Supporting Information:

J- vs. H-type assembly: pentamethine cyanine (Cy5) as near IR chiroptical reporter

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1. General

Absorption spectra were recorded in 1-cm quartz cells at 20 °C on Varian Cary-100 Bio-UV/VIS spectrophotometer equipped with a Varian Cary-block temperature controller.

Emission spectra and quantum yields ($\Phi_F$) were measured in 1-cm quartz cells at 20 °C on Varian Cary Eclipse fluorescence spectrophotometer equipped with a Varian Cary-block temperature controller.

CD spectra were recorded on a JASCO J-715 spectropolarimeter using quartz cuvettes with an optical path of 1 cm.

HPLC purity determination was performed with a Shimadzu LC system equipped with a Shimadzu-block temperature controller.

Oligonucleotides ON1 and ON2 containing Cy5 molecule in the backbone were from Microsynth (Balgach, Switzerland) and BaseClick (Tutzing, Germany). Not modified oligonucleotides ON3 and ON4 were from Microsynth (Balgach, Switzerland). Oligonucleotide ON5 containing squaraine (Sq) molecule in the backbone was synthesized according to [1].

2. Experimental procedures

The purity of the oligonucleotides was determined by reverse phase HPLC: column LiChrospher® 100 RP-18, 250 mm × 4 mm, Merck; mobile phase A = (Et$_3$NH)OAc (0.1 M, pH 7.4); mobile phase B = MeCN; elution at 20 °C; gradient 0 – 40% B over 22 min, then 40-100% B over 5 min.

Measurements of spectra in aqueous solutions (except quantum yields determination) were performed at a concentration of 1.5 μM (10 mM PB, pH = 7.4, 100 mM NaCl) for oligonucleotides and at a concentration of 1.5 μM + 1.5 μM of each strand (10 mM PB, pH = 7.4, 100 mM NaCl) for duplexes. Concentration of oligonucleotides was determined using molar absorbivities of $\varepsilon_{260}$(ON1) = 227 400 M$^{-1}$ cm$^{-1}$, $\varepsilon_{260}$(ON2) = 206 200 M$^{-1}$ cm$^{-1}$, $\varepsilon_{260}$(ON3) = 231 400 M$^{-1}$ cm$^{-1}$, $\varepsilon_{260}$(ON4) = 216 500 M$^{-1}$ cm$^{-1}$ and $\varepsilon_{260}$(ON5) = 212 200 M$^{-1}$ cm$^{-1}$; a value of $\varepsilon_{260}$(Cy5) = 5 000 M$^{-1}$ cm$^{-1}$ was applied for calculation of $\varepsilon_{260}$(ON1) and $\varepsilon_{260}$(ON2) and a value of $\varepsilon_{260}$(Sq) = 11 000 M$^{-1}$ cm$^{-1}$ was applied for calculation of $\varepsilon_{260}$(ON5).

Thermal denaturation experiments were carried out on a Varian Cary-100 Bio-UV/VIS spectrophotometer and data were collected at 260, 590 and 645 nm for duplex ON1*ON2 with internal Cy5 modifications in the backbone and at 260 nm for non-modified duplex ON3*ON4 (cooking-heating cycles in the temperature range of 20-90 °C, temperature gradient of 0.5 °C/min). Melting temperature ($T_m$) values were determined as the maximum of the first derivative of the melting curve.

For the determination of the quantum yields, the integrated relative intensities of the samples were measured against Cy5 (quantum yield, $QY_{Cy5}$ = 27% in water [2]) as the reference. Absorbance of the solutions at the excitation wavelength (600 nm for the ON1, ON2 and ON5, and 625 nm for the

duplexes ON1*ON2 and ON1*ON5) was between 0.04–0.06 measured in a 1-cm cell. The emission spectra of the solutions were recorded and the quantum yields of the samples were determined as described in [3] according to formula:

\[
QY = QY_{Cy5} \times \left( \frac{F}{F_{Cy5}} \right) \times \left( \frac{A_{Cy5}}{A} \right) \times \left( \frac{n^2}{n^2_{Cy5}} \right),
\]

where \( F \) and \( F_{Cy5} \) are the integrated areas of the fluorescence spectra, \( A \) and \( A_{Cy5} \) are the absorbance at the excitation wavelength and \( n \) and \( n_{Cy5} \) are the refraction indices of solvents used for the sample under examination and Cy5, respectively.

3. Mass-spectrometry and HPLC data of the cyanine (Cy5) modified ON1 and ON2 and squaraine (Sq) modified ON5

Table S1. Mass spectrometry data of the ON1, ON2 (Qualitative analysis report) and ON5 (ESI-MS, negative mode, MeCN/H2O/0.5%, Et3N)

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Sequence</th>
<th>Molecular formula</th>
<th>Mol Weight</th>
<th>Found mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON1</td>
<td>5’ AGCTCGGTCACy5CGAGAGTGCA</td>
<td>C226H282N83O120P20</td>
<td>6700.62</td>
<td>6700.24</td>
</tr>
<tr>
<td>ON2</td>
<td>3’ TCGAGCCAGT Cy5GCTCTCACGT</td>
<td>C224H284N73O124P20</td>
<td>6602.54</td>
<td>6602.17</td>
</tr>
<tr>
<td>ON5</td>
<td>3’ TCGAGCCAGTSqGCTCTCACGT</td>
<td>C231H293N73O126P20</td>
<td>6724.35</td>
<td>6724.34</td>
</tr>
</tbody>
</table>

Figure S1. HPLC data of the oligonucleotides ON1 (left) and ON2 (right) recorded at 260 and 650 nm.

Figure S2. HPLC data of the oligonucleotide ON5 recorded at 260 and 630 nm.

Qualitative Analysis Report – ESI-TOF

Data Filename: 20524A15D01 1-2d  
Acq Method: ESI_Standard_0.m  
DA Method: baseclick_4-15kDa.m  
Position: P1-D4  
Acquired Time: 06/01/2012 12:43:50 PM  
Comment: HPLC

Sample Group
Info
Contact Person: Larysa Markova  
Order ID: 120509-195  
Sequence: 5'-AGC TCG GTC A6C GAG AGT GCA -3'  
# Bases: 20  
Expected MW: 6701

Deconvoluted Spectra

--- End Of Report ---
Qualitative Analysis Report – ESI-TOF

Data Filename: 20522A853E02 2-2d
Acq Method: ESI_Standard_1.m
DA Method: baseclick_4-15kDa.m
Position: P1-D6
Acquired Time: 06/01/2012 1:25:01 PM
Comment: HPLC

Sample Group Info
Contact Person: Larysa Markova
Order ID: 120509-195
Sequence: 5'-TGC ACT CTC G6T GAC C6A C6A GCT-3'
# Bases: 20
Expected MW: 6603

Deconvoluted Spectra

--- End Of Report ---
Figure S3. HR-MS of the ON5.

4. Excitation spectra

Figure S4. Excitation spectra of the oligonucleotide ON2 (left) and duplex ON1*ON2 (right). $\lambda_{em} = 670$ nm.
5. Influence of the temperature on the absorption and emission spectra of Cy5 containing oligonucleotide ON2 and duplex ON1*ON2

Figure S5. Influence of the temperature on the absorption and emission spectrum of the Cy5 containing oligonucleotide ON2 in the temperature range 20-80 ºC.

Figure S6. Influence of the temperature on the absorption spectrum of the Cy5 containing duplex ON1*ON2 in the temperature range 20-80 ºC.
6. Tm experiments

![Graph showing absorbance and temperature relationship for Tm determination](image)

**Figure S7.** Example of Tm determination procedure: duplex ON3*ON4, melting curves at 260 nm (left) and first derivative curve (right), Tm = 70.7 °C (right).

**Table S2.** Hybridization data of the cyanine containing duplex ON1*ON2 and its non-modified analog ON3*ON4. (Cy5-Cy5 vs A-T base pair)*.

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Duplex</th>
<th>Tm, °C</th>
<th>ΔTm, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON3</td>
<td>5’ AGCTCGGTCATCGAGAGTGCA 3’ TCGAGCCAGTAGCTCTCACGT</td>
<td>70.7</td>
<td>–</td>
</tr>
<tr>
<td>ON4</td>
<td>5’ AGCTCGGTCATCGAGAGTGCA 3’ TCGAGCCAGTAGCTCTCACGT</td>
<td>66.7</td>
<td>- 4.0</td>
</tr>
</tbody>
</table>

* from the first derivative of the cooling (90 °C – 20 °C) curve
7. Quantitative analysis of asymmetry of exciton couplet:

7.1 Fitting of absorbance and CD bands shape.

**Figure S8.** Fitting of absorbance band shape of Cy-Cy containing hybrid.
**Figure S9.** Fitting of CD shape of Cy-Cy containing hybrid.

### 7.2 Integration of CD signals.

<table>
<thead>
<tr>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
<th>Integral Result of B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index</strong></td>
<td><strong>Area</strong></td>
<td><strong>Area IntgP (%)</strong></td>
<td><strong>Row Index</strong></td>
<td><strong>Beginning X</strong></td>
<td><strong>Ending X</strong></td>
<td><strong>FWHM</strong></td>
<td><strong>Center</strong></td>
<td><strong>Height</strong></td>
</tr>
<tr>
<td>1</td>
<td>45.8718</td>
<td>3.42315</td>
<td>120</td>
<td>228</td>
<td>309.5</td>
<td>23.70212</td>
<td>280</td>
<td>5.7309</td>
</tr>
<tr>
<td>3</td>
<td>-99.23928</td>
<td>-7.40566</td>
<td>826</td>
<td>622</td>
<td>645</td>
<td>19.21005</td>
<td>633</td>
<td>-5.62839</td>
</tr>
<tr>
<td>4</td>
<td>607.59975</td>
<td>45.3417</td>
<td>895</td>
<td>645</td>
<td>710.5</td>
<td>24.58379</td>
<td>667.5</td>
<td>23.32792</td>
</tr>
</tbody>
</table>
Ratio of g-factors

Circular dichroism is defined as difference in absorbance of left and right circularly polarized light beams, \( CD = A^L - A^R \)

g-factor, is defined as [4]:

\[
g = \frac{\Delta \varepsilon}{\varepsilon} = \frac{A^L - A^R}{A}
\]

Where \( A^L \) and \( A^R \) are the absorptions of left and right circularly polarized light; and \( A \) represents the absorbance of nonpolarized light.

Output of CD instrument is usually presenting in ellipticity in mdeg, \( \Theta \) (mdeg):

\[
\Theta \ (\text{mdeg}) = \sim 33000 \ CD = \sim 33000(A^L - A^R)
\]

Therefore value of CD, or \( (A^L - A^R) \) can be obtained from experimental data as \( \Theta \ (\text{mdeg}) / 33000 \)

Importantly, g-value calculation as \( g = (A^L - A^R) / A \) does not required concentration or extinction coefficient (\( \varepsilon \)), when CD and absorbance are measured for the same sample. This is very valuable, because concentration and/or exact \( \varepsilon \) are often barely defined, especially for aggregated materials, polymers, etc.

g-factor is also called as anisotropy or dissymetry factor, and was applied in our work to characterize an asymmetry of exciton coupllet.

Experimental data for ON1-ON2 hybrid at 20 °C:

\[
\begin{align*}
A(668) &= 0.59678 \quad A(633) = 0.34578 \quad A(601) = 0.32854 \\
CD(668) &= 23.3657 \quad CD(633) = -5.53441 \quad CD(601) = -9.03216 \\
g(668) &= 7.01 \times 10^{-4} \quad g(633) = -1.68 \times 10^{-4} \quad g(601) = -2.74 \times 10^{-4}
\end{align*}
\]

that gives \( g_{(668\text{nm})}/g_{(633\text{nm})} = 4.17 \) and \( g_{(668\text{nm})}/g_{(601\text{nm})} = 2.56 \)

8. Titration experiment.

Titration of the oligonucleotide ON1 (starting conc. 1.5 µM, 10 mM phosphate, pH = 7.4, 100 mM NaCl, 20 ºC, V = 1000 µL) by addition of stock solution of oligonucleotide ON2 (C_stock sol. = 7.5×10⁻⁵ M) in 40 µL steps. At first step, 20 µL of ON2 stock solution was used.

First step corresponds to the 7.4×10⁻⁸ M of duplex formed, assuming a total shift of equilibrium to the hybrid at 20 ºC. Linear response of CD signal intensity on ON2 concentration reveals a fast kinetic of hybridization and correlates with formation of one chiral product upon Cy-Cy assembly within hybrid ON1-ON2.
9. CD spectra of the oligonucleotides ON2 and ON5 and hybrids ON3*ON5 and ON3*ON4

![CD spectra of ON2 and ON5](Image)

**Figure S10.** The CD spectra of the oligonucleotides **ON2** and **ON5** (conditions: 1.5 µM single strand conc., 10 mM phosphate, pH = 7.4, 100 mM NaCl).
**Figure S11.** The CD spectra of the hybrids ON3*ON5 and ON3*ON4 (conditions: 1.5 μM each strand conc., 10 mM phosphate, pH = 7.4, 100 mM NaCl).