

Supporting Information

Metal ion triggers for reversible switching of DNA polymerase

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Experimental Section

Materials

All DNA oligonucleotides used in this study were synthesized and purified by Bioneer® (Daejeon, Korea), except for the template DNA (purified by PAGE) and TaqMan probe labeled with FAM and BHQ1 (purified by HPLC). The oligonucleotide sequences are listed in Table S1. The metal salts, cysteine and SYBR green I were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure DNase/RNase-free distilled water was purchased from Bioneer® (Daejeon, Korea). *Thermus aquaticus* DNA polymerase (Taq DNA polymerase, 550 nM) was purchased from NEB (New England Biolabs). All other chemicals were of analytical grade and used without further purification.

General reaction conditions

The reaction mixtures were separately prepared as part A and part B. Part A (total volume of 20 μ L) composed of 1X Taq reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM MgCl₂), 500 μ M dNTPs, and 500 nM DNA aptamer was heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C/s) and incubated at 25 °C for 20 min. Metal ions (10 μ M) or cysteine (20 μ M) was added to this solution, followed by incubation at 25 °C for 20 min. Taq DNA polymerase (11 nM) was next added to the solution which was then incubated for 20 min. Part B (total volume of 20 μ L) composed of 1X Taq reaction buffer, 500 nM template, 500 nM primer, and 400 nM TaqMan probe was heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C/s) and incubated at 25 °C for 60 min. Parts A and B were mixed, and the fluorescence was measured on a C1000™ thermal cycler (Bio-Rad, CA, USA). The fluorescence signal from the TaqMan probe during the primer extension reaction was monitored every 2 min at 25 °C.

Reversible switching of DNA polymerase activity

The reaction mixtures were separately prepared as part A and part B. Part A (total volume of 20 μ L) composed of 1X Taq reaction buffer, 500 μ M dNTPs, and 500 nM DNA aptamer was heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C/s) and incubated at 25 °C for 20 min. Taq DNA polymerase (11 nM) was then added to each solution which was incubated for 20 min. Part B (total volume of 20 μ L) composed of 1X Taq reaction buffer, 1 μ M template, 1 μ M primer, and 800 nM TaqMan probe was heated at 90 °C for 5 min, cooled slowly to 25 °C

(0.1 °C/s) and incubated at 25 °C for 60 min. Parts A and B were mixed, and the fluorescence was measured on a C1000™ thermal cycler (Bio-Rad, CA, USA). Metal ions (5 μM) and cysteine (5 μM) were successively added every 30 min after mixing Parts A and B, and the fluorescence signal was measured every 2 min at 25 °C.

Polyacrylamide gel electrophoresis (PAGE)

Sample solutions were prepared as described in the “General reaction conditions” except that the TaqMan probe was excluded in Part B. The product solutions obtained after the reaction for 200 min were mixed with 6X loading buffer (Bioneer®, Daejeon, Korea) and subjected to electrophoresis analysis on a 15% precast polyacrylamide gel (Bio-Rad, CA, USA). The analysis was done in 1X TBE (89 mM Tris, 89 mM Borate, and 2 mM EDTA, pH 8.3) at 120 V for 170 min. After SYBR gold (Invitrogen, CA, USA) staining, gels were scanned with a UV transilluminator.

Confirmation of the metal ion-induced structural change of the DNA aptamer

A solution (total volume of 40 μL) containing 250 nM DNA aptamer in a 1X Taq reaction buffer was heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C/s) and incubated at 25 °C for 20 min. Metal ions (5 μM) or cysteine (10 μM) was added to this solution, followed by incubation at 25 °C for 20 min. The solution was next mixed with 2X SYBR green I and incubated for 30 min. The resulting fluorescence signal was measured on a C1000™ thermal cycler (Bio-Rad, CA, USA) at 25 °C.

Table S1 DNA sequences used in this work.

Strand name	DNA sequence (5'→ 3')
Template DNA	CAG AAA TCT CAG GGA CTC TAA AGC TCA ACT TGC ATA AAC TTC TGA GGA
Primer DNA	TCC TCA GAA GTT TAT GCA
TaqMan probe	FAM-TAG AGT CCC TGA GAT TTC TG-BHQ1
TQ21D aptamer ^(a)	GC GGT CGG CTC GGG GCA TTC TTA GCG TTT TGC CCC GAG CCG ACC GC
Control TQ21D aptamer ^(a)	GC GGT CGG CTC GGG GCA AAA AAA AAA AAA TGC CCC GAG CCG ACC GC
Hairpin-structured DNA of TQ30D aptamer	ACA TTG ATC TTC ATA TAG TGA CAA TGT ACA GTA TTG
Blocker DNA of TQ30D aptamer	TCA CTA TAT GAA GAG CAA
C1-blocker DNA of C1-TQ30D aptamer ^(b)	TCA CTA TAT C AA GAG CAA
C2-blocker DNA of C2-TQ30D aptamer ^(b)	TCA CTA TAT CA A C AG CAA
Homoadenine blocker DNA of control TQ30D aptamer	AAA AAA AAA AAA AAA AAA
FAM-labeled hairpin-structured DNA of FQ-C2-TQ30D aptamer	FAM-ACA TTG ATC TTC ATA TAG TGA CAA TGT ACA GTA TTG
BHQ1-labeled C2-blocker DNA of FQ-C2-TQ30D aptamer ^(b)	TCA CTA TAT CA A C AG CAA-BHQ1

^(a) The loop region of the TQ21D aptamer is depicted in green color.

^(b) # in C#-blocker DNA indicates the number of cytosine nucleobases that form the C-C mismatched base pairs with the hairpin-structured DNA of the TQ30D aptamer, which is depicted in red color.

Fig. S1 The effect of the TQ21D aptamer on fluorescence signal change ($F_{200}-F_0$). The solution containing DNA polymerase (5.5 nM) was treated with the TQ21D aptamer at varying concentrations.

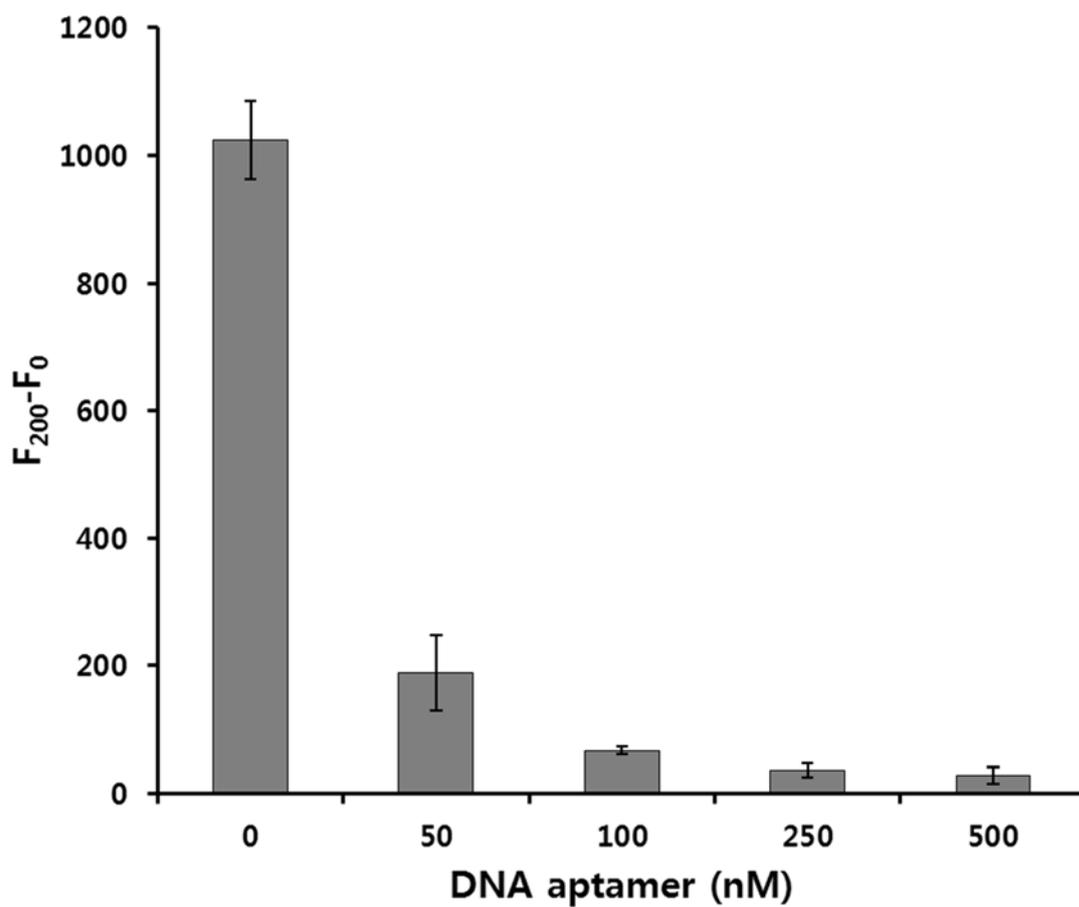


Fig. S2 The effect of Hg^{2+} ions on fluorescence signal change ($F_{200}-F_0$). The solution containing DNA polymerase (5.5 nM) and TQ21D aptamer (250 nM) was treated with Hg^{2+} ions at varying concentrations.

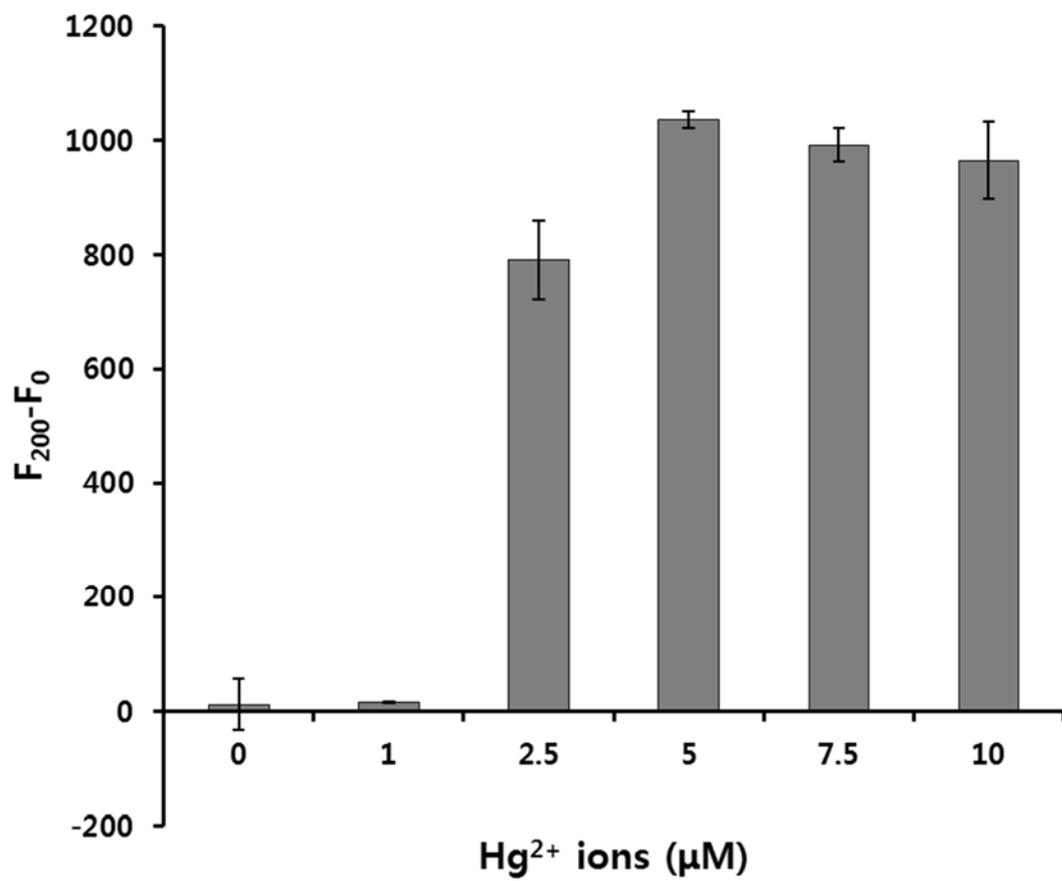


Fig. S3 The confirmation of the structural change of the TQ21D aptamer induced by Hg^{2+} ions. (a) Fluorescence intensities from SYBR green I incubated with free TQ21D aptamer (1) and TQ21D aptamer in the presence of cysteine (2), Hg^{2+} ions (3), and Hg^{2+} ions and cysteine (4). (b) Degree of fluorescence increase ($F_{\#}-F_1$), for which F_1 and $F_{\#}$ are the fluorescence intensities from the free TQ21D aptamer (1) and TQ21D aptamer in the presence of cysteine (2), Hg^{2+} ions (3), and Hg^{2+} ions and cysteine (4), respectively. The final concentrations of the DNA polymerase, TQ21D aptamer, cysteine and Hg^{2+} ions were 5.5 nM, 250 nM, 10 μM and 5 μM , respectively.

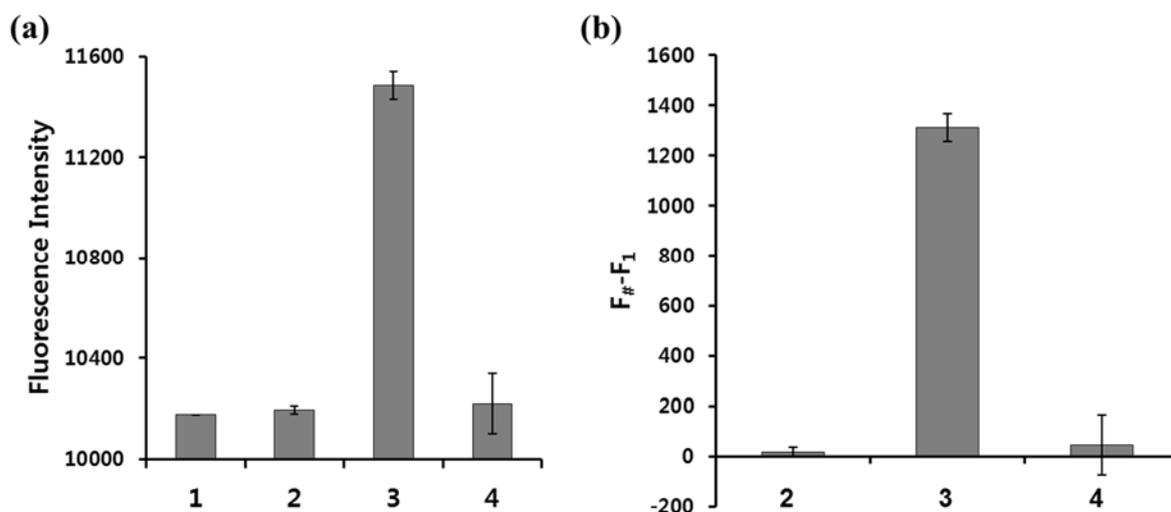
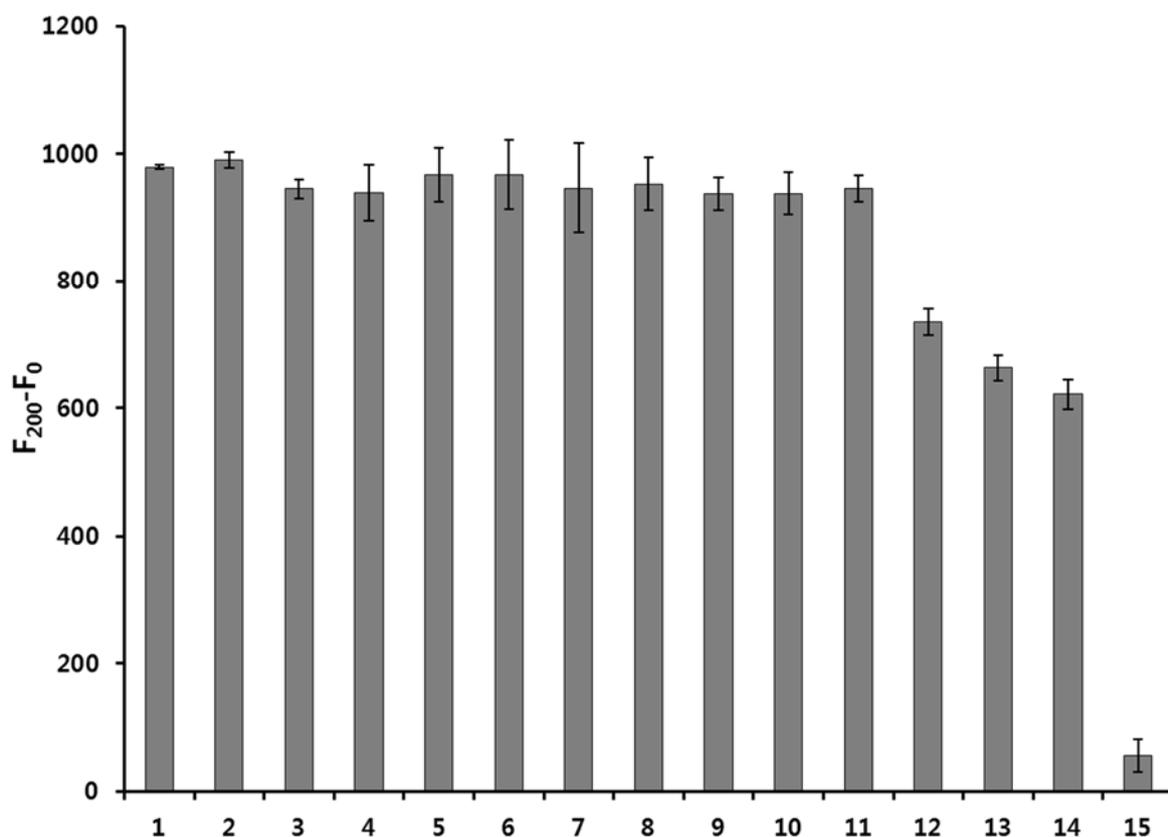


Fig. S4 The direct effect of metal ions on DNA polymerase activity. The solution containing DNA polymerase (1) was treated with different metal ions: Ag^+ (2), Hg^{2+} (3), Ca^{2+} (4), Fe^{3+} (5), Ni^{2+} (6), Li^+ (7), K^+ (8), Na^+ (9), Mg^{2+} (10), Fe^{2+} (11), Cu^{2+} (12), Cd^{2+} (13), Pb^{2+} (14) and Zn^{2+} (15). The final concentrations of the DNA polymerase and metal ions were 5.5 nM and 10 μM , respectively.



Scheme S1 Schematic illustration of C2-TQ30D aptamer-mediated reversible switching of DNA polymerase activity achieved by applying Ag^+ and cysteine triggers.

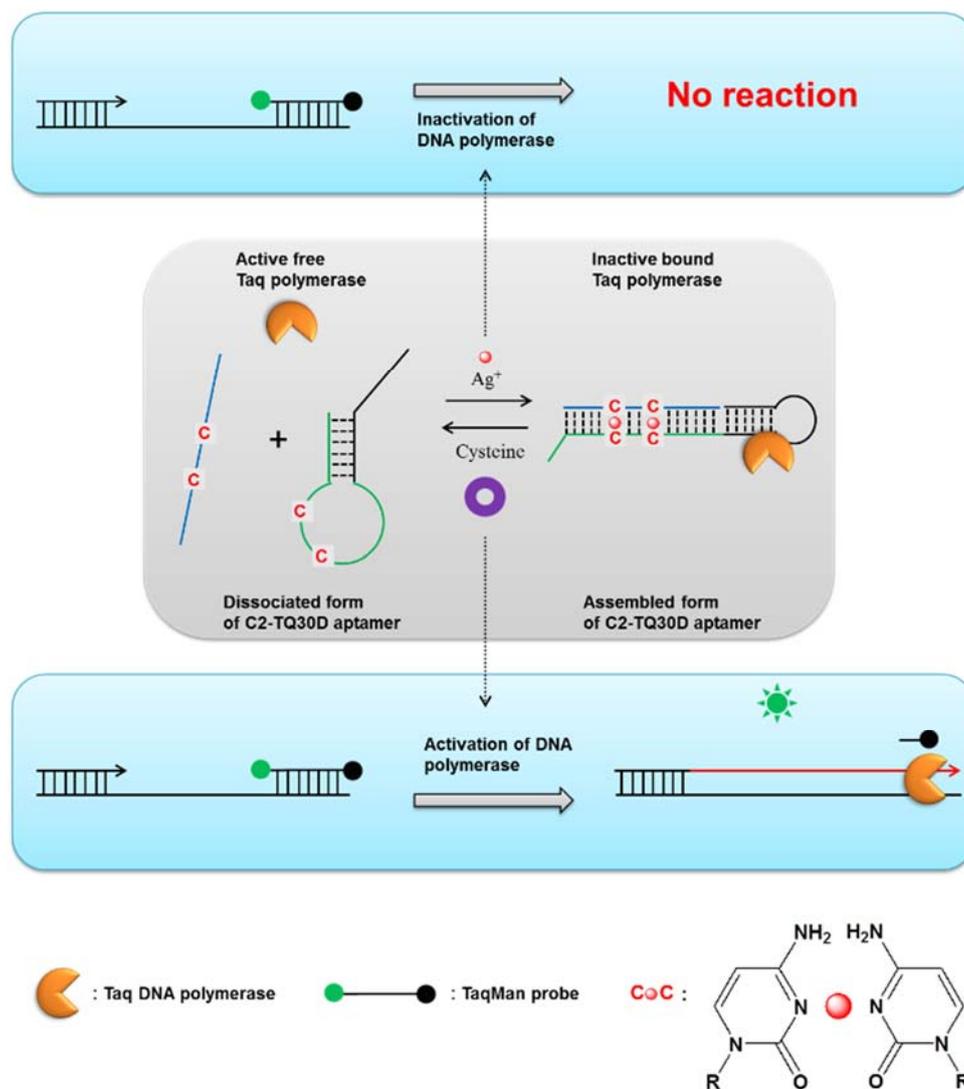


Fig. S5 The effect of C-C mismatched base pairing on the modified TQ30D aptamer-induced inhibition of DNA polymerase activity in the presence of Ag^+ ions. Negative control (NC) and positive control (PC) are solutions without and with DNA polymerase, respectively. The modified TQ30D aptamer (1-6) comprised of hairpin-structured DNA and blocker DNA was added to the solution containing DNA polymerase (PC). Different blocker DNAs such as blocker DNA (1 and 2), C1-blocker DNA designed to form one C-C mismatched base pair with the hairpin-structured DNA (3 and 4) and C2-blocker DNA designed to form two C-C mismatched base pairs with the hairpin-structured DNA (5 and 6) were used to determine the optimal blocker DNA which effectively suppresses DNA polymerase activity only in the presence of Ag^+ ions. Odd and even numbers represent the solutions in the absence and presence of Ag^+ ions, respectively. The final concentrations of the DNA polymerase, hairpin-structured DNA and blocker DNA of modified TQ30D aptamer, and Ag^+ ions were 5.5 nM, 250 nM and 300 nM, and 5 μM , respectively.

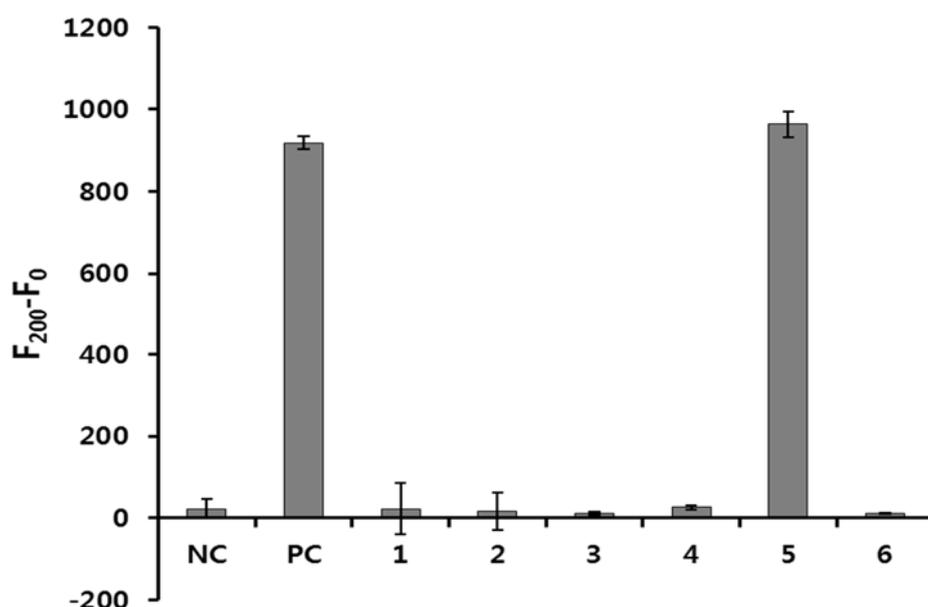
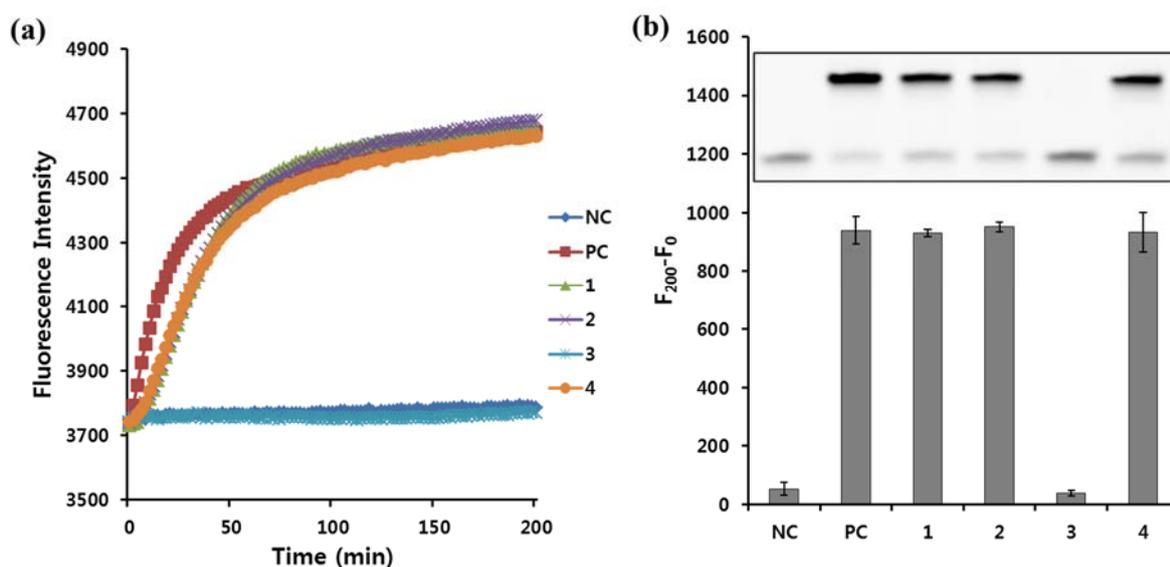
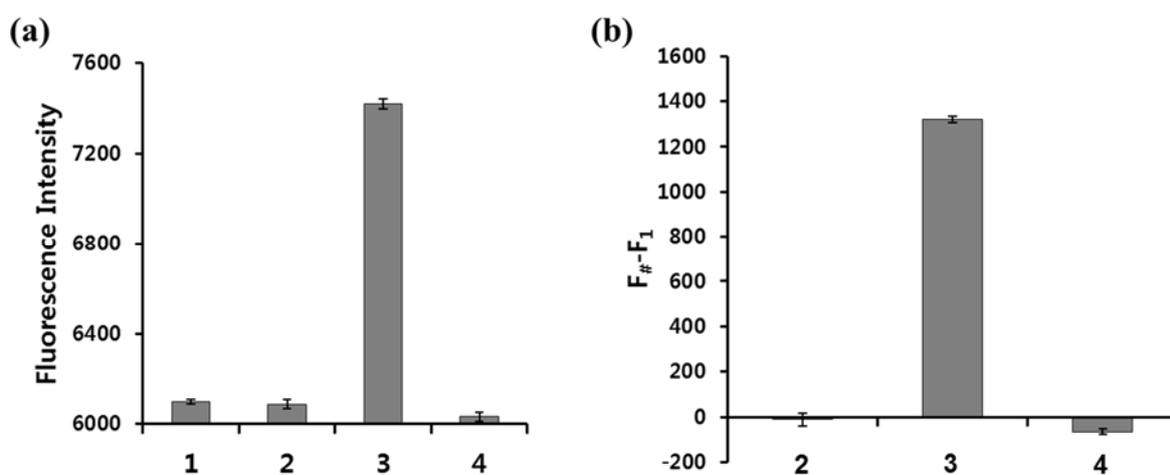


Fig. S6 The effects of the C2-TQ30D aptamer, Ag⁺ ions and cysteine on DNA polymerase activity. (a) Time-dependent fluorescence intensities from the TaqMan probe during a primer extension reaction. Negative control (NC) and positive control (PC) are solutions without and with DNA polymerase, respectively. Free C2-TQ30D aptamer (1) or C2-TQ30D aptamer together with cysteine (2), Ag⁺ ions (3), and Ag⁺ ions and cysteine (4) was added to the solution containing DNA polymerase (PC). (b) Fluorescence signal change ($F_{200}-F_0$). Inset in (b): polyacrylamide gel electrophoresis image. The final concentrations of the DNA polymerase, hairpin-structured DNA and C2-blocker DNA of C2-TQ30D aptamer, cysteine and Ag⁺ ions were 5.5 nM, 250 nM and 300 nM, 10 μ M and 5 μ M, respectively.



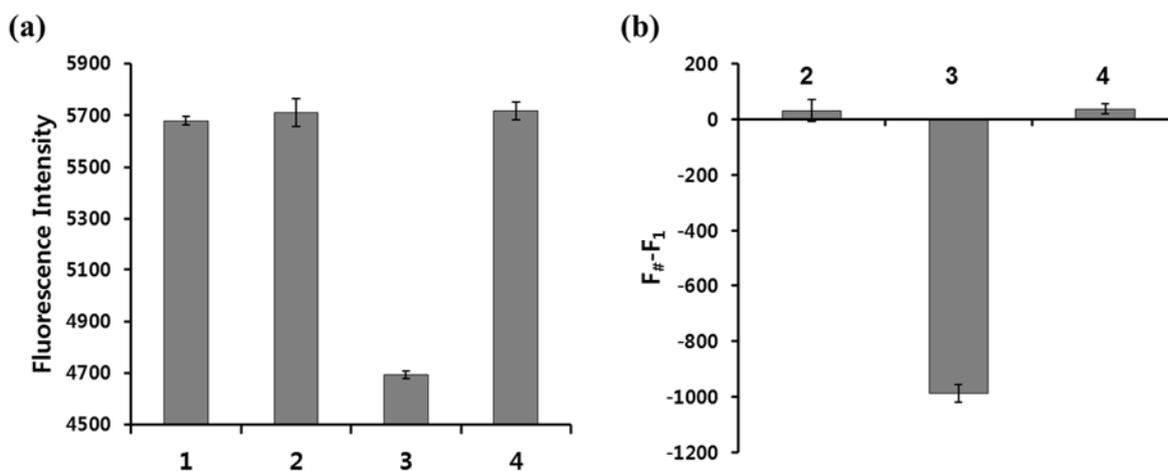
As shown in Fig. S6, free DNA polymerase resulted in a high fluorescence enhancement (PC, Fig. S6), which was not inhibited by the C2-TQ30D aptamer (1, Fig. S6). However, the application of Ag⁺ ions enabled the C2-TQ30D aptamer to effectively inhibit DNA polymerase, consequently resulting in quite low fluorescence enhancement (3, Fig. S6) comparable to that from the negative control without DNA polymerase (NC, Fig. S6). Most importantly, cysteine applied together with Ag⁺ ions completely reversed the Ag⁺-induced inhibition of DNA polymerase and produced the high fluorescence enhancement again (4, Fig. S6). The results also show that cysteine alone has no direct effect on the C2-TQ30D aptamer-regulated DNA polymerase activity (2, Fig. S6). These results were quite consistent with those from the electrophoretic analysis, in which gel bands corresponding to extension products were obtained only from the four cases with the non-inhibited DNA polymerase (PC, 1, 2, and 4, Inset of Fig. S6 (b)).

Fig. S7 The confirmation of Ag^+ ion-induced structural change of C2-TQ30D aptamer. (a) Fluorescence intensities from SYBR green I incubated with free C2-TQ30D aptamer composed of hairpin-structured DNA and C2-blocker DNA (1) and C2-TQ30D aptamer in the presence of cysteine (2), Ag^+ ions (3), and Ag^+ ions and cysteine (4). (b) Degree of fluorescence increase ($F_{\#}-F_1$), for which F_1 and $F_{\#}$ are the fluorescence intensities from the free C2-TQ30D aptamer (1) and C2-TQ30D aptamer in the presence of cysteine (2), Ag^+ ions (3), and Ag^+ ions and cysteine (4), respectively. The final concentrations of the DNA polymerase, hairpin-structured DNA and C2-blocker DNA of C2-TQ30D aptamer, cysteine and Ag^+ ions were 5.5 nM, 250 nM and 300 nM, 10 μM and 5 μM , respectively.



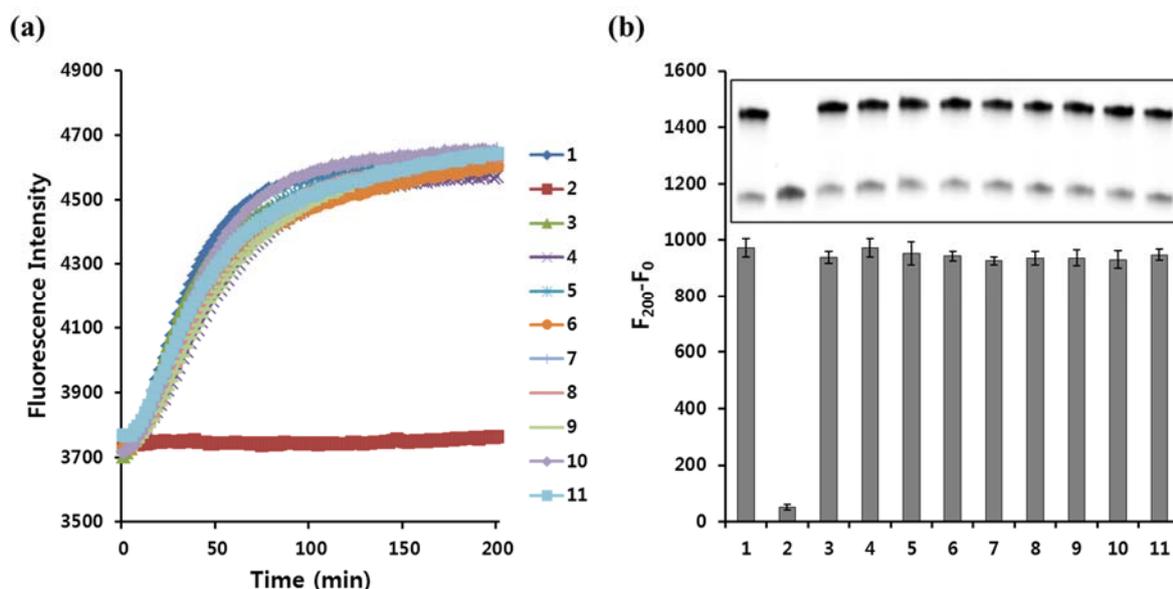
As shown in Fig. S7, the fluorescence signal from SYBR green I was enhanced only in the presence of the Ag^+ ions, which indicates that the hairpin-structured DNA hybridized with the mismatched blocker DNA through the formation of C- Ag^+ -C.¹

Fig. S8 The confirmation of the Ag^+ ion-induced structural change of the C2-TQ30D aptamer. (a) Fluorescence intensities of free FQ-C2-TQ30D aptamer comprised of FAM-labeled hairpin-structured DNA and BHQ1-labeled C2-blocker DNA (1) and FQ-C2-TQ30D aptamer in the presence of cysteine (2), Ag^+ ions (3), and Ag^+ ions and cysteine (4). (b) Degree of fluorescence decrease ($F_{\#}-F_1$), for which F_1 and $F_{\#}$ are the fluorescence intensities from the free FQ-C2-TQ30D aptamer (1) and FQ-C2-TQ30D aptamer in the presence of cysteine (2), Ag^+ ions (3), and Ag^+ ions and cysteine (4), respectively. The final concentrations of the DNA polymerase, FAM-labeled hairpin-structured DNA and BHQ1-labeled C2-blocker DNA of FQ-C2-TQ30D aptamer, cysteine and Ag^+ ions were 5.5 nM, 250 nM and 300 nM, 10 μM and 5 μM , respectively.



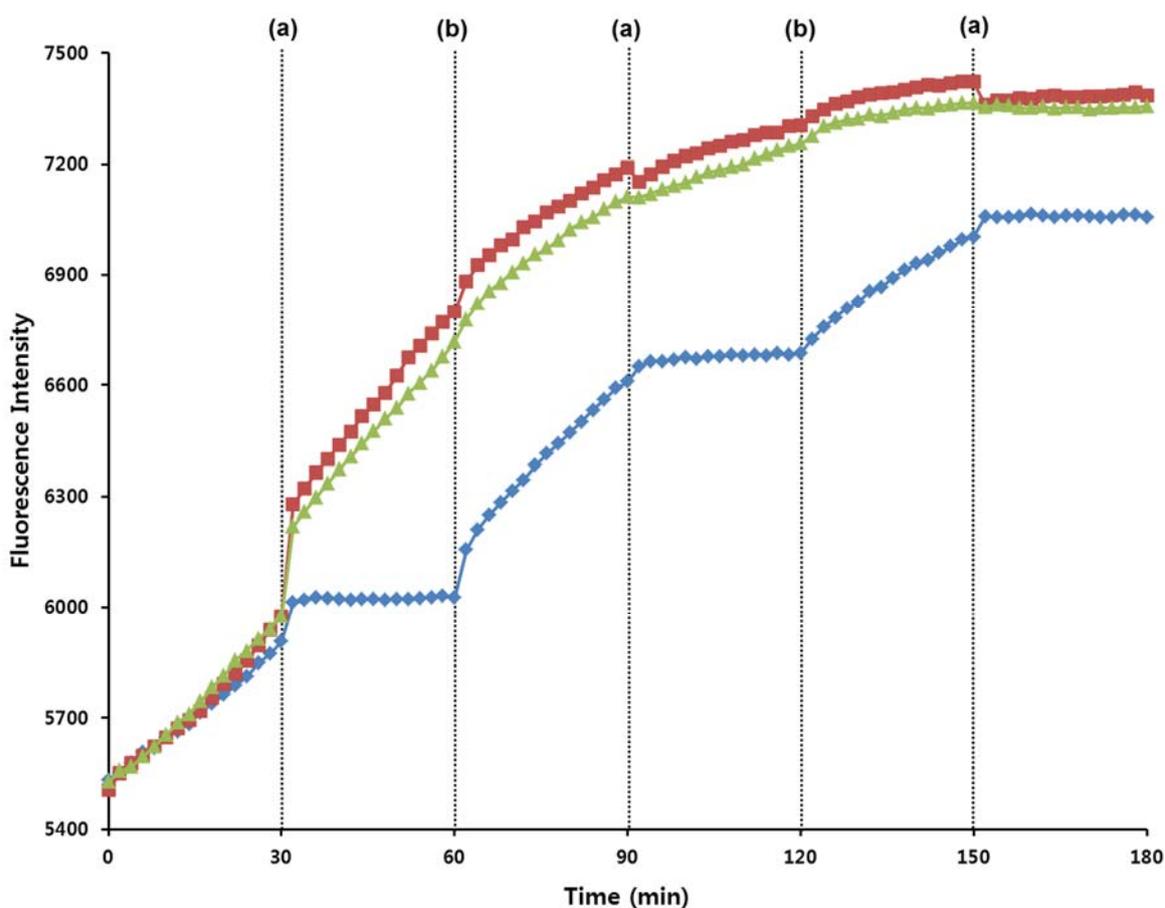
As shown in Fig. S8, the fluorescence signal from FAM within the hairpin-structured DNA was greatly reduced only in the presence of the Ag^+ ions as a result of the fluorescence resonance energy transfer between FAM and BHQ1, which indicates that the hairpin-structured DNA and blocker DNA are hybridized through the formation of C- Ag^+ -C.²

Fig. S9 Selectivity of the metal ions to induce the C2-TQ30D aptamer-mediated inhibition of DNA polymerase. (a) Time-dependent fluorescence intensities from the TaqMan probe during a primer extension reaction. The solution containing DNA polymerase and C2-TQ30D aptamer (1) was treated with different metal ions: Ag^+ (2), Hg^{2+} (3), Ca^{2+} (4), Fe^{3+} (5), Ni^{2+} (6), Li^+ (7), K^+ (8), Na^+ (9), Mg^{2+} (10) and Fe^{2+} (11). (b) Fluorescence signal change ($F_{200}-F_0$). Inset in (b): polyacrylamide gel electrophoresis image. The final concentrations of the DNA polymerase, hairpin-structured DNA and C2-blocker DNA of C2-TQ30D aptamer, and Ag^+ ions were 5.5 nM, 250 nM and 300 nM, and 5 μM , respectively.



As shown in Fig. S9, only the Ag^+ ions induce the inhibition of DNA polymerase, evidenced by the low fluorescence enhancement and no extension products. On the other hand, high fluorescence enhancement was observed when other metal ions were used, indicating the exceptionally high specificity of Ag^+ ions to activate the C2-TQ30D aptamer to inhibit DNA polymerase.

Fig. S10 Time-dependent fluorescence intensities from the TaqMan probe obtained during C2-TQ30D aptamer-mediated reversible switching cycles of DNA polymerase activity. (Blue curve) The system containing DNA polymerase (5.5 nM) and C2-TQ30D aptamer comprised of hairpin-structured DNA (250 nM) and C2-blocker DNA (300 nM) was successively supplied with Ag^+ ions (5 μM) at point (a) and cysteine (5 μM) at point (b); (Red curve) The system containing DNA polymerase (5.5 nM) and C2-TQ30D aptamer was treated with Hg^{2+} ions at point (a) instead of Ag^+ ions and cysteine (5 μM) at point (b); (Green line) The system containing the control TQ30D aptamer (Table S1) was supplied with Ag^+ ions (5 μM) at point (a) and cysteine (5 μM) at point (b), in which the control TQ30D aptamer consisted of hairpin-structured DNA (250 nM) and homoadenine blocker DNA (300 nM).



As shown in Fig. S10, DNA polymerase produced high fluorescence enhancement in the absence of Ag^+ ions during the first 30 min time period (Blue curve, Fig. S10). The application of Ag^+ ions at point (a) induced the inhibition of DNA polymerase by forming the assembled structure of the C2-TQ30D aptamer and switched off the DNA polymerase activity,

evidenced by no fluorescence enhancement during the second 30 min time period. Remarkably, the application of cysteine at point (b) switched on the DNA polymerase activity by extracting the Ag^+ ions from the C2-TQ30D aptamer and separating the C2-TQ30D aptamer into two DNAs. This reversible on-off switching of DNA polymerase activity was repeatedly achieved by successively applying Ag^+ ions and cysteine (Blue curve, Fig. S10).

Control experiments indicate that the addition of Hg^{2+} ions in place of Ag^+ ions did not have any effect on the C2-TQ30D aptamer-mediated inhibition of DNA polymerase, and thus, DNA polymerase remained active to promote multiple extension reactions (Red curve, Fig. S10). When we used the control TQ30D DNA aptamer comprised of hairpin-structured DNA and homoadenine blocker DNA which does not form C- Ag^+ -C base pairs, DNA polymerase activity was not influenced at all by treatment with either Ag^+ ions or cysteine (Green curve, Fig. S10). These results clearly show that the specific interaction of Ag^+ /cysteine with the C2-TQ30D aptamer is exclusively responsible for the reversible switching of DNA polymerase activity.

REFERENCES

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2. K. S. Park, M. W. Seo, C. Jung, J. Y. Lee and H. G. Park, *Small*, 2012, **8**, 2203-2212.