Special Feature: Tutorial

Linking Molecular Models with Ion Mobility Experiments.

Illustration with a rigid nucleic acid structure

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MATERIALS AND METHODS

MD simulation in aqueous solution. The crystal structure of the parallel [dTGGGGT]$_4$ G-quadruplex (PDB entry 352D)[1] was relaxed performing a 50 ns MD run in explicit water. The system was immersed in a truncated octahedron box of TIP3P[2] water molecules, with a minimum distance of one edge of the box and one solute atom equal to ca. 10 Å; 17 additional sodium ions were added to neutralize the negative charge of the quadruplex. To optimize and equilibrate the system, we design a multistep protocol consisting of 5000 steps of minimization and 300-ps of equilibration, where we performed a gradual heating of the system and removal of positional restraints on DNA. Going into details, the minimization step was split in three rounds: in the first one we performed 500 steps of steepest descendent followed by 1500 steps of conjugate gradient, applying an harmonic restraints of 100 kcal/mol$Å^2$ on the solute; in the second round we decreased the conjugate gradient to 1000 steps and the harmonic restraints on the solute to 50 kcal/mol$Å^2$ and in the final round we decreased it to 10 Kcal/mol$Å^2$. As regard the equilibration step, it was split in five 20-ns rounds of restricted MD simulations, followed by a final round of 200-ns of unrestricted MD simulation. In the first 20-ps round, the system was kept at constant volume and gently heated up to 300 K, while the DNA was weakly restrained by a 10 Kcal/mol$Å^2$ harmonic potential. The following four 20-ns rounds were performed at isothermal isobaric ensemble (P= 1 atm, T= 300 K), with the DNA constraints gradually decreasing to 10, 5, 2.5, and 1 Kcal/mol$Å^2$, until no constraints were applied to the final 200-ps equilibration round. Finally, the 50-ns unrestrained MD production was performed with *pmemd* module of AMBER12. Periodic boundary conditions and the Particle Mesh Ewald (PME) method [3] were used, together with a 2.0 fs time step, with bonds involving hydrogens constrained by the SHAKE algorithm.[4] The radial cut-off for the non-bonded interaction was 8 Å in both equilibration and production runs.

MD simulation in gas phase. Four snapshots (specifically those at 1, 5, 10 and 50 ns) of the solution simulation were selected to create the starting structures needed for four independent gas phase MD runs. Water molecules and external counter ions were removed; then, the charge state of the models was adapted with that one observed in ESI-MS experiments (corresponding to -5 for the predominant charge state), by applying the “distributed model” described by Rueda et al.,[5] that requires the equal distribution of the total charge along all the phosphates. After that, the structures were relaxed with 5000 steps of steepest descendent, followed by 15000 steps of conjugate gradient, without applying any harmonic restraints. A thermalization period of 1-ns was then performed, and followed by a 500-ns unrestrained MD production run with the *sander* module of AMBER12. The temperature was kept constant at 298 K using a weak-coupling algorithm.[6] No non-bonded interactions radial cut-off was applied; SHAKE [4] algorithm was used to constrain all the bonds involving hydrogen atoms, and the integration time step was 1.0 fs.

Figure S1: Key simulation results obtained from 50-ns MD simulation of [dTGGGGT]$_4$ in solution. Plots following the stability of the models during the dynamic run, through the variation of the structural descriptors of the system: **(a)** radii of gyration; **(b)** root mean square deviations of all backbone atoms, the thymines, and the G-tetrads; **(c)** root mean square fluctuations of the thymines (black), and the guanines (white); **(d)** interatomic distances between all guanine N1-O6 (grey) and N2-N7 atoms (black) participating to the Hoogsteen H-bonds of the G-tetrads.

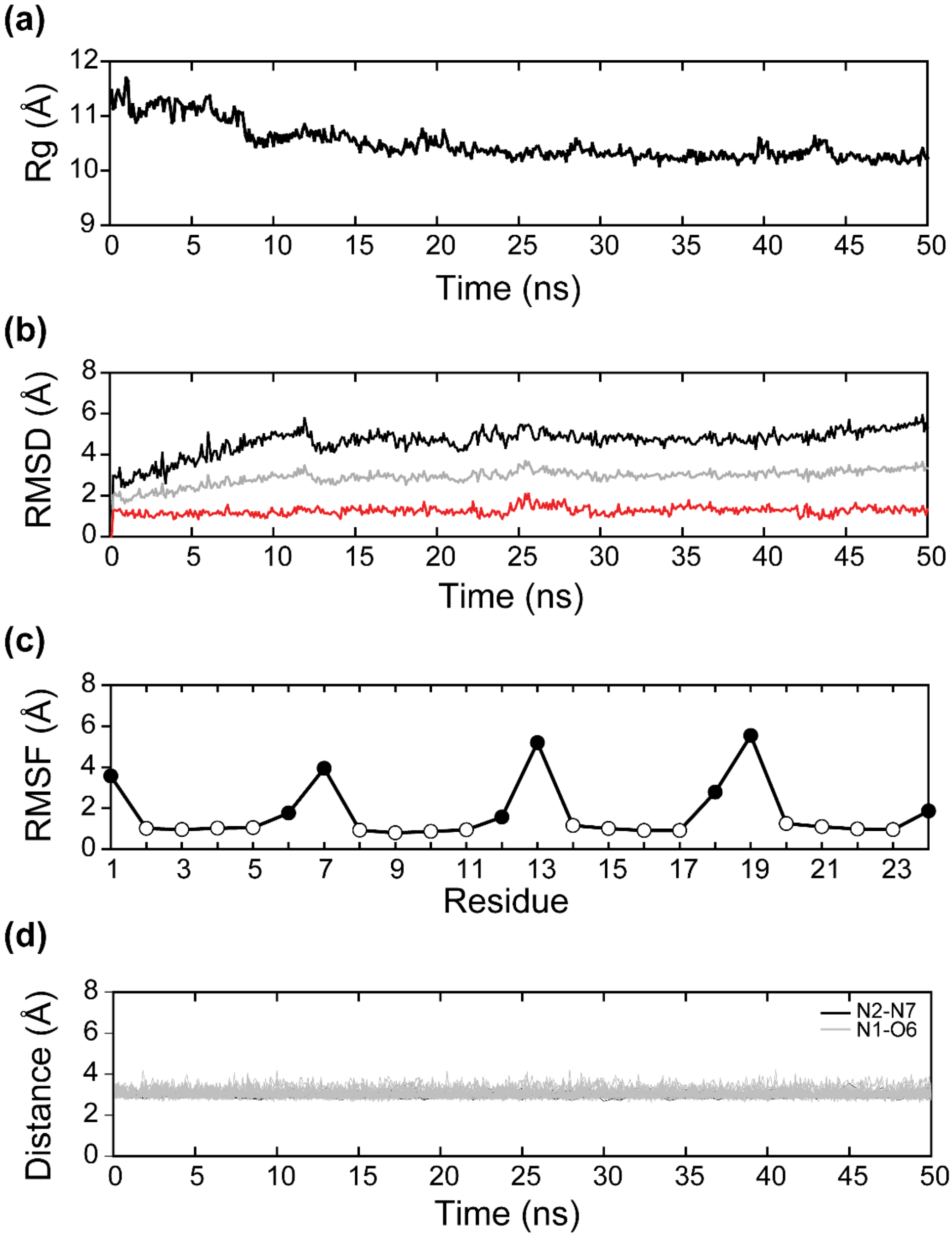


Figure S2: Key simulation results obtained from 500-ns MD simulation of replica 1 of $[d(TGGGGT)_4 + 3NH_4]^{5-}$ in the gas-phase. Plots following the stability of the models during the dynamic run, through the variation of the structural descriptors of the system: **(a)** radii of gyration; **(b)** root mean square deviations of all backbone atoms, the thymines, and the G-tetrads; **(c)** root mean square fluctuations of the thymines (black), and the guanines (white); **(d)** interatomic distances between all guanine N1-O6 (grey) and N2-N7 atoms (black) participating to the Hoogsteen H-bonds of the G-tetrads.

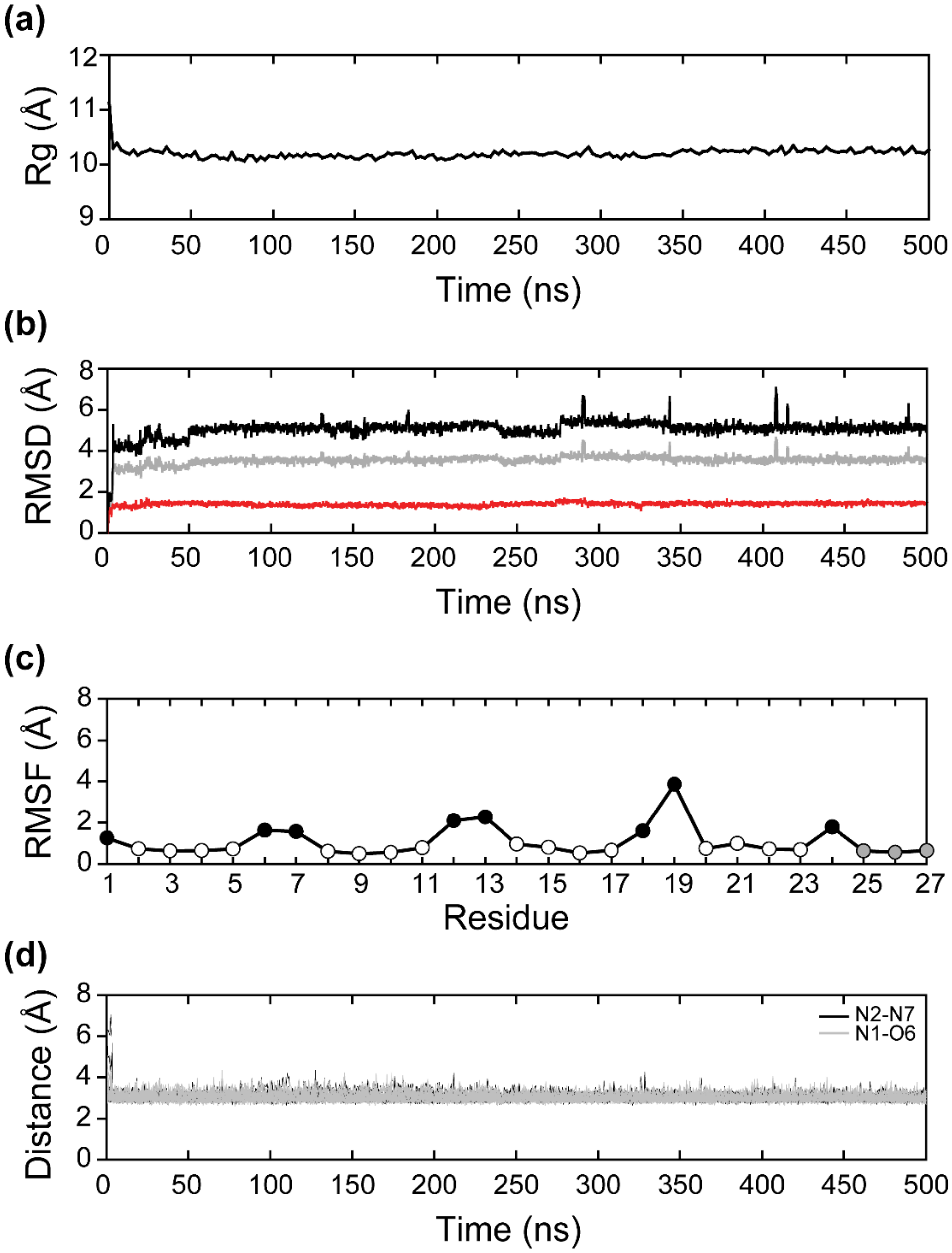


Figure S3: Key simulation results obtained from 500-ns MD simulation of replica 2 of $[d(TGGGGT)_4 + 3NH_4]^{5-}$ in the gas-phase. Plots following the stability of the models during the dynamic run, through the variation of the structural descriptors of the system: **(a)** radii of gyration; **(b)** root mean square deviations of all backbone atoms, the thymines, and the G-tetrads; **(c)** root mean square fluctuations of the thymines (black), and the guanines (white); **(d)** interatomic distances between all guanine N1-O6 (grey) and N2-N7 atoms (black) participating to the Hoogsteen H-bonds of the G-tetrads..

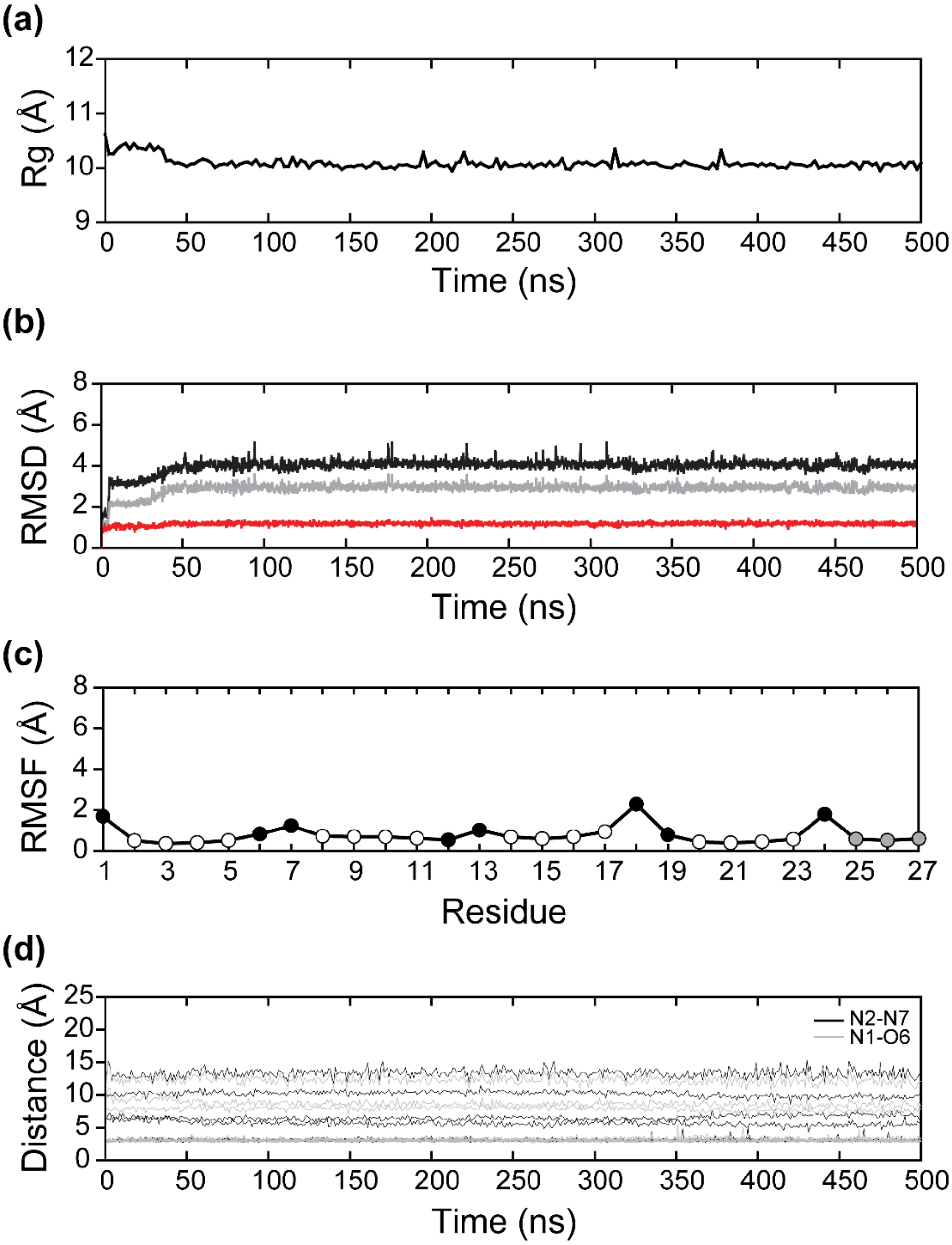


Figure S4: Key simulation results obtained from 500-ns MD simulation of replica 3 of $[d(TGGGGT)_4 + 3NH_4]^{5-}$ in the gas-phase. Plots following the stability of the models during the dynamic run, through the variation of the structural descriptors of the system: **(a)** radii of gyration; **(b)** root mean square deviations of all backbone atoms, the thymines, and the G-tetrads; **(c)** root mean square fluctuations of the thymines (black), and the guanines (white); **(d)** interatomic distances between all guanine N1-O6 (grey) and N2-N7 atoms (black) participating to the Hoogsteen H-bonds of the G-tetrads.

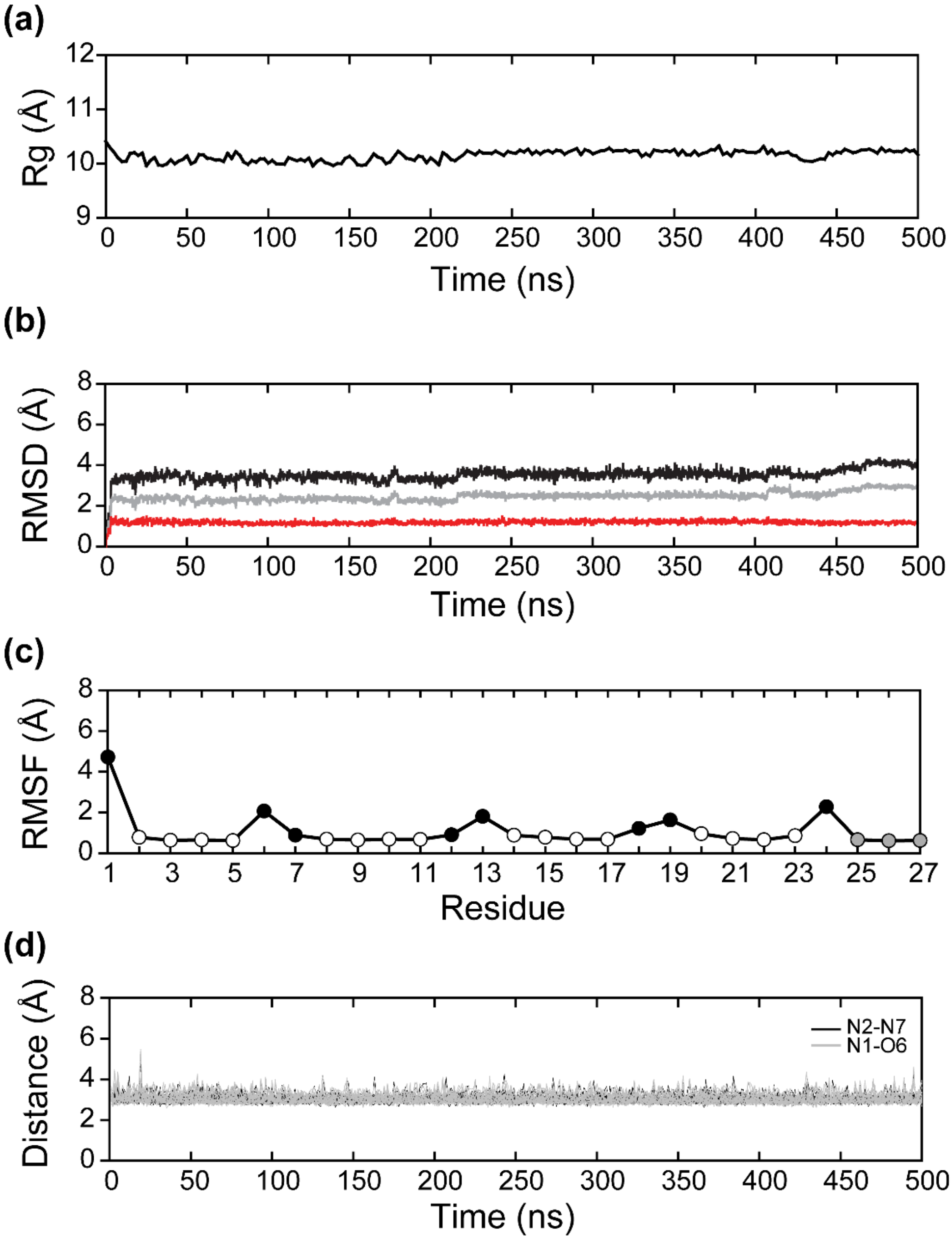
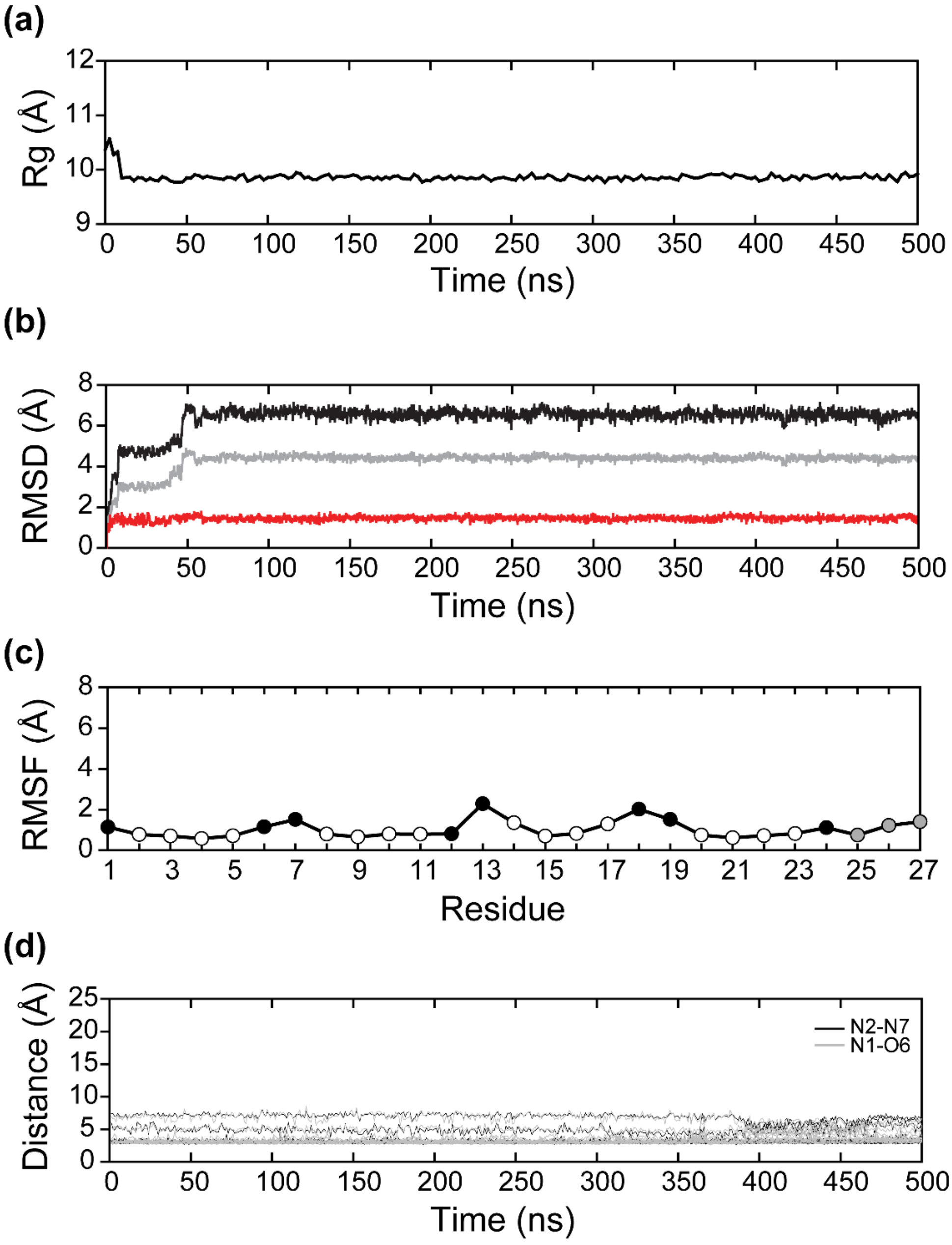


Figure S5: Key simulation results obtained from 500-ns MD simulation of replica 4 of $[d(TGGGGT)_4 + 3NH_4]^{5-}$ in the gas-phase. Plots following the stability of the models during the dynamic run, through the variation of the structural descriptors of the system: **(a)** radii of gyration; **(b)** root mean square deviations of all backbone atoms, the thymines, and the G-tetrads; **(c)** root mean square fluctuations of the thymines (black), and the guanines (white); **(d)** interatomic distances between all guanine N1-O6 (grey) and N2-N7 atoms (black) participating to the Hoogsteen H-bonds of the G-tetrads.



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