

## Electronic Supplementary Information

### DNA-Catalyzed Reactivity of a Phosphoramidate Functional Group and Formation of an Unusual Pyrophosphoramidate Linkage

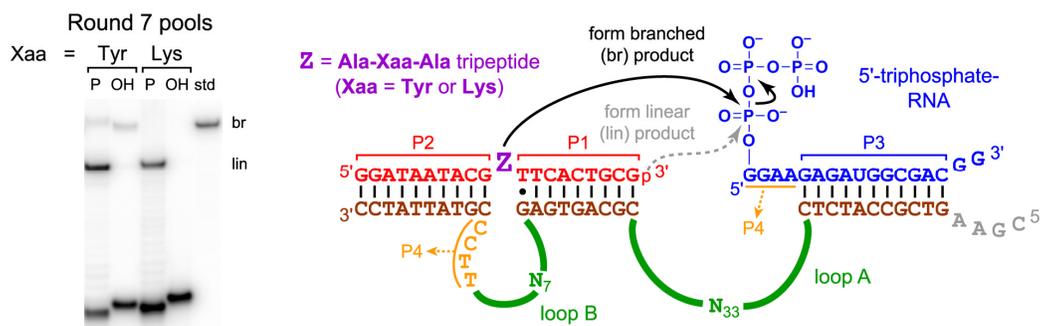
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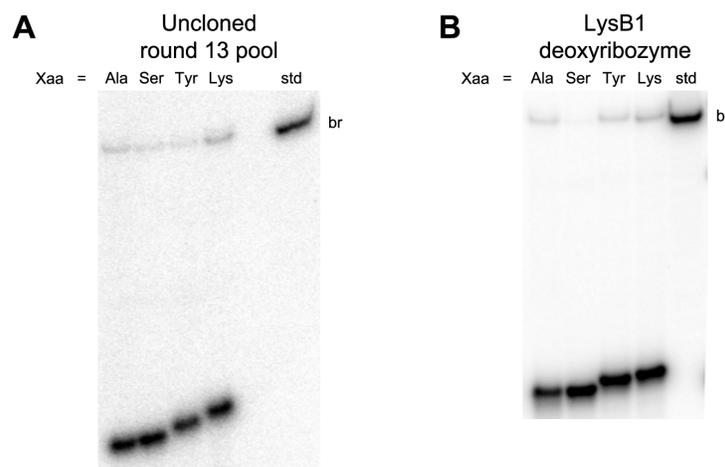
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Assay of uncloned round 7 pool from Lys selection with 3'-phosphate substrate



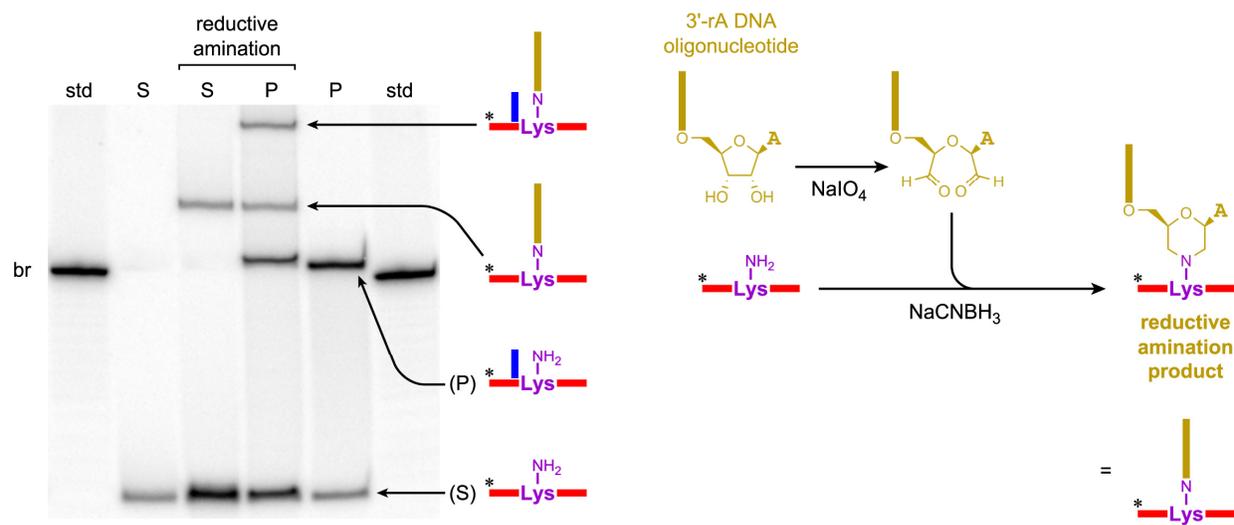
**Figure S1.** Assay of round 7 selection products formed using either 3'-phosphate (P) or 3'-OH tripeptide-containing (OH) substrate. These assays were performed with pool not covalently joined to the RNA substrate (i.e., covalent loop of Fig. 1 absent). Assays were performed in 50 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM KCl and 20 mM  $Mn^{2+}$ , and 40 mM  $Mg^{2+}$  at 37 °C for 21 h. Std = branched standard, prepared using the 9HR17 deoxyribozyme that forms a 2',5'-branched nucleic acid product from DNA-rA-DNA and 5'-triphosphate-RNA substrates.<sup>1</sup> The 9HR17 product has similar molecular weight and branched architecture as the expected Lys nucleopeptide ligation product. Also shown are corresponding round 7 assays for the previously reported selection experiment with the Tyr-containing substrate, where the products also predominantly involved nucleophilic reactivity of the 3'-phosphate rather than the Tyr side chain.<sup>2</sup> For both the Lys and Tyr pools, the products are predominantly “linear” (lin) rather than “branched” (br), from reaction of the DNA 3'-phosphate with the RNA 5'-triphosphate.

Assays for uncloned round 13 pool from Lys selection and the 13LS3 deoxyribozyme



**Figure S2.** Ligation assays for the uncloned round 13 pool and the 13LS3 deoxyribozyme. These assays were performed with pool or 13LS3 not covalently joined to the RNA substrate (i.e., covalent loop of Fig. 1 absent). (A) Ligation reactions with the uncloned round 13 pool, using the Ala-Xaa-Ala 3'-ddC substrate with Xaa = Ala, Ser, Tyr, or Lys. Assays were performed in 50 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM KCl, 20 mM  $MnCl_2$ , and 40 mM  $MgCl_2$  at 37 °C for 20 h. Ligation yields (left to right) were 4%, 2%, 2%, and 6%. (B) Analogous ligation reactions with the 13LS3 deoxyribozyme. Ligation yields (left to right) were 4%, <0.5%, 2%, and 6%. Similar results were observed when 20 mM  $Mn^{2+}$  alone was used in place of 20 mM  $Mn^{2+}$  and 40 mM  $Mg^{2+}$  (data not shown).

Reductive amination assay of the 13LS3 ligation product



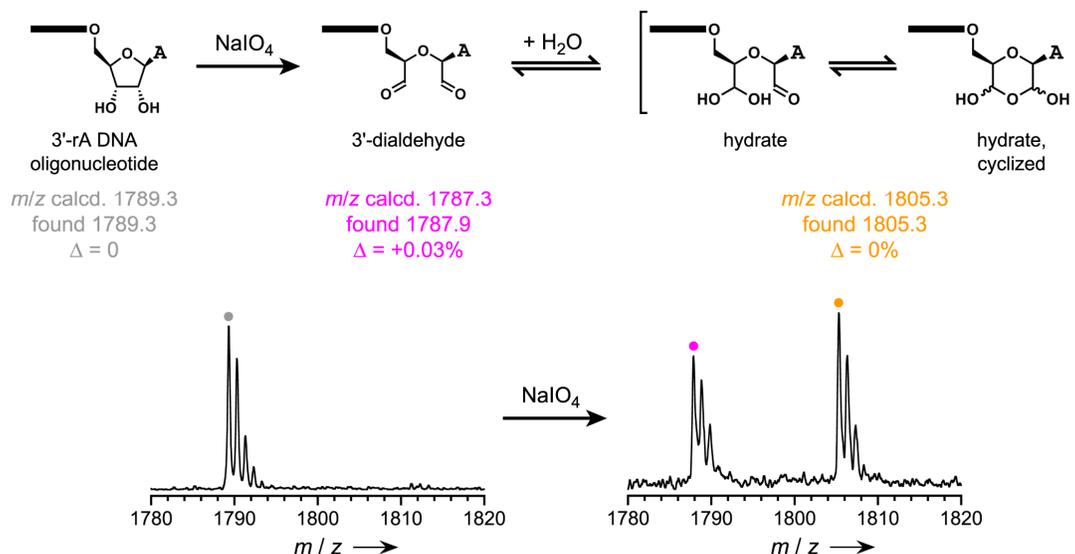
**Figure S3.** Reductive amination of the 13LS3 ligation product (prepared from the Ala-Lys-Ala 3'-ddC substrate), demonstrating that the product retains an aliphatic amine functional group. S = substrate; P = product. Lys denotes the central amino acid of the Ala-Lys-Ala tripeptide. Assay procedure: 10 pmol of either the 5'-<sup>32</sup>P-radiolabeled 13LS3 substrate or product (as indicated) was joined with a 3'-rA-terminated DNA oligonucleotide that was oxidized with NaIO<sub>4</sub> to create a 3'-dialdehyde oligonucleotide (see mass spectrometry data in Fig. S4). A 100 pmol portion of the 35-mer 3'-rA-terminated DNA oligonucleotide [(AAC)<sub>4</sub>ACGTCGGAAGTCTCATGTACTT-rA] was oxidized in 100 μL containing 100 mM HEPES, pH 7.5, and 10 mM NaIO<sub>4</sub> at 25 °C for 1 h. After ethanol precipitation, the 3'-dialdehyde oligonucleotide was incubated with the 13LS3 substrate or product in 20 μL containing 100 mM NaOAc, pH 5.2, 50 mM NiCl<sub>2</sub>, and (added last) 10 mM NaCNBH<sub>3</sub> at 37 °C for 12 h, followed by 20% PAGE. Analogous reductive amination reactions with an amino-bearing control oligonucleotide reproducibly provided ~30% yield, similar to what is observed here for both substrate (lane 3) and product (lane 4).

In Fig. S3, note that instability of the 13LS3 ligation product was evident, leading to regeneration of a variable amount of the original substrate (i.e., the tripeptide-containing substrate not attached to its RNA ligation partner) by cleavage of the pyrophosphate linkage in the final product sample used for these assays. This product instability is associated with the gel extraction process used after product synthesis by 13LS3 and separation by PAGE, as described in the main text.

Periodate oxidation of 3'-rA oligonucleotide assayed by mass spectrometry

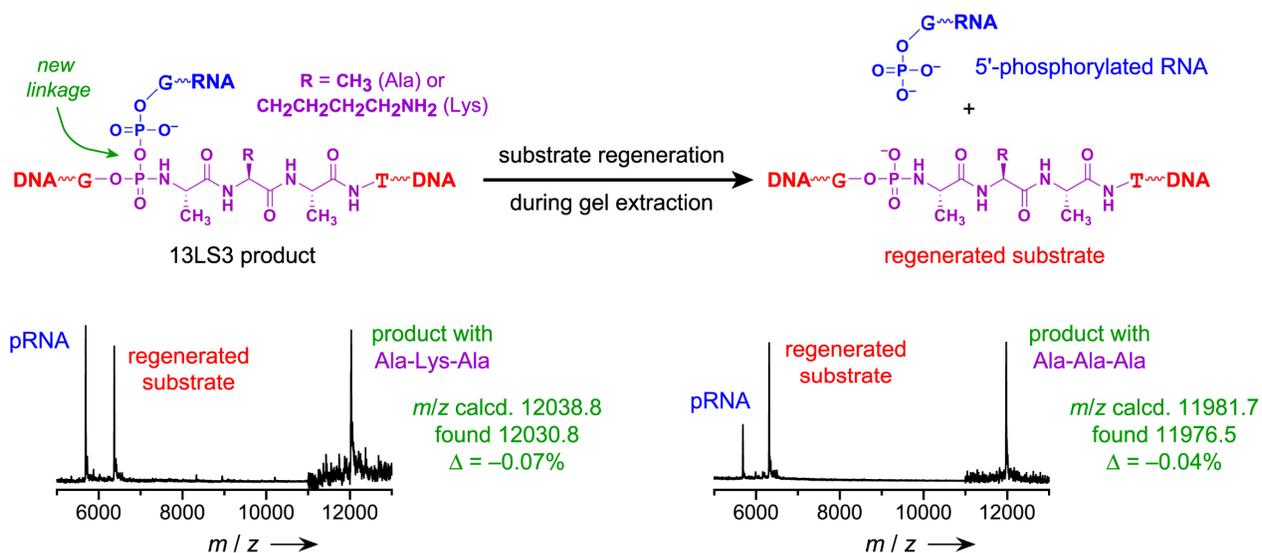
The reductive amination assay of Fig. S3 relies upon the use of a 3'-dialdehyde DNA oligonucleotide, formed by periodate ( $\text{NaIO}_4$ ) oxidation of a 3'-rA oligonucleotide. Periodate oxidation of 3'-ribonucleotide-terminated oligonucleotides is extremely well-precedented.<sup>3-5</sup> Nevertheless, to provide direct evidence of such oxidation in the present situation, the model hexamer 5'-ACTATrA-3' (corresponding to the 3'-terminal six nucleotides of the full-length 20 nt 3'-rA oligonucleotide) was analyzed by MALDI mass spectrometry both without and with periodate treatment. The observed mass spectrometry data was fully in accord with periodate oxidation to form a 3'-dialdehyde (Fig. S4A). The oxidized 3'-dialdehyde oligonucleotide was observed partially in the hydrated form, as expected for aldehydes.

The full-length 20 nt 3'-rA oligonucleotide was also assayed by mass spectrometry after periodate oxidation and ethanol precipitation. Only the hydrated form of the 3'-dialdehyde was observed (calcd. 6140.1, found 6138.6,  $\Delta = -0.02\%$ ); a similar result was observed after periodate oxidation without ethanol precipitation (calcd. 6140.1, found 6140.0,  $\Delta = -0.002\%$ ). Observation of the hydrated form compels the conclusion that oxidation occurred.



**Figure S4.** Periodate oxidation of hexamer 3'-rA oligonucleotide assayed by MALDI mass spectrometry. Observed is a mixture of 3'-dialdehyde and its hydrated form, presumably cyclized to the bis-hemiacetal as illustrated. The ratio of 3'-dialdehyde to hydrated form depended on the details of sample preparation (data not shown). Procedure: a 1 nmol portion of the 3'-rA oligonucleotide was oxidized in 100  $\mu\text{L}$  containing 100 mM HEPES, pH 7.5, and 10 mM  $\text{NaIO}_4$  at 25  $^\circ\text{C}$  for 1 h. The sample was desalted by passage through a Sephadex G-10 spin column, concentrated to 10  $\mu\text{L}$  volume by SpeedVac, desalted by  $\text{C}_{18}$  ZipTip, and assayed by MALDI mass spectrometry.

### MALDI mass spectrometry of the 13LS3 product



**Figure S5.** MALDI mass spectrometry of the 13LS3 reaction product, formed using either the Ala-Lys-Ala or Ala-Ala-Ala substrate and purified by PAGE followed by gel extraction. Peaks arising from substrate regeneration are also evident, as expected (see main text for explanation and Experimental Section for mass values).

### References for Supporting Information

1. P. I. Pradeepkumar, C. Höbartner, D. A. Baum and S. K. Silverman, *Angew. Chem. Int. Ed.*, 2008, **47**, 1753-1757.
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5. O. W. Odom, Jr., D. J. Robbins, J. Lynch, D. Dottavio-Martin, G. Kramer and B. Hardesty, *Biochemistry*, 1980, **19**, 5947-5954.