5-3-2018

Effects of Ionic Liquids on Nucleic Acid Secondary Structures

Miranda L. Shaine
The College at Brockport, miranda.shaine2@gmail.com

Follow this and additional works at: https://digitalcommons.brockport.edu/honors

Part of the Chemistry Commons

Repository Citation
https://digitalcommons.brockport.edu/honors/214

This Honors Thesis is brought to you for free and open access by the Master’s Theses and Honors Projects at Digital Commons @Brockport. It has been accepted for inclusion in Senior Honors Theses by an authorized administrator of Digital Commons @Brockport. For more information, please contact kmyers@brockport.edu.
Effects of Ionic Liquids on Nucleic Acid Secondary Structures

A Senior Honors Thesis
Submitted in Partial Fulfillment of the Requirements
for Graduation in the Honors College

By:
Miranda L. Shaine
Chemistry Major

The College at Brockport – State University of New York
May 3, 2018

Thesis Director:
Dr. Joshua Blose, Assistant Professor, Department of Chemistry and Biochemistry

Educational use of this paper is permitted for the purpose of providing future students a model example of an Honors senior thesis project.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>3</td>
</tr>
<tr>
<td>Abstract</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>8</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>10</td>
</tr>
<tr>
<td>Conclusion</td>
<td>15</td>
</tr>
<tr>
<td>References</td>
<td>16</td>
</tr>
<tr>
<td>Figures</td>
<td>18</td>
</tr>
</tbody>
</table>
Acknowledgements

I would like to thank my research advisor, Dr. Joshua Blose, for all his investment in me over the past two years. Without Dr. Blose’s commitment to success and support, I would not have achieved as much as I have while at Brockport. Thank you for your dedication to this discipline and guiding me throughout these years.

Thank you to Department of Chemistry and Biochemistry at The College at Brockport for giving me the opportunity and chemical foundation to perform this biochemistry research. This experience has molded me to be a better student, for today and in the future.

Finally, I would like to thank my family and most importantly my mother for supporting me through this experience and pushing me to become a better individual.
Abstract

The effects of ionic liquids (ILs) on DNA are not well understood. Ionic liquids are important to the chemistry of DNA because they could serve as a long-term storage buffer for DNA, contribute to the use of DNA in nanotechnology and sensors, and influence the use of DNA as a platform for designing catalysts. In this research, imidazolium chloride ionic liquids with varying alkyl chain lengths were tested for their effects on the stability and structure on a representative DNA duplex. Thermal denaturation and ionic liquid titration experiments using Circular Dichroism spectroscopy were performed to assess the stability and conformation of DNA duplexes. Results of the experiments suggested that the imidazolium ionic liquids were interacting directly with the DNA, altering its structure and causing it to precipitate out of solution by the end of our titrations. This behavior was dependent on both the alkyl chain length of the ionic liquid as well as the concentration of the DNA and suggests that precipitation of the DNA comes after ionic-liquids are saturating DNA interaction sites.
Introduction

Deoxyribonucleic acid (DNA) is made up of phosphate groups, sugars, and nitrogenous bases. The macromolecule occurs naturally in the form of a right-handed double helical structure with the bases projecting into the helix. The double helix is stabilized by Watson-Crick hydrogen bonding, stacking interactions, and charge-charge interactions. The nucleotide base pairs for DNA are guanine and cytosine (G-C) and adenosine and thymine (A-T). A-T base pairs stabilize the helical structure less than the G-C base pairs due to only two hydrogen bonds linking A and T, compared to three hydrogen bonds linking G to C. The hydrophobic effect, which is the burying of the hydrophobic bases in the interior of the helix while the hydrophilic sugar and phosphate groups are on the exterior interacting with water, helps stabilize the DNA.

In addition, the stacking of the bases induces Van der Waals forces which also stabilize the structure as the number of interactions increases. The negatively charged phosphate groups are shielded by a positive cation, which further stabilizes the structure.

Nucleic acids are very sensitive to the solution that they are in and generally fall under three classes: A-, B-, or Z-form. RNA is typically found in the A-form and DNA is almost solely found in the B-form. DNA folds to the A-form when under dehydrating conditions such as crystal formation. The formation of Z-DNA is unfavorable; however, alternating purine-pyrimidine sequences and high salt conditions or cations may drive the DNA to Z-form. Despite being reasonably stable in aqueous solution, the DNA B-form helix can denature at non-physiological temperatures and pH, and varying ionic strength.

Co-solutes and osmolytes can also affect the stability of the structure. Co-solutes interact with the nucleic acid while it is unfolding and can be used as quantitative probes of structural conformational changes. The more ions bound to the DNA has been studied to have a direct effect on the stability of the DNA. Similarly, in this experiment, DNA-IL interactions are
studied. An ionic liquid is a salt in its liquid state, with a melting point less than 100 °C, and made up of ions. Ionic liquids are unique due to their near-zero vapor pressure, low flammability, good thermal conductivity, and high ionic conductivity. These compounds have a relatively high melting temperature due to the lattice structures formed from the cations and anions. ILs are practical in the lab because they are generally inexpensive, can be recycled easily, and usually involve a simple reaction process.

Ionic liquids are mainly used for their benign environmental advantages compared to typical organic or highly acidic catalysts. Due to their varying hydrophilic and hydrophobic properties, ILs can be used for enzymatic reaction and long-term stability of nucleic acids. In previous studies, IL ions disrupted the water cage around DNA causing partial dehydration in the minor groove. This helps stabilize DNA for long-term because the partial dehydration may prevent the hydrolytic reactions that can denature DNA. Small molecule ionic liquids interact with DNA either by binding to the minor groove or by intercalating between base pairs. ILs can be alternative solvents for DNA binding molecular interactions. Thus, the thermodynamic parameters for these events must be understood.

In this experiment, ILs with imidazolium cations and chloride anions are used. In recent studies, imidazolium-based ILs were used for successful gene delivery. The ILs that are studied are 1-hexyl-3-methylimidazolium chloride ([C₆MIM][Cl]) and 1-hexadecyl-3-methylimidazolium chloride ([C₁₆MIM][Cl]), which differs only in the length of the alkyl chain. ([C₁₆MIM][Cl]) belongs to a much larger group called the long chain imidazolium ionic liquids (LILs) in which there are more than eight carbons in the alkyl chain. Imidazolium-based chloride ILs with varying alkyl chain lengths have been studied with calf thymus DNA by UV
absorption spectroscopy and fluorescence spectroscopy.\textsuperscript{11} According to the literature, as the chain length of the IL increased, so did the stability of the DNA structure.\textsuperscript{11}

Spectroscopic analysis of DNA is very useful in studying the macromolecule. DNA absorbs at a wavelength similar to UV light at around 260 nm due to the aromaticity of the bases. DNA denaturation can be identified measuring absorbance because a single strand of DNA will absorb more light than double-stranded DNA, which is known as the hyperchromic effect.\textsuperscript{12} Native, double-stranded DNA absorbs less because the nitrogenous bases are still left in-tact and stacked, thus, there is less exposure to light. We are using imidazolium chloride ILs with alkyl chain lengths of 6 and 16 carbons and using Circular Dichroism (CD) to determine the secondary structural conformations and observing melt curves to determine structural stability at different concentrations. Circularly polarized light results from two plane waves of equal amplitude but differing in phase by 90°. CD is the difference in the absorption of left-handed circularly polarized light (L-CPL) and right-handed circularly polarized light (R-CPL) and is detected when a molecule contains at least one chiral group, as seen in Figure 1. In this research, CD is used to look at the B-form conformation of DNA and its variability upon changing solution conditions. A characteristic B-form DNA CD signal has a positive peak around 280 nm and a negative peak around 250 nm. CD signal is very dependent on the nucleic acid sequence and the solution conditions. DNA with more A-T base pairs are portrayed by a larger negative band around 245-250 nm.\textsuperscript{13} Therefore, changes in CD signal, especially at the two peak wavelength ranges, gives valuable information about the structural conformational changes of the DNA. Ionic liquid titrations and thermal DNA denaturation were performed to estimate the concentration of the IL at which it starts to crash out of solution to further understand the structure of the DNA under these conditions.
Materials and Methods

**Duplex Synthesis**

Integrated DNA Technologies performed solid phase synthesis to synthesize the DNA sequences studied in these experiments:

- 5'- GCT CAC TAT GTA GCG -3’ (labeled as DNA Strand, Purine 47)
- 5'- CGC TAC ATA GTG AGC -3’ (labeled as DNA Strand, Purine 53)

The solutions were diluted to 1 mM using 1X TE buffer for storage. The secondary structure of a DNA double helix is shown in Figure 2.

**Ionic Liquid Solution Preparation**

Varying concentrations of 1-hexyl-3-methylimidazolium chloride and 1-hexadecyl-3-methylimidazolium chloride were made in 2mM MES/MOPS pH 7.0 buffer.

**CD Full Scans**

CD spectra were recorded on a CD/ORD/LD Photomultiplier at 20 °C, 50 °C, and 80 °C using a rectangular quartz cell of 1 cm path length ranging from 330 nm to 200 nm at a bandwidth of 2.0 nm. Spectra shown were averaged over three successive scans and recorded at a scan speed of 5 sec/nm. Respective buffers were subtracted from each spectrum and the data were subjected to the noise reduction analysis.

**CD Melting Experiments**

Melting experiments ranged from 8 °C to 88 °C at 280 nm, at 1 °C per minute. Samples were prepared with 2 µL of each 1 µM DNA strand solution (3 µM DNA Duplex) and varying ionic liquid solution. The data was normalized by dividing each absorbance by the highest signal
recorded, factoring in the extinction coefficient, and respective buffers were subtracted from each spectra and the data were subjected to the noise reduction analysis.

**CD Titrations**

Titration experiments were performed at 280 nm at 20 °C. Data were averaged over ten successive wavelength scans and recorded at a scan speed of 10 sec/point. Titrations were performed with a fixed concentration of DNA duplex (3 µM and 6 µM) and increasing concentrations of [C₆MIM][Cl] and [C₁₆MIM][Cl]. The data was normalized by dividing each absorbance by the highest signal recorded, factoring in the extinction coefficient, and respective buffers were subtracted from each spectra and the data were subjected to the noise reduction analysis.
Results and Discussion

In Figure 3, the chemical structures of the two imidazolium chloride ionic liquids studied in this research are given. These ionic liquids are structurally similar but differ in the length of the alkyl chain on the imidazolium cation.

Figure 4 shows the full spectra of the DNA with increasing [C\textsubscript{16}MIM][Cl]. It can be seen that the addition of [C\textsubscript{16}MIM][Cl] IL has a significant impact on the intensity of the 280 nm peak, altering the structure upon each IL addition. However, the B-form conformation might generally be maintained since there is not observed shifts, but there are changes within that structure due to the IL.\textsuperscript{13,14} The decrease in CD signal at high IL concentration may be due to the strong interactions between the DNA and the ionic liquid cations, which causes the DNA to take up more space due to DNA-IL and duplex-duplex interactions.\textsuperscript{14} The time dependence of [C\textsubscript{16}MIM][Cl] in Figure 5 shows that the signal at 280 nm does not change over a 15 minute period. The data was put on a scale that is consistent to the scale of the full scans, depicting how the deviations would be displayed on the full scan spectrum. At 0 minutes, the signal fluctuates a little due to the nitrogen background equilibrating, but the signal is otherwise stable. Therefore, it is reasonable to say that changes in the DNA spectra in the presence of the IL do not have a kinetic component on the time scale of our measurements.

As heat is added to the DNA Duplex, there is a significant structural change in the full CD spectral scans, shown in Figure 6. These are depicted as “mini melts”. The lower amplitude of the peaks at 280nm and about 250nm suggests that there is disruption in the base stacking interactions and the helical structure of the DNA, respectively. Thus, the addition of heat without any ionic liquid denatures and changes the confirmation of the DNA. With the addition of the [C\textsubscript{6}MIM][Cl], the addition of heat also changes the structure of the DNA and the CD
signal. Therefore, heat denaturation is also still occurring with ionic liquid. The large amount of noise at about 240 nm is due to high absorbance, making subtraction difficult.

Figure 7 depicts the full CD spectral scans at constant temperature and increasing \([\text{C}_6\text{MIM}][\text{Cl}]\) concentration. Most strongly portrayed at 20 °C, the spectral scan of the DNA with no ionic liquid is significantly higher at 280 nm than with the \([\text{C}_6\text{MIM}][\text{Cl}]\) ionic liquid. The decrease in the peak represents a structural change in the DNA solely with ionic liquid added, not taking other parameters or factors in consideration. The ionic liquid changes the structure at various temperatures too, indicating that this is ionic liquid driven and also temperature dependent.

Similarly, in Figure 8, the full spectral scans of \([\text{C}_{16}\text{MIM}][\text{Cl}]\) at increasing temperature with various concentrations is depicted. Again, the change in the positive and negative peaks at 280 and 250 nm, respectively, indicate a structural change. Therefore, even at varying concentrations of both ionic liquids, temperature causes denaturation. In Figure 9, the longer chain ionic liquid also changes the structure of the DNA at constant temperature and increasing concentrations. More prominently seen with the \([\text{C}_{16}\text{MIM}][\text{Cl}]\) ionic liquid, a blue shift is observed, suggesting that there is some sort of change in state of the DNA. In comparison, we do not see a bathochromic change (red shift) which is particularly dominant for intercalators.\(^8\) We predict that there is more groove binding rather than intercalation due to the lack of red shift in our CD spectra with increasing concentration of IL. Interestingly at 50 °C, there a positive peak overlap of 100 µM with 0 µM and 50 µM \([\text{C}_{16}\text{MIM}][\text{Cl}]\) which reason is unknown at this time. At 80 °C, the scans and thus structures are practically the same, meaning hear could be an equalizer.
Melting of the DNA gives further information of the structure of the DNA with ionic liquid as it heats up and unfolds. In Figure 10, the starting points of all three [C_{16}MIM][Cl] concentrations are different; therefore, the DNA is coming from completely different structures initially. The 0 µM and 50 µM show a nice melting curve with a smooth transition at around 35°C. In the melt, we can also see the crossover of 100 µM at 280 nm which is consistent with the data in Figure 9. The reason for this overlap is unknown for the time being but it suggests a completely new state formed. For [C_{6}MIM][Cl], the melting curves are a little more difficult to produce. The curves at 70 and 100 mM are much less prominent than the melting curve with no ionic liquid. Tests were done to see how far out the concentration for the shorter strand IL would go. It seems that at 120 mM [C_{6}MIM][Cl], we are on the brink of the curve and the signal is not useful. For both ionic liquids, we can say that the melting temperature potentially increases with increasing IL concentration based on estimations; however, it is difficult to conclude due to different starting points and new state formations. It is important to note the difference in concentration of the [C_{6}MIM][Cl] and [C_{16}MIM][Cl] because it takes millimolar amounts and micromolar amounts, respectively. This is due to the different chain lengths and suggests that the IL stability is based on the number of binding sites.

Titrations at 280 nm were performed to understand the interactions occurring between the DNA and the IL in Figure 11. The [C_{16}MIM][Cl] titration was done at different concentrations of DNA. Upon addition of IL, there is a constant decrease in signal until the IL finally crashes out of solution at about 125 µM and 180 µM [C_{16}MIM][Cl] for 3.3 µM and 6.1 µM DNA duplex, respectively. Due to the need for more IL with more DNA to drive the solution to precipitation, it can be concluded that this transition is ionic liquid driven and not DNA driven. If it was DNA driven, it would take less IL to cause precipitation for more DNA. The crashing
out of solution can be seen from 0 to 100 µM [C_{16}MIM][Cl], about 50% of the signal has already been lost. Interestingly, there is a linear trend from the starting point to right before the crashing point of the addition of IL. Therefore, we can conclude that there is a direct relationship between the amount of IL added and the CD signal (structural change of the DNA). It is predicted that the ionic liquid binds to the grooves of the DNA until it is full saturated and crashes out of solution.

The [C_{6}MIM][Cl] titration at 280 nm in Figure 12 depicts a different story with the shorter alkyl chain, observing two transitions rather than one. The IL crashes out of solution at about 325 mM [C_{6}MIM][Cl] and particulates are seen in the cuvette. The two transitions pose an interesting question for us. According to previous research using [C_{4}MIM][Cl] and calf thymus DNA, low concentrations (<60 mM) of IL formed a coil-to-globule structure and higher concentrations (>60 mM) formed larger coil and globular structures eventually forming a micelle-like structure at 500 mM IL.\textsuperscript{14} These structures are given in Figure 13. The IL cations electrostatically associate around the negatively charged phosphates on the DNA and the IL hydrocarbon chains interact with the hydrophobic DNA bases to form DNA-IL complexes. Due to the limited number of the short four carbon alkyl chain on the cation of the IL, the hydrophobic tails are able to favorably interact with each other, forming a coil structure. Eventually with more IL added but not exceeding 60 mM, there is a globule structure formed due to increasing hydrophobic interactions of the tails. These electrostatic and hydrophobic forces alter the DNA in which it structurally transitions from coil-to-globule forms, both coexisting in solution. With higher concentrations of [C_{4}MIM][Cl] exceeding 60 mM, both the coil and globule structures grow in size due to the hydrophobic interactions of the added IL and the already DNA-IL complexes. At 500 mM IL, there is so much IL in the solution and all the
binding spots on the phosphates are taken up that the IL new interactions take place. Therefore,
a micelle structure is predicted to form with all the hydrophobic tails interacting with each other
and forming a hydrophobic center. This two-step transition seen with [C₄MIM][Cl] is what we predict we are seeing in the titration with [C₆MIM][Cl] because ours is also a short alkyl chain length with only two more carbons attached. Thus, the transition seen at about 50 mM we predict is the first structure forming and the second transition at about 300 mM is the second structure forming. Although the DNA Duplex used in this experiment is only 15 nucleotides long, about 1.5 turns of the double helix, there is evidence that the DNA aggregates and can stack on top of each other to form a longer strand.¹⁵ At the high concentration of [C₆MIM][Cl],
the formation of the larger coexisting coil and globule structures of the DNA and IL can potentially be related to the crashing out of solution once these form large macromolecule structures.

However, with the [C₁₆MIM][Cl] IL, we do not observe this two-step transition because the alkyl chain length is so much longer than the other IL that it is not favorable for the hydrophobic ails to interact. Therefore, the tails interact directly with the major and minor grooves of the DNA and wraps itself and intertwines itself around the DNA, seen in Figures 14 and 15. Once all the binding spots are taken and the DNA is considered fully saturated, that is when we see the particulates and it crashes out of solution. This conclusion is consistent with results from previous tests using TAT Triplex in which the third DNA strand was bound to the major groove of the helix. In that study, [C₆MIM][Cl] would crash out of solution at 280 mM, compared to 325 mM in these studies. This may be due to the lack of binding sites in the major groove and gives further reasoning to our stipulation.
Conclusion

Our results suggest that ionic liquids stabilize DNA by binding in the major and minor grooves and electrostatically associate around the negatively charged phosphates of the DNA. Both \([C_6\text{MIM}]\)[Cl] and \([C_{16}\text{MIM}]\)[Cl] change the DNA structure even at low temperatures. This is seen by the significant loss in signal at 280nm and 250nm. The stability of the DNA is dependent not only on the length of the alkyl chain of the ionic liquid, but also the concentration of the DNA. Our results reflect that this transition is not DNA driven, but ionic liquid driven, causing participate formation at high concentrations of IL. The melting temperature potentially increases with increasing concentration of the ionic liquids; however, the formation of a new state with \([C_{16}\text{MIM}]\)[Cl] must be further evaluated. The stability and states are reflected upon the amount of binding sites available in the DNA. The double transition of the \([C_6\text{MIM}]\)[Cl] titration suggests are connection to previous literature in which a coil-to-globule formation occurs at low concentrations of similar short-stranded alkyl chain length (\([C_4\text{MIM}]\)[Cl]) and a micelle-like structure at higher concentrations. For \([C_{16}\text{MIM}]\)[Cl] with the longer alkyl chain, the stability is dependent on the saturation limit and the amount of binding spots available. The initial association of DNA is seen only in \([C_6\text{MIM}]\)[Cl], but both generate precipitation states.

Future studies will utilize fluorescence tests to confirm the structures of the DNA and the inter-duplex interactions while in contact with these ionic liquids. This will aid in the understanding of unknown transitions in the melting data and grasp an idea of the structure of the DNA in the titration data. In addition, we will conduct similar tests as described in this Thesis using \([C_4\text{MIM}]\)[Cl] to try to reproduce the results in the literature. Computational studies and models of the DNA interacting with these ionic liquids may be performed in the future and can be utilized to predict DNA-IL interactions and conformational changes.
References


**Images**


(2) [http://upload.wikimedia.org/wikipedia/commons/thumb/6/6e/Chiral_molecules_example.svg/2231px-Chiral_molecules_example.svg.png](http://upload.wikimedia.org/wikipedia/commons/thumb/6/6e/Chiral_molecules_example.svg/2231px-Chiral_molecules_example.svg.png)
Figures

Figure 1. Circular Dichroism. Circular Dichroism occurs when a compound absorbs left-handed (LH) and right-handed (RH) circularly polarized light differently. Only chiral molecules display CD. Spectrum can be normalized by residue (amino acid or nucleotide).\textsuperscript{1,2}

Figure 2. Nucleic Acid Helical B-Form. The most common DNA form which is an extended right-handed double helix. The structure was generated using Discovery Studio Visualizer 4.5 (BioNova) with a solvent surface using a radius of 1.4 Å.
Figure 3. Ionic Liquid Structures. 1-hexyl-3-methylimidazolium chloride and 1-hexadecyl-3-methylimidazolium chloride. The molecular weights are listed below each structure.

![Chemical structures of 1-hexyl-3-methylimidazolium chloride and 1-hexadecyl-3-methylimidazolium chloride](image)

- 1-hexyl-3-methylimidazolium chloride: [C₆MIM][Cl]
  - Molecular weight: 202.79 g mol⁻¹
- 1-hexadecyl-3-methylimidazolium chloride: [C₁₆MIM][Cl]
  - Molecular weight: 343.00 g mol⁻¹

Figure 4. Example [C₁₆MIM][Cl] CD Spectra. Full Normalized CD spectra from 240 nm to 320 nm for 3.3 µM DNA Duplex in the absence of ionic liquid and the presence of increasing concentrations of [C₁₆MIM][Cl]. The loss of signal at 280 nm and 253 nm qualitatively suggests that the IL is changing the base-stacking interactions and the helical structure, respectively.

![CD spectra of [C₁₆MIM][Cl]](image)
Figure 5. Example [C_{16}MIM][Cl] Time Dependency Data. Normalized CD signal at 280 nm is plotted vs. time for 3.3 μM DNA Duplex at various [C_{16}MIM][Cl] concentrations. The signal does not fluctuate over time.

Figure 6. Example [C_{6}MIM][Cl] Full Spectrum CD Scan Data. Normalized CD signal from 240 nm to 320 nm is plotted for 3.3 μM DNA Duplex at various [C_{6}MIM][Cl] concentrations with increasing temperatures. The spectrum changes qualitatively suggests that heat is destabilizing the duplex.
Figure 7. Example [C₆MIM][Cl] Full Spectrum CD Scan Data. Normalized CD signal from 240 nm to 320 nm is plotted for 3.3 µM DNA Duplex at various temperatures with increasing [C₆MIM][Cl] concentrations. The spectrum changes qualitatively suggests that the ionic liquid is destabilizing the duplex.
**Figure 8.** Example [C\(_{16}\)MIM][Cl] Full Spectrum CD Scan Data. Normalized CD signal from 220 nm to 320 nm is plotted for 3.3 µM DNA Duplex at various [C\(_{16}\)MIM][Cl] concentrations with increasing temperatures. The spectrum changes qualitatively suggests that heat is destabilizing the duplex.
Figure 9. Example [C_{16}MIM][Cl] Full Spectrum CD Scan Data. Normalized CD signal from 220 nm to 320 nm is plotted for 3.3 µM DNA Duplex at various temperatures with increasing [C_{16}MIM][Cl] concentrations. The spectrum changes qualitatively suggests that the ionic liquid is destabilizing the duplex.

Figure 10. Example Melting Data. Normalized CD signal from 280 nm is plotted vs. temperature for 3.3 µM DNA Duplex at increasing [C_{16}MIM][Cl] and [C_{18}MIM][Cl] concentrations.
Figure 11. Example $[\text{C}_16\text{MIM}][\text{Cl}]$ Titration Data. Normalized CD signal at 280 nm is plotted vs. $[\text{C}_16\text{MIM}][\text{Cl}]$ concentration for 3.3 µM and 6.1 µM DNA Duplex. There is a linear relationship present for 3.3 µM and 6.1 µM ($R^2 = 0.9922$, 0.9745, respectively) before the crash point is reached. The signal changes observes one phase transition and qualitatively suggests that the ionic liquid is altering the structural conformation of the duplex.

Figure 12. Example $[\text{C}_6\text{MIM}][\text{Cl}]$ Titration Data. Normalized CD signal at 280 nm is plotted vs. $[\text{C}_6\text{MIM}][\text{Cl}]$ concentration for 3.3 µM DNA Duplex. The signal changes observes two phase transitions and qualitatively suggests that the ionic liquid is altering the structural conformation of the duplex at different concentrations.
Figure 13. DNA and Ionic Liquid Complexes. [C₄MIM][Cl] ionic liquid interacting with the phosphates and nucleobases on the DNA at concentrations smaller and larger than 60 mM. This qualitatively suggests that the concentration of short-chained alkyl lengths directly affects the structure of the DNA.¹⁴

Figure 14. DNA and Ionic Liquid Interaction Sites. [C₄MIM][Cl] ionic liquid interacting with the major groove, minor groove, and phosphates on the DNA. This qualitatively suggests that once all the binding spots are filled, precipitation occurs due to saturation of DNA.⁸
Figure 15. DNA and Ionic Liquid Interaction Site. More realistic sizing and spacing of the ionic liquid interacting with the major groove of the DNA.\textsuperscript{11}