



Supplementary Materials

Disclosing the Potential of Fluorinated Ionic Liquids as Interferon-Alpha 2b Delivery Systems

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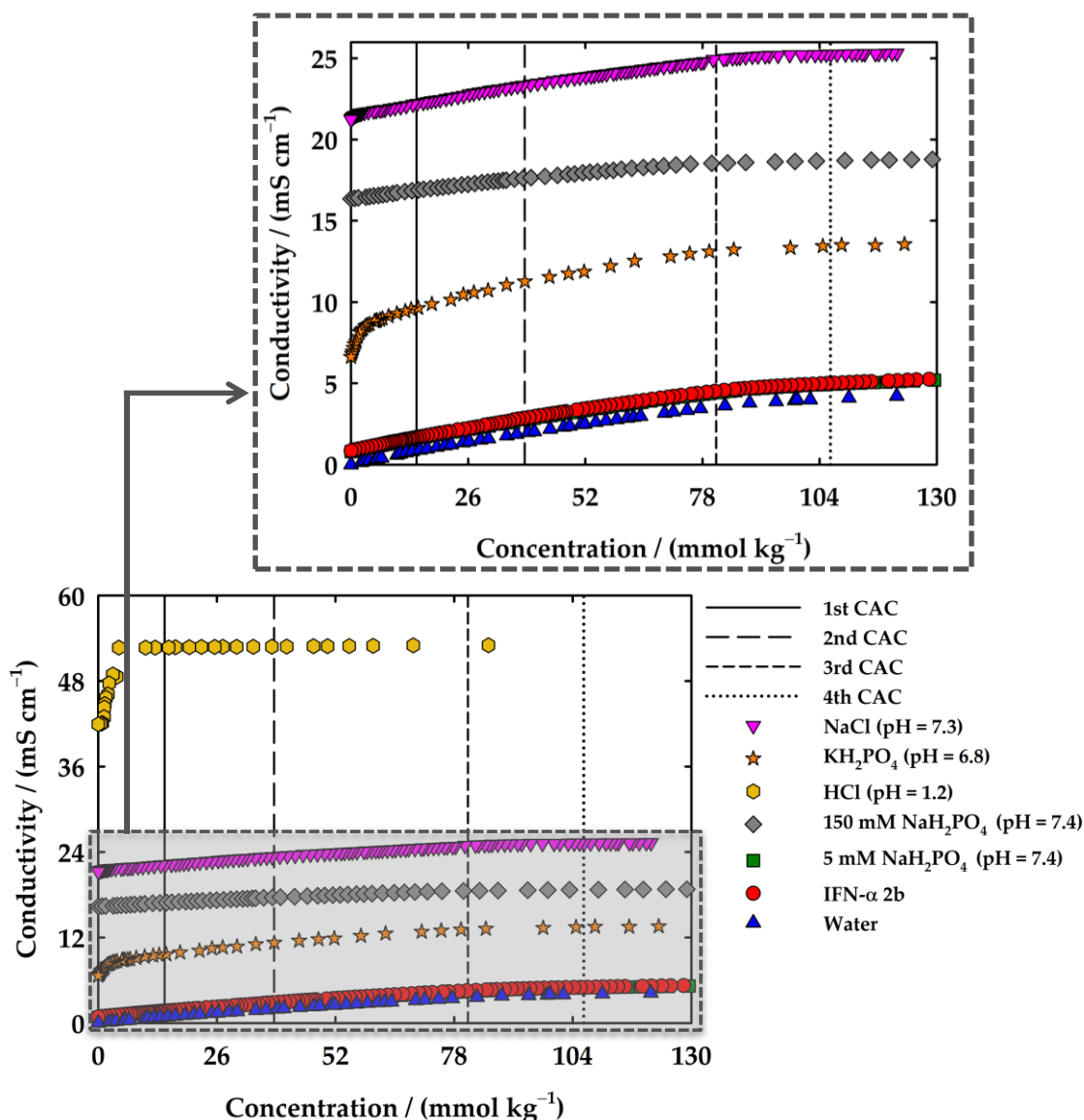


Figure S1. Conductivity profile of $[C_2C_1Im][C_4F_9SO_3]$ at 25°C from 0 to ~130 mM at different conditions: (i) 150 mM of NaCl at pH=7.3 (pink, \blacktriangledown); (ii) 25 mM of KH_2PO_4 at pH=6.8 (orange, \star); (iii) 100 mM of HCl at pH 1.2 (yellow, \bullet); (iv) 150 mM of NaH_2PO_4 at pH 7.4 (grey, \blacklozenge); (v) 5 mM of NaH_2PO_4 at pH 7.4 (green, \blacksquare); (vi) 5 mM of NaH_2PO_4 at pH 7.4 with 10 $\mu g/mL$ of IFN- α 2b (red, \bullet); and (vii) water (blue, \blacktriangle) [1] to comparison purposes. The lines represent the different CACs of $[C_2C_1Im][C_4F_9SO_3]$ in water [1].

Table S1. Critical aggregation concentrations, CACs, ionization degree, α , and Gibbs free energy of aggregation, ΔG^0_{agg} , of the systems with [C₂C₁Im][C₄F₉SO₃] determined by conductometry at 25°C

		150 mM NaCl (pH=7.3)	25 mM KH ₂ PO ₄ (pH=6.8)	100 mM HCl (pH=1.2)	150 mM NaH ₂ PO ₄ (pH=7.4)	5 mM NaH ₂ PO ₄ (pH=7.4)	10 µg/mL IFN- α 2b + 5 mM NaH ₂ PO ₄ (pH=7.4)	Water ^(a)
First	w_{FIL}	0.0014	0.0008	0.0019	0.0060	0.0056	0.0062	0.0060
	CAC							
	α							
	ΔG^0_{agg} [kJ mol ⁻¹]							
Second	w_{FIL}	0.0138	0.0158		0.0135	0.0189	0.0179	0.0158
	CAC							
	α							
	ΔG^0_{agg} [kJ mol ⁻¹]							
Third	w_{FIL}	0.0342	0.0293		0.0282	0.0341	0.0342	0.0332
	CAC							
	α							
	ΔG^0_{agg} [kJ mol ⁻¹]							

(a) Data from reference [1].

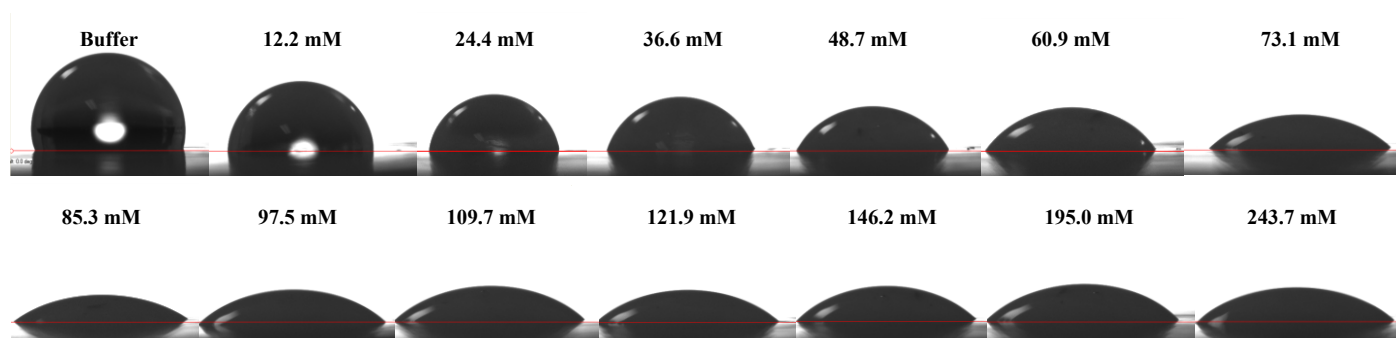
Table S2. Density, ρ , and mass fraction, w_{FIL} , for the binary system [C₂C₁Im][C₄F₉SO₃] + water at 25°C.

w_{FIL}	$\rho / \text{g} \cdot \text{cm}^{-3}$	Reference
1.0000	1.5458	[1]
0.9804	1.5257	[1]
0.9593	1.5060	[1]
0.9418	1.4949	[1]
0.9212	1.4765	[1]
0.8990	1.4615	[1]
0.8686	1.4421	[1]
0.8398	1.4270	[1]
0.7997	1.4049	[1]
0.7801	1.3914	[1]
0.7398	1.3603	[1]
0.6998	1.3417	[1]
0.6496	1.3081	[1]
0.5999	1.2773	[1]
0.5489	1.2462	[1]
0.5001	1.2177	[1]
0.2989	1.1135	This work
0.1000	1.0353	This work
0.0384	1.0104	This work
0.0245	1.0048	This work
0.0109	0.9994	This work
0.0030	0.9965	This work

Table S3. Critical aggregation concentration (CAC), surface properties, and critical packing parameters for [C₂C₁Im][C₄F₉SO₃] aqueous solutions determined by the tensiometer and goniometer at 25°C.

		5 mM NaH ₂ PO ₄ (pH=7.4)	5 µg/mL IFN-α 2b + 5 mM NaH ₂ PO ₄ (pH=7.4)				Water ^(a)
			Breakpoints				
			1 st	2 nd	3 rd	4 th	
Tensiometer	CAC (w_{FIL})	0.0330	0.0008	0.0025	0.0089	0.0335	0.0375
	CAC (mmol·kg ⁻¹)	80.35	1.963	6.105	21.61	81.59	91.39
	γ_{CAC} [mN m ⁻¹]	24.6	51.4	48.2	38.3	24.3	24.5
	$10^6 \Gamma_{max}$ [mol m ⁻²]	3.26	–	–	–	4.41	2.60
	A_{min} [nm ²]	0.51	–	–	–	0.38	0.64
	Π_{CAC} [mN m ⁻¹]	46.4	–	–	–	37.8	44.4
	ΔG^0_{ad} [kJ mol ⁻¹]	-41.0	–	–	–	-32.36	-44.5
Critical packing parameters	$P_{eq\ 8}^{(b)}$	0.550	–	–	–	0.7439	0.439
	$P_{eq\ 9}^{(b)}$	0.553	–	–	–	0.7481	0.442
Goniometer	CAC (w_{FIL})	0.0329	0.0009	0.0030	0.0157	0.0430	–
	CAC (mmol·kg ⁻¹)	80.27	2.101	7.288	38.18	104.7	–

(a) Parameters from reference [1]; (b) the critical packing parameters were obtained by Equations 8 and 9 of the paper.

**Figure S2.** Drops measured in goniometer at 25°C of [C₂C₁Im][C₄F₉SO₃] aqueous solutions at different concentrations in 5 mM NaH₂PO₄ (pH = 7.4). The red line indicates the surface where the drop was deposit.

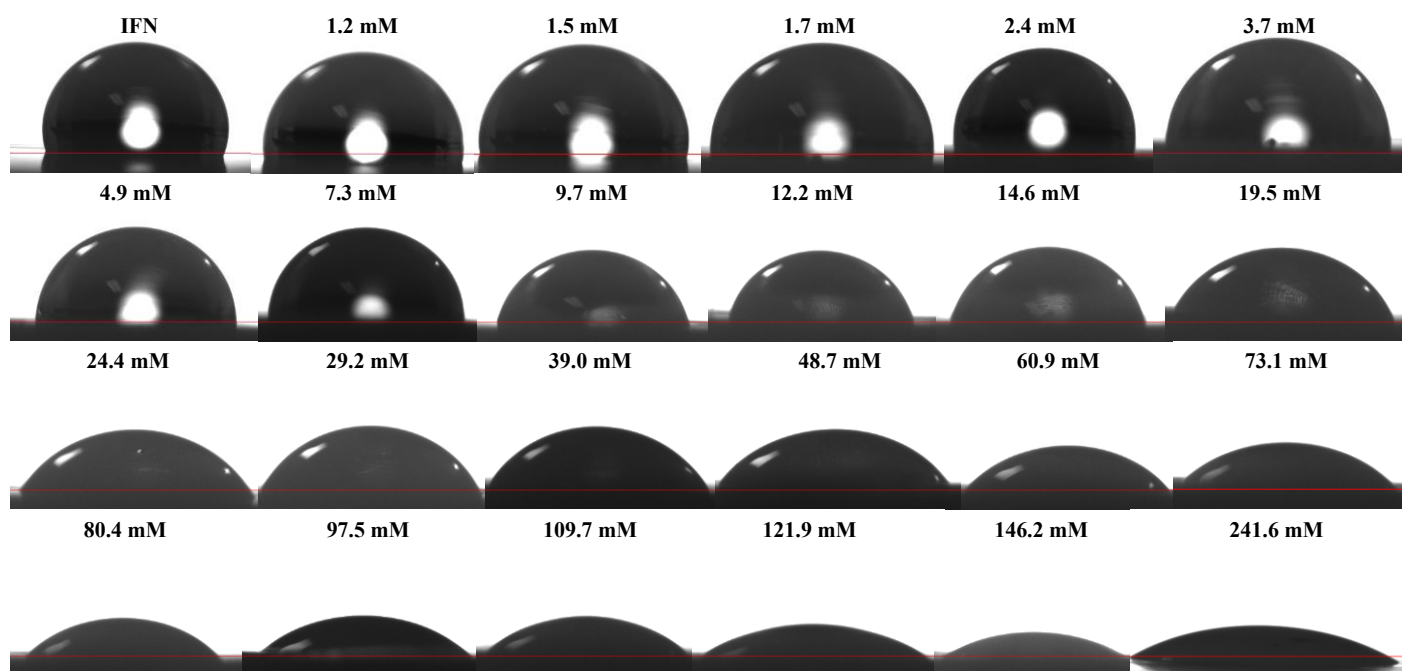


Figure S3. Drops measured in goniometer at 25°C of $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$ aqueous solutions at different concentrations with 5 µg/mL of IFN- α 2b in 5 mM NaH_2PO_4 (pH=7.4). The red line indicates the surface where the drop was deposit.

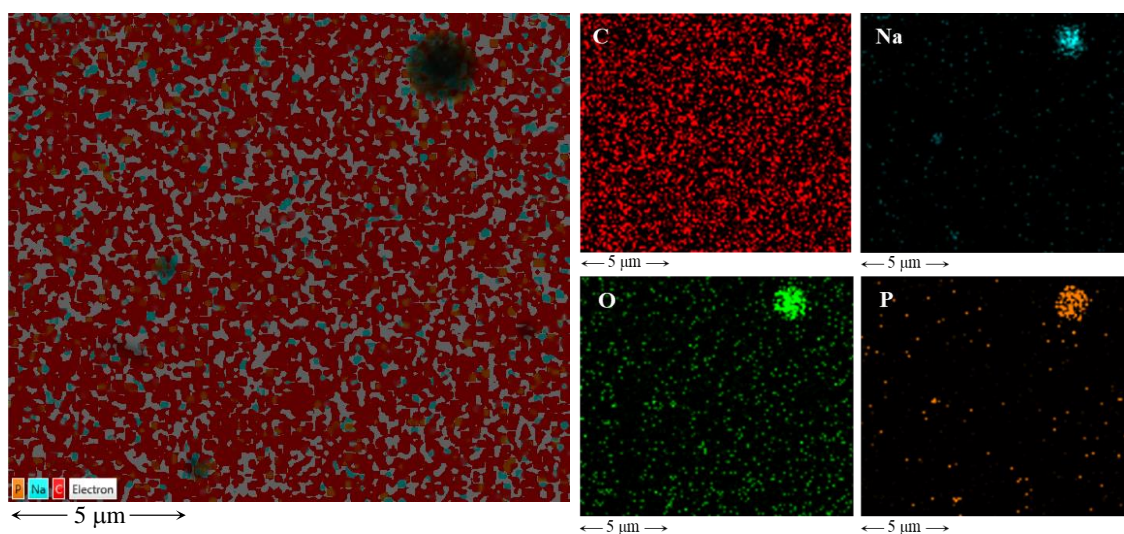


Figure S4. EDS analysis of IFN- α 2b at 0.0001 µg/mL in 150 mM NaH_2PO_4 measured at 25°C.

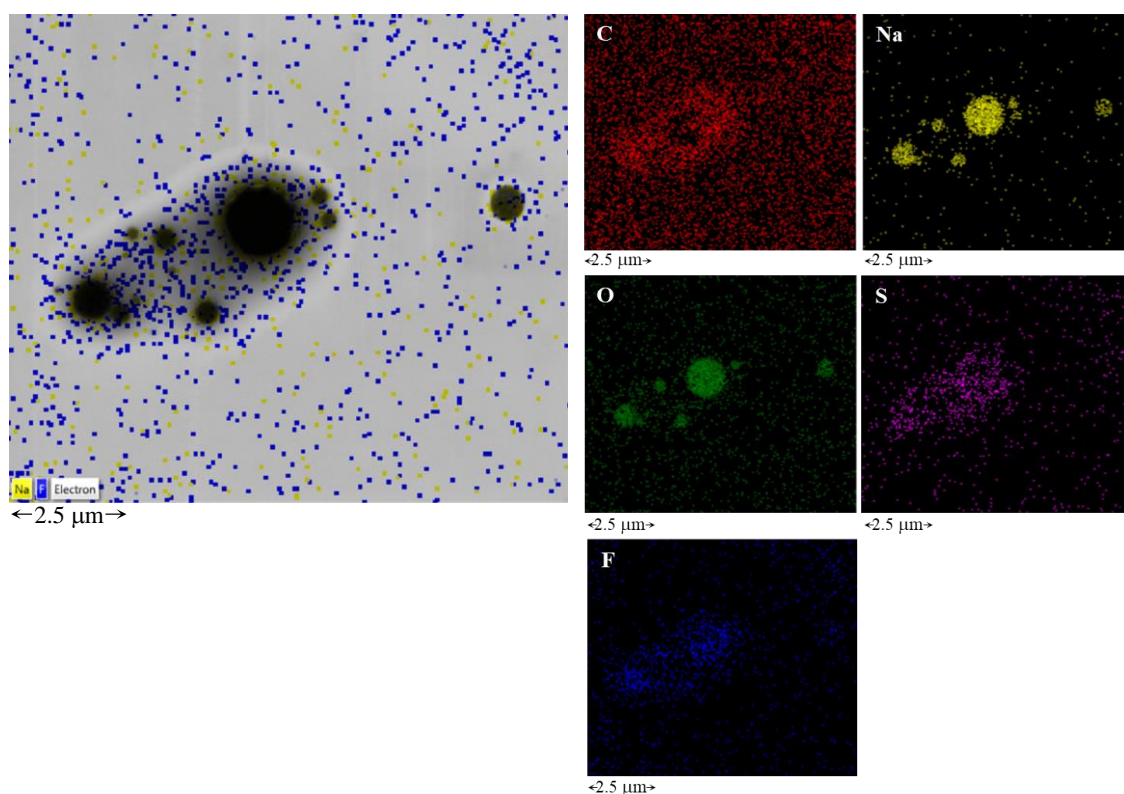


Figure S5. EDS analysis of $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$ at 29.2 mM in 150 mM NaH_2PO_4 measured at 25°C.

Table S4. Polydispersity index (PDI) obtained from DLS measurements in 150 mM NaH_2PO_4 measured at 25°C.

Sample	PDI
50 $\mu\text{g/mL}$ IFN α -2b	0.427
29.3 mM $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$	1.000
29.3 mM $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$ + 50 $\mu\text{g/mL}$ IFN α -2b	0.630
243.8 mM $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$	0.157
243.8 mM $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$ + 50 $\mu\text{g/mL}$ IFN α -2b	0.279
29.8 mM $[\text{N}_{1112}(\text{OH})][\text{C}_4\text{F}_9\text{SO}_3]$	0.784
29.8 mM $[\text{N}_{1112}(\text{OH})][\text{C}_4\text{F}_9\text{SO}_3]$ + 50 $\mu\text{g/mL}$ IFN α -2b	0.628
248.0 mM $[\text{N}_{1112}(\text{OH})][\text{C}_4\text{F}_9\text{SO}_3]$	0.543
248.0 mM $[\text{N}_{1112}(\text{OH})][\text{C}_4\text{F}_9\text{SO}_3]$ + 50 $\mu\text{g/mL}$ IFN α -2b	0.849

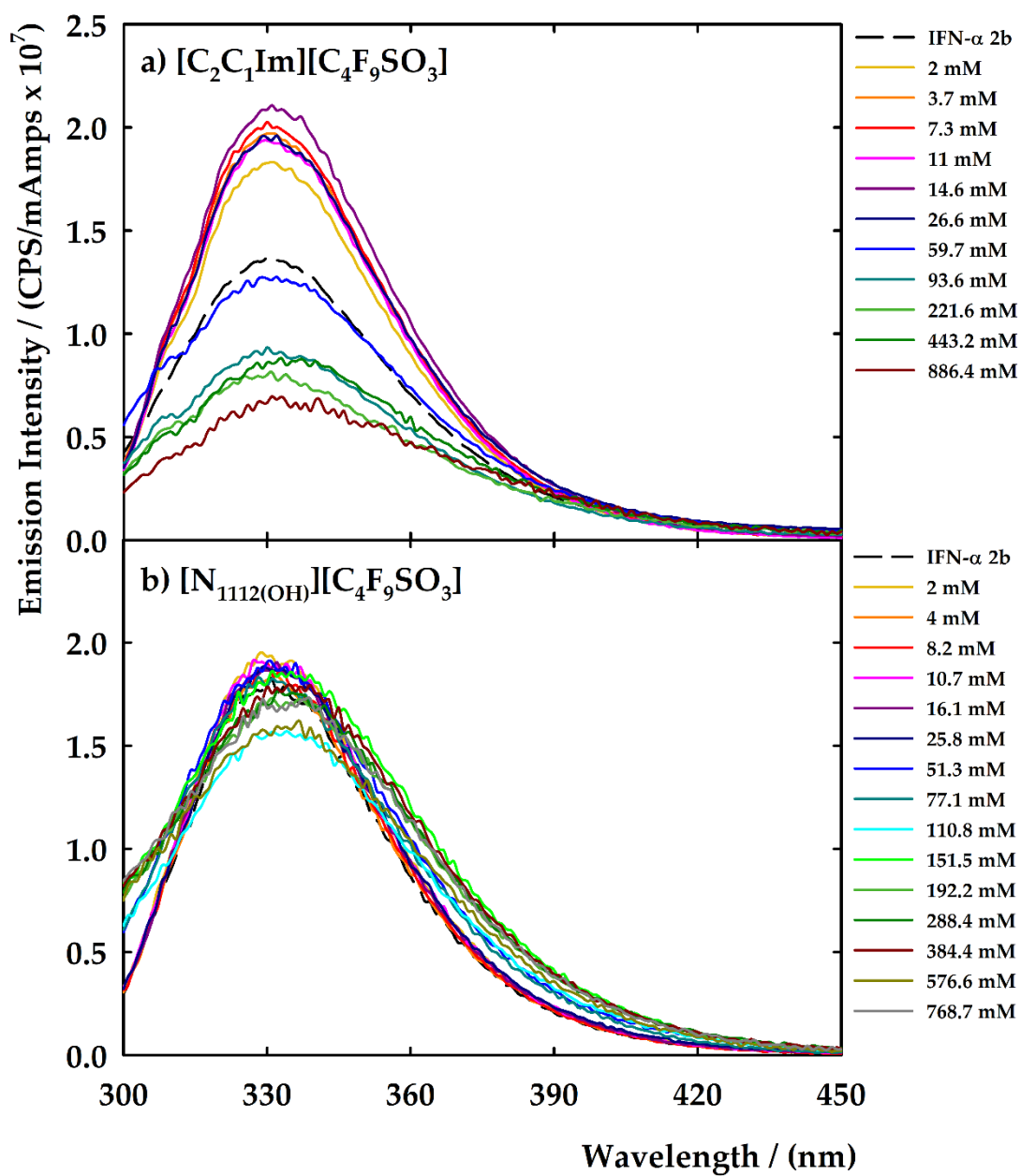


Figure S6. Fluorescence spectra of IFN- α 2b (20 μ g/mL, black dashed line) with a) $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$ and b) $[\text{N}_{1112}(\text{OH})][\text{C}_4\text{F}_9\text{SO}_3]$ at different concentrations. All samples were prepared in 5 mM NaH_2PO_4 at pH = 7.4 and measured at 25°C.

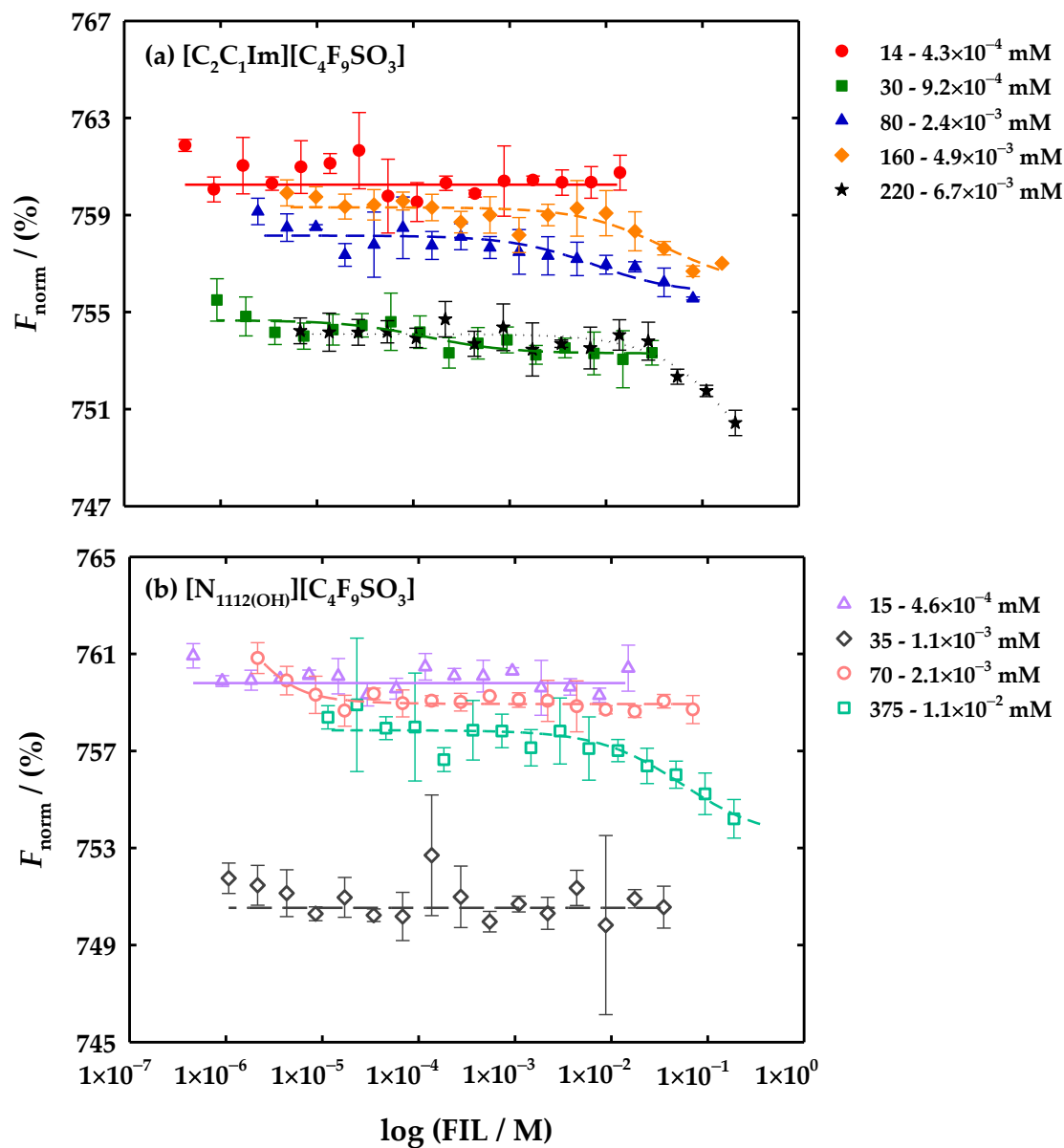


Figure S7. Dose-response curve from MST of labelled IFN- α at 2.7 μM in the presence of different ranges of concentrations of a) $[\text{C}_2\text{C}_1\text{Im}][\text{C}_4\text{F}_9\text{SO}_3]$ and b) $[\text{N}_{1112}(\text{OH})][\text{C}_4\text{F}_9\text{SO}_3]$. All the samples were measured in 5 mM NaH_2PO_4 (pH = 7.4) at 25°C. The error bars represent the standard deviations from the triplicate assays.

References

1. Pereiro, A.B.; Araújo, J.M.M.; Teixeira, F.S.; Marrucho, I.M.; Piñeiro, M.M.; Rebelo, L.P.N. Aggregation behavior and total miscibility of fluorinated ionic liquids in water. *Langmuir* **2015**, *31*, 1283–1295. <https://doi.org/10.1021/la503961h> (reference 38 of the paper)