Supplementary information

Building expanded structures from tetrahedral DNA branching elements, RNA and TMV protein

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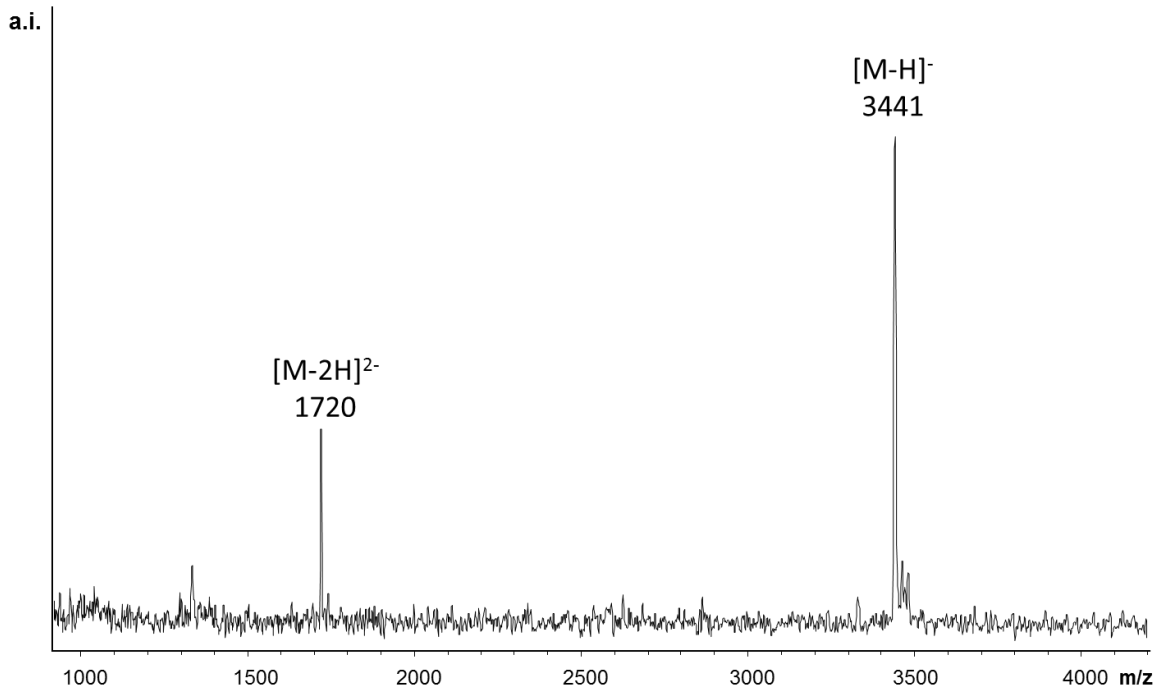

Figure S1 **MALDI-TOF mass spectrum of (5'-pCC)$_4$TBA hybrid (1).** MALDI-TOF MS: 2,4,6-trihydroxyacetophenon (THAP, 0.2 M in ethanol) and diammonium citrate (2:1), linear negative modus, calc. [M-H]-: 3441, found: 3441.

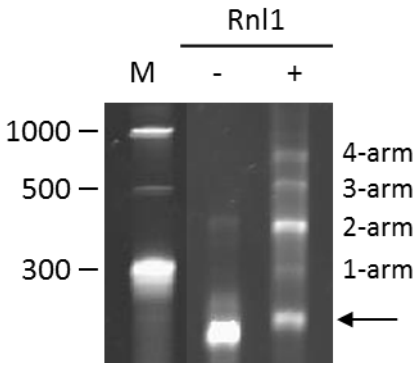

Figure S2 **Analysis of phosphorylation status of $(N_{30}CC)_4$TBA hybrid.** Denaturing gel electrophoretic separation of the products (1-arm to 4-arm) from suspensions of $(N_{30}CC)_4$ TBA hybrid with possible one to four 5'-phosphorylated DNA linker arms (arrow) and RNA oligomers without (-) or with (+) Rnl1.

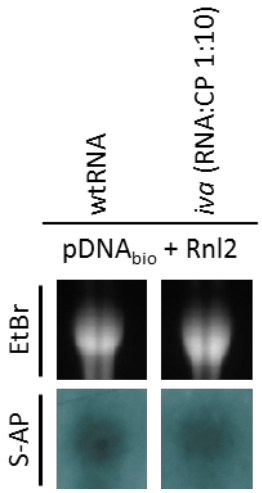

Figure S3 **Analysis of RNA termini accessibility in TLPs assembled with substoichiometric CP to RNA ratio.** $pDNA_{bio}$ could be ligated to the 3' RNA termini of bare wtRNA (wtRNA) and of TLPs partially *in vitro* assembled (*iva*) using a substoichiometric molar CP to RNA ratio of 10:1. The respective educts (bare wtRNA or TLPs assembled with substoichiometric CP to RNA ratio, respectively) were incubated with $pDNA_{bio}$ and Rnl2 in the presence of DNA splint wt_$pDNA_{bio}$. Products of each ligation reaction were purified and the RNA (potentially ligated to $pDNA_{bio}$) separated by denaturing gel electrophoresis and visualized (EtBr). Blotting and detection with S-AP revealed successful ligation.

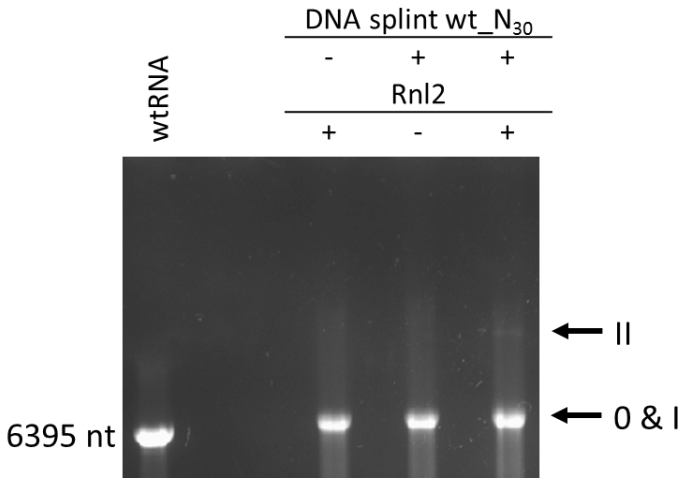

Figure S4 **Initial ligation efficiencies between 5'-phosphorylated DNA termini of $(pN_{30}CC)_4$TBA hybrids and 3' RNA termini of pre-fabricated TMV elements, yielding long-armed nucleoprotein structures under non-optimized reaction conditions.** Denaturing gel electrophoretic separation of the nucleic acid products purified from suspensions of TMV elements with protruding 3' RNA termini combined with $(pN_{30}CC)_4$TBA hybrids in the absence (-) or presence (+) of Rnl2 and DNA splints wt_N_{30}, respectively. DNA-RNA-interlinked hybrid nucleic acids from two-armed (II) products migrate with reduced velocity compared to the educt wtRNA (0). Nucleic acid-hybrid scaffolds from potential one-armed products (I) were not separated sufficiently from educt wtRNA (0) due to the small difference in size. Products with more arms were not detectable with this method.

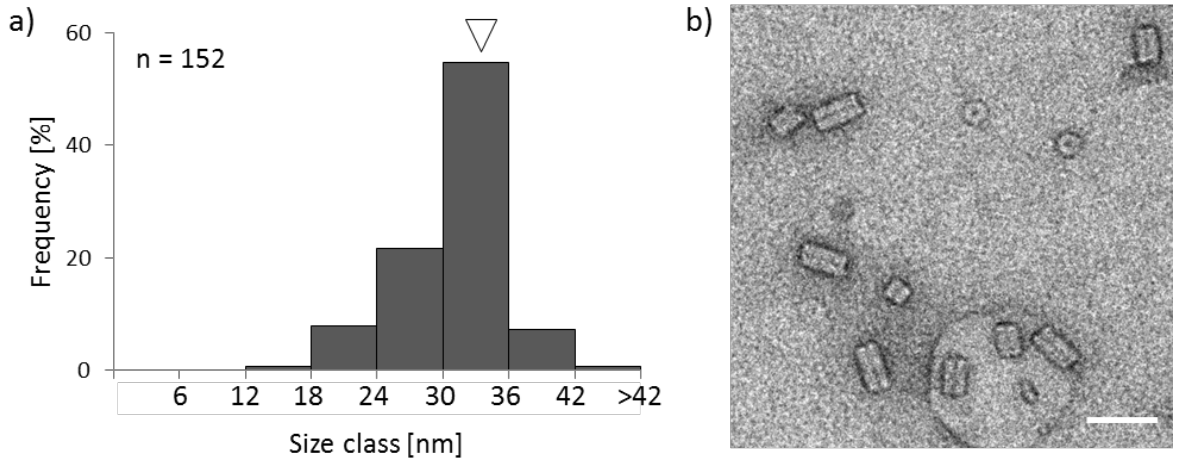

Figure S5 **Nanotube length distribution of *in vitro* assembled RNA720-based TLPs.** a) Length distribution histogram with *n* structures analysed. The triangle indicates the size class of the expected nanotube length of 34 nm; median length = 31 nm. b) Representative TEM image of the corresponding *in vitro* assembled TLPs. UAc negative stain, scale bar = 50 nm.

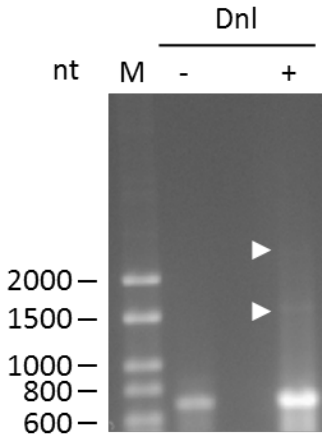

Figure S6 **DnI also catalysed the ligation of RNA720 to $(pN_{30}CC)_4$TBA hybrid.** RNA720 was incubated with $(pN_{30}CC)_4$TBA hybrid without (-) or with (+) DnI in the presence of DNA splint 720_N_{30}. In the sample that contained DnI, some product formation could be observed (arrowheads), but to a lesser extent than with Rnl2.

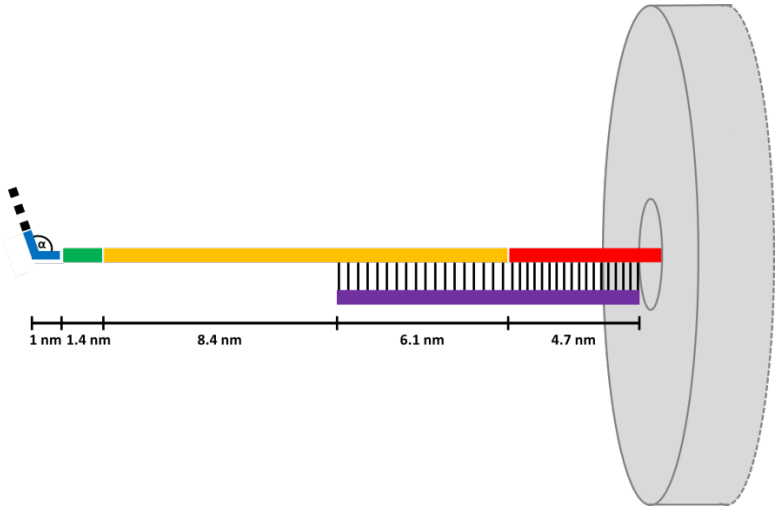

Figure S7 **Calculation of the theoretical distances between the RNA and the distinct linkage and attachment sites to the TBA core.** Indicated are the lengths of the TBA "arm" (blue) and CC dimer (green), the DNA linker's ssDNA (yellow) or dsDNA portion, respectively (yellow/purple) due to hybridization with the DNA splint (purple) as well as the length of the dsRNA/DNA hybrid (red/purple). All components, including the TMV protein disk (grey) are drawn to scale. For clarity, only one arm of the structure is shown, the dashed black line indicates a second arm. The tetrahedral angle α of 109° was used to calculate the distance between the RNAs' attachment sites determined as 35 nm, using the indicated lengths (21.6 nm in total). The distance between the centre of the TBA core and the terminal oxygen of one biphenyl arm (blue) was calculated as described in the Experimental section. The lengths of the nucleic acid strands were determined using the following values: contour length per nucleotide in the fully extended, ssDNA conformation: 0.7 nm (determined by using a random DNA sequence in the program CNS[1]); helix rise per bp for B-form dsDNA: 0.34 nm[2] and helix rise per bp for A-form dsRNA/DNA: 0.26 nm[2].

Table S1 **Sequences of nucleic acid strands used in this work.** Lower case and upper case letters denote DNA and RNA residues, respectively. Coloured underlining shows complementary sequences of DNA splints to respective nucleic acid strands. The sequence of the DNA stopper serving as toehold for potential release is indicated in italics. Groups attached to the 5' or 3' ends are indicated at the respective end. NH_2 = amino group, p = phosphate group, biotinTEG = biotin moiety connected to DNA strand via triethylene glycol (TEG) spacer.

Name	**Sequence 5' to 3'**
N_{30} DNA linker	tctttctttttttttttctttctcgttcag-NH_2
DNA splint N_{30}_CC	ggctgaacgaga
DNA splint wt_$pDNA_{bio}$	cacacagacagcttgggcccctaccg
DNA splint wt_N_{30}	gaaaaaaaaaaagaaagaagggcccctaccggg
DNA splint 720_N_{30}	gaaaaaaaaaaagaaagatatggtcgacctgcaggc
DNA stopper	*ctgacttc* gcatcttgactagctcaagttgc
$pDNA_{bio}$	p-agctgtctgtgtgtatgcca-biotinTEG
wtRNA 3' end	[…] CCCCCCGUUACCCCCGGUAGGGGCCCA
RNA720 3' end	[…] AGUGCGGCCGCCUGCAGGUCGACCAUA

Supplementary references

1. A. T. Brünger, P. D. Adams, G. M. Clore, W. L. DeLano, P. Gros, R. W. Grosse-Kunstleve, J. S. Jiang, J. Kuszewski, M. Nilges, N. S. Pannu, R. J. Read, L. M. Rice, T. Simonson and G. L. Warren, *Acta Crystallogr D*, 1998, **54**, 905–921.
2. W. Saenger, *Principles of Nucleic Acid Structure*, Springer New York, New York, NY, 1984.