Overview

Polarizable force fields for molecular dynamics simulations of biomolecules

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Molecular dynamics simulations are well established for the study of biomolecular systems. Within these simulations, energy functions known as force fields are used to determine the forces acting on atoms and molecules. While these force fields have been very successful, they contain a number of approximations, included to overcome limitations in computing power. One of the most important of these approximations is the omission of polarizability, the process by which the charge distribution in a molecule changes in response to its environment. Since polarizability is known to be important in many biochemical situations, and since advances in computer hardware have reduced the need for approximations within force fields, there is major interest in the use of force fields that include an explicit representation of polarizability. As such, a number of polarizable force fields have been under development: these have been largely experimental, and their use restricted to specialized researchers. This situation is now changing. Parameters for fully optimized polarizable force fields are being published, and associated code incorporated into standard simulation software. Simulations on the hundred-nanosecond timescale are being reported, and are now within reach of all simulation scientists. In this overview, I examine the polarizable force fields available for the simulation of biomolecules, the systems to which they have been applied, and the benefits and challenges that polarizability can bring. In considering future directions for development of polarizable force fields, I examine lessons learnt from non-polarizable force fields, and highlight issues that remain to be addressed.

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INTRODUCTION

Computational studies employing molecular dynamics simulation are firmly established as a tool for the study of biomolecular systems.[1] Especially when combined with relevant experimental data, they have provided new insights into everything from small drug-like compounds[2–4] to proteins,[5] DNA,[6,7] and even entire viruses.[8–10] These molecular dynamics simulations are based on 'force fields', empirically parameterized sets of equations that describe the energies of the interacting atoms and molecules within the system being simulated.[11] The more accurately these force fields are able to describe physical reality, the more accurate the output from any simulations will be. An important caveat that comes with this statement is that, in general, the more accurately a force field describes physical reality, the more complex it is and the more computationally demanding simulations become. For this reason, during the development of force fields for molecular dynamics simulation, there has been a continuing need to trade-off accuracy against computational efficiency. To ensure that molecular dynamics simulations can be applied on biologically meaningful timescales, a

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number of simplifying approximations have been introduced into force fields.

One important approximation involves the description of the electrostatic properties of atoms and molecules. Within the majority of force fields applied to biomolecular systems, the electrostatic properties of atoms are treated by placing a point partial charge at the site of the atomic nucleus. The magnitude of this partial charge, and its position at the nucleus, then remain fixed throughout any subsequent simulation. This is an approximation because, in reality, we know that the charge distribution of a molecule can change in response to that molecule's environment; we know that molecules are polarizable. As a simple example, it is well known that the dipole moment of a water molecule varies significantly according to its environment, from 1.9 D in the gas phase[12] to 2.1 D in a small cluster[13] and 2.9 D in bulk liquid.[14] Moreover, these effects are expected to be very important in biomolecular systems. It is known, for example, that the electronic distribution of a peptide varies according to its conformation,[15] and that the electronic distribution of a ligand changes upon binding to a protein.[16] Neither of these effects would be captured by a non-polarizable force field.

The development of polarizable force fields for molecular dynamics simulation began in the 1970s,[17] but limitations in the computing resources available at the time meant that polarizable simulations were not widely adopted. As computer-power has increased, however, interest in simulations with polarizable force fields has also increased. The last 15 years, in particular, have seen an increasing number of publications describing the development of polarizable force fields: a SciFinder[18] search indicates that 2 papers were published on the topic 'polarizable force field' in 2000, compared to 55 in 2013. The development of these polarizable force fields has required the introduction of novel theoretical methods, and extensive parameterization of new potential functions. Important progress has been made in both of these areas, and biomolecular simulations employing polarizable force fields have now been published. These force fields have also been shown to be robust, and have been made available within well-known molecular dynamics software packages such as CHARMM,[19] AMBER,[20] NAMD,[21] and GROMACS.[22] As a result, simulations with polarizable force fields are no longer the preserve of specialist developers, but are increasingly accessible to the wider simulation community. For the non-specialist researcher, this raises a number of questions: what polarizable force fields are available? Which should be used for simulating which systems? What are the specific challenges that polarizable force fields bring? Are they worth the additional computational cost that comes with polarizability? These are the questions that I seek to address in this overview.

TABLE 1 | The Polarizable Force Fields Discussed in this Overview

Force Field	Biomolecule Coverage	Accessible Via
AMBER ff02	Proteins	AMBER
	Nucleic Acids	
AMOEBA	Proteins	AMBER; TINKER
CHARMM Drude	Proteins	CHARMM; NAMD
	Nucleic Acids	
	Lipids (partial coverage)	
	Carbohydrates (partial coverage)	
CHEQ	Proteins	CHARMM
	Lipids (partial coverage)	
	Carbohydrates (partial coverage)	

The 'Biomolecule Coverage' column includes only biomolecules for which parameters have been published; the 'Accessible via' column includes only molecular dynamics software packages that are publically available.

THEORETICAL METHODS FOR INCLUDING POLARIZABILITY

The objective of this overview is not to discuss in detail the theoretical methods by which polarizability can be included into force fields: there are a number of excellent reviews that have previously described these theoretical methods in some detail.[23–25] This overview will focus on the use of polarizable force fields for the simulation of biomolecular systems. Nonetheless, it is instructive to consider the basic details of the methods available for including polarizability. With an understanding of the basic concepts behind these force fields, we can begin to understand more clearly their strengths and weaknesses, and any issues that need to be addressed before they are more widely adopted. It is also important to note that this overview will consider only polarizable force fields that have been used for the simulation of biomolecular systems, and that have been implemented within publically available molecular dynamics software packages. In particular, I will consider polarizable force fields implemented within the most widely used simulation packages: CHARMM; AMBER, and GROMACS (Table 1). While this excludes much excellent work, it is a necessary step to maintain the focus of the overview.

Fluctuating Charges

Fluctuating charge models retain the same underlying structure as traditional non-polarizable force fields: the partial charge of each atom is placed at the site of the atomic nucleus, and electrostatic interactions are calculated using a standard Coulomb potential. Where they differ from non-polarizable force fields is that the magnitude of the individual atomic partial charges can change over the course of a simulation. This is achieved by assigning fictitious masses to each of the charges and treating them as additional degrees of freedom in the equations of motion, with charges flowing between atoms until instantaneous electronegativities are equalized.[26] This type of approach is attractive because it does not require the inclusion of any additional interaction terms into the force field, and so attracts a relatively small computational overhead. The major drawback of the approach is that it cannot easily represent polarization that does not occur in the direction of bonds: for example, a planar molecule (such as benzene) cannot be polarized perpendicular to the plane, though in principle this problem could be solved relatively easily via the inclusion of additional point charges to represent charge density not directly associated with the atomic nuclei.[27–31] From a practical perspective, the fluctuating charge (FQ)/charge equilibration (CHEQ) force field (its name has changed over the course of its development) developed by Patel and co-workers within the CHARMM program has been the fluctuating charge model most heavily applied to the simulation of biomolecules.[32,33]

Drude Oscillators (also known as Charges on Springs)

Methods based on Drude oscillators also use point charges and Coulomb potentials to describe electrostatic interactions; where they differ from other point charge-based methods is that each polarizable atom is represented by a pair of point charges.[34–36] The first of these charges sits at the site of the atomic nucleus, and the second is associated with a massless particle (a 'Drude particle') attached to the nucleus by a spring. The total partial charge of the atom is the sum of these two individual charges, and the magnitude of each charge remains fixed throughout the simulation. The polarizability within the model comes from the fact that the Drude particle is free to move anywhere around the nucleus of its parent atom in response to the external field, giving rise to an induced dipole moment. Advantages to this approach are that it is relatively easy to implement within existing force fields, and that it is chemically intuitive: the two particles can be considered to represent the nucleus and the electron density, respectively. The major disadvantage is that the inclusion of many extra charges means more interactions must be calculated. There are several groups working on the development of Drude oscillator-based polarizable force fields, but to date the only one available for the simulation of biomolecules is that due to MacKerell, Roux, and co-workers.[37–39] In this implementation, Drude particles are added to all non-hydrogen atoms and the atomic polarizability, α, of a given atom is given by equation 1.

$$\alpha = \frac{q_{\mathrm{D}}^2}{k_{\mathrm{D}}} \tag{1}$$

where q_{D} is the charge on a Drude particle and k_{D} the force constant for the spring that connects this Drude particle to its parent nucleus. In practice, a constant value of k_{D} is used for all atoms, meaning that q_{D} determines the polarizability of an individual atom. For atoms that can act as H bond acceptors, k_{D} is treated as a vector rather than a scalar, allowing for an anisotropic representation of polarizabilities. Additional anisotropy is included in the model via the addition of point charges to represent lone pairs. These extra point charges are fixed in magnitude and position, but allow for a better representation of the directionality of H bonding interactions.

Inducible Dipoles

A third method for including polarizability into force fields involves the use of inducible dipoles. This approach retains the framework of fixed atomic partial charges found in a non-polarizable force field, and adds to it a set of inducible point dipoles,[40] where the induced dipole at each site is determined by the electric field at that site. The presence of these induced dipoles means that the electrostatic interactions can no longer be calculated by using a Coulomb potential to evaluate the charge–charge interactions: extra terms must be included into the force field to account for the charge–dipole and dipole–dipole interactions. Practically, the inducible point dipoles are most usually placed at the sites of the atomic nuclei,[41] but have also been implemented on bonds between atoms.[42] This method is attractive because it is relatively straightforward to parameterize, but suffers from being more challenging to implement within existing packages for molecular dynamics simulation.[34] Such an approach has, however, been implemented within the AMBER package, and the AMBER ff02 force field[43] is probably the most widely used polarizable force field based on inducible dipoles.

Methods Including Multipole Electrostatics

While the three approaches described above all include an explicit representation of polarizability, they all use point charge models to describe the permanent electrostatics of the systems of interest. This approach is inherently limited because point charges are intrinsically isotropic, when real atoms are not[44]: examples of anisotropy in the electron distributions of real atoms include lone pairs, π-clouds, and σ-holes. When using point charge models, these effects can only be accurately described by including extra point charges at sites other than the atomic nuclei. A more rigorous solution is to represent atoms not as point charges, but using multipole moments that include not only monopoles (charges) but also higher order terms such as dipoles and quadrupoles. These multipole moments are anisotropic, and naturally capture any non-spherical components of the atomic charge density. In terms of polarizable force fields targeting biomolecular simulation, AMOEBA[45,46] (atomic multipole optimized energetics for biomolecular applications) is the most widely used force field including multipole electrostatics. In this force field, permanent electrostatics are represented by point charges, dipole vectors, and quadrupole tensors at the atomic sites, with polarization included via inducible point dipoles. Both multipole moments and inducible dipoles are located at all atomic sites within AMOEBA, including hydrogens. The intrinsic anisotropy of the permanent multipole moments used in AMOEBA removes the need to include any additional point charges, and also provides a much more general solution to the problem of anisotropy. In the CHARMM Drude model mentioned above, for example, extra point charges are included to represent anisotropy due to lone pairs. No extra charges are included to represent anisotropy due to π-clouds or σ-holes. When using multipole moments, all of these (and any other) instances of anisotropy will automatically be reproduced. In the AMOEBA model of polarizability, the dipoles induced at each of the polarizable sites are calculated using an 'atomic dipole induction scheme'. In this approach, the field produced by the permanent multipoles induces a dipole at each of the polarizable sites, and this induced dipole then induces further polarization at other sites. This is implemented in a 'group-based' way. Atoms are placed into groups, and permanent multipoles do not polarize other atoms within the group; mutual polarization of induced dipoles, however, occurs between all atoms. This scheme allows larger molecules to be built directly from smaller, explicitly parameterized, fragments.

BIOMOLECULAR SIMULATIONS

Proteins

Combining a multipolar description of fixed electrostatics with an induced dipole model of polarizability, AMOEBA is the most theoretically rigorous polarizable force field that is currently available to the general researcher. Its development to date has been largely focused on small molecules and proteins, where results obtained using the AMOEBA-2013 parameter set are very encouraging. Following optimization of the force field parameters, Shi et al.[47] used AMOEBA-2013 to calculate x, y, and z components of the gas phase dipole moments of multiple conformations of each amino acid dipeptide. On comparing their results to those from high-level QM calculations, they obtained a correlation coefficient of 0.998. For a non-polarizable force field targeted at simulating condensed phase properties, such a result would be impossible. The authors themselves note that 'no other force field has demonstrated to be able to represent peptide electrostatic properties … to such accuracy.'[47] Good results were also obtained when the same model was applied to the simulation of ten different proteins in aqueous solution. No molecule showed a large deviation from a reference experimental crystal structure (Figure 1), and calculated order parameters were also in good agreement with experimental data, indicating that the force field was not simply overstabilizing the protein conformations. One caveat with these results, however, is that they were obtained from relatively short simulations (10 ns) of relatively small proteins (20–129 residues). This illustrates one of the key challenges of force field development: the more accurate a potential function, the greater the computational burden and the shorter the possible simulations. With current hardware, simulations of large systems on the 100 ns timescale may not be feasible using AMOEBA. There are still, however, important biological problems that can be addressed. As Jiao et al. have noted, 'electrostatics and polarization play important roles in molecular recognition'[48] and using an earlier version of the AMOEBA force field, it has been possible to obtain quantitative agreement in the calculation of protein-ligand binding free energies.[48,49]

As in the AMOEBA force field, the AMBER ff02 model incorporates polarizability via an inducible point dipole approach. The AMBER ff02 representation of permanent electrostatics, however, uses only point charges. This makes the ff02 force field much less computationally demanding than AMOEBA, meaning that ff02 presents an attractive option for simulating timescales inaccessible to AMOEBA. Following initial optimization of small molecule parameters,[43]

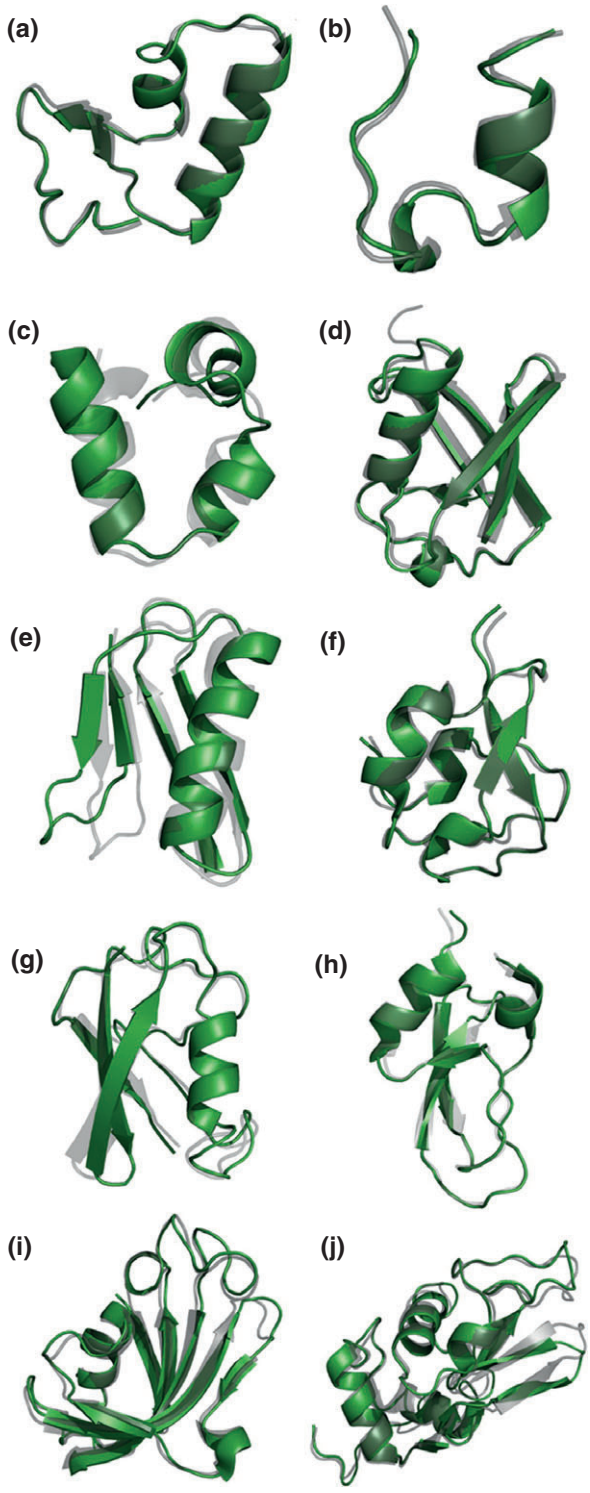

FIGURE 1 | Protein structures obtained from simulations with the AMOEBA polarizable force field (green) superimposed on reference crystal structures (gray). (a) Crambin, 1EJG. (b) Trp cage, 1L2Y. (c) Villin headpiece (1VII). (d) Ubiquitin, 1UBQ. (e) GB3 domain, 2OED. (f) RD1 antifreeze protein, 1UCS. (g) SUMO-2 domain, 1WM3. (h) BPTI, 1BPI. (i) FK binding protein, 2PPN. (j) Lysozyme, 6LYT. (Reprinted with permission from Ref 47. Copyright 2013 American Chemical Society)

torsion parameters for use with proteins were optimized within the ff02 framework, yielding the ff02r1 parameter set.[50] With these optimized parameters, replica exchange simulations of Ace-Ala-NMe and Ace-$(Ala)_7$-Nme, with around 30 replicas and 8 ns of simulation per replica, gave conformational distributions in good agreement with experimental data. Since then, however, the ff02r1 force field has been only infrequently applied to the study of proteins. In simulations of the jun-fos dimerization process, Zuo et al. found that the ff02r1 model provided a more accurate estimate of the binding affinity than comparable non-polarizable force fields.[51] In a study of the energetics of a cyclic decapeptide, however, Doemer et al. found that the ff02 model performed worse than either of the non-polarizable AMBER ff96 and ff99SB force fields[52]; in the same test, AMOEBA was the best performing of all force fields.

Parameters for the simulation of proteins have also been developed within the CHARMM FQ force field[53,54] and used to run simulations of multiple proteins for several nanoseconds, at the time the longest polarizable simulations of proteins. Although short compared to current state-of-the-art simulations, these calculations did reveal that the atomic charges within amino acids were sequence dependent. This effect could never be captured by a fixed-charge force field, but agrees with results from QM/MM calculations, indicating that the polarizable model is providing a more accurate representation of the underlying biophysics. Despite this evidence that the FQ model could provide new insights into the physics of proteins, it has also suffered from a relatively small uptake. Patel et al. have used it in the simulation of channels in membranes, and this work will be discussed in greater detail below. Kucukkal and Stuart performed 38 ns simulations of Gly-Ala, Gly-Pro, and Ala-Pro dipeptides in mixture with water.[55] They concluded that, while the polarizable water model was more realistic than the corresponding non-polarizable model, the peptide aggregation properties agreed less well with experiment.

The Drude polarizable force field of MacKerell, Roux, and co-workers, termed Drude-2013,[37] has been used to simulate proteins up to 224 amino acids in length over periods of 100–200 ns. For smaller proteins, simulations of 1 μs have been reported.[56] These simulations have shown the force field to be stable and to give structural and dynamic properties on a par with those obtained from state-of-the-art non-polarizable force fields. While this may not sound impressive for a force field designed to give a more physically realistic description of biomolecules, existing non-polarizable force fields have been subject

to years of careful optimization. It is reasonable to assume that with similar attention, there will be significant improvements in the current generation of polarizable force fields. Lopes et al. also observed significant differences in the dipole properties of both proteins and water molecules in their simulations with the Drude-2013 model, arguing that 'the variations of the electronic structure do impact the dynamics of the system and the microscopic forces dictating the structural and dynamical properties of proteins.'[37]

Nucleic Acids

Polarizability is expected to be important in the study of the nucleic acids. These molecules are highly charged and large parts of their structure are determined by hydrogen bonding, meaning that any conformational change will be associated with large changes in charge distribution. Moreover, because of their high charge density, nucleic acids are also strongly polarizing, which will influence the properties of surrounding molecules, including solvents or binding partners.

The first polarizable force field to include parameters for the nucleic acids was AMBER ff02.[43] This force field has been applied to the study of DNA in both crystal[57,58] and solution[59] environments, in direct comparison with the non-polarizable AMBER ff99 force field. Running simulations of DNA oligomers from ideal B-form geometries, the authors monitored how well these simulations were able to reproduce local distortions from B-form geometry observed in experimental structures. They found that, particularly in the crystal environment, the polarizable ff02 force field could 'reproduce sequence-specific features better than the simpler ff99 on the nanosecond time scale.'[59] More recently, simulations on the 50–100 ns timescale identified important deficiencies in the ability of the non-polarizable AMBER ff99 force field to represent DNA structure.[60] As a result, a number of updates to the ff99 force have been published,[61,62] significantly improving its performance. To date, the polarizable ff02 force field has not been directly compared to these updated versions of ff99.

A polarizable model for DNA has also been developed as part of the Drude polarizable force field within CHARMM.[38,63,64] Using this model, solution-phase molecular dynamics simulations of duplex DNA on the 100–200 ns timescale have been performed.[38] These are the longest polarizable DNA simulations reported to date, and yielded structural and dynamic results of equivalent quality to those obtained with the most recent version of the non-polarizable CHARMM force field for DNA.[65] At the same time, the polarizable force field provides a more accurate representation of counterion condensation on DNA than is seen with the non-polarizable CHARMM force field,[66] suggesting that it includes a more accurate representation of electrostatics. The model has also been used to calculate potentials of mean force for DNA base flipping.[67] In agreement with a previous study[68] (Figure 2), simulations with the polarizable force field showed that the bases undergo significant changes in dipole moment as they progress along the flipping pathway.[67] This is an effect that is not captured with non-polarizable force fields[68] (Figure 2). The developers of this force field do note, however, a number of limitations. These specifically relate to 'some underestimation of the BII population and representation of some terminal DNA nucleotides.'[38]

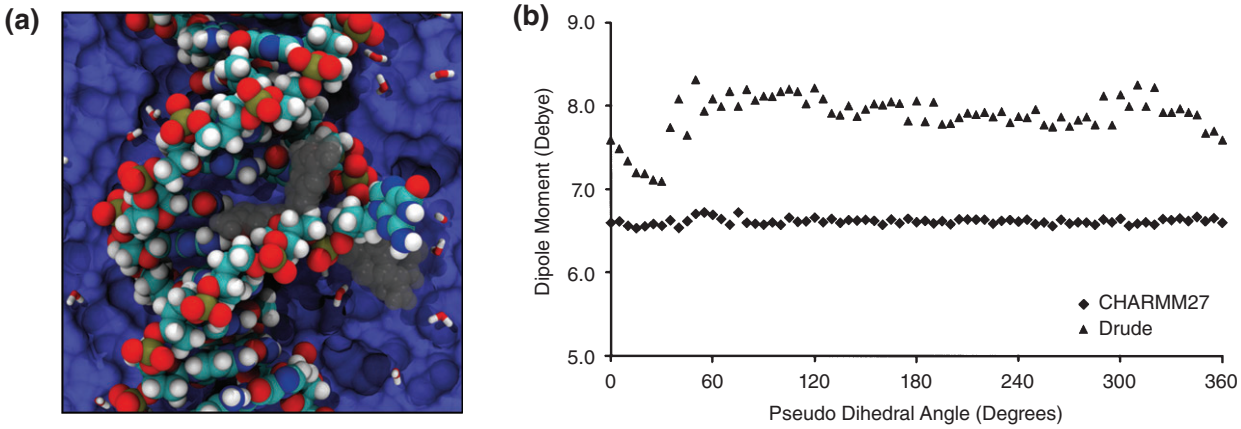

FIGURE 2 | The influence of polarizability on the calculated dipole moment of a guanine base as it undergoes base flipping. (a) Schematic description of the base flipping process. (b) The dipole moment of the guanine base calculated using polarizable (Drude) and non-polarizable (CHARMM27) force fields; at a pseudo-dihedral angle of 0° the base is in the canonical Watson–Crick H bonded arrangement, at 60° it is fully exposed to the solvent.[68] (Reproduced by permission of The Royal Society of Chemistry)

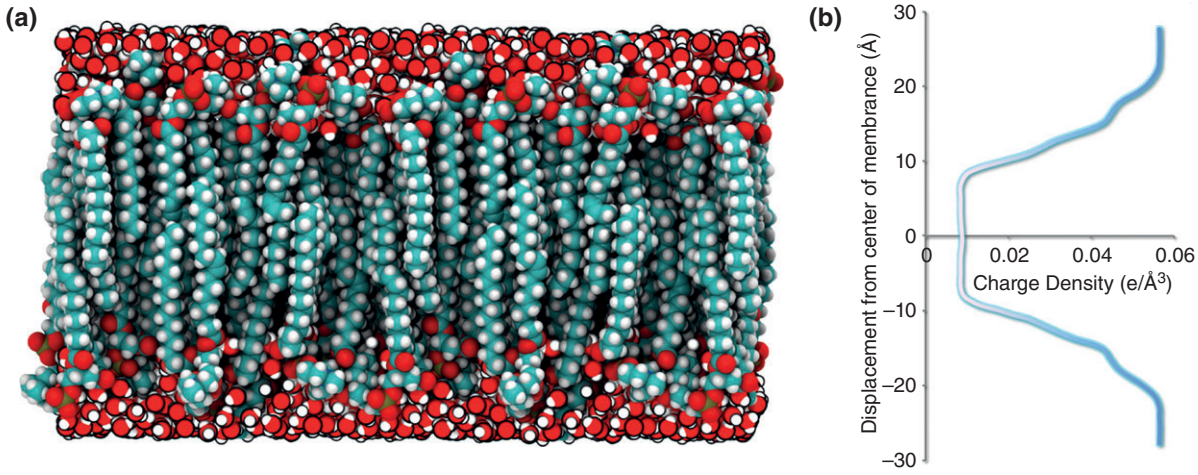

FIGURE 3 | The change in electrostatic environment across a lipid bilayer. (a) The structure of a solvated lipid bilayer. (b) The corresponding charge density profile across the membrane. The charge density profile is calculated as described by White and Wimley[69]: initially, absolute partial charge densities are calculated for groups of atoms, with group definitions and volumes taken from Ref 70 and atomic partial charges taken from Ref 71. The average charge density across the membrane is then obtained by weighting these group charge densities by the number density of groups within the bilayer and the group volume.

It is notable that, to date, no simulations of RNA molecules with polarizable force fields have been published, though parameters for uracil have been developed for both the ff02 and CHARMM Drude models. It is unclear whether this lack of RNA simulations relates to fundamental difficulties in the development and parameterization of polarizable force fields for RNA, or a comparative lack of interest in the simulation of RNA molecules.

Lipids

Lipid bilayers are among the most important biomolecular complexes found in nature. Forming the membranes that surround cells and sub-cellular structures, these constructs provide impermeable barriers that exist to maintain ions, proteins, and other molecules at the concentrations required for biological activity in specified locations. The structure of these membranes consists of two-layered sheets, or bilayers, with outward-facing hydrophilic headgroups and inward-facing hydrophobic tails (Figure 3). The result of this structure is that the dielectric environment at the membrane surface is very different to that in its interior (Figure 3), and that molecules within the membrane may behave very differently to those on the surface of the bilayer, due to their different polarization.

As a result, there has been significant interest in the development of polarizable force fields for the simulation of lipids. The most concerted efforts have come from Patel and co-workers, who have developed a polarizable force field for lipids based on the CHEQ charge equilibration approach.[32] The work has been reviewed in detail elsewhere,[33] but as well as performing simulations of hydrated lipid monolayers and bilayers, they have extended their approach to consider channels within membranes.[72,73] In the case of water permeation, initial simulations with the polarizable model predicted that water was better able to penetrate the lipid bilayer than was suggested by non-polarizable simulations.[73] This was because the dipole moment of the polarizable water molecules could deviate from the value it possesses in the strongly polar bulk water environment. This effect was reduced following a reoptimization of the CHEQ parameters to better match hydration free energy data (emphasizing the important role of careful parameterization in force field development). Simulations with the revised parameter set, however, still showed more water molecules in the membrane interior than did equivalent simulations with non-polarizable force fields.[74] Simulations of hydrated DPPC monolayers also suggested that the inclusion of polarizability results in an improved representation of the monolayer-water potential difference relative to the water–air interface.[75] Simulations with the CHEQ force field have also been used to probe the transfer of the methyl-guanidinium cation across the bilayer-water interface, giving results in approximate agreement with those obtained from previous, non-polarizable, simulations.[76]

Drude models have also been applied to the study of lipids, initially by Allen and co-workers.[77–79] This work did not employ a force field specifically

optimized for the study of lipids, but rather applied Drude parameters optimized for alkanes to the hydrophobic tails of the lipids being studied. This allowed for estimates of the correction required due to the omission of polarizability when calculating potentials of mean force for the permeation of charged groups through membranes. In this permeation of charged groups through membranes, the membrane dipole potential plays an important role, but is poorly reproduced by non-polarizable force fields.[80] Using a more sophisticated model, in which Drude particles were added to all heavy atoms, Harder et al. showed that the inclusion of polarizability resulted in a quantitative reproduction of the membrane dipole potential.[81] The polarizable model also gave a much improved representation of the dielectric constant of the bulk hydrocarbon regions. More recently, optimized parameters for Drude models of dipalmitoylphosphatidylcholine (DPPC),[39] cholesterol,[82] and sphingomyelin[82] have been published. In all cases, an improved representation of the membrane dipole potential was identified as a key benefit of the polarizable model.

Carbohydrates

The development of force fields for carbohydrates, whether polarizable or not, presents a number of challenges.[83] The monosaccharide building blocks upon which carbohydrates are built are conformationally flexible, rich in polar groups and usually possess multiple chiral centers (Figure 4). Each monosaccharide also contains multiple sites at which it can be connected to other monosaccharides to form polysaccharides. These polysaccharides can be either branched or linear and contain multiple rotatable bonds, meaning that they are able to access a large number of different conformations. The result of this conformational flexibility is that, in solution, oligosaccharides cannot be 'characterized in terms of their three-dimensional or secondary structural motifs'[85] and are more accurately described by an ensemble of conformations.[86]

These complexities make the development of carbohydrate force fields challenging, and polarizable force fields for carbohydrates are not as advanced as those for other biomolecules. Following optimization of parameters for acyclic polyalcohols,[87] the developers of the CHARMM Drude polarizable force field have developed polarizable models of the hexapyranose monosaccharides.[88] Within the CHEQ force field, Patel and co-workers optimized parameters for *N*-acetyl-β-glucosamine: they found that they were able to obtain a better description of solvent and solute diffusion than is possible with a non-polarizable model.[89] This is indicative of an improved representation of the electrostatic interactions (in particular, hydrogen bonds) between the solute and solvent. Subsequent work has used this polarizable model to study the binding of an *N*-acetyl-β-glucosamine trimer to the hen egg white lysozyme protein.[90]

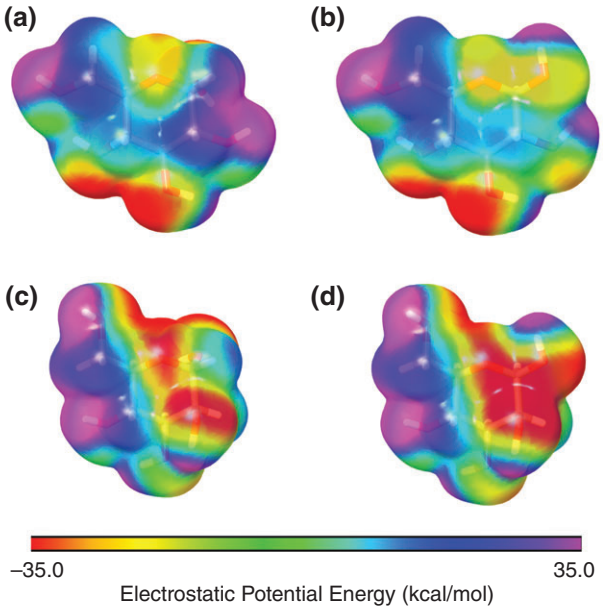

FIGURE 4 | Electrostatic potential energy surfaces around carbohydrate molecules. (a) α-glucose. (b) β-glucose. (c) α-mannose. (d) β-mannose. Each carbohydrate differs from each of its neighbors only by the inversion of one stereocenter. The surfaces were obtained from quantum mechanics calculations: the structure of each molecule was initially optimized, and the electron density calculated. Surfaces are drawn at electron density contours of 0.002 e/Å^3, and are colored according to the electrostatic potential energy at every point on the surface. All calculations were performed at the MP2/6-31G** level of theory using the program Jaguar.[84]

CHALLENGES AND FUTURE DIRECTIONS

Table 1 describes the current scope of the polarizable force fields discussed in this overview. As always, however, it is necessary to exercise some caution: just because parameters have been published for a particular force field, does not mean that they are perfect for the system at hand, or even necessarily fit for purpose. While the discussion above can provide a starting point in deciding which polarizable force fields are most appropriate for which situations, it is important to carefully monitor simulation results. For if there is one unequivocal lesson to be learnt from 20+ years of development of non-polarizable force fields, it is that weaknesses in current polarizable

force fields will be identified once those force fields are pushed beyond the limits of the simulations used in their parameterization. In short, once they become more widely used.

Parameterization

One of the challenges facing the current generation of polarizable force field developers, will be in responding to the identification of any weaknesses in parameter sets. Compared to their non-polarizable analogs, polarizable force fields contain more individual parameters per atom, by virtue of their more complicated potential functions. However, if polarizable force fields are more physically realistic, atoms should be able to adapt to their specific environments. This should mean that fewer distinct atom types are required in a polarizable force field than in a non-polarizable force field. This is not the case in the CHARMM Drude polarizable force field, where as many distinct atom types are required as in non-polarizable analogs,[91] and this may point to weaknesses in the Drude model for polarizability. Regardless of whether polarizable force fields are more or less difficult to parameterize than non-polarizable force fields, the development of parameters for polarizable force fields is a challenging problem that typically requires significant manual involvement. Such a situation is not tenable on an indefinite timeframe, and several groups have been working to develop automated schemes for the optimization of force field parameters.[92,93] The force balance method, for example, is independent of functional form and allows for a systematic and reproducible optimization of parameters, shown to converge to single parameter set regardless of starting point.[94] This approach has already been used to optimize parameters for a simplified AMOEBA water model.[95] Huang and Roux have proposed an alternative approach, based on QM target data, focusing on the automated optimization of parameters for small molecules within either non-polarizable or polarizable force fields.[96]

Sampling

Another challenge facing polarizable force fields is that of simulation time. Polarizable force fields have a reputation for being slow. While this is no longer the case in absolute terms, it is true that polarizable force fields are not as fast as their non-polarizable counterparts, simply because of their more complicated functional forms. The question for the individual researcher then becomes: are the gains in accuracy obtained with a polarizable force field sufficient to offset the concomitant decrease in sampling? It is a question with no simple answer. In simulations where sampling is less important, polarizable force fields are likely to be extremely useful. There are some situations, however, in which it may be impossible to obtain adequate sampling from equilibrium simulations with a polarizable force field. This is most likely to be true when studying dynamic properties of large, highly flexible molecules, as in protein folding[97] or coupled folding and binding of intrinsically disordered proteins.[98] In these cases, the local environment of an individual protein residue will be constantly changing, and polarizability is expected to be extremely important. As such, it will be necessary to find ways to use polarizable force fields without compromising sampling. In the long term, application of enhanced sampling schemes[99] may help to solve this problem. Baker and Best, for example, have shown that a non-polarizable force field matched to a polarizable analog allows Hamiltonian replica exchange between simulations with the two different force fields.[100] Although only small molecules were considered, this greatly increased sampling with the polarizable force field, indicating that it may, in future, be possible to combine the computational performance of a non-polarizable model with the increased accuracy of a polarizable model.

Protein-Ligand Binding

Within the pharmaceutical and agrochemical industries, computational chemistry plays an important role in the rational design of small molecules that interact with specific protein targets.[101] Molecular dynamics simulation has the potential to play a key role in this process.[102] Due to their dynamic nature, molecular dynamics simulations automatically account for flexibility in proteins, ligands, and protein-ligand complexes; the ensemble of structures obtained from molecular dynamics simulations allows for the direct calculation of binding free energies via statistical thermodynamics. To date, however, the challenge has always been to obtain estimates of binding free energies that are accurate enough to provide meaningful design cues to a synthetic chemistry program. The challenges come from both the accuracy of the force fields and the quantity of sampling. If either of these is inadequate, inaccurate free energy estimates will be obtained. There are now indications, however, that the sampling problem has been overcome, at least for binding sites that are relatively inflexible. Using free energy perturbation methods, and running calculations on GPU clusters with enhanced sampling algorithms, it has been possible to achieve near quantitative agreement with experimental binding free energies.[103] Polarization, however, is expected

to contribute 10–20% of the total interaction energy within a molecular complex, or more when the systems are charged.[104] As such, if well-optimized polarizable force fields can be included within such calculations, we can expect to see further increases in both the scope and accuracy of the methods. At present, polarizable force fields are not being applied in such cases due to the lack of parameters for a diverse range of small molecules; where small molecule parameters are available, the AMOEBA method has been shown to produce reliable estimates of experimental binding free energies.[104] When automated methods for generating force field parameters for small molecules become available within polarizable force fields, it is likely that our ability to accurately calculate binding free energies will be further enhanced.

CONCLUSION

Molecular dynamics simulation is widely use to study the structure and function of biological macromolecules. Electrostatic interactions play a crucial role in determining these structures and functions, but there are important deficiencies in the way that electrostatic interactions are represented within current molecular dynamics simulations. Specifically, the force fields used in molecular dynamics simulations do not allow the charge distribution of a molecule to change in response to its environment. This lack of an explicit representation of polarizability is well recognized as a weakness of the current generation of force fields used for molecular dynamics simulations of biomolecules. In response to this weakness, there have been concerted efforts to develop force fields that do include an explicit representation of polarizability. As discussed in this overview, we have now reached the point where polarizable force fields have been published for all major classes of biomolecules, and made available within a variety of different simulation packages. There are still a number of issues that remain to be overcome with these force fields: as they are applied more widely, it is likely that weaknesses in current parameter sets will be identified; and the relative slowness of simulations employing polarizable force fields remains an issue. Already, however, these polarizable force fields are being applied across a range of biochemical systems. In the systems being studied, polarizable force fields provide better agreement with experimental data, and access to a level of detail not achievable with non-polarizable force fields. Given this success, it is inevitable that polarizable force fields will eventually become the method of choice for molecular dynamics simulations of biomolecules. When combined with high-quality experimental data, the improved physical representation provided by these polarizable simulations will lead to an improved understanding of a range of biological phenomena.

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