Supplementary Information

Protein-coated dsDNA nanostars with high structural rigidity and high enzymatic and thermal stability

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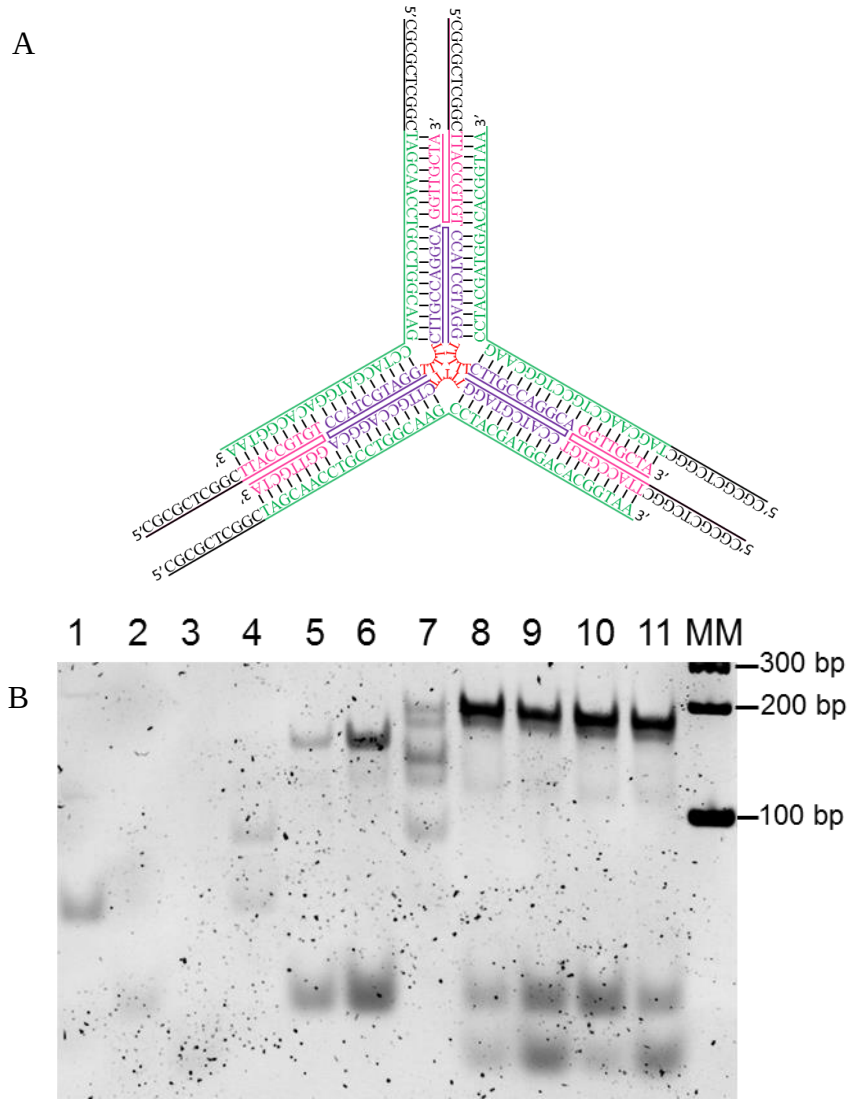

Figure S1. Electrophoresis gel of thermal annealing of CLS DNA junction. 1) Oligo C, 2) Oligo L, 3) Oligo S, 4) CL 1:1, 5) CL 1:3, 6) CL 1:6, 7) CLS 1:1:1, 8) CLS 1:3:3, 9) CLS 1:3:6, 10) CLS 1:6:3, 11) CLS 1:6:6. MM: Molecular markers. Total time of annealing was 151 minutes (see details in Methods and Materials Section of the main manuscript).

Table S1. Sequences of oligos

Name	Sequence 5' → 3'
CLS dsDNA NANOSTARS	
	CLS junction
C	CCATCGTAGGTTTTTCTTGCCAGGCACCATCGTAGGTTTTTCTTGCCAGGCACCATCG TAGGTTTTTCTTGCCAGGCA
L-OH_{10}	CGCGCTCGGCTAGCAACCTGCCTGGCAAGCCTACGATGGACACGGTAA
S-OH_{10}	CGCGCTCGGCTTACCGTGTGGTTGCTA
L-OH_{15}	TCCCACGCGCTCGGCTAGCAACCTGCCTGGCAAGCCTACGATGGACACGGTAA
S-OH_{15}	TCCCA CGCGCTCGGCTTACCGTGTGGTTGCTA
L-OH_{20}	GCTGCTCCCACGCGCTCGGCTAGCAACCTGCCTGGCAAGCCTACGATGGACACGGTA A
S-OH_{20}	GCTGCTCCCACGCGCTCGGCTTACCGTGTGGTTGCTA
	CLS dsDNA Fragments (with sticky-end overhangs)
FwOH_{10}-S-P	GCCGAGCGCG(PEG_3)CGCAACTGTTGGGAAGGGCG
FwOH_{15}-S-P	GCCGAGCGCGTGGGA(PEG_3)CGCAACTGTTGGGAAGGGCG
FwOH_{20}-S-P	GCCGAGCGCGTGGGAGCAGC(PEG_3)CGCAACTGTTGGGAAGGGCG
FwOH_{10}-P	GCCGAGCGCGCGCAACTGTTGGGAAGGGCG
FwP	CGCAACTGTTGGGAAGGGCG
Rv211bp	TCTGGACAAGACACGTGGCC
Rv367bp	CACCACCACAACCACCACCG
Rv539bp	GCAACACGTTTTGCAACCTGTTTG
Rv722bp	TCAGTGAGCGAGGAAGCGGA
Rv722bp-OH_{10}-S-P	AAAGGCCCCC(PEG_3)TCAGTGAGCGAGGAAGCGGA
Stv-Bt dsDNA NANOSTARS (biotinylated dsDNA fragments)	
Fw201bp	GGTTGCAAAACGTGTTGCAGCA
Fw368bp	GGTTGTGGTGGTGAAATTCGTGC
Fw555bp	GGCGAATTGAAGGAAGGCCG
Fw732bp	GCCGAGCGCGCGCAACTGTTGGGAAGGGCG
Rvbiotin	Biotin-TCAGTGAGCGAGGAAGCGGA

Table S2. Sequence of plasmid template used to amplify the dsDNA

5’CTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAA
ATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATTCGCCAT
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CTGCAAGAACTGGCAGTTCAGAAAGGTTGGCGTCTGCCGGAATATACCGTTGCACAAGAAAGCGGTCCGCCTCATAAACG
TGAATTTACCATTACCTGTCGTGTTGAAGGTGGTAGCGGTGGTGGTTGTGGTGGTGAAATTCGTGCACTGAAATATGAAAT
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ACGATAAAACGCAATACGCTGGCTATCCGGTGCCGCAATGCCATACAGCACCAGAAAACGATCCGCCCATTCGCCGCCCA
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CGCTTCCATACGGGTACGCGCACGTTCAATACGATGTTTCGCCTGATGATCAAACGGACAGGTCGCCGGGTCCAGGGTAT
GCAGACGACGCATGGCATCCGCCATAATGCTCACTTTTTCTGCCGGCGCCAGATGGCTAGACAGCAGATCCTGACCCGGC
ACTTCGCCCAGCAGCAGCCAATCACGGCCCGCTTCGGTCACCACATCCAGCACCGCCGCACACGGAACACCGGTGGTGGC
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ACCCTGCGCGCTCAGACGAAACACCGCCGCATCAGAGCAGCCAATGGTCTGCTGCGCCCAATCATAGCCAAACAGACGTT
CCACCCACGCTGCCGGGCTACCCGCATGCAGGCCATCCTGTTCAATCATACTCTTCCTTTTTCAATATTATTGAAGCATTTAT
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CGAAAAGTGCCAC-3’

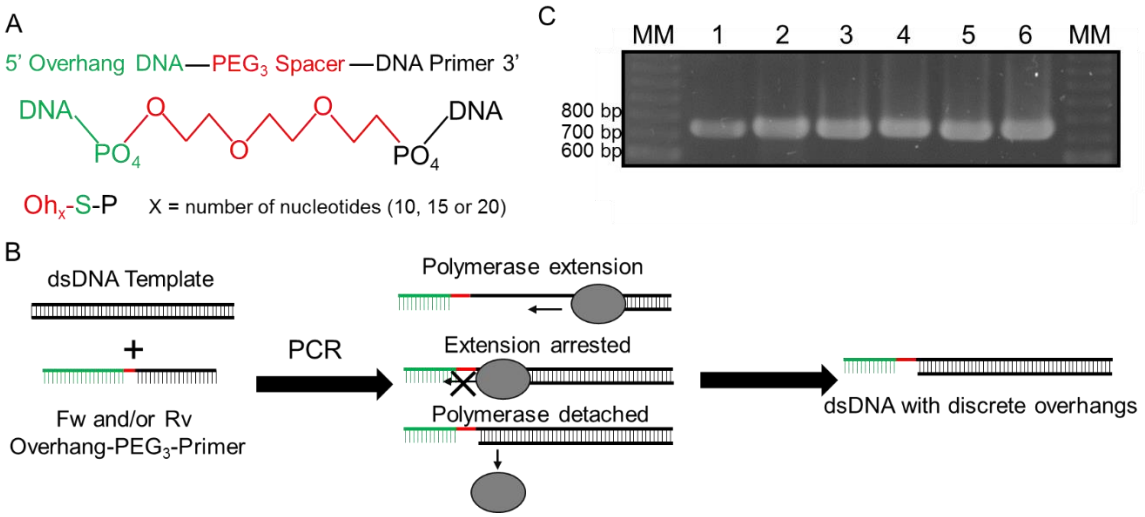

Figure S2. PCR production of dsDNA fragments with discrete sticky-end overhangs. A) Design of the modified PCR oligos: Oh_X-S-P, where "Oh_X" is the sticky-end overhang with *x* number of nucleotides, "S" is the PEG_3 spacer and "P" the Fw primer. B) Diagram explaining the PCR production of the dsDNA fragments with overhangs. C) Electrophoresis in agarose gel of dsDNA fragments produced with PCR end point using different oligos, 1) Fw P (without overhang and spacer) + Rv P, 2) Fw Oh_{10}-P + Rv P, 3) Fw Oh_{10}-S-P + Rv P, 4) Fw Oh_{10}-S-P + Rv Oh_{10}-S-P, 5) Fw Oh_{15}-S-P + Rv P, and 6) Fw Oh_{20}-S-P + Rv P. MM: molecular markers. Table S1 has the sequences used.

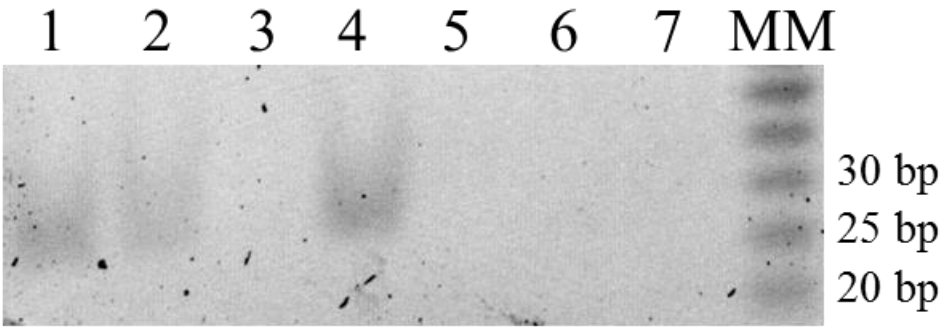

Figure S3. dsDNA fragment with discrete overhangs binding to oligo with complementary sequence. Oligo L-OH_{10} mixed with dsDNA fragment bearing: 1) not spacer (S) nor Overhang (Oh) (blunt ends), 2) overhang Oh_{10} but not spacer (blunt ends), 3) overhang Oh_{10} and Spacer S, 4) oligo L-OH_{10}, 5) dsDNA fragment bearing Oh_{10}-S in both ends 6) overhang Oh_{15} and spacer S, and 7) overhang Oh_{20} and spacer S. MM: molecular markers.

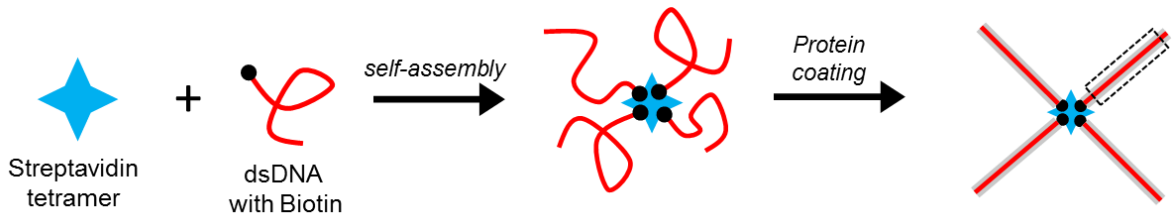

Figure S4. Preparation of DNA nanostars through self-assembly of biotinylated dsDNA fragments and streptavidin tetramer.

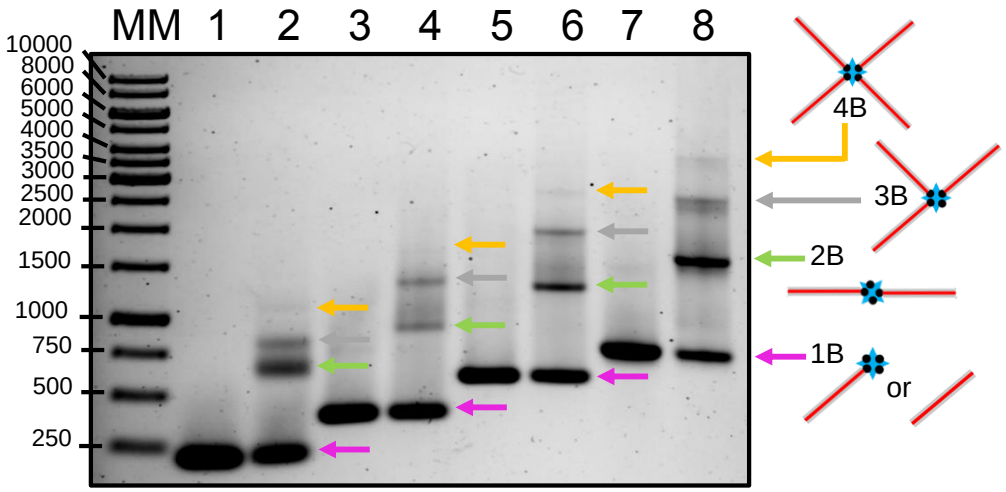

Figure S5. Self-assembly of branched DNA nanostars. Electrophoretic Mobility Shift Assays showing the results of the complexes formed after incubation of streptavidin (represented as blue coloured center in the cartoons) incubated with biotinylated linear dsDNA fragments in a 1:4 molar excess. MM: Molecular marker, dsDNA 201 bp alone (1) and with streptavidin (2), dsDNA 368 bp alone (3) and with streptavidin (4), dsDNA 555 bp alone (5) and with streptavidin (6) and dsDNA 732 bp alone (7) and with streptavidin (8). MM: Molecular markers.

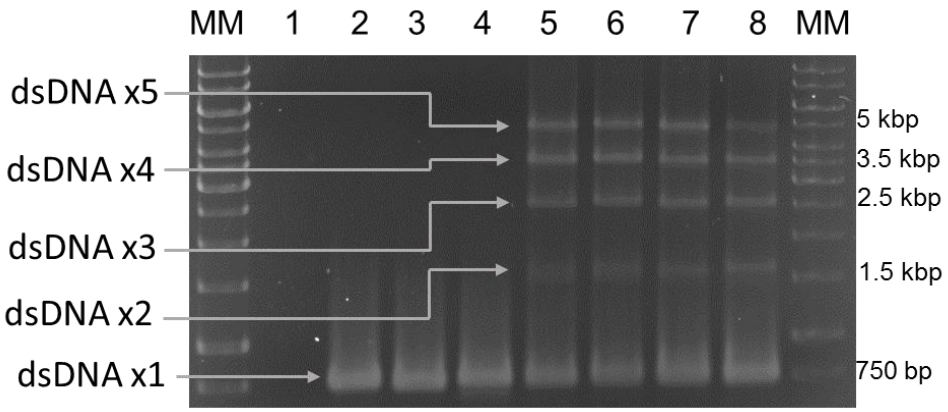

Figure S6. Self-assembly of branched DNA nanostars. A six-arm junction was incubated with linear dsDNA of 722 bp bearing discrete overhangs. CLS junction (1), 5' Oh_{10}-L-DNA (2), 5' DNA + CLS junction (3), 5' Oh_{10}-DNA + CLS junction (4), 5' Oh_{10}-L-DNA + CLS junction (5), 5' and 3' Oh_{10}-L-DNA + CLS junction (6), 5' Oh_{15}-L-DNA + CLS junction (7), and 5' Oh_{20}-L-DNA + CLS junction (8). MM: molecular markers.

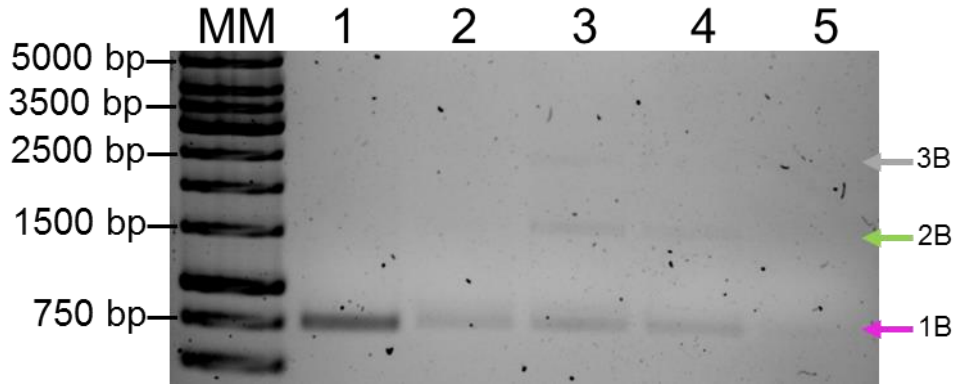

Figure S7. Disassembly of purified CLS dsDNA nanostars. Agarose gel of purified bands (by electroelution) of CLS dsDNA nanostars with 1 branch (1), 2 branches (2), 3 branches (3), 4 branches (4) and 5 branches (5). MM: molecular markers. Bands with lower branching than the original are observed, suggesting their disassembly.

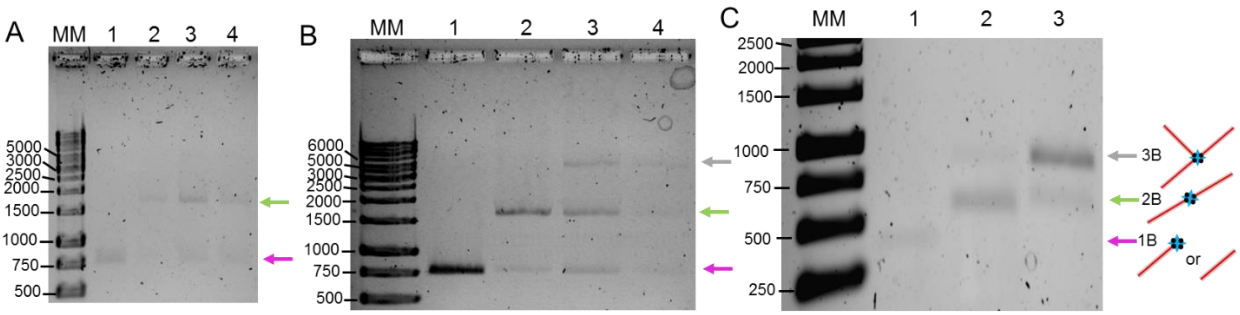

Figure S8. Electrophoresis in agarose gel of bands of Stv-Bt dsDNA nanostars purified using: A) electroelution in dialysis membrane (Stv-Bt dsDNA 732 bp),[1] B) electroelution in sacarose solution[2] (Stv-Bt dsDNA 732 bp), and C) Freeze 'N Squeeze™ DNA Gel Extraction Spin Columns (Bio-Rad Laboratories, Inc., Cal) (Stv-Bt dsDNA 368 bp). Top number indicates the number of arms of the purified dsDNA nanostructure. MM: molecular markers. Bands with lower valency than the original are observed, suggesting their disassembly.

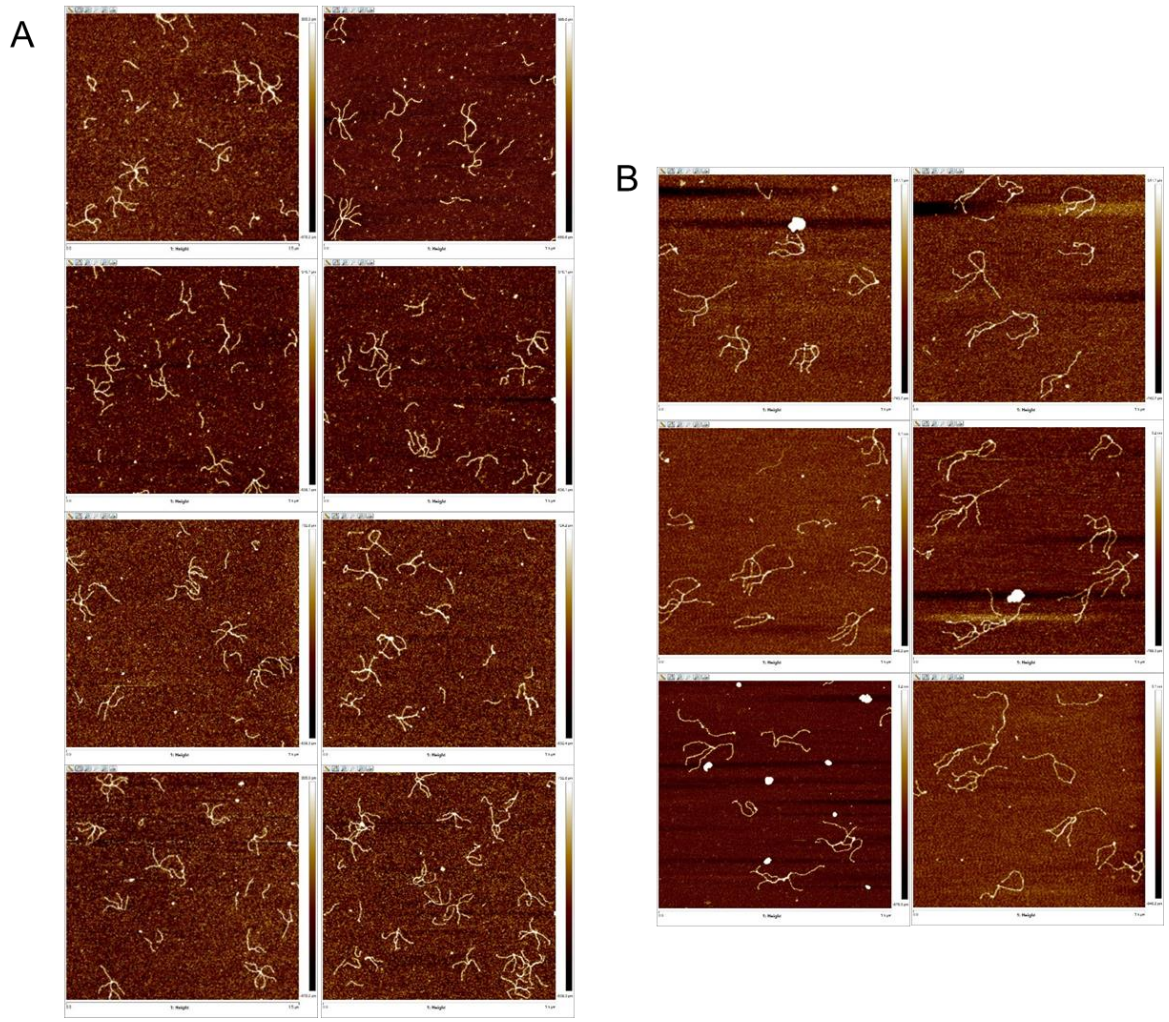

Figure S9. AFM images of CLS dsDNA nanostars bearing arms with length of A) 367 bp and B) 722 bp. The image sizes are 1.5 x 1.5 µm.

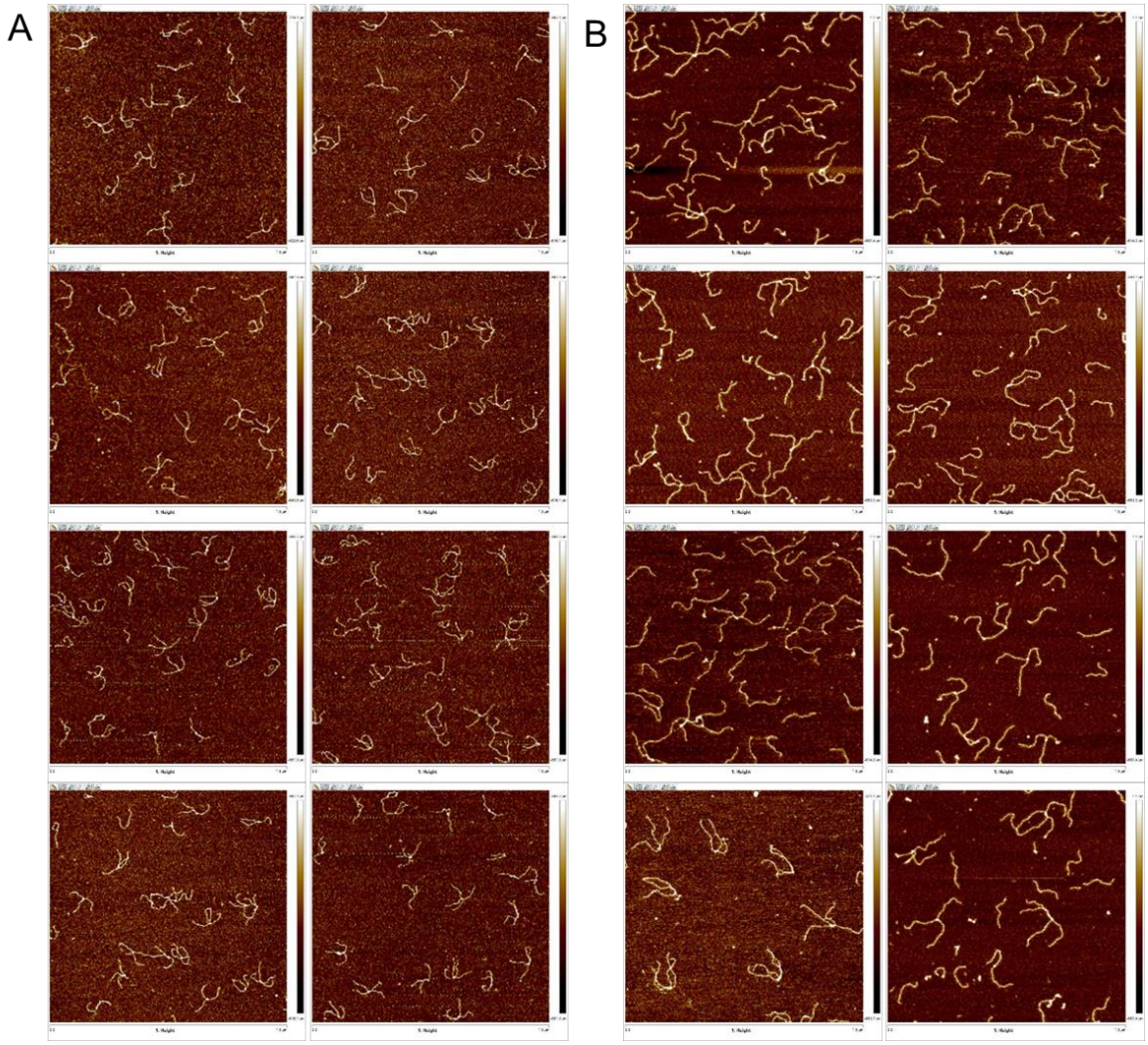

Figure S10. AFM images of Stv-Bt dsDNA nanostars bearing arms with length of A) 368 bp and B) 732 bp. The images are 1.5 x 1.5 µm.

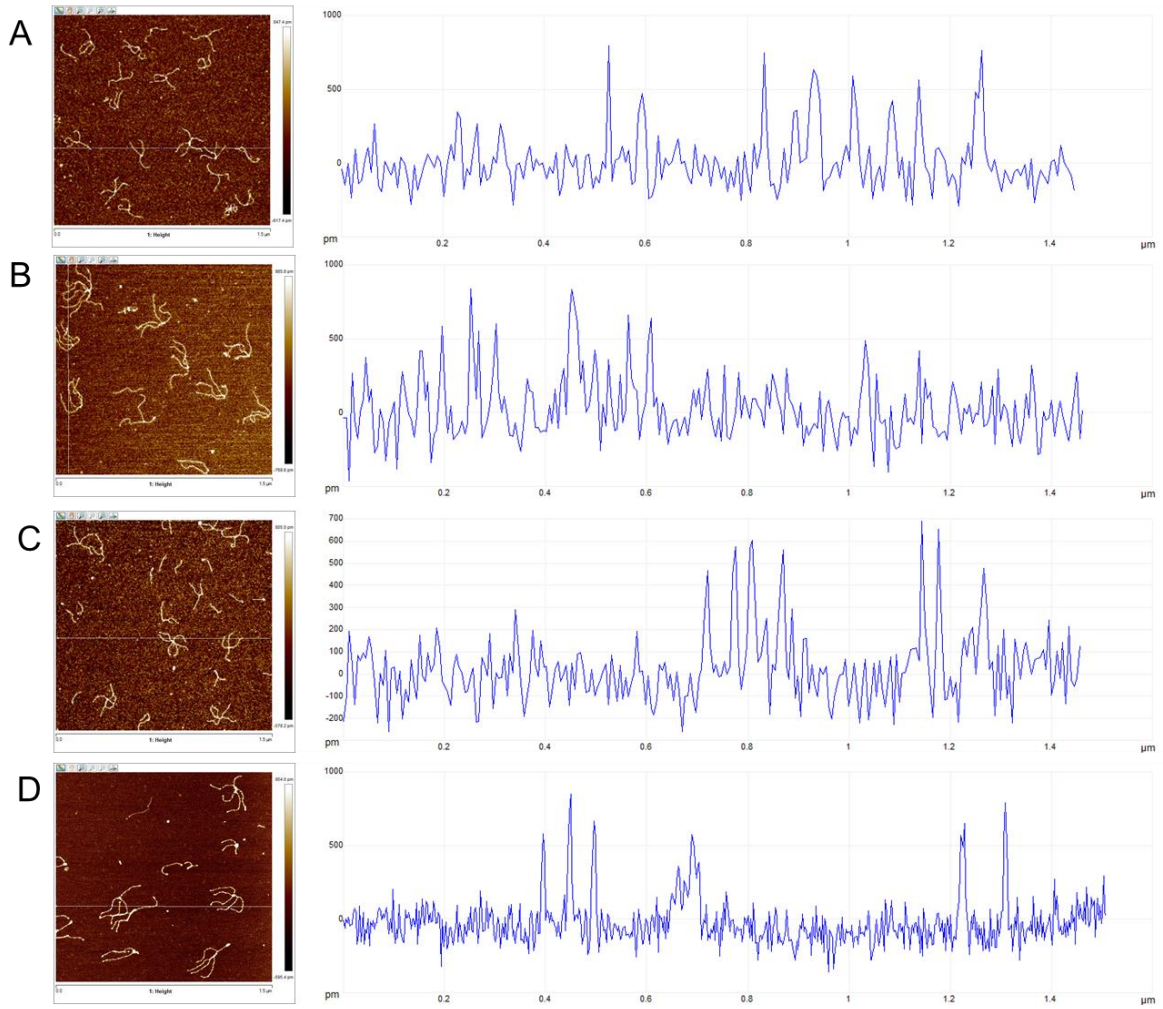

Figure S11. Z-height profiles from AFM images of naked dsDNA nanostars. Stv-Bt dsDNA nanostars bearing arms with length of 368 bp (A) and 732 bp (B). CLS dsDNA nanostars bearing arms with length of 367 bp (C) and 722bp (D). Size of images is 1.5 x 1.5 µm.

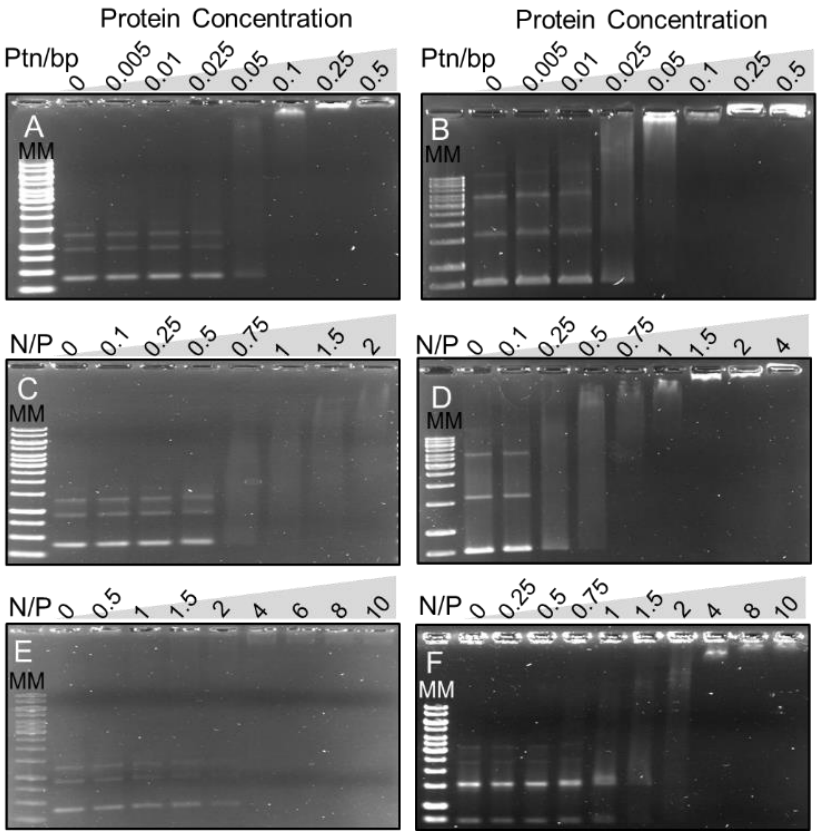

Figure S12. Binding Stv-Bt dsDNA nanostars with virus-like proteins. Electrophoretic Mobility Shift Assays showing complexes formed between streptavidin and biotinylated dsDNA fragments of 368 bp (left column) and 732 bp (right column) being bound by protein protein C_8-B^{Sso7d} (A-B), C_4-B^{K12} (C-D) and C_4-S_{10}-B^{K12} (E-F).

Table S3. Protein to DNA bp ratio used for maximum charge neutralization of DNA nanostars

	CLS NS		Stv-Bt NS	
Protein	367 bp	722 bp	368 bp	732 bp
C_8-B^{Sso7d}	0.25	0.5	0.25	0.25
C_4-B^{K12}	0.33 (2)[a)]	>0.33 (>2)	>0.33 (>2)	0.67 (4)
C_4-S_{10}-B^{K12}	0.67 (4)	>1.33 (>8)	0.67 (4)	1.33 (8)

[a)]In parentheses is N/P

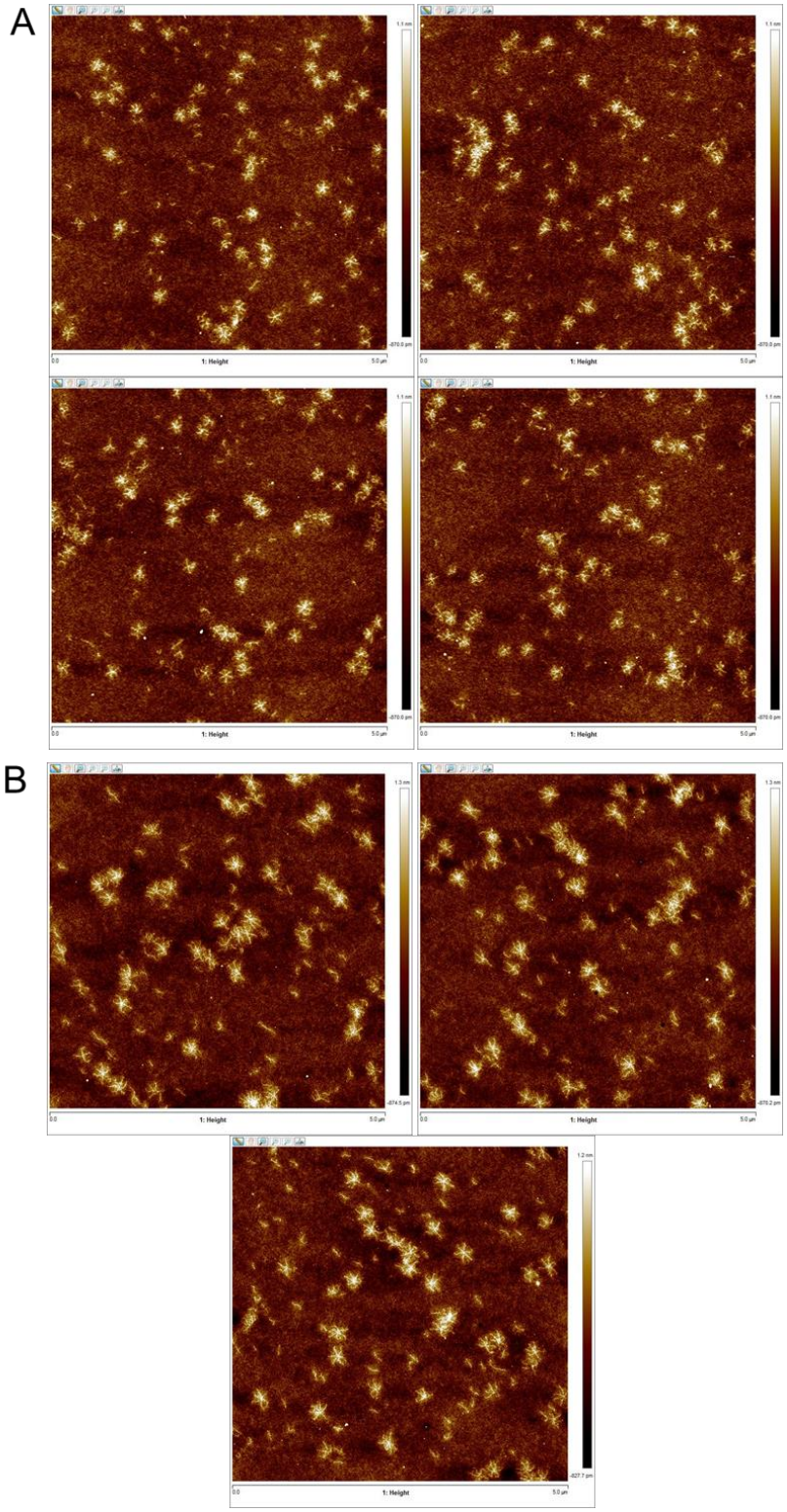

Figure S13. Low magnification AFM images of CLS dsDNA nanostars coated with protein C_8B^{Sso7d} bearing arms with length of 368 bp (A) and 539 bp (B). The image sizes are 5 x 5 µm.

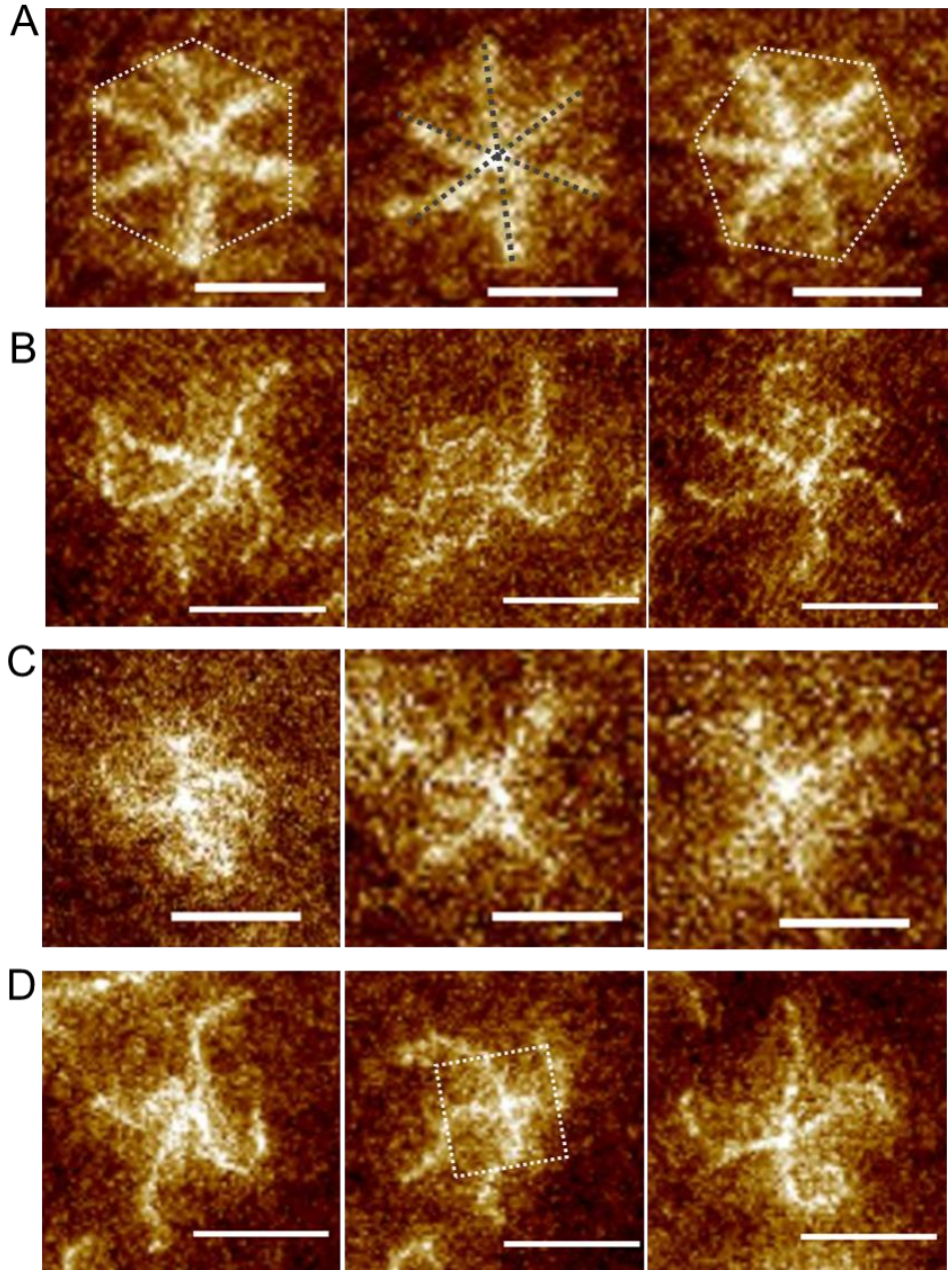

Figure S14. Zoom AFM images of dsDNA nanostars coated with protein C_8B^{Sso7d}. CLS dsDNA nanostars bearing arms with length of 367 bp (A) and 722 bp (B). Stv-Bt dsDNA nanostars bearing arms with length of 368 bp (C) and 732 bp (D). Scale bar is 125 nm (for stars with 367 bp arms) and 250 nm (for stars with 722 and 732 bp arms). Geometric images were superposed to visualize the structural order of the nanostars.

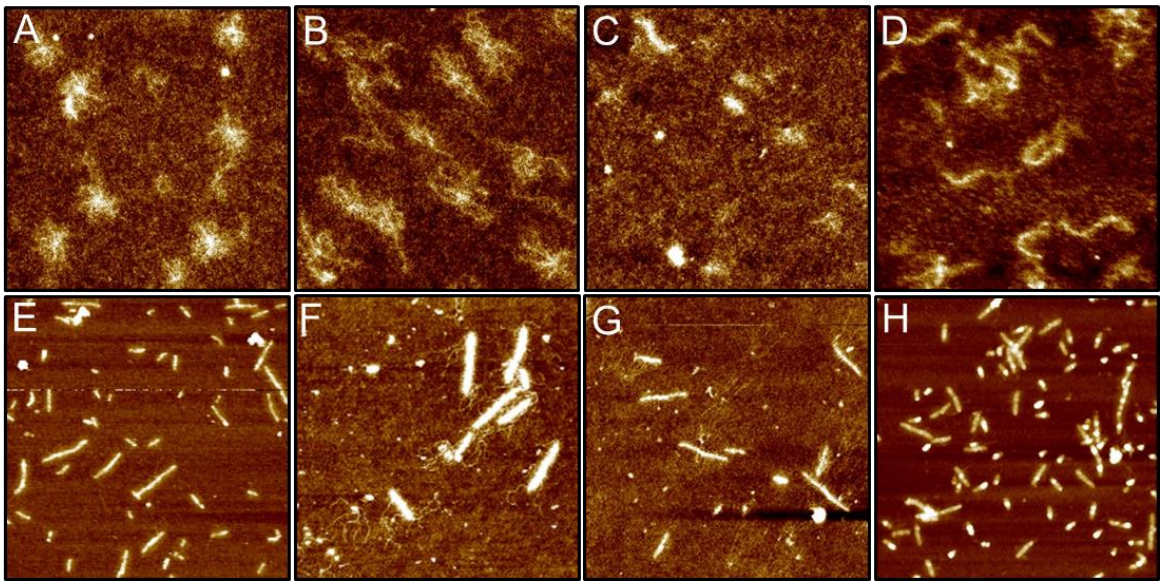

Figure S15. AFM images of dsDNA nanostars coated with virus-like protein C_4-B^{K12} (N/P = 4) (top row) and C_4-S_{10}-B^{K12} (N/P = 8 for 722 & 732 bp and N/P = 10 for 368 bp) (bottom row). Single CLS dsDNA nanostars bearing arms with length of 367 bp (A & E) and 722 bp (B & F). Single Stv-Bt dsDNA nanostars bearing arms with length of 368 bp (C & G) and 732 bp (D & H). Size of images is 1.5 x 1.5 µm.

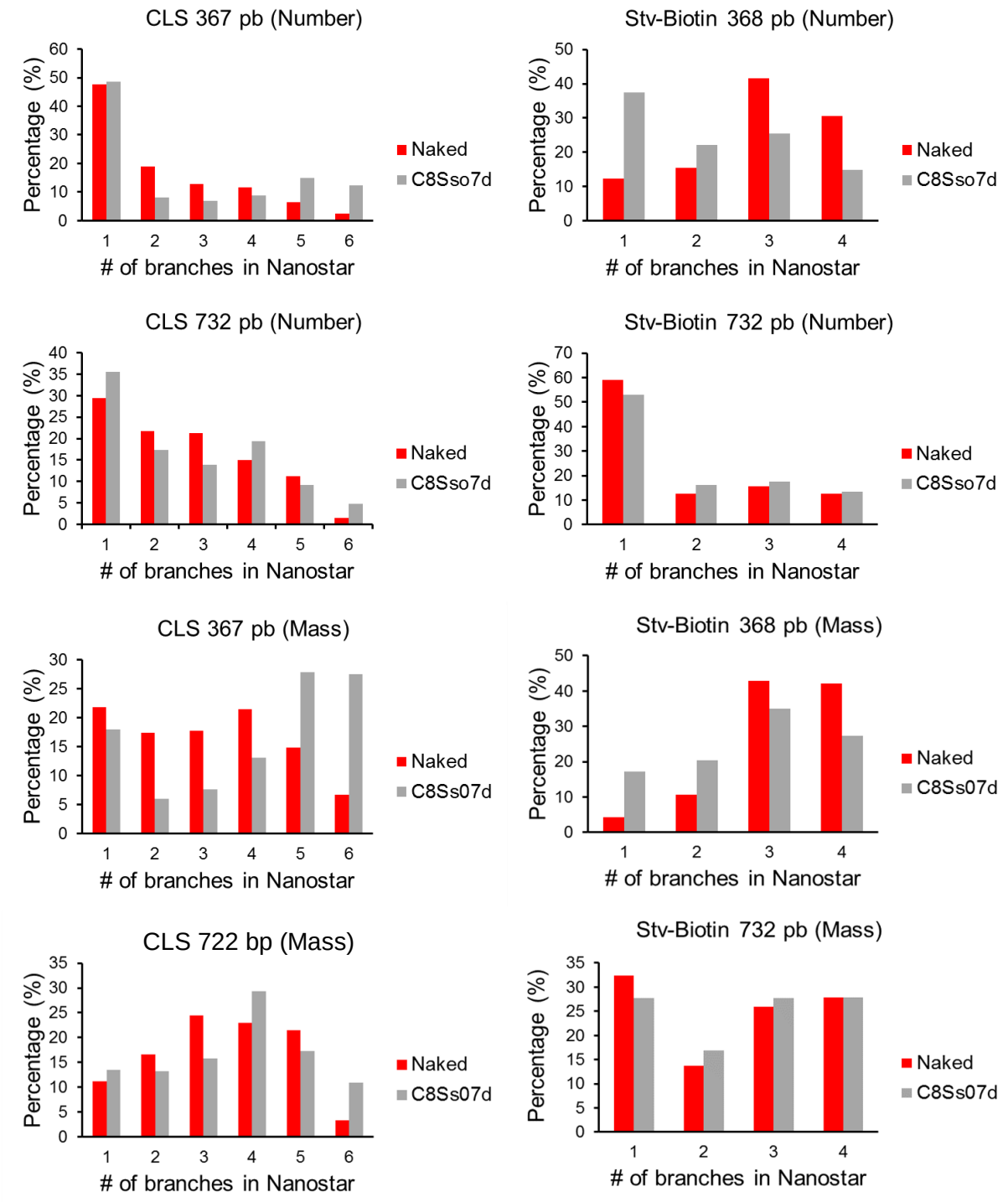

Figure S16. Percentage distributions of CLS and Stv-Biotin dsDNA nanostars by number of branches observed in AFM images when naked or coated with protein C_8-B^{sso7d}. Distribution of dsDNA nanostars by number (top two rows) and by mass (bottom two rows).

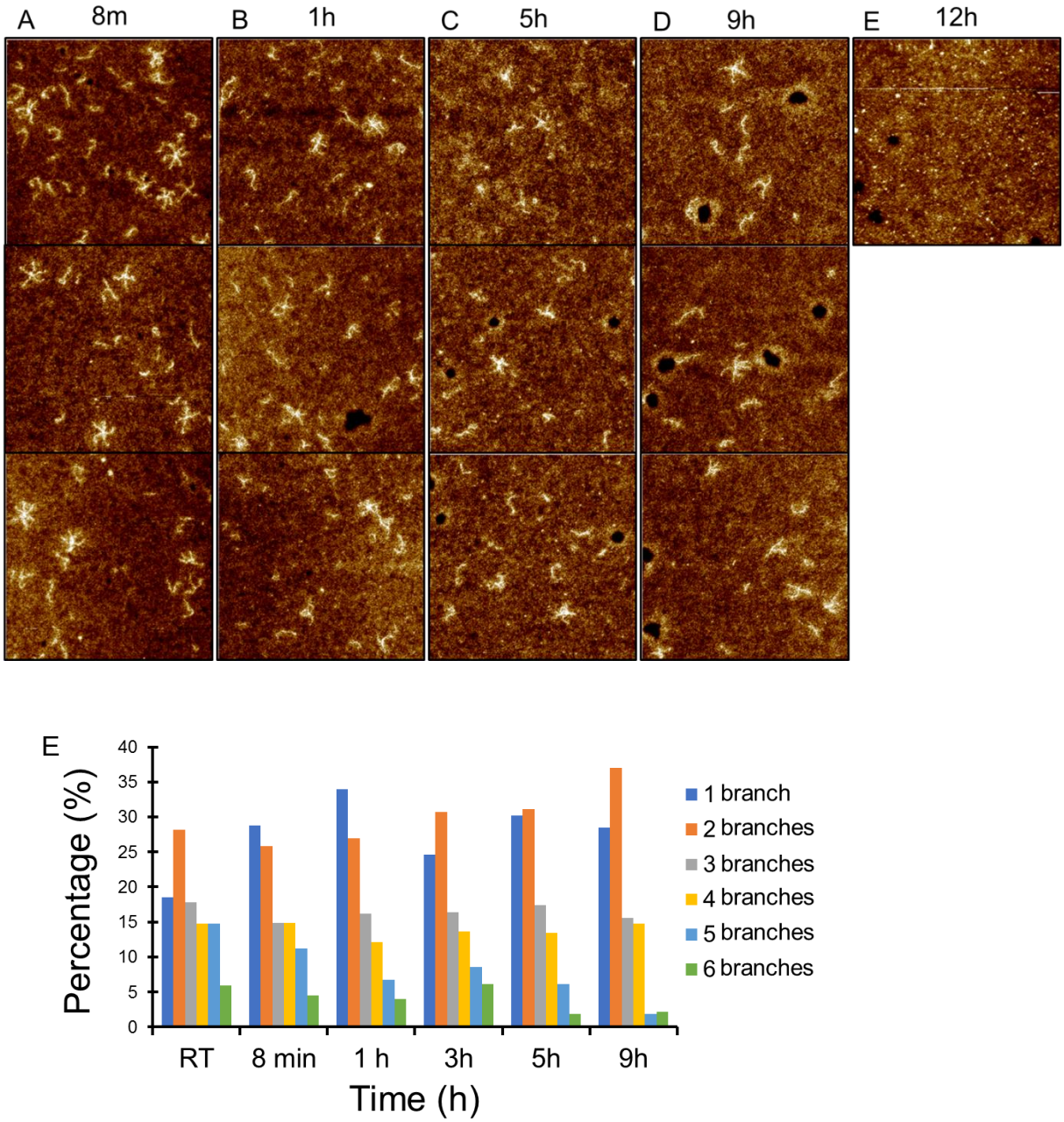

Figure S17. AFM images of CLS dsDNA nanostars bearing arms with length of 367 bp coated with protein C_8-B^{Sso7d} (protein/bp = 0.5) incubated at 37°C for 8 minutes (A), 1h (B), 5h (C), 9h (D) and 12h (E). Images are 1.5 x 1.5 µm. (E) Percentage distributions of protein C_8-B^{sso7d} coated CLS dsDNA nanostars by number of branches observed in AFM images when incubated at 37°C. Distribution of dsDNA nanostars is by mass.

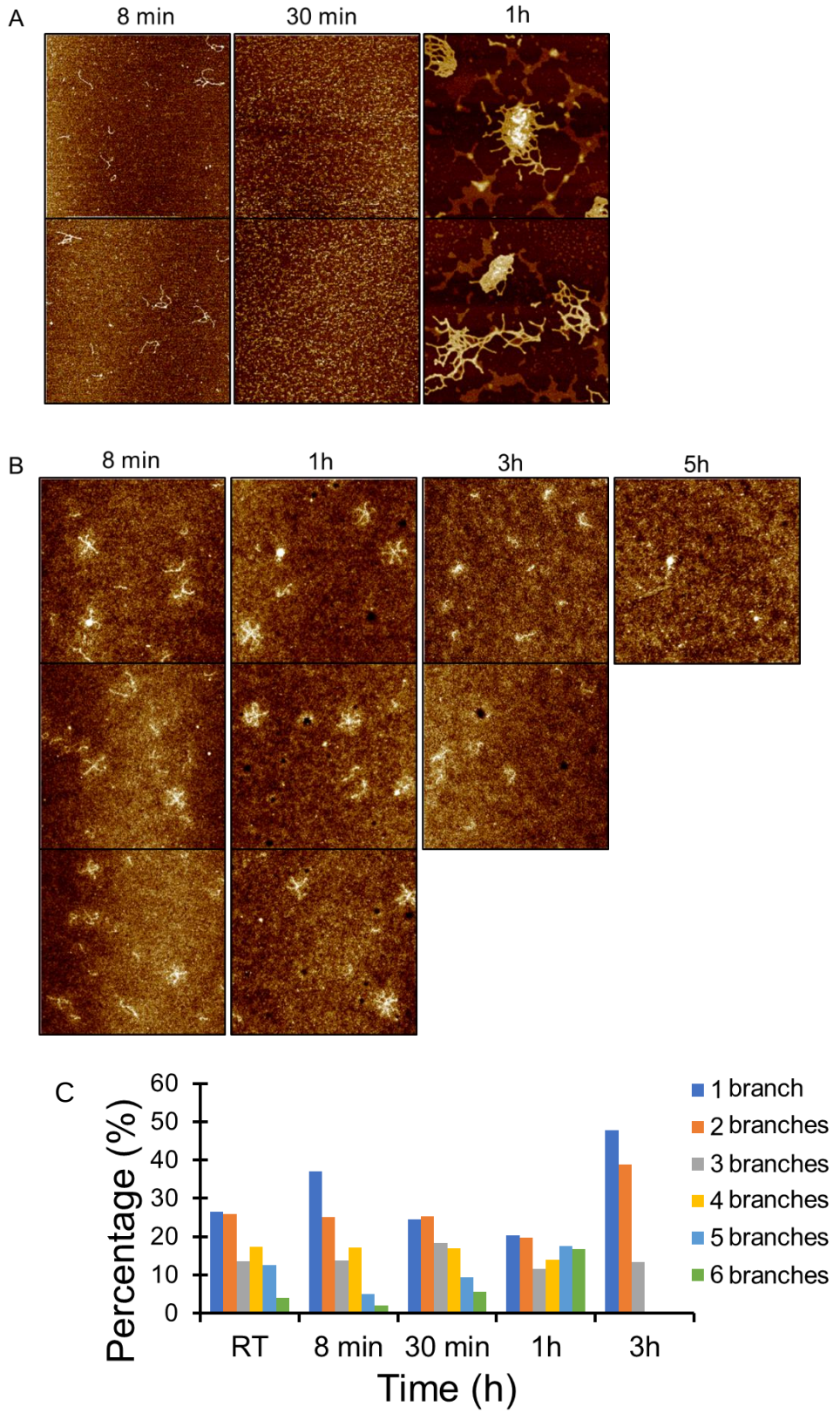

Figure S18. Thermal stability. AFM images of CLS dsDNA nanostars bearing arms with length of 367 bp incubated at 50°C at different times. (A) Naked CLS dsDNA nanostars. CLS dsDNA nanostars coated with protein C_8-B^{Sso7d} (protein/bp = 0.5) (B). The size of images are 1.5 x 1.5 µm. (C) Percentage distributions of protein C_8-B^{sso7d} coated CLS dsDNA nanostars by number of branches observed in AFM images when incubated at 50°C. Distribution of dsDNA nanostars is by mass.

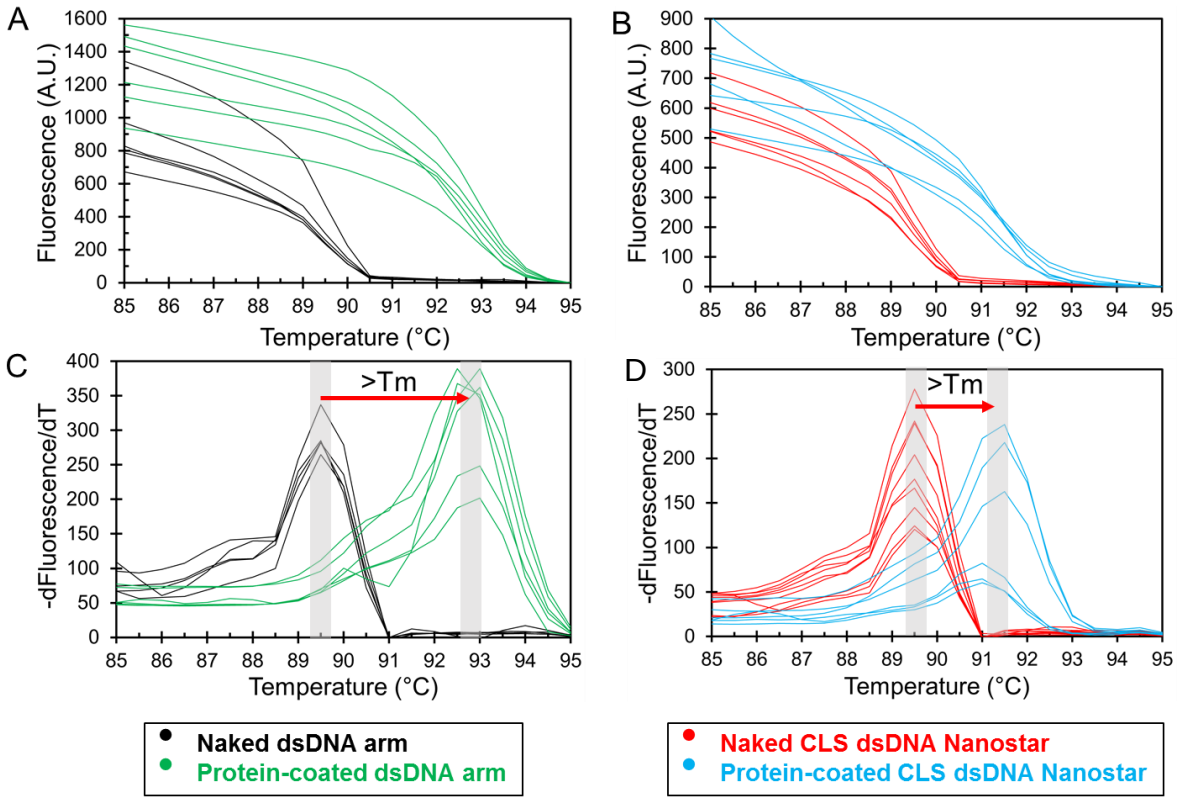

Figure S19. Thermal melting curves of dsDNA nanostars with arm length of 367 bp. Fluorescence of naked and protein-coated dsDNA arm (A) and naked and protein-coated CLS dsDNA nanostar (B). First derivative of the melting curves of naked and protein-coated dsDNA arm (C) and naked and protein-coated CLS dsDNA nanostar (D).

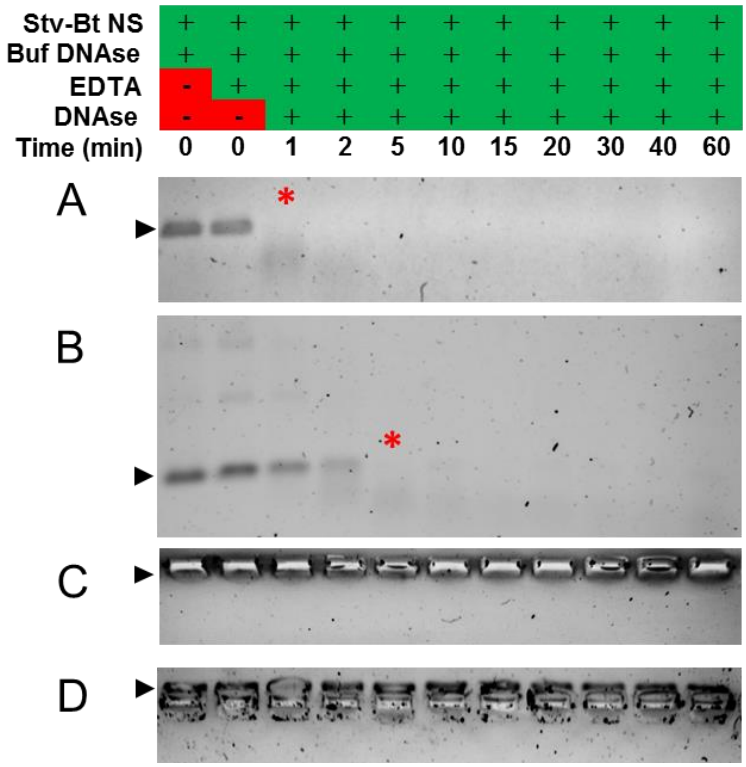

Figure S20. Enzymatic stability of protein-dsDNA nanostars. (A) naked biotinylated dsDNA fragment of 368 bp. (B) Naked Stv-Bt dsDNA nanostars. Protein C_8-B^{Sso7d} coated lineal CLS (C) and biotinylated lineal dsDNA fragment (D) (Ptn/bp = 0.5). Black arrow indicates the band that was followed and red asterisk indicates it was completely digested.

References

(1) Tian, Y.; He, Y.; Ribbe, A. E.; Mao, C. Preparation of Branched Structures with Long DNA Duplex Arms. *Org. Biomol. Chem.* **2006**, *4* (18), 3404–3405. https://doi.org/10.1039/B605464A.

(2) Bellot, G.; McClintock, M. A.; Lin, C.; Shih, W. M. Recovery of Intact DNA Nanostructures after Agarose Gel–based Separation. *Nat. Methods* **2011**, *8*, 192.