Temperature-Dependent Conformational Equilibrium of RNA 2'-OH Groups in Molecular Dynamics

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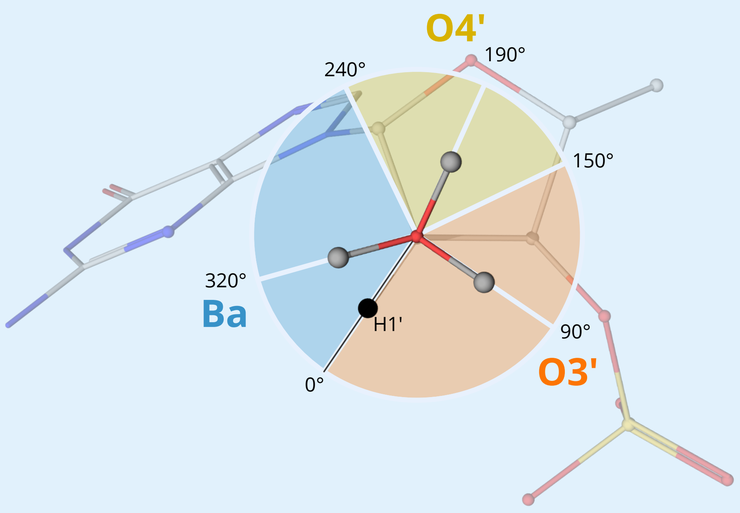
2'-OH torsion

The 2'-OH group enables the forming of hydrogen bonds in the RNA backbone, supporting the stability of many non-canonical motifs. The group adopts three preferred orientations:



Studies based on different NMR methods show mixed results in helices but agree in non-canonical regions. A recent joint X-ray/ neutron diffraction study on the Sarcin-Ricin Loop can resolve hydrogens directly. Crystallization, however, introduces artifacts due to crystal contacts and low temperatures (100 K), which is important to take into account when comparing experiments with MD simulations.

Low temperature extrapolation

Simulations of the two structures were conducted at 7 temperatures in the range between 160 to 300 K.
ΔG along with ΔH and ΔS were extrapolated from the linear fit of $\ln K$ to the inverse temperatures.

$$\ln\left(\frac{[\text{base}]}{[\text{O3'}]}\right) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

In 23 of the 25 non-terminal residues in the SRL the transition from O3' to the base orientation is entropically disfavored at 100 K. The enthalpic contributions is dependent on the environment of the hydroxyl group.
Below 200 K, sampling is limited not by water freezing but by excessively strong binding of K^+ ions to the RNA backbone, which restricts structural fluctuations, leading to non-equilbrium distributions. These low-temperature points were therefore excluded from the fit. At 200 K and above the distributions converge in a maximum timeframe of 10 µs.
O4' orientations are excluded in the fit due to their insignificant contribution in all but one residue (G_{55} of the SRL).

Example of the cumulative distribution function (CDF) of the minimal distance between K^+ ions and O2' for residue 50 in 7UCR. The CDFs converge for temperatures above 200 K.

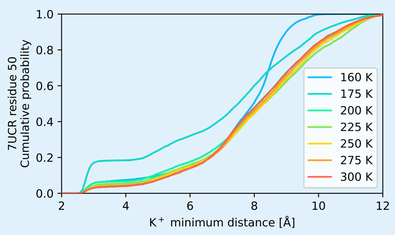
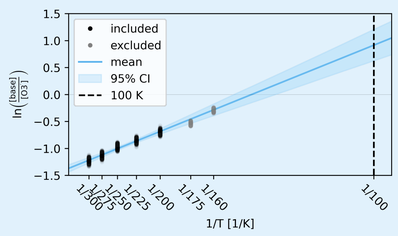


Illustration of linear fit for residue 131 in 1QC0. Data points at 160 K and 175 K were excluded from the fit. The linear regression was bootstrapped 5000 times to estimate confidence intervals.



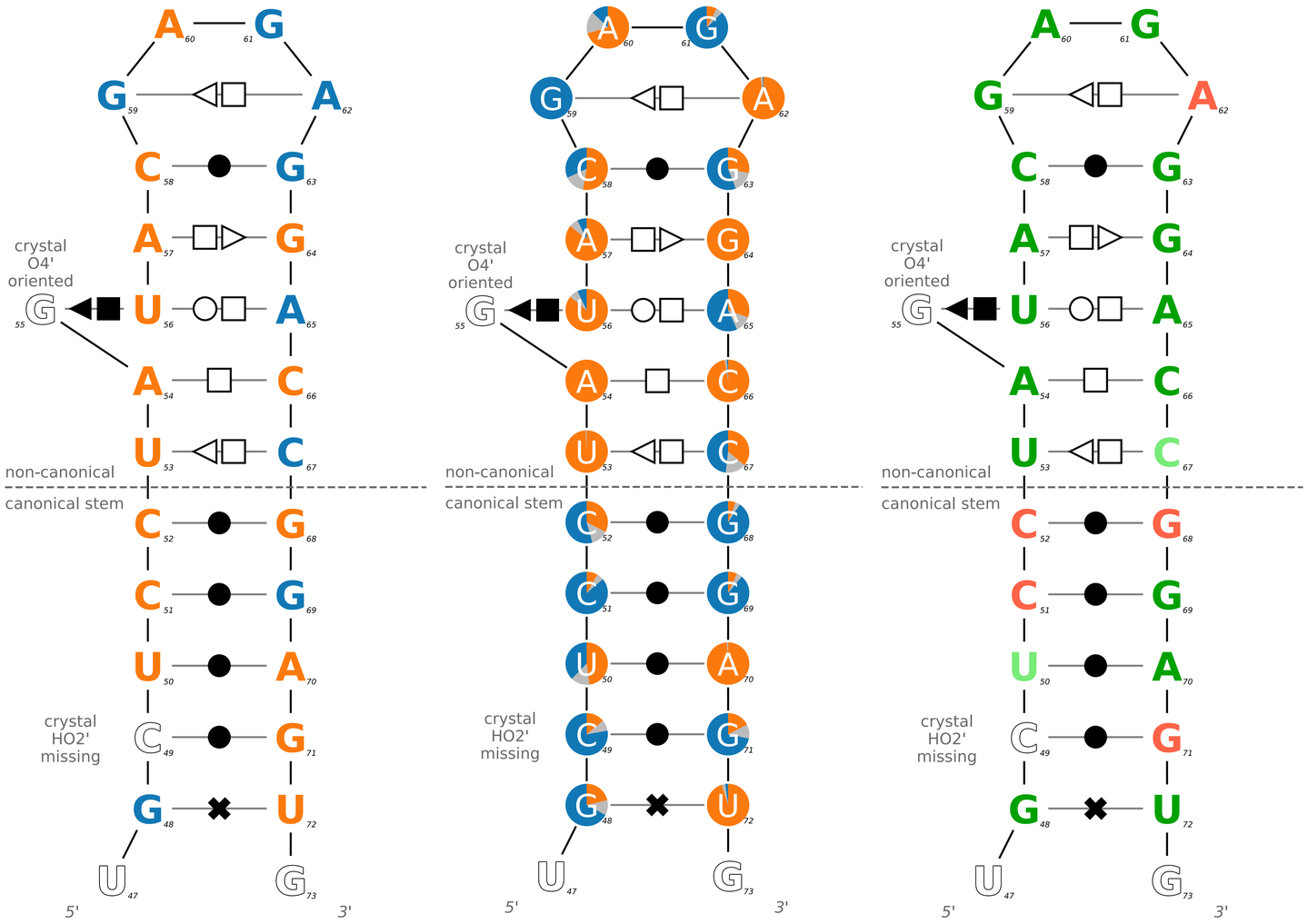
Methods

MD simulations:

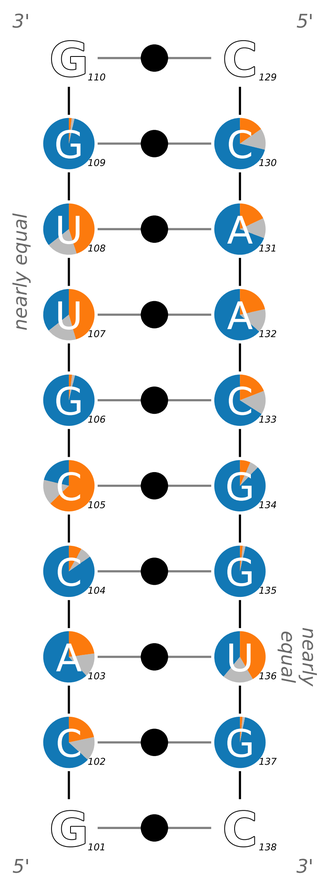
force field:	ff99bsc0χOL3 (OL3)
time:	5 - 10 µs with 4 fs timestep
solvent:	TIP3P water, 150 mmol/l KCl (Joung & Cheatham)
	CD (part of 1QC0): 4190 WAT, 29 K^+, 11 Cl^-
	SRL (7UCR): 6461 WAT, 44 K^+, 18, Cl^-
conditions:	NpT, p = 1 atm, T = 160 - 300 K (7 values)
restraints:	backbone dihedrals: αβγδεζχ
	flat-bottom harmonic at crystal value ± 15 °, k = 150 kcal·mol^{-1}·rad^{-2}

Extrapolation:
linear fit of ln K as a function of 1/T (5000 boostraps).
Residues were assigned according to 95% bootstrap confidence intervals of extrapolated base:O3' population ratios at 100 K. Assignments were made when the entire interval was above (base) or below (O3') the 1:1 threshold; intervals including the threshold were designated as nearly equal.

Sarcin-Ricin Loop (7UCR)



X-ray / neutron @ 100 K
MD extrapolation to 100 K



MD extrapolation to 100 K
- base oriented
- O3' oriented
- 95 % CI
- excluded

Comparison of experiment and model
- correct: CI within region
- consistent: CI spans threshold
- incorrect: CI opposite region
- excluded

Sarcin-Ricin Loop

OL3 in good agreement with experimental 2'-OH torsions at 100 K.

Strong agreement in the Sarcin–Ricin Loop non-canonical region.
Only residue A_{60} deviates.

Greater discrepancies in the canonical stem of the SRL:
Four residues experimentally adopting O3'-oriented conformations predicted as base-oriented.

Indicates a slight bias of the force field toward base orientation in helices.

Canonical Duplex

Base-oriented tendency observed in larger canonical double helix (part of PDB ID 1QC0). *Lack of resolved hydrogen positions prevents direct comparison to experiment.*

At 100 K, 12 of 16 residues adopt a base-oriented state. Three uracils show near-equal populations of base and O3' orientations.

Only C_{105} favors the O3' orientation.

Conclusions

- OL3 generally in good agreement with experiment
- slightly overestimates base-oriented conformations

→ stabilization of base-orientation not justified

- temperature-dependent biases in cryogenic structures

→ comparison with room-temperature MD with caution

References

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Acknowledgement

This work is supported by the IGA_PrF_2025_003 grant of the Internal Grant Agency of Palacký University Olomouc.