Supporting Information

Building a Multifunctional Aptamer-Based DNA Nanoassembly for Targeted Cancer Therapy

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Inventory of Supplemental Information

Supporting information text

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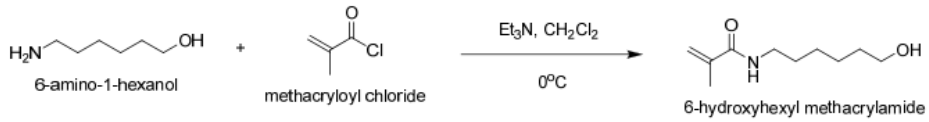
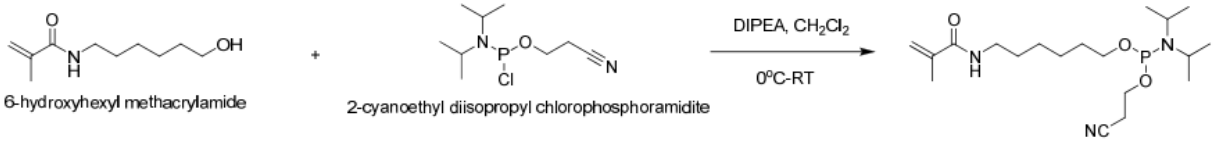
1. Table of DNA sequences

Name	Detailed sequence information
Connector a	ATG AAT AGC GGT CAG ATC CGT ACC TAC TCG
Connector b	CAT ACG TAC AGC ACC GCT ATT CAT CGG TCG TGG TGG GTT ATA AT
Connector c	GTT CGC AAT ACG GCT GTA CGT ATG GTC TCG CGT CTC TAC CTG AT
Connector d	CGA GTA GGT ACG GAT CTG CGT ATT GCG AAC GAA TCA GCT GTG AAC CAA G
Functional domain 1-a (w/ acrydite)	Acrydite-$(PEG)_6$-ATG GAT CCG CAT GAC ATT CGC CGT AAG ATT ATA ACC CAC CCG ACC G
Functional domain 1-b (w/ sgc8)	CTT ACG GCG AAT GAC CGA ATC AGC CTT TTT TTT TTT ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA
Functional domain 1-c	AGG CTG ATT CGG TTC ATG CGG ATC CA-Biotin
Functional domain 2-a	TGG ATC CGC ATG ACA TTC GCC GTA AGA TCA GGT AGA GAC GCG AGA C
Functional domain 2-b (w/ sgc8)	AGG CTG ATT CGG TTC ATG CGG ATC CAT TTT TTT TTT ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA
Functional domain 2-c	CTT ACG GCG AAT GAC CGA ATC AGC CT-Biotin
Functional domain 3-a (w/ acrydite)	Acrydite-$(PEG)_6$-TGG ATC CGC ATG ACA TTC GCC GTA AGC TTG GTT CAC AGC TGA TTC
Functional domain 3-b (w/ sgc8)	AGG CTG ATT CGG TTC ATG CGG ATC CA TTT TTT TTT T ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA
Functional domain 3-c	CTT ACG GCG AAT GAC CGA ATC AGC CT-Biotin
Functional domain 1-b (w/ KK1B10)	CTT ACG GCG AAT GAC CGA ATC AGC CT TTT TTT TTT T AC AGC AGA TCA GTC TAT CTT CTC CTG ATG GGT TCC TAT TTA TAG GTG AAG CTG T
Functional domain 2-b (w/ MDR AS)	AGG CTG ATT CGG TTC ATG CGG ATC CA TTT TTT TTT T *TTC* ***AAG ATC CAT CCC GAC CTC GCG***
Functional domain 2-b (w/ Random oligonucleotide)	AGG CTG ATT CGG TTC ATG CGG ATC CA TTT TTT TTT T ***CGC GAG GTC GGG ATG GAT CTT GAA***

Italic bold letters denote phosphorothioated bases.

2. Synthesis of acrydite phosphoramidite





The acrylic used in DNA sequences was synthesized by two steps. First, 6-amino-1-hexanol (9.32g, 0.08mol) and TEA (16.16g, 0.16mol) were mixed in 100mL dichloromethane, and the solution was cooled to 0 °C. Methacryloyl chloride (10g, 0.0957mol) was added slowly, and the reaction was stirred at 0 °C for 2 hours, after which 100mL of water were added to quench the reaction. The organic layer was washed with 5% HCl and dried. After evaporation of all solvent, the crude 6-hydroxyhexyl methacrylamide was used for the next step without further purification. To a solution containing 6-hydroxyhexyl methacrylamide (2 g, 10.8 mmol) in anhydrous CH_3CN (40 mL) at 0 °C, N, N' Diisopropylethylamine (DIPEA) (3.9 g, 30.0 mmol) was added in 15 minutes. Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (2.9 ml, 13 mmol) was added dropwise, and the reaction mixture was stirred at 0 °C for 5 h. After removing the solvent, the residue was dissolved in ethyl acetate, and the organic phase was washed with $NaHCO_3$ standard solution and NaCl solution and dried over anhydrous magnesium sulfate. The solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate/hexane/triethylamine 40:60:3) and dried to afford the title compound (3.33 g, 8.64 mmol, 80%) as a colorless oil. 1 H NMR (CDCl 3): δ 5.92 (br, 1H), 5.63 (m, 1H), 5.27 (m. 1H), 3.86-3.72 (m, 2H), 3.66-3.49 (m, 4H), 3.30-3.23 (m, 2H), 2.61 (t, 2H), 1.92 (m, 3H), 1.58-1.50 (m, 4H) 1.37-1.32 (m, 4H) 1.17-1.13 (m, 12H). 13C NMR (CDCl 3): δ 168.6, 140.4, 119.3, 118.0, 63.8, 63.6, 58.6, 58.3, 43.2, 43.1, 39.8, 31.3, 29.7, 26.8, 25.8, 24.9, 24.8, 24.7, 19.0. 31P (CDCl 3): δ 148.

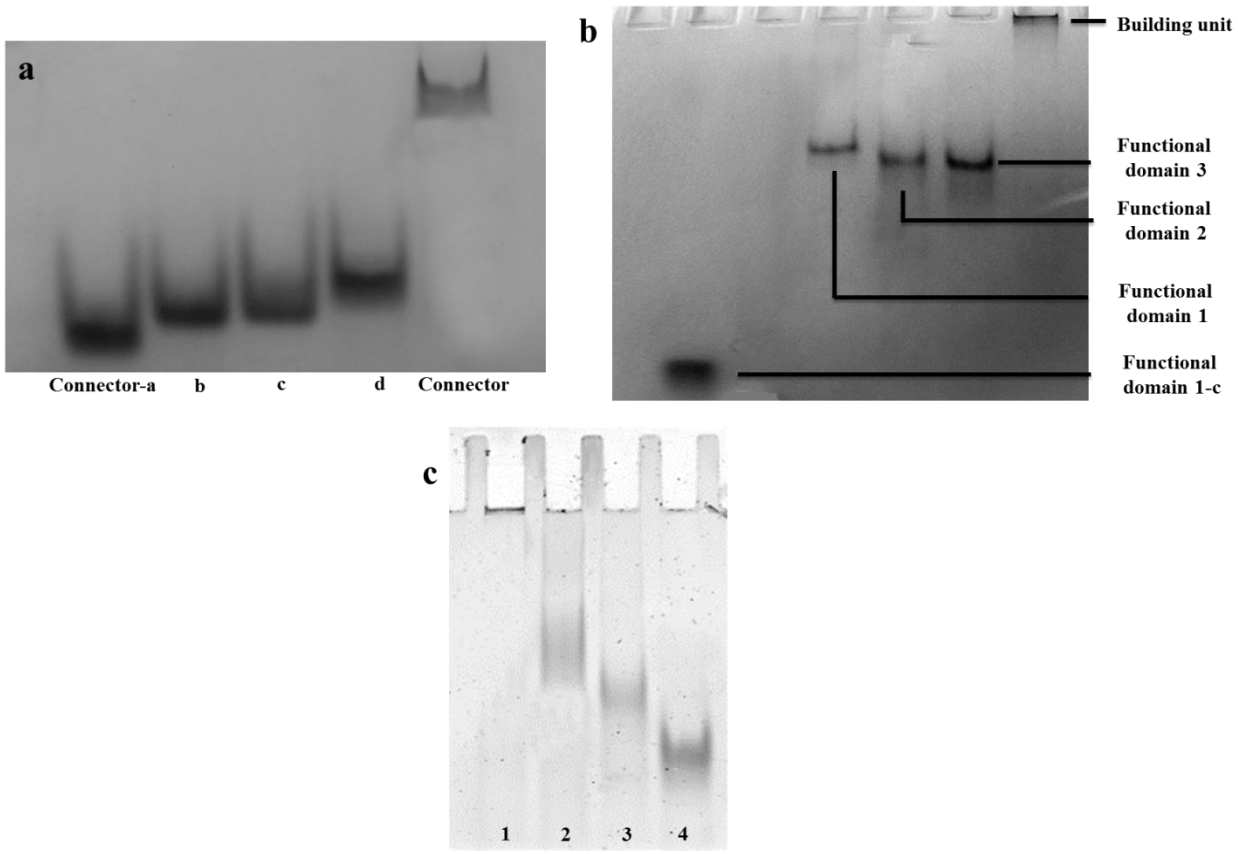


Figure S1. Gel electrophoresis image of formation of X-shaped connector (a) and nanoassembly building unit (b). (c) Precise self-assembly of nanoassembly structure in contolled ratio of functional domains. Lane 1: X-shaped connector + functional domain a + functional domain b + functional domain c; Lane 2: X-shaped connector + functional domain a + functional domain b; Lane 3: X-shaped connector + functional domain a; Lane 4: X-shaped connector.

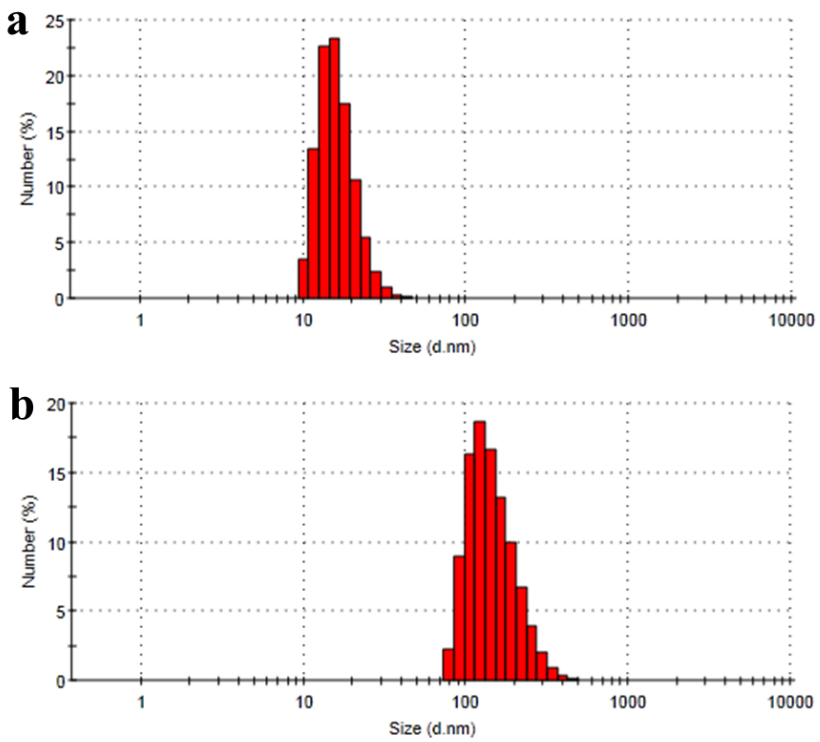


Figure S2. Size distribution of AptNAs obtained by dynamic light scattering measurement. (a) Diameter of AptNA building unit (17 nm). (b) Diameter of AptNAs (218 nm).

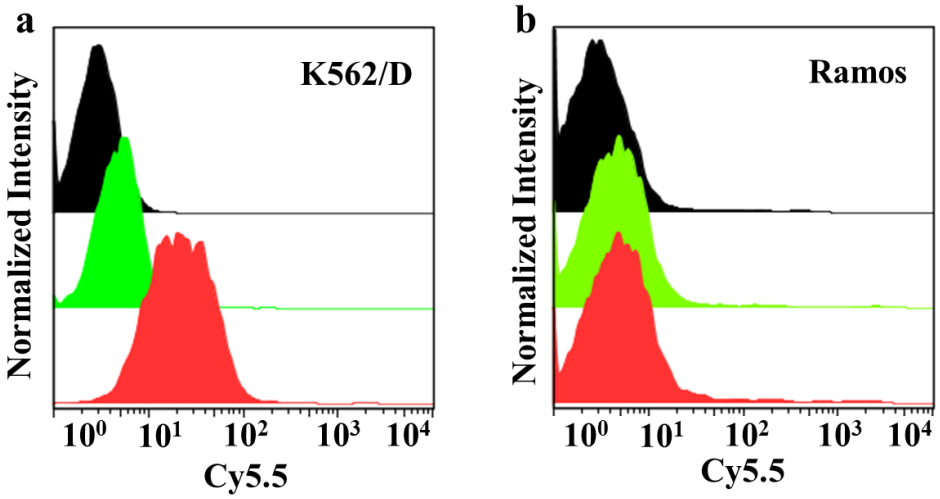


Figure S3. Flow cytometric analysis of specific binding of KK1B10-NAs to target drug-resistant K562 cells (a), but not to nontarget Ramos cells (b). Black peak: Cells only; Green peak: Library DNA-NAs; Red peak: KK1B10-NAs.

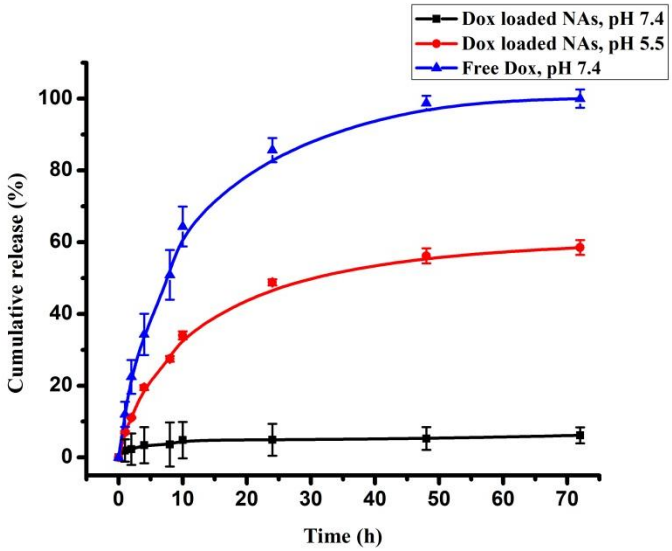


Figure S4. Cumulative release evaluation of Dox loaded in AptNAs through Dox diffusion assay in physiological and acidic environment (pH 7.4 and 5.5) relative to free Dox (pH 7.4). The maximum release was normalized according to the release amount of free Dox during the same time.

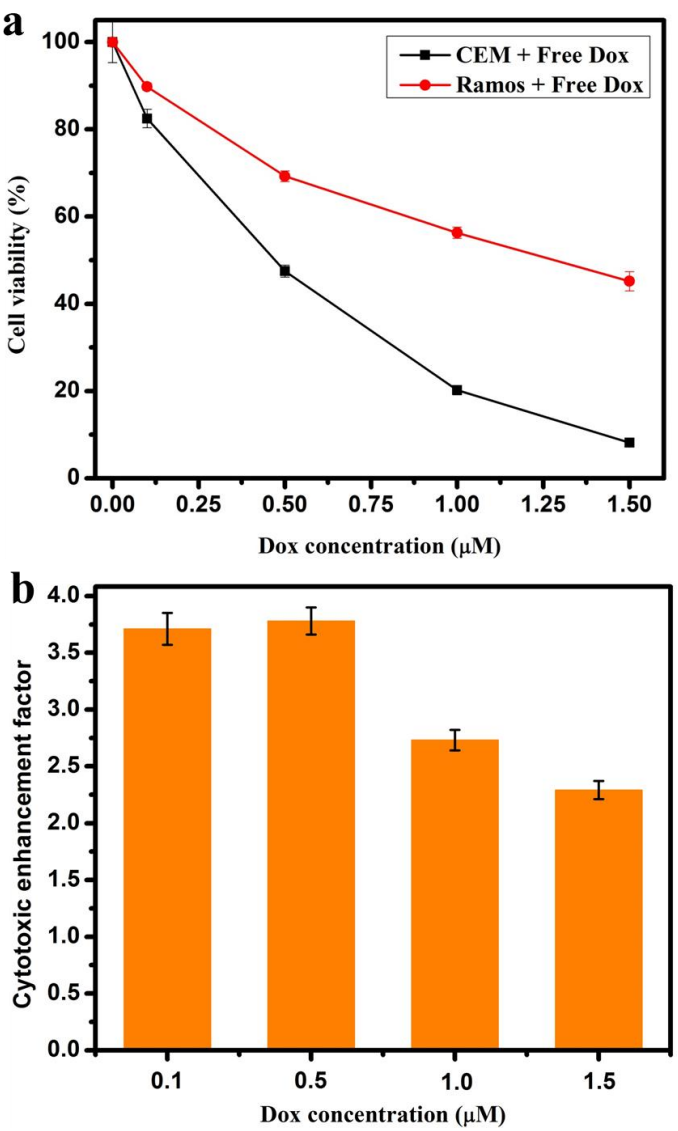


Figure S5. (a) Cytotoxicity assay of CEM and Ramos cells treated with different concentrations of free Dox (0.10, 0.50, 1.00, and 1.50 μM). (b) Cytotoxic enhancement factor of target CEM cells over nontarget Ramos cells treated with varied Dox concentrations loaded sgc8-NAs. The cytotoxicity of Dox loaded sgc8-NAs has been normalized to CEM and Ramos cells sensitivity treated with free Dox.

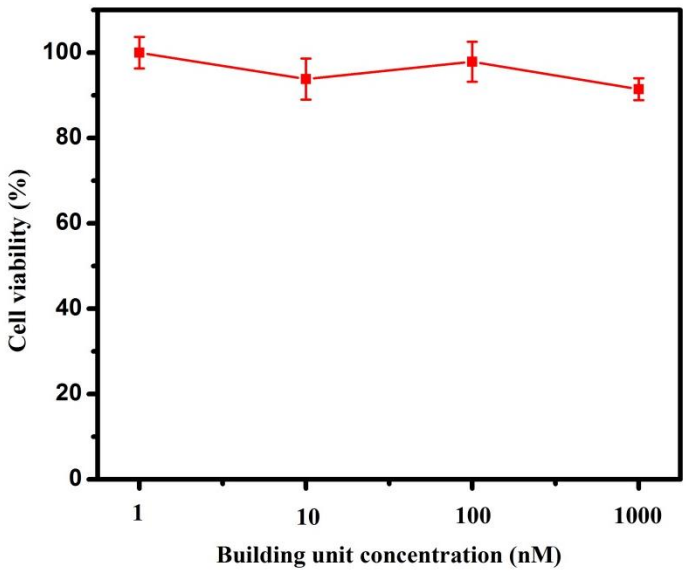


Figure S6. Cytotoxicity study of AptNAs. Error bars represent standard deviations from three replicates.

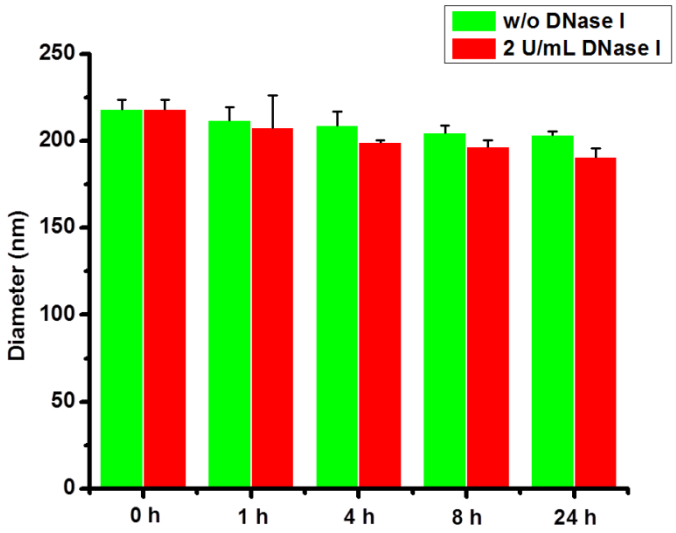


Figure S7. Stability of AptNAs tested after treatment with 2 units/mL DNase I for 1 h, 4 h, 8 h, and 24 h.

References:

[1] J. Lee, Y. Roh, S. Um, H. Funabashi, W. Cheng, J. Cha, P. Kiatwuthinon, D. Muller, D. Luo. *Nat. Nano*, **2009**, 4, 430-436.

[2] G. Zhu, J. Zheng, E. Song, M. Donovan, K. Zhang, C. Liu, W. Tan. *Proc. Natl. Acad. Sci. U. S. A.*, **2013**, 110, 7998-8003.