Structure, Function and Dynamics of Modified Oligonucleotides

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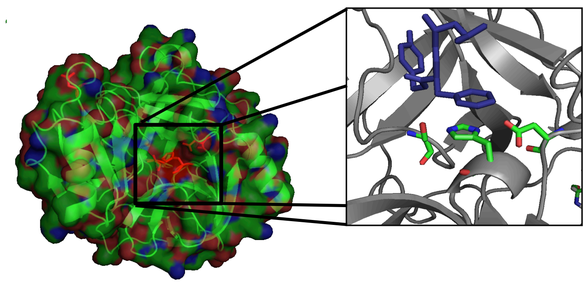
In the development of new types of **synthetic enzymes**, the DNA scaffold has recently been shown to be a **suitable scaffold** for the acceleration of a range of reactions by providing a hydrophobic and chiral environment. One of the most attractive features of a DNA sequence is the **mutual recognition** of two complementary strands with the formation of a double helix structure in a predictable and programmed manner. Contrary to the use of intercalated ligands or in vitro selection procedures, recent developments within OBCR have made the introduction of **amino-acid like side-chain functionalities** on the thymine base possible. In addition, it was demonstrated both by NMR and UV-melting experiments that their incorporation leaves **duplex stability unaltered** or even enhanced, therefore opening new possibilities for the development of two types of new synthetic enzymes: **serine protease** and **esterase like DNAzymes**. The aim of the first system is to successfully mimic the catalytic triad of α-chymotrypsin while the second type of systems is inspired by the synthetic enzyme developed by Baltzer and co-workers[1].



α-chymotrypsin and it's active site
(Asp102, His 57 and Ser195; PDB ID 1AFQ)

Systems of Interest

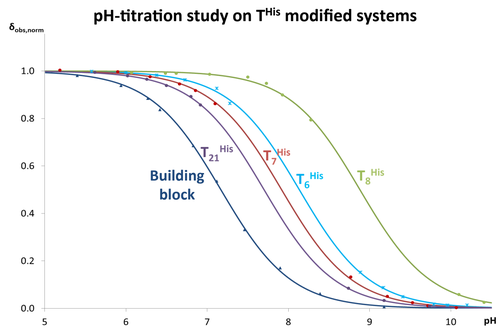
1 2 3 4 5 6 7 8 9 10 11 12 13 14
5′-GACCATTTGCAGCG-3′
3′-CTGGTAATCGTCGC-5′
28 27 26 25 24 23 22 21 20 19 18 17 16 15

Wild type

1 2 3 4 5 6 7 8 9 10 11 12 13 14
5′-GACCATTT^{His}GCAGCG-3′
3′-CTGGTAAT CGTCGC-5′
28 27 26 25 24 23 22 21 20 19 18 17 16 15

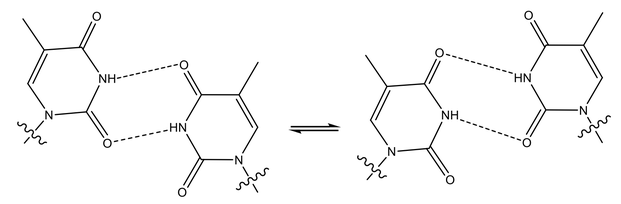
T_8^{His}

Sequence	Tm (°C, UV-VIS)
Wild type	48,6 ± 0,3
T_6^{His}	50,5 ± 0,2
T_7^{His}	50,1± 0,3
T_8^{His}	54,6± 0,1
T_{21}^{His}	53,3± 0,3

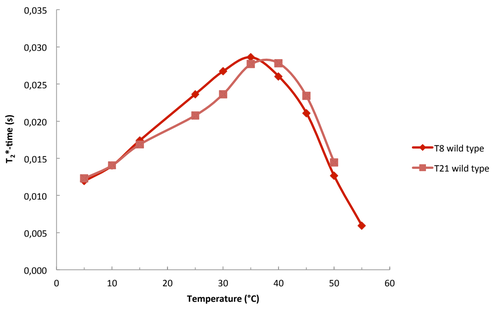


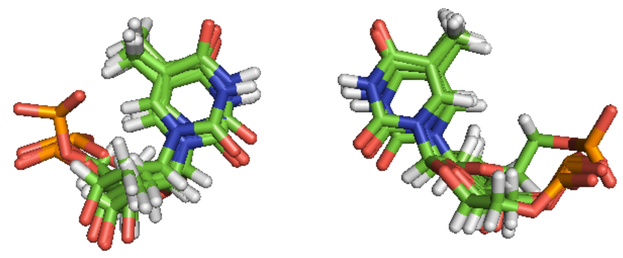
- Melting temperatures (Tm) determined by NMR and UV-VIS independently show a noticeable increase in stability of T_8^{His} and T_{21}^{His}
- Increase in pKa of modified nucleotide inserted in duplex: stabilization of protonated His-like modification through electrostatic interaction with negatively charged DNA backbone ?
- Increase in pKa is far higher for T_8^{His}
  ➔ Additional, specific electrostatic interaction

T-T mismatch



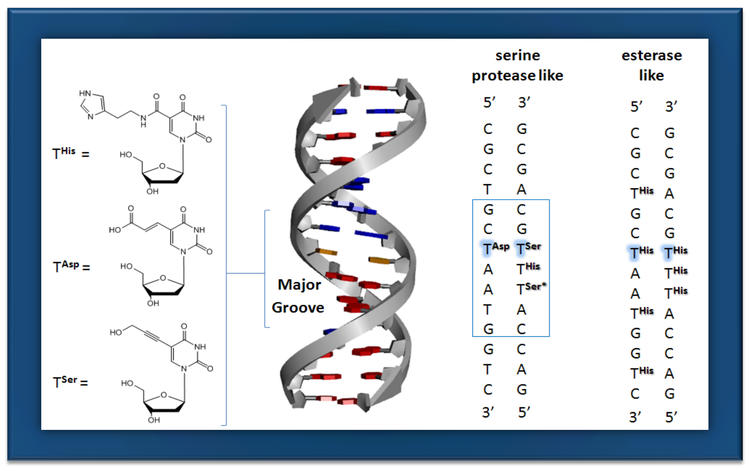
T-T mismatch = wobble type base pairing
W↑ and W↓ in fast exchange ~ $5x10^{10}$ Hz





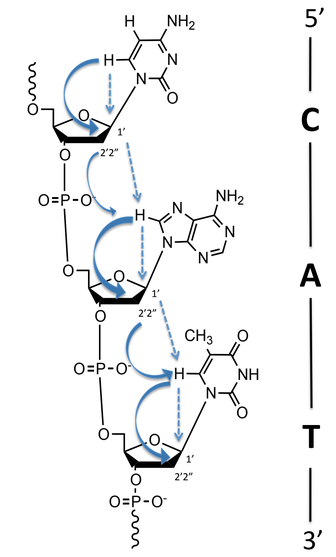
Average T-T mismatch from nOe-restrained based modeling (T8-T21) superposition of five structures with the lowest energy[3,4]

Since both types of the wobble type of base pairing are already in fast exchange at 25°C, the nOe-contacts of this base pair are averaged over both conformations. The T_2* profile indicates this as well as the influence of exchange with water at ± 45°C.



Assignment Wild Type

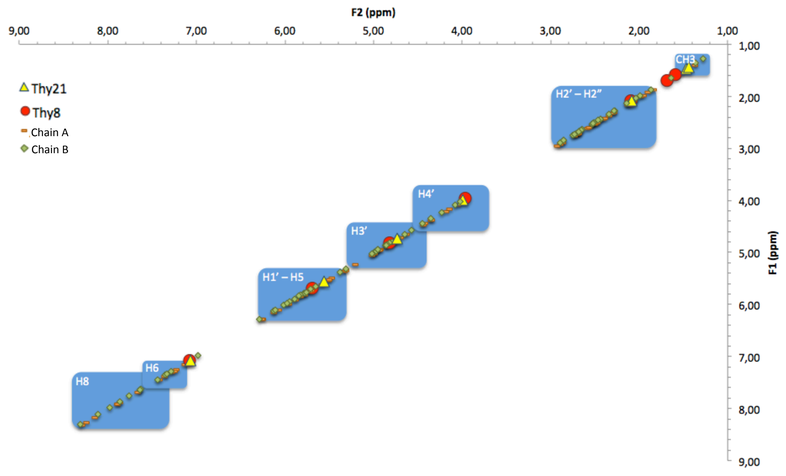
Assignments were performed mainly by nonlabile H1'-H6/H8 and H2'/H2"-H6/H8 sugar-base through space contacts. H5-H6, H6-CH_3 and sugar protons were assigned using TOCSY/COSY spectra, granting additional confirmation of each H1' and H2'/H2" belonging to the same deoxyribose spin system[2].



Pictorial presentation of short ^{1}H-^{1}H distances d_i(6,8;2') and d_s(2";6,8) (solid arrows), d_i(6,8;1') and d_s(1';6,8) (dashed arrows)

1 2 3 4 5 6 7 8 9 10 11 12 13 14
5′-GACCATTTGCAGCG-3′
3′-CTGGTAATCGTCGC-5′
28 27 26 25 24 23 22 21 20 19 18 17 16 15

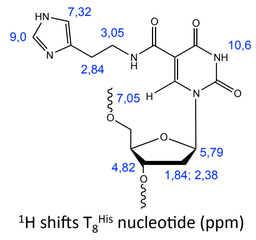
Wild Type Chain A & B



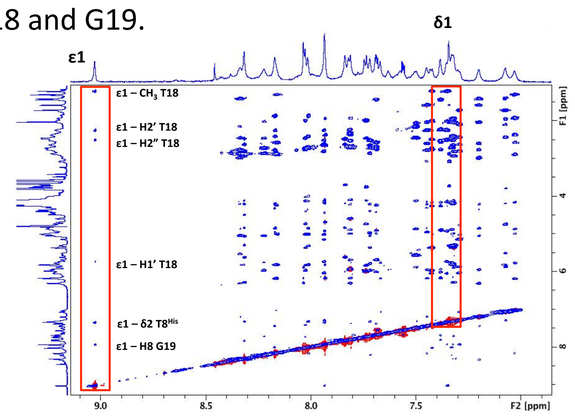
Schematic representation assignment Wild Type; All residues are located in standard ^{1}H chemical shift regions for DNA/RNA except H2'/H2" and H6 protons of the T-T mismatch

Assignment T_8^{His}

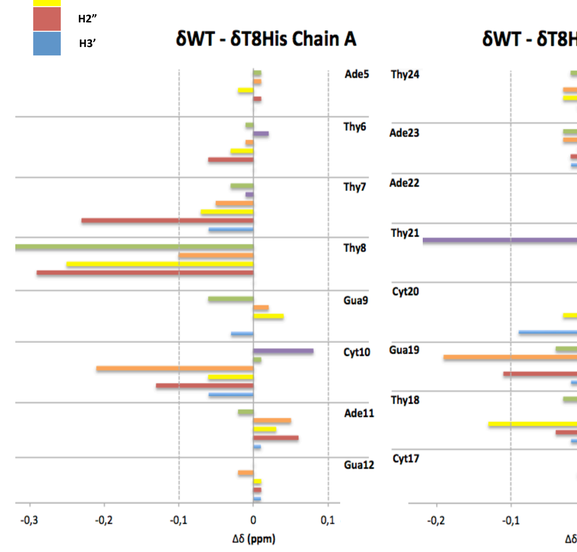
Special attention is given to the assignment of the T^{His} functionality and its position. Differences in chemical shift δ as well as the nOe-contacts present, suggest the linker is positioned inside the major groove, with the imidazole moiety situated between T18 and G19.

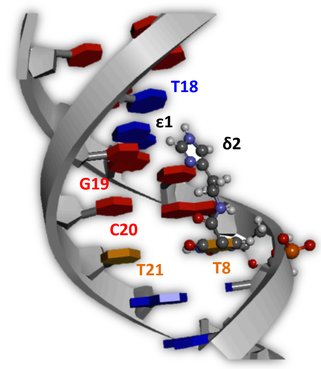


^{1}H shifts T_8^{His} nucleotide (ppm)



2D NOESY tm 250ms, pH 5,0 @ 700MHz, 25°C





Preliminary proposal position of T_8^{His} modified functionality, this orientation could permit a H-bond with T18 or G19 explaining the higher increase in pKa

As shown above, valuable information regarding assignment and determination of the precise state of the modifications introduced inside the scaffold is available via NMR. In later stages, the T_{21}^{His} system as well as the additivity of the other modifications will be investigated while the cooperativity between the various functional groups may also be obtained, providing valuable information on the catalytic site. Restrained based modeling on the T_8^{His} system will be used to visualize the position of the modification and confirm the hypothesis of H-bond formation of the imidazole functionality with the T18 or G19 base on the opposite strand of the DNA duplex.

Acknowledgements

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References

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