

Journal of Materials Chemistry B

Materials for biology and medicine

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: W. Wang, X. Li, K. Tang, Z. Song and X. Luo, *J. Mater. Chem. B*, 2020, DOI: 10.1039/D0TB00666A.



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.

AuNPs-capped cage fluorescent biosensor based on controlled-release and cyclic enzymatic amplification for ultrasensitive detection of ATP

View Article Online
DOI: 10.1039/D0TB00666A

Wei Wang,* Xin Li, Kai Tang, Zhiling Song, and Xiliang Luo*

Designed gold nanodevices have attracted extensive interest in detecting specific targets within cells. However, constructing gold sensing devices that can be activated by the simulation of remote applications remains a huge challenge. Here, we report an Au nanoparticles (AuNPs)-capped cage fluorescent biosensor based on controlled-release and Exonuclease III (Exo III) assisted cyclic enzymatic amplification that can be activated by Adenosine triphosphate (ATP). In the system, AuNPs were employed as building blocks to cap the pores of Au Nanocages (AuNCs) loaded with Rhodamine B (RhB) molecules through hybridization of DNA. The RhB fluorescent molecules were finally released with the help of Exo III in the presence of ATP for detection purpose. Ultimately, the biosensor leads to a wide linear ATP detection range from 1.0×10^{-9} to 1.0×10^{-7} M with a limit of detection (LOD) down to 0.88 nM. In addition, it also has a good selectivity for ATP to distinguish between ATP and ATP analogues such as Cytidine triphosphate (CTP), Guanosine triphosphate (GTP), and Uridine triphosphate (UTP). Therefore, as a convenient and sensitive biosensor, it is expected to be widely used in the biomedical field.

1. Introduction

Applications of gold nanostructures in electronics¹, sensors^{2–4}, catalysis^{5,6} and biomedical science, especially in cancer diagnose and therapy^{7–10}, have been tremendous in recent years due to their intriguing optical, electrical, and chemical properties¹¹. Many properties of nanoparticles are determined by its size, morphology, and composition¹¹. Different morphologies of gold nanostructures including nanoparticles, nanorods, nanowires, nanostars and branched nanostructures can be formed depending on the different synthesis methods^{12–15}. To control the morphologies, different methods have been reported, including employing selective adsorbates and adjusting the reaction conditions^{16,17}. Despite tremendous advances acquired in this area, the production of gold nanostructures with layers or complex structures remains challenging and it is more difficult to systematically adjust the morphology of these complex nanostructures.

Among the many Au nanostructures, AuNCs have been used extensively in imaging^{18,19}, diagnosis²⁰, drug delivery²¹ and therapy^{22,23} owing to their hollow interiors, porous surface and attractive optical properties. The key feature of AuNCs for biomedical applications is the scattering and absorption of light at resonant wavelengths, which is commonly known as localized surface plasmon resonance (LSPR)²⁴. Moreover, the size, shape and geometry of the nanostructures determine the resonance wavelength²⁵. In addition to the properties described

above, AuNCs still hold other excellent characteristics involving their compact sizes, large absorption cross sections (nearly five orders of magnitude higher than those of conventional organic dyes), and their bio-inertness, as well as a stable and straightforward procedure for surface modification based on Au-thiolate bonding²⁶. Besides, AuNPs have found application in a variety of assays, can be synthesized with high monodispersity and attached easily by biomolecules. Many studies have reported that the nucleic acid was coupled to AuNPs and has the ability to retain selectively and reversibly hybridize to the complementary sequence^{27–29}. That is AuNPs can effectively protect miRNA molecules from nuclease-induced degradation^{30,31}.

Adenosine triphosphate (ATP), a regulated energy vector, powers cellular machinery, drives metabolic reactions and acts an emitting molecule in many physiological and pathological bodily functions³². ATP plays a vital role in regulating cellular functions (such as catalyzing various enzymatic reactions, maintaining cellular breathing, providing energy, and conducting extracellular signals)^{33–35} and mediating physiological activity (such as muscle contraction³⁶, vascular tone³⁷, platelet aggregation³⁸, and ion channel³⁹). Furthermore, abnormal levels of ATP are thought to be associated with several malignancies. Therefore, many biosensors for ATP have been developed including aptamer biosensor⁴⁰, fluorescent probes^{33,41–43}, high-performance liquid chromatography (HPLC)⁴⁴ or capillary electrophoresis (CE)⁴⁵. However, most reported detection methods

do not show high sensitivity and selectivity simultaneously. This defect is mainly manifested in the fact that either a lower level of ATP cannot be detected or ATP and ATP analogs such as CTP, GTP, and UTP cannot be distinguished well.

Herein, we developed a novel ATP detection method employing an AuNPs-capped cage fluorescent biosensor based on controlled-release and Exo III assisted cyclic enzymatic amplification. DNA S2-modified AuNPs were used to block the pores on the surface of AuNCs to form an AuNPs-capped cage system through partial hybridization of long-strand DNA S2 and short-strand DNA S1, which causing the fluorescent molecules to be blocked inside AuNCs. Once the target ATP appeared, the system could be identified by the primer S3, which was released from a double-stranded hybrid formed with ATP aptamer owing to the ATP-triggered competitive substitution reaction. The released primer S3 hybridized to the remaining unhybridized part of long-strand DNA S2 hybridized with DNA S1 and formed a double-stranded structure with a blunt 3' terminus. Then, Exo III catalyzed the stepwise removal of mononucleotides from this terminus, eventually resulting AuNPs divorced from the surface of AuNCs and the fluorescent molecules were released. The liberated primer S3 then hybridized with other long-strand DNA S2, whence the cycle started anew. Therefore, the primer S3 produced by a very small amount of ATP could release a large number of fluorescent molecules to achieve ultra-sensitive detection of ATP.

2. Experimental section

2.1 Chemicals and reagents

Hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from Alfa Aesar. Rhodamine B (RhB), Dithiothreitol (DTT), Adenosine triphosphate (ATP), Cytosine triphosphate (CTP), Guanosine triphosphate (GTP), and Uridine triphosphate (UTP) were obtained from Shanghai Aladdin Chemistry Corp (China). Exonuclease III (Exo III) was procured from Thermo Scientific, USA. Carboxyl modified magnetic beads (COOH-MNPs) and Thiol-modified magnetic beads (SH-MNPs) were obtained from BaseLine Chomtech Research Center of Tianjin. Polyvinylpyrrolidone K-30 (PVP) was purchased from Sinopharm Chemical Reagent, China. Sodium chloride (NaCl), Silver nitrate (AgNO_3), and Sodium sulfide nonahydrate ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) were obtained from Tianjin Guangcheng Chemical Reagent. Ethylene glycol was purchased from Yuandong Chemicals of Yantai. Acetone and Ethanol were obtained from Sanhe Chemical Reagent of Yantai. The synthetic oligonucleotides were purchased from Sangon Biotech. Their sequences are as follows:

ATP-aptamer:

5'-NH₂-TTTAAACCTGGGGGGAGTATTGCGGAGGAAG-3' View Article Online
DOI: 10.1039/D0TB00666A

S1: 5'-CACTGGGTTGGGCGGGATTTTTTTTTT-SH-3'

S2:

5'-SH-TTTTTTTTTATCCCGCCCAACCCAGTGATTGCGGA-3'

S3: 5'-CCTCCGCAAT-3'

2.2 Synthesis of AuNCs

AuNCs were synthesized by the galvanic replacement reaction between Ag nanocubes and chloroauric acid (HAuCl_4) with some modifications⁹. Briefly, add 200 μL of silver nanocubes to a 50 mL round-bottomed flask with 10 mL water containing 1 mg mL^{-1} PVP under magnetic stirring. Heat the solution to a mild boil for approximately 10 min, and then, 0.2 mM HAuCl_4 aqueous solution was slowly added to the solution. A series of color changes would be observed and can be used to estimate the peak position of the LSPR of the AuNCs while the HAuCl_4 solution was added to the flask. Stop adding HAuCl_4 solution until the color occurred similar to blue when the LSPR peak had moved to approximately 744 nm. Continue heating and refluxing for ten minutes until the color is stable, then cool the solution to room temperature. The whole process was carried out under vigorous stirring. The sample was centrifuged and washed through adding saturated NaCl solution to dissolve and remove AgCl generated during the chemical reaction. The obtained AuNCs can be dispersed in deionized water and saved in the dark for the next step.

2.3 Modification of AuNCs with SH-DNAs

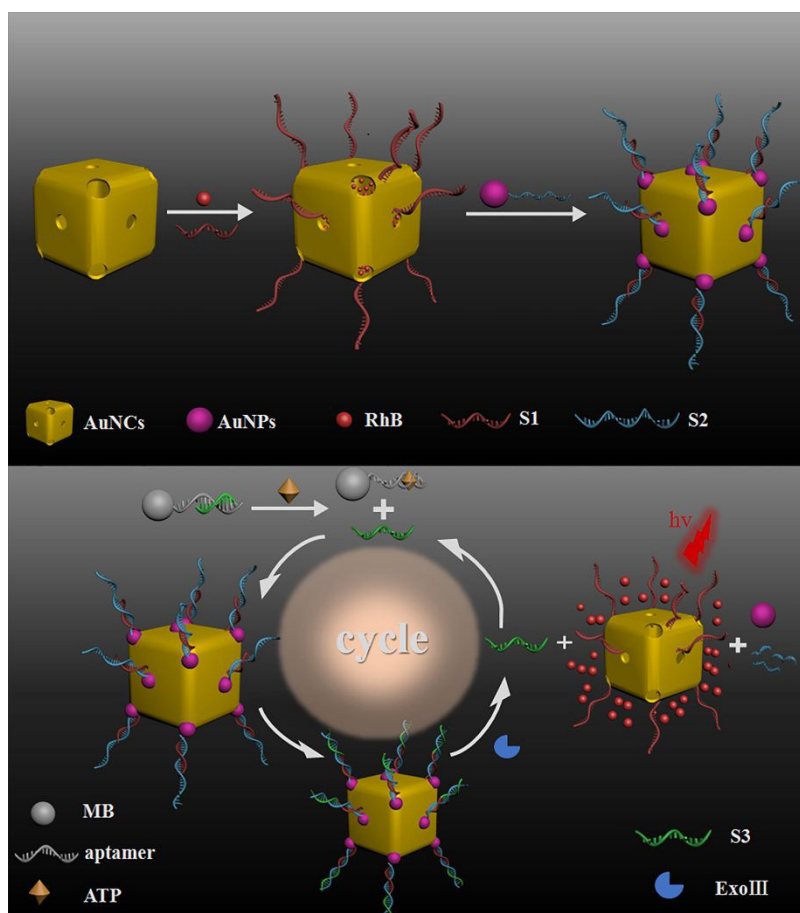
In order to improve the separation process, MNPs modified with sulfhydryl groups (SH-MNPs) were used to combine with the as-prepared AuNCs. 20 μL SH-MNPs washed repeatedly with 200 μL PBS buffer (pH 7.4), and it was added into 400 μL of the as-prepared AuNCs suspension, then the mixture was agitated for 12 h at 37 °C. Magnetic separation was implemented and the acquired MNPs-combined AuNCs were washed thrice by 100 μL PBS. Then, 10 μL of the 1.0×10^{-5} M SH-DNAs S1, 10 μL of the 1.0×10^{-3} M DTT and 80 μL PBS were added and agitated for 12 h at 37 °C. At this moment, the DNA-AuNC-MNP conjugates were formed.

2.4 Loading the DNA S1-modified AuNCs with RhB

In order to load RhB into the hollow interiors of the DNA S1-modified AuNCs, magnetic separation was implemented and the acquired DNA-AuNC-MNP was washed thrice by 100 μL PBS. Then, 2 μL of 5.0×10^{-4} M RhB solution and 98 μL PBS were added separately. The mixture was agitated for 12 h at 37 °C.

2.5 Synthesis of AuNPs

AuNPs were synthesized by the classical sodium citrate reduction method. Typically, 385 μL of 2.4×10^{-2} M HAuCl_4 and 3.5 mL of 1% sodium citrate were



Scheme 1 Schematic illustration of the AuNPs-capped cage fluorescent biosensor system for the detection of ATP.

added into a flask containing 40 mL water under stirring. Then, 4.0 mL of 0.05% sodium borohydride was dripped slowly into the mixture. The solution was kept stirring for an additional 10 min. Thus, the newly synthesized bare AuNPs were stored at 4 °C for future use.

2.6 Modification of the synthesized AuNPs with SH-DNAs S2

To obtain AuNPs modified with DNA, 10 μL of 1.0×10^{-5} M SH-DNAs S2 and 10 μL of 1.0×10^{-3} M DTT were added into 400 μL of the as-prepared AuNPs. The mixture was agitated for 12 h at 37 °C.

2.7 Fabrication of the controlled-release biosensor

In order to block the holes of AuNCs loading with RhB molecules, the DNA S2-modified AuNPs were added into the above well-decorated AuNCs. Respectively, the AuNPs could cap the holes of AuNCs due to the hybridization between DNA strand S1 and S2. The mixture was agitated for 2 h at 37 °C. Afterwards, magnetic separation was implemented and the controlled-release biosensor was fabricated.

2.8 Fabrication of the ATP recognition probe

The ATP recognition probe was a double-stranded

structure constructed by the hybridization between the ATP aptamer and the primer S3. Briefly, 10 μL of the activated COOH-MNPs (10 mg mL^{-1}), 10 μL of 1.0×10^{-5} M amino-modified ATP aptamer and 80 μL PBS were mixed and agitated for 12 h at 37 °C. In the next step, 10 μL of 1.0×10^{-5} M primer S3 was added after magnetic separation and agitated for 2 h at 37 °C. Next, magnetic separation was performed and washed repeatedly with PBS. Until now, the ATP recognition probe was formed for the next step.

2.9 Detection of target ATP

In order to evaluate the performance of the controlled-release biosensor for in vitro detection, we used the prepared biosensor for ATP detection. Firstly, 10 μL of different concentrations of ATP was added to the as-prepared ATP recognition probe and the mixture was then diluted to 100 μL with PBS. With ATP present, the aptamers from the recognition probes will combine with ATP under 37 °C for 1 h. As a result, the hybrid double strand previously formed by the ATP aptamer and the primer S3 was separated by competitive substitution reaction, causing the primer S3 to be released into the solution. After magnetic separation, the separated primer S3 was

added to the above controlled-release biosensor as well as 2 μL Exo III, followed by reaction for 2 h at 37 $^{\circ}\text{C}$. Ultimately, magnetic separation was implemented and the supernatant was abstracted for fluorescence detection.

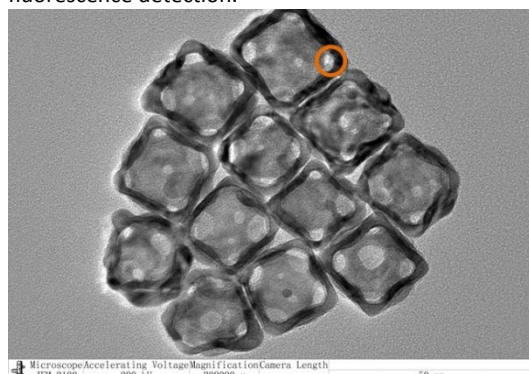


Fig.1 Transmission electron microscopy (TEM) image of the synthesized AuNCs with marked hole.

2.10 Determination of ATP in cultured cell extracts

Finally, Hela cells (cervical cancer cells) were applied to assess the capability of the proposed biosensor in biological samples. Firstly, a suspension of 1.0 mL Hela cells dispersed in RPMI cell media buffer was centrifuged at 3000 rpm for 5 min. Then, the cells

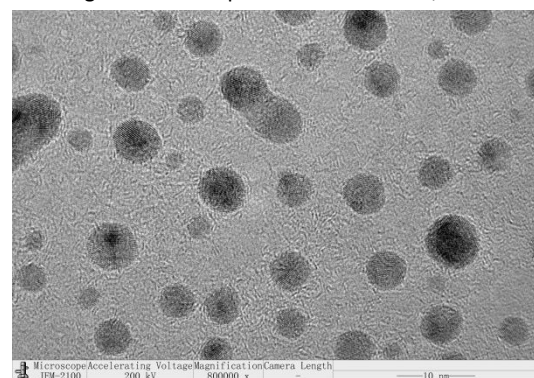


Fig.2 Transmission electron microscopy (TEM) image of the synthesized AuNPs.

were resuspended in 200 μL of PBS (pH 7.4) after washing with PBS. Next, the cells were disrupted by sonication and centrifuged at 3000 rpm for 5 min to remove cell debris. The obtained cell lysate was added into the ATP recognition probe, incubating at 37 $^{\circ}\text{C}$ for 1 h to ensure the full reaction between ATP and ATP aptamers. The supernatant containing the released primer S3 was introduced into the proposed biosensor as well as 2 μL Exo III for the enzymatic amplification process after magnetic separation and PBS was applied as a control. Finally, magnetic separation was performed after the mixture reacting at 37 $^{\circ}\text{C}$ for 2 h, and the supernatant was collected and diluted to 2.0 mL with water for fluorescence detection.

3. Results and discussion

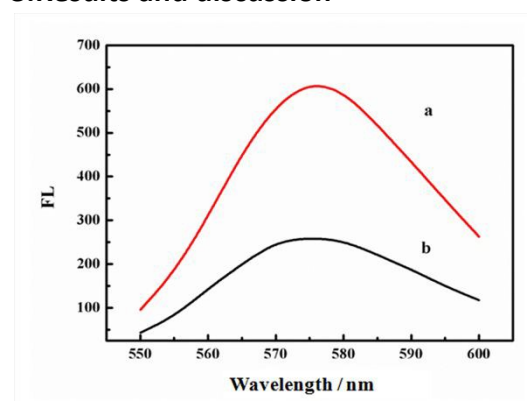


Fig. 3 Fluorescence intensity of the solution in the absence (b) and in the presence of 5.0×10^{-8} M ATP (a).

3.1 Principle of the assay

In this work, we developed a novel ATP detection method employing an AuNPs-capped cage fluorescent biosensor based on controlled-release and Exo III assisted cyclic enzymatic amplification, which was illustrated in **Scheme 1**. Briefly, AuNPs attached with a long-strand SH-DNA S2 were immersed into the solution of the RhB-loaded AuNCs modified with a short-strand SH-DNA S1. The AuNPs-capped cage system was constructed by the self-assembly of AuNPs onto AuNCs through the hybridization between the long-strand DNA S2 and the short-strand DNA S1, which causing the fluorescent molecules RhB to be blocked inside AuNCs. The long-strand S2 comprises two parts, which are complementary to the short-strand S1 and the primer strand S3, respectively. Once the target ATP appeared, the system could be identified by the primer S3, which was released from a double-stranded hybrid formed with the ATP aptamer owing to the ATP-triggered competitive substitution reaction. The released primer S3 hybridized to the remaining unhybridized part of the long-strand DNA S2 hybridized with DNA S1 and formed a double-stranded structure with a blunt 3' terminus. Then, Exo III catalyzed the stepwise removal of mononucleotides from this terminus, eventually resulting AuNPs divorced from the surface of AuNCs and the fluorescent molecules were released. The liberated primer S3 then hybridized with other long-strand DNA S2 containing unhybridized part, whence the cycle started anew. Thus, the primer S3 produced by a very small amount of ATP could release a large number of fluorescent molecules to achieve ultra-sensitive detection of ATP.

3.2 Characterization of the synthesized AuNCs

Transmission electron microscopy (TEM) images

clearly demonstrated the synthesized AuNCs with hollow interior and porous sidewalls as shown in **Fig. 1**. In addition, the average size was 40.2 ± 3.8 nm in edge length and an average pore size of 5.3 ± 0.5 nm as measured. The UV-visible absorbance spectrum (**Fig. S1**) proved that the synthesized AuNCs had an LSPR peak at 744.0 nm, further indicating that AuNCs had a hollow structure. These results indicated that the morphology and size of the synthesized AuNCs were consistent with those reported in the literature⁹.

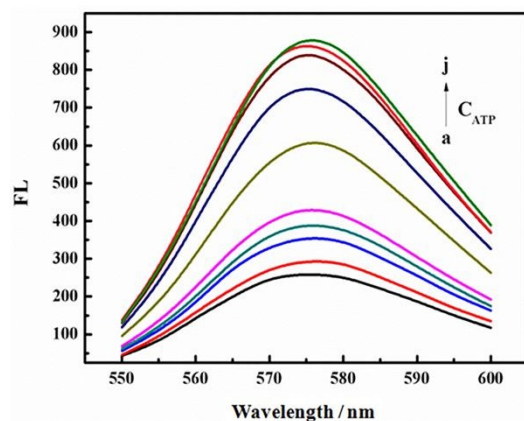


Fig. 4 The Fluorescence intensity corresponding to the different concentrations of ATP (a $\rightarrow$ j: 0 ; 1.0×10^{-9} ; 5.0×10^{-9} ; 1.0×10^{-8} ; 2.0×10^{-8} ; 5.0×10^{-8} ; 8.0×10^{-8} ; 1.0×10^{-7} ; 2.5×10^{-7} ; 5.0×10^{-7} M).

3.3 Characterization of the synthesized AuNPs

The prepared AuNPs showed a spherical shape with an average size 5.6 ± 0.9 nm according to TEM image in **Fig. 2**. Meanwhile, the UV-visible absorbance spectrum showed that the synthesized AuNPs had an absorption peak at 508.0 nm (**Fig. S2**). The results indicated that it can be employed to block the holes of AuNCs (**Fig. S3**).

3.4 Fluorescence response of the biosensor

In order to test the feasibility of the AuNPs-capped cage system, control experiments were firstly conducted. **Fig. 3** exhibited the fluorescence intensity of RhB released from AuNCs capped by DNA S2-modified AuNPs. In the absence of ATP (curve b), the fluorescence intensity was weak because AuNPs were immobilized still on the pores of AuNCs and prevented the release of RhB from AuNCs. In contrast to curve b, a significantly fluorescence intensity appeared in the presence of ATP due to the detachment of AuNPs from the surface of AuNCs and the resulting release of RhB. The results proved that the pores of AuNCs could be blocked with AuNPs through the hybridization between DNA S1 and DNA S2. As long as ATP exists, the primer S3 that has been

eliminated by competition would hybridize with the unhybridized part of DNA S2. Then, Exo III catalyzed the stepwise removal of mononucleotides from the 3'-hydroxyl termini of DNA S2, resulting in the

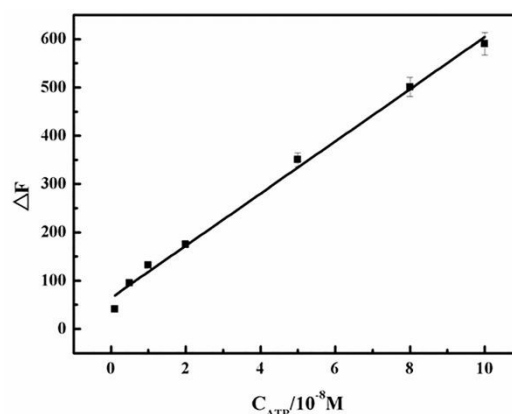


Fig.5 The linear relationship of ATP concentrations and the fluorescence enhancements. ΔF is the fluorescence intensity after blank deduction.

liberation of primer S3 and the detachment of AuNPs from the surface of AuNCs. The liberated primer S3 participated in the cycles of hybridization and enzymatic cleavage, which resulting in more and more fluorescent molecules being released. As a result, ATP could be detected sensitively through the release of RhB. Therefore, the proposal based on the proposed AuNPs-capped cage system is feasible.

3.5 Optimization of reaction conditions

It is necessary to seek the optimal experimental conditions involving incubation temperature and reaction time for the purpose of the optimum sensing performance. Firstly, we optimized the reaction temperature of Exo III. It could be clearly found that the fluorescence intensity at 37 °C was significantly stronger than that at 25 °C (**Fig. S4**) in the presence of 1.0×10^{-7} M ATP. Therefore, we chose 37 °C as the optimal reaction temperature for the experiment. **Fig. S5** exhibited the relationship between the fluorescence intensity and the incubation time in the presence of 1.0×10^{-7} M ATP. Obviously, the fluorescence intensity of RhB increased appreciably with the increasing incubation time from 0 to 3 h. When the incubation time reached 2 h, the fluorescence of RhB was basically unchanged. Therefore, 2 h was used in the following experiments.

3.6 Quantitative measurement of target ATP

Under the optimal conditions, the quantitative measurements of ATP by the proposed

AuNPs-capped cage fluorescent biosensor based on controlled-release and Exo III assisted cyclic enzymatic amplification were implemented. **Fig. 4** showed the fluorescent signals of RhB released from the hollow interiors of AuNCs in the presence of different concentrations of ATP from 0 to 5.0×10^{-7} M (a-j). With the increase of the concentration of ATP, more termini of duplex DNAs were cleaved, thus the

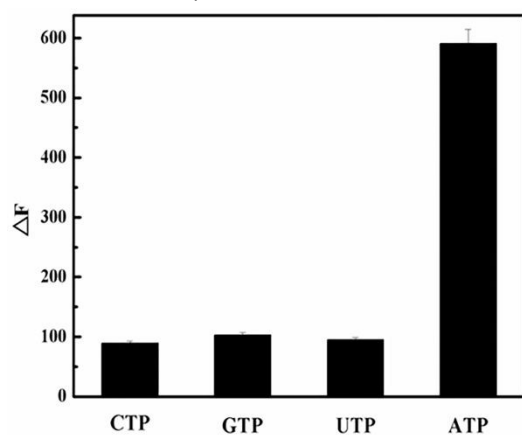


Fig. 6 Selectivity of the fluorescence biosensor for CTP, GTP, UTP and ATP (1.0×10^{-6} M), respectively. ΔF is the fluorescence intensity after blank deduction.

more RhB molecules were released leading to the significant enhancement of the fluorescence intensity. As depicted in **Fig. 5**, the fluorescence enhancement exhibited a good positive correlation with the ATP concentrations from 1.0×10^{-9} to 1.0×10^{-7} M, and the regression equation of $\Delta F = 64.1209 + 54.0283 C_{ATP}$ (10^{-8} M) was obtained with a correlation coefficient of 0.9933. The detection limit (LOD) was evaluated to be 8.8×10^{-10} M by calculations based on the standard deviation of the measurements (s , $n=11$) and the slope (S) of the calibration curve at the levels approaching the limits according to equation $LOD = 3s/S$. The results demonstrated that the proposed AuNPs-capped cage fluorescent biosensor based on controlled-release and Exo III assisted cyclic enzymatic amplification was an efficient detection system (Table S1). The presence of ATP can trigger continuous cycles of hybridization and enzymatic cleavage and the resulting magnified release of many fluorescent molecules RhB from the porous AuNCs, so that its concentration can be sensitively detected by the significantly enhanced fluorescent signal. Even in the presence of trace amounts of ATP, more cycles can occur and more fluorescent molecules can be released. Therefore, the sensitivity is greatly improved, which ensured ultra-sensitive and highly selective detection of ATP.

3.7 Selectivity of the fluorescence biosensor

To investigate the selectivity of the as-prepared biosensor, the ATP analogues including CTP ($1.0 \times$

10^{-6} M), GTP (1.0×10^{-6} M), and UTP (1.0×10^{-6} M) were employed in control experiments. As shown in **Fig. 6**, when the target ATP was replaced by CTP, GTP, or UTP, the enhancement of the fluorescence signal generated in the presence of ATP (1.0×10^{-6} M) is much higher than in the presence of CTP, GTP, and UTP, respectively. The proposed sensing system showed excellent selectivity for ATP, which was attributed to the specific binding of ATP with its aptamer.

3.8 Preliminary analysis of the biosensor in tumor cells

In order to evaluate the feasibility of the developed biosensor in actual samples, the lysate of Hela cells (cervical cancer cells) with high expression of ATP was used for the detection of intracellular ATP. **Fig. S6** showed the fluorescence intensity of RhB released from the hollow interiors of AuNCs toward the Hela cell lysate (curve a) and PBS (curve b). Obviously, the fluorescence response was enhanced significantly in the presence of the Hela cell lysate compared with PBS control, which implied the high expression of ATP in the Hela cells. The result showed that the concentration of ATP in the lysates of Hela cells was 3.27×10^{-6} M.

4. Conclusion

In summary, an AuNPs-capped cage fluorescent biosensor based on controlled-release and Exo III assisted cyclic enzymatic amplification was developed. Systemically, AuNPs were employed as building blocks to cap the pores of AuNCs loaded with RhB molecules through hybridization of DNA. The RhB fluorescent molecules were finally released with the help of Exo III in the presence of ATP for detection purpose. The results demonstrated that the system ensured ultra-sensitive and highly selective detection of ATP. Moreover, the obtained bio-responsive controlled-release system was an effective biosensor for intracellular ATP detection in cancer cells through aptamer-target binding and cyclic enzyme signal amplification processes. Obviously, the proposed strategy can be further extended to a therapeutic system with multiple applications in molecular imaging as well as chemical and photothermal treatments.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research was supported by the National Nature Science Foundation of China (21675093, 21603099), the Taishan Scholar Program of Shandong Province, China (ts20110829).

References

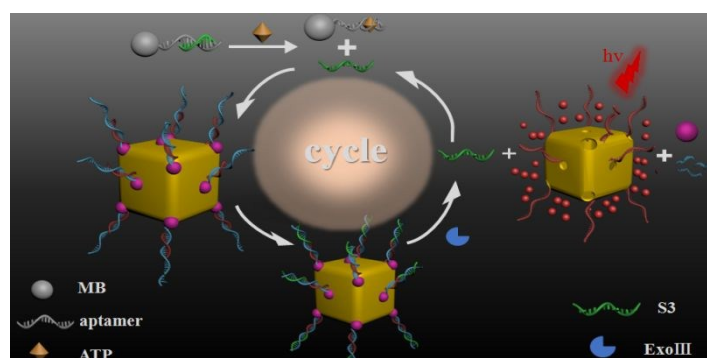
- 1 P. Banerjee, D. Conklin, S. Nanayakkara, T. H. Park, M. J. Therien and D. A. Bonnell, *ACS Nano*, 2010, **4**, 1019–1025.
- 2 T. Kang, S. M. Yoo, I. Yoon, S. Y. Lee and B. Kim, *Nano Lett.*, 2010, **10**, 1189–1193.
- 3 S. Pandey and K. K. Nanda, *ACS Sensors*, 2016, **1**, 55–62.
- 4 W. Kubo and S. Fujikawa, *Nano Lett.*, 2011, **11**, 8–15.
- 5 Z. Wu, G. Hu, D. E. Jiang, D. R. Mullins, Q. F. Zhang, L. F. Allard, L. S. Wang and S. H. Overbury, *Nano Lett.*, 2016, **16**, 6560–6567.
- 6 S. C. Hsu, Y. C. Chuang, B. T. Sneed, D. A. Cullen, T. W. Chiu and C. H. Kuo, *Nano Lett.*, 2016, **16**, 5514–5520.
- 7 Z. Zhang, Y. Wang, S. Xu, Y. Yu, A. Hussain, Y. Shen and S. Guo, *J. Mater. Chem. B*, 2017, **5**, 5464–5472.
- 8 Z. Zhang, S. Xu, Y. Wang, Y. Yu, F. Li, H. Zhu, Y. Shen, S. Huang and S. Guo, *J. Colloid Interface Sci.*, 2018, **509**, 47–57.
- 9 L. Au, D. Zheng, F. Zhou, Z. Y. Li, X. Li and Y. Xia, *ACS Nano*, 2008, **2**, 1645–1652.
- 10 J. G. Piao, D. Liu, K. Hu, L. Wang, F. Gao, Y. Xiong and L. Yang, *ACS Appl. Mater. Interfaces*, 2016, **8**, 2847–2856.
- 11 M. C. Daniel and D. Astruc, *Chem. Rev.*, 2004, **104**, 293–346.
- 12 J. Wu, A. S. Babadi, D. Jacobsson, J. Colvin, S. Yngman, R. Timm, E. Lind and L. E. Wernersson, *Nano Lett.*, 2016, **16**, 2418–2425.
- 13 X. Huang, X. Qi, Y. Huang, S. Li, C. Xue, C. L. Gan and F. Boey, *ACS Nano*, 2010, **4**, 6196–6202.
- 14 P. Scodeller, V. Flexer, R. Szamocki, E. J. Calvo, N. Tognalli, H. Troiani and A. Fainstein, *J. Am. Chem. Soc.*, 2008, **130**, 12690–12697.
- 15 S. Sasidharan, D. Bahadur and R. Srivastava, *ACS Appl. Mater. Interfaces*, 2016, **8**, 15889–15903.
- 16 H. Wang, S. Ming, L. Zhang, X. Li, W. Li and Z. Bo, *Nanoscale*, 2018, **10**, 11745–11749.
- 17 M. Grzelczak, J. Pérez-Juste, P. Mulvaney and L. M. Liz-Marzán, *Chem. Soc. Rev.*, 2008, **37**, 1783–1791.
- 18 J. Chen, F. Saeki, B. J. Wiley, H. Cang, M. J. Cobb, Z. Y. Li, L. Au, H. Zhang, M. B. Kimmey, X. Li and Y. Xia, *Nano Lett.*, 2005, **5**, 473–477.
- 19 Y. Wang, Y. Liu, H. Luehmann, X. Xia, P. Brown, C. Jarreau, M. Welch and Y. Xia, *ACS Nano*, 2012, **6**, 5880–5888.
- 20 X. Xia, M. Yang, L. K. Oetjen, Y. Zhang, Q. Li, J. Chen and Y. Xia, *Nanoscale*, 2011, **3**, 950–953.
- 21 H. Sun, J. Su, Q. Meng, Q. Yin, L. Chen, W. Gu, Z. Zhang, H. Yu, P. Zhang, S. Wang and Y. Li, *Adv. Funct. Mater.*, 2017, **27**, 1604300.
- 22 J. Chen, D. Wang, J. Xi, L. Au, A. Siekkinen, A. Warsen, Z. Y. Li, H. Zhang, Y. Xia and X. Li, *Nano Lett.*, 2007, **7**, 1318–1322.
- 23 J. Chen, C. Glaus, R. Laforest, Q. Zhang, M. Yang, M. Gidding, M. J. Welch and Y. Xia, *Small*, 2010, **6**, 811–817.
- 24 X. Xia, Q. Zhang, M. Yang and E. U. N. C. Cho, *Accounts Chem. Res.*, 2011, **44**, 914–924.
- 25 S. E. Skrabalak, L. Au, X. Li and Y. Xia, *Nano Lett.*, 2007, **7**, 2182–2190.
- 26 C. Kim, E. C. Cho, J. Chen, K. H. Song, L. Au, C. Favazza, Q. Zhang, C. M. Cobley, F. Gao, Y. Xia and L. V. Wang, *ACS Nano*, 2010, **4**, 4559–4564.
- 27 M. Luan, L. Yu, Y. Li, W. Pan, X. Gao, X. Wan, N. Li and B. Tang, *Anal. Chem.*, 2017, **89**, 10601–10607.
- 28 J. Wang, Z. Lu, H. Tang, L. Wu, Z. Wang, M. Wu, X. Yi and J. Wang, *Anal. Chem.*, 2017, **89**, 10834–10840.
- 29 S. R. Nicewarner Peña, S. Raina, G. P. Goodrich, N. V. Fedoroff and C. D. Keating, *J. Am. Chem. Soc.*, 2002, **124**, 7314–7323.
- 30 D. S. Seferos, A. E. Prigodich, D. A. Giljohann, P. C. Patel and C. A. Mirkin, *Nano Lett.*, 2009, **9**, 308–311.
- 31 Y. Wu, J. A. Phillips, H. Liu, R. Yang and W. Tan, *ACS Nano*, 2008, **2**, 2023–2028.
- 32 G. Dahl, *Philos. Trans. R. Soc. B Biol. Sci.*, 2015, **370**, 1–11.
- 33 K. Y. Tan, C. Y. Li, Y. F. Li, J. Fei, B. Yang, Y. J. Fu and F. Li, *Anal. Chem.*, 2017, **89**, 1749–1756.
- 34 R. Mailhot, T. Traviss-Pollard, R. Pal and S. J. Butler, *Chem. -Eur. J.*, 2018, **24**, 10745–10755.
- 35 S. V. Khlyntseva, Y. R. Bazel', A. B. Vishnikin and V. Andrich, *J. Anal. Chem.*, 2009, **64**, 657–673.
- 36 F. W. M. Lu and J. M. Chalovich, *Biochemistry*, 1995, **34**, 6359–6365.
- 37 M. Imai, E. Kaczmarek, K. Koziak, J. Sévigny, C. Goepfert, O. Guckelberger, E. Csizmadia, J. Schulte Am Esch and S. C. Robson, *Biochemistry*, 1999, **38**, 13473–13479.
- 38 B. D. Palmer, A. J. Kraker, B. G. Haitl, A. D. Panopoulos, R. L. Panek, B. L. Batley, G. H. Lu, S. Trumpp-Kallmeyer, H. D. H. Showalter and W. A. Denny, *J. Med. Chem.*, 1999, **42**, 2373–2382.
- 39 U. Roy Chowdhury, K. B. Viker, K. L. Stoltz, B. H. Holman, M. P. Fautsch and P. I. Dosa, *J. Med. Chem.*, 2016, **59**, 6221–6231.
- 40 J. Zhao, J. Gao, W. Xue, Z. Di, H. Xing, Y. Lu and L. Li, *J. Am. Chem. Soc.*, 2018, **140**, 578–581.
- 41 W. Wang, N. Zhao, X. X. Li, J. Wan and X. L. Luo, *Analyst*, 2015, **140**, 1672–1677.
- 42 V. R. Singh and P. K. Singh, *J. Mater. Chem. B*, 2020, **8**, 1182–1190.
- 43 Y. Zhong and T. Yi, *J. Mater. Chem. B*, 2019, **7**, 2549–2556.
- 44 M. Von Papen, S. Gambaryan, C. Schütz and J. Geiger, *Transfus. Med. Hemotherapy*, 2013, **40**, 109–116.
- 45 J. X. Liu, J. T. Aerts, S. S. Rubakhin, X. X. Zhang and J. V. Sweedler, *Analyst*, 2014, **139**, 5835–5842.

AuNPs-capped cage fluorescent biosensor based on controlled-release and cyclic enzymatic amplification for ultrasensitive detection of ATP

View Article Online
DOI: 10.1039/C9TB00666A

Wei Wang,* Xin Li, Kai Tang, Zhiling Song, and Xiliang Luo*

Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science, Ministry of Education, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China.



AuNPs-capped cage fluorescent biosensor based on controlled-release and cyclic enzymatic amplification for ultrasensitive detection of ATP.