**Supporting Information**

# Self-assembled "DNA Nanocentipede" as Multivalent Drug Carrier for Targeted Delivery

Wenshan Li, Xiaohai Yang*, Leiliang He, Kemin Wang*, Qing Wang, Jin Huang, Jianbo Liu, Bin Wu, and Congcong Xu

*State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha 410082, China*

**To whom correspondence should be addressed:*
*Phone: +86-731-88821566. Fax: +86-731-88821566.*
*E-mail: yangxiaohai@hnu.edu.cn, kmwang@hnu.edu.cn*

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## Experimental Section

### S1 Chemicals and Materials

Doxorubicin (Dox) was purchased from Hualan Chemistry Technology Co. Ltd. (Shanghai, China). 3-(4, 5-Dimethylthiazol-2-y1)-2, 5-diphenyltetrazolium bromide (MTT) and Streptavidin were purchased from Sigma-Aldrich (USA). LysoTracker Blue DND-22, SYBR Green I and SYBR Gold were purchased from Invitrogen (USA). Bull serum albumin (BSA) and yeast tRNA were purchased from Dingguo Changsha Biotechnology Co., Ltd (Beijing, China). All DNA oligonucleotides were synthesized and HPLC purified by Sangon Biotech. Co. Ltd. (Shanghai, China) and dissolved in phosphate buffered saline (PBS) solution (10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4). The sequences of the oligonucleotides were listed in Table 1. All other reagents were of the highest grade available. All solutions were prepared and diluted using ultrapure water (≥ 18.2 MΩ cm) from the Millipore Milli-Q system (Barnstead/Thermolyne NANO-pure, Dubuque, IA). Washing buffer contained glucose (4.5 g/L) and $MgCl_2$ (5 mM) in PBS solution. Binding buffer was prepared by adding yeast tRNA (0.1 mg/mL) and BSA (1 mg/mL) to the washing buffer.

### S2 Cell Culture

SMMC-7721 cells (human hepatocellular carcinoma), L02 cells (normal human hepatocytes) were purchased from the Shanghai Institute of Cell Biology of the Chinese Academy of Science. All cells were cultured in RPMI medium 1640 supplemented with 10% fetal bovine serum (FBS; heat-inactivated), 100 units/mL penicillin and 100 μg/mL streptomycin. Both cultures were incubated at 37 °C under a 5% $CO_2$ atmosphere.

### S3 Preparation of DNA Nanocentipedes

H1 and H2 were dissolved with PBS to a common final concentration of 100 μM, heated to 96 °C for 3 min, immediately cooled on ice for 3 min, and then left at room temperature for 2 h before use. Various concentrations of Trigger ([Trigger] = 5000, 2500, 1250, 500 and 250 nM) were incubated with H1 (25 μM), and H2 (25 μM) at 25 $^{o}$C for 24 h, then dilute with PBS, followed multiple streptavidin and biotinylated Zy1 were added and incubated at 25 $^{o}$C for 2 h.

### S4 Agarose Gel Electrophoresis

A 2% agarose gel was prepared using a 1 × TAE buffer (40 mM Tris AcOH, 2.0 mM $Na_2EDTA$, pH 8.5). The 100 × SYBR Gold (2 μL) was used as an oligonucleotide dye and mixed with samples. The gel was run at 100 V for 60 min in a 1 × TAE buffer at room temperature, and then photographed in Gel Imaging (Tanon 2500 R, Tianneng Ltd., Shanghai, China).

### S5 Fluorescence Anisotropy

The fluorescence anisotropy signals were measured on a modular spectrofluorometer (Photon Technology International Inc.). The 100 μL of H1-FITC

solution (100 μM) was added to 100 μL of H2-FITC (100 μM) solution and 1 μL of trigger (100 μM), the mixture was hybridized at room temperature for 24 h, followed by the addition of streptavidin and biotinylated Zy1, the fluorescence anisotropy was recorded. Excitation was set to 488 nm and emission was detected at 520 nm. All experiments were carried out at room temperature.

**S6 Atomic Force Microscopy**

Trigger (250 nM) was incubated with H1 (25 μM) and H2 (25 μM) at 25 $^{o}$C for 24 h, then dilute 250-fold with TAE-$Mg^{2+}$ (40 mM Tris AcOH, 2.0 mM $Na_2EDTA$, 12.5 mM $Ac_2Mg$, pH 8.5), followed streptavidin (10 nM) and biotinylated Zy1 (10 nM) were added and incubated at 25 $^{o}$C for 2 h. The sample was pipetted on a freshly cleaved mica. Then, the surface was washed 10 times by 20 μL of purified water and gently blown dry by nitrogen gas. The prepared sample was scanned by ScanAsyst-air tips in ScanAsyst Imaging Mode on Multimode 8 Atomic Force Microscope with a NanoScope V controller (Bruker Inc.).

**S7 Stability of Zy1-Nces**

For stability assay, the Zy1-Nces were incubated with 0.05 unit/μL Exonuclease III in PBS at 37 °C for difference times. The reaction was then stopped with a final concentration of 20 mM EDTA. Finally, the samples were then loaded on a 2% agarose gel and subjected to electrophoresis as described above.

**S8 Multivalent Binding Ability**

Flow cytometry was used to evaluate the binding ability of DNA nanocentipedes conjugates toward specific cells. Briefly, anchorage-dependent cells were firstly harvested with 0.02% EDTA and 0.5% trypsin to prepare cell suspensions. 200 000 cells were incubated with varying concentrations of free Zy1 or DNA nanocentipedes in 200 μL binding buffer at 4 $^{o}$C for 30 min. Zy1 was labeled with FITC. After incubation, the cells were washed three times with 200 μL of washing buffer, and 10 000 counts cells were suspended in binding buffer (200 μL) prior to flow cytometry analysis (BD Biosciences, Mountain View, CA, USA). The FITC-labeled random DNA library was used as a negative control to determine nonspecific binding. All of the experiments for the binding assay were repeated three times. Data were analyzed with the FlowJo software. The mean fluorescence intensity of target cells labeled by aptamers was used to calculate the specific binding by subtracting the mean fluorescence intensity of nonspecific binding from the random DNA library. The equilibrium dissociation constants (Kd) of the aptamer-cell interaction were obtained by fitting the dependence of fluorescence intensity of specific binding on the concentration of the aptamers to the equation $Y = Bmax\ X / (Kd + X)$, where Y is the fluorescence intensity and X is the concentration of aptamers.

**S9 Selective Recognition Ability**

Flow cytometry was used to evaluate the selective recognition ability of DNA nanocentipedes in binding buffer at 4 $^{o}$C. Briefly, anchorage-dependent cells were firstly harvested with 0.02% EDTA and 0.5% trypsin to prepare cell suspensions. In binding buffer (200 μL), 200 000 cells were incubated with Lib, Lib-Nces, free Zy1

and Zy1-Nces for 30 min, respectively. Lib and Zy1 were all labeled with FITC. After incubation, the cells were washed three times with 200 μL of washing buffer, and 10 000 counts cells were suspended in binding buffer (200 μL) prior to flow cytometry analysis (BD Biosciences, Mountain View, CA, USA).

Flow cytometry was also used to evaluate the selective recognition ability of DNA nanocentipedes in binding buffer or cell culture medium (containing 10% FBS) at 37 $^{o}$C. The experimental details were similar to the above procedures.

**S10 Time-Dependent Internalization**

Cells were plated in a 35 mm confocal dish and grown to around 80% confluency before the experiment. The cells were incubated with Zy1-Nces (200 nM FITC-labeled Zy1 equivalents) and free Zy1 (200 nM) at 37 °C for various time points, after the medium was removed, the cells were washed twice with washing buffer and LysoTracker Blue was added for specific staining of the lysosomes for 10 min. The cells were washed with washing buffer twice, and then binding buffer (200 μL) was added. All cellular fluorescent images were collected on a FV 500-IX70 confocal microscope (Olympus America Inc., Melville, NY) with a 100 × oil immersion objective (Olympus, Melville, NY). Excitation wavelength and emission filters: LysoTracker Blue channel: excitation 405 nm, emission bandpass (430–460 nm) filter; FITC channel: excitation 488 nm, emission bandpass (505−545 nm) filter.

**S11 SYBR Green I Loading and Cellular Internalization**

A fixed concentration of SYBR Green I (1 ×) was incubated with different concentrations of Zy1-Nces ([Zy1-Nces] = 0, 1.25, 2.5, 5, 10 and 20 nM) at room temperature for 2 h, and the SYBR Green I loading was monitored by fluorescence spectrometry [excitation (Ex): 488 nm], using a Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan).

Cells were plated in a 35 mm confocal dish and grown to around 80% confluency before the experiment. The cells were incubated with SYBR Green I (1 ×) solutions stained Zy1-Nces (5 nM) and Mono-Zy1-Nces (5 nM) at 37 °C for 2 h, after the medium was removed, the cells were washed twice with washing buffer and LysoTracker Blue was added for specific staining of the lysosomes for 10 min. The cells were washed with washing buffer twice, and then binding buffer (200 μL) was added. All cellular fluorescent images were collected on a FV 500-IX70 confocal microscope (Olympus America Inc., Melville, NY) with a 100 × oil immersion objective (Olympus, Melville, NY). Excitation wavelength and emission filters: LysoTracker Blue channel: excitation 405 nm, emission bandpass (430–460 nm) filter; SYBR Green I channel: excitation 488 nm, emission bandpass (505−545 nm) filter.

**S12 Drug Loading and Selective Transport**

Dox was chosen as a model drug and loaded into Zy1-Nces. A fixed concentration of Dox (2 μM) was incubated with different concentrations of Zy1-Nces ([Zy1-Nces] = 0, 0.2, 0.4, 1, 2, 4 and 10 nM) at room temperature for 2 h, and the Dox loading was monitored by fluorescence spectrometry [excitation (Ex): 488 nm], using a Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan).

Cells were plated in a 35 mm confocal dish and grown to around 80% confluency before the experiment. The cells were incubated with free Dox (2 μM) and Zy1-Nces-Dox (2 μM Dox equivalents) at 37 °C for 2 h, after the medium was removed, the cells were washed twice with washing buffer and LysoTracker Blue was added for specific staining of the lysosomes for 10 min. The cells were washed with washing buffer twice, and then binding buffer (200 μL) was added. All cellular fluorescent images were collected on a FV 500-IX70 confocal microscope (Olympus America Inc., Melville, NY) with a 100 × oil immersion objective (Olympus, Melville, NY). Excitation wavelength and emission filters: LysoTracker Blue channel: excitation 405 nm, emission bandpass (430–460 nm) filter; Dox channel: excitation 488 nm, emission bandpass (505 nm long-pass) filter.

Cells were seeded into 24-well plate and grown to around 80% confluency before the experiment. The cells were incubated with free Dox (2 μM) and Zy1-Nces-Dox (2 μM Dox equivalents) at 37 °C for 2 h, after the medium was removed, the cells were washed twice with washing buffer, harvested, and washed three times with washing buffer. Then, the fluorescence intensity of the cells was determined by flow cytometry (BD Biosciences, Mountain View, CA, USA).

**S13 Cytotoxicity Assay**

In vitro cytotoxicity was determined using MTT assay. Briefly, cells were seeded at $1 \times 10^4$ cells per well into 96-well plates for 24 h. Then, cells were treated with Zy1-Nces, free Dox, or Dox-loaded on Zy1-Nces at varied concentrations ([Zy1-Nces] = 0, 0.1, 0.2, 0.5, 1, 2, 5 and 10 nM; [Dox]=0, 0.05, 0.1, 0.25, 1, 2.5 and 5 μM) in medium (without FBS, 37 °C; 5% $CO_2$) for 2 h. The supernatant medium was removed, and fresh medium (10% FBS, 200 μL) was added for further cell growth (48 h). After removing cell medium, 20 μL of MTT solution (5 mg/mL) diluted in fresh medium (100 μL) was added to each well and incubated at 37 °C for 4 h. The precipitated formazan violet crystals were dissolved in 150 mL of DMSO. The absorbance value at 570 nm was recorded using a multi-function plate reader Infinite® M1000 (Tecan, Switzerland). Each concentration was tested at least three times.

## Figures

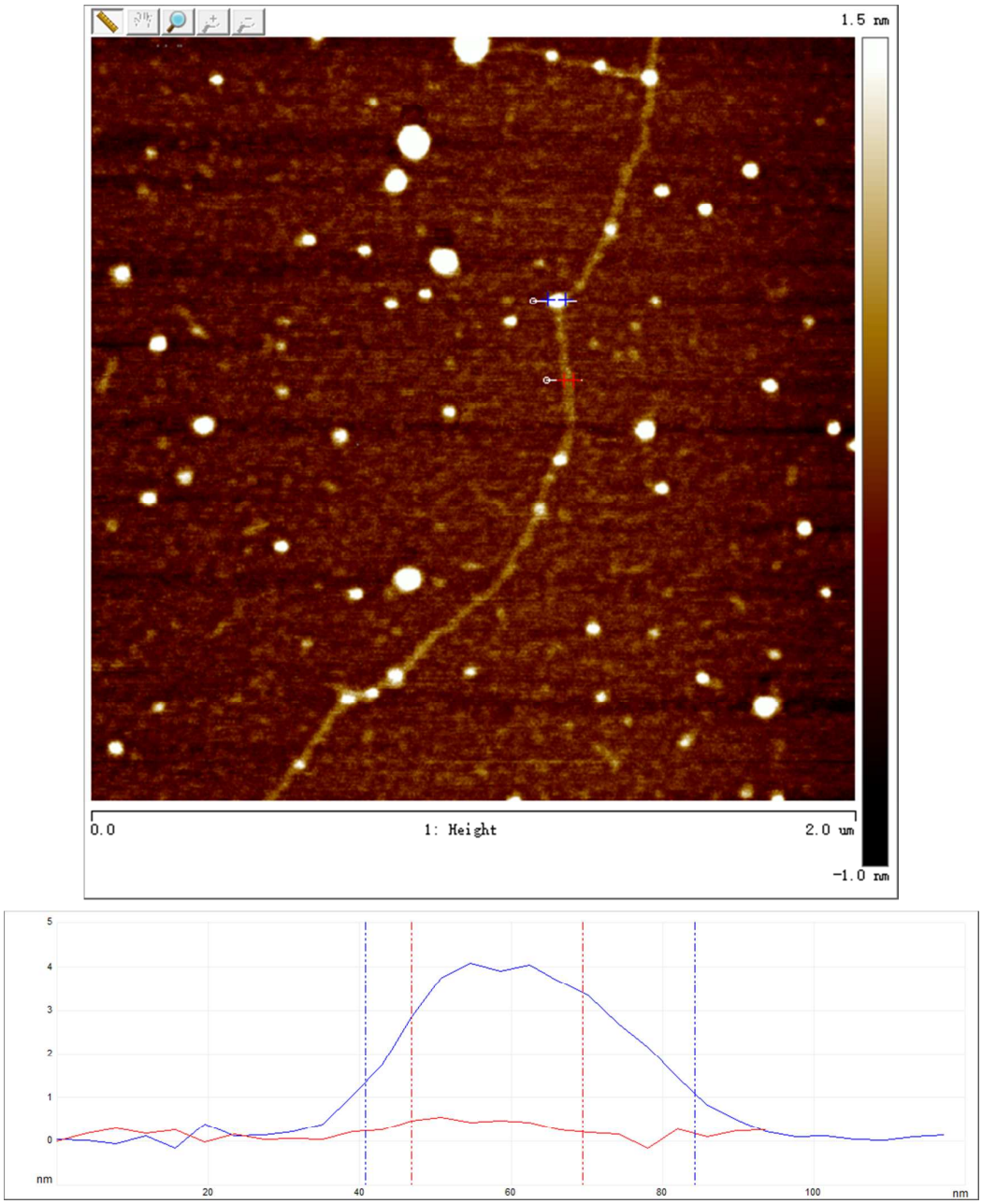


**Figure S1. Atomic force microscopy image and cross-section analysis of DNA nanocentipedes.**

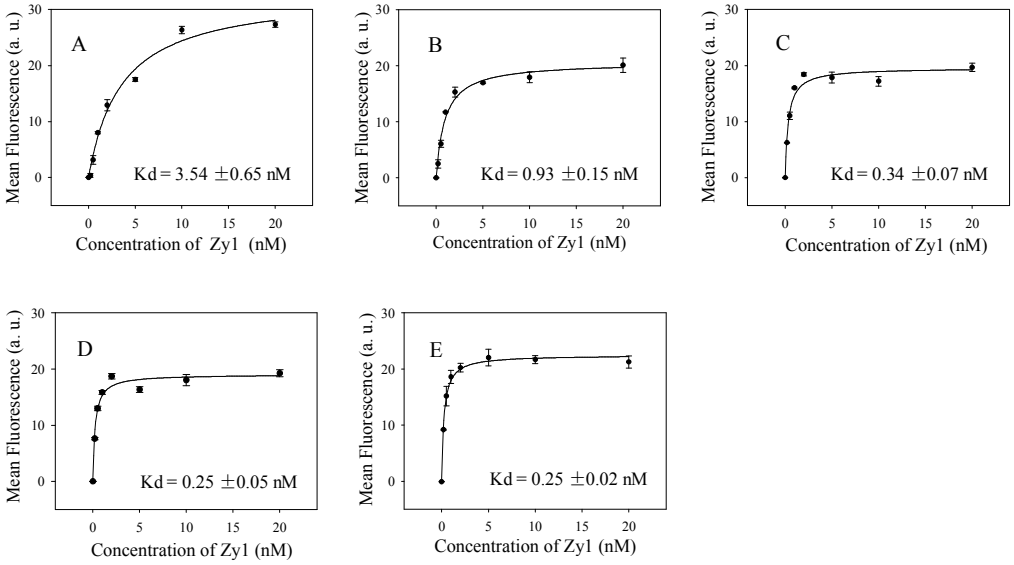


**Figure S2. Effects of trigger concentration on the dissociation constant (Kd).** At a given concentration of H1/H2 (25 μM each), a series of concentration of trigger (5000 nM, 2500 nM, 1250 nM, 500 nM, 250 nM, from A to E) was added to obtain different length of HCR products, lower concentration of trigger induced longer trunk of nanocentipede. SMMC-7721 cells were treated with different concentration of nanocentipedes (FITC-labeled Zy1 equivalents) at 4 $^{o}$C for 30 min. The mean fluorescence intensity of cells was determined by flow cytometry. The error bars indicated the standard deviations of three experiments.

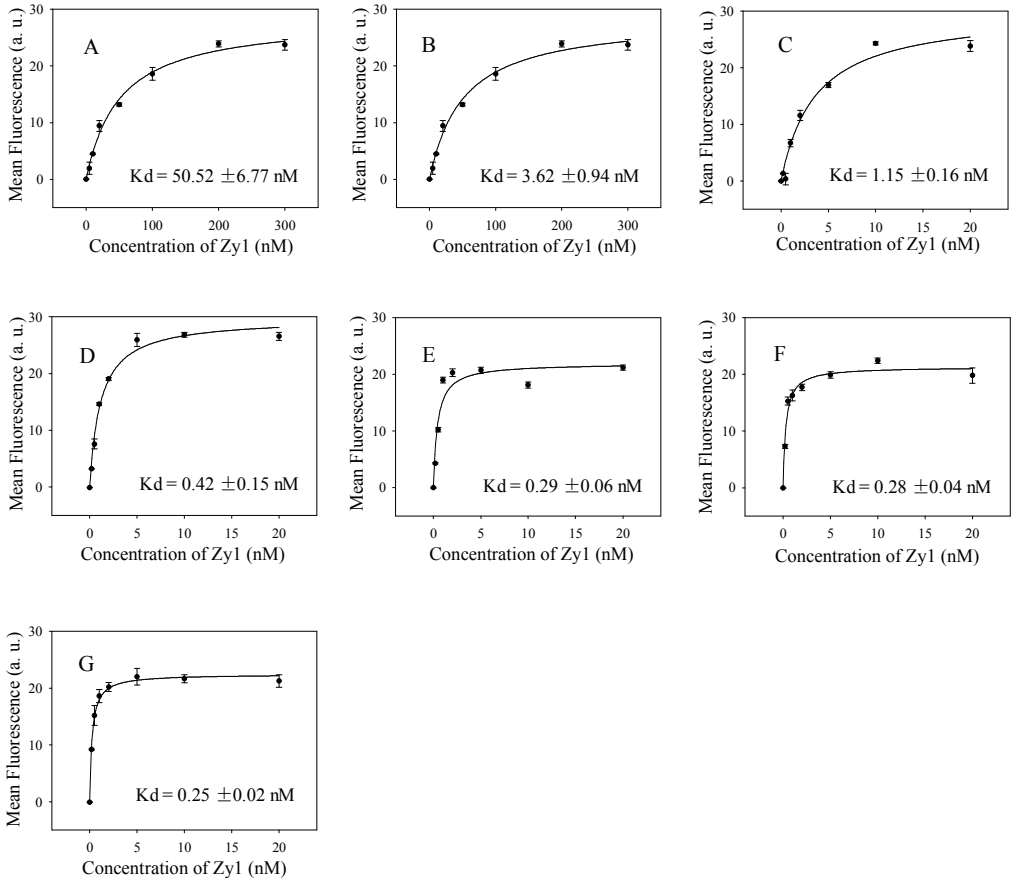


**Figure S3. Effects of the numbers of aptamer per nanocentipede on Kd.** At a given concentration of H1/H2 (25 μM each) and trigger (250 nM), higher concentration of aptamer Zy1 (0.25 μM, 1.25 μM, 2.5 μM, 5 μM, 12.5 μM, 25 μM, 50 μM, from A to G) induced larger numbers of Zy1 per Zy1-Nces. SMMC-7721 cells were treated with different concentration of nanocentipedes (FITC-labeled Zy1 equivalents) at 4 $^{o}$C for 30 min. The mean fluorescence intensity of cells was determined by flow cytometry. The error bars indicated the standard deviations of three experiments.

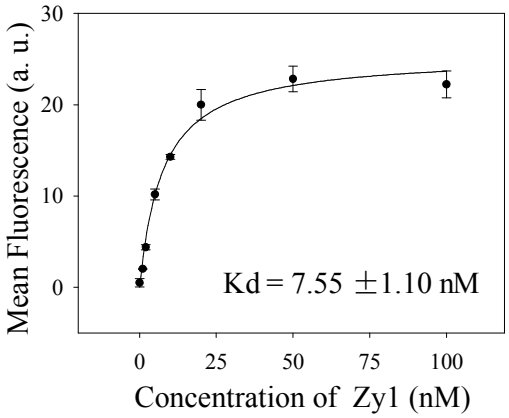


**Figure S4. Characterization of the binding affinity of free Zy1 to SMMC-7721 cells.** SMMC-7721 cells were treated with different concentration of free Zy1 (FITC-labeled Zy1) at 4 oC for 30 min. The mean fluorescence intensity of cells was determined by flow cytometry. The error bars indicated the standard deviations of three experiments.

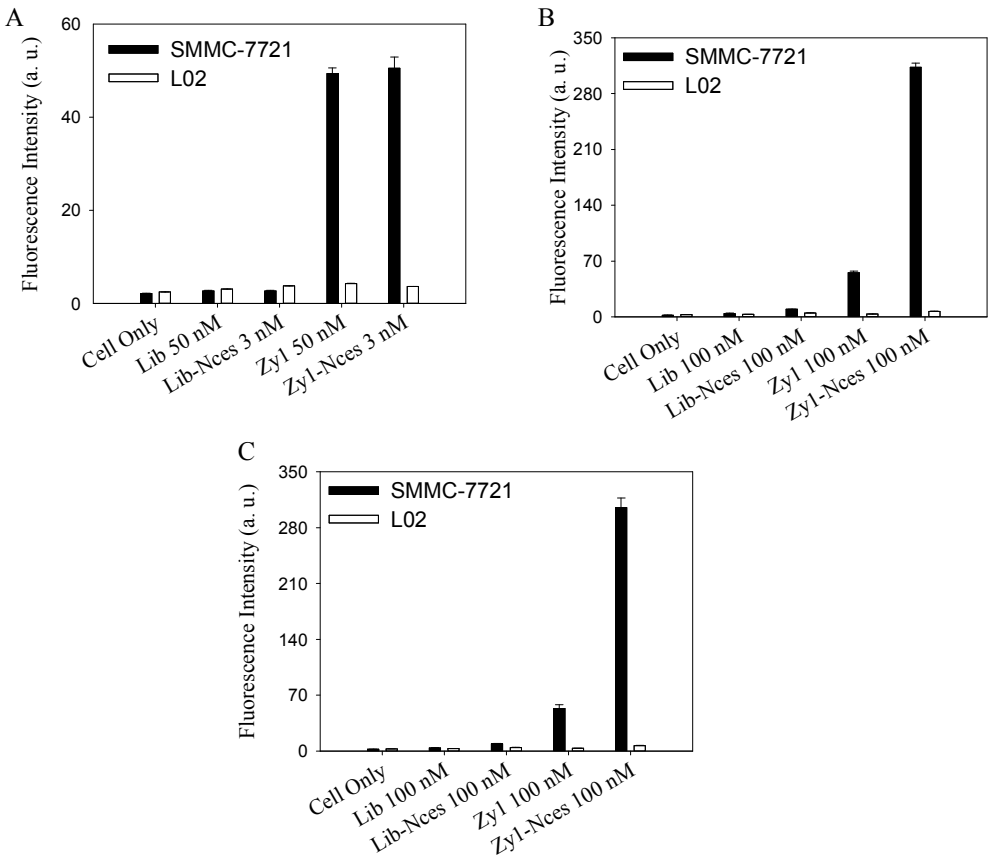


**Figure S5. Characterization of the binding of Zy1-Nces with target SMMC-7721 cells at 4 $^{o}$C (A) or 37 $^{o}$C (B and C).** Cells were incubated with random DNA library (Lib, 50 nM or 100 nM FITC-labeled Lib), random DNA library-based DNA nanocentipede (Lib-Nces, 3 nM or 100 nM FITC-labeled Lib equivalents), free Zy1 (Zy1, 50 nM or 100 nM FITC-labeled Zy1) and Zy1-Nces (3 nM or 100 nM FITC-labeled Zy1 equivalents) in binding buffer (A and B) or in cell culture medium (C, containing 10% FBS), respectively. The fluorescence intensity of cells was determined by flow cytometry. The error bars indicated the standard deviations of three experiments.

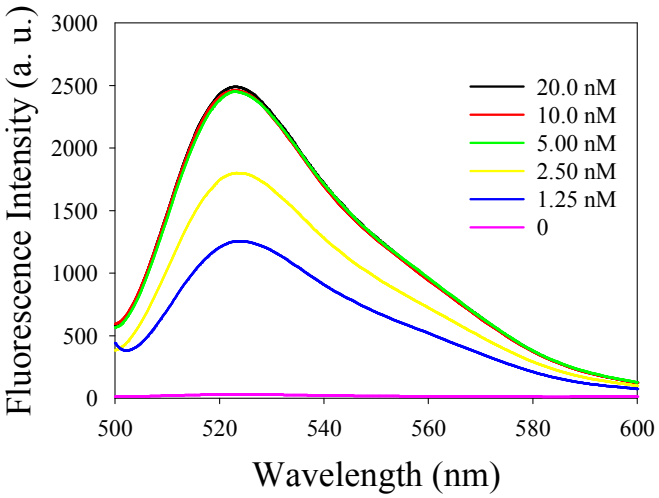


**Figure S6. Optimization of concentration of Zy1-Nces.** Fluorescence spectra of Sybr Green I (1× ) with increasing equivalents of Zy1-Nces (shown by values from bottom to top). The fluorescence enhancement indicates fluorogenic dyes loading into Zy1-Nces. The apparent Sybr Green I fluorescence enhancing with as high as 5.00 nM Zy1-Nces reflects high payload capacity.

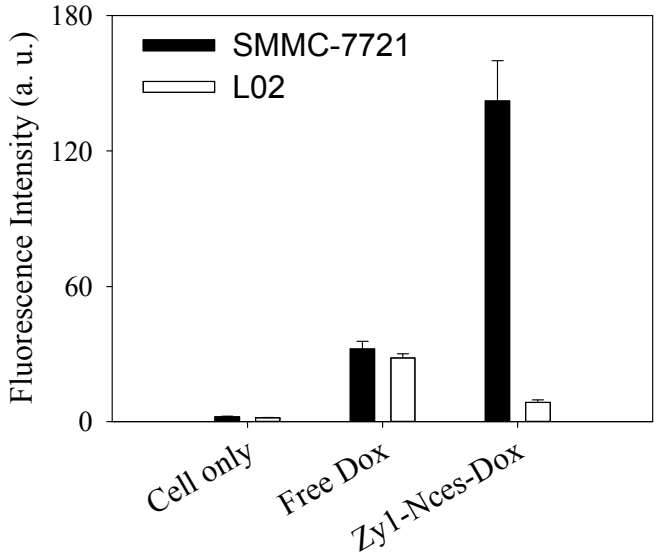


**Figure S7. Characterization of the fluorescence intensity of intracellular Dox.** Cells were incubated with Zy1-Nces-Dox (2 μM Dox equivalents) or free Dox (2 μM) at 37 $^{o}$C for 2 h. The fluorescence intensity of cells was determined by flow cytometry. The error bars indicated the standard deviations of three experiments.