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Effect of antifouling dendrimers and Au DENPs on the enhancement of PCR amplification

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Abstract: The polymerase chain reaction (PCR) has been considered as one of the most fundamental techniques to amplify and analyze specific DNA fragments in the field of molecular biology and clinical medicine. Recently, a variety of nanoparticles (NPs) have been regarded as a novel method to enhance quality and yield of PCR technique. Herein, we report the use of generation 5 (G5) poly(amidoamine) (PAMAM) dendrimers and dendrimer-entrapped gold nanoparticles (Au DENPs) modified with polyethylene glycol (PEG) moieties and/or acetyl groups as a novel class of enhancers to improve the PCR amplification. We set up the nonspecific PCR and two-round PCR as model systems to investigate mechanisms of enhanced PCR. Our results show that dendrimer-based derivatives seem to enhance the PCR specificity. It is worth noting that the modification of antifouling PEG significantly lowered the optimization capability of the corresponding dendrimers, while this inhibition effect can be remarkably compromised by the entrapment of Au NPs. Furthermore, we found that in the presence of Au NPs, the Au NPs-induced thermal conductivity could play the dominant role in the PCR optimization, while in the absence of Au NPs, electrostatic interaction of the dendrimers with PCR components may be the major factor affecting the PCR system. Our results also showed that the optimal concentrations of the materials in the two test systems were very close, which indicates that the developed dendrimer derivatives with good thermal stability may be the efficient PCR additives for enhancing different PCR systems.

Keywords: Polymerase chain reaction; Thermal conductivity; Antifouling; Dendrimers; Au DENPs

1. Introduction

It is essential to develop an accurate and rapid method for amplifying and analyzing gene segments in terms of disease diagnosis.¹ The advent of polymerase chain reaction (PCR) has attracted much attention from scientists.² PCR has been one of the most important techniques in modern molecular biology³ and has widespread applications in many areas, for example, DNA cloning,⁴ diagnosis therapy,⁵ functional analysis of genes.⁶ The amplification technique can increase the number of copies of target genes by a magnification of several millions within hours, which allows detection and analysis of DNA sequences. However, the main defects of PCR such as poor quality and low productivity could not be avoided because of its *in vitro* amplification.

Recently, nanomaterials-assisted PCR (nanoPCR) is considered as a new and efficient experimental strategy to improve PCR products. Compared with the conventional PCR additives, such as dimethyl sulfoxide (DMSO), betain and single-stranded DNA binding proteins (SSB),⁷⁻⁸ nanoparticles (NPs)-based enhancers have become more attractive due to their exceptional physicochemical properties like large surface area and volume ratio, surface charge density, and excellent thermal conductivity.⁹ A variety of nanomaterials including gold NPs (Au NPs),¹⁰ quantum dots (QDs),¹¹ carbon nanotubes (CNTs),¹² carbon nanopowder (CNPs),¹³ poly(amidoamine) (PAMAM) dendrimers and dendrimer-entrapped gold NPs (Au DENPs)¹⁴ have been proven to enhance efficiency and specificity of PCR amplification. And several underlying mechanisms for the effects of nanomaterials have also been suggested. For instance, Li et al.¹⁵ reported that Au NPs as additives could improve the performance of PCR reaction and postulated that this process could be a result of the excellent thermal conductivity of the Au NPs. Vu et al.¹⁶ and Mi et al.¹⁷ postulated that Au NPs adsorb onto DNA polymerases and dynamically interact with it to reduce nonspecific fragment in PCR solution. Cao et al.¹⁸⁻¹⁹ reported that the

electrostatic interaction between the PCR components and NPs may play the primary role in the PCR enhancing effect. All these studies suggest that thermal conductivity of nanomaterials, concentration of DNA polymerases as well as electrostatic interaction between the NPs and PCR components may play a prominent role in PCR enhancing. In order to further study the mechanisms involved in this process, the development of various NPs with different surface characteristics and thermal conductivity as well as antifouling properties would be necessary.

Polyethylene glycol (PEG) is a water soluble polymer with low toxicity and one of the most studied antifouling polymers.²⁰ PEGylation is the process of attaching the strands of the polymer PEG to molecules, most typically peptides, proteins, and antibody fragments. It produces alterations in the physicochemical changes in conformation, electrostatic binding, hydrophobicity.²¹ PEG coatings have been considered as the subject of considerable interest as a means of reducing protein adsorption.²² In a recent report,²³ PEG has been shown to reduce the interaction of the nanomaterials/DNA compound with serum proteins as well as to increase the blood circulation time of the compound in gene delivery applications.

In this study, we explore a new application of dendrimer derivatives as PCR enhancers, including positively charged amine-terminated generation 5 PAMAM dendrimers (G5.NH₂) and Au DENPs modified with PEG, and the acetylated counterpart materials with nearly neutral surface charge. We systematically investigated the effect of surface charges, structure and protein absorption capacity as well as thermal conductivity of dendrimer derivatives on the specificity and efficiency of the PCR products using non-specific and two-round PCR systems, and possible molecular mechanisms were also discussed for each respective PCR system. We believe that these findings will offer meaningful insights into the application of dendrimer derivatives and other NPs in nanoPCR.

2. Experimental section

2.1 Materials

Ethylenediamine core G5.NH₂ dendrimers with a polydispersity index less than 1.08 were purchased from Dendritech (Midland, MI). G5.NH₂ dendrimers with defined PEGylation (G5.NH₂-*m*PEG) and acetylation (G5.NHAc-*m*PEG) degrees, Au DENPs ($\{(Au^0)_{100}\text{-G5.NH}_2\}$), PEGylated Au DENPs ($\{(Au^0)_{300}\text{-G5.NH}_2\text{-}m\text{PEG}\}$), and PEGylated Au DENPs modified with acetyl groups ($\{(Au^0)_{300}\text{-G5.NHAc-}m\text{PEG}\}$) were synthesized and characterized following our previous work.²⁴⁻²⁵ The gold salt (HAuCl₄)/dendrimer molar ratio are at 100:1 and 300:1 respectively. The structures of the dendrimer derivatives or nanocomposites were schematically illustrated in **Figure 1**. The water used in all the experiments was purified using a Mili-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18 MΩ cm.

2.2 UV-Vis spectrometry

UV-Vis spectrometry was used to compare the stability of the Au DENPs before and after the PCR process without addition of PCR components. The spectra were collected using a Lambda 25 UV-Vis spectrometer (Perkin Elmer, Waltham, MA). The tested samples were dissolved in water at a concentration of 0.1 mg/mL. For comparison, the G5.NH₂ dendrimers without Au NPs entrapped with the concentration of 0.1 mg/mL were also measured.

2.3 Non-specific amplification test system

PCR was carried out with 40 ng of genomic DNA template, isolated from mouse tail (presented by Prof. Xiao Junhua group, Donghua University), 0.2 μM primers (Shanghai Sangon Biological Engineering and Technology and Service Co.Ltd), 0.25 mM each deoxy- ribonucleoside triphosphate, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl₂, 0.05 U/μL Taq DNA polymerase (Takara Bio. Inc.) in a final volume of 25 μL. The primers (primer 1: ATG AAC CCA GCC ATC AGC G,

primer 2: GGG TAA GGA CCT TGA TAT AGG) were designed to amplify an 869-bp fragment of mouse Thy-1 gene sequences. The PCR procedure was: 1 min at 94 °C for pre-denaturation, followed by 30 cycles of: 15 s at 94 °C, 15s at 56 °C, 30 s at 72 °C. Then cycling was terminated after 1 min incubation at 72 °C. The amplification was performed using S1000TM Thermal Cycler (Bio-Rad Inc.).

2.4 Two-round PCR test system

The two-round PCR was similar to the model described in our previous work.¹⁹ In the first round PCR, 369-bp DNA segment was amplified from the *Pseudomonas aeruginosa* genome DNA (extracted in our laboratory) by using one pair of primers with high specificity. Then, the 1000-fold diluted 369-bp PCR products of the first round were used as the template for the second round PCR amplification with the same primers. PCR amplifications were performed in 25 µL reaction volumes using the similar conditions mentioned above, except that 0.025 U/µL Taq DNA polymerase was added into the mixture. The PCR procedure was: 2 minutes at 94 °C for pre-denaturation, followed by 27 cycles of: 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C. This was terminated by extension at 72 °C for 5 minutes. The amplification was performed using S1000TM Thermal Cycler.

2.5 Evaluation methods of PCR

The PCR products (3µL) were mixed with 0.6 µL loading buffer (Takara Bio. Inc.), and then analyzed by the agarose gel electrophoresis (1.5 W/V %) stained with ethidium bromide, finally visualized and photographed on a UV transilluminator. Marker II (Tiangen Biotech Inc.) and DL2000 Marker (Takara Bio. Inc.) were employed to mark the DNA size. The ability of the tested additives to increase the efficiency of amplification was defined as a ratio of the densitometric value of the target DNA band determined after PCR to 700 bp of Marker II or to 500 bp of DL2000 Marker. The specificity of amplification was calculated as a ratio of the densitometric value of the specific band

and that of all bands amplified by PCR. The specificity in the absence of non-specific bands was defined as the maximal value that equals to 1.0. The concentration of each additive that made PCR produce the maximal specificity and the brightest target band was identified to be the optimum concentration. All the PCR products in the absence or presence of the different additives were sequenced to confirm the fidelity of the PCR, and our result shows the sequence of the PCR product in the presence of the enhancers is quite consistent with that in the absence of the enhancers.

2.6 Dynamic light scattering (DLS) and zeta potential measurements

The size and zeta potential measurements were carried out using a Zetasizer Nano ZS system (Malvern, UK) equipped with a standard 633 nm laser at room temperature of 25 °C. Solutions (1000 μ L) of G5.NH₂ derivatives or nanocomposites with the optimum concentrations used to optimize the PCR in the absence and presence of PCR components were prepared before the DLS measurement. 800 μ L solutions of each material with concentration of 0.05mg/mL were prepared for the zeta potential measurements. These measurements were test in triplicate for mean values.

3. Results and Discussion

3.1 Thermal stability of additives

Considering three steps of the PCR reaction process: denaturation, annealing and extension, which can be repeated for 30-40 cycles and the denaturation temperature is as high as 94 °C, it may make the Au DENPs unstable and aggregate in the PCR mixture, thereby decreasing the PCR enhancing effect. Therefore, it is very important to explore the thermal stability of Au DENPs. The thermal stability of $\{(Au^0)_{100}\text{-G5.NH}_2\}$, $\{(Au^0)_{300}\text{-G5.NH}_2\text{-}m\text{PEG}\}$ and $\{(Au^0)_{300}\text{-G5.NHAc-}m\text{PEG}\}$ before and after the PCR process without addition of the PCR components were measured by the UV-Vis spectrometry (**Figure 2**). It is clear that the surface plasmon resonance (SPR) peaks at

around 510 nm indicates the existence of the Au NPs. After the PCR process, the absorption feature and the SPR peaks do not have any significant changes, indicating that the Au DENPs are stable, which is essential for enhancement of different PCR systems. As suggested above, all the tested additives are very stable during the PCR process, which is essential for PCR improvement.

3.2 Effects of additives on non-specific PCR test system

In our non-specific PCR system, both the specific fragment of Thy-1 gene and several other non-specific fragments (**Figure 3**, Lane 1 for each lane) were amplified following the conventional process. On the basis of the published reports, these non-specific amplification could not be improved by optimizing the annealing temperature, the concentration of Mg^{2+} ions or DNA polymerase.¹⁵ Therefore, it is an appropriate model to evaluate if the NPs could enhance the specificity of PCR amplification.

To determine whether or not the dendrimer derivatives have the ability to improve the specificity and efficiency of PCR, six kinds of additives with different surface charges (see **Table 1**) were separately added into the PCR mixture at a series of different concentrations. The PCR products were analyzed by agarose gels electrophoresis. As shown in **Figure 3**, it is clear that the non-specific and smear tailed bands gradually decreased as the concentration of additives increased, suggesting that all the dendrimer derivatives can strengthen the specificity of the nonspecific PCR system. However, all the additives tended to suppress PCR reaction when the concentrations exceeded their corresponding optimum concentration, in agreement with our previous reports.¹⁸⁻¹⁹

The PCR specificity and efficiency in the presence of each additive were calculated by semi-quantitative analysis according to ImageJ software (**Table 2**). It shows that for the dendrimers without Au NPs entrapped, the optimum concentrations used for optimizing this nonspecific PCR system follow the order of $G5.NH_2$ (14.39 nM) < $G5.NH_2-mPEG$ (17.92 nM) < $G5.NHAc-mPEG$

(19.23 μM). It is obvious that the modification of PEG moieties significantly lowers the optimization capability of G5.NH₂ dendrimers. This may be due to the antifouling properties of PEG, compromising the interaction with the PCR components. For example, Vu et al.¹⁶ reported that the effect of Au NPs on PCR can be duplicated simply by reducing DNA polymerase concentration and the effect can be reversed by increasing the polymerase concentration or by adding bovine serum albumin (BSA). Mi et al.¹⁷ postulated that Au NPs adsorb onto DNA polymerases and dynamically interact with it to reduce nonspecific fragment in PCR solution. The non-fouling properties of PEG are primarily attributed to their high chain mobility, large exclusion volume, and steric hindrance effect. Therefore, we believe that the reduction in G5.NH₂-*m*PEG optimization capability may result from the fact that PEG has the ability to resist protein adsorption, so it may resist the interaction between the DNA polymerase and dendrimers, and weaken opportunities of the dynamic contact between DNA polymerase and other components.^{22-23, 26}

In addition, it is noted that the optimum concentration of G5.NHAc-*m*PEG is much higher than that of G5.NH₂-*m*PEG or G5.NH₂. Previously, our findings indicated that the enhancement of the PCR using nanomaterials may be caused by surface-charge-mediated electrostatic interactions.²⁷ Cao et al.¹⁹ reported that positively charged CNT/PEI could accumulate the PCR elements locally on the NPs backbones by electrostatic interaction to increase opportunities of the dynamic contact among each component. According to **Table 1**, the ζ -potential follows the order of G5.NH₂ (22.7 mV) > G5.NH₂-*m*PEG (22.2 mV) > G5.NHAc-*m*PEG (1.2 mV). In the PCR conditions (pH 7.4), the used templates, primers, and the DNA polymerase with an isoelectric point of 6.03 are all negatively charged, which enables effective electrostatic interaction with the positively charged additives. As the surface charges of NPs decreasing, the interaction of dendrimers with the PCR components should become weaker. Therefore, the optimal concentrations of the enhancers follow opposite order

of surface potential of nanomaterials. For the G5.NHAc-*m*PEG with the terminal amine groups neutralized by acetyl groups, the optimal concentration is three orders of magnitude higher than that of G5.NH₂-*m*PEG or G5.NH₂, which suggests that the electrostatic interaction between the dendrimers and PCR components should play a main role in the optimization of non-specific PCR system, comparing with protein absorption capability.

Besides, we further measured the hydrodynamic sizes of all additives under their corresponding optimum concentrations of PCR optimization before and after addition of PCR components by DLS (**Table 1**), to determine the interaction between the additives and PCR components. The size of PCR mixture was 634.15 nm and the Au DENPs seem to have smaller sizes compared with the identical dendrimers without Au NPs. This phenomenon may be because of the entrapped of Au NPs, the dendrimers have more reserved 3D spherical morphology to provide more binding sites for stronger interaction with the PCR components. The PEGylated dendrimer has a larger size than G5.NH₂ dendrimer to some extent. This is due to the fact that long linear polymer chain (PEG) modified on the surface of dendrimer makes the size larger than dendrimer alone. After addition of PCR components, the sizes of the formed complexes are much smaller than those of the corresponding materials. The results indicate that there may be strong interaction between the additives and the PCR components.

For the Au DENPs materials, the optimal concentrations follow the order of {(Au⁰)₁₀₀-G5.NH₂} (10.76 nM) < {(Au⁰)₃₀₀-G5.NH₂-*m*PEG} (12.51 nM) < {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} (22.72 nM), and they were much lower than that of the corresponding dendrimers without Au NPs entrapped. It seems that the entrapment of Au NPs can be more effective to improve the PCR specificity than the materials without Au NPs entrapped. It is worth emphasizing that the inhibition effect of antifouling dendrimers can be significantly compromised by the entrapment of Au NPs. In our previous works,

we proposed that interaction sites of the flexible soft G5.NH₂ are much less than those of the Au DENPs with reserved 3D spherical morphology, which offers less opportunity to interact with the PCR elements.^{14, 28} Furthermore, the optimal concentration of {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} is three orders of magnitude less than that of G5.NHAc-*m*PEG. Since both materials are nearly neutral charged, the optimizing effects may result from the Au NPs-induced thermal conductivity in the PCR mixture.

3.3 Effects of additives on two-round amplification test system

In order to further verify our findings that the thermal conductivity of the nanomaterials may be a vital factor in the PCR optimization, we also introduced the two-round PCR system as a new model to test the PCR enhancing effect when the surface charges of NPs are close to zero. The two-round PCR was similar to our previous error-prone two-round PCR system,¹⁴ and the DNA template was replaced with the specific band of 396-bp DNA segment. Because the same primers were used in two-round of PCR, the nonspecific bands (**Figure 4**, Lane 1 for each electropherogram) below the target band are always visible in the second round. The surface charges of the G5.NHAc-*m*PEG and {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} are both nearly neutral, so we can compare the improvement effect of the additives with or without Au NPs entrapped and test whether or not the enhanced PCR depends on the thermal conductivity of nanomaterials. As shown in **Figure 4** and **Table 3**, it is clear that both additives can enhance the PCR specificity. The optimization concentrations of G5.NHAc-*m*PEG and {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} are 16.03 μM and 24.69 nM, respectively. There are three orders of magnitude difference between the optimum concentrations of both additives, which is in accordance with our previous work.¹⁴ Our results further confirm that in the presence of Au NPs, the Au NPs-induced thermal conductivity may play the major role in the PCR enhancement.

4. Conclusion

In summary, we have used antifouling dendrimers and Au DENPs as a novel class of additives to investigate their effects on the specificity and efficiency of PCR amplification. Our results indicate that all the materials can increase the specificity of PCR at their optimal concentrations in the nonspecific PCR and two-round PCR systems. The modification of antifouling PEG significantly lowered the optimization capability of G5.NH₂ dendrimer. With the entrapment of Au NPs, dendrimer enhance the PCR specificity more effectively than the corresponding dendrimer without Au NPs entrapped, and the inhibition effect of antifouling dendrimers can be significantly compromised by the entrapment of Au NPs. Our results suggest that in the presence of Au NPs, the Au NPs-induced thermal conductivity may play the main role in the PCR optimization, while in the absence of Au NPs, electrostatic interaction of the dendrimers with PCR components may be the crucial factor affecting the PCR system. With good thermal stability, the developed dendrimer derivatives or nanocomposites may be used as the efficient PCR additives for enhancing different PCR systems.

Notes

The authors declare no competing financial interest.

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Table 1. Zeta potential and Hydrodynamic size of G5.NH₂ dendrimers derivatives or composites before and after interaction with the PCR components

Sample	Zeta potential (mV)	Hydrodynamic size/nm	
		Before addition of PCR components	After addition of PCR components
G5.NH ₂	22.7	1855.25	815.48
G5.NH ₂ - <i>m</i> PEG	22.2	1991.80	891.45
G5.NHAc- <i>m</i> PEG	1.2	645.23	402.43
{(Au ⁰) ₁₀₀ -G5.NH ₂ }	33.8	1801.67	628.12
{(Au ⁰) ₃₀₀ -G5.NH ₂ - <i>m</i> PEG}	17.5	1260.15	572.47
{(Au ⁰) ₃₀₀ -G5.NHAc- <i>m</i> PEG}	-4.5	811.17	334.90

Table 2. Efficiency, specificity, and optimum concentration of additives in a nonspecific PCR.

Additive	Optimum	Maximal	Maximal
	concentration	efficiency	specificity
G5.NH ₂	14.39 nM	1.13	0.45
G5.NH ₂ - <i>m</i> PEG	17.92 nM	1.03	0.46
G5.NHAc- <i>m</i> PEG	19.23 μM	1.21	0.47
{(Au ⁰) ₁₀₀ -G5.NH ₂ }	10.76 nM	1.11	0.46
{(Au ⁰) ₃₀₀ -G5.NH ₂ - <i>m</i> PEG}	12.50 nM	1.23	0.53
{(Au ⁰) ₃₀₀ -G5.NHAc- <i>m</i> PEG}	22.72 nM	1.08	0.50

Table 3. Efficiency, specificity, and optimum concentration of additives in a two-round PCR.

Additive	Optimum concentration	Maximal efficiency	Maximal specificity
G5.NHAc- <i>m</i> PEG	16.03 μ M	1.00	0.47
{(Au ⁰) ₃₀₀ -G5.NHAc- <i>m</i> PEG}	24.69 nM	1.04	0.45

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Figure captions:

Figure 1. Schematic illustration of the structure of G5.NH₂, Au DENPs, and their derivatives with defined acetylation and PEGylation degrees.

Figure 2. UV-Vis spectra of {(Au⁰)₃₀₀-G5.NH₂-*m*PEG} (1), {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} (2), {(Au⁰)₁₀₀-G5.NH₂} (3) and G5.NH₂ dendrimers (4) before (a) and after the PCR process (b).

Figure 3. The effect of the additives on the specificity of nonspecific PCR. In every image, lane M is for marker, the last lane is the negative control. (a) G5.NH₂ was added into the PCR mixture, and from lane 1 to lane 6, its final concentration was 0, 4.80, 9.60, 14.39, 19.19, and 26.39 nM, respectively. (b) G5.NH₂-*m*PEG was added into the PCR mixture, and from lane 1 to 7, its final concentration was 0, 5.97, 7.17, 11.94, 17.92, 29.86, and 35.83 nM, respectively. (c) G5.NHAc-*m*PEG was added into the PCR mixture, and from lane 1 to 6, its final concentration was 0, 12.82, 19.23, 38.48, 89.76, and 128.23 μM, respectively. (d) {(Au⁰)₁₀₀-G5.NH₂} was added into the PCR mixture, from lane 1 to 6, its final concentration was 0, 7.18, 10.76, 14.35, 21.52, and 35.87 nM, respectively. (e) {(Au⁰)₃₀₀-G5.NH₂-*m*PEG} was added into the PCR mixture, from lane 1 to 7, its final concentration was 0, 7.50, 10.00, 12.51, 18.76, 37.52, and 62.53 nM respectively. (f) {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} was added into the PCR mixture, from lane 1 to 7, its final concentration was 0, 7.75, 15.15, 22.72, 30.29, 37.86, and 60.58 nM, respectively.

Figure 4. The effect of the additives on the specificity of two-round PCR. In every image, lane M is for marker, the last lane is the negative control. (a) G5.NHAc-*m*PEG was added into the PCR mixture, and from lane 1 to 8, its final concentration was 0, 3.21, 9.62, 16.03, 22.44, 28.85, 57.80, and 70.52 μM, respectively. (b) {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} was added into the PCR mixture, from lane 1 to 8, its final concentration was 0, 16.46, 24.69, 32.92, 41.16, 49.39, 65.85, and 82.31 nM, respectively.

Figure 1. Schematic illustration of the structure of G5.NH₂, Au DENPs, and their derivatives with defined acetylation and PEGylation degrees.

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Figure 2. UV-Vis spectra of $\{(\text{Au}^0)_{300}\text{-G5.NH}_2\text{-}m\text{PEG}\}$ (1), $\{(\text{Au}^0)_{300}\text{-G5.NHAc-}m\text{PEG}\}$ (2), $\{(\text{Au}^0)_{100}\text{-G5.NH}_2\}$ (3) and G5.NH₂ dendrimers (4) before (a) and after the PCR process (b).

Figure 3. The effect of the additives on the specificity of nonspecific PCR. In every image, lane M is for marker, the last lane is the negative control. (a) G5.NH₂ was added into the PCR mixture, and from lane 1 to lane 6, its final concentration was 0, 4.80, 9.60, 14.39, 19.19, and 26.39 nM, respectively. (b) G5.NH₂-*m*PEG was added into the PCR mixture, and from lane 1 to 7, its final concentration was 0, 5.97, 7.17, 11.94, 17.92, 29.86, and 35.83 nM, respectively. (c) G5.NHAc-*m*PEG was added into the PCR mixture, and from lane 1 to 6, its final concentration was 0, 12.82, 19.23, 38.48, 89.76, and 128.23 μ M, respectively. (d) {(Au⁰)₁₀₀-G5.NH₂} was added into the PCR mixture, from lane 1 to 6, its final concentration was 0, 7.18, 10.76, 14.35, 21.52, and 35.87 nM, respectively. (e) {(Au⁰)₃₀₀-G5.NH₂-*m*PEG} was added into the PCR mixture, from lane 1 to 7, its final concentration was 0, 7.50, 10.00, 12.51, 18.76, 37.52, and 62.53 nM respectively. (f) {(Au⁰)₃₀₀-G5.NHAc-*m*PEG} was added into the PCR mixture, from lane 1 to 7, its final concentration was 0, 7.75, 15.15, 22.72, 30.29, 37.86, and 60.58 nM, respectively.

Figure 4. The effect of the additives on the specificity of two-round PCR. In every image, lane M is for marker, the last lane is the negative control. (a) G5.NHAc-*m*PEG was added into the PCR mixture, and from lane 1 to 8, its final concentration was 0, 3.21, 9.62, 16.03, 22.44, 28.85, 57.80, and 70.52 μM , respectively. (b) $\{(\text{Au}^0)_{300}\text{-G5.NHAc-}m\text{PEG}\}$ was added into the PCR mixture, from lane 1 to 8, its final concentration was 0, 16.46, 24.69, 32.92, 41.16, 49.39, 65.85, and 82.31 nM, respectively.

Graphical abstracts:

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