

Binding of cations to phospholipid bilayers

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Despite of vast amount of experimental and theoretical studies the binding affinity of cations, especially biologically relevant Na^+ and Ca^{2+} ions, into a phospholipid bilayer is not agreed in the literature. Here we directly compare the measured choline headgroup order parameters to the simulations with different models in the presence of different cations. We conclude that the least complicated explanation for the experimental and theoretical observations is that the Na^+ ions do not penetrate into a phosphatidylcholine lipid bilayer in mM concentrations, in contrast to Ca^{2+} . Further, the binding affinity of Na^+ is overestimated in almost all molecular dynamics simulation models. However, the electrometer concept connecting the choline order parameter changes to the penetrating charges is valid also in simulations. This work has been, and continues to be, progressed and discussed through the blog: nmrlipids.blogspot.fi. Everyone is invited to join the discussion and make contributions through the blog. The manuscript will be eventually submitted to an appropriate scientific journal. Everyone who has contributed to the work through the blog will be offered coauthorship. For more details see: nmrlipids.blogspot.fi.

I. INTRODUCTION

The cation interactions with phospholipid membranes are present in large amount of physiological processes, nerve cell signalling being the prime example. Thus, the interactions between different cations and phospholipid bilayers have been very widely studied experimentally and theoretically. It is practically agreed that the relative binding affinity of different ions follows the Hofmeister series [2–10], however the quantitative binding affinities of different ions are not agreed in the literature. The extensive reviews about work done prior 1990 [3, 4] concluded that monovalent cations (lithium being an exception) interact only weakly with phospholipid bilayers, while the interactions are significant for multivalent ions. This conclusion has been supported by further studies where the bilayer properties have been intact with increasing monovalent salt concentration [5, 11, 12]. On the other hand, the weak interactions of monovalent ions, especially sodium, has been also questioned in several more recent studies suggesting stronger binding [7–10, 13–19]. In this work we concentrate on the interactions between phospholipid bilayer and two widely studied and biologically relevant cations, monovalent sodium and divalent calcium.

The experimental studies supporting the weak interactions between sodium ions and phospholipid bilayer have shown that NaCl with mM concentrations has a negligible effect on choline headgroup order parameters [20], area per molecule [11], dipole potential [21] and lipid lateral diffusion [12] while these properties were significantly affected by the presence of CaCl_2 or other multivalent ions. In addition, water sorption isotherm for POPC/NaCl system was essentially similar to NaCl in pure water indicating only weak interaction between ion and lipid [5]. Only minor changes in

POPC infrared spectra were observed in the presence of NaCl compared to the significant changes in the presence of CaCl_2 and other multivalent ions, and it was again concluded that the Na^+ -lipid interactions are weak [5].

In contrast, experimentally observed decrease in fluorescent probe rotational and translational dynamics in lipid bilayer due to addition of NaCl has suggested significant ion binding also with mM concentrations [8, 10, 13]. However, the reduced lateral diffusion is not observed in NMR experiments without probes suggesting that it would arise from interactions between probe and ion, not between lipid and ion [12]. NaCl binding to phospholipids leading to the change of the strength of bilayer against rupture and area per lipid reduction has been also suggested from Atomistic Force Microscopy (AFM) [15–19]. Interpretation of calorimetric measurements has been controversial: Previously the small effect of monovalent ions (except Lithium) on phase transition temperature compared to multivalent ions was interpreted such that only multivalent ions and Lithium specifically bind to phospholipid bilayer [3], however more recently the small changes in calorimetric experiments have been interpreted to indicate also Na^+ binding [9, 13]. In electrophoresis measurements of phosphatidylcholine vesicles the NaCl can increase the originally negative zeta potential close to zero, however positive zeta potential can be typically reached only with multivalent ions or Lithium ions [2, 9, 15, 16, 22]. The lack of significant positive electrophoretic mobility in the presence of NaCl has been recognized to contradict with suggested strong binding of Na^+ and the contradiction has been explained by the effect of Cl^- ions to the electrophoretic mobility [23, 24].

In atomistic resolution molecular dynamics simulations all the generally used models seems to predict binding of Na^+ ions into a phosphatidylcholine lipid bilayer, but the strength of binding depends on the force field [13, 14, 23, 25–28]. The reduction of lipid lateral diffusion due to Na binding in simulations is in agreement with fluorescent probe measurements [8, 10, 13], but not with the NMR experiments [12]. The AFM experiments are interpreted to agree with the area



FIG. 1: Chemical structure of POPC.

per lipid reduction due to Na binding in simulations [15–19], however compared to the scattering experiments the reduction is observed with too low concentrations in simulations compared to experiments [11]. The simulations has been suggested to predict larger positive electrophoretic mobility than experimentally measured with NaCl, which has been explained by choride ion behaviour [23, 24].

It is agreed in the literature that the Ca^{2+} ions do penetrate in the phosphatidylcholine bilayer and signifncantyl affect membrane properties already with mM concentrations of CaCl_2 , however the strength of the binding is not agreed [14, 22, 29].

One of the experimental methods used to measure the ion binding is the so called "electrometer concept" where the changes of the C-H bond order parameters for the choline α and β segments (see Fig. 1 for definitions and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) chemical structure) are used to determine the amount of penetrating charges into a bilayer [20, 29–31]. The concept is based on the findings that the absolute value of the β order parameter increases and the α order parameter decreases with increasing amount of penetrating charges [20, 29–31]. From the simulation point of view the electrometer concept is particularly interesting since one can directly compare the actual measurable quantity, i.e. order parameters, to the simulations. In this work we compare the ion induced changes in phosphatidylcholine headgroup order parameters between different simulation models to resolve the above discussed controversies in the literature.

In our recent work we showed that the experimental order parameters for choline and glycerol backbone were not quantitatively reproduced by any available lipid model in fully hydrated conditions, however, the response of the choline order parameters to the dehydration were qualitatively correct in all models and the response to the cholesterol concentration was correct for the CHARMM36 model [32]. In this work, we show that the qualitative response of order parameters to penetrating cations is qualitatively correct but the partition of ions is too strong in all the studied models.

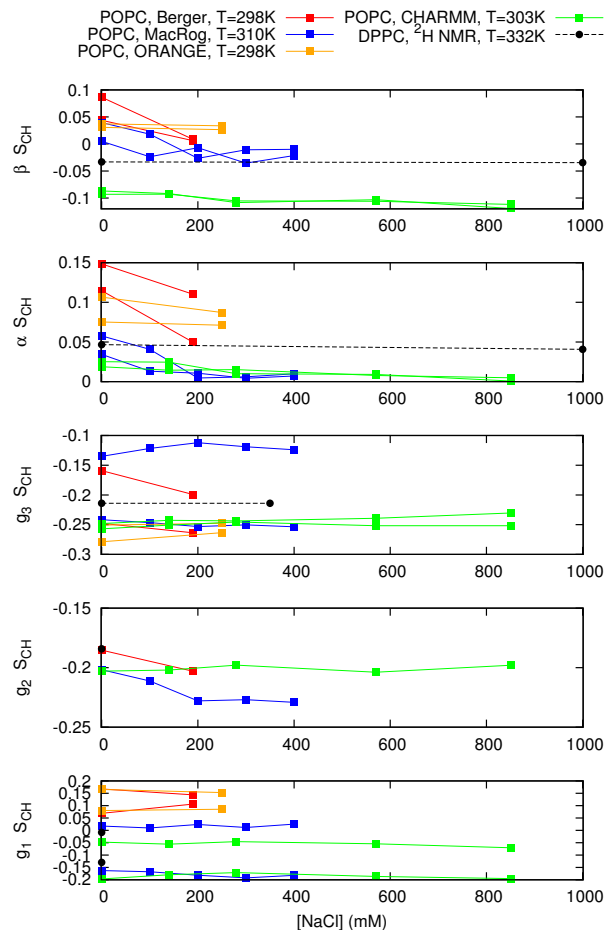


FIG. 2: Order parameters as a function of NaCl concentration from simulations with the Berger, CHARMM36, MacRog and Orange force fields compared to the experiments by Akutsu et al. [20]. The signs are assumed to be the same as measured by Hong et al. [33], Hong et al. [33] and Gross et al. [34]. The effect of ions to the g_2 and g_3 were not measured, thus only the values without ions are shown. The straight line between the results with and without ions is plotted to guide the eye.

II. RESULTS AND DISCUSSION

The effect of NaCl on the choline and glycerol backbone order parameters from experiments by Akutsu et al. [20] and various simulations are shown in Fig. 2. The simulated systems are described in the table I and further details are given in Supplementary Information. The experimental results are shown for DPPC bilayer due to more extensive data set [20], however the measurements for POPC gave essentially the same results [29].

The most important observation is that the experimental order parameters for the headgroup and glycerol backbone g_3

TABLE I: Simulated lipid bilayers with ions.

Force field	lipid	[Ion] mM	^a N _l	^b N _w	^c N _{Na}	^d N _{Ca}	^e N _{Cl}	^f T (K)	^g t _{sim} (ns)	^h t _{anal} (ns)	Files
Berger-POPC-07[35]	POPC	0	128	7290	0	0	0	298	270	240	[36]
Berger-POPC-07[35], ionFF [?] 1.	POPC	190 (NaCl)	128	7202	44	0	44	298	110	50	?2.
Berger-POPC-07[35], ionFF [?] 3.	POPC	190 (CaCl ₂)	128	7157	0	44	88	298	110	50	?4.
CHARMM36[37]	POPC	0	72	2242	0	0	0	303	30	20	[38]
CHARMM36[37], ionFF [?] 5.	POPC	140 (NaCl)	72	2085	13	0	13	303	80	60	?6.
CHARMM36[37], ionFF [?] 7.	POPC	280 (NaCl)	72	2085	26	0	26	303	80	60	?8.
CHARMM36[37], ionFF [?] 9.	POPC	570 (NaCl)	72	2085	52	0	52	303	80	60	?10.
CHARMM36[37], ionFF [?] 11.	POPC	85 (NaCl)	72	2085	78	0	78	303	73	60	?12.
MacRog[39] 13.	POPC	0	?	?	0	0	0	?	?	?	?
MacRog[39] 14. , ionFF [?] 15.	POPC	?(NaCl)	?	?	?	?	?	?	?	?	?
MacRog[39] 16. , ionFF [?] 17.	POPC	?(NaCl)	?	?	?	?	?	?	?	?	?
MacRog[39] 18. , ionFF [?] 19.	POPC	?(NaCl)	?	?	?	?	?	?	?	?	?
MacRog[39] 20. , ionFF [?] 21.	POPC	?(NaCl)	?	?	?	?	?	?	?	?	?
Orange, ionFF [?] 22.	POPC	0	?	?	0	0	0	298	?	?	?23.
Orange, ionFF [?] 24.	POPC	250 (NaCl)	72	2802	26	0	26	298	120	60	?25.
Orange, ionFF [?] 26.	POPC	240 (CaCl ₂)	72	2802	0	26	52	298	120	60	? 27.
Slipid[40]	DPPC	0	128	3840	0	0	0	323	150	100	[41]
Slipid[40], ionFF [?] 28.	DPPC	150 (NaCl)	?	?	?	?	?	323	?	?	?

^a The number of lipid molecules^b The number of water molecules^c The number of Na⁺ molecules^d The number of Ca²⁺ molecules^e The number of Cl molecules^f Simulation temperature^g The total simulation time^h Time frames used in the analysis

segment are practically unchanged even though 1M NaCl concentration was added to the system. Thus, the presence of mM concentrations of NaCl do not affect the structure of these parts of lipids. On the other hand, the presence of multivalent ions or charged amphiphiles changes the α and β order parameters in a systematic way suggesting that the amount and sign of charge penetrated into a bilayer can be measured from the changes of these parameters [20, 29–31]. This is known as the "electrometer concept". Thus, the most straightforward explanation for the experimental results shown in Fig. 2 is that the Na⁺ and Cl⁻ ions do not essentially penetrate into a phospholipid bilayer below 1M concentrations.

The response of order parameters to the NaCl concentration in simulations depends on the used model in Fig. 2: The addition of NaCl leads to a significant changes for choline and g_2 and g_3 segments in glycerol backbone in Berger and MacRog models, while only moderate changes are seen in CHARMM36 and Orange force fields. The experimental data for glycerol backbone as a function of NaCl concentration is available only for the g_3 segment. The changes in g_1 and g_2 are unlikely since all the other order parameters are unaffected and ¹H NMR data indicates that multivalent cations binding to phospholipid interact mainly with choline group leaving glycerol backbone conformations intact [42, 43].

The core idea of the electrometer concept is that the de-

crease (increase) of β - and α -carbon order parameters is related to cation (anion) penetration into a lipid bilayer. It should be noted that in the original experiments the absolute value of β order parameter was increasing with the presence of cations, however it was shown later on that the β order parameter is negative [33, 34, 44], thus the value is actually increasing (becoming less negative). The order parameter changes for β - and α -segments are plotted in Fig. 3 in the presence of NaCl and CaCl₂ from experiments and different simulations. The clear decrease seen with CaCl₂ compared to the negligible effect of NaCl. Interpreted in terms of electrometer concept, the result indicates that the Ca²⁺ ions significantly penetrate into PC bilayer in contrast to Na⁺ [20, 29].

The differences in NaCl behaviour between different models already seen in Fig. 2 are seen more clearly in Fig. 3 showing the changes. **32.The discussion about simulation results with CaCl₂ to be written once we have all the results.**

To study the correlation between order parameter changes and ion partitioning into a bilayer, suggested by the electrometer concept [20, 29–31], the density distributions as a function of membrane normal are shown in Fig. 4 for lipids and cations from different simulation models. It is evident from the figure that the Na⁺ density peaks at the lipid bilayer interface are higher (indicating stronger ion binding) for the models experiencing also larger changes in order parameters with NaCl

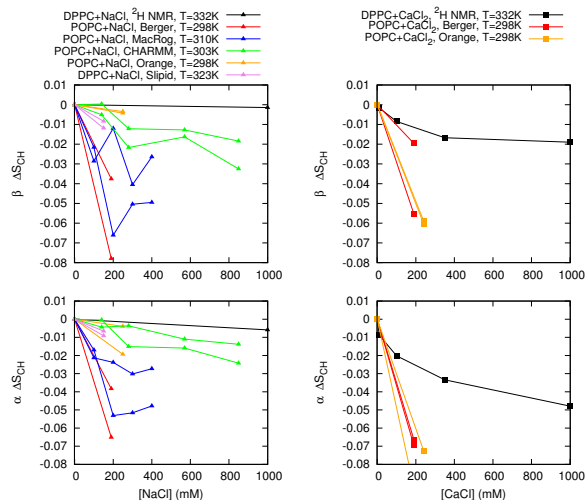


FIG. 3: The change of order parameters for β and α segments as a function of NaCl (left column) and CaCl_2 (right column) concentrations from simulations and experiments by Akutsu et al. [20]. The signs of the order parameters without ions are taken from [33, 34, 44] and it is reasonable to assume that the sign do not change with concentrations represented here [29]. Note that the β order parameter with negative sign decreases here with cation penetration in contrast to measured absolute value increase in the original studies [20, 29]. Regarding the actual electrometer concept this not relevant, however. The experimental accuracy of order parameter changes is much higher than the accuracy of quantitative values due to high resolution of ^2H NMR spectroscopy [?].

29. I think that we have to run Orange simulation with higher NaCl concentration. I will do it.

30. I think that we have to run CHARMM36 with CaCl_2 . If anyone happen to have the data and is willing to share, let us know. If not, then I will do it.

31. I think that it would be very interesting to test the modified CHARMM ions: Venable et al. [dx.doi.org/10.1021/jp401512z](https://doi.org/10.1021/jp401512z), J. Phys. Chem. B 2013, 117, 1018310192. According to the paper, these parameters improve the ion binding to the charged lipid bilayers. Even though I think that testing this would be highly relevant, I do not have time to do it now.

concentration shown in Fig. 2. The both Na^+ binding affinity and order parameter changes are smallest, thus closest to the experiments, for the Orange model, and then increase in order CHARMM36, MacRog, and Berger having the highest binding affinity and order parameter changes. Thus, the correlation between decreasing choline order parameters and amount of penetrating cations (i.e. the electrometer concept) is also seen in simulations, independently of the quantitative quality of the used simulation model.

The most straightforward explanation for our results is that Na^+ ions do not practically penetrate into a PC lipid bilayer with mM concentrations, thus the presence of NaCl does not affect the bilayer properties as observed in various experiments [5, 11, 12, 20, 21, 29]. Consequently, the Na^+ penetration and concomitant changes in order parameters, area

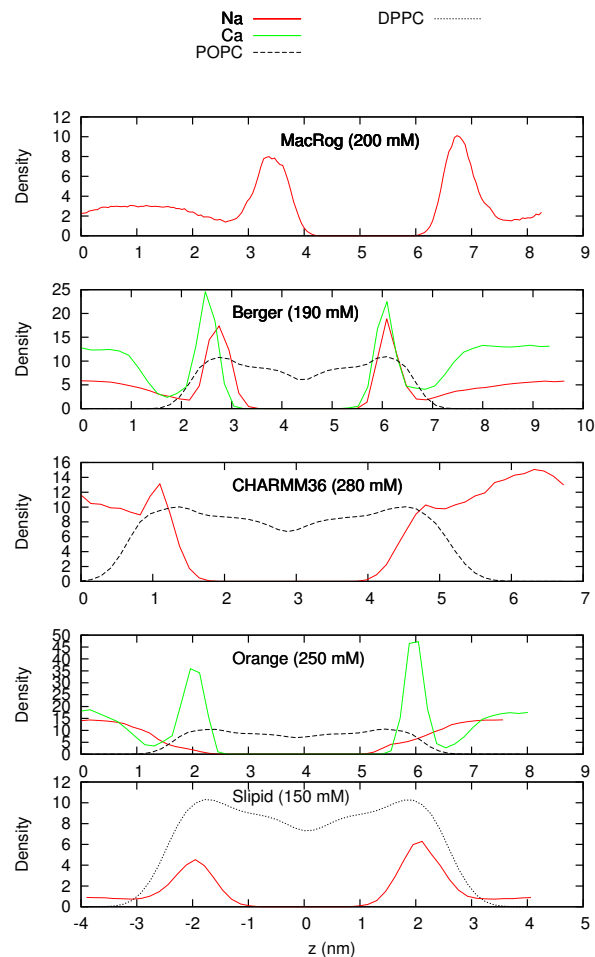


FIG. 4: Mass density profiles for lipids and ions from simulations with different force fields. The lipid densities are divided with 100 to make them visible with the used y-axis scale.

33. This figure is in progress, however, the main point can be seen.
- I think that we should use number densities instead of mass densities to make the difference between Na and Ca distributions more clear. Now the difference comparison is little bit distracted due to the different mass of the atoms.
 - The lipid density from MacRog simulation is missing. Maybe Matti Javanainen could provide this? If the data will be uploaded to Zenodo, it also trivial to calculate it with script.
 - It seems that relative integrated (total) ion density in different models do not correspond the reported relative concentrations. For example, total Na density seems bigger in Berger than in MacRog even though there is only minor difference in concentration, and into the wrong direction.
 - I think that CHARMM36 simulation with CaCl_2 should be done
 - Figure editing will be finished (centering of the graphs, labels, etc.) will be done once the above issues have been sorted out.

per molecule and lateral diffusion seen in almost all simulation models would be artefact due to overestimated attraction between ions and lipid bilayer. Even though this would also explain the absence of positive zeta potential in electrophoresis experiments [2, 9, 15, 16, 22], the presented data do not rule out the suggested possibility of equal binding of Na^+ and Cl^- ions [24], however, this equal binding should happen in

such a way that the bilayer properties are unaffected. Negligible binding of Na^+ with mM concentrations suggested here differs from the conclusions made from measurements of fluorescent probe dynamics [8, 10, 13], membrane hardness with AFM [15–19] and calorimetry [9, 13]. However, the fluorescent measurement results may arise from direct interactions between probe and ions, as already suggested by Filippov et al. [12]. Further, the calorimetric results have been also interpreted to support negligible binding [3], and AFM result is relatively indirect, thus there may be alternative explanations as well.

The effect of Ca^{2+} on the α and β order parameters is overestimated in all the simulation models, however, qualitatively the change is correct, i.e. order parameters are decreasing. The overestimation of the effect could be due to too strong partitioning or too sensitive headgroup model to the presence of ions. **34. The discussion should be finished when we have CHARMM results with CaCl_2 .**

35. The ^1H NMR experiments suggest that the N- β - α -O dihedral is only in gauche conformation in the absence of ions, but in the presence of multivalent ions there would be also anti conformations present [Hauser et al. BBA 508, 450 (1978), Hauser et al. Chem. Phys. Lipids 29, 103 (1981)]. I think that we should check if this happens in simulations.

The origin for suggested inaccuracies in lipid-ion interactions in simulation models is unknown. In principle, the incorrect choline structure [32], lack of polarizability [45] or the used water model could cause such results. The effect of changes in lipid and ion model or the ion partition is discussed in supplementary material. **36. In the Orange simulation only lipid model is changed, respect to Berger, and Jukka tested the effect of 0.7 charge scaling for Na ion (suggested by Leontyev et al. [45] to compensate to the lack of electronic polarizability in the model). I think we should discuss these things in supplementary material. Even though we cannot be fully conclusive, there is some essential**

information also in these results.

III. CONCLUSIONS

We have compared phospholipid bilayer interactions with NaCl and CaCl_2 between different molecular dynamics simulation models and ^2H NMR experiments. The comparison led to the following conclusions

- The most straightforward explanation for the various experimental observations is that there is no Na^+ ion binding into the phospholipid bilayer with mM concentrations, in contrast to Ca^{2+} which specifically binds.

- The Na^+ is overestimated in almost all molecular dynamics simulation models, however, from the publicly available models the CHARMM36 has the most realistic description.

- The electrometer concept suggesting connection between α and β order parameter decrease and cation partitioning [20, 29–31] works also in simulations, despite of inaccuracies in actual atomistic resolution structures.

- **37. Final conclusions about the structural response to be written once we have all the results**

This work has been, and will be further, progressed and discussed through the blog: nmrlipids.blogspot.fi. Everyone is invited to join the discussion and make contributions through the blog. The manuscript will be eventually submitted to an appropriate scientific journal. Everyone who has contributed to the work through the blog will be offered coauthorship. For more details see: nmrlipids.blogspot.fi.

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SUPPLEMENTARY INFORMATION

b. CHARMM36

Appendix A: methods

1. Simulated systems

All simulations are ran with a standard setup for planar lipid bilayer in zero tension with periodic boundary conditions with Gromacs software package (version numbers 4.5-X-4.6.X).

2. Simulation details

a. Berger

The simulation without ions is the same as in [32]. The starting structures for simulations with ions is made by replacing water molecules with appropriate amount of ions under study. **38.Samuli, finalize and check the methods.**

The Berger force field was used for the POPC [46], with the dihedral potential next to the double bond taken from [47]. The simulations are identical to previous publications [35, 48, 49]. Timestep of 2 fs was used with leap-frog integrator. Covalent bond lengths were constrained with LINCS algorithm [50, 51]. Coordinated were written every 10 ps. PME with real space cut-off 1.0 nm was used for electrostatics. Plain cut-off was used for the Lennart-Jones interactions with a 1.0 nm cut-off. The neighbour list was updated every 5th step with cut-off 1.0 nm. Temperature was coupled separately for lipids and water to 298 K with the velocity-rescale method [52] with coupling constant 0.1 ps^{-1} . Pressure was semi-isotropically coupled to the atmospheric pressure with the Berendsen method [53].

The simulation without ions is the same as in [32]. The starting structures for simulations with ions is made by replacing water molecules with appropriate amount of ions under study. **39.Samuli, finalize and check the methods.**

Timestep of 1 fs was used with leap-frog integrator. Covalent bonds with hydrogens were constrained with LINCS algorithm [50, 51]. Coordinated were written every 5 ps. PME with real space cut-off 1.4 nm was used for electrostatics. Lennart-Jones interactions were switched to zero between 0.8 nm and 1.2 nm. The neighbour list was updated every 5th step with cut-off 1.4 nm. Temperature was coupled separately for lipids and water to 303 K with the velocity-rescale method [52] with coupling constant 0.2 ps. Pressure was semi-isotropically coupled to the atmospheric pressure with the Berendsen method [53].

c. MacRog

40.Matti Javanainen, please deliver the details.

d. Orange

41.Jukka Maatta and Luca Monticelli, please deliver as much details as you can.

e. Slipids

The simulation without ions is the same as in [32]. **42.Andrea Catte, please deliver the details.**

3. analysis

The order parameters were calculated from simulation trajectories directly applying the equation $S_{\text{CH}} = \frac{3}{2} \langle \cos^2 \theta - 1 \rangle$. For united atom models the hydrogen locations were regenerated for each molecule in each frame after the simulation trajectory was created. ??The statistical error estimate for each order parameter calculated from simulation was roughly 0.01, which is much smaller than the differences discussed in this work.??

TODO

	P.
1. Appropriate reference for the ion model?	3
2. Samuli put to Zenodo	3
3. Appropriate reference for the ion model?	3
4. Samuli put to Zenodo	3
5. Appropriate reference for the ion model?	3
6. Samuli put to Zenodo	3
7. Appropriate reference for the ion model?	3
8. Samuli put to Zenodo	3
9. Appropriate reference for the ion model?	3

10. Samuli put to Zenodo	3
11. Appropriate reference for the ion model?	3
12. Samuli put to Zenodo	3
13. Is this the same as in the first manuscript for full hydration?	3
14. Matti Javanainen, please check the numbers and let us know if you share some files through Zenodo	3
15. Appropriate reference for the ion model?	3
16. Matti Javanainen, please check the numbers and let us know if you share some files through Zenodo	3
17. Appropriate reference for the ion model?	3
18. Matti Javanainen, please check the numbers and let us know if you share some files through Zenodo	3
19. Appropriate reference for the ion model?	3
20. Matti Javanainen, please check the numbers and let us know if you share some files through Zenodo	3
21. Appropriate reference for the ion model?	3
22. Samuli check the numbers	3
23. Jukka Mtt and Luca Monticelli, please let us know if we can share some files. This is unpublished model.	3
24. Appropriate reference for the ion model?	3
25. Jukka Mtt and Luca Monticelli, please let us know if we can share some files through Zenodo. This is unpublished model.	3
26. Appropriate reference for the ion model?	3
27. Jukka Mtt and Luca Monticelli, please let us know if we can share some files. This is unpublished model.	3
28. Andrea Catte, please check the numbers and let us know if you share some files through Zenodo	3
32. The discussion about simulation results with CaCl_2 to be written once we have all the results.	3
29. I think that we have to run Orange simulation with higher NaCl concentration. I will do it.	4
30. I think that we have to run CHARMM36 with CaCl_2 . If anyone happen to have the data and is willing to share, let us know. If not, then I will do it.	4
31. I think that it would be very interesting to test the modified CHARMM ions: Venable et al. dx.doi.org/10.1021/jp401512z , J. Phys. Chem. B 2013, 117, 1018310192. According to the paper, these parameters improve the ion binding to the charged lipid bilayers. Even though I think that testing this would be highly relevant, I do not have time to do it now.	4
33. This figure is in progress, however, the main point can be seen. - I think that we should use number densities instead of mass densities to make the difference between Na and Ca distributions more clear. Now the difference comparison is little bit distracted due to the different mass of the atoms. - The lipid density from MacRog simulation is missing. Maybe Matti Javanainen could provide this? If the data will be uploaded to Zenodo, it also trivial to calculate it with script. - It seems that relative integrated (total) ion density in different models do not correspond the reported relative concentrations. For example, total Na density seems bigger in Berger than in MacRog even though there is only minot difference in concentration, and into the wrong direction. - I think that CHARMM36 simulation with CaCl_2 should be done - Figure editing will be finished (centering of the graphs, labels, etc.) will be done once the above issues have been sorted out.	4
34. The discussion should be finished when we have CHARMM results with CaCl_2	5
35. The ^1H NMR experiments suggest that the N- β - α -O dihedral is only in gaughe conformation in the absense of ions, but in the presense of multivalent ions there would be also anti conformations present [Hauser et al. BBA 508 , 450 (1978), Hauser et al. Chem. Phys. Lipids 29 , 103 (1981)]. I think that we should check if this happens in simulations.	5
36. In the Orange simulation only lipid model is changed, respect to Berger, and Jukka tested the effect of 0.7 charge scaling for Na ion (suggested by Leontyev et al. [45] to compensate to the lack of electronic polarizability in the model). I think we should discuss these things in supplementary material. Even though we cannot be fully conclusive, there is some essential information also in these results.	5
37. Final conclusions about the structural response to be written once we have all the results	5
38. Samuli, finalize and check the methods.	7
39. Samuli, finalize and check the methods.	7
40. Matti Javanainen, please deliver the details.	7
41. Jukka Maatta and Luca Monticelli, please deliver as much details as you can.	7
42. Andrea Catte, please deliver the details.	7