Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Supplementary information

## PNA-NLS conjugates as single-molecular activators of target sites in double-stranded DNA for site-selective scission

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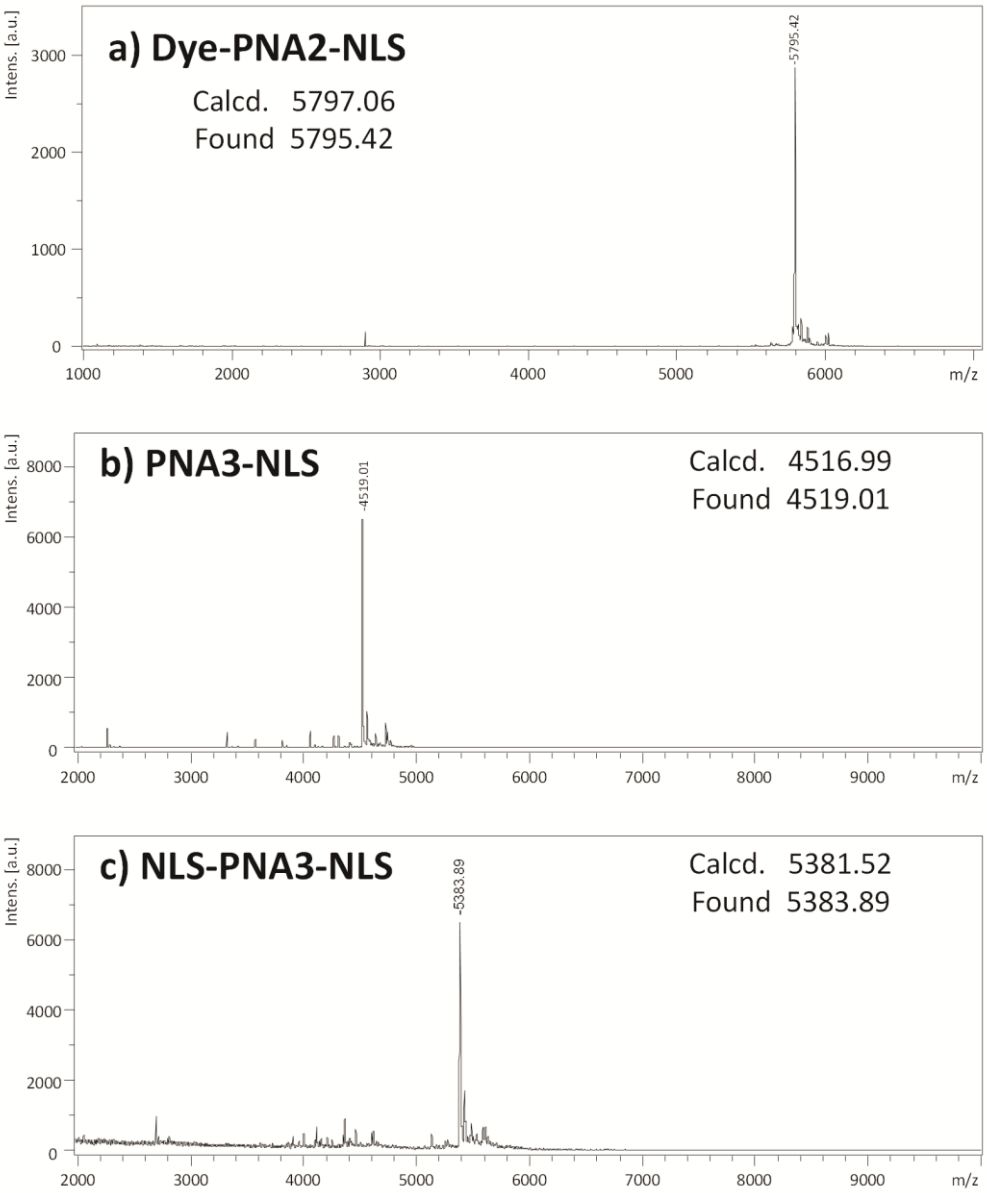
# 1. Synthesis of the PNA-NLS conjugates

The synthesis of PNA 1 and PNA1-NLS was described in reference 12. PNA2-NLS, Dye-PNA2-NLS, PNA3-NLS and NLS-PNA3-NLS were synthesized using standard Fmoc- or Boc-chemistry-based solid phase peptide synthesis (see reference 11) and characterized by MALDI TOF-MS. Typical results are shown in Supplementary Figure 1.

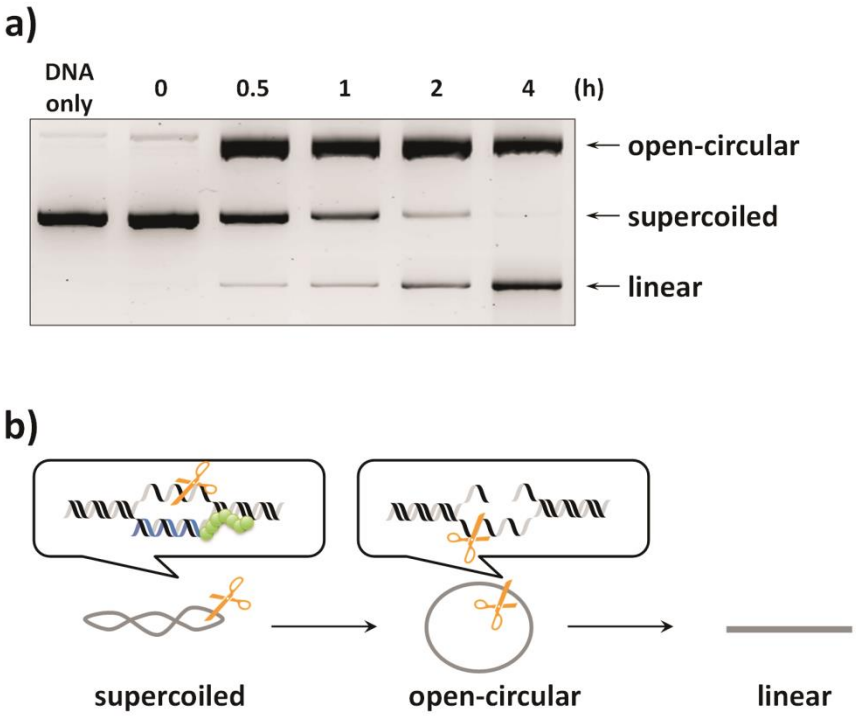



***Supplementary Figure 1.*** MALDI-TOF-MS spectra of (a) Dye-PNA2-NLS, (b) PNA3 and (c) NLS-PNA3-NLS.

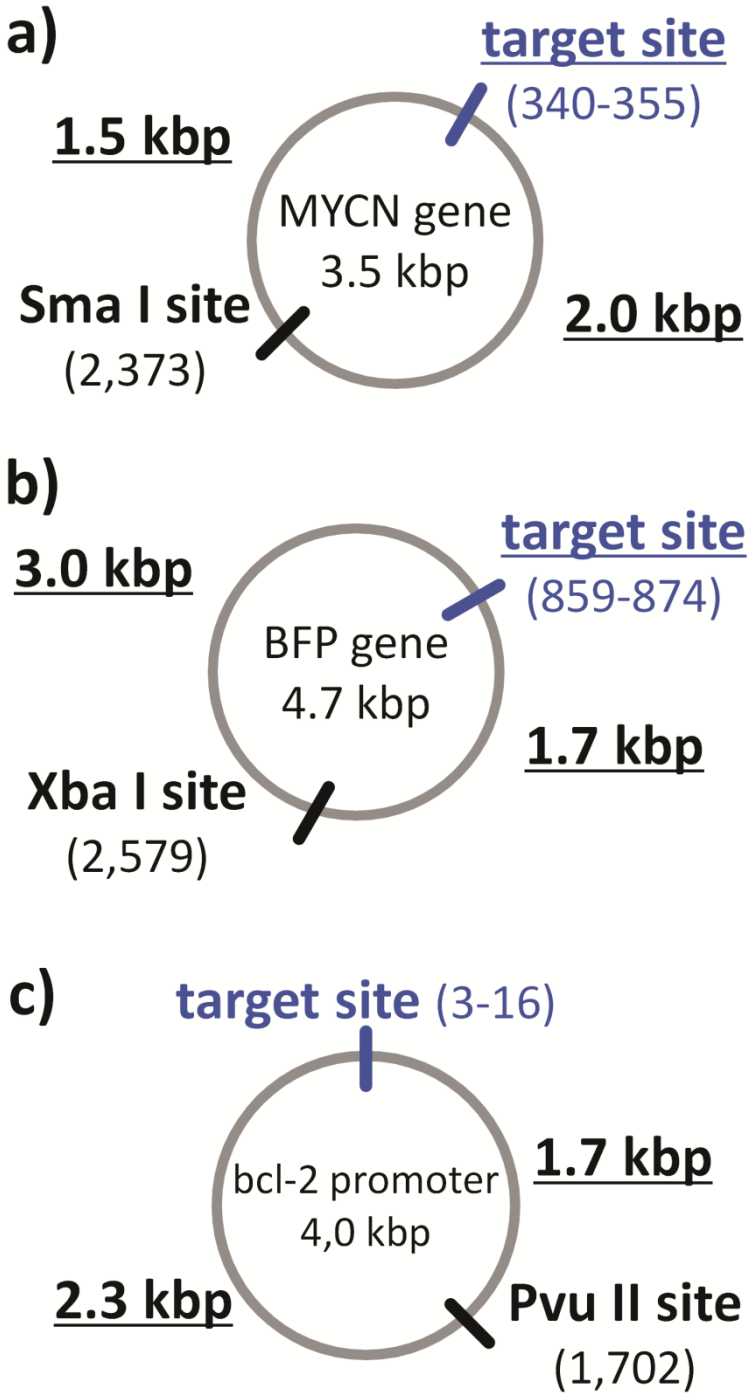
## 2. Site-selective DNA hydrolysis by Ce(IV)-EDTA

The target DNA was first incubated with the PNA-NLS conjugate at 50°C for 1 h. Then, Ce(IV)/EDTA and NaCl solutions were added to final concentrations of 200 μM and 100 mM, respectively. After a predetermined time, the reaction was quenched by adding the solution of ethylenediamine-*N*,*N*,*N'*,*N'*-tetrakis(methylenephosphonic acid). Typical gel electrophoresis patterns are presented in Supplementary Figure 2.

As described in the text, the products were further treated with the corresponding restriction enzyme, and analyzed by 0.8% agarose gel electrophoresis (the sites of the scissions by Ce(IV)-EDTA and the restriction enzymes are presented in Supplementary Figure 3).



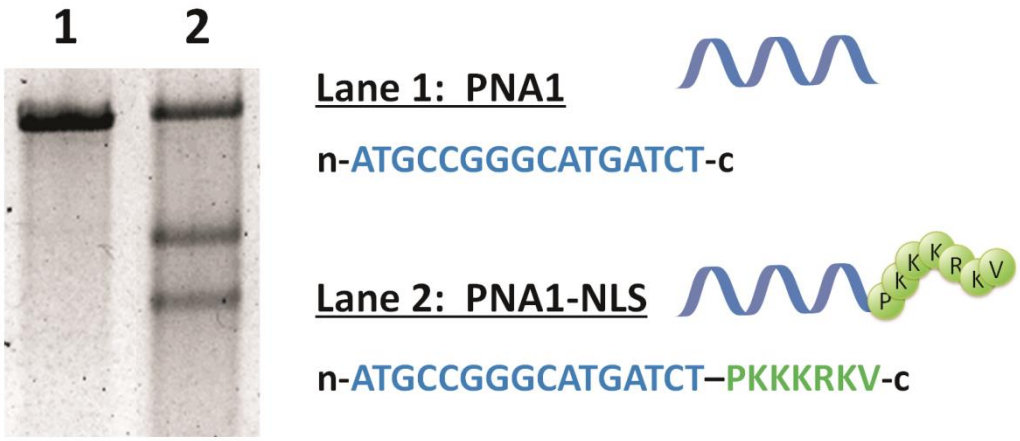

***Supplementary Figure 2.*** (a) Typical time-course of the DNA hydrolysis by PNA1-NLS/Ce(IV)-EDTA system. (b) Schematic depiction of the DNA scission reaction from supercoiled plasmid DNA (form I) to linearized one (form III). Conditions: [DNA] = 4.0 nM, [PNA-NLS] = 200 nM, [Ce(IV)-EDTA] = 200 μM, [HEPES (pH 7.0)] = 5.0 mM and [NaCl] = 100 mM at 50°C.




***Supplementary Figure 3.*** The cleavage maps of the present artificial DNA cutter (PNA-NLS conjugate + Ce(IV)-EDTA) and the corresponding restriction enzyme. The target sequences of the cutter are (a) *MYCN* gene, (b) *BFP* gene, and (c) *bcl-2* promoter.

# 3. Comparison of DNA-cutting activity of PNA1/Ce(IV)-EDTA with PNA1-NLS/Ce(IV)-EDTA

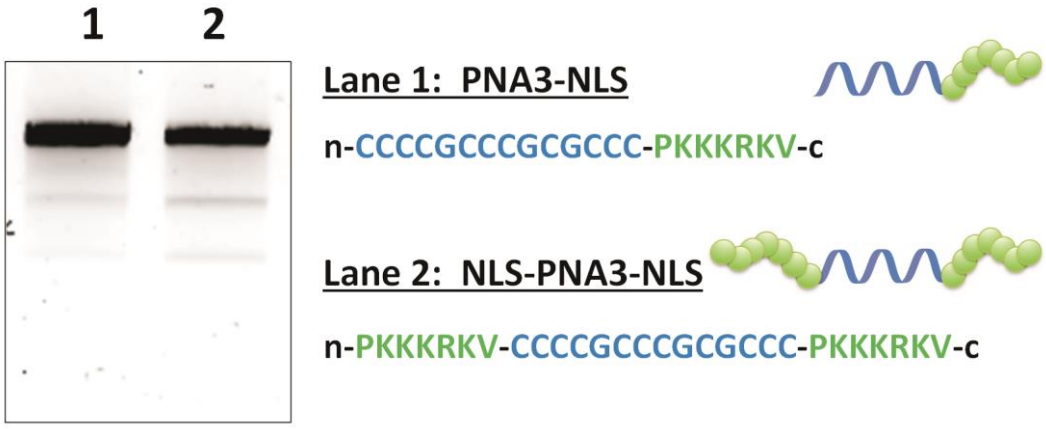
When PNA-1 bearing no NLS was combined with Ce(IV)-EDTA, no DNA scission was detectable as shown in Supplementary Figure 4 (lane 1). The essential role of the NLS moiety was confirmed.




***Supplementary Figure 4.*** Comparison of the PNA1-NLS conjugate and unmodified PNA1 for the efficiency of the site-selective DNA scission by Ce(IV)/EDTA. Conditions: [DNA] = 4.0 nM, [PNA or PNA-NLS] = 200 nM, [Ce(IV)/EDTA] = 200 μM, [HEPES (pH 7.0)] = 5.0 mM and [NaCl] = 100 mM at 37°C for 3 days.

# 4. Site-selective scission of highly GC-rich sequence (bcl-2 gene) by NLS-modified PNA/Ce(IV)-EDTA

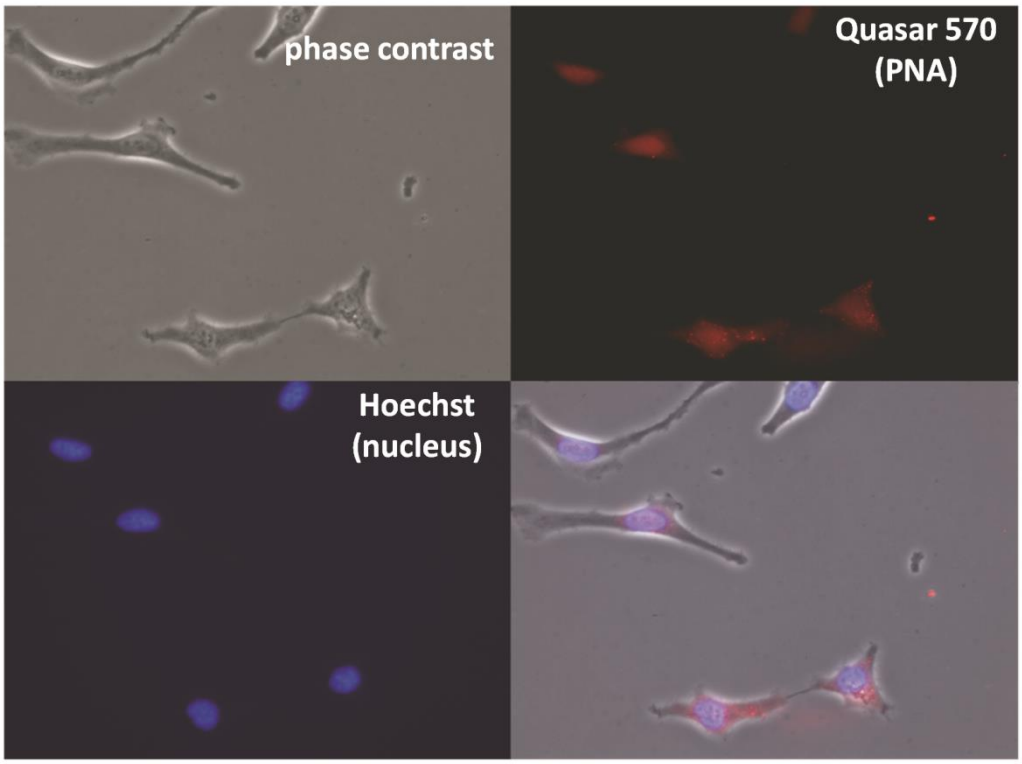
A consecutive 14-bp GC sequence in *bcl-2* gene, which is hard to be targeted by pcPNAs, was selectively cleaved by the NLS-modified PNAs. Both PNA3-NLS (with one NLS) and NLS-PNA3-NLS (with two NLS) showed the desired two scission bands and NLS-PNA3-NLS was slightly more effective than PNA3-NLS (Supplementary Figure 5).




***Supplementary Figure 5.*** Comparison of the PNA3-NLS and NLS-PNA3-NLS for the efficiency of the site-selective scission of highly GC-rich sequence. Conditions: [DNA] = 4.0 nM, [PNA or PNA-NLS] = 200 nM, [Ce(IV)/EDTA] = 200 μM, [HEPES (pH 7.0)] = 5.0 mM and [NaCl] = 100 mM at 37°C for 3 days.

# 4. Behavior of dye-labeled PNA-NLS conjugate in human cells

Intense localization of PNA-NLS conjugates in the nuclei of human cells was confirmed by fluorescence microscopy in Supplementary Figure 5 (other applications of NLS-modified PNA in cell; a) L. J. Branden, A. J. Mohamed and C. I. E. Smith, *Nat. Biotechnol.*, 1999, **17**, 784-787, b) G. Cutrona, E. M. Carpaneto, M. Ulivi, S. Roncella, O. Landt, M. Ferrarini and L. C. Boffa, *Nat. Biotechnol.*, 2000, **18**, 300-303).




***Supplementary Figure 6.*** Intracellular localization of PNA-NLS conjugate in human cells. PNA2-NLS was labeled with Quasar 570, and was introduced into HeLa cells by electroporation (Neon Neon$^{TM}$ Transfection System from Invitrogen). Then the cells were stained with Hoechst, and then observed by a fluorescence microscope.

# 5. Experimental in the fluorescence lifetime measurement

The fluorescence spectra and lifetimes were measured by a photon counting method with a streak scope (Hamamatsu Photonics, C4334-01) using the second harmonic generation (SHG, 395 nm) of a Ti:sapphire laser (Spectra-Physics, Tsunami 3950-L2S, fwhm = 150 fs) as an excitation source.

**Supplementary Table 1.**
The fluorescence parameters obtained by the fluorescence decay measurements.

| | $\tau_1$/ns | $A_1$ | $\tau_2$/ns | $A_2$ | $\chi^2$ |
|---|---|---|---|---|---|
| Dye-PNA2-NLS alone | 0.14 | 0.82 | 0.91 | 0.18 | 1.33 |
| Dye-PNA2-NLS + 16-mer complementary oligonucleotide | 0.15 | 0.47 | 1.35 | 0.53 | 1.17 |
| Dye-PNA2-NLS + the plasmid | 0.18 | 0.27 | 1.72 | 0.73 | 1.27 |