SUPPORTING INFORMATION

Enhanced specificity against misfolding in a thermostable mutant of the *Tetrahymena* ribozyme

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Measuring stability of the native $E^{\Delta P5abc}$ *ribozyme by UV melting and TGGE.* To determine the effects of the R14C mutations on the stability of the native conformer of $E^{\Delta P5abc}$, we performed UV melting and TGGE experiments for the $R14C^{\Delta P5abc}$ mutant and wild-type $E^{\Delta P5abc}$. Surprisingly, temperature gradient gel electrophoresis (TGGE) did not reveal transitions at substantially lower temperatures than the corresponding full-length ribozyme versions, and UV absorbance measurements gave similar dependences, with transitions for both the wild-type and variant P5abc-deleted ribozymes at only slightly lower temperatures than the corresponding full-length ribozymes (data not shown), suggesting that they reflect principally the loss of secondary structure rather than functional tertiary structure. In both our work and previous work (*1*), the transitions for $E^{\Delta P5abc}$ were much broader than those of the full-length wild-type ribozyme (ref. *2* and data not shown). This is most likely because the full-length ribozyme unfolds more cooperatively, with close coupling between global loss of tertiary structure and substantial losses of secondary structure, whereas the transitions become much less closely coupled in the P5abc-deleted mutants because the tertiary structure is destabilized substantially (*3, 4*).

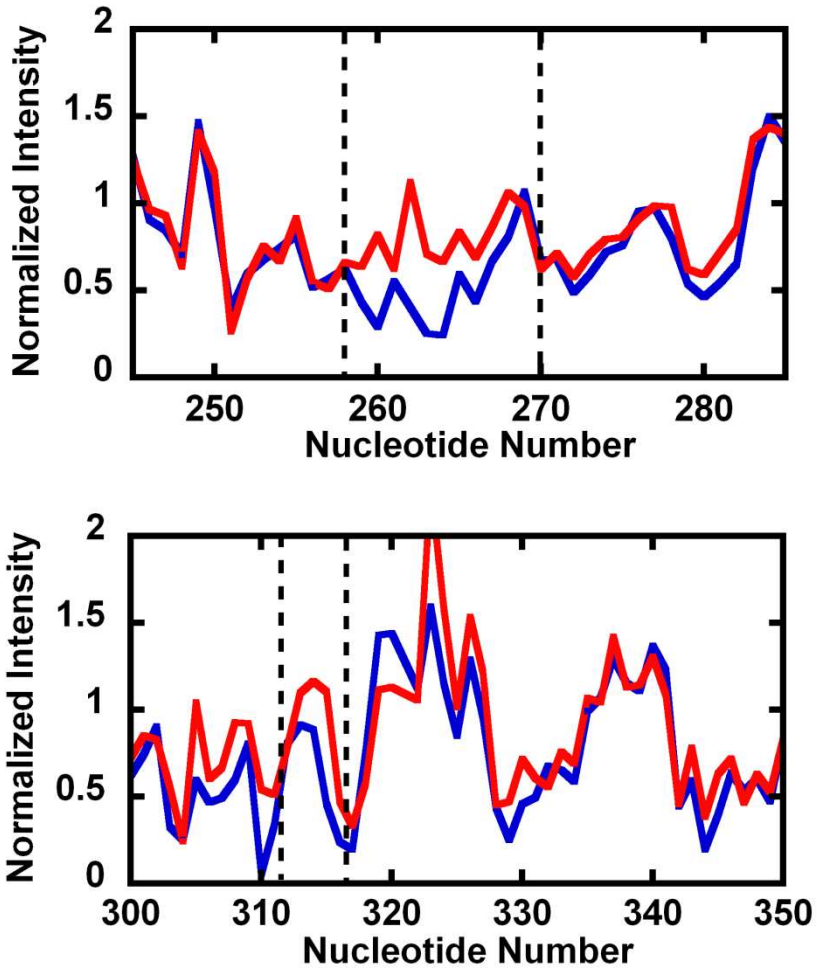

Figure S1. Hydroxyl radical footprinting of native and misfolded conformers of the full-length R14C ribozyme. Normalized band intensities from experiments probing predominantly native (blue) or misfolded (red) ribozyme were averaged from two independent experiments. The misfolded R14C ribozyme showed features of the protection pattern that are characteristic of the long-lived misfolded species of the wild-type ribozyme (*5*). In particular, the misfolded R14C ribozyme displayed greater exposure than the native ribozyme in the regions from nucleotide 258 to 270 and from 313 to 315 (indicated by dashed lines). Footprinting experiments were performed as described previously (*5, 6*).

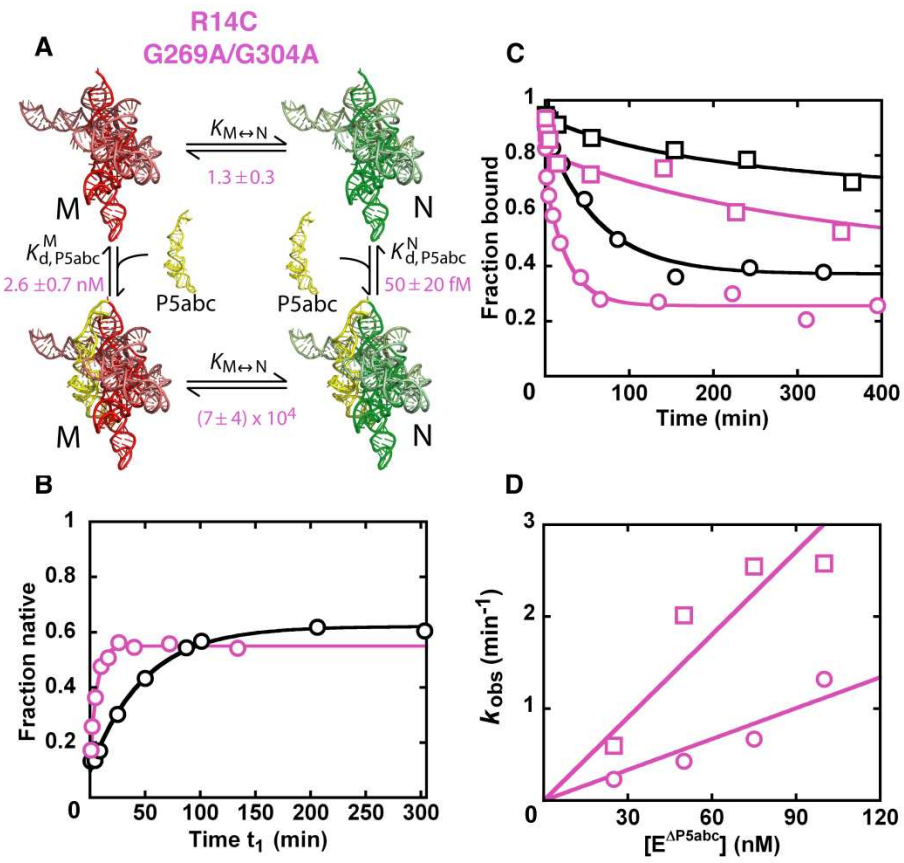

Figure S2. A reversion mutant in the R14C background (G269A/G304A) loses the enhanced specificity for native folding. (A) Thermodynamic cycle for the R14C G269A/G304A variant. (B) Equilibrium folding of the P5abc-deleted, R14C G269A/G304A ribozyme. The observed rate constant is 0.14 ± 0.02 min^{-1} and the equilibrium value is 1.3 ± 0.3. (C) Dissociation of P5abc from the P5abc-deleted R14C G269A/G304A variant (violet). As in Figure 3B, dissociation was followed from a solution of predominantly native ribozyme (violet squares) or a mixture of native and misfolded ribozyme (violet circles). Data from wild-type $E^{\Delta P5abc}$ ribozyme are shown in black for comparison. (D) P5abc association kinetics. Analogous to experiments in Figure 3D, association was measured for a population of largely native R14C G269A/G304A$^{\Delta P5abc}$ variant ribozyme (violet squares) or a population of predominantly misfolded ribozyme (violet circles) (*7*). The calculated values from these measurements are (2.8 ± 0.6) x 10^7 M^{-1} min^{-1} for binding to the native ribozyme and (9.9 ± 1.6) x 10^6 M^{-1} min^{-1} for binding to the misfolded ribozyme.

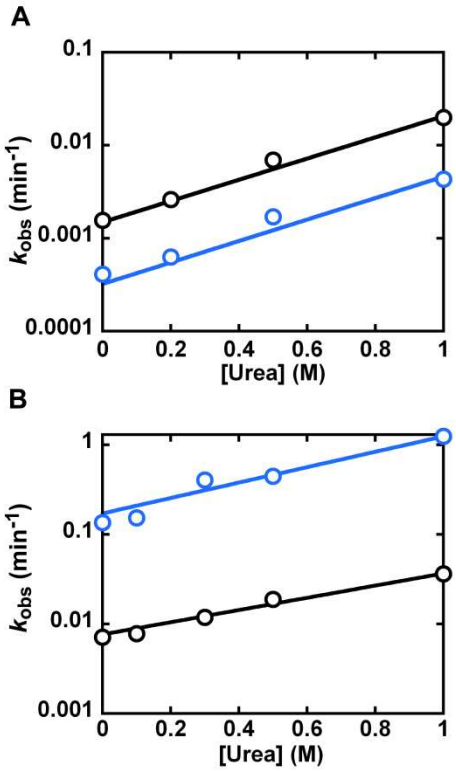

Figure S3. Folding transitions of the full-length and P5abc-deleted ribozymes. (A) Urea dependence of refolding from the misfolded conformation for full-length R14C (blue) and wild-type (black) ribozymes (37 ℃, 10 mM Mg^{2+}, 50 mM Na-Mops, pH 7.0). The R14C ribozyme gives an *m*-value of 1.6 kcal mol^{-1} M^{-1}, indistinguishable from that for the wild-type ribozyme obtained here and previously (*5*). (B) Urea dependence for refolding of the $R14C^{\Delta P5abc}$ (blue) and $E^{\Delta P5abc}$ (black) ribozymes (25 ℃, 50 mM Mg^{2+}, 50 mM Na-Mops, pH 7.0). The dependence for $R14C^{\Delta P5abc}$ gives an *m*-value of 1.2 kcal mol^{-1} M^{-1}, similar to the value of 0.93 kcal mol^{-1} M^{-1} for $E^{\Delta P5abc}$ measured in parallel and the value of 1.0 kcal mol^{-1} M^{-1} measured previously for $E^{\Delta P5abc}$ (*4*).

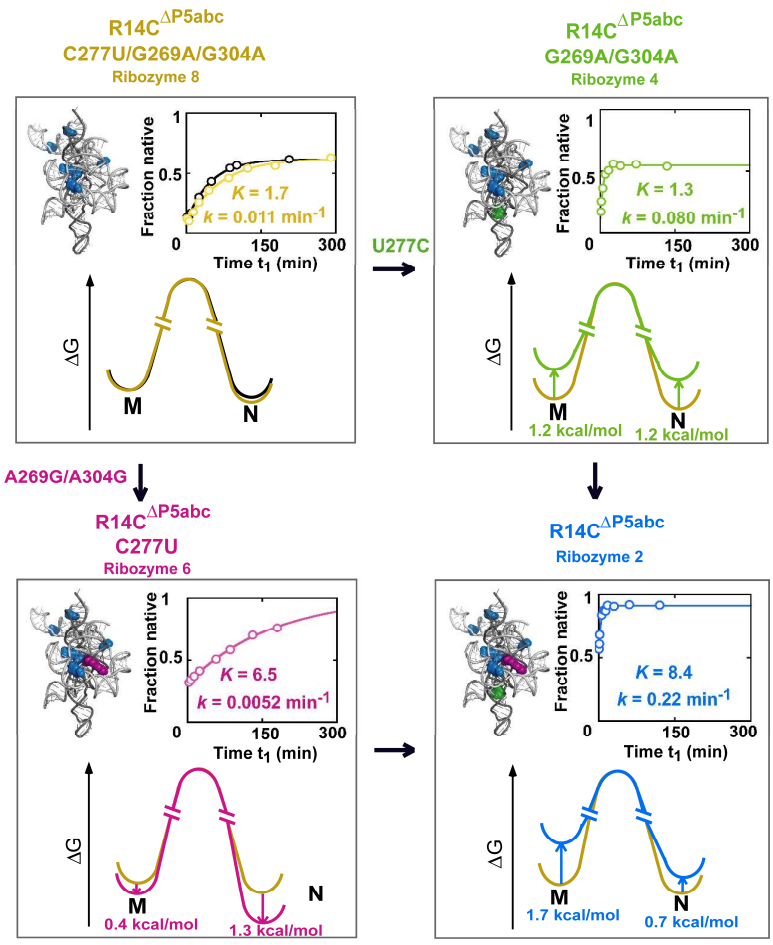

Figure S4. Mutations of nucleotides 269/304 and 277 in the R14C$^{\Delta P5abc}$ background. At top left, results are shown for a mutant in which nucleotides 269, 304 and 277 are reversed to their identities in the wild-type ribozyme, whereas the other six positions correspond to the R14C$^{\Delta P5abc}$ ribozyme (blue in the structure schematic). Folding of this mutant is very similar to that of the wild-type E$^{\Delta P5abc}$ ribozyme (black), as shown in the data plot (insets) and corresponding free energy profile. When nucleotides 269 and 304 are mutated to G (the 'forward' direction toward the R14C mutant), the equilibrium shifts toward the native state while the refolding rate is not affected (bottom left, magenta). The behavior of the mutant is similar to the corresponding A269G/A304G$^{\Delta P5abc}$ mutant in the wild-type background (see Figure 7). A U277C mutant in this R14C$^{\Delta P5abc}$ background (top right) also gives similar behavior as in the wild-type background. The basic effects of the isolated substitutions are also present in the behavior of the R14C$^{\Delta P5abc}$ ribozyme (bottom right, blue).

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