Electronic Supplementary Material (ESI) for Biomaterials Science
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Supporting Information for

Novel stem-loop RNA and drug-bearing DNA hybrid nanostructures specific to LNCaP prostate carcinoma

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Supporting Methods

Preparation of RNA and DNA precursors All RNA and DNA fragments were annealed using a PCR heating block. To prepare double-stranded DNA, two single-stranded DNAs that were 40 and 44 bases long, respectively, were mixed together at a 1:1 molar ratio. The DNAs were exposed to the following sequential heat program: 95°C for 4 minutes for denaturation, 70°C for 10 minutes for annealing, and 25°C for 60 minutes for cooling. To prepare RNA aptamers (A10 or Pan), RNA molecules were mixed with 5X annealing buffer solution. The final volume was 75 μl, and the final concentration of RNA aptamer was 40 μM. The RNA was placed in a 90°C heat block for 1 minute. The temperature was slowly decreased over about 4 hours to hybridize the RNAs.

Ligation of RNA and DNA precursors The prepared RNA and DNA molecules were mixed with nuclease-free water. Next, 10X diluted ligase buffer and T4 ligase were sequentially dropped into the mixtures. The solutions were kept at room temperature for over 4 hours or at the other experimental temperatures and times evaluated in the ligation optimization studies. To characterize the resultant hybrids, 3% agarose gels were used. The samples were electrophoresed at 100 V for 40 minutes. After electrophoresis, the gels were stained with ethidium bromide solution at a final concentration of 2 μg/ml for about 10 or 15 min. Bands

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in the gel were observed using a GelDoc-IT imaging system (UVP, CA). To evaluate the relative band intensity, Image J software, provided by the NIH, was used.

Supporting Results

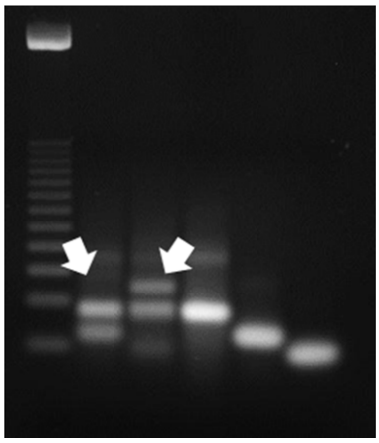

Fig. S1. Gel electrophoretic mobility shift assay (GEMSA) image of A10-ds40 hybrids and Pan-ds40 hybrid nanostructures. The A10-ds40 hybrid, Pan-ds40 hybrid, ds40, A10, and Pan, were loaded in lanes 2, 3, 4, 5, and 6 respectively. Although A10 and Pan have the same overhang sequence (GCTA), the RNA-DNA hybrid band intensities indicate that Pan forms RNA-DNA hybrid nanostructures more efficiently than A10.

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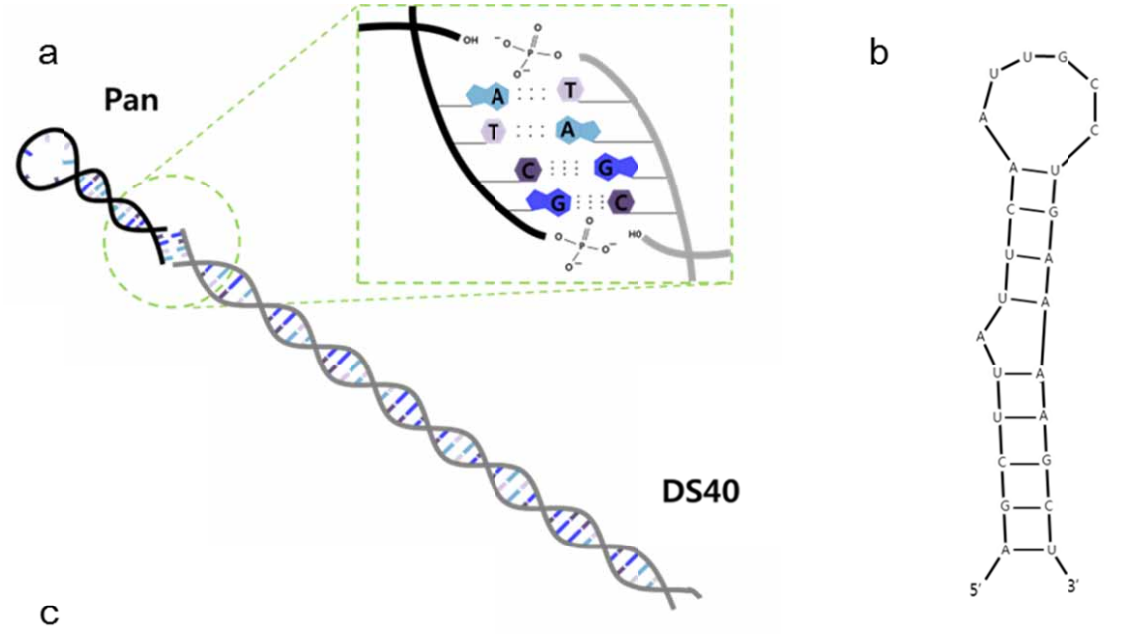

	M.W. (g/mole)	Length	Sequence	Loop size
Pan	9243.6	29 bases	5'- /Phosphate/TAG CAG CUU AUU CAA UUG CCU GAA AA G CU-3'	6 bases
ds40	26239.2	40 base pairs with 4 bases overhang	5'- /Thiol/GTC GCA GGC ACA TCA GTA CAG CCA GAT CCG TAC CTA CTC G -3' 5'-/Phosphate/GCT ACG AGT AGG TAC GGA TCT GGC TGT ACT GAT GTG CCT GCG AC -3'	

Fig. S2. Schematic of the two nucleic acid segments used for ligation. (a) Ligation of the Pan RNA aptamer with 40-bp DNA (ds40). (b) Structure of Pan. (c) Detailed information for Pan and ds40. Red colors in the sequence body represent specific overhang sequences for DNA-RNA hybridization.

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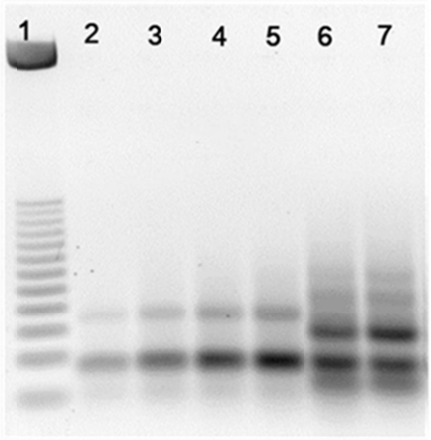
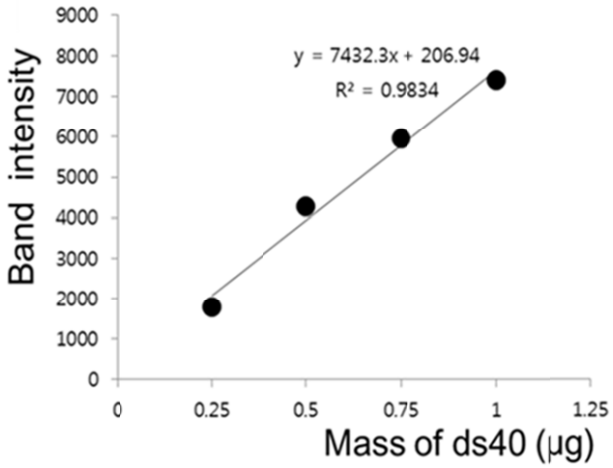
a



b



c

	Lane #2 (ds40 0.25 μg)	Lane #3 (ds40 0.5 μg)	Lane #4 (ds40 0.75 μg)	Lane #5 (ds40 1 μg)	Lane #6	Lane #7
Band intensity	1774.912	4268.861	5959.811	7404.811	5505.326	4687.326
Left-over ds40 (μg)					0.738	0.593
A10-ds40 hybrids yield (%)					26.12	40.68

Fig. S3. Quantification of A10-ds40 hybrid samples. (a) Gel electrophoresis analysis to quantify the yields of A10-ds40 hybrids. Different amounts of ds40 were loaded in lanes 2, 3, 4, and 5 at 0.25 μg, 0.5 μg, 0.75 μg, and 1 μg, respectively, as standards. A10-ds40 hybrid samples were loaded in lanes 6 and 7 and the band intensity was compared to that of 1 μg of ds40. (b) A standard curve of ds40 was obtained by calibrating the band intensities of various masses of ds40 from lanes 2 to lane 5. (c) A10-ds40 hybrids yield was quantitatively evaluated by interpolating the band intensity of unhybridized ds40 in each sample.

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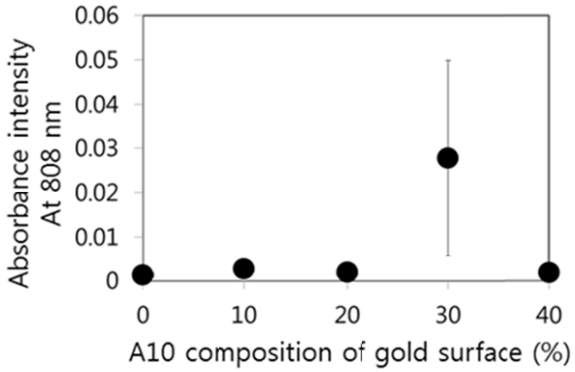

Fig. S4. Measurement for the presence of gold nanorods in the cell debris. The absorbance intensity at 808 nm of gold nanorods was evaluated. Sudden peak intensity, which indicates nanoparticles containing 30 % A10 compositions, would be correlated with relative reductions of its absorbance intensities in the plated cells.

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	A10 ratio	ds40 ratio	Time	Temperature	Total volume	RPM	Total mass	Sequence (GCAT/GCTA)	RNA Size
Lane 2	38.8	43.6	32.5	40.9	69.2	56.3	53.6	9.4	14.8
Lane 3	38.4	29.0	39.9	46.8	60.1	48.2	45.5	40.7	26.9
Lane 4	31.7	17.6	40.5	41.9	59.6				

Table S1. Ligation yield results obtained by band intensity analysis of RNA (either A10 or Pan)-DNA hybrid nanostructures. Molar ratios of 1:1, 1:2 or 1:4 of RNA:DNA, incubation temperatures of 4~8 ^{o}C, 16 ^{o}C, and 24 ^{o}C, the two different 5-prime overhang sequences of GCAT and GCTA, a total mass of nucleic acids of 3 μg or 6 μg, a RPM of 0 and 300, a total volume of 12 μl, 24 μl, or 48 μl, an incubation time of 12 hours, 24 hours, or 48 hours, as well as different sizes of RNA (A10 and Pan, at 12121.5 g/mole and 7527.8 g/mole, respectively) were evaluated.

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	Mixed molar ratio of [Ligation sample] / [ds40] (%)				
components	0	25	50	75	100
A10-ds40 hybrids ligation stock (Lane #7)	-	0.0845 nmol	0.169 nmol	0.254 nmol	0.338 nmol
Ds40	0.338 nmol	0.254 nmol	0.169 nmol	0.0845 nmol	-
Mixed molar ratio of [A10] / [ds40] (%)	0	10	20	30	40

Table S2. Preparation of [ligated A10-ds40] / [ds40] mixed feeding solution. Initial A10-ds40 hybrid ligation stock (Lane#7 of Fig. S3) contained 40.68% A10-ds40 hybrid. By mixing ds40 and ligation stock, various mixed solutions containing both A10-ds40 and ds40 were prepared. The final mixed molar ratios of [A10]/[ds40] were 0%, 10%, 20%, 30%, and 40%, respectively.