A modular tyrosine kinase deoxyribozyme with discrete aptamer and catalyst domains

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Oligonucleotides, peptides, and DNA-anchored peptide conjugates

DNA oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA) or prepared by solid-phase synthesis on an ABI 394 instrument using reagents from Glen Research. 5′-Triphosphorylated RNA (pppRNA) oligonucleotides were prepared by *in vitro* transcription using synthetic DNA templates and T7 RNA polymerase.[1] All oligonucleotides were purified by 7 M urea denaturing PAGE with running buffer 1× TBE (89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3) as described previously.[2] Peptides were synthesized on the solid phase as described.[3] DNA-anchored peptide conjugates were synthesized by disulfide formation between a DNA HEG-tethered 3′-thiol and the N-terminal cysteine side chain of the peptide, following the procedure shown in Fig. S1. The experimental procedure is provided in our recent report.[4]

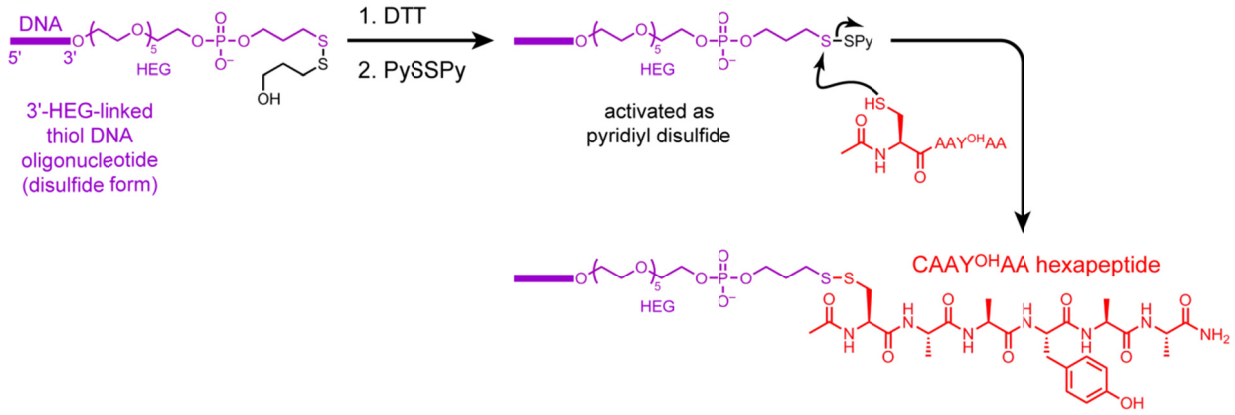

Figure S1. Synthesis and structure of the DNA-anchored CAAYAA hexapeptide phosphorylation substrate. Note the hexa(ethylene glycol), or HEG, tether connecting the DNA oligonucleotide anchor to the peptide.

In vitro selection procedure

The selection procedure was performed essentially as described,[4] but with different DNA and RNA sequences to suppress contamination of the new selection experiments by existing deoxyribozymes. The nucleotide details are shown in Fig. S2. All steps of the selection process were performed as reported,[4] except that Pfu polymerase was used in place of Taq polymerase because the latter led to substantially greater yield of the desired PCR product.

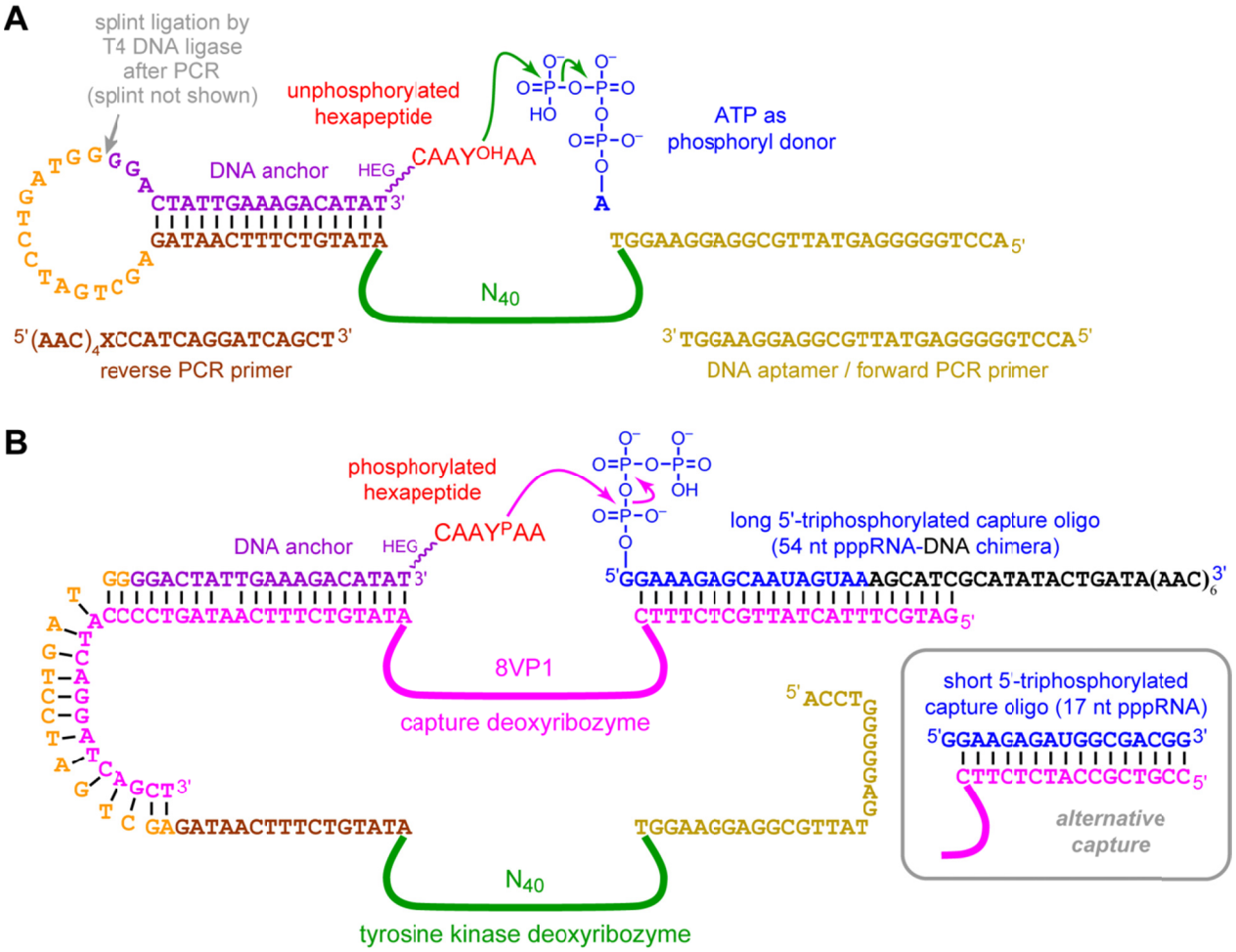

Figure S2. Nucleotide details of the selection and capture steps of *in vitro* selection. (A) Selection step. (B) Capture step. See our previous report for a full explanation of all components.[4] The splint sequence for the ligation step in all rounds was 5′-ATATGTCTTTCAATAGTCCCCATCAGGATCAGCTCTATTGAAAGACATAT-3′.

Selection progression

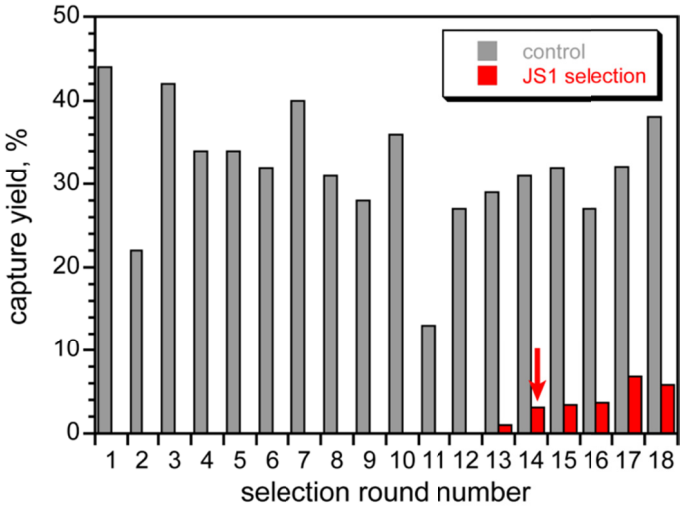

Figure S3. Progression of the *in vitro* selection experiment. The arrow marks the cloned round, number 14.

Divalent metal ion dependence of the 14JS101 deoxyribozyme

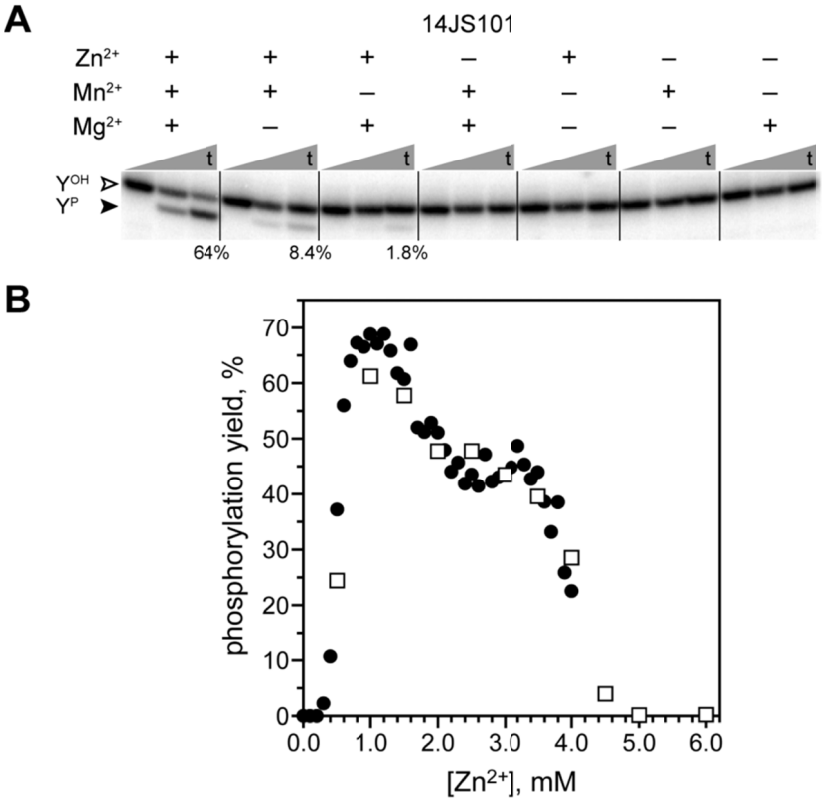

Figure S4. Divalent metal ion dependence of the 14JS101 deoxyribozyme. (A) Ascertaining the requirements for inclusion of Zn^{2+}, Mn^{2+}, and Mg^{2+}. (B) Determining the optimum Zn^{2+} concentration. For all samples, the Tris concentration was held constant at 40 mM. Different symbols correspond to different data sets. The origin of the interesting and rather unusually shaped [Zn^{2+}] dependence curve is unknown.

MALDI mass spectrometry of the 14JS101 phosphorylation product

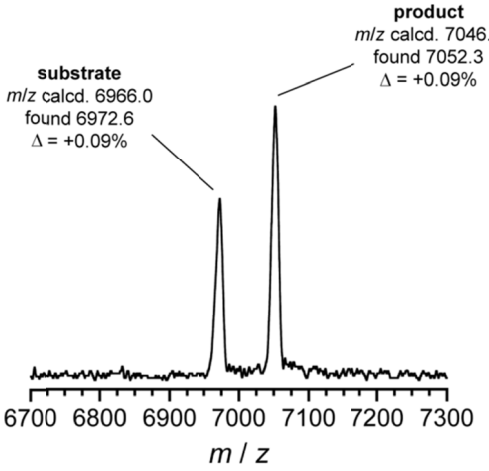

Figure S5. MALDI mass spectrometry of the 14JS101 phosphorylation product. The DNA-anchored CAAY^{P}AA phosphorylation product was prepared from a 10 µL sample containing 50 pmol of DNA-anchored HEG-tethered CAAY^{OH}AA substrate (Fig. S1), 80 pmol of 14JS101 deoxyribozyme, and 2 nmol of ATP, which were annealed in 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating at 95 °C for 3 min and cooling on ice for 5 min. The DNA-catalyzed phosphorylation reaction was initiated by bringing the sample to 20 µL total volume containing 40 mM Tris, pH 7.5, 1.0 mM $ZnCl_2$, 10 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h, quenched with EDTA, desalted by Millipore C_{18} ZipTip and analyzed by MALDI mass spectrometry (Bruker `UltrafleXtreme; matrix 3-hydroxypicolinic acid, positive ion mode).

References for Electronic Supplementary Information

1. J. F. Milligan, D. R. Groebe, G. W. Witherell and O. C. Uhlenbeck, *Nucleic Acids Res.*, 1987, **15**, 8783-8798.
2. (*a*) A. Flynn-Charlebois, Y. Wang, T. K. Prior, I. Rashid, K. A. Hoadley, R. L. Coppins, A. C. Wolf and S. K. Silverman, *J. Am. Chem. Soc.*, 2003, **125**, 2444-2454; (*b*) Y. Wang and S. K. Silverman, *Biochemistry*, 2003, **42**, 15252-15263.
3. J. Chandrasekar and S. K. Silverman, *Proc. Natl. Acad. Sci. USA*, 2013, **110**, 5315-5320.
4. S. M. Walsh, A. Sachdeva and S. K. Silverman, *J. Am. Chem. Soc.*, 2013, **135**, 14928-14931.