Supporting Information

A rapid and concise setup for the fast screening of FRET pairs using bioorthogonalized fluorescent dyes

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S1. Spectral characteristics of modified Cy3 and Cy5 dyes

Fluorescent spectra of the cyanine dyes only slightly changed upon modification of the core framework with -methyltetrazine attached through a vinylene linker - or upon conjugated to BCN and DNA oligomers.

**Fig. S1** Fluorescent excitation and emission spectra of the core scaffold of Cy3 and Cy5 dyes, the tetrazine harboring Cy3T and Cy5T dyes, BCN conjugated Cy3T and Cy5T and the Clarwt3’ oligonucleotide modified by Cy3T and Cy5T
S2. Spectral overlap of the selected dyes

Fig. S2 Spectral overlap of the selected dye pairs
S3. Sequential labeling of the amino modified DNA followed by capillary electrophoresis

Fig. S3 Capillary electrophoretic follow up of the fluorescent labeling of the amino modified DNA oligomer
Conditions 64.5 cm total and 56.5 cm effective length fused silica capillary ID 50 µm,
BGE: 200 mM sodium borate buffer (pH 9.0); injection 50 mbar×6 sec; 30 kV; monitored at 260 nm
Fig. S4 Predicted secondary structure of the single stranded DNA oligomers (\textit{Clar}^{\text{wt}5'} \text{ and } \textit{Clar}^{\text{wt}3'} )
S4. Identification of fluorescently labeled DNA oligomers by LC-MS

Fig. S5 Negative ESI-MS spectrum of Clar"5' oligonucleotide (Mw = 5338 Da)

Fig. S6 Negative ESI-MS spectrum of Clar"3' oligonucleotide (Mw = 5368 Da)
**Fig. S7** Negative ESI-MS spectrum of Clar<sup>wt</sup><sup>5′</sup> oligonucleotide (Mw = 5401 Da)

**Fig. S8** Negative ESI-MS spectrum of Clar<sup>wt</sup><sup>5′</sup> oligonucleotide labeled with Cy1A dye (Mw = 5958.74 Da)
Fig. S9 Negative ESI-MS spectrum of Clar wt 5’ oligonucleotide labeled with Cy3T dye (Mw = 6071.96 Da)

Fig. S10 Negative ESI-MS spectrum of Clar wt 3’ oligonucleotide labeled with Cy3T dye (Mw = 6101.96 Da)
Fig. S11 Negative ESI-MS spectrum of ClarK\textsuperscript{wt}\textsuperscript{5}' oligonucleotide labeled with Cy3T dye (Mw = 6134.96 Da)

Fig. S12 Negative ESI-MS spectrum of ClarK\textsuperscript{wt}\textsuperscript{5}' oligonucleotide labeled with Cy5T dye (Mw = 6097.99 Da)
Fig. S13 Negative ESI-MS spectrum of Clar3' oligonucleotide labeled with Cy5T dye (Mw = 6127.99 Da)

Fig. S14 Negative ESI-MS spectrum of Clar5' oligonucleotide labeled with CBRD1A dye

(Mw = 5919.69 Da)
Fig. S15 Negative ESI-MS spectrum of Clar\textsuperscript{3}' oligonucleotide labeled with CBRD1A dye

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\begin{align*}
&M-11H^{11-} \quad 5949.69 \\
&M-10H^{10-} \quad 593.7 \\
&M-9H^{9-} \quad 559.6 \\
&M-8H^{8-} \quad 548.6 \\
&M-7H^{7-} \quad 538.6 \\
&M-6H^{6-} \quad 528.6 \\
&M-5H^{5-} \quad 518.2 \\
&M-4H^{4-} \quad 508.2 \\
&M-3H^{3-} \quad 498.2 \\
\end{align*}
\]

S5. Purity of fluorescently labeled DNA oligomers measured by CE

The purity of the fluorescently labeled DNA oligomers was analyzed by capillary electrophoresis. The average purity of the end products is above 90%. In the samples the initial or BCN conjugated DNA can be found as impurity in negligible quantity. In some of these samples the DNA can be detected in folded structure (cf. Fig. S4) which resulted in multiple peaks in the electropherograms but the product peaks can be identified by the characteristic absorbance peak of the conjugated dyes.
**Fig. S16** Electropherogram of Clari\textsuperscript{wt}K5′ oligonucleotide

**Fig. S17** Electropherogram of Clari\textsuperscript{wt}3′ oligonucleotide
**Fig. S18** Electropherogram of Clar\textsuperscript{wt}5' oligonucleotide

**Fig. S19** Electropherogram of Clar\textsuperscript{wt}K5' oligonucleotide labeled with Cy1A dye
**Fig. S20** Electropherogram of Clar\textsuperscript{5'} oligonucleotide labeled with Cy3T dye

**Fig. S21** Electropherogram of Clar\textsuperscript{3'} oligonucleotide labeled with Cy3T dye
**Fig. S22** Electropherogram of Clar\(^w\)K5' oligonucleotide labeled with Cy3T dye

**Fig. S23** Electropherogram of Clar\(^w\)S' oligonucleotide labeled with Cy5T dye
Fig. S24 Electropherogram of Clar\textsuperscript{wt3} oligonucleotide labeled with Cy5T dye

Fig. S25 Electropherogram of Clar\textsuperscript{wt5} oligonucleotide labeled with CBRD1A dye
Fig. S26 Electropherogram of Clari<sup>3'</sup> oligonucleotide labeled with CBRD1A dye
**S6. Estimation of distances of 17-mer double stranded DNA oligomer**

**Table S1** Calculated distances (5'-phosphate-P to 5'-phosphate-P in trans position) of different DNA structures from PDB database

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Description</th>
<th>Chain A</th>
<th>Chain B</th>
<th>Distance (Å)</th>
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<tbody>
<tr>
<td>4Q45</td>
<td>(damaged DNA)</td>
<td>Chain:B</td>
<td>Chain:C</td>
<td>59.4</td>
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<tr>
<td>1D66</td>
<td>(bound to TFs)</td>
<td>Chain:D</td>
<td>Chain:E</td>
<td>51.9</td>
</tr>
<tr>
<td>1U0T</td>
<td>(telomeric DNA)</td>
<td>Chain:C</td>
<td>Chain:D</td>
<td>52.2</td>
</tr>
<tr>
<td>1W0U</td>
<td>(telomeric DNA)</td>
<td>Chain:C</td>
<td>Chain:D</td>
<td>50.2</td>
</tr>
<tr>
<td>5CDP</td>
<td>(broken DNA)</td>
<td>Chain:F</td>
<td>Chain:H</td>
<td>42.1</td>
</tr>
<tr>
<td>1O3R</td>
<td>(deformed DNA)</td>
<td>Chain:B</td>
<td>Chain:C</td>
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<tr>
<td>3L2P</td>
<td>(nicked DNA)</td>
<td>Chain:B</td>
<td>Chain:D</td>
<td>57.5</td>
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<tr>
<td>4E54</td>
<td>(damaged DNA)</td>
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<td>Chain:G</td>
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<td>4E5Z</td>
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<td>Chain:G</td>
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<td>1A02</td>
<td>(bound to TFs)</td>
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<td>Chain:B</td>
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<td>1DCT</td>
<td>(crosslinked DNA)</td>
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<td>Chain:M</td>
<td>56.4</td>
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<td>1HF0</td>
<td>(bound to TFs)</td>
<td>Chain:M</td>
<td>Chain:N</td>
<td>50.4</td>
</tr>
<tr>
<td>1IGN</td>
<td>(telomeric DNA)</td>
<td>Chain:C</td>
<td>Chain:D</td>
<td>50.1</td>
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<td>1T2K</td>
<td>(bound to TFs)</td>
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<td>Chain:F</td>
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<td>Chain:B</td>
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<td>2AS5</td>
<td>(bound to TFs)</td>
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<td>Chain:I</td>
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<tr>
<td>2RBA</td>
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<td>(bound to TFs)</td>
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<td>49.7</td>
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<tr>
<td>5CQQ</td>
<td>(bound to TFs)</td>
<td>Chain:C</td>
<td>Chain:D</td>
<td>44.5</td>
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</table>

**Average** 50.8 Å  
**SE** 5.2 Å
S7. Temperature controlled denaturation of fluorescently labeled DNA oligomer pairs

**Fig. S27** Changes in FRET efficiencies as a function of temperature in case of the oligonucleotide pairs labeled at proximal (black squares) and distant termini (red circles); A: Cy3T/Cy5T; B: Cy1A/Cy3T; C: Cy1A/CBRD1A;
S8. Flow cytometric analysis

For flow cytometric analysis HEK293T cells were transferred into 24-well plate (Greiner-bio-one 662160) (40,000 cell/well) and incubated for 48 h at 37°C in a 5% CO₂ atmosphere. Cells were transfected with 25 pmol fluorescently labeled DNA using Lipofectamine-3000 (Invitrogen L3000-001) and Optimem medium (Gibco 70011-044) and incubated for 4 h at 37°C in a 5% CO₂ atmosphere. Before measurements cells were harvested using 10% Trypsin-EDTA solution (Sigma T3924) and complete DMEM. Cells were washed twice with PBS solution (400 g, 5 min). Flow cytometric analysis were carried out with BD FACSCanto II system (Beckton Dickinson) equipped with 405, 488, 532 and 633 nm lasers with AmCyan (510 ± 50nm), PE (585 ± 42 nm) and PerCP (LP 670) filters.

Fig. S28 Flow cytometric analysis of HEK293T cells labelled by fluorescent DNA oligomers
S9. NMR spectra of Cy1A dye