Methods

HPLC Composition Analysis of Crystals. The strand composition of AP8 and DAP8 crystals was assessed by HPLC. Separation of oligonucleotide strands obtained from Dharmacon Inc. was performed using a 3.9 x 300 mm μBondapack C18 column (Waters Corp.) buffered by a TEAA/TEA ion pairing system (*1, 2*) and operated at 0.50 ml min^{-1}. Buffer A consisted of 0.10 M TEAA, *p*H 7.0 containing 0.5% acetonitrile. Buffer B was identical to buffer A, except for the presence of 50% (*v/v*) acetonitrile. Linear gradients of 15 to 30% buffer B were used to elute samples in 70 min. For control experiments, purified RNA strands were docked to form the hairpin ribozyme (as described for crystallization) and then mixed with synthetic mother liquor to mimic the pre-crystallization state. After incubation, the complex was disrupted by heating for 5 min at 90^{o} C. Hot samples (20-50μl) were injected into the HPLC and separated immediately. The separation was monitored at 260 nm as a function of time (Supporting Figure 3). For crystal composition analysis, 4-8 single crystals were removed from hanging drop experiments and transferred to a microfuge tube containing 50 μl of well solution. The tube was microcentrifuged (14K x g) for 15 sec, followed by 3x 50μl washes with synthetic mother liquor. The mother liquor was removed and crystals were dissolved in 20-50 μl water, heated to 90^{o} C and immediately injected into the HPLC column for separation. The percentage of cleaved substrate in crystals (matched in age to those of x-ray diffraction experiments) was calculated to be 6% for the AP8 and 15% for DAP8 variants. The amount of cleaved substrate strand was calculated based on the peak area of products relative to uncleaved substrate or by comparison to controls comprising pre-cleaved 5'- and 3'-product analogs (i.e. the 5-mer possessed a ribose at A-*1*, devoid of the cyclic-2',3'-phosphodiester). Areas of substrate and product strands between HPLC samples were normalized to the peak area of the late-eluting 19-

mer RNA (Figure 1A, blue strand). The AP8 modification was reported by the manufacturer to be light sensitive. Therefore caution was exercised in the treatment of this strand.

References

1. Grum-Tokars, V., Milovanovic, M., and Wedekind, J. E. (2003) Crystallization and X-ray diffraction analysis of an all-RNA U39C mutant of the minimal hairpin ribozyme, *Acta Crystallogr. D59*, 142-5.
2. Wedekind, J. E. & McKay, D. B. (2000) Purification, crystallization, and X-ray diffraction analysis of small ribozymes, *Methods Enzymol. 317*, 149-68.

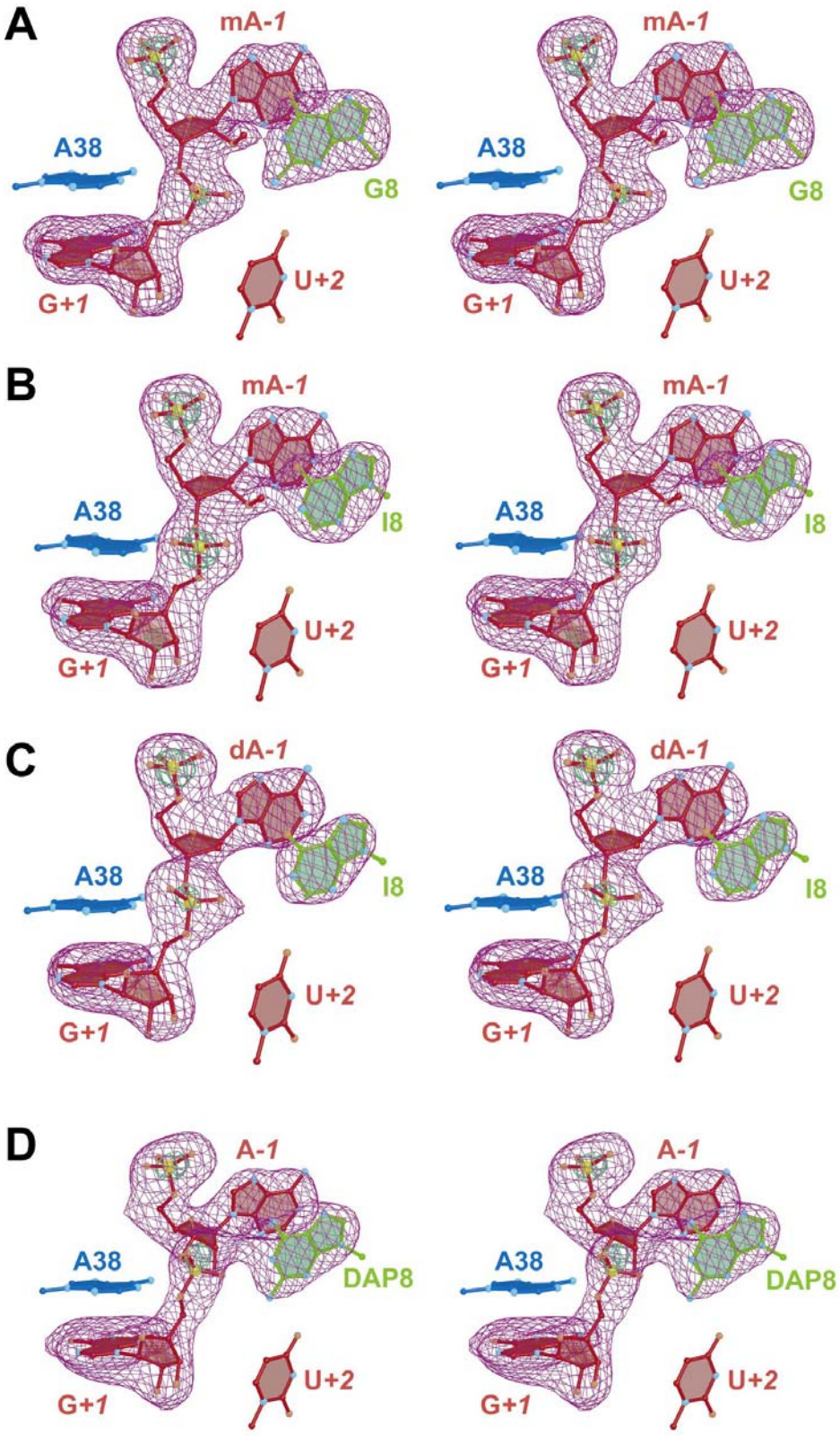
Legends of Supporting Figures

SUPPORTING FIGURE 1: Representative stereo diagrams of simulated annealing omit electron density maps for the scissile bond and position 8 of the hairpin ribozyme. Maps have σA coefficients (m$|F_o|$ - D$|F_c|$). Electron density is contoured at the 3σ (purple) and 9σ levels (green) unless specified otherwise. (*A*) The native structure at 2.05 Å resolution. The green contour level is 10σ. (*B*) The G8I structure at 2.33 Å resolution. The green contour level is 10.5 σ. (*C*) The G8/2'-deoxy A-*1* structure at 2.40 Å resolution. (*D*). The G8DAP structure at 2.40 Å resolution. The green contour level is 6.0 σ. (*E*) The G8AP structure at 2.70 Å resolution. The green contour level is 7.0 σ. (*F*) The G8A structure at 2.40 Å resolution. (*G*) The G8U structure at 2.38 Å resolution. The green contour level is 10.0 σ.

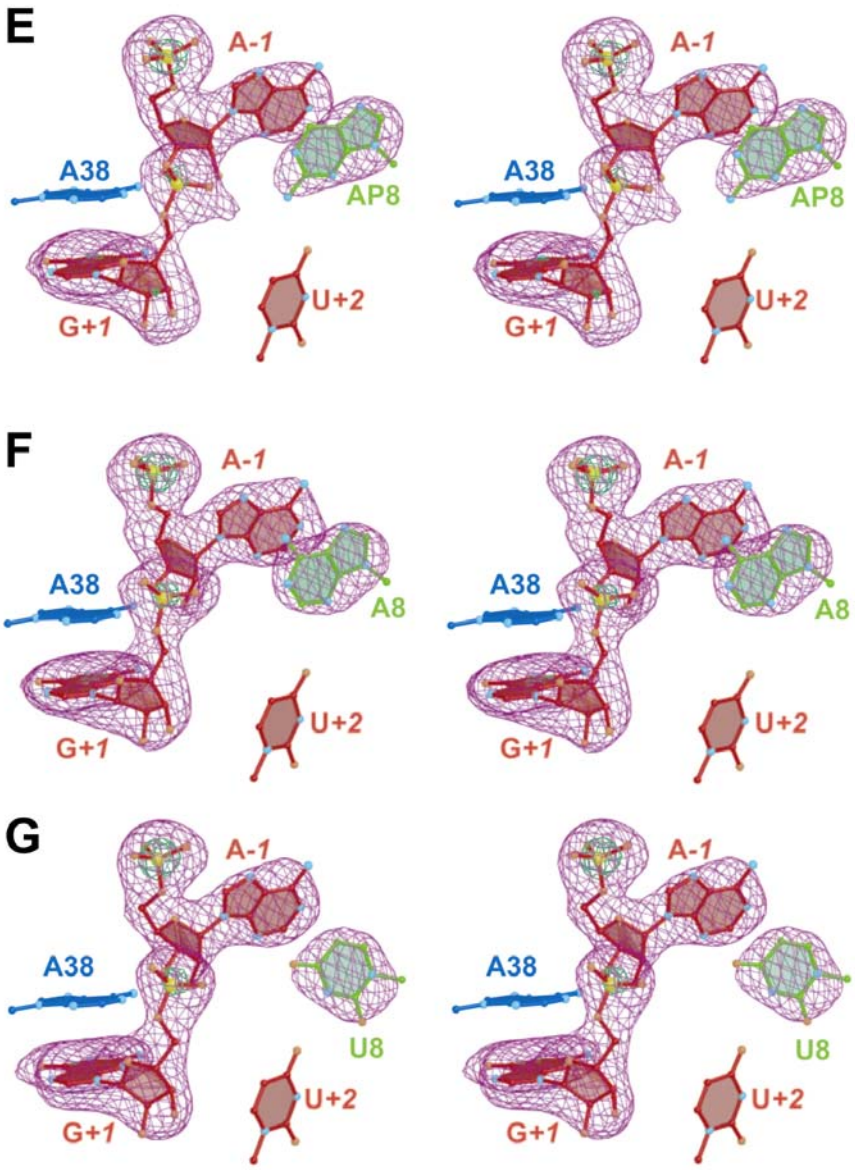
SUPPORTING FIGURE 2: Stereographic views of the G8I/2'-deoxy A-*1* structure. (*A*) Schematic view of the G8I/2'-deoxy A-*1* structure (ball-and-stick) superimposed upon the native G8 structure (black). (*B*) Superposition of the G8I/2'-deoxy A-*1* (ball-and-stick) variant upon the G8I structure (black bonds).

SUPPORTING FIGURE 3: Representative elution profiles for G8AP and G8DAP variants of the hairpin ribozyme showing crystals and control samples separated by reverse phase HPLC chromatography. The ordinate represents absorption at 260 nm; the abscissa represents time. The elution time for major peaks is listed in minutes. (*A*) Control sample run in synthetic mother liquor comprising the 12-mer (G8AP), 17-mer and 19-mer depicted in Figure 1A (green, purple and blue strands, respectively). The 13-mer substrate strand was omitted. (*B*) Control

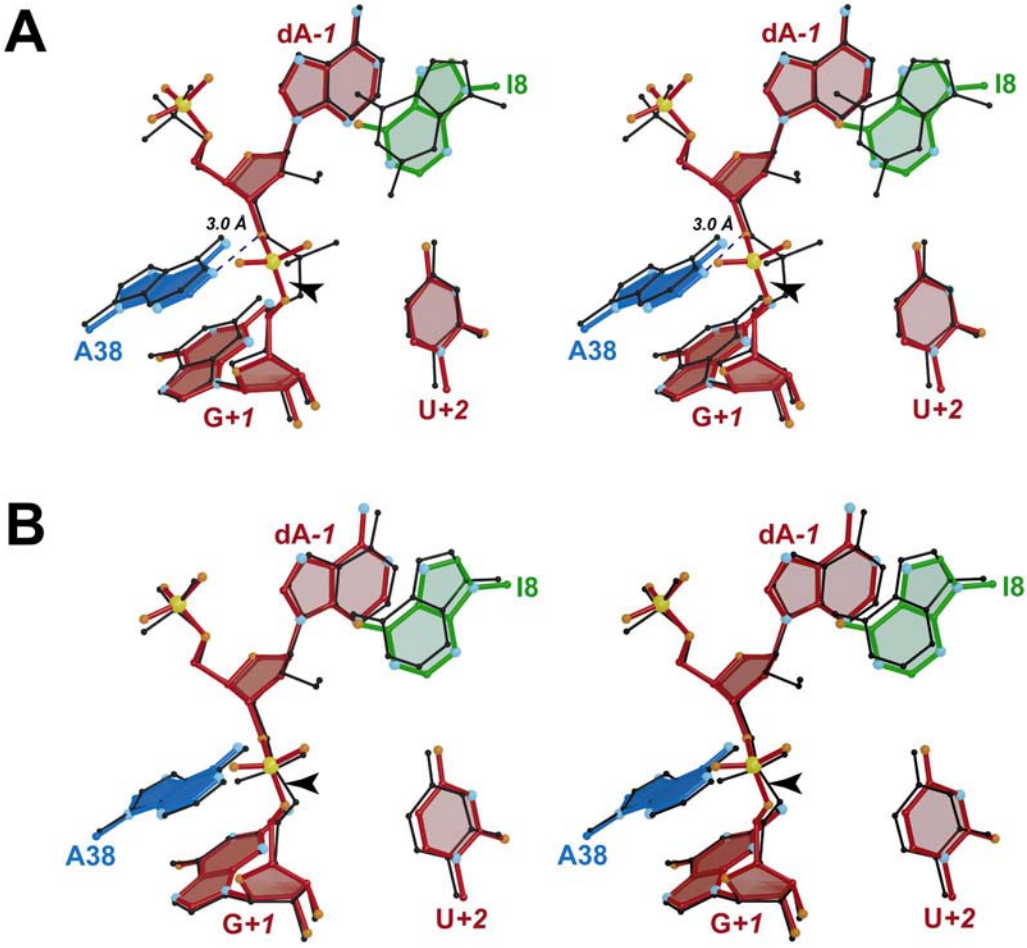
G8AP sample run in synthetic mother liquor comprising all four oligonucleotide strands depicted in Figure 1A. (*C*) Crystals of G8AP grown at *p*H 8.8 (<1 month old), representative of those used for X-ray diffraction experiments in this study. The arrows show the locations of cleavage products. (*D*) Control G8AP sample run in synthetic mother liquor comprising five oligonucleotide strands. The substrate strand was replaced with 5-mer and 8-mer RNA strands whose sequences are identical to the 5'- and 3'-cleavage products. (*E*) Crystals of G8AP grown at *p*H 8.8. The substrate 12-mer contains an inert 2'-deoxyribose group at position A-*1*. (*F*) Crystals of G8DAP grown at *p*H 8.6 (<1 month old), representative of those used for X-ray diffraction experiments in this study. Arrows show the locations of cleavage products. (*G*) Crystals of G8DAP grown at *p*H 8.6 and harvested after 10 months. The first two peaks at 16 and 25.5 min match the control experiment in (*D*), consistent with genuine product formation in crystals. The 12-mer strand appears as a doublet at 40.9 and 42.2 min, suggesting the DAP nucleotide may have undergone light mediated degradation since no effort was made to protect this analogue in the manner used for AP8.



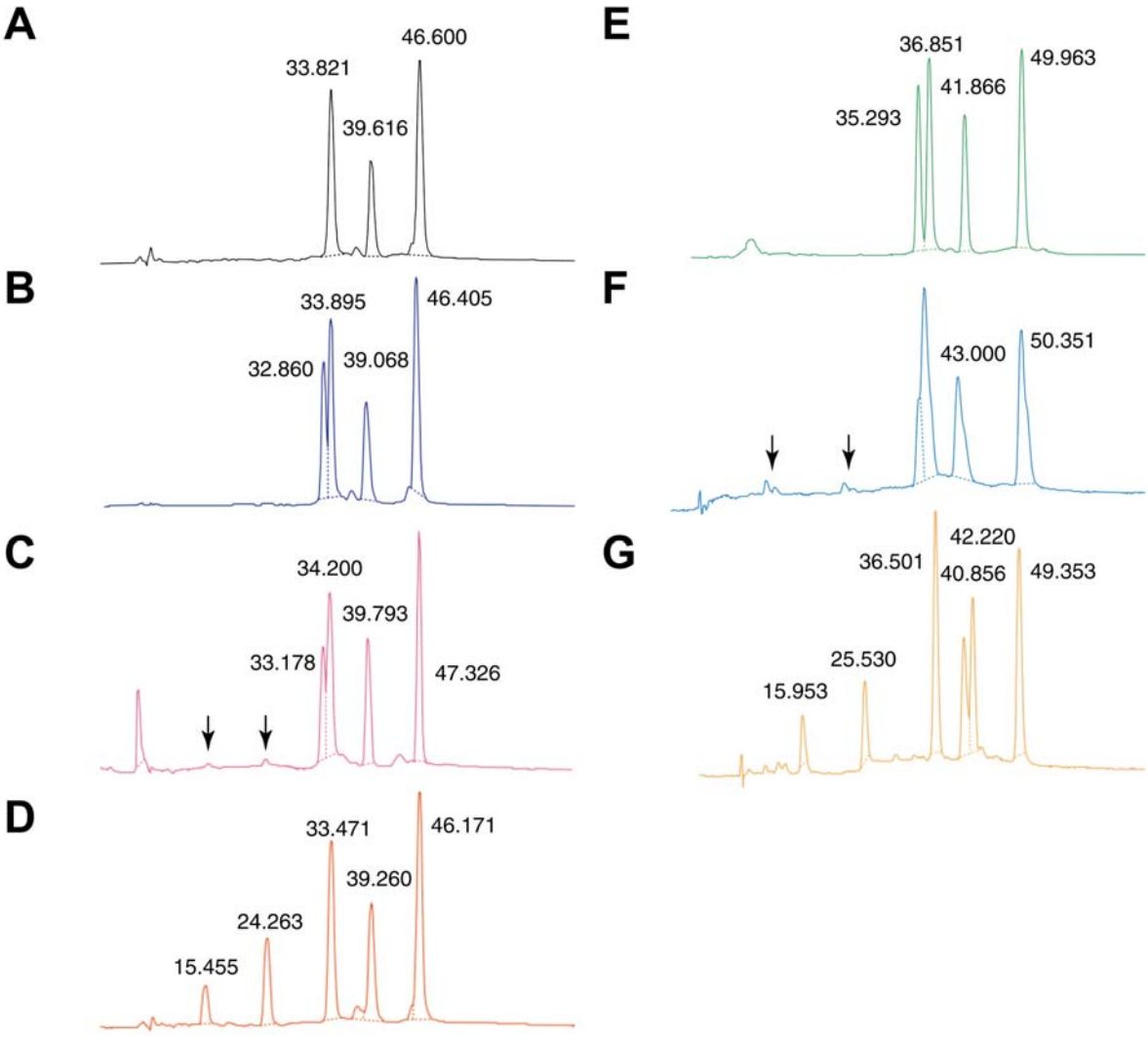
Supporting Figure 1 (part 1)



Supporting Figure 1 (part 2)



Supporting Figure 2



Supporting Figure 3