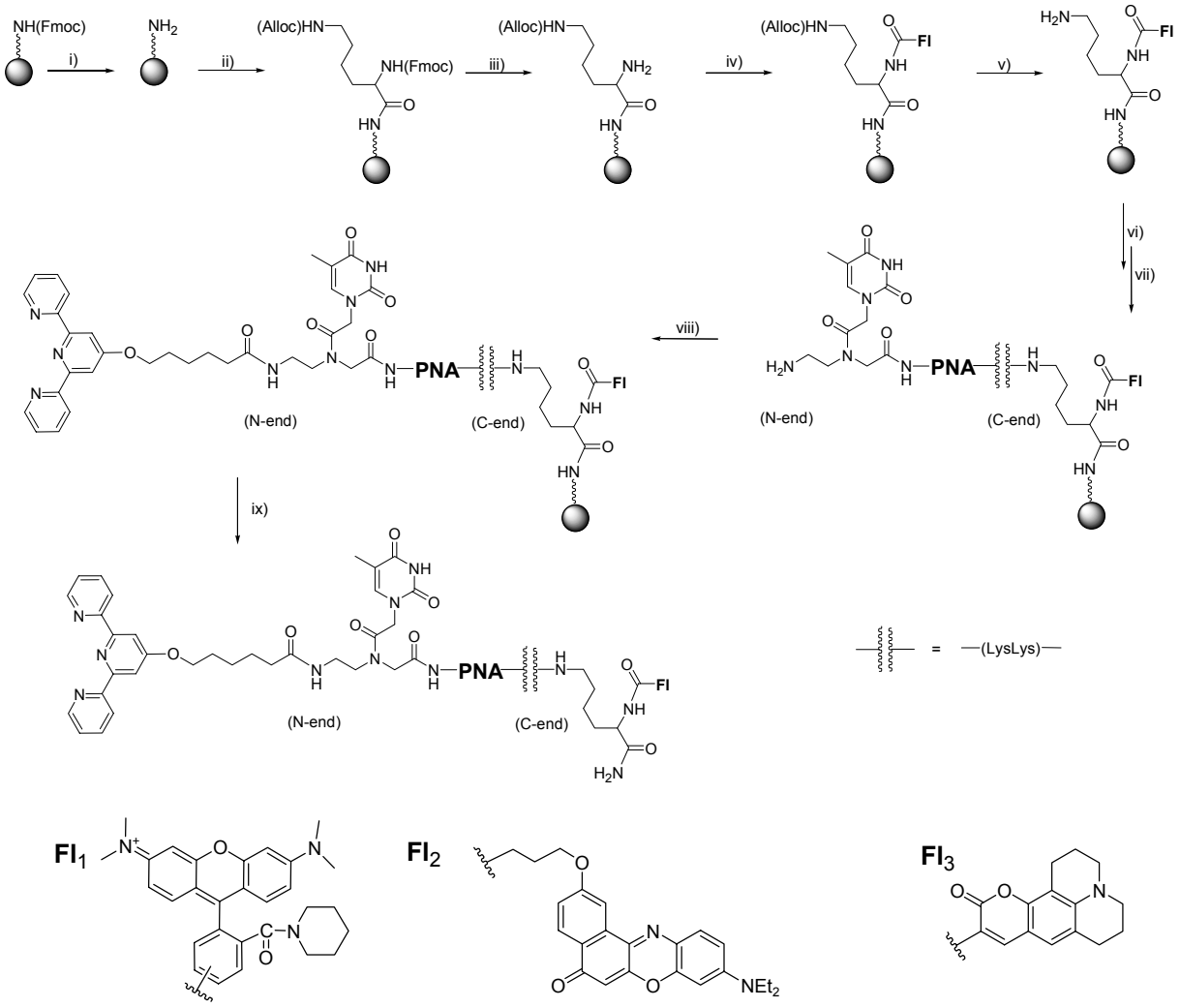
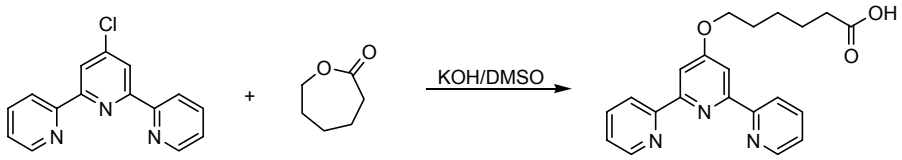
Supporting Information

PNA synthesis and characterization



Scheme 1s a: i) Piperidine, DMF ii) (Fmoc)-Lys(Alloc)OH, HOBT, HBTU, DIEA, DMF
iii) Piperidine, DMF iv) a) 5-(and-6)-carboxytetramethylrhodamine succinimidyl ester (TAMRA) (**Fl$_1$**), DIEA, DMF
b) Nile red butyric acid pentafluorophenyl ester (**Fl$_2$**), DIEA, DMF
c) Coumarin 343 (**Fl$_3$**), HOBT, HBTU, DIEA, DMF
v) $Pd(PPh_3)_4$, PPh_3, $[Et_2NH_2^+HCO_3^-]$, CH_2Cl_2
vi) Incorporation of two Lysines (FmocLys(Boc)OH) via automated synthesis
vii)PNA-synthesis
viii) 2,2′:6′,2′′-terpyridine carboxylic acid, HBTU, HOBT, DIEA, DMF
ix) TFA/*m*-Cresole



Scheme 1s b: 4′-Chloro-2,2′:6′,2′′-terpyridine; ε-caprolactone; KOH; DMSO
Ligand was synthesized according to Lit [P. R. Andres, R. Lunkwitz, G. R. Pabst, K. Boehm, D. Wouters, S. Schmatloch, U. S. Achuberth, *Eur. J. Org. Chem.* **2003**, 19, 3769-3776]

General remarks

Solid support bound PNA was synthesized on an Expedite 8909 PNA/DNA synthesizer according manufacturer´s recommendations for 2 μmolar synthesis using commercially available PNA building blocks (Fmoc-chemistry) and Fmoc-Lys(Boc)-OH amino acids.
1-Hydroxybenzotriazole (HOBT) and diisopropylethylamine (DIEA) were purchased from Acros Organics. O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU), 4′-chloro-2,2′:6′,2′′-terpyridine and Coumarin 343 were purchased from Sigma-Aldrich. 5-(and-6)-Carboxytetramethylrhodamine succinimidyl ester (TAMRA) was purchased from Molecular Probes. Nile red butyric acid pentafluorophenyl ester was kindly provided by S. Black.

MALDI-TOF analysis of PNA probes. A solution of 3,5-dimethoxy-4-hydroxycinnamic acid (27mM, TFA 0.1 %, CH_3CN 33%, MeOH 33% in water) was used as a matrix for MALDI-TOF analysis of the PNA conjugates. MALDI-TOF mass spectra were recorded on a Bruker BIFLEX III spectrometer. Mass accuracy with external calibration was 0.1% of the peak mass, i.e. +/- 3.0 at m/z 3000.

HPLC purification of PNA probes. HPLC was performed at 49°C on a Shimadzu liquid chromatograph equipped with a UV-VIS detector and column oven. Machery-Nagel Nucleosil C4 250 x 4.6 mm column with gradients of CH_3CN (0.1% TFA, solvent B) in water (0.1 % TFA, solvent A) was used (gradient A: 49°C, 0 % B for 5 min, in 30 min to 35 % B, in 10 min to 90 %B, 90 % B for 10 min; gradient B: 22°C, 0-2 % B for 5 min, in 23 min to 20% B in 7 min to 95 % B, 95 % B for 10 min).

PNA characterization.

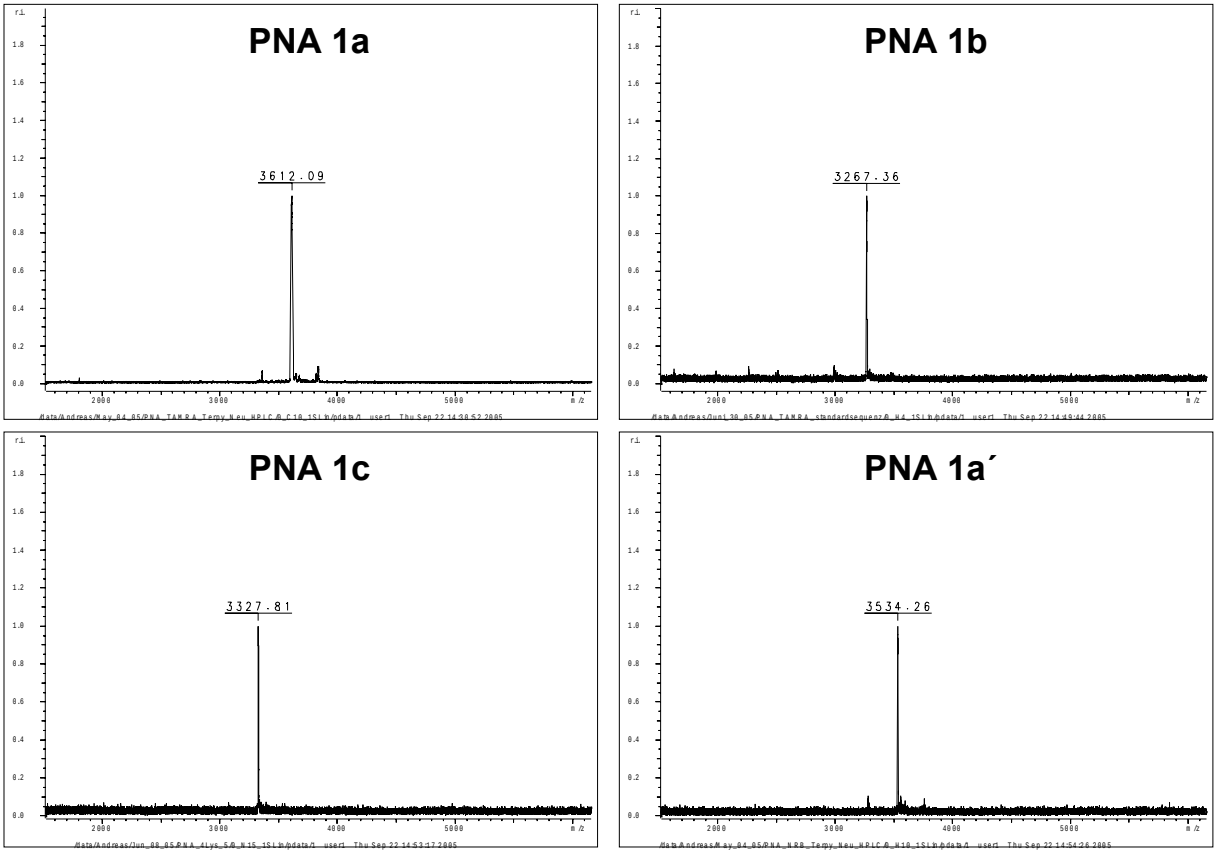
PNA1a (M_{calc}: 3613.2, M_{found}: 3612.1) **PNA1b** (M_{calc}: 3267.8, M_{found}: 3267.4)

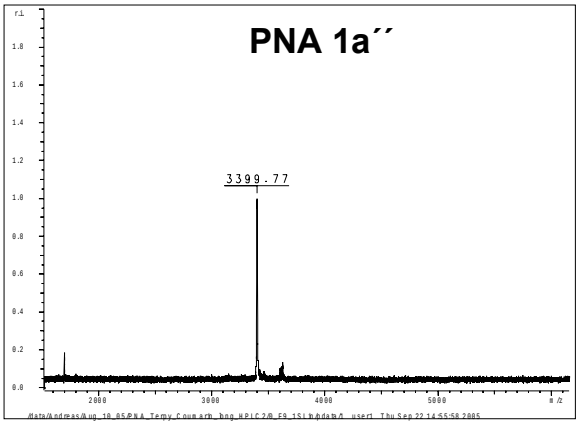
PNA1c (M_{calc}: 3328.8, M_{found}: 3327.8) **PNA1a´** (M_{calc}: 3335.1, M_{found}: 3534.3)

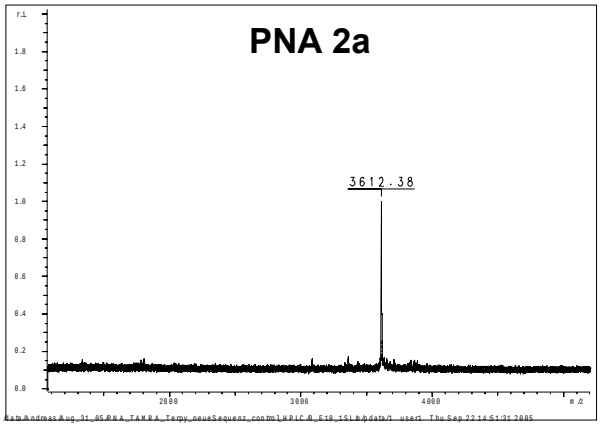
PNA1a´´ (M_{calc}: 3398.7, M_{found}: 3399.8)

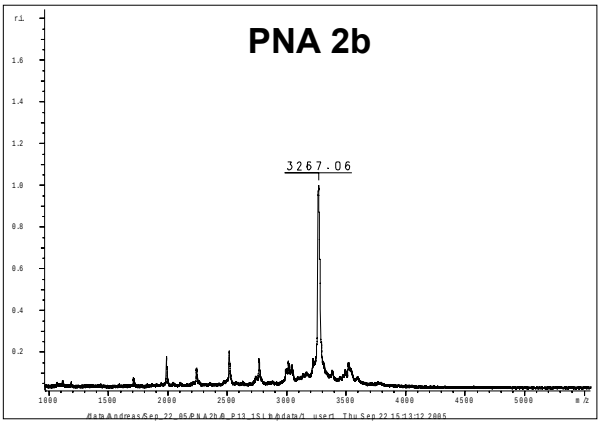
PNA2a (M_{calc}: 3613.2, M_{found}: 3612.4) **PNA2b** (M_{calc}: 3267.8, M_{found}: 3266.1)

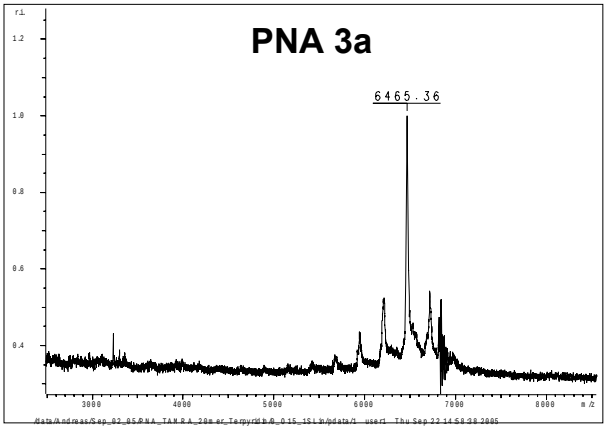
PNA3a (M_{calc}: 6468.7, M_{found}: 6465.4) **PNA3b** (M_{calc}: 6123.3, M_{found}: 6123.8)

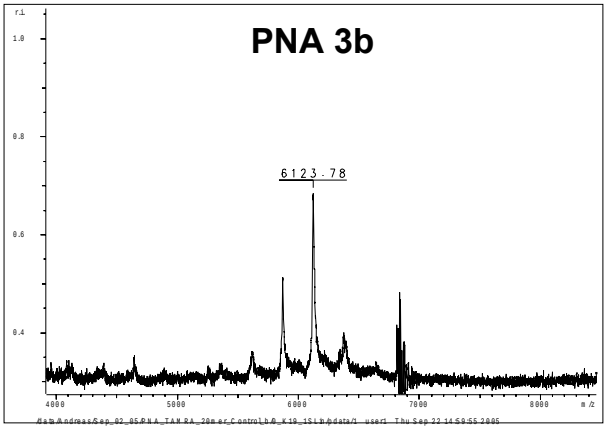



PNA 1a´´
3399.77

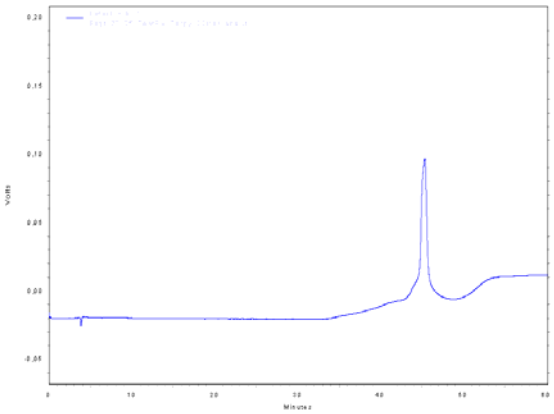

PNA 2a
3612.38


PNA 2b
3267.06

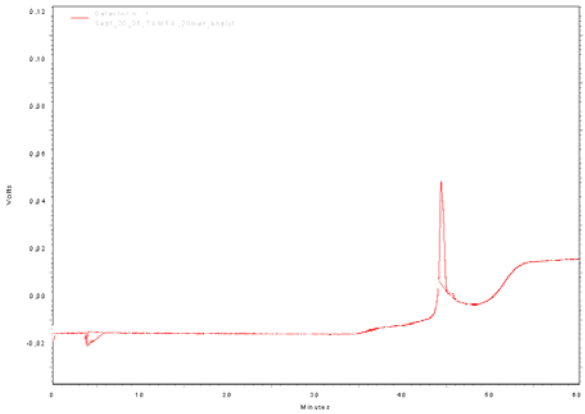

PNA 3a
6465.36


PNA 3b
6123.78

Analytical HPLC of PNA 3a and 3b



PNA 3a (R_t = 45.37 min)



PNA 3b (R_t = 44.55 min)

UV-melting experiments

UV-melting curves were measured between 20 and 80 °C for PNA:DNA duplexes in the absence and presence of Zn(II) using a Varian Carey 100 Bio UV-Vis spectrophotometer. Heating and cooling rates were 0.5 °C/ min. Melting points were averages of the extrema from the first derivative of 41 point smoothed melting curves calculated from at least 2 cooling and 2 heating experiments.

	absence of Zn^{2+}	1 eq Zn^{2+}	2eq Zn^{2+}
PNA 1a/DNA4	50.1°C	54.6°C	55.6°C
PNA 1b/DNA4	44.4°C	44.5°C	44.5°C

TableS1: Melting temperatures of **PNA1a/b** (2µM) with complementary **DNA 4** (2µM) in 10 mM MOPS buffer pH 7, 100 mM NaCl

Experimental details of flow cytometry

HeLA cells (CCl-2) were cultured according to vendor´s recommendations (LPC Promochem). For flow cytometry analysis, cells were plated in 6 well plates (Nunc) and grown to 80% confluency. Cells were washed twice with warm HEPES buffer (150 mM NaCl, 1.2 mM CaCl, 1.2 mM MgCl, 2.4 mM K_2HPO_4, 20 mM HEPES, pH 7.4) and incubated with PNA 1-3 (final concentration 2.5 µM, in HEPES/Glucose) for 1 h at 37°C. cells were washed subsequently twice with warm HEPES and suspended using trypsin/EDTA. Cells were resuspended in 1.5 ml HEPES buffer and subjected to cell sorting.
Flow cytometry was performed on a DAKOCytomation MoFlo high speed cell sorter equipped with a Coherent Innova 90C-6 Argon ion laser. The Laser was tuned to 488 nm at 100 mW. Forward (FSC) and side scatter (SSC) laser light was collected. The fluorescence signal was collected through a 630/40 bandpass filter. Data analysis was performed using Summit software using FSC and SSC to gate out debris. The resultant histograms were saved as a tab delimited file for export into Excel.

Dependence of PNA 1a uptake on Zn^{2+} concentration

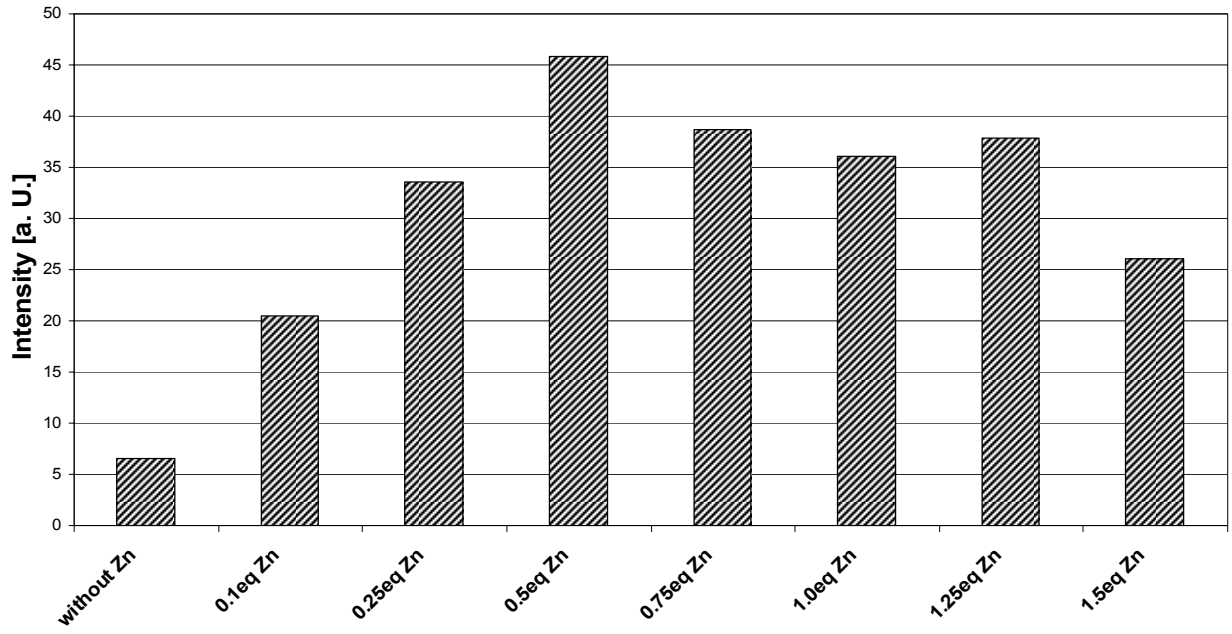


Figure 1s: Flow cytometry analysis of HeLa cells incubated with PNA 1a (2.5µM) for 1h with varying equivalents of Zn^{2+}, (Mean cellular fluorescence) corrected for autofluorescence

UV-titration of PNA 1a with Zn^{2+}

UV-vis curves were measured using a Varian Carey 100 Bio UV-Vis spectrophotometer.

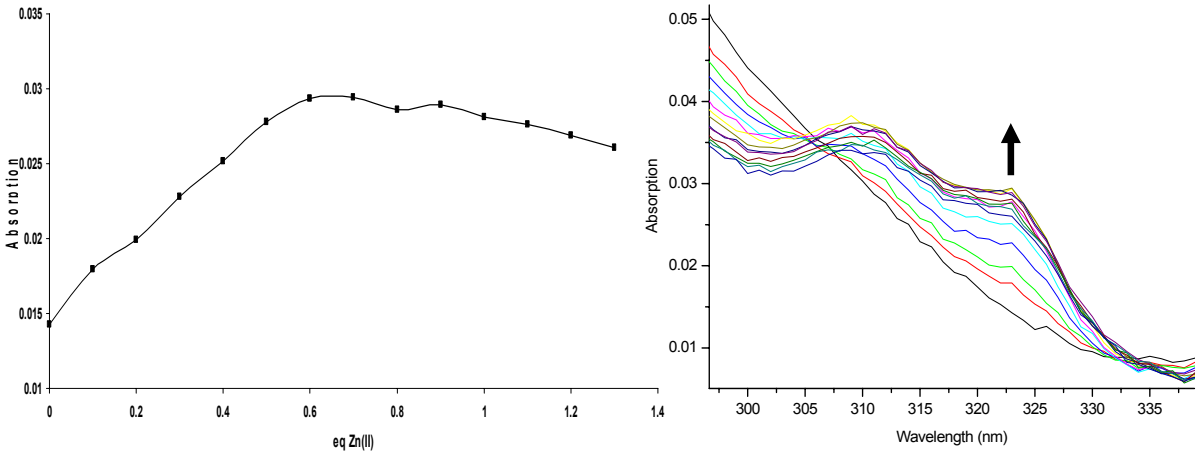


Figure 2s: Titration of PNA **1a** (2µM) with a solution of $ZnSO_4$. Right: Dependence of 323nm absorbance on 0-1.3 eq Zn(II). Left: Spectra on addition of 0-1.3 eq Zn(II), pH 7.0, 10 mM HEPES buffer, 100 mM NaCl, T=25°C.

Similar spectral changes were observed in a titration of 2,2´:6´,2´´-terpyridine (tpy) with $ZnSO_4$. Significant changes of absorbance from 0 to ca. 0.5 equiv. Zn(II) indicated formation of a complex $(\mathbf{1a})_2Zn$ (or $(tpy)_2Zn$ respectively). Upon addition of more Zn, formation of a 1:1 complex was expected, but the spectral changes were insignificant and this transition could not be followed by spectrophotometry.

It cannot be concluded from Figure 1s, whether species $(\mathbf{1a})_2Zn$ or $(\mathbf{1a})Zn$, is preferentially taken up by HeLa cells.

Confocal microscopy of HeLa cells incubated with PNA 1d, e

For confocal microscopy cells were plated in 8 well LabTek chambers and 35 mm MatTek chambers and grown to 80% confluency. Cells were washed twice with warm HEPES buffer and incubated with PNA **1-3** (final concentration 2 µM, in HEPES/Glucose) for 1 h at 37°C. Then cells were washed twice with warm HEPES, and imaged in HEPES /Glucose. Confocal microscopy was performed on a Zeiss LSM 510 Meta confocal microscope. Fluorescence detection channels were set to Ch1: (Ex: 532 nm) LP 570 nm, Ch2: (Ex 488 nm) LP 500 nm. Imaging was performed using a 10x air objective and a 63x oil objective. Image processing was performed using Zeiss LSM 5 image browser.

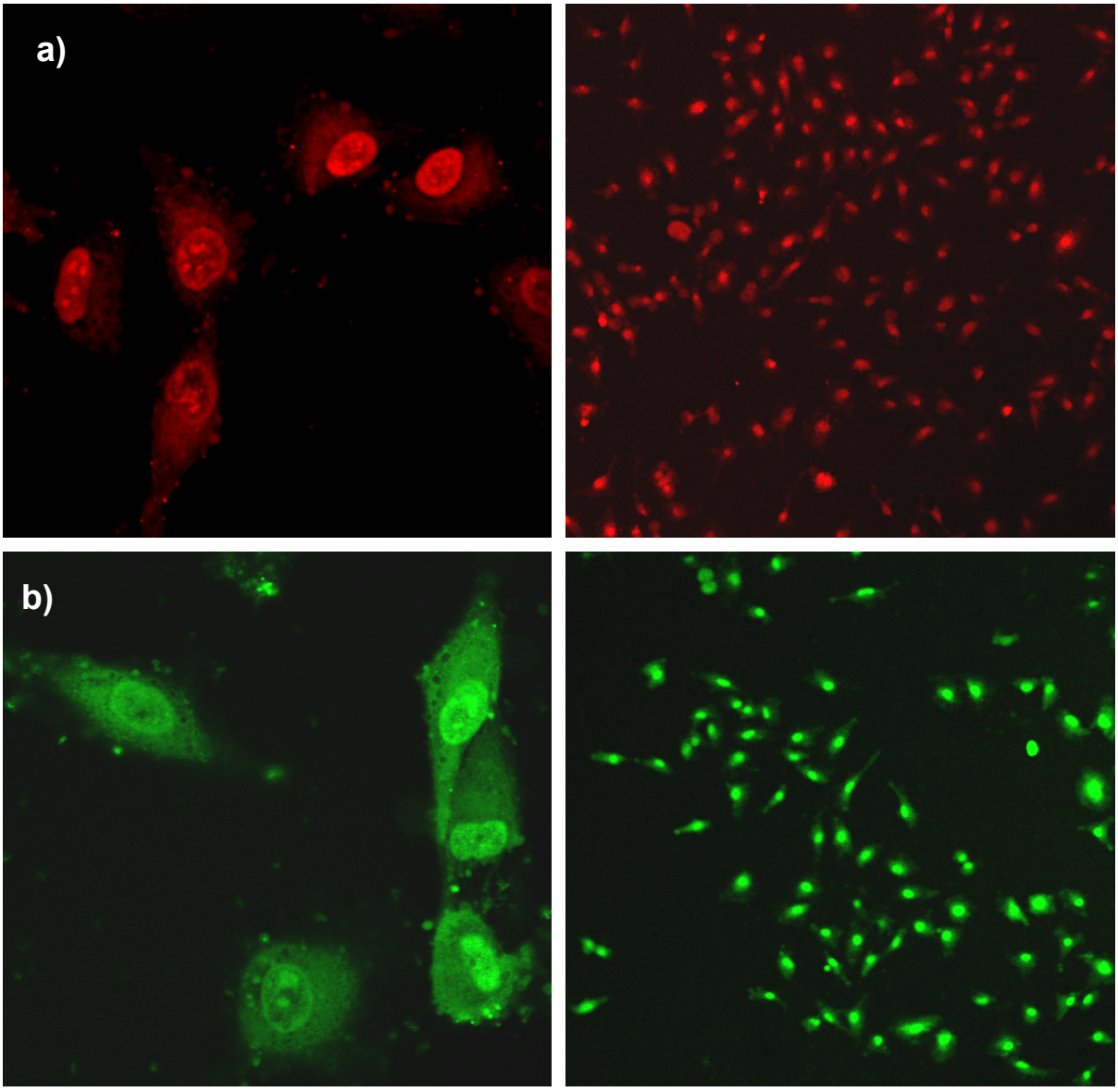


Figure 3s : Confocal image of living Hela cells directly after incubation of 2 µM PNA for one hour. a) PNA **1a'** + 1 eq. Zn^{2+} b) PNA **1a''** + 1 eq. Zn^{2+}, left panels show pictures taken with 63x, right panels with 10x objectives.