

## Electronic Supporting Information

### Efficient photoactivation of a Diels-Alderase ribozyme

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#### List of Abbreviations:

1-AB = anthracen-1-yl-BODIPY, BODIPY = 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene, CEP-Cl = 2-cyanoethyl diisopropylphosphoramidochloridite, DAse = Diels-Alderase ribozyme, DEAD = diethyl azodicarboxylate, DMAP = *N,N*-dimethylpyridin-4-amine, DMF = dimethylformamide, DMT-Cl = 4,4'-dimethoxytrityl chloride, EtOH = ethanol, FRET = Förster resonance energy transfer, MeCN = acetonitrile, NPE = (*S*)-1-(2-nitrophenyl)ethyl, NPM = *N*-pentylmaleimide, *R*-NPE = *R*-(1-(2-nitrophenyl)ethanol, *S*-NPE = *S*-(1-(2-nitrophenyl)ethanol, TEAA = triethylammonium acetate, THF = tetrahydrofurane, TOM = [(triisopropylsilyl)oxy]methyl, TOM-Cl = [(triisopropylsilyl)oxy]methyl chloride, *wt* = wild-type.

## 1. Materials and general procedures

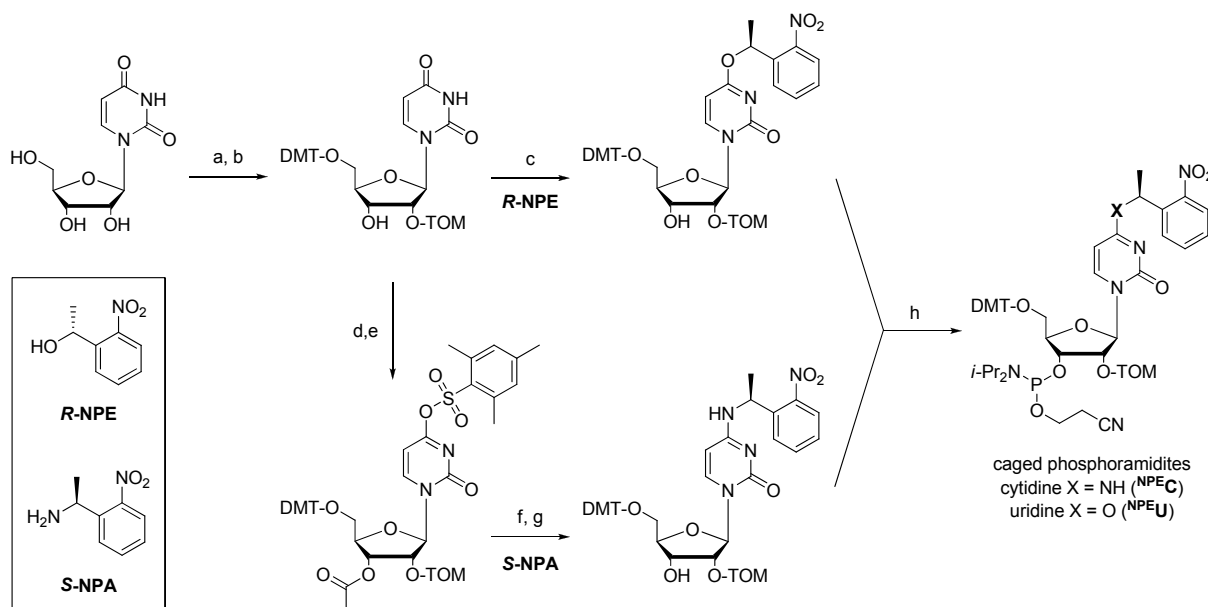
Chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany) or Acros Organics (Geel, Belgium) and used without further purification. All chemical reactions were carried out under argon atmosphere with dry solvents (stored over molecular sieves). NMR spectra were recorded on a Varian 300 MHz NMR Mercury Plus instrument ( $^1\text{H}$ : 300 MHz,  $^{31}\text{P}$ : 202 MHz), and referenced using the residual proton signal of the deuterated solvent.  $\text{CDCl}_3$  was stored over dry  $\text{K}_2\text{CO}_3$ . The following abbreviations are used to explain the multiplicities in  $^1\text{H}$ : s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet. The chemical shifts are reported in parts per million ( $\delta$ ) and the coupling constant values  $J$  are given in Hz.

TBE buffer (10x, 1.0 M Tris-HCl/borate buffer, pH 8.3, 20 mM EDTA) was purchased from Carl Roth (Karlsruhe, Germany). Standard DAse buffers (1x: 300 mM NaCl, 30 mM Tris-HCl, pH 7.4) with 0, 20 and 80 mM  $\text{MgCl}_2$  were prepared as 5x stock solutions and diluted as required. RNA concentrations were measured in 2.0  $\mu\text{l}$  sample volumes with a NanoDrop ND-1000 spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany) using the theoretical extinction coefficient of the respective sequence.<sup>1</sup>

Oligonucleotide fragments of the full-length constructs (49mers), carrying the modifications Cy3, Cy5, biotin and/or rIsoC, as well as the 49mer splint DNA were obtained from IBA GmbH (Göttingen, Germany) as 100  $\mu\text{M}$  stock-solutions and were used as received: 5'-CGA GGU CGU GCC A-3' (13mer), 5'-CAA UAC UCG AC-biotin-3' (11mer), 5'-Cy3 -GGA GCU CGC UUC GGC GAG GUC GUG CCA-3' (27mer), and 5'-GCU CU(dU<sup>Cy5</sup>) CGG AGC AA(rIsoC) ACU CGA C-biotin-3' (22mer), 5'-Fluorescein-GTC GAG TAT TGC TCC GAA GAG CTG GCA CGA CCT CGC CGA AGC GAG CTC C-3' (49mer DNA splint). Oligonucleotides containing the photo-cleavable caging group were self-made by implementing the phosphoramidite of cytidine (<sup>NPE</sup>C) or uridine (<sup>NPE</sup>U) in the solid-phase RNA synthesis protocol: 5'-CGA GGU CGU G(<sup>NPE</sup>C)C A-3' (13mer), 5'-CAA (<sup>NPE</sup>U)AC UCG AC-biotin-3' (11mer). A biotin-loaded resin (IBA GmbH) was used for the 3'-derivatized sequences. All Enzymes were obtained from Fermentas GmbH (St. Leon-Rot, Germany). The preparative purification of self-made RNA was performed by reversed phase HPLC (Agilent 1100 Series system) on a Phenomenex Luna<sup>®</sup> C18 column (5  $\mu\text{m}$ , 250 x 15 mm at 6.0 ml/minute). Appropriate gradients of buffers A (0.1 M TEAA in  $\text{H}_2\text{O}$ , pH 7.4) and B (0.1 M TEAA in MeCN/ $\text{H}_2\text{O}$ , 5:1, pH 7.4) were used for separation. Prior to injection, samples were filtered through a 0.22  $\mu\text{m}$  teflon syringe filter.

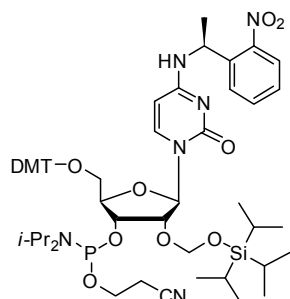
## 2. Synthesis of NPE derivatized phosphoramidites

The phosphoramidite synthesis was conducted according to Höbartner *et al.*,<sup>2</sup> starting from Uridine (Figure S1). TOM-Cl was synthesized according to a method by Pitsch *et al.*<sup>3</sup> Enantiopure *R*-(1-(2-nitrophenyl)ethanol (*R*-NPE) and *S*-(1-(2-nitrophenyl)ethanol (*S*-NPE) were obtained by literature known procedures.<sup>2, 4</sup>



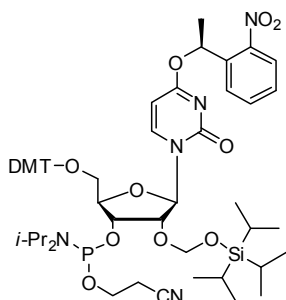
**Figure S1** Synthesis of the NPE protected phosphoramidites of cytidine and uridine according to Höbartner *et al.*:<sup>2</sup> a) DMT-Cl, pyridine; b)  $\text{Bu}_2\text{SnCl}_2$ , *i*-Pr<sub>2</sub>EtN, TOM-Cl,  $\text{CH}_2\text{Cl}_2$ ; c) *R*-NPE, DEAD,  $\text{PPh}_3$ , THF; d)  $\text{Ac}_2\text{O}$ , DMAP, pyridine; e) Mesitylenesulfonylchloride, Et<sub>3</sub>N, DMAP,  $\text{CH}_2\text{Cl}_2$ ; f) *S*-NPE, DMF,  $\Delta$ ; g)  $\text{MeNH}_2$ , EtOH; h) CEP-Cl,  $\text{Me}_2\text{NEt}$ ,  $\text{CH}_2\text{Cl}_2$ .

*5'*-*O*-(4,4'-Dimethoxytrityl)-*N*<sup>4</sup>-[(*S*)-1-(2-nitrophenyl)ethyl]-2'-*O*-{[(triisopropylsilyloxy)methyl]cytidine-3'-[(2-cyanoethyl)-*N,N*-(diisopropyl)phosphoramidite]} (<sup>NPE</sup>C)



$\delta_{\text{H}}$ (300 MHz; acetone-*d*<sub>6</sub>) 0.98-1.03 (21H, m), 1.57 (3H, d, *J* 6.9), 2.67-2.78 (2H, m), 3.35 (1H, dd, *J* 3.9 and 10.8), 3.51-3.64 (3H, m) 3.78 (6H, s), 3.82-3.95 (2H, m), 4.17-4.19 (1H, m), 4.38 (1H, t, *J* 4.7), 4.44-4.48 (1H, m), 5.08 (2H, s), 5.66 (1H, d, *J* 7.5), 5.72-5.78 (1H, m), 6.12 (1H, d, *J* 4.3), 6.87-6.89 (4H, m), 7.22-7.35 (8H, m) 7.46-7.52 (4H, m), 7.66-7.74 (2H, m), 7.79 (1H, d, *J* 7.5) and 7.92 (1H, d, *J* 8.1);  $\delta_{\text{P}}$ (202 MHz;  $\text{CDCl}_3$ ) 149.45 and 149.54.

5'-O-(4,4'-Dimethoxytrityl)-O<sup>4</sup>-[(S)-1-(2-nitrophenyl)ethyl]-2'-O-[[triisopropylsilyl]oxy]-methyl}uridine-3'-[(2-cyanoethyl-N,N-(diisopropyl)phosphoramidite] (<sup>NPE</sup>U)



$\delta_{\text{H}}$ (300 MHz; CDCl<sub>3</sub>) 0.95-1.16 (66H, m), 1.70 (1H, d, *J* 6.4), 2.35 (1H, t, *J* 6.5), 2.55-2.61 (2H, m), 3.37-3.68 (8H, m), 3.80-3.81 (14H, m), 3.84-3.93 (1H, m), 4.22-4.29 (4H, m), 4.34-4.51 (1H, m), 5.07-5.19 (3H, m), 5.45 (1H, d, *J* 7.4), 6.11 (1H, d, *J* 2.6), 6.72-6.78 (1H, m), 6.81-6.86 (8H, m), 7.26-7.33 (14H, m), 7.37-7.45 (6H, m), 7.58-7.69 (4H, m), 7.92-7.95 (2H, m), 8.20 (1H, d, *J* 7.4) and 8.29 (1H, d, *J* 7.4);  $\delta_{\text{P}}$ (202 MHz; CDCl<sub>3</sub>) 149.79 and 149.92 ppm.

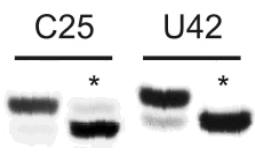
### 3. Splinted ligation

Three RNA ligation systems were used to assemble the final 49mer constructs: <sup>NPE</sup>C<sub>25</sub> (3'-14mer+13mer+22mer-5'), <sup>NPE</sup>U<sub>42</sub> (3'-27mer+11mer+11mer-5') and the rIsoC mutant (3'-27mer+22mer-5'). The procedure was based upon protocols as described in reference <sup>5</sup>. In the optimized version the scale of preparative two-way and the three-way ligations was 3.0 nmol. The 5'-phosphorylation reaction of the 3'-fragments as well as the ligation reaction of the RNA/DNA hybrids were performed in ligation buffer (50 mM Tris·HCl, pH 7.4, 10 mM MgCl<sub>2</sub>, 2.0 mM ATP and 5 mM DTT). Each 3'-RNA fragment (1.0 eq., final: 30 μM) was phosphorylated for 1.0 h at 37°C using 75 U T4 polynucleotide kinase (7.5 μl, 10 U/μl). The kinase was deactivated by short heating (5.0 minutes, 75°C). Subsequently additional ligation buffer, as well as the 5'-RNA fragment (1.0 eq.) and the complementary DNA-splint (1.0 eq.) were added and hybridized (30 s, 75°C and controlled cooling within 30 minutes to 16°C). The T4-DNA ligase (600 U, 20.0 μl, 30 U/μl) was added and the mixture was incubated over night at 16°C (final concentration of RNA: 10 μM). The DNA was digested by adding 200 U DNase I (4.0 μl, 50 U/μl) and incubating the sample for 60 minutes at 37°C. The mixture was diluted with 225 μl loading buffer (90% formamide, 1x TBE buffer), heated for 2.0 minutes 75°C and quickly chilled on ice for 20 minutes. Three-way ligations were performed accordingly by analogous 5'-phosphorylation of the additional strand (40 μM).

The ligation products were purified on denaturing PAGE gels (15%). The positions of the dye-labelled constructs (~1.0 nmol) were localized visually. The single, purple coloured bands were excised, eluted, precipitated and dissolved in 20-25 μl H<sub>2</sub>O to obtain stock-solutions (20 μM) that were stored at -20°C. Final yields of ligated and purified constructs were 12-18%.

#### 4. Photocleavage of the NPE group

Photocleavage of the NPE derivatized RNA (2.0  $\mu$ M in standard DAse buffer) was performed with a Nd:YAG solid-state laser (355 nm, 10 Hz). Quantitative deprotection was achieved by irradiating the RNA for 60 s. The protected and deprotected 11mer and 13mer RNA ligation fragments were analyzed by gel electrophoresis and ESI high resolution mass spectrometry (Figure S2). High-resolution mass spectra of oligonucleotides were recorded on a Bruker micrOTOF-Q II (ESI) in negative mode.



Sample	Chem. formula	Calc. mass [M-H] <sup>-4</sup>	Meas. mass [M-H] <sup>-4</sup>	Deviation [ppm]
<sup>NPE</sup> C <sub>25</sub> (13mer)	C <sub>132</sub> H <sub>162</sub> N <sub>52</sub> O <sub>91</sub> P <sub>12</sub>	1074.9059	1074.9024	3.3
C <sub>25</sub> (13mer)	C <sub>124</sub> H <sub>155</sub> N <sub>51</sub> O <sub>89</sub> P <sub>12</sub>	1037.6439	1037.6460	2.0
<sup>NPE</sup> U <sub>42</sub> (11mer)	C <sub>128</sub> H <sub>166</sub> N <sub>45</sub> O <sub>82</sub> P <sub>11</sub> S <sub>1</sub>	1003.6693	1003.6709	1.6
U <sub>42</sub> (11mer)	C <sub>120</sub> H <sub>159</sub> N <sub>44</sub> O <sub>80</sub> P <sub>11</sub> S <sub>1</sub>	966.4074	966.4072	0.2

**Figure S2** Photolysis of the NPE derivatized oligonucleotide ligation fragments with a Nd:YAG laser (60 s at 355 nm, 10 Hz). 20% denaturing PAGE (left) and ESI mass spectrometry results (right) for the 11mer (C<sub>25</sub>) and the 13mer (U<sub>42</sub>).

#### 5. Fluorescence kinetic measurements

Fluorescence measurements were conducted with a Jasco FP-6500 fluorophotometer (Jasco Inc., Easton, MD, USA) with a built-in thermoelectric controller (ETC-273T) and an external Julabo F25 thermostat. All samples were always kept in brown vials and on ice. Prior to any measurements the RNA constructs were dissolved in standard DAse buffer and properly refolded by heating for 2.0 minutes at 75°C and controlled cooling in the thermo shaker (Eppendorf) within 20 minutes to RT. Measurements of laser-irradiated 49mer RNA constructs were performed without additional refolding. Fluorescence kinetic measurements with the 1-AB fluorogenic probe (2.0 mM stock solution) were carried out essentially as described in Nierth *et al.*,<sup>6</sup> with the exception of the wavelengths for excitation and emission of the BODIPY moiety. These were shifted slightly to 460 nm/510 nm, as to ensure no interference with the Cy3/Cy5 labels on the RNA. The fluorophotometer settings were: response time (2.0 s), data pitch (1.0 s), sensitivity (high), excitation bandwidth (3 nm) and emission bandwidth (5 nm).

Fluorescence curves were recorded and three consecutive measurements were accumulated using the fluorophotometer software. Mathematical fitting of the kinetic curves to monoexponentials was performed with GraphPad Prism v5.0 (GraphPad Software, La Jolla, California, USA) and plotted using Origin Pro v8.0 (OriginLab, Northampton,

Massachusetts, USA). Linear regression was used for the background reactions. The relative catalytic activity was calculated by comparing the initial slopes (at the reaction time = 0 s) to the value for the *wt* DAse.

## 6. Bulk FRET efficiencies

The bulk FRET efficiencies  $E_{FRET}$  of the RNA constructs were measured by comparing the fluorescence emission intensities of the donor (Cy3) and acceptor (Cy5) fluorophores. The following equation was used, estimating a correction factor  $\gamma$  of 0.75, with  $I_D$  and  $I_A$  corresponding to the donor and acceptor fluorescence intensities respectively:

$$E = \frac{I_A}{I_A + \gamma \cdot I_D}$$

Prior to the measurements, the RNA constructs were refolded, by dissolving 10 pmol in water, heating to 75°C for 2.0 minutes and controlled cooling in the thermo shaker to reach room temperature within 20 minutes. 3.0  $\mu$ l DAse buffer (5x) with varying concentrations MgCl<sub>2</sub> (0, 20 and 80 mM) were added to give final volumes of 15.0  $\mu$ l. Each sample was transferred into a cuvette and equilibrated for 2.0 minutes at 25°C in the thermostat controlled cell holder. Fluorescence emission spectra (550-750 nm) were recorded by exciting the Cy3 fluorophore at 532.0 nm. Three consecutive scans were accumulated using the following device settings: response time (0.5 s), data pitch (1.0 nm), sensitivity (high), scanning speed 1000 nm/minute, excitation bandwidth (3 nm) and emission bandwidth (3 nm).

## 7. References

1. [http://www.ambion.com/techlib/misc/oligo\\_calculator.html](http://www.ambion.com/techlib/misc/oligo_calculator.html).
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