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Diffusional Dynamics of Tetraalkylphosphonium Ionic Liquid Films Measured by Fluorescence Correlation Spectroscopy

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Disciplines
Physical Chemistry

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Abstract:
Fluorescence correlation spectroscopy (FCS) is applied to investigate the diffusional dynamics of hydrophilic (Atto 590) and amphiphilic (DiD) fluorophores in a series of alkylphosphonium ionic liquid (IL) films ([P4448][Cl], [P6668][Cl], [P66614][Cl], and [P66614][NTf2]) in order to determine diffusional parameters and to elucidate nanoscale structural heterogeneities within the IL. From the measured correlation functions, the diffusion coefficients of the fluorescent molecules are estimated, rendering values that span from 0.39-1.2 and 0.146-5.2 µm²/s, for Atto 590 and DiD, respectively. A decrease in the diffusion coefficients is measured as the alkyl chain increases, coincident with a decrease in their viscosity. Interestingly, deviations from the Brownian diffusion behavior of the fluorescent probes in the ILs are observed, showing a time-dependent diffusion coefficient in most of the cases. These deviations can be attributed to the presence of nanoscale structural heterogeneities in the tetraalkylphosphonium ILs. These results confirm experimentally the presence of nanosegregation in tetraalkylphosphonium ILs, which has previously been observed in molecular dynamics (MD) studies.
Introduction

Ionic liquids (ILs) are generally defined as organic salts that have a melting point below 100 °C. They are typically composed of an organic cation paired with an inorganic or organic anion. These liquids exhibit an assortment of useful and interesting physicochemical properties, such as low-to-negligible vapor pressure, wide solvating power, and good thermal stability.\textsuperscript{1} Moreover, some IL properties can be tuned by choosing the appropriate cationic and anionic components. Consequently, ILs have attracted considerable attention for the extraction and separation of organic compounds, biomolecules, and metal ions. ILs have been used as solvents for liquid-liquid and liquid-phase microextraction, buffer additives, capillary-wall-supported coatings in capillary electrophoresis, sorbents for solid-phase extraction, and sorbent coatings for solid-phase microextraction.\textsuperscript{2-5} The amphiphilic nature in combination with the good thermal stability of ILs can be particularly exploited in chromatographic techniques,\textsuperscript{6-8} in which they are used as stationary phases to separate both polar and nonpolar compounds. Furthermore, they have been employed as matrices for matrix-assisted laser desorption/ionization mass spectrometry.\textsuperscript{1}

Several experimental and computational studies have revealed that some of the properties observed in ILs at the macroscopic scale are the result of structural heterogeneities at the molecular level, which affect the solvation and rotational dynamics of a solute in ILs.\textsuperscript{9-12} This structural heterogeneity is a consequence of the aggregation of the alkyl tail of the cations, formed mainly through attractive van der Waals interactions (nonpolar domains), and charge ordering (polar domains).\textsuperscript{13, 14} The formation of domains is facilitated by an increase in the alkyl chain length,\textsuperscript{10, 15} and their presence significantly affects the transport behavior and ionic conductivity of solutes in IL matrices.

Using a multiscale coarse-grained model, Wang \textit{et al.}\textsuperscript{15} explored the effect of the cation side-chain length in a series of alkylimidazolium nitrate ILs. These simulations showed that, with sufficient side-chain length (C≥4), neutral tail groups of cations aggregate to form nonpolar domains, while the charged head groups of cations and anions homogenously distribute due to the collective short-range electrostatic interactions forming
polar domains. Using small-wide angle x-ray scattering (SWAXS), Russina et al.\textsuperscript{16} accessed intermolecular dynamic features that suggest the existence of nanoscale structure in [C\textsubscript{n}MIM][NTf\textsubscript{2}] for n ≥3. Furthermore, using molecular dynamics (MD) simulations, Canongia \textit{et al.}\textsuperscript{10} and Pádua \textit{et al.}\textsuperscript{17} observed nanoorganization in 1-alkyl-3-methylimidazolium-based ILs. An important conclusion is that aggregation of nonpolar domains was observed for alkyl side chains as small as C\textsubscript{4}, in agreement with the results from Wang and Voth.\textsuperscript{15} Margulis and co-workers investigated the slow dynamics of an imidazolium IL by means of MD simulations and predicted the existence of heterogeneity.\textsuperscript{11,14,18} They concluded that this heterogeneity is the underlying microscopic cause of the red-edge effect, wherein the fluorescence emission wavelength shifts to longer wavelengths as the excitation wavelength also shifts to longer wavelengths.

Several experimental studies have been performed to study the diffusion of organic dyes in neat or IL-water systems using FCS.\textsuperscript{19-25} To mention a few, Guo \textit{et al.}\textsuperscript{20} provided experimental evidence for chain-length-dependent self aggregation in a homogeneous series of N-alkyl-N-methyl-pyrrolidinium bis(trifluoromethylsulfonyl) imide ILs of varying alkyl chain length (n= 3, 4, 6, 8, 10) using Rhodamine 6G. Similarly, Patra \textit{et al.}\textsuperscript{22} studied the translational diffusion of charged (R123) and neutral (DCM, 4NBD) fluorescent probes via FCS. Their study showed evidence for chain-length-dependent self aggregation in a series of 1-alkyl-3-methylimidazolium ILs. The FCS experiments indicated that the fluorescent probes exhibited anomalous diffusion in the ILs tested, suggesting the presence of two distinct domains. Additionally, they found that by increasing the alkyl chain length in the cation, the fast and slow diffusion rate decreased; and the contribution of the slow diffusion increased, suggesting an increase in the domain size as the alkyl chain in the imidazolium ring increased. Interestingly, a biomodal diffusion behavior of the fluorescent probes was observed even with a small alkyl chain length ([EMIM][NTf\textsubscript{2}]), in agreement with previous studies.\textsuperscript{16}

Recently, there has been an increasing interest in employing phosphonium ILs due to their lower cost of synthesis, higher thermal stability, and solubilization of low polarity compounds in comparison to analogous ILs with nitrogen-based cations.\textsuperscript{26} These features render them excellent materials for separation applications.\textsuperscript{27} In the specific case of quaternary phosphonium salts, the existence of structural heterogeneities has been
observed through MD simulations. Gontrani et al.\textsuperscript{28} showed the presence of nanostructures in trihexyl(tetradecyl)phosphonium chloride ([P\textsubscript{66614}][Cl]) by MD simulations and X-ray scattering data, where the charged domains are formed by phosphorous and chloride atoms while the nonpolar regions correspond to the alkyl chains. Their studies described the morphology of the segregated domains as a sponge-like structure rather than a lamellar organization. Furthermore, Hettige et al.\textsuperscript{29} studied the structural behavior of the same cation ([P\textsubscript{66614}\textsuperscript{+}]) coupled with six different anions, and found that the structure of the ILs is defined by charge and polar alternation. They observed that a temperature increase in the IL system results in a better defined polar network. Wang et al.\textsuperscript{30} applied MD simulations to a series of tetraalkylphosphonium cations coupled with a chloride anion in order to study the effect of the aliphatic chain in the microscopic ionic structures. They reported that for ILs consisting of cations with shorter alkyl chains, the liquid morphologies are characterized by sponge-like interpenetrating polar and nonpolar networks. When the aliphatic chain length is increased, however, the polar network is partially broken or totally segregated within the nonpolar framework due to the progressive expansion of the nonpolar domains.

Nanoconfinement, or short-range ordering, has been reported by several authors for an IL film coated on a solid substrate. This short-range ordering can extend from 0.25 to 60 nm away from the substrate.\textsuperscript{31-34} Additionally, Anaredy et al.\textsuperscript{35} reported long-range ordering (ca. 1000 nm) in a set of IL films under the influence of shearing. By studying the rotational dynamics of anionic, cationic, and neutral fluorophores as a function of distance from a silica surface, Ma et al.\textsuperscript{36} reported organization within the IL over ca. 100 \textmu m in a [BMIM][BF\textsubscript{4}] IL film supported on a negatively-charged silica substrate. They raised the question if this long-range ordering could be observed in a wider range of ILs, where more bulky cations and a different set of anions are present in the IL structure.

In order to gain further insight into the structural heterogeneities observed by molecular simulations in tetraalkylphosphonium ILs, we used fluorescence correlation spectroscopy to study molecular diffusion in a series of tetraalkylphosphonium ILs films. The basic motivation is to understand how the nanostructural organization affects the diffusional behavior of fluorophores of different polarity (Atto 590 and DiD) when the cation and anion in tetraalkylphosphonium ILs are altered.
Scheme 1. Structure of fluorophores and constituent cations and anions of ILs used in this study. (a) Tributyl(octyl)phosphonium cation, [P$_{4448}^+$]; (b) trihexyl(octyl)phosphonium cation, [P$_{6668}^+$]; (c) Trihexyl(tetradecyl)phosphonium cation, [P$_{66614}^+$]; (d) bis[(trifluoromethyl)sulfonyl]imide, [NTf$_2^-$]; (e) chloride, [Cl$^-$], (f) Atto 590 (mixture of 5- and 6-isomers); (g) DiD (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine, 4-Chlorobenzenesulfonate Salt).

2. Experimental Section
2.1. Sample Preparation.

Scheme 1 shows the structure of the phosphonium ILs and fluorophores. Tributyl(octyl)phosphonium chloride, [P$_{4448}$][Cl$^-$]), and trihexyl(octyl)phosphonium chloride, [P$_{6668}$][Cl$^-$], were supplied as a gift by CYTEC (West Paterson, NJ, USA). Trihexyl(tetradecyl)phosphonium chloride, [P$_{66614}$][Cl$^-$], (98%) was purchased from Iolitec (Tuscaloosa, AL, USA). Trihexyl(tetradecyl)phosphonium bis[(trifluoromethyl)sulfonyl]imide, [P$_{66614}$][NTf$_2^-$], was synthesized as described in the SI. The ILs used in this study do not absorb light at 594 nm (SI Fig. S1). They were rigorously dried under high vacuum for 48 h at 40 °C before use, rendering their water content to be ~1000 ppm. The water content was determined by coulometric titration using a DL39 Karl Fischer Coulometric Titrator (Mettler Toledo, Columbus, OH). The position of the IR
bands in the OH region is sensitive to the water environment and extent of the H-bonding network, however, attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra (Fig. S2) showed no peaks in the region 3000 – 3800 cm\(^{-1}\), where the antisymmetric (\(\nu_1\)) and symmetric (\(\nu_3\)) stretching modes of water are expected, at the low water concentrations used in this study. Viscosity measurements of the dry ILs were performed at 25 °C by the cone-plate method using a DV1 Brookfield digital viscometer with a CPE-51 spindle at 6 rpm. The water content and viscosity of the dried ILs are presented in Table 1.

**Table 1.** Viscosity, water content, and film thickness for the indicated tetraalkylphosphonium IL samples.

<table>
<thead>
<tr>
<th>Ionic Liquid</th>
<th>Viscosity (cP)</th>
<th>Water content (ppm)(^a)</th>
<th>Film Thickness (nm)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>734</td>
<td>1110 ± 90</td>
<td>1150 ± 60</td>
</tr>
<tr>
<td>[P(_{4448})][Cl]</td>
<td>3961</td>
<td>1120 ± 5</td>
<td>1040 ± 30</td>
</tr>
<tr>
<td>[P(_{6668})][Cl]</td>
<td>3228</td>
<td>1080 ± 60</td>
<td>940 ± 50</td>
</tr>
<tr>
<td>[P(_{66614})][Cl]</td>
<td>2270</td>
<td>570 ± 10</td>
<td>840 ± 60</td>
</tr>
<tr>
<td>[P(_{66614})][NTf(_2)]</td>
<td>304</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Average of 3 measurements  
\(^b\) Average of 15 measurements (3 measurements for each film)

To avoid the sorption of environmental moisture, all handling and sample preparation were performed in a nitrogen-filled glove box (relative humidity <5%). A 100 mg/mL solution of dried IL was prepared in dichloromethane, and an appropriate amount of the stock fluorophore solution (Atto 590 and DiD, Sigma Aldrich, prepared in dichlomethane) was added to reach a final concentration of 15 nM. For all reported results, the fluorophore concentration in the samples was maintained at 15 nM. This provided sufficient signal with an average of approximately 10 molecules in the confocal volume at any time. The experimental conditions ensured that the fluctuations in the fluorescence intensity are dominated by the diffusion of molecules through the confocal volume.

To prepare the film of the IL containing fluorophore, 200 µL of solution was deposited on a 18 mm\(^2\) amino-modified (full details are provided in the SI) cover glass (No. 1.5, Corning Inc., Corning, NY) and then spun for 180 s at 8500 rpm using a KW-4A spin coater (Chemat Technology, Northridge, CA). A homogeneous film was obtained and
transferred to a home-made sample holder to avoid water intake from the environment during the FCS measurements. Fig. S3A shows an optical image of one IL film. Additionally, Raman spectroscopy was used to confirm the formation of the IL film on the glass substrate (SI Fig. S3B). To compensate for cover glass thickness and refractive index mismatch, careful adjustment of the objective correction collar was performed for each measurement, by finding the maximum of the counts per molecule for each set of experiments. In order to minimize effects from short-range layering due to surface confinement, FCS experiments were performed approximately 100-200 nm above the IL-glass interface. This was accomplished by moving the laser focus within the sample using a motorized microscope stage. Using a high numerical aperture objective, it is not possible to move the focus further away from the substrate, and it is possible that long-range organization may be present within these IL films.

The thickness of the IL films was measured using a spectroscopic ellipsometer (J.A. Woollam α -SE, J.A. Woollam Co. Inc., Lincoln, NE, USA), operating at the wavelength range of 380-900 nm at 70°. The IL film thickness was tracked as a function of time by acquiring Psi(ψ) and Delta (Δ), values at different time points. Data analysis was performed using CompleteEase software. Fig. S4 shows example ellipsometry data for one IL film. The thickness of the IL film was approximated using the Cauchy model. Thickness measurements were performed at five different locations on the sample and an average thickness and standard deviation of the IL films were computed (Table 1).

2.2 Instrumentation.

The FCS apparatus was based on an inverted confocal microscope (Nikon, Ti) equipped with a 594-nm pulsed picosecond (fwhm=500 ps, repetition rate 20 MHz) diode laser (BDS-SMY, Becker & Hickel) as the excitation beam. The output of the laser (0.7 mW) was coupled to a polarization-maintaining single-mode optical fiber (KineMATIXP2, Qioptiq). The laser beam was expanded to overfill slightly the back aperture of the 100× oil-immersion microscope objective (Apo TIRF, NA. 1.49, Nikon) with a correction collar (0.13-0.20), and a neutral density filter (OD 1.5) was used to reduce the laser power at the sample. Fluorescence generated by the focused laser beam was collected by the same objective and isolated by a dichroic mirror (zt488/594rpc-uf5, Chroma Technology) and a
long-pass emission filter (ET 645/75m, Chroma Technology) located in front of the detector. Fluorescence was then guided through a 100 µm pinhole to block the out-of-focus light. Photons were detected with a single photon counting detector (SPCM-AQR-16, Excellitas). A piezo stage (Mad City Lab) was used to control the movement of the sample. The output signal from the detector was sent to a time-correlated single-photon counting board (SPC-830, Becker&Hickel). Data were analyzed with the built-in functions of IgorPro 6.37 (Wavemetrics, Lake Oswego, OR). All of the FCS measurements were done at 22 °C.

2.3 Data Analysis.

The time-dependent fluorescence intensity, $I(t)$, is analyzed in terms of its autocorrelation function $G(\tau)$, which compares the fluorescence intensity at time $t$ with the intensity after a time delay, equation 1:

$$G(\tau) = \frac{\langle \delta F(t) \cdot F(t + \tau) \rangle}{\langle F(t) \rangle^2}$$  \hspace{1cm} (1)

where $\delta F = I(t) - \langle F(t) \rangle$; $\langle F(t) \rangle$ is the time-average of the fluorescence intensity. One advantage of using FCS is that different models can be used to extract diffusion parameters. For example, for measurements in solution, the probe volume is defined by a 3D Gaussian profile, in which a 3D Brownian diffusion model (equation 2) can be used. The calibration measurement using a solution of fluorescent polymeric beads and glycerol used the 3D diffusion model.

$$G(\tau) = \frac{1}{N} \left[ 1 + \left( \frac{\tau}{\tau_d} \right)^{\alpha} \right]^{-1} \left[ 1 + \frac{1}{\kappa \tau_d} \left( \frac{\tau}{\tau_d} \right)^{\alpha} \right]^{-1/2} + 1$$  \hspace{1cm} (2)

For a 2D diffusion model, equation 3 can be applied. All measurements of IL films used the 2D model.

$$G(\tau) = \frac{1}{N} \left[ 1 + \left( \frac{\tau}{\tau_d} \right)^{\alpha} \right]^{-1}$$  \hspace{1cm} (3)

$N$ is the average number of fluorescence molecules in the confocal volume, $\tau_d$ is the characteristic diffusion time, $\kappa$ describes the confocal volume and is given by $\kappa = \omega_z / \omega_{xy}$, where $\omega_z$ and $\omega_{xy}$ correspond to the $1/e^2$ values in the axial and longitudinal directions, respectively. For Brownian diffusion, the exponent ($\alpha$) is unity. For anomalous diffusion, however, the mean-squared displacement of the fluorophore is no longer proportional to
time, $t$, but rather to $t^\alpha$, where $\alpha$ may assume values less than 1 or greater than 1, for sub-diffusion and super-diffusion, respectively.

The structure parameter, $\kappa$, of the excitation volume was calibrated using 20-nm fluorescent beads (580/605 fluorescent FluoSpheres 0.02 µm, Invitrogen/Molecular Probes, Eugene, OR) in water with a diffusion coefficient of 2.17 µm$^2$/s. Experimentally measured correlation data on the diffusion of FluoroSpheres were fit with the autocorrelation function for a 3D diffusion model (Equation 2) rendering a $\omega_{xy}$ value of 450 nm, $\kappa = 3$, and an effective focal volume of 1.5 fL. The confocal volume in the longitudinal direction, $w_{xy}$, was corroborated by collecting confocal images of 100-nm fluorescent beads (580/605 fluorescent FluoSpheres 0.1 µm, Invitrogen/Molecular Probes, Eugene, OR) in different media with varying refractive index ($n=1.5150$ (immersion oil), 1.49551 ([P$_{4448}$][Cl]), 1.49245 ([P$_{6668}$][Cl]), 1.48326 ([P$_{66614}$][Cl]), 1.45164 ([P$_{66614}$][NTf$_2$]). The lateral cross-sections of the beads were fitted using a Gaussian function and rendered an average $w_{xy}$ value of 450 ± 30 nm, which is in good agreement with the value obtained from the aforementioned instrument calibration. This shows that the refractive index difference among the IL samples as well as the calibration medium did not affect the accurate measurement of the diffusion parameters.

Correlation data for ILs films was fit using equation 2, and the obtained $\tau_d$ was used to calculate the diffusion coefficient (D) using equation 4. When $\alpha < 1$, the diffusion coefficient is time dependent. All reported diffusion coefficients correspond to a time of 1 s.

$$\tau_d = \frac{w_{xy}^2}{4D} \quad (4)$$

3. Results and discussion

Using FCS, the effects of alkyl chain length and anion on the diffusion of the cationic fluorophore (Atto 590) and amphiphilic fluorophore (DiD) in alkylphosphonium ILs were measured. Specifically, the $\alpha$-exponent in equations 2 and 3 was used to quantify the degree of deviation from Brownian diffusion, which is measured in homogeneous media. Figure 1 depicts the normalized fluorescence autocorrelation curves of the fluorophores in the [P$_{4448}$][Cl], [P$_{6668}$][Cl], and [P$_{66614}$][Cl] films. The quality of the fits was assessed by the
distribution of the residuals. Summarized in Table 2 are the average values of the diffusion parameters obtained from the best fit of the autocorrelation curves for glycerol and tetraalkylphosphonium chloride ILs.

![Normalized autocorrelation curves](image)

**Figure 1.** Normalized autocorrelation curves for (left) Atto 590 and (right) DiD in (red) [P_{4448}][Cl], (green) [P_{6668}][Cl], and (blue) [P_{66614}][Cl] tetraalkylphosphonium ILs. The corresponding fits to equation 3 are shown as black lines. The lower panels show the residuals between the experimental data and fit using the same color scheme.

**Table 2.** Translational diffusion parameters measured by FCS for the indicated tetraalkylphosphonium ILs and for the homogenous glycerol sample.

<table>
<thead>
<tr>
<th>Ionic Liquid</th>
<th>Atto 590 Diffusion Coefficient (µm²/s)</th>
<th>Atto 590 α</th>
<th>DiD Diffusion Coefficient (µm²/s)</th>
<th>DiD α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>2.9 ± 0.2</td>
<td>0.96 ± 0.03</td>
<td>0.146 ± 0.009</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>[P_{4448}][Cl]</td>
<td>0.39 ± 0.05</td>
<td>0.72 ± 0.05</td>
<td>0.90 ± 0.03</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>[P_{6668}][Cl]</td>
<td>0.73 ± 0.05</td>
<td>0.73 ± 0.04</td>
<td>0.98 ± 0.09</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td>[P_{66614}][Cl]</td>
<td>1.2 ± 0.1</td>
<td>0.97 ± 0.04</td>
<td>0.98 ± 0.09</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td>[P_{66614}][NTf₂]</td>
<td>3.2 ± 0.3</td>
<td>0.87 ± 0.04</td>
<td>5.2 ± 0.6</td>
<td>0.65 ± 0.04</td>
</tr>
</tbody>
</table>

Figure 2 plots the diffusion coefficients of Atto 590 and DiD as a function of the
IL’s viscosity. Generally, the fluorophore’s diffusion coefficient decreases due to the increase in viscosity. However, the diffusion of fluorophores within the ILs of varying viscosity does not follow the Stokes-Einstein equation (dotted line within Figure 2). This deviation is expected since Stokes law was generated for macroscopic particles in motion in a continuous medium. The diffusion coefficients of Atto 590 are smaller than the diffusion coefficient of DiD, which is unexpected given the smaller size of Atto 590. As reported by Kaintz et al. \textsuperscript{40} “the dependence on relative size alone is insufficient to provide useful quantitative predictions of diffusion coefficients in ILs.” In addition, the differences in the diffusion coefficients between Atto 590 and DiD are not consistent across all the ILs. This would be expected for a homogenous medium when the only factors influencing diffusion are the size of the fluorophore and viscosity of the IL. When combined, these observations suggest that the ILs have nanoscale heterogeneities and that the two fluorophores are diffusing in different environments within the ILs. These experimental results highlight the complexity of the IL system, in part due to the presence of different local environments as the fluorophores diffuse in the focal volume. The presence of nanoscale heterogeneities is consistent with several computational studies of tetraalkylphosphonium ILs, including [P\textsubscript{4448}][Cl], showing polar entities consisting of chloride anions and central P(CH\textsubscript{2})\textsubscript{4} groups and nonpolar domains composed of the remaining alkyl chain.\textsuperscript{29} The solvation properties of ILs are quite complex, and it is not possible to state the chemical nature of the domains in which the fluorophores are diffusing within these ILs with the available data. Additionally, it is not possible to state that the fluorophores have access of all domains within the IL framework.
Figure 2. Atto 590 and DiD diffusion coefficients as a function of IL viscosity (red symbols, left axis). The error bars are the standard deviation from 20 replicate measurements. The dotted line and black symbols (right axis) correspond to the expected diffusion coefficient values calculated based on the Stokes-Einstein equation and assuming a fluorophore diameter of 1 nm.

As expected for a homogenous sample that does not contain structural heterogeneities, the diffusion of Atto 590 in glycerol is Brownian, with $\alpha \sim 1$ (SI Fig. S5, Table 1). This demonstrates the sensitivity of the FCS measurements to distinguish homogeneous environments in high viscosity media. On the other hand, $\alpha$ for [P$_{4448}$][Cl] and [P$_{6668}$][Cl] are less than 1 for both fluorophores; and diffusion is not Brownian. In general, the deviation of the $\alpha$ value from unity suggest that the means square displacement of the fluorophores follows an anomalous sub-diffusive behavior (with $\alpha < 1$), due to the structural heterogeneity present in these complex media. The data in Table 2 show the $\alpha$ value is lower for DiD than Atto 590 within the same type of IL, indicating that DiD experience higher local dynamic heterogeneity. For DiD, $\alpha$ reaches a constant value of 0.65 as the alkyl chain length in the IL increases. Given the amphiphilic nature of the DiD molecules, it might interact with both polar and nonpolar entities of the ILs. The characteristic size of nanoscopic structural heterogeneities in pyrrolidinium, pyridinium and imidazolium ILs extend over a few nanometers and linearly scale with the aliphatic chain length.$^{16, 41, 42}$ Given the presence of four aliphatic alkyl chains in the alkylphosphonium cation, the nonpolar nanostructures are expected to be larger in comparison with the nitrogen-based ILs (i.e pyrrolidinium, pyridinium and imidazolium), as demonstrated by MD simulations.$^{30}$

Surprisingly, $\alpha$ for Atto 590 in [P$_{66614}$][Cl] is similar to that in glycerol and is approximately 1. This indicates that the fluorophore experiences Brownian diffusion in this IL. Several computational studies, however, have demonstrated the presence of polar and nonpolar domains in [P$_{66614}$][Cl]. The volume fraction of the nonpolar domains is significantly larger in comparison with the volume fraction occupied by the polar parts of the [P$_{66614}$][Cl] IL, as Wang et al.$^{30}$ demonstrated by MD simulations. Wang’s studies revealed that as the alkyl chain length in the cation increases, the nonpolar domain
expanded, while the polar network lost part of its connectivity. This resulted in a segregated distribution of polar domains within the nonpolar structure. The discontinuity of the polar network might prohibit Atto 590 molecules from moving among different domains present in [P$_{66614}$][Cl] while producing Brownian diffusion within the domains it can access.

**Figure 3.** Experimental normalized autocorrelation curves of Atto 590 (left) and DiD (right) in (blue) [P$_{66614}$][Cl] and (pink) [P$_{66614}$][NTf$_2$]. The corresponding fits to equation 3 are shown as black lines. The lower panels show the residuals between the experimental data and fit using the same color scheme.

The effects of the same cation, [P$_{66614}^+$], coupled with a different anion on the diffusion properties of Atto 590 and DiD were analyzed (Figure 3). When the anion is changed from [Cl$^-$] to [NTf$_2^-$], the diffusion coefficient increases with the reduction in the viscosity of the IL. On the other hand, $\alpha < 1$ when the cation is kept constant and the anion size is increased. Hettige *et al.*$^{29}$ observed by MD simulations that there is a more accentuated positive-negative alternation of charge, as well as a more polar-nonpolar alternation, in [P$_{66614}$][NTf$_2$] in comparison with [P$_{66614}$][Cl]. Furthermore, Gardas *et al.*$^{43}$ estimated the cation and anion volume for a series of ILs, where the predicted volume for [P$_{66614}^+$], [Cl$^-$], and [NTf$_2^-$] was 947, 47, and 248 Å$^3$, respectively. Thus, the relative volume
fraction of the polar domain is larger for [P$_{66614}$][NTf$_2$], and the alpha value smaller, compared to [P$_{66614}$][Cl]. Comparing all the ILs in this study, this indicates that the alpha value of Atto 590 is smaller for the ILs with the largest relative volume fraction of the polar domains.

Conclusions
The translational diffusion dynamics of Atto 590 and DiD in alkylphosphonium ILs films was studied using FCS. The results are in agreement with several experimental and MD simulations for a variety of phosphonium-based and nitrogen-based ILs that have demonstrated that IL structure defines solute dynamics.$^{11, 16}$ Anomalous diffusion of the fluorophores was observed in most of the IL systems under study, which can be attributed to the presence of structural heterogeneities that result from the phase segregation of the alkyl carbon chains. Interestingly, however, it was observed that when the size of the nonpolar domains became significantly larger in comparison to the polar entities (i.e., [P$_{66614}$][Cl]), FCS experiments using a hydrophilic Atto 590 fluorophore may not give insight about the presence of these heterogeneities. These results provide new experimental understanding into the structural complexity of tetraalkylphosphonium ILs and will be useful as a stepping stone for stimulating theoretical and computational investigations of the factors that affect this structural heterogeneity.

Associated Content
Supporting Information
Synthesis, experimental details, and supporting figures.

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References


Table of Contents (TOC) Image
Scheme 1. Structure of fluorophores and constituent cations and anions of ILs used in this study. (a) Tributyl(octyl)phosphonium cation, \([\text{P}_{4448}^+]\); (b) trihexyl(octyl)phosphonium cation, \([\text{P}_{6668}^+]\); (c) Trihexyl(tetradecyl)phosphonium cation, \([\text{P}_{66614}^+]\); (d) bis\([(\text{trifluoromethyl})\text{ sulfonyl}]\text{imide}, \text{[NTf}_2^-\text{]}\); (e) chloride, \([\text{Cl}^-]\); (f) Atto 590 (mixture of 5- and 6-isomers); (g) DiD (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine, 4-Chlorobenzenesulfonate Salt).

165x135mm (600 x 600 DPI)
Figure 1. Normalized autocorrelation curves for (left) Atto 590 and (right) DiD in (red) [P_{4448}][Cl], (green) [P_{6668}][Cl], and (blue) [P_{66614}][Cl] tetraalkylphosphonium ILs. The corresponding fits to equation 3 are shown as black lines. The lower panels show the residuals between the experimental data and fit using the same color scheme.

177x127mm (300 x 300 DPI)
Figure 2. Atto 590 and DiD diffusion coefficients as a function of IL viscosity (red symbols, left axis). The error bars are the standard deviation from 20 replicate measurements. The dotted line and black symbols (right axis) correspond to the expected diffusion coefficient values calculated based on the Stokes-Einstein equation and assuming a fluorophore diameter of 1 nm.
Figure 3. Experimental normalized autocorrelation curves of Atto 590 (left) and DiD (right) in (blue) [P₆₆₆₁₄][Cl] and (pink) [P₆₆₆₁₄][NTf₂]. The corresponding fits to equation 3 are shown as black lines. The lower panels show the residuals between the experimental data and fit using the same color scheme.