ChemComm

SUPPORTING INFORMATION

Facile immobilization of DNA using an enzymatic his-tag mimic

Pascal Röthlisberger,[a] Fabienne Levi-Acobas,[a] Ivo Sarac,[a] Bruno Baron,[b] Patrick England,[b] Philippe Marlière,[c] Piet Herdewijn[d] and Marcel Hollenstein*[a]

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Methods for immobilization of DNA on solid supports are in high demand. Herein, we present a generally applicable enzymatic method for the immobilization of DNA without any prior chemical derivatization. This strategy relies on the homopolymerization of the modified triphosphate dImTP by the TdT. The resulting enzymatic his-tag mimic ensures binding of DNA on Ni-NTA agarose. The usefulness of this method is highlighted by the immobilization of functional nucleic acids without impairing their specific activities.

TABLE OF CONTENTS

[a] *Laboratory for Bioorganic Chemistry of Nucleic Acids, Department of Structural Biology and Chemistry, Institut Pasteur, CNRS UMR3523, 28, rue du Docteur Roux, 75724 Paris Cedex 15, France. E-mail: marcel.hollenstein@pasteur.fr .*
[b] *Institut Pasteur, Plate-Forme de Biophysique des Macromolecules et de leurs Interactions, 28, rue du Docteur Roux, 75015 Paris, France.*
[c] *University of Paris Saclay, CNRS, iSSB, UEVE,Genopole, 5 Rue Henri Desbrueres, 91030 Evry, France*
[d] *KU Leuven, Rega Institute for Medical Research, Medicinal Chemistry Minderbroedersstraat 10, 3000 Leuven, Belgium*

ChemComm

SUPPORTING INFORMATION

1. Experimental Procedures

A) General Procedures:

The modified triphosphate **dImTP** was synthesized as reported previously[1] and the corresponding phosphoramidite **dIm** was synthesized by application of literature protocols.[2] DNA oligonucleotides without imidazole modifications were purchased from Microsynth. DNA oligonucleotides with imidazole modifications were synthesized on an H-8 DNA synthesizer from K&A on a 0.2 µmol scale. Natural DNA phosphoramidites (dT, dC4bz, dG2DMF, dA6Bz) and solid support (dA6Bz-lcaa-CPG 500Å) were all purchased from ChemGenes. Natural DNA phosphoramidites as well as the **dIm** phosphoramidite were prepared as 0.07 M solutions in MeCN and were coupled using 50 sec and 490 sec steps, respectively. 5-(ethylthio)-1*H*-tetrazole (0.25 M in MeCN) was used as coupling agent. Capping, oxidation, and detritylation were performed using standard conditions. Cleavage from the solid support and deprotection of oligonucleotides was achieved by treatment with concentrated ammonia at 55°C for 16 h. After centrifugation, the supernatants were collected and the resulting solutions were evaporated to dryness on a speed-vac. Crude oligonucleotides were purified by anion exchange HPLC (Dionex - DNAPac PA100). Buffer solutions of 25 mM Tris-HCl in H_2O, pH 8.0 (buffer A) and 25 mM Tris-HCl, 1.25 M NaCl in H_2O, pH 8.0 (buffer B) were used. The purified oligonucleotides were then desalted with SepPack C-18 cartridges. Oligonucleotide concentrations were quantitated by UV spectroscopy using a UV5Nano spectrophotometer (Mettler Toledo). The chemical integrity of oligonucleotides was assessed by UPLC-MS analysis: UPLC was performed on a BEH C18 column (130 Å, 1.7 µm, 2.1 mm x 50 mm) from Waters, installed on an ACQUITY UPLC H-Class System (SQ Detector 2). A Buffer containing 20 mM TEA and 400 mM HFIP in H_2O was used with a linear gradient from 18 to 31% Methanol within 5 minutes and a flow rate of 0.3 mL/min.
The terminal deoxynucleotidyl transferase was purchased from New England Biolabs. Ni-NTA Agarose was purchased from Macherey-Nagel and Ni-NTA Agarose magnetic particles were obtained from Yena Bioscience. Metal salts ($EuCl_3$, $CoCl_2$, $NiCl_2$), ABTS, H_2O_2, hemin, and sulforhodamine B were all purchased from Sigma Aldrich. Acrylamide/bisacrylamide (29:1, 40%) was obtained from Fisher Scientific. Visualization of PAGE gels was performed by fluorescence imaging using a Storm 860 phosphorimager with the ImageQuant software (both from GE Healthcare).

B) General Protocol for the TdT tailing reactions:

A solution containing 40 pmol of the appropriate single-stranded DNA primer and 10 U of TdT, was added to a mixture composed of of **dImTP** (200 µM final), 10x TdT reaction buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, pH 7.9), the adequate metal cofactor (0.25 mM Co^{2+} or 1 mM Mn^{2+}, final concentrations), and H_2O (for a final reaction volume of 10 µL). The reaction mixtures were then incubated at 37°C for various time periods. For gel analysis, the reactions mixtures were quenched by addition of 10 µL of loading buffer (formamide (70%), ethylenediaminetetraacetic acid (EDTA; 50 mM), bromophenol (0.1%), xylene cyanol (0.1%)). The reaction products were then resolved by electrophoresis (PAGE 20%) containing trisborate-EDTA (TBE) 1× buffer (pH 8) and urea (7 M). Visualization was performed by fluorescence imaging using a Storm 860 phosphorimager. For fixation on the agarose resin or magnetic particles, the polymerase was heat deactivated (20 min at 75°C) after the tailing reaction and the modified oligonucleotides bound to the solid support by application of the general protocol C.

C) General Protocol for the fixation of modified oligonucleotides on Eu-NTA Agarose:

200 µL of Ni-NTA Agarose were centrifuged and the flow-through was discarded. The agarose was washed with 10 bed volumes (1 mL) of H_2O. The Ni^{2+} ions were stripped off by washing the agarose with 10 bed volumes (1 mL) of EDTA 100 mM (pH 8.0). After a wash with 10 bed volumes (1 mL) of H_2O, the agarose was incubated with 10 bed volumes of an aqueous solution of $EuCl_3$ (100 mM) for 10 min at room temperature. After removal of the flow-through, the resin was washed with 10 bed volumes (1 mL) of H_2O. For an immediate use, the resin was equilibrated with 10 volumes of equilibration buffer (100 mM Tris-HCl, pH 8.0). The Eu^{3+}-NTA resins can also be stored in 30% EtOH and stored at 4°C. After equilibration, the resin was incubated and constantly mixed at 37°C for 60 min with the TdT tailing reaction (40 µL) and 360 µL of equilibration buffer. The resin was then washed twice with 10 bed volumes of equilibration buffer. Elution of the bound oligonucleotides was done by incubation of the resin with 10 bed volumes of EDTA 100 mM (pH 8.0) for Eu-NTA agarose resins and with 10 bed volumes of an imidazole buffer (250

mM imidazole, 150 mM NaCl, 100 mM Tris-HCl, pH 8.0). The eluted oligonucleotides were purified with NucleoSpin (Macherey-Nagel) clean-up kit. A similar protocol was applied using the Ni-NTA agarose magnetic particles (using 150 µL of the slurry) instead of the resin.

D) Fixation of DNAzyme and catalytic oxidation of ABTS:

After the TdT tailing reaction with primer **P2** (general protocol B), a total of 240 pmol of the modified oligonucleotide were immobilized on the Eu-NTA agarose magnetic particles by application of the general protocol C). The slurry was then incubated at room temperature for 60 min in the presence of 20 mM KCl, 25 mM Tris-HCl (pH 8.0), 100 mM ABTS, 1 mM hemin (dissolved in DMSO), 200 mM NaCl, and 0.05% Triton X-100 in a total volume of 17 µL. The reactions were initiated by addition of 3 µL of H_2O_2 (60 mM) and the color of the reaction mixtures was recorded by a digital camera, while the absorption intensity was monitored using a UV5Nano (Mettler Toledo) UV–Vis spectrophotometer at room temperature. The experiment was carried out in triplicate.

E) Capture of the anti-sulforhodamine B aptamer on solid support:

Method A:
After the TdT tailing reaction with primer **P3** (general protocol B), a total of 160 pmol of the modified oligonucleotide was evaporated to dryness on a speed-vac and incubated at 37°C for 30 min in 100 mM KCl and 100 mM Tris-HCl (pH 8.0). A large excess of sulforhodamine B (4 mg, 7.16 µmol) —to ensure that a large proportion of the aptamers are bound to the target (K_d = 660 nM)[3]— was then added to the slurry which was allowed to stir at 37°C for another 30 min. The aptamer-dye complex was then incubated with 50 µL of Eu-NTA agarose resin at 37°C for 60 min. The unbound sulforhodamine B dye was then washed off with multiple additions of 500 µL of KCl (10 mM) until disappearance of the color. The color of the immobilized aptamer-target complex was recorded with a digital camera. The aptamer-target complex was eluted from the resin with EDTA (see general protocol C). The color of the eluted dye was recorded by a digital camera, while the absorption intensity was monitored using a UV5Nano (Mettler Toledo) UV–Vis spectrophotometer at room temperature. The experiment was carried out in triplicate.

Method B:
After the TdT tailing reaction with primer **P3** (general protocol B), a total of 160 pmol of the modified oligonucleotide was immobilized on Eu-NTA agarose magnetic particles (50 µL) by application of the general protocol C). The bound aptamer was then incubated in 70 µL of incubation buffer (100 mM KCl, 100 mM Tris-HCl, pH 8.0) for 30 min at 37°C. A large excess of sulforhodamine B (12 mg, 21.5 µmol) was then added to the immobilized aptamer and incubated at 37°C for 30 min to ensure complete formation of the aptamer-dye complex. The unbound sulforhodamine B dye was then washed off with multiple additions of 500 µL of KCl (10 mM) until disappearance of the color. The color of the immobilized aptamer-target complex was recorded with a digital camera. The aptamer-target complex was eluted from the resin with EDTA (see general protocol C). The color of the eluted dye was recorded by a digital camera, while the absorption intensity was monitored using a UV5 Nano (Mettler Toledo) UV–vis spectrophotometer at room temperature.

F) Biophysical analysis of binding:

Microscale Thermophoresis:
A 1 µM solution of the fluorescein labelled oligonucleotide **S2** was prepared in buffer (100 mM Tris-HCl pH 8.2, 150 mM NaCl). 10 µl (10 pmol) of this solution were incubated for 5 min. with 10 µL of the Ni-NTA magnetic agarose beads that were diluted prior to use in the concentration range of 2 mM down to 4.57 nM (i.e. 16 times a 2/1 dilution of a 2 mM stock solution). The resulting suspension was thoroughly stirred and then transferred into standard MST capillaries. The MST measurements were performed at 25°C with LED power of 80% and MST power of 20 % on a Monolith NT.115 blue/red Microscale Thermophoresis instrument from Nanotemper technologies. 5 independent repeats of this experiment were carried out.
Similar experiments were performed using primer **P1** devoid of the **dIm**-tag as a negative control.

2. Additional Figures

Additional gel images, ESI-MS spectra, fluorescence quenching experiments as well as tables summarizing the different oligonucleotides used in this study along with their characterization are reported in the following section:

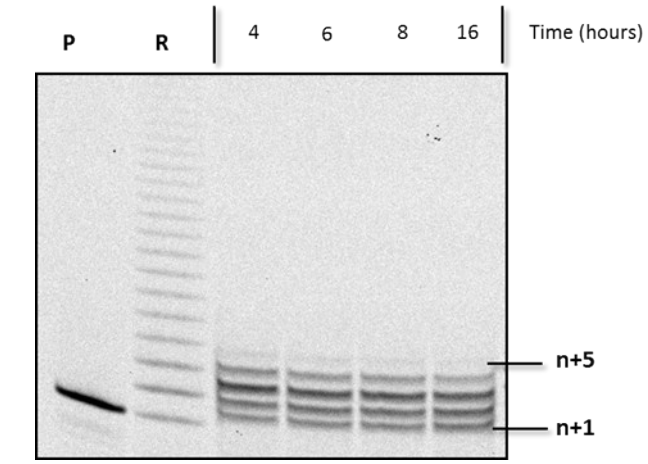


Fig. S1 Gel analysis (PAGE 20%) of the TdT catalyzed tailing reactions with **dImTP** with primer **P1** and Co^{2+} as a cofactor. Lane **P**: unreacted oligonucleotide **P1**; lane **R**: control reaction with natural dTTP (100 μM, 1h reaction time).

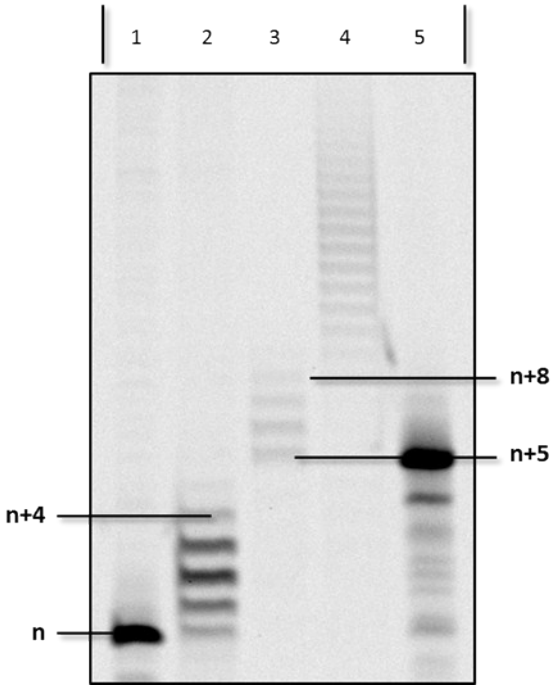


Fig. S2 Gel analysis (PAGE 20%) of the TdT catalyzed tailing reactions with **dImTP**. Lane 1: unreacted primer **P1**; lane 2: 8h reaction with primer **P1** and Co^{2+} cofactor; lane 3: reaction with oligonucleotide **S1** and Co^{2+} cofactor; lane 4: 8h reaction with primer **P1** and Mn^{2+} cofactor; lane 5: unreacted oligonucleotide **S1**.

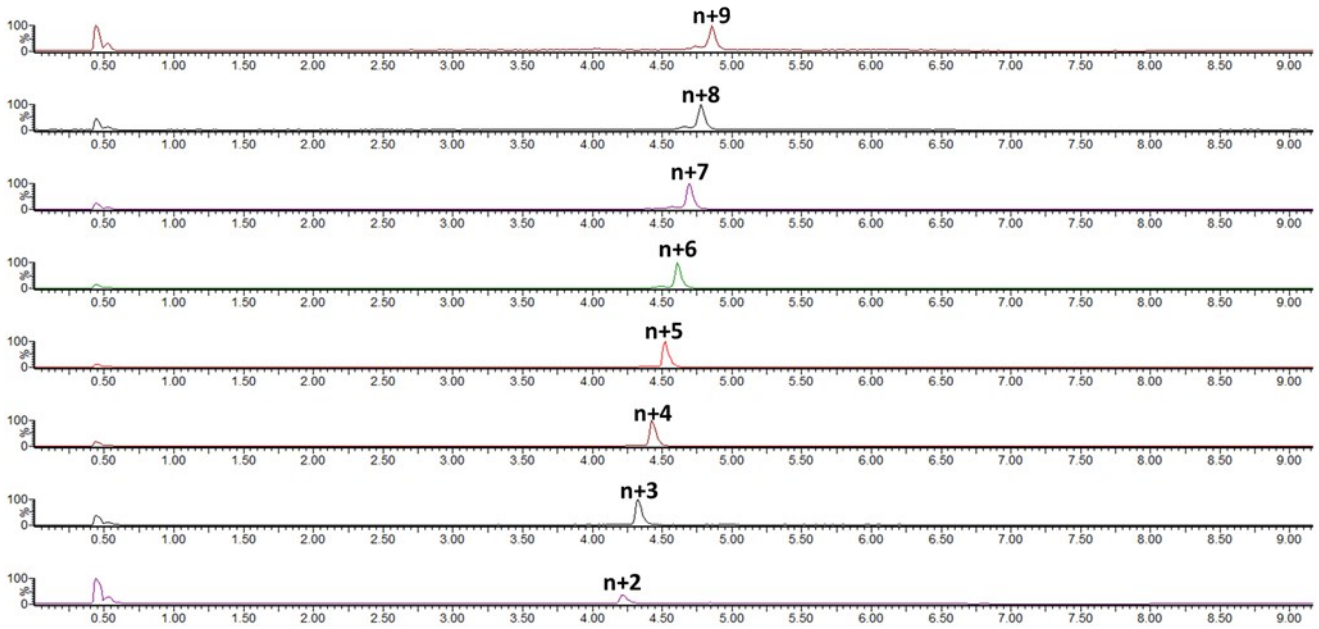


Fig. S3 UPLC-MS analysis of the TdT tailing reaction with **dImTP**: detail of the selected ion monitoring recorded at the charge state -4.

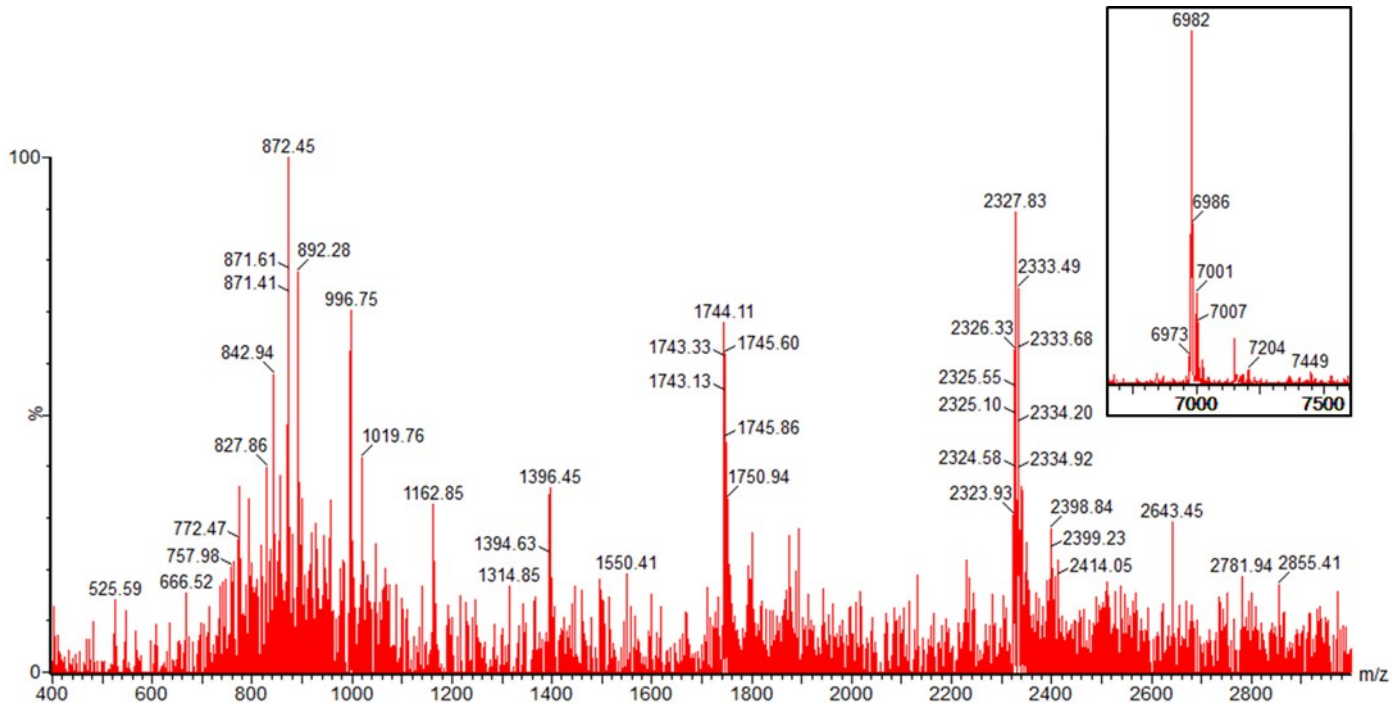


Fig. S4 ESI-MS spectrum corresponding to the n+3 product. The insert shows the deconvoluted mass spectrum (calcd: 6984; found: 6982).

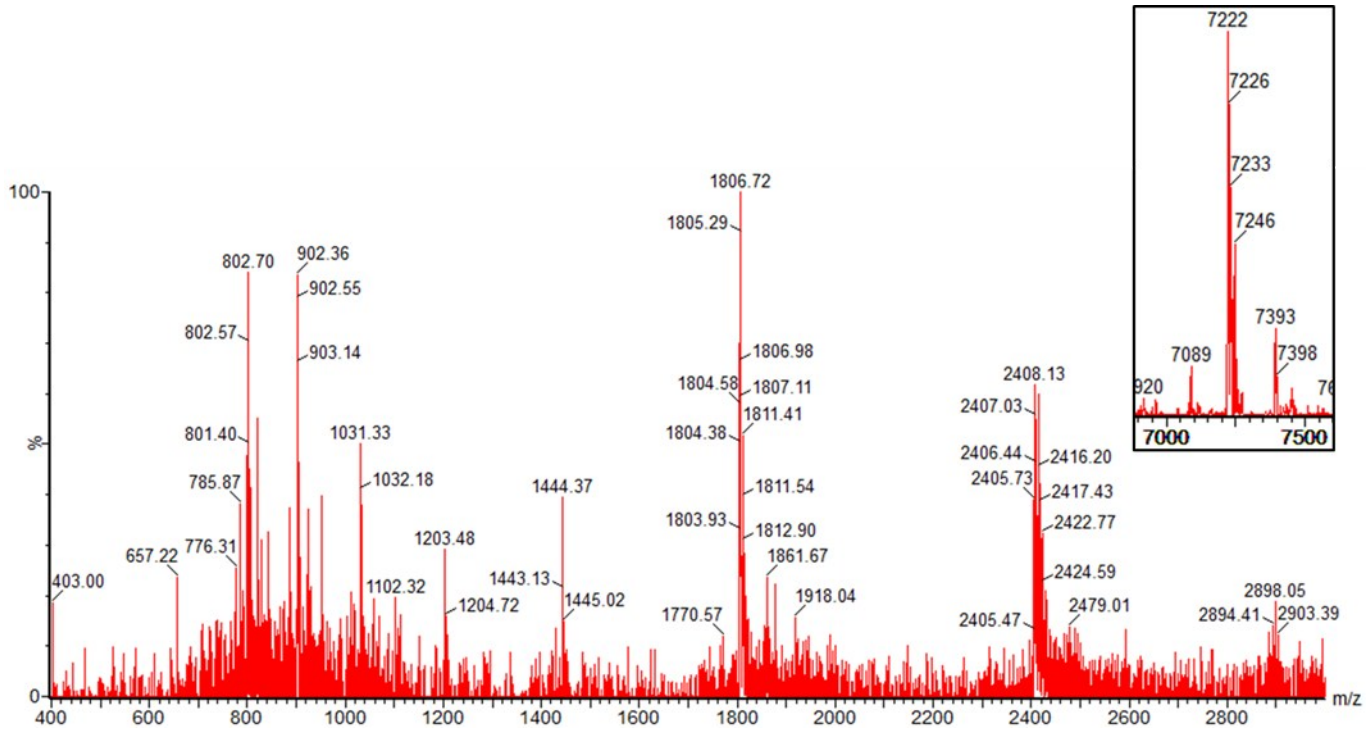


Fig. S5 ESI-MS spectrum corresponding to the n+4 product. The insert shows the deconvoluted mass spectrum (calcd: 7230; found: 7222).

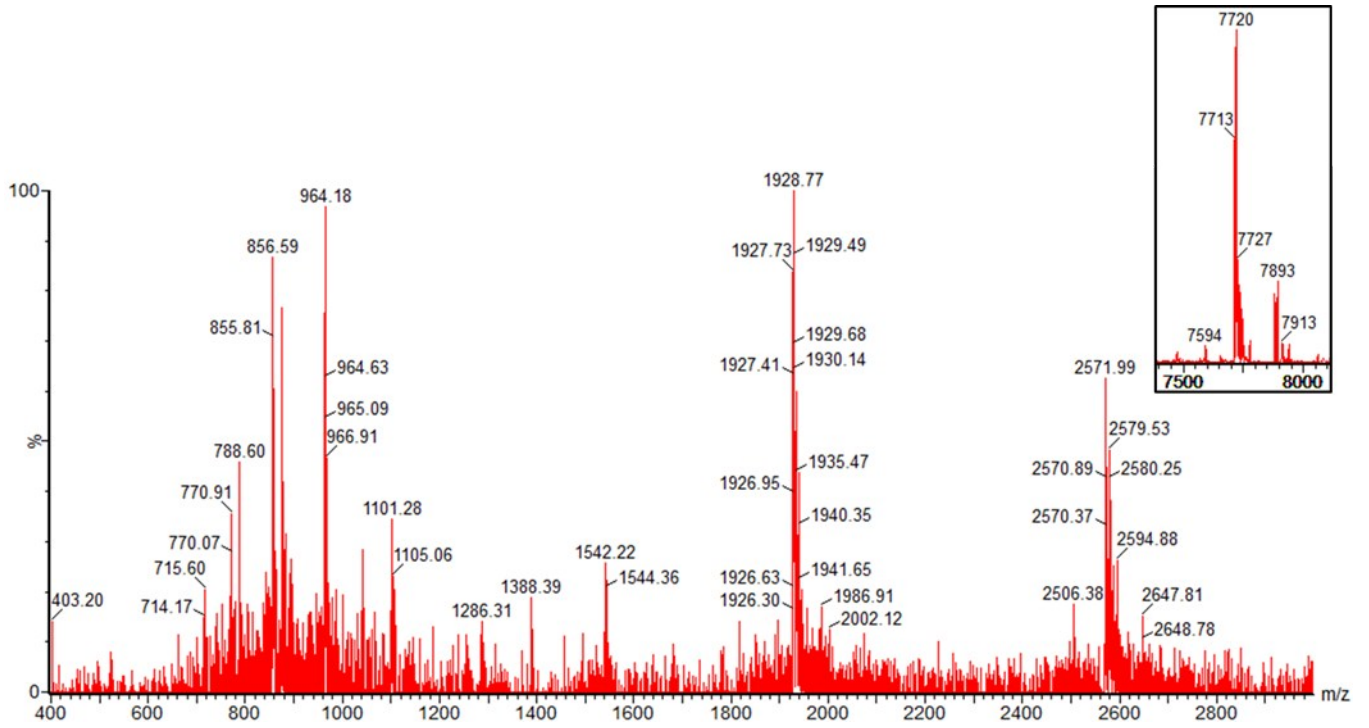


Fig. S6 ESI-MS spectrum corresponding to the n+6 product. The insert shows the deconvoluted mass spectrum (calcd: 7722; found: 7720).

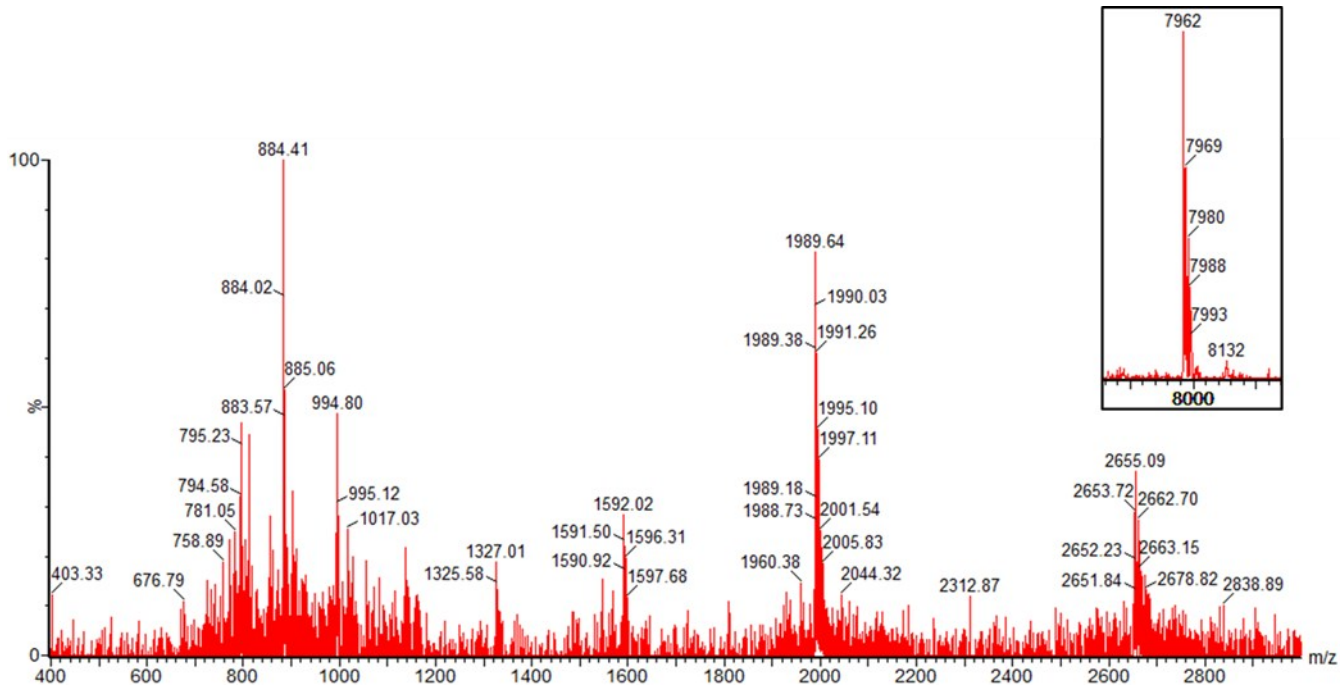


Fig. S7 ESI-MS spectrum corresponding to the n+7 product. The insert shows the deconvoluted mass spectrum (calcd: 7968; found: 7962).

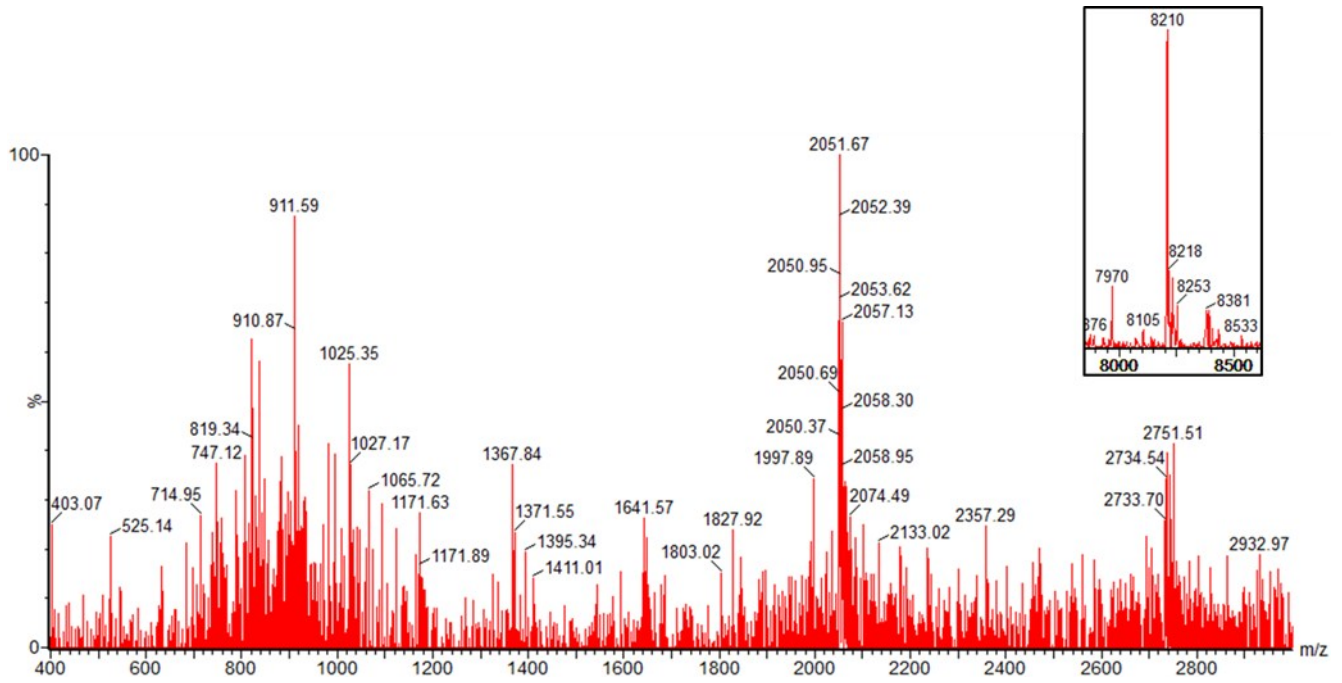


Fig. S8 ESI-MS spectrum corresponding to the n+8 product. The insert shows the deconvoluted mass spectrum (calcd: 8214; found: 8210).

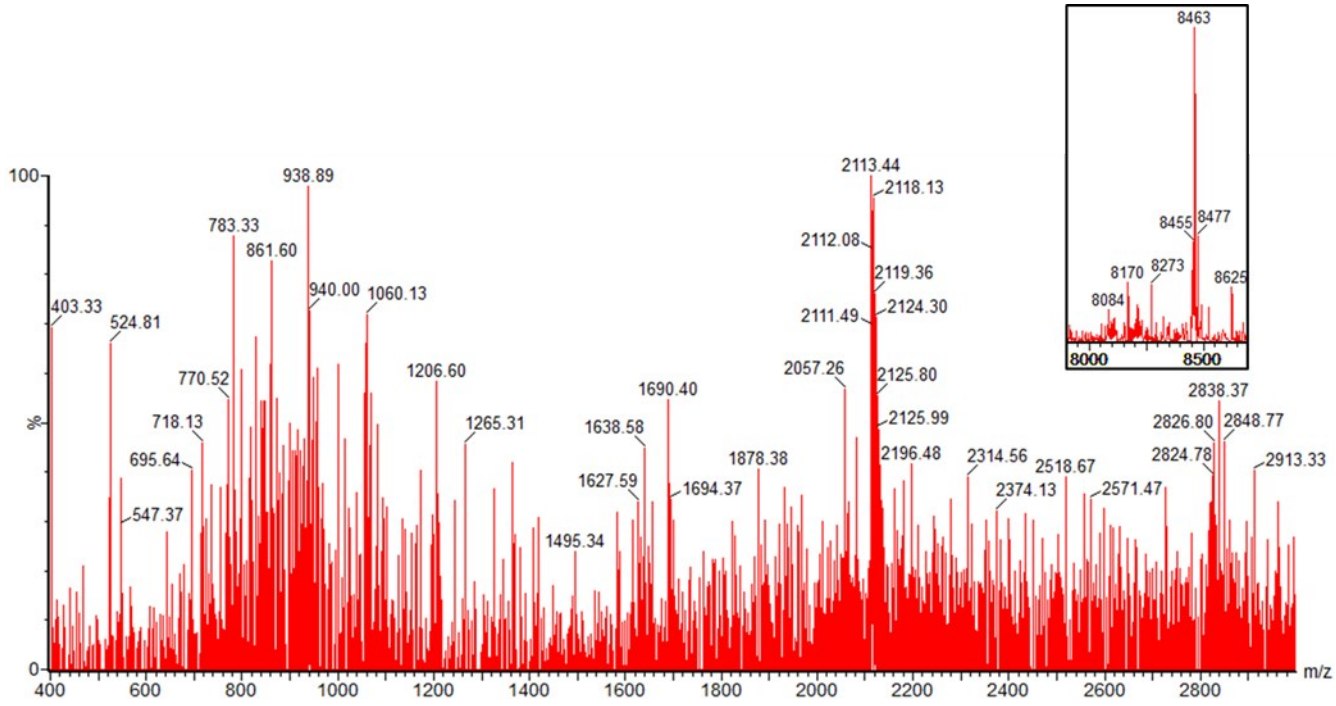


Fig. S9 ESI-MS spectrum corresponding to the n+9 product. The insert shows the deconvoluted mass spectrum (: calcd: 8461; found: 8463).

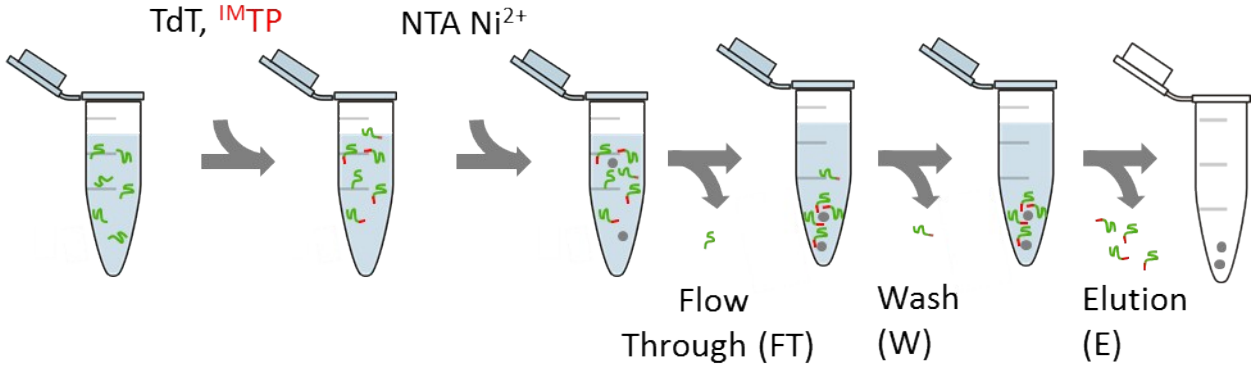


Fig. S10 Schematic representation of all the steps required in the enzymatic method for the immobilization of oligonucleotides on a solid support.

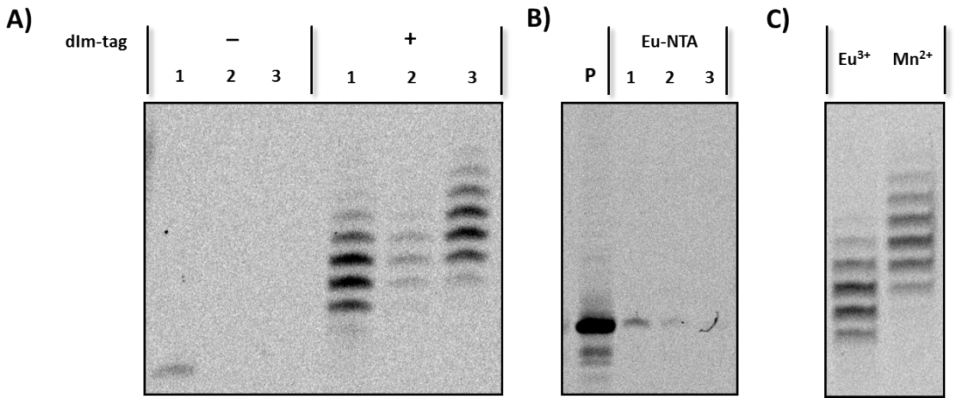
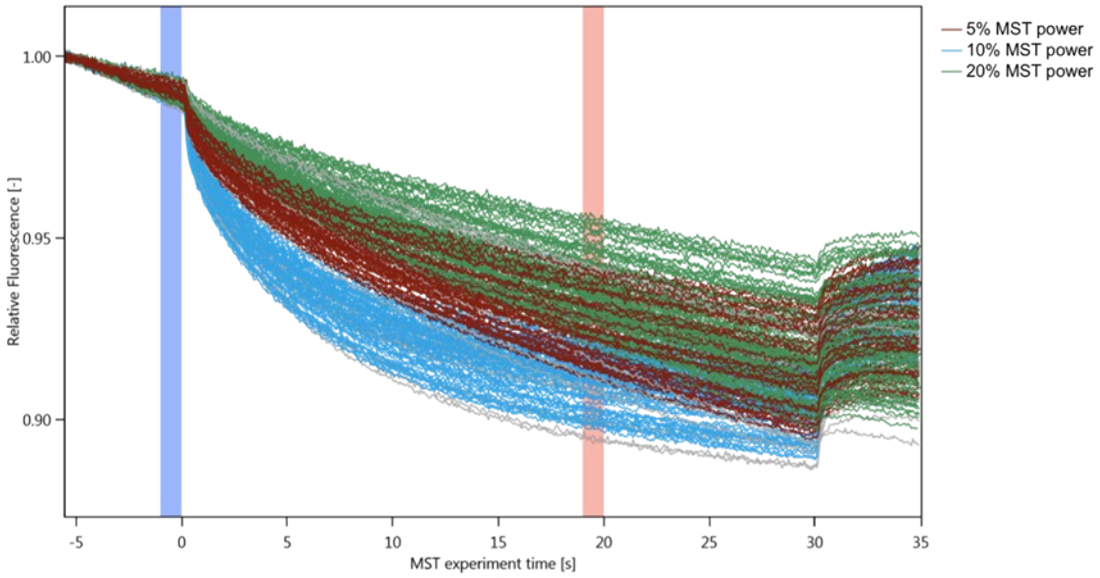


Fig. S11 Gel analysis (PAGE 20%) of the presence and absence of a **dIm**-tag as well as reaction time on the immobilization efficiency. A) influence of the presence (right-hand side) and absence (left-hand side) of the **dIm**-tag on the immobilization of primer **P1**. The **dIm**-tag was created by an 8-hour long incubation of primer **P1** with TdT and **dImTP** (200 µM). B) Immobilization of unmodified primer **P1** on Eu-NTA agarose. lanes 1: flow through after binding; lanes 2: washes after binding; lanes 3: elution of the modified oligonucleotides from the solid support with 250 mM imidazole (100 mM EDTA for Eu-NTA agarose); lane P: primer **P1**. C) Effect of the cofactor (Eu^{3+} or Mn^{2+}) on the efficiency of the TdT-mediated tailing reaction with **dImTP** and primer **P1** (reactions were incubated at 37°C for 5h).

A)



B)

Name:	RPON41 500um 5	RPON41 500um 5	RPON41 500um 5
Graph Color:	●	●	●
Target Name:	Target	Target	Target
Target Concentration:	500	500	500
Ligand Name:	Ligand	Ligand	Ligand
Ligand Concentration:	2E+06 to 4.57E+03	2E+06 to 4.57E+03	2E+06 to 4.57E+03
n:	5	5	5
Comments:			
Excitation Power:	80%	80%	80%
MST Power:	5%	10%	20%
Temperature:	25.0°C	25.0°C	25.0°C
Kd:	3.2055E+05	1.6673E+05	38581
Kd Confidence:	± 63151	± 22964	± 7274.4
Response Amplitude:	23.335445	20.420208	15.991346
TargetConc:	500[Fixed]	500[Fixed]	500[Fixed]
Unbound:	937.28	926.99	909.74
Bound:	960.62	947.41	925.73
Std. Error of Regression:	1.1226967	0.78020061	0.79357776
Reduced χ^2:	0.45959263	0.10696696	0.32609111
Signal to Noise:	22.326857	28.114336	21.645592

Fig. S12 A) Raw MST data corresponding to the binding of fluorescein-labeled oligonucleotide **S2** on Ni-NTA agarose magnetic beads. The highlighted areas in blue and red represent the fluorescence readout zones. B) Summary of the MST data analysis.

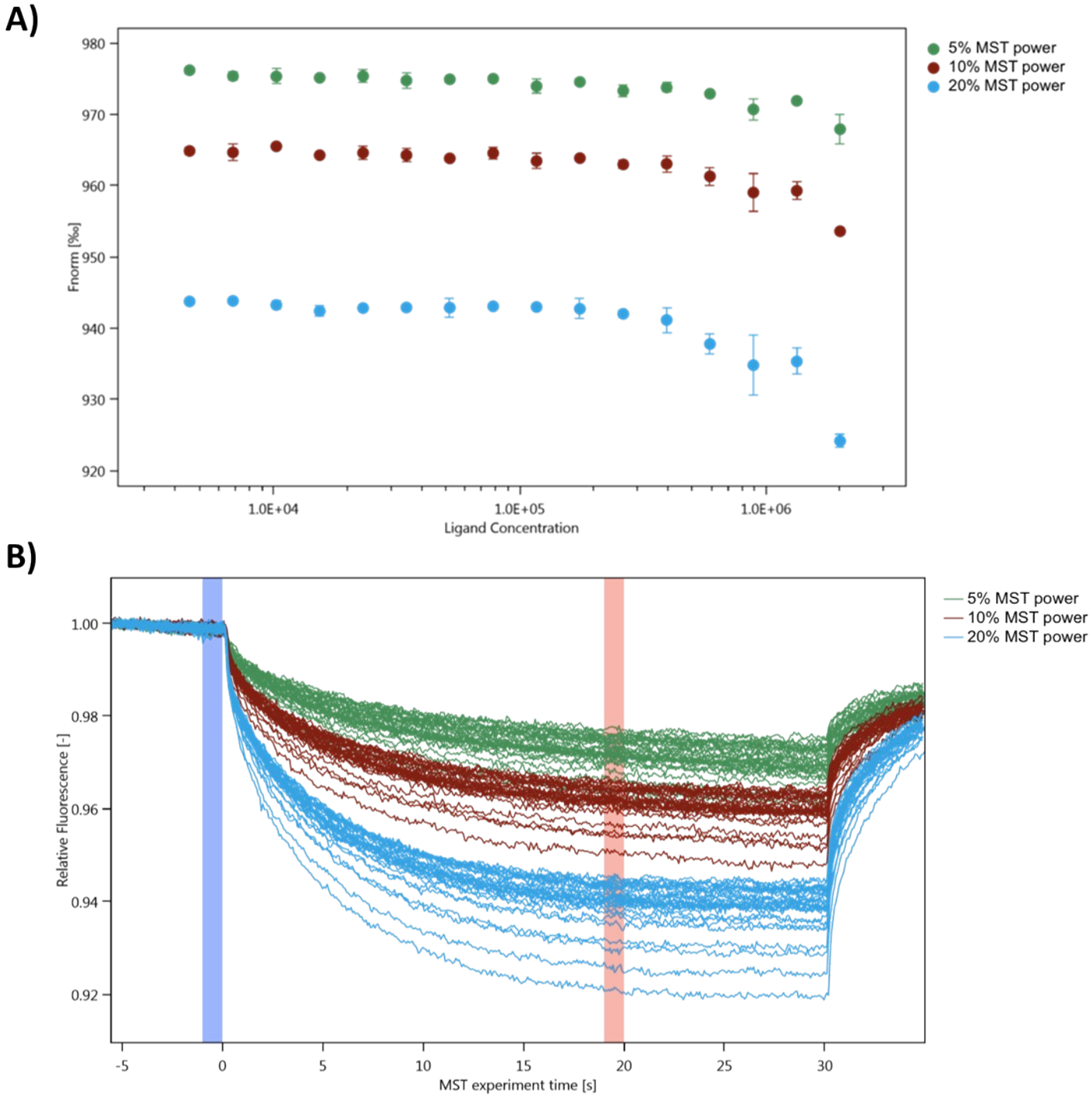


Fig. S13 Summary of MST data obtained for the interaction of primer **P1** lacking dIm and Ni-NTA agarose magnetic particles. A) Bead concentration-dependence saturation curves showing that unlabeled primer **P1** did not bind on Ni-NTA agarose magnetic particles B) Raw MST data.

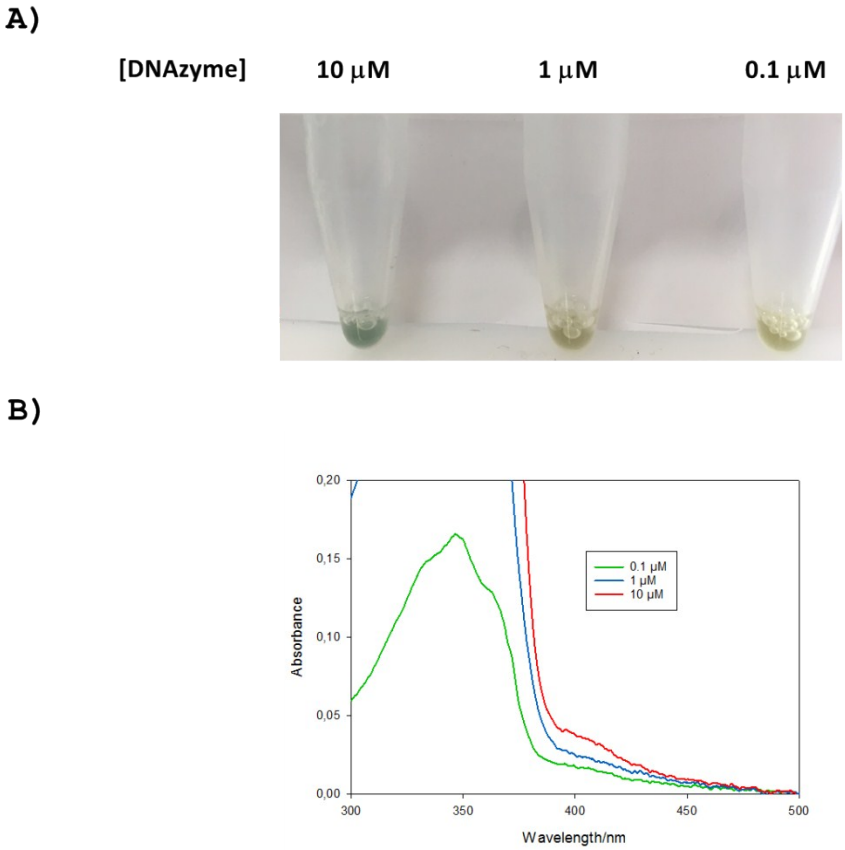


Fig. S14 Reaction of soluble, non-immobilized DNAzyme **P2** at three different concentrations (10 µM, 1 µM, and 0.1 µM). A) Photographs of the color change. B) UV/Vis absorption spectra for analysis of the formation of the oxidation product ($ABTS^{\bullet+}$). Absorption at 420 nm of the reaction with the immobilized DNAzyme was 0.1205 (Figure 4C).

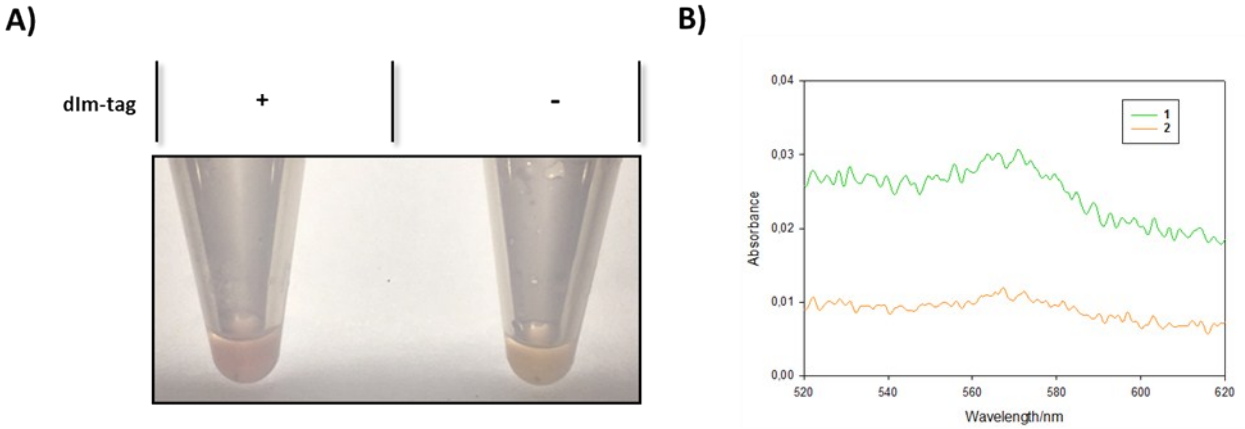
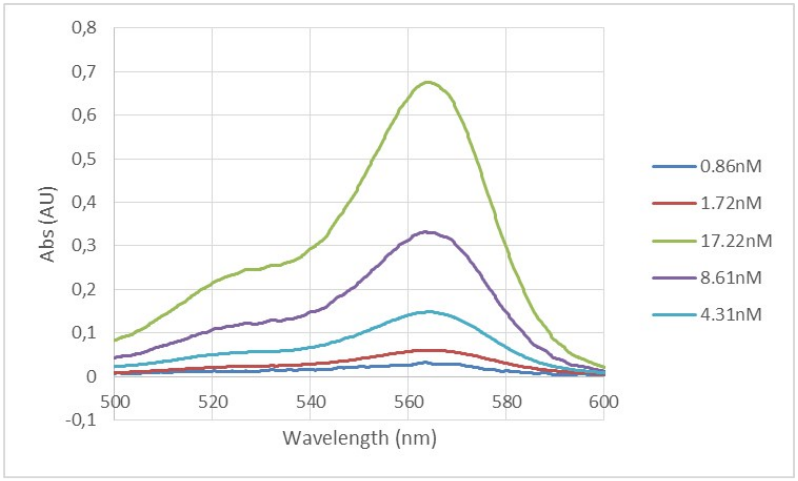


Fig. S15 Analysis of immobilization of primer **P3** by immobilizing the **dIm**-modified oligonucleotide first and capturing the sulforhodamine B dye in a second step. A) Photographs of samples prior elution from the solid support in the presence and absence of the **dIm**-tag; B) UV/Vis absorption spectra of eluted sulforhodamine B on sample with (**1**) and without (**2**) the **dIm**-tag.

A)



B)

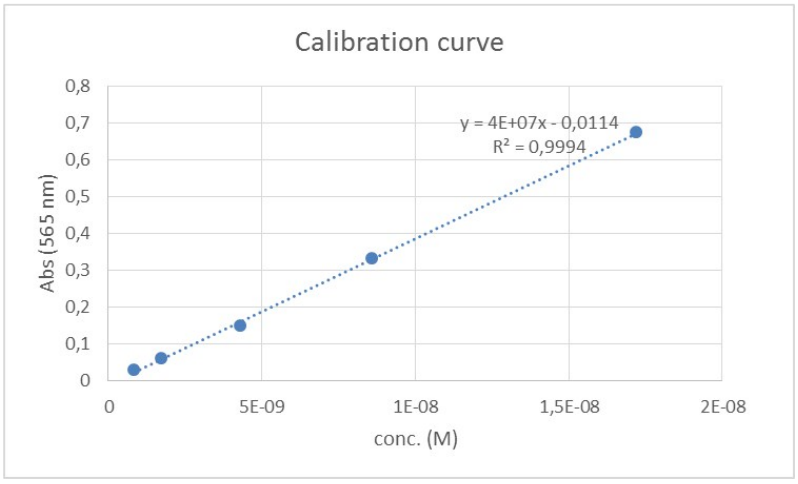


Fig. S16 Calibration curve of sulforhodamine B: A sulforhodamine B solution of 1 µM in H_2O was used to get solution with a concentration from 17.2 nM to 0.86 nM in order to establish a calibration curve. For the calibration curve the absorptions at 565nm were plotted against the concentration in order to get a linear regression to determine the concentration of sulforhodamine bound by the aptamer. The absorbance measured with the immobilized aptamer-target complex (Figure 5C of the manuscript) was 0,04796, hence a concentration of 1.5 nM.

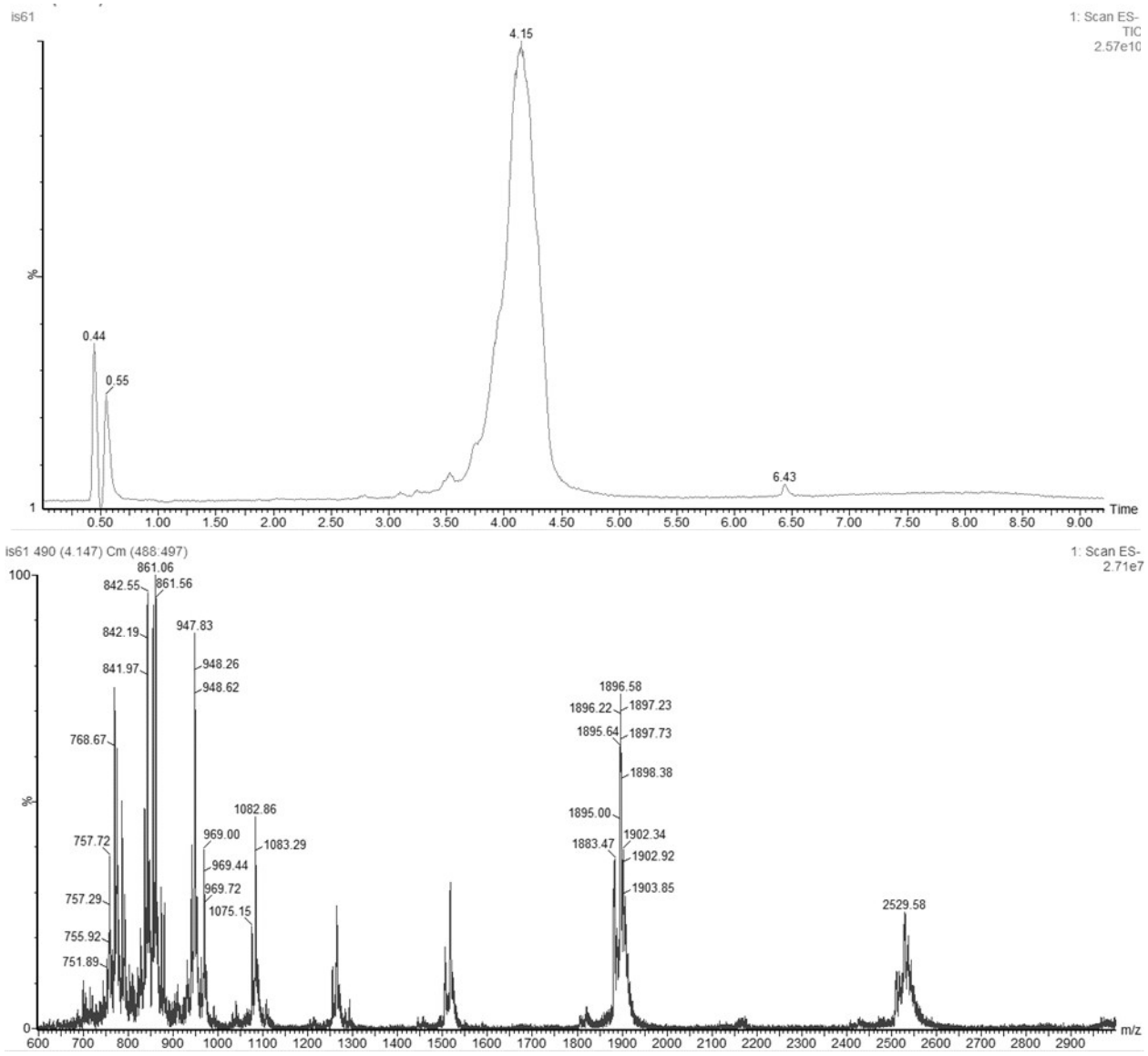
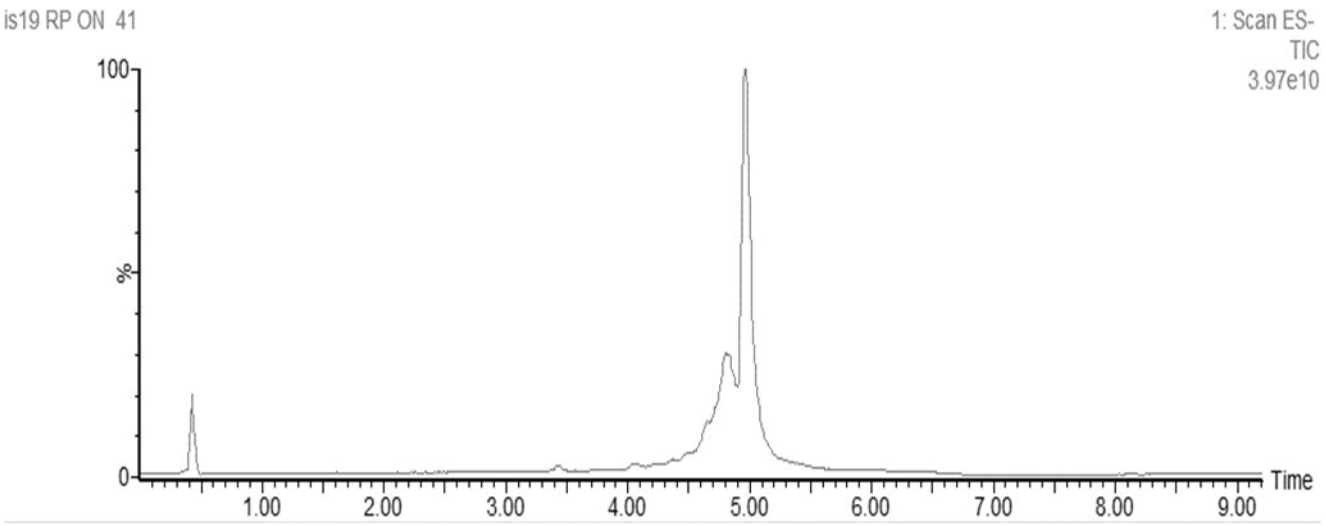


Fig. S17 UPLC-MS analysis of oligonucleotide **S1**.



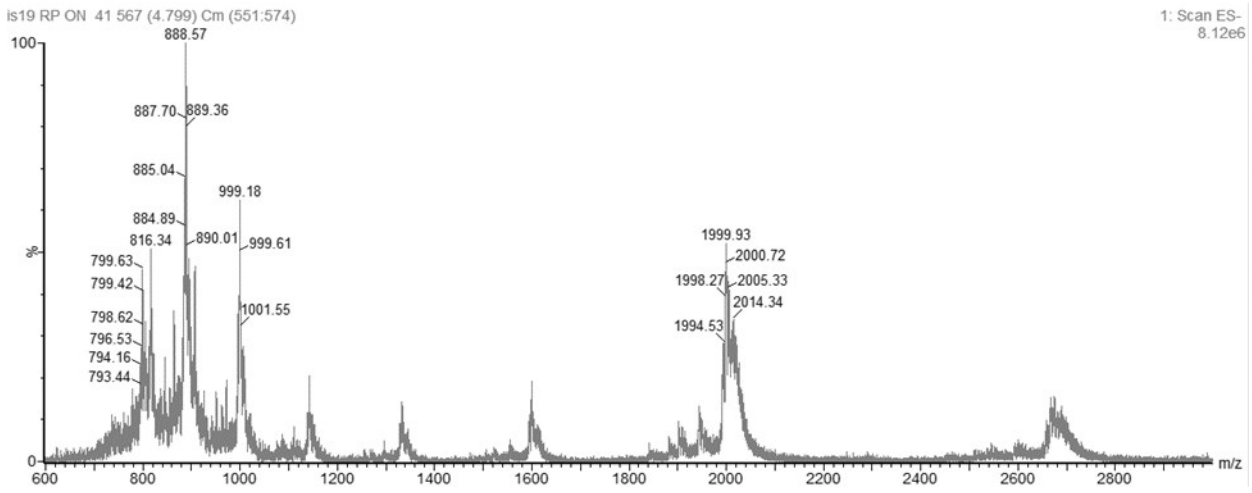
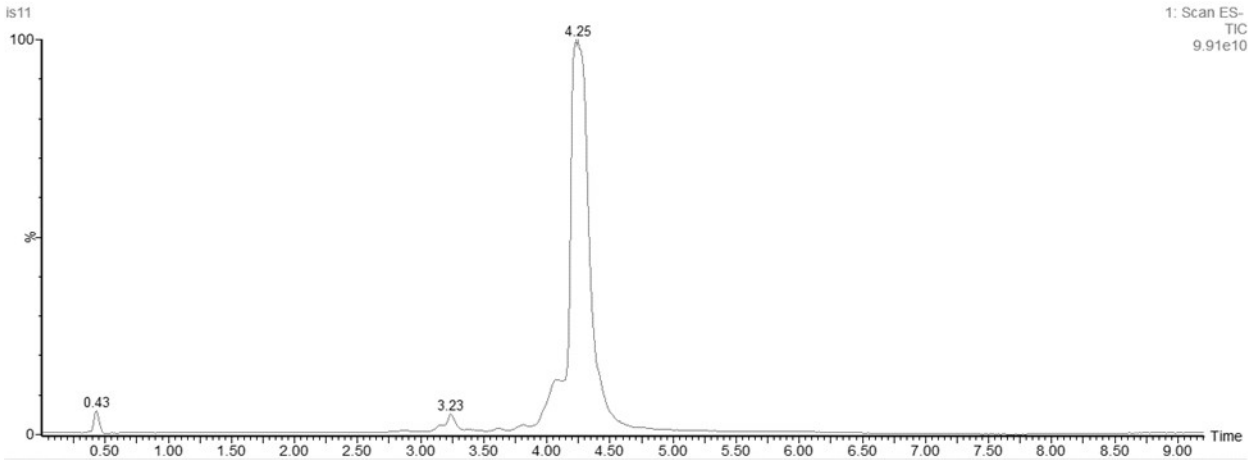


Fig. S18 UPLC-MS analysis of oligonucleotide **S2**.



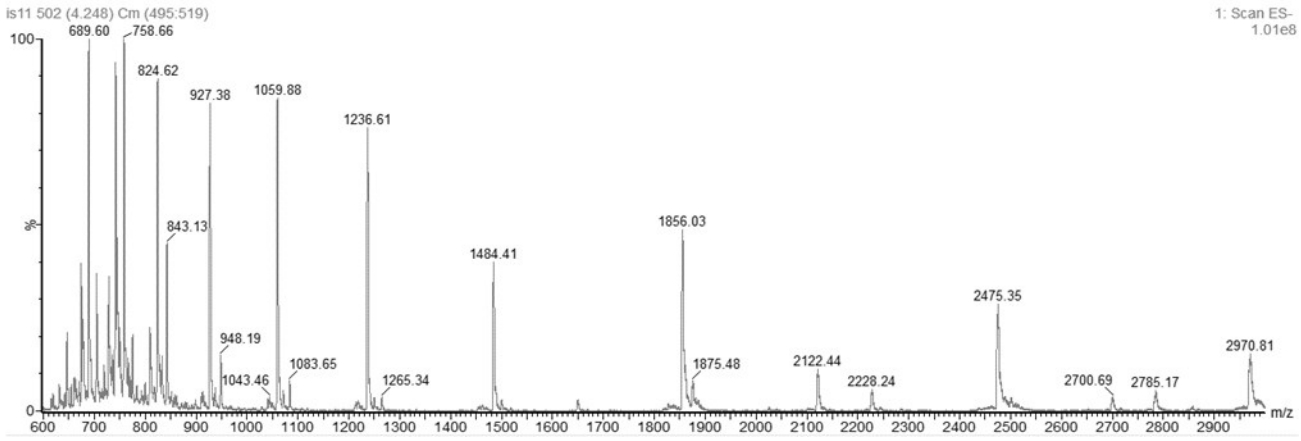


Fig. S19 UPLC-MS analysis of oligonucleotide **S3**.

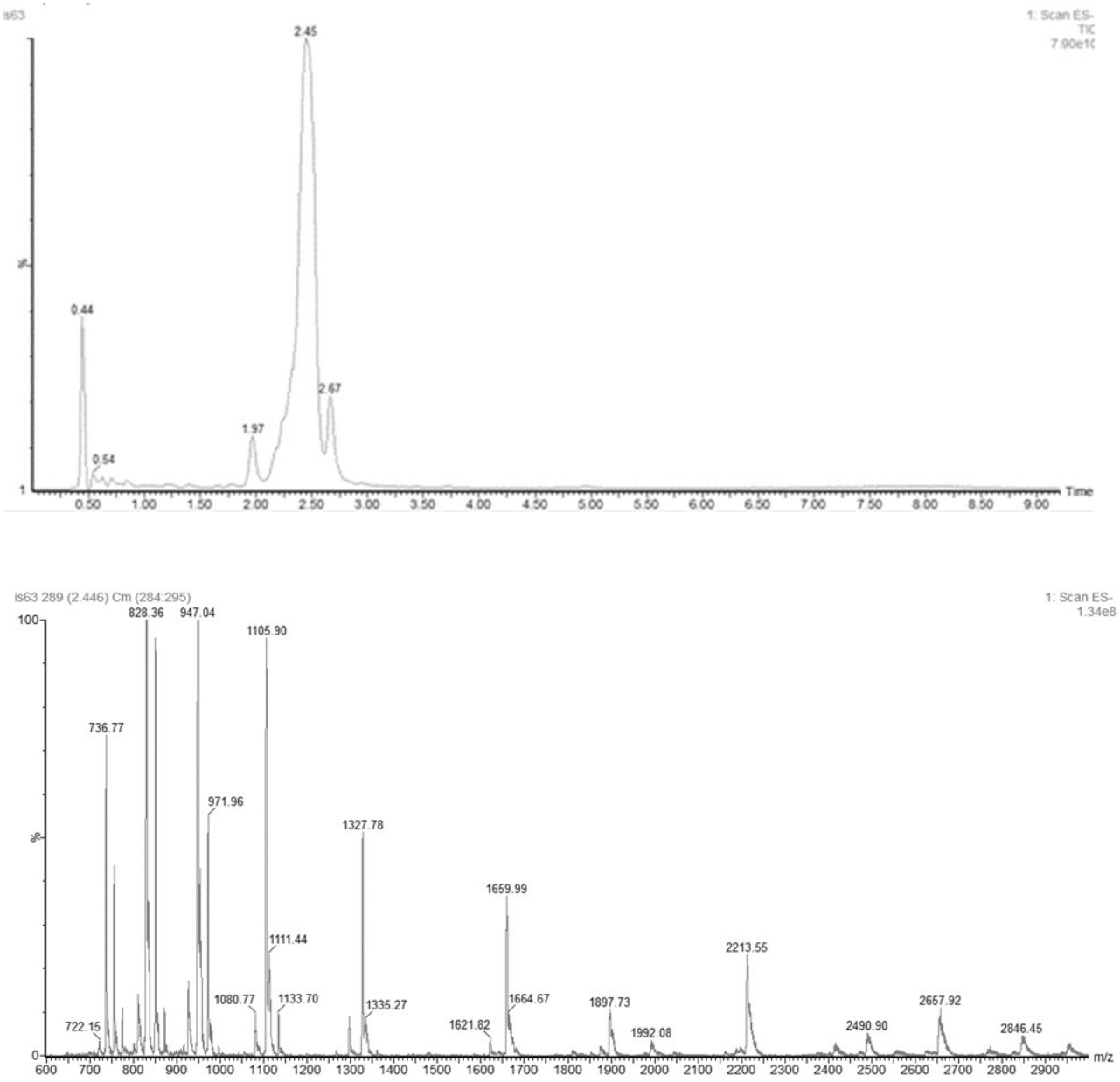


Fig. S20 UPLC-MS analysis of oligonucleotide **P2**.

3. Additional tables:

Table S1 DNA oligonucleotides templates for the TdT tailing reactions (shown 5' to 3').

Oligonucleotide	Sequence	*m/z* (calcd.)	*m/z* (found)
P1	FAM-TAC GAC TCA CTA TAG CCT C	n.d.[a]	n.d.[a]
P2	TTG TGG GTA GGG CGG GTT GGG	6645.31	6643.65
P3	CCG GCC AAG GGT GGG AGG GAG GGG GCC GG	9155.92	9154.08
S1	FAM-TAC GAC TCA CTA TAG CCT C**lmlm lmlmlm**	7594.32[b]	7590.32[b]
S2	FAM-TAC GAC TCA CTA TAG CCT C**lmlm lmlmlm lmlm**	8013.38	8006.72
S3	TAC GAC TCA CTA TAG CCT C**lmlm lmlmlm lmlm**	7429.28	7428.12

[a] n.d. = not determined since the oligonucleotide was purchased in a purified form from Microsynth; [b] corresponds to the iso-butyryl-protected fluorescein.

Table S2 Summary of the UPLC-MS analysis of the TdT-tailing reaction

z	n+3		n+4		n+5		n+6		n+7		n+8		n+9	
	Calcd.	**Found**	**Calcd.**	**Found**	**Calcd.**	**Found**	**Calcd.**	**Found**	**Calcd.**	**Found**	**Calcd.**	**Found**	**Calcd.**	**Found**
***m/z*[b]**	**6984**	**6982**	**7230**	**7222**	**7476**	**7476**	**7722**	**7720**	**7968**	**7962**	**8214**	**8210**	**8462**	**8463**
-3	2327.09	2327.09	2409.10	2408.13	2491.11	2489.02	2573.13	2571.99	2655.14	2655.09	2737.15	2734.54	2819.50	2824.78
-4	1745.06	1744.11	1806.57	1806.72	1868.08	1867.00	1929.59	1928.77	1991.10	1989.64	2052.61	2051.67	2114.38	2113.44
-5	1395.85	1396.45	1445.06	1444.37	1494.27	1492.74	1543.48	1542.22	1592.68	1592.02	1641.89	1641.57	1691.30	1690.40
-6	1163.04	1162.85	1204.05	1203.48	1245.06	1244.31	1286.06	1286.31	1327.07	1327.01	1368.08	1367.84	1409.25	1408.35
-7	996.75	996.75	1031.90	1031.33	1067.05	1066.11	1102.20	1101.28	1137.35	1137.82	1172.49	1171.63	1207.79	1206.60
-8	872.03	872.45	902.79	902.36	933.54	933.43	964.30	964.18	995.05	994.80	1025.81	1027.17	1056.69	1060.13
-9	775.03	774.75	802.37	802.70	829.70	829.16	857.04	856.59	884.38	884.41	911.72	911.59	939.17	938.89
-10	697.43	n.d.[c]	722.03	n.d.[c]	746.63	n.d.[c]	771.24	n.d.[c]	795.84	795.23	820.45	819.34	845.15	n.d.[c]

[a] the n, n+1, and n+2 products were present in too low amounts to be characterized by ESI-MS and the n+10 could only partially be characterized (deconvoluted spectrum: *m/z* (calcd.): 8708, *m/z* (found): 8708; 3- charge state: *m/z* (calcd.): 2901.51, *m/z* (found): 2906.77; 4- charge state: *m/z* (calcd.): 2175.89, *m/z* (found): 2175.61). [b] deconvoluted spectra. [c] not detectable.

Table S3 Gel quantification of the products formed during the TdT-tailing reaction (4h reaction time).

	n+2	n+3	n+4	n+5	n+6	n+7	n+8	n+9	n+10
Fraction	0.2%	5.4%	15.0%	27.8%	24.4%	15.2%	7.5%	3.3%	1.2%

4. References

1. P. Röthlisberger, F. Levi-Acobas, I. Sarac, P. Marlière, P. Herdewijn and M. Hollenstein, *Org. Biomol. Chem.*, 2017, **15**, 4449-4455.
2. S. Johannsen, N. Megger, D. Bohme, R. K. O. Sigel and J. Muller, *Nat. Chem.*, 2010, **2**, 229-234.
3. X. R. Zhang, D. Chabot, Y. Sultan, C. Monreal and M. C. DeRosa, *ACS Appl. Mater. Interfaces*, 2013, **5**, 5500-5507.

