Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Electronic Supplementary Information

Co-delivery of DNAzyme and chemotherapy drug using DNA tetrahedron for enhanced anticancer therapy through synergistic effects

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Strand	Sequences (5′-3′)
S1	GGGTTAGTGTTATGGTTAGGGAGGGTTAGGGTTATGGTTAG TGAGGGTTAGTGTTAGGGTTAGTG
S2	CCCTAACCCTAACCCTAACCCATGACGAAGACCTTCTCGGC CGAGAGCGCATGGTGGTTCGGCCA
S3	CGGCCGAGAAGGTCTTCGTCAACCCTAACCCTAACCCTAAC CCACACCCTCCGTCCCGGGCGCTC
S4	CCCTAACCCTAACCCTAACCCATGGCCGAACCACCATGCGC TCAGAGCGCCCGGGACGGAGGGTG
S2-tail	CCCTAACCCTAACCCTAACCCATGACGAAGACCTTCTCGGC CGAGAGCGCATGGTGGTTCGGCCATTTTTTTTTT
S3-tail	CGGCCGAGAAGGTCTTCGTCAACCCTAACCCTAACCCTAAC CCACACCCTCCGTCCCGGGCGCTCTTTTATTTTT
Aptamer	ACGCGCGCGCGCATAGCGCGCTGAGCTGAAGATCGTACCGT GAGCGCGTAAAAAAAAAA
DNAzyme	TCAACATCAGTTCCGAGCCGGTCGAAGATAAGCTAAAAATA AAAA
Cy5-S1	Cy5-GGGTTAGTGTTATGGTTAGGGAGGGTTAGGGTTATGGT TAGTGAGGGTTAGTGTTAGGGTTAGTG
miR-21 analogue	TAGCTTATC/rA//rG/ACTGATGTTGA

Table S1. Sequences of DNA nanostructures. The /rA/, /rG/ denote RNA bases.

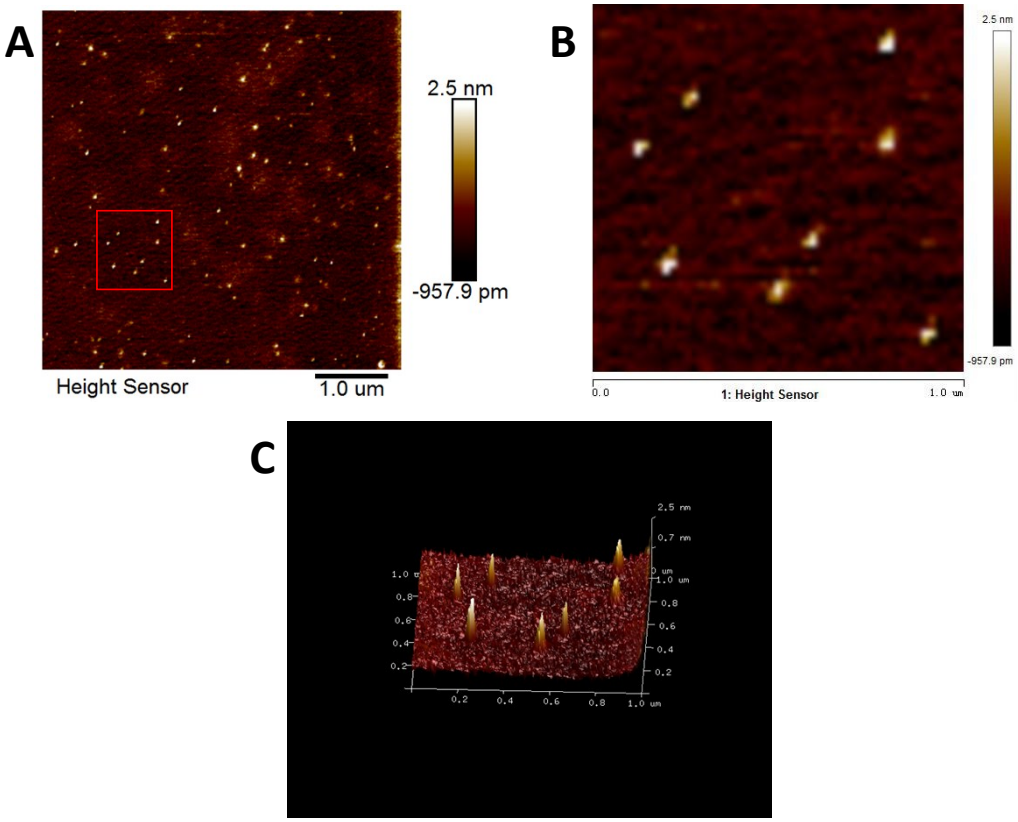


Figure S1. AFM images of Ap-Td-Dz. (A) 2D AFM image of Ap-Td-Dz (scale bar: 1 μm). (B) Enlargement of red frame in A with average size 17.3 nm. (C) 3D AFM image of Ap-Td-Dz with height about 2.3 nm.

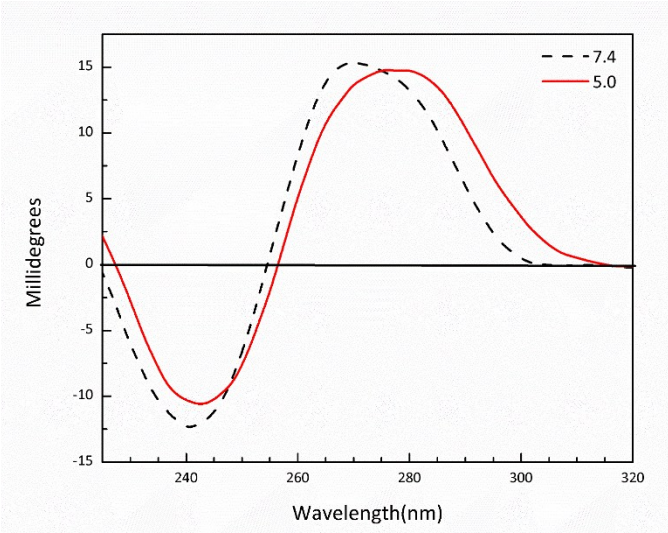


Figure S2. CD spectra of DNA tetrahedron at diverse pH. At pH 7.4, the Ap-Td-Dz has a positive peak approximately 265 nm and a negative peak approximately 240 nm. At pH 5.0, the formation of i-motif structure was revealed in a red-shift, with a positive peak around 280 nm and a negative peak around 250 nm.

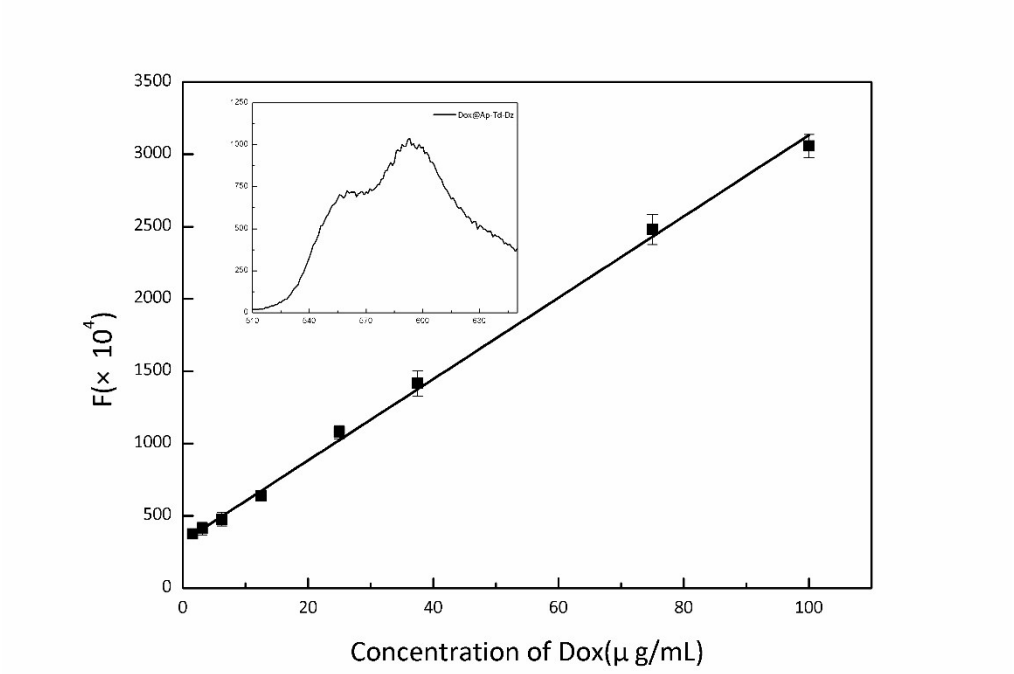


Figure S3. Standard curve of Dox derived from measuring the fluorescence of known concentrations of Dox. Fluorescence spectra of Dox@Ap-Td-Dz (initial concentration of Dox: 50 μg/mL) inside the figure.

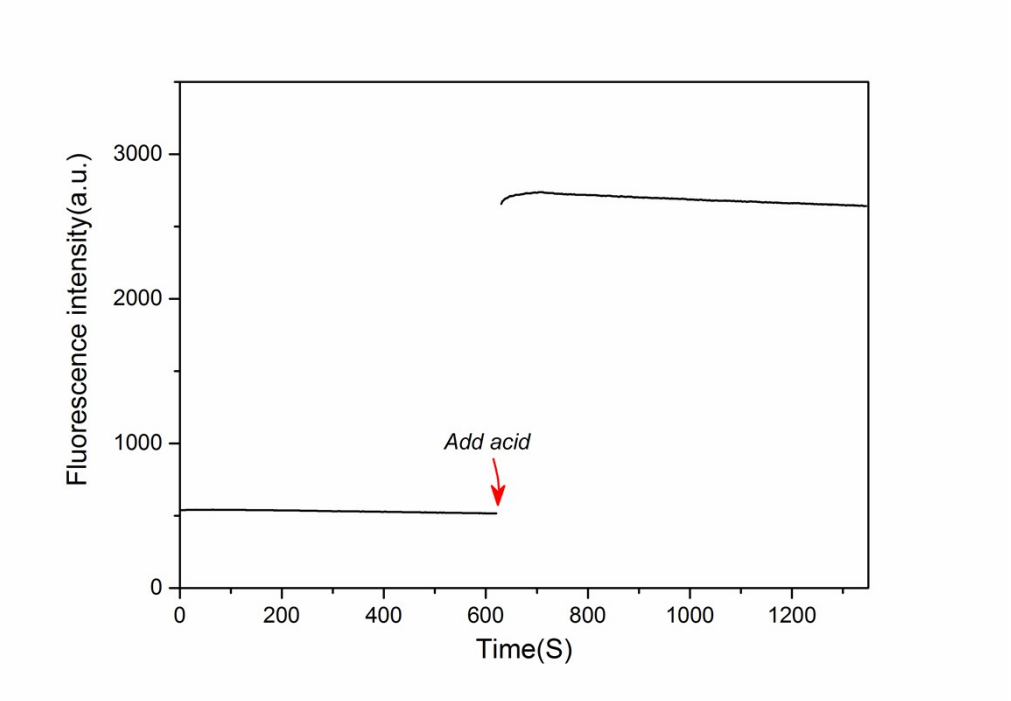


Figure S4. Drug release curve in acidic condition with time.

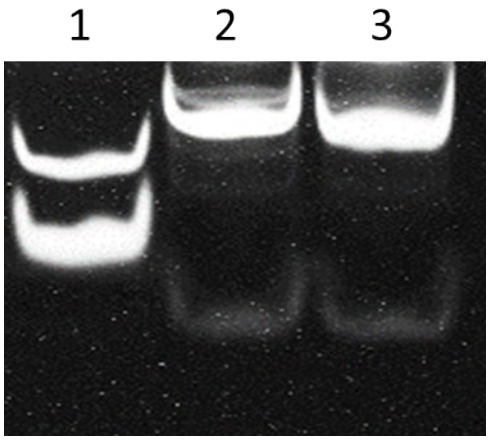


Figure S5. 15% PAGE analysis at 4 ℃. Lane 1: Td+miR-21 analogue; Lane 2: Ap-Td-Dz+miR-21 analogue; Lane 3: Td-Dz+miR-21 analogue. The results showed distinct cleavage products in lane 2 and 3 separately, while no cleavage was observed in lane 1.

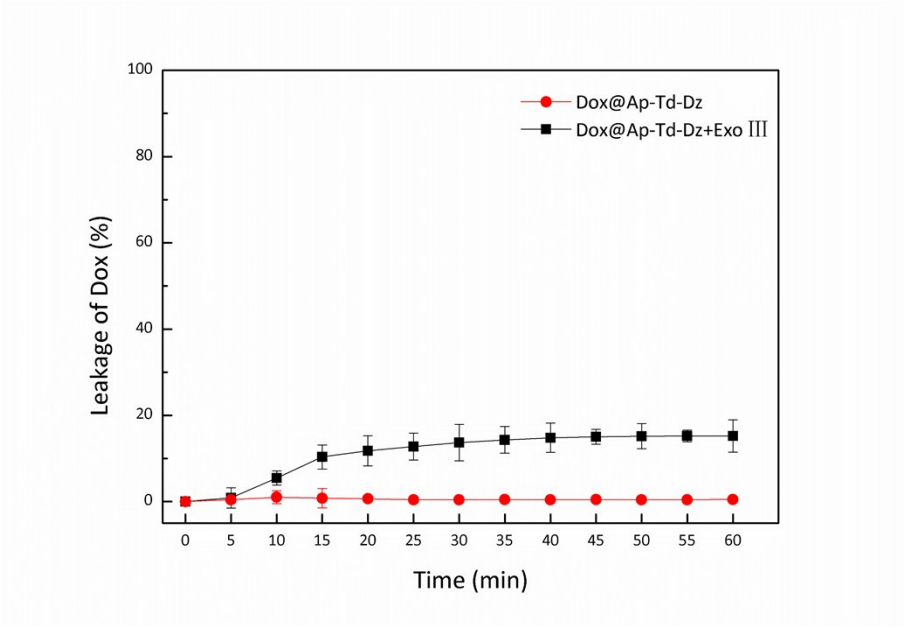
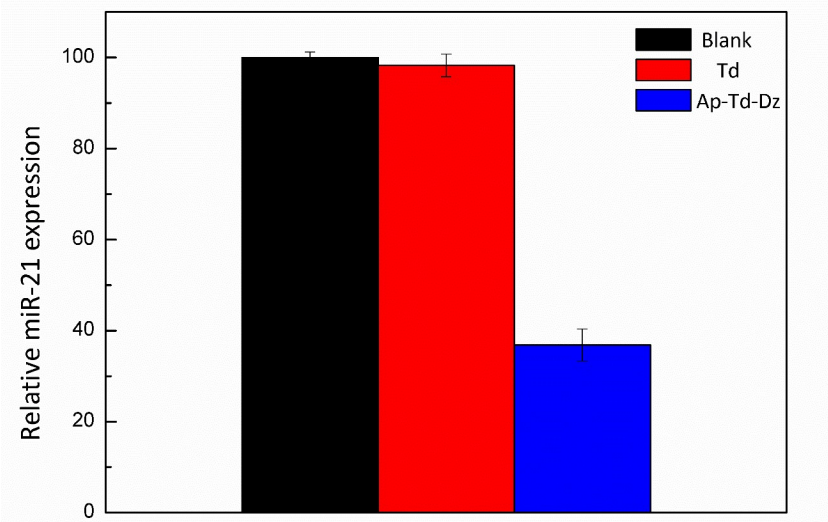


Figure S6. Study of nuclease digestion resistance. Investigation for nuclease (Exo Ⅲ) digestion resistance of DNA drug delivery system.

A



B

Gene / Sample	miRNA21	U6	ΔCt	ΔΔCt	$2^{-(\Delta\Delta Ct)}$
Blank	16.6893101	7.81009054	8.87921953	0	1
Td	15.9470034	7.04271173	8.90429163	0.0250721	0.98277148
Ap-Td-Dz	17.9881732	7.66830969	10.3198635	1.440644	0.36840292

Figure S7. Analysis of miR-21 in SMMC-7721 cells by qRT-PCR. (A) The histogram of the qRT-PCR analysis that represents the relative miR-21 level after treated with PBS, Td or Ap-Td-Dz. (B) Average Ct values in qRT-PCR assay of miR-21.The mRNA expression was evaluated by normalizing to the expression of U6 and using the $2^{-(\Delta\Delta Ct)}$ method.