Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information for:

The hierarchical assembly of multi-level DNA ring-based nanostructure in a precise order and its application for screening tumor cells

Jing-Ting Wu,[#] Ran Liu,[#] Yan-Ru Chen, Xiao-Qi Zheng, Zai-Sheng Wu*

Cancer Metastasis Alert and Prevention Center, Fujian Provincial Key Laboratory of Cancer Metastasis Chemoprevention and Chemotherapy, State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou, 350002, China

Corresponding Author:
Email: wuzaisheng@163.com (Z.-S. Wu)

Table S1. Sequences of DNA oligonucleotides designed in this work.[a]

Name	Sequence (5'-3')
Padlock Probe A (PPA)	pGACACACA**CCAC***GATGCAAGACCTCACAACTC***GCCACAGAGACAATCT**AGAGCAGGCTAAACA GTAACACGAGCTGGACATGGAACCG
Padlock Probe B (PPB)	pCCATAGAA**CTTGGGTCGTCTCATAGTCTTGGTGAATGTTGTACTAGAA**GAACTCTGCAGCTCGT GTTACTGTTTAGAACCATTGTACG
FAM-labeled Padlock Probe B (FAM-PPB)	pCCATAGAACTTGGGTCGTCT/FAM/CATAGTCTTGGTGAATGTTGTACTAGAAGAACTCTGCAGC TCGTGTTACTGTTTAGAACCATTGTACG
Linker A (LA)	TGTGTGTCCGGTTCCATGTCCAGAGTTCCCAGTTCTACGTTGAGTCAGATGATC
Linker B (LB)	TTCTATGGCGTACAATGGTTCCTGCTCTGATCATCTGACTCAACGTAGAACTGG
Linker C (LC)	TGTGTGTCCGGTTCCATGTC----------CCAGTTCTACGTTGAGTCAGATGATC
Linker D (LD)	TTCTATGGCGTACAATGGTT----------GATCATCTGACTCAACGTAGAACTGG
Linker-Sgc8 (LS)	*GAGTTGTGAGGTCTTGCATC*TTTTTTTTATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGT

[a]The regions with gray background in PPA and PPB denote the two single-stranded domains in the basic structural units (S-ring) of hierarchical DNA ring-based nanostructures (h-Nanoring). LA and LB are the ligation templates for the cyclization of PPA and PPB, respectively, while the middle regions indicate the additional hybridization domains (Ahd) to the adjacent ring. LC and LD respectively have the same sequences as the LA and LB but without the Ahd fragment. Linker-Sgc8 (LS) is designed via prolonging the original sequence of aptamer Sgc8 (boxed, capable of specifically binding to PTK7 protein) by adding a linker fragment in italic at the 5'-end, which is able to be positioned to the single-stranded domain of PPA because of partly complementary fragment. Lower-case letter 'p' indicates phosphate group. More details are shown in Figure S1.

Supporting figures

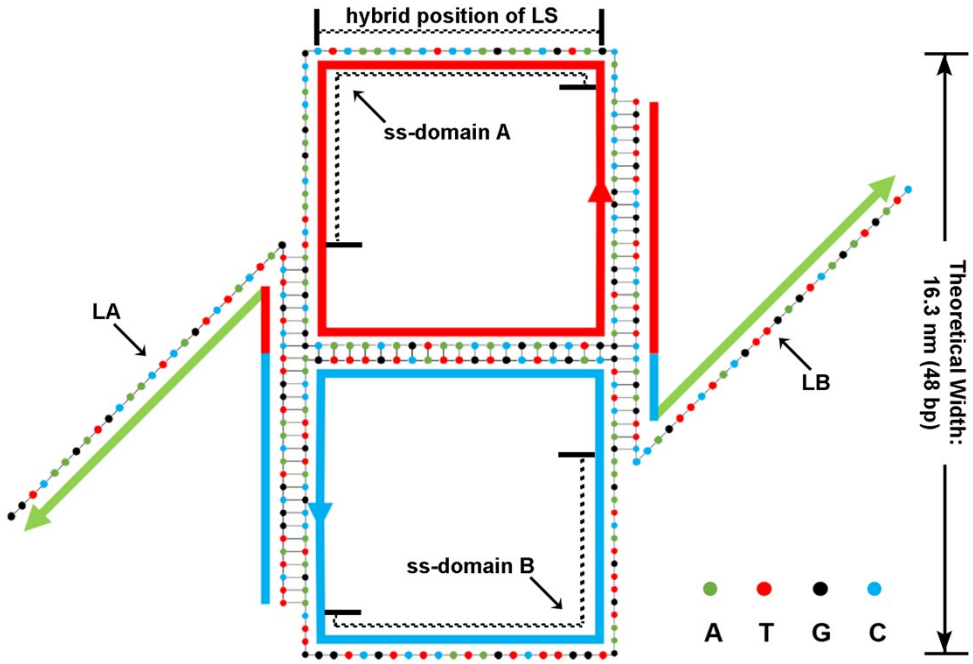

Figure S1. The molecular structure of the second-level ring (S-ring) that severs as the basic textural unit of hierarchical DNA ring-based nanostructures (h-Nanoring).

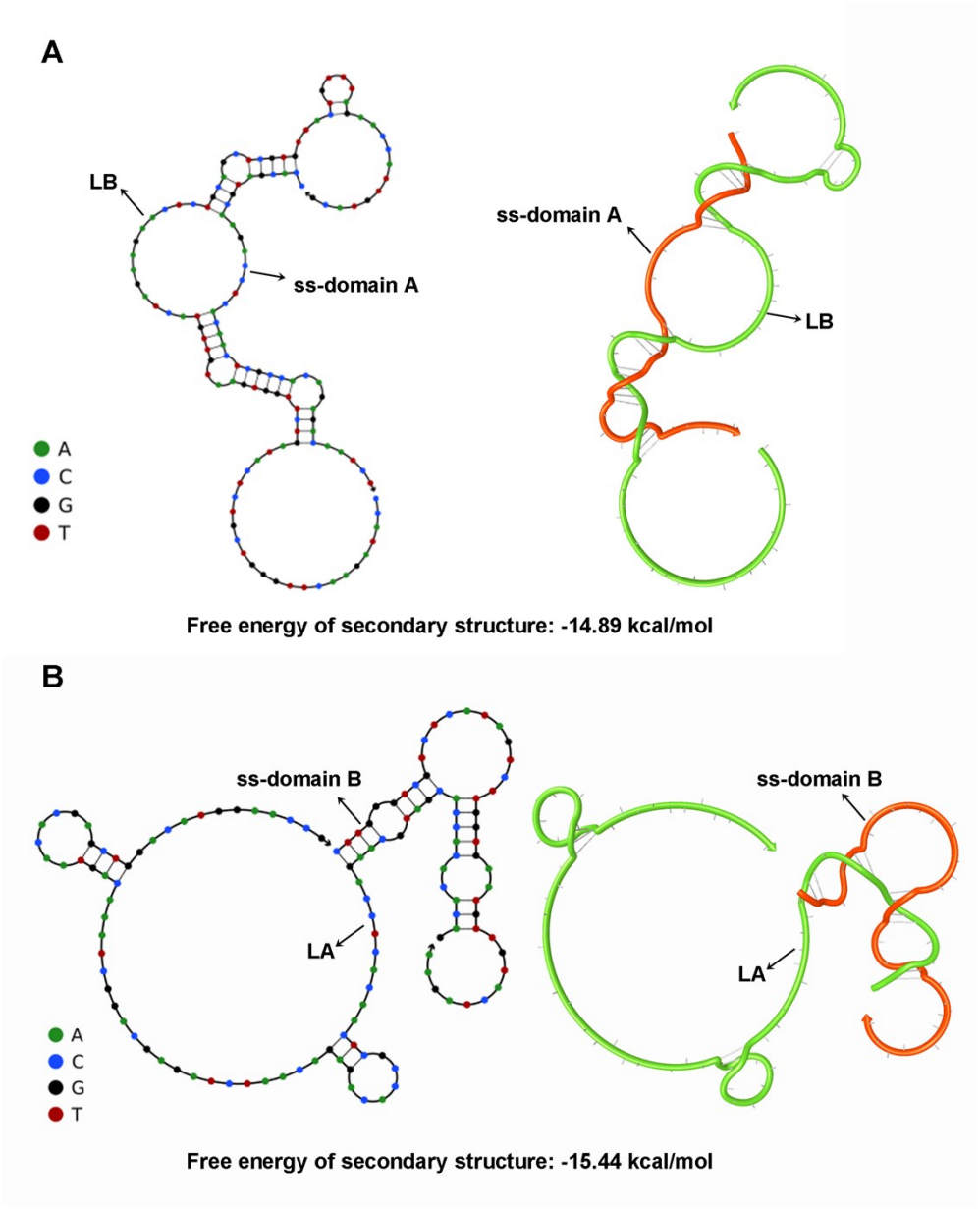

Figure S2. The secondary structure of DNA hybrids analyzed with NUPACK software. (A) The interaction between ss-domain A in S-ring with LB. (B) The interaction between ss-domain B in S-ring with LA strand. Minimum free energy (MFE)-based structure and predicted helicity are shown in the left side and in the right side, respectively.

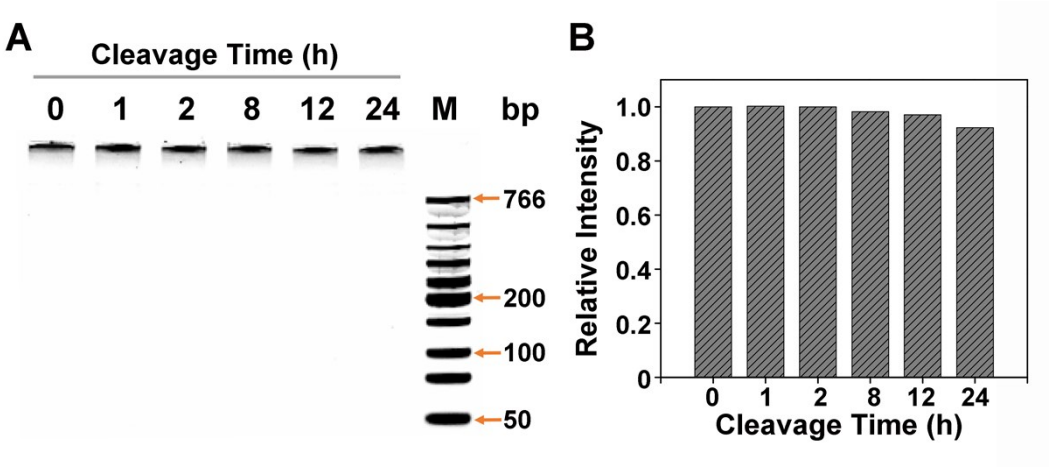

Figure S3. (A) Serum stability assay of h-Nanoring by native-PAGE analysis. The final serum concentration is 10% (v/v). (B) The cleavage products are fluorescently quantified where the fluorescence intensity of DNA band of 0 h is defined as 1.

Experimental procedure:

The sample was prepared by adding 2 μL of fetal bovine serums into 18 μL of DNA h-Nanorings and mixing thoroughly. After incubation at 37°C for 0 h, 1 h, 2 h, 8 h, 12 h and 24 h respectively, the mixtures were immediately used for native-PAGE analysis. The final serum concentration is 10% (v/v).

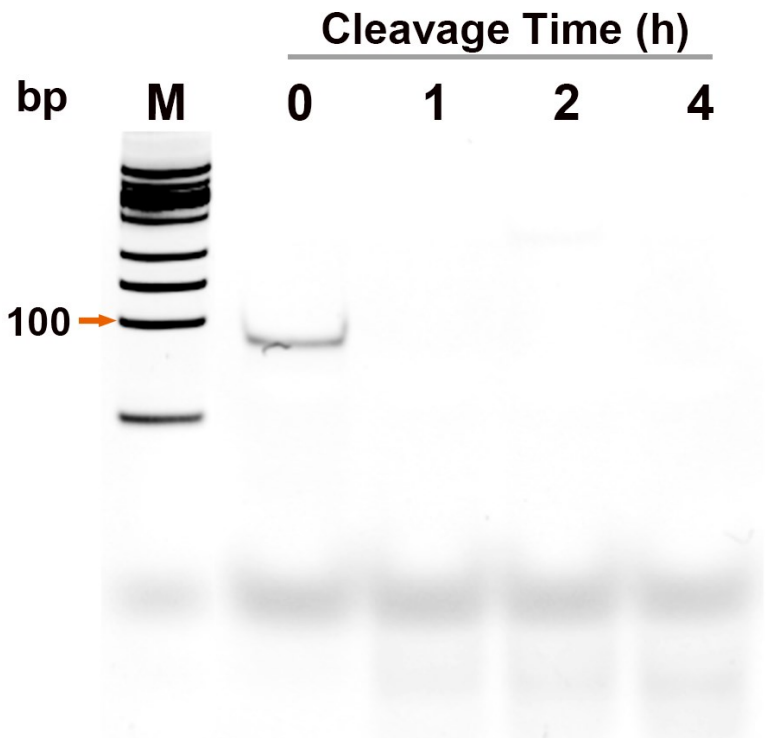

Figure S4. Characterization of DNase I stability of FAM-PPB by native PAGE analysis.

Experimental procedure:

The sample was prepared by adding 2 μL of DNase I (50 units/mL) into 18 μL of FAM-PPB and mixing fully. After incubation at 37°C for a given time period (0 h, 1 h, 2 h or 4 h), the resulting solution was immediately characterized by performing native-PAGE electrophoresis. The final concentration of FAM-PPB and DNase I were 0.25 μM and 5 units/mL, respectively.