We have characterized the role of Watson-Crick hydrogen bonding in the 3′-terminal base pair on the 3′-5′ exonuclease activity of the human mitochondrial DNA polymerase. Nonpolar nucleoside analogs of thymidine (dF) and deoxyadenosine (dQ) were used to eliminate hydrogen bonds while maintaining base pair size and shape. Exonuclease reactions were examined using pre-steady state kinetic methods. The time dependence of removal of natural nucleotides from the primer terminus paired opposite the nonpolar analogs dF and dQ were best fit to a double exponential function. The double exponential kinetics as well as the rates of excision (3–6 s⁻¹ fast phase, 0.16–0.3 s⁻¹ slow phase) are comparable with those observed during mismatch removal of natural nucleotides even when the analog was involved in a sterically correct base pair. Additionally, incorporation of the next correct base beyond a nonpolar analog was slow (0.04–0.22 s⁻¹), so that more than 95% of terminal base pairs were removed rather than extended. The polymerase responds to all 3′-terminal base pairs containing a nonpolar analog as if it were a mismatch regardless of the identity of the paired base, and kinetic partitioning between polymerase and exonuclease sites failed to discriminate between correct and incorrect base pairs. Thus, steric alone are insufficient, whereas hydrogen bond formation is essential for proper proofreading selectivity by the mitochondrial polymerase. The enzyme may use the alignment and prevention of fraying provided by proper hydrogen bonding and minor groove hydrogen bonding interactions as critical criteria for correct base pair recognition.

Accurate replication by a DNA polymerase relies upon discrimination against incorrect nucleotides during polymerization, and the overall fidelity can be improved by the selective removal of mismatches by the 3′-5′ exonuclease activity. As shown in Fig. 1, during each round of processive synthesis the polymerase can bind and incorporate the next nucleotide, or the 3′-terminal base can be removed by the proofreading exonuclease. Earlier studies employing the T7 DNA polymerase (1–3) and subsequent work on the human mitochondrial DNA polymerase (4) showed that the kinetic partitioning between polymerase and exonuclease sites governs the selectivity of the proofreading exonuclease. During successive rounds of correct base pair incorporation, polymerization is fast relative to the slow migration of the DNA to the exonuclease site, and so, excision occurs infrequently, and the cost of proofreading is low. However, after incorporation of a mismatch, forward polymerization stalls, thereby inverting the kinetic partitioning to favor excision rather than extension of the DNA. Moreover, enzyme trapping experiments established that the DNA can migrate from the polymerase site to the exonuclease site and back again without dissociating from the enzyme.

These studies showed that the decision to continue incorporation or remove a nucleotide is made at the polymerase site by recognition of a proper base pair after incorporation. The enzyme somehow recognizes a mismatch at the primer terminus and markedly reduces the rate of incorporation of the next nucleotide. However, little is known about how the polymerase recognizes a mismatch at the 3′-terminus and how the rate of polymerization is inhibited. Because proofreading is an important contributor to overall fidelity of DNA replication, it is of interest to determine the physical origins of this enzymatic selectivity. In principle, the recognition of a mismatch could be due to loss of base pair hydrogen bonding and lower free energy of formation of the terminal base pair, or it could be a function of selection for proper base pair geometry. To evaluate the relative contributions of these effects in the human mitochondrial polymerase, we have employed nonpolar nucleoside analogs in the terminal base pair of primer-template duplexes.

We have used the nonpolar analogs shown in Fig. 2. The analog difluorotoluene deoxyriboside (dF) is a non-hydrogen bonding isostere of thymidine (5), whereas 9-methyl-1-H-imidazo[(4,5)-b]pyridine deoxyribose (dQ) is an isostere of deoxyadenosine that also lacks Watson-Crick hydrogen bonding ability (6). Thus, the analogs can serve as useful probes of the relative roles of hydrogen bonding and shape in governing the selectivity of proofreading by examining their effects on rates of excision and extension.

We have employed the human mitochondrial DNA polymerase (Pol γ), a high fidelity A family DNA polymerase, to assay...
Hydrogen Bonds in Proofreading by Pol γ

In the accompanying paper we reported on the influence of wild type and exonuclease-deficient polymerase were performed as reported previously (8–11). Purification and labeling of oligonucleotides, polymerase assay conditions, product analysis, and data fitting have been documented in the accompanying paper (8).

Reagents—Unmodified oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Nucleoside triphosphates were purchased from Sigma-Aldrich. [γ-32P]ATP was purchased from PerkinElmer Life Sciences. The nucleoside analogs dF and dQ were prepared as described (5, 6). The nucleoside triphosphate analog difluorotoluene deoxynucleoside triphosphate (dFTP) was prepared as described by Moran et al. (12). The 45-mer oligonucleotides containing dF and dQ were also prepared as described (13, 14). All other reagents were purchased from Fisher unless otherwise noted.

Formation of a dFMP-terminated Primer—To assay the kinetics of incorporation after a 3’ dFMP:dA pair (where dA was the templating base, a dFMP-terminated primer was used. During the final reaction, concentrations were noted.

Exonuclease Assay Conditions—The final reaction conditions for the exonuclease experiments were the same as for the polymerase experiments described previously (8), except that the dNTP was omitted so the exonuclease reaction could be observed. The holoenzyme-DNA complex was assembled in a buffer free of MgCl₂. Oligonucleotides used in this study were shown in Table 1. This solution was mixed (1:1) with a combined solution was rapidly mixed (1:1) with a solution containing 25 mM MgCl₂ and varying concentrations of dCTP to assay the nucleotide concentration dependence of extension on top of dFMP. During the final reaction, concentrations were as follows: 100 nM catalytic subunit, 500 nM accessory subunit, 90 nM 28pF/45A, 25 mM MgCl₂, 50 μM dFTP, and 10–500 μM dCTP.

The 3’-5’ exonuclease activity of the polymerase can enhance the overall fidelity of the enzyme by as much as 200-fold (4). In this study, pre-steady state kinetic methods have been employed to assay the effect of nonpolar analogs on the selectivity of the exonuclease activity for removing correct and incorrect base pairs. We have also assayed the kinetics of polymerization of the next correct nucleotide beyond each base pair to assess the kinetic partitioning between extension versus excision.

In the accompanying paper we reported on the influence of hydrogen bonding during nucleotide incorporation by Pol γ (8). Here we show that Watson-Crick hydrogen bonding plays a central role in exonuclease proofreading and in subsequent rounds of polymerization, as seen by the lack of selectivity exhibited by Pol γ in removing and/or burying correct and incorrect 3’ terminal bases when hydrogen bonding groups are absent.

MATERIALS AND METHODS

Expression, purification, reconstitution, and characterization of wild type and exonuclease-deficient polymerase were performed as reported previously (8–11). Purification and labeling of oligonucleotides, polymerase assay conditions, product analysis, and data fitting have been documented in the accompanying paper (8).

Reagents—Unmodified oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Nucleoside triphosphates were purchased from Sigma-Aldrich. [γ-32P]ATP was purchased from PerkinElmer Life Sciences. The nucleoside analogs dF and dQ were prepared as described (5, 6). The nucleoside triphosphate analog difluorotoluene deoxynucleoside triphosphate (dFTP) was prepared as described by Moran et al. (12). The 45-mer oligonucleotides containing dF and dQ were also prepared as described (13, 14). All other reagents were purchased from Fisher unless otherwise noted.

Formation of a dFMP-terminated Primer—To assay the kinetics of incorporation after a 3’ dFMP:dA pair (where dA was the templating base, a dFMP-terminated primer was required. These reactions were carried out by stepwise synthesis to first incorporate dFMP and then examine incorporation on top of the product. Catalytic subunit (400 nM) was preincubated with a 2 μM accessory subunit and 360 nM 27p/45A primer/template in a buffer lacking MgCl₂. Oligonucleotides used in this study were shown in Table 1. This solution was mixed (1:1) with one containing 200 μM dFTP and 50 mM MgCl₂. After 10 s, this combined solution was rapidly mixed (1:1) with a solution containing 25 mM MgCl₂ and varying concentrations of dCTP to assay the nucleotide concentration dependence of extension on top of dFMP. During the final reaction, concentrations were as follows: 100 nM catalytic subunit, 500 nM accessory subunit, 90 nM 28pF/45A, 25 mM MgCl₂, 50 μM dFTP, and 10–500 μM dCTP.

Exonuclease Assay Conditions—The final reaction conditions for the exonuclease experiments were the same as for the polymerase experiments described previously (8), except that the dNTP was omitted so the exonuclease reaction could be observed. The holoenzyme-DNA complex was assembled in a buffer free of MgCl₂ at twice the final concentration and then examine incorporation on top of the product. Catalytic subunit (400 nM) was preincubated with a 2 μM accessory subunit and 360 nM 27p/45A primer/template in a buffer lacking MgCl₂. Oligonucleotides used in this study were shown in Table 1. This solution was mixed (1:1) with one containing 200 μM dFTP and 50 mM MgCl₂. After 10 s, this combined solution was rapidly mixed (1:1) with a solution containing 25 mM MgCl₂ and varying concentrations of dCTP to assay the nucleotide concentration dependence of extension on top of dFMP. During the final reaction, concentrations were as follows: 100 nM catalytic subunit, 500 nM accessory subunit, 90 nM 28pF/45A, 25 mM MgCl₂, 50 μM dFTP, and 10–500 μM dCTP.

Exonuclease Assay Conditions—The final reaction conditions for the exonuclease experiments were the same as for the polymerase experiments described previously (8), except that the dNTP was omitted so the exonuclease reaction could be observed. The holoenzyme-DNA complex was assembled in a buffer free of MgCl₂ at twice the final concentration and then rapidly mixed 1:1 with a solution containing MgCl₂. The concentration of product was plotted against the time course of the reaction, and the subsequent curve was fit to a single or double exponential equation to determine the rate of exonuclease proofreading by Pol γ as described (4).
Nonpolar Analogs

pairs at the DNA terminus, the Pol mismatch (Table 2). Thus, for the non-hydrogen-bonded base nearly indistinguishable from the removal of mismatched base natural nucleotide paired opposite the nonpolar analog was parameters describing the time course of hydrolysis in each case, fit best to a double exponential equation. The fitted distinguish between correct and incorrect base pairs and removed results shown in Fig. 3. The kinetics of removal are nearly indis-sion over several of the base pairs containing nonpolar analogs.

RESULTS

Exonuclease Removal of Natural Nucleotides Paired with Nonpolar Analogs—We used single turnover kinetic methods to examine the removal of each of the four possible nucleotides paired opposite the nonpolar analogs dF and dQ to get the results shown in Fig. 3. The kinetics of removal are nearly indistinguishable irrespective of the base pair assayed and, in each case, fit best to a double exponential equation. The fitted parameters describing the time course of hydrolysis in each case are presented in Table 2. The kinetics of removal of each natural nucleotide paired opposite the nonpolar analog was nearly indistinguishable from the removal of mismatched base pairs described previously (4) and repeated here for a dG:dT mismatch (Table 2). Thus, for the non-hydrogen-bonded base pairs at the DNA terminus, the Pol γ exonuclease failed to distinguish between correct and incorrect base pairs and removed any nucleotide paired with a nonpolar analog in the template strand as if it were a mismatch.

These data suggest that hydrogen bonding is absolutely essential for recognition of correct terminal base pair. However, the major contributor to selectivity of the proofreading exonuclease is not the rate of excision but, rather, the rate of extension over a mismatch. Therefore, we measured the kinetics of extension over several of the base pairs containing nonpolar analogs.

Incorporation of the Next Correct Base—A series of experiments was carried out to determine the effects of a 3’ terminal base pair containing a nonpolar analog on the incorporation of the next correct nucleotide by Pol γ. Representative data are shown in Fig. 4 for the incorporation of dCTP over a 3’dTMP:dQ (primer:template) base pair. The time courses of product formation (Fig. 4A) were fit to a single exponential to obtain the rate of polymerization at several concentrations of dCTP (the next correct base). Fitting the concentration dependence of the rate to a hyperbola (Fig. 4B) defines the maximum rate $k_{\text{pol}}$ and apparent dissociation constant for dCTP binding, $K_p$. The rate of nucleotide incorporation was reduced by ~400-fold relative to the rate of extension of a dT:dA base pair.

The results of similar experiments using several different 3’-terminal base pairs (3’dFMP:dA, 3’dAMP:dF, 3’dTMP:dQ, and 3’dCMP:dQ) are summarized in Table 3. In each case the kinetics of primer extension are considerably slower than the rate of extension of a correct natural base pair and also slower than the rate of extension to bury a natural mismatch (4). For example, the efficiency of extension beyond a 3’dFMP:dA cor-
rates of 3.3 and 0.46 s^{-1} for matched nucleotides are removed in a biphasic reaction with binding and faster incorporation of the next correct nucleotide. Rather than extension of a mismatch. Similar analysis using Pol γ after a mismatch is the major contributor to exonuclease selection of the proofreading function and that stalling of the polymerase between polymerase and exonuclease sites governs selectivity using T7 polymerase showed that the kinetic partitioning (15) that the 3'-terminal base pair mimic was 680,000-fold lower than that observed for a native 3’dTMP:dA pair (Table 3). Interestingly, the extension of the converse 3’dAMP:dF pair was 130-fold more efficient than the converse 3’dFMP:dA pair due to both tighter binding and faster incorporation of the next correct nucleotide.

**DISCUSSION**

In this report we have employed the nonpolar analogs dF and dQ and used pre-steady state kinetic methods to test the roles of hydrogen bonding within the 3’-terminal base pair on selectivity during exonuclease removal and primer extension. The present data suggest that base pair hydrogen bonding plays a central role in error correction. We observed a complete loss of the ability of the enzyme to distinguish between correctly and incorrectly paired bases at the terminus when hydrogen bonding was eliminated. This is in marked contrast with naturally hydrogen bonded pairs and mismatches. Thus, we conclude that terminal pair hydrogen bonding has a strong influence on the partitioning of the primer terminus to the exonuclease site and in the recognition of a mismatch at the polymerase site. Possible reasons for this are discussed below.

**Exonuclease Removal of Natural Nucleotides Paired Opposite Nonpolar Analogos**—It was first shown by Brutlag and Kornberg (15) that the 3’-5’ exonuclease activity of a DNA polymerase shows selectivity for mismatched DNA. Subsequent analysis using T7 polymerase showed that the kinetic partitioning between polymerase and exonuclease sites governs selectivity of the proofreading function and that stalling of the polymerase after a mismatch is the major contributor to exonuclease selectivity (1) by changing the kinetic partitioning to favor excision rather than extension of a mismatch. Similar analysis using Pol γ showed that correct nucleotides are removed by the 3’-5’ exonuclease domain at a rate of 0.02 s^{-1} (4), whereas mismatched nucleotides are removed in a biphasic reaction with rates of 3.3 and 0.46 s^{-1} (Table 2). The biphasic nature of the removal of incorrectly paired nucleotides suggests a model whereby the rates of removal represent partitioning of the primer strand between the polymerase site and the exonuclease site and subsequent exonuclease removal of the 3’ terminal nucleotide. Because the reaction was initiated by the addition of Mg^{2+} to a preformed enzyme-DNA complex, it is not possible to assign these observed rates to individual steps in the pathway. Nonetheless, the observed rate of 3.3 s^{-1} sets a lower limit of the rate of excision, and the large amplitude of this fast phase indicates that the majority of the DNA was excised at this rate. Previous analysis of the rates of hydrolysis of DNA containing a frayed primer stand of varying lengths suggested that the slow phase may represent the rate of migration of the primer strand from the polymerase to the exonuclease site, whereas the fast phase sets a lower limit on the rate of hydrolysis once the primer strand is at the exonuclease site (4). Regardless of the details of the model with which we interpret the observed kinetics, it is apparent that the primer:template analogs containing nonpolar analogs are excised at rates comparable with the rates of removal of mismatches. The rates of excision are independent of the identity of the base pair in that any 3’ terminal nucleotide involved in a base pair with a nonpolar analog is removed as if it were a mismatch.

In an earlier study of proofreading by the Klenow fragment of DNA Pol I, it was shown that the rate of removal of dA paired opposite dF was much faster than the rate of removal of dA paired opposite dT (13). In the present study with polymerase γ this is also the case; moreover, we show that the rate of removal of dA paired opposite dF was roughly equivalent to the rates of removal of the mismatches dC, dG, and dT paired opposite dT. In addition, we observe that the kinetics of removal of dA, dC, dG, and dT paired opposite dF are all biphase and that the rates observed are nearly equivalent. We have also shown that the kinetics of removal of most of these nucleotides paired opposite dQ do not deviate significantly from those nucleotides paired opposite dF, and they are not changed by the presence of bases of varied sizes and shapes opposite these analogs. One minor but interesting exception is dG opposite dQ, which is edited 3-fold more slowly than a G-T mismatch (although still 50-fold more rapidly than the control T-A pair). A possible explanation for this is that dQ, when flipped to the syn orientation, is isosteric with C and could in principle form a hydrogen bond with G analogous to the central bond in a G-C pair (Fig. 5).

Our data for polymerase γ show that in the absence of hydrogen bonding, simple structural mimicry is not sufficient to suppress rapid editing by the proofreading exonuclease. NMR data and solution studies have shown that dF is a good shape mimic of dT and that the presence of dF opposite dA in a DNA duplex distorts the structure of the duplex very little (16), and yet the dA:dF pair is proofread as if it were a mismatch.

Taken together, the current data suggest that partitioning to the exonuclease site in Pol γ is influenced strongly by hydrogen bonding in the terminal pair. We hypothesize that the unwinding of the terminus of the primer-template duplex starts with the terminal pair, and so fraying rates of that terminal pair could well directly affect exonuclease rates. Because they lack hydrogen bonds that could provide an energetic or kinetic constraint on initial separation of the pair, it seems quite possible that pairs involving dF and dQ may well fray at an increased rate and have an equilibrium for fraying that lies further to the separated side. NMR studies of DNA duplexes containing F-A and F-Q pairs show evidence of rapid motion around the nonnatural bases, consistent with the possibility that the lack of hydrogen bonds increases fraying rates (16, 17). Although the nonpolar bases of dF and dQ in fact stack more strongly in DNA than their natural counterparts thymine and adenine (17), pairs involving dF and dQ are weakly destabilizing to short synthetic duplexes containing 5’ overhangs as occurs in proofreading (13). The same is true of natural mismatched pairs. Although

**TABLE 3**

Incorporation beyond a nonpolar analog of a “correct” base pair

The concentration dependence of the rate of incorporation of dCTP as the next correct base (dG in the template) was measured for each primer:template analog combination to obtain estimates for $K_a$ and $k_{cat}$ governing incorporation (see Fig. 4). The -fold effect was calculated as the ratio of the specificity constant for dCTP incorporation, $47 \mu M^{-1} s^{-1}$ (4) divided by $K_{pol}/K_{ex}$ for each analog base pair.

| Terminal base pair (P/T) | $K_a$ | $k_{cat}$ | Fold effect |
|--------------------------|-------|-----------|-------------|
| 3’dFMP:dA                | 550 ± 110 | 0.038 ± 0.005 | 680,000     |
| 3’dAMP:dF                | 24 ± 4   | 0.22 ± 0.02  | 5,100       |
| 3’dTMP:dQ                | 250 ± 60 | 0.22 ± 0.04  | 53,000      |
| 3’dCMP:dQ                | 180 ± 40 | 0.21 ± 0.03  | 40,000      |
Roles of Hydrogen Bonding in Extension of Pairs—The frequency of editing of a given base pair depends strongly on the rate of primer extension. As summarized in Table 3, the effect of a nonpolar analog in the primer terminus ranged from a 5,100- to 680,000-fold effect on $k_{pol}/K_d$. Base pairs containing one nonpolar partner either in the primer or template strand were extended at markedly reduced rates. For example, Pol $\gamma$ extends a 3’dAMP:dF pair ~5000-fold less efficiently than a natural 3’dAMP:dT pair. Conversely, Pol $\gamma$ extends the converse 3’dFMP:dA pair 680,000-fold less efficiently than the analogous 3’dTMP:dA pair. It is interesting to note the asymmetry in that dF in the primer strand has a much greater effect than dF in the template strand, paralleling the effects seen during incorporation (8). These results are reminiscent of the asymmetry in the dG:dT wobble base pair incorporation kinetics (7) and suggest a difference in interactions with the enzyme in the primer versus template strand.

We suggest two possible explanations for this poor extension of pairs containing a nonpolar base. One is that the dF is more free to “wobble” into the major groove when it is on the primer side of the duplex and that hydrogen bonds play a critical role in preventing this motion with native nucleotides. A second possibility (not mutually exclusive) is that Pol $\gamma$ may form energetically important minor groove hydrogen bonds both to the primer and template sides of the groove at the extended pair. Thus, a nonpolar base on either side would result in a costly desolvation upon extension.

Previous results with the Klenow polymerase and nonpolar analogs showed extensions that were less efficient by 110-fold when nonpolar bases were in the template strand and by 3000-fold when in the primer strand; this latter poor extension could be rescued by the addition of a minor groove hydrogen bond acceptor (13, 19–21). This suggested an important minor groove interaction on the primer side of the extended pair. Other studies of nonpolar base pairs have also reported poor extension by the Klenow polymerase and improved extension with the addition of hydrogen bond acceptors in the groove (22, 23). We expect that geometry of the A-F pair is not likely to cause this poor extension with Pol $\gamma$, since the dF analog mimics any effects of geometry of the base pair by closely mimicking the shape of thymidine (24).

Taken together, these data suggest that either the alignment of base pairs afforded by hydrogen bonding or the minor groove hydrogen bonds (or both) are critical elements leading to fast primer extension. In the absence of hydrogen bonding, the resulting misalignment of the 3’-terminal base and/or the cost of desolvation of the enzyme may slow the rate of extension.

Cost of Proofreading and Error Correction—The cost of proofreading is a measure of the frequency with which a correctly paired nucleotide is removed by the exonuclease and is defined as $k_{exo}/(k_{extend} + k_{exo})$, where $k_{exo}$ is the rate of excision, and $k_{extend}$ is the rate of primer extension. One would expect highly efficient DNA polymerases to exhibit a low rate of removal of correct nucleotides relative to removal of incorrect nucleotides. Indeed, this is the case for Pol $\gamma$. The cost of proofreading for Pol $\gamma$ is 0.14%, whereas between 75 and 99.5% of mismatches are removed rather than extended (4). T7 DNA polymerase shows even greater selectivity (1).

When a nonpolar analog is present in the template, the rate of extending the 3’ terminal pair is only 0.022 s$^{-1}$. The rate of
removal of the nucleotide from the primer opposite such an analog is between 4.1 and 6 s\(^{-1}\), leading to a cost of proofreading between 95 and 97%, a value comparable with the efficiency seen for mismatch removal. Thus, the processive synthesis of base pairs containing nonpolar bases is quite inefficient with this polymerase.

**Summary**—Combined with the results from the companion study of nucleotide incorporation by Pol\(\gamma\) (8), the data show that the synthesis of base pairs by the mitochondrial polymerase depends significantly on hydrogen bonds in three ways. First, the companion study revealed that the fidelity and efficiency of nucleotide insertion was significantly greater for natural nucleotides compared with non-hydrogen-bonding mimics. Second, the current study shows that extension beyond the nonpolar analogs by this enzyme is a factor of at least 10\(^3\)-fold less efficient than beyond natural polar ones, suggesting a possible role of hydrogen bonds in alignment of the 3’-terminal base of the primer. Third, the current data also show that exonuclease editing of pairs lacking hydrogen bonds is rapid, suggesting that hydrogen bonds play an important role in lowering the cost of proofreading, possibly by slowing the fraying of the DNA terminus.

**REFERENCES**
1. Donlin, M. J., Patel, S. S., and Johnson, K. A. (1991) Biochemistry 30, 538–546
2. Patel, S. S., Wong, I., and Johnson, K. A. (1991) Biochemistry 30, 511–525
3. Wong, I., Patel, S. S., and Johnson, K. A. (1991) Biochemistry 30, 526–537
4. Johnson, A. A., and Johnson, K. A. (2001) J. Biol. Chem. 276, 38097–38107
5. Schweitzer, B. A., and Kool, E. T. (1994) J. Org. Chem. 59, 7238–7242
6. Guckian, K. M., Morales, J. C., and Kool, E. T. (1998) J. Org. Chem. 63, 9652–9656
7. Lee, H. R., and Johnson, K. A. (2006) J. Biol. Chem. 281, 36236–36240
8. Lee, H. R., Helquist, S. A., Kool, E. T., and Johnson, K. A. (2008) J. Biol. Chem. 283, 14402–14410
9. Johnson, A. A., Tsai, Y. C., Graves, S. W., and Johnson, K. A. (2000) Biochemistry 39, 1702–1708
10. Johnson, A. A., and Johnson, K. A. (2001) J. Biol. Chem. 276, 38090–38096
11. Graves, S. W., Johnson, A. A., and Johnson, K. A. (1998) Biochemistry 37, 6050–6058
12. Moran, S., Ren, R. X. F., and Kool, E. T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10506–10511
13. Morales, J. C., and Kool, E. T. (2000) Biochemistry 39, 2626–2632
14. Moran, S., Ren, R. X. F., Rurney, S., and Kool, E. T. (1997) J. Am. Chem. Soc. 119, 2056–2057
15. Brutlag, D., and Kornberg, A. (1972) J. Biol. Chem. 247, 241–248
16. Guckian, K. M., Krugh, T. R., and Kool, E. T. (1998) Nat. Struct. Biol. 5, 954–959
17. Guckian, K. M., Schweitzer, B. A., Ren, R. X., Sheils, C. J., Tahmassebi, D., and Kool, E. T. (2000) J. Am. Chem. Soc. 122, 2213–2222
18. Kouchakdjian, M., Li, B. F., Swann, P. F., and Patel, D. J. (1988) J. Mol. Biol. 202, 139–155
19. Morales, J. C., and Kool, E. T. (1999) J. Am. Chem. Soc. 121, 2323–2324
20. Spratt, T. E. (1997) Biochemistry 36, 13292–13297
21. Spratt, T. E. (2001) Biochemistry 40, 2647–2652
22. Henry, A. A., and Romesberg, F. E. (2003) Curr. Opin. Chem. Biol. 7, 727–733
23. Leconte, A. M., Matsuda, S., and Romesberg, F. E. (2006) J. Am. Chem. Soc. 128, 6780–6781
24. Guckian, K. M., Krugh, T. R., and Kool, E. T. (2000) J. Am. Chem. Soc. 122, 6841–6847