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Interactions of RNA and Water probed by 2D-IR Spectroscopy

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Abstract. Combined experimental-theoretical investigation of ultrafast hydration dynamics of an A-form RNA double helix in water reveals an ordered arrangement of water molecules and provides boundary conditions for the ion atmosphere around the polyanionic RNA.

1 RNA backbone vibrational modes as a sensitive probe of solvent environment

Ribonucleic acid (RNA) as an elementary constituent of biological cells exhibits more complex biochemical functionality than DNA, including the transmission of information in the form of mRNA, RNA-mediated catalytic function in ribosomes, and the encoding of genetic information in viruses. Interactions of RNA with the surrounding water shell, as well as counterions, determine the formation of such three-dimensional RNA structures but are understood only insufficiently and are hard to access by experiment. In principle, atomistic molecular dynamics (MD) simulations can provide guidance but challenges arise due to macromolecular size, uncertainties on force field accuracy and equilibration time scales, as well as the lack of direct comparison to spectroscopic observables.

We report the first ultrafast pump-probe and 2D-IR study of an RNA double helix in a water environment augmented by in depth theoretical modelling of the RNA vibrational response. RNA backbone vibrational modes of phosphate and sugar groups in the frequency range 900-1300 cm^{-1} are utilized to monitor solvation dynamics and molecular couplings (Fig. 1). The presence of an OH group attached to the 2' position of the ribose units has a strong impact on the vibrational spectrum and the hydration pattern in which the phosphate and sugar groups serve as distinct interaction sites for water molecules. The action of the fluctuating electric force generated by low-frequency, e.g., librational motion of solvating water molecules is directly manifested in the vibrational lineshape of the backbone modes that report on local field fluctuations in the 2-3 neighboring water layers [1,2].

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2 Mode Character, Vibrational Dynamics and Couplings of the RNA backbone

Pump-probe and 2D-IR spectra are recorded in the range of RNA backbone modes where prominent peaks appear for the asymmetric $(PO_2)^-$ stretch vibration P1 (doublet), the symmetric $(PO_2)^-$ stretch vibration P2 and backbone linker modes L1/2. Characteristic RNA bands at 1120 cm^{-1} with a shoulder at 1135 cm^{-1} arise from the OH group in the 2′ position with contributions from C4'−O4'−C1' stretching modes located on the ribose units (Fig. 1a) that are absent in DNA. Vibrational $\nu = 1$ lifetimes are found to be in the 0.3 – 1.1 ps range with the fastest population decay for P1 modes (Fig. 1b). A 2D-IR spectrum of RNA is plotted as a function of excitation (ν_1) and detection frequency (ν_3) in Fig. 1c and compared to the corresponding spectrum of DNA. As the 2D signal strength is proportional to the fourth power of the vibrational transition dipole moment ($S_{2D} \propto \mu^4$), the 2D signal provides higher contrast than the infrared absorption spectrum, making visible the distinct diagonal peak of RNA linker mode L1 that appears as a shoulder in the linear absorption.

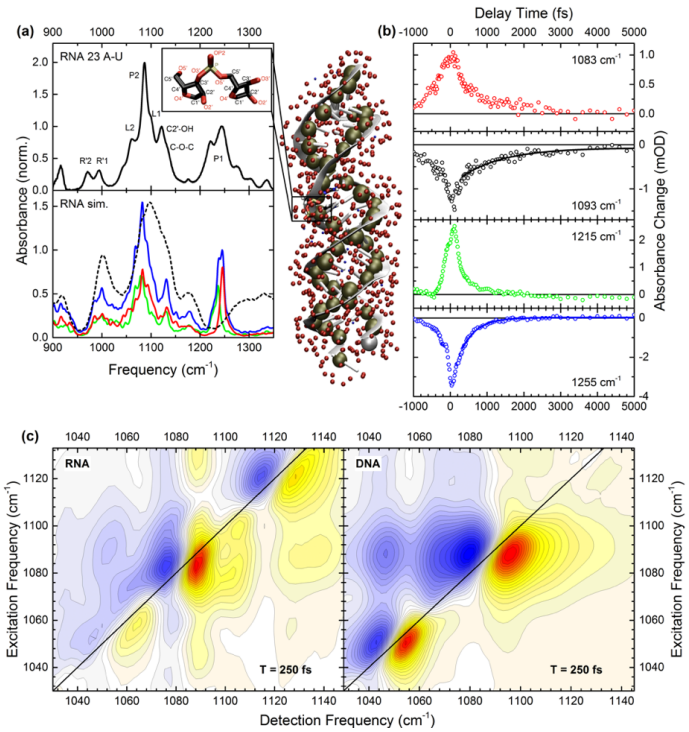

Fig. 1. a) Experimental (top) and simulated (bottom) linear absorption spectrum, obtained by QM/MM normal mode analysis along molecular dynamics trajectories (3• 200 ns = 0.6 µs total simulation time) of an (A-U)23 A-RNA double helix; inset: sugar-phosphate backbone. (b) Pump-probe transients recorded at the frequency position of symmetric P2 and asymmetric P1 phosphate stretch vibrations. (c) 2D-IR spectra of RNA (left) and DNA (right) revealing profound differences in mode number, coupling pattern and lineshape (normalized to the maximum positive signal).

Cross peaks are particularly pronounced for P2 - L2 modes and P2 - C2'-OH modes. Notably, coupling between the dublet of P1 modes at 1247 and 1220 cm^{-1} is minor while the latter mode strongly couples to the 1120 cm^{-1} 2′–OH mode and the P2 mode (data not shown). The comparison of 2D lineshapes of P2 modes of RNA and DNA reveals that for RNA contours are oriented almost parallel to the ν_1 axis, i.e., predominant homogeneous broadening, while the P2 line shape of DNA is tilted with respect to the ν_1 axis, suggesting more inhomogeneity. These findings are further quantified by theoretical simulations employing a third order nonlinear vibrational response function formalism with a biexponential Kubo ansatz for the frequency fluctuation correlation function (FFCF). The fast time scale τ_1 = 300 fs in the FFCF accounts for ultrafast spectral diffusion caused by field fluctuations due to adjacent water molecules and the slow τ_2 = 50 ps time scale represents inhomogeneity in local solvation structure along the helix. The amplitude Δ_2 of the slow components is found to be reduced compared to the inhomogeneous broadening of DNA [1,3]. The findings correlate with X-ray diffraction data [4] where individual water molecules link the 2′–OH group with ribose oxygen atoms and oxygen atoms of the phosphate group. Moreover, neighboring phosphate groups are bridged by individual water molecules, forming a more ordered hydration shell around RNA.

3 Boundary conditions for ion atmosphere around polyanionic RNA

Quantum mechanical molecular mechanics (QM/MM) normal-mode analyses performed for the alternating 23-mer A-U RNA helix along snapshots of MD trajectories (0.6 μs simulation time) provide a clear assignment of backbone modes to structural units of the backbone. While frequency filtering of P1 modes (ν_{P1} < 1250 cm^{-1}) provides spectra in good agreement with the experimental absorption spectra in the entire range of backbone modes (blue line, Fig 1a), the prominent P1 mode with a doublet due to asymmetric $(PO_2)^-$ stretching vibrations is found to be particularly sensitive to the local water hydration geometries and the presence of ions (cf. dashed and blue line in Fig 1a). As the latter induce a blue-shift to values > 1250 cm^{-1}, the unfiltered ensemble of QM/MM derived P1 mode frequencies appears broad and structureless (dashed line, Fig 1c) which is not fully consistent with the experimental IR spectrum. The extensive sampling of molecular dynamics simulation on the microsecond time scale allows us to dissect RNA-ion atmosphere force field deficiencies from partial equilibration, a persistent challenge in MD simulations. The profound sensitivity on local hydration structures of RNA P1 modes facilitates a quantification of the local ion atmosphere in computer simulations where the high sensitivity of 2D vibrational spectra impose boundary conditions for realistic solvation geometries that are hard to quantify experimentally.

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