Supporting Information for "Tightening of Active Site Interactions En-route to the Transition State Revealed by Single-Atom Substitution in the Guanosine-Binding Site of the *Tetrahymena* Group I Ribozyme"

Marcello Forconi, Rishi H. Porecha, Joseph A. Piccirilli, and Daniel Herschlag

A. The G264deaza substitution introduces clashes with the exocyclic amino group of the nucleophilic guanosine

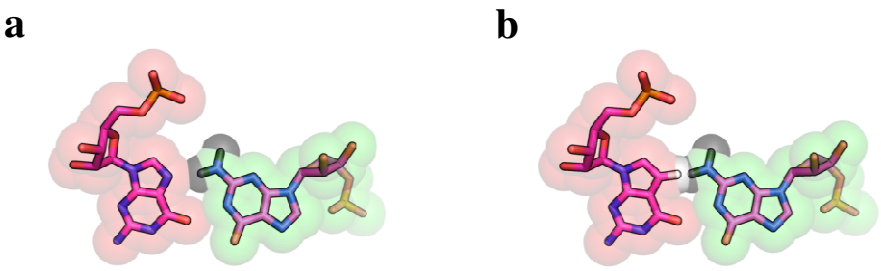

Figure S1. Space-filling models derived from the *Azoarcus* crystal structure (3BO3) suggest steric clashes in the G264deaza ribozyme. The guanosine nucleophile is in green; residue G264 is in red. Hydrogen atoms are shown only for the exocyclic amino group of G (in gray) and for the CH group introduced in the G264deaza ribozyme (in white). (A) Space-filling model of the WT ribozyme using van der Waals radii. (B) Modeling a CH group in place of N7 of G264 reveals steric clashes between the hydrogen atom of 7-deazaguanosine at position 264 and one of the hydrogen atoms of the exocyclic amino group of the guanosine nucleophile.

B. The sum of the coupling energies from the individual reaction steps is consistent with the overall coupling energy.

We tested the consistency of the values of the coupling energies determined for the individual steps (shown in Figure 5) with the overall coupling energy of 7.5 kcal/mol (Figure 3), which represents the coupling energy on reactions starting from the $(E•S)_o$ complex (Figures 1A and S2). As shown in Figure S2, the overall coupling energy on the second-order rate constant $[(k_{cat} / K_M)^{AUCX}_{open}]$ is given by the sum of the coupling energies for the docking step without AUCX bound ($K^{E•S}_{dock}$), the AUCX binding step with docked E•S $[(K_d^{AUCX})_c]$, and the chemical step (k_C). The coupling energy on the docking step without AUCX bound is zero because if AUCG or ACUI are not bound they can not contribute to the docking equilibria. The coupling energies for the AUCX binding step with docked E•S and for the chemical step are shown in Figures 5B and 5C and correspond to 4.3 and 3.0 kcal/mol, respectively. Therefore, the calculated overall coupling energy is equal to 0+4.3+3.0 = 7.3 kcal/mol, which is essentially identical to the 7.5 kcal/mol value shown in Figure 3.

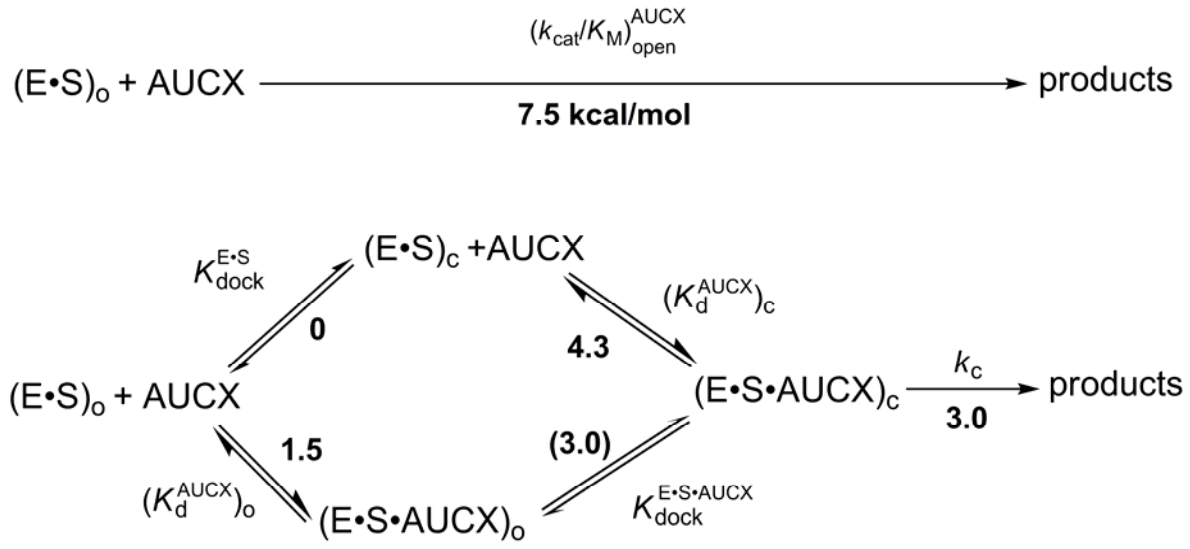

Figure S2. Coupling energy for the individual reaction steps.

Because the individual reaction steps are part of a cycle (Figure S2), it also follows that the overall coupling energy of 7.5 kcal/mol equals the sum of the coupling energies for the AUCX binding to the $(E•S)_o$ complex step [$(K_d^{AUCX})_o$, corresponding to 1.5 kcal/mol as shown in Figure 5A], the docking step with AUCX bound ($K_{dock}^{E•S•AUCX}$), and the chemical step (k_C corresponding to 3.0 kcal/mol as explained above and shown in Figure 5C). Thus, we can infer the coupling energy for the docking step with AUCX bound as 7.5-1.5-3.0 = 3.0 kcal/mol.

C. The G264deaza ribozyme binds AUCG more weakly than the WT ribozyme

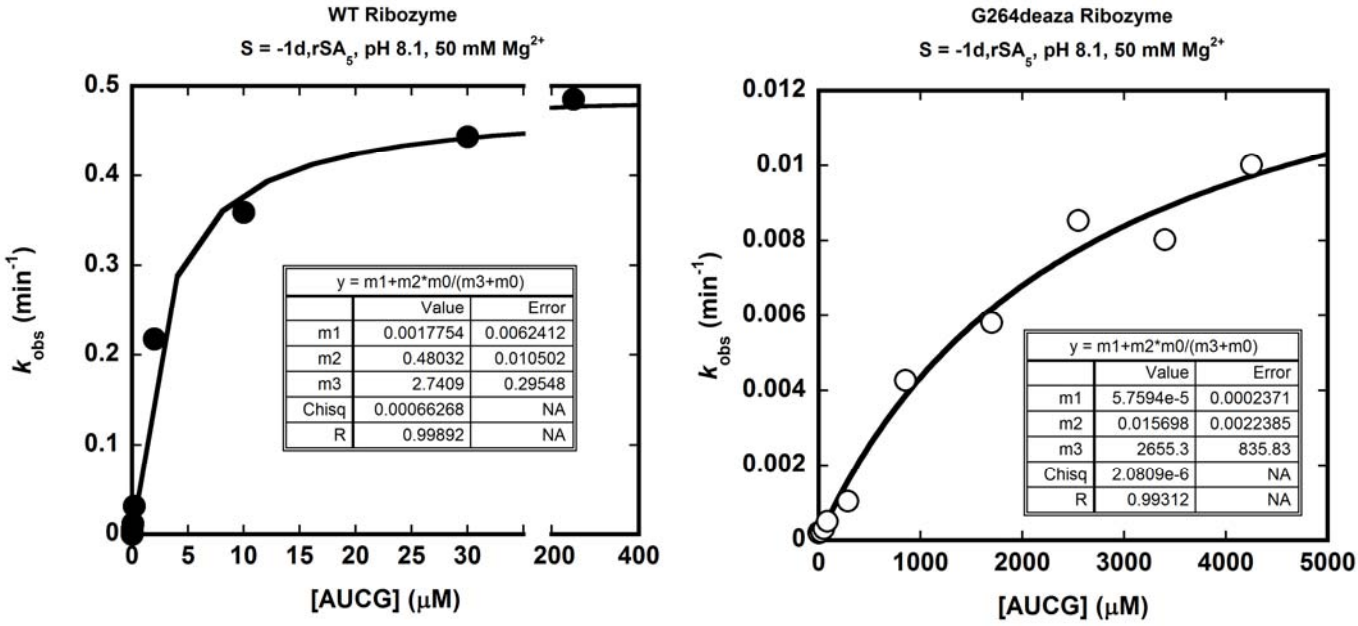

Figure S3. Plot of the observed rate constant versus the concentration of AUCG for the WT and the G264deaza ribozymes. Fitting of data according to a single binding event (see Figure 1A or Figure S2) gives values of k_{max} = (0.48 ± 0.01) min^{-1} and of $(K_d^{AUCG})_c$ = (2.7 ± 0.3) μM for the WT ribozyme, and of k_{max} = (0.016 ± 0.002) min^{-1} and of $(K_d^{AUCG})_c$ = (2600 ± 800) μM for the G264deaza ribozyme.

D. The contact with M_c is altered in the G264deaza ribozyme.

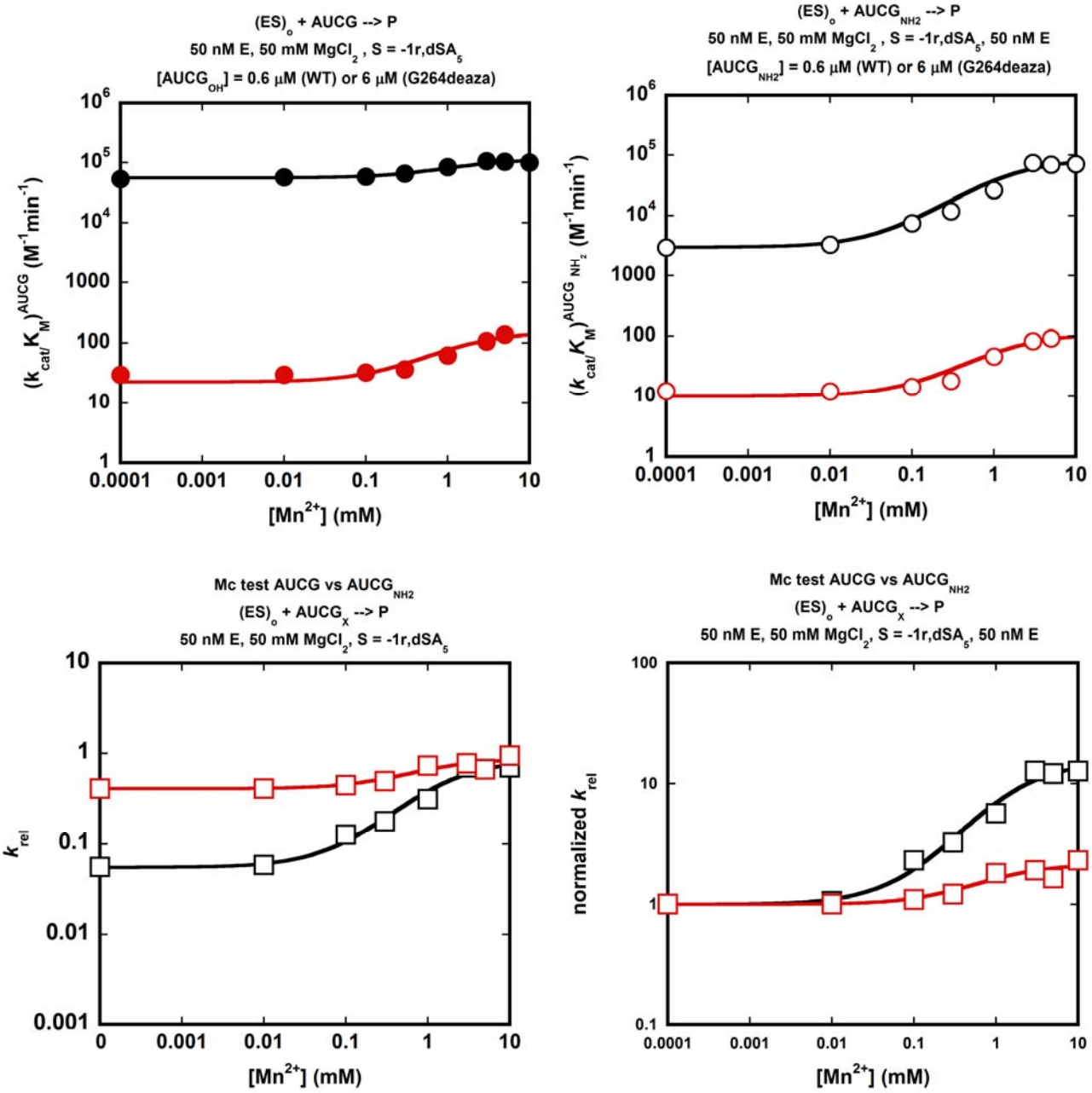

Figure S4. M_C rescue of -1r,dSA5 cleavage with saturating $AUCG_{2'\text{-}NH_2}$ by wild-type and modified ribozymes. Rescue reactions were carried out as indicated in the Experimental Section and in Figure 7 legend.