Electronic Supplementary Information

Plasma membrane permeabilisation by ionic liquids: a matter of charge

Diego O. Hartmann,[a] Karina Shimizu,[b†] Filipa Siopa,[c†] M. Cristina Leitão,[a]
Carlos A. M. Afonso,[c] José N. Canongia Lopes[a,b] and Cristina Silva Pereira[*a]

[a] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

[b] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal

[c] iMed.ULisboa, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

[†] Equally contributing authors

[*] Corresponding author: spereira@itqb.unl.pt

Experimental

General remarks

All chemicals, reagents and solvents for the synthesis of the compounds were of analytical grade, purchased from commercial sources, namely Sigma-Aldrich®, Merck and Alfa Aesar and these were used without further purification. The synthesis of the alkyl-(2-hydroxyethyl)-dimethylammonium bromides ($[N_{1\,1\,n\,2OH}]$, *n* = 2, 4, 6 ,8 and 12) has been previously described by us.[1] ^1H and ^{13}C NMR spectra were measured on an Ultrashield Bruker Avance II 300 spectrometer and Ultrashield Bruker Avance II 400 spectrometer. Splitting patterns are indicated as *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet; *br*, broad peak. The low resolution electrospray ionization mass spectra were recorded using a Micromass® Quattro Micro triple quadruple (Waters®, Ireland) with an electrospray (ESI) ion source. Elemental analysis was performed in a Flash 2000 CHNS-O analyzer (Thermo Scientific, UK). Melting points were determined on a Stuart SMP10 apparatus**.**

General method for the synthesis of alkyl-(2-hydroxyethyl)-dimethylammonium bromides

In a Aldrich ace pressure tube (Z181064) at room temperature, a solution of the correspondent brominated derivative (45 mmol) dissolved in MeCN (5 mL) was added to a solution of 2-(dimethylamino)ethanol (4.1 g, 45 mmol) in MeCN (5 mL) and heated overnight at 60 ºC, unless stated. For the compounds $[N_{1\,1\,2\,2OH}]$Br, $[N_{1\,1\,4\,2OH}]$Br, the salt precipitated during the reaction, while for the compound $[N_{1\,1\,12\,2OH}]$Br, the salt precipitated when the Aldrich ace pressure tube was cooling to room temperature. Diethyl ether was added and the salt was filtered and dried under vacuum. For the compounds $[N_{1\,1\,6\,2OH}]$Br, $[N_{1\,1\,8\,2OH}]$Br, and $[N_{1\,1\,10\,2OH}]$Br, in which the reaction mixture was liquid, the solvent was evaporated and diethyl ether was added to precipitate the product. The ionic liquid was then filtered and dried under vacuum.

Ethyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,2\,2OH}]$Br)**.** The reaction mixture was heated for 20 min. The product was collected as a white solid in 96 % yield, m.p. 280-282ºC (Lit. 282 - 284ºC)[2]. ^1H NMR (300 MHz, D_2O) δ 4.02-3.97 (m, 2H), 3.47 – 3.39 (m, 4H), 3.08 (s, 6H), 1.35 – 1.28 (m, 3H). ^{13}C NMR (75 MHz, D_2O) δ 64.41, 61.04, 55.39, 50.91, 50.86, 7.73. Anal. Calcd for $C_6H_{16}BrNO$: C, 36.38; H, 8.14; N, 7.07. Found: C, 36.13; H, 8.24; N, 7.24. Spectral data ^1H and ^{13}C NMR identical to reported one.[3]

Butyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,4\,2OH}]$Br)**.** The salt was recovered as a white solid in 96 %, m.p. 110-113 ºC. ^1H NMR (300 MHz, D_2O) δ 3.97 (s, 2H), 3.44 – 3.40 (m, 2H), 3.34 - 3.29 (m, 2H), 3.07 (s, 6H), 1.75 – 1.64 (m, 2H), 1.38 – 1.25 (m, 2H), 0.88 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, D_2O) δ 65.30, 64.83, 55.39, 51.46, 51.41, 23.98, 19.10, 12.91. Anal. Calcd for $C_8H_{20}BrNO$: C, 42.49; H, 8.91; N, 6.19. Found: C,42.64; H, 9.22; N, 6.43. Spectral data ^1H and ^{13}C NMR identical to reported one.[3]

Hexyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,6\,2OH}]$Br)**.** The reaction mixture was heated during 72 h. The product was obtained as a white gummy in 95 %. ^1H NMR (300 MHz, $CDCl_3$) δ 3.79 (s, 2H), 3.44 – 3.41 (m, 2H), 3.33 – 3.27 (m, 2H), 3.08 (s, 6H), 1.48 (s, 2H), 1.05 – 1.01 (m, 6H), 0.59 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.25, 65.86, 55.17, 51.49, 30.60, 25.27, 22.17, 21.79, 13.37. Spectral data ^1H and ^{13}C NMR identical to reported one.[3]

Octyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,8\,2OH}]$Br)**.** A white salt was obtained in 93 %, m.p. 107-110 ºC (Lit. 116 – 118 ºC)[4]. ^1H NMR (300 MHz, CDCl3) δ 4.07 (s, 2H), 3.71 - 3.70 (m, 2H), 3.54 – 3.49 (m, 2H), 3.33 (s, 6H), 1.71 (s, 2H), 1.31 - 1.22 (m, 10H), 0.83

(t, J = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 66.00, 65.56, 55.82, 52.07, 31.61, 29.12, 29.02, 26.24, 22.85, 22.54, 14.05. Spectral data ^{1}H NMR identical to reported one.[4]

Decyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,10\,2OH}]Br$**).** The product was obtained as a white solid in 95 %, m.p. 167-168 ºC. ^{1}H NMR (400 MHz, D_2O) δ 3.95 (s, 2H), 3.43 (s, 2H), 3.34 – 3.32 (m, 2H), 3.08 (s, 6H), 1.70 (s, 2H), 1.28 – 1.20 (m, 14H), 0.78 (d, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 65.25, 64.95, 55.37, 51.51, 31.69, 29.18, 28.83, 25.95, 22.47, 22.29, 13.77. Anal. Calcd for $C_{14}H_{32}BrNO$: C, 54.19; H, 10.39; N, 4.51. Found: C, 54.19; H, 10.30; N, 4.53. Spectral data ^{1}H identical to reported one.[5]

Dodecyl-(2-hydroxyethyl)-dimethylammonium bromide ($[N_{1\,1\,12\,2OH}]Br$**).** A white solid was recovered in 95 %, m.p. 190-193 ºC (Lit 193 - 196 ºC)[6]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.04 (s, 2H), 3.69 – 3.66 (m, 2H), 3.52 – 3.46 (m, 2H), 3.30 (s, 6H), 1.68 (s, 2H), 1.28 – 1.18 (m, 18H), 0.80 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 66.01, 65.55, 55.80, 52.06, 31.85, 29.56, 29.46, 29.40, 29.29, 29.21, 26.26, 22.87, 22.64, 14.09. Spectral data ^{1}H NMR identical to reported one.[5]

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Table S1. Liquid chromatography conditions for the quantification of ionic liquids organic ions in the culture media. The tested ionic liquids were: 1-alkyl-3-methylimidazolium chlorides ($[C_n mim]Cl$, n = 2, 4, 6 8 or 10), cholinium alkanoates (anion = $[eth]^-$, $[but]^-$, $[hex]^-$, $[oct]^-$ or $[dec]^-$) and alkyl-(2-hydroxyethyl)-dimethylammonium bromides ($[N_{1\,1\,n\,2OH}]Br$, n = 2, 4, 6)[a].

Ion	Detection / nm	Column	Mobile phase[b]	Flow rate / mL min^{-1}
$[C_2mim]^+$	218	Synergi Polar-RP column (150 × 4.6 mm) packed with polar endcapped particles (4 μm, pore size 80 Å) (Phenomenex), set at 26 °C	98% A1 + 2% B1	0.8
$[C_4mim]^+$			94% A1 + 6% B1	1.2
$[C_6mim]^+$			80% A1 + 20% B1	1.2
$[C_8mim]^+$			50% A1 + 50% B1	1.0
$[C_{10}mim]^+$			40% A1 + 60% B1	1.0
$[N_{1\,1\,1\,2OH}]^+$	215	Synergi Polar-RP column (150 × 4.6 mm) packed with polar endcapped particles (4 μm, pore size 80 Å) (Phenomenex), set at 26 °C	98% A2 + 2% B2	1.0
$[N_{1\,1\,2\,2OH}]^+$			95% A2 + 5% B2	
$[N_{1\,1\,4\,2OH}]^+$			70% A2 + 30% B2	
$[N_{1\,1\,6\,2OH}]^+$			45% A2 + 55% B2	
$[eth]^-$	212	Acquity UPLC HSS C18 (2.1 × 150 mm), 1.8 μm particle size column (Waters), set at 25 °C	Linear gradient from 10% to 95% of B3 (90% to 5% of A3) in 5.7min, then to 100% of B3in 1.3 min. Return to initial conditions in 1.5 min, maintaining for 1.5 min	0.4
$[but]^-$				
$[hex]^-$				
$[oct]^-$				
$[dec]^-$				

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[a] The $[N_{1\,1\,8\,2OH}]^+$, $[N_{1\,1\,10\,2OH}]^+$ and $[N_{1\,1\,12\,2OH}]^+$ cations could not be quantified since their initial concentrations were below the detection limits of the established method. [b] A1) aqueous solution of 5 mM phosphate buffer, pH 3; B1) acetonitrile; A2) aqueous solution of 0.1% (v/v) heptafluorobutyric acid, pH 6; B2) methanol, A3) aqueous solution of 0.1% (v/v) phosphoric acid; B3) acetonitrile.

Table S2. List of the designed *q*RT-PCR oligonucleotides (forward and reverse) used in this study. Oligonucleotides were designed based on the sequences of *A. nidulans* genes, obtained from the *Aspergillus* Genome Database (http://www.aspergillusgenome.org/). The selected genes are involved in plasma membrane biosynthesis of *Aspergillus nidulans*. The γ-actin (*actA*) gene was selected as housekeeping (internal control).

Name	**Gene (Code)**	**Sequence (5′ to 3′)**
HMGR1f	*HMGR1* (AN3817)	AAGCACTCCATGTCATGGCTA
HMGR1r	*HMGR1* (AN3817)	TAATAGCCTCAGCCACAACTGA
AN9407F	*fasA* (AN9407)	CTTCAAAGCAAGGTCTTCCTGA
AN9407R	*fasA* (AN9407)	CGCCAGGGACTCAATCACA
AN9408F	*fasB*(AN9408)	TTGGAAGACGTGAAGGCTCA
AN9408R	*fasB* (AN9408)	GGTACGTCGATTCCCTTCAGA
barAf	*barA* (AN4332)	GTGGTGCTCAACCTGATGGA
barAr	*barA* (AN4332)	TGGGTACATGATATGGCGTGA
lagAf	*lagA* (AN2464)	TCCCCACAGAGAGCACGAA
lagAr	*lagA* (AN2464)	GATGATGTGGTGGCCCACA
ACTF	*actA* (AN6542)	CTGGGACGACATGGAGAAGAT
ACTR	*actA* (AN6542)	GTAGATGGGGACGACGTGAG

Table S3. Minimal inhibitory and fungicidal concentrations (MIC and MFC, respectively) of 1-alkyl-3-methylimidazolium chlorides ([$C_n$mim]Cl, n = 2, 4, 6, 8 or 10), cholinium alkanoates (anion = $[eth]^-$, $[but]^-$, $[hex]^-$, $[oct]^-$ or $[dec]^-$) and alkyl-(2-hydroxyethyl)-dimethylammonium bromides ([$N_{1\ 1\ n\ 2OH}$]Br, n = 2, 4, 6, 8, 10 or 12) defined for *Aspergillus nidulans*.

Ionic liquid	**MIC / mM**	**MFC / mM**
1-alkyl-3-methylimidazolium chlorides		
[C_2mim]Cl	1390	>4000*
[C_4mim]Cl	445	450
[C_6mim]Cl	7	7
[C_8mim]Cl	0.9	0.9
[C_{10}mim]Cl	0.2	0.2
cholinium alkanoates		
[$N_{1\ 1\ 1\ 2OH}$][eth]	1450	>4000*
[$N_{1\ 1\ 1\ 2OH}$][but]	345	1000
[$N_{1\ 1\ 1\ 2OH}$][hex]	56	59
[$N_{1\ 1\ 1\ 2OH}$][oct]	19.8	22.8
[$N_{1\ 1\ 1\ 2OH}$][dec]	4.3	5
alkyl-(2-hydroxyethyl)-dimethylammonium bromides		
[$N_{1\ 1\ 2\ 2OH}$]Br	>2000*	>2000*
[$N_{1\ 1\ 4\ 2OH}$]Br	890	>2000*
[$N_{1\ 1\ 6\ 2OH}$]Br	105	105
[$N_{1\ 1\ 8\ 2OH}$]Br	16.5	16.5
[$N_{1\ 1\ 10\ 2OH}$]Br	2	2
[$N_{1\ 1\ 12\ 2OH}$]Br	0.2	0.2

*MIC or MFC could not be determined and is estimated to be above the maximum concentrations tested

Table S4. Percentage of membrane-damaged conidia after one hour of incubation with distinct concentrations of 1-alkyl-3-methylimidazolium chlorides ([C$_n$mim]Cl, n = 2, 4, 6 8 or 10), cholinium alkanoates (anion = [eth]$^-$, [but]$^-$, [hex]$^-$, [oct]$^-$ or [dec]$^-$) or alkyl-(2-hydroxyethyl)-dimethylammonium bromides ([N$_{1\,1\,n\,2OH}$]Br, n = 2, 4, 6, 8, 10 or 12). Values were obtained as: (number of propidium iodide-stained conidia / total number of conidia) × 100. Percentage in the saline solution control was 5.87%. Emboldened values were obtained with concentrations above the minimal fungicidal concentration.

Ionic liquid	**Concentration / mM**						
	0.01	0.1	1	10	100	1000	2000
1-alkyl-3-methylimidazolium chlorides							
[C$_2$mim]Cl	n.d.	n.d.	n.d.	n.d.	n.d.	7.8 ± 3.6	8.0 ± 3.7
[C$_4$mim]Cl	n.d.	5.6 ± 0.5	6.3 ± 1.8	6.3 ± 1.1	32.3 ± 2.0	**32.4 ± 2.1**	n.d.
[C$_6$mim]Cl	7.1 ± 0.7	9.2 ± 1.5	11.4 ± 0.6	**25.0 ± 3.7**	**34.6 ± 1.0**	n.d.	n.d.
[C$_8$mim]Cl	8.2 ± 2.2	10.5 ± 2.0	**16.8 ± 3.6**	**46.7 ± 3.6**	**92.6 ± 3.8**	n.d.	n.d.
[C$_{10}$mim]Cl	9.8 ± 1.8	13.6 ± 1.5	**50.0 ± 6.3**	**91.4 ± 4.6**	**94.7 ± 4,1**	n.d.	n.d.
cholinium alkanoates							
[N$_{1\,1\,1\,2OH}$][eth]	n.d.	n.d.	n.d.	n.d.	n.d.	12.4 ± 6.2	27.9 ± 1.9
[N$_{1\,1\,1\,2OH}$][but]	n.d.	5.6 ± 0.2	6.9 ± 1.0	8.1 ± 1.4	9.2 ± 0.9	**31.2 ± 4.1**	n.d.
[N$_{1\,1\,1\,2OH}$][hex]	5.8 ± 0.9	5.7 ± 0.8	6.1 ± 0.3	6.6 ± 1.3	**8.2 ± 1.6**	n.d.	n.d.
[N$_{1\,1\,1\,2OH}$][oct]	5.2 ± 0.9	5.9 ± 1.1	5.7 ± 1.3	6.5 ± 1.1	**9.7 ± 1.9**	n.d.	n.d.
[N$_{1\,1\,1\,2OH}$][dec]	5.3 ± 1.0	7.1 ± 1.0	6.9 ± 2.7	**14.2 ± 1.3**	**25.9 ± 2.7**	n.d.	n.d.
alkyl-(2-hydroxyethyl)-dimethylammonium bromides							
[N$_{1\,1\,2\,2OH}$]Br	n.d.	n.d.	n.d.	n.d.	n.d.	12.8 ± 1.0	n.d.
[N$_{1\,1\,4\,2OH}$]Br	n.d.	5.9 ± 1.0	5.9 ± 0.7	7.5 ± 0.5	11.3 ± 1.4	11.6 ± 2.0	n.d.
[N$_{1\,1\,6\,2OH}$] Br	6.0 ± 2.0	5.9 ± 0.3	4.6 ± 0.5	11.0 ± 0.8	25.8 ± 0.5	n.d.	n.d.
[N$_{1\,1\,8\,2OH}$] Br	5.0 ± 0.8	7.6 ± 1.7	7.8 ± 0.8	20.7 ± 2.4	**91.3 ± 2.6**	n.d.	n.d.
[N$_{1\,1\,10\,2OH}$] Br	5.8 ± 1.7	6.5 ± 2.0	33.2 ± 0.6	**82.0 ± 3.0**	**93.8 ± 1.7**	n.d.	n.d.
[N$_{1\,1\,12\,2OH}$] Br	8.8 ± 1.5	25.8± 0.5	**86.8 ± 5.1**	**94.8 ± 2.1**	**95.2 ± 0.7**	n.d.	n.d.

n.d. = not determined.

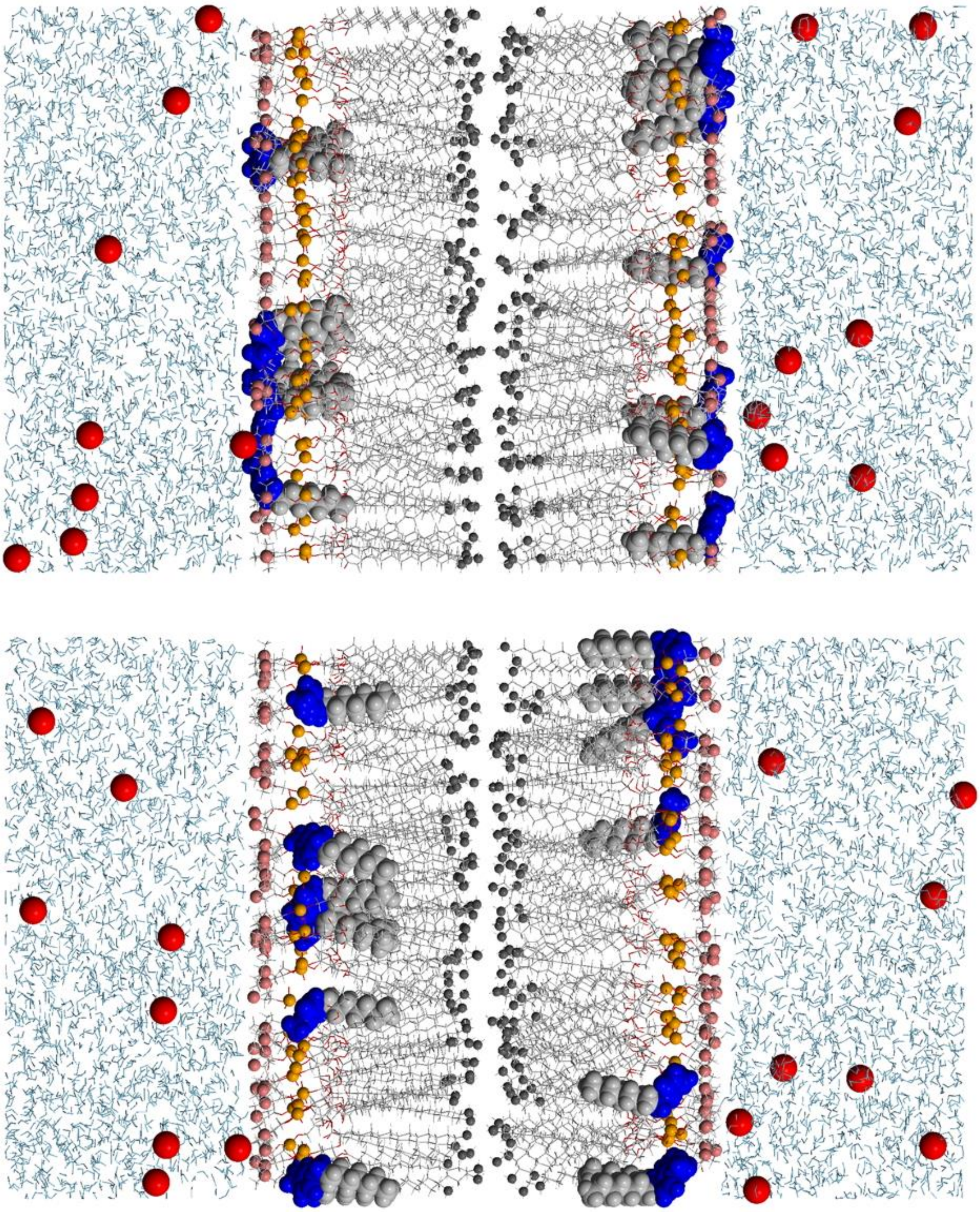

Figure S4. Initial configuration snapshots of a section of a mixed phospholipids bilayer in water with 16 ion pairs (1-octyl-3-methylimidazolium chloride, [C$_8$mim]Cl) initially incorporated in the bilayer: (top) charged cation headgroups close to the ammonium groups in the phospholipids; (bottom) charged cation headgroups close to the phosphate groups in the phospholipids.

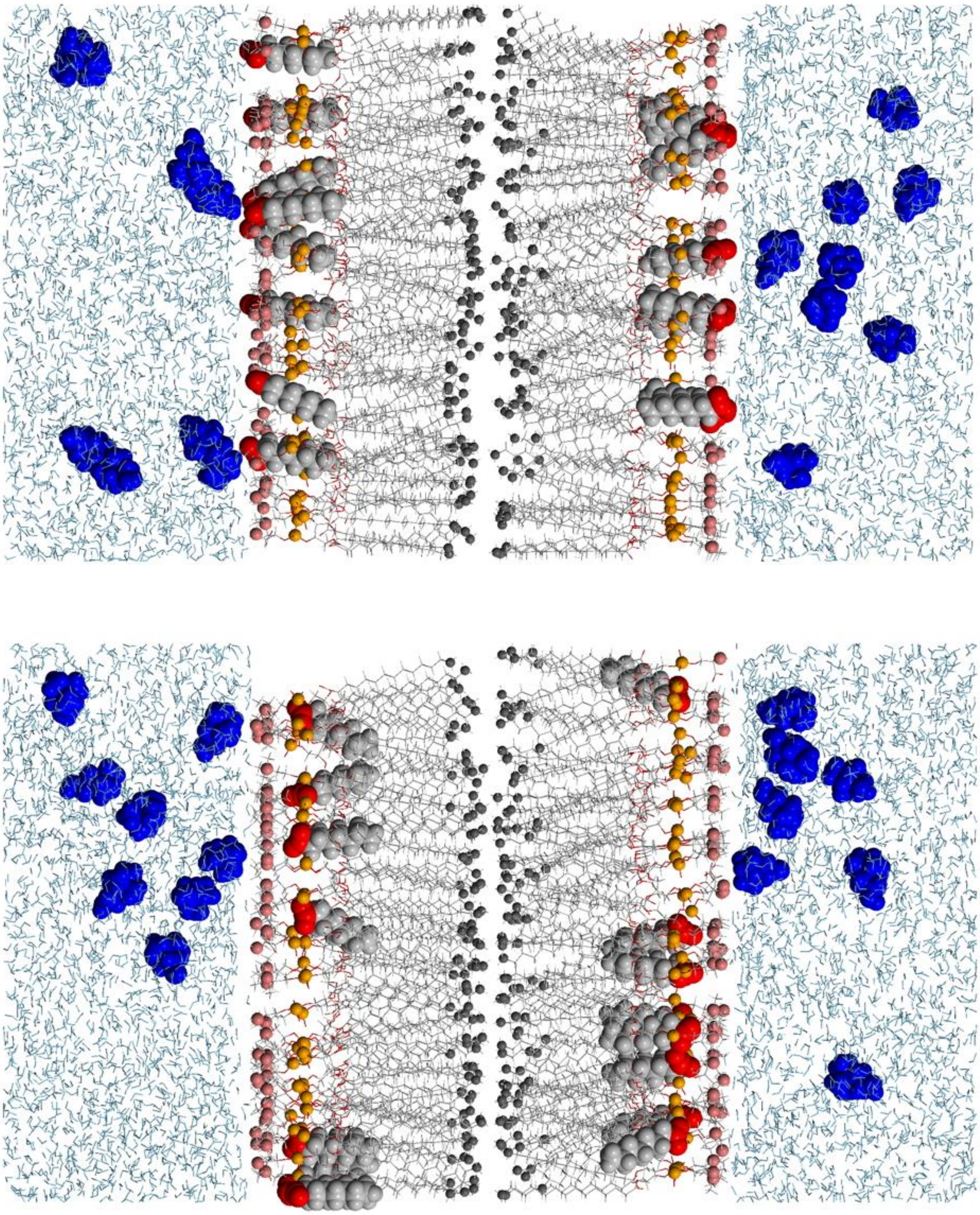

Figure S5. Initial configuration snapshots of a section of a mixed phospholipids bilayer in water with 16 ion pairs (cholinium octanoate, $[N_{1\,1\,1\,2OH}]$[oct]) initially incorporated in the bilayer: (top) charged anion headgroups close to the ammonium groups in the phospholipids; (bottom) charged anion headgroups close to the phosphate groups in the phospholipids.