Supporting Information

DNA framework carrier with asymmetric hydrophobic drug pattern for enhanced cellular cytotoxicity

Yan Zhao,†[ab] Linjie Guo,†[bc] Shuting Cao,[b] Mo Xie,[d] Hongzhen Peng,[c] Jiang Li,[bc] Shihua Luo,[e] Lan Ma,[af] and Lihua Wang*[bcg]

[a] *Institute of Biomedical Health Technology and Engineering, Shenzhen Bay Laboratory, Shenzhen 518132, China.*
[b] *Division of Physical Biology Department, CAS Key Laboratory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China. E-mail: wanglihua@sinap.ac.cn*
[c] *The Interdisciplinary Research Center, Shanghai Synchrotron Radiation Facility, Zhangjiang Laboratory, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China.*
[d] *Key Laboratory for Organic Electronics and Information Displays (KLOEID) & Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials (IAM), National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts and Telecommunications, Nanjing, Jiangsu 210023, China.*
[e] *Department of Traumatology, Rui Jin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China*
[f] *Institute of Biopharmaceutical and Health Engineering, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen 518055, China*
[g] *Shanghai Key Laboratory of Green Chemistry and Chemical Processes, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200241, China*
† These authors contributed equally to this work.
*Corresponding authors
E-mail: wanglihua@sinap.ac.cn

Experiment

Materials. All DNA strands were purchased from Sangon Biotech (China) and purified by high-performance liquid chromatography. Maytansine (DM1) was bought from Xi'an Ruixi Biological Technology (China). DMEM medium was purchased from Gibco (Grand Island, NY, USA). Cell counting kit-8 and Annexin V-FITC apoptosis detection kits were purchased from Beyotime.

Conjugation, purification, and characterization of ssDNA-DM1. The amino-modified ssDNA was mixed with DM1-SMCC at a ratio of 1:20 in PBS buffer, and 50% (v/v) DMSO was added to completely dissolve DM1-SMCC. The mixture was reacted at room temperature for 2 hours. The ssDNA-DM1 was purified through a NAP-5 desalting column to remove excess DM1-SMCC and DMSO. The product was characterized through 12% PAGE analysis and Liquid Chromatograph Mass Spectrometer (LC-MS) which was conducted by Sangon Biotech (Shanghai).

HPLC characterization of ssDNA-DM1. A C18 column (Waters XBridge^{TM}C18, 4.6*150 mm) was used and DNA was recorded at 260 nm, while DM1 was at 254 nm. Reversed-phase chromatography gradient elution method list as below. Mobile phase A: 0.1 mol/L Triethylammonium bicarbonate buffer, phase B: acetonitrile, flow rate: 1mL/min.

Table S1 Reversed-phase chromatography gradient elution method

Procedure	Time (min)	Phase A (%)	Phase B (%)
1	1	98	2
2	10	70	30
3	25	2	98
4	26	98	2
5	36	98	2

Preparation of 6HB nanostructures. The fabrication of 6HB nanostructures is a one-step process. Twenty strands (6-helix-1~6-helix-20, as listed in Table S1, shown in Figure S1) were mixed equally (1 μM) in 100 μL synthetic buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 50 mM magnesium acetate, pH 8.0), with the temperature slowly cooled from 95°C to room temperature in a water bath (in a 1L styrofoam box).

Gel electrophoresis Characterization. 6% PAGE gel was used to verify the formation of the expected 6HB nanostructures. The electrophoresis was carried out on a Bio-Red gel electrophoresis system under 100 V for 1 h at room temperature in TAE buffer (40 mM Tris-HCl, 2 mM EDTA, 20 mM acetic acid, and 12.5 mM Mg Acetate). DNA was stained with GelRed (Biotium, USA) for analysis on a chemiluminescence imaging system.

AFM characterization. *AFM image was collected using tapping mode on Bruker atomic force microscope. Fluid+ tips (Veeco Inc., USA) with an elastic coefficient (k) of 0.7 N m^{-1} were used. 10 nM of the 6-helix sample in 8 μL synthetic buffer were spotted onto the mica surface which was pretreated by aminopropyltriethoxysilane (APTES) and adsorbed for 5 min. Images were*

flattened and analyzed using NanoScope Analysis software.

Stability of 6HB in cell medium. To analyze the stability of 6HB in the cell medium, the structures were dispersed in a DMEM culture medium containing 10% (v/v) fetal bovine serum (FBS) to achieve a final structure concentration of 100 nM. The resulting mixture was incubated at 37 °C for 0, 2, 6, 12, and 24 h and analyzed by gel electrophoresis.

Cellular uptake of 6HB by 4T1 cells. For quantification analysis, 4T1 cells were seeded in 24-well culture plates at a density of 1×10^5 cells/well, and incubated overnight (DMEM medium supplemented with 10% (v/v) FBS) before being used. Then, 4T1 cells were incubated with Alexa Fluor 488-labeled 6HB for 1, 2, 3, and 4 h at 37 ℃. After washing three times with PBS, cells were harvested, and the fluorescence intensity of the cells was determined by flow cytometry (Beckman, USA).

For imaging analysis, 4T1 cells were seeded into confocal plates and incubated overnight. Then Alexa Fluor-488 modified 6HB nanostructures incubated with cells for 4 h. After washing with PBS three times, cells were fixed with 4% paraformaldehyde for 30 min, stained with DAPI for 15 min, and imaged by a confocal laser scanning microscope (Zeiss LSM980, German).

Cell viability assay. 4T1 cells were seeded in 96 well plates at a density of 1×10^4 cells/well (n=3) and allowed to incubate overnight. Cells were treated with 6HB, 6HB-$DM1_{in}$, 6HB-$DM1_{out}$-center, and 6HB-$DM1_{out}$-side for 4 h and subsequently changed for fresh medium. Then at a different time point, cells were washed with PBS and treated with CCK8 solution. After incubation at 37 ℃ for 1 h, absorbance was measured with a microplate reader at 450 nm.

Supporting Tables

Table S2. DNA Sequences

Name	Sequence (5'-3')
6HB-1	CAGTTGACTGCTAGTACCTGAGCACTGAATGCGATGTAGAAGTAGCTCTG CTCCATC
6HB-2	CGACTTGATGGAGCAGACCTATCGTCAC
6HB-3	AGGCAGATACGAAGAGCGAGGCTATGTCGTAGATAGTTCTCGCACGACG CTAGACAC
6HB-4	GCAGTCAACTG
6HB-5	CGGTACGTGACGATAGGACACATCAGATGTCTTAGGAGAGGTCACAGTA ACCTTCGACAATCT
6HB-6	AGATTGTCGAACGTATCTGCCT
6HB-7	GAACTATGACATCTGATGTGTGCTACTTGGCTGCCTAGAC
6HB-8	CGCTCTTGGTTACTGTGACCTGTGCTCAGCCGCTGTGAAT
6HB-9	GCATTCACTCCTAACTACGAC
6HB-10	TCTAGCGTGTCTAGCGT
6HB-11	TGTCCGTGCAACCGATCAATCC
6HB-12	GCCTAGCGATCCAATGGAACGACCGTATTGCTGAGGTGAGTGTATGTATC ACTTGCACGGACA
6HB-13	CTGTACCGTTG
6HB-14	GGATTGATCGGATGCCAGACGCATCGGATTCGATGAGCCTACTCGACCAA CTCAACG
6HB-15	GGCAATGTCCACCATTGGATCG
6HB-16	CAACGGTACAGGGCAGCCTCCAACCTTGTAACCAGCGGCATAACGCTGGA CATTGCC
6HB-17	GGAGGCGTGCGACTGGCATGTGATACATACACTGGTTGGA
6HB-18	GGATGATAGCCTGGCTCATGCAATACGGTCGTTGCGTTAT
6HB-19	GTTACAACACCTCACGAATCC
6HB-20	GCACCACGTTGAGTTGG
6HB-4-Alexa Fluor 488	Alexa Fluor 488-GCAGTCAACTG
6HB-3-in	AGGCAGATACGAAGAGCGAGGC/iNH2C6dT/ATGTCGTAGA/iNH2C6dT/ AGTTCTCGCACGACGCTAGACAC
6HB-9-in	GCAT/iNH2 C6dT/CACTCCTAACTACGAC
6HB-14-in	GGATTGATCGGA/iNH2C6dT/GCCAGACGCATCGGATTCGA/iNH2C6dT/ GAGCCTACTCGACCAACTCAACG
6HB-16-in	CAACGGTACAGTGGTGACTCCAACCTTGTAACG/iNH2C6dT/CCTCGATAA CGCTGGACATTGCC
6HB-2-side	/5'NH2 C6/CGACTTGATGGAGCAGACCTATCGTCAC
6HB-5-side	/5'NH2C6/CGGTACGTGACGATAGGACACATCAGATGTCTTAGGAGAGGT CACAGTAACCTTCGACAATCT

6HB-10-side	/5’NH2 C6/TCTAGCGTGTCTAGCGT
6HB-11-side	/5’NH2 C6/TGTCCGTGCAACCGATCAATCC
6HB-12-side	GCCTAGCGATCCAATGGAACGACCGTATTGCTGAGGTGAGTGTATGTATC ACTTGCACGGACA/3’NH2 C6/
6HB-13-side	CTGTACCGTTG/3’NH2 C6/
6HB-8-center	CGCT/iNH2 C6dT/CTTGGTTACTGT/iNH2 C6dT/GACCTGTGCTCACGAGGACCGGCC
6HB-9-center	/5’NH2 C6/GCATTCACTCCTAACTACGAC
6HB-18-center	GGATGATAGCCTGGCT/iNH2 C6dT/CATGCAATACGGTCGTTGCGT/iNH2 C6dT/TAT
6HB-19-center	GTTACAACACCTCACGAATCC/3’NH2C6/

Supporting Figures

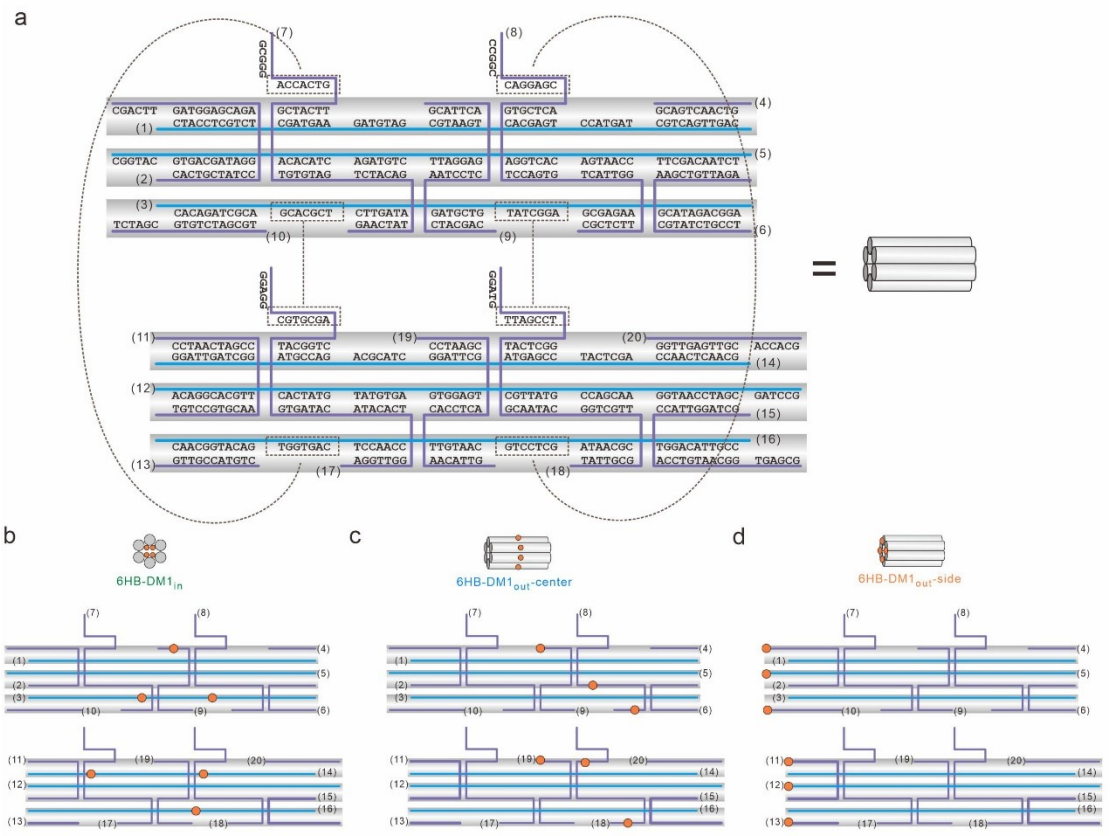


Figure S1. Design of DNFs in this study. a) Schematic of 6HB with detailed sequences. b) Schematic of 6HB-DM1$_{in}$. c) Schematic of 6HB-DM1$_{out}$-center. d) Schematic of6HB-DM1$_{out}$-side. The orange spots indicate the positions of DM1.

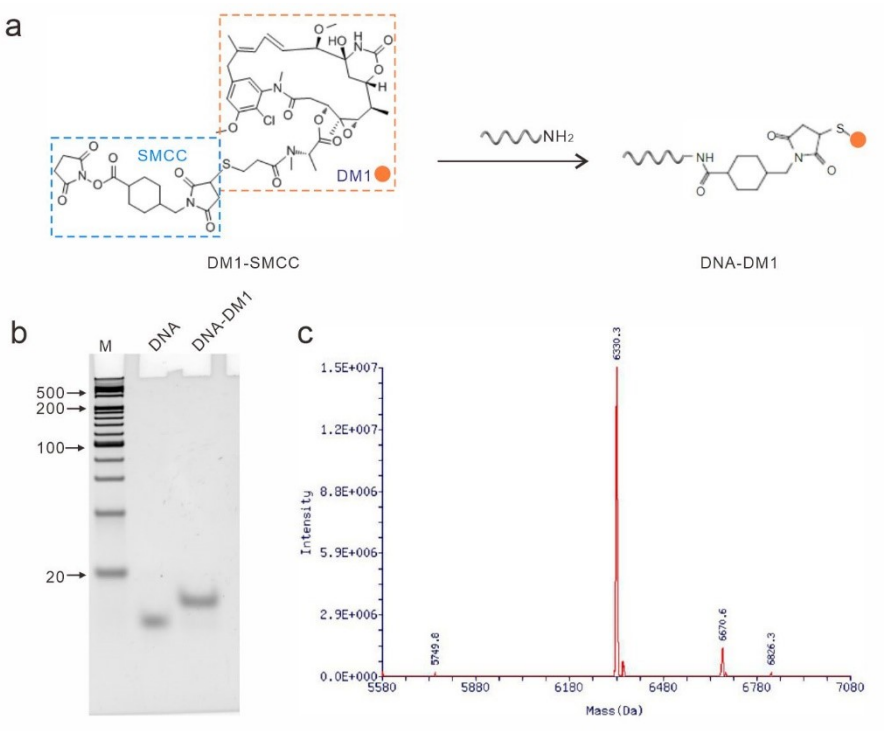


Figure S2. Conjugation ssDNA with DM1. a) Roadmap for the synthesis of DNA-DM1. b)12% PAGE analysis of DNA-DM1 conjugation. c) Mass spectra of ssDNA-DM1 conjugation. Before conjugation, the molecular weight of amino-ssDNA is 5371.6. The MS results presented a total molecule weight of 6330.3, consistent with the theoretical value ($\mathbf{M}_{ssDNA\text{-}NH2}$**+**$\mathbf{M}_{DM1\text{-}SMCC}$**-**$\mathbf{M}_{displaced}$=5371.59+1072.61-114=6330.2), indicating the successful conjugation of DM1.

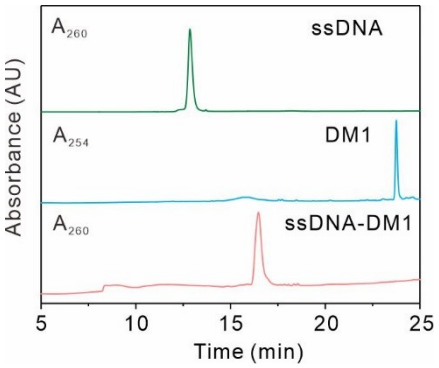


Figure S3. Reverse-HPLC characterization of ssDNA and DM1 conjugates.

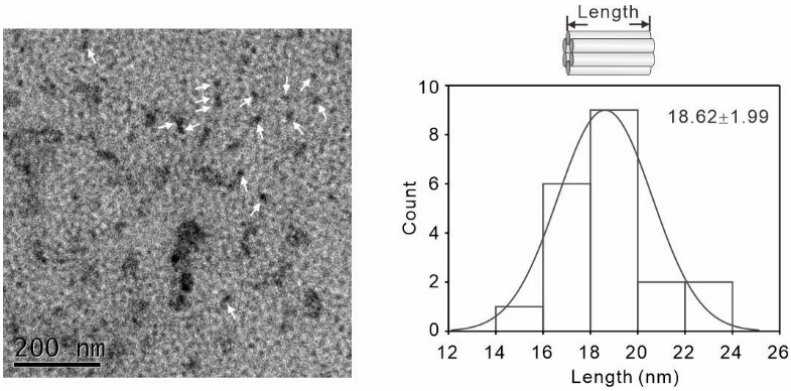
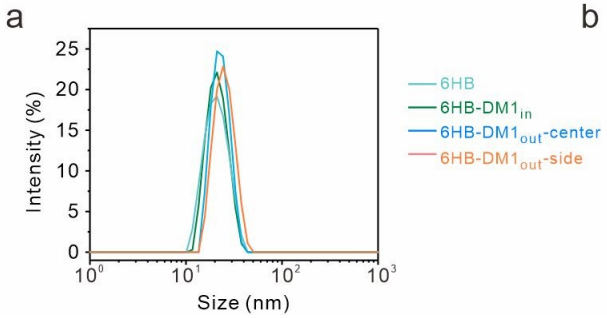


Figure S4. TEM images of 6HB and distributions of lengths taken from 20 individual particles.

a



b

group	PDI
6HB	0.227±0.023
6HB-DM1$_{in}$	0.207±0.010
6HB-DM1$_{out}$-center	0.196±0.024
6HB-DM1$_{out}$-side	0.183±0.038

Figure S5. (a) Dynamic Laser Scattering (DLS) in intensity of four structures and their (b) Polydisperse index (PDI) value.

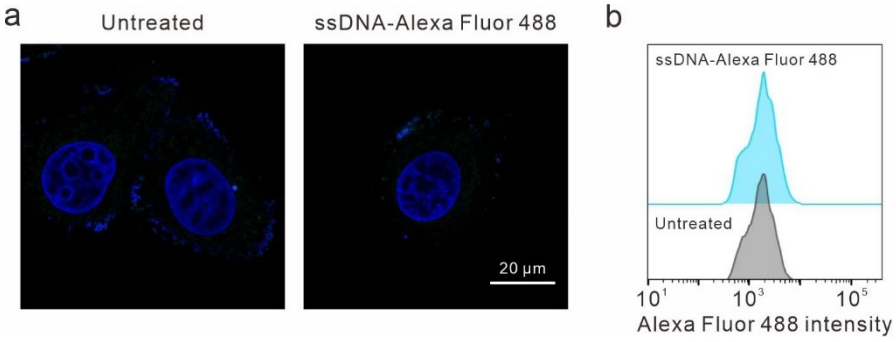


Figure S6. Confocal images (a) and flow cytometry (b) of Alexa Fluor 488-ssDNA uptakes into cells after incubation for 4 hours.

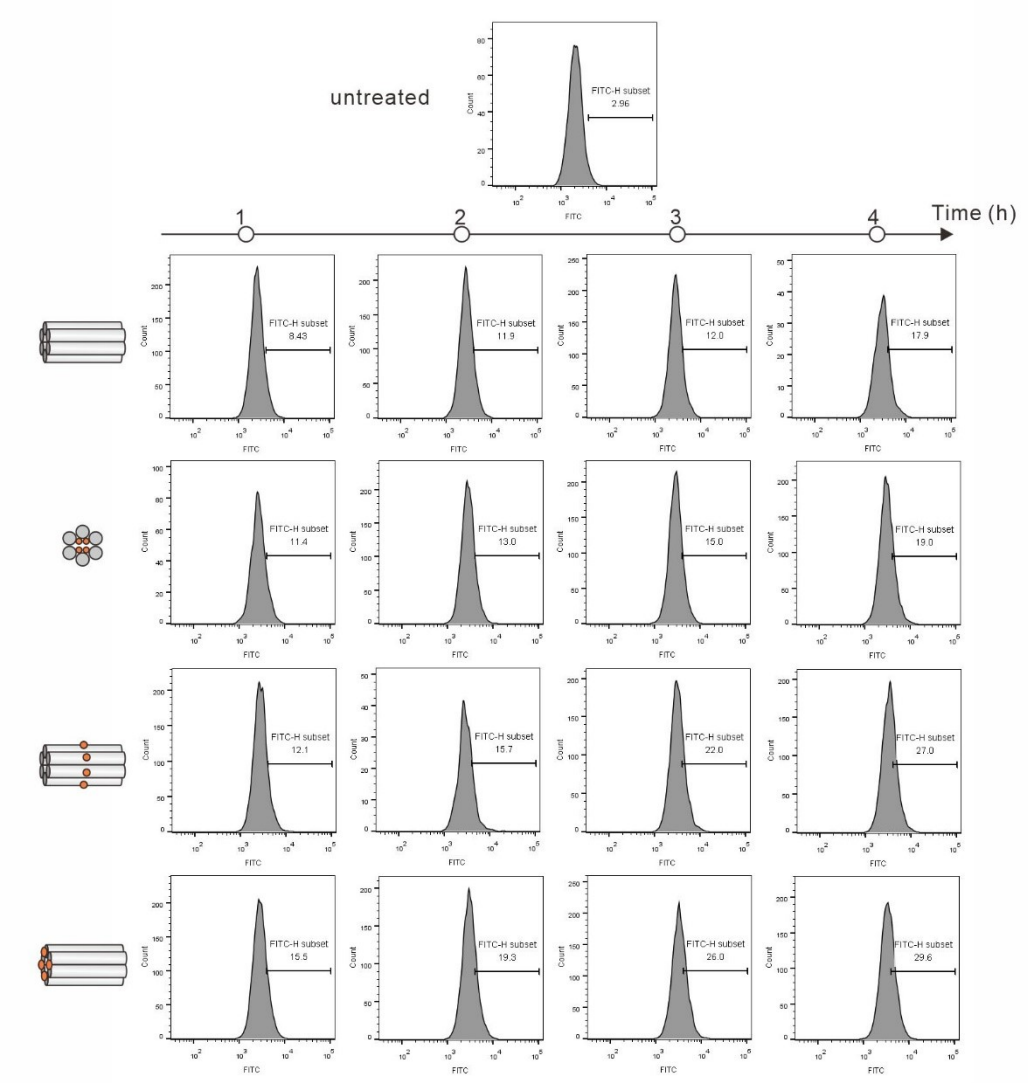


Figure S7. Flow cytometry time-course analysis of 4T1 cells incubated with 6HB, 6HB-DM1$_{in}$, 6HB-DM1$_{out}$-center, and 6HB-DM1$_{out}$-side from 1 to 4 h.