nature portfolio

Peer Review File

An Open Quantum Systems approach to proton tunnelling in DNA

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors provided a theoretical study on the structure of DNA base pair, with a special emphasis on how quantum tunneling of the protons impacts on it. A one-dimensional model based on the Wigner-Moyal Master equation is chosen. It was found that proton tunneling (double) can induce much faster interconversions between the canonical and the tautomeric forms of the G-C base pair, than that from the classical description. The tautomeric occupation number is estimated. Overall, I think the manuscript presents a self-contained story, worth to be published. Two weak aspects are also clear, as:

1. Rigorousness of the model.
a) A one-dimensional model is a much too simplified model for a complex system like DNA base pair. Even in systems with much lower dimensionality, corner cutting effects in quantum tunneling had been shown important. In the system studied here, two protons are involved in the tunneling. Rearrangement of the heavy atom skeleton will play a central on determining the tunneling barrier height. I doubt the possibility of getting a reliable number from such a simplified treatment.
b) Even for the simplified model, a renormalized effective mass should be used to described the process when two protons tunneling. But it seems like the mass of proton is used directly in Eq. 1.
c) The potential energy surface is defined using a model. Nowadays, there are many quantum chemistry methods available. At least parameters should be achieved from a high-level quantum chemistry calculation, instead of being defined manually.

2. There is no comparison between the theoretical results with available experimental observations. By changing one or two parameters used above, e.g. barrier height, effective mass, the final occupation probability can change by orders of magnitude. Even when the experimental results become available, what sense does it make to compare an adhoc result with these experimental observations.

The picture presented will be of interest to readers who have research interest in the behavior of quantum tunneling in biosystems, or behaviors of DNA. I think it is worthy to be published on some specialized journal, but maybe not on this one.

Reviewer #2 (Remarks to the Author):

The authors have examined the tautomerisation of the G=C base-pair using an open quantum systems approach. They find that the contribution of quantum tunnelling to the reaction rate far outweighs classical barrier-hopping. They interpret their determination of the equilibrium occupation probability of the tautomeric state, 1.7e-4, as "[a] surprising result[] that could have far-reaching consequences", based on the supposition that the tautomeric state will "survive the DNA cleavage process in the helicase" and therefore contribute to mutation (ultimately depending on the efficacy of later DNA sequence repair mechanisms).

The results are (apparently) carefully calculated and well-presented but there are fundamental misconceptions in their presentation and interpretation, as well as important aspects of the design of the model system not being adequately justified.

It is assumed at the outset that the two (potentially tunnelling) proton exchanges required for tautomerisation are asynchronous, but no timescale is defined for the definition of "synchronicity": the authors' final conclusions would seem to require that movement of the second proton in the opposite direction (to preserve electroneutrality) occurs on a timescale much shorter than the lifetime of the tautomeric state. However there is no calculation, estimation or discussion of the energy or lifetime of the "double ion" state that is initially produced by the transfer of a single proton along just one of the hydrogen bonds. The energy of this state is in all probability quite high, but actual calculations can produce surprising results. The reader needs reassurance that the authors' single-proton-transfer calculation of the tautomerisation energetics is justified. This is an important problem which could upset the entire enterprise because the high energy of the "double ion" state could be equally easily "relieved" by rapid reversal of the first proton transfer rather than a second "asynchronous" proton transfer (the other one), the event needed to produce the tautomer. Furthermore, it is then necessary to ask "Under what assumptions would quantum considerations of the energy states of a tandem hydrogen bond system give the same results?" If one hydrogen bond transition causes enough of a disturbance in the other to cause its electron to transition to the other side, then there must be significant coupling between the two subsystems. Some argument justifying the authors' approach should be presented to allay such concerns.

The discussion of thermodynamic equilibrium (end of page 1 into page 2) is confused. The definition of the equilibrium constant under conditions of thermodynamic ideality in terms of the ratio of G=C state occupation probabilities (* = tautomer; 0 = canonical), K = p*/p0, is said to "assume[] perfect thermodynamic equilibrium, which is not necessarily true". This is an elementary misconception. There is only one form of equilibrium and that is equilibrium. It either exists within whatever thermodynamic constraints prevail or it does not. In that sense equilibrium is either perfect or it does not exist. This is not pedantic nitpicking. It goes to the heart of the biological significance of the authors' calculations (see later).

The authors' Gamma is not an "activity coefficient". Gamma is the ratio of the activity coefficients of the tautomeric (*) and canonical (0) states, gamma*/gamma0. The authors state that "without an accurate estimate of [Gamma], we cannot determine the quantity of interest, [p*/p0]". Thermodynamic non-ideality (gamma ≠ 1) arises when the chemical potential of a subsystem has a contribution due to interactions between the elementary entities or with the surroundings, usually involving changes in molecular potential energy. In the current calculation, the only interactions considered consist of energy exchanges between the G=C base-pair and the "bath" of harmonic oscillators (representing a simplified aqueous environment). I have no quibble with this approach, but it is obvious that Gamma can deviate from unity only if the interaction between the tautomer (*) and the bath is for some reason quantitatively different from the interaction between the canonical state (0) and the bath. The authors cite Cramer (2013) as justification for considering "e.g., quantum mechanical effects such as thermally enhanced tunnelling and zero-point energy", but I am not aware of Cramer ever having considered these effects as contributing to differential effects on the thermodynamic activity of alternative states of a system. In any case, any change in zero-point energy could, by its very nature, only make a contribution to the ideal activity of a state, not to some activity coefficient (gamma ≠ 1) embodying non-ideality. Even for a 2 component system (G=C base-pair plus bath) the thermodynamic activity of either component cannot be defined without specifying the constraints under which the chemical potential of that component is altered. All of this was carefully worked over by Terrell Hill in the 1950s (see below). In the current case, neither the tautomer nor the canonical state is able preferentially to store potential energy (+ve or –ve) in

the bath, in which case both the base-pair and the bath behave ideally, giving gamma* = gamma0 = Gamma = 1.

The authors then state that "we carry out a fully quantum mechanical calculation to obtain the G*-C* occupation probability within an open quantum system approach". Excellent, but we are then told that "proton delocalisation causes the strength of the hydrogen bonds in DNA base pairs to follow an anomalous temperature dependence due to the subtle balance of several quantum effects involved in the binding". The reader looks forward to a description of how proton delocalisation and other quantum phenomena affect the strength (free energy) of hydrogen bonds in DNA. At this stage the reader is led to believe that the results will shed light on the strength of the hydrogen bond rather than the dynamics of tautomerisation. But then the problem is divided into two independent parts, the first being "the potential energy landscape of the double H-bond between the C and G bases" (the strength of the hydrogen bonds) and the second being a model of "the proton dynamics using an open quantum systems (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment" (the dynamics of exchange between the alternative * and 0 states). Thus, two quite different aspects of the problem (energetics and dynamics) are obfuscated.

Next there is an admirably clear visualisation of the underlying problem (Figure 1) and its formal representation according to the authors' assumptions (Figure 2). It is not made clear, as it should be, that the energy change (0.435 eV) between the * and 0 states absolutely dictates that the classical thermal equilibrium constant for the model system would have to be K = exp[(–0.435 eV)/kT] = 5.4e-8 (at T = 300 K, kT = 0.026 eV). However, with the spacing between the eigenstates of the system being much greater than kT, the validity of an "exp(–Delta_E/kT) thermalisation" calculation of the p*/p0 ratio cannot be assumed, not because of any "non-ideality", rather because of the energy separation (>> kT) of the eigenstates (analogous to way the vibrational states in an ideal gas of diatomic molecules are occupied at ordinary temperatures). The * and 0 probabilities under any circumstances presumably depend on the non-zero amplitudes of the wave-functions in the * and 0 regions of the reaction coordinate q and the fractional occupations of each of the eigenstates under those circumstances. At equilibrium (single electron Maxwell-Boltzmann distribution over the states of the system) we cannot expect the ratio p*/p0 to be the same as it would be in the high temperature (classical) limit, exp[-Delta-E(reaction)/kT] = 5.4e-8. The authors calculate a value of p* = 1.73e-4 (and therefore p0 = 1 – p*) which is 3200 times greater than the naïve classical result, 5.4e-8.

It is, in principle, possible to calculate the equilibrium distribution p*/p0 without any dynamic considerations whatsoever. However, the dynamic calculations conducted in this study are analogous to observations made in temperature-jump experiments and the like when the system is placed in an initial non-equilibrium state and the time evolution of its rapid approach (return) to equilibrium is monitored. The perturbation from equilibrium chosen by the authors is the non-thermalised p0 = P(E_omega) = 1 distribution. The transition to equilibrium requires the absorption of heat from the bath until a final stationary distribution between the eigenstates of the system (equilibrium) is reached and k_f x p0 = k_r x p*. (We can take this to be a definition of the forward and reverse rate constants in terms of the necessary equality of the forward and reverse rates at equilibrium.) We observe that the calculated tunnelling factor, 10^5, increases both k_f and k_r by the same factor according to Eq.(10) and similarly shortens the lifetime of both the reactant and product by the same factor of 10^5. This means that something has gone horribly wrong. If k_f and k_r increase by the same factor, then p*/p0 at equilibrium must remain the same, but the authors'

calculation of this ratio is not the same as the classical value.

My opinion is that the value of p*/p0 obtained by the authors is an improvement over previous calculations, and that the difference is not because of tunnelling or non-ideality, rather because of the equilibrium distribution of eigenstate occupations. Nor do I doubt that the tunnelling factor calculation is reasonable, but I question the validity of using Eq.(10) in which the rate constants depend only on the heights of the activation barriers E_f,r and take no account of the radically discrete quantum character of the eigenstates that transverse both sides of the reaction pathway; nor the distribution of their fractional occupations in the * and 0 staes of base-pairing. (This may also cause problems with Eq.(8) and the calculation of the tunnelling factor, but I leave that to the authors.) That is to say, having justified and taken a "low temperature" approach to the dynamic problem, the authors should take a similar approach to the description of the equilibrium distribution over the individual states of the system and the manner in which this affects the dynamics of forward and reverse reactions (which is exactly what they have implicitly calculated to find the equilibrium endpoint).

Last of all I will comment on the biological significance of these results. The authors discuss this in relation to helicase catalysis of double-stranded unwinding, which requires the breaking of the hydrogen bonds that are simulated in this study. The argument presented is that the tautomerisation exchange is so rapid that the helicase breaking of hydrogen bonds simply "traps" the equilibrium p*/p0 ratio in the single-stranded product. However, I think a much more sophisticated argument is needed, one which takes into account the manner in which the potential energy surface (on which the rapid tautomerisation takes place) is distorted as the hydrogen bond is, on a timescale much longer than electron tunnelling, stretched and finally broken. The answer may end up being the same, but it will be much better justified, and may differ between (i) thermal melting of the bonds, (ii) passive "Brownian ratchet" helicase action and (iii) dissipative "stepped motor" helicase action. These need to be considered to do justice to the real physical situation in any discussion of its biological significance. Furthermore, the authors' result may be relevant to the origin of life. According to Eigen & Schuster (Naturwissenschaften 64, 541 565 (1977)), single stranded RNA phage replicases have error rates ~5e–4, suggesting that the early enzyme-controlled replication of nucleic acids may have been accuracy-limited by the tautomerisation reaction studied by the current authors.

This paper presents two interesting predictions that will ultimately have to be confirmed or falsified empirically: p*/p0 = 1.73e-4 and KEI = 30. These predictions are interesting enough to warrant publication, but I think all of the problems of interpretation I have pointed out need to be dealt with carefully.

Minor typographical and stylistic anomalies

1. 7th line of abstract "accounts" instead of "account".

2. page 1, RH column, 5th line of paragraph, "sine" instead of "since"

3. page 2, RH column, 5th line of paragraph "proton" not "protons"

4. There is a minor discrepancy between the stated height of the forward reaction energy barrier in

the Figure 2 caption (0.705 eV) and the text: page 3, line 9 (0.704 eV).

5. It is not clear why Eq.(2) has been written as it is with the exponential factor appearing twice.

6. The Heaviside function appears in Eq.(2) but is not defined until much later.

References:

Hill TL (1956) J. Am. Chem. Soc., 1956, 78 (17), 4281-4284 DOI: 10.1021/ja01598a025

Hill TL (1957) J. Am. Chem. Soc., 1957, 79 (18), 4885-4890 DOI: 10.1021/ja01575a016

Eigen M & Schuster P (1977) Naturwissenschaften 64, 541-565 doi: 10.1007/BF00450633

Reviewer #3 (Remarks to the Author):

The article "An Open Quantum Systems approach to proton tunnelling in DNA" is an interesting paper in which authors have investigated the proton dynamics in the H-bond between the Guanine-Cytosine (G-C) DNA base pair using an open quantum systems approach based on the Wigner-Moyal Master equation to accounts for the interaction of the proton with the cellular environment.

Authors have corrected the Master equation, which works right at high temperatures, to employ it at biological temperature (low temperatures). To implement this low temperature correction in the Master equation, authors have replaced the thermal energy term of the equation with the zero-point energy. Assuming and environment such as “aqueous solution”, authors have stablished the phenomenological friction constant of the master equation with the collision spectrum of water. Authors have found several surprising results, such as quantum corrected half-lives which are much shorter than the classical ones, very high tunneling effect for the proton transfer ~105 and high kinetic isotope effects, 30.

Comments:

1) The methodology of this work is interesting and could offer some interesting perspectives, however, the validity of the Master equation low temperature correction should be analyzed more carefully. In this way, more than one system (pair G-C in this case) should be studied to provide robustness to their results.

2) Authors have obtained very different results (several orders of magnitude) higher than the classical ones for the G-C proton transfer. In addition, they do not have experimental results to support their findings. In biological systems, tunneling effects higher than 10 are rare, and values of ~100000 are too high even for pure gas phase reactions. Professor Warshel and coworkers have stablished a very limited effect of the tunneling effect in biological process (see J Phys Chem B. 2008 May 15;112(19):5950-4 and J. Phys. Chem. B 2001, 105, 33, 7887–7907). These are examples of enzymatic catalysis but could be extended to these DNA systems as well.

3) Authors should check this correction of the Master equation in a biological system in which the

rate constant is very well know and their results could be validated. Afterwards, they could extend the “algorithm” to these systems much more complicated.

Recommendation:
Employ the low temperature correction of the Master equation in one system with experimental data in order to validate it. Afterwards, authors could apply this new correction in biological systems without experimental data. Otherwise, their findings could be an artefact of the correction included.

Response to reviewer reports: An Open Quantum Systems approach to proton tunnelling in DNA

L. Slocombe, M. Sacchi, J. S. Al-Khalili

February 8, 2022

Thank you for giving us the opportunity to submit a revised draft of the manuscript "An Open Quantum Systems approach to proton tunnelling in DNA". We thank all the referees for their comprehensive reports. The level of detail in the referee's responses is extremely appreciated, as is their time and effort to provide insightful comments and suggestions. In light of the concerns raised by the referees, we have made significant improvements to the content and structure of the paper and have incorporated all of their suggestions. They raise several common themes, the most prominent of which is the adoption and the treatment of the double-well potential we use in the system Hamiltonian, which have now been carefully explained and justified. However, we clearly had not provided sufficient context to the reader concerning the origins of the potential derived from accurate *ab initio* quantum chemistry calculations consistent with the wider literature.

We have typeset parts of the referees' comments to clarify our responses. We have highlighted the changes we have made to address their comments. Outlined below are our responses and changes to the paper in line with their suggestions. Our response is formatted in the following way:

> *The referees' comments are in italics and coloured blue.*

Response Our response is written unindented adjacent the bold lettering.

- Explanation and location of the changes are shown here.
  The changes made to the revised manuscript are in red.

1 Referee 1

In this manuscript, the authors provided a theoretical study on the structure of DNA base pair, with a special emphasis on how quantum tunneling of the protons impacts on it. A one-dimensional model based on the Wigner-Moyal Master equation is chosen. It was found that proton tunneling (double) can induce much faster interconversions between the canonical and the tautomeric forms of the G-C base pair, than that from the classical description. The tautomeric occupation number is estimated. Overall, I think the manuscript presents a self-contained story, worth to be published.

Response We thank Referee 1 for their interest in the manuscript and their detailed review. We will now highlight the following key improvements made to the revised manuscript as motivated by the referee's comments:

- Adjusted the text to highlight the origins and the rigorousness of the potential.
- Improved the discussion on the mass of the system in context of other work.
- Added more discussion of the results in light of experimental evidence.
- Added supplementary calculations on the sensitivity of the tunnelling to changes to the potential and the mass. Please see the new supplementary information file.

1.1 Point 1

Rigorousness of the model: A one-dimensional model is a much too simplified model for a complex system like DNA base pair. Even in systems with much lower dimensionality, corner cutting effects in quantum tunneling had been shown important. In the system studied here, two protons are involved in the tunneling. Rearrangement of the heavy atom skeleton will play a central on determining the tunneling barrier height. I doubt the possibility of getting a reliable number from such a simplified treatment.

Response We apologise for not making our methods clearer. We do not employ a simplified one-dimensional model for describing the double proton tunnelling but have a fully unconstrained *ab initio* model (previously described in [1]). Indeed, we completely agree with the referee about the importance of the heavy atoms rearrangements during the proton transfer. The influence of the molecular restructuring and the motion of the carbon, nitrogen and oxygen atoms has been incorporated into the DFT calculation of the potential energy surface. The apparent single dimensionality, which results in the plot in Fig. 2, is due to the application of transition state theory to this system, which results in a single effective reaction coordinate [2] being displayed to represent the minimum energy reaction pathway for the tautomerisation. Using the potential energy surface from *ab initio* quantum chemical calculations [1], we have included the rearrangements of the rest of the base and not just the atoms involved in the hydrogen bonds. During the determination of the reaction coordinate, all atoms are unrestricted, and their motion is projected onto the reaction coordinate. Previously, the energetics of the proton transfer scheme in the A-T and G-C base pairs have been well studied by *ab initio* quantum chemical methods [1,3–9]. Furthermore, our potential energy surface has been extensively compared to other computational results reported in the literature [3–9] with excellent agreement.

We agree that explicitly including more degrees of freedom into the open quantum system Hamiltonian could be of interest. However, exploring the role of the fuller degrees of freedom of such a many-body system in enabling double proton transfer would increase the complexity of the model and the computational cost prohibitively and suggests that it is out of the scope of this work. Therefore, following Referee 1's comment, we have:

- We have updated the description of how we obtained the potential and its apparent single-dimensionality. Page 7, third paragraph on the left.
  The apparent single-dimensionality of the PES is due to the application of transition state theory to this system, which results in a single effective reaction coordinate being displayed to represent the minimum energy reaction pathway for tautomerisation.
- Updated the text in the methods section to stress that this approach has been used successfully in literature before. Page 7, third paragraph on the left.
  The approach of inserting the PES calculated from density functional theory into an OQS Hamiltonian has been successfully used before for proton transfer reactions [6].

- Updated the caption of Fig. 2 (showing the potential) to reflect the text better. See page 3.
  G-C double proton transfer energy landscape obtained from density functional theory calculations converted into a back-to-back double Morse potential. The first ten eigenstates are displayed. With values $E_0 = 0.049\,\mathrm{eV}$, $E_\mathrm{f} = 0.705\,\mathrm{eV}$, $\Delta E = 0.435\,\mathrm{eV}$.
- Described the potential 'corner-cutting' effects in the conclusion section as requested by the referee. See page 5, third paragraph.
  We would also like to highlight that adding further dimensions into the system Hamiltonian could lead to potential corner-cutting effects [10] which could further enhance the rate.
- In the manuscript, we neglected to define the full potential explicitly. Therefore, in light of Referee 1's comments, we have added the following text to the methods section on page 7, first paragraph on the left side.
  The double proton transfer reaction can be described as a PES obtained from *ab initio* quantum chemical methods (determined in Ref. [1] - see discussions of the calculations within). We described this PES using a back-to-back double Morse potential given by

$$\begin{aligned} V(q) =& V_1 \left\{ \exp\left(-2a_1\left[q - r_1\right]\right) - 2\exp\left(a_1\left[q - r_1\right]\right)\right\} \\ &+ V_2 \left\{ \exp\left(-2a_2\left[r_2 - q\right]\right) - 2\exp\left(a_2\left[r_2 - q\right]\right)\right\}. \end{aligned}$$

1.2 Point 2

Even for the simplified model, a renormalized effective mass should be used to described the process when two protons tunneling. But it seems like the mass of proton is used directly in Eq. 1.

Response The choice of the mass is related to the previous comment on dimensionality. At the transition state, the motion of a single proton dominates, and the collective classical rearrangement of the rest of the atoms of both bases can be neglected [6]. Thus, the dynamics of one proton dominates the tunnelling through the barrier [6] as the reaction is an asynchronous concerted process [3]. It has previously been determined that the rearrangements of the rest of the base and the DNA structure play a role in setting up the proton transfer reaction and the heavy-ion movements only dominate the outer regions of potential energy surface away from the transition state [1]. Ideally, one could incorporate several more dimensions where the additional mass ions could be explicitly included in the open system Hamiltonian. However, extending the OQS equation to 3N-6 degrees of freedom would make the model both theoretical and computationally intractable. In order to clarify the point raised by the referee, we have made the following changes:

- We added an additional reference and further clarified the adoption of the mass for this system. See page 6, the first section under methods.
  Studies on other proton transfer reactions suggest that the reduced mass at the transition state is dependent on the proton transfer process and can fluctuate to a much heavier mass far from the transition state [11]. Depending on the reaction pathway, if the two protons transfer simultaneously or not, the reduced mass of the system can tend to one or two proton masses [11]. In our case, at the transition state, the motion of a single proton dominates [1]. Consequently, it is acceptable to assume that the mass of the proton mass is in, a very good approximation, the effective mass of the tunnelling particle in the asynchronous concerted process [3]. Furthermore, the collective classical rearrangement of the rest of the atoms of both bases can be neglected.

1.3 Point 3

The potential energy surface is defined using a model. Nowadays, there are many quantum chemistry methods available. At least parameters should be achieved from a high-level quantum chemistry calculation, instead of being defined manually.

Response In fact, this is precisely what we have done. Clearly, we have not adequately explained this in the paper. Our potential energy surface is indeed determined from high-level quantum chemistry calculations (density functional theory) [1]. We are interested in how the two bonded DNA bases inter-convert between canonical and tautomeric states. However, most standard approaches exploring the importance of quantum tunnelling neglect the influence of the surrounding environment. Therefore, our approach is a two-step process whereby having obtained a potential energy surface from density functional theory calculations, this is then inputted into an open quantum system calculation to account for the decoherent and dissipative biological environment. As far as we know, there is no full conjunction of open quantum systems into density functional theory approaches yet. Additionally, if we directly adopt the results from the *ab initio* calculation, we neglect that outer edges of the potential would follow a Morse-like relation. Therefore, we believe our method of inserting a modified potential energy surface, obtained from *ab initio* calculations, into the open quantum system Hamiltonian is appropriate.

- We have clarified that the potential we use comes from high-level density functional theory calculations. See page 2 second to bottom paragraph.
  We use an accurate description of the potential energy landscape of the double H-bond between the C and G bases [1]; we model the proton dynamics using an open quantum system (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment.
- We further clarify our modifications of the potential from the density functional theory calculations. The clarifying comments can be found on page 7 below the equation defining the potential.
  In the limits of the reaction coordinate away from the well minima, we require that the potential rapidly increases, creating a bounding wall. The wall corresponds to the strong repulsive force as the bond between the hydrogen and the hydrogen-bond donator atoms shorten. Here, the back-to-back double Morse potential captures these requirements while maintaining the reaction profile obtained from high-level density functional theory calculations. The bounded potential can then be inserted into our open quantum system approach and solve the dynamics and the steady-state solution.

- The section title in the Methods section is misleading. We have updated it to reflect that the potential is not just a model. The title now reads,
  The Proton Transfer Potential

1.4 Point 4

We have split this point into two and addressed each part individually.

1.4.1 Comparison to Experimental Observations

> *There is no comparison between the theoretical results with available experimental observations. . . . Even when the experimental results become available, what sense does it make to compare an adhoc result with these experimental observations.*

Response We apologise that our wording was not clear; the potential is not *ad hoc*, but it has been obtained by performing accurate first-principles quantum chemical calculations [1]. We believe our previous responses have addressed the ambiguity of the origins of the potential.

While the proton transfer potential parameters (height, width, and reaction asymmetry) are well informed from high-level quantum chemistry calculations, currently, there are no available experimental observations on the nature of the potential energy surface connecting the canonical and tautomeric forms of DNA.

- To address the contention in the literature on the accuracy and nature of the proton transfer energy landscape, we have added an additional sentence on page 2, second paragraph on the right.
  Further discussion on the contention in the literature on the accuracy and nature of the proton transfer process in DNA can be found in Ref. [12], and [13].
- Regarding the lack of comparison between the theoretical results with available experimental observations, we have added discussion on wobble mismatch incorporation via tautomerism into the conclusion. See page 6, second paragraph on the left.
  The first [14] structural evidence for the rare tautomer hypothesis was observed using X-ray crystallographic analysis of a DNA polymerase. Whereby the polymerase was observed mismatching C with A. The mismatch adopts a Watson-Crick-like configuration which can only be obtained via the movement of a single proton on one of the mismatched bases. The proton transfer product alters the hydrogen-bonding pattern such that the mispair forms an overall shape that is virtually indistinguishable from a canonical, Watson-Crick base pair in double-stranded DNA. It remains an open question if the proton transfer has contributions from quantum effects.

  More recently, tautomerisation in DNA has been experimentally observed using NMR relaxation dispersion. Here, hairpin DNA duplex structures were observed to interchange between a wobble G-T and a Watson-Crick-like structure via tautomerisation (neutral) or ionisation (charged) [15,16]. This experimentally confirms that tautomerisation in DNA is a non-trivial effect.

  A positive indication that tautomerisation might be facilitated by tunnelling was provided recently by Rangadurai *et al.* [17]. They extensively 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_2O and D_2O. The authors reported a KIE as the rate of exchange between the two conformations of the mismatch was $\sim$ 3-fold slower in D_2O than in H_2O. This result provides the first experimental evidence supporting a transition state for tautomerisation involving proton tunnelling. However, before the observed KIE can be unequivocally

attributed to proton tunnelling, further measurements will need to be conducted to investigate its temperature dependence [18]. Since a definitive experimental hallmark of tunnelling is a temperature-independent rate at low temperature, the rate of classical over-the-barrier hopping becomes negligible at low temperatures, leaving only the quantum contribution [19, 20].

Experimental measurements of tautomerisation rates for G-C double proton transfer reaction are still not available. Nevertheless, we believe that our theoretical results force us to radically revise our understanding of the likelihood of point mutations in DNA, and we hope they will encourage new experimental measurements of the proton transfer reaction. Perhaps, a temperature jump experiment, similar to investigations conducted on intramolecular proton transfer in heterocycles [21], could elucidate the proton transfer rates.

One can note that our model predicts a rate of tautomerisation much higher than the overall rate of spontaneous mutations ($\sim 10^{-8}$) [22], but this is consistent with the well-known presence of highly efficient DNA repair mechanisms. However, it is also difficult to comment on the overall efficiency of these repair mechanisms because other spontaneous mutations will also take place in DNA aside from tautomerisation [23, 24].

1.4.2 Sensitivity of the Potential

... By changing one or two parameters used above, e.g. barrier height, effective mass, the final occupation probability can change by orders of magnitude. ...

Response We thank the referee for raising this important point. Changing the barrier height has less effect on the final occupation probability but more on the proton transfer rate. On the other hand, the reaction asymmetry has a much more significant effect on the occupation probability. For example, a smaller energy difference between the reactant and product results in a more equal occupation distribution between the two. We know the reaction asymmetry and barrier with a high degree of confidence (subject to the limitations of our quantum chemistry calculations).

More recent evidence has suggested that free energy interactions can provide a diverse range of energetic scenarios [7], suggesting that an ensemble of possible potentials can explain the proton transfer process. We agree that exploring a sensitivity analysis of the potential energy surface on the proton transfer reaction would be of general interest. However, this would require significant further work using ensemble multi-scale modelling combined with a correction for tunnelling.

- We have explored the dependence on the mass and determined that it significantly changes the rate and the occupation. Additional calculations on the sensitivity of the barrier and the mass can be found in a new supplementary information file. In summary, we determine that increasing the barrier height significantly impacts the dynamics; namely, it causes a striking reduction in classical rate and affects the tunnelling much less. As a result, if the proton transfer barrier were to be much larger, we would see an increase in the quantum-to-classical contribution to the rate. Nevertheless, the conclusion would still stand; the rates are so high that the equilibrium populations of the canonical and tautomeric forms would quickly be reached. We have updated the discussion to reflect this comment. See page 4, third paragraph on the right of the "Lifetime of the Tautomer" section.

  We performed a sensitivity analysis of the reaction barrier (see the supplemental information (SI) file) and determined that increasing the barrier height significantly impacts the rate of the thermally activated proton 'over-the-barrier' hopping dynamics; namely, it causes a striking reduction in classical rate, but it affects the quantum tunnelling rate much less. Furthermore, the tunnelling correction remains

so high that the equilibrium populations of the canonical and tautomeric forms would quickly be reached even if the barrier height was 50% higher than in our study.

2 Referee 2

The authors have examined the tautomerisation of the G=C base-pair using an open quantum systems approach. They find that the contribution of quantum tunnelling to the reaction rate far outweighs classical barrier-hopping. They interpret their determination of the equilibrium occupation probability of the tautomeric state, 1.7e-4, as "[a] surprising result that could have far-reaching consequences", based on the supposition that the tautomeric state will "survive the DNA cleavage process in the helicase" and therefore contribute to mutation (ultimately depending on the efficacy of later DNA sequence repair mechanisms). The results are (apparently) carefully calculated and well-presented but there are fundamental misconceptions in their presentation and interpretation, as well as important aspects of the design of the model system not being adequately justified.

Response We sincerely thank Referee 2 for their interest in the manuscript and their detailed review. We will now highlight the following key improvements made to the revised manuscript as motivated by the referee's comments:

- Expanded discussion on the proton transfer reaction profile and our potential in context of the wider literature.
- Clarified the wording on the thermodynamic equilibrium and the hydrogen bond strength.
- Added discussion on the phage replicases.
- Fixed the highlighted typographical errors.

2.1 Point 1

It is assumed at the outset that the two (potentially tunnelling) proton exchanges required for tautomerisation are asynchronous, but no timescale is defined for the definition of "synchronicity": the authors' final conclusions would seem to require that movement of the second proton in the opposite direction (to preserve electroneutrality) occurs on a timescale much shorter than the lifetime of the tautomeric state. However there is no calculation, estimation or discussion of the energy or lifetime of the "double ion" state that is initially produced by the transfer of a single proton along just one of the hydrogen bonds. The energy of this state is in all probability quite high, but actual calculations can produce surprising results. The reader needs reassurance that the authors' single-proton-transfer calculation of the tautomerisation energetics is justified. This is an important problem which could upset the entire enterprise because the high energy of the "double ion" state could be equally easily "relieved" by rapid reversal of the first proton transfer rather than a second "asynchronous" proton transfer (the other one), the event needed to produce the tautomer. Furthermore, it is then necessary to ask "Under what assumptions would quantum considerations of the energy states of a tandem hydrogen bond system give the same results?" If one hydrogen bond transition causes enough of a disturbance in the other to cause its electron to transition to the other side, then there must be significant coupling between the two subsystems. Some argument justifying the authors' approach should be presented to allay such concerns.

Response We thank the referee for pointing out that the specifics of the transfer process has not been made evident. These questions are crucial to the process, but we failed to make this clear to the reader. The use of the asynchronous tautomerisation process is well justified. The exact chemical procedure is highlighted in a previous paper [1] and is a topic that has been discussed in detail by several studies [3–5, 7, 9, 12, 13] but has not been adequately covered in the paper. The

term asynchronous is used in literature to describe the proton transfer process whereby the motion of a single proton dominates the transition state and is closely followed by a second proton.

- The asynchronous tautomerisation process is not merely "assumed" but is well established in the literature. However, the additional context behind the adoption of the mechanism had not been included in the paper; consequently, we have added a few sentences to provide further context. See the last paragraph on the first page.
  We assume, as previously observed [6, 25], that the two protons undergo asynchronous transfer, suggesting that the rate-determining process is the transfer of a single proton across the barrier, with the other proton then moving in the opposite direction to preserve electroneutrality. There is a substantial agreement in the literature that the proton transfer in GC is an asynchronous process, while the stepwise proton transfer pathway (in which a proton transfer before the second one) has been ruled out in favour of the double proton transfer scheme. The argument is that in the standard Watson-Crick configuration, the formation of the single proton transfer is either unstable or energetically unfavourable [3–5, 7].
- We added further signposting to existing literature, discussing the proton transfer mechanism and why the other mechanisms are not preferred. See page 2, second paragraph on the right.
  Further discussion on the contention in the literature on the accuracy and nature of the proton transfer process in DNA can be found in Ref. [12], and [13].

2.2 Point 2

The discussion of thermodynamic equilibrium (end of page 1 into page 2) is confused. The definition of the equilibrium constant under conditions of thermodynamic ideality in terms of the ratio of G=C state occupation probabilities (= tautomer; 0 = canonical), $K = p^*/p_0$, is said to "assume perfect thermodynamic equilibrium, which is not necessarily true". This is an elementary misconception. There is only one form of equilibrium and that is equilibrium. It either exists within whatever thermodynamic constraints prevail or it does not. In that sense equilibrium is either perfect or it does not exist. This is not pedantic nitpicking. It goes to the heart of the biological significance of the authors' calculations (see later).*

Response Correction accepted; we agree this needs to be adequately addressed. The non-ideality we mentioned was attributable to the "perfect ideality (as of ideal behaviour such as that of an ideal gas) of the reactant and product of this reaction". Therefore,

- We corrected our confusing statement in the text according to the comment made by the referee. See the last paragraph on the first page.
  The thermodynamic equilibrium constant, K_{eq}, is generally written as K_{eq} = [G*-C*]/[G-C] (the ratio between the concentration of products – tautomeric pair – and reactants – canonical pair – present at chemical equilibrium). However, the concentrations are modified by an activity coefficient.

2.3 Point 3

We split this point into two and address each part individually.

2.3.1 Regarding the activity coefficient

The authors' Gamma is not an "activity coefficient". Gamma is the ratio of the activity coefficients of the tautomeric (*) and canonical (0) states, gamma/gamma0. The*

authors state that "without an accurate estimate of [Gamma], we cannot determine the quantity of interest, [p^*/p_0]". Thermodynamic non-ideality (gamma ≠ 1) arises when the chemical potential of a subsystem has a contribution due to interactions between the elementary entities or with the surroundings, usually involving changes in molecular potential energy. In the current calculation, the only interactions considered consist of energy exchanges between the G=C base-pair and the "bath" of harmonic oscillators (representing a simplified aqueous environment). I have no quibble with this approach, but it is obvious that Gamma can deviate from unity only if the interaction between the tautomer (*) and the bath is for some reason quantitatively different from the interaction between the canonical state (0) and the bath. . . . In any case, any change in zero-point energy could, by its very nature, only make a contribution to the ideal activity of a state, not to some activity coefficient (gamma ≠ 1) embodying non-ideality. Even for a 2 component system (G=C base-pair plus bath) the thermodynamic activity of either component cannot be defined without specifying the constraints under which the chemical potential of that component is altered. All of this was carefully worked over by Terrell Hill in the 1950s (see below). In the current case, neither the tautomer nor the canonical state is able preferentially to store potential energy (+ve or –ve) in the bath, in which case both the base-pair and the bath behave ideally, giving gamma = gamma0 = Gamma = 1.*

Response We thank the referee for raising this point. In this study, we calculated the ratio between the product and reactant, [GC*]/[GC] directly by integrating over the Wigner distribution when $t \to \infty$ and compared our results with the equilibrium constant for this reaction reported by other authors. Since the calculated potential energy surface and the ΔG of the reaction are comparable to the literature results, the difference between our treatment and all previous calculations is that we do not assume the equilibrium to obey the standard thermodynamic equality $\Delta G = -RT\log(K_{\text{eq}})$. Since $K_{\text{eq}} = P/R \cdot \gamma_P/\gamma_R$, we made the assumption that all the non-ideal activity behaviour of GC and GC* can be incorporated into γ_P/γ_R. We understand and accept that this caused confusion. We corrected our analysis by assuming that the new "quantum" equilibrium constant $K_{\text{eq-quantum}} = K_{\text{eq}} \cdot \Gamma_{\text{qm}}$, with Γ_{qm} being a coefficient that takes care of the non-ideal behaviour of the system due exclusively to non-classical activity (tunnelling, zero-point energy, decoherence and dissipation). Since, as the referee correctly points out, there is no reason to assume that the coupling between GC and GC* with the thermal bath would be radically different, Γ_{qm} (not gamma-ratio) should likely be the source of most of the "non-ideal behaviour (where non-ideal means simply a behaviour far from that of an ideal solution).

- We changed the term used to describe the equilibrium discrepancy to clarify that this is exclusively a non-classical activity coefficient. See the first paragraph on the left on the second page.
  More correctly, a quantum activity coefficient, Γ_{qm}, needs to be included as a factor such that $K_{\text{eq-qm}} = \Gamma_{\text{qm}} \times$[G*-C*]/[G-C]. Which is exclusively a non-classical activity coefficient containing effects such as thermally enhanced tunnelling, zero-point energy, decoherence and dissipation, leading to an activity coefficient that is far from unity, . . .

2.3.2 The Cramer Citation

. . . The authors cite Cramer (2013) as justification for considering "e.g., quantum mechanical effects such as thermally enhanced tunnelling and zero-point energy", but I am not aware of Cramer ever having considered these effects as contributing to differential effects on the thermodynamic activity of alternative states of a system. . . .

Response Thank you for bringing this to our attention. We intended to provide a relatively recent textbook reference for calculations of thermodynamic properties from *ab initio* quantum chemical calculations. However, as pointed out, a reference from Hill's work would be a better fit.

- We have added a reference to Hill's work on statistical thermodynamics as this provides a better-suited reference for the fundamental descriptions of thermodynamic equilibrium. We have amended the manuscript accordingly. See the first paragraph on the left on the second page.
  ... in the same way that solvent effects can shift equilibrium [26].

2.4 Point 4

The authors then state that "we carry out a fully quantum mechanical calculation to obtain the G-C* occupation probability within an open quantum system approach". Excellent, but we are then told that "proton delocalisation causes the strength of the hydrogen bonds in DNA base pairs to follow an anomalous temperature dependence due to the subtle balance of several quantum effects involved in the binding". The reader looks forward to a description of how proton delocalisation and other quantum phenomena affect the strength (free energy) of hydrogen bonds in DNA. At this stage the reader is led to believe that the results will shed light on the strength of the hydrogen bond rather than the dynamics of tautomerisation. But then the problem is divided into two independent parts, the first being "the potential energy landscape of the double H-bond between the C and G bases" (the strength of the hydrogen bonds) and the second being a model of "the proton dynamics using an open quantum systems (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment" (the dynamics of exchange between the alternative * and 0 states). Thus, two quite different aspects of the problem (energetics and dynamics) are obfuscated.*

Response Thank you for pointing this out; the language used at the start could leave the reader confused. We do not explicitly calculate the hydrogen bond strength and variation in our work. However, it is possible to draw a parallel between the facility of the proton transfer from donor to the acceptor and the hydrogen bond "weakening". The relationship between the proton transfer facility and the strength of the hydrogen bond has been previously discussed in several theoretical papers, including Wei *et al.* [27] and Lu *et al.* [28]. For "weakening", we, therefore, mean the overall delocalisation of the protons involved in the hydrogen bond. Or alternatively, the facility of the bond to fluctuate between the two potential configurations, the canonical and tautomeric. We need to make this reasoning clearer and directly address the change of bond "strength" or energy due to quantum effects.

- We accept the suggestion and have amended the second paragraph on the right-hand side, page 2.
  Therefore, we employ in this work a fully open quantum system treatment of the dynamics, and subsequent populations, of the proton transfer in the G-C tautomerisation reaction.
- We reworded the signposting paragraph in the introduction to clarify the origins and usage of the potential energy landscape. See page 2, third paragraph on the right and the comments from Referee 1, point 3.
  We use an accurate description of the potential energy landscape of the double H-bond between the C and G bases [1]; we model the proton dynamics using an open quantum system (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment.

2.5 Point 5

*Next there is an admirably clear visualisation of the underlying problem (Figure 1) and its formal representation according to the authors' assumptions (Figure 2). It is not made clear, as it should be, that the energy change (0.435 eV) between the * and 0 states absolutely dictates that the classical thermal equilibrium constant for the model system would have to be* $K = \exp[(-0.435\,\text{eV})/kT] = 5.4e-8$ *(at* $T = 300\,\text{K}$, $kT = 0.026\,\text{eV}$*). However, with the spacing between the eigenstates of the system being much greater than kT, the validity of an "*$\exp(-\Delta E/kT)$ *thermalisation" calculation of the* p^*/p_0 *ratio cannot be assumed, not because of any "non-ideality", rather because of the energy separation (*$\gg kT$*) of the eigenstates (analogous to way the vibrational states in an ideal gas of diatomic molecules are occupied at ordinary temperatures). The * and 0 probabilities under any circumstances presumably depend on the non-zero amplitudes of the wave-functions in the * and 0 regions of the reaction coordinate q and the fractional occupations of each of the eigenstates under those circumstances. At equilibrium (single electron Maxwell-Boltzmann distribution over the states of the system) we cannot expect the ratio* p^*/p_0 *to be the same as it would be in the high temperature (classical) limit,* $\exp[-\Delta E_{reaction}/kT] = 5.4 \times 10^{-8}$*. The authors calculate a value of* $p^* = 1.73 \times 10^{-4}$ *(and therefore* $p_0 = 1\text{–}p^*$*) which is 3200 times greater than the naïve classical result,* 5.4×10^{-8}*.*

Response We want to bring to your attention Fig. 4, which shows the occupation probability as a function of temperature for both the low-temperature and high-temperature approximation. We stress that we are using the term "low-temperature approximation" in the context of the open quantum system Master Equation. Our Master equation is based on the Caldeira-Leggett model, which uses the limit $k_bT \gg \hbar\omega$ in their influence functional description of the system bath correlation. Consequently, we define "low-temperature" conditions as those in which the thermal energy k_bT becomes comparable to $\hbar\omega$.

Furthermore, we distinguish a low-temperature value because it violates the condition versus a biologically low temperature. In the particular case of G-C tautomerisation, the low-temperature limit is readily reached even at standard biological temperatures. Hence, requiring an extension to standard Caldeira-Leggett formalism to low temperature. The low-temperature correction causes the difference between the approach of $K_{\text{eq}} = \exp(-\Delta G/k_{\text{b}}T)$ and the quantum approach presented in this work.

- We make the classical thermal equilibrium constant clear in the text, see page 5, second paragraph after Eq. 4.
  The energy change ($\Delta E = 0.435\,\text{eV}$, see Fig. 4) between the canonical and tautomeric states dictates that the classical thermal equilibrium constant for the model system would have to be $K_{\text{eq}} = \exp(-(0.435\,\text{eV})/k_{\text{b}}T) = 5.4 \times 10^{-8}$. As we observe from the calculations of Fig. 4, there is a factor of $\sim 10^4$ difference between the thermalised tautomer occupation probability obtained from the two treatments.

- We have added additional background information and calculations on the low-temperature correction in a new supporting information document.

2.6 Point 6

It is, in principle, possible to calculate the equilibrium distribution p ∗ /p0 without any dynamic considerations whatsoever. However, the dynamic calculations conducted in this study are analogous to observations made in temperature-jump experiments and the like

when the system is placed in an initial non-equilibrium state and the time evolution of its rapid approach (return) to equilibrium is monitored. The perturbation from equilibrium chosen by the authors is the non-thermalised $p0 = P(E_{omega}) = 1$ distribution. The transition to equilibrium requires the absorption of heat from the bath until a final stationary distribution between the eigenstates of the system (equilibrium) is reached and $k_f \cdot p_0 = k_r \cdot p^*$. (We can take this to be a definition of the forward and reverse rate constants in terms of the necessary equality of the forward and reverse rates at equilibrium.) We observe that the calculated tunnelling factor, 10^5, increases both k_f and k_r by the same factor according to Eq.(10) and similarly shortens the lifetime of both the reactant and product by the same factor of 10^5. This means that something has gone horribly wrong. If k_f and k_r increase by the same factor, then p^*/p_0 at equilibrium must remain the same, but the authors' calculation of this ratio is not the same as the classical value.

Response We can determine directly the ratio p^*/p_0 by either taking a thermal average over the system Hamiltonian (see the section entitled "The Thermal Solution" in the new supplementary information file) and then applying Eq. 4, or by obtaining $W^{\mathrm{eq}}(q,p) = W(q,p,t \to \infty)$ by integrating Eq. 1 and then applying Eq. 4. Both approaches return the same numerical result within the bounds of reasonable numerical errors. We were unaware of these types of experiments; thanks for raising this. We agree that they may be relevant for addressing our results.

- We modified the third to last paragraph in the conclusion section to suggest, according to your comment, future temperature-jump experimental measurements that could further corroborate our calculations. See page 6.
  Experimental measurements of tautomerisation rates for G-C double proton transfer reaction are still not available. Nevertheless, we believe that our theoretical results force us to radically revise our understanding of the likelihood of point mutations in DNA, and we hope they will encourage new experimental measurements of the proton transfer reaction. Perhaps, a temperature jump experiment, similar to investigations conducted on intramolecular proton transfer in heterocycles [21], could elucidate the proton transfer rates.

2.7 Point 7

*My opinion is that the value of p^*/p_0 obtained by the authors is an improvement over previous calculations, and that the difference is not because of tunnelling or non-ideality, rather because of the equilibrium distribution of eigenstate occupations. Nor do I doubt that the tunnelling factor calculation is reasonable, but I question the validity of using Eq.(10) in which the rate constants depend only on the heights of the activation barriers $E_{f,r}$ and take no account of the radically discrete quantum character of the eigenstates that transverse both sides of the reaction pathway; nor the distribution of their fractional occupations in the * and 0 staes of base-pairing. (This may also cause problems with Eq.(8) and the calculation of the tunnelling factor, but I leave that to the authors.) That is to say, having justified and taken a "low temperature" approach to the dynamic problem, the authors should take a similar approach to the description of the equilibrium distribution over the individual states of the system and the manner in which this affects the dynamics of forward and reverse reactions (which is exactly what they have implicitly calculated to find the equilibrium endpoint).*

Response Thanks for raising this important point. We agree that the equilibrium distribution of eigenstate occupations is different in the low-temperature approach than simply taking the thermal average of the system Hamiltonian.

- To address this point, we included additional information on the low-temperature correction. The additional information is provided in a new supporting document. We believe this is best placed here to not distract from the paper's main aim. Within the supporting information document, see the section entitled "Examining the Low-Temperature Correction" and the subsection "The Thermal Solution" contained within.

2.8 Point 8

Last of all I will comment on the biological significance of these results. The authors discuss this in relation to helicase catalysis of double-stranded unwinding, which requires the breaking of the hydrogen bonds that are simulated in this study. The argument presented is that the tautomerisation exchange is so rapid that the helicase breaking of hydrogen bonds simply "traps" the equilibrium p/p0 ratio in the single-stranded product. However, I think a much more sophisticated argument is needed, one which takes into account the manner in which the potential energy surface (on which the rapid tautomerisation takes place) is distorted as the hydrogen bond is, on a timescale much longer than electron tunnelling, stretched and finally broken. The answer may end up being the same, but it will be much better justified, and may differ between (i) thermal melting of the bonds, (ii) passive "Brownian ratchet" helicase action and (iii) dissipative "stepped motor" helicase action. These need to be considered to do justice to the real physical situation in any discussion of its biological significance. Furthermore, the authors' result may be relevant to the origin of life. According to Eigen & Schuster (Naturwissenschaften 64, 541 565 (1977)), single stranded RNA phage replicases have error rates* $\sim 5.0 \times 10^{-4}$*, suggesting that the early enzyme-controlled replication of nucleic acids may have been accuracy-limited by the tautomerisation reaction studied by the current authors.*

Response These are excellent points. We completely agree with the referee's statement; we would like to add that this point has been largely overlooked in the literature. The potential energy surface will become significantly distorted as the hydrogen bonds dissociate during the cleavage process. We are currently in the process of preparing another manuscript on this process. Unfortunately, it is well out of the remit of this paper.

- Thanks for your suggestion on the reference. We were not aware of these types of replicases. We added this point to the last paragraph of the conclusion.

  The high tautomer occupation probability might also be relevant to address the role of mutations in several theories of the origin of life, including the RNA-mechanism proposed by Eigen *et al.* [29], in which single-stranded RNA phage replicases have error rates on the order of 10^{-4}, suggesting that the early enzyme-controlled replication of nucleic acids may have been accuracy-limited by the tautomerisation reaction.

2.9 Typographical 1

7th line of abstract "accounts" instead of "account".

Response Correction accepted.

2.10 Typographical 2

page 1, RH column, 5th line of paragraph, "sine" instead of "since"

Response Correction accepted.

2.11 Typographical 3

page 2, RH column, 5th line of paragraph "proton" not "protons

Response Correction accepted.

2.12 Typographical 4

*There is a minor discrepancy between the stated height of the forward reaction energy barrier in the Figure 2 caption (*0.705 eV*) and the text: page 3, line 9 (*0.704 eV*).*

Response Thanks for pointing out the discrepancy, this has now been fixed. 0.704 eV is the correct value.

2.13 Typographical 5

It is not clear why Eq.(2) has been written as it is with the exponential factor appearing twice.

Response Correction accepted.

2.14 Typographical 6

The Heaviside function appears in Eq.(2) but is not defined until much later.

Response Thanks for pointing this out. We have moved the definition to after Eq. 2.

3 Referee 3

The article "An Open Quantum Systems approach to proton tunnelling in DNA" is an interesting paper in which authors have investigated the proton dynamics in the H-bond between the Guanine-Cytosine (G-C) DNA base pair using an open quantum systems approach based on the Wigner-Moyal Master equation to accounts for the interaction of the proton with the cellular environment. Authors have corrected the Master equation, which works right at high temperatures, to employ it at biological temperature (low temperatures). To implement this low temperature correction in the Master equation, authors have replaced the thermal energy term of the equation with the zero-point energy. Assuming and environment such as "aqueous solution", authors have stablished the phenomenological friction constant of the master equation with the collision spectrum of water. Authors have found several surprising results, such as quantum corrected half-lives which are much shorter than the classical ones, very high tunneling effect for the proton transfer ~ 105 *and high kinetic isotope effects, 30.*

Response We sincerely thank Referee 3 for their interest in the manuscript and their detailed review. We will now highlight the following key improvements made to the revised manuscript as motivated by the referee's comments:

- Added further supporting calculations on the low-temperature correction.
- Expanded discussion and comparison to available experimental literature.
- Included further calculations comparing our model of tunnelling to the results of a benchmark potential.

3.1 Point 1

The methodology of this work is interesting and could offer some interesting perspectives, however, the validity of the Master equation low temperature correction should be analyzed more carefully. In this way, more than one system (pair G-C in this case) should be studied to provide robustness to their results.

Response We appreciate the referee's concern about the validity of the Master equation. As suggested, we have added additional calculations to provide further robustness to our results. The new content covers a numerical comparison of our model to well established analytical solutions of the motion of a particle in a harmonic potential in the low-temperature case. The comparison between our numerical solutions to the analytical solutions should allay concerns regarding the validity of the low-temperature correction.

- The additional calculations are provided in a new supporting information document. We believe this is best placed here to not distract from the paper's main aim. See the section entitled "Examining the Low-Temperature Correction" and the subsections contained within the supporting information document.

3.2 Point 2

Authors have obtained very different results (several orders of magnitude) higher than the classical ones for the G-C proton transfer. In addition, they do not have experimental results to support their findings. In biological systems, tunneling effects higher than 10 are rare, and values of $\sim 100\,000$ *are too high even for pure gas phase reactions. Professor Warshel and coworkers have established a very limited effect of the tunneling effect in biological process (see J Phys Chem B. 2008 May 15;112(19):5950-4 and J. Phys. Chem.*

B 2001, 105, 33, 7887–7907). These are examples of enzymatic catalysis but could be extended to these DNA systems as well.

Response We accept the correction and have expanded the discussion of our result compared to available experiments. Unfortunately, there is no direct experimental measure for the degree of quantum tunnelling during a reaction, but the magnitude of the primary kinetic isotope effect (KIE) is likely to be a reasonable first approximation. Consequently, we have added experimental comparisons to this metric.

Currently, there are no available experimental observations on the nature of the potential energy surface connecting the canonical and tautomeric forms of DNA or the rate of proton transfer. However, in light of the referees' comment, we have included additional discussion on other proton transfer reactions in DNA.

We agree that the methods of Warshel *et al.* [30, 31] could be applied to the DNA system. However, the quantitative comparison is meaningless without directly calculating the tunnelling effects as their potential is wildly different. The tunnelling effects are dependent on the potential barrier parameters, height and width, potential minima separation distance [32]. We suggest that the reaction barrier is sufficiently low and thin for the proton transfer in the DNA system that the quantum rate through the barrier is high. We believe that the high quantum rate is high compared to the classical rate due to the nature of the potential energy surface. Here, we have the case that the classical rate is low, but the barrier is sufficiently narrow that the proton can tunnel from one side of the hydrogen bond to the other. The degree of tunnelling during an H-transfer reaction is susceptible to the barrier through which tunnelling occurs and depends on the specific reaction.

- Taking the referee's comment on board we restructured our presentation of the kinetic isotope effect calculation. See the fourth paragraph on page 4. In addition, at the end of the "Lifetime of the Tautomer" section, we highlighted that our KIE value is comparable to experimental values of other biological systems.
  We investigated the impact of the kinetic isotope effect (KIE) as a function of temperature (doubling the proton's mass if replaced by a deuteron). See the SI and Methods section. A strong dependence of the reaction rate on the reduced mass of the system can indicate tunnelling. Hence the kinetic isotope can be used to probe if quantum effects dominate [18–20].

  At low temperatures, the KIE rapidly increases, providing further evidence that the transfer process becomes increasingly dependent on the quantum (tunnelling) contribution. Meanwhile, at high temperatures, the KIE exponentially tails off. At biological temperatures the KIE = 30.

  A high tunnelling factor and KIE has been observed in other models of open quantum systems [33, 34] probing proton transfer reactions. Furthermore, in a proton transfer reaction catalysed by enzyme soybean lipoxygenase, in the wild type, a high KIE of $\sim$ 80 [35] has been experimentally observed and verified using theory [36]. Furthermore, the KIE increased to the values from 500–800 in the case of various mutants [18,37–39]. Consequently, the value of KIE reported here is within sensible bounds of experimentally observed values.

- We updated the methods section on the KIE to incorporate the changes above. See methods the section entitled "Kinetic Isotope Effect" on page 8.
  We investigated the impact of the kinetic isotope effect to determine possible quantum effects. We investigated the kinetic isotope effect using the same parameters before but doubled the mass.

$$\mathrm{KIE} = \frac{k_{\mathrm{QM}}^{\mathrm{p}}}{k_{\mathrm{QM}}^{\mathrm{d}}}, \tag{1}$$

where $k_{\mathrm{QM}}^{\mathrm{p}}$ ($k_{\mathrm{QM}}^{\mathrm{d}}$) is the rate for a proton (deuteron). We neglect any changes to the transfer energy landscape due to isotopic substitutions, such as zero-point energy and free energy contributions, and consider only the effect on the reaction rate due to modifications of the mass terms in Eq. 1.

- See the section on the mass dependence ('Sensitivity to the Mass and the Kinetic Isotope Effect') in the new supplementary file.
- In the conclusion we have incorporated several new points referencing our work to experimental evidence on the G-T wobble proton transfer mechanism. See the new parts of the conclusion spanning pages 5 to 6. See also the discussion raised by Referee 1, point 4.

  The first [14] structural evidence for the rare tautomer hypothesis was observed using X-ray crystallographic analysis of a DNA polymerase. Whereby the polymerase was observed mismatching C with A. The mismatch adopts a Watson-Crick-like configuration which can only be obtained via the movement of a single proton on one of the mismatched bases. The proton transfer product alters the hydrogen-bonding pattern such that the mispair forms an overall shape that is virtually indistinguishable from a canonical, Watson-Crick base pair in double-stranded DNA. It remains an open question if the proton transfer has contributions from quantum effects.

  More recently, tautomerisation in DNA has been experimentally observed using NMR relaxation dispersion. Here, hairpin DNA duplex structures were observed to interchange between a wobble G-T and a Watson-Crick-like structure via tautomerisation (neutral) or ionisation (charged) [15,16]. This experimentally confirms that tautomerisation in DNA is a non-trivial effect.

  A positive indication that tautomerisation might be facilitated by tunnelling was provided recently by Rangadurai *et al.* [17]. They extensively 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_2O and D_2O. The authors reported a KIE as the rate of exchange between the two conformations of the mismatch was $\sim$ 3-fold slower in D_2O than in H_2O. This result provides the first experimental evidence supporting a transition state for tautomerisation involving proton tunnelling. However, before the observed KIE can be unequivocally attributed to proton tunnelling, further measurements will need to be conducted to investigate its temperature dependence [18]. Since a definitive experimental hallmark of tunnelling is a temperature-independent rate at low temperature, the rate of classical over-the-barrier hopping becomes negligible at low temperatures, leaving only the quantum contribution [19, 20].

  Experimental measurements of tautomerisation rates for G-C double proton transfer reaction are still not available. Nevertheless, we believe that our theoretical results force us to radically revise our understanding of the likelihood of point mutations in DNA, and we hope they will encourage new experimental measurements of the proton transfer reaction. Perhaps, a temperature jump experiment, similar to investigations conducted on intramolecular proton transfer in heterocycles [21], could elucidate the proton transfer rates.

  One can note that our model predicts a rate of tautomerisation much higher than the overall rate of spontaneous mutations ($\sim 10^{-8}$) [22], but this is consistent with the well-known presence of highly efficient DNA repair mechanisms. However, it is also difficult to comment on the overall efficiency of these repair mechanisms because other spontaneous mutations will also take place in DNA aside from tautomerisation [23, 24].
- We compared our rates to observations of the femtosecond dynamics of tautomeri-

sation of a DNA base analogue. See the second paragraph in the "Lifetime of the Tautomer" section, page 4.
In an analogue system (the 7-azaindole dimer), ultraviolet femtosecond pulsed time-of-flight spectroscopy indicates that proton transfer can occur on a timescale of a few hundred femtoseconds [40]. Here, light is used to induce proton transfer and probe the dimer's dynamics. In addition, Douhal *et al.* [40] demonstrate that the process is isotopically sensitive, indicating that quantum tunnelling can play a role in the dynamics. The rates experimentally reported are much faster than the rates reported here, which is likely due to the initial ultraviolet pulse modifying the reaction profile. However, the experiment suggests that the proton transfer reaction rate can be fast and have a quantum component.

3.3 Point 3

Authors should check this correction of the Master equation in a biological system in which the rate constant is very well know and their results could be validated. Afterwards, they could extend the "algorithm" to these systems much more complicated.

Response We accept the suggestion and have added additional calculations. We compare our model of tunnelling to the results of a benchmark potential used by several authors to check the validity of their model. We agree with other authors about the classical-to-quantum contribution to the transmission through the benchmark potential's barrier within a factor of two.

- The additional calculations are provided in the supporting information document. See the section entitled "Tunnelling Comparison to a Benchmark Potential".

4 Further corrections

This section covers additional minor corrections that the referees have not directly suggested. However, we believe they will improve the general quality of the paper. Please see the changes below.

4.1 Further correction 1

To improve the clarity of the paper, we made minor adjustments the abstract to improve readability. One of the most important topics in molecular biology is the genetic stability of DNA. One threat to this stability is proton transfer along the hydrogen bonds of DNA that could lead to tautomerisation, hence creating point mutations. We present a theoretical analysis of the hydrogen bonds between the Guanine-Cytosine (G-C) nucleotide, which includes an accurate model of the structure of the base pairs, the quantum dynamics of the hydrogen bond proton, and the influence of the decoherent and dissipative cellular environment. We determine that the quantum tunnelling contribution to the proton transfer rate is several orders of magnitude larger than the classical over-the-barrier hopping. Due to the significance of the quantum tunnelling even at biological temperatures, we find that the canonical and tautomeric forms of G-C inter-convert over timescales far shorter than biological ones and hence thermal equilibrium is rapidly reached. Furthermore, we find a large tautomeric occupation probability of 1.73×10^{-4}, suggesting that such proton transfer may well play a far more important role in DNA mutation than has hitherto been suggested. Our results could have far-reaching consequences for current models of genetic mutations.

4.2 Further correction 2

We have observed that "ohmic" should read as "Ohmic". All occurrences of this have now been corrected for.

4.3 Further correction 3

We changed the annotation and x-axis caption on Fig. 3 to match the style of the other figures.

4.4 Further correction 4

We changed the wording in the third paragraph in the conclusion to highlight an example system. The paragraph now reads:
Attempting to identify isotope effects in this system is problematic since investigating biological assays in deuterated solvents comes with a whole host of problems, for example, viscosity effects inhibiting correct protein function [41]. In addition, the impairment of gene expression at the transcription or translation level could preclude the measurement of the KIE discussed in the Methods section. Therefore, more direct measurements via nuclear magnetic resonance shifts, such as those applied to the G-T wobble mismatch, need to be applied [16].

References

[1] L Slocombe, JS Al-Khalili, and M Sacchi. Quantum and classical effects in dna point mutations: Watson–crick tautomerism in at and gc base pairs. *Physical Chemistry Chemical Physics*, 23(7):4141–4150, 2021.

[2] D. Wales, R. Saykally, A. Zewail, and D. King. *Energy Landscapes: Applications to Clusters, Biomolecules and Glasses*. Cambridge University Press, 2003.

[3] Ol'ha O Brovarets' and Dmytro M Hovorun. Atomistic mechanisms of the double proton transfer in the h-bonded nucleobase pairs: Qm/qtaim computational lessons. *Journal of Biomolecular Structure and Dynamics*, 37(7):1880–1907, 2019.

[4] Ol'ha O. Brovarets' and Dmytro M. Hovorun. Why the tautomerization of the g·c watson–crick base pair via the dpt does not cause point mutations during dna replication? qm and qtaim comprehensive analysis. *Journal of Biomolecular Structure and Dynamics*, 32(9):1474–1499, 2014. PMID: 23909623.

[5] Ol'ha O. Brovarets and Dmytro M. Hovorun. Can tautomerization of the a·t watson–crick base pair via double proton transfer provoke point mutations during dna replication? a comprehensive qm and qtaim analysis. *Journal of Biomolecular Structure and Dynamics*, 32(1):127–154, 2014. PMID: 23383960.

[6] AD Godbeer, JS Al-Khalili, and PD Stevenson. Modelling proton tunnelling in the adenine–thymine base pair. *Physical Chemistry Chemical Physics*, 17(19):13034–13044, 2015.

[7] A Gheorghiu, PV Coveney, and AA Arabi. The influence of base pair tautomerism on single point mutations in aqueous dna. *Interface focus*, 10(6):20190120, 2020.

[8] Radek Pohl, Ondřej Socha, Petr Slavíček, Michal Šála, Paul Hodgkinson, and Martin Dračínskỳ. Proton transfer in guanine–cytosine base pair analogues studied by nmr spectroscopy and pimd simulations. *Faraday discussions*, 212:331–344, 2018.

[9] Diego Soler-Polo, Jesús I Mendieta-Moreno, Daniel G Trabada, Jesús Mendieta, and José Ortega. Proton transfer in guanine-cytosine base pairs in b-dna. *Journal of chemical theory and computation*, 15(12):6984–6991, 2019.

[10] Jingzhi Pu, Jiali Gao, and Donald G. Truhlar. Multidimensional tunneling, recrossing, and the transmission coefficient for enzymatic reactions. *Chemical Reviews*, 106(8):3140–3169, 2006. PMID: 16895322.

[11] Stefan Schweiger and Guntram Rauhut. Plateau reactions: Double proton-transfer processes with structureless transition states. *The Journal of Physical Chemistry A*, 107(45):9668–9678, 2003.

[12] Youngchan Kim, Federico Bertagna, Edeline M. D'Souza, Derren J. Heyes, Linus O. Johannissen, Eveliny T. Nery, Antonio Pantelias, Alejandro Sanchez-Pedreño Jimenez, Louie Slocombe, Michael G. Spencer, Jim Al-Khalili, Gregory S. Engel, Sam Hay, Suzanne M. Hingley-Wilson, Kamalan Jeevaratnam, Alex R. Jones, Daniel R. Kattnig, Rebecca Lewis, Marco Sacchi, Nigel S. Scrutton, S. Ravi P. Silva, and Johnjoe McFadden. Quantum biology: An update and perspective. *Quantum Reports*, 3(1):80–126, 2021.

[13] Ruby Srivastava. The role of proton transfer on mutations. *Frontiers in chemistry*, 7:536, 2019.

[14] Weina Wang, Homme W Hellinga, and Lorena S Beese. Structural evidence for the rare tautomer hypothesis of spontaneous mutagenesis. *Proceedings of the National Academy of Sciences*, 108(43):17644–17648, 2011.

[15] Isaac J Kimsey, Katja Petzold, Bharathwaj Sathyamoorthy, Zachary W Stein, and Hashim M Al-Hashimi. Visualizing transient watson–crick-like mispairs in dna and rna duplexes. *Nature*, 519(7543):315–320, 2015.

[16] Isaac J Kimsey, Eric S Szymanski, Walter J Zahurancik, Anisha Shakya, Yi Xue, Chia-Chieh Chu, Bharathwaj Sathyamoorthy, Zucai Suo, and Hashim M Al-Hashimi. Dynamic basis for dg• dt misincorporation via tautomerization and ionization. *Nature*, 554(7691):195–201, 2018.

[17] Atul Rangadurai, Eric S Szymanski, Isaac Kimsey, Honglue Shi, and Hashim M Al-Hashimi. Probing conformational transitions towards mutagenic watson–crick-like g· t mismatches using off-resonance sugar carbon r 1ρ relaxation dispersion. *Journal of Biomolecular NMR*, pages 1–15, 2020.

[18] Judith P Klinman and Adam R Offenbacher. Understanding biological hydrogen transfer through the lens of temperature dependent kinetic isotope effects. *Accounts of chemical research*, 51(9):1966–1974, 2018.

[19] Linus O. Johannissen, Sam Hay, and Nigel S. Scrutton. Nuclear quantum tunnelling in enzymatic reactions – an enzymologist's perspective. *Phys. Chem. Chem. Phys.*, 17:30775–30782, 2015.

[20] Linus O. Johannissen, Andreea I. Iorgu, Nigel S. Scrutton, and Sam Hay. What are the signatures of tunnelling in enzyme-catalysed reactions? *Faraday Discuss.*, 221:367–378, 2020.

[21] O Bensaude, M Dreyfus, G Dodin, and JE Dubois. Intramolecular nondissociative proton transfer in aqueous solutions of tautomeric heterocycles: A temperature-jump kinetic study. *Journal of the American Chemical Society*, 99(13):4438–4446, 1977.

[22] Wen-Jin Wu, Wei Yang, and Ming-Daw Tsai. How dna polymerases catalyse replication and repair with contrasting fidelity. *Nature Reviews Chemistry*, 1(9):1–16, 2017.

[23] Thomas A Kunkel and P Sue Alexander. The base substitution fidelity of eucaryotic dna polymerases. mispairing frequencies, site preferences, insertion preferences, and base substitution by dislocation. *Journal of Biological Chemistry*, 261(1):160–166, 1986.

[24] Walter J Zahurancik, Seth J Klein, and Zucai Suo. Significant contribution of the 3'→ 5' exonuclease activity to the high fidelity of nucleotide incorporation catalyzed by human dna polymerase epsilon. *Nucleic acids research*, 42(22):13853–13860, 2014.

[25] Alejandro Pérez, Mark E Tuckerman, Harold P Hjalmarson, and O Anatole Von Lilienfeld. Enol tautomers of watson- crick base pair models are metastable because of nuclear quantum effects. *Journal of the American Chemical Society*, 132(33):11510–11515, 2010.

[26] T.L. Hill. *Thermodynamics of Small Systems*. Dover Books on Chemistry. Dover Publications, 1994.

[27] Wei Fang, Ji Chen, Mariana Rossi, Yexin Feng, Xin-Zheng Li, and Angelos Michaelides. Inverse temperature dependence of nuclear quantum effects in dna base pairs. *The journal of physical chemistry letters*, 7(11):2125–2131, 2016.

[28] Lu Wang, Stephen D Fried, Steven G Boxer, and Thomas E Markland. Quantum delocalization of protons in the hydrogen-bond network of an enzyme active site. *Proceedings of the National Academy of Sciences*, 111(52):18454–18459, 2014.

[29] Manfred Eigen and Peter Schuster. A principle of natural self-organization. *Naturwissenschaften*, 64(11):541–565, 1977.

[30] Janez Mavri, Hanbin Liu, Mats HM Olsson, and Arieh Warshel. Simulation of tunneling in enzyme catalysis by combining a biased propagation approach and the quantum classical path method: application to lipoxygenase. *The Journal of Physical Chemistry B*, 112(19):5950–5954, 2008.

[31] Jordi Villa and Arieh Warshel. Energetics and dynamics of enzymatic reactions. *The Journal of Physical Chemistry B*, 105(33):7887–7907, 2001.

[32] Sam Hay and Nigel S Scrutton. Good vibrations in enzyme-catalysed reactions. *Nature chemistry*, 4(3):161–168, 2012.

[33] Yanying Liu, Yaming Yan, Tao Xing, and Qiang Shi. Understanding the large kinetic isotope effect of hydrogen tunneling in condensed phases by using double-well model systems. *The Journal of Physical Chemistry B*, 125(22):5959–5970, 2021. PMID: 34033714.

[34] Weiwei Xie, Yang Xu, Lili Zhu, and Qiang Shi. Mixed quantum classical calculation of proton transfer reaction rates: From deep tunneling to over the barrier regimes. *The Journal of chemical physics*, 140(17):174105, 2014.

[35] Michael H Glickman, Jeffrey S Wiseman, and Judith P Klinman. Extremely large isotope effects in the soybean lipoxygenase-linoleic acid reaction. *Journal of the American Chemical Society*, 116(2):793–794, 1994.

[36] Pengfei Li, Alexander V Soudackov, and Sharon Hammes-Schiffer. Impact of mutations on the binding pocket of soybean lipoxygenase: Implications for proton-coupled electron transfer. *The journal of physical chemistry letters*, 9(22):6444–6449, 2018.

[37] Pengfei Li, Alexander V Soudackov, and Sharon Hammes-Schiffer. Fundamental insights into proton-coupled electron transfer in soybean lipoxygenase from quantum mechanical/molecular mechanical free energy simulations. *Journal of the American Chemical Society*, 140(8):3068–3076, 2018.

[38] Shenshen Hu, Alexander V Soudackov, Sharon Hammes-Schiffer, and Judith P Klinman. Enhanced rigidification within a double mutant of soybean lipoxygenase provides experimental support for vibronically nonadiabatic proton-coupled electron transfer models. *ACS catalysis*, 7(5):3569–3574, 2017.

[39] Alexander V Soudackov and Sharon Hammes-Schiffer. Proton-coupled electron transfer reactions: analytical rate constants and case study of kinetic isotope effects in lipoxygenase. *Faraday discussions*, 195:171–189, 2017.

[40] A Douhal, SK Kim, and AH Zewail. Femtosecond molecular dynamics of tautomerization in model base pairs. *Nature*, 378(6554):260–263, 1995.

[41] Luisa S Hohlefelder, Tobias Stögbauer, Madeleine Opitz, Thomas M Bayerl, and Joachim O Rädler. Heavy water reduces gfp expression in prokaryotic cell-free assays at the translation level while stimulating its transcription. *BioMed research international*, 2013, 2013.

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have addressed my questions in a satisfactory manner. I suggest acceptance.

Reviewer #2 (Remarks to the Author):

I have reviewed the revised manuscript and studied the point by point justifications that the authors have provided. I am satisfied that the authors have carefully and respectfully addressed all of the issues raised and that any outstanding matters can be considered differences of scientific opinion, not matters of scientific rectitude. I therefore recommend publication of this article in its current form, subject to the consent of my fellow learned reviewers.

Signed: Peter Wills (University of Auckland)

Reviewer #3 (Remarks to the Author):

All questions raised have been properly answered by the authors. In any case, I consider that the application of the Master Equation should have been tested in biological systems with verified experimental data. I think this is essential in science

Response to reviewer reports: An Open Quantum Systems approach to proton tunnelling in DNA

L. Slocombe, M. Sacchi, J. S. Al-Khalili

February 8, 2022

Thank you for giving us the opportunity to submit a revised draft of the manuscript "An Open Quantum Systems approach to proton tunnelling in DNA". We thank all the referees for their comprehensive reports. The level of detail in the referee's responses is extremely appreciated, as is their time and effort to provide insightful comments and suggestions. In light of the concerns raised by the referees, we have made significant improvements to the content and structure of the paper and have incorporated all of their suggestions. They raise several common themes, the most prominent of which is the adoption and the treatment of the double-well potential we use in the system Hamiltonian, which have now been carefully explained and justified. However, we clearly had not provided sufficient context to the reader concerning the origins of the potential derived from accurate *ab initio* quantum chemistry calculations consistent with the wider literature.

We have typeset parts of the referees' comments to clarify our responses. We have highlighted the changes we have made to address their comments. Outlined below are our responses and changes to the paper in line with their suggestions. Our response is formatted in the following way:

> *The referees' comments are in italics and coloured blue.*

Response Our response is written unindented adjacent the bold lettering.

- Explanation and location of the changes are shown here.
  The changes made to the revised manuscript are in red.

1 Referee 1

In this manuscript, the authors provided a theoretical study on the structure of DNA base pair, with a special emphasis on how quantum tunneling of the protons impacts on it. A one-dimensional model based on the Wigner-Moyal Master equation is chosen. It was found that proton tunneling (double) can induce much faster interconversions between the canonical and the tautomeric forms of the G-C base pair, than that from the classical description. The tautomeric occupation number is estimated. Overall, I think the manuscript presents a self-contained story, worth to be published.

Response We thank Referee 1 for their interest in the manuscript and their detailed review. We will now highlight the following key improvements made to the revised manuscript as motivated by the referee's comments:

- Adjusted the text to highlight the origins and the rigorousness of the potential.
- Improved the discussion on the mass of the system in context of other work.
- Added more discussion of the results in light of experimental evidence.
- Added supplementary calculations on the sensitivity of the tunnelling to changes to the potential and the mass. Please see the new supplementary information file.

1.1 Point 1

Rigorousness of the model: A one-dimensional model is a much too simplified model for a complex system like DNA base pair. Even in systems with much lower dimensionality, corner cutting effects in quantum tunneling had been shown important. In the system studied here, two protons are involved in the tunneling. Rearrangement of the heavy atom skeleton will play a central on determining the tunneling barrier height. I doubt the possibility of getting a reliable number from such a simplified treatment.

Response We apologise for not making our methods clearer. We do not employ a simplified one-dimensional model for describing the double proton tunnelling but have a fully unconstrained *ab initio* model (previously described in [1]). Indeed, we completely agree with the referee about the importance of the heavy atoms rearrangements during the proton transfer. The influence of the molecular restructuring and the motion of the carbon, nitrogen and oxygen atoms has been incorporated into the DFT calculation of the potential energy surface. The apparent single dimensionality, which results in the plot in Fig. 2, is due to the application of transition state theory to this system, which results in a single effective reaction coordinate [2] being displayed to represent the minimum energy reaction pathway for the tautomerisation. Using the potential energy surface from *ab initio* quantum chemical calculations [1], we have included the rearrangements of the rest of the base and not just the atoms involved in the hydrogen bonds. During the determination of the reaction coordinate, all atoms are unrestricted, and their motion is projected onto the reaction coordinate. Previously, the energetics of the proton transfer scheme in the A-T and G-C base pairs have been well studied by *ab initio* quantum chemical methods [1,3–9]. Furthermore, our potential energy surface has been extensively compared to other computational results reported in the literature [3–9] with excellent agreement.

We agree that explicitly including more degrees of freedom into the open quantum system Hamiltonian could be of interest. However, exploring the role of the fuller degrees of freedom of such a many-body system in enabling double proton transfer would increase the complexity of the model and the computational cost prohibitively and suggests that it is out of the scope of this work. Therefore, following Referee 1's comment, we have:

- We have updated the description of how we obtained the potential and its apparent single-dimensionality. Page 7, third paragraph on the left.
  The apparent single-dimensionality of the PES is due to the application of transition state theory to this system, which results in a single effective reaction coordinate being displayed to represent the minimum energy reaction pathway for tautomerisation.
- Updated the text in the methods section to stress that this approach has been used successfully in literature before. Page 7, third paragraph on the left.
  The approach of inserting the PES calculated from density functional theory into an OQS Hamiltonian has been successfully used before for proton transfer reactions [6].

- Updated the caption of Fig. 2 (showing the potential) to reflect the text better. See page 3.
  G-C double proton transfer energy landscape obtained from density functional theory calculations converted into a back-to-back double Morse potential. The first ten eigenstates are displayed. With values $E_0 = 0.049\,\mathrm{eV}$, $E_\mathrm{f} = 0.705\,\mathrm{eV}$, $\Delta E = 0.435\,\mathrm{eV}$.
- Described the potential 'corner-cutting' effects in the conclusion section as requested by the referee. See page 5, third paragraph.
  We would also like to highlight that adding further dimensions into the system Hamiltonian could lead to potential corner-cutting effects [10] which could further enhance the rate.
- In the manuscript, we neglected to define the full potential explicitly. Therefore, in light of Referee 1's comments, we have added the following text to the methods section on page 7, first paragraph on the left side.
  The double proton transfer reaction can be described as a PES obtained from *ab initio* quantum chemical methods (determined in Ref. [1] - see discussions of the calculations within). We described this PES using a back-to-back double Morse potential given by

$$\begin{aligned} V(q) =& V_1 \left\{ \exp\left(-2a_1\left[q - r_1\right]\right) - 2\exp\left(a_1\left[q - r_1\right]\right)\right\} \\ &+ V_2 \left\{ \exp\left(-2a_2\left[r_2 - q\right]\right) - 2\exp\left(a_2\left[r_2 - q\right]\right)\right\}. \end{aligned}$$

1.2 Point 2

Even for the simplified model, a renormalized effective mass should be used to described the process when two protons tunneling. But it seems like the mass of proton is used directly in Eq. 1.

Response The choice of the mass is related to the previous comment on dimensionality. At the transition state, the motion of a single proton dominates, and the collective classical rearrangement of the rest of the atoms of both bases can be neglected [6]. Thus, the dynamics of one proton dominates the tunnelling through the barrier [6] as the reaction is an asynchronous concerted process [3]. It has previously been determined that the rearrangements of the rest of the base and the DNA structure play a role in setting up the proton transfer reaction and the heavy-ion movements only dominate the outer regions of potential energy surface away from the transition state [1]. Ideally, one could incorporate several more dimensions where the additional mass ions could be explicitly included in the open system Hamiltonian. However, extending the OQS equation to 3N-6 degrees of freedom would make the model both theoretical and computationally intractable. In order to clarify the point raised by the referee, we have made the following changes:

- We added an additional reference and further clarified the adoption of the mass for this system. See page 6, the first section under methods.
  Studies on other proton transfer reactions suggest that the reduced mass at the transition state is dependent on the proton transfer process and can fluctuate to a much heavier mass far from the transition state [11]. Depending on the reaction pathway, if the two protons transfer simultaneously or not, the reduced mass of the system can tend to one or two proton masses [11]. In our case, at the transition state, the motion of a single proton dominates [1]. Consequently, it is acceptable to assume that the mass of the proton mass is in, a very good approximation, the effective mass of the tunnelling particle in the asynchronous concerted process [3]. Furthermore, the collective classical rearrangement of the rest of the atoms of both bases can be neglected.

1.3 Point 3

The potential energy surface is defined using a model. Nowadays, there are many quantum chemistry methods available. At least parameters should be achieved from a high-level quantum chemistry calculation, instead of being defined manually.

Response In fact, this is precisely what we have done. Clearly, we have not adequately explained this in the paper. Our potential energy surface is indeed determined from high-level quantum chemistry calculations (density functional theory) [1]. We are interested in how the two bonded DNA bases inter-convert between canonical and tautomeric states. However, most standard approaches exploring the importance of quantum tunnelling neglect the influence of the surrounding environment. Therefore, our approach is a two-step process whereby having obtained a potential energy surface from density functional theory calculations, this is then inputted into an open quantum system calculation to account for the decoherent and dissipative biological environment. As far as we know, there is no full conjunction of open quantum systems into density functional theory approaches yet. Additionally, if we directly adopt the results from the *ab initio* calculation, we neglect that outer edges of the potential would follow a Morse-like relation. Therefore, we believe our method of inserting a modified potential energy surface, obtained from *ab initio* calculations, into the open quantum system Hamiltonian is appropriate.

- We have clarified that the potential we use comes from high-level density functional theory calculations. See page 2 second to bottom paragraph.
  We use an accurate description of the potential energy landscape of the double H-bond between the C and G bases [1]; we model the proton dynamics using an open quantum system (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment.
- We further clarify our modifications of the potential from the density functional theory calculations. The clarifying comments can be found on page 7 below the equation defining the potential.
  In the limits of the reaction coordinate away from the well minima, we require that the potential rapidly increases, creating a bounding wall. The wall corresponds to the strong repulsive force as the bond between the hydrogen and the hydrogen-bond donator atoms shorten. Here, the back-to-back double Morse potential captures these requirements while maintaining the reaction profile obtained from high-level density functional theory calculations. The bounded potential can then be inserted into our open quantum system approach and solve the dynamics and the steady-state solution.

- The section title in the Methods section is misleading. We have updated it to reflect that the potential is not just a model. The title now reads,
  The Proton Transfer Potential

1.4 Point 4

We have split this point into two and addressed each part individually.

1.4.1 Comparison to Experimental Observations

> *There is no comparison between the theoretical results with available experimental observations. . . . Even when the experimental results become available, what sense does it make to compare an adhoc result with these experimental observations.*

Response We apologise that our wording was not clear; the potential is not *ad hoc*, but it has been obtained by performing accurate first-principles quantum chemical calculations [1]. We believe our previous responses have addressed the ambiguity of the origins of the potential.

While the proton transfer potential parameters (height, width, and reaction asymmetry) are well informed from high-level quantum chemistry calculations, currently, there are no available experimental observations on the nature of the potential energy surface connecting the canonical and tautomeric forms of DNA.

- To address the contention in the literature on the accuracy and nature of the proton transfer energy landscape, we have added an additional sentence on page 2, second paragraph on the right.
  Further discussion on the contention in the literature on the accuracy and nature of the proton transfer process in DNA can be found in Ref. [12], and [13].
- Regarding the lack of comparison between the theoretical results with available experimental observations, we have added discussion on wobble mismatch incorporation via tautomerism into the conclusion. See page 6, second paragraph on the left.
  The first [14] structural evidence for the rare tautomer hypothesis was observed using X-ray crystallographic analysis of a DNA polymerase. Whereby the polymerase was observed mismatching C with A. The mismatch adopts a Watson-Crick-like configuration which can only be obtained via the movement of a single proton on one of the mismatched bases. The proton transfer product alters the hydrogen-bonding pattern such that the mispair forms an overall shape that is virtually indistinguishable from a canonical, Watson-Crick base pair in double-stranded DNA. It remains an open question if the proton transfer has contributions from quantum effects.

  More recently, tautomerisation in DNA has been experimentally observed using NMR relaxation dispersion. Here, hairpin DNA duplex structures were observed to interchange between a wobble G-T and a Watson-Crick-like structure via tautomerisation (neutral) or ionisation (charged) [15,16]. This experimentally confirms that tautomerisation in DNA is a non-trivial effect.

  A positive indication that tautomerisation might be facilitated by tunnelling was provided recently by Rangadurai *et al.* [17]. They extensively 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_2O and D_2O. The authors reported a KIE as the rate of exchange between the two conformations of the mismatch was $\sim$ 3-fold slower in D_2O than in H_2O. This result provides the first experimental evidence supporting a transition state for tautomerisation involving proton tunnelling. However, before the observed KIE can be unequivocally

attributed to proton tunnelling, further measurements will need to be conducted to investigate its temperature dependence [18]. Since a definitive experimental hallmark of tunnelling is a temperature-independent rate at low temperature, the rate of classical over-the-barrier hopping becomes negligible at low temperatures, leaving only the quantum contribution [19, 20].

Experimental measurements of tautomerisation rates for G-C double proton transfer reaction are still not available. Nevertheless, we believe that our theoretical results force us to radically revise our understanding of the likelihood of point mutations in DNA, and we hope they will encourage new experimental measurements of the proton transfer reaction. Perhaps, a temperature jump experiment, similar to investigations conducted on intramolecular proton transfer in heterocycles [21], could elucidate the proton transfer rates.

One can note that our model predicts a rate of tautomerisation much higher than the overall rate of spontaneous mutations ($\sim 10^{-8}$) [22], but this is consistent with the well-known presence of highly efficient DNA repair mechanisms. However, it is also difficult to comment on the overall efficiency of these repair mechanisms because other spontaneous mutations will also take place in DNA aside from tautomerisation [23, 24].

1.4.2 Sensitivity of the Potential

... By changing one or two parameters used above, e.g. barrier height, effective mass, the final occupation probability can change by orders of magnitude. ...

Response We thank the referee for raising this important point. Changing the barrier height has less effect on the final occupation probability but more on the proton transfer rate. On the other hand, the reaction asymmetry has a much more significant effect on the occupation probability. For example, a smaller energy difference between the reactant and product results in a more equal occupation distribution between the two. We know the reaction asymmetry and barrier with a high degree of confidence (subject to the limitations of our quantum chemistry calculations).

More recent evidence has suggested that free energy interactions can provide a diverse range of energetic scenarios [7], suggesting that an ensemble of possible potentials can explain the proton transfer process. We agree that exploring a sensitivity analysis of the potential energy surface on the proton transfer reaction would be of general interest. However, this would require significant further work using ensemble multi-scale modelling combined with a correction for tunnelling.

- We have explored the dependence on the mass and determined that it significantly changes the rate and the occupation. Additional calculations on the sensitivity of the barrier and the mass can be found in a new supplementary information file. In summary, we determine that increasing the barrier height significantly impacts the dynamics; namely, it causes a striking reduction in classical rate and affects the tunnelling much less. As a result, if the proton transfer barrier were to be much larger, we would see an increase in the quantum-to-classical contribution to the rate. Nevertheless, the conclusion would still stand; the rates are so high that the equilibrium populations of the canonical and tautomeric forms would quickly be reached. We have updated the discussion to reflect this comment. See page 4, third paragraph on the right of the "Lifetime of the Tautomer" section.

  We performed a sensitivity analysis of the reaction barrier (see the supplemental information (SI) file) and determined that increasing the barrier height significantly impacts the rate of the thermally activated proton 'over-the-barrier' hopping dynamics; namely, it causes a striking reduction in classical rate, but it affects the quantum tunnelling rate much less. Furthermore, the tunnelling correction remains

so high that the equilibrium populations of the canonical and tautomeric forms would quickly be reached even if the barrier height was 50% higher than in our study.

2 Referee 2

The authors have examined the tautomerisation of the G=C base-pair using an open quantum systems approach. They find that the contribution of quantum tunnelling to the reaction rate far outweighs classical barrier-hopping. They interpret their determination of the equilibrium occupation probability of the tautomeric state, 1.7e-4, as "[a] surprising result that could have far-reaching consequences", based on the supposition that the tautomeric state will "survive the DNA cleavage process in the helicase" and therefore contribute to mutation (ultimately depending on the efficacy of later DNA sequence repair mechanisms). The results are (apparently) carefully calculated and well-presented but there are fundamental misconceptions in their presentation and interpretation, as well as important aspects of the design of the model system not being adequately justified.

Response We sincerely thank Referee 2 for their interest in the manuscript and their detailed review. We will now highlight the following key improvements made to the revised manuscript as motivated by the referee's comments:

- Expanded discussion on the proton transfer reaction profile and our potential in context of the wider literature.
- Clarified the wording on the thermodynamic equilibrium and the hydrogen bond strength.
- Added discussion on the phage replicases.
- Fixed the highlighted typographical errors.

2.1 Point 1

It is assumed at the outset that the two (potentially tunnelling) proton exchanges required for tautomerisation are asynchronous, but no timescale is defined for the definition of "synchronicity": the authors' final conclusions would seem to require that movement of the second proton in the opposite direction (to preserve electroneutrality) occurs on a timescale much shorter than the lifetime of the tautomeric state. However there is no calculation, estimation or discussion of the energy or lifetime of the "double ion" state that is initially produced by the transfer of a single proton along just one of the hydrogen bonds. The energy of this state is in all probability quite high, but actual calculations can produce surprising results. The reader needs reassurance that the authors' single-proton-transfer calculation of the tautomerisation energetics is justified. This is an important problem which could upset the entire enterprise because the high energy of the "double ion" state could be equally easily "relieved" by rapid reversal of the first proton transfer rather than a second "asynchronous" proton transfer (the other one), the event needed to produce the tautomer. Furthermore, it is then necessary to ask "Under what assumptions would quantum considerations of the energy states of a tandem hydrogen bond system give the same results?" If one hydrogen bond transition causes enough of a disturbance in the other to cause its electron to transition to the other side, then there must be significant coupling between the two subsystems. Some argument justifying the authors' approach should be presented to allay such concerns.

Response We thank the referee for pointing out that the specifics of the transfer process has not been made evident. These questions are crucial to the process, but we failed to make this clear to the reader. The use of the asynchronous tautomerisation process is well justified. The exact chemical procedure is highlighted in a previous paper [1] and is a topic that has been discussed in detail by several studies [3–5, 7, 9, 12, 13] but has not been adequately covered in the paper. The

term asynchronous is used in literature to describe the proton transfer process whereby the motion of a single proton dominates the transition state and is closely followed by a second proton.

- The asynchronous tautomerisation process is not merely "assumed" but is well established in the literature. However, the additional context behind the adoption of the mechanism had not been included in the paper; consequently, we have added a few sentences to provide further context. See the last paragraph on the first page.
  We assume, as previously observed [6, 25], that the two protons undergo asynchronous transfer, suggesting that the rate-determining process is the transfer of a single proton across the barrier, with the other proton then moving in the opposite direction to preserve electroneutrality. There is a substantial agreement in the literature that the proton transfer in GC is an asynchronous process, while the stepwise proton transfer pathway (in which a proton transfer before the second one) has been ruled out in favour of the double proton transfer scheme. The argument is that in the standard Watson-Crick configuration, the formation of the single proton transfer is either unstable or energetically unfavourable [3–5, 7].
- We added further signposting to existing literature, discussing the proton transfer mechanism and why the other mechanisms are not preferred. See page 2, second paragraph on the right.
  Further discussion on the contention in the literature on the accuracy and nature of the proton transfer process in DNA can be found in Ref. [12], and [13].

2.2 Point 2

The discussion of thermodynamic equilibrium (end of page 1 into page 2) is confused. The definition of the equilibrium constant under conditions of thermodynamic ideality in terms of the ratio of G=C state occupation probabilities (= tautomer; 0 = canonical), $K = p^*/p_0$, is said to "assume perfect thermodynamic equilibrium, which is not necessarily true". This is an elementary misconception. There is only one form of equilibrium and that is equilibrium. It either exists within whatever thermodynamic constraints prevail or it does not. In that sense equilibrium is either perfect or it does not exist. This is not pedantic nitpicking. It goes to the heart of the biological significance of the authors' calculations (see later).*

Response Correction accepted; we agree this needs to be adequately addressed. The non-ideality we mentioned was attributable to the "perfect ideality (as of ideal behaviour such as that of an ideal gas) of the reactant and product of this reaction". Therefore,

- We corrected our confusing statement in the text according to the comment made by the referee. See the last paragraph on the first page.
  The thermodynamic equilibrium constant, K_{eq}, is generally written as K_{eq} = [G*-C*]/[G-C] (the ratio between the concentration of products – tautomeric pair – and reactants – canonical pair – present at chemical equilibrium). However, the concentrations are modified by an activity coefficient.

2.3 Point 3

We split this point into two and address each part individually.

2.3.1 Regarding the activity coefficient

The authors' Gamma is not an "activity coefficient". Gamma is the ratio of the activity coefficients of the tautomeric (*) and canonical (0) states, gamma/gamma0. The*

authors state that "without an accurate estimate of [Gamma], we cannot determine the quantity of interest, [p^*/p_0]". Thermodynamic non-ideality (gamma ≠ 1) arises when the chemical potential of a subsystem has a contribution due to interactions between the elementary entities or with the surroundings, usually involving changes in molecular potential energy. In the current calculation, the only interactions considered consist of energy exchanges between the G=C base-pair and the "bath" of harmonic oscillators (representing a simplified aqueous environment). I have no quibble with this approach, but it is obvious that Gamma can deviate from unity only if the interaction between the tautomer (*) and the bath is for some reason quantitatively different from the interaction between the canonical state (0) and the bath. . . . In any case, any change in zero-point energy could, by its very nature, only make a contribution to the ideal activity of a state, not to some activity coefficient (gamma ≠ 1) embodying non-ideality. Even for a 2 component system (G=C base-pair plus bath) the thermodynamic activity of either component cannot be defined without specifying the constraints under which the chemical potential of that component is altered. All of this was carefully worked over by Terrell Hill in the 1950s (see below). In the current case, neither the tautomer nor the canonical state is able preferentially to store potential energy (+ve or –ve) in the bath, in which case both the base-pair and the bath behave ideally, giving gamma = gamma0 = Gamma = 1.*

Response We thank the referee for raising this point. In this study, we calculated the ratio between the product and reactant, [GC*]/[GC] directly by integrating over the Wigner distribution when $t \to \infty$ and compared our results with the equilibrium constant for this reaction reported by other authors. Since the calculated potential energy surface and the ΔG of the reaction are comparable to the literature results, the difference between our treatment and all previous calculations is that we do not assume the equilibrium to obey the standard thermodynamic equality $\Delta G = -RT\log(K_{\text{eq}})$. Since $K_{\text{eq}} = P/R \cdot \gamma_P/\gamma_R$, we made the assumption that all the non-ideal activity behaviour of GC and GC* can be incorporated into γ_P/γ_R. We understand and accept that this caused confusion. We corrected our analysis by assuming that the new "quantum" equilibrium constant $K_{\text{eq-quantum}} = K_{\text{eq}} \cdot \Gamma_{\text{qm}}$, with Γ_{qm} being a coefficient that takes care of the non-ideal behaviour of the system due exclusively to non-classical activity (tunnelling, zero-point energy, decoherence and dissipation). Since, as the referee correctly points out, there is no reason to assume that the coupling between GC and GC* with the thermal bath would be radically different, Γ_{qm} (not gamma-ratio) should likely be the source of most of the "non-ideal behaviour (where non-ideal means simply a behaviour far from that of an ideal solution).

- We changed the term used to describe the equilibrium discrepancy to clarify that this is exclusively a non-classical activity coefficient. See the first paragraph on the left on the second page.
  More correctly, a quantum activity coefficient, Γ_{qm}, needs to be included as a factor such that $K_{\text{eq-qm}} = \Gamma_{\text{qm}} \times$[G*-C*]/[G-C]. Which is exclusively a non-classical activity coefficient containing effects such as thermally enhanced tunnelling, zero-point energy, decoherence and dissipation, leading to an activity coefficient that is far from unity, . . .

2.3.2 The Cramer Citation

. . . The authors cite Cramer (2013) as justification for considering "e.g., quantum mechanical effects such as thermally enhanced tunnelling and zero-point energy", but I am not aware of Cramer ever having considered these effects as contributing to differential effects on the thermodynamic activity of alternative states of a system. . . .

Response Thank you for bringing this to our attention. We intended to provide a relatively recent textbook reference for calculations of thermodynamic properties from *ab initio* quantum chemical calculations. However, as pointed out, a reference from Hill's work would be a better fit.

- We have added a reference to Hill's work on statistical thermodynamics as this provides a better-suited reference for the fundamental descriptions of thermodynamic equilibrium. We have amended the manuscript accordingly. See the first paragraph on the left on the second page.
  ... in the same way that solvent effects can shift equilibrium [26].

2.4 Point 4

The authors then state that "we carry out a fully quantum mechanical calculation to obtain the G-C* occupation probability within an open quantum system approach". Excellent, but we are then told that "proton delocalisation causes the strength of the hydrogen bonds in DNA base pairs to follow an anomalous temperature dependence due to the subtle balance of several quantum effects involved in the binding". The reader looks forward to a description of how proton delocalisation and other quantum phenomena affect the strength (free energy) of hydrogen bonds in DNA. At this stage the reader is led to believe that the results will shed light on the strength of the hydrogen bond rather than the dynamics of tautomerisation. But then the problem is divided into two independent parts, the first being "the potential energy landscape of the double H-bond between the C and G bases" (the strength of the hydrogen bonds) and the second being a model of "the proton dynamics using an open quantum systems (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment" (the dynamics of exchange between the alternative * and 0 states). Thus, two quite different aspects of the problem (energetics and dynamics) are obfuscated.*

Response Thank you for pointing this out; the language used at the start could leave the reader confused. We do not explicitly calculate the hydrogen bond strength and variation in our work. However, it is possible to draw a parallel between the facility of the proton transfer from donor to the acceptor and the hydrogen bond "weakening". The relationship between the proton transfer facility and the strength of the hydrogen bond has been previously discussed in several theoretical papers, including Wei *et al.* [27] and Lu *et al.* [28]. For "weakening", we, therefore, mean the overall delocalisation of the protons involved in the hydrogen bond. Or alternatively, the facility of the bond to fluctuate between the two potential configurations, the canonical and tautomeric. We need to make this reasoning clearer and directly address the change of bond "strength" or energy due to quantum effects.

- We accept the suggestion and have amended the second paragraph on the right-hand side, page 2.
  Therefore, we employ in this work a fully open quantum system treatment of the dynamics, and subsequent populations, of the proton transfer in the G-C tautomerisation reaction.
- We reworded the signposting paragraph in the introduction to clarify the origins and usage of the potential energy landscape. See page 2, third paragraph on the right and the comments from Referee 1, point 3.
  We use an accurate description of the potential energy landscape of the double H-bond between the C and G bases [1]; we model the proton dynamics using an open quantum system (OQS) model to account for dissipation and decoherence effects due to the surrounding cellular environment.

2.5 Point 5

*Next there is an admirably clear visualisation of the underlying problem (Figure 1) and its formal representation according to the authors' assumptions (Figure 2). It is not made clear, as it should be, that the energy change (0.435 eV) between the * and 0 states absolutely dictates that the classical thermal equilibrium constant for the model system would have to be* $K = \exp[(-0.435\,\text{eV})/kT] = 5.4e-8$ *(at* $T = 300\,\text{K}$, $kT = 0.026\,\text{eV}$*). However, with the spacing between the eigenstates of the system being much greater than kT, the validity of an "*$\exp(-\Delta E/kT)$ *thermalisation" calculation of the* p^*/p_0 *ratio cannot be assumed, not because of any "non-ideality", rather because of the energy separation (*$\gg kT$*) of the eigenstates (analogous to way the vibrational states in an ideal gas of diatomic molecules are occupied at ordinary temperatures). The * and 0 probabilities under any circumstances presumably depend on the non-zero amplitudes of the wave-functions in the * and 0 regions of the reaction coordinate q and the fractional occupations of each of the eigenstates under those circumstances. At equilibrium (single electron Maxwell-Boltzmann distribution over the states of the system) we cannot expect the ratio* p^*/p_0 *to be the same as it would be in the high temperature (classical) limit,* $\exp[-\Delta E_{reaction}/kT] = 5.4 \times 10^{-8}$*. The authors calculate a value of* $p^* = 1.73 \times 10^{-4}$ *(and therefore* $p_0 = 1\text{–}p^*$*) which is 3200 times greater than the naïve classical result,* 5.4×10^{-8}*.*

Response We want to bring to your attention Fig. 4, which shows the occupation probability as a function of temperature for both the low-temperature and high-temperature approximation. We stress that we are using the term "low-temperature approximation" in the context of the open quantum system Master Equation. Our Master equation is based on the Caldeira-Leggett model, which uses the limit $k_bT \gg \hbar\omega$ in their influence functional description of the system bath correlation. Consequently, we define "low-temperature" conditions as those in which the thermal energy k_bT becomes comparable to $\hbar\omega$.

Furthermore, we distinguish a low-temperature value because it violates the condition versus a biologically low temperature. In the particular case of G-C tautomerisation, the low-temperature limit is readily reached even at standard biological temperatures. Hence, requiring an extension to standard Caldeira-Leggett formalism to low temperature. The low-temperature correction causes the difference between the approach of $K_{\text{eq}} = \exp(-\Delta G/k_{\text{b}}T)$ and the quantum approach presented in this work.

- We make the classical thermal equilibrium constant clear in the text, see page 5, second paragraph after Eq. 4.
  The energy change ($\Delta E = 0.435\,\text{eV}$, see Fig. 4) between the canonical and tautomeric states dictates that the classical thermal equilibrium constant for the model system would have to be $K_{\text{eq}} = \exp(-(0.435\,\text{eV})/k_{\text{b}}T) = 5.4 \times 10^{-8}$. As we observe from the calculations of Fig. 4, there is a factor of $\sim 10^4$ difference between the thermalised tautomer occupation probability obtained from the two treatments.

- We have added additional background information and calculations on the low-temperature correction in a new supporting information document.

2.6 Point 6

It is, in principle, possible to calculate the equilibrium distribution p ∗ /p0 without any dynamic considerations whatsoever. However, the dynamic calculations conducted in this study are analogous to observations made in temperature-jump experiments and the like

when the system is placed in an initial non-equilibrium state and the time evolution of its rapid approach (return) to equilibrium is monitored. The perturbation from equilibrium chosen by the authors is the non-thermalised $p0 = P(E_{omega}) = 1$ distribution. The transition to equilibrium requires the absorption of heat from the bath until a final stationary distribution between the eigenstates of the system (equilibrium) is reached and $k_f \cdot p_0 = k_r \cdot p^*$. (We can take this to be a definition of the forward and reverse rate constants in terms of the necessary equality of the forward and reverse rates at equilibrium.) We observe that the calculated tunnelling factor, 10^5, increases both k_f and k_r by the same factor according to Eq.(10) and similarly shortens the lifetime of both the reactant and product by the same factor of 10^5. This means that something has gone horribly wrong. If k_f and k_r increase by the same factor, then p^*/p_0 at equilibrium must remain the same, but the authors' calculation of this ratio is not the same as the classical value.

Response We can determine directly the ratio p^*/p_0 by either taking a thermal average over the system Hamiltonian (see the section entitled "The Thermal Solution" in the new supplementary information file) and then applying Eq. 4, or by obtaining $W^{\mathrm{eq}}(q,p) = W(q,p,t \to \infty)$ by integrating Eq. 1 and then applying Eq. 4. Both approaches return the same numerical result within the bounds of reasonable numerical errors. We were unaware of these types of experiments; thanks for raising this. We agree that they may be relevant for addressing our results.

- We modified the third to last paragraph in the conclusion section to suggest, according to your comment, future temperature-jump experimental measurements that could further corroborate our calculations. See page 6.
  Experimental measurements of tautomerisation rates for G-C double proton transfer reaction are still not available. Nevertheless, we believe that our theoretical results force us to radically revise our understanding of the likelihood of point mutations in DNA, and we hope they will encourage new experimental measurements of the proton transfer reaction. Perhaps, a temperature jump experiment, similar to investigations conducted on intramolecular proton transfer in heterocycles [21], could elucidate the proton transfer rates.

2.7 Point 7

*My opinion is that the value of p^*/p_0 obtained by the authors is an improvement over previous calculations, and that the difference is not because of tunnelling or non-ideality, rather because of the equilibrium distribution of eigenstate occupations. Nor do I doubt that the tunnelling factor calculation is reasonable, but I question the validity of using Eq.(10) in which the rate constants depend only on the heights of the activation barriers $E_{f,r}$ and take no account of the radically discrete quantum character of the eigenstates that transverse both sides of the reaction pathway; nor the distribution of their fractional occupations in the * and 0 staes of base-pairing. (This may also cause problems with Eq.(8) and the calculation of the tunnelling factor, but I leave that to the authors.) That is to say, having justified and taken a "low temperature" approach to the dynamic problem, the authors should take a similar approach to the description of the equilibrium distribution over the individual states of the system and the manner in which this affects the dynamics of forward and reverse reactions (which is exactly what they have implicitly calculated to find the equilibrium endpoint).*

Response Thanks for raising this important point. We agree that the equilibrium distribution of eigenstate occupations is different in the low-temperature approach than simply taking the thermal average of the system Hamiltonian.

- To address this point, we included additional information on the low-temperature correction. The additional information is provided in a new supporting document. We believe this is best placed here to not distract from the paper's main aim. Within the supporting information document, see the section entitled "Examining the Low-Temperature Correction" and the subsection "The Thermal Solution" contained within.

2.8 Point 8

Last of all I will comment on the biological significance of these results. The authors discuss this in relation to helicase catalysis of double-stranded unwinding, which requires the breaking of the hydrogen bonds that are simulated in this study. The argument presented is that the tautomerisation exchange is so rapid that the helicase breaking of hydrogen bonds simply "traps" the equilibrium p/p0 ratio in the single-stranded product. However, I think a much more sophisticated argument is needed, one which takes into account the manner in which the potential energy surface (on which the rapid tautomerisation takes place) is distorted as the hydrogen bond is, on a timescale much longer than electron tunnelling, stretched and finally broken. The answer may end up being the same, but it will be much better justified, and may differ between (i) thermal melting of the bonds, (ii) passive "Brownian ratchet" helicase action and (iii) dissipative "stepped motor" helicase action. These need to be considered to do justice to the real physical situation in any discussion of its biological significance. Furthermore, the authors' result may be relevant to the origin of life. According to Eigen & Schuster (Naturwissenschaften 64, 541 565 (1977)), single stranded RNA phage replicases have error rates* $\sim 5.0 \times 10^{-4}$*, suggesting that the early enzyme-controlled replication of nucleic acids may have been accuracy-limited by the tautomerisation reaction studied by the current authors.*

Response These are excellent points. We completely agree with the referee's statement; we would like to add that this point has been largely overlooked in the literature. The potential energy surface will become significantly distorted as the hydrogen bonds dissociate during the cleavage process. We are currently in the process of preparing another manuscript on this process. Unfortunately, it is well out of the remit of this paper.

- Thanks for your suggestion on the reference. We were not aware of these types of replicases. We added this point to the last paragraph of the conclusion.

  The high tautomer occupation probability might also be relevant to address the role of mutations in several theories of the origin of life, including the RNA-mechanism proposed by Eigen *et al.* [29], in which single-stranded RNA phage replicases have error rates on the order of 10^{-4}, suggesting that the early enzyme-controlled replication of nucleic acids may have been accuracy-limited by the tautomerisation reaction.

2.9 Typographical 1

7th line of abstract "accounts" instead of "account".

Response Correction accepted.

2.10 Typographical 2

page 1, RH column, 5th line of paragraph, "sine" instead of "since"

Response Correction accepted.

2.11 Typographical 3

page 2, RH column, 5th line of paragraph "proton" not "protons

Response Correction accepted.

2.12 Typographical 4

*There is a minor discrepancy between the stated height of the forward reaction energy barrier in the Figure 2 caption (*0.705 eV*) and the text: page 3, line 9 (*0.704 eV*).*

Response Thanks for pointing out the discrepancy, this has now been fixed. 0.704 eV is the correct value.

2.13 Typographical 5

It is not clear why Eq.(2) has been written as it is with the exponential factor appearing twice.

Response Correction accepted.

2.14 Typographical 6

The Heaviside function appears in Eq.(2) but is not defined until much later.

Response Thanks for pointing this out. We have moved the definition to after Eq. 2.

3 Referee 3

The article "An Open Quantum Systems approach to proton tunnelling in DNA" is an interesting paper in which authors have investigated the proton dynamics in the H-bond between the Guanine-Cytosine (G-C) DNA base pair using an open quantum systems approach based on the Wigner-Moyal Master equation to accounts for the interaction of the proton with the cellular environment. Authors have corrected the Master equation, which works right at high temperatures, to employ it at biological temperature (low temperatures). To implement this low temperature correction in the Master equation, authors have replaced the thermal energy term of the equation with the zero-point energy. Assuming and environment such as "aqueous solution", authors have stablished the phenomenological friction constant of the master equation with the collision spectrum of water. Authors have found several surprising results, such as quantum corrected half-lives which are much shorter than the classical ones, very high tunneling effect for the proton transfer ~ 105 *and high kinetic isotope effects, 30.*

Response We sincerely thank Referee 3 for their interest in the manuscript and their detailed review. We will now highlight the following key improvements made to the revised manuscript as motivated by the referee's comments:

- Added further supporting calculations on the low-temperature correction.
- Expanded discussion and comparison to available experimental literature.
- Included further calculations comparing our model of tunnelling to the results of a benchmark potential.

3.1 Point 1

The methodology of this work is interesting and could offer some interesting perspectives, however, the validity of the Master equation low temperature correction should be analyzed more carefully. In this way, more than one system (pair G-C in this case) should be studied to provide robustness to their results.

Response We appreciate the referee's concern about the validity of the Master equation. As suggested, we have added additional calculations to provide further robustness to our results. The new content covers a numerical comparison of our model to well established analytical solutions of the motion of a particle in a harmonic potential in the low-temperature case. The comparison between our numerical solutions to the analytical solutions should allay concerns regarding the validity of the low-temperature correction.

- The additional calculations are provided in a new supporting information document. We believe this is best placed here to not distract from the paper's main aim. See the section entitled "Examining the Low-Temperature Correction" and the subsections contained within the supporting information document.

3.2 Point 2

Authors have obtained very different results (several orders of magnitude) higher than the classical ones for the G-C proton transfer. In addition, they do not have experimental results to support their findings. In biological systems, tunneling effects higher than 10 are rare, and values of $\sim 100\,000$ *are too high even for pure gas phase reactions. Professor Warshel and coworkers have established a very limited effect of the tunneling effect in biological process (see J Phys Chem B. 2008 May 15;112(19):5950-4 and J. Phys. Chem.*

B 2001, 105, 33, 7887–7907). These are examples of enzymatic catalysis but could be extended to these DNA systems as well.

Response We accept the correction and have expanded the discussion of our result compared to available experiments. Unfortunately, there is no direct experimental measure for the degree of quantum tunnelling during a reaction, but the magnitude of the primary kinetic isotope effect (KIE) is likely to be a reasonable first approximation. Consequently, we have added experimental comparisons to this metric.

Currently, there are no available experimental observations on the nature of the potential energy surface connecting the canonical and tautomeric forms of DNA or the rate of proton transfer. However, in light of the referees' comment, we have included additional discussion on other proton transfer reactions in DNA.

We agree that the methods of Warshel *et al.* [30, 31] could be applied to the DNA system. However, the quantitative comparison is meaningless without directly calculating the tunnelling effects as their potential is wildly different. The tunnelling effects are dependent on the potential barrier parameters, height and width, potential minima separation distance [32]. We suggest that the reaction barrier is sufficiently low and thin for the proton transfer in the DNA system that the quantum rate through the barrier is high. We believe that the high quantum rate is high compared to the classical rate due to the nature of the potential energy surface. Here, we have the case that the classical rate is low, but the barrier is sufficiently narrow that the proton can tunnel from one side of the hydrogen bond to the other. The degree of tunnelling during an H-transfer reaction is susceptible to the barrier through which tunnelling occurs and depends on the specific reaction.

- Taking the referee's comment on board we restructured our presentation of the kinetic isotope effect calculation. See the fourth paragraph on page 4. In addition, at the end of the "Lifetime of the Tautomer" section, we highlighted that our KIE value is comparable to experimental values of other biological systems.
  We investigated the impact of the kinetic isotope effect (KIE) as a function of temperature (doubling the proton's mass if replaced by a deuteron). See the SI and Methods section. A strong dependence of the reaction rate on the reduced mass of the system can indicate tunnelling. Hence the kinetic isotope can be used to probe if quantum effects dominate [18–20].

  At low temperatures, the KIE rapidly increases, providing further evidence that the transfer process becomes increasingly dependent on the quantum (tunnelling) contribution. Meanwhile, at high temperatures, the KIE exponentially tails off. At biological temperatures the KIE = 30.

  A high tunnelling factor and KIE has been observed in other models of open quantum systems [33, 34] probing proton transfer reactions. Furthermore, in a proton transfer reaction catalysed by enzyme soybean lipoxygenase, in the wild type, a high KIE of $\sim$ 80 [35] has been experimentally observed and verified using theory [36]. Furthermore, the KIE increased to the values from 500–800 in the case of various mutants [18,37–39]. Consequently, the value of KIE reported here is within sensible bounds of experimentally observed values.

- We updated the methods section on the KIE to incorporate the changes above. See methods the section entitled "Kinetic Isotope Effect" on page 8.
  We investigated the impact of the kinetic isotope effect to determine possible quantum effects. We investigated the kinetic isotope effect using the same parameters before but doubled the mass.

$$\mathrm{KIE} = \frac{k_{\mathrm{QM}}^{\mathrm{p}}}{k_{\mathrm{QM}}^{\mathrm{d}}}, \tag{1}$$

where $k_{\mathrm{QM}}^{\mathrm{p}}$ ($k_{\mathrm{QM}}^{\mathrm{d}}$) is the rate for a proton (deuteron). We neglect any changes to the transfer energy landscape due to isotopic substitutions, such as zero-point energy and free energy contributions, and consider only the effect on the reaction rate due to modifications of the mass terms in Eq. 1.

- See the section on the mass dependence ('Sensitivity to the Mass and the Kinetic Isotope Effect') in the new supplementary file.
- In the conclusion we have incorporated several new points referencing our work to experimental evidence on the G-T wobble proton transfer mechanism. See the new parts of the conclusion spanning pages 5 to 6. See also the discussion raised by Referee 1, point 4.

  The first [14] structural evidence for the rare tautomer hypothesis was observed using X-ray crystallographic analysis of a DNA polymerase. Whereby the polymerase was observed mismatching C with A. The mismatch adopts a Watson-Crick-like configuration which can only be obtained via the movement of a single proton on one of the mismatched bases. The proton transfer product alters the hydrogen-bonding pattern such that the mispair forms an overall shape that is virtually indistinguishable from a canonical, Watson-Crick base pair in double-stranded DNA. It remains an open question if the proton transfer has contributions from quantum effects.

  More recently, tautomerisation in DNA has been experimentally observed using NMR relaxation dispersion. Here, hairpin DNA duplex structures were observed to interchange between a wobble G-T and a Watson-Crick-like structure via tautomerisation (neutral) or ionisation (charged) [15,16]. This experimentally confirms that tautomerisation in DNA is a non-trivial effect.

  A positive indication that tautomerisation might be facilitated by tunnelling was provided recently by Rangadurai *et al.* [17]. They extensively 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_2O and D_2O. The authors reported a KIE as the rate of exchange between the two conformations of the mismatch was $\sim$ 3-fold slower in D_2O than in H_2O. This result provides the first experimental evidence supporting a transition state for tautomerisation involving proton tunnelling. However, before the observed KIE can be unequivocally attributed to proton tunnelling, further measurements will need to be conducted to investigate its temperature dependence [18]. Since a definitive experimental hallmark of tunnelling is a temperature-independent rate at low temperature, the rate of classical over-the-barrier hopping becomes negligible at low temperatures, leaving only the quantum contribution [19, 20].

  Experimental measurements of tautomerisation rates for G-C double proton transfer reaction are still not available. Nevertheless, we believe that our theoretical results force us to radically revise our understanding of the likelihood of point mutations in DNA, and we hope they will encourage new experimental measurements of the proton transfer reaction. Perhaps, a temperature jump experiment, similar to investigations conducted on intramolecular proton transfer in heterocycles [21], could elucidate the proton transfer rates.

  One can note that our model predicts a rate of tautomerisation much higher than the overall rate of spontaneous mutations ($\sim 10^{-8}$) [22], but this is consistent with the well-known presence of highly efficient DNA repair mechanisms. However, it is also difficult to comment on the overall efficiency of these repair mechanisms because other spontaneous mutations will also take place in DNA aside from tautomerisation [23, 24].
- We compared our rates to observations of the femtosecond dynamics of tautomeri-

sation of a DNA base analogue. See the second paragraph in the "Lifetime of the Tautomer" section, page 4.
In an analogue system (the 7-azaindole dimer), ultraviolet femtosecond pulsed time-of-flight spectroscopy indicates that proton transfer can occur on a timescale of a few hundred femtoseconds [40]. Here, light is used to induce proton transfer and probe the dimer's dynamics. In addition, Douhal *et al.* [40] demonstrate that the process is isotopically sensitive, indicating that quantum tunnelling can play a role in the dynamics. The rates experimentally reported are much faster than the rates reported here, which is likely due to the initial ultraviolet pulse modifying the reaction profile. However, the experiment suggests that the proton transfer reaction rate can be fast and have a quantum component.

3.3 Point 3

Authors should check this correction of the Master equation in a biological system in which the rate constant is very well know and their results could be validated. Afterwards, they could extend the "algorithm" to these systems much more complicated.

Response We accept the suggestion and have added additional calculations. We compare our model of tunnelling to the results of a benchmark potential used by several authors to check the validity of their model. We agree with other authors about the classical-to-quantum contribution to the transmission through the benchmark potential's barrier within a factor of two.

- The additional calculations are provided in the supporting information document. See the section entitled "Tunnelling Comparison to a Benchmark Potential".

4 Further corrections

This section covers additional minor corrections that the referees have not directly suggested. However, we believe they will improve the general quality of the paper. Please see the changes below.

4.1 Further correction 1

To improve the clarity of the paper, we made minor adjustments the abstract to improve readability. One of the most important topics in molecular biology is the genetic stability of DNA. One threat to this stability is proton transfer along the hydrogen bonds of DNA that could lead to tautomerisation, hence creating point mutations. We present a theoretical analysis of the hydrogen bonds between the Guanine-Cytosine (G-C) nucleotide, which includes an accurate model of the structure of the base pairs, the quantum dynamics of the hydrogen bond proton, and the influence of the decoherent and dissipative cellular environment. We determine that the quantum tunnelling contribution to the proton transfer rate is several orders of magnitude larger than the classical over-the-barrier hopping. Due to the significance of the quantum tunnelling even at biological temperatures, we find that the canonical and tautomeric forms of G-C inter-convert over timescales far shorter than biological ones and hence thermal equilibrium is rapidly reached. Furthermore, we find a large tautomeric occupation probability of 1.73×10^{-4}, suggesting that such proton transfer may well play a far more important role in DNA mutation than has hitherto been suggested. Our results could have far-reaching consequences for current models of genetic mutations.

4.2 Further correction 2

We have observed that "ohmic" should read as "Ohmic". All occurrences of this have now been corrected for.

4.3 Further correction 3

We changed the annotation and x-axis caption on Fig. 3 to match the style of the other figures.

4.4 Further correction 4

We changed the wording in the third paragraph in the conclusion to highlight an example system. The paragraph now reads:
Attempting to identify isotope effects in this system is problematic since investigating biological assays in deuterated solvents comes with a whole host of problems, for example, viscosity effects inhibiting correct protein function [41]. In addition, the impairment of gene expression at the transcription or translation level could preclude the measurement of the KIE discussed in the Methods section. Therefore, more direct measurements via nuclear magnetic resonance shifts, such as those applied to the G-T wobble mismatch, need to be applied [16].

References

[1] L Slocombe, JS Al-Khalili, and M Sacchi. Quantum and classical effects in dna point mutations: Watson–crick tautomerism in at and gc base pairs. *Physical Chemistry Chemical Physics*, 23(7):4141–4150, 2021.

[2] D. Wales, R. Saykally, A. Zewail, and D. King. *Energy Landscapes: Applications to Clusters, Biomolecules and Glasses*. Cambridge University Press, 2003.

[3] Ol'ha O Brovarets' and Dmytro M Hovorun. Atomistic mechanisms of the double proton transfer in the h-bonded nucleobase pairs: Qm/qtaim computational lessons. *Journal of Biomolecular Structure and Dynamics*, 37(7):1880–1907, 2019.

[4] Ol'ha O. Brovarets' and Dmytro M. Hovorun. Why the tautomerization of the g·c watson–crick base pair via the dpt does not cause point mutations during dna replication? qm and qtaim comprehensive analysis. *Journal of Biomolecular Structure and Dynamics*, 32(9):1474–1499, 2014. PMID: 23909623.

[5] Ol'ha O. Brovarets and Dmytro M. Hovorun. Can tautomerization of the a·t watson–crick base pair via double proton transfer provoke point mutations during dna replication? a comprehensive qm and qtaim analysis. *Journal of Biomolecular Structure and Dynamics*, 32(1):127–154, 2014. PMID: 23383960.

[6] AD Godbeer, JS Al-Khalili, and PD Stevenson. Modelling proton tunnelling in the adenine–thymine base pair. *Physical Chemistry Chemical Physics*, 17(19):13034–13044, 2015.

[7] A Gheorghiu, PV Coveney, and AA Arabi. The influence of base pair tautomerism on single point mutations in aqueous dna. *Interface focus*, 10(6):20190120, 2020.

[8] Radek Pohl, Ondřej Socha, Petr Slavíček, Michal Šála, Paul Hodgkinson, and Martin Dračínskỳ. Proton transfer in guanine–cytosine base pair analogues studied by nmr spectroscopy and pimd simulations. *Faraday discussions*, 212:331–344, 2018.

[9] Diego Soler-Polo, Jesús I Mendieta-Moreno, Daniel G Trabada, Jesús Mendieta, and José Ortega. Proton transfer in guanine-cytosine base pairs in b-dna. *Journal of chemical theory and computation*, 15(12):6984–6991, 2019.

[10] Jingzhi Pu, Jiali Gao, and Donald G. Truhlar. Multidimensional tunneling, recrossing, and the transmission coefficient for enzymatic reactions. *Chemical Reviews*, 106(8):3140–3169, 2006. PMID: 16895322.

[11] Stefan Schweiger and Guntram Rauhut. Plateau reactions: Double proton-transfer processes with structureless transition states. *The Journal of Physical Chemistry A*, 107(45):9668–9678, 2003.

[12] Youngchan Kim, Federico Bertagna, Edeline M. D'Souza, Derren J. Heyes, Linus O. Johannissen, Eveliny T. Nery, Antonio Pantelias, Alejandro Sanchez-Pedreño Jimenez, Louie Slocombe, Michael G. Spencer, Jim Al-Khalili, Gregory S. Engel, Sam Hay, Suzanne M. Hingley-Wilson, Kamalan Jeevaratnam, Alex R. Jones, Daniel R. Kattnig, Rebecca Lewis, Marco Sacchi, Nigel S. Scrutton, S. Ravi P. Silva, and Johnjoe McFadden. Quantum biology: An update and perspective. *Quantum Reports*, 3(1):80–126, 2021.

[13] Ruby Srivastava. The role of proton transfer on mutations. *Frontiers in chemistry*, 7:536, 2019.

[14] Weina Wang, Homme W Hellinga, and Lorena S Beese. Structural evidence for the rare tautomer hypothesis of spontaneous mutagenesis. *Proceedings of the National Academy of Sciences*, 108(43):17644–17648, 2011.

[15] Isaac J Kimsey, Katja Petzold, Bharathwaj Sathyamoorthy, Zachary W Stein, and Hashim M Al-Hashimi. Visualizing transient watson–crick-like mispairs in dna and rna duplexes. *Nature*, 519(7543):315–320, 2015.

[16] Isaac J Kimsey, Eric S Szymanski, Walter J Zahurancik, Anisha Shakya, Yi Xue, Chia-Chieh Chu, Bharathwaj Sathyamoorthy, Zucai Suo, and Hashim M Al-Hashimi. Dynamic basis for dg• dt misincorporation via tautomerization and ionization. *Nature*, 554(7691):195–201, 2018.

[17] Atul Rangadurai, Eric S Szymanski, Isaac Kimsey, Honglue Shi, and Hashim M Al-Hashimi. Probing conformational transitions towards mutagenic watson–crick-like g· t mismatches using off-resonance sugar carbon r 1ρ relaxation dispersion. *Journal of Biomolecular NMR*, pages 1–15, 2020.

[18] Judith P Klinman and Adam R Offenbacher. Understanding biological hydrogen transfer through the lens of temperature dependent kinetic isotope effects. *Accounts of chemical research*, 51(9):1966–1974, 2018.

[19] Linus O. Johannissen, Sam Hay, and Nigel S. Scrutton. Nuclear quantum tunnelling in enzymatic reactions – an enzymologist's perspective. *Phys. Chem. Chem. Phys.*, 17:30775–30782, 2015.

[20] Linus O. Johannissen, Andreea I. Iorgu, Nigel S. Scrutton, and Sam Hay. What are the signatures of tunnelling in enzyme-catalysed reactions? *Faraday Discuss.*, 221:367–378, 2020.

[21] O Bensaude, M Dreyfus, G Dodin, and JE Dubois. Intramolecular nondissociative proton transfer in aqueous solutions of tautomeric heterocycles: A temperature-jump kinetic study. *Journal of the American Chemical Society*, 99(13):4438–4446, 1977.

[22] Wen-Jin Wu, Wei Yang, and Ming-Daw Tsai. How dna polymerases catalyse replication and repair with contrasting fidelity. *Nature Reviews Chemistry*, 1(9):1–16, 2017.

[23] Thomas A Kunkel and P Sue Alexander. The base substitution fidelity of eucaryotic dna polymerases. mispairing frequencies, site preferences, insertion preferences, and base substitution by dislocation. *Journal of Biological Chemistry*, 261(1):160–166, 1986.

[24] Walter J Zahurancik, Seth J Klein, and Zucai Suo. Significant contribution of the 3'→ 5' exonuclease activity to the high fidelity of nucleotide incorporation catalyzed by human dna polymerase epsilon. *Nucleic acids research*, 42(22):13853–13860, 2014.

[25] Alejandro Pérez, Mark E Tuckerman, Harold P Hjalmarson, and O Anatole Von Lilienfeld. Enol tautomers of watson- crick base pair models are metastable because of nuclear quantum effects. *Journal of the American Chemical Society*, 132(33):11510–11515, 2010.

[26] T.L. Hill. *Thermodynamics of Small Systems*. Dover Books on Chemistry. Dover Publications, 1994.

[27] Wei Fang, Ji Chen, Mariana Rossi, Yexin Feng, Xin-Zheng Li, and Angelos Michaelides. Inverse temperature dependence of nuclear quantum effects in dna base pairs. *The journal of physical chemistry letters*, 7(11):2125–2131, 2016.

[28] Lu Wang, Stephen D Fried, Steven G Boxer, and Thomas E Markland. Quantum delocalization of protons in the hydrogen-bond network of an enzyme active site. *Proceedings of the National Academy of Sciences*, 111(52):18454–18459, 2014.

[29] Manfred Eigen and Peter Schuster. A principle of natural self-organization. *Naturwissenschaften*, 64(11):541–565, 1977.

[30] Janez Mavri, Hanbin Liu, Mats HM Olsson, and Arieh Warshel. Simulation of tunneling in enzyme catalysis by combining a biased propagation approach and the quantum classical path method: application to lipoxygenase. *The Journal of Physical Chemistry B*, 112(19):5950–5954, 2008.

[31] Jordi Villa and Arieh Warshel. Energetics and dynamics of enzymatic reactions. *The Journal of Physical Chemistry B*, 105(33):7887–7907, 2001.

[32] Sam Hay and Nigel S Scrutton. Good vibrations in enzyme-catalysed reactions. *Nature chemistry*, 4(3):161–168, 2012.

[33] Yanying Liu, Yaming Yan, Tao Xing, and Qiang Shi. Understanding the large kinetic isotope effect of hydrogen tunneling in condensed phases by using double-well model systems. *The Journal of Physical Chemistry B*, 125(22):5959–5970, 2021. PMID: 34033714.

[34] Weiwei Xie, Yang Xu, Lili Zhu, and Qiang Shi. Mixed quantum classical calculation of proton transfer reaction rates: From deep tunneling to over the barrier regimes. *The Journal of chemical physics*, 140(17):174105, 2014.

[35] Michael H Glickman, Jeffrey S Wiseman, and Judith P Klinman. Extremely large isotope effects in the soybean lipoxygenase-linoleic acid reaction. *Journal of the American Chemical Society*, 116(2):793–794, 1994.

[36] Pengfei Li, Alexander V Soudackov, and Sharon Hammes-Schiffer. Impact of mutations on the binding pocket of soybean lipoxygenase: Implications for proton-coupled electron transfer. *The journal of physical chemistry letters*, 9(22):6444–6449, 2018.

[37] Pengfei Li, Alexander V Soudackov, and Sharon Hammes-Schiffer. Fundamental insights into proton-coupled electron transfer in soybean lipoxygenase from quantum mechanical/molecular mechanical free energy simulations. *Journal of the American Chemical Society*, 140(8):3068–3076, 2018.

[38] Shenshen Hu, Alexander V Soudackov, Sharon Hammes-Schiffer, and Judith P Klinman. Enhanced rigidification within a double mutant of soybean lipoxygenase provides experimental support for vibronically nonadiabatic proton-coupled electron transfer models. *ACS catalysis*, 7(5):3569–3574, 2017.

[39] Alexander V Soudackov and Sharon Hammes-Schiffer. Proton-coupled electron transfer reactions: analytical rate constants and case study of kinetic isotope effects in lipoxygenase. *Faraday discussions*, 195:171–189, 2017.

[40] A Douhal, SK Kim, and AH Zewail. Femtosecond molecular dynamics of tautomerization in model base pairs. *Nature*, 378(6554):260–263, 1995.

[41] Luisa S Hohlefelder, Tobias Stögbauer, Madeleine Opitz, Thomas M Bayerl, and Joachim O Rädler. Heavy water reduces gfp expression in prokaryotic cell-free assays at the translation level while stimulating its transcription. *BioMed research international*, 2013, 2013.