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Supporting Information

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A Generalizable DNA-Catalyzed Approach to Peptide–Nucleic Acid Conjugation

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Procedure for preparation of 3'-³²P-radiolabeled 5'-ImpDNA

The 3'-³²P-radiolabeled 5'-ImpDNA for single-turnover kinetic assays was prepared via a multi-step procedure. The unlabeled DNA oligonucleotide was 3'-³²P-radiolabeled by incubating 20 pmol of oligonucleotide, 20 μCi of α-³²P-dCTP (800 Ci/mmol), and 10 units of terminal deoxytransferase (Fermentas) in 20 μL of 1× TdT reaction buffer (200 mM potassium cacodylate, 25 mM Tris, pH 7.2, 0.01% Triton X-100, and 1 mM CoCl₂) for 30 min at 37 °C. The TdT was inactivated by heating at 75 °C for 20 min. The 3'-³²P-radiolabeled oligonucleotide was 5'-phosphorylated in the same tube by adding 5 units of T4 polynucleotide kinase (Fermentas) and ATP to 1 mM in a total volume of 30 μL, followed by incubation at 37 °C for 1 h and purification by 20% PAGE. The resulting sample was assumed to contain 20 pmol of PAGE-purified 5'-phosphorylated 3'-³²P-radiolabeled oligonucleotide. To form 5'-Imp, a 5 pmol portion was incubated in a total volume of 25 μL containing 100 mM 1-ethyl 3-(3-dimethylaminopropyl) carbodiimide (EDC), 100 mM imidazole (pH 6.0 with HCl), and 5 pmol of a 5'-phosphorylated decoy oligo of unrelated sequence (included to suppress nonspecific sticking to the subsequent desalting column) at room temperature for 2 h. A Micro Bio-Spin P-6 desalting column (Bio-Rad) was prepared by centrifuging at 1000 g for 1 min and rinsing 4× by adding 500 μL of water followed by centrifuging at 1000 g for 1 min. The 25 μL sample was applied to the column and eluted by centrifuging at 1000 g for 4 min. The eluant was dried by SpeedVac and redissolved in 25 μL of water. The resulting sample was assumed to contain 5 pmol of 3'-³²P-radiolabeled 5'-ImpDNA in 25 μL of water (0.2 μM).

Procedures for in vitro selection and cloning

An overview of the key selection and capture steps of each selection round is shown in Fig. 1b of the main text. Table S1 lists all relevant oligonucleotides.

Selections with 5'-pppRNA substrates

Procedure for ligation step in round 1. A 50 μL sample containing 1 nmol of DNA pool, 1 mM ATP, 1× T4 PNK buffer A, and 10 units of T4 polynucleotide kinase (Fermentas) was incubated at 37 °C for 2.5 h. T4 polynucleotide kinase was removed by phenol/chloroform extraction followed by ethanol precipitation. The sample was dissolved in 20 μL of 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA containing 1.2 nmol of 5'-pppRNA substrate and annealed by heating at 95 °C for 3 min and cooling on ice for 5 min. The ligation reaction was initiated by bringing the sample to a final volume of 30 μL containing 50 mM Tris, pH 7.5, 10 mM DTT, 5 mM MgCl₂, 0.05 mM ATP and 20 units of T4 RNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h and purified by 8% PAGE.

Procedure for ligation step in subsequent rounds. A 10 μL sample containing the PCR-amplified DNA pool (~5–10 pmol) and 100 pmol of 5'-pppRNA 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. The ligation reaction was initiated by bringing the sample to a final volume of 20 μL containing 50 mM Tris, pH 7.5, 10 mM DTT, 5 mM MgCl₂, 0.05 mM ATP and 10 units of T4 RNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h and purified by 8% PAGE.

Procedure for selection step in round 1. Each selection experiment was initiated with 200 pmol of the ligated N₄₀ pool. A 20 μL sample containing 200 pmol of ligated N₄₀ pool 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 XJ and XK selections additionally included 250 pmol of 19 nt oligonucleotide complementary to the 3'-binding arm). The selection reaction was initiated by bringing the sample to 40 μL total volume containing 70 mM HEPES, pH 7.5, 1 mM azido-AYA or azido-GPYSGN peptide (added from 50 mM stock solution in DMF), each of 40 mM MgCl₂ and 20 mM MnCl₂ if included, 1 mM ZnCl₂, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h followed by ethanol precipitation.

Procedure for selection step in subsequent rounds. A 10 μL sample containing the ligated pool 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 XJ and XK selections additionally included 50 pmol of 19 nt oligonucleotide complementary to the 3'-binding arm in odd rounds and 40 nt oligonucleotide complementary to the 3'-

binding arm in even rounds). The selection reaction was initiated by bringing the sample to 20 μL total volume containing 70 mM HEPES, pH 7.5, 1 mM or 100 μM or 10 μM azido-AYA or azido-GPYSGN peptide (added from a stock solution of 50 mM peptide in DMF, 1 mM peptide in 2% aqueous DMF, or 100 μM peptide in 0.2% aqueous DMF), each of 40 mM MgCl_2 and 20 mM MnCl_2 if included, 1 mM ZnCl_2 , and 150 mM NaCl. The sample was incubated at 37 $^\circ\text{C}$ for 14 h followed by ethanol precipitation.

Procedure for CuAAC capture step in round 1. The precipitated selection sample was brought to 20 μL total volume containing 200 pmol of DNA splint for capture, 300 pmol of 49 nt 3'-alkyne capture oligonucleotide, 50 mM HEPES, pH 7.5, 150 mM NaCl, 2.8 mM tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), 0.8 mM sodium ascorbate, and 0.4 mM CuSO_4 . Concentrations of THPTA, sodium ascorbate, and CuSO_4 were optimized through preliminary experiments (not shown). The three reagents were added from 28 mM (10 \times), freshly prepared 20 mM (25 \times), and 4 mM (10 \times) stock solutions, respectively; the three reagents were mixed together and then added at once to the other components. The sample was incubated at 37 $^\circ\text{C}$ for 1 h and separated by 8% PAGE. Including an annealing step before adding the THPTA, sodium ascorbate, and CuSO_4 did not increase the yield of the capture reaction.

Procedure for CuAAC capture step in subsequent rounds. The precipitated selection sample was brought to 20 μL total volume containing 50 pmol of DNA splint for capture, 100 pmol of 16 nt (even rounds) or 49 nt (odd rounds) 3'-alkyne capture oligonucleotide, 50 mM HEPES, pH 7.5, 150 mM NaCl, 2.8 mM tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), 0.8 mM sodium ascorbate, and 0.4 mM CuSO_4 . THPTA, sodium ascorbate, and CuSO_4 were added from 28 mM (10 \times), freshly prepared 20 mM (25 \times), and 4 mM (10 \times) stock solutions, respectively; the three reagents were mixed together and then added at once to the other components. The sample was incubated at 37 $^\circ\text{C}$ for 1 h and separated by 8% PAGE.

Procedure for PCR. 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 5'-phosphorylated forward primer, 50 pmol of reverse primer, 20 nmol of each dNTP, and 10 μL of 10 \times Taq polymerase buffer (1 \times = 20 mM Tris-HCl, pH 8.8, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 10 mM KCl, 2 mM MgSO_4 , and 0.1% Triton X-100). This sample was cycled 10 times according to the following PCR program: 94 $^\circ\text{C}$ for 2 min, 10 \times (94 $^\circ\text{C}$ for 30 s, 47 $^\circ\text{C}$ for 30 s, 72 $^\circ\text{C}$ for 30 s), 72 $^\circ\text{C}$ 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 5'-phosphorylated 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 \times Taq polymerase buffer. This sample was cycled 30 times according to the following PCR program: 94 $^\circ\text{C}$ for 2 min, 30 \times (94 $^\circ\text{C}$ for 30 s, 47 $^\circ\text{C}$ for 30 s, 72 $^\circ\text{C}$ for 30 s), 72 $^\circ\text{C}$ for 5 min. The sample was separated by 8% PAGE.

Selections with 5'-ImpDNA substrates

Procedure for ligation and 5'-phosphorylation steps in round 1. A 50 μL sample containing 1 nmol of DNA pool, 1 mM ATP, 1 \times T4 PNK buffer A, and 10 units of T4 polynucleotide kinase (Fermentas) was incubated at 37 $^\circ\text{C}$ for 2.5 h. T4 polynucleotide kinase was removed by phenol/chloroform extraction followed by ethanol precipitation. The sample was dissolved in 20 μL of 5 mM Tris, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA containing 1.2 nmol of DNA splint and 1.5 nmol of DNA substrate and annealed by heating at 95 $^\circ\text{C}$ for 3 min and cooling on ice for 5 min. The ligation reaction was initiated by bringing the sample to a final volume of 40 μL containing 1 \times T4 DNA ligase buffer and 5 units of T4 DNA ligase (Fermentas). The sample was incubated at 37 $^\circ\text{C}$ for 12 h, and T4 DNA ligase was inactivated by heating at 65 $^\circ\text{C}$ for 30 min. To avoid adenylation of the 5'-phosphorylated DNA substrate by T4 DNA ligase, the 5'-phosphate was incorporated onto the DNA substrate (now attached to the DNA pool) after the ligation step. The ligation product was 5'-phosphorylated in 50 μL volume containing 1 mM ATP, 1 \times T4 PNK buffer A, and 10 units of T4 polynucleotide kinase (Fermentas). The sample was incubated at 37 $^\circ\text{C}$ for 5 h and purified by 8% PAGE.

Procedure for ligation and 5'-phosphorylation steps in subsequent rounds. A 17 μL sample containing the PCR-amplified DNA pool (~5–10 pmol), 50 pmol of DNA splint, and 100 pmol of DNA 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. The ligation reaction was initiated by bringing the sample to a final volume of 20 μ L containing 1 \times T4 DNA ligase buffer and 1 unit of T4 DNA ligase (Fermentas). The sample was incubated at 37 °C for 12 h, and T4 DNA ligase was inactivated by heating at 65 °C for 30 min. The ligation product was 5'-phosphorylated in 30 μ L volume containing 1 mM ATP, 1 \times T4 PNK buffer A, and 5 units of T4 polynucleotide kinase (Fermentas). The sample was incubated at 37 °C for 1 h and purified by 8% PAGE.

Procedure for 5'-phosphorimidazolide (5'-Imp) formation. Immediately prior to performing the selection step, a 25 μ L sample containing the 5'-phosphorylated ligation product, 100 mM 1-ethyl 3-(3-dimethylaminopropyl) carbodiimide (EDC), and 100 mM imidazole (pH 6.0 with HCl) was incubated at room temperature for 2 h. A Micro Bio-Spin P-6 desalting column (Bio-Rad) was prepared by centrifuging at 1000 g for 1 min and rinsing 4 \times by adding 500 μ L of water followed by centrifuging at 1000 g for 1 min. The 25 μ L sample was applied to the column and eluted by centrifuging at 1000 g for 4 min. For round 1, the eluant was quantified by NanoDrop. For subsequent rounds, the eluant was dried by SpeedVac.

Procedure for selection step in round 1. Each selection experiment was initiated with 200 pmol of the 5'-Imp ligated N₄₀ pool. A 20 μ L sample containing 200 pmol of 5'-Imp ligated N₄₀ pool 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 μ L total volume containing 50 mM ($-Zn^{2+}$) or 70 mM ($+Zn^{2+}$) HEPES, pH 7.5, 1 mM azido-AYA peptide (added from 50 mM stock solution in DMF), each of 40 mM MgCl₂, 20 mM MnCl₂, 1 mM ZnCl₂ if included, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h followed by ethanol precipitation.

Procedure for selection step in subsequent rounds. A 10 μ L sample containing the 5'-Imp ligated N₄₀ pool 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 μ L total volume containing 50 mM ($-Zn^{2+}$) or 70 mM ($+Zn^{2+}$) HEPES, pH 7.5, 1 mM or 100 μ M azido-AYA peptide (added from 50 mM peptide in DMF or 1 mM peptide in 2% aqueous DMF), each of 40 mM MgCl₂, 20 mM MnCl₂, 1 mM ZnCl₂ if included, and 150 mM NaCl. The sample was incubated at 37 °C for 14 h followed by ethanol precipitation.

Procedure for CuAAC capture step in round 1. The procedure for the 5'-pppRNA substrate was used.

Procedure for CuAAC capture step in subsequent rounds. The procedure for the 5'-pppRNA substrate was used.

Procedure for PCR. The procedure for the 5'-pppRNA substrate was used.

Cloning and screening of individual deoxyribozymes

PCR primers used for cloning are listed in Table S1 (the forward cloning primer was same as the forward PCR primer for selection, but without the 5'-phosphate). The 10-cycle PCR product from the appropriate selection round was diluted 10³-fold. A 50 μ L sample was prepared containing 1 μ L of the diluted 10-cycle PCR product from the appropriate selection round, 100 pmol of forward cloning primer, 25 pmol of reverse cloning primer, 10 nmol of each dNTP, and 5 μ L of 10 \times Taq polymerase buffer. This sample was cycled 30 times according to the following PCR program: 94 °C for 2 min, 30 \times (94 °C for 30 s, 47 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. The sample was separated by 1% agarose gel and extracted using a GeneJET Gel Extraction Kit (Fermentas). The extracted product was quantified by absorbance (A₂₆₀) and diluted to 4–8 ng/ μ L. A 1 μ L portion of the diluted PCR product was inserted into the pCR2.1-TOPO vector using a TOPO TA cloning kit (Life Technologies). Individual *E. coli* colonies harboring plasmids with inserts were identified by blue-white screening and grown in LB/amp media. Miniprep DNA was prepared using a GeneJET Plasmid Miniprep Kit (Fermentas) and screened for properly sized inserts by EcoRI digestion and agarose gel analysis. Before sequencing, assays of individual deoxyribozyme clones were performed with PAGE-purified DNA strands prepared by PCR from the miniprep DNA, using the single-turnover assay procedure described in the main text.

oligonucleotide purpose	oligonucleotide sequence
XJ/XK pool ^{a,b}	CGAACGAAAGCCTCCTTC-N ₄₀ -ATACGCATAAAGGTAGAGCTGATCCTGATGG
EM1 pool ^c	CGAAATAGATTATCATTC-N ₄₀ -ATAATTAGTAACCTGAAGCTGATCCTGATGG
EN1 pool ^d	CGAACATTGCAACAGTTC-N ₄₀ -ATATCATCAGTGGCTCAGCTGATCCTGATGG
EP1 pool ^e	CGAAGTATAAACCTGTTC-N ₄₀ -ATATTTGTATCCAGGAAGCTGATCCTGATGG
EN1 splint for ligation	GAACTGTTGCAATGTTCCGTCCTTGCACAGTTC
EP1 splint for ligation	GACAGGTTTATACTTCGTCCTATAAACCTGTTC
XJ/XK 5'-pppRNA substrate	GGAAGGAGGCUUUCGGG
EM1 5'-pppRNA substrate	GGAAUGAUAUUAUUGG
EN1 5'-ImpDNA substrate	GGAAGTGTGCAATGGGA
EP1 5'-ImpDNA substrate	GGAACAGGTTTATACGGA
decoy oligo for 5'-Imp formation (5'-p)	CGAACGAAAGCCTCCTTC
XJ/XK complement to 3'-binding arm (19 nt)	GGACTACCTTTATGCGTAT
XJ/XK complement to 3'-binding arm (40 nt)	(AAC) ₇ GGACTACCTTTATGCGTAT
XJ/XK splint for capture	TTTCGCCGAAAGCCTCCTTCCCGTGTCTGATGCGGC
EM1 splint for capture	TTTCGCCATAGATTATCATTCCTCCGCTGTCTGATGCGGC
EN1 splint for capture	TTTCGTCCTTGCACAGTTCCTCCGCTGTCTGATGCGGC
EP1 splint for capture	TTTCGTCCTATAAACCTGTTCCTCCGCTGTCTGATGCGGC
3'-alkyne capture oligo (16 nt)	GCCGCATCAGACAGCG
3'-alkyne capture oligo (49 nt)	(AAC) ₁₁ GCCGCATCAGACAGCG
XJ/XK forward PCR primer for selection (5'-p)	CGAACGAAAGCCTCCTTC
EM1 forward PCR primer for selection (5'-p)	CGAAATAGATTATCATTC
EN1 forward PCR primer for selection (5'-p)	CGAACATTGCAACAGTTC
EP1 forward PCR primer for selection (5'-p)	CGAAGTATAAACCTGTTC
reverse PCR primer for selection	(AAC) ₄ XCCATCAGGATCAGCT
reverse PCR primer for cloning	TAATTAATTAATTACCCATCAGGATCAGCT
decoy oligo for assay of 8XJ105	TTATGCGTATCCATTCCGTAAGTTTCCGCTGAGAGACATCTCCCGAAGGAGGCT

Table S1. Oligonucleotides used during the in vitro selection, cloning, and assay procedures. All sequences are written 5' to 3' and are DNA unless indicated explicitly as RNA. The 3'-alkyne was introduced using 3'-alkyne serinol CPG (Glen Research). In the reverse PCR primer for selection, X denotes the HEG spacer to stop Taq polymerase.

^a XJ denotes the selection with azido-AYA peptide, pppRNA, and Mg²⁺/Mn²⁺/Zn²⁺.

^b XK denotes the selection with azido-AYA peptide, pppRNA, and Zn²⁺.

^c EM1 denotes the selection with azido-GPYSGN peptide, pppRNA, and Mg²⁺/Mn²⁺/Zn²⁺.

^d EN1 denotes the selection with azido-AYA peptide, ImpDNA, and Mg²⁺/Mn²⁺.

^e EP1 denotes the selection with azido-AYA peptide, ImpDNA, and Mg²⁺/Mn²⁺/Zn²⁺.

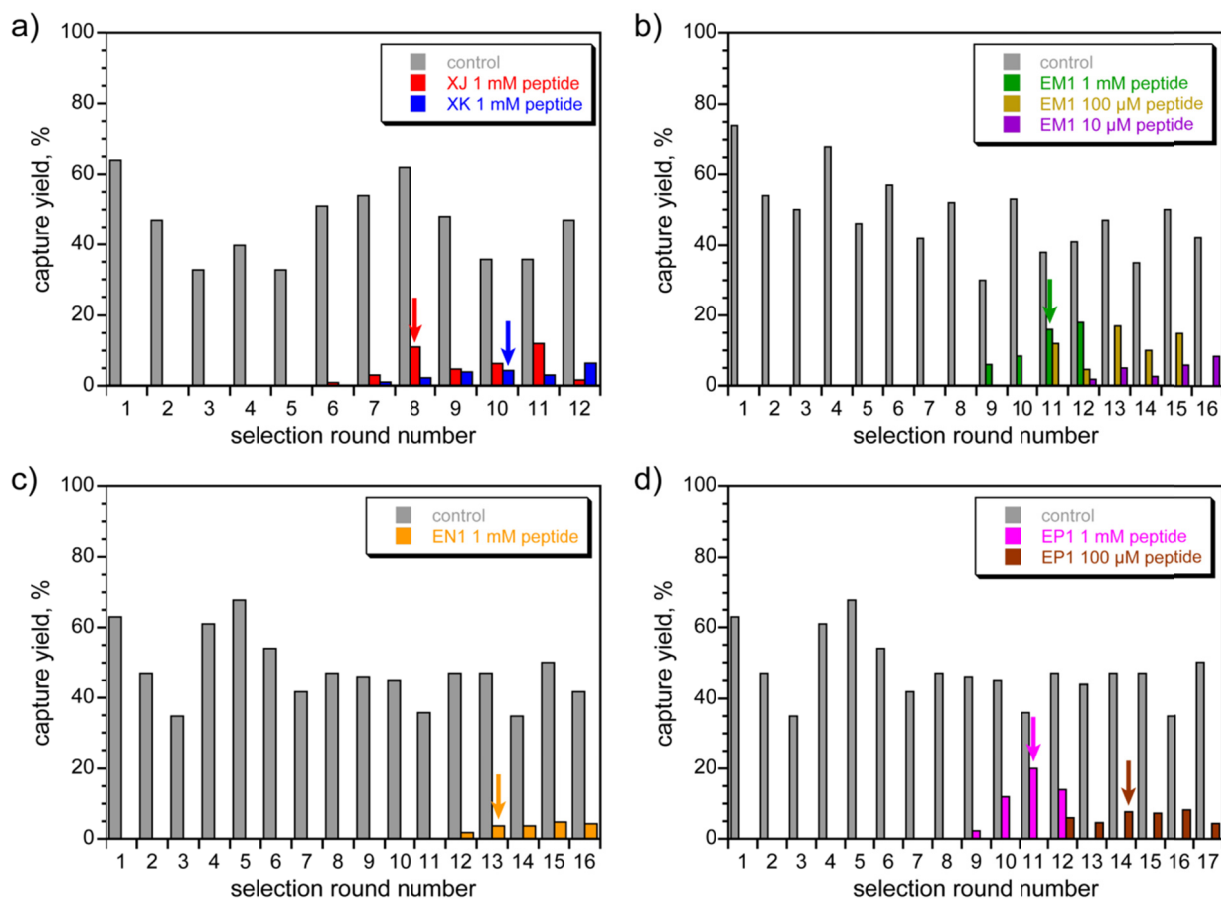
In vitro selection progressions

Figure S1. Progressions of the in vitro selection experiments. “Control” refers to the yield for the CuAAC standard capture reaction, which used a random DNA pool ligated to a conjugate between an azido-peptide and RNA. This conjugate was prepared using the 15MZ36 deoxyribozyme, the azido-AYA peptide, and 5'-pppRNA for the XJ/XK selections (Table S1). “Selection” refers to the yield for the CuAAC capture reaction using the deoxyribozyme pool for that round. Arrows mark the cloned rounds. See main text and Table S1 footnotes for descriptions of selection designations. a) XJ and XK selections. b) EM1 selection. c) EN1 selection. d) EP1 selections.

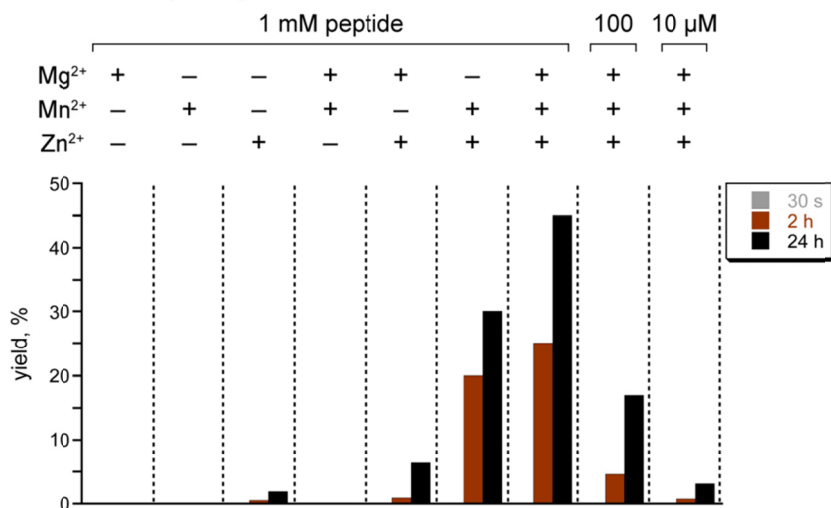
Sequences of individual deoxyribozymes

	1	10	20	30	40	
8XJ105	GGGAGATGTC	TCTCAGACGG	AAACTTTTCAG	TACGGAATGG	40	(1)
10XK22	AC . . TT . . AA	GACT . AGT . .	CT . GGGAG . A	GTGAA . C . C .	40	(4)
10XK35	AC . . CT . . AA	GA . T . AGT . .	CTCGGG . A . T	. TTTAGG . TA	40	(2)
11EM103	GTCGCCAGTC	TCTGCTGCCT	TGGTCATCAA	CCTTTCTGTC	40	(20)
11EP101	TGTGGGCCGT	AAATCGCTTG	CGGTGCTTTT	TGGATGGGGT	40	(6, 5)
11EP103	A . GTT . GG . G	CGTAGTTGCT	TTTG . GCGAA	. ATGCTT . . C	40	(2, 1)
11EP104	G . GA . TAG . G	CCTGG . GCAC	TT . C . GCCCG	. . AGACA . CA	40	(1, 0)
11EP111	G . A . TC . T . .	TTGAGT . GGC	TATCC . G . . A	AT . GC . . . TA	40	(7, 17)
11EP119	G . CT . TTG . C	TC . . . ATA . T	AT . ATTGAG .	. C . . . TC . G	40	(1, 1)
11EP126	A . GTT . GG . G	CGT . ACTGCT	TAA . . TGA . .	CAAT . . . CA .	40	(1, 0)
14EP125	. CG . . . AATC	GTGGGTGGCC	. AAATG . G . .	AATGAA . AAG	40	(0, 2)

Figure S2. Sequences of the deoxyribozymes described in this study. Only the initially random (N_{40}) sequences are shown. All deoxyribozymes were used as 5'-CCCGAAAGCCTCCTTC- N_{40} -ATACGCATAAAGGTAG-3' (XJ/XK selections), 5'-CCATAGATTATCATTTC- N_{40} -ATAATTAGTAACCTGA-3' (EM1 selection), or 5'-CCGTATAAACCTG-TTC- N_{40} -ATATTTGTATCCAGGAAGCTGATCCTGATGG-3' (EP1 selection; the underlined segment is the primer binding region, mentioned below). In each alignment, a dot denotes conservation, i.e., the same nucleotides as in the uppermost sequence; a dash denotes a gap. On the far right is shown the sequence length and (in parentheses) the number of times that the particular sequence was found during cloning. For the EP1 selection, the two numbers in parentheses correspond to rounds 11 and 14, respectively. For the 8XJ and 10XK deoxyribozymes, where the selection rounds were each performed in the presence of a short oligonucleotide complementary to the 3'-binding arm (Table S1), this short oligonucleotide was included in two-fold excess relative to the deoxyribozyme in all single-turnover assays. Omission of the short oligonucleotide led to six-fold decrease in yield for 8XJ105 and two-fold decrease in yield for each of 10XK22 and 10XK35. Each of the 11EP and 14EP deoxyribozymes was assayed both with and without its 3'-terminal primer binding region, which was single-stranded during each selection round. For 11EP104, 11EP111, and 14EP125, the primer binding region could be omitted without loss of rate or yield; each of these deoxyribozymes was assayed in detail without the primer binding region. The other four 11EP deoxyribozymes were inactive without the primer binding region; each of these deoxyribozymes was assayed in detail with the primer binding region.

Quantification of PAGE data for metal ion dependence in Fig. 2a

8XJ105 deoxyribozyme



11EM103 deoxyribozyme

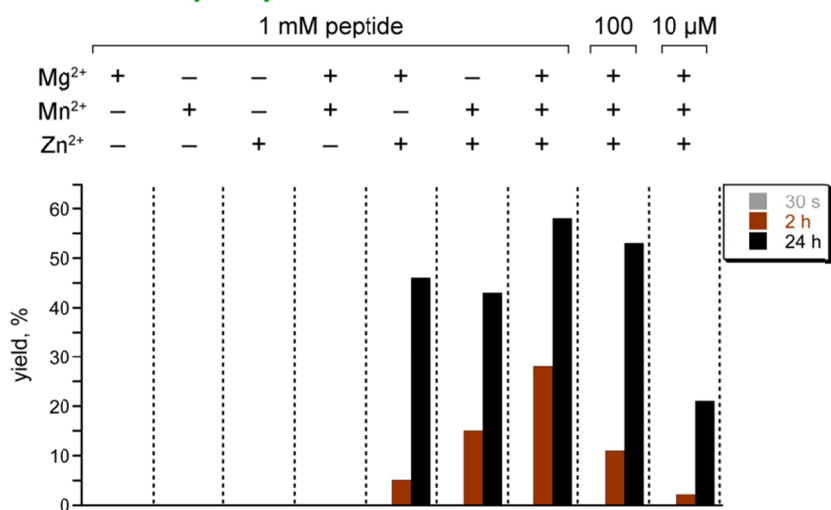


Figure S3. Quantification of PAGE data for metal ion dependence experiments in Fig. 2a.

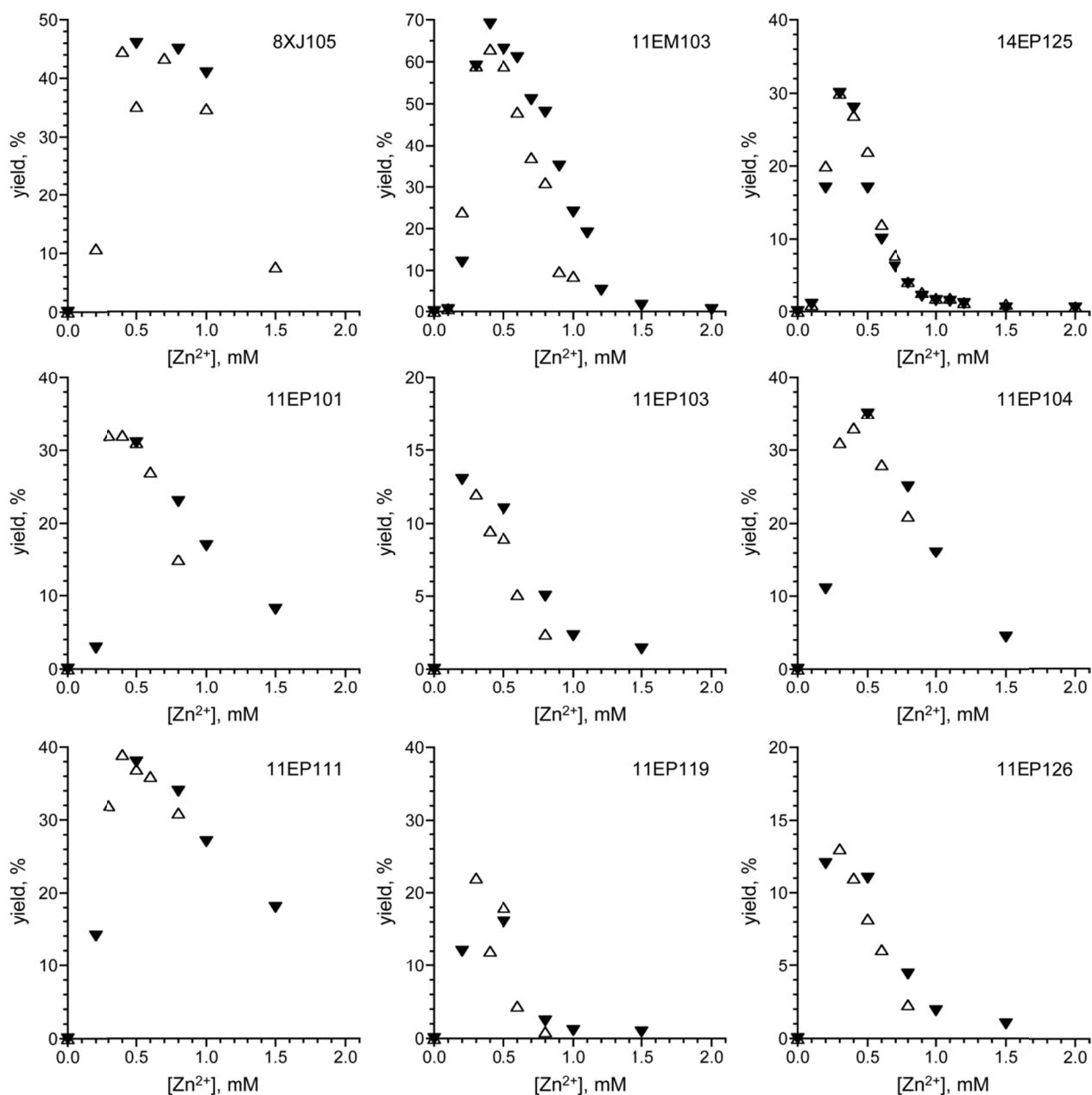
Dependence of deoxyribozymes on Zn^{2+} concentration

Figure S4. Dependence of deoxyribozymes on Zn^{2+} concentration. Incubation conditions: 70 mM HEPES, pH 7.5, 40 mM $MgCl_2$, 20 mM $MnCl_2$, 0–2 mM $ZnCl_2$, and 150 mM NaCl at 37 °C for 14 h. Assays were performed independently of Fig. 3. Data were quantified as yield at 14 h, where the reaction had progressed to its maximal extent. Therefore, these data do not allow determination of precise values of apparent K_d . The annealing buffer contributed a final EDTA concentration of 0.05 mM; the Zn^{2+} concentration in the plots was uncorrected for EDTA chelation. Different symbols correspond to different data sets.

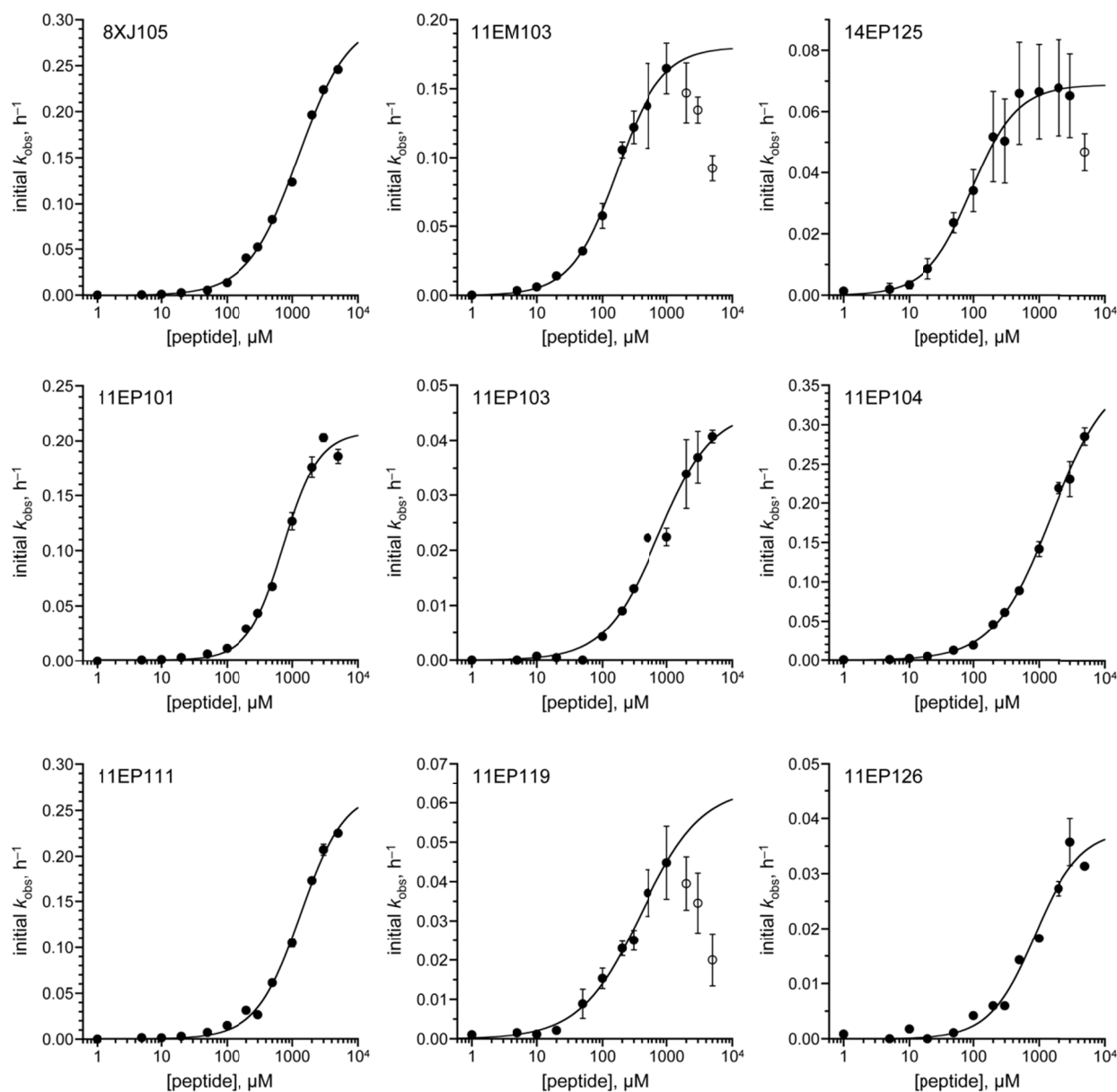
Apparent K_m values for peptide substrates

Figure S5. Determination of apparent K_m values for peptide substrates. All k_{obs} values used to construct these plots were determined by initial-rate kinetics, using time points from 0–2 h. Therefore, these k_{obs} values do not match precisely the analogous k_{obs} values obtained by fitting the full progress curves as shown in Figs. 2 and 3. Incubation conditions: 70 mM HEPES, pH 7.5, 40 mM MgCl_2 , 20 mM MnCl_2 , 1 mM ZnCl_2 (8XJ105) or 0.4 mM ZnCl_2 (all others), and 150 mM NaCl at 37 °C. Peptide was added from a stock solution as follows: 50 mM peptide in DMF (5, 3, and 2 mM peptide), 10 mM peptide in 20% aqueous DMF (1 mM and 500, 300, and 200 μM peptide), 1 mM peptide in 2% aqueous DMF (100, 50, and 20 μM peptide), 0.1 mM peptide in 0.2% aqueous DMF (10 and 5 μM peptide), or 0.01 mM peptide in 0.02% aqueous DMF (1 μM peptide). Apparent K_m values were obtained by fitting to $k_{\text{obs}} = k_{\text{max}} \cdot ([\text{peptide}]^n / (K_m^n + [\text{peptide}]^n))$, where n is the Hill coefficient. Open data points in three of the plots indicate inhibition at very high peptide concentrations and were omitted from the fit. For 11EM103, 14EP125, and 11EP119, error bars are standard deviation ($N = 3$). For 11EP101, 103, 104, 111, and 126, error bars represent half of range ($N = 2$) for $[\text{peptide}] \geq 1$ mM; all other $[\text{peptide}]$ are $N = 1$. For 8XJ105, $N = 1$. Fit apparent K_m values in μM (Hill coefficients): 8XJ105 1230 ± 140 (1.12 ± 0.07); 11EM103 168 ± 16 (1.24 ± 0.09); 14EP125 93 ± 10 (1.24 ± 0.13); 11EP101 731 ± 71 (1.60 ± 0.17); 11EP103 741 ± 196 (1.05 ± 0.17); 11EP104 1570 ± 280 (0.99 ± 0.07); 11EP111 1340 ± 170 (1.28 ± 0.10); 11EP119 392 ± 149 (0.93 ± 0.13); 11EP126 879 ± 270 (1.25 ± 0.28). In all cases except for 14EP125, for which the data points turn over at higher $[\text{peptide}]$, the fit apparent K_m value is an approximate lower limit on the actual value.

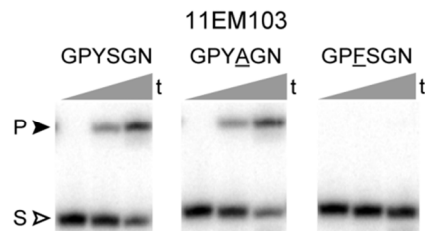
Assays of 11EM103 with variants of the GPYSGN peptide

Figure S6. Assays of 11EM103 with variants of the GPYSGN peptide, to establish the site of nucleophilic reactivity. Both azido-GPYAGN and azido-GPFESGN were prepared using the same procedure as for azido-GPYSGN. The azido-GPFESGN peptide was less soluble in DMF than was azido-GPYSGN, and therefore 25 mM (rather than 50 mM) stock solutions of each peptide in DMF were prepared. The peptide concentration in each assay was 1 mM (4% DMF). Incubation conditions: 70 mM HEPES, pH 7.5, 40 mM MgCl₂, 20 mM MnCl₂, 0.4 mM ZnCl₂, and 150 mM NaCl at 37 °C (*t* = 30 s, 2 h, 24 h).

Assays of stability of the Imp functional group

Several experiments were performed to assess the stability of the Imp functional group. These experiments used 14EP125 and 11EP111 as representative deoxyribozymes (Fig. S7). We first assessed the DNA-catalyzed reactions either without or with the standard annealing step in which all nucleic acid components are heated in 5 mM HEPES, pH 7.5, 15 mM NaCl, and 0.1 mM EDTA at 95 °C for 3 min and then cooled on ice for 5 min before adding the reaction buffer and all requisite metal ion cofactors (70 mM HEPES, pH 7.5, 40 mM MgCl₂, 20 mM MnCl₂, 0.4 mM ZnCl₂, and 150 mM NaCl for both 14EP125 and 11EP111) and incubating at 37 °C. Immediately after the start of the incubation period, the 5'-ImpDNA in the annealed samples was already substantially (~50%) degraded to 5'-phosphate, whereas the 5'-ImpDNA in the nonannealed samples was less degraded (~35%; Fig. S7, PAGE images in panel a). As one consequence, the final reaction yield in the annealed sample was ~15% lower than the yield in the nonannealed sample (Fig. S7, first two data sets in each kinetic plot of panel b). Preincubation of the 5'-ImpDNA in a variety of conditions for 10 h, including merely water at -20 °C or 37 °C (i.e., simply storing the 5'-ImpDNA after its preparation), led to substantial suppression of the reaction yield, indicating degradation of the 5'-ImpDNA. After storage of the 5'-ImpDNA at 37 °C, essentially complete suppression was observed, corresponding to complete degradation of the 5'-ImpDNA. Our conclusion from these experiments is that 5'-ImpDNA is rather unstable, which makes it modestly difficult to handle but renders it sufficiently reactive to enable useful DNA-catalyzed preparation of peptide-nucleic acid conjugates.

All assays in Fig. 3, Fig. S4, and Fig. S5 that used the 5'-ImpDNA substrate were performed with the annealing step. No special precaution was taken to use the sample of 5'-ImpDNA substrate as soon as possible, although this substrate was always used within at most 2 h after its preparation and not stored for any longer period of time.

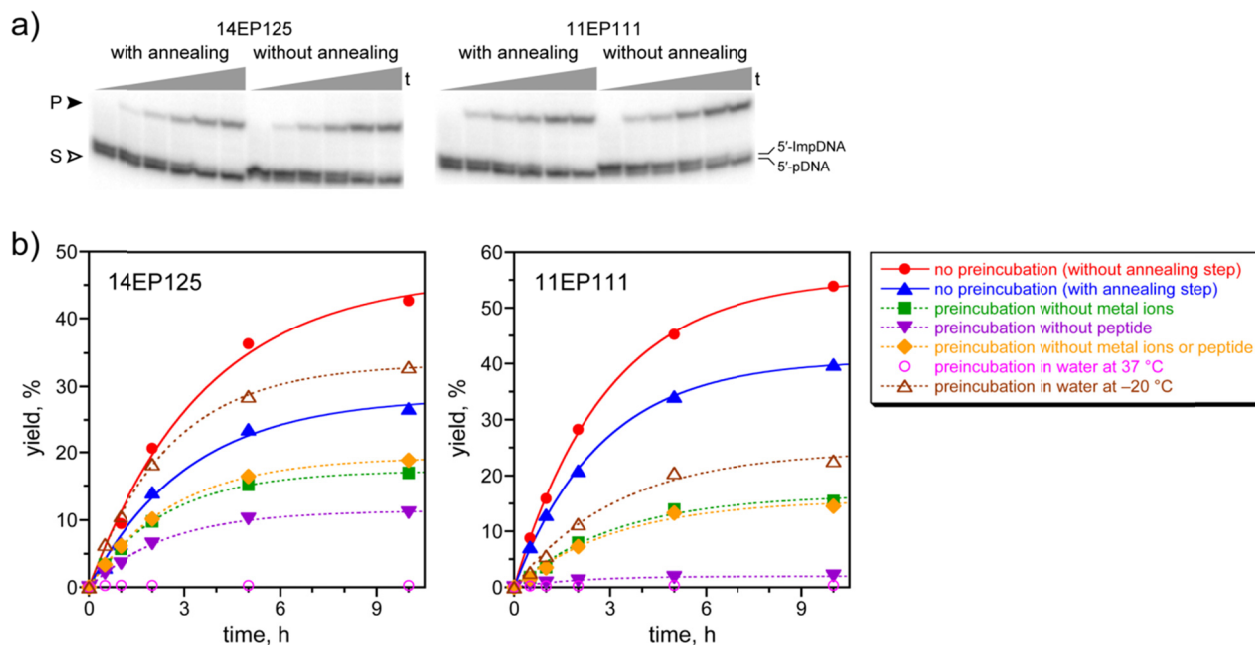


Figure S7. Assays on stability of the Imp functional group. a) PAGE images for assays of 14EP125 and 11EP111 either with or without the standard annealing step. b) Kinetic plots for assays of 14EP125 and 11EP111, either with or without the standard annealing step, and with various preincubations of the 5'-ImpDNA substrate. Each preincubation other than in water was in the reaction buffer (70 mM HEPES, pH 7.5, 40 mM MgCl₂, 20 mM MnCl₂, 0.4 mM ZnCl₂, and 150 mM NaCl) either including or excluding the three divalent metal ions or 1 mM peptide as indicated. Note the ~15% difference in yield between the red and blue curves of each panel (no preincubation; without and with annealing, respectively).

Assays of peptide substrate sequence dependence of the new deoxyribozymes

Each of the nine deoxyribozymes mentioned in Fig. 2 and Fig. 3 was assayed for activity with the “parent” peptide substrate sequence used in its selection as well as four unrelated heptapeptide sequences (Fig. S8). These heptapeptides were derived from the sequence of human α -thrombin (Russo Krauss et al., *Nucleic Acids Res.* **2011**, *39*, 7858-7867), which we are using as a test substrate in other experiments. The results reveal a range of reactivity, from 8XJ105 that is highly tolerant of the alternative peptide sequences, to 11EP104 that is moderately tolerant, to the other seven deoxyribozymes that are much less tolerant or intolerant.

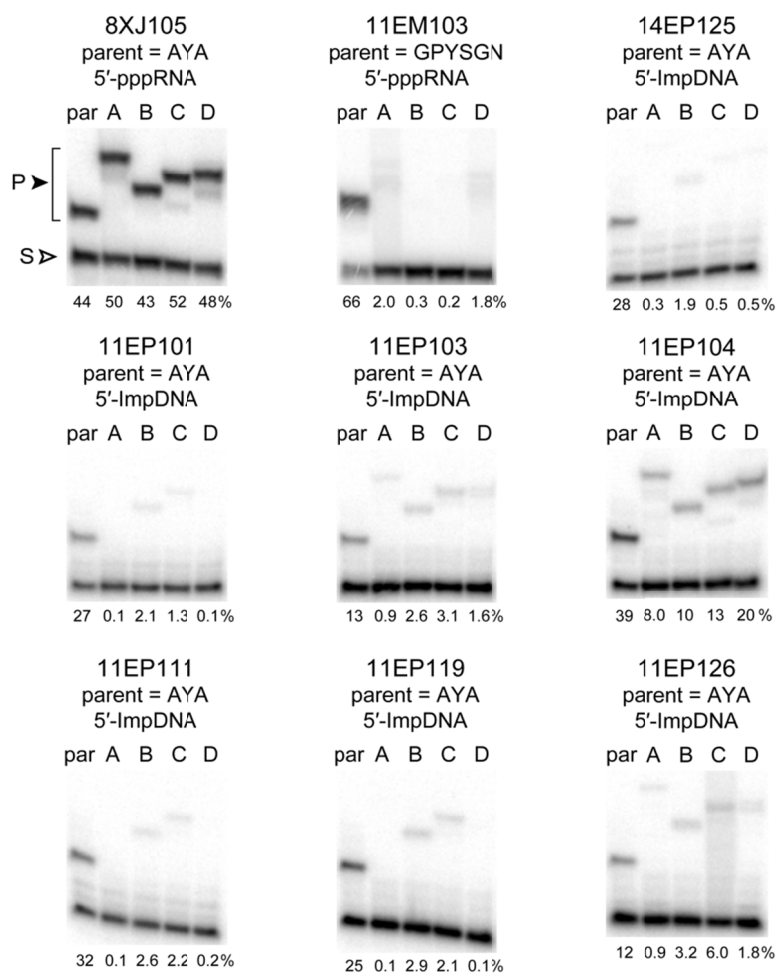


Figure S8. Assays of peptide substrate sequence dependence of the new deoxyribozymes. For each deoxyribozyme, the parent (par) peptide sequence was as indicated. The four variant heptapeptides A–D were as follows: A = RTRYERN; B = FSDYIHP; C = EKIIYHP; D = QAGYKGR. The parent peptide substrate included the azido group as used during selection, whereas the four variant heptapeptides were synthesized with a simple *N*-acetyl group. Assay conditions: 20 nM 3'-³²P-radiolabeled 5'-pppRNA or 5'-ImpDNA, 0.5 μ M deoxyribozyme, 1 mM peptide, 70 mM HEPES, pH 7.5, 40 mM MgCl₂, 20 mM MnCl₂, 1.0 mM (8XJ105) or 0.4 mM (all other deoxyribozymes) ZnCl₂, and 150 mM NaCl at 37 °C. *t* = 16 h. Yield at this timepoint is given below each lane.

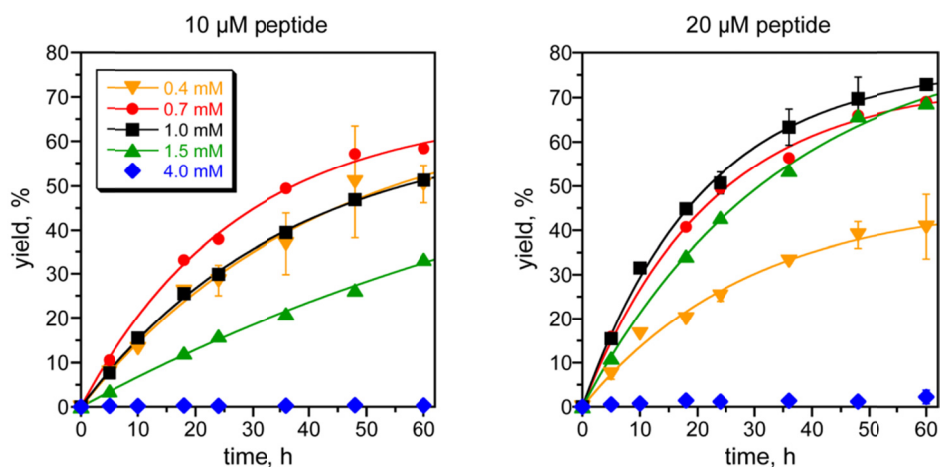
Smaller-scale assays to optimize $[\text{Zn}^{2+}]$ for the 11EM103 preparative experiments

Figure S9. Smaller-scale assays to optimize $[\text{Zn}^{2+}]$ for the 11EM103 preparative experiments of Fig. 4. Assay conditions: 10 μL total volume with 10 or 20 μM azido-GPYSGN, 15 or 30 μM 11EM103 deoxyribozyme, 20 or 40 μM 5'-pppRNA (with 20 nM 3'-³²P-radiolabeled 5'-pppRNA), 70 mM HEPES, pH 7.5, 40 mM MgCl_2 , 20 mM MnCl_2 , 0.4–4 mM ZnCl_2 , and 150 mM NaCl at 37 $^\circ\text{C}$. For 0.4, 1.0, and 4.0 mM Zn^{2+} , error bars represent half of range ($N = 2$). For 0.7 and 1.5 mM Zn^{2+} , $N = 1$. Because the pppRNA was included in two-fold excess relative to the peptide, to mimic the intended preparative conditions in which the peptide is the limiting reagent, the maximum yield based on observing the radiolabeled RNA was 50%. Therefore, the observed yields were normalized to a maximum of 50% by multiplying by 2 before the plots were constructed. First-order curve fits are provided merely to guide the eye, noting that concentrations of both peptide and pppRNA are changing substantially as the reaction progresses, and therefore simple first-order kinetics are not obeyed.