Towards DNA based esterases: uncovering Hoogsteen face regulation of the pK<sub>A</sub> of a tethered imidazole functionality by NMR and molecular dynamics

Dieter Buyss<sup>1,2</sup>, Vicky Gheerardij<sup>2</sup>, Bjorn Van Gasse<sup>1</sup>, Jos Van den Begin<sup>2</sup>, Annemieke Madder<sup>2</sup> & José C. Martins<sup>1</sup>

1 OBCR, Department of Organic Chemistry, University of Ghent, Krijgslaan 281 S4, B-9000, Ghent, Belgium
2 OBCR, Department of Organic Chemistry, University of Ghent, Krijgslaan 281 S4, B-9000, Ghent, Belgium

1. General concept & design

In the development of esterase like DNAzymes, recent advances in OBCR made the introduction of amino acid-like side chain functionalities on the thymine base possible. The hydrophobic and chiral environment of a B-DNA helix is used as a new scaffold for the development of synthetic enzymes. Both first generation, single histidine modified systems and second generation, double modified systems are subjected to a systematic study.

2. First glimpse of the pK<sub>A</sub> regulating motif

**Thermal stability**

<table>
<thead>
<tr>
<th>System</th>
<th>A+T base pair</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; (°C)</th>
<th>∆T&lt;sub&gt;m&lt;/sub&gt; at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>T&lt;sub&gt;m&lt;/sub&gt;&lt;sup&gt;CTGGTAAR&lt;/sup&gt;</td>
<td>48.4±0.6</td>
<td>4.6±0.4</td>
</tr>
<tr>
<td>+mod</td>
<td>T&lt;sub&gt;m&lt;/sub&gt;&lt;sup&gt;CTGGTAAR&lt;/sup&gt;</td>
<td>53.0±0.3</td>
<td>6.5±0.1</td>
</tr>
</tbody>
</table>

From melting temperatures:
- No loss of stability due to modification
- Increase in stability is dependent on position of modification: T<sub>m</sub><sup>CTGGTAAR</sup>=5°-6°C

**Specific noe contacts**

- Solely for T<sub>m</sub><sup>CTGGTAAR</sup> there’s a clear set of noe contacts to position n+3 (A<sub>1</sub>-T<sub>15</sub> base pair)
- T<sub>m</sub><sup>CTGGTAAR</sup> and T<sub>m</sub><sup>CTGGTAAR</sup> prove that an AT base pair is tolerated as well as that the motif is robust and sequence-independent

**NMR pH titration: follow ε<sub>1</sub> and δ<sub>2</sub>**

- 6°±
- 7°±
- 8°±

3. Molecular Dynamics

**Starting structure**

50ns of MD trajectory shows persistent H-bridging in T<sub>m</sub><sup>CTGGTAAR</sup> and close interproton contacts validated in noe’s

**Thermal stability**

<table>
<thead>
<tr>
<th>System</th>
<th>pHunit</th>
<th>∆pK&lt;sub&gt;A&lt;/sub&gt;</th>
</tr>
</thead>
</table>
| T<sub>m</sub><sup>CTGGTAAR</sup> | 7.2±0.07 | -0.10
| T<sub>m</sub><sup>CTGGTAAR</sup> | 8.3±0.05 | -

4. Application of the motif in double modified systems

- Increase in melting temperature is consistent with presence of the pK<sub>A</sub> regulating motif for T<sub>m</sub><sup>CTGGTAAR</sup> in both systems
- Increase in melting temperature is less pronounced with increasing vicinity of the imidazole functionalities

**References**


Acknowledgements

The 700MHz is part of the INMRF jointly operated by UGent, UA and VUB.