How to design a prebiotic self-replicating system: A Simulation-Guided Conformational Space Study of the Hairpin Ribozyme

FORGET Sélène;[A] **DUBOUE-DJION Esise**;[B] **STIRNEMANN Guillaume**[A]

A) Laboratoire PASTEUR, Département de Chimie, Ecole Normale Supérieure, PSL University; B) Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimique, PSL University
selene.forget@ens.psl.eu

A key question for the RNA world hypothesis[1] is the emergence of autocatalytic networks in abiotic conditions without protein-based enzymes. **Ribozymes** (RNA enzymes) are likely components. However, large ribozymes, which are known to form self-replicating networks[2], cannot have self-assembled from the short RNA fragments available in abiotic conditions. On the other hand, typical short ribozymes tend to favor the cleavage reaction over the required ligation for the formation of larger molecules. In this study, we propose to **investigate the link between size, sequence, and catalytic properties** by studying a model system, the hairpin ribozyme. Indeed, this ribozyme can be found in various sizes, all sharing a common catalytic core, and experimental studies have shown accelerated ligation for the longest version when compared to the minimal structure[3].

Here, we aim to provide a molecular understanding of the tertiary structure effect on the ligation/cleavage equilibrium using all-atom molecular dynamics with enhanced sampling[4], and to clarify the reaction thermodynamics and mechanism. Before shedding light on the behavior of the hairpin ribozyme, we demonstrate the crucial importance of using specific enhanced sampling techniques to provide a **reliable conformational landscape of self-reactive species -**like ribozymes-, for which pre-catalytic structures are not experimentally available[5].

When comparing hairpin ribozymes of different sizes but with identical active site, and consistently with the experiments, our simulations indicate an increased **accessibility of reactive conformations** as the ribozyme gets larger. We identify differences in the S-turn structural motif between each system which we relate to the rearrangement of the catalytic site, suggesting the importance of **long-range effects.**

By capturing the free energy landscape of different versions of the hairpin ribozyme, this study lays the groundwork for 1) elucidating the cleavage/ligation mechanism and 2) investigating at a quantum level whether the hairpin accelerated reactivity can be attributed to the **stabilization of mechanistic intermediate species**.

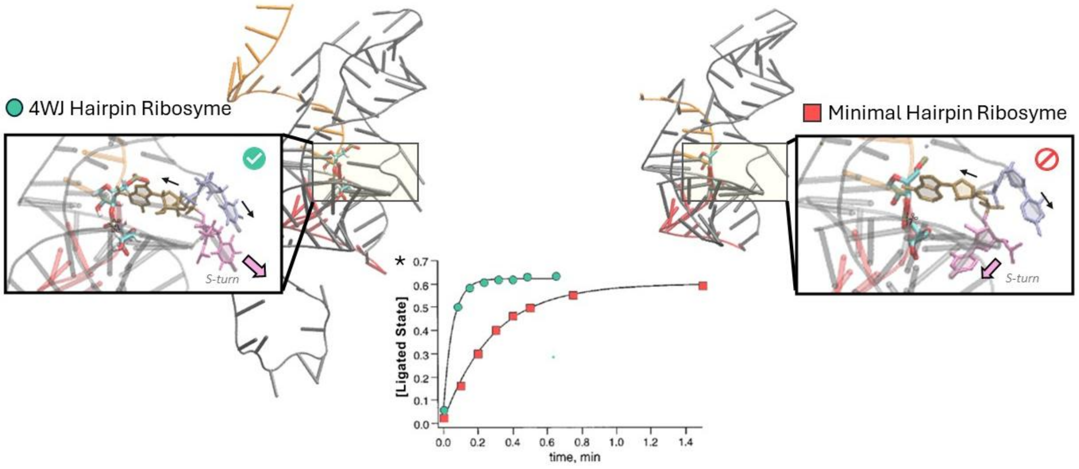

Figure 1. The Difference in Ligation Reactivity between the Hairpin Ribozyme Sequences is Related to the Arrangement of their S-turn Structural Motifs. *(* adapter from[3])*.

Keywords: Ribozymes, Enhanced Sampling Molecular Dynamics, Tertiary Structure Effects, Reactivity,

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