



Cite this: DOI: 10.1039/c5cp00206k

High performance surface-enhanced Raman scattering from molecular imprinting polymer capsulated silver spheres†

Yan Guo,^a Leilei Kang,^a Shaona Chen^a and Xin Li^{*ba}

Driven by the ultrasensitivity of the surface-enhanced Raman scattering (SERS) technique and the directive selection of molecular imprinting polymers (MIPs), core-shell silver-molecularly imprinted polymer (Ag@MIP) hybrid structure was synthesized to serve as a novel SERS platform. The results show that as prepared Ag@MIPs wrapped over a thin shell of MIPs are sensitive to the template molecule (Rhodamine 6G). To investigate the selectivity of Ag@MIPs, the structurally related molecules, such as Rhodamine B and crystal violet, were chosen as controls. Notably, the high sensitivity of Ag@MIPs is restrained by the non-specific recognition of Rhodamine B and crystal violet. This high SERS enhancement of the template molecule can be interpreted by the "gate effect" and/or "dummy hot spots". We believe that the sensitivity of SERS coupled with the selectivity of MIPs could induce a promising chemosensor or biosensor for practical applications.

Received 14th January 2015,
Accepted 6th February 2015

DOI: 10.1039/c5cp00206k

www.rsc.org/pccp

Introduction

Surface-enhanced Raman scattering (SERS) has witnessed a tremendous advance in theoretical and experimental results due to the rapid development of computational science and nanotechnology since this available phenomenon was discovered in 1974.^{1,2} Based on the SERS method, the detection limit has achieved an unprecedented level of single molecule detection.^{3–6} Besides scientific research, a great deal of effort has been made in order to apply this promising tool to our real life, such as food,^{7,8} environment,⁹ medicine,^{10,11} and so on.^{12,13} To meet these demands, all kinds of SERS substrates with super-hydrophobicity,¹⁴ high affinity¹⁵ and intense plasmonic nanostructures¹⁶ have been fabricated in recent years. However, it is still a challenge for these developed SERS platforms to fulfil the requirements relevant to significant social problems.^{17,18}

The molecular imprinting technique (MIT) has been developed as one of the most important tools for the specific identification, separation and analyte detection in chemistry, biology, materials, and medicine.^{19–23} Due to their stability, specific recognition and reusability, molecular imprinting polymers (MIPs) are of great interest. Hence, the combination of the advantages of SERS and MIPs would be expected to immensely promote the development of sensitive and selective detection. However, the number of reported

papers relevant to the marriage of SERS and MIPs is very limited to date. MIPs were explored to integrate with a SERS-active film as the sensing layer to detect the explosive, 2,4,6-trinitrotoluene (TNT).²⁴ A SERS-based sensor for the determination of theophylline had been developed by imprinting the template molecules on the SERS active surface of Ag nanoparticles, showing a good reproducibility and a dose-response relationship.²⁵ Long and coworkers reported that surface-imprinted core-shell Au nanoparticles can be explored for highly selective detection of bisphenol A (BPA) based on SERS. The detection limit can reach 0.12 mg L⁻¹ owing to the obviously specific identification, which furnishes a promising tool for sensitive and selective detection in real samples.²⁶ Recently, we have reported that the core-shell silver-molecularly imprinted polymer (Ag@MIPs) hybrid as a SERS platform has potential for ultrasensitive sensing and analytical applications.²⁷

Here, we introduce the synthesis of the Ag@MIPs hybrid system using Rhodamine 6G (R6G) as the template molecule. The developed Ag@MIP complex is sensitive to the template molecule due to the specific recognition. Moreover, the ability of its exclusively selective detection of R6G was carefully investigated by comparing with other analogues, such as Rhodamine B (RB) and Crystal violet (CV).

Experimental

Synthesis of Ag microspheres

Ag particles were synthesized by integrating the reported methods with some improvements.^{28,29} In a typical liquid-phase reduction

^a Department of Chemistry, Harbin Institute of Technology, Harbin 150001, P. R. China

^b State Key Lab of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, P. R. China. E-mail: lixin@hit.edu.cn

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5cp00206k

synthesis, 2 mL of AgNO_3 aqueous solution (1 M) and 20 mL of polyvinyl pyrrolidone aqueous solution (0.2 M, $M_w \approx 40\,000$ – $50\,000$) were added into 100 mL of ultrapure water under stirring in an ice-water bath. Then, 2 mL of ascorbic acid (1 M) was quickly injected into the mixture and stirred continually for 15 min. The resulting products were collected by centrifugation and then dried in a vacuum at $40\text{ }^\circ\text{C}$ for 24 h.

Synthesis of the Ag@MIPs hybrid

To modify the surface of Ag particles with 3-methacryloxypropyltrimethoxysilane (MPS), 500 mg of Ag particles and 8 mL of MPS were added into 40 mL solution of ethanol–water (4 : 1, v/v). After the sample dissolved completely, the mixture was magnetically stirred at $40\text{ }^\circ\text{C}$ for 24 h under nitrogen protection. The products (Ag@MPS) were washed with ethanol several times and then dried under vacuum. In order to synthesize the Ag@MIPs hybrid, 0.5 mmol of R6G, 5 mmol of acrylamide, 25 mmol of ethylene glycol dimethacrylate (EGDMA), 20 mg of azobisisobutyronitrile (AIBN) and 200 mg of modified Ag particles (Ag@MPS) were dispersed into 40 mL of acetonitrile and stirred for 24 h at $65\text{ }^\circ\text{C}$ under the protection of nitrogen. The sample was washed with methanol–acetic acid (4 : 1, V/V) solution until no template molecule was detected in the washing solution by UV-vis spectroscopy. Finally, the resulting products were washed with ethanol and then dried under vacuum at $40\text{ }^\circ\text{C}$ for 24 h.

Characterization

The products were characterized using a X-ray diffractometer (XRD, Shimadzu XRD, 6000) with Cu K α radiation ($\lambda = 1.5418\text{ \AA}$), a transmission electron microscope (TEM, FEI, Tecnai G² F20), and a scanning electron microscope (SEM, Hitachi S4800 HSD). Fourier transform infrared spectra (FT-IR) were recorded on an Avatar 360 (Nicolet) instrument by dispersing the sample in a KBr pellet (0.1%, w/w). UV-vis absorption spectroscopy was conducted on T6 (Beijing Purkinje General Instrument Co., Ltd). SERS spectra were collected on a Renishaw inVia confocal micro-Raman spectroscopy system ($\lambda = 633\text{ nm}$). The incident laser power and exposure time were 0.1 mW and 10 s, respectively.

Results and discussion

The detection of specific and low-affinity molecules is one of the problems, which is to be urgently solved in the SERS field. Polymers resulting from the polymerization of monomers retain populations of complementary matching sites in the presence of target molecules or a fragment due to the collective interactions between monomers and templates, which provides a possible way to overcome the drawbacks of SERS technology.²³ In our previous work, we have successfully synthesized an Ag@MIP hybrid with high sensitivity.²⁷ However, its selectivity has not yet been investigated. It is well-known that the SERS phenomenon is an obvious surface effect. Especially for core-shell structure, the shell thickness is considered to be the key factor that affects SERS performance and the Raman signal decreases exponentially with increasing shell thickness.^{30,31} Therefore, the fabrication

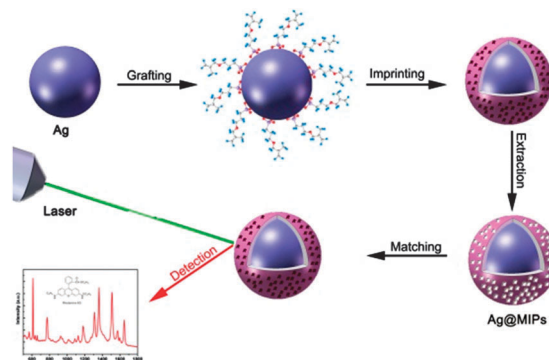


Fig. 1 Schematic illustration of the fabrication and detection process of Ag@MIPs.

of Ag@MIPs with thin MIPs should be beneficial to improving SERS performance. We herein prepared Ag@MIPs residing short-distance recognition sites onto the Ag core. Schematic illustration of the fabrication and detection process of Ag@MIPs is presented in Fig. 1.

As shown in Fig. 2, SEM and TEM images were conducted to evaluate the morphology and nanostructures of Ag and Ag@MIPs. Ag particles with a smooth surface were produced, which would be harmful to SERS response, but offer a good opportunity to wrap a uniform shell of MIPs. And the distribution of the particle size is narrowed to $\sim 800\text{ nm}$ in diameter (Fig. 2a). There are no obvious changes in surface morphology of Ag and Ag@MIP spheres, except for a small amount of scattering aggregates which possibly come from the self-polymerization of functional monomers (Fig. 2c). Compared with the TEM image of Ag spheres, a shell with a thickness of about 16 nm clearly appears on the Ag@MIP surface, indicating that Ag spheres have been successfully encapsulated by MIPs (Fig. 2b and d). It is worthy to point out that the self-polymerization of the monomer is inevitable in the polymerization process, which slightly disturbs the quality of the MIP shell.

X-ray diffraction (XRD) patterns of Ag and Ag@MIPs in Fig. 3 show the typical face-centered cubic (fcc) Ag crystal phase, where the diffraction peaks can be well indexed to the (111), (200), (220) and (311) crystal planes. The relative intensity ratio

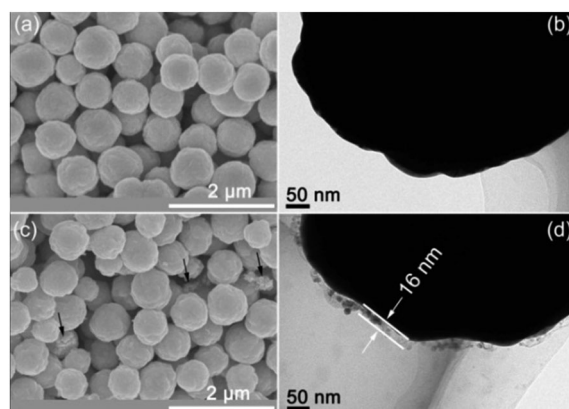


Fig. 2 SEM and TEM images of Ag spheres (a, b) and Ag@MIPs (c, d). Arrows pointed scattering aggregates originated from the self-polymerization of the functional monomer.

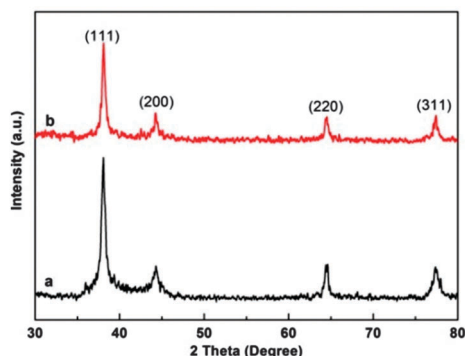


Fig. 3 XRD patterns of Ag (a) and Ag@MIPs (b).

of diffraction peaks is almost identical but the corresponding peak intensity of Ag@MIPs is weaker than that of the pure Ag phase, suggesting that the encapsulation process would impair the intensity of diffraction peaks. Furthermore, the energy dispersive spectrum (EDS) from Ag@MIPs shows the existence of C and O which is absent in naked Ag spheres (Fig. S1, ESI[†]). Also, there are lots of newly formed functional groups in the Fourier transform infrared (FT-IR) spectrum collected from Ag@MIPs. The detailed analysis shows that these vibrational modes come from the functional monomer (acrylamide) and the cross linking agent (EGDMA) (Fig. S2, ESI[†]). These data, taken together, indicate that MIPs have been successfully wrapped over the Ag cores.

The removal of template molecules is one of the most important procedures to realize the quantitative determination of target molecules for MIPs. Fig. 4 shows the UV-visible absorption spectra of R6G in supernatant liquid as a function of cleaning time scale. It can be seen that the initially prominent absorption peak gradually declines as the washing process continues, and completely disappears after 4 h, which manifests that R6G molecules have been thoroughly eliminated from Ag@MIPs. The weak and ambiguous Raman peaks collected from washed Ag@MIPs demonstrate that these formerly imprinted recognition sites on MIPs were created after a long period of washing time (Fig. S3, ESI[†]).

R6G, the template molecule in our system, was used as the first target analyte to examine the SERS performance of the prepared Ag@MIPs. As shown in Fig. S4 (ESI[†]), Raman spectra

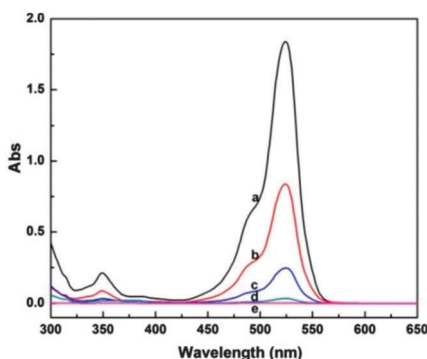


Fig. 4 UV-visible spectra of R6G in supernatant liquid as a function of rinsing time scale of (a) 0 h, (b) 1 h, (c) 2 h, (d) 3 h and (e) 4 h.

of R6G at a concentration of 10^{-5} M were collected from Ag particles and Ag@MIPs, respectively. It can be seen that high-quality Raman spectra of R6G were obtained. The characteristic Raman fingerprints of R6G molecules at 1313 cm^{-1} , 1360 cm^{-1} , 1508 cm^{-1} , 1570 cm^{-1} and 1648 cm^{-1} can be assigned to C=C stretching vibration of the benzene ring. And a prominent peak at 611 cm^{-1} is responsible for C-C resonance. Moreover, Raman bands at 774 cm^{-1} and 1184 cm^{-1} can be ascribed to stretching and bend vibration of C-H. These peak positions are well consistent with the reported studies employing R6G as the model analyte.^{32–34} One can find that the corresponding Raman peaks collected from Ag@MIPs are much stronger than that from Ag spheres. This phenomenon indicates that recognition sites residing on Ag@MIPs could be more efficient as so called “hot spots” than gaps between pure Ag particles. The presence of MIPs avoids the target molecules from directly contacting with plasmonic metal, which protects the SERS active core from contaminating by what is being probed.

Concentration-dependent SERS spectra were recorded to test the SERS performance of Ag@MIPs by ascertaining the limit of detection. As shown in Fig. 5a, the intensity of characteristic peaks declines with decreasing concentration of R6G. However, the distinguishable Raman fingerprints can still be clearly observed even when the concentration of R6G was diluted to 10^{-14} M. Fig. 5b shows the plots of SERS intensity depending on the Raman peaks at 1184 cm^{-1} and 1508 cm^{-1} as a function of the concentration of R6G. Of note is that the SERS intensity is obtained by subtracting that of the blank sample. One can see that SERS intensity increases gradually with the increment of the concentration of R6G. There is a linear relationship

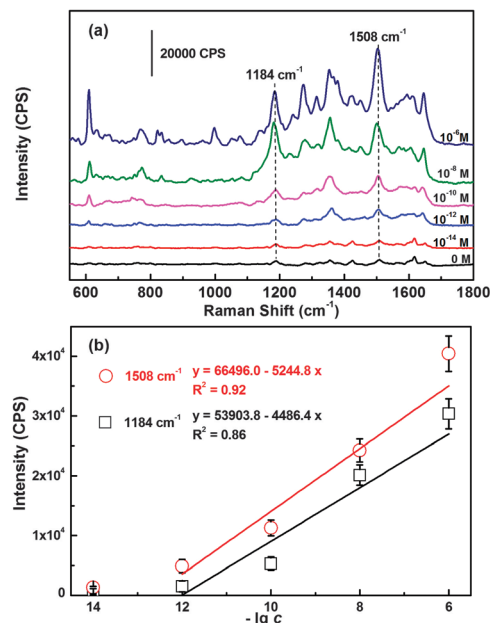


Fig. 5 (a) Concentration-dependent SERS spectra of R6G obtained from Ag@MIPs. (b) Linear relationship of Raman intensity as a function of the R6G concentration for the bands at 1184 cm^{-1} and 1508 cm^{-1} . Each data point is the average result of three SERS spectra.

between SERS intensity and the concentration of R6G in the range from 10^{-12} M to 10^{-6} M. Based on the given linear equations, as prepared Ag@MIPs can be explored for quantitative detection. Interestingly, the SERS intensity of R6G does not drop linearly when the concentration of R6G is 10^{-14} M. It could be interpreted that the imprinted cavities could be highly efficient to capture the template molecule even when the number of the molecules is very limited. Bearing this in mind, the detection limit of Ag@MIPs for R6G can be estimated as 10^{-14} M. The enhancement factor (EF) was calculated to be $\sim 10^8$ using the R6G peak at 1184 cm^{-1} (ESI†). However, Ag spheres without MIPs can only detect R6G with a concentration above 10^{-7} M. (Fig. S5, ESI†).

The specific recognition is the most important property of MIPs. After removal of the template molecules, the imprinted cavities with spatial characteristics and chemical functionalities were created. As a consequence, template molecules with a specific size and functional groups will be anchored into cavities by chemical bonding in the penetration process. RB and CV, possessing similar molecular structures with R6G, were selected for investigating the specific recognition of Ag@MIPs. The results show that almost identical SERS intensity of them was obtained when Ag particles were utilized as SERS platforms (Fig. S6, ESI†). As expected, it can be seen in Fig. 6 that the Raman signal of R6G on Ag@MIPs is much stronger than that of RB and CV. This phenomenon is rationalized that R6G molecules can be captured into the cavities of MIPs because of the compatibility of the size and the bonding effect. The only difference between R6G and RB in the molecular formula is their functional group. Therefore, the weak non-covalent and/or reversible covalent interactions between functional groups of RB and active sites of MIPs lead to an unstable state for RB in the R6G formed sites, which may be responsible for a subdued SERS response. Further analysis shows that Raman intensity of RB is stronger than that of CV, which would originate from the mismatch in both the size and functional groups. These results suggest that the Raman signal of the template molecule can be tremendously amplified on Ag@MIPs by the specific recognition. In contrast, the analytes mismatching in the spatial characteristic and/or bonding effect will obtain weak SERS signals. Notably, the SERS signals of RB and CV

cannot completely vanish from Raman spectra. Even though the SERS technique is a very sensitive tool, a very small amount of analytes is non-specifically absorbed onto the surface of Ag@MIPs.

This sensitive and selective performance would be responsible for the core-shell structure and near-surface recognition sites. To date, charge transfer (chemical enhancement) and electromagnetic enhancement (physical enhancement) have been widely accepted as SERS enhancement mechanisms. However, the thickness of the MIP shell in our case is up to 16 nm, which is difficult to interpret by the classic theory (*i.e.* Förster resonance energy transfer).³⁵ The possible reason for MIPs based SERS mechanism is the existence of unobserved tunnel leading chemical species to the near surface of plasmonic metal, which is named the “gate effect”.^{36–38} In our case, the template molecule (R6G) will have access to the cavities and could move close to the Ag core with the assistance of the gate effect. It will be easy to understand that the high performance of Ag@MIPs is contributed by both chemical and physical enhancements.

Recently, researchers found that laser light can be stored in a bottle-like microresonator for about ten nanoseconds. The interaction between light and single atoms will happen during this period.³⁹ Inspired by this phenomenon, we suggest that some of the recognition sites on MIPs can capture not only the spatial scale and/or chemical interaction matched species but also a large amount of photons, which will be available as “dummy hot spots” for SERS enhancement. Once matched species were anchored onto these cavities, the Raman signals will be immensely amplified. But mismatch of species in size and chemical bonding will lead to a bleak SERS response. Combining both of them, the extremely high enhancement of Ag@MIPs could be more rationalized by the synergetic effect of the “gate effect” and “dummy hot spots”. However, the deep insight into the MIP related SERS enhancement mechanism needs to be further explored in future work.

Conclusions

In summary, we have successfully synthesized an Ag@MIP hybrid system with a thin MIP shell (~ 16 nm). As-prepared core-shell nanostructure shows a high sensitivity and selectivity toward the template molecule. In this regard, it can be exactly interpreted by the “gate effect” and “dummy hot spots”, or the synergetic effect of both of them. However, there are many problems to handle before the MIP related SERS method could serve as a robust technique in practical applications. Therefore, future research efforts need to be continually made.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

The authors would like to thank the financial support from the National Natural Science Foundation of China (No. 21176052

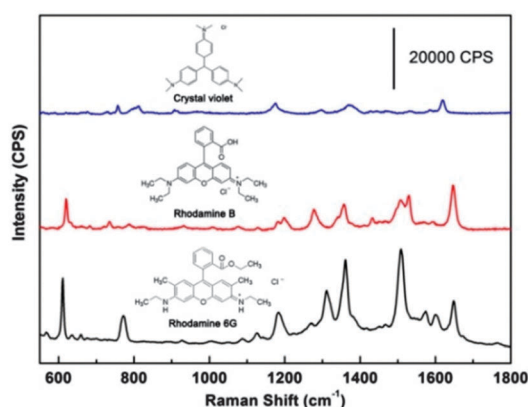


Fig. 6 SERS spectra of R6G, RB and CV recorded from Ag@MIPs at a concentration of 10^{-5} M and their corresponding molecular structures.

and 51379052) and the State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (2013TS05).

Notes and references

- 1 M. Fleischmann, P. J. Hendra and A. J. McQuillan, *Chem. Phys. Lett.*, 1974, **26**, 163–166.
- 2 M. Fan, G. F. S. Andrade and A. G. Brolo, *Anal. Chim. Acta*, 2011, **693**, 7–25.
- 3 K. Kneipp, Y. Wang, H. Kneipp, L. T. Perelman, I. Itzkan, R. Dasari and M. S. Feld, *Phys. Rev. Lett.*, 1997, **78**, 1667–1670.
- 4 S. M. Nie and S. R. Emery, *Science*, 1997, **275**, 1102–1106.
- 5 L. Brus, *Acc. Chem. Res.*, 2008, **41**, 1742–1749.
- 6 R. Zhang, Y. Zhang, Z. C. Dong, S. Jiang, C. Zhang, L. G. Chen, L. Zhang, Y. Liao, J. Aizpurua, Y. Luo, J. L. Yang and J. G. Hou, *Nature*, 2013, **498**, 82–86.
- 7 M. Harz, P. Rosch, K. D. Peschke, O. Ronneberger, H. Burkhardt and J. Popp, *Analyst*, 2005, **130**, 1543–1550.
- 8 M. Lin, L. He, J. Awika, L. Yang, D. R. Ledoux, H. Li and A. Mustapha, *J. Food Sci.*, 2008, **73**, T129–T134.
- 9 S. L. Truong, G. Levi, F. Bozon-Verduraz, A. V. Petrovskaya, A. V. Simakin and G. A. Shafeev, *Appl. Phys. A: Mater. Sci. Process.*, 2007, **89**, 373–376.
- 10 X. Wang, C. Wang, L. Cheng, S.-T. Lee and Z. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 7414–7422.
- 11 L. Xu, H. Kuang, C. Xu, W. Ma, L. Wang and N. A. Kotov, *J. Am. Chem. Soc.*, 2012, **134**, 1699–1709.
- 12 W. Ren, Y. Fang and E. Wang, *ACS Nano*, 2011, **5**, 6425–6433.
- 13 M. P. Cecchini, V. A. Turek, J. Paget, A. A. Kornyshev and J. B. Edel, *Nat. Mater.*, 2013, **12**, 165–171.
- 14 F. De Angelis, F. Gentile, F. Mecarini, G. Das, M. Moretti, P. Candeloro, M. L. Coluccio, G. Cojoc, A. Accardo, C. Liberale, R. P. Zaccaria, G. Perozziello, L. Tirinato, A. Toma, G. Cuda, R. Cingolani and E. Di Fabrizio, *Nat. Photonics*, 2011, **5**, 683–688.
- 15 X. Liu, L. Cao, W. Song, K. Ai and L. Lu, *ACS Appl. Mater. Interfaces*, 2011, **3**, 2944–2952.
- 16 S. M. Ansar, R. Haputhanthri, B. Edmonds, D. Liu, L. Yu, A. Sygula and D. Zhang, *J. Phys. Chem. C*, 2011, **115**, 653–660.
- 17 R. A. Alvarez-Puebla and L. M. Liz-Marzan, *Chem. Soc. Rev.*, 2012, **41**, 43–51.
- 18 Y. Wang, B. Yan and L. Chen, *Chem. Rev.*, 2013, **113**, 1391–1428.
- 19 F. Lai, U. A. Orom, M. Cesaroni, M. Beringer, D. J. Taatjes, G. A. Blobel and R. Shiekhattar, *Nature*, 2013, **494**, 497–501.
- 20 C. Malitesta, E. Mazzotta, R. A. Picca, A. Poma, I. Chianella and S. A. Piletsky, *Anal. Bioanal. Chem.*, 2012, **402**, 1827–1846.
- 21 A. P. F. Turner, *Chem. Soc. Rev.*, 2013, **42**, 3184–3196.
- 22 X. Xing, S. Liu, J. Yu, W. Lian and J. Huang, *Biosens. Bioelectron.*, 2012, **31**, 277–283.
- 23 Y. Hoshino, H. Koide, T. Urakami, H. Kanazawa, T. Kodama, N. Oku and K. J. Shea, *J. Am. Chem. Soc.*, 2010, **132**, 6644–6645.
- 24 E. L. Holthoff, D. N. Stratis-Cullum and M. E. Hankus, *Sensors*, 2011, **11**, 2700–2714.
- 25 P. Liu, R. Liu, G. Guan, C. Jiang, S. Wang and Z. Zhang, *Analyst*, 2011, **136**, 4152–4158.
- 26 J. Q. Xue, D. W. Li, L. L. Qu and Y. T. Long, *Anal. Chim. Acta*, 2013, **777**, 57–62.
- 27 C. Limin, D. Yan and L. Xin, *Biosens. Bioelectron.*, 2013, **50**, 106–110.
- 28 B. Zhang, P. Xu, X. Xie, H. Wei, Z. Li, N. H. Mack, X. Han, H. Xu and H. L. Wang, *J. Mater. Chem.*, 2011, **21**, 2495–2501.
- 29 H. Liang, Z. Li, W. Wang, Y. Wu and H. Xu, *Adv. Mater.*, 2009, **21**, 4614–4618.
- 30 J. A. Dieringer, A. D. McFarland, N. C. Shah, D. A. Stuart, A. V. Whitney, C. R. Yonzon, M. A. Young, X. Y. Zhang and R. P. Van Duyne, *Faraday Discuss.*, 2006, **132**, 9–26.
- 31 D. Li, S. Wu, Q. Wang, Y. Wu, W. Peng and L. Pan, *J. Phys. Chem. C*, 2012, **116**, 12283–12294.
- 32 X. Zhu, L. Shi, M. S. Schmidt, A. Boisen, O. Hansen, J. Zi, S. Xiao and N. A. Mortensen, *Nano Lett.*, 2013, **13**, 4690–4696.
- 33 J. T. Bahns, Q. Guo, J. M. Montgomery, S. K. Gray, H. M. Jaeger and L. Chen, *J. Phys. Chem. C*, 2009, **113**, 11190–11197.
- 34 X. Li, H. Hu, D. Li, Z. Shen, Q. Xiong, S. Li and H. J. Fan, *ACS Appl. Mater. Interfaces*, 2012, **4**, 2180–2185.
- 35 Y. Chen, M. B. O'Donoghue, Y. F. Huang, H. Kang, J. A. Phillips, X. Chen, M. C. Estevez, C. J. Yang and W. Tan, *J. Am. Chem. Soc.*, 2010, **132**, 16559–16570.
- 36 Y. Yoshimi, K. Sato, M. Ohshima and E. Piletska, *Analyst*, 2013, **138**, 5121–5128.
- 37 S. A. Piletsky, T. L. Panasyuk, E. V. Piletskaya, I. A. Nicholls and M. Ulbricht, *J. Membr. Sci.*, 1999, **157**, 263–278.
- 38 Y. Yoshimi, A. Narimatsu, K. Nakayama, S. Sekine, K. Hattori and K. Sakai, *J. Artif. Organs*, 2009, **12**, 264–270.
- 39 <http://phys.org/news/2013-05-helicopter-light-beams-tool-quantum-optics.html>.