Minimal Hammerhead Ribozymes with Uncompromised Catalytic Activity:

Supplemental Materials

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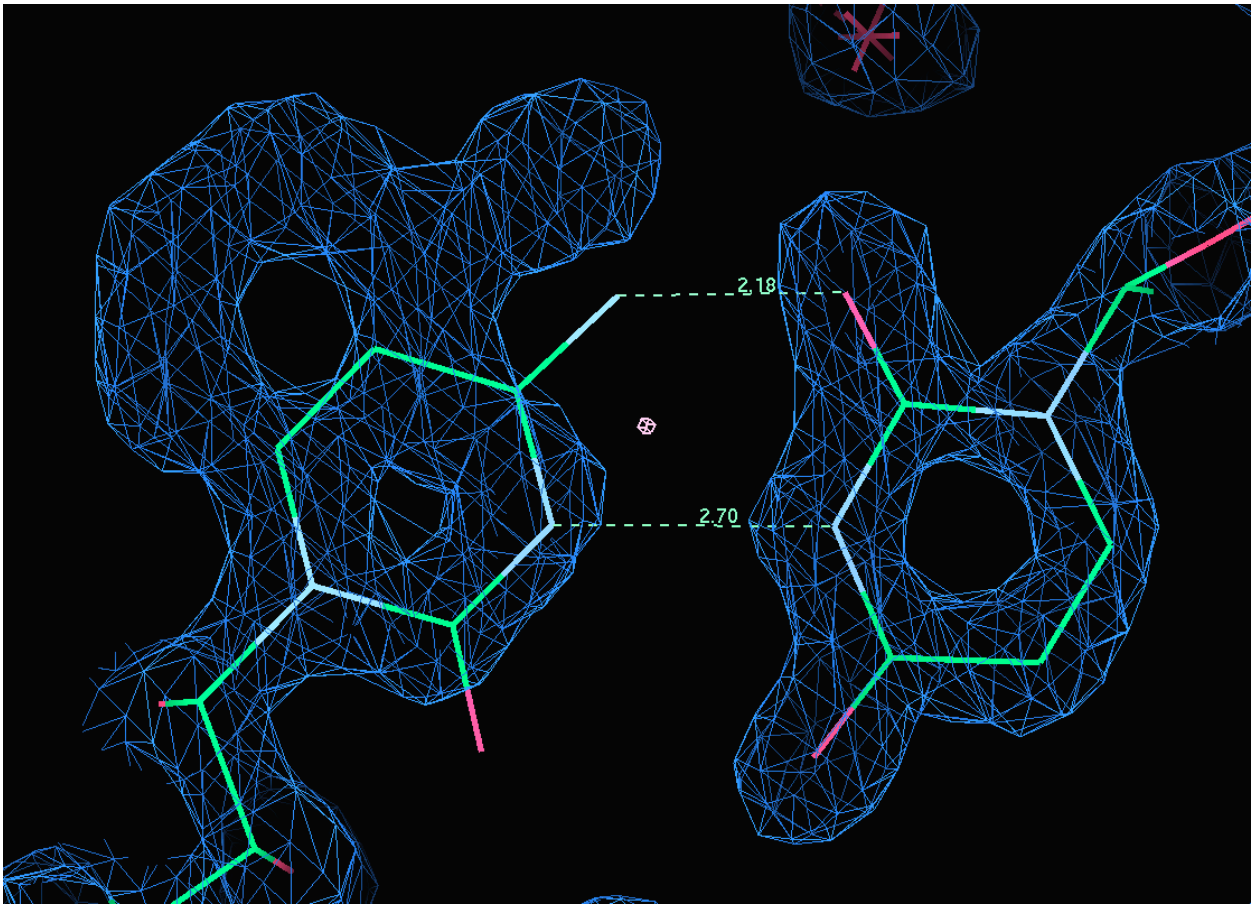
Contents



Supplemental Figure A: *In silico* modeling of the AL4C mutation using COOT†. The 1.55 Å resolution electron density corresponds to the observed AL4-U1.7 *trans*-Hoogsteen base pair observed in 3ZP8. The A was simply changed to a C, while retaining the N1 position of the nucleotide base as well as the ribose and phosphate positions. A 180° manual rotation about the glycosylic bond was performed to transform the nucleotide from the *anti*-conformation to the *syn*-conformation. No positional refinement or optimizations were performed. The result illustrated here demonstrates that the AL4C mutation is an isostructural substitution that preserves the conformation and hydrogen bonding pattern observed in the *trans*-Hoogsteen AU base pair.

† Emsley P, Lohkamp B, Scott WG, Cowtan K. (2010). Features and development of Coot. *Acta Crystallogr* ***D*** **66**, 486-501.

Animated morphing of less-active Minimal Hammerhead Ribozyme to Highly Active Minimal Hammerhead Ribozyme

The following is (an unmonitored) link to an animated gif that depicts the structural transformation that accompanies formation of the U1.7-AL4 *trans*-Hoogsteen base pair that we observed to be both necessary and sufficient to change the minimal hammerhead into a fully-active form:

http://www.chem.ucsc.edu/~wgscott/gifs/morphing_labeled.gif

Materials and Methods

RNA Preparation and purification

The hammerhead RNA enzyme strands used in this study were prepared using T7 RNA polymerase runoff transcription. The substrate RNA used in this study was obtained as a commercially prepared synthesis.

DNA templates for transcription were obtained as follows: 2nM each of partially overlapping complementary DNA oligomers (IDT Coralville, IA.) designed to incorporate a 5' T7 promotor binding sequence and hammerhead RNA , were annealed and extended using 10 U/µl MMLV reverse transcriptase (Life Technologies, Carlsbad, CA.) in a mixture containing 0.5 mM each dNTP, 3 mM $MgCl_2$, 75 mM KCl, 10 mM dithiothreitol (DTT), and 50 mM Tris-HCl (pH 8.3), which was incubated at 42^0C for 1 hr.

The RNAs were transcribed and purified as follows: The extended products created using the above procedure were transcribed using 15 U/µl T7 RNA polymerase, 0.001 U/µl inorganic pyro-phosphatase (NEB, Ipswich, MA.), 5 mM each NTP (NEB), 25 mM $MgCl_2$, 2 mM spermidine, 10 mM DTT, and 40 mM Tris-HCl (pH 7.9), which was incubated at 37^0C for 2 hr. Then, 0.5 U/µl DNase I (Roche Applied Science, Pleasanton CA.) was added, and the mixture was incubated at 37^0C for an additional 30 mins. Transcribed RNA was purified on a 15% denaturing PAGE gel. RNA eluted from the gel was purified by ethanol precipitation, and resuspended at a concentration of 100µM in water.

Hammerhead Ribozyme Activity Assays

Single turnover cleavage assays were performed using conditions similar to those previously published[15], except that the ribozyme enzyme strand was in >50-fold excess to the synthetic Cye3 5′-labeled substrate strand (IDT), with an enzyme concentration of 20 µM. Assay conditions for the fast-cleaving constructs were performed (as has been done previously) at lower pH, *i.e.,* 50 mM MES pH 5.6, 100 mM NaCl, 0.1 mM EDTA, and 10 mM $MgCl_2$ at 27°C, or under standard higher-pH reaction conditions for minimal hammerheads, *i.e.*, 50 mM Tris-HCl pH 7.4, 100 mM NaCl, 0.1 mM EDTA, and 10 mM $MgCl_2$ at 27°C, for the slower-cleaving ribozymes. Prior to adding $MgCl_2$, the enzyme and substrate were heated to 95°C for 2 min, then 65°C for 2 min, then allowed to equilibrate at 27°C for 5 min. At this point, a sample was removed and designated as the zero time point. The reaction was initiated by adding $MgCl_2$, and the subsequent triplicate time points (at 15 seconds, 30 seconds, 45 seconds, 1 minute, 2 minutes, 3 minutes, 5 minutes, 15 minutes, 30 minutes, and 60 minutes) samples were collected and flash frozen in

gel loading buffer (47.5% formamide, 10mM EDTA, 0.01% SDS, 0.01% bromophenol blue) immersed in liquid nitrogen. (During our initial characterization experiments, additional time points ranging to several hours were obtained, but these did not indicate any further increases in the cleavage fraction, and the dye label appears to deteriorate.)

Cleavage products were separated on a 20% denaturing PAGE gel, and quantified on a Typhoon Trio phosphorimager (GE Healthcare UK) using Image Jay (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014.)

Triplicate data were then fitted to a standard three-parameter exponential rise function in GraphPad Prism6 (San Diego, California, www.graphpad.com) using robust nonlinear regression as shown in Figure 3 in the main text, and as described in ref #12.

$$F(t) = F_o + F_{sat}\left(1 - e^{-k_{obs}\,t}\right)$$

The first parameter, the fraction of cleavage at t=0, F_o, was constrained to be less than or equal to the average initial fraction cleaved observed in each dataset, and the second parameter, F_{sat}, (saturation, or estimated extent of cleavage), was constrained to be greater than or equal to the average value of the greatest extent of cleavage observed in the dataset. The third parameter, k_{obs}, was then obtained via nonlinear regression analysis implemented within Prism6. We observed that adhering to this procedure, described and recommended in ref #12, yielded the most consistent and conservative estimates of k_{obs}, and enabled comparison with the previously published results given in the last three rows of Table 1 in the main text.