

# **CHEMISTRY**

---

## **A EUROPEAN JOURNAL**

### Supporting Information

© Copyright Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, 2012

#### **Interfacing Click Chemistry with Automated Oligonucleotide Synthesis for the Preparation of Fluorescent DNA Probes Containing Internal Xanthene and Cyanine Dyes**

**I. Kira Astakhova\* and Jesper Wengel<sup>[a]</sup>**

chem\_201202621\_sm\_miscellaneous\_information.pdf

## Supporting Information

# Interfacing click chemistry with automated oligonucleotide synthesis for preparation of fluorescent DNA probes containing internal xanthene and cyanine dyes

*I. Kira Astakhova\*<sup>1</sup> and Jesper Wengel*

### Contents

RP HPLC profile and MALDI-TOF spectrum of the crude product <b>ON2</b> (Figure S1)	S3
HPLC profile and MALDI-TOF spectrum of the crude product <b>ON4</b> (Figure S2)	S3
IE HPLC profiles of the purified Dmt-Off products <b>ON1</b> and <b>ON2</b> (B) (Figure S3)	S4
Photophysical properties of single molecule fluorescent dyes used in this study (Table S1)	S5
Single-labeled reference oligonucleotides <b>ON20–ON37</b> (Table S2)	S6
IE HPLC profiles of double-labeled oligonucleotides <b>ON6–ON17</b> (Figure S4)	S7
Representative thermal denaturation curves of the reference unmodified and double-labeled duplexes (Figure S5)	S8
Thermal denaturation temperatures for duplexes of <b>ON4</b> , <b>ON19</b> and DNA/RNA complements containing single-base mismatches (Table S3)	S9
Representative CD spectra of oligonucleotides and duplexes prepared in this study (Figure	S10

---

<sup>1</sup> Corresponding author. E-mail: [ias@sdu.dk](mailto:ias@sdu.dk)

S6)

Photophysical characteristics of single-stranded modified oligonucleotides and their duplexes: UV-visible absorption and fluorescence maxima (Table S4) S11

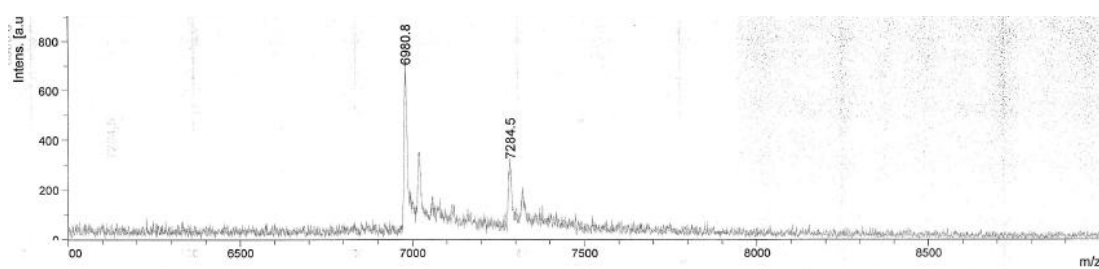
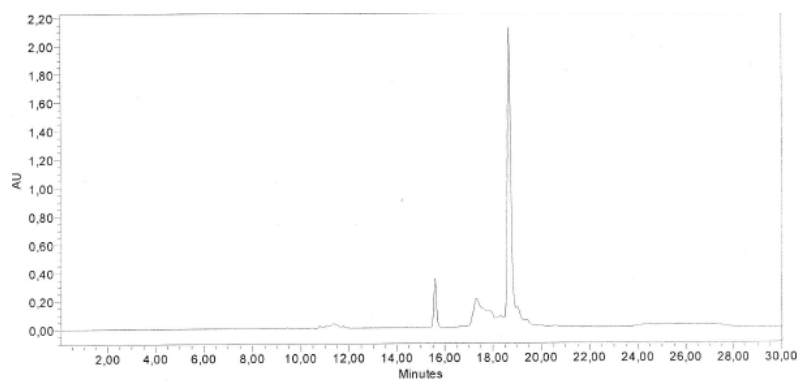
Photophysical characteristics of single-stranded modified oligonucleotides and their duplexes: fluorescence quantum yields and fluorescence brightness values (Table S5) S12

Molar extinction coefficients and fluorescence brightness values of double-labeled oligonucleotides and their duplexes with complementary DNA and RNA targets (Table S6) S13

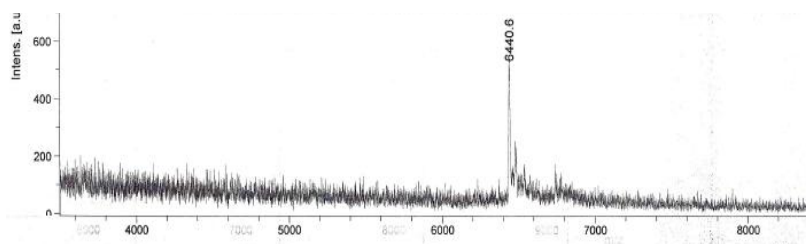
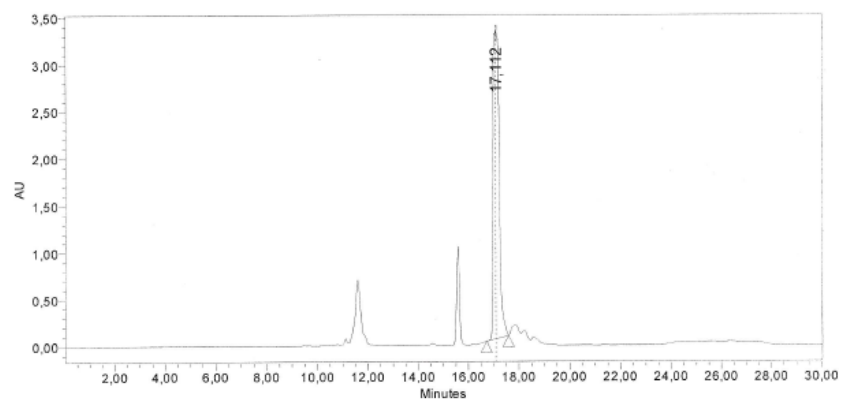
Representative visible absorbance and excitation spectra of double-labeled oligonucleotides and their duplexes with complementary DNA/RNA (Figure S7) S14

Representative steady-state emission spectra of double-labeled oligonucleotides and their duplexes with complementary DNA and RNA (Figure S8) S15

**Figure S1. RP HPLC profile (254 nm) and MALDI-TOF spectrum of the crude product ON2.**

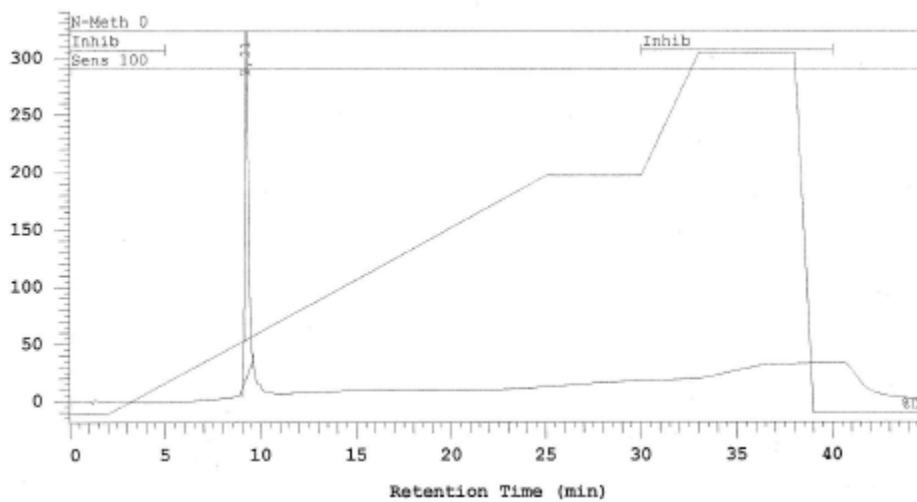


**Figure S2. RP HPLC profile (254 nm) and MALDI-TOF spectrum of the crude product ON4.**

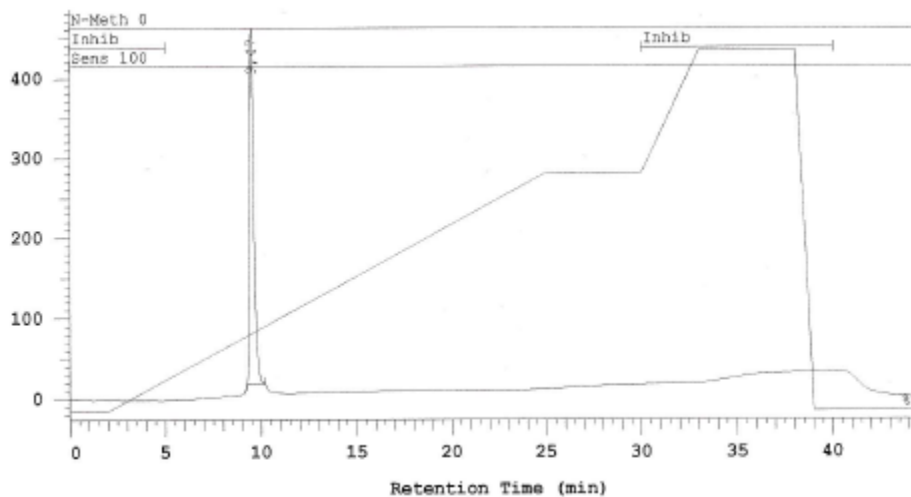


**Figure S3. IE HPLC profiles (254 nm) of the purified Dmt-Off products ON1 (A) and ON2 (B).**

A)



B)



**Table S1.** Photophysical characteristics of single molecule dyes used in this study.<sup>a</sup>

Dye	$\lambda^{\text{ex}}$ , nm	$\lambda^{\text{fl}}_{\text{max}}$ , nm	$\epsilon_{\text{max}}$ , $\text{cm}^{-1}\cdot\text{M}^{-1}$	$\Phi_{\text{F}}$
Cy3	546	563	150.000	0.1
5-R110	496	520	80.000	0.9
5-JOE	520	548	71.000	0.6
6-ROX	570	591	82.000	0.7
Cy3.5	591	604	116.000	0.35
Cy5	646	662	250.000	0.2
Cy5.5	673	707	209.000	0.2

<sup>a</sup>This information is available online, e.g. on web-page of fluorescent dyes supplier Lumiprobe LLC: <http://www.lumiprobe.com/>

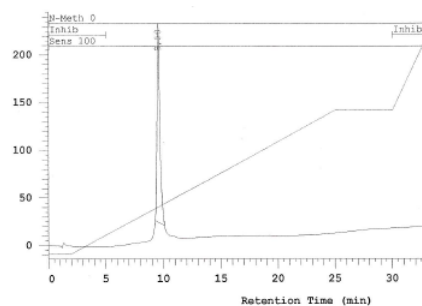
**Table S2. Single-labeled reference oligonucleotides ON20–ON37.\***

code	Sequence, 5'→3'
ON20	TGC ACT CTA M <sup>1</sup> GT CTG TAT CAT
ON21	TGC ACM <sup>1</sup> CTA TGT CTG TAT CAT
ON22	TGC ACT CTA TGT CM <sup>1</sup> G TAT CAT
ON23	TGC ACT CTA TGT CTG TAM <sup>1</sup> CAT
ON24	TGC ACT CTA M <sup>3</sup> GT CTG TAT CAT
ON25	TGC ACT CTA M <sup>4</sup> GT CTG TAT CAT
ON26	TGC ACT CTA M <sup>5</sup> GT CTG TAT CAT
ON27	TGC ACT CTA M <sup>6</sup> GT CTG TAT CAT
ON28	TGC ACT CTA M <sup>7</sup> GT CTG TAT CAT
ON29	TGC ACT CTA M <sup>8</sup> GT CTG TAT CAT
ON30	TGC ACM <sup>3</sup> CTA TGT CTG TAT CAT
ON31	TGC ACM <sup>4</sup> CTA TGT CTG TAT CAT
ON32	TGC ACM <sup>5</sup> CTA TGT CTG TAT CAT
ON33	TGC ACM <sup>6</sup> CTA TGT CTG TAT CAT
ON34	TGC ACM <sup>7</sup> CTA TGT CTG TAT CAT
ON35	TGC ACM <sup>8</sup> CTA TGT CTG TAT CAT
ON36	TGC ACT CTA TGT CM <sup>2</sup> G TAT CAT
ON37	TGC ACT CTA TGT CTG TAM <sup>2</sup> CAT

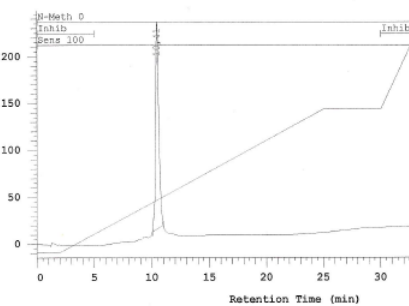
\* Oligonucleotides **ON20–ON23** were used as starting material for preparation of the corresponding fluorescent probes **ON24–ON37**.

**Figure S4. IE HPLC profiles (254 nm) of double-labeled oligonucleotides ON6–ON17.**

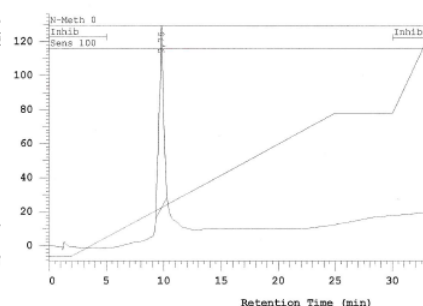
**ON6**



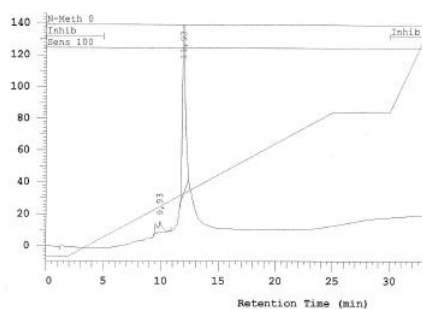
**ON7**



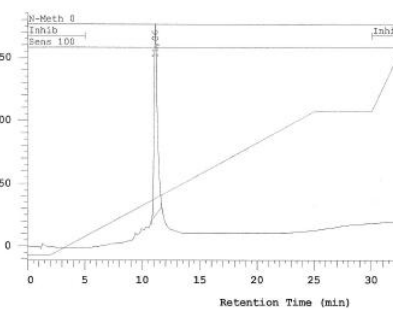
**ON8**



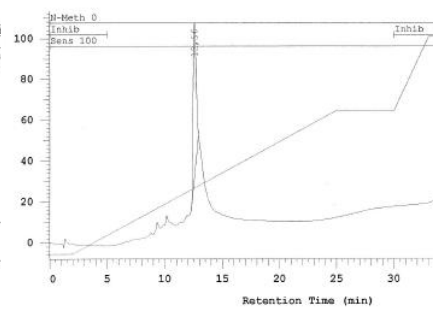
**ON9**



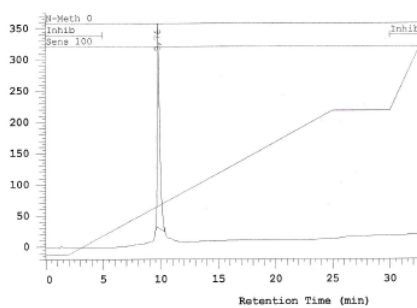
**ON10**



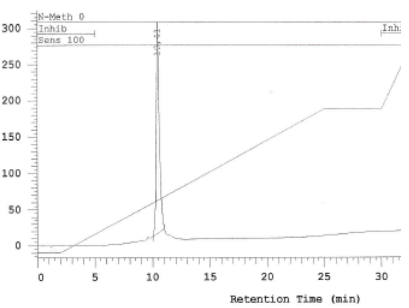
**ON11**



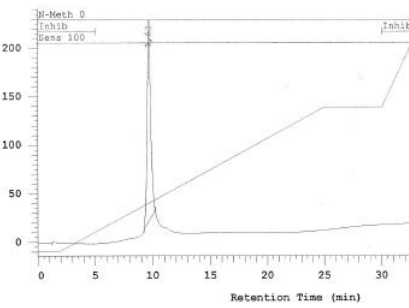
**ON12**



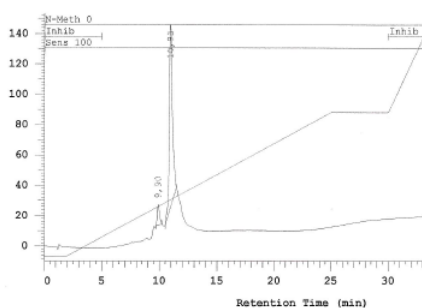
**ON13**



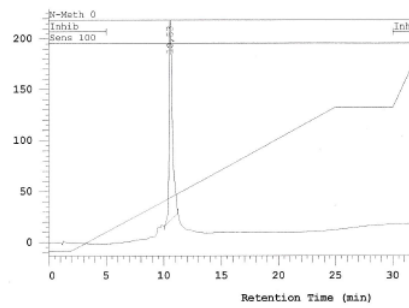
**ON14**



**ON15**



**ON16**



**ON17**

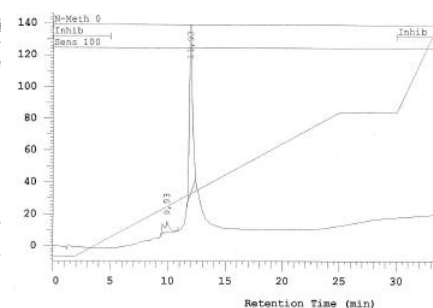
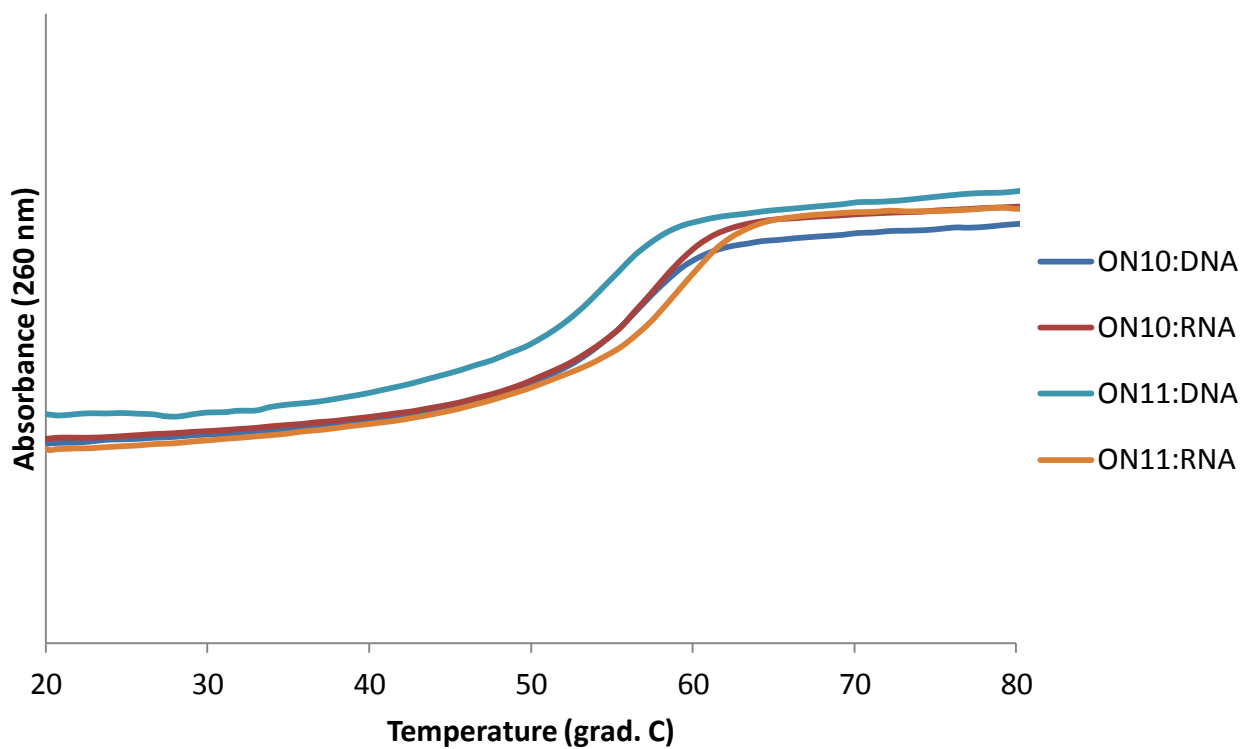
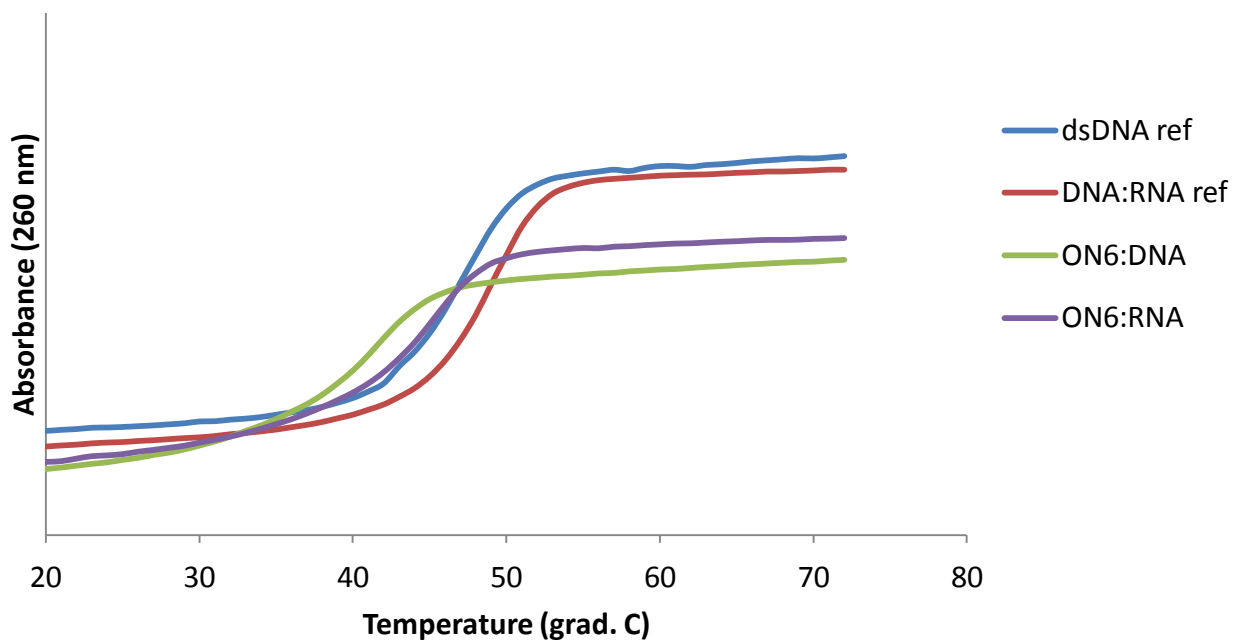




Figure S5. Representative thermal denaturation curves of the reference unmodified and double-labeled duplexes.

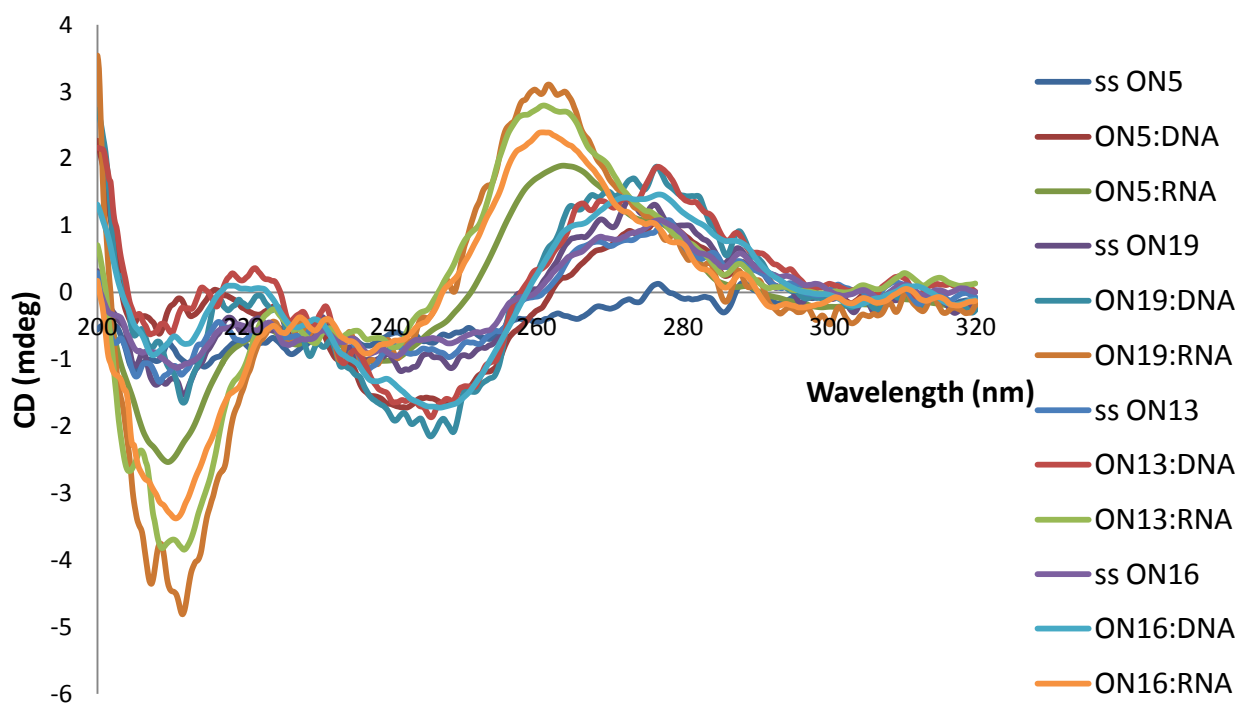


**Table S3. Thermal denaturation temperatures for duplexes of ON4, ON19 and DNA/RNA targets containing single-base mismatches.<sup>a</sup>**

ON:TARGET*	$T_m / ^\circ\text{C}$							
	DNA target				RNA target			
	B:	A	C	T	G	A	C	U
<b>ON4</b>								
5' - TGC ACM <sup>1</sup> CTA TGT CTG TAM <sup>1</sup> CAT 3' - ACG TGA GAT ACA GAC ATA <b>B</b> TA	52.5	52.5	52.5	56.0	57.0	58.0	57.0	61.0
5' - TGC ACM <sup>1</sup> CTA TGT CTG TAM <sup>1</sup> CAT 3' - ACG TGA GAT ACA GAC AT <b>B</b> GTA	56.0	51.0	50.0	51.5	61.0	57.0	57.0	58.5
5' - TGC ACM <sup>1</sup> CTA TGT CTG TAM <sup>1</sup> CAT 3' - ACG TGA GAT AC <b>B</b> GAC ATA GTA	56.0	48.0	48.0	52.5	61.0	53.0	53.0	58.0
5' - TGC ACM <sup>1</sup> CTA TGT CTG TAM <sup>1</sup> CAT 3' - ACG TGA <b>B</b> AT ACA GAC ATA GTA	47.0	45.0	46.0	56.0	50.0	47.0	48.0	61.0
5' - TGC ACM <sup>1</sup> CTA TGT CTG TAM <sup>1</sup> CAT 3' - ACG TG <b>B</b> GAT ACA GAC ATA GTA	56.0	48.0	50.0	51.0	61.0	52.0	52.0	57.5
<b>ON19</b>								
5' - TGC ACM <sup>9</sup> CTA TGT CTG TAM <sup>9</sup> CAT 3' - ACG TGA GAT ACA GAC ATA <b>B</b> TA	53.0	53.0	53.0	55.0	58.0	58.0	58.0	58.0
5' - TGC ACM <sup>9</sup> CTA TGT CTG TAM <sup>9</sup> CAT 3' - ACG TGA GAT ACA GAC AT <b>B</b> GTA	55.0	52.0	52.0	53.0	58.0	58.0	58.0	59.0
5' - TGC ACM <sup>9</sup> CTA TGT CTG TAM <sup>9</sup> CAT 3' - ACG TGA GAT AC <b>B</b> GAC ATA GTA	55.0	55.0	55.0	54.0	58.0	51.0	53.0	58.0
5' - TGC ACM <sup>9</sup> CTA TGT CTG TAM <sup>9</sup> CAT 3' - ACG TGA <b>B</b> AT ACA GAC ATA GTA	55.0	55.0	55.0	55.0	51.0	48.0	48.0	58.0
5' - TGC ACM <sup>9</sup> CTA TGT CTG TAM <sup>9</sup> CAT 3' - ACG TG <b>B</b> GAT ACA GAC ATA GTA	55.0	49.0	50.0	53.0	58.0	51.0	53.0	58.0

<sup>a</sup> The melting temperatures of the unmodified double stranded DNA and DNA:RNA are 59.5 °C and 61.0 °C, respectively. Melting temperatures of complementary complexes are indicated in green. \* Herein, the sequences of DNA target variants are presented.

**Figure S6. Representative CD spectra of oligonucleotides and duplexes prepared in this study.**



**Table S4. Photophysical characteristics of single-stranded modified oligonucleotides (SSP) and their duplexes: UV-visible absorption and fluorescence maxima.\***

#	$\lambda_{\max}^{abs}$ (nm)			$\epsilon_{\max}$ , $\text{cm}^{-1}\cdot\text{M}^{-1}$			$\lambda_{\max}^f$ (nm)		
	SSP	DNA	RNA	SSP	DNA	RNA	SSP	DNA	RNA
<b>ON24</b>	511	509	509	70.300	67.100	65.100	530	526	525
<b>ON25</b>	528	522	522	63.900	56.000	71.100	553	546	548
<b>ON26</b>	586	586	586	59.700	60.100	56.400	605	602	604
<b>ON27</b>	595, 557	589, 550	589, 550	83.400	65.900	83.100	605	602	602
<b>ON28</b>	646, 600	646, 600	644, 600	197.700	213.100	191.800	–	–	–
<b>ON29**</b>	691, 638	684, 637	684, 637	102.000	100.000	94.400	–	–	–
<b>ON30</b>	508	508	508	72.700	64.600	73.400	528	523	525
<b>ON31</b>	528	521	521	69.700	74.300	77.600	553	547	548
<b>ON32</b>	589	589	584	69.400	71.900	55.800	606	606	606
<b>ON33</b>	594, 554	593, 550	589, 552	86.800	60.300	84.200	606	601	599
<b>ON34</b>	647	647	642	207.000	197.500	188.400	–	–	–
<b>ON35**</b>	689, 635	684, 637	684, 637	109.500	94.000	90.400	–	–	–
<b>ON36</b>	550, 518	548, 516	548, 516	118.000	128.000	100.100	560	560	560
<b>ON37</b>	548, 516	547, 516	547, 516	119.000	127.000	109.000	560	560	560

\* UV-visible absorbance and fluorescence studies were performed as described for oligonucleotides **ON6–ON17** using excitation wavelengths: 500 nm (monomer  $\text{M}^3$ : **ON24**, **ON30**), 520 nm (monomers  $\text{M}^4$  and  $\text{M}^5$ : **ON25–ON26** and **ON31–ON32**), and 546 nm (monomers  $\text{M}^6$ – $\text{M}^8$ : **ON27–ON29** and **ON33–ON35**). **ON36** and **ON37** were studied using excitation wavelengths 500, 520 and 546 nm, since they represent references as both donors and acceptors FRET. Resulting photophysical data was similar for abovementioned excitation wavelengths. \*\* No fluorescence emission band was observed even upon increasing probes' concentration up to 10  $\mu\text{M}$  and using excitation wavelength of Cy5.5 dye at 673 nm.

**Table S5. Photophysical characteristics of single-stranded modified oligonucleotides (SSP) and their duplexes: fluorescence quantum yields and fluorescence brightness values.\***

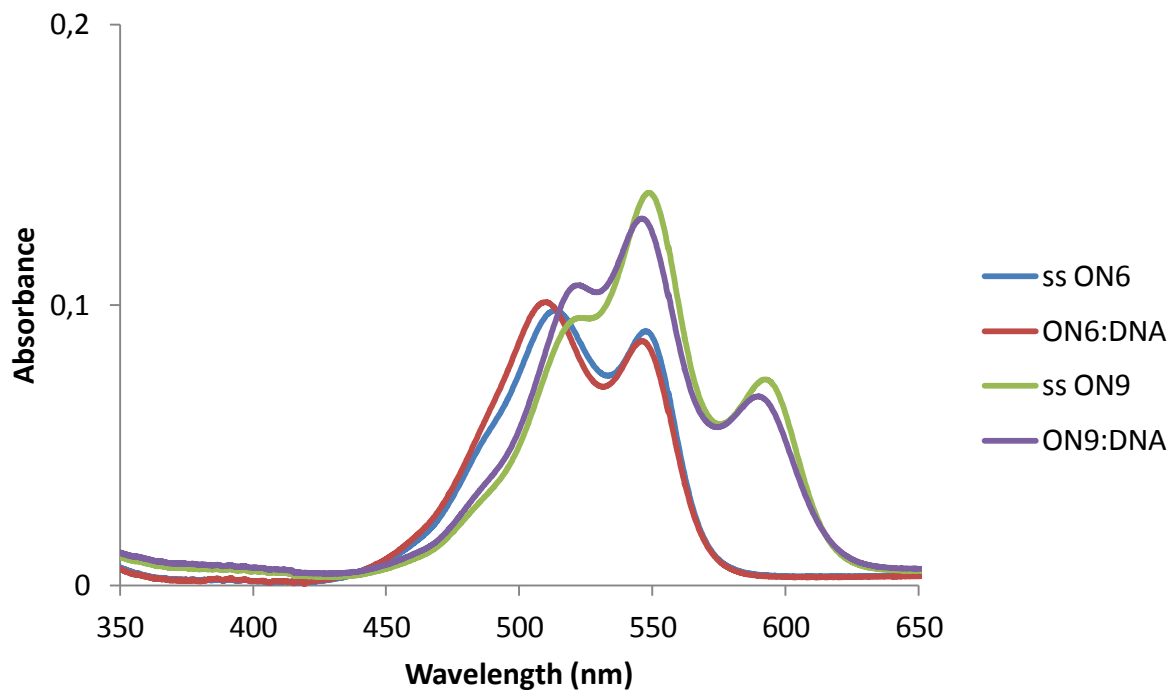
#	$\Phi_F$			FB		
	<i>SSP</i>	<i>DNA</i>	<i>RNA</i>	<i>SSP</i>	<i>DNA</i>	<i>RNA</i>
<b>ON24</b>	0.53	0.47	0.65	38	32	42
<b>ON25</b>	0.57	0.74	0.64	36	42	45
<b>ON26</b>	0.10	0.10	0.07	6	6	4
<b>ON27</b>	0.07	0.06	0.04	6	4	4
<b>ON28</b>	0.01	0.02	0.01	20	42	19
<b>ON29</b>	0.02	0.01	0.01	20	10	9
<b>ON30</b>	0.78	0.73	0.95	57	47	70
<b>ON31</b>	0.68	0.75	0.63	47	55	49
<b>ON32</b>	0.11	0.09	0.01	8	7	1
<b>ON33</b>	0.04	0.07	0.03	4	4	2
<b>ON34</b>	0.01	0.02	0.02	20	40	38
<b>ON35</b>	0.02	0.02	0.03	22	18	27
<b>ON36</b>	0.27	0.12	0.12	32	15	12
<b>ON37</b>	0.21	0.22	0.17	25	28	18

**Table S6. Molar extinction coefficients and fluorescence brightness (FB) values of double-labeled oligonucleotides (SSP) and their duplexes with complementary DNA and RNA targets.**

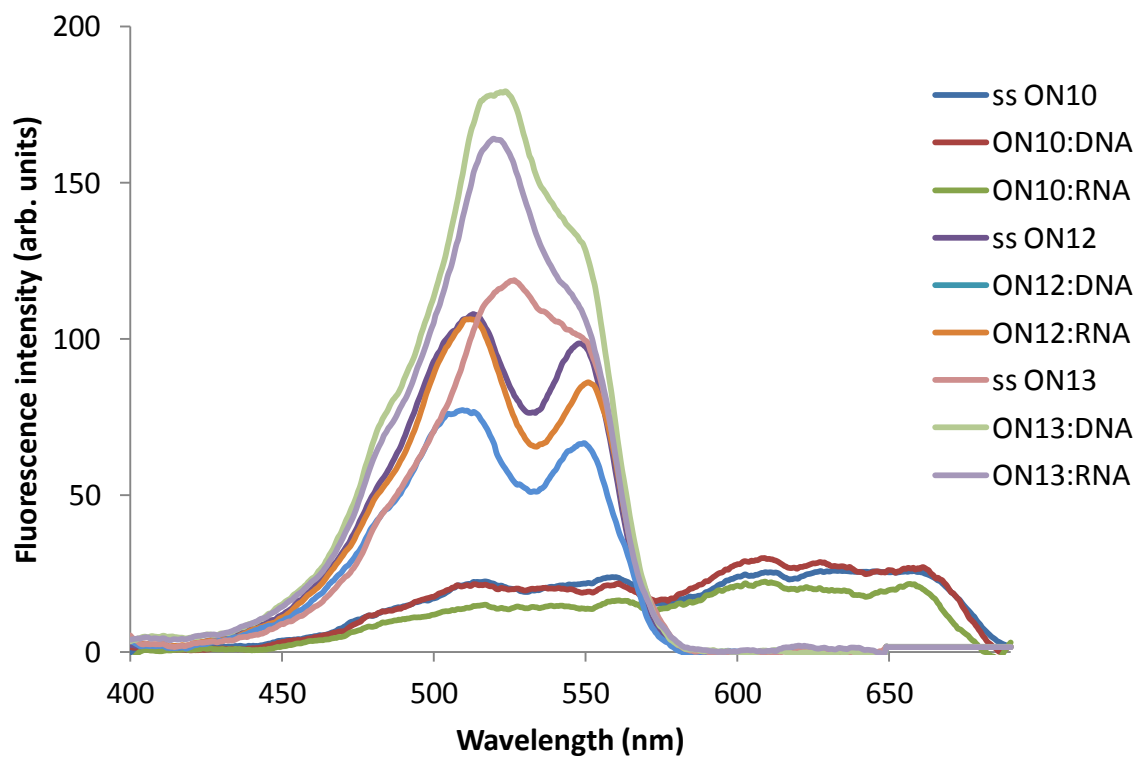
#	$\epsilon_{\max}, \text{cm}^{-1} \cdot \text{M}^{-1}$			FB		
	<i>SSP</i>	<i>DNA</i>	<i>RNA</i>	<i>SSP</i>	<i>DNA</i>	<i>RNA</i>
<b>ON6</b>	78.800	78.900	78.400	14	8	5
<b>ON7</b>	98.200	98.000	98.200	13	16	15
<b>ON8</b>	76.700	76.700	76.500	6	4	5
<b>ON9</b>	120.100	120.100	120.100	9	10	5
<b>ON10</b>	142.300	142.200	142.300	5	6	4
<b>ON11</b>	133.200	133.000	133.300	4	6	3
<b>ON12</b>	105.300	105.600	106.000	10	20	20
<b>ON13</b>	112.000	129.000	129.000	11	26	21
<b>ON14</b>	92.800	103.000	103.000	1	9	11
<b>ON15</b>	116.000	116.100	116.000	10	16	13
<b>ON16</b>	147.000	147.200	147.400	4	18	16
<b>ON17</b>	135.000	165.000	165.000	3	10	3

**Figure S7. Representative visible absorbance (A) and excitation (B) spectra of double-labeled oligonucleotides and their duplexes with complementary DNA/RNA.**

A)



B)



**Figure S8. Representative fluorescence spectra of double-labeled oligonucleotides and their duplexes with complementary DNA and RNA.**

