Electronic Supplementary Material (ESI) for Nanoscale.

DNA Conformational Equilibrium Enables Continuously Changing of Curvatures

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Supporting Information

Oligonucleotides:

All oligonucleotides were purchased from IDT Inc. and directly used without further purification.

DNA strand sequences (extra chemical group marked as red):

h: CTAG ACGCGT GGATCC GGCC;

hC: CTAG ACGCGT GGATCC GGCC **C;**

hA: CTAG ACGCGT GGATCC GGCC **A;**

hG: CTAG ACGCGT GGATCC GGCC **G;**

hT: CTAG ACGCGT GGATCC GGCC **T;**

hAA: CTAG ACGCGT GGATCC GGCC **AA;**

hF: CTAG ACGCGT GGATCC GGCC **FAM;**

hd: TTTT ACGCGT GGATCC TTTT.

Buffers compositions:

TAE buffer: 40 mM tris base, 2 mM EDTA and 20 mM acetic acid, pH 8.0.

TAE/Mg^{2+} (incubation buffer): 40 mM tris base, 2 mM EDTA, 20 mM acetic acid, and 12.5 mM magnesium acetate, pH 8.0.

TA/Mg^{2+}/Ni^{2+} (AFM imaging buffer): 40 mM tris base, 20 mM acetic acid, 10 mM magnesium acetate, and 5 mM nickel (II) chloride, pH 8.0.

Polyacrylamide Gel Electrophoresis (PAGE):

DNA was diluted to 20 μM or 2 μM in TAE/Mg^{2+}, then sequentially annealed the solutions: 95 °C for 5 min, 65 °C for 30 min, 50 °C for 30 min, 37 °C for 30 min, and 22 °C for 30 min. 10% native PAGE [10% acrylamide/bisacrylamide (19:1) in TAE/Mg^{2+}, pH 8.0] was used to examine the DNA polymerization. Gels were run on a FB-VE10-1 electrophoresis unit (FisherBiotech) at 22 °C (200 V, constant voltage) in TAE/Mg^{2+} for 3.5 hours. After electrophoresis, the gels were stained with Stains-all dye (Sigma) and scanned.

Assemble structure on mica:

For morphology study, DNA was diluted in TAE/Mg^{2+} with designated concentration. Then, 5 μL DNA solution was deposited on a freshly-cleaved mica and incubated for 10 min. After incubation, the DNA solution was removed and 25 μL TA/Mg^{2+}/Ni^{2+} solution was added for further AFM imaging.

For Mg^{2+} concentration study, DNA was diluted to 2 μM in TAE buffer with a designated concentration of Mg^{2+} (magnesium acetate). Then, 5 μL DNA solution was dropped on a fresh-cleaved mica and incubated for 10 min. After incubation, the DNA solution was removed and 25 μL TA/Mg^{2+}/Ni^{2+} solution was added for further AFM imaging.

For kinetic study, DNA was diluted to a designated concentration in TAE/Mg^{2+}. Then, 5 μL DNA solution was deposited on a freshly-cleaved mica and incubated for a designated time. After incubation, the DNA solution was removed. The mica surface was washed with 25 μL 10 mM magnesium acetate solution. Finally, the mica surface was blown dry by compressed air. The washing and drying should be within 3 seconds to minimize potential DNA structure change.

For thermodynamic slow anneal study, DNA was diluted to 2 μM in TAE/Mg^{2+}. Then, a freshly-cleaved mica was immersed in the DNA solution. The mica-containing solution was heated to 95 °C and slowly decreased to 22 °C in 48 h. After annealing, the mica was removed out from the solution. Finally, 25 μL TA/Mg^{2+}/Ni^{2+} solution was added onto the mica surface for AFM imaging.

AFM imaging:

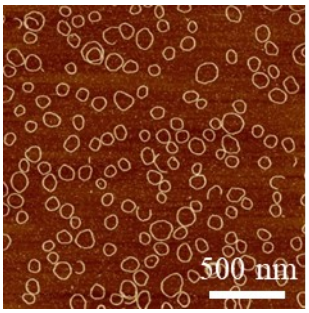
AFM images were captured by MultiMode 8 (Bruker) in tapping mode either in fluid with ScanAsyst-fluid mode and ScanAsyst-fluid+ probes (Bruker) or in air with ScanAsyst-air mode and ScanAsyst-air probes (Bruker). The tip-surface interaction was automatically adjusted to optimize the scan set-point, gain and Z-limit. All AFM scanning were carried out at 22 °C.

Bending angle calculation:

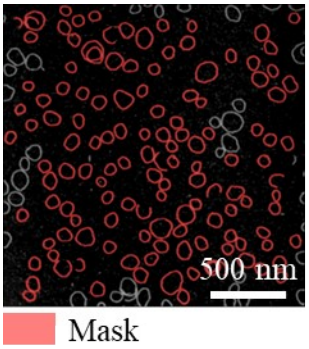
AFM images were processed by TopoStats[1] to sketch the individual DNA fiber contour length and end-to-end length. The DNA closed rings were picked when end-to-end distance is zero. The bending angles were calculated as described in Fig. 3a. To

maximize the picking of closed rings, the ring area and deviation were set to 200 nm^2 and 0.5-10, respectively. Following is the detail protocol.

1. Install the TopoStats, run it in Docker as a container.
2. Prepare the AFM original file. For Bruker MultiMode 8 AFM, the file extension is .000.



3. Set appropriate parameters. In our study, to maximize the picking of closed rings, the ring area and deviation were set to 200 nm^2 and 0.5-10, respectively
4. Using pygwytracing.py to analyze the AFM image.
5. The software will mask all trace by height.



6. In the data report, each trace has its contour length and end-to-end length. In our study, we only selected traces when the end-to-end length was zero. This step can pick all circular DNA ladders.
7. The contour length from all circular DNA ladders is used in angle calculation as ***L***.

Reference:

1. Joseph G. Beton, Robert Moorehead, Luzie Helfmann, Robert Gray, Bart W. Hoogenboom, Agnel Praveen Joseph, Maya Topf and Alice L.B. Pyne. TopoStats – A program for automated tracing of biomolecules from AFM images. *Methods*. **193**, 68–79 (2021).

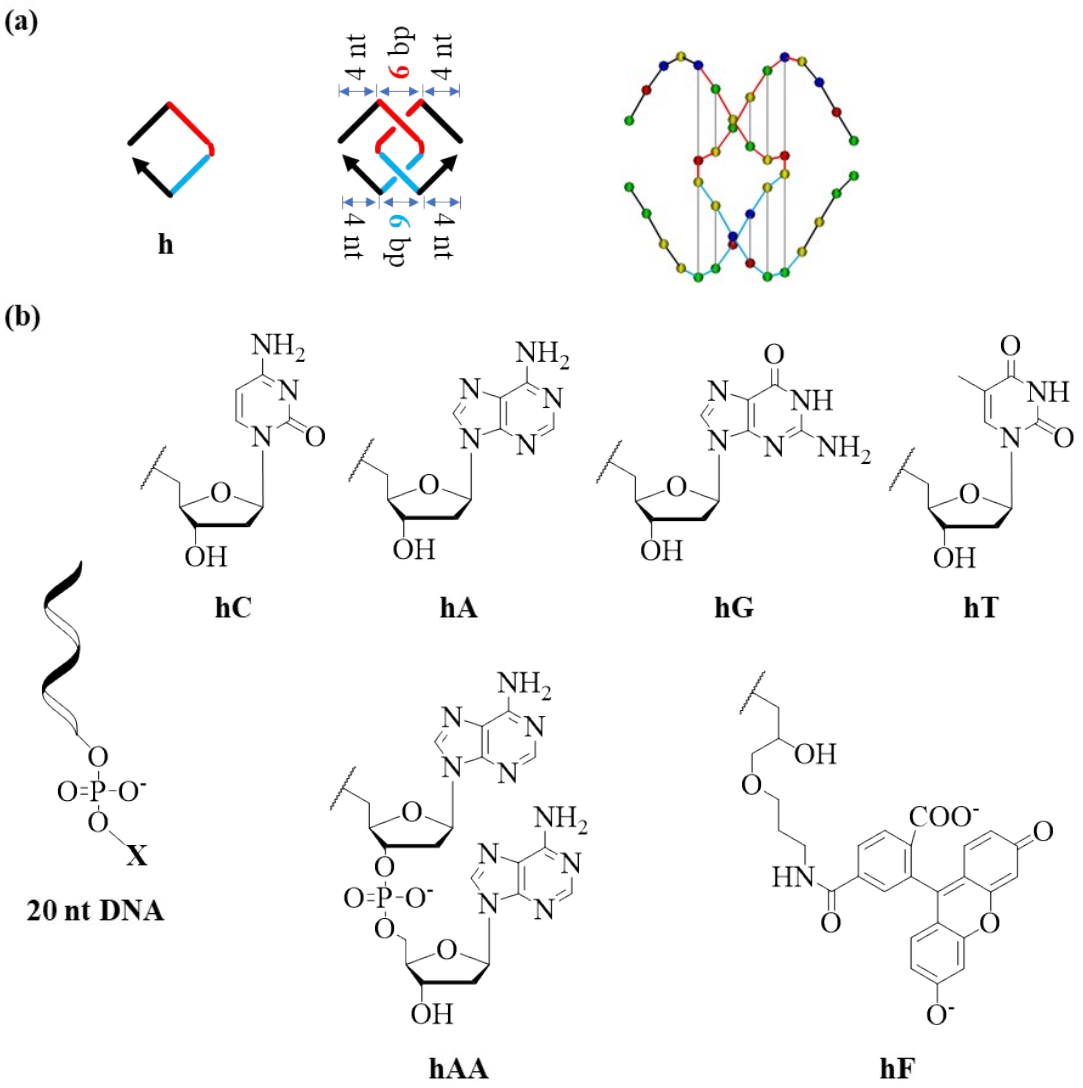


Figure S1. Supramolecular homopolymerization of DNA (a) Two copies of DNA strand **h** consisting of four palindromes (color different) can dimerize (b) The chemical structure of extra functional groups on the DNA 3' end.

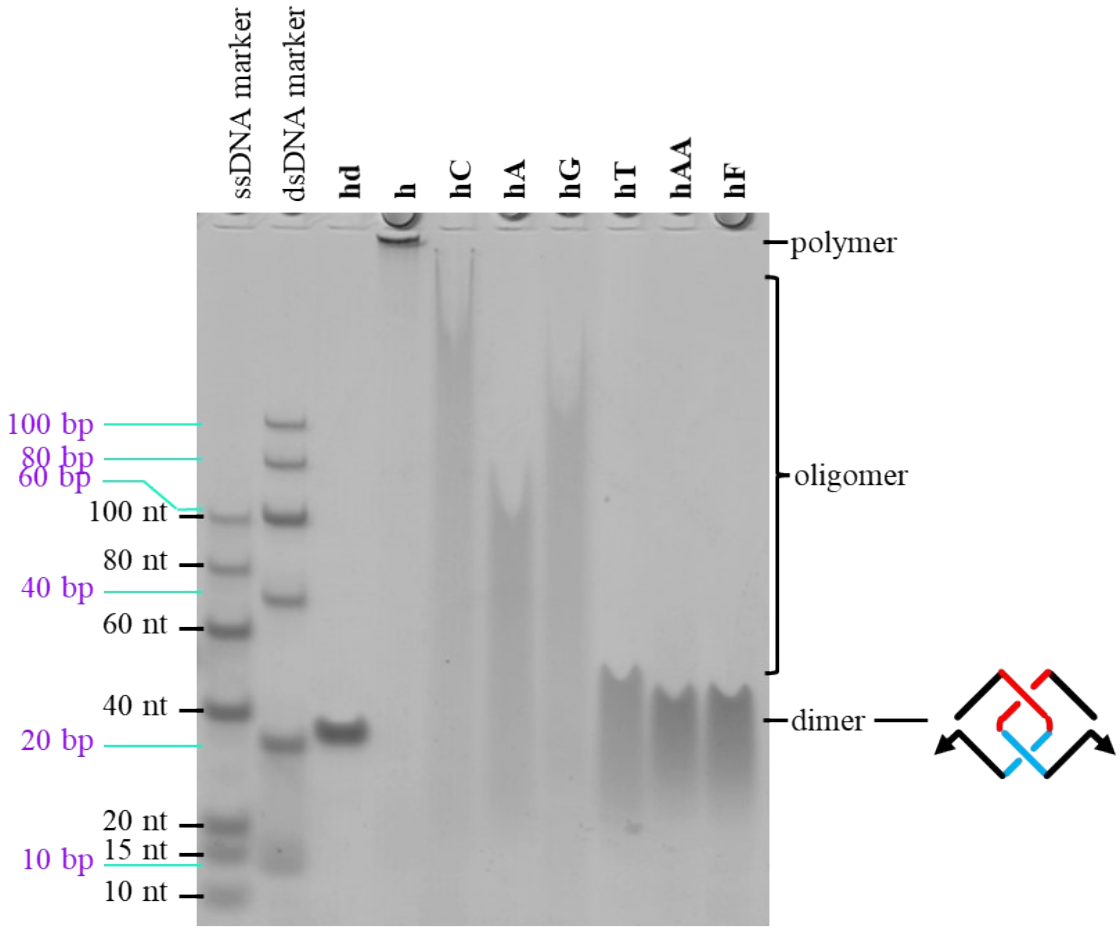


Figure S2. Native PAGE (10%) analysis of DNA assembly in TAE/Mg^{2+} at 22 °C. [DNA] = 20 μM. Note that sample "**hd**" is a control molecule that can only form homodimer but can't further polymerize. In these designs, individual DNA dimer motif is not particularly stable at the PAGE temperature. Such dimers will be further stabilized by inter-motif interactions.. But overall, all inter-strand and inter-motif interactions are not particular stable. So under experienced shear force during PAGE, DNA assembly slowly, but constantly dissociate; thus smear.

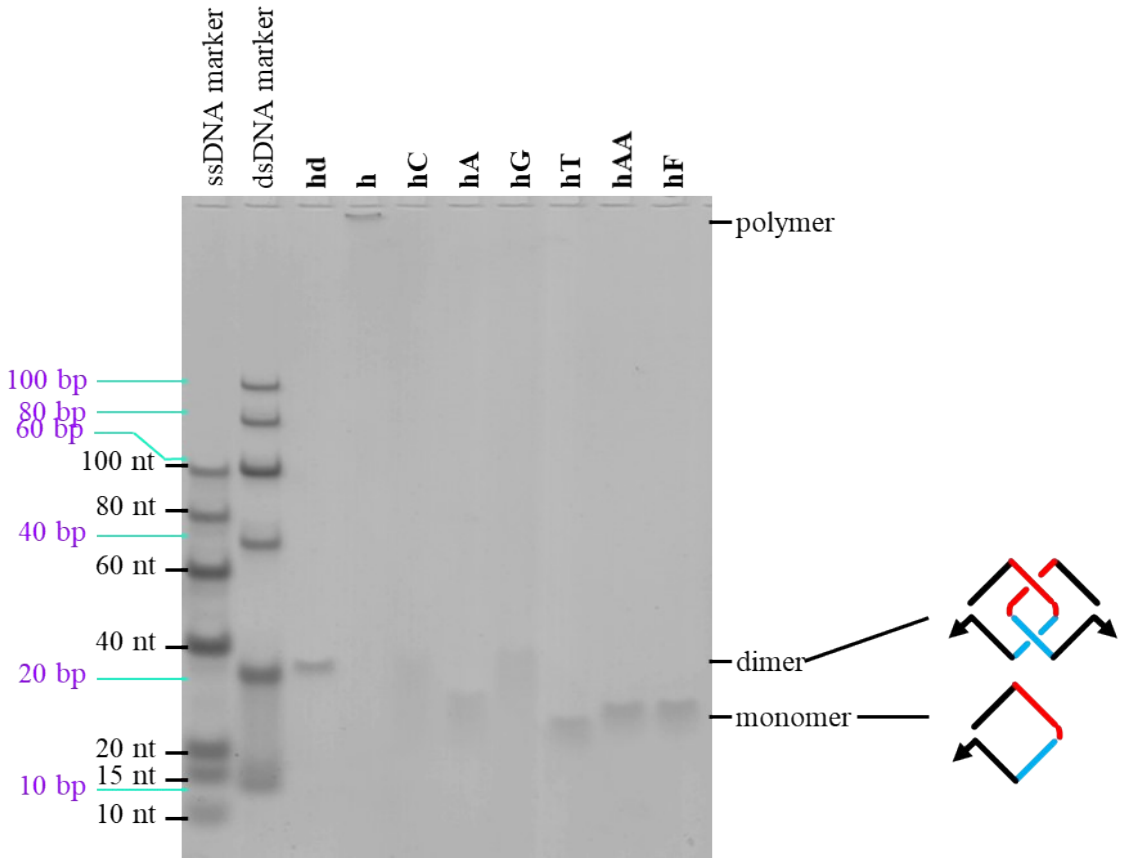


Figure S3. Native PAGE (10%) analysis of DNA assembly in TAE/Mg^{2+} at 22 °C. [DNA] = 2 μM. Note that sample "**hd**" is a control molecule that can only form homodimer but can't further polymerize.

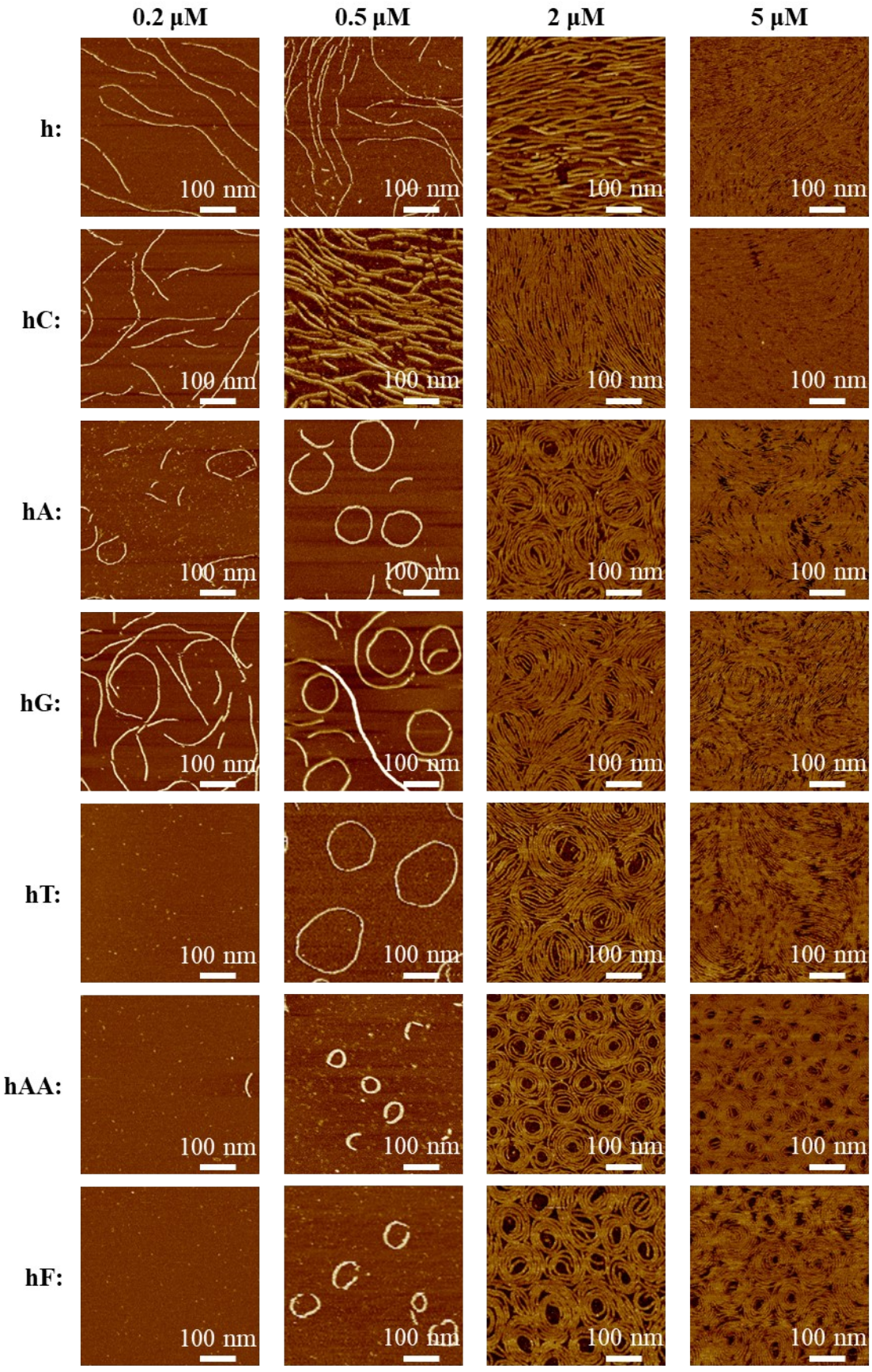

0.2 µM
0.5 µM
2 µM
5 µM
h:
hC:
hA:
hG:
hT:
hAA:
hF:
100 nm

Figure S4. AFM images of the DNA homopolymer structures under different DNA concentrations in TAE/Mg^{2+} solution.

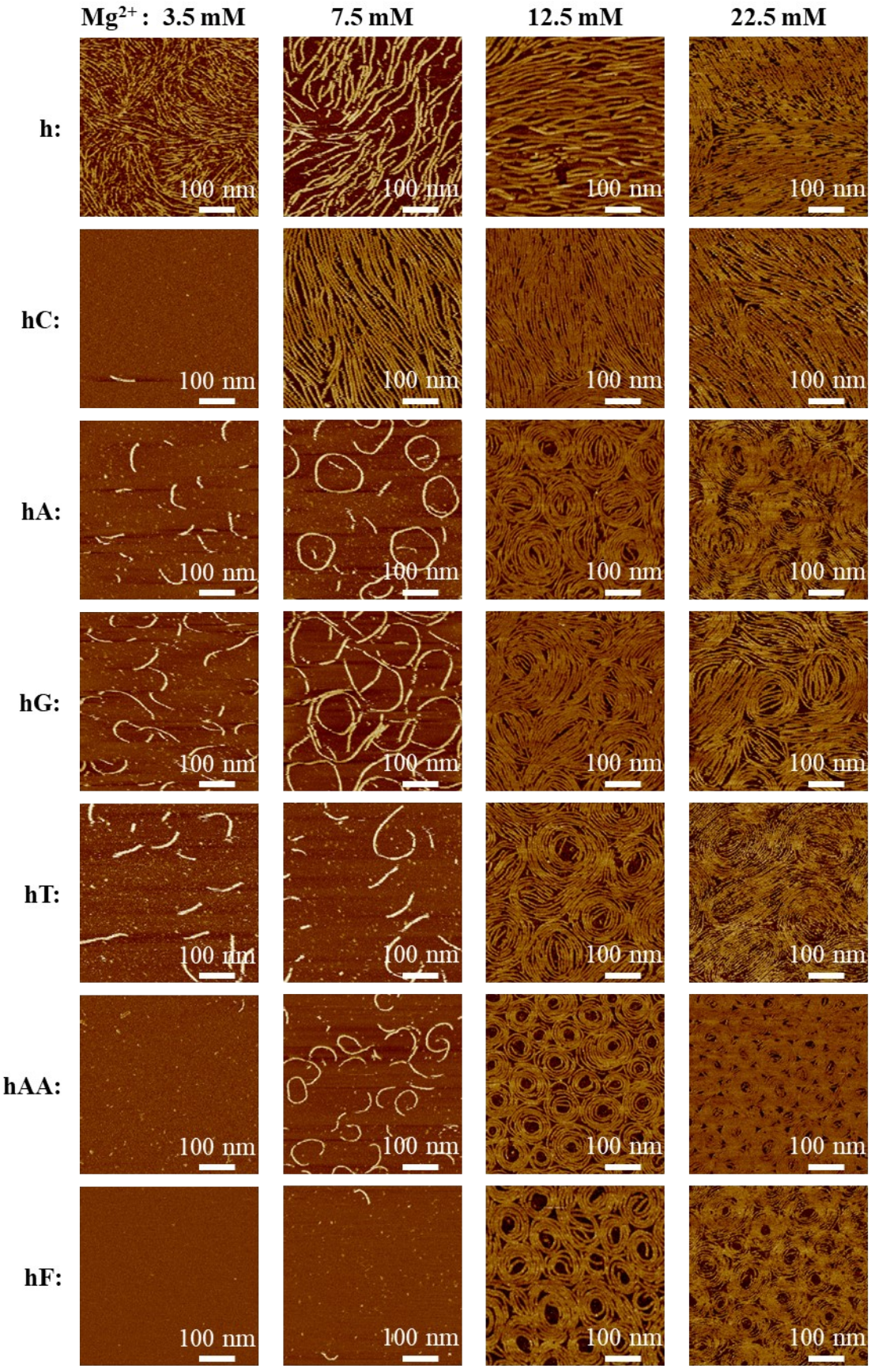

Mg2+ : 3.5 mM
7.5 mM
12.5 mM
22.5 mM
h:
hC:
hA:
hG:
hT:
hAA:
hF:
100 nm

Figure S5. AFM images the DNA homopolymer structures under TAE buffer with different Mg^{2+} concentrations, [DNA] = 2 μM.

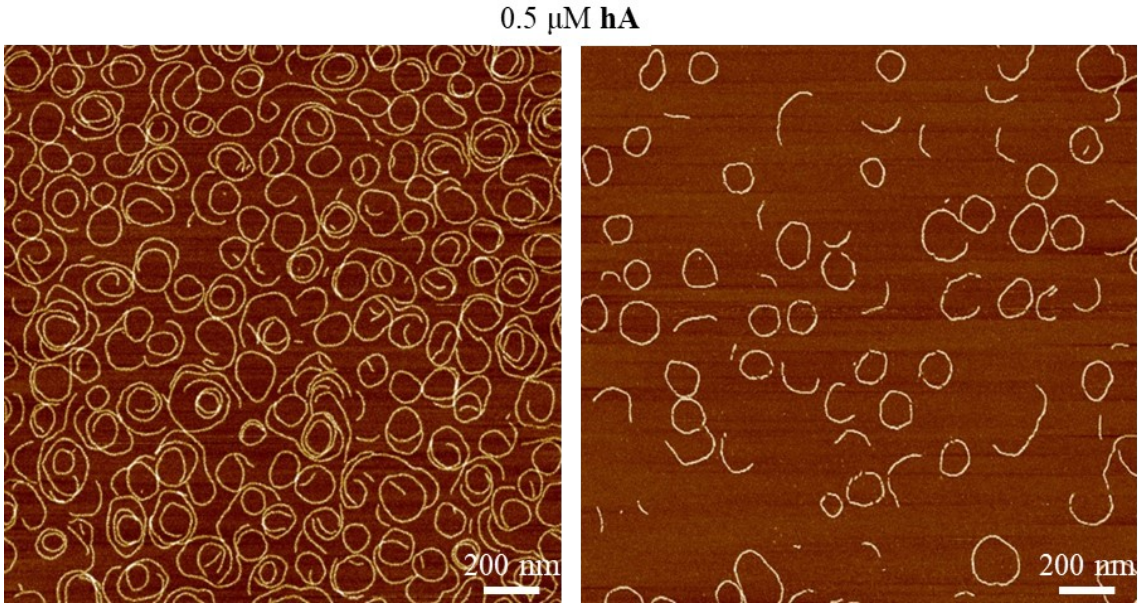


Figure S6. AFM images of DNA circles from 0.5 μM **hA**.

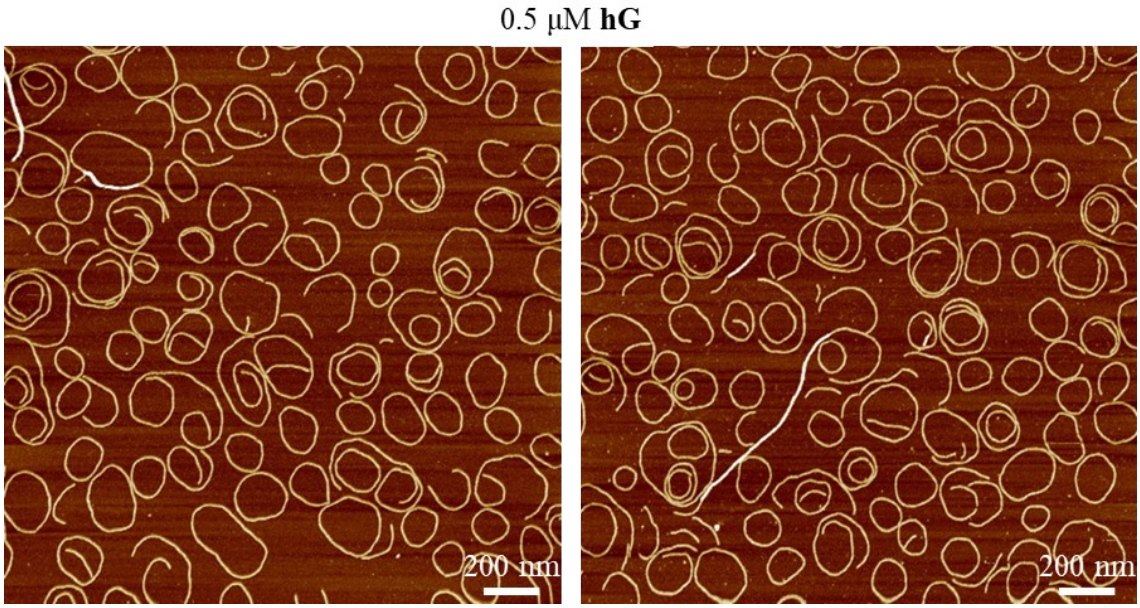


Figure S7. AFM images of DNA circles from 0.5 μM **hG**.

0.5 μM **hT**

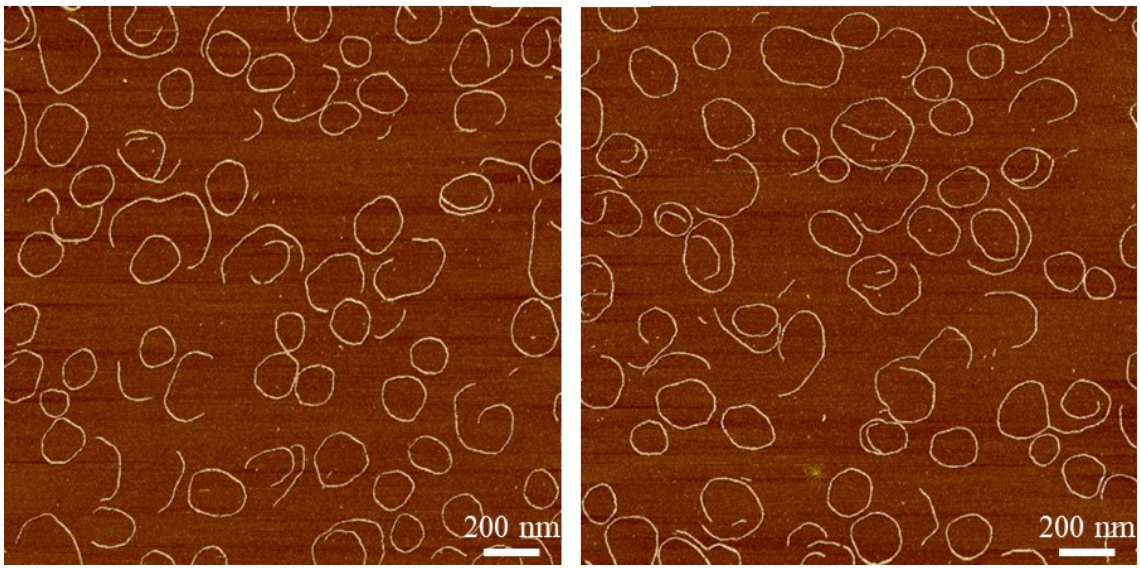


Figure S8. AFM images of DNA circles from 0.5 μM **hT**.

1 μM **hAA**

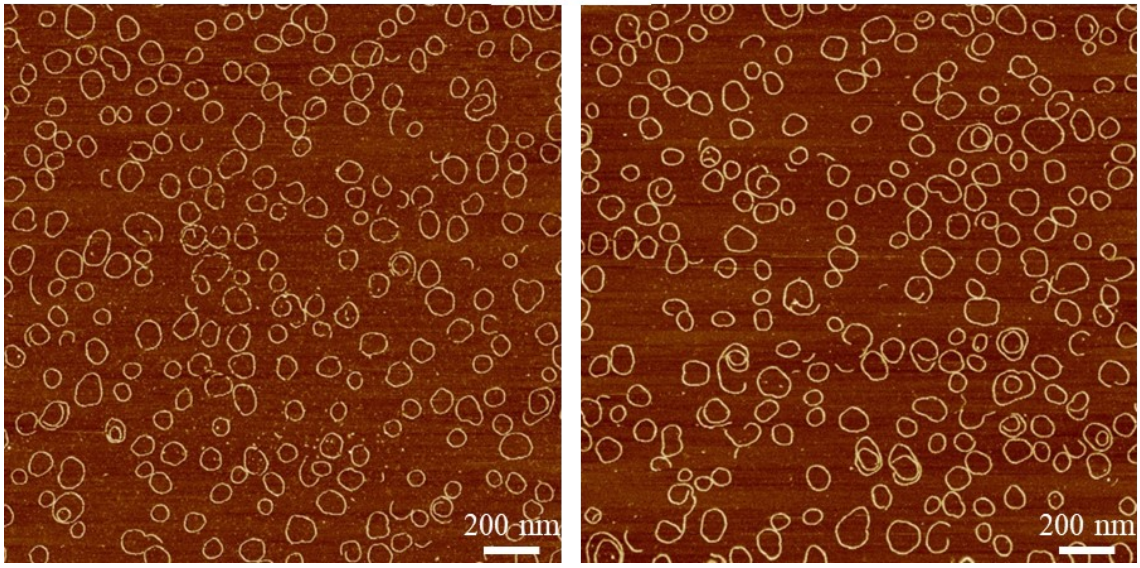


Figure S9. AFM images of DNA circles from 1 μM **hAA**.

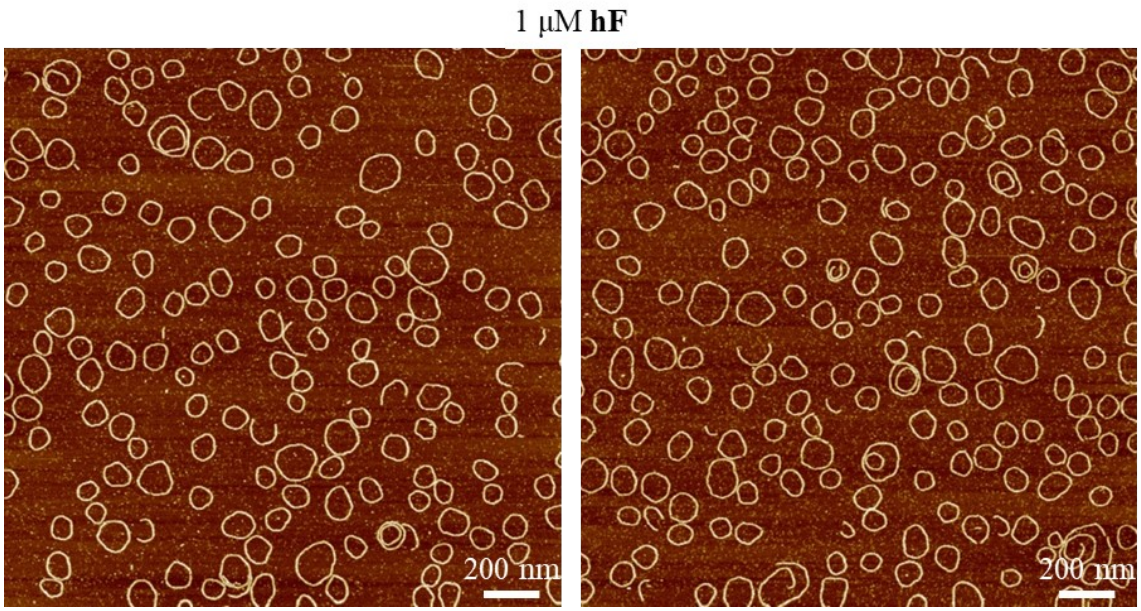


Figure S10. AFM images of DNA circles from 1 μM **hF**.

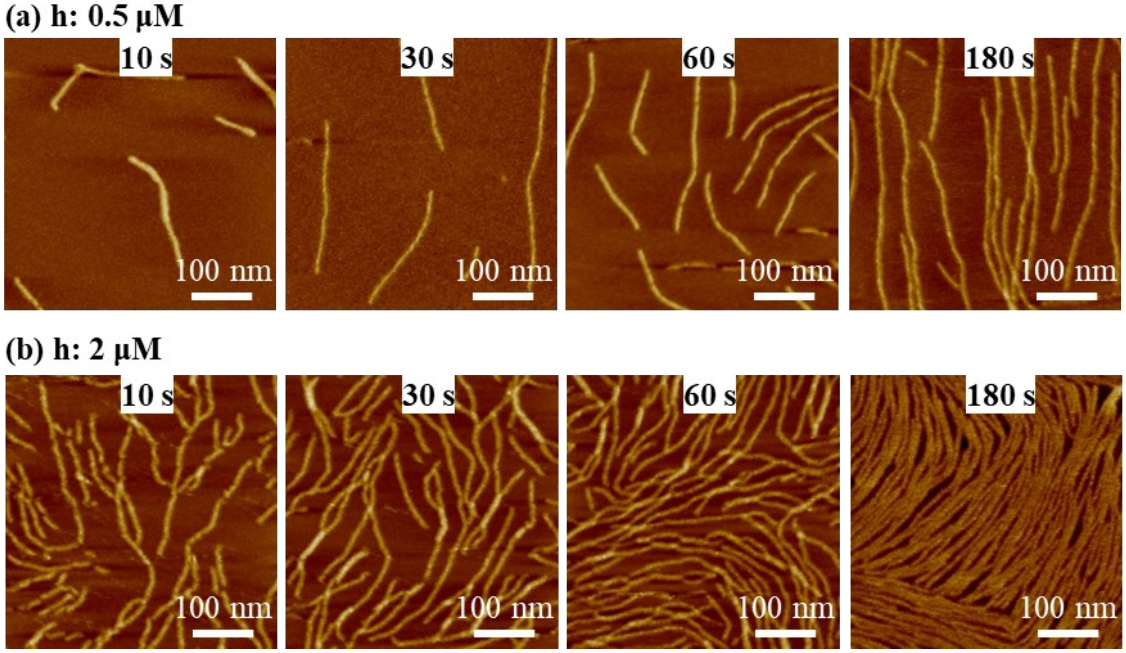


Figure S11. The assembly kinetics of **h** at different DNA concentrations. (a) at 0.5 μM (b) at 2 μM.

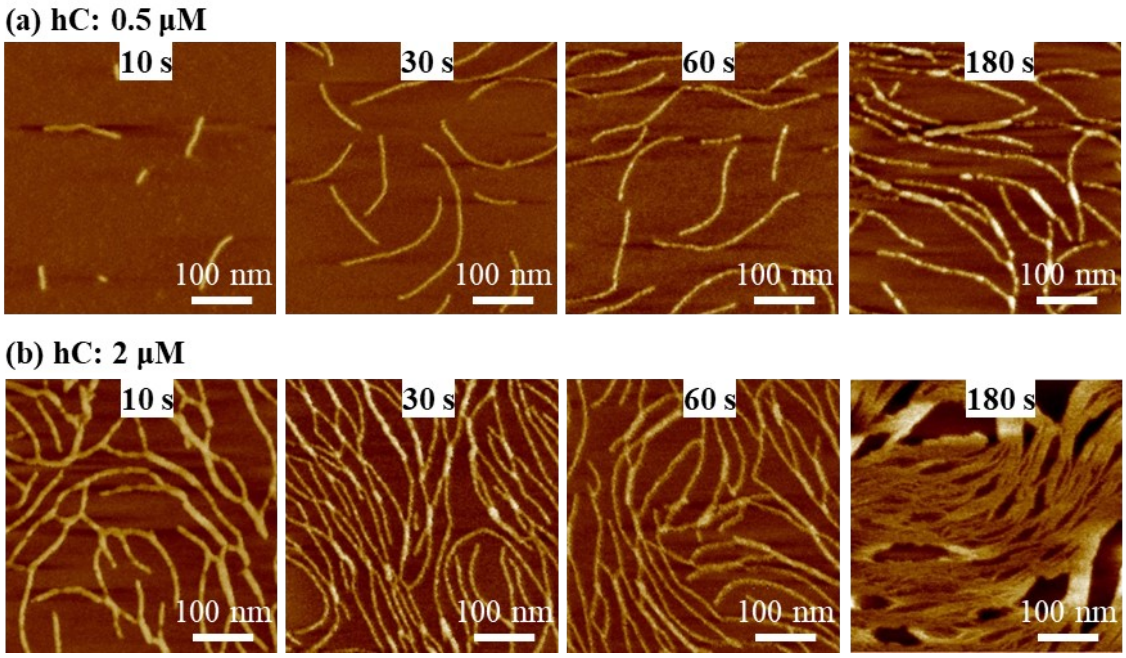


Figure S12. The assembly kinetics of **hC** at different DNA concentrations. (a) at 0.5 µM (b) at 2 µM.

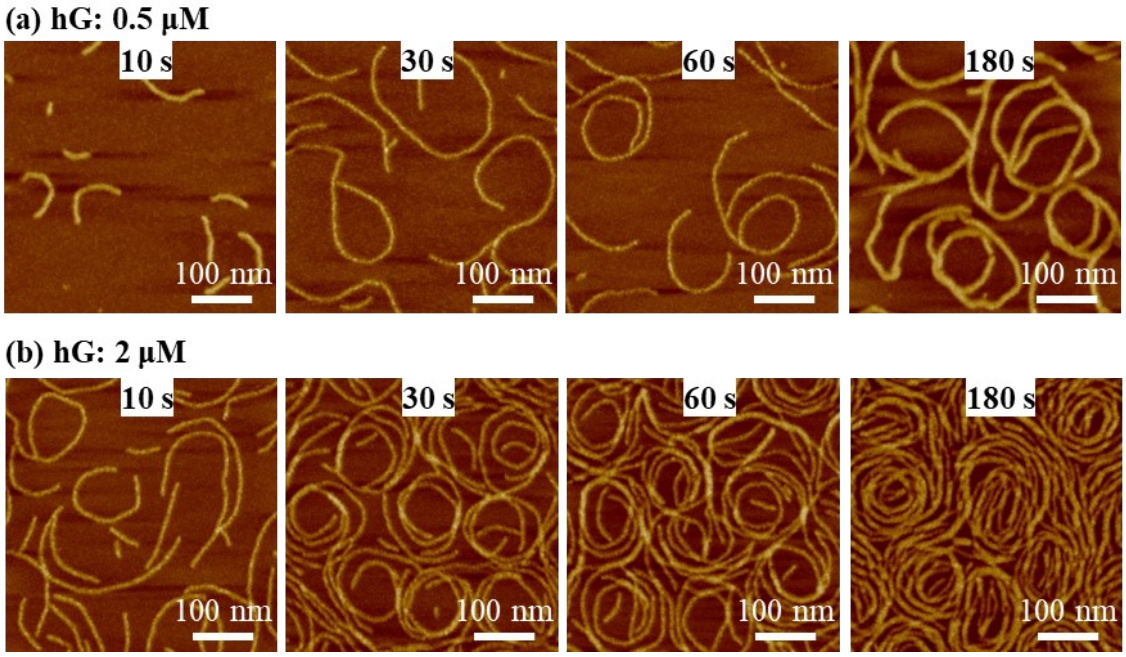


Figure S13. The assembly kinetics of **hG** at different DNA concentrations. (a) at 0.5 µM (b) at 2 µM.

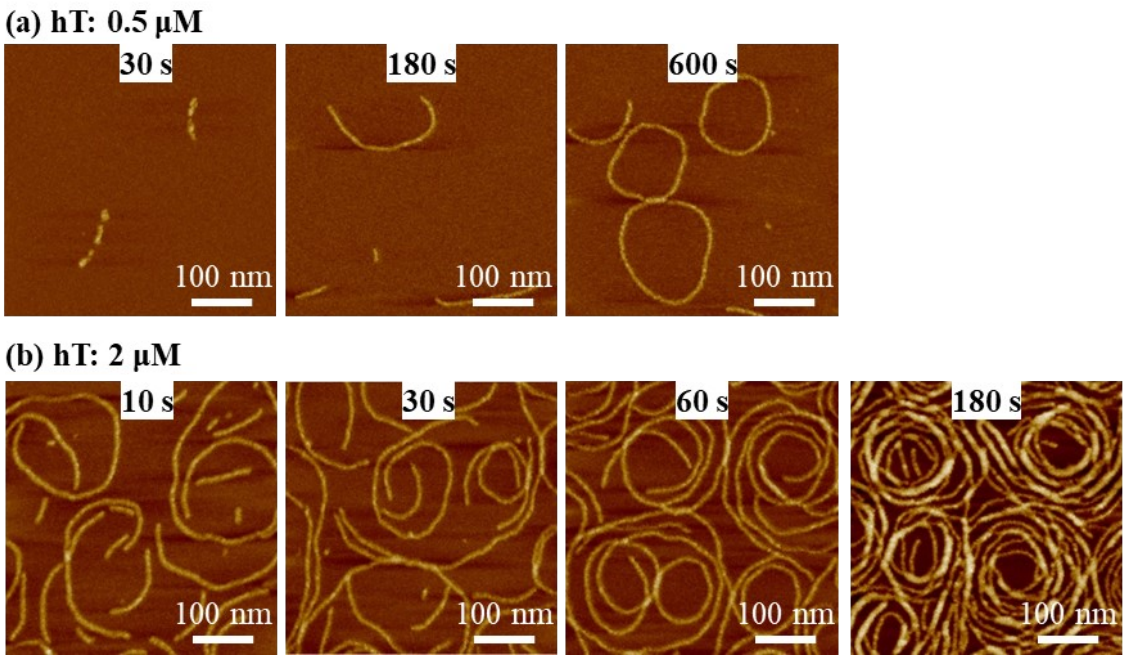


Figure S14. The assembly kinetics of **hT** at different DNA concentrations. (a) at 0.5 μM (b) at 2 μM.

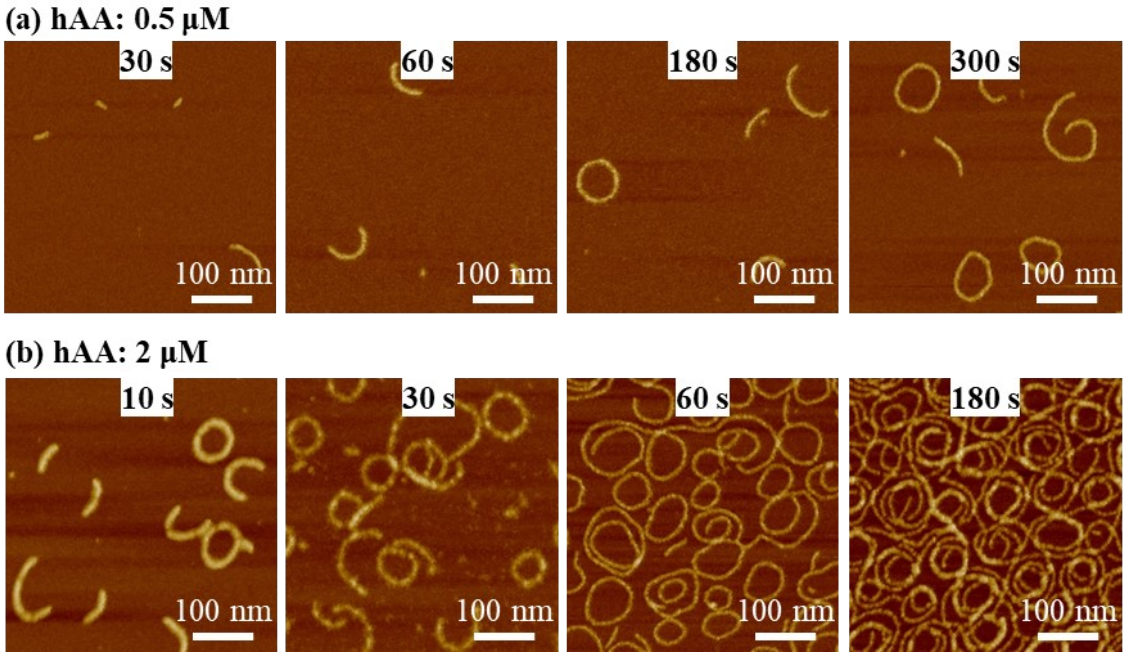


Figure S15. The assembly kinetics of **hAA** at different DNA concentrations. (a) at 0.5 μM (b) at 2 μM.

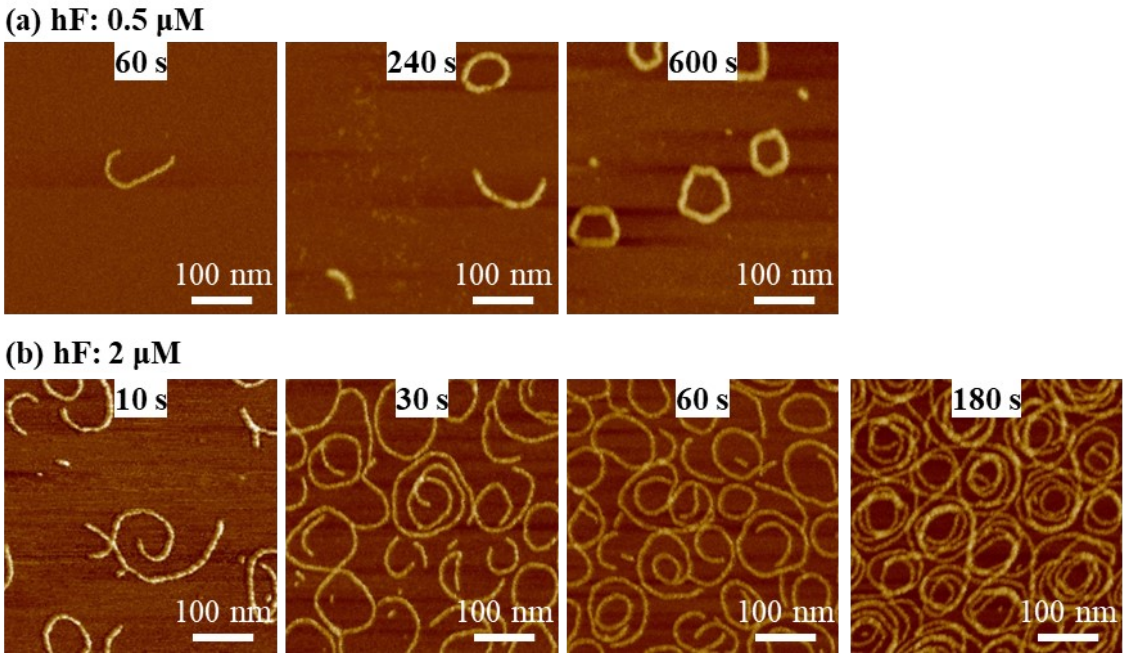


Figure S16. The assembly kinetics of **hF** at different DNA concentrations. (a) at 0.5 μM (b) at 2 μM.

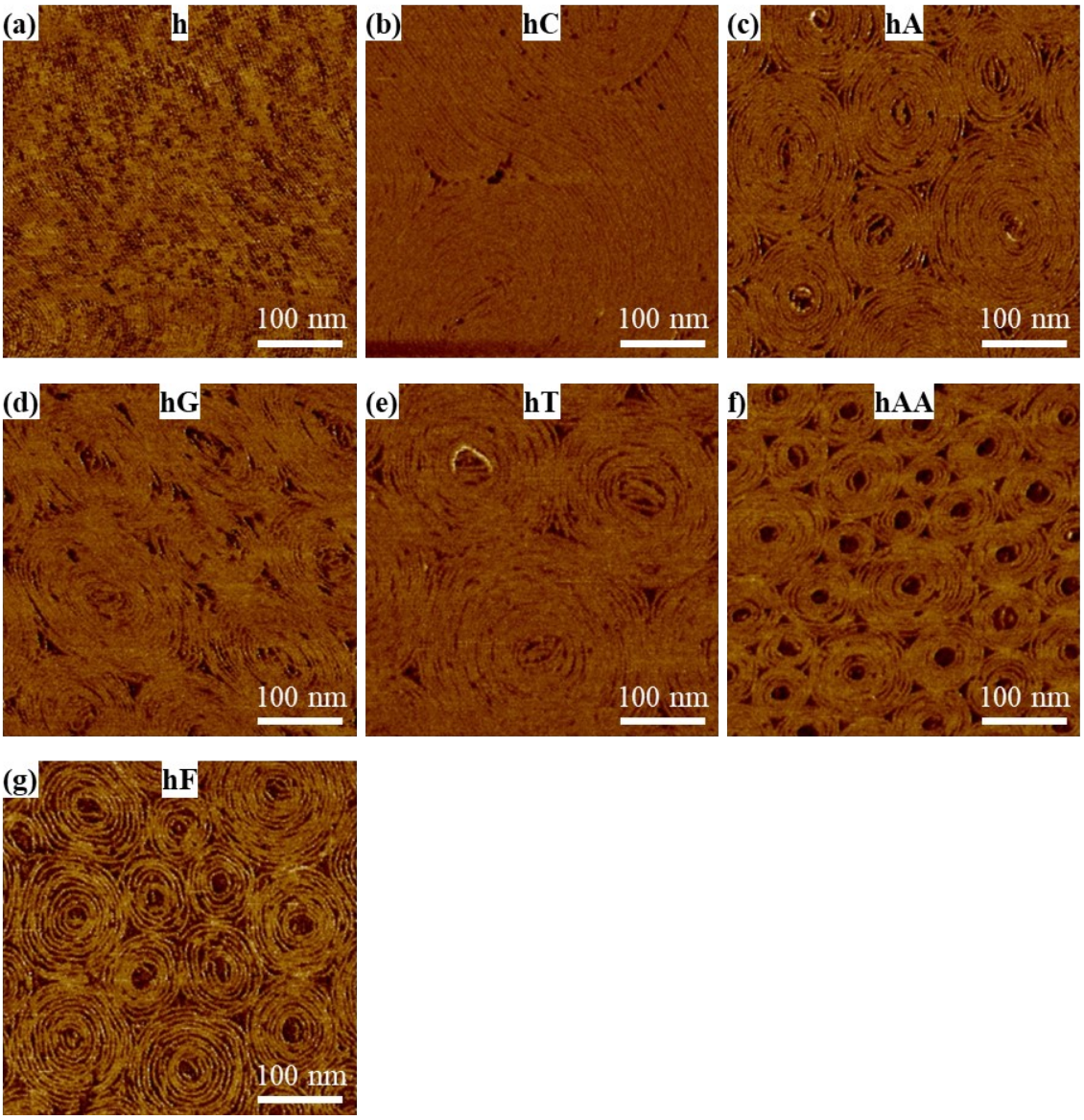


Figure S17. AFM images of thermodynamic slow annealing structures. (a) **h,** (b) **hC,** (c) **hA,** (d) **hG,** (e) **hT,** (f) **hAA,** and (g) **hF.**