# The influence of base-pair tautomerism on single point mutation rates in aqueous DNA

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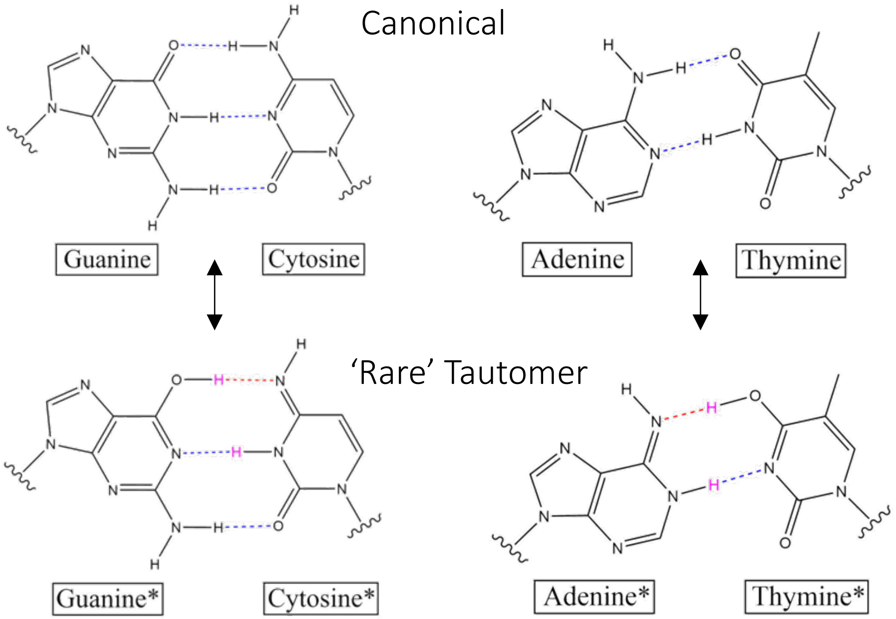
# Mutations in DNA

- External agents such as external electric fields, or other carcinogenic compounds are known to facilitate DNA mutations[1]

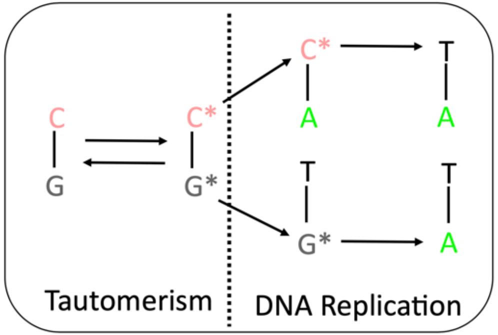
- … But what about *spontaneous mutations?*


(1) Loft, Steffen, and Henrik E. Poulsen. "Cancer risk and oxidative DNA damage in man." *Journal of molecular medicine* 74, no. 6 (1996): 297-312.

# Watson-Crick Base-Pairs and Löwdin's Hypothesis




- Protons obey the laws of quantum theory, behaving like 'wave packets'.
- Due to the quantum-mechanical tunnelling effect, there is always a small probability of proton transfer within any given base-pair.

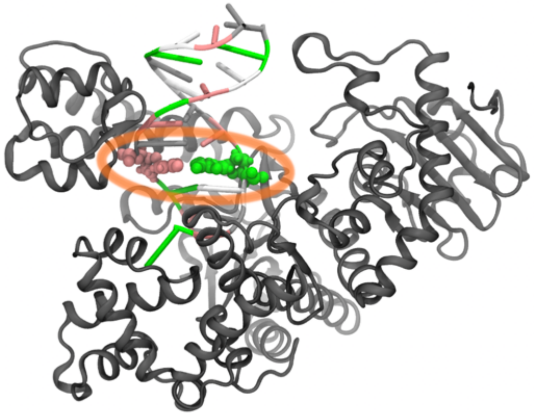



*'...this transfer of proton over distances less than 1 A may be the driving force for genetic mutations in all living organisms...'* – Löwdin[2]



[2] Löwdin, Per-Olov. "Proton tunneling in DNA and its biological implications." *Reviews of Modern Physics* 35.3 (1963): 724.

# Experimental Evidence and Previous Simulation



- DNA polymerase caught red-handed!
- Structural evidence of a C:A mismatch within crystallised DNA polymerase

- An overall **G:C → A:T** mutation bias has been reported in a variety of organisms

## QM-only gas-phase studies

- Proton transfer in G:C is energetically more favourable than in A:T[3]
- Double proton transfer; step-wise or concerted mechanism? [4]
- Little to no quantification of errors, due to lack of replicas
- A gas-phase base-pair is an idealised structure – is it truly representative?
- Provide a good starting point

[3] Jacquemin, Denis, et al. "Assessing the importance of proton transfer reactions in DNA." *Accounts of chemical research* 47.8 (2014): 2467-2474.
[4 ]Sekiya, H., & Sakota, K. (2008). Excited-state double-proton transfer in a model DNA base pair: Resolution for stepwise and concerted mechanism controversy... *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, *9*(2), 81-91.

# Considerations when modelling aqueous DNA

Reproducibility?

A realistic model; solvated, large enough DNA sample, >10,000 atoms

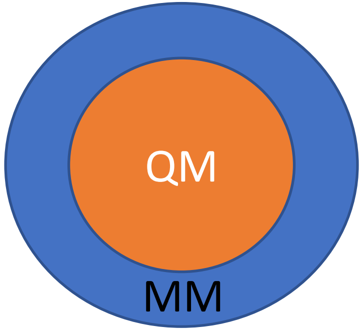
Proton transfer is both a bond breaking/ forming effect

Requires quantum mechanics (**QM**) to model: Expensive at high accuracy

DNA is a large, complex biomolecule molecule

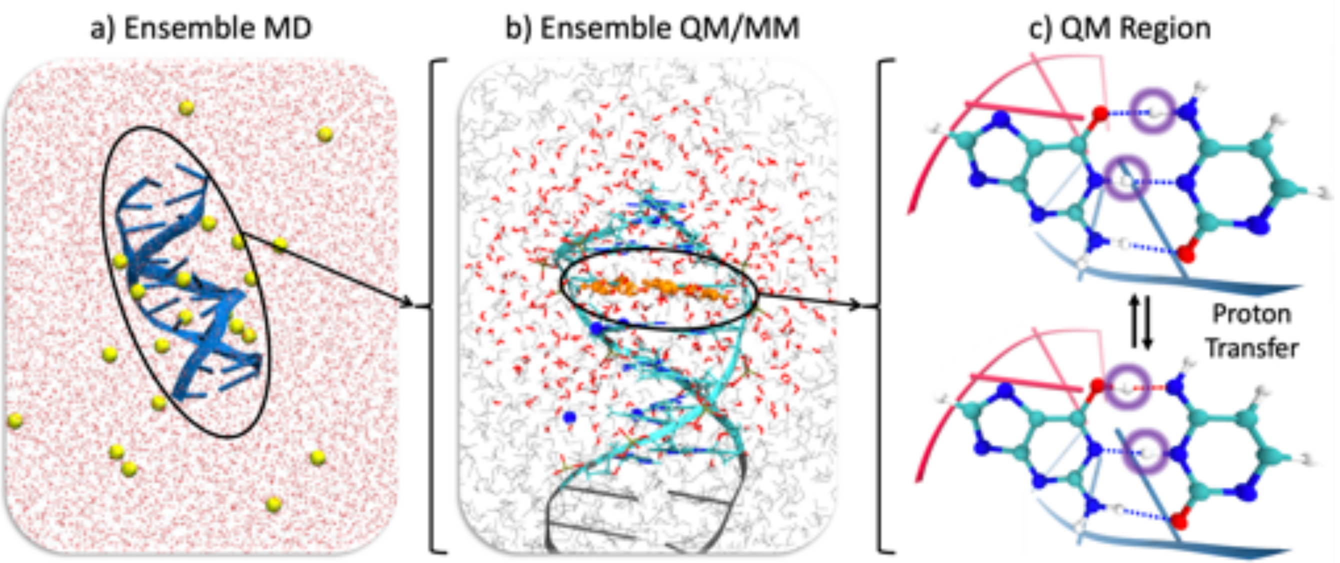
Use forcefield molecular mechanics (**MM**): Computationally cheaper

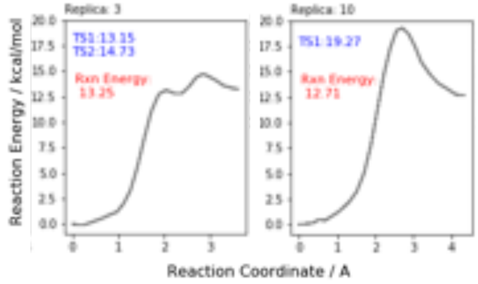
We can utilise both of these methods simultaneously[5]: (**QM/MM**)




[5] Lu, You, Zhenggang Lan, and Walter Thiel. "Hydrogen bonding regulates the monomeric nonradiative decay of adenine in DNA strands." *Angewandte Chemie International Edition* 50.30 (2011): 6864-6867.

# Modelling Proton Transfer: Our Multiscale Workflow




25 QM/MM reaction pathways mapped with nudged elastic band per base-pair

a) MD Code: NAMD
AMBER *parmbsc1* force field at 300 K, 1 atm. **10** replicas of 10 ns simulation to thermalize DNA effectively.

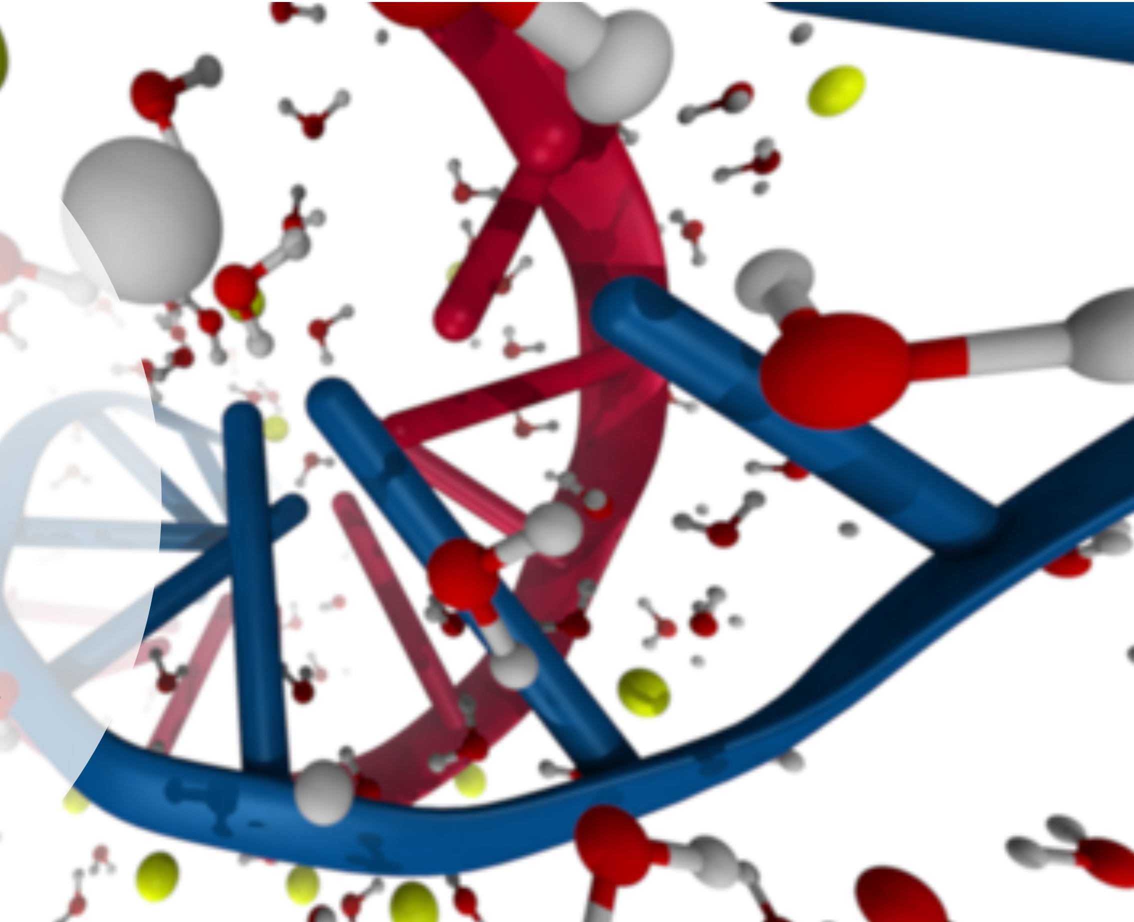
b) QM/MM Code: ChemShell
Linking NWChem (QM) with DL-POLY (MM). Ensemble QM/MM, using configurations drawn from MD*. **25 replicas** for a given base-pair.

c) QM Code: NWChem
One base pair (~30 atoms).
QM approximation:
B3LYP+XDM/aug-cc-pvdz

* Snapshots are chosen on an average base-pair distance criteria

# Advantages to using Ensemble QM/MM

- Inclusion of explicit solvation and DNA structure - more realistic than gas-phase!
- Observe multiple reaction paths for the same base-pair
- Determine the probability of each pathway occurring
- Comment on the statistical variance of the rates for each pathway



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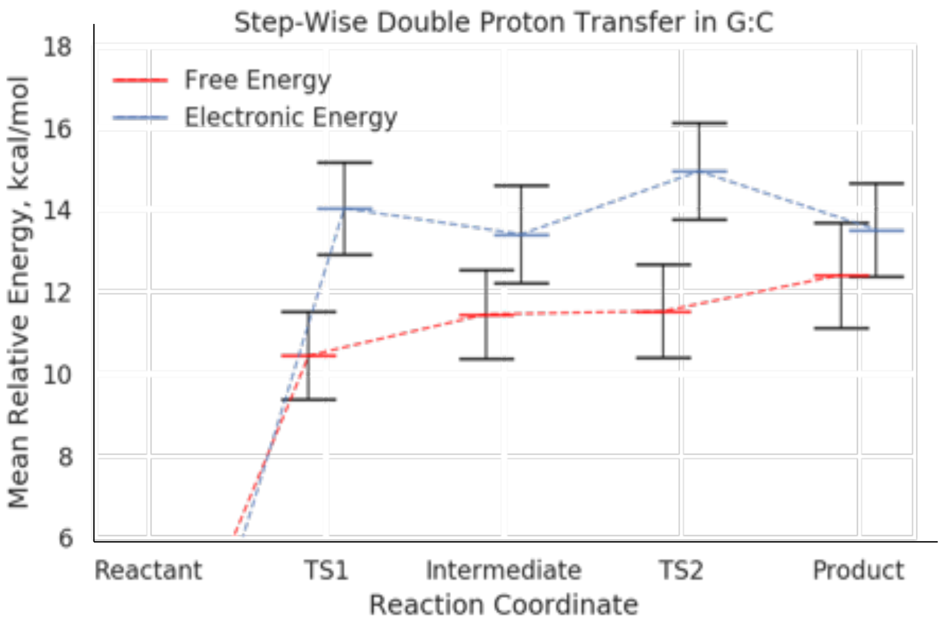
| Base-Pair | No proton transfer | SPT Concerted | DPT | |
|---|---|---|---|---|
| | | | Step-Wise | Concerted |
| % of G:C replicas | 0 | 4 | 80 | 16 |
| % of A:T replicas | 56 | 36 | 0 | 8 |

# Step-Wise Double Proton Transfer in G:C

- The most probable type of proton transfer in G:C is step-wise, 80% of the time
- Step-wise, two transition states and an intermediate

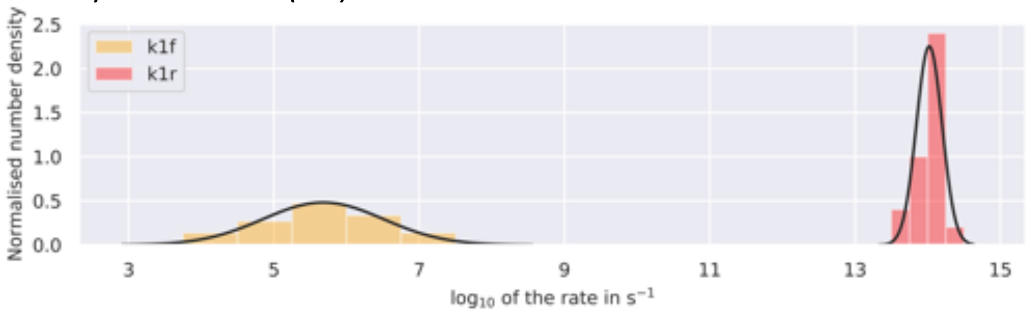



The reaction coordinate for the step-wise double proton transfer mechanism in the G:C base-pair. Error bars are standard deviation. Data points calculated from 20 replicas out of 25 total

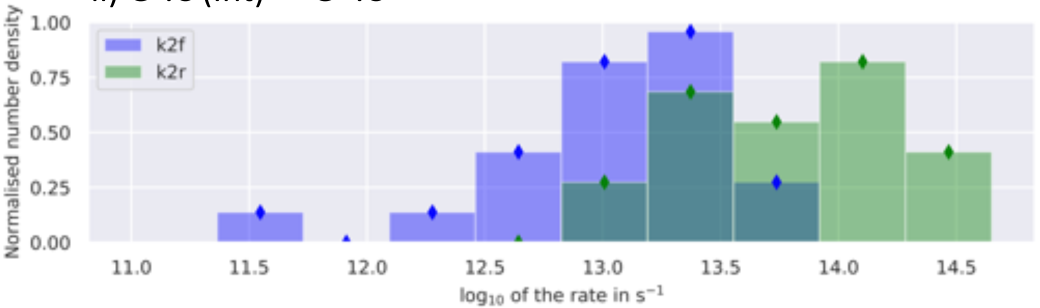
# Step-Wise Double Proton Transfer in G:C

- Rate determining step is $k_{1f}$ , many orders of magnitude slower than $k_{1r}$
- The use of ensemble QM/MM gives insight to the statistical variance
- The second step is much closer to equilibrium than the first
- Tautomer has a lifetime of ~50 fs (1/ $k_{1r}$)

i) G:C -> $G^{+}$:$C^{-}$(Int)




ii) $G^{+}$:$C^{-}$(Int) -> G*:C*




The normalized histogram of log proton transfer rates for the step-wise double proton transfer mechanism in the G:C base-pair

# Step-Wise Double Proton Transfer in G:C

$$G_{taut} = \left( \sum_{K_{DPT}} K_{eq,weighted} \right) \times G \quad (1)$$

where;

$G$ is size of genome in base-pairs ($3 \times 10^9$)

The probability of step-wise double proton transfer is 0.8

$K_{eq}$ is the calculated average equilibrium constant ($1.54 \times 10^{-8}$)

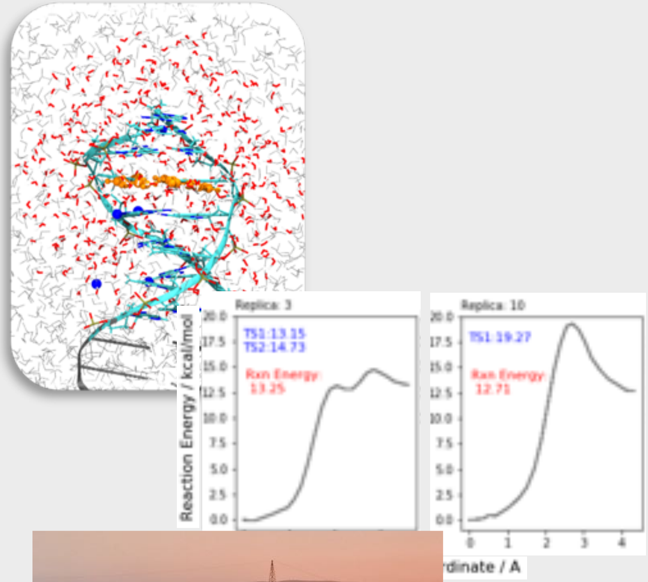
$K_{eq,weighted}$ is $K_{eq}$ multiplied by it's probability of occurrence

$G_{taut}$ is the number of tautomeric base-pairs in the genome at any given time

- Assuming;
  - Genome has 100% G:C content
  - Every tautomerism leads to mismatch
- $G_{taut}$ = 0 – 114 base-pairs per-genome
- 1 in 3.8 x $10^{-8}$ mutations per nucleotide site.
- This value is within the estimation of mutation rate per nucleotide in humans ($10^{-11}/10^{-8}$) [6]

[6] Nachman, Michael W., and Susan L. Crowell. "Estimate of the mutation rate per nucleotide in humans." *Genetics* 156.1 (2000): 297-304.

# Conclusions

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- Proton transfer in biological systems is very sensitive to its environment, necessitating multiscale modelling
- Multiple reaction pathways for each base-pair are observed, with varying probabilities
  - G:C double proton transfer 96% of the time
  - A:T single proton transfer 36% of the time
- The frequency of a tautomer in human genome is $< 0.001$ %
- The life-times of the tautomers are *fs*, while DNA unwinding occurs on the *ns* scale

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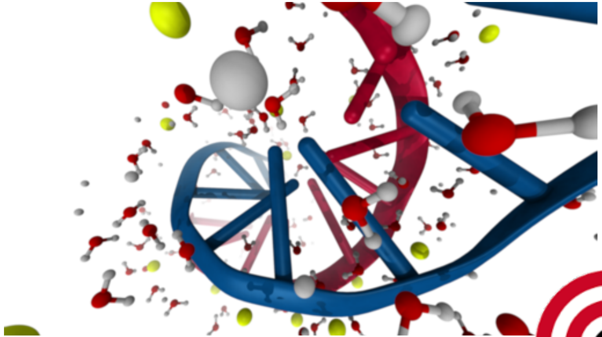
# *Thanks for listening!*



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