Supporting Information:

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

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TABLE OF CONTENTS

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 (Φ_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_3NH)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 absorbtivities of ε_{260}(**ON1**) = 227 400 M^{-1} cm^{-1}, ε_{260}(**ON2**) = 206 200 M^{-1} cm^{-1}, ε_{260}(**ON3**) = 231 400 M^{-1} cm^{-1}, ε_{260}(**ON4**) = 216 500 M^{-1} cm^{-1} and ε_{260}(**ON5**) = 212 200 M^{-1} cm^{-1}; a value of ε_{260}(**Cy5**) = 5 000 M^{-1} cm^{-1} was applied for calculation of ε_{260}(**ON1**) and ε_{260}(**ON2**) and a value of ε_{260}(**Sq**) = 11 000 M^{-1} cm^{-1} was applied for calculation of ε_{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** (cooling-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

1 L.I. Markova, V.L. Malinovskii, L.D. Patsenker, R.Häner, Synthesis and properties of squaraine-modified DNA. Org. Biomol. Chem. 2012, **10**, 8944-8947.
2 R.B. Mujumdar, L.A. Ernst, S.R. Mujumdar, C.J. Lewis, A.S. Waggoner, Cyanine dye labeling reagents: sulfoindocyanine succinimidyl esters. Bioconjug. Chem. 1993, **4**, 105–111.

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:

$$QY = QY_{Cy5} \times (F/F_{Cy5}) \times (A_{Cy5}/A) \times (n^2/n^2_{Cy5}),$$

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/H_2O/0.5%, Et_3N)

Oligo-mer	Sequence	Molecular formula	Mol Weight	Found mass
ON1	5’ AGCTCGGTCA**Cy5**CGAGAGTGCA	$C_{226}H_{282}N_{83}O_{120}P_{20}^-$	6700.62	6700.24
ON2	3’ TCGAGCCAGT**Cy5**GCTCTCACGT	$C_{224}H_{284}N_{73}O_{124}P_{20}^-$	6602.54	6602.17
ON5	3’ TCGAGCCAGT**Sq**GCTCTCACGT	$C_{231}H_{293}N_{73}O_{126}P_{20}$	6724.35	6724.34

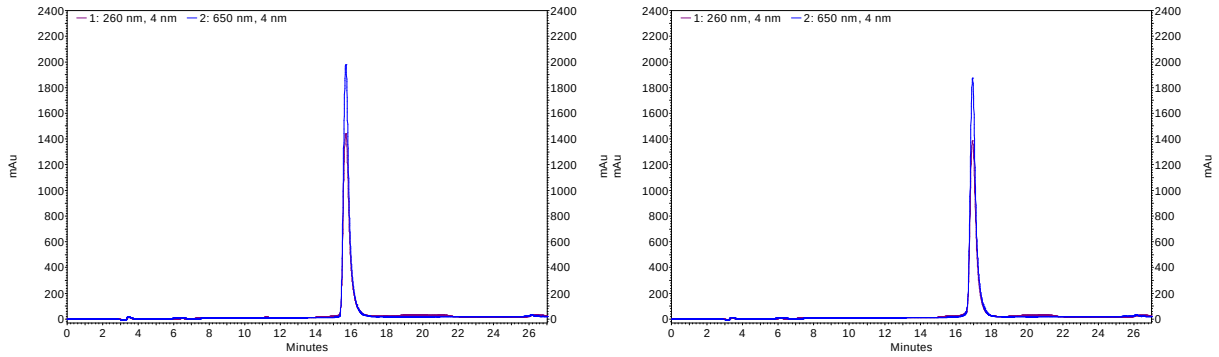


Figure S1. HPLC data of the oligonucleotides **ON1** (left) and **ON2** (right) recorded at 260 and 650 nm.

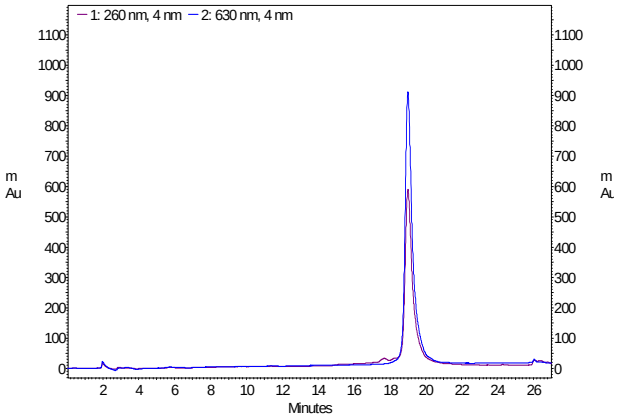


Figure S2. HPLC data of the oligonucleotide **ON5** recorded at 260 and 630 nm.

3 C.A. Parker, Photoluminescence of Solutions, Elsevier Publishing Company, Amsterdam, 1968.

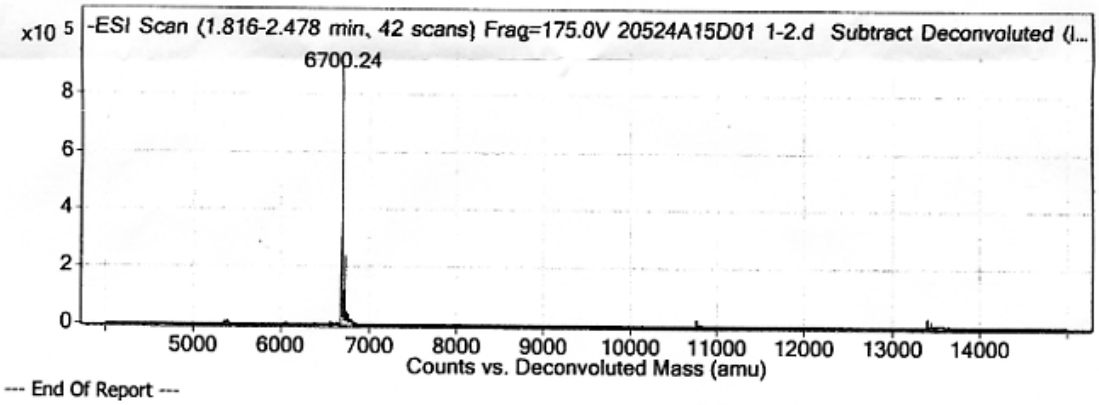
baseclick

Qualitative Analysis Report – ESI-TOF

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



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baseclick

Qualitative Analysis Report – ESI-TOF

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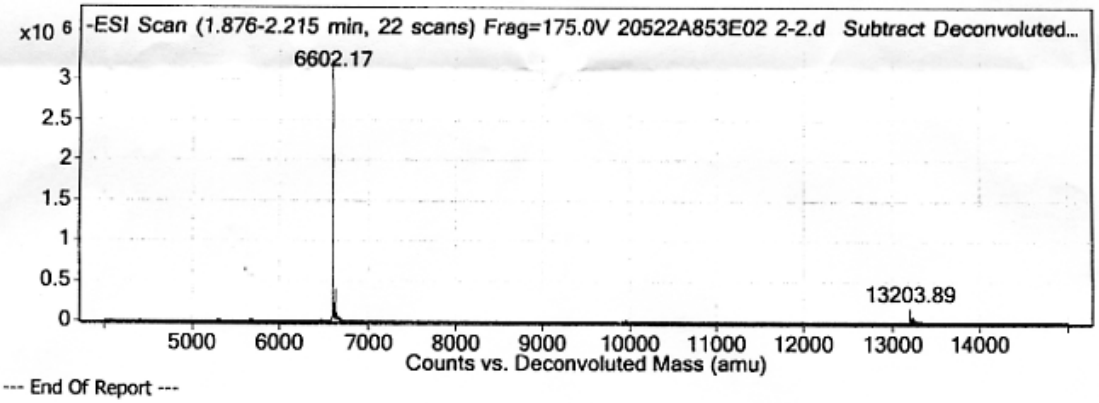
DA Method	baseclick_4-15kDa.m	**Comment**	HPLC

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

Deconvoluted Spectra



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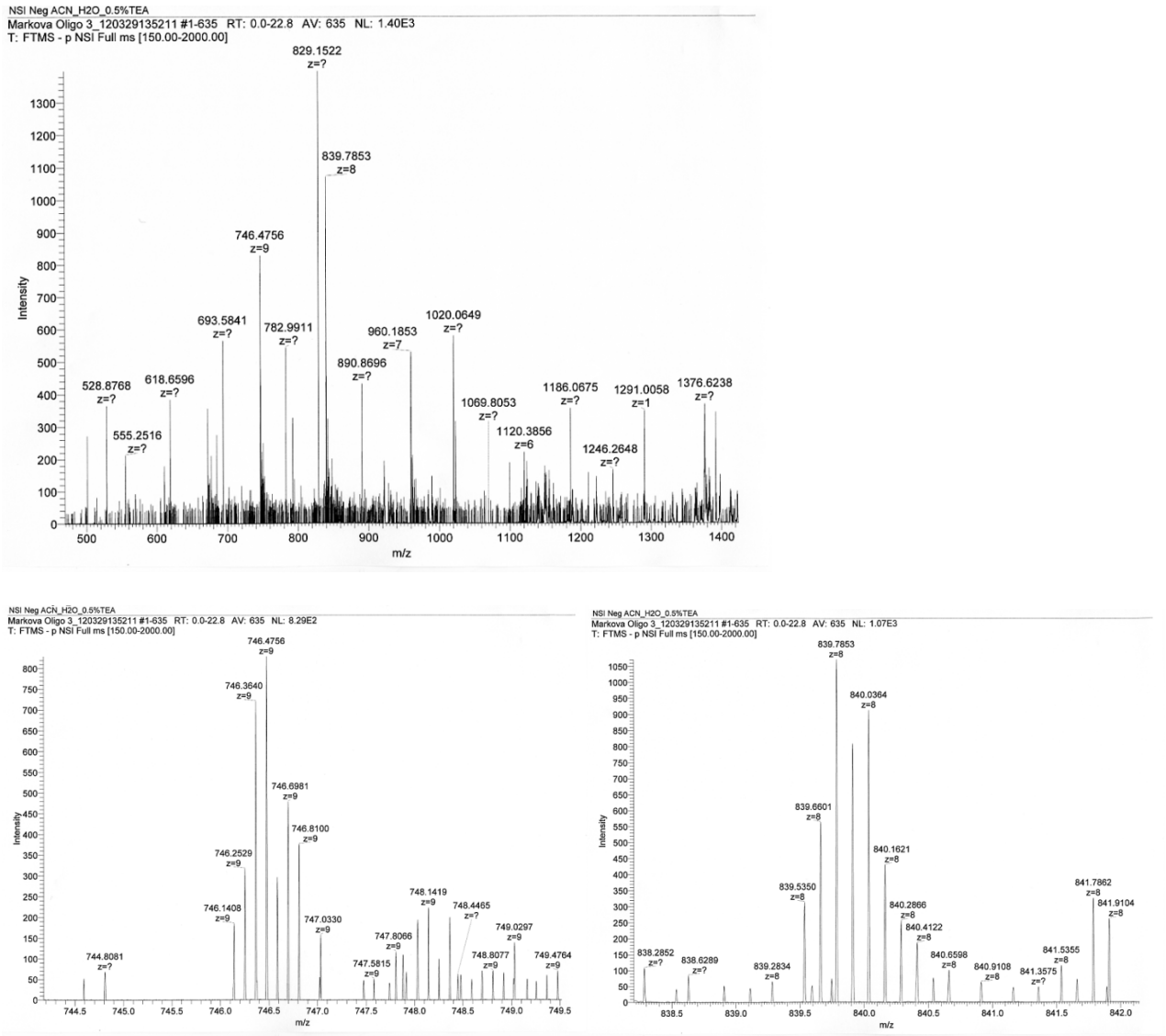


Figure S3. HR-MS of the **ON5.**

4. Excitation spectra

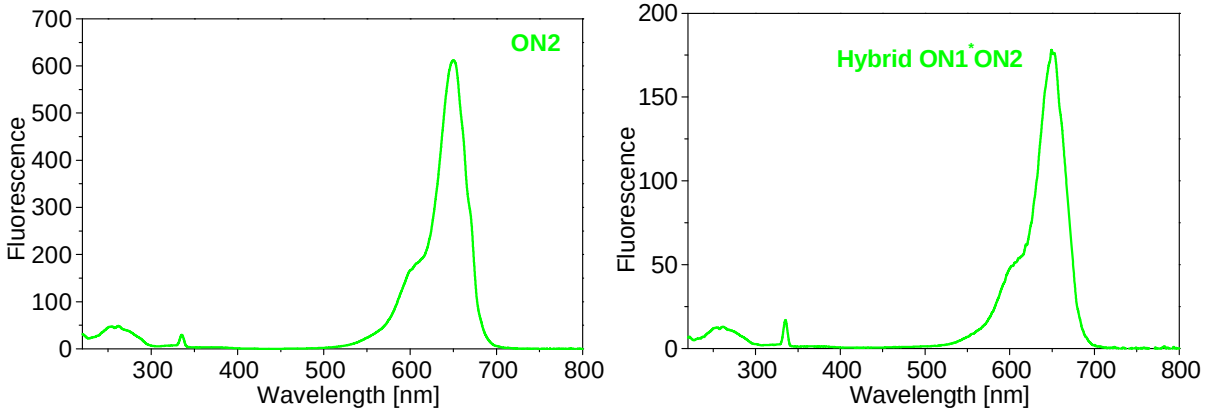


Figure S4. Excitation spectra of the oligonucleotide **ON2** (left) and duplex **ON1*ON2** (right). λ_{em} = 670 nm.

5. Influence of the temperature on the absorption and emission spectra of Cy5 containing oligonucleotide ON2 and duplex ON1*ON2

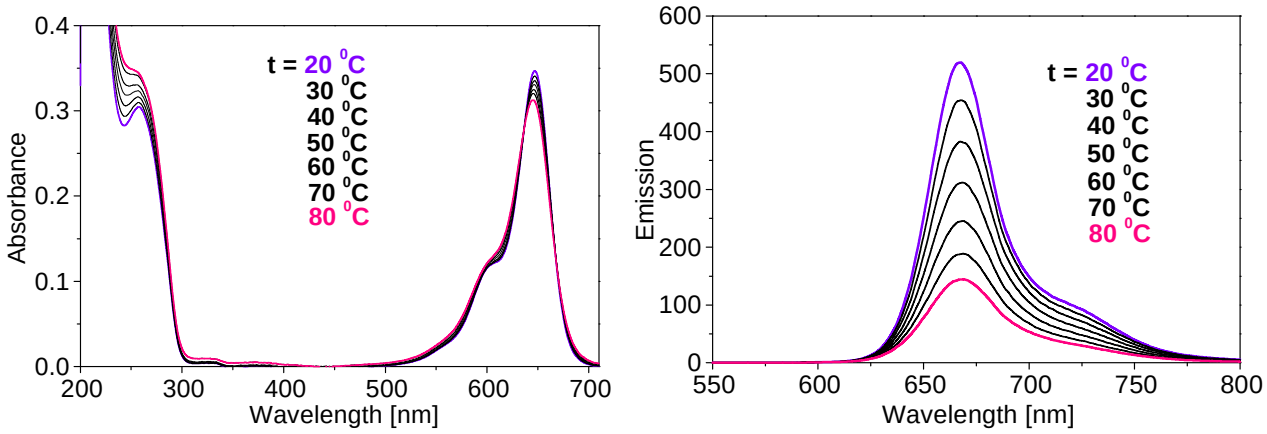


Figure S5. Influence of the temperature on the absorption and emission spectrum of the **Cy5** containing oligonucleotide **ON2** in the temperature range 20-80 ºC.

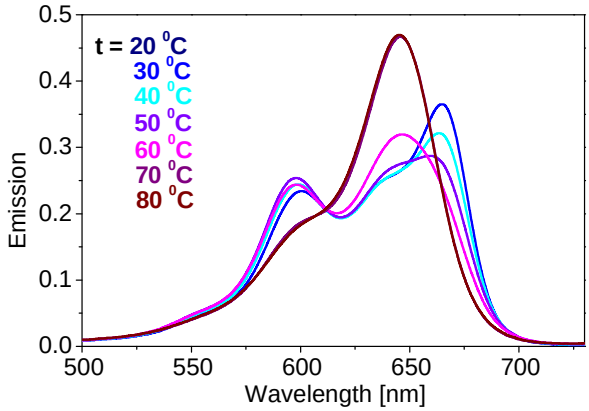


Figure S6. Influence of the temperature on the absorption spectrum of the **Cy5** containing duplex **ON1*ON2** in the temperature range 20-80 ºC.

6. Tm experiments

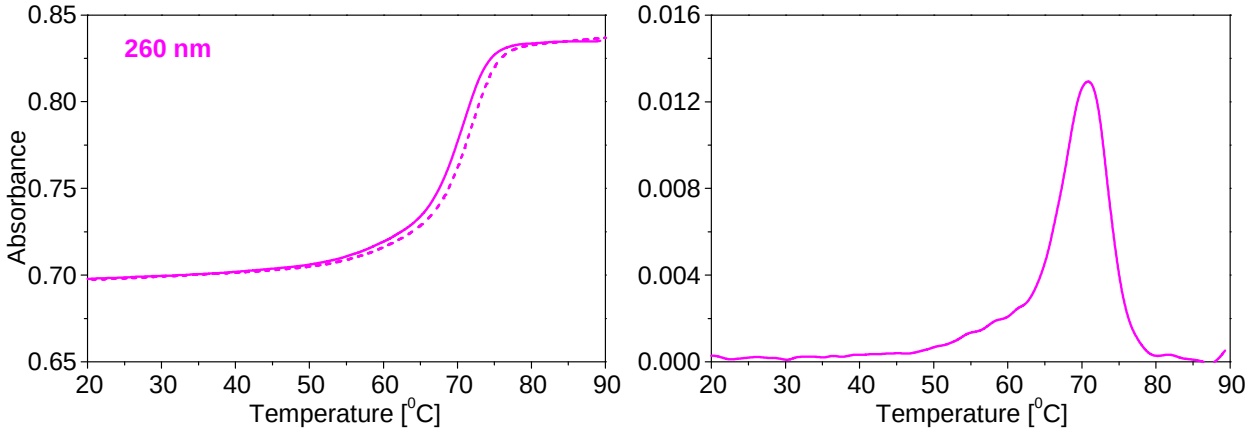


Figure S7. Example of Tm determination procedure: duplex **ON3*ON4**, melting curves at 260 nm (left) and first derivative curve (right), Tm = 70.7 °C (right).

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)*.

Oligomer	Duplex	Tm, °C	ΔTm, °C
ON3	5’ AGCTCGGTCA**T**CGAGAGTGCA	70.7	–
ON4	3’ TCGAGCCAGT**A**GCTCTCACGT		
ON1	5’ AGCTCGGTCA**Cy5**CGAGAGTGCA	66.7	- 4.0
ON2	3’ TCGAGCCAGT**Cy5**GCTCTCACGT		

* from the first derivative of the cooling (90 °C – 20 ºC) curve

7. Quantitative analysis of asymmetry of exciton couplet:

7.1 Fitting of absorbance and CD bands shape.

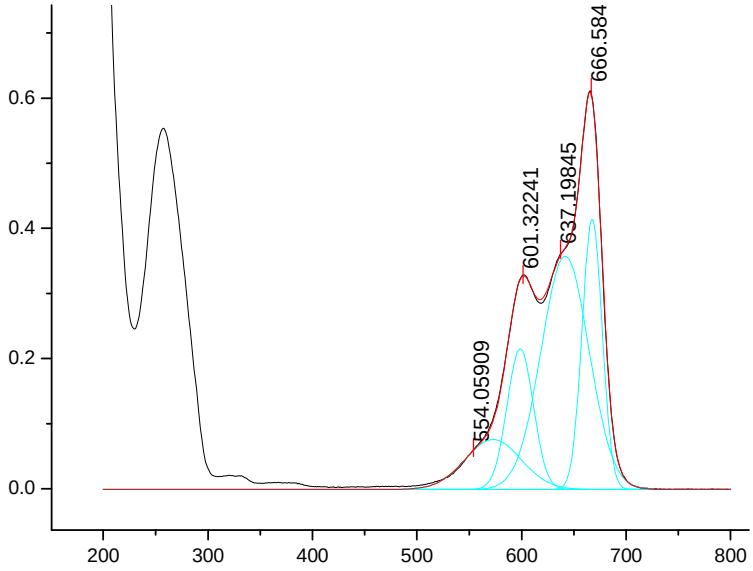
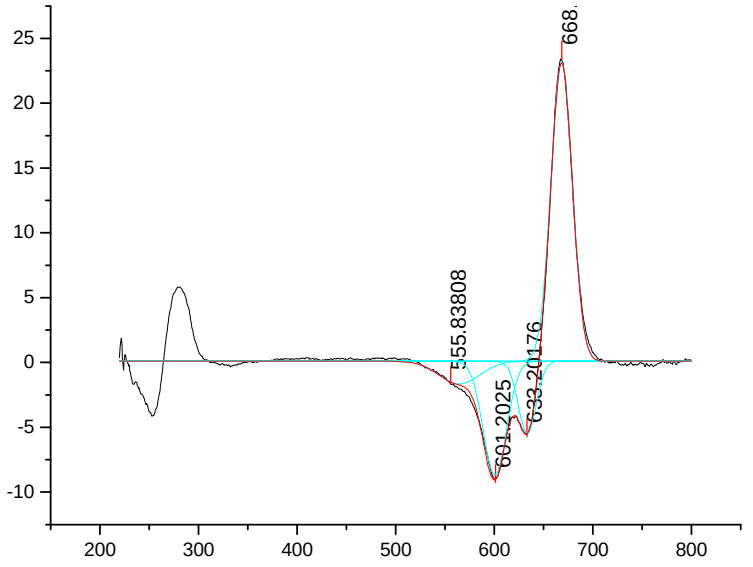


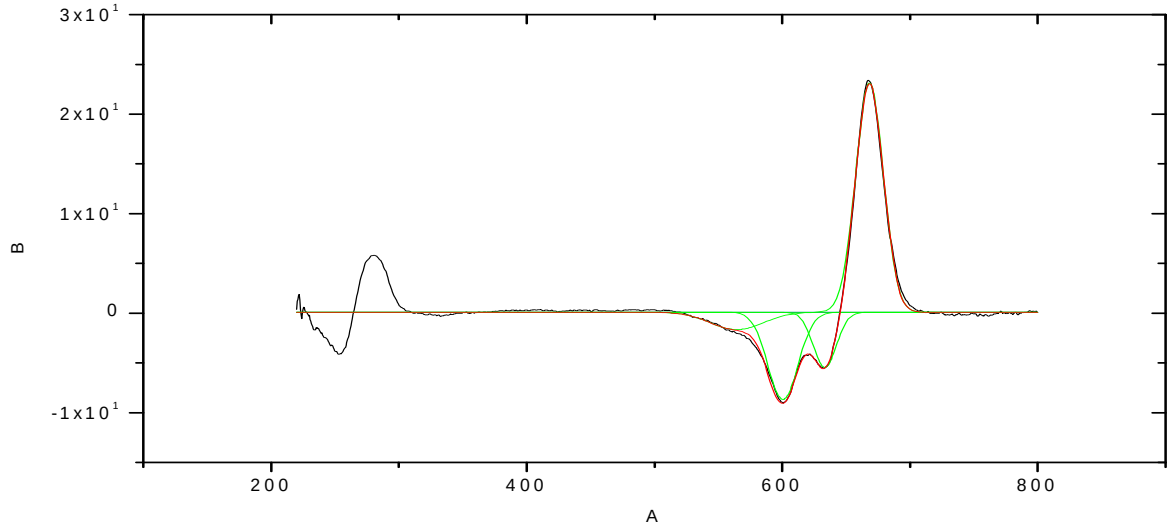
Figure S8. Fitting of absorbance band shape of **Cy-Cy** containing hybrid.



Peak Analysis

Data Set:[DUPL20CCOR]Sheet1!B Date:17.03.2013

Chi^2=1.57595E+000 Adj. R-Square=9.31557E-001 # of Data Points=1161
SS=1.81076E+003 Degree of Freedom=1149.

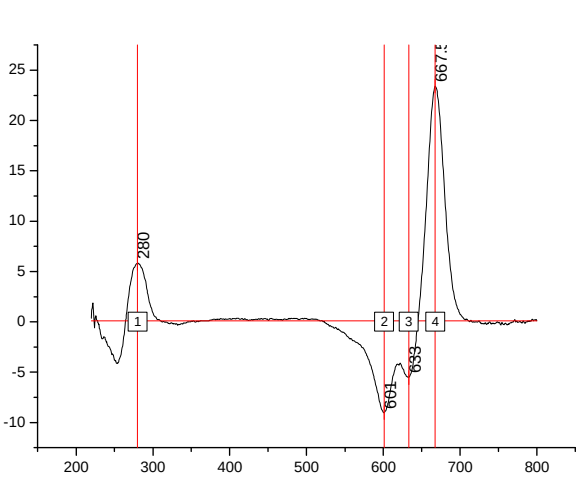


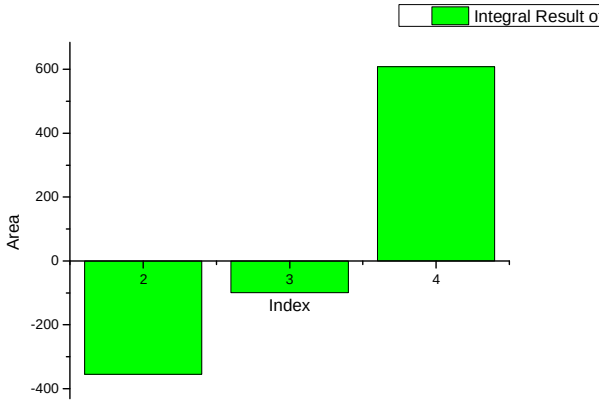
Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1.	Gaussian	-90.69806	48.77989	-1.74673	564.54777	-8.60082
2.	Gaussian	-259.87933	27.87716	-8.75772	600.55963	-24.64415
3.	Gaussian	-125.87017	21.3651	-5.53459	633.34685	-11.93617
4.	Gaussian	622.59554	25.40534	23.02231	668.2994	59.04023

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

7.2 Integration of CD signals.





Integral Result of B	Integral Result of B	Integral Result of B	Integral Result of B	Integral Result of B	Integral Result of B	Integral Result of B	Integral Result of B	Integral Result of B
Index	Area	AreaIntgP (%)	Row Index	Beginning X	Ending X	FWHM	Center	Height
1	45.8718	3.42315	120	228	309.5	23.70212	280	5.7309
2	-354.14384	-26.42773	762	521	622	31.77151	601	-9.12614
3	-99.23928	-7.40566	826	622	645	19.21005	633	-5.62839
4	607.59975	45.3417	895	645	710.5	24.58379	667.5	23.32792

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]:

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

$$g = \frac{CD}{A}$$

Where $\mathbf{A^L}$ and $\mathbf{A^R}$ 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, **Θ** (mdeg):

Θ (mdeg) = ~33000 CD = ~33000($A^L - A^R$)

Therefore value of CD, or **($A^L - A^R$)** can be obtained from experimental data as **Θ (mdeg) / 33000**

Importantly, **g**-value calculation as **g = ($A^L - A^R$) /A** does not required concentration or extinction coefficient (ε), when CD and absorbance are measured for the same sample. This is very valuable, because concentration and/or exact ***ε*** 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 couplet.

Experimental data for **ON1-ON2** hybrid at 20 ºC:

A(668)= 0.59678 ; A(633)= 0.34578 A(601)= 0.32854

CD(668)= 23.3657 ;CD(633)= -5.53441 ; CD(601)= -9.03216

g(668)= 7.01 x 10^{-4} ; g(633)= -1.68 x 10^{-4} ; g(601)= -2.74 x 10^{-4}

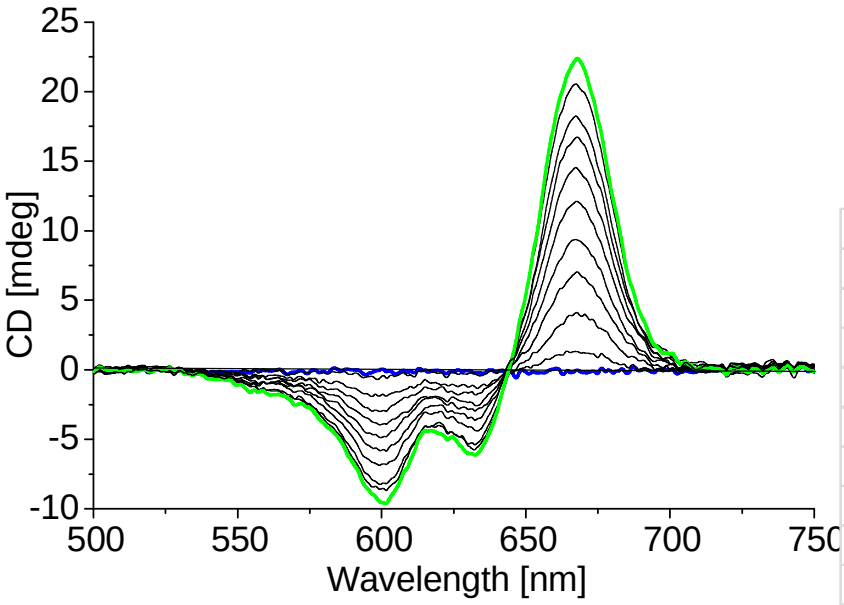
that gives $\mathbf{g}_{(668nm)}/\mathbf{g}_{(633nm)}$= 4.17 and $\mathbf{g}_{(668nm)}/\mathbf{g}_{(601nm)}$ = 2.56

4 N. Berova, L. Di Bari, G. Pescitelli, Chem.Soc.Rev., 2007, 36, 914-931.

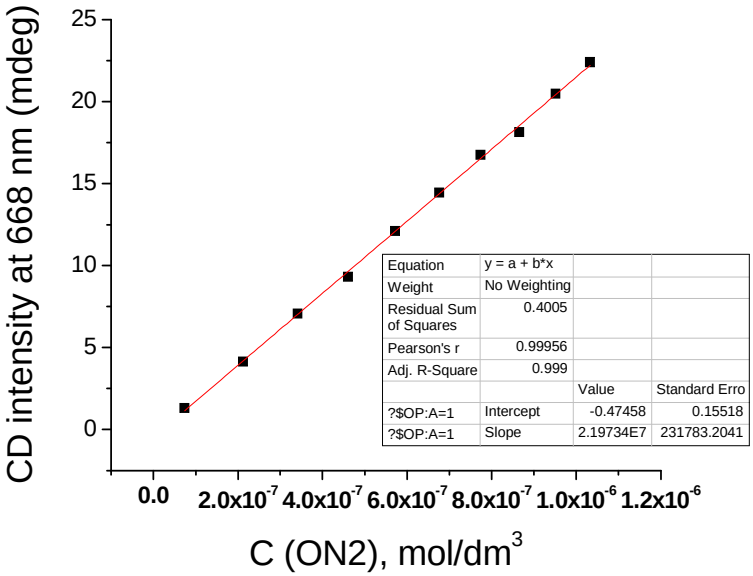
8. Titration experiment.

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**.



7.35294E-08	1.318126
2.12264E-07	4.140826
3.40909E-07	7.066873
4.60526E-07	9.312865
5.72034E-07	12.10704
6.7623E-07	14.44468
7.7381E-07	16.76052
8.65385E-07	18.14449
9.51493E-07	20.48726
1.03261E-06	22.40637



9. CD spectra of the oligonucleotides ON2 and ON5 and hybrids ON3*ON5 and ON3*ON4

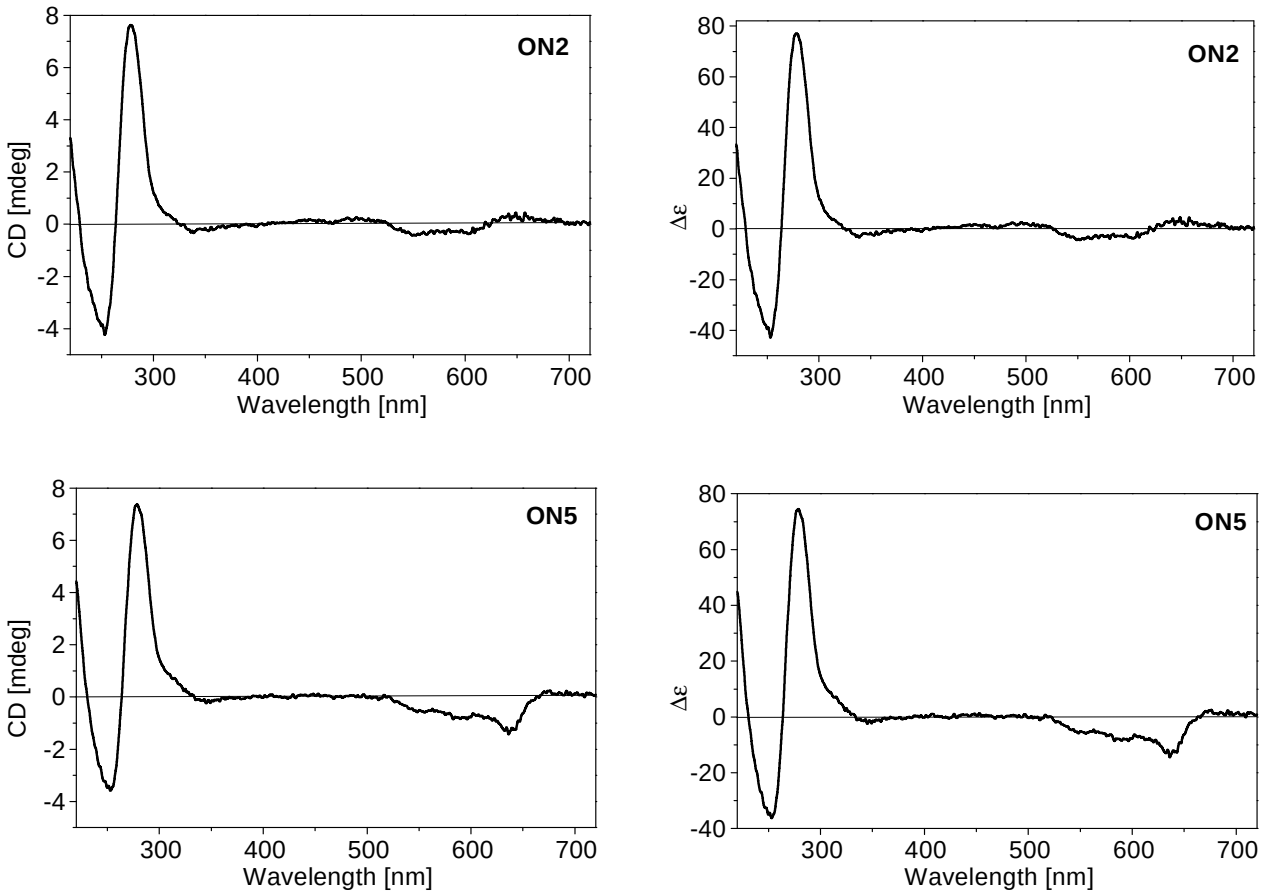


Figure S10. The CD spectra of the oligonucleotides **ON2** and **ON5** (conditions: 1.5 μM single strand conc., 10 mM phosphate, pH = 7.4, 100 mM NaCl).

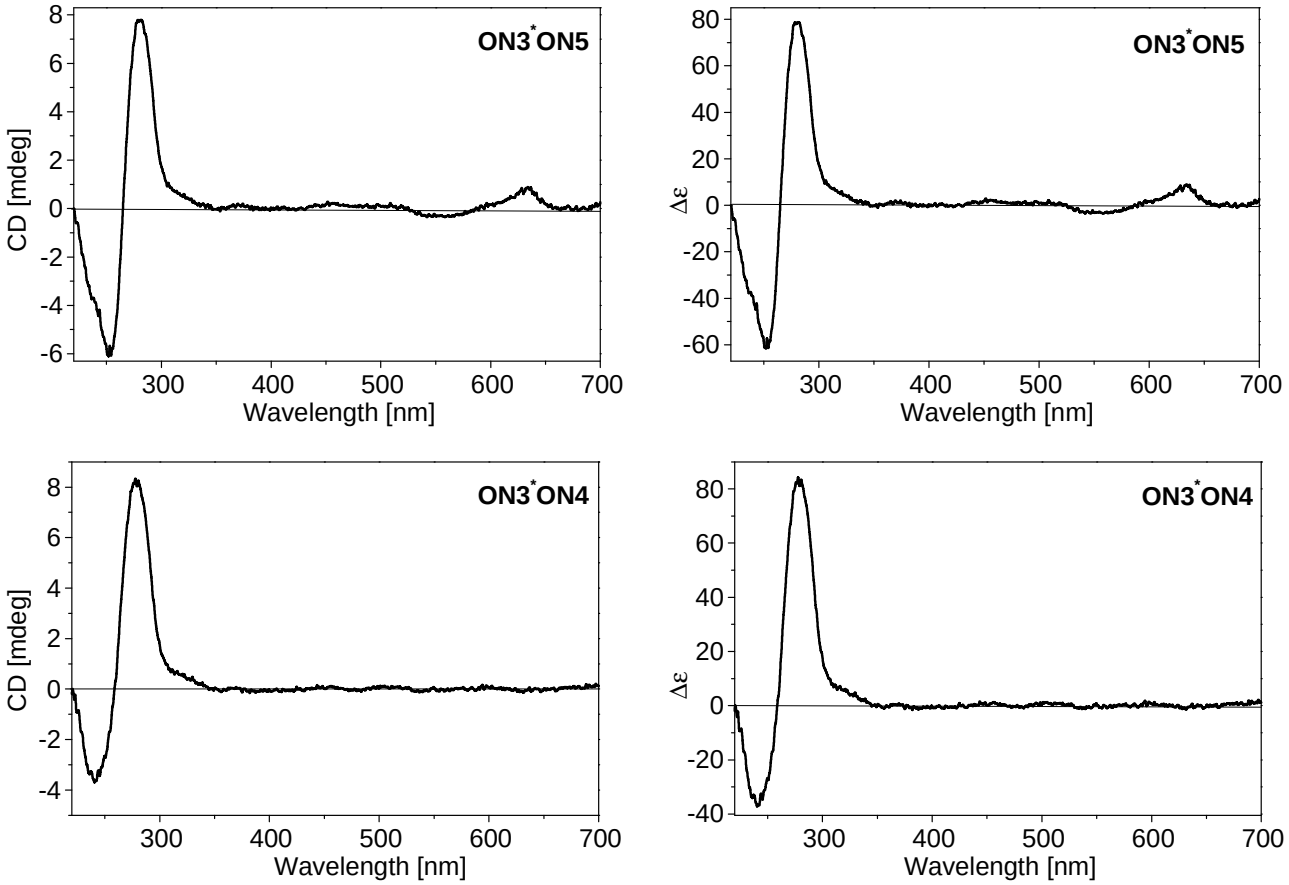


Figure S11. The CD spectra of the hybrids **ON3*ON5** and **ON3*ON4** (conditions: 1.5 μM each strand conc., 10 mM phosphate, pH = 7.4, 100 mM NaCl).