Functional Compromises Among pH Tolerance, Site Specificity, and Sequence Tolerance for a DNA-Hydrolyzing Deoxyribozyme

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Characterization of the 8NLJ1 deoxyribozyme and its cleavage product

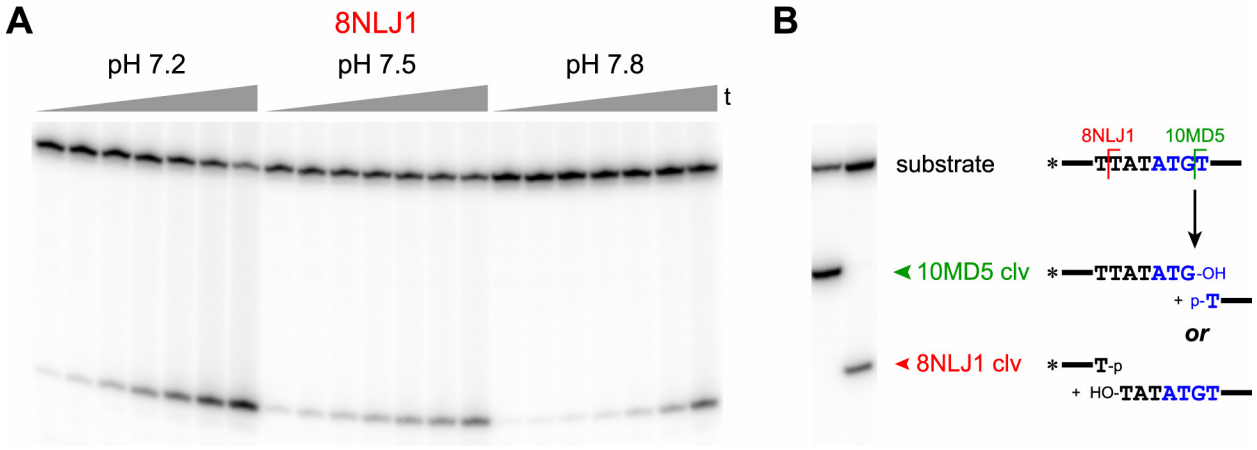

Figure S1. Characterization of the 8NLJ1 deoxyribozyme and its cleavage product. (A) Activity of 8NLJ1 at three different pH values. Incubation was under the standard conditions of 70 mM HEPES, 20 mM $MnCl_2$, 1 mM $ZnCl_2$, and 150 mM NaCl at 37 °C (t = 7, 15, 30 min; 1, 2, 5, and 20 h). The 20 h yields (left to right) were 71, 33, and 25%. (B) 8NLJ1 cleavage site within the DNA substrate, as assigned by comparing the PAGE migration rate of the pH 7.5 cleavage product (t = 20 h) with those of standard oligonucleotides (not shown) and the 10MD5 product. Note that 8NLJ1 leaves a 3′-phosphate, rather than a 5′-phosphate as for 10MD5 and 9NL27. The assigned 8NLJ1 cleavage-site location was confirmed by MALDI mass spectrometry (Table S1).

pH tolerance of 9NL27 with changes to substrate sequence

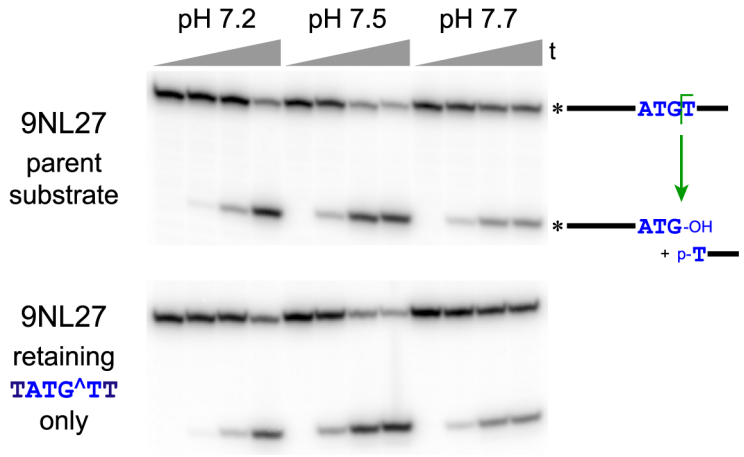

Figure S2. Assay of pH tolerance of 9NL27 when the substrate sequence is changed. Incubation was under the standard conditions of 70 mM HEPES, 20 mM $MnCl_2$, 1 mM $ZnCl_2$, and 150 mM NaCl at 37 °C (t = 0, 15 min, 2 h, 22 h). The upper experiment used the parent substrate sequence (equivalent to the experiment with 9NL27 in Figure 3). The lower experiment used the substrate sequence with Tv2 changes in all nucleotides except TATG^TT at the cleavage site.

Sequence specificity of 9NL27 with expanded recognition site

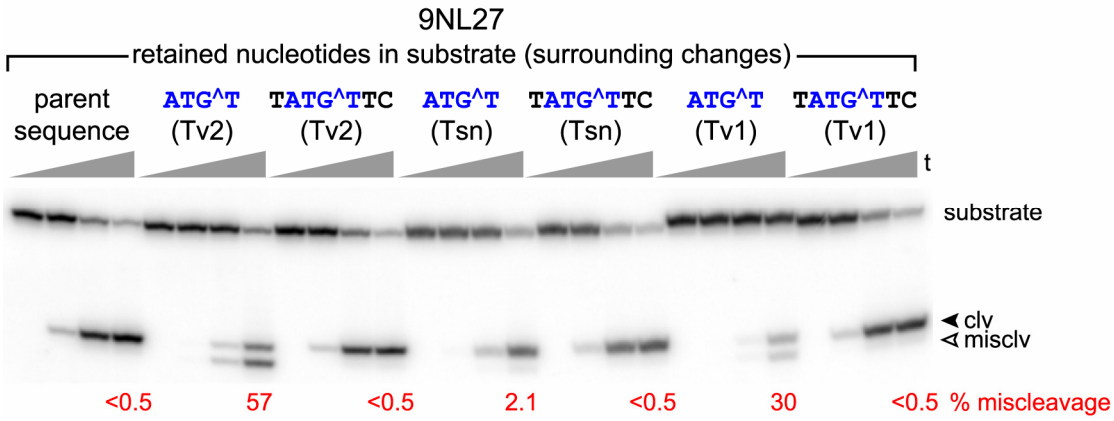

Figure S3. Establishing sequence specificity of 9NL27 by systematic variation of substrate nucleotides outside of ATG^T or TATG^TTC to all three alternative base identities (Tsn = A↔G, T↔C; Tv1 = A↔C, G↔T; Tv2 = A↔T, G↔C). In all cases, Watson-Crick base pairing was retained between the DNA substrate and the deoxyribozyme binding arm. Incubation was under the standard conditions of 70 mM HEPES, 20 mM $MnCl_2$, 1 mM $ZnCl_2$, and 150 mM NaCl at 37 °C (t = 0, 15 min, 2 h, 22 h).

Site specificities of 9NL1, 9NL12, and 9NL33

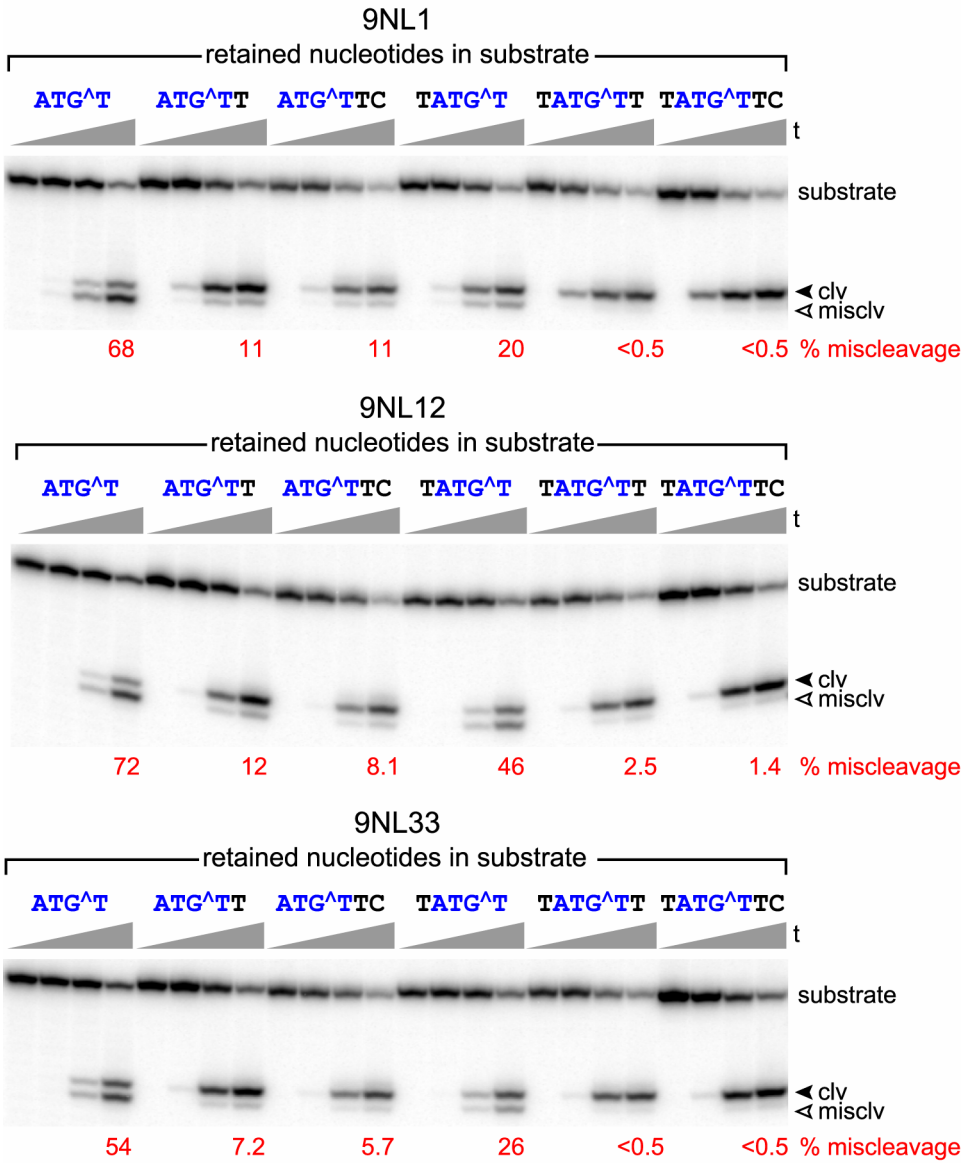

Figure S4. Expansion of the recognition site beyond ATG^T (i.e., reducing the sequence tolerance) restores site specificity for 9NL1, 9NL12, and 9NL33. Incubation was under the standard conditions of 70 mM HEPES, 20 mM $MnCl_2$, 1 mM $ZnCl_2$, and 150 mM NaCl at 37 °C (t = 0, 15 min, 2 h, 22 h). The data shown here are analogous to the images shown in Figure 7 for 9NL27.

MALDI mass spectrometry analyses of the cleavage products

deoxyribozyme		mass L calcd.	mass L found	L error, % (found – calcd.)	mass R calcd.	mass R found	R error, % (found – calcd.)
10MD5 [a,b]		6418.9	6420.9	+0.03	3741.3	3742.6	+0.03
9NL1 [a]		6418.9	6417.4	–0.02	3741.3	3739.9	–0.04
9NL12 [a]		6418.9	6415.9	–0.05	3741.3	3740.6	–0.02
9NL27 [a]		6418.9	6411.0	–0.12	3741.3	3735.2	–0.16
9NL33 [a]		6418.9	6418.1	–0.01	3741.3	3739.8	–0.04
8NLJ1 [a]		4630.8	4635.8	+0.11	5529.4	5536.0	+0.12
10MD5 (#5) [c]	clv	6378.9	6382.1	+0.05	3741.3	3744.2	+0.08
	misclv	6089.7	6093.1	+0.06	4030.5	4033.5	+0.07
10MD5 (#8) [c]	clv	6378.9	6383.3	+0.07	3741.3	3746.3	+0.13
	misclv 1	6683.0	6686.9	+0.06	3437.1	3441.9	+0.14
	misclv 2	6987.2	6991.2	+0.06	3133.0	3137.8	+0.15
10MD5 (#9) [c]	clv	6418.9	6424.2	+0.08	3741.3	3745.8	+0.12
10MD5 (#10) [c]	clv	6378.9	6382.9	+0.06	3741.3	3744.0	+0.07
	misclv 1	6683.0	6686.1	+0.05	3437.1	3439.2	+0.06
	misclv 2	6987.2	6990.6	+0.05	3133.0	3135.1	+0.07
9NL27 [d]	clv	6477.0	6475.6	–0.02	3710.3	3710.8	+0.01
	misclv	6147.8	6146.8	–0.02	4039.5	4039.9	+0.01

Table S1. MALDI mass spectrometry analysis of the deoxyribozyme cleavage products. L and R respectively denote the left-hand (5′) and right-hand (3′) cleavage products. All MALDI mass spectra were obtained in the mass spectrometry laboratory of the UIUC School of Chemical Sciences.

[a] Data obtained using the indicated deoxyribozyme and the parent DNA substrate, for which site-specific cleavage is observed.

[b] Data for the parent 10MD5 deoxyribozyme and DNA substrate is from (*1*) and is provided for comparison.

[c] Data obtained using 10MD5 and the DNA substrate in the indicated experiment # from Figure 8, where non-site-specific cleavage is observed. Tabulated are values for L and R products corresponding to cleavage at the original ATG^T position as well as L and R products of miscleavage at the position(s) indicated in Figure 8, each with formation of 5′-phosphate + 3′-hydroxyl termini. For #9, the small amount of miscleavage product was not detectable by mass spectrometry.

[d] Data obtained using 9NL27 and the DNA substrate for which ATG^T was retained, but all other nucleotides were changed by A↔T or G↔C. With this substrate, non-site-specific cleavage is observed (Figure 6 and Figure 7). Tabulated are values for L and R products as for 10MD5 when non-site-specific.