Supporting Information for

Tumor-Targeted, Cytoplasmic Delivery of Large, Polar Molecules using a pH-Low Insertion Peptide

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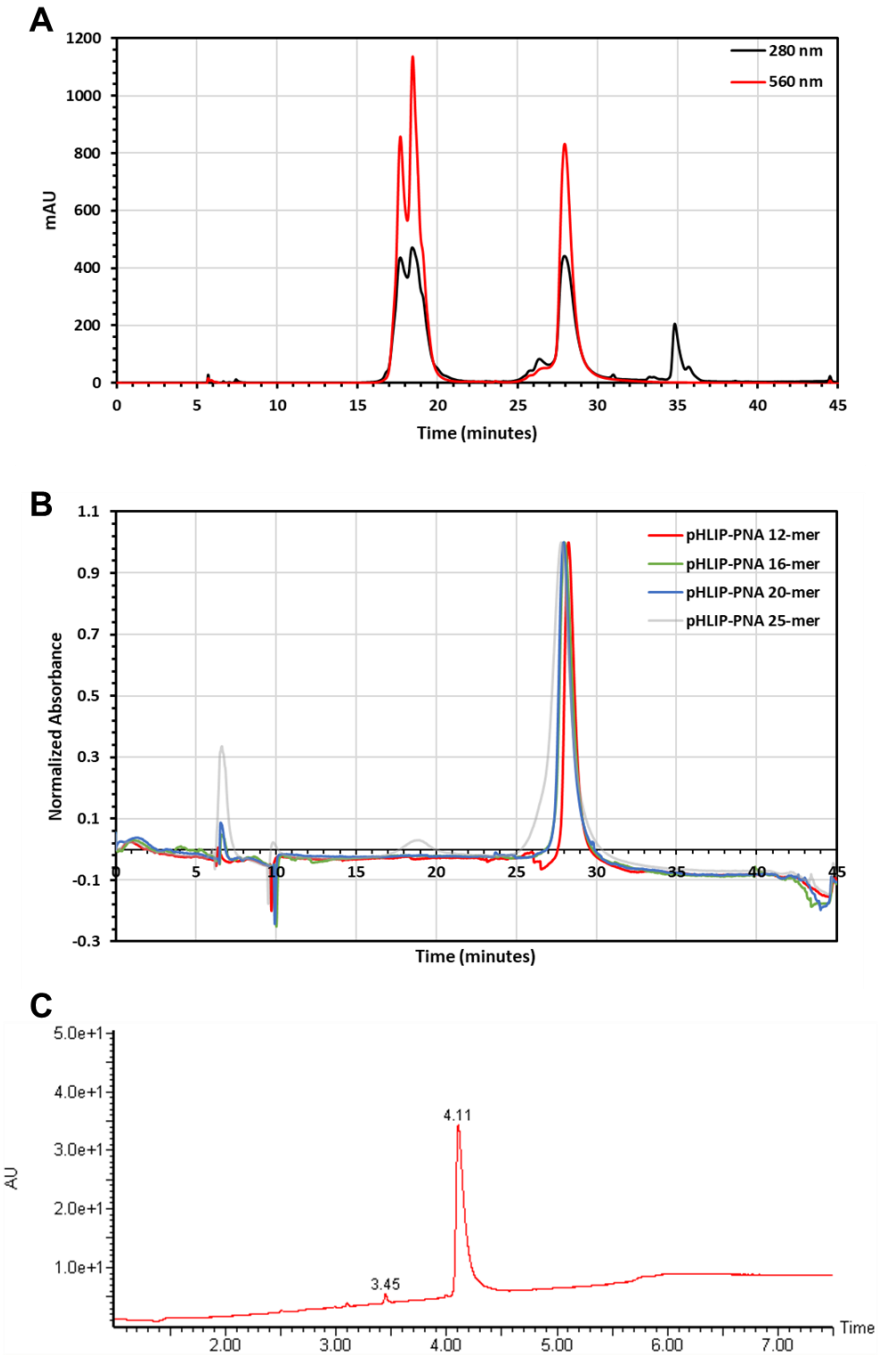

Figure S1: Chromatograms of pHLIP Conjugates. A) Representative HPLC chromatogram depicting the products of a typical pHLIP-PNA conjugation reaction. The split peaks at ~17.5 and ~18.5 minutes represent unreacted PNA and PNA dimer, respectively. The peak at ~28 minutes represents pHLIP-PNA. The peak at ~35 minutes represents unreacted pHLIP. B) HPLC chromatograms of purified pHLIP-PNAs. The peaks at ~6.5 minutes and negative peaks at ~10 minutes and ~44 minutes are artifacts. C) LC-MS chromatogram of purified pHLIP-TAMRA conjugate.

Table S1: Mass Spectrometry Results:

Construct	Description	Expected Mass	Observed Mass
pHLIP-$PNA_{12\text{-}mer}$	pHLIP-Cys-Cys-ooo-$PNA_{12\text{-}mer}$-ooo-TAMRA	$[M+H^+]$: 8313.67	8312.08
pHLIP-$PNA_{16\text{-}mer}$	pHLIP-Cys-Cys-ooo-$PNA_{16\text{-}mer}$-ooo-TAMRA	$[M+H^+]$: 9363.08	9362.18
pHLIP-$PNA_{20\text{-}mer}$	pHLIP-Cys-Cys-ooo-$PNA_{20\text{-}mer}$-ooo-TAMRA	$[M+H^+]$: 10486.51	10491.92
pHLIP-$PNA_{25\text{-}mer}$	pHLIP-Cys-Cys-ooo-$PNA_{25\text{-}mer}$-ooo-TAMRA	$[M+Na^+]$: 11899.04	11897.20
pHLIP-TAMRA	pHLIP-Cys-TAMRA	$[M+2H^+]$: 2346.13 $[M+3H^+]$: 1564.42	2346.11 1564.41

TAMRA = 5-Carboxytetramethylrhodamine
Cys = cysteine
ooo = $-NH-(CH_2CH_2O)_3CH_2CO-$
Cys-Cys denotes disulfide bond
Cys-TAMRA denotes thioether bond between cysteine thiol and maleimide on TAMRA

* MALDI-TOF mass spectrometry was used for pHLIP-PNA constructs. Liquid chromatography-mass spectrometry was used for the pHLIP-TAMRA construct.

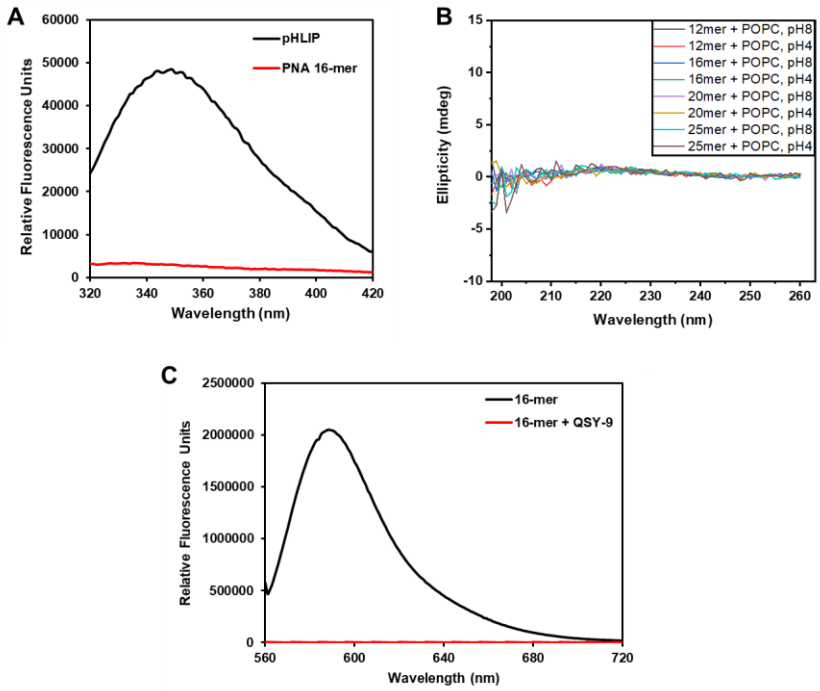

Figure S2: Control Fluorescence Emission and CD Spectra. A) Comparison of fluorescence within tryptophan emission range for 1 µM pHLIP alone vs. 1 µM $PNA_{16\text{-}mer}$ alone (no pHLIP) upon excitation at 280 nm. Notably, the PNA exhibits negligible fluorescence emission in this range compared to pHLIP. B) CD spectroscopy of PNAs alone when incubated with POPC vesicles at pH 8 or pH 4, respectively. Notably, no significant CD signal was observed. C) TAMRA fluorescence emission of 2 µM pHLIP-$PNA_{16\text{-}mer}$ before and after the addition of 8 µM QSY-9 quencher in the absence of POPC liposomes. Notably, the fluorescence signal was completely quenched.

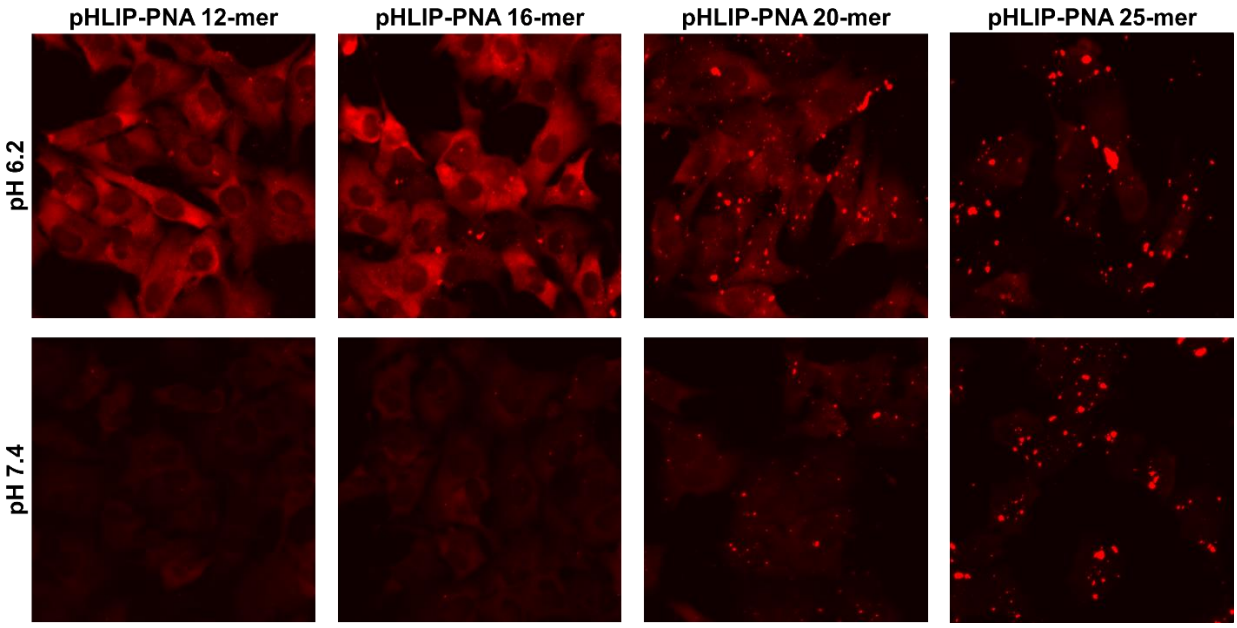

Figure S3: pHLIP Delivery of PNA Cargoes to Cultured Cells. A549 cells were incubated with 500 nM of each length pHLIP-PNA, respectively, at either pH 6.2 (top) or pH 7.4 (bottom) for 2 hours. Confocal microscopy images of the PNA TAMRA fluorescence signal are shown.