Electronic Supplementary information

Self-assembled multifunctional DNA nanospheres for biosensing and drug delivery into specific target cells

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Additional experimental.

Materials and apparatus. All oligonucleotides used in this study were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China), and their sequences are listed in Table S1. The T4 DNA ligase and Klenow fragment exo- DNA polymerase were ordered from Fermentas (Canada). Lysozyme, thrombin, BSA, streptavidin, and doxorubicin (Dox) were purchased from Sigma-Aldrich (America). The deoxyribonucleoside triphosphates (dNTPs) mix was obtained from SBS Genetech Co., Ltd. (Beijing, China). All regents were of analytical grade, which were used as received unless otherwise stated. Double-distilled deionized ultrapure water was used in all experiments.

Table S1. Sequences of oligonucleotides used in this work.

No.	Sequences (from 5′ to 3′)
(1)	P-ATCAGTAGTCGATGGCTTCCACAACATACAC
(2)	TTTTACGATAAGGATGCGGTGTATGTTGTGGATCCATTGACGAGAGAGG
(2')	FAM-TTTTACGATAAGGATGCGGTGTATGTTGTGGATCCATTGACGAGAGAGG
(3)	P-ACACGATTGACGACCCTCATCGACTACTGATAGCGCGACTACATA
(4)	P-CGCATCCTTATCGTCCTCTCTCGTCAATCCAGTCGTCAATCGTGTTATGTAGTCGCGCT
(5)	P-CGCGCTATCAGTAGTCGATGGCTAATGGATCCACTAAGTAACT
(6)	Dabcyl-TGGATCCATTTCC ATTGACGAGAGAGG
(7)	ATCTACGAATTCATCAGGGCTAAAGAGTGCAGAGTTACTTAG-FAM
(8)	P-ACACGATTGACGACCCTCATCGACTACTGATAGCGCGACTACATAGG
(9)	P-CTGCACTCTTCCTCTCTCGTCAATCCAGTCGTCAATCGTGTCCTATGTAGT
(10)	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGACATCGCTACTGTAGCCAATCCT GTCAGTACT
(11)	P-AGTACTGACAGGATCCCTCATCGACTACTGATAGCGCGACTACATA
(12)	TTTTACGATAAGGATGCGGTGTATGTTGTGGATCCCTACAGTAGCGATG
(12')	FAM-TTTTACGATAAGGATGCGGTGTATGTTGTGGATCCCTACAGTAGCGATG

Transmission electron microscopy (TEM) images were obtained by using a JEM2100 transmission electron microscope (JEOL, Japan) with an acceleration voltage of 200 kV. Scan electron microscopy (SEM) images were recorded with a JSM-6700F instrument (JEOL, Japan) operating at a working voltage of 8 kV. Atomic Force Microscopy (AFM) image was performed on a Being Nano-Instruments CSPM-4000 (Benyuan, China) operated under tapping mode. Fluorescence measurements were carried out using a Hitachi F-4600 fluorescence spectrometer (Hitachi, Japan). Fluorescent images were collected on a Leica TCS SP8 confocal laser scanning microscope (Leica, Germany).

Polyacrylamide gel electrophoresis (PAGE). The reaction pathways of DNA NSs and lysozyme sensing were estimated by 15% acrylamide gel (containing 29/1 acrylamide/bisacrylamide (w/w)) in 1× TAE and run at 170 V for 5 min and 110 V for 1 hours at room temperature. The samples were soaked with 1× loading buffer beforehand. The gels were stained with Ethidium Bromide (EB) for 30 min and imaged under UV irradiation. The pictures were captured using a WD-9413B gel imaging system (Beijing Liuyi Instument Factory, Beijing, China).

Cell culture. CCRF-CEM and Ramos cell lines were cultured in RPMI 1640 medium (American Type Culture Collection, ATCC) supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin (ATCC) at 37 ^{o}C in a humid atmosphere with 5% CO_2 stmosphere. Cancer cells were grown to 90% confluence, and cell density was determined by a hemocytometer prior to experiments.

Polyacrylamide gel electrophoresis (PAGE) of the self-assembly pathways of the DNA NSs. Fig. S1 lane a suggests stands (1) and (4) can stably coexist. Meanwhile, a small mobility of basic building units was observed corresponding to that consisting of DNAs (1), (2) and (3) (lane c). Upon the introduction of connector (4), the building units anisotropically and spontaneously linked to the connectors via the predesigned arm sequences (lane e). Thus, much less mobility was observed due to the self-assembly of building units into DNA nanostructures with collective increased molecular weight (lane e). Additionally, according to our design, nanoassembly structure can also be achieved by the assembly of DNAs (2)/(3)/(4) (land b) and DNAs (1)/(3)/(4) (lane d).

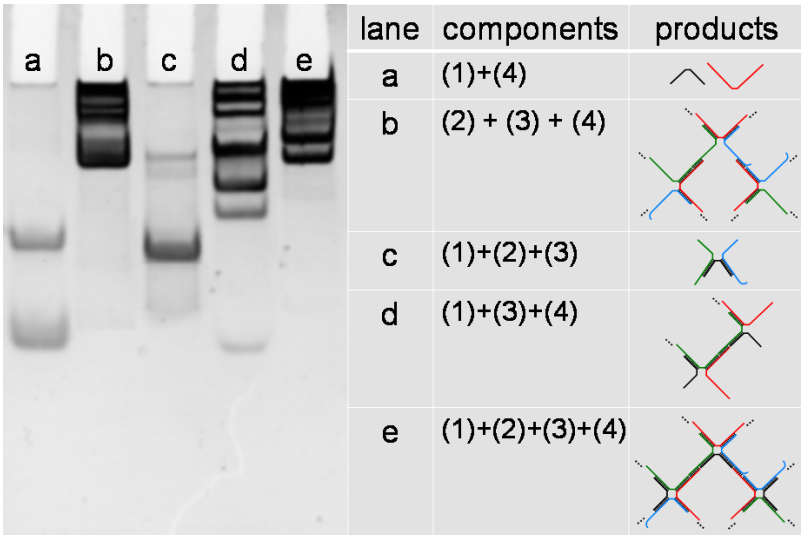

Fig. S1 Polyacrylamide gel electrophoresis (PAGE) of the self-assembly pathways of the DNA nanostructures.

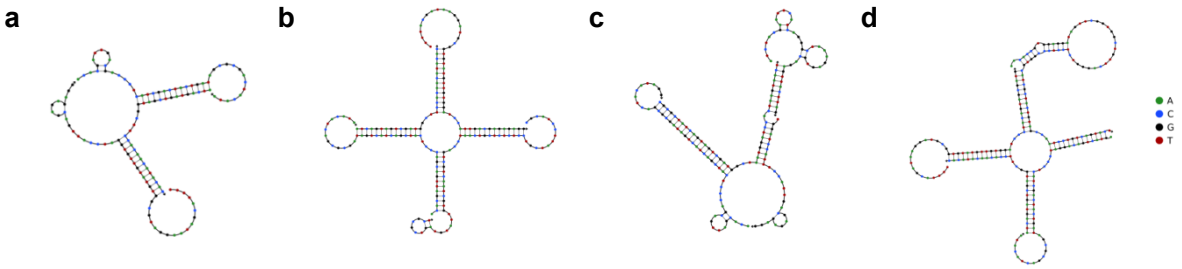

Fig. S2 Predicted hybridization structures of the assemblies of (a) (1)/(2)/(3), (b) (1)/(2)/(3)/(4), (c) (5)/(6)/(7)/(8), and (d) (1)/(10)/(11)/(12), which were predicted using the Nupack software (www.nupack.org).

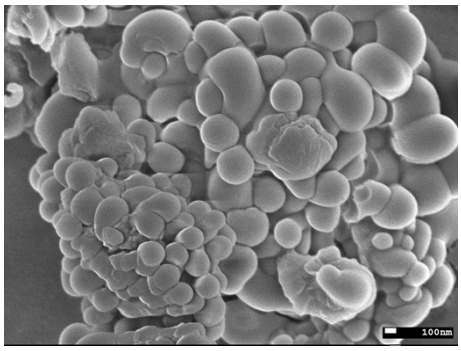

Fig. S3 SEM image of preliminary structure of DNA spherical structure without washing. The morphology of the DNA assemblies exhibited interconnected bulky structures without washing, which were pinched off and separate into monodispersed individual spherical particles with diameters of ~200 nm after washing (Fig. 1d).

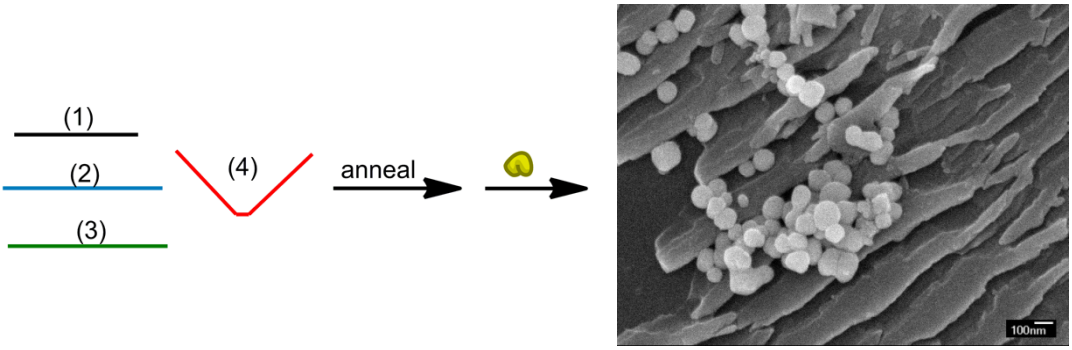

Fig. S4 SEM image of the DNA nanostructures obtained by mixing strands (1), (2), (3), and (4) in one-pot for annealing in the presence of T4 DNA ligase. Irregular spherical structures were observed by annealing the strands (1), (2), (3), and (4) in one-pot accompanied by gel-like morphology.