

Supporting Information:

**Accurate binding of sodium and calcium to
phospholipid bilayers by effective inclusion of
electronic polarization**

Josef Melcr,[†] Hector Martinez-Seara,[†] Jiří Kolafa,[‡] Pavel Jungwirth,^{†,¶} and O. H.
Samuli Ollila^{*,†,§}

[†]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech
Republic, Prague 6, Czech Republic*

[‡]*Department of Physical Chemistry, Institute of Chemical Technology, Prague 6, Czech
Republic*

[¶]*Department of Physics, Tampere University of Technology, P.O. Box 692, FI-33101
Tampere, Finland*

[§]*Institute of Biotechnology, University of Helsinki*

E-mail: samuli.ollila@helsinki.fi

S1 Simulation details

Table S1: Simulation parameters

simulation property	parameter
time-step	2 fs
equilibration time	100 ns
total simulation time	300 ns
temperature	313 K
thermostat	v-rescale ¹
barostat	Parrinello-Rahman, semi-isotropic ²
long-range electrostatics	PME ³
cut-off scheme	Verlet ⁴
Coulomb and VdW cut-off	1.0 nm
constraints	LINCS, only hydrogen atoms ⁵
constraints for water	SETTLE ⁶

S2 Area per molecule and calcium binding with different water models

Table S2: Area per lipid (APL) from different models of POPC with no ions
Joe, please deliver the citations for all experimental area per molecule values or remove them.

model	APL (\AA^2)	Temperature [K]
Lipid14	65.1 ± 0.6	300
Lipid14 ⁷	65.6 ± 0.5	303
ECC-lipids		
SPC/E	63.2 ± 0.6	300
SPC/E	65.1 ± 0.6	313
OPC3	62.2 ± 0.6	300
OPC3	64.2 ± 0.6	313
OPC	64.4 ± 0.6	313
TIP4p/2005	66.8 ± 0.6	313
TIP3p	66.2 ± 0.6	313
TIP3p-FB	64.8 ± 0.6	313
TIP4p-FB	65.6 ± 0.6	313
experiment	62.7	293
experiment ⁸	64.3	303
experiment	67.3	323
experiment	68.1	333

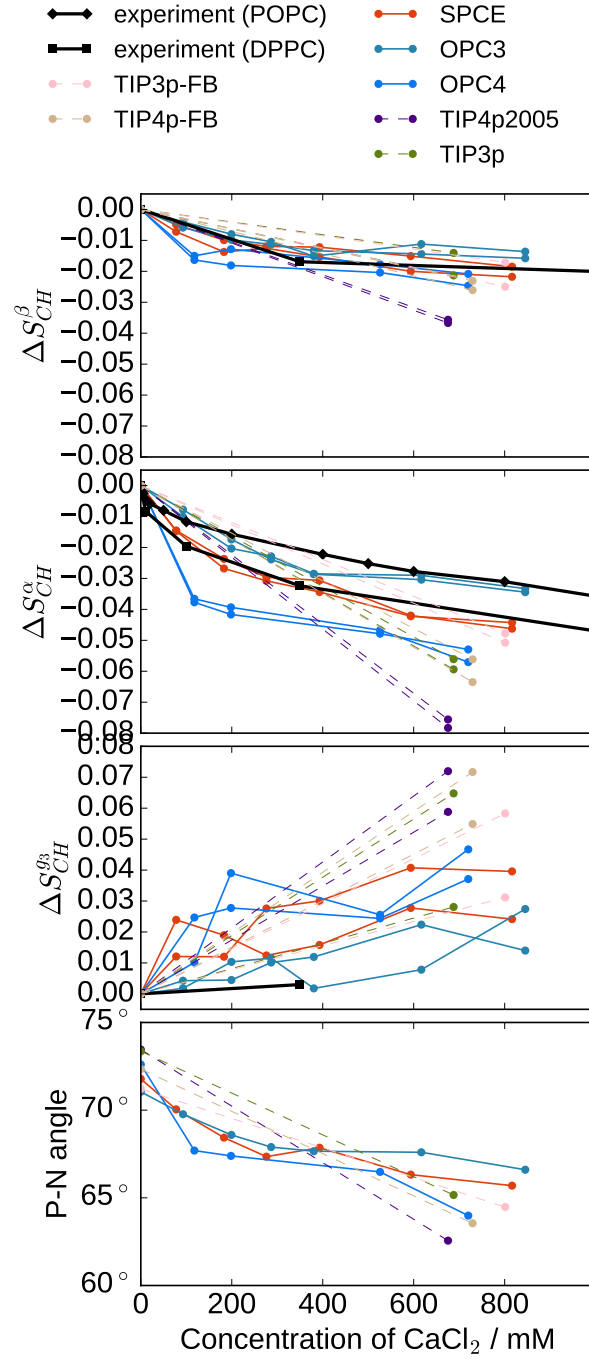


Figure S1: Changes of head group and glycerol carbon g_3 order parameters, and P-N vector orientation of POPC bilayer as a function of CaCl_2 concentrations in bulk (C_b) from ECC-POPC simulations at 313K with different water models (OPC,⁹ OPC3,¹⁰ TIP3P,¹¹ TIP3p-FB and TIP4p-FB,¹² and TIP4p/2005¹³) together with experimental data (DPPC (323 K)¹⁴ and POPC (313 K)¹⁵). Ion concentrations (C_b) are calculated from the cation number density C_{np} at the farthest point from the lipid bilayer in the aqueous phase as $[\text{ion}] = C_{np}/0.602$.

S3 Sodium binding to POPC with the glycerol carbon g_3 order parameters and the head group orientation

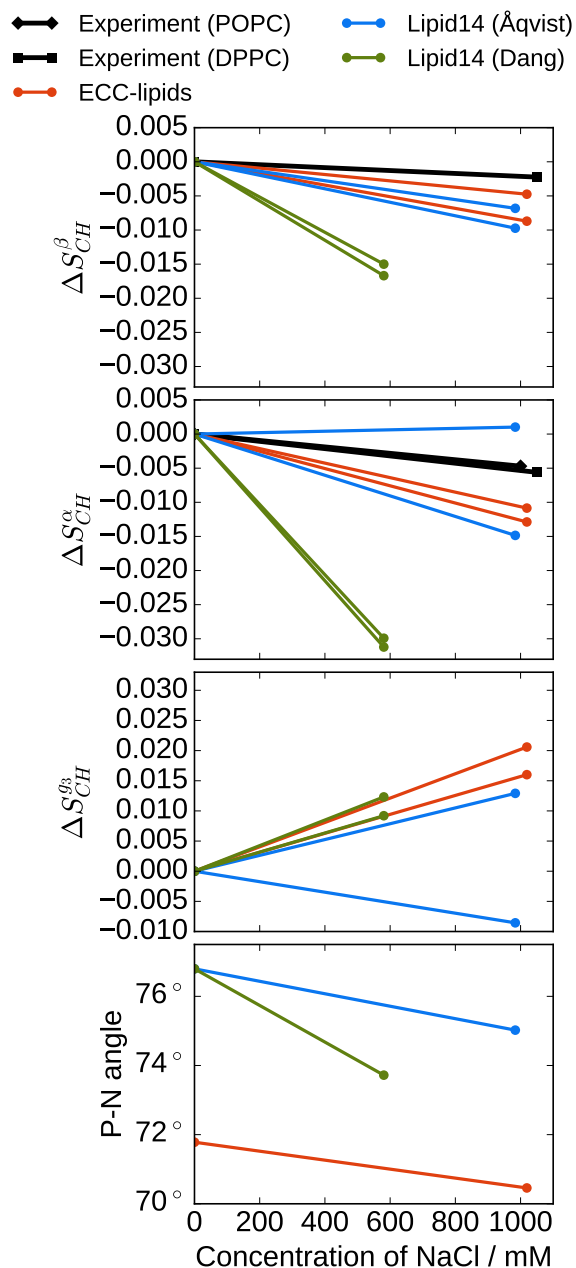


Figure S2: Changes of head group and glycerol carbon g_3 order parameters, and the P-N vector orientation of POPC bilayer as a function of NaCl concentrations in bulk (C_b) from simulations with different force fields at 313 K together with experimental data (DPPC (323 K)¹⁴ and POPC (313 K)¹⁵). Simulation data with Lipid14 and Åqvist ion parameters at 293 K is taken directly from Refs. 16–18.

S4 Ternary complex model in simulations

The NMR data about PC headgroup order parameters and atomic absorption spectroscopy data were previously best explained using a ternary complex binding model.¹⁵ In this model, one calcium is assumed to form complexes with two lipids, i.e. with the binding stoichiometry of 1 Ca^{2+} :2 POPC. The model predicts a linear relationship between quantities C_b and $\sqrt{C_b/C_I}$, where C_b is the mole fraction of bound Ca^{2+} per POPC and C_I is the concentration of free cations at the plane of ion binding.¹⁵ Experimentally determined C_b from NMR measurements and atomic absorption spectroscopy together with C_I calculated from the Poisson-Boltzmann equation gave a good agreement with the predictions of the ternary complex model.¹⁵

To compare ECC-POPC simulations to the ternary complex model, we calculated C_b from simulations (as defined in the main text), and C_I from the minimum CaCl_2 concentration at membrane-water interface, locating around 2.6 nm from the membrane center in the density profiles in Fig. 5 in the main text.

The results from simulations are shown in Fig. S3 together with the line fitted to experimental data by Altenbach and Seelig.¹⁵ Both results are in agreement with the prediction of the ternary complex model. The small discrepancy between the results from experiments and simulations probably arise from difference in the evaluation of the concentrations and inaccuracy of Poisson-Boltzmann theory for divalent cations like Ca^{2+} .¹⁹ In conclusion, the results suggest that the almost equal probabilities for Ca^{2+} to form complexes with 1-3 lipids observed in the main text is, indeed, in line with the experimental observations supporting the ternary complex model.

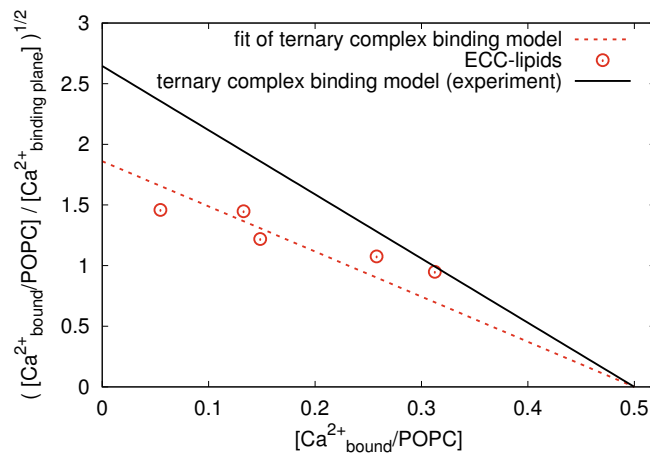


Figure S3: Fits of experimental¹⁵ and ECC-POPC simulation data to the prediction of the ternary complex model. Joe, please change the axes labels to correspond the notation in the text, i.e. C_b and C_I , and write in the title of simulation fit (red dashed line) that it is the fit to simulation.

S5 Histograms of residence times

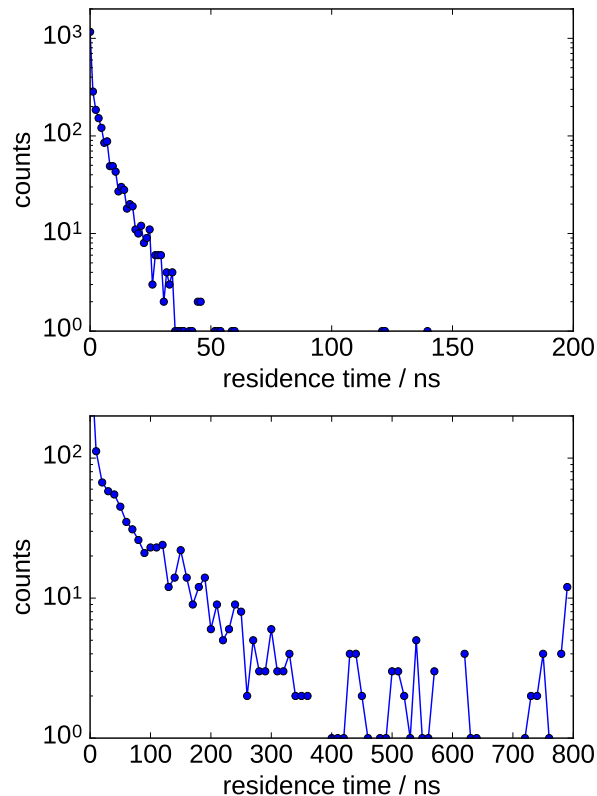


Figure S4: Histograms of residence times of Ca^{2+} in a POPC bilayer from ECC-POPC (top) and CHARMM36 (bottom) simulations with ECC-ions. Both simulations had the same concentration of CaCl_2 respect to water ($C_w = 450$ mM). CHARMM36 simulation was directly taken from Refs. 20,21. Scales of x-axes represent the lengths of the simulations used for analysis. In ECC-POPC simulation, 90% of the residence times are shorter than 60 ns, with the longest observed residence time being 141 ns, which is well below the total length of the simulation (200 ns). This is, however not the case in CHARMM36 simulation, where residence times of several calcium cations are apparently limited by the length of the simulation. Less than 60% of the residence times are shorter than the half of the simulation length (400 ns) in CHARMM36 simulation.

S6 Comparison between Gromacs and openMM

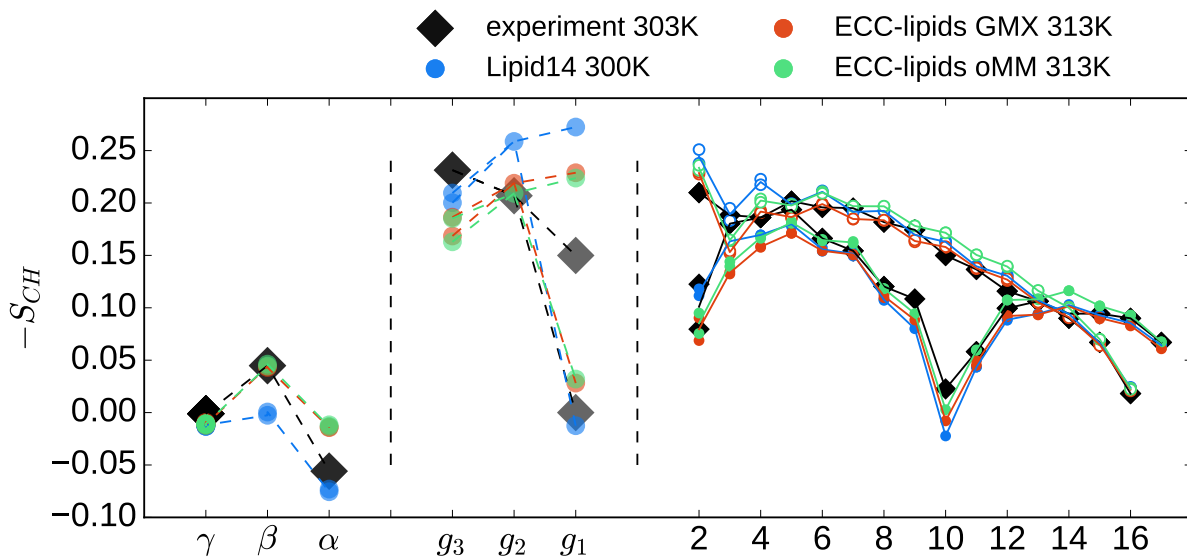


Figure S5: Order parameters of POPC head group, glycerol backbone and acyl chains from Lipid14⁷ and ECC-POPC simulations ran with GROMACS 5.1.4²² and openMM 7²³ together with experiments.²⁴ The size of the markers for the head group order parameters correspond to the error estimate ± 0.02 for experiments,^{25,26} while the error estimate for simulations is ± 0.005 . The size of the points for acyl chains are decreased by a factor of 3 to improve the clarity of the plot.

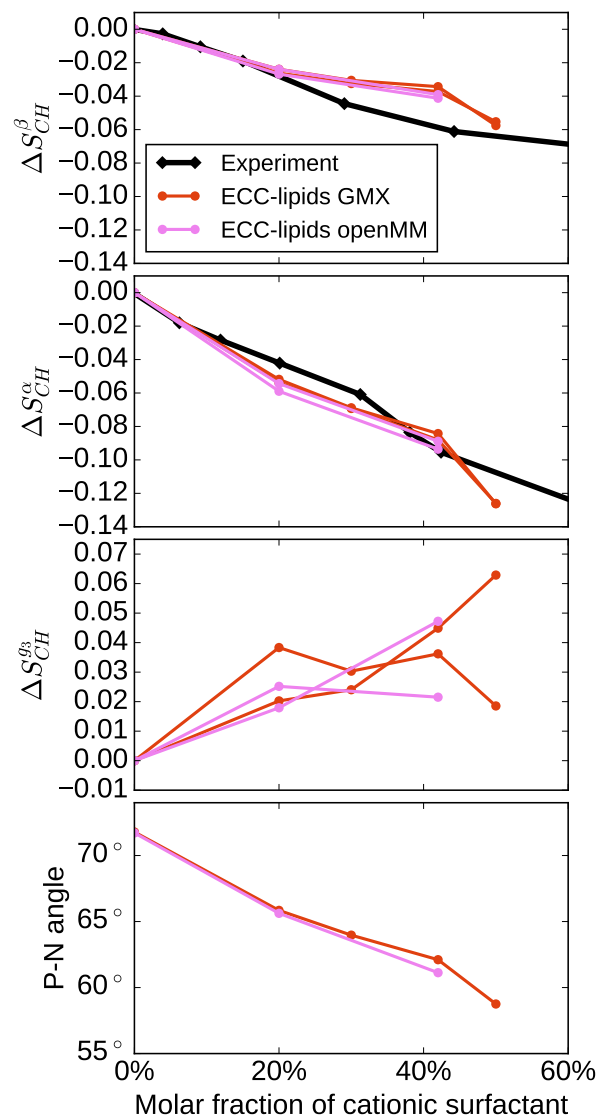


Figure S6: Changes of headgroup and glycerol carbon g_3 order parameters, and P-N vector orientation as a function of a molar fraction of the cationic surfactant dihexadecyldimethylammonium in a POPC bilayer from simulations with the ECC-lipids models simulated with GROMACS 5.1.4²² and openMM 7²³ compared with the experimental values from.²⁷ The size of the markers for the head group order parameters correspond to the error estimate ± 0.02 for experiments,^{25,26} while the error estimate for simulations is ± 0.005 .

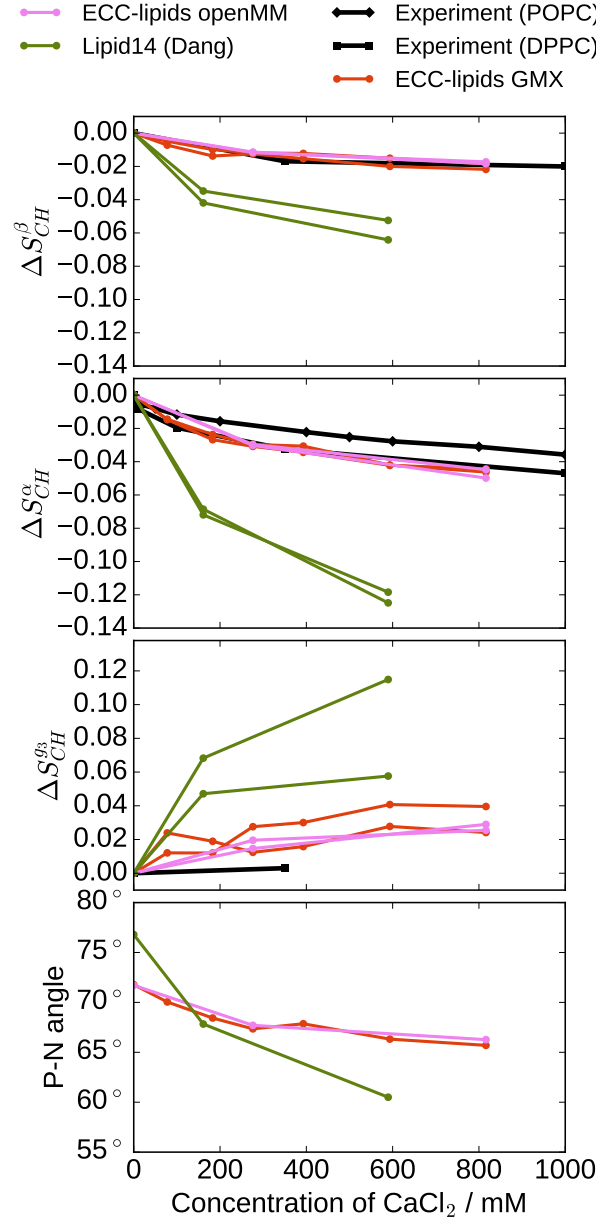


Figure S7: Changes of the head group and glycerol carbon g_3 order parameters, and P-N vector orientation of a POPC bilayer as a function of the CaCl_2 concentration in bulk (C_b) from Lipid14⁷ and ECC-POPC simulations ran with GROMACS 5.1.4²² and openMM 7²³ at 313 K together with experiments (DPPC (323 K)¹⁴ and POPC (313 K)¹⁵). Bulk concentrations from simulations are calculated from the farthest point from the lipid bilayer in the aqueous phase with an error estimate of 10 mM.

References

- (1) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys* **2007**, *126*.
- (2) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* **1981**, *52*, 7182–7190.
- (3) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An $N \log(N)$ method for Ewald sums in large systems. *J. Chem. Phys* **1993**, *98*, 10089–10092.
- (4) Páll, S.; Hess, B. A flexible algorithm for calculating pair interactions on {SIMD} architectures. *Computer Physics Communications* **2013**, *184*, 2641 – 2650.
- (5) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. LINCS: a linear constraint solver for molecular dynamics simulations. *J. Comput. Chem.* **1997**, *18*, 1463–1472.
- (6) Miyamoto, S.; Kollman, P. A. SETTLE: An analytical Version of the SHAKE and RATTLE Algorithm for Rigid Water Models. *J. Comput. Chem* **1992**, *13*, 952–962.
- (7) Dickson, C. J.; Madej, B. D.; Skjevik, Å. A.; Betz, R. M.; Teigen, K.; Gould, I. R.; Walker, R. C. Lipid14: The Amber Lipid Force Field. *J. Chem. Theory Comput.* **2014**, *10*, 865–879.
- (8) Kučerka, N.; Nieh, M. P.; Katsaras, J. Fluid phase lipid areas and bilayer thicknesses of commonly used phosphatidylcholines as a function of temperature. *Biochim. Biophys. Acta* **2011**, *1808*, 2761–2771.
- (9) Izadi, S.; Anandakrishnan, R.; Onufriev, A. V. Building Water Models: A Different Approach. *The Journal of Physical Chemistry Letters* **2014**, *5*, 3863–3871.
- (10) Izadi, S.; Onufriev, A. V. Accuracy limit of rigid 3-point water models. *Journal of Chemical Physics* **2016**, *145*, 074501.

- (11) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys* **1983**, *79*, 926–935.
- (12) Wang, L. P.; Martinez, T. J.; Pande, V. S. Building force fields: An automatic, systematic, and reproducible approach. *Journal of Physical Chemistry Letters* **2014**, *5*, 1885–1891.
- (13) Abascal, J. L.; Vega, C. A general purpose model for the condensed phases of water: TIP4P/2005. *The Journal of chemical physics* **2005**, *123*, 234505.
- (14) Akutsu, H.; Seelig, J. Interaction of metal ions with phosphatidylcholine bilayer membranes. *Biochemistry* **1981**, *20*, 7366–7373.
- (15) Altenbach, C.; Seelig, J. Calcium binding to phosphatidylcholine bilayers as studied by deuterium magnetic resonance. Evidence for the formation of a calcium complex with two phospholipid molecules. *Biochemistry* **1984**, *23*, 3913–3920.
- (16) Catte, A.; Girych, M.; Javanainen, M.; Loison, C.; Melcr, J.; Miettinen, M. S.; Monticelli, L.; Maatta, J.; Oganessian, V. S.; Ollila, O. H. S. et al. Molecular electrometer and binding of cations to phospholipid bilayers. *Phys. Chem. Chem. Phys.* **2016**, *18*.
- (17) Girych, M.; Ollila, O. H. S. POPC_AMBER_LIPID14_Verlet. 2015; <http://dx.doi.org/10.5281/zenodo.30898>.
- (18) Girych, M.; Ollila, O. H. S. POPC_AMBER_LIPID14_NaCl_1Mol. 2015; <http://dx.doi.org/10.5281/zenodo.30865>.
- (19) Andelman, D. *Handbook of biological physics*; Elsevier Science, 1995; Vol. 1; Chapter 12, pp 603–642.
- (20) Javanainen, M.; Melcrova, A.; Magarkar, A.; Jurkiewicz, P.; Hof, M.; Jungwirth, P.; Martinez-Seara, H. Two cations, two mechanisms: interactions of sodium and calcium with zwitterionic lipid membranes. *Chem. Commun.* **2017**, *53*, 5380–5383.

- (21) Javanainen, M. POPC with varying amounts of cholesterol, 450 mM of CaCl₂. Charmm36 with ECC-scaled ions. 2017; <https://doi.org/10.5281/zenodo.259376>.
- (22) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindah, E. Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, *1-2*, 19–25.
- (23) Eastman, P.; Swails, J.; Chodera, J. D.; McGibbon, R. T.; Zhao, Y.; Beauchamp, K. A.; Wang, L.-P.; Simmonett, A. C.; Harrigan, M. P.; Stern, C. D. et al. OpenMM 7: Rapid development of high performance algorithms for molecular dynamics. *PLOS Computational Biology* **2017**, *13*, e1005659.
- (24) Ferreira, T. M.; Coreta-Gomes, F.; Ollila, O. H. S.; Moreno, M. J.; Vaz, W. L. C.; Topgaard, D. Cholesterol and POPC segmental order parameters in lipid membranes: solid state 1H-13C NMR and MD simulation studies. *Phys. Chem. Chem. Phys.* **2013**, *15*, 1976–1989.
- (25) Botan, A.; Favela-Rosales, F.; Fuchs, P. F. J.; Javanainen, M.; Kanduč, M.; Kulig, W.; Lamberg, A.; Loison, C.; Lyubartsev, A.; Miettinen, M. S. et al. Toward Atomistic Resolution Structure of Phosphatidylcholine Headgroup and Glycerol Backbone at Different Ambient Conditions. *J. Phys. Chem. B* **2015**, *119*, 15075–15088.
- (26) Ollila, O. S.; Pabst, G. Atomistic resolution structure and dynamics of lipid bilayers in simulations and experiments. 2016; <http://dx.doi.org/10.1016/j.bbamem.2016.01.019>, In Press.
- (27) Scherer, P. G.; Seelig, J. Electric charge effects on phospholipid headgroups. Phosphatidylcholine in mixtures with cationic and anionic amphiphiles. *Biochemistry* **1989**, *28*, 7720–7728.