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Fabrication and Characterization of PNA-DNA Hybrid Nanostructures†

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Materials and Methods

Free solution annealing. High-Performance Liquid Chromatography (HPLC) purified synthetic oligonucleotides of DNA and PNA were purchased from BIONEER (Daejeon, Korea) and PANAGENE (Daejeon, Korea), respectively. Complexes were formed by mixing a stoichiometric quantity of each strand in a physiological 1× TAE/Mg^{2+} buffer [40 mM Tris base, 20 mM Acetic acid, 1 mM EDTA (pH 8.0), and 12.5 mM magnesium acetate] for each structure. We used the 1-step conventional free solution annealing method for DNA nanostructures and the 2-step annealing method for PNA-DNA hybrid DNA nanostructures. For the 2-step, the first step involved high-temperature annealing of equimolar mixtures of strands of DX-1 and DX-2 tiles of 400 nM tile concentration, each in two different AXYGEN test tubes. They were cooled slowly from 95°C to 25°C by placing AXYGEN tubes in a Styrofoam box containing 2 L of boiled water for at least 24 hours to facilitate hybridization. The second step involved low-temperature annealing, with the same volume of DX-1 and DX-2 tiles. Each tile concentration became 200 nM; they were cooled slowly from 40°C to 25°C by placing an AXYGEN tube in a Styrofoam box containing 2 L of boiled water (initial temperature, 40°C) for 24 hours to assemble PNA-DNA hybrid nanostructures. The second step of the annealing process prevented PNA aggregation.

Pretreatment of glass substrates. The 5 × 5 mm^2 glass substrate was cleansed with piranha solution (1:2 ratios of H_2O_2 and H_2SO_4) for 30 min., followed by rinsing with deionized (DI) water. The cleaned substrates were immersed into a Petri dish filled with DI water for a few hours before use.

PNA-DNA hybrid nanostructure growth on a substrate (mica or glass). Synthetic oligonucleotides, purified by high performance liquid chromatography (HPLC), were formed by mixing 1× TAE/Mg^{2+} buffer solution, containing an equimolar mixture of 6 for 5HR and 8 different strands for DX. For annealing, a substrate and DNA and PNA strands were inserted into an AXYGEN-tube with total sample volume of 250 µL at a proper concentration. The tube was then placed in a Styrofoam box with 2 L of boiled water and cooled slowly from 95°C to 25°C over a period of at least 24 hours to facilitate hybridization. During the annealing process, DX(5HR) strands formed PNA-DNA hybrid lattices(ribbons) on the substrate. Consequently, these PNA-DNA hybrid structures covered the mica surface completely when the monomer concentration was over 20 nM.

AFM imaging. For AFM imaging, a surface assisted grown DNA sample was placed on a metal puck using instant glue. Then, 30 µL of 1× TAE/Mg^{2+} buffer was pipetted onto the substrate and another 10 µL of 1× TAE/Mg^{2+} buffer was dispensed into the AFM tip (Veeco Inc. USA). AFM images were obtained by Multimode Nanoscope (Veeco Inc., USA) in the liquid tapping mode.

Raman measurement. Before measuring the Raman spectra, all glass assisted samples were rinsed with DI water. This was followed by fine blowing with nitrogen gas to remove residues from the surface. To avoid optical loss due to transmission through the transparent glass, the PNA-DNA samples were placed on a metal puck using instant glue. The measurements were performed at room temperature with a confocal Raman microscope (WITEC, alpha 300 R) at 532 nm. Spectral readings were obtained at 600 gr/mm diffraction grating and 500 nm blade height.

Fig. S1 Schematic diagram of unit 5HR DNA strands.

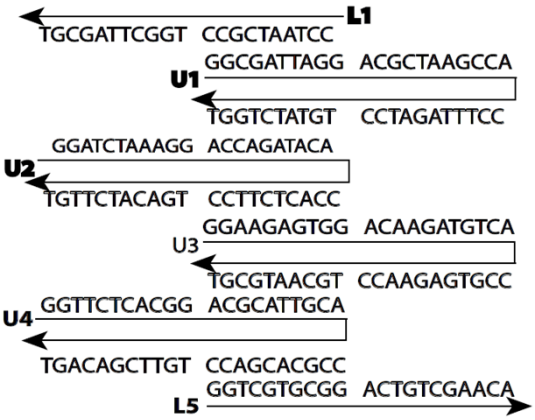

Fig. S2 Schematic diagram of unit 5HR (with L1P) PNA-DNA hybrid ribbon (PNA strands shown by red line)

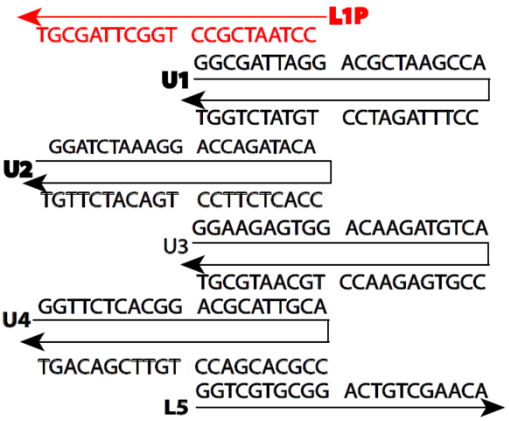

Fig. S3 Schematic diagram of unit DX tiles.

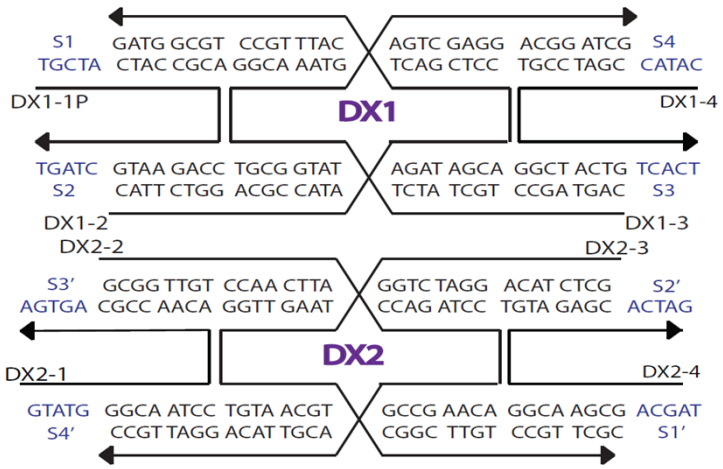

Fig. S4 Schematic diagram of unit DX (with DX1-1P) PNA-DNA hybrid and DNA tiles (PNA shown by red line).

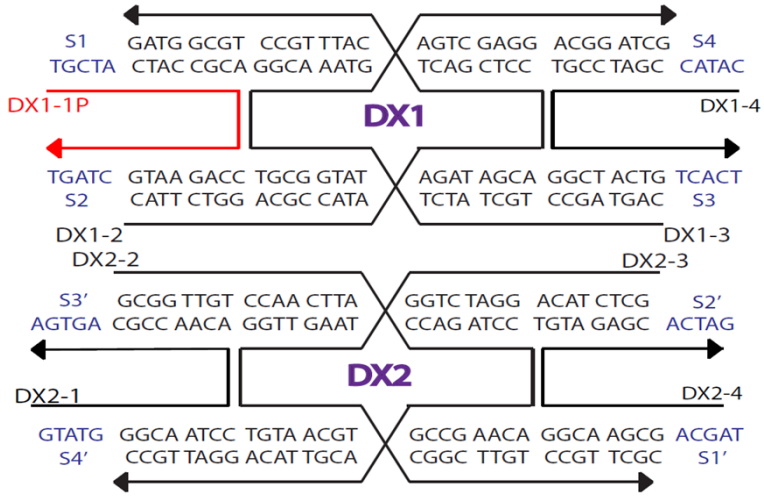

Fig. S5 Schematic diagram of unit DX (with DX1-1P and DX2-1P) PNA-DNA hybrid tiles (PNA shown by red lines).

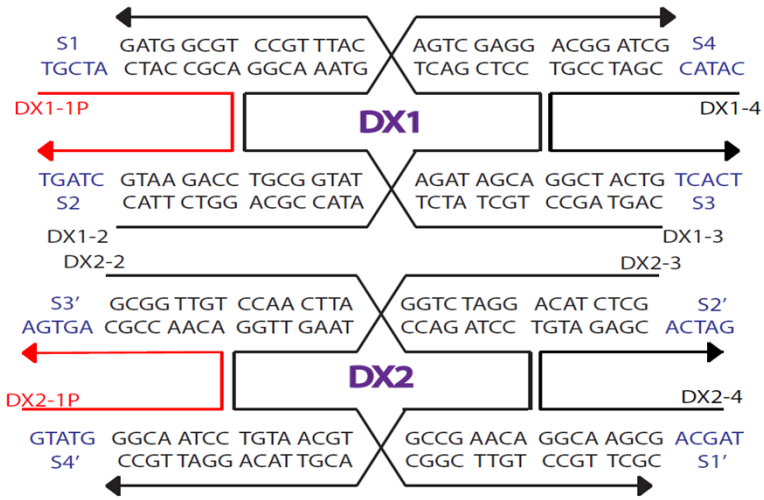

Fig. S6 Schematic diagram of unit DX (with DX1-1P and DX2-4P) PNA-DNA hybrid tiles (PNA shown by red lines).

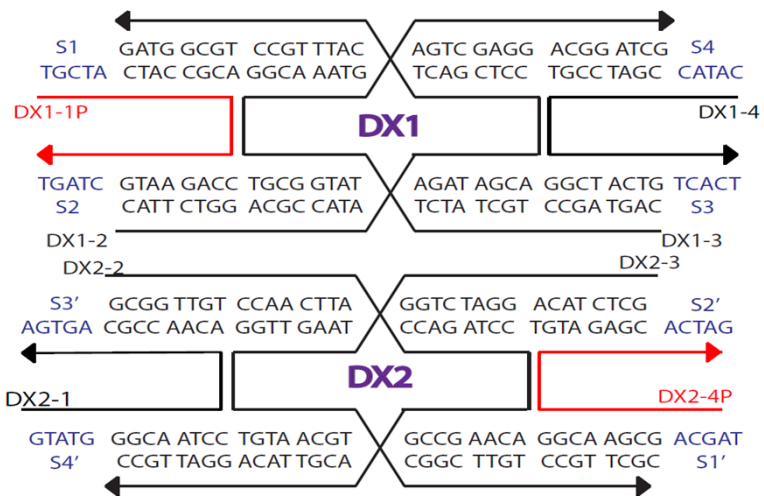

Fig. S7 Schematic diagram of unit DX (with DX1-1P, DX1-4P, DX2-1P and DX2-4P) PNA-DNA hybrid tiles (PNA shown by red lines).

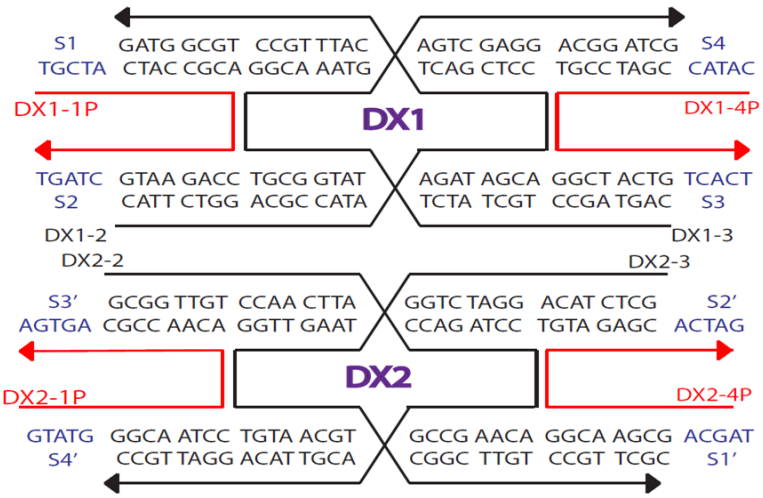

Table S1 Sequencing pool for 5HR ribbons. Sequences in red indicates PNA strands.

Strand	Base #	Sequence(5' to 3')
L1	21	CCTA ATCG CCTG GCTT AGCGT
L1P	21	CCTA ATCG CCTG GCTT AGCGT
U1	42	GGCG ATTA GGAC GCTA AGCC ACCT TTAG ATCC TGTA TCTG GT
U2	42	GGAT CTAA AGGA CCAG ATAC ACCA CTCT TCCT GACA TCTT GT
U3	42	GGAA GAGT GGAC AAGA TGTC ACCG TGAG AACC TGCA ATGC GT
U4	42	GGTT CTCA CGGA CGCA TTGC ACCG CACG ACCT GTTC GACA GT
L5	21	GGTC GTGC GGAC TGTC GAACA

Table S2 Sequencing pool for DX lattices. Sequences in red indicates PNA strands.

Strand	Base #	Sequence(5' to 3')
DX1-1	26	TGCTA CTACCGCA CCAGAATG CTAGT
DX1-1P	26	TGCTA CTACCGCA CCAGAATG CTAGT
DX1-2	48	CATTCTGG ACGCCATA AGATAGCA CCTCGACT CATTTGCC TGCGGTAG
DX1-3	48	CAGTAGCC TGCTATCT TATGGCGT GGCAAATG AGTCGAGG ACGGATCG
DX1-4	26	CATAC CGATCCGT GGCTACTG TCACT
DX1-4P	26	CATAC CGATCCGT GGCTACTG TCACT
DX2-1	26	GTATG GGCAATCC ACAACCGC AGTGA
DX2-1P	26	GTATG GGCAATCC ACAACCGC AGTGA
DX2-2	48	GCGGTTGT CCAACTTA CCAGATCC ACAAGCCG ACGTTACA GGATTGCC
DX2-3	48	GCTCTACA GGATCTGG TAAGTTGG TGTAACGT CGGCTTGT CCGTTCGC
DX2-4	26	TAGCA GCGAACGG TGTAGAGC ACTAG
DX2-4P	26	TAGCA GCGAACGG TGTAGAGC ACTAG

Fig. S8 Raman spectra of glass, mica, and DNA on mica.