Supporting Information

DNA-Organized Artificial LHCs – Testing the Limits of Chromophore Segmentation

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General Methods

All reagents and solvents were purchased from commercial suppliers and used without further purification. Mass-spectrometric data were obtained on Thermo Fisher LTQ Orbitrap XL using Nano Electrospray Ionization (NSI). UV-vis spectra were measured on a Cary 100 Bio spectrophotometer. Fluorescence and excitation spectra were measured on a Cary Eclipse spectrofluorimeter, excitation slit width was set to 2.5 nm and emission slit width was set to 5 nm if not mentioned otherwise. Fluorescence integrals and quantum yields were determined in three independent triplicate experiments.

DNA Synthesis and Purification

DNA strands without modifications were purchased from Microsynth (Switzerland). Syntheses of 1,8-dialkynylpyrene phosphoramidite and 3,6-dialkynylphenanthrene phosphoramidite were described previously.[1–3] Oligomer strands

S1 – **S18** were prepared on an Applied Biosystems 394 DNA/RNA synthesizer. A standard cyanoethyl phosphoramidite coupling protocol was used beginning with nucleoside-loaded controlled pore glass (CPG) support. After synthesis, the CPG-bound oligomers were cleaved and deprotected by treatment with 28-30% NH_4OH (aq) at 55°C overnight. The supernatants were collected and the debris' were washed two times with 1 ml H_2O. After lyophilization the crude oligomers were purified by reversed phase HPLC (Merck LiChroCART 250-4; LiChrospher 100, RP-18, 5 µm); Solvent A: 0.1 M aqueous ammonium acetate; Solvent B: CH_3CN; 1 ml/min; T = 40°C.

	Gradient 1	Gradient 2	Gradient 3
Time [min]	**Solvent B in Solvent A** [%]	**Solvent B in Solvent A** [%]	**Solvent B in Solvent A** [%]
0.01	0	0	0
2.00	0	0	5
22.00	30	40	50
24.00	100	100	100
26.00	100	100	100
28.00	0	0	0

Table S1: HPLC solvent gradients used for oligomer purification

Gradient 1 was applied for purification of oligomer strands **S1**, **S2**, **S3**, and **S4**; Gradient 2 was applied for purification of oligomer strands **S5** and **S6**; Gradient 3 was applied for purification of oligomer strands **S7** - **S15**. Oligomer purity was confirmed by analytical re-injection.

The samples were measured in negative ion mode in mixtures of water/acetonitrile/triethylamine. The purified oligomers were dissolved in 1 ml Milli-Q H_2O. Samples of the stock solutions were diluted and the absorbance at 326 nm was measured to determine the concentrations of oligomer strands **S2** – **S15**, using the ε_{326} value of 34'500 for 3,6-dialkynylphenanthrene.[1] To determine the concentration of oligomer strand **S1**, which does not contain 3,6-dialkynylphenanthrene, the molar absorption coefficient at 260 nm was calculated using the ε_{260} values of 15'300, 11'700, 7'400 and 9'000 for A, G, C and T bases, respectively, and 30'000 for 1,8-dialkynylpyrene.[2]

Strand	Sequence	Calcd. mass	Found mass
S1	5' GGC TAA **Y**TA AAT TTA AAT CGC 3'	6524.1548	6524.0712
S2	3' CCG ATT **α**AT TTA AAT TTA GCG 5'	6482.1280	6482.0520
S3	5' GGC TAA **Y**T**α** AAT TTA AAT CGC 3'	6587.0412	6587.1432
S4	3' CCG ATT **α**A**α** TTA AAT TTA GCG 5'	6554.0278	6554.1216
S5	5' GGC TAA **Y**T**α** A**α**T TTA AAT CGC 3'	6649.9276	6649.1470
S6	3' CCG ATT **α**A**α** T**α**A AAT TTA GCG 5'	6625.9276	6625.1470
S7	5' GGC TAA **Y**T**α** A**α**T **α**TA AAT CGC 3'	6721.8274	6722.1693
S8	3' CCG ATT **α**A**α** T**α**A **α**AT TTA GCG 5'	6688.8140	6689.1672
S9	5' GGC TAA **Y**T**α** A**α**T **α**T**α** AAT CGC 3'	6784.7138	6785.2350
S10	3' CCG ATT **α**A**α** T**α**A **α**A**α** TTA GCG 5'	6760.7138	6760.2222
S11	5' GGC TAA **Y**α**α** **αα**T TTA AAT CGC 3'	6784.7138	6784.1983
S12	3' CCG ATT **ααα** **αα**A AAT TTA GCG 5'	6760.7138	6761.2182
S13	3' CCG ATT **α**T**α** T**α**A **α**AT TTA GCG 5'	6679.8006	6680.1760
S14	3' CCG ATT **α**A**α** T**α**T **α**AT TTA GCG 5'	6679.8006	6680.1570
S15	3' CCG ATT **α**T**α** T**α**T **α**AT TTA GCG 5'	6670.7872	6670.1316

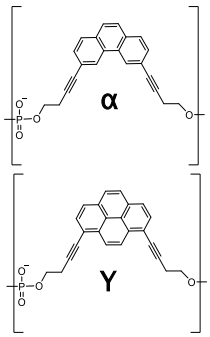

Table S2: Calculated and found masses (negative ion mode) of oligomer strands.

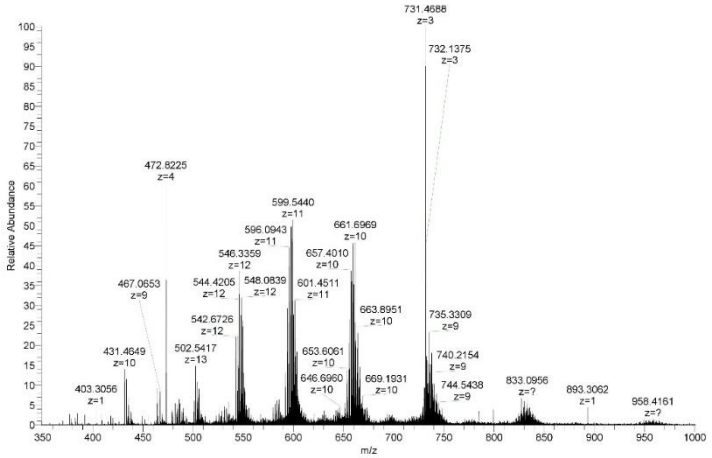

Figure S1: Mass spectrum of single strand **S1**.

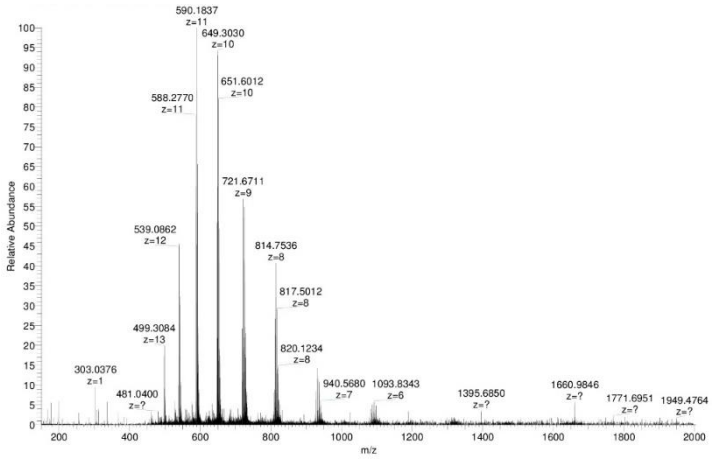

Figure S2: Mass spectrum of single strand **S2**.

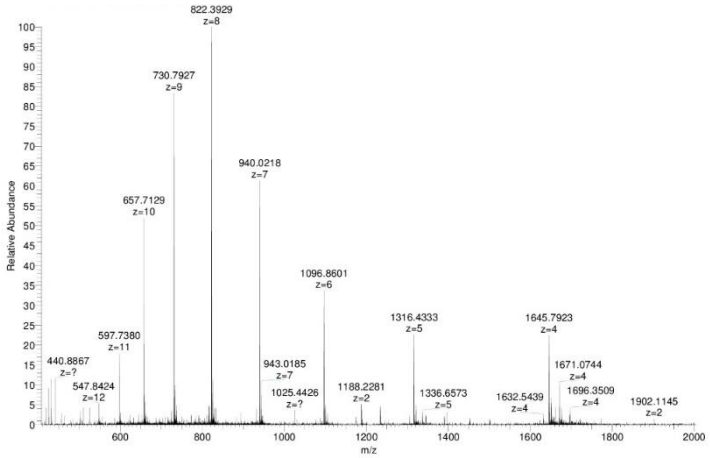

Figure S3: Mass spectrum of single strand **S3**.

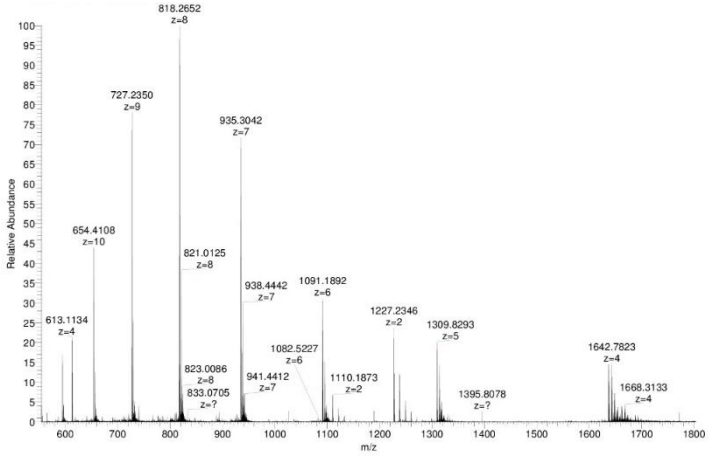

Figure S4: Mass spectrum of single strand **S4**.

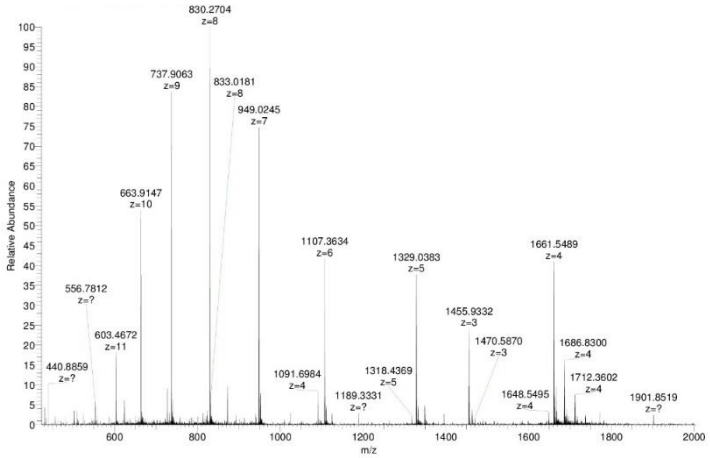

Figure S5: Mass spectrum of single strand **S5**.

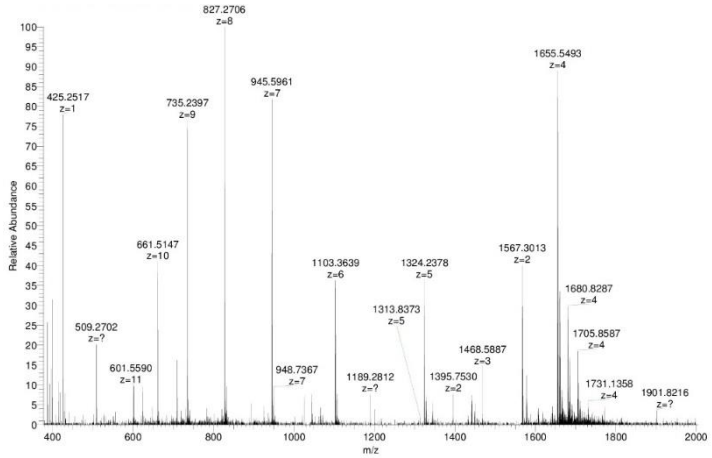

Figure S6: Mass spectrum of single strand **S6**.

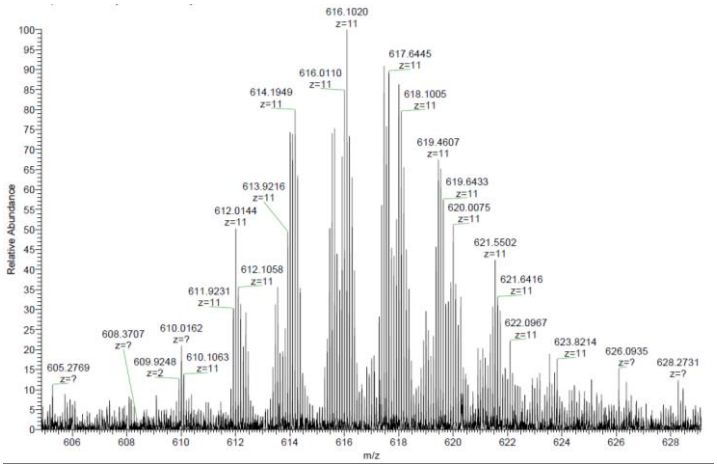

Figure S7: Mass spectrum of single strand **S7**.

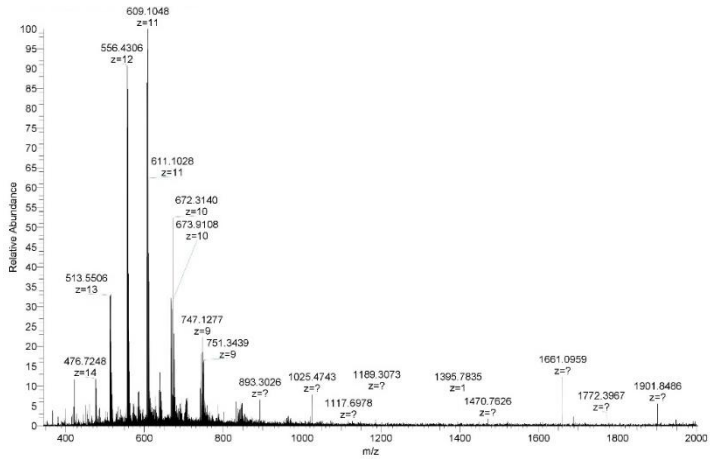

Figure S8: Mass spectrum of single strand **S8**.

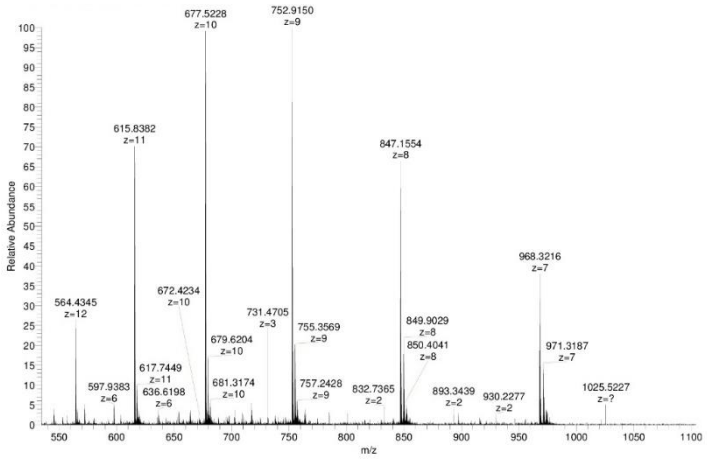

Figure S9: Mass spectrum of single strand **S9**.

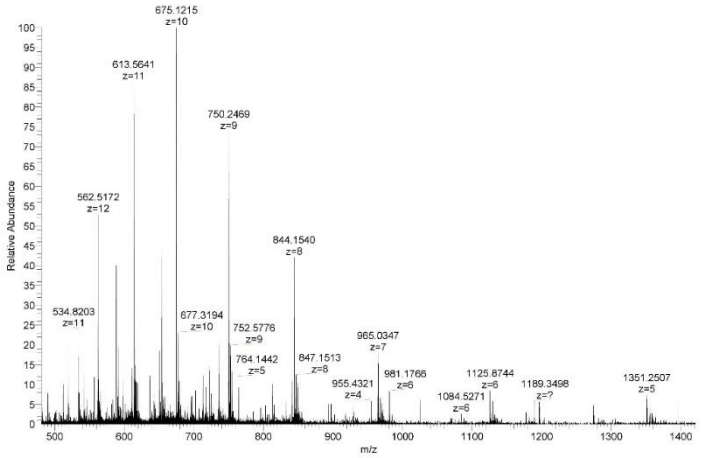

Figure S10: Mass spectrum of single strand **S10**.

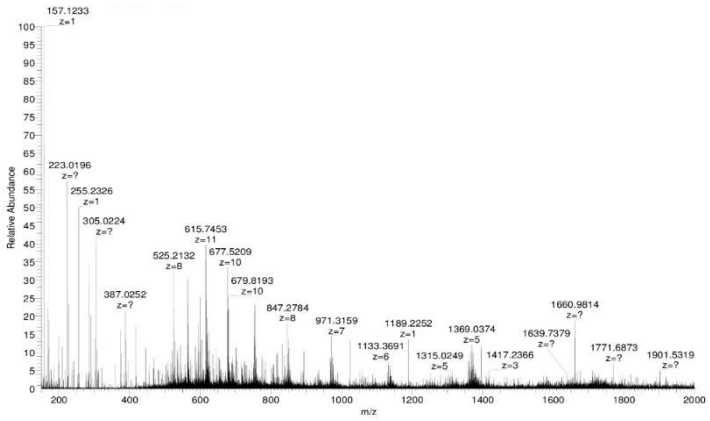

Figure S11: Mass spectrum of single strand **S11**.

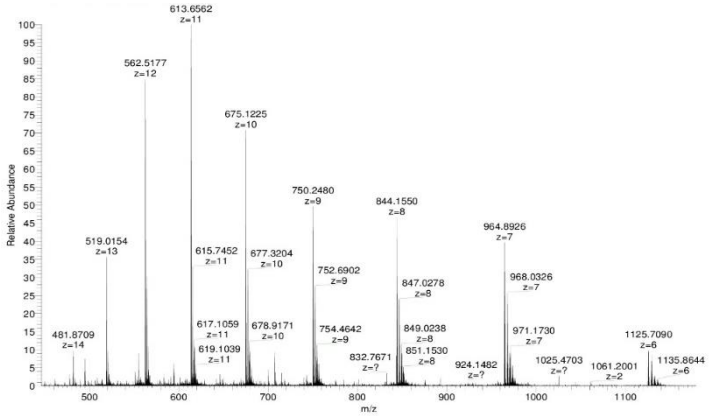

Figure S12: Mass spectrum of single strand **S12**.

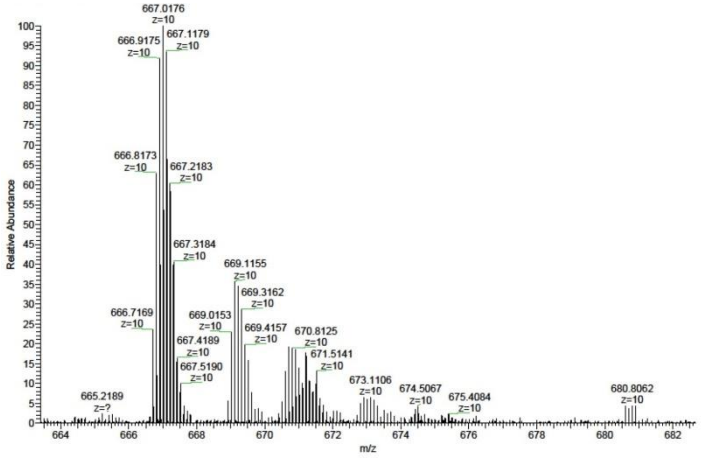

Figure S13: Mass spectrum of single strand **S13**.

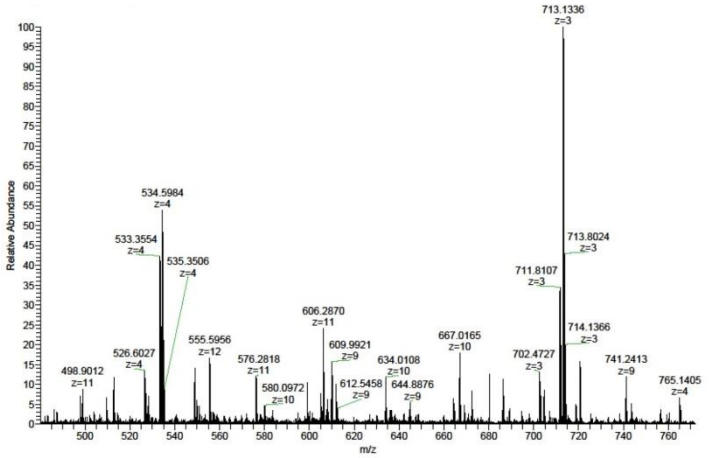

Figure S14: Mass spectrum of single strand **S14**.

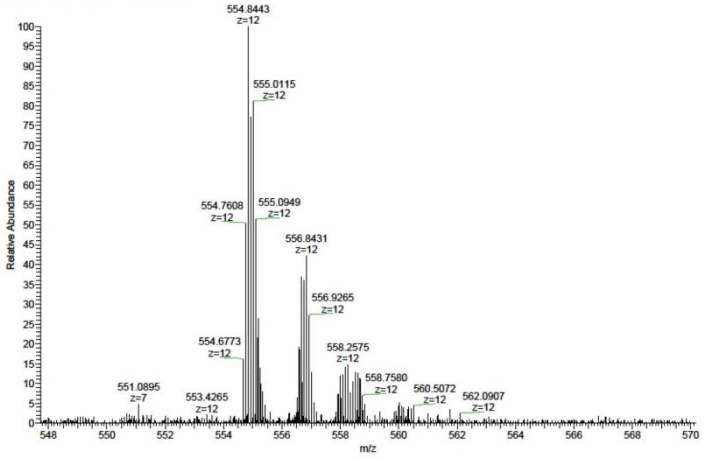

Figure S15: Mass spectrum of single strand **S15**.

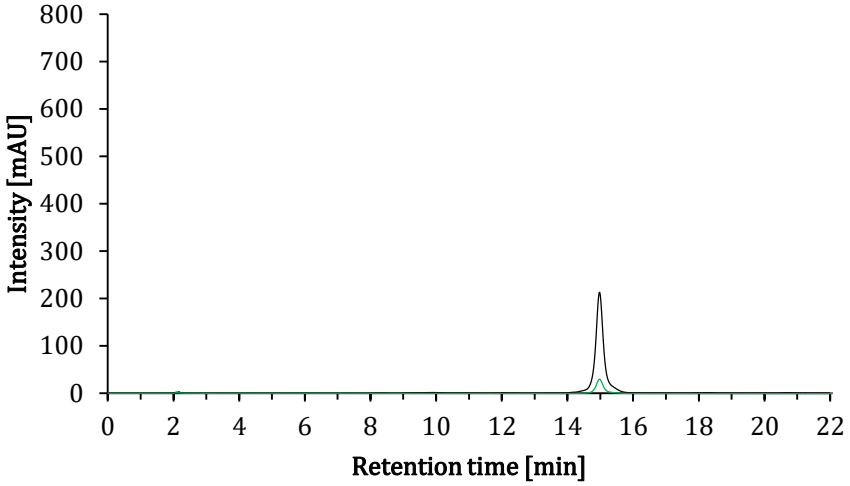

Figure S16: HPLC trace of oligomer strand **S1**. Black curve: Abs. 260 nm, green curve: Abs. 365 nm

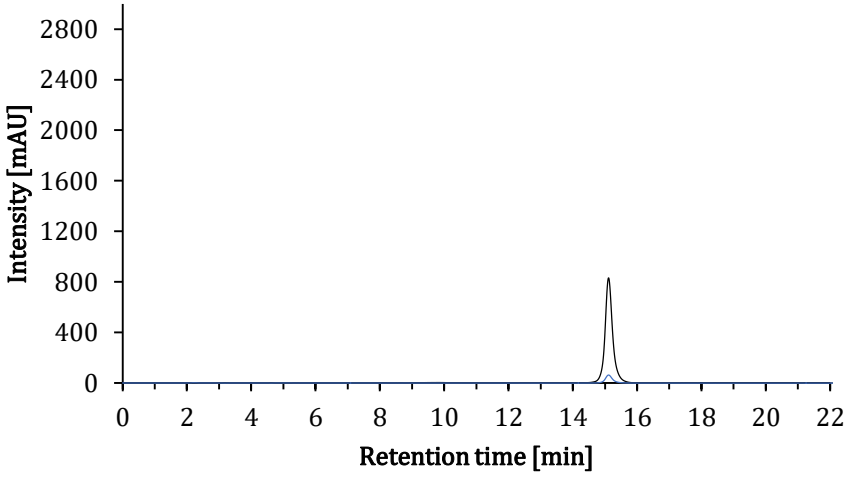

Figure S17: HPLC trace of oligomer strand **S2**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

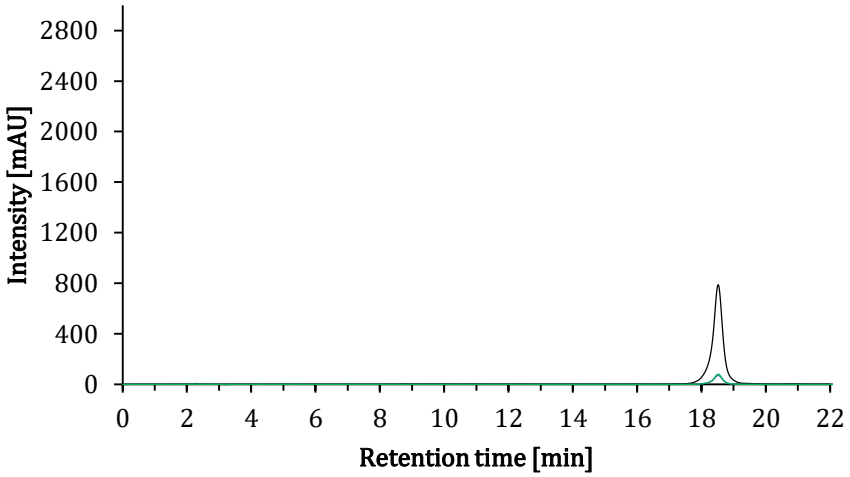

Figure S18: HPLC trace of oligomer strand **S3**. Black curve: Abs. 260 nm, green curve: Abs. 365 nm

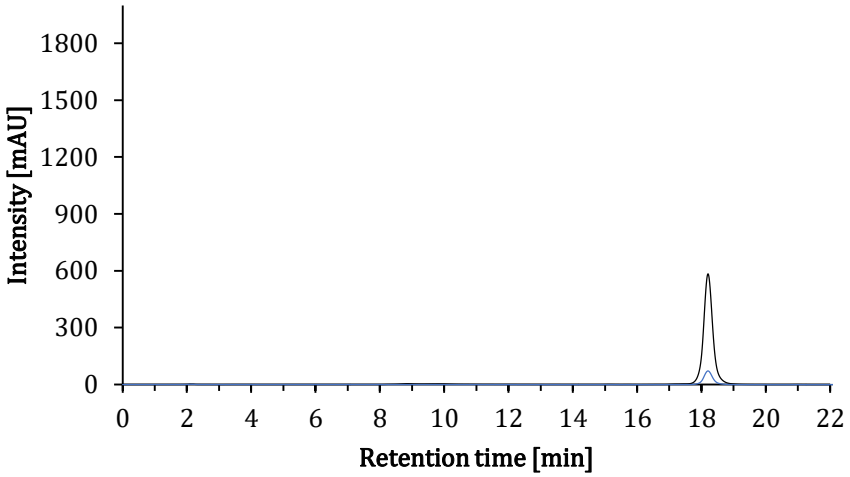

Figure S19: HPLC trace of oligomer strand **S4**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

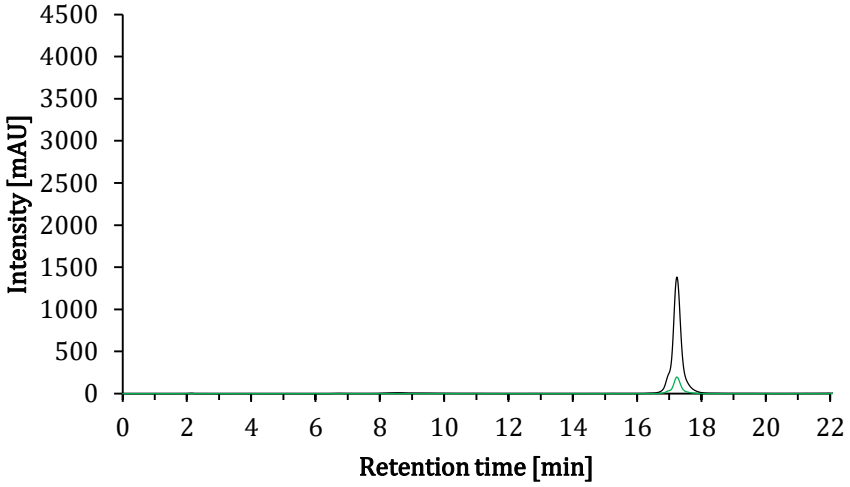

Figure S20: HPLC trace of oligomer strand **S5**. Black curve: Abs. 260 nm, green curve: Abs. 365 nm

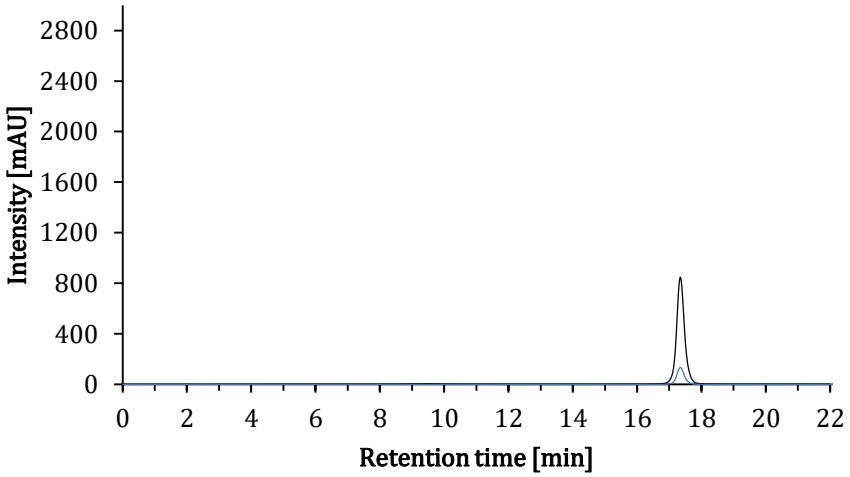

Figure S21: HPLC trace of oligomer strand **S6**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

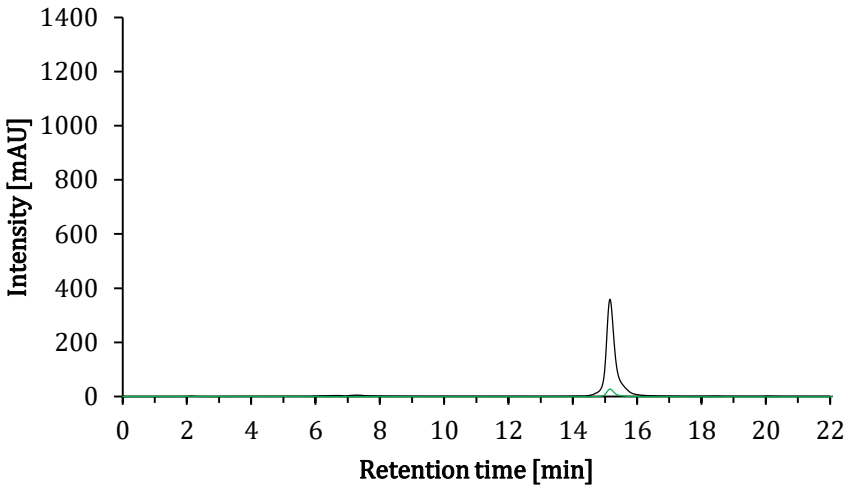

Figure S22: HPLC trace of oligomer strand **S7**. Black curve: Abs. 260 nm, green curve: Abs. 365 nm

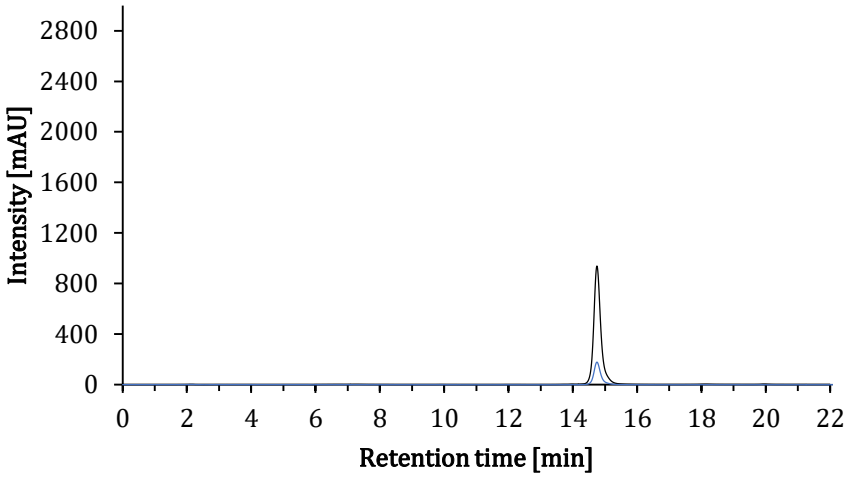

Figure S23: HPLC trace of oligomer strand **S8**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

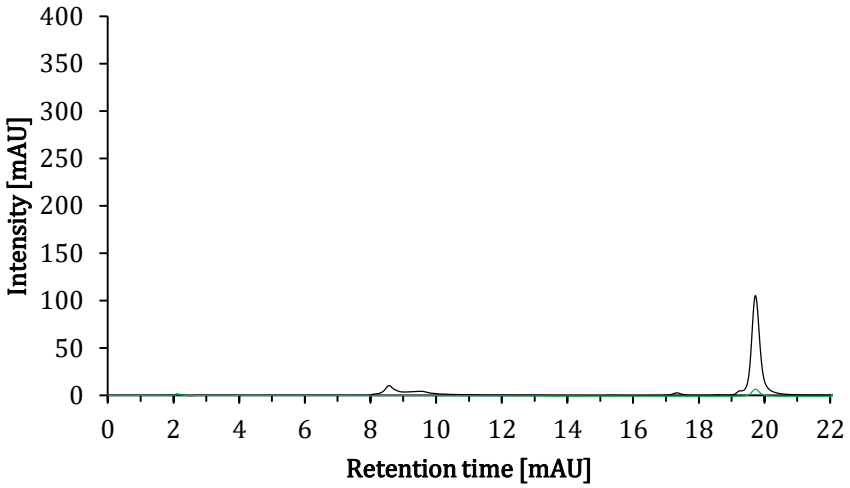

Figure S24: HPLC trace of oligomer strand **S9**. Black curve: Abs. 260 nm, green curve: Abs. 365 nm

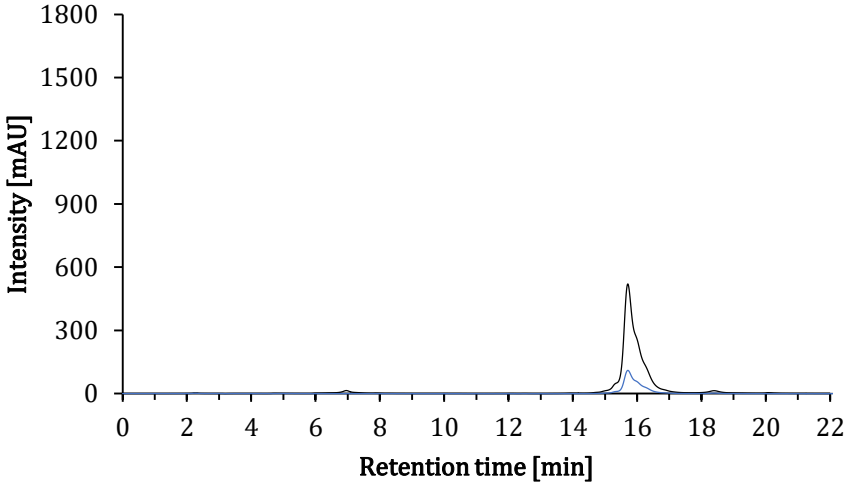

Figure S25: HPLC trace of oligomer strand **S10**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

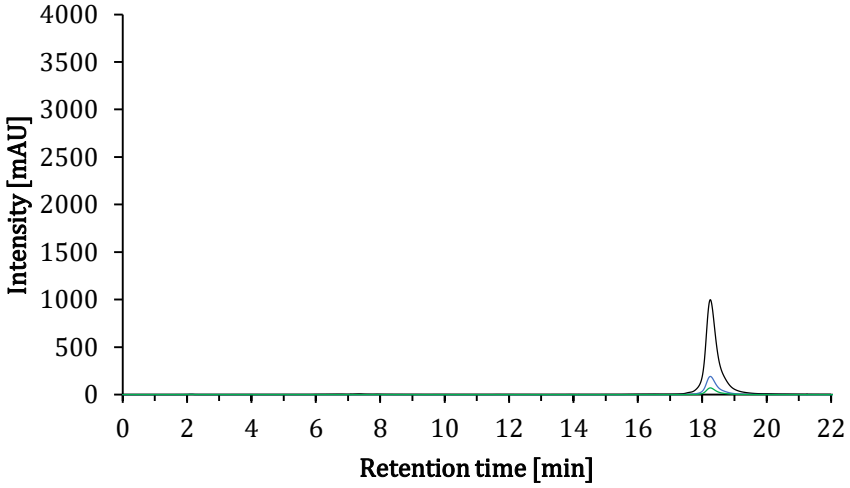

Figure S26: HPLC trace of oligomer strand **S11**. Black curve: Abs. 260 nm, green curve: Abs. 365 nm

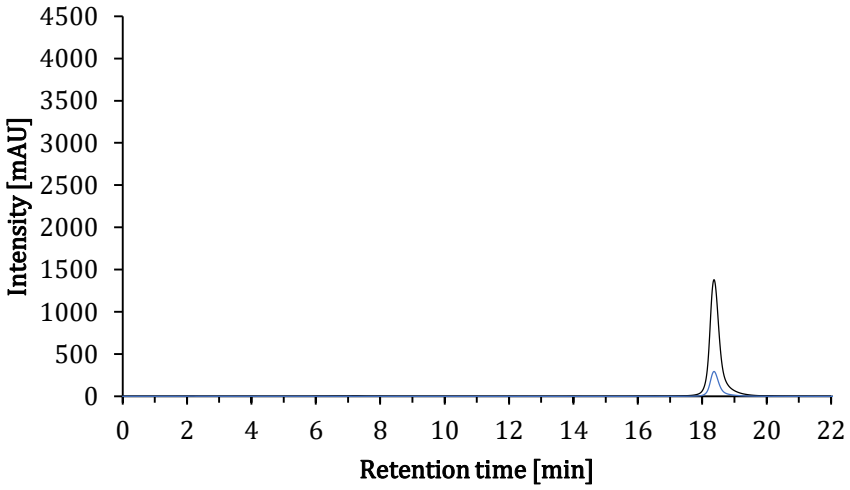

Figure S27: HPLC trace of oligomer strand **S12**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

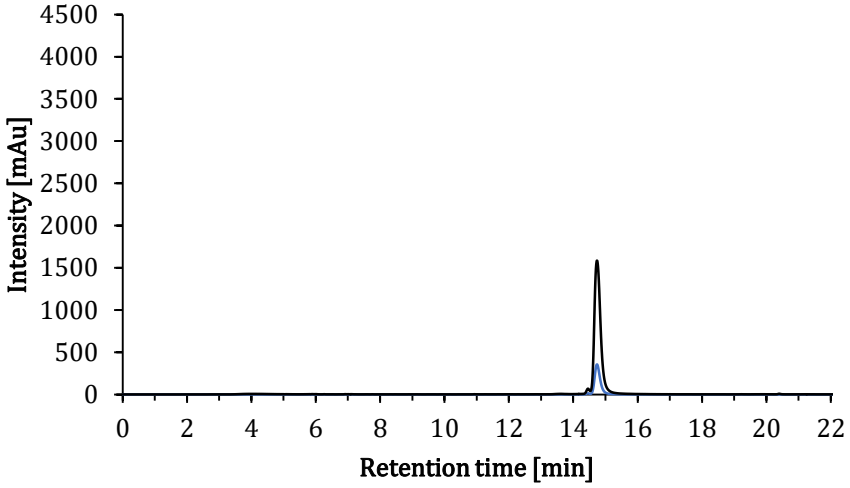

Figure S28: HPLC trace of oligomer strand **S13**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

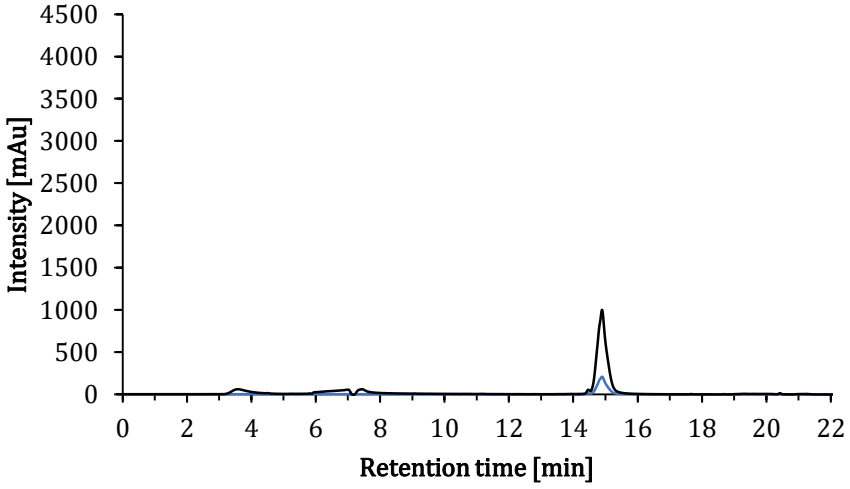

Figure S29: HPLC trace of oligomer strand **S14**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

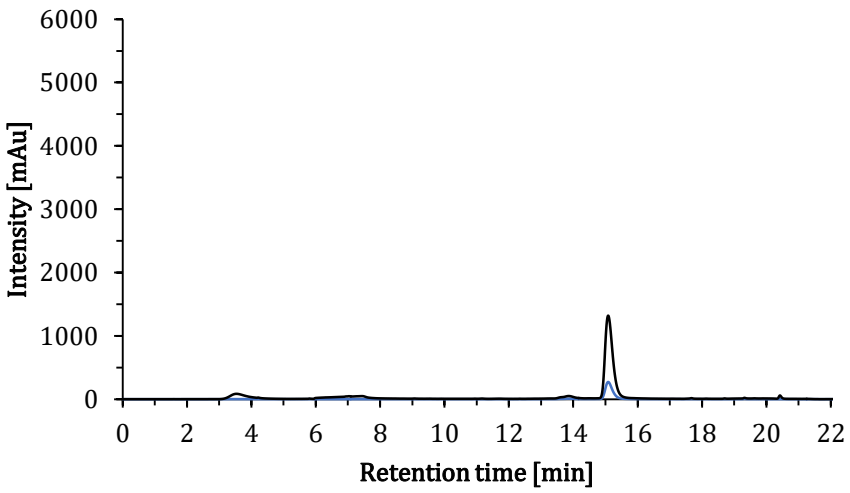

Figure S30: HPLC trace of oligomer strand **S15**. Black curve: Abs. 260 nm, blue curve: Abs. 326 nm

Spectroscopic Data of Single Strands

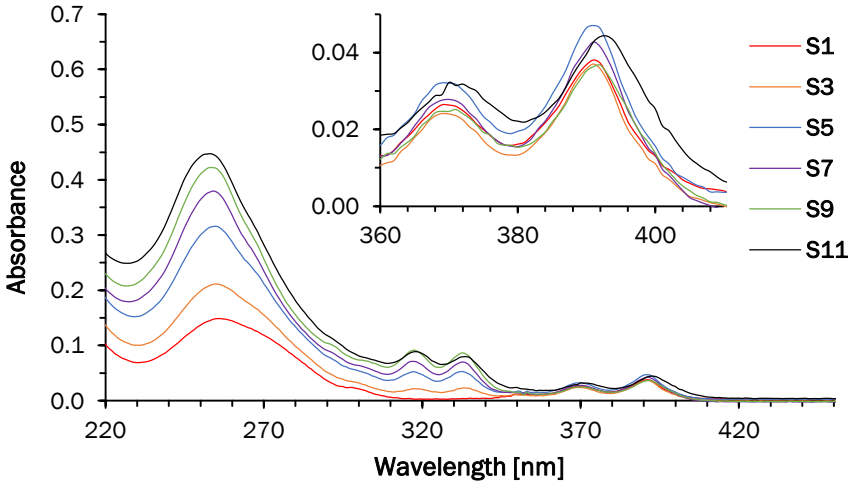

Figure S31: Absorption spectra of single strands with the pyrene acceptor. Conditions: 0.5 µM each strand, 10 mM sodium phosphate buffer pH=7.0, 400 mM NaCl, 20°C.

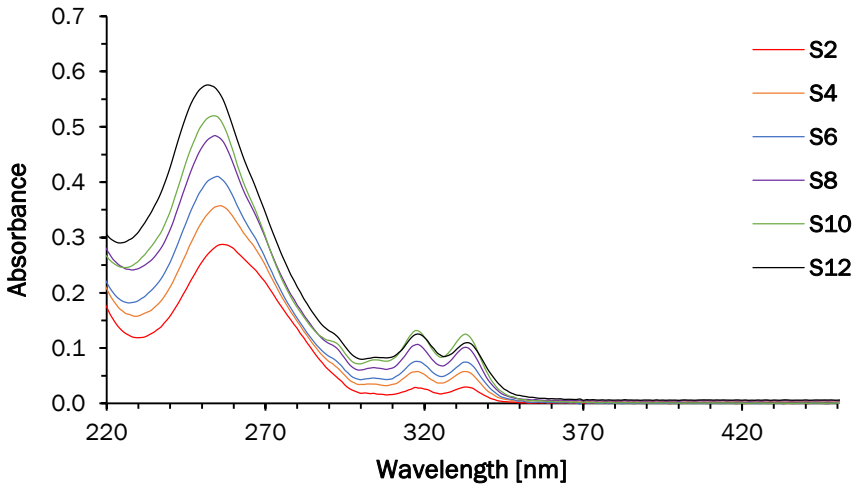

Figure S32: Absorption spectra of single strands without the pyrene acceptor. Conditions: 0.5 µM each strand, 10 mM sodium phosphate buffer pH=7.0, 400 mM NaCl, 20°C.

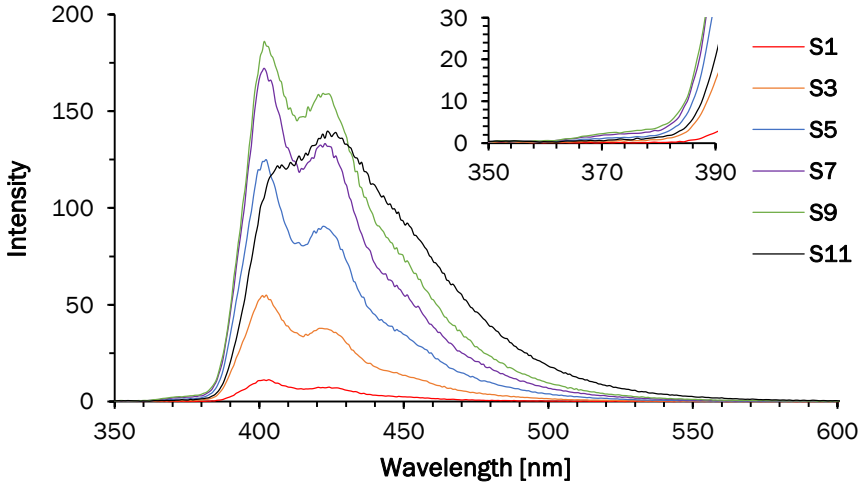

Figure S33: Emission spectra of single strands with the pyrene acceptor. Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl, $\lambda_{exc.}$ 316 nm, 20°C. Excitation slit: 2.5 nm, emission slit: 5 nm.

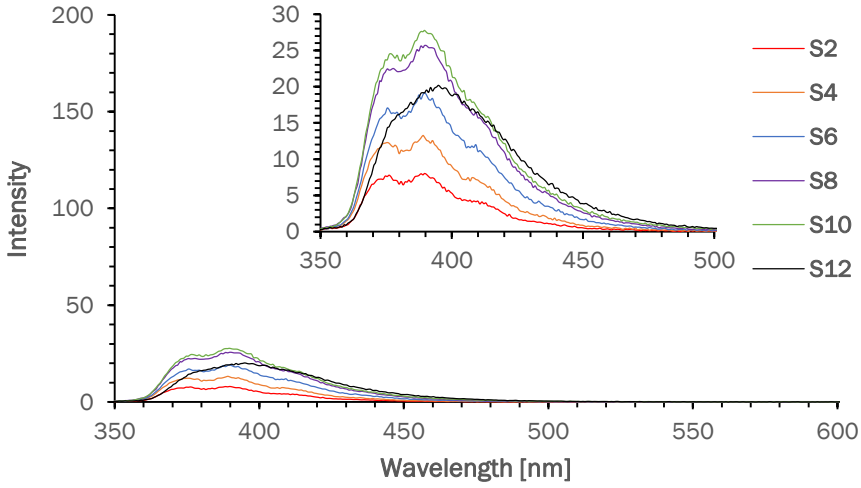

Figure S34: Emission spectra of single strands without the pyrene acceptor. Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl, $\lambda_{exc.}$ 316 nm, 20°C. Excitation slit: 2.5 nm, emission slit: 5 nm.

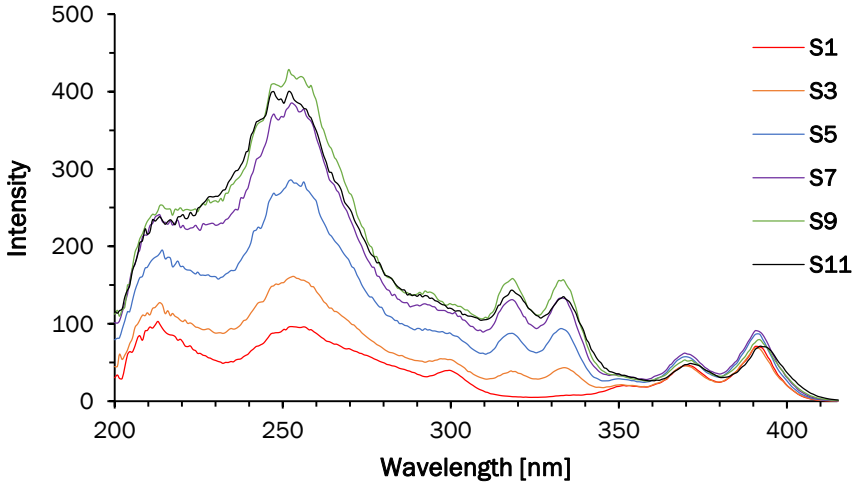

Figure S35 Excitation spectra of single strands with the pyrene acceptor. Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl, $\lambda_{em.}$ 425 nm, 20°C. Excitation slit: 2.5 nm, emission slit: 5 nm.

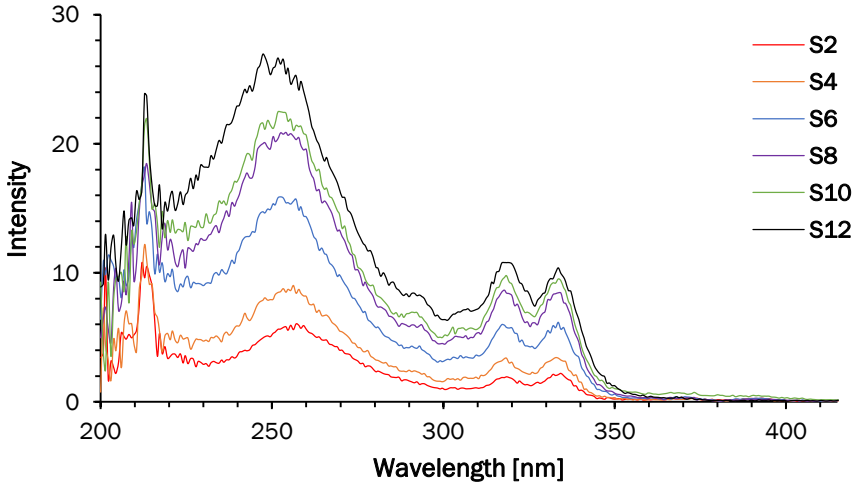

Figure S36: Excitation spectra of single strands without the pyrene acceptor. Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl, $\lambda_{em.}$ 425 nm, 20°C. Excitation slit: 2.5 nm, emission slit: 5 nm.

Melting Curves and Melting Temperatures of Duplexes

To measure melting temperatures of duplexes, cooling-heating curves were recorded after heating duplexes to 80°C in the thermo-shaker for about 30 min. Monitored was absorbance at 260 nm, where nucleobases as well as dialkynylphenanthrene and -pyrene absorb. Cooling stage (80 – 20°C) is depicted as blue curve, heating stage (20 – 80°C) as orange curve. General conditions, if not stated otherwise were: Cooling/heating rate= 0.5°C/min, 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0 and 400 mM NaCl. Melting temperatures were calculated from the first derivation of melting curves.

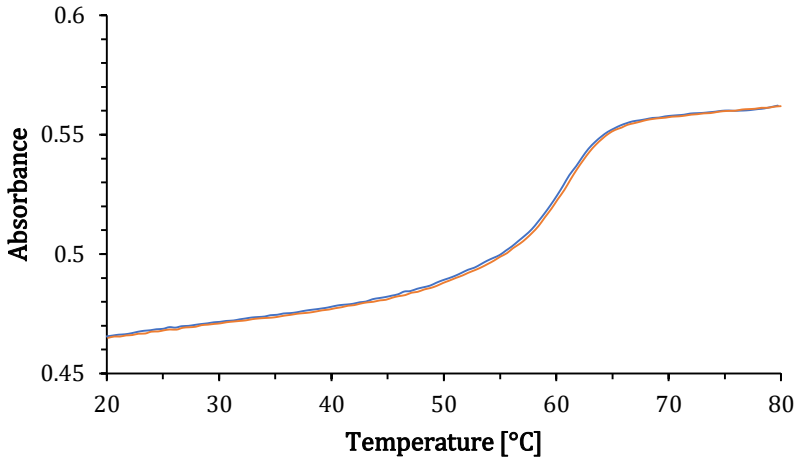

Figure S37: Cooling-heating curves of duplex **1**.

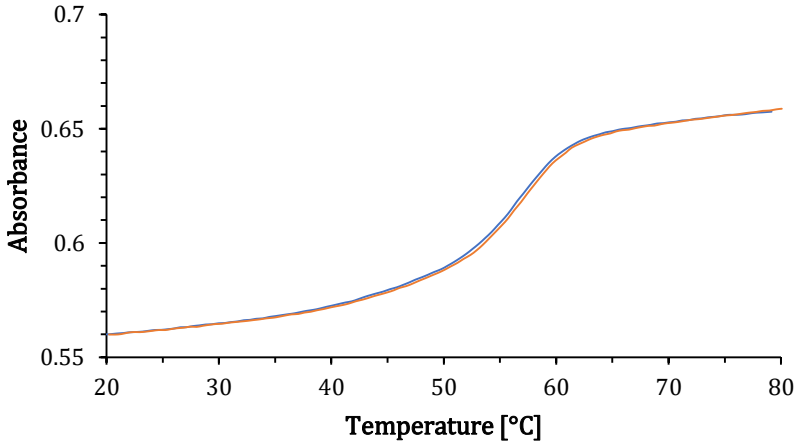

Figure S38: Cooling-heating curves of duplex **2**.

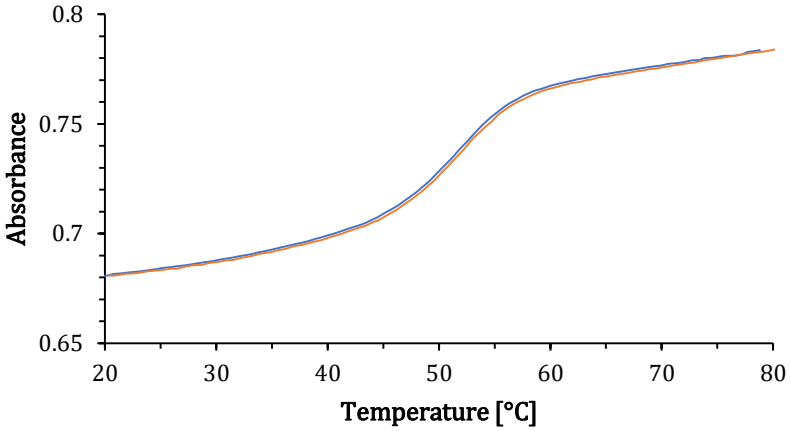

Figure S39: Cooling-heating curves of duplex **3**.

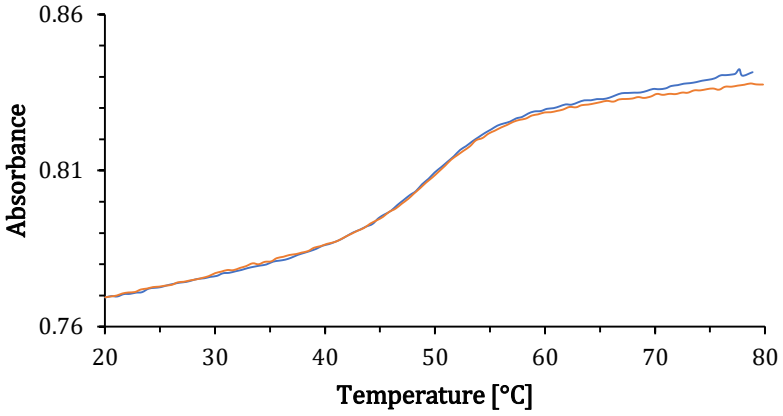

Figure S40: Cooling-heating curves of duplex **4**.

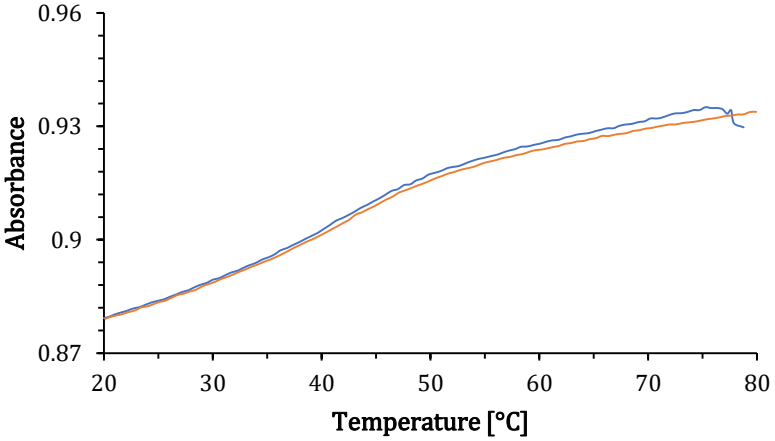

Figure S41: Cooling-heating curves of duplex **5**.

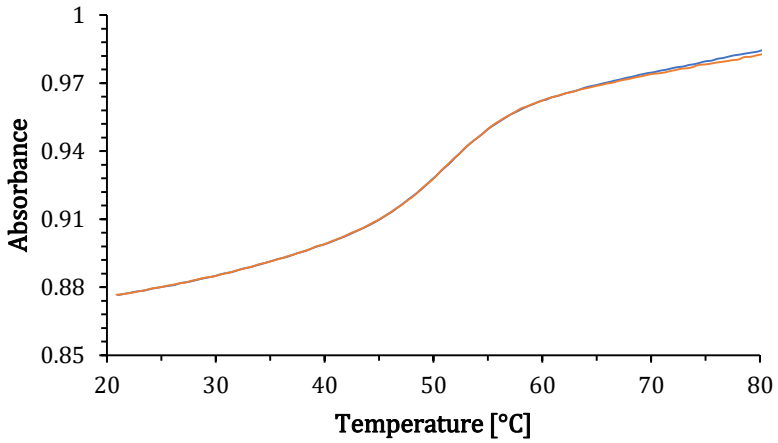

Figure S42: Cooling-heating curves of duplex **6**.

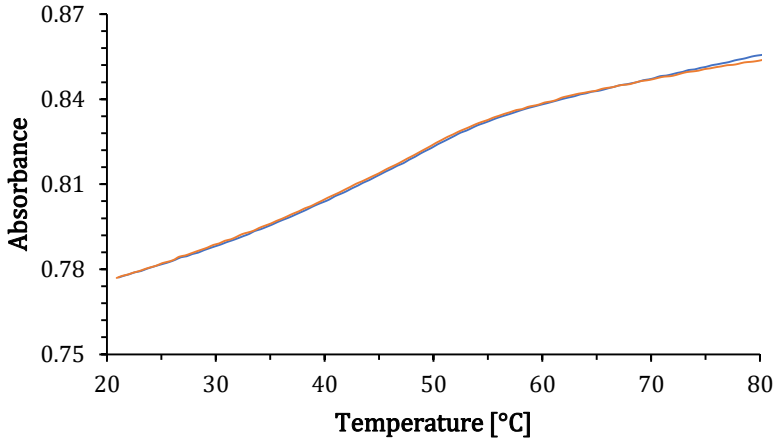

Figure S43: Cooling-heating curves of duplex **7**.

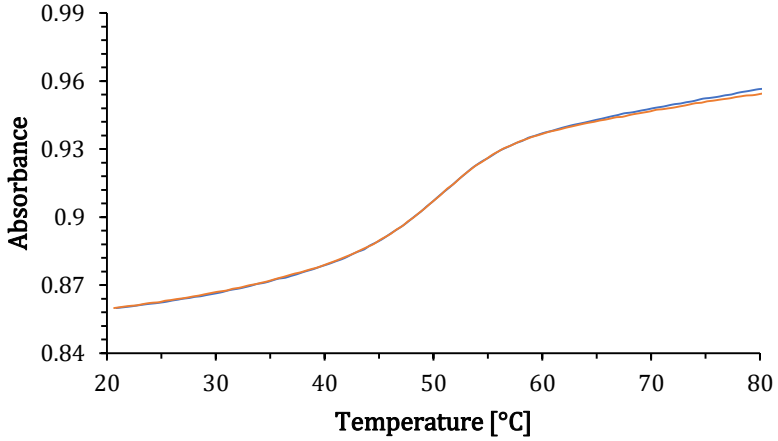

Figure S44: Cooling-heating curves of duplex **8**.

Duplex	Sequence	T_m [°C]
1	5' GGC TAA YTA AAT TTA AAT CGC 3' 3' CCG ATT αAT TTA AAT TTA GCG 5'	60.7
2	5' GGC TAA YTα AAT TTA AAT CGC 3' 3' CCG ATT αAα TTA AAT TTA GCG 5'	57.4
3	5' GGC TAA YTα AαT TTA AAT CGC 3' 3' CCG ATT αAα TαA AAT TTA GCG 5'	52.5
4	5' GGC TAA YTα AαT αTA AAT CGC 3' 3' CCG ATT αAα TαA αAT TTA GCG 5'	49.8
5	5' GGC TAA YTα AαT αTα AAT CGC 3' 3' CCG ATT αAα TαA αAα TTA GCG 5'	42.6
6	5' GGC TAA YTα AαT αTA AAT CGC 3' 3' CCG ATT αTα TαA αAT TTA GCG 5'	50.7
7	5' GGC TAA YTα AαT αTA AAT CGC 3' 3' CCG ATT αAα TαT αAT TTA GCG 5'	48.3
8	5' GGC TAA YTα AαT αTA AAT CGC 3' 3' CCG ATT αTα TαT αAT TTA GCG 5'	51.3

Table S3: Melting temperatures of duplexes **1**, **2**, **3**, **4**, **5**, **6**, **7** and **8**. Conditions: Cooling/heating rate= 0.5 °C/min, 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0 and 400 mM NaCl.

Additional Spectroscopic Data of Duplexes

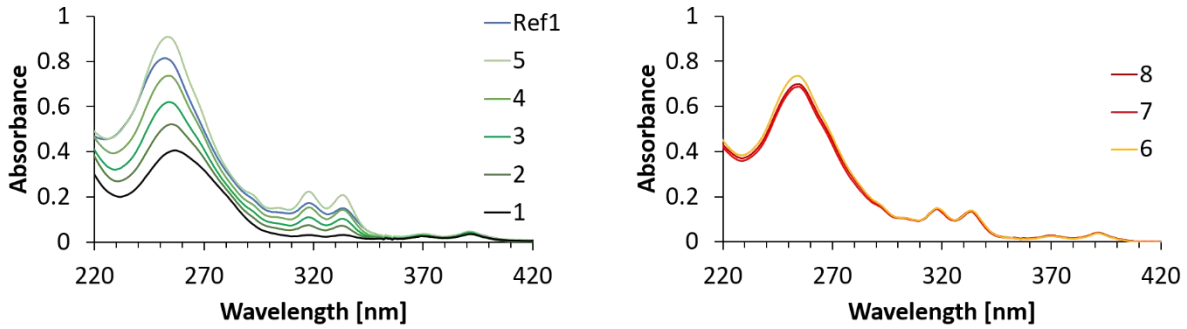

Figure S45: Absorption spectra of duplexes **Ref1**, **1** – **5** (left) and **6** -**8** (right). Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl.*

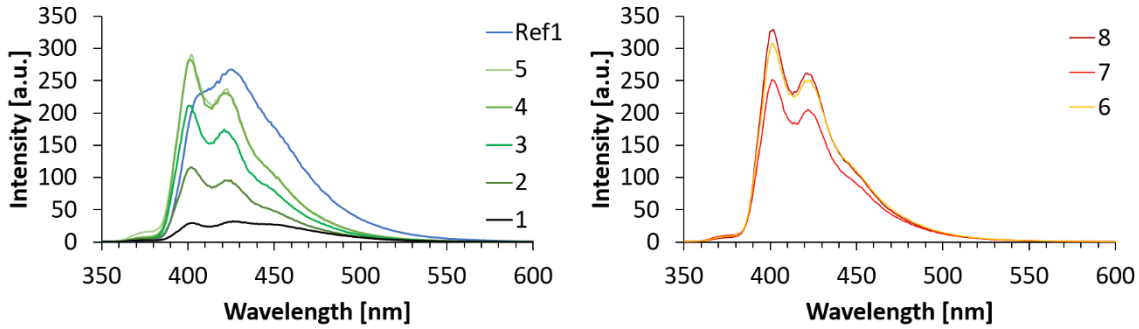

Figure S46: Emission spectra of duplexes **Ref1**, **1** – **5** (left) and **6** -**8** (right). Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl, λ_{ex} 316 nm, 20 °C. Excitation slit: 2.5 nm, emission slit: 5 nm.*

*Figures S39 and S40 correspond to Figures 2 and 3, respectively, in the main text. For increased readability, they are here grouped into two sets of curves.

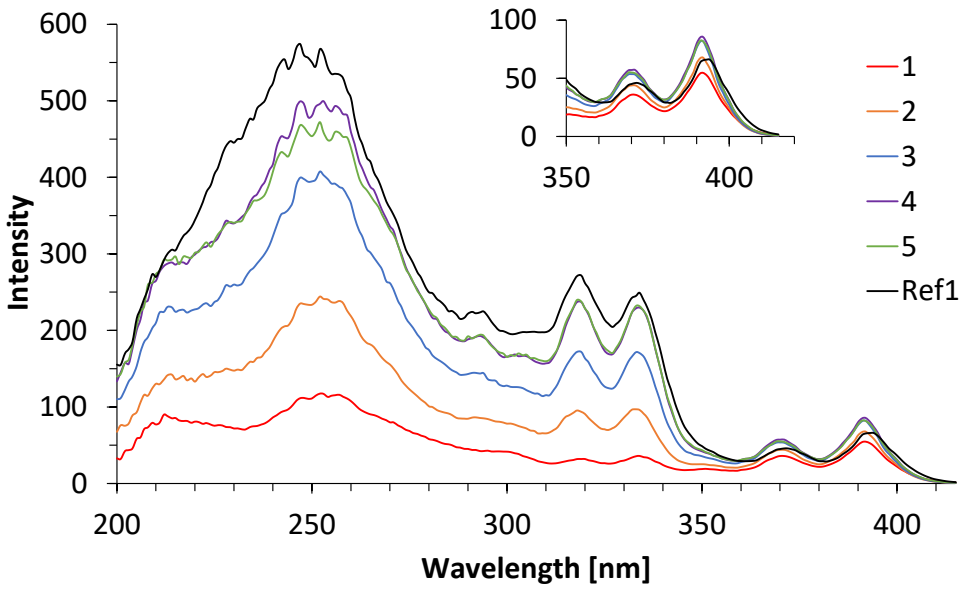

Figure S47: Excitation spectra of duplexes **Ref1**, **1** – **5**. Conditions: 0.5 µM each strand, 10mM sodium phosphate buffer pH=7.0, 400mM NaCl, λ_{em} 425 nm, 20 °C. Excitation slit: 2.5 nm, emission slit: 5 nm.

Quantum Yield Determination

Quantum yields φ_F were determined relative to quinine sulfate in 0.5 M H_2SO_4 as a standard following the procedure described in literature.[4] By integration of the fluorescence area from 350 – 600 nm the quantum yield could be determined. The Absorption of the duplexes and quinine sulfate at the excitation wavelength 316 nm were measured. The formula for the calculation was:

$$\varphi_F = \frac{I_c \cdot A_R}{A_c \cdot I_R} \cdot \varphi_R$$

I_c: fluorescent area (350 – 600 nm) of the samples
I_R: fluorescent area (350 – 600 nm) of quinine sulfate
A_c: Absorption of the samples at 316 nm
A_R: Absorption of the quinine sulfate at 316 nm
φ_R: quantum yield of quinine sulfate = 0.546

References

[1] C. B. Winiger, S. Li, G. R. Kumar, S. M. Langenegger, R. Häner, *Angew. Chemie - Int. Ed.* **2014**, *53*, 13609–13613.

[2] H. Bittermann, D. Siegemund, V. L. Malinovskii, R. Häner, *J. Am. Chem. Soc.* **2008**, *130*, 15285–15287.

[3] M. Vybornyi, A. V. Rudnev, S. M. Langenegger, T. Wandlowski, G. Calzaferri, R. Häner, *Angew. Chemie - Int. Ed.* **2013**, *52*, 11488–11493.

[4] S. Fery-Forgues, D. Lababre, J. Chem. Educ. **1999**, *76*, 1260-1264.