How many tautomerization pathways connect Watson–Crick-like G*·T DNA base mispair and wobble mismatches?

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In this study, we have theoretically demonstrated the intrinsic inability of the wobble G·T(w)/G*·T*(w) and Watson–Crick-like G*·T(WC) DNA base mispairs to interconvert into each other via the DPT tautomerization. We have established that among all these transitions, only one single G·T(w) ↔ G*·T(WC) pathway is eligible from a biological perspective. It involves short-lived intermediate – the G·T*(WC) base mispair – and is governed by the planar, highly stable, and zwitterionic TSG G–T(w)→G·T*(WC) transition state stabilized by the participation of the unique pattern of the five intermolecular O6–H⋯O4, O6–H⋯N3, N1–H⋯N3, N1–H⋯O2, and N2–H⋯O2 H-bonds. This non-dissociative G·T(w) ↔ G*·T(WC) tautomerization occurs without opening of the pair: Bases within mispair remain connected by 14 different patterns of the specific intermolecular interactions that successively change each other along the IRC. Novel kinetically controlled mechanism of the thermodynamically non-equilibrium spontaneous point GT/TG incorporation errors has been suggested. The mutagenic effect of the analogues of the nucleotide bases, in particular 5-bromouracil, can be attributed to the decreasing of the barrier of the acquisition by the wobble pair containing these compounds of the enzymatically competent Watson–Crick’s geometry via the intrapair mutagenic tautomerization directly in the hydrophobic recognition pocket of the replication DNA-polymerase machinery. Proposed approaches are able to explain experimental data, namely growth of the rate of the spontaneous point incorporation errors during DNA biosynthesis with increasing temperature.

Keywords: spontaneous point mutation; incorporation error; mutagenic tautomerization; structural switching; DPT; zwitterionic transition state

Introduction

The G*·T(WC) and G·T*(WC) DNA base mispairs with Watson–Crick (WC) geometry (Bebenek, Pedersen, & Kunkel, 2011; Brovarets’ & Hovorun, 2015a; Brovarets’, Kolomiets’, & Hovorun, 2012; Danilov, Anisimov, Kurita, & Hovorun, 2005; Kimsey, Petzold, Sathyamoorthy, Stein, & Al-Hashimi, 2015; Topal & Fresco, 1976) (here and below rare mutagenic tautomers (Brovarets’, Zhurakivsky, & Hovorun, 2014d; Kosenkov et al., 2009) are marked with an asterisk), each of which is covered by three classical intermolecular H-bonds (Brovarets’ & Hovorun, 2015a; Brovarets’ et al., 2012), occupy a special place in the theory of the spontaneous point mutagenesis (Watson & Crick, 1953). In particular, transitions that among all possible spontaneous point mutations occur most frequently (Friedberg et al., 2006; Lee, Popodi, Tang, & Foster, 2012; Lynch, 2010) are associated with these pairs (Topal & Fresco, 1976), which geometry is very close to the structure of the canonical G·C(WC) and A·T(WC) Watson–Crick DNA base pairs (Brovarets’ & Hovorun, 2014a, 2014d; Giulia Rossetti et al., 2015). Nowadays, there are no well-established conceptions, in what way the G* and T* mutagenic tautomers of the DNA bases, which themselves are long-lived structures (Brovarets’ & Hovorun, 2010a), are generated in the process of the genome functioning.

Significant step in this biologically important direction has been performed in the pioneer work (Brovarets’ & Hovorun, 2009a), where for the first time it was theoretically shown the unique ability of the wobble G·T(w) base pair, stabilized by two antiparallel N3H⋯O6 and N1H⋯O2 H-bonds, to transform through the dynamically unstable intermediate – the G·T*(WC) mismatch – into the G*·T(WC) DNA base mispair, which is the global minimum, and vice versa. Herewith the zwitterionic planar TSG G·T(w)→G·T*(WC) (“+” means protonation and “−” deprotonation of the G and T bases by the O6 and N3 sites, respectively), transition state of this conversion is stabilized by the participation of the original pattern of the five O6–H⋯O4, O6–H⋯N3, N1–H⋯N3, N1–H⋯O2, 

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The main goal of this work was to answer on the fundamental question, whether the biologically important pathway of the G·T(w) ↔ G*·T(WC) tautomeric transition presented for the first time in the work (Brovarets & Hovorun, 2009a) is the sole. In this study, in addition to the already known G·T(w) ↔ G*·T(WC) tautomeration pathway (Brovarets’ & Hovorun, 2009a), that has sparked a great interest last time (Bebenek, Pedersen, & Kunkel, 2011; Kimsey et al., 2015; Nomura et al., 2013; Kimsey, Petzold, Sathyamoorthy, Stein, & Al-Hashimi, 2015), we managed to reveal three new routes of the tautomerization, namely G*·T(WC) ↔ G·T(w1), G*·T(WC) ↔ G·T(w2), and G*·T(WC) ↔ G*·T*(w), occurring via the sequential DPT and rearrangement of the bases, respectively, each other within the pair and concerning a novel wobble structures formed by the G and T bases in their canonical and rare tautomeric forms (Brovarets’, Yurenko, Dubey, & Hovorun, 2012), and to establish their structural, energetic and kinetic properties. A key finding of this work is that eventually it turned out that two novel uncovered G·T(WC) ↔ G·T(w1) and G*·T(WC) ↔ G·T(w2) tautomerization reactions are too slow events comparably with the time of the DNA replication in the cell (see works (Alberts et al., 2002; Friedberg et al., 2006) and references therein) and the third one – G*·T(WC) ↔ G*·T*(w) – is low probable, or, in other words, all of them are unrealistic and cannot be considered as an acceptable alternative to the path that was previously revealed (Brovarets’ & Hovorun, 2009a). Possible biological implications for the results, which we obtained here and previously, have been also critically discussed in the brief form.

Computational methods

All calculations have been performed using Gaussian’09 package (Frisch et al., 2010), employing B3LYP DFT (Lee, Yang, & Parr, 1988; Parr & Yang, 1989; Tirado-Rives & Jorgensen, 2008) functional combined with Pople’s 6-311++G(d,p) basis set for calculations of the geometries and harmonic vibrational frequencies of the base mispairs and transition states of their tautomerization. This functional is capable of handling physico-chemical characteristics for similar systems (Arabi & Matta, 2011; Brovarets’ & Hovorun, 2010c, 2011a, 2011b; El-Sayed, Tamara Molina, Álvarez-Ros, & Alcolea Palafoux, 2015; Matta, 2010; Palafoux, 2014; Samijlenko, Yurenko, Stepanyugin, & Hovorun, 2012). A scaling factor that is equal to 0.9668 has been used in the present work to correct harmonic frequencies of all studied base pairs (Brovarets’ & Hovorun, 2014c, 2015b; Brovarets’, Zhurakivsky, & Hovorun, 2013b, 2014a, 2014c). We have confirmed the minima and TS, located by means of Synchronous Transit-guided Quasi-Newton method (Peng,
Ayala, Schlegel, & Frisch, 1996), on the potential energy landscape by the absence or presence, respectively, of the imaginary frequency in the vibrational spectra of the pair.

In order to consider electronic correlation effects as accurately as possible, we followed geometry optimizations with single-point energy calculations using MP2 functional (Frisch, Head-Gordon, & Pople, 1990) and a wide variety of basis sets, in particular, Pople’s basis sets of valence triple-ζ quality (Harijaran & Pople, 1973; Krishnan, Binkley, Seeger, & Pople, 1980), as well as Dunning’s cc-type basis sets (Kendall, Dunning, & Harrison, 1992), augmented with polarization and/or diffuse functions: 6-311++G(2df, pd), 6-311++G(3df, 2pd), cc-pVTZ, and cc-pVQZ.

Reaction pathway was established by following intrinsic reaction coordinate (IRC) in the forward and reverse directions from each TS using Hessian-based predictor–corrector integration algorithm (Hratchian & Schlegel, 2005) with tight convergence criteria. These calculations eventually ensure that the proper reaction pathway, connecting the expected reactants and products on each side of the TS, has been found. We have investigated the evolution of the energetic, geometric, polar, and electronic-terological characteristics of the H-bonds and base pairs along the pathway of the G·T(w) ↔ G*·T(WC) tautomeration reaction establishing them at each point of the IRC (Brovarets’ & Hovorun, 2014a, 2014c, 2015b; Brovarets’ et al., 2013, 2014a).

Electronic interaction energies \( E_{\text{int}} \) have been calculated at the MP2/6-311++G(2df, pd)//B3LYP/6-311++G(d, p) level of theory as the difference between the total energy of the base mispair and the energies of the isolated monomers. Gibb’s free energy of interaction has been obtained using similar equation. In each case, the interaction energy was corrected for the basis set superposition error (Boys & Bernardi, 1970; Gutowski, Van Lenthe, Verbeek, Van Duijneveldt, & Chalasinski, 1986) through the counterpoise procedure (Sordo, 2001; Sordo, Chin, & Sordo, 1988).

The Gibbs free energy \( G \) for all structures was obtained in the following way:

\[
G = E_{\text{el}} + E_{\text{corr}} \tag{1}
\]

where \( E_{\text{el}} \) – electronic energy, while \( E_{\text{corr}} \) – thermal correction. We applied the standard TS theory (Atkins, 1998) to estimate activation barriers of the tautomeration reactions.

The time \( t_{99.9\%} \) necessary to reach 99.9% of the equilibrium concentration of the initial and terminal base mispairs in the system of reversible first-order forward \( (k_f) \) and reverse \( (k_r) \) reactions can be estimated by formula (Atkins, 1998):

\[
t_{99.9\%} = \ln 10^3 \frac{k_r}{k_f + k_r} \tag{2}
\]

To estimate the values of the rate constants \( k_f \) and \( k_r \).

\[
k_{f,r} = \frac{k_B T}{h} \cdot e^{-\Delta G_{f,r}^s} \tag{3}
\]

we applied standard TS theory (Atkins, 1998), in which quantum tunneling effect is accounted by Wigner’s tunneling correction (Wigner, 1932), that has been successfully used for the DPT reactions (Brovarets’ & Hovorun, 2013a, 2013b; Brovarets’ et al., 2013a, 2014b):

\[
\Gamma = 1 + \frac{1}{24} \left( \frac{\nu v_i}{k_B T} \right)^2 \tag{4}
\]

where \( k_B \) – Boltzmann’s constant, \( h \) – Planck’s constant, \( \Delta G_{f,r}^s \) – Gibb’s free energy of activation for the tautomeration reaction in the forward (f) and reverse (r) directions, \( \nu_i \) – magnitude of the imaginary frequency associated with the vibrational mode at the TS.

Bader’s quantum theory of atoms in molecules was applied to analyze the electron density distribution (Bader, 1990; Lecomte, Espinosa, & Matta, 2015; Matta, 2014; Matta, Huang, & Massa, 2011). The topology of the electron density was analyzed using program package AIMAll (Keith, 2010) with all default options. The presence of a bond critical point (BCP), namely the so-called \((3,-1)\) BCP, and a bond path between hydrogen donor and acceptor, as well as the positive value of the Laplacian at this BCP \( (\Delta \rho > 0) \), were considered as criteria for the H-bond formation (Brovarets’, Yureno, & Hovorun, 2014a, 2014b). Wave functions were obtained at the level of theory used for geometry optimization.

The energies of the weak CH⋯O H-bonds (Brovarets’ et al., 2014a, 2014b) were calculated by the empirical Espinosa–Molins–Lecomte (EML) formula (Espinosa, Molins, & Lecomte, 1998; Mata, Alkorta, Espinosa, & Molins, 2011), which has been first successfully applied by Matta (Matta, Castillo, & Boyd, 2006) for the estimation of the individual energetic contributions of the separate H-bonds in the two Watson–Crick DNA base pairs:

\[
E_{\text{CH⋯O}} = 0.5 \cdot V(r) \tag{5}
\]

where \( V(r) \) – value of a local potential energy at the (3, −1) BCP.

The energies of the AH⋯B (A, B designate O, or N atoms) conventional H-bonds were evaluated by the empirical Logansen’s formula (Logansen, 1999):

\[
E_{\text{AH⋯B}} = 0.33 \cdot \sqrt{\Delta v - 40} \tag{6}
\]

where \( \Delta v \) – magnitude of the redshift (relative to the free molecule) of the stretching mode of the AH H-bonded group involved in the AH⋯B H-bond. The partial deuteration was applied to minimize the effect of vibrational resonances (Brovarets’ & Hovorun, 2014c; Brovarets’ et al., 2013b).
The energies of the OH···O and NH···O/N H-bonds in the selected TSs containing loosened covalent bridges were estimated by the Nikolaienko-Bulavin-Hovorun formulas (Nikolaienko, Bulavin, & Hovorun, 2012):

\[ E_{\text{OH} \cdots \text{O}} = -3.09 + 239 \cdot \rho, \]

\[ E_{\text{NH} \cdots \text{O/N}} = -2.03 + 225 \cdot \rho, \]

where \( \rho \) – the electron density at the (3, -1) BCP of the H-bond.

The atomic numbering scheme for the G and T bases is conventional (Saenger, 1984).

Results and their discussion

Novel pathways of the tautomerization of the G*·T (WC) base mispair into the G·T(w) and G·T(w2) wobble mismatches and vice versa

In this study, for the first time, we have detected three additional ways of the tautomeric conversion of the Watson–Crick-like G*·T(WC) base mispair into the wobble G·T(w1), G·T(w2), and G*·T*(w) base mispairs and vice versa, that have not been presented in the literature by this time (Figures 1–10 and Tables 1–7). Let us analyze them one after another.

The first pathway – the G*·T(WC) ↔ G*·T*(w) tautomerization reaction (Figure 1) – has among all investigated processes the lowest barrier of the transformation (\( \Delta G_{\text{TS}} = 12.39 \text{ kcal mol}^{-1} \) (Tables 1–3)) and therefore passes very fast, initially converting via the rapid DPT (\( t_{99.9\%} = 4.98 \times 10^{-9} \text{ s} \)) (Table 3 and Figure 1a) into the dynamically unstable intermediate – the G·T*(WC) base mispair (Brovarets & Hovorun, 2015a; Brovarets et al., 2012) (Figure 1a). Here, we would not describe in detail this process, because it has been profoundly elucidated in our previous paper (Brovarets’ & Hovorun, 2015a). Then, this reaction continues with the transformation of the G·T*(WC) base mispair through the wobble-like TSG_{G·T*(WC)}^{G·T*(w)} transition state (\( \nu_1 = -43.0 i \text{ cm}^{-1} \)), stabilized by the participation of the O6–H···O4 (10.68), N1+H···N3 (8.50), N2+H···N3 (1.76), and N2+H···O2 (3.24 kcal mol\(^{-1}\)) H-bonds, into the dynamically unstable intermediate – the G·T (w) ion pair (the value of the reverse barrier \( \Delta E = 5.18 \text{ kcal mol}^{-1} \) is negative (Table 3)), joined by the participation of the O6–H···O4 (5.29), N1+H···O4 (11.77), and N2+H···N3 (6.86 kcal mol\(^{-1}\)) H-bonds (Figure 1b, Table 1). And further the G*·T (w) base mispair quickly crosses via the transfer of the single proton localized at the N1+ nitrogen atom of the G+ protonated base to the O4- oxygen atom of the T- deprotonated base through the TSG_{G·T*(WC)}^{G·T*(w)}(w) ion pair (\( \nu_1 = -575.2i \text{ cm}^{-1} \)), bound by the two O6H···O4 (1.58) and N2H···N3 (5.32 kcal mol\(^{-1}\)) H-bonds and single N1–H···O4 covalent bridge, into the short-lived (lifetime \( \tau = 2.97 \times 10^{-12} \text{ s} \)) wobble G*·T*(w) base mispair (Figure 1c). This mismatch, that has not been previously described in the literature, is stabilized by the two anti-parallel O4H···N1 (9.90) and N2H···N3 (4.77 kcal mol\(^{-1}\)) H-bonds (Table 1). It has a considerably high energy relatively the G*·T*(WC) base mispair (\( \Delta G = 12.84 \text{ kcal mol}^{-1} \) (Table S1)) and therefore – too low population (3.8 \times 10^{-8} \%) in the selected TSs containing loosened covalent bridges (Brovarets et al., 2012), and its negligible population put out this mismatch outside the limits of the biological significance.

The second new route – G*·T(WC) ↔ G·T*(w1) (Tables 1, 2, 4, and Figures 1a, 3a, and 4), passing through the dynamically unstable intermediate – the G·T*(WC) base mispair (Brovarets’ & Hovorun, 2015a; Brovarets et al., 2012), occurs without opening of the pair that is tautomerized and is accompanied by the substantial changes in the base pair geometry.

The G*·T(WC) ↔ G·T*(w1) tautomerization reaction includes two stages – very fast one, from which the tautomerization process starts, and a very slow final phase. Tautomerization of the G*·T(WC) base pair begins with a very rapid process of the G*·T (WC) → G·T*(WC) DPT tautomerization (Table 3 and Figure 1a); at this, the G·T*(WC) base pair acts as a dynamically unstable intermediate (Brovarets’ & Hovorun, 2015a; Brovarets et al., 2012). Terminal, very slow (\( t_{99.9\%} = 2.31 \times 10^{10} \text{ s} \)) stage of the G*·T (WC) → G·T(w1) tautomerization passes (Figures 3a, 4, and Table 4) through the substantially non-planar zwitterionic TSG_{G·T*(WC)}^{G·T*(w1)} transition state (\( \angle \text{C6N1N3C5} = 83.7^\circ \)) with the relative Gibbs free energy 32.28 kcal mol\(^{-1}\) (Table S1) under normal conditions. It is stabilized by the participation of the two parallel H-bonds – O4–H···N1 (8.39) and N3+H···N1 (13.14 kcal mol\(^{-1}\)) (Table 1, Figure 3a), which are centered on the one and the same N1 nitrogen atom of the G base. The N9H and N1H glycicpic bonds of the G- and T+ bases in the TSG_{G·T*(WC)}^{G·T*(w1)} are in the syn-orientation relatively each other (\( \angle \text{N9H}H_2\text{N1} = 67.6^\circ \)). It is formed by the proton transfer from the N1 nitrogen atom of the G base along the intermolecular NH···N3 H-bond to the N3 nitrogen atom of the T* base in the G·T*(WC) base mispair and is accompanied by the mutation of the bases around the N1–N3 imaginary axis almost at a right angle. Vibrational spectra of the TSG_{G·T*(WC)}^{G·T*(w1)} contains mode with the imaginary frequency \( \nu_2 = 674.72 \text{ cm}^{-1} \).

This tautomerization process results in the formation of the substantially non-planar (\( \angle \text{C6N1N3C5} = 87.7^\circ \)) wobble G·T(w1) base mispair, which is stabilized by three intermolecular C5MeH···O6 (1.85), N1H···O4...
(2.90), and N2H⋯O4 (0.71 kcal mol\(^{-1}\)) H-bonds (Tables 1, 2, and Figure 3a). This tautomerization process is also accompanied by the significant change in the geometry of the base pair. However, at this, the base pair does not decompose into the monomers, remaining joined by the different patterns of the intermolecular H-bonds that successively change each other. To the best of our knowledge, the wobble G·T\(_{(w1)}\) DNA base mispair has not been mentioned in the literature so far. \(\text{Cis}\)-orientation of the N1H and N9H glycosidic bonds is characteristic for the wobble G·T\(_{(w1)}\) base pair (\(\angle\text{N9HHN1} = 39.7^\circ\)). This pair can be easily planarized (\(\Delta\Delta G_{\text{TS}} = 1.23\) kcal mol\(^{-1}\)) by turning of the Me-group at an angle of 60° and a flattening of the NH\(_2\) amino group of the G base (Figure 3b). Only under this circumstance, the wobble G·T\(_{(w1)}\) base mispair can be incorporated into the DNA double helix.

Figure 1. Geometrical structures of the base mispairs and TSs involved in the (a) G\(^*\)·T(WC) ↔ G·T\(^*\)(WC) (Brovarets' & Hovorun, 2015a), (b) G·T\(^*\)(WC) ↔ G\(^\ast\)·T\(^\ast\)(w), and (c) G\(^\ast\)·T\(^\ast\)(w) ↔ G\(^*\)·T\(^*\)(w) reaction pathways obtained at the B3LYP/6-311++G(d, p) level of theory. Notes: Here and below, dotted lines indicate AH⋯B H-bonds, while continuous lines show covalent bonds (their lengths are presented in angstroms). Carbon atoms are in light-blue, nitrogen – in dark-blue, hydrogen – in gray, and oxygen – in red. \(\nu_1\) – imaginary frequency.
It should be noted that the low-energy wobble G·T (w1) base mispair is connected with the high-energy wobble G·T(w2) base mispair (ΔG = 1.16 kcal mol\(^{-1}\)) under normal conditions) by the fast (τ99.9% = 1.56 × 10\(^{-11}\) s) conformational transformation (Table 4 and Figure 3c) via the substantially non-planar TS\(_{G·T(w1)←→G·T(w2)}\) transition state (∠C6N1N3C5 = 122.3°) stabilized by the N1H···O4 (1.98) and N2H···O4 (0.78 kcal mol\(^{-1}\)) H-bonds (Table 1) that is centered on the one and the same O4 oxygen atom of the T base. This G·T(w1)←→G·T(w2) conformational conversion corresponds to the vibrational mode with the imaginary frequency \(v_1 = -15.2i\) cm\(^{-1}\) and occurs without opening of the pair — bases remain bound by various H-bonds, which replace each other.

It is interesting to note that mirror-symmetric enantiomers of the G·T(w2) base mispair (∠C6N1N3C5 = ± 62.8°), that is presented here for the first time and has not been published elsewhere in the literature before, interconvert between each other through the planar TS\(_{G·T(w1)←→G·T(w2)}\) transition state (ΔΔG\(_{TS}\) = 6.89 kcal mol\(^{-1}\)) under normal conditions) (Table S2 and Figure 3d), to which corresponds the torsion vibration of the NH2 group of the G base with imaginary frequency \(v_1 = -178.2i\) cm\(^{-1}\). This fast (τ99.9% = 6.09 × 10\(^{-8}\) s) (Table S2) and large amplitude quantum oscillation of the G·T(w2) base mispair allows it to be incorporated into the structure of the DNA double helix, since its energy is significantly less than the energy of the stacking interactions of the neighboring Watson–Crick DNA base pairs (Jissy & Datta, 2014).

The third novel G*·T(WC) ↔ G·T(w2) pathway (Table 5 and Figures 5 and 6) is controlled by the slightly non-planar (∠C6N1N3C5 = 13.5°) TS\(_{G·T(WC)←→G·T(w2)}\) transition state, that is an ion pair involving G\(^{-}\) base, deprotonated by the N1 site, and T\(^{+}\) base, protonated by the N3 site, stabilized by the participation of the four intermolecular O4\(^{-}\)H···O6\(^{-}\) (4.70), O4\(^{-}\)H···N1\(^{-}\) (5.19), N3\(^{-}\)H···N1\(^{-}\) (5.00), and N3\(^{-}\)H···N2\(^{-}\) (2.02 kcal mol\(^{-1}\)) H-bonds (Table 1). The vibrational mode with imaginary frequency \(v_1 = -291.2i\) cm\(^{-1}\) corresponds to it. The TS\(_{G·T(WC)←→G·T(w2)}\) transition state is formed by the transfer of the proton localized at the O6 oxygen atom of the G base along the intermolecular O6H···O4 H-bond to the O4 oxygen atom of the T base and is accompanied by the significant displacement of the bases relatively each other (Figure 5).

This very slow tautomerization process (τ99.9% = 8.53 × 10\(^{12}\) s) (Table 5) is completed with the transition to the essentially non-planar wobble G·T(w2) base pair, which is stabilized by three H-bonds: N1H···O4 (2.43) and N2H···O4 (1.89) with the joint center — the O4 atom of the T base — and N3H···N2 (2.82 kcal mol\(^{-1}\)) (Table 1). The N9H and N1H glycosidic bonds of this base pair are in cis-orientation relative to each other (∠N9HN1H1N1 = 57.1°). The G·T (WC) ↔ G·T(w2) tautomerization process described here is accompanied by the significant change in geometry of the pair that is tautomerized; at this, the pair does not decay — it remains stabilized by the different patterns of the intermolecular H-bonds sequentially changing each other.

The data obtained in this study allow us to conclude that previously described biologically important route of the G*·T(w) ↔ G·T* (WC) tautomerization (Brovarets’ & Hovorun, 2009a) has no reasonable alternatives from the standpoint of the spontaneous point mutagenesis, since three new reaction paths that were discussed above — G*·T(WC) ↔ G·T(w1), G*·T(WC) ↔ G·T(w2), and G*·T(WC) ↔ G·T* (w) — are either much slower processes than even the process of the DNA replication in the cell (~10\(^{6}\) s (Alberts et al., 2002; Friedberg et al., 2006)) (in the case of the G*·T(WC)↔G·T(w1) and G*·T(WC)↔G·T(w2) tautomerization reactions), or low probable (3.8 × 10\(^{-10}\)) (in the case of the G*·T (WC)↔G*·T* (w) pathway). However, in our opinion, these pathways take a keen interest in the study of the mechanisms of the mutagenic tautomerization of the other biologically important wobble pairs involving G and T nucleotide bases with the cognate architecture of the H-bonding.

**Biological significance of the obtained results: a critical discussion**

So, making sure that there are no alternatives to the pathway of the G·T(w)↔G*·T(WC) tautomerization (Brovarets’ & Hovorun, 2009a), we can briefly discuss its primary biological importance.
| Complex                        | \(\Delta G^a\) | \(\Delta H^b\) | \(\Delta S^c\) | \(b_{\text{AH}}\) | \(b_{\text{B}}\) | \(d_{\text{AH}}\) | \(d_{\text{B}}\) | \(E_{\text{HH}}\) | \(\mu^d\) |
|-------------------------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|----------------|-----------|
| \(\text{G}^*\cdot\text{T}(\text{WC})\) | 0.00          | N&H−N1       | 0.040         | 0.012         | 2.66           | 2.723          | 1.742          | 0.020         | 171.3     | 400.5     | 6.27     | 5.77     |
| G−T*\(\text{WC})\) | 1.22          | N2H−O2       | 0.022         | 0.078         | 5.82           | 3.022          | 2.009          | 0.007         | 177.3     | 134.0     | 3.20     |          |
| G−\(\mathbb{1})\) | 1.69          | N3H−O6       | 0.030         | 0.117         | 5.23           | 2.860          | 1.828          | 0.021         | 174.2     | 360.0     | 5.90     | 8.37     |
| TS\(_G\cdot\text{T}+(\text{WC})\) | 2.63          | N2H−O2       | 0.035         | 0.136         | 6.25           | 2.801          | 1.776          | 0.015         | 173.4     | 278.8     | 5.10     |          |
| G−T(\(\mathbb{1})\) | 6.01          | N1H−O4       | 0.017         | 0.155         | 2.20           | 2.520          | 1.496          |          | 174.1     |          | 15.07**  | 3.70     |
| G−T(\(\mathbb{2})\) | 7.34          | N2H−O4       | 0.012         | 0.049         | 4.07           | 3.098          | 2.231          | 0.001         | 143.0     | 11.4      | 2.83     | 6.69     |
| TS\(_G\cdot\text{T}+(\text{WC})−\text{G}^*\cdot\text{T}(\text{WC})\) | 12.58         | N3H−O2       | 0.026         | 0.094         | 3.40           | 2.938          | 1.910          | 0.017         | 178.9     | 323.8     | 5.56     |          |
| \(\text{TSG}−\text{T}^*(\text{WC})\) | 13.56         | O6H−O4       | 0.058         | 0.098         | 4.93           | 2.671          | 1.675          | 0.049         | 164.5     | 940.7     | 9.90     | 2.51     |
| \(\text{TSG}−\text{T}+(\text{WC})−\text{T}^*(\text{WC})\) | 18.73         | O6H−O4       | 0.076         | 0.178         | 1.29           | 2.474          | 1.467          | 0.063         | 164.5     | 1087.7    | 10.68    | 2.56     |
| \(\text{TSG}−\text{T}+(\text{WC})−\text{T}^*(\text{WC})\) | 22.88         | O4H−N1       | 0.160         | 0.057         | 6.03           | 2.770          | 1.858          | 0.017         | 148.5     | 296.9     | 5.29     | 3.01     |
| \(\text{TSG}−\text{T}+(\text{WC})−\text{T}^*(\text{WC})\) | 39.67         | O4H−O6       | 0.054         | 0.191         | 10.49          | 2.766          | 1.830          | 0.036         | 153.5     | 685.7     | 8.39     | 8.70     |

\(^a\)The relative Gibbs free energy of the complex obtained at the MP2/cc−pVQZ/B3LYP/6−311++G(d,p) level of theory under normal conditions (\(T = 298.15\) K), kcal mol\(^{-1}\).
\(^b\)The energy of the H-bonds, calculated by Iogansen (\(\text{AH}−\text{B}\)).
\(^c\)The redshift of the stretching vibrational mode \(\nu(\text{AH})\) of the AH−B bonding group, cm\(^{-1}\).
\(^d\)The electron density at the (3, −1) BCP of the H-bond, \(\rho\).
Table 2. Interbase interaction energies (in kcal mol\(^{-1}\)) for the base mispairs and TSs of their mutual transformations via the sequential DPT obtained at the MP2\(6/311++G(2df, p)//B3LYP/6-311++G(d, p)\) level of theory.

| Complex | \(-\Delta E_{\text{int}}^{a}\) | \(\sum E_{\text{H}}^{b}\) | \(\Sigma E_{\text{H}}/\Delta E_{\text{int}}^{a}, \%\) | \(-\Delta G_{\text{int}}^{c}\) |
|---------|-----------------|-----------------|-----------------|-----------------|
| \(G^*\cdot T(WC)^{\S}\) | 19.79            | 17.35           | 87.7            | 7.09            |
| \(G\cdot T(WC)^{\S}\)     | 33.40            | 20.48           | 61.3            | 20.66           |
| \(G\cdot T(w)^{\S}\)      | 16.71            | 11.00           | 65.8            | 5.06            |
| \(G\cdot T(w_1)\)         | 12.93            | 5.46            | 42.2            | 1.75            |
| \(G\cdot T(w_2)\)         | 11.12            | 7.14            | 64.2            | -2.22           |
| \(TSG\cdot T(w_1)\rightarrow G\cdot T(w_2)\) | 10.35            | 6.83            | 66.0            | -0.93           |
| \(TSG\cdot T(w_2)\rightarrow G\cdot T(w_1)\) | 10.92            | 10.50           | 96.1            | -1.13           |
| \(G^*\cdot T*(w)\)        | 18.15            | 14.67           | 80.8            | 7.05            |
| \(TSG\cdot T*(w)\rightarrow G^*\cdot T*(w)\) | 143.80           | 24.18           | 16.8            | 127.44          |
| \(G^*\cdot T*(WC)\)       | 141.94           | 23.92           | 16.9            | 128.29          |
| \(TSG\cdot T*(WC)\rightarrow G^*\cdot T*(WC)\) | 135.84           | 18.15           | 13.4            | 120.59          |
| \(G^*\cdot T*(WC)\)       | 135.40           | 21.53           | 15.9            | 124.76          |
| \(TSG\cdot T*(WC)\rightarrow G^*\cdot T*(WC)\) | 122.58           | 16.91           | 13.8            | 110.82          |

\(^{a}\text{The BSSE-corrected electronic interaction energy.}\)

\(^{b}\text{The total energy of the intermolecular H-bonds (see Table 1).}\)

\(^{c}\text{The BSSE-corrected Gibbs free energy of interaction (T=298.15 K).}\)

\(^{\S}\text{and}^{\S}\text{Data are taken from the works (Brovarets’ \\& Hovorun, 2015a) and (Brovarets’ \& Hovorun, 2009a), respectively.}\)

Thus, in accordance with our results, the microstructural mechanism of the origin of the spontaneous transitions – incorporation errors – boils down to the tautomerization of the wobble G·T(w) mispair into the G*·T(WC) DNA mispair via the zwitterionic transition state \(TSG\cdot T(WC)\rightarrow G^*\cdot T*(WC)\), which is stabilized by the participation of the unique pattern of the five intermolecular H-bonds including bifurcated ones with a joint center localized at the O6\(^+\) and N1\(^+\) protonated atoms – O6\(^+\)H\(\cdots\)O4\(^-\) (2.69), O6\(^+\)H\(\cdots\)N3\(^-\) (5.21), N1\(^+\)H\(\cdots\)N3\(^-\) (2.61), N1\(^+\)H\(\cdots\)O2\(^-\) (3.20), and N2\(^+\)H\(\cdots\)O2\(^-\) (4.44 kcal mol\(^{-1}\)) (Figures 7–10 and Tables 1, 2, 6, 7), and involving short-lived (\(\tau = 8.13 \times 10^{-11}\) s) intermediate – the G*·T* (WC) base pair with Watson–Crick geometry, directly in the essentially hydrophobic recognition pocket of the high-fidelity replicative DNA-polymerase. The rapid (\(\tau_{99\%} = 4.98 \times 10^{-12}\) s (Table 6)) interconversion between the G*·T(WC) and G·T*(WC) base mispairs is defined by the transition state \(TSG\cdot G\cdot T*(WC)\rightarrow G^*\cdot T*\cdot T(WC)\) (\(v_i = -1134.2\) cm\(^{-1}\)) and was discussed in details in the work (Brovarets’ & Hovorun, 2015a). The G·T (w)\(\rightarrow\)G·T(WC) tautomerization transformation (Figure 7a) proceeds without the rupture of the H-bonds: The N3H\(\cdots\)O6 (5.90) and N1H\(\cdots\)O2 (5.10) H-bonds in the wobble G·T(w) base mispair rearrange into the O6H\(\cdots\)O4 (6.27), N3H\(\cdots\)N1 (7.89), and N2H\(\cdots\)O2 (3.20) H-bonds in the G*·T(WC) base mispair with Watson–Crick geometry (Table 1). Herewith, it has been observed 14 unique patterns of the intermolecular interactions including from 2 to 5 H-bonds and 3 loosened covalent bridges (Figure 9 and Table 7).

Detailed microstructural information about the course of the G·T(w)\(\rightarrow\)G·T(WC) tautomerization via the sequential DPT is presented in Figures 7, S2–S5 and Tables 6, 7.

Based on these data, we can arrive to the following important conclusions:

1. Both extrema of the first derivative of the electron energy with respect to the IRC – dE/dIRC (Figure S2 in the Supplementary Files), the so-called reaction force (Brovarets’ & Hovorun, 2014a, 2014d), are consistent with the key points of the G·T(w)\(\leftrightarrow\)G·T*(WC) tautomerization reaction, where values of the \(\Delta\rho_{(T)N3H\cdots O6(G)}\) and \(\Delta\rho_{(T)N3H\cdots N1(G)}\) become equal to 0 (Figures S2 and S4b in the Supplementary Files);

2. Investigated tautomerization process is dipole-active process, that can potentially be used for the laser control of this chemical reaction (Bandrauk, Sedik, & Matta, 2004, 2006; Matta, Sowlati-Hashjin, & Bandrauk, 2013) (Figure S3 in the Supplementary Files);

3. In the wide range of the IRC values (\(-11.41\) to \(23.03\) Bohr) tautomeration is assisted by the N2H\(\cdots\)O2 H-bond (Figures 9, S4–S5 in the Supplementary Files and Table 7).

It is logical to suggest that the barrier of the G·T (w)\(\rightarrow\)G·T*(WC) mutagenic tautomerization of the wobble G·T(w) base mispair in the recognition pocket of the DNA-polymerase reduces comparably with the isolated state, in particular, due to the stacking interactions (Jissy & Datta, 2014). At this, the values of the Gibbs free energy of the G*·T(WC) base mispair relatively to the G·T(w) mismatch together with just mentioned barrier
Figure 3. Geometrical structures of the base mispairs and TSs involved in the (a) G·T*(WC) ↔ G·T(w1), (b) G·T(w1) ↔ G·T*sym(w1), (c) G·T(w1) ↔ G·T(w2) and (d) G·T(w2) ↔ G·T*sym(w2) reaction pathways obtained at the B3LYP/6-311++G(d, p) level of theory (see also Figure S1). For definitions see Figure 1.
Figure 4. Profile of the relative electronic energy $\Delta E$ of the base mispair, that tautomerizes, along the IRC of the G$^*$·T(WC) ↔ G·T*(WC) tautomerization via the sequential DPT obtained at the B3LYP/6-311++G(d, p) level of theory. See also Figure S1 in the Supplementary Files for the profile of the relative electronic energy $\Delta E$ of the G·T*(w2) ↔ G·T*$^\text{sym}$(w2) conformational interconversion of the mirror-symmetric enantiomers of the wobble G·T(w2) base mispair via the rotation of the NH$_2$ amino group of the G base along the IRC obtained at the B3LYP/6-311++G(d, p) level of theory.

also decrease comparably with the corresponding values in the isolated state due to the interaction of the invariant N3/O2 atomic groups of the G$^*$·T(WC) base mispair with the appropriate amino acid residues of the recognition pocket of the high-fidelity DNA-polymerase (Brovarets’ & Hovorun, 2015a; Brovarets et al., 2012; Poltev & Bruskov, 1977; Poltev, Shulyupina, & Bruskov, 1998).

Oddly enough, but the replicative DNA-polymerase accelerates the G·T(w) → G$^*$·T(WC) process of the acquisition by the wobble pair of the Watson–Crick geometry comparably with the isolated state.

It is very eloquently that this tautomerization process occurs without the destruction of the pair (Figures 9 and 10) and hence without the direct participation in it of the endogenous water molecules as a chemical agent: This is due to the fact that tautomerization is controlled by the zwiterionic transition state $\text{TS}^0_{G^*\text{-}T(WC)\rightarrow G\text{-}T^*}$, which energy of stabilization ($\Delta E^0_{\text{ST}}$) is much greater than the energy of the bases hydration by the corresponding sites of the interaction (Danilov et al., 2009; Furmanchuk et al., 2011; Zubatik, Shishkin, Gorb, Hovorun, & Leszczynski, 2015). Incidentally, the base pair, that is tautomerized, stays in the zwiterionic state in the fairly wide range of the IRC values: −5.20 to 10.14 Bohr.

Table 3. Energetic and kinetic characteristics of the G$^*$·T(WC) ↔ G·T*(WC) (Brovarets’ & Hovorun, 2015a), G·T*(WC) ↔ G$^*$·T*(w) and G$^*$·T*(w) ↔ G·T*$^\text{sym}$(w) tautomerization reactions obtained at the different levels of theory for the geometry calculated at the B3LYP/6-311++G(d, p) level of theory.

| Level of theory | $\Delta G^a$ | $\Delta E^b$ | $\Delta G_{TS}^c$ | $\Delta E_{TS}^d$ | $\Delta G^e$ | $\Delta E^f$ | $t_{99.9\%}^g$ |
|-----------------|------------|------------|-----------------|-----------------|------------|------------|-------------|
| G$^*$·T(WC) ↔ G·T*(WC) (Brovarets’ & Hovorun, 2015a) | | | | | | | |
| MP2/6-311++G(2df, pd) | 1.16 | 1.14 | 2.33 | 5.31 | 1.17 | 4.17 | $4.38 \times 10^{-12}$ |
| MP2/6-311++G(3df, 2pd) | 1.27 | 1.24 | 2.76 | 5.74 | 1.49 | 4.50 | $7.69 \times 10^{-12}$ |
| MP2/cc-pVTZ | 1.40 | 1.38 | 2.47 | 5.44 | 1.07 | 4.07 | $3.82 \times 10^{-12}$ |
| MP2/cc-pVQZ | 1.22 | 1.19 | 2.63 | 5.61 | 2.63 | 5.61 | $4.98 \times 10^{-12}$ |
| G·T*(WC) ↔ G$^*$·T*(w) | | | | | | | |
| MP2/6-311++G(2df, pd) | 14.84 | 16.56 | 12.35 | 11.36 | −2.50 | −5.20 | $1.64 \times 10^{-14}$ |
| MP2/6-311++G(3df, 2pd) | 14.96 | 16.68 | 12.39 | 11.40 | −2.57 | −5.28 | $1.44 \times 10^{-14}$ |
| MP2/cc-pVTZ | 14.95 | 16.67 | 12.39 | 11.40 | −2.56 | −5.27 | $1.47 \times 10^{-14}$ |
| MP2/cc-pVQZ | 14.82 | 16.53 | 12.34 | 11.36 | −2.47 | −5.18 | $1.70 \times 10^{-14}$ |
| G$^*$·T*(w) ↔ G·T*$^\text{sym}$(w) | | | | | | | |
| MP2/6-311++G(2df, pd) | −3.30 | −3.38 | −1.48 | −0.29 | 1.82 | 3.09 | $7.03 \times 10^{-14}$ |
| MP2/6-311++G(3df, 2pd) | −3.22 | −3.30 | −1.35 | −0.15 | 1.88 | 3.14 | $8.80 \times 10^{-14}$ |
| MP2/cc-pVTZ | −3.45 | −3.52 | −1.47 | −0.28 | 1.97 | 3.24 | $7.11 \times 10^{-14}$ |
| MP2/cc-pVQZ | −3.19 | −3.27 | −1.31 | −0.12 | 1.88 | 3.15 | $9.32 \times 10^{-14}$ |

$^a$The Gibbs free energy of the product relatively the reactant of the tautomerization reaction ($T = 298.15$ K), kcal mol$^{-1}$.

$^b$The electronic energy of the product relatively the reactant of the tautomerization reaction, kcal mol$^{-1}$.

$^c$The Gibbs free energy barrier for the reverse reaction of tautomerization, kcal mol$^{-1}$.

$^d$The Gibbs free energy barrier for the forward reaction of tautomerization, kcal mol$^{-1}$.

$^e$The Gibbs free energy barrier for the reverse reaction of tautomerization, kcal mol$^{-1}$.

$^f$The electronic energy barrier for the forward reaction of tautomerization, kcal mol$^{-1}$.

$^g$The time necessary to reach 99.9% of the equilibrium concentration between the reactant and the product of the tautomerization reaction, s.

See also summary Table S1 for the Gibbs and electronic energies of the mispairs and TSs relatively the global minimum – the Watson–Crick-like G$^*$·T(WC) base mispair.
Since the characteristic parameter $\tau$ of the G·T (w) ↔ G·T*(WC) tautomeration (2.42 s (Table 6)) is much greater than the time $\Delta t_{pol} = 8.3 \times 10^{-4}$ s, which spends DNA-polymerase machinery for the one act of incorporation of the incoming nucleotide into the structure of the DNA double helix that is synthesized (Kirmizialtin et al., 2012), it implies with necessity that the emergence of the spontaneous transitions – thermodynamically non-equilibrium incorporation errors – is under the kinetic control. This observation automatically explains the well-known experimental fact – the growth of the rate of the spontaneous point mutations (as it is known, transitions make up the bulk among them (Brovarets et al., 2012; Friedberg et al., 2006; LeeTable 4. Energetic and kinetic characteristics of the G·T*(WC) ↔ G·T(w1) tautomeration reaction and G·T(w1) ↔ G·T(w2) conformation transition obtained at the different levels of theory for the geometry calculated at the B3LYP/6-311++G(d, p) level of theory.

| Level of theory             | $\Delta G$ | $\Delta E$ | $\Delta G_{TS}$ | $\Delta E_{TS}$ | $\Delta G_{\tau 99.9\%}$ | $\Delta E_{\tau 99.9\%}$ |
|-----------------------------|------------|------------|------------------|-----------------|---------------------------|---------------------------|
| G·T*(WC) ↔ G·T(w1)          |            |            |                  |                 |                           |                           |
| MP2/6-311++G(2df, pd)       | 3.14       | 4.69       | 30.04            | 32.79           | 26.90                     | 28.10                     |
| MP2/6-311++G(3df, 2pd)      | 3.14       | 4.69       | 30.48            | 33.23           | 27.34                     | 28.54                     |
| MP2/cc-pVTZ                 | 3.48       | 5.03       | 30.85            | 33.59           | 27.37                     | 28.56                     |
| MP2/cc-pVQZ                 | 3.33       | 4.87       | 31.06            | 33.80           | 27.73                     | 28.93                     |
| G·T(w1) ↔ G·T(w2)           |            |            |                  |                 |                           |                           |
| MP2/6-311++G(2df, pd)       | 1.20       | 1.05       | 2.66             | 2.58            | 1.46                      | 1.53                      |
| MP2/6-311++G(3df, 2pd)      | 1.21       | 1.05       | 2.71             | 2.62            | 1.50                      | 1.56                      |
| MP2/cc-pVTZ                 | 0.67       | 0.52       | 3.01             | 2.92            | 2.34                      | 2.40                      |
| MP2/cc-pVQZ                 | 1.16       | 1.00       | 2.80             | 2.71            | 1.64                      | 1.71                      |

Table 5. Energetic and kinetic characteristics of the G·T*(WC) ↔ G·T(w2) tautomeration reaction obtained at the different levels of theory for the geometry calculated at the B3LYP/6-311++G(d, p) level of theory.

| Level of theory             | $\Delta G$ | $\Delta E$ | $\Delta G_{TS}$ | $\Delta E_{TS}$ | $\Delta G_{\tau 99.9\%}$ | $\Delta E_{\tau 99.9\%}$ |
|-----------------------------|------------|------------|------------------|-----------------|---------------------------|---------------------------|
| MP2/6-311++G(2df, pd)       | 5.50       | 6.87       | 39.23            | 40.83           | 33.72                     | 33.95                     |
| MP2/6-311++G(3df, 2pd)      | 5.62       | 6.99       | 39.47            | 41.08           | 33.86                     | 34.09                     |
| MP2/cc-pVTZ                 | 5.55       | 6.92       | 39.73            | 41.33           | 34.18                     | 34.41                     |
| MP2/cc-pVQZ                 | 5.70       | 7.07       | 39.67            | 41.27           | 33.97                     | 34.20                     |

Note: For footnote definitions see Table 3.

Since the characteristic parameter $\tau_{99.9\%}$ of the G·T (w) ↔ G·T*(WC) tautomeration (2.42 s (Table 6)) is much greater than the time $\Delta t_{pol} = 8.3 \times 10^{-4}$ s, which spends DNA-polymerase machinery for the one act of incorporation of the incoming nucleotide into the structure of the DNA double helix that is synthesized (Kirmizialtin et al., 2012), it implies with necessity that

Figure 5. Geometrical structures of the base mispairs and TSs involved in the G·T*(WC) ↔ G·T(w2) reaction pathway obtained at the B3LYP/6-311++G(d, p) level of theory. For definitions, see Figure 1.

![Figure 5](image-url)
Figure 6. Profile of the relative electronic energy $\Delta E$ of the base mispair, that tautomerizes, along the IRC of the $G^*\cdot T(WC) \leftrightarrow G\cdot T(w_2)$ tautomerization via the sequential DPT obtained at the B3LYP/6-311++G(d, p) level of theory.

Figure 8. Profile of the relative electronic energy of the base mispair, that tautomerizes, along the IRC of the $G\cdot T(w) \leftrightarrow G^*\cdot T(WC)$ tautomerization via the sequential DPT obtained at the B3LYP/6-311++G(d, p) level of theory.

Figure 7. Geometrical structures of the base mispairs and TSs involved in the (a) $G\cdot T(w) \leftrightarrow G^*\cdot T(WC)$ (Brovarets’ & Hovorun, 2009a) and (b) $G^*\cdot T(WC) \leftrightarrow G^*\cdot T(WC)$ (Brovarets’ & Hovorun, 2015a) reaction pathways obtained at the B3LYP/6-311++G(d, p) level of theory. For definitions, see Figure 1.
et al., 2012; Lynch, 2010)) with increasing temperature (see Refs. (Auerbach, 1976; Lindgren, 1972) and bibliography presented there).

Most interesting fact follows from the phenomenon of the kinetic control of the spontaneous point mutations, namely GT/TG incorporation errors: If from one or another reason DNA-polymerase machinery as a part of replisome (Ligasová & Koberna, 2011) slows its operation (time $\Delta t_{\text{pol}}$ increases), it once again automatically leads to the increasing of the frequency of the spontaneous transitions. In this way, in particular, the nature of the so-called error catastrophe (Li et al., 2014) can be explained.

Further, since endogenous water is not directly included in the processes of the mutagenic tautomerization that underlie the occurrence of the spontaneous transitions – incorporation errors, so the dependency of the frequency of the latters on pH can be explained by the immediate influence of this physico-chemical factor per se on the course of the G·T(w)→G·T*(WC) tautomerization reaction, that is controlled by the zwitterionic transition state $TS_{G\cdot T(w)\rightarrow G\cdot T*(WC)}^{G\cdot T}$ but not only by the ionization of the isolated bases, incorporated into these pairs (Koag, Nam, & Lee, 2015).

By the way, comparison of our data, including lengths of the intermolecular H-bonds, with experimental data (Bebenek et al., 2011) unequivocally testifies to the fact that exactly the quasi-planar G·T*(WC) base mispair is enzymatically competent base pair, but not the others possible G·T*(WC) (Brovarets’ & Hovorun, 2015a), G·T, or G·T+ pseudo-Watson–Crick mispairs (Brovarets’, Zhurakivsky, & Hovorun, 2010), since namely for it the best coincidence of the theoretical results with experimental data is observed (see Table 6 in Brovarets’ & Hovorun, 2015a).

And finally the latter. It is completely logical to associate the mutagenic action of the modified nucleotide bases with the reduction of the energetic barrier of the tautomerization of the wobble mispairs into the Watson–Crick mismatches by their participation within the

| Patterns | IRC range, Bohr | Intermolecular interactions, forming patterns |
|----------|----------------|---------------------------------------------|
| I        | $[-15.81$ to $-11.41]$ | (T)N3H⋯O6(G), (G)N1H⋯O2(T) |
| II       | $[-11.41$ to $-5.71]$  | (T)N3H⋯O6(G), (G)N1H⋯O2(T), (G)N2H⋯O2(T) |
| III      | $[-5.71$ to $-5.20]$   | (T)N3H⋯O6(G), (G)N1H⋯O2(T), (G)N2H⋯O2(T) |
| IV       | $[-5.20$ to $-2.08]$   | (G)O6H⋯N3(T), (G)N1H⋯O2(T), (G)N2H⋯O2(T) |
| V        | $[-2.08$ to $-0.13]$   | (G)O6H⋯N3(T), (G)N1H⋯O2(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| VI       | $[-0.13$ to $2.73]$    | (G)O6H⋯O4(T), (G)O6H⋯N3(T), (G)N1H⋯O2(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| VII      | $[2.73$ to $3.38]$     | (G)O6H⋯O4(T), (G)O6H⋯N3(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| VIII     | $[3.38$ to $10.14]$    | (G)O6H⋯O4(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| IX       | $[10.14$ to $10.65]$   | (G)O6H⋯O4(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| X        | $[10.65$ to $16.72]$   | (T)O4H⋯O6(G), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| XI       | $[16.72$ to $17.03]$   | (T)O4H⋯O6(G), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| XII      | $[17.03$ to $18.53]$   | (G)O6H⋯O4(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| XIII     | $[18.53$ to $18.92]$   | (G)O6H⋯O4(T), (G)N1H⋯N3(T), (G)N2H⋯O2(T) |
| XIV      | $[18.92$ to $23.03]$   | (G)O6H⋯O4(T), (T)N3H⋯N1(G), (G)N2H⋯O2(T) |

Table 6. Energetic and kinetic characteristics of the G·T(w) ↔ G·T*(WC) (Brovarets’ & Hovorun, 2009a) and G·T*(WC) ↔ G·T wobble one at the different levels of theory for the geometry calculated at the B3LYP/6-311++G(d, p) level of theory.

| Level of theory | $\Delta G$ | $\Delta E$ | $\Delta G_{TS}$ | $\Delta E_{TS}$ | $\Delta G$ | $\Delta E$ | $\tau_{99.9\%}$ |
|----------------|------------|------------|-----------------|-----------------|------------|------------|----------------|
| G·T(w) ↔ G·T*(WC) |            |            |                 |                 |            |            |                |
| MP2/6-311++G(2df, pd) | $-0.54$  | $-1.33$  | 17.47            | 16.80            | 18.01     | 18.13     | 5.06           |
| MP2/6-311++G(3df, 2pd) | $-0.27$  | $-1.06$  | 17.41            | 16.74            | 17.68     | 17.80     | 3.93           |
| MP2/cc-pVTZ          | $-0.65$  | $-1.44$  | 17.13            | 16.46            | 17.78     | 17.90     | 3.04           |
| MP2/cc-pVQZ           | $-0.48$  | $-1.27$  | 17.04            | 16.37            | 17.51     | 17.64     | 2.42           |
| G·T*(WC) ↔ G·T wobble |            |            |                 |                 |            |            |                |
| MP2/6-311++G(2df, pd) | $-1.16$  | $-1.14$  | 1.17             | 4.17             | 2.33      | 5.31      | $3.28 \times 10^{-12}$ |
| MP2/6-311++G(3df, 2pd) | $-1.27$  | $-1.24$  | 1.49             | 4.50             | 2.76      | 5.74      | $5.76 \times 10^{-12}$ |
| MP2/cc-pVTZ          | $-1.40$  | $-1.38$  | 0.10             | 4.07             | 2.47      | 5.44      | $2.85 \times 10^{-12}$ |
| MP2/cc-pVQZ           | $-1.22$  | $-1.19$  | 1.41             | 4.42             | 2.63      | 5.61      | $4.98 \times 10^{-12}$ |

For footnote definitions see Table 3.
framework of the proposed model of the origin of the spontaneous transitions – incorporation errors.

Indeed, the probability $P$ of the origin of the spontaneous transitions – incorporation errors – can be estimated by the formula $P = P_w \cdot P_w \rightarrow WC$, where $P_w$ – the probability of the wobble base mispair formation in the recognition pocket of the replicative DNA-polymerase and $P_w \rightarrow WC$ – the probability of the conversion of the wobble base mispair into the Watson–Crick-like base mispair in the recognition pocket of the replicative DNA-polymerase. Whereas both multipliers included to this formula, especially $P_w \rightarrow WC$, are much smaller than 1, so the low frequency of the spontaneous point mutations – incorporation errors – can be explained by this fact.

Unfortunately, experimental values of the $P_w$ and $P_w \rightarrow WC$ parameters are not available in the literature now. However, the growth of the rate of the transitions induced by the modification of the bases $P_{ind}/P_{spont} = P_w \rightarrow WC/P_w \rightarrow WC$ (the titles of the pairs involving modified bases-mutagens are highlighted in bold) can be estimated by this formula in the first approximation. An elementary calculation of the $P_{ind}/P_{spont}$ ratio by the known formulas of the physico-chemical kinetics (Podolyan, Gorb, & Leszczynski, 2003) using numerical data for the corresponding values of the reaction rates ($k_f = 0.97/k_r = 0.39 \text{s}^{-1}$ for the $G\cdot T(w) \rightarrow G^*\cdot T(WC)$ tautomerization (Brovarets’ & Hovorun, 2009a) and $k_f = 34.20/k_r = 19.59 \text{s}^{-1}$ for the $G\cdot 5\text{BrU}(w) \rightarrow G^*\cdot 5\text{BrU}(WC)$ tautomerization (Brovarets’ & Hovorun, 2010, 2013; Brovarets’ & Hovorun, 2009b)) gives the value equals to 35, which despite the simplified model agrees well with experimental data which constitute from 20 (Lasken & Goodman, 1985) to 29 (Kaufman & Davidson, 1978) times.

This is the convincing evidence of the adequacy of the proposed and substantiated by us microstructural model of the emergence of the spontaneous, as well as induced by the modified nucleotide bases incorporation errors that occur during DNA biosynthesis.

Conclusions and perspectives

To the best of our knowledge, this study is the first report, where it was revealed for the first time that biologically important process of the $G\cdot T(w) \leftrightarrow G^*\cdot T(WC)$ mutagenic tautomerization is carried out by one single route, controlled by the zwitterion planar transition state $TSG^+\cdot C1/G\cdot C1(T)$, which is stabilized by the participation of the unique pattern of the five intermolecular H-bonds. The $G\cdot T(w) \leftrightarrow G^*\cdot T(WC)$ tautomerization process

Figure 9. Profiles of the $E_{AH-B}$ energies of the intermolecular H-bonds estimated by the EML formula at the (3, –1) BCPs along the IRC of the $G\cdot T(w) \leftrightarrow G^*\cdot T(WC)$ tautomerization via the sequential DPT obtained at the B3LYP/6-311++G(d, p) level of theory (see Tables 1 and 7).

Figure 10. Profiles of: (a) the distance $R(H_1-H_9)$ between the $H_1$ and $H_9$ glycosidic hydrogens of the $T$ and $G$ bases, respectively, and (b) the $\alpha_1$ (ζN9H9(G)H1(T)) and $\alpha_2$ (ζN1H1(T)H9(G)) glycosidic angles along the IRC of the $G\cdot T(w) \leftrightarrow G^*\cdot T(WC)$ tautomerization via the sequential DPT obtained at the B3LYP/6-311++G(d, p) level of theory (see Figure 7).
occurs without opening of the pair, that tautomerizes, and ensures the acquisition by the wobble G·T(w) base pair of the geometric mimicry with the Watson–Crick base pair. This means that this process is intrapair (i.e., in this case, Nature exploits the ability of the wobble G·T(w) base pair to tautomerize spontaneously in the process of thermal fluctuations into the G·T(WC) base mispair with Watson–Crick geometry as its immanency) and therefore does not require for its implementation the endogenous water molecules. In the previously studied systems, water affects the tautomerization process intervening directly into the process itself (Danilov et al., 2009; Furmanchuk et al., 2011; Zubatiuk et al., 2015). Throughout the entire G·T(w) → G·T*(WC) tautomerization process bases within pairs remain connected by 14 different patterns of the intermolecular interactions including from 2 to 5 AH···B H-bonds and 3 loosened A-H-B covalent bridges that successively change each other along the IRC.

In the course of the G·T(w) → G·T*(WC) intermolecular mutagenic tautomerization G base as a molecule-partner of interaction plays the role of the catalyst, significantly accelerating this reaction in comparison with the intramolecular mutagenic tautomerization of the T base. At this, the ΔΔGTS Gibbs free energy barrier of the tautomerization is reduced to 17.47 kcal mol⁻¹ (Table 7), when T base tautomerizes via the intermolecular DPT within the G·T(w) base pair, in comparison with the tautomerization within the isolated base via the intramolecular SPT, for which this value is 39.22 kcal mol⁻¹ under normal conditions (Brovarets’ & Hovorun, 2010a).

In this study, novel kinetically controlled mechanism of the spontaneous point mutations – incorporation errors – that are thermodynamically non-equilibrium events has been suggested.

Moreover, in the framework of this model, the nature of the mutagenic activity of the 5·XU (X = F, Cl, Br) compounds becomes clear: It is connected with the decreasing of the barrier of the G·5XU(w) → G·5XU*(WC) tautomerization reaction. Simple estimation by the formulas of the physico-chemical kinetics (Podolyan et al., 2003) gives a value equal to 7.9 × 10⁻⁴ and 2.8 × 10⁻² for the frequencies of the GT and G·BrU incorporation errors, respectively. At this, it was established that BrU as the most strong mutagen among halogen derivatives of the U provides the exceeding of the background value corresponding to the T base in 35 times, that coincide well with the experimental data (Kauffman & Davidson, 1978; Lasken & Goodman, 1985).

Proposed approaches are able to explain quite naturally the growth of the rate of the spontaneous transitions with increasing temperature (Auerbach, 1976; Lindgren, 1972).

Discoveries presented above give impetus to a variety of implications for future investigations in the field of the spontaneous and induced point mutations and their reparation. Notably, the outlined theoretical concepts of the mutagenic tautomerization of the wobble G·T(w) base pair as its intrinsic property into the G·T*(WC) base pair with Watson–Crick geometry (Brovarets’ & Hovorun, 2015a) have not only outstripped the experiment (Kimsey et al., 2015) by a few years, but also allowed to reasonably plan and interpret it.

Based on the theoretical data that have worthily passed the experimental verification of their viability, we can assume that reparation enzymes, “sharpened” for the wobble G·T(w) base mispair, cannot cope completely with their intended purpose. The point is that the intrinsic ability of this mismatch to switch into the G·T*(WC) base mispair with Watson–Crick geometry, representing itself “hiding place” from the enzyme that cannot be recognized by it, principally restricts the ultimate accuracy of the reparation process. Obviously, this assumption requires further experimental confirmation.

In addition, the foregoing discussion in principle allows to understand the mechanism according to which the G·T mutagenic tautomer, which in themselves is a long-lived structure with a lifetime (Brovarets’ & Hovorun, 2010a) that is by orders of magnitude greater than the time of the DNA replication in the cell (~10⁶ s (Alberts et al., 2002; Friedberg et al., 2006)), can be eliminated from the genome. It is important to mention here once again, that the intrinsic ability of the G·T*(WC) base mispair to transform into the wobble G·T(w) base mispair allows to eliminate the G·T tautomers from the genome by the reparation systems, “sharpened” for the G·T(w) base pair, by several cycles of DNA replication.

Results obtained in our present and previous studies (Brovarets’, 2012, 2013; Brovarets’ & Hovorun, 2009a, 2009b, 2015c) are able to drastically change the existing conceptions according the general mechanisms of the origin of the spontaneous point mutations as at the DNA replication, so at the protein synthesis. We anticipate that our results would significantly influence the comprehension of the universal microstructural principles of the regulation of the DNA replication and transcription.

Supplementary material
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**References**

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Chapter 5. The initiation and completion of DNA replication in chromosomes. In John H. Wilson & Tim Hunt (Eds.), Molecular biology of the cell (4th ed.). New York, NY: Garland Science. ISBN-10: 0-8153-3218-1, ISBN-10: 0-8153-4072-9.

Arabi, A. A., & Matta, C. F. (2011). Effects of external electric fields on double proton transfer kinetics in the formic acid dimer. *Physical Chemistry Chemical Physics, 13*, 13738–13748.

Atkins, P. W. (1998). *Physical chemistry*. Oxford: Oxford University Press.

Auerbach, C. (1976). *Mutation research: Problems, results, and perspectives*. New York, NY: Wiley.

Bader, R. F. W. (1990). *Atoms in molecules: A quantum theory*. Oxford: Oxford University Press.

Bandrauk, A. D., Sedik, E. S., & Matta, C. F. (2004). Effect of absolute laser phase on reaction paths in laser-induced chemical reactions. *The Journal of Chemical Physics, 121*, 7764–7775.

Bandrauk, A. D., Sedik, E. S., & Matta, C. F. (2006). Laser control of reaction paths in ion–molecule reactions. *Molecular Physics, 104*, 95–102.

Bebenek, K., Pedersen, L. C., & Kunkel, T. A. (2011). Replication infidelity via a mismatch with Watson–Crick geometry. *Proceedings of the National Academy of Sciences, 108*, 1862–1867.

Boys, S. F., & Bernardi, F. (1970). The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors. *Molecular Physics, 19*, 553–566.

Brovarets’, O. O. (2010). Physico-chemical nature of the spontaneous and induced by the mutagens transitions and transformations (PhD thesis). Taras Shevchenko National University of Kyiv, Kyiv.

Brovarets’, O. O. (2012). Mutagenic properties of 5-halogen derivatives of uracil: Quantum-chemical investigation. *Ukrainica Bioorganica Acta, 10*, 17–24.

Brovarets’, O. O. (2013). Impact of the uracil modification on the barrier of the tautomerisation of the wobble Gua•5XUra pair into the Gua•3XUra pair with Watson–Crick geometry: Quantum-chemical study. *Reports of the National Academy of Sciences of Ukraine, 4*, 154–158.

Brovarets’, O. O., & Hovorun, D. M. (2009a). Physicochemical mechanism of the wobble DNA base pairs Gua•Thy and Ade Cyt transition into the mismatched base pairs Gua•Thy and Ade-Cyt* formed by the mutagenic tautomers. *Ukrainica Bioorganica Acta, 8*, 12–18.

Brovarets’, O. O., & Hovorun, D. M. (2009b). The new physicochemical mechanism of the mutagenic action of 5-bromouracil. *Ukrainica Bioorganica Acta, 2*, 19–23.

Brovarets’, O. O., & Hovorun, D. M. (2010a). How stable are the mutagenic tautomers of DNA bases? *Biopolymers and Cell, 26*, 72–76.

Brovarets’, O. O., & Hovorun, D. M. (2010b). Quantum-chemical investigation of the elementary molecular mechanisms of pyrimidine–purine transversions. *Ukrains’kyi Biokhimichniy Zhurnal, 82*, 57–67.

Brovarets’, O. O., & Hovorun, D. M. (2010c). Stability of mutagenic tautomers of uracil and its halogen derivatives: The results of quantum-mechanical investigation. *Biopolymers and Cell, 26*, 295–298.

Brovarets’, O. O., & Hovorun, D. M. (2011a). Intramolecular tautomeration and the conformational variability of some classical mutagens – cytosine derivatives: Quantum chemical study. *Biopolymers and Cell, 27*, 221–230.

Brovarets’, O. O., & Hovorun, D. M. (2011b). IR Vibrational spectra of H-bonded complexes of adenine, 2-aminopurine and 2-aminopurine* with cytosine and thymine: Quantum-chemical study. *Optics and Spectroscopy, 111*, 750–757.

Brovarets’, O. O., & Hovorun, D. M. (2013a). Atomistic nature of the DPT tautomerisation of the biologically important C•C* DNA base mispair containing amino and imino tautomers of cytosine: A QM and QTAIM approach. *Physical Chemistry Chemical Physics, 15*, 20091–20104.

Brovarets’, O. O., & Hovorun, D. M. (2013b). Atomistic understanding of the C•T mismatched DNA base pair tautomeration via the DPT: QM and QTAIM computational approaches. *Journal of Computational Chemistry, 34*, 2577–2590.

Brovarets’, O. O., & Hovorun, D. M. (2014a). 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*, 127–154.

Brovarets’, O. O., & Hovorun, D. M. (2014b). Does the G•G* syn DNA mismatch containing canonical and rare tautomers of the guanine tautomerise through the DPT? A QM/QTAIM microstructural study. *Molecular Physics, 112*, 3033–3046.

Brovarets’, O. O., & Hovorun, D. M. (2014c). How does the long G•G* Watson–Crick DNA base mispair comprising keto and enol tautomers of the guanine tautomerise? The results of a QM/QTAIM investigation. *Physical Chemistry Chemical Physics, 16*, 15886–15899.

Brovarets’, O. O., & Hovorun, D. M. (2014d). 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*, 1474–1499.

Brovarets’, O. O., & Hovorun, D. M. (2015a). The nature of the transition mismatches with Watson–Crick architecture: The G•T or G•T* DNA base mispair or both? A QM/QTAIM perspective for the biological problem. *Journal of Biomolecular Structure and Dynamics, 33*, 925–945.

Brovarets’, O. O., & Hovorun, D. M. (2015b). The physicochemical essence of the purine pyrimidine transition mismatches with Watson–Crick geometry in DNA: A C* versa A* C. A QM and QTAIM atomistic understanding. *Journal of Biomolecular Structure and Dynamics, 33*, 28–55.
TautomORIZATION PATHWAYS BETWEEN WATSON–CRICK-LIKE G*−T DNA BASE MISPAIR AND WOBBLE MISMATCHES

Brovarets', O. O., & Hovorun, D. M. (2015c). Tautomeric transition between wobble A·C DNA base mispair and Watson-Crick-like A·C* mismatch: Microstructural mechanism and biological significance. *Physical Chemistry Chemical Physics*. doi:10.1039/C5CP01568E

Brovarets', O. O., Kolomieits', I. M., & Hovorun, D. M. (2012). Elementary molecular mechanisms of the spontaneous point mutations in DNA: A novel quantum-chemical insight into the classical understanding. In T. Tada (Ed.), *Quantum chemistry – Molecules for innovations* (pp. 59–102). Rijeka: In Tech Open Access.

Brovarets', O. O., Yurenko, Y. P., Dubey, I. Y., & Hovorun, D. M. (2012). Can DNA-binding proteins of replisome tautomerize nucleotide bases? *Ab initio* model study. *Journal of Biomolecular Structure and Dynamics*, 29, 1101–1109.

Brovarets', O. O., Yurenko, Y. P., & Hovorun, D. M. (2014a). Intermolecular CH·−·O/N H-bonds in the biologically important pairs of natural nucleobases: A thorough quantum-chemical study. *Journal of Biomolecular Structure & Dynamics*, 32, 993–1022.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2010). Is there adequate ionization mechanism of the spontaneous transitions? Quantum-chemical investigation. *Biopolymers and Cells*, 26, 398–405.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2013a). DPT tautomerization of the long A·A* Watson–Crick base pair formed by the amino and imino tautomers of adenine: combined QM and QTAIM investigation. *Journal of Molecular Modeling*, 19, 4223–4237.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2013b). The physico-chemical mechanism of the tautomerisation via the DPT of the long Hyp···Hyp Watson–Crick base pair containing rare tautomer: A QM and QTAIM detailed look. *Chemical Physics Letters*, 578, 126–132.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2014a). A QM/QTAIM microstructural analysis of the tautomerisation via the DPT of the hypoxanthine-adenine nucleobase pair. *Molecular Physics*, 112, 2005–2016.

Brovarets', O. O., Yurenko, Y. P., & Hovorun, D. M. (2014b). The significant role of the intermolecular CH···O/N hydrogen bonds in governing the biologically important pairs of the DNA and RNA modified bases: a comprehensive theoretical investigation. *Journal of Biomolecular Structure and Dynamics*. doi:10.1080/07391102.2014.968623

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2014b). Does the tautomer status of the adenine bases change upon the dissociation of the A···A*···A Asyn Topal–Fresco DNA mismatch? A combined QM and QTAIM atomistic insight. *Physical Chemistry Chemical Physics*, 16, 3715–3725.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2014c). Is the DPT tautomerization of the long A·G Watson–Crick DNA base mispair a source of the adenine and guanine mutagenic tautomers? A QM and QTAIM response to the biologically important question. *Journal of Computational Chemistry*, 35, 451–466.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2014d). Structural, energetic and tautomeric properties of the T···T Watson–Crick DNA mismatch involving mutagenic tautomers of thymine: A QM and QTAIM insight. *Chemical Physics Letters*, 592, 247–255.

Brovarets', O. O., Zhurakivsky, R. O., & Hovorun, D. M. (2015). DPT tautomisation of the wobble guanine thymine DNA base mispair is not mutagenic: QM and QTAIM arguments. *Journal of Biomolecular Structure and Dynamics*, 33, 674–689.

Danilov, V. I., Anisimov, V. M., Kurita, N., & Hovorun, D. (2005). MP2 and DFT studies of the DNA rare base pairs: The molecular mechanism of the spontaneous substitution mutations conditioned by tautomerism of bases. *Chemical Physics Letters*, 412, 285–293.

Danilov, V. I., van Mourik, T., Kurita, N., Wakabayashi, H., Tsukamoto, T., & Hovorun, D. M. (2009). On the mechanism of the mutagenic action of 5-bromouracil: A DFT study of uracil and 5-bromouracil in a water cluster. *The Journal of Physical Chemistry A*, 113, 2233–2235.

Duderstadt, K. E., Reyes-Lamote, R., van Oijen, A. M., & Sherratt, D. J. (2014). Replication-fork dynamics. *Cold Spring Harbor Perspectives in Biology*, 6, 1–17.

El-Sayed, A. A., Tamara Molina, A., Alvarez-Ros, M. C., & Alcolea Palafox, M. (2015). Conformational analysis of the anti-HIV Nikavir produg: Comparisons with AZT and Thymidine, and establishment of structure-activity relationships/tendencies in other 6-derivatives. *Journal of Biomolecular Structure and Dynamics*, 33, 723–748.

Espinosa, E., Molins, E., & Lecomte, C. (1998). Hydrogen bond strengths revealed by topological analyses of experimentally observed electron densities. *Chemical Physics Letters*, 283, 170–173.

Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., & Ellenberger, T. (2006). *DNA repair and mutagenesis*. Washington, DC: ASM Press.

Friis, M. J., Head-Gordon, M., & Pople, J. A. (1990). Semi-direct algorithms for the MP2 energy and gradient. *Chemical Physics Letters*, 166, 281–289.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., … Pople, J. A. (2010). *Gaussian 09* (Revision B.01). Wallingford: CT: Gaussian.

Furmanchuk, A., Isayev, O., Gorb, L., Shishkin, O. V., Hovorun, D. M., & Leszczynski, J. (2011). Novel view on the mechanism of water-assisted proton transfer in the DNA bases: bulk water hydration. *Physical Chemistry Chemical Physics*, 13, 4311–4317.

Giulia Rossetti, G., Dans, P. D., Gomez-Pinto, I., Ivani, I., Gonzalez, G., & Orozco, M. (2015). The structural impact of DNA mismatches. *Nucleic Acids Research*, 43, 4309–4321. doi:10.1093/nar/gkv254

Gutowski, M., Van Lenthe, J. H., Verbeek, J., Van Duijneveldt, F. B., & Cha, H. (2005). Determination of the stretching vibration of the S(II) vibration in infrared spectra. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 55, 1585–1612.
Saenger, W. (1984). Principles of nucleic acid structure. New York, NY: Springer.

Samijlenko, S. P., Yurenko, Y. P., Stepanyugin, A. V., & Hovorun, D. M. (2012). Tautomeric equilibrium of uracil and thymine in model protein–nucleic acid contacts. Spectroscopic and quantum chemical approach. Journal of Physical Chemistry B, 114, 1454–1461.

Sordo, J. A. (2001). On the use of the Boys–Bernardi function counterpoise procedure to correct barrier heights for basis set superposition error. Journal of Molecular Structure: THEOCHEM, 537, 245–251.

Sordo, J. A., Chin, S., & Sordo, T. L. (1988). On the counterpoise correction for the basis set superposition error in large systems. Theoretica Chimica Acta, 74, 101–110.

Tirado-Rives, J., & Jorgensen, W. L. (2008). Performance of B3LYP density functional methods for a large set of organic molecules. Journal of Chemical Theory and Computation, 4, 297–306.

Topal, M. D., & Fresco, J. R. (1976). Complementary base pairing and the origin of substitution mutations. Nature, 263, 285–289.

Watson, J. D., & Crick, F. H. C. (1953). The structure of DNA. Cold Spring Harbor Symposia on Quantitative Biology, 18, 123–131.

Wigner, E. (1932). Über das Überschreiten von Potentialschwellen bei chemischen Reaktionen [Crossing of potential thresholds in chemical reactions]. Zeitschrift für Physikalische Chemie, B19, 203–216.

Zubatiuk, T., Shishkin, O., Gorb, L., Hovorun, D., & Leszcynski, J. (2015). Structural waters in the minor and major grooves of DNA – A major factor governing structural adjustments of the A–T mini-helix. The Journal of Physical Chemistry B, 119, 381–391.