




Cite this: *RSC Adv.*, 2017, 7, 25746

# Facile synthesis of Au/Al<sub>2</sub>O<sub>3</sub> nanocomposites for improving the detection sensitivity of adenosine triphosphate†

Li Xu, Qin Xu, Xiaoyu Guo, Ye Ying, Yiping Wu,\* Ying Wen and Haifeng Yang \*

Alumina is widely recognized as chemically inert, and resistant to oxidation and high temperature. In this study, Au/Al<sub>2</sub>O<sub>3</sub> nanocomposites (NCs) were prepared by a facile method and were used as a surface enhanced Raman scattering (SERS) substrate for detection of adenosine triphosphate (ATP). This Au/Al<sub>2</sub>O<sub>3</sub>-based SERS substrate demonstrated good sensitivity with the lowest detectable concentration of  $5 \times 10^{-9}$  M for ATP and a good linear relationship ranging from  $5 \times 10^{-5}$  to  $5 \times 10^{-9}$  M. The strategy of improvement of SERS detection sensitivity for ATP was based on the consideration of ATP captured by such SERS substrate *via* chelation of aluminum and phosphates, which was validated by X-ray photoelectron spectroscopy. In addition, alumina as a supporting material could be expected to prolong the stability of such a SERS substrate.

Received 30th March 2017  
 Accepted 26th April 2017

DOI: 10.1039/c7ra03683c

[rsc.li/rsc-advances](http://rsc.li/rsc-advances)

## 1 Introduction

Surface enhanced Raman scattering (SERS) was discovered in the 1970s<sup>1–3</sup> and SERS effects are generally attributed to chemical (CHEM) enhancement<sup>2</sup> and electromagnetic (EM) enhancement.<sup>3</sup> SERS is ultrasensitive and can detect some analytes at single molecular level.<sup>4–6</sup> SERS can also be utilized in multiplex detection due to structural and fingerprint information with excellent frequency resolution.<sup>7,8</sup> Therefore, SERS spectroscopy, as a non-destructive and *in situ* technique, has been widely applied in food safety,<sup>9,10</sup> biomarker detection,<sup>11–13</sup> forensic science,<sup>14,15</sup> environment monitoring<sup>16–18</sup> and other trace level analysis fields.<sup>19,20</sup>

Alumina is well known as being chemically inert and tolerant of oxidation and high temperature. Nanocomposites (NCs) formed with alumina and noble metals have been widely studied. Alumina was used to support ultrafine gold particles to act as Al<sub>2</sub>O<sub>3</sub>-supported Au catalysts.<sup>21,22</sup> Alumina nanoporous layers have demonstrated great potential as stable SERS platforms. For example, porous anodic aluminum oxide (AAO) with a well-arranged hexagonal order was synthesized as a SERS-active substrate.<sup>23–26</sup> Au/Al<sub>2</sub>O<sub>3</sub> NCs and Ag/Al<sub>2</sub>O<sub>3</sub> NCs synthesized *via* the sonoelectrochemical method improved the

thermal stability of the SERS substrates and exhibited excellent sensitivity.<sup>27–29</sup>

In addition, alumina is widely employed as a polar adsorbent in chromatographic separations and has high affinity for molecules with strong polarity, indicating that alumina has the potential to selectively bind molecules. Based on this principle, Van Duyn *et al.*<sup>30</sup> deposited a sub-1 nm alumina layer on silver film-over-nanosphere (AgFON) substrates and exploited the high adsorption affinity of carboxylic acids to alumina-modified AgFON surfaces to detect calcium dipicolinate, a bacillus spore biomarker. In addition to carboxylic acid, aluminum can adsorb phosphorus compounds, which is critical for soil fertility. Feng *et al.*<sup>31</sup> studied the size-dependent sorption of myo-inositol hexakisphosphate (IHP) and inorganic phosphate (KH<sub>2</sub>PO<sub>4</sub>, Pi) on nano- $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, and they found that surface complexation is the main mechanism for IHP and Pi sorption on large size  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> (35 nm and 70 nm), while there also exists a surface precipitation mechanism for very small size  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> (5 nm). Chen *et al.*<sup>32</sup> used pigeon ovalbumin, a phosphate protein to functionalize Fe<sub>3</sub>O<sub>4</sub>@Al<sub>2</sub>O<sub>3</sub> magnetic NPs through aluminum phosphate chelation to selectively detect Shiga-like toxin-1B.

Adenosine triphosphate (ATP) acts as a universal energy carrier in biological systems and hydrolyzes to generate energy for muscle contraction and several cytological processes.<sup>33,34</sup> A Raman spectroscopic experiment was conducted to study the interaction of divalent metal ions with the adenine moiety of ATP in 1979.<sup>35</sup> SERS spectra of ATP adsorbed on silver electrodes and the influence of voltage, pH value, and divalent metal ions on the SERS spectra of ATP were further studied in 1989.<sup>36</sup> In recent years, an aptamer biosensor with gold nanostar@Raman label@SiO<sub>2</sub> core-shell nanoparticles was constructed to detect ATP.<sup>37</sup> A 3D SERS structure made of Au/cicada wing was recently

*The Education Ministry Key Lab of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials, Shanghai Municipal Education Committee, Key Laboratory of Molecular Imaging Probes and Sensors, Department of Chemistry, Shanghai Normal University, Shanghai, 200234, China. E-mail: hfyang@shnu.edu.cn; yipingwu@shnu.edu.cn; Tel: +86-21-64321701*

† Electronic supplementary information (ESI) available: SERS spectra of  $10^{-4}$  M ATP with different pH (Fig. S1). XPS patterns of P 2p of ATP adsorbed on Al<sub>2</sub>O<sub>3</sub> and pure ATP (Fig. S2). SERS spectra of  $10^{-5}$  M ATP and  $10^{-5}$  M adenosine on Au/Al<sub>2</sub>O<sub>3</sub> NCs, respectively (Fig. S3). See DOI: 10.1039/c7ra03683c



developed for the quantitative determination of ATP in a mixture of ATP and ADP (adenosine diphosphate).<sup>38</sup> However, the above approaches to detect ATP are either laborious or require special materials.

In this study, we synthesize Au/Al<sub>2</sub>O<sub>3</sub> NCs by a facile chemical pathway for the sensitive SERS detection of ATP. ATP can be adsorbed onto the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> surface to form surface complexation *via* aluminum phosphate chelation. The high adsorption affinity of ATP molecules to Au/Al<sub>2</sub>O<sub>3</sub> NCs will further increase SERS detection sensitivity and improve the ultimate detection

limit. The acquired lowest detectable concentration is  $5 \times 10^{-9}$  M with a linear range from  $5 \times 10^{-5}$  to  $5 \times 10^{-9}$  M.

## 2 Experimental

### 2.1. Materials

Chloroauric acid (HAuCl<sub>4</sub>·4H<sub>2</sub>O, 99.9%) and sodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·2H<sub>2</sub>O, 99.8%) were obtained from Sinopharm Chemical Reagent Co., Ltd.  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> (20 nm) was obtained from Aladdin Industrial Corporation. ATP was purchased from Sigma-

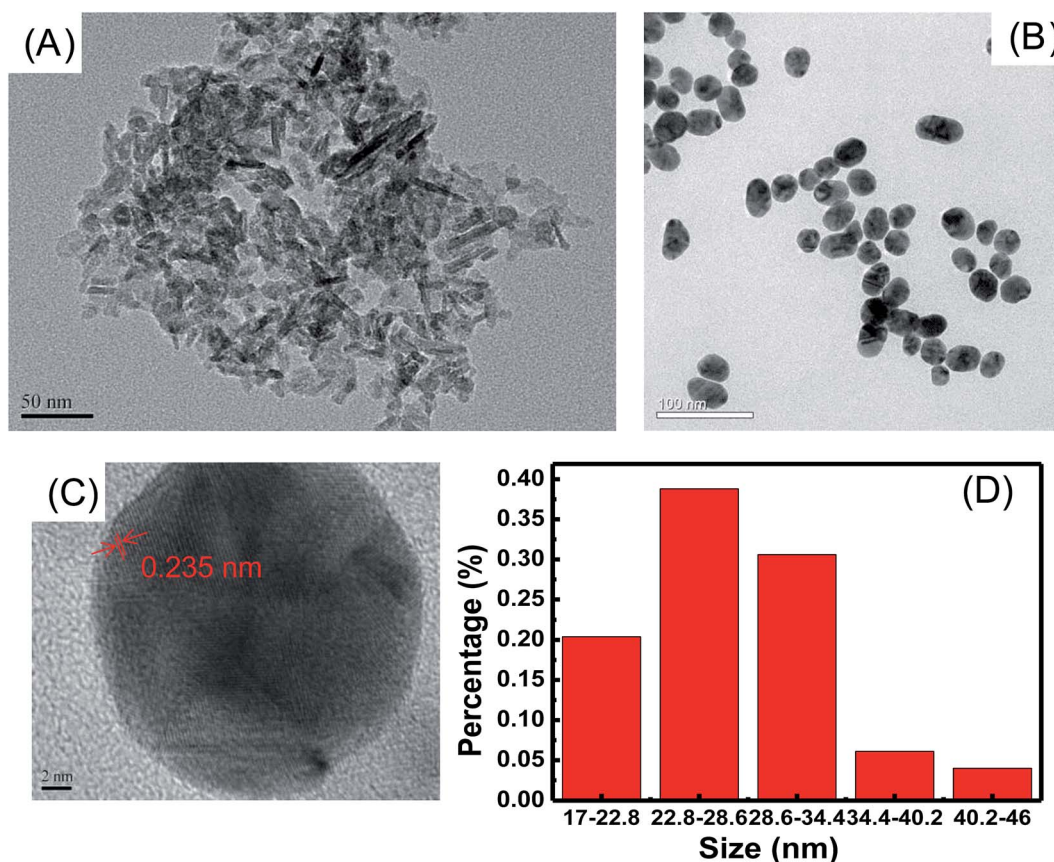


Fig. 1 TEM images of (A)  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, (B) Au NPs, (C) HR-TEM image of Au NPs and (D) size distribution of Au NPs.

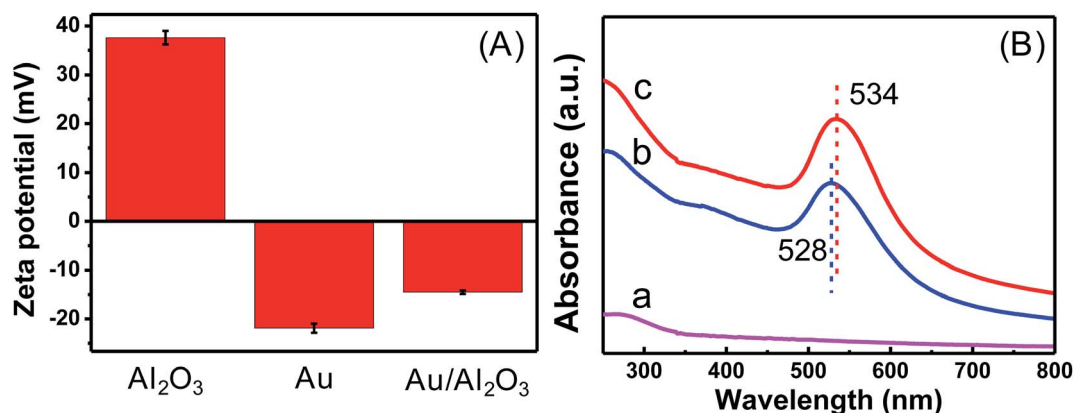


Fig. 2 (A) Zeta potential of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, Au NPs and Au/Al<sub>2</sub>O<sub>3</sub> NCs; (B) UV-vis absorption spectra of (a)  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>, (b) Au NPs and (c) Au/Al<sub>2</sub>O<sub>3</sub> NCs.



Aldrich and stored at 4 °C. All the chemicals were of analytical reagent grade and used without further purification. Ultrapure water (18.2 M $\Omega$  cm) was produced using a Millipore water purification system and used for all solution preparations. Unless otherwise noted, the synthesis reactions were carried out at room temperature (20 °C–25 °C). Phosphate buffer solution

(PBS, 0.1 M, pH = 7) was used and the pH value of PBS buffer was adjusted with dropwise addition of 0.1 M KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>.

## 2.2. Instruments

Morphologies of Au colloid and Au/Al<sub>2</sub>O<sub>3</sub> NCs were observed with a JEOL JEM-2000 FX transmission electron microscope

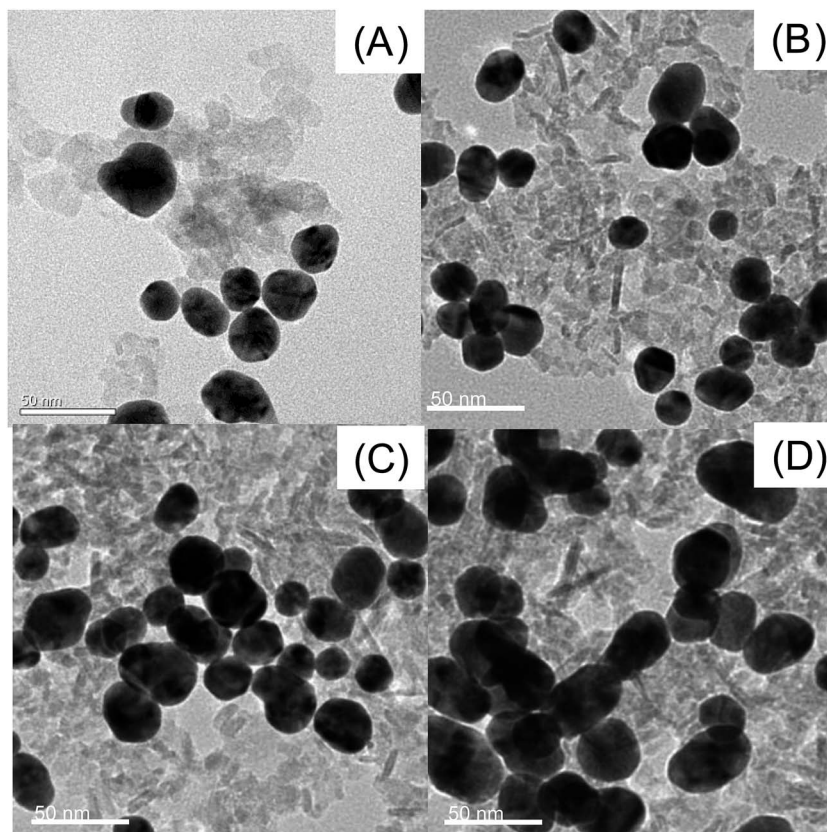


Fig. 3 TEM images of Au/Al<sub>2</sub>O<sub>3</sub> NCs synthesized with a fixed amount of Au colloid (10 mL, 0.35 mM) and different volumes of 0.1 M Al<sub>2</sub>O<sub>3</sub>: (A) 10  $\mu$ L; (B) 30  $\mu$ L; (C) 50  $\mu$ L and (D) 70  $\mu$ L. Scale bar: 50 nm.

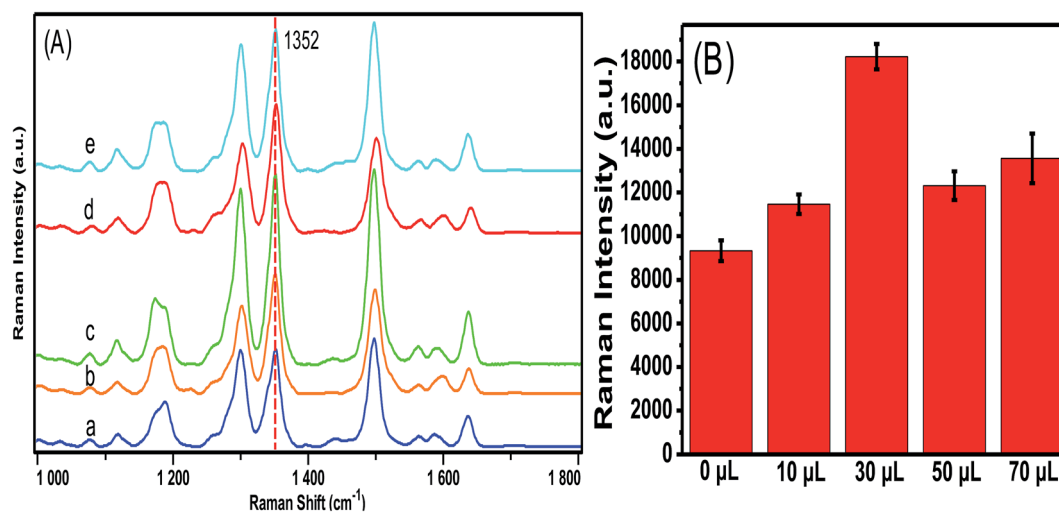


Fig. 4 (A) SERS spectra of 10<sup>-6</sup> M R6G with Au/Al<sub>2</sub>O<sub>3</sub> NCs synthesized with different volumes of 0.1 M Al<sub>2</sub>O<sub>3</sub>: (a) 0  $\mu$ L; (b) 10  $\mu$ L; (c) 30  $\mu$ L, (d) 50  $\mu$ L and (e) 70  $\mu$ L. (B) SERS intensity of the peak at 1352 cm<sup>-1</sup> for R6G absorbed on Au/Al<sub>2</sub>O<sub>3</sub> NCs prepared with different amounts of Al<sub>2</sub>O<sub>3</sub>.



(TEM) operating at an acceleration voltage of 200 kV. Surface plasmon resonance (SPR) spectra were acquired in a range of 200–800 nm using a UV-7504 UV-visible spectrophotometer (Shanghai XinMao Instrument Co., Ltd.). Zeta potential was monitored with a Zetasizer Nano ZS ZEN3600 (Malvern, UK). Raman measurements were conducted using a Portable Stabilized R. Laser Analyzer (Enwave, USA) with a narrow line width diode laser at 785 nm and an adjustable power with the maximum of 300 mW. X-ray photoelectron spectroscopy (XPS, PHI 5000 VersaProbe) was performed to identify the chemical composition of the surface.

### 2.3. Preparation of Au/Al<sub>2</sub>O<sub>3</sub> NCs as SERS substrate

Colloidal gold was prepared *via* reduction of HAuCl<sub>4</sub> by sodium citrate according to the Frens method.<sup>39</sup> Briefly, 0.25 mL of

0.1 M HAuCl<sub>4</sub> was added to 100 mL of ultrapure water and heated to boil under vigorous stirring. Then, 1.5 mL of 1% sodium citrate solution was injected into the mixture quickly. The colloid was kept boiling and was stirred for 30 min until it turned wine red.

Afterwards, different volumes (10, 30, 50, 70  $\mu$ L) of 0.1 M  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> suspension liquid were added into 10 mL of 0.35 mM Au colloid and stirred for 1 h to form Au/Al<sub>2</sub>O<sub>3</sub> NCs *via* the electrostatic interaction between Au and Al<sub>2</sub>O<sub>3</sub>. The usage of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> was optimized by the SERS performance of the as-prepared Au/Al<sub>2</sub>O<sub>3</sub> NCs with R6G as a model analyte.

### 2.4. SERS measurements

To get the optimal pH of ATP solution for Raman tests, PBS buffer (PBS, 0.1 M, pH = 7) was adjusted with dropwise addition

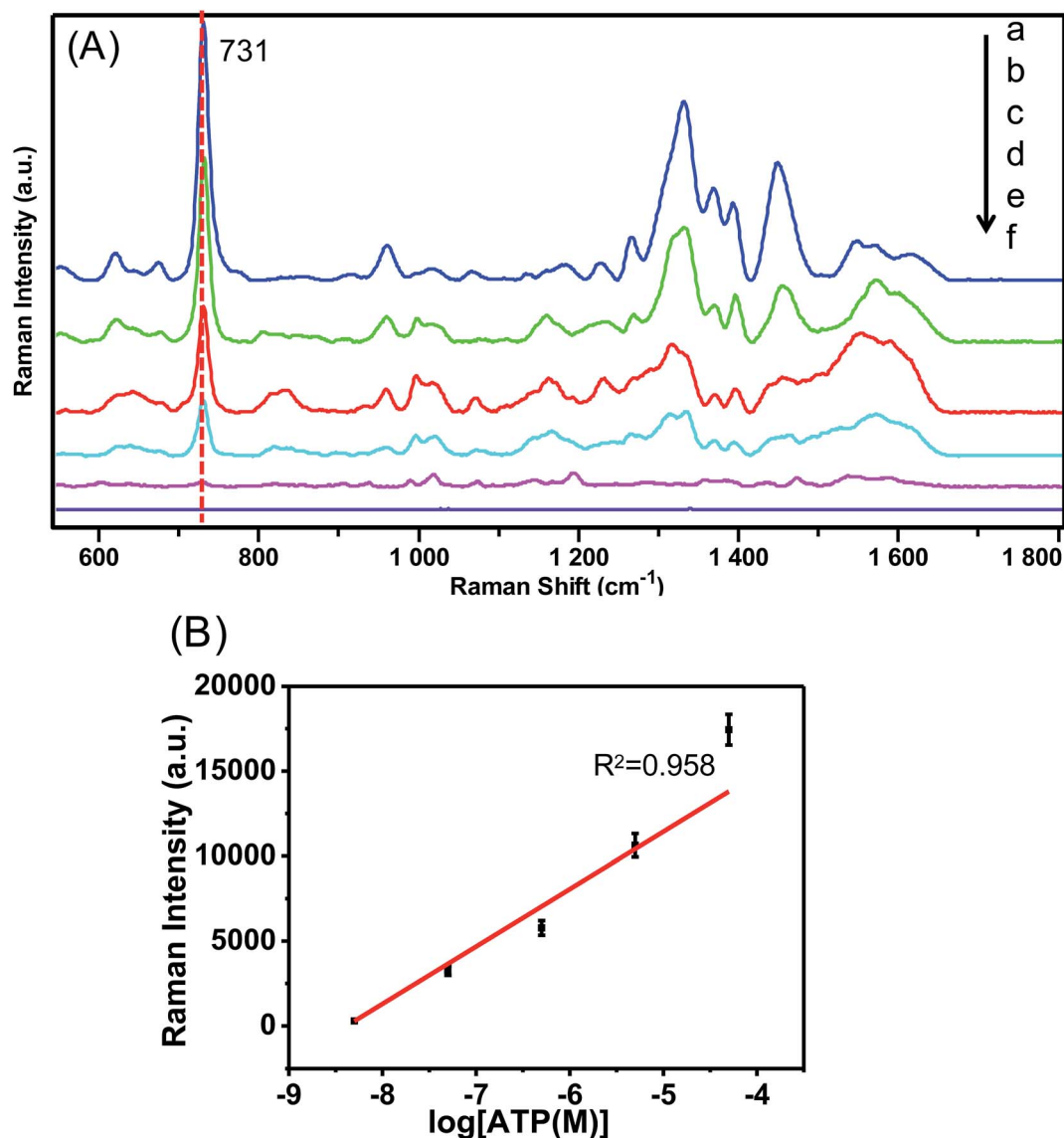


Fig. 5 (A) Concentration-dependent SERS spectra of ATP bound to the optimized Au/Al<sub>2</sub>O<sub>3</sub> NCs in a neutral environment (pH = 7). Spectra (a–f) represent the concentrations of ATP being (a)  $5 \times 10^{-5}$ , (b)  $5 \times 10^{-6}$ , (c)  $5 \times 10^{-7}$ , (d)  $5 \times 10^{-8}$ , (e)  $5 \times 10^{-9}$  M and (f) bare substrate as blank control. (B) The linear correlation of Raman intensities at 731  $\text{cm}^{-1}$  with the logarithm of ATP concentrations.



of 0.1 M  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$  to pH 3 and pH 10, respectively. After pH optimization, ATP solutions with optimal pH were prepared in a concentration range from  $10^{-4}$  to  $10^{-8}$  M. Then, 5  $\mu\text{L}$   $\text{Au}/\text{Al}_2\text{O}_3$  NCs and 5  $\mu\text{L}$  ATP solution were mixed together, dropped onto an aluminium foil and dried in the ambient environment for the subsequent Raman test. All the spectra were recorded with the acquisition time of 1 s and accumulation times of 3 s. Unless otherwise mentioned, the accumulation time and the laser power were the same for all Raman measurements.

## 3 Results and discussion

### 3.1. Characterization

The morphologies of  $\gamma\text{-Al}_2\text{O}_3$  and Au NPs were examined by TEM. The crystallite shape of 20 nm  $\gamma\text{-Al}_2\text{O}_3$  is acicular, as shown in Fig. 1A. Fig. 1B shows that Au NPs are uniform and well dispersed on a large scale. Fig. 1C is the HR-TEM image of Au NPs, which shows the crystallinity of the Au NPs. Apparent lattice fringes can be seen and the lattice spacing of two adjacent planes is 0.235 nm, corresponding to the Au (111) plane.<sup>40</sup> The size distribution of Au NPs is displayed in Fig. 1D and the average size is  $28 \pm 6$  nm.

Zeta potential was monitored to observe changes in the surface characteristics during the formation of  $\text{Au}/\text{Al}_2\text{O}_3$  NCs. Au NPs prepared by Frens' method were stabilized by citrate, thus making the surface negatively charged with a measured zeta potential value around  $-22$  mV. The iso-electric point (IEP) of 20 nm  $\gamma\text{-Al}_2\text{O}_3$  was around 9,<sup>31</sup> and the surface was positively charged at a pH lower than  $\text{pH}_{\text{IEP}}$ . The zeta potential value of  $\gamma\text{-Al}_2\text{O}_3$  dispersed in distilled water was tested and was found to be about 38 mV, which proved that the surface carried the

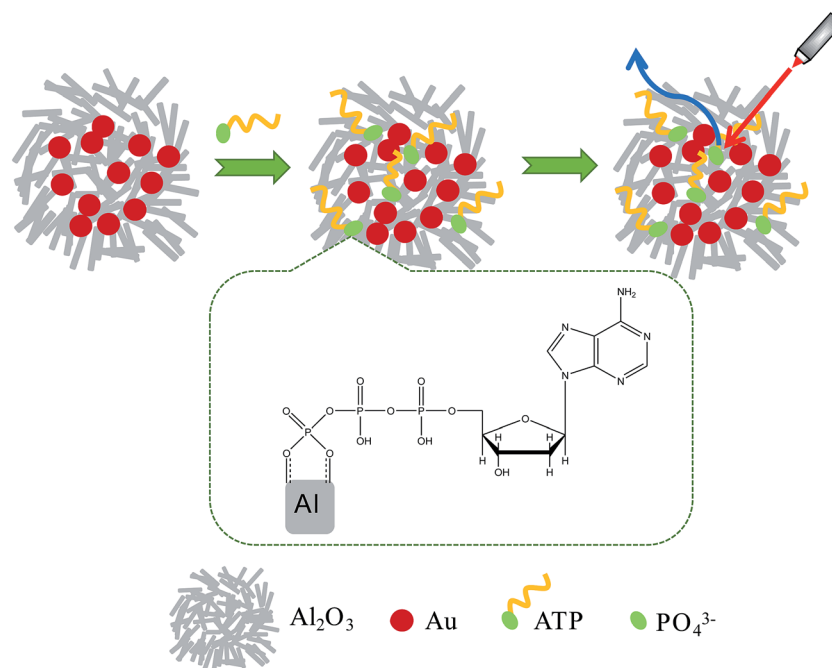
positive charge. Therefore, Au NPs and  $\gamma\text{-Al}_2\text{O}_3$  bound together due to electrostatic interaction, and the formation of  $\text{Au}/\text{Al}_2\text{O}_3$  NCs led to a final zeta potential of about  $-15$  mV (see Fig. 2A). UV-visible absorption spectra are revealed in Fig. 2B.  $\gamma\text{-Al}_2\text{O}_3$  displays a featureless band as can be seen from curve a. Curve b presents a characteristic SPR band of the prepared Au NPs at 528 nm. After the formation of  $\text{Au}/\text{Al}_2\text{O}_3$  NCs, the SPR band red-shifts to 534 nm, which can be ascribed to the formation of a bigger agglomerate of Au NPs.<sup>41</sup>  $\text{Au}/\text{Al}_2\text{O}_3$  NCs prepared with 30  $\mu\text{L}$  of 0.1 M  $\text{Al}_2\text{O}_3$  and 10 mL of 0.35 mM Au colloid were used for both zeta potential measurement and the UV-visible absorption test.

### 3.2. Optimization of SERS performance

Fig. 3 shows TEM images of  $\text{Au}/\text{Al}_2\text{O}_3$  NCs synthesized with different dosage ratios of Au to  $\text{Al}_2\text{O}_3$ . As can be seen, Au NPs are deposited onto the surface of  $\text{Al}_2\text{O}_3$  and tend to form large agglomerations with the increase in volume of 0.1 M  $\text{Al}_2\text{O}_3$  from 10 to 70  $\mu\text{L}$ . SERS performance of these four substrates was further analyzed with R6G as the Raman probe and the results are revealed in Fig. 4. It is clear that the highest SERS signal is observed with the addition of 30  $\mu\text{L}$  of 0.1 M  $\text{Al}_2\text{O}_3$ , which can be ascribed to the partial aggregation of Au NPs, but not severe aggregation as observed for the 50 and 70  $\mu\text{L}$  volumes of  $\text{Al}_2\text{O}_3$ . Therefore,  $\text{Au}/\text{Al}_2\text{O}_3$  NCs prepared with 30  $\mu\text{L}$  of 0.1 M  $\text{Al}_2\text{O}_3$  is chosen as the optimized SERS substrate and used for the following SERS determination of ATP.

### 3.3. Raman detection of ATP

Before the SERS test of ATP, the effect of pH of the ATP solution on the SERS signal was studied, and the results are given in



Scheme 1 Schematic of the procedure to detect ATP with  $\text{Au}/\text{Al}_2\text{O}_3$  NCs.



Fig. S1 (ESI†). Raman peaks show a negative shift at pH 7 compared to that at pH 3 due to deprotonation.<sup>42</sup> However, there is no significant shift when pH varies from 7 to 10. Raman intensity of ATP reaches the highest signal at pH 7, and hence SERS determination of ATP is conducted in a neutral environment. Fig. 5A shows the concentration-dependent SERS spectra of ATP at various concentrations from  $5 \times 10^{-5}$  to  $5 \times 10^{-9}$  M with the optimized Au/Al<sub>2</sub>O<sub>3</sub> NCs as SERS substrate. The intensities of characteristic peaks increase with an increase in the ATP concentration. The main peaks of ATP molecules are assigned as follows: 680 cm<sup>-1</sup> (out-of plane wagging of NH<sub>2</sub>), 731 cm<sup>-1</sup> (ring-breathing of adenine ring), 1332 cm<sup>-1</sup> (C5–N7 stretching), 1450 cm<sup>-1</sup> (C=N stretching).<sup>35</sup> The lowest detectable concentration is determined as  $5 \times 10^{-9}$  M. Fig. 5B depicts the relationship between SERS intensity of the peak at 713 cm<sup>-1</sup> and the logarithm of ATP concentration, which demonstrates a good linear relationship ( $R^2 = 0.958$ ) from  $5 \times 10^{-5}$  to  $5 \times 10^{-9}$  M.

In this study, the lowest detectable concentration is lower than that of previously reported methods by 1 order of magnitude.<sup>38</sup> The possible reasons could be inferred as illustrated in Scheme 1 that  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> provides a platform to adsorb more ATP molecules onto the SERS substrate area due to aluminum phosphate chelation.<sup>31</sup> The chelation contribution is further proven by careful comparison of XPS patterns of P 2p of ATP bound on Al<sub>2</sub>O<sub>3</sub> and pure ATP (see Fig. S2 in ESI†). After ATP binding with Al<sub>2</sub>O<sub>3</sub>, a positive shift of P 2p binding energy indicates the interaction of the phosphate group with Al<sub>2</sub>O<sub>3</sub>. Au/Al<sub>2</sub>O<sub>3</sub> NCs have also demonstrated a better SERS intensity in detecting ATP than adenosine at the same concentration (see Fig. S3 in ESI†). This chemical control selectivity provided by the supporting material of Al<sub>2</sub>O<sub>3</sub> not only helps to further lower the detection limit of ATP but also has the possibility to distinguish ATP and adenosine if multiple elutions are applied in a flow injection combined with SERS detection system. Finally, the partial aggregation of Au NPs on the Al<sub>2</sub>O<sub>3</sub> surface may also be responsible for the enhancement of the SERS signal.<sup>29</sup>

## 4 Conclusions

In summary, a facile protocol was proposed for ATP detection with Au/Al<sub>2</sub>O<sub>3</sub> NCs as SERS substrates. Au/Al<sub>2</sub>O<sub>3</sub> NCs were prepared *via* the electrostatic interaction between  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> and Au NPs and demonstrated good sensitivity with a lowest detectable concentration of  $5 \times 10^{-9}$  M and a good linear relationship from  $5 \times 10^{-5}$  to  $5 \times 10^{-9}$  M. With alumina as the supporting material, the SERS substrate acquired both stability and sensitivity. The low detection limit can be attributed to the partial aggregation of Au NPs onto the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> surface and aluminum phosphate chelation between  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> and the phosphate group of ATP. Considering their easy preparation, good sensitivity and short analysis time, Au/Al<sub>2</sub>O<sub>3</sub> NCs have potential as a promising SERS substrate for ATP detection.

## Acknowledgements

This work is supported by the National Natural Science Foundation of China (No. 21475088) and PCSIRT (IRT-16R49).

## References

- 1 M. Fleischmann, P. J. Hendra and A. J. McQuillan, *Chem. Phys. Lett.*, 1974, **26**, 163–166.
- 2 M. G. Albrecht and J. A. Creighton, *J. Am. Chem. Soc.*, 1977, **99**, 5215–5217.
- 3 D. L. Jeanmaire and R. P. Van Duyne, *J. Electroanal. Chem.*, 1977, **84**, 1–20.
- 4 S. M. Nie and S. R. Emory, *Science*, 1997, **275**, 1102–1106.
- 5 K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. R. Dasari and M. S. Feld, *Phys. Rev. Lett.*, 1997, **78**, 1667–1670.
- 6 D. Radziuk and H. Moehwald, *Phys. Chem. Chem. Phys.*, 2015, **17**, 21072–21093.
- 7 A. H. Nguyen, Y. Shin and S. J. Sim, *Biosens. Bioelectron.*, 2016, **85**, 522–528.
- 8 H. Jang, E. Y. Hwang, Y. Kim, J. Choo, J. Jeong and D. W. Lim, *J. Biomed. Nanotechnol.*, 2016, **12**, 1938–1951.
- 9 X. Hu, P. Zheng, G. Meng, Q. Huang, C. Zhu, F. Han, Z. Huang, Z. Li, Z. Wang and N. Wu, *Nanotechnology*, 2016, **27**, 384001.
- 10 M. Jahn, S. Patze, T. Bocklitz, K. Weber, D. Cialla-May and J. Popp, *Anal. Chim. Acta*, 2015, **860**, 43–50.
- 11 T. Yang, X. Guo, Y. Wu, H. Wang, S. Fu, Y. Wen and H. Yang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 20985–20993.
- 12 B. Tang, J. Wang, J. A. Hutchison, L. Ma, N. Zhang, H. Guo, Z. Hu, M. Li and Y. Zhao, *ACS Nano*, 2016, **10**, 871–879.
- 13 W. A. El-Saida, D. M. Fouada and S. A. El-Safty, *Sens. Actuators, B*, 2016, **228**, 401–409.
- 14 Z. Han, H. Liu, J. Meng, L. Yang and J. Liu, *Anal. Chem.*, 2015, **87**, 9500–9506.
- 15 B. Sägmüller, B. Schwarze, G. Brehm and S. Schneider, *Analyst*, 2001, **126**, 2066–2071.
- 16 D. Han, S. Y. Lim, B. J. Kim, L. Piao and T. D. Chung, *Chem. Commun.*, 2010, **46**, 5587–5589.
- 17 S. S. R. Dasary, A. K. Singh, D. Senapati, H. Yu and P. C. Ray, *J. Am. Chem. Soc.*, 2009, **131**, 13806–13812.
- 18 S. J. Lee and M. Moskovits, *Nano Lett.*, 2011, **11**, 145–150.
- 19 A. Barhoumi and N. J. Halas, *J. Am. Chem. Soc.*, 2010, **132**, 12792–12793.
- 20 R. Li, S. Li, M. Dong, L. Zhang, Y. Qiao, Y. Jiang, W. Qi and H. Wang, *Chem. Commun.*, 2015, **51**, 16131–16134.
- 21 J. Jia, K. Haraki, J. N. Kondo, K. Domen and K. Tamaru, *J. Phys. Chem. B*, 2000, **104**, 11153–11156.
- 22 J. Radnik, L. Wilde, M. Schneider, M. M. Pohl and D. Herein, *J. Phys. Chem. B*, 2006, **110**, 23688–23693.
- 23 M. Pisarek, R. Nowakowski, A. Kudelski, M. Holdynski, A. Roguska, M. Janik-Czachor, E. Kurowska-Tabor and G. D. Sulka, *Appl. Surf. Sci.*, 2015, **357**, 1736–1742.
- 24 P. Nielsen, S. Hassing, O. Albrechtsen, S. Foghmoes and P. Morgen, *J. Phys. Chem. C*, 2009, **113**, 14165–14171.
- 25 K. Malek, A. Brzózka, A. Rygula and G. D. Sulka, *J. Raman Spectrosc.*, 2014, **45**, 281–291.
- 26 K. D. Jernshøj, S. Hassing, R. S. Hansen and P. Krohne-Nielsen, *J. Chem. Phys.*, 2011, **135**, 124514.



- 27 C. C. Chang, C. C. Yu, Y. C. Liu and K. H. Yang, *J. Electroanal. Chem.*, 2013, **696**, 38–44.
- 28 F. D. Mai, K. H. Yang, Y. C. Liu and T. C. Hsu, *Analyst*, 2012, **137**, 5906–5912.
- 29 F. D. Mai, C. C. Yu, K. H. Yang and M. Y. Juang, *J. Phys. Chem. C*, 2011, **115**, 13660–13666.
- 30 X. Zhang, J. Zhao, A. V. Whitney, J. W. Elam and R. P. Van Duyne, *J. Am. Chem. Soc.*, 2006, **128**, 10304–10309.
- 31 Y. Yan, L. K. Koopal, W. Li, A. Zheng, J. Yang, F. Liu and X. Feng, *J. Colloid Interface Sci.*, 2015, **451**, 85–92.
- 32 F. Y. Kuo, B. Y. Chang, C. Y. Wu, K. K. T. Mong and Y. C. Chen, *Anal. Chem.*, 2015, **87**, 10513–10520.
- 33 J. Ross, *J. Phys. Chem. B*, 2006, **110**, 6987–6990.
- 34 T. Tenório, A. M. Silva, J. M. Ramos, C. D. Buarque and J. Felcman, *Spectrochim. Acta, Part A*, 2013, **105**, 88–101.
- 35 A. Lanir and N. T. Yu, *J. Biol. Chem.*, 1979, **254**, 5382–5887.
- 36 T. T. Chen, C. S. Kuo, Y. C. Chou and N. T. Liang, *Langmuir*, 1989, **5**, 887–891.
- 37 M. Li, J. Zhang, S. Suri, L. J. Sooter, D. Ma and N. Wu, *Anal. Chem.*, 2012, **84**, 2837–2842.
- 38 H. Fang, H. J. Yin, M. Y. Lv, H. J. Xu, Y. M. Zhao, X. Zhang, Z. L. Wu, L. Liu and T. W. Tan, *Biosens. Bioelectron.*, 2015, **69**, 71–76.
- 39 G. Frens, *Nature*, 1973, **241**, 20–22.
- 40 T. Yang, X. Guo, H. Wang, S. Fu, Y. Wen and H. Yang, *Biosens. Bioelectron.*, 2015, **68**, 350–357.
- 41 Y. Lin, C. Chen, C. Wang, F. Pu, J. Ren and X. Qu, *Chem. Commun.*, 2011, **47**, 1181–1183.
- 42 R. A. Alberty, *J. Biol. Chem.*, 1968, **243**, 1337–1343.

