

Supplementary Material

Metallacarborane complex boosts the rate of DNA oligonucleotide hydrolysis in the reaction catalyzed by snake venom phosphodiesterase

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Figure S1. Analytical RP HPLC profiles and MALDI-TOF or ESI-Q-TOF (*) mass spectra of **1**, **P1a**, **P1b**, **P1c**, **1a**, **1b**, **1c**, **2**, **P2a**, **P2b**, **2a**, **2b**, **FL-1** and **FL-2**.

RP-HPLC conditions were as follow: the buffer A (0.1 M CH₃COONH₄) and buffer B (100% CH₃CN). The buffer B gradient: 0→2 min 0%; 2→25 min 0-48%; 25→30 min 48-60%; 30→35 min 60-0%; 35→38 min 0%.

Figure S2. PAGE analysis of the **1**, **1a**, **1b**, **1c** and **2**, **2a**, **2b** oligonucleotides (20%/7 M urea)

Figure S3. svPDE - assisted hydrolysis of reference oligonucleotide **1** with and without magnesium ions, monitored by MALDI-TOF mass spectrometry.

Figure S4. Original MALDI-TOF MS spectra of hydrolysis of **1c** in the presence of svPDE (in triplicate).

Figure S5. MALDI-TOF mass spectrometry analysis of the 1:1:1 mixture of **1**, **1a** and oligonucleotide **1** containing two FESAN clusters (synthetic data not shown). Intensity of peaks is given on the Figure.

Figure S6. Original MALDI-TOF MS spectra of the hydrolysis mixture of **1+1a** in the presence of svPDE (in triplicate).

Figure S7. Figure S7. MALDI-TOF MS analysis of the reaction mixture of svPDE-assisted hydrolysis of unmodified oligonucleotides **1** and **2** (0.1 OD) in the presence of a free metallacarborane (FESAN, 182 nM) at 0-60 min.

Figure S8. MALDI-TOF MS analysis of the hydrolysis rate of oligonucleotide **1** in the svPDE-assisted reaction carried out in the presence of 0, 1, 2 or 3 equivalents of metallacarborane (in triplicate)

Figure S9. PAGE analysis of crude snake venom.

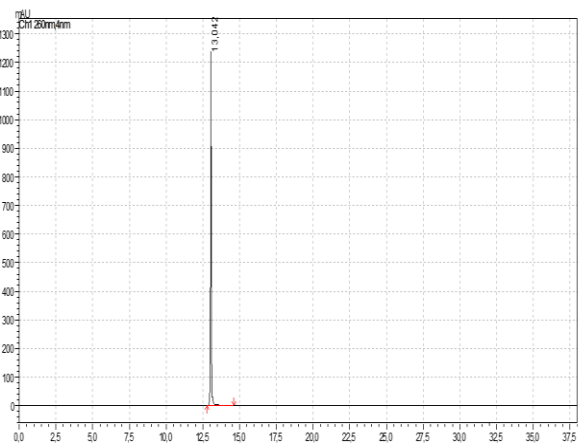
Figure S10. The protein samples capillary scan from microscale thermophoresis (the venom proteins fluorescently labeled with RED-NHS 2nd generation).

Figure S11. MST data for K_d determination of affinity of FESAN (ferra(III) bis(dicarbollide)) to **FL-1** ($K_d=5.48\pm 0.20$ μ M).

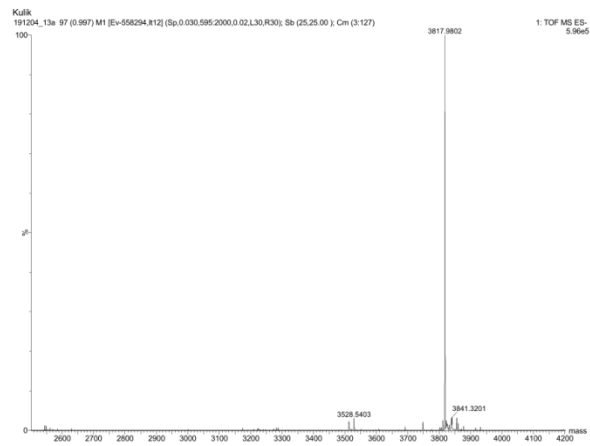
Figure S1. Analytical RP HPLC profiles and MALDI-TOF or ESI-Q-TOF (*) mass spectra of **1**, **P1a**, **P1b**, **P1c**, **1a**, **1b**, **1c**, **2**, **P2a**, **P2b**, **2a**, **2b**, **FL-1** and **FL-2**.

RP-HPLC conditions were as follow: the buffer A (0.1 M CH₃COONH₄) and buffer B (100% CH₃CN). The buffer B gradient: 0→2 min 0%; 2→25 min 0-48%; 25→30 min 48-60%; 30→35 min 60-0%; 35→38 min 0%.

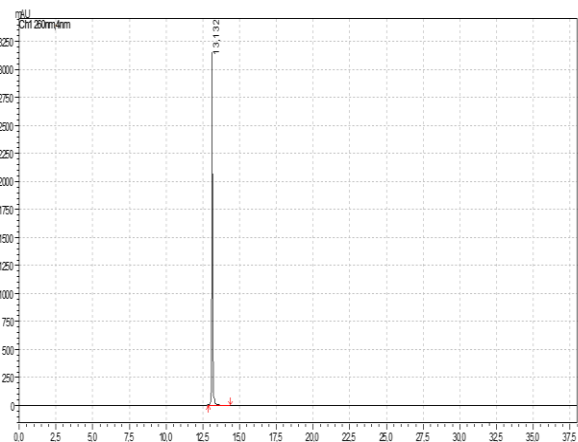
1 5'-d(TTT CTT TTC CTC C)-3'



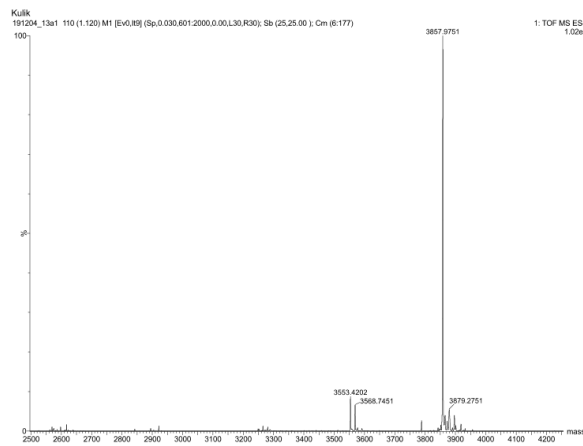
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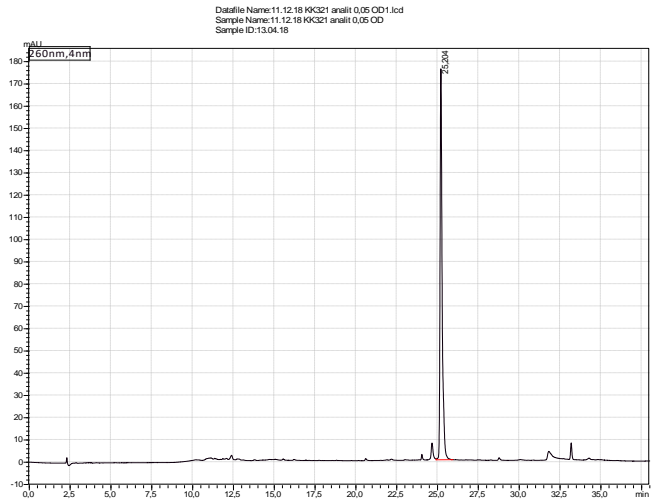
P1a 5'-d(U_{Pr}TT CTT TTC CTC C)-3'



(*)

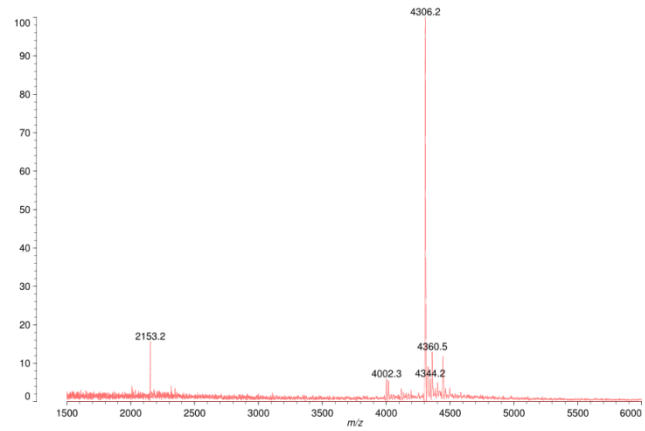


1a 5'-d(U_BTT CTT TTC CTC C)-3'

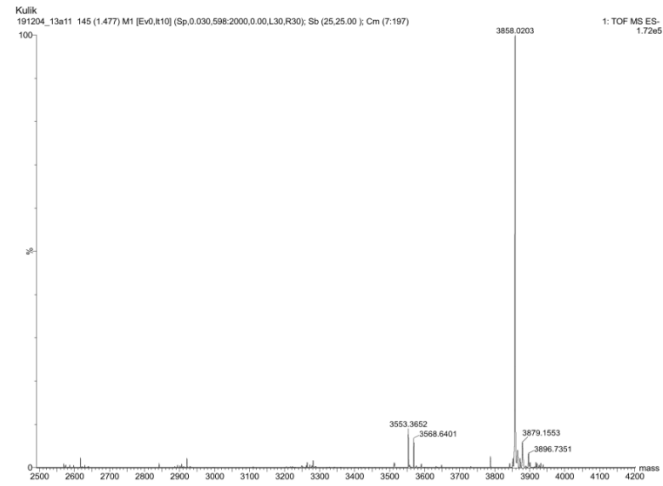
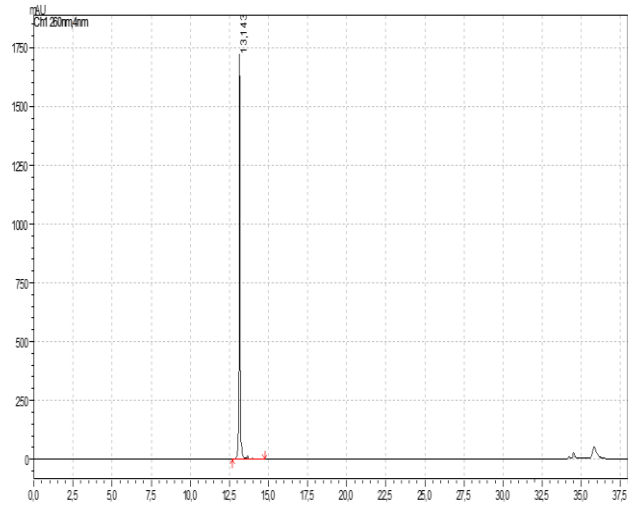


P1b 5'-d(TTT CTT TTC CU_{Pi}C)-3'

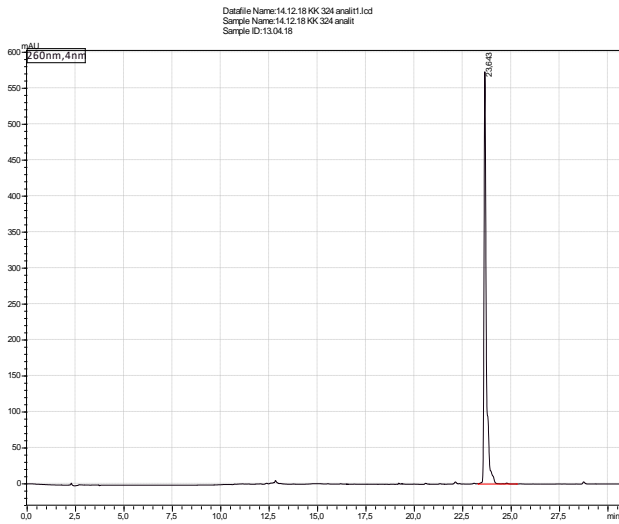
Kulik K, KK-321, linear neg
HPA 50 mg/mL, H₂O/ACN 1:1 v/v, AC 50 mg/mL, H₂O/ACN 1:1 v/v; HPA/AC 8:1
Data: he280003.L11[c] 3 Dec 2018 15:03 Cal: HPA_T5_T18 31 Oct 2018 15:33
Shimadzu Biotech Avima Performance 2.9.1.20100121: Mode Linear_neg_2018, Power: 118, Blanked, P.Ext. @ 3800 (bin 88)
%Int.: 33 mV[sum= 6576 mV] Profiles 1-200 Smooth Av 20 -Baseline 60



(*)

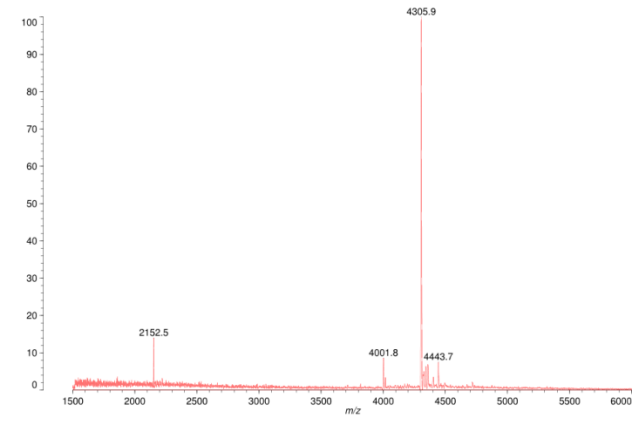


1b 5'-d(TTT CTT TTC CU_BC C)-3'

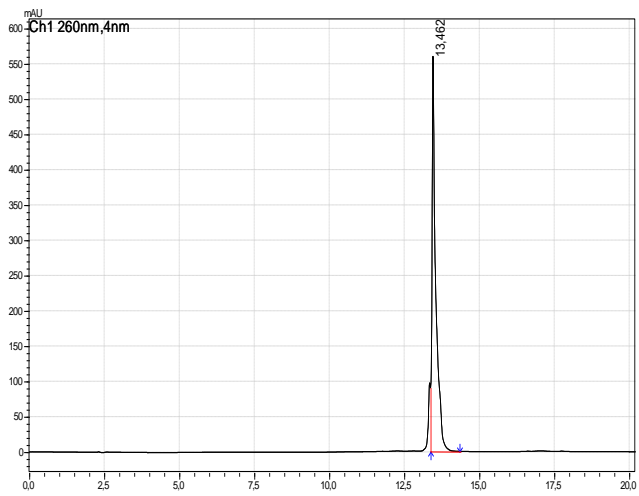


Datafile Name:14.12.18 KK-324 analit1.fcd
 Sample Name:14.12.18 KK-324 analit
 Sample ID:13.04.18

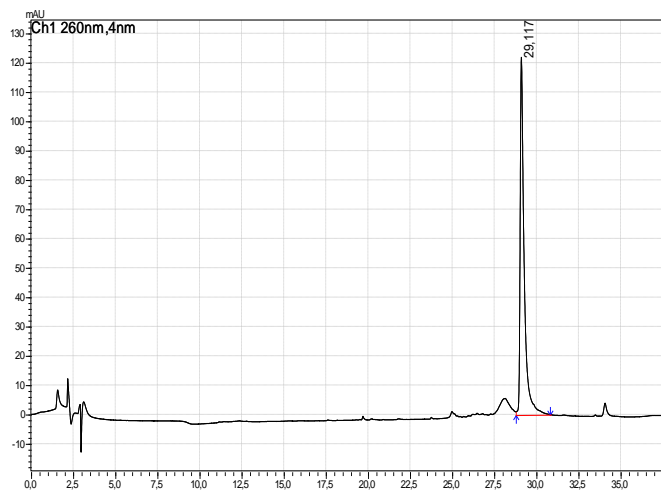
Kulik K, KK-324, linear neg
 HPA 50 mg/mL, H₂O/ACN 1:1 v/v, AC 50 mg/mL, H₂O/ACN 1:1 v/v, HPA/AC 8:1
 Data: he290002.L [3c] 3 Dec 2018 15:08 Cal: HPA_T5_T18 31 Oct 2018 15:33
 Shimadzu Biotech Asima Performance 2.9:1.20100121, Mode Linear, neg, 2018, Power: 112, Blanked, P.Ext. @ 3500 (bin 85)
 %Int. 65 mV[sum= 9830 mV] Profiles 1-152 Smooth Av 20 -Baseline 60



P1c 5'-d(U_{Pr}TT CTT TTC CTC U_{Pr})-3'

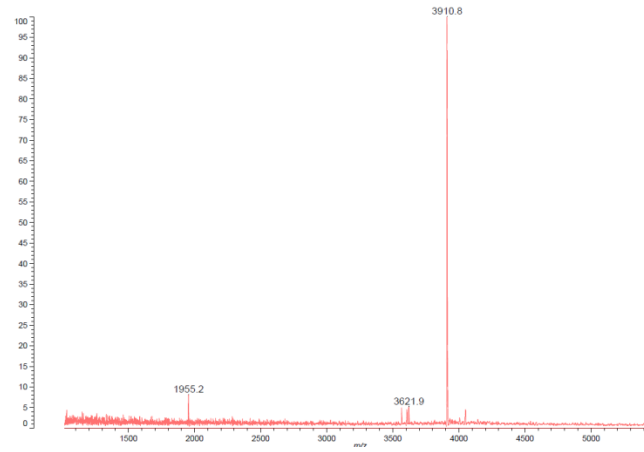


1c 5'-d(U_BTT CTT TTC CTCU_B)-3'

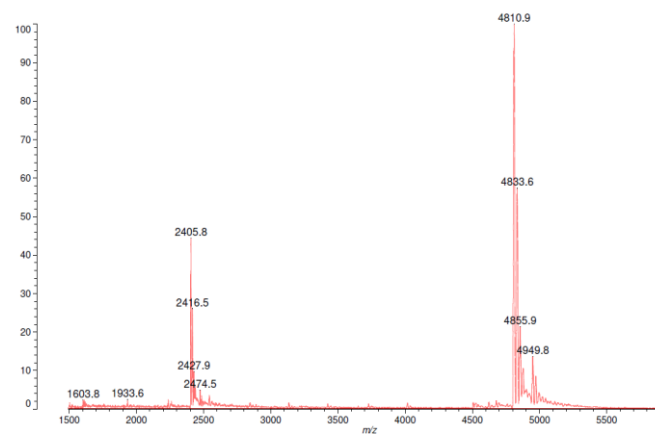


2 5'-d(CTC CAG AGC CCG A)-3'

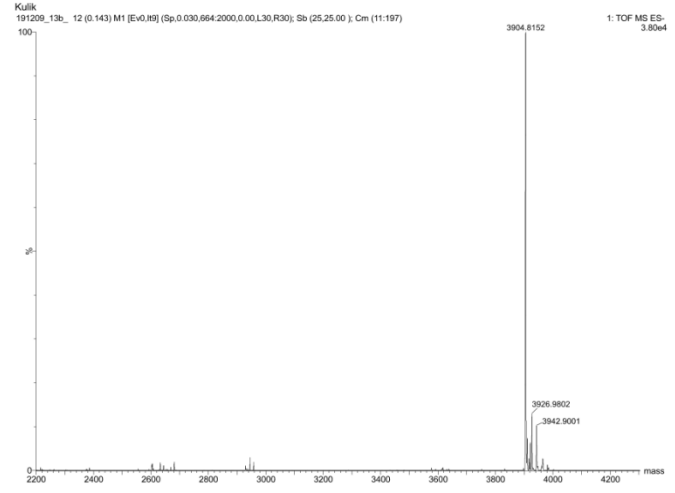
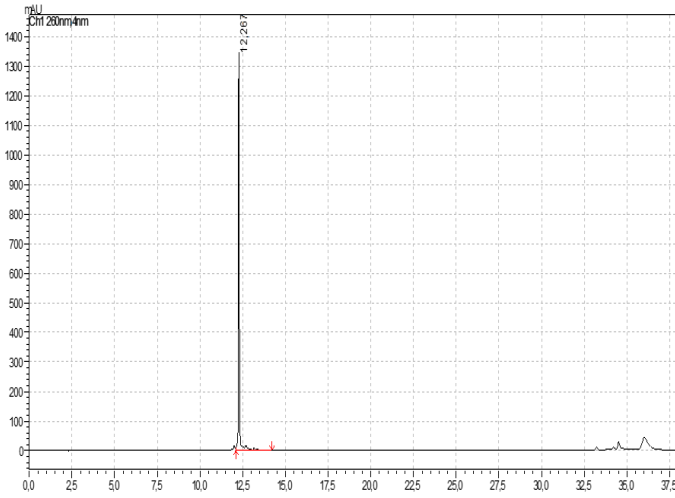
DK 13a1.13_linear_neg
P: CH2O2N6CH3_SAMMeOH:H2O : PM 20/10 v/v
Data: h005001 M1 [c] 6 Apr 2020 9:48 Cal: HPA_T5_T124 31 Oct 2018 15:37
Shimadzu Biotech Axima Performance 2.9 1.20100121: Mode Linear_neg_2018: Power: 106, Blanked, P.Ext. @ 3500 (bin 85)
%Int: 3.3 mV[sum= 669 mV] Profiles 1-200 Smooth Gauss 20-Baseline 75



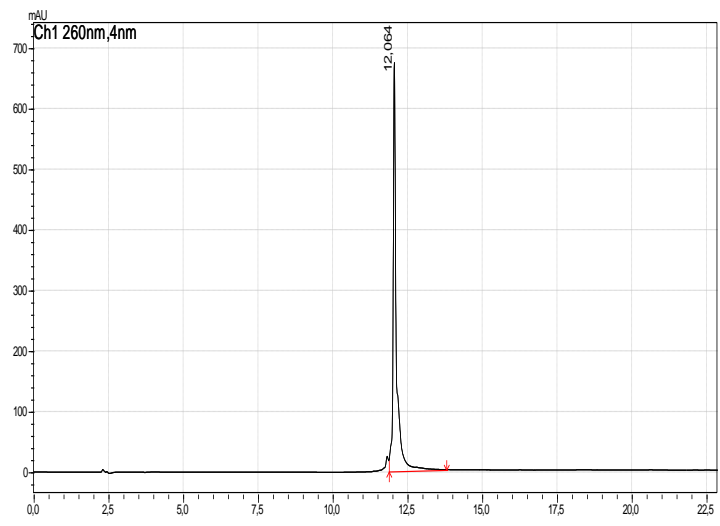
13a1.13_f1=0
HPA
Data: hm870004.F7 [c] 4 Mar 2020 15:03 Cal: HPA_T5_T18 31 Oct 2018 15:33
Shimadzu Biotech Axima Performance 2.9 1.20100121: Mode Linear_neg_2018: Power: 111, Blanked, P.Ext. @ 2800 (bin 76)
%Int: 62 mV[sum= 2227 mV] Profiles 1-36 Smooth Gauss 35-Baseline 75



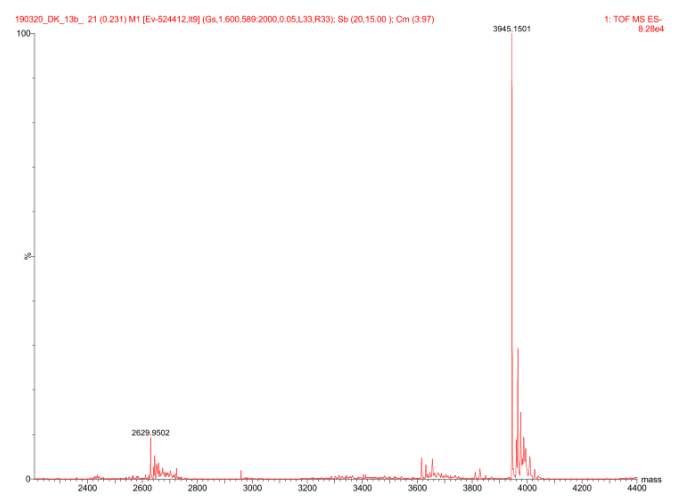
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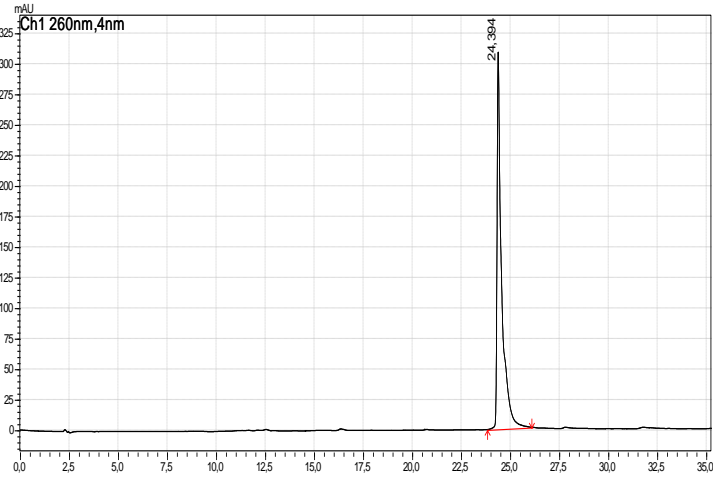
P2a 5'-d(CPC CAG AGC CCGA)-3'



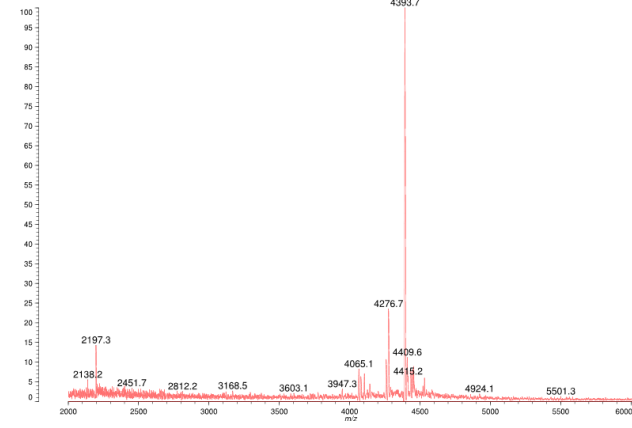
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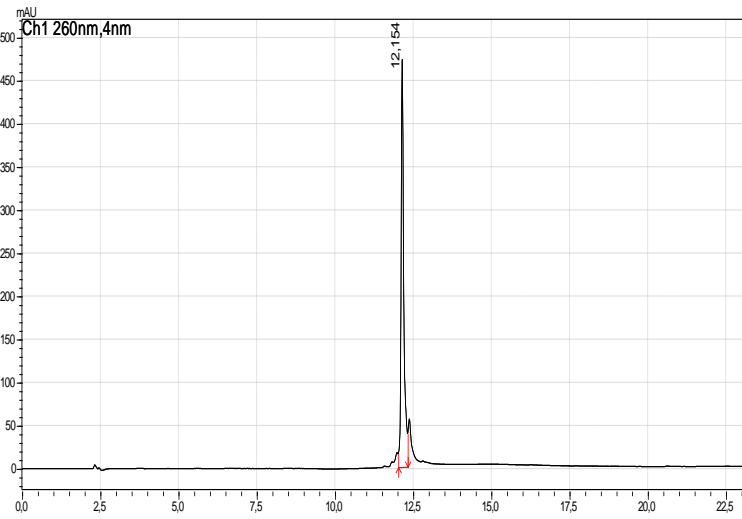
2a 5'-d(CBC CAG AGC CCGA)-3'



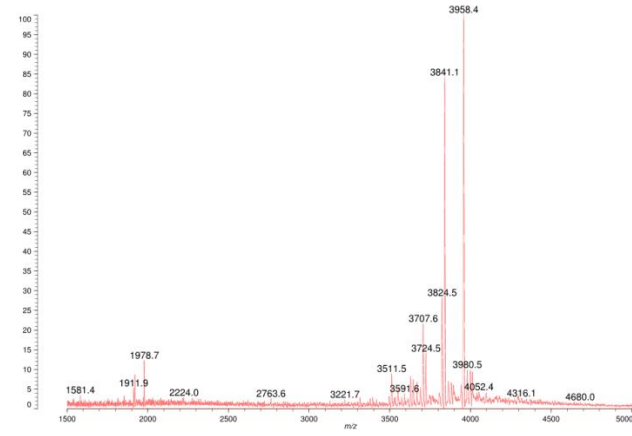
Eberlyter-Obitnska_K_608022_infer_neg
 HPA 50 mg/mL H2O/ACN 1:1 v/v; AC 50 mg/mL H2O/ACN 1:1 v/v; HPA/AC 8:1
 Data: hg60003.N13[c] 18 Mar 2019 12:27 Cal: HPA_T5_T18 31 Oct 2018 15:33
 Shimadzu Biotech Avanti Performance 2.3.1.20100121; Mode Linear; neg; 2018; Power: 113; Blanked; P.Ext. @ 3800 (bin 88)
 /Unit: 21 mV[um=1933 mV] Profiles 1-90 Smooth Gauss 15 -Baseline 60



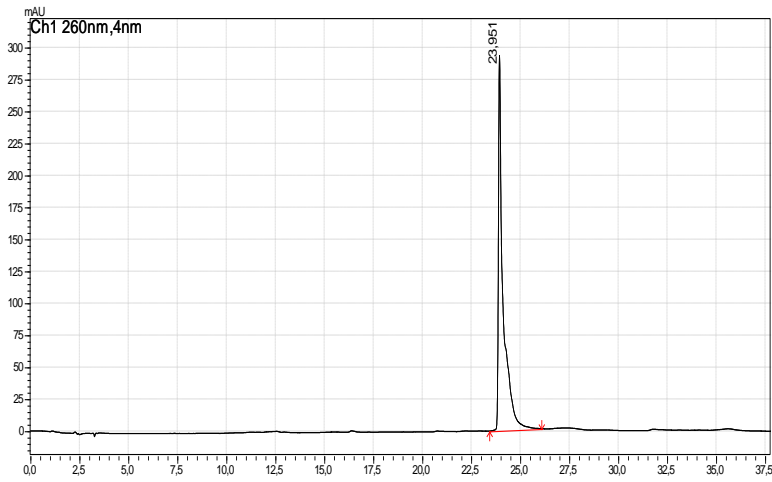
P2b 5'-d(CTC CAG AGC CPGA)-3'



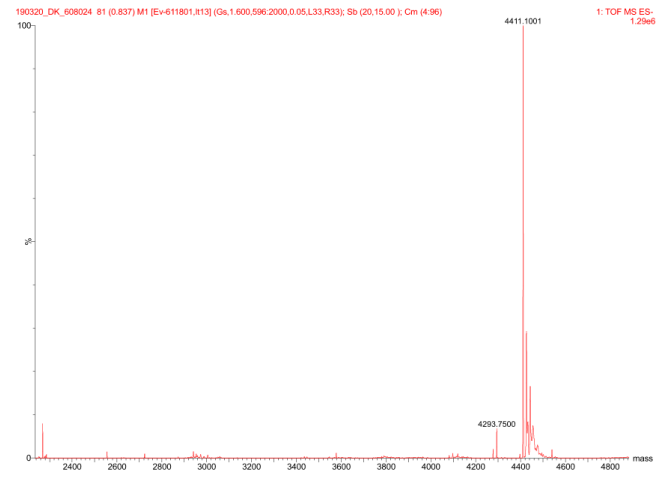
Eberlyter-Obitnska_K_13011_infer_neg
 HPA 50 mg/mL H2O/ACN 1:1 v/v; AC 50 mg/mL H2O/ACN 1:1 v/v; HPA/AC 8:1
 Data: hg70001.D8[c] 13 Mar 2019 15:22 Cal: HPA_T5_T124 31 Oct 2018 15:37
 Shimadzu Biotech Avanti Performance 2.3.1.20100121; Mode Linear; neg; 2018; Power: 115; Blanked; P.Ext. @ 2800 (bin 76)
 /Unit: 24 mV[um=4825 mV] Profiles 1-200 Smooth Gauss 15 -Baseline 60



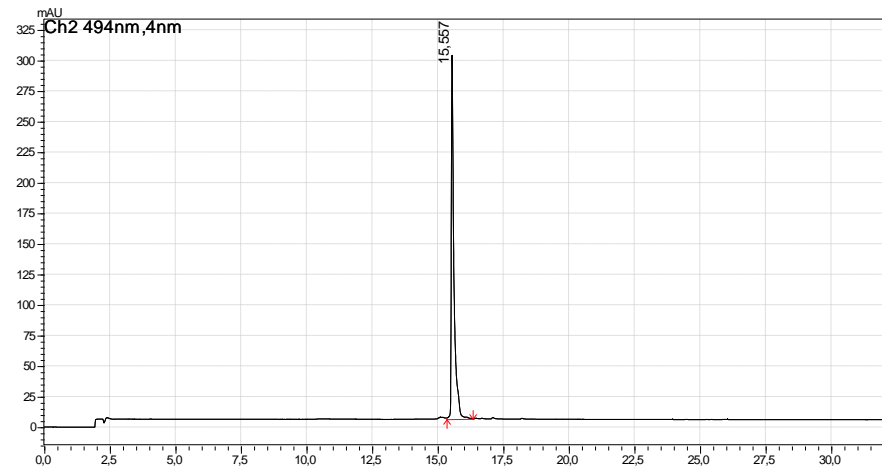
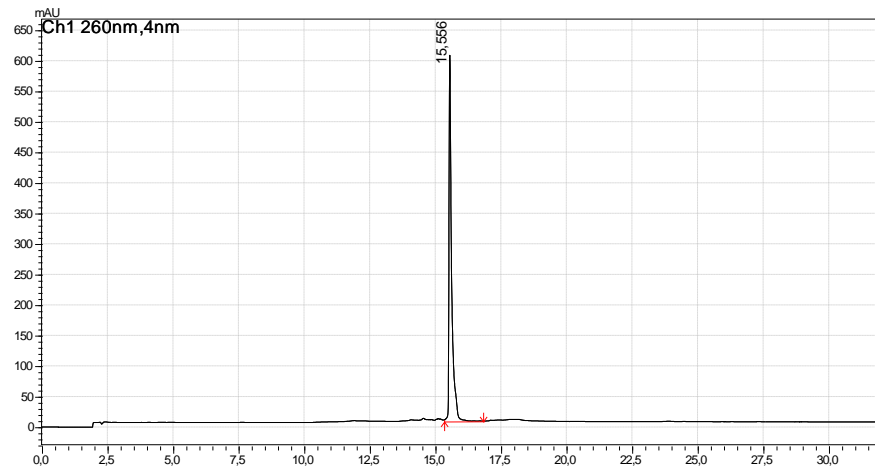
2b 5'-d(CTC CAG AGC CBGA)-3'



(*)



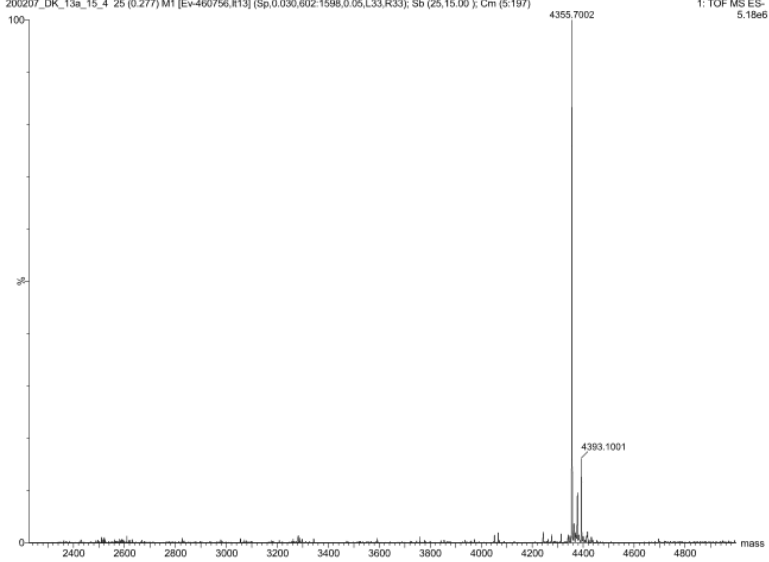
FL-1 5'-6FAM-d(TTT CTT TTC CTC C)-3'



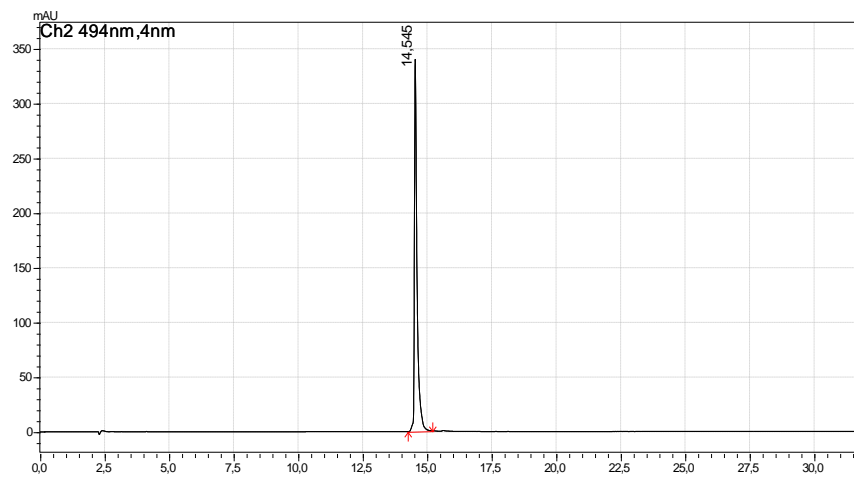
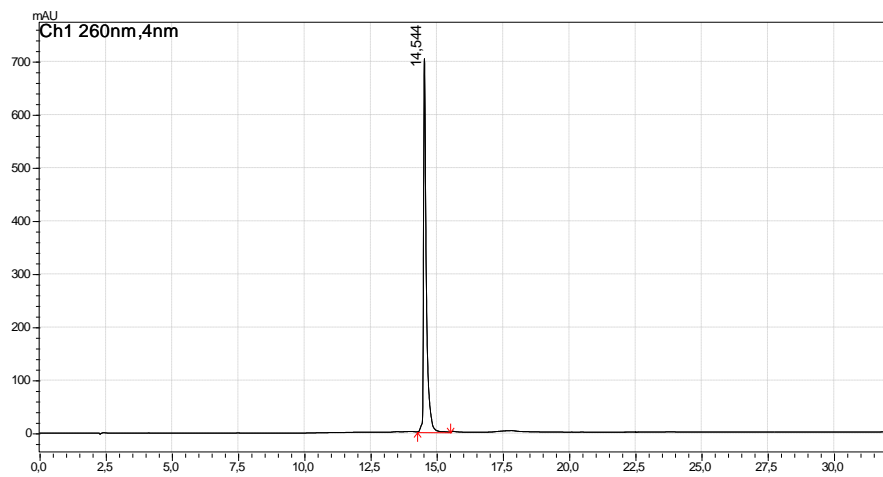
(*)

Ebenryter_Oblinska_K
200207_DK_13a_15_4_25 (0.277) M1 [Ev=460756.113] (Sp,0.030,602:1598,0.05,L33,R33); Sb (25,15.00); Cm (5:197)

1: TOF MS ES-
5.18e6



FL-2 5'-6FAM-d(CTC CAG AGC CCG A)-3'



(*)

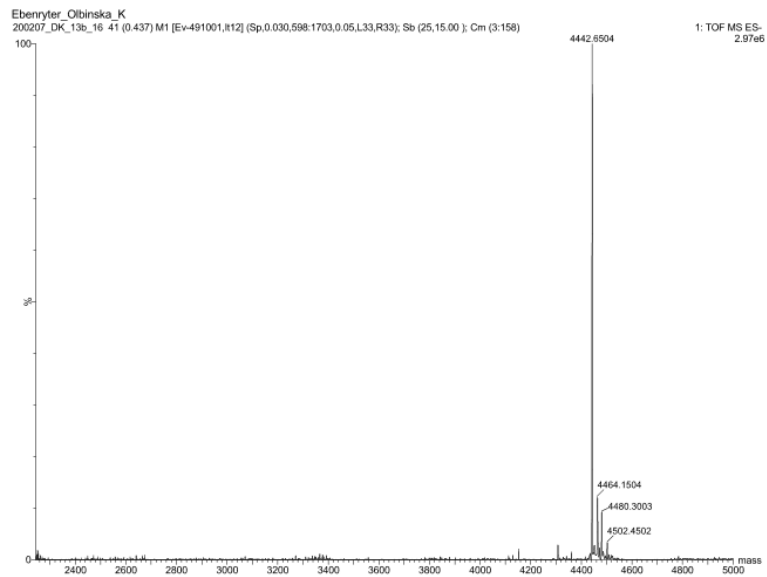


Figure S2. PAGE analysis of the **1**, **1a**, **1b**, **1c** and **2**, **2a**, **2b** oligonucleotides (20%/7 M urea)

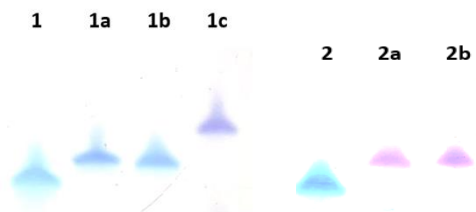


Figure S3. svPDE - assisted hydrolysis of reference oligonucleotide **1** with and without magnesium ions, monitored by MALDI-TOF mass spectrometry.

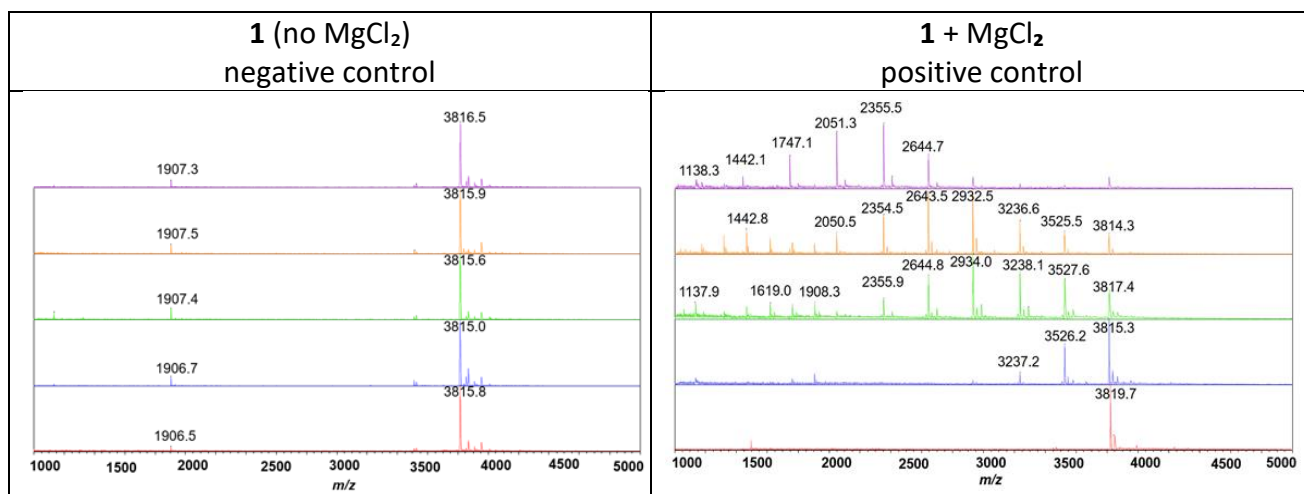
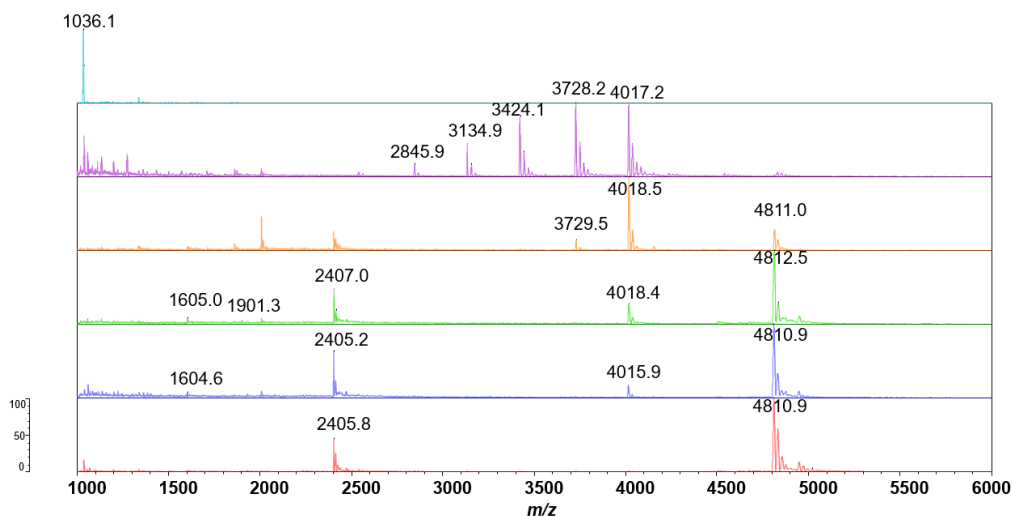
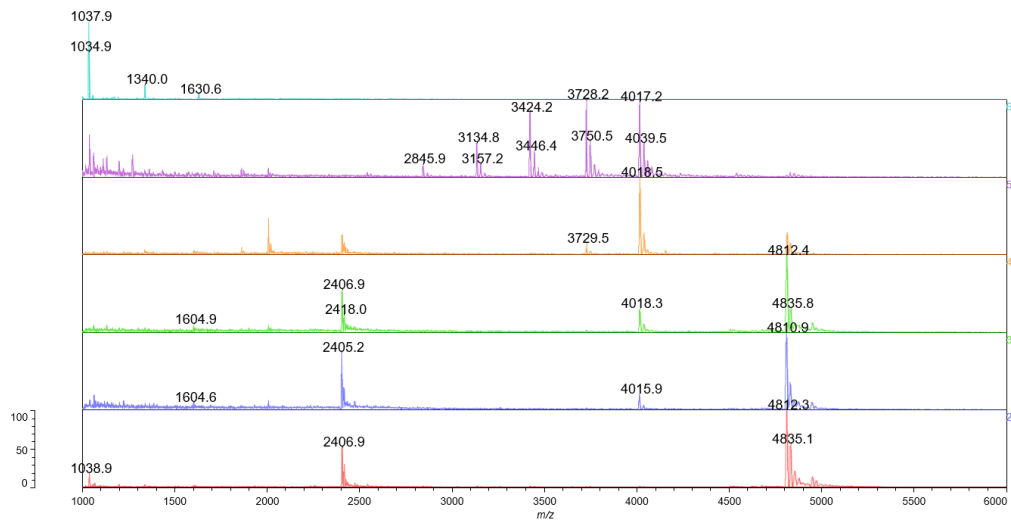


Figure S4. Original MALDI-TOF MS spectra of hydrolysis of **1c** in the presence of svPDE (in triplicate)

i) First experiment



ii) second experiment



iii) third experiment

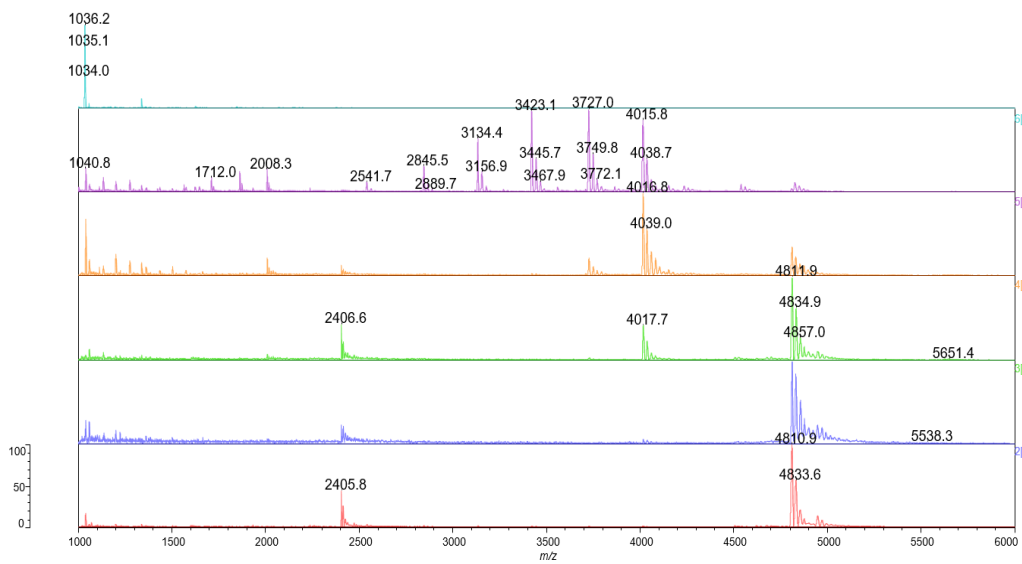


Figure S5. MALDI-TOF mass spectrometry analysis of the 1:1:1 mixture of **1**, **1a** and oligonucleotide **1** containing two FESAN clusters (synthetic data not shown). Intensity of peaks is given on the Figure.

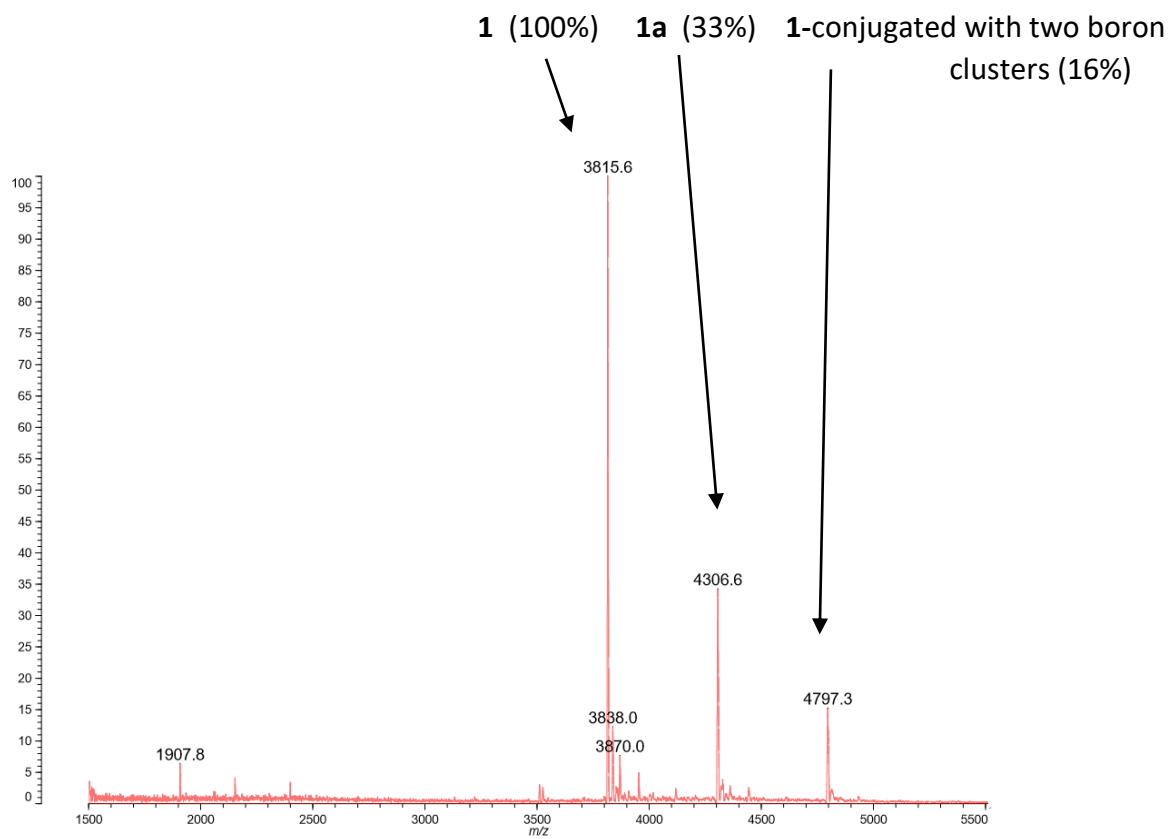
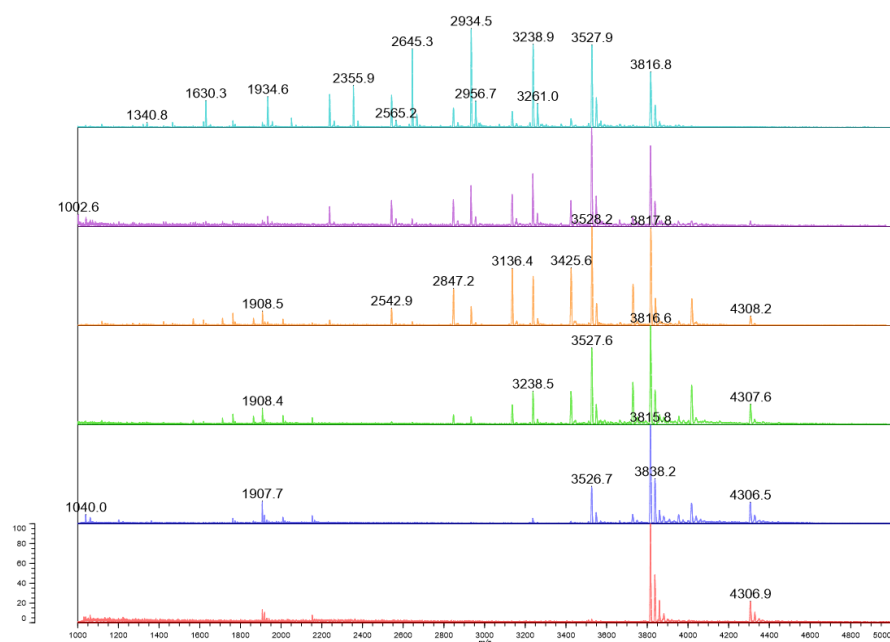
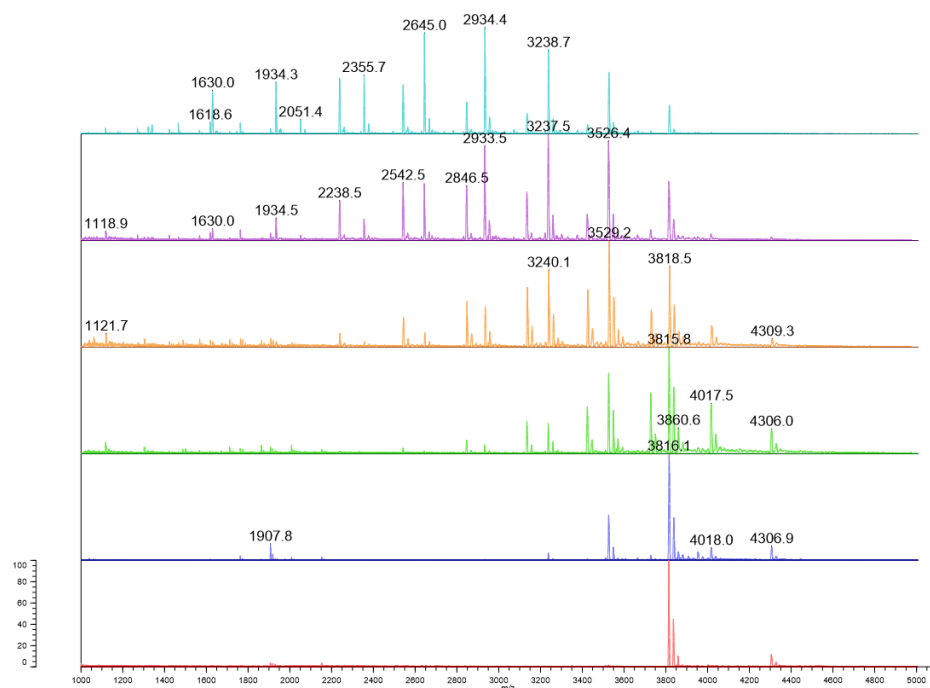


Figure S6. Figure S6. Original MALDI-TOF MS spectra of the hydrolysis mixture of **1+1a** in the presence of svPDE (in triplicate).

first experiment



i) second experiment



ii) third experiment

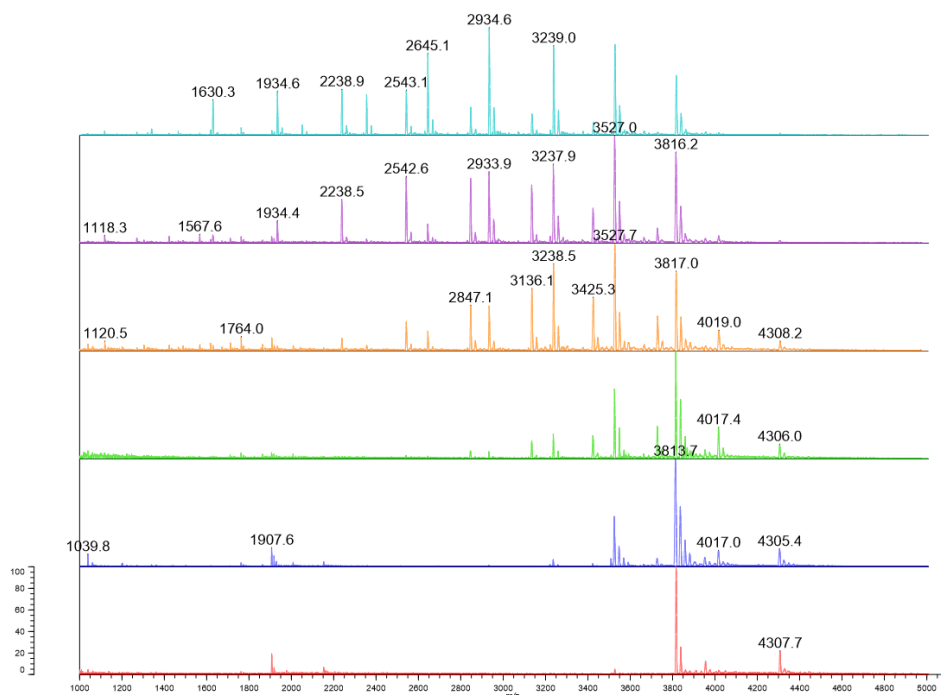


Figure S7. MALDI-TOF MS analysis of the reaction mixture of svPDE-assisted hydrolysis of unmodified oligonucleotides **1** and **2** (0.1 OD) in the presence of a free metallacarborane (FESAN, 182 nM) at 0-60 min.

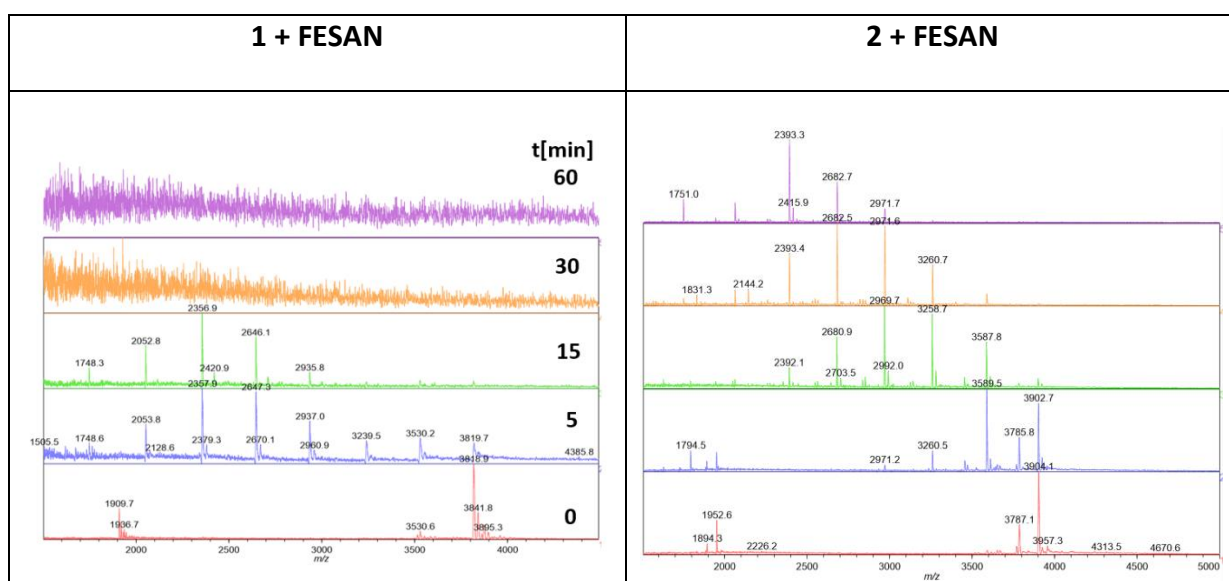
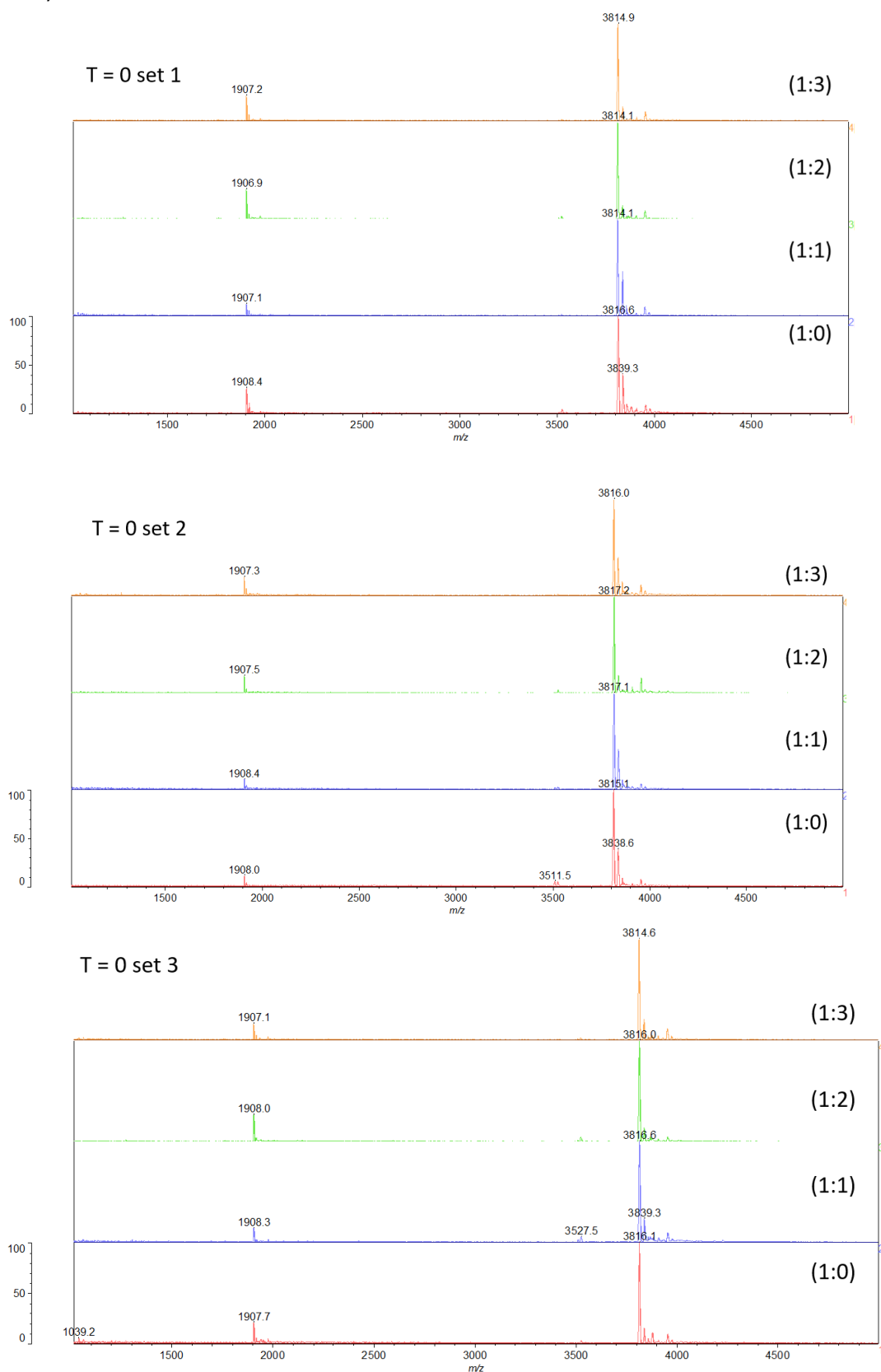
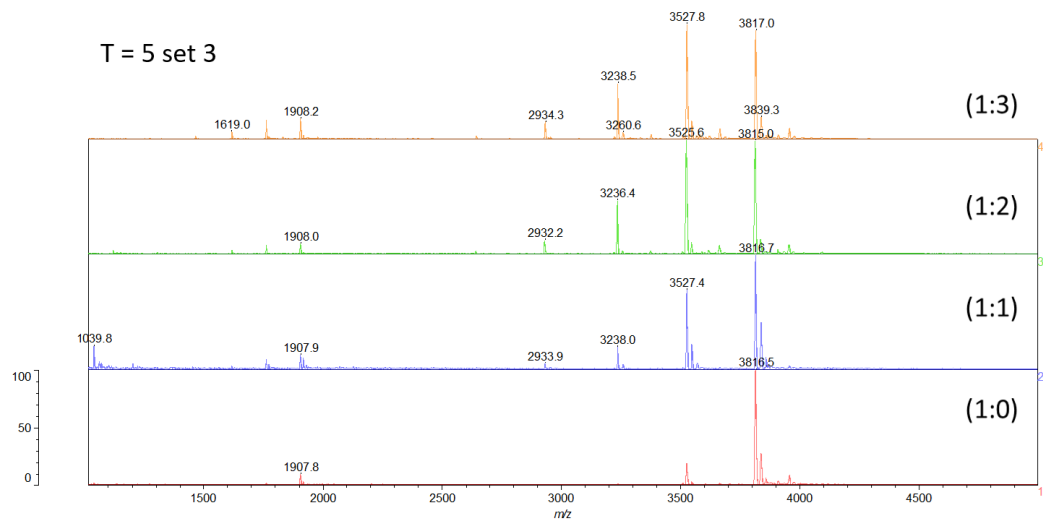
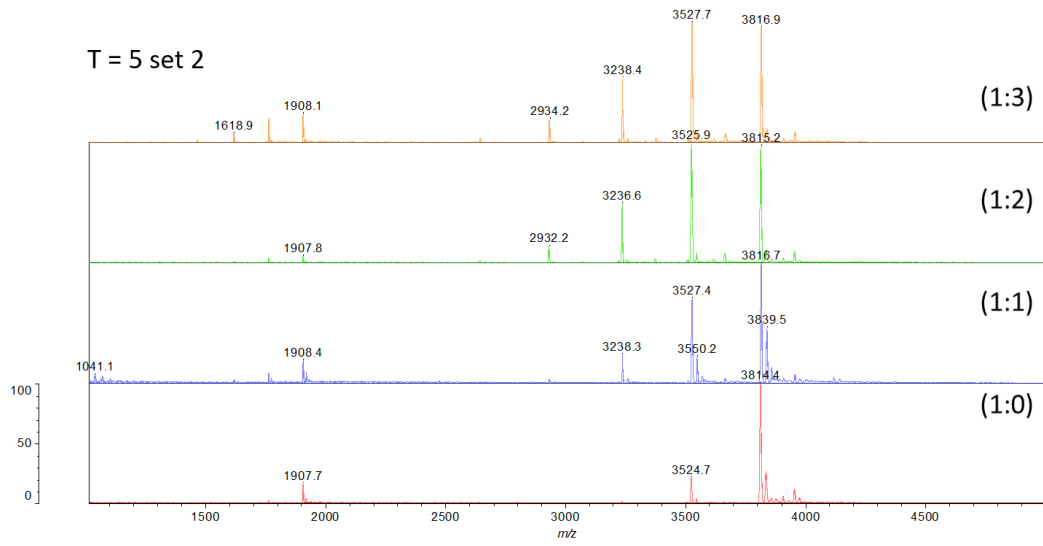
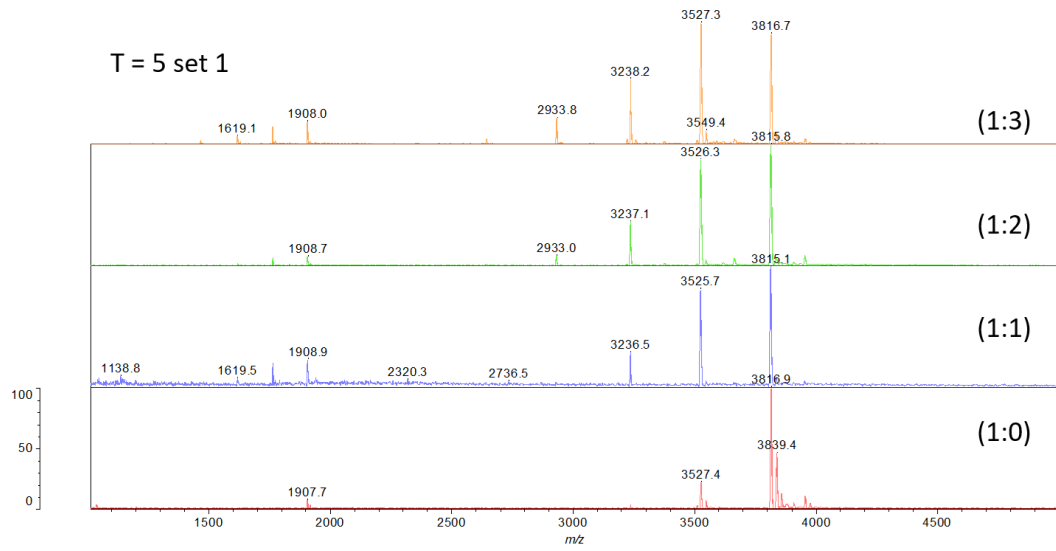


Figure S8. MALDI-TOF analysis of the hydrolysis rate of oligonucleotide **1** in the svPDE-assisted reaction carried out in the presence of 0, 1, 2 or 3 equivalents of metallacarborane (in triplicate)

i) measurements at 0 min.



ii) Measurements at 5 min.



iii) Measurements at 15 min.

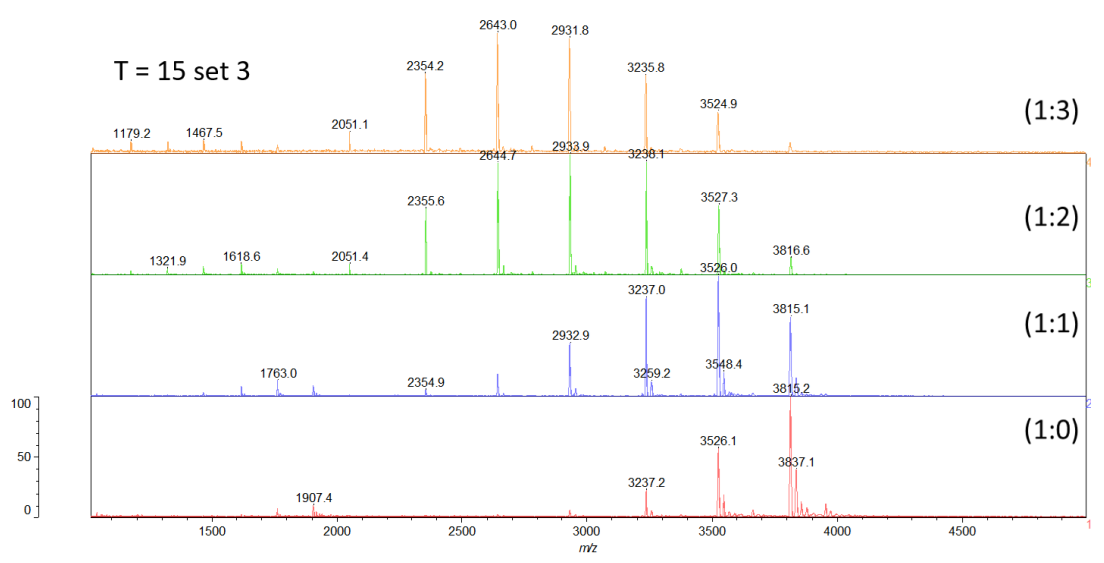
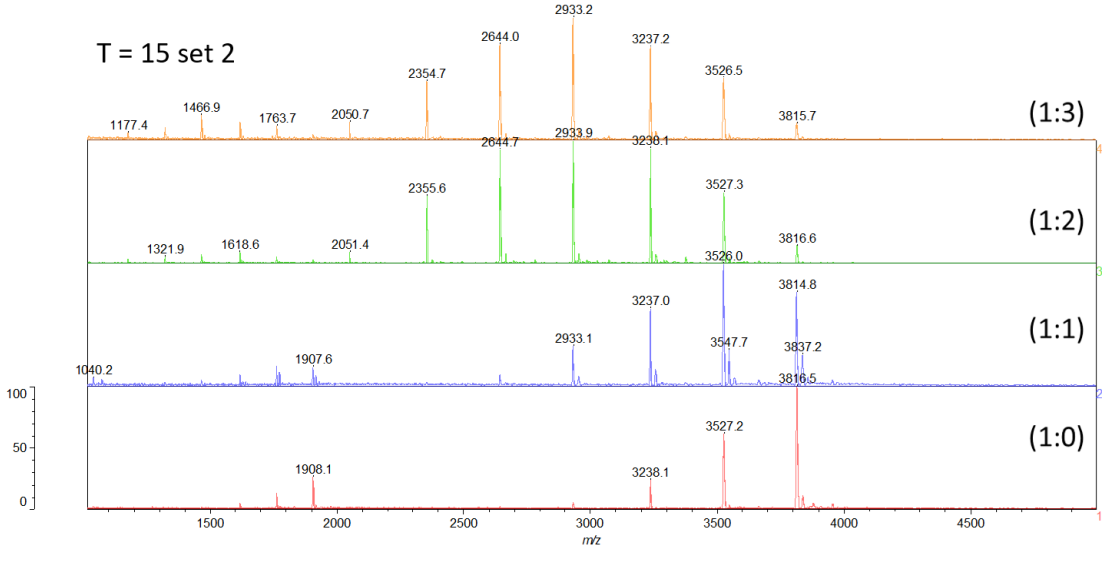
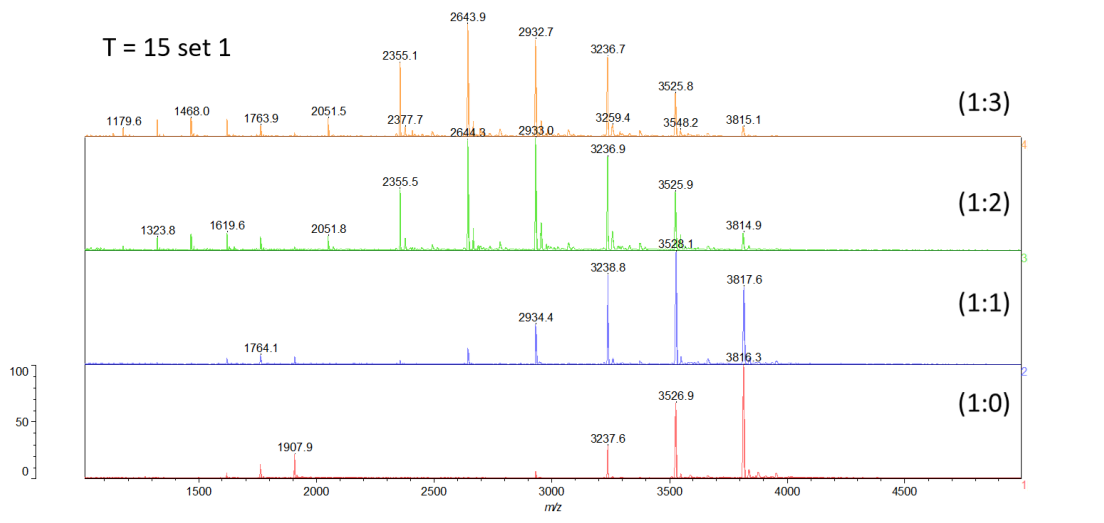


Figure S9. PAGE analysis of crude snake venom.

Lane 1 – molecular weight ladder,

lane 2 – the sample of crude svPDE of MW 112 kDa (marked).

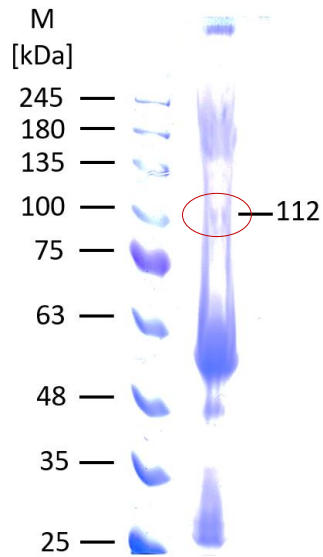


Figure S10. The protein samples capillary scan from microscale thermophoresis (the venom proteins fluorescently labeled with RED-NHS 2nd generation).

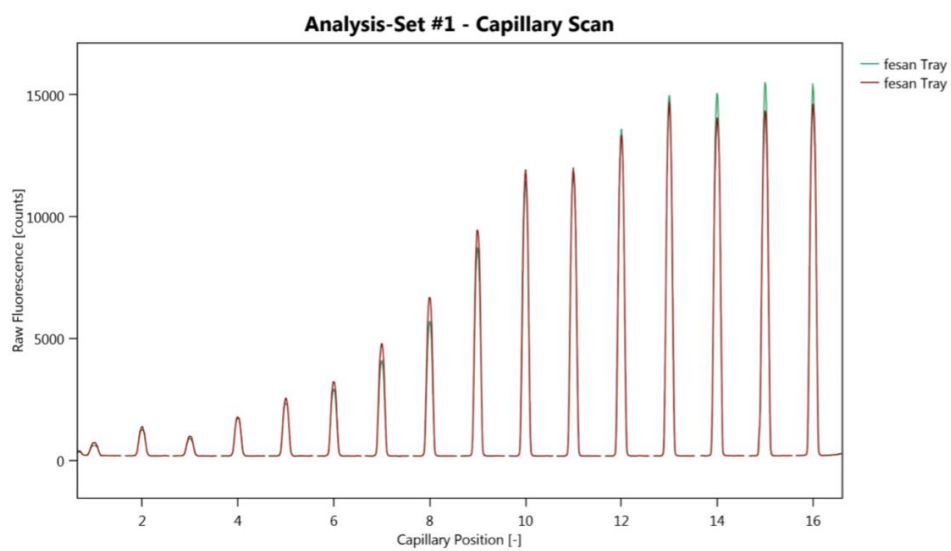



Figure S11. MST data for K_d determination of affinity of FESAN (ferra(III) bis(dicarbollide)) to FL-1 ($K_d=5.48\pm0.20 \mu\text{M}$).

Sample Information	
Merge-Set Name:	Fesan Tray
Date of Measurement:	2020-02-07 11:51:19.022 → 2020-02-07 15:36:37.773
Capillary Type:	Monolith NT.115 Standard Treated Capillary
Target:	1-FL
TargetConcentration:	0,00000001
Ligand:	Fesan
Measurement Settings	
MST-Power:	Medium
Excitation-Power:	20%
Excitation type:	Green
Thermostat Setpoint:	22,0°C
Fit Results (Kd)	
Fit Model:	Kd
Bound	903,4445704
Unbound	923,7399589
Kd	5,4834E-06
TargetConc	0,00000001 
Standard Deviation:	0,207569137
Kd Confidence:	±4,34262E-06