Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting information

A mini DNA-RNA hybrid origami nanobrick

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Methods and materials

***In vitro* transcription of RNA scaffold.** The RNA scaffold was obtained by *in vitro* transcription from a dsDNA template (Table S1), a gift from Dr. Prashanth Rangan's lab in at SUNY Albany. Briefly, 40 ng dsDNA template was used in the T7 *in vitro* transcription by following the instruction of HiScribe™ T7 High Yield RNA Synthesis Kit (New England BioLabs, inc., E2040S). Following the transcription, 1 μl DNase I (2,000 units/ml, New England BioLabs, Inc., M0303S) was added to digest the DNA templates and primers. The obtained RNA scaffold was purified using RNA Clean & Concentrator™-5 kit (Zymo Research, Inc.).

Folding and purification of DNA-RNA hybrid origami nanostructures. Each 10 μl reaction sample contained final concentrations of 50 nM RNA scaffold, 500 nM DNA staples strands, 0.5× Tris-EDTA buffer (5 mM Tris, 1 mM EDTA, pH 7.5) and different concentrations of NaCl (0-60 mM). Samples were incubated in a 12-hour thermal annealing protocol from 65 °C to 20 °C (Table S3) in a thermal cycler (Bio-Rad, C1000). After annealing, 10 μl of the sample was mixed with 1 μl of 10× GelRed to stain the nanostructures. Before loading the gel, 2 μl of 6× loading dye was added to the sample. Then, 10 μl of the prepared sample was loaded to each well in a 2.5% agarose gel that was run in 0.5× TBE buffer at 55 V for 2 h at 4 °C. The gel bands containing the folded nanostructures were excised and chopped into small pieces by stainless steel razor blade. Then the gel pieces were transferred to a Freeze 'N Squeeze DNA Gel Extraction Spin Column (Bio-RAD) and the nanostructures were extracted by spinning down at 13000 g for 5 min. The DNA-RNA hybrid duplexes were made using the same protocol of the origami nanostructures.

Atomic force microscopy. Purified nanostructures were imaged by an Asylum MFP-3D atomic force microscope with SNL-10 tip from Bruker. First, 2 μl of 2 mM aqueous $NiCl_2$ was dropped on freshly cleaved mica and incubated for ~1 min. Then 3 μl of 0.25 nM aqueous nanostructure was mixed with 3 μL of the 2 mM $NiCl_2$ solution and placed on the mica surface and incubated for about 10 min before scanning by the tip.

Biostability assays. For RNase H assay, DNA-RNA hybrid origami and duplex control structures were first mixed with 1× RNase H buffer (final) followed by the addition of various amounts of the enzyme. Samples were incubated at 37 °C. For testing in cell culture medium, we used Dulbecco's Modified Eagle's Medium (DMEM) with high glucose (Millipore Sigma, D6429) spiked with 5% FBS and 1% L-glutamine. Annealed structures were mixed with DMEM at 1% and 5% final concentrations in a 10 μl reaction.

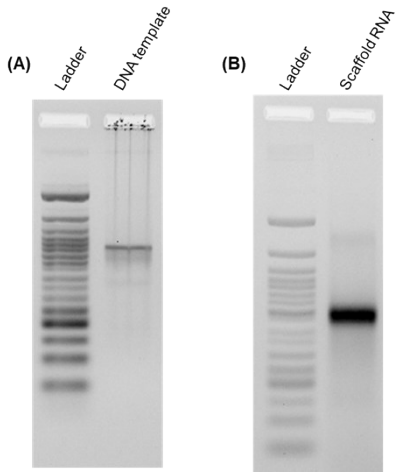

Figure S1. RNA scaffold preparation. (A) Gel image of the dsDNA template of the scaffold (2.5% agarose gel, 0.5× TBE buffer, 55 V for 1 h at room temperature). (B) Gel image of the in vitro transcribed RNA scaffold (2.5% agarose gel, 0.5x TBE buffer, 55 V for 2 h at 4 °C).

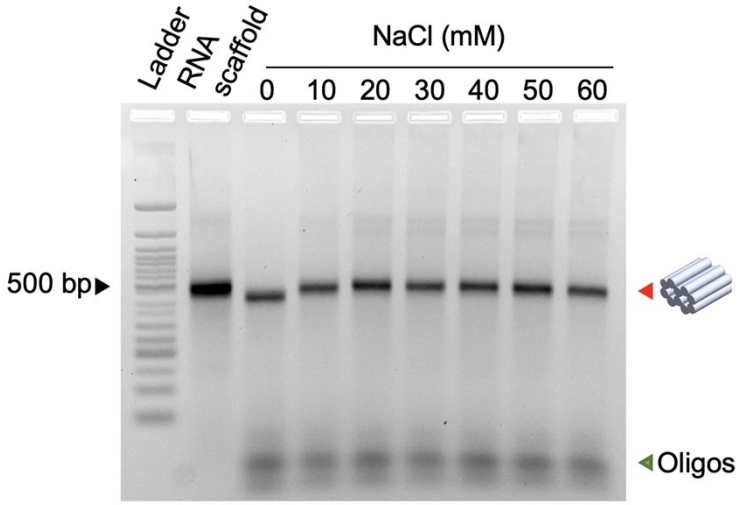

Figure S2. Agarose gel image of the nanostructure folded with different concentrations of NaCl. The red arrow indicates the bands corresponding to the folded origami structure and the green arrow indicates the excess DNA staples strands.

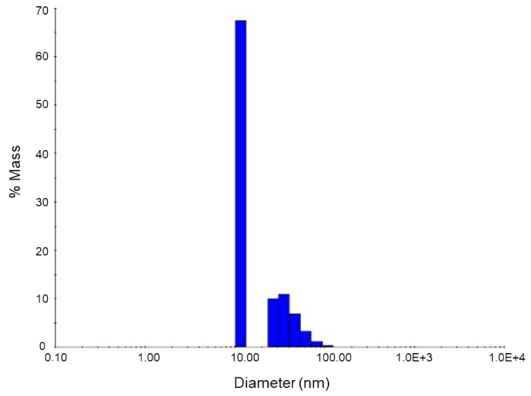

Figure S3. DLS test result of the diameter of the self-assembled origami nanostructures.

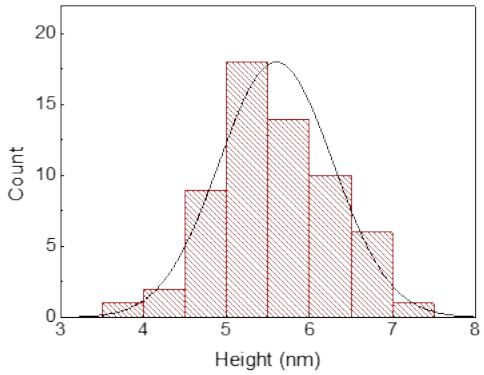

Figure S4. AFM analysis of origami nanobrick average height profiles over multiple assembled nanostructures.

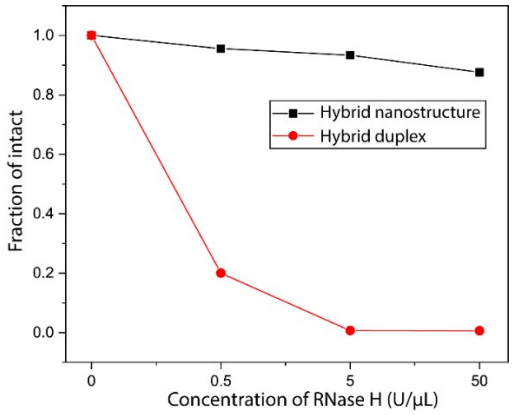

Figure S5. Analysis of the fraction of the undigested structures from the gel images shown in Figure 3A and 3B.

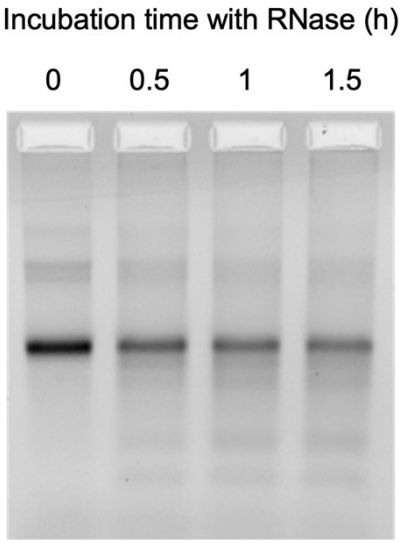

Figure S6. Full image of the RNase H digestion gel shown in Figure 3C.

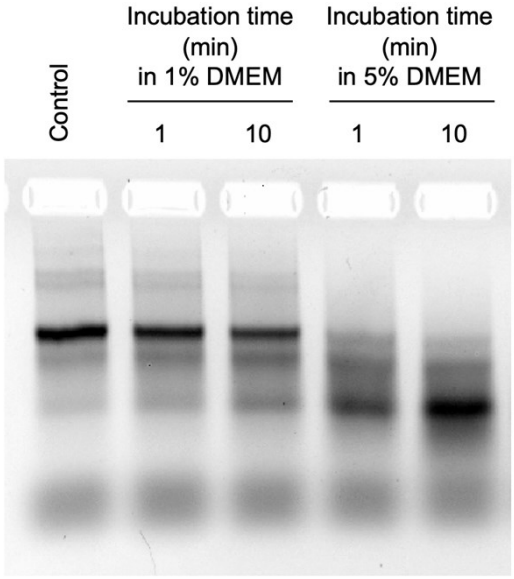

Figure S7. Stability of DNA-RNA hybrid origami in 1% DMEM cell culture media containing 5% FBS.

Table S1. Sequence of the RNA scaffold.

Name	Sequence (5' to 3')	Length
RNA scaffold	CUGGACCUCCCAAAAGCCAACUUAUUGUGAUAUUUGUAAAUUAUAGUUUUAG CAGUUCGUUUGCCACAUGAGUGGAACAUCGUGAAUGCACUUUUGAUAAGUGC UCGGUUAUUUUAUAUUGUAACUACCAGCCUUCAGAGGCGAUCGUAUGCAUAG UUUCUUGAAGUCAAUUUGUCCGUGUAUUCAAAUGUUUGCUUUCGUGAAAACU CGCAUUGUUUUGUCACUCUACCAAGUAAUCAAUUUGUACCAAUCAAUCGCAU AUGGUUGUCCUAGAUCUAAAAAUGGCAAUAAUUUGCCUUCGGUAUUGCACCU AAUGUAUUCAAGAACAAGUAGGGAAGCUCGAAAUUUCUCAAAUACUUACCCA AAAAAUAGAUAGAAAUAUAUUUUCGAUUCGCAAUCGU	401

Table S2. DNA staple strands used to fold the RNA scaffold.

Name	Sequence (5' to 3')	Length
Oligo1	CACGATGCTTATCATGTTCTTGACAACC	28
Oligo2	TATCACATCTATCTTAAGTAT	21
Oligo3	GGGAGGTATTGCGAGCTTCCC	21
Oligo4	TGCATACTGCGAGTATTACTT	21
Oligo5	CAAATTGACATTTGGCGATTG	21
Oligo6	ATATATTATAAGTTCATGTGGCAAACGAACAATATCAATACCTATTGCC	49
Oligo7	AAAACAAGATCGCCGGTAGTTACTGCTATTACAAA	35
Oligo8	AAAGCAAACTTCAACCGAGCATTCCACTGGCTTTT	35
Oligo9	TTGAGAATTAGGTGAAAATAAGAAACTA	28
Oligo10	GGTAGAGATCAAATGAAGGATATTTGGGATTTTAT	35
Oligo11	ATTGGTAGATCTAGGAATACAATTTCGAATCGAAA	35
Oligo12	ATTTTTACAAATTGTTTCACG	21

Table S3. PCR thermal annealing program for DNA-RNA hybrid nanostructure.

Step	Temperature (°C)	Description	Time (min:sec)	Cycles	ΔT (°C)
1	65	Preheating	00:30	-	-
2	65	Anneal	01:30	49 ×	-0.1
3	60	Anneal	01:30	99 ×	-0.1
4	50	Anneal	01:30	99 ×	-0.1
5	40	Anneal	01:30	99 ×	-0.1
6	30	Anneal	02:00	99 ×	-0.1
7	20	Anneal	Pause	-	-

Table S4. Complementary DNA strands used to form the single DNA-RNA hybrid duplex.

Name	Sequence (5' to 3')	Length
RNA Scaffold_comp_1	ATTTCTATCTATTTTTTGGGTAAGTATTTGAGAAATTTCGAG	42
RNA Scaffold_comp_2	CTTCCCTACTTGTTCTTGAATACATTAGGTGCAATACCGAAG	42
RNA Scaffold_comp_3	GCAAATTATTGCCATTTTTAGATCTAGGACAACCATATGCGA	42
RNA Scaffold_comp_4	TTGATTGGTACAAATTGATTACTTGGTAGAGTGACAAAACAA	42
RNA Scaffold_comp_5	TGCGAGTTTTCACGAAAGCAAACATTTGAATACACGGACAAA	42
RNA Scaffold_comp_6	TTGACTTCAAGAAACTATGCATACGATCGCCTCTGAAGGCTG	42
RNA Scaffold_comp_7	GTAGTTACAATATAAAATAACCGAGCACTTATCAAAAGTGCA	42
RNA Scaffold_comp_8	TTCACGATGTTCCACTCATGTGGCAAACGAACTGCTAAAACT	42
RNA Scaffold_comp_9	ATAATTTACAAATATCACAATAAGTTGGCTTTTGGGAGGTCCAG	44
RNA Scaffold_comp_10	ACGATTGCGAATCGAAAATAT	21