Supporting Information

Ten-input Cube Root Logic Computation with Rational Designed DNA nanoswitches coupled with DNA Strand Displacement Process

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Table S1 Sequences of the oligonucleotides used in this work. The toehold sites in P1, P2, P3 and P4 were colored in red.

Switch	Strand	DNA Sequence (from 5' to 3')
S1	P_1	ATGGT TTTTA ATATG TCACA ATTAA TCAAT ATTTT T
	P_{11}	GTGAC ATATT AAA
	P_{12}	AAAAA TATTG ATT
S2	P_2	TTTGA TTGGATTAAGA ACATTA GGCTGAGATGTAAAC TTGCT
	P_{21}	TCTTA ATCCA GAAA
	P_{22}	TTTAC ATCTC AGCC
S3	P_3	CATTCA GAGT CTCAC CGGAA TTAAA GCGCG TTCGT ATA
	P_{31}	TTCCG GTGAG ACTC
	P_{32}	CGATA ATATA CGAAC GCGCT
S4	P_4	TTGACG GTCT CCGAA ATACA TAATT ACAAT AAGTG TCAC
	$P4_1$	ATGTA TTTCG GAGAC
	P_{42}	GTGAC ACTTA TTGT
Inputs	A	GTGAC ATATT AAA AACCAT
	B	TTAAC TTATA ACATT TCCAA
	C	AGCAAG TTTACATCT CAGCC
	D	TCTTA ATCCA ATCAA A
	E	TTATA ACATT TTAAC TCCAA
	F	TGGAT TAAGA GAGCCAT GGCTGAGA TGTAA A
	G	TTCCG GTGAG ACTC TGAATG
	H	TTTACATCTCAGCC ATGGCTC
	I	TTTAC ATCTC AGCC TAATGT
	J	ATGTA TTTCG GAGAC CGTCA A ATTA CATTCA GAGT CTATTA TTTGAT TGGATT

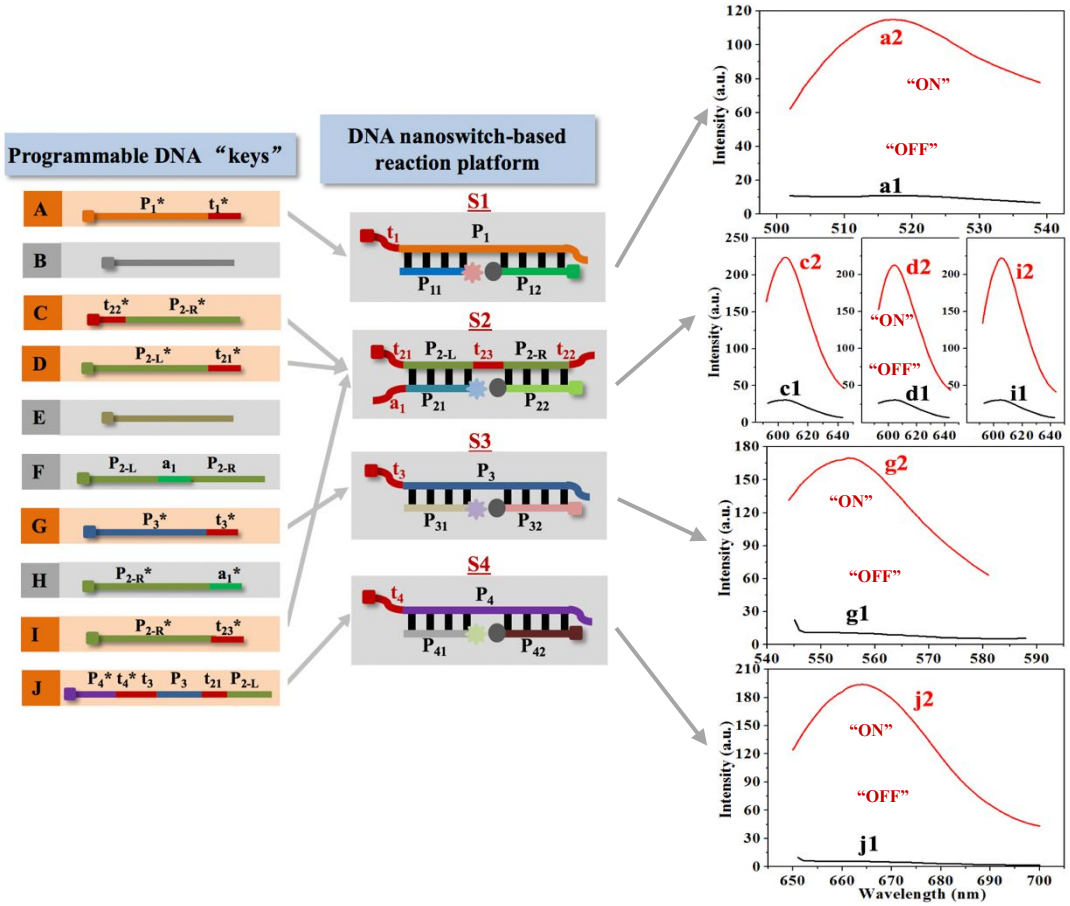

Figure S1. The designed four DNA nanoswitches can be turned on and lit up by specific input signals, performing the "ON" and "OFF" fluorescent output signals (FAM, ROX, HEX and cy5).

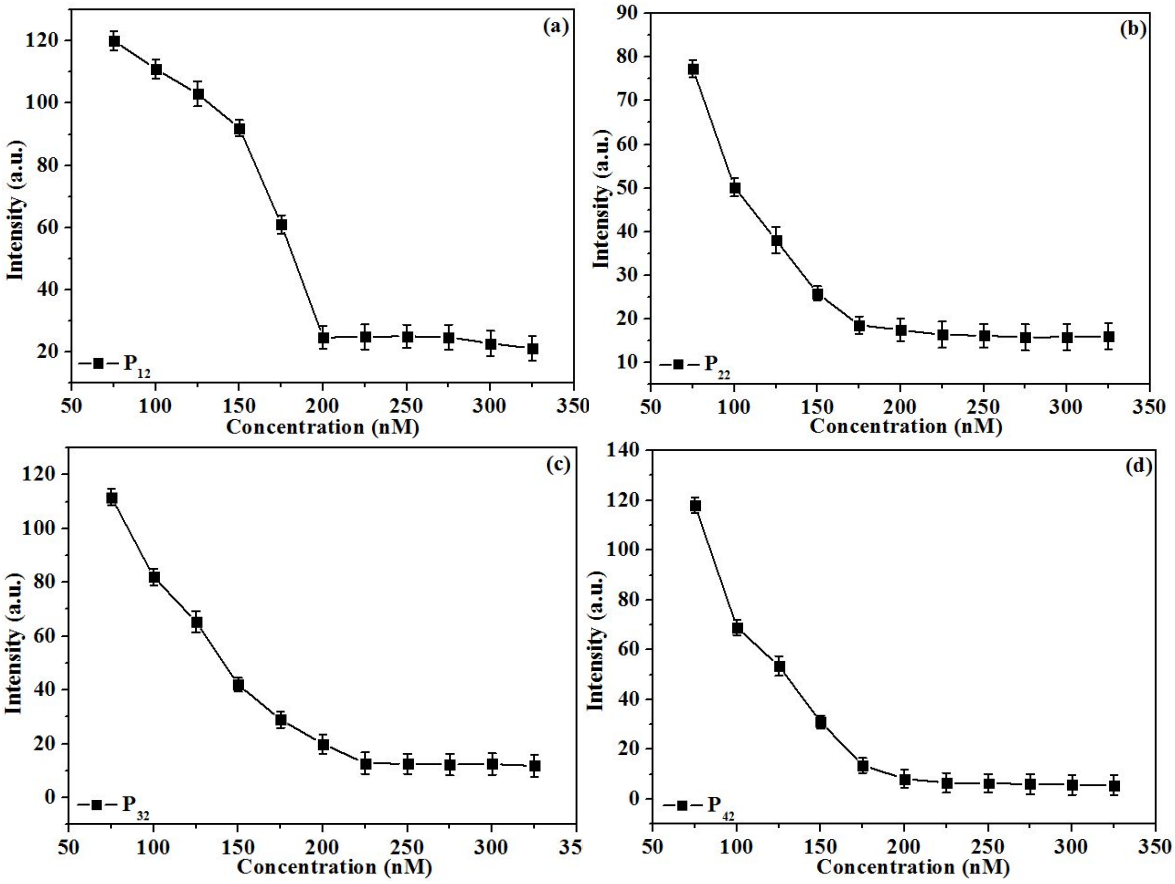

Figure S2. The fluorescence responses of FAM, ROX, HEX and Cy5 that modified on P_{11}, P_{21}, P_{31} and P_{41} in DNA-based nanoswitches (S1, S2, S3 and S4) with increasing the concentrations of P_{12} (a), P_{22} (b), P_{32} (c) and P_{42} (d). The concentrations of P_{11}, P_{21}, P_{31} and P_{41} were fixed at 125 nM, and the concentrations of P_1, P_2, P_3 and P_4 were fixed at 250 nM, which was a little bit higher in order to fully react.

Before the operation of 10-bit cube root logic circuit, the optimum conditions for the formation of nanoswithches should be determined first. In order to optimize the concentrations of the three DNA components of the nanoswitch, the concentrations of two DNA components should be fixed first, and then the third DNA component can be optimized. Take S1 as an example, in order to optimize the concentrations of P_1, P_{11} and P_{12}, the concentration of P_{11}, which was modified by FAM fluorescence, was first fixed at 125 nM, and the concentration of P_1 was defined as 250 nM, which was a little bit higher in order to fully react. Then, as shown in Fig. S2a, with increasing the concentrations of P_{12}, which was modified by quencher, the trend of fluorescence

intensity reached the minimum at 200 nM and remained basically the same via FRET, showing the optimized concentration of P_{12} was 200 nM in 10-bit cube root logic operation. As shown in Figure S2b, with the increase concentration of P_{22}, the trend of fluorescence intensity reached the minimum at 175 nM and remained basically the same, which showed the optimized concentration of P_{22} was 175 nM. As shown in Figure S2c, with the increase concentration of P_{32}, the trend of fluorescence intensity reached the minimum at 225 nM and remained basically the same, which showed the optimized concentration of P_{32} was 225 nM. As shown in Figure S2d, with the increase concentration of P_{42}, the trend of fluorescence intensity reached the minimum at 175 nM and remained basically the same, which showed the optimized concentration of P_{42} was 175 nM.

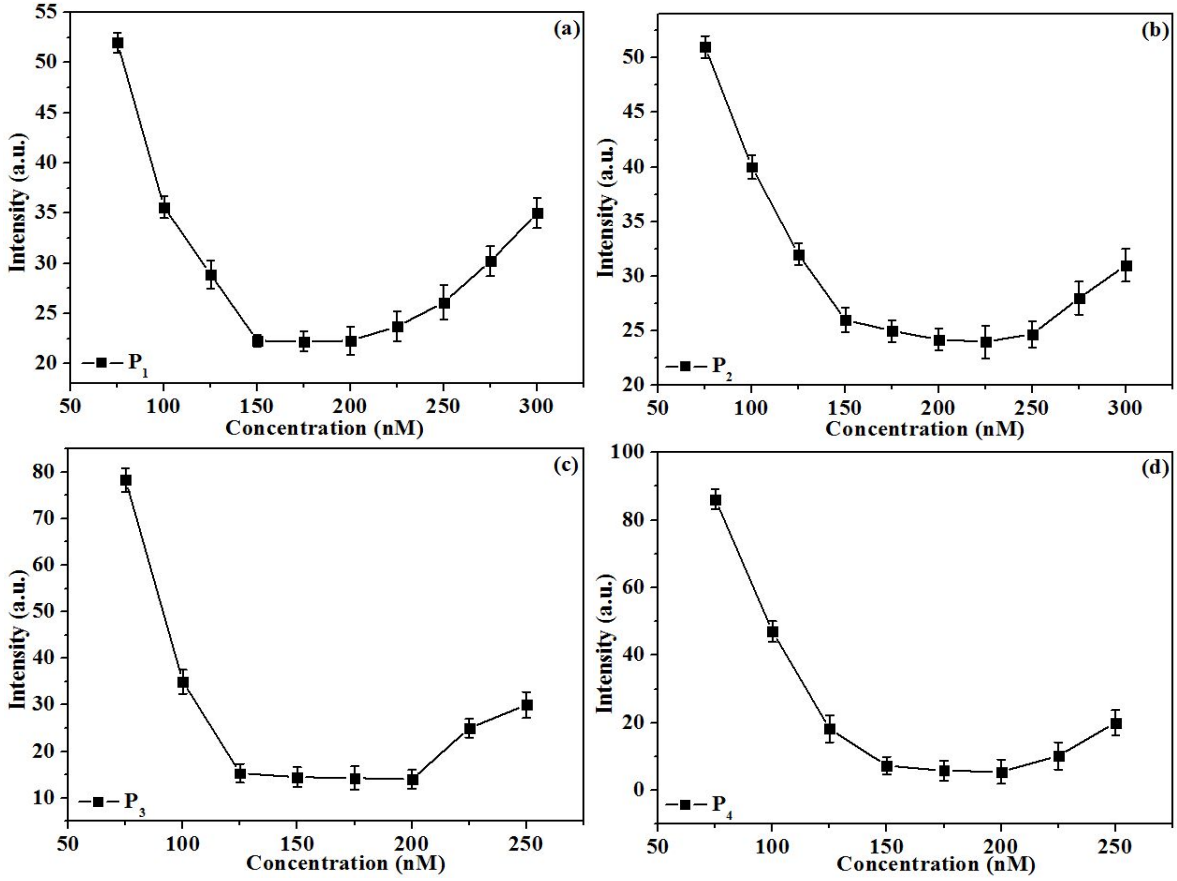

Figure S3. The fluorescence responses of FAM, ROX, HEX and Cy5 that modified on P_{11}, P_{21}, P_{31} and P_{41} in DNA-based nanoswitches (S1, S2, S3 and S4) with increasing the concentrations of P_1 (a), P_2 (b), P_3 (c) and P_4 (d). The concentrations of P_{11}, P_{21}, P_{31} and P_{41} were fixed at 125 nM, and the concentrations of P_{12}, P_{22}, P_{32} and P_{42} were optimized at 200 nM, 175 nM, 225 nM and 175 nM.

As mentioned above, in order to optimize the concentrations of the three DNA components of the nanoswitch, the concentrations of P_{12}, P_{22}, P_{32} and P_{42} were optimized at 200 nM, 175 nM, 225 nM and 175 nM first. Next, after the concentrations of P_{12}, P_{22}, P_{32} and P_{42} were confirmed to optimized values, the concentrations of P_1, P_2, P_3 and P_4 should be optimized since these fixed concentrations were higher during the process of optimizing P_{12}, P_{22}, P_{32} and P_{42}. As shown in Figure S3a, with the increase concentration of P1, the trend of fluorescence intensity reached the minimum at 150 nM and remained basically the same via FRET, which showed the optimized concentration of P_1 was 150 nM in 10-bit cube root logic

operation. As shown in Figure S3b, with the increase concentration of P_2, the trend of fluorescence intensity reached the minimum at 150 nM and remained basically the same via FRET, which showed the optimized concentration of P_2 was 150 nM in 10-bit cube root logic operation. As shown in Figure S3c, with the increase concentration of P_3, the trend of fluorescence intensity reached the minimum at 125 nM and remained basically the same, which showed the optimized concentration of P_3 was 125 nM. As shown in Figure S3d, with the increase concentration of P_4, the trend of fluorescence intensity reached the minimum at 150 nM and remained basically the same, which showed the optimized concentration of P_4 was 150 nM.

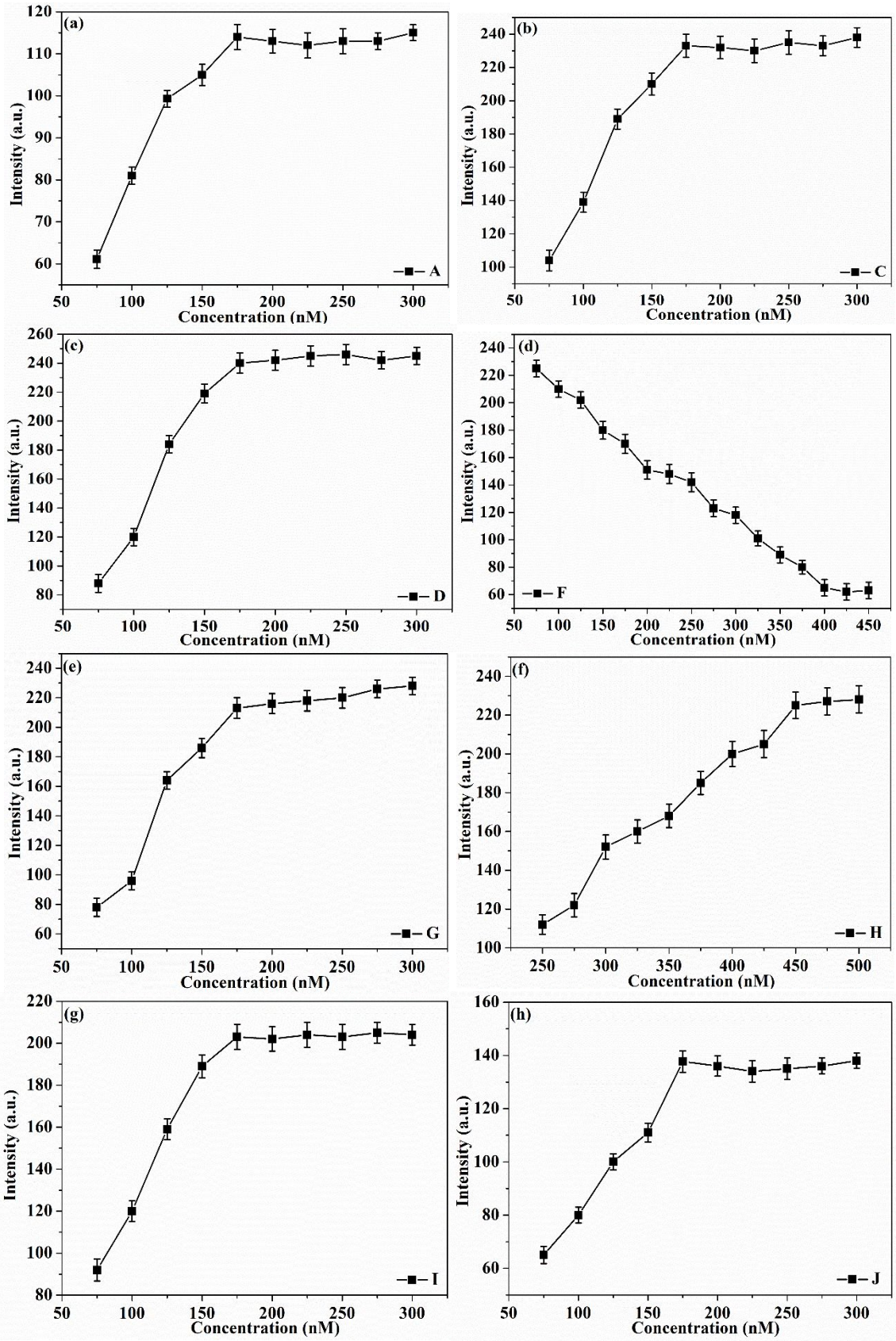

Figure S4. The fluorescence responses of DNA-based nanoswitches (S1, S2, S3 and S4) with increasing the concentrations of A (a), C (b), D (c), F (d), G (e), H (f), I (g), J (h).

Before the operation of 10-bit cube root logic circuit, different concentrations of inputs involved in hybridization including A, C, D, F, G, H, I and J have been optimized with DNA-based nanoswitch platform based on the fluorescence of FAM, ROX, HEX and Cy5 that modified on P11, P21, P31 and P41. As shown in Fig. S4a, with the increase concentration of A, the trend of fluorescence intensity reaches the maximum at 175 nM and remains basically the same via FRET, showing the optimized concentration of A is 175 nM in 10-bit cube root logic operation. As shown in Fig. S4b, with the increase concentration of C, the trend of fluorescence intensity reaches the maximum at 175 nM and remains basically the same, which shows the optimized concentration of C is 175 nM. As shown in Fig. S4c, with the increase concentration of D, the trend of fluorescence intensity reaches the maximum at 175 nM and remains basically the same via FRET, showing the optimized concentration of Dis 175 nM. As shown in Fig. S4d, the task of input F is that in the calculation of "$\sqrt[3]{125}$=5", it is programmed to hybridize with input C and D to inhibit the enhancement of Y2. With the increase concentration of F, the trend of fluorescence intensity reaches to the minimum at 400 nM and remains basically the same via FRET, showing the optimized concentration of F is 400 nM in 10-bit cube root logic operation. As shown in Fig. S4e, with the increase concentration of G, the trend of fluorescence intensity reaches to the maximum at 175 nM and remains basically the same via FRET, showing the optimized concentration of G is 175 nM in 10-bit cube root logic operation. As shown in Figure S4f, the task of input H is that in the calculation of "$\sqrt[3]{1000}$=10", it is programmed to hybridize with input F, which can hybridize with input I and affect the enhancement of Y2. Therefore, with the increase concentration of input H, the trend of fluorescence intensity reaches to the maximum at 450 nM and remains basically the same via FRET, showing the optimized concentration of input H is 450 nM in 10-bit cube root logic operation. As shown in Figure S4g, with the increase concentration of I, the trend of fluorescence intensity reaches to the maximum at 175 nM and remains basically the same via FRET, showing the optimized concentration of I is 175 nM. As shown in Figure S4h, with

the increase concentration of J, the trend of fluorescence intensity also reaches to the maximum at 175 nM and remains basically the same, which showed the optimized concentration of J is 175 nM.

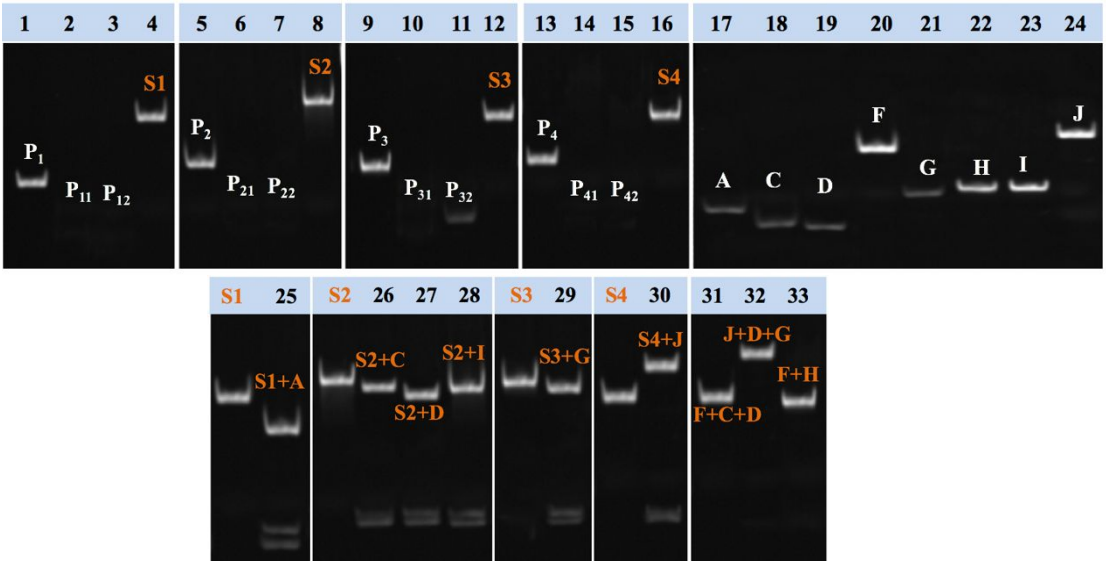

Figure S5. PAGE analysis of the interaction between DNA nanoswitch-based platform and inputs in the operation of 10-bit cube root logic circuit. Different lanes have been labeled in the figure.

In order to further identify the DNA hybridizations in the realization of the 10-bit cube root logic operation, the native polyacrylamide gel electrophoresis (PAGE) experiments are performed as shown in Figure S5. All the DNA sequences involved in the 10-bit cube root logic operation have been determined by different belts. First of all, the hybridizations of the corresponding DNA components in the formation of each DNA nanoswitch are demonstrated. From Lane 1 to Lane 3, the bands show the single-stranded of P1, P11 and P12. When the three components are mixed together, a new band appears in Lane 4, indicating the hybridization happens and forms the duplex of P1/(P11+P12), which is the nanoswitch S1. From Lane 5 to Lane 7, the bands show the single-stranded P2, P21 and P22. When they are mixed together, a new band appears in Lane 8, indicating the hybridization happens and forms the duplex of P2/(P21+P22), which is the nanoswitch S2. From Lane 9 to Lane 11, the bands show the single-stranded P3, P31 and P32. A new band appears in Lane 12

when they are mixed together, which proves the hybridization happens among P3, P31 and P32 and forms the duplex of P3/(P31+P32), which is the nanoswitch S3. From Lane 13 to Lane 15, the bands show the single-stranded P4, P41 and P42. A new band appear in Lane 16 when they are mixed together, which proves the hybridization happens among P4, P41 and P42 and forms the duplex of P4/(P41+P42), which is the nanoswitch S4. From Lane 17 to Lane 24, the bands show the input strands involved in hybridization reactions, including input A, C, D, F, G, H, I and J. As mentioned above, the designed DNA nanoswitches (S1, S2, S3 and S4) can be turned on by specific inputs through toehold-mediated strand displacement reaction, which can be further confirmed by PAGE experiments. When input A is added to S1, a new band appears in Lane 25 since the input A is programmed to hybridize with S1. When input C, D or I is added to S2, a new band appears in each Lane of 26, 27 and 28, which confirms that inputs C, D and I can hybridize with S2 respectively. When input G is added to S3, a new band appears in Lane 29 since the input G is designed to turn on S3. When the addition of input J to S4, input J can turn on S4 and form a new band in Lane 30. In addition, it is mentioned that in the calculation of the cube root of decimal “125”, input F has the priority to hybridize with C and D. Thus, when input C, D and F are mixed together, a new band appears in Lane 31, demonstrating the formation of the duplex of F/(C+D). From the calculation of cube roots of decimal “729” and “1000”, it is designed that the input J can not only turn on S4, but also hybridize with inputs D and G. Therefore, when they are added to the platform, a new band appears in Lane 32. Also, when inputs F and H mixed together, a new band appears in Lane 33 since they have the priority to hybridize and form the duplex of F/H. The bands of P11, P12, P21, P22, P31, P41 and P42 are barely saw in Fig. S5. That is because these DNA sequences have too few bases (P11: 13 bases; P12: 13 bases; P21: 14 bases; P22: 14 bases; P31: 14 bases; P41: 15 bases; P42: 14 bases), and the chain size is too small that they passed directly through the gap in the gel and cannot stay in it. The PAGE results validate the DNA reactions involve in 10-bit cube root logic circuit occur as expected, which are consistent with the fluorescence results in Figure 3.