

Supporting Information

Epitope mapping of imidazolium cation in ionic liquid-protein interactions unveils the balance between hydrophobicity and electrostatics towards protein destabilisation

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Table S1 – NMR determined self-diffusion coefficient value of HDO in IL solutions with different HSA concentrations

STD-NMR experiments

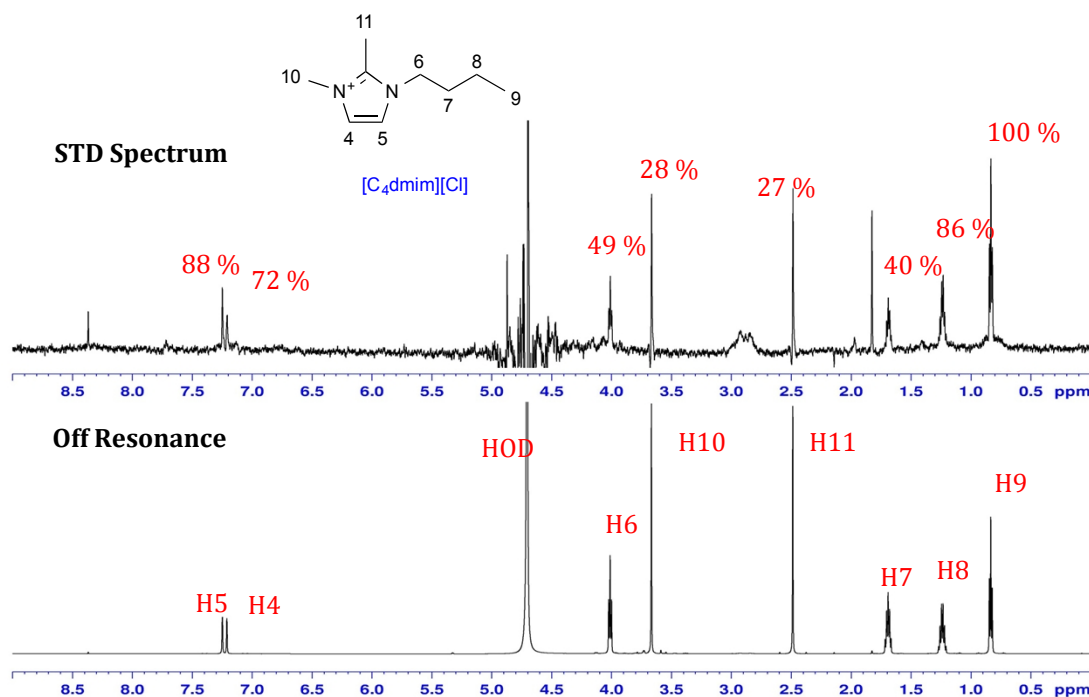


Figure S1 – Top – STD NMR spectrum of 5 mM $[C_4dmim][Cl]$ with 50 μ M human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments.

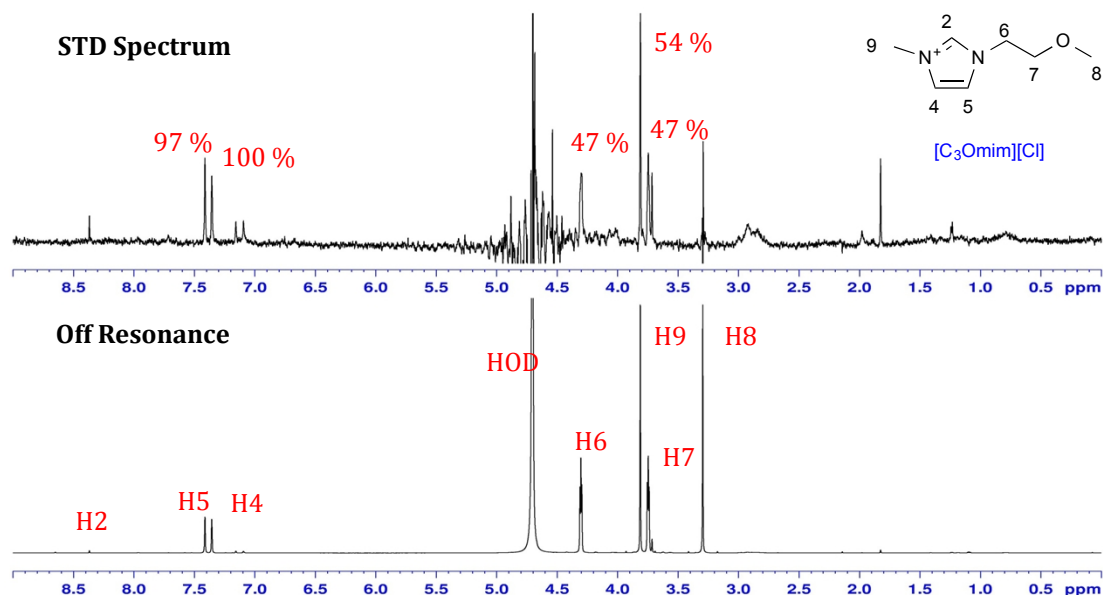
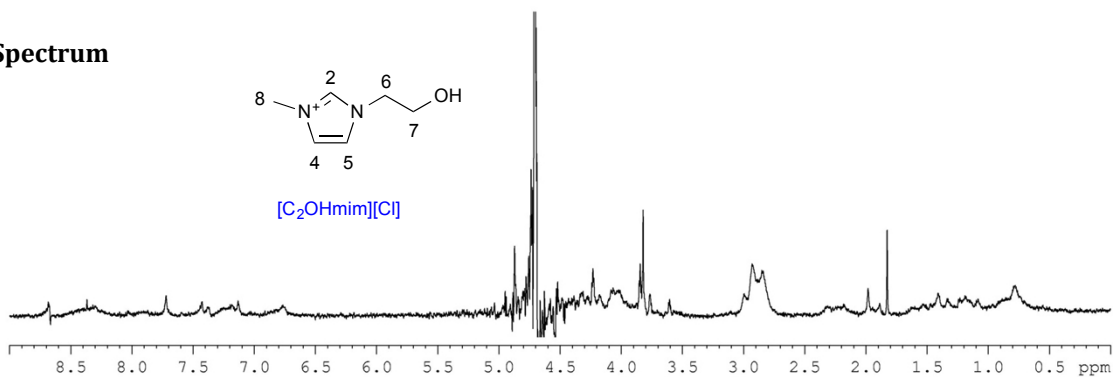
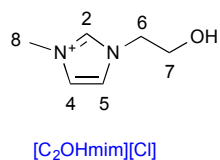


Figure S2 – Top – STD NMR spectrum of 5 mM $[C_3Omim][Cl]$ with 50 μ M human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments. H2 exchanges with D_2O over time and therefore its STD response can not be accurately determined.

STD Spectrum



Off Resonance

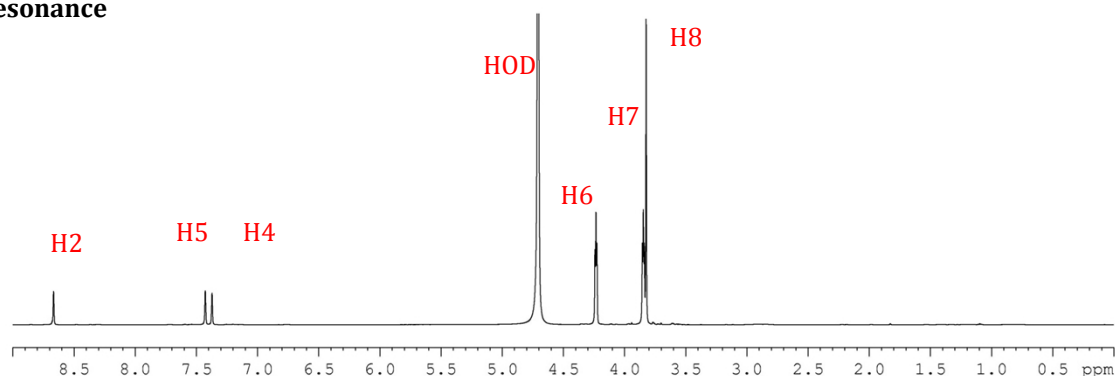
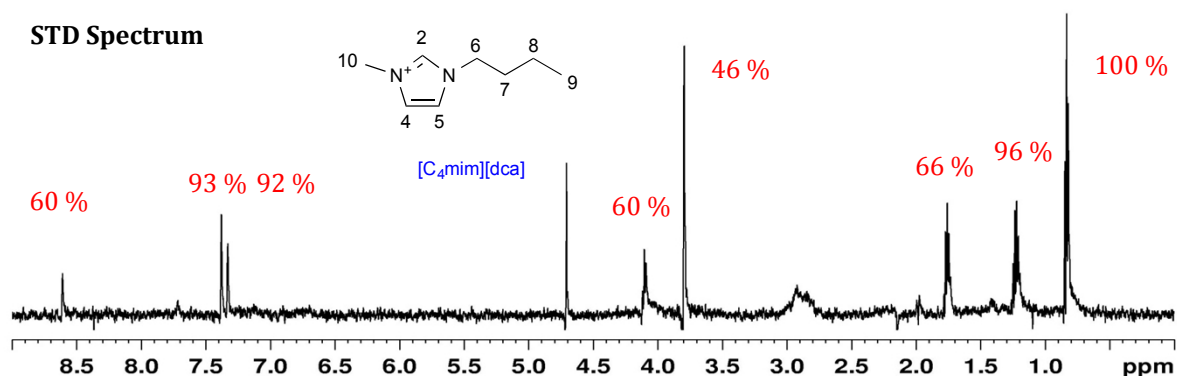
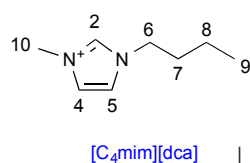


Figure S3 – Top – STD NMR spectrum of 5 mM $[C_2OHmim][Cl]$ with 50 μM human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments.

STD Spectrum



Off Resonance

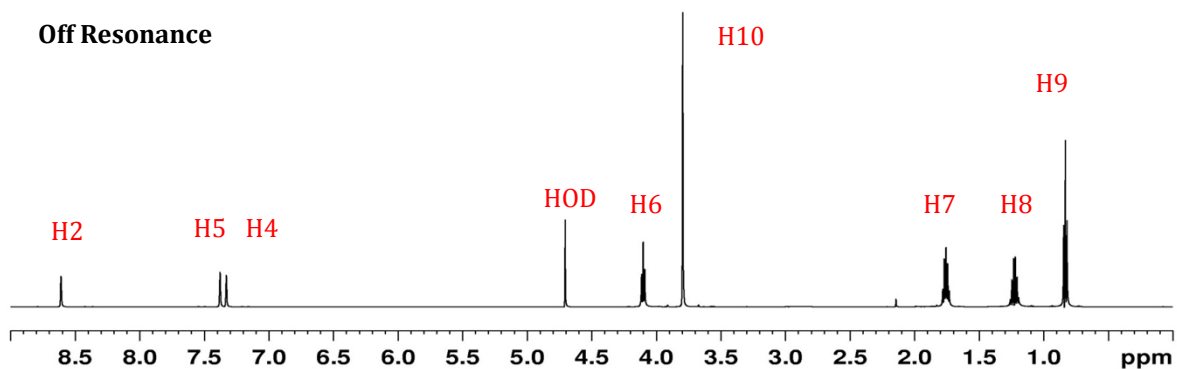


Figure S4 – Top – STD NMR spectrum of 5 mM $[C_4mim][dca]$ with 50 μM human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments. H2 exchanges with D_2O over time and therefore its STD response can not be accurately determined.

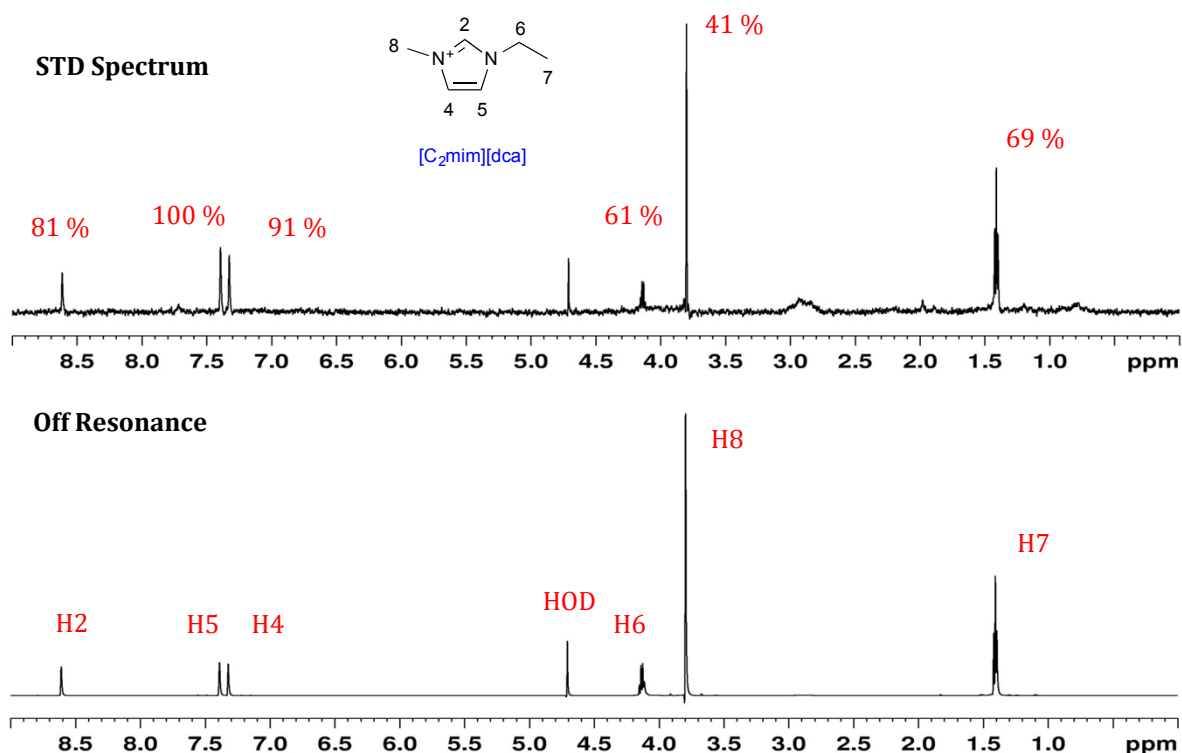


Figure S5 – Top – STD NMR spectrum of 5 mM $[C_2mim][dca]$ with 50 μM human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments. H2 exchanges with D_2O over time and therefore its STD response can not be accurately determined.

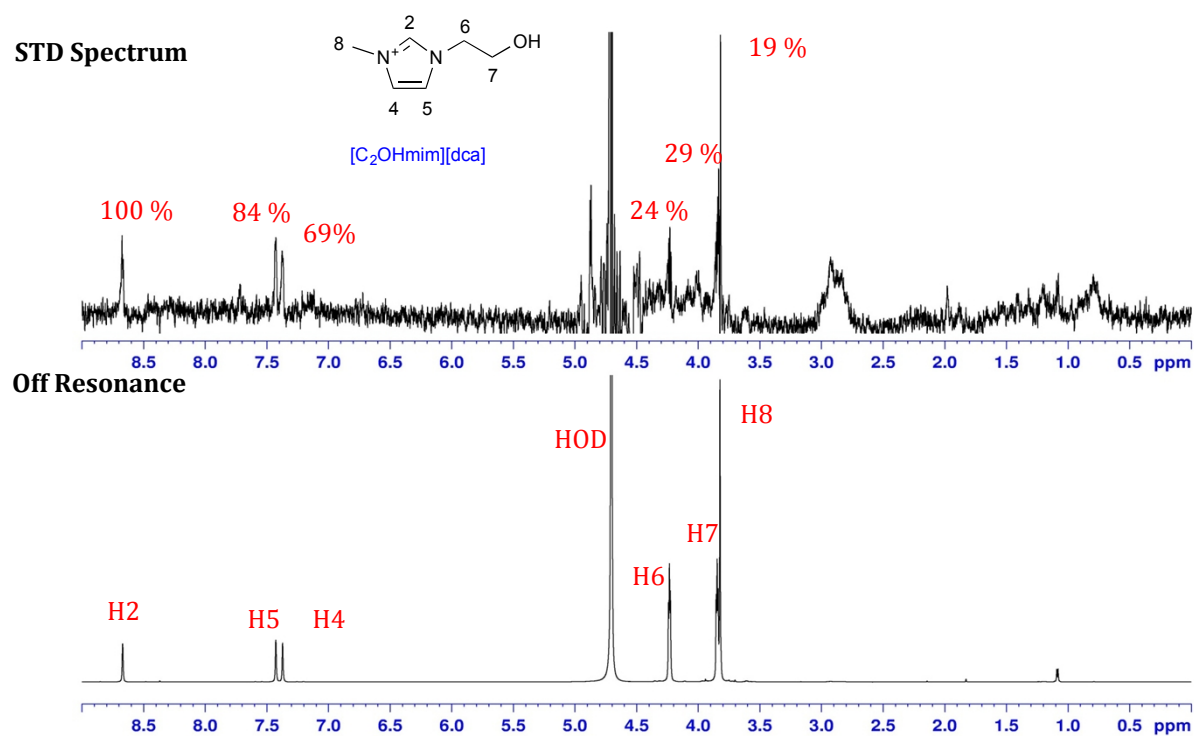
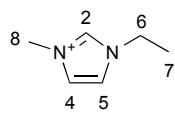
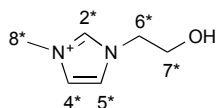


Figure S6 – Top – STD NMR spectrum of 5 mM $[C_3Omim][Cl]$ with 50 μM human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments.

Competition STD-NMR experiments

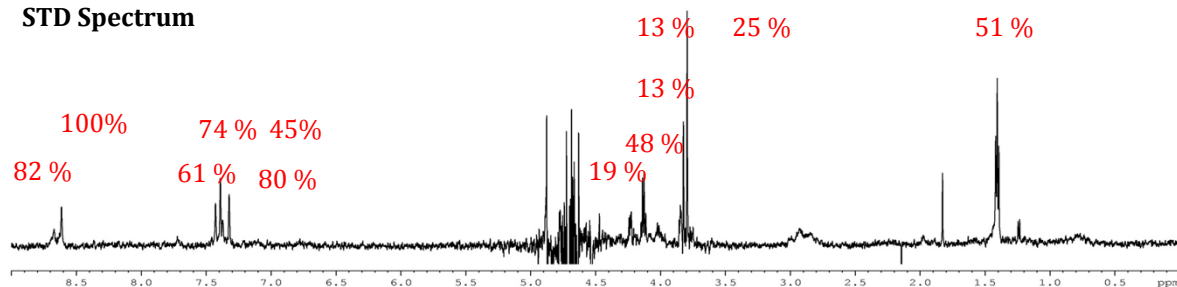


[C₂mim][Cl]



[C₂OHmim][Cl]

STD Spectrum



Off Resonance

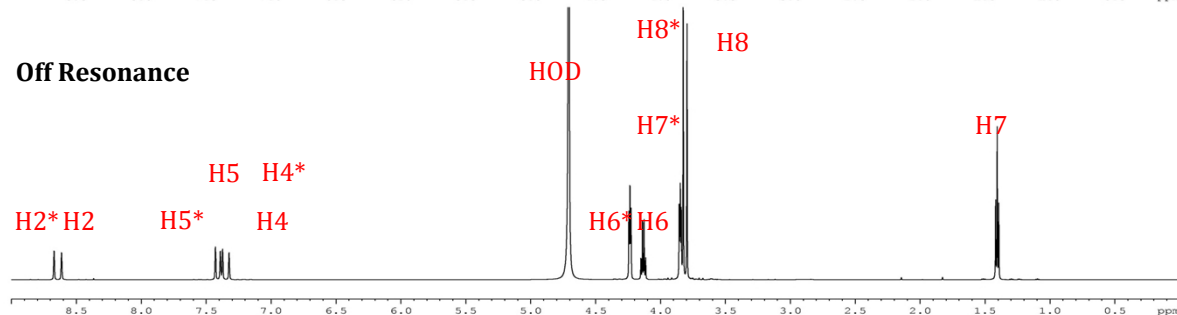
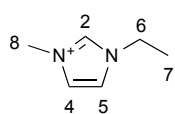
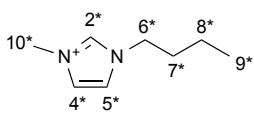


Figure S7 – Top – STD NMR spectrum of 2.5 mM [C₂mim][Cl] and [C₂OHmim][Cl] with 50 μ M human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments.

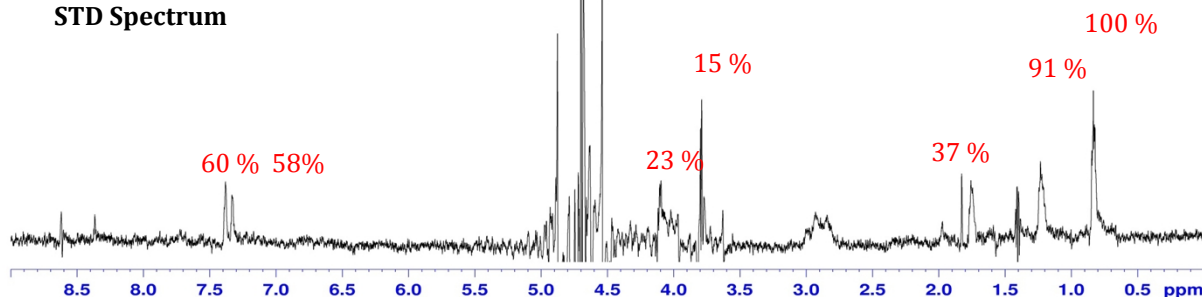


[C₂mim][Cl]



[C₄dmim][Cl]

STD Spectrum



Off Resonance

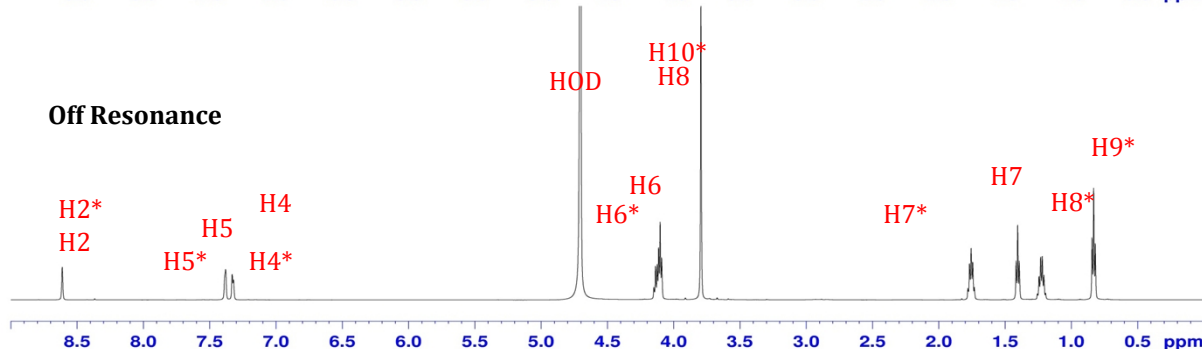


Figure S8 – Top – STD NMR spectrum of 2.5 mM [C₂mim][Cl] and [C₄mim][Cl] with 50 μ M human HSA and relative STD NMR intensities in percentage; Bottom – Reference spectrum (off resonance) with resonance assignments.

Anion-protein interaction - IL titrations with HSA followed by ^{35}Cl NMR

$[\text{C}_2\text{mim}][\text{Cl}]$

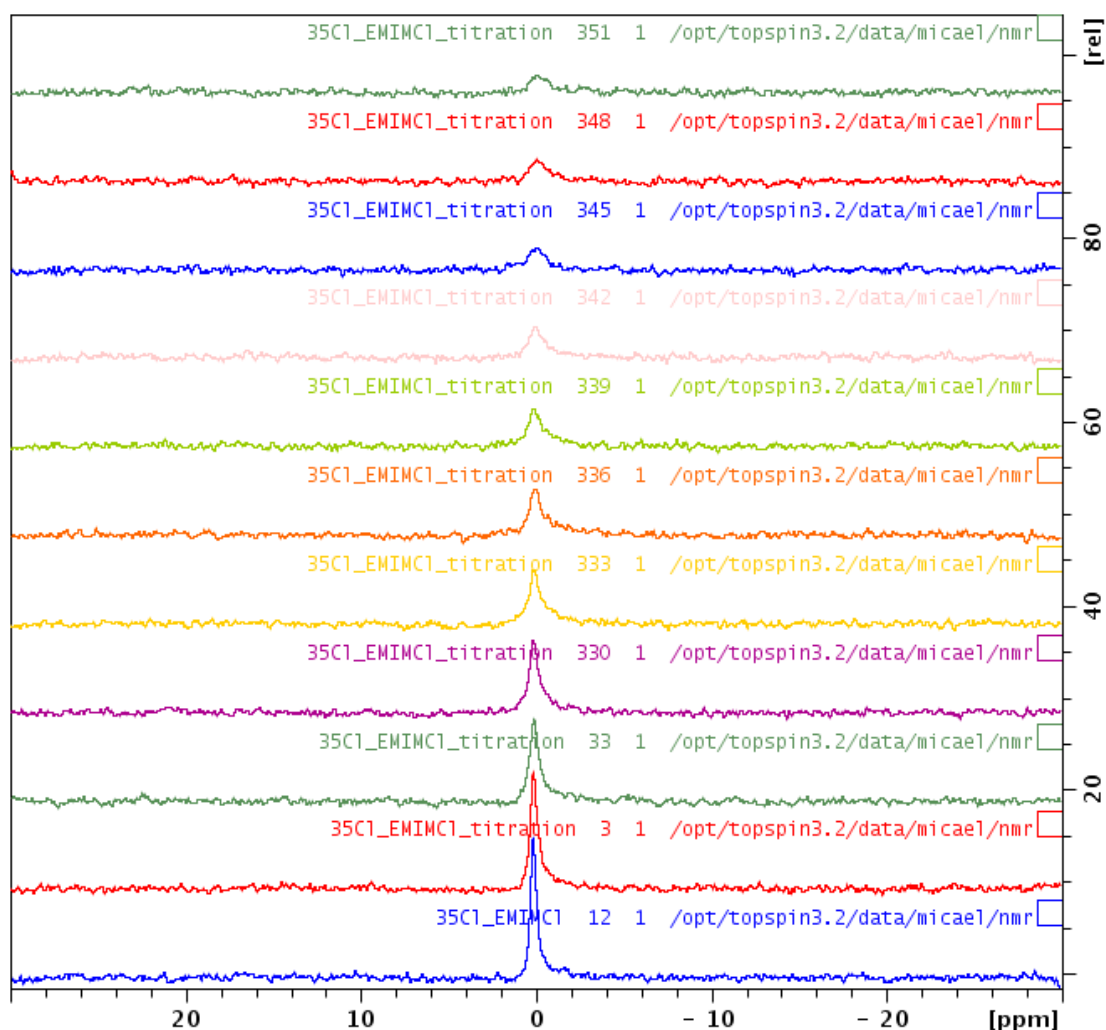


Figure S9 – Effect of HSA addition on the ^{35}Cl resonance of a sample containing 5 mM $[\text{C}_2\text{mim}][\text{Cl}]$ in D_2O (500 μL). Increased HSA concentration was achieved through the addition of small volumes of 50 μM HSA, 5 mM $[\text{C}_2\text{mim}][\text{Cl}]$ to maintain the total concentration of chlorine constant. From bottom to top: volumes added in μL (HSA concentration in μM): 0 (0), 10 (0.98), 20 (1.92), 30 (2.83), 50 (4.55), 75 (6.52), 100 (8.33), 150 (11.54), 225 (15.52), 300 (18.75), and 360 (20.93). Top spectrum: 50 μM HSA, 5 mM $[\text{C}_2\text{mim}][\text{Cl}]$.

[C₄mim][Cl]

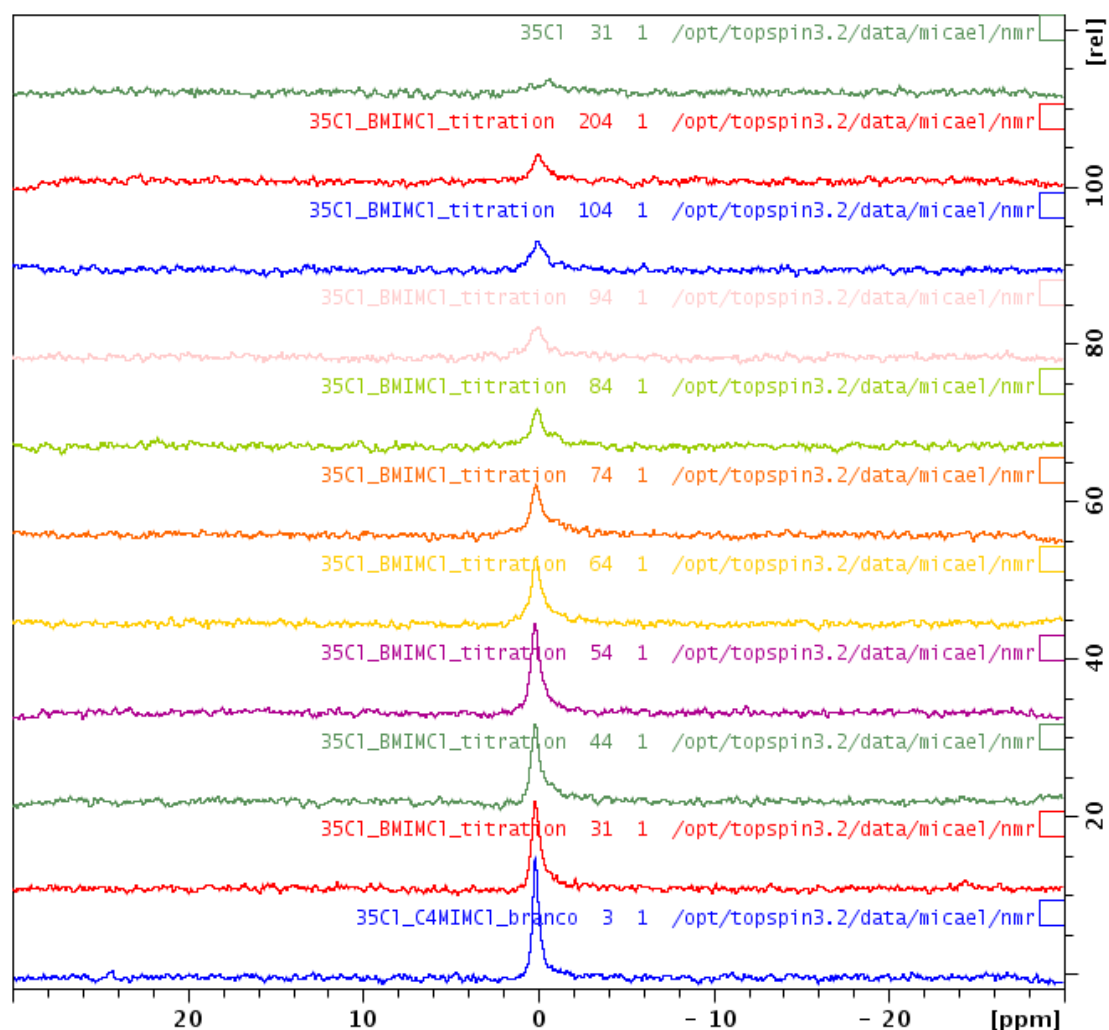


Figure S10 – Effect of HSA addition on the ³⁵Cl resonance of a sample containing 5 mM [C₄mim][Cl] in D₂O (500 μL). Increased HSA concentration was achieved through the addition of small volumes of 50 μM HSA, 5 mM [C₄mim][Cl] to maintain the total concentration of chlorine constant. From bottom to top: volumes added in μL (HSA concentration in μM): 0 (0), 10 (0.98), 20 (1.92), 30 (2.83), 50 (4.55), 100 (8.33), 150 (11.54), 225 (15.52), 300 (18.75), and 385 (21.75). Top spectrum: 50 μM HSA, 5 mM [C₄mim][Cl].

[C₄dmim][Cl]

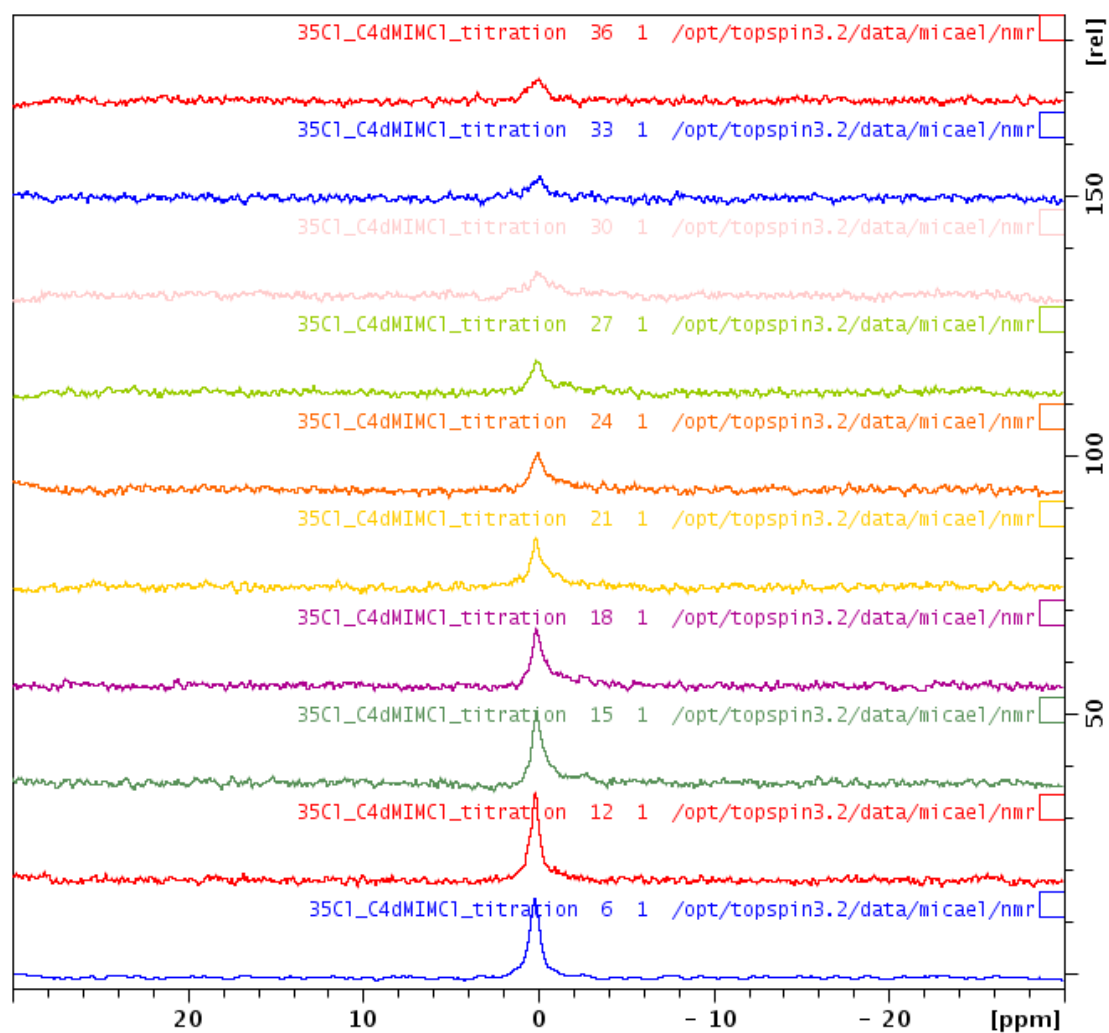


Figure S11 – Effect of HSA addition on the ³⁵Cl resonance of a sample containing 5 mM [C₄dmim][Cl] in D₂O (500 μL). Increased HSA concentration was achieved through the addition of small volumes of 50 μM HSA, 5 mM [C₄dmim][Cl] to maintain the total concentration of chlorine constant. From bottom to top: volumes added in μL (HSA concentration in μM): 0 (0), 10 (0.98), 20 (1.92), 30 (2.83), 50 (4.55), 75 (6.52), 100 (8.33), 150 (11.54), 225 (15.52), 300 (18.75), and 360 (20.93). Top spectrum: 50 μM HSA, 5 mM [C₄dmim][Cl].

[C₃Omim][Cl]

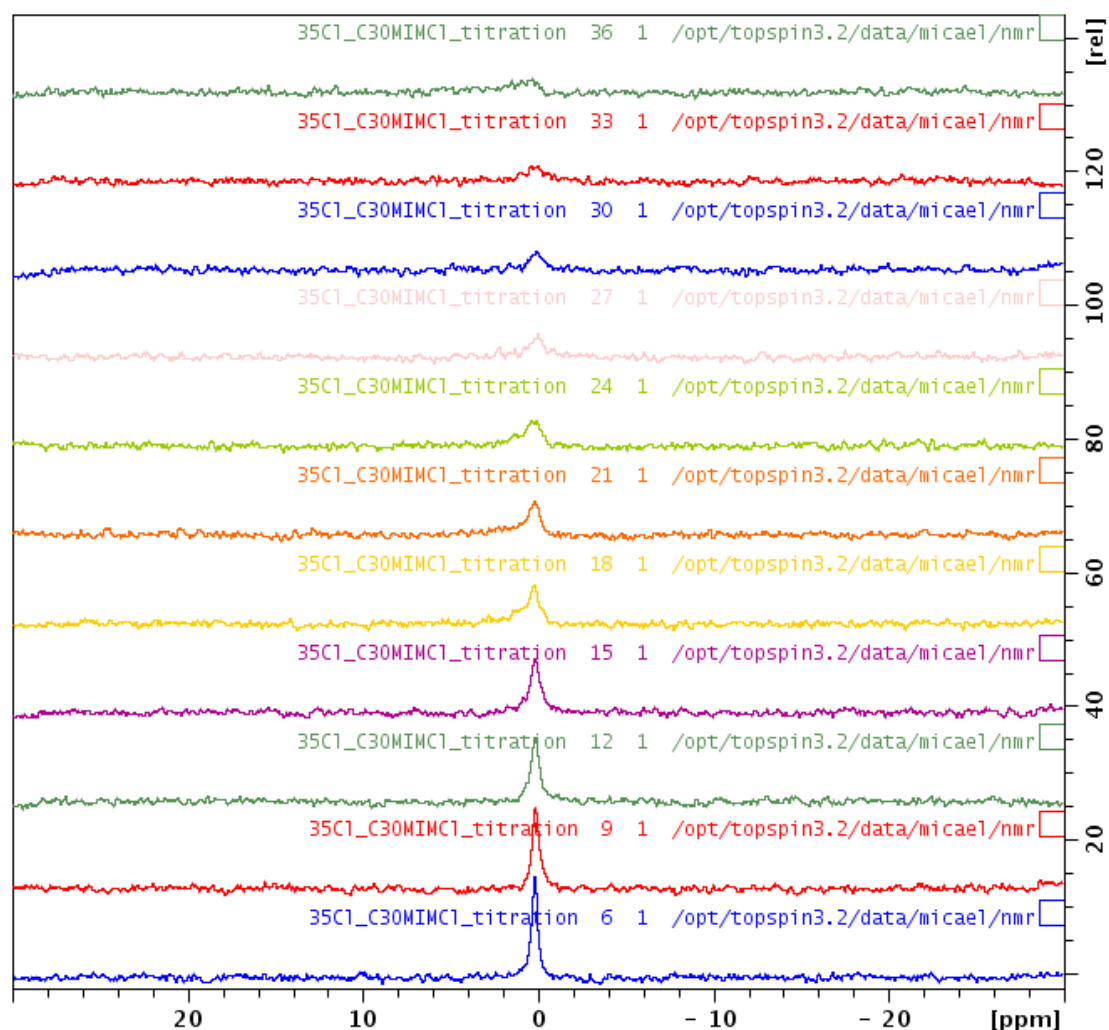


Figure S12 – Effect of HSA addition on the ³⁵Cl resonance of a sample containing 5 mM [C₃Omim][Cl] in D₂O (500 μL). Increased HSA concentration was achieved through the addition of small volumes of 50 μM HSA, 5 mM [C₃Omim][Cl] to maintain the total concentration of chlorine constant. From bottom to top: volumes added in μL (HSA concentration in μM): 0 (0), 10 (0.98), 20 (1.92), 30 (2.83), 50 (4.55), 75 (6.52), 100 (8.33), 150 (11.54), 225 (15.52), 300 (18.75), and 360 (20.93). Top spectrum: 50 μM HSA, 5 mM [C₃Omim][Cl].

[C₂OHmim][Cl]

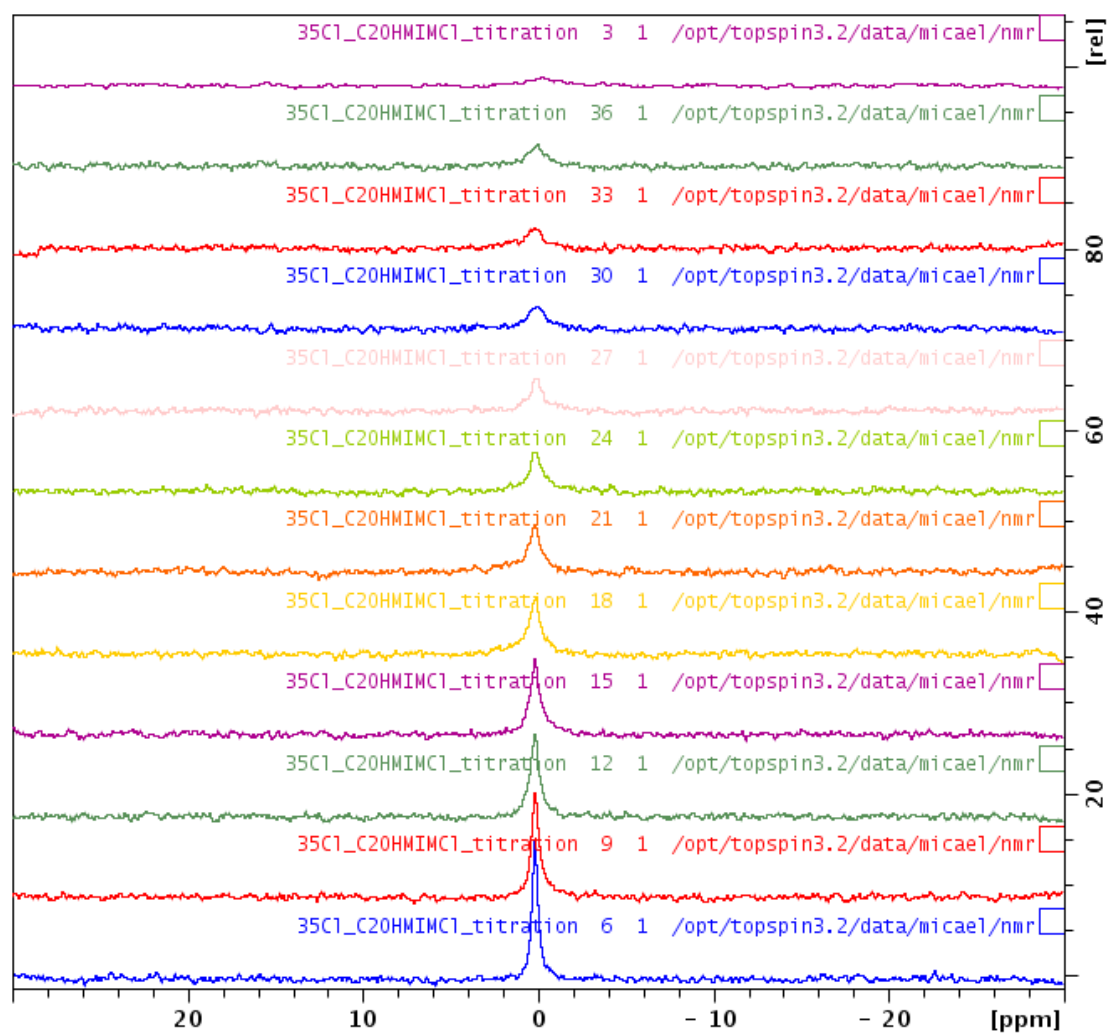


Figure S13 – Effect of HSA addition on the ³⁵Cl resonance of a sample containing 5 mM [C₂OHmim][Cl] in D₂O (500 μL). Increased HSA concentration was achieved through the addition of small volumes of 50 μM HSA, 5 mM [C₃Omim][Cl] to maintain the total concentration of chlorine constant. From bottom to top: volumes added in μL (HSA concentration in μM): 0 (0), 10 (0.98), 20 (1.92), 30 (2.83), 50 (4.55), 75 (6.52), 100 (8.33), 150 (11.54), 225 (15.52), 300 (18.75), and 360 (20.93). Top spectrum: 50 μM HSA, 5 mM [C₂OHmim][Cl].

Table S1 - NMR determined self-diffusion coefficient of HDO in IL solutions with different HSA concentrations

	<i>D ($\times 10^{-9} \text{ m}^2/\text{s}$)</i>		
	<i>0 μM HSA</i>	<i>21.75 μM</i>	<i>50 μM</i>
[C ₄ mim][Cl]	1.68 $\pm$ 0.01	1.68 $\pm$ 0.01	1.67 $\pm$ 0.01
[C ₄ dmim][Cl]	1.67 $\pm$ 0.01	1.68 $\pm$ 0.01	1.67 $\pm$ 0.01
[C ₂ mim][Cl]	1.69 $\pm$ 0.01	1.68 $\pm$ 0.01	1.65 $\pm$ 0.01
[C ₃ Omim][Cl]	1.69 $\pm$ 0.01	1.68 $\pm$ 0.01	1.66 $\pm$ 0.01
[C ₂ OHmim][Cl]	1.69 $\pm$ 0.01	1.68 $\pm$ 0.01	1.68 $\pm$ 0.01