

Molecular simulations to investigate the molecular mechanism of *B. subtilis* rRNA maturation

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In *Bacillus subtilis*, the final maturation of the 5S RNA is performed by RNase M5, a 190 residue enzyme mostly found in Firmicutes, which cleaves the double stranded rRNA precursor on both sides of a double stranded stem. This reaction requires a ribosomal protein cofactor (UL18), that allows the rRNA to adopt the proper conformation for recognition and cleavage. Previous studies have suggested that the cleavage occurs in two steps, starting with the removal of the 3' extension before cleavage of the 5' strand ¹. In a recent work performed by C. Tisné's team ², details of the interaction between M5 and the 50S ribosome particle were obtained combining the cryo-EM structure of the complex and X-ray structures of the two domains of M5. The aim of our study is first to assess the stability of the complex (active site and interface) and then to investigate its catalytic mechanism. The observation of two Mg²⁺ ions in a recent a crystal structure of the protein suggests a **two ion-mechanism**, comparable to that of RNase H ³. Capturing the interaction between divalent cations and nucleic acids is a challenge for simulations, due to electronic polarization and possible charge transfer effects. We have thus tested different force fields to **model this complex active site**, and shown that our scaled charge ECC approach is very promising ⁴. Structural characterization and the study of RNA-protein interface dynamics were carried out using 4 replicas of 500 ns in standard simulation as well as **advanced sampling methods** ⁵. We characterized the fluctuations of this relatively small interface, and showed that they give rise to distinct active site geometries, prone to react along different reaction pathways. From the main identified conformations, the mechanistic study was set up using a mixed QM/MM-MD description called "**adaptive string method**" ⁶, which dynamically samples possible reaction paths.

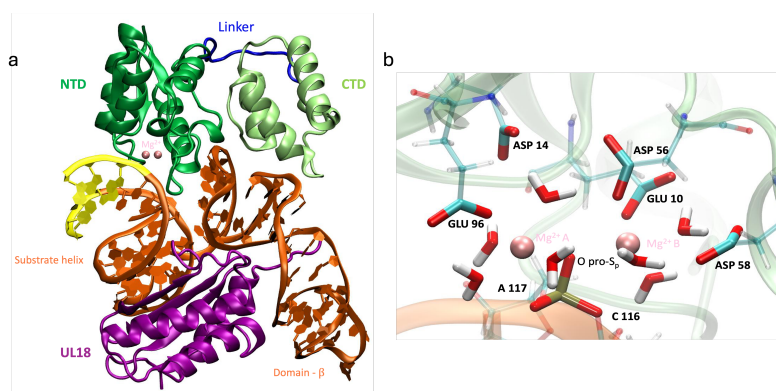


Figure 1: **a)** Simulated complex between M5, a rRNA fragment, and the protein uL18 cofactor **b)** Structure of M5 active site after repositioning of the scissile phosphate in between the two magnesium ions

Keywords: Ions-RNA/Protein interactions, enhanced sampling method, enzyme mechanism, QM/MM-MD.

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