Proton Transfer and Mutations in DNA Replication Dynamics
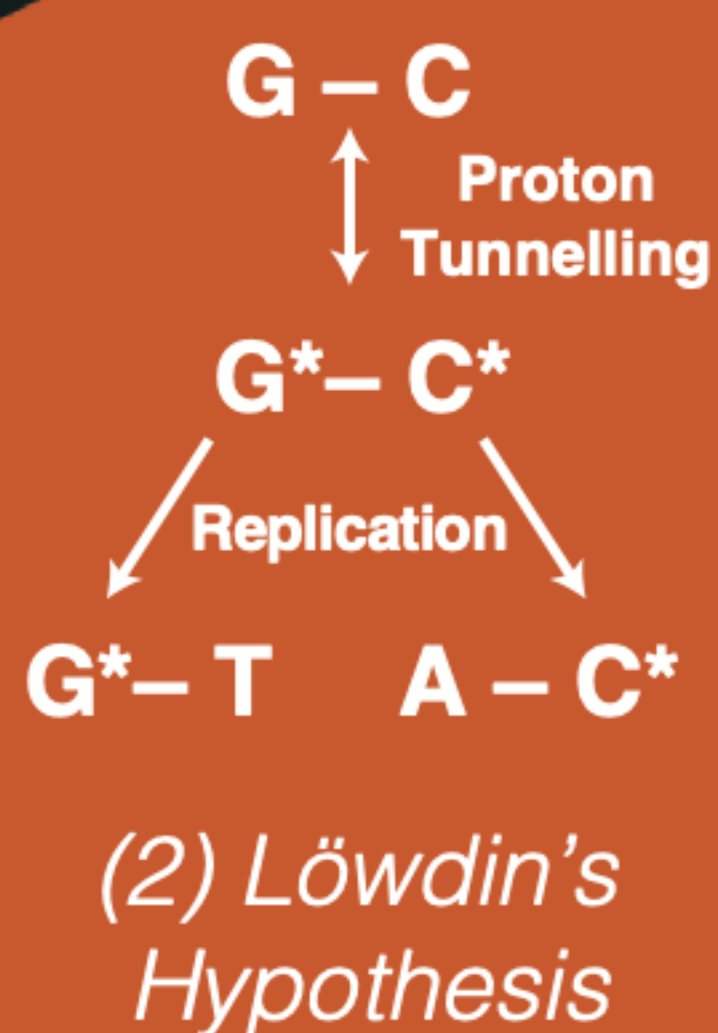
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Helicase induced strand separation

Background

$G - C$
Proton Tunnelling
$G^* - C^*$
Replication
$G^* - T \quad A - C^*$

(2) Löwdin's Hypothesis

Double proton transfer (DPT) in static G-C has been described by density functional theory.[1] Löwdin's Hypothesis (1) provides a mechanism for DPT to lead to mutations.[2] Literature has dismissed the plausibility of tautomers surviving DNA replication due to their short life time.[3]
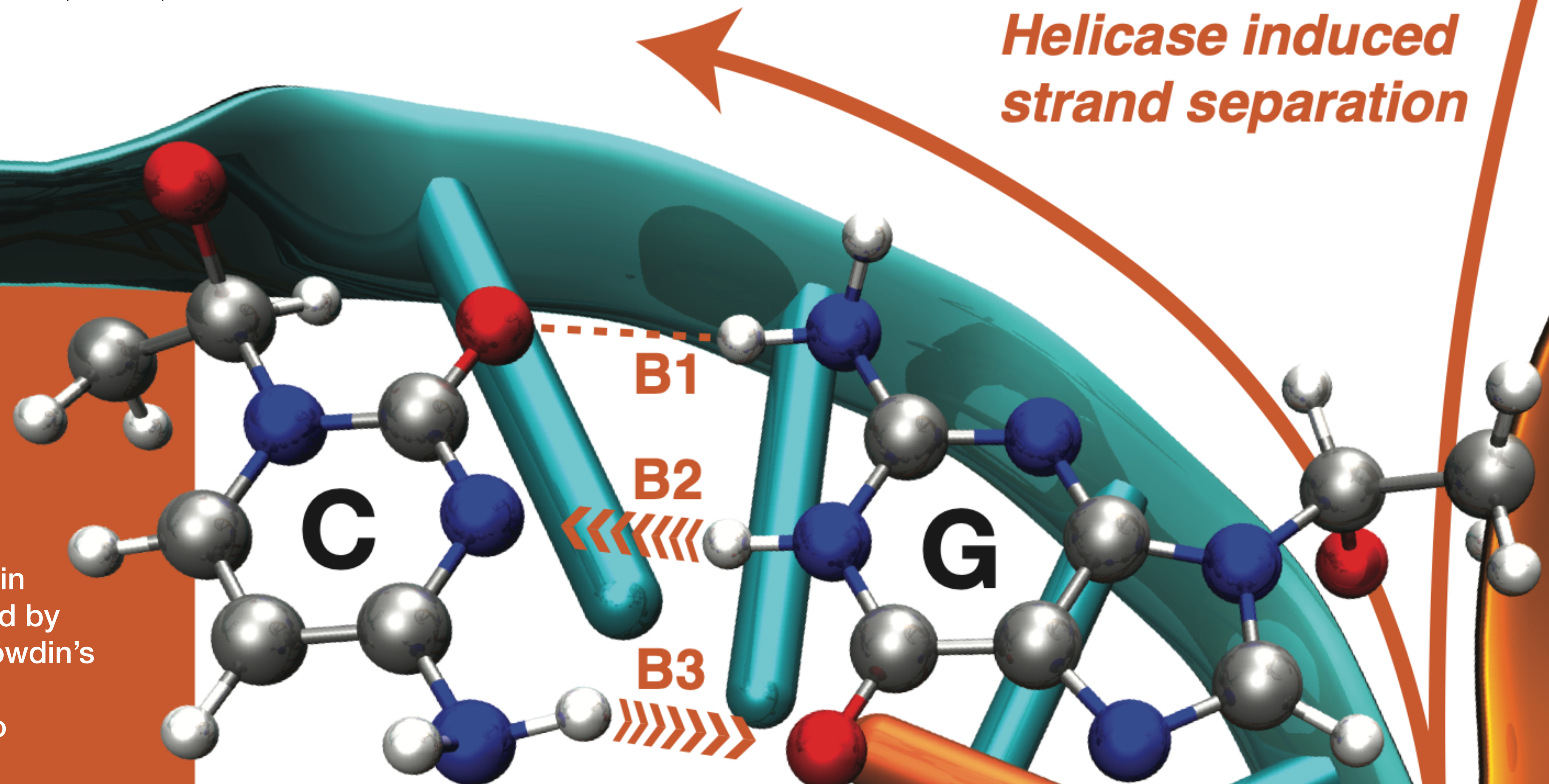
(1) Site of proton transfer during DNA unwinding

Modelling the Double Proton Transfer

- Density Functional Theory (DFT) provides an accurate model of quantum chemistry, yielding forces and relative energies for DNA mutagens.[1]

- A Nudged Elastic Band (ML-NEB) method is employed map the energetic landscape of the double proton transfer (DPT) reaction in G-C.

- For the first time we determine the effect of base pair separation on the DPT energetics in (3), we see that separation increases the tautomer stability. This separation arises in biology during unwinding.
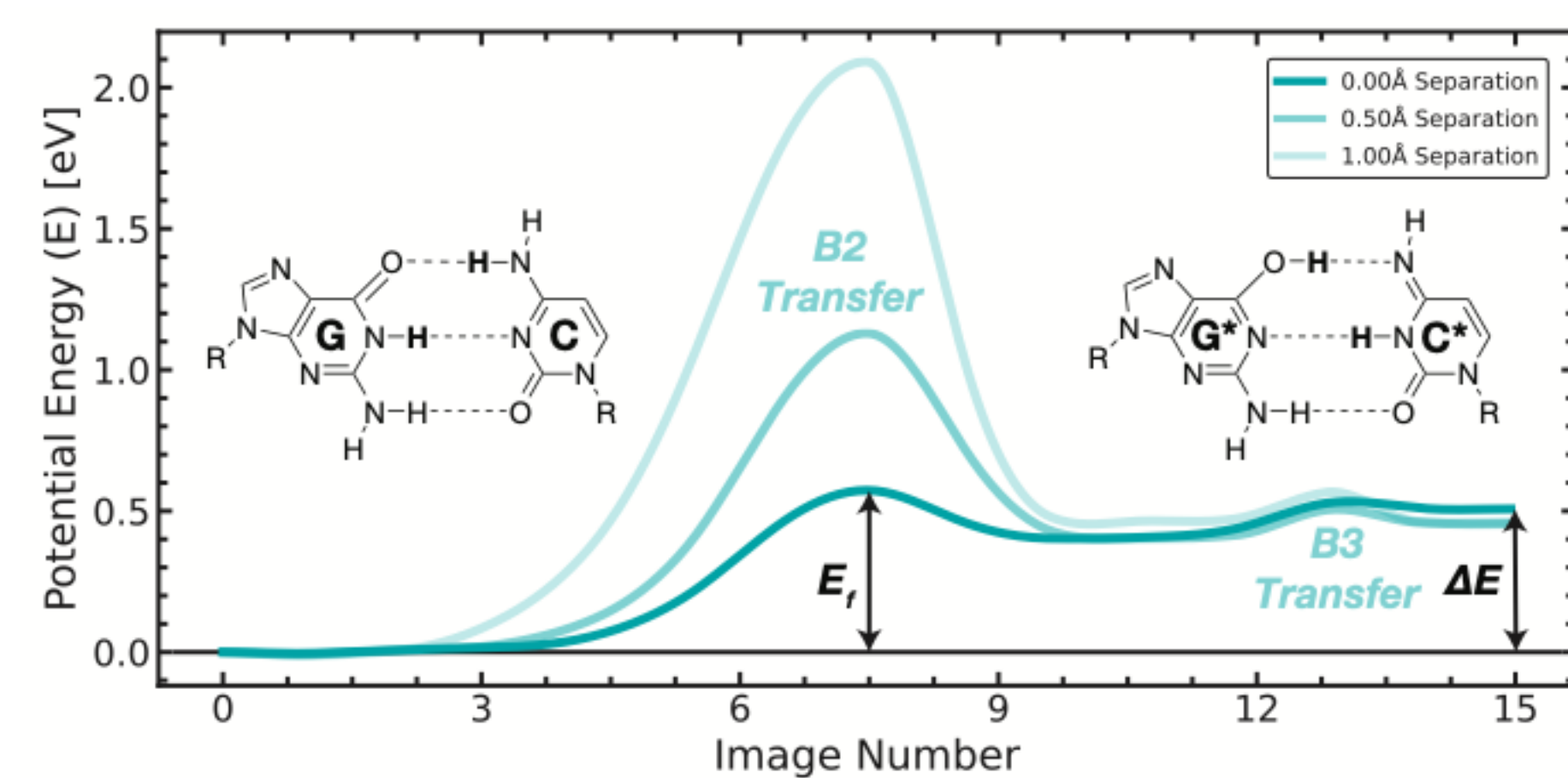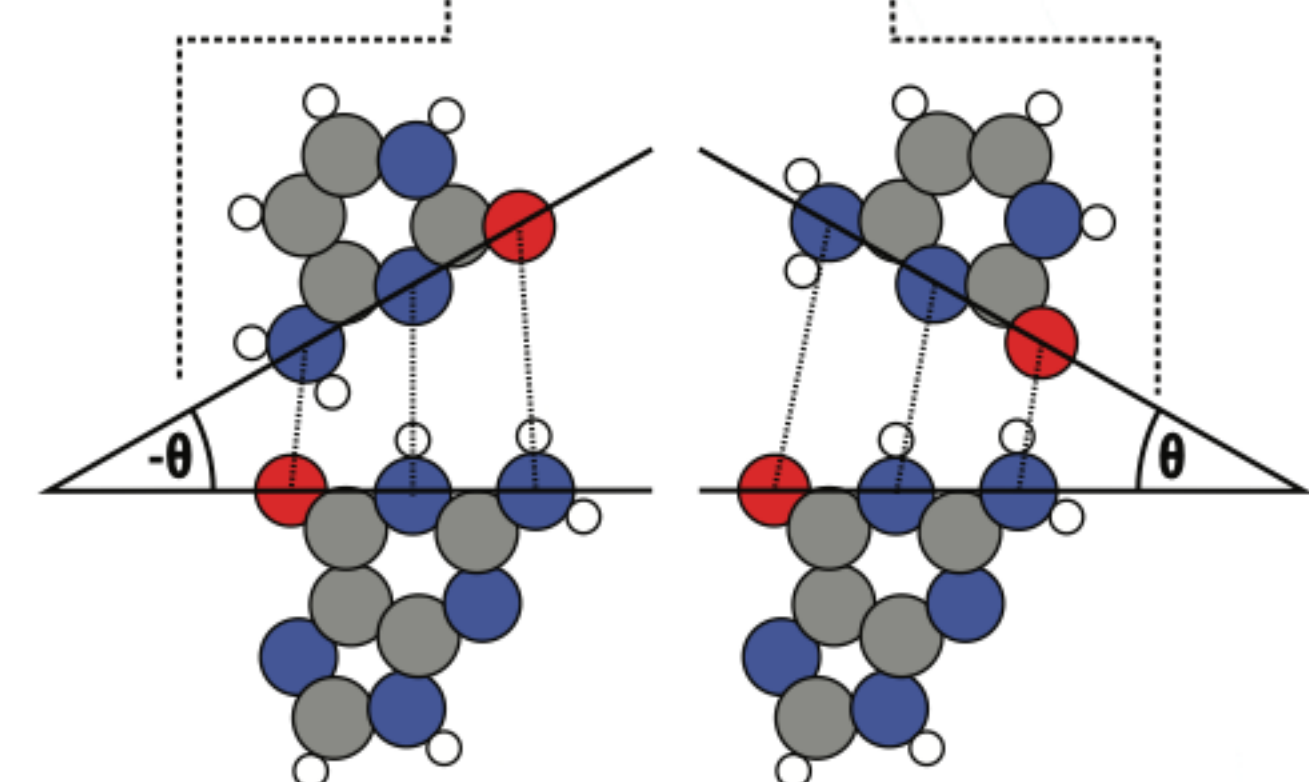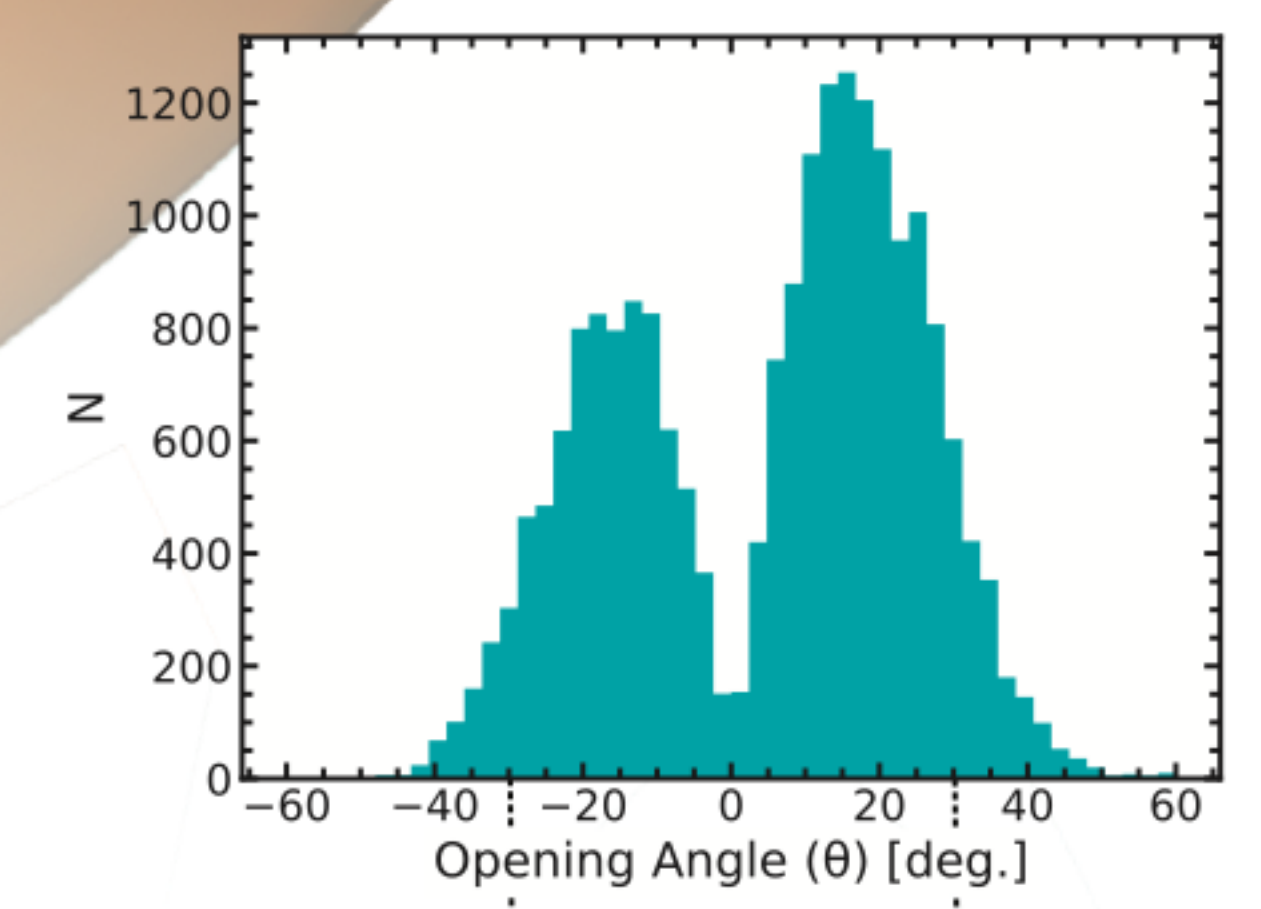

(3) DPT Energetics w/ Separation
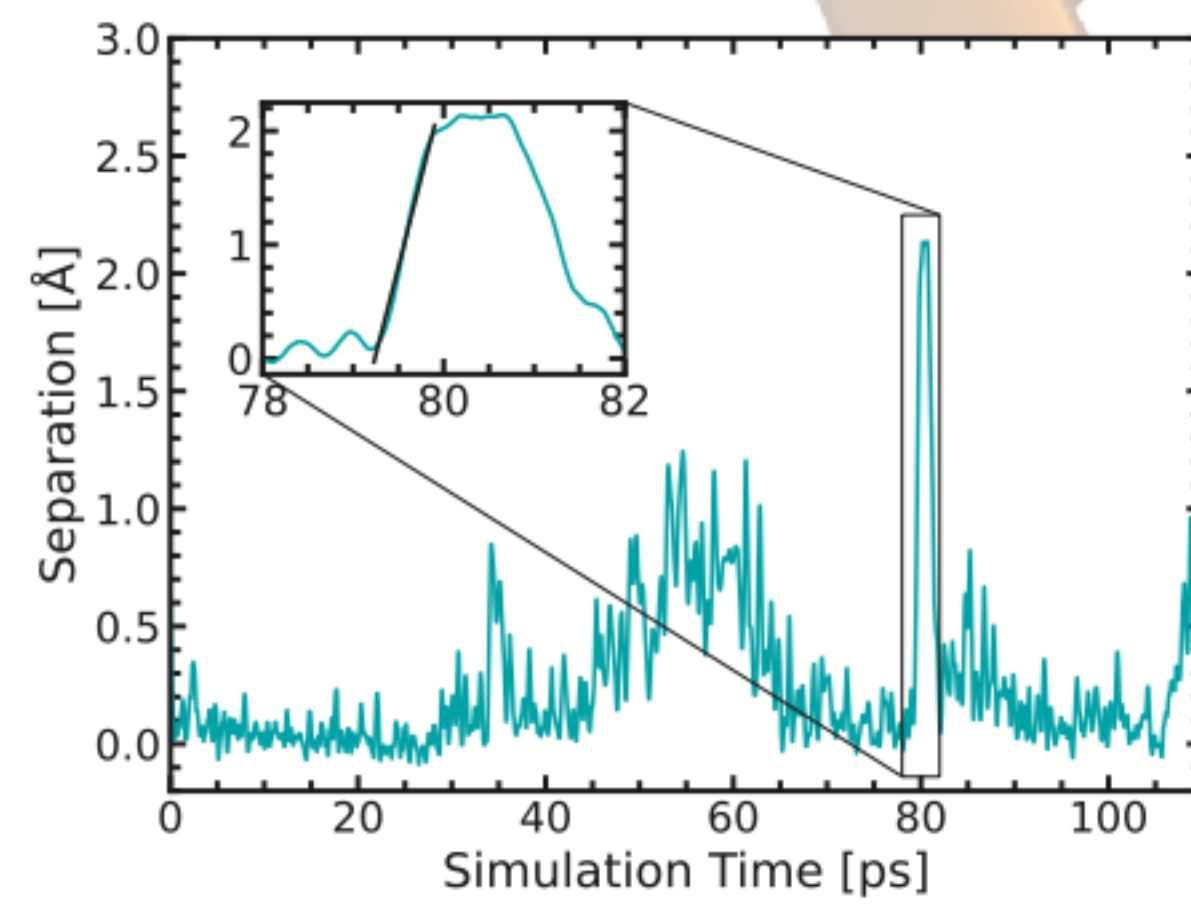
(4) H-bonds breaking in MD
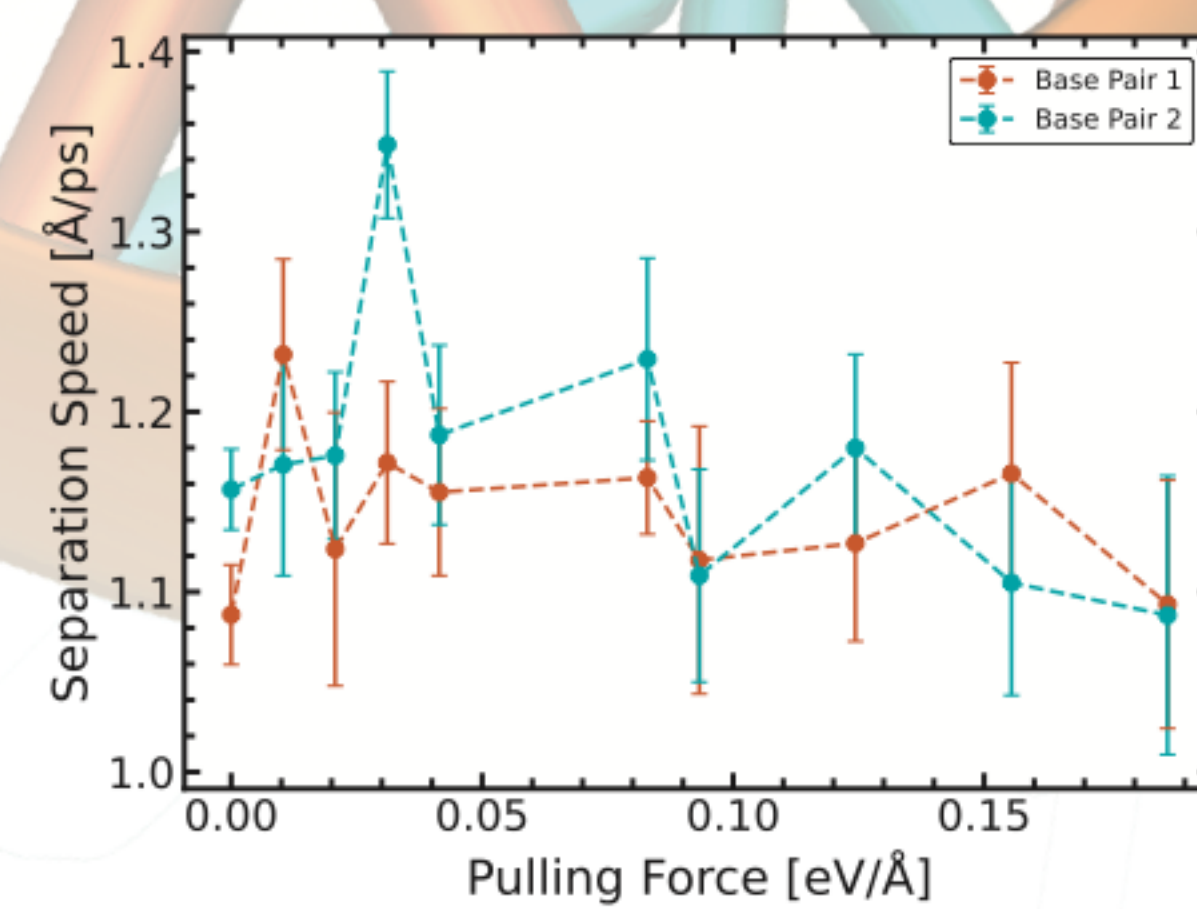

(5) Speed of GC separation

(6) Asymmetry of separation

Dynamical Simulations of Strand Separation

- Steered molecular dynamics (SMD) is used to simulate aqueous double stranded DNA (1). To model the action of Helicase, a force is applied to the terminal base pair.

- The dynamics of strand separation are revealed by measuring variation in hydrogen bond lengths (B1, B2, B3). An algorithm applies linear fits to the separation events (4).

- Figure (5) provides novel insight in to the atomistic speed of separation, largely unbiased by force and base pair choice, centring on 1.2 Å/ps.

- We find a bimodal distribution in the opening angle (6) peaked at 18.9±0.1 and -17.4±0.1 degrees.

Explicitly Modelling the Replisome

The bacterial enzyme PcrA Helicase (7) is a good starting point for exploring the DPT in the replisome. It is a small and well-studied ATPase that acts to unwind and separate DNA via a stepping motor-like action. To study the dynamics of this enzyme, we again choose to employ Steered Molecular Dynamics, applying forces as seen in (7). Additionally, we have applied multiscale reaction mapping techniques to reveal the effect of the local protein environment on the DPT at the active site of the enzyme. Our preliminary results indicate that the local environment plays an important role.

Force
Force
Site of Proton Transfer
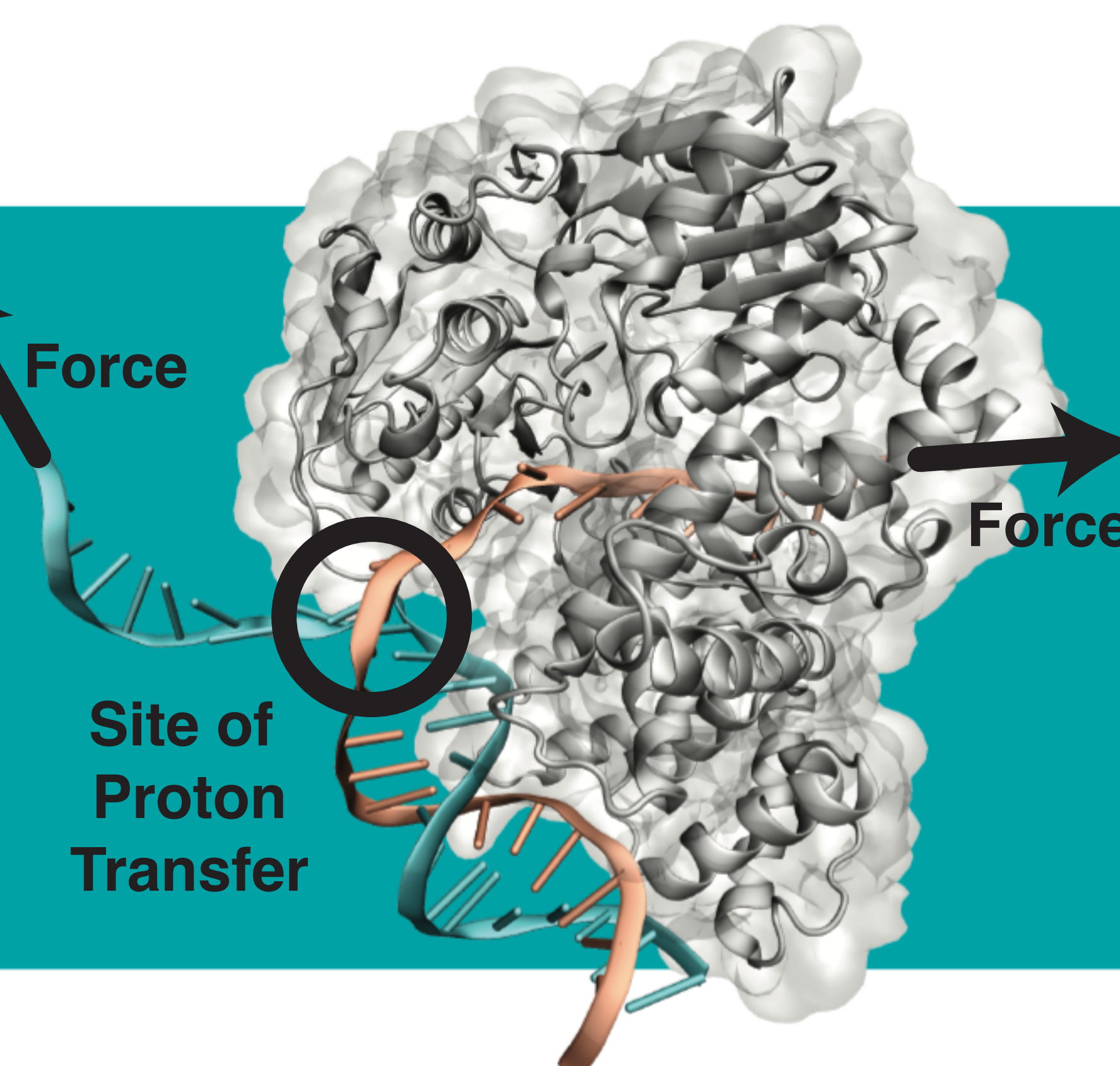
(7) PcrA Helicase-DNA Complex

Conclusion & Outlook

Our results show that DNA strand separation occurs at two magnitudes faster than experimental Helicase translocation speed, and that the tautomeric state is stabilised by such separation. We also indicate that the environment affects the DPT. Future work in G-C tautomerism must include such dynamics and an explicit protein environment. Fundamental physics can offer insight into the nature of the Life Code and cellular biology.

[1] L. Slocombe, J. S. Al-Khalili, M. Sacchi, Phys. Chem. Chem. Phys., (2021), 23(7), pp.4141-4150.
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[3] O. Brovarets', D. Hovorun, J. Biomol. Struct. Dyn., (2018), 37(7), pp.1880-1907.
[4] J. Yu, T. Ha, L. Schulten. Biophysical J., (2006), 91, pp. 2097.