Dependence of aptamer activity on opposed terminal extensions: improvement of light-regulation efficiency

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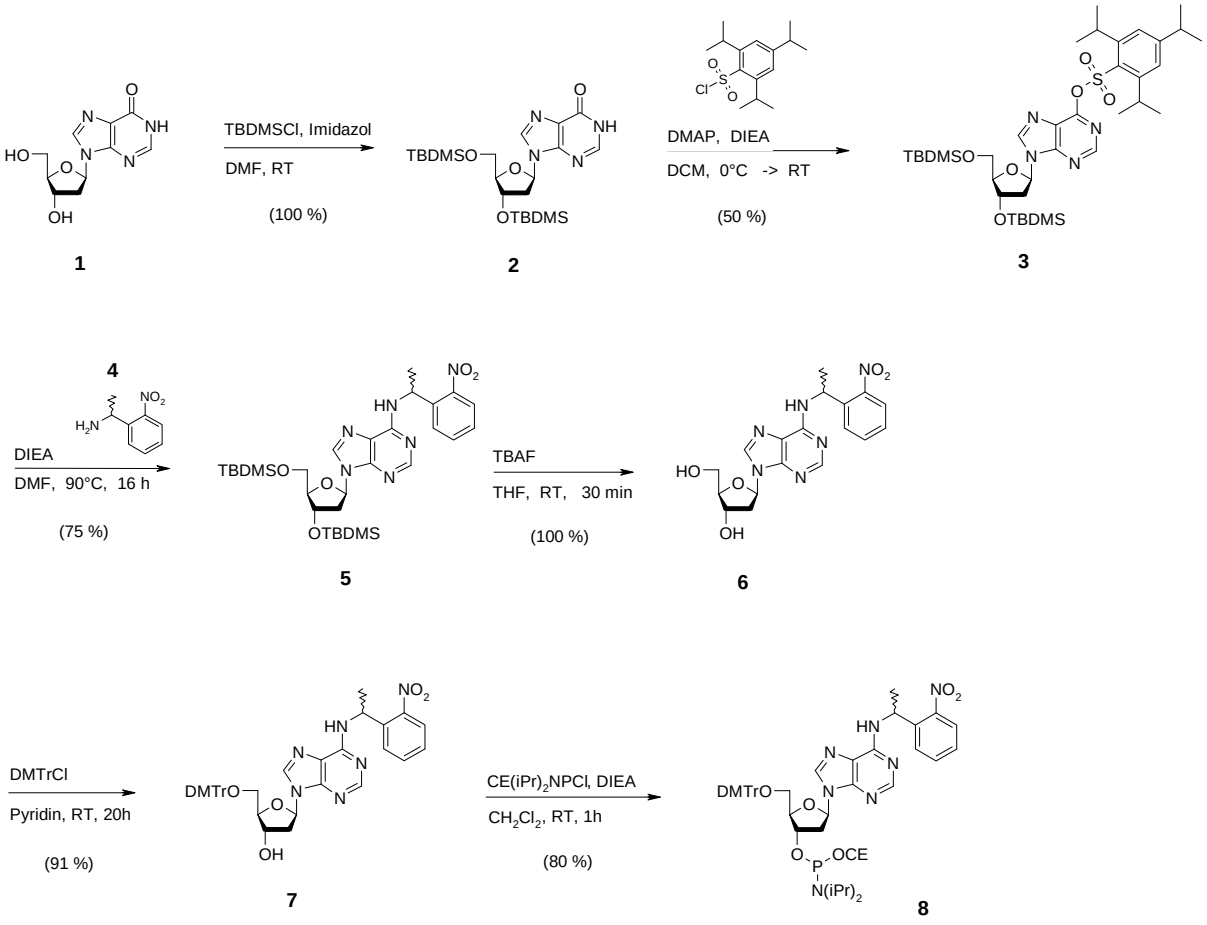
SUPPLEMENTARY DATA

1. Details of the synthesis shown in Figure 3.



Compounds **2** and **3** were synthesized in analogy to Kieselyov,A.S., Steinbrecher,T. and Harvey,R.G. (1995) Synthesis of the Fjord-region cis and trans-amino triol derivatives of the carcinogenic hydrogencarbon benzo[g]chrysene and utilization for the synthesis of a deoxyadenosine adduct linked to the N6-amino group. *J. Org. Chem.*, **60**, 6129-6134 and compound **4** in analogy to Salerno,C.P., Madge,D. and Patron,A.P. (2000) Enzymatic synthesis of caged NADP cofactors: aqueous NADP photorelease and optical properties. *J. Org. Chem.*, **65**, 3971-3981.

3',5'-*O*-Di(*tert*butyldimethylsilyl)-2'-deoxyinosine (2):

2'-Deoxyinosine (**1**) (5.0 g, 19.8 mmol), imidazole (9.43 g, 138 mmol, 7 eq.) and *tert*-butylchlorodimethylsilane (13.1 g, 79.3 mmol, 4 eq.) were placed in a 250 mL round bottom flask under argon atmosphere. The compounds were dissolved in dry DMF (12 mL) and the resulting solution was stirred at room temperature over night. After the addition of ethanol (15 mL) the reaction mixture was stirred additional 15 minutes. Then the solvent was evaporated, the residue was dissolved in dichloromethane and was washed consecutively with aq. HCl (1 M), sat. aq. $NaHCO_3$

and sat. aq. NaCl, then dried with $MgSO_4$. After evaporation of the solvent, the product (**2**) was obtained as a colorless solid and was used for the following synthesis without further purification.
Yield: 9.53 g, quantitative yield.
TLC (dichloromethane/methanol 9:1): R_f = 0.68
^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.01, 0.03, 0.11 (12 H, 4 $SiCH_3$); 0.85, 0.89 (18H, 2 $SiC(CH_3)_3$); 2.30-2.36 (m, 1H, 2'-H); 2.75-2.82 (m, 1H, 2'-H); 3.64-3.68 (m, 1H, 5'-H); 3.74-3.78 (m, 1H, 5'-H); 3.83-3.87 (m, 1H); 4.57-4.60 (m, 1H); 6.27-6.32 (m, 1H, 1'-H); 8.04 (s, 1H); 8.25 (s, 1H); 12.36 (s, 1H, NH) ppm.

3',5'-*O*-Di(*tert*butyldimethylsilyl)-6-*O*-(2,4,6-triisopropylbenzenesulfonyl)-2'-deoxyinosine (3):
3',5'-*O*-Di(*tert*butyldimethylsilyl)-2'-deoxyinosine (**2**) (9.45 g, 19.7 mmol) and *N,N*-dimethylaminopyridine (241 mg, 1.97 mmol, 0.1 eq.) were dissolved in dry dichloromethane (135 mL) under argon atmosphere. The solution was cooled in an ice bath to 0°C. *N*-Ethyldiisopropylamine (16.9 mL, 98.5 mmol, 5 eq.) was added, followed by 2,4,6-triisopropylbenzenesulfonylchloride (10.7 g, 35.5 mmol, 1.8 eq.). The solution was stirred 10 minutes at 0°C. The ice bath was removed and the solution was stirred additional 45 minutes at room temperature. TLC showed there was no more starting material. The solvent was evaporated and the residue was purified via flash chromatography with cyclohexane/ethylacetate 93:7 → 9:1.
Yield: 7.45 g of **3** as light yellow foam (50 %) and 6.35 g of the 1-*N*-sulfonylated isomer as light yellow foam (43 %).
TLC (cyclohexane/ethylacetate 9:1): R_f = 0.31, 1-*N*-sulfonylated isomer R_f = 0.15
^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.06-0.12 (m, 12H, 4 $SiCH_3$); 0.88-0.92 (m, 18H, 2 $SiC(CH_3)_3$); 2.42-2.50 (m, 1H); 2.59-2.67 (m, 1H); 2.87-2.98 (m, 1H); 3.74-3.89 (m, 2H, 2 5'-H); 4.01-4.05 (m, 1H); 4.19-4.40 (m, 2H, iPr-CH); 4.59-4.63 (m, 1H); 6.47-6.51 (m, 1H, 1'-H); 7.21 (s, 2H); 7.22 (s, 1H); 8.38 (s, 1H); 8.56 (s, 1H) ppm.

3',5'-*O*-Di(*tert*butyldimethylsilyl)-6-*N*-(1-(2-nitro)phenyl)ethyl-2'-deoxyadenosine (5):
Compound **3** (7.35 g, 9.84 mmol), (1-(2-nitro)phenyl)ethylamine (**4**) (3.27 g, 19.7 mmol, 2 eq.) and *N*-ethyldiisopropylamine (3.37 mL, 19.7 mmol, 2 eq.) were dissolved in dry DMF (60 mL) under argon atmosphere. The solution was heated for 24 hours to 90°C. The solvent was evaporated and the residue was chromatographed with cyclohexane/ethylacetate 8:2.
Yield: 4.78 g of **5** as light yellow foam (75 %).
TLC (cyclohexane/ethylacetate 8:2): R_f = 0.06
^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.03-0.10 (m, 12H, 4 $SiCH_3$); 0.80-0.88 (m, 18H, 2 $SiC(CH_3)_3$); 1.63-1.65 (m, 3H, NPE-CH_3); 2.21-2.30 (m, 1H, 2'-H); 2.83-2.90 (m, 1H, 2'-H); 3.60-3.65 (m, 1H, 5'-H); 3.76-3.83 (m, 2H, 4'-H + 5'-H); 4.57-4.62 (m, 1H, 3'-H); 5.71-5.78 (m, 1H, NPE-CH); 6.25-6.32 (m, 1H, 1'-H); 7.39-7.43 (m, 1H, NPE(ar)); 7.64 (t, J = 7.6 Hz, 1H, NPE(ar)); 7.84 (d, J = 7.8 Hz, 2H, NPE(ar)); 8.01 (s, 1H); 8.33 (s, 1H); 8.59-8.65 (m, 1H, NH) ppm.
^{13}C NMR (75 MHz, DMSO-d_6): δ = -5.54; -4.96; -4.77; 17.72; 17.96; 21.35; 22.89; 25.69; 25.74; 26.35; 29.63; 44.66; 62.34; 62.51; 71.83; 72.02; 83.27; 83.39; 86.90; 87.02; 119.57; 123.56; 127.76; 127.94; 133.48; 139.50; 139.65; 140.45; 148.59; 148.85; 152.10; 153.27 ppm.
MALDI-HRMS: *m/*z calcd. for $C_{30}H_{49}O_5N_6Si_2$ $[M+H]^+$ 629.32975, found 629.33003 (Δm 0.00028, error 0.45 ppm)

6-*N*-(1-(2-nitro)phenyl)ethyl-2'-deoxyadenosine (6):
Compound **5** (4.68 g, 7.26 mmol) was dissolved in THF (120 mL). Tetrabutylammoniumfluoride solution (1 M in THF) (14.5 mL, 14.5 mmol, 2 eq.) was added. The reaction mixture was stirred for 30 minutes. TLC showed complete consumption of the starting material. The solvent was evaporated and the residue was purified by flash chromatography (dichloromethane/methanol 97:3 → 95:5 → 93:7).
Yield: 2.91 g of **6** as light yellow foam (quantitative yield).
TLC (dichloromethane/methanol 95:5): R_f = 0.05
^{1}H NMR (400 MHz, acetone-d_6): δ = 1.79 (d, J = 7.2 Hz, 3H, NPE-CH_3); 2.27-2.32 (m, 1H, 2'-H); 2.81-2.88 (m, 1H, 2'-H); 3.62-3.68 (m, 1H, 5'-H); 3.73-3.78 (m, 1H, 5'-H); 4.04-4.06 (m, 1H, 4'-H); 4.42-4.44

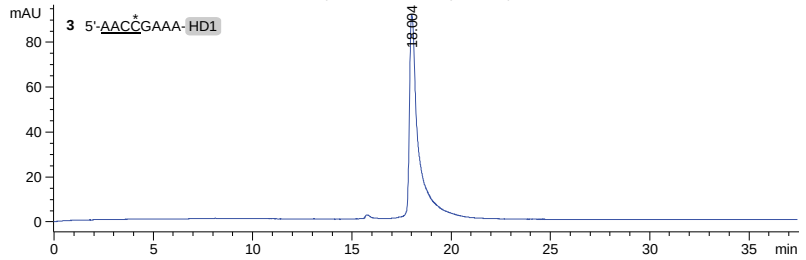
(m, 1H, 3'-OH); 4.58-4.61 (m, 1H, 3'-H); 5.38-5.44 (m, 1H, 5'-OH); 5.89-5.97 (m, 1H, NPE-CH); 6.37-6.41 (m, 1H, 1'-H); 7.41-7.46 (m, 1H, NPE(ar)); 7.61-7.65 (m, 2H, NPE(ar) + NH); 7.87-7.93 (m, 2H, NPE(ar)); 8.06 (s, 1H); 8.16-8.21 (m, 1H) ppm.
^{13}C NMR (75 MHz, acetone-d_6): δ = 20.58; 22.00; 41.21; 46.40; 54.97; 63.70; 73.19; 87.26; 90.14; 124.68; 128.62; 134.11; 141.13; 150.40; 152.72; 172.53 ppm.
MALDI-HRMS: *m/*z calcd. for $C_{18}H_{21}O_5N_6$ $[M+H]^+$ 401.15679, found 401.15727 (Δm 0.00048, error 1.19 ppm)

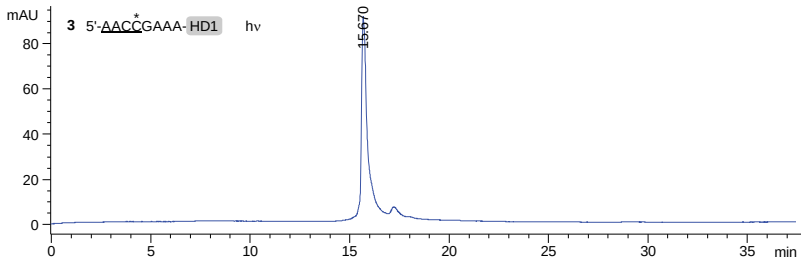
5'-*O*-(4,4'-Dimethoxytrityl)-6-*N*-(1-(2-nitro)phenyl)ethyl-2'-deoxyadenosine (7):
Compound **6** (2.81 g, 7.02 mmol) was coevaporated from pyridine (200 mL) and dissolved in pyridine (250 mL). The solution was cooled to 0°C under argon atmosphere and 4,4'-dimethoxytritylchloride (2.85 g, 8.42 mmol, 1.2 eq.) was added. The ice bath was removed and the solution was stirred over night at room temperature. TLC showed complete conversion of the starting material. Methanol (60 mL) was added and the solution was stirred for additional 10 minutes at room temperature. The solvents were evaporated. The residue was dissolved in dichloromethane and the organic layer was washed consecutively with aq. citric acid (5 %), sat. aq. $NaHCO_3$ and sat. aq. NaCl, then dried with $MgSO_4$. The solvent was evaporated and the residue was purified by flash chromatography (dichloromethane/methanol 99:1 → 98:2 → 97:3). The column was packed with the initial solvent additionally containing 0.5 % Triethylamine.
Yield: 4.47 g of **7** as light yellow foam (91 %).
TLC (dichloromethane, 1 % methanol, 0.5 % *N*-Ethyldiisopropylamine): R_f = 0.13
^{1}H NMR (400 MHz, acetone-d_6): δ = 1.78 (d, J = 7.1 Hz, 3H, NPE-CH_3); 2.39-2.45 (m, 1H, 2'-H); 2.88-3.00 (m, 1H, 2'-H); 3.29-3.35 (m, 2H, 2 x 5'-H); 3.76, 3.77 (2s, 6H, 2 OMe); 4.10-4.14 (m, 1H, 4'-H); 4.51-4.53 (m, 1H, 3'-OH); 4.65-4.71 (m, 1H, 3'-H); 5.87-5.97 (m, 1H, NPE-CH); 6.39-6.43 (m, 1H, 1'-H); 6.78-6.83 (m, 4H, DMTr(ar)); 7.16-7.25 (m, 3H, DMTr(ar)); 7.28-7.31 (m, 4H, DMTr(ar)); 7.40-7.45 (m, 3H, 2 DMTr(ar) + NPE(ar)); 7.47-7.50 (m, 1H, NH); 7.58-7.63 (m, 1H, NPE(ar)); 7.86-7.93 (m, 2H, NPE(ar)); 8.00-8.02 (m, 1H); 8.08-8.15 (m, 1H) ppm.
^{13}C NMR (100 MHz, acetone-d_6): δ = 40.22; 40,44; 54.96; 55.49; 65.05; 72.62; 85.10; 86.91; 87.39; 113.82; 124.64; 127.45; 128.50; 128.69; 128.95; 130.84; 130.97; 134.13; 136.73; 136.86; 136.99; 140.17; 146.13; 150.39; 153.20; 159.56 ppm.
MALDI-HRMS: *m/*z calcd. for $C_{39}H_{39}O_7N_6$ $[M+H]^+$ 703.28747, found 703.28746 (Δm 0.00001, error 0.02 ppm)

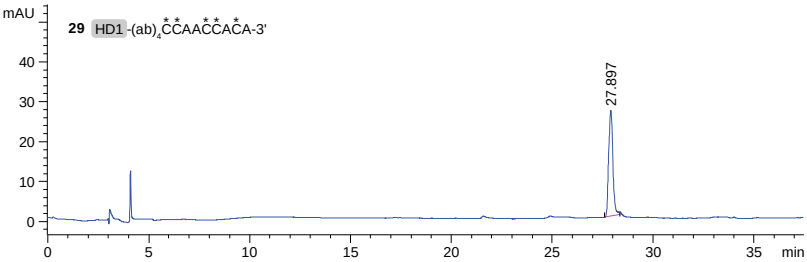
5'-*O*-(4,4'-Dimethoxytrityl)-6-*N*-(1-(2-nitro)phenyl)ethyl-2'-deoxyadenosine-3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (8):
Compound **7** (150 mg, 213 µmol) was dissolved in dry dichloromethane (6 mL) under argon atmosphere. *N*-Ethyldiisopropylamine (182 µL, 1.07 mmol, 5 eq.) was added, followed by 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (95 µL, 426 µmol, 2 eq.). After stirring for 90 minutes at room temperature, TLC showed that the reaction was complete. The solution was diluted with dichloromethane, and the organic layer was washed with sat. aq. $NaHCO_3$ and dried with $MgSO_4$. The solvent was evaporated and the crude product was chromatographed with cyclohexane/acetone 2:1. The column was packed with the initial solvent additionally containing 0.5 % Triethylamine.
Yield: 155 mg of **8** as light yellow foam (80 %).
TLC (cyclohexane/acetone 2:1, 0.5 % *N*-Ethyldiisopropylamine): R_f = 0.38
^{1}H NMR (400 MHz, dmso-d_6): δ = 1.01-1.13 (m, 12H, 2 iPr-CH_3); 1.64 (d, J = 7.2 Hz, 3H, NPE-CH_3); 2.39-2.48 (m, 1H, 2'-H); 2.66 (t, J = 6.1 Hz, 1H, Cyanoethyl-CH_2); 2.75 (t, J = 6.1 Hz, 1H, Cyanoethyl-CH_2); 2.96-3.07 (m, 1H, 2'-H); 3.14-3.26 (m, 2H, 2 x 5'-H); 3.48-3.59 (m, 2H, 2 iPr-CH); 3.62-3.75 (m, 8H, 2 OMe + Cyanoethyl-CH_2); 4.05-4.13 (m, 1H, 4'-H); 4.70-4.82 (m, 1H, 3'-H); 5.72-5.79 (m, 1H, NPE-CH); 6.25-6.38 (m, 1H, 1'-H); 6.75-6.81 (m, 4H, DMTr(ar)); 7.15-7.21 (m, 7H, DMTr(ar)); 7.27-7.33 (m, 2H; DMTr(ar)); 7.38-7.44 (m, 1H, NPE(ar)); 7.60-7.65 (m, 1H, NPE(ar)); 7.83-7.87 (m, 2H, NPE(ar)); 7.91-7.96 (m, 1H); 8.29-8.33 (m, 1H); 8.57-8.66 (m, 1H, NH) ppm.
^{31}P NMR (162 MHz, dmso-d_6): δ = 147.03, 147.61 ppm.

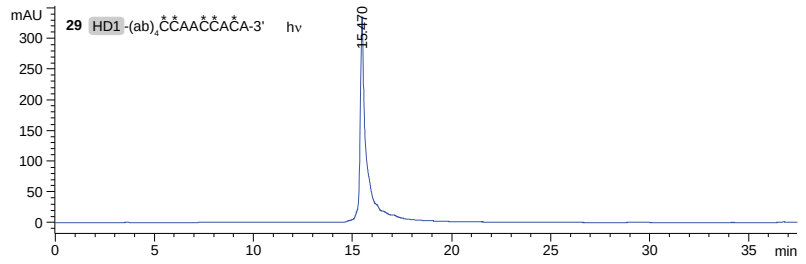
2. HPLC-traces of the caged aptamers 3, 29 and 30

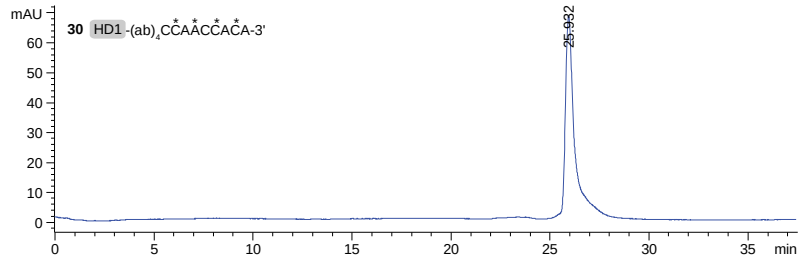
Column: Nucleosil 100 5 C 18, 0.1 M triethylammonium acetate pH 7, acetonitrile, gradient: 0% MeCN for 2 min, 0% to 40% MeCN in 33 min, flow 1 ml/min, detection: 254 nm

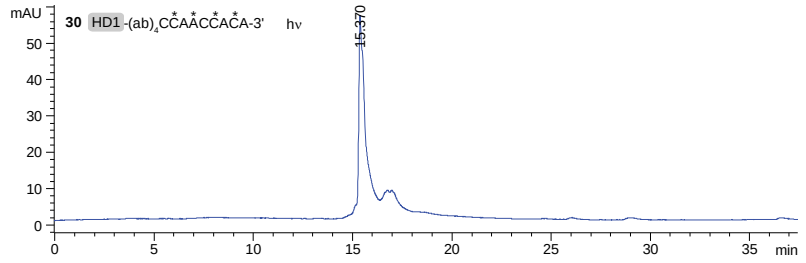




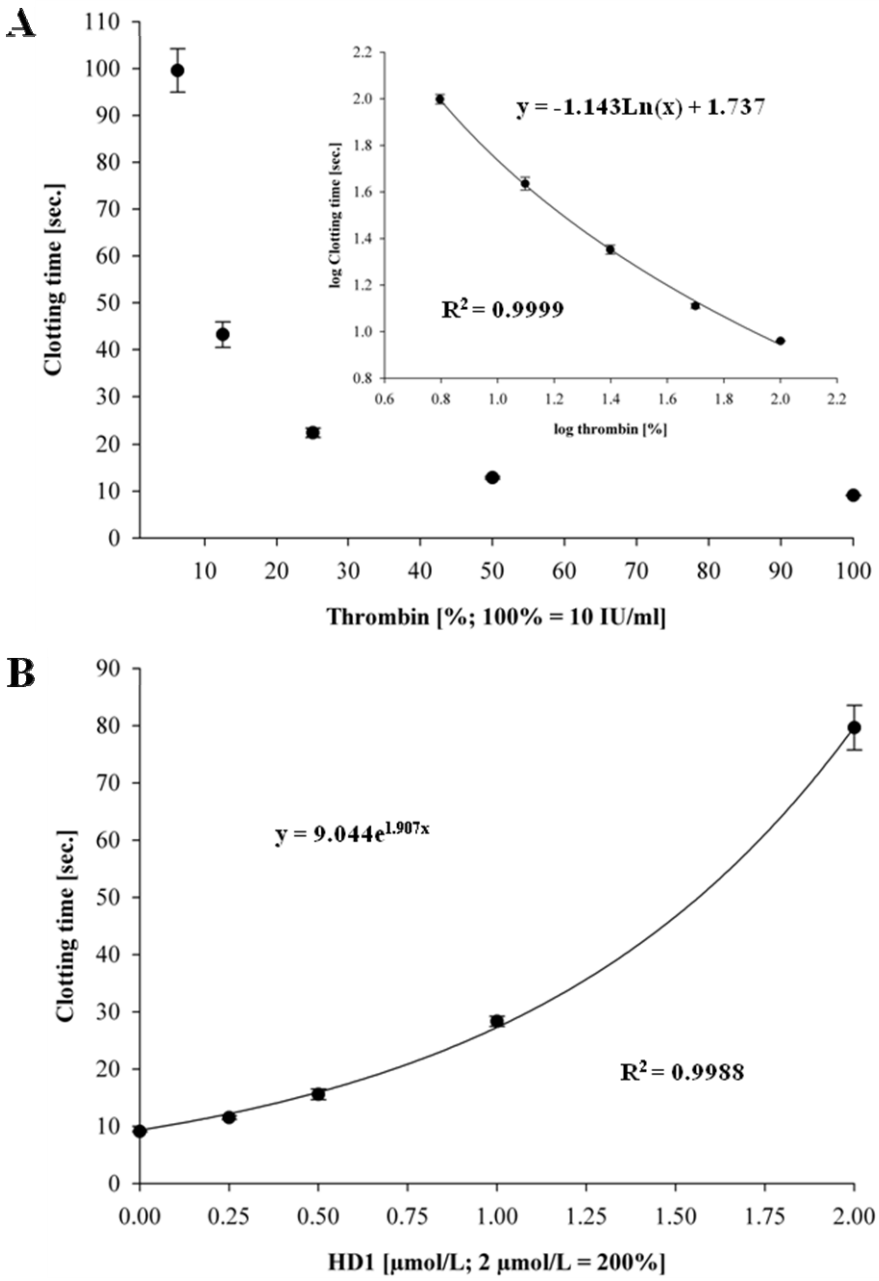








3. Normalization of clotting times



Relation between applied thrombin/**HD1**-concentrations and observed TT-clotting times. Lowering thrombin-activity from a starting level of 10 IU/ml (100%) led to an exponential increase of clotting times (A). Best fit of data for the generation of thrombin standard curves was achieved on log-transformed data (A, inlet). Increasing concentrations of **HD1** also led to an exponential increase of clotting times. Interpolation was performed by exponential curve fit (B). Error-bars represent the standard deviations calculated from four independent experiments, demonstrating the high reproducibility of the applied TT-assay.

4. Saturation levels in the filter binding studies (cf. Table 1)

aptamer	saturation level [%]
HD1	9
1	<2
5	19
8	11
7	17
12	16
14	12
15	9
18	<2
19	<2
23	7
24	5
25	<2
26	45
26 hv	3
27	25
27 hv	2
28	20
28 hv	<2
29	6
29 hv	<2
30	15
30 hv	<2