was used for the later experiments.

The sensitivity of the colorimetric sensor for bacillus subtilis assay

Various concentrations of bacillus subtilis were added into the lysozyme-capped AuNPs solution. The color of the solution and the UV-Vis absorption intensity were monitored. As presented in Fig. 4, the color of the solution gradually changed from purple to purplish red with increasing of bacillus subtilis concentration, which could be discernable by the naked eye conspicuously. And the lowest detectable concentration by the naked eye was as low as 4.5×10^3 CFU/mL (Fig. 4A). Meanwhile, the corresponding UV-Vis absorption intensity increased at around 521 nm

progressively, while the other absorption band shifted from 630 nm to 550 nm, and accompanied with the upward intensity as well (Fig. 4B). The value of A_{521}/A_{602} increased sharply at the range of 4.5×10^3 CFU/mL to 1.5×10^5 CFU/mL, and reached a plateau at over 1.5×10^5 CFU/mL (Fig. 4C). Furthermore, a good linear relationship was observed between the value of A_{521}/A_{602} and bacillus subtilis concentration in the range of 7.5×10^3 CFU/mL to 1.5×10^5 CFU/mL. The regression equation was expressed as:

$$A_{521}/A_{602} = 1.75\times10^{-6}\, C_{Bacillus} + 0.92$$

Where A_{521} and A_{602} represented the UV-Vis absorption intensities of the sensing system at 521 nm and 602 nm, respectively, $C_{Bacillus}$ was the concentration of bacillus subtilis. The correlation coefficient (R) was 0.9825, and the limit of detection (LOD) was estimated to 2.57×10^3 CFU/mL by the UV-Vis absorption measurement (signal/noise = 3). These results indicated that the presented sensor provided a reliable method to determine the bacteria.

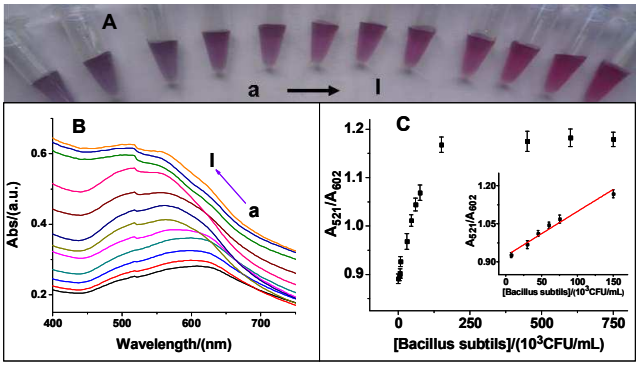

Fig. 4 Sensitivity of this assay for bacillus subtilis by the color change and UV-Vis spectroscopy. (A) Color change with increasing concentrations of bacillus subtilis, from a to l were 0.0 CFU/mL, 4.5×10^3 CFU/mL, 6.0×10^3 CFU/mL, 7.5×10^3 CFU/mL, 3.0×10^4 CFU/mL, 4.5×10^4 CFU/mL, 6.0×10^4 CFU/mL, 7.5×10^4 CFU/mL, 1.5×10^5 CFU/mL, 4.5×10^5 CFU/mL, 6.0×10^5 CFU/mL and 7.5×10^5 CFU/mL, respectively. (B) Their corresponding absorbance responses and (C) plots of the A_{521}/A_{602} ratio value versus bacillus subtilis concentrations, where A_{521} and A_{602} indicated the UV/Vis absorption intensities at 521 nm and 602 nm, respectively.

The specificity of the colorimetric sensor

Taken into account the fact that the sensor was designed based on the interaction between lysozyme and bacteria through the electrostatic interaction, which may suffer from the interference of other common proteins and amino acids with different kinds of charge, thus some common proteins and amino acids were selected to demonstrate the selectivity of the presented sensor. As shown in Fig. 5A, the color had no significant variation for most of solutions (which remained purple color) except low-pI proteins (BSA, OB and HSA) and bacterias (bacillus subtilis, escherichia coli and staphylococcus aureus) whose colors were red or purplish red. We reasoned that these species except proteins and bacterias were high-pI, and could not couple with lysozyme because of the electrostatic repulsion force between them, resulting in the AuNPs solution was induced to aggregate together by lysozyme, a purple color was remained in these mixed solutions. While the low-pI species including BSA, OB and HSA which has a strong ability of electrostatic interaction with the positive lysozyme was capable of removing lysozyme from the AuNPs surfaces, inducing the AuNPs to disperse well in the solution and display a red color. However, only the color of bacterial solution was purplish red. In view of the bacterial surface containing a large number of proteins with low pI, contributed to bacteria allowing the same binding ability with lysozyme. However, owing to the large size of bacteria, a part of bacteria was induced to combine directly with lysozyme that kept on the surface of the aggregated AuNPs. Another part of them was attached onto lysozyme which was removed from the AuNPs surfaces, induced AuNPs was dispersed well in the solution. As a result, a color of purplish red including both red and purple was observed in the solution. In order to distinguish the results for better, the resulting data from UV-Vis absorption spectra was analyzed through statistical analysis using the SPSS 17.0 package,[36] and transformed the raw data into three canonical factors. The variation of the three canonical factors containing 70.165%, 28.85% and 0.896% were presented in the canonical score plot of the Fig. 5C, where each dot represented the UV-Vis absorbance response pattern of a single amino acid or protein by the proposed sensor. It was revealed that three distinct clusters which were corresponding to the three colors of the solutions (purplish red, purple and red) were observed clearly in the canonical plot. These results indicated that the proposed colorimetric sensor was selective enough to differentiate bacteria from other common amino acids and proteins.

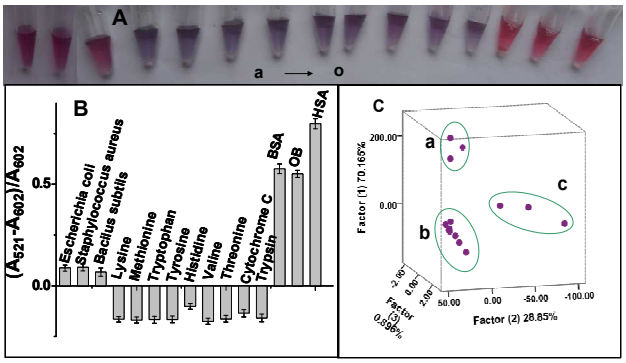

Fig. 5 Effects of several amino acids and proteins on the sensing system. (A) Color change with several common amino acids and proteins. From a to o were escherichia coli, staphylococcus aureus, bacillus subtilis, lysine, methionine, tryptophan, tyrosine, histidine, valine, cytochrome C, hemoglobin, trypsin, ovalbumin (OB), bovine serum albumin (BSA) and human serum albumin (HSA), respectively. (B) The corresponding variation of the $(A_{521}- A_{602})/A_{602}$ value, where A_{521} and A_{602} indicated the UV/Vis absorption intensities at 521 nm and 602 nm, respectively. (C) Canonical score plot of the three factors of the UV-Vis absorbance response patterns, area a was attributed to bacillus subtilis, escherichia coli and staphylococcus aureus; area b was attributed to other amino acids and proteins. area c was attributed to OB, BSA and HSA. Concentration of all species was 50 μg/mL except bacillus subtilis, escherichia coli and staphylococcus aureus (1.5×10^5 CFU/mL)

Conclusions

We have demonstrated a simple and selective colorimetric sensor for the determination of bacteria based on the protein-capped

AuNPs. And bacillus subtilis was used as a model to demonstrate the feasibility of this sensing system. In the presence of bacillus subtilis, the color of the solution changed from purple to purplish red, and accompanied by the absorption intensity upward. Moreover, this process allowed a good proportional response to bacillus subtilis concentrations through the UV-Vis absorption measurement. And the lowest detectable concentration by the naked eye was 4.5×10^3 CFU/mL. Additionally, statistical analysis revealed that the sensing system can differentiate bacteria from other common amino acids and proteins readily. Taking into account the numerous advantages of the proposed colorimetric sensor including simplicity, rapid response, easy operation and cost effectiveness, this sensor could be explored as other bacteria sensing system, and may have widely potential applications in clinical diagnosis of infectious diseases.

Acknowledgements

This project was financially supported by the National Basic Research Program of China (No. 21175024, 41076059), Program for Changjiang Scholars and Innovative Research Team in University (No. IRT1116), the innovation fund of Jiangxi Academy of Agricultural Sciences (No. 2013CBS001) and National projects for Quality & Safety Risk Assessment of Lives tock and Poultry Products of China (No. GJFP2014007).

Notes and references

[a] *MOE Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350002, China.*
Fax/Tel: 86-591-22866135; E-mail: zylin@fzu.edu.cn (Zhenyu Lin).
[b] *Institute for Quality & Safety and Standards of Agricultural Products Research, Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi, 330200, China.*
Fax/Tel: 86-0791-87090293: E-mail: luolinguang@126.com (Linguang Luo).

† Electronic Supplementary Information (ESI) available: [Raman measurement, TEM image of the Au nanoparticles, the result of zeta potential (ζ) and the optimization of lysozyme concentration]. See DOI: 10.1039/b000000x/

1 O. R. Miranda, X. Li, L. Garcia-Gonzalez, Z. Zhu, B. Yan, U. H. F. Bunz and V. M. Rotello, *J. Am. Chem. Soc.* 2011, **133**, 9650-9653.
2 H. W. Cheng, Y. Y. Chen, X. X. Lin, S. Y. Huan, H. L. Wu, G. L. Shen and R. Q. Yu, *Anal. Chim. Acta* 2011, **707**, 155-163.
3 D. Ivnitski, I. Abdel-Hamid, P. Atanasov and E. Wilkins, *Biosens. Bioelectron*. 1999, **14**, 599-624.
4 S. Pashtiban, M. A. Javed, Arutyunov D., A. Singh, G. Banting, C. M. Szymanski and S. Evoy, *Analyst* 2013, **138**, 5619-5626.
5 M. Magliulo, P. Simoni, M. Guardigli, E. Michelini, M. Luciani, R. Lelli and A. Roda, *J. Agric. Food Chem.* 2007, **55**, 4933-4939.
6 Z. Shen, M. Huang, C. Xiao, Y. Zhang, X. Zeng and P. G. Wang, *Anal. Chem.* 2007, **79**, 2312-2319.
7 J. D. Besant, D. Jagotamoy, E. H. Sargent and S. O. Kelley, *ACS Nano* 2013, **7**, 8183-8189.
8 M. D. Morales, B. Serra, A. G. Prada, Á. J. Reviejo and J. M. Pingarrón, *Analyst* 2007, **132**, 572-578.
9 Q. Li, K. Sun, K. Chang, J. Yu, D. T. Chiu, C. Wu and W. Qin, *Anal. Chem.* 2013, **85**, 9087-9091.
10 C. McDonagh, C. S. Burke and B. D. MacCraith, *Chem. Rev.* 2008, **108**, 400-422.
11 E. Jentzsch and A. Mokhir, *Inorg. Chem.* 2009, **48**, 9593-9595.
12 V. Chandrasekhar, S. Das, R. Yadav, S. Hossain, R. Parihar, G. Subramaniam and P. Sen, *Inorg. Chem.* 2012, **51**, 8664-8666.
13 C. Männel-Croisé, C. Meister and F. Zelder, *Inorg. Chem.* 2010, **49**, 10220-10222.
14 P. Das, A. Ghosh and A. Das, *Inorg. Chem.* 2010, **49**, 6909-6916.
15 D. Maity and T. Govindaraju, *Inorg. Chem.* 2011, **50**, 11282-11284.
16 W. Qu, Y. Liu, D. Liu, Z. Wang and X. Jiang, *Angew. Chem. Int. Ed*. 2011, **50**, 3442-3445.
17 K. Saha, S. S. Agasti, C. Kim, X. Li and V. M. Rotello, *Chem. Rev.* 2012, **112**, 2739-2779.
18 Y. Leng, Y. Li, A. Gong, Z. Shen, L. Chen and A. Wu, *Langmuir* 2013, **29**, 7591-7599.
19 Y. Zhang, Y. Leng, L. Miao, J. Xin and A. Wu, *Dalton Trans.* 2013, **42**, 5485-5490.
20 Y. Leng, F. Zhang, Y. Zhang, X. Fu, Y. Weng, L. Chen and A. Wu, *Talanta* 2012, **94**, 271-277.
21 A. Makhal, S. Sarkar and S. K. Pal, *Inorg. Chem.* 2012, **51**, 10203-10210.
22 J. M. Lim, C. O. Jeon, S. M. Song and C. J. Kim, *Int. J. Syst. Evol. Microbiol*. 2005, **55**,165-170.
23 J. L. Sagripanti, M. Carrera, J. Insalaco, M. Ziemski, J. Rogers and R. Zandomeni, *J. Appl. Microbiol.* 2007, **102**, 11-21.
24 J. Chenau, F. Fenaille, E. Ezan, N. Morel, P. Lamourette, P. L. Goossens and F. Becher, *Anal. Chem*. 2011, **83**, 8675-8682.
25 S. Yitzhaki, E. Zahavy, C. Oron, M. Fisher and A. Keysary, *Anal. Chem*. 2006, **78**, 6670-6673.
26 I. C. Fernández-No, M. Guarddon, K. Böhme, A . Cepeda, P. Calo-Mata and J. Barros-Velázquez, *Food Microbiol.* 2011, **28**, 605-610.
27 P. G. Luo, H. Wang, L. Gu, F. Lu, Y. Lin, K. A. Christensen, S. Yang and Y. Sun, *ACS Nano* 2009, **3**, 3909-3916.
28 H. Zhen, S. Qiu, K. Xu, L. Luo, Y. Song, Z. Lin, L. Guo, B. Qiu and G. Chen, *Analyst* 2013, **138**, 6517-6522.
29 D. C. McPherson, H. Kim, M. Hahn, R. Wang, P. Grabowski, P. Eichenberger and A. Driks, *J Bacteriol*. 2005, **187**, 8278-8290.
30 D. Liu, W. Chen, J. Wei, X. Li, Z. Wang and X., Jiang, *Anal. Chem*. 2012. **84**, 4185-4191.
31 C. Edwards, *J. Gen. Microbiol.* 1980, **119**, 277-279.
32 G. A. Wilson and K. F. Bott, *BBA*. 1970, **199**, 464-475.
33 M. Nomura, J. Hosoda and S. Nishimura, *BBA*. 1958, **29**, 161-167.
34 B. D. Westmacott and H. R. Perkins, *J. Gen. Microbiol.*1979, **115**, 1-11.
35 R. Kuwana, T. Okumura, H. Takamatsu and K. Watabe, *FEMS Microbiol. Lett*. 2005, **242**, 51-57.
36 Y. Zhao, B. Zhang, G. Chen, A. Chen, S. Yang and Z. Ye, *J. Agric. Food Chem*. 2013, **61**, 7055-7060.