PcrA Helicase Protects Against Quantum Point Mutations

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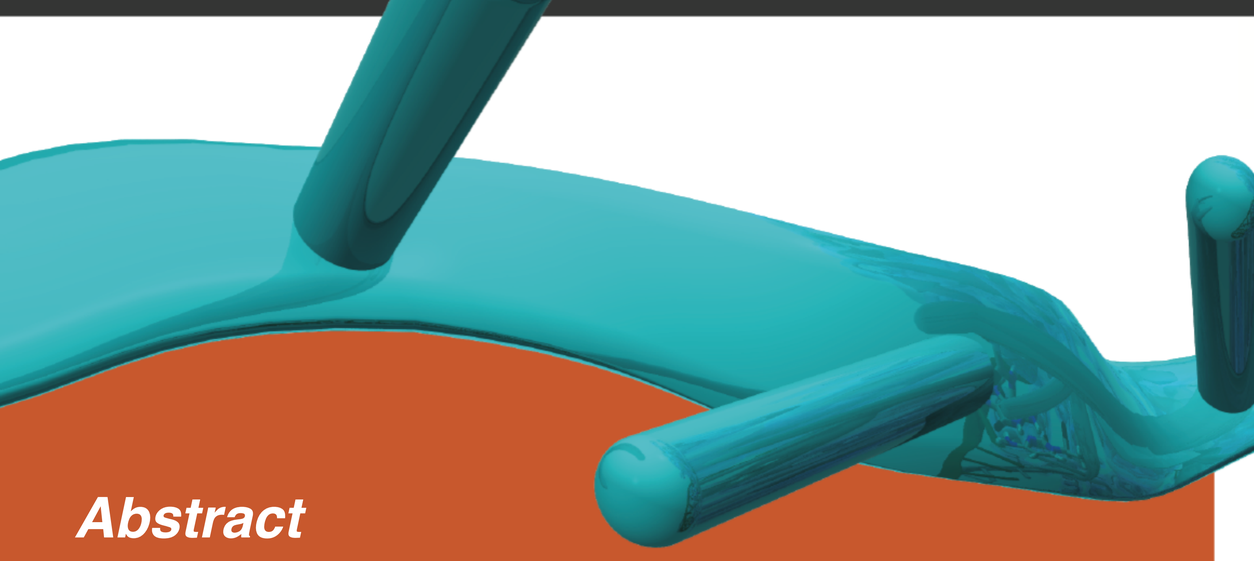
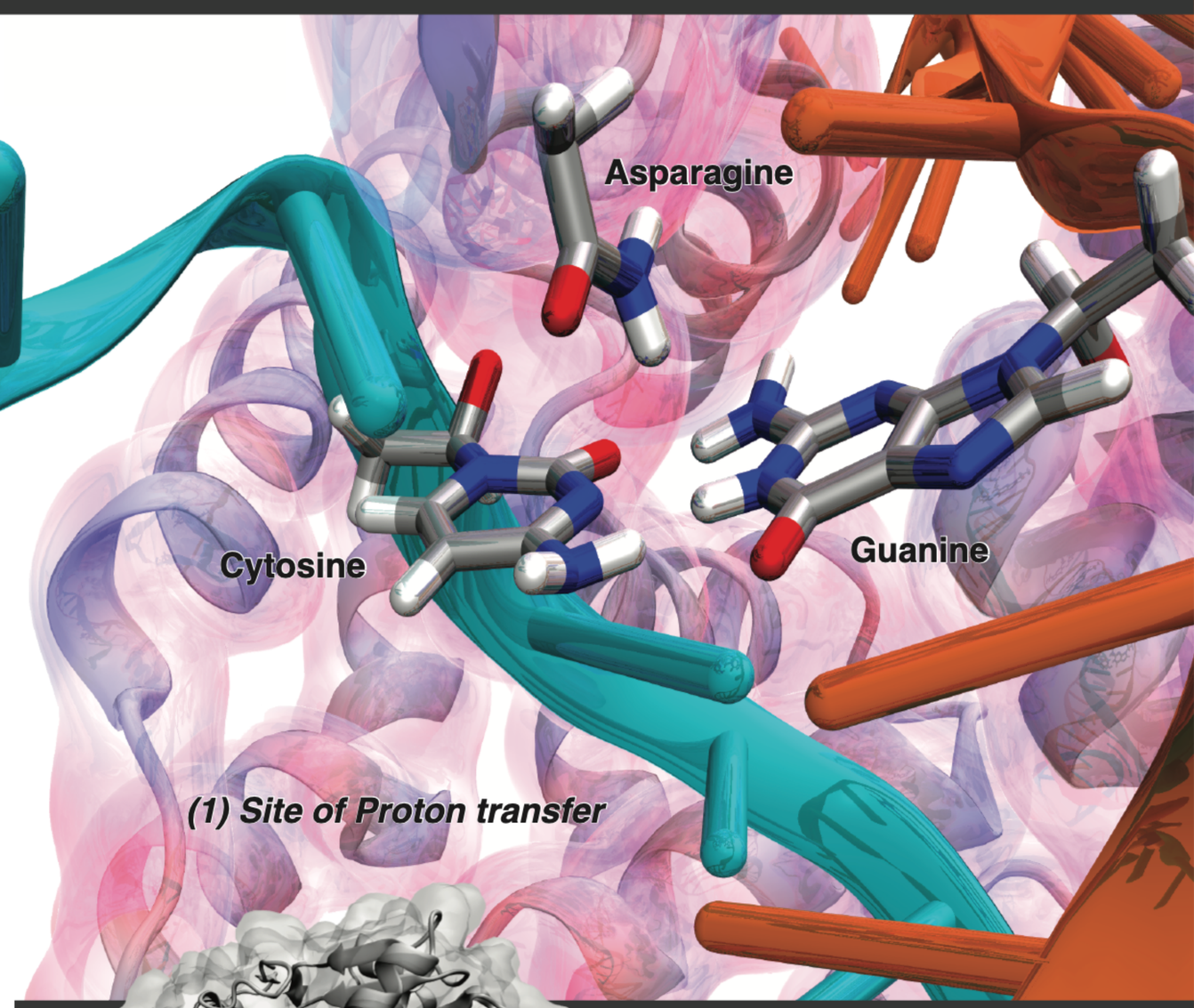
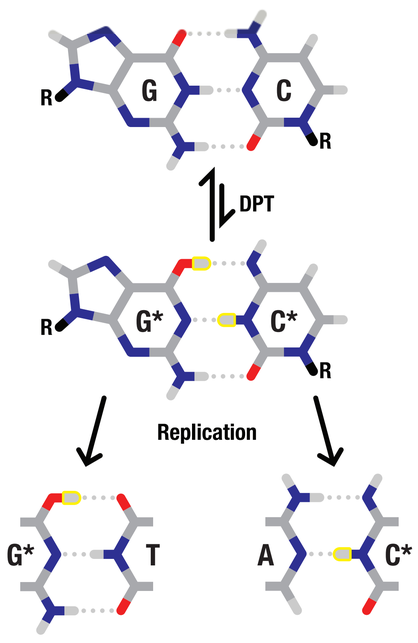
Abstract

Löwdin's hypothesis suggests a mechanism for single-point mutations in DNA, via the proton transfer across hydrogen bonds to produce unstable non-canonical nucleobase dimers.[1] Previous computational models have revealed this process to be feasible between G-C and G*C*, but the tautomeric product is short-lived.[2,3] To the best of our knowledge, the effect of replisome enzymes on the proton transfer has been computationally modelled for the first time. In this work, multi-scale QM/MM umbrella sampling simulations reveal the effect of the bacterial PcrA Helicase on the energetics of the double proton transfer in the guanine-cytosine base pair. It is shown that the presence of the local protein environment drastically increases the forward energy barrier and reaction asymmetry. This highlights the importance of explicit environmental models and suggests that PcrA Helicase could have evolved to reduce spontaneous mutations.



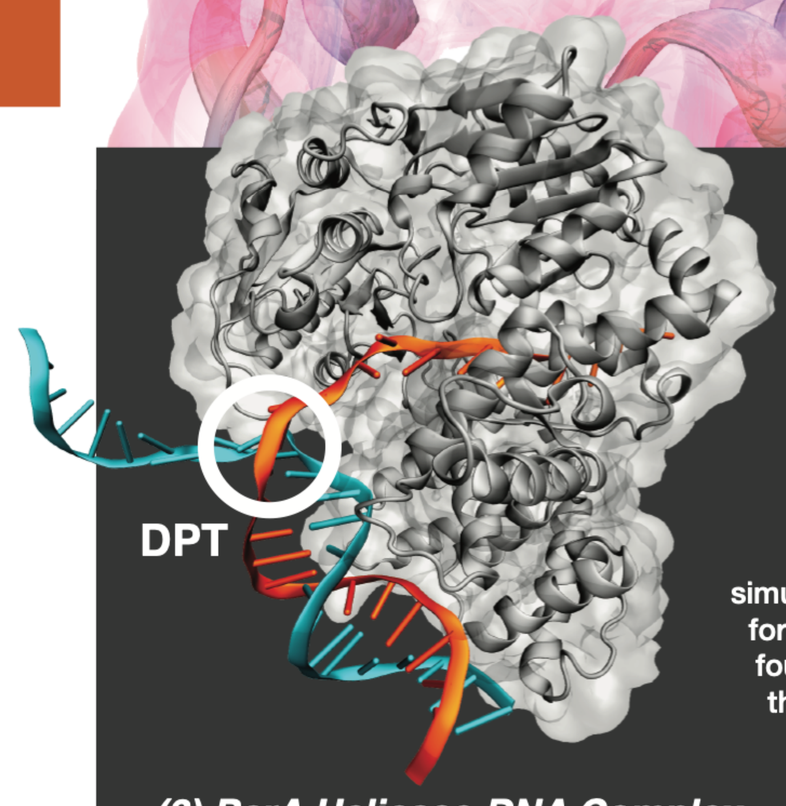




(2) Path to Mutations

Background

Double proton transfer (DPT), can change the steric profile of nucleobases, creating metastable tautomeric forms of DNA dimers.[1] If these tautomers survive replication they will cause mismatches and result in point mutations. Previous work has shown; the DPT in duplex DNA leads to a stable G*C* product;[2] that an asynchronous DPT pathway is energetically preferred;[3] quantum tunnelling dominates the DPT;[4] and that mechanical separation stabilises G*C*.[5] But what about the chemical interaction of Helicase on the DPT landscape?



(3) PcrA Helicase-DNA Complex

Multiscale

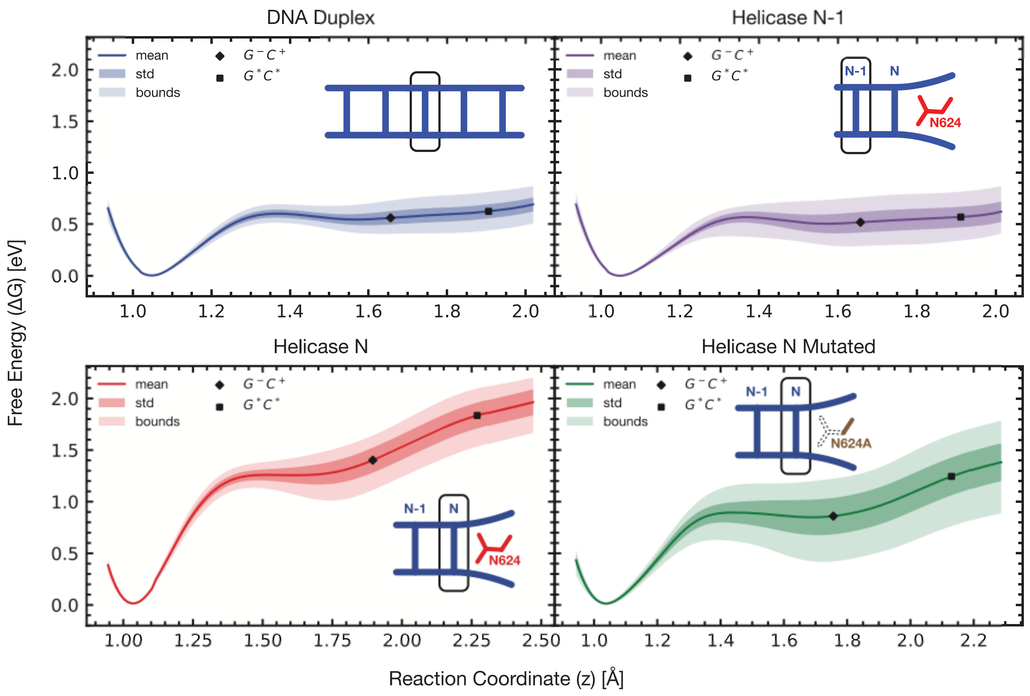
To explicitly model the replisome environment we employ QM/MM simulations where the GC dimer (DFT/BLYP) is embedded in the PcrA Helicase-DNA complex.

Reaction Mapping

Snapshots from molecular dynamics simulations (MD) are taken as starting points for umbrella sampling (US) simulations with four reaction coordinates (RC's) describing the DPT. The weighted histogram analysis method (WHAM) produces free energy profiles from the US trajectories.

Results

Our asymmetry and barrier energies for aqueous DNA agree with Gheorghiu,[3] and we also find an asynchronous pathway to be energetically preferred. As we approach the Helicase, the DPT behaviour is not modified. This is shown in our results for the penultimate (N-1) base pair whose DPT profile is near-indistinguishable from the duplex DNA case. This confirmes that a single base pair is enough to model stacking effects. Once the final base pair (N) is reached, the asparagine N624 interacts with the GC base pair and destabilises the G*C* tautomer. If we mutate the arsparagine into an alanine (effectively removing the amino acid's sidechain), the destabilising effect is reduced. DPT is no longer possible via quantum tunnelling as there is no stable right-hand well.



(4) GC DPT in various helicase environments

Implications

We are the first to study the DPT in a realistic replication environment, and find asparagine N624 to play a role in reducing the stability of the G*C* mutagen. N624 does not contribute to the stepping-motor strand separation function of PcrA helicase and is present in the majority of PcrA helicase sequences across 59 species of bacterium.[6] A finite but low rate of mutations is necessary for evolution, and our results indicate that asparagine N624 may have been preferentially selected to reduce the rate of quantum point mutations. Our results demonstrate the need for dynamics and realistic biological environments when assessing the likelihood of quantum effects influencing biological systems.

Conclusion and Outlook

Multiscale umbrella sampling simulations reveal that the DPT within guanine-cytosine (GC) is highly sensitive to the local biological environment. Novel free energy profiles for the GC DPT for DNA in complex with the bacterial PcrA helicase show that asparagine N624 specifically destabilises the G*C* mutagen. We recommend similar methodology for assessing the role of similar PT's in the replisome including intrabase transfer and the rejection of mismatches by polymerase. Asparagine N624's role in suppressing mutations can be tested experimentally. Further work is needed model the proton delocalisation in a time-dependent environemnt with an OQS treatment.

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