Surface Review and Letters, Vol. 15, No. 6 (2008) 757–762


World Scientific
www.worldscientific.com

# SIZE AND SURFACE EFFECT OF GOLD NANOPARTICLES (AuNPs) IN NANOGOLD-ASSISTED PCR

JIEHUAN HUANG*, XIAODONG ZHANG†,‡, CHUNMEI WANG*, LIHUA WANG†, HAIKUO LI*, XUEYAN CAO*, AILI ZHANG*, XUELING LI*, CHUNHAI FAN*,† and JUN HU*,†

*College of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, P. R. China

†Shanghai Institute of Applied Physics, Chinese Academy of Sciences, P.O. Box 800-204, Shanghai 201800, P. R. China

‡zxdzjh@133sh.com

Received 9 May 2008

Recently, gold nanoparticles (AuNPs) were reported to increase the specificity and efficiency of the polymerase chain reaction (PCR). In this paper, we tested the enhancement of AuNPs with five different sizes on the specificity of two-round PCR. The results showed that, except 5.02 nm AuNPs, the AuNPs that could achieve the similar enhancement happened to have nearly the same total surface area. The surface effect seems to be the key factor of nanogold-assisted PCR.

*Keywords*: PCR; gold nanoparticles; size effect.

## 1. Introduction

Gold nanoparticles (AuNPs) are nontoxic, biocompatible nanomaterials that can be obtained commercially or prepared in laboratories. Owing to their special characters such as small size, unique optical and electronic properties, and the easily modified surface, AuNPs have been found important in multifarious applications in biomedical science.[1–7] For instance, imaging of AuNPs markers on cells has been employed in immunocytochemistry for about 35 years.[1,2] Recently, it was shown that AuNPs were more likely to absorb single-strand DNA rather than double-strand DNA. Based on this unique behavior, AuNPs have been used in sequence assaying or detecting single-base-pair mismatch.[3–5] AuNPs were also proven as an effective drug carrier to deliver tumor necrosis factor (TNF) targeting solid tumor in mice,[6] or as a platform to assemble DNA molecules for amplifying the signals of DNA sensor.[7]

In our previous work, we found that AuNPs could also provide an important function in enhancing the specificity and efficiency of the polymerase chain reaction (PCR).[8,9] With the help of an appropriate amount of 10 nm AuNPs, only the target fragment was amplified while nearly all non-specific amplifications were inhibited in an error-prone two-round PCR.[8] The specificity of PCR could be hold even up to seventh round with the help of AuNPs.[10] Li *et al.* also reported that AuNPs could increase the reaction rate of real-time PCR.[11] However, all these researches used AuNPs with diameter only about 10 nm. The influence of AuNPs with different sizes, or the size effect, has not been studied.

‡Corresponding author.

As it is known, size always plays a crucial role in the quantum effects, surface effects, or confinement effects of nanoparticles. For instance, changing the sizes of nanoparticles would influence various functions such as catalytic efficiency and quantum dot illuminescent characteristics as well.[12–14] AuNPs have been proven to affect the PCR procedures distinctly; however, the mechanism is still unclear. And from a practical point of view, the study on the influence of different sizes of AuNPs is important to optimize the PCR performance for future applications.

In this work, we first obtained the optimum concentration for each kind of AuNPs with different sizes to enhance the specificity of PCR, then calculated several relevant parameters of AuNPs under such condition, and analyzed whether there were some correlations among them. We found that the total surface area of AuNPs happened to be about the same to achieve the similar enhancement, which favors a kind of surface-based mechanism.

## 2. Experimental Sections

### 2.1. *Materials*

AuNPs (5, 10, and 20 nm, 0.01% $HAuCl_4$) were purchased from Sigma-Aldrich Corp. Two other AuNps in larger sizes (samples 1 and 2, 0.01% $HAuCl_4$) were homemade based on the standard citrate reduction method reported by Frens.[15]

Lamda DNA, Ex Hotstart Taq DNA polymerase, dNTPs, 10× PCR buffer of Ex Hotstart Taq DNA polymerase, and DNA marker DL2000 (2000, 1000, 750, 500, 250, and 100 bp) were obtained from TaKaRa Bio. Inc. Primers were synthesized by Shanghai Sangon Biological Engineering & Technology and Service Co. Ltd. The sequences of the primers were as follows: Primer 1: 5′-GGCTTCG-GTCCCTTCTGT-3′, Primer 2: 5′-CACCACCTG-TTCAAACTCTGC-3′. Sterile deionized water was got from Milli Q instrument (18.2 MΩ).

### 2.2. *Methods*

To detect the diameters of AuNPs, the atomic force microscopy (AFM) (MultiMode, Nanoscope IIIa, Veeco Corp.) equipped with J-scanner was employed. Silicon probes (MikroMasch) with spring constant of 48 N/m were used for imaging in air. The solution of AuNPs was dropped onto the mica substrate, which was modified by (3-aminopropyl) triethoxysilane (APS, purchased from United Chemical Corp.)[16] and kept for several minutes. Then the mica surface was rinsed by Milli Q water and dried with a flow of clean air. All images were captured in tapping mode (TM-AFM) and the heights of over 200 nanoparticles were measured for obtaining the average size of each kind of nanoparticle samples.

The error-prone two-round PCR model employed in this paper was the same as the model in our previous report.[8] Briefly, in the first round PCR, 283-bp target DNA was amplified from Lamda DNA template by using Primers 1 and 2 with high specificity. Then the DNA products of first round were diluted 1000 fold as the template in the second round PCR, followed by amplification using the same primers.

PCR was carried out under the conditions listed in Table 1. The PCR conditions were as follows: 2 min at 94°C for pre-denaturation, followed by 35 cycles: 20 s at 94°C, 30 s at 55°C, and 30 s at 72°C. Then after a final elongation at 72°C for 5 min, PCR tubes were maintained at 4°C. All amplifications were performed on the ABI 9600 PCR Thermal Cyclers. PCR products were analyzed by pre-stained EtBr agarose gel electrophoresis (1.5%).

A serial amount of AuNPs with different sizes were added into the reaction, and the concentration of AuNPs that helped the PCR produce single and brightest target band (maximum DNA yield) on the gel was selected as the optimum amount of AuNPs with this size. The DNA yields of PCR products were calculated based on the ratio of the densitometric value of the desired DNA band (283 bp) to the

Table 1. PCR reaction mixture.

| Reagent | Volume or weight |
|---|---|
| Takara Ex Taq polymerase (5 U/μL) | 0.125 μL |
| 10× PCR buffer (with $Mg^{2+}$) | 2.5 μL |
| dNTPs (10 mM) | 1.0 μL |
| Primer 1 (1 μM) | 2.0 μL |
| Primer 2 (1μM) | 2.0 μL |
| Template DNA (lamda DNA in the first round; purified 283 bp PCR product in the second round) | 0.5 ng |
| AuNPs (with difference sizes) | Variable |
| Sterile deionized water | Added up to 25 μL |

densitometric value of 500 bp band (50 ng) in marker in the same image.

## 3. Results and Discussion

The diameters of nanoparticles could be characterized by TEM/SEM, photon correlation spectroscope (PCS), or AFM.[17,18] While AFM was used, only the height from the peak to valley measured from the AFM images was chosen to represent the diameter of nanoparticles because the lateral dimension might be affected by broadening effect caused by AFM tip's shape or size. In this paper, the diameter $d_i$ of each AuNPs sample was measured in average height by AFM in tapping mode, as presented in Table 2. The results showed that three measured diameters $5.02 \pm 0.16$, $10.28 \pm 0.35$, and $21.32 \pm 0.32$ nm were in good agreement with the commercial reference values 5, 10, and 20 nm, respectively.

To evaluate the amount of AuNPs with different sizes, we assumed that the density ($\rho$) of AuNPs was the same as that of metal gold ($19.37\,\mathrm{g/cm^3}$); then we converted the weight percent (0.01% $HAuCl_4$) of the AuNPs samples to the molarity $M_i$ based on the formula as follows:

$$M_i = (W/W_i)/N_a, \tag{1}$$

where $W$ is the total weight of the Au atom in the 1 L solution, which is equal to $HAuCl_4$ weight in 1 L/$HAuCl_4$ molecular weight × Au molecular weight $= 0.01 \times 10^{-2} \times 10^3/340 \times 197 = 5.79 \times 10^{-2}$ (g); $W_i$ is the weight of one Au nanoparticle with the diameter of $d_i$ nm, which is equal to $4/3 \times \pi \times (d_i \times 10^{-9}/2)^3 \times \rho = 1.014 \times 10^{-20} \times d_i^3$ (g); $N_a$ is Avogadro Constant, which is equal to $6.02 \times 10^{23}$/mole. Therefore, the molarity of each AuNP sample could be calculated as shown in Table 2.

Table 2. Statistic data of height and molarity of five different sizes of AuNPs samples.

| Gold nanoparticles | $d_i$ = height (nm) | Number of particles in AFM images | Molarity (nmol/L) |
|---|---|---|---|
| Sigma 5 nm | $5.02 \pm 0.16$ | 322 | 75.1 |
| Sigma 10 nm | $10.28 \pm 0.35$ | 260 | 8.77 |
| Sigma 20 nm | $21.32 \pm 0.32$ | 272 | 0.982 |
| Homemade sample 1 | $44.41 \pm 1.20$ | 316 | 0.187 |
| Homemade sample 2 | $57.02 \pm 1.86$ | 213 | 0.0513 |

The error-prone two-round PCR model was employed in this paper. Generally, many nonspecific products would be amplified in the second round PCR, showing broad molecular size distribution in agarose gel electrophoresis. However, an appropriate amount of AuNPs could help produce a single predominant band corresponding to the original 283-bp target DNA.[8] Hence, it is easy to detect if AuNPs with different sizes could get the similar optimizing effect using this error-prone two-round PCR model. For each kind of AuNPs, serial amount samples were added to the second round PCR mixture. As demonstrated in Figs. 1(a)–1(e), with increasing concentrations of the samples, there was an increase in the amount of the specific product along with a decrease in nonspecific bands. Within their appropriate concentrations, all kinds of AuNPs with different sizes were capable of dramatically inhibiting nonspecific amplification in second-round PCR. Being consistent with other reports,[8–10] excess AuNPs would inhibit the PCR amplification to some extent.

The measured curves in Fig. 1(f) from Figs. 1(a)–1(e) showed the ability of the tested AuNPs to improve PCR specificity. As illustrated in Fig. 1(f), corresponding to the AuNPs with average size of 5.02, 10.28, 21.32, 44.41, and 57.02 nm, the effective range of AuNPs for optimizing PCR was 0.24–0.42, 0.28–0.49, 0.055–0.071, 0.022–0.039 and 0.012–0.021 nM, respectively. And within these ranges, optimum concentration of AuNPs was 0.24, 0.35, 0.063, 0.022, 0.014 nM, respectively. These results indicate that there is a size effect in nanogold-assisted PCR. Hence, we calculated several parameters including surface-to-volume ratio ($S/V$ ratio), the weights, and the total surfaces of AuNPs with different sizes in their optimum amount in 25 $\mu$L PCR mixture. All the results were showed in Table 3.

From Table 3, we could find that the smaller the AuNP was, the larger the $S/V$ was, the more the optimum molarity needed for optimizing PCR and the lighter the optimum weight was. It can be easily found that the total surface areas of the AuNPs with 10.28, 21.32, 44.41, and 57.02 nm required for optimizing PCR were close to the same level, showing that the surface effect seems to be the key factor of

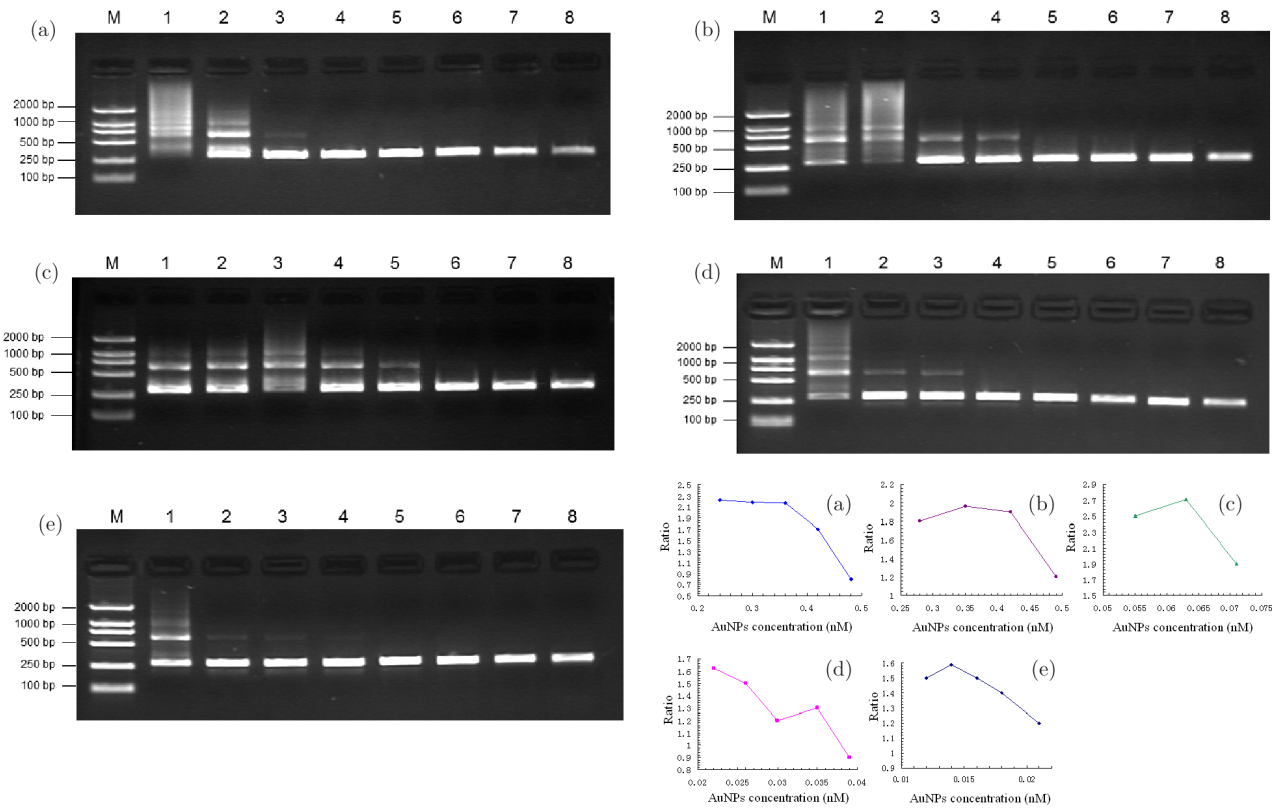


Fig. 1. The size effect of gold nanoparticle on the specificity second round PCR. Lane M in every image is for marker. (a) The 5.02 nm gold nanoparticle was added into PCR mixture, and for lane 1 to lane 8 its final concentration was 0, 0.09, 0.18, 0.24, 0.3, 0.36, 0.42, 0.48 nM, respectively. (b) The 10.28 nm gold nanoparticle was added into PCR mixture, and for lane 1 to lane 8 its final concentration was 0, 0.07, 0.14, 0.21, 0.28, 0.35, 0.42, 0.49 nM, respectively. (c) The 21.32 nm gold nanoparticle was added into PCR mixture, and for lane 1 to lane 8 its final concentration was 0, 0.024, 0.031, 0.039, 0.047, 0.055, 0.063, 0.071 nM, respectively. (d) The 44.41 nm gold nanoparticle was added into PCR mixture, and for lane 1 to lane 8 its final concentration was 0, 0.013, 0.017, 0.022, 0.026, 0.030, 0.035, 0.039 nM, respectively. (e) The 57.02 nm gold nanoparticle was added into PCR mixture, and for lane 1 to lane 8 its final concentration was 0, 0.0082, 0.010, 0.012, 0.014, 0.016, 0.018, 0.021 nM, respectively. (f) The DNA yields of PCR products as a ratio of the densitometric value of the desired DNA band (283-bp) and the densitometric value of 500 bp band (50 ng) of marker in the same image. Only the 283-bp DNA yeilds of those lanes which did not include nonspecific amplification band were collected (Lanes 4–8 in (a), lanes 5–8 in (b), lanes 6–8 (c), lanes 4–8 in (d), and lanes 4–8 in (e)).

nanogold-assisted PCR. It should be noted that the total surface of 5.02 nm AuNPs required for optimizing PCR was distinctively smaller than others. This might be due to its different preparation method based on $NaBH_4$ reduction,[19,20] or another unknown mechanism plays a role in such small size.

In our previous works, we proposed two possibilities on the mechanism of nanogold-assisted PCR. One is based on the interaction between AuNPs and single-stranded DNA in PCR,[8] while the other is due to the interaction between AuNPs and polymerase.[9] Both of these interactions should take place on the surface of AuNPs. While combining with the results in this work, a kind of surface-based mechanism is preferred. However, it is still difficult to get a clear deduction on the mechanism of nanogold-assisted PCR from the current data and knowledge. In fact, before AuNPs were found as a dramatic optimizing additive in PCR, many other additives such as formamide,[21] nonionic detergents,[22] DMSO,[23] glycerol,[24] TMA,[25] betaine;[26] etc. had been proven to be effective to some extent in enhancing specificity or yields of PCR, but most of their optimizing mechanisms were still in hypotheses. The difficulty is

Table 3. Weight and total surface of optimum amount of different size gold nanoparticles.

| Gold nanoparticles | Average height (nm) | Surface-to-volume ratio ($m^2/m^3$)[a] | Effective molarity range (nM) | Optimum molarity (nM) | Optimum AuNPs weight (ng)[b] | Total surface ($mm^2$)[c] |
|---|---|---|---|---|---|---|
| Sigma 5 nm | 5.02 | $1.20 \times 10^9$ | 0.24–0.42 | 0.24 | 4.63 | 0.287 |
| Sigma 10 nm | 10.28 | $5.84 \times 10^8$ | 0.28–0.49 | 0.35 | 57.90 | 1.752 |
| Sigma 20 nm | 21.32 | $2.81 \times 10^8$ | 0.055–0.071 | 0.063 | 92.64 | 1.351 |
| Homemade sample 1 | 44.41 | $1.35 \times 10^8$ | 0.022–0.039 | 0.022 | 289.5 | 2.027 |
| Homemade sample 2 | 57.02 | $1.05 \times 10^8$ | 0.012–0.021 | 0.014 | 405.3 | 2.210 |

[a]Value calculated based on the formula: $S/V = 4\pi\ (d_i/2)^2/[4/3\pi(d_i/2)^3] = 6 \times 10^9/d_i\ (\mathrm{m}^{-1})$, where $d_i$ is equal to the average height.
[b]Value calculated based on the formula: optimum AuNPs weight = Optimum molarity $\times\ V_P \times N_a \times W_i$ (ng), where $V_P$ is equal to one PCR volume 25 $\mu$L; $W_i$ is the weight of one Au nanoparticles with diameter $d_i$ nm.
[c]Value calculated based on the formula: total surface = optimum molarity $\times\ V_P \times N_a \times 4\pi\ (d_i/2)^2$, where $V_P$ is equal to 25 $\mu$L.

mainly due to the complexity of PCR system, which includes many kinds of molecules such as ds-DNA template, polymerase, dNTPs, ss-DNA primers, etc. In terms of AuNPs, there are many reports about the interaction between AuNPs and DNA,[3–5] protein,[1,2] or dNTPs.[27] So far no systematic research work has been performed on competitive absorption among AuNPs and all these molecules. Therefore, the detailed mechanism of nanogold-assisted PCR still requires further investigation.

## 4. Conclusions

In summary, we tested three kinds of commercial AuNPs with 5.02, 10.28, and 21.32 nm in diameter and two kinds of homemade AuNPs with 44.41 nm and 57.02 nm in diameter in enhancing the specificity of the error-prone two-round PCR. Based on their different optimum concentrations, several parameters were calculated including $S/V$, the weights, and the total surfaces of AuNPs samples with different sizes. It was found that the total surface areas of the AuNPs were on the same level for achieving the same optimum effect except the sample with 5.02 nm in diameter. This seemed to favor a kind of surface-based mechanism in nanogold-assisted PCR.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (10335070, 10674147), the Committee of Science and Technology of Shanghai (0652nm006, 0752nm021), and National Basic Research Program of China (973 Program 2007CB936000, 2006CB933000).

## References

1. W. P. Faulk and G. M. Taylor, *Immunochemistry* **8** (1971) 1081.
2. J. M. Polak and S. V. Noorden, *Immunocytochemistry: Modern Methods and Applications* (John Wright and Sons, Bristol 1986).
3. H. Li and L. Rothberg, *Proc. Natl. Acad. Sci.* **101** (2004) 14036.
4. H. Li and L. J. Rothberg, *J. Am. Chem. Soc.* **126** (2004) 10958.
5. L. Wang, X. Liu, X. Hu, S. Song and C. Fan, *Chem. Commun.* **18** (2006) 3780.
6. G. Paciotti, L. Myer, D. Weinreich, D. Goia, N. Pavel, R. McLaughlin and L. Tamarkin, *Drug Deliv.* **11** (2004) 169.
7. J. Zhang, S. Song, L. Zhang, L. Wang, H. Wu, D. Pan and C. Fan, *J. Am. Chem. Soc.* **128** (2006) 8575.
8. H. Li, J. Huang, J. Lv, H. An, X. Zhang, Z. Zhang, C. Fan and J. Hu, *Angew. Chem. Int. Ed.* **44** (2005) 4989.
9. M. Lijuan, Z. Hongping, Z. Xiaodong, H. Jun and F. Chunhai, *Chin. Sci. Bull.* **52** (2007) 2345.
10. J. Pan, H. Li, X. Cao, J. Huang, X. Zhang, C. Fan and J. Hu, *J. Nanosci. Nanotechnol.* **7** (2007) 4428.
11. M. Li, Y. C. Lin, C. C. Wu and H. S. Liu, *Nucleic Acids Res.* **33** (2005) 1093.
12. R. Narayanan and M. A. El-Sayed, *J. Phys. Chem. B* **107** (2003) 12416.
13. M. A. EL-Sayed, *Acc. Chem. Res.* **37** (2004) 326.

14. E. Cottancin, J. Lermé, M. Gaudry, M. Pellarin, J.-L. Vialle and M. Broyer, *Phys. Rev. B* **62** (2000) 5179.
15. G. Frens, *Nature Phys. Sci.* **241** (1973) 20.
16. J. Hu, M. Wang, H.-U. G. Weier, P. Frantz, W. Kolbe, D. F. Ogletree and M. Salmeron, *Langmuir* **12** (1996) 1697.
17. Ch. Wong, P. E. West, K. S. Olson, M. L. Mecartney and N. Starostina, *J. Metal* **59** (2007) 12.
18. W. Rui-Bin, H. Lin, L. Gang, N. Xiao-Ming, H. Dan-Nong and L. Qing-Hua, *J. Instrument. Anal.* (in Chinese) **24** (2005) 119.
19. J. Tschopp, E. R. Podack and H. J. Miiller-Eberhard, *Proc. Natl. Acad. Sci.* **79** (1982) 7474.
20. K. C. Grabar, K. J. Allison, B. E. Baker, R. M. Bright, K. R. Brown, R. G. Freedman, A. P. Fox, M. D. Keating, M. D. Musick and M. J. Natan, *Langmuir* **12** (1996) 2353.
21. G. Sarkar, S. Kapelner and S. S. Sommer, *Nucleic Acids Res.* **18** (1990) 7465
22. B. Bachmann, W. Luke and G. Hunsmann, *Nucleic Acids Res.* **18** (1990) 1309.
23. P. R. Winship, *Nucleic Acids Res.* **17** (1989) 266.
24. M. Nagai, A. Yoshida and N. Sato, *Biochem. Mol. Biol. Int.* **44** (1998) 157.
25. M. Kovarova and P. Draber, *Nucleic Acids Res.* **28** (2000) E70.
26. W. A. Rees, T. D. Yager, J. Korte and P. H. von Hippel, *Biochemistry* **32** (1993) 137.
27. L. M. Demers, M. Ostblom, H. Zhang, N.-H. Jang, B. Liedberg and C. A. Mirkin, *J. Am. Chem. Soc.* **124** (2002) 11249.