Supporting Information

Perfluoroalkylation as a New Tactic for Enhancing Cell Penetration of Peptide Nucleic Acids (PNA) via reducing the Nanoparticle size

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General procedure: All PNAs were synthesized on MBHA solid support using Boc-peptide synthesis protocol under microwave conditions (25 W, 75 °C, 5 min). PNA-conjugates were synthesized using commercially available Boc-PNA monomers, undecanoic acid and perfluoro undecanoic acid. All PNAs were cleaved from solid support using TFA/TFMSA in the presence of thioanisole and 1,2 ethane dithiol. The free PNA oligomers were purified by reverse phase high performance liquid chromatography (RP-HPLC) on a semi-preparative C18 column using acetonitrile/water linear gradient elution. For PNAs 1 & 2, 5% ACN: H₂O and 50% ACN: H₂O were used. Whereas, in case of PNAs 3 & 4, 5% ACN: H₂O and 95% ACN: H₂O were used. The integrity of PNA oligomers was confirmed by MALDI-TOF/TOF using DHB matrix. PNA oligomers were purified by reverse phase HPLC system using semi-preparative BEH130 C18 (10 X 250 mm) column.
HPLC of PNA 4-CF

[Graph showing chromatograms at 15.2, 11.9, and 13.1 minutes]
MALDI of PNA 1

Calcd: 3101.4214 [M+H]
Obsvd: 3101.6819 [M+H]
MALDI of PNA 2

Calcd: 3430.2504 [M+H]
Obsvd: 3430.3914 [M+H]

MALDI of PNA 3-CF

Calcd: 3460.4769 [M+2H]
Obsvd: 3460.2451 [M+2H]

MALDI of PNA 4-CF

Calcd: 3765.3084 [M+H]
Obsvd: 3765.8383 [M+H]
MALDI of PNA 5

Calcd: 3151.4469 [M + 2H]
Obsvd: 3151.3916 [M + 2H]

MALDI of PNA 6-CF

Calcd: 3456.2789 [M + H]
Obsvd: 3456.2004 [M + H]

MALDI of PNA 7-CF

Calcd: 3508.4868 [M + H]
Obsvd: 3508.3051 [M + H]
Sample preparation for confocal microscopy experiment

NIH 3T3 or HeLa cells were plated in 8-well chambered cover glass in 200 µL Dulbecco's Modified Eagle Medium (DMEM) containing 10 % Fetal Bovine Serum (FBS) at the concentration of 1.5 x 10^4 to 2 x 10^4 cells per well. The cells were grown by maintaining at 37 °C in a humidified atmosphere containing 5 % CO₂ for 12 h. The required amounts of 5(6)-carboxyfluorescein tagged PNAs were added to the corresponding wells to achieve the desired final concentration of 2 µM. The cells incubated with tagged PNA oligomers were maintained at 37 °C in a humidified atmosphere containing 5 % CO₂ for 24 h.

After 24 h incubation the medium was aspirated and the cells were washed thrice with ice-cold PBS. The cells were then replenished with 200 µL of DMEM medium containing Hoechst 33342 and ER-red of 1 µM final concentration of each, and incubated for 30 minutes at 37 °C. The excess nuclear stain Hoechst and ER-red were removed by washing twice with cold PBS. Then fresh OPTIMEM medium was added to the cells and the cells were immediately visualized using 60 X objective of Zeiss LSM 710 laser scanning confocal microscope. The confocal microscopy imaging has been repeated at least twice for each PNA.
Confocal images of PNA 3-CF in NIH 3T3 cells

Fig. S1 Confocal images of PNA 3-CF in NIH 3T3 cells: (A) Nuclear stain from Hoechst 33442 (B) Red fluorescence image from ER-red, (C) Green fluorescence image from PNA, and (D) Superimposed image of A-C.

Confocal images of PNA 4-CF in NIH 3T3 cells

Fig. S2 Confocal images of PNA 4-CF in NIH 3T3 cells: (A) Nuclear stain from Hoechst 33442 (B) Red fluorescence image from ER-red, (C) Green fluorescence image from PNA, and (D) Superimposed image of A-C.
Fig. S3 Confocal images of PNA 3-CF in HeLa cells: (A) Nuclear stain from Hoechst 33442 (B) Red fluorescence image from ER-red, (C) Green fluorescence image from PNA, and (D) Superimposed image of A-C.

Fig. S4 Confocal images of PNA 4-CF in HeLa cells: (A) Nuclear stain from Hoechst 33442 (B) Red fluorescence image from ER-red, (C) Green fluorescence image from PNA, and (D) Superimposed image of A-C.
**Fig. S4** Confocal images of PNA 7-CF in HeLa cells: (A) bright field image, (B) Nuclear stain from Hoechst 33442 (C) Green fluorescence image from PNA, and (D) Superimposed image of A-C.

**Fig. S4** Confocal images of PNA 8-CF in HeLa cells: (A) bright field image, (B) Nuclear stain from Hoechst 33442 (C) Green fluorescence image from PNA, and (D) Superimposed image of A-C.
**FACS sample preparation**

NIH 3T3 or HeLa cells were plated in 9 X 60 mm dishes in 2.0 mL DMEM medium at a concentration of 2 x 10^6 cells per dish. The cells were maintained at 37 °C in a humidified atmosphere containing 5 % CO₂ for 12 h. The required amounts of 5(6)-carboxyfluorescein tagged PNA stock solutions were added to the corresponding wells to achieve the final concentration of 1 µM. The cells incubated with tagged PNA oligomers were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 h.

After the incubation period was over, the medium was aspirated and cells were washed twice with ice-cold PBS. Cells were collected by trypsinization using 0.5 mL of 0.05 % trypsin-EDTA for each dish. After trypsinization 2 mL of DMEM medium was added and the cells were transferred to 15 mL falcon tubes. The tubes containing cells were centrifuged for 5 min at 4 °C, the supernatant was aspirated and the cells were washed twice with ice-cold PBS. To the cell suspension 1.0 mL of PBS containing 1.0 MM EDTA and 25 MM HEPES was added and washed for 5 min at 4 °C.

To the cell suspension 200 µL of 1% PFA was added and incubated on ice for 10 min for fixation of the cells. The cell suspension was again washed once with ice cold PBS for 5 min at 4 °C, to the cell suspension 1 mL of ice cold PBS was added and filtered through 70 µm cell strainers. The samples were transferred to FACS tubes and were analysed on BD Biosciences FACS Calibur flow cytometer. The data obtained from FACS was processed using Cell Quest Pro acquisition software. The cell permeation of PNA oligomers has been confirmed by histograms obtained from the experiment. The Percent positive cells for each individual PNA were plotted on Microcal origin 8.
Fig. S5 Overlay histogram of mean fluorescence: (A) & (B) HeLa cells; black) control cells without dye; green) PNA 3-CF/PNA 7-CF and red) PNA 4-CF/PNA 8-CF. (C) NIH 3T3 cells; black) control cells without dye; green) PNA 3-CF and red) PNA 4-CF.

(A) 3T3 cells; black) control cells without dye; green) PNA 3-CF and red) PNA 4-CF.

Fig. S6 Percent positive cells in 3T3 and HeLa cells.

Fig. S7 FESEM images of (A) PNA 1 and (B) PNA 2.

Fig. S8 FESEM images of (A) PNA 5 and (B) PNA 6.
Fig. S8 DLS of (A) PNA 1 and (B) PNA 2.

Fig. S9 $^{19}$F-NMR of PNA 2.

Fig. S10 $^{19}$F-NMR of 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecanoic acid.