Supplementary Materials: The Effect of Small Cosolutes that Mimic Molecular Crowding Conditions on the Stability of Triplexes Involving Duplex DNA

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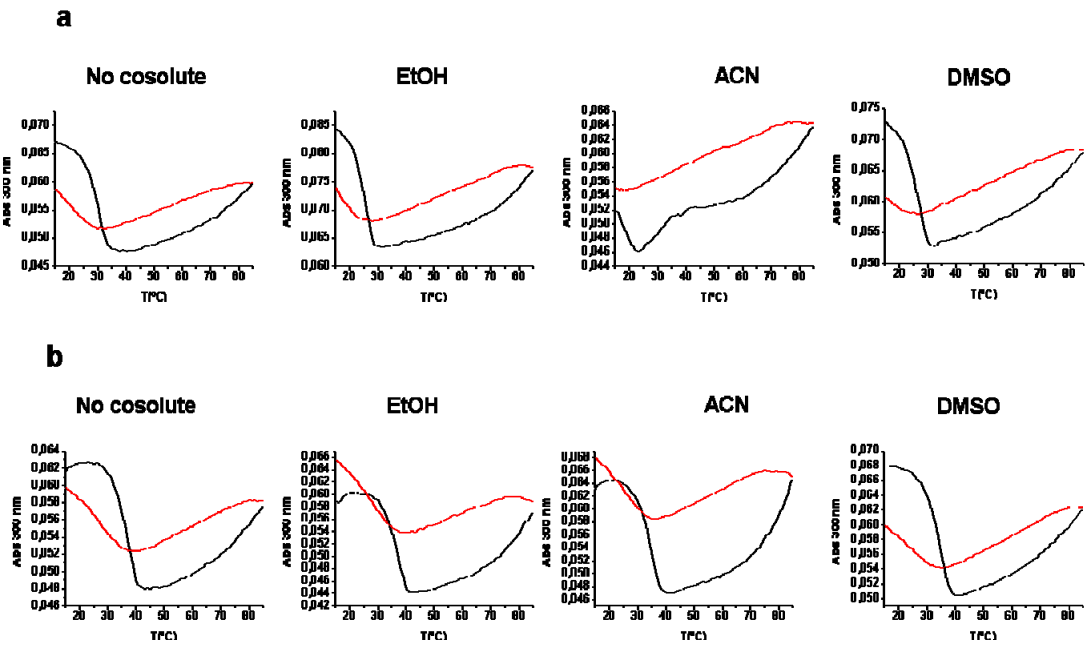

Figure S1. Melting (in black) and annealing (in red) curves of triplex formed with hairpin with (**a**) TFA DNA 2 (**b**) TFA RNA 2 in the presence of 20% (*v*/*w*) of cosolutes, 10 mM phosphate buffer 100 mM NaCl pH 6 at 295 nm.

Table S1. Melting temperatures (T_m, °C) [a] of the expected triplexes formed with hairpin 2 and TFA DNA 2 and TFO RNA 2 at 295 nm.

T_m (°C)	Expected Transition	No Cosolute	20% EtOH	20% ACN	20% DMSO
Hairpin duplex 2 + DNA TFO 2	Triplex to Duplex	26.5	24.7	18.2	26.2
Hairpin duplex 2 + RNA TFO 2	Triplex to Duplex	36.6	36.6	34.4	34.3

[a] 295 nm, 10 mM phosphate buffer 100mM NaCl pH 6 and the 20% *w*/*v* of cosolute; Heating rate: 0.5 °C/min.

Table S2. Apparent thermodynamic parameters of hairpin and the expected triplexes formed with hairpins 1 and 2 and the corresponding TFO DNAs and RNAs.

ΔH Kcal/mol	**Expected Transition**	**No Cosolute**	**20% EtOH**	**20% ACN**	**20% DMSO**
Hairpin 1	Duplex to SS	−69 ± 5	−80 ± 1	−78 ± 1	−71 ± 3
Hairpin 1 + TFO	Triplex to Duplex	−134 ± 19	–	–	–
DNA 1	Duplex to SS	−72 ± 3	−79 ± 2	−74 ± 1	−69 ± 2
Hairpin 1 + TFO	Triplex to Duplex	−150±15	−130 ± 14	−168 ± 24	−116±7
RNA 1	Duplex to SS	−73 ± 4	−80 ± 3	−71 ± 2	−73 ± 3
Hairpin 2	Duplex to SS	−77 ± 5	−81 ± 6	−71 ± 4	−72 ± 6
Hairpin 2 + TFO	Triplex to Duplex	−140 ± 3	−168 ± 4	−177 ± 5	−144 ± 5
DNA 2	Duplex to SS	−93 ± 5	−114 ± 2	−99 ± 2	−106 ± 3
Hairpin 2 + TFO	Triplex to Duplex	−147 ± 4	−130 ± 3	−83 ± 4	−109 ± 5
RNA 2	Duplex to SS	−112 ± 2	−115 ± 3	-102 ± 2	−122 ± 2
ΔS° Kcal/mol	**Expected Transition**	**No Cosolute**	**20% EtOH**	**20% ACN**	**20% DMSO**
Hairpin 1	Duplex to SS	−205 ± 15	−247 ± 3	−239 ± 4	−212 ± 9
Hairpin 1 + TFO	Triplex to Duplex	−435 ± 66	–	–	–
DNA 1	Duplex to SS	−213 ± 9	−241 ± 5	−227 ± 3	−207 ± 6
Hairpin 1 + TFO	Triplex to Duplex	−487 ± 53	−412 ± 47	−547 ± 81	−371±26
RNA 1	Duplex to SS	−215 ± 13	−243 ± 7	−213 ± 5	−218 ± 9
Hairpin 2	Duplex to SS	−230 ± 16	−249 ± 19	−215 ± 12	−214 ± 17
Hairpin 2 + TFO	Triplex to Duplex	−428 ± 10	−528 ± 13	−568 ± 16	−450 ± 15
DNA 2	Duplex to SS	−268 ± 15	−343 ± 7	−299 ± 5	−313 ± 7
Hairpin 2 + TFO	Triplex to Duplex	−446 ± 12	−393 ± 9	−244 ± 14	−328 ± 18
RNA 2	Duplex to SS	−324 ± 5	−343 ± 10	−310 ± 4	−361 ± 7
ΔG°_{37} Kcal/mol	**Expected Transition**	**No Cosolute**	**20% EtOH**	**20% ACN**	**20% DMSO**
Hairpin 1	Duplex to SS	−6.3	−3.4	−4.0	−5.2
Hairpin 1 + TFO	Triplex to Duplex	0.8	–	–	–
DNA 1	Duplex to SS	−6.5	−4.6	−3.5	−5.0
Hairpin 1 + TFO	Triplex to Duplex	1.0	−2.6	1.3	−1.5
RNA 1	Duplex to SS	−6.9	−4.7	−3.4	−5.2
Hairpin 2	Duplex to SS	−5.9	−4.3	−3.7	−5.5
Hairpin 2 + TFO	Triplex to Duplex	−7.2	−3.7	−0.6	−4.3
DNA 2	Duplex to SS	−9.4	−7.8	−5.9	−8.6
Hairpin 2 + TFO	Triplex to Duplex	−9.2	−8.9	−7.5	−7.6
RNA 2	Duplex to SS	−11.2	−8.3	−6.2	−10.0

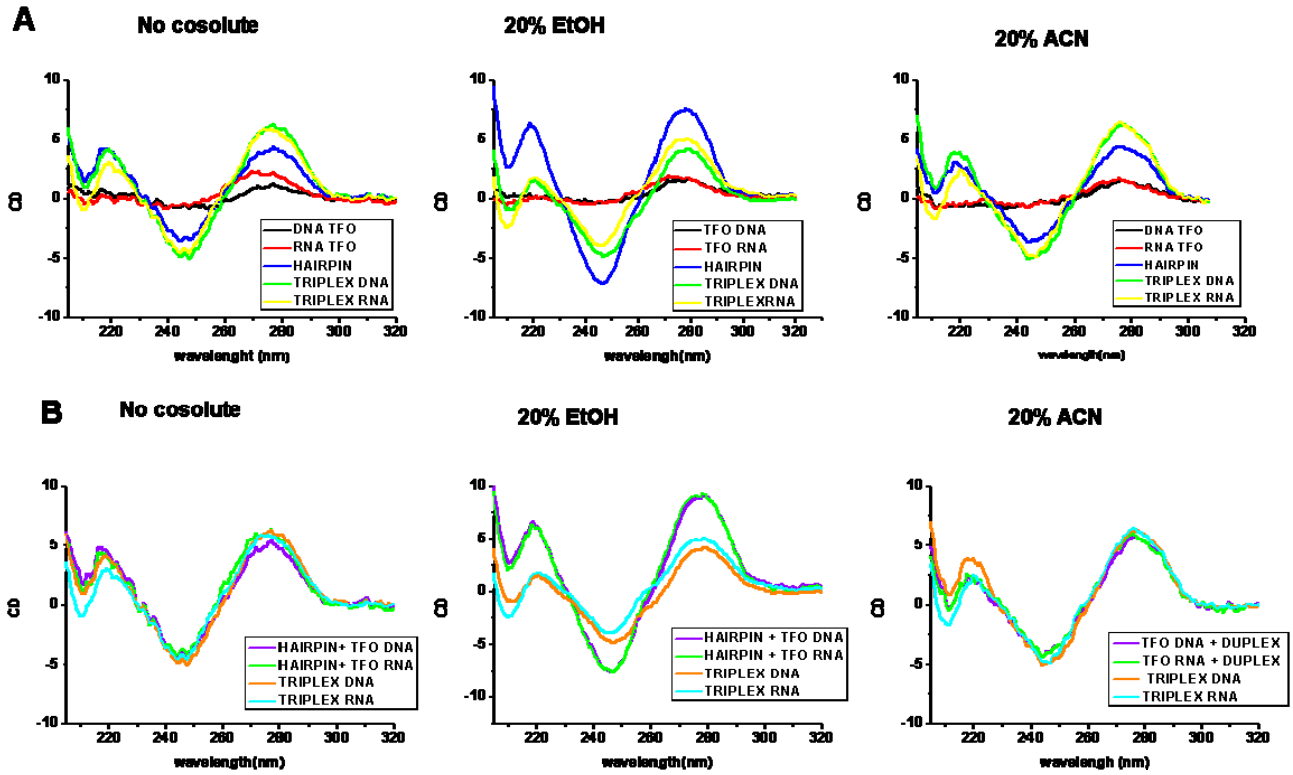

Figure S2. (**A**) CD spectra of TFOs, hairpin 1 and the triplexes formed by the addition TFOs to hairpin 1 without cosolute and in 20% EtOH and ACN; (**B**) Comparison of CD spectra of the arithmetic sum of CD spectra of hairpin 2 and TFOs and the CD spectra of the triplex structures. Buffer 10 mM phosphate buffer, 100 mM NaCl, pH 6.

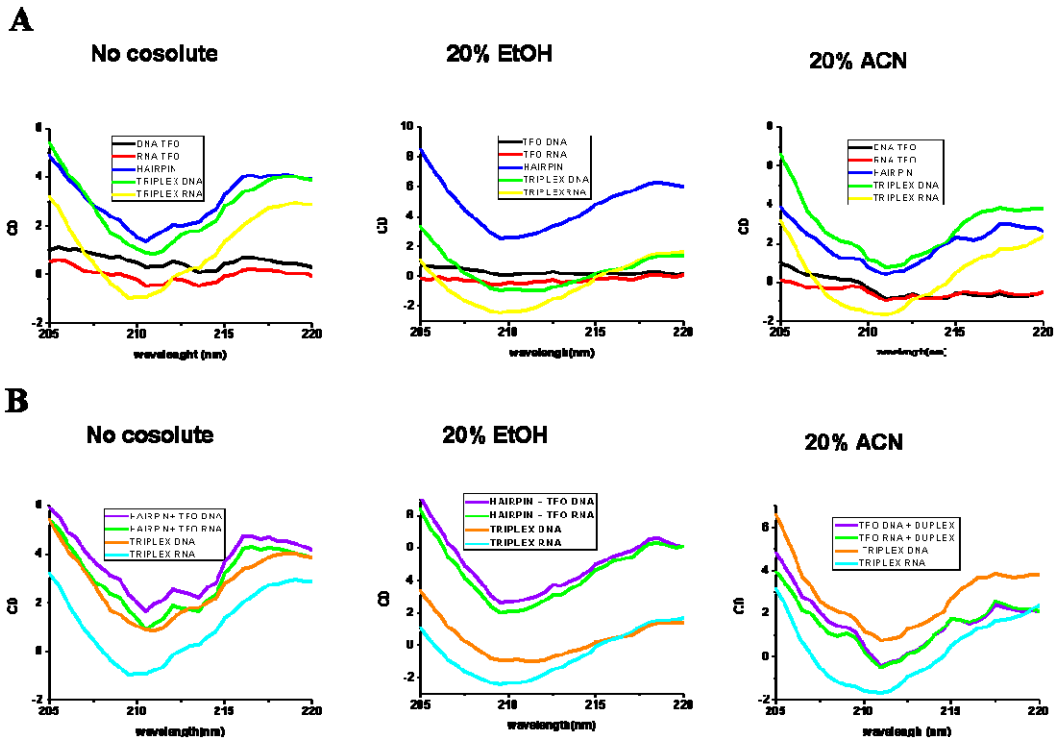

Figure S3. Amplification of the 205–220 nm region of CD spectra shown in Figure S2. (**A**) CD spectra of TFOs, hairpin 1 and the triplexes formed by the addition TFOs to hairpin 1; (**B**) Comparison of CD spectra of the arithmetic sum of CD spectra of hairpin 2 and TFOs and the CD spectra of the triplex structures.

Table S3. Oligonucleotide sequences and mass spectrometry data (MALDI) for the oligonucleotides used in this work.

Name	Sequence	M (Expected)	M (Found)
Hairpin 1	GAAAAGGAAGGA 5' (EG)6 CTTTTCCTTCCT 3'	7695.8	7691.3
TFO DNA1	5′TCCTTCCTTTTC3′	3513.4	3514.3
TFO RNA 1	5′UCCUUCCUUUUC3′	3607.4	3608.9
Hairpin 2	AGAAAAGAGGAAAGG 5' (EG)6 TCTTTTCTCCTTTCC 3'	9549.0	9549.8
TFO DNA 2	5′CCTTTCCTCTTTTCT3′	4411.0	4413.9
TFO RNA 2	5′CCUUUCCUCUUUUCU3′	4525	4528.4