Electronic Supplementary Material (ESI) for Chemical Communications

5-methylcytosine enhance the substrate activity of DNA polymerase

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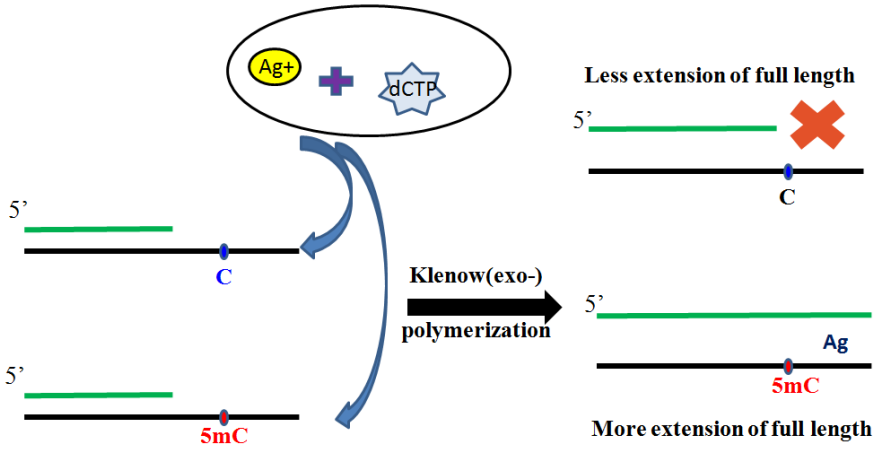

Here, we first demonstrated that 5-MedCTP could be incorporated into diverse synthetic DNA templates by the exonuclease deficient Klenow Fragment with a much higher efficiency than dCTP and 5-Hydroxymethyl-dCTP. And the efficiency gap of incorporation could be increased in presence of different amounts of Ag^{I}. Further, we first conducted a comparable study of primer extension reaction using templates containing deoxycytidine (dC) or 5-methyldeoxycytidine (5-mdC) for incorporating different triphosphates of dCTP, 5-MedCTP and 5-Hydroxymethyl-dCTP. Based on our finding, 5-methyldeoxycytidine could enhance the substrate activity of Klenow Fragment (exo-) and this feature could be potentially used in DNA methylation analysis.

Supporting Information

5-methylcytosine enhance the substrate activity of DNA polymerase

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General information: The oligonucleotides and dCTPs were purchased from Invitrogen Technology(Shanghai, China). Klenow Fragment(exo-), Klenow Fragment, T4 DNA polymerase, Taq DNA polymerase and 5-MedCTPs were purchased from New England Biolabs, Inc. 5-Hydroxymethyl-dCTPs were purchased from Bioline, Inc. Tris base, $MgCl_2$, KCl, NaCl, formamide(deionized) and dithiothreitol(DTT) were purchased from Sigma Inc. PAGE analyses were performed with denaturing 20%(19:1) polyacrylamide gel in presence of 8 M urea.

DNA polymerization by polymerase: The reaction mixtures (20 μL) containing 100 nM primer, 150 nM template, 20 μM dNTPs, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of polymerase and 1 mM dithiothreitol(DTT), in the presence or absence of various concentrations of metal ions were incubated at 37 ^{o}C for 1 h. The reactions were quenched by adding 100 μL 80% aqueous formamide(deionized), and the solutions were immediately heated at 90 ^{o}C for 10 min. After cooling down to 4 ^{o}C, the solutions were analyzed by denaturing 20% PAGE. FAM labeled 15-primer, 19-mer and 24-mer were used as the markers in gel analysis.

Kinetic studies of 5-MedCTP or dCTP incorporation by Klenow Fragment (exo-). The reaction mixtures (20 μL) containing 500 nM primer, 1.0 μM template, 20 μM dATP, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 1.0 unit of Klenow Fragment (exo-) and 1 mM dithiothreitol(DTT), in the presence of various concentrations of 5-MedCTP or dCTP were incubated at 37 ^{o}C for 6 mins. The reactions were quenched by adding 100 μL 80% aqueous formamide(deionized), and the solutions were immediately heated at 90 ^{o}C for 10 min. After cooling down to 4 ^{o}C, the solutions were analyzed by denaturing 20% PAGE. FAM labeled 15-primer, 19-mer and 24-mer were used as the markers in gel analysis. Kinetic data were derived as described in known literature[1].

Table S1 Sequences of oligomers used for polymerization.

Oligomer	Sequence(from 5'to 3')
Template	GGTGCGTGGCTATAGTGAGTCGTA
Template Me	GGTG（MeC）GTGGCTATAGTGAGTCGTA
FAM-15mer	5'-FAM-TACGACTCACTATAG
FAM-19mer	5'-FAM-TACGACTCACTATAGCCACA
FAM-24mer	5'-FAM-TACGACTCACTATAGCCACACACC

Table S2 Kinetic parameters of 5-MedCTP or dCTP incorporation by Klenow Fragment (exo-).

Incorporation	K_M (μM)	kcat (min^{-1})	k_{cat}/K_M (min^{-1} μM^{-1})
5-MedCTP	0.95	3.9	4.10
dCTP	8.9	2.7	0.30

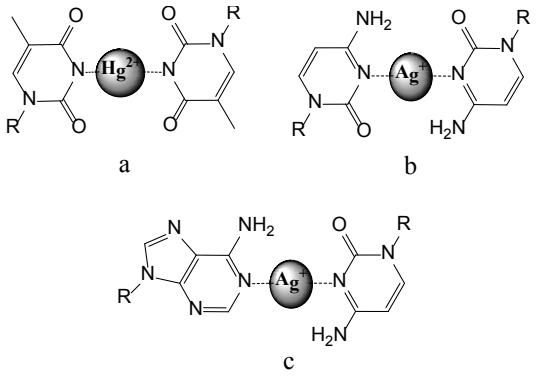

Figure S1. Illustration of different metal ion-base complex. a, C-Ag^{+}-C; b, T-Hg^{2+}-T, c, A-Ag^{+}-C.

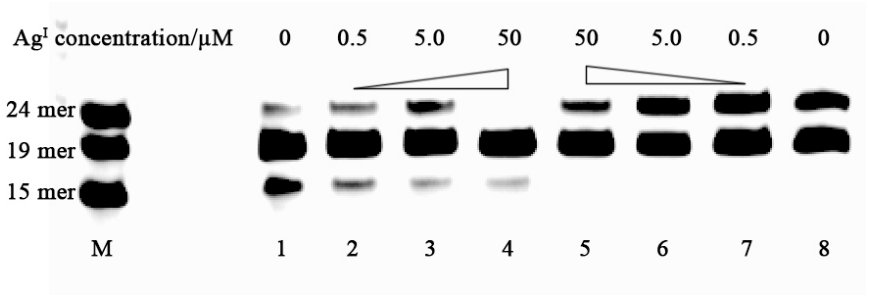

Figure S2. The polymerization reaction by the Klenow Fragment(exo-) in the absence or presence of Ag^{I} ions. The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of Klenow Fragment(exo-), 1 mM dithiothreitol(DTT) and 20 μM dNTPs (dATP + dCTP or dATP + 5-MedCTP), in the presence or absence of different amounts of $AgNO_3$. M: mixtures of FAM-15mer, FAM-19mer and FAM-24mer; 1-4: 20 μM dCTP together with dATP was used; 5-8: 20 μM 5-MedCTP together with dATP was used.

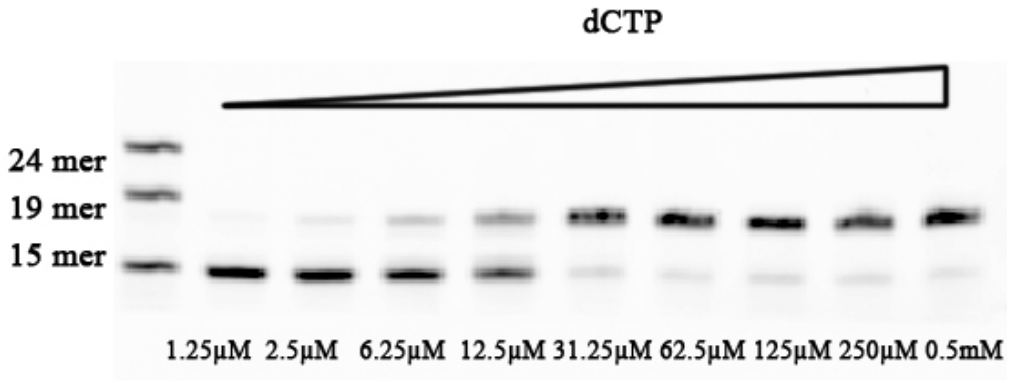

Figure S3. The reaction mixtures (20 μL) containing 500 nM primer, 1.0 μM template, 20 μM dATP, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 1.0 unit of Klenow Fragment (exo-) and 1 mM dithiothreitol(DTT), in the presence of various concentrations of dCTP were incubated at 37 ^oC for 6 mins. The reactions were quenched by adding 100 μL 80% aqueous formamide(deionized), and the solutions were immediately heated at 90 ^oC for 10 min. After cooling down to 4 ^oC, the solutions were analyzed by denaturing 20% PAGE. FAM labeled 15-primer, 19-mer and 24-mer were used as the markers in gel analysis.

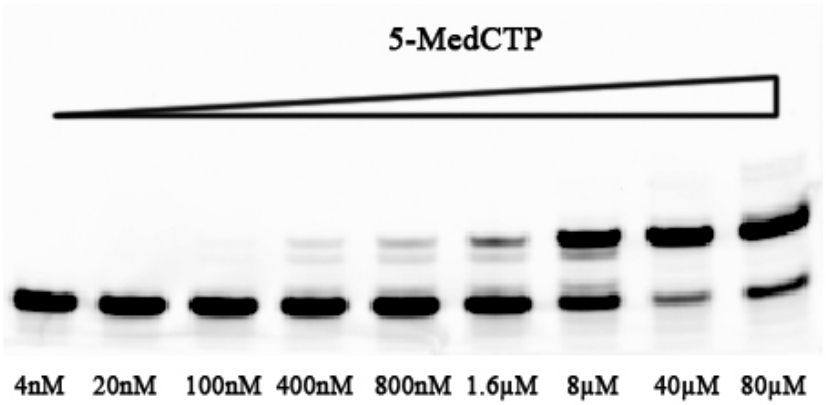

Figure S4. The reaction mixtures (20 μL) containing 500 nM primer, 1.0 μM template, 20 μM dATP, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 1.0 unit of Klenow Fragment (exo-) and 1 mM dithiothreitol(DTT), in the presence of various concentrations of 5-MedCTP were incubated at 37 ^oC for 6 mins. The reactions were quenched by adding 100 μL 80% aqueous formamide(deionized), and the solutions were immediately heated at 90 ^oC for 10 min. After cooling down to 4 ^oC, the solutions were analyzed by denaturing 20% PAGE. FAM labeled 15-primer, 19-mer and 24-mer were used as the markers in gel analysis.

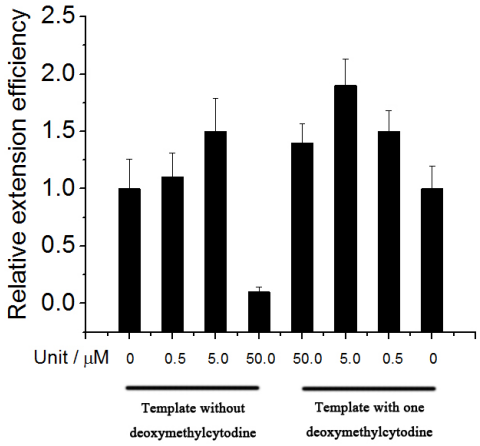

Figure S5. Effects of Ag^{I} ions on the polymerization reaction by the Klenow fragment(exo-). The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of Klenow Fragment (exo-), 1 mM dithiothreitol (DTT) and 20 μM dNTPs, in the presence or absence of different amounts of $AgNO_3$. And dATP + dCTP was used in the assay. Bars showed the relative amounts of the full-length product of Ag^{I} mediated reactions over the stopped product of the reactions compared with control reaction without Ag^{I} ions. Values are averages and the standard deviation determined by three independent experiments.

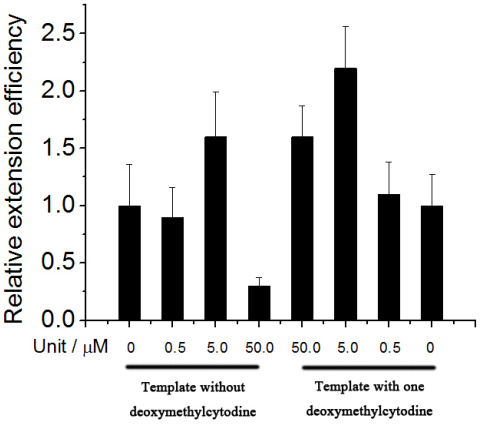

Figure S6. Effects of Ag^{I} ions on the polymerization reaction by the Klenow fragment(exo-). The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of Klenow Fragment (exo-), 1 mM dithiothreitol (DTT) and 20 μM dNTPs, in the presence or absence of different amounts of $AgNO_3$. And dATP + 5-Hydroxymethyl-dCTP was used in the assay. Bars showed the relative amounts of the full-length product of Ag^{I} mediated reactions over the stopped product of the reactions compared with control reaction without Ag^{I} ions. Values are averages and the standard deviation determined by three independent experiments.

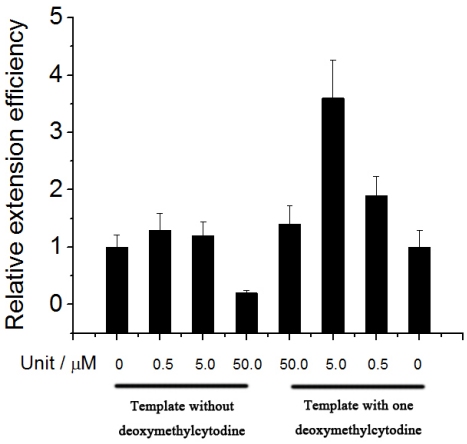

Figure S7. Effects of Ag^I ions on the polymerization reaction by the Klenow fragment(exo-). The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of Klenow Fragment (exo-), 1 mM dithiothreitol (DTT) and 20 μM dNTPs, in the presence or absence of different amounts of $AgNO_3$. And dATP + 5-MedCTP was used in the assay. Bars showed the relative amounts of the full-length product of Ag^I mediated reactions over the stopped product of the reactions compared with control reaction without Ag^I ions. Values are averages and the standard deviation determined by three independent experiments.

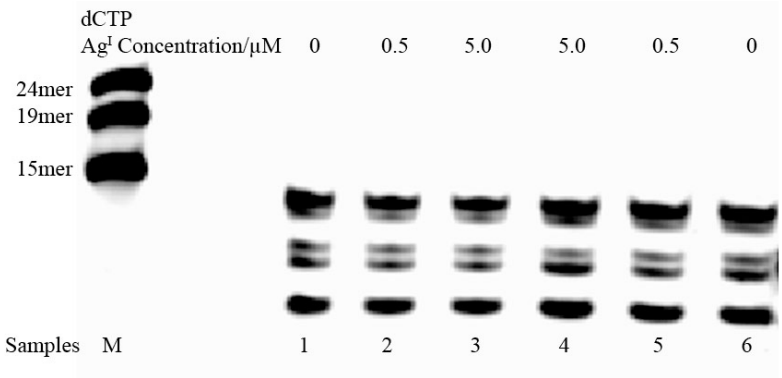

Figure S8. Effects of Ag^I ion concentrations on the polymerization reaction by the Klenow Fragment with exonuclease activity. The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of Klenow Fragment, 1 mM dithiothreitol(DTT) and 20 μM dNTPs (dATP + dCTP), in the presence or absence of different amounts of $AgNO_3$. M: mixtures of FAM-15mer, FAM-19mer and FAM-24mer; 1-3: template without 5-methyldeoxycytodine was used; 4-6: template with one 5-methyldeoxycytodine was used.

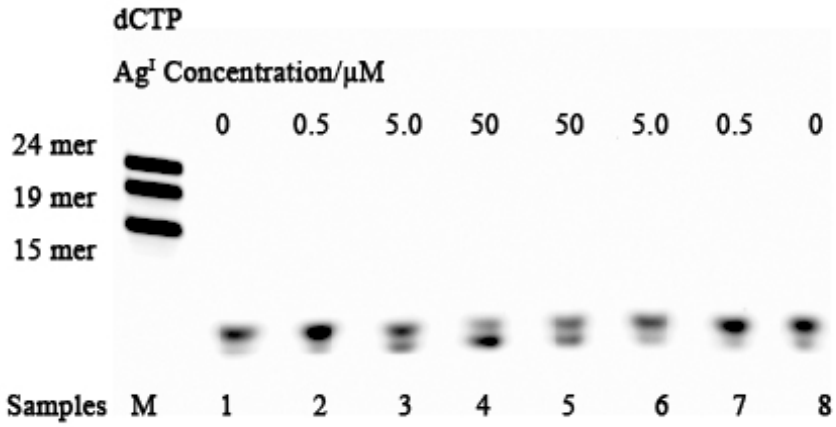

Figure S9. Effects of Ag^{I} ion concentrations on the polymerization reaction by T4 DNA polymerase. The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol, 0.6 units of T4 DNA polymerase, 1 mM dithiothreitol(DTT) and 20 μM dNTPs (dATP + dCTP), in the presence or absence of different amounts of $AgNO_3$. M: mixtures of FAM-15mer, FAM-19mer and FAM-24mer; 1-4: template without 5-methyldeoxycytodine was used; 5-8: template with one 5-methyldeoxycytodine was used.

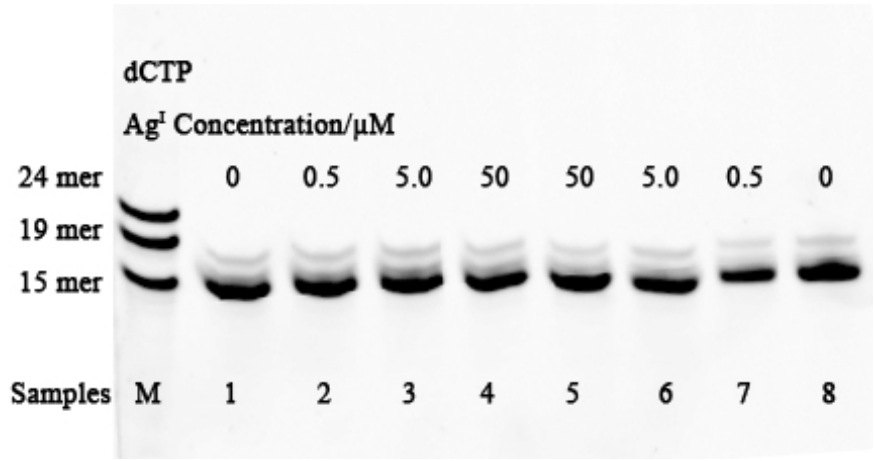

Figure S10. Effects of Ag^{I} ion concentrations on the polymerization reaction by Taq DNA polymerase. The reaction was performed in a buffer consisting of 100 nM primer, 150 nM template, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 4 mM $MgCl_2$, 0.08% (v/v) Nonidet P40, 0.6 units of Taq DNA polymerase, 1 mM dithiothreitol(DTT) and 20 μM dNTPs (dATP + dCTP), in the presence or absence of different amounts of $AgNO_3$. M: mixtures of FAM-15mer, FAM-19mer and FAM-24mer; 1-4: template without 5-methyldeoxycytodine was used; 5-8: template with one 5-methyldeoxycytodine was used.

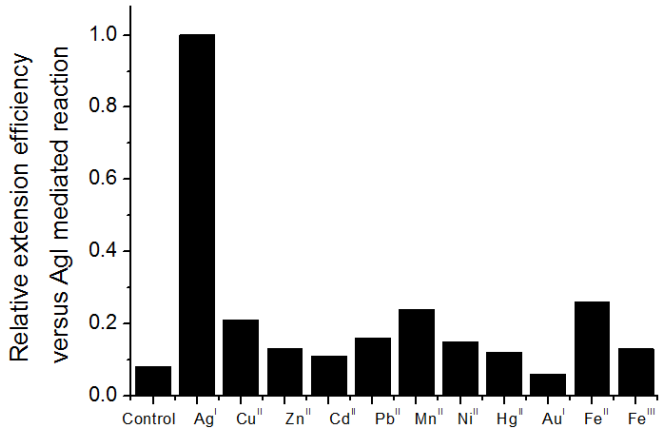

Figure S11. Effects of different metal ions on the polymerization reaction by the Klenow fragment(exo-). Bars showed the relative amounts of the full-length product over the stopped product of the reactions compared with Ag^{I}.

References:

[1] S. Creighton, L. B. Bloom and M. F. Goodman, *Methods Enzymol* **1995**, *262*, 232-256.