Supplementary Information

Custom-shaped metal nanostructures based on DNA origami silhouettes

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Contents

1. Materials

All reagents are commercially available and applied without any further purification. In all procedures the water used was Milli-Q purified or double-distilled (ddH_2O). TEOS was purchased from Sigma-Aldrich and NH_4OH from Baker Analyzed.

2. DNA origami

2.1. DNA origami annealing

DNA origamis (Rothmund rectangle [S1] (see Fig. S1) and Seeman tile [S2]) were prepared by folding single stranded DNA from virus M13mp18 (100 nM, New England Biolabs) with a set of short staple strands (100 µM scale, Integrated DNA Technologies) see refs. [S1,S2].

Origamis were folded using Finnzymes Instruments Piko Thermal Cycler or G-Storm thermal cycler. The annealing ramps have been reported in [S1,S2]. After folding the structures were stored at 4 °C.

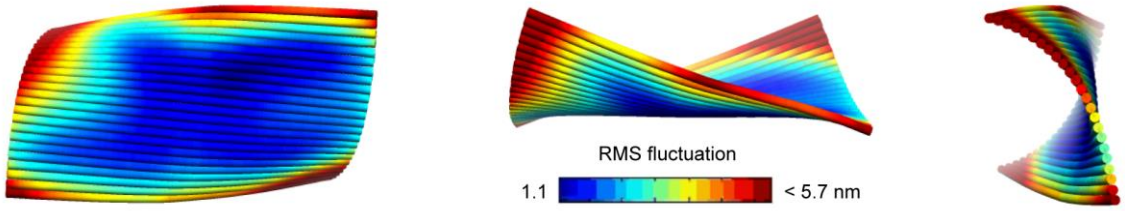

Figure S1: *Predicted solution shape of a rectangular origami [S1] without side strands. The simulation was performed using caDNAno design file [S3] as an input for CanDo modeling [S4,S5]. Three views along different orthogonal directions are presented. The rigid-beam simulation indicates that a rectangular origami is highly flexible and adopts a significantly twisted shape in the solution. Due to this, some rectangles appear twisted on the silicon substrate after their deposition. Thus, the final patterning yield decreases and the created metallic objects are not that regular.*

2.2. Optional purification of DNA origami

The excess amount of staple strands was removed in a non-destructive spin-filtering process. For filtering, we used either Millipore Amicon Ultra YM-100 filter columns with 100 kDa molecular weight cut-off. Filtration steps for 100 kDa filter are described below.

- 50 µl of DNA solution was mixed with 450 µl of folding buffer and injected into the filter.
- Solution was spun with 14,000 rcf for 3 min at room temperature.
- Flowthrough was discarded and 450 µl of folding buffer was added to the filter.
- Sample was spun in total 4 times repeating the procedure described above, expect for the last round the centrifugation time was set to 5 min.

- After the last spinning, the filter was turned upside down in a fresh container and was spun 2 min at 1,000 rcf to collect the solution.

After filtration the volume of the solution was typically brought from 500 µl down to 17-20 µl. Based on the observations throughout the whole fabrication procedure, it was noticed that the purification is not necessarily needed. Therefore, the origamis can be directly deposited onto the Si surface after the folding. However, the spin-filtering procedure enables the buffer exchange, which might be useful if the deposition is done for the specific substrates (e.g. the salt concentration of the buffer solution can be freely adjusted).

3. Agarose gel electrophoresis

The quality of origami folding was verified by agarose gel electrophoresis using BIO-RAD Power Pac Basic equipment. 1-2 % agarose gels were prepared by dissolving 1-2 g of agarose (Sigma-Aldrich) into 100 ml of 1x TAE buffer (40 mM Tris, 19 mM acetic acid, 1 mM EDTA) with 11 mM Mg++. The gel was stained with 30 µl of ethidium bromide (EthBr) solution (0.625 mg/l). 1x TAE + 11 mM Mg++ as used as a running buffer. Samples were stained with 6x Blue Loading Dye (New England Biolabs). As a reference we used an M13mp18 scaffold strand. The gels were run with a constant voltage of 90 V for 45-90 minutes.

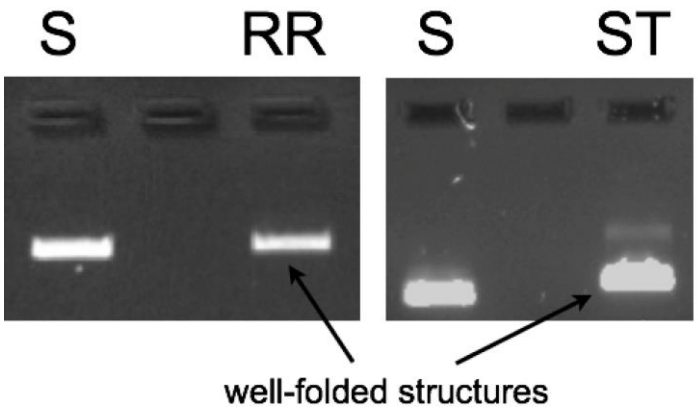

Figure S2: *Agarose gel electrophoresis for rectangular (RR) and cross-shaped (ST) origamis. S means a scaffold strand (M13mp18), which is used as a reference sample. RR and ST samples run with a different speed compared to the reference. This indicates proper folding. The faint band in an ST lane represents dimers.*

4. Fabrication procedure of the metallic nanostructrues

4.1 Step 1: Immobilization of DNA origami on Si substrate

- A slightly boron-doped *p*-type silicon chip (6 x 6 mm) was cleaned with hot acetone and isopropanol followed by a sonication (2 min) with an ultrasonic cleaner. Finally, the chip was dried by N_2 flow.
- The cleaned Si chip was treated with O_2 plasma (oxygen flow 50 sccm, plasma power 200 W, temperature 30 °C and time 20 min) in a RIE chamber (Oxford Plasmalab 80 Plus).
- 5 µl of DNA origami solution in 1x TAE buffer (40 mM Tris, 19 mM acetic acid, 1 mM EDTA) with 100 mM Mg++ was pipetted onto the Si chip (within 30 min after the plasma treatment).
- After incubation in a closed chamber for 5 min, the chip was washed 3 times with 50 µl ddH_2O and gently dried with a N_2 gas flow.

4.2 Step 2: DNA origami silhouettes formation

- 80 grams of silica gel was conditioned overnight in a humidity chamber (Climatic test chamber, Weiss) at 80 % relative humidity (at room temperature).
- The silica gel was positioned at the bottom of a 1.5-liter glass desiccator. On top of it the Si chip with DNA origami was placed between two small vials containing tetraethyl orthosilicate (TEOS, ≥99.0 %) and ammonium hydroxide (NH_4OH, 25 % NH_3 in H_2O).
- After incubation for 16-22 hours, the sample was taken out of the desiccator in order to stop the SiO_2 growth.

4.3 Step 3: RIE etching of Si and SiO_2

- Depending on the thickness of the growth SiO_2, 2-4 nm of the SiO_2 layer was etched by RIE (CHF_3 flow 25 sccm, Ar flow 25 sccm, plasma power 100 W, temperature 25 °C and time 12-24 s).
- Without taking the sample out of the RIE chamber, 57 nm of Si was subsequently etched (SF_6 flow 100 sccm, O_2 flow 8 sccm, plasma power 50 W, temperature 30 °C and time 30 s).

4.4 Step 4: Metal deposition

- The sample was fixed on a sample stage and loaded into a custom-built UHV evaporation chamber (~ 10^{-9} mbar).
- The metal to be deposited (Au, Cu or Ag) was heated by an electron beam until the appropriate evaporation rate (0.04 nm/s for Cu, 0.06 nm/s for Au and Ag) was reached and stabilized.
- Evaporation was controlled by a mechanical shutter and monitored by a quartz crystal microbalance sensor. About 20 nm of metal (Au, Cu or Ag) film was deposited perpendicularly to the sample.

4.5 Step 5: Lift-off

- After immersing the sample into a lift-off solution (HF (38%) / HCl (38%) as 12:1 mixture for Au or 4% HF aqueous solution for Cu and Ag), the suspended metal film was removed by shaking the sample or gently rubbing the surface of the chip with a cotton stick in the lift-off solution.
- Finally the sample was rinsed with ddH_2O and gently dried by N_2 flow.

5. Imaging with AFM and SEM

The sample was characterized either by AFM (after Steps 1 & 2) or SEM (throughout the Steps 2-5).

5.1 AFM imaging

The Si chip with immobilized DNA origami or origami-shaped silhouettes was imaged with AFM (Veeco Dimension 3100) in tapping mode with a scan rate of 1.0-1.5 Hz.

5.2 SEM imaging

SEM images were taken using a SEM/e-beam lithography device (Raith eLine) with 10 kV acceleration voltage and 30 µm aperture with a pixel noisy reduction mode.

6. Fabrication yield of the gold nanostructures

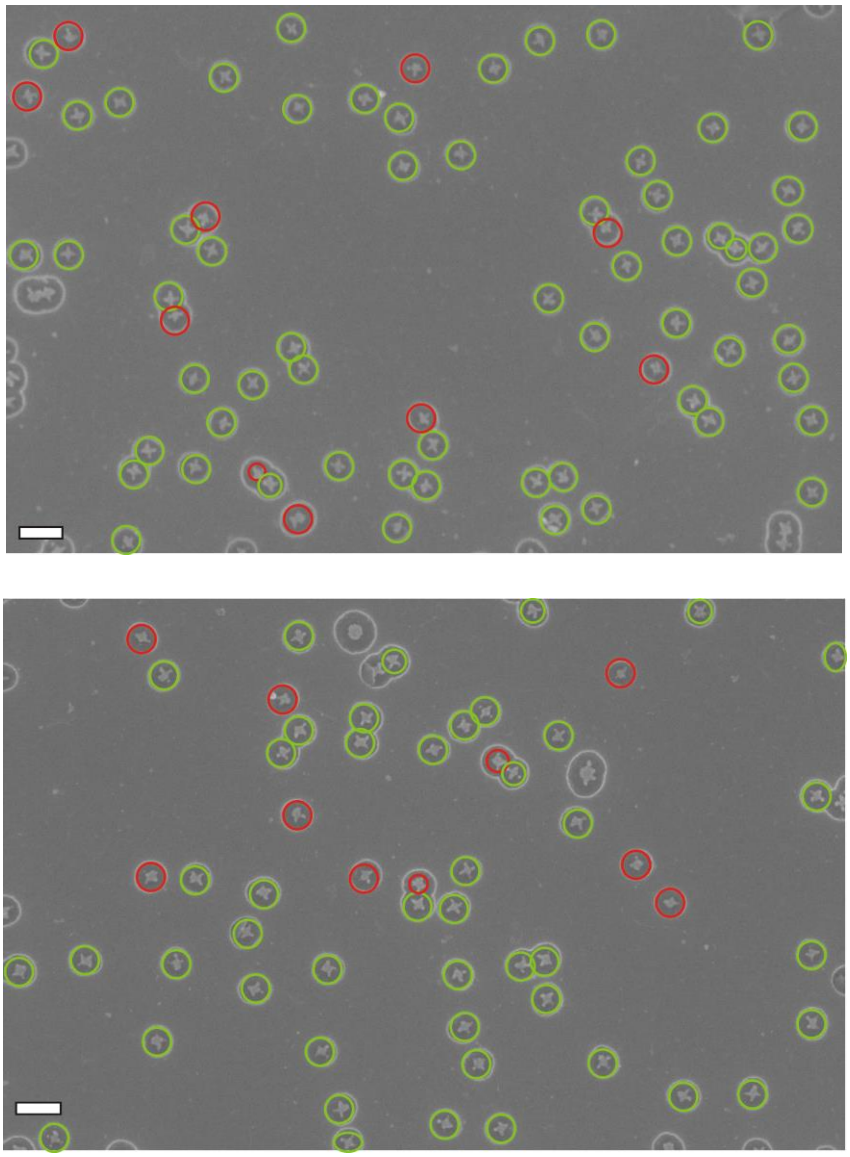

Figure S3: *SEM images of the gold cross-shaped structures. After lift-off, 86 % of the observed structures have the desired shape (marked with green circles) and 14 % are misshaped structures (marked with red circles). The scale bars are 200nm.*

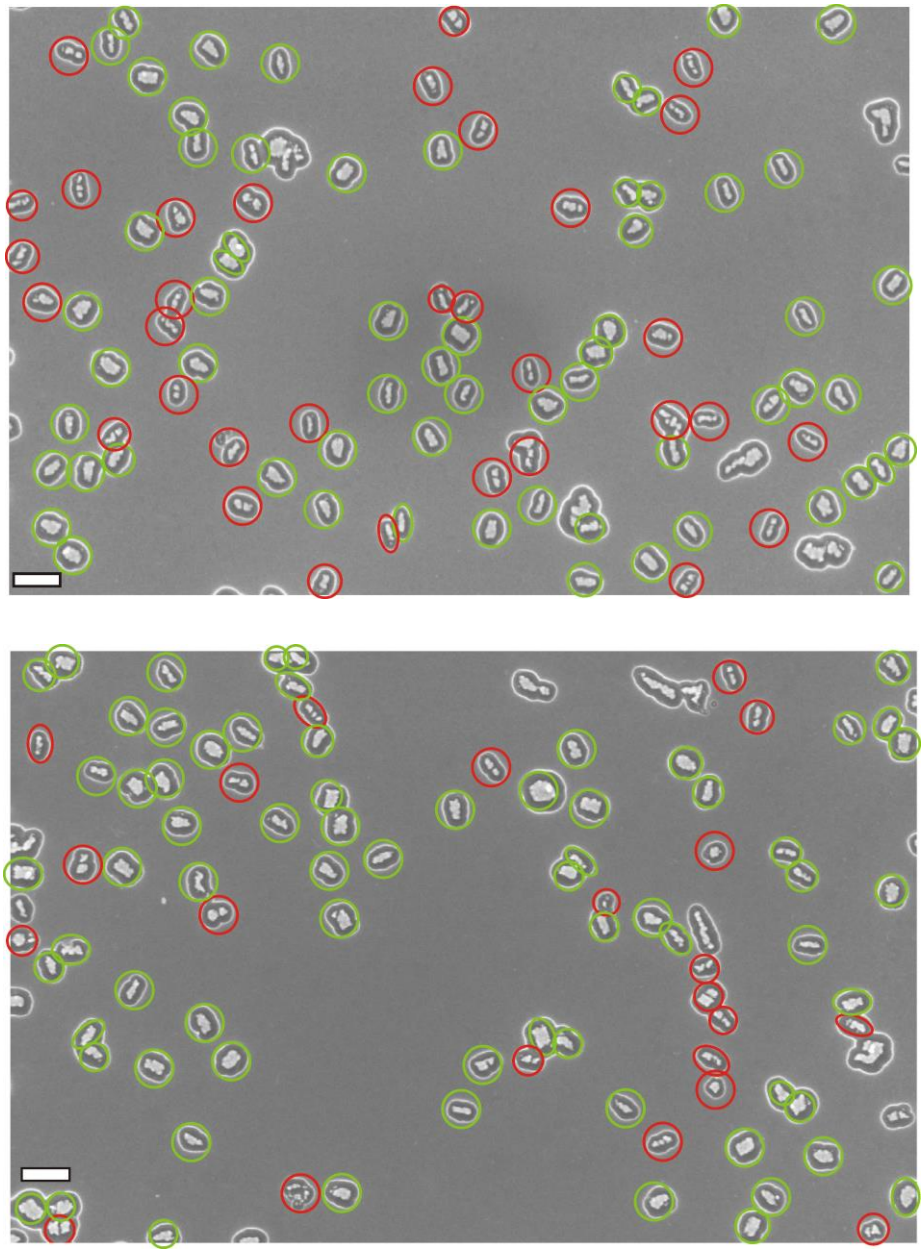

Figure S4: *SEM images of rectangular origami -shaped metal nanostructures. The yield for desired structures after lift-off is 65 % (marked with green circles), and 35 % are misshaped or broken structures (marked with red circles). Note that due to the twisted shape of the rectangle, the dimensions of the nanostructures can vary. The scale bars are 200nm.*

7. Dimensions of the gold nanostructures

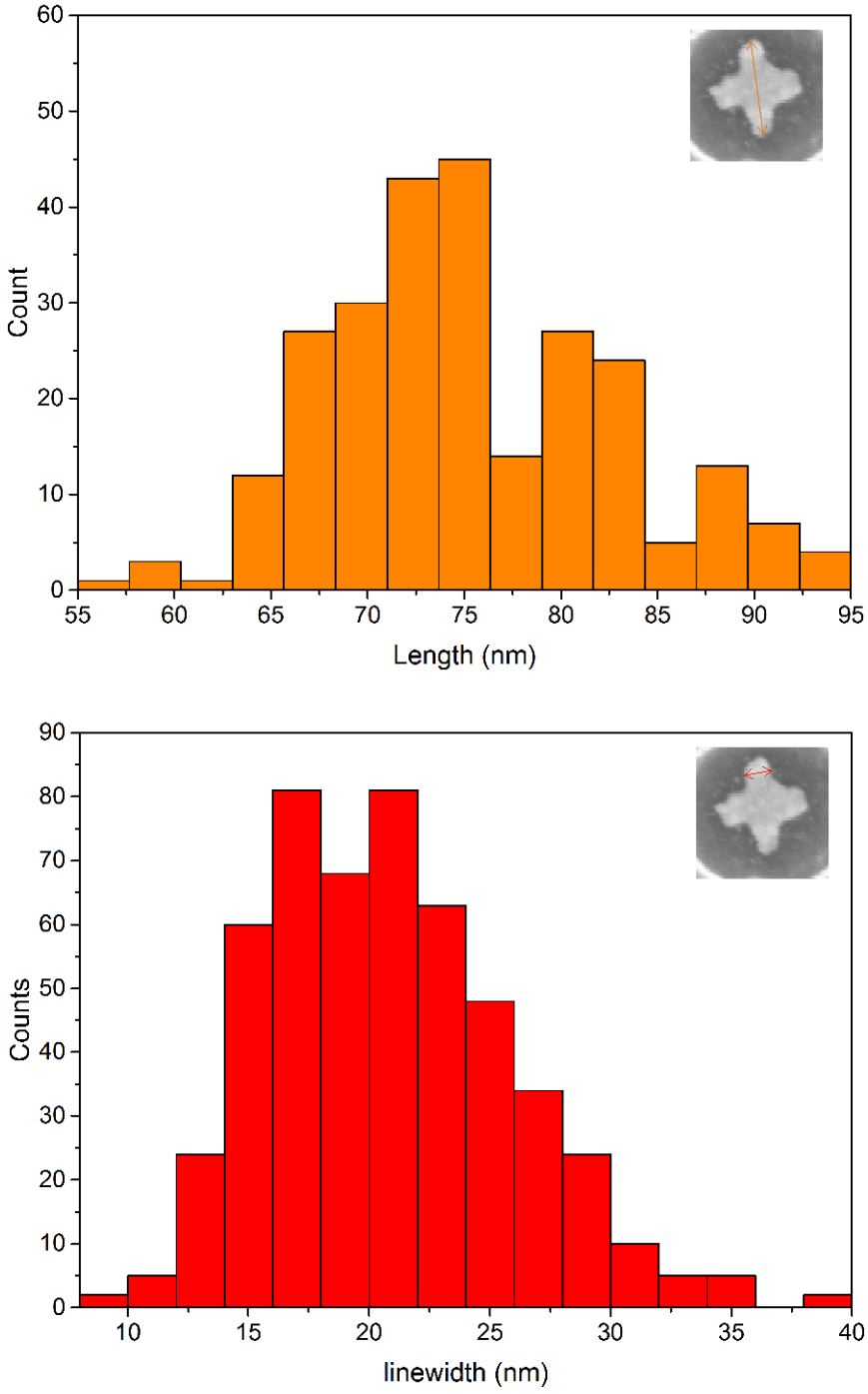

Figure S5: *The distribution of dimensions (length and width) of the Seeman tile -shaped structures. The average linewidth is about 20 nm. The average distance between the metallic structures using optimized parameters was 380 ± 190 nm.*

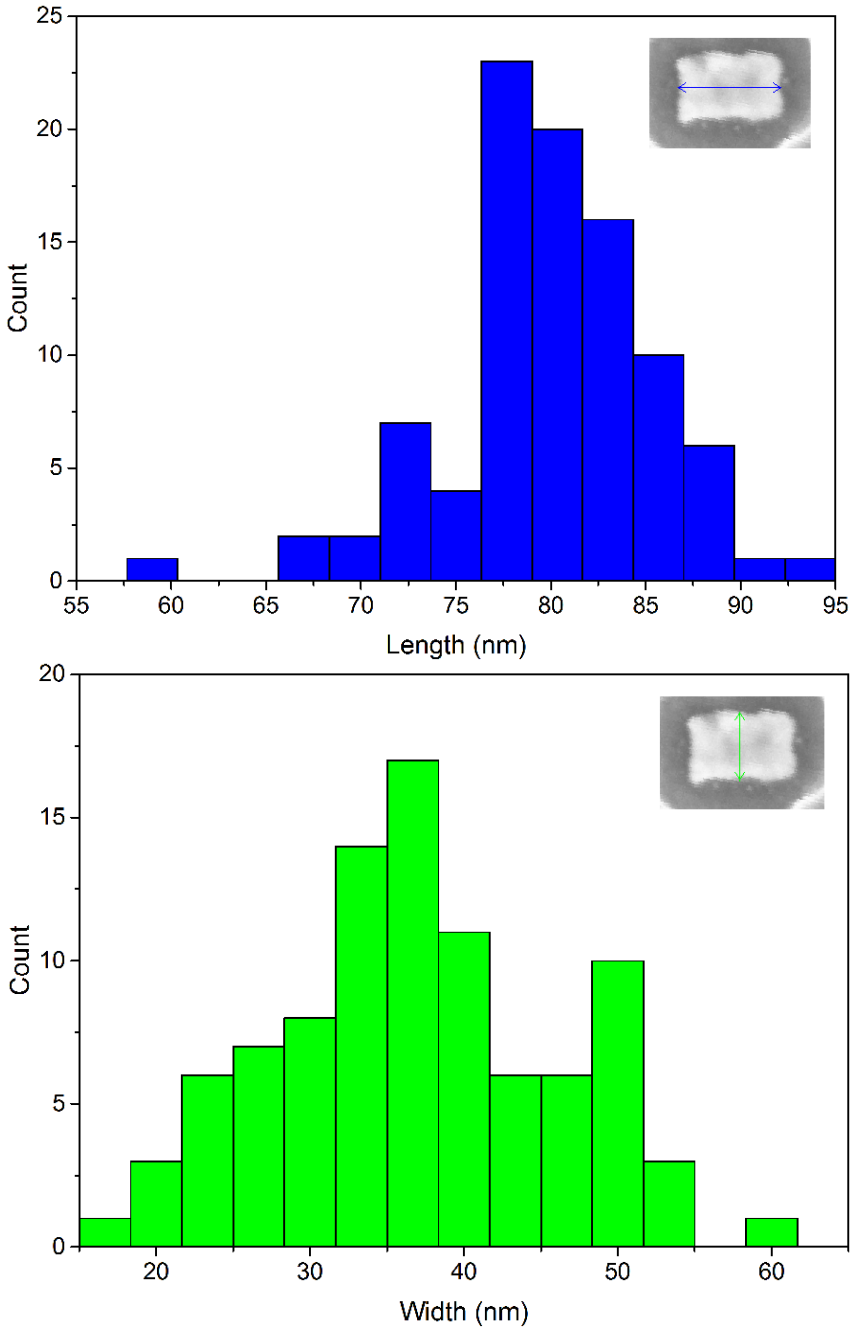

Figure S6: *The length and the width distributions of Rothemund rectangle -shaped metal structures. The length of the RR origami (92 nm) roughly correspond to the length of the metal structures, but the average width of the metal structure is about half of the origami width (72 nm). The average distance between the metallic structures using optimized parameters was 270 ± 130 nm.*

8. SEM montages of the gold nanostructures

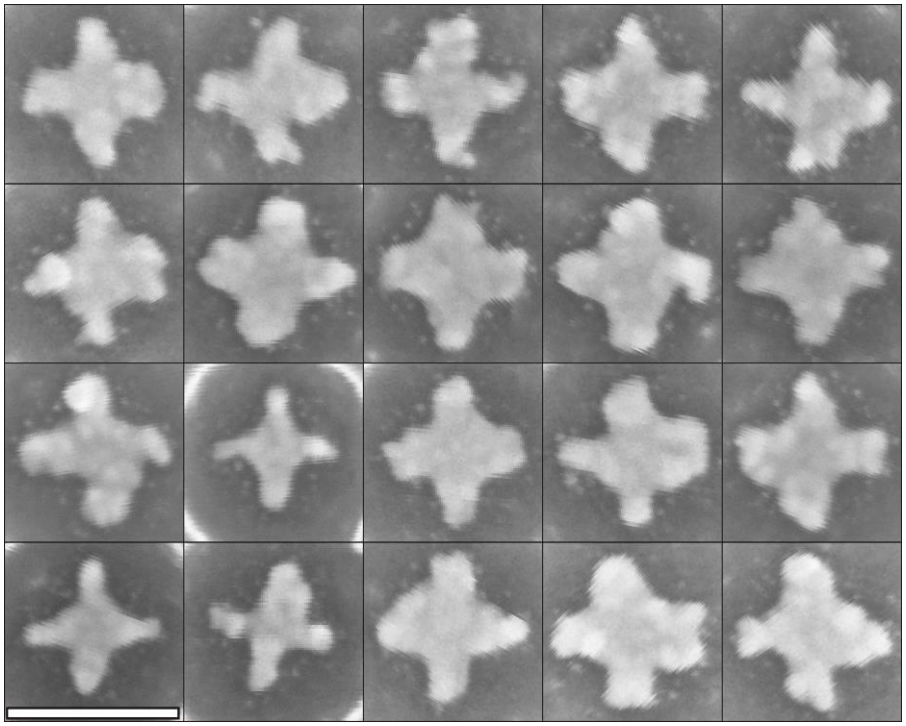

Figure S7: *Collection of typical SEM images of gold cross-shaped nanostructures. The scale bar is 100 nm.*

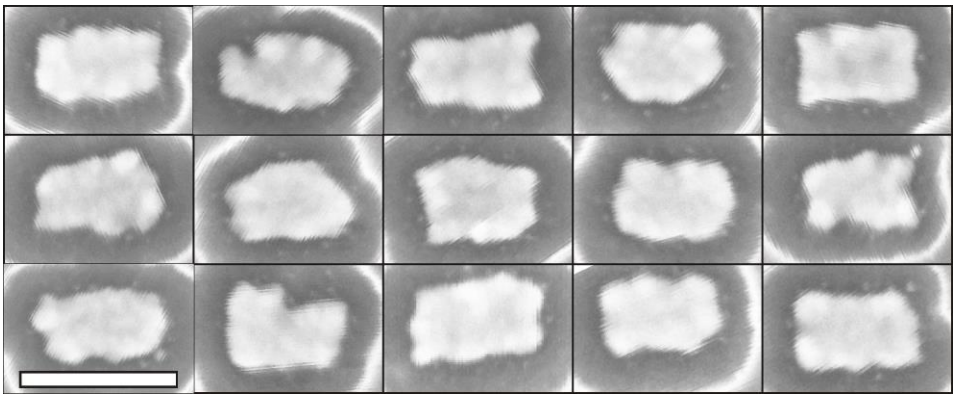

Figure S8: *Collection of typical SEM images of gold rectangular-shaped nanostructures. The size distribution is wide due to the twisted shape of the rectangle. The scale bar is 100 nm.*

9. Supplementary information references

[S1] P. W. K. Rothemund, *Nature,* 2006, **440**, 297-302.

[S2] W. Liu, H. Zhong, R. Wang and N. C. Seeman, *Angew. Chem. Int. Ed.,* 2011, **50**, 264-267.

[S3] S. M. Douglas, A. H. Marblestone, S. Teerapittayanon, A. Vazquez, G. M. Church and W. M. Shih, *Nucleic Acids Res.,* 2009, **37**, 5001-5006.

[S4] C. E. Castro, F. Kilchherr, D.-N. Kim, E. L. Shiao, T. Wauer, P. Wortmann, M Bathe and H. Dietz, *Nat. Methods,* 2011, **8**, 221-229.

[S5] D.-N. Kim, F. Kilchherr, H. Dietz and M. Bathe, *Nucleic Acids Res.,* 2012, **40**, 2862-2868.