Identification of dynamical hinge points of the L1 ligase molecular switch

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I. SUPPLEMENTARY INFORMATION

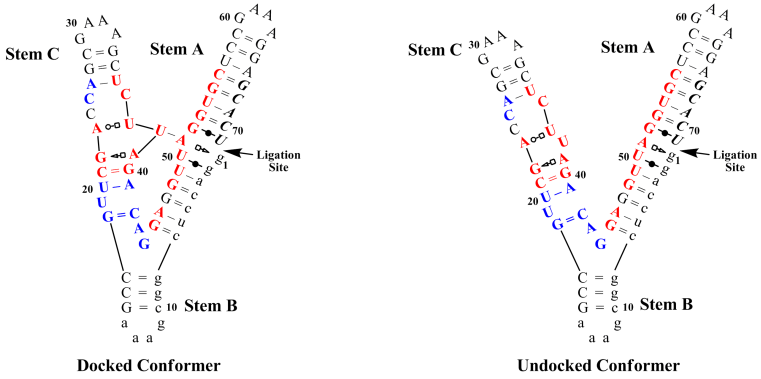

FIG. 1 Secondary structure of the L1 ligase ribozyme conformers as found in the crystal structure. With the exception of the interactions responsible for docking stem C into stem A (represented by U_{38}–A_{51} base pair found in the docked conformer) and several hydrogen bonds located mainly in the terminal GAAA tetraloops, the internal base pairing scheme of L1 ligase is conserved between the two conformers. Notations for base-pairing schemes follow reference Leontis et al., 2002. Lower case letters indicate regions that were not targeted for the in vitro evolutionary optimization. Residue in blue were more than 85% conserved within the L1 ligase family, residues in red were invariant. The substrate is italicized.

References

Leontis NB, Stombaugh J, Westhof E. 2002. The non-watson-crick base pairs and their associated isostericity matrices. *Nucleic Acids Res* **30**:3497–3531.

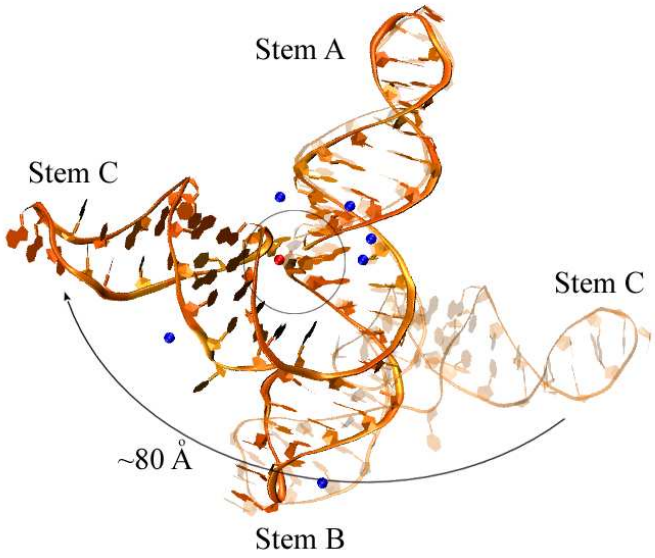

FIG. 2 The two L1 Ligase crystallized conformers with stems A and B superimposed. The undocked conformer is shown in shaded representation. Magnesium ions are shown in a sphere representation, colored with blue with the exeption of the one closest to the active site that is colored in red. This figure supports the fact that the tip of stem C has to travel ~ 80 Å from oner conformation to the other and the fact that the RMSD(AB,C) has a value of 48 Å. For the definition of the RMSD(FS, MS) measure see the text.

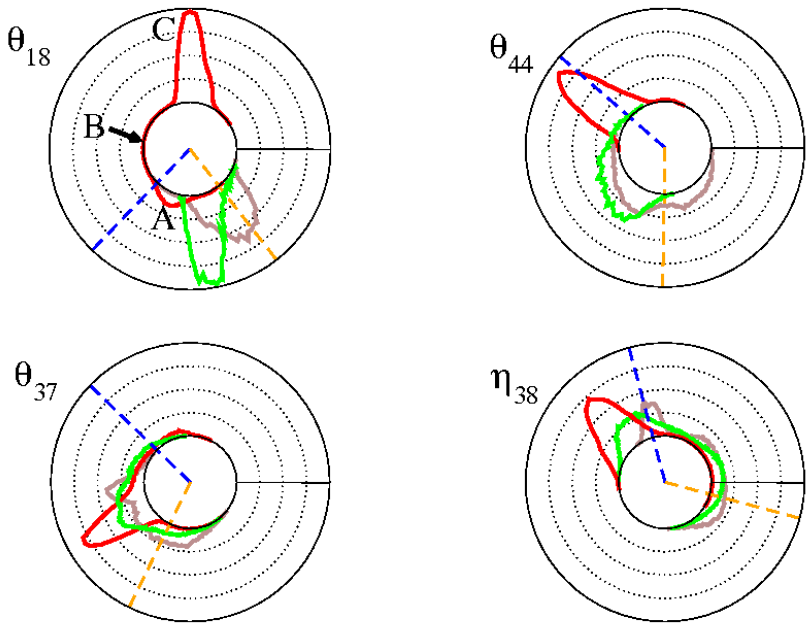

FIG. 3 Exploring the L1 ligase conformational transition in the space of the four virtual torsions angles for the Prod-D (black), Prod-U (red) and Prod-D-UF (green) simulations. The values of the the virtual torsions in the two crystal conformers are indicated with vertical dashed lines: orange and blue for the docked and undocked conformers, respectively. (Upper Left) As suggested by the structures of the two crystallized conformers θ_{18} has to travel an arc of length 277.6 degrees. Moving along the complimentary shorter arc is prevented by steric clashes between stem A and C. This is confirmed by the Prod-U simulation in red that shows θ_{18} sampling space along the longer arc towards to docked structure. (Upper right) θ_{44} spans two distinct regions in the Prod-D and Prod-U simulations. Similarly with θ_{18}, θ_{44} must travel on an arc of 231.8 degrees in order for the docked $\rightleftharpoons$ undocked transformation to take place. (Lower Left and Right) θ_{37} and η_{38} can be used follow the interchange between the two states of the U_{38} loop, as suggested by the crystal structures and confirmed by the simulations. If during the Prod-D and Prod-U the two torsions cover two distinct regions, during the Prod-D-UF simulation the interchange between the two states is observed. For a detailed depiction of the two states on the correlation plot of the two virtual along with their structures see the main text.

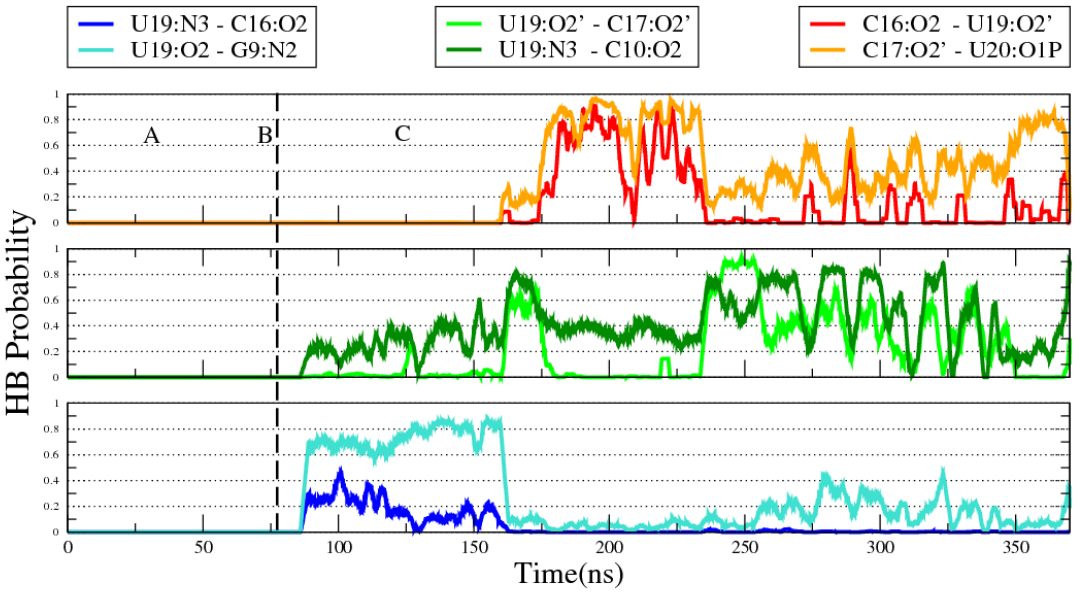

FIG. 4 Time series of specific hydrogen bond probabilities (see text) between U_{19} and stem B. The dashed line represents the moment at which the backbone undergoes a transition along the θ_{18} virtual torsion angle passing from structure A to structure C illustrated in the main text.

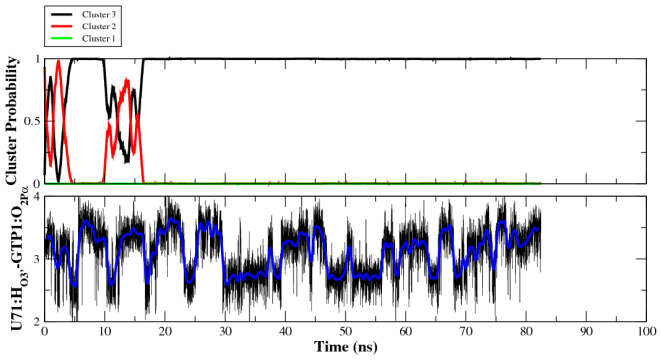

FIG. 5 The non-canonically base-paired ligation site exhibits a high degree of conformational variability, passing through a series of three states (clusters) (1, 2 and 3) characterized by specific hydrogen bond patterns between GTP_1/G_1 and the ligation site (see main text). Prec-XTP simulation differs only in the arangement of the tri-phosphate group and the absence of Mg^{2+} B. The time evolution of probabilities of the three states, and their correlation with the formation of specific U71:$H_{O3'}$ – GTP1:$O_{2P\alpha}$ interactions are shown for the Prec-D-XTP simulation.