Quantum Tunnelling Effects in the Guanine-Thymine Wobble Misincorporation via Tautomerisation

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Abstract

The misincorporation of a non-complimentary DNA base in the polymerase active site is a critical source of replication errors that can lead to genetic mutations. In this work, we model the mechanism of wobble mispairing and the subsequent rate of misincorporation errors by coupling first-principles quantum chemistry calculations to an open quantum systems master equation. This methodology allows us to accurately calculate the proton transfer between bases, allowing the misincorporation and formation of mutagenic tautomeric forms of DNA bases. Our quantum mechanic model predicts the existence of a short-lived “tunnelling-ready” configuration along the wobble reaction pathway, effectively compressing the energy barrier for this reaction and dramatically increasing the rate of mismatch formation by a hundredfold. Further, we calculate rates of genetic error formation that are in excellent agreement with experimentally
observed mutation rates, demonstrating that quantum tunnelling plays a critical role in determining the transcription error frequency of the polymerase.

DNA polymerase is an enzyme that catalyses the synthesis of DNA molecules by matching complimentary deoxyribonucleoside triphosphates (dNTP) to the template DNA strand using the standard Watson-Crick (WC) base pair rules. However, when a non-complimentary dNTP diffuses into the active site during the polymerase dNTP sampling, the polymerase domain will transition from an open to an ajar conformation. Thus, forming a different non-standard hydrogen-bonded base-pairing arrangement called a wobble mispair. While the usual (canonical) A-T and G-C base pairs have a characteristic WC geometries, wobble mispairs, such as wobble(G-T) and wobble(A-C), distort this geometry which is normally correctly identified by the polymerase. While there are other sources of replication errors, the fidelity of replication primarily depends on the ability of polymerases to select and incorporate the correct complementary base (see Fig. 1) and reject these wobble mispairs. However, it is proposed\textsuperscript{1–6} that the wobble mismatch can form alternative tautomeric configurations that can mimic the WC geometry and lead to erroneous DNA base matches, such as wobble(G-T)→G*-T, where G* is the tautomeric (enol) form of the G base.\textsuperscript{7} Watson-Crick-like mispairs have been observed in the active sites of DNA polymerases,\textsuperscript{8–10} and ribosomes in enzymatically competent conformations.\textsuperscript{11–13} Both NMR relaxation dispersion experiments and simulations\textsuperscript{1–3} indicate that the concentration of tautomeric mismatches in the cellular environment is significant and has a considerable impact on the replication fidelity of the polymerase. They have all demonstrated that the population of Watson-Crick-like G-T mispairs depends on the local environment, such as the base sequence and the local solvation environment.

Recent theoretical work on the Watson-Crick bonded bases entering the helicase enzyme has shown that quantum effects lead to the formation of meta-stable tautomeric forms of DNA.\textsuperscript{14,15} Quantum chemical models of G-C and A-T base pairs\textsuperscript{14} describe the potential energy surface (PES) of the double proton transfer in both canonical base pairs. The main
difference between the A-T and G-C PES is that A-T has a considerable forward barrier for the tautomer formation, but a small reverse barrier that causes its tautomeric form to be highly unstable. On the other hand, G-C has a sizeable reverse barrier, giving a tautomeric lifetime that is comparable to the replication process. Moreover, quantum tunnelling leads to a fast proton exchange between the bases, such that the timescale of the helicase cleavage is much slower than the proton transfer dynamics. Consequently, using a semi-classical interpretation, the potentially mutagenic tautomeric form is continuously formed and destroyed over timescales several orders of magnitude quicker than the helicase cleavage, after which the bases are split into their monomeric forms. However, using a quantum interpretation, the tunnelling proton’s wave functions evolve on a shorter timescale such that two clear probability distributions (in the canonical and tautomeric configuration) emerge. As demonstrated in previous paper, once the tautomer is formed and the DNA is opened, it is stabilised and is unlikely to revert to its canonical form due to a prohibitively large reaction barrier.

Recent NMR experiments using isotopic substitution suggests that when the DNA enters the polymerase active site, the wobble tautomerisation reaction might be facilitated by tunnelling. Rangadurai et al. investigated the dynamics of the transition between a wobble and Watson-Crick-like G-T in duplex DNA by performing NMR relaxation dispersion in both H$_2$O and D$_2$O. The authors reported that the kinetic isotope effect (KIE) in the
exchange rate between the two conformations of the mismatch was threefold slower in heavy water. This result provides the first experimental evidence supporting the hypothesis that quantum effects are involved in wobble tautomerisation.

In the replication machinery, during the polymerase dNTP sampling, the sample is rejected if G is mismatched against T. We investigate a pathway that connects the wobble mismatch to a Watson-Crick-like pairing (shown as pathway 1 in Fig. 1), leading to base misincorporation through a phosphodiester bond formation. In this scheme, proton transfer must occur for the bases to slide to a Watson-Crick-like pairing, either classically, via an “over the barrier” mechanism, or via quantum tunnelling. To avoid replication errors the polymerase should reject such mismatches; otherwise, the wrong base pairing can undergo further proton transfer, connecting two Watson-Crick-like tautomeric forms (shown as pathway 2). Additional pathways are explored in supplementary note 3.

We investigate the reactions: wobble(G-T) ⇌ G-T* (reaction 1) and wobble(G-T) ⇌ G*-T, whereby the reactants start as a wobble mismatch and, via proton transfer, result in a Watson-Crick-like conformation. We determine that the reaction wobble(G-T) to G*-T proceeds through a G-T* intermediate state in a step-wise mechanism. The minimum energy pathway can therefore be described by two steps; in the first step, the wobble(G-T) passes through the transition state of wobble(G-T) ⇌ G-T*. In the second step, through an intermediate local minimum and, the G-T* intermediate converts to G*-T. In comparison, the G-T* reaction (reaction 1) contains one transition state with no intermediate minimum.

Fig. 2 shows the minimum energy path of this reaction, for which the forward barrier is 0.926 eV and the reverse barrier is 0.680 eV. We perform a normal mode analysis to calculate the free energy values of the reactant, transition state, and product. We determine that the free energy values are smaller than the electronic energy barriers. The free energy contributions reduce the forward by 20% and the reverse barrier by 30%.

On the wobble(G-T) ⇌ G-T* (reaction 1) reaction path we observe three regions (see Fig. 2). The first region (0-4 Å) largely corresponds to the collective movement of the bases
Figure 2: Minimum energy path of the wobble(G-T) ⇌ G-T* proton transfer reaction pathway. The reaction paths are obtained using a machine learning approach to the nudged elastic band method. The reaction path contains classical rearrangement of the bases and a high reaction barrier where we suppose the proton can tunnel through. Relative to each other as they drift to a Watson-Crick-like bonding angle. In this region, the $\Delta E$ is essentially constant as the molecules move over a flat PES in which weak Van der Waals interactions dominate. The fast and activated proton transfer occurs between 4-7.5 Å. In this region, the proton of the Thimine N-H bond first transfers to the oxygen of the carboxylic group of G (as described by the arrow in the transition state of Fig. 2). The same proton subsequently hops back to the nearest oxygen of T. Finally, the region of the reaction path closest to the product (> 7.5 Å) corresponds to a further collective translation of the bases toward a Watson-Crick-like configuration, with little rearrangement in the bond of the transferred proton.

Despite several previous attempts to model the creation of G-T wobble mismatches,\textsuperscript{3,4,22} the presence and role of quantum effects in this reaction have not been addressed, with previously reported semi-classical models severely underestimating the experimental reaction rates. In the following, we introduce a first-principles based quantum dynamic approach for modelling proton tunnelling in a realistic cellular environment, which accounts for the
noise and thermal fluctuations of the biological system. We then employ this method to calculate the G-T wobble mismatch reaction pathway to the Watson-Crick-like configuration and the double proton transfer scheme in the Watson-Crick-like configuration (see Fig. 1). Quantum and classical contributions to the reaction rate are determined, and we discuss the contribution of proton tunnelling in forming Watson-Crick-like tautomers within the polymerase active site.

The open quantum systems (OQS) approach employed in this study is based on Caldeira and Leggett’s quantum Brownian Motion model in which the protons in the hydrogen bonds are embedded in an Ohmic bath of quantum oscillators, which represent the cellular environment. The interactions between the DNA and the environment are integrated over time using the path integral formalism introduced by Feynman and Vernon. The equivalent phase-space version is given by the Wigner-Moyal Caldeira and Leggett equation (WM-CL),

$$\frac{\partial W}{\partial t} = -\frac{p}{\mu} \frac{\partial W}{\partial q} + \frac{\partial V}{\partial q} \frac{\partial W}{\partial p} - \frac{\hbar^2}{24} \frac{\partial^3 V}{\partial q^3} \frac{\partial^3 W}{\partial p^3} + O(h^4) + \gamma \frac{\partial p W}{\partial p} + \gamma \mu k_B \tilde{T} \frac{\partial^2 W}{\partial p^2}. \tag{1}$$

Here, $W$ is a quasi-probability density encapsulating the proton’s quantum state as a function of both position ($q$) and momentum ($p$). The first set of terms in Eq. 1 corresponds to the Schrödinger dynamics of a particle with effective mass $\mu$. The subsequent two terms correspond to the dissipation and decoherence arising from the coupling to the quantum bath. Here, $\gamma$ is the phenomenological friction constant that describes the strength of the coupling to the bath, $k_B$ is Boltzmann’s constant and $\tilde{T}$ represents the effective bath temperature.

Assuming that the system-to-environment coupling constant is dominated by the thermal fluctuations of the surrounding water molecules, we can estimate the value of $\gamma$. Water has a vibrational spectrum in the range $3300-3900 \text{ cm}^{-1}$. So, assuming that the fastest oscillators in this range dominate, we use an Ohmic spectral density for our environment oscillators having a coupling parameter $\gamma = 3900 \text{ cm}^{-1}$. We determine the quantum contribution to the
reaction rate by monitoring the flux of the density passing through the transition state, see supplementary note 2 for further details. The forward and reverse reaction rate constants, $k_f$ and $k_r$, are obtained from,

$$k_{f,r} = \frac{\kappa}{h\beta} e^{-G_{f,r}\beta},$$  

(2)

where $\beta = 1/(k_B T)$ and $G_{f,r}$ corresponds to the Gibbs free energy barrier of the forward and reverse reaction, respectively. The tunnelling factor, $\kappa$, encapsulates the quantum-to-classical contribution to the rate, incorporating quantum effects such as tunnelling and non-classical reflections.

Firstly, we determine the quantum and classical rates for reaction 1 using our open quantum systems approach. Reaction 1 has a prohibitively high and wide reaction barrier (see Fig. 2) resulting in a low classical and quantum reaction rate. We evaluate that the quantum-to-classical ratio is small, $\kappa = 1.48$, suggesting that tunnelling is negligible. We find that the overall reaction rate is $1.907 \times 10^{-3} \text{s}^{-1}$ and is therefore many orders of magnitude smaller than the dNTP unbinding rate, which is of the order $70,000 \text{s}^{-1}$. Furthermore, we determine the effect of isotopic substitutions on the reaction rate and found that the reaction rate is essentially unaffected by deuterium substitution (KIE=1.1). Due to the slow reaction rate, the dNTPs unbinding rate and subsequent base rejection compete with the proton transfer mechanism. As a result, statistically, some of the wobble mismatches will eventually diffuse from the polymerase’s active site before proton transfer occurs. Since the diffusion timescale competes with the proton transfer timescale, the final population of tautomers incorporated will be reduced as accounted for by the kinetic network in Ref. 2.

If one applies classical transition state theory to the PES described in figure 2, the tunnelling rate would be negligible because the barrier width would be on the order of 4.0 Å. Thus, the overall (classical) reaction rate would be $1.907 \times 10^{-3} \text{s}^{-1}$, much slower than experimentally measured ($0.6-68 \text{s}^{-1}$). However, we note that the PES in Fig. 2 describes three fundamentally different molecular motions, and only the inner barrier (section 2 in Fig. 2) corresponds to the proton transfer between the bases. In contrast, regions 1 and 3
correspond to overall translations of the bases without significant changes in the hydrogen bond length. This PES topology is compatible with a tunnelling-ready state composed of the reactant’s activated structure seen at the end of region 1. The activation process concerns the reorganisation of the heavy atoms where thermal energy is required for the reactants to reach an activated tunnelling-ready state, whereby the reactant and product states become similar in energy.

The subsequent minimum energy pathway is shown in Fig. 3. Here, the reaction pathway shows three pseudo-minima corresponding to the bases already part way slid into a Watson-Crick-like shape, the second, where the proton has transferred to the other base, and the third, the return of the proton back to the same base. The first minima have an energy difference of 0.621 eV compared to the ground state. Assuming this configuration is explored with a penalty in the form \( \exp(-E/k_B T) \) gives a weighting of \( \sim 3.18 \times 10^{-11} \). The last two pseudo-minima indicates that, if we assume that the proton transfer is much faster than the rest of the atomic motion during the reaction, that a bifurcation of the reaction pathway is possible. In fact, after the first initial proton transfer, the rest of the atoms could rearrange, trapping the population in the middle well. This expected distribution of outcomes is consistent with the NMR experiments, which suggests the fast interchange between the Watson-Crick-like configurations but a slow initial formation due to the prohibitively high initial rearrangement cost to facilitate the transfer.

In order to calculate the contribution of quantum tunnelling in this activated tunnelling-ready state, we re-evaluate the PES considering only where the proton is transferring. We then calculate the rate of proton tunnelling from the tunnelling ready state through region 2. We calculate the inner barrier section (between 4-7.5 Å) by taking the image of the start of the barrier from reaction 1 and assuming that the local polymerase environment has thermally induced this conformation change. Using this approach, we calculate the quantum contribution to the reaction rate and find that the overall rate is much larger. The rate is now \( 1.279 \times 10^{-1} \text{s}^{-1} \), with a \( \kappa = 99.0 \) indicating a large contribution from tunnelling. By
substituting hydrogen with deuterium, we find that the tunnelling-corrected reaction rate exhibits a KIE of 10.15, which is compatible with experimental results\(^2\) that predict a three-fold reduction in rate. Consequently, by adopting the tunnelling-ready state we are able to bridge the timescale gap between experimental observation (0.6-68 s\(^{-1}\)) and the rate predicted by theory (1.279 \times 10^{-1} s^{-1}). The tunnelling-ready mechanism for proton transfer could be facilitated via a concerted mechanism between the transition of the polymerase domain from the ajar to the closed conformation, which is consistent with Li et al.\(^3\) The conformation change could sample the short-lived tunnelling-ready state where the reactant and products become similar in energy, increasing the quantum contribution to the rate.

For the G*-T ⇌ G-T* (reaction 2 in Fig. 1), the barrier is considerably smaller than for the wobble transfer reaction (reaction 1 in Fig. 1), 0.356 eV vs 0.926 eV. As shown in Fig. 4, the region of the PES comprised between 0.0 and 0.5 Å corresponds to the small translation of the two bases towards each other to facilitate the transfer. First, the middle proton in the N-H-N bond transfers (denoted by the arrow in Fig. 4). Then, the O-H-O proton transfers, observed in the secondary hump after the transition state. We determine that the reduced
mass of the double proton transfer is 1.49 $m_p$ at the transition state (see supplementary note 4).

Figure 4: Minimum energy path of the $G^*\rightleftharpoons G-T^*$ reaction. The double proton transfer reaction pathway, reaction 2, assuming the conversion to a Watson-Crick-like state has already taken place.

Here, the forward reaction barrier, $E_f = 0.356$ eV, and asymmetry $\Delta E = 0.017$ eV are small compared to the wobble-to-enol transfer and compares well with previous calculations ($E_f = 0.34$ eV$^3$). The low reaction barrier leads to a fast proton transfer with a large forward and reverse reaction rate on the order of $10^8$ s$^{-1}$. We use the OQS quantum dynamics model to determine a quantum-to-classical rate ratio of $\kappa = 18.1$ and a KIE of 4.25. The high $\kappa$ and KIE for this reaction suggest that quantum effects play a significant role in reaction 2 and that, due to the fast proton transfer timescale, the system can quickly reach equilibrium. After a single proton transfer successfully forms the tautomeric Watson-Crick-like form, the protons can continue to transfer between the bases (reaction 2) via a fast double proton transfer. Consequently, following another step of replication in the polymerase, an error will likely be induced on both daughter strands, as the enol forms will readily mismatch with the wrong base.$^{16}$

In summary, we have employed quantum chemical calculations to determine the reaction pathway of several reactions for generating tautomers of the G-T wobble mispair. We applied an open quantum systems approach to account for the bases’ decoherent and dissipative
local environment and identified quantum and classical contributions to the reaction rates. For the wobble(G-T) ⇌ G*-T mechanism, we find that the reaction proceeds via a step-wise process involving G-T*. Consequently, we focused on wobble(G-T) ⇌ G-T*. The proton transfer reaction from the wobble to the Watson-Crick pathway has a significantly high and broad reaction barrier, which implies an insignificant contribution from quantum tunnelling and a slow classical rate. By including a tunnelling ready state, where the wobble mispair has already partly translated into a Watson-Crick-like configuration, the reactant and products become similar in energy, enhancing the tunnelling probability. Our model predicts tunnelling rates that match the experimental NMR observed rates to a high degree of accuracy—opening the possibility that quantum mechanics is required to explain the biologically relevant functionality of polymerase.

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Data availability

The data for the reaction pathway is available on Github. Additional, data presented in this article are available from the corresponding authors upon reasonable request.

Author contributions

L.S., M.S., and J.A-K. conceived and designed this research. L.S. built the computational apparatus. All the authors contributed to the preparation of the manuscript.

Competing interests

The authors declare no competing interests.

Supporting Information Available

The Supporting Information files are available free of charge.

- GT wobble tunnelling ESI: The supplementary contains a detailed description of the methods and theory used to generate potential energy reaction surfaces and the parameters used to model the tunnelling.

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