Supporting Information for

Multiplexed DNA Detection Based on Positional Encoding/Decoding with Self-Assembled DNA Nanostructures

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1. Materials

All DNA stands were purchased from Sangon Biotech (Shanghai) Co. Ltd and DL2000 DNA molecular weight marker was from TaKaRa Biotechnology (Dalian) Co. Ltd. Freeze ’N Squeeze column was purchased from Bio-Rad Laboratories, Inc. and uranyl formate was from Polysciences, Inc. Gel electrophoresis was performed on a Bio-Rad system using 2% agarose gel. UV-vis absorption values were obtained using a Eppendorf Biophotometer Plus facility. Transmission electron microscopy (TEM) imaging was performed using a JEOL JEM-1011 facility.

2. Design of Self-Assembled DNA Nanostructures

DNA sequences for the nanostructures were generated by program Sequin.[S1] To reduce undesired interaction patterns, criton size is set to 7, which means any continuous sequence of 7 or more nucleotides (nt) appears at most once.

DNA nanostructures were designed by following Peng Yin’s LEGO-like model (for all the DNA sequence information, please see Section 14 of the Supporting Information).[S2,S3] In our design of the core cuboid part of **CU**, DNA double helices were arranged as square lattice bundles, as shown in Figure S1. First, we used Sequin to generate sequences for 36 DNA double helices, each of which is 128 BP (base pairs) in length. Then a sequence of 8Ts (eight continuous thymidines) were added to both ends of one strand of each helix (5’-3’ strand for odd helices, 3’-5’ strand for even helices, refer to Figure S1 for the odd and even numbering of helices) to prevent non-specific blunt-end stacking. Second, a nick site was created for each helix every 16 nt. Because of the different numbers of nt for the two strands of each helix, the nick sites are staggered by 8 nt. Third, the nick sites of each odd helix were linked with the corresponding sites of neighboring even helix clockwise. Take H9 (helix 9) as an example, its protruding 8Ts on the 5’ end is linked to the protruding 8Ts on the 3’ end of H14, then the first nick site of H9 should be linked to the first one of H10, the second site of H9 to the second one of H2, the third site of H9 to the third one of H8, and the fourth site of H9 to the fourth one of H14. After the linkage, the remaining 16 nt strands should be merged to 32 nt ones to improve the stability of the nanostructures. The registry marker of **CU** is direct extension of the 9 helices, each of which is 64 BP in length, at one corner of the core cuboid.

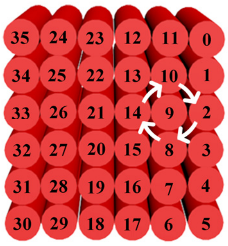

Figure S1. The design of **CU** viewed from the cross-section of the end of core cuboid opposite to the registry marker. This view gives a top left corner location for the registry marker.

The locations of 14 capture probe strands at the top and bottom surfaces of **CU** are shown in Figure S2. The locations of 15 detection probe strands of **DU** are shown in Figure S3 and S4.

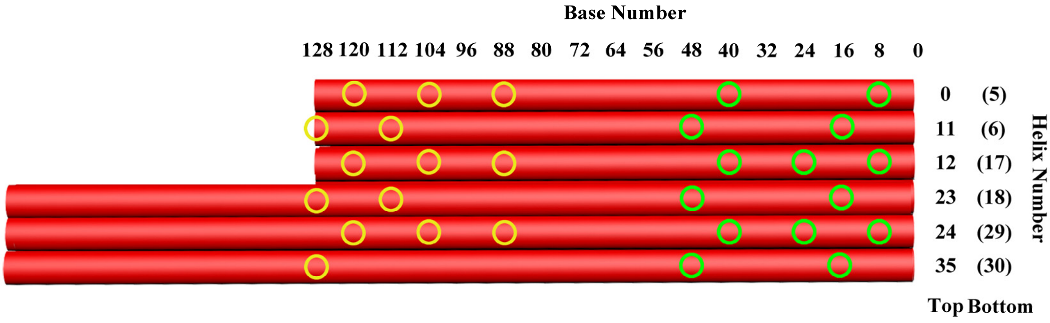

Figure S2. The locations of 14 capture probe strands at the top and bottom surfaces of **CU**, with base numbers and helix numbers specified (circle-marked sites at right: for **TT1** and **TB1**; circle-marked sites at left: for **TT2** and **TB2**).

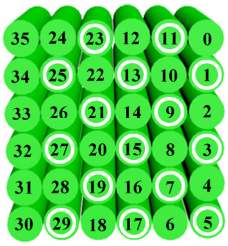

Figure S3. The locations of 15 detection probe strands at **DUT1** (identical for **DUT2**), with the base number (64 BP) and helix numbers (circle-marked sites) specified.

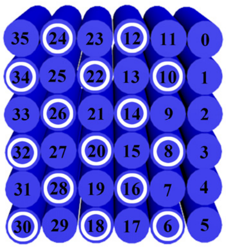

Figure S4. The locations of 15 detection probe strands at **DUB2** (identical for **DUB1**), with the base number (64 BP) and helix numbers (circle-marked sites) specified.

DCU is assembled by **CU** and a cuboid. There are 10 linker strands at the 5’ end of **CU** (linker 1: H0, H2, H4, H8, and H10; linker 2: H24, H26, H28, H32, and H34) and they are complementary to the other 10 strands dangling from the 3’ end of the cuboid (linker 1: H1, H3, H5, H7, and H9; linker 2: H25, H27, H29, H31, and H33). The locations of 10 linker strands are shown in Figures S5 and S6.

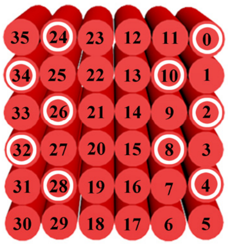

Figure S5. The locations of 10 linker strands at **CU**, with the base number (1 BP) and helix numbers (circle-marked sites) specified.

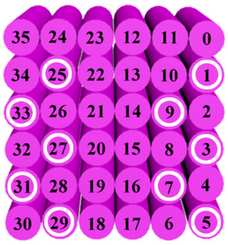

Figure S6. The locations of 10 linker strands at cuboid, with the base number (128 BP) and helix numbers (circle-marked sites) specified.

3. Experimental Procedures

Preparation of nanostructures: Hundreds of single-strand DNA (ssDNA) were mixed and then freeze-dried. After the dissolution of ssDNA in 0.5×TE buffer (5 mM Tris, 1 mM EDTA, pH=8.0, supplemented with 40 mM $MgCl_2$) to a final concentration of 200 nM per strand, the solution was annealed from 90 ^{o}C to 60 ^{o}C at a cooling rate of 5 min/^{o}C and from 60 ^{o}C to 24 ^{o}C at a rate of 2 h/^{o}C.

Purification of nanostructures: The annealed samples were loaded to a native 2% agarose gel with 0.5 μg/mL ethidium bromide (running buffer: 0.5×TBE buffer, containing 44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA, supplemented with 11 mM $MgCl_2$) and gel electrophoresis was performed at 80 volts for 2 h in an ice bath. Target bands were excised and cut into small pieces. The gel pieces were placed into Freeze 'N Squeeze columns, frozen at -20 ^oC for 5 min and then centrifuged at 7000g at 4 ^oC for 5 min.

Hybridization assay: DNA Nanostructures were quantified by measurements of UV-vis absorption values at 260 nm. Different DNA nanostructures were mixed in a molar ratio of 1:1 and target DNA (3 μL) was then added to the mixture. The solution was diluted with 0.5×TBE buffer to a final volume of 10 μL (5 nM for the final concentration of each nanostructure). The hybridization was allowed to proceed at 30 ^oC.

TEM imaging: A 2.1 μL of hybridization solution was mixed with 0.3 μL of ssDNA (with a sequence of 5'-GCCTGAAGTCTGGTGCTTAGGCCTTGAAATCA -3' for the generation of a hydrophilic TEM grid surface) and the whole solution was loaded onto a glass slide. On top of the solution was covered with a carbon-coated TEM grid and the contact between the solution and grid was allowed to proceed for 2 min. The TEM grid was then stained with a 2% uranyl formate aqueous solution (containing 25 mM NaOH) for 2 min followed by twice wash with water. TEM imaging was performed at 100 kV.

4. Screening of $MgCl_2$ Concentration for DNA Hybridization

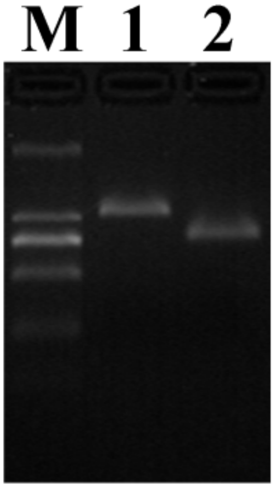

Figure S7. Gel electrophoresis bands for purified **CU** and **DUT1**. Lane M: DL2000 molecular weight marker (same for all the following gel electrophoresis images); Lane 1: **CU**; Lane 2: **DUT1**.

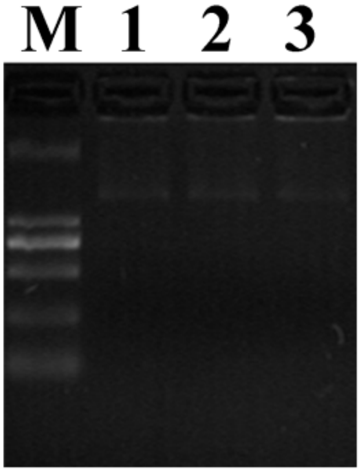

Figure S8. Gel electrophoresis bands for **CU**, **DUT1**, and **TT1** in the presence of different concentrations of $MgCl_2$. Lane 1: 11 mM $MgCl_2$; Lane 2: 20 mM $MgCl_2$; Lane 3: 30 mM $MgCl_2$. The concentration of **TT1** is 300 nM. The non-penetrating material in the gel is the non-specific aggregation of the hybridization product, which probably appears after storage at 4 ^{o}C and could be reduced by pre-heating the sample at 30 ^{o}C before gel electrophoresis (same for all the following gel electrophoresis images).

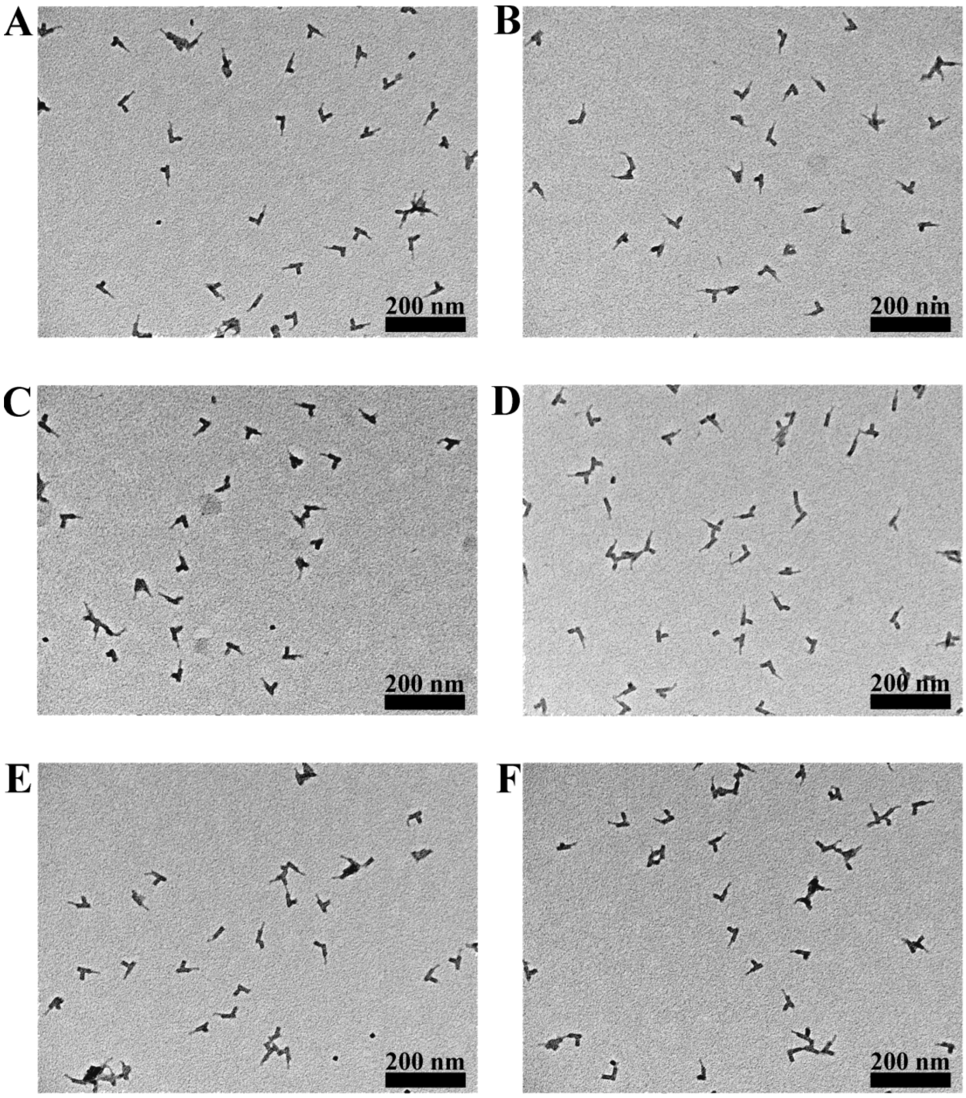

Figure S9. Representative TEM images of **CU**, **DUT1**, and **TT1** in the presence of different concentrations of MgCl$_2$. A) and B): 11 mM MgCl$_2$; C) and D): 20 mM MgCl$_2$; E) and F): 30 mM MgCl$_2$. The concentration of **TT1** is 300 nM.

5. Screening of DNA Hybridization Time

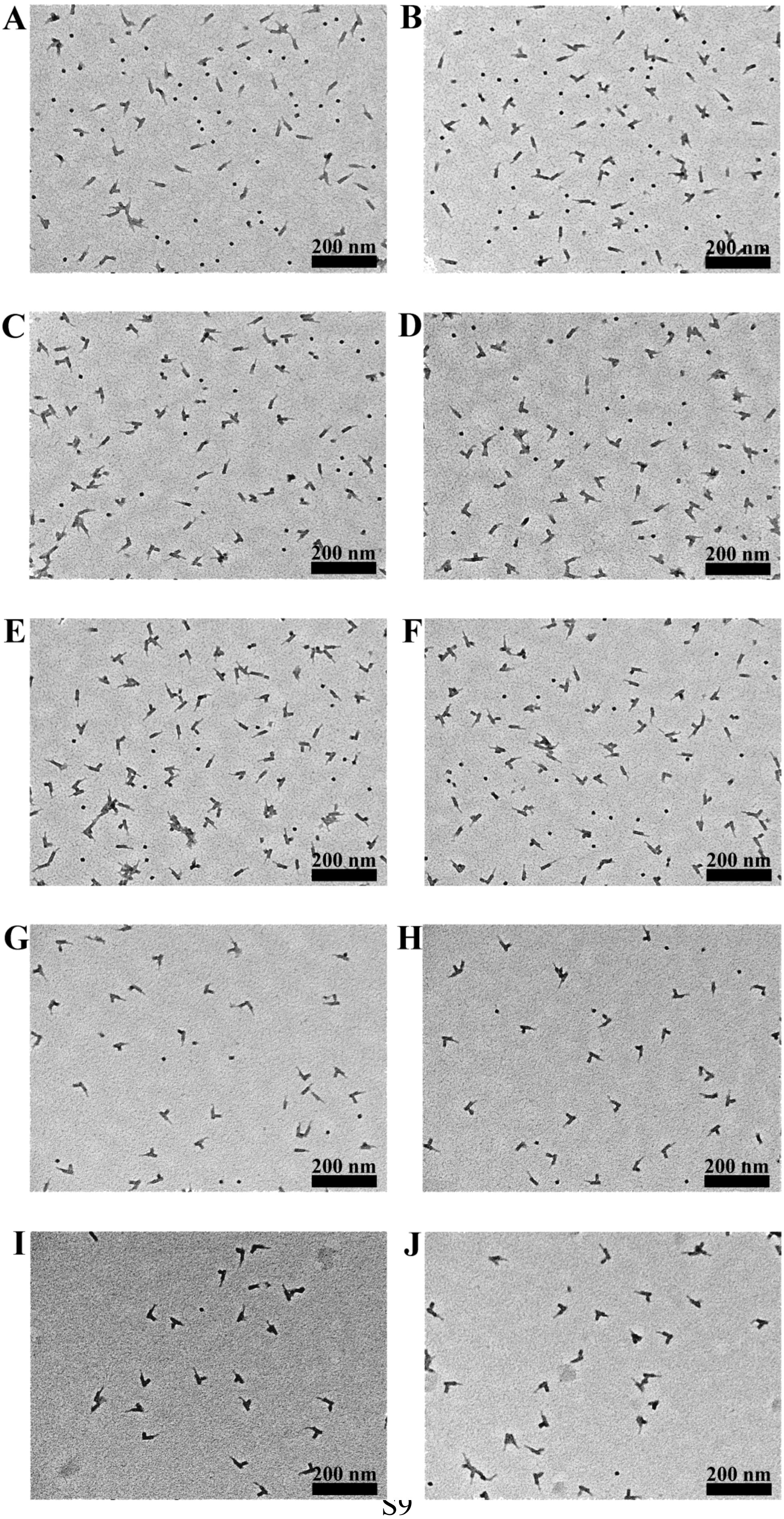
A
200 nm
B
200 nm
C
200 nm
D
200 nm
E
200 nm
F
200 nm
G
200 nm
H
200 nm
I
200 nm
J
200 nm

Figure S10. Representative TEM images of **CU**, **DUT1**, and **TT1** after hybridization for different durations of time. A) and B): 5 min (hybridization percentage, or HP: 41%); C) and D): 10 min (HP: 63%); E) and F): 20 min (HP: 71%); G) and H): 1 h (HP: 88%); I) and J): 8 h (HP: 91%). HP is defined as the percentage of observed **CU**-**DUT1** over all structurally resolved **CU** (calculated from ~300 **CU**). The concentration of **TT1** is 300 nM. The small dark square objects in the images are individual **DUT1** which prefer head-on settlement on the carbon grid instead of side-on settlement as hybridized **DUT1**. All kinds of individual **DU** (**DUT1**, **DUT2**, **DUB1**, **DUB2** and **DUB3)** show head-on settlement in the following TEM images.

6. Single-Target DNA Detection

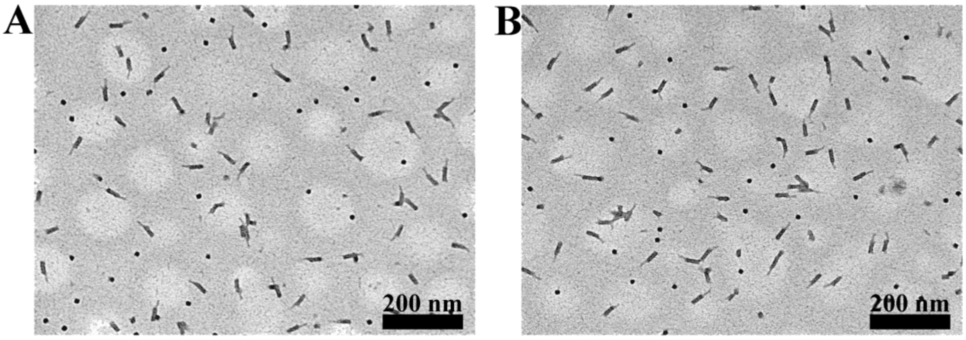

Figure S11. Representative TEM images of **CU** and **DUT1** in the absence of **TT1**.

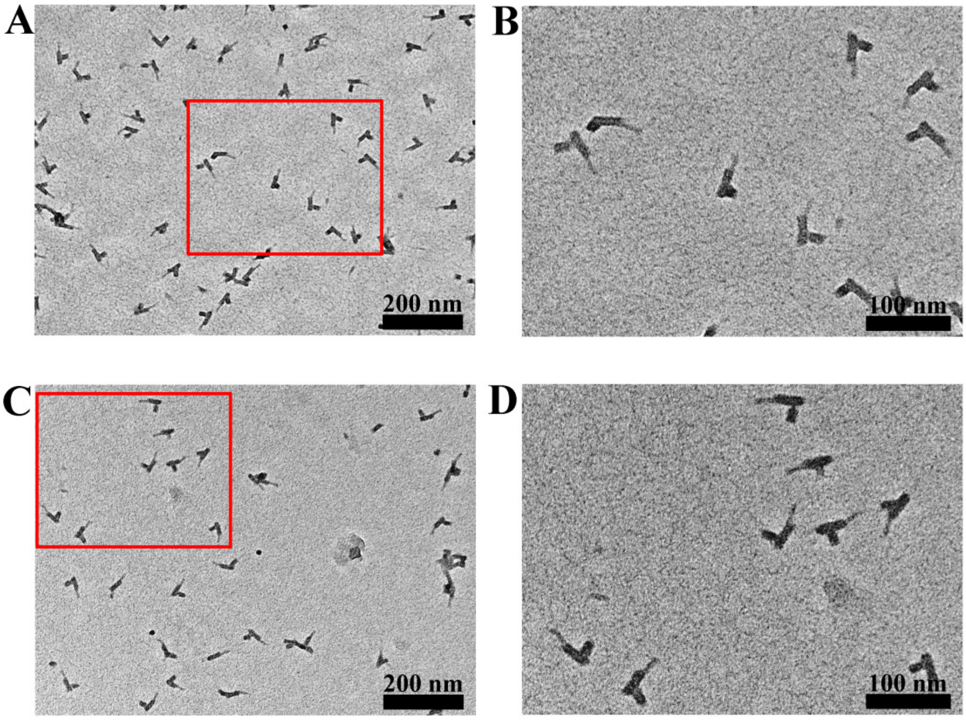

Figure S12. Representative TEM images of **CU** and **DUT1** in the presence of **TT1**. B) and D) are enlarged view images of A) and C), respectively (rectangles marked in red serve only as an approximate viewing guide for the enlarged area; same for all the following TEM images if applicable). The concentration of **TT1** is 300 nM.

7. DNA Detection Limit

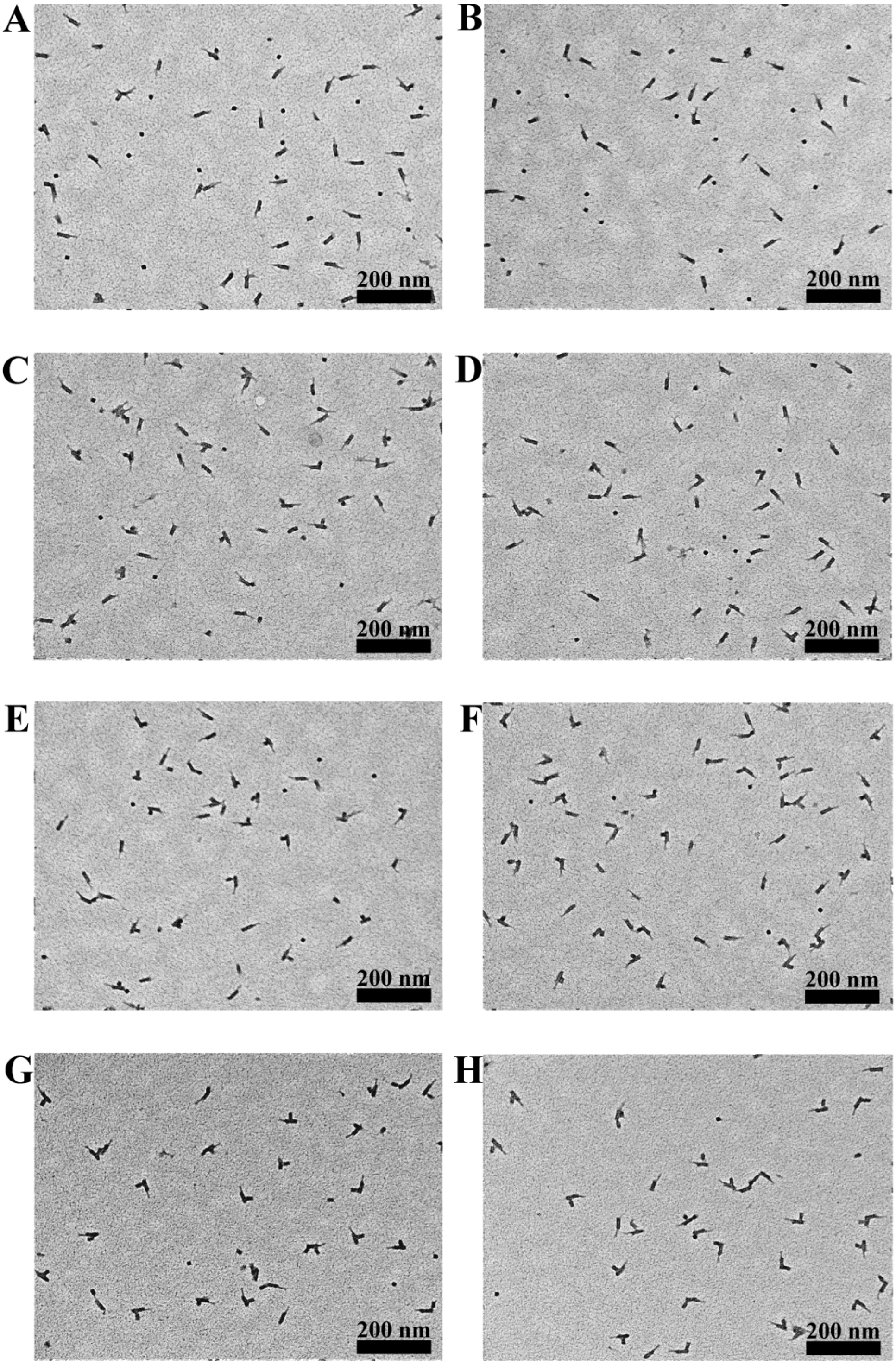

Figure S13. Representative TEM images of **CU** and **DUT1** in the presence of different concentrations of **TT1**. A) and B): 5 nM (HP: 10%); C) and D): 10 nM (HP:

37%); E) and F): 20 nM (HP: 65%). G) and H): 30 nM (HP: 88%). HP was determined by calculation from ~250 **CU**.

8. PCU DNA Detection System

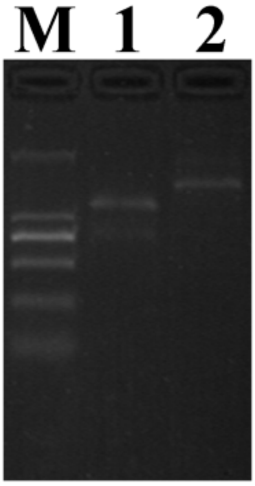

Figure S14. Gel electrophoresis bands for **PCU** and **DUT1** in the absence (lane 1) and presence (lane 2) of **TT1**. The concentration of **TT1** is 300 nM.

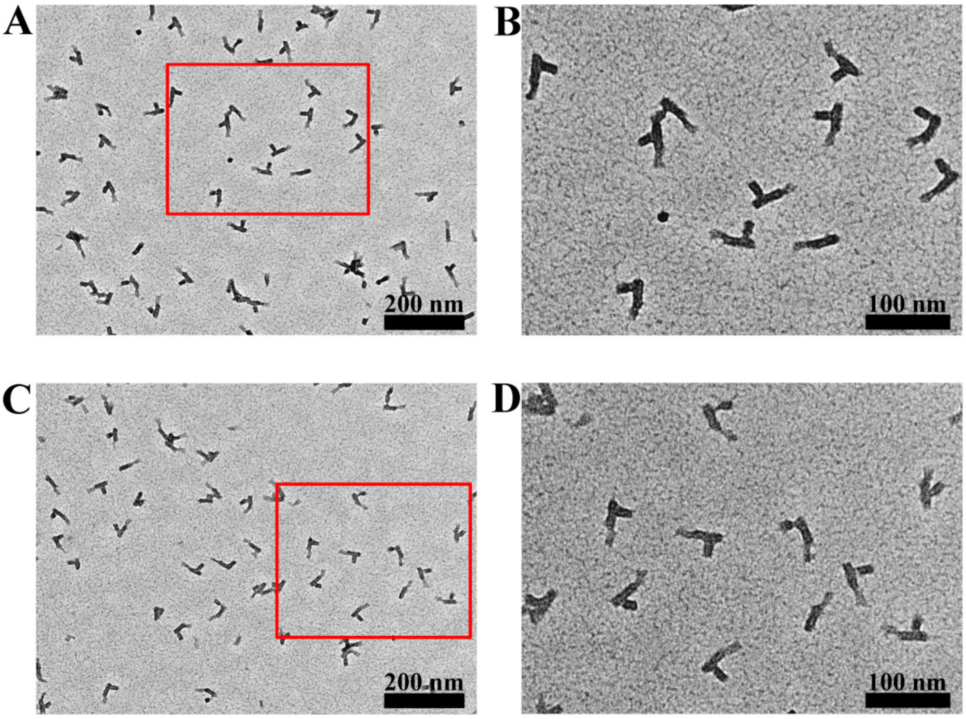

Figure S15. Representative TEM images of **PCU** and **DUT1** in the presence of **TT1**. B) and D) are enlarged view images of A) and C), respectively. The concentration of **TT1** is 300 nM.

9. Detection of RNA

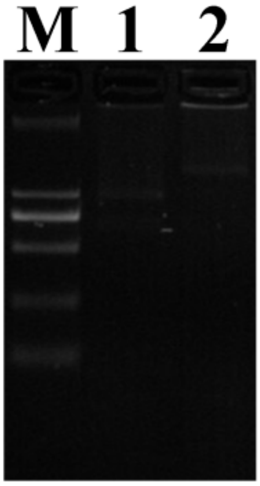

Figure S16. Gel electrophoresis bands for **CU** and **DUT1** in the absence (lane 1) and presence (lane 2) of **RTT1**. The concentration of **RTT1** is 300 nM.

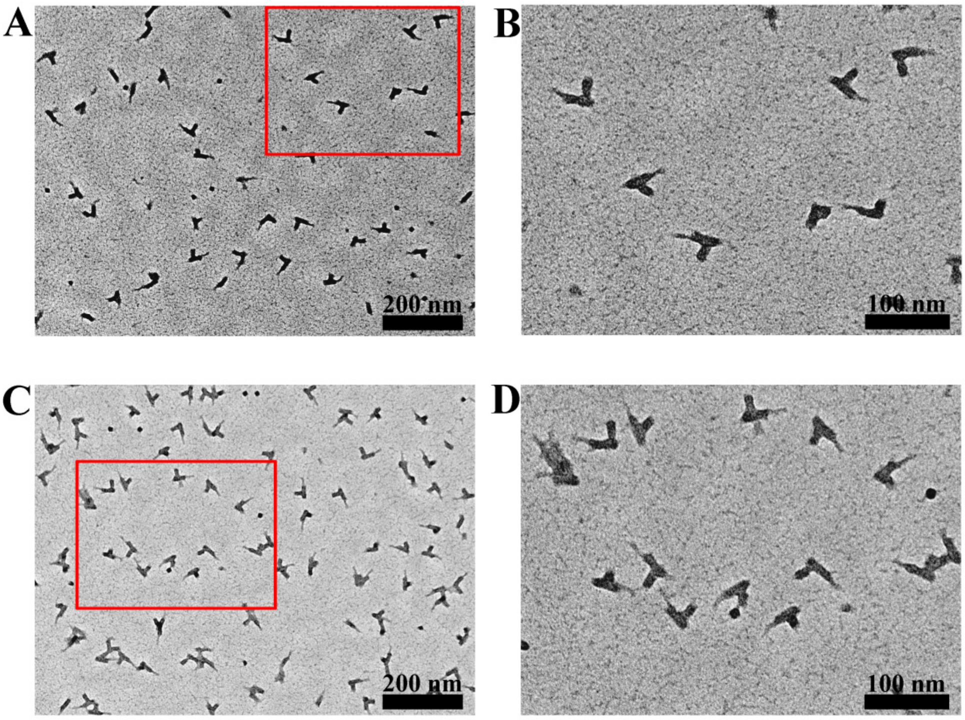

Figure S17. Representative TEM images of **CU** and **DUT1** in the presence of **RTT1**. B) and D) are enlarged view images of A) and C), respectively. The concentration of **RTT1** is 300 nM.

10. Two-Target DNA Detection

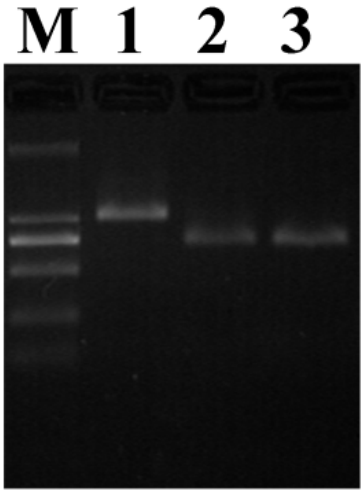

Figure S18. Gel electrophoresis bands for purified **CU**, **DUT1**, and **DUB2**. Lane 1: **CU**; Lane 2: **DUT1**; Lane 3: **DUB2**.

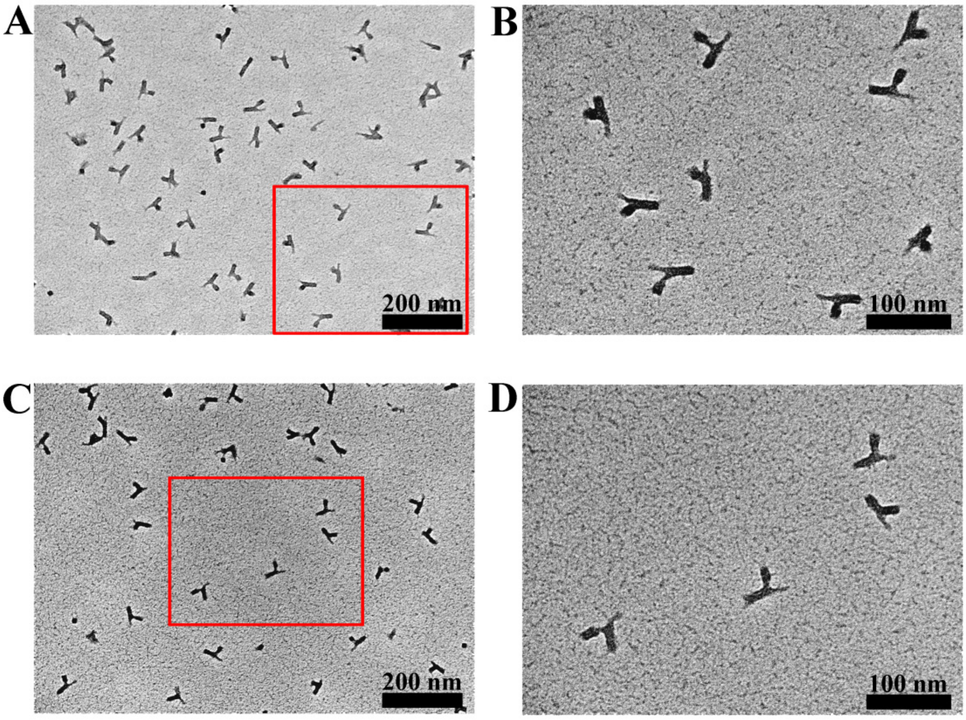

Figure S19. Representative TEM images of **CU** and **DUB2** in the presence of **TB2**. B) and D) are enlarged view images of A) and C), respectively. The concentration of **TB2** is 300 nM.

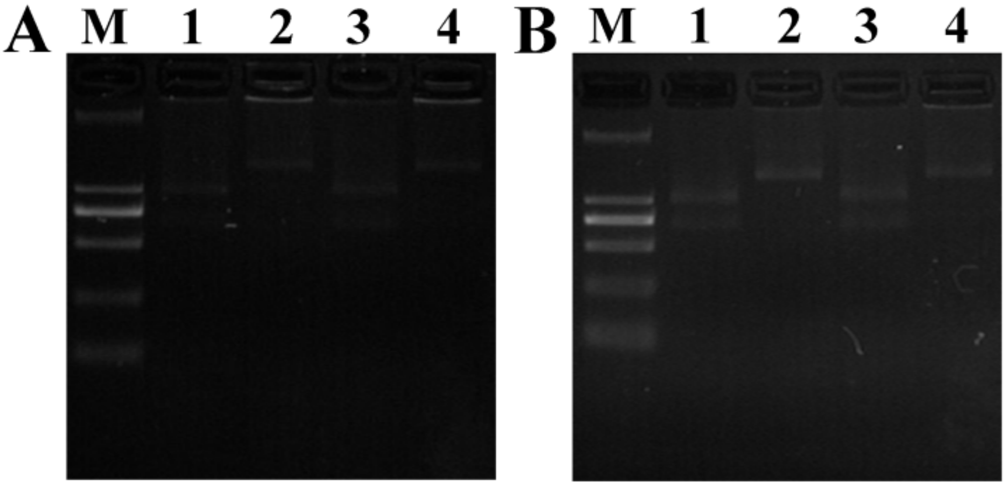

Figure S20. Gel electrophoresis bands for two-target detection (separate hybridization). A) **CU** and **DUT1** in the absence (lane 1) and presence (lane 2) of **TT1**, **CU** and **DUT1** in the presence of **TB2** (lane 3), **CU** and **DUT1** in the presence of both **TT1** and **TB2** (lane 4); B) **CU** and **DUB2** in the absence (lane 1) and presence (lane 2) of **TB2**, **CU** and **DUB2** in the presence of **TT1** (lane 3), **CU** and **DUB2** in the presence of both **TT1** and **TB2** (lane 4). The concentrations of **TT1** and **TB2** are 300 nM.

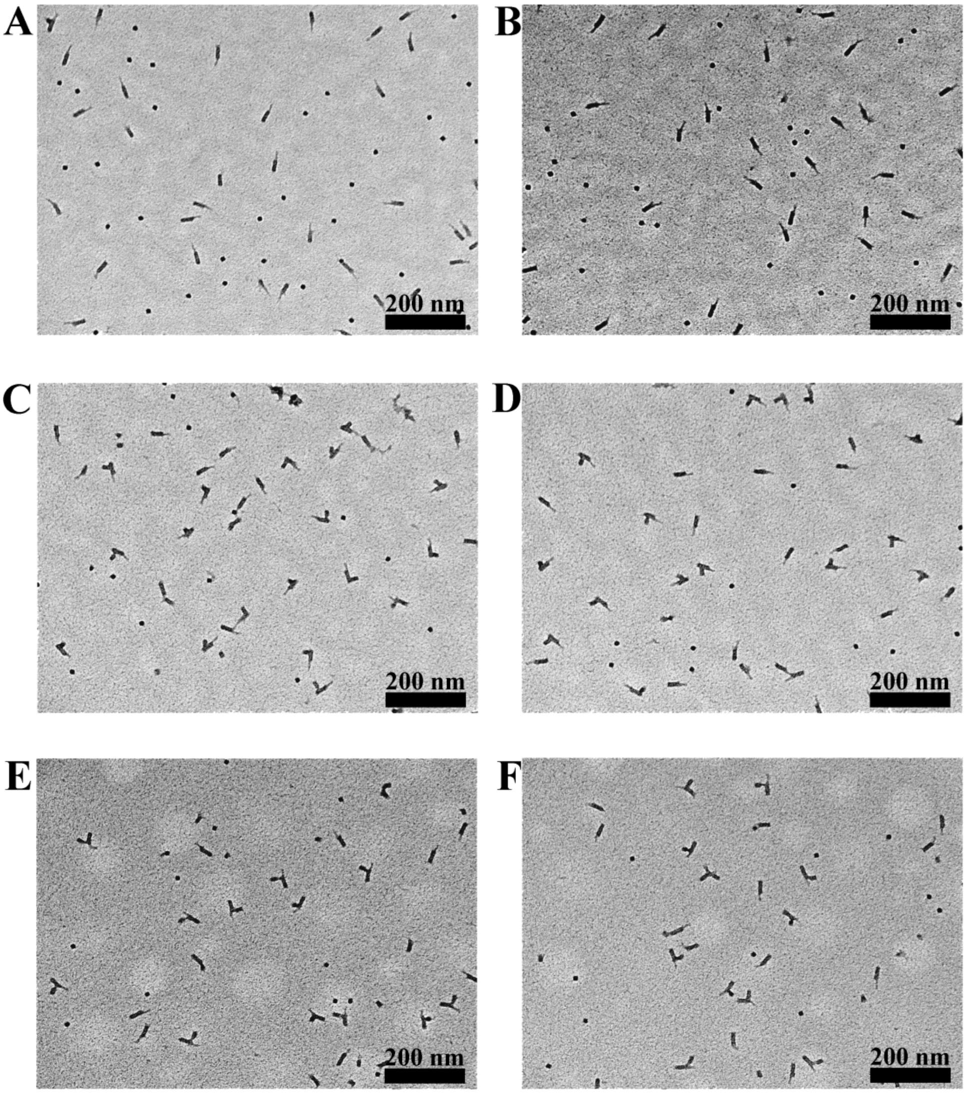

Figure S21. Representative TEM images for two-target detection (in the presence of one target). A) and B) **CU** and **DUT1**, **CU** and **DUB2** in the presence of neither **TT1** nor **TB2**; C) and D) **CU** and **DUT1**, **CU** and **DUB2** in the presence of **TT1** (HP: 50%); E) and F) **CU** and **DUT1**, **CU** and **DUB2** in the presence of **TB2** (HP: 48%). HP was determined by calculation from ~250 **CU**. The concentrations of **TT1** and **TB2** are 300 nM.

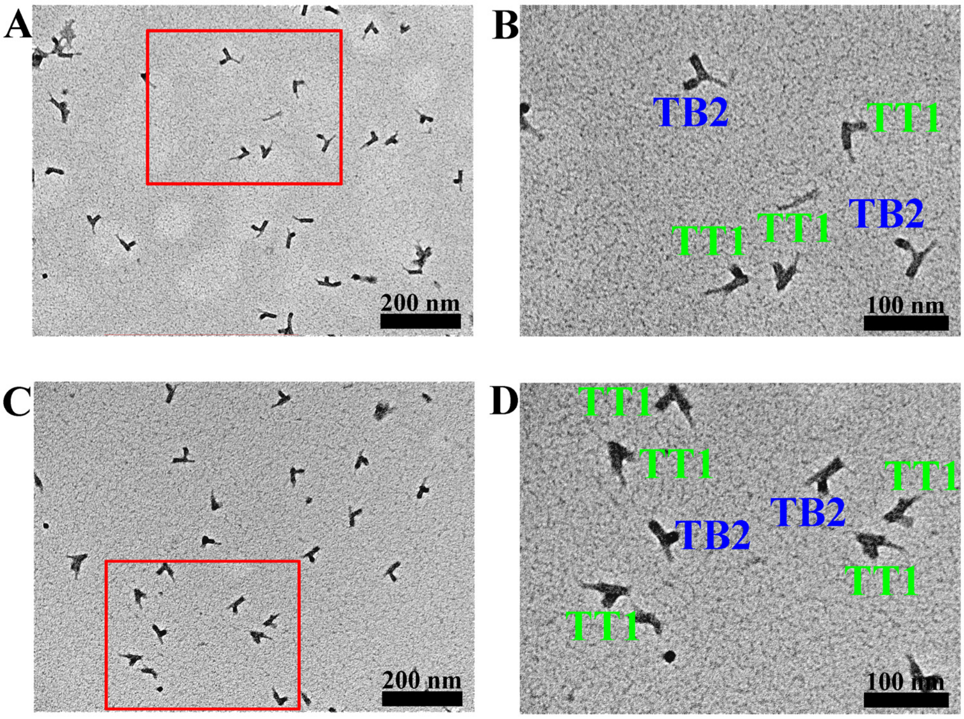

Figure S22. Representative TEM images for two-target detection (separate hybridization, in the presence of two targets). **CU** and **DUT1**, **CU** and **DUB2** in the presence of both **TT1** and **TB2** (HP for **CU-DUT1**: 46%; HP for **CU-DUB2**: 45%). B) and D) are enlarged view images of A) and C), respectively. HP was determined by calculation from ~250 **CU**. The concentrations of **TT1** and **TB2** are 300 nM.

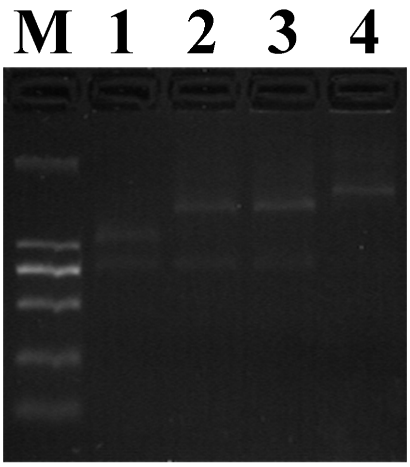

Figure S23. Gel electrophoresis bands for two-target detection (simultaneous hybridization). Lane 1: **CU**, **DUT1**, and **DUB2** in the absence of either **TT1** or **TB2**; Lane 2: **CU**, **DUT1**, and **DUB2** in the presence of **TT1**; Lane 3: **CU**, **DUT1**, and **DUB2** in the presence of **TB1**; Lane 4: **CU**, **DUT1**, and **DUB2** in the presence of both **TT1** and **TB2**. The concentrations of **TT1** and **TB2** are 300 nM.

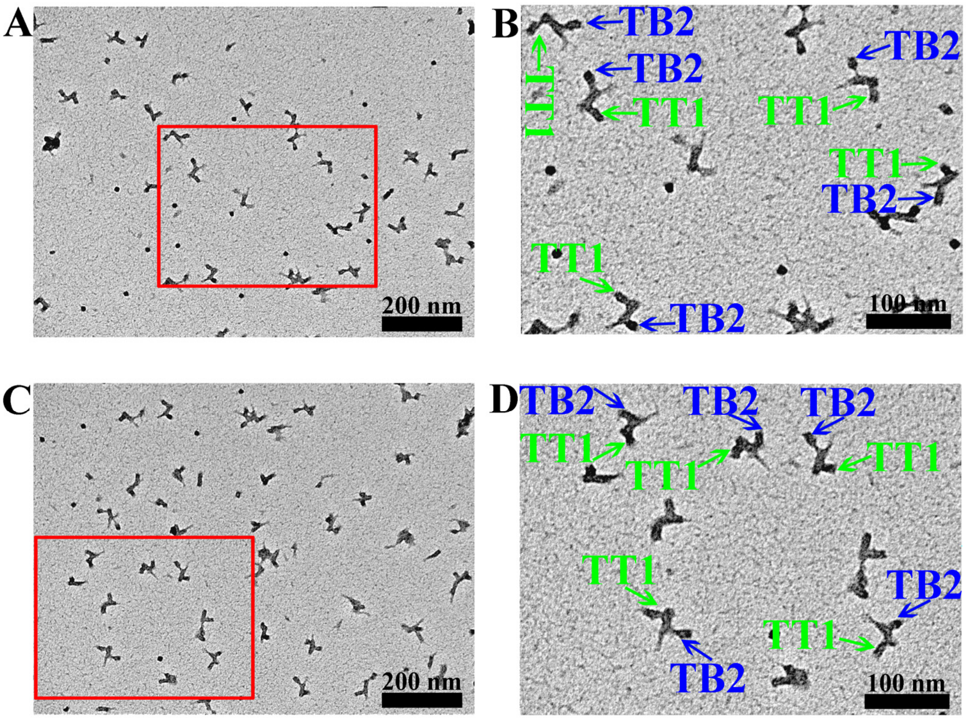

Figure S24. Representative TEM images for two-target detection (simultaneous hybridization). **CU**, **DUT1**, and **DUB2** in the presence of both **TT1** and **TB2** (HP for **CU-DUT1**: 80%; HP for **CU-DUB2**: 83%; for calculation of HP in the case of simultaneous hybridization, **CU-DUT1** and **CU-DUB2** are counted for any structure containing **CU-DUT1** and **CU-DUB2**, respectively). B) and D) are enlarged view images of A) and C), respectively. HP was determined by calculation from ~200 **CU**. The concentrations of **TT1** and **TB2** are 300 nM.

11. Four-Target DNA Detection

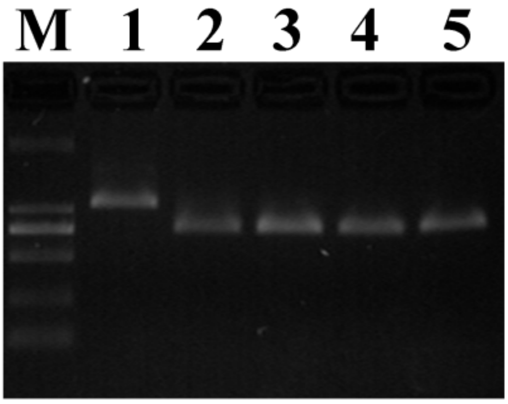

Figure S25. Gel electrophoresis bands for purified **CU**, **DUT1**, **DUT2**, **DUB1**, and **DUB2**. Lane 1: **CU**; Lane 2: **DUT1**; Lane 3: **DUT2**; Lane 4: **DUB1**; Lane 5: **DUB2**.

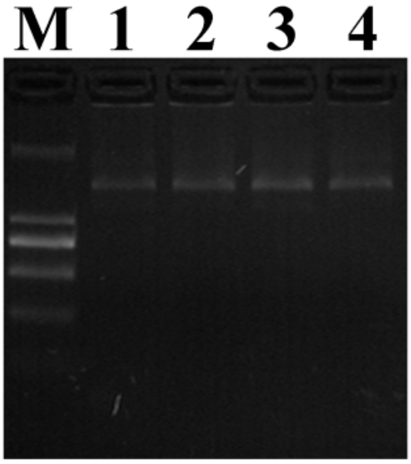

Figure S26. Gel electrophoresis bands for four-target (**TT1**, **TT2**, **TB1**, **TB2**; separate hybridization) detection. Lane 1: **CU** and **DUT1** in the presence of four targets; Lane 2: **CU** and **DUT2** in the presence of four targets; Lane 3: **CU** and **DUB1** in the presence of four targets; Lane 4: **CU** and **DUB2** in the presence of four targets.

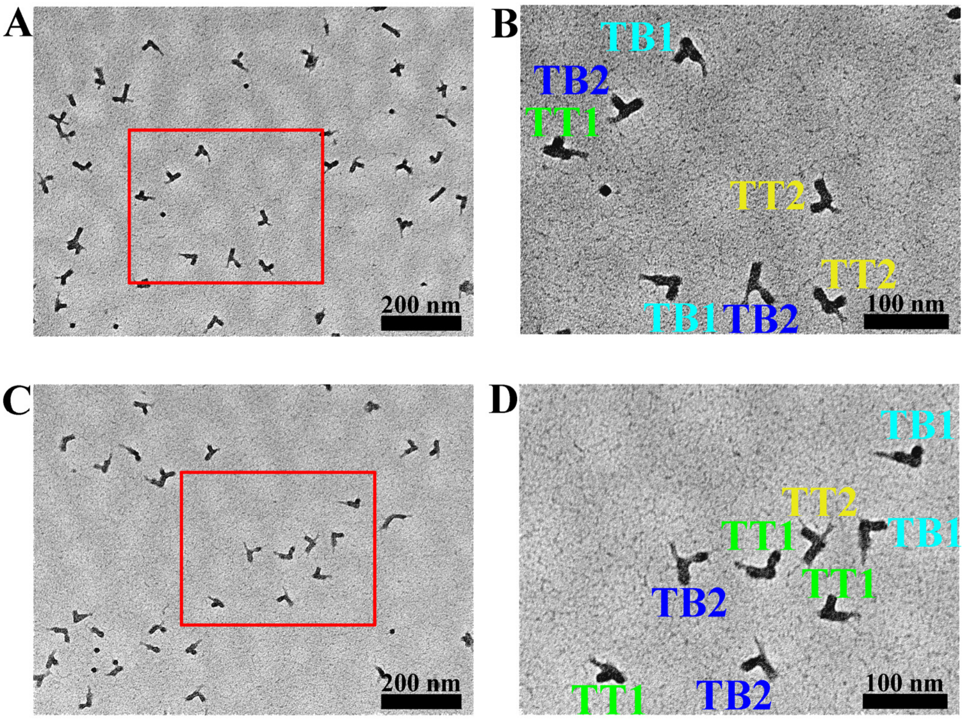

Figure S27. Representative TEM images for four-target detection (separate hybridization). **CU** and **DUT1**, **CU** and **DUT2**, **CU** and **DUB1**, **CU** and **DUB2** in the presence of four targets (**TT1**, **TT2**, **TB1**, **TB2**) (HP for **CU-DUT1**: 22%; HP for **CU-DUT2**: 23%; HP for **CU-DUB1**: 25%; HP for **CU-DUB2**: 24%). B) and D) are enlarged view images of A) and C), respectively. HP was determined by calculation from ~300 **CU**. The concentrations of four targets are all 300 nM.

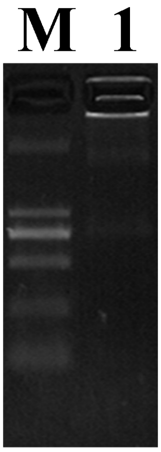

Figure S28. Gel electrophoresis bands for four-target (**TT1**, **TT2**, **TB1**, **TB2**; simultaneous hybridization) detection. Lane 1: **CU**, **DUT1**, **DUT2**, **DUB1**, and **DUB2** in the presence of four targets.

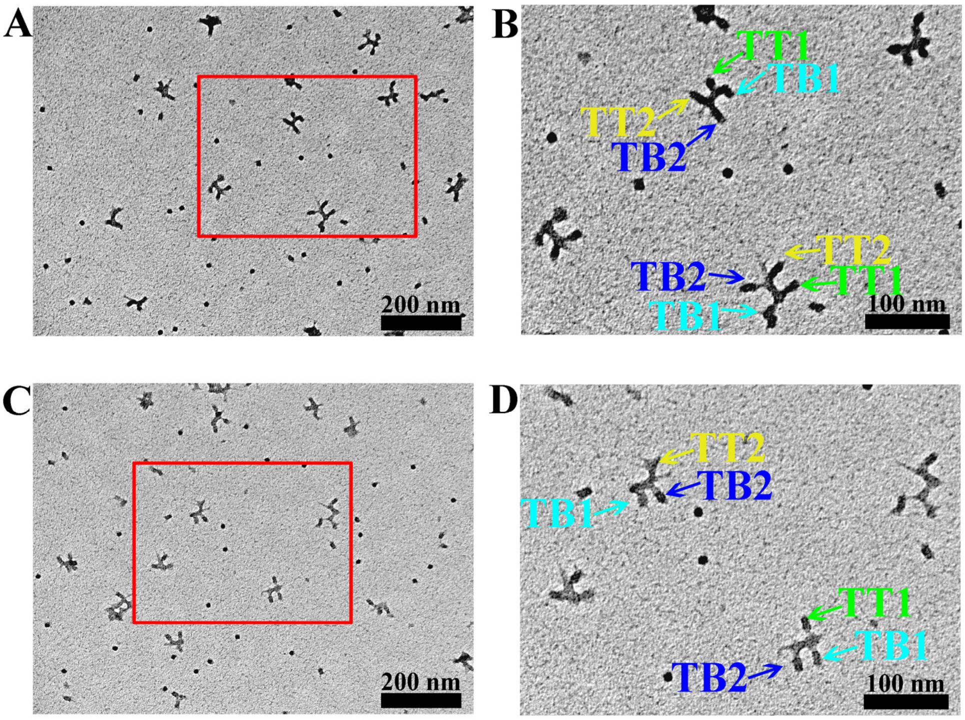

Figure S29. Representative TEM images for four-target detection (simultaneous hybridization). **CU**, **DUT1**, **DUT2**, **DUB1**, and **DUB2** in the presence of four targets (**TT1**, **TT2**, **TB1**, **TB2**) (HP for **CU-DUT1**: 85%; HP for **CU-DUT2**: 75%; HP for **CU-DUB1**: 79%; HP for **CU-DUB2**: 86%). B) and D) are enlarged view images of A) and C), respectively. HP was determined by calculation from ~200 **CU**. The concentrations of four targets are all 300 nM.

12. Assembly of DCU

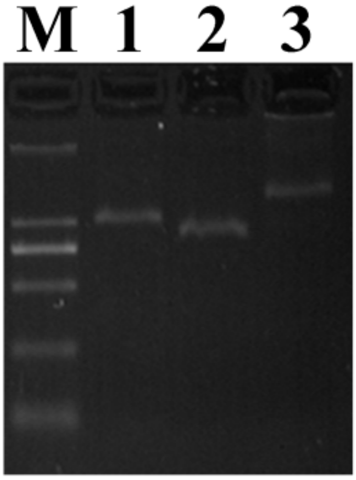

Figure S30. Gel electrophoresis bands for purified **CU**, a purified cuboid with a size identical to the core cuboid part of **CU**, and **DCU**. Lane 1: **CU**; Lane 2: cuboid; Lane 3: **DCU**.

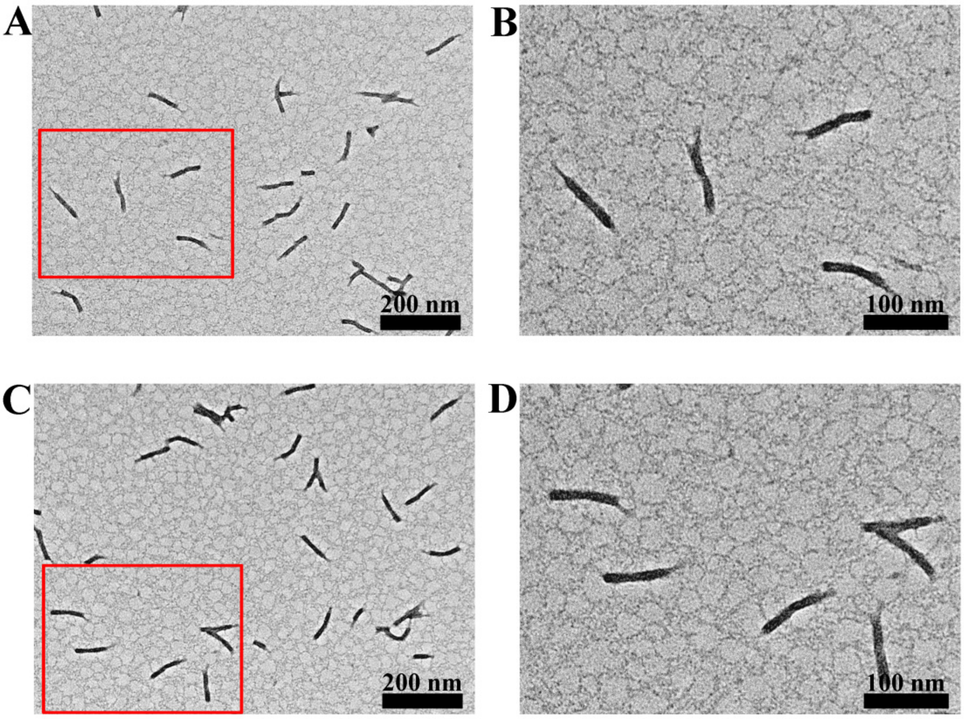

Figure S31. Representative TEM images of **DCU** (HP: 86%). HP is defined as the percentage of observed **DCU** over all structurally resolved **CU** (calculated from ~250 **CU**).

13. Single-Target and Two-Target DNA Detection by DCU Detection System

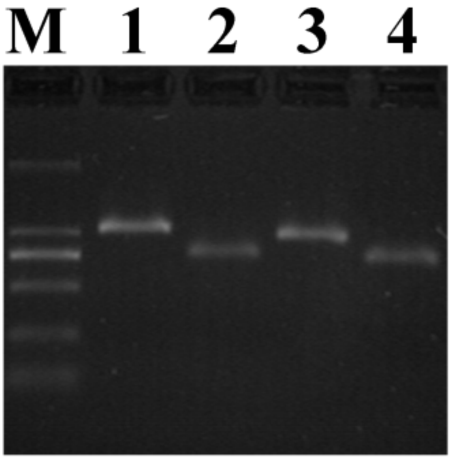

Figure S32. Gel electrophoresis bands for purified **CU**, **DUT1**, cuboid, and **DUB3**. Lane 1: **CU**; Lane 2: **DUT1**; Lane 3: cuboid; Lane 4: **DUB3**.

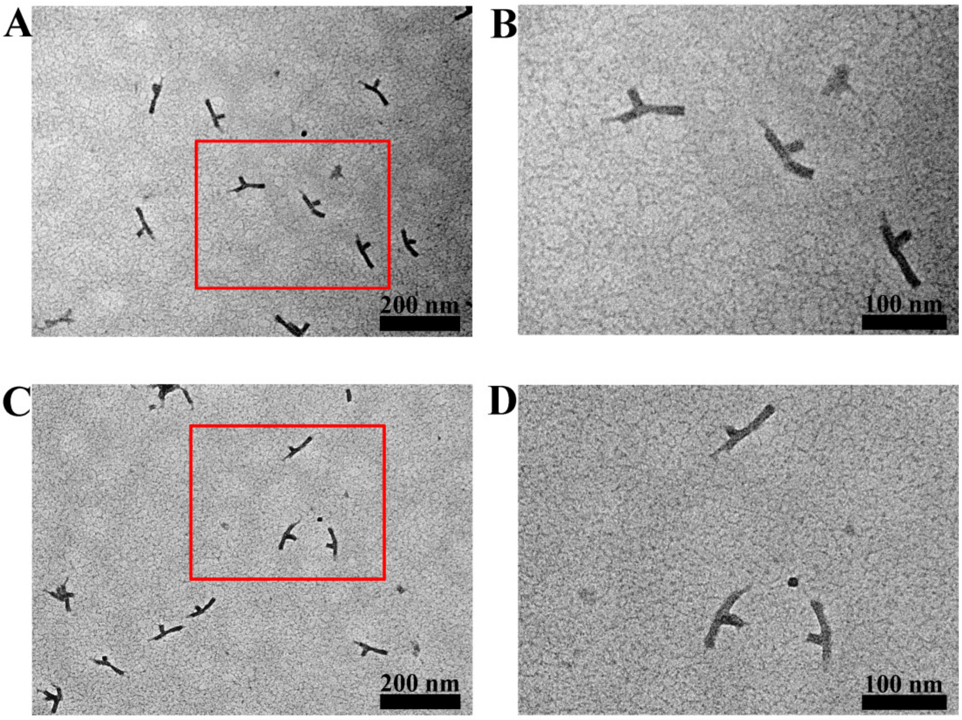

Figure S33. Representative TEM images of **DCU** and **DUT1** in the presence of **TT1** (HP: 92%). B) and D) are enlarged view images of A) and C), respectively. HP is defined as the percentage of observed **DCU-DUT1** over all structurally resolved **DCU** (calculated from ~200 **DCU**). The concentration of **TT1** is 300 nM.

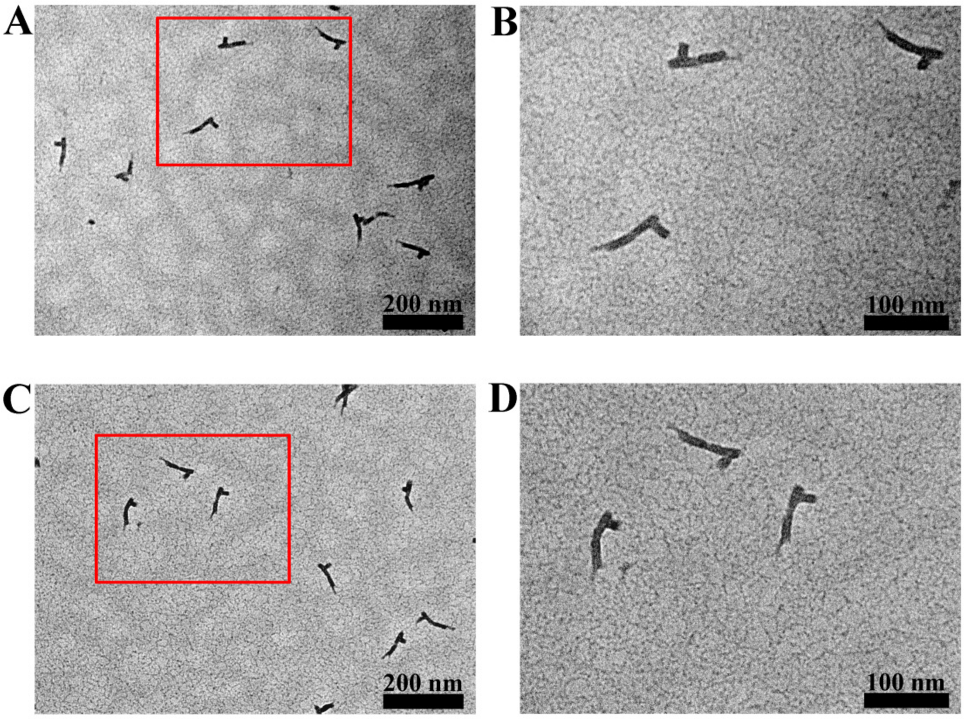

Figure S34. Representative TEM images of **DCU** and **DUB3** in the presence of **TB3** (HP: 93%). B) and D) are enlarged view images of A) and C), respectively. HP is defined as the percentage of observed **DCU-DUB3** over all structurally resolved **DCU** (calculated from ~200 **DCU**). The concentration of **TB3** is 300 nM.

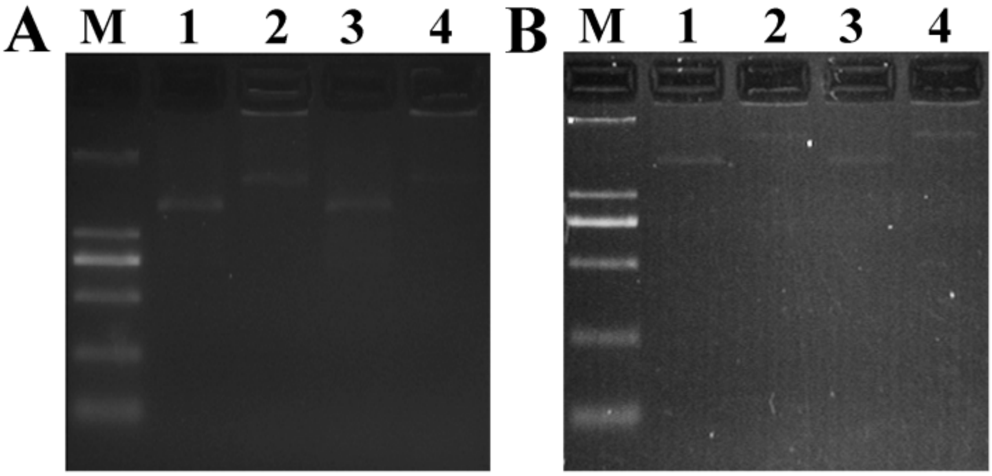

Figure S35. Gel electrophoresis bands for two-target detection. A) **DCU** and **DUT1** in the absence (lane 1) and presence (lane 2) of **TT1**, **DCU** and **DUT1** in the presence of **TB3** (lane 3), **DCU** and **DUT1** in the presence of both **TT1** and **TB3** (lane 4); B) **DCU** and **DUB3** in the absence (lane 1) and presence (lane 2) of **TB3**, **DCU** and **DUB3** in the presence of **TT1** (lane 3), **DCU** and **DUB3** in the presence of both **TT1** and **TB3** (lane 4). The concentrations of **TT1** and **TB3** are 300 nM.

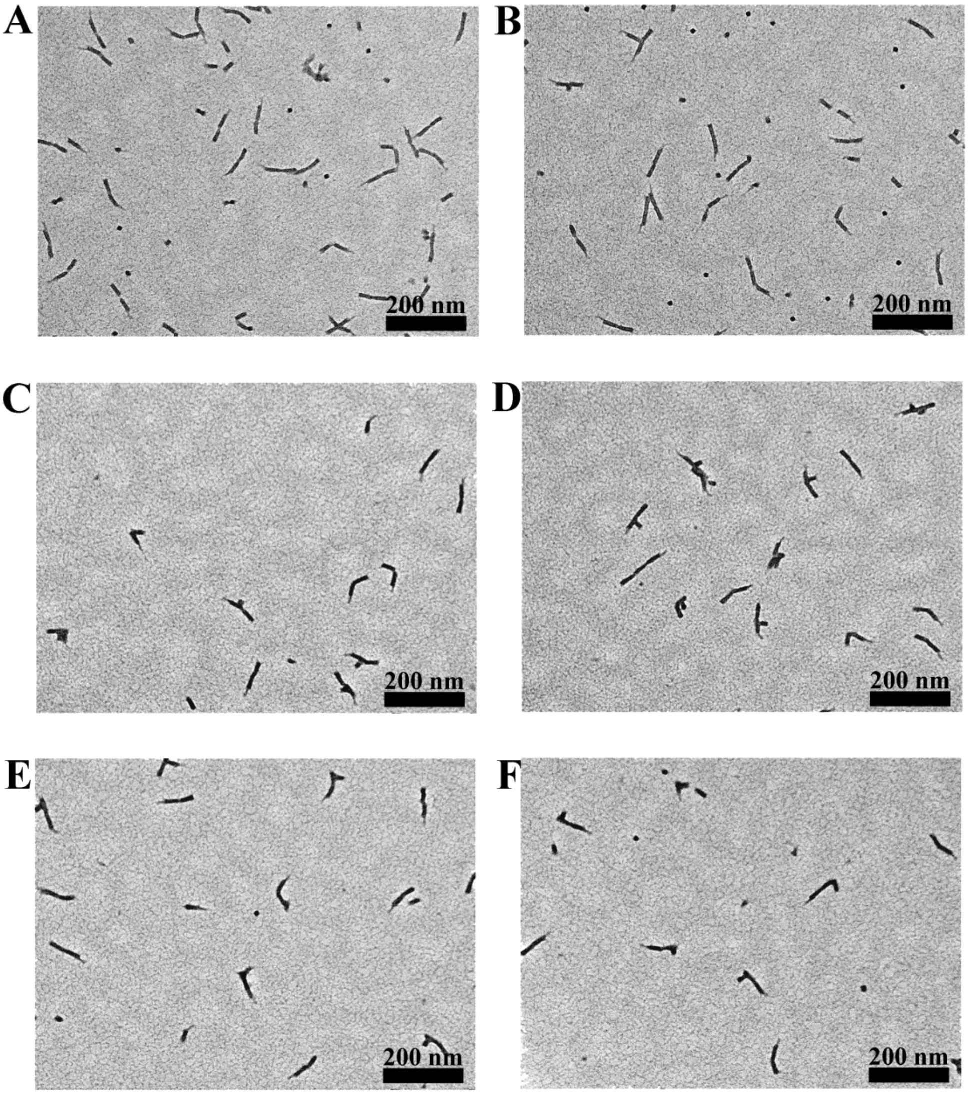

Figure S36. Representative TEM images for two-target detection (in the presence of one target). A) and B) **DCU** and **DUT1**, **DCU** and **DUB3** in the presence of neither **TT1** nor **TB3**; C) and D) **DCU** and **DUT1**, **DCU** and **DUB3** in the presence of **TT1** (HP for **DCU-DUT1**: 47%); E) and F) **DCU** and **DUT1**, **DCU** and **DUB3** in the presence of **TB3** (HP for **DCU-DUB3**: 51%). HP was determined by calculation from ~200 **DCU**. The concentrations of **TT1** and **TB3** are 300 nM.

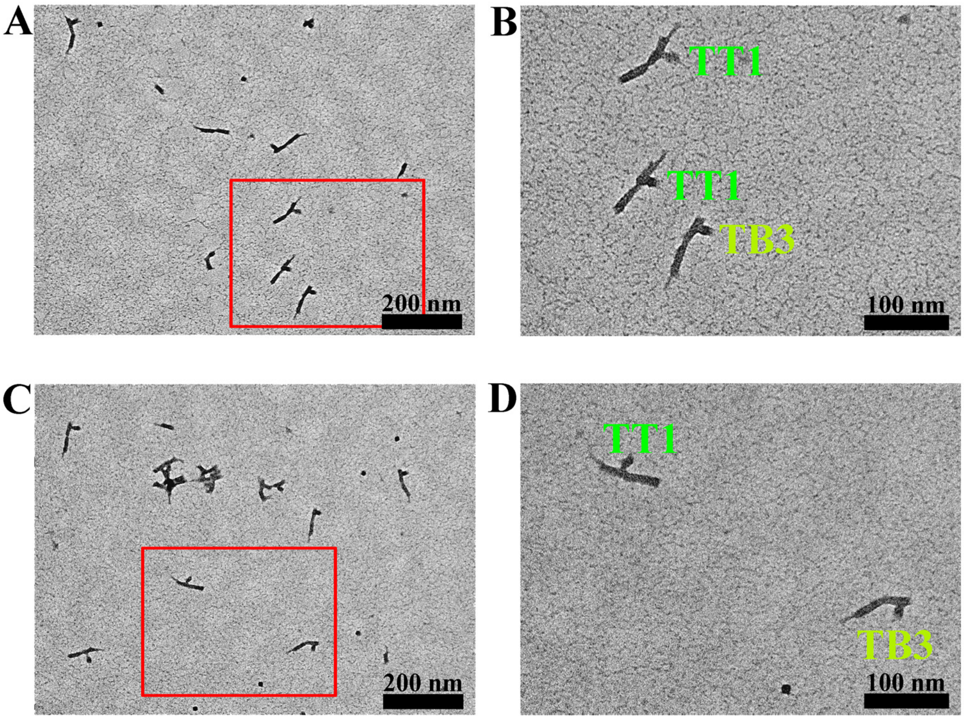

Figure S37. Representative TEM images for two-target detection (separate hybridization, in the presence of two targets). A)-D) **DCU** and **DUT1**, **DCU** and **DUB3** in the presence of both **TT1** and **TB3** (HP for **DCU-DUT1**: 44%; HP for **DCU-DUB3**: 48%). B) and D) are enlarged view images of A) and C), respectively. HP was determined by calculation from ~200 **DCU**. The concentrations of **TT1** and **TB3** are 300 nM.

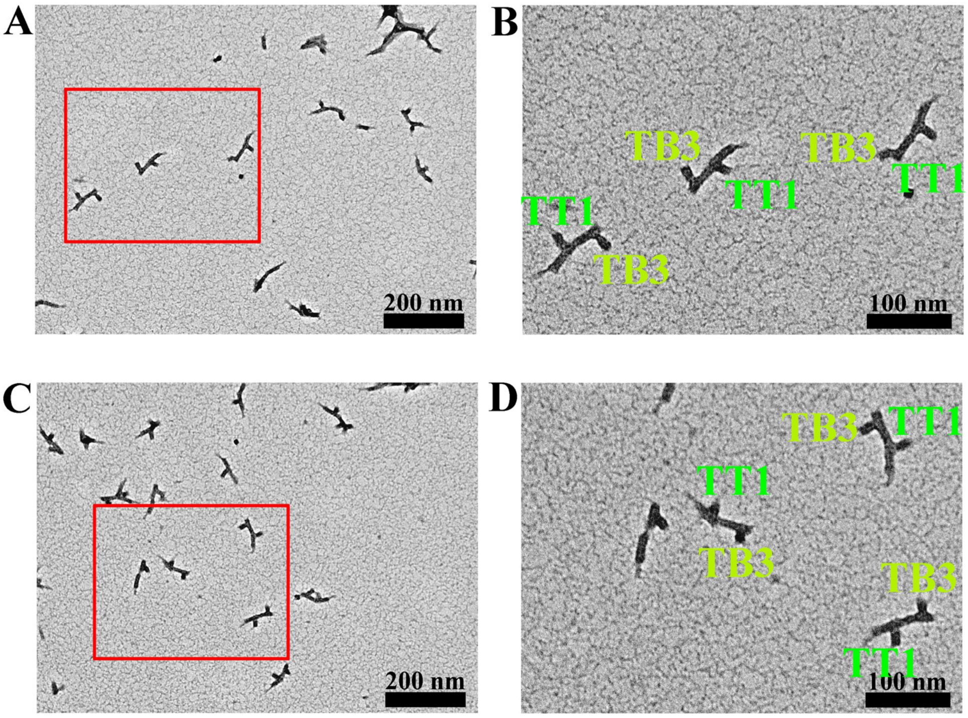

Figure S38. Representative TEM images for two-target detection (simultaneous hybridization). **DCU**, **DUT1**, and **DUB3** in the presence of both **TT1** and **TB3** (HP for **DCU-DUT1-DUB3**: 76%). B) and D) are enlarged view images of A) and C), respectively. HP was determined by calculation from ~200 **DCU**. The concentrations of **TT1** and **TB3** are 300 nM.

14. DNA Sequence Information

Table S1. Probe and target sequences

Name	Sequence (5'-3')	Length
CU TT1 capture probe	GGCTTCTAAAG	11
CU TT2 capture probe	AACGGCAGGAA	11
CU TB1 capture probe	GAATTATGAGT	11
CU TB2 capture probe	GCAAGGGTCAC	11
DCU TB3 capture probe	CGATGTGTCGC	11
DUT1 detection probe	GGTTGTTGGATTTCA	15
DUT2 detection probe	GGCTGGCAGGATGCT	15
DUB1 detection probe	GTCGGTTCACGGAGC	15
DUB2 detection probe	GACCGAGTTACTGTT	15
DUB3 detection probe	GCAGCTCGTGGACCA	15
DCU linker-1 on **CU**	TAAGAGCTATGGG	13
DCU linker-2 on **CU**	CAACAGAGGCAGA	13
DCU linker-1 on cuboid	CCCATAGCTCTTA	13
DCU linker-2 on cuboid	TCTGCCTCTGTTG	13
TT1	CTTTAGAAGCCTGAAATCCAACAACC	26

TT2	TTCCTGCCGTTAGCATCCTGCCAGCC	26
TB1	GCTCCGTGAACCGACACTCATAATTC	26
TB2	AACAGTAACTCGGTCGTGACCCTTGC	26
TB3	TGGTCCACGAGCTGCGCGACACATCG	26
RTT1	CUUUAGAAGCCUGAAAUCCAACAACC	26

Table S2. DNA sequences for the assembly of nanostructures (the sequences used for each nanostructure is marked in the corresponding color: **CU**, **PCU**, **CU for DCU**, **cuboid for DCU**, **DUT1**, **DUT2**, **DUB1**, **DUB2**, **DUB3**)

Name	**Sequence (5’-3’)**	**Length**									
1	CCGCTTCCTTTTTTTTTTTTTTTTGTGCTTGC	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
2	TTGACACCTTTTTTTTTTTTTTTTGGTTCGGA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
3	ACGCGGCCTTTTTTTTTTTTTTTTGGTGAGGA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
4	CCGCCTCCTTTTTTTTTTTTTTTTGGAGCAAG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
5	GCCACGACGCAAGCACGTATTATGGCGAAGAT	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
6	GGCTCAGCTCCGAACCGGAAGGGCTATGATAA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
7	CATATCCCTCCTCACCGGAAGCGGGAGGATGG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
8	CATCCGTCTTTGTCCCGGAGGCGGGTGCGAGA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
9	ATTATCCTATGTCCTCGGCCGCGTGAGTTAGA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
10	TAACTATTACAATACCGAGATCGAGGTCTTAG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
11	CTCATTTCTTATCATAGGGATATGGAAACTAG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
12	GTACCGTCATCTTCGCGGAATTAAACCGGGGT	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
13	AATTAGATCCGGGTCGAGGATAATGGATGTTG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
14	TACTACCTCCATCCTCGACGGATGAGTATATT	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
15	CCACAGATTCTAACTCAATAGTTAATGTCAGA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
16	ATCCACGCTCTCGCACTGGCCGGGACGAAGGC	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
17	GATCCCGTATCGTAATGTCGTGGCATTACGAT	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
18	AACCCTTTACCCCGGTAGGTAGTAGTTGAAAG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
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20	ATTTCGTGAATATACTATCTGTGGGGCAGATG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
21	CCCCGTGCGCCTTCGTGTTGTGAAGTTCGAGC	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
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24	TGCCGCCCACCCTACCATGTGACAAGGTTTGA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
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26	CGTCCGTACCCGTCCAAAAGGGTTGGAGATAG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
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28	ATTAGCCGTGACTTGTGTGCCGACAATGCAGT	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
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30	GCTTAGCTTCAAACCTTAGGCGTAGTGTGGTG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
31	CCTCTACCCTGAAAACAAGAATAAGGTATGGG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
32	CCAGCACCTGCTTTCTTACGGACGCTGACGTA	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩
33	TTACAGCTACTGCATTTAGATGCGGGCGCGAG	32	🟥	🟧	🟥	🟪	🟩	🟨	🟦	🟦	🟩

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41	AACTCCACTTCTACTCTTGTAGCGAGGCAACG	32
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43	TACTTCCACCTCTCTTCGTTATATGCGATGTC	32
44	GCACTCGCCCTCGTCACATGATCGCGGAGACA	32
45	CAATATGTATTCGTTTAACATGGGCTCGGGTT	32
46	GTTCAGACCCACGTTCTTGCGGATACAAGAGC	32
47	TCGATCTCTTTTTTTTTTTTTTTTGGGACAAA	32
48	ATTTGCGCTTTTTTTTTTTTTTTTGAGGACAT	32
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66	ACCCCTGATTACCTGTCACCCGTAATCCGCCCGGTGTCAACGACCCGG	48
67	CCCGGCCACCGGACCCGGGGGCCTGGGGCCCGAGGTTTTAGAGGTCCG	48
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72	CACGCCCAGCTATCCGTTACCCGGTCTTCTGGGGGCGGCAGCAATGTA	48
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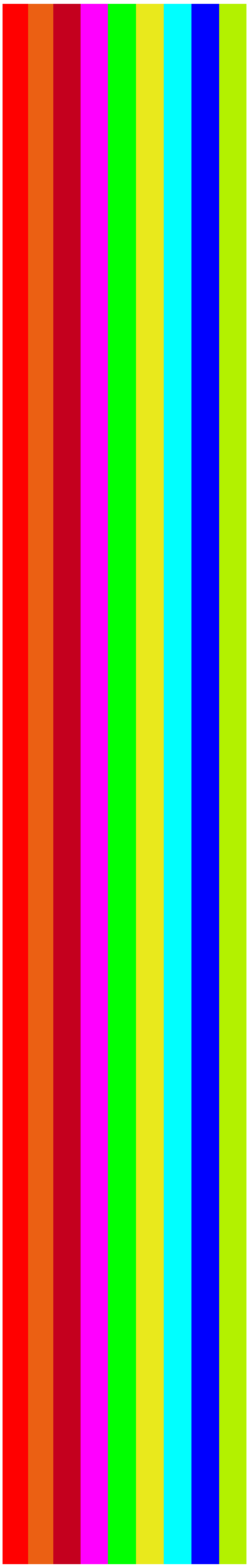

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92	CAAACGCCTTGTGTCTGCGCGGTGTGTATTGA	32
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112	TACCTGCCCTCTATTCAGTTGGTTGGCGGTCA	32
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115	CTCGACCACCGACCCCAGTGAAAAGATATGTG	32
116	GACTTCCGTTTACCGCGGGACACGTAGAGCGC	32
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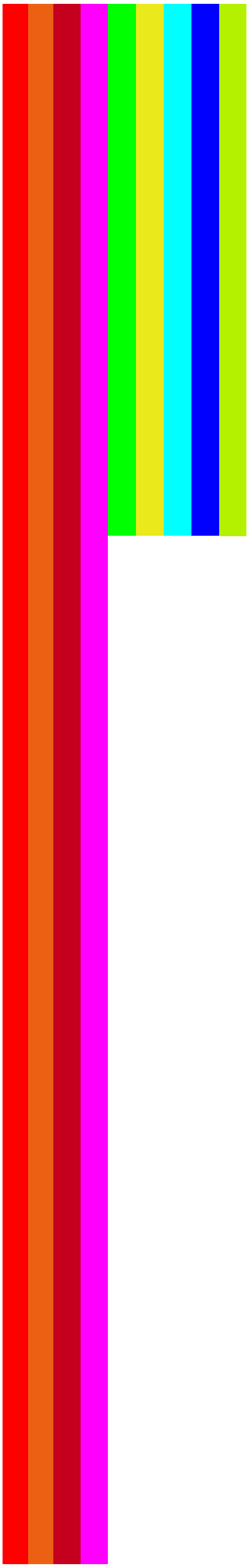

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125	CCCGTCAACCTTTAGCAAGGTGCAGTGAATGA	32	🟥	🟧	🟫	🟪
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128	CAATCGCGCACTACACAAGGCGACAAGAAGGT	32	🟥	🟧	🟫	🟪
129	TCCTGGATTCTTACTGGGTGGACGAGCCGGGT	32	🟥	🟧	🟫	🟪
130	TCGCTTCTCGACTGCTGACGCAGGAAGCTGTT	32	🟥	🟧	🟫	🟪
131	TGCATTCATATCACTTTTGACGGGGGACGTAA	32	🟥	🟧	🟫	🟪
132	TGCAATCCCCAGTCTCTAGGACCGAGGGGCGA	32	🟥	🟧	🟫	🟪
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142	TTTTTTTTTAATCTATGGCTTGAATTTTTTTT	32	🟥	🟧	🟫	🟪
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144	TTCGTCTCCGTTGCCTGAACTTGGGGAACCAAGAGTCATAAGGGTCTT	48	🟥	🟧	🟫	🟪
145	CATTGACCCACGCTACCGAGGTACGGACATTG	32	🟥	🟧	🟫	🟪
146	CATGAACCGCAACCTGTGGGCGTGGGTTAAAT	32	🟥	🟧	🟫	🟪
147	TGTACACCTACCGGATGCGCGGATGGTGAACA	32	🟥	🟧	🟫	🟪
148	CCACGCGCCAGTTTCTTGATTGCCACCCGACGTGTGGTTAGGTTCACG	48	🟥	🟧	🟫	🟪
149	TTACTGACACACAGCACACGGGCCTCAATACAGTCTGAACGGATGGTA	48	🟥	🟧	🟫	🟪
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155	CCCATGTTCTTGCCCTAAGTCAAGAGACACAAGGCGTTTGGGTCGACG	48	🟥	🟧	🟫	🟪
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158	CCAGCGACCAATGTCCGTACCTCGCATCCTGCATGGTTGAGTGAACGA	48	🟥	🟧	🟫	🟪
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162	CTGTTAACTGCAACTCCTTCCGATCGCCCGTAAATAGTGGTACGAAGT	48	🟥	🟧	🟫	🟪
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164	TTCGTTCCTTTCGCAGGTGGAGTTGGAAGACG	32	🟥	🟧	🟫	🟪
165	CACTGTCCGACATCGCGCCAAGACGGACGGCA	32	🟥	🟧	🟫	🟪

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168	CTTGTTCCAACCCGAGGCGAGTGCGGTTCGCG	32
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170	CACAAGCCACATGACTTCTGTCAAACGTCACAAGAGACGGTGGGTGGT	48
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173	CGCGCCTCGTATCACGATCGATGATCTGTAGAACAATGACATGGATCA	48
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176	TTTTTTTTGCCTTAATAGCCTGTAGCCCAGACGATGGAAGCAGTAAGA	48
177	TTTTTTTTAGCCGTCAGGTTCCCGTGGCCCGCGAGGTTATGGGTTTAG	48
178	GGTCTTTTGTTCGTCGGCCGCCGGCCGTTTGTGACCAGGGGACTAAAG	48
179	TCGTATAACATTACTTGAGCTACATGCTACACTTATATAGATGGATTA	48
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182	TGAGCCCCAACACTCCGGTCAATGTGCAAAAGGCCATAGGCCGCTGGA	48
183	CGCTATTTACTCAGTGCTCTATGCATTTAACCGGACAGTGTGCGAATG	48
184	CCCTGTAACCCGGGCCGGTGTACAGAGTACTGAAATACAGAACGCTGT	48
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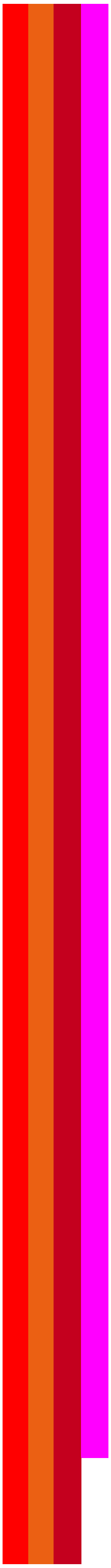

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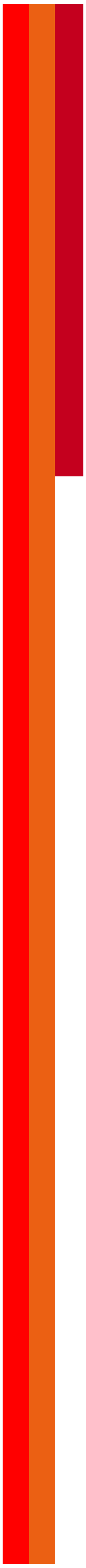

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DUT1-9	TTTTTTTTTCCGTCACGTTTAAGGTTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨			
DUT1-10	TTTTTTTTCACGCTACCGAGGTACTTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨			

DUT1-11	TTTTTTTTGCAACCTGTGGGCGTGTTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨		
DUT1-12	TTTTTTTTTACCGGATGCGCGGATTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨		
DUT1-13	TTTTTTTTACTTCGTAACATATTGTTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨		
DUT1-14	TTTTTTTTTCAATACAGTCTGAACTTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨		
DUT1-15	TTTTTTTTACCCGACGTGTGGTTATTTTTTTTGGTTGTTGGATTTCA	47	🟩	🟨		
DUT2-1	TTTTTTTTTTTCGCAGGTGGAGTTTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-2	TTTTTTTTTGTCTCCGTGGAAGTATTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-3	TTTTTTTTAACCCGAGGCGAGTGCTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-4	TTTTTTTTGACATCGCGCCAAGACTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-5	TTTTTTTTACCGTTGGGATAAATGTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-6	TTTTTTTTGCTCTTGTTGGACACATTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-7	TTTTTTTTTCGTTCACGAGACATATTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-8	TTTTTTTTCGTTGCCTGAACTTGGTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-9	TTTTTTTTTCCGTCACGTTTAAGGTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-10	TTTTTTTTCACGCTACCGAGGTACTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-11	TTTTTTTTGCAACCTGTGGGCGTGTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-12	TTTTTTTTTACCGGATGCGCGGATTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-13	TTTTTTTTACTTCGTAACATATTGTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-14	TTTTTTTTTCAATACAGTCTGAACTTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUT2-15	TTTTTTTTACCCGACGTGTGGTTATTTTTTTTGGCTGGCAGGATGCT	47		🟨		
DUB1-1	GTCGGTTCACGGAGCTTTTTTTTTTTCGCAGGTGGAGTTTTTTTTTT	47			🟦	
DUB1-2	GTCGGTTCACGGAGCTTTTTTTTTGTCTCCGTGGAAGTATTTTTTTT	47			🟦	
DUB1-3	GTCGGTTCACGGAGCTTTTTTTTAACCCGAGGCGAGTGCTTTTTTTT	47			🟦	
DUB1-4	GTCGGTTCACGGAGCTTTTTTTTGACATCGCGCCAAGACTTTTTTTT	47			🟦	
DUB1-5	GTCGGTTCACGGAGCTTTTTTTTACCGTTGGGATAAATGTTTTTTTT	47			🟦	
DUB1-6	GTCGGTTCACGGAGCTTTTTTTTGCTCTTGTTGGACACATTTTTTTT	47			🟦	
DUB1-7	GTCGGTTCACGGAGCTTTTTTTTTCGTTCACGAGACATATTTTTTTT	47			🟦	
DUB1-8	GTCGGTTCACGGAGCTTTTTTTTCGTTGCCTGAACTTGGTTTTTTTT	47			🟦	
DUB1-9	GTCGGTTCACGGAGCTTTTTTTTTCCGTCACGTTTAAGGTTTTTTTT	47			🟦	
DUB1-10	GTCGGTTCACGGAGCTTTTTTTTCACGCTACCGAGGTACTTTTTTTT	47			🟦	
DUB1-11	GTCGGTTCACGGAGCTTTTTTTTGCAACCTGTGGGCGTGTTTTTTTT	47			🟦	
DUB1-12	GTCGGTTCACGGAGCTTTTTTTTTACCGGATGCGCGGATTTTTTTTT	47			🟦	
DUB1-13	GTCGGTTCACGGAGCTTTTTTTTACTTCGTAACATATTGTTTTTTTT	47			🟦	
DUB1-14	GTCGGTTCACGGAGCTTTTTTTTTCAATACAGTCTGAACTTTTTTTT	47			🟦	
DUB1-15	GTCGGTTCACGGAGCTTTTTTTTACCCGACGTGTGGTTATTTTTTTT	47			🟦	
DUB2-1	GACCGAGTTACTGTTTTTTTTTTTTTCGCAGGTGGAGTTTTTTTTTT	47				🟦
DUB2-2	GACCGAGTTACTGTTTTTTTTTTTGTCTCCGTGGAAGTATTTTTTTT	47				🟦
DUB2-3	GACCGAGTTACTGTTTTTTTTTTAACCCGAGGCGAGTGCTTTTTTTT	47				🟦
DUB2-4	GACCGAGTTACTGTTTTTTTTTTGACATCGCGCCAAGACTTTTTTTT	47				🟦
DUB2-5	GACCGAGTTACTGTTTTTTTTTTACCGTTGGGATAAATGTTTTTTTT	47				🟦
DUB2-6	GACCGAGTTACTGTTTTTTTTTTGCTCTTGTTGGACACATTTTTTTT	47				🟦
DUB2-7	GACCGAGTTACTGTTTTTTTTTTTCGTTCACGAGACATATTTTTTTT	47				🟦
DUB2-8	GACCGAGTTACTGTTTTTTTTTTCGTTGCCTGAACTTGGTTTTTTTT	47				🟦
DUB2-9	GACCGAGTTACTGTTTTTTTTTTTCCGTCACGTTTAAGGTTTTTTTT	47				🟦

DUB2-10	GACCGAGTTACTGTTTTTTTTTTCACGCTACCGAGGTACTTTTTTTT	47	🟦
DUB2-11	GACCGAGTTACTGTTTTTTTTTTGCAACCTGTGGGCGTGTTTTTTTT	47	
DUB2-12	GACCGAGTTACTGTTTTTTTTTTTACCGGATGCGCGGATTTTTTTTT	47	
DUB2-13	GACCGAGTTACTGTTTTTTTTTTACTTCGTAACATATTGTTTTTTTT	47	
DUB2-14	GACCGAGTTACTGTTTTTTTTTTTCAATACAGTCTGAACTTTTTTTT	47	
DUB2-15	GACCGAGTTACTGTTTTTTTTTTACCCGACGTGTGGTTATTTTTTTT	47	
DUB3-1	GCAGCTCGTGGACCATTTTTTTTTTTCGCAGGTGGAGTTTTTTTTTT	47	🟩
DUB3-2	GCAGCTCGTGGACCATTTTTTTTTGTCTCCGTGGAAGTATTTTTTTT	47	
DUB3-3	GCAGCTCGTGGACCATTTTTTTTAACCCGAGGCGAGTGCTTTTTTTT	47	
DUB3-4	GCAGCTCGTGGACCATTTTTTTTGACATCGCGCCAAGACTTTTTTTT	47	
DUB3-5	GCAGCTCGTGGACCATTTTTTTTACCGTTGGGATAAATGTTTTTTTT	47	
DUB3-6	GCAGCTCGTGGACCATTTTTTTTGCTCTTGTTGGACACATTTTTTTT	47	
DUB3-7	GCAGCTCGTGGACCATTTTTTTTTCGTTCACGAGACATATTTTTTTT	47	
DUB3-8	GCAGCTCGTGGACCATTTTTTTTCGTTGCCTGAACTTGGTTTTTTTT	47	
DUB3-9	GCAGCTCGTGGACCATTTTTTTTTCCGTCACGTTTAAGGTTTTTTTT	47	
DUB3-10	GCAGCTCGTGGACCATTTTTTTTCACGCTACCGAGGTACTTTTTTTT	47	
DUB3-11	GCAGCTCGTGGACCATTTTTTTTGCAACCTGTGGGCGTGTTTTTTTT	47	
DUB3-12	GCAGCTCGTGGACCATTTTTTTTTACCGGATGCGCGGATTTTTTTTT	47	
DUB3-13	GCAGCTCGTGGACCATTTTTTTTACTTCGTAACATATTGTTTTTTTT	47	
DUB3-14	GCAGCTCGTGGACCATTTTTTTTTCAATACAGTCTGAACTTTTTTTT	47	
DUB3-15	GCAGCTCGTGGACCATTTTTTTTACCCGACGTGTGGTTATTTTTTTT	47	

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