Single Amino Acid Mutation Controls Hole Transfer Dynamics in DNA-Methyltransferase HhaI Complexes

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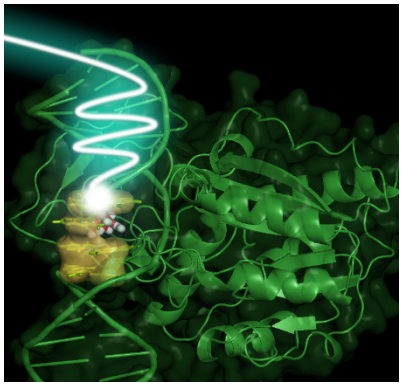
Different mutagenic effects are generated by DNA oxidation that implies the formation of radical cation states (so-called holes) on purine nucleobases. The interaction of DNA with proteins may protect DNA from oxidative damage owing to hole transfer (HT) from the stack to aromatic amino acids. However, how protein binding affects HT dynamics in DNA is still poorly understood. Here, we report a computational study of HT in DNA complexes with methyltransferase HhaI with the aim of elucidating the molecular factors that explain why long-range DNA HT is inhibited when the glutamine residue inserted in the double helix is mutated into a tryptophan. We combine molecular dynamics, quantum chemistry and kinetic Monte Carlo simulations, and find that protein binding stabilizes the energies of the guanine radical cation states and significantly impacts the corresponding electronic couplings, thus determining the observed behavior, whereas the formation of a tryptophan radical leads to less efficient HT.

TOC GRAPHIC



KEYWORDS DNA damage • charge transfer • methyltransferase HhaI • molecular dynamics • INDO/S

Charge transport through the DNA base stack has been a subject of considerable interest in recent years[1–5], with possible implications in areas as diverse as carcinogenesis and molecular electronics[6]. It has been shown that radical cation states (electron holes) generated in DNA can migrate through nucleobases over distances up to 200 Å from the initial oxidized point. This ability of DNA can be employed for redox sensing and signaling in the genome, as long as it may provide new insight into the direction of mutations upon oxidative stress. Extensive studies, both theoretical and experimental, suggest that long-range HT occurs as a multistep hopping process between adjacent guanines.[7–10] Although HT through DNA strands is well known, how this process occurs in DNA-protein systems, where the hole can migrate from DNA to aromatic amino acid residues protecting the genome, is still poorly understood.[7,11,12] The formation of tryptophan and tyrosine radicals arising from HT initiated in DNA has been observed using the flash-quench technique upon the addition of DNA-bound peptides to ruthenated DNA assemblies.[13,14] Quantum chemical calculations suggest that both electronic coupling and the driving force of HT between nucleobases and aromatic amino acids depend strongly on the mutual arrangement of donor and acceptor sites.[15,16]

The Barton group has probed charge migration and radical trapping in DNA assemblies in the presence of the specifically bound enzyme methyltransferase HhaI using a combination of gel electrophoretic assays and transient spectroscopy.[11,12] The enzyme carries out an alkylation process after flipping out the central cytosine of its binding domain 5'-GCGC-3' and inserting a glutamine side chain into the resultant DNA pocket (See Fig 1). In the present study we do not address this enzymatic reaction, but rather investigate the molecular factors that explain the interesting behaviour unveiled by the experiments performed in the Barton group. Such experiments indicated that binding of the wild type (WT) enzyme decreases long-range oxidative

damage at distal 5'-GG-3' sites substantially, by 42%. In contrast, distal oxidative damage was retained in a Q237W mutant, which inserts a tryptophan (W) side chain instead of a glutamine (Q) within the DNA π-stack.[11] These studies also revealed the involvement of both guanine and tryptophan radicals in the process through detection of transient spectroscopic signals, and gel electrophoresis experiments indicated considerable oxidative damage at the G 5' to the W inserted amino acid, suggesting a stabilization mechanism of the G cation state due to stacking with the W reminiscent of the stabilization of 5'-GG-3' motifs.[12]

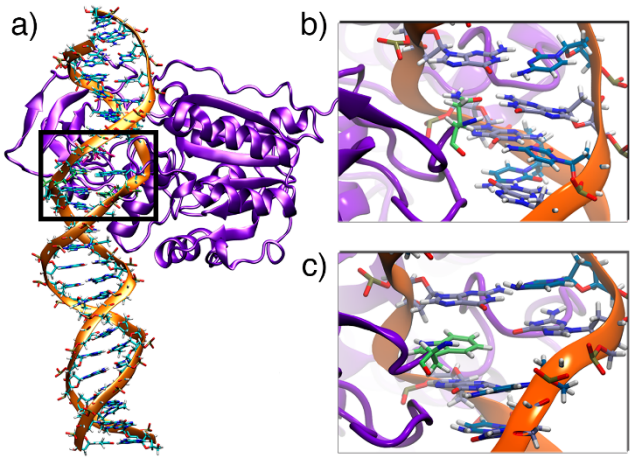


Figure 1. a) Structure of the DNA-HhaI complex considered in this study. Detailed view of the binding mode of the a) glutamine in the wild-type system and b) of the tryptophan in the Q237W mutant.

In order to establish the microscopic basis for the observed HT behaviour, here we combine classical molecular dynamics simulations with INDO/S quantum mechanical calculations of HT energetics and electronic couplings in the binding domain (see Fig. 2), to explore how the interaction of the DNA duplex with the wild-type (WT) or mutant enzyme affects its HT properties. This approach allows a realistic description of the effect of structural fluctuations on

the HT parameters[17], which are strongly dependent on the arrangement of donor and acceptor. We use Marcus theory and a Kinetic Monte-Carlo (KMC) scheme to estimate HT rates and various pathways along the binding domain of both assemblies. In Figure 3 we show the HT reactions included in the simulations of the overall HT kinetics. Our results indicate that protein binding strongly affects the HT characteristics of DNA. In particular, the modulation of G site energies and electronic couplings are responsible for the observed HT behaviour, whereas the formation of a W^+ radical leads to less efficient HT.

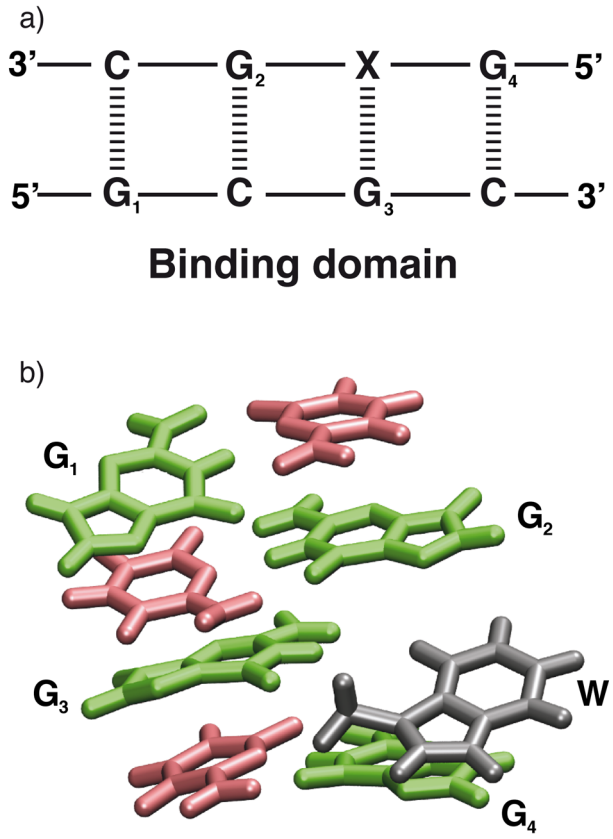


Figure 2. a) Binding domain of the DNA stack in the DNA-HhaI complex. b) Structure of the model system, which includes the nucleobases in the binding domain (except the X abasic site) plus the Q (wild-type) or W (Q237W mutant) residues inserted into the DNA pocket.

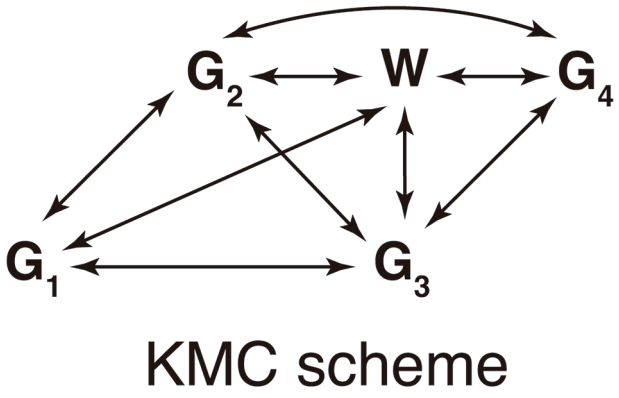


Figure 3. Scheme of relevant pathways and states included in the Kinetic Monte Carlo simulations of the HT dynamics.

It is well-known that the HT properties of DNA are highly sensitive to disruptions of the π-stack. In HhaI-DNA complexes, the local structure of the DNA in the binding domain is significantly distorted.[18,19] In the DNA-protein assemblies we address, the presence of an abasic site X, i.e. the absence of a nucleobase attached to the sugar, can further impact conformational changes within the π-stack. In order to understand the impact of these two effects in the HT dynamics, we have performed MD simulations on the DNA and DNA-X systems, as well as on the DNA-X system bound to the WT and Q237W mutant HhaI enzymes. Our results indicate that the structural parameters of the double helix change significantly upon the protein binding and the presence of the abasic site. Earlier, it was suggested that in contrast to the WT protein, the Q237W mutant, retains long-range oxidative damage at distal 5'-GG-3' sites because the inserted W fulfills the π-gap in the DNA stack by adopting a stacked configuration relative to the nucleobases.[11,12] Our simulations confirm this hypothesis, and indicate that W and G_3 compete for the abasic site space, as shown by the degree of insertion shown in Fig. 4. We define the degree of insertion as the ratio between the distance among upper and lower stacked guanines and the sum of the

distances among the inserted amino acid side chain and such guanines. Thus, this value ranges from 0 to 1, the latter indicating complete insertion of the amino acid in the π-stack (see Supporting Information for further details on the structural analysis).

Regarding the impact of the abasic site and protein binding on the parameters determining the HT behaviour, we find that the formation of the abasic site in the DNA stack increases V_{24} and V_{34} couplings, which change from 7 to 15 meV and from ~8 to ~66 meV by passing from DNA to DNA-X. This dramatic change arises from the displacement of G_3 allowing a better stacking between guanines G_3 and G_4 (See Fig. S9 in Supporting Information). The conformational changes also significantly destabilize the radical cation state of G_3 in contrast to the DNA, where all sites have similar energies. When, however, the DNA-X helix binds the WT HhaI protein the insertion of the Q residue in the π-gap precludes the rearrangement of G_3 site and thereby leads to a dramatic attenuation of V_{24} and V_{34} (2 and 4 meV). Also, the V_{23} coupling decreases from 31 to 12 meV. The energies of the G_2 and G_4 hole states are destabilized compared to the other sites (see Fig. 5) causing a potential inhibition of long-range oxidative damage as observed experimentally. Interestingly, the overall picture for HT in the Q237W mutant reminds that observed for the unbound DNA-X system. Compared to the WT complex, in Q237W both G_2 and G_4 sites are stabilized due to the insertion of the stacked tryptophan, thus recovering the fast HT toward the final G_4 site in the binding domain. Moreover, the mutation of the inserted Q residue to W enhances strongly the couplings between guanine sites, as shown in Table S1, due to the superexchange interaction of the nucleobases mediated by the W residue.

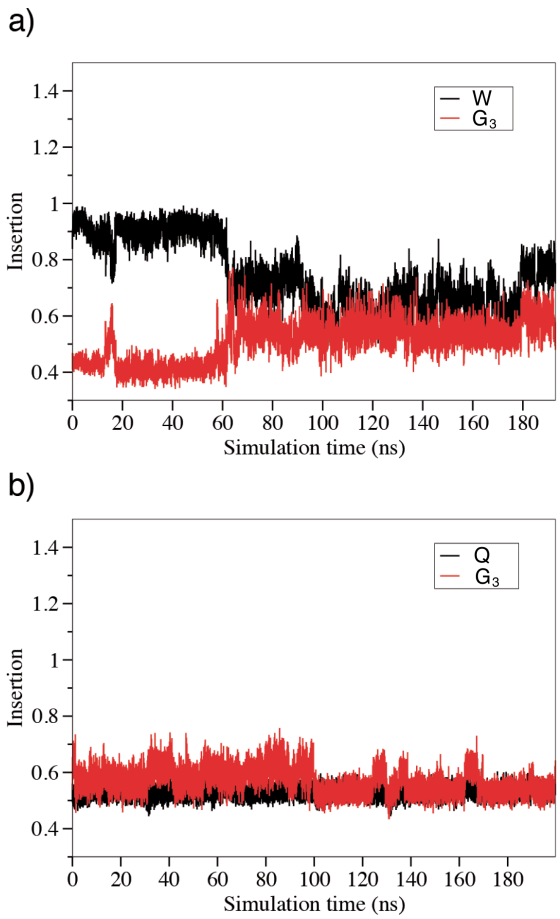


Figure 4. Degree of insertion of a) W/G_3 and b) Q/G_3 residues between the G_2 and G_4 sites in the Q237W and WT assemblies, respectively (see text for details).

In the mutant, W237 may act also as an intermediate state in the HT process. Our additional simulations performed on single guanine and tryptophan in water predict that a correction of -0.61 eV to the INDO/S energy of the W state should be applied to reproduce the data derived from experimental oxidation potentials at neutral pH.[20] The correction has been applied in the simulation of HT within the Q237W complex.

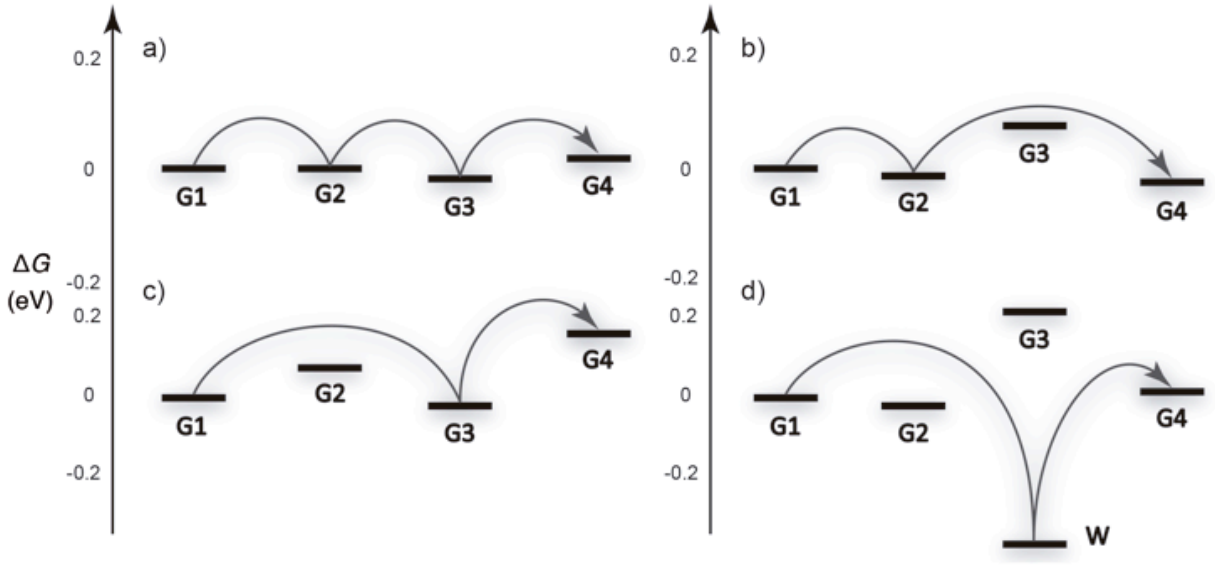


Figure 5. Site energies of the radical cation states localized on the guanines and the tryptophan for the a) DNA, b) DNA-X, c) WT and d) Q237W systems. The most frequent HT pathways according to KMC simulations are indicated by arrows.

In Fig. 5 we show the preferred pathway for HT estimated from the KMC simulations for each system, which include the pathways illustrated in Fig. 3 (more details are provided in Fig. S11-S12). The rates for the individual HT steps and the overall process in each system are presented in Fig. 6. Due to a small energetic disorder among guanines in the DNA system, no particular pathway is preferred and most transfer steps occur with a time constant, i.e. the inverse of the rate, of τ~1 ns, leading to the overall HT time of ~2.4 ns. In the DNA-X system, the $G_1G_2G_4$ pathway is preferred in 54% of the KMC realizations, due to the G_3 site destabilization. However, a better stacking between guanines G_3 and G_4 leads to stronger electronic couplings in the binding domain resulting in faster overall HT with a characteristic time of ~710 ps.

The binding of DNA-X to the HhaI complex further modulates its HT dynamics. Binding to the WT enzyme, which destabilizes the G_4 site and attenuates V_{24} and V_{34}, leads to much slower

hopping rates from G_2/G_3 to G_4 (τ = 94 and 124 ns), resulting in a longer overall HT time of 178 ns. Because of the relatively close energies of G_1, G_2 and G_3, a variety of paths contribute to the transfer in this case, the $G_1G_3G_4$ one being slightly preferred due to the lower energy of G_3 compared to G_2 (see Supporting Information). In Q237W, we can separate the impact of the binding into two effects (1) modulation of site energies and electronic couplings change the rate of individual hopping steps and (2) new pathways appear that imply the formation of W^+. The insertion of the tryptophan clearly destabilizes the G_3 site, but it strongly mediates all couplings involving G_2, G_3 and G_4. If we simulate the overall HT kinetics excluding the eventual formation of W^+, the path $G_1G_2G_4$ is preferred in 90% of the realizations, and the overall HT process becomes much faster (τ = 220 ps). Transient absorption experiments, however, confirm the participation of the W^+ state in the hopping process,[12] so the eventual formation of W^+ needs to be included in the simulations. In this case, our KMC simulations indicate that the G_1WG_4 pathway is preferred in 26% of the realizations, in agreement with experiments.

Here it is worth noting that gel electrophoresis experiments indicated that G_4 is a better trapping site than G_2, in accord with results found for the 5'-G_4WG_2-3' sequence.[12] Our simulations rather predict a slightly higher energy for G_4. However, some damage was also experimentally observed in the G_2 site, suggesting that the site energies of G_2 and G_4 are comparable, in accord with our simulations. So, the insertion of the stacked tryptophan at the sequence 5'-G_4WG_2-3' stabilizes the G_2 and G_4 sites compared to the WT system (Fig. 5). This effect is similar to that observed in 5'-GG-3' or 5'-GGG-3' motifs, where the electrostatic interaction with neighboring GC pairs stabilizes a hole trap.[21,22] In contrast, only minor damage at G_2 and no damage at G_4 is observed in the WT complex in agreement with the predicted destabilization G_2 and G_4. We note

here that these experiments only assessed the oxidative damage in the labeled strand carrying the G_2 and G_4 sites.

Because the energy of the W is predicted to be considerably lower than those of the guanine sites, its participation in the hopping process leads to a trapping effect which slows down the G_1 → G_4 transfer, leading to an overall HT time of 39 ns. As mentioned above, in this case the preferred path through the binding domain is G_1WG_4. This estimate still describes a significantly faster HT process in the Q237W mutant compared to the WT enzyme, in agreement with experimental observations.

Table 1. Time constants for the $G_1 \rightarrow G_4$ HT transfer and the contribution of preferred pathways in the studied systems.

	DNA	DNA-X	WT	Q237W	
τ (ns)	2.41	0.71	178	0.22	39.2[a]
	G1G2G3G4 (2%)	G1G2G4 (54%)	G1G3G4 (1%)	G1G2G4 (90%)	G1WG4 (26%)[a]

[a] Total time and corresponding pathway in Q237W including all possible forward/backward HT reactions involving W.

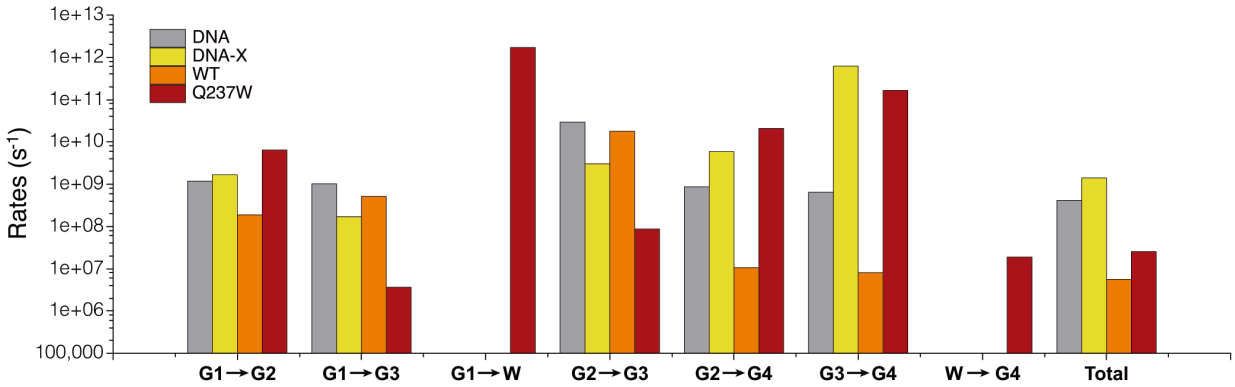


Figure 6. HT rates obtained from the Kinetic Monte Carlo simulations corresponding to the reference DNA stack, the DNA-X with an abasic site, and the DNA-X bound to the WT and Q237W *Hha*I protein.

We conclude that structural deformation of the DNA stack upon protein binding strongly affects its HT characteristics. The Q residue inserted in the π-stack in the WT enzyme destabilizes neighboring guanine sites (G 3' and G 5' to Q) and thereby inhibits the long-range oxidative damage, in accord with experimental findings. In contrast, the HT is estimated to be quite fast in a complex of the Q237W mutant where W237 stabilizes hole states on the adjacent G bases and enhances the electronic coupling of these sites. An alternative HT pathway that implies the formation of a W^+ radical, also occurs in agreement with recent transient absorption experiments. But it is found to slow overall HT dynamics along the DNA stack. Overall, our study provides a consistent molecular picture on how long-range HT in DNA is controlled by the nature of amino acid closely interacting with the nucleobases in DNA/HhaI complexes.

Computational Methods

We have performed extensive MD simulations at room temperature (300K) of the DNA Ru-1 duplex described in Ref.[12], both including or not the abasic X site (DNA and DNA-X systems),

as well as for the complex of DNA-X with the WT and Q237W mutant enzymes and a single guanine nucleobase and a tryptophan in water solution (see Supporting Information for a detailed description of the simulations). In the synthetic DNA-X system, the abasic site did not contain any nucleobase attached to the sugar. Because of the considerable size of the system studied, quantum-chemical calculations on the model system illustrated in Fig. 2 were performed using the semiempirical INDO/S method[23] on 1000 structures extracted along the MD trajectories. The INDO/S method has been shown to provide a reliable description of HT energies and electronic couplings of stacked nucleobases using Koopmans' approximation by comparison to high-level CASPT2 calculations, especially in the calculation of electronic couplings as compared to other semiempirical approaches.[24] However, our results indicated that it systematically overestimates the tryptophan radical cation state energy, which was corrected *a posteriori* based on the experimental one-electron oxidation potentials of guanine and tryptophan (see Supporting Information for details) Electrostatic embedding effects exerted by the environment, including protein, DNA, solvent and counterions, were considered adopting the charges as defined in the force field used for the MD simulations.[25–27] For structures of the WT and Q237W mutant systems, the hole states on the G sites and the W were represented by four and five highest-occupied molecular orbitals (HOMOs) of the neutral species (HOMO→HOMO-3/HOMO-4), respectively. HT couplings between the states were estimated using the Fragment Charge Difference method (FCD)[28]. From these calculations, HT rates were estimated for all forward and reverse HT reactions using Marcus theory. Finally, the effective rates and the paths for the overall HT processes G_1 to G_4 for each system were simulated using a Kinetic Monte Carlo algorithm[29]. In the KMC simulations, HT occurs in a stochastic manner and the probability to find the hole in a given site is reproduced by averaging over multiple realizations. In some cases,

like in the DNA and WT systems, the similar energies of several sites leads to multiple back and forth transfers of the hole among the sites, thus leading to many pathways that ultimately transfer the hole to the final G_4 site, as illustrated in Fig. S11 and S12 in the Supporting Information. Further details on the methodology adopted, and the analysis of structural and energetic parameters, are provided in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information. Detailed methods description. RMSD deviations, stacking overlap and base pair step parameters from the MD simulations. Structure of the binding domain in the DNA-X stack, distribution of HT pathways from KMC simulations, and table of electronic couplings, free energy differences, and time constants for the individual HT steps. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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