SUPPLEMENTARY DATA

Engineering a DNA triple helix into an octahedral DNA nanostructure for a reversible opening/closing switching mechanism: a computational and experimental integrated study

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Oligonucleotides Section

T-cage

O1	*CCTCTT*TCCTTCTCTCGTTTGCTCTCTTCCT**TTCTCC**TCGGACCGTGATTCCATGACCTTAGAGTTGCCACCAGGTTTTTCGATGTCTAAGCTGACCGTTTTTTTC**GGAGAA**
O2	TGGCTACAGTTTTTCGGTCAGCTTAGACATCGTTTTTGAATCCTATGCTCGGACGTTTTTGGCTCACAT
O3	TCACGGTCCTTTTTCTATCCGATCGAGGCATGTTTTTCATACTGAGAGCGTTCCGTTTTTGTCATGGAA
O4	*CCTCTT*TCCTTCTCTCGTTTGCTCTCTTCCT**TTCTCC**TCCTGTAGCCAATGTGAGCCTGTCGCAGTTCAGATACGCTTTTTCATGCCTCGATCGGATAGTTTTTTTC**GGAGAA**
O5	CTCAGTATGTTTTTCGGTTACGGTACAATGCCTTTTTCGCAAGACGTTAGTGTCCTTTTTCGGAACGCT
O6	GGTGTATCGTTTTTGGCATTGTACCGTAACCGTTTTTGCGTATCTGAACTGCGACTTTTTCCACCGAAT
O7	CGTCTTGCGTTTTTGTATGACGCAGCACTTGCTTTTTCCTGGTGGCAACTCTAAGTTTTTGGACACTAA
O8	ATAGGATTCTTTTTGCAAGTGCTGCGTCATACTTTTTCGATACACCATTCGGTGGTTTTTCGTCCGAGC

LT-cage

O1	*CCTCTT*TCCTTCTCTCGTTTGCTCTCTTCC*T***TTCTCC**TC**TTTTTTT**GGACCGTGATTCCATGACTTTTTTCTTAGAGTTGCCACCAGGTTTTTCGATGTCTAAGCTGACCG**TTTTTTT**TC**GGAGAA**
O2	TGGCTACAGTTTTTCGGTCAGCTTAGACATCGTTTTTGAATCCTATGCTCGGACGTTTTTGGCTCACAT
O3	TCACGGTCCTTTTTCTATCCGATCGAGGCATGTTTTTCATACTGAGAGCGTTCCGTTTTTGTCATGGAA
O4	*CCTCTT*TCCTTCTCTCGTTTGCTCTCTTCCT**TTCTCC**TC**TTTTTTT**CTGTAGCCAATGTGAGCCTTTTTTGTCGCAGTTCAGATACGCTTTTTCATGCCTCGATCGGATAG**TTTTTTT**TC**GGAGAA**
O5	CTCAGTATGTTTTTCGGTTACGGTACAATGCCTTTTTCGCAAGACGTTAGTGTCCTTTTTCGGAACGCT
O6	GGTGTATCGTTTTTGGCATTGTACCGTAACCGTTTTTGCGTATCTGAACTGCGACTTTTTCCACCGAAT
O7	CGTCTTGCGTTTTTGTATGACGCAGCACTTGCTTTTTCCTGGTGGCAACTCTAAGTTTTTGGACACTAA
O8	ATAGGATTCTTTTTGCAAGTGCTGCGTCATACTTTTTCGATACACCATTCGGTGGTTTTTCGTCCGAGC

For the above sequences, the bases in bold represent the duplex forming portion of the switch. The underlined bases represent the random loop sequences and the italic bases represent the triplex forming portion. Red characters indicate the seven-thymidine spacers.

pH-independent cage

O1	GCCACCAGGTTTTTCGATGTCTAAGCTGACCGTCGAATATTTCCCCCCCCCCCAGAAACCTTCCTGGACCGTGATTCCATGACTTTTTCTTAGAGTT
O2	TGGCTACAGTTTTTCGGTCAGCTTAGACATCGTTTTTGAATCCTATGCTCGGACGTTTTTGGCTCACAT
O3	TCACGGTCCTTTTTCTATCCGATCGAGGCATGTTTTTCATACTGAGAGCGTTCCGTTTTTGTCATGGAA
O4	CAGATACGCTTTTTCATGCCTCGATCGGATAGTCGAATATTTCCCCCCCCCCCAGAAACCTTCCTCTGTAGCCAATGTGAGCCTTTTTGTCGCAGTT
O5	CTCAGTATGTTTTTCGGTTACGGTACAATGCCTTTTTCGCAAGACGTTAGTGTCCTTTTTCGGAACGCT
O6	GGTGTATCGTTTTTGGCATTGTACCGTAACCGTTTTTGCGTATCTGAACTGCGACTTTTTCCACCGAAT
O7	CGTCTTGCGTTTTTGTATGACGCAGCACTTGCTTTTTCCTGGTGGCAACTCTAAGTTTTTGGACACTAA
O8	ATAGGATTCTTTTTGCAAGTGCTGCGTCATACTTTTTCGATACACCATTCGGTGGTTTTTCGTCCGAGC

Non-functionalized octahedral cage

OL1	GCCACCAGGTTTTTCGATGTCTAAGCTGACCGTTTTTGGACCGTGATTCCATGACTTTTTCTTAGAGTT
OL2	TGGCTACAGTTTTTCGGTCAGCTTAGACATCGTTTTTGAATCCTATGCTCGGACGTTTTTGGCTCACAT
OL3	TCACGGTCCTTTTTCTATCCGATCGAGGCATGTTTTTCATACTGAGAGCGTTCCGTTTTTGTCATGGAA
OL4	CAGATACGCTTTTTCATGCCTCGATCGGATAGTTTTTCTGTAGCCAATGTGAGCCTTTTTGTCGCAGTT
OL5	CTCAGTATGTTTTTCGGTTACGGTACAATGCCTTTTTCGCAAGACGTTAGTGTCCTTTTTCGGAACGCT
OL6	GGTGTATCGTTTTTGGCATTGTACCGTAACCGTTTTTGCGTATCTGAACTGCGACTTTTTCCACCGAAT
OL7	CGTCTTGCGTTTTTGTATGACGCAGCACTTGCTTTTTCCTGGTGGCAACTCTAAGTTTTTGGACACTAA
OL8	ATAGGATTCTTTTTGCAAGTGCTGCGTCATACTTTTTCGATACACCATTCGGTGGTTTTTCGTCCGAGC

Unimolecular clamp-switch

CCTCTT-TCCTTCTCTCGTTTGCTCTCTTCCT-**TTCTCC**-TTTT-**GGAGAA**

Control duplex

TTCTCC-TTTT-**GGAGAA**

For the above sequences, the bases in bold represent the duplex forming portion. In the clamp-switch, the underlined bases represent the random loop sequences and the italic bases represent the triplex forming portion.

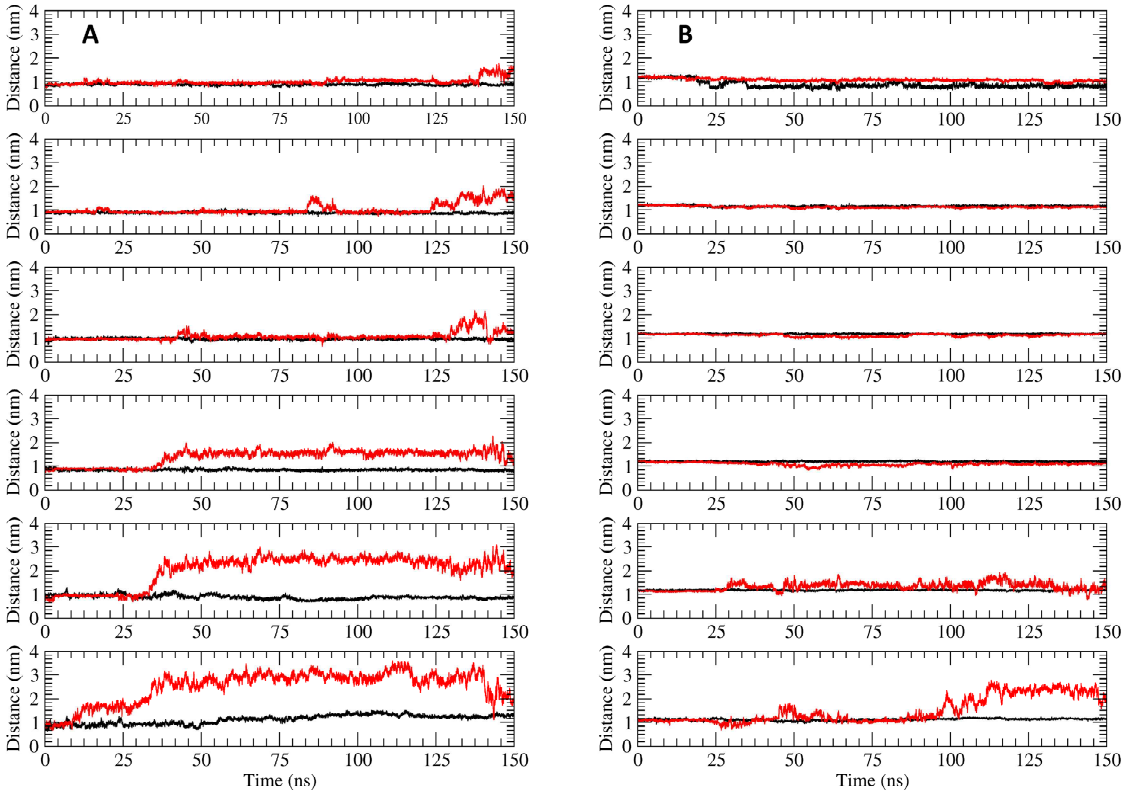

Figure S1. Time dependent evolution of base pairs distances**.** (A) Time dependence of the distance between the mass centers of the base pairs involved in the Hoogsteen interactions at pH 8.0 for the T- (black line) and LT-cage (red line). B) Time dependence of the distance between the mass centers of the base pairs involved in W-C HBs at pH 8.0 for the T- (black line) and LT-cage (red line).

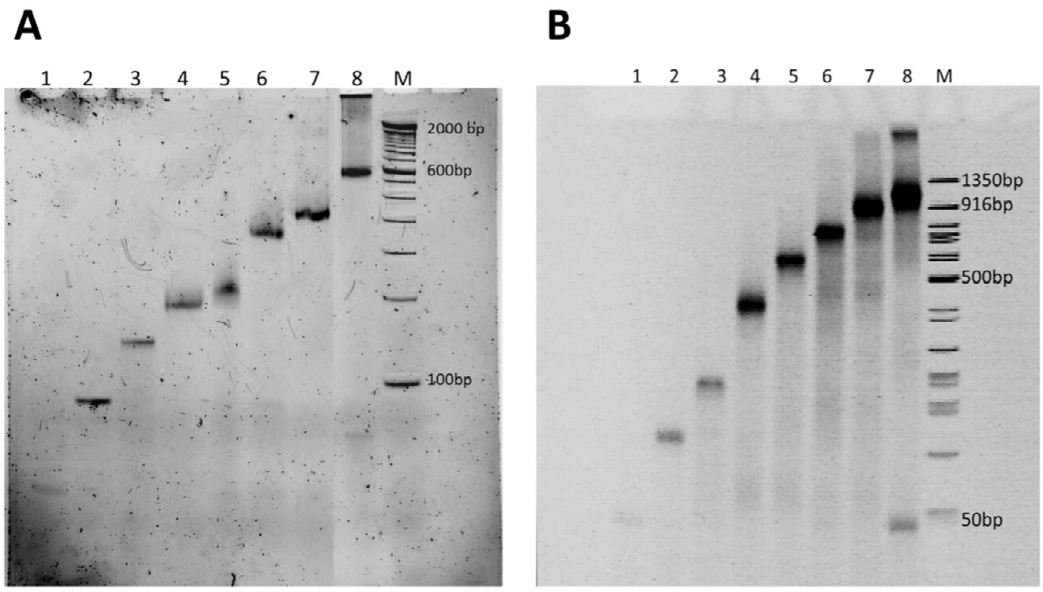

Figure S2. Gel-electrophoretic analyses of the cage assembly**.** Ladder experiments obtained increasing the numbers of DNA oligonucleotides that form the T (A) and LT (B) cages Lane M: DNA marker. Lanes 2-9 show the results of the assemby, increasing the number of oligonucleotides (i.e. O1; O1-O2; O1-O2-O3; O1-O2-O3-O4; O1-O2-O3-O4-O5; O1-O2-O3-O4-O5-O6; O1-O2-O3-O4-O5-O6-O7; O1-O2-O3-O4-O5-O6-O7-O8).

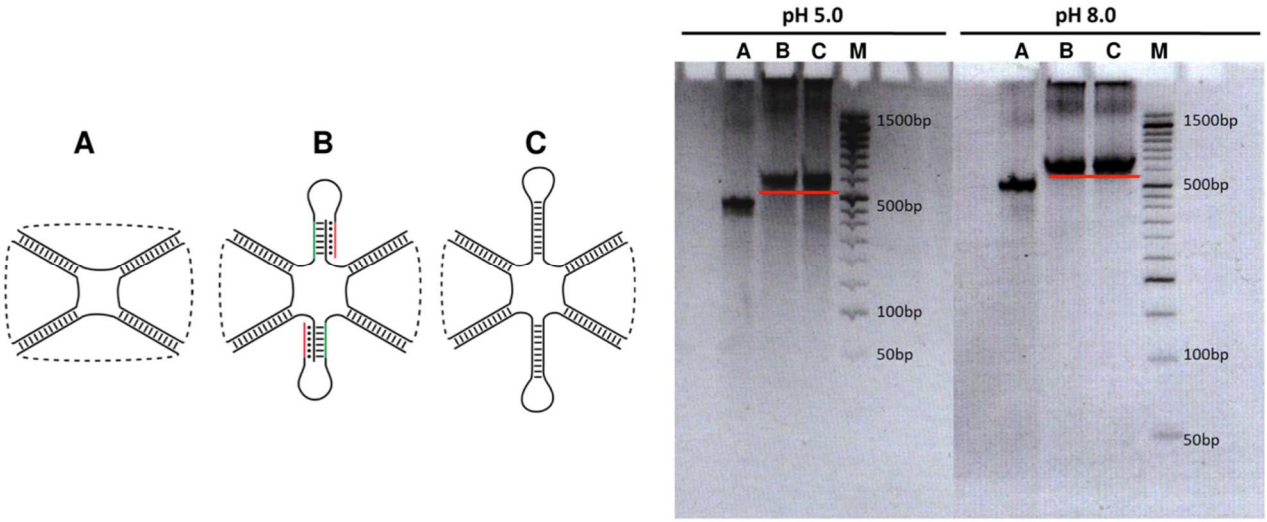

Figure S3. Gel-analysis of purified cages at pH 5.0 and 8.0. Lane M: DNA marker. Lane A: non-functionalized octahedral DNA cage, Lane B: T-cage, Lane C: pH-independent cage.

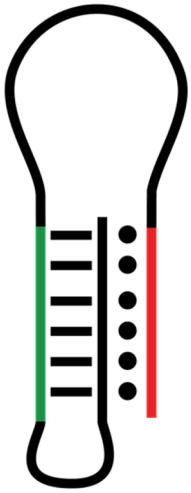

Figure S4. The isolated switch is composed by an intramolecular hairpin formed by the W-C hybridization of two six- base complementary sequences (black and green strands connected by thick lines), separated by a five bases loop. The duplex DNA is able to form a triplex structure via the formation of a second hairpin through Hoogsteen parallel interactions with the triplex forming sequence of the switch (red and black strands connected by dashes).

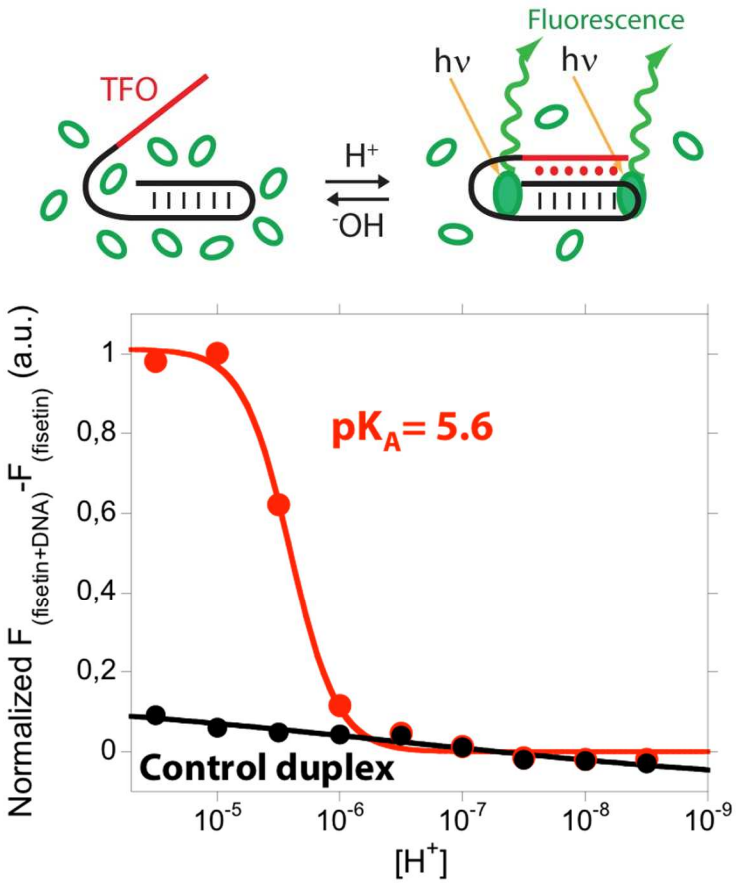

Figure S5. pH curves of the clamp triplex (red) and control duplex (black) by measuring the fluorescence fisetin signal at different pH values (see experimental section for details).