1. INTRODUCTION

AMBER, CHARMM, OPLS, and GROMOS are the subject of this literature review. They all, use so called Class I additive potential energy function that has a general form shown below:

$$U(R) = \sum_{bonds} K_b(b - b_0)^2 + \sum_{angles} K_\theta(\theta - \theta_0)^2 + \sum_{dihedral} \times K_\chi(1 + cos(n\chi - \delta)) +$$

$$+ \sum_{impropers}^{n} K_{imp}(\varphi - \varphi_0)^2 + \sum_{nonbond} (\varepsilon_{ij}[(\frac{Rmin_{ij}}{r_{ij}})^{12} - (\frac{Rmin_{ij}}{r_{ij}})^{6}]) + \frac{q_i q_j}{\varepsilon r_{ij}}$$

Where b is a bond length, θ is the valence angle, □ is the dihedral angle, φ is the improper angle and r_{ij} is the distance between atoms i and j.

It should be noted that while the general form is the same for the four force fields, there are some difference in how the energy is calculated. For example, CHARMM and AMBER use geometric mean to calculate ε_{ij} of atoms i and j, while arithmetic mean is used to calculate $Rmin_{ij}$. OPLS, on the other hand, uses geometric means for both. Similarly, AMBER and CHARMM use Rmin (radius at which L-J potential is at its minimum) while OPLS uses σ (radius at which L-J potential is zero).

Even though the potential energy is calculated in a similar manner, it is the parameters and the logic used to obtain them that decide how applicable and accurate a force field is. More details on those as well as applications of force fields will be discussed in the following chapters dedicated to each force field individually.

2. AMBER

AMBER ff94 was the first force field capable of all-atom simulations of proteins in water and was the foundation for many parameter optimizations since then. The most recent work on improving the agreement with experimental data focused on protein secondary structure representation by refitting of the dihedral parameters. For example, the ff99 version introduced explicit four atom dihedral terms. The ff99SB model also fixed the problem with overestimated stability of α-helices and improved the conformations of glycines. It was achieved by changing the ψ,Φ dihedral parameters and charge distribution for the entire protein force field. It should be noted that ff99SB was found to predict accurate equilibrium structures, however was not successful when simulations of conformational changes were attempted. The reason for that was found to be overestimated steepness of potential energy basins. Modifications of backbone potential that resulted in better energetic balance between helix and coil conformations yielded AMBER ff99SB*. Explicit side chain parameters were introduced for Ile, Leu, Asp, and Asn in

one of the newer models, AMBER ff99SB-ILDN. Another important difference between the original and newer AMBER force fields is the treatment of 1,4 nonbonded interactions. The original ff94 used a scale factor of 0.5 which was increased to 0.83 in the later versions, including those meant for nucleic acids.

Even with all the improvements mentioned above, the temperature dependence of partial folding remained the main limitation. AMBER-FB15 was developed in response to this problem. This force field together with TIP3P-FB water model is the best choice for general-purpose simulations of proteins, especially when fluctuations away from equilibrium and temperature dependence play an important role as the parameters were reoptimized specifically to address these problems. AMBER-FB15 was also tested for its accuracy when simulating Denaturated State Ensemble that is used to model intrinsically disordered proteins. The results showed that further improvements are still needed as the radius of gyration was underestimated. This is a common problem that all tested force fields ran into. The accuracy improved with newer water models, but more work on parameter optimization is still needed for more reliable simulations of such ensemble.

The initial versions of AMBER were intended to model proteins. It should be noted that there exist variants, such as AMBER PARM94, designed specifically for nucleic acid simulations. With the exception of the LJ terms (which were taken from the models developed for proteins), the parameters were developed from small molecules by reproduction of geometries, vibrational spectra, and conformational energies. Such parameters were then applied and adjusted to larger nucleic acids. In general, this type of AMBER force fields tends to underestimate the helical twist but provides stable DNA structures in solutions. Simulations of B forms in high water activity concentrations, however, seem to be problematic and better results were obtained with other popular force fields, such as CHARMM.

3. CHARMM

CHARMM22 is a different force field that in many ways resembles AMBER. Both had their intramolecular parameters derived from the experimental and quantum mechanical data for small molecules. CHARMM models are even more versatile as they can be used to for proteins, nucleic acids, lipids, small drug-like molecules, or even carbohydrates with a commercial variant of CHARMM package called CHEAT. It should be noted that one of the earliest CHARMM models, CHARMM19 is a united-atom model rather than an all-atom force field like AMBER or CHARMM22. This review intends to provide an overview of the newest available force fields, so that particular version will not be discussed here.

One of the newer versions of this force field is CHARMM22/CMAP where CMAP stands for optimization of an additional term for the backbone ψ, Φ torsion angles - the two-dimensional cubic spline potential. Main advantage of this model is inclusion of the 2D dihedral energy grid correction that allows for accurate treatment of both low and high energy regions of the ψ,Φ space. It has been shown that many fast folding proteins, such as for example Villin headpiece

subdomain, will reach their native state starting from unfolded configuration if sufficient simulation time using CHARMM22/CMAP is provided. It is worth noting that a study[3] for GB3 protein found that CHARMM22 failed to come up with a stable native state and the protein unfolded during the simulation, while CHARMM22/CMAP did not ran into that problem. The same study also showed that CHARMM22/CMAP and AMBER ff99SB-ILDN seem to describe folded proteins with comparable accuracy. However, when flexible peptides were investigated it the difference between these two force fields were much more pronounced, pointing out how important choosing the right force field for a particular system is. CHARMM22/CMAP has several known disadvantages. Firstly, even though Villin reaches its native state at the end of the simulation, it has been shown that the folding mechanism differs from the one determined experimentally. Some structures, such as pin WW domain, are misfolded in long simulations that start from a completely unfolded state. When this problem was investigated it was found that the misfolded states had lower free energy than the native state most likely due to small errors in the potential for the backbone that propagated over the simulation and resulted in completely incorrect results.

CHARMM36 was developed to address the aforementioned problems. The major improvements involved a new backbone CMAP potential, optimization of side-chain dihedral potential against dipeptide quantum mechanical data and NMR information for unfolded proteins, update of LJ parameters for aliphatic hydrogens, reparametrization of tryptophan and the guanidinium moiety of arginine. This force field showed to largely improve CHARMM22 tendency to overestimate α-helices. Side chain scalar coupling data for the χ_1 torsion showed better results than CHARMM22/CMAP for both folded and unfolded proteins. The study by Huang[5] compared computational results to the experimental NMR data that were not used in parametrization of CHARMM36 in hopes to provide independent benchmarking. A major improvement in hydrogen bond scalar couplings was shown compared to CHARMM22/CMAP. This study also demonstrated better accuracy in properties of both folded and partially disordered peptides. Efforts to improve this force field specifically for intrinsically disordered proteins have been made and resulted in CHARMM36m.

4. OPLS

OPLS stands for Optimized Potentials for Liquid Simulations. It was the first model specifically optimized for small organic molecules and their thermodynamic properties in the liquid state. The original version was a united-atom model, which means instead of accounting for each single atom, atoms were grouped together and their mechanical properties considered per group rather than individually. This review focuses on more recent force fields so those variants will not be discussed. Instead the focus will be on all-atom models that have more flexibility for charge distributions and torsional energetics, and thus greater accuracy. The internal part of the all-atom force field was developed by taking the AMBER PARM94 model and optimizing the torsion parameters according to quantum mechanical data. Several all-atom versions of OPLS resulted from subsequent parameter reoptimizations, most notably OPLS2.0, OPLS2.1, OPLS2005, and OPLS3.

OPLS3 is the newest from the discussed all-atom force fields that is superior to any of its predecessors. The major improvements as compared to the older OPLS versions include more accurate valence and torsional parametrizations, advancements in charge model to better represent lone pair and sigma hole charge distributions, and corrected protein force field. All those developments lead to a highly accurate model that can be used for small molecules, proteins, and in the studies of protein-ligand interactions. It has been shown[7] that unlike older versions, the OPLS3 is a competitive alternative to AMBER and CHARMM when it comes to simulations of ordered states of proteins. The same study has also shown that this variant of OPLS provides high level of accuracy data on protein-ligand interactions for a broad range of targets and ligands. Important changes that lead to such improvements of accuracy include changing back the scaling factor of the 1,4 nonbonded interactions to 0.5 (instead of 0.83 as it had place in OPLS2 or newer versions of AMBER) and better coverage of the phase space of the quantum chemical data used to fit the backbone tortions. Another recent study[8] looked into the potential use of simulations in rational drug design and investigated the conformers generated by several different force fields (including OPLS3, OPLS_2005, and AMBER*) for the desired bioactive conformations. The success of all the force fields was comparable, however OPLS3, the most recent of the investigated ones, showed the best success rate. Its high accuracy was attributed to the parameter set covering a wide range of dihedral angles.

It should be also noted that some models for carbohydrates have been developed that are basically an extension of OPLS[1]. The major difference between those and the standard OPLS force field is scaling of the 1,5 and 1,6 electrostatic interactions to account for the vicinal hydroxyl groups. One of the major drawbacks of this force field is lack of parameters for many sugar derivatives and protein linkages for monosaccharides that take part in molecular recognition mechanism. This OPLS-derived model, nonetheless, yields decent results for simple carbohydrates, however there is no general consensus empirical force field with more broad applications for this class of compounds.

5. GROMOS

GROMOS stands for GROningen MOlecular Simulation referring to the location of the research group that developed it. There has been several different releases of the force field and its parameters, but in this review GROMOS 54A8 is the main focus, even but many statements will be true for older versions as well.

GROMOS and OPLS share many similarities in their philosophy since they were developed in a similar manner. Both models had their parameters derived mainly from experimental spectroscopic and thermodynamic data for small molecules in form of liquids and solutions with the assumption that parameters determined for small molecules are applicable to corresponding fragments in larger molecules. Both GROMOS and OPLS put emphasis on the description of torsional angle properties, non-bonded interactions, and solvations effects which makes them most relevant to condensed phase and biomolecular systems. In fact, GROMOS

has been used in simulations of peptides, proteins, nucleic acids, carbohydrates, and lipids, so it is a very versatile force field.

The main difference between GROMOS and all force fields discussed so far is the fact that this is the only the only model that only has a united-atom version. Specifically, the aliphatic hydrocarbons (CH, CH_2, CH_3, and CH_4) are treated as one pseudo-atom rather than several atoms with individual properties. Other features of this force field include a quadratic bond-stretching, cosine-harmonic bond angle bending, cosine-series dihedral angle torsion, and harmonic improper dihedral angle distortion terms. Atomic charges are consistent with condensed-phase (polar) environments and electronic polarization is represented by a mean field. Atomic partial charges are freely adjustable, meaning they are not determined by the atom type. The first and second neighbors are excluded from nonbonded interaction calculations. A special set of parameters reduces van der Waals interaction for third covalent neighbor. Van der Waals interactions are also adjusted to distinguish between non-hydrogen bonding, uncharged hydrogen bonding, and charged hydrogen bonding interactions.

Some of the issues with GROMOS include the fact that the molecular ions analogues to charged amino acid side chain functionalities, such as e.g. guanidinium for arginine, were developed for ideal Coulombic electrostatic interactions in a macroscopic nonperiodic system, but it is not possible to conduct a simulation with agreement to such assumption due to computing power limitations. This discrepancy may result in a bias towards ion-ion binding in a solution or underestimated interactions with the solvent. Another simulation artefact was found in lipid bilayer simulations[10]. The area per lipid matched the experimental results, however volume per lipid and bilayer thickness were both underestimated. The most likely reason was the truncated electrostatic potential. In proteins simulations, a slight bias towards α-helices was found. When proteins in aqueous electrolyte solutions were investigated, some minor disagreement with experimental data was found for radial distribution function of water molecules as well as sodium and chloride counterions around charged functional groups of the protein. With all those drawbacks in mind, GROMOS 54A8 was still found to be an appropriate force field for simulations of electrolyte solutions, lipids, and proteins.

6. References

1. MacKerell, A. D. Jr. Empirical Force fields for Biological Macromolecules: Overview and Issues. *J. Comput. Chem.* **2004**, 25(13), 1584-1604.
2. Wang, L. P.; McKiernan, K. A; Gomes, J.; Beauchamp, K. A.; Head-Gordon, T.; Rice, J. E.; Swope, W. C.; Martinez, T. J.; Pande, V. S. Building a More Predictive Protein Force Field: A Systematic and Reproducible Route to AMBER-FB15. *J. Phys. Chem. B.* **2017**, 121, 4023-4039.
3. Lindorff-Larsen, K.; Maragakis, P.; Piana, S.; Eastwood, M. P.; Dror, R. O.; Shaw, D. E. Systematic Validation of Protein Force Fields against Experimental Data. *PloS ONE* **2012**, 7(2), e32131.

4. Best, R. B.; Zhu, X.; Shim, J.; Lopes, P. E. M.; Mittal, J.; Feig, M.; MacKerell, A. D. Optimization of the Additive CHARMM All-Atom Protein Force Field Targeting Improved Sampling of the Backbone ψ,Φ and Side-Chain χ_1 and χ_2 Dihedral Angles. *J. Chem. Theory Comput.* **2012**, 8, 3257-3273.
5. Huang, J.; MacKerell, A. D. CHARMM36 All-Atom Additive Protein Force Field: Validation Based on Comparison to NMR Data. *J. Comput. Chem.* **2013**, 34(25), 2135-2145.
6. Martin-Garcia, F.; Papaleo, E.; Gomez-Puertas, P.; Boomsma, W.; Lindorff-Larsen, K. Comparing Molecular Dynamics Force Fields in the Essential Subspace. *PLoS ONE* **2015**, 10(3), E0121114.
7. Harder, E.; Damm, W.; Maple, J.; Wu, C.; Reboul, M.; Xiang, J. Y.; Wang, L.; Lupyan, D.; Dahlgren, M. K.; Knight, J. L.; Kaus, J. W.; Cerutti, D. S.; Krilov, G.; Jorgensen, W. L.; Abel, R.; Friesner, R. A. OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins. *J. Comput. Chem.* **2016**, 12, 281-296.
8. Gursoy, O.; Smiesko, M. Searching for Bioactive Conformations of Drug-Like Ligands with Current Force Fields: How Good Are We? *J. Cheminform.* **2017**, 9, 29.
9. Horta, B. A. C.; Fuchs, P. F. J.; van Gunsteren, W. F.; Hunenberger, P. H. New Interaction Parameters for Oxygen Compounds in the GROMOS Force Field: Improved Pure-Liquid and Solvation Properties for Alcohols, Ethers, Aldehydes, Ketones, Carboxylic Acids, and Esters. *J. Chem. Theory Comput.* **2011**, 7, 1016-1031.
10. Reif, M. M.; Winger, M.; Oostenbrink, C. Testing of the GROMOS Force-Field Parameter Set 54A8: Structural Properties of Electrolyte Solutions, Lipid Bilayers, and Proteins. *J. Chem. Theory Comput.* **2013**, 9, 1247-1264.