DNAmazing: RNA cleavage mechanism of catalytically active DNA and the effect of non-natural nucleobases on it

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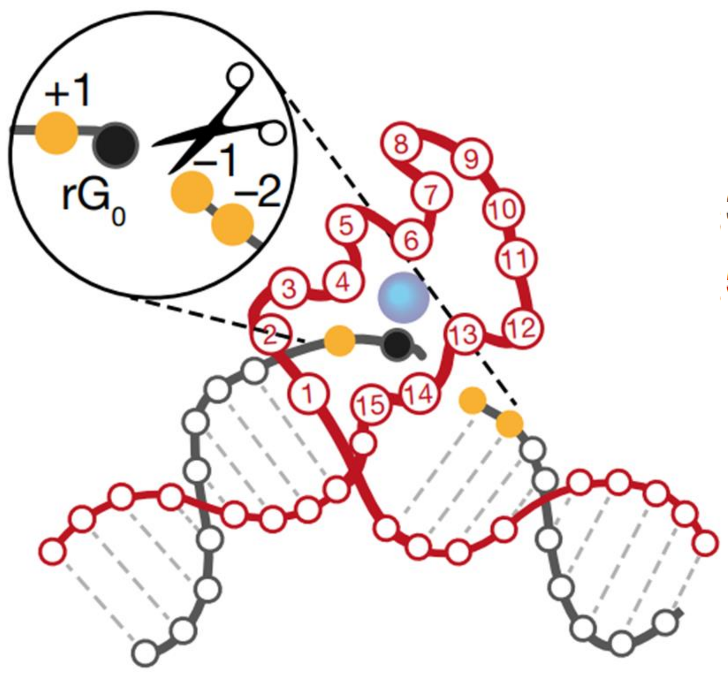
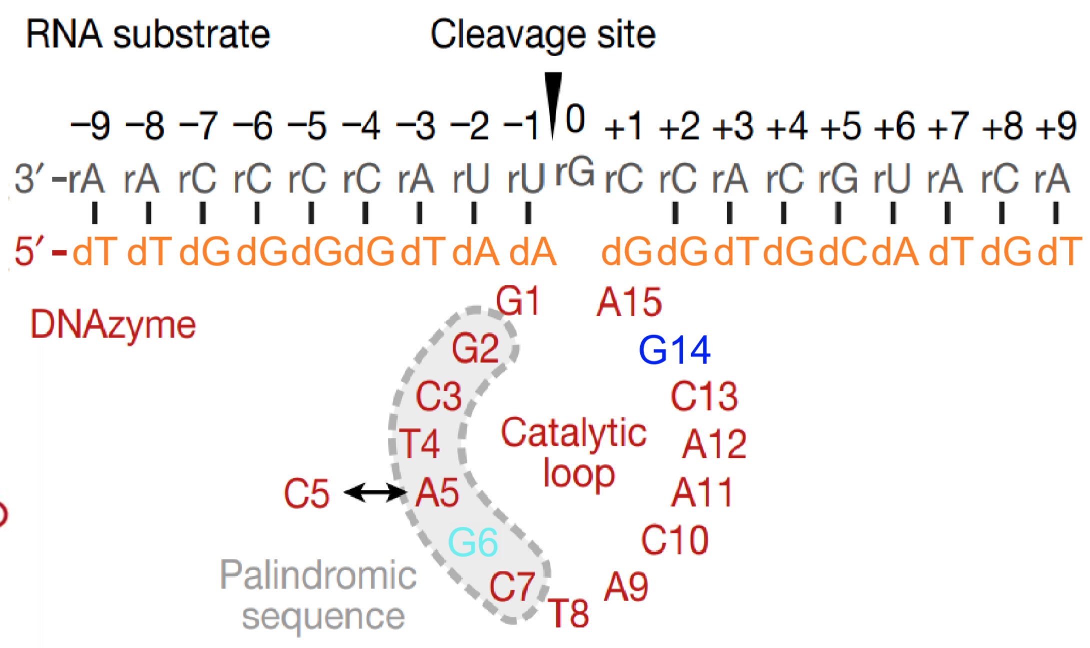
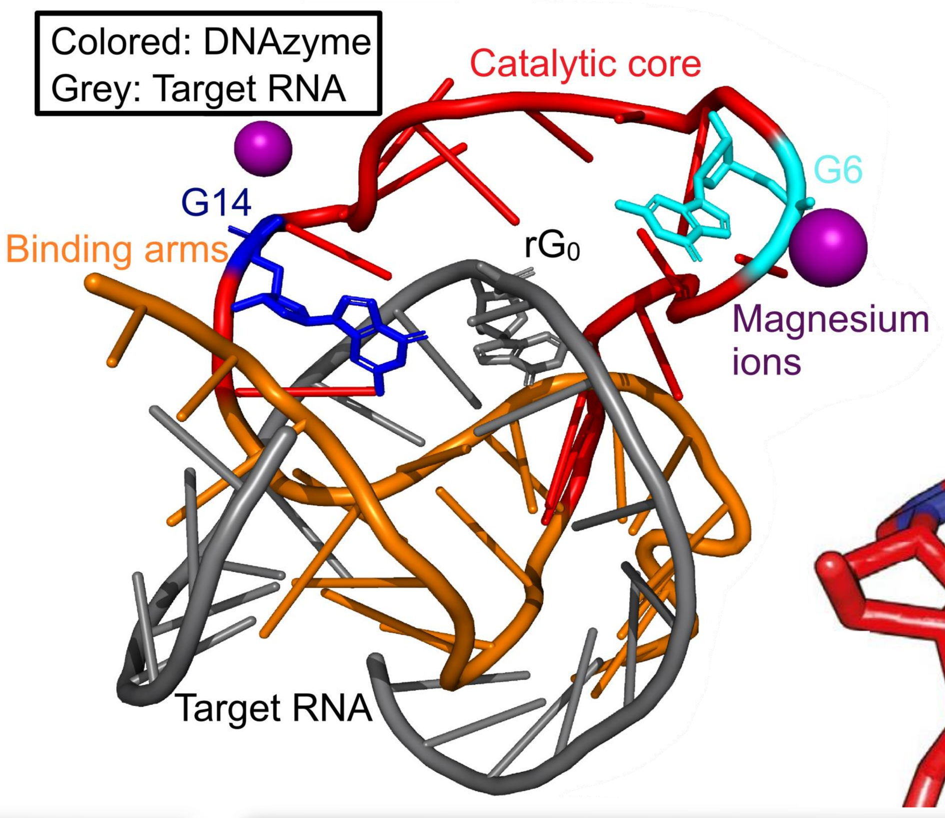
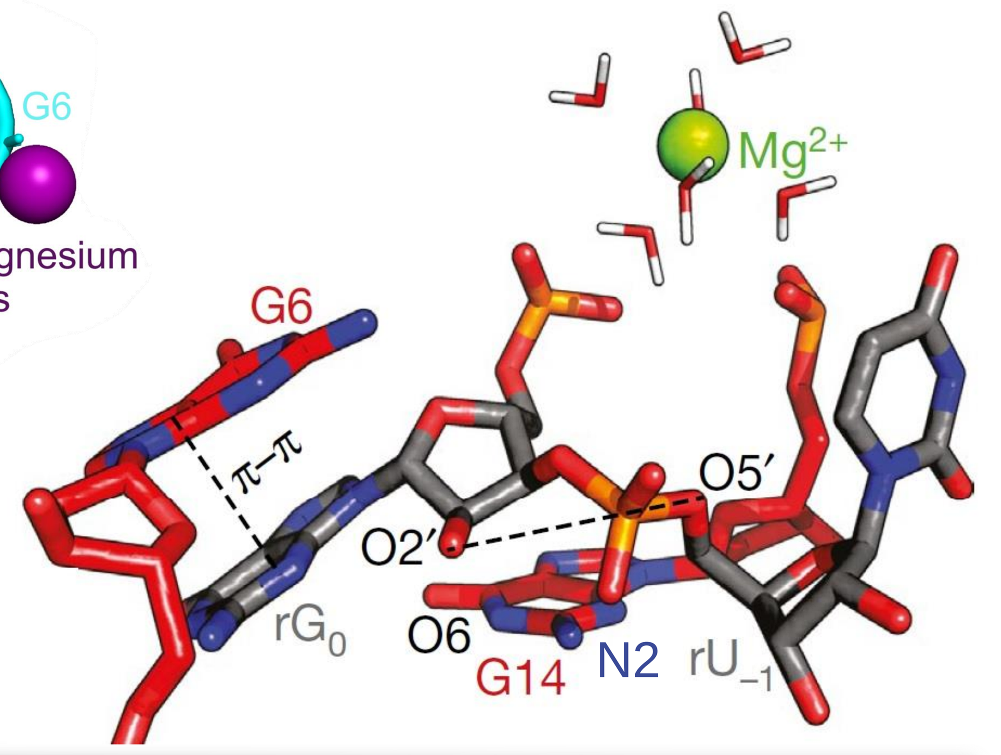
Introduction

The DNA is most known as the blueprint of protein synthesis, being the very first step into our life. Recently, a new function and the mechanism of **DNA** strands with catalytic activity, that cleave RNA, dubbed **DNAzymes**, has been revealed.[1] By molecular dynamics (MD) simulations in connection with experimental validation, we are investigating **how this cleavage conformation, the in-line attack (ILA), is formed and how non-natural nucleobases can be used to stabilize it**. This question is addressed on **DNAzyme 10-23, a DNAzyme with a wide range of RNA targets, able to cleave them with high selectivity**.[2] To increase the *in vivo* activity, we investigate the effect of non-natural nucleobases on the ILA formation, the molecular prerequisites of the ILA formation, the Mg^{2+} binding, and the $\pi\pi$-stacking of involved bases. In combination with experimental validations, we provide **insights into the cleavage mechanism of DNAzyme 10-23, improvements made by the insertion of non-natural nucleobases, and predictions for further modifications.**







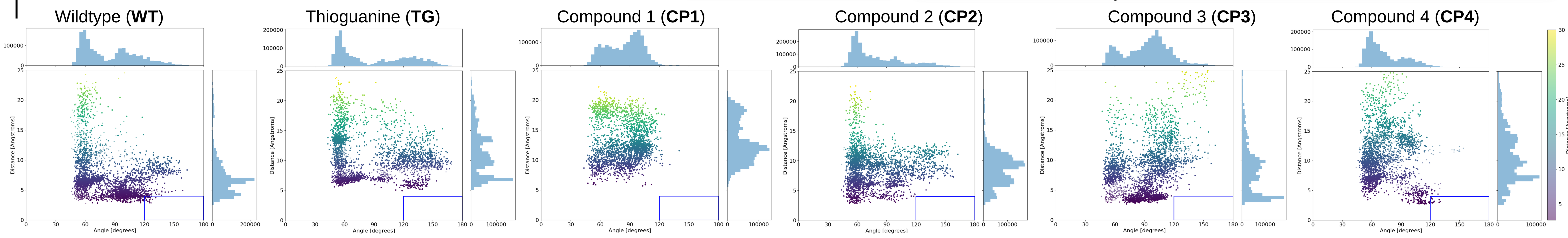


Distances and angles involved in in-Line attack formation

The **angle** between O2′ of **rG$_0$** of the RNA and both the phosphate and an oxygen **of rU$_{-1}$** must be **>120°** while the **distance between the O2′ of rG$_0$ and N2 of G14** is less than 4 Å.

In-line attack needs both requirements met (blue box).

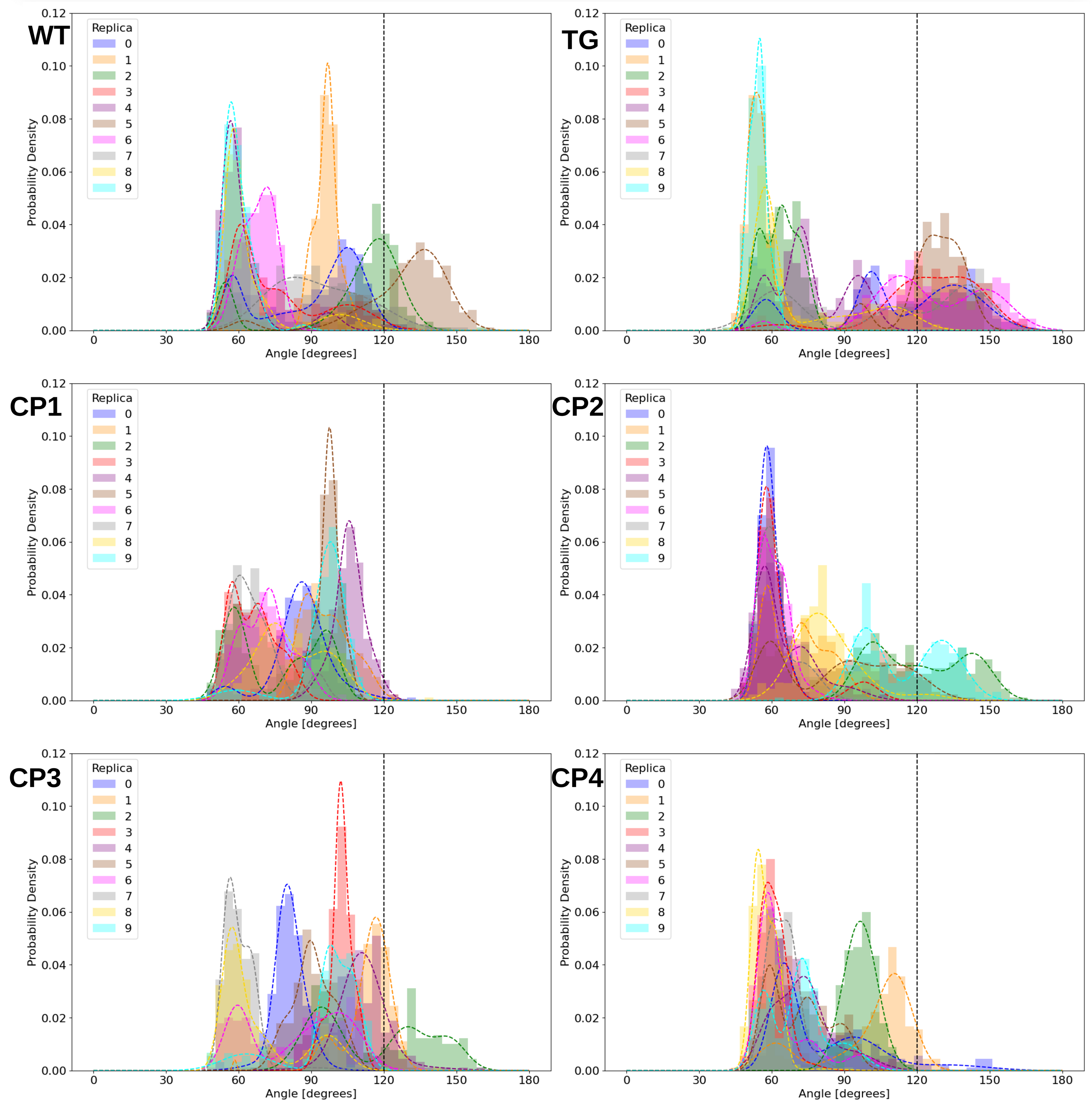
Both G14 and G6 are involved in the ILA formation. 4 modifications exchanging those were run (CP1-CP4), while an **unmodified DNAzyme 10-23 (WT)** and a thioguanine at G14 (**TG**) were used as references with previous works, yielding similar results.[1] **Only in WT and CP4 an ILA formation was observed.**



ILA:	0.96±0.08%	0%	0%	0%	0%	0.51±0.02%

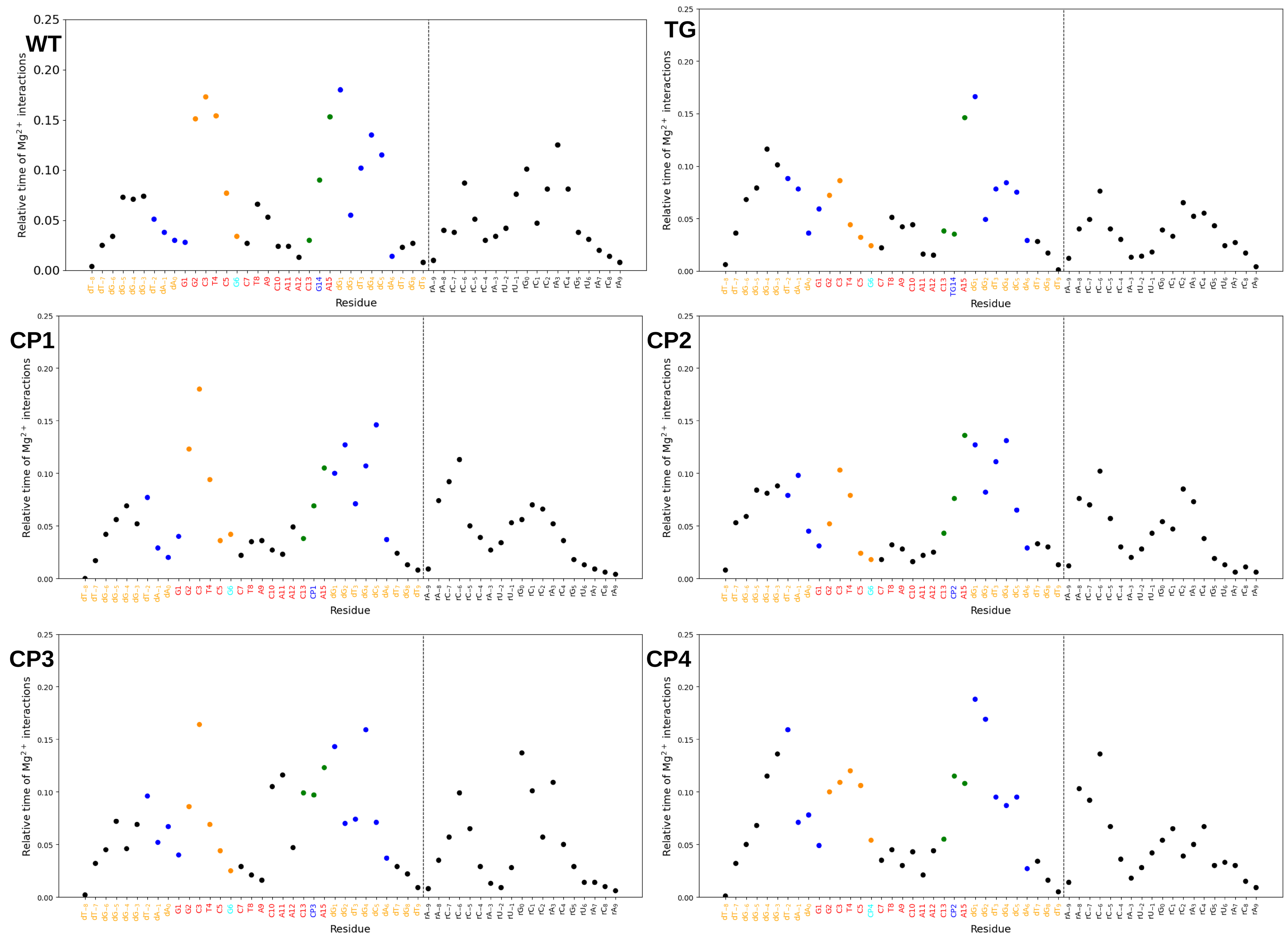
Potential attack angle distribution

The **distribution of attack angles** was investigated to see how any modification changed them. The experimentally **most active modification (TG)** showed the **most potential attack angles** but lacked the distance to initiate ILA.



Magnesium ion distribution in the DNAzyme

The Mg^{2+} binding was investigated, were the **metal binding sites of the unmodified DNAzyme** have been colored blue, orange and green. A **new metal binding site** is observed in modification **CP4**, potentially leading to a **reduction of Mg^{2+} dependency of the DNAzyme** due to being more exposed to the solvent in this region.



Summary

Unbiased MD show **that the introduction of non-natural modification can significantly change the behavior of DNAzymes.** While the unmodified DNAzyme 10-23 (**WT**) showed the highest ILA formation, the **experimentally 6-times more active thioguanine modification (TG) showed no ILA**. This is in line with previous simulations, were also no ILA formation was detected for thioguanine.[1] Both the In-line attack formation and Mg^{2+} binding change upon the insertion of non-natural nucleobases, aiding **in decreasing their strong cofactor dependency**.[3] Given the current simulations, the greatest challenge is closing the distance between O2′ and N2 to initiate the ILA formation. Most modification can reach proper attack angles and even form an ILA but simply can't close the distance. **Experimental verification showed that beneficial effects observed in the formation of the ILA also stabilize the ground state**, increasing the energy barrier to cleave the RNA. To overcome this energy barrier, further modifications of the involved atoms may be necessary.

References

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