

# A polarized charge model for RNA: enhanced stability of G- quadruplexes and improved folding free energy landscapes

**Shuaizhen Tian**

Jining University

**Nana Wang**

Shandong Police College

**Xinxin Du**

Jining University

**Mengxi Qiu**

Jining University

**John Z.H. Zhang**

`john.zhang@suat-sz.edu.cn`

Shenzhen University of Advanced Technology

---

## Research Article

**Keywords:** Polarized force field, RNA G-quadruplexes, Molecular dynamics simulations, Free energy landscape

**Posted Date:** October 21st, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-11229196/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

**Additional Declarations:** No competing interests reported.

---

# A polarized charge model for RNA: enhanced stability of G-quadruplexes and improved folding free energy landscapes

Shuaizhen Tian<sup>1</sup>, Nana Wang<sup>2</sup>, Xinxin Du<sup>1</sup>, Mengxi Qiu<sup>1</sup>, John Z.H. Zhang<sup>3\*</sup>

<sup>1</sup> *School of Life Science and Engineering, Jining University, Jining 273199, China.*

<sup>2</sup> *Department of Criminal Science and Technology, Shandong Police College, Jina 250200, China*

<sup>3</sup> *Faculty of Synthetic Biology, Shenzhen University of Advanced Technology, Shenzhen 518000, China*

## Abstract

Ribonucleic acid (RNA) plays essential roles in numerous biological processes, and molecular dynamics (MD) simulations have become indispensable for probing RNA structure and function at atomic resolution. However, current RNA force fields exhibit notable deficiencies in describing hydrogen bonding, ion stability, and folding pathways, largely because they neglect the electrostatic screening effect of aqueous solvent. To address these limitations, we introduce a set of polarized nucleic acid-specific charges (PNC) derived from fragment-based quantum chemistry calculations and apply them to two representative RNA systems. The PNC method explicitly incorporates polarization effects into electrostatic interactions. For the G-quadruplex (GQ) system, PNC appreciably enhances structural stability, particularly by preserving hydrogen bonds between G-tetrads, and retains cations within the central channel across nearly all tested ion models. In contrast, standard AMBER force fields without polarization allow terminal cations to escape into bulk solvent within a few nanoseconds. For folding of a single-stranded RNA into a hairpin motif, none of the five conventional AMBER force fields yields a physically reasonable free-energy landscape, whereas four show clear improvement when combined with PNC. Despite its structural dependence—a recognized limitation—the PNC method offers excellent compatibility: it requires no modification to the original force-field functional form and incurs no additional computational cost, making it a practical and efficient tool for RNA simulations

**Keywords:** Polarized force field; RNA G-quadruplexes; Molecular dynamics simulations; Free energy landscape.

\*Correspondence to: john.zhang@suat-sz.edu.cn

## 1. Introduction

RNA systems play a pivotal role in pathological and pharmacological research, including disease pathogenesis and drug target identification [1, 2]. Deciphering the structure–dynamics–function relationships of nucleic acids are equally critical for advancing life sciences and pharmaceutical R&D [3]. Accordingly, molecular dynamics (MD) simulations of nucleic acid systems were introduced into computational biology in the 1980s [4, 5]. Over decades, MD simulations have evolved from rudimentary tools into core techniques capable of capturing the dynamic conformations of biological macromolecules at atomic resolution and elucidating the mechanisms of intermolecular interactions [6-9]. Using this approach, researchers have systematically studied key topics such as RNA folding mechanisms, conformational regulatory rules, and RNA–ligand recognition and binding [10-16]. These studies have not only revealed microscopic processes inaccessible to conventional experiments but also yielded pivotal theoretical findings that support RNA-targeted drug design and disease diagnostic technologies.

Nevertheless, MD simulations suffer from inherent limitations, primarily in two aspects: the simulation timescale, which requires exhaustive conformational sampling, and the accuracy of empirical force fields. Driven by continuous advances in enhanced sampling methods—including replica exchange MD (REMD) [17], accelerated MD (aMD) [18], umbrella sampling [19], and metadynamics (MetaD) [20, 21]—and by the widespread use of high-performance GPUs that greatly boost computational efficiency, the capability of MD simulations to characterize structural rearrangements has been substantially expanded. The accessible timescale has extended from femtoseconds to milliseconds [22, 23], gradually overcoming the sampling challenges. Despite these advances, the development of more accurate force fields has emerged as the primary bottleneck restricting the predictive power of contemporary MD simulations [14, 24-26].

To date, a variety of RNA force fields have been developed, such as AMBER, CHARMM, OPLS, GROMOS, Drude, and AMOEBA [27-35]. The complexity of RNA force fields stems from the intricate intramolecular interactions within RNA molecules. The 2'-hydroxyl (2'-OH) group on the ribose endows RNA with high structural flexibility, allowing interactions among nucleobases, ribose moieties, and phosphate groups [14]. For instance, in addition to hydrogen bonds between complementary base pairs, hydrogen bonds can also form between bases and ribose moieties, or between bases and phosphate groups. As documented previously, existing RNA force fields have inherent limitations in characterizing these interactions [7, 26, 36, 37]. They tend to underestimate hydrogen bonding between base pairs [26, 36, 38]. Meanwhile, inaccurate backbone dihedral parameters drive RNA hairpin structures to adopt

unfolded conformations [36, 38]. Additionally, these force fields often overestimate repulsive interactions between charged ions [7, 37, 39]. Such defects further lead to structural instability, inadequate conformational sampling, and distorted free-energy landscapes. A range of strategies have been proposed to address these issues, including polarizable force field approaches such as Drude oscillator models and the AMOEBA force field [30, 40, 41], as well as nonpolarizable schemes with revised van der Waals parameters, modified backbone dihedral terms, or adjusted hydrogen-bond energies [26, 38, 42-50]. These approaches have proven effective for diverse RNA systems.

In most force fields, identical atoms of the same residue are assigned equal partial charges. For instance, in the AMBER force field, the phosphorus atom in every RNA phosphate group carries a fixed positive charge of 1.17 e. In practice, these atoms possess distinct charges in different chemical environments. This renders the AMBER force field incapable of fully accounting for solvent electrostatic screening effects, leading to deficiencies in electrostatic interaction treatment—for example, overestimating interactions between phosphate groups and bases while underestimating hydrogen-bond interactions formed by bases. If this screening effect were incorporated into the force field, solvent-exposed phosphate groups would be solvated by water molecules in aqueous solution, theoretically weakening phosphate–base interactions. Likewise, for hydrogen bonds buried inside macromolecules and shielded from water, their electrostatic interactions should be strengthened when such an effect is considered. Based on this electrostatic screening effect, our research group established a polarized charge model [51-53]. This method divides biomacromolecules into individual fragments, derives atomic charges for each fragment separately, and then assembles these fragment charges to obtain the charge distribution of the full-length macromolecule. However, this model has not yet been applied to RNA systems. Considering its ability to capture background charges and solvent surface charges, this model can potentially characterize polarization interactions in RNA with high accuracy. On this basis, we extended this polarized charge model to RNA systems and validated its performance via MD simulations on two representative complex RNA constructs.

The first system investigated is an RNA GQ as shown in Fig. 1. GQs are non-canonical secondary structures formed by guanine-rich sequences harboring tandem guanine repeats from one or more nucleic acid strands, in the presence of suitable monovalent cations [54]. Within the GQ architecture (Fig. 1), four adjacent guanine residues constitute a planar G-tetrad through Hoogsteen hydrogen bonding; consecutive G-tetrads stack tightly against each other, thereby stabilizing the overall GQ scaffold. In addition, a central channel is formed between stacked G-tetrads, the inner wall of which is lined with electronegative oxygen atoms. Consequently, the accommodation of monovalent cations—most notably  $K^+$  ions—within this channel drastically neutralizes the electronegativity of the channel interior, further strengthening the

structural stability of GQs [55-57]. Potential G-quadruplex motifs have been identified in the genomes of both DNA and RNA viruses, and GQ-binding ligands can significantly reduce viral titers generated by cells infected with these viruses [58, 59]. Given the relatively complex structure of GQ and their status as vital therapeutic targets for diseases, it is highly essential to employ the recently resolved high-resolution structure of the NS5-B quadruplex from the West Nile virus genome to benchmark the PNC method and make a systematic comparison with the standard AMBER force fields.

The second system investigated is an RNA hairpin motif (Fig. 2), which represents the most prevalent and fundamental secondary structural unit of single-stranded RNA [60, 61]. Formed by the intramolecular folding and complementary base pairing of the RNA strand, this hairpin architecture arises from the presence of inverted complementary sequences within the polynucleotide chain. During the folding process, intrastrand hydrogen-bonding interactions dominate over interstrand interactions; meanwhile, the loop region adopts an optimal length (excessively short loops introduce severe steric strain, whereas overly long loops confer structural instability). Under ideal conditions, the folded hairpin conformation corresponds to the global minimum on the Gibbs free energy landscape. Despite its structural simplicity, the RNA hairpin governs the global folding, structural stability, and biological functions of numerous non-coding RNAs. Accordingly, it serves as an excellent benchmark system for evaluating the reliability of RNA force fields [36, 42, 62].

We performed conventional MD simulations on the first system (RNA GQ), mainly to evaluate the stability of hydrogen bonds, metal ions and the overall structure. The simulation results demonstrate that the standard AMBER force fields fail to adequately describe the RNA G-quadruplex, leading to poor structural stability. Cations including  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{NH}_4^+$  dissociate from the interior of the GQ to the external environment; some force fields give rise to non-native bifurcated hydrogen bonds, while others even fail to preserve the native hydrogen-bonding network. In contrast, the force fields modified with the PNC model maintain relatively stable structures, in which the cations remain stably localized and the native hydrogen bonds are well preserved. Furthermore, we carried out folding simulations on the second system using the MetaD enhanced sampling technique. The results reveal that none of the five conventional AMBER force fields yield reasonable free energy landscapes, whereas the incorporation of the PNC model enables four force fields (LJbb, ROC, OL3 and Shaw) to reproduce relatively accurate free energy profiles. Collectively, extensive tests and comparisons across multiple systems validate that the PNC method is applicable to RNA systems and can improve the performance of the current AMBER force fields to a certain extent.

Furthermore, our folding simulations of the RNA hairpin reveal that, within the framework of the LJbb force field, the PNC parameters derived from distinct

conformations of the same structure exert a substantial influence on the resulting free energy landscape. A notable limitation of the PNC method lies in its structural dependence: the PNC values calculated from different structural conformations are inherently non-identical. Nonetheless, the initial structures employed in standard MD simulations are generally equilibrated conformations that correspond to the global minimum on the Gibbs free energy profile, and such conformations occur with the highest frequency during simulations. In this context, it is reasonable to compute and adopt the PNC parameters based on these equilibrated structures. Meanwhile, the advantages of the PNC method are pronounced. This method delivers relatively fast computational speeds, with detailed data presented in Section 3.1 of the Results. In addition, it exhibits good compatibility with mainstream MD software packages. Compared with other polarization models, the PNC approach preserves the original functional form of the AMBER force field: it only requires the replacement of the native charge parameters without compromising the computational efficiency of AMBER simulations.

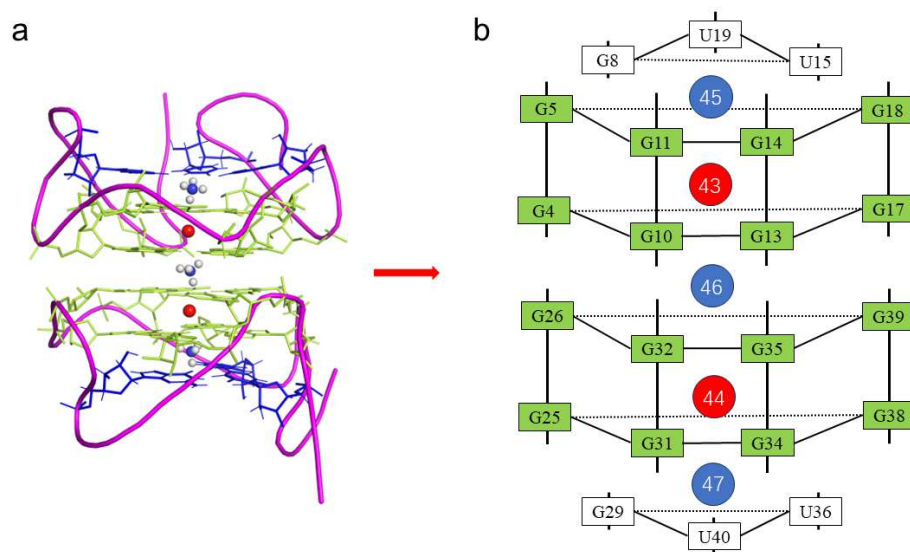


**Figure 1.** Structural features of RNA GQ. **(a)** The system is comprised of two strands (as shown in purple cartoon): each strand folds into two mutually stacked G-tetrads (as shown in green sticks). Meanwhile, the G-tetrads originating from the two strands further stack against one another, and an additional triad (as shown in blue sticks) is situated at each end of the assembly to further reinforce the structural stability. Red spheres: K<sup>+</sup>; Blue-white spheres: NH<sub>4</sub><sup>+</sup>. **(b)** Structure with loop regions omitted, for clarity, featuring four G-tetrads and two triads. The core channel accommodates two K<sup>+</sup> ions (represented by red circles, id 43 and 44) and one NH<sub>4</sub><sup>+</sup> ion (represented by blue circles, id 46), with an additional two NH<sub>4</sub><sup>+</sup> ions (id 45 and 47) located at the stem-loop junctions.

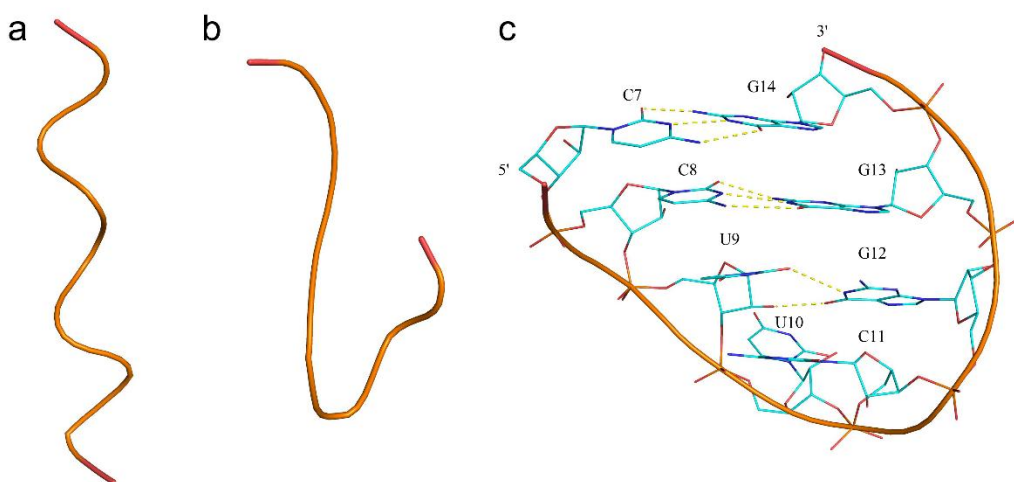


**Figure 2.** Structures utilized in MD and MetaD simulations are displayed with both cartoon and stick styles. (a) The structure generated by the Tleap module was used in conventional MD simulations. (b) Unfold state structure, this structure taken from the last snapshot from the conventional MD simulations as for the starting structure of MetaD simulation. (c) Folded GQs hairpin structure from experiment with protein data bank (PDB) id 1F7Y, and the hydrogen bond is given by yellow dash line.

## 2. Methods

### 2.1 Generation of PNC

Accurately computing the atomic charges for large biomolecular systems via direct quantitative methodologies entails an extraordinarily high computational cost. A pragmatic and efficient alternative involves decomposing the target system into a set of discrete segments on a residue-by-residue basis, with this fragmentation protocol depicted comprehensively in supplementary information (SI) Fig. S1. Taking a RNA system as a paradigm, if it consists of  $N$  residues, it can be partitioned into  $N$  independent segments by breaking the phosphodiester linkages between the O3' and P atoms of adjacent nucleotide units. Notably, to cap the severed chemical bonds and retain the intrinsic physicochemical properties of the original structure, specialized capping moieties are introduced at the cleavage sites: a  $-\text{PO}_3\text{CH}_3$  group is attached to the O3' atom, whereas a complementary  $-\text{OCH}_3$  group is appended to the adjacent P atom at each cut position.

The initial stage of this workflow focuses on acquiring the preliminary atomic charges of the system in the vacuum phase. Based on the above fragmentation criteria, the biomolecular system is divided into multiple segments and modified with the corresponding capping groups. The electronic density and electrostatic potential of each segmented unit are subsequently calculated using the Gaussian16 software at the B3LYP/6-31G\* theoretical level [63]. The derived electrostatic potential data is then fitted through the RESP algorithm to determine the atomic charges of individual

segments, which are ultimately assembled to generate the complete charge distribution for the full biomolecular system [64].

Once the preliminary atomic charges of the system are obtained, the subsequent procedure adopts an iterative computational workflow as depicted in Fig. S2. Initially, the Delphi program is employed to compute the full solvent-induced surface charge distribution [65]. Subsequently, the electrostatic potential of each fragment is calculated in sequence, where the surface charges and the atomic charges of all other fragments serve as the background electrostatic environment. The derived potential is utilized to re-optimize the charge parameters of each fragment, which are further assembled to construct the complete atomic charge set for the entire system. This iterative routine is performed for 12 cycles until the fluctuations in the system's total energy and atomic charges achieve convergence.

This entire computational protocol fundamentally follows the self-consistent reaction field (SCRF) theory. The PNC method bears considerable resemblance to the RESP charge fitting strategy adopted for small molecules in the AMBER force field yet possesses a core difference: the explicit incorporation of background charges throughout the PNC charge derivation process. Additionally, the fundamental principle of charge parameterization in the PNC model is highly consistent with that of the AMBER force field, which guarantees favorable compatibility between PNC charges and other standard AMBER parameters, supporting the direct implementation of the PNC approach in the AMBER simulation platform. For MD simulations, the default partial charges in the conventional AMBER force field can be directly substituted with the PNC-derived charges, whereas all other force field parameters remain unchanged.

In the native 8UTG system, the G-tetrad incorporates  $\text{NH}_4^+$ . We first computed the PNC for this native RNA system containing the  $\text{NH}_4^+$ . Subsequently, the  $\text{NH}_4^+$  was substituted with  $\text{K}^+$ , and the PNC for this modified system were also recalculated. Notably, the PNC calculation is not required for the system in which the  $\text{NH}_4^+$  is replaced by a  $\text{Na}^+$ , as its charge parameters are identical to those of the  $\text{K}^+$ -substituted system. This is because both  $\text{Na}^+$  and  $\text{K}^+$  are treated as point charges with a single positive charge during the PNC parameterization process, meaning the PNC results are independent of the specific type of monovalent cation. For the 1F7Y system, we calculated PNC parameters not only for its native hairpin conformation but also for its other two distinct conformations. Further details are provided in Section 2.4.

For clear identification and systematic comparison, we designate the polarized force field derived by replacing the partial charges in the LJbb force field with PNC as the LJbb-PNC force field. This naming convention is also applied to all other modified force fields generated in this manner, such as OL3 vs. OL3-PNC, ROC vs. ROC-PNC, Shaw vs. Shaw-PNC and YIL vs. YIL-PNC.

## 2.2 Description of ions in force field

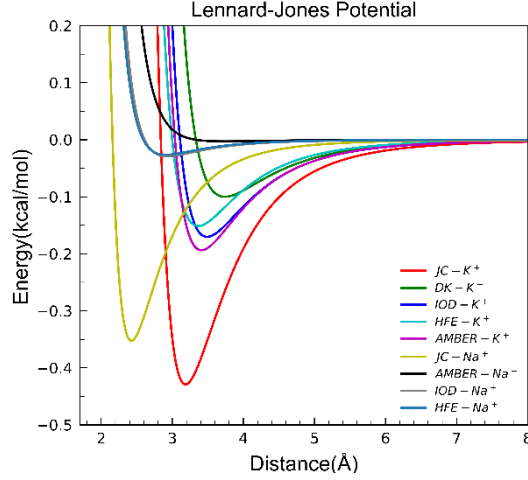


**Figure 3.** Lennard-Jones potentials between two  $K^+$  or two  $Na^+$  under different parameter models.

In most of the traditional force fields, the interaction between molecules or ions consists of only two terms, namely electrostatic interaction and van der Waals interaction. The former is expressed in terms of Coulomb potential energy, and the latter in terms of Lennard-Jones potential. Van der Waals interaction involves two types of forces: the first is long-range attractive London dispersion force, and the second is short-range repulsion arising from the Pauli exclusion principle. The functional form of the corresponding LJ potential energy model is as follows:

$$V_{LJ}(r) = 4\varepsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6 \right]$$

$\sigma$  is the distance between two ions where the potential energy is zero, and  $\varepsilon$  is the depth of the potential well, which controls the strength of the interaction. In essence,  $\varepsilon$  measures the strength of the mutual attraction between two atoms.  $r_m$  stands for the distance where the LJ potential energy has a minimum value, and the transformation between  $\sigma$  and  $r_m$  is  $\sigma = r_m / 2^{1/6}$ . In addition to the AMBER  $K^+$  and  $Na^+$  models, we collected a variety of  $K^+$  and  $Na^+$  models that have been optimized to reproduce experimental hydration free energies or ion-oxygen distance, they are defined as JC [66], DK [67], IOD and HFE [68]. Because  $K^+$  and  $Na^+$  have fixed masses and charges, only the Lennard-Jones parameters differ between those ion sets, as shown in Fig. 3 and Table S1.

## 2.3 Material and simulation protocol

In this study, one system (the PDB id is 8UTG) was selected for conventional MD simulations, as shown in Fig. 1. This system is an intermolecular assembly composed of two individual strands, each folding into a GQ. The two GQ units are further stacked together through G-tetrad  $\pi$ - $\pi$  stacking, with one  $NH_4^+$  sandwiched between them. Two additional  $K^+$  ions and  $NH_4^+$  are also located inside the ionic channel, respectively.

MD simulation of LJbb, OL3, ROC, Shaw and YIL force fields and their corresponding PNC force fields were carried out using the Amber22 code and employing the pmemd CUDA implementation [22, 23, 69, 70]. The AMBER force field was loaded using the Tleap module to generate GQ parameters [71]. For  $\text{NH}_4^+$ , the corresponding Amber parameters were generated via the general AMBER force field (GAFF) in the Antechamber module [72], with atomic charges derived from the AM1-BCC charge model [73, 74]. The PNC calculation was based on the confirmed 8UTG structure, as described in Section 2.1. Each system was immersed in a truncated octahedron of TIP3P water molecules [75], with the nearest distance from the solute to the edge of the box set to 12 Å, and AMBER  $\text{K}^+$  ions were added to neutralize the system or represent the ions originally present in the system. Prior to MD simulation, all systems were optimized with a 5000-step steepest descent algorithm followed by a 5000-step conjugate gradient algorithm to remove undesirable contacts within the structure generated during the initialization step. After structural optimization, each system was heated from 0 to 300K over 500ps in the NVT ensemble, and then equilibrated in 500ps in the NPT ensemble. Finally, the systems were simulated for 1000ns under the NPT ensemble. In MD simulation, the integral time step was set to 2 fs, Langevin dynamics was used to maintain the temperature at 300K [76], the collision frequency was set to 1  $\text{ps}^{-1}$ , and the constant pressure was set to 1atm. Particle mesh Ewald (PME) method was used to handle long-range electrostatic interaction [77], and the cutoff value for van der Waals interactions was set to 12 Å. In addition, all bonds containing hydrogen atoms are constrained using the Shake algorithm [78].

In MD simulations of biomacromolecular, the convergence behavior evaluated by Root-mean-square deviation (RMSD) must be taken into consideration. In practice, most small nucleic acid systems can readily reach dynamical convergence within 2–20 ns [79-81]. Therefore, the 200 ns or 1000 ns MD simulation timescale adopted for each force field is reasonable and sufficient. Furthermore, besides the standard AMBER ion model, four additional  $\text{K}^+$  ion models (JC, DK, IOD, HFE) and three  $\text{Na}^+$  parameter sets (JC, IOD, HFE) were considered. For the 8UTG system, each force field was combined with one ion parameter set, and a 200 ns MD simulation was performed for each combination, under the corresponding PNC force field. Finally, in this work, all MD trajectory files generated from RNA simulation systems were post-processed and analyzed using the CPPTRAJ module [82].

Meanwhile, to verify the electrostatic-screening effect in aqueous solution, we performed conventional MD simulations for the 1F7Y system (Fig. 2c) under both the AMBER and PNC force fields. Three distinct dielectric constants (1, 2, and 4) were adopted following the same simulation protocols and parameter settings described above, with each simulation lasting 1000 ns.

## 2.4 Well-tempered metadynamics

The well-tempered metadynamics (MetaD) method is commonly employed to characterize the free energy landscape and other state functions of a system [20, 21]. During the simulation, the state of the system is assumed to be described by one or more reaction coordinates, also referred to as collective variables (CVs). By projecting the free energy of the system onto the dimensions corresponding to these CVs, the high-dimensional free energy landscape can be effectively subjected to dimensionality reduction. Throughout the simulation, Gaussian repulsive potentials are deposited along these dimensions to prevent the system from revisiting the previously sampled states. As the Gaussian potentials accumulate continuously, the system gradually surmounts the free energy barriers, thereby enabling comprehensive sampling of the configurational space.

The original coordinates of the unfolded RNA chain (5'-ccUUCGgg-3') of the 1F7Y (PDB id) system were taken from the Tleap program in Amber22 software [71, 83]. The initial system was solvated in a solvent box and neutralized by adding AMBER  $K^+$  ions (Fig. 2(a)). The ParmEd tool was used to convert the AMBER parameter/topology and coordinate files into the GROMACS topology and coordinate formats [84]. All subsequent MD simulations were performed using GROMACS. Energy minimization was performed under the OL3 force field, followed by 50 ns of pre-equilibration in both NVT and NPT ensembles to stabilize the system at 300 K and 1 atm. Subsequently, using identical coordinates and velocities (Fig. 2(b)), 10  $\mu$ s MetaD simulations were carried out with PLUMED under the LJbb, OL3, ROC, Shaw, and YIL force fields, as well as their corresponding polarized force fields (LJbb-PNC, OL3-PNC, ROC-PNC, Shaw-PNC and YIL-PNC). The PNC values here were calculated based on the experimental hairpin structure (Fig. 2(c)), which only residues 7 to 14 (5'-ccUUCGgg-3') were truncated from 1F7Y following the recommendations from reference [36].

eRMSD is a metric specifically designed to quantify the similarity between three-dimensional RNA structures [85]. It operates by measuring the distance between structures based exclusively on the relative positions and orientations of nucleobases. Conceptually, eRMSD can be regarded as a vectorial representation of contact maps. In MetaD simulations of DNA or RNA folding, eRMSD is recognized as a highly effective reaction coordinate and has been validated [36, 86], while Distance is a classical and widely adopted CV. It is also worth mentioning that residues 1 and 8 are located at the two ends of the hairpin, respectively. The CV "Distance" is defined as the distance between the C7@C1' atom and the G14@C1' atom. It essentially characterizes the width of the hairpin structure, and this distance measures approximately 10.40 Å in the experimental hairpin structure. Thus, both eRMSD and Distance were employed as two CVs, with the crystal structure of 1F7Y used as the reference structure for these two CVs (Fig. 2(c)), the specific PLUMED parameters can be found in Fig. S3. In summary,

we performed two-dimensional (2D) 10  $\mu$ s MetaD simulations under different force fields.

To investigate the impact of PNC parameters acquired from distinct structural states on the free energy profile, we developed two customized force field variants: LJbb-chain-PNC, which integrates PNC parameters from the pristine RNA chain built by the Tleap module (Fig. 2(a)), and LJbb-npt-PNC, which adopts PNC parameters from the conformation equilibrated via MD simulations (Fig. 2(b)). These two reparameterized force fields were utilized to carry out 10  $\mu$ s MetaD simulations under identical simulation settings. To additionally corroborate the performance of the PNC model with diverse CVs, we performed supplementary 2D MetaD simulations with the original LJbb and PNC-corrected LJbb force fields, employing eRMSD and RMSD as the dual CVs. The relevant input parameters configured in the PLUMED code are presented in Fig. S4.

While Replica Exchange Metadynamics (RE-MetaD) delivers enhanced performance in conformational sampling [20], its practical deployment is drastically restricted by demanding hardware prerequisites, especially the need for extensive high-performance GPU resources. Owing to the shortage of available GPU computing capacity, we chose standard MetaD as a pragmatic and cost-efficient alternative. This computational strategy effectively elevated the simulation efficiency, permitting us to conduct adequately prolonged simulations to verify the convergence and dependability of the acquired data within our accessible computational resources. Meanwhile, we also note that previous studies only performed 3  $\mu$ s MetaD simulations for the 1F7Y system [38]. By contrast, we extended the simulation to 10  $\mu$ s in the present work, which provides a sufficiently long sampling time.

In summary, two distinct systems were subjected to MD and MetaD simulations in this study. The simulation durations and force field parameters employed for each system are comprehensively summarized in Table 1.

**Table 1** Summary of MD or MetaD simulation protocols of two systems under different force fields and parameters

| Systems                 | Protocols and parameters                                                                         |
|-------------------------|--------------------------------------------------------------------------------------------------|
| MD simulation           |                                                                                                  |
| 8UTG <sup>a,b</sup>     | 1000 ns with NH <sub>4</sub> <sup>+</sup> and AMBER K <sup>+</sup> model                         |
| 8UTG <sup>a,b</sup>     | 200 ns with AMBER, IOD, JC, HFE, DK K <sup>+</sup> models, respectively                          |
| 8UTG <sup>a,b</sup>     | 200 ns with AMBER, IOD, JC, HFE Na <sup>+</sup> models, respectively                             |
| 1F7Y <sup>a,b</sup>     | 1000 ns with AMBER K <sup>+</sup> model, under three distinct dielectric constants, respectively |
| MetaD simulation        |                                                                                                  |
| 1F7Y <sup>a,b,c</sup> , | 10 $\mu$ s with CVs (eRMSD and Distance)                                                         |
| 1F7Y <sup>d</sup>       | 10 $\mu$ s with CVs (eRMSD and RMSD)                                                             |

<sup>a</sup> LJbb, OL3, ROC, Shaw, and YIL force fields

<sup>b</sup> LJbb-PNC, OL3-PNC, ROC-PNC, Shaw-PNC and YIL-PNC force fields

<sup>c</sup> LJbb-chain-PNC and LJbb-npt-PNC force fields

<sup>d</sup> LJbb and LJbb-PNC force fields

### 3. Results and Discussion

#### 3.1 Convergence of the PNC parameters

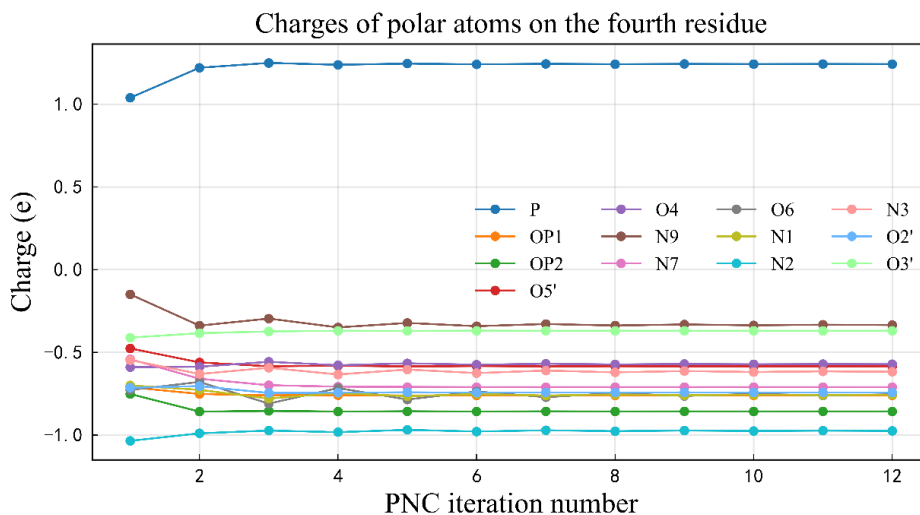


**Figure 4.** Charge evolution of polar atoms on residue G4 in the 8UTG structure during PNC parameter calculation.

We calculated PNC for three distinct systems, namely the 1F7Y system, the native 8UTG system containing  $\text{NH}_4^+$ , and a modified 8UTG system where  $\text{NH}_4^+$  were substituted with alternative monovalent metal ions. Owing to the substantial total number of atoms in the system, only the charges of nitrogen, oxygen and phosphorus atoms within a single representative residue are displayed here. Figure 4 and Fig. S5 illustrate the charge variations of polar atoms belonging to hydrogen-bond-forming residues in the first two systems throughout the PNC fitting procedure. After five iterative cycles, the atomic charges converge, demonstrating that the PNC method is reliable. Meanwhile, this method achieves high computational efficiency. A single fragment takes merely three minutes per calculation cycle on a 24-core Central Processing Unit (CPU). For the 8-residue 1F7Y system, the entire 12-round iterative calculation consumes approximately only 4.80 hours in total.

#### 3.2 Stability of the structures

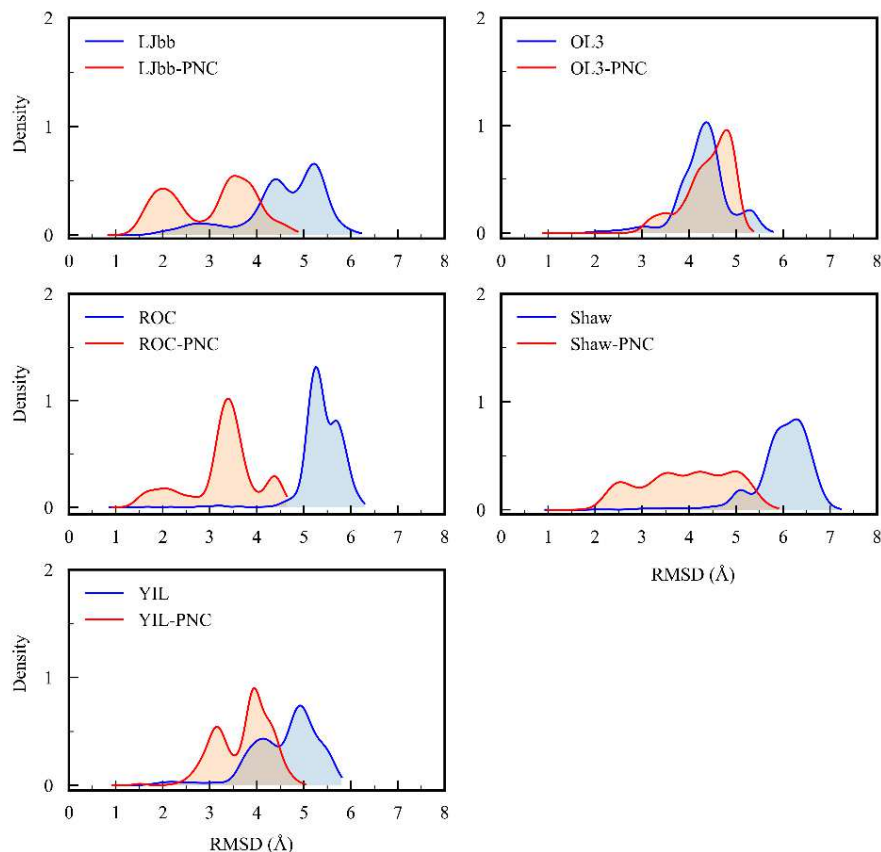


**Figure 5.** Probability density function of RMSD for 8UTG system in the 1000ns MD simulations using different force filed.

During 1000 ns MD simulations, the overall conformations of GQs gradually evolve toward an energetically optimized state. RMSD quantifies the structural deviation of dynamic conformations relative to the initial crystal structure, serving as a robust metric for evaluating structural stability and monitoring global conformational fluctuations throughout MD simulations. In this work, we calculated the RMSD of non-hydrogen atoms for the entire structure, as well as the RMSD of G-tetrads with loops excluded.

As observed in Fig. S6, the RMSD values of all simulated systems reach convergence either rapidly or gradually for both polarizable and non-polarizable force fields. Figure 5 presents the probability density distribution of the corresponding RMSD values, except for the very close distribution between OL3 and OL3-PNC, RMSD polarizable force fields produce lower average RMSD values than their corresponding non-polarizable counterparts. In addition, while the RMSD profiles under several force fields show evident time-dependent fluctuations, the G-tetrad RMSD exhibits substantially higher stability relative to the global system RMSD (as shown in Fig. S7). This indicates that the fluctuations in RMSD are mainly caused by conformational changes in the loop structures, which is consistent with the subsequent

root mean square fluctuation (RMSF) analysis. Notably, the RMSD values of G-tetrads region under polarizable force fields are consistently lower than those under the corresponding non-polarizable force fields. These results demonstrate that the incorporation of polarization effects effectively stabilizes the GQ structures during MD simulations.

### 3.3 Stability of Individual Nucleotides

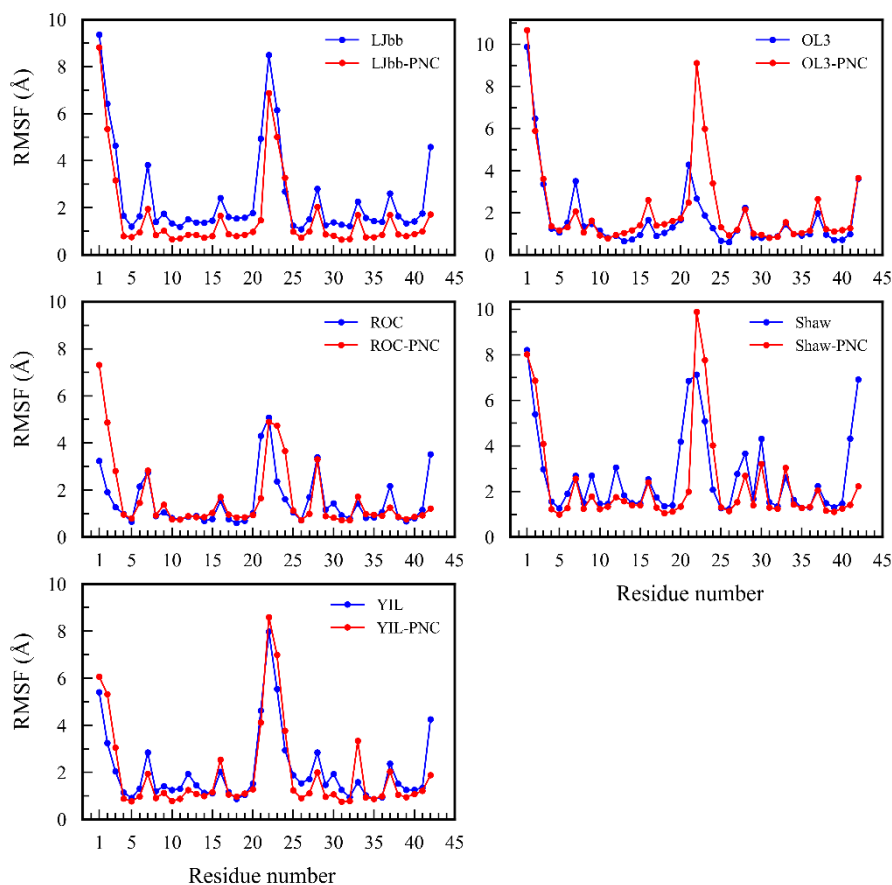


**Figure 6.** RMSF values calculated under different force fields. The red and blue scatter points correspond to the non-polarized and polarized force fields, respectively.

RMSF can quantitatively characterize the local dynamic flexibility of each residue within the molecule, identify the rigid core and flexible functional regions, uncover the sources of conformational fluctuations, and compare the structural stability and dynamic differences between distinct systems. Examination of the RMSF profiles for the 8UTG system over 1000 ns simulations reveals that, as this system consists of two strands, the responding results shown in Fig. 6, regions with relatively high RMSF values (indicating pronounced flexibility) are mainly concentrated in the terminal loop regions (U1A2U3 and U19A20U21, U22A23U24 and U40A41U42). In contrast, regions with low RMSF values correspond to the guanine residues that constitute the G-tetrads of the GQ.

Notably, within this G-tetrad core region, the RMSF values computed with the LJbb-PNC and YIL-PNC force fields (incorporating polarization effects) are significantly lower than those obtained with their corresponding non-polarizable force fields. In fact, direct comparison of the RMSD values exclusively for the G-tetrads shows that all PNC-modified force fields yield smaller RMSD values than the standard AMBER force fields as shown in Fig. S7. This observation confirms that the PNC charge model effectively enhances the structural stability of the internal G-quadruplex core.

### 3.4 Stability of $\text{NH}_4^+$ , $\text{K}^+$ or $\text{Na}^+$ in GQs

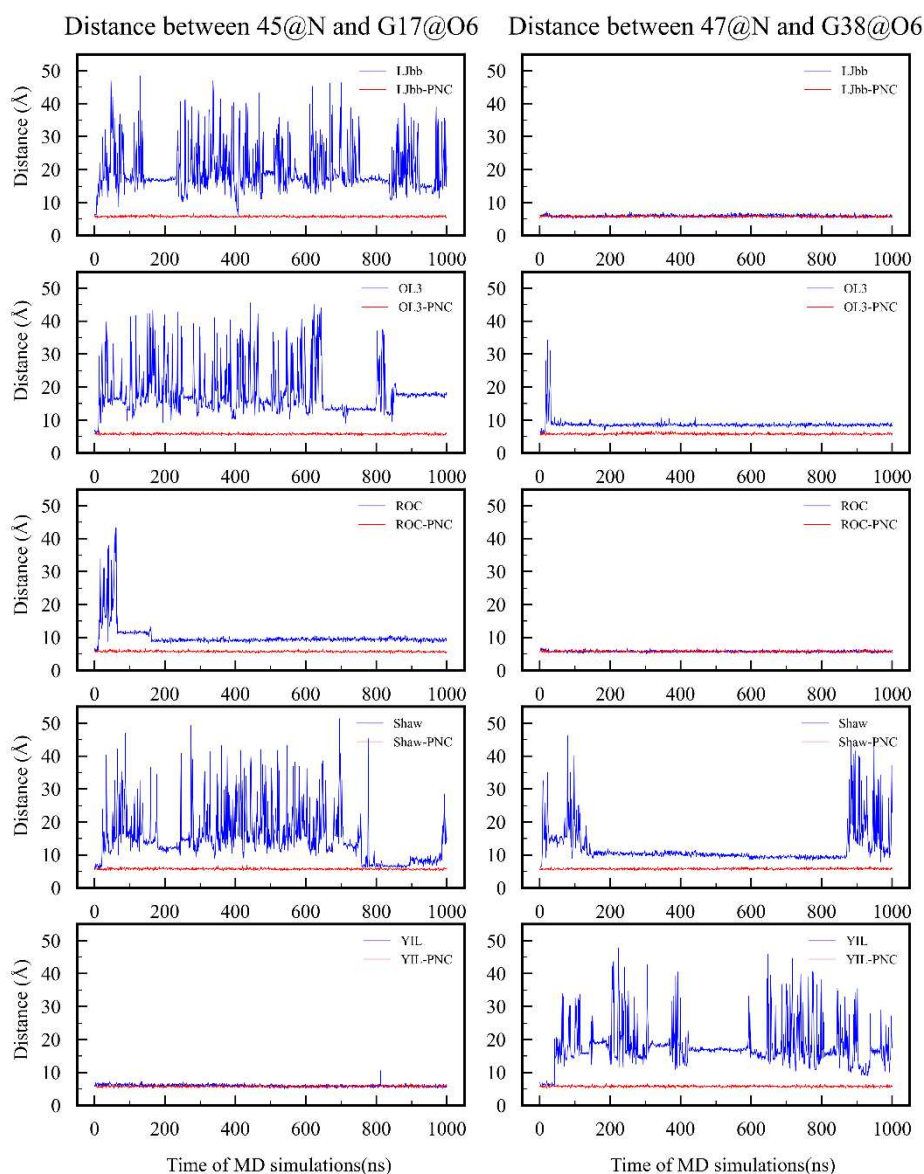


**Figure 7.** Using AMBER  $\text{K}^+$  parameters, the distance of 45@N-G17@O6 and 47@N-G38@O6 as a function of simulation time under different force field simulations.

Within the channel of the 8UTG GQ reside three  $\text{NH}_4^+$  and two  $\text{K}^+$  ions (Fig. 1). These monovalent cations are indispensable, as they effectively reduce the negative

electrostatic potential inside the channel via electrostatic interactions with many surrounding O6 atoms from guanine residues, thereby enhancing structural stability. To examine whether various force fields can accurately describe ion–RNA interactions, we calculated the time-dependent distances between nitrogen atom of the 45NH<sub>4</sub><sup>+</sup> ion and G17@O6, as well as between the 47NH<sub>4</sub><sup>+</sup> ion and G38@O6 throughout the simulation. For reference, these two distances in the experimental structure are 5.5 Å and 5.4 Å, respectively.

As shown in Fig. 7, under non-polarizable force fields, at least one NH<sub>4</sub><sup>+</sup> rapidly escapes into the aqueous solvent within the initial stage of the simulation. Although the distance between one NH<sub>4</sub><sup>+</sup> and the O6 atom remains stable at approximately 10 Å in the OL3, ROC or Shaw force field, this NH<sub>4</sub><sup>+</sup> has already moved away from its original coordination site, interacting with phosphate groups of the backbone and stabilizing in the outer groove of the GQ (Fig. S8). In contrast, under all polarizable force fields, the distances between the terminal ions and O6 atoms remain stable at around 6.00 Å throughout the 1000 ns simulation.

Meanwhile, we replaced all ions with either Na<sup>+</sup> or K<sup>+</sup> and performed 200 ns MD simulations using different ion parameter models respectively. For the five non-polarizable force fields combined with nine ion models in all combinations, consistent escape of ions into the solvent was observed in every case (Fig. S9-S17). But under the PNC force field, K<sup>+</sup> ions remain stably bound at their original coordination sites across all five K<sup>+</sup> models. Among the four Na<sup>+</sup> ion models, Na<sup>+</sup> ions remained stable at their initial positions in most cases. Exceptions were observed for the HFE and IOD models under the YIL-PNC force field, as well as the HFE model under the LJbb-PNC force field. Among the 45 combinations of nine ion models and five force fields, 42 exhibited satisfactory performance. These results indicate that polarizable force fields can better describe the electrostatic interactions between cations ions and negatively charged atoms.

Structural analysis reveals that K45 engages in strong electrostatic interactions with the O6 atoms of G5, G11, G14, and G18, as well as with the O6 or O4 atoms of G8, U15 and U19. Similarly, K47 interacts with the O6 atoms of G25, G31, G34, and G38, along with the O6 or O4 atoms of G29, U36 and U40 (Fig. 1). Under PNC force fields, there are marked increase in the electronegativity of these important oxygen atoms. Specifically, the partial charges on O6 and O4 atoms increase to approximately -0.68~-0.74 and -0.66~-0.68, respectively, compared to a uniform value of -0.56 and -0.58 for O6 and O4 atom types in non-polarized force fields (Table S2). This observed charge enhancement directly strengthens the electrostatic attraction between these oxygen atoms and monovalent cations (K<sup>+</sup>/Na<sup>+</sup>). Consequently, in PNC force fields, water molecules are unable to displace the coordinated ions, which is attributed to the substantially improved ion stability within the GQ core.

We quantitatively analyze the energy variations at different ion distances for the 8UTG system containing five  $K^+$  ions, in which  $NH_4^+$  ions are replaced by  $K^+$ . Given that the five  $K^+$  ions adopt a near-linear arrangement, we displaced the K45 ion outward along the K43→K45 direction starting from the original position of K43 at an interval of 0.1 Å per step as shown in Fig. S18. At each displaced position, the electrostatic potential between K45 and all other atoms was computed in the gas phase. An equivalent calculation for K47 was performed at the opposite end of the GQ. Since the charge parameters are identical for the LJbb, OL3, ROC, Shaw and LJbb force fields, the resulting data and their PNC counterparts are presented in Fig. 8. It should be noted that van der Waals interactions need not be discussed here, as they are identical between the standard AMBER force fields and their corresponding PNC versions.

It can be observed that under the PNC force field, the electrostatic potential energy for K45 (or K47) reaches a global minimum value at a distance about 2.50 Å from K25 (or K29). Slopes are observed on both sides of the minimum, which suggests that ions are likely stabilized around 2.5 Å with the PNC force field. In addition, this 2.50 Å distance is highly favorable, as it is very close to the sum of the van der Waals radii of the two  $K^+$  ions. However, at the same position under the AMBER non-polarized force field, the energy profile is very smooth in the region beyond 2.50 Å. This may indicate that K45 or K47 ions are more readily displaced by water molecules in the AMBER force field.

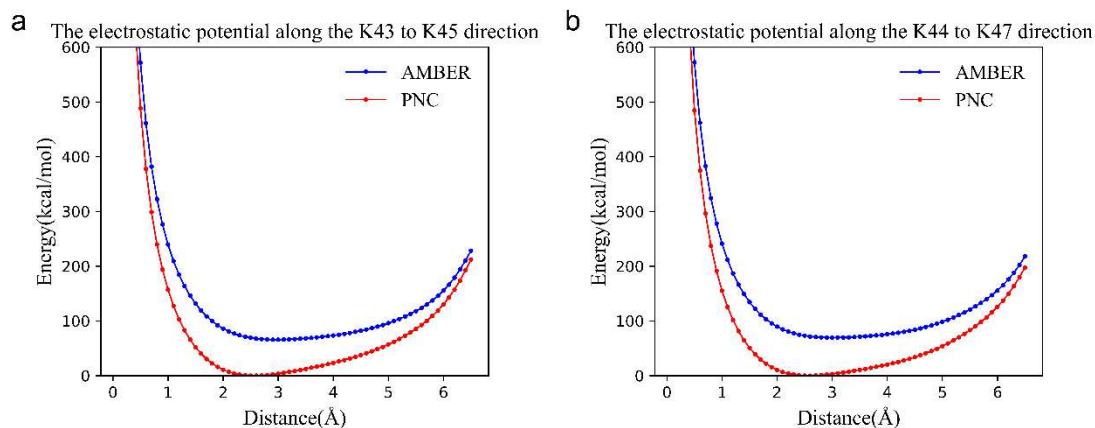


**Figure 8.** The relative electrostatic interaction energy between GQ and  $K^+$  in the channel. **(a)** Calculated electrostatic potential between K45 and all other atoms in the AMBER and PNC force fields during K45 ion moving away from the K43 position along the K43 → K45 direction toward outside (step size = 0.1 Å). **(b)** Calculated electrostatic potential for K47 in the AMBER and PNC force fields using the identical computational procedure described above.

### 3.5 Formation of Bifurcated Hydrogen Bonds

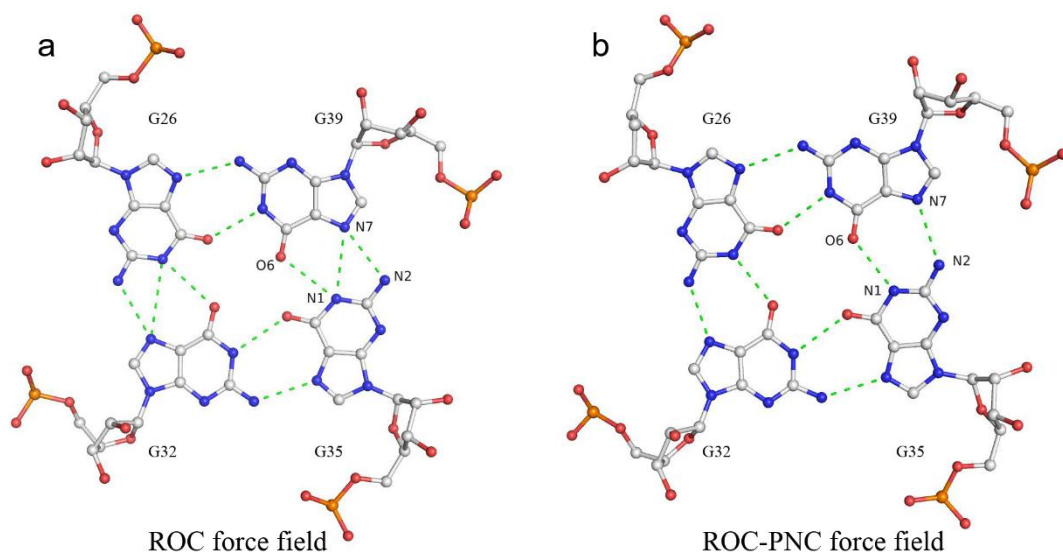


**Figure 9.** Hoogsteen GG base pair. (a) G35 and G39 formed bifurcated bonding geometry from 1JRN simulated structure under ROC force field. (b) G35 and G39 from the ROC-PNC force field.

**Table 2** The average distance of, N1-O6, N1-N7 and N2-N7 atoms in G35 and G39, respectively, these heavy atoms formed hydrogen bonds during the MD simulations

| Force fields     | N1-O6 (Å) | N1-N7 (Å) | N2-N7 (Å) |
|------------------|-----------|-----------|-----------|
| exp <sup>a</sup> | 2.85      | 3.85      | 2.82      |
| LJbb             | 3.05      | 3.58      | 2.91      |
| LJbb-PNC         | 2.92      | 3.70      | 2.83      |
| OL3              | 3.13      | 3.50      | 2.93      |
| OL3-PNC          | 2.91      | 3.72      | 2.83      |
| ROC              | 3.08      | 3.54      | 2.99      |
| ROC-PNC          | 2.87      | 3.80      | 2.85      |
| Shaw             | 2.94      | 3.77      | 2.91      |
| Shaw-PNC         | 2.87      | 3.73      | 2.79      |
| YIL              | 3.11      | 3.54      | 2.95      |
| YIL-PNC          | 2.92      | 3.71      | 2.83      |

<sup>a</sup> Denoted as experimental results

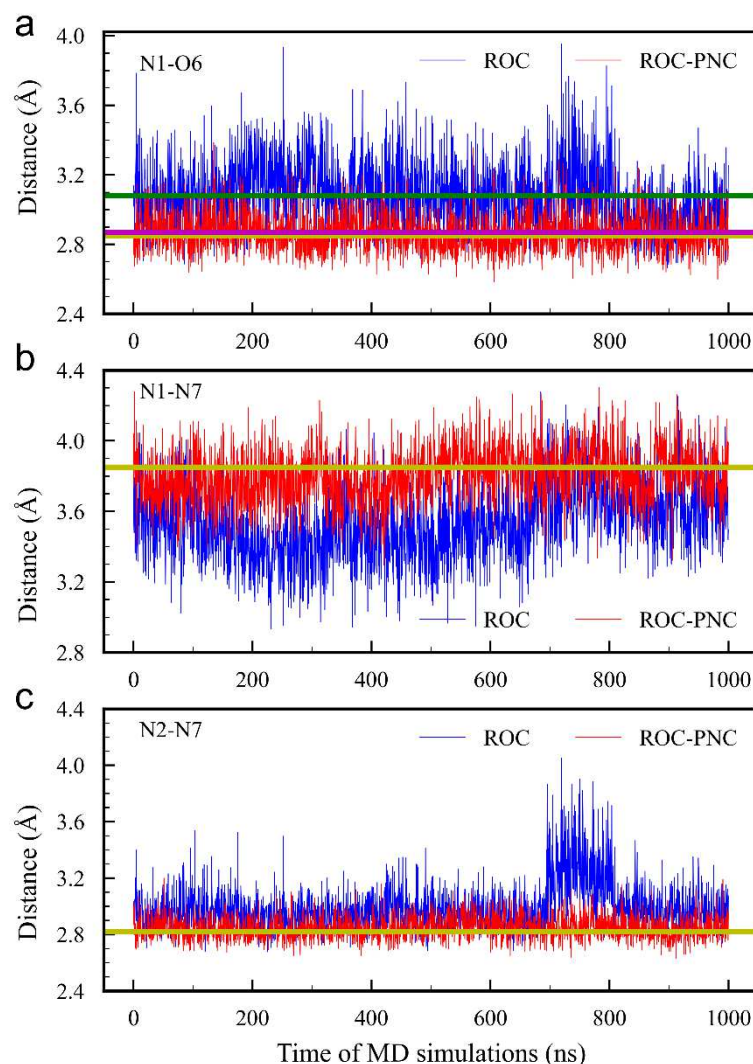


**Figure 10.** The heavy atoms distance from G35, G39 on 1000ns MD simulation under ROC force field. **(a)** the hydrogen bond distance formed by N1 and O6. **(b)** N1 and N7 distance. **(c)** the hydrogen bond distance formed by N2 and N7. The averaged distance under ROC-PNC is given by the purple line while that of ROC by the green line. The yellow line is the distance from the experimental structure.

Base pairing and base stacking constitute two qualitatively distinct noncovalent interactions that govern RNA structural stability, and hydrogen bonding acts as the dominant stabilizing interaction in base pairing. Within canonical RNA conformations, the C–U base pair is stabilized by three hydrogen bonds, while the A–T base pair contains two hydrogen bonds. Comparable base pairs are also present in RNA GQs, two native hydrogen bonds are formed between G–G base pairs (Fig. 9), corresponding to the N1–O6 and N2–N7 atomic pairs. These hydrogen bonds cross-link four guanine bases into a planar G-tetrad, which further interacts with the cations residing in the central channel. Given the indispensable role of hydrogen bonds in maintaining the local architectures of G-tetrads, an accurate description of hydrogen bond energetics in

MD simulations is crucial for advancing our fundamental understanding of the dynamic structures of G-RNAs. If inaccurate force field parameters underestimate the stability of GG base pairs, this would lead to the bifurcated hydrogen bonding geometry in the MD simulations [87], as shown in Fig. 9(a).

To evaluate whether the native hydrogen-bonding geometry was preserved in MD simulations, we calculated the average distances for three key atomic pairs (N2-N7, N1-O6, N1-N7; as shown in Fig. 9) between the two G35-G39 base pairs across trajectories obtained from different force fields. The corresponding experimental distances are 2.85, 2.84, and 3.71 Å, respectively, as listed in Table 2.

MD simulations carried out using the standard nonpolarizable AMBER force field reveal the formation of an extra hydrogen bond between the N1 and N7 atoms in the G-tetrad consisting of G26–G32–G35–G39 (Fig. 9), beyond the two native hydrogen bonds. As the N2–N7 and N1–O6 distances were consistently longer than the experimental values, while the N1–N7 distance was shortened (Table 2, Fig. 10 and S19-S22). In contrast to non-polarized force fields, as shown in Table 2, MD simulations employing the four PNC force fields showed N2–N7 and N1–O6 distances in close agreement with the experimental values. Although the average N1–N7 distance was slightly increased compared to the experimental reference, the overall geometry maintained proper hydrogen-bonding characteristics throughout the simulation.

**Table 3** The average distance of, N1-O6, N1-N7 and N2-N7 atoms in G4 and G10, respectively, these heavy atoms formed hydrogen bonds during the MD simulations

| Force fields     | N1-O6 (Å) | N1-N7 (Å) | N2-N7 (Å) |
|------------------|-----------|-----------|-----------|
| exp <sup>a</sup> | 2.84      | 3.86      | 2.89      |
| LJbb             | 3.03      | 4.44      | 3.13      |
| LJbb-PNC         | 2.84      | 3.98      | 2.90      |
| OL3              | 4.84      | 6.08      | 5.33      |
| OL3-PNC          | 2.85      | 3.97      | 2.91      |
| ROC              | 2.95      | 4.51      | 3.22      |
| ROC-PNC          | 2.83      | 3.91      | 2.87      |
| Shaw             | 2.93      | 3.97      | 2.96      |
| Shaw-PNC         | 2.79      | 3.92      | 2.86      |
| YIL              | 2.94      | 4.03      | 3.02      |
| YIL-PNC          | 2.83      | 3.98      | 2.89      |

<sup>a</sup> Denoted as experimental results

In addition to the formation of non-native bifurcated hydrogen bonds, Table 3 further demonstrates that under the OL3 and ROC force fields, the average distance of the hydrogen bond between G10@N2 and G23@N7 exceeds or approaches 3.2 Å,

resulting in the rupture and considerable instability of this specific hydrogen bond. Conversely, for the five force fields integrated with the PNC model, the distance of this hydrogen bond closely matches the experimental value. These results demonstrate that the polarized force fields effectively preserve the native hydrogen-bonding interactions, thereby contributing to the stability of the G-tetrad planar configuration.

### 3.6 Current force fields neglect the electrostatic screening effect in aqueous solution

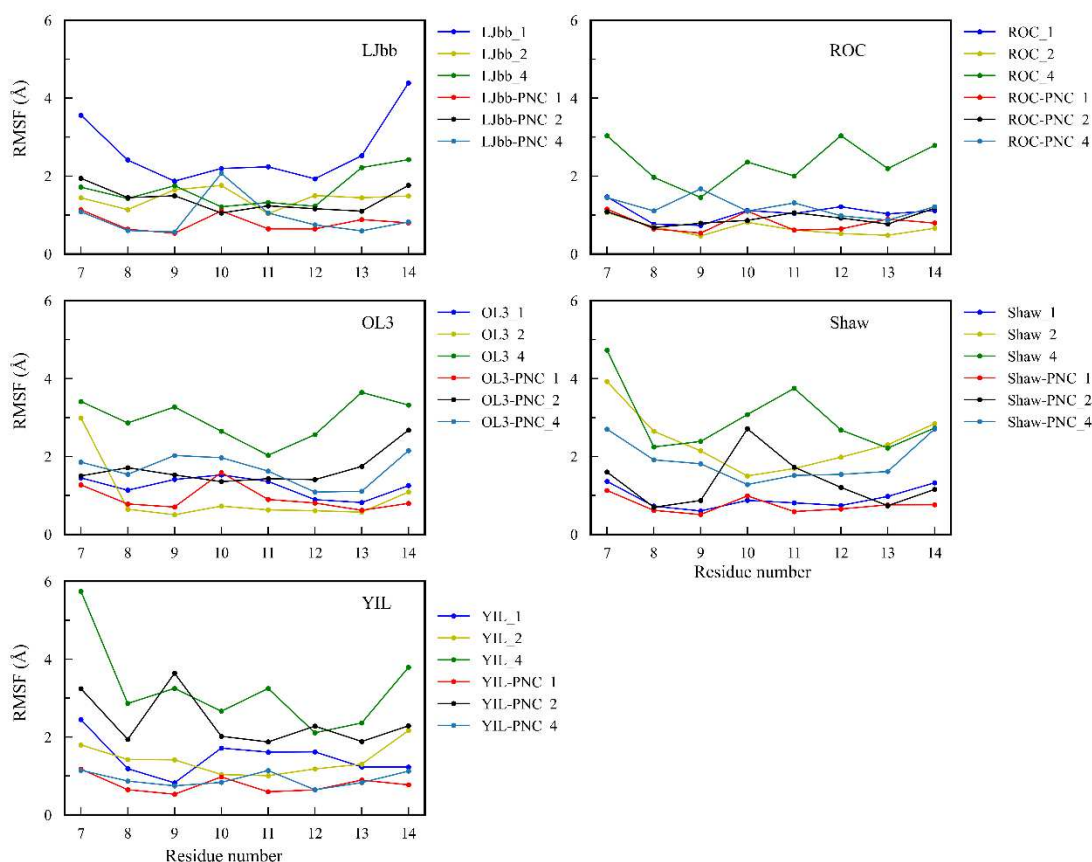


**Figure 11.** The RMSF values calculated under three distinct dielectric constants during conventional MD simulations employing the AMBER and PNC force fields.

The dielectric constant modulates the weighting of electrostatic interactions within the force field. Conventional MD simulations of the RNA hairpin structure were performed sequentially with three dielectric constant values (1, the default parameter; 2; and 4) under the PNC and AMBER force fields. RMSF values were calculated against the original crystal structure to characterize the stability of trajectories, and the corresponding results are presented in Fig. 11. As observed, for the LJbb, OL3 and ROC force fields, the RMSF values are generally substantially lower when a dielectric constant of 2 is adopted compared with the default value of 1 or the value of 4. This

finding confirms the inherent limitations of the OL3 force field: it overestimates the interactions between phosphate groups and bases as well as those between ribose moieties and bases, while underestimating hydrogen-bonding interactions [38]. This phenomenon arises because the default dielectric constant of 1 neglect the electrostatic screening effect induced by water molecules. In aqueous solution, the charged phosphate groups on the RNA surface are solvated by water molecules, and surrounding ions further screen electrostatic interactions, which weakens the electrostatic attraction between phosphate groups and other moieties. Meanwhile, we observed that the PNC force field with the default dielectric constant of 1 exhibited the best performance. Furthermore, since the PNC scheme intrinsically accounts for the electrostatic-screening effect of solvent, the PNC force field with a dielectric constant of 1 achieves optimal performance. By contrast, adjusting the dielectric constant to 2 or 4 disrupts this balance and instead introduces instability to the trajectories obtained with the PNC force field.

### 3.7 Free-Energy Landscape of RNA Chain Folding into a Hairpin

RNA hairpins are common and crucial conformations in RNA molecules. During the folding of single-stranded RNA into hairpin conformation, the system experiences a continuous decrease in entropy, which represents an unfavorable thermodynamic contribution to the total free energy. Nonetheless, this entropic loss is compensated by a corresponding reduction in structural potential energy, resulting in a gradual decline of the overall free energy throughout the folding process and the ultimate achievement of a stable conformational state. Recent experimental determination of the three-dimensional structure of a 8-nucleotide hairpin has furnished an opportunity to explore the folding mechanism of this RNA molecule into the hairpin conformation, and more importantly, to assess and validate the accuracy of modern state-of-the-art force fields.

First, we evaluated the convergence of simulations for all tested force fields. A total of 20 million Gaussian kernels were accumulated over the course of 10000 ns MetaD simulations. Specifically, the free energy profile along each one-dimensional CV—including eRMSD, RMSD, and interatomic distance—was estimated every million Gaussian kernels. This approach yielded 20 independent datasets per CV, with the final 10 datasets plotted as individual curves in the corresponding figures. As illustrated in Fig. S23–S36, the overlapping profiles confirm that satisfactory convergence was generally attained across all force fields.

Next, we quantified the state transition events occurring during the RNA folding simulations. As summarized in Table S3, multiple bidirectional transitions between the folded and unfolded conformational states were observed, verifying that the simulations were not trapped in any single metastable state. Finally, the 2D free energy surfaces for each force field were reconstructed using the complete set of accumulated Gaussian

kernels.

**Table 4** The free energy difference between the folded and unfolded states under different force fields (kcal/mol)

| Force fields   | Unfolded state                |       | Foiled state     |       | $\Delta G_{u \rightarrow f}$ |
|----------------|-------------------------------|-------|------------------|-------|------------------------------|
|                | (eRMSD,Distance) <sup>a</sup> | $G_u$ | (eRMSD,Distance) | $G_f$ |                              |
| LJbb           | (1.73,16.20)                  | 1.19  | (0.51,10.55)     | 6.58  | 5.39                         |
| LJbb-PNC       | (2.12,20.11)                  | 2.14  | (0.40,10.23)     | 0.45  | -1.69                        |
| OL3            | (2.07,20.83)                  | 0.90  | (0.48,10.59)     | 7.32  | 6.42                         |
| OL3-PNC        | (1.92,15.85)                  | 5.26  | (1.00,10.20)     | 2.45  | -2.81                        |
| ROC            | (1.80,20.27)                  | 0.18  | (0.54,10.07)     | 6.46  | 6.28                         |
| ROC-PNC        | (1.83,20.10)                  | 7.19  | (0.95,10.57)     | 0.00  | -7.19                        |
| Shaw           | (1.82,20.41)                  | 1.41  | (0.44,10.54)     | 2.86  | 1.45                         |
| Shaw-PNC       | (1.82,19.78)                  | 2.27  | (0.41,10.60)     | 0.92  | -1.35                        |
| YIL            | (2.03,20.07)                  | 1.19  | (0.44,10.52)     | 10.13 | 8.94                         |
| YIL-PNC        | (2.09,20.80)                  | 3.39  | (0.97,10.56)     | 11.26 | 7.87                         |
| LJbb-chain-PNC | (2.02,14.43)                  | 0.00  | (1.14,10.55)     | 10.22 | 10.22                        |
| LJbb-npt-PNC   | (2.08,20.84)                  | 0.25  | (0.41,10.59)     | 15.46 | 15.21                        |
|                | Unfolded state                |       | Foiled state     |       |                              |
|                | (eRMSD,RMSD) <sup>a</sup>     | $G_u$ | (eRMSD,RMSD)     | $G_f$ |                              |
| LJbb           | (1.76,4.94)                   | 0.00  | (0.40,1.43)      | 11.69 | 11.69                        |
| LJbb-PNC       | (2.06,7.70)                   | 0.00  | (0.86,2.47)      | 4.02  | 4.02                         |

<sup>a</sup>The unit is Ångström (Å)

Figure 12 shows the calculated free energy profile using the standard AMBER LJbb, OL3, ROC, Shaw and YIL force fields and their corresponding PNC force fields, with respect to two CVs (eRMSD, Distance) at  $T = 300$  K. The free energy difference between the folded ( $G_f$ ) and unfolded ( $G_u$ ) states was defined as  $\Delta G_{u \rightarrow f} = G_f - G_u$ . Since all the MetaD simulations under different force fields share the same initial structure, the  $G_u$  state corresponds to eRMSD = 2.08 and Distance = 20.00 Å. As shown in Table 4, compared to the standard AMBER force fields, some PNC force fields yield more reasonable free energy differences. For instance, the LJbb force field predicts an unfavorable positive  $\Delta G_{u \rightarrow f}$  of 5.39 kcal/mol, while the LJbb-PNC force field yields a more physically meaningful value of -1.69 kcal/mol. Furthermore, under the ROC-PNC force field, the folded state corresponds to the global free energy minimum, and under the OL3-PNC force field, the free energy of the folded state is also near the global minimum. This indicates that the RNA chain can spontaneously fold into a hairpin under ROC-PNC force field. These relatively accurate results demonstrates that PNC can improve the accuracy of the AMBER force field. It is noteworthy that lower eRMSD values generally reflect closer agreement with the experimental folded hairpin conformation. Within an acceptable range, the folded state under the ROC-PNC force field exhibits a slightly higher eRMSD (0.95) than the standard ROC force field (0.54).

Concurrently, we observed that the introduction of the PNC charge model does not bring about a particularly significant improvement to the YIL force field. This phenomenon is presumably ascribed to the substantial deficiencies existing in the other parameters of this force field beyond atomic charges.

To probe how PNC parameters derived from distinct conformational states shape the free energy landscape, we calculated PNC sets for the unfolded RNA single strand and the conformation equilibrated by MD simulations (Fig. 2). Each PNC dataset was combined with the LJbb force field to construct two unique polarized force fields, termed LJbb-chain-PNC and LJbb-npt-PNC (Section 2.4, Methods). Using the identical pre-equilibrated conformation as the starting structure, we carried out MetaD simulations under those two force fields. The resultant free energy profiles are plotted in Fig. S37, which lack a local minimum assigned to the folded RNA conformation in the bottom-left area of the free energy surface. Collectively, these observations demonstrate that PNC parameters parameterized from either the bare initial single strand or the equilibrated unfolded structure fail to accurately reproduce RNA folding dynamics.

Finally, we also investigate the impacts of varying CVs (eRMSD and RMSD) on the practical performance of the PNC model. Fig. S35,36 verifies the full convergence of the performed MetaD simulations, while the free energy results are displayed in Table 4 and Fig. S38, respectively. As illustrated in Fig. S38, no local minimum corresponding to the folded conformation is observed in the bottom-left region of the free energy surface under the standard LJbb force field, whereas such a characteristic energy minimum emerges in the LJbb-PNC polarized force field system. Furthermore, despite the positive free energy differences being retained in both force field systems, the incorporation of polarization effects substantially reduces the energy gap. Specifically, the free energy difference decreases from 11.69 kcal/mol in the conventional LJbb force field to 4.02 kcal/mol in the optimized LJbb-PNC force field. These analytical results solidly confirm that the incorporation of PNC parameters can effectively mitigate the computational errors of free energy landscapes, regardless of the types of CVs adopted for simulation.

By observing the experimental 1F7Y hairpin structure, we identified more than seven key stabilizing hydrogen bonds between residue pairs C7–G14, C8–G13 and U9–G12 (Fig. 2, S39–S41). Notably, the six participating residues exhibit state-dependent variations in the partial atomic charges of their polar atoms. PNC derived from the folded conformation inherently incorporates polarization effects induced by hydrogen bonding, whereas charges computed for the unfolded state where these hydrogen bond interactions are absent, lack such polarization explicitly. As quantitatively shown in Table S4, the O6 atomic charges of G14, G13 and G12 determined by the PNC method are  $-0.62$ ,  $-0.62$ , and  $-0.63$  in the single-stranded state, shifting to  $-0.66$ ,  $-0.66$ , and

−0.71 in the folded state, respectively. A similar trend is observed for the H1 atom too, for example, the charge of G14@H1 increases from 0.43 to 0.46, G7@N3 increases from -0.92 to -1.08. This systematic increase in electronegativity reflects the inclusion of polarization effects in the folded state compared with single-stranded state. All in all, the five AMBER standard force fields share identical charge parameters for each residue (Table S4), with the O6 and H1 atoms carrying fixed charges of −0.56 and 0.34, respectively. In contrast to these non-polarized force fields, the PNC method increases the negative charge on O6 by 0.10–0.15 and the positive charge on H1 by 0.10–0.12. The refined PNC force fields, which incorporate charges derived from the folded state that account for critical factors such as hydrogen bonding and solvation effects, demonstrate improved accuracy to a certain extent.

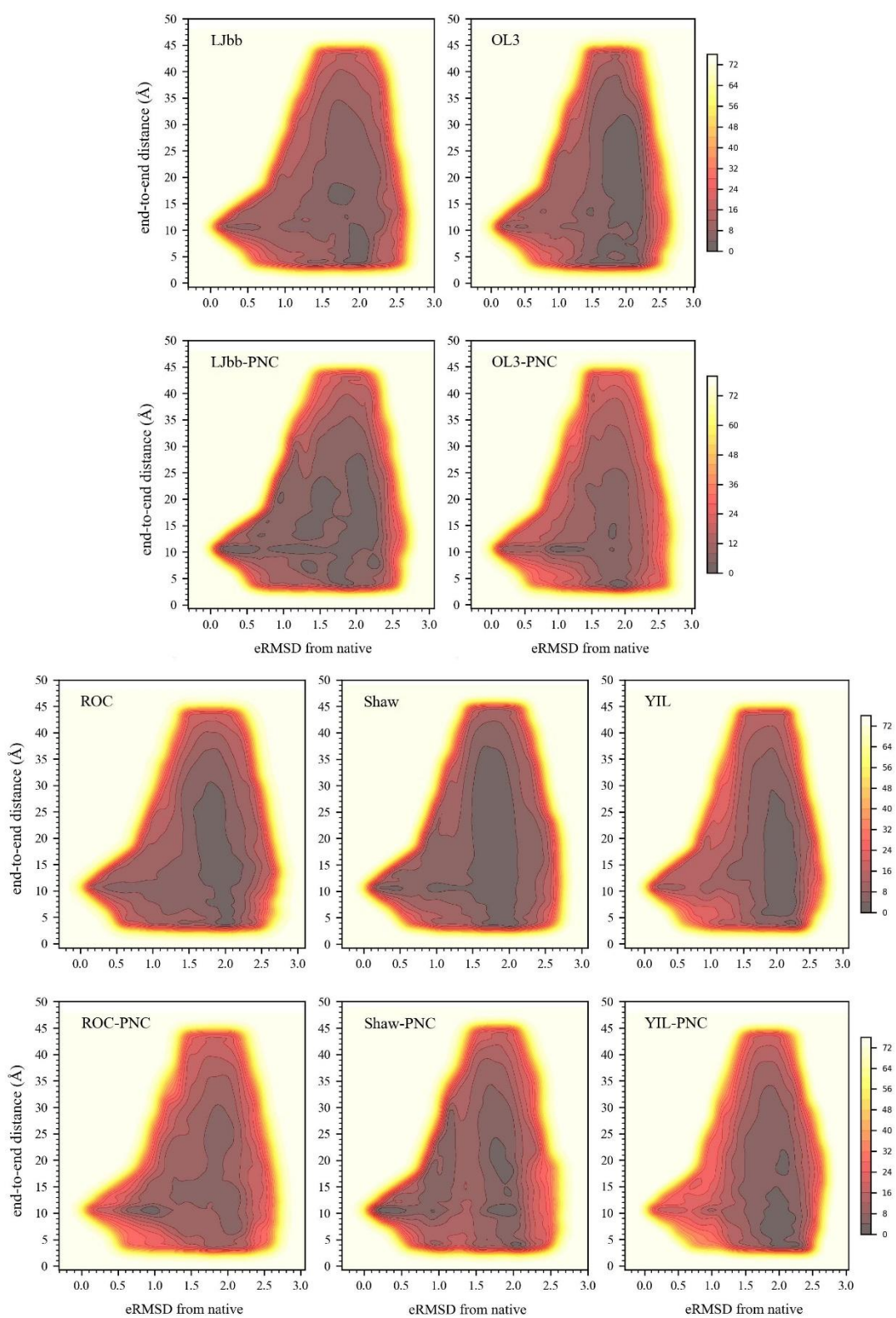


**Figure 12.** Free energy profiles obtained from MetaD simulations at 300 K using different force fields.

## 4. Conclusion

In this work, we developed and evaluated a new computational strategy for MD simulations of RNA systems using a polarized nucleic acid-specific charge (PNC) force field. The approach combines nucleotide-specific fragmentation with quantum mechanical calculations on RNA fragments in an implicit solvent, thereby capturing the solvent electrostatic-screening effect in a self-consistent manner. The resulting PNC parameters were applied to explore the dynamic conformations of a G-quadruplex (GQ) and an RNA hairpin. By directly comparing MD-simulated configurations with experimental structures and analyzing free-energy landscapes across different force fields, we comprehensively examined the impact of nucleotide polarization on the structural and dynamic properties of these RNA systems.

Monovalent metal cations are indispensable for GQs, as they are essential for both formation and structural stability. Our results show that polarization of guanine bases in the G-tetrad layers is critical for keeping these metal ions stably confined within the central channel. Conventional AMBER force fields, which lack explicit polarization effects, severely underestimate the electrostatic attraction between cations and nucleobases. This limitation ultimately causes the two outer cations to diffuse out of the channel into the bulk solvent.

For the folding of a single-stranded RNA into a hairpin structure, comparative MD simulations show that the PNC approach improves the performance of existing force fields to a certain extent. The resulting free-energy landscape profiles indicate that explicit consideration of electronic polarization systematically improves sampling efficiency in MD simulations. Although the PNC method exhibits excellent compatibility—allowing straightforward charge replacement in AMBER force fields—its adaptability varies among different PNC-derived force field variants. For the 8UTG system, all five PNC-modified force fields perform moderately better than their non-polarizable parent force fields. For the 1F7Y system, the PNC-modified force fields exhibit variable performance, with four showing clear improvement. The mechanisms underlying this variability warrant further investigation.

The LJbb-chain-PNC and LJbb-npt-PNC force fields failed to produce a physically meaningful free-energy surface. This likely arises from large deviations between the structural models used for PNC parameterization and the native experimental conformation. In practice, PNC charges are generally derived from a reference RNA structure, which in most cases is an experimentally resolved equilibrium geometry. Using such a single equilibrium conformation is usually the most computationally efficient strategy for MD investigations.

Compared with the Drude and AMOEBA models [31,41], the PNC model does not alter or extend the functional form of the force field; it merely replaces the original AMBER charges with PNC charges. This feature gives the PNC model favorable

compatibility while retaining AMBER's inherent computational advantage over Drude and AMOEBA.

Notably, these atomic charges are conformation-dependent and may introduce an artificial bias that stabilizes configurational sampling near the reference structure used for PNC fitting. Using PNC parameters obtained from non-native conformations can indeed shift sampling away from the equilibrium state. A promising direction would be to implement dynamic on-the-fly updates of PNC charges during simulation, generating new parameters from the final snapshot of each sampling window. However, such a procedure would substantially increase computational cost. In addition, although standard metadynamics alone can generate free-energy profiles for RNA folding, the resulting data may contain inherent artifacts or unrecognized drawbacks relative to the more reliable replica-exchange metadynamics (RE-MetaD) framework. These issues warrant systematic and in-depth exploration in future work.

### **Data Availability**

The PDB files store the charges calculated based on the PNC method, and all input files related to the 8UTG and 1F7Y systems (including PLUMED parameter files, reference structure PDB file, GROMACS MD protocol files, parameter files of different force fields, topology files, and script execution file) have been uploaded to GitHub at: <https://github.com/sztian-jn/PNC-RNA>. Other related figures and tables can be found in the supplementary file.

### **Acknowledgements**

All the authors are thankful to Jining University for providing necessary support to conduct this research.

### **Author Contributions**

Shuaizhen Tian and Nana Wang performed the MD simulations, drafted the original manuscript, and prepared all figures. Xinxin Du and Mengxi Qiu assisted with data analysis and revised the manuscript. John ZH Zhang conceptualized and designed this study.

### **Funding Information**

Shuaizhen Tian and Nana Wang acknowledge the support received from Jining University (Grant No. 000022/220116) and Shandong Provincial Natural Science Foundation (Grant No. ZR2024QA199). This work was also supported by the National Natural Science Foundation of China (Grant nos. 22333006, 92270001), Shanghai Municipal Science and Technology Commission (Grant No. 25DX2800500), and Guangdong Provincial Pearl River Talent Program (Grant No. 2023ZT10L045).

## Declarations

### Ethics approval and consent to participate

This study did not involve experiments using animals, human subjects, human-derived tissues, or genetically modified living organisms; hence, ethical approval is not applicable.

### Consent for publication

This work does not employ any copyright-protected materials; therefore, publication consent is not required.

### Competing interests

The authors declare no competing interests.

## References

1. Dong, J., et al., *Inhaled nucleic acid delivery systems for the treatment of pulmonary interstitial diseases: Challenges and opportunities*. Acta Pharm Sin B. 2026. **16**(4): p. 2093-2118. <https://doi.org/10.1016/j.apsb.2025.12.047>
2. Jin, K., et al., *In Vitro and In Vivo Evaluation of the Pathology and Safety Aspects of Three- and Four-Way Junction RNA Nanoparticles*. Mol. Pharm. 2024. **21**(2): p. 718-728. <https://doi.org/10.1021/acs.molpharmaceut.3c00845>
3. Genna, V., et al., *Nucleic acids in modern molecular therapies: A realm of opportunities for strategic drug design*. Curr. Opin. Struct. Biol. 2024. **87**: p. 102838. <https://doi.org/https://doi.org/10.1016/j.sbi.2024.102838>
4. Harvey, S.C., et al., *Phenylalanine transfer RNA: molecular dynamics simulation*. Science, 1984. **223**(4641): p. 1189-91. <https://doi.org/10.1126/science.6560785>
5. Harvey, S.C., M. Prabhakaran, and J.A. McCammon, *Molecular-dynamics simulation of phenylalanine transfer RNA. I. Methods and general results*. Biopolymers, 1985. **24**(7): p. 1169-1188. <https://doi.org/https://doi.org/10.1002/bip.360240706>
6. Li, N., et al., *Benchmark force fields for the molecular dynamic simulation of G-quadruplexes*. Molecules. 2021. **26**(17): p. 5379. <https://doi.org/10.3390/molecules26175379>
7. Šponer, J., et al., *Folding of guanine quadruplex molecules—funnel-like mechanism or kinetic partitioning? An overview from MD simulation studies*. Biochimica et Biophysica Acta (BBA)-General Subjects, 2017. **1861**(5): p. 1246-1263. <https://doi.org/10.1016/j.bbagen.2016.12.008>
8. Stadlbauer, P., et al., *Hairpins participating in folding of human telomeric sequence quadruplexes studied by standard and T-REMD simulations*. Nucleic Acids Res. 2015. **43**(20): p. 9626-9644. <https://doi.org/10.1093/nar/gkv994>
9. Gresh, N., et al., *Channeling through two stacked guanine quartets of one and two*

- alkali cations in the Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, and Rb<sup>+</sup> series. Assessment of the accuracy of the SIBFA anisotropic polarizable molecular mechanics potential.* J. Phys. Chem. B. 2017. **121**(16): p. 3997-4014. <https://doi.org/10.1021/acs.jpcc.7b01836>
10. Tao, P., et al., *Transcription reshapes RNA hairpin folding pathways revealed by all-atom molecular dynamics simulations.* PLOS Comput. Biol. 2025. **21**(9): p. e1013472. <https://doi.org/10.1371/journal.pcbi.1013472>
  11. Lazzeri, G., et al., *RNA folding pathways from all-atom simulations with a variationally improved history-dependent bias.* Biophys. J. 2023. **122**(15): p. 3089-3098. <https://doi.org/10.1016/j.bpj.2023.06.012>
  12. Liu, Y. and A.T. Frank, *Using Selectively Scaled Molecular Dynamics Simulations to Assess Ligand Poses in RNA Aptamers.* J. Chem. Theory Comput. 2022. **18**(9): p. 5703-5709. <https://doi.org/10.1021/acs.jctc.2c00123>
  13. Berdnikova, D.V., et al., *Role and Perspective of Molecular Simulation-Based Investigation of RNA-Ligand Interaction: From Small Molecules and Peptides to Photoswitchable RNA Binding.* Molecules, 2021. **26**(11). <https://doi.org/10.3390/molecules26113384>
  14. Sponer, J., et al., *RNA structural dynamics as captured by molecular simulations: a comprehensive overview.* Chem. Rev. 2018. **118**(8): p. 4177-4338. <https://doi.org/10.1021/acs.chemrev.7b00427>
  15. Hegde, S., et al., *Mechanistic studies of small molecule ligands selective to RNA single G bulges.* Nucleic Acids Res. 2025. **53**(12): p. gkaf559. <https://doi.org/10.1093/nar/gkaf559>
  16. Bekker, G.-J., et al., *Assessment of RNA Force Fields for Dynamic Docking of Small Molecules Using Multicanonical MD Simulations* J. Chem. Theory Comput. 2025. **21**(21): p. 11259-11270. <https://doi.org/10.1021/acs.jctc.5c01114>
  17. Sugita, Y. and Y. Okamoto, *Replica-exchange molecular dynamics method for protein folding.* Chem. Phys. Lett. 1999. **314**(1-2): p. 141-151. [https://doi.org/10.1016/S0009-2614\(99\)01123-9](https://doi.org/10.1016/S0009-2614(99)01123-9)
  18. Pierce, L.C., et al., *Routine access to millisecond time scale events with accelerated molecular dynamics.* J. Chem. Theory Comput. 2012. **8**(9): p. 2997-3002. <https://doi.org/10.1021/ct300284c>
  19. Torrie, G.M. and J.P. Valleau, *Nonphysical sampling distributions in Monte Carlo free-energy estimation: Umbrella sampling.* J. Comput. Phys. 1977. **23**(2): p. 187-199. [https://doi.org/https://doi.org/10.1016/0021-9991\(77\)90121-8](https://doi.org/https://doi.org/10.1016/0021-9991(77)90121-8)
  20. Bussi, G., et al., *Free-energy landscape for  $\beta$  hairpin folding from combined parallel tempering and metadynamics.* J. Am. Chem. Soc. 2006. **128**(41): p. 13435-13441. <https://doi.org/10.1021/ja062463w>
  21. Barducci, A., G. Bussi, and M. Parrinello, *Well-tempered metadynamics: a smoothly converging and tunable free-energy method.* Phys. Rev. Lett. 2008. **100**(2): p.

020603. <https://doi.org/10.1103/PhysRevLett.100.020603>
22. Götz, A.W., et al., *Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 1. Generalized Born*. J. Chem. Theory Comput. 2012. **8**(5): p. 1542-1555. <https://doi.org/10.1021/ct200909j>
  23. Salomon-Ferrer, R., et al., *Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 2. Explicit Solvent Particle Mesh Ewald*. J. Chem. Theory Comput. 2013. **9**(9): p. 3878-3888. <https://doi.org/10.1021/ct400314y>
  24. Knappeová, B., et al., *Comprehensive Assessment of Force-Field Performance in Molecular Dynamics Simulations of DNA/RNA Hybrid Duplexes*. J. Chem. Theory Comput. 2024. **20**(15): p. 6917-6929. <https://doi.org/10.1021/acs.jctc.4c00601>
  25. Mlýnský, V., et al., *Can We Ever Develop an Ideal RNA Force Field? Lessons Learned from Simulations of the UUCG RNA Tetraloop and Other Systems*. J. Chem. Theory Comput. 2025. **21**(8): p. 4183-4202. <https://doi.org/10.1021/acs.jctc.4c01357>
  26. Kührová, P., et al., *Improving the Performance of the Amber RNA Force Field by Tuning the Hydrogen-Bonding Interactions*. J. Chem. Theory Comput. 2019. **15**(5): p. 3288-3305. <https://doi.org/10.1021/acs.jctc.8b00955>
  27. Zgarbová, M., et al., *Refinement of the sugar-phosphate backbone torsion beta for AMBER force fields improves the description of Z-and B-DNA*. Theory Comput. 2015. **11**(12): p. 5723-5736. <https://doi.org/10.1021/acs.jctc.5b00716>
  28. Zgarbová, M., et al., *Refinement of the Cornell et al. nucleic acids force field based on reference quantum chemical calculations of glycosidic torsion profiles*. J. Chem. Theory Comput. 2011. **7**(9): p. 2886-2902. <https://doi.org/10.1021/ct200162x>
  29. Pérez, A., et al., *Refinement of the AMBER force field for nucleic acids: improving the description of  $\alpha/\gamma$  conformers*. Biophys. J. 2007. **92**(11): p. 3817-3829. <https://doi.org/10.1529/biophysj.106.097782>
  30. Huang, J., et al., *Mapping the Drude polarizable force field onto a multipole and induced dipole model*. J. Chem. Phys. 2017. **147**(16): p. 161702. <https://doi.org/10.1063/1.4984113>
  31. Zhang, C., et al., *AMOEBa polarizable atomic multipole force field for nucleic acids*. J. Chem. Theory Comput. 2018. **14**(4): p. 2084-2108. <https://doi.org/10.1021/acs.jctc.7b01169>
  32. Brooks, B.R., et al., *CHARMM: the biomolecular simulation program*. J. Comput. Chem. 2009. **30**(10): p. 1545-1614. <https://doi.org/10.1002/jcc.21287>
  33. McDowell, S.E., et al., *Molecular dynamics simulations of RNA: an in silico single molecule approach*. Biopolymers. 2007. **85**(2): p. 169-84. <https://doi.org/10.1002/bip.20620>
  34. Cornell, W.D., et al., *A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules*. J. Am. Chem. Soc. 1995. **117**(19): p. 5179-5197. <https://doi.org/10.1021/ja00124a002>

35. Cheatham Iii, T.E. and D.A. Case, *Twenty-five years of nucleic acid simulations*. Biopolymers. 2013. **99**(12): p. 969-977. <https://doi.org/https://doi.org/10.1002/bip.22331>
36. Bottaro, S., et al., *Free energy landscape of GAGA and UUCG RNA tetraloops*. J. Phys. Chem. Lett. 2016. **7**(20): p. 4032-4038. <https://doi.org/10.1021/acs.jpcclett.6b01905>
37. Havrila, M., et al., *Effect of monovalent ion parameters on molecular dynamics simulations of G-quadruplexes*. J. Chem. Theory Comput. 2017. **13**(8): p. 3911-3926. <https://doi.org/10.1021/acs.jctc.7b00257>
38. Kührová, P., et al., *Computer Folding of RNA Tetraloops: Identification of Key Force Field Deficiencies*. J. Chem. Theory Comput. 2016. **12**(9): p. 4534-4548. <https://doi.org/10.1021/acs.jctc.6b00300>
39. Fadrná, E., et al., *Single stranded loops of quadruplex DNA as key benchmark for testing nucleic acids force fields*. J. Chem. Theory Comput. 2009. **5**(9): p. 2514-2530. <https://doi.org/10.1021/ct900200k>
40. Huang, J., et al., *Molecular dynamics simulations using the drude polarizable force field on GPUs with OpenMM: Implementation, validation, and benchmarks*. 2018, Wiley Online Library. <https://doi.org/10.1002/jcc.25339>
41. Sengul, M.Y. and A.D. MacKerell Jr, *Accurate modeling of RNA hairpins through the explicit treatment of electronic polarizability with the classical Drude oscillator force field*. J. Chem. Theory Comput. 2022. **21**(04): p. 461-471. [https://doi.org/10.1142/9789819808199\\_0003](https://doi.org/10.1142/9789819808199_0003)
42. Aytenfisu, A.H., et al., *Revised RNA Dihedral Parameters for the Amber Force Field Improve RNA Molecular Dynamics*. J. Chem. Theory Comput. 2017. **13**(2): p. 900-915. <https://doi.org/10.1021/acs.jctc.6b00870>
43. Bergonzo, C. and T.E. Cheatham, III, *Improved Force Field Parameters Lead to a Better Description of RNA Structure*. J. Chem. Theory Comput. 2015. **11**(9): p. 3969-3972. <https://doi.org/10.1021/acs.jctc.5b00444>
44. Yang, C., et al., *Predicting RNA Structures via a Simple van der Waals Correction to an All-Atom Force Field*. J. Chem. Theory Comput. 2017. **13**(2): p. 395-399. <https://doi.org/10.1021/acs.jctc.6b00808>
45. Yoo, J. and A. Aksimentiev, *Improved Parameterization of Amine–Carboxylate and Amine–Phosphate Interactions for Molecular Dynamics Simulations Using the CHARMM and AMBER Force Fields*. J. Chem. Theory Comput. 2016. **12**(1): p. 430-443. <https://doi.org/10.1021/acs.jctc.5b00967>
46. Mlýnský, V., et al., *Reactive Conformation of the Active Site in the Hairpin Ribozyme Achieved by Molecular Dynamics Simulations with  $\epsilon/\zeta$  Force Field Reparametrizations*. J. Phys. Chem. B. 2015. **119**(11): p. 4220-4229. <https://doi.org/10.1021/jp512069n>

47. Zgarbová, M., et al., *Noncanonical  $\alpha/\gamma$  Backbone Conformations in RNA and the Accuracy of Their Description by the AMBER Force Field*. J Phys Chem B, 2017. **121**(11): p. 2420-2433. <https://doi.org/10.1021/acs.jpcb.7b00262>
48. Tan, D., et al., *RNA force field with accuracy comparable to state-of-the-art protein force fields*. . Proc. Natl. Acad. Sci. U. S. A. 2018. **115**(7): p. E1346-e1355. <https://doi.org/10.1073/pnas.1713027115>
49. He, W., et al., *Refining the RNA Force Field with Small-Angle X-ray Scattering of Helix–Junction–Helix RNA*. J. Phys. Chem. Lett. 2022. **13**(15): p. 3400-3408. <https://doi.org/10.1021/acs.jpcllett.2c00359>
50. Keedakkatt Puthenpeedikakkal, A.M., C.E. Cavender, and D.H. Mathews, *BPS2025 - Refinement of the amber force field for RNA: Improving the description of non-bonded interactions*. Biophys. J. 2025. **124**(3): p. 488a. <https://doi.org/10.1016/j.bpj.2024.11.2573>
51. Song, J., C. Ji, and J.Z. Zhang, *The critical effect of polarization on the dynamical structure of guanine quadruplex DNA*. Phys. Chem. Chem. Phys. 2013. **15**(11): p. 3846-3854. <https://doi.org/10.1039/c2cp44100d>
52. Chen, X.H. and J.Z. Zhang, *Theoretical method for full ab initio calculation of DNA/RNA-ligand interaction energy*. J Chem Phys, 2004. **120**(24): p. 11386-91. <https://doi.org/10.1063/1.1737295>
53. Ji, C., Y. Mei, and J.Z. Zhang, *Developing polarized protein-specific charges for protein dynamics: MD free energy calculation of pKa shifts for Asp26/Asp20 in thioredoxin*. Biophys J, 2008. **95**(3): p. 1080-8. <https://doi.org/10.1529/biophysj.108.131110>
54. Blackburn, E. and J. Szostak, *The molecular structure of centromeres and telomeres*. Annu. Rev. Biochem. 1984. **53**(1): p. 163-194. <https://doi.org/10.1146/annurev.bi.53.070184.001115>
55. Largy, E., J.-L. Mergny, and V. Gabelica, *Role of alkali metal ions in G-quadruplex nucleic acid structure and stability*, in *The Alkali Metal Ions: Their Role for Life*. 2016, Springer. p. 203-258. [https://doi.org/10.1007/978-3-319-21756-7\\_7](https://doi.org/10.1007/978-3-319-21756-7_7)
56. Wang, Z.-F., et al., *Structural basis of sodium–potassium exchange of a human telomeric DNA quadruplex without topological conversion*. Nucleic Acids Res. 2014. **42**(7): p. 4723-4733. <https://doi.org/10.1093/nar/gku083>
57. Plavec, J., *Metal ion coordination in G-quadruplexes*. Metal Complex–DNA Interactions, 2009: p. 55-93. <https://doi.org/10.1002/9781444312089.ch3>
58. Neidle, S., *The structures of quadruplex nucleic acids and their drug complexes*. Curr. Opin. Struct. Biol. 2009. **19**(3): p. 239-250. <https://doi.org/10.1016/j.sbi.2009.04.001>
59. Terrell, J.R., et al., *Structure of an RNA G-quadruplex from the West Nile virus genome*. Nat Commun, 2024. **15**(1): p. 5428. <https://doi.org/10.1038/s41467-024->

49761-5

60. Svoboda, P. and A. Di Cara, *Hairpin RNA: a secondary structure of primary importance*. Cell Mol Life Sci. 2006. **63**(7-8): p. 901-8. <https://doi.org/10.1007/s00018-005-5558-5>
61. Davis, P.W., W. Thurmes, and I. Tinoco, Jr., *Structure of a small RNA hairpin*. Nucleic Acids Res. 1993. **21**(3): p. 537-545. <https://doi.org/10.1093/nar/21.3.537>
62. Smith, L.G., et al., *Chemically Accurate Relative Folding Stability of RNA Hairpins from Molecular Simulations*. J. Chem. Theory Comput. 2018. **14**(12): p. 6598-6612. <https://doi.org/10.1021/acs.jctc.8b00633>
63. Frisch, M.e., et al., *Gaussian 16*. 2016, Gaussian, Inc. Wallingford, CT.
64. Bayly, C.I., et al., *A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model*. J. Phys. Chem. 1993. **97**(40): p. 10269-10280. <https://doi.org/10.1021/j100142a004>
65. Rocchia, W., et al., *Rapid grid-based construction of the molecular surface and the use of induced surface charge to calculate reaction field energies: Applications to the molecular systems and geometric objects*. J. Comput. Chem. 2002. **23**(1): p. 128-137. <https://doi.org/10.1002/jcc.1161>
66. Joung, I.S. and T.E. Cheatham III, *Determination of alkali and halide monovalent ion parameters for use in explicitly solvated biomolecular simulations*. J. Phys. Chem. B. 2008. **112**(30): p. 9020-9041. <https://doi.org/10.1021/jp8001614>
67. Dang, L.X. and P.A. Kollman, *Free energy of association of the K<sup>+</sup>: 18-crown-6 complex in water: a new molecular dynamics study*. J. Phys. Chem. 1995. **99**(1): p. 55-58. <https://doi.org/10.1021/j100001a011>
68. Li, P., L.F. Song, and K.M. Merz Jr, *Systematic parameterization of monovalent ions employing the nonbonded model*. J. Chem. Theory Comput. 2015. **11**(4): p. 1645-1657. <https://doi.org/10.1021/ct500918t>
69. D.A. Case, R.M.B., D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke, A.W. Goetz, N. Homeyer, S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, C. Lin, T. Luchko, R. Luo, B. Madej, D. Mermelstein, K.M. Merz, G. Monard, H. Nguyen, H.T. Nguyen, I. Omelyan, A. Onufriev, D.R. Roe, A. Roitberg, C. Sagui, C.L. Simmerling, W.M. Botello-Smith, J. Swails, R.C. Walker, J. Wang, R.M. Wolf, X. Wu, L. Xiao and P.A. Kollman. *AMBER 2016*. University of California, San Francisco, 2016. <https://doi.org/10.1002/jcc.20290>
70. Le Grand, S., A.W. Götz, and R.C. Walker, *SPFP: Speed without compromise—A mixed precision model for GPU accelerated molecular dynamics simulations*. Comput. Phys. Commun. 2013. **184**(2): p. 374-380. <https://doi.org/10.1016/j.cpc.2012.09.022>
71. Case, D.A., et al., *AmberTools*. J. Chem. Inf. Model. 2023. **63**(20): p. 6183-6191.

- <https://doi.org/10.1021/acs.jcim.3c01153>
72. Wang, J. and P.A. Kollman, *Automatic parameterization of force field by systematic search and genetic algorithms*. J. Comput. Chem. 2001. **22**(12): p. 1219-1228. <https://doi.org/10.1002/jcc.1079>
  73. Jakalian, A., et al., *Fast, efficient generation of high-quality atomic Charges. AM1-BCC model: I. Method* J. Comput. Chem. 2000. **21**(2): p. 132-146. [https://doi.org/10.1002/\(SICI\)1096-987X\(20000130\)21:2%3C132::AID-JCC5%3E3.0.CO;2-P](https://doi.org/10.1002/(SICI)1096-987X(20000130)21:2%3C132::AID-JCC5%3E3.0.CO;2-P)
  74. Jakalian, A., D.B. Jack, and C.I. Bayly, *Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation*. J. Comput. Chem. 2002. **23**(16): p. 1623-1641. <https://doi.org/10.1002/jcc.10128>
  75. Jorgensen, W.L., et al., *Comparison of simple potential functions for simulating liquid water*. J. Chem. Phys. 1983. **79**(2): p. 926-935. <https://doi.org/10.1063/1.445869>
  76. Pastor, R.W., B.R. Brooks, and A. Szabo, *An analysis of the accuracy of Langevin and molecular dynamics algorithms*. Mol. Phys. 1988. **65**(6): p. 1409-1419. <https://doi.org/10.1080/00268978800101881>
  77. Darden, T., D. York, and L. Pedersen, *Particle mesh Ewald: An  $N \cdot \log(N)$  method for Ewald sums in large systems*. J. Chem. Phys. 1993. **98**(12): p. 10089-10092. <https://doi.org/10.1063/1.464397>
  78. Ryckaert, J.-P., G. Ciccotti, and H.J. Berendsen, *Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes*. J. Comput. Phys. 1977. **23**(3): p. 327-341. [https://doi.org/10.1016/0021-9991\(77\)90098-5](https://doi.org/10.1016/0021-9991(77)90098-5)
  79. Ponomarev, S.Y., K.M. Thayer, and D.L. Beveridge, *Ion motions in molecular dynamics simulations on DNA*. Proc. Natl. Acad. Sci. U. S. A. 2004. **101**(41): p. 14771-14775. <https://doi.org/10.1073/pnas.0406435101>
  80. Rueda, M., et al., *Exploring the counterion atmosphere around DNA: what can be learned from molecular dynamics simulations?* Biophys. J. 2004. **87**(2): p. 800-811. <https://doi.org/10.1529/biophysj.104.040451>
  81. Várnai, P. and K. Zakrzewska, *DNA and its counterions: a molecular dynamics study*. Nucleic Acids Res. 2004. **32**(14): p. 4269-4280. <https://doi.org/10.1093/nar/gkh765>
  82. Roe, D.R., T.E.J.J.o.c.t. Cheatham III, and computation, *PTRAJ and CPPTRAJ: software for processing and analysis of molecular dynamics trajectory data*. J. Chem. Inf. Model. 2013. **9**(7): p. 3084-3095. <https://doi.org/10.1021/ct400341p>
  83. Case, D.A., et al., *Recent Developments in Amber Biomolecular Simulations*. J. Chem. Inf. Model. 2025. **65**(15): p. 7835-7843. <https://doi.org/10.1021/acs.jcim.5c01063>
  84. Shirts, M.R., et al., *Lessons learned from comparing molecular dynamics engines on the SAMPL5 dataset*. J. Comput. Aided Mol. 2017. **31**(1): p. 147-161. <https://doi.org/10.1007/s10822-016-9977-1>

85. Bottaro, S., F. Di Palma, and G. Bussi, *The role of nucleobase interactions in RNA structure and dynamics*. Nucleic Acids Res. 2014. **42**(21): p. 13306-13314. [https://doi.org/ 10.1093/nar/gku972](https://doi.org/10.1093/nar/gku972)
86. Yang, C., et al., *In silico direct folding of thrombin-binding aptamer G-quadruplex at all-atom level*. Nucleic Acids Res. 2017. **45**(22): p. 12648-12656. [https://doi.org/ 10.1093/nar/gkx1079](https://doi.org/10.1093/nar/gkx1079)
87. Špačková, N.a., I. Berger, and J. Šponer, *Nanosecond molecular dynamics simulations of parallel and antiparallel guanine quadruplex DNA molecules*. J. Am. Chem. Soc. 1999. **121**(23): p. 5519-5534. <https://doi.org/10.1021/ja984449s>

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.docx](#)