Considerations of Recent All-atom Lipid Force Field Development

Jeffery B. Klauda[1,2,*]

[1]Biophysics Graduate Program, [2]Department of Chemical and Biomolecular Engineering,
University of Maryland,
College Park, Maryland 20742, USA

*Corresponding Author: jbklauda@umd.edu

Abstract

Molecular simulations of biological molecules require an accurate description of molecular interactions through a force field (FF). The focus of this *Perspectives* article is on all-atom lipid FFs. Recent additions to the CHARMM36 lipid FF continue to expand a researcher's ability to probe membrane structure and function with a wide variety of biologically important lipids. Currently, there is an effort to reduce the assumptions in all-atom lipid FFs. The inclusion of long-range dispersion interaction through particle-mesh Ewald is allowing for more accurate descriptions of lipid bilayer and monolayer properties without additional computational cost. Soon, simulations with lipid FFs will no longer depend on short-range cutoffs and will accurately represent long-range dispersion. This requires efficient FF parameterization with an automated approach due to FF complexity. In addition, polarizable FFs for lipids will be important for the next generation of simulations that accurately represent how molecule interactions respond to a varied environment.

1. Introduction

Lipids are an essential biological building block that provide structure to cellular membranes and limit the movement of molecules across the membrane to that primarily controlled by transmembrane proteins. Although a strong focus on disease has been how proteins and their mutations play an important role in health, lipids play a critical role in biological processes and mechanisms.[1] From a biotechnological perspective, lipids are important to vaccine development and have been used as delivery vehicles and promoting an immune response with lipid nanoparticles and are key to the efficacy of the messenger RNA-based SARS-CoV-2 vaccines.[2-4]

Molecular simulations of biomolecules, such as lipids, rely on accurate descriptions of interactions that yield forces between molecules to probe the natural movement of molecules in their environment. This requires an accurate mathematical representation of molecular forces known as a force field (FF). This *Perspectives* article will briefly introduce the complexity of all-atom pair-wise additive lipid FFs. The focus here will be on summarizing key FFs used by the AMBER and CHARMM communities and how these have recently been applied to ether lipids and modeling bacterial membranes.

Until recently, lipid FFs assumed that long-range van der Waals interactions need not be included. However, the development of methods to incorporate long-range dispersion energies efficiently in simulations has opened the door to consider developing a lipid FF that includes long-range dispersion. Since it has been shown that lipid FFs cannot simultaneously represent lipid bilayer and monolayer properties,[5] the inclusion of this long-range interaction is crucial for a well-balanced FF. With many experimental observables as targets for FF optimization with lipid assemblies, the development of automated approaches to parameter optimization will guide the improvement of FFs. This *Perspectives* article will summarize some recent work on this with a focus in using thermodynamic reweighting to guide the lipid parameter optimization.[6, 7]

Finally, this *Perspectives* article will present the next generation of lipid FFs that include electronic polarization. The CHARMM community approach using the Drude Oscillator will be introduced and its current limitations. The methods developed for the automization of parameter fitting in the pairwise-additive FF are incorporated in optimization of the Drude FF to improve its current inaccuracies in membrane mechanical properties.

2. All-atom Pair-wise Additive Lipid Force Fields

This section briefly presents the common mathematical description of the interaction between atoms in a lipid system, i.e., the force field, and a summary how these are obtained. More details can be found in a past review article.[8] The FF used to describe the all-atom CHARMM36 (C36) lipid FF[9] generally follows the classical Class I FF with harmonic bonded terms, dihedrals, and non-bonded terms (van der Waals and electrostatics) as shown

in eq. 1. However, C36 contains a Urey-Bradley cross-term for a few triplicates so has a Class II functional form for these cases.

$$
\begin{aligned}
U(r_1, r_2, \cdots, r_N) \\
&= \sum_{bonds} k_{b,ij}\left(r_{ij} - r_{0,ij}\right)^2 + \sum_{angles} k_{\theta,ijk}\left(\theta_{ijk} - \theta_{0,ijk}\right)^2 + \sum_{cross} k_{ub,i(i+2)}\left(r_{ii(i+2)} - r_{0,ii(i+2)}\right)^2 \\
&+ \sum_{improper} k_{\phi,ijkl}\left(\phi_{ijkl} - \phi_{0,ijkl}\right)^2 + \sum_{dihedrals} \sum_{\forall n} k_{\varphi,n}\left(1 + cos\left(n\varphi_{ijkl} - \delta_n\right)\right) \\
&+ \sum_{\substack{non-bonded \\ pairs}} \varepsilon_{ij}\left[\left(\frac{R_{min,ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{min,ij}}{r_{ij}}\right)^{6}\right] + \sum_{electrostatic} \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}
\end{aligned}
\tag{1}
$$

The bond and angle terms in eq. (1) are typically obtained to match structural and spectroscopic data of small molecules and if none is available then quantum mechanical calculations are performed to fit these parameters. The equilibrium distance (r_0), angles (θ_0, ϕ_0), and associated force constants (k_b, k_θ, k_{ub}, k_ϕ) are treated as universal for each atom type. The improper terms are typically added to maintain chiral or planar centers. The C36 lipid FF does not contain CMAP terms that are present for proteins.[10]

The last three terms in eq. (1) are the most integral to bilayer properties and careful optimization is required to develop an accurate lipid FF. For most widely-used all-atom lipid FFs, the dihedral term is a correction to the non-bonded potential as interactions separated by three chemical bonds (1-4 interactions) include the electrostatic and Lennard-Jones (LJ) interactions (with or without a scaling factor depending on the family of the force field, i.e., AMBER and CHARMM).[8] These are typically fit to high-level quantum mechanical (QM) calculations of torsional profiles for relevant small molecules. The Slipid[11, 12] and Lipid14[13] FFs follow the AMBER community use of the restricted electrostatic potential approach (RESP)[14] to obtain partial charges using QM and LJ parameters are optimized to experimental observables. However, the CHARMM-community takes the supramolecular approach with charges and Lennard-Jones terms obtained from small molecule-water interactions and additional fits to experimental observables.[15-17]

Traditionally the optimization of FF parameters took advantage of the transferability of functional group parameters so that small molecules can act as a guide in developing an accurate FF for the macromolecule, here phospholipids. The last three terms in eq. (1) are coupled and thus optimization requires simultaneous fitting or procedure for parameter refinement. Simulations of small molecules (alkanes, alkenes, ethers, phosphates, etc.) compared to bulk experiments (densities, heats of vaporization, free energy of solvation, diffusion, etc.) are used to fit or refine atomic non-bonded parameters (ε, R_{min}, q). Then, the dihedral potential can be fit to QM torsional profiles based on the non-bonded parameters. This approach had a benefit to not require expensive all-atom lipid bilayer simulations in the optimization of FF parameters assuming that the small molecule parameters were

transferrable to the larger lipid. For the alkane to lipid-acyl chain, this transferability was a good assumption. However, considering the lipid head group and the glycerol region connecting the two acyl chains, transferability was more difficult and ultimately required some parameter optimization based on bilayer simulations for the C36 lipid FF.[9] With the current computational speed, optimization procedures of the lipid FF are moving towards more automated and lipid-bilayer focused to allow for more accurate parameters (see Section 4).

Although there is always room for improvement in FF parameters, the general careful optimization of the C36, Slipid, and Lipid14 FFs has allowed for accurate simulations of lipid bilayers ranging from one lipid type to multiple lipids to systems with proteins. The details of lipid diversity can be found in a past review,[8] but overall the Lipid14 FF is limited to phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol lipids, Slipids has additional diversity including phosphatidylglycerol (PG), phosphatidylserine (PS), sphingolipids and polyunstaturated lipids. However, the C36 FF has been developed for a wide range of lipid types including phosphitylinositols, various sterols, gylcolipids, ether lipids, etc. Our recent addition of plasmalogen lipids with their vinyl ether chain (Fig 1A)[18] will allow for development of membrane models for neuronal membranes that have a significant proportion of these lipids. The lipid diversity and testing accuracy across varied temperatures and lipid compositions[19-21] has allowed for development of accurate model membranes such as that of the plasma membrane of *Psudomonas aeruginosa* (Fig 1B).[22] For example, these model membranes have been used to probe peptide binding to yeast organelles[23] and the mechanism of the serotonin receptor in a model neuronal membrane (Fig 1C).[24]

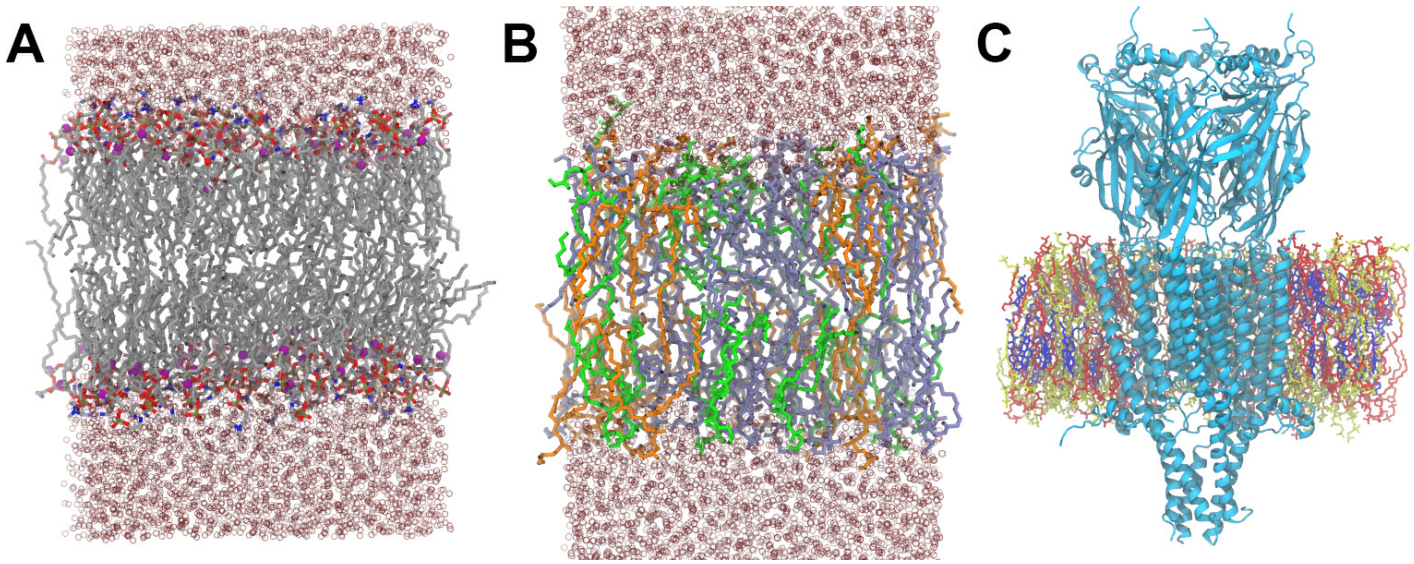


Figure 1. A. PLAPE (C18(Plasm-18:1PE)) with POPC bilayer at 2:1 ratio with water in red hollow spheres, carbon in silver, oxygen in red, phosphorous in gold, nitrogen in blue and the ether oxygen in magenta.[18] **B.** Plasma membrane of *P. aeruginosa* in the biofilm condition with PE lipids in blue, PG lipids in orange, and lipids (PE and PG) with cyclopropane-containing chains in green.[22] **C**. 5-HT3 (light blue) embedded in a membrane of cholesterol (blue), SDPC (red) and POPC (yellow).[24]

3. Importance in including Long-range van der Waals Interactions

The FF function described above is half of the story with regards to simulating a condensed phase system. In reality, molecules interact based on position and if a distance is large then its interaction is essentially zero. This can be used to reduce the number of calculations for the non-bonded parts to eq (1) (last two terms). Traditionally, to save computational time, cutoffs were used in which interactions beyond a cutoff are ignored. Although the strongest interaction is local, long-range (LR) effects can have a significant contribution to the energy state of a molecule in a liquid. This is especially important for the slowly decaying Coulombic term of the FF and thus it is widely accepted that the use of particle-Mesh Ewald[25] for electrostatics is required for simulations.[26]

Since the dispersion term in the LJ potential decays $\propto r^{-6}$ compared to the electrostatic slower decay $\propto r^{-1}$, ignoring LR LJ dispersion might appear to be a reasonable assumption. For some examples this is the case when electrostatics dominate the interaction between molecules like the density of water. However, if you consider the water/air surface tension, there is a slight dependence in this value for cutoffs at 10 Å (cutoff value used with some FFs) with PME electrostatics, which are 5-7% off compared to values from 25 Å cutoffs (Figure 2).[27] This cutoff dependance is even stronger with molecules dominated by weaker LJ dispersion interactions, e.g., alkanes. The hexadecane/air surface tension shows a strong dependence on cutoff with 60% reduction in surface tension at 10 Å cutoff compared to 24 Å. The FF developer has two options: 1. Fit a FF that ignores the LR LJ and is an effective fit lumping the LR effects into the shorter range or 2. Fit a FF that explicitly incorporates the LR LJ. Although Option #1 could be used, the accuracy of the short-range interaction is sacrificed. Option #2 has been the choice in developing FF parameters based on small molecule studies and was used in the development of the C36 lipid FF but not used until recently in lipid simulations, i.e., the long-range correction was used for small molecule fits but then standard LJ cutoffs are used in simulations with bilayers.

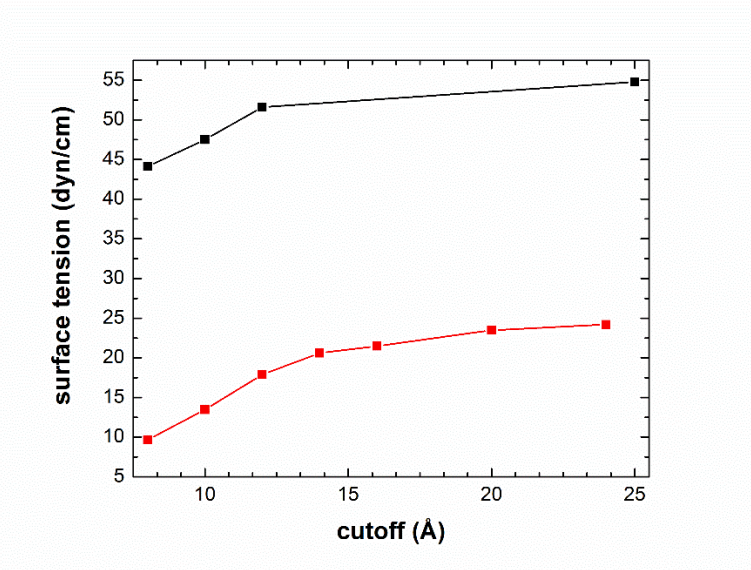


Figure 2. Surface tension of water (black) and hexadecane (red) with air taken from past work.[27, 28]

For simple isotropic systems, the inclusion of LR LJ can be incorporated with a radial distribution function approximation for the LR contribution. However, for systems of biological interest, especially lipid membranes, this isotropic assumption is invalid. Lagüe et al.[29] used a pressure-based LR correction (LRC) for LJ that increased the pressure of the system based on the LR forces. This worked well for simple alkanes and some bilayers and monolayers, but is a correction based on the average pressure effect due to the LR effects. A more rigorous approach focusing on the LR effect at a given state would require extending PME to include LJ dispersion. In 1995, Essmann et al.[30] demonstrated the use of Ewald dispersion in PME but not until recently has a form been developed for popular simulation programs like GROMACs[31] and CHARMM[28] (with the method referred to as LJ-PME here). These methods allow for efficient LR LJ calculations that are effectively at the cost of standard cutoffs used for LJ potentials.

Although recent inclusion of LR LJ to simulation programs has allowed for the use of LJ-PME, current lipid FFs are generally tuned a represent experiment with a cutoff for the LJ interactions. For the C36 lipid FF, lipid bilayers should be run with a force-based switching function that brings the force to zero at 12 Å, so as to reproduced the published accuracy of this FF on various lipids.[9, 18, 20, 32-37] However, not including the LR LJ in lipid monolayers is known to result in inaccurate property prediction, i.e., pressure-area isotherm.[5] This leads to an unsatisfying result that requires a different approach for bilayers and monolayers to agree with experimental observables. With a multi-dimensional parameter space and many experimental observables, the use of an automated approach for optimizing parameters and predicting how parameter changes influence membrane properties now allows[6, 7] for a more efficient reaching of an improved FF to universally represent the lipid bilayer and monolayer with LJ-PME.

4. Focus on Semi-Automated Parameter Optimization

Force field optimization requires a detailed focus on all contributions to the interaction and one that should accurately represent interactions beyond the initial fit. The ability to transfer FF parameters to common functional groups is ideal and allows for consistency between other portions of the FF. For example, parameters developed for the lipids with optimization to properties of bilayers and monolayers must be also accurate for other biomolecules that interact with the membrane, i.e., proteins, nucleic acids, or carbohydrates. Care must be taken in automatic parameter optimization that overfitting could lead to potential issues with predicting properties.

Automated FF development has expanded recently and includes some initial efforts to the lipid FF.[6, 7] The ForceBalance method was used that utilizes a regularized least-squared objective function which prevents overfitting by incorporating a penalty term to reduce large variations. This limited the change of the parameters and resulted in a RMS of change from the initial values to 10.9%.[7] Analytical gradients were used in a Levenberg-Marquardt algorithm for optimization based on an ensemble average of a thermodynamic property and the energy with a given parameter set. The optimization was done on to match area per lipid and order parameters and appears to work well for the optimizing parameter and agrees with experiments for a single lipid, but the computational cost for this approach is not ideal for developing a model based on a larger set of experimental observables and varied lipids.

Instead, in collaboration with Drs. Richard Pastor, Andreas Krämer, and Bernard Brooks, thermodynamic reweighting with regularization was used to predict parameter sets that best match experimental observables without the need to calculate membrane properties for various parameter sets.[6, 7] Moreover, with the inclusion of LJ-PME, optimization can be performed with the aim to match bilayer and monolayer properties. This approach essentially reduces the time required to optimize a FF down from years to a manner of a couple months. The key is to use statistical thermodynamics of parameter sensitivity estimated with thermodynamic reweighting,[7]

$$\boldsymbol{S}_{prop}(\boldsymbol{\lambda}, \boldsymbol{\delta\lambda}) = \langle \boldsymbol{f}^{sim} \rangle_{\boldsymbol{\lambda}+\boldsymbol{\delta\lambda}} - \langle \boldsymbol{f}^{sim} \rangle_{\boldsymbol{\lambda}} = \frac{\langle \boldsymbol{f}^{sim} e^{-\beta(U_{\boldsymbol{\lambda}+\boldsymbol{\delta\lambda}} - U_{\boldsymbol{\lambda}})} \rangle_{\boldsymbol{\lambda}}}{\langle e^{-\beta(U_{\boldsymbol{\lambda}+\boldsymbol{\delta\lambda}} - U_{\boldsymbol{\lambda}})} \rangle_{\boldsymbol{\lambda}}} - \langle \boldsymbol{f}^{sim} \rangle_{\boldsymbol{\lambda}} \qquad (2)$$

where $\boldsymbol{S}_{prop}$ is the sensitivity matrix of properties, $\boldsymbol{\lambda}$ is the original parameter set and $\delta\boldsymbol{\lambda}$ is the perturbation of that parameter set. $\langle \cdot \rangle_{\boldsymbol{\lambda}}$ denotes the average over trajectory, which is also the ensemble average of parameter set $\boldsymbol{\lambda}$, approximately. As long as the underlying description of the partition function in $\boldsymbol{\lambda}$ is correct with the $\delta\boldsymbol{\lambda}$ perturbation, this approach can use simulations of a starting parameter set to predict changes in parameters to better match experimental observations. Details of the methods to optimize can be found in reference [7], but perturbations are limited to small changes in parameter space, several hundredths of a partial atomic charge, 3% for $R_{min}/2$ and 10% for ε.[6] Multiple passes in the optimization are typically required to result in a well fit parameter set but with C36 as a starting point only two to three passes are required for convergence. This is approach is

semi-automated as weight factors and parameter restraints can be adjusted during the optimization process.[6] Ultimately with the use of GPUs, a single pass for optimization requires only a few days.

One of the key aspects in optimization was to consider the range of parameter space optimization. This approach can certainly be used to optimize much of the lipid head group, but is this necessary and would this lead to overfitting? We tested optimizations of the entire PC head group (*Global*) versus that of the glycerol backbone (*Linkage*).[6] The phosphate parameters in the CHARMM FF were carefully parameterized based on nucleic acids and adjustments to these parameters should only be made if it was deemed necessary for the lipid FF. Maintaining transferability and balance in how the phosphate interacts with other biological molecules and water is important for a general FF for all biological molecules. Our investigation on the *Global* versus *Linkage* optimization demonstrated that the reduced optimization set resulted in fits that were within statistical errors of the *Global* set (see Table 4 of reference [6]) and thus the *Linkage* optimization was deemed to be appropriate for optimizing parameters for phospholipids.[6] The optimization for our initial training set of dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer properties (area per lipid, chain order parameters, area compressibility) and DPPC monolayer isotherm showed convergence in three optimization steps for the *Global* and two steps for the *Linkage* parameter sets. The change in the optimized non-bonded properties for the *Global* set was more monotonic compared to the *Linkage* but both showed convergence.[6] Consequently, the *Linkage* optimization resulted in excellent agreement for tested bilayer and monolayer properties with LJ-PME.

The next test on optimization was to extend this approach to lipids besides the saturated and monounsatured PC lipids. Further optimizations was done for PC lipids with an ether linkage and validation of transferability of parameters was tested for PE and PG lipids.[7] The optimization of the ether linkage resulted in excellent agreement with scattering-based experimental properties. The PC lipids (saturated and monounsaturated tails) were tested across a wider range of temperatures (303-333K) on x-ray and neutron form factors and associated estimates in membrane thickness and area per lipid and the agreement was excellent.[7] The area compressibility and deuterium order parameters also showed strong agreement with experiment for C36 LJ/PME. However, the PG lipids showed some inaccuracy in the order parameter suggesting further optimization of the glycerol group is needed to obtain a less packed membrane and decrease the order parameters to better match experiment. For some lipids, like PE, C36 parameters can be transferred but other lipids (PG and likely PS) require additional optimization.[7] C36 LJ/PME is likely compatible with the CHARMM protein FF but further validation is necessary.

5. Next Generation Force Fields that Include Polarization

Although the development of pair-wise additive FF has continued, the assumption of a fixed partial charge that is not influenced by the environment can be a severe assumption. The distribution of partial charges of a

molecule in water compared to in an alkane-like environment will change and is related to the polarizability. Not including the polarization effect will have inaccuracies when considering residues exposed to water versus a membrane core, estimating the free energy of solvation of molecules in water compared to an organic solvent, permeation of drugs across a bilayer, etc. Although including polarization is important, the computational cost has been high and consequently limited in application.

There are many approaches to consider the polarization of molecules in molecular simulation and these have been used and developed over the last several decades. Traditionally, polarization has been incorporated using fluctuating charge (FQ) or induced dipole/multipole approaches,[38] but these have traditionally used self-consistent iterative approaches that are computationally demanding. Original developments in the lipid polarizable FF, considered this approach and Patel and co-workers developed a working FF for some common lipids.[39, 40] The importance of considering larger lipid membrane patches and the inclusion of proteins in membrane requires an approach that is computationally more efficient.

The CHARMM community has moved to using a Drude Oscillator approach[41, 42] to include polarization with a more efficient approach. A virtual particle (Drude particle) has a charge $q_{D,i}$ that is connected to a polarizable atom *i* and is harmonically restrained with a force of k_D. The Drude particle fluctuates due to the variation in the electrostatic environment to represent the electron cloud response to its local environment. Therefore, the Drude FF can use a Class I energy function (eq. (3)) with an additional term to describe the energy associated with the Drude particle:[43]

$$U_{Drude}(r_1,\cdots,r_N,r_{D1},\cdots,r_{Dn}) = \frac{1}{4\pi\varepsilon_0}\left(\sum_{i<j}\frac{q_{D,i}q_j}{\|r_{Di}-r_j\|}+\sum_{i<j}\frac{q_{D,i}q_{D,j}}{\|r_{Di}-r_{Dj}\|}\right)+\frac{1}{2}\sum_{\substack{polarizable\\ atoms\ i}} k_D\|r_{Di}-r_i\|^2$$

(3)

The charge of the Drude particle is represented by its polarizability (α_i) with $q_{D,i}=\sqrt{k_D\alpha_i}$. To save computational time, an extended Lagrangian method is used where the Drude particle has 0.4 amu in mass taken from the associated heavy atom.[44, 45] This way the simulation can proceed like a pairwise additive simulation and only requires the cost of an additional particle for each heavy atom. Due to frequency of motion, simulations require a 1 fs timestep compared to generally a 2 fs timestep used with all-atom pairwise additive simulations.

The development of the Drude polarizable FF for lipids for applications to lipid bilayers started with water and alkanes.[41-43] In 2013,[46] the first Drude polarizable FF was developed for DPPC and further optimized and extended to other PC lipids and PE lipids.[47] This most up-to-date Drude FF utilized a restrained ensemble-maximum entropy methodology to obtain accurate representations of the NMR order parameters of the head group. For the tested lipids, the structural properties of lipid bilayers are in reasonable agreement with experiment (area per lipid, NMR order parameters, bilayer density profiles, and thicknesses) and the pairwise additive model.

However, the lateral area compressibility for the Drude lipids was at least twice that of experiment suggesting further improvement is warranted that will likely use the thermodynamic reweighting approach as discussed above.

6. Conclusions

Our initial headway into including long-range Lennard-Jones into parametrization and simulations for lipid bilayers has been successful for PC and PE lipids. The C36/LJ-PME FF can now represent bilayer and monolayers simultaneously and remove the dependence of LJ cutoff on properties by including LJ with PME. The use of thermodynamic reweighting is an attractive approach to optimize parameters, especially with a reasonable starting point that the original C36 FF provides. As noted here, the anionic lipids (PG and PS) will require additional parameterization to match the experimental observables. Since C36 without LJ-PME has an exhaustive set of lipids, C36/LJ-PME will need to be tested and parameters optimized for the varied set of lipids. Other important lipids, i.e., cholesterol, sphingolipids, and lipids polyunsaturated chains, will be the initial focus before optimizing lipids that are less common. The use of thermodynamic reweighing is certainly not limited to lipid FF development and can be applied to the development of any molecular force field.

In addition to new optimization routines, the use of hydrogen mass repartitioning (HMR)[48, 49] or virtual sites[50] has been shown to be important to maintain FF accuracy but allows for simulations with 4-5 fs timesteps that will allow for roughly twice the speedup compared to standard approaches. These approaches will continue to be tested to determine if these can be routinely used for membrane simulations.

Finally, the next generation of lipid FFs will need to include polarization to accurately represent many properties. A collaborative effort is currently underway between various labs including mine, Alex MacKerell (U. Maryland – Baltimore), Richard Pastor (NIH), Benoît Roux (U. Chicago), in optimizing the Drude FF. The thermodynamic reweighing approach is currently being used to improve the general agreement in properties and focusing on obtaining an accurate description of lipid bilayers and improving the representation of the area compressibility modulus. As with C36/LJ-PME, this will require an extensive effort to optimize the FF for a wide range of lipid types. With an updated and accurate Drude lipid FF, molecule partitioning in membranes relative to water will be better described compared to a pairwise additive FFs that cannot represent the change in environment. Simulating how drugs partition into cellular membranes will benefit from an accurate Drude FF. Expect in the next few years more updates and improved lipid FFs for both the pairwise additive and polarizable force fields!

7. Acknowledgements

This research was supported by two NSF grants: MCB-1951425 and CHE-2003912. I would like to acknowledge important current and former students involved in the research presented above: Nick Guros, Alison Leonard, and Yalun Yu.

8. References

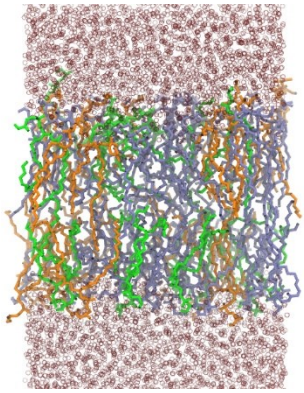
1. van Meer, G.; Voelker, D. R.; Feigenson, G. W., Membrane lipids: where they are and how they behave. *Nature Reviews Molecular Cell Biology* **2008,** *9* (2), 112-124.
2. Elia, U.; Ramishetti, S.; Rosenfeld, R.; Dammes, N.; Bar-Haim, E.; Naidu, G. S.; Makdasi, E.; Yahalom-Ronen, Y.; Tamir, H.; Paran, N., et al., Design of SARS-CoV-2 hFc-Conjugated Receptor-Binding Domain mRNA Vaccine Delivered via Lipid Nanoparticles. *ACS Nano* **2021**.
3. Guevara, M. L.; Persano, F.; Persano, S., Advances in Lipid Nanoparticles for mRNA-Based Cancer Immunotherapy. *Frontiers in Chemistry* **2020,** *8* (963).
4. Eygeris, Y.; Patel, S.; Jozic, A.; Sahay, G., Deconvoluting Lipid Nanoparticle Structure for Messenger RNA Delivery. *Nano Letters* **2020,** *20* (6), 4543-4549.
5. Pastor, R. W.; MacKerell, A. D., Development of the CHARMM Force Field for Lipids. *The Journal of Physical Chemistry Letters* **2011,** *2* (13), 1526-1532.
6. Yu, Y.; Krämer, A.; Venable, R. M.; Simmonett, A. C.; MacKerell, A. D.; Klauda, J. B.; Pastor, R. W.; Brooks, B. R., Semi-automated Optimization of the CHARMM36 Lipid Force Field to Include Explicit Treatment of Long-Range Dispersion. *Journal of Chemical Theory and Computation* **2021,** *17* (3), 1562-1580.
7. Yu, Y.; Krämer, A.; Venable, R. M.; Brooks, B. R.; Klauda, J. B.; Pastor, R. W., CHARMM36 Lipid Force Field with Explicit Treatment of Long-Range Dispersion: Parametrization and Validation for Phosphatidylethanolamine, Phosphatidylglycerol, and Ether Lipids. *Journal of Chemical Theory and Computation* **2021,** *17* (3), 1581-1595.
8. Leonard, A. N.; Wang, E.; Monje-Galvan, V.; Klauda, J. B., Developing and Testing of Lipid Force Fields with Applications to Modeling Cellular Membranes. *Chemical Reviews* **2019,** *119* (9), 6227-6269.
9. Klauda, J. B.; Venable, R. M.; Freites, J. A.; O'Connor, J. W.; Mondragon-Ramirez, C.; Vorobyov, I.; Tobias, D. J.; MacKerell, A. D.; Pastor, R. W., Update of the CHARMM all-atom additive force field for lipids: Validation on six lipid types. *Journal of Physical Chemistry B* **2010,** *114* (23), 7830-7843.
10. Feig, M.; MacKerell, A. D., Jr.; Brooks, C. L., Force field influence on the observation of π-helical protein structures in molecular dynamics simulations. *Journal of Physical Chemistry B* **2003,** *107* (12), 2831-2836.
11. Jämbeck, J. P. M.; Lyubartsev, A. P., An Extension and Further Validation of an All-Atomistic Force Field for Biological Membranes. *Journal of Chemical Theory and Computation* **2012,** *8* (8), 2938-2948.
12. Jämbeck, J. P. M.; Lyubartsev, A. P., Another Piece of the Membrane Puzzle: Extending Slipids Further. *Journal of Chemical Theory and Computation* **2013,** *9* (1), 774-784.
13. 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. *Journal of Chemical Theory and Computation* **2014,** *10* (2), 865-879.
14. Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Kollman, P. A., Application of RESP Charges to Calculate Conformational Energies, Hydrogen-Bond Energies, and Free-Energies of Solvation. *Journal of the American Chemical Society* **1993,** *115* (21), 9620-9631.
15. Yin, D. X.; MacKerell, A. D., Ab initio calculations on the use of helium and neon as probes of the van der Waals surfaces of molecules. *Journal Of Physical Chemistry* **1996,** *100* (7), 2588-2596.
16. Yin, D. X.; MacKerell, A. D., Jr., Combined ab initio empirical approach for optimization of Lennard-Jones parameters. *Journal of Computational Chemistry* **1998,** *19* (3), 334-348.

17. Klauda, J. B.; Venable, R. M.; MacKerell, A. D.; Pastor, R. W., Considerations for lipid force field development. In *Computational Modeling of Membrane Bilayers*, Scott, E. F., Ed. Elsevier: London, UK, 2008; Vol. 60, pp 1-48.
18. West, A.; Zoni, V.; Teague, W. E.; Leonard, A. N.; Vanni, S.; Gawrisch, K.; Tristram-Nagle, S.; Sachs, J. N.; Klauda, J. B., How do ethanolamine plasmalogens contribute to order and structure of neurological membranes? *Journal of Physical Chemistry B* **2020,** *124* (5), 828-839.
19. Khakbaz, P.; Klauda, J. B., Investigation of phase transitions of saturated phosphocholine lipid bilayers via molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2018,** *1860* (8), 1489-1501.
20. Zhuang, X.; Dávila-Contreras, E. M.; Beaven, A. H.; Im, W.; Klauda, J. B., An extensive simulation study of lipid bilayer properties with different head groups, acyl chain lengths, and chain saturations. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2016,** *1858* (12), 3093-3104.
21. Boughter, C. T.; Monje-Galvan, V.; Im, W.; Klauda, J. B., Influence of Cholesterol on Phospholipid Bilayer Structure and Dynamics. *The Journal of Physical Chemistry B* **2016,** *120* (45), 11761-11772.
22. Yu, Y.; Klauda, J. B., Modeling Pseudomonas Aeruginosa Inner Plasma Membrane in Planktonic and Biofilm Modes. *Journal of Chemical Physics* **2018,** *149*, 215102
23. Monje-Galvan, V.; Klauda, J. B., Preferred Binding Mechanism of Osh4's ALPS Motif, Insights from Molecular Dynamics. *Journal of Physical Chemistry B* **2018,** *122* (42), 9713-9723.
24. Guros, N. B.; Balijepalli, A.; Klauda, J. B., Microsecond-timescale simulations suggest 5-HT–mediated preactivation of the 5-HT_{3A} serotonin receptor. *Proceedings of the National Academy of Sciences of the United States of America* **2020,** *117* (1), 405-414.
25. Darden, T.; York, D.; Pedersen, L., Particle Mesh Ewald - an NLog(N) Method for Ewald Sums in Large Systems. *Journal of Chemical Physics* **1993,** *98* (12), 10089-10092.
26. York, D. M.; Darden, T. A.; Pedersen, L. G., The Effect of Long-Range Electrostatic Interactions in Simulations of Macromolecular Crystals - a Comparison of the Ewald and Truncated List Methods. *Journal of Chemical Physics* **1993,** *99* (10), 8345-8348.
27. Klauda, J. B.; Wu, X. W.; Pastor, R. W.; Brooks, B. R., Long-range Lennard-Jones and Electrostatic Interactions in Interfaces: Application of the Isotropic Periodic Sum Method. *Journal of Physical Chemistry B* **2007,** *111* (17), 4393-4400.
28. Leonard, A. N.; Simmonett, A. C.; Pickard, F. C. t.; Huang, J.; Venable, R. M.; Klauda, J. B.; Brooks, B. R.; Pastor, R. W., Comparison of Additive and Polarizable Models with Explicit Treatment of Long-Range Lennard-Jones Interactions Using Alkane Simulations. *J Chem Theory Comput* **2018,** *14* (2), 948-958.
29. Lagüe, P.; Pastor, R. W.; Brooks, B. R., Pressure-based long-range correction for Lennard-Jones interactions in molecular dynamics simulations: Application to alkanes and interfaces. *Journal of Physical Chemistry B* **2004,** *108* (1), 363-368.
30. Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G., A smooth particle mesh Ewald method. *J. Chem. Phys.* **1995,** *103* (19), 8577-8593.
31. Wennberg, C. L.; Murtola, T.; Pall, S.; Abraham, M. J.; Hess, B.; Lindahl, E., Direct-space corrections enable fast and accurate Lorentz-Berthelot combination rule Lennard-Jones lattice summation. *Journal of Chemical Theory and Computation* **2015,** *11* (12), 5737-5746.
32. Klauda, J. B.; Monje, V.; Kim, T.; Im, W., Improving the CHARMM Force Field for Polyunsaturated Fatty Acid Chains. *Journal of Physical Chemistry B* **2012,** *116*, 9424-9431.
33. Lim, J. B.; Rogaski, B.; Klauda, J. B., Update of the Cholesterol Force Field Parameters in CHARMM. *Journal of Physical Chemistry B* **2012,** *116* (1), 203-210.
34. Wu, Emilia L.; Engström, O.; Jo, S.; Stuhlsatz, D.; Yeom, Min S.; Klauda, Jeffery B.; Widmalm, G.; Im, W., Molecular Dynamics and NMR Spectroscopy Studies of E. coli Lipopolysaccharide Structure and Dynamics. *Biophysical Journal* **2013,** *105* (6), 1444-1455.
35. Venable, R. M.; Sodt, A. J.; Rogaski, B.; Rui, H.; Hatcher, E.; MacKerell, A. D.; Pastor, R. W.; Klauda, J. B., CHARMM All-Atom Additive Force Field for Sphingomyelin: Elucidation of Hydrogen Bonding and of Positive Curvature. *Biophysical Journal* **2014,** *107* (1), 134-145.

36. Zhuang, X.; Makover, J.; Im, W.; Klauda, J. B., A systematic molecular dynamics simulation study of temperature dependent bilayer structural properties. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2014,** *1838* (10), 2520-2529.
37. Wang, E.; Klauda, J. B., Simulations of Pure Ceramide and Ternary Lipid Mixtures as Simple Interior Stratum Corneum Models. *The Journal of Physical Chemistry B* **2018,** *122* (10), 2757-2768.
38. Lemkul, J. A.; Huang, J.; Roux, B.; MacKerell, A. D., An Empirical Polarizable Force Field Based on the Classical Drude Oscillator Model: Development History and Recent Applications. *Chemical Reviews* **2016,** *116* (9), 4983-5013.
39. Davis, J. E.; Patel, S., Charge Equilibration Force Fields for Lipid Environments: Applications to Fully Hydrated DPPC Bilayers and DMPC-Embedded Gramicidin A. *The Journal of Physical Chemistry B* **2009,** *113* (27), 9183-9196.
40. Davis, J. E.; Rahaman, O.; Patel, S., Molecular Dynamics Simulations of a DMPC Bilayer Using Nonadditive Interaction Models. *Biophysical Journal* **2009,** *96* (2), 385-402.
41. Lamoureux, G.; MacKerell, A. D.; Roux, B., A simple polarizable model of water based on classical Drude oscillators. *Journal Of Chemical Physics* **2003,** *119* (10), 5185-5197.
42. Lamoureux, G.; Roux, B., Modeling induced polarization with classical Drude oscillators: Theory and molecular dynamics simulation algorithm. *The Journal of Chemical Physics* **2003,** *119* (6), 3025-3039.
43. Vorobyov, I. V.; Anisimov, V. M.; MacKerell, A. D., Polarizable empirical force field for alkanes based on the classical drude oscillator model. *Journal Of Physical Chemistry B* **2005,** *109* (40), 18988-18999.
44. Vanommeslaeghe, K.; MacKerell, A. D., Jr., CHARMM additive and polarizable force fields for biophysics and computer-aided drug design. *Biochim Biophys Acta* **2015,** *1850* (5), 861-71.
45. Lamoureux, G.; Roux, B. Ì. t., Modeling induced polarization with classical Drude oscillators: Theory and molecular dynamics simulation algorithm. *J. Chem. Phys.* **2003,** *119* (6), 3025-3039.
46. Chowdhary, J.; Harder, E.; Lopes, P. E. M.; Huang, L.; MacKerell, A. D.; Roux, B., A Polarizable Force Field of Dipalmitoylphosphatidylcholine Based on the Classical Drude Model for Molecular Dynamics Simulations of Lipids. *The Journal of Physical Chemistry B* **2013,** *117* (31), 9142-9160.
47. Li, H.; Chowdhary, J.; Huang, L.; He, X.; MacKerell, A. D., Jr.; Roux, B., Drude Polarizable Force Field for Molecular Dynamics Simulations of Saturated and Unsaturated Zwitterionic Lipids. *J Chem Theory Comput* **2017,** *13* (9), 4535-4552.
48. Balusek, C.; Hwang, H.; Lau, C. H.; Lundquist, K.; Hazel, A.; Pavlova, A.; Lynch, D. L.; Reggio, P. H.; Wang, Y.; Gumbart, J. C., Accelerating Membrane Simulations with Hydrogen Mass Repartitioning. *Journal of Chemical Theory and Computation* **2019,** *15* (8), 4673-4686.
49. Hopkins, C. W.; Le Grand, S.; Walker, R. C.; Roitberg, A. E., Long-Time-Step Molecular Dynamics through Hydrogen Mass Repartitioning. *Journal of Chemical Theory and Computation* **2015,** *11* (4), 1864-1874.
50. Loubet, B.; Kopec, W.; Khandelia, H., Accelerating All-Atom MD Simulations of Lipids Using a Modified Virtual-Sites Technique. *Journal of Chemical Theory and Computation* **2014,** *10* (12), 5690-5695.

$$f = m \cdot a$$

$$f = -\left(\frac{\partial U}{\partial \vec{r}}\right)$$



TOC GRAPHIC