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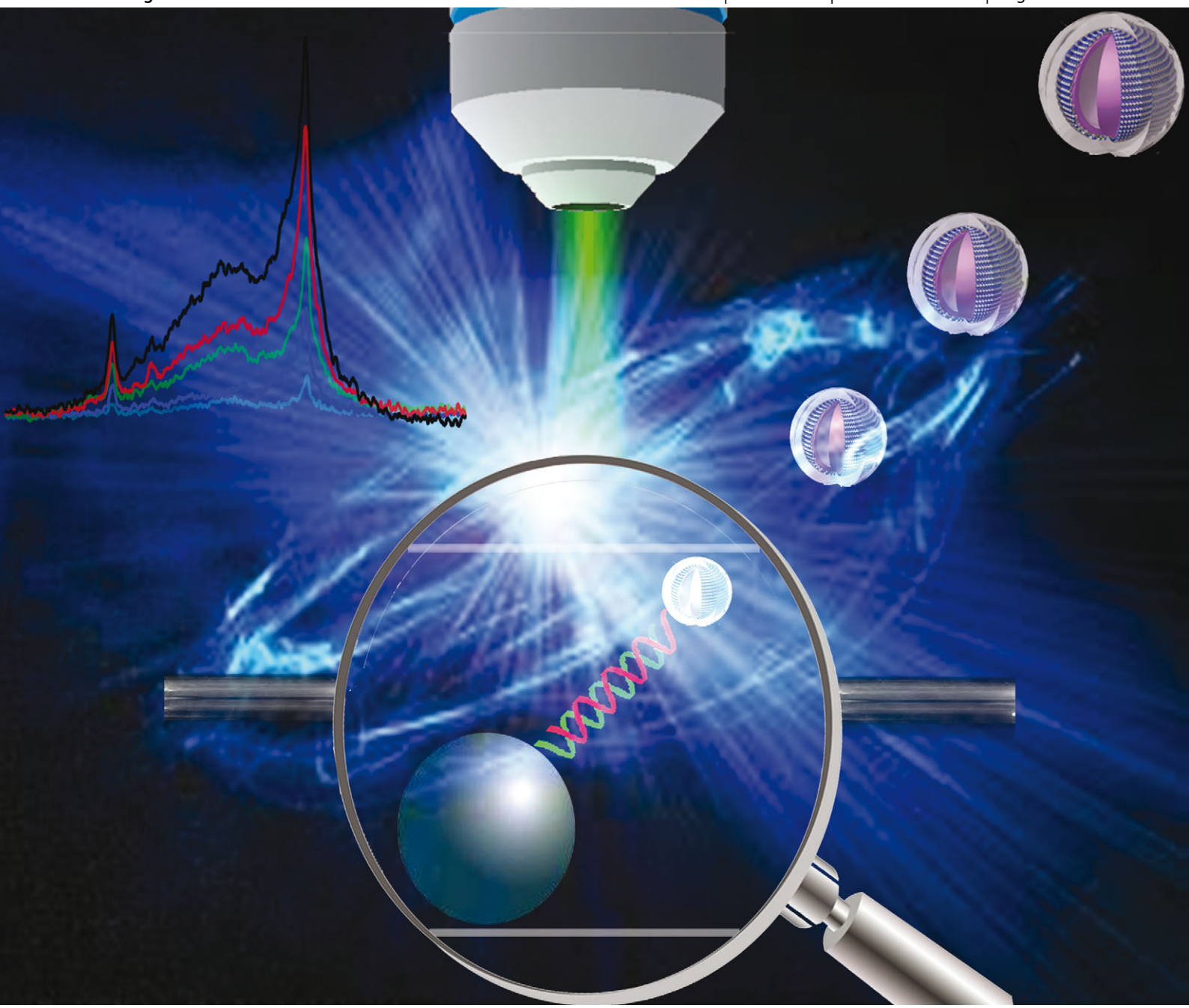

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Raman spectroscopic detection of sub-picomolar DNA by coupling silver catalyzed silver deposition with circular strand-replacement polymerization on magnetic nanoparticles†

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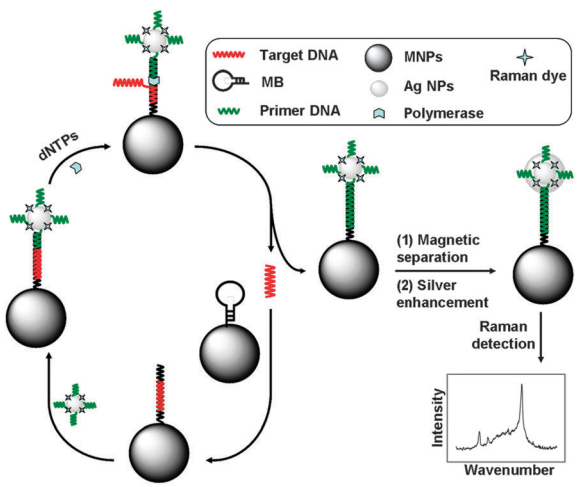
A novel strategy of dual signal amplification was developed using circular strand-replacement DNA polymerization with a molecular beacon as a switch and silver enhancement on magnetic nanoparticles for the specific Raman spectroscopic detection of DNA down to the sub-picomolar level.

Recently DNA sensors have undergone rapid development due to the need for trace DNA detection in molecular diagnostics,[1a] pathogen detection,[1b] forensic investigations,[1c] and environmental monitoring.[1d] DNA sensors can be used to perform simple, reliable and high throughput detection of multiple DNA employing various analytical techniques such as the measurement of fluorescence,[2a] surface plasmon resonance,[2b] electric signals,[2c] and mass changes.[2d] Among these DNA sensing methods, fluorescence-based assays are currently the preferred technique for multiplex DNA detection,[3] but they suffer from photobleaching and the need for elaborate manipulation. Thus novel sensing techniques are still urgently needed. Surface-enhanced Raman spectroscopic (SERS) detection can avoid the limitations of fluorescent biosensing due to its unique characteristics such as an enormous informational spectrum, narrow spectral band (<1 nm, half-widths), and lower susceptibility to both photobleaching and self-quenching of the fluorophore. These distinct advantages have led to the development of a number of ingenious SERS sensing platforms for DNA detection.[4]

In order to improve the sensitivity of the SERS method, in this work we designed a dual signal amplification strategy by combining circular strand-replacement polymerization (CSRP) with silver enhancement on the surface of magnetic nanoparticles (MNPs). The CSRP is a process using a single DNA target molecule to cyclically interact with a nucleic acid-based signal probe. The target recycling approach has previously been demonstrated to be efficient in signal amplification using endonuclease[5a] or exonuclease.[5b] Polymerase-based CSRP has also recently attracted considerable attention in target recycling amplification without the need for a specific recognition site.[5c] This technique uses a lengthening new strand to replace the target sequence, and thus releases the target to initiate a new polymerization cycle. It can be performed at a constant temperature with a short reaction time of as little as 1–2 h and may achieve signal amplification with about a 10^3 to 10^5-fold increase.[5c]

Silver enhancement has been widely used for improving the sensitivity of DNA detection in scanning electrochemical microscopic,[6a] quartz crystal microbalance,[6b] electrochemical stripping,[6c] or even Raman spectroscopic[6d] analysis. Here, the silver enhancement was performed by silver nanoparticle (AgNP) catalyzed silver deposition on MNPs. The AgNPs were attached to MNPs by the recognition of an immobilized molecular beacon (MB) for a DNA target and hybridization of the opened stem part of the MB with primer co-assembled with a Raman dye, 4-mercaptobenzoic acid (MBA), on AgNPs (reporter AgNPs), which initiates a polymerization of the DNA strand in the presence of polymerase. The polymerization led to the release of target and another recognition and polymerization cycle on the MNP surface (Scheme 1). The CSRP process produced the multiplication of target-related reporter AgNPs and thus carried more Raman dye molecules to the MNPs. Upon the silver enhancement after separation of the CSRP product from the reaction mixture, the dual amplification of the SERS signal was achieved. This proposed SERS



Scheme 1 Schematic illustration of dual signal amplification by CSRP and silver enhancement for SERS detection of DNA.

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method could sensitively detect DNA down to the sub-picomolar level. The good analytical performance showed its potential application in DNA analysis and in extending this strategy to other biorecognition events.

UV-vis spectra were used to demonstrate the binding of the SH-modified primer to AgNPs (Fig. 1A). The size of the AgNPs could be estimated to be 20 nm from the absorption peak at 435 nm (curve a). Compared with AgNPs, the UV-vis spectrum of primer attached AgNPs showed a slight red shift of the absorption peak to 440 nm (curve b), indicating that the latter possessed better dispersion due to the presence of negatively charged oligonucleotides. Meanwhile, the primer attached AgNPs showed a strong absorption peak at about 260 nm (curve b), which could be attributed to the adsorption of the DNA strand, indicating the successful binding of primer to the surfaces of AgNPs.

MNPs were used as a solid support due to their advantages such as free suspension in water, large surface area to accelerate reaction and bind more MBs for enhanced sensitivity, easy and rapid isolation in a magnetic field, and effective concentration of the CSRP product when the MNPs are collected. The XPS spectrum of the MNPs showed an N (1s) peak at 399 eV (Fig. 1B, curve a), indicating the existence of nitrogen on the MNPs surface, which should come from the activation process.[7] After MB was covalently linked to the MNPs, the height of the N (1s) peak at 399 eV increased by 2.0 times (Fig. 1B, curve b), which was obviously due to the covalent binding of MB on the surfaces of MNPs.

As shown in Fig. 2A, both the blank solution which was measured in the absence of target, polymerase and dNTPs (curve a) and the polymerase/dNTPs solution (curve b) did not show any Raman signal, indicating that the primer and Raman dye assembled AgNPs could not non-specifically adsorb on the surfaces of MB functionalized MNPs. In contrast, after target DNA was added to MB functionalized MNPs and following silver deposition, the resulting MNPs showed a weak Raman peak at around 1586 cm^{-1} (curve c), which was obviously attributed to the ν_{8a} aromatic ring vibration of MBA. Thus the primer and Raman dye assembled AgNPs could bind to MB functionalized MNPs in the presence of target. This result demonstrated that the DNA target could hybridize with the immobilized MB to open the cycle of the MB and the opened stem part of MB could then combine the primer and MBA co-assembled AgNPs *via* Ag–S chemistry to the surfaces of MNPs. In the presence of polymerase and dNTPs, upon addition of target to the MB modified MNPs solution, the peak attributed to the ν_{8a} aromatic ring vibration of MBA greatly increased (curve d). Thus under these conditions the

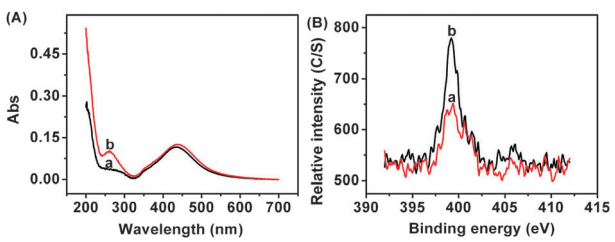

Fig. 1 (A) UV-vis absorption spectra of (a) AgNPs and (b) DNA functionalized AgNPs, and (B) XPS analysis of (a) MNPs and (b) DNA immobilized MNPs.

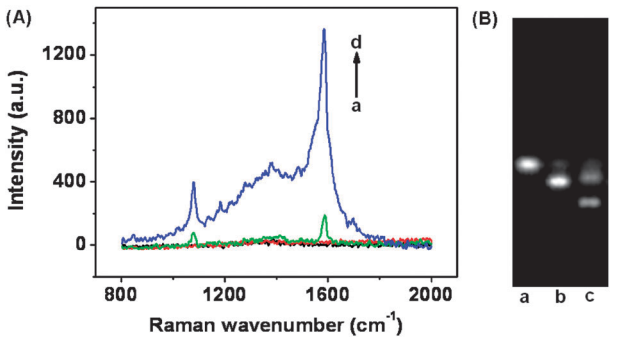

Fig. 2 (A) SERS spectra of (a) blank, (b) polymerase/dNTPs, (c) target, and (d) target and polymerase/dNTPs after incubation for 100 min at 37 °C and silver deposition time for 2 min, and (B) PAGE analysis of (a) 1.0×10^{-7} M MB, (b) 1.0×10^{-7} M MB, 0.5×10^{-7} M primer and target and (c) 1.0×10^{-7} M MB, 0.5×10^{-7} M primer and target with polymerase/dNTPs after CSRP for 30 min at 37 °C.

recognition of the immobilized MB for target DNA initiated the polymerization of the DNA strand and produced CSRP amplification. After CSRP for 100 min at 37 °C, the Raman intensity increased by about 6.7 times, which could greatly improve the sensitivity of Raman detection.

The polymerization reaction was further confirmed with polyacrylamide gel electrophoresis (PAGE) analysis (Fig. 2B). The MB showed only one band (lane a) at a position different from the mixture of MB, primer and target (lane b). The difference resulted from the hybridization of MB with target and then the opened stem with primer added in the mixture. The formed double stranded DNA led to a new band. In the presence of polymerase and dNTPs, the mixture of MB, target and primer showed two bands with a band at a slower migration rate (lane c). This result could be the contribution of an intermediate product of MB–target–polymerization DNA complex existing in the process of primer extension before the target was displaced, verifying the polymerization process.

Under optimal conditions, with an increasing concentration of target DNA the SERS spectra after CSRP and silver enhancement showed increased Raman intensity of MBA at 1586 cm^{-1} (Fig. 3). The plot of Raman intensity *vs.* the logarithm of target DNA concentration showed a good linearity in the range from 1.0×10^{-13} to 1.0×10^{-8} mol L^{-1} (inset of Fig. 3). The corresponding detection limit for the target was calculated to be 5.6×10^{-14} mol L^{-1} by evaluating the average response of the blank plus 3 times standard deviation, which corresponded to 0.56 amol in 10.0 μL solution. The detection limit is much lower than those of 5.0×10^{-7} to 1.0×10^{-12} mol L^{-1} in other Raman works.[4]

In order to confirm the contribution of the CSRP reaction and silver enhancement to the high sensitivity, control experiments were also carried out in the absences of polymerase/dNTPs or/and silver enhancement. The Raman intensity increased with the increasing concentration of target in the range from 1.0×10^{-9} to 1.0×10^{-5} mol L^{-1} with a detection limit of 0.89 nM in the absence of both polymerase/dNTPs and silver enhancement (Fig. S2, ESI†). The sensitivity was about 10 000 times lower than that obtained in the presence of both polymerization and silver enhancement. In the absence of only silver enhancement, the plot of Raman intensity *vs.* the

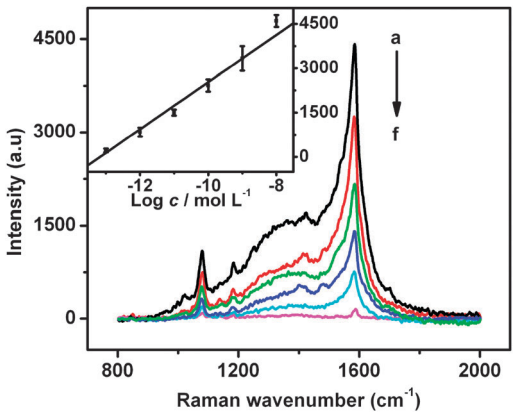

Fig. 3 SERS spectra and Raman intensity (inset) after CSRP and silver enhancement at target concentrations from 10^{-13} to 10^{-8} mol L^{-1}.

logarithm of target DNA concentration also showed a good linearity in the range from 1.0×10^{-12} to 1.0×10^{-8} mol L^{-1} (Fig. S3, ESI†). The corresponding detection limit for the target was calculated to be 0.48 pM, which was about 10 times higher than that obtained in the presence of both polymerization and silver enhancement. Thus the high sensitivity of this method could be attributed to two factors: (1) the amplification of the CSRP process, which increased the number of MBA on the surface of MB modified MNPs, and (2) the silver deposition process, which grew a layer of AgNPs outside the MBA and primer co-assembled AgNPs and made the MBA exist in the interstices between the assembled nanostructures, thus enhancing the Raman signal.[4f]

The selectivity of the proposed SERS method was studied using three kinds of DNA sequence including perfectly complementary target, a single-base mismatched strand and a non-complementary strand at concentrations of 10 pmol L^{-1}. A comparison of the three responses and background is shown in Fig. S4, ESI.† The single-base mismatch sequence showed a response 4.8 times lower than that of the perfectly complementary target, while the responses to the non-complementary strand and the background were further lower than that of the single-base mismatch sequence, indicating good selectivity for the sequence detection of target DNA. This high specificity arose from the specific recognition of MB for target[8] and the conformational constraint of the stem–loop structure of MB, which led to thermodynamically unfavorable binding of the stem to primer assembled on the reporter AgNPs. These results demonstrate that this proposed method is able to detect the target effectively with high specificity, and has great potential for single nucleotide polymorphism analysis.

This work proposed a new approach for enhancing the sensitivity of SERS detection by the combination of CSRP with silver enhancement. The dual signal amplification was performed on the surfaces of MB modified MNPs, which brought not only the specific recognition of MB for target DNA but also the convenient separation of the CSRP product from the reaction mixture. The number of Raman dye molecules assembled on the primer attached MNPs could greatly increase after the CSRP process, and the silver deposition process could further enhance the Raman scattering, which led to a detection limit down to 56 fmol L^{-1}, four orders of magnitude lower than that in the absence of dual signal amplification. The surface CSRP process was highly specific, thus this method could discriminate perfectly matched target DNA from single-base mismatched DNA with high selectivity. Different from PCR based SERS,[9] the proposed CSRP-based SERS could be performed at a constant temperature, which greatly simplified the process of DNA detection. Thus this method possesses promising applications in trace DNA detection.

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