

## Supplementary Material

### **Stability of 2',3' and 3',5' cyclic nucleotides in formamide and in water: a theoretical insight into the factors controlling accumulation of nucleic acid building blocks in the prebiotic pool**

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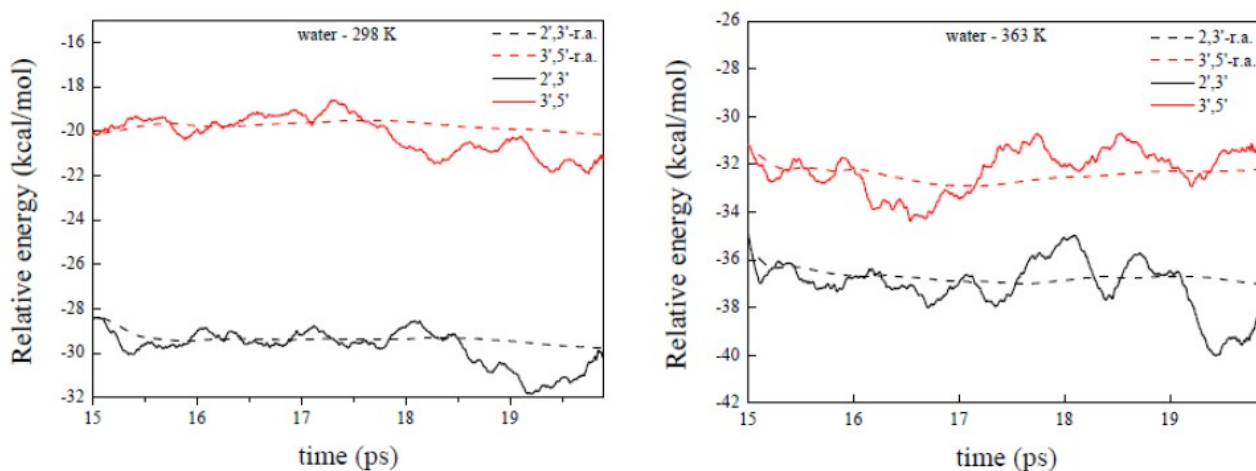
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## Total energy error bars

Although the results shown in the main text of the current investigation are widely supported by the collateral analysis of the average H-bond number solvating the cyclic nucleotides models, a specific check on the errors involved in the evaluation of the average total energies is in order. Therefore, with the aim of quantitatively clarify this delicate aspect, a systematic analysis of the instantaneous energies and of the respective running averages has been performed. This way, we determined the error bars of the estimated relative energy shown in Table 1 and Table 2 of the main text.

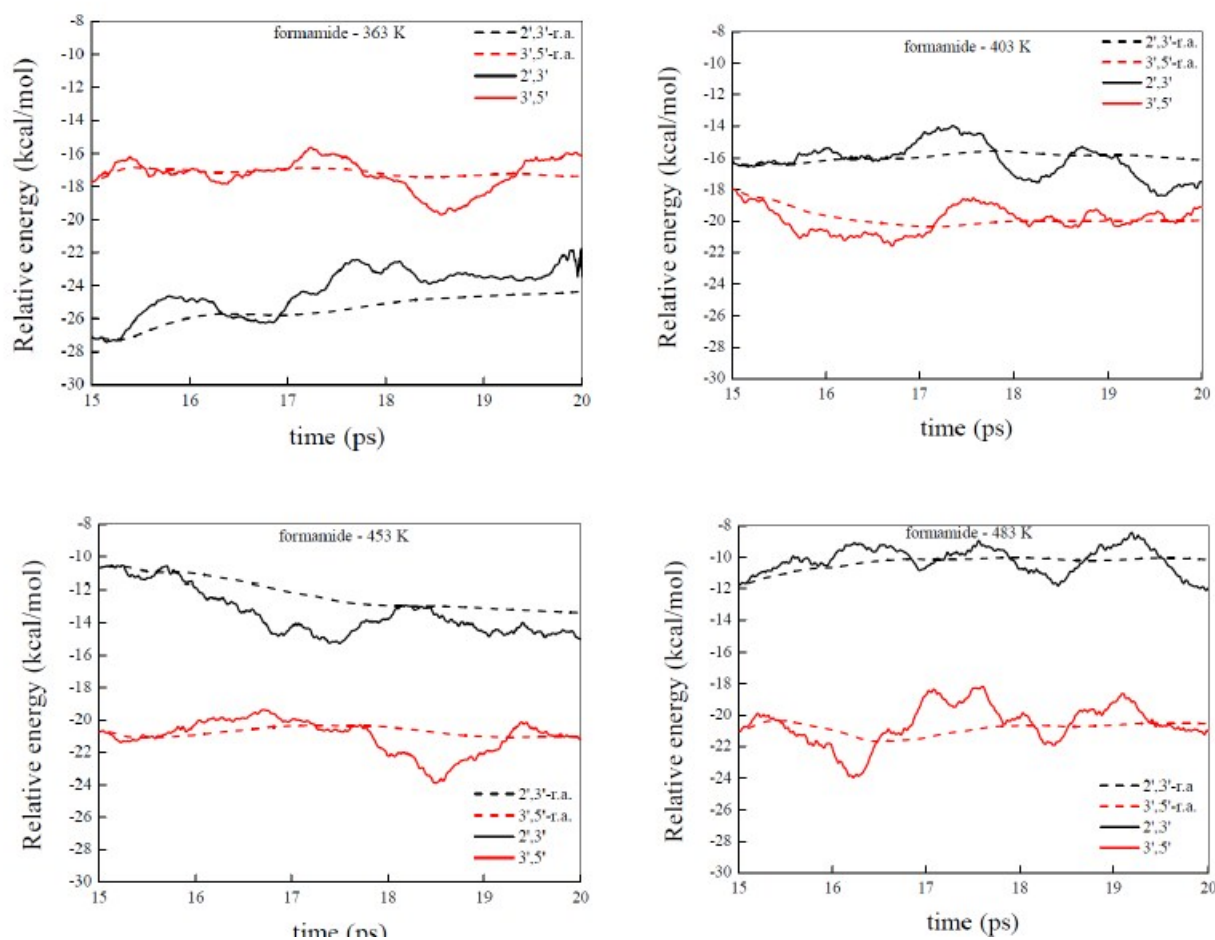
In Fig. S1 the instantaneous total energies and the respective running averages of the “numerical samples” composed of 2',3' and, separately, 3',5' cyclic nucleotides in liquid water both at 298 K and at 363 K are displayed. In particular, the running averages have been sampled during the last 5 ps of each simulation by performing a progressive average of the instantaneous energy values each 0.1 ps.



**Figure S1.** Instantaneous total energies (continuous lines) and respective running averages (dashed curves) of 2',3' (black) and 3',5' (red) in liquid water at 298 K (left panel) and at 363 K (right panel).

From the difference between the maximum and the minimum value of each running averages curve the evaluation of the errors of Table 1 of the main text is straightforward.

At the same way, this kind of error analysis has been performed for the formamide cases as shown in Fig. S2. All these data prove that even if the thermodynamic convergence of the total energy of a system, theoretically achievable only in infinite simulations or by means of advanced metadynamics techniques<sup>1,2</sup>, cannot be ensured, the error estimate falls in a satisfactory range of about 3 kcal/mol. Moreover, the correlation curve shown in Fig. 2 of the main text makes us confident about the reliability of the results referred to the average energy values. Indeed, the lifetime of an H-bond in these kind of polar solvents falls in the range [0.1-1.0] ps.<sup>3</sup> These values are actually from 2 to 1 orders of magnitude smaller than the time-length of the numerical simulations here presented, suggesting that the H-bond analysis, which almost rigorously correlates with the total energy investigation (see Fig. 2 of the main text), has fully achieved its statistical convergence.



**Figure S2.** Instantaneous total energies (continuous lines) and respective running averages (dashed curves) of 2',3' (black) and 3',5' (red) in liquid formamide at 363 K (top left panel), at 403 K (top right panel), at 453 K (bottom left panel), and at 483 K (bottom right panel).

## Bibliography

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