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## Introduction

Recently difluorotoluene (**F**) has been placed as a non-polar isostere of thymine (**T**) opposite to adenine (**A**) within DNA without having a measurable effect on DNA synthesis [1]. This has led to the conclusion that shape complementarity and not hydrogen bonding may be the key to reliable copying of DNA. In order to gain deeper insight into the electronic and structural features of the **A-T** and **A-F** base pairs (Fig. 1) we have performed quantum chemical calculations for a comparison of interaction energies, electrostatic potentials and NMR spectra.

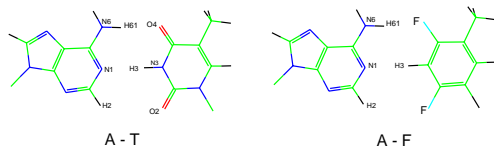


Fig. 1. The **A-F** and **A-T** base pairs

## Methods

The **A-F** and **A-T** Watson-Crick base pairs have been optimized at HF/6-31G(d,p) and B3LYP/6-31G(d,p) levels with a subsequent Hessian calculation. Interaction energies corrected for the basis set superposition error (BSSE) have been determined using the MP2/6-31G(d,p)//HF/6-31G(d,p) and B3LYP/6-31G(d,p) methods. The Gauge Independent Atomic Orbital (GIAO) method [2] has been used to estimate NMR shifts using the 6-311+G(2d,2p) basis set.

For comparison with the calculated intermolecular distances, structural parameters of **A-T** base pairs in 21 DNA X-ray structures refined to high resolution have been analyzed using HBxplorer [3].

## Results

Both base pairs are planar and their other structural parameters are very similar except for an opening of the **A-F** pair relative to **A-T** as a consequence of a missing hydrogen bond between C2-H...F4 (Fig. 2). The rms deviation of the superimposed complex coordinates is only 0.79 Å. Donor acceptor atom distances calculated for the **A-T** pair at the B3LYP level are in better agreement with experimental X-ray distances than the somewhat longer distances from HF optimizations (Tab. 1).

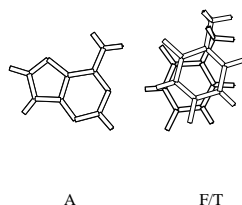


Fig. 2. Superposition of **A** in the **A-T** (bold) and **A-F** base pair structures.

Tab. 1. Structural parameters of the **A-F** and **A-T** base pairs<sup>a)</sup>

	<b>A-F</b>		<b>A-T</b>		exp. <sup>c)</sup>
	HF <sup>b)</sup>	B3LYP <sup>b)</sup>	HF	B3LYP	
r(H2...F/O2)	4.20	3.98	2.80	2.96	-
r(N1...H3)	2.57	2.37	1.98	1.80	-
r(H61...O4/F)	2.25	2.09	2.09	1.92	-
r(C2...O2/F2)	4.91	4.58	3.77	3.62	3.52
r(N1...N3)	3.25	3.10	2.99	2.85	2.82
r(N6...O4/F4)	3.63	3.44	3.09	2.94	2.98

a) distances in Å; b) 6-31G(d,p); c) average from 21 high resolution X-ray structures

The total interaction energy taking into account BSSE, electron correlation and deformation for **A-F** (-3.0 kcal/mol) is smaller than the **A-T** interaction energy (-10.3 kcal/mol). The difference between MP2 and B3LYP interaction energies is small (Tab. 2).

The calculated NMR shifts of H61 (**A-F**: 1.2, **A-T**: 3.0 ppm) and N1 (**A-F**: -3.0, **A-T**: -12.5 ppm) in the complexes relative to isolated **A** indicate a much smaller interaction between **A** and **F** than between **A** and **T**.

The dipole moments 2.0 and 1.6 D of the **A-T** and **A-F** pairs are not very different. The electrostatic potential shows minor differences for the atoms in the contact region (F,O), Fig. 3.

Tab. 2. Energies of the **A-F** and **A-T** base pairs<sup>a)</sup>

	<b>A-F</b>	<b>A-T</b>
EMP2	-3.9	-12.3
EB3LYP	-3.3	-14.0
EDEF	0.1	0.6
ZPE	0.8	1.4
E <sub>0</sub>	-3.0	-10.3

a) EMP2, EB3LYP interaction energies (kcal/mol); EDEF deformation energy; ZPE zero point energy change; E<sub>0</sub> = EMP2 + EDEF + ZPE total interaction energy.

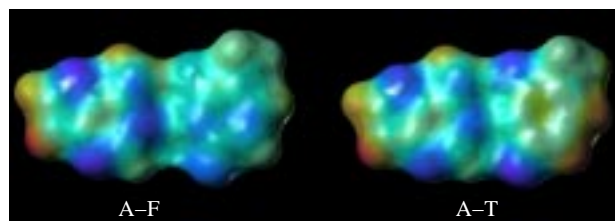


Fig. 3. Electrostatic potential at the Conolly surface of the **A-F** and **A-T** pairs (blue: negative potential, red: positive potential).

## Summary

The structure of the hydrogen bonded **A-T** base pair determined with B3LYP appears to be more accurate than the structure obtained from HF calculations.

**A** can form planar complexes with **T** and **F**. Both complex structures are similar except for a base pair opening found for the **A-F** pair (Fig. 2, 3). The interaction energy between **A** and **F** is much smaller than between **A** and **T**. The **A-F** interaction energy E<sub>0</sub> = -3.0 kcal/mol is even smaller than the corresponding energies found for any other Watson-Crick or non-canonical pairs of standard bases, the smallest ab initio interaction energy being -9.6 kcal/mol for **G-A** [4]. In spite of this weak interaction the distances N6-H61...F and N1...H3-C3 are below the sum of the corresponding van der Waals radii, which means that the interaction cannot be governed by van der Waals forces alone. The quantum chemical study provides detailed information on similarities and differences between the **A-T** and **A-F** pairs. The experimental result that **T** can be replaced by **F** in DNA indicates that the weak interaction energy in **A-F** is still sufficient for retaining a high fidelity of DNA polymerase. This finding may be important for the design of other non-standard bases.

## Acknowledgements

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## References

1. S. Moran, R. X.-F. Ren, S. Rumney, E. T. Kool, J. Am. Chem. Soc. **119**, 2056 (1997).
2. R. Krishnan, J. S. Binkley, R. Seeger, J. A. Pople, J. Chem. Phys. **72**, 650 (1980).
3. K. Lindauer, C. Bendic, J. Sühnel, Comput. Appl. Biosci. **12**, 281 (1996).
4. J. Sponer, J. Leszczynski, P. Hobza, J. Biomol. Struct. Dyn. **4**, 117 (1997).