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SUPPLEMENTARY DATA

Nonenzymatic Catalytic Assembly of Valency-Controlled DNA Architectures for Nanoparticles and Live Cell Assembly

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1 Experimental Section

1.1 Materials and instruments

Oligonucleotides (Table S1) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography. All the DNA strands and hybrids were examined by the mfold web server (http://unafold.rna.albany.edu/?q=mfold) to make sure that they had no unwanted secondary structures or unwanted intermolecular interactions. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Alfa Aesar (China). Bis(*p*-sulfonatophenyl)-phenylphosphine dihydrate dipotassium salt (BSPP) was purchased from Sigma-Aldrich (USA). N-Azidoacetylmannosamine-tetraacylated (Ac_4ManNAz) was purchased from Click Chemistry Tools (USA). Human acute lymphoblastic leukemia cell line (CCRF-CEM) was obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). CellTracker™ Green Dye was purchased from Keygen biotechnology (Jiangsu, China). RPMI 1640, fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco (Thermo Fisher Scientific). 1×DPBS and trypsin-EDTA solution were bought from BBI (Sangon Biotech, Shanghai, China) and used according to manufacturer's specifications. The laser confocal dishes were purchased from NEST Biotechnology Co., Ltd (Wuxi, China). Deionized and sterilized water (resistance >18 MΩ·cm) was used throughout the experiments.

pH values of corresponding solutions were measured by a Mettler−Toledo Delta 320 pH meter. UV-Vis spectral measurements were performed on an Agilent Technologies Cary 60 UV/Vis spectrometer. All fluorescence measurements were carried out on a Shimadzu RF-5301 PC fluorescence spectrometer (Shimadzu Ltd., Japan). Transmission electron microscopy (TEM) was recorded with HITACHI HT7700 Exalens (Hitachi, Ltd, Japan) operated at an accelerating voltage of 100 kV. Atomic force microscopy (AFM) characterization was observed by Bruker Multimode V8 Scanning Probe Microscopy (USA). The images of cells were visualized on an inverted confocal microscope (A1R Confocal System, Nikon).

Table S1. Sequences of all oligonucleotides used in this work

Oligonucleotides	Sequence (5'-3')
a	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA

b	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
c	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
d	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
a'	CCAAAAAAAAAAAAAAAAAAAAAAACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
b'	CCAAAAAAAAAAAAAAAAAAAAAATATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
c'	CCAAAAAAAAAAAAAAAAAAAAAATCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
d'	CCAAAAAAAAAAAAAAAAAAAAAATTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
a#	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATACGAGTGGAGAATCCTGAATGCGACTGTT
b#	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATACGAGTGGAGAATCCTGAATGCGACTGTT
c#	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTACGAGTGGAGAATCCTGAATGCGACTGTT
d#	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTACGAGTGGAGAATCCTGAATGCGACTGTT
HS-Link	HS-C6-TACATATCTATACATATCTATACATATCTATACATATCTATACATATCTAAACAGTCGCATTCAGGATTCTCCACTCGTA
DBCO-link	DBCO-TACATATCTATACAAACAGTCGCATTCAGGATTCTCCACTCGTA
T5	HS-C6-TTTTTT
T1 (initiator)	TAGCTTATCAGACTGATGTTGA
H1	TTTTTTTTTTTTTTTTTTTTTTTTGGTCAACATCAGTCTGATAAGCTACATTGGATGCTCTAGCTTATCAGACTG
H2	TTTTTTTTTTTTTTTTTTTTTTTTGGTAAGCTAGAGCATCCAATGTAGCTTATCAGACTGCATTGGATGCTC
H1*	TTTTTTTTTTTTTTTTTTTTTTTTGGTCAACATCAGTCTGATAAGCTACA/iCy3dT/TGGATGCTCTAGCTTATCAGACTG
H2*	TTTTTTTTTTTTTTTTTTTTTTTTGGTAAGCTAGAGCATCCAATGTAGC/iCy5dT/TATCAGACTGCATTGGATGCTC-Cy5
H2**	TTTTTTTTTTTTTTTTTTTTTTTTGGTAAGC/iBHQ1dT/AGAGCATCCAATGTAGCTTATCAGACT

	GCATTGGATGCTC-FAM
T2 (initiator)	TGAGGTAGTAGGTTGTATAGTT
H4**	TTTTTTTTTTTTTTTTTTTTTTGGCTACC/iBHQ2dT/CATCACCTTCTTCTTGAGGTAGTAGCAA GAAGAAGGTGAT-ROX

1.2 Preparation of TDN-based building blocks and probes

Differently valent TDN-based building blocks and probes were prepared by mixing corresponding oligonucleotide strands (Table S2) in TM buffer (20 mM Tris-HCl, 50 mM $MgCl_2$, pH=8.0). Unless otherwise stated, the concentration of a or a' is 1 μM while the concentration of hairpins depends on the valence of blocks. For instance, to synthesize P1, the concentrations of a', b', c', d' were 1 μM, and H1, H3 were 2 μM. The mixtures were heated at 95 °C for 5 min and then cooled to 4 °C within 1 min and stayed at 4 °C for 3 min. The obtained probes were then directly used for characterization without further fractionation or purification.

Table S2. Preparation of TDN-based building blocks and probes

	Ratio of used oligonucleotides			
TDN	referred to all kinds of tetrahedrons from zerovalent to quadrivalent			
TDNH1	referred to all kinds of TDNs containing H1 from monovalent to quadrivalent			
TDNCH2	referred to all kinds of TDNs containing H2 from monovalent to quadrivalent			
Zerovalent blocks	zTDN	a:b:c:d=1:1:1:1		
Monovalent blocks	mTDNH1	a':b:c:d:H1=1:1:1:1:1	mTDNH1*	a':b:c:d:H1*=1:1:1:1:1
	mTDNH2	a':b:c:d:H2=1:1:1:1:1	mTDNH2*	a':b:c:d:H2*=1:1:1:1:1
Bivalent blocks	bTDNH1	a':b':c:d:H1=1:1:1:1:2	bTDNH1*	a':b':c:d:H1*=1:1:1:1:2
	bTDNH2	a':b':c:d:H2=1:1:1:1:2	bTDNH2*	a':b':c:d:H2*=1:1:1:1:2
Trivalent blocks	tTDNH1	a':b':c':d:H1=1:1:1:1:3	tTDNH1*	a':b':c':d:H1*=1:1:1:1:3
	tTDNH2	a':b':c':d:H2=1:1:1:1:3	tTDNH2*	a':b':c':d:H2*=1:1:1:1:3
Quadrivalent blocks	qTDNH1	a':b':c':d':H1=1:1:1:1:4	qTDNH1*	a':b':c':d':H1*=1:1:1:1:4
	qTDNH2	a':b':c':d':H2=1:1:1:1:4	qTDNH2*	a':b':c':d':H2*=1:1:1:1:4
Probe 1 (P1)	a':b':c':d':H1:H3=1:1:1:1:2:2			
Probe 2 (P2)	a':b':c':d':H2**:H4**=1:1:1:1:2:2			

1.3 Initiator-triggered catalytic assembly

According to Table S2, differently valent building blocks or probes were mixed with initiator (T1 or T2) of varied concentrations. The mixture was then isothermally incubated at 37 °C for proper time.

1.4 Electrophoresis characterization

Polyacrylamide gel electrophoresis (PAGE) was used to characterize traditional CHA amplification reaction. Reaction solutions were loaded on 10% native polyacrylamide gel, and the electrophoresis analysis was performed in 1×TBE buffer (89 mM Tris−boric acid, 2 mM EDTA, pH 8.3) at a 120 V constant voltage for 80 min at 4 °C. After staining with Goldgreen (Keygen Biotech, China) for 15 min at room temperature, the gel was visualized via a gel image system (Bio-Rad Laboratories, USA). Agarose gel electrophoresis (AGE) was applied to characterize the successful preparation of TDN-based blocks and the formation of catalytic assembly products. The reaction solutions were separated by 1% and 2% agarose gel (80 V, 30 min). The gels were stained with Goldgreen in advance and visualized with the gel image system. The concentration of a or a' is 1 μM and H1, H2 changes with different valences in the electrophoresis characterization

1.5 Atomic force microscopy imaging

Proper concentrations of blocks and assembly products were deposited onto a freshly cleaved mica surface and left to adsorb to the surface for 5 min, washed with 30 μL of water for more than 10 times and dried with compressed air. A MultiMode V8 AFM (Bruker) system was used to image the samples under ScanAsyst-Air mode.

1.6 Fluorescence resonance energy transfer (FRET) measurement

Unless otherwise stated, all of the fluorescent labelled hairpins were kept at a concentration of 50 nM while the concentrations of a or a' and so on depend on their compositions. To achieve the *in vitro* FRET sensing of initiator, different concentrations of initiator were added in corresponding catalytic assembly-based sensing systems containing Tris-HCl buffer (20 mM Tris-HCl with 10 mM $MgCl_2$, pH=7.4), followed by incubation for proper time at 37 °C. Then, the fluorescence spectrum of the mixture was collected from 550 to 800 nm with 540 nm excitation. All experiments were repeated at least three times. To investigate the reaction kinetics of assembly process, real-time monitoring of fluorescence intensity at 660 nm (λ_{ex} = 540 nm) was performed after initiator

addition. The fluorescence spectrum of FAM or ROX were collected at 520 nm (with 488 nm excitation) and at 610 nm (with 587 nm excitation), respectively. All experiments were repeated at least three times.

1.7 Preparation of AuNPs

Au nanoparticles (AuNPs) with ~5 nm average diameters were synthesized using the following way.[1] First, 100 mL of 0.01% $HAuCl_4$ solution was gently stirred for 3 min. Next, 2 mL of 38.8 mM sodium citrate was added, and the mixture was stirred for several minutes. Thereafter, 1 mL of freshly prepared 0.075% $NaBH_4$ in 38.8 mM sodium citrate was quickly added, and the reaction mixture was stirred for 5 min. The solution color turned from pale yellow to bright red. For further use, the solution was filtered using a 0.22 μm Millipore syringe filter. The molar concentration of AuNPs in filtrate was measured to be about 100 nM according to molar absorption coefficient of 5 nm AuNPs at 520 nm ($\varepsilon_{520} = 9.3 \times 10^6\ M^{-1}\ cm^{-1}$). The prepared AuNPs were stored at 4 °C for the subsequent experiments.

1.8 Phosphination of AuNPs

3 mg BSPP was added in 10 mL above-prepared AuNPs solution, and the mixture was shaken overnight at room temperature.[2] Then, NaCl was slowly added under stirring until the solution color was changed from deep burgundy to light purple. The resulting mixture was centrifuged at 10000 rpm for 10 min, and the supernatant was removed. AuNPs were then resuspended in 0.5 mL of BSPP solution (2.5 mM) and mixed with 0.5 mL methanol. The mixture was centrifuged again, the supernatant was removed and the AuNPs were resuspended in 100 μL of BSPP solution (2.5 mM). The concentration of the ~ 100-fold concentrated AuNPs was estimated from the optical absorbance at 520 nm. Phosphine coating increases the negative charge of particle surface and therefore stabilizes high-density AuNPs in high concentration of electrolyte solution.

1.9 Preparation of AuNP/HS-Link conjugates

Sulfydryl-modified DNA strand (HS-Link, Table S1) was incubated with equimolar phosphinated AuNPs in 0.5×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) containing 50 mM NaCl overnight at room temperature.[3] AuNP/HS-Link conjugates containing different numbers of HS-Link were separated by 3% agarose gel (running buffer: 0.5×TBE; loading buffer: 50% glycerol; 15 V/cm). The band of AuNP/HS-Link conjugates with the AuNP/HS-Link ratio of 1:1 was

electroeluted into the glass fiber filter membrane, backed by dialysis membrane (MWCO 10000). AuNP/HS-Link conjugates were recovered using a 0.45 μm centrifugal filter device. The concentration of the obtained AuNP/HS-Link conjugates was estimated from the optical absorbance at ~ 520 nm.

1.10 Coating AuNP/HS-Link conjugates with short oligonucleotides

The AuNP/HS-Link conjugates were further stabilized by thiolated single-stranded T5 (Table S1). To achieve this, the above-prepared conjugates were mixed with T5 ([T5]/[AuNP]=100) in 0.5×TBE buffer containing 50 mM NaCl. The mixtures were incubated for 12 h at room temperature. The presence of high-density T5 strands on the AuNP surface could provide additional stability against high concentrations of electrolytes that were required for subsequent DNA assembly reactions.

1.11 Preparation of AuNP-modified building blocks

TDN-based building blocks were prepared as above using the oligonucleotides listed in Table S1 and Table S3. The blocks were then mixed with above-prepared AuNP/HS-Link conjugate (abbreviated as HS-Link in Table S3) in TM buffer (20 mM Tris-HCl, 10 mM $MgCl_2$, pH=8.0) according to the ratio given in Table S3. The mixtures were heated to 45 °C and then cooled slowly from 45 °C to 25 °C over 24 h.

1.12 TEM characterization of AuNP-modified building blocks and corresponding assembly products

AuNP-modified building blocks were mixed with 20 nM initiator. The mixture was isothermally incubated at 37 °C for proper time. Each solution (10 μL) was directly deposited on the grid for 10 min, the excess solution was wicked away with filter paper and washed three times with deionized water, and then submitted for TEM characterization.

Table S3. Preparation of AuNP-modified building blocks

	Composition ratio of used oligonucleotides
TDN-1Au[a]	a#:b:c:d:HS-Link=1:1:1:1:1
TDN-2Au	a#:b#:c:d:HS-Link=1:1:1:1:2
TDN-3Au	a#:b#:c#:d:HS-Link=1:1:1:1:3
TDN-4Au	a#:b#:c#:d#:HS-Link=1:1:1:1:4

mTDNH1-1Au	a′:b#:c:d:H1:HS-Link =1:1:1:1:1:1
mTDNH1-2Au	a′:b#:c#:d:H1:HS-Link=1:1:1:1:1:2
mTDNH1-3Au	a′:b#:c#:d#:H1:HS-Link=1:1:1:1:1:3
bTDNH1-2Au	a′:b′:c#:d#:H1:HS-Link=1:1:1:1:2:2
tTDNH1-1Au	a′:b′:c′:d#:H1:HS-Link=1:1:1:1:3:1
mTDNH2-1Au	a′:b#:c:d:H2:HS-Link=1:1:1:1:1:1
mTDNH2-2Au	a′:b#:c#:d:H2:HS-Link=1:1:1:1:1:2
mTDNH2-3Au	a′:b#:c#:d#:H1:HS-Link=1:1:1:1:1:3
bTDNH2-2Au	a′:b′:c#:d#:H2:HS-Link=1:1:1:1:2:2
tTDNH2-1Au	a′:b′:c′:d#:H2:HS-Link=1:1:1:1:3:1

[a]The valence states of TDNs are calculated according to the number of hairpins. AuNPs do not participate in the CHA assembly reaction and cannot be used for the calculation of valence state.

1.13 Preparation of DBCO-modified TDNs

TDN-based building blocks were prepared as above using the oligonucleotides listed in Table S1 and Table S4. The blocks were then mixed in TM buffer (20 mM Tris-HCl, 10 mM $MgCl_2$, pH=8.0) according to the ratio given in Table S4. The mixtures were heated to 95 °C for 5 min and then cooled slowly from 4 °C over 24 h. The obtained TDNs were then directly used for characterization without further fractionation or purification.

Table S4. Preparation of DBCO-modified building blocks

	Composition ratio of used oligonucleotides
DBCO-mTDNH1	a′:b#:c:d:H1:DBCO-Link =1:1:1:1:1:1
DBCO-mTDNH2	a′:b#:c:d:H2:DBCO-Link =1:1:1:1:1:1
DBCO-bTDNH1	a′:b#:c′:d:H1:DBCO-Link =1:1:1:1:2:1
DBCO-bTDNH2	a′:b#:c′:d:H2:DBCO-Link =1:1:1:1:2:1
DBCO-tTDNH1	a′:b#:c′:d′:H1:DBCO-Link =1:1:1:1:3:1
DBCO-tTDNH2	a′:b#:c′:d′:H2:DBCO-Link =1:1:1:1:3:1

[a]The valence states of TDNs are calculated according to the number of hairpins. DBCO do not participate in the CHA assembly reaction and cannot be used for the calculation of valence state.

1.14 Manipulation of cell-cell interaction and CLSM imaging

CCRF-CEM cells were cultured in RPMI-1640 (GIBCO) medium with 10% FBS and 1% penicillin-streptomycin (10000 IU penicillin and 10000 μg/mL streptomycin). The cells were harvested by aspirating the supernatant after centrifugation. To stain cells with green color, cells (10^6) were first washed 3 times with 1×DPBS buffer and then incubated with prewarmed CellTracker™ working solution (1 μM) at 37 °C for 15 min. After removing the CellTracker™ working solution by centrifugation, CCRF-CEM cells were cultured in RPMI-1640 medium containing 40 μM Ac_4ManNAz for three days.

To manipulate cell assembly, green-stained CEM cells (10^6) were incubated with DBCO-modified TDNH1 (DBCO-xTDNH1, DBCO concentration: 250 nM, 100 μL) and unstained CEM cells (10^6) were incubated with DBCO-xTDNH2 (DBCO concentration: 250 nM, 100 μL) at room temperature for 30 min. The incorporation of DBCO-xTDNH1 and DBCO-xTDNH2 on cell membrane was realized through the covalent linkage. DBCO-xTDNH1 and DBCO-xTDNH2-modified cells were washed with 1×DPBS buffer and mixed together at a 1:1 ratio in 1×DPBS buffer supplemented with 5 mM $MgCl_2$. Initiator was added to trigger the assembly reaction between DBCO-xTDNH1 and DBCO-xTDNH2. The mixture was shaken at 37 °C with the speed of 300 rpm for proper time.

Cell surface fluorescence was observed through confocal laser scanning microscopy (CLSM). CellTracker™ Green fluorescence was collected from 500 nm to 550 nm under 488 nm excitation. Cy3 fluorescence was collected from 560 nm to 600 nm under 545 nm excitation. Cy5 fluorescence was collected from 640 nm to 680 nm under 630 nm excitation.

2 Working mechanism of traditional CHA

The principle of the traditional CHA is shown in Fig. S1a. In CHA, two complementary nucleic acid hairpins (H1, H2, Table S1) are designed. By inserting complementary regions in the hairpin stems, their spontaneous hybridization is kinetically hindered. Only an initiator strand is added, H1 can be opened based on the toehold-mediated strand displacement, which will further enable the assembly of both H1 and H2. Eventually, the initiator can be displaced and recycled, acting as a catalyst to induce more hairpin assembly events.

The performance of traditional CHA reaction was investigated by PAGE. As shown in Fig. S1b, H1 and H2 exhibited a single DNA band, respectively. As for the H1/H2 mixture, a weak band with a slower migration, which was associated with the interaction of H1 and H2, was observed even in the absence of initiator, implying the low level of spontaneous and undesired hybridization interactions between the two hairpins despite elaborate design and optimization. Upon the addition of the initiator, a more centralized band could clearly be seen on the gel. These results demonstrated that the initiator can promote the assembly of H1 and H2.

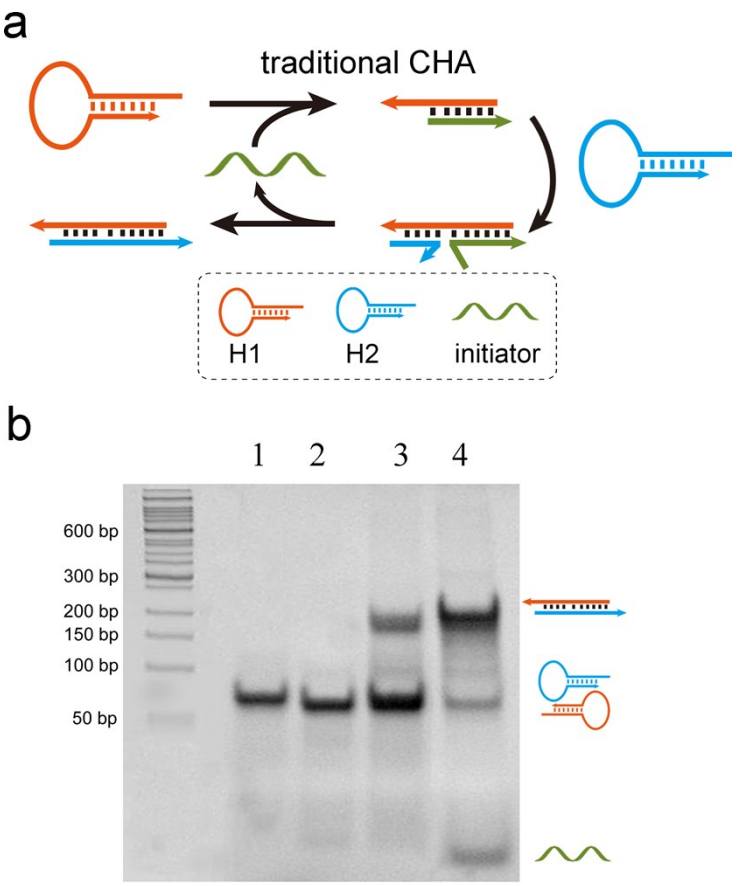

Fig. S1 Schematic illustration and characterization of traditional CHA (a) The operating mechanism of traditional CHA. (b) PAGE (10%) analysis of traditional CHA. Lanes 1→4: H1 alone; H2 alone; H1+H2; H1+H2+initiator.

3 Construction of differently valent building blocks

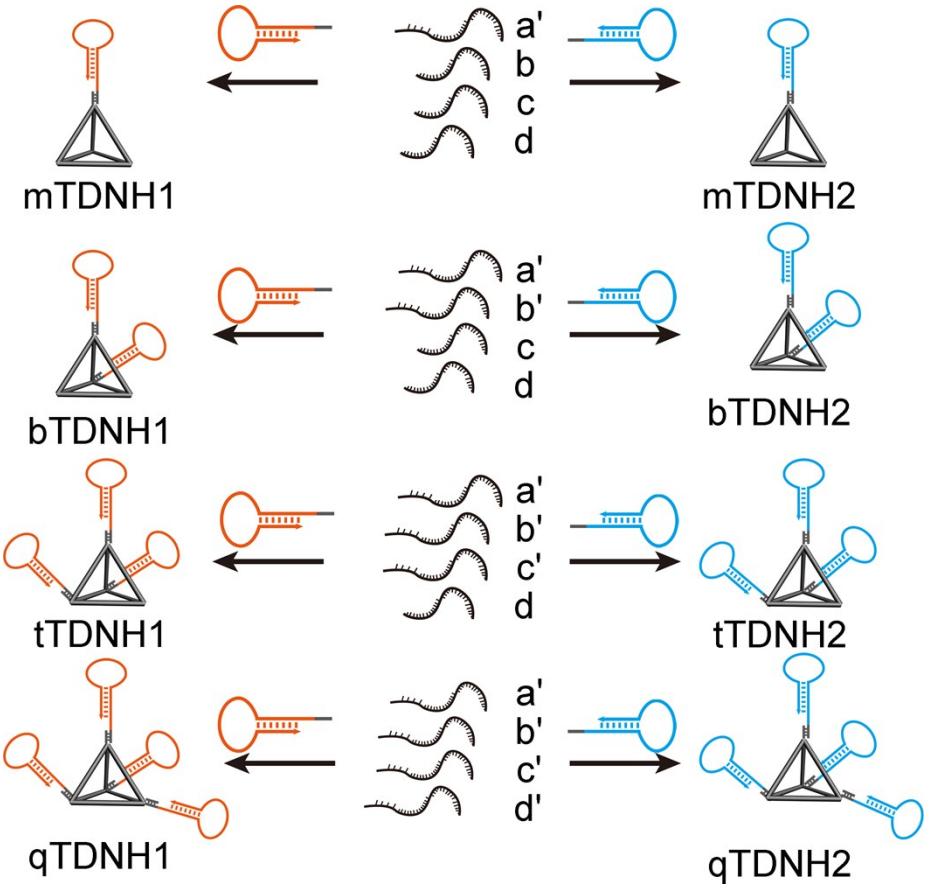

Fig. S2 Schematic illustration for the construction of TDN-based building blocks with different valences.

4 Electrophoretic characterization of differently valent building blocks

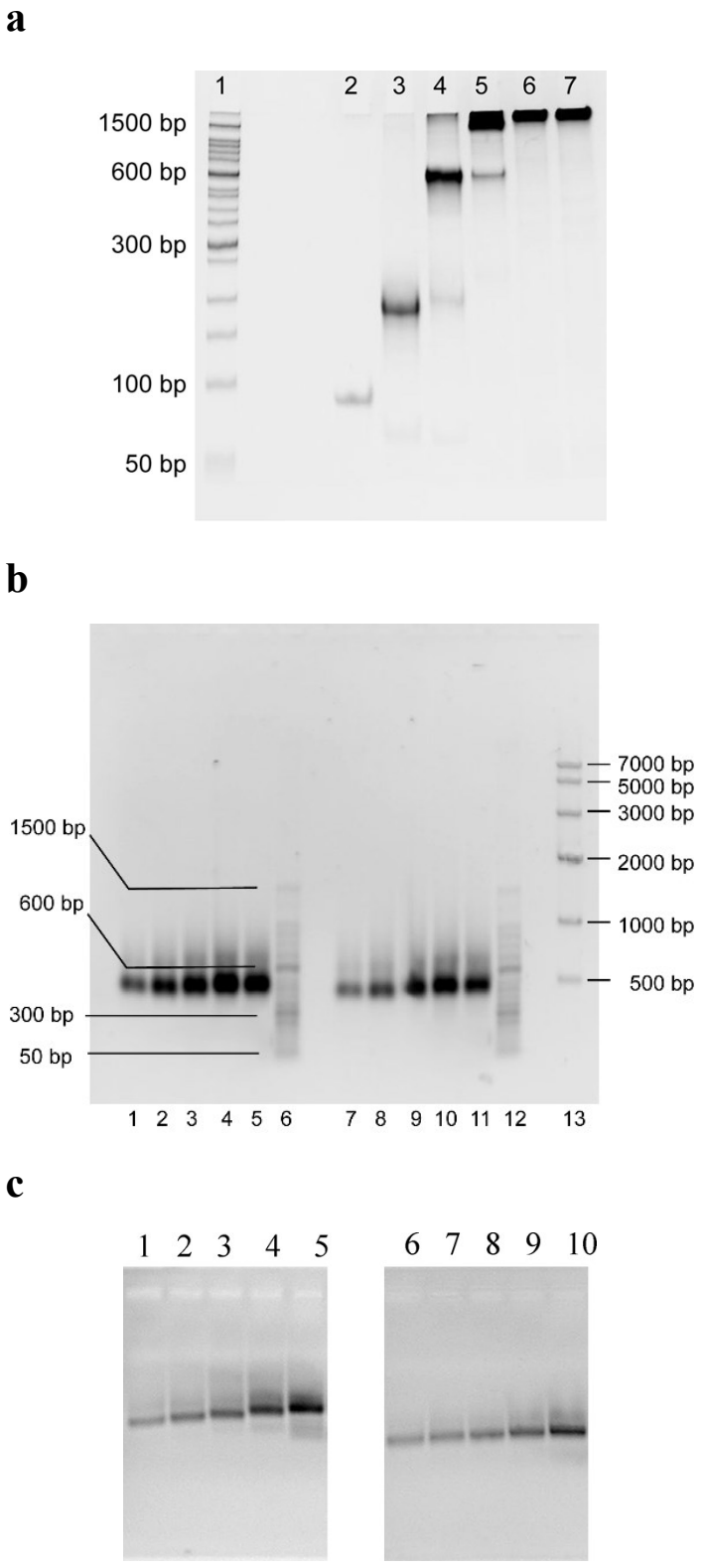

Fig. S3 Electrophoretic characterization of TDN-based building blocks. (a) PAGE analysis. Lanes (1→7): 50 bp DNA Ladder, a'; a'+b'; a'+b'+c'; a'+b'+c'+d'; a'+b'+c'+d'+H1; a'+b'+c'+d'+H2. (b) AGE analysis. Lanes (1→13): TDN; mTDNH1; bTDNH1; tTDNH1; qTDNH1; 50 bp DNA Ladder; TDN; mTDNH2; bTDNH2; tTDNH2; qTDNH2, 50 bp DNA Ladder and DNA Ladder IV. (c) AGE analysis without DNA ladder. Lanes (1→10): TDN; mTDNH1; bTDNH1; tTDNH1; qTDNH1; TDN; mTDNH2; bTDNH2; tTDNH2 and qTDNH2. The concentration of a or a' is 1 μM and H1, H2 changes with different valences in the electrophoresis characterization.

5 Construction of P1/P2 and synergistic effect within polyvalent CHA

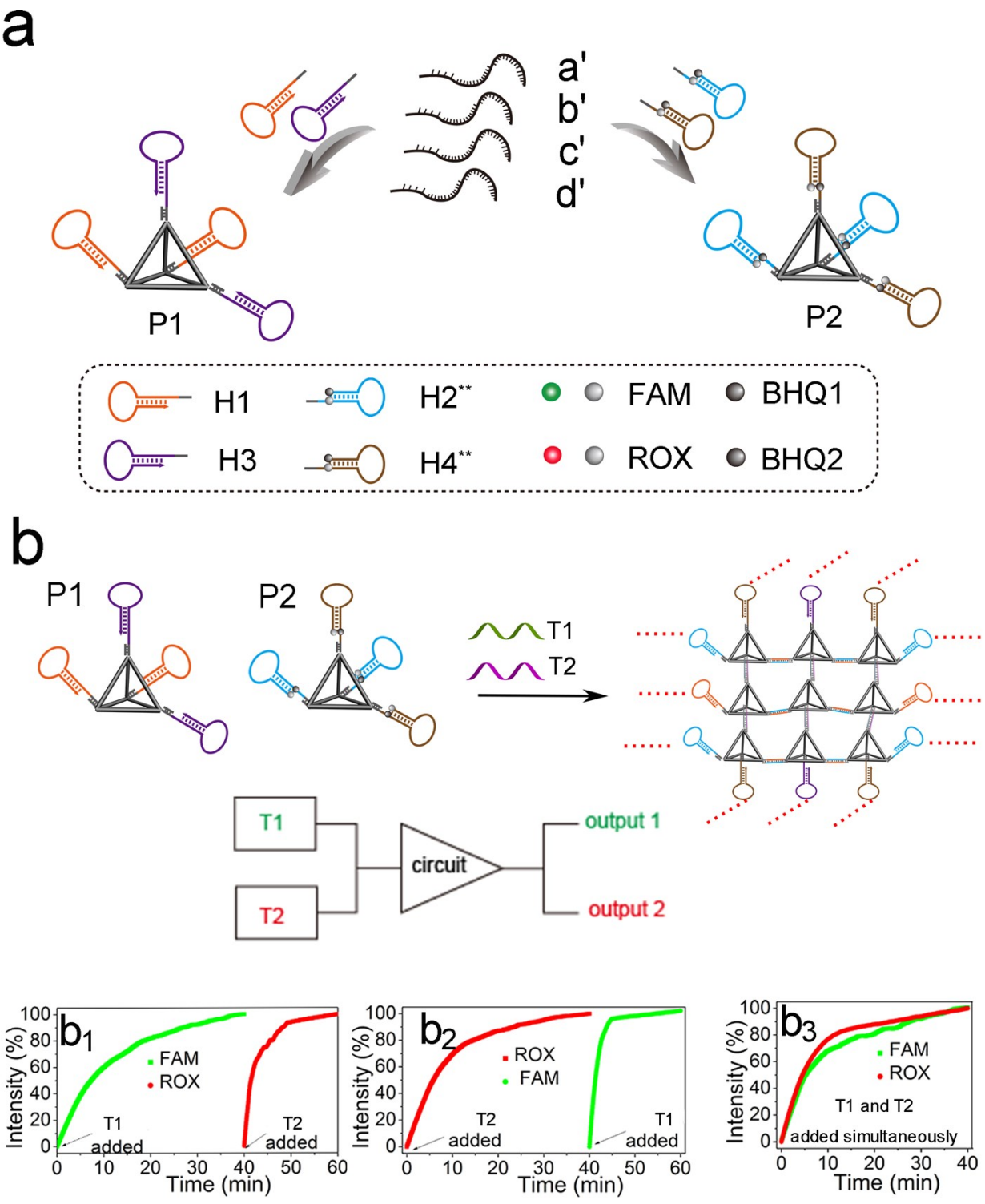

Fig. S4 (a) Schematic illustration for the construction of P1 and P2. (b) Time-dependent FAM and ROX fluorescence percentage changes of the heterogenous TDN-mediated CHA. (b_1) 20 nM of T1 and T2 were successively added; (b_2) 20 nM of T2 and T1 were successively added; (b_3) 20 nM of T1 and T2 were added simultaneously.

6 TEM characterization of HS-Link-modified AuNPs

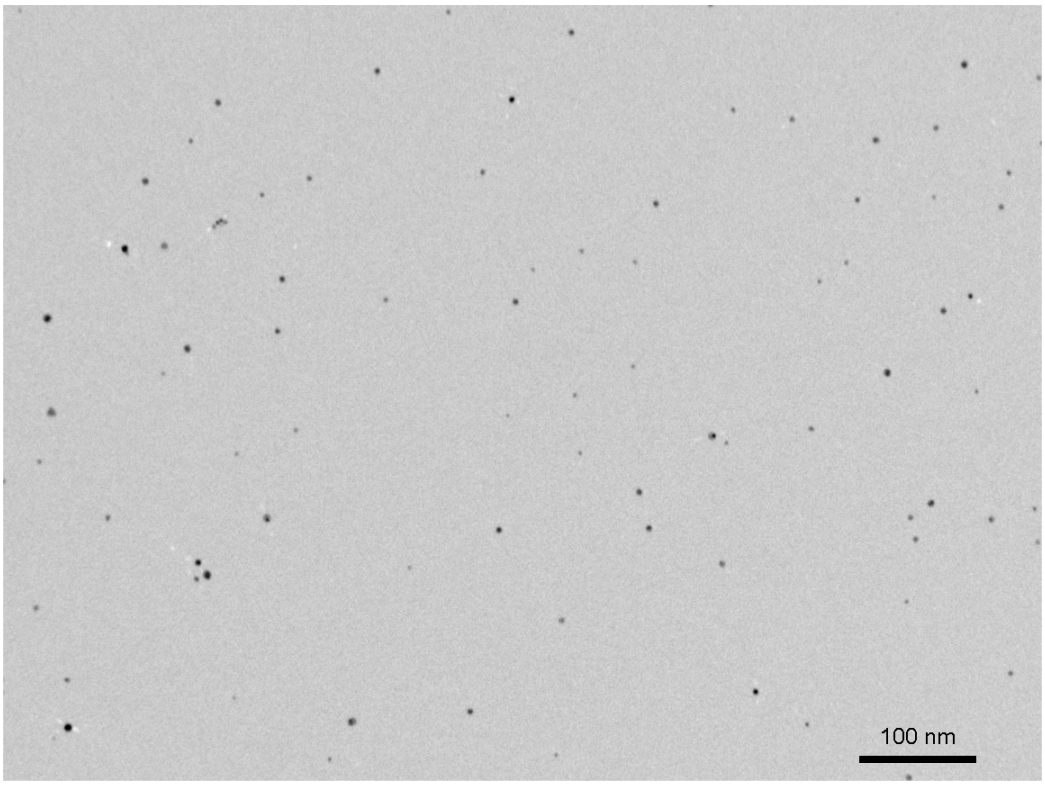

Fig. S5 Typical TEM image of monodispersed HS-Link-modified AuNPs without the modification of TDNs.

7 Supplementary TEM characterization of TDNs modified with different numbers of AuNPs

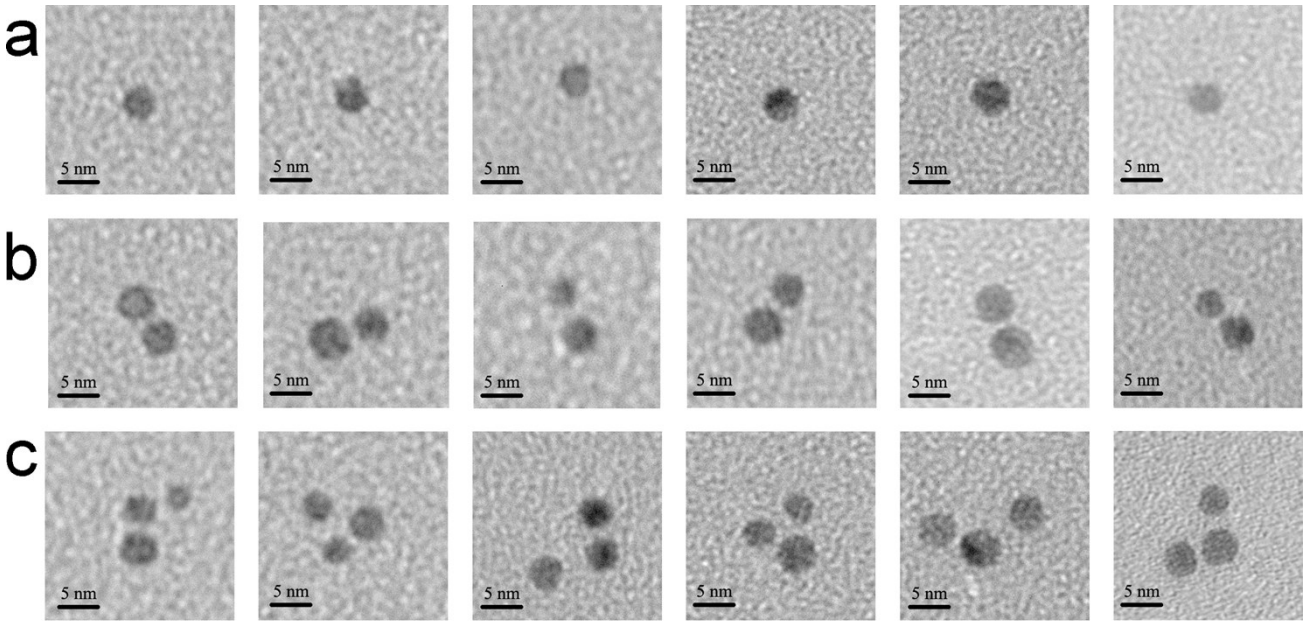

Fig. S6 Typical supplementary images of mono-, bi- and trivalent building blocks without initiator picked out from TEM. The numbers of AuNPs on each TDN are (a) 1. (b) 2. or (c) 3.

8 Supplementary TEM characterization for trivalent and bivalent TDN-mediated assembly reactions

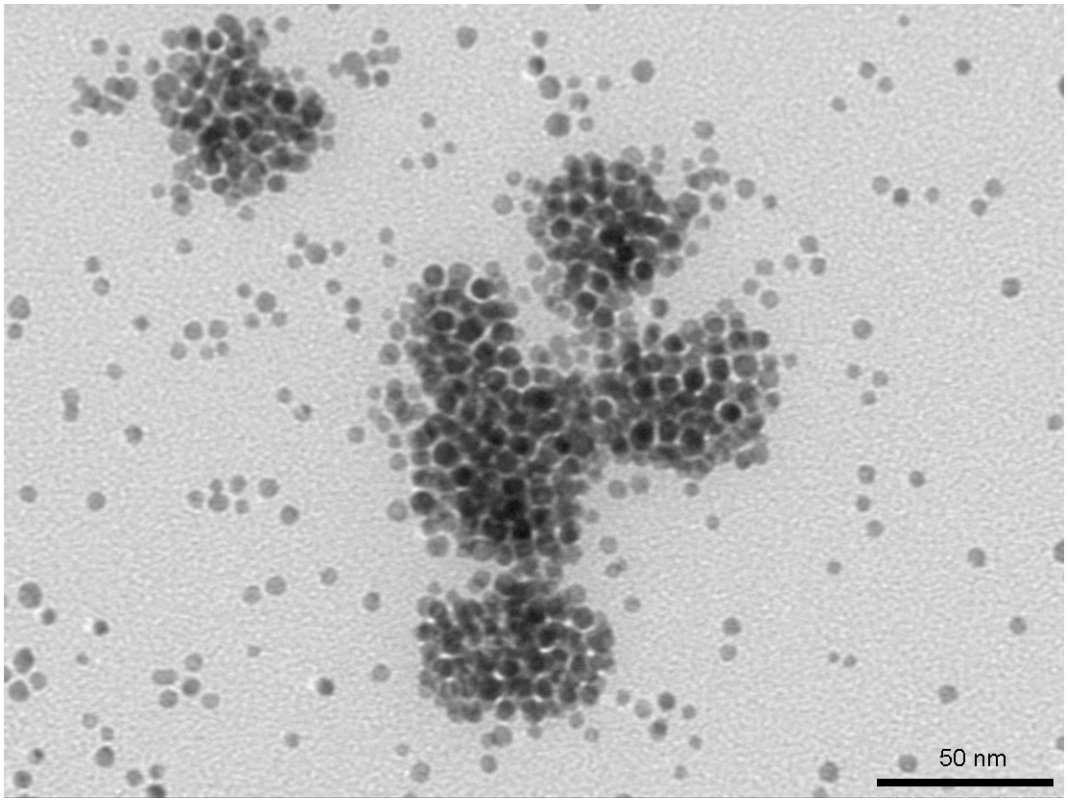

Fig. S7 Additional TEM image of the assembly products of trivalent blocks (tTDNH1 + tTDNH2). Each block was modified with one AuNP.

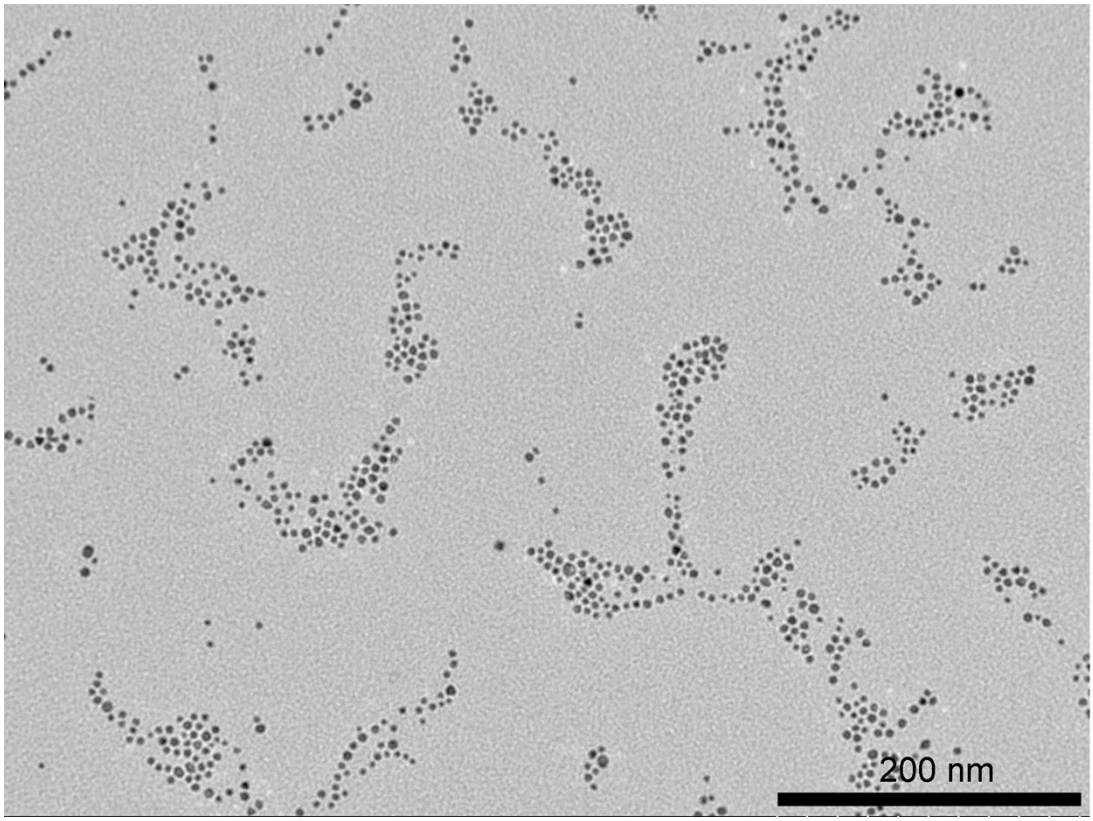

Fig. S8 Additional TEM image of the assembly products of bivalent blocks (bTDNH1 + bTDNH2). Each block was modified with two AuNPs.

9 Surface modification on live cell membrane

We modified TDN-based probes on live cell membrane using a covalent method. As demonstrated by CLSM characterization (Fig. S9), we can see that the two probes (DBCO-TDNH1* and DBCO-TDNH2*) could be successfully modified on the cell membrane. On the contrary, little signal could be detected for the control sample of TDNH1* or TDNH2* containing no DBCO-link. The engineered live cells facilitated subsequent research.

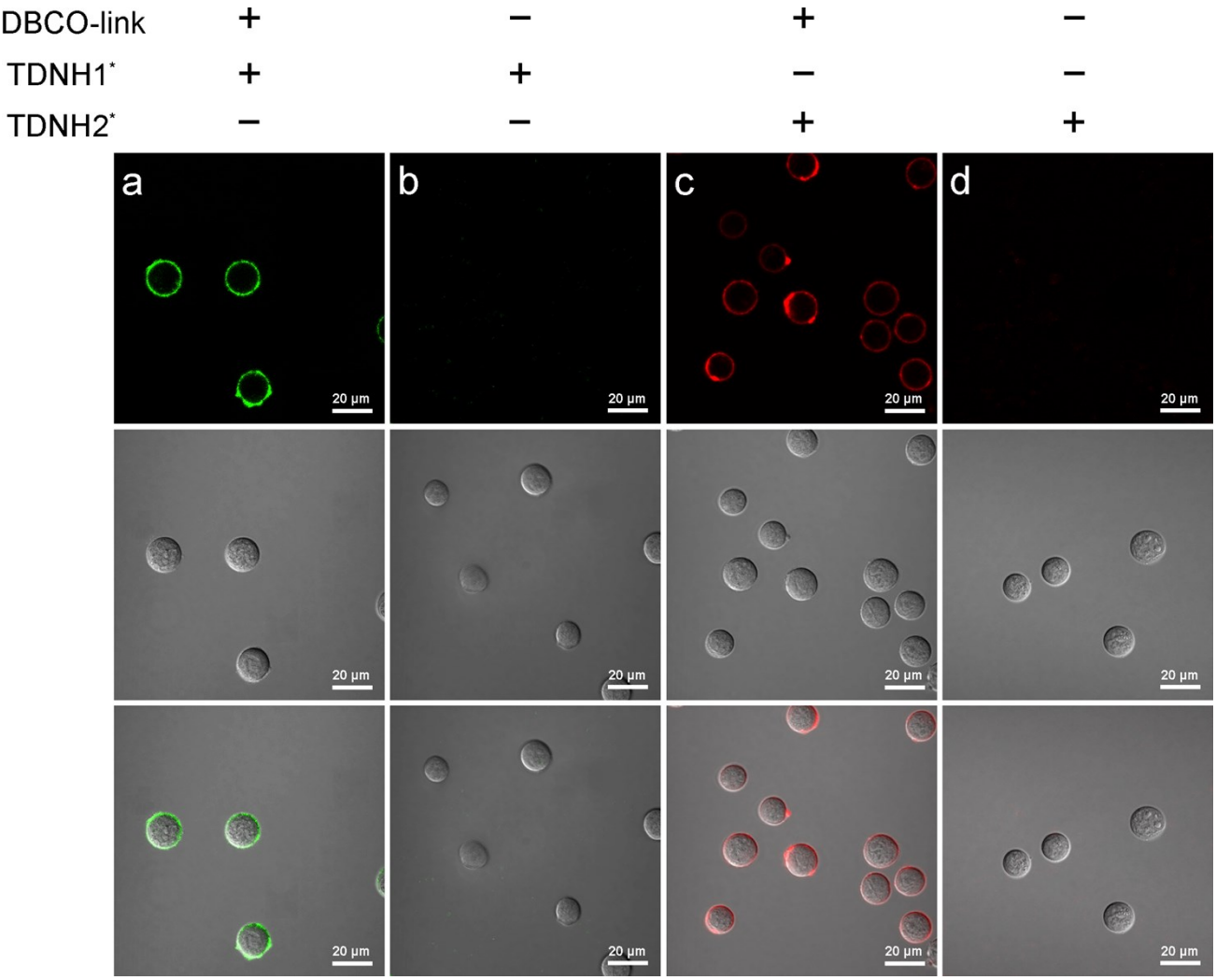

Fig. S9 CLSM imaging of the CEM cells treated with DBCO modified TDN-based probes. Scale bar: 20 μm.

10 Biostability characterization of probes on cell membrane

After being successfully labeled on the membrane of live cells, the DBCO-modified probes should be able to stably stay on the cell membrane for a period of time for further use. CEM cells were treated with fluorescent DBCO-TDNH1* for 30 min at room temperature. After that, they were washed with DPBS buffer, resuspended in culture medium containing 10% FBS, and further incubated at 37 °C in 5% CO_2 atmosphere for different time. The experimental results showed that TDN-based probe could stay for at least 12 h on the cell membrane.

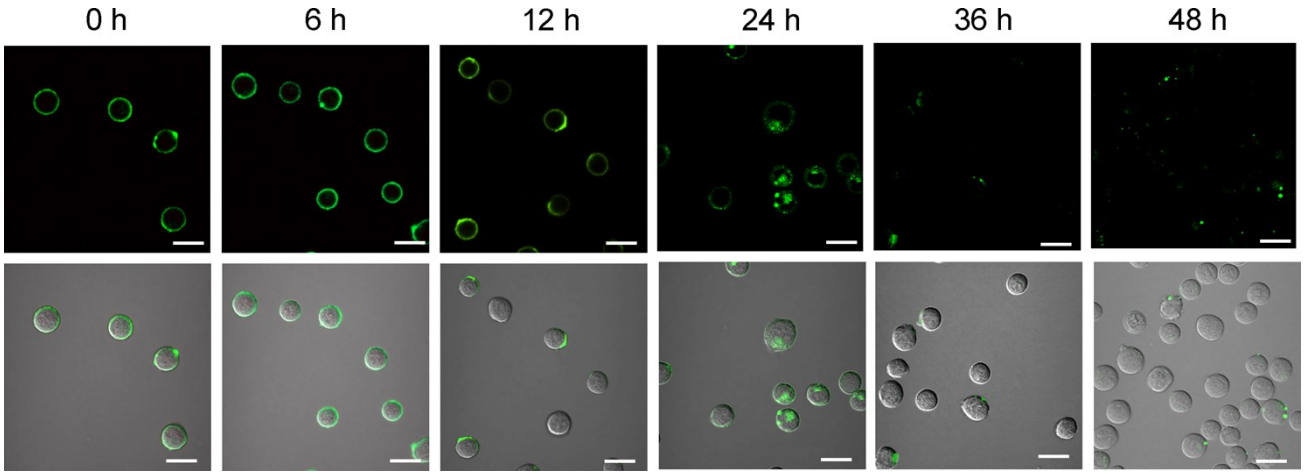

Fig. S10 Time-dependent CLSM images of CEM cells treated with DBCO modified TDN-based probe. Scale bar: 20 μm.

11 CLSM characterization of cells modified by TDN-based probes without initiator

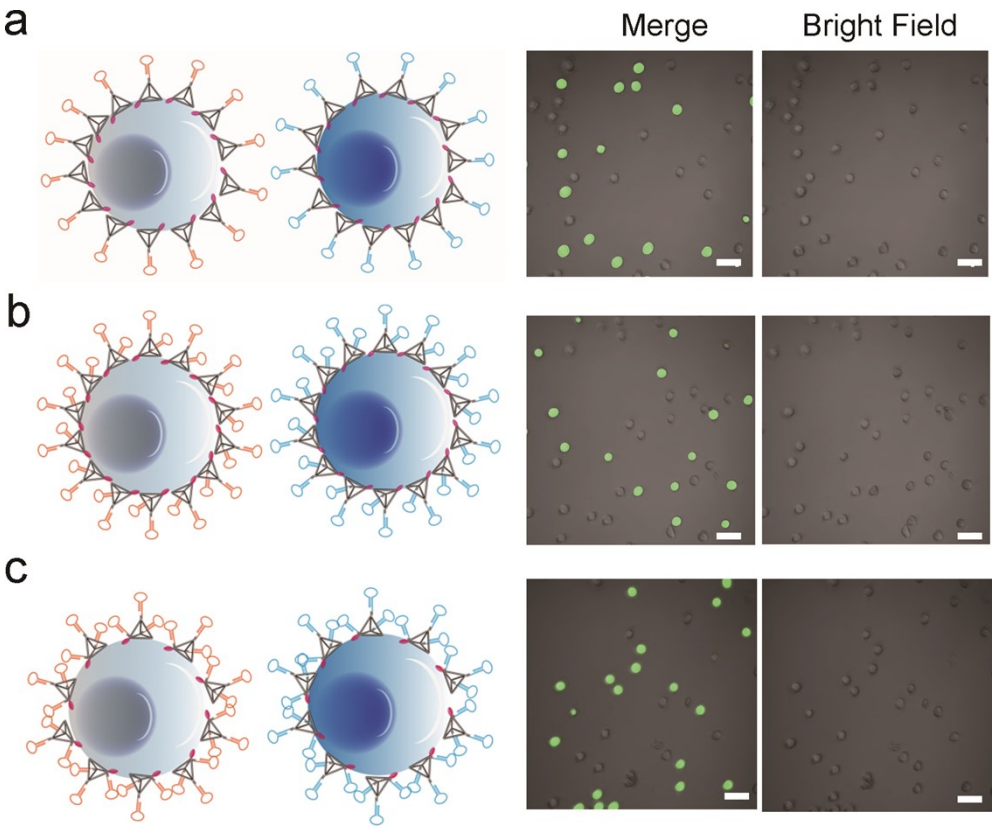

Fig. S11 Schematic illustration (left) and CLSM characterization (right) of the mixtures of DBCO-xTDNH1-modified cells (colorless) and DBCO-xTDNH2-modified cells (green) in the absence of initiator. Scale bar: 40 μm. Merge channel (green channel and bright field were mixed).

12 Aggregation percentage of cells modified by TDN-based probes

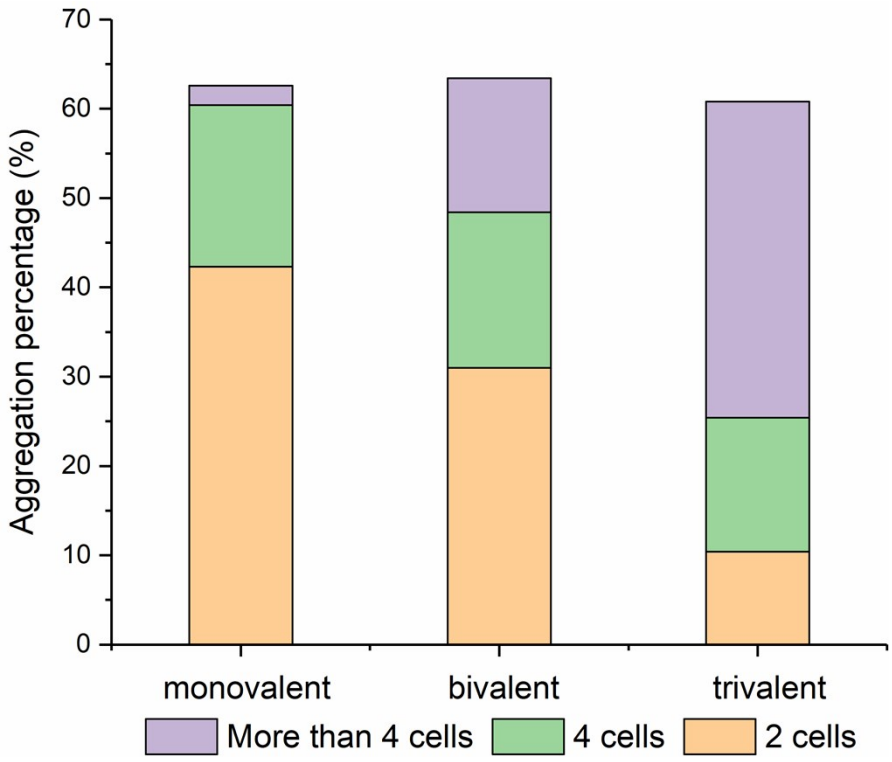

Fig. S12 Aggregation percentage of cells modified by differently valent TDN-mediated assembly reactions. Over 1000 green cells were measured for each sample group. The aggregation percentage was calculated based on the percentage of green cells bound with at least one colorless cell.

References

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