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Nucleic acid sensing by an orthogonal reporter system based on homo-DNA

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Supplementary information

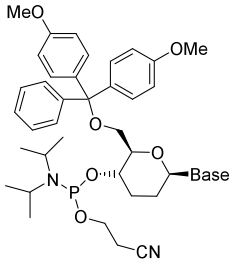
Experimental procedures

General

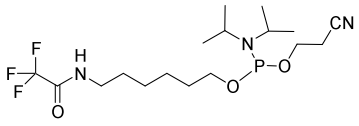
Flash chromatography (FC) was performed with silica gel 60 (230-400 mesh) from Fluka. ^{1}H NMR spectra were recorded at 300 MHz on a *Bruker AC-300* or on a *Bruker Avance* spectrometer. Chemical shifts are reported in ppm using the residual undeuterated solvent as an internal reference. Multiplicities are abbreviated as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. ^{31}P NMR spectra were recorded at 121 MHz on a *Bruker Avance* spectrometer using 85% H_3PO_4 as an external standard.

Phosphoramidite synthesis

For the synthesis of the four phosphoramidites **1**-**4** we followed the published procedures of Eschenmoser and coworkers.[1] The phosphoramidite of the C_6-aminolinker **5** was purchased from Glen Research.

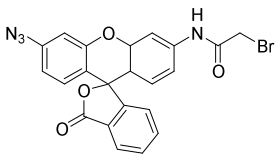


1-4 (with A, T, C, G as base)

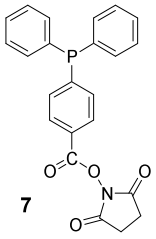


5

Building block synthesis for templated chemistry



6



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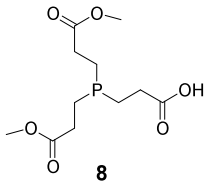

8

Building block **6** and **8** were synthesized according to literature procedures.[2,3] Triphenylphosphine NHS-ester **7** was synthesized as described.[4]

Figures

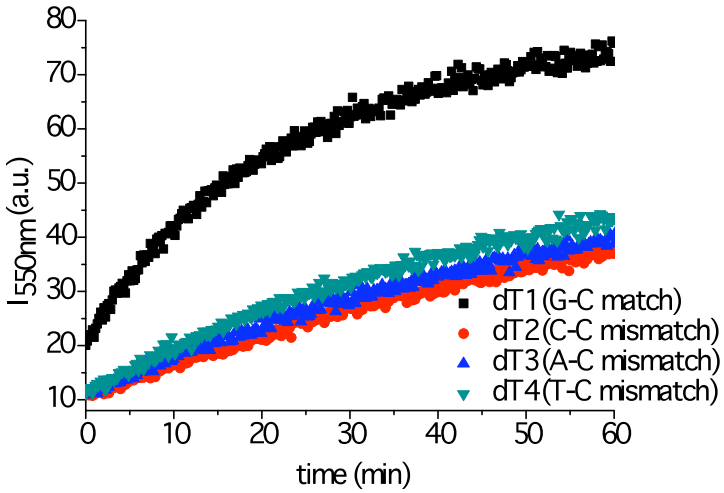

Figure S1. Templated Rhodamine-azide reduction of **R2** by **R1** on **O1** for mismatch discrimination on DNA. The experiments were performed at 20°C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with **R2** (400 nM), **R1** (225 nM), **O1** (200 nM) and the indicated targets (200 nM).

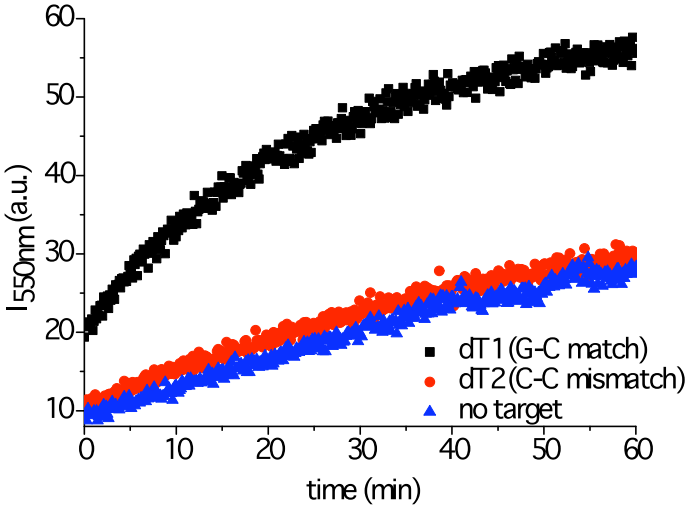

Figure S2. Mismatch discrimination on DNA targets with the Ω-probe **O1** compared to the background. The experiments were performed at 20°C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with **R1** (400 nM), **R2** (400 nM), **O1** (200 nM) and the indicated targets (200 nM).

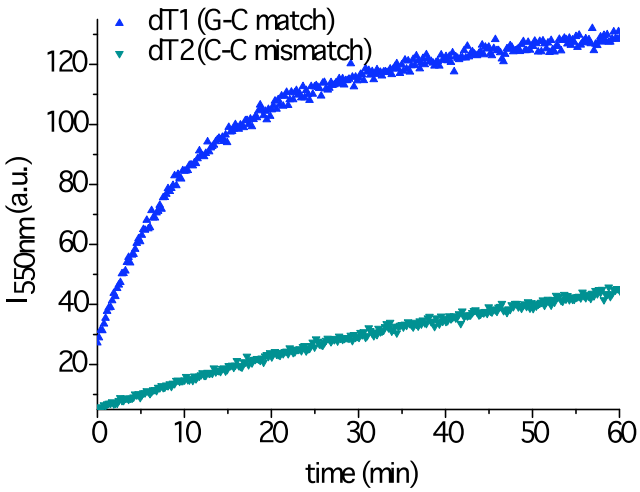

Figure S3. Reaction of **R1** with **R2** on **O2**. Experiments were performed at 20°C in buffer 1 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 8.0) with **R1** (400 nM), **R2** (400 nM), **O2** (200 nM) and the indicated targets (200 nM).

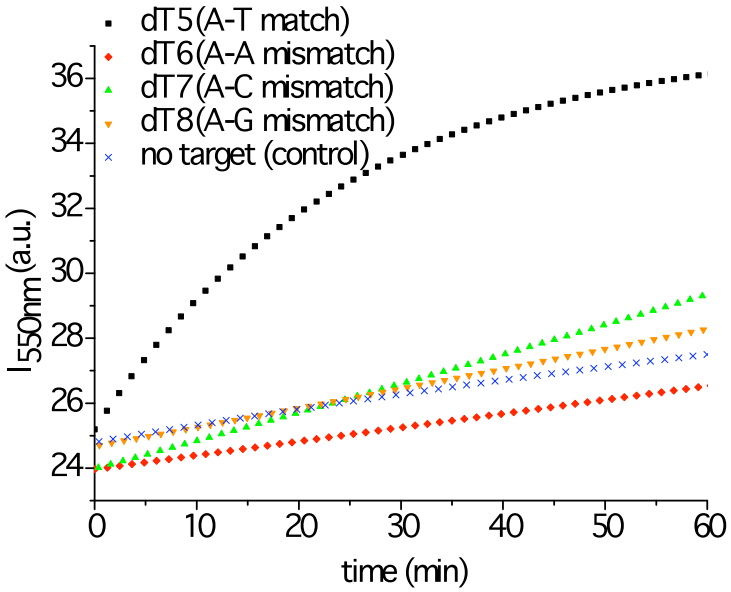

Figure S4. Reaction of **R1** with **R3** on **L1** and **L2** in presence of matched and mismatched DNA targets (**dT5-8**). The experiments were performed at 25°C in buffer 2 (50 mM KCl, 10 mM Tris, 3.5 mM $MgCl_2$, pH 7.0) with **R1** (600 nM), **R3** (400 nM), **L1, L2** (400 nM) and the indicated targets (400 nM).

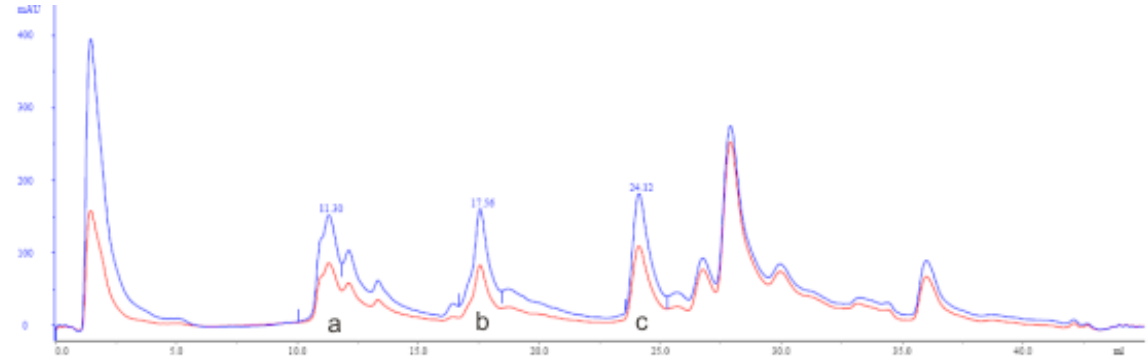

Figure S5. HPLC trance of crude **P1** after post synthetic modification. The peak **a** eluting at 11 min is assigned to the non-conjugated oligonucleotide. The oxidized TPP product is eluting at 18 min (peak **b**) and the desired TPP product at 24 min (peak **c**). Assignments are based on ESI-MS.

Oligonucleotide purification and analysis

Deprotection conditions

A) 55°C, 16 h, 33% NH_3
B) 55°C, 60 h, 33% NH_3
C) r.t., 3 h then 55°C, 16 h, 33% NH_3
D) r.t., 4 h, DBU (1.5 ml) then 55°C, 60 h, 33% NH_3 (before ammonia treatment the DBU solution was removed by centrifuging and washing 3x with 1 ml ACN)

HPLC gradients and buffers

RP20: 0%→20% B in 30’ (Flow: 1 ml/min)
RP30: 0%→30% B in 30’ (Flow: 1 ml/min)
RP40: 0%→40% B in 30’ (Flow: 1 ml/min)
RP50: 0%→50% B in 30’ (Flow: 1 ml/min)

Buffer A: 0.1 M Et_3N/CH_3COOH in H_2O, pH 7.0.
Buffer B: 0.1 M Et_3N/CH_3COOH in H_2O/ACN (1:4), pH 7.0.

Columns

Either column A (colA) or column B (colB) were used as indicated in Table S1
ColA: Source 15 RPC ST 4.6/100 from Pharmacia Biotech
ColB: VA 150/4.6 Nucleogel RP 300-5 from Macherey Nagel

Table S1 Synthesis and analytical data of homo-DNA and hybrid oligonucleotides

Entry	Sequence [a)]	RP-HPLC method, retention time, column [b)]	ESI$^-$-MS m/z found (m/z calcd) [c)]	Deprotection conditions [d)]
R1	6' - TPP - gcc tat g - 4'	RP50, 24 min, colA	2661.0 and 2677.0 (70% ox.) (2662.0)	D
R2	6' - ggc acg t - Rhd-N_3 - 4'	RP50, 23 min, colA	2712.0 (2713.0)	C
R3	6' - gca cgt - Rhd-N_3 - 4'	RP50, 24 min, colA	2368.5 (2368.8)	C
R4	6' - TCEP - gcc tat g - 4'	RP50, 15 min, colA	2649.0 (100% ox.) (2633.9)	D
R5	6' - ggc acg t - Rhd-NH_2 - 4'	RP50, 18min, colA	2686.0 (2687.0)	C
O1	5'- GTC ACT GA acg tgc ctt tca tag gc GAC CAT TTA CGT - 3'	RP30, 19 min, colA	n.d. (11553.4)	A
O2	5' - GTC ACT GA acg tgc c $(glyc18)_3$ cat agg c GAC CAT TTA CGT - 3'	RP30, 19 min, colA	11628.0 (11631.8)	A
L1	5' - CCA AGT TAA G acg tgc c - 4'	RP30, 17 min, colA	5277.0 (5277.4)	B
L2	6' - cat agg c ACC TAT GCA T - 3'	RP30, 18 min, colA	5252.0 (5252.4)	A
L3	5' - TAG GGA ACA AGG GCA acg tgc c - 4'	RP30, 19 min, colA	6916.0 (6916.4)	B
L4	6' - cat agg c AGA GTG TCT GGC TAG - 3'	RP30, 16min, colA	6914.0 (6913.4)	A

a) Homo-DNA: lower case letters; natural DNA: capital letters.
b) For details about buffers and columns see the section about oligonucleotide purification.
c) TPP and TCEP modified oligonucleotides were detected partially in the oxidized form (see also the comments in the section about the purification of post synthetic modified oligonucleotides).
d) For details about the deprotection conditions see the section about the oligonucleotides synthesis.

References

1. Böhringer, M.; Roth, H. J.; Hunziker, J.; Göbel, M.; Krishnan, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* 1992, **75**, 1416-1477.
2. Pianowski, Z.; Gorska, K.; Oswald, L.; Merten, C. A.; Winssinger, N. *J. Am. Chem. Soc.* 2009, **131**, 6492-6497.
3. Abe, H.; Wang, J.; Furukawa, K.; Oki, K.; Uda, M.; Tsuneda, S.; Ito, Y. *Bioconjugate Chem.* 2008, **19**, 1219-1226.
4. Stoop, M.; Leumann, C. J. *Chem. Comm.* 2011, **47**, 7494-7496.