

Supporting Information

In-stem labelling allows visualization of DNA strand displacements by distinct fluorescent colour change

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1. Preparation and purification of modified oligonucleotides

Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. Reagents and controlled pore glass (CPG) (1 μ mol) were purchased from ABI and Glen Research. DNA synthesis of thiazole orange (TO) and thiazole red (TR) modified DNA was performed using a modified protocol. Activator solution (0.45 M tetrazole in acetonitrile) was pumped simultaneously with the building block (0.1 M in acetonitrile). The coupling time was extended to 61.0 min with an intervening step after 30.8 min for washing and refreshing the activator/phosphoramidite solution in the CPG vial. The CPG vial was flushed with acetonitrile after the coupling. After preparation, the oligonucleotides were cleaved from the resin and deprotected by treatment with conc. NH_4OH at 45 °C for 20 h.

The modified oligonucleotides were purified by HPLC on a semi preparative RP-C18 column (300 Å, Supelco) using the following conditions: A) NH_4OAc buffer (50 mM), pH = 6.5; B) acetonitrile; gradient 0 – 20 % B over 45 min; flow rate 2.5 mL/min; UV/vis detection at 260 nm, 512 nm for TO modified DNA and 630 nm for TR modified DNA. Purification was verified by MS (MALDI) on a Biflex-IV spectrometer from Bruker Daltonics in the linear negative mode (matrix: 2:1 mixture of 2,4,6- trihydroxyacetophenone (0.3 M in EtOH) and diammoniumcitrate (0.1 M in H_2O)). Finally the oligonucleotides were lyophilized and quantified by their absorbance in 10 mM sodium phosphate buffer at 260 nm on a ND-1000 spectrophotometer from NanoDrop in the nucleic acid mode. Double strands were annealed by heating up to 90 °C for 10 min and then cooling down slowly to room temperature over 18 h.

DNA1

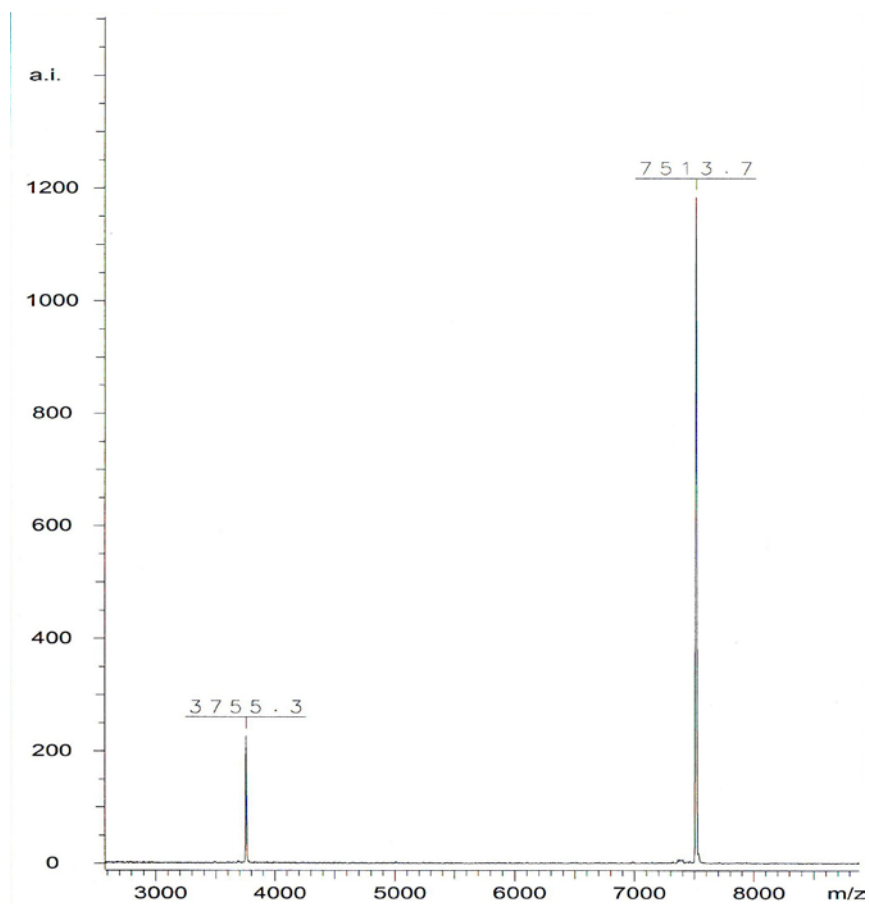
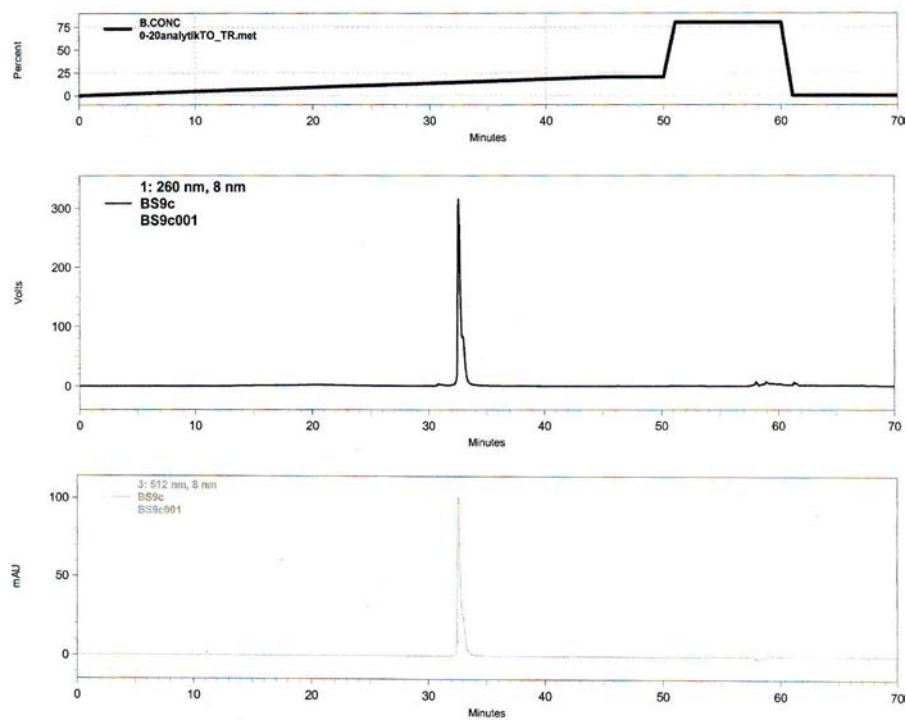


Figure S1. Images of HPLC and MS (MALDI) analysis of **DNA1** (calculated = 7507.3; found = 7513.7).

DNA10

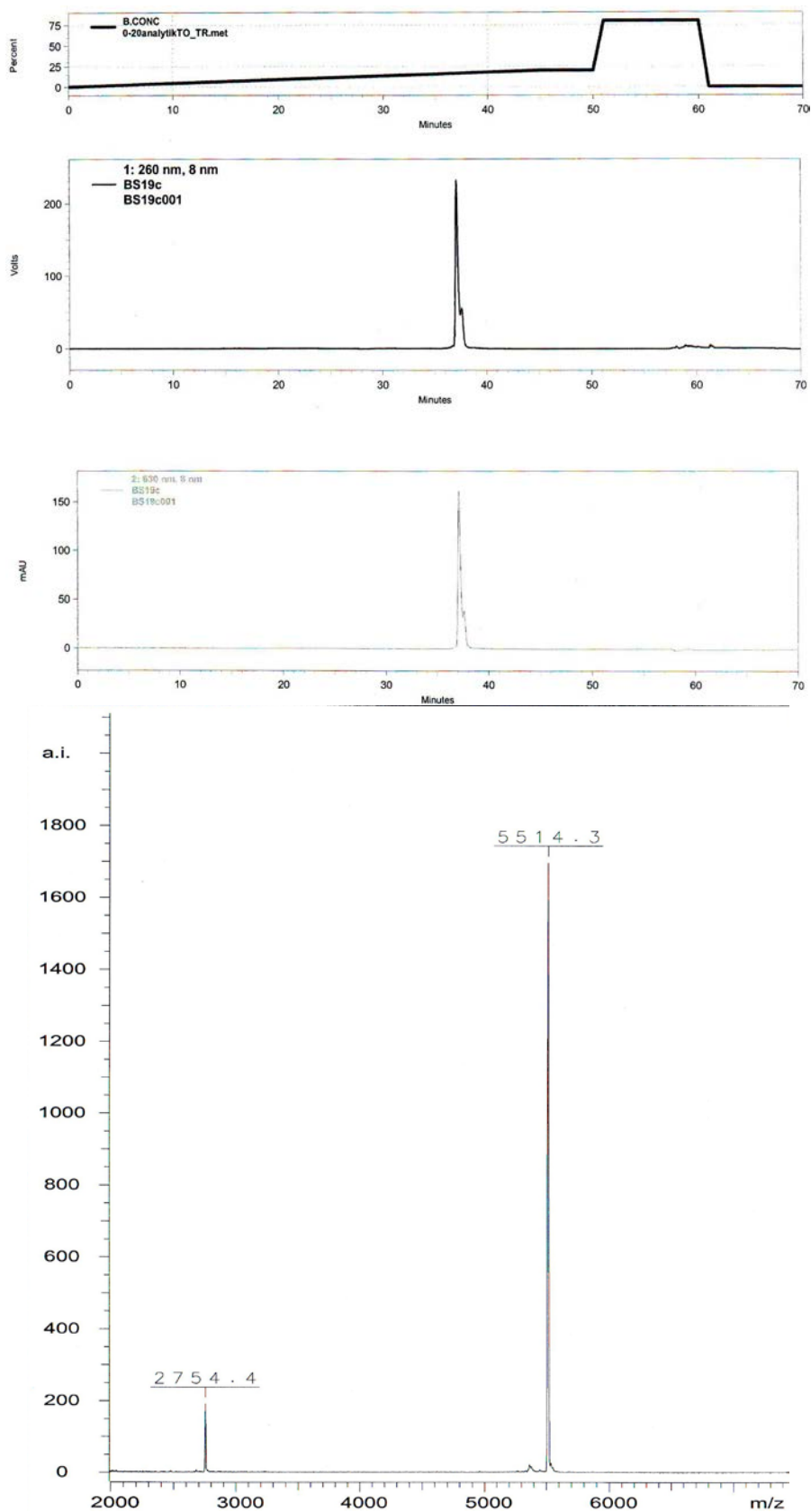
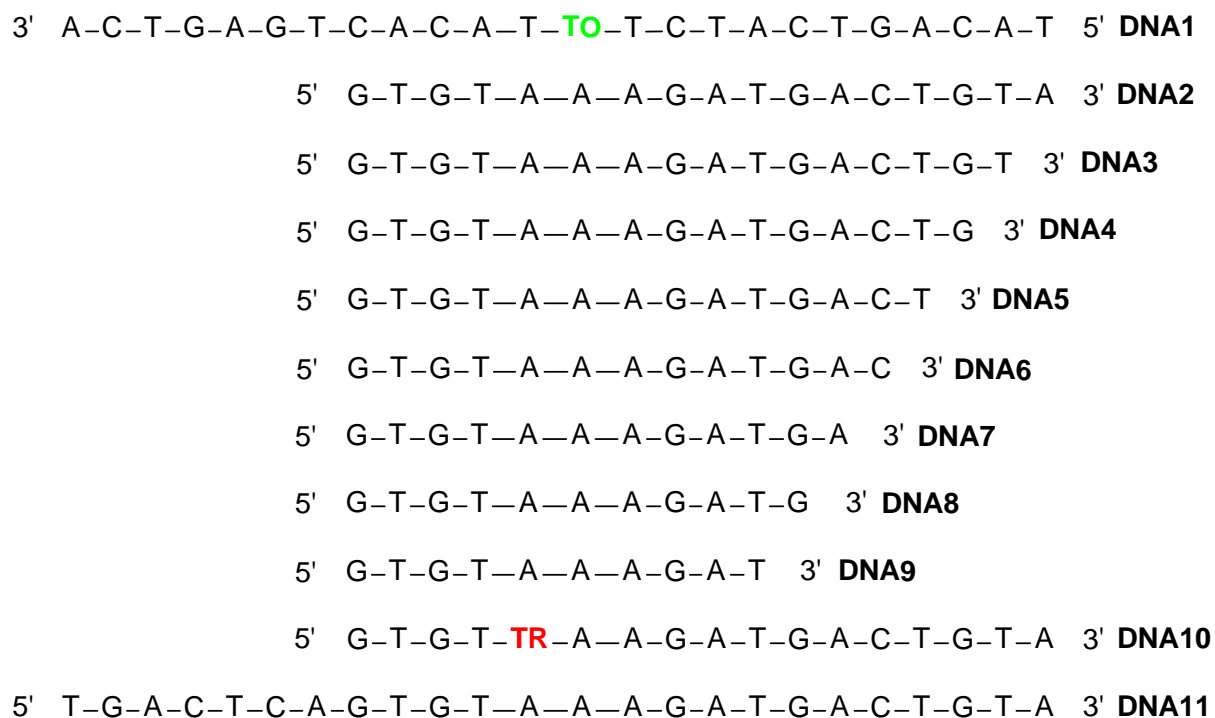


Figure S2. Images of HPLC and MS (MALDI-TOF) analysis of **DNA10** (calculated = 5512.0; found = 5514.3).

2. DNA sequences and melting temperatures (T_m)

Unmodified DNA strands were purchased from Metabion international AG (Martinsried, Germany).



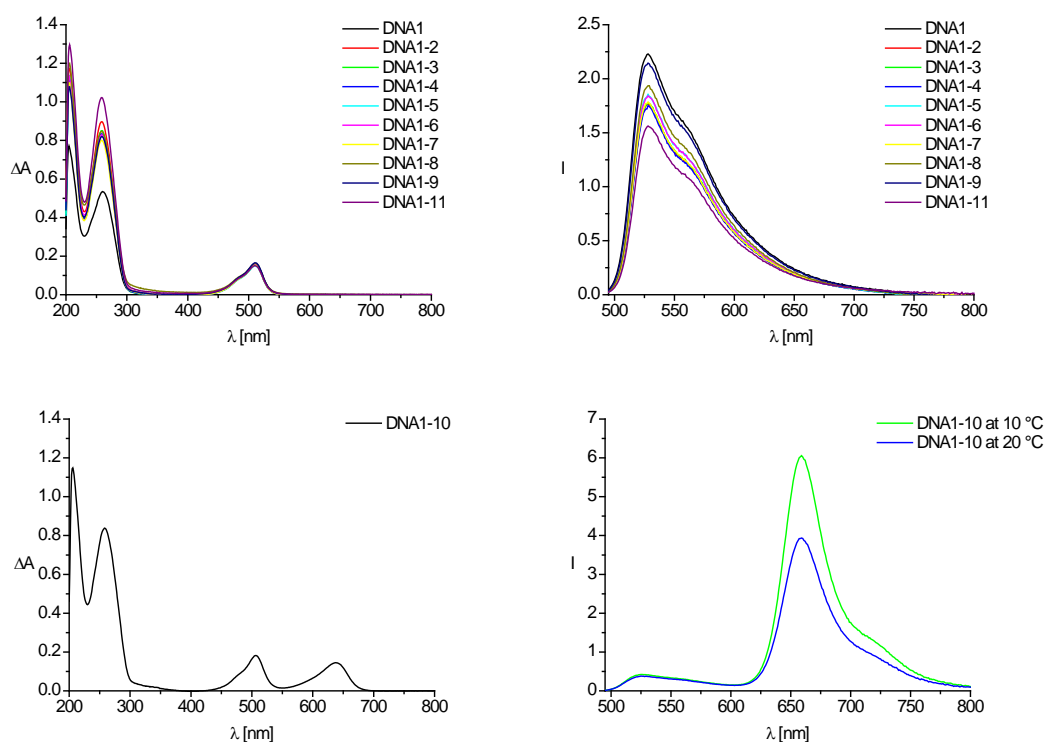
Scheme S1. Sequences of oligonucleotides.

Table S1. Melting temperatures of **DNA1-2** to **DNA1-11**: $\lambda = 260$ nm, 10-90 °C, interval: 0.7 °C/min, 2.5 μ M DNA in 10 mM NaPi-buffer (pH = 7.0), 250 mM NaCl.

| DNA | T_M [°C] |
|-------------|------------|
| 1-2 | 53.8 |
| 1-3 | 52.3 |
| 1-4 | 49.3 |
| 1-5 | 44.6 |
| 1-6 | 42.2 |
| 1-7 | 32.4 |
| 1-8 | 23.1 |
| 1-9 | 12.4 |
| 1-10 | 53.2 |
| 1-11 | 67.8 |

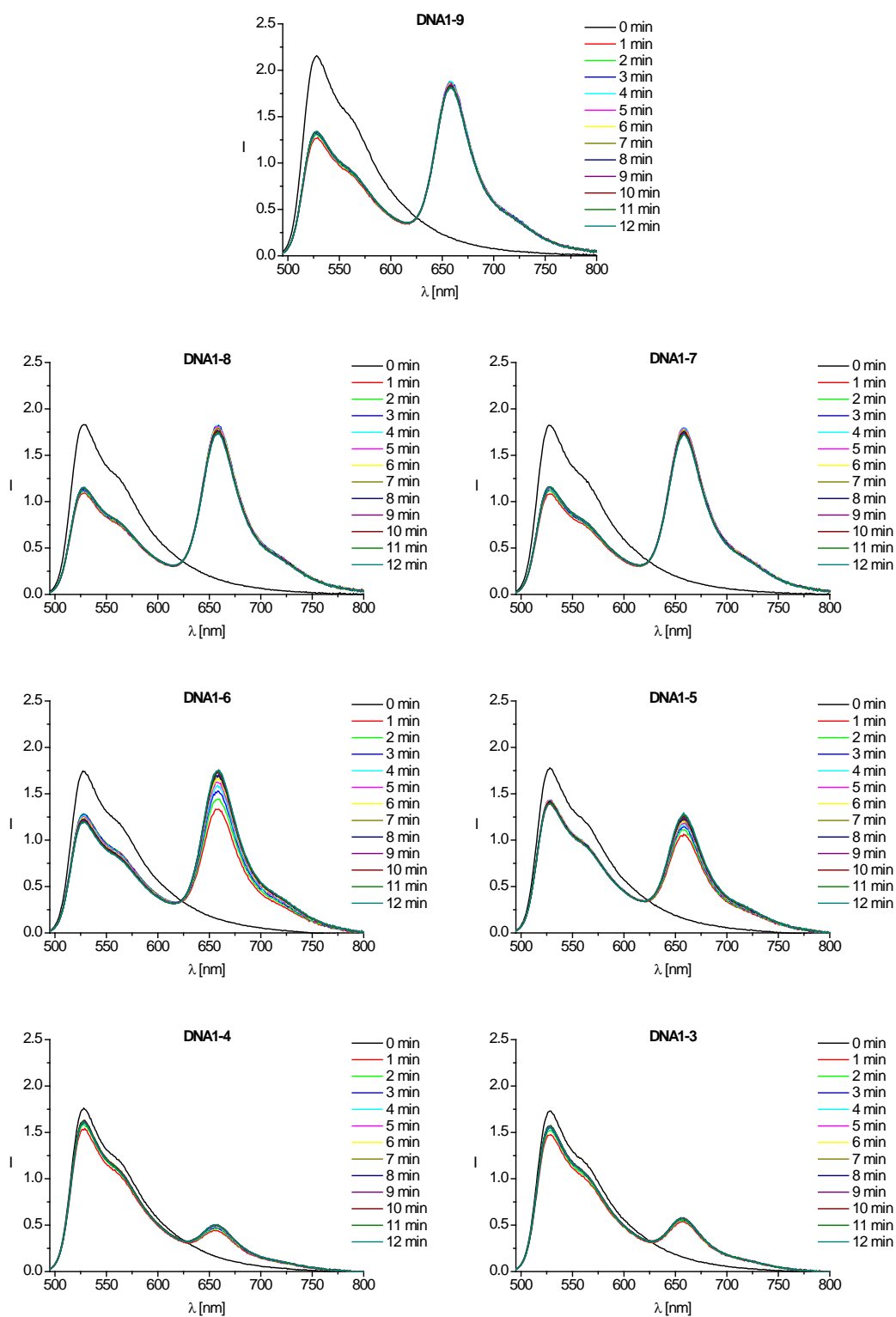
3. Spectroscopic data

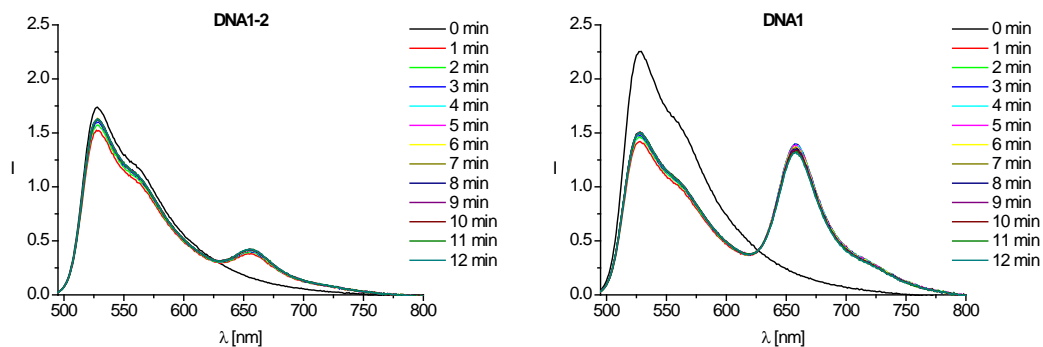
Spectroscopic measurements were recorded in Na-Pi buffer solution (10 mM, pH = 7) with 250 mM NaCl using quartz glass cuvettes (10 mm) at 20 °C, if not other mentioned. Absorption spectra were recorded with a Varian Cary 100 spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured with a Jobin–Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation band pass of 3 nm, an emission band pass of 2 nm, an excitation wavelength of 490 nm and are corrected for Raman emission from the buffer solution.



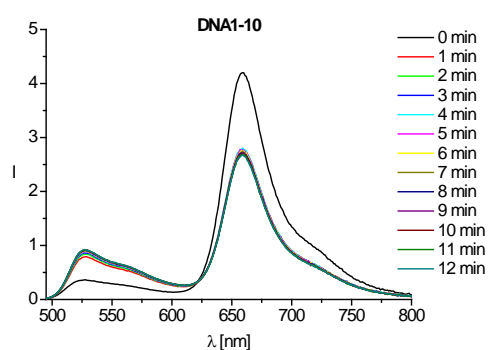
3.1 Time-dependent experiments

For the time-dependent measurements 0.5 equiv. **DNA10** were added to a 2.5 μM solution containing **DNA1-2** to **DNA1-9** and **DNA1** and fluorescence spectra were recorded in 1 min steps.

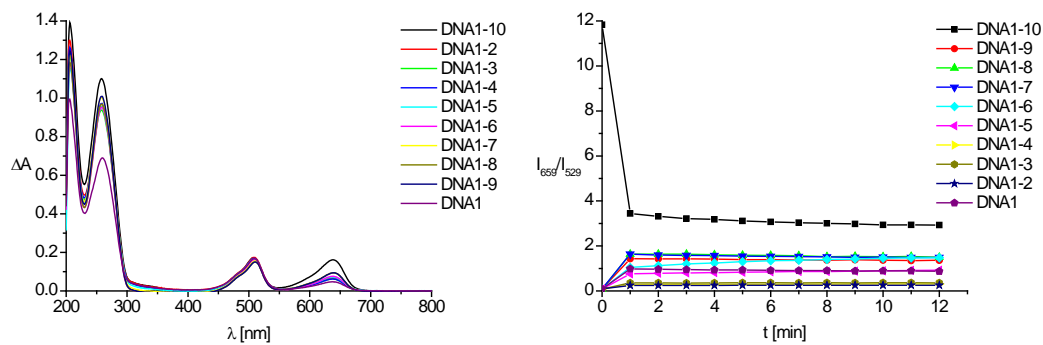




The additional time-dependent measurement was carried out with 0.5 equiv. **DNA11** + **DNA1-10**.

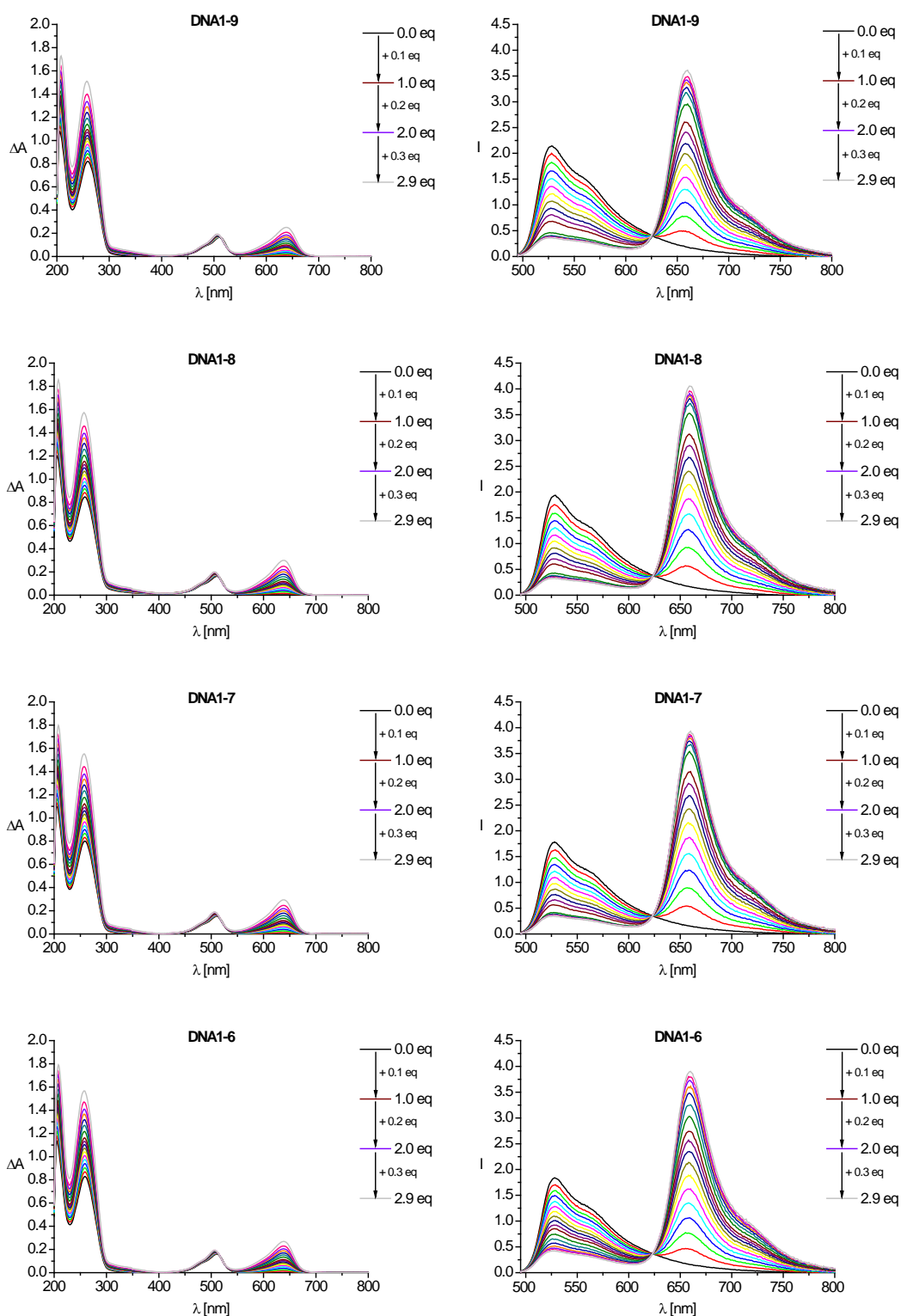


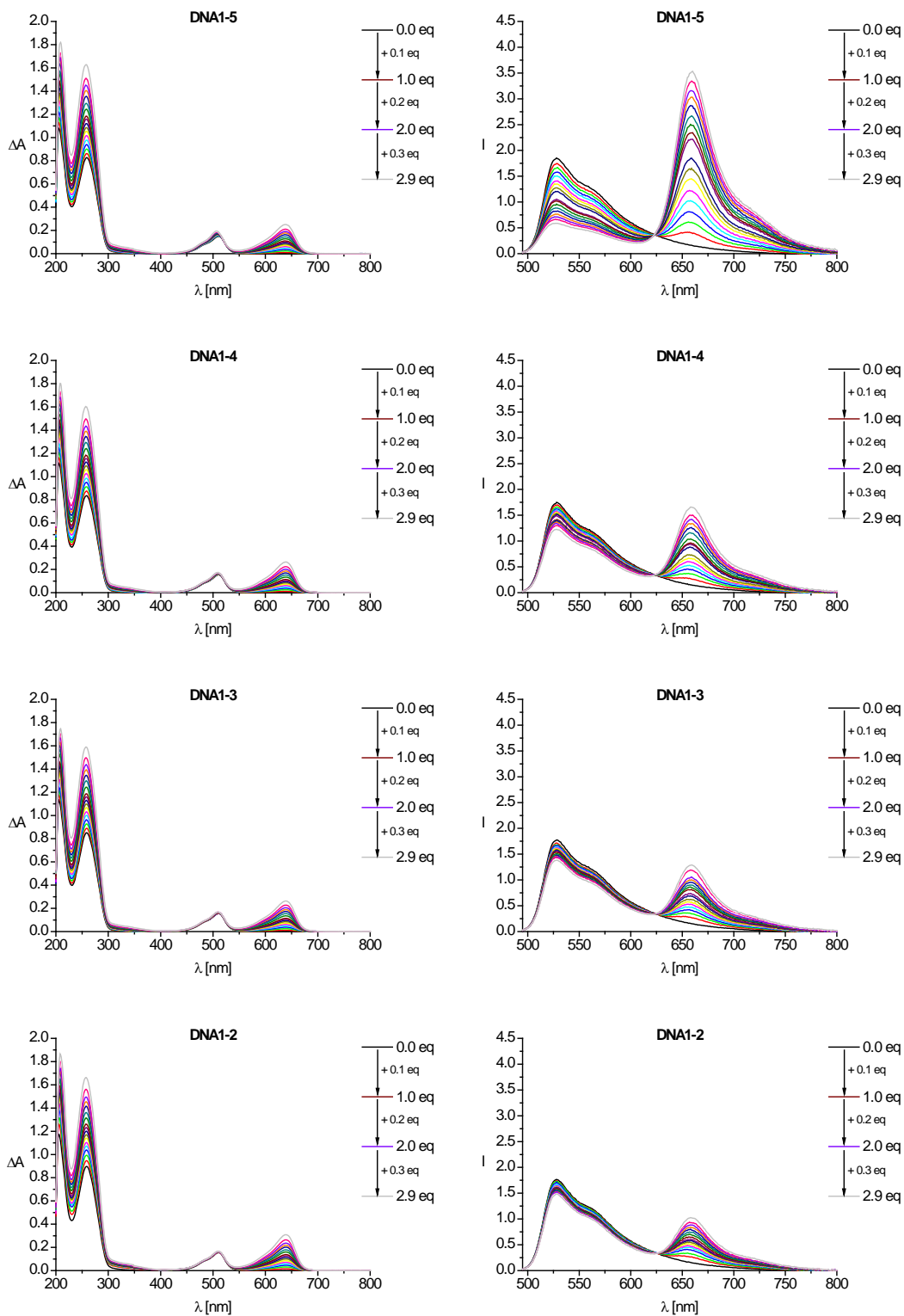
Summary of the time-dependent measurement (left) and the changes of the red:green (I_{659}/I_{529}) ratio during the measurements (right).

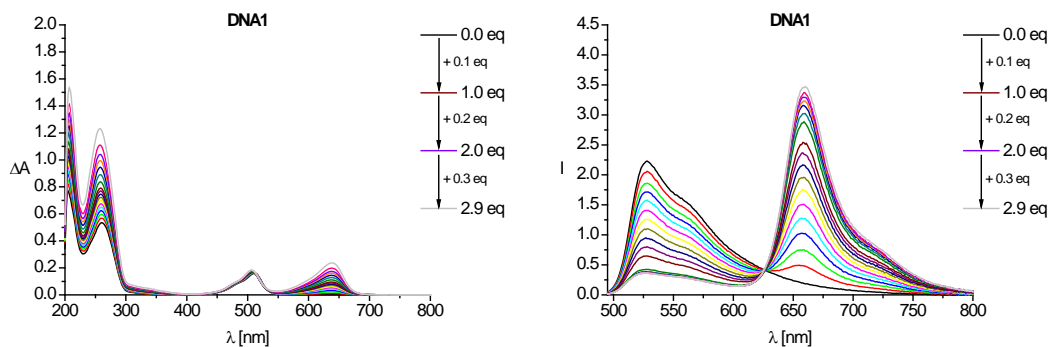


3.2 Strand displacement experiments

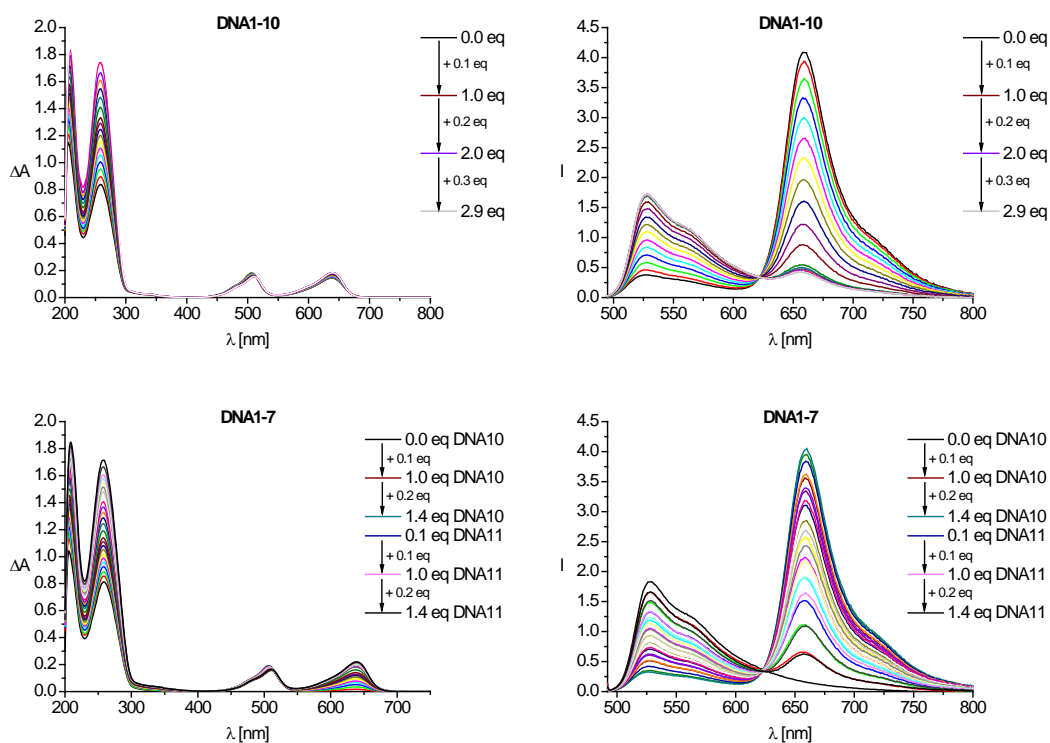
Each 2.5 μM solution containing **DNA1-2** to **DNA1-9** and **DNA1** was titrated with up to 2.9 equiv. of **DNA10**; each optical measurement was performed after 10 min incubation time.



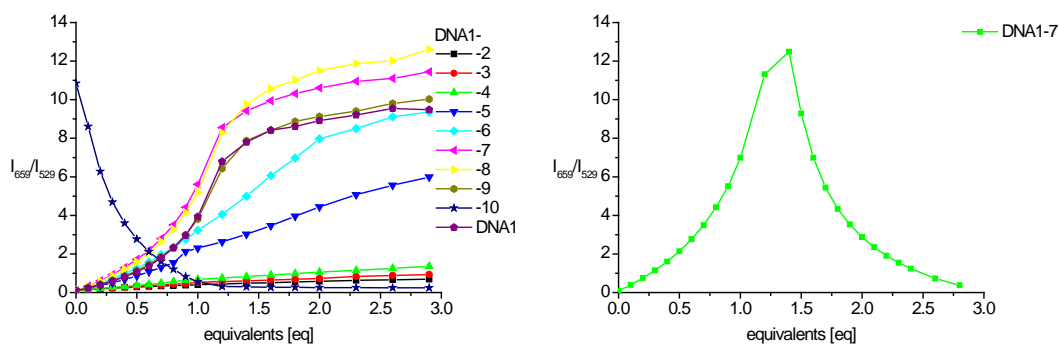




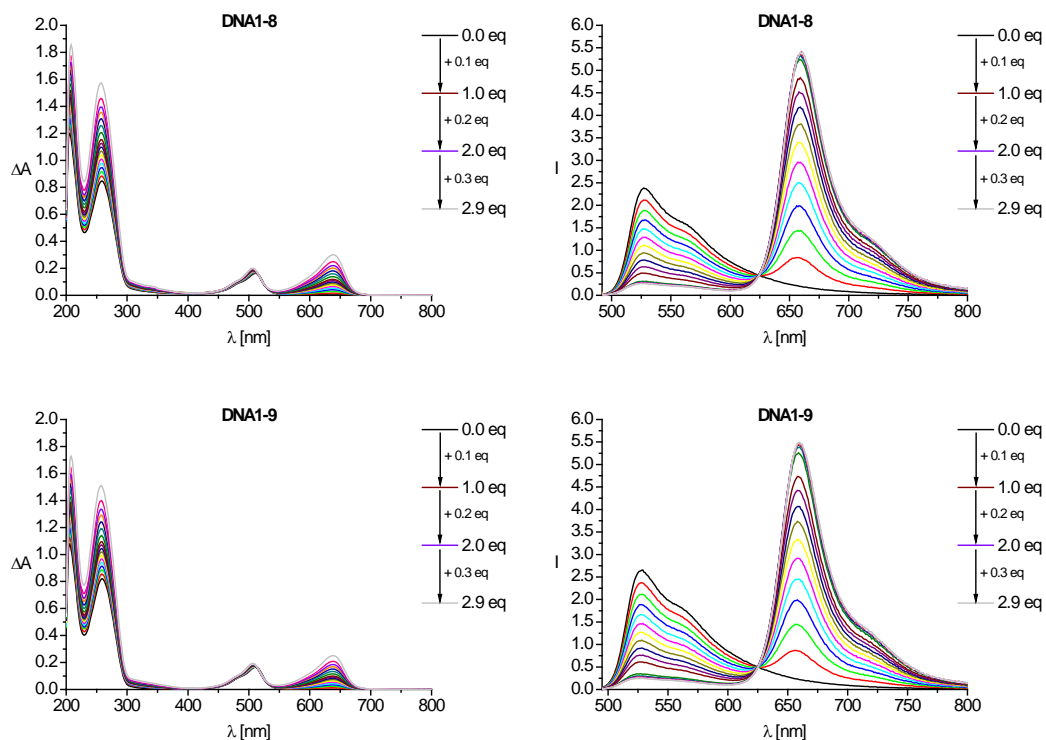
The additional titrations were carried out with **DNA1-10 + DNA11** (with up to 2.9 equiv. of **DNA11**) and **DNA1-7 + DNA10 + DNA11** (with up to 1.4 equiv. of **DNA10** respectively **DNA11**).



Summary of the changes of the red:green (I_{659}/I_{529}) ratio during the titration of **DNA1-2** to **DNA1-9** and **DNA1** with **DNA10**, **DNA1-10** with **DNA11** (left) and the two step titration of **DNA1-7** with **DNA10** and **DNA11** (right).



Additionally, the titrations of **DNA1-8** and **DNA1-9** with up to 2.9 equiv. of **DNA10** were carried out at 10 °C.



Summary of the changes of the red:green (I_{659}/I_{529}) ratio during the titration of **DNA1-8** (green line) and **DNA1-9** (blue line) with **DNA10** at 10 °C (continuous) compared to the changes at 20°C (dashed).

