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69451 Weinheim, Germany

Complex Reconfiguration of DNA Nanostructures**

Bryan Wei,* Luvena L. Ong, Jeffrey Chen, Alexander S. Jaffe, and Peng Yin*

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Supporting Information 1

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S1 Methods

S1.1 DNA sequence design

DNA sequences of component strands for the 24 helix \times 29 turns (24 \times 29T) rectangular canvas were adopted from an earlier study.¹ A toehold segment (GGCCTCG) was added to the 3' end of specific component strands of the 24H \times 29T rectangular canvas for a control test. Sequences of the full set of carving strands, which are respectively reverse complementary to the full set of component strands, were generated (the sequences of strands which are reverse complementary to the specific component strands with toeholds were also generated). DNA sequences of component strands for the 10H \times 10H \times 80 base-pair (B) cuboid were adopted from another earlier study.² Sequences of a full set of carving strands which are respectively reverse complementary to the full set of component strands were generated. DNA strands were synthesized by Integrated DNA Technology, Inc (http://www.idtdna.com).

S1.2 Sample preparation.

- To assemble the structure of 24H×29T rectangular canvas, component DNA strands were mixed in a roughly equimolar concentration of 100 nM in 0.5×TE buffer (5 mM Tris, pH 7.9, 1 mM EDTA) supplemented with 15 mM MgCl₂. The mixtures was then annealed in a PCR thermo cycler by the following protocol: 65 °C for 15 min and then 48 °C for 15 hours. The product of rectangle canvas was then analyzed using a 2% native agarose gel electrophoresis, which resulted in one dominant band. Depending on the desired subsequent carving step, the canvas was either directly imaged under atomic force microscopy (AFM) imaging or imaged after excision and purification from the gel band.
 - 1. For carving of intaglio set of alphabet (and all the control tests used in Fig. 3), a set of carving strands that are complementary to the specific set of component strands were added to the annealed sample in equimolar ratio (some control experiments used an excess amount of carving strands) with an incubation at 45 °C for 15 hours. The annealed samples of the canvas and the carved shapes were then subjected to 2% agarose gel electrophoresis (gel prepared in 0.5×TBE buffer supplemented with 10 mM MgCl₂ and pre-stained with SYBR safe) in an ice water bath. The target gel bands were excised out and put into a Freeze N' Squeeze column (Bio-Rad). The gel pieces were crushed using a microtube pestle in the column and the column was then directly subjected to centrifuge at 438 g for 3 minutes. Purified samples were collected in the eluate, and concentrations were determined by Nanodrop absorption at 260 nm prior to AFM imaging.
 - 2. For carving of relief set of alphabet, the canvas was annealed as described in point 1. The annealed canvas were then subjected to 2% agarose gel electrophoresis (gel prepared in 0.5×TBE buffer supplemented with 10 mM MgCl₂ and pre-stained with SYBR safe) in an ice water bath. The target gel band containing canvas was excised out and put into a Freeze N' Squeeze column (Bio-Rad). The gel pieces were crushed using a microtube pestle in the column and the column was then directly subjected to centrifuge at 438 g for 3 minutes. Purified samples were collected in the eluate, and concentrations were determined by Nanodrop absorption at 260 nm. A set of carving strands which are complementary to the specific set of component strands were added to the annealed sample in equimolar ratio with an incubation at 35 °C for 15 hours. The samples after carving were subjected to AFM imaging.
- To assemble the structure of 10H×10H×80B cuboid, component DNA strands were mixed to a roughly equal molar concentration of 200 nM in 0.5×TE buffer (5 mM Tris, pH 7.9, 1 mM EDTA) supplemented with 40 mM MgCl₂. The mixture was then annealed in a PCR thermo cycler by cooling from 80 °C to 60 °C over 2 hours, followed by cooling from 60 °C to 25 °C over 72 hours. The annealed samples were then subjected to 2% agarose gel electrophoresis (gel prepared in 0.5×TBE buffer supplemented with 10 mM MgCl₂ and prestained with SYBR safe) in an ice water bath. The target gel band of the cuboid was excised out and put into a Freeze N' Squeeze column (Bio-Rad). The gel pieces were crushed using a microtube pestle in the column and the column was then directly subjected to centrifuge at 700 g for 5 minutes. Purified samples were collected in the eluate, and concentrations were determined by Nanodrop absorption at 260 nm. A set of carving strands which are complementary to the specific set of component strands respectively were added to the annealed sample

in equimolar ratio with an incubation at 28 °C for 3 hours. The samples after carving were subjected to TEM imaging.

S1.3 AFM imaging.

AFM images were obtained using an SPM Multimode with Digital Instruments Nanoscope V controller (Vecco). A 5 μ L droplet (2 to 10 nM) of annealed (or purified) sample and then a 40 μ L drop of $0.5 \times \text{TE}$ / 10mM MgCl₂ solution was applied to a freshly cleaved mica surface and left for approximately 2 min. Sometimes, additional dilution of the sample was performed to achieve the desired sample density. As for the structures of relief set of alphabet, 20 μ L supplemental 10mM NiCl₂ was added to increase the strength of DNA-mica binding.³ The images were taken under the liquid tapping mode, with C-type triangular tips (resonant frequency, $f_0 = 40 - 75kHz$; spring constant, $k = 0.24Nm^{-1}$) from the SNL-10 silicon nitride cantilever chip (Bruker Corparation).

S1.4 TEM imaging.

For TEM imaging, 2.5 μ L sample (1-5 nM) was adsorbed onto glow discharged carbon-coated TEM grids for 2 minutes and then stained for a few seconds using a 2% aqueous uranyl formate solution containing 25 mM NaOH. Imaging was performed using a JEOL JEM-1400 TEM operated at 80 kV.

S1.5 Yield quantification by gel electrophoresis and AFM.

Yield was estimated by analysis using native agarose gel electrophoresis, pre-stained with SYBR Safe DNA stain. The ratio between the fluorescence intensity of the product band and that of the entire lane was taken as an estimate of the gross yield of structural formation. Yield was also estimated by analyzing AFM image of a certain carved shape (annealed sample without purification). The ratio between the number of well-formed shapes and the total number of identifiable shapes was defined as AFM yield.

S2 Carving of two sets of alphabets

S2.1 Schematics and AFM images of carved alphabets

Fig. S1 shows the indexing of component strands set.



Figure S1. Index of the 2D canvas for carving. (a) Strand diagram with index. (b) Brick view diagram (as shown in the computer program for designing structures, Fig. S2 and S3) with index. The numbers on the leftmost or rightmost refer to the row numbers and the numbers at the bottom refer to the column numbers. x-y is the coordinate index of a specific component strand (x: column number; y: row number). For example, component strand 2-7 refers to strand in column 2 and row 7. The carving strand set shares the same index with the component strand set.

Fig. S2 and S3 shows the diagrams and AFM images of two sets of carved alphabets.



Figure S2. Diagrams and AFM images for the carving of a set of alphabet (intaglio). Diagrams are shown in top panels (SSTs to be carved out are highlighted in blue) and the corresponding AFM images are shown at the bottom (scanning size: $150 \text{ nm} \times 150 \text{ nm}$).



Figure S3. Diagrams and AFM images for the carving of a set of alphabet (relief). Diagrams are shown in top panels (SSTs to be carved out are highlighted in blue) and the corresponding AFM images are shown at the bottom (scanning size: $150 \text{ nm} \times 150 \text{ nm}$).

S2.2 Agarose gel electrophoresis results

Fig. S4 shows the results of native agarose gel electrophoresis for the carved set of alphabet (intaglio). The gel-purified samples after annealing were subjected to AFM imaging, with results shown in Fig. S6 to Fig. S11.



Figure S4. Agarose gel electrophoresis analysis for the carving of a set of alphabet (intaglio). Labels above gel lanes indicate letters carved out (e.g. letter a refers to the shape 'a' carved out); DL: 1 kb DNA ladders; CV: the plain canvas. Mixtures containing 100 nM of each component strand were annealed in $0.5 \times$ TE buffer supplemented with 15 mM MgCl₂ at 48°C for 15 hours. A specific set of carving strands was added to the annealed canvas sample with molar ratio between component strands and carving strands to be 1:1 and the carving sample was incubated in 45°C for 15 hours. Then, a 10 μ L carving sample (mixed with 2 μ L 6× bromophenol blue loading dye) was loaded into a 2% native agarose gel and subjected to electrophoresis in an ice water bath with 0.5× TBE running buffer (supplemented with 10 mM MgCl₂). Gross assembly yields calculated from the intensity ratio between the target band of plain canvas and the entire lane are 28% and 34% respectively for lanes CV. In principle, the carving yield can be calculated by the intensity ratio between a target band after carving and the plain canvas band. However, a carved shape shows no apparent mobility difference to the intact canvas and the target band after carving is likely a mixture of carved shape, partially carved shape and un-carved shape, which makes the yield analysis challenging. Therefore, quantitative gel analysis is not provided.

Fig. S5 shows the results of native agarose gel electrophoresis for carved structures of a set of alphabet (relief). The gel-purified samples after annealing were subjected to AFM imaging shown in Fig. S12 to Fig. S15.



Figure S5. Agarose gel electrophoresis analysis for the carving of a set of alphabet (relief). Labels above gel lanes indicate letters carved out (e.g. letter a refers to the shape 'a' carved out). DL: 1 kb DNA ladders; CV: the plain canvas. Mixtures containing 100 nM of each component strand were annealed in $0.5 \times$ TE buffer supplemented with 15 mM MgCl₂ at 48°C for 15 hours. The target band was extracted and purified by centrifugation, with the concentration estimation by the measurement of ultraviolet absorption at 260 nm. A specific set of carving strand was added to the purified canvas sample with molar ratio between component strands and carving strands to be 1:1 and the carving sample was incubated in 35° C for 15 hours. Then, a 10 μ L carving sample (mixed with 2 μ L 6× bromophenol blue loading dye) was loaded into a 2% native agarose gel and subjected to electrophoresis in an ice water bath with $0.5 \times$ TBE running buffer (supplemented with 10 mM MgCl₂). In principle, the carving yield can be calculated by the intensity ratio between a target band after carving and the plain canvas band. However, some carved shapes show very little mobility difference to the plain canvas, which makes the yield analysis challenging. Therefore, quantitative gel analysis is not provided.

S2.3 AFM imaging results

Figs. S6-S11 show large AFM images for carved structures of a set of alphabet (relief).



Figure S6. AFM image of the carved structures of a set of alphabet (intaglio) 1 (scale bar: 500 nm).



Figure S7. AFM image of the carved structures of a set of alphabet (intaglio) 2 (scale bar: 500 nm).



Figure S8. AFM image of the carved structures of a set of alphabet (intaglio) 3 (scale bar: 500 nm).



Figure S9. AFM image of the carved structures of a set of alphabet (intaglio) 4 (scale bar: 500 nm).



Figure S10. AFM image of the carved structures of a set of alphabet (intaglio) 5 (scale bar: 500 nm).



Figure S11. AFM image of the carved structures of a set of alphabet (intaglio) 6 (scale bar: 500 nm).

Figs. S12-S15 show large AFM images for carved structures of a set of alphabet (intaglio).



Figure S12. AFM image of the carved structures of a set of alphabet (relief) 1 (scale bar: 500 nm).



Figure S13. AFM image of the carved structures of a set of alphabet (relief) 2 (scale bar: 500 nm).



Figure S14. AFM image of the carved structures of a set of alphabet (relief) 3 (scale bar: 500 nm).



Figure S15. AFM image of the carved structures of a set of alphabet (relief) 4 (scale bar: 500 nm).

S3 Mechanism study of carving

S3.1 Agarose gel electrophoresis results

Fig. S16 shows the results of native agarose gel electrophoresis for a certain shape carved from canvas with and without predesigned external toeholds for specific components to be displaced. The carved samples were subjected to AFM imaging shown in Fig. S19 and Fig. S20. Fig. S17 shows the results of native agarose gel electrophoresis for certain shapes carved from canvas without external toeholds for specific components to be displaced in different carving patterns. The carved samples were subjected to AFM imaging shown in Fig. S21 and Fig. S23. Fig. S18 shows the results of native agarose gel electrophoresis for reversible carving. The respective samples were subjected to AFM imaging shown in Fig. S25 and Fig. S26.

Note that the number depicted below a target band indicates the ratio between the intensity of the target band and that of the canvas in the control lane. This number provides a rough approximation of the carving yield. When interpreting this yield number, we should note that it does not capture the following factors. First, the analysis considers only the intensity of the canvas and does not include multimers that may be present in the mixture. Second, the analysis does not account for the size difference between the carved shape and the intact canvas. In principle, the yield could be further adjusted by accounting for the number of components present in the carved structure versus that in the original canvas; however, such adjustment would assume that the intensity is linearly proportional to the size of the structure. As such, this intensity ratio is likely not an accurate measure for the absolute yield, but should be still useful as a relative metric for comparing carving yields for carving systems that produce similar target shapes carved from similar canvas.



Figure S16. Agarose gel electrophoresis analysis for the carving with and without external toeholds. DL: 1 kb DNA ladders; CV': the canvas with external toeholds. 1'-1: carving in pattern 1, with toeholds, molar ratio between carving strands and component strands to be 1:1; 1'-2: carving in pattern 1, with toeholds, molar ratio between carving strands and component strands to be 2:1; 1'-3: carving in pattern 1, with toeholds, molar ratio between carving strands and component strands to be 1:1; 1-2: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 1:1; 1-2: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 1:1; 1-2: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 1:1; 1-2: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 2:1; 1-3: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 2:1; 1-3: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 2:1; 1-3: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 2:1; 1-3: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 2:1; 1-3: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 2:1; 1-3: carving in pattern 1, without toeholds, molar ratio between carving strands and component strands to be 3:1. Numbers indicate yields calculated from the intensity ratio between the target band and that of the canvas from lane CV. Mixtures containing 100 nM of each component strand were annealed in $0.5 \times$ TE buffer supplemented with 15 mM MgCl₂ at 48°C for 15 hours. A specific set of carving strands were added to the annealed canvas sample with molar ratio between component and carving strands to be 1:1, 2:1



Figure S17. Agarose gel electrophoresis analysis for the carving without external toeholds in different carving patterns. DL: 1 kb DNA ladders; CV: the canvas without toeholds. For subsequent samples, the first number indicates the carving pattern and the second number indicates the ratio between carving and component strands. For example, 1-2: carving in pattern 1, molar ratio between carving strands and component strands to be 2:1. Mixtures containing 100 nM of each component strand were annealed in $0.5 \times$ TE buffer supplemented with 15 mM MgCl₂ at 48°C for 15 hours. A specific set of carving strands were added to the annealed canvas sample with molar ratio between component strands and carving strands to be 1:1, 2:1 or 3:1 and the carving sample was incubated in 45° C for 15 hours. Then, a 10 μ L carving sample (mixed with 2 μ L 6× bromophenol blue loading dye) was loaded into a 2% native agarose gel and subjected to electrophoresis in an ice water bath with 0.5× TBE running buffer (supplemented with 10 mM MgCl₂).



Figure S18. Agarose gel electrophoresis analysis for the reversible carving . DL: 1 kb DNA ladders; CV: the canvas without toeholds. 4: carving in pattern 4; CVR: canvas reconfigured from the carved sample in pattern 4; R5: carving in pattern 5 from the reconfigured canvas shown in lane CVR; 5: carving in pattern 5; Mixture containing 100 nM of each component strand was annealed in $0.5 \times$ TE buffer supplemented with 15 mM MgCl₂ at 48°C for 15 hours. A specific set of carving strands were added to the annealed canvas sample with molar ratio between component strands and carving strands to be 1:1 and the carving sample was incubated in 45°C for 15 hours. The component strands to be 2:1. Such a sample was incubated in 45°C for 15 hours for canvas re-assembly. Then another set of carving strands to be 1:1 and the carving sample with molar ratio between component strands added to the re-assembled canvas sample with molar ratio between shows added to the re-assembled canvas sample with molar ratio between component strands to be 2:1. Such a sample was incubated in 45°C for 15 hours for canvas re-assembly. Then another set of carving strands to be 1:1 and the carving sample with molar ratio between component strands added to the re-assembled canvas sample with molar ratio between component strands and carving strands to be 1:1 and the carving sample was incubated in 45°C for 15 hours. Carving in pattern 4 from the plain canvas was adopted as controls (molar ratio between component strands and carving strands to be 1:1 and the carving sample or ones with equivalent molar concentration (mixed with 2 μ L 6× bromophenol blue loading dye) was loaded into a 2% native agarose gel and subjected to electrophoresis in an ice water bath with 0.5× TBE running buffer (supplemented with 10 mM MgCl₂).



Figure S19. AFM image of the carved structures in pattern 1 from canvas without external toeholds (scale bar: 500 nm).



Figure S20. AFM image of the carved structures in pattern 1 from canvas with external toeholds (scale bar: 500 nm).



Figure S21. AFM image of the carved structures in pattern 2 from canvas without external toeholds (scale bar: 500 nm).



Figure S22. AFM image of the carved structures in pattern 3 from canvas without external toeholds (scale bar: 500 nm).



Figure S23. AFM image of the carved structures in pattern 4 from canvas without external toeholds (scale bar: 500 nm).



Figure S24. AFM image of the carved structures in pattern 5 from canvas without external toeholds (scale bar: 500 nm).



Figure S25. AFM image of the re-assembled canvas from carved structures in pattern 4 (scale bar: 500 nm).



Figure S26. AFM image of the carved structures in pattern 5 from re-assembled canvas (scale bar: 500 nm).

S3.3 Yield analysis based on AFM imaging

Figs. S27-S32 give the carving yield analysis for structures carved in different patterns.



Figure S27. Yield calculation for AFM image of the carved structures in pattern 1 from canvas without external toeholds (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of carved structures was 14% (N = 177). This yield value is higher than the yield calculated from agarose gel electrophoresis (0% in Fig. S16). Note that AFM yields appeared higher than gel yields (50% in Fig. S17), which might reflect that partially carved structures co-migrated with intact canvas on the gel, but broke apart under AFM (e.g. during deposition or imaging process). Such an elevated AFM yield was also observed in carving patterns 1', 2 and 4.



Figure S28. Yield calculation for AFM image of the carved structures in pattern 1 from canvas with external toeholds (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of carved structures was 81% (N = 97).



Figure S29. Yield calculation for AFM image of the carved structures in pattern 2 from canvas without toeholds (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of carved structures was 36% (N = 131).



Figure S30. Yield calculation for AFM image of the carved structures in pattern 3 from canvas without external toeholds (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of carved structures was 99 % (N = 202). This yield value is higher than the yield calculated from agarose gel electrophoresis (50% in Fig. S16). The difference is due to different standards of estimation. The gel yield is based on the fluorescent intensity between the target band and the canvas band. Although the band intensity of the target band appears lower, it is the only prominent band in that gel lane. This indicates a very limited partial carving which is consistent with the AFM yield study.



Figure S31. Yield calculation for AFM image of the carved structures in pattern 4 from canvas without external toeholds (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of carved structures was 89 % (N = 174).



Figure S32. Yield calculation for AFM image of the carved structures in patten 5 from canvas without external toeholds (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of well-formed structures was 79% (N = 72).



Figure S33. Yield calculation for AFM image of the re-assembled canvas from the carved structure in pattern 4 (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were not fully reconfigured and the structures marked with red circles filled with blue dots were reconfigured. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of well-formed structures was 78% (N = 214).



Figure S34. Yield calculation for AFM image of the carved structures in pattern 5 from re-assembled canvas (scanning size: 2 μ m × 2 μ m). The structures marked with empty red circles were non-carved or partially carved and the structures marked with red circles filled with blue dots were carved. The yield was calculated as the ratio between the number of carved and the total number of selected structures. According to our analysis, the yield of well-formed structures was 72% (N = 237).

S4 3D carving

Fig. S35 and Fig. S36 show the indexing of component strands set.



Figure S35. Index of the 3D cuboid for carving. (a) Voxel index. Different voxel planes are indexed from 0-11according to the positions along the helical direction. (b) Helix index. Different helices are indexed from 0-99. (c) 3D view. A specific strand is indexed using by four or more of its constituent domains. For example, index [21, 7, 21, 8, 22, 7, 22, 8] means that the strand contains a first domain in helix 21 and voxel plane 7, a second domain in helix 21 and voxel plane 8, a third domain in helix 22 and voxel plane 7, and a fourth domain in helix 22 and voxel plane 8. The carving strands set share the same index with the component strands set.



Figure S36. Strand diagram the 3D cuboid for carving. The numbers on the left and right indicate the helix number and the numbers on the top and bottom indicate the voxel plane number.

S4.1 Agarose gel electrophoresis results

Fig. S37 shows the results of native agarose gel electrophoresis for the 3D carved structures from the cuboid. The carved samples from gel-purified cuboid after annealing were subjected to TEM imaging shown in Fig. S39 to Fig. S41.



Figure S37. Agarose gel electrophoresis analysis for 3D carving. DL: 1 kb DNA ladders; CB: the plain cuboid. 1: carved structure 1 (a corner off); 2: carved structure 2 (a tunnel through); 3: carved structure 3 (cut in halves). Numbers above and below bands indicate the yield of the band relative to the total intensity of the lane. Mixtures containing 200 nM of each component strand were annealed in $0.5 \times$ TE buffer supplemented with 40 mM MgCl₂ from 90°C to 25°C over 72 hours. The target band was extracted and purified by centrifugation, with the concentration estimation by the measurement of ultraviolet absorption at 260 nm. A specific set of carving strand was added to the purified canvas sample with molar ratio between component strands and carving strands to be 1:1 and the carving sample was incubated in 27°C for 3 hours. Then, a 10 μ L sample (mixed with 2 μ L 6× bromophenol blue loading dye) was loaded into a 2% native agarose gel and subjected to electrophoresis in an ice water bath with $0.5 \times$ TBE running buffer (supplemented with 10 mM MgCl₂).

S4.2 TEM imaging results

Figs. S38-S41 show TEM images for structures the cuboid for carving and the carved structures.



Figure S38. TEM images of the cuboid for 3D carving $(scale \ bars: \ 100 \ nm).$



Figure S39. TEM images of a carved structure from the cuboid (a corner off) (scale bars: 100 nm).



Figure S40. TEM images of a carved structure from the cuboid (a tunnel through) (scale bars: 100 nm).



Figure S41. TEM images of a carved structure from the cuboid (cut in halves) (scale bars: 100 nm).

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