Supporting information

for

Labelling of nucleosides and oligonucleotides by solvatochromic 4-aminophthalimide fluorophore for studying DNA-protein interactions

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1. Additional figures

**Figure S1.** Excitation (left) and fluorescence spectra (right, quantum distribution over wavenumbers) of dA^API (4) in several solvents (dee – diethyl ether, ea – ethyl acetate, thf – tetrahydrofurane, acn – acetonitrile, dmf – dimethyl formamide, meoh – methanol, w – water) upon excitation at 340 nm in all cases.

**Figure S2.** Excitation (left) and fluorescence spectra (right, quantum distribution over wavenumbers) of dA^DAPI (6) in several solvents (dee – diethyl ether, ea – ethyl acetate, thf – tetrahydrofurane, acn – acetonitrile, dmf – dimethyl formamide, meoh – methanol, w – water) upon excitation at 340 nm in all cases. Excitation at 380 nm was chosen for water solution.
**Figure S3.** Excitation (left) and fluorescence spectra (right, quantum distribution over wavenumbers) of dC\textsuperscript{DAPI} (7) in several solvents (dee – diethyl ether, ea – ethyl acetate, thf – tetrahydrofuran, acn – acetonitrile, dmf – dimethyl formamide, meoh – methanol, w – water) upon excitation at 340 nm in all cases. Excitation at 380 nm was chosen for water solution.

**Figure S4.** a) Sovatochromism of dA\textsuperscript{DAPI} (4). Shown are the peak positions of the excitation (labelled abs) and emission (fls) bands of Figure S1 against a measure of solvent polarity. b) Sovatochromism of dA\textsuperscript{DAPI} (6). Shown are the peak positions of the excitation (labelled abs) and emission (fls) bands of Figure S2 against a measure of solvent polarity. Regression lines were calculated from the solvents dee, ea, thf, dmf and acn. Dashed lines represent the behavior of unsubstituted 4-amino-phthalimide.
Figure S5. Sovatochromism of dC\textsuperscript{DAPI} (7). Shown are the peak positions of the excitation (labelled abs) and emission (fis) bands of Figure S4 against a measure of solvent polarity. Regression lines were calculated from the solvents 
dee, ea, thf, dmf and acn. Dashed lines represent the behavior of unsubstituted 4-amino-phthalimide.

Figure S6. Comparison of fluorescence excitation (solid lines at left) and absorption bands (dashed) for the dA\textsuperscript{APP}TP (8) and dA\textsuperscript{DAP}TP (10) in water. Fluorescence excitation bands are structured and appear slightly red-shifted, compared to the unstructured absorption bands (see text).
Study of p53 binding at 1 μM concentration

Figure S7. Increase of fluorescence intensity of 1 μM dC<sup>API</sup>-labeled DNA (pex<sup>p53</sup> dC<sup>API</sup>) upon p53 binding. The API-labeled DNA was titrated by 0.5 and 1 equivalent of p53 protein.
2. p53-DNA binding assay

Binding of p53 protein to API-modified 50-nt ds oligonucleotide substrate (pex^{p53}) prepared from temp^{p53} template using dC^{API}TP (9), see sequence in figure below: top strand is template temp^{p53}, primer stretch is denoted by *italics*, p53 recognition site is *bold*, cytosines bearing the API labels are *red* and extension of the primer by arrow) was checked by electrophoretic mobility shift assay (EMSA). The p53 protein was incubated with pexit{p53} in 50 mM KCl, 5 mM Tris (pH 7.6), 2 mM DTT, 0.01% Triton X-100 for 30-min at 10 °C. The reaction mixture contained 20 ng of the ^{32}P-labeled pexit{p53} and 120 ng of p53 protein. The protein– pexit{p53} complexes were detected by EMSA in 5% native polyacrylamide gel followed by autoradiography. Unmodified pexit{p53} was prepared using mixture of all four natural dNTPs.

As evident from the autoradiogram below, the p53 protein formed stable complexes with both unmodified and API-modified DNA substrates.

![Electrophoretic mobility shift assay of the binding of p53 to unmodified and modified DNA.](image)

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3. Full emission spectrum

![Full emission spectrum of API during SSB-DNA binding study](image)

**Figure S9.** Full emission spectra of API during SSB-DNA binding study.
4. Copies of $^1$H, $^{31}$P-NMR spectra

$^1$H NMR spectrum of P-API (2)

$^1$H NMR spectrum of P-DAPI (3)
$^1$H NMR spectrum of dA$^\text{DAPI}$ (4)

$^1$H NMR spectrum of dA$^\text{DAPI}$ (6)
$^1$H NMR spectrum of dC$^{API}$ (5)

$^1$H NMR spectrum of dC$^{DAPI}$ (7)
$^1$H NMR spectrum of dA$^{API}$TP (8)

$^{31}$P NMR spectrum of dA$^{API}$TP (8)
$^1$H NMR spectrum of dA$^{DAPI}$TP (10)

$^{31}$P NMR spectrum of dA$^{DAPI}$TP (10)
$^1$H NMR spectrum of dC$^{\text{AP1TP}}$ (9)

$^{31}$P NMR spectrum of dC$^{\text{AP1TP}}$ (9)
$^1$H NMR spectrum of dC$^{\text{DAPI TP}}$ (11)

$^{31}$P NMR spectrum of dC$^{\text{DAPI TP}}$ (11)
5. Copies of MALDI-TOF spectra

MALDI-TOF spectrum of pex<sup>C</sup>(dC<sup>API</sup>) oligonucleotide

MALDI-TOF spectrum of pex<sup>rnd16</sup>(dC<sup>API</sup>) oligonucleotide, peak at 5205.8 represents double charged ion-radical.

Both spectra contain fragmentation products which have lower molecular weight by 125 Da as result of single loss of uracil.