

**FACULTY  
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**SUMMARY OF DOCTORAL THESIS**

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**Simulation of processes in cellular  
membranes**

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# Preface

Cellular membranes are important and evolutionarily very old biological structures. [Alberts et al., 2008] The first primitive predecessors of cells already bear hints of membranes separating their inner environment from the outer world. Current organisms often contain a multitude of immensely complex membranes, each serving many functions. Processes in cellular membranes are thus crucial for life.

My work is motivated by processes, which involve interactions of biologically relevant ions with cellular membranes. For instance in neurons, the fusion of synaptic vesicles containing neurotransmitter with neuronal cell membranes is controlled by a divalent cation  $\text{Ca}^{2+}$ . [Berridge et al., 2003, Clapham, 2007] This process is triggered by a change in the transmembrane potential across the neuronal plasma membrane, which is modulated by the exchange of the monovalent cations  $\text{Na}^+$  and  $\text{K}^+$ . [Sten-Knudsen, 2002] The transmembrane potential in atomistic simulations can be modeled by two approaches, the constant electric field method, and the ion-imbalance method. The methodological differences between them raise the following questions: Do they provide the same results? Can they be used interchangeably? What happens to the membrane under voltage?

Until recently, there was no consensus on the binding of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  cations to biological membranes – simulations and some experiments [Berkowitz and Vacha, 2012, Vacha et al., 2009, Harb and Tinland, 2013] do not reproduce other experiments [Roux and Bloom, 1990, Pabst et al., 2007, Akutsu and Seelig, 1981]. From the point of simulations, all currently available models require improvements to reproduce quantitatively structures and interactions with cations. Here, the following questions arise: Is the missing electronic polarization in standard non-polarizable simulations responsible for the discrepancy? If yes, how crucial is it for the interactions of phospholipid bilayers with biologically relevant cations, and can it be effectively accounted for by rescaling charges? Can we obtain in this way realistic structures of phospholipid bilayers with interacting cations at atomistic resolution?



# Simulation of processes in cellular membranes

## Interactions of ions with phospholipid bilayers

Cellular membranes are surrounded by weak electrolytic solutions of KCl on the intracellular side, and of NaCl on the extracellular side. The biological relevance of these ions reaches from relatively simple osmotic effects to the complex processes in neural signalling. [Sten-Knudsen, 2002] Calcium is an important cation in biology, which takes part in many signalling pathways and processes.

Interactions of cations like  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  with cellular membranes play a key role in many biological processes. Although the binding of these cations to model biological membranes has been widely studied both in experiments and simulations [Catte et al., 2016, Bacle et al., 2018], the details of the interactions are, however, not fully consistent in the literature. Interpretations of mostly spectroscopic methods, like nuclear magnetic resonance (NMR), x-ray scattering, and infrared spectroscopy suggest that monovalent alkali cations (with the exception of  $\text{Li}^+$ ) exhibit negligible binding to PC lipid bilayers, while multivalent cations interact more strongly [Cevc, 1990, Tocanne and Teissié, 1990, Hauser et al., 1976, 1978, Herbette et al., 1984, Altenbach and Seelig, 1984, Uhríková et al., 2008]. In contrast, much stronger interactions between monovalent cations and phospholipids are reported from microscopy measurements [Harb and Tindall, 2013, Garcia-Manyes et al., 2006, Fukuma et al., 2007, Ferber et al., 2011, Redondo-Morata et al., 2012], and atomistic molecular dynamics (MD) simulations [Cordomí et al., 2009, Valley et al., 2011, Berkowitz and Vacha, 2012, Catte et al., 2016, Bacle et al., 2018, Melcr et al., 2018]. My work solves this controversy by introducing a fundamental physics-based improvement into MD simulations making them for the first time *quantitatively* agreeing with the works predicting the weaker binding.

Ion binding to lipid bilayers can be experimentally quantified by measuring the changes of the head group order parameters in the lipids using solid state NMR. In the case of the head group order parameters  $S^\alpha$  and  $S^\beta$  in phosphatidylcholine (see Fig. 1 for the definition of the order parameters) such changes are known under the term "electrometer concept" [Seelig et al., 1987, Catte et al., 2016, Ollila and Pabst, 2016]. It is experimentally observed that the C-H bond order parameters of  $S^\alpha$  and  $S^\beta$  carbons in a PC lipid head group are proportional to the amount of charge, positive or negative, bound per lipid [Seelig et al., 1987]. The change of the order parameters measured with varying concentrations of aqueous ions can be then related to the amount of bound ions.

In our publications [Catte et al., 2016, Bacle et al., 2018], we have shown that such order parameters can be accurately determined also from MD simulations and their changes correlate with the amount of bound charge in phospholipid bilayers, despite the inaccuracies in their actual structures without salts [Botan et al., 2015]. It was found, however, that none of the force fields examined in those works provided a sufficient accuracy for interpreting the experimentally measured structural changes induced by salt concentrations and cation-lipid stoichiometries.

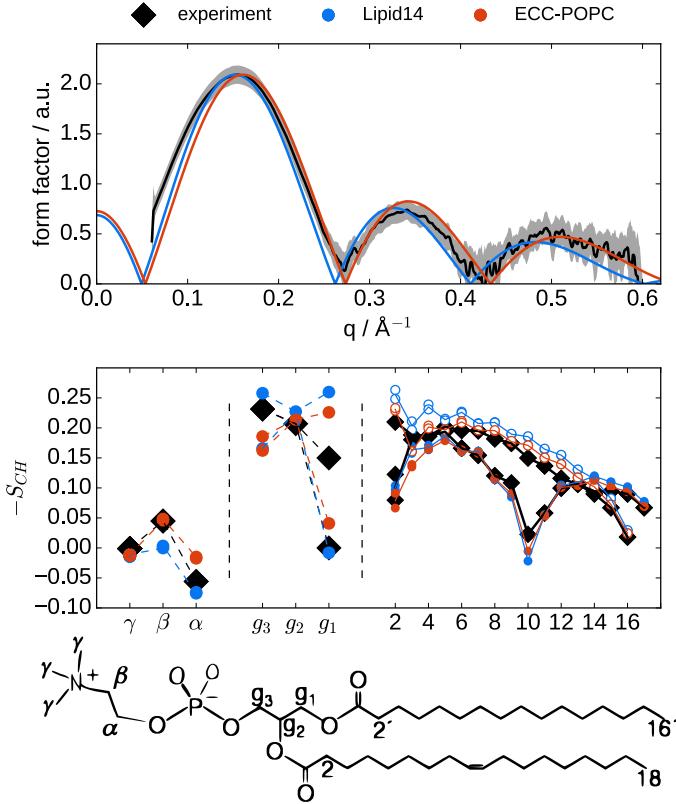


Figure 1: Top: X-ray scattering form factors from simulations with the Lipid14 [Dickson et al., 2014] and the ECC-POPC [Melcr et al., 2018] models compared with experiments [Kučerka et al., 2011] at 303 K. Middle: Order parameters of POPC head group, glycerol backbone and acyl chains from simulations with the Lipid14 and the ECC-POPC models compared with experiments [Ferreira et al., 2013] at 300 K. The size of the markers for the head group order parameters correspond to the error estimate  $\pm 0.02$  for experiments [Botan et al., 2015, Ollila and Pabst, 2016], while the error estimate for simulations is  $\pm 0.005$  (Bayesian estimate of 95% confidence interval [Jones et al., 2001–2018]). The size of the points for acyl chains are decreased by a factor of 3 to improve the clarity of the plot. Open/closed symbols are used for palmitoyl/oleoyl chains of POPC. Bottom: The chemical structure of POPC and the labeling of the carbon segments.

A major improvement over currently available non-polarizable force fields was achieved by developing new models of phospholipids, the so called ECC-lipids. We developed models of POPC, POPE and POPS lipids, which account for electronic polarization through electronic continuum correction (ECC), and which yield accurate response of the head group order parameters to various monovalent and divalent cations. [Melcr et al., 2018] In short, ECC is an implicit model of electronic polarizability, which can be straightforwardly implemented into current force fields by scaling charges. Moreover, simulations with ECC-lipids, an *implicitly polarizable* force field, come without any extra computational costs compared to standard fixed-charge simulations. Our new model, ECC-lipids, provides a proof of concept of the applicability of ECC to charged as well as neutral molecules.

We compared X-ray scattering form factors and NMR order parameters of

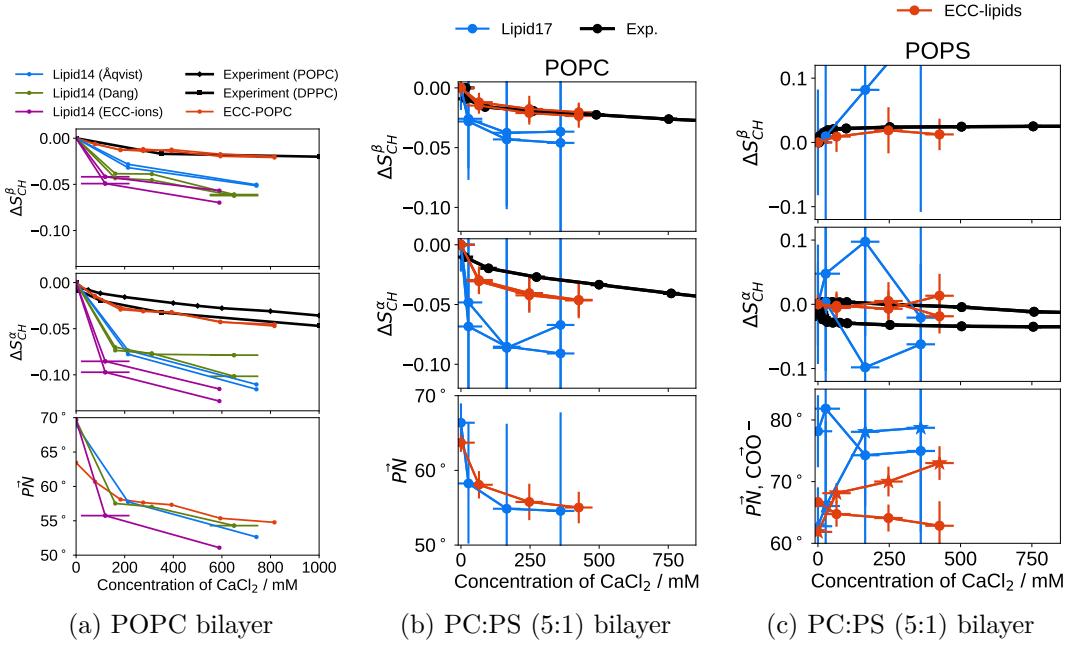


Figure 2: Changes of the head group order parameters  $S^\alpha$  and  $S^\beta$  and the orientations of the carboxylate group and the P-N vector of POPC (left, middle) and POPS (right) in phospholipid bilayers as a function of  $\text{CaCl}_2$  concentration in bulk ( $C_{ion}$ ) from simulations with different force fields and experiments (DPPC (323 K) [Akutsu and Seelig, 1981], POPC (313 K) [Altenbach and Seelig, 1984], PC:PS [Roux and Bloom, 1990]). Results from a pure POPC bilayer are shown in the left figure; results from a mixed PC:PS (5:1) bilayer are shown in the middle and right figure. The orientation of the  $\text{COO}^-$  group is defined as the connector from the  $\beta$  carbon to the carbon in  $\text{COO}^-$  (stars, bottom right).

bilayers in pure water without any ions (or only counterions) from simulations and experiments as the first step in the assessment of the quality of the model. The experimental X-ray scattering form factors of a bilayer are well reproduced for all lipids employing the presented ECC-lipids model, as shown in Fig. 1 for POPC. The head group order parameters  $S^\alpha$  and  $S^\beta$  are highly relevant for this work, as they are being used in the lipid electrometer concept. For POPC in pure water, the order parameter  $S^\beta$  agrees well with the experiment, while the order parameter  $S^\alpha$  is somewhat lower.

The changes of the head group order parameters  $S^\alpha$  and  $S^\beta$  from simulations and experiments are shown in Fig. 2a for a neutral POPC bilayer, and in Figs. 2b and 2c for a negatively charged bilayer with the composition 5 PC:1 PS. For a direct comparison and a connection to our works [Catte et al., 2016, Bacle et al., 2018], we show simulation results from ECC-lipids and also from Lipid17, a standard non-polarizable fixed-charge force field [Gould et al., 2018]. This also highlights the improvements in ECC-lipids over Lipid17 arising from the electronic polarization. The effect is probably the most striking for POPS, for which also the structure of a pure POPS bilayer with only counterions is dramatically improved with the augmentation.

Increasing concentrations of  $\text{CaCl}_2$  induce a systematic decrease of the order

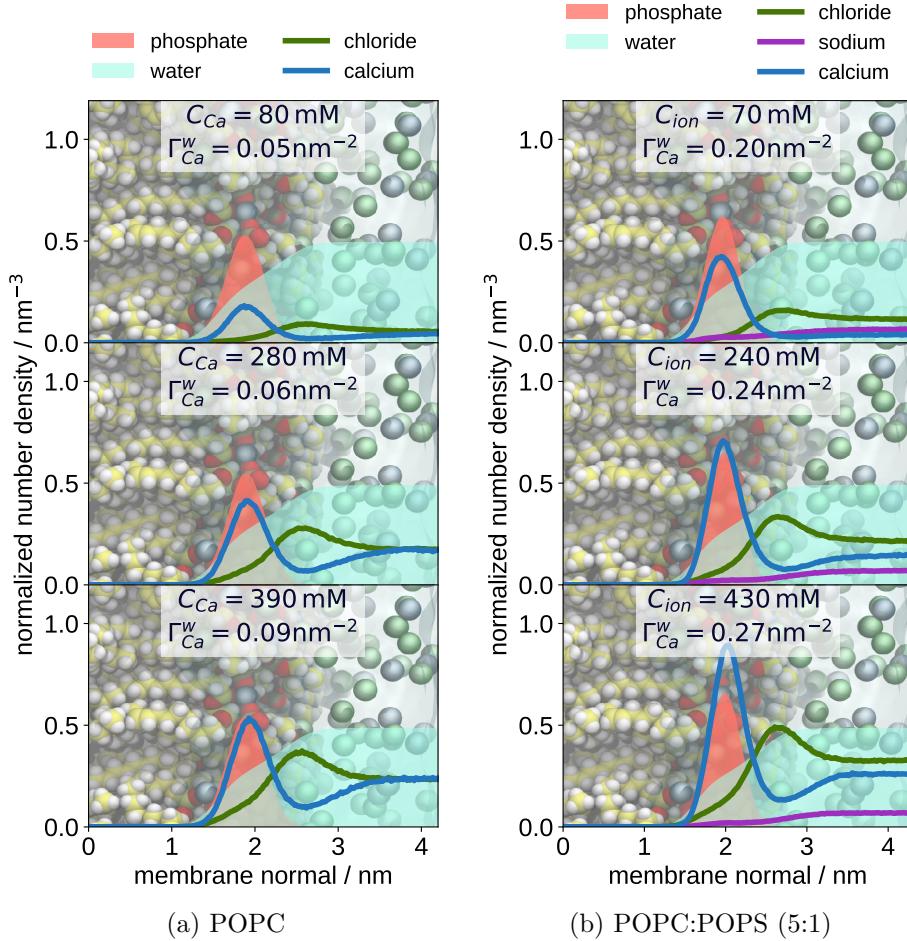


Figure 3: Number density profiles of  $\text{Ca}^{2+}$  and  $\text{Na}^+$ ,  $\text{Cl}^-$  along the normal of the phospholipid bilayers starting at the centre for different concentrations of  $\text{CaCl}_2$  from simulations with ECC-lipids. In order to visualize the density profiles with a scale comparable to the profile of  $\text{Ca}^{2+}$ , the density profiles of  $\text{Na}^+$  and  $\text{Cl}^-$  ions are divided by 2, and the density profiles of phosphate groups and water are divided by 5 and 200, respectively.

parameters  $S^\alpha$  and  $S^\beta$  in POPC. Although the total magnitude of the response of the PC head group order parameters is only slightly higher in the negatively charged bilayers than in the neutral bilayers, the shape of the changes in the latter shows a steeper onset at low concentrations. This is apparently due to the presence of POPS, which has a higher affinity to  $\text{Ca}^{2+}$  compared to POPC. ECC-lipids is the first model, which achieves a *quantitative* agreement with the changes induced by  $\text{CaCl}_2$  in experiments.

The increase in the amount of bound calcium cations from pure POPC to the mixed negatively charged bilayer containing POPS is well demonstrated using the relative surface excess,  $\Gamma_{Ca}^w$ . Distributions of  $Ca^{2+}$ ,  $Na^+$  counterions and also  $Cl^-$  are plotted in Fig. 3a for the neutral POPC bilayer, and in Fig. 3b for the negatively charged bilayer. The increasing concentration of  $CaCl_2$  and, hence, a higher amount of bound  $Ca^{2+}$  attracts  $Cl^-$  anions to the bilayer as can be seen from its growing density at the interface. The density profiles of ions suggest that the dominant contribution to the binding of  $Ca^{2+}$  to phospholipid bilayers comes

from the interactions with the phosphate groups in both POPC and POPS. This is also reflected in the shift of the mean orientation of the  $\text{COO}^-$  group from  $62^\circ$  to  $73^\circ$  (420 mM  $\text{CaCl}_2$ ) which was measured as the connector of the carbon atoms forming the bond between the group and the  $\beta$ -carbon of the phospholipid. The interactions of the carboxylate group in PS with calcium and other phosphate groups shed light into the qualitatively different response of the head group order parameters  $S^\alpha$  and  $S^\beta$  in PS compared to PC (see Figs. 2a and 2b). We find that the complex response of the head group order parameters of POPS is affected by the conformational changes of the carboxylate group, which is attracted more towards the phosphate region, where the calcium cations dominantly bind. In line with the experimental work by Browning and Seelig [1980], this is also very likely the reason, why the magnitude of the P-N vector change in POPS is diminished compared to POPC, which is not restrained by an additional cation binding group like  $\text{COO}^-$  in POPS.

In summary, treatment of the electronic polarization was shown to have a dramatically positive impact on the accuracy of the description of interactions between phospholipids and cations. The presented application of ECC to lipids constitutes a pivotal work for its future adaptations to also other compounds, especially charged and zwitterionic, e.g., proteins or nucleic acids, for which we expect improvements in computational description in a similar range.

## Modeling of transmembrane potential

The intracellular environment of cells contains a weak electrolytic solution of KCl, while there is a similarly weak solution of NaCl on the extracellular side. The concentrations of these salts, usually around 150 mM, are regulated and maintained out of equilibrium through specific channels and pumps to provide cellular functions and general homeostasis conditions [Bezanilla, 2008, Sten-Knudsen, 2002]. The unequal distribution of ions on either side of the membrane gives rise to a transmembrane potential. For instance, the concerted action of voltage-gated ion channels and pumps in neurons modulates their transmembrane potential providing a fast signal transduction through the axons [Sten-Knudsen, 2002, Storace et al., 2015, Sung et al., 2015]. A common magnitude of the transmembrane potential in cells is in the range of 10–100 mV.

The transmembrane potential in simulations can be modeled by several methods. [Tieleman et al., 2001, Sin et al., 2015, Roux, 1997, Sachs et al., 2004]. In particular, there are two approaches for the modeling of the membrane potential in atomistic molecular dynamics simulations – the constant electric field method [Roux, 1997, 2008, Gumbart et al., 2012], and the ion-imbalance method [Sachs et al., 2004, Delemonette et al., 2008]. Both of these methods have been successfully used to study membrane electroporation or voltage-sensitive proteins [Vargas et al., 2012, Böckmann et al., 2008, Gumbart et al., 2012, Kutzner et al., 2011, Casciola et al., 2014]. These two practically independent developments were compared and connected together in our work [Melcr et al., 2016], where we prove them to be equivalent models of the transmembrane potential yielding indistinguishable results, at least for electrolytes formed by the same monovalent ions on both sides of the membrane. We used simulations in which we simultaneously applied both methods with the same magnitude but opposite polarity yielding

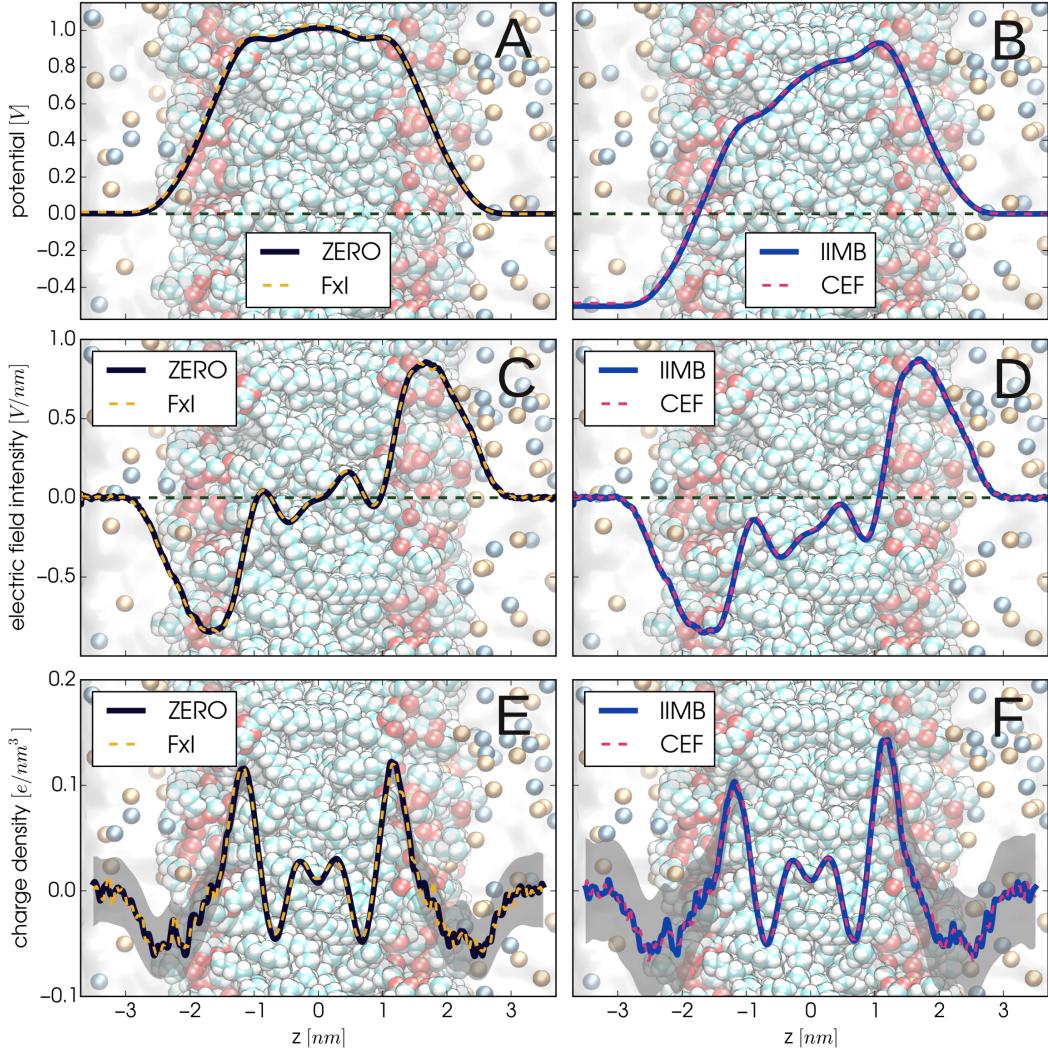


Figure 4: The transmembrane potential (A,B), the electric field intensity (C,D), and the charge density (E,F) profiles for simulations of a POPC bilayer with 150 mM concentration of KCl. ZERO stands for simulations without any applied voltage, IIMB stands for the ion imbalance method, CEF stands for the constant electric field method, and FxI denotes the special setup, in which the two methods are applied simultaneously. The gray area shows the standard error. The voltage drop across the membrane is 499 mV for IIMB and 486 mV for CEF with the error of about 7 mV.

zero transmembrane voltage in total to highlight possible artifacts. The comparison in Fig. 4 shows that such a setup is indistinguishable from simulations without voltage within the achievable accuracy. The electric field induced by the voltage exists exclusively in the hydrophobic region of the membrane, where it has an almost constant strength. This finding provides clues to understanding the evolutionary design of voltage-gated proteins. [Vargas et al., 2012] Moreover, the structure of the bilayer is preserved even at high voltages at the time scales of our simulations, unlike that of water at the interface with the hydrophobic core of the bilayer underlining its importance in electroporation. [Bu et al., 2017]

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# List of publications

- I Transmembrane Potential Modeling: Comparison between Methods of Constant Electric Field and Ion Imbalance. *Journal of Chemical Theory and Computation*, 12(5), (2016). **Josef Melcr**, Daniel Bonhenry, Štěpán Timr, and Pavel Jungwirth.
- II Molecular electrometer and binding of cations to phospholipid bilayers. *Phys. Chem. Chem. Phys.*, (2016). Andrea Catte, Mykhailo Girych, Matti Javanainen, Claire Loison, **Josef Melcr**, Markus S. Miettinen, Luca Monticelli, Jukka Maatta, Vasily S. Oganesyan, O. H. Samuli Ollila, Joona Tynkkynen, and Sergey Vilov.
- III Accurate Binding of Sodium and Calcium to a POPC Bilayer by Effective Inclusion of Electronic Polarization. (2018) *The Journal of Physical Chemistry B*, 122(16), (2018). **Josef Melcr**, Hector Martinez-Seara, Ricky Nencini, Jiří Kolafa, Pavel Jungwirth, and O. H. Samuli Ollila.
- IV Head group and glycerol backbone structures, and cation binding in bilayers with PS lipids. Submitted (2018). Amelie Bacle, Pavel Buslaev, Lukasz Cwiklik, Fernando Favela, Tiago Ferreira, Patrick Fuchs, Ivan Gushchin, Matti Javanainen, Batuhan Kav, Jesper Madsen, **Josef Melcr**, Markus Miettinen, Ricky Nencini, Chris Papadopoulos, Thomas Piggot and O. H. Samuli Ollila.

