Minor Corrections and Additions to a Computational Study on Catalytic Strategies

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The Bevilacqua lab published a paper in 2018 that described the development and use of a computational pipeline to study the catalytic strategies of various small ribozymes [Seith, D. D.; Bingaman, J. L.; Veenis, A. J.; Button, A. C.; Bevilacqua, P. C. *ACS Catal.* **2018**, *8*, 314-327]. After publication, we discovered several minor issues. The Author Guidelines for *ACS Catalysis* specifies that corrections and additions that are minor will not be published. Because the issues identified do not significantly impact the conclusions presented in the paper, we decided to present our corrections and additions in this document and post it to our website. This includes several updated plots for the twister and hairpin ribozymes as well as corrections and clarifications to some of the content presented in the methods section and in Table S1 of the paper.

Three crystal structures of the twister ribozyme (PDB IDs 5DUN, 4RGF, and 4RGE) include divalent cations close to the *pro*-S_P NBO and the O5' of the scissile phosphate, yet they were not depicted in the corresponding distance plots in our publication. We found that an outdated version of the γ, β, and δ Scissile Phosphate Plugin, which did not consider metals ions, was used to collect data on these crystal structures. Data were recollected on these crystal structures and the relevant plots were remade (Figures 1 and 2). As seen in these figures, the divalent cations are the closest contact for both the *pro*-S_P NBO and the O5' of the twister ribozyme.

Upon studying the distance plots for the hairpin ribozyme, we noted some oddities regarding how the nucleobase variants were plotted. For instance, G8DAP was often plotted twice for a given contact atom, yet only one of the hairpin ribozyme crystal structures considered in the paper contains this modification. The Scissile Phosphate Downstream Processing Script was inspected, and corrections were made to how the code managed nucleobase variants for the hairpin ribozyme. The updated script was then used to remake the relevant hairpin ribozyme plots (Figures 3, 4, 5, and 6) which, as anticipated, exhibited changes in how the nucleobase variants were depicted. Additionally, two of the plots (Figures 4 and 5) revealed minor differences beyond how the nucleobase variants were depicted. These additional small changes are likely due to differences between the original and the current sets of data files that were used to create these plots.

We now turn to address the methods section and Table S1 of the paper. In the methods section, it was stated that atoms belonging to conformation A in the crystal structures studied always have an occupancy factor of at least 0.5. While this is the case for the majority of the crystal structures, four structures (PDB IDs 3I2R, 3I2S, 4G6P, and 4G6R) contain atoms that belong to conformation A and have occupancy factors less than 0.5. The remainder of our comments pertain to how some of the structures were detailed and categorized in Table S1. They are listed below.

- For PDB ID 3B4B, the ribozyme contains a G33A nucleobase variant in addition to the 2′,5′ linkage.
- For PDB ID 3G8S, the ribozyme contains a 2′-methoxy at A–1 in addition to being unliganded.
- For PDB ID 2OUE, the ribozyme contains a U39C nucleobase variant in addition to the 2′-methoxy at A–1.
- For PDB ID 3GS8, the ribozyme contains a 2′,5′ linkage but does not contain a N1-deazaadenosine at A38.
- For PDB ID 1X9K, we did not state that the crystal structure contains a 2′-methoxy at A–1. While this functional group is indeed absent in the PDB file, Alam and colleagues specified in their paper that this modification was included to inhibit self-cleavage.[1]
- For PDB ID 4G6R, this structure was inadvertently not considered as vanadate-like even though it contains a 2′,5′ linkage.
- For PDB ID 4G6S, this structure was inadvertently not considered as vanadate-like even though it contains a 2′,5′ linkage.

In conclusion, we report here several minor issues with our catalytic strategies paper that were discovered after publication. Two updated plots for the twister ribozyme are presented that now include divalent metal ions. Furthermore, four updated plots for the hairpin ribozyme are shown which depict a variety of differences when compared with the original plots of our publication. Lastly, we comment on some observations regarding the content of the methods section and Table S1. None of these changes alter the major conclusions of the paper. Our catalytic strategies paper presents a powerful tool for comparing complicated structural details of different ribozymes. We hope that readers find value in the information presented and that our paper stimulates the creation of new ideas on how ribozymes may function.

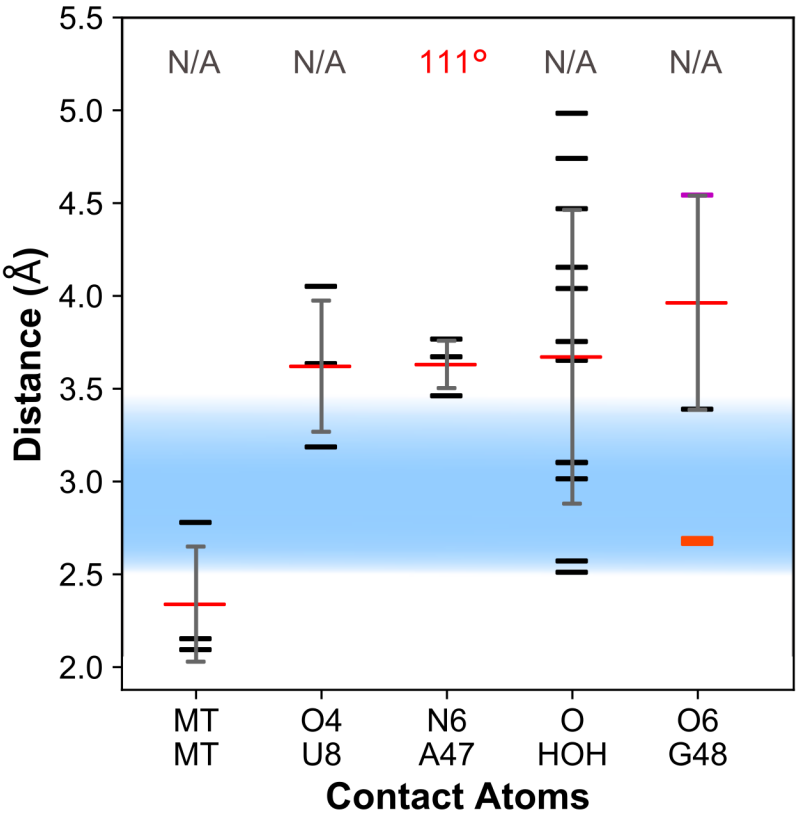

Figure 1. Top five contact atoms to the *pro*-S_P NBO of the twister ribozyme. This corrected plot corresponds to Figure 5C (left) in the paper. The black, purple, and orange bars represent distances from wild-type, intermediate-mimic, and non-catalytically relevant structures, respectively. The red and grey bars represent the average and standard deviation, respectively, of all black and purple bars. The blue shaded region represents optimal distances for hydrogen bonding. When applicable for the particular contact atom, the average hydrogen bonding angle is depicted at the top of the plot. Values below 140° are considered suboptimal and shown in red.

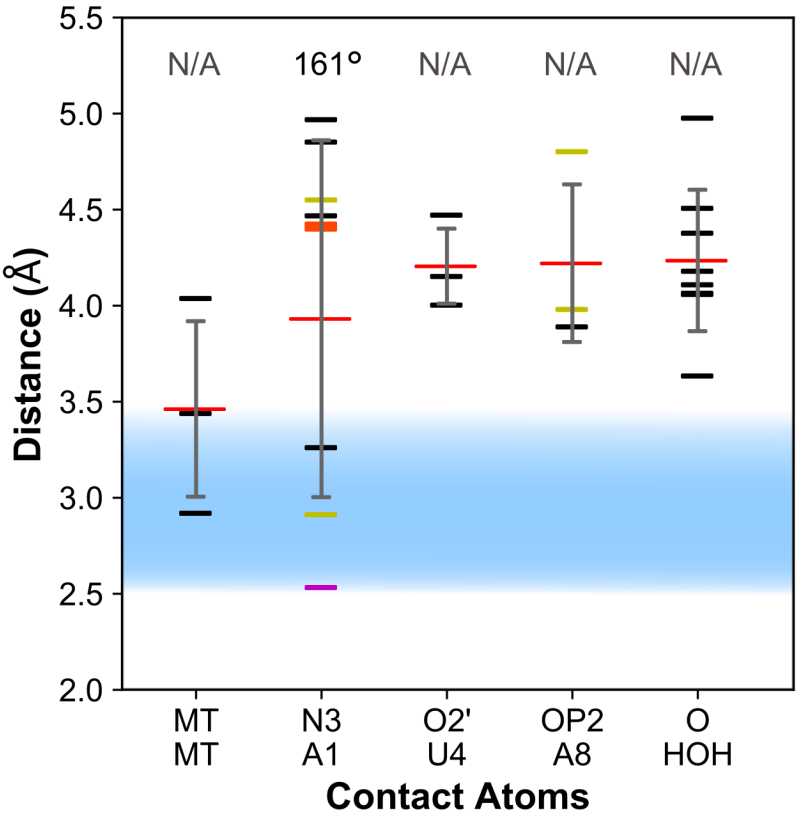

Figure 2. Top five contact atoms to the O5′ of the twister ribozyme. This corrected plot corresponds to Figure 6C in the paper. The black, purple, orange, and yellow bars represent distances from wild-type, intermediate-mimic, non-catalytically relevant, and computationally modeled structures, respectively. The red and grey bars represent the average and standard deviation, respectively, of all black, purple, and yellow bars. The blue shaded region represents optimal distances for hydrogen bonding. When applicable for the particular contact atom, the average hydrogen bonding angle is depicted at the top of the plot. Values below 140° are considered suboptimal and shown in red.

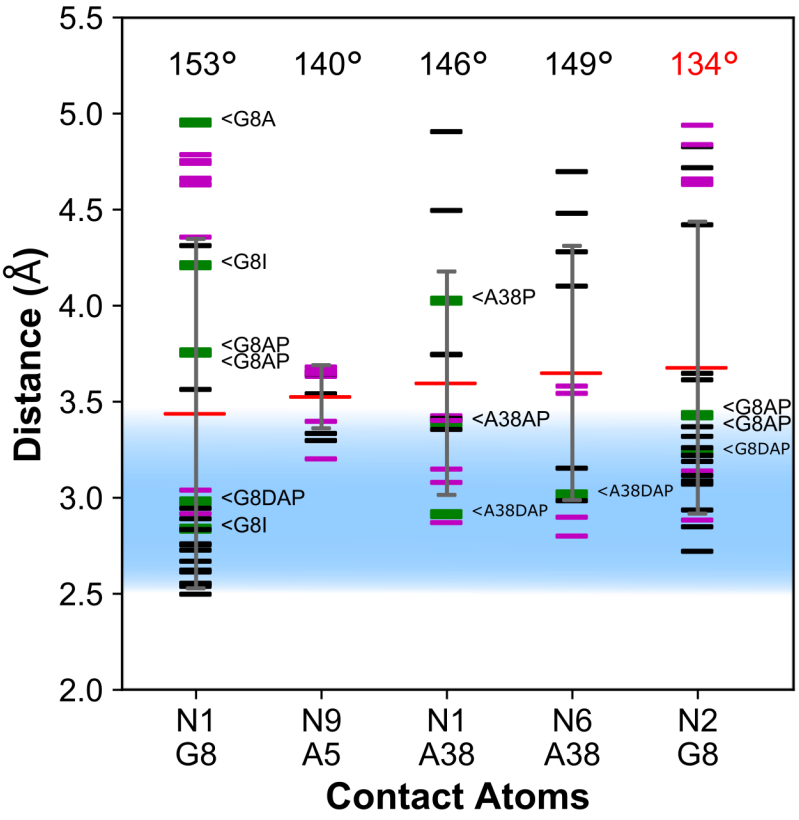

Figure 3. Top five contact atoms to the O2′ of the hairpin ribozyme. This corrected plot corresponds to Figure 4D in the paper. The black and purple bars represent distances from wild-type and intermediate-mimic structures, respectively. The green bars represent distances involving contact atoms that belong to nucleobase variants. The identity of the nucleobase variant is depicted to the right of each green bar. The red and grey bars represent the average and standard deviation, respectively, of all black and purple bars. The blue shaded region represents optimal distances for hydrogen bonding. When applicable for the particular contact atom, the average hydrogen bonding angle is depicted at the top of the plot. Values below 140° are considered suboptimal and shown in red.

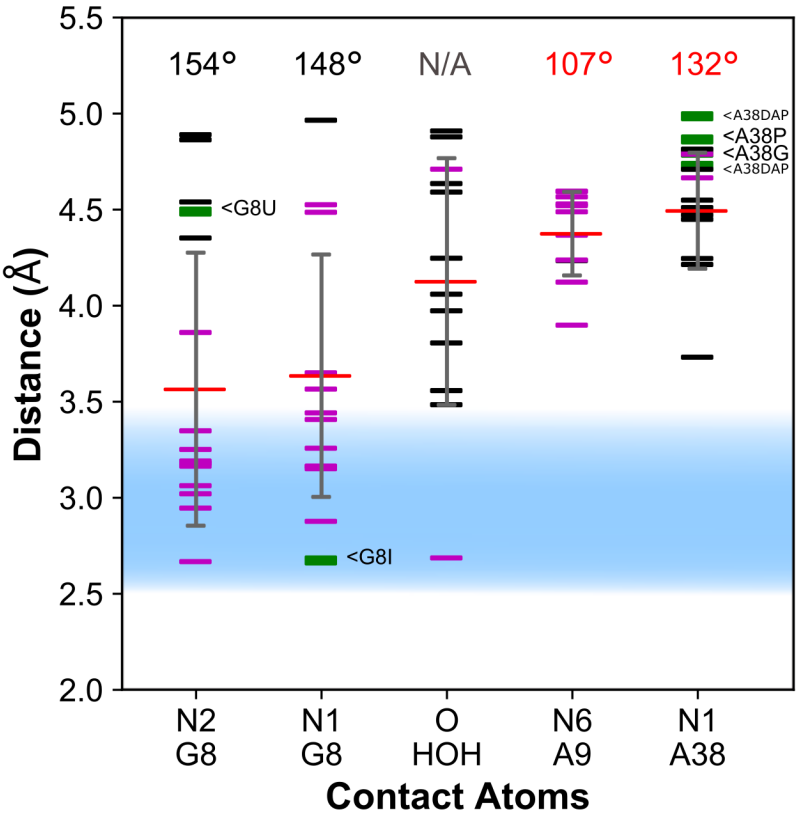

Figure 4. Top five contact atoms to the *pro-S*$_P$ NBO of the hairpin ribozyme. This corrected plot corresponds to Figure 5D (left) in the paper. The black and purple bars represent distances from wild-type and intermediate-mimic structures, respectively. The green bars represent distances involving contact atoms that belong to nucleobase variants. The identity of the nucleobase variant is depicted to the right of each green bar. The red and grey bars represent the average and standard deviation, respectively, of all black and purple bars. The blue shaded region represents optimal distances for hydrogen bonding. When applicable for the particular contact atom, the average hydrogen bonding angle is depicted at the top of the plot. Values below 140° are considered suboptimal and shown in red.

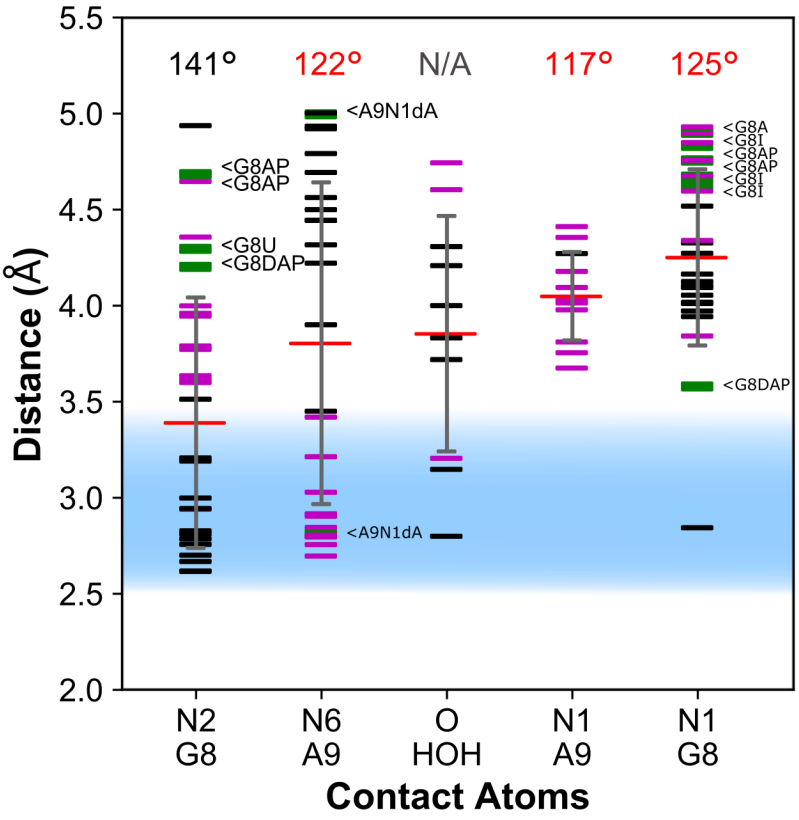

Figure 5. Top five contact atoms to the *pro-R*$_P$ NBO of the hairpin ribozyme. This corrected plot corresponds to Figure 5D (right) in the paper. The black and purple bars represent distances from wild-type and intermediate-mimic structures, respectively. The green bars represent distances involving contact atoms that belong to nucleobase variants. The identity of the nucleobase variant is depicted to the right of each green bar. The red and grey bars represent the average and standard deviation, respectively, of all black and purple bars. The blue shaded region represents optimal distances for hydrogen bonding. When applicable for the particular contact atom, the average hydrogen bonding angle is depicted at the top of the plot. Values below 140° are considered suboptimal and shown in red.

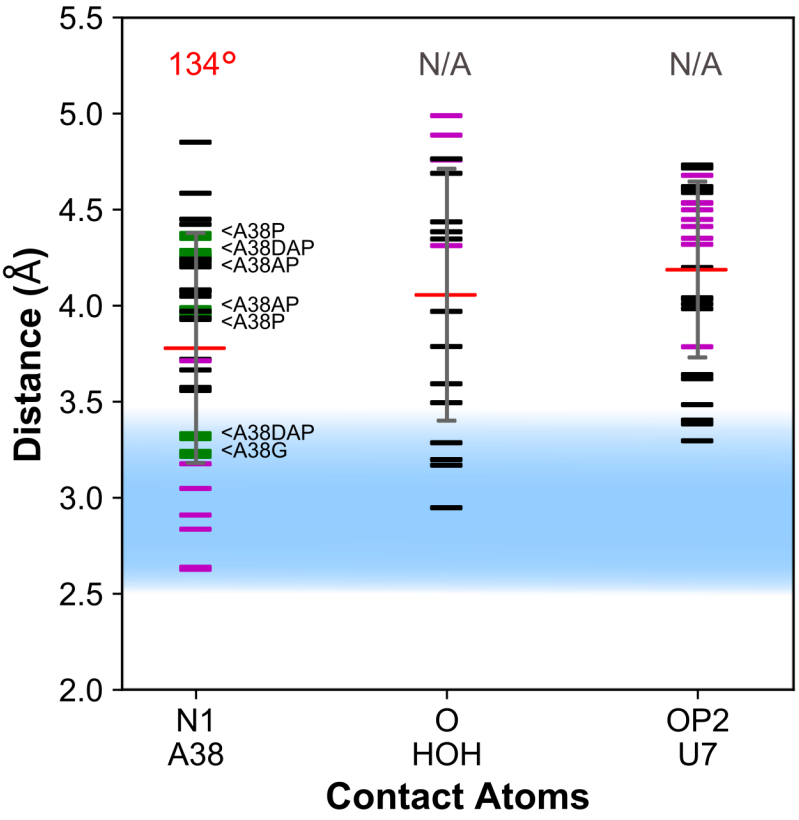

Figure 6. Top five contact atoms to the O5′ of the hairpin ribozyme. This corrected plot corresponds to Figure 6D in the paper. The black and purple bars represent distances from wild-type and intermediate-mimic structures, respectively. The green bars represent distances involving contact atoms that belong to nucleobase variants. The identity of the nucleobase variant is depicted to the right of each green bar. The red and grey bars represent the average and standard deviation, respectively, of all black and purple bars. The blue shaded region represents optimal distances for hydrogen bonding. When applicable for the particular contact atom, the average hydrogen bonding angle is depicted at the top of the plot. Values below 140° are considered suboptimal and shown in red. Only three contact atoms met the criteria detailed in the paper needed to be included in the top five.

References

1. Alam, S.; Grum-Tokars, V.; Krucinska, J.; Kundracik, M. L.; Wedekind, J. E., *Biochemistry* **2005,** *44*, 14396-14408.