## Supporting Information

# Backbone-Branched DNA Building Blocks for Facile Angular Control in Nanostructures 

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Table S1. DNA sequences used in this study.

| Name | Sequence | Source |
| :--- | :--- | :---: |
| DNA1 | $5^{\prime}-$ ctc gat cgg tct ccA(2'- $O$-propargyl $)$ gcc tgg -3' | synthesized |
| DNA2 | $5^{\prime}-\mathrm{N}_{3}-$ cac tag gcg cct agt g-3' | synthesized |
| bbDNA3 | $5^{\prime}-$ ctc gat cgg tct ccA(2'-cac tag gcg cct agt g) gcc tgg -3' | CuAAC |
| DNA4 | $5^{\prime}-$ cca ggc tgg aga ccg atc gag-3' | IDT |
| DNA5 | $5^{\prime}-$-acc gat cga gcc agg ctg gag-3' | IDT |
| DNA6 | $5^{\prime}-$ cca ggc tgg aga ccg atc gag cca ggc tgg aga ccg atc gag -3' | IDT |
| DNA7 | $5^{\prime}-$-cgc tagU(2'- $O$-propargyl)cat gca gU(2'- $O$-propargyl)ccacgc-3' ${ }^{a}$ | synthesized |
| bbDNA8 | $5^{\prime}-$-cgc tagU(2'- cac tag gcg cct agt g)cat gca gU(2'- cac tag gcg cct agt g)cca cgc-3' ${ }^{a}$ | CuAAC |
| DNA9 | $5^{\prime}-$-gcg tgg act gca tga cta gcg -3' | IDT |
| DNA10 | $5^{\prime}-$ cat gac tag cgg cgt gga cag -3' | IDT |
| DNA11 | $5^{\prime}-$ gcg tgg act gca tga cta gcg gcg tgg act gca tga cta gcg -3' | IDT |

${ }^{a}$ The branching residue base is 'U' rather than ' $t$ ' (i.e., 5-methyl-U) as the 2 '- $O$-propargyl necessitates a ribosyl (RNA) phosphoramidite.

Table S2. Dihedrals between backbone extensions at the 2'-atom in B-form helix based on 10.5 residues per turn $\left(360^{\circ}\right)$ or helical pitch of $34.29^{\circ}$ per residue. For angles greater than $180^{\circ}, \Theta$ is calculated in the other direction (i.e., the smaller angle corresponding to $360-\Theta)$. Thus for $\mathrm{N}=7, \Theta=274.32^{\circ} \equiv(360-274.32)=85.68^{\circ}$

| Number of residues between branches ( N ) | Dihedral between branches ( $\Theta$; degrees) |
| :---: | :---: |
| 0 | 34.29 |
| 1 | 68.57 |
| 2 | 102.86 |
| 3 | 137.14 |
| 4 | 171.43 |
| 5 | 154.29 |
| 6 | 120 |
| 7 | 85.68 |
| 8 | 51.39 |
| 9 | 17.10 |
| 10 | 17.10 |
| 11 | 51.39 |
| 12 | 85.68 |
| 13 | 120 |
| 14 | 154.29 |
| 15 | 171.43 |
| 16 | 137.14 |
| 17 | 102.86 |
| 18 | 68.57 |
| 19 | 34.29 |
| 20 | 0 |

A
Time


B


Figure S1. Optimizing timing for the click-branching reaction to obtain bbDNA3. A. A $20 \%$ polyacrylamide ( 8 M urea) gel used to resolve click branching reaction mixtures over time. The bbDNA3 (upper band) forms with disappearance of DNA1 over time ( $\mathrm{t}=0$ to 5 hrs ). B. Graph of bbDNA3 formed over time as quantified from the gel, indicates maximal labeling is achieved in 3 h with marginal improvement after 2 h .


Figure S2. Time-temperature trace of the step-wise annealing for nanoassembly formation.


Figure S3. AFM scans of DNA nanoassembly based on co-planar branches and associated line profile


Figure S4. AFM scans of DNA nanoassembly based on dual perpendicular branches and associated line profile.

