DNA Catalysts with Tyrosine Kinase Activity

Shannon M. Walsh, Amit Sachdeva, and Scott K. Silverman*

Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, United States

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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 Figure S1 and given below as adapted from our recent report.[3]

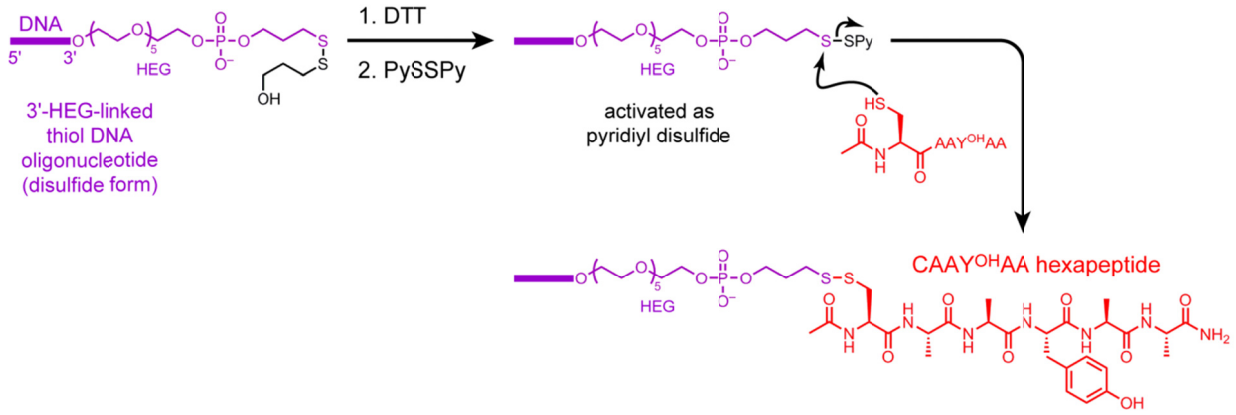

Figure S1. Synthesis and structure of the DNA-anchored CAAYAA hexapeptide phosphorylation substrate. Note the hexa(ethylene glycol), or HEG, tether connecting the DNA oligonucleotide anchor to the peptide.

Procedure for synthesis of DNA-anchored peptide conjugate. The DNA anchor oligonucleotide was 5′-GGATAATACGACTCACTAT-HEG-p-C_3-SS-C_3-OH-3′ (see first structure in Figure S1), where the 3′-disulfide linker was introduced via standard solid-phase DNA synthesis and unmasked to a 3′-thiol by DTT treatment. A 50 µL sample containing 5 nmol of DNA anchor oligonucleotide in 100 mM HEPES, pH 7.5, and 50 mM DTT was incubated at 37 °C for 2 h. The reduced product was precipitated to remove excess DTT by addition of 50 µL of water, 10 µL of 3 M NaCl, and 300 µL of ethanol. The precipitated product (DNA-HEG-p-C_3-SH) was dissolved in 35 µL of water and 10 µL of 100 mM triethylammonium acetate, pH 7.0. Activation as the pyridyl disulfide was achieved by adding 5 µL of 100 mM 2,2′-dipyridyl disulfide in DMF and incubating at 37 °C for 2 h. The product (DNA-HEG-p-C_3-SSPy) was precipitated by addition of 50 µL of water, 10 µL of 3 M NaCl and 300 µL of ethanol and dissolved in 25 µL of water. Conjugation to the peptide was performed by adding 20 µL of 50 mM triethylammonium acetate, pH 7.0, and 5 µL of 20 mM peptide (100 nmol, 20 equivalents). The sample was incubated at 37 °C for 12 h, and the DNA-anchored peptide was purified by 20% PAGE. A typical yield was 3.5–4.5 nmol.

In vitro selection procedure

The selection procedure, cloning, and initial analysis of individual clones were performed essentially as described previously,[2a,4] but with a different ligation step[3,5] and with a new capture step based on the 8VP1 deoxyribozyme.[5] An overview of the key selection and capture steps of each round is shown in Figure 2, and a depiction with nucleotide details is shown in Figure S2.

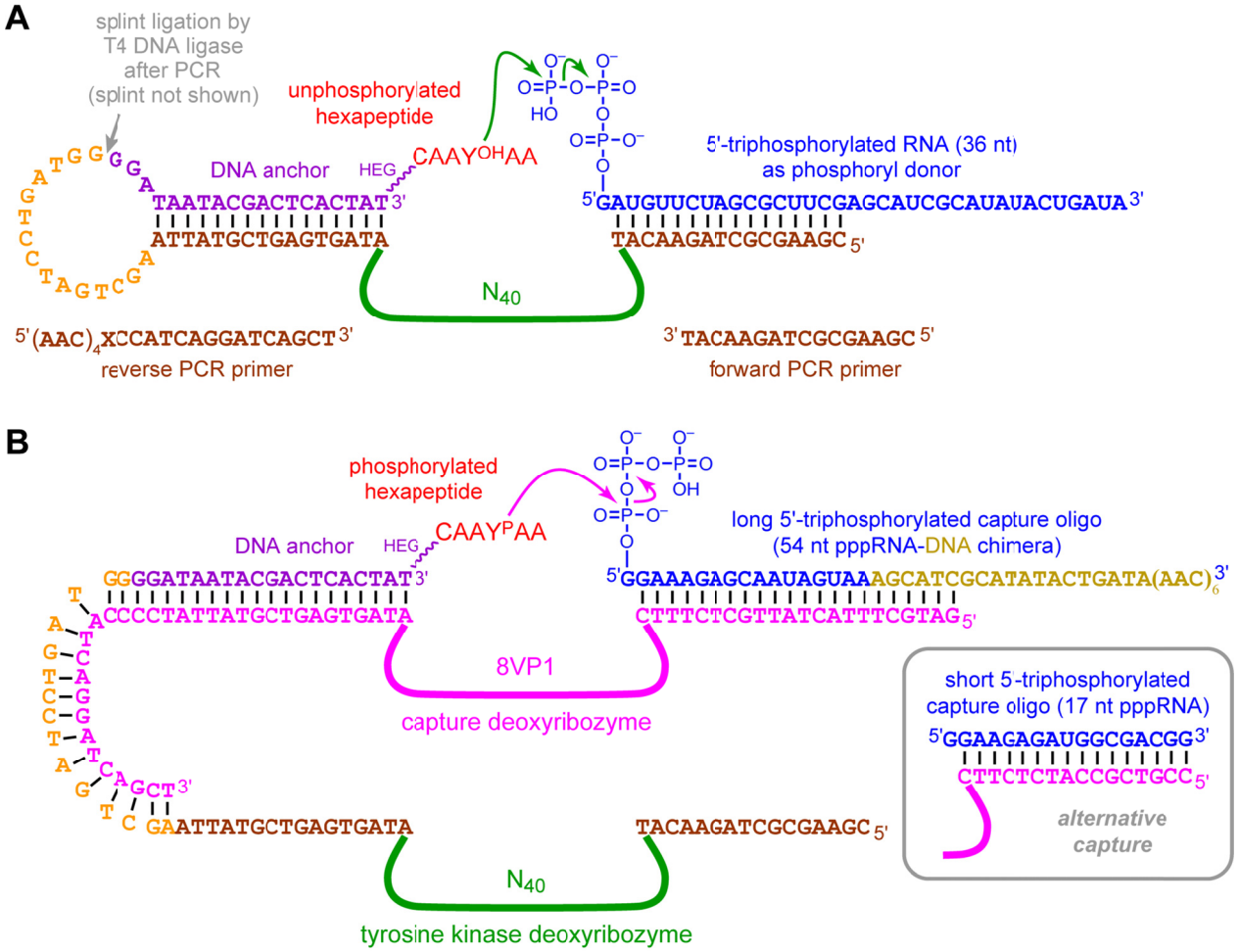

Figure S2. Nucleotide details of the selection and capture steps of in vitro selection. (A) Selection step. The length of the 5′-triphosphorylated RNA oligonucleotide (36 nt pppRNA; phosphoryl donor) was chosen such that any unwanted deoxyribozymes that catalyze covalent attachment of the Tyr hydroxyl group to the RNA via attack of Tyr at the α-phosphorus atom migrate differently on PAGE than does the desired capture product of panel B. In the selections for which free GTP replaced pppRNA as the phosphoryl donor, the RNA oligonucleotide was omitted entirely; therefore, the left-hand DNA binding arm (i.e., the pool segment to the 5′-side of the random region) was not base-paired to anything. In the reverse PCR primer, X is the HEG spacer (Glen Spacer 18) that stops extension by Taq polymerase and leads to a size difference between the two PCR product strands. (B) Capture step. See our previous report for validation that 8VP1 is highly selective for reaction of phosphorylated Tyr (Tyr^P) over unphosphorylated Tyr (Tyr^{OH}).[5] The sequence of the 8VP1 catalytic region was 5′-GGACACGATGAGTGACTAAGTG-GAATGAGGAAAGCACGAG-3′. To avoid emergence of noncatalytic DNA sequences that migrate aberrantly at a fixed PAGE position, the length of the capture oligo reaction partner was alternated in successive rounds between a short version (17 nt pppRNA, even-numbered rounds) and a long version (54 nt pppRNA-DNA chimera, odd-numbered rounds including round 1). The pppRNA-DNA chimera was prepared by splint ligation of the pppRNA component to the 5′-phosphorylated DNA component using a complementary DNA splint and T4 DNA ligase. The short and long reaction partners had different sequences to avoid emergence of deoxyribozymes that catalyze unwanted covalent attachment of Tyr^{OH} to RNA during the capture step (this would be facilitated if the DNA random region had a sequence that allows substantial Watson-Crick base pairing to the constant capture oligo sequence). For the long capture oligo, the 8VP1 binding arm was extended several nucleotides past the RNA-DNA junction, which suppressed nonspecific cleavage (transesterification) of the RNA nucleotides near the junction.

Procedure for ligation step in round 1. The splint sequence was 5′-ATAGTGAGTCGTATTATCCTCCATC-AGGATCAGCTTAATACGACTCACTAT-3′, where the underlined T is included to account for the untemplated A nucleotide that is added at the 3′-end of each PCR product by Taq polymerase. This T nucleotide was omitted from the splint used for ligation of the initially random N_{40} pool, which was prepared by solid-phase synthesis without the untemplated A. A 35 μL sample containing 1 nmol of DNA pool, 800 pmol of DNA splint, and 500 pmol of 5′-phosphorylated DNA-anchored hexapeptide substrate was 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. To this solution was added 4 μL of 10× T4 DNA ligase buffer (400 mM Tris, pH 7.8, 100 mM $MgCl_2$, and 5 mM ATP) and 1 μL of 5 U/μL T4 DNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h and separated by 8% PAGE.

Procedure for ligation step in subsequent rounds. The same splint was used as in the round 1 ligation procedure. A 17 μL sample containing the PCR-amplified DNA pool (~5–10 pmol), 25 pmol of DNA splint, and 50 pmol of 5′-phosphorylated DNA-anchored hexapeptide substrate was 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. To this solution was added 2 μL of 10× T4 DNA ligase buffer and 1 μL of 1 U/μL T4 DNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h and separated by 8% PAGE.

Procedure for selection step in round 1. Each selection experiment was initiated with 200 pmol of the ligated pool. A 20 μL sample containing 200 pmol of ligated pool and 300 pmol of 36 nt pppRNA phosphoryl donor was annealed in 5 mM HEPES, 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 selection reaction was initiated by bringing the sample to 40 μL total volume containing 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. The Mn^{2+} was added from a 10× stock solution containing 200 mM $MnCl_2$. The Zn^{2+} was added from a 10× stock solution containing 10 mM $ZnCl_2$, 20 mM HNO_3, and 200 mM HEPES at pH 7.5; this stock solution was freshly prepared from a 100× stock of 100 mM $ZnCl_2$ in 200 mM HNO_3. The metal ion stocks were added last to the final sample. For selections with GTP as the phosphoryl donor, the pppRNA was omitted and 1 mM GTP was added from a 10 mM stock solution after annealing. The sample was incubated at 37 °C for 14 h and separated by 8% PAGE.

Procedure for selection step in subsequent rounds. A 10 μL sample containing the ligated pool and 50 pmol of 36 nt pppRNA phosphoryl donor was annealed in 5 mM HEPES, 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 selection reaction was initiated by bringing the sample to 20 μL total volume containing 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. For selections with GTP as the phosphoryl donor, the pppRNA was omitted and 1 mM GTP was added from a 10 mM stock solution after annealing. The sample was incubated at 37 °C for 14 h and separated by 8% PAGE.

Procedure for capture step in round 1. A 28 μL sample containing ligated pool, 500 pmol of 54 nt 5′-triphosphorylated RNA-DNA chimera substrate, and 300 pmol of 8VP1 capture deoxyribozyme was annealed in 5 mM HEPES, 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 capture reaction was initiated by bringing the sample to 40 μL total volume containing 50 mM HEPES, pH 7.5, 20 mM $MnCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h. Before PAGE, to the sample was added 500 pmol of a 60 nt decoy oligonucleotide complementary to the 40 nt 8VP1 catalytic region and 10 nt of binding arm on each side, to ensure complete removal of the capture deoxyribozyme from the pool. The sample was separated by 8% PAGE.

Procedure for capture step in subsequent rounds. A 14 μL sample containing ligated pool, 50 pmol of 17 nt pppRNA substrate (in even rounds) or 54 nt 5′-triphosphorylated RNA-DNA chimera substrate (in odd rounds), and 25 pmol of 8VP1 capture deoxyribozyme was annealed in 5 mM HEPES, 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 capture

reaction was initiated by bringing the sample to 20 μL total volume containing 50 mM HEPES, pH 7.5, 20 mM $MnCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h. To the sample was added 100 pmol of a 60 nt decoy oligonucleotide complementary to the 40 nt 8VP1 catalytic region and 10 nt of binding arm on each side (note that the decoy sequence is different in even-numbered and odd-numbered rounds). The product was separated by 8% PAGE.

Procedure for PCR in subsequent rounds. In each selection round, two PCR reactions were performed, 10-cycle PCR followed by 30-cycle PCR. First, a 100 μL sample was prepared containing the PAGE-separated capture product, 200 pmol of forward primer, 50 pmol of reverse primer, 20 nmol of each dNTP, and 10 μL of 10× Taq polymerase buffer (200 mM Tris-HCl, pH 8.8, 100 mM $(NH_4)_2SO_4$, 100 mM KCl, 20 mM $MgSO_4$, and 1% Triton X-100). This sample was cycled 10 times according to the following PCR program: 94 °C for 2 min, 10× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. Taq polymerase was removed by phenol/chloroform extraction. Second, a 50 μL sample was prepared containing 1 μL of the 10-cycle PCR product, 100 pmol of forward primer, 25 pmol of reverse primer, 10 nmol of each dNTP, 20 μCi of α-^{32}P-dCTP (800 Ci/mmol), and 5 μL of 10× Taq polymerase buffer. This sample was cycled 30 times according to the following PCR program: 94 °C for 2 min, 30× (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. Samples were separated by 8% PAGE.

Selection progressions

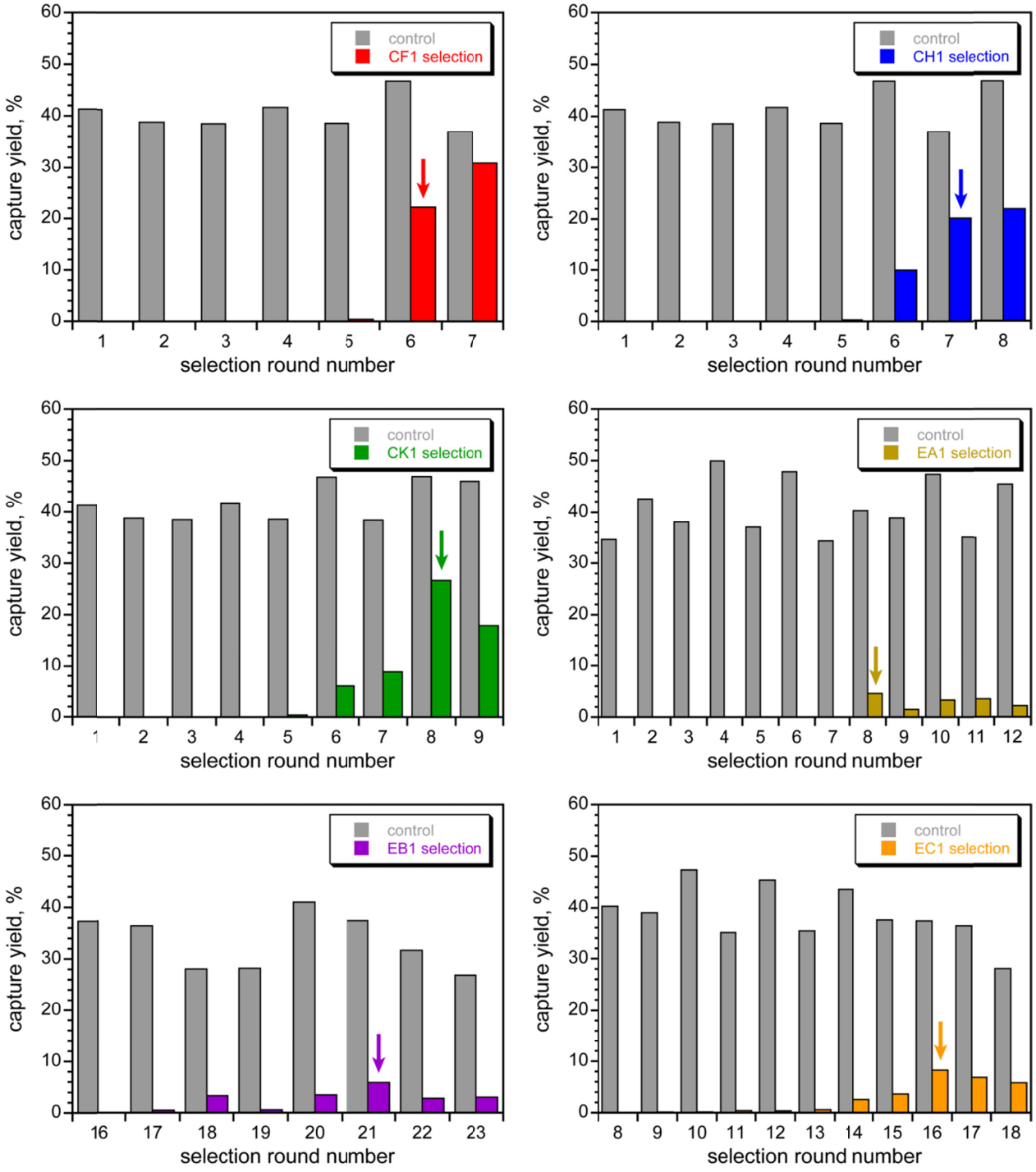

Figure S3. Progressions of the in vitro selection experiments. In each round, "control" refers to the yield for the 8VP1-catalyzed capture reaction using the CAAY^{P}AA substrate as illustrated in Figure S2B, and "selection" refers to the yield for the 8VP1-catalyzed capture reaction using the deoxyribozyme pool for that round. Arrows mark the cloned rounds. The CF1, CH1, and CK1 selections were for CAAY^{OH}AA phosphorylation using an N_{30}, N_{40}, and N_{50} random region, respectively, and pppRNA as the phosphoryl donor. The EA1, EB1, and EC1 selections were for CAAY^{OH}AA phosphorylation using an N_{30}, N_{40}, and N_{50} random region, respectively, and free GTP as the phosphoryl donor. For the EB1 and EC1 selections, the activities are shown beginning with the last round in which activity was undetectable (<0.5%; 16EB1 and 8EC1).

Mass spectrometry and 8VP1 reactivity to establish phosphorylation product identities

deoxyribozyme	$[M+H]^+$ calcd.	$[M+H]^+$ found	error, % (found – calcd.)
6CF127	6982.9	6982.6	–0.004
6CF134	6982.9	6982.0	–0.01
7CH102	6982.9	6981.7	–0.02
7CH104	6982.9	6984.8	+0.03
8CK105	6982.9	6982.7	–0.003
8CK128	6982.9	6981.4	–0.02
8EA101	6982.9	6984.7	+0.03
21EB121	6982.9	6982.2	–0.01
16EC103	6982.9	6986.3	+0.05

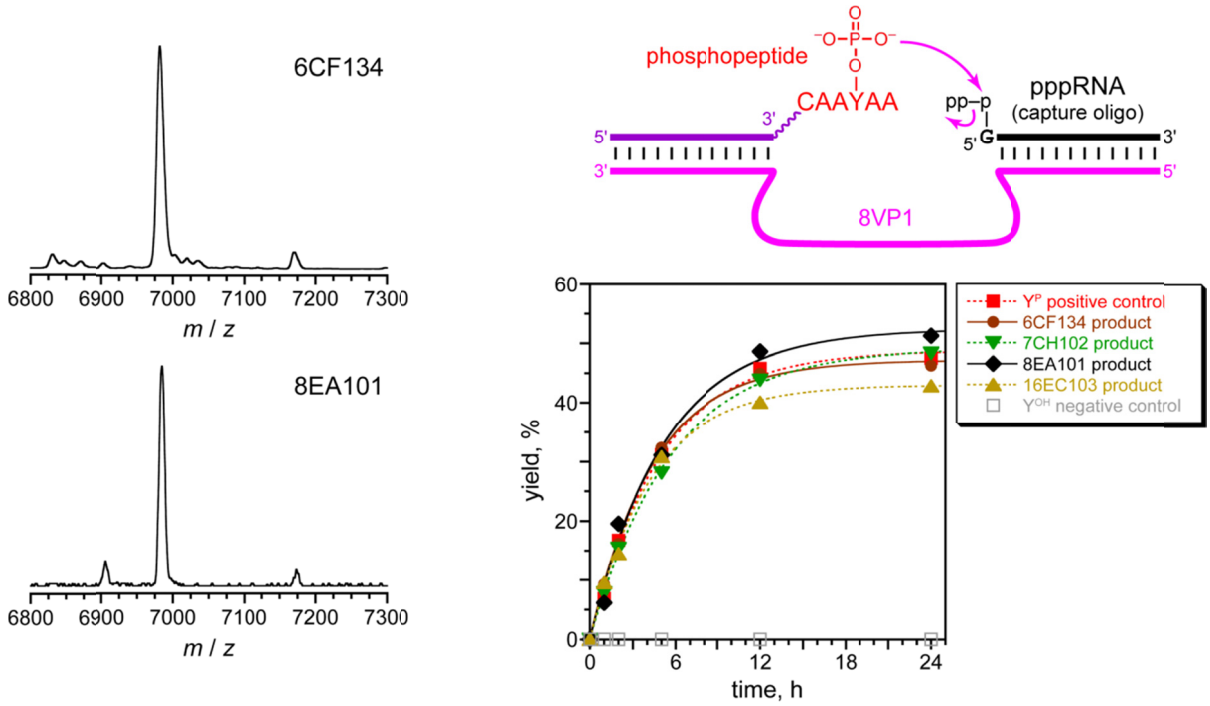

Table S1. MALDI mass spectrometry and 8VP1 reactivity data for individual tyrosine kinase deoxyribozymes. Masses are tabulated for the DNA-HEG-CAAY^{P}AA product. The DNA-anchored CAAY^{P}AA phosphorylation product was prepared from a 10 µL sample containing 300 pmol of DNA-anchored HEG-tethered CAAY^{OH}AA substrate (Figure S1), 350 pmol of deoxyribozyme, and 400 pmol of pppRNA phosphoryl donor, which were annealed in 5 mM HEPES, 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. For samples with GTP as the phosphoryl donor, the pppRNA was omitted and 1 mM GTP was added from a 10 mM stock solution after annealing. The DNA-catalyzed phosphorylation reaction was initiated by bringing the sample to 20 µL total volume containing 70 mM HEPES, pH 7.5, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h and separated by 8% PAGE (only the lower one-fourth to one-half of the product band was excised, to suppress extraneous signals due to unreacted substrate). The PAGE-purified sample was desalted by Millipore C_{18} ZipTip and analyzed by MALDI mass spectrometry (Bruker UltrafleXtreme; matrix 3-hydroxypicolinic acid). Four of the individual phosphorylation products were isolated by PAGE and subjected to 8VP1-catalyzed reaction with the pppRNA capture oligonucleotide. In each case as shown in the plot, essentially the same rate and yield of product formation were observed as for the Y^P positive control (50 mM HEPES, pH 7.5, 20 mM $MnCl_2$, and 150 mM NaCl at 37 °C; k_{obs} ca. 0.2 h^{-1}). These data directly demonstrate that the tyrosine hydroxyl group is the attachment site for the phosphoryl group that was joined to Y^{OH} by the indicated kinase deoxyribozyme.

Sequences of individual deoxyribozymes

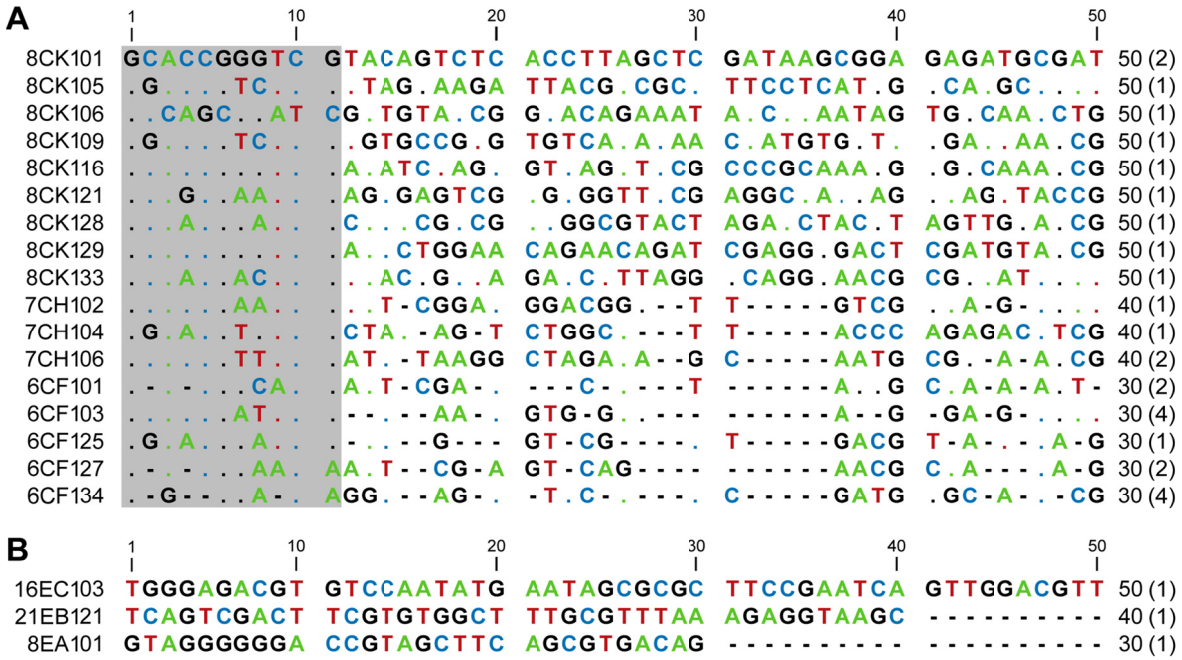

Figure S4. Sequences of the initially random regions of the new tyrosine kinase deoxyribozymes. All deoxyribozymes were used as 5′-CGAAGCGCTAGAACAT-N_x-ATAGTGAGTCGTATTA-3′, where N_x represents the specific x nucleotides (x = 30, 40, or 50) of the initially random region as determined through the selection process. All alignments show only the initially random region. A dot denotes conservation, i.e., the same nucleotide as in the uppermost sequence; a dash denotes a gap. In both sets of sequences, next to the sequence length in nucleotides on the far right is shown (in parentheses) the number of times that sequence was found during cloning. (A) Sequence alignment of all initially obtained N_{30}, N_{40}, and N_{50} deoxyribozymes that use pppRNA as the phosphoryl donor. Alignment was performed using CLC Sequence Viewer using standard parameters. Note the strongly conserved 11 nt segment present at the 5′-end of the sequences (boxed in grey). (B) Unaligned sequences of the deoxyribozymes that use GTP as the phosphoryl donor.

Single-turnover deoxyribozyme assay procedure

The DNA-anchored hexapeptide substrate was 5′-^{32}P-radiolabeled using γ-^{32}P-ATP and T4 polynucleotide kinase (Fermentas), using 10× buffer that lacks DTT (500 mM Tris, pH 7.6, 100 mM $MgCl_2$, and 1 mM spermidine). A 10 μL sample containing 0.25 pmol of 5′-^{32}P-radiolabeled DNA-anchored phosphopeptide substrate, 10 pmol of deoxyribozyme, and 20 pmol of pppRNA phosphoryl donor was annealed in 5 mM HEPES, 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. For assays with GTP as the phosphoryl donor, the pppRNA was omitted and 2 μL of 1 mM GTP was added from a 10 mM stock solution after annealing in 8 μL volume. The DNA-catalyzed phosphorylation reaction was initiated by bringing the sample to 20 μL total volume containing 70 mM HEPES, pH 7.5, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl (or other ion concentrations as appropriate). The Mn^{2+} was added from a 10× stock solution containing 200 mM $MnCl_2$. The Zn^{2+} was added from a 10× stock solution containing 5 mM $ZnCl_2$, 10 mM HNO_3, and 100 mM HEPES at pH 7.5; this stock solution was freshly prepared from a 200× stock of 100 mM $ZnCl_2$ in 200 mM HNO_3. The metal ion stocks were added last to the final sample, which was divided into 2 μL aliquots that were all incubated at 37 °C. At appropriate time points, 2 μL aliquots were quenched with 5 μL stop solution (80% formamide, 1× TBE [89 mM each Tris and boric acid and 2 mM EDTA, pH 8.3], 50 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol). Samples were separated by 20% PAGE and quantified with a PhosphorImager. Values of k_{obs} were obtained by fitting the yield versus time data directly to first-order kinetics; i.e., yield $Y = Y_{max}\bullet(1 - e^{-kt})$, where $k = k_{obs}$ and Y_{max} is the final yield. Errors in k_{obs} values were calculated as the standard deviation from the indicated number of independent

determinations. When k_{obs} was sufficiently low such that an exponential fit was not meaningful, the initial points were fit to a straight line, and k_{obs} was taken as the slope of the line.

Additional data for deoxyribozyme kinetics and metal dependence

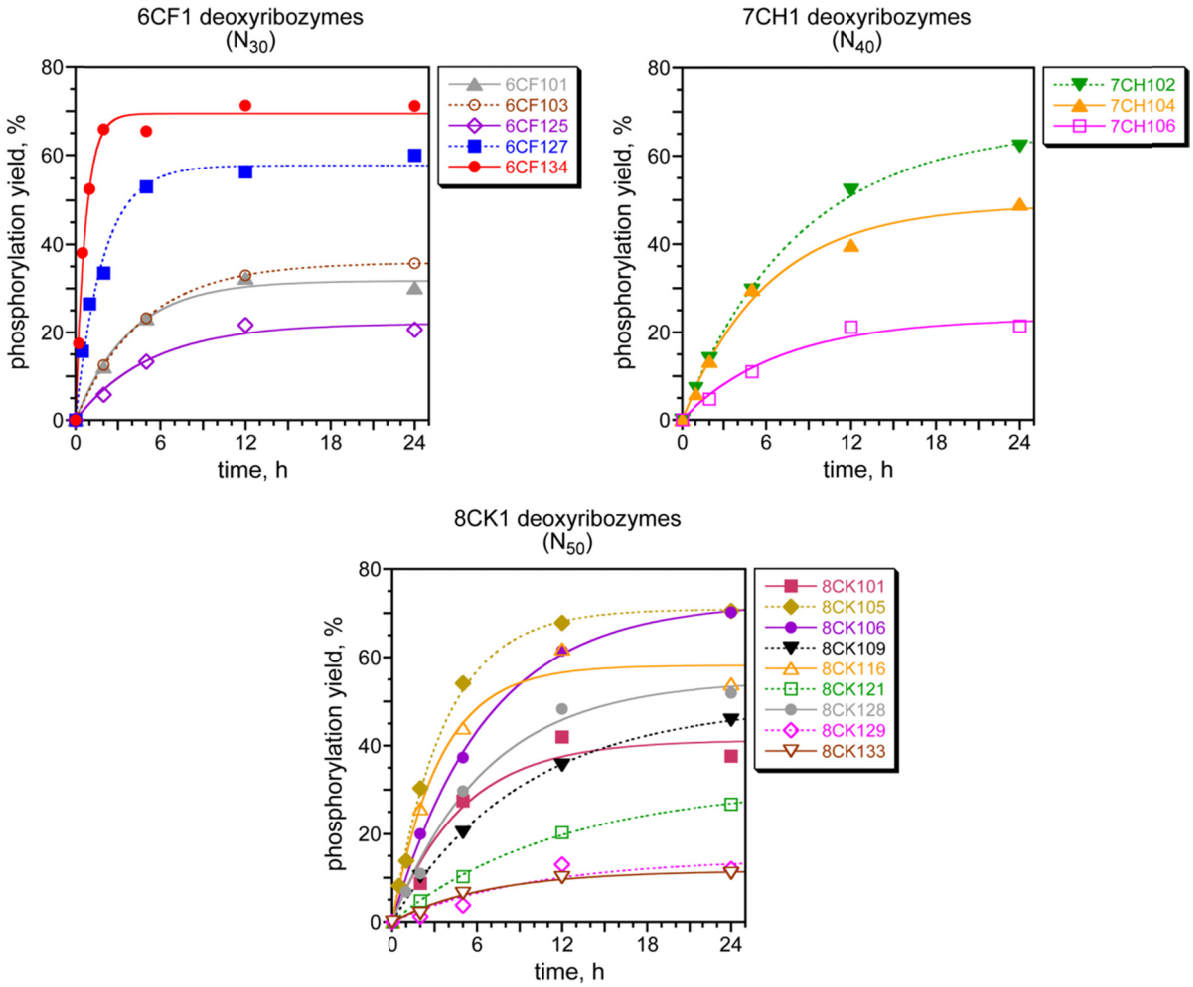

Figure S5. Kinetic data for tyrosine kinase deoxyribozymes that use pppRNA as the phosphoryl donor. Incubation conditions: 70 mM HEPES, pH 7.5, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl at 37 °C. Data for the six deoxyribozymes of Figure 3 are included for completeness and to facilitate comparisons. k_{obs} values (h^{-1}) were as follows. 6CF1 deoxyribozymes (N_{30}): 6CF101 0.26, 6CF103 0.21, 6CF125 019, 6CF127 0.52, and 6CF134 1.42. 7CH1 deoxyribozymes (N_{40}): 7CH102 0.12, 7CH104 0.16, and 7CH106 0.14. 8CK1 deoxyribozymes (N_{50}): 8CK101 0.20, 8CK105 0.27, 8CK106 0.15, 8CK109 0.11, 8CK116 0.30, 8CK121 0.085, 8CK128 0.15, 8CK129 0.10, and 8CK133 0.15.

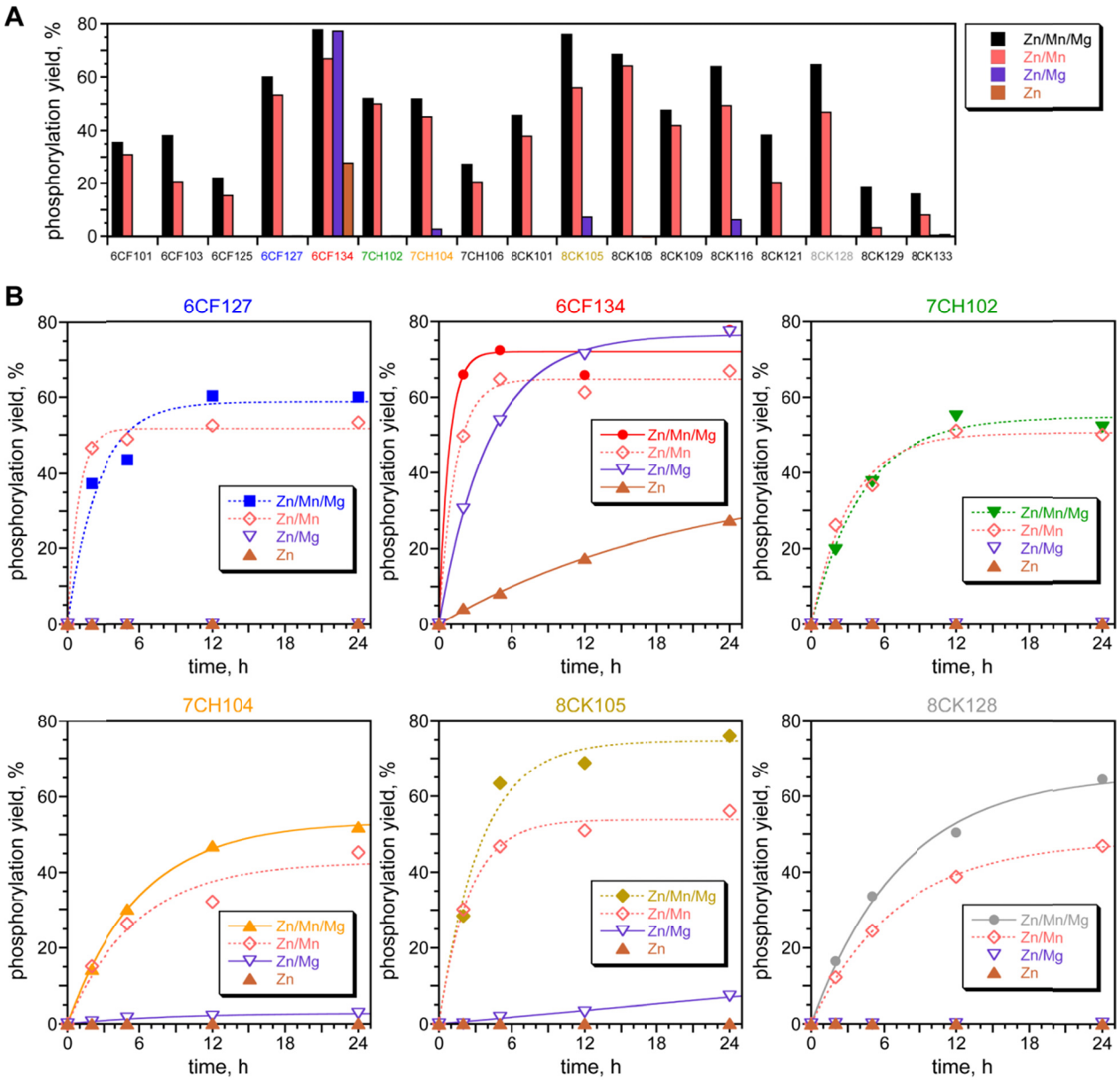

Figure S6. Metal dependence data for tyrosine kinase deoxyribozymes that use pppRNA as the phosphoryl donor. Incubation conditions: 70 mM HEPES, pH 7.5, combinations of 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, and 40 mM $MgCl_2$ as indicated, and 150 mM NaCl at 37 °C. (A) Data at $t = 24$ h as in Figure 3B for all deoxyribozymes. The combination Mn/Mg had no activity in all cases (<0.5%; data not shown). (B) Kinetic plots for the six deoxyribozymes of Figure 3B.

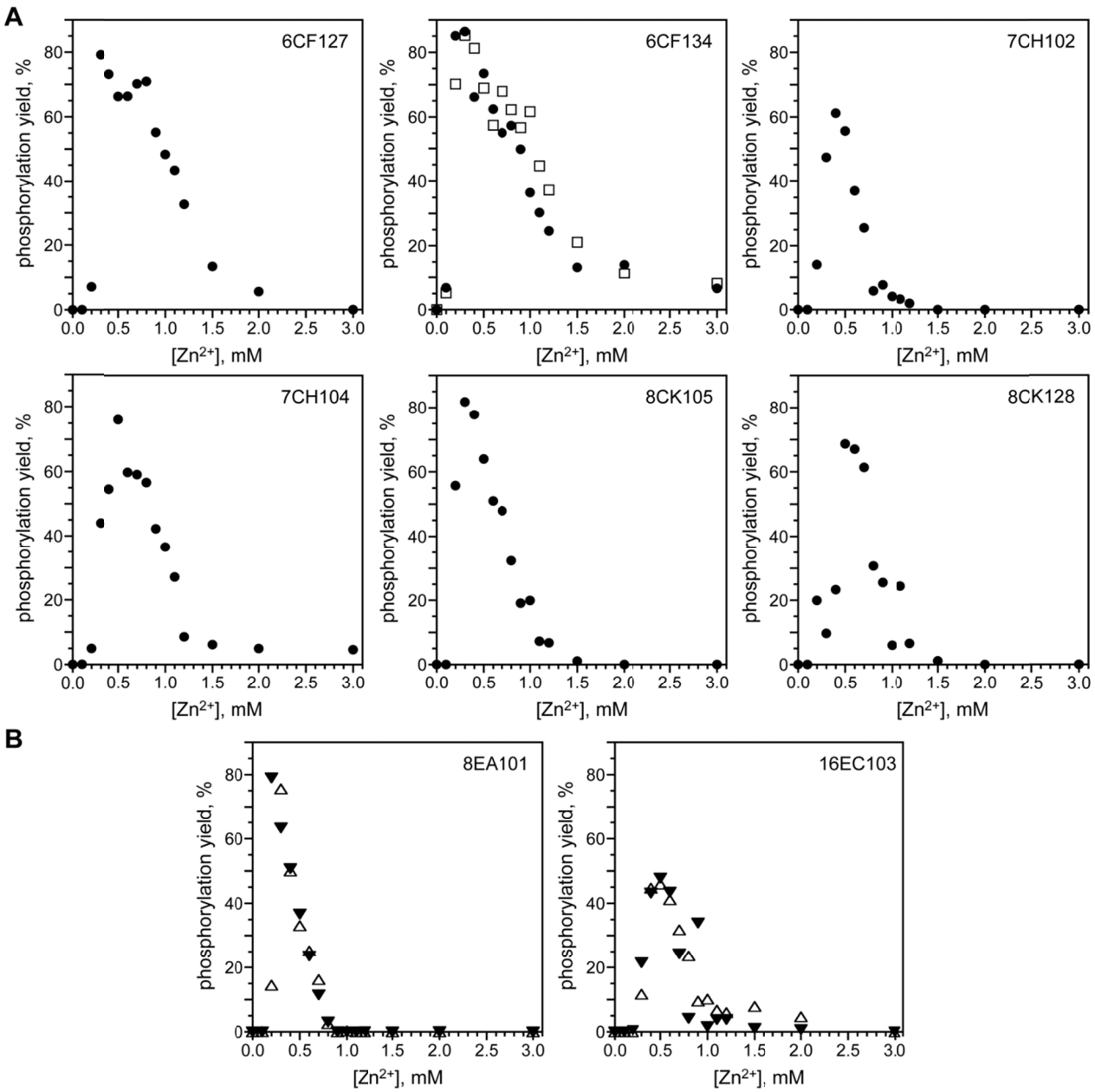

Figure S7. Dependence of tyrosine kinase deoxyribozymes on Zn^{2+} concentration. Incubation conditions: 70 mM HEPES, pH 7.5, 0–3 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl at 37 °C for 24 h. The annealing buffer contributed a final EDTA concentration of 0.05 mM (for samples with pppRNA) or 0.04 mM (for samples with GTP); the Zn^{2+} concentration in the plots was uncorrected for EDTA chelation. Different symbols correspond to different data sets. (A) Deoxyribozymes that use pppRNA as the phosphoryl donor. (B) Deoxyribozymes that use GTP as the phosphoryl donor.

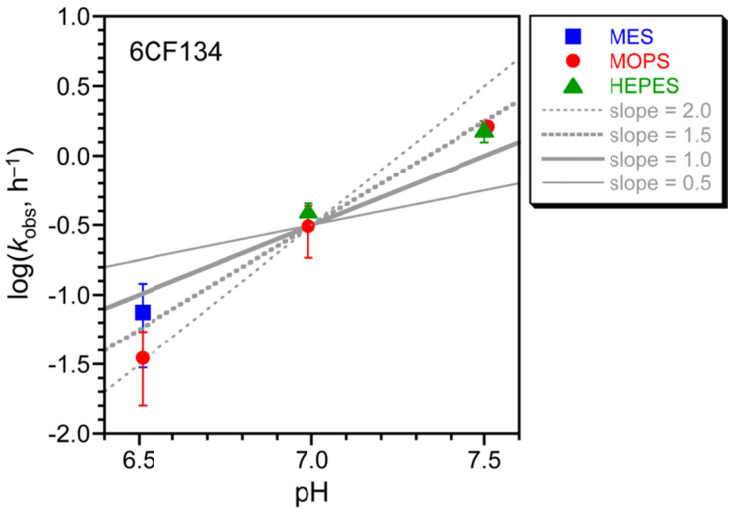

Figure S8. pH dependence data for the 6CF134 deoxyribozyme. Incubation conditions: 70 mM MOPS, MES, or HEPES at the indicated pH for the 1 M stock solution, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl at 37 °C. Each data point is plotted as mean ± standard deviation (n = 4). Because of the apparent dependence of k_{obs} on buffer compound identity (compare MES versus MOPS at pH 6.5), assigning a single slope from these data is challenging. The straight lines are for reference and have slopes of 0.5, 1.0, 1.5, and 2.0. The best estimate of the slope is between 1.0 and 1.5.

Dependence of deoxyribozyme activities on peptide and tether

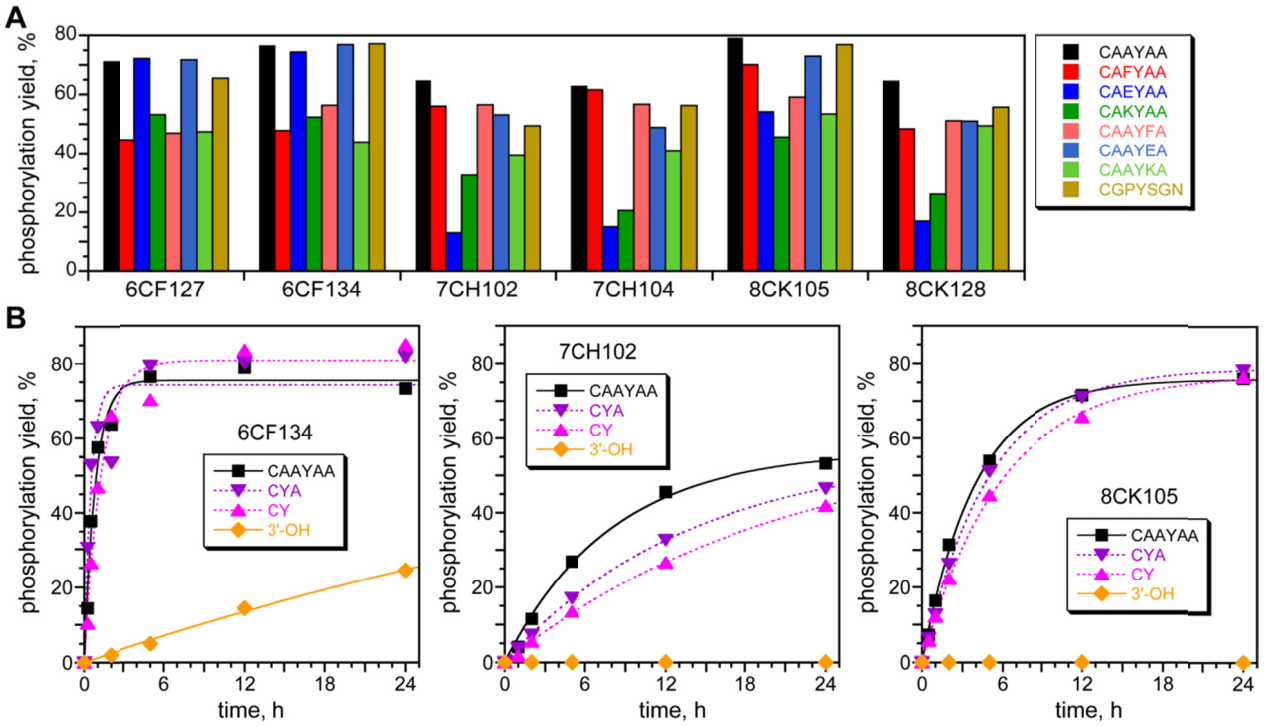

Figure S9. Dependence of deoxyribozyme activity on peptide sequence and length. Incubation conditions: 70 mM HEPES, pH 7.5, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl at 37 °C. (A) Peptide sequence dependence. The substrate included the indicated peptide on a HEG tether (t = 24 h). Data are shown here for all six deoxyribozymes of Figure 3 (data for 6CF134 and 7CH102 are also in Figure 4). 6CF127 and 8CK105 behave similarly to 6CF134; all three deoxyribozymes tolerate each of Phe (F), Glu (E), or Lys (K) in place of Ala (A) at both positions flanking the Tyr (Y) residue. The substrate with GPYSGN was evaluated because these amino acids are found at the N-terminus of the natural human rhinovirus 14 sequence, where the Tyr is attached to an RNA strand;[6] here this sequence serves as a test substrate that has a Tyr flanked by many non-Ala residues. (B) Peptide length dependence. The substrate included the indicated peptide on a HEG tether, or the substrate provided only a DNA 3′-hydroxyl group for phosphorylation. Full kinetic plots are shown for the 6CF134, 7CH102, and 8CK105 deoxyribozymes, which are representative of the three types of behavior illustrated in Figure 4 (6CF134: not selective among the three peptide lengths and also slightly active with the DNA 3′-hydroxyl substrate; 7CH102: modestly selective for the longer parent CAAYAA substrate over the shorter CYA and CY substrates; 8CK105: not selective among the three peptide lengths). k_{obs} values (h^{-1}) were as follows, listed top to bottom for each data set. 6CF134: 1.23, 2.09, 0.80, 0.012. 7CH102: 0.13, 0.071, 0.047, ~0. 8CK105: 0.25, 0.20, 0.17, ~0.

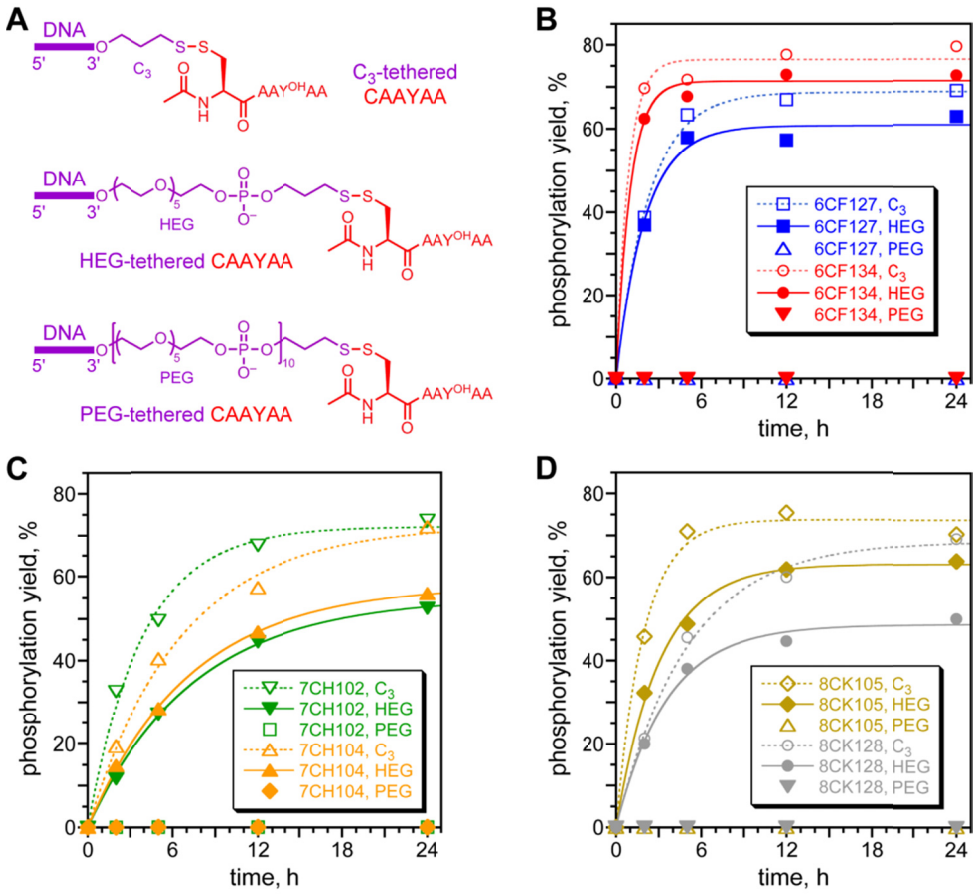

Figure S10. Dependence of deoxyribozyme activity on length of tether connecting the DNA anchor to the CAAYAA hexapeptide substrate. Incubation conditions: 70 mM HEPES, pH 7.5, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, and 150 mM NaCl at 37 °C. (A) Structures of the three substrates. (B) 6CF127 and 6CF134 deoxyribozymes. (C) 7CH102 and 7CH104 deoxyribozymes. (D) 8CK105 and 8CK128 deoxyribozymes.

Apparent K_m(GTP) of 8EA101 and 16EC103 deoxyribozymes

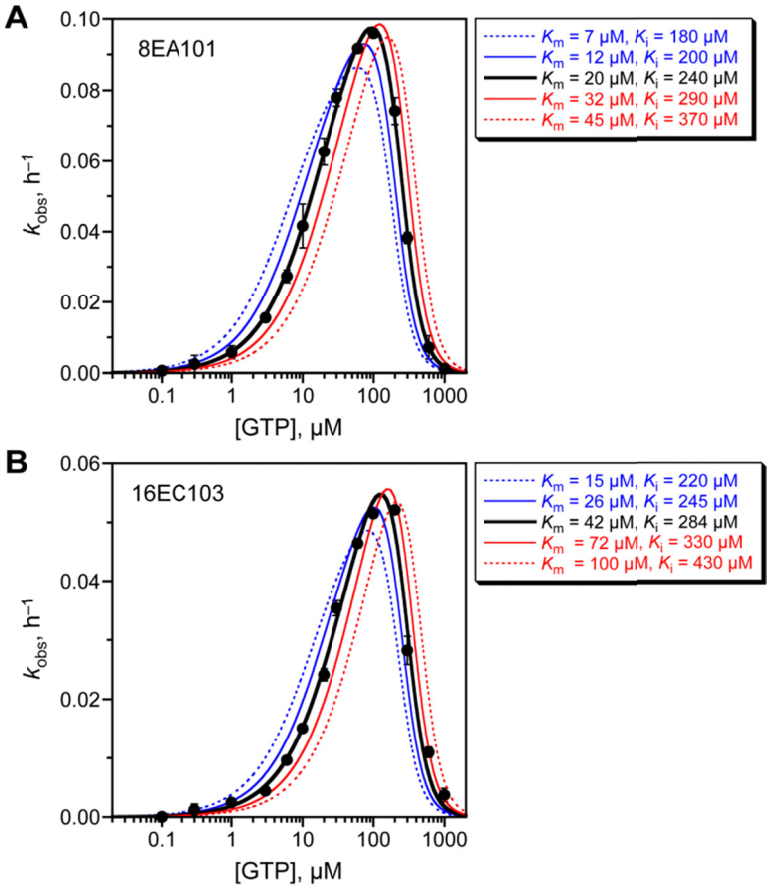

Figure S11. Determining the apparent K_m(GTP) of the 8EA101 and 16EC103 deoxyribozymes. Each deoxyribozyme was assayed under incubation conditions of 70 mM HEPES, pH 7.5, 0.5 mM $ZnCl_2$, 20 mM $MnCl_2$, 40 mM $MgCl_2$, 150 mM NaCl, and 0.1–1000 μM GTP at 37 °C, with k_{obs} values determined from initial-rate kinetics (linear fits to plots of yield versus time from 0 to 2 h; $n = 3$ at each GTP concentration). The k_{obs} values are plotted as mean ± standard deviation, noting that some error bars are smaller than the sizes of the data points. The numerical k_{obs} values from initial-rate kinetics are slightly different from those obtained by fitting the full progress curves (e.g., left-hand plot in Figure 5), but all of the apparent K_m values were determined using internally consistent k_{obs} values from initial-rate kinetics. Data were fit to $k_{obs} = k_{cat} \bullet [C^n/(K_m{}^n + C^n)] \bullet [1 - C^m/(K_i{}^m + C^m)]$, where k_{cat} is the apparent k_{cat} value (i.e., k_{obs} at saturating GTP concentration), C is the GTP concentration, K_m is the apparent K_m(GTP) value for productive GTP binding, K_i is the inhibition constant for unproductive GTP binding, and n and m are Hill coefficients for productive and unproductive GTP binding, respectively. Data for each deoxyribozyme fit well to $n = 1$ and $m = 3$; the corresponding fit values of apparent K_m and K_i did not depend strongly on the precise values of n and m. (A) 8EA101. Fitting both apparent K values simultaneously gave $K_m = 20 \pm 1$ μM and $K_i = 240 \pm 4$ μM. However, other combinations of apparent K_m and K_i also led to acceptable curve fits, as illustrated. From these data, we conservatively estimate that apparent K_m is between 12–32 μM and apparent K_i is between 200–290 μM. (B) 16EC103. Fitting both apparent K values simultaneously gave $K_m = 42 \pm 7$ μM and $K_i = 284 \pm 17$ μM. However, other combinations of apparent K_m and K_i also led to acceptable curve fits, as illustrated. From these data, we conservatively estimate that apparent K_m is between 26–72 μM and apparent K_i is between 245-330 μM.

References for Supporting Information

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