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Interactions of the Primers and Mg^{2+} with Graphene Quantum Dots Enhance PCR Performance

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ABSTRACT: It was reported that graphene oxide (GO), a novel carbon materials with unique structural and chemical/physical properties, could improve the performance of polymerase chain reaction (PCR), similar to many nanomaterials. However, GO that with a large lateral size, exhibits poor biocompatibility, and the mechanism of its effect on PCR remains unclear. Using graphene quantum dots (GQDs) that inherit the basic properties of GO, but with nanometer lateral sizes and much better biocompatibility, we systematically investigated their functional roles in PCR enhancement. The overall performance of PCR in terms of its yield, sensitivity, and specificity can be improved with much less amount of GQDs than that of GO. It is found that the stacking of the primers on GQDs improves the sensitivity and specificity of PCR through improving efficiency of base-pairing between the primer and the template. The yield of PCR is improved primarily by GQDs *via* increasing the activity of polymerase, which is tuned by GQDs through chelating magnesium ions with their peripheral carboxylic groups. The adsorption of the polymerase on GQDs affects marginally the activity of polymerase that is different from the proposed function of GO to PCR. The superb performance of GQDs in PCR exhibits their practical potential in PCR, thus can be used in biology and medical applications.

Introduction

The DNA amplification method, polymerase chain reaction (PCR), is one of the most important techniques in basic and applied medical research and biology.[1-3] The performance of the PCR thus has been kept improving since it was developed in 1985.[4-6] Nanoparticles (NPs), as one category of PCR enhancers, have attracted extensive attention due to their unique structural and physical properties.[7-11] Many different NPs including metallic and non-metallic NPs were employed to improve the efficacy, sensitivity and fidelity of PCR. For instances, single-walled carbon nanotubes could increase yield of PCR,[12] enhance the specificity of PCR with long DNA fragment,[13] carbon nanopowder could enhance the efficiency of PCR,[14] gold NPs could improve the specificity and yield of PCR,[9] Ag, Pt, CdTe Quantum Dots,[15-16] nano-alloys and upconversion NPs[17] have also been investigated for their beneficial effect on PCR. Recently, graphene oxide (GO), single carbon atomic, micrometer-sized sheets that are are rich with oxygen containing groups, new member of the carbon nanomaterials family, was found to enhance PCR specificity as well, which was believed to be attributed by the great affinity of GO to DNA and polymerase, and high thermal conductivity of GO.[8] Several optimization mechanisms by NPs have been proposed, some believe that NPs interact with DNA polymerase in PCR system to regulate its catalytic activity, thus improve PCR yield;[18] some proposed that NPs interact selectively with single-stranded DNA; others believed that metallic NPs might use its excellent heat transfer or catalytic property to improve the PCR efficiency;[12] and some suggested that NPs enhanced PCR specificity by increasing the T_m (melting temperature) difference between the matched and mismatched primers.[19] Though NPs generally can improve the performance of PCR in certain extent, their functional mechanisms remains unclear.

We have found in our previous work that graphene quantum dots (GQDs) that maintain single atomic-layered structural motif, with better conjugate state, and lateral dimension of less than 100 nm, exhibited potentials in many biological applications.[20] GQDs could interact with double helical DNA and the DNA that has unique structure motif,[21-22] and we also found that GQDs have much better biocompatibility than that of GO.[23] Here, we systemically investigate the functional role of GQDs in PCR by inspecting the interaction of GQDs with the major components of PCR reaction system. We found that GQDs could improve the yield, sensitivity and specificity of PCR with much smaller amount than that of GO.[8] More importantly, the functional mechanism of GQDs in PCR is different from that of GO or other NPs. GQDs improve the specificity of PCR primarily through selective interaction with the primers and single stranded DNA (ssDNA), which enhances base-pairing efficacy between the primer and template. And GQDs improved the yield of PCR predominantly through tuning the activity of polymerase *via* chelating magnesium ions, the cofactor of the polymerase in PCR reaction system, with their peripheral carboxylic groups. The superb performance of GQDs in PCR exhibits their potential in biology and medical practical applications.

Experimental Section

Materials. GQDs were prepared through photo-Fenton reactions of graphene oxide and were generated as described in our previous work.[20] The generated GQDs were characterized by AFM, XPS, and FT-IR, as we described in our previous work. GQDs-NH_2 were synthesized by modified the GQDs with 1, 2 - ethylene diamine using EDC/NHS cross linking reagents, and characterized similar to that for the GQDs. The reagents for PCR were purchased in Sangon (Shanghai, China).The PBR322 plasmid and calf thymus DNA was purchased in Sigma. The GC-rich DNA was synthesized by Sangon.

PAGE purified primers were purchased from Sangon and used without further purification. The first letter in primer names indicates the template (P stands for PBR322; C for calf thymus; and G for GC-rich template), the number indicates the length of the primer. The letters F and R indicate the forward or reverse primers, respectively. P309-F: 5'-CGC TAA CGG ATT CAC CAC-3' and P309-R: 5'-CAC GGA AAC CGA AGA CCA-3' were employed to amplify a 309 bp fragment from the PBR322 plasmid DNA (from Thermo Scientific). P134-F: 5'-ATT AAG GAC ATC TTA GGG GCC CTC T-3' and P134-R: 5'-GGG TTT GAT GTG AGG GGG TGT GTT G-3' were employed to amplify a 134bp fragment from the calf thymus DNA (from Sigma). P80-F: 5'-ATC TAC CTG AGT CCA GCC-3' and P80-R: 5'-ACT ATC ACC TAA TCC GCT G-3' were employed to amplify a 80bp fragment from a GC-rich DNA purchased from Sangon. The P309-F10 was labeled with 2-Ap by Sangon too.

Methods. The PCR was carried out on a T100TM Thermal Cycler (BIO-RAD, USA). The optimal amount of all reagents in the PCR system were mixed in a final volume of 25 μL in a PCR tube in the first round according to the following final conditions: 1×PCR buffer, 0.2 mM of dNTP , 0.25 μM of each primer, template DNA (2×10^{-5} ng μL^{-1} of PBR322 DNA, 0.8 ng μL^{-1} of calf thymus DNA or 0.05 ng μL^{-1} of GC-rich DNA), 0.06 U μL^{-1} DNA polymerase; the sample was made up to the final volume with GQDs and ultrapure water. In the round-2 PCR, the reagents used were the same as in the first round, except that the previous-round PCR product (0.4 μL) was substituted for plasmid DNA. If there was no special emphasis, the PCR of PBR322 DNA conditions were as follows: 1) 5 min at 94 °C; 2) 30 s at 95 °C; 3) 20 s at 52 °C; 4) 30 s at 72 °C; 5) 36 cycles for step 2-4; 6) 5 min at 72 °C; 7) 10 min at 12 °C. For calf thymus DNA, the PCR conditions were as follows: 1) 8 min at 95 °C; 2) 30 s at 95 °C; 3) 25 s at 68 °C; 4) 30 s at 72 °C; 5) 30 cycles for step 2-4; 6) 10 min at 72 °C; 7) 10 min at 12 °C. For GC-rich DNA, the PCR conditions were as same as

PBR322, except the step 3 and 7 were 25 s at 51 °C and 10 min at 72 °C, respectively. PCR products were analyzed by agarose gel with DNA 100bp Ladder to analyze product sizes, and the yields were analyzed by software of Gel Image System (Tanon 2500, Version 4.1.2).

Fluorescence measurements were carried out on a Cary Eclipse spectrofluorometer (Agilent Technologies, USA). Fluorescence spectra of 2-Ap-labeled DNA were measured by using an excitation wavelength of 305 nm and emission from 325 to 550 nm in the absence or presence of different amounts of GQDs in different temperature. The relative samples were heating to 25, 52, 72°C respectively in water bath. For the experiment GQDs effect with Mg^{2+}, the fluorescence emission wavelength was recorded from 350 to 650 nm with an excitation wavelength of 345 nm. Taq DNA polymerase was excitation in 280 nm, the emission wavelength was from 300 to 550 nm. The model of Taq polymerase was performed using Discovery Studio software (Accelrys, Version-4.0). The crystal structure of Taq polymerase was downloaded from the PDB protein databank (PDB ID: 1TAU).

Results and Discussion

GQDs Enhance the Yield, Sensitive, and Specificity of PCR. Effect of GQDs on PCR was first examined. PCR products were visualized using agarose gel electrophoresis, and the intensity of the bands in the gel is a relative measure of the efficiency of the PCR. Figure 1a showed that the yield of PCR can be improved by GQDs with different templates, and under the best condition the PCR yield could be improved about 2 folds. However, with the different templates the amount of GQDs needed for optimization is slightly different. For instances, for the PCR with PBR322 plasmid template, 0.06 ng μL^{-1} of GQDs is necessary to obtain the best result, while for the calf thymus template, 0.08 ng μL^{-1} of GQDs is required under the same reaction condition. Even for GC-rich DNA template (GC%, 67.5%), PCR product can be improved 1.4 times with 0.08 ng μL^{-1} of GQDs.

Notably, the amount of the GQDs used in optimization of PCR is much less comparing to that of GO reported by Mi *et al*,[8, 24] which might be due to higher biocompatibility of GQDs.[25] Quantifying the PCR product with RT-PCR failed, because GQDs quenched the fluorescence of the dye that used for labeling in the experiment. Apparently, similar to many nanoparticles and GO,[8, 13, 17, 26] GQDs are able to improve the yield of PCR efficiently.

Similarly, the sensitivity of PCR in the presence of GQDs was also studied. To better display their effect, the PCR was carried out with 5×10^{-6} ng of the template, at which there is little product amplified (Figure 1b, lane 1). In the presence of GQDs, more products were found and it was also

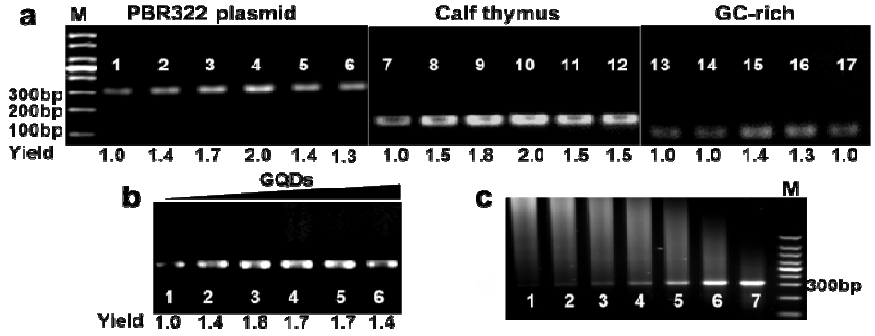


Figure 1. GQDs improve the performance of PCR. a) GQDs improve the yield of PCR with different templates. In each case, PCR product yield of the first lane without GQDs (lanes 1, 7 and 13) was taken as 1, the yield of others are relative to it, respectively. Lanes 1-6 contain 0, 0.2, 1, 1.5, 2 and 2.5 ng of GQDs, lanes 7-12 contain 0, 0.4, 0.6, 0.8, 2 and 4 ng GQDs, lane 13-17 contain 0, 1, 2, 3 and 4 ng GQDs, respectively. The templates are PBR322 plasmid DNA (2.5×10^{-4} ng, 309 bp), calf thymus DNA (5×10^{-3} ng, 134 bp), and GC-rich DNA (0.05 ng, 80 bp). b) GQDs enhance the sensitivity of PCR. Lanes 1-6 contain 0, 0.4, 0.6, 0.8, 2, and 4 ng of GQDs, and PBR322 was 2.5×10^{-6} ng. c) The effect of GQDs (from left to right: 0, 0.5, 1, 1.5, 2, 2.5, and 3 ng) on the specificity

of the round-2 PCR. PCR conditions are described in the experimental section. M represents maker. The templates used in Figure 1b and 1c were PBR322 plasmid.

proportional to the amount of GQDs added, and the sensitivity reached 1.8 fold (Figure 1b, lanes 2-6). Likewise, the amount of GQDs needed for such optimization is only one tenth of the GO used.[8]

The effect of GQDs on the PCR specificity was illustrated by using the first round PCR product as the template to amplify PBR322 plasmid DNA. As shown in Figure 1c, the second round PCR product without GQDs appeared as smeared bands (Figure 1c, lane 1), large amount of non-specific bands were generated. However, when GQDs were included in the PCR reaction system, the non-specific bands disappeared gradually with the increase of the GQDs amount, and only the target band appeared when GQDs was 3 ng (Figure 1c, lane 7). This result is similar to the effect by reduced GO, however, the amount of the reduced GO was 100 folds higher.[8] This difference is possibly contributed by the better biocompatibility of the GQDs.

Interaction of GQDs with Primer and Template. It has been generally believed that ssDNA interact with GO using its bases *via* π-π stacking,[27] while for dsDNA, its bases are wrapped inside the double helix, therefore their interaction with GO is weak.[28] Such difference in the interaction with GO or GQDs between ssDNA and dsDNA has been extensively used for the detection of DNA or other species.[29] Accordingly, in the PCR reaction system, such difference may also be existed for primers and template when GQDs are present. To inspect the interaction of the primer with GQDs, one base A in the primer P309-F10 was labeled with 2-Ap that employed frequently to label DNA molecules without disturbing the DNA structure too much.[30-31] As shown in Figure 2a, when GQDs were added to the solution of 2-Ap labeled primer, the fluorescence of the primer declines with the

increase of GQDs, which is highly in consistent with the fact that GQDs could quench efficiently the fluorescence of the small molecules through π-π stacking.[32] For comparison, the interaction of GQDs with dsDNA was also examined with 2-Ap labeled double helix. Figure S1 showed the little decrease of the fluorescence of the labeled dsDNA upon the addition of the GQDs. The results confirm that the primer and ssDNA more tend to stack to GQDs in PCR system, but the interaction of template and double stranded PCR products with GQDs is much weaker. GQDs have selectivity to the primer and ssDNA in the PCR reaction system. Hu *et al* believed because Au nanoparticles have such selectivity to ssDNA, the mechanism of Au nanoparticle is similar to ssDNA binding protein (SSB).[9, 16]

Figure 2b compared the stacking between GQDs and the primer at different temperatures. Different curves of the fluorescence intensity of the primer *vs* the concentration of GQDs that obtained at three different temperatures revealed that the GQDs quenched the fluorescence of the primer more prominent at a lower temperature. At 25 °C, the fluorescence of the labeled primer

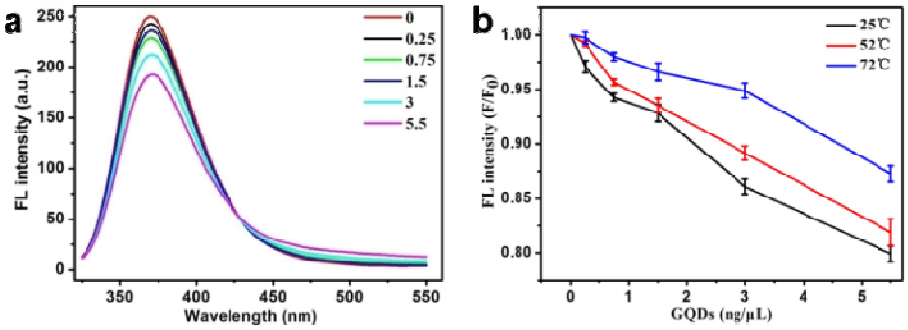


Figure 2. GQDs interact with the primer. a) GQDs (0, 0.25, 0.75, 1.5, 3, and 5.5 ng μL^{-1}) quenched the fluorescence of 2-Ap labeled primer P309-F10 (5'-CGCTAACGG2ApTTCACCAC-3', 25 mM) at room temperature; b) GQDs quench the fluorescence of the primer at 25°C (black), 52°C

(red) and 72°C (blue). The primer was dissolved in ddH_2O. F: fluorescence intensity of the primer with different concentration of GQDs. F_0: fluorescence intensity of the primer without GQDs.

was decreased about 20% with 5.5 ng μL^{-1} of GQDs, and it is only about 10% decrease at 72°C. Clearly at a lower temperature, the primers stacked to GQDs more strongly, which suggests that the interaction of the primer or ssDNA with GQDs probably only affect the base-paring step of primer with the template in PCR, because the temperatures are higher at denature or extension steps of PCR, and the interaction between GQDs and the primer or ssDNA at these two steps is weaker.

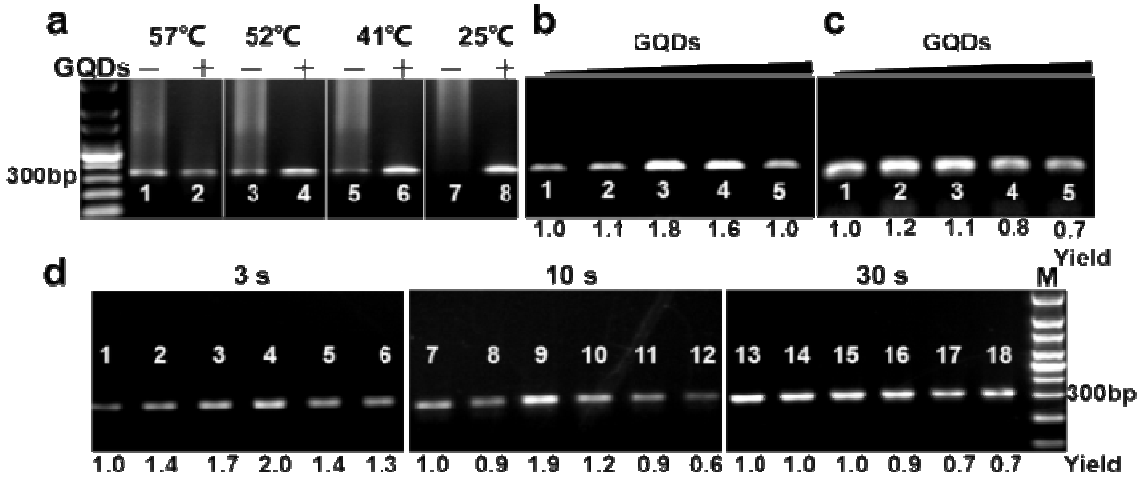


Figure 3. Effect of the GQDs on PCR at different annealing temperatures and annealing times. a) The round-2 PCR amplification at different annealing temperatures (57°C, 52 °C, 41°C, 25°C) in the presence of GQDs (+, 2.5 ng of GQDs) and in the absence of GQDs (-). b) and c) PCR products with different amount of GQDs at annealing temperature of 25 and 57°C, respectively. Lanes 1-5 in b) and c) contain 0, 1, 1.5, 2 and 2.5 ng of GQDs, respectively. d) PCR product with different annealing times (3, 10, and 30 s) in the presence of different amount of GQDs. In each case, PCR product yield of the first lane without GQDs (lanes 1, 7, and 13) was taken as 1, the yields of the

others are relative to them, respectively. GQDs are from 0, 0.2, 1, 1.5, 2, to 2.5 ng, respectively. The template in b), c), and d) was PBR322 plasmid (2.5×10^{-4} ng). The template in a) was the first round PCR product and the target sequence in all figures was 309 bp.

To support this hypothesis, we first examined the effect of the GQDs to the specificity of PCR with different annealing temperatures. Figure 3a showed that at different annealing temperatures, there are many nonspecific products generated in the round-2 PCR product in the absence of GQDs, but these non-specific smeared bands disappeared in the presence of GQDs. Importantly, the enhancement to PCR specificity by GQDs is much better at lower annealing temperature, as evidenced by the increasing intensity of the product band from 57 to 25°C (Figure 3a, lanes 2, 4, and 6). The effect of the GQDs on the PCR with the annealing temperature of 25 °C and 57°C were depicted in detail in Figure 3b and Figure 3c, respectively. PCR with lower annealing temperature (25 °C) apparently was better than that at 57 °C under the same condition; about 1.8 fold of the product at 25 °C was obtained with 0.06 ng μL^{-1} of GQDs. The results clearly reveal that GQDs are favorable to PCR at lower annealing temperature under the same condition. When the annealing temperature is lifted, the stacking between GQDs and the primer is getting weaker (Figure 2b), thus the effect of GQDs on the specificity of PCR is less evident (Lanes at 57 *vs* 52 °C in Figure 3a). The result was further reinforced by the experiment with different annealing times as shown in Figure 3d. When the annealing time is shorter, the enhancement of GQDs is better. Taken these results together, the selective stacking of the primers and ssDNA to the GQDs at a lower annealing temperature strengthens the annealing step of PCR, which possibly due to the confinement of GQDs to the primer and ssDNA, thus minimizing their self-folding or formation of the secondary structures that lead to mis-pairing. This mechanism is different from the mechanism that proposed for Au nanoparticles, in which Au particles were believed decreasing the T_m of dsDNA, equivalent

to increasing the annealing temperature, which led to high base-pairing specificity.[19] However, we found previously that GO could increase T_m of dsDNA.[33] Hence, the lower annealing temperature is favorable to PCR indicated that GQDs did not affect PCR through annealing temperature. Our finding also can explain that the reduced GO exhibited better PCR optimization ability than that of GO,[8] because reduced GO have higher hydrophobicity, thus can stack better with the primers and ssDNA. Our mechanism also agrees well with the findings of Hu *et al*, who showed that soft ssDNA with a much greater degree of freedom is likely to wrap around the quantum dots, thus greatly minimizing mis-pairing between primer and template during DNA replication.[34] Notably, in the term of specificity, GQDs should be much better than other quantum dots due to their unique single atomic layered structure resulting in its great affinity to the primers and ssDNA.[9, 14, 16] In principle, dNTPs also could stack on GQDs like the primers, however, such stacking is negligible, because there is only one base in each dNTP molecule, and in addition, the phosphate group will be repulsed by GQDs for they both negatively charged.

Interaction of GQDs with Taq Polymerase. It was previously reported that GO affect PCR mainly by absorbing the polymerase, template and primer to impetus their interactions, reducing the base mismatch etc..[8] However, double strand template has a low affinity to GQDs, and we also found that PCR enhancement only occurred at a certain concentration range of GQDs, excessive GQDs led to the inhibition to PCR (Figure 4a). With 3 ng of GQDs, the PCR product was improved dramatically (Figure 4a, lane 4), further increasing GQDs up to 6 ng, no more PCR product was found (Figure 4a, lane 7). Interestedly, such suppression by GQDs could be reversed by adding Taq polymerase, and more PCR products were recovered with the increase of the concentration of Taq (Figure 4b, middle group). These findings suggest that the interaction between GQDs and Taq polymerase also occurred in PCR reaction system. For comparison, the template and primers in the

PCR reaction system were examined as well. As shown in Figure 4b, under the same reaction and experimental conditions, increasing the concentration of the primers from 0.2 to 6 mM did not change the yield of PCR;

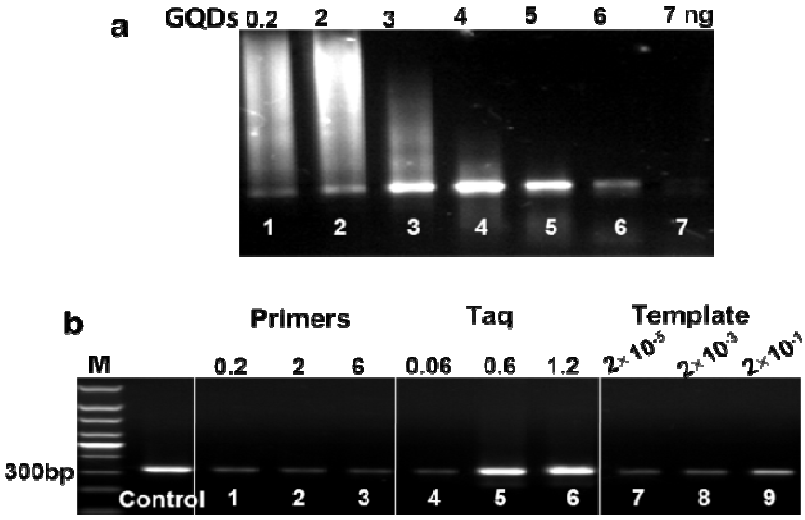


Figure 4. a) GQDs could improve the performance of PCR, but excess of GQDs induce the suppression to PCR. Lanes 1-7 contain 0.2, 2, 3, 4, 5, 6 and 7 ng of GQDs, respectively. All lanes contained 0.06 U μL^{-1} of Taq, 0.2 mM of the primer, and diluted Round-1 product as template. b). Taq polymerase reverses the suppression induced by excessive GQDs, but the primers and template can not. The control sample contains 0.06 U μL^{-1} of Taq, 0.2 mM of the primer, and 2×10^{-5} ng μL^{-1} of the template (PBR322 plasmid), but without GQDs. All lanes contain 4 ng of GQDs. Lanes 1-3, 4-6, and 7-9 are the concentration gradient of the primer (mM), Taq polymerase (U μL^{-1}), and template (ng μL^{-1}) as indicated, respectively; other conditions are the same as the control sample.

increasing the concentration of template to 10^4 folds also did not affect the yield of the PCR (Figure 4, right group). No effect of the primer concentration on PCR also supported afore mentioned

conclusion that the interaction between the primers and GQDs is responsible for the specificity optimization (Figure 3a). Similar phenomenon was reported with C60 in PCR, in which increasing the amount of Taq polymerase in the PCR reaction system, the inhibition induced by C60 was eliminated.[35] The author proposed that C60 bound to the active site of Taq polymerase thus decreased its activity, eventually led to the inhibition of PCR. We however, suggest that instead of direct blocking the active site, the interaction between GQDs and Taq enzyme possibly is through other avenues that lead to the decrease of the activity of Taq, because the average size of GQDs (~ 40 nm) is too large to the active site of Taq.[36]

To further understand these results, the fluorescence of Taq was measured in the presence of GQDs in the PCR reaction buffer. As expected, the intrinsic fluorescence of Taq was dramatically quenched with 20 ng μL^{-1} of GQDs (Figure 5a), confirming the interaction between GQDs and Taq. This result is similar to that GQDs quench the fluorescence of the primers as described in Figure 2.

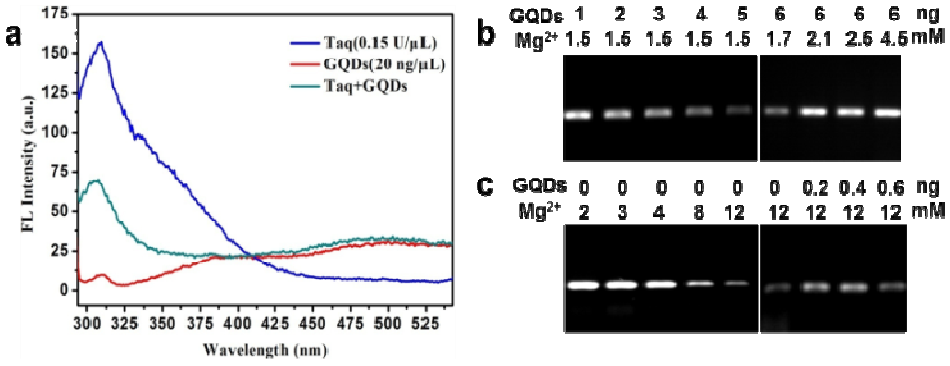


Figure 5. a) GQDs quench the intrinsic fluorescence of Taq (Ex: 280 nm). b) Excessive GQDs inhibited PCR (left part), and increase the concentration of Mg^{2+} can reverse the suppression to PCR induced by the GQDs (right part). c) High concentration of Mg^{2+} also inhibit PCR (left part),

and GQDs can reverse the suppression to PCR induced by the excessive Mg^{2+} (right part).The templates used in Figure 5b and 5c were PBR322 plasmid.

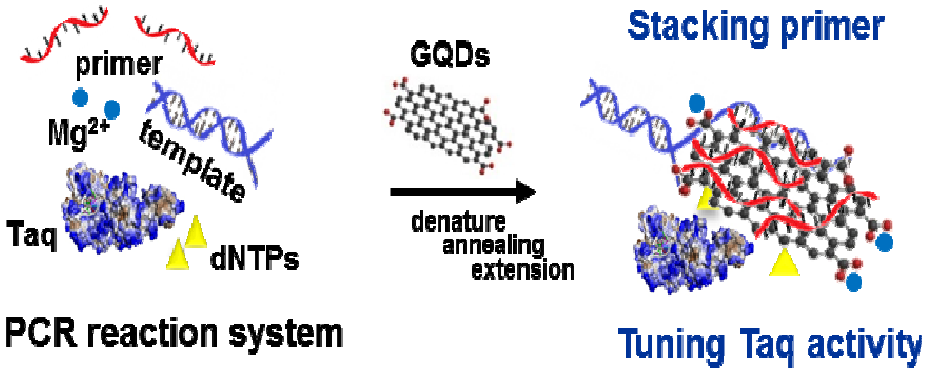
There are many hydrophobic and hydrophilic domains on the surface of Taq polymerase (Figure S2), thus weak interactions, such as electrostatic, hydrogen bonding, Van der Waals interaction, and hydrophobic interaction between GQDs and Taq should be expected. And the interaction of GO with proteins has been proved to be existed by AFM, and Zeta potential.[37] Although GQDs are smaller comparing to GO, they are still very large relative to the Taq enzyme, adsorption of Taq through weak interactions on the planar surface of GQDs should exist. Interaction of enzymes with solid substrates usually decreases their activities; therefore it is possible that PCR is inhibited by the high concentration of GQDs. If this is a major contribution to the effect of GQDs on PCR, then GQDs will inhibit PCR whenever their concentration is high or low. On the other hand, if adsorption of the Taq on the GQDs is a major contribution to the effect of GQDs on PCR, inclusion of other proteins or enzymes in PCR may affect the inhibition induced by GQDs. Figure S3 showed that BSA (which has a similar molecular weight and isoelectric point to Taq polymerase) reversed the inhibition caused by GQDs. However, the amount of BSA that needed for such reversion is hundred times more than that of Taq (0.15 μg of Taq *vs* 14 μg of BSA) under the same condition. Such big difference in the amount of BSA and Taq suggests that the adsorption of Taq to the GQDs is not a major contribution to its enhancement effect on PCR, and excessive GQDs will decrease the activity of Taq polymerase. However, adsorption of Taq on GQDs may get the reaction components in PCR system to be closer.

Interaction of the GQDs with Magnesium Ions. Generally, the activity of Taq can be modulated by the reaction condition, such as temperature and cofactor Mg^{2+}. Temperature is not very sensitive because Taq has high thermo stability. Hence, to further understand effect of GQDs on the activity

of Taq, interaction of GQDs with cofactor Mg^{2+} was investigated.[38] As shown in Figure 5b, when Mg^{2+} was 1.5 mM, 2 ng of GQDs started to inhibit PCR, and 7 ng of GQDs almost inhibit 80% of PCR; increasing Mg^{2+} from 1.5 to 4.5 mM, even with 6 ng of GQDs, PCR proceeds quite normal, indicating high concentration of Mg^{2+} could alleviate the suppression to PCR generated by the excessive GQDs. Importantly, such phenomenon could occurred in the other way around, GQDs reversed the inhibition generated by the excess of Mg^{2+} as shown in Figure 5c. 0.4 ng of GQDs relieved the inhibition generated by 12 mM of Mg^{2+}. The interaction between Mg^{2+} and GQDs must be occurred. There are peripheral carboxylic groups in GQDs,[11] they in principle can chelate Mg^{2+}. When the GQDs are present under normal PCR condition, Mg^{2+} ions will be chelated thus the activity of Taq decreased (Figure 5b, left half), but the activity lose of Taq can be rescued by adding more Mg^{2+} (Figure 5c, right half). On the other hand, when excessive Mg^{2+} are present, the activity of Taq was inhibited (Figure 5c, left half); increasing the concentration of GQDs could chelate excessive Mg^{2+} and recover the activity of Taq, which resembles to that high concentration of dNTPs requires high concentration of Mg^{2+} for success PCR. This result also is similar to the function of chelating reagent EDTA (Figure S4), where the presence of EDTA will decrease the yield of PCR or completely inhibit it. In order to confirm the chelation of GQDs to Mg^{2+}, GQDs were replaced with amine group modified GQDs (GQDs-NH_2). Figure S5 showed that the amount of GQDs-NH_2 needed for optimization of PCR is about 30 ng, while only 3 ng of GQDs necessary under the same condition, implying that peripheral carboxylic groups of GQDs are involved in Mg^{2+} chelation. The result was further supported by that the amount of Mg^{2+} that needed to reverse the inhibition induced by the GQDs-NH_2 (2.1 mM of Mg^{2+}) is much less than that of GQDs (4.5 mM of Mg^{2+}) under the same PCR condition. Together, these results reveal that the GQDs could tune the activity of Taq *via* interacting with Mg^{2+}. Importantly, this finding is in along with the

published work that the carboxyl modified CdTe could better enhance PCR, though the authors proposed that the carboxyl modified CdTe selectively bound to the ssDNA rather than dsDNA.[16] As matter of the fact, effect of Mg^{2+} in PCR with carbon nanotubes was reported, however in that case, author believed that the functional role of the carbon nanotubes is to replace Mg^{2+},[12] which we did not observe with GQDs.

To summary, the GQDs enhance the performance of PCR through several ways as shown in Scheme 1. Primers can selectively stack on the GQDs to enforce their pairing with the template,



Scheme 1. GQDs enhance the performance of PCR through stacking the primers to improve the base-pairing between the primers with template, tuning the activity of Taq polymerase, and adsorbing polymerase and other components to narrow their distance.

and eventually improve the selectivity and specificity of PCR. The interaction GQDs with polymerase itself may also increase the extension step of PCR because the proximity of the primers, template and enzyme. However, the adsorption of Taq polymerase on GQDs did not enhance its activity, on the contrary the activity of Taq polymerase was modified by GQDs through chelating

polymerase cofactor Mg^{2+} with their peripheral carboxylic groups, which ultimately affect the yield of the PCR.

Conclusions

We demonstrated that GQDs can improve overall performance of PCR by increasing yield, sensitivity, and specificity with a very low concentration. The mechanism of the optimization by GQDs was systematically investigated by inspecting the interaction of GQDs with major components of the PCR reaction system. We found that the primers and dsDNA selectively stack to GQDs, which prevents their self-folding and thus improves the sensitivity and specificity of PCR by enhancing efficiency of the base-pairing between the primers and template. The yield of PCR, on the other hand, was improved primarily by the ability of GQDs that can tune the activity of Taq polymerase by chelating the magnesium ions with their peripheral carboxylic groups. Interaction of GQDs with polymerase and dNTPs marginally affects the activity of polymerase, thus contributes less to the enhancement. GQDs may also enhance PCR by the increase of the proximity of the PCR reaction components. Combining with GQDs super biocompatibility, the understanding of the mechanism of their function in PCR made its possible to develop practical applicable GQDs formulation for PCR, especially to improve the performance of those unsatisfied PCR systems such as amplifying GC rich DNA fragments.

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Table of Contents

GQDs enhance PCR performance through stacking the primers selectively, tuning the activity of polymerase activity *via* chelating magnesium ions, and accelerating the PCR reaction by adsorbing polymerase and other components to increase their proximity.