Supplementary Information

Bridge DNA guided Assembly of Nanoparticles to Program Chemical Reaction Networks

Jianing Zhang[a, b], Jiayu Yu[a, b], Jing Jin[*a], Xiang Zhou[b], Haojun Liang[b], Feng Zhou[c], Wei Jiang[*a, b]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China

[b] University of Science and Technology of China, Hefei, Anhui 230026, PR. China

[c] Personalized Prescribing Inc., Toronto, ON M3C 3E5, Canada

[*] Corresponding authors: Fax: +86-431-85262126; Tel: +86-431-85262151

E-mail: jjin@ciac.ac.cn (J. Jin); wjiang@ciac.ac.cn (W. Jiang)

This supplementary file includes:

S1. The DNA sequences used in the study.

Table S1. The DNA sequences used in simultaneous detection in Figure 3D.

Name	Sequences in simultaneous detection
Sequences attached onto AuNPs in simultaneous detection	
T-DNA-1	5'- GGTGGTCTTCGTATGAATACAAAAAAAA-HS-SH-3'
T-DNA-2	5'-HS-SH-AAAAAAAATTCAGGCGTGTAAGCTCGTTTC-3'
Sequences used in the formation of bridge DNA in simultaneous detection	
T-bridge DNA-1	5'-TTCATACGAAGACCACCCGATCCAAGTGAAACGAGCTTACACGCCT-3'
T-bridge DNA-2	5'-TTCATACGAAGACCACCACTTGGATCGGAAACGAGCTTACACGCCT-3'
Other sequences used in simultaneous detection	
T-DNA-1 target	5'-GTATTCATACGAAGACCACC-3'
T-DNA-2 target	5'-GAAACGAGCTTACACGCCTGAA-3'
flare DNA-1	5'-cy3-GTATTCATACGAAG-3'
flare DNA-2	5'-cy5-ACACGCCTGAA-3'

The color coding is the same as that used in Figure 3D.

Table S2. The DNA sequences used in the multi-step reaction with different toehold lengths in Figure 6.

Name	Sequences in the multi-step reaction with different toehold lengths
Toehold length-1=6 Toehold length-2=6	
Sequences used in the formation of bridge DNA with different toehold lengths	
bridge DNA-1	5'-ACTCCAACTCTACCCTCACCGATCCAAGTCTACTCCTACCACTCATCA-3'
bridge DNA-2	5'-ACTCCAACTCTACCCTCACACTTGGATCGCTACTCCTACCACTCATCA-3'
Other sequences used in the multi-step reaction	
ssDNA linker	5'-ACTCCAACTCTACCCTCACCGATCCAAGTCTACTCCTACCACTCATCA-3'
protector-1	5'-GTAGAGTTGGA-3'
protector-2	5'-ATGAGTGGTAG-3
catalyst-1	5'-GTGAGGGTAGAG-3'
catalyst-2	5'-TGGTAGGAGTAG-3'
Toehold length-1=6 Toehold length-2=7	
Sequences used in the formation of bridge DNA with different toehold lengths	
bridge DNA-1	5'-CACTCCAACTCTACCCTCACCGATCCAAGTCTACTCCTACCACTCATCAC-3'
bridge DNA-2	5'-CACTCCAACTCTACCCTCACACTTGGATCGCTACTCCTACCACTCATCAC-3'
Other sequences used in the multi-step reaction	
ssDNA linker	5'-CACTCCAACTCTACCCTCACCGATCCAAGTCTACTCCTACCACTCATCAC-3'
protector-1	5'-GTAGAGTTGGAG-3'
protector-2	5'-GATGAGTGGTAG-3
catalyst-1	5'-GTGAGGGTAGAGT-3'

catalyst-2	5’-ATGGTAGGAGTAG-3’
Toehold length-1=7 Toehold length-2=7	
Sequences used in the formation of bridge DNA with different toehold lengths	
bridge DNA-1	5’-CACTCCAACTCTACCCTCACCCGATCCAAGTCCTACTCCTACCACTCATCAC-3’
bridge DNA-2	5’-CACTCCAACTCTACCCTCACCACTTGGATCGCCTACTCCTACCACTCATCAC-3’
Other sequences used in the multi-step reaction	
ssDNA linker	5’-CACTCCAACTCTACCCTCACCCGATCCAAGTCCTACTCCTACCACTCATCAC-3’
Toehold length-1=7 Toehold length-2=8	
bridge DNA-1	5’-CCACTCCAACTCTACCCTCACCCGATCCAAGTCCTACTCCTACCACTCATCACC-3’
bridge DNA-2	5’-CCACTCCAACTCTACCCTCACCACTTGGATCGCCTACTCCTACCACTCATCACC-3’
Other sequences used in the multi-step reaction	
ssDNA linker	5’-CCACTCCAACTCTACCCTCACCCGATCCAAGTCCTACTCCTACCACTCATCACC-3’
Toehold length-1=7 Toehold length-2=9	
bridge DNA-1	5’-CCCACTCCAACTCTACCCTCACCCGATCCAAGTCCTACTCCTACCACTCATCACCC-3’
bridge DNA-2	5’-CCCACTCCAACTCTACCCTCACCACTTGGATCGCCTACTCCTACCACTCATCACCC-3’
Other sequences used in the multi-step reaction	
ssDNA linker	5’-CCCACTCCAACTCTACCCTCACCCGATCCAAGTCCTACTCCTACCACTCATCACCC-3’
Toehold length-1=5 Toehold length-2=8	
bridge DNA-1	5’-CCACTCCAACTCTACCCTCACGATCCAAGTTACTCCTACCACTCATCACC-3’
bridge DNA-2	5’-CCACTCCAACTCTACCCTCAACTTGGATCGTACTCCTACCACTCATCACC-3’
Other sequences used in the multi-step reaction	
ssDNA linker	5’-CCACTCCAACTCTACCCTCACGATCCAAGTTACTCCTACCACTCATCACC-3’
catalyst-1	5’-TGAGGGTAGAGTT-3
catalyst-2	5’-AGTGGTAGGAGTA-3’

The color coding is the same as that used in Figure 7.

Table S3. The DNA sequences used in DNA logic gate in Figure 8.

Name	DNA sequences
Sequences used in the formation of bridge DNA in DNA logic gate	
L-bridge DNA-1	5’-FAM-CCACTCCAACTCTACCCTCACCGATCCAAGTCTACTCCTACCACTCATCACC-3’

L-bridge DNA-2	5’-CCACTCCAACTCTACCCTCACACTTGGATCGCTACTCCTACCACTCATCACC-Cy5-3’
Sequences attached onto AuNPs in DNA logic gate	
L-DNA-1	5’-HS-SH-TTTTTTTTTTTTTTGGTAGAGTTGGAGTGG-3’
L-DNA-2	5’-GGTGATGAGTGGTAGGTTTTTTTTTTTTTT-HS-SH-3’
Other sequences used in DNA logic gate	
L-protector-1	5’-GTAGAGTTGGAGT-3’
L-protector-2	5’-TGATGAGTGGTAG-3
L-catalyst-1	5’-GTGAGGGTAGAGTT-3’
L-catalyst-2	5’-AGTGGTAGGAGTAG-3’

The color coding is the same as that used in Figure 8.

S2. Characterization of the DNA modified AuNP.

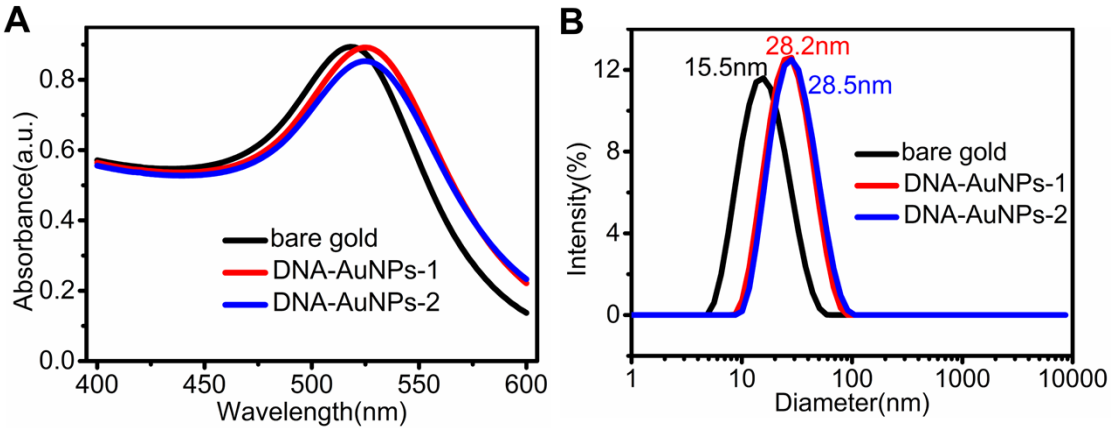

Figure S1. UV-vis spectra (A) and hydrodynamic sizes (B) of bare AuNP and functional DNA-AuNPs with DNA-1 and DNA-2, respectively.

To get more information on the preparation of the DNA functionalized AuNP, the solutions of bare AuNP and functional DNA-AuNPs with DNA-1 and DNA-2 are characterized by UV-vis spectroscopy and DLS, respectively. As shown in Figure S1A, the UV-vis extinction spectra of the bare gold (black line) have an absorption maximum at a wavelength of 518 ± 1 nm, and the DNA-AuNPs-1 (red line) and DNA-AuNPs-1 (blue line) show the absorption maximum at a wavelength of 521 ± 1 nm, these redshifts prove the successful preparation of DNA-AuNPs. In Figure S1B, the direct proof of DNA modified AuNPs is provided by the DLS results. The presence of the diameter peaks at around 28.2 ± 0.6 nm and 28.5 ± 0.4 nm demonstrate the increase in size compared with the diameter of bare gold being 15.5 ± 0.5 nm, implying that DNA can specifically bind to AuNP. Therefore, DNA-AuNP with two kinds of DNA has been successfully prepared, respectively.

S3. Characterization of the DNA modified AuNP with different ratios of two types of DNA strands.

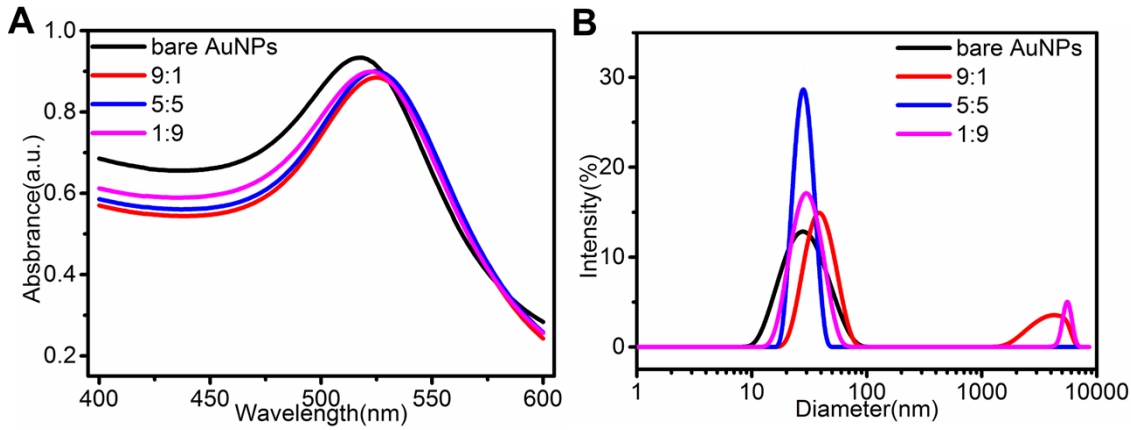

Figure S2. UV-vis spectra (A) and hydrodynamic sizes (B) of bare AuNP and functional DNA-AuNPs. The ratios of DNA-1 to DNA-2 were 9:1, 5:5, and 1:9.

To verify the successful assembly of AuNPs, UV-vis spectra and DLS were used to characterize the preparation of DNA-AuNPs modified by two types of DNA strands with different ratios. As shown in Figure S2A, the spectra of the DNA-AuNPs modified by two types of DNA strands with different ratios display clear redshifts compared to that of the bare gold, which is attributable to the adsorption of DNA on the surface of AuNPs. Figure S2B shows the results of DLS. Specifically, the diameters of DNA-AuNPs modified by two types of DNA strands with different ratios show a more pronounced increase than that of the bare gold, demonstrating that DNA strands have been successfully modified to the surface of nanoparticles. Evaluation of DLS data also gives the same particle size which indicates a monodisperse size distribution of DNA-AuNPs. Therefore, the successful preparation of DNA-AuNPs with two types of DNA strands with different ratios has also been proved.

S4. TEM images of the assembly of nanoparticles in a one-step reaction.

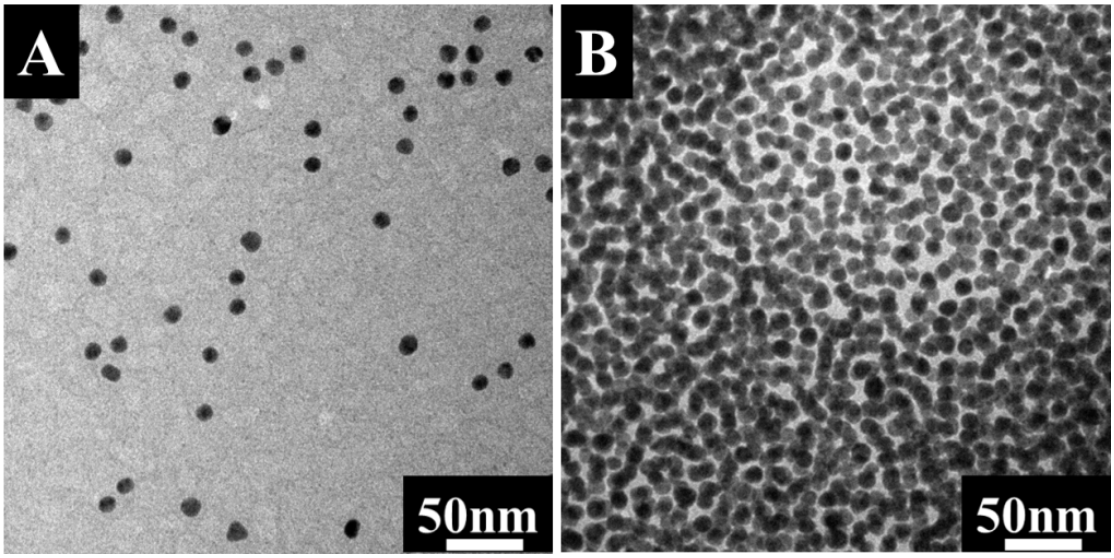

Figure S3. TEM images of the solution before (A) and after (B) the assembly of DNA-AuNPs mixture in a one-step reaction.

S5. TEM images of the assembly of nanoparticles with different ratios of DNA grafted on AuNPs in one-step reaction.

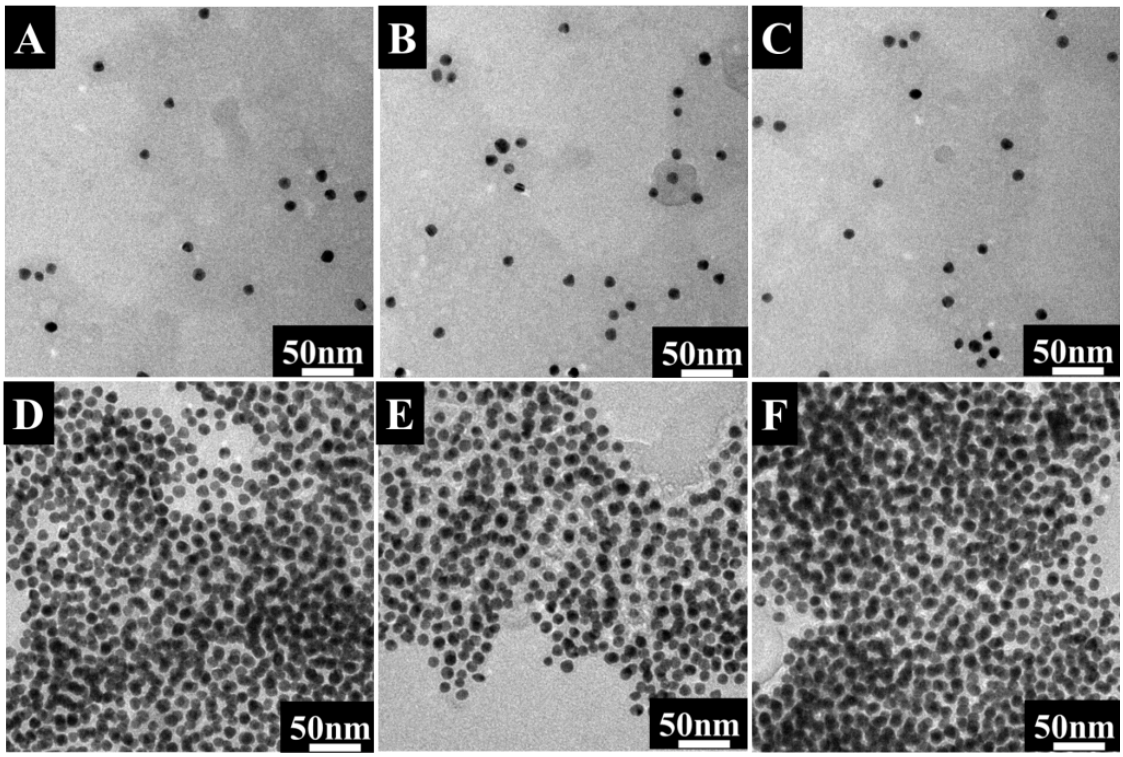

Figure S4. TEM images of the solution before (A-C) and after (D-F) the assembly of AuNP in a one-step reaction with the ratios of DNA-1 and DNA-2 being 9:1 (A and D), 5:5 (B and E), and 1:9 (C and F).

S6. DLS measurement of the assembly of DNA-AuNPs in a one-step reaction.

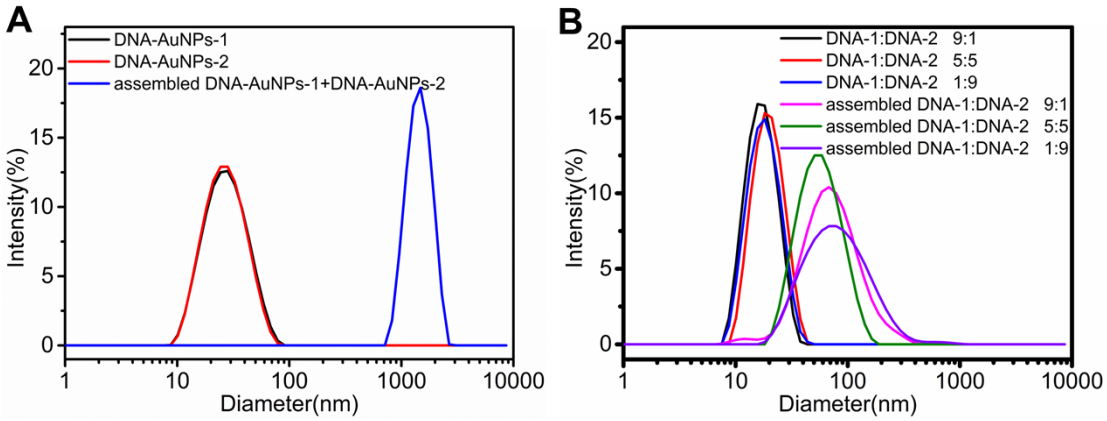

Figure S5. DLS measurement of the one-step reaction programmed by bridge DNA and DNA-AuNPs mixture (A) and different ratios of two types of DNA grafted on AuNP (B).

DLS measurement is an important tool to prove the assembly of bridge DNA and DNA-AuNPs by analyzing size evolution. Dispersed DNA-AuNPs and the products of the one-step reaction are measured to compare the hydrodynamic diameters. As a result, the DLS data exhibit a gradually increasing tendency when the ratio of DNA strands grafted on AuNP increases (Figure S5B). However, the average diameter of the product of DNA-AuNPs-1 and DNA-AuNPs-2 mixtures is larger (about 1500 nm) than those of different ratios of DNA strands grafted on AuNP.

S7. DLS measurement of the assembly of DNA-AuNPs with different ratios of bridge DNA in a one-step reaction.

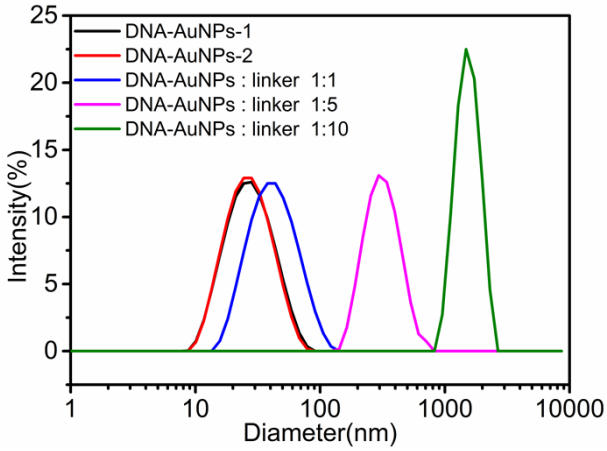

Figure S6. DLS measurement of the assembly of DNA-AuNPs with different ratios of bridge DNA linker in a one-step reaction.

DLS measurement is applied to study the size evolution of the products with different ratios of bridge DNA linker in the one-step reaction. Figure S6 indicates that the products in the one-step reaction show an increased hydrodynamic radius as the ratios of bridge DNA linker increase. When the ratio of bridge DNA linker is 1:10, the hydrodynamic diameters are the largest at 1520 nm (Figure S6, green curve), indicating that this ratio of bridge DNA is proper to program one-step reaction.

S8. TEM images of the assembly of nanoparticles with different ratios of bridge DNA in one-step reaction.

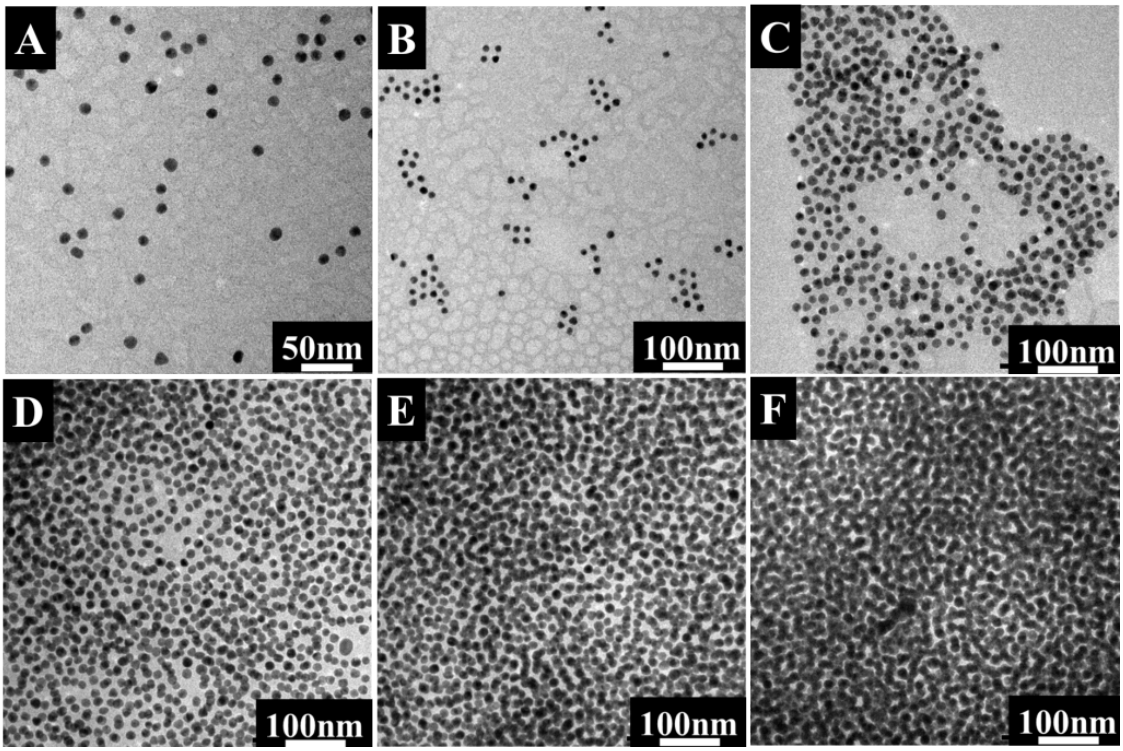

Figure S7. TEM images of the solution before (A) and after the assembly of AuNP in a one-step reaction with different ratios of bridge DNA being 1:5 (B), 1:10 (C), 1:25 (D), and 1:100 (F).

S9. Real-time reaction dynamics of both ssDNA sequences of the bridge DNA in multi-step reaction.

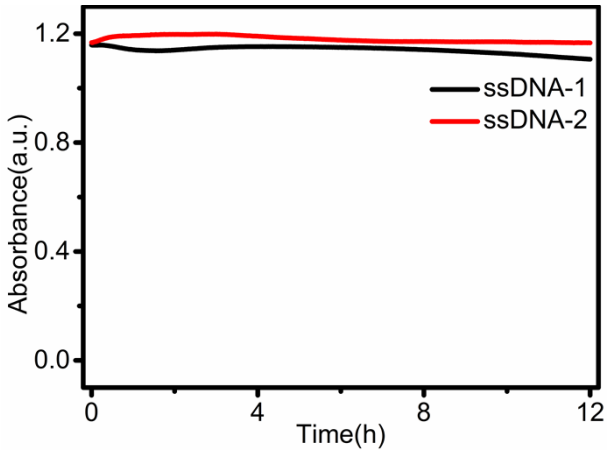

Figure S8. Real-time reaction dynamics of ssDNA guided the assembly of nanoparticles in multi-step reactions.

The appropriate catalyst concentration (75nM) in bridge DNA driving the multi-step reaction is chosen to run both ssDNA sequences of bridge DNA. The flat lines (Figure S8) indicate that the multi-step reaction cannot be triggered by both ssDNA sequences of the bridge DNA.

S10. Real-time reaction dynamics of ssDNA guided the assembly of nanoparticles in multi-step reactions with varied catalyst concentrations.

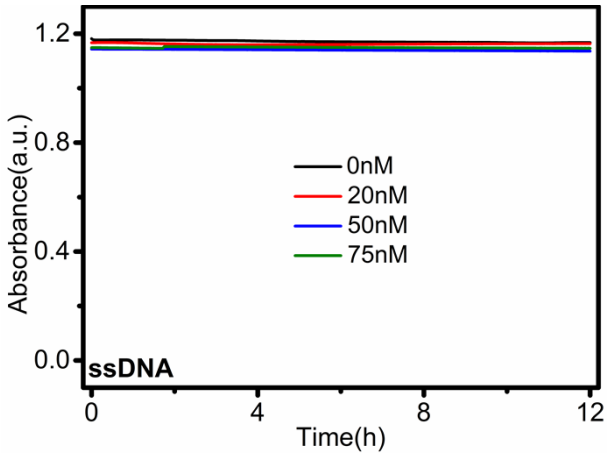

Figure S9. Real-time reaction dynamics of ssDNA guided the assembly of nanoparticles in multi-step reactions with varied catalyst concentrations.

Since the structures of the two ssDNA sequences in bridge DNA are almost similar except for the formed bridge part in the middle, one of the ssDNA sequences is selected to study the kinetic at different catalyst concentrations (Figure S9). No absorbance changes are detected with increasing catalyst concentrations, proving that ssDNA still does not trigger a reaction even though increasing the concentrations of catalyst.

S11. The universality of the bridge DNA and toehold length influences.

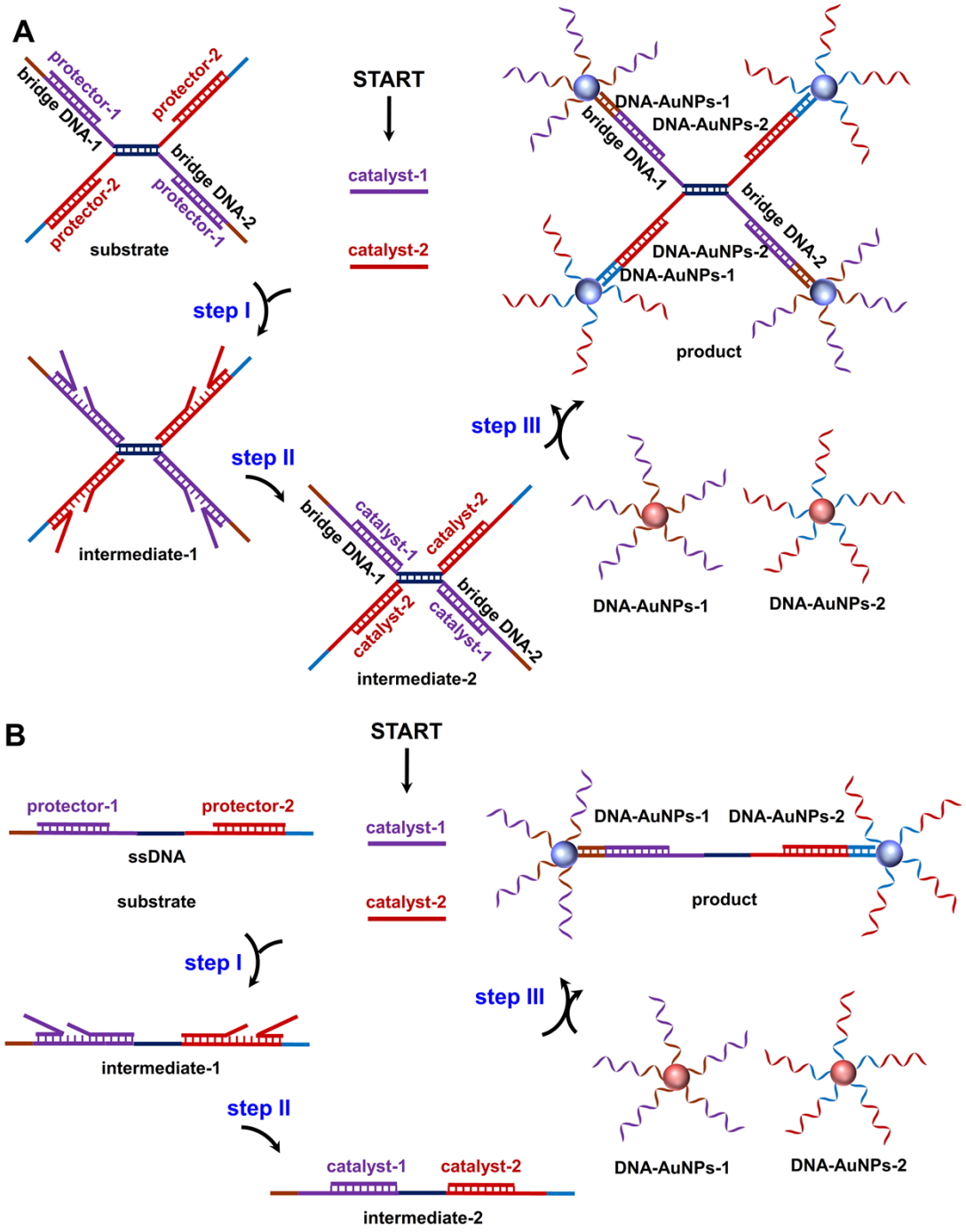

Figure S10. A schematic of the detailed reaction processes in other reactions programmed by bridge DNA (A) and ssDNA (B) with different toeholds.

Table S4. The DNA sequences used in other multi-step reactions with different toehold length-1 being seven and toehold length-2 being eight and nine.

Name	DNA sequences
Sequences used in the formation of bridge DNA in other multi-step reactions	
bridge DNA-1	5'-TTCATCTACCTCCACCTCTACTCGATCCAAGTCACACAAATCCAACCTATTCCC-3'
bridge DNA-2	5'-TTCATCTACCTCCACCTCTACTACTTGGATCGCACACAAATCCAACCTATTCCC-3'
Sequences attached onto AuNPs in other multi-step reactions	
DNA-1	5'-HS-SH-TTTTTTTTTTTTTTTGGTGGAGGTAGATGAA-3'
DNA-2	5'-GGGAATAGGTTGGATTTTTTTTTTTTTTTT-HS-SH-3'
Other sequences used in other multi-step reactions	
ssDNA linker	5'-TTCATCTACCTCCACCTCTACTCGATCCAAGTCACACAAATCCAACCTATTCCC-3'
protector-1	5'-GTAGAGTTGGAGT-3'
protector-2	5'-TGATGAGTGGTAG-3'
catalyst-1	5'-GTGAGGGTAGAGT-3'
catalyst-2	5'-GTGGTAGGAGTAG-3'

The color coding is the same as that used in Figure S10.

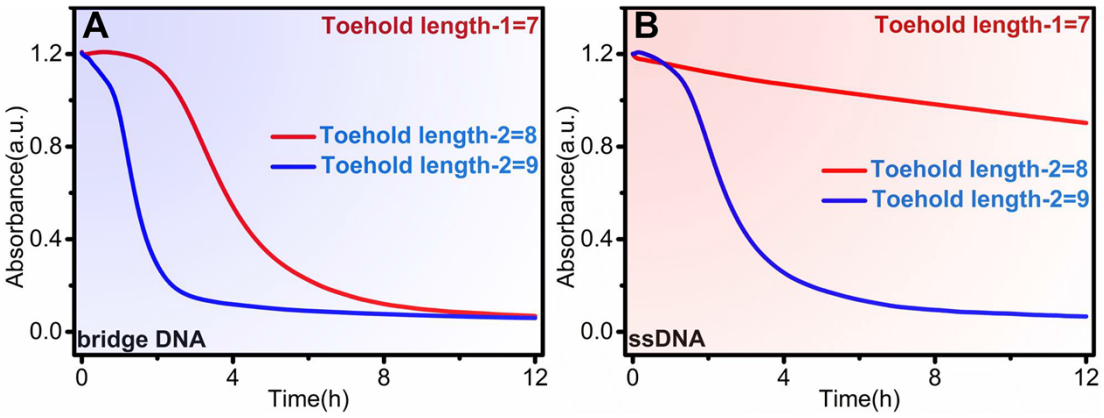

Figure S11. Real-time reaction dynamics of other multi-step reactions programmed by bridge DNA (A) and ssDNA (B) with different toehold lengths.

The detailed steps are shown here in Figure S10. Specifically, linker (bridge DNA and ssDNA) hybridized with protector-1 and protector-2 is prepared first to form a substrate. Then, catalyst and DNA-AuNPs are added to this system simultaneously. Since these two types of linkers have the same type of sticky ends (toehold-1) which is complementary to catalyst strands, the intermediate-1 and intermediate-2 are obtained by mixing these two catalysts. Then, DNA-AuNPs hybridize with intermediate-2 through toehold-2. Finally, the aggregates in this multi-step reaction

are achieved. The result of the obtained AuNPs aggregates is measured by a UV-vis spectrophotometer. The DNA sequences used in these part is shown in Table S4. In Figure S11A, the curves of multi-strep reaction based on bridge DNA driving with toehold length-2 being eight and nine show an apparent decrease. However, only the curve of multi-step reaction based on ssDNA driving with toehold length-2 being nine (Figure S11B, blue line) shows the same trend with bridge DNA, suggesting the aggregation of AuNPs. Therefore, for bridge DNA, the multi-step reaction can be well programmed when the toehold length-1 is seven and the toehold length-2 is great than or equal to eight. For ssDNA, the multi-step reaction can be programmed when the toehold length-1 is seven and the toehold length-2 is equal to nine. These results also validate the effect of toehold length.

S12. A 2D plot based on toehold length and the change of absorbance in the multi-step reaction.

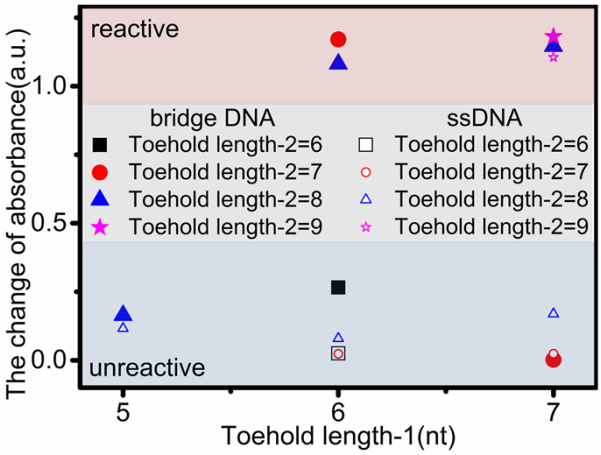

Figure S12. A 2D plot of the multi-step reaction programmed by bridge DNA (A) and ssDNA (B) with varied toehold lengths.

Figure S12 shows a 2D plot for a multi-step reaction programmed by bridge DNA and ssDNA with varied toehold lengths. The reactive and unreactive regions are backgrounded with light pink pane and dusty blue pane, respectively, to explicitly and visually track the reaction feasibility driven by bridge DNA and ssDNA. For bridge DNA, the multi-step chemical reaction can be programmed when toehold length-1 is more than or equal to six bases and toehold length-2 is not less than seven bases. For ssDNA, the multi-step chemical reaction can be programmed only when toehold length-1 is seven bases and toehold length-2 is nine bases.