

## Methods

**DNA strand design, synthesis, and purification:** DNA sequences were designed using the program *SEQUIN*.<sup>24</sup> The DNA strands with modifications, for example, biotinylated, were synthesized on an Applied Biosystems 394, removed from the support, and deprotected using routine phosphoramidite procedures. Other strands were purchased from Integrated DNA Technologies, Inc. All strands have been purified by denaturing gel electrophoresis (PAGE); bands were cut out of 15–20% denaturing gels and eluted in a solution containing 500 mM ammonium acetate, 11 mM magnesium acetate, and 1 mM EDTA. This is the only purification step in the entire procedure.

**Formation of Hydrogen-Bonded BTX Complexes:** The strands of each tile were mixed stoichiometrically as estimated by  $OD_{260}$  and dissolved to 0.5  $\mu\text{M}$  in TAE/ $\text{Mg}^{2+}$  buffer (40 mM Tris-HCl, 20 mM Acetic Acid, 2 mM EDTA, 12.5 mM Magnesium Acetate, pH 8.0). The solutions were slowly annealed from 90 °C to room temperature (RT) over 48 hours in a 2-litre water bath insulated in a Styrofoam box.

**Formation of Self-Replication Seeds:** Seven individual seed tiles were prepared using the protocol described above and were mixed stoichiometrically. The mixed solution was slowly annealed from 45 °C to RT over 24 hours in a 2-litre water bath insulated in a Styrofoam box. Seeds (seven tiles in length in specific sequence) with biotin and without biotin were prepared separately in the same condition. Seeds with biotin were used for AFM imaging only; seeds without biotin were used as starting material in the first step of self-replication. Before AFM imaging, seeds with biotin were incubated with streptavidin (biotin:streptavidin = 1:1) at room temperature for at least 6 hours.

**First Step of Self-Replication (Formation of First-Generation):** (1) Three first-generation tiles (I', A', and B') were prepared as described above. (2) Annealed seeds were mixed with annealed first-generation tiles (seeds:I':A':B'=1:2:4:8), slowly annealed

from 45 °C to RT over 24 hours in a 2-litre water bath insulated in a Styrofoam box. (3) Dynabeads (Invitrogen) were washed with ddH<sub>2</sub>O and TAE/Mg buffer, mixed with beads linker in TAE/Mg buffer, slowly annealed from 55 °C to RT by 5 °C/hour on a rotator in a programmable incubator, mixed with DNA solution prepared in step (2). (4) The above solution containing dynabeads was annealed from 33 °C to 23 °C by 1 °C/hour on a rotator in a programmable incubator. (5) Solution from (4) was placed on magnetic stand for two minutes and washed with TAE/Mg buffer. (6) Link 2, link 6 and link 9 were then added. (7) Solution was cooled down from 33 °C to 23 °C by 1 °C/hour on a rotator in a programmable incubator, placed on magnetic stand for two minutes and washed with TAE/Mg buffer and excess linkers washed away. (8) Dynabeads in TAE/Mg buffer were kept at 37 °C for one hour, placed on magnetic stand for two minutes at 37 °C. The solution was removed from dynabeads and stored in a clean tube for AFM imaging

**Second Step of Self-Replication (Formation of Second-Generation):** Formation of the second generation starts from initial seed preparation, followed by formation of the first generation in which steps (6) and (7), described above, were performed before step (5). (1) Three second-generation tiles (I'', A'', and B'') were prepared as described above. A strand fully complementary to 6C was added to the solution of first step self-replication and incubate at 25 °C for 2 hours. (2) Repeat step (2)-(8) described in formation of first-generation except that (6) and (7) were performed before (5).

**AFM Imaging:** All AFM imaging was performed in tapping-mode AFM in air. A 5-7 µL of DNA sample was spotted on freshly cleaved mica (Ted Pella, Inc.) and was left to absorb for 1 minute. Mica was washed with 3-5 drops of ddH<sub>2</sub>O three times and excess water was removed by blotting the mica with filter paper. The mica was then blown dry using compressed air. All AFM imaging was performed on a NanoScope IV MultiMode SPM (Digital Instruments), using commercial cantilevers with Silicon tips (Veecoprobes).