Stabilization of nucleic acid secondary structures results from a subtle balance of multiple interactions. Base pairing, base stacking, and cation binding have been extensively studied for decades, whereas the role of other interactions remains poorly understood. Among them, nonconventional C–H···O hydrogen bonds are especially interesting. The importance of these interactions in proteins was recognized more than 40 years ago, and during the last few years, several studies have shown their relevance in multiple biological systems. In nucleic acids, most studies have focused on aromatic C–H···O interactions, and in particular, on the effect of nonconventional hydrogen bonds in base pairing and in base-sugar interactions. Backbone C–H···O hydrogen bonds have received less attention, although they have been proposed to play a role in the stabilization of four-stranded i-motif structures, and in tight packing of RNA and DNA duplexes. Although these interactions are considered weak in natural nucleic acids, their strength can be increased through chemical modifications. Fluorine substitutions are believed to be especially interesting. The importance of these effects has been well-characterized in small molecular systems. Recently, Kakshoor et al. have observed fluorine-enhanced aromatic C–H···N interactions that significantly affect the strength of 2,4-difluorotoluene–adenine base pairs. The prevalence of 2'-fluorinated oligonucleotides in nucleic acids research motivated us to examine the role of enhanced nonconventional hydrogen bonding in duplex stability.

Effects contributing to the enhanced thermal stability of 2'-deoxy-2'-fluororibonucleic acid (2F-RNA) duplexes have been the subject of recent studies. Recently, work by Egli and co-workers has demonstrated that 2F-RNA duplex stabilization is primarily enthalpic in origin, through enhanced base stacking and pairing arising from long-range fluorine inductive effects on the nucleobases. Stabilizing effects arising from fluorine have also been observed in the 2'-epimer of 2F-RNA, 2'-deoxy-2'-fluororibonucleic acid (2F-ANA). The geometry of 2F-ANA:RNA duplexes are such that close 2F–purine (H8) contacts are provoked, providing a very favorable geometry for hydrogen bond formation.

Herein, we identify an additional stabilizing factor in A-form duplexes modified with 2F-ANA and 2F-RNA. Results demonstrate that fluorine-enhanced FC–H···O backbone interactions can have a strong stabilizing effect on oligonucleotide duplexes. Although close FC–H···O interactions have gone largely unnoticed, we propose that these interactions exist in other recently reported fluorinated duplexes and complement the enhanced base stacking and pairing effects stabilizing 2F-RNA duplexes.

Two chimeric self-complementary duplexes containing continuous tracts (duplex H) and alternating regions (duplex S) of 2F-ANA and 2F-RNA residues (Figure 1A and B) were used to carry out these structural, thermodynamic, and computational studies.

Self-complementary duplex formation and melting was monitored by UV absorbance at 260 nm and 1H and 19F NMR (Supporting Information, Figure S1 and S2). Melting temperature ($T_m$) values are shown in Table 1, showing that the $T_m$ of H is over 20°C greater than that of S, a trend in agreement with previous reports.

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**Table 1: $T_m$ values and thermodynamic parameters for the self-complementary duplexes**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5'-3')</th>
<th>$T_m$ [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>5'-d(CGGGAATTCGGG)-3'</td>
<td>58.4</td>
</tr>
<tr>
<td>R</td>
<td>5'-r(CGGGAUUGGGG)-3'</td>
<td>65.1</td>
</tr>
<tr>
<td>aF</td>
<td>5'-af(CGGGAATTCGGG)-3'</td>
<td>76.1</td>
</tr>
<tr>
<td>rF</td>
<td>5'-rf(CGGGAUUGGGG)-3'</td>
<td>&gt;85</td>
</tr>
<tr>
<td>H</td>
<td>5'-r(CGGGA)-af(TCTCGG)-3'</td>
<td>70.4</td>
</tr>
<tr>
<td>S</td>
<td>5'-r(CGGGA)-af-rfu-afc-rfC-rfG-3'</td>
<td>48.8</td>
</tr>
</tbody>
</table>

[a] αM oligonucleotide concentration, buffer conditions: 140 mM KCl, 1 mM MgCl$_2$ and 5 mM Na$_2$HPO$_4$, pH 7.2. [b] aF and rF represent 2F-ANA and 2F-RNA units, respectively.

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The structures of H and S are intermediate between canonical A and B forms and retain structural features of both families of double helical structures (Figure 1; Supporting Information, Figure S8). The geometry of 2F-RNA nucleotides in the continuous tracts of H and S is very similar to that expected for an RNA homoduplex, with riboses adopting the expected C3'-endo conformation (phase angles about 40–60°; Figure 1; Supporting Information, Figure S9). Interestingly, 2F-ribose conformations of alternating 2F-ANA/2F-RNA steps in S fall within the east domain, with pseudorotation phase angles between 80° and 95°. This unexpected ribose conformational switch arising in the steps region is fully consistent with the experimental J-coupling data (Figure 1). The 2F-arabinoses adopt south/east conformation, with pseudorotation phase angles between 75° and 140° in H, and 100° to 160° in S. Geometrical parameters are shown in the Supporting Information, Tables S4–S9.

As 2F-ANA and 2F-RNA nucleotides are considered, respectively, as DNA and RNA analogues, it is interesting to compare the structures of the 2F-ANA:2F-RNA segments of H with that of 2F-ANA:RNA hybrids. In the structure of a 2F-ANA:RNA hybrid decamer (PDB ID: 2KP4),[21] 2F-ANA nucleotides adopt an east sugar conformation and glycosidic angles between 110° and 120°, provoking a close 2F–H8 contact, with a 2F–H8–C angle of about 150° that favors hydrogen bond formation.[21] In contrast, glycosidic angles of contiguous tracts of 2F-ANA residues in H are between 130° and 140° (Supporting Information, Figure S9). These higher anti values in H are typical of a pure A duplex and prevent the appropriate orientation for 2F–H8–C hydrogen bond formation. The experimental evidence for this interaction in 2F-ANA:RNA hybrids arises from strong sequential 2F-H8 HOE cross-peaks and heteronuclear 19F–1H J-couplings for inter and intra interactions, respectively. None of these are observed in the 2F-ANA:2F-RNA chimeric duplex H (Supporting Information, Figure S7).

On the other hand, very close contacts between H2 in 2F-RNA and H2' in 2F-ANA and their 3'-neighbor O4'-sugar and O5'-backbone atoms are observed in the continuous tracts of 2F-ANA or 2F-RNA (Figure 2). The H2'–O distances are particularly short in 2F-ANA tracts (about 2.8 Å excluding the terminal residue), where the C–H2'–O4' angles are close to linearity (average of 160°; see Table 2 and Supporting Information, Tables S10 and S11 for details). Owing to the high fluorine electronegativity, the geminal H2/F-ANA residues in 2F-RNA segments of H are positively polarized, provoking a favorable electrostatic interaction with the surrounding electron dense oxygen atoms in the oligonucleotide backbone (Figure 2). In particular, the C–H–O4' geometry (Table 2) resembles that of nonconventional C–H–O hydrogen bonds observed in different contexts.[26] In our case, the positive charge polarization of H2/H2' protos induced by the geminal 2F is supported by their NMR chemical shifts, which in 2F-ANA and 2F-RNA exhibit significant downfield shifts (5–6 ppm) compared to those typically observed in RNA (4 ppm).

The dramatic impact of these sequential C–H–O interactions on duplex stability becomes apparent when the structures of the H and S duplexes are compared. The
Table 2: Average values of H\textsuperscript{2}--H\textsuperscript{2} and H\textsuperscript{2}--O\textsuperscript{4} distances [Å], angles between C--H\textsuperscript{2}--H\textsuperscript{2}--O\textsuperscript{4} in different structures, and topological properties of the bond critical points (BCP) detected between the H\textsuperscript{2}--H\textsuperscript{2}--O\textsuperscript{4}.

<table>
<thead>
<tr>
<th>Step</th>
<th>Structure</th>
<th>Distance H\textsuperscript{2}--H\textsuperscript{2}--O\textsuperscript{4}</th>
<th>Angle C2H\textsuperscript{2}--H\textsuperscript{2}--O\textsuperscript{4}</th>
<th>ρ [au]</th>
<th>∇\textsuperscript{2}ρ [au]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA/RNA\textsubscript{ VIDEO}</td>
<td>Pure RNA (PDB: 1RXB)</td>
<td>2.5</td>
<td>144</td>
<td>0.005435</td>
<td>0.020660</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>Pure 2F-RNA (PDB: 3P4A)</td>
<td>2.6</td>
<td>140</td>
<td>0.010307</td>
<td>0.034542</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>H, S</td>
<td>2.9</td>
<td>110</td>
<td>0.007085</td>
<td>0.027376</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>S</td>
<td>2.8</td>
<td>160</td>
<td>0.004617</td>
<td>0.017697</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>S</td>
<td>2.5</td>
<td>147</td>
<td>0.007612</td>
<td>0.027338</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>S</td>
<td>4.4</td>
<td>73</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>2F-RNA:RNA hybrid</td>
<td>2.9</td>
<td>146</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>Standard A-type</td>
<td>2.9</td>
<td>120</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>2F-RNA/2F-RNA\textsubscript{ VIDEO}</td>
<td>Standard B-type</td>
<td>4.7</td>
<td>107</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
</tbody>
</table>

[a] n.d.: not detected; n.c.: not calculated.

Favorable H\textsuperscript{2}--O\textsuperscript{4} contacts (2.9 Å) observed in the continuous tracts are disrupted in some of the 2F-RNA/2F-ANA\textsubscript{ VIDEO} junctions (8–9 and 10–11) of duplex S (H\textsuperscript{2}--O\textsuperscript{4} distance around 4.4 Å) (Figure 2 and Table 2). These distances are closely connected with the sugar conformations in 2F-ANA/2F-RNA and in 2F-RNA/2F-ANA junctions. Alternation of south 2-fluororiboses and east 2-fluororiboses favors C-H\textsuperscript{2}--O\textsuperscript{4} contacts in 2F-ANA/2F-RNA\textsubscript{ VIDEO} steps, and prevents their formation in the adjacent 2F-RNA/2F-ANA\textsubscript{ VIDEO} step (Figure 2D). Furthermore, since fluorine inductive effects might be dependent on the 1,2-diaxial disposition of the fluorine,\cite{11} the induction felt by the nucleobases of east conformers of 2F-RNA (and south conformers of 2F-ANA) could be reduced. Thus, the east 2F-RNA residues observed in the S duplex may lack both enhanced stacking and an FC-H-O interaction with the 3' neighboring 2F-ANA nucleotide, both of which contribute to the large difference in stability between H and S (ΔT\textsubscript{m} = 21.6°C).

Interestingly, as seen in the S duplex, flanking 2F-ANA modifications can invoke an unprecedented east conformation in 2F-RNA. The pseudorotation energy profile resulting from QM calculation indicates that such conformation is unfavorable (Supporting Information, Figure S10 and S11). However, east puckered 2F-RNA residues allows the formation of optimal FC-H\textsuperscript{2}--O\textsuperscript{4} contacts in 2F-ANA/2F-RNA\textsubscript{ VIDEO} steps (Table 2).

Close FC-H\textsuperscript{2}--O contacts most likely contribute to the enhanced stability reported in the crystallographic structures of a pure 2F-RNA duplex and a chimeric 2F-RNA/RNA duplex.\cite{9} H2\textsuperscript{2}--O\textsuperscript{4} angles are about 140° (Supporting Information, Figure S12); both are consistent with hydrogen bond formation. The two structures are pure A form with all of the sugars in the north conformation. Similarly, close H2\textsuperscript{2}--O\textsuperscript{4} distances and a co-linear arrangement of C-H\textsuperscript{2}--O\textsuperscript{4} is observed in the solution structure of a 2F-ANA:RNA hybrid (Table 2; Supporting Information, Table S15). In this case, the overall structure of the duplex is intermediate between A and B forms, and 2F-arabinoses adopt the east conformation.\cite{11}

These contacts are not observed in the structure of 2F-RNA/2F-ANA duplexes, where the global structure is the B-form and the arabinoses adopt the south conformation (Table 2).\cite{11} Taking together all of these observations, we conclude that sequential FC-H-O interactions are favored in the A-form (such as 2F-RNA:2F-RNA) or A-like hybrids (such as 2F-ANA:RNA).

To address whether these FC-H-O interactions are real hydrogen bonds, we carried out quantum-mechanical calculations in dinucleotide model systems that mimic the four possible step combinations that occur in H and S, that is, 2F-RNA/2F-RNA\textsubscript{ VIDEO}, 2F-ANA/2F-ANA\textsubscript{ VIDEO}, 2F-RNA/2F-ANA\textsubscript{ VIDEO}, and 2F-ANA/2F-RNA\textsubscript{ VIDEO}. We have also included 2F-RNA/2F-RNA\textsubscript{ VIDEO}, and RNA/RNA\textsubscript{ VIDEO} dinucleotides from the high-resolution X-ray structures of a pure 2F-RNA duplex and a RNA duplex, respectively (Supporting Information, Figure S12–13 and Tables S12 and S14).\cite{9,15}

To detect potential stabilizing interactions in these systems, we analyzed the electron density of the optimized dinucleotide steps by means of the theory of "atoms in molecules".\cite{16} Values of the electron density (ρ) and the Laplacian of the electron density (∇\textsuperscript{2}ρ) for the observed BCPs in each dinucleotide step are listed in Table 2. The presence of BCPs is a necessary condition to identify stabilizing noncovalent interactions. In the case of hydrogen bonds, the electron density in the BCP of a hydrogen bond ranges from 0.002 to 0.011 atomic units (au), and the

![Figure 2](image-url)
Laplacian of the charge density is known to be positive and in the range of 0.014 to 0.139 au.[17] Values shown in Table 2 are within this range, indicating the presence of a sequential FC–H···O hydrogen bond, in all steps except 2’F-RNA/2’F-ANA$_i$. These values are in agreement with those reported for small-molecular-weight systems,[7a] and indicate that FC stabilizing than C H···O hydrogen bonds in 2’F-RNA/2’F-ANA$_i$ and 2’F-AN/A/2’F-RNA$_i$ steps are between 1 to 2 kcal mol$^{-1}$ more stabilizing than C–H···O hydrogen bonds in RNA/RNA$_i$ steps.

In conclusion, the results presented herein reveal that FC–H···O hydrogen bonds have a strong stabilizing effect on 2’-fluorinated duplexes. The detailed structural analysis of two oligonucleotides containing alternate and contiguous tracts of 2’F-RNA and 2’F-ANA shows that the disruption of FC–H···O hydrogen bonds in 2’F-RNA/2’F-ANA steps destabilize the duplex, providing a means to tune siRNA thermal stability and gene silencing activity.[12] It is worth mentioning that these FC–H···O interactions are not restricted to duplex structures, but can also provoke a strong stabilization of parallel G-quadruplexes.[18] This FC–H···O effect might be involved in the stabilization of other noncanonical structures, such as i-motifs where sugar–sugar contacts are of particular relevance.[5] Clearly, the existence of noncanonical H-bonds in these fluorinated structures appears to make a beneficial contribution to stability, providing additional insight on the structural nature of fluorinated oligonucleotides.

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