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Khavani, Mohammad; Mehranfar, Aliyeh; Vahid, Hossein

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### **Application of Amino Acid Ionic Liquids for Increasing the Stability of**

### **DNA in Long Term Storage**

Mohammad Khavani<sup>1,2\*</sup>, Aliyeh Mehranfar<sup>1</sup>, Hossein Vahid<sup>1,3</sup>

<sup>1</sup>Department of Chemistry and Materials Science, School of Chemical Engineering, Aalto University, P.O. Box 16100, FI-00076 Aalto, Finland

<sup>2</sup>Molecular Cell Biomechanics Laboratory, Departments of Bioengineering and Mechanical Engineering, University of California Berkeley, Berkeley, California 94720, USA

<sup>3</sup>Department of Applied Physics, Aalto University, P.O. Box 11000, FI-00076 Aalto, Finland <u>Mohammad.khavanisariani@aalto.fi</u>

khavanim@yahoo.com

# Abstract

The structural stability of DNA is important because of its biological activity. DNAs due to their inherent chemical properties are not stable in an aqueous solution, therefore, a long period of storage of DNA at the ambient condition in bioscience is of importance. Ionic liquids (ILs) as interesting alternatives compared to organic solvents and water due to their considerable properties can be used as new agents to increase the stability of DNA for a long period of storage. In this article, molecular dynamics (MD) simulations and quantum chemistry calculations were applied to investigate the effects of amino acid ionic liquids ([BMIM][Ala], [BMIM][Gly], [BMIM][Val], [BMIM][Pro] and [BMIM][Leu]) on the dynamical behavior and the structural stability of calf thymus DNA. Based on the obtained MD results ILs enter into the solvation shell of the DNA and push away the water molecules from the DNA surface. Structural analysis shows that [BMIM]<sup>+</sup> cations can occupy the DNA minor groove without

disturbing the double-helical structure of DNA. ILs due to strong electrostatic and van der Waals (vdW) interactions with the DNA structure contribute to the stability of the double-helical structure. Quantum chemistry calculations indicate that the interactions between the [BMIM]<sup>+</sup> cation and DNA structure has an electrostatic character. Moreover, this cation forms a more stable complex with the CGCG region of the DNA in comparison with AATT base pairs. Overall, the results of this study can provide new insight into the application of ILs for maintaining DNA stability during long-term storage.

**Keywords:** DNA; Amino acid ionic liquids; Electrostatic interactions; Stability; MD Simulation; Nucleic acid



# **Graphical Abstract**

Theoretical methods reveal that amino acid ionic liquids can be good candidates for increasing the stability of DNA in long-term storage.

# Introduction

Nucleic acid (DNA and RNA) based materials have been considered in recent years due to their interesting applications in different fields such as drug delivery (Li et al., 2013), nanosensors (Choi et al., 2018; Khavani et al., 2019; Khavani et al., 2018), electrochemistry (Silva et al., 2018), and chemistry (Lopez et al., 2018). The structural stability of DNA is important because of its biological activity. They can be used as a detector for single nucleotide polymorphism and as microarrays for gene expression analyses (Ott et al., 2003; Tateishi et al., 2014; Koch et al., 2018). There are many reports in the literature about the application of DNA and RNA structures as molecular computing devices and molecular transport agents (Phadke et al., 2001; Yurke et al., 2000; Li et al., 2013; Girard et al., 2017; Wang et al., 2017). DNAs due to their inherent chemical properties are not stable in an aqueous solution, which is a great challenge in the progress of nanotechnology based on nucleic acids (Chandran et al., 2012; Lukin et al., 2006; Marchand et al., 2018). Moreover, pH, ionic strength, salt concentration, and nonphysiological temperature have important effects on DNA properties and can denature the DNA structure easily (Vijayaraghavan et al., 2010; Oprzeska et al., 2018; Stellwagen et al., 2019). Therefore, long period storage of DNA at the ambient condition in nanotechnology is of importance.

Recently, scientists and researchers have considered ionic liquids (ILs) as interesting alternatives compared to organic solvents and water due to their

considerable properties such as low toxicity, low vapor pressure, high chemical, and thermal stability, and high conductivity (He et al., 2006; Huddleston et al., 2001; Welton et al., 1999). ILs can be used in different areas like synthesis (Zhou et al., 2003), catalysis (Olivier et al., 2010; Wasserscheid et al., 2000), separation (Anderson et al., 2002; Blanchard et al., 2001), electrochemistry (Fukumoto et al., 2005; Leone et al., 2001) and gas adsorption (Sistla et al., 2015). Low cytotoxicity and biodegradability of ionic liquids make them an ideal candidate for interaction with biomolecules. There are many experimental and theoretical studies in the literature about the effects of ionic liquids on the stability, activity, and properties of biomolecules like DNAs, proteins, and enzymes (Pillai et al., 2018; Reslan et al, 2018; Haque et al., 2017). For example, Kaar and coworkers studied the effects of dialkylimidazolium and pyrrolidinium-based ionic liquids with different anions including  $[PF_6]^-$ ,  $[OAC]^-$ ,  $[BF_4]^-$  and  $[NO_3]^-$  on the lipase activity and stability (Kaar et al., 2003). Their obtained results indicated that lipase has greater activity in ionic liquids with  $[BF_4]^-$  and  $[PF_6]^-$  in comparison with ILs containing  $[NO_3]^-$  anion. Micaelo et al. by employing molecular dynamics (MD) simulations investigated the effect of 1-butyl-3methylimidazolium hexafluorophosphate ([BMIM][PF<sub>6</sub>]) and 1-butyl-3methylimidazolium nitrate ([BMIM][NO<sub>3</sub>]) on the stability of protease structure (Micaelo et al., 2008). Based on MD simulation data, the native structure of the protease is more stable in [BMIM][PF<sub>6</sub>] than [BMIM][NO<sub>3</sub>] and shows better stability at higher temperatures in the presence of this IL.

The experimental study on the effects of  $[BMIM][PF_6]$  ionic liquid on the ethidium bromide interaction with DNA indicates that [BMIM]<sup>+</sup> interacts with phosphate groups in the DNA, which leads to the transformation of DNA conformation and reduces the interaction of ethidium bromide with the DNA (Cheng et al., 2007). Moreover, Ding and coworkers reported that [BMIM][Cl] ionic liquid increases the stability of DNA structure, which the reason for this stability is the electrostatic and hydrophobic interactions between DNA and [BMIM]<sup>+</sup> cation (Ding et al., 2010). Another experimental study by Sharma and coworkers revealed that choline-based bioionic liquids such as choline glycolate and choline pyruvate have remarkable effects on the chemical stability of DNA (Sharma et al., 2015). According to their obtained results, significant electrostatic interactions and hydrogen bonding between ionic liquids and DNA are the main factors for this stability. Sahoo et al. by employing theoretical methods investigated the choline amino acids ILs interactions with ct-DNA and reported that the corresponding ILs by hydrogen bond (H-bond) formation with phosphate groups and van der Waals (vdW) interactions stabilize the ct-DNA (Sahoo, et al., 2018). Based on the reported results by Chandran and coworkers electrostatic and hydrophobic interactions between cations of ILs and the DNA backbone can increase DNA stability significantly (Chandran et al., 2012). Besides, ILs can prevent the hydrolytic interactions that denature the DNA by disturbing the water cage around the DNA structure through the dehydration process.

In this study, by employing molecular dynamics (MD) simulations and quantum chemistry calculations the dynamical behavior and the structural stability of calf thymus DNA (as a case study) in water and different amino acid ionic liquids were investigated. The results of this study can provide new insight into the amino acid ionic liquid interactions with DNA from the molecular viewpoint, which can be useful for maintaining DNA stability during long-term storage.

# **Computational details**

#### **Molecular dynamics simulation**

To study the effect of amino acid ionic liquids (ILs) on the stability and dynamical behavior of DNA, full atomistic molecular dynamics (MD) simulations were applied. The initial structure of the well-characterized Dickerson-Drew dodecamer (5'-(CGCGAATTCGCG)<sub>2</sub>-3') as a model in MD simulations was obtained from the protein data bank (PDB) with 1BNA code. This DNA structure is a very stable B-DNA duplex that has a full helical turn. The studied ionic liquids composed of 1-butyl-3-methylimidazolium ([BMIM]<sup>+</sup>) as the cation and glycinate ([Gly]<sup>-</sup>), alanate ([Ala]<sup>-</sup>), valinate ([Val]<sup>-</sup>), leucinate ([Leu]<sup>-</sup>) and prolinate ([Pro]<sup>-</sup>) as anions.



Figure 1. The optimized structures of the cation and anions of the studied ILs.

The ff99SB (Hornak et al., 2006) and OL15 (Zgarbova et al., 2013) force field parameters were applied for the ILs and DNA, respectively. The structures of the cation and anions of the ionic liquids were optimized at M06-2X-D3/6-311++G(d,p) (Zhao et al., 2006; Zhao et al., 2006; Frisch et al., 2009; Khavani et al., 2015) and atomic charges were calculated by the CHelpG method at the same level. Figure 1 shows the optimized cation and anion structures of the studied ILs. To examine the validity of the employed force field for the ionic liquids, the appropriate number of ILs (Table S1) were added in a cubic box  $(5 \times 5 \times 5 \text{ nm}^3)$  and 50 ns NPT MD simulations were applied. At the first step of the MD simulation, energy minimization was performed in 50000 steps. Then, the temperature of the IL systems increased from 0 to 298.15 K during 10 ns with 1 fs time step by employing an NVT ensemble. In the next step, all the ILs were equilibrated during 10 ns (1 fs time step) in an NPT ensemble at 298.15 and 1 bar. Finally, 50 ns MD simulations (with 2 fs time step) were performed on the equilibrated structures as the product step. According to Table S1, the calculated density values for the corresponding ILs from MD simulations are in good agreement with experimental data (Sistla et al., 2015), which confirms that the ff99SB force field can reproduce the amino acid ionic liquids properties.

The dynamical behavior of the DNA in pure water and in presence of 10, 20, 30, 40, and 50 wt% of ILs was examined. The DNA structure was fully solvated by pure water (TIP3P water model (Jorgensen et al., 1983)) and water-IL mixtures in a cubic box  $(8 \times 8 \times 8 \text{ nm}^3)$ . The number of water and ionic liquid molecules is reported in Table 1. The systems were kept neutral by adding 22 Na<sup>+</sup> ions (Joung et al., 2008). After initial energy minimization during 100000 steps, the temperature of the systems increased from 0 to 298.15 K during 10 ns (with 1 fs time step) in an NVT ensemble by employing the restraining force constant of 2.5 kcal.mol<sup>-1</sup>.A<sup>2</sup> for the solute structure. Then all the systems were equilibrated in an NPT ensemble (1 bar and 298.15 K) during 10 ns with 1 fs time step. After this step, 200 ns NPT MD simulations as the product step (with 2 fs time step) were performed on the results of the equilibration step. The periodic boundary conditions were applied in all directions for the studied systems. Based on Table 1, in addition to the pure water, the dynamical behavior of the DNA was investigated in twenty-five water-IL mixtures.

In the NPT simulations, the temperature was controlled by employing the Langevin thermostat and the pressure was regulated by the isotropic Berendsen method with a collision frequency of 2 ps<sup>-1</sup> and a relaxation time of 1 ps for the

temperature and pressure, respectively (Sindhikara et al., 2009; Beruaga et al., 2004). To calculate the long-range electrostatic interactions, Particle Mesh Ewald (PME) method with 10 A° direct cutoff was applied (Ryckaert et al., 1977). The shake constraints were employed on all bonds involving hydrogen atoms (Tobias et al., 1997). All the MD simulations were performed using the Amber 14 software package (Case et al., 2014). To calculate the binding Gibbs energies for the ionic liquid interactions with DNA, the molecular mechanics-Poisson-Boltzmann surface area (MM-PBSA) method was employed (Miller et al., 2012; Gohlke et al., 2003; Khavani et al., 2015; Izadyar et al., 2015; Khavani et al., 2017; Khavani et al., 2020). The binding Gibbs energies were calculated from the final 50 ns of the product step.

Table 1. The number of water and ionic liquid molecules in the simulation box of the DNA.											
IL weight		[BMIM][Ala]	[BMIM][Gly]	[BMIM][Leu]	[BMIM][Pro]	[BMIM][Val]					
percent											
10 wt%	IL:H <sub>2</sub> O	136:15212	145:15212	114:15074	123:15318	120:15138					
20 wt%	IL:H <sub>2</sub> O	247:13558	292:13558	227:13313	249:13749	241:13426					
30 wt%	IL:H <sub>2</sub> O	413:11888	440:11888	339:11566	378:12143	362:11714					
40 wt%	IL:H <sub>2</sub> O	553:10202	590:10202	450:9834	510:10498	483:10002					
50 wt%	IL:H <sub>2</sub> O	695:8499	741:8499	561:8116	646:8813	604:8290					

# Quantum chemistry calculations

To study the nature and strength of [BMIM]<sup>+</sup> cation interactions with the DNA structure density functional theory (DFT) and density functional theory dispersion corrected (DFT-D3) calculations were applied. The corresponding DNA structure is composed of two parts including AATT and CGCG base pairs. Therefore, quantum chemistry calculations were employed to compare the

[BMIM]<sup>+</sup> cation interactions with AATT (A-T) and CGCG (C-G) base pairs. The [BMIM]<sup>+</sup> complexes with AATT and CGCG regions were designed based on the obtained structure of the DNA in the presence of ionic liquids after 200 ns MD simulations.

The structures of the [BMIM]<sup>+</sup> complexes with the A-T and C-G base pairs were optimized using BP86 (Perdew, 1986) and BP86-D3 (Austin et al., 2012) functionals within 6-31G(d) basis set (Petersson et al., 1991). The counterpoise correction method was employed to calculate the basis set superposition error (E<sub>BSSE</sub>) to eliminate the effect of basis set incompleteness (Simon et al., 1996). In contrast to the BP86, the PB86-D3 functional can describe dispersion interactions, therefore the energy difference obtained by these functionals was reported as the dispersion interaction energy. Frequency calculations were performed to calculate the thermodynamic parameters and structural stability of [BMIM]<sup>+</sup> complexes with A-T and C-G base pairs. The frequency analysis confirmed the nature of the stationary points as minima with real frequencies, which indicates the stability of the corresponding structures. To analyze the electrostatic, donor-acceptor and molecular orbital interactions between [BMIM]<sup>+</sup> cation and the base pairs natural bond orbital (NBO) analysis was performed at the BP86-D3/6-31G(d) of the theory (Reed et al., 1988; Khavani et al., 2018; Khavani et al., 2018). Gaussian 09 computational package (Frisch et al., 2009) was employed for all the quantum chemistry calculations. In order to

compare and determine the nature of the interactions between [BMIM]<sup>+</sup> cation and the A-T and C-G base pairs 2D and 3D non-covalent interaction (NCI) analysis using MultiWFN 3.8 was performed (Lu et al., 2012; Izadyar et al., 2017; Khavani et al., 2018).

# **Results and discussion**

#### **DNA** stability in water

The dynamical behavior of DNA in water was investigated during 200 ns MD simulations. The structural analysis confirms that DNA maintains its native B-conformation and has considerable stability along simulation trajectories (Figure 2-a). Moreover, the obtained structure of DNA in water shows that Na<sup>+</sup> ions condense around phosphate groups of the DNA backbone. According to Figure S1, the obtained DNA structure after MD simulation in water is very close to the crystal structure, which confirms considerable stability of the DNA during the simulation time.

The root mean square deviation (RMSD) values of DNA in water during 200 ns MD simulations were calculated and reported in Figure 2-b. According to this figure, the calculated average value of RMSD of DNA is 3.45 A° ( $\pm$ 0.41). This value is small and indicates the remarkable stability of DNA in water. The calculated radii of gyration (Rg) values of DNA (Figure 2-c) confirmed RMSD results and indicate that DNA is stable along the MD simulations. To analyze the flexibility of DNA, root mean square fluctuation (RMSF) values were calculated.

According to Figure 2-d, central residues of DNA have lower fluctuation or more stability in comparison to the terminal residues.



Figure 2. The obtained DNA structure (Na<sup>+</sup> presented in green color) after simulation time in water (a) and the calculated RMSD (b), Rg (c), RMSF (d), radial distribution function for the H-bond interactions inside the DNA (e) and the number of internal H-bonds of the DNA (f) during 200 ns MD simulations.

Moreover, to study the possibility of the hydrogen bond (H-bond) formation between base pairs inside the DNA structure, radial distribution function (RDF) analysis was applied. Figure 2-e shows the probability of finding a pair of NH<sup>...</sup>N or NH<sup>…</sup>O in terms of the intermolecular distance. According to figure 2-e, the base pair of DNA can form two types of H-bonds, in which NH<sup>…</sup>O interactions are stronger than NH<sup>…</sup>N hydrogen bonds. The RDF plots show a sharp peak at 1.85 and 1.95 A°, for the NH<sup>…</sup>O and NH<sup>…</sup>N hydrogen bonds, respectively, which is in good agreement with the calculated distances in the crystal structure (NH<sup>…</sup>O= 1.87 A° and NH<sup>…</sup>N= 1.94 A°). H-bond interactions between base pairs have a key role in the structural stability of the DNA. The calculated number of H-bonds (Figure 2-f) along the simulation time reveals that base pairs inside the DNA have considerable interactions with each other. Actually, due to strong H-bond interactions between base pairs, the DNA can maintain its B-conformation during the MD simulations.

#### Ionic liquid effects on the dynamics of DNA

To investigate the effect of ionic liquids on the DNA structure its dynamical behavior was analyzed in the presence of [BMIM][Ala], [BMIM][Gly], [BMIM][Val], [BMIM][Pro], and [BMIM][Leu] ionic liquids through 200 ns MD simulations. A high concentration of ionic liquids increases the viscosity of the solution, which can have important effects on the flexibility and dynamics of the DNA. Therefore, MD simulations were performed in the water-ionic liquid mixtures with 10 to 50 wt% concentration.

Figure 3 shows the obtained DNA structures in the presence of various ILs after 200 ns MD simulations. According to this figure [BMIM]<sup>+</sup> cation makes

complexes with the minor and major grooves of the DNA and anions lie around the DNA due to the H-bond interactions. Structural analysis reveals that [BMIM]<sup>+</sup> cation prefers the minor groove of the DNA because at the lower concentration [BMIM]<sup>+</sup> first occupies the minor groove. Then by increasing the IL concentration, this cation interacts with DNA from the major groove. Moreover, due to the considerable interactions between anions of ILs and Na<sup>+</sup> the condensation of Na<sup>+</sup> reduced around the DNA backbone (Figure 3). For example, in the presence of 50 wt% of [BMIM][Val] and [BMIM][Ala] ionic liquids, there is no Na<sup>+</sup> in the first solvation shell of the DNA.

The calculated RMSD and Rg values of DNA in the presence of different ILs indicate that ILs have remarkable effects on the stability of the DNA structure (Figures S2 and S3). According to Figure 4-a, the calculated average values of RMSD for the DNA in water-IL mixtures are lower than 2 Å, which is smaller than that of in the water. This result indicates that ILs elevate the structural stability of the DNA in comparison with water. Based on the calculated RMSD values, there is no logical trend between the concentration of ILs and the structural stability of the DNA. For example, DNA has a greater fluctuation in the presence of 10 wt% of [BMIM][Pro], while this behavior is observable in the presence of 50 wt% of [BMIM][Ala]. The calculated RMSD values are too small and confirm that in the presence of ILs the DNA structure has considerable stability.



Figure 3. The obtained structures of DNA in various water-ionic liquid mixtures after 200 ns MD simulations. The [BMIM]<sup>+</sup>, [Ala]<sup>-</sup>, [Gly]<sup>-</sup>, [Leu]<sup>-</sup>, [Pro]<sup>-</sup> and [Val]<sup>-</sup> ions are shown in gray, orange, blue, cyan, tan and silver colors, respectively.

A comparison between the calculated Rg values for the DNA in the presence and absence of ILs reveals that ILs increase the compaction of the DNA structure. According to Figure 4-b, the DNA structure has the minimum and maximum compaction in the presence of [BMIM][Gly](40 wt%) and [BMIM][Leu](50 wt%), respectively. Based on the Rg analysis ILs decrease the DNA fluctuations, in other words, ionic liquids can increase the structural stability of DNA in comparison with water. Because of small differences, a conclusion based on the calculated RMSD and Rg values cannot show meaningful results, therefore other analyses were applied.





ILs can penetrate into the solvent shell around the DNA and increase its stability due to interaction with DNA structure. Figure 4-c shows the calculated solvent accessible surface area (SASA) of the DNA in water and different ILs. This figure clearly shows that ILs reduce the SASA of the DNA. Moreover, the trend of the calculated SASA is according to the calculated Rg values. For example, in the presence of [BMIM][Leu] (40 and 50 wt%) the DNA structure is more accessible by water molecules in comparison with other ILs, therefore DNA structure has a greater Rg in the presence of this ionic liquid. This means that water-DNA interactions can decrease DNA compaction and make the DNA unstable, while ILs by pressing the DNA elevate the intermolecular interactions and DNA stability.

The calculated number of internal H-bonds of the DNA in water and different water-IL mixtures confirms that ILs can improve the internal interactions of the DNA (Figure 4-d). ILs make DNA stabilize by increasing the internal H-bond interactions, therefore DNA has greater stability in the presence of the ILs in agreement with Rg and RMSD results. The calculated RMSF values (Figure S4) indicate that ILs change the flexibility of base pairs inside the DNA. According to Figure S4, the concentration and anion of the ILs have important effects on the flexibility of the base pair. Terminal base pairs of the DNA have greater fluctuations in the presence of 10 wt% of [BMIM][Ala] compared to water. Besides, the central part of the DNA in mixtures of [BMIM][Leu] (20 wt%) and [BMIM][Pro] (30 wt%) shows more stability than other water-IL mixtures. Based on RMSF results central base pairs have lower fluctuation in comparison with the

terminal residues in water, while according to Figure S4 central and terminal residues of the DNA in the presence of 40 and 50 wt% of ILs have approximately similar flexibility. In other words, the ILs by elevating the structural compaction reduce the base pair flexibility, therefore DNA is more stable in ILs than in water.

### Ionic liquid interactions with the DNA structure

Structural analysis revealed that DNA has a more stable structure in IL-water mixtures in comparison with pure water. Therefore, analyzing the IL interactions with the DNA structure can provide interesting results from the molecular viewpoint to elucidate the effects of ILs on the stability of the DNA structure. The calculated average number of contacts (cutoff 5 Å) between ILs (cations and anions) and the DNA structure shows that ILs have strong interactions with the dodecamer structure (Figure 5-a). Furthermore, by increasing the concentration of the ILs the interaction between ILs and DNA elevates, which is due to the entrance of ILs into the solvation shell of the DNA. Based on the calculated values of contacts, [BMIM][Leu] has more contacts with the DNA among the other ILs. Due to the considerable IL contacts with DNA, structural analysis shows that [BMIM]<sup>+</sup> cations can occupy the DNA minor groove. This cation entered the minor groove and bound to the groove without disturbing the doublehelical structure of DNA.



Figure 5. The calculated average values of the number of contacts (a), distance between ILs and the DNA (b), the number of H-bonds (c), average EIE (d), and average vdW interaction energy of the IL interactions with the DNA structure (e) and the number of ILs around the structure of DNA (cutoff 5 Å).

Even in the presence of 10 wt% of ILs at least three cations occupy the minor groove of the DNA. Figure 5-b shows the calculated average distance between the ILs and the structure of the dodecamer. According to this figure by increasing the concentration of ILs their distance from the DNA decreases confirming that ILs can enter into the first solvation shell of the DNA. In other words, ILs and water molecules compete with each other to interact with the DNA structure. In comparison with water molecules, cations of the ILs have greater interaction with the negatively charged DNA structure and the minor and major grooves, which are appropriate places for the H-bond interactions.

H-bond interaction is one of the important factors that has key role effects on the structural stability of the DNA. The cation and anions of the studied ILs are able to form H-bonds with the backbone and grooves of the DNA. According to Figure 5-c, the [BMIM][Gly] has greater H-bond interactions with the DNA in comparison with other ILs. Based on the structural analysis there is a considerable number of H-bonds between the DNA grooves and IL cations, including the guanine (O and N atoms) and thymine (O atoms) of the DNA as the acceptors and the carbon atoms in the [BMIM]<sup>+</sup> ring as donors. The carbon atoms in the ring of the cation have a perpendicular direction relative to the groove. In this orientation, the C-H bond of the cation ring forms H-bond with the nucleotides. Based on the obtained results the cation has more H-bond interactions with adenine and cytosine in comparison with guanine in this direction. According to Figure 5-c, [BMIM][Ala] and [BMIM][Gly] have a greater number of H-bonds with the DNA structure than other ILs. This result can be explained due to the smaller size of the anion of the ILs because these anions can better enter into the solvation shell and make H-bonds with the DNA in comparison with [Leu]<sup>-</sup>, [Pro]<sup>-</sup> and [Val]<sup>-</sup> anions.

The calculated interaction energies using the linear interaction energy (LIE) method show that the electrostatic and van der Waals (vdW) interactions are a combination of hydrophobic and polar interactions between the ILs and the backbone and grooves of the DNA, which contribute to the DNA stability. Figure 5-d shows the calculated average electrostatic interaction energy (EIE) values between the corresponding ILs and DNA structure. According to this figure, [BMIM][Gly] has the maximum EIE with the DNA among the studied ILs, which is a result of the greater H-bond interactions between this IL and the dodecamer. Based on Figure 5-d, irrespective of the IL concentration, [BMIM][Gly] and [BMIM][Ala] have greater electrostatic interactions with the DNA than other ILs, because a certain amount of the ILs enter into the grooves and solvation shell of the DNA. Since [Leu]<sup>-</sup> and [Val]<sup>-</sup> anions have hydrophobic side chains in comparison with [Gly]<sup>-</sup> and [Ala]<sup>-</sup> anions, the [BMIM][Leu] and [BMIM][Val] ionic liquids have lower electrostatic interactions with the DNA.

The calculated vdW interaction energy (Figure 5-e) reveals that [BMIM][Gly] and [BMIM][Pro] have the minimum and maximum vdW interactions with the DNA, respectively, which is in contrast to the electrostatic interaction energy analysis. This result clearly shows that the strength of the IL interactions with DNA depends on the nature of the anions of the ILs. Moreover, the calculated values of the electrostatic and vdW interactions reveal that electrostatic interactions contribute more to the stability of the DNA than vdW interactions. Due to the strong electrostatic interactions between ILs and the DNA, ILs can increase the stability of the dodecamer structure. For example, RMSF analysis (Figure S4) revealed that the DNA structure has the maximum stability in the presence of [BMIM][Gly] (50 wt%), which this stability can be due to the considerable electrostatic interactions (Figure 5-d) between this IL and the DNA.

Hydration has important effects on structural stability, dynamical behavior and the form of the double helix of the DNA. To study the hydration properties of the DNA in the IL-water mixtures the distribution of ILs around this structure was analyzed. Figure 5-f shows the calculated number of ILs around the structure of DNA (cutoff 5 A). According to this figure, there are more [BMIM][Gly] in the hydration shell of DNA, which confirms that this IL has greater interactions with the DNA structure in comparison with the other ILs. As the concentration of ILs increases the IL population elevates in the solvation layer. In other words, ILs enter the solvation shell of the DNA and push away the water molecules from the DNA surface (Figure S5). It is well worth mentioning that the trend of the calculated number of ILs around the DNA structure is in agreement with the calculated EIE values. This result indicates that [BMIM][Gly] and [BMIM][Ala] ionic liquids have more contribution to the stability of the DNA, through strong electrostatic interactions. Because the ions of the corresponding ILs can better enter into the solvation shell of the DNA. Irrespective of IL concentration the order of ILs in the solvation shell of the DNA is as follows: [BMIM][Gly]>

[BMIM][Ala]> [BMIM][Pro]> [BMIM][Val]> [BMIM][Leu], which confirms that the type of anions of IL can have a considerable role on the hydration properties of biomolecules.

Trajectory analysis shows that a considerable amount of water molecules lies in the spine of the DNA. In the presence of ILs the probability of finding water molecules in the spine of DNA decreases. In other words, the cation of ILs disturbs the spine of hydration by entering into the DNA minor groove. Figure 6a clearly shows that [BMIM]<sup>+</sup> cation competes with water molecules to lie in the spine of the DNA. Besides, structural analysis confirms that at least 4 or 5 [BMIM]<sup>+</sup> cations can be found in the spine of the DNA in the presence of 50 wt% of ILs. To further analyze the effect of ILs in the solvation shell of DNA the number of water molecules that were stripped from the DNA solvation shell was calculated.

According to Figure 6-b, by increasing the concentration of ILs the number of water molecules around the DNA structure reduces. Based on the calculated values, 50 wt% of ILs, can strip about 35-45% of water molecules from the DNA structure. Therefore, the obtained results confirm that [BMIM][Gly] and [BMIM][Leu] ionic liquids have the maximum and minimum effects on the solvation shell of the DNA, which is in agreement with the EIE analysis.



Figure 6. The water molecules and [BMIM]<sup>+</sup> cations in the spine of the DNA (a) and the frequency of observed water molecules in the solvation shell of the DNA (b). The structures were obtained in pure water and IL-water mixtures (50 wt%) after simulation time)

To understand the distribution of cation and anion of the ILs around the DNA structure RDF analysis was applied. Figure S6 shows the calculated RDF plots for the cation and anion of ILs with the O2 and N3 atoms of the base pairs. According to this figure, [BMIM]<sup>+</sup> cation has more interactions with the DNA structure in comparison with the anion of the ILs. In addition, the quality of the obtained RDF peaks at almost the same distances reveals the intrusion of the ILs into the DNA grooves. It can be concluded that ILs due to strong electrostatic and

vdW interactions can increase the stability of the DNA structure by entering into the solvation shell of the DNA structure. Furthermore, the type of anions of ILs can be essential for the stability of the DNA structure. However, the cation of ILs plays a more crucial role because the cation has stronger interactions than the anion with the dodecamer structure.

### **Binding energies of the IL-DNA complexes**

To have a quantitative insight into the IL interactions with the DNA structure, Gibbs binding energies were calculated using the MM-PBSA method. Based on the obtained results the DNA structure remains its B-conformation in the presence of different ILs, which is due to the considerable IL interactions with the backbone and grooves of the DNA.

The calculated values of electrostatic ( $\Delta E_{ele}$ ) and van der Waals ( $\Delta E_{vdW}$ ) interactions confirm that electrostatic interactions have more effects on the stability of the DNA structure in comparison with vdW interactions (Table 2). According to the calculated Gibbs binding energy in the gas phase ( $\Delta G_{gas}$ ), the complex formation between ILs and the DNA structure is favorable from the thermodynamic viewpoint. Moreover, [BMIM][Ala] ionic liquid makes the most stable complex with the DNA among the studied ILs in the gas phase. Based on the structural analysis the [BMIM]<sup>+</sup> cation lies in the grooves of the DNA irrespective of the anion type, while the calculated values of  $\Delta G_{gas}$  reveal that anions can be important in the strength of the IL interactions with the double-

helical structure.

Table 2. The calculated contributions of the energy components (kcal.mol <sup>-1</sup> ) of the IL interactions with the DNA.												
ILs	$\Delta E_{vdW}$	$SEM^*$	$\Delta E_{ele}$	SEM	$\Delta G_{gas}$	SEM	$\Delta G_{sol}$	SEM	$\Delta G_{tot}$	SEM		
[BMIM][Ala]												
10 wt%	-89.68	2.22	-2696.03	116.33	-2785.72	119.14	2762.90	117.45	-22.81	1.85		
20 wt%	-155.36	3.09	-4305.69	114.06	-4461.05	116.55	4435.33	115.62	-25.71	1.49		
30 wt%	-154.43	2.50	-3199.87	99.17	-3362.55	100.29	3354.31	98.58	-8.24	2.35		
40 wt%	-121.32	3.60	-2331.61	95.15	-2462.15	96.07	2452.94	97.58	-9.20	1.85		
50 wt%	-373.25	1.66	-6998.20	80.80	-7408.59	79.23	7398.23	80.72	-10.36	2.68		
[BMIM][Gly]												
10 wt%	-81.65	2.76	-2680.36	103.67	-2762.01	105.88	2736.96	104.62	-25.08	1.49		
20 wt%	-127.56	3.18	-3226.17	108.02	-3353.74	109.89	3347.76	108.61	-5.97	1.74		
30 wt%	-159.01	3.11	-2984.05	135.73	-3157.91	135.95	3143.06	137.43	-14.84	1.95		
40 wt%	-290.50	3.59	-6226.38	81.24	-6516.88	82.84	6511.54	81.76	-5.34	1.88		
50 wt%	-373.19	2.13	-5435.28	72.87	-5912.34	72.91	5903.00	73.92	-9.34	2.51		
[BMIM][L	eu]											
10 wt%	-81.13	2.17	-2449.81	81.68	-2530.61	83.08	2511.59	81.88	-19.01	1.47		
20 wt%	-95.65	2.39	-2318.22	88.32	-2413.88	89.98	2403.91	88.95	-9.96	1.39		
30 wt%	-181.42	2.51	-5443.42	111.09	-5624.84	113.11	5598.99	112.80	-25.84	1.39		
40 wt%	-169.78	2.62	-4605.02	99.81	-4787.82	101.46	4774.80	101.84	-13.01	1.85		
50 wt%	-225.82	3.74	-4804.88	134.28	-5042.31	134.09	5030.71	136.67	-11.60	3.06		
[BMIM][Pi	ro]											
10 wt%	-114.61	2.21	-3002.10	92.60	-3116.71	94.00	3098.85	93.04	-17.44	1.33		
20 wt%	-150.73	1.33	-4681.90	86.78	-4832.63	87.46	4784.98	86.76	-47.65	1.19		
30 wt%	-89.51	3.45	-1483.37	89.75	-1579.61	91.13	1572.89	92.44	-6.72	1.65		
40 wt%	-161.40	2.42	-4098.14	130.42	-4267.53	130.91	4259.55	132.44	-7.98	1.24		
50 wt%	-343.33	1.15	-6309.44	57.49	-6675.51	56.30	6652.77	57.97	-22.73	3.36		
[BMIM][V	al]											
10 wt%	-117.33	1.00	-3756.52	64.27	-3873.85	64.76	3841.38	64.34	-32.47	1.03		
20 wt%	-154.41	1.77	-5020.67	50.58	-5175.08	51.51	5135.37	51.29	-39.70	1.11		
30 wt%	-191.24	1.81	-4502.43	62.01	-4693.67	62.78	4685.65	61.79	-8.02	1.47		
40 wt%	-176.09	4.17	-4692.82	134.15	-4870.91	137.72	4867.05	136.35	-3.86	2.00		
50 wt%	-242.77	1.94	-6049.00	71.40	-6291.78	72.20	6286.43	71.34	-5.34	2.04		
*SEM= Sta	*SEM= Standard Error of Mean											

In contrast to the gas phase, the IL-DNA complexation is not favorable in the solution phase ( $\Delta G_{sol}$ ) thermodynamically ( $\Delta G$ > 0). In other words, water molecules reduce the IL interactions with the DNA, since they compete with ILs for H-bond formation with DNA base pairs. The calculated total Gibbs binding energy ( $\Delta G_{tot}$ ) confirmed that the concentration and anion type of ILs are important factors in the stability of the IL-DNA complexes. Figure 7 shows the

calculated  $\Delta G_{tot}$  versus concentration of different ILs. According to this figure, it can be claimed that by increasing the concentration of ILs the stability of the IL-DNA complexes reduces. Because of the high concentration of ionic liquids the density of [BMIM]<sup>+</sup> cation on the surface and solvation shell of the DNA increases, which can elevate the repulsion interactions between the bound cation in the grooves of the DNA. Moreover, Figure 7 shows that the [BMIM][Pro] (20 wt%) and [BMIM][Val] (40 wt%) complexes with the DNA have maximum and minimum stability in comparison with other IL-DNA complexes. The calculated results by the MM-PBSA method are in agreement with the results of LIE analysis, both methods confirmed that electrostatic interactions between ILs and the DNA have a key role in the stability and structural properties of the DNA. Moreover, both cations and anions have effects on DNA stability but cations play a more important role.



Figure 7. The correlation between the concentration of the ILs and the calculated  $\Delta G_{tot}$  (kcal.mol<sup>-1</sup>) of the IL-DNA complexes.

#### Quantum chemistry aspects of the IL-DNA interactions

MD simulations revealed that IL can increase the stability of the DNA structure due to strong interactions with the base pairs in comparison with water molecules. Moreover, [BMIM]<sup>+</sup> cation has more effects on the stability of the double-helical structure than anions. Therefore, quantum chemistry calculations were applied to investigate the nature and strength of the cation interactions with the DNA structure. In the DFT and DFT-D3 calculations, the complexation process between [BMIM]<sup>+</sup> cation and AATT (A-T) and CGCG (C-G) base pairs were investigated.

The optimized structures (Figure S7) of the base pair complexes with the cation show that the cation has a stable position in the minor groove of the DNA, which is according to the MD simulation results. The calculated thermodynamic parameters such as binding energy ( $\Delta E$ ) and Gibbs binding energy ( $\Delta G$ ) of the base pair complexes with the [BMIM]<sup>+</sup> cation confirm that DNA forms a stable complex with this cation and the complexation process is favorable from the thermodynamic viewpoint ( $\Delta G < 0$ ). According to Table 3, dispersion interactions between this cation and the base pairs have a remarkable role in the stability of the IL-DNA complexes since the calculated thermodynamic parameters by the DFT-D3 method show greater stability for the corresponding complexes in comparison with the DFT method. Moreover, based on the calculated values by

both	methods	the ca	ation 1	makes	a mo	re	stable	complex	with	the	C-G	base	pairs
than	A-T.												

Table cation	<ol> <li>The cal</li> </ol>	culated the	ermodyn	amic para	meters an	d stabili	zation end	ergy of the	e base pair complexe	s with the [BMIM] <sup>+</sup>
		DFT-D3			DFT			ersion	$\sum E(2)$	$\sum E(2)$
	(kcal.mol <sup>-1</sup> )			(kcal.mol <sup>-1</sup> )			Energy		(kcal.mol <sup>-1</sup> )	(kcal.mol <sup>-1</sup> )
	-ΔΕ	-ΔG	$E_{BSSE}$	<b>-</b> ΔE	-ΔG	$E_{BSSE}$	$-\Delta E_{dis}$	$-\Delta G_{dis}$	[BMIM] <sup>+</sup> →DNA	$DNA \rightarrow [BMIM]^+$
A-T	258.61	238.87	12.30	209.87	195.18	12.30	48.74	43.68	12.27	45.24
C-G	263.74	248.29	14.07	227.08	209.57	14.07	36.65	38.72	11.61	57.02

To determine the nature of the cation interactions with the DNA, NCI analysis was applied at the BP86-D3/6-31G(d) level of theory. By employing this method, it is possible to determine the attractive and repulsive interactions based on the electron density  $(\rho)$  and the sign of the second derivative in the perpendicular direction of the bond ( $\lambda_2$ ). The sign of  $\lambda_2$  can be either negative or positive depending on the type of interaction. The positive and negative values of  $\lambda_2$  show the non-bonding (steric repulsion) and bonding (H-bond) interactions, respectively, while the negligible values of  $\lambda_2$  ( $\lambda \approx 0$ ) reveal the vdW interactions. Figure 8-a shows the calculated 2D NCI plots of the C-G and A-T base pairs and their complexes with the [BMIM]<sup>+</sup> cation. These plots show the reduced density gradient (RDG) versus the electron density. Figure 8-a shows three definite regions, which are specified by blue, red and green colors indicating H-bond, repulsion and vdW interactions, respectively. The 2D NCI plot of the C-G base pairs shows a greater aggregation of blue dots in the negative region ( $\lambda_2 < 0$ ), which indicates that C-G base pairs have more internal H-bonds in comparison with A-T base pairs. Moreover, the peaks of the RDG at  $\lambda_2 \approx$  [-0.05,-0.04] au in the plot of C-G confirm strong internal H-bonds between these base pairs.

The comparison between 2D NCI plots of the base pairs in the presence and absence of [BMIM]<sup>+</sup> cation reveals that the cation has considerable vdW interactions with the surface of DNA. In other words, the corresponding graphs show the aggregation of green dots in vdW regions in the presence of [BMIM]<sup>+</sup> cation, which confirms that this cation through the vdW interactions contributes to the stability of the DNA. Moreover, the extent of the aggregation of green dots about  $\lambda_2 \approx$  -0.02 au indicates that [BMIM]<sup>+</sup> cation have greater vdW interactions with the C-G than A-T base pairs.



Figure 8. The calculated plots of 2D (a) and 3D (b) NCI for the C-G and A-T base pairs and their complexes with the  $[BMIM]^+$  cation at the BP86-D3/6-31G(d) level of theory.

To have a better insight into the [BMIM]<sup>+</sup> cation interactions with the DNA surface, a 3D NCI plot is also represented in Figure 8-b. This figure shows the bonding, vdW and non-bonding interactions by blue, green and red color-filled isosurfaces, respectively. According to Figure 8-b, the dark blue isosurfaces between CO and NH groups of the base pairs confirm strong internal H-bonds. Moreover, the green isosurfaces between the base pairs clearly show the  $\pi$ stacking inside the DNA structure. 3D NCI analysis indicates that [BMIM]<sup>+</sup> cation has both vdW and H-bond interactions with the DNA surface. The dark blue and green isosurfaces confirm H-bond and vdW interactions between C-H (ring of the cation) and oxygen atoms of the base pairs and ring and chain of the cation with the surface of the DNA, respectively. Moreover, Figure 8-b shows that the cation ring has more vdW interactions with the C-G base pairs compared to A-T. This result is in agreement with the calculated thermodynamic parameters. In other words, [BMIM]<sup>+</sup> cation makes the most stable complex with the C-G region of the DNA through strong vdW interactions. NCI analysis confirmed that IL can increase the stability of the DNA through electrostatic (Hbond) and vdW interactions with the surface of the double-helical structure, which is in agreement with the MD simulation results. The obtained results are in good agreement with the previously reported studies. Rezki and coworkers reported that hydrogen bond formation and hydrophobic interactions between DNA and IL are responsible for IL-DNA complexation process (Rezki et al., 2020; Al-Sodies et al., 2020). On the other hand, the reported results by Celik et

al. confirmed that there are considerable H-bond interactions between the DA5 and DA6 residues of DNA and the ionic liquid (Celik et al., 2018; Celik et al., 2019). Moreover, based on the quantum chemistry calculations the cation of the IL forms a more stable complex with the C-G base pairs than the A-T base pairs, which is in good agreement with the reported results by Hodyna and coworkers (Hodyna et al., 2022).

To study the charge transfer, donor-acceptor and molecular orbital interactions between the [BMIM]<sup>+</sup> cation and the DNA structure stabilization energy, E(2), was calculated by employing NBO analysis at BP86-D3/6-31G(d) level of theory. According to the obtained results, the main interactions are related to the lone pair electrons of nitrogen and oxygen atoms of the base pairs as donors and antibonding orbital of C-H ( $\sigma^*_{CH}$ ), C=C ( $\pi^*_{CC}$ ) and C=N ( $\pi^*_{CC}$ ) bonds as acceptors. According to the calculated  $\Sigma E(2)$  values for the [BMIM]<sup>+</sup> cation interactions with the DNA ([BMIM]<sup>+</sup> $\rightarrow$ DNA, cation as the donor and the DNA as acceptor) the cation has more interactions with A-T in comparison with the C-G base pairs. This result reveals that in the presence of the cation the A-T base pairs are better acceptors than C-G. On the other hand, the calculated  $\Sigma E(2)$  for the  $DNA \rightarrow [BMIM]^+$  interaction (DNA as donor and cation as acceptor) indicates that there is a greater charge transfer from the C-G to the cation than A-T base pairs. Overall, based on the calculated electronic interaction energies it can be concluded that [BMIM]<sup>+</sup> cation prefers to form a complex with the DNA from the C-G region due to greater molecular orbital interactions, which is according to the previous results. Moreover, molecular orbital and donor-acceptor interactions may be considered the driving force for the IL-DNA complex formation.

# Conclusion

Molecular dynamics simulations and quantum chemistry calculations were applied to investigate the effect of amino acid ionic liquids on the stability and dynamical behavior of DNA structure. Based on the obtained results, DNA structure has more stability in the presence of ionic liquids in comparison with pure water. The structural analysis confirmed that [BMIM]<sup>+</sup> cation lies in the minor and major grooves of the DNA and anions of the ILs distribute around the backbone of the dodecamer structure. Moreover, the DNA structure in the presence of [BMIM][Gly] (50 wt%) has the maximum structural stability in comparison with other ionic liquids and pure water. The cation and anions of the studied ILs are able to form H-bonds with the backbone and grooves of the DNA. According to the obtained results, the [BMIM][Gly] has greater H-bond interactions with the DNA than other ILs, irrespective of the IL concentration. Because a certain amount of the ILs enter into the grooves and solvation shell of the DNA, therefore this IL has the maximum electrostatic interactions with the DNA. On the other hand, the distribution of ILs in the hydration shell of the DNA reveals that there are more [BMIM][Gly], which confirms that this IL has greater interactions with the DNA structure in comparison with the other ILs. ILs due to strong electrostatic and vdW interactions with the DNA structure contribute to the stability of the double-helical structure. It is well worth mentioning that the type of anions of ILs can be important in the stability of the DNA structure. However, the cation of ILs plays a more crucial role because the cation has stronger interactions than the anion with the dodecamer structure. The DFT-D3 calculations indicated that the ring of the cation has more vdW interactions with the C-G base pairs in comparison with A-T. In other words, the [BMIM]<sup>+</sup> cation makes a more stable complex with DNA from the CGCG region than AATT base pairs. Moreover, donor-acceptor interaction analysis confirmed that molecular orbital interactions may be considered the driving force for the IL-DNA complex formation. The results of this study can provide new insight into the ILs interactions with DNA from the molecular viewpoint.

# **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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