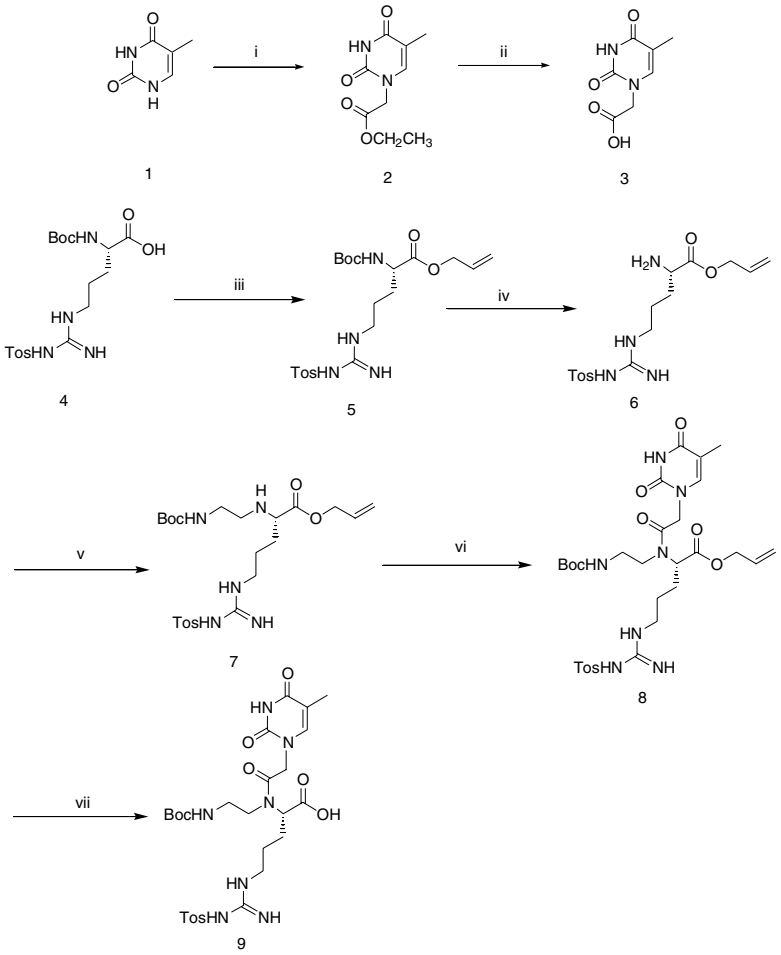
Novel Binding and Efficient Cellular Uptake of Guanidine-Based Peptide Nucleic Acids (G-PNA)

Supporting Information



Scheme S1. Synthesis of GPNA monomers *N*-(2-Boc-aminoethyl)-N-(thymin-yl-1-acetyl)- arginine. *Reaction conditions*: (i) $BrCH_2COOCH_2CH_3$, K_2CO_3/DMF, room temperature, 24 hours; (ii) NaOH/H_2O, room temperature, 2 hours; then adjusted pH to 4 by 4M HCl at 0^oC; (iii) Allyl bromide, Na_2CO_3/DMF, 35 ^oC, 24 hours; (iv) TFA/CH_2Cl_2, 0^oC, 30min; (v) *t*-Butoxycarbonyl aminoacetaldehyde/MeOH, 0^oC, 30min, then addition of $NaBH_3CN$, 16hour; (vi) Thymine-1-acetic acid **3,** DCC, DhbtOH/DMF, room temperature, 16hours; (vii) $Pd(PPh_3)_4$, morpholine/THF, room temperature, 30min.

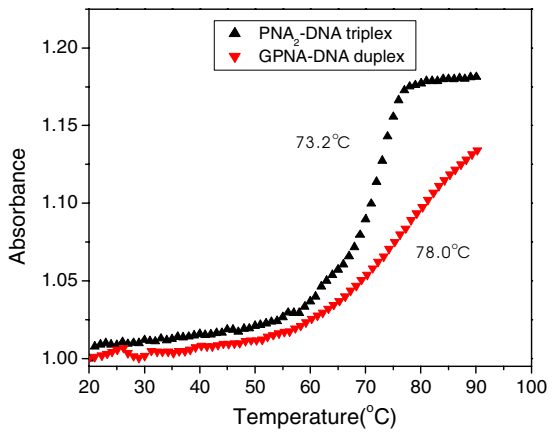
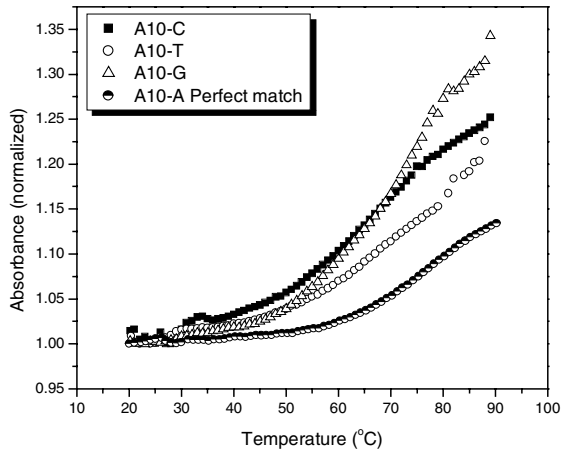


Figure S1. Melting curves of GPNA-DNA duplex and PNA_2-DNA triplex. The strand concentrations of DNA, PNA, and GPNA were 2μM, 4μM, and 2μM, respectively. Buffer contained 10mM NaPi (pH 7.0) and 20mM NaCl. The samples were first pre-annealed by incubating at 90˚C for 5min, followed by gradual-cooling to room temperature. UV-absorption was monitored at 260nm from 20 to 90˚C at the rate of 0.5˚C per minute.



X	T_m(˚C)	ΔT_m(˚C)	Description
A	78.0		Perfect match
C	67.9	10.1	T-C mismatch
G	73.5	4/5	T-G mismatch
T	68.3	9.7	T-T mismatch

Figure S2. UV-melting curves of GPNA $(T_g)_{10}$ and DNA (5'-AAAAAXAAAA-3') duplexes, where X corresponds to the four nucleobases indicated in the table on the right side. Experiments were performed under identical condition described in Fig. S2. Our data indicated that introduction of base-pairing mismatches lowered the T_m by ~10˚C for T-C and T-T, and ~5˚C for G-T.

Localization of GPNA

In order to demonstrate that GPNA translocated into the cell nucleus, we took DIC (Differential Interference Contrast) and fluorescent images of HCT116 cells after incubating with 0.1μM GPNA at 37˚C for 10min, followed by several washes with PBS buffer. Superimposition of DIC and fluorescent images shows that the fluorescent intensity predominantly strongest in the nucleus (Fig. S2). Cells incubated with negative controls (fluorescein alone and unmodified PNA covalently linked to fluorescein), however, did not significant fluorescent intensity. The residual staining observed with these reagents was found on the periphery of the cells, indicating binding to the cell surface.

In a separate experiment, DAPI, a fluorescent dye that localizes specifically to the nucleus, was co-incubated with GPNA. Overlay of the fluorescence images from DAPI and FITC channel showed a precise overlap of the fluorescent intensity—again indicating that GPNA localized specifically to the cell nucleus (Fig. S4).

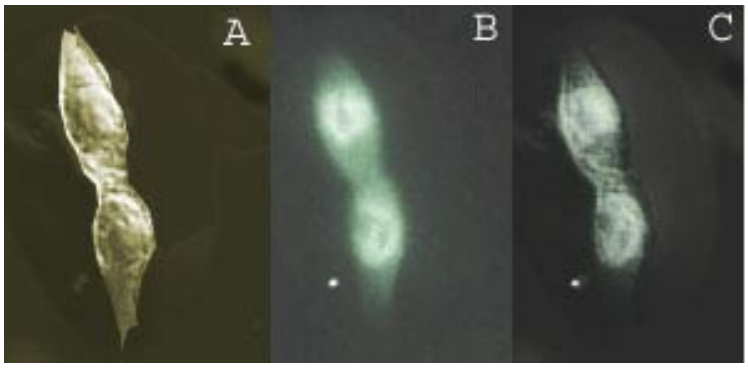


Figure S3. DIC and fluorescent images of HCT 116 cells incubated for 5 minutes at 37 ^{o}C with 0.10 μM of G-PNA FI-$(T_g)_{10}$, followed by a thorough wash with PBS. A: DIC image, B: fluorescent image, C: an overlapping image of A and B.

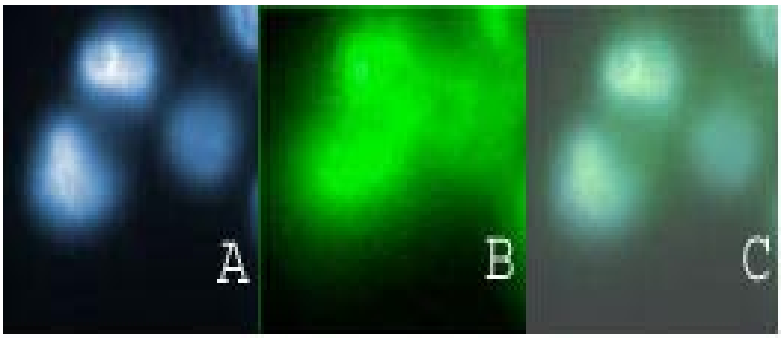
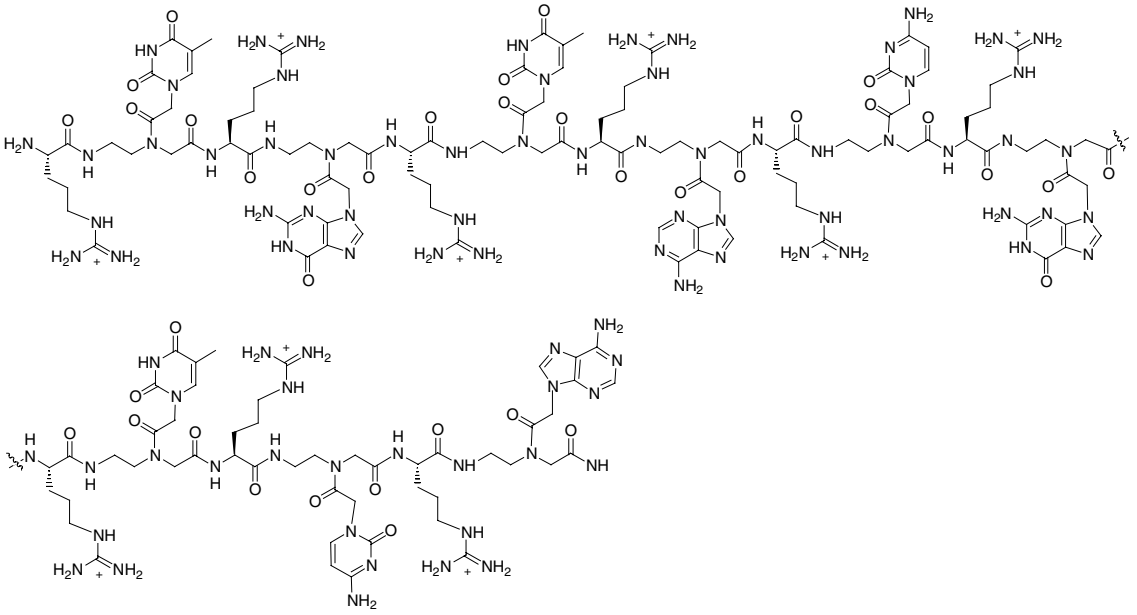


Figure S4. Fluorescent images of HCT 116 cells following: (i) incubation with 1 μM GPNA for 5min at 37°C, (ii) thoroughly washed with PBS, (iii) fixed with 4% paraformadehyde for 30min at room temperature, (iv) permeabilized with 1% Triton-X for 30min, (v) incubate with 1uM DAPI for 30min, (vi) thoroughly washed and mounted on the microscope slide. A: image taken with DAPI channel, B: image taken with FITC channel (emission from fluorescein covalently linked to GPNA), and C: an overlay between A and B.



Scheme S2. Structure of PNA containing alternating PNA units and arginine amino acids. NH_2-Arg-T-Arg-G-Arg-T-Arg-A-Arg-C-Arg-G-Arg-T-Arg-C-Arg-A