Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Signal Control by Self-Assembly of Fluorophores in a Molecular Beacon – A Model Study.

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Supporting Information

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Synthetic and analytical procedures

The building blocks alkynylpyrene (**Y**)[1] and perylenediimide (**E**)[2] were synthesized as previously described. Oligonucleotides **T1**–**T4** were obtained commercially from Microsynth, Balgach, Switzerland. **MB1–MB5** were prepared via automated oligonucleotide synthesis on a 394-DNA/RNA synthesizer (Applied Biosystems). For the coupling of the PDI building block, a solution of 5-(ethylthio)-1H-tetrazole in tetrahydrofurane (0.3M) was used. The PDI phosphoramidite was used as a 0.06M solution in dichloromethane. A coupling time of 2 min was applied for this building block. Cleavage from the solid support and final deprotection was done by treatment with 30% NH_4OH solution at 55°C overnight.

Purification was performed by reverse phase HPLC (LiChrospher 100 RP-18, 5 um, Merck; Shimadzu LC-20AT and Kontron Instruments). Mass spectrometry was done with a Scienx QSTAR pulsar (hybrid quadrupole time-of-flight mass spectrometer, Applied Biosystems); ESI-TOF MS (negative mode, acetonitrile/H_2O/triethylamine).

Temperature-dependent UV/Vis spectra were carried out on a Varian Cary-100 Bio-UV/Vis spectrophotometer equipped with a Varian Cary-block temperature controller and data were collected with Varian WinUV software, over the range of 200-700 nm at 10-90°C in 10°C increments. CD spectra were recorded on a JASCO J-715 spectrophotometer using quartz cuvettes with an optic path of 1 cm. Fluorescence spectra were performed on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Varian Cary-block temperature controller using 1 cm x 1 cm quartz cuvettes.

HPLC chromatogrammes and masses

MB1

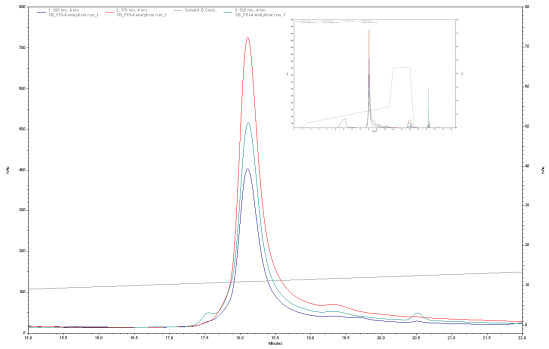


Fig. S1: HPLC chromatogram of **MB1**. Eluent A = $(Et_3NH)OAc$ (0.1 M, pH 7.4), B = MeCN; elution at 30°C; gradient 5 – 20 % B over 20 min, t_R = *18.7* min. Inset: chromatogram from 0 to 39 min.

MB2

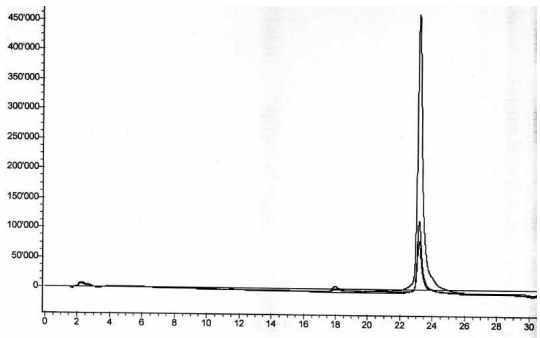


Fig. S2: HPLC chromatogram of **MB2**. Eluent A = $(Et_3NH)OAc$ (0.1 M, pH 7.4), B = MeCN; elution at 60°C; gradient 5 – 40 % B over 30 min, t_R = *23.1* min.

MB3

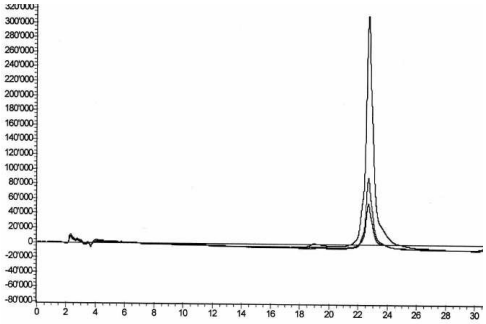


Fig. S3: HPLC chromatogram of **MB3**. Eluent A = $(Et_3NH)OAc$ (0.1 M, pH 7.4), B = MeCN; elution at 60°C; gradient 5 – 50 % B over 30 min, t_R = *22.7* min.

MB4

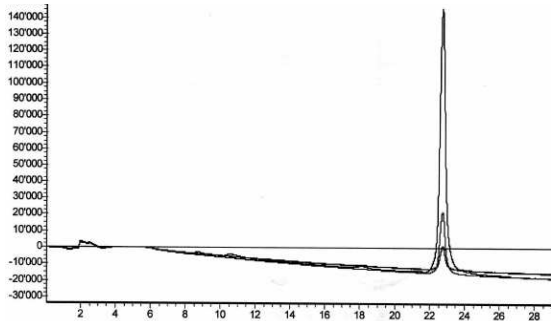


Fig. S4: HPLC chromatogram of **MB4**. Eluent A = $(Et_3NH)OAc$ (0.1 M, pH 7.4), B = MeCN; elution at 60°C; gradient 5 – 40 % B over 30 min, t_R = *22.8* min.

MB5

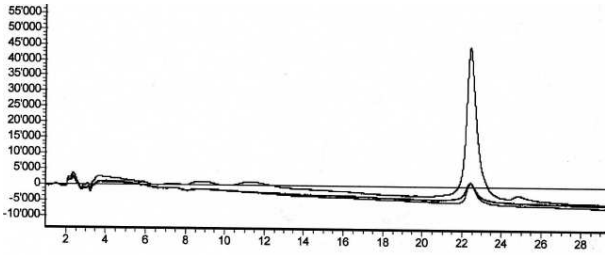


Fig. S5: HPLC chromatogram of **MB5**. Eluent A = $(Et_3NH)OAc$ (0.1 M, pH 7.4), B = MeCN; elution at 60°C; gradient 5 – 30 % B over 30 min, t_R = *22.5* min.

Table S1: Molecular formula and masses of **MB1**–**MB5**.

ON	*molecular formula*	*calcd. avg. mass*	*found avg. mass*
MB1	$C_{363}H_{393}N_{112}O_{176}P_{29}$	10039.0	10039.6
MB2	$C_{325}H_{345}N_{96}O_{152}P_{25}$	8802.1	8802.6
MB3	$C_{286}H_{296}N_{81}O_{128}P_{21}$	7566.3	7565.8
MB4	$C_{295}H_{324}N_{94}O_{144}P_{24}$	8233.6	8232.8
MB5	$C_{329}H_{380}N_{117}O_{176}P_{29}$	9687.4	9680.8

Thermal denaturation of **MB1**

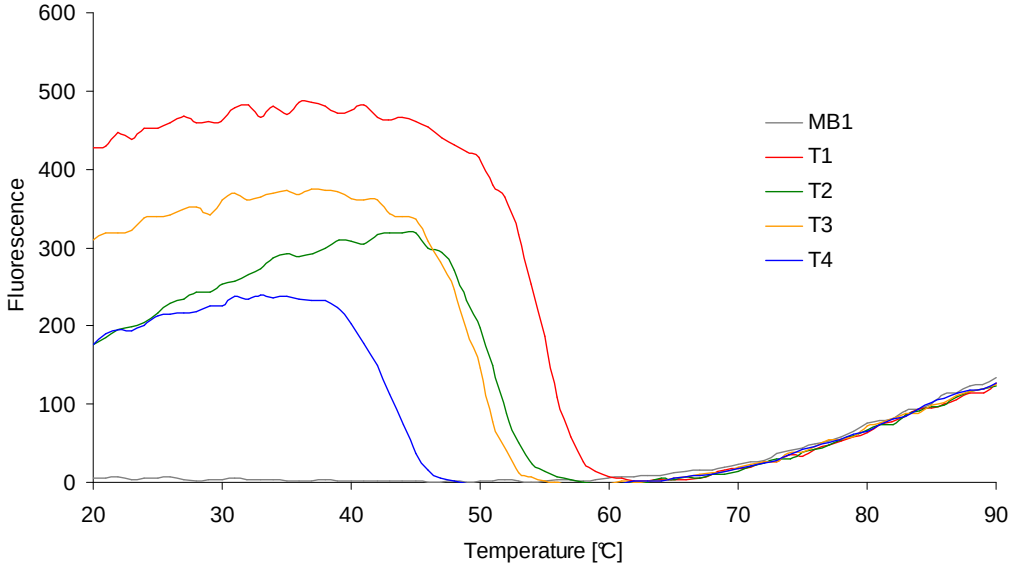


Fig. S6: Conditions: **MB1** 0.1 µM, **Tn** 0.1 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Excitation: 370 nm; detection at 520 nm (excimer); excitation slit width: 5 nm; emission slit width: 5 nm; PMT voltage: 800 V; ramp: heating; heating/cooling rate: 0.5 °C/min.

Thermal denaturation of **MB2**

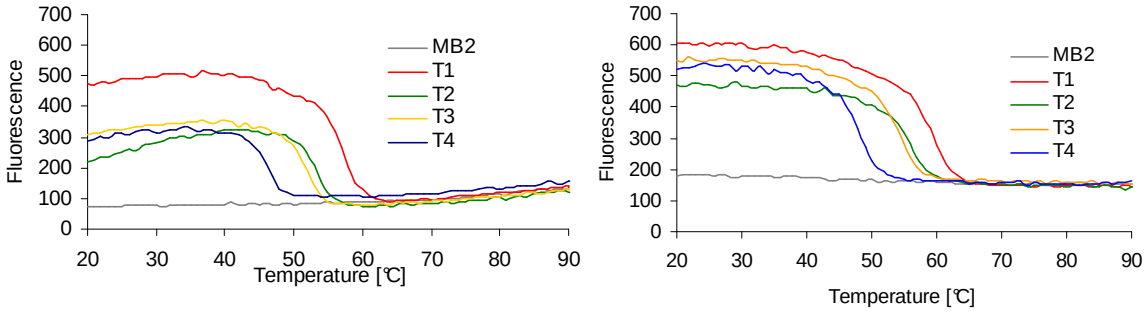


Fig. S7: Conditions: **MB2** 0.1 µM, **Tn** 0.1 µM, 10 mM phosphate buffer pH 7.0 (left) or 10 mM Tris-HCl buffer pH 7.4 (right), 100 mM NaCl. Excitation: 370 nm; detection at 520 nm (excimer); excitation slit width: 5 nm; emission slit width: 5 nm; PMT voltage: 800 V; ramp: heating; heating/cooling rate: 0.5 °C/min.

Thermal denaturation of **MB5**

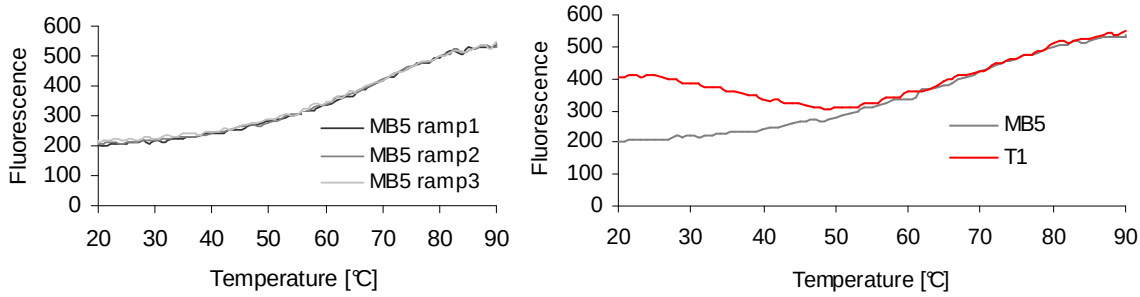


Fig. S8: Conditions: **MB5** 0.1 µM, 100 mM NaCl, 10 mM phosphate buffer pH 7.0 (left). Fluorescence characteristics of **MB5** hybridization with DNA. Conditions: **MB5** 0.1 µM, **T1** 0.1 µM, 100 mM NaCl, 10 mM phosphate buffer pH 7.0. Excitation: 370 nm; detection at 400 nm; excitation slit width: 10 nm; emission slit width: 5 nm; PMT voltage: 800 V; ramp1 and ramp3: cooling, ramp2: heating, heating/cooling rate: 0.5 °C/min.

T_M values

Table S2: T_M values (phosphate buffer was used if not otherwise indicated).

hybrid	T_M [°C]
MB1	n.d.
MB1*T1	55
MB1*T2	51
MB1*T3	50
MB1*T4	44
MB2	n.d.
MB2*T1	57
MB2*T2	54
MB2*T3	52
MB2*T4	46
MB2 (in Tris-HCl buffer)	n.d.
MB2*T1 (in Tris-HCl buffer)	59
MB2*T2 (in Tris-HCl buffer)	56
MB2*T3 (in Tris-HCl buffer)	54
MB2*T4 (in Tris-HCl buffer)	47
MB3	n.d.
MB4	n.d.
MB5	62
MB5*T1	47

n.d.: could not be determined

Fluorescence measurements

Spectroscopic response of **MB1** (1.0 µM) upon hybridization to target **T1** (0 to 10 equiv.)

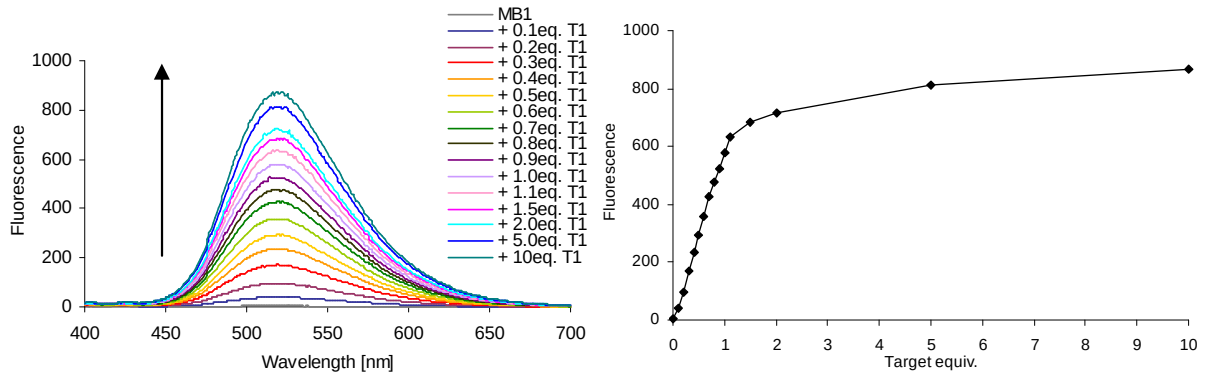


Fig. S9: Fluorescence spectra at 1.0 µM against 0 to 10 eq. target. Conditions: **MB1**1.0 µM, **T1** 0 to 10 equiv., 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Excitation: 370 nm; excitation slit width: 10 nm; emission slit width: 5 nm; PMT voltage: 600 V; Temp.: 37°C; **MB1** sample without target was heated to 90°C and cooled down to 20°C prior to measurement; target titration: data were collected immediately after adding the target (Increasing target concentration illustrated with the arrow: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.5, 2.0, 5.0, 10 equiv.). Right: Concentration dependent fluorescence intensities at 520 nm.

Spectroscopic response of **MB2** (1.0 µM) upon hybridization to target **T1** (0 to 10 equiv.)

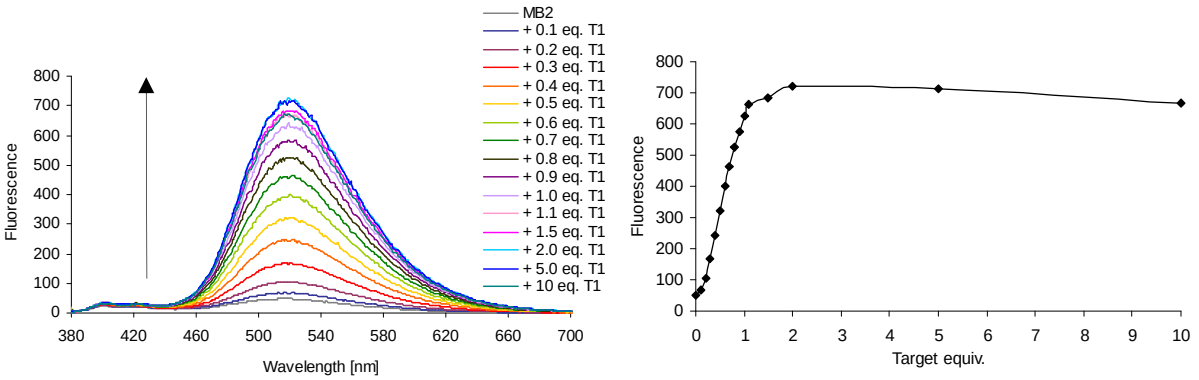
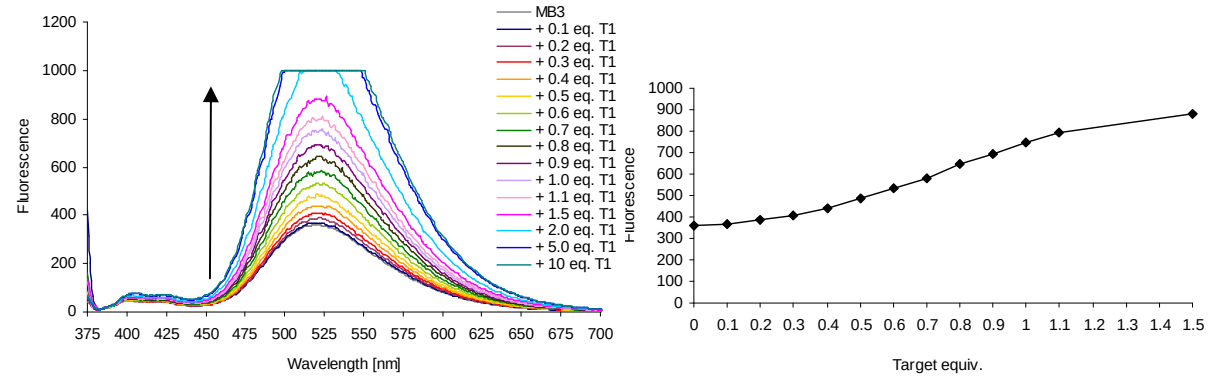


Fig. S10: Fluorescence spectra at 1.0 µM against 0 to 10 eq. target. Conditions: **MB2** 1.0 µM, **T1** 0 to 10 equiv., 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Excitation: 370 nm; excitation slit width: 10 nm; emission slit width: 5 nm; PMT voltage: 600 V; Temp.: 37°C; **MB2** sample without target was heated to 90°C and cooled down to 20°C prior to measurement; target titration: data were collected immediately after adding the target (Increasing target concentration illustrated with the arrow: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.5, 2.0, 5.0, 10 equiv.). Right: Concentration dependent fluorescence intensities at 520 nm.

Spectroscopic response of **MB3** (1.0 µM) upon hybridization to target **T1** (0 to 10 equiv.)

a)



b)

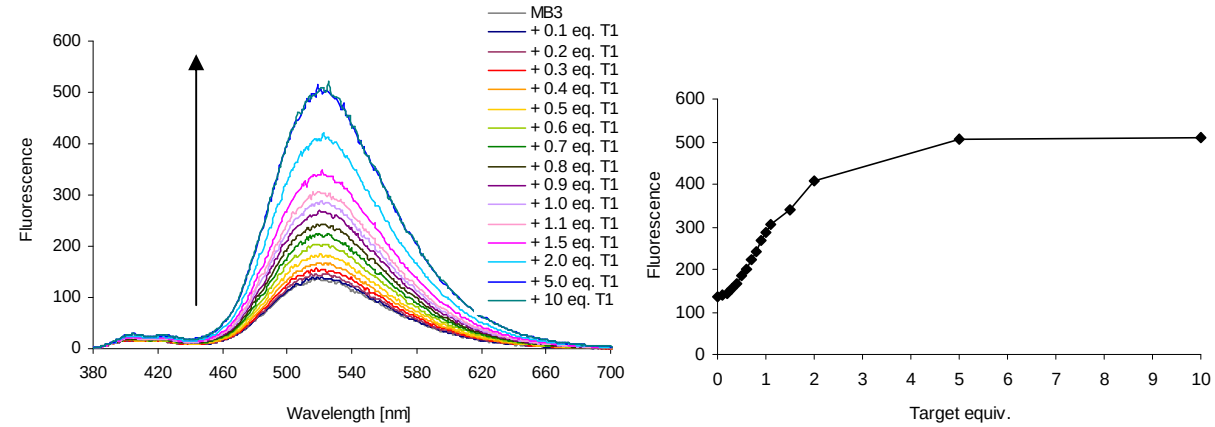


Fig. S11: Fluorescence spectra at 1.0 µM against 0 to 10 eq. target. Conditions: **MB3** 1.0 µM, **T1** 0 to 10 equiv., 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Excitation: 370 nm; a) excitation slit width: 10 nm; emission slit width: 5 nm; PMT voltage: 600 V; b) excitation slit width: 5 nm; emission slit width: 5 nm; PMT voltage: 600 V Temp.: 37°C; **MB3** sample without target was heated to 90°C and cooled down to 20°C prior to measurement; target titration: data were collected immediately after adding the target (Increasing target concentration illustrated with the arrow: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.5, 2.0, 5.0, 10 equiv.). Right: Concentration dependent fluorescence intensities at 520 nm.

Concentration-dependent fluorescence read-out at of **MB1**, **MB2** and **MB3**

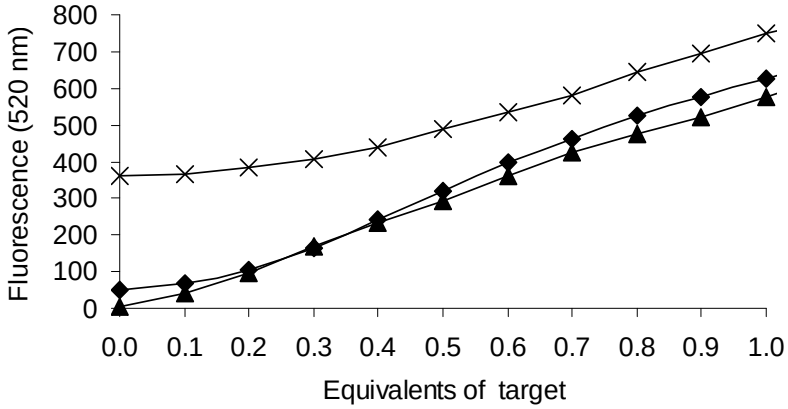


Fig. S12: Concentration-dependent fluorescence read-out at 520 nm obtained with **MB1** (▲), **MB2** (♦) and **MB3** (×) (1.0 µM) on hybridization with the DNA target **T1** (0 to 1 equiv. in 0.1 steps). Conditions: Excitation at 370 nm, slits 10/5 nm; 100 mM NaCl, 10 mM phosphate buffer, pH 7.0, 37°C.

Spectroscopic response of **MB4** (1.0 µM) upon hybridization to target **T1** (0 to 10 equiv.)

a)

b)

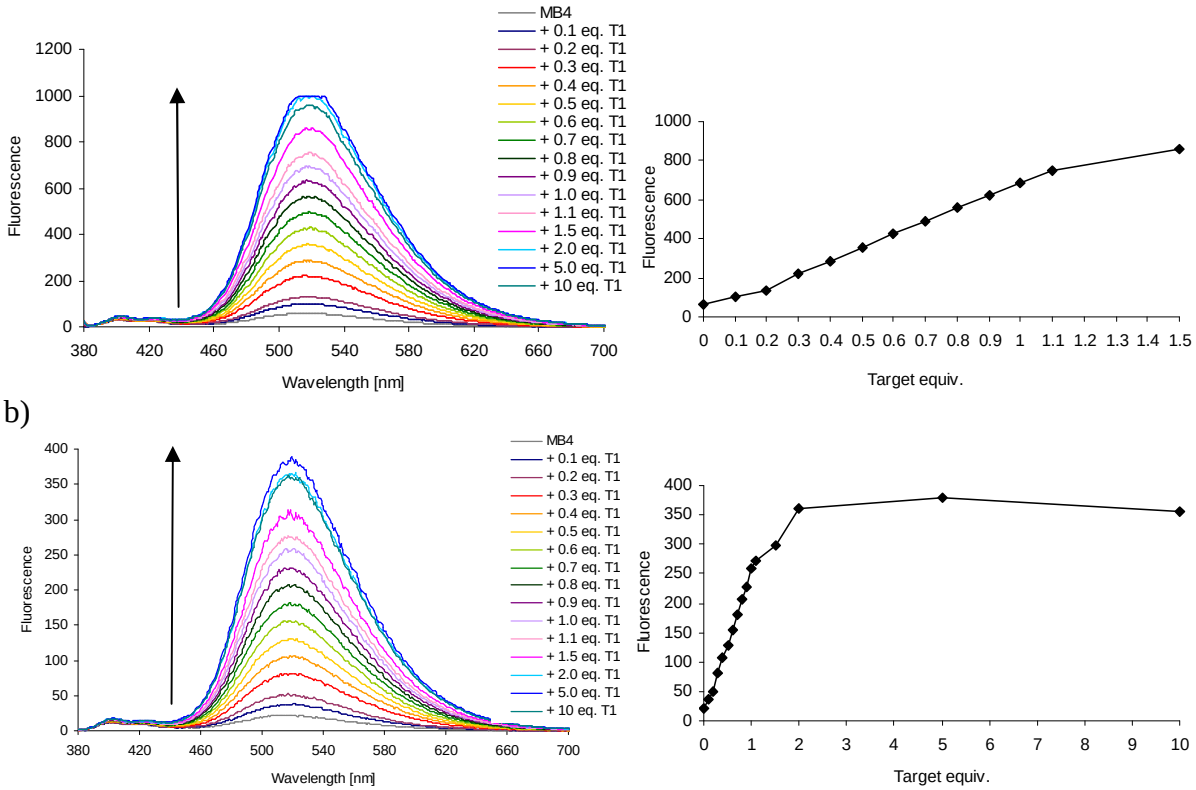


Fig. S13: Fluorescence spectra at 1.0 µM against 0 to 10 eq. target. Conditions: **MB4** 1.0 µM, **T1** 0 to 10 equiv., 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Excitation: 370 nm; a) excitation slit width: 10 nm; emission slit width: 5 nm; PMT voltage: 600 V; b) excitation slit width: 5 nm; emission slit width: 5 nm; PMT voltage: 600 V; Temp.: 37°C; **MB4** sample without target was heated to 90°C and cooled down to 20°C prior to measurement; target titration: data were collected immediately after adding the target (Increasing target concentration illustrated with the arrow: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.5, 2.0, 5.0, 10 equiv.). Right: Concentration dependent fluorescence intensities at 520 nm.

Concentration-dependent fluorescence read-out at of **MB1**, **MB2** and **MB4**

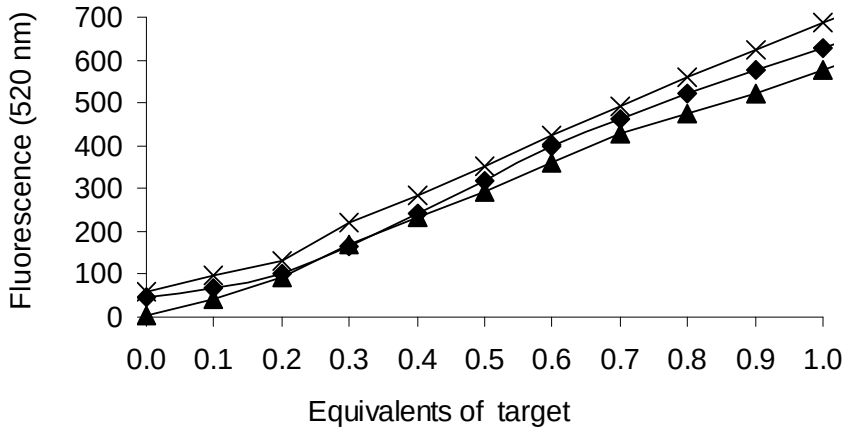


Fig. S14: Concentration-dependent fluorescence read-out at 520 nm obtained for MB1 (▲), MB2 (♦) and MB4 (×) (1µM) on hybridization with the DNA target T1 (0 to 1 equiv. in 0.1 steps). Conditions: Excitation at 370 nm, slits 10/5 nm; 100 mM NaCl, 10 mM phosphate buffer, pH 7.0, 37°C.

Spectroscopic response of **MB5** (0.1 µM) upon hybridization to target **T1** (0 to 10 equiv.)

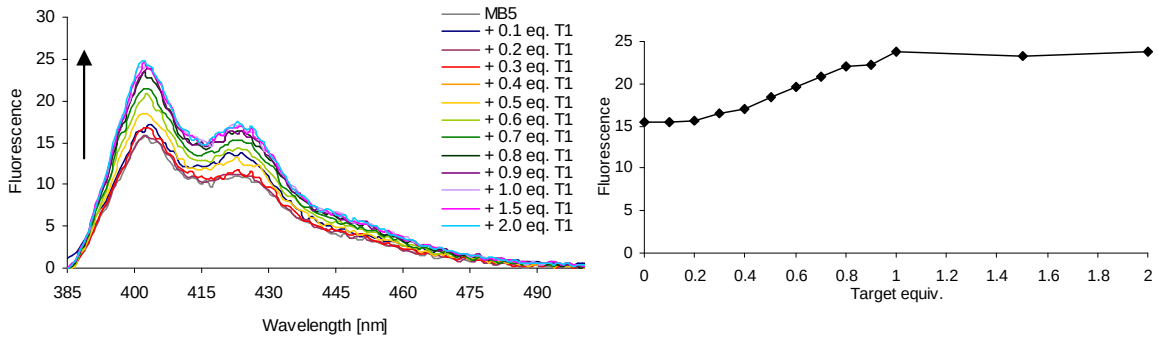


Fig. S15: Fluorescence spectra at 0.1 µM against 0 to 2 eq. target. Conditions: **MB5** 0.1 µM, **T1** 0 to 2 equiv., 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Excitation: 370 nm; excitation slit width: 10 nm; emission slit width: 5 nm; PMT voltage: 600 V; Temp.: 37°C; **MB5** sample without target was heated to 90°C and cooled down to 20°C prior to measurement; target titration: data were collected 10 min after adding the target (Increasing target concentration illustrated with the arrow: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0 equiv.). Right: Concentration dependent fluorescence intensities at 400 nm.

Temperature dependent absorbance spectra of **MB1** (2 µM)

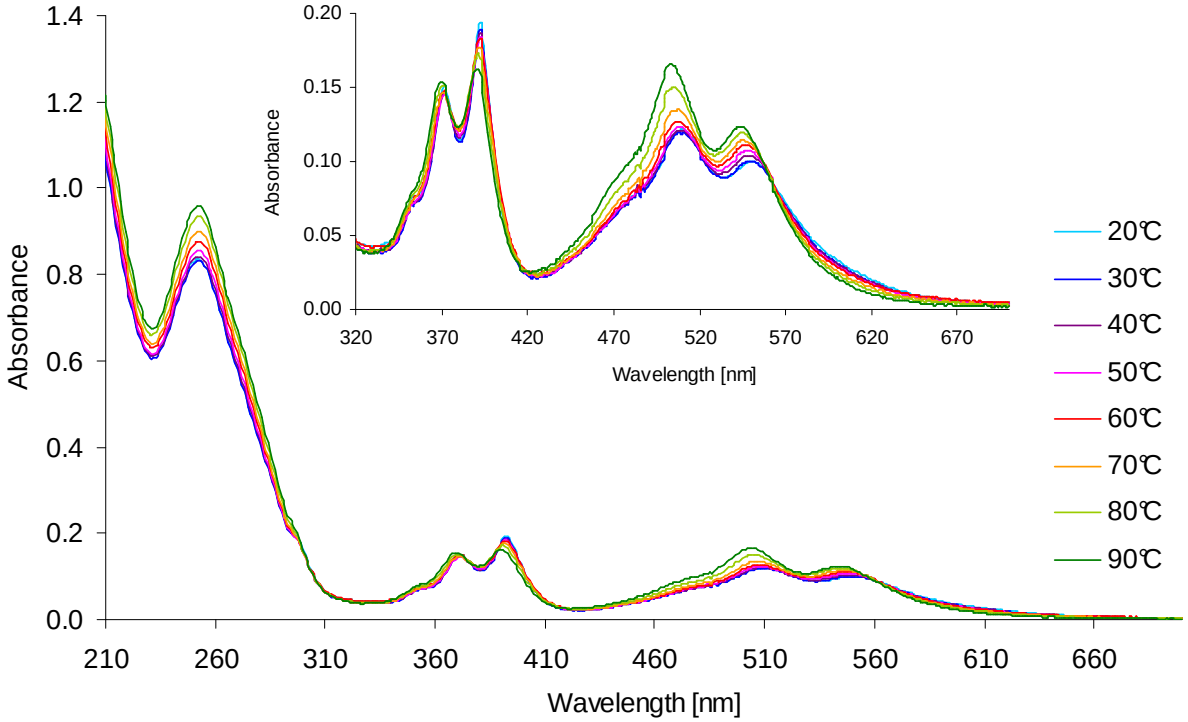


Fig. S16: Temperature dependent absorbance spectra. Conditions: **MB1** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 20°C, temperature range: 20 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB1** (2 µM)

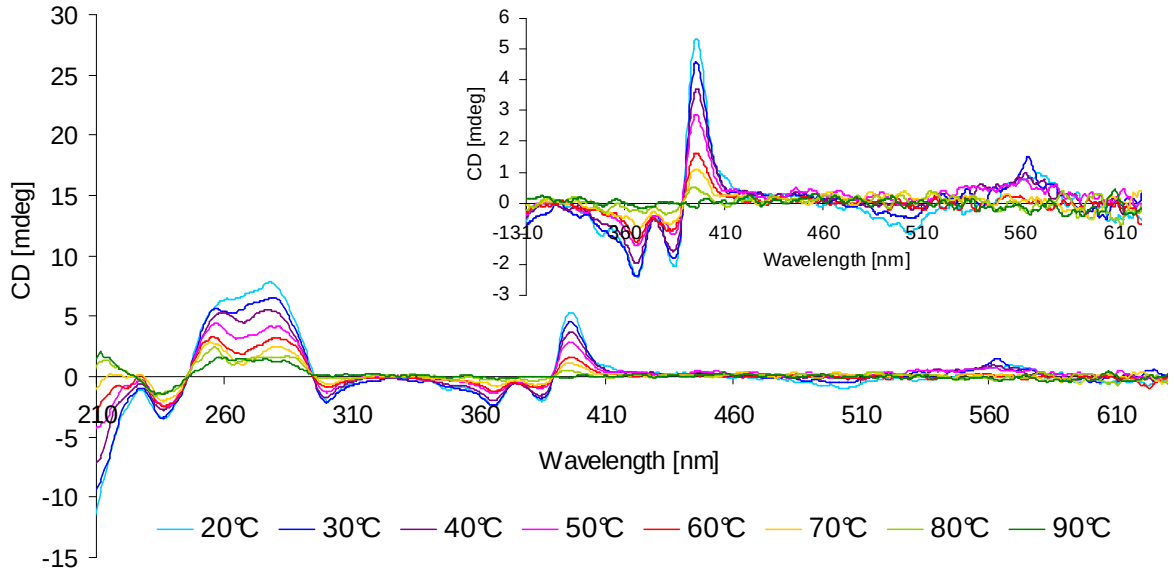


Fig. S17: Temperature dependent CD spectra. Conditions: **MB1** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 20°C, temperature range: 20 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 620 nm.

Temperature dependent absorbance spectra of **MB1** (5 µM) with the complementary (**T1**) target (6 µM)

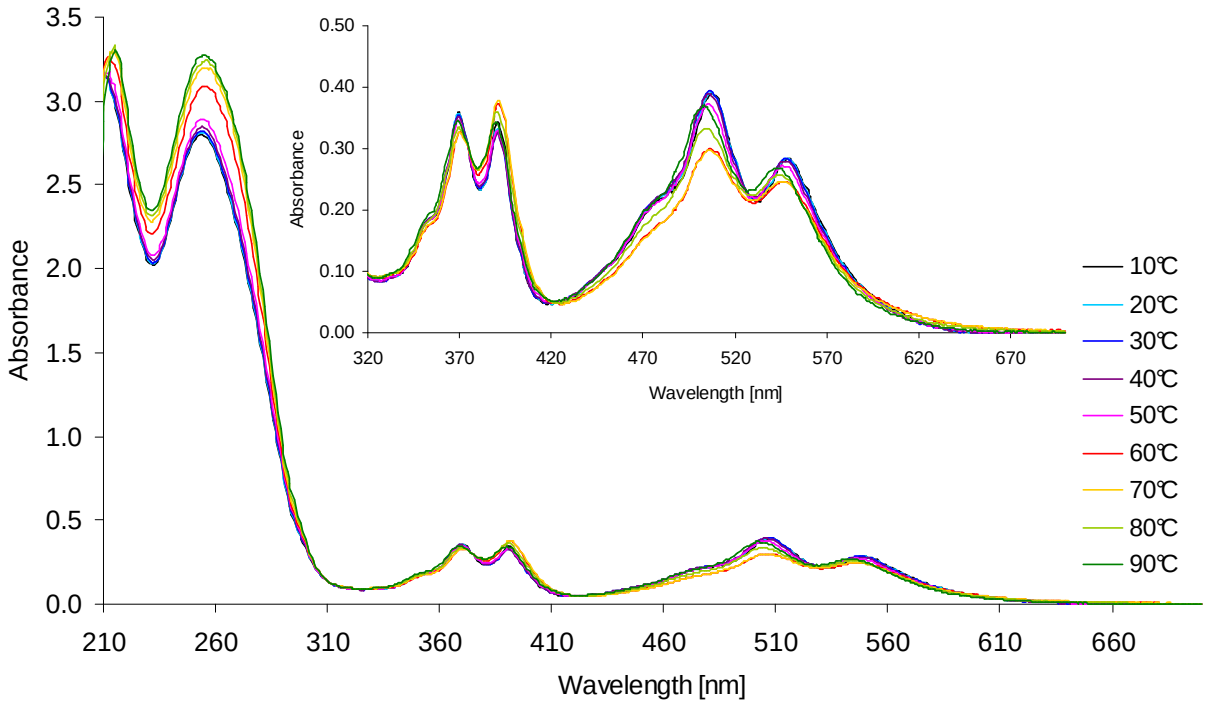


Fig. S18: Temperature dependent absorbance spectra. Conditions: **MB1** 5 µM, **T1** 6 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 10°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min, except for 10°C: the equilibration time was 20 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB1** (5 µM) with the complementary (**T1**) target (6 µM)

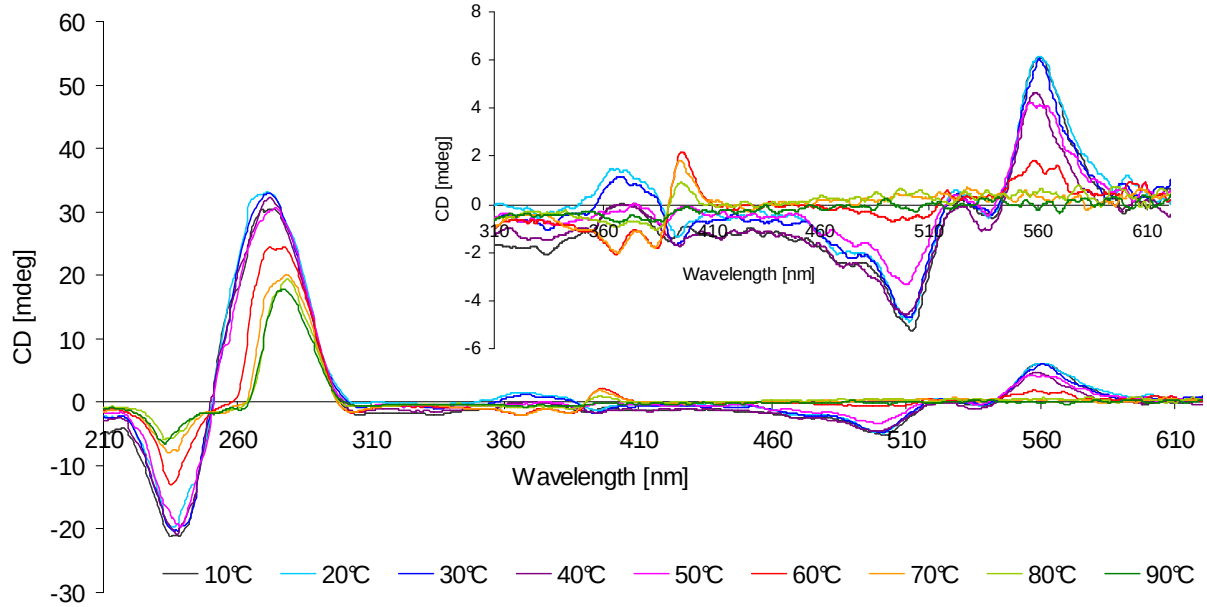


Fig. S19: Temperature dependent CD spectra. Conditions: **MB1** 5 µM, **T1** 6 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min, except for 10°C: the equilibration time was 20 min. Inset: enlarged CD spectra from 310 – 620 nm.

Temperature dependent absorbance spectra of **MB2** (2 µM)

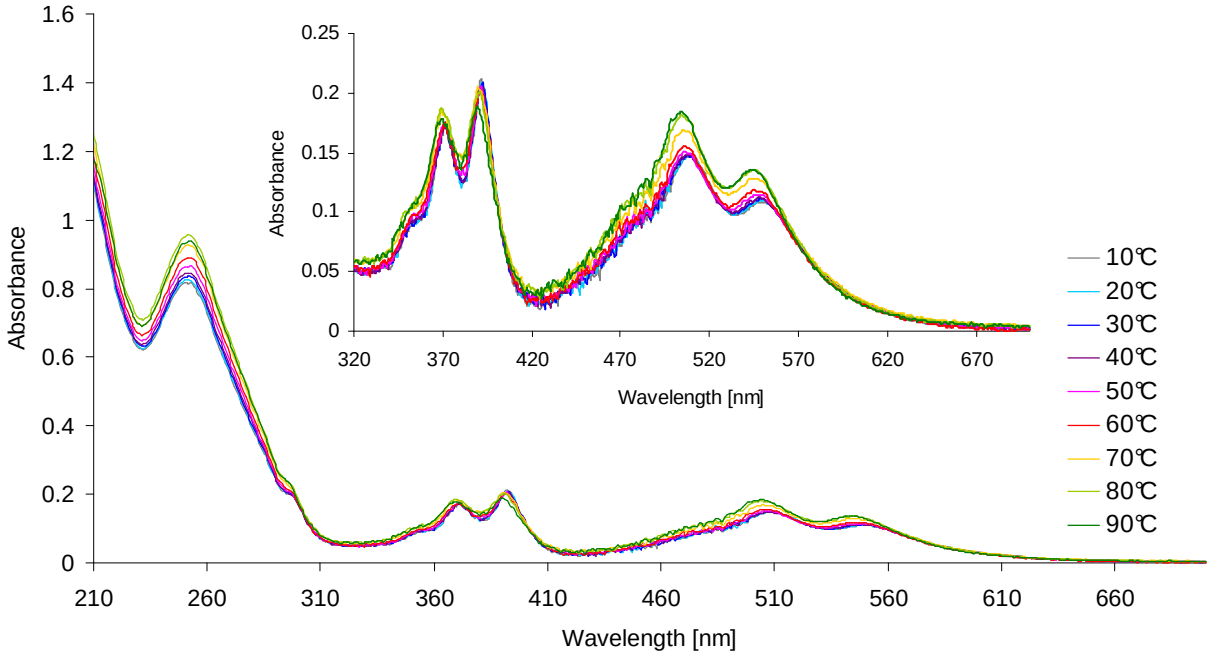


Fig. S20: Temperature dependent absorbance spectra. Conditions: **MB2** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB2** (5 µM)

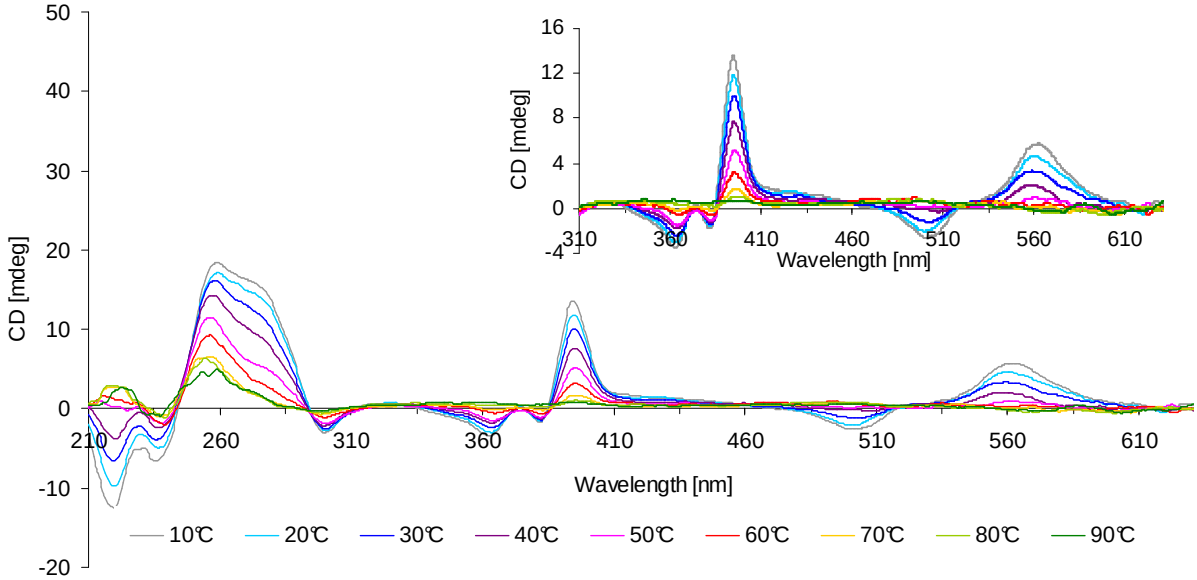


Fig. S21: Temperature dependent CD spectra. Conditions: **MB2** 5 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 20°C, temperature range: 20 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Temperature dependent absorbance spectra of **MB2** (2 µM) with the complementary (**T1**) target (2.4 µM)

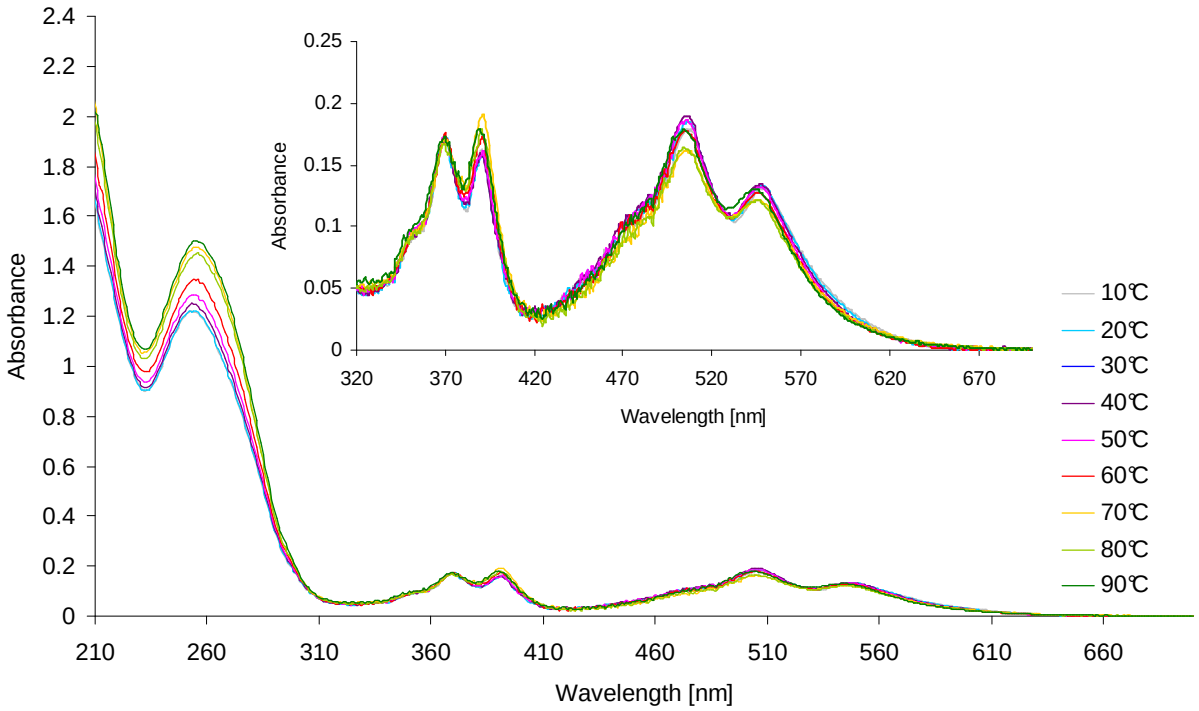


Fig. S22: Temperature dependent absorbance spectra. Conditions: **MB2** 2 µM, **T1** 2.4 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB2** (5 µM) with the complementary (**T1**) target (6 µM)

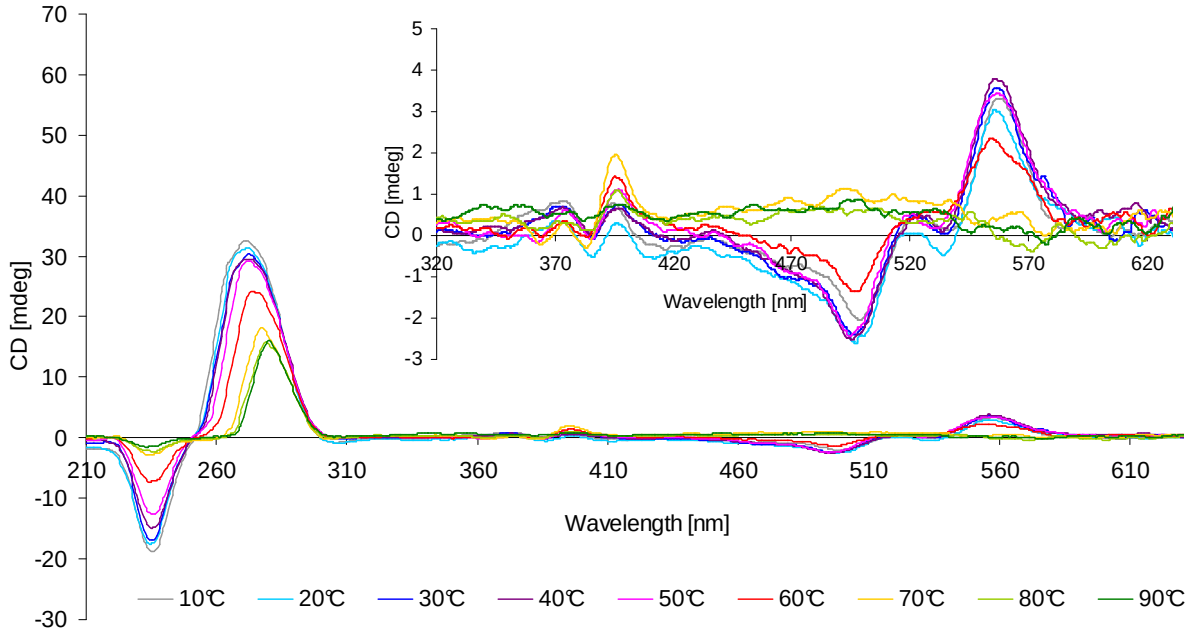


Fig. S23: Temperature dependent CD spectra. Conditions: **MB2** 5 µM, **T1** 6 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Temperature dependent absorbance spectra of **MB3** (2 µM)

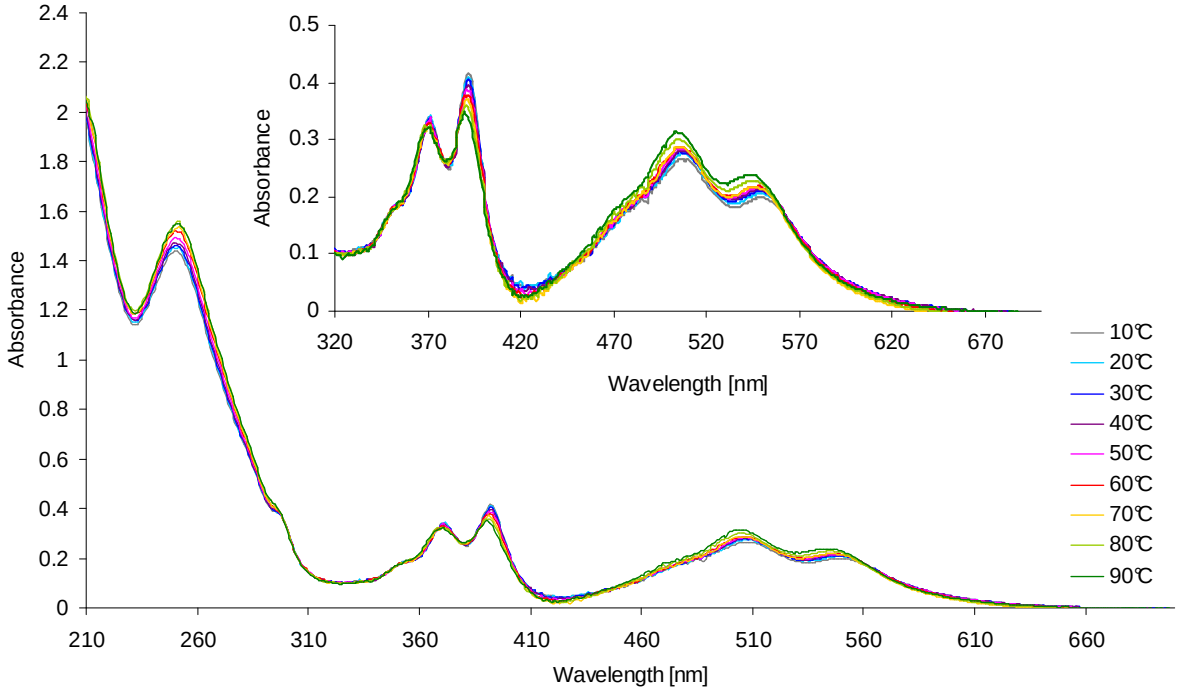


Fig. S24: Temperature dependent absorbance spectra. Conditions: **MB3** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB3** (2 µM)

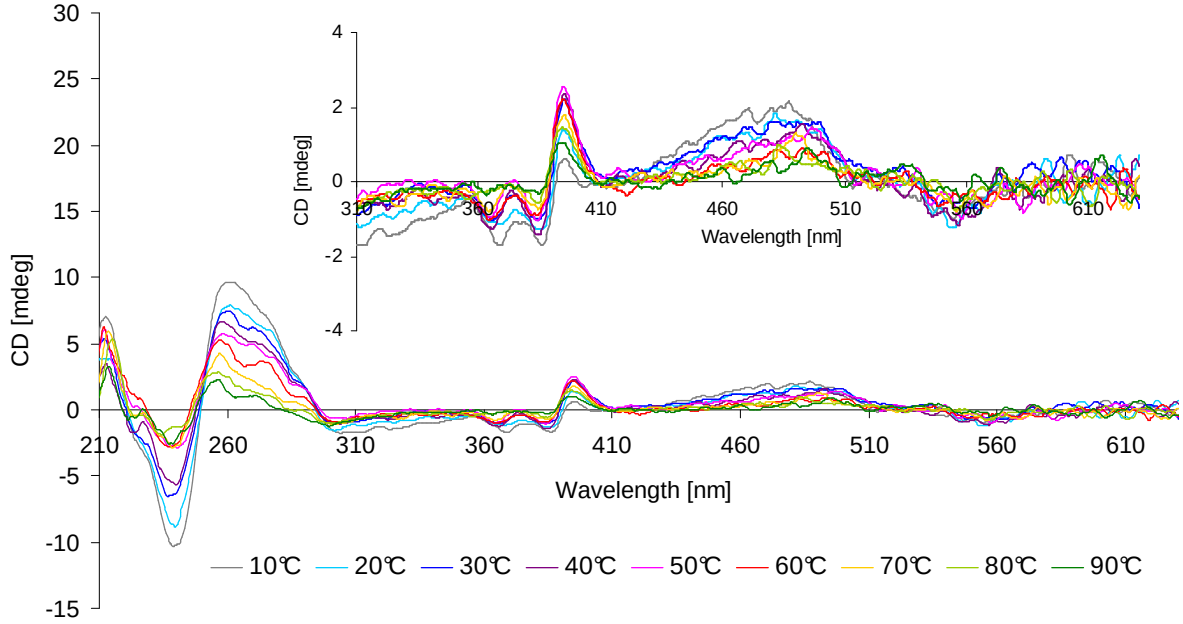


Fig. S25: Temperature dependent CD spectra. Conditions: **MB3** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Temperature dependent absorbance spectra of **MB3** (2 µM) with the complementary (**T1**) target (2.4 µM)

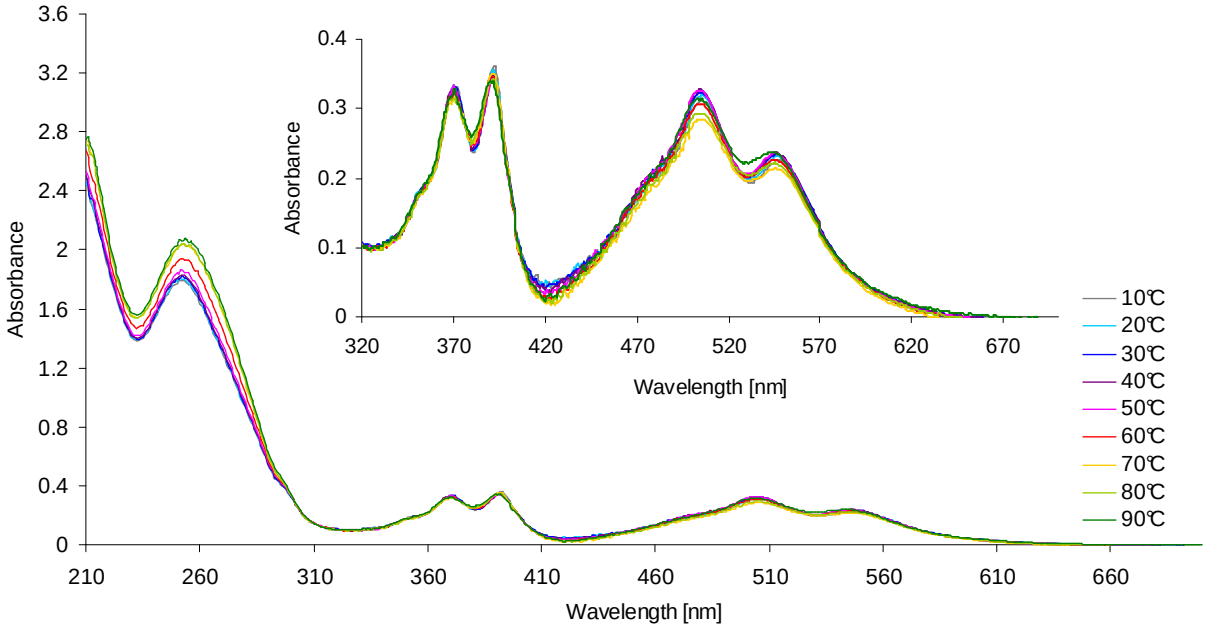


Fig. S26: Temperature dependent absorbance spectra. Conditions: **MB3** 2 µM, **T1** 2.4 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Bottom: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB3** (2 µM) with the complementary (**T1**) target (2.4 µM)

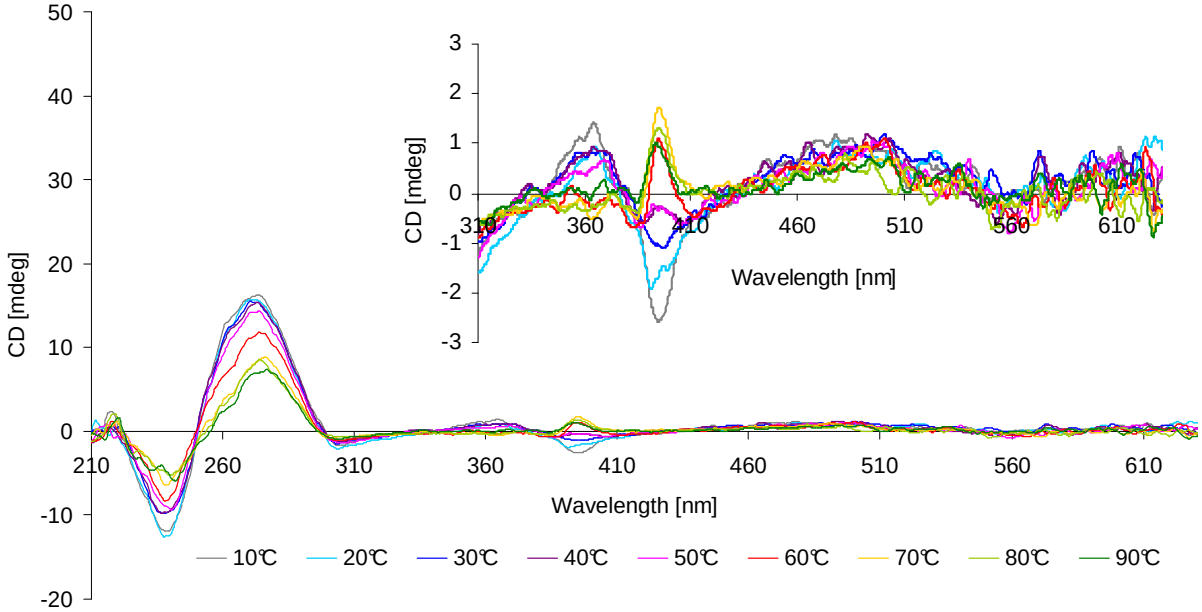


Fig. S27: Temperature dependent CD spectra. Conditions: **MB3** 2 µM, **T1** 2.4 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Temperature dependent absorbance spectra of **MB4** (2 µM)

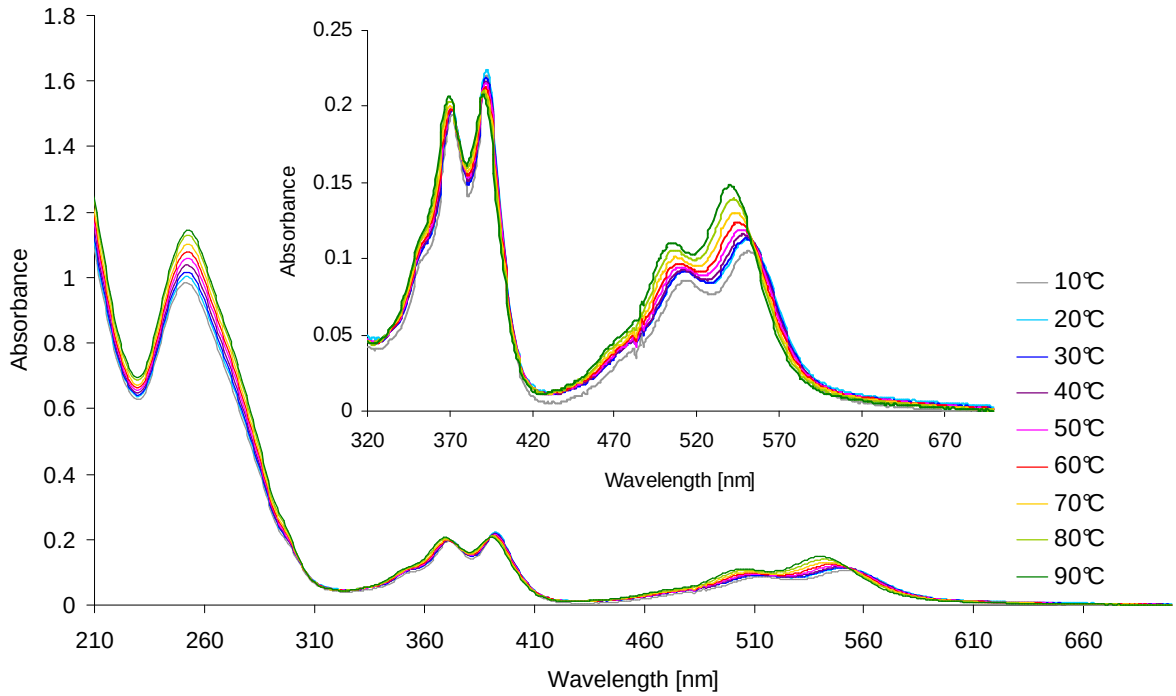


Fig. S28: Temperature dependent absorbance spectra. Conditions: **MB4** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB4** (5 µM)

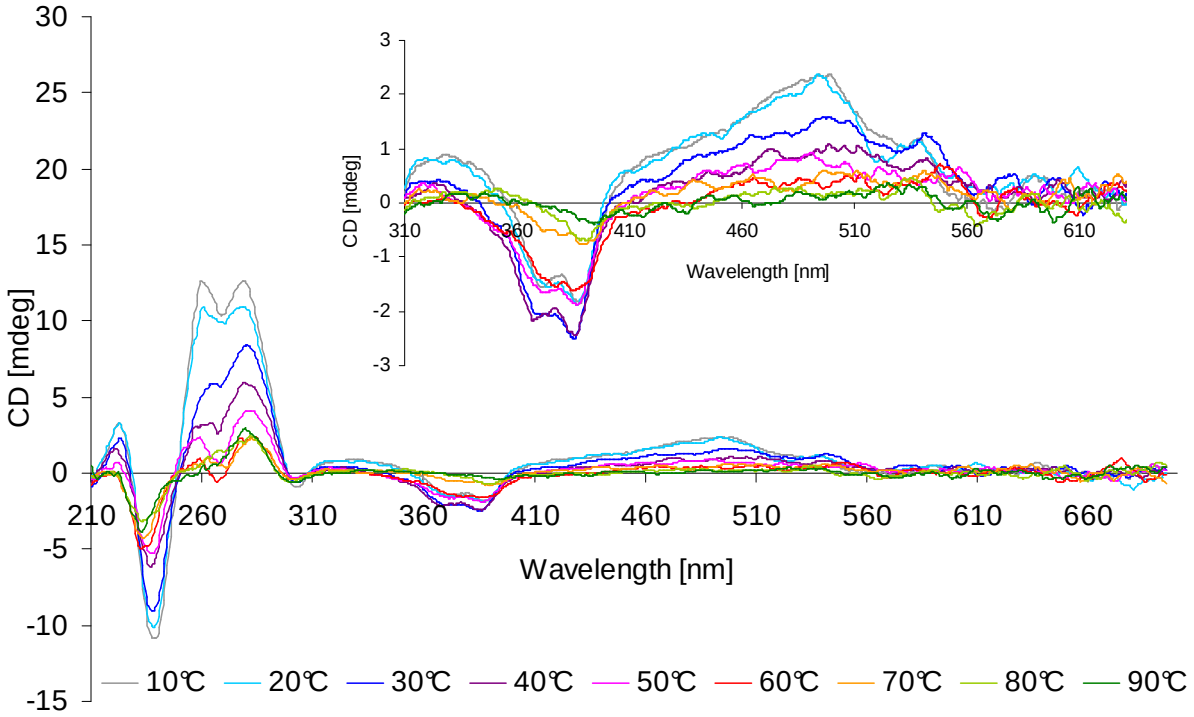


Fig. S29: Temperature dependent CD spectra. Conditions: **MB4** 5 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 620 nm.

Temperature dependent absorbance spectra of **MB4** (2 µM) with the complementary (**T1**) target (2.4 µM)

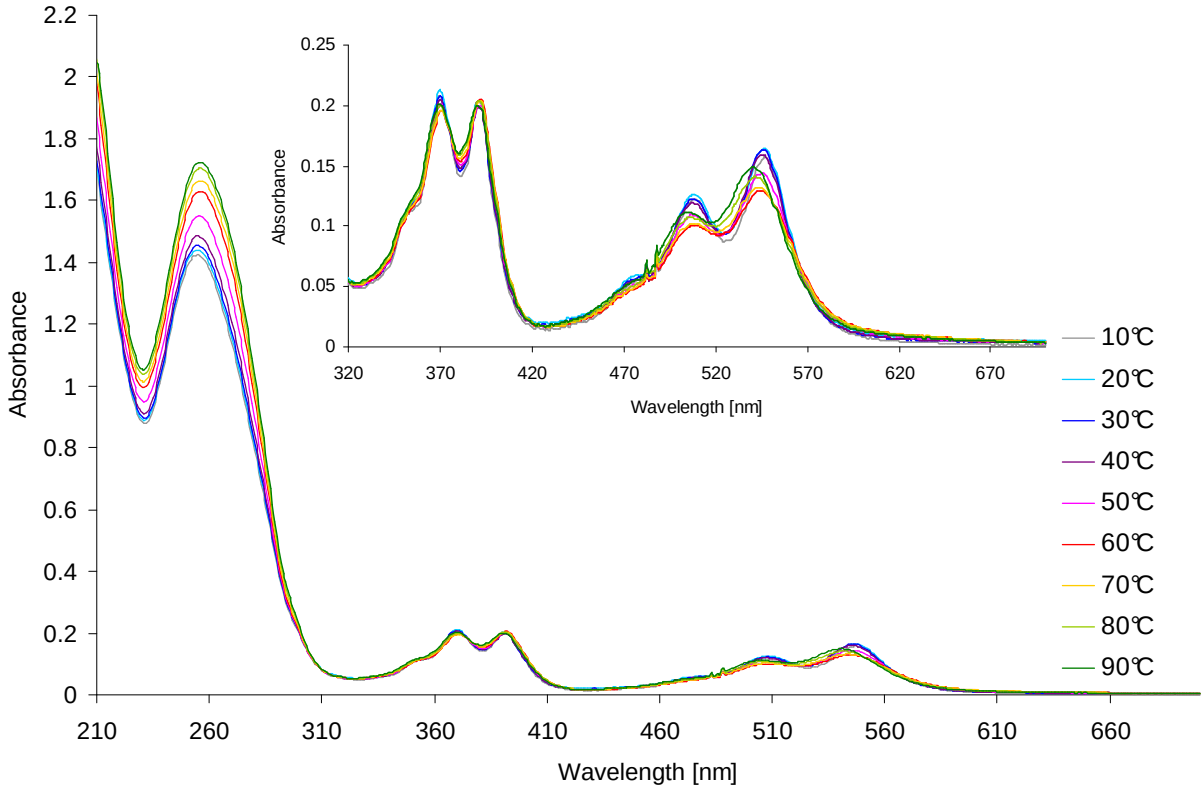


Fig. S30: Temperature dependent absorbance spectra. Conditions: **MB4** 2 µM, **T1** 2.4 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min, Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB4** (5 µM) with the complementary (**T1**) target (6 µM)

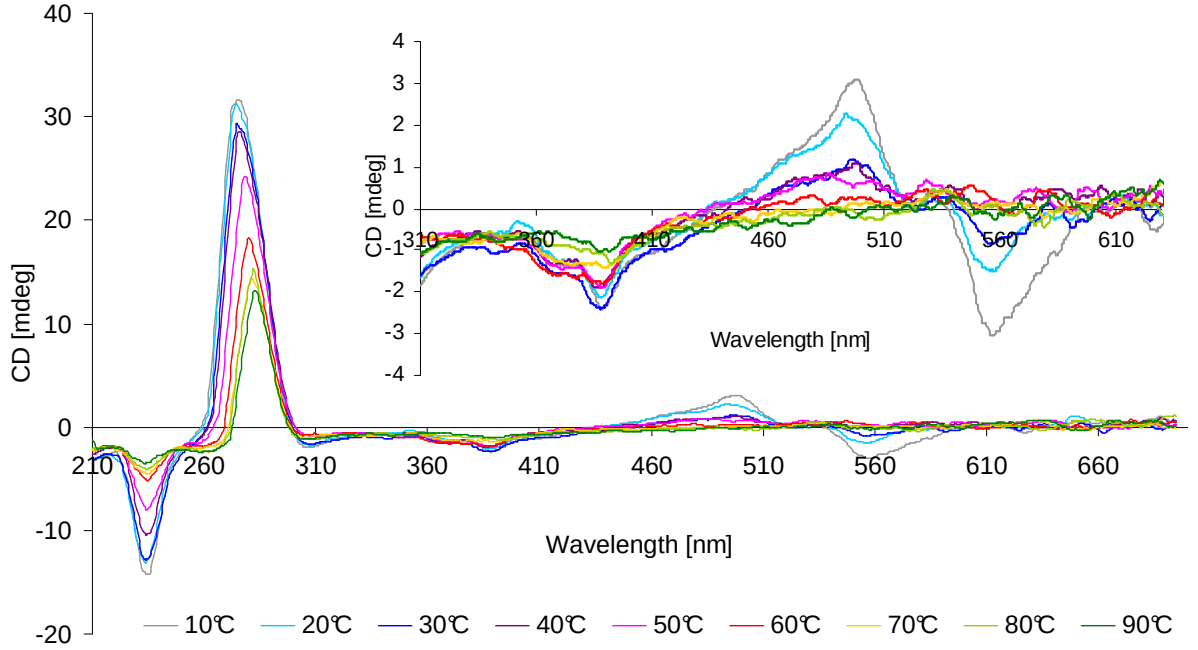


Fig. S31: Temperature dependent CD spectra. Conditions: **MB4** 5 µM, **T1** 6 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Temperature dependent absorbance spectra of **MB5** (2 µM)

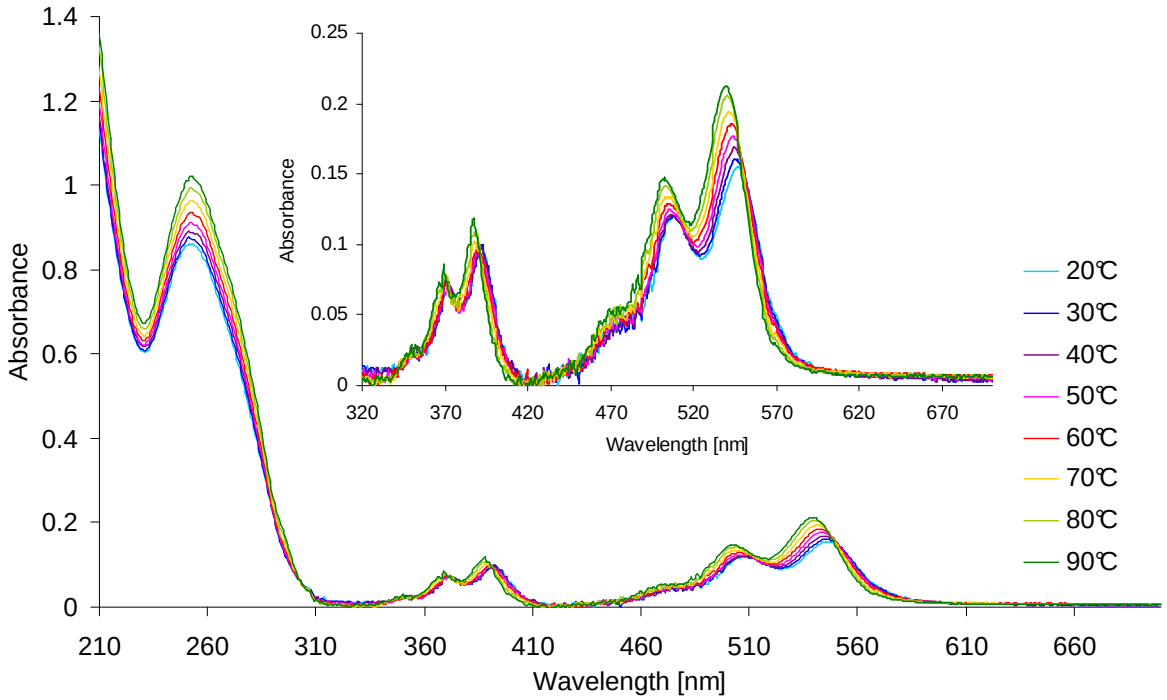


Fig. S32: Temperature dependent absorbance spectra. Conditions: **MB5** 2 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 20°C, temperature range: 20 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB5** (4 µM)

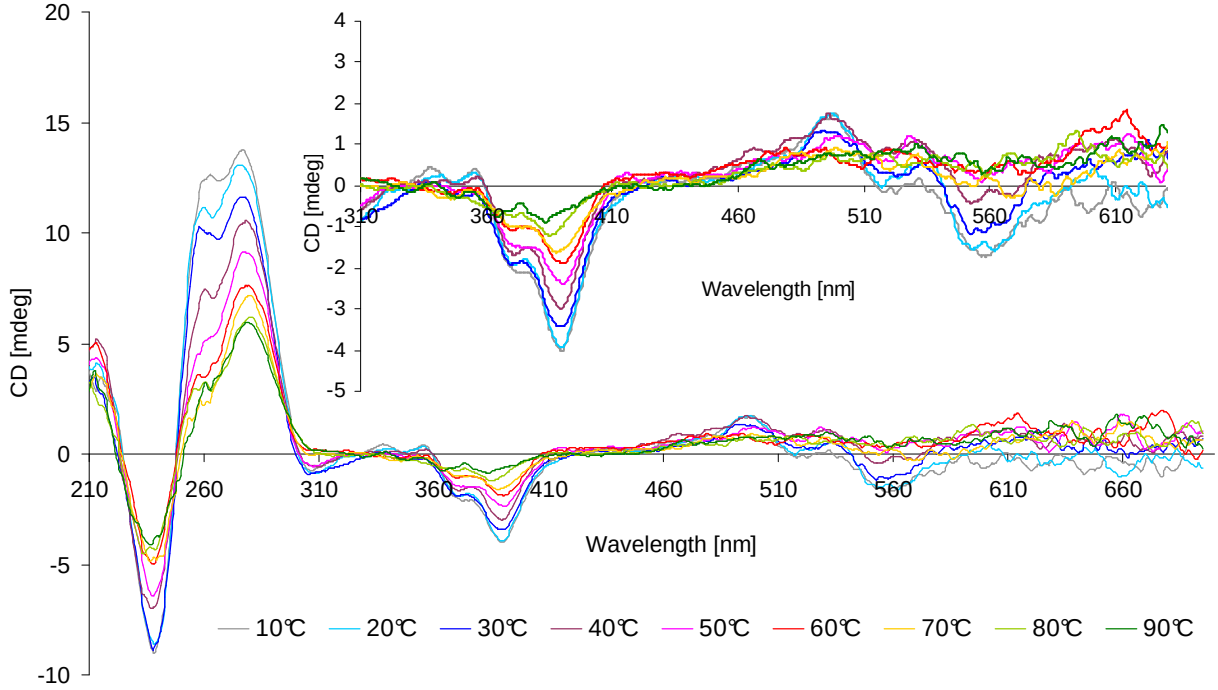


Fig. S33: Temperature dependent CD spectra. Conditions: **MB5** 4 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Temperature dependent absorbance spectra of **MB5** (2 µM) with the complementary (**T1**) target (2.4 µM)

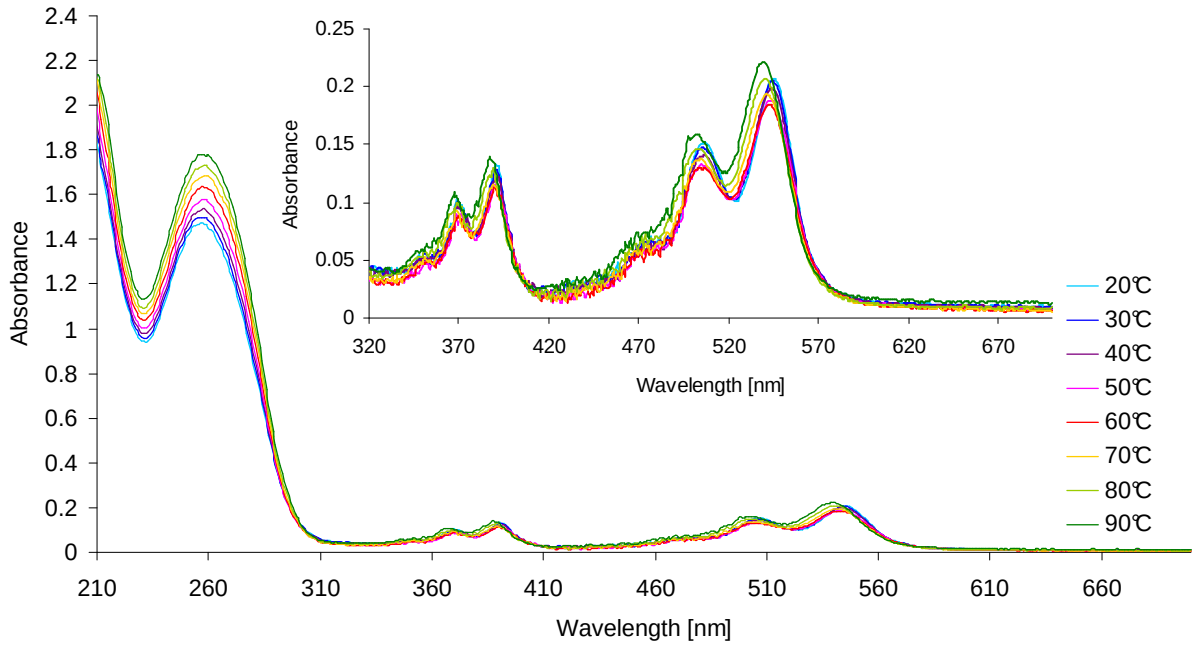


Fig. S34: Temperature dependent absorbance spectra. Conditions: **MB5** 2 µM, **T1** 2.4 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 20°C, temperature range: 20 – 90°C, equilibration time: 10 min. Inset: Enlarged part of the spectra between 320 and 700 nm.

Temperature dependent CD spectra of **MB5** (4 µM) with the complementary (**T1**) target (4.8 µM)

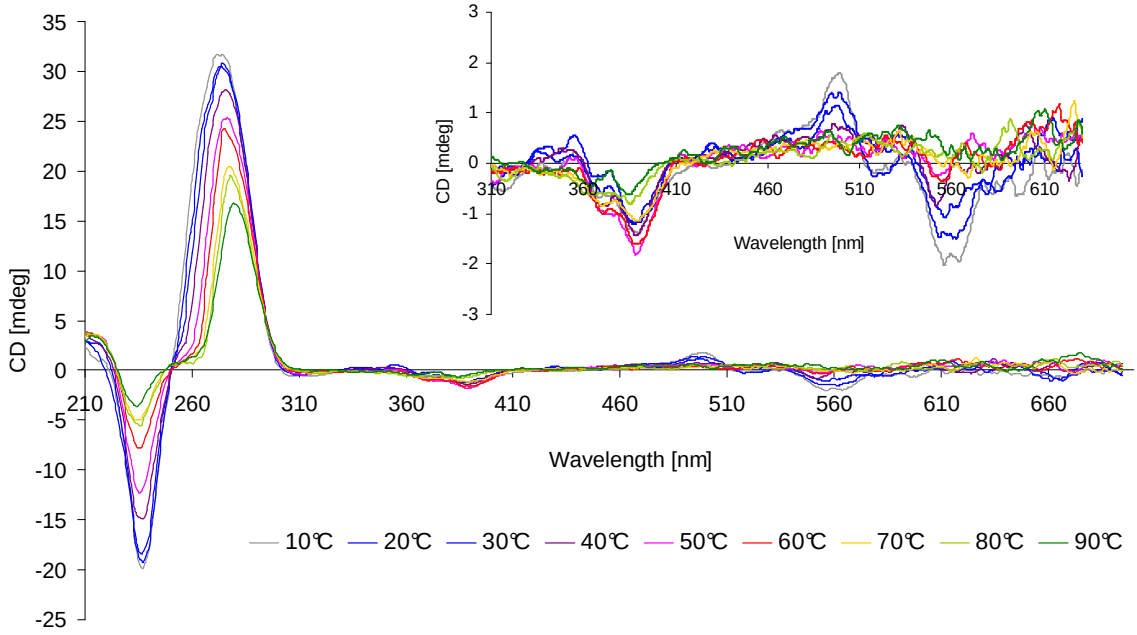


Fig. S35: Temperature dependent CD spectra. Conditions: **MB5** 4 µM, **T1** 4.8 µM, 10 mM phosphate buffer pH 7.0, 100 mM NaCl. Sample was heated to 90°C and cooled down to 20°C prior to measurement; Starting temperature: 10°C, temperature range: 10 – 90°C, equilibration time: 10 min. Inset: enlarged CD spectra from 310 – 630 nm.

Determination of the signal-to-background (S/B) ratio and the quenching efficiency (Q%)

Signal-to-background (S/B) and quenching efficiency (Q%) values were determined according to the following formulas (from: Vet, J. A. M.; Marras, S. A. E. Design and Optimization of Molecular Beacon Real-Time Polymerase Chain Reaction Assays. In *Oligonucleotide Synthesis: Methods and Applications*; Humana Press Inc.: Totowa, NJ, 2004; Chapter 17):

$$S/B = (F_{hybrid} - F_{buffer}) / (F_{MB} - F_{buffer})$$

$$Q\% = 100 \times \{1-((F_{MB} - F_{buffer}) / (F_{hybrid} - F_{buffer}))\}$$

Values obtained for **MB1-MB5** in the presence of 1 equiv. of target **T1**[a]:

	MB1[b]	**MB2**[b]	**MB3**[c]	**MB4**[c]	**MB5**[d]
S/B	309	35.6	2.1	11.8	1.6
Q%	99.7	97.2	53.1	91.5	36.9

[a] Conditions: 100 mM NaCl, 10 mM phosphate buffer, pH 7.0, 37°C, Excitation: 370 nm, PMT voltage: 600 V, [b] **MB1** and **MB2**: 1.0 μM, Ex/Em slit width: 10/5 nm, [c] **MB3** and **MB4**: 1.0 μM, Ex/Em slit width: 5/5 nm, [d] **MB5**: 0.1 μM, Ex/Em slit width: 10/5 nm.

Values obtained for **MB** in the presence of 1, 2 and 5 equiv. of target **T1**:

	MB1 + 1eq. **T1**	**MB1** + 2eq. **T1**	**MB1** + 5eq. **T1**
Q%	99.7	99.7	99.8
	MB2 + 1eq. **T1**	**MB2** + 2eq. **T1**	**MB2** + 5eq. **T1**
Q%	97.2	97.6	97.5
	MB3 + 1eq. **T1**	**MB3** + 2eq. **T1**	**MB3** + 5eq. **T1**
Q%	53.1	66.9	73.5
	MB4 + 1eq. **T1**	**MB4** + 2eq. **T1**	**MB4** + 5eq. **T1**
Q%	91.5	93.9	94.2
	MB5 + 1eq. **T1**	**MB5** + 2eq. **T1**	
Q%	36.9	37.6	

References

1 H. Bittermann, D. Siegemund, V. L. Malinovskii, R. Häner, *J.Am.Chem.Soc.*, 2008, **130**, 15285-15287.

2 N. Rahe, C. Rinn, T. Carell, *Chem Commun.*, 2003, 2119-2121.