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Enhanced PCR Amplification of GC-Rich DNA Templates by Gold Nanoparticles

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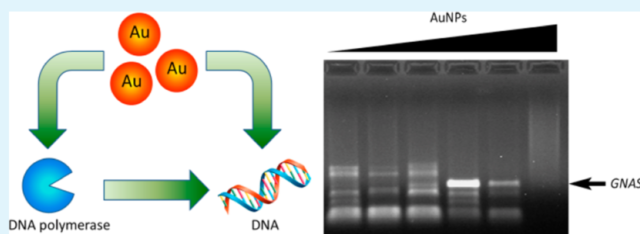
Supporting Information

ABSTRACT: Gold nanoparticles (AuNPs) have been reported to facilitate double-stranded DNA dissociation and improve performance of several PCR systems. Here we investigated AuNPs' effect on GC-rich DNA amplification. We found that AuNPs could enhance PCR amplification of the *GNAS1* promoter region (~84% GC) mediated by *Pfu* or *Taq* DNA polymerase. However, under optimal concentrations of AuNPs, higher amounts of *Taq* were required. Furthermore, the GC-rich *FMR1* (80.4% GC) gene of *Homo sapiens* as well as *exoT* (67.3% GC), *exsE* (71% GC) and *pqqF* genes (74% GC) of *Pseudomonas aeruginosa* were also efficiently amplified. AuNPs can become an effective additive in GC-rich PCR and facilitate analysis of challenging genomic sequence in basic and clinical research.



KEYWORDS: *gold, nanoparticles, GC-rich DNA template, DNA amplification, PCR*

INTRODUCTION

Amplification of GC-rich DNA templates is of great importance because of the fact that many regions of the genome, such as house-keeping genes or tumor-suppressor genes and many cis regulatory elements, contain GC-rich DNA sequences.[1] However, efficient amplification of these GC-rich templates by PCR is often impeded by their high melting temperature and secondary structures,[2] resulting in the failure of a PCR reaction. To date, many investigators have devoted considerable resources to improve such amplifications, and several strategies have been proposed, including novel additives,[3,4] combination of some well-known enhancers,[5−8] optimization of PCR parameters,[9−12] and new primer design strategy.[13] Even though these approaches have been successful under some conditions, the efficacies of these strategies are often unpredictable.[2]

NanoPCR, first developed to improve the specificity in an error-prone two-round PCR,[14] has attracted some attention,[15,16] because of its unexpected enhancing performance of PCR under various conditions. Among the various nanoparticles experimented, gold nanoparticles (AuNPs) have been shown to be the most consistent in performance, and are biocompatible.[17−20] Li et al. found that AuNPs could enhance the efficiency of PCR amplification,[19] which was further supported by the improved diagnosis of Japanese encephalitis virus infection by AuNP-based RT-PCR and real-time quantitative RT-PCR.[21] AuNPs have also been used to improve the specificity in multiround PCR reactions[22] and to achieve a hot-start-like effect.[23] Besides, the sensitivity of the telomere repeat amplification protocol (TRAP)[24] and the performance of allele-specific PCR in genotyping and haplotyping[25] could be greatly improved by AuNPs.

In this study, we have investigated the effect of AuNPs on GC-rich DNA amplification. We have found that for DNA polymerases, *Taq* and *Pfu*, AuNPs are a generally applicable additive and have enabled the efficient amplification of templates with a GC content greater than 80% (the *GNAS1* promoter region). Therefore, we demonstrate that a proper use of AuNPs as an additive under regular PCR conditions can significantly improve its performance with GC-rich DNA templates. This simple approach should find broad applications in molecular genomics, such as diagnosis of inherited diseases[6] and the study of functions and regulations of various genes.[10]

EXPERIMENTAL SECTION

Materials. *Ex Taq* DNA polymerase, dNTPs, lambda DNA, and DNA marker DL2000 were obtained from TaKaRa. Native *Taq* DNA polymerase was obtained from Sino-American Biotechnology. Recombinant and native *Pfu* DNA polymerases were purchased from TIANGEN Biotech and Shanghai Sangon Biological Engineering & Technology and Service, respectively. Human genomic DNA was purchased from Beijing BioDev-Tech. Scientific & Technical Co., Ltd. AuNPs (10 nm, 0.01% $HAuCl_4$) were purchased from Sigma. The primers used are shown in the Supporting Information, Table 1, and were all synthesized by Shanghai Sangon Biological Engineering & Technology and Service.

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PCR Amplification. All reactions were carried out in an Eppendorf Mastercycler Gradient (Hamburg, Germany). Each reaction was performed with a final volume of 25 μL, which commonly contained 0.2 μM each primer, 0.2 mM each dNTP (dATP, dCTP, dTTP and dGTP), 100 ng of *Homo sapiens* genomic DNA or 200 pg of *Pseudomonas aeruginosa* genomic DNA, DNA polymerase, and corresponding 1× reaction buffer. The concentration of DNA polymerase and AuNPs would be stated in detail for each reaction later.

For the amplification of the *GNAS1* promoter using *Taq* DNA polymerase, a "touchdown" PCR program followed predenaturation at 94 °C for 5 min, which consisted of 25 cycles of amplification: 1 min at 94 °C, 30 s at annealing temperature and 40 s at 72 °C. The annealing temperature decreased 0.5 °C every cycle from 72 to 60 °C. Then a standard PCR program was performed for 20 cycles (1 min at 94 °C, 30 s at 60 °C and 40 s at 72 °C). As to *Pfu* DNA polymerase-mediated amplification, almost the same PCR program was used except that the denaturation process was changed to 45 s at 98 °C and the time for extension at 72 °C was prolonged to 1 min for each cycle.

When amplifying the *FMR1* gene from *Homo sapiens* genomic DNA, predenaturation at 94 °C for 5 min was followed by 40 cycles of 1 min at 94 °C, 30 s at 60 °C, and 40 s at 72 °C. For the amplification of GC-rich genes from *Pseudomonas aeruginosa* genome, predenaturation at 94 °C for 5 min was followed by 40 cycles of 30 s at 94 °C, 6 s at 55 °C, and 15 s (*exsE* gene) or 2 min (*pqqF* and *exoT* gene) at 72 °C.

RESULTS AND DISCUSSION

To test AuNPs' ability to improve the PCR amplification of GC-rich DNA templates, we first chose the complete *GNAS1* promoter in the human genome as a model template. The *GNAS1* promoter contains extremely GC-rich regions with a regional GC content of up to 86% and unsuccessful attempts to amplify this region have been reported.[2] Although 7-deaza-2′-deoxyguanosine (dc^{7}GTP) was effective for the amplification of the *GNAS1* promoter, a rather time-consuming "slowdown" PCR program designed upon "touchdown" PCR was still essential.[2,9]

Because of its excellent heat stability, *Pfu* DNA polymerase was preferred for the amplification of GC-rich DNA fragments with high denaturing temperatures (>95 °C).[26] Therefore, we first examined the effect of AuNPs on the amplification of the *GNAS1* promoter mediated by recombinant *Pfu* DNA polymerase. We found that the recombinant *Pfu* DNA polymerase can effectively amplify the GC-rich *GNAS1* promoter with the addition of 0.456 nM∼0.608 nM AuNPs while no band of the target DNA fragment could be detected without the addition of AuNPs (Figure 1A). Therefore, AuNPs were able to enhance the amplification of the GC-rich *GNAS1* promoter by the recombinant *Pfu* DNA polymerase. Such an improving effect could be due to AuNPs' ability to facilitate the dissociation of double-stranded DNA.[27,28] However, probably because of their interactions with the DNA polymerase, excessive AuNPs can have an inhibitive effect on the amplification (lanes 5 and 6 in Figure 1A).[29,30]

Because previous studies showed that the effect of AuNPs on PCR amplification can be influenced by the type of DNA polymerase used,[19,31] we also investigated the effect of AuNPs on the amplification of the GNAS1 promoter by the native *Pfu* DNA polymerase. As showed in Figure 1B, when the concentration of AuNPs reached 2.280 nM, the yield of the target DNA fragment was also greatly improved with other nonspecific amplification products diminished. When compared with the native *Pfu* DNA polymerase, we found that the recombinant *Pfu* DNA polymerase required a lower amount of AuNPs. Although the exact mechanism may require further investigation, we speculate that the often used tags on the recombinant protein for the ease of purification could have a significant influence on the interaction between the recombinant polymerase and the AuNPs.[32] Because different suppliers might have used different strategies in their purification procedure, the amount of AuNPs, as well as the enzyme concentration, in the specific reaction may require its own optimization.

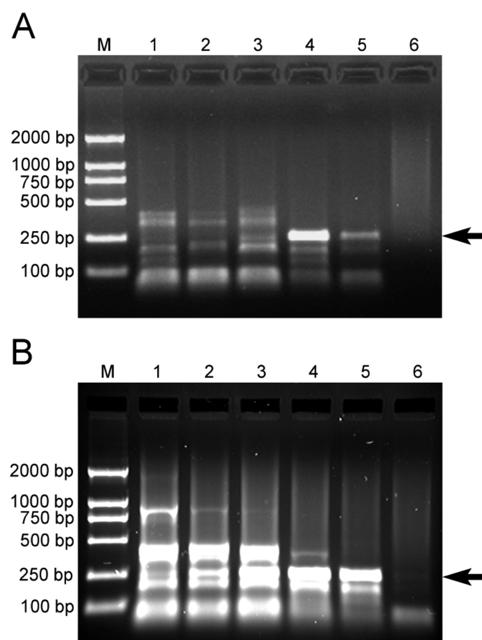

Figure 1. Optimization of *Pfu* DNA polymerase-mediated PCR amplification of the GNAS1 promoter by AuNPs. (A) Optimization by AuNPs when using 1.25U recombinant *Pfu* DNA polymerase. Lane M: DNA marker DL2000. The final concentration of AuNPs for lane 1 to lane 6 was 0.000, 0.152, 0.304, 0.456, 0.608, and 0.760 nM. (B) Optimization by AuNPs when using 1.25U native *Pfu* DNA polymerase. Lane M: DNA marker DL2000. The final concentration of AuNPs for lane 1 to lane 6 was 0.000, 1.140, 1.520, 1.900, 2.280, and 2.660 nM. The arrows indicated the positions of the target DNA fragment.

To determine whether AuNPs are also effective with other commonly used DNA polymerases, we further characterized the amplification of the *GNAS1* promoter by another commonly used DNA polymerase, *Taq*. Because of its lower thermal stability, the denaturation temperature used in the PCR reaction was set at 94 °C.[26] Using the concentrations of *Taq* DNA polymerase recommended by the supplier, the addition of AuNPs could not rescue the failure of the PCR amplification of the *GNAS1* promoter by *Ex Taq* (Figure 2A). However, because it has been reported that a higher concentration of AuNPs might increase their interaction with the templates, thus enhancing the denaturation effect, we have therefore decided to search for possible improvements by increasing the concentration of AuNPs.[28] Here, to counter the inhibitory effect of the large amount of AuNPs on the DNA polymerase, additional *Taq* DNA polymerase was also added in the reactions.[29,30] As Figure 2B showed, the addition of 0.304 nM∼0.380 nM AuNPs

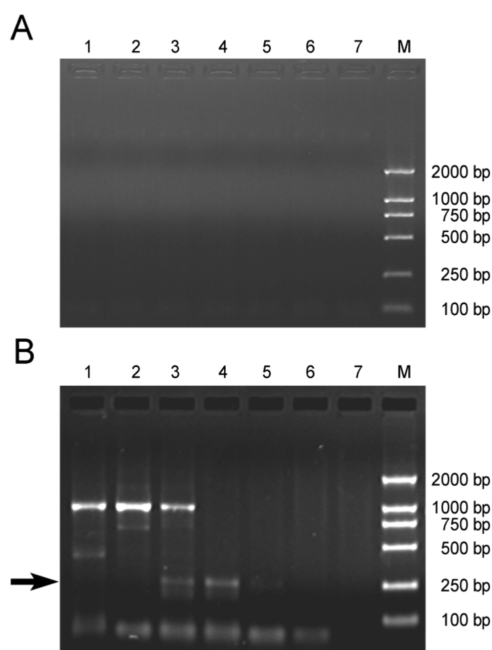

Figure 2. Optimization of *Ex Taq* DNA polymerase-mediated PCR amplification of the *GNAS1* promoter by AuNPs. (A) Optimization of reactions by AuNPs when 1.25 U *Ex Taq* DNA polymerase was used. Lane M: DNA marker DL2000. The final concentration of AuNPs for lane 1 to lane 7 was 0.000, 0.038, 0.114, 0.190, 0.266, 0.342, and 0.418 nM. (B) Optimization of reactions by AuNPs when 3.75 U *Ex Taq* DNA polymerase was used. Lane M: DNA marker DL2000. The final concentration of AuNPs for lane 1 to lane 7 was 0.000, 0.228, 0.304, 0.380, 0.456, 0.532, and 0.608 nM. The arrows indicated the positions of the target DNA fragment.

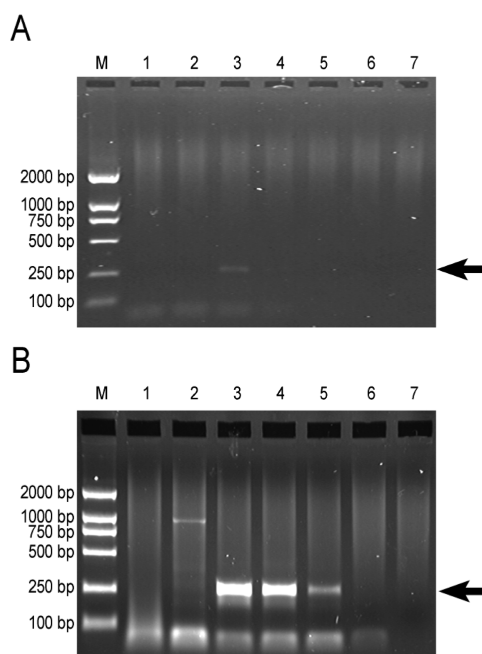

Figure 3. Optimization of native *Taq* DNA polymerase-mediated PCR amplification of the *GNAS1* promoter by AuNPs. (A) Optimization of reactions by AuNPs when 1.25 U native *Taq* DNA polymerase was used. Lane M: DNA marker DL2000. The final concentration of AuNPs for lane 1 to lane 7 was 0.000, 0.152, 0.228, 0.304, 0.380, 0.456, and 0.532 nM. (B) Optimization of reactions by AuNPs when 3.75U native *Taq* DNA polymerase was used. Lane M: DNA marker DL2000. The final concentration of AuNPs for lane 1 to lane 7 was: 0.000, 0.760, 1.140, 1.520, 1.900, 2.280, and 2.660 nM. The arrows indicated the positions of the target DNA fragment.

have resulted in the successful amplification of the *GNAS1* promoter when 3.75U *Ex Taq* DNA polymerase was used. These results suggested that although the improvement of GC-rich PCR by AuNPs could be attributed to AuNPs' ability to facilitate the dissociation of double-stranded DNA,[27,28] their interaction with the DNA polymerase could also play an important role. The details of this interaction require additional investigation in order to further delineate the mechanistic principle of this system.

Because of the important differences found with native and recombinant *Pfu*, we further tested this reaction with native *Taq* DNA polymerase (Figure 3). As Figure 3A illustrated, with increasing concentrations of AuNPs, only 0.228 nM AuNPs resulted in a faint band of the target DNA fragment where the native *Taq* DNA polymerase concentration remained 1.25 U (lane 3). Because AuNPs could exert an inhibitory effect on DNA polymerases, we further increased the amount of *Taq* DNA polymerase in the reaction to improve the amplification efficiency. As expected, when the native *Taq* DNA polymerase was increased to 3.75 U, optimal amplification of the *GNAS1* promoter was achieved at an AuNP concentration between 1.140 and 1.900 nM (lanes 3−5 in Figure 3B). It is interesting to note that although AuNPs could interact with both DNA polymerase and the DNA template,[17,18,33] the lowered denaturation temperature required by *Taq* DNA polymerase does not require a higher concentration of AuNPs when compared to that of *Pfu*, a result somewhat unexpected. However, we also noted that even at a much higher concentration, *Ex Taq* DNA polymerase is much less efficient than that of native *Taq* DNA polymerase (lane 4 in Figure 2B compared with lane 3 and 4 in Figure 3B), indicating again that the effect of AuNPs in PCR reactions must be individually optimized to achieve the optimal outcome.[33]

To test whether AuNPs could exert a similar enhancing effect on the amplification of non-GC-rich DNA templates, we chose a non-GC-rich DNA fragment from lambda DNA as a control. In the corresponding PCR systems mediated by the aforementioned four types of DNA polymerase, the addition of optimal amounts of AuNPs, which was previously determined during the amplification of the *GNAS1* promoter, did not show any obvious enhancing effect (see the Supporting Information, Figure 1s). Therefore, the addition of AuNPs is mostly beneficial for the amplification of GC-rich DNA templates. To further demonstrate that the enhancing effect of AuNPs on GC-rich PCR is not due to unknown specific properties of the *GNAS1* sequences, we further examined the effect of AuNPs in the PCR amplification of several other GC-rich regions using the *Ex Taq* DNA polymerase. As shown in Figure 4, AuNPs are equally effective for *FMR1* (80.4% GC) gene of *Homo sapiens* as well as *exoT* (67.3% GC), *exsE* (71.1% GC), and *pqqF* genes (74.1% GC) of *Pseudomonas aeruginosa* (Figure 4). However, the optimal range of AuNP concentration should be individually adjusted to achieve the best result.

CONCLUSION

In summary, we have demonstrated that AuNPs are an effective reagent to improve GC-rich DNA amplification using regular PCR conditions. For both *Pfu* and *Taq* DNA polymerase, AuNPs enabled the success amplification of several GC-rich templates from two different organisms. However, we also

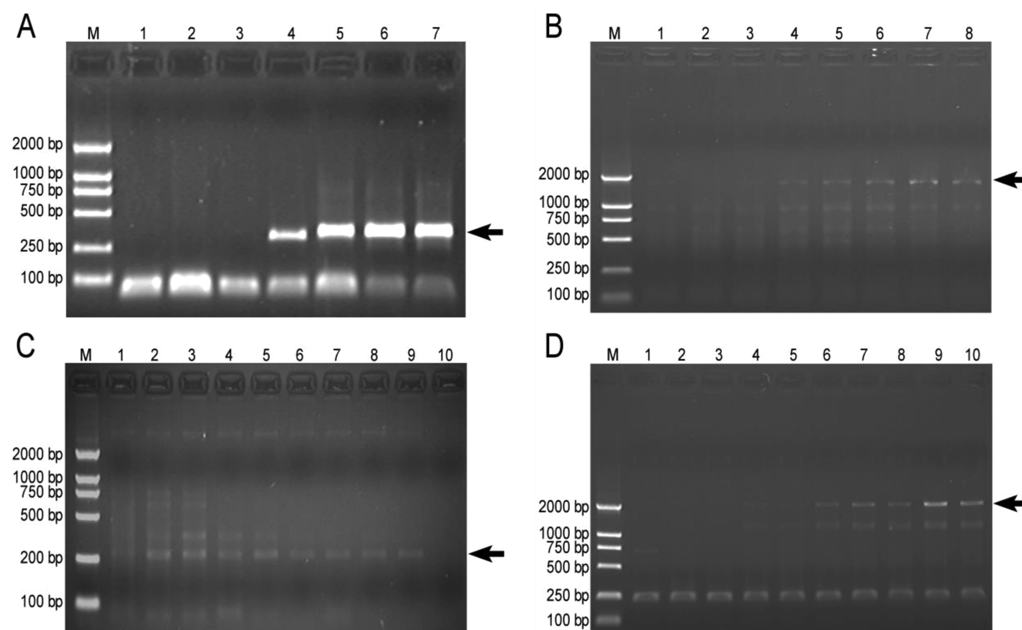

Figure 4. Optimization of PCR amplification of *FMR1* gene of (A) *Homo sapiens* as well as (B) *exoT*, (C) *exsE*, and (D) *pqqF* genes of *Pseudomonas aeruginosa* by AuNPs when using 3.75 U *Ex Taq* DNA polymerase. In each panel, the final concentration of AuNPs represented by lane 1 to lane 10 was 0.000, 0.228, 0.304, 0.380, 0.456, 0.532, 0.608, 0.684, 0.760, and 0.836 nM. The arrows indicated the positions of the target DNA fragment.

show that AuNPs not only interact with the DNA template, presumably mediating its dissociation during the PCR cycles, but also interact with the DNA polymerases in the reactions. The effect of the latter is highly enzyme-specific. As such, for each specific enzyme, the optimal AuNP concentration should be determined individually. These results firmly establish AuNPs as a general additive in PCR amplification of GC-rich DNA templates and such protocols provide an additional strategy in the repertoire of genomics techniques for both basic and clinical applications.

ASSOCIATED CONTENT

Supporting Information

Primers used for the amplification of all GC-rich genes mentioned in this manuscript and the influence of AuNPs on the PCR amplification of a non-GC-rich DNA fragment showed by gel electrophoresis of corresponding amplification products. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Mamedov, T. G.; Pienaar, E.; Whitney, S. E.; Termaat, J. R.; Carvill, G.; Goliath, R.; Subramanian, A.; Viljoen, H. J. *Comput. Biol. Chem.* **2008**, *32*, 452−7.
(2) Bachmann, H. S.; Siffert, W.; Frey, U. H. *Pharmacogenetics* **2003**, *13*, 759−66.
(3) Zhang, L. F.; Liang, Y.; Meng, L. Z.; Lu, X. J.; Liu, Y. H. *Chem. Biodiversity* **2007**, *4*, 163−74.
(4) Schnoor, M.; Voss, P.; Cullen, P.; Boking, T.; Galla, H. J.; Galinski, E. A.; Lorkowski, S. *Biochem. Biophys. Res. Commun.* **2004**, *322*, 867−72.
(5) Ralser, M.; Querfurth, R.; Warnatz, H. J.; Lehrach, H.; Yaspo, M. L.; Krobitsch, S. *Biochem. Biophys. Res. Commun.* **2006**, *347*, 747−51.
(6) Musso, M.; Bocciardi, R.; Parodi, S.; Ravazzolo, R.; Ceccherini, I. *J. Mol. Diagn.* **2006**, *8*, 544−50.
(7) Zhang, Z.; Yang, X.; Meng, L.; Liu, F.; Shen, C.; Yang, W. *Biotechniques* **2009**, *47*, 775−9.
(8) Jensen, M. A.; Fukushima, M.; Davis, R. W. *PLoS One* **2010**, *5*, e11024.
(9) Frey, U. H.; Bachmann, H. S.; Peters, J.; Siffert, W. *Nat. Protoc.* **2008**, *3*, 1312−7.
(10) Wei, M. C.; Deng, J.; Feng, K.; Yu, B. Y.; Chen, Y. J. *Anal. Chem.* **2010**, *82*, 6303−7.
(11) Orpana, A. K.; Ho, T. H.; Stenman, J. *Anal. Chem.* **2012**, *84*, 2081−7.
(12) Pratyush, D. D.; Tiwari, S.; Kumar, A.; Singh, S. K. *Gene* **2012**, *497*, 269−72.
(13) Li, L. Y.; Li, Q.; Yu, Y. H.; Zhong, M.; Yang, L.; Wu, Q. H.; Qiu, Y. R.; Luo, S. Q. *Clin. Biochem.* **2011**, *44*, 692−8.
(14) Li, H.; Huang, J.; Lv, J.; An, H.; Zhang, X.; Zhang, Z.; Fan, C.; Hu, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 5100−3.
(15) Chen, J.; Cao, X.; Guo, R.; Shen, M.; Peng, C.; Xiao, T.; Shi, X. *Analyst* **2012**, *137*, 223−8.
(16) Tong, W.; Cao, X.; Wen, S.; Guo, R.; Shen, M.; Wang, J.; Shi, X. *Int. J. Nanomed.* **2012**, *7*, 1069−78.
(17) Pan, D.; Mi, L.; Huang, Q.; Hu, J.; Fan, C. *Integr. Biol.* **2012**, *4*, 1155−63.
(18) Pan, D.; Wen, Y.; Mi, L.; Fan, C.; Hu, J. *Curr. Org. Chem.* **2011**, *15*, 486−97.

(19) Li, M.; Lin, Y. C.; Wu, C. C.; Liu, H. S. *Nucleic Acids Res.* **2005**, *33*, e184.
(20) Cao, X.; Shen, M.; Zhang, X.; Hu, J.; Wang, J.; Shi, X. *Electrophoresis* **2012**, *33*, 2598−603.
(21) Huang, S. H.; Yang, T. C.; Tsai, M. H.; Tsai, I. S.; Lu, H. C.; Chuang, P. H.; Wan, L.; Lin, Y. J.; Lai, C. H.; Lin, C. W. *Nanotechnology* **2008**, *19*, 405101.
(22) Pan, J.; Li, H.; Cao, X.; Huang, J.; Zhang, X.; Fan, C.; Hu, J. *J. Nanosci. Nanotechnol.* **2007**, *7*, 4428−33.
(23) Mi, L.; Wen, Y.; Pan, D.; Wang, Y.; Fan, C.; Hu, J. *Small* **2009**, *5*, 2597−600.
(24) Xiao, Y.; Dane, K. Y.; Uzawa, T.; Csordas, A.; Qian, J.; Soh, H. T.; Daugherty, P. S.; Lagally, E. T.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* **2010**, *132*, 15299−307.
(25) Chen, P.; Pan, D.; Fan, C.; Chen, J.; Huang, K.; Wang, D.; Zhang, H.; Li, Y.; Feng, G.; Liang, P.; He, L.; Shi, Y. *Nat. Nanotechnol.* **2011**, *6*, 639−44.
(26) Dutton, C. M.; Paynton, C.; Sommer, S. S. *Nucleic Acids Res.* **1993**, *21*, 2953−4.
(27) Haber, A. L.; Griffiths, K. R.; Jamting, A. K.; Emslie, K. R. *Anal. Bioanal. Chem.* **2008**, *392*, 887−96.
(28) Yang, J.; Pong, B. K.; Lee, J. Y.; Too, H. P. *J. Inorg. Biochem.* **2007**, *101*, 824−30.
(29) Mi, L. J.; Zhu, H. P.; Zhang, X. D.; Hu, J.; Fan, C. H. *Chin. Sci. Bull.* **2007**, *52*, 2345−9.
(30) Vu, B. V.; Litvinov, D.; Willson, R. C. *Anal. Chem.* **2008**, *80*, 5462−7.
(31) Yang, W. C.; Mi, L. J.; Cao, X. Y.; Zhang, X. D.; Fan, C. H.; Hu, J. *Nanotechnology* **2008**, *19*, 255101.
(32) Dabrowski, S.; Kur, J. *Protein Expression Purif.* **1998**, *14*, 131−8.
(33) Lou, X.; Zhang, Y. *ACS Appl. Mater. Interfaces* **2013**, *5*, 6276−84.