NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

Journal Name

ARTICLE

Colorimetric β-lactamase inhibitor assay with double catalyzed signal amplification

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chen Chen[a,b], Jun Lv[b], Wanyi Chen[a], Yang Xiang*[c], Juan Zhang*[a,b]

A colorimetric method is developed for the determination of the activity of β-lactamase (β-Lac) and the screening of its inhibitors based on cysteine-induced gold nanoparticles (AuNPs) aggregation. The widely used penicillin is served as β-Lac substrate, and the strong reduction capability of its corresponding product which can react with Cu(II) to form Cu(I) conjugates, is utilized in the method. The double catalyzed signal amplification is accomplished via the addition of both β-Lac and Cu(II) which can catalyze the oxidization of cysteine into cystine. The activity of β-Lac is determined at levels as low as 2.36 U/mL. The inhibitory effects of clavulanic acid and sulbactam are tested and give 0.92 and 0.06 μM, respectively. The assay is simple, rapid, easy-operated, and selective, and the proposed method has a great potential not only for the detection of β-Lac activity but also for the screening of the inhibitors.

Introduction

Currently, the increased resistance of bacterial infections to antibiotic treatment has been extensively documented[1]. β-Lactamases (β-Lac) are a family of bacterial enzymes that can hydrolyze the β-lactam ring in penicillins and cephalosporins with high catalytic efficiency and render the bacteria resistant to the β-lactam antimicrobial reagents[2]. Therefore, detecting β-Lac activity and screening their inhibitors in biological samples before conducting the efficient antibiotic therapy is extremely important clinically.

Up to now, different methods, such as fluorescence spectrometry[3], colorimetric method[4], and titration method[5], have been used for the detection of β-Lac activity and the screening of the inhibitors. Liu et al. synthesized fluorescent β-Lac substrate containing a linker, and detected the enzyme activity by using gold nanoparticles (AuNPs) aggregation induced by the linker which can be released after β-Lac hydrolysis[6]. Zhang et. al. synthesized a methoxyimino cephalosporin derivative containing a pair of fluorescence resonance energy transfer fluorophores, and rapidly distinguished bacterial cells that are either sensitive or resistant to broad-spectrum β-lactam antibiotics[7]. Those methods have some major drawbacks, such as laborious manipulation, time-consuming processes, high reliance on specific instrumentation, and limited chemical stability and aqueous solubility of the individual substrates. Therefore, it is highly desirable to establish a simple and economical method for the determination of β-Lac activity and the screening of the inhibitors.

AuNPs have served as a versatile platform for exploring many facets of basic science because of its interesting optical and electronic properties, for instance, plasmon coupling that may red-shift the resonance wavelength[8, 9]. Normally, the exact plasmon resonance band can be determined by the size, shape, medium, and the relative distance between the particles[10, 11]. The different agglomeration states of AuNPs can result in distinctive colorchanges[12]. This extraordinary optical phenomenon makes AuNPs ideal chromogenic probes for reporting molecular recognition events, for example, colorimetric detection of DNA hybridization[13, 14], carbohydrate sensing[15, 16], sensitive measurement of metal ions, optical study of a single virus, and monitoring special enzyme activities such as alkaline phosphatases, kinases, proteases, etc.[14, 17, 18].

Numerous experiments have demonstrated that Cu(II) can catalyze the oxidation of cysteine (Cys) into cystine, resulting in the transformation of AuNPs agglomeration states from aggregation to dispersion[19]. Meanwhile, β-Lac can catalyze the hydrolysis of penicillin (Pen) into penicilloic acid which reacts with Cu(II) to form Cu(I) conjugates, leading to the decrease of the quantity of Cu(II) in the reaction system. Based on these, we have developed a novel and easily operational chromogenic assay based on AuNPs aggregation. This method can be used to rapidly identify the β-Lac activity and screen the enzyme inhibitors in a high throughput fashion by a simple colorimetric reader. On the one hand, the widely used substrate, Pen, has been utilized in the method and it does not need to synthesize any complex substrate. On the

[a] State Key Laboratory of Dairy Biotechnology, Technology Center of Bright Dairy & Food Co., Ltd., Shanghai 200436, P. R. China.
[b] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, P. R. China.E-mail: xiangy@nju.edu.cn; Fax:+86 25 89685616
[c] School of Life Sciences, Nanjing University, Nanjing 210093, P. R. China.E-mail: juanzhang@shu.edu.cn; Fax:+86 21 66137037
* Corresponding authors.

other hand, in this detection system, enzymes freely react with substrates, escaping from the steric hindrance and slow enzyme kinetics caused by the bulky conjugation of enzyme substrates with AuNPs. This type of assay is less sterically hindered, exhibits fast enzyme kinetics, and is more efficient in monitoring the enzyme activity without functionalization of the AuNPs. More importantly, both enzyme catalysis and Cu(II) catalysis have been well utilized and the double catalysis undoubtedly amplifies the detection signal to a great extent so as to enhance the sensitivity of the proposed method.

Experimental section

Materials and reagents

Cys, sulfate copper, gold acid chloride trihydrate, citric acid trisodium salt dehydrate, clavulanic acid, and sulbactam were purchased from Sigma-Aldrich Co. (Shanghai, China). Pen and β-Lac (Blas, EC 3.5.2.6, 1000 U/mg) were obtained from Aladdin Co. (Shanghai, China). All buffers and aqueous solutions were prepared with ultrapure water, which was purified with a Millipore Milli-Q water purification system (Branstead, USA) to a specific resistance of 18 MΩ cm.

Synthesis of AuNPs

AuNPs were synthesized according to a published procedure[20]. Briefly, 10 mL trisodium citrate (38.8 mM) was added to a boiling solution of $HAuCl_4$ (100 mL, 1 mM), and the resulting solution was kept continuously boiling for another 30 min to give a wine red mixture which was cooled to room temperature. Then the mixture was filtrated through a Millipore syringe (0.45 μm) to remove the precipitate and the filtrate was stored in a refrigerator at 4 °C for use.

β -Lac activity assay

β -Lac and Cys solution were obtained through dissolution into the phosphate buffer solution (10 mM, pH 7.4). 10 μL β-Lac solution with different concentrations from 0.1 to 11 U/mL was added into 20 μL Pen aqueous solution (20 mM) and the resulting mixture was allowed to stand by at 30 °C for 30 min. Then 20 μL sulfate copper aqueous solution (10 μM) was added into the reaction mixture. After 10 min, 40 μL cysteine solution (1 mM) was further mixed and continuously incubated for 10 min, followed by the addition of AuNPs solution (50 μL). After 10 min, the reaction solution were photographed with a digital camera (NET-3NL, SONY, Japan) and used for UV-vis spectroscopic measurements (Shimadzu Co., Kyoto, Japan).

Inhibition efficiency evaluation

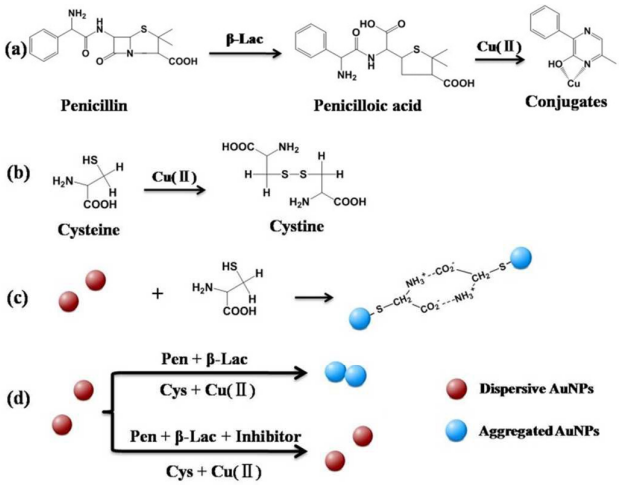
For the inhibition assay, clavulanic acid or sulbactam with different concentrations (5 μL) were firstly premixed with β-Lac (5μL) for 10 min at 30 °C. Then Pen (20 μL, 20 mM) was added into the mixed solution and continuously incubated for 30 min at 30°C. Subsequently, sulfate copper (20 μL) was added into the resulting mixture to obtain its final concentration of 10 μM. After 10 min, Cys solution (40 μL, 1 mM) was mixed with the mixture and successively reacted for 10 min, followed by the addition of AuNPs solution (50 μL). After 10 min, the reaction solution was photographed and the absorbance of each sample was recorded using UV-vis spectroscopy with the IC_{50} value calculated. The inhibitory ratio (%) of clavulanic acid or sulbactam on β-Lac was expressed as follows:

$$\text{Inhibitory ratio (\%)} = \frac{A_{657}/A_{533} - A^{*}_{657}/A^{*}_{533}}{A^{0}_{657}/A^{0}_{533} - A^{*}_{657}/A^{*}_{533}} \times 100$$

where A_{657}/A_{533} was the ratio of the absorbance value at 657 nm to that at 533 nm in the presence of both the inhibitor and the enzyme, A^{0}_{657}/A^{0}_{533} was the ratio of the absorbance value at 657 nm to that at 533 nm without the enzyme and inhibitor, A^{*}_{657}/A^{*}_{533} was the ratio of the absorbance value at 657 nm to that at 533 nm in the presence of enzyme only.

Results and discussions

Mechanism investigation for β-Lac activity assay



Scheme 1 Schematic illustration of the mechanism for (a) the catalytic reaction of β-Lac and the transformation of Cu(II) into Cu(I), (b) the catalytic reaction of Cu(II), (c) AuNPs aggregation induced by Cys through Au-S bond and electrostatic interaction, and (d) colorimetric determination of β-Lac activity and the inhibitors.

The principle of the colorimetric method for the detection of β-Lac activity and the screening of the inhibitors is shown in Scheme 1. Cys can bind to the surface of AuNPs through Au-S bonds and so the aggregation of AuNPs occurs through

electrostatic interactions between the Cys bound AuNPs, and the solution color changes from red to blue (Scheme 1(c)). Since Cu(II) can catalyze oxidation of Cys into cystine (Scheme 1(b)), a decrease in the concentration of Cu(II) can increase Cys-induced AuNPs aggregation by decreasing the oxidation of Cys into cystine. In presence of β-Lac, Pen, a usual substrate, can be catalyzed into its corresponding product, penicilloic acid, which has a strong reduction ability, and can react with Cu(II) to produce Cu(I) conjugates, resulting in the decrease of Cu(II) concentration in the detection system (Scheme 1(a)). So when Pen, β-Lac, Cys, and Cu(II) exist in the testing solution, the Cys-induced aggregation of AuNPs will occur due to the decrease of the number of Cu(II) which can be reduced into Cu(I) by penicilloic acid, β-Lac-catalyzed reaction product, with Pen as enzyme substrate (Scheme 1(d)). In contrast, when the detection system contains the inhibitor, penicilloic acid and the resultant Cu(I) conjugates can not form as a result of the inhibition of β-Lac activity, so AuNPs remain the dispersive state and the corresponding color of the testing solution keeps red owing to the disappearance of Cys which can be catalyzed into cystein by Cu(II) (Scheme 1(d)). Considering that the enzyme or inhibitor concentration is related to the degree of aggregation of AuNPs, a new method for monitoring β-Lac activity or screening the inhibitors through the change in the UV-vis spectrum of the AuNPs dispersion, can be developed.

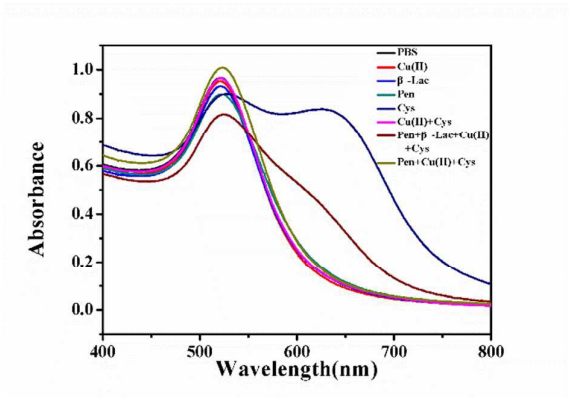


Figure 1 UV-vis spectra and photographs (inset) of the mixture prepared by separate adding PBS buffer (90 μL, 10 mM, pH 7.4) (blank curve), Cu(II) (20 μL, 10 μM) (red curve), β-Lac (10 μL, 10 U/mL) (light blue curve), Pen (20 μL, 20 mM) (cyan curve), Cys (40 μL, 1 mM) (deep blue curve), Cu(II) (20 μL, 10 μM) + Cys (40 μL, 1 mM) (pink curve), Pen (20 μL, 20 mM) + β-Lac (10 μL, 10 U/mL) + Cu(II) (20 μL, 10 μM) + Cys (40 μL, 1 mM) (deep red curve), Pen (20 μL, 20 mM) + Cys (40 μL, 1 mM) + Cu(II) (20 μL, 10 μM) (green curve), into AuNPs solution (50 μL, 8.4 nM) to give the final volume of 140 μL with the addition of PBS buffer.

As exhibited in Figure 1, upon adding PBS buffer into AuNPs solution as a control experiment, the color of the testing solution exhibits a strong absorption peak at 533 nm (blank curve), giving the feature of well-dispersive AuNPs[20]. With the separate addition of Cu(II), β-Lac, and Pen into AuNPs, the absorption peak keep unchanged (red curve, light blue curve, and cyan curve). This suggests that the three substances could not induce the occurrence of AuNPs aggregated state. Nevertheless, it can be noticed that the addition of Cys may essentially trigger the aggregation of AuNPs, resulting in the production of a new absorption peak at 657 nm (deep blue curve), confirmed by transmission electron microscopy (TEM) (Figure S1). This phenomenon can be explained by the formation of Au-S bond between AuNPs and Cys and electrostatic interaction between Cys fraction at outer layer of AuNPs (Scheme 1(c)). Upon the addition of both Cu(II) and Cys into the AuNPs, the testing solution keeps UV-vis spectrum of well dispersed AuNPs (pink curve). It can well demonstrate the conversion of Cys into cystine due to Cu(II) catalytic oxidation (Scheme 1(b)). However, it can be observed that the aggregated AuNPs appears to some extent after the successive addition of Pen, β-Lac, Cu(II), and Cys into the aqueous dispersion of the AuNPs (deep red curve). In this detection system, the transformation of Cys decreases owing to the decreasing number of Cu(II) which is reduced into Cu(I) by penicilloic acid, the product of β-Lac catalyzed reaction using Pen as substrate (Scheme 1(a)). In absence of β-Lac, the testing solution exhibits the UV-vis spectrum of dispersive AuNPs and no aggregation happens (green curve), due to the oxidation of Cys catalyzed by Cu(II) which can not be converted into Cu(I) as a result of no formation of penicilloic acid.

Optimization of colorimetric assay

Cys can induce AuNPs aggregation through the formation of Au-S bond and electrostatic interaction between Cys bound AuNPs and so its increasing amount in the testing solution can benefit AuNPs aggregation so as to improve the sensitivity and linear range of the proposed method. As depicted in Figure S2 in Supplementary date, the absorbance values of the peak at 657 nm increase along with the increasing concentrations from 0.08 mM to 30 mM. Meanwhile, it is found that no significant change in the UV-vis spectra can be observed with the addition of 1 mM Cys. Thus, 1 mM is chosen in this work as the optimal concentration of Cys for AuNPs aggregation.

Cu(II) can catalyze the oxidation of Cys into cystine which has no inductive ability on AuNPs aggregation, thus its increasing number can prevent AuNPs aggregation so as to advance the sensitivity of the method. It can be observed that the aggregated extent decreases with the increase of AuNPs concentrations from 5 μM to 100 μM (Figure S3 in Supplementary date). In the meantime, the spectra almost keep nearly unchanged when 10 μM of Cu(II) is added in the detection system. So 10 μM is used as optimal concentration for colorimetric method.

In the presence of β-Lac, Pen can be hydrolyzed into penicilloic acid which can reduce Cu(II) into Cu(I) and its increased amount benefits AuNPs aggregation in order to enhance the sensitivity of the proposed method. On the other

hand, the increasing number of Pen in the testing solution could result in AuNPs aggregation. Therefore, AuNPs can be employed as the probe to investigate the impact of Pen concentration. As shown in Figure S4 in the Supplementary data, the addition of Pen more than 10 mM can lead to the AuNPs aggregation. Thus 20 mM is selected as the optimal Pen concentration in the detection system.

Selective assay

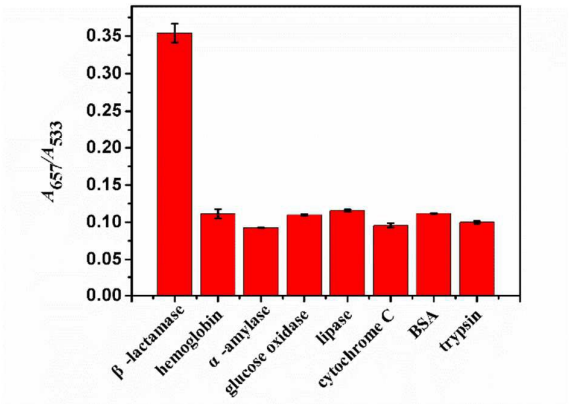


Figure 2 The A_{657}/A_{533} ratios versus eight different substrates including β-Lac (0.01 mg/mL), hemoglobin (1 mg/mL), α-amylase (1 mg/mL), glucose oxidase (1 mg/mL), lipase (1 mg/mL), cytochrome C (1 mg/mL), bovine serum albumin (BSA) (1 mg/mL), and trypsin (1 mg/mL). The main paragraph text follows directly on here.

The selectivity of the proposed method has been evaluated by UV-vis spectroscopy with AuNPs as probe in the presence of other proteins including hemoglobin, α-amylase, glucose oxidase, lipase, cytochrome C, BSA, and trypsin. As depicted in Figure 2, these proteins at one hundred times β-Lac concentration have negligible influences on the AuNPs aggregation and will not cause observable interference. It suggests that the proposed methods have high specificity for the determination of β-Lac activity.

β -Lac activity assay

Along with the increase of β-Lac concentrations from 0.1 U/mL to 11 U/mL, the changes happen in the UV-vis spectra of the dispersion (Figure 3 (a)) and the values of A_{657}/A_{533} increase (Figure 3 (b)), indicating the increased extent of AuNPs aggregation. The increasing amount of β-Lac leads to the efficient conversion of Pen into penicilloic acid with high reduction capability. Subsequently, penicilloic acid can react with Cu(II) to form Cu(I) conjugate, resulting in the decreased number of Cu(II) in the detection system. The catalyzed oxidation of Cys decreases correspondingly due to low concentration of Cu(II) in the testing mixture. As a result, high amount of Cys will induce the aggregation of AuNPs through the formation of Au-S bond and electrostatic interaction.

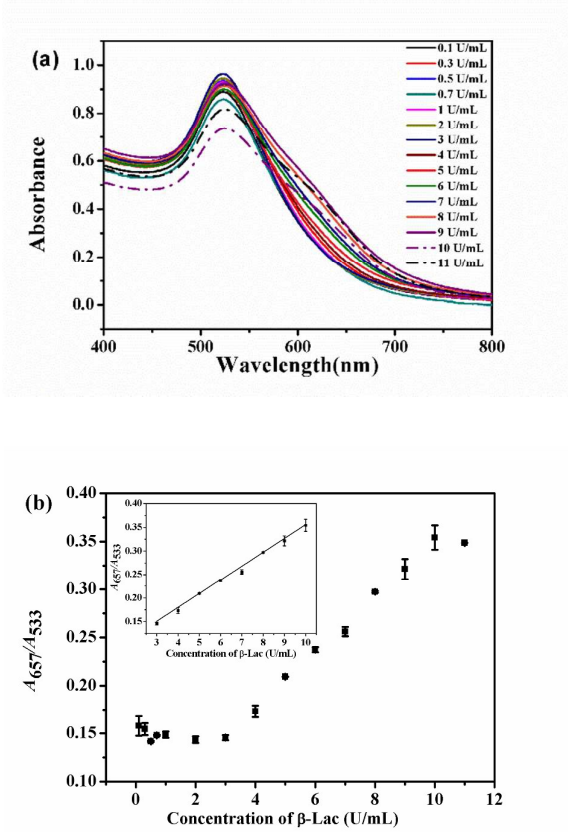


Figure 3 (a) The UV-vis spectra of the mixtures containing various concentrations of β-Lac. (b) Calibration curve corresponding to A_{657}/A_{533} against β-Lac concentrations. Inset shows the linear relationship between the A_{657}/A_{533} values and β-Lac concentrations.

The calibration curve obtained for the β-Lac detection with different concentrations is exhibited in Figure 3 (b). The ratios of A_{657}/A_{533} show a linear response toward β-Lac concentration ranging from 2 U/mL to 10 U/mL and follows the regression equation of A_{657}/A_{533}=0.0293 C + 0.0628 (U/mL, R^2=0.9984). The detection limit has been calculated to be 2.36 U/mL by the interpolation of the mean plus three times the standard deviation of the zero standards [21]. It is lower than those obtained using existing rapid detection methods, such as 8.92 U/mL for the pH-stat alkalimetric titration method[22], 4 U/mL for the modified cylinder plate method[23], 20 U/mL for the chromogenic cephalosporin method[24], and 4 U/mL for high-performance liquid chromatography[25]. The detection precision has been investigated according to the slope of the regression equation of β-Lac (from 2 U/mL to 10 U/mL) obtained from the three independent assay processes. The *RSD* of the three slope is 2.1 %, suggesting that the precision and reproducibility of the proposed method are acceptable.

Inhibition efficiency evaluation

Combinations of β-lactam antibiotics and β-Lac inhibitors have been proved successful for the treatment of the infections caused by bacteria producing β-Lac[26]. So it is of great importance to search β-Lac inhibitors. For the sake of demonstrating that the proposed method can be employed for the screening of β-Lac inhibitors, two compounds, clavulanic acid and sulbactam, have been selected for this study. Firstly, in order to exclude the possibility that the inhibitors react with Cu(II), the control assays have been carried out and the corresponding results exhibit in Figure S5. The addition of both sulbactam and clavulanic acid have no influence on the spectrum of the complex solution. It indicates that the inhibitors cannot react with Cu(II).

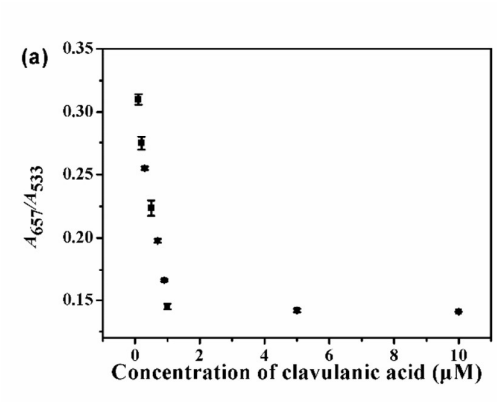


Figure 4 The ratios of A_{657}/A_{533} versus various concentrations of (a) clavulanic acid and (c) sulbactam. Inhibition ratio versus the concentrations of (b) clavulanic acid and (d) sulbactam.

Clavulanic acid has excellent inhibitory activity against β-Lac of Richmond classes II, III, IV, and V, and the inhibition is a time-dependent reaction and irreversible[27]. As shown in Figure 4 (a), with the increase of clavulanic acid concentrations from 0.1 μM to 10 μM, the value of A_{657}/A_{533} first decreases gradually and then levels off, suggesting that the inhibition by clavulanic acid has a dose-dependent manner. It has been reported that If clavulanic acid and other penicillins are present simultaneously, there is completion for receptor sites, however, with previous exposure of enzyme to clavulanic acid, it has bound irreversibly to the hydrolytic site[27]. In our experiment, clavulanic acid were premixed with β-Lac, so it can be well predicted that the hydrolytic site of β-Lac has been irreversibly bound by clavulanic acid. The disappearance of β-Lac activity results in the decreasing production of penicilloic acid, the increase of Cu(II) remaining in the detection system, and the corresponding rising of Cys conversion into cystine. Therefore, the aggregation of AuNPs and the ratios of A_{657}/A_{533} decrease. It can be found that 1 μM clavulanic acid can evidently inhibit the β-Lac activity because of no noticeable change observed in Figure 4 (a). 97.5% of maximum inhibition ratio can be tested for clavulanic acid with the IC_{50} value of 0.92 μM (Figure 4 (b)), which is in agreement with the values reported previously[26, 28].

Similar to clavulanic acid, sulbactam has also been widely applied as β-Lac inhibitor[27]. As shown in Figure 4 (c), along with the increase in sulbactam concentrations from 0.01 μM to 0.1 μM, the ratios of A_{657}/A_{533} increase and the ratios remain almost unchangeable with the further increase of sulbactam concentrations from 0.1 μM to 1 μM. It implies that the inhibition of sulbactam is dose-independent. Sulbactam has been proved to be a time-dependent irreversible inhibitor, and this inhibition occurs in two principal steps: (1) formation of a reversible enzyme-

inhibitor complex, (2) evolution of this complex into one, or more, inactive protein(s)[29]. It can be observed that β-Lac activity can be significantly inhibited by 0.1 μMsulbactam (Figure 4 (c)). The maximum inhibition ratio of sulbactam is 87.9% with the IC_{50} value of 0.06 μM (Figure 4 (d)), in accordance with the previous report[26, 28]. These results demonstrate that the proposed method can also be well used to evaluate the inhibitor efficiency.

Conclusions

A simple and yet effective colorimetric method has been established for the detection of β-Lac activity and the screening of the inhibitors based on both β-Lac and Cu(II) catalyzed signal amplification. The β-Lac activity can be analyzed linearly from 2 U/mL to 10 U/mL. Meanwhile, the method can well evaluate the inhibitory activities of model β-Lac inhibitors, clavulanic acid and sulbactam, with IC_{50} values of 0.92 μM and 0.06 μM, respectively. In term of its low technical and instrumental demands, and high sensitivity, the method can be developed as a simple and convenient high throughput screening of β-Lac inhibitors.

Acknowledgements

This work is supported by the Open Project Program of State Key Laboratory of Dairy Biotechnology, Bright Dairy & Food Co. Ltd., (No. SKLDB2015-003), the National Natural Science Foundation of China (Grant Nos. 31101354 and 21235003) and the Innovation Program of Shanghai Municipal Education Commission (No. 15ZZ048).

References

1. C. Walsh, *American Society for Microbiology*, 2003.
2. E. Jackson, J. Hardman, L. Limbird and A. Gilman,*The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, 1995.
3. V. Grimm, S. Ezaki, M. Susa, C. Knabbe, R. D. Schmid and T. T. Bachmann, *Journal of Clinical Microbiology*, 2004, **42**, 3766-3774.
4. A. Galarneau, M. Primeau, L.E. Trudeau and S. W. Michnick, *Nature Biotechnology*, 2002, **20**, 619-622.
5. S. N. Olsen, *Thermochimica Acta*, 2006, **448**, 12-18.
6. R. Liu, R. Liew, J. Zhou and B. Xing, *Angewandte Chemie*, 2007, **119**, 8955-8959.
7. J. Zhang, Y. Shen, S. L. May, D. C. Nelson and S. Li, *Angewandte Chemie*, 2012, **124**, 1901-1904.
8. N. Nath and A. Chilkoti, *Analytical Chemistry*, 2002, **74**, 504-509.
9. D. A. Schultz, *Current Opinion in Biotechnology*, 2003, **14**, 13-22.
10. B. Nikoobakht and M. A. El-Sayed, *Chemistry of Materials*, 2003, **15**, 1957-1962.
11. K.H. Su, Q.H. Wei, X. Zhang, J. Mock, D. R. Smith and S. Schultz, *Nano Letters*, 2003, **3**, 1087-1090.
12. C. M. Niemeyer and C. A. Mirkin, *Nanobiotechnology: concepts, applications and perspectives*, John Wiley & Sons, 2006.
13. X.Zhu, Y.Liu, J.Yang, Z.Liang and G.Li, *Biosensors & Bioelectronics*, 2010, **25**, 2135-2139.
14. T. Gao, L. Ning, C. Li, H. Wang and G. Li, *Analytica Chimica Acta*, 2013, **788**, 171-176.
15. J. De la Fuente, A. Barrientos, T. Rojas, J. Rojo, J. Canada, A. Fernandez and S. Penades, *Angewandte Chemie (International ed. in English)*, 2001, **40**, 2257.
16. C. L. Schofield, A. H. Haines, R. A. Field and D. A. Russell, *Langmuir*, 2006, **22**, 6707-6711.
17. C. Li, Y. Yang, L. Wei, X. Wang, Z. Wang, Y. Yin and G. Li, *Theranostics*, 2015, **5**, 62-70.
18. Y. Xu, J. Wang, Y. Cao and G. Li, *Analyst*, 2011, **136**, 2044-2046.
19. C.H. Lu, Y.W. Wang, S.L. Ye, G.N. Chen and H.H. Yang, *Npg Asia Materials*, 2012, **4**.
20. J. Deng, P. Yu, L. Yang and L. Mao, *Analytical chemistry*, 2013, **85**, 2516-2522.
21. J. Zhang, Y. Liu, J. Lv and G. Li, *Nano Research*, 2014, 1-11.
22. S. Liu, Z. Zhou, W. Zhou, Z. Zhang and J. Ma, *Food Science*, 2010, **31**, 216-218.
23. S. Cui, J. Li, C. Hu, S. Jin and Y. Ma, *Journal of AOAC International*, 2007, **90**, 1128-1132.
24. C. H. O'Callaghan, A. Morris, S. M. Kirby and A. H. Shingler, *Antimicrobial Agents and Chemotherapy*, 1972, **1**, 283-288.
25. H. Chen, W. J. Ma, J. H. Tian and J. Li, *Animal Husbandry and Feed Science*, 2010, **1**, 029.
26. D. J. Kim, J. A. King, L. Zuccarelli, C. F. Ferris, G. A. Koppel, C. T. Snowdon and C. H. Ahn, *Pharmacology Biochemistry and Behavior*, 2009, **93**, 112-120.
27. H. C. Neu and K. P. Fu, *Antimicrobial Agents and Chemotherapy*, 1978, **14**, 650-655.
28. G. Valle, L. M. Quirós, M. T. Andrés and J. F. Fierro, *FEMS Microbiology Letters*, 1998, **158**, 191-194.
29. R. Labia, V. Lelievre and J. Peduzzi, *Biochimica et Biophysica Acta*, 1980, **611**, 351-357.

Table of Contents

A simple and effective AuNPs-based colorimetric method with both β-lactamase and Cu(II) catalyzed signal amplification.