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Supporting Information

Highly Fluorescent Conjugated Pyrenes in Nucleic Acid Probes: (Phenylethynyl)pyrenecarbonyl-Functionalized LNA

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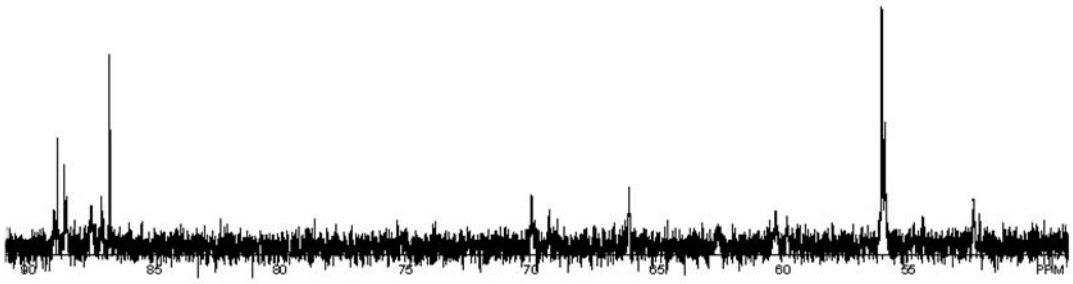
Contents

A)



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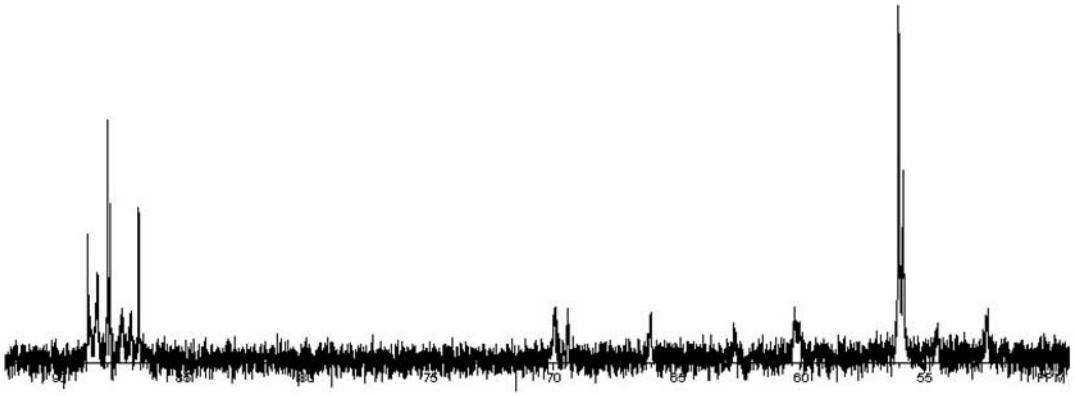


Figure S1. Sugar parts of ^{13}C NMR spectra of compounds **22a** (A) and **22e** (B) (75 MHz, DMSO-d_6, 8196 scans).

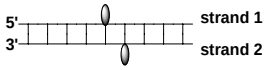
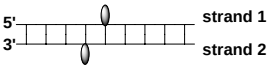
Table S1. MALDI-MS of synthesized oligonucleotides.[a]

#	Sequence, 5′→3′	Found *m/z* [M–H]	Calc. *m/z* [M–H]
ON1	GTG A**M**^{1}A TGC	3107	3109
ON2	GCA **M**^{1}AT CAC	3035	3038
ON3	GCA TA**M**^{1} CAC	3035	3038
ON4	GTG A**M**^{1}A **M**^{1}GC	3461	3465
ON5	GCA **M**^{1}A**M**^{1} CAC	3392	3394
ON6	GTG A**M**^{2}A TGC	3108	3109
ON7	GCA **M**^{2}AT CAC	3037	3038
ON8	GCA TA**M**^{2} CAC	3037	3038
ON9	GTG A**M**^{2}A **M**^{2}GC	3463	3465
ON10	GCA **M**^{2}A**M**^{2} CAC	3394	3394
ON11	GTG A**M**^{3}A TGC	3207	3209
ON12	GCA **M**^{3}AT CAC	3139	3138
ON13	GCA TA**M**^{3} CAC	3135	3138
ON14	GTG A**M**^{3}A **M**^{3}GC	3666	3665
ON15	GCA **M**^{3}A**M**^{3} CAC	3641	3644
ON16	GTG A**M**^{4}A TGC	3208	3209
ON17	GCA **M**^{4}AT CAC	3138	3138
ON18	GCA TA**M**^{4} CAC	3137	3138
ON19	GTG A**M**^{4}A **M**^{4}GC	3665	3665
ON20	GCA **M**^{4}A**M**^{4} CAC	3663	3661
ON21	GTG A**M**^{5}A TGC	3310	3309
ON22	GCA **M**^{5}AT CAC	3239	3238
ON23	GCA TA**M**^{5} CAC	3239	3238
ON24	GTG A**M**^{5}A **M**^{5}GC	3865	3866
ON25	GCA **M**^{5}A**M**^{5} CAC	3794	3794
ON26	CG^{L}T TT^{L}A T^{L}A**M**^{5} AT^{L}C A^{Me}C^{L} G	5255	5251
ON27	CG^{L}T TT^{L}A **M**^{5}A**M**^{5} AT^{L}C A^{Me}C^{L} G	5780	5779
ON28	CG^{L}T T**M**^{5}A T^{L}AT^{L} A**M**^{5}C A^{Me}C^{L} G	5803	5803

ON29	GTG A**M**^{6}A TGC	3306	3309
ON30	GCA **M**^{6}AT CAC	3234	3238
ON31	GCA TA**M**^{6} CAC	3237	3238
ON32	GTG A**M**^{6}A **M**^{6}GC	3868	3866
ON33	GCA **M**^{6}A**M**^{6} CAC	3791	3794
ON34	CG^{L}T TT^{L}A T^{L}A**M**^{6} AT^{L}C A^{Me}C^{L} G	5253	5251
ON37	CG^{L}T TT^{L}A T^{L}AT AT^{L}C A^{Me}C^{L} G	4679	4681
ON38	CG^{L}T TT^{L}A TAT AT^{L}C A^{Me}C^{L} G	4650	4653
ON39	CG^{L}T TTA T^{L}AT^{L} ATC A^{Me}C^{L} G	4680	4681
ON40	CG^{L}T GAT^{L}A**M**^{5}A TAA ACG	5184	5183
ON41	CG^{L}T GA**M**^{5}AT^{L}A TAA ACG	5179	5183

[a] T^{L} = thymin-1-yl LNA monomer, ^{Me}C^{L} = 5-methylcytosin-1-yl LNA monomer, G^{L} = guanin-9-yl LNA monomer; see Scheme 2 for the structures of monomers **M**^{1}–**M**^{6}.

Table S2. Thermal denaturation properties for "+1" and "–1 zipper" duplexes containing combined monomers $\mathbf{M^1}$–$\mathbf{M^6}$.[a]

	T_m/ºC	
	5′-d(GTG A**M**A TGC) 3′-d(GCA **M**AT CAC) 	5′-d(GTG A**M**A TGC) 3′-d(GCA TA**M** CAC) 

M:	strand 1											
strand 2	$\mathbf{M^1}$	$\mathbf{M^2}$	$\mathbf{M^3}$	$\mathbf{M^4}$	$\mathbf{M^5}$	$\mathbf{M^6}$	$\mathbf{M^1}$	$\mathbf{M^2}$	$\mathbf{M^3}$	$\mathbf{M^4}$	$\mathbf{M^5}$	$\mathbf{M^6}$
$\mathbf{M^1}$	**< 10**	24.0	34.0	37.0	< 10	38.0	**31.9**	38.0	29.0	35.5	37.0	36.0
$\mathbf{M^2}$	34.0	**39.5**	38.5	31.0	38.5	51.0	40.0	**60.0**	33.5	no clear transition	24.0	41.0
$\mathbf{M^3}$	37.0	30.0	**30.5**	23.0	44.0	no clear transition	30.0	< 10	**33.5**	no clear transition	42.0	34.0
$\mathbf{M^4}$	no clear transition	29.0	25.5	**31.0**	24.0	46.0	32.0	no clear transition	32.0	**34.0**	42.0	36.5
$\mathbf{M^5}$	35.0	28.0	44.0	31.0	**34.3**	37.5	31.0	40.0	33.0	36.0	**50.5**	42.0
$\mathbf{M^6}$	no clear transition	26.5	40.0	34.0	43.0	**36.5**	34.0	< 10	40.0	32.0	34.0	**40.0**

[a] Thermal denaturation temperatures recorded using the same conditions as described in Table 1. T_m values of the duplexes modified with the same fluorochrome in the both *strands 1, 2* are placed on the diagonal and set off in bold. The droplets represent monomers $\mathbf{M^1}$–$\mathbf{M^6}$.

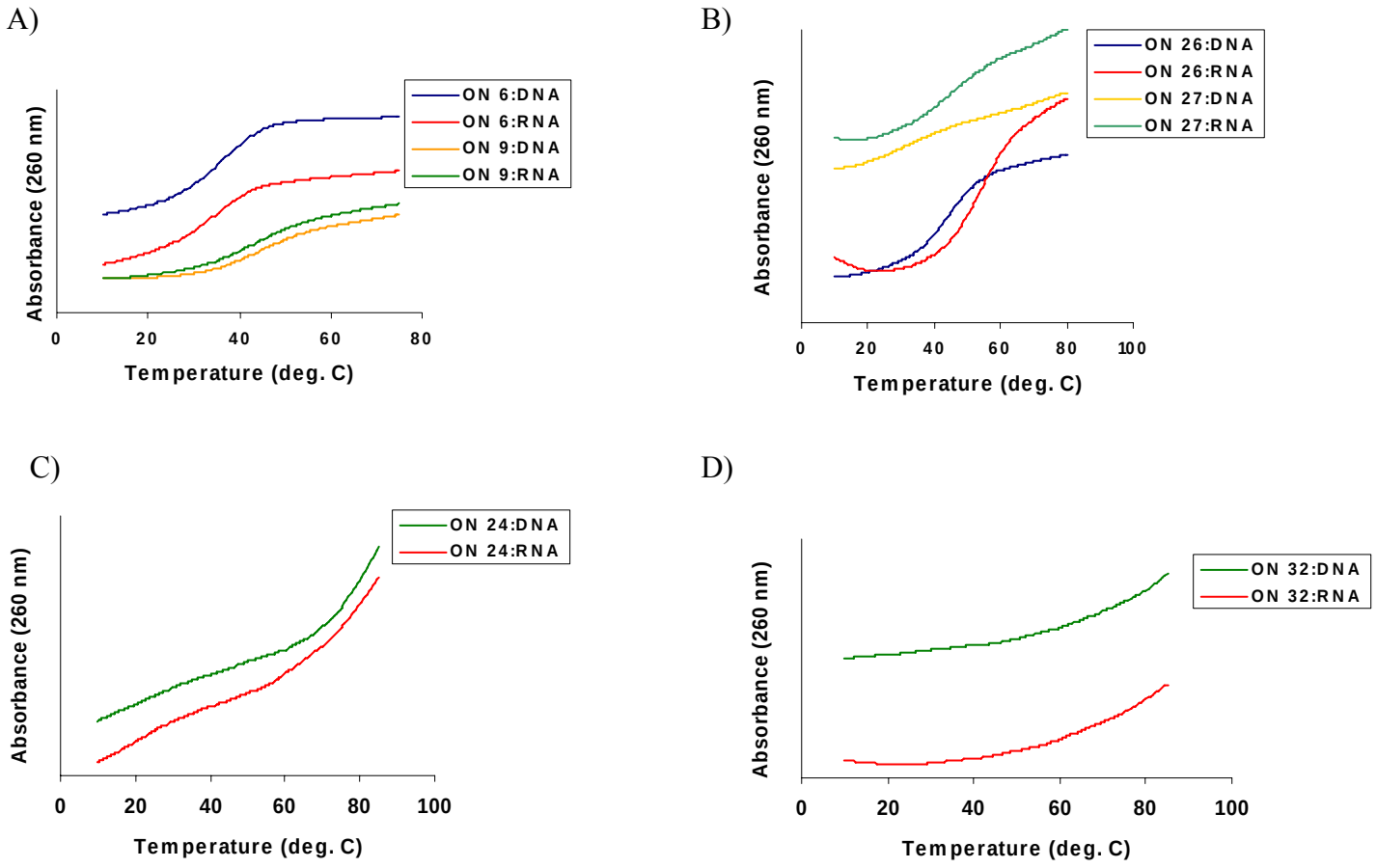


Figure S2. Thermal denaturation curves of duplexes containing monomers $\mathbf{M}^2$ (A), $\mathbf{M}^4$ (B), $\mathbf{M}^5$ (C) and $\mathbf{M}^6$ (D). Recorded in medium salt buffer.

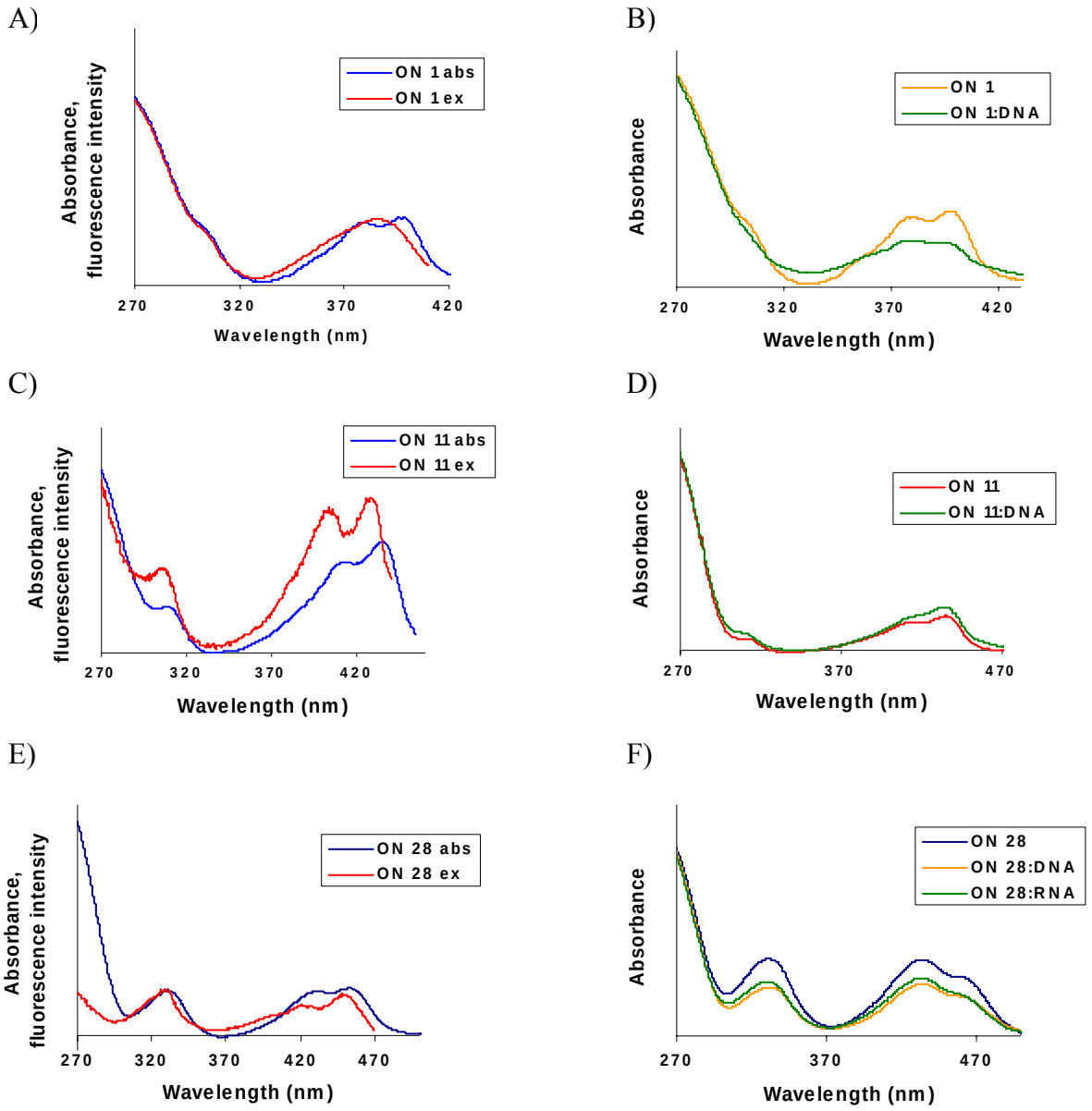


Figure S3. UV-visible absorption and excitation spectra of **ON1** (A), **ON 11** (C) and **ON28** (E); UV-visible absorption spectra of **ON1** (B), **ON11 (**D), **ON28** (E) and corresponding duplexes with complementary DNA (**ON1**, **ON11**) and DNA and RNA (**ON28**). Spectra recorded in medium salt buffer at 19 °C; for the excitation spectra emission wavelengths of 420 nm (**ON1**), 450 nm (**ON11**) and 480 nm (**ON28**) were used. Absorption and excitation spectra were obtained using 0.5 μM and 0.1 μM concentrations, respectively, and not normalized.

Fluorescence steady-state emission studies and quantum yield determinations

Fluorescence spectra were obtained in a medium salt buffer using a PerkinElmer LS 55 luminiscence spectrometer equipped with a Peltier temperature controller. For recording of fluorescence spectra 0.1 μM concentrations of the ss probe or corresponding duplex were used. For weakly fluorescent samples concentration was increased to 0.5 μM. Excitation spectra were obtained recording emission at 420 nm (monomers **M**^{1}, **M**^{2} and **M**^{4}), 440 nm (monomer **M**^{3}), or 460 nm (monomers **M**^{5}, **M**^{6}). The fluorescence quantum yields (Φ_F) were measured by the relative method using standards of highly diluted solution of 9,10-diphenylantracene (Φ_F 0.95)[1] in cyclohexane as a first standard and perylene (Φ_F 0.93)[2] in cyclohexane and 5-(pyren-1-ylethynyl)-2′-deoxyuridine[3] in abs. EtOH as second standards. For fluorescence quantum yields determination 0.5 μM solutions were used. In all cases, absorption in the range 310–520 nm did not exceed 0.1, and was not less than 0.01. Control experiments with an elimination of dissolved oxygen in the buffer solution did not show significant change of fluorescence intensity compared to non-degassed solutions. Thus, the samples used in quantum yield measurements were not degassed. Steady-state fluorescence emission spectra were obtained as an average of 5 scans using an excitation wavelength of 375 nm (**M**^{1}), 370 nm (**M**^{2}), 415 nm (**M**^{3}), 395 nm (**M**^{4}), 425 nm (**M**^{5} ^{6}), or 420 nm (**M**^{6}), excitation slit of 4.0 nm, emission slit of 2.5 nm and scan speed of 120 nm/min. The fluorescence quantum yield of 5-(pyrene-1-ylethynyl)-2′-deoxyuridine[3] in abs. EtOH and perylene (Φ_F 0.93)[2] in cyclohexane relative to 9,10-diphenylantracene in cyclohexane (lit. (1) Φ_F 0.95) in these experimental settings were measured to be 0.45 and 0.93, respectively.

Emission quantum yields of modified ONs Φ_F *(ON)* were determined according to:[4]

$$\Phi_F(ON) = \Phi_F(ref) \times \frac{I(ON)}{I(ref)} \times \frac{OD_{\lambda_{ex}}(ref)}{OD_{\lambda_{ex}}(ON)} \times \frac{n^2_{buffer}}{n^2_{ref}}$$

where Φ_F (*ref*) is the cross-calibrated (by using of 9,10-diphenylantracene in cyclohexane as a first standard) value of the fluorescence quantum yield of 5-(pyren-1-ylethynyl)-2′-deoxyuridine in abs. EtOH (monomers **M**^{1} and **M**^{2}), or perylene in cyclohexane (monomers **M**^{3}–**M**^{6}); *I (ON)* is the area of

the fluorescence emission spectra of the sample, *I (ref)* is the area of the second standard's emission spectra at the same region, n_{buffer} and n_{ref} are the refractive indexes of the buffer (1.334), ethanol (1.361), or cyclohexane (1.426), respectively. High quantum yields ($\Phi_F > 0.2$) were determined as the average of two measurements within ±10%. Determinations of low quantum yields ($\Phi_F < 0.1$) may be associated with considerably larger error.

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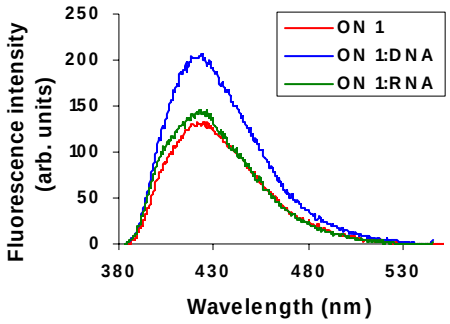

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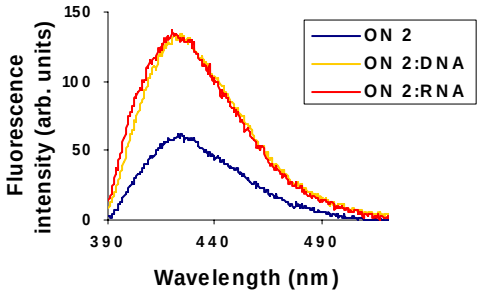

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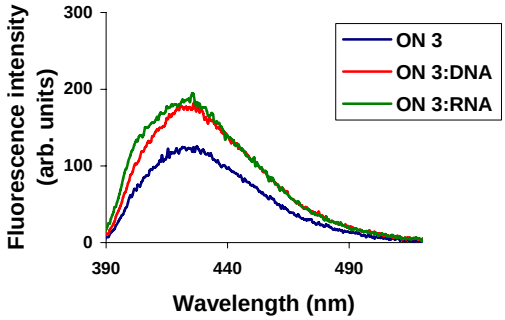

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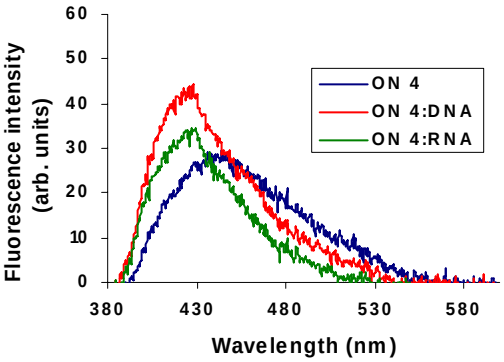

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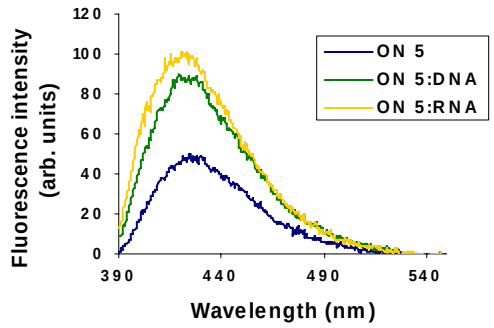

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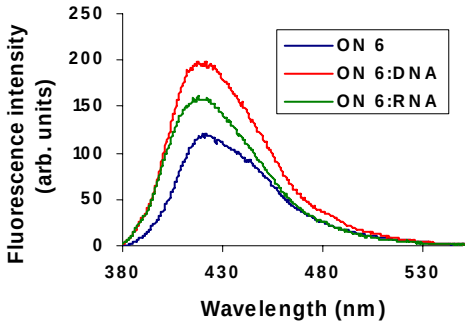
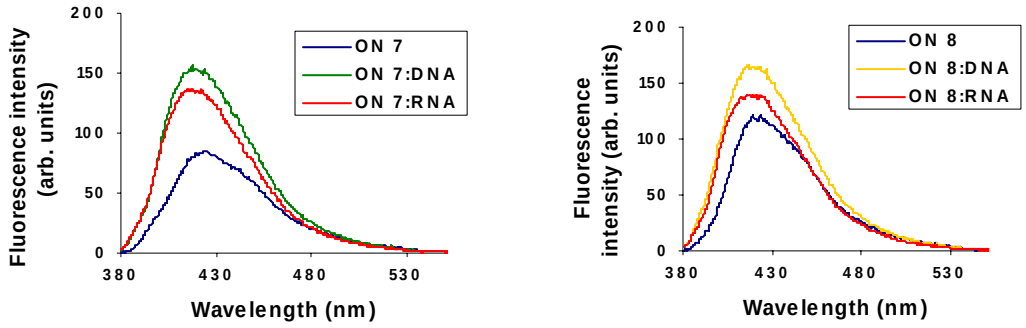


G) H)



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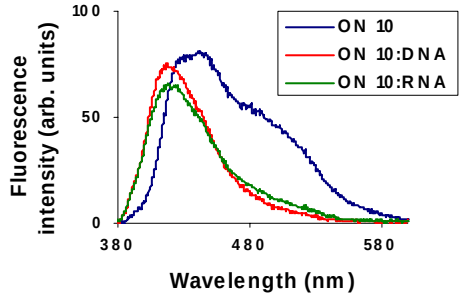
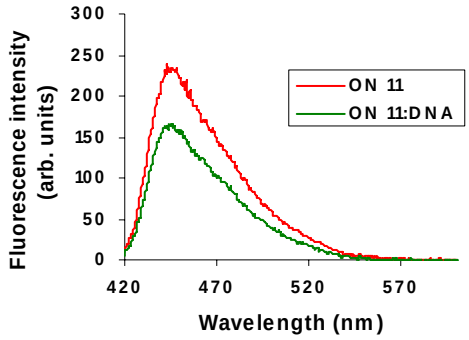
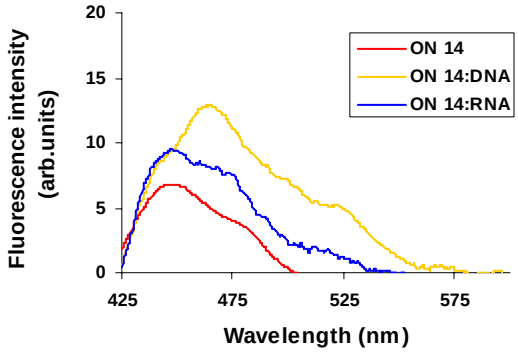


Figure S4. Steady-state fluorescence emission spectra of $\mathbf{M}^1$- and $\mathbf{M}^2$-modified oligonucleotides and of the corresponding duplexes with complementary DNA/RNA. For conditions see Figure 3.

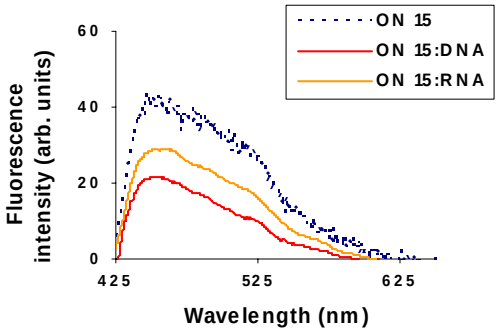
A)


Fluorescence intensity (arb. units)
300
250
200
150
100
50
0
420
470
520
570
Wavelength (nm)
ON 11
ON 11:DNA

B)


Fluorescence intensity (arb.units)
20
15
10
5
0
425
475
525
575
Wavelength (nm)
ON 14
ON 14:DNA
ON 14:RNA

C)


Fluorescence intensity (arb. units)
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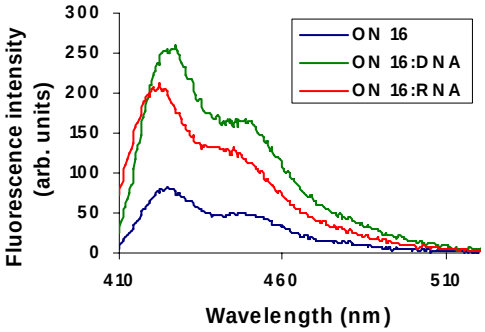
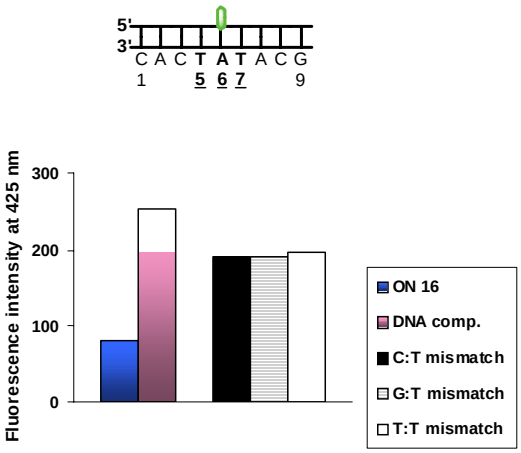
40
20
0
425
525
625
Wavelength (nm)
ON 15
ON 15:DNA
ON 15:RNA

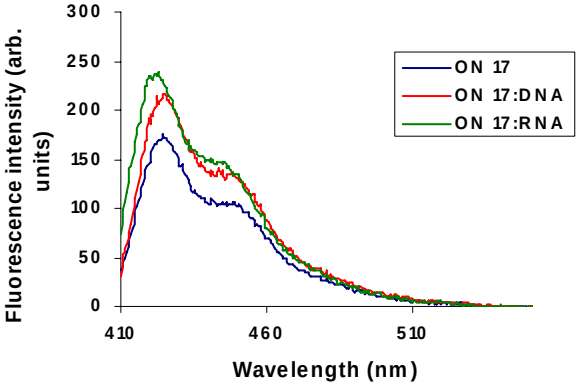
D)


Fluorescence intensity (arb. units)
300
250
200
150
100
50
0
410
460
510
Wavelength (nm)
ON 16
ON 16:DNA
ON 16:RNA

E)


5'
3'
C A C T A T A C G
1 5 6 7 9
Fluorescence intensity at 425 nm
300
200
100
0
ON 16
DNA comp.
C:T mismatch
G:T mismatch
T:T mismatch

F)


Fluorescence intensity (arb. units)
300
250
200
150
100
50
0
410
460
510
Wavelength (nm)
ON 17
ON 17:DNA
ON 17:RNA

G)

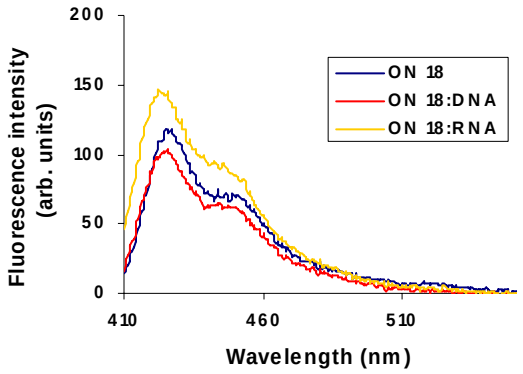
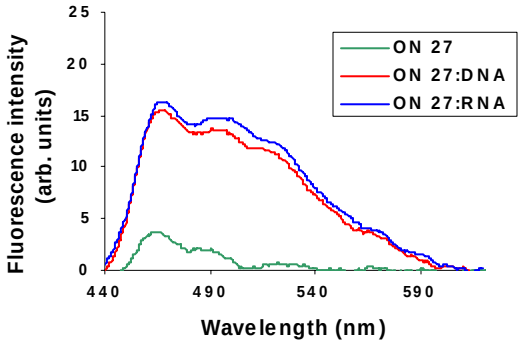


Figure S5. Steady-state fluorescence emission spectra of $\mathbf{M^3}$- and $\mathbf{M^4}$-modified oligonucleotides and of the corresponding duplexes with complementary DNA/RNA. For conditions see Figure 3.

A)



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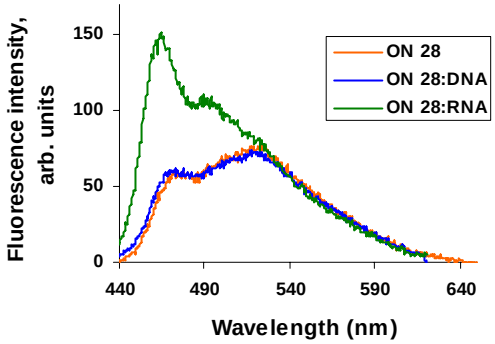

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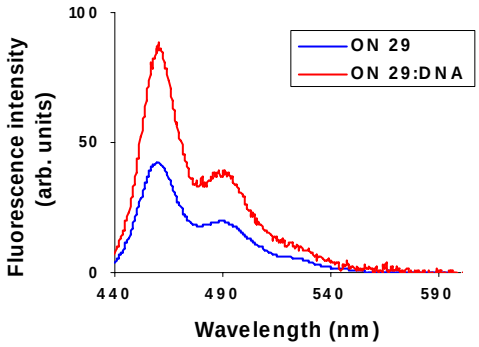


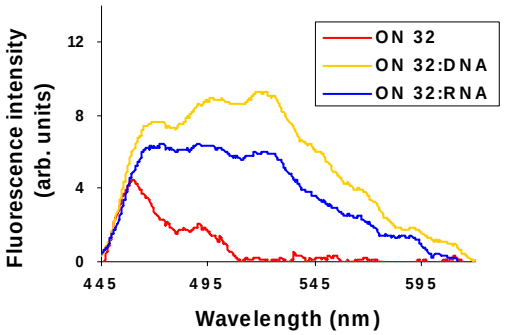
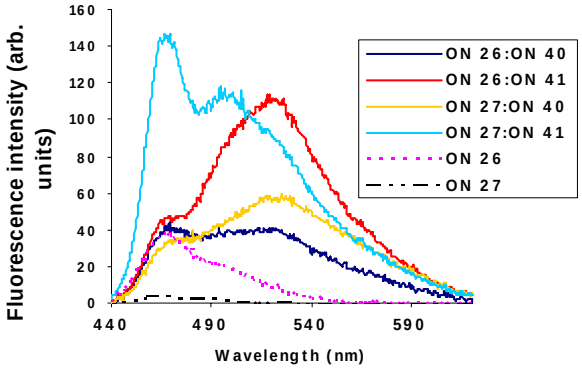
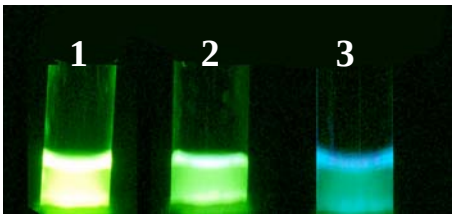


Figure S6. Steady-state fluorescence emission spectra of $\mathbf{M}^5$- and $\mathbf{M}^6$-modified oligonucleotides and of the corresponding duplexes with complementary DNA/RNA. For conditions see Figure 3.





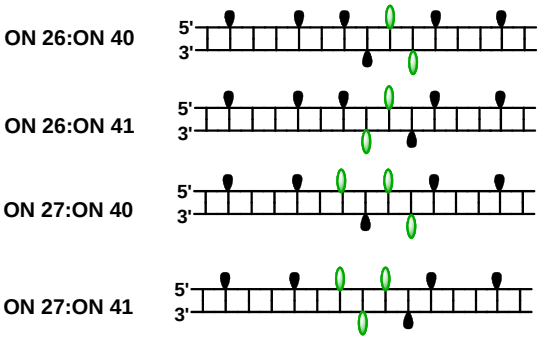
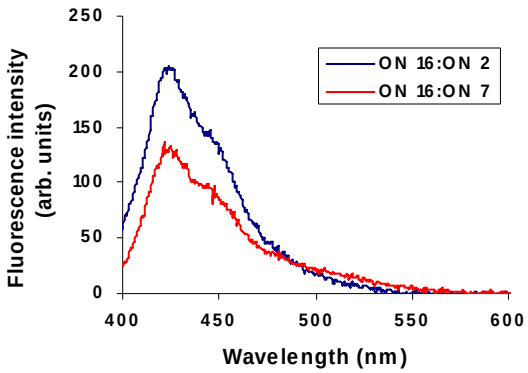


Figure S7. Left: steady-state fluorescence emission spectra of the 15-mer duplexes containing monomer **M**^{5} in both complementary strands. For conditions see Figure 3. Right: photographs of the fluorescence in medium salt buffer of **ON26:ON41** (vial 1); **ON16:ON18** (vial 2) and single stranded **ON4** (vial 3) (2.0 μM solutions). For conditions see Figure 4. The green and black droplets represent monomer **M**^{5} and LNA monomers, respectively.

A)



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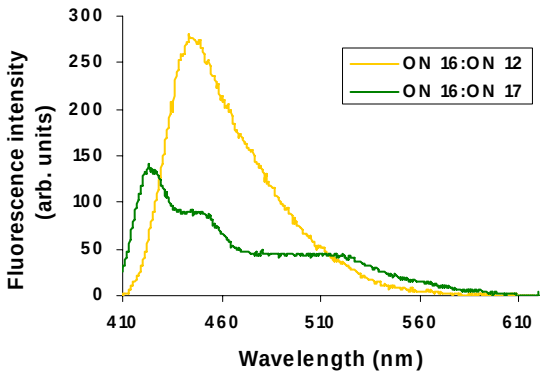

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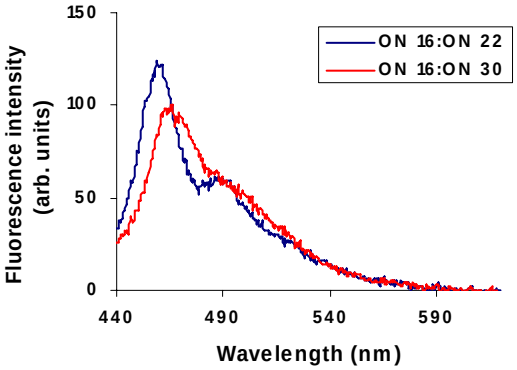
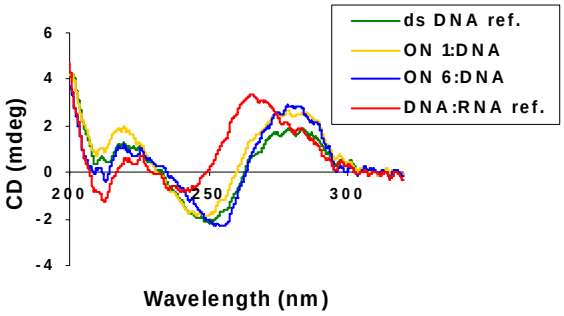


Figure S8. Representative steady-state fluorescence emission spectra of "+1 zipper" duplexes containing combined monomers $\mathbf{M^1}$–$\mathbf{M^6}$ in both complementary strands. For conditions see Figure 3.

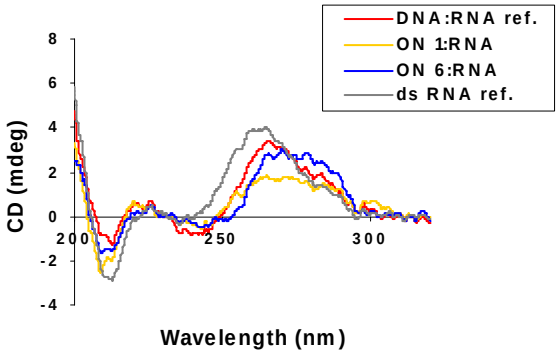
CD measurements

CD spectra were recorded on JASCO J-815 CD Spectrometer equipped with CDF 4265/15 temperature controller. Samples for CD measurements were prepared as described in the thermal denaturation studies section except that a concentration of 2.0 μM of both the complementary strands was used. Quartz optical cells with a path-length of 0.5 cm were used.

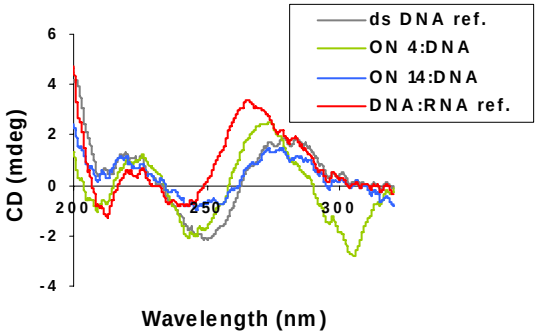
A)



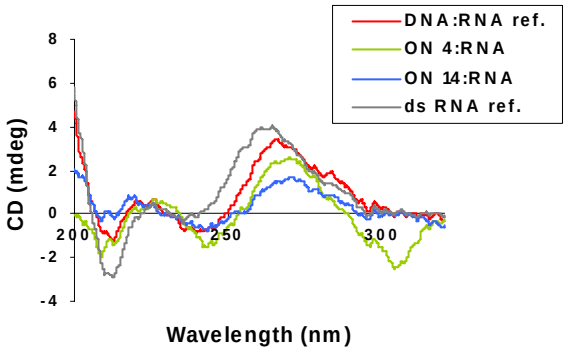
B)



C)



D)



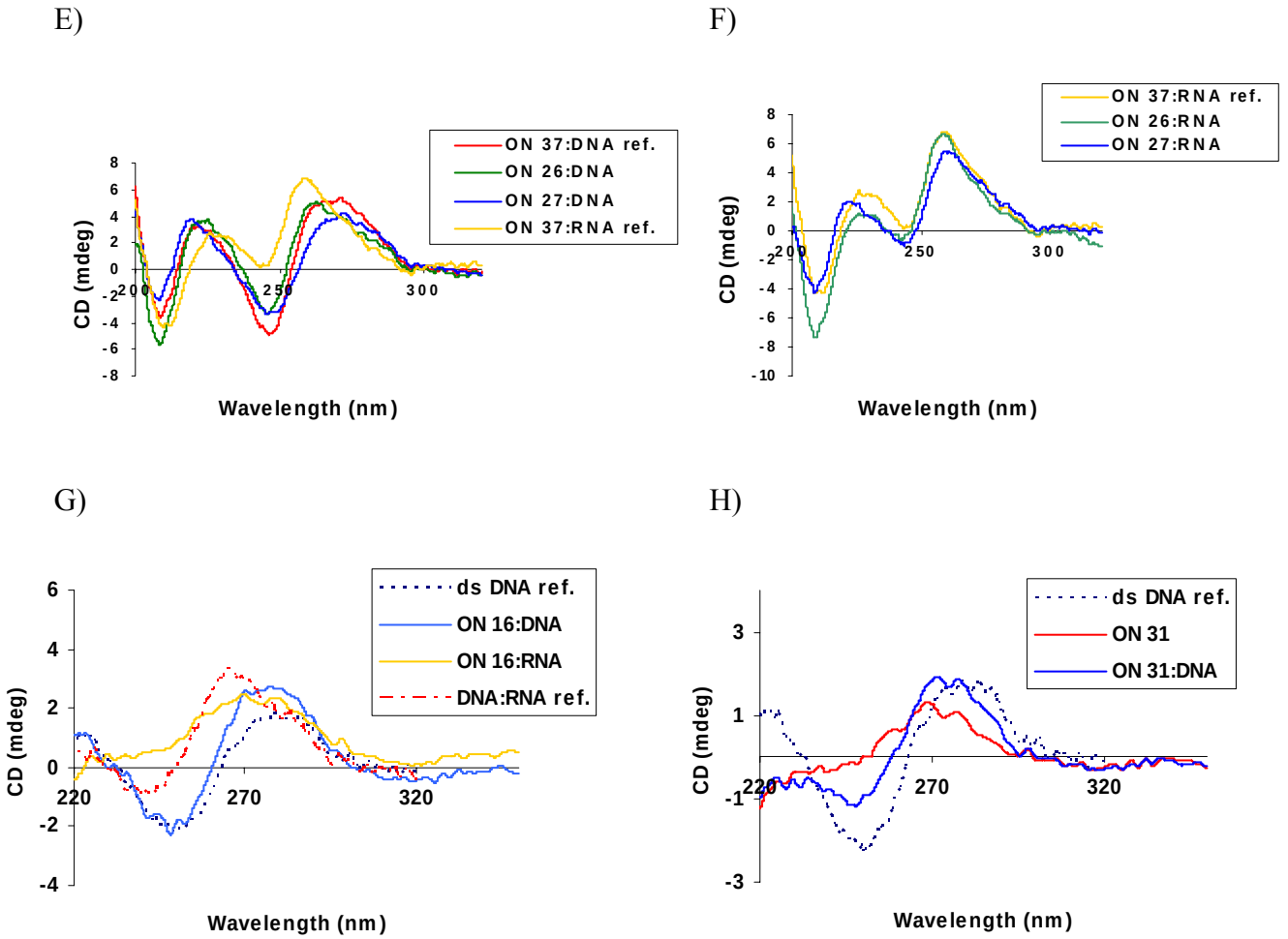


Figure S9. CD spectra of modified duplexes. The spectra were recorded in medium salt buffer at 19 ºC using 1.5 µM concentration of complementary strands.

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