Supplementary Material (ESI) for Chemical Communications
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RNA-DNA Hybrid Origami: Folding a Long RNA Single Strands into Complex Nanostructures

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Supplementary Information

Materials and Methods

Oligonucleotides:

Forward primer (P1): 5'-ttctaatacgactcactataggtctgacagttaccaat-3' (the T7 promoter sequence is underlined.); Reverse primer (P2): 5'-gacgaaagggcctcgt-3'. The sequences for RNA scaffold and DNA staples are given in Figure S1. Both primers and DNA staple strands were purchased from Integrated DNA technology (IDT) and used without further purification.

PCR amplification of DNA template:

The dsDNA template containing T7 promoter sequence was prepared by polymerase chain reaction (PCR) with a DNA plasmid pUC19 (New England Biolabs), primers (P1 and P2), and GoTaq Flexi DNA polymerase (Promega).

In vitro RNA transcription:

AmpliScribe™ T7-Flash™ Transcription Kit (Epicentre) was used to transcribe single-stranded RNA from DNA template, following the protocol provided with the kit. Briefly, a total of 20 uL mixture, including DNA template, ribonucleotides, RNase inhibitor together with T7-Flash RNA polymerase, was prepared in the provided reaction buffer, and then incubated at 42 °C for 120 mins. Then, transcription product was treated by DNase I to remove DNA template by incubating at 37 °C for 15 mins.

Quantification of RNA scaffold:

Purified RNA strands were quantified by a UV-Vis spectrophotometer. RNA strands without purification after in vitro transcription were indirectly quantified through agarose gel electrophoresis by using purified RNA as the calibrator. 1 μg of purified RNA was loaded into the gel, and unpurified RNA with known volume was loaded into the same gel. To compare the intensity of the two bands after staining with EtBr, the concentration of unpurified RNA could be obtained relatively based on the purified RNA with known concentration.

Regular assembly of RNA-DNA hybrid origami:

10 nM of RNA scaffold was mixed with DNA staples (at a ratio of 1:10 or specifically indicated value) in 1×TAE/Mg^{2+} buffer, which contains 40 mM Tris base (pH 8.0), 20 mM Acetic acid, 2 mM ethylenediamine tetraacetic acid (EDTA), and 12.5 mM $Mg(OAc)_2$. The mixed aqueous solution was incubated under the following protocol: 10 mins each at 65, 50, 37 and 25 °C. All RNA-DNA hybrid origami structures were assembly by this method unless specifically indicated by isothermal assembly.

Isothermal assembly of RNA-DNA hybrid origami:

10 nM of RNA scaffold was mixed with DNA staples (at a ratio of 1:10) in 1×TAE/Mg^{2+} buffer, which contains 40 mM Tris base (pH 8.0), 20 mM Acetic acid, 2 mM ethylenediamine tetraacetic acid (EDTA), and 12.5 mM $Mg(OAc)_2$. The mixed aqueous solution was incubated at one specified temperature for a specified duration. The assembly process was cryogenically stopped by being quenched into dry ice.

RNase H treatment

50 μL RNA-DNA hybrid origami sample (assembled from 10 nM RNA and 100 nM DNA staples) was incubated with 5 units of RNase H (New England Biolabs) in the provided RNase H buffer for 30 mins at 37 °C.

Agarose gel electrophoresis:

1% (for PCR amplification and RNA transcription characterization) and 2.5% (for RNA-DNA hybrid origami characterization) agarose gels were prepared with 1×TBE buffer (89 mM Tris base, 2 mM EDTA, 89 mM Boric acid) and run in the same buffer under constant voltage 8 V/cm. The gels were stained with EtBr solution and then visualized under a UV illuminator.

AFM imaging:

5 uL of RNA-DNA hybrid origami sample was deposited onto freshly cleaved mica for 20 seconds, washed with 30 uL of water and then dried with compressed air. A MultiMode 8 AFM (Bruker) was used to image the samples under ScanAsyst-Air mode, using a ScanAsyst-Air probe (Bruker).

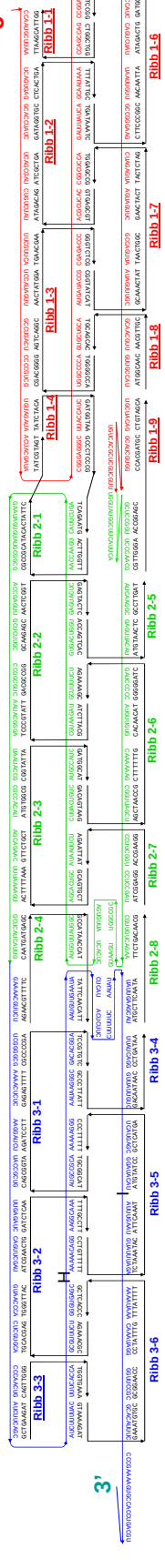

Figure S1. Detailed design of the ribbon origami. Colored strand is the RNA scaffold strand and black strands are DNA staple strands.

Figure S2. Detailed design of the rectangle origami. The red strand is the RNA scaffold strand and black strands are the DNA staple strands.

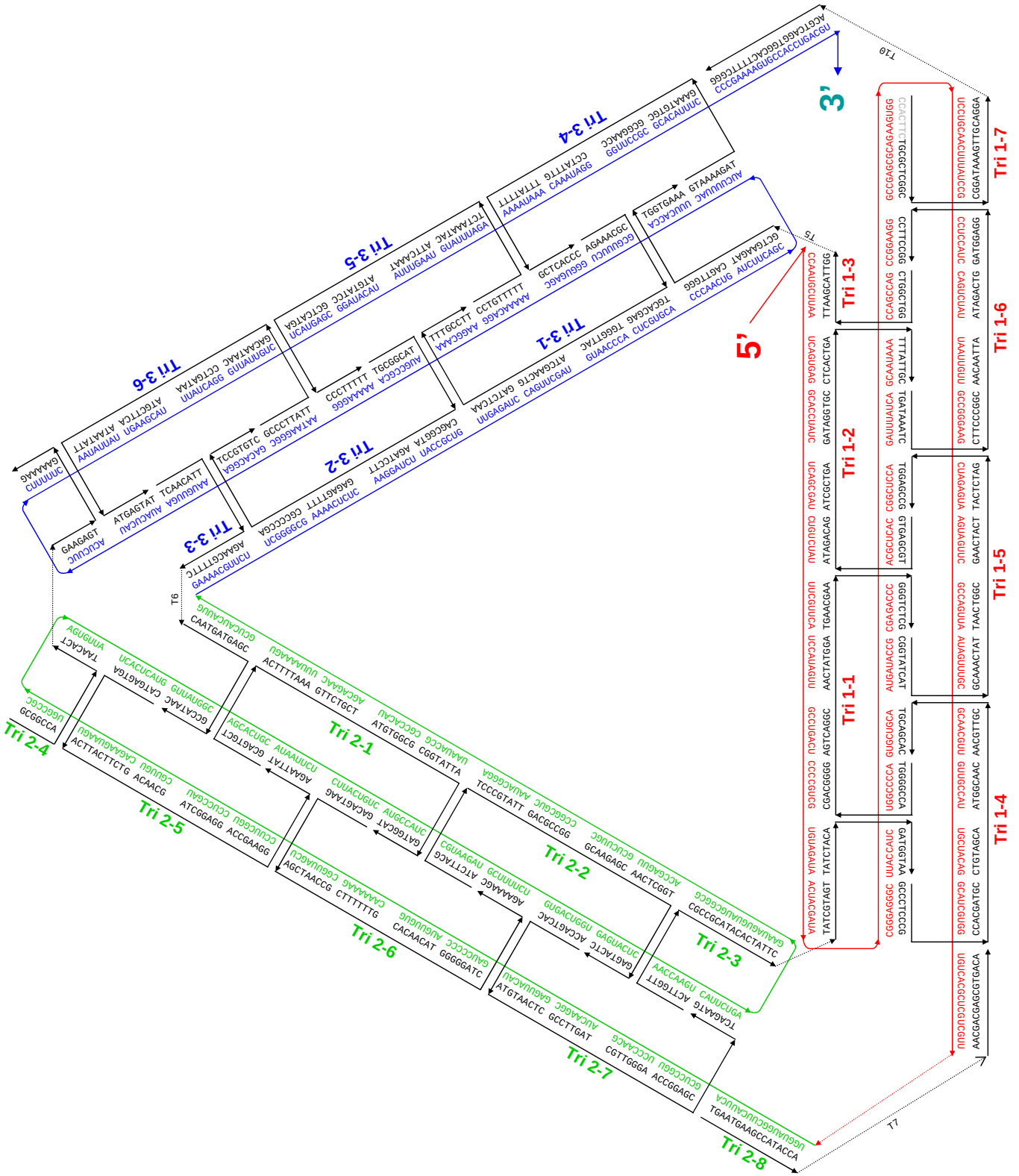

Figure S3. Detailed design of the triangle origami. Colored strand is the RNA scaffold strand and black strands are DNA staple strands.

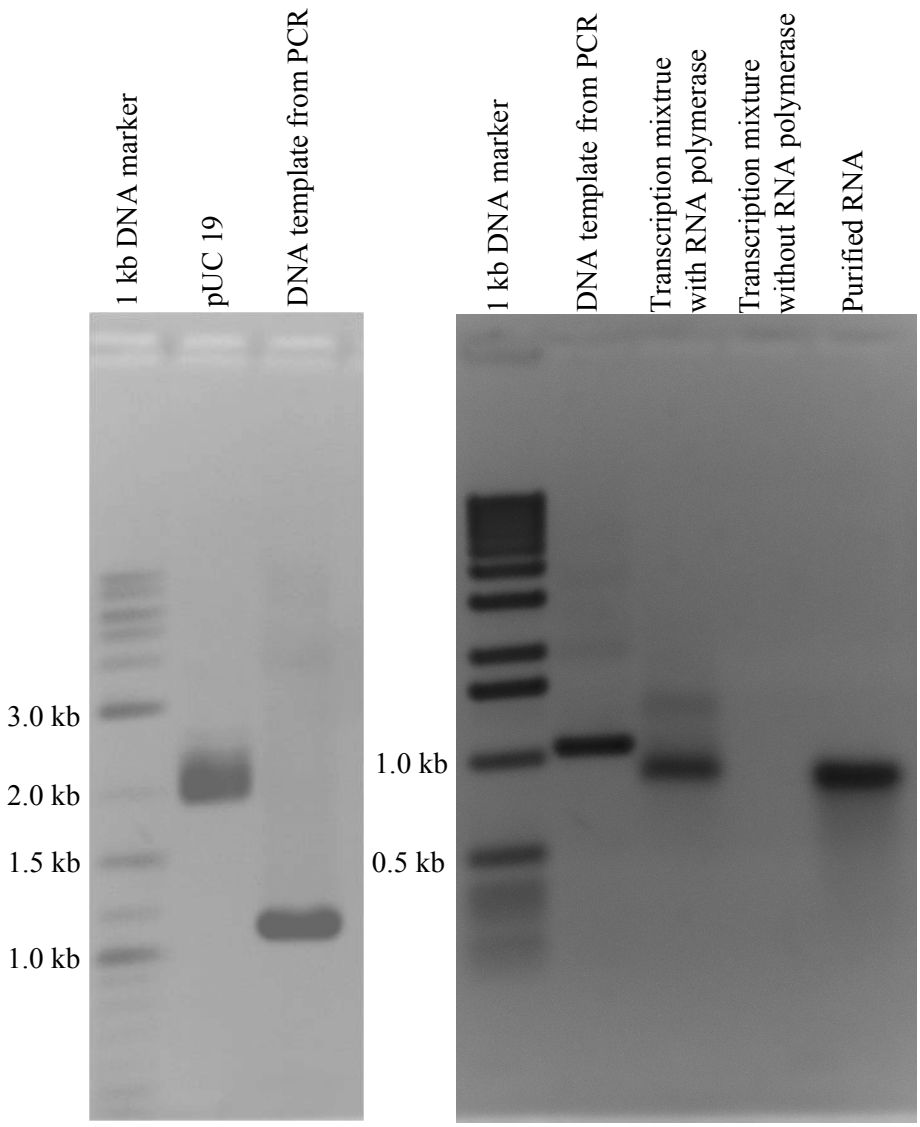

Figure S4. Agarose gel analysis of the preparation of DNA template, RNA scaffold strand, and RNA-DNA hybrid origami structures. The RNase H experiments further confirm that the origami structures are hybrid assembly of RNA scaffold and DNA staples.

Table S1. Yield quantification of three origami structures (All counts are the observed objects in AFM images)

Origami structures	Length (nm)	Yield (%)	Total count (N)
Ribbon	70.3±11.4	67.3	401
Rectangle	NA	60.1	642
Triangle	NA	77.8	771

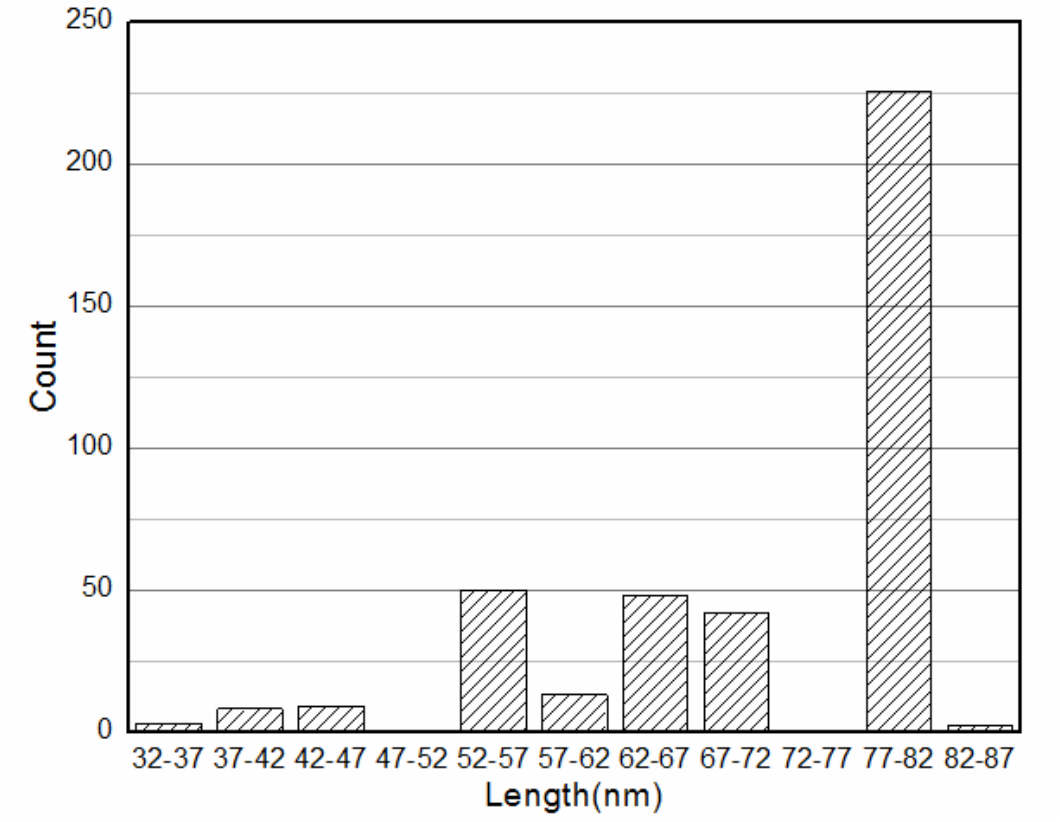

Figure S5. Length distribution of the ribbon origami measured from AFM images. Note that ribbons can stack onto each other and leads to ribbons longer than the expected value, 77 nm.

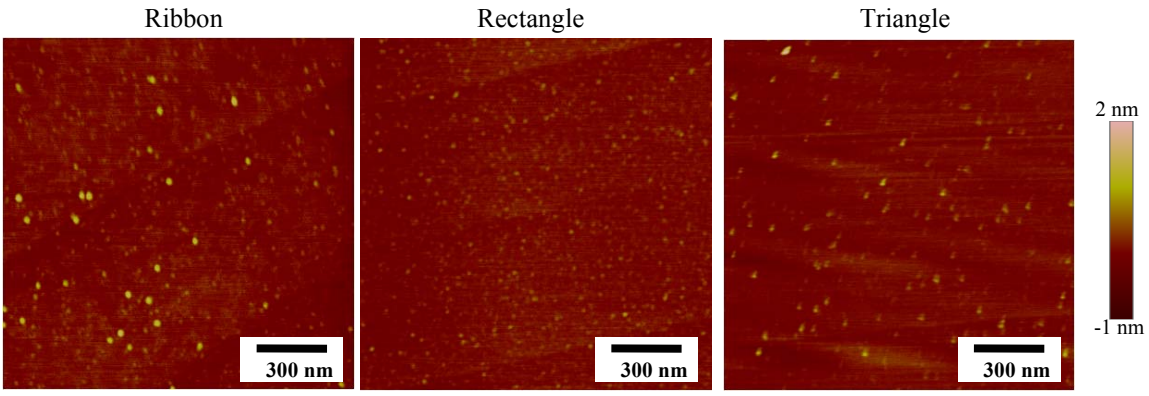

Figure S6. A negative control: assemblies from the transcription mixture without T7 RNA polymerase. No origami structure has been observed. This experiment confirms that the reported origami structures are indeed from RNA scaffolds instead of potential DNA template contaminations.

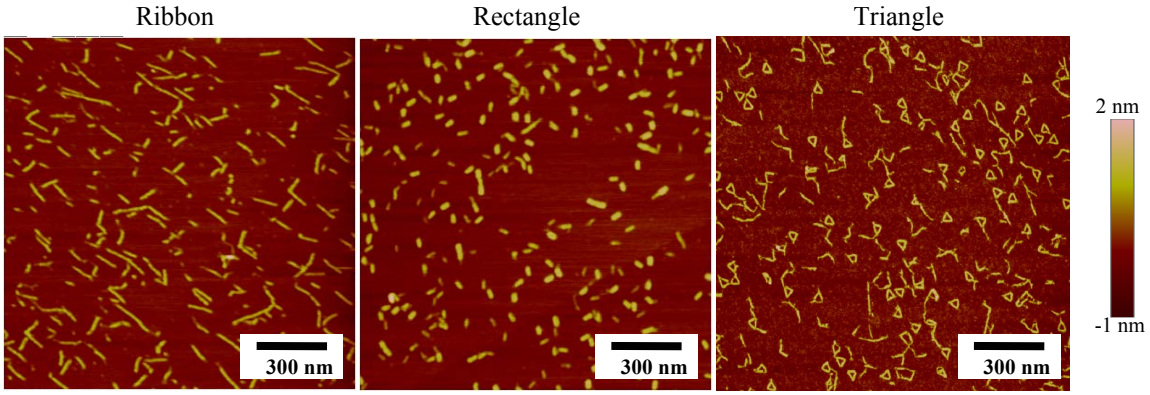

Figure S7. RNA origami assembled from purified RNA strand.

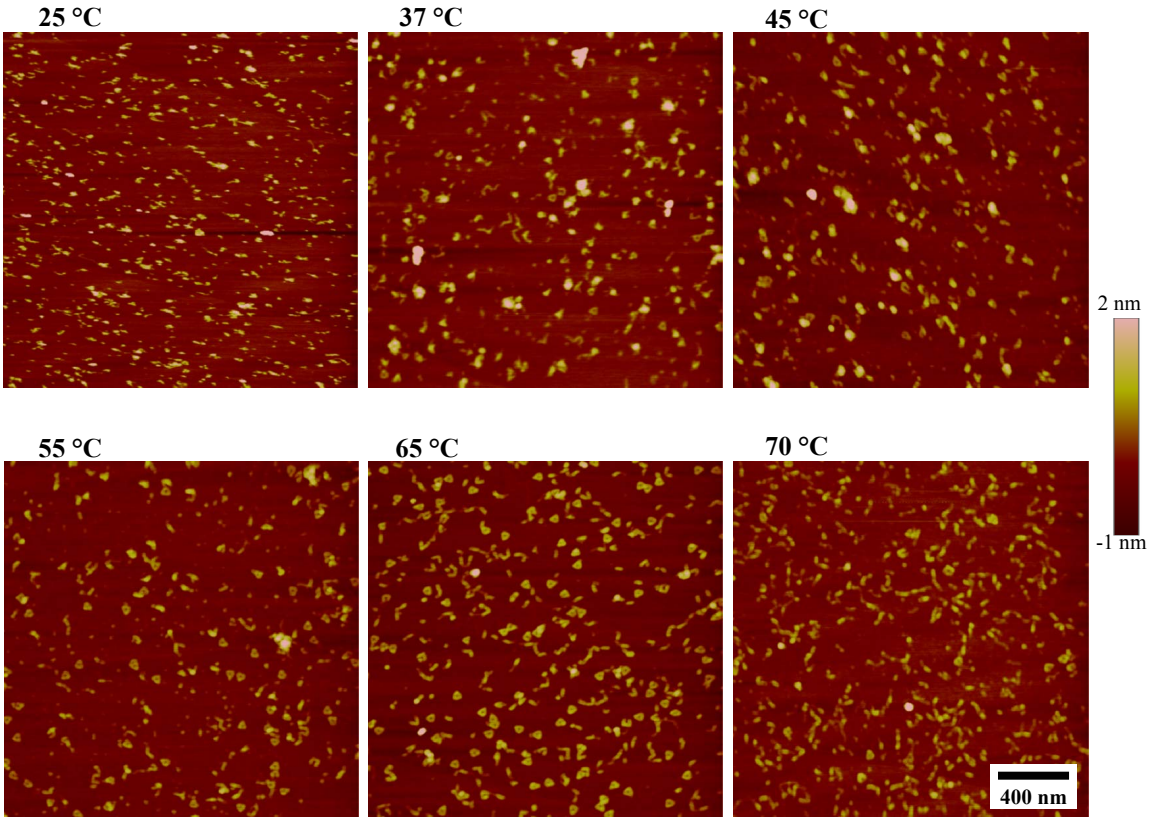

Figure S8. Isothermal assembly of the triangle origami at different temperature for 1 hour incubation (RNA:DNA = 1:10).

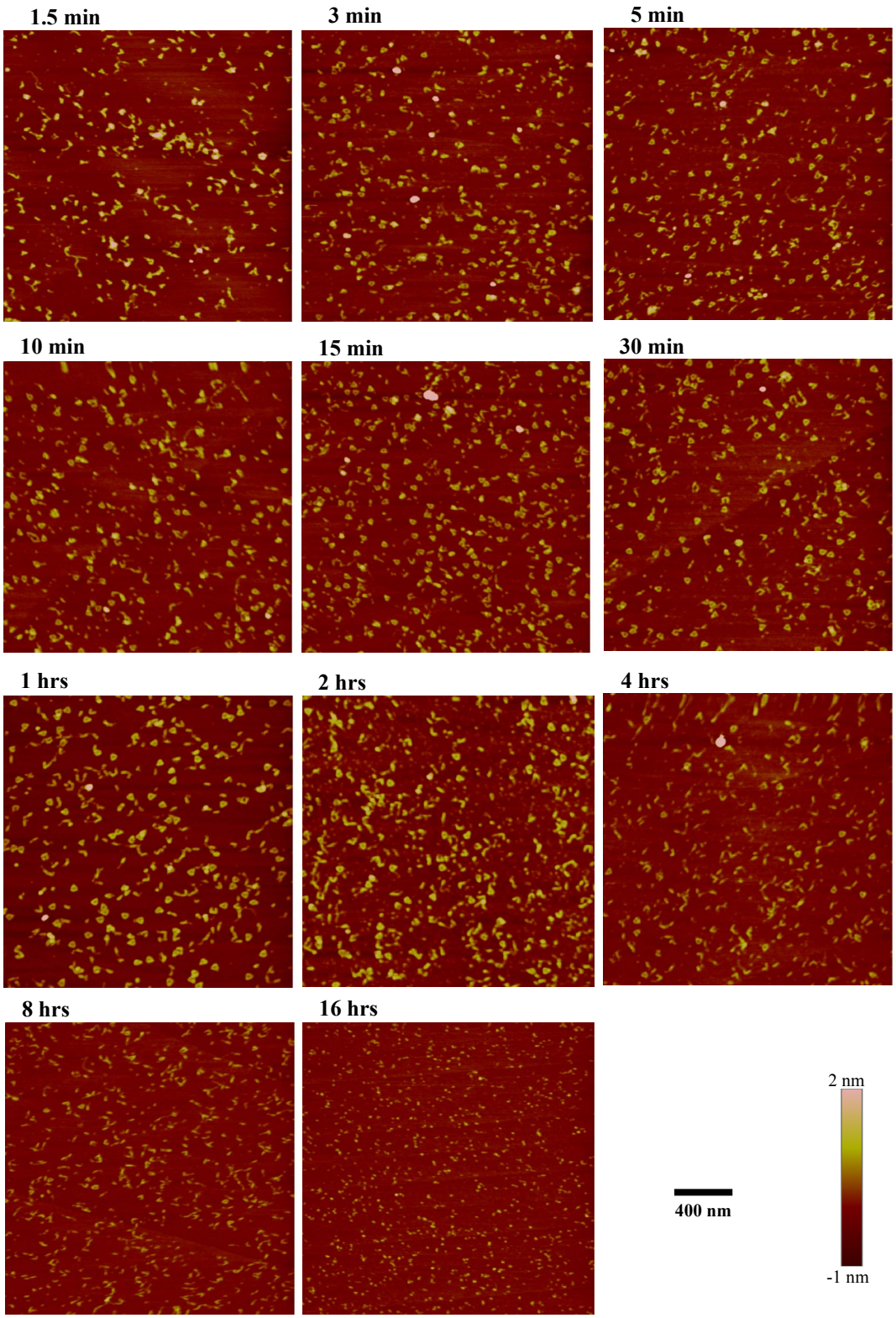

Figure S9. Isothermal assembly of triangle origami at 65 ºC for different incubation time (RNA:DNA = 1:10).

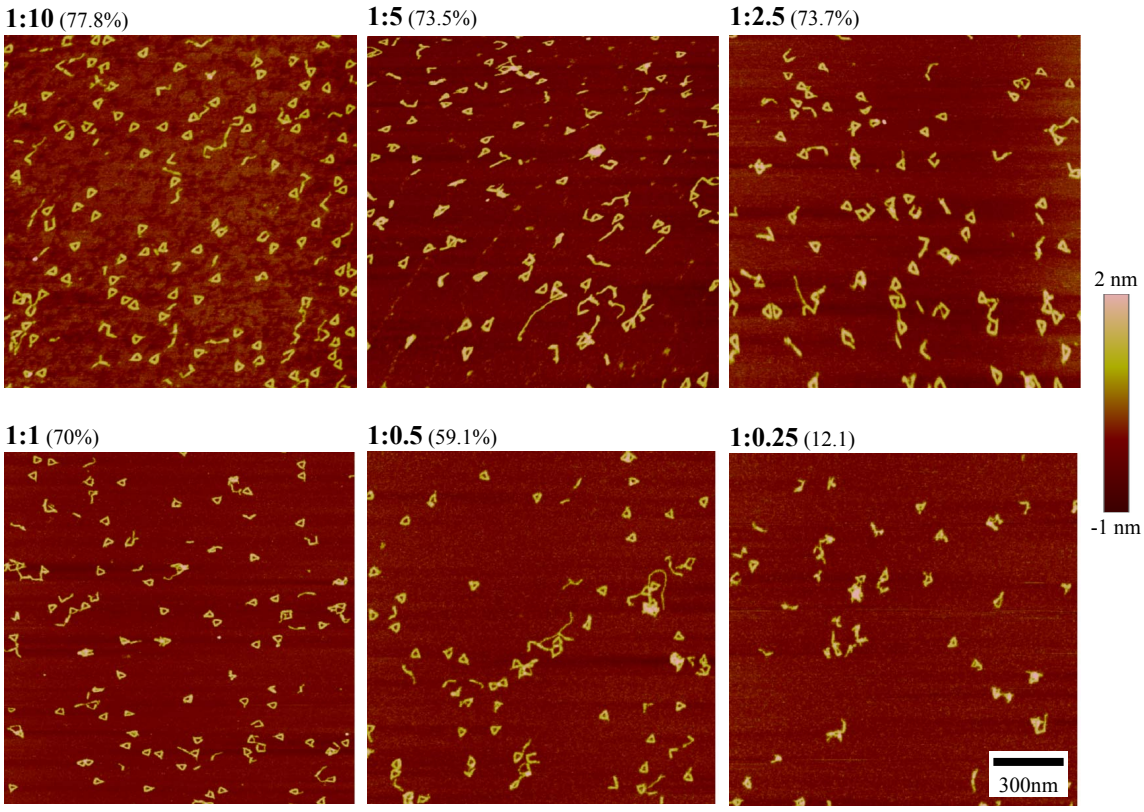

Figure S10. Assembly the triangle origami with different molecular ratio (RNA:DNA). For each condition, the assembly yield was calculated based on the observed objects in AFM imaging and given in parentheses.