

Electronic Supplementary Information

Lanthanide Ions as Required Cofactors for DNA Catalysts

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Selection progressions

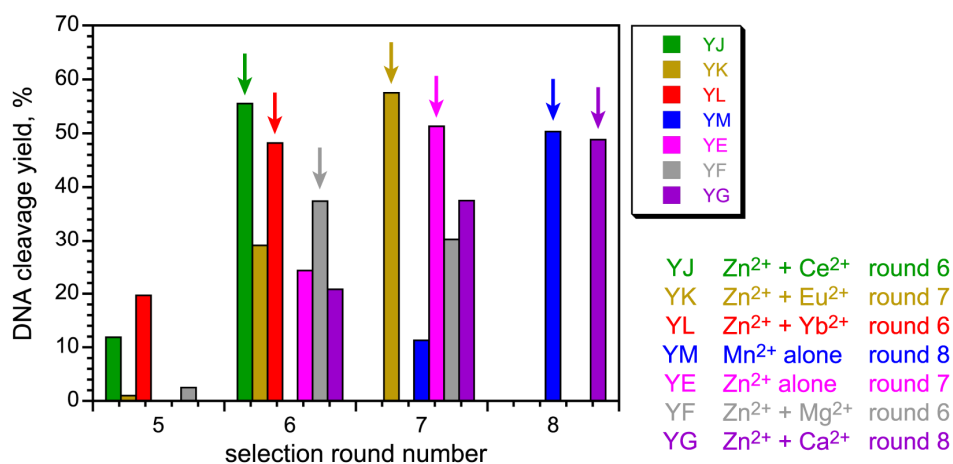


Figure S1. Progressions of the *in vitro* selection experiments in this study (DNA cleavage yield as a function of selection round number). For each selection, the round at which individual deoxyribozymes were cloned is marked with an arrow on the plot. The cloned round number is also stated on the right. All incubation times were 12 h (see Experimental Section in the main text).

Deoxyribozyme sequences

	1	10	20	30	40																																					
6YJ10	C	G	C	A	G	C	G	G	G	G	T	G	A	A	C	G	C	G	G	T	G	A	G	A	C	T	C	G	A	A	T	G	T	C	G	T	T	T				
6YJ14	G	T	G	G	T	A	C	G	G	G	G	T	G	T	A	G	T	A	G	G	C	A	C	G	A	C	G	C	C	C	T	G	C	T	C	C	A	T	G	G		
7YK24	G	C	G	A	G	G	G	G	C	A	A	A	G	C	G	C	G	A	G	A	A	C	C	A	T	G	A	T	A	C	T	C	G	C	G	A	C	T	A	A	G	
7YK34	C	C	C	C	C	G	G	T	C	A	T	G	G	G	T	C	A	A	G	C	A	A	G	C	A	G	A	G	G	A	G	A	A	T	G	A	A	A	G	G		
7YK35	C	C	C	C	C	G	C	A	A	A	G	A	A	G	C	A	A	G	G	G	G	A	C	C	G	C	T	T	T	G	A	A	T	A	C	G	G					
6YL4	T	G	G	G	G	G	C	T	A	G	C	C	G	A	G	A	G	C	A	A	A	G	C	C	A	T	G	C	A	A	A	C	G	A	G	G	A	C				
6YL11	G	C	A	G	G	C	G	A	G	T	A	C	G	G	C	A	C	A	A	C	A	A	G	A	C	C	T	A	T	A	C	A	A	C	T	A	G	G	T	C		
6YL24	C	A	A	G	G	T	C	C	A	A	C	A	G	C	C	A	G	G	T	C	A	A	G	T	T	G	C	C	C	G	C	A	G	C	T	G	G	C	C			
6YL34	G	C	A	G	G	C	G	A	G	C	A	C	G	G	C	A	T	G	A	A	A	G	A	A	T	G	T	T	A	A	T	A	C	C	A	T	T	C	T			
8YM4	T	A	G	A	G	A	G	C	C	C	A	G	A	A	T	T	A	G	T	C	A	G	T	C	C	C	G	G	G	T	T	A	C	G	A	A	A	A	G			
8YM17	A	A	G	T	G	T	A	C	G	G	A	T	G	C	C	C	C	C	T	G	C	G	C	T	C	G	G	T	C	A	A	A	T	G	T	A	G	G	C			
8YM20	A	A	C	C	G	T	G	G	C	A	G	C	A	T	C	C	T	C	A	A	G	C	G	G	G	G	A	A	A	G	C	A	G	C	A	G	T	A				
8YM26	A	A	G	C	C	A	G	A	T	G	T	A	T	T	C	C	T	C	T	A	C	C	G	G	A	C	C	G	A	C	G	A	G	G	A	G	G	A				
7YE2	C	C	C	C	G	T	C	C	G	C	C	C	T	G	A	A	T	T	C	C	G	A	T	T	C	C	G	A	T	T	G	G	A	T	T	G	G					
7YE5	C	A	G	A	C	G	T	G	C	G	A	T	C	G	G	A	T	C	A	A	G	T	C	G	C	T	C	G	A	G	A	A	G	T	C	C	C	C	C			
7YE8	C	C	A	A	T	C	C	A	A	G	T	A	C	A	A	T	T	G	G	A	T	G	T	C	C	A	G	A	G	A	A	G	T	C	C	C	C	C	G	A		
7YE19	C	A	G	C	G	A	G	A	C	A	C	G	T	A	G	T	G	A	T	C	A	G	G	C	T	C	G	A	G	A	A	G	T	C	C	C	C	C	C			
6YF2	C	C	C	A	C	A	C	C	A	T	A	T	A	C	A	A	G	G	G	A	A	G	A	T	G	G	G	C	G	T	C	C	G	A	G	G	G	C	T			
6YF13	A	A	G	C	C	G	G	A	A	T	A	G	C	C	G	A	T	G	A	G	C	C	C	A	G	A	G	A	A	A	G	T	C	C	C	C	C	G	T			
6YF15	G	T	C	G	C	A	G	C	C	C	G	A	C	C	C	G	G	G	T	C	T	A	C	C	G	A	G	T	G	C	A	T	G	T	G	G	C	G	G			
6YF16	C	C	C	A	C	C	T	C	A	A	T	G	T	T	G	T	A	T	G	A	G	C	A	A	G	A	G	A	C	G	A	C	G	A	G	G	G	T				
6YF20	C	C	C	A	C	T	G	T	G	C	C	A	A	T	G	C	G	C	C	A	T	G	C	A	A	C	A	A	C	T	G	C	C	G	A	G	G	G	C			
6YF27	C	C	C	A	G	A	T	C	G	G	C	A	A	C	G	G	T	C	G	T	T	C	A	C	G	A	T	G	C	T	A	C	G	A	G	G	G	T				
8YG1	C	T	C	C	C	G	A	C	C	A	A	C	G	A	G	C	T	A	A	G	G	G	T	A	C	A	T	T	C	T	T	A	G	G	T	G	G	C				
8YG7	C	C	G	G	C	G	C	C	C	A	A	G	T	G	C	T	T	A	A	A	C	G	C	A	T	C	C	G	T	A	T	A	C	G	C	A	A					
8YG24	G	A	G	C	A	G	G	T	A	G	A	T	G	A	G	C	A	C	C	G	C	C	G	A	A	A	T	G	A	A	T	A	T	A	T	G	C	C	G			
8YG29	A	C	G	C	A	A	G	T	C	C	C	T	G	T	C	G	T	C	A	A	T	G	G	G	G	C	G	G	A	C	C	G	C	A	T	T	T					

Figure S2. Sequences of the initially random (N_{40}) catalytic regions of the deoxyribozymes in this study. Each functional deoxyribozyme comprises the listed catalytic region, surrounded by two Watson-Crick binding arms that interact with the single-stranded DNA substrate. For the *in trans* (intermolecular) assays, the sequence to the 5'-side of each catalytic region was 5'-CCCGAAAGCCTCCTTC-3', and the sequence to the 3'-side of each catalytic region was 5'-ATACGCATAAAGGTAG-3'.

Assays of individual deoxyribozymes that cleave DNA by hydrolysis

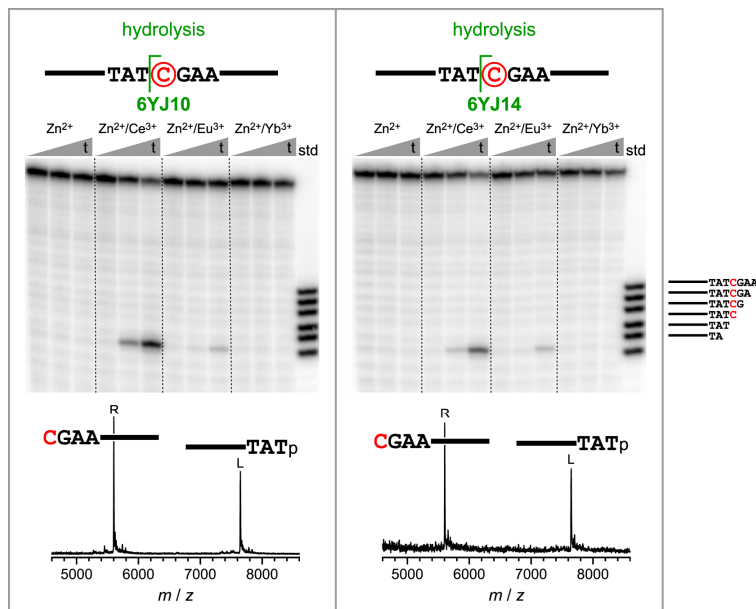


Figure S3. Assays of individual deoxyribozymes that catalyze DNA hydrolysis from the selection experiment that used $Zn^{2+} + Ce^{2+}$ ($t = 30$ s, 1 h, 16 h). Assays were in 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 10 μM $LnCl_3$ (if included), and 150 mM NaCl at 37 °C. The PAGE image on the left is also shown in Figure 3a; the mass spectrometry image on the left is also shown in Figure 4b. See Table S2 for all mass spectrometry data values (for products formed using Ce^{3+}). In all cases, the assigned hydrolysis sites were consistent with the PAGE standard ladders (see Experimental Section); definitive assignment of reaction products was made on the basis of the mass spectrometry data.

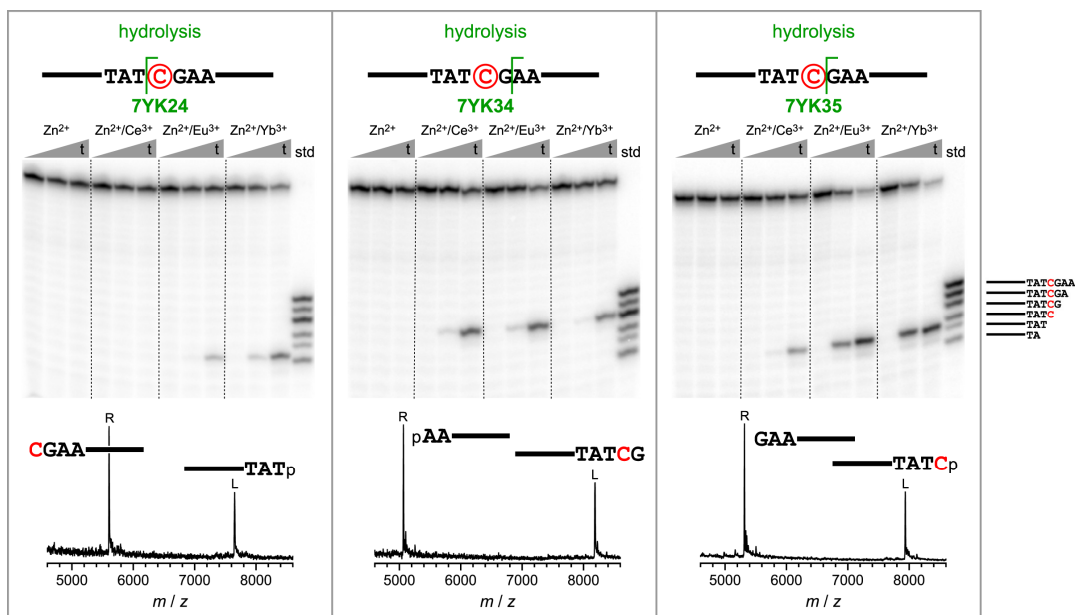


Figure S4. Assays of individual deoxyribozymes that catalyze DNA hydrolysis from the selection experiment that used $Zn^{2+} + Eu^{2+}$ ($t = 30$ s, 1 h, 16 h). Assays were in 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 10 μM $LnCl_3$ (if included), and 150 mM NaCl at 37 °C. See Table S2 for all mass spectrometry data values (for products formed using Eu^{3+}). In all cases, the assigned hydrolysis sites were consistent with the PAGE standard ladders (see Experimental Section); definitive assignment of reaction products was made on the basis of the mass spectrometry data.

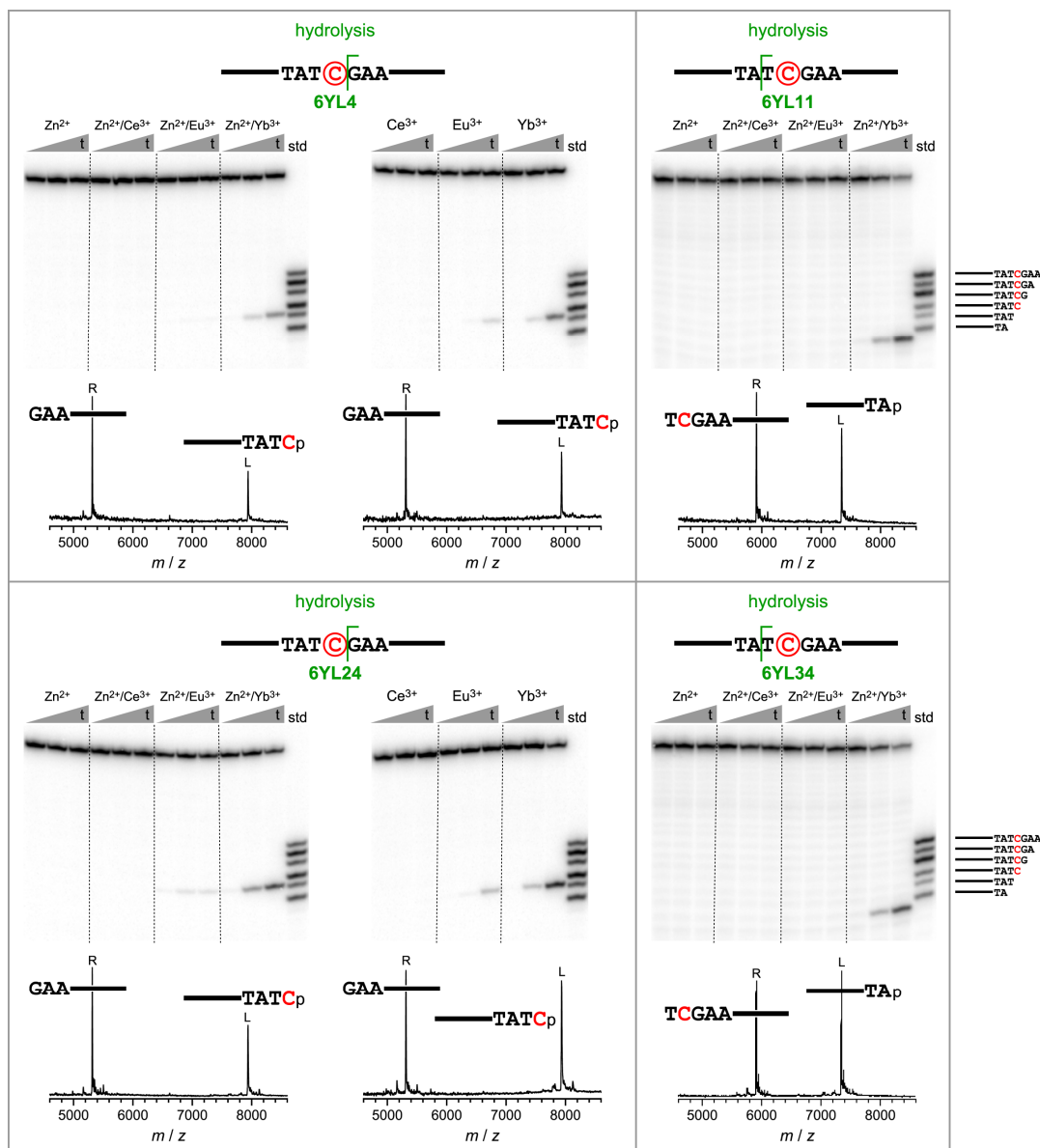


Figure S5. Assays of individual deoxyribozymes that catalyze DNA hydrolysis from the selection experiment that used Zn²⁺ + Yb²⁺ (t = 30 s, 1 h, 16 h). Assays that included Zn²⁺ were performed in 70 mM HEPES, pH 7.5, 1 mM ZnCl₂, 10 μM LnCl₃ (if included), and 150 mM NaCl at 37 °C. Assays that omitted Zn²⁺ were performed in 50 mM HEPES, pH 7.5, 10 μM LnCl₃, and 150 mM NaCl at 37 °C. The PAGE images for the two lanthanide-only assays are also shown in Figure 5a. See Table S2 for all mass spectrometry data values (for products formed using Yb³⁺). In all cases, the assigned hydrolysis sites were consistent with the PAGE standard ladders (see Experimental Section); definitive assignment of reaction products was made on the basis of the mass spectrometry data.

Tabulation of k_{obs} and yield values for the data in Figure 3 and Figure 5

deoxyribozyme	metal ions	k_{obs} , h ⁻¹	yield, %
6YJ10	Zn ²⁺ /Ce ³⁺	0.38	69
	Zn ²⁺ /Eu ³⁺	0.0060 ^a	10
6YJ14	Zn ²⁺ /Ce ³⁺	0.11	67
	Zn ²⁺ /Eu ³⁺	0.0072 ^a	27
7YK24	Zn ²⁺ /Eu ³⁺	0.028	28
	Zn ²⁺ /Yb ³⁺	0.098	63
7YK34	Zn ²⁺ /Ce ³⁺	0.091	59
	Zn ²⁺ /Eu ³⁺	0.071	55
	Zn ²⁺ /Yb ³⁺	0.044	40
7YK35	Zn ²⁺ /Ce ³⁺	0.096	53
	Zn ²⁺ /Eu ³⁺	1.46	79
	Zn ²⁺ /Yb ³⁺	1.15	72
6YL4	Zn ²⁺ /Yb ³⁺	0.055	25
6YL11	Zn ²⁺ /Yb ³⁺	0.19	55
6YL24	Zn ²⁺ /Eu ³⁺	<0.003 ^a	2
	Zn ²⁺ /Yb ³⁺	0.18	53
6YL34	Zn ²⁺ /Yb ³⁺	0.17	64
6YL4	Eu ³⁺	0.0015 ^a	5
6YL4	Yb ³⁺	0.038 ± 0.009 ^b	48 ± 3 ^b
6YL24	Eu ³⁺	0.0036 ^a	15
6YL24	Yb ³⁺	0.057 ± 0.020 ^b	71 ± 11 ^b

Table S1. k_{obs} and yield values for the data plotted in Figure 3 and Figure 5. k_{obs} values are from first-order kinetic fits unless otherwise noted. Yield values were observed at the 48 h timepoints.

^a k_{obs} values are from linear fits to the initial time points.

^b Values represent mean ± sd from four independent experiments.

Mass spectrometry assays of cleavage products for DNA-hydrolyzing deoxyribozymes

deoxyribozyme ^a	metal ion cofactor(s)	mass L calcd.	mass L found	L error, % (found – calcd.)	mass R calcd.	mass R found	R error, % (found – calcd.)
6YJ10	Zn ²⁺ /Ce ³⁺	7648.0	7648.0	0	5603.7	5602.4	-0.02
6YJ14	Zn ²⁺ /Ce ³⁺	7648.0	7648.2	+0.003	5603.7	5604.6	+0.02
7YK24	Zn ²⁺ /Eu ³⁺	7648.0	7652.2	+0.05	5603.7	5606.4	+0.05
7YK34	Zn ²⁺ /Eu ³⁺	8186.4	8188.0	+0.02	5065.3	5066.2	+0.02
7YK35	Zn ²⁺ /Eu ³⁺	7937.2	7937.4	+0.003	5314.5	5315.6	+0.02
6YL4	Zn ²⁺ /Yb ³⁺	7937.2	7940.2	+0.04	5314.5	5316.1	+0.03
6YL4	Yb ³⁺	7937.2	7934.9	-0.03	5314.5	5312.1	-0.05
6YL11	Zn ²⁺ /Yb ³⁺	7343.8	7345.4	+0.02	5907.9	5909.8	+0.03
6YL24	Zn ²⁺ /Yb ³⁺	7937.2	7939.0	+0.02	5314.5	5315.7	+0.02
6YL24	Yb ³⁺	7937.2	7936.2	-0.01	5314.5	5313.4	-0.02
6YL34	Zn ²⁺ /Yb ³⁺	7343.8	7346.1	+0.03	5907.9	5910.0	+0.04
7YE11	Zn ²⁺	7568.0	7568.4	+0.01	5683.7	5684.3	+0.01
8YG11	Zn ²⁺	8186.4	8189.7	+0.04	5065.3	5067.4	+0.04

Table S2. Mass spectrometry assays of cleavage products for DNA-hydrolyzing deoxyribozymes.

^a See Figure S3 (6YJ deoxyribozymes, Zn²⁺/Ce³⁺), Figure S4 (7YK deoxyribozymes, Zn²⁺/Eu³⁺), Figure S5 (6YL deoxyribozymes, Zn²⁺/Yb³⁺ or Yb³⁺ alone), and Figure 7 (7YE11, Zn²⁺; 8YG11, Zn²⁺/Ca²⁺) for each deoxyribozyme's assigned hydrolysis site.

Lanthanide ion concentration dependence of the lanthanide-dependent deoxyribozymes

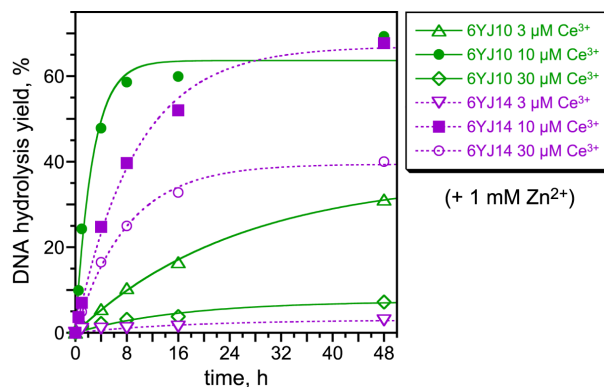


Figure S6. Assays of 6YJ10 and 6YJ14 at 1 mM Zn²⁺ and 3, 10, or 30 μM Ce³⁺. The data at 10 μM Ce³⁺ are the same as shown for these two deoxyribozymes in Figure 3b.

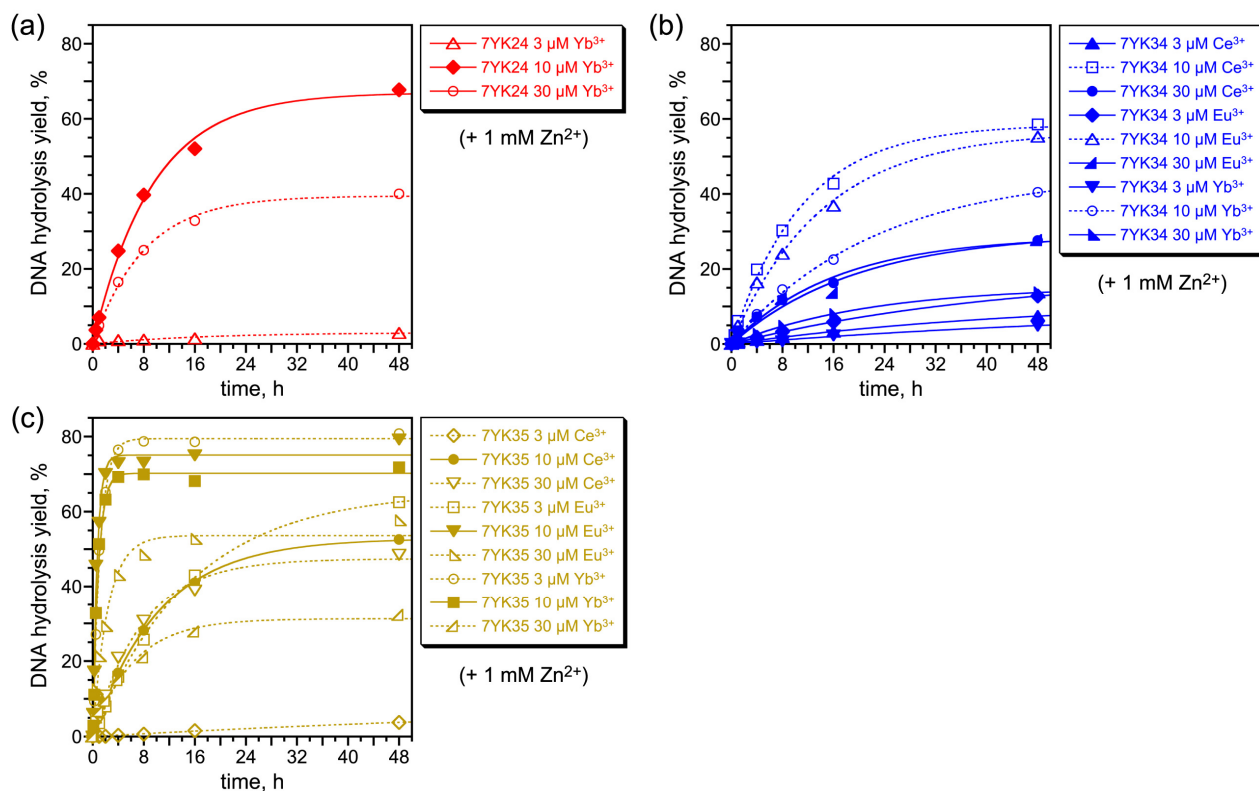


Figure S7. Assays of 7YK24, 7YK34, and 7YK35 at 1 mM Zn²⁺ and 3, 10, or 30 μM Ce³⁺, Eu³⁺, or Yb³⁺. For 7YK24, the data at 10 μM Yb³⁺ shown here are from a different experiment than is shown in Figure 3c (note that the concentration dependence for Yb³⁺ was determined because Yb³⁺ was a more effective cofactor than Eu³⁺). For 7YK34 and 7YK35, the data at 10 μM Ce³⁺, Eu³⁺, or Yb³⁺ shown here are from the same experiments as shown in Figure 3c.

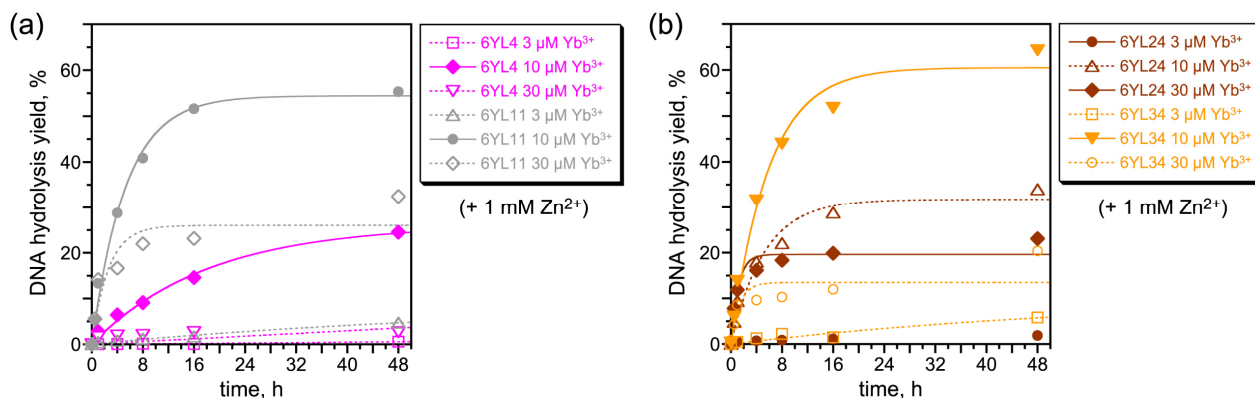


Figure S8. Assays of 6YL4, 6YL11, 6YL24, and 6YL34 at 1 mM Zn²⁺ and 3, 10, or 30 μM Yb³⁺. For 6YL24, the data at 10 μM Yb³⁺ shown here are from a different experiment than is shown in Figure 3d. For 6YL4, 6YL11, and 6YL34, the data at 10 μM Yb³⁺ shown here are from the same experiments as shown in Figure 3d.

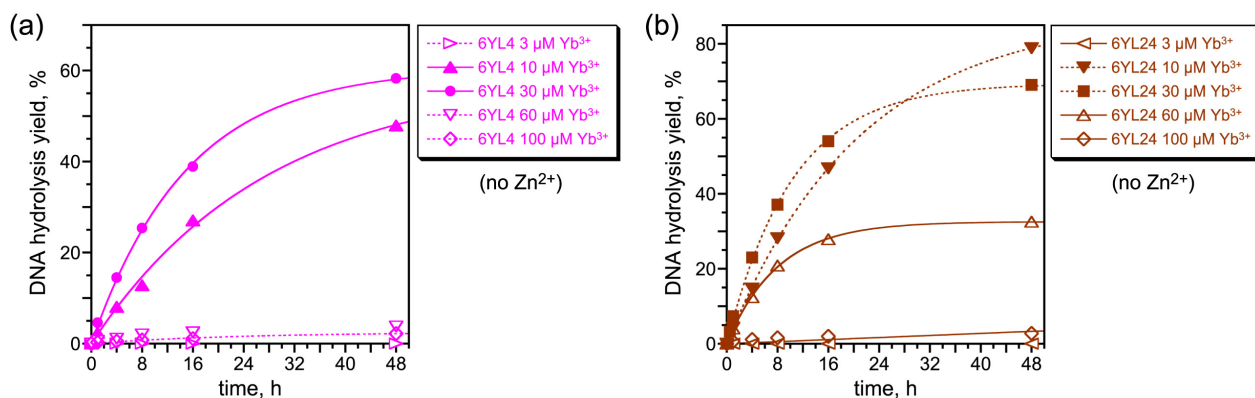


Figure S9. Assays of 6YL4 and 6YL24 at 3, 10, 30, 60, or 100 μM Yb³⁺ in the absence of Zn²⁺. The data at 10 μM Yb³⁺ shown here are from a different experiment than is shown in Figure 5.

Assays of individual deoxyribozymes that cleave DNA by deglycosylation

In several cases, the left-hand (L) product formed upon deglycosylation and β-elimination was observed as an L+58 peak, *i.e.*, a peak with mass 58 higher than expected for the L product with a simple 3'-phosphate group. Typically both the L and L+58 peaks were observed. Per Figure 1b, the first β-elimination reaction after deglycosylation releases the right-hand (R) oligonucleotide product, which is always observed cleanly by mass spectrometry in its simple 5'-phosphate form. The ensuing, second β-elimination reaction should release L. However, if the excised nucleoside sugar ring (red in Figure 1b) is partially fragmented rather than fully released from the L product fragment, then the L product fragment will have slightly higher mass than expected. There is precedent for such partial sugar fragmentation, at least for oxidatively induced DNA cleavage reactions (Joshi, R. R.; Likhite, S. M.; Kumar, R. K.; Ganesh, K. N. *Biochim. Biophys. Acta* **1994**, *1199*, 285-292; Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089-1107). In the present examples, although the available metal ions—either Zn²⁺ alone or Zn²⁺ with Mg²⁺ or Ca²⁺—are not redox-active, we speculate that a similar sugar ring fragmentation occurs (*via* unknown mechanism), leading to the observed L+58 = L+C₂H₂O₂ products.

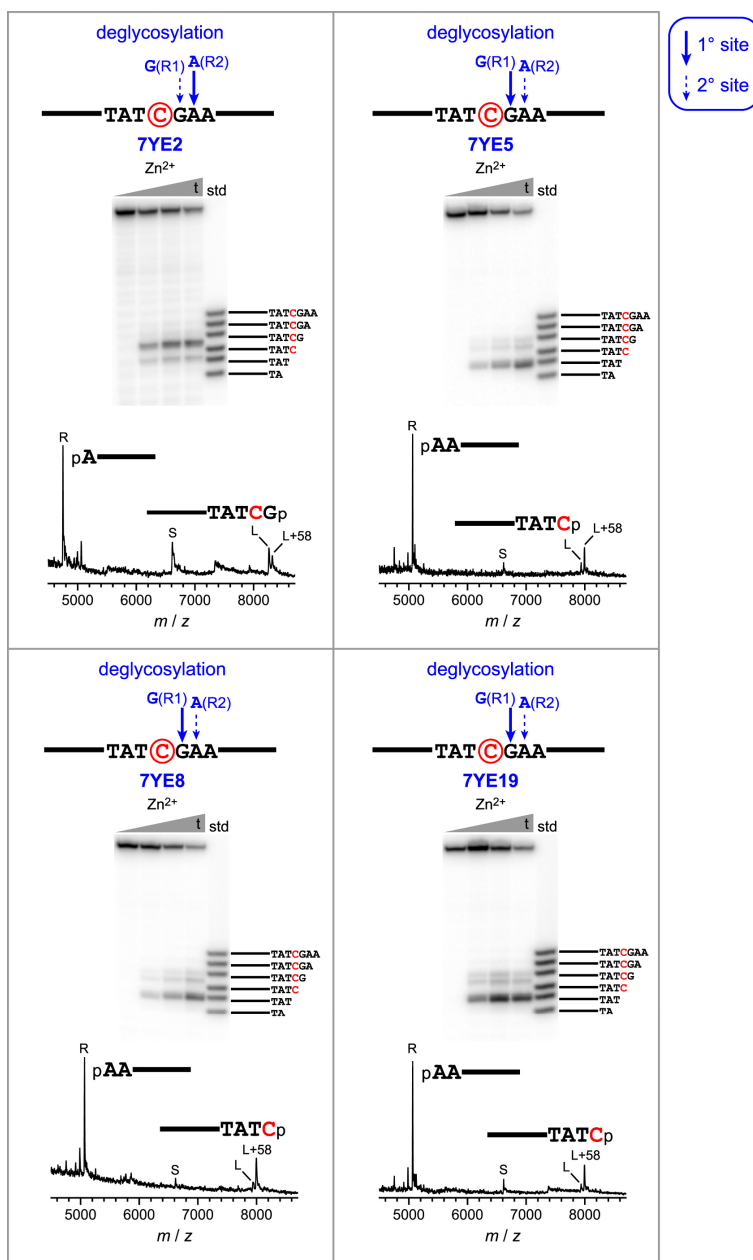


Figure S10. Assays of individual deoxyribozymes that catalyze DNA deglycosylation from the selection experiment that used Zn²⁺ (t = 30 s, 30 min, 2 h, 16 h). Assays were in 70 mM HEPES, pH 7.5, 1 mM ZnCl₂, and 150 mM NaCl at 37 °C. The arrows above each sequence mark the site(s) of deglycosylation, as assigned on the basis of the MALDI mass spectrometry data. Nucleotides are numbered as part of either the L (left) or R (right) portion of the substrate, counting outward. See Table S3 for all mass spectrometry data values. Mass spectrometry peaks labeled S correspond to uncleaved substrate (z = 2). In all cases, the assigned deglycosylation sites were consistent with the PAGE standard ladders (see Experimental Section); definitive assignment of reaction products was made on the basis of the mass spectrometry data.

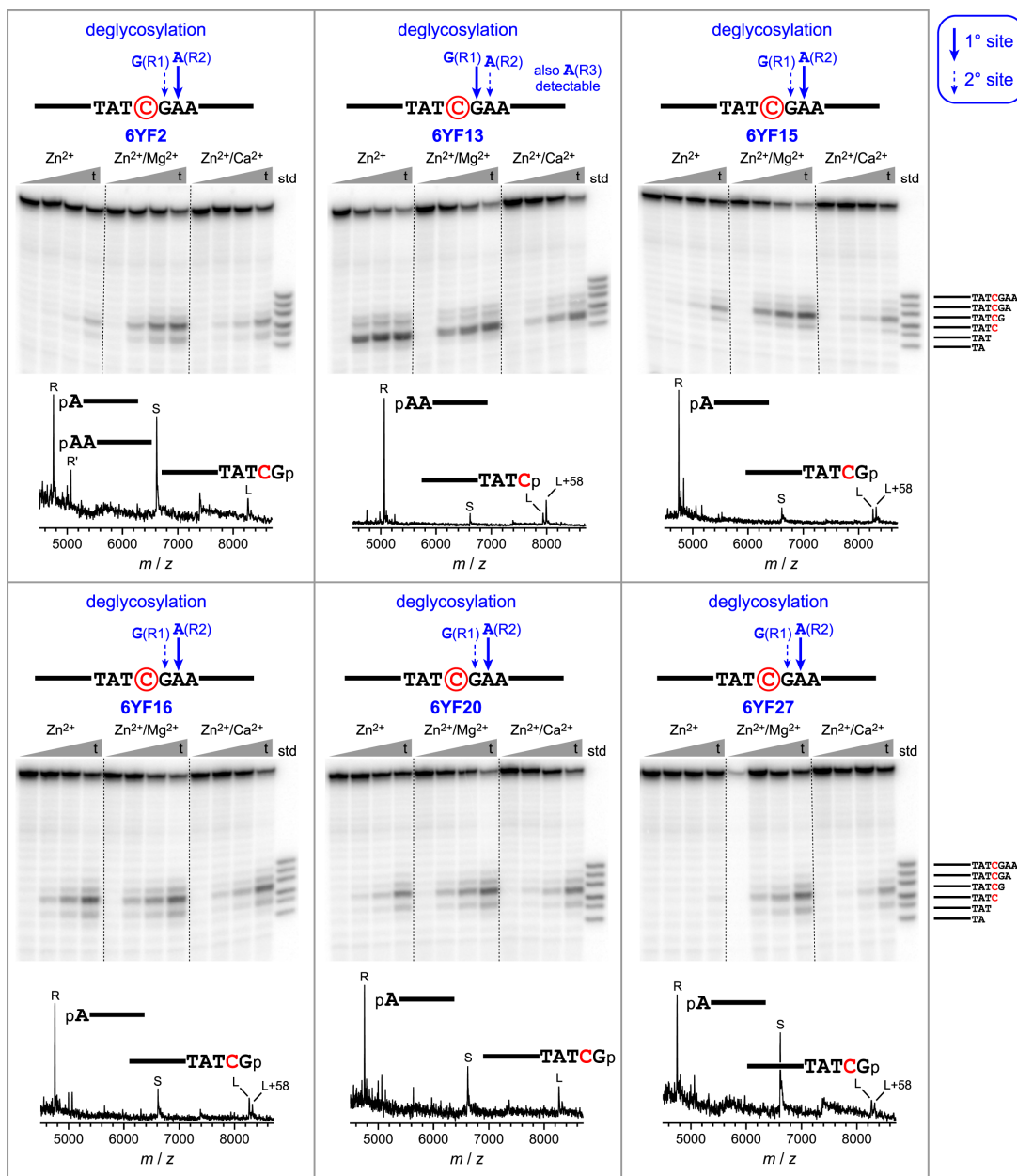


Figure S11. Assays of individual deoxyribozymes that catalyze DNA deglycosylation from the selection experiment that used Zn²⁺ + Mg²⁺ (t = 30 s, 30 min, 2 h, 16 h). Assays were in 70 mM HEPES, pH 7.5, 1 mM ZnCl₂, 40 mM MgCl₂ or CaCl₂ (if included), and 150 mM NaCl at 37 °C. 6YF2, 15, and 27 are clearly more active with Zn²⁺ + Mg²⁺ than with Zn²⁺ alone or Zn²⁺ + Ca²⁺. In contrast, 6YF13, 16, and 20 have nearly equivalent activity with Zn²⁺ alone and Zn²⁺ + Mg²⁺, and they are slightly suppressed by Ca²⁺. The arrows above each sequence mark the site(s) of deglycosylation, as assigned on the basis of the MALDI mass spectrometry data. Nucleotides are numbered as part of either the L (left) or R (right) portion of the substrate, counting outward. See Table S3 for all mass spectrometry data values (for products formed using Zn²⁺ + Mg²⁺). Mass spectrometry peaks labeled S correspond to uncleaved substrate (z = 2). In all cases, the assigned deglycosylation sites were consistent with the PAGE standard ladders (see Experimental Section); definitive assignment of reaction products was made on the basis of the mass spectrometry data.

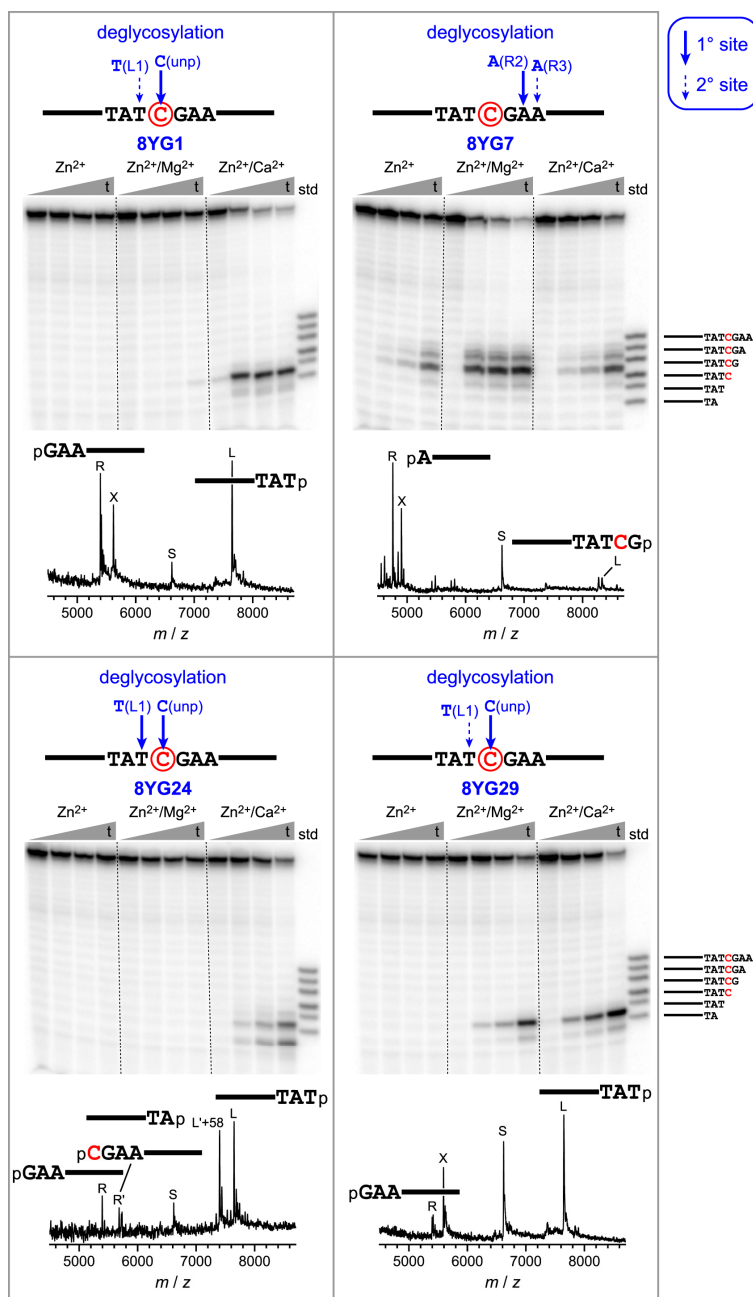


Figure S12. Assays of individual deoxyribozymes that catalyze DNA deglycosylation from the selection experiment that used $Zn^{2+} + Ca^{2+}$ ($t = 30$ s, 30 min, 2 h, 16 h). Assays were in 70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 40 mM $MgCl_2$ or $CaCl_2$ (if included), and 150 mM NaCl at 37 °C. 8YG1 and 24 clearly require $Zn^{2+} + Ca^{2+}$ for activity. 8YG7 is more active with $Zn^{2+} + Mg^{2+}$ than with Zn^{2+} alone or $Zn^{2+} + Ca^{2+}$. 8YG29 is inactive with Zn^{2+} alone and approximately equally active with $Zn^{2+} + Mg^{2+}$ and $Zn^{2+} + Ca^{2+}$. The arrows above each sequence mark the site(s) of deglycosylation, as assigned on the basis of the MALDI mass spectrometry data. Nucleotides are numbered as part of either the L (left) or R (right) portion of the substrate, counting outward. See Table S3 for all mass spectrometry data values (for products formed using $Zn^{2+} + Ca^{2+}$). Mass spectrometry peaks labeled S correspond to uncleaved substrate ($z = 2$); mass spectrometry peaks labeled X were unassigned and not reproducibly observed. In all cases, the assigned deglycosylation sites were consistent with the PAGE standard ladders (see Experimental Section); definitive assignment of reaction products was made on the basis of the mass spectrometry data.

Mass spectrometry assays of cleavage products for deglycosylating deoxyribozymes

deoxyribozyme	deglycos. site(s) ^a	mass L calcd.	mass L found	L error, % (found – calcd.)	mass R calcd.	mass R found	R error, % (found – calcd.)
8YM4	G(R1): L, R	7937.2	7936.2	-0.01	5065.3	5065.0	-0.01
8YM17	T(L1): L, R	7343.8	7343.8	0	5683.7	5682.8	-0.02
	A(L2): L', R'	7030.6	7029.9	-0.01	5987.9	5987.8	-0.002
8YM20	G(R1): L, R	7937.2	7940.9	+0.05	5065.3	5067.2	+0.04
	A(R2): L', R'	8266.4	not obs.	—	4752.1	4754.2	+0.04
	A(R3): L'', R''	8579.6	8574.5	-0.06	4438.9	4441.1	+0.05
8YM26	T(L1): L, R	7343.8	7347.2	+0.05	5683.7	5686.5	+0.05
	A(L2): L', R'	7030.6	7034.0	+0.05	5987.9	5991.6	+0.06
7YE2	A(R2): L, R	8266.4	8262.6	-0.05	4752.1	4749.9	-0.05
	G(R1): L', R'	7937.2	not obs.	—	5065.3	5062.6	-0.05
7YE5	G(R1): L, R ^b	7995.2	7993.9	-0.02	5065.3	5064.8	-0.01
7YE8	G(R1): L, R ^b	7995.2	7997.4	+0.03	5065.3	5064.3	-0.02
7YE19	G(R1): L, R ^b	7995.2	7994.7	-0.01	5065.3	5065.7	+0.01
6YF2	A(R2): L, R	8266.4	8267.6	+0.01	4752.1	4752.8	+0.01
	G(R1): L', R'	7937.2	not obs.	—	5065.3	5066.5	+0.02
6YF13	G(R1): L, R ^b	7995.2	7992.2	-0.04	5065.3	5064.4	-0.02
6YF15	A(R2): L, R	8266.4	8258.9	-0.09	4752.1	4747.4	-0.10
6YF16	A(R2): L, R	8266.4	8266.2	-0.002	4752.1	4752.7	+0.01
	G(R1): L', R' ^b	7995.2	not obs.	—	5065.3	5066.1	+0.02
6YF20	A(R2): L, R	8266.4	8268.5	+0.03	4752.1	4753.2	+0.02
6YF27	A(R2): L, R	8266.4	8264.4	-0.02	4752.1	4750.8	-0.03
8YG1	C(unn): L, R	7648.0	7650.6	+0.03	5394.5	5396.9	+0.04
8YG7	A(R2): L, R ^b	8324.4	8334.2	+0.12	4752.1	4756.9	+0.10
8YG24	C(unn): L, R	7648.0	7648.6	+0.01	5394.5	5394.5	0
	T(L1): L', R' ^b	7401.8	7402.0	+0.003	5683.7	5684.1	+0.01
8YG29	C(unn): L, R	7648.0	7650.8	+0.04	5394.5	5396.5	+0.04

Table S3. Mass spectrometry assays of cleavage products for DNA-deglycosylating deoxyribozymes.

^a See Figure 6 (8YM deoxyribozymes, Mn²⁺), Figure S10 (7YE deoxyribozymes, Zn²⁺), Figure S11 (6YF deoxyribozymes, Zn²⁺/Mg²⁺), and Figure S12 (8YG deoxyribozymes, Zn²⁺/Ca²⁺) for assigned deglycosylation site(s). Some of the secondary deglycosylation sites observed by PAGE were sufficiently weak such that no corresponding product peaks could be observed by mass spectrometry; a row is omitted altogether for these secondary sites. In addition, the L peaks for several of the secondary sites were not observed, although the R peaks were observed; these instances are denoted by “not obs.” in the table.

^b In this case, the L product peak was observed primarily in the L+58 form (see discussion at start of Supplementary Information section entitled “Assays of individual deoxyribozymes that cleave DNA by deglycosylation”). The L calcd. value was computed from the initially expected L value by adding the mass of C₂H₂O₂.

Piperidine treatment to assess deglycosylation without β -elimination

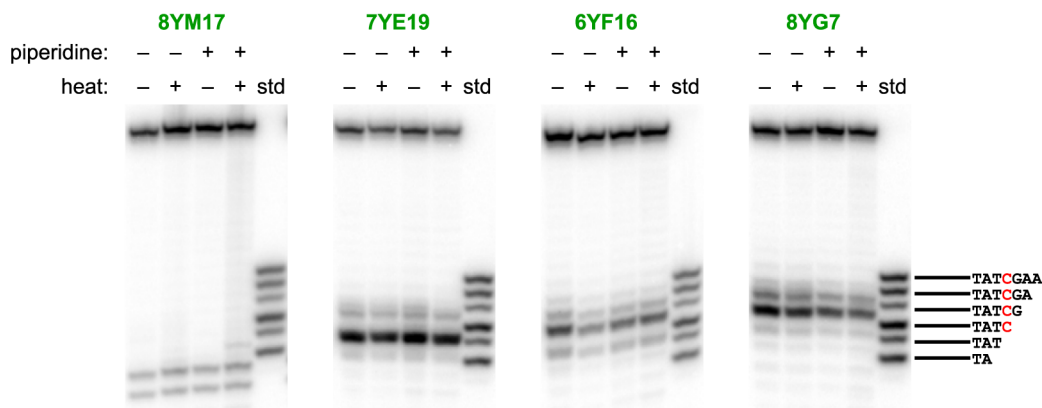


Figure S13. Treatment of the DNA-catalyzed cleavage products from individual deoxyribozymes with piperidine and heat offers no evidence that β -elimination following deglycosylation is incomplete. In no case were yields reproducibly different among any of the variously treated samples for the same deoxyribozyme. Assay data is shown for four representative deoxyribozymes; six others were also tested, with similar lack of piperidine effect (8YM26, 7YE5, 6YF2, 6YF15, 8YG1, and 8YG24). Procedure: An 8 μ L sample was prepared according to the standard DNA cleavage assay procedure as described in the main text, using the appropriate metal ions (8YM17: 20 mM Mn^{2+} ; 7YE19: 1 mM Zn^{2+} ; 6YF16: 1 mM Zn^{2+} + 40 mM Mg^{2+} ; 8YG7: 1 mM Zn^{2+} + 40 mM Ca^{2+}). After 16 h incubation at 37 $^{\circ}C$, the sample was brought to 10% (v/v) piperidine or treated with an equivalent volume of water and heated at 95 $^{\circ}C$ for 30 min or kept at room temperature, followed by 20% PAGE.