Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Data

Hybrid DNA/RNA nanostructures with 2'-5' linkages

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MATERIALS AND METHODS

Oligonucleotide synthesis

DNA strands were purchased from Integrated DNA Technologies (IDT) with standard desalting. Native RNA strands and RNA strands with 2′-5′ linkages were chemically synthesized at the 1.0 μmol scale by an automated Oligo-800 synthesizer that uses standard phosphoramidite chemistry. 3'-TBDMS-2'-CE phosphoramidites were purchased from Glen Research and used as 0.1 M solution in acetonitrile. All the other reagents are standard solutions obtained from ChemGenes Corporation. After synthesis, the oligos were cleaved from the solid support and fully deprotected with AMA (ammonium hydroxide:methylamine solution = 1:1) at 65 °C for 45 min. The amines were removed by Speed-Vac concentrator followed by Triethylamine trihydrofluoride ($Et_3N.3HF$) treatment to remove the TBDMS protecting groups. Purification of RNA strands were performed at room temperature using 15% denaturing polyacrylamide gel electrophoresis (PAGE) in 1X Tris-Borate-EDTA (TBE) buffer run for 2.5 h at 500 V. Samples were then desalted, concentrated and lyophilized before re-dissolving to RNase free water.

DNA nanostructure assembly

The component strands were annealed in 1× TAE/Mg^{2+} buffer (40 mM Tris base, pH 8.0; 20 mM acetic acid and 12.5 mM magnesium acetate) at a final concentration of 1 μM. For duplex, 4-arm junction, DX and asymmetric triangle structures, the component strands were mixed in equimolar ratios. For the symmetric triangle structure, strands 3TS1, 3TS2 and 3TS3 were mixed in 3:3:1 ratio. DNA mixtures for duplex, 4-arm junction and DX were annealed 70 °C to 20 °C over two hours in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). The tensegrity triangle was annealed from 70 °C to 20 °C by placing the tubes in 2 liters of hot water in a beaker placed in a Styrofoam box for 48 hours.

Polyacrylamide gel electrophoresis (PAGE)

Non-denaturing gels containing 6-12% polyacrylamide (29:1 acrylamide/bisacrylamide) were run at 4 °C (100 V, constant voltage) in 1× TAE/Mg^{2+} running buffer. Samples were mixed with gel loading dye containing bromophenol blue and 1× TAE/Mg^{2+} before loading on gels. Gels were stained in 1× TAE/Mg^{2+} buffer containing 0.5× GelRed (Biotium, Fremont, CA, USA). Denaturing gels (15% polyacrylamide and 8.3 M urea) were run at 20 °C (500 V, constant voltage). Running buffer consisted of 89 mM Tris, 89 mM boric acid, 2 mM EDTA (TBE). Imaging was done on a Bio-Rad Gel Doc XR+ imager using the default settings for GelRed with UV illumination. Gel

images were exported as 12-bit images and quantified using ImageJ. Quantification was done using the highest exposure image that did not contain saturated pixels in the band of interest.

UV melting experiments

Experiments were performed in a Cary 300 UV-Visible Spectrophotometer equipped with a temperature controller, using 1 μM annealed complexes. Melting curves (four cycles) were acquired at 260 nm by heating and cooling from 5 °C to 85 °C at a rate of 1 °C/min.

Circular dichroism experiments

CD spectra were collected on 5 μM samples annealed in 1× TAE/Mg^{2+}. Experiments were performed on a Jasco-815 CD spectrometer at room temperature in a quartz cell with a 1 mm path length. CD spectra were collected from 320 to 200 nm with a scanning speed of 100 nm/min and with 3 accumulations. The bandwidth was 1.0 nm, and the digital integration time was 1.0 s. All CD spectra were baseline-corrected for signal contributions due to the buffer.

RNase H assay

Annealed DX complexes (at 1 μM) were first mixed with RNase H reaction buffer (final of 1×). Dilutions of RNase H enzyme (New England Biolabs) to different units was made in nuclease-free water. For the assay, 1 μL of the enzyme was added to 10 μL of the sample containing RNase H reaction buffer. Samples were incubated at 37 °C for different time intervals. Incubated samples were mixed with gel loading dye containing bromophenol blue and 1× TAE/Mg^{2+} and run on non-denaturing PAGE to analyze degradation over time.

Molecular dynamics simulation

The initial structure for all duplexes were generated and modified with respect to 2′-5′ linkage positions using the MOE package.[1] All-atom molecular dynamics simulations were performed using GROMACS.2016[2] and the modified Chen-Garcia force field for RNA[3] including backbone phosphate modifications of Steinbrecher et al,[4] combined with AmberBSC1 for DNA parameters.[5] The parametrization regarding to 2′-5′ linkages were carried out by switching charges between O3′ and O2′ to maintain the total -1 charge of middle nucleotide consistent with the Chen-Garcia force field parameters for RNA. Each duplex was solvated with 104405 TIP4P-Ew[6] waters in a 14.7 nm cubic box and neutralized using 80 K^+ and 8 Cl^- ions using the activity-coefficient calibrated parameters of Cheatham and Joung.[7] Initial structures were minimized for 500000 steps using Steepest Descent minimization algorithm and equilibrated at 300 K and 1 atm

constant pressure with random initial velocities drawn from Boltzmann distribution. Long-range interactions were treated using the particle-mesh Ewald (PME) method. All bonds were constrained with the SHAKE algorithm. More than 36 ns were run saving 18000 coordinates every 2 ps for each simulation.

We used GROMACS to calculate the root-mean-square deviations (RMSD) of all duplexes from their corresponding initial structure. We used the average RMSD of each trajectory as a cut-off to cluster them. The Daura et al. algorithm[8] was used to perform cluster conformation analysis on all six trajectories excluding the first 10 ns. The bending angle distribution of the main cluster of each duplex was calculated by assigning 3 points, from the middle, top and bottom of each duplex to two unit vectors and finding the angle between these two vectors. Each point is representing the average of middle hydrogen bond distance in base pairs 1, 19 and 37. Chimera was used for visualization and figure representations.[9]

SUPPLEMENTARY REFERENCES

1. Molecular Operating Environment (MOE), 2019.01; Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2019.

2. Abraham,M.J., Murtola,T., Schulz,R., Páll,S., Smith,J.C., Hess,B. and Lindahl,E. (2015) GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, **1–2**, 19–25.

3. Chen,A.A. and García,A.E. (2013) High-resolution reversible folding of hyperstable RNA tetraloops using molecular dynamics simulations. *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 16820–16825.

4. Steinbrecher,T., Latzer,J. and Case,D.A. (2012) Revised AMBER Parameters for Bioorganic Phosphates. *J. Chem. Theory Comput.*, **8**, 4405–4412.

5. Ivani,I., Dans,P.D., Noy,A., Pérez,A., Faustino,I., Hospital,A., Walther,J., Andrio,P., Goñi,R., Balaceanu,A., *et al.* (2016) Parmbsc1: a refined force field for DNA simulations. *Nat. Methods*, **13**, 55–58.

6. Horn,H.W., Swope,W.C., Pitera,J.W., Madura,J.D., Dick,T.J., Hura,G.L. and Head-Gordon,T. (2004) Development of an improved four-site water model for biomolecular simulations: TIP4P-Ew. *J. Chem. Phys.*, **120**, 9665–9678.

7. Joung,I.S. and Cheatham,T.E. (2008) Determination of Alkali and Halide Monovalent Ion Parameters for Use in Explicitly Solvated Biomolecular Simulations. *J. Phys. Chem. B*, **112**, 9020–9041.

8. Daura,X., Gademann,K., Jaun,B., Seebach,D., Gunsteren,W.F. van and Mark,A.E. (1999) Peptide Folding: When Simulation Meets Experiment. *Angew. Chem. Int. Ed.*, **38**, 236–240.

9. Pettersen,E.F., Goddard,T.D., Huang,C.C., Couch,G.S., Greenblatt,D.M., Meng,E.C. and Ferrin,T.E. (2004) UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.*, **25**, 1605–1612.

Table S1. Full sequences of oligonucleotides used, written from 5′ to 3′ end. 2′-5′ linkages in RNA strands are indicated by *. 1L to 4L = 1 to 4 linkages.

Duplex: *DX-A comp + DX-A (equimolar ratio)*		
DX-A comp	AATGCTCAGGCTGTGTAATCATAGCGGCAGGATGTCG	37
4-arm junction: *DX-A + DX-B + DX-C + J-D (equimolar ratio)*		
J-D	AATGCTCAGGCTGTGTAATCATAGCGGCACCAACTGGCATGTAGTATCGTCCGATCAA	58
DX motif: *DX-A + DX-B + DX-C + DX-D + DX-E (equimolar ratio)*		
DX-A	CGACATCCTGCCGCTATGATTACACAGCCTGAGCATT	37
DX-B	TTGATCGGACGATACTACATGCCAGTTGGACTAACGG	37
DX-C	CCGTTAGTGGATGTCG	16
DX-D	AATGCTCACCGATCAA	16
DX-E	TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTGGCA	42
DX-A-RNA	CGACAUCCUGCCGCUAUGAUUACACAGCCUGAGCAUU	37
DX-A-RNA1L	CGACAUCCUGCCGCUAUG*AUUACACAGCCUGAGCAUU	37
DX-A-RNA2L	CGACAUCCUGCCGCUAUG*AU*UACACAGCCUGAGCAUU	37
DX-A-RNA3L	CGACAUCCUGCCGCUA*UG*AU*UACACAGCCUGAGCAUU	37
DX-A-RNA4L	CGACAUCCUGCCGCUA*UG*AU*UA*CACAGCCUGAGCAUU	37
3-turn symmetric (3TS) triangle: *3TS1 + 3TS2 + 3TS3 (in 3:3:1 ratio)*		
3TS1	GAGCAGCCTGTACTCGGCTTGTCGGACATCA	31
3TS2	TCTGATGTGGCTGC	14
3TS3	CCGAGTACACCGACAAGCCGAGTACACCGACAAGCCGAGTACACCGACAAG	51
3TS1-RNA	GAGCAGCCUGUACUCGGCUUGUCGGACAUCA	31
3TS1-RNA1L	GAGCAGCCUGUACUCG*GCUUGUCGGACAUCA	31
3TS1-RNA2L	GAGCAGCCUGUACUCG*GC*UUGUCGGACAUCA	31
3TS1-RNA3L	GAGCAGCCUGUACU*CG*GC*UUGUCGGACAUCA	31
3TS1-RNA4L	GAGCAGCCUGUACU*CG*GC*UU*GUCGGACAUCA	31
3-turn asymmetric (3TA) triangle: *3TS1 + 3TA2 + 3TA3 + 3TA4 + 3TA5 + 3TA6 + 3TA7 (equimolar ratio)*		
3TA2	TGCGTAGTGGCTGC	14
3TA3	GTCCTACCTGGTGAGTACGTTCAGGACGACT	31
3TA4	TCTGATGTGGTAGG	14
3TA5	CAGATACCTGATAATAGGTTGACGGACTACG	31
3TA6	ACAGTCGTGGTATC	14
3TA7	CTATTATCACCTGAACGTACTCACCACCGACAAGCCGAGTACACCGTCAAC	51

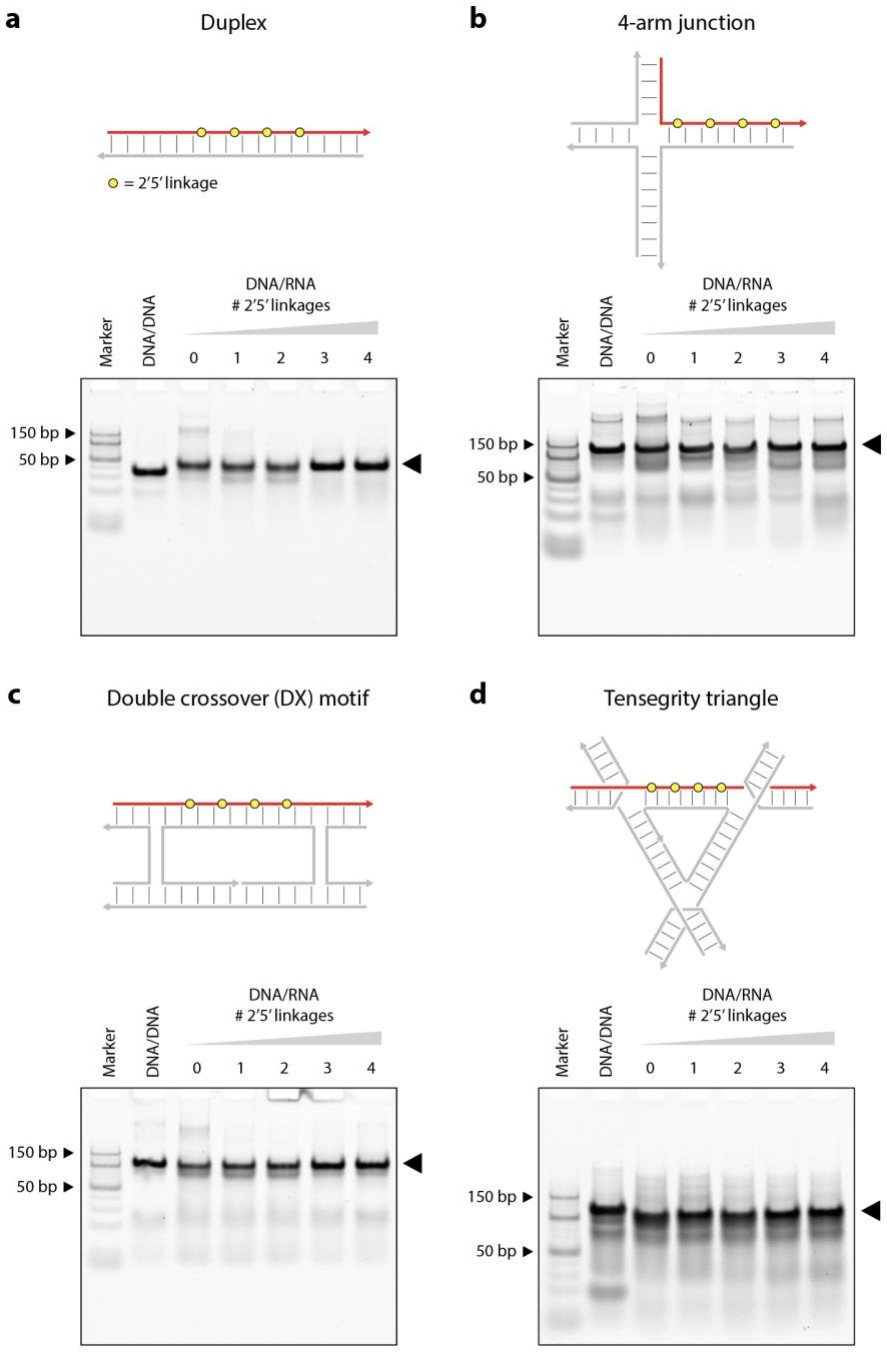

Figure S1. PAGE analysis of (a) duplex (no branch points), (b) 4-arm junction (single branch point), (c) double crossover (DX) motif (two branch points) and (d) tensegrity triangle motif (each vertex of the triangle is a junction, thus three branch points). Full gels of images shown in Figure 2b.

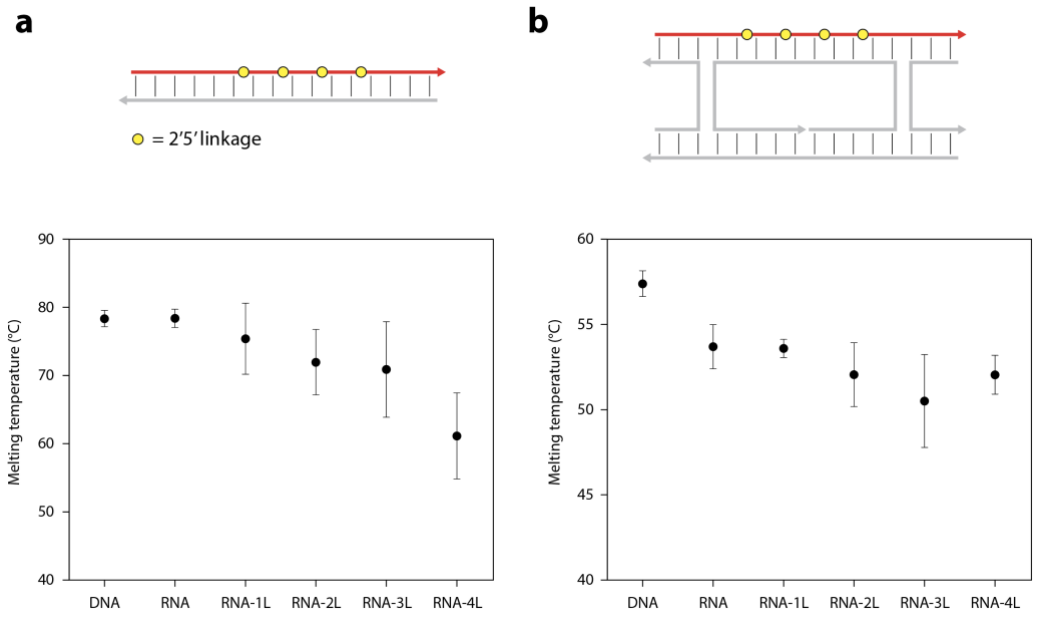

Figure S2. UV melting analysis and melting temperatures of duplex and double crossover (DX) motif containing one to four 2′-5′ linkages.

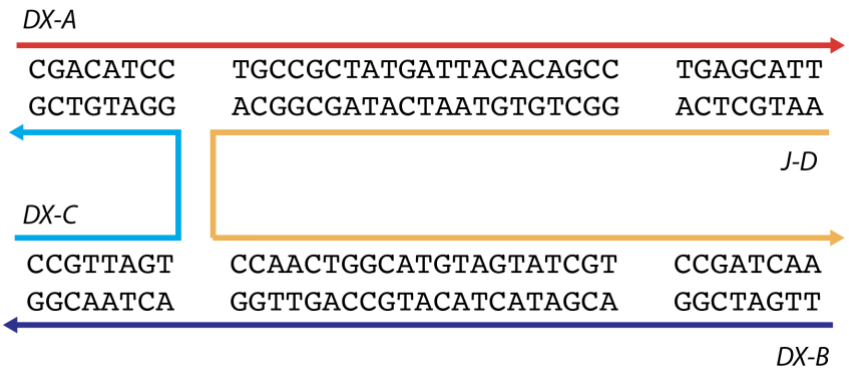

Figure S3. Strand diagram for the 4-arm junction showing strand identities. Strand DX-A was modified to be an RNA strand containing one to four 2′-5′ linkages. Arrows indicate 3′ end of strands.

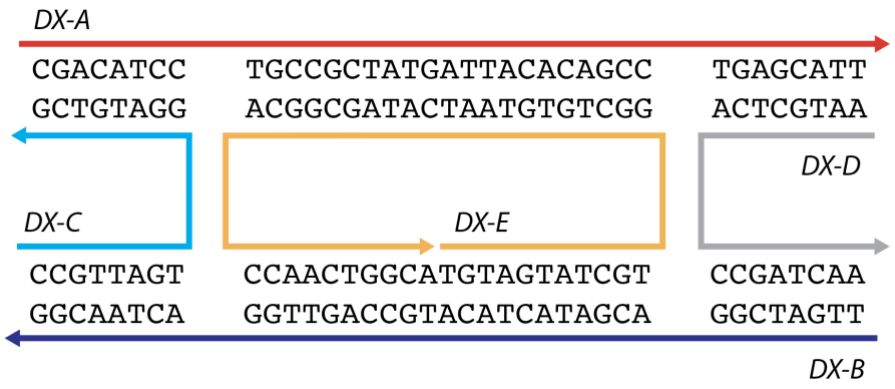

Figure S4. Strand diagram for the double crossover (DX) motif showing strand identities. Strand DX-A was modified to be an RNA strand containing one to four 2′-5′ linkages. Arrows indicate 3′ end of strands.

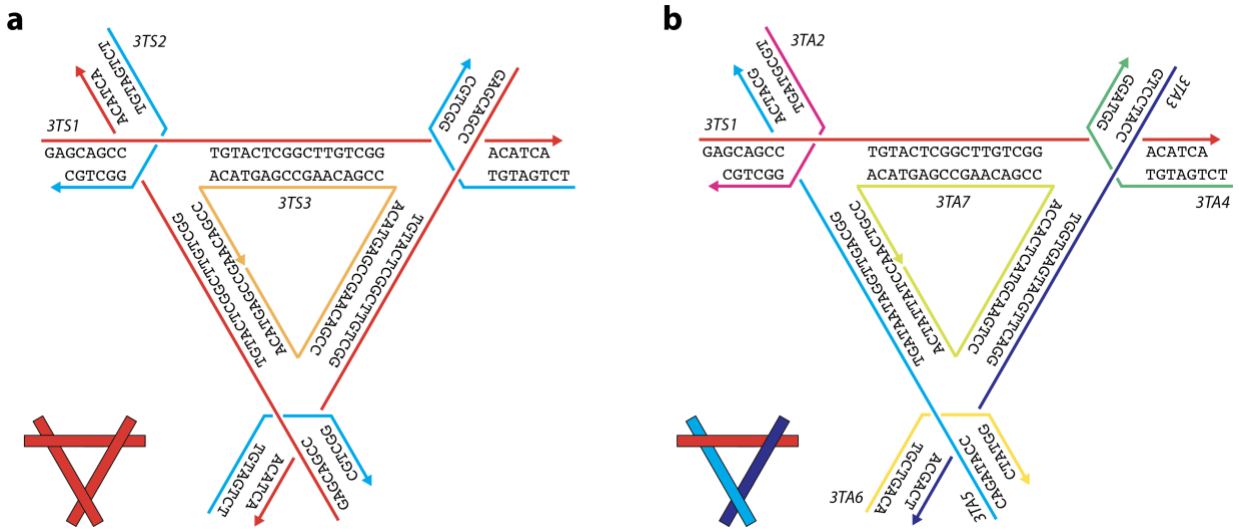

Figure S5. Strand diagram for the tensegrity triangle motif showing strand identities. (a) Symmetric motif where all three edges have the same sequences. (b) Asymmetric motif where all three edges have unique sequences. Strand 3TS1 is common to both the symmetric and asymmetric design, and was modified to be an RNA strand containing one to four 2′-5′ linkages. Arrows indicate 3′ end of strands.

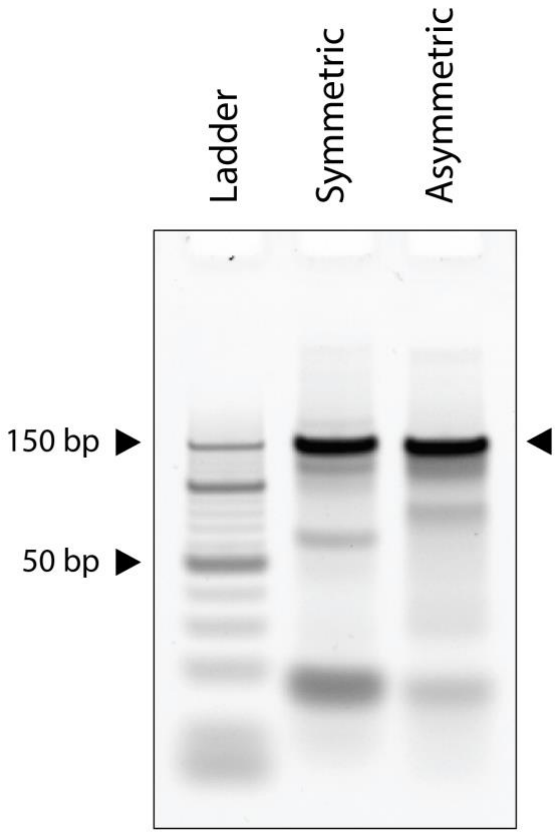

Figure S6. Formation of symmetric and asymmetric versions of the tensegrity triangle motif.

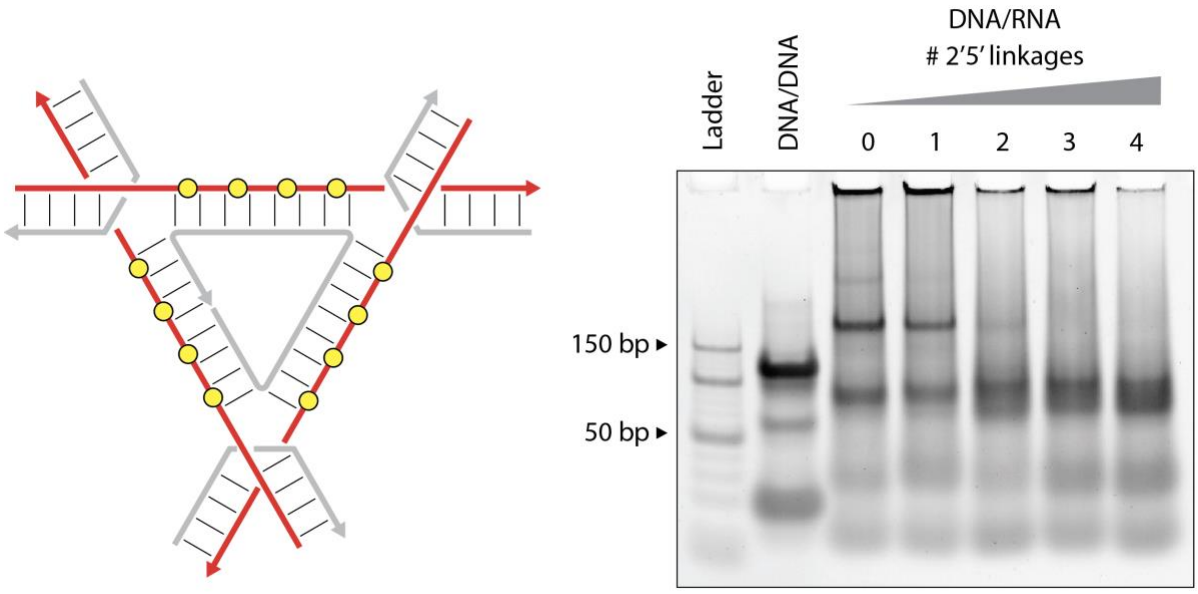

Figure S7. Symmetric tensegrity triangle motif with RNA strand containing 2′-5′ linkages.

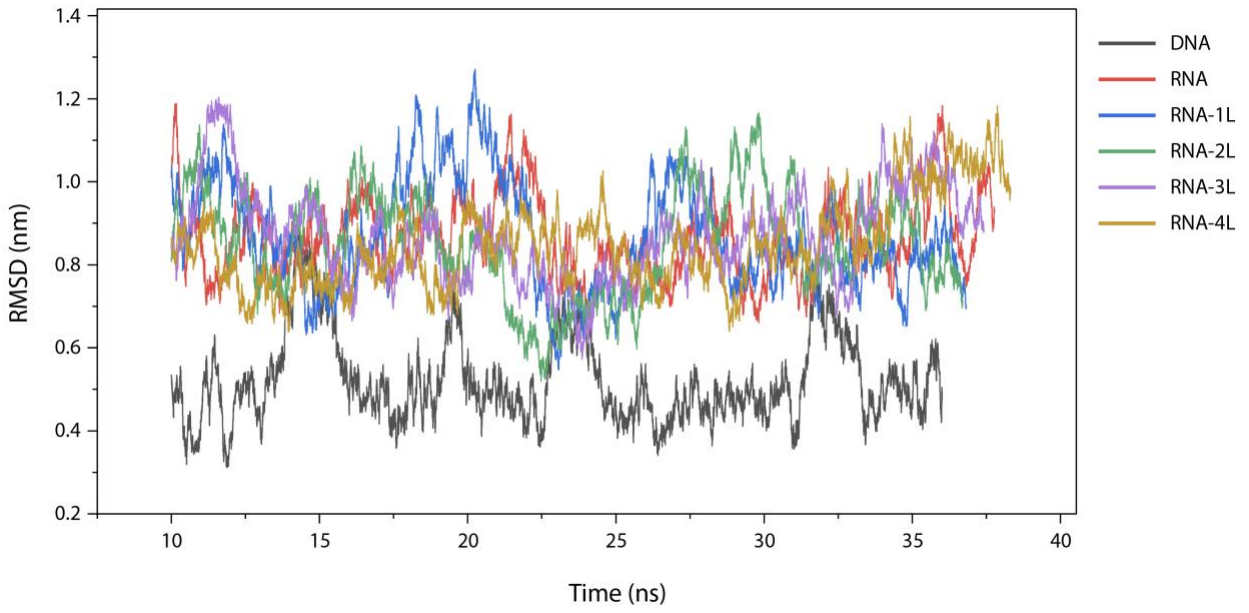

Figure S8. Time evolution RMSD of duplexes with respect to their initial structure. 1L to 4L = 1 to 4 linkages.

Figure S9. Superposition of 10 representatives of the main cluster of each duplex structure.

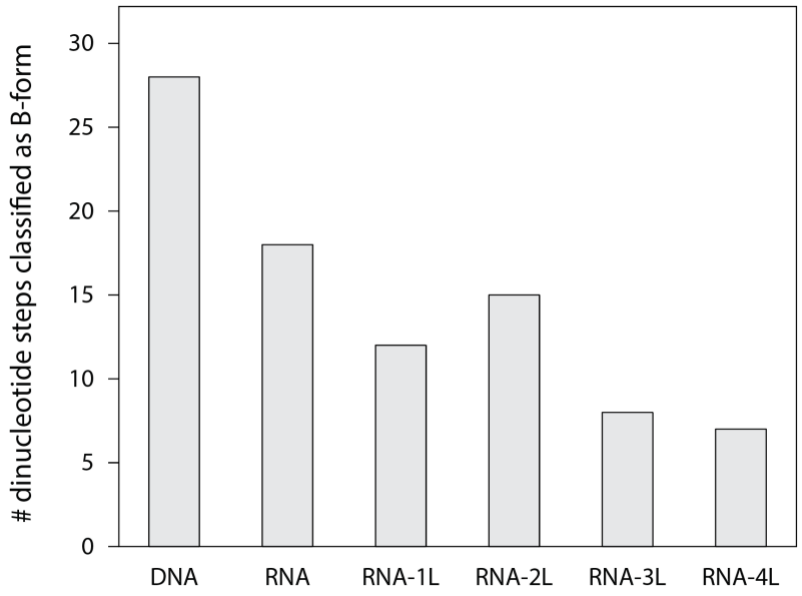

Figure S10. B-form conformational transition from DNA/DNA duplex to DNA/RNA with none to four 2′-5′ linkages.

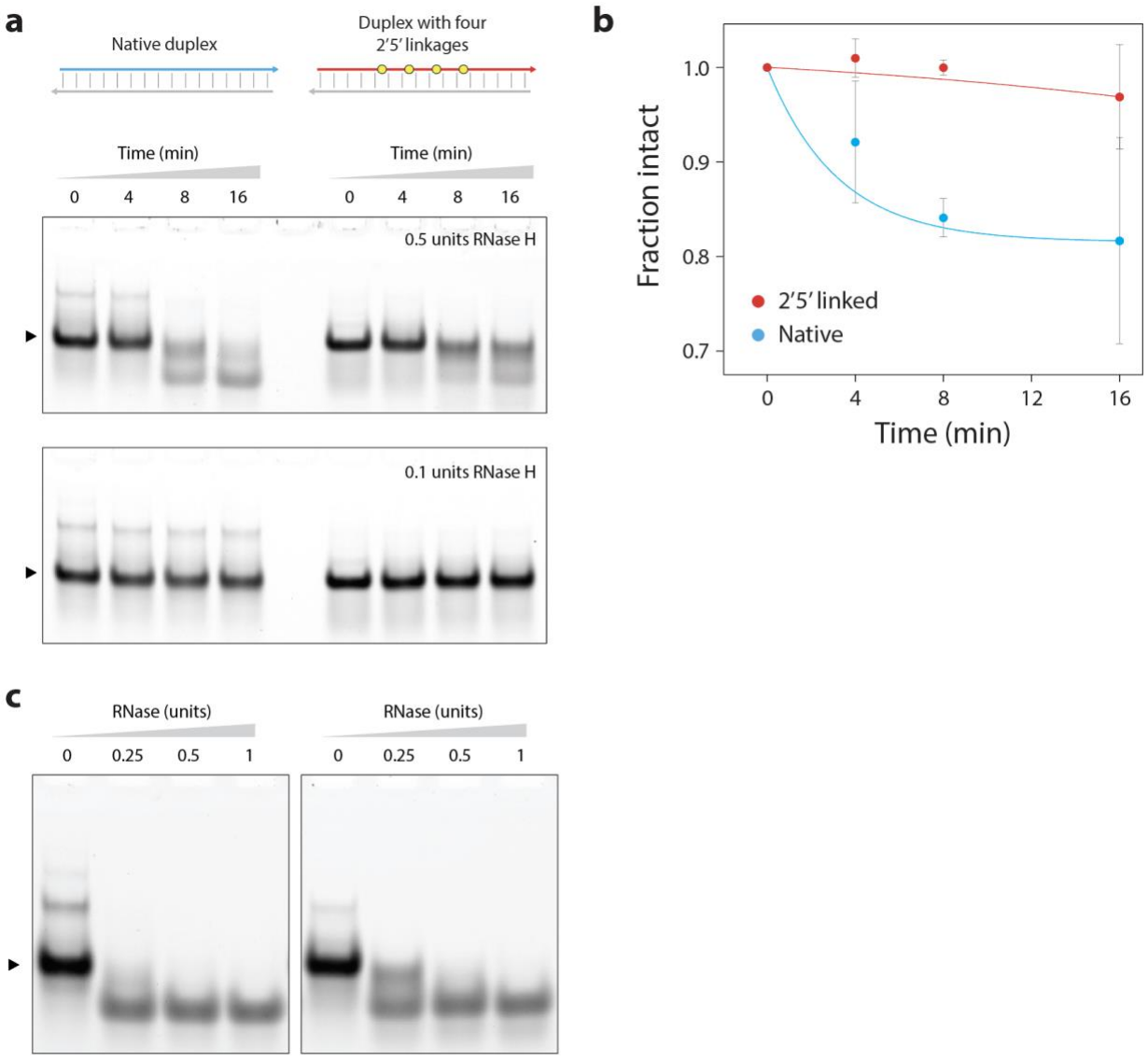

Figure S11. RNase H digestion of native and 2′-5′-linked duplexes. (a) Time series of RNase H treatment at 0.5 units (plot shown in Figure 4c) and 0.1 unit enzyme. (b) Plot of digestion with 0.1 unit RNase H. (c) Treatment of native and modified duplexes at different enzyme amounts (plot shown in Figure 4b).