The mutagenic action of 5-bromouracil
The structure of DNA

DNA bases

- Cytosine (C)
- Thymine (T)
- Guanine (G)
- Adenine (A)

oxygen

nitrogen

carbon

hydrogen
Hydrogen bonds
DNA replication

picture from wikipedia
Mutations: changes in the nucleotide sequence may cause changes in the protein encoded by the gene.

Incorporation into DNA of unusual bases may cause mutations.

For example, 5-bromouracil (5BrU) is known to cause mutations.
However, the mutagenic mechanism responsible for the mutagenic action of 5BrU is not known

Experimental evidence is ambiguous

Can theory help to explain the mutagenic activity of 5BrU?
5-bromouridine is mutagenic => 5BrU must be able to bind with G

Proposed mechanisms to explain pairing of 5BrU with G:

1. deprotonation of 5BrU
   => unlikely

2. wobble hydrogen bonds
   => unlikely, due to unfavourable arrangement of oxygen atoms

3. involvement of excited states
   => unlikely, as mutation is a dark reaction
   => unlikely, as formation should not depend on bromine

4. keto → enol

5BrU*G
Proposed mechanism of 5BrU mutagenicity

(Hu et al. Biochemistry 43, 6361 (2004))

5BrU incorporated into DNA

5BrU

A

T

A

DNA replication

A

5BrU

A

5BrU*

G

5BrU* 

A

5BrU

G*

5BrU

A

T

G* 

A

T

mutations

G

C

G*

5BrU*

G

C

G

5BrU*

G

C

A

T

A

T
Electronic structure quantum chemistry

Time-independent Schrödinger equation: $H\Psi = E\Psi$
Tautomerisation energies

$E$ more negative $\Rightarrow$ molecule is more stable

$$\Delta E_{\text{tautomerisation}} = E_{\text{enol}} - E_{\text{keto}}$$
Is the conversion from 5BrU to 5BrU* likely?

\[
\begin{align*}
U & \rightarrow U^* & 50.9 \text{ kJ/mol} \\
5\text{BrU} & \rightarrow 5\text{BrU}^* & 52.5 \text{ kJ/mol}
\end{align*}
\]

gas phase

B3LYP/6-31G(d,p)^1

Calculations on isolated bases: the conversion from keto to enol form is energetically very unfavourable for both U and 5BrU

Can a hydrated environment change the results?

^1 M Hanus, M Kabeláč, D Nachtigallová and P Hobza
Biochemistry 44, 1701 (2005)
Intermezzo: hydration methods

- explicit hydration
- continuum solvation
Explicit hydration
Continuum solvation models
Continuum solvation: the conversion from keto to enol form is energetically very unfavourable for both U and 5BrU.

However, does the continuum solvation model describe the solvated phase sufficiently accurately?

1 M Hanus, M Kabeláč, D Nachtigallova and P Hobza
Biochemistry 44, 1701 (2005)
Hydrated clusters of U and 5BrU

Calculations on U, U*, 5BrU, 5BrU*

Monte Carlo simulations with 400 waters; NVT ensemble

Used as starting structures for DFT calculations

B3LYP/6-31G(d,p) and BLYP/6-31G(d,p) with 50 and 100 waters

CP-corrected interaction energies with BLYP, B3LYP and M05-2X
U, U*, 5BrU, 5BrU* hydrated by 50 and 100 waters
**keto→enol tautomerisation energies**
(B3LYP/6-31G(d,p) calculations)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Energy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U → U*</td>
<td>50.9</td>
</tr>
<tr>
<td>5BrU → 5BrU*</td>
<td>52.5</td>
</tr>
<tr>
<td>U-W50 → U*-W50</td>
<td>16.1</td>
</tr>
<tr>
<td>5BrU-W50 → 5BrU*-W50</td>
<td>-55.7</td>
</tr>
<tr>
<td>U-W100 → U*-W100</td>
<td>56.3</td>
</tr>
<tr>
<td>5BrU-W100 → 5BrU*-W100</td>
<td>-36.5</td>
</tr>
</tbody>
</table>
Water reverses the tautomeric preference of 5BrU, causing the rare tautomer to be preferred.

However, will the conversion from the enol to the keto tautomer actually take place?


T van Mourik, VI Danilov, VV Dailidonis, N Kurita, H. Wakabayashi and T Tsukamoto, Theor Chem Acc 125, 233-244 (2010)
**Car-Parinello Molecular Dynamics**

U and 5BrU with 49 water molecules

BLYP-D and plane-wave basis set (kinetic energy cut-off 70 Ry)

Microcanonical ensemble, periodic boundary conditions

Constrained dynamics with the N3-H3 distance as constraint

Increase N-H distance in U-(H₂O)₄⁹ and 5BrU-(H₂O)₄⁹ from 1.05 (1.04) Å to 1.94 (2.17) Å, until proton binds to nearby water molecule

At each distance, perform CPMD simulation for 4-6 ps
U-(H$_2$O)$_{49}$ with N3H3 fixed at 1.94 Å
BrU-(H₂O)$_{49}$ with N₃H₃ fixed at 2.17 Å
**Conclusions**

**Static DFT calculations:**

Water reverses the tautomeric preference of 5BrU, causing the rare tautomer to be preferred.

**Dynamic DFT calculations:**

The keto-enol tautomerisation is favourable for 5BrU in aqueous solution, but does not happen for hydrated U.
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