Force fields for simulating the interaction of surfaces with biological molecules

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The interaction of biomolecules with solid interfaces is of fundamental importance to several emerging biotechnologies such as medical implants, anti-fouling coatings and novel diagnostic devices. Many of these technologies rely on the binding of peptides to a solid surface, but a full understanding of the mechanism of binding, as well as the effect on the conformation of adsorbed peptides, is beyond the resolution of current experimental techniques. Nanoscale simulations using molecular mechanics offer potential insights into these processes. However, most models at this scale have been developed for aqueous peptide and protein simulation, and there are no proven models for describing biointerfaces. In this review, we detail the current research towards developing a non-polarizable molecular model for peptide–surface interactions, with a particular focus on fitting the model parameters as well as validation by choice of appropriate experimental data.

1. Introduction

The physical interface between biological tissue and abiotic surfaces has long been exploited in nature, and it is a major determinant of the performance of a wide range of modern technologies. For example, in marine environments biofouling of ships can become a significant economic burden, and there is a demand for a cheap and non-toxic anti-fouling coating [1]. Implantable medical devices are often associated with poor tissue integration due to bio-incompatibility, leading to scarring, sub-optimal performance and in some cases rejection. The idea that these adverse medical outcomes could be remedied with a biofunctionalized interface [2] is driving a large sector of modern biomaterials research. Biomolecule–surface interactions are also being explored on gold nanoparticles for cancer diagnostics [3] and on polymers to construct protein screening microarrays [4]. Yet, there is still debate about exactly what properties of a surface make it biocompatible or not—for example, the hydrophilicity [5] and surface topology [6] have been shown to strongly influence the behaviour of proteins and cells on surfaces. Our incomplete understanding of the roles, both individual and in combination, of chemical and physical structures of surfaces makes the rational design of optimum biological interfaces challenging. A better understanding of how biology interacts with surfaces is needed to facilitate progress in the field.

For mammalian cells, adhesion occurs through receptor proteins that are normally used to bind to specific external motifs such as protein constituents of the body’s extracellular matrix (figure 1). Hence the question of how proteins react to different surfaces is central to the problem. Proteins tend to bind weakly to hydrophilic surfaces, but can maintain their native conformation, whereas they typically unfold and adhere strongly to hydrophobic surfaces [8]. This is because energy minimization of the surface protein system is favoured by contact of the hydrophobic interior segments of the protein to the hydrophobic surface. Unfolding or denaturation of proteins at surfaces needs to be avoided as unfolding proteins signal the presence of a foreign body to the host tissues and initiate harmful immune reactions and foreign body responses [9]. Molecules from the extracellular matrix can be incorporated into the surface to mimic mammalian tissues [10] and provide appropriate biological signals to cells at the interface. Although immobilization of extracellular matrix proteins has been shown to be beneficial [11] for promoting healthy cell integration attention is...
shifting to peptides and protein segments. Biomimetic peptides are preferable to whole proteins in manufacturing environments because they can be cheaply synthesized, require less demanding purification and are more robust during processing and handling than entire protein molecules that need to maintain fragile conformations. The rapidly growing body of knowledge in biochemistry that ascribes functions performed by protein molecules to particular segments and peptides is opening up the possibility of driving biological responses through surface immobilized fragments/peptides that target single recognition sites [12] with fewer non-specific effects.

Progress in this field will require a deeper understanding of the adsorption of peptides to surfaces. Different-sized silica nanoparticles, for instance, can attract peptides with only 20% sequence similarity [13] and the mechanism for different specificity is unclear. Experimental methods operating at their current limits of resolutions are largely inadequate in this sphere. Single molecule force microscopy, one of the highest resolution experimental probes available, can measure the interactions of whole peptides, but cannot interrogate the roles of individual amino acids [14] in the process. While there have been recent advances in experimental studies of surface-adsorbed protein structure [15] and conformation [16] using ensemble averaging techniques such as nuclear magnetic resonance (NMR) and circular dichroism, nanoscale modelling is proving to be an invaluable complementary tool as it is able to achieve a resolution higher than any experimental method currently available.

Recognizing the absence of an ‘ultimate microscope’ with the capability to measure the position and identity of individual atoms at femtosecond speed, atomic-scale resolution is only available by using molecular modelling simulations. However, making the simulations computationally tractable typically requires some assumptions and some restrictions in the number of degrees of freedom of the system being modelled. Theoretically, the most detailed model describes the quantum mechanical (QM) nature of the valence electrons that take part in forming covalent bonds within molecules and surfaces. Ab initio approaches, such as density functional theory, approximate solutions to the Schrödinger equation and can be used to determine electron densities within molecules [17]. The computational effort of QM modelling methods limits their application in practice to systems of up to hundreds of atoms. These approaches do not scale well computationally so the simulation of a solvated peptide interacting with a surface (tens of thousands of atoms) is not yet feasible at this level of accuracy. By treating electron density implicitly as a force field (FF) between atoms, a significantly less computationally intensive molecular model can be formulated using Newton’s equations of motion to govern atomic interactions [18]. The degrees of freedom can be further reduced by using coarse-grained models [19] (where groups of atoms are treated as a single particle) or with continuum models [20] (where water is represented by a continuous dielectric medium rather than individual molecules). These methods reduce the computational load and increase the system size scale and timescale that can be explored by compromising on resolution.

While coarse-grain and continuum methods are suitable for modelling the behaviour of bulk materials, accurate calculation of molecular interaction energies from a single peptide molecule requires an all-atom representation, meaning all atoms are accounted for individually. All-atom simulations are based on molecular mechanics, which is a method of generating physically realistic conformations of a system made up of atoms bound into molecules. These conformations are generated by using a potential energy function, known as an FF, which can be used to calculate the force on each atom at small time intervals in order to generate a time evolution of conformations. Within any FF, there are two considerations to gauge the accuracy of a simulation: sampling and parametrization. Sampling refers to how closely the conformations represent a Boltzmann-weighted ensemble average, and thus measures the confidence in their thermodynamic accuracy, and for protein–surface interactions this has been reviewed thoroughly by Latour [14]. Parametrization is the process of
assigning accurate values for atomic properties such as charge or bond length to the atoms within a simulation.

Several FFs already exist that have rigorously determined parameter sets for aqueous protein simulations—the CHARMM [21], AMBER [22], GROMOS [23] and OPLS [24]. FFs are among the best known, and thus well tested, and they have been shown to fairly accurately reproduce aqueous protein dynamics. In this review, we focus mainly on the CHARMM FF, because it has been used in many of the efforts to develop new FFs for simulating proteins and solid surfaces. In molecular dynamics simulations, there is a time evolution of a molecular system whereby integration of the potential energy function determines the force on each atom at small time intervals (femtoseconds) and allows for iterative updates of their positions. The biophysical FFs mentioned above all use a similar potential energy function for these calculations. Because of its prevalence in parametrizing interfacial systems, the potential energy function as it is expressed in the CHARMM FF is an appropriate choice to demonstrate this method. The potential energy function in the CHARMM FF is

\[
U_{\text{total}} = \sum_{\text{bonds}} K_b \left( b - b_0 \right)^2 + \sum_{\text{angles}} K_\alpha \left( \theta - \theta_0 \right)^2 + \sum_{\text{improper}} K_u \left( S - S_0 \right)^2 + \sum_{\text{LJ}} \epsilon_{ij} \left[ \left( \frac{r_{\text{min}}}{r_{ij}} \right)^{12} - 2 \left( \frac{r_{\text{min}}}{r_{ij}} \right)^{6} \right] + \sum_{\text{coulomb}} \frac{q_i q_j}{4 \pi \epsilon_0 r_{ij}}
\]

where the adjustable intramolecular (bonded) parameters are \(b\) (bond length), \(\theta\) (bond angle), \(S\) (Urey-Bradley), \(\chi\) (bond rotation), \(\varphi\) (improper term for planar ring moieties). For intermolecular (non-bonded) interactions, van der Waals interactions are modelled with the Lennard-Jones (LJ) potential [25] with parameters \(\epsilon\) for well depth, \(r_{\text{min}}\) for the point of minimum energy, while coulombic attraction is calculated using partial charge \(q\). The subscript ‘0’ refers to the equilibrium value. In this paradigm, atoms of an element in a given chemical environment are classified as an ‘atom type’ that shares values of these parameters characteristic to that environment. A change in environment will necessitate either new parameters, or that the original parameter set incorporates the average of both environments. Most protein FFs today have fixed values for the partial charge and are thus referred to as ‘additive’ FFs, as the total electrostatic potential energy is the sum of all the two-body potentials. Non-additive FFs (also known as polarizable FFs), in which the molecular charge distribution can change in response to the local environment, are also being developed [26]. While additive FFs are computationally less expensive they perform best in one particular dielectric medium, which will be illustrated later using water behaviour at phase interfaces. Another consideration for the success of any biophysical FF is the rigorous parametrization of atom types and molecules for the potential energy function, which ensures that it is both balanced and properly validated.

A balanced FF can accurately reproduce molecular behaviour at the atomic scale, while also being capable of reproducing macroscale thermodynamic quantities. This is as important for simulating small-molecule drugs binding to proteins as it is for peptides binding to surfaces, because a binding event is an emergent property that is the sum of not only the individual atomic interactions (an enthalpic quality) but also the entropic change in bulk water (if the net change favours binding, this is known as the hydrophobic effect). Balance is ingrained into an FF at the parametrization stage by carefully choosing a broad range of experimental measures to use as target data for fitting the parameters. In the CHARMM FF, this means satisfying structural data like QM torsional potential energy scans as well as bulk properties like the enthalpy of vaporization. The non-trivial nature of the parameter solution means this is a laborious process with a lot of scope for parameter correlation—where errors in a parameter set cancel, allowing the erroneous parameters to nevertheless fit the target data. A multiscale set of target data can alleviate this issue.

When a parameter set for a molecule is determined, its utility must be evaluated by reproducing some experimental measure that is outside the training set—this is known as validation. Ideally, validation should integrate many degrees of freedom to demonstrate their aggregate behaviour—for the protein FFs, folding into native conformations relies upon all of the bond vibrations and rotations, electronic charges and implicitly treated dispersion (van der Waals) forces. The validation of the CHARMM lipid parameter set followed a similar process [27]. For solid surfaces with limited dynamic behaviour, the validation must rely mostly on the non-bonded parameters—combining the effect of both the enthalpic potential and the entropic effect of surface-adsorbed water structure.

For interfacial systems, parametrizing an FF to accurately represent these contributions is difficult because of the extra attraction afforded by polarization on the surface due to charged regions of the peptide [28]. Polarization effects have to be handled implicitly in additive FFs—as an example, the dipole moment of water is 1.85 D in the gas phase [29] and it increases to 3.0 D in liquid water due to polarization. The TIP3P water model [30] used in many biophysical FFs has a dipole moment of 2.35 D, which represents a middle ground that accommodates both pure aqueous water as well as solute behaviour. To our knowledge, no measurements of water dipole moment at solid surfaces are available, but one would expect the dipole moment to decrease near proteins or surfaces with lower dielectric constants. Herein lies the difficulty of simulating interfacial systems with a fixed-charge FF. The fixed partial charges used in the current FFs are optimized for protein interactions in bulk aqueous environments and incorporate the effects of polarization through a mean-field approximation. Such an implicit treatment of polarization seems to work well in bulk, but polarization effects are dependent on the environment, and this could lead to problems in inhomogeneous systems as amply demonstrated in the narrow gramicidin channel [31]. In the light of this, there has been some debate about the ability of existing FFs to model an interfacial system [32], but there are promising signs that with rigorous parametrization it is possible, and that it can be assessed using proper validation. In this review, we will cover some of the efforts at parametrizing a fixed-charge FF for interfacial peptide–surface interactions.

2. Interfacial force fields and surface models

There have been several attempts at FF development for the purpose of peptide–surface interactions. We will cover three materials, gold, silica and polymers, because they are important for a range of biotechnological applications and they illustrate the primary concerns of FF development
such as parameter balance and validation, but there is ongoing work on FFs for other materials like metals [33,34], metal oxides [35], minerals [36,37] and graphene [38,39]. For gold surfaces, there is a strong polarization component to binding, which increases with peptide charge [28]. This is a consequence of mobile electrons that contribute to ‘image-charge’ effects [40]—an oppositely charged pseudo-molecule in the metal that is induced within the metal surface. As a consequence, many FFs include some degree of polarization in the surface. The first such FF for use with proteins, GolP [41], used a rotating dipolar rod as a method of reproducing image charge effects, with an additional potential to account for the chemisorption of sulfur. With polarization accounted for, the other terms (the LJ parameters) were tuned to reproduce both QM calculations [41] and experimental adsorption energies of model alkane molecules determined by helium atom reflectivity [42]. Validation of the FF with further alkane molecules produced reasonable agreement, and while analysis of the free energy of adsorption correlated with experiment, the resolution of the experimental measures was not fine enough to determine a quantitative comparison—more approaches to validation with adsorption free energy are discussed later. The next advance in gold–protein interactions was the GolP-CHARMM FF [43] that was integrated with the existing biophysical CHARMM FF.

The GolP-CHARMM FF reparametrized GolP from first principles and extended its use to both Au(111) and Au(100) surfaces, while retaining the capability for polarizability all within the existing CHARMM FF. While not completely consistent, requiring several new atom types to be added to the existing parameters for proteins, it successfully showed quantitative agreement with amino acid adsorption energies (but not free energies, which include entropic effects). The parameterization effort was based mostly on ab initio results for the (111) surface, and entirely on ab initio data for the extension to the (100) surface, owing to the lack of experimental data. In this case, where there are no bonded parameters (the gold atoms were fixed) and the charge parameters are handled by the rotating rod model, the main consideration is the LJ well depth, which was fitted to QM interaction energies of alkanes approaching a gold surface in the gas phase. Ordinarily, interaction energies from gas-phase QM approximations are not applicable in condensed-phase fixed-charge FFs, because the electron density varies so much from the gas phase to the condensed phase (and condensed-phase QM simulations are computationally prohibitive). The polarizable model used here accounted for this effect. One concern is that the LJ cutoff was changed during simulation, as it is agreed that parameterization of existing FFs incorporates, and relies upon, the native cutoff [27]. In this respect, it will be interesting to see whether this FF can be rigorously validated in protein adsorption free energy simulations. It is implied that the fitting of enthalpic adsorption energies with model molecules (including water) will translate to an accurate thermodynamic treatment of the interface, and this is best tested with free energy simulations. Recent results measuring the free energy of adsorption of the gold-binding peptide AuBP1 on three different gold facets compare favourably with experimental data and also help explain facet selectivity and the role of water in binding [44]. Fitting the FF to the bulk material properties of the surface, by removing the fixed atom restraint on gold atoms, will also allow for validation using interfacial energies.

Another thoroughly parametrized interfacial FF is also integrated with biomolecular FFs and is also fixed-charge. The INTERFACE FF [45] of Heinz et al. [46] is a collection of parameter sets for inorganic materials, having started with the CHARMM-METAL FF and subsequently being extended to several clay minerals, silica, sulfates and phosphates. Parametrization of these materials followed a nuanced approach that took into account broad sources of data that are applicable to each material to create a thermodynamically consistent FF. As an example, the CHARMM-METAL FF aimed to accurately simulate face-centred cubic metals. Prior to this FF, existing biomolecular FFs were parametrized using vaporization energies of model alkane compounds as target data. However, the temperature-dependence of the LJ approximation means it is not applicable to parametrizing metals at their boiling points an order of magnitude above the temperature of interest [46], so vaporization energy was no longer applicable. Heinz et al. thus used experimental material properties like density and surface energy as target data to fit the LJ atomic radii and well depth, respectively (in fixed-charge FFs for metals, the partial charge can be set to zero). This meant incorporating the attractive effect of polarization into the LJ well depth, which was significantly higher than other atom types from the biomolecular FFs.

Highly polarizable surfaces expose the main question about fixed-charge FFs in interfacial systems. The net polarization in peptide adsorption is the polarization of the surface by the peptide, minus the loss of its polarization by displaced water [28]. Fixed-charge FFs, with averaged parameters to implicitly incorporate polarization effects, may accurately determine thermodynamic quantities because in simulation these values are calculated using the interaction of a single molecule, whereas in experiment millions or billions of molecules are measured. Small inaccuracies resulting from the averaging of parameters can lead to large inaccuracies in the absolute value of the quantity being measured. To our knowledge, there has not been a thermodynamic investigation of peptide adsorption using the CHARMM-METAL FF, so this could be a valuable next step in validating its place within the CHARMM FF.

On insulating surfaces, there is less of an attractive force from polarization as there are no image charge effects. We will now consider one such surface, silica, and its parametrization [47]. For silica, a crystal, there is a useful set of target data from X-ray diffraction. For the INTERFACE silica parameters, electron densities from X-ray diffraction of alpha-quartz were integrated within the silicon and oxygen atomic radii to determine atomic partial charges [48]. On the surface, hydroxyl and silic oxide groups were given partial charges rationalized by their similar pH to already parametrized functional groups. By then fitting the bonded and LJ parameters to X-ray and Raman spectra target data, the FF was fitted to bulk material properties (in this case, heat of immersion). Validation included computed water contact angles and adsorption isotherms. This procedure is an ideal example of balanced parametrization of a surface—there is detailed scrutiny of surface morphology at the atomic scale, as well as on bulk properties. A companion paper attempted to validate the FF using peptide adsorption [49]. This exercise demonstrated qualitative agreement of binding strength to silica surfaces with different surface groups, and allowed for an atomistic understanding of the trends controlling binding. However, the comparison of experimental adsorption data with the simulated percentage of time the
peptides spent on surfaces (which share a logarithmic, not linear, relationship) was an uncommon measure not suitable for quantitative comparisons. There are enhanced sampling methods available to extend the effective sampling beyond 5 ns, allowing for the calculation of free energies [50]. Given that a single set of partial charge parameters were used across several different FFs, each with different water molecules, there is the possibility of parameters not being suitable for the water model and associated protein parameters on the atomic scale, despite good agreement at the bulk level. The extent of agreement with measured free energies of adsorption can be used to assess accuracy at the atomic scale. This approach to validation is covered below.

The most thorough work in validating FFs for interfacial systems comes from the Latour laboratory working with peptides on self-assembled monolayers (SAMs). While metal and crystal surfaces have a well-defined structure, for most organic surfaces such as polymers there is the added complication of a high level of heterogeneity in the surface composition. Their bulk properties are best considered with coarse-grain approaches [19], inherently unsuitable for determining the effect of specific functional group interactions. An added difficulty in simulating these systems is the presence of contaminants, and also the heterogeneity introduced by surface treatments, such as by plasma [51]. Given that there is no atomic structure for these materials, researchers have turned to SAMs of alkanes that can be more easily controlled in experiment, and more explicitly defined in simulation. These can be a proxy for polymer surfaces because the surface-tethered alkanes can be functionalized with specific functional groups that are presented on the surface and mimic those groups that would be found on the surface of a polymer. This has been used by Wei and Latour as a powerful source of both experimental and simulated data to facilitate a comparison for parametrization and validation purposes [52].

Latour is the first, and to our knowledge only, investigator to consider the free energy of peptide adsorption as a property for the validation of an interfacial FF [53]. For these studies, Latour used a simulated model of SAMs using parameters for similar moieties in the CHARMM FF. First, Sun et al. tried calculating the adsorption free energy of peptides using implicit water, but it became clear from this work that explicit water is necessary for accurately calculating the free energy of peptide adsorption [54]. The extra computational load of explicit water was addressed using a novel enhanced sampling method [55,56]. Since that work, computer power has increased and the need for enhanced sampling has diminished—longer periods of simulation can now adequately sample slower degrees of freedom [14]. Experimentally measured adsorption data were used as a validation dataset for the parameters transferred from the CHARMM FF and showed that the existing CHARMM parameters were not completely transferable to an SAM surface [32]. Specifically, there was good agreement for the calculated free energy of adsorption for the test peptide on SAM surfaces with hydrophilic end groups, but poor agreement for the same system with hydrophobic end groups.

The failure of the existing CHARMM parameters to simulate an SAM surface led to the development of a new FF, the Dual-FF, that scaled the interactions between the solid and liquid phases such that the adsorption free energies from experiment were reproduced in the simulation [32]. In particular, the effective LJ well depth of the water and the SAM carbons was altered, as well as the effective charge on regions of the peptide. These changes had no effect on the interactions within each phase separately (and so preserved the peptide solution-phase behaviour), but scaled the potential energy calculated between phases to compensate for errors in peptide behaviour over hydrophobic SAMs. This method successfully reproduced the free energy of adsorption on hydrophobic SAMs without affecting the same measure over hydrophilic SAMs. The Dual-FF approach has also been applied to silica [57] and high-density polyethylene [58] to reproduce experimental adsorption data. While this solution achieves the goal of reproducing the desired experimental data, it is a practical solution, which neglects the possibility of underlying errors in the parametrization of the surface. Additionally, by using peptide adsorption free energies as the target data for parametrizing the FF, the reproduction of peptide adsorption free energies becomes a foregone conclusion and the experimental data available for validation is reduced. Another approach, detailed below, is to first parametrize the surface based on external datasets and then to test its behaviour in the target system.

The CHARMM FF has adopted the principle of transferability, whereby parameters for atoms in a class of functional groups can be transferred to similar groups without further optimization. The experimental and simulation work by Latour et al. shows that for parametrizing interfacial systems, the transferability of existing CHARMM parameters is compromised. In agreement with this, it has been determined by the CHARMM developers that monolayers cannot be simulated self-consistently by the latest lipid bilayer CHARMM parameter set [27]. This might not preclude simulating peptide–surface interactions consistently with the CHARMM FF, but it may require additional atom typing and parametrization. Given that the system in question is an alkane monolayer, it is instructive to look at the parametrization of a similar system, the lipid bilayer.

Following the parametrization philosophy adopted in CHARMM [59], the initial step to parametrizing a new molecule is to develop internal degrees of freedom to reproduce lowest energy molecular configurations, usually determined by QM modelling. Following this, a first guess of the partial charges is made and then LJ parameters are determined (figure 2). This is an iterative process, which can be reiterated from any stage according to how well the model reproduces the experimental target data (figure 2). Some simulation data have shown monolayer structure to be sensitive to the LJ terms [60], so this may provide an avenue to address parameter correlation and guide the parametrization process. As asserted by Zhu et al. [59], the task can be approached by degrees—having validated the non-bonded terms, the torsional terms can be refit to reproduce accurate dihedral behaviour and ensure the correct material dynamics are preserved.

A thorough parametrization process such as this can potentially produce a surface model that is balanced—reproducing both monolayer material properties as well as bulk thermodynamic quantities. Then, validation using the experimental peptide adsorption data can assess the rigour of the parameters.

As demonstrated on the gold and silica surfaces, there is no hard and fast rule for experimental target data, because the nuances of each material will be different. In the following section, we will describe some of the experimental tools that can be used to characterize surfaces for simulation,
particularly those data that can be reproduced by simulation so that the validity of the model can be tested.

3. Experimental target data and validation data

All surfaces will require some measure of experimental data to be used in parametrization. For some materials, explicit topological data can be determined. These surfaces have either a crystal or lattice structure that can be probed by X-ray diffraction. Others, such as monolayers or lipid bilayers, do not have a single atomic-level description and must instead be understood by dynamic means, based on a statistical distribution function. Even still, contamination of the experimental surface can frustrate parametrization and validation, so a careful understanding of the experimental conditions is required. This section will demonstrate how these considerations have been addressed by the choice of experimental target data, as well as covering some simulation techniques for validation.

The LJ terms and electrostatic terms, thought of as non-bonded terms, are not typically expected to influence the structure of bonded molecules. However, in most FFs, non-bonded interactions are ignored only between atoms that are bonded by up to three bonds. This means the non-bonded parameters can still affect the torsional behaviour of a dynamic molecule, and thereby the structure of mobile phases such as mono- or bilayers. For the CHARMM lipid FF, this meant that parameter correlation in the non-bonded terms was an issue that affected lipid structure. Ordinarily, in biomolecular FFs, after partial charges were determined, the LJ parameters were developed by fitting to experimental heats of vaporization and relative free energies of solvation [61,62]. However, the solutions to these fits were non-trivial, with scope for parameter correlation, such that errors in one parameter could be compensated by another. The solution was to use QM modelling of model molecules interacting with helium and neon, which carry no partial charge to interfere with the calculations, as a means to determine the relative van der Waals minimum interaction distances. This reduced the number of possible combinations of parameters, informing the choice of the correct set.

Dynamic materials like mono- and bilayers are usually described using the surface area per lipid, thickness of the bilayer or acyl chain order parameters. Despite the differing timescales of simulation and experiment (nanoseconds for simulation compared to microseconds for $^1$H NMR [63]), these measures have been used successfully for the parametrization of lipid bilayers [29]. A specific consideration that has emerged from this process is the importance of using the particle mesh Ewald, a method of calculating long-range electrostatics, rather than a cutoff, because the surface area of lipid is sensitive to the balance of forces acting on the headgroup and alkane tails [64]. Whether or not this is true for alkanethiol SAMs such as those commonly used in peptide binding experiments remains to be determined.

The type and quantity of the functional groups appearing on the surface of a material will significantly affect the ability of peptides to bind. For example, by varying the chemistry of silica surfaces with pH changes, adsorbant peptides have less than 5% sequence similarity [47]. As there are 20 natural amino acids, this equates to random sequence homology, and the reasons for this binding pattern are unexplained at the atomic level. There are a wide range of methods to determine the identity and the area density of these surface chemical groups, and they can be used to inform the development of the surface model. To some extent the atomic composition of the surface will be known a priori, but X-ray photoelectron spectroscopy (XPS) can be used in this context to ascertain the presence of contaminants that might affect peptide interactions at the surface [47,65,66]. On silica, specific surface area in combination with thermogravimetric analysis, in which the loss of mass during heating is measured, were used in tandem to determine the area density of surface siloxide groups [47]. Particularly where pH effects are concerned, potentiometric titration and zeta potential are useful for measuring the degree of ionization on the surface.

Attenuated total reflection infrared spectroscopy and XPS typically penetrate further into the substrate than is desired for surface characterization, where only the first few atomic layers are of interest [67]. These techniques thus combine information about the chemistry of the bulk material with that of the surface, but peptide interactions are dominated by the chemical groups immediately on the surface. For this reason, in cases where there are charged groups on the surface, atomic force microscopy has been used to determine the surface potential and through this, the area density of surface charges [68]. Similarly, for SAMs where the composition is known a priori, but can also be confirmed with XPS, surface plasmon resonance (SPR) can measure the pKa of the surface functional groups and help in modelling the correct area density of charges under different pH conditions [69].

The presence of specific functional groups on a surface is necessary for any all-atom model of an interface with peptides in solution to function, but for a balanced representation, the surface must also reproduce bulk material properties. For solid surfaces such as metals or polymers, where there is very little dynamic behaviour, it is likely that the main determinants of peptide binding will be the structure of water on the surface. This is because in bulk aqueous solution, water will form a dynamic network of freely moving water molecules, with a large number of stabilizing hydrogen bonds [70]. This is an enthalpically and entropyfavourable situation. However on hydrophilic surfaces, this network is disturbed and water molecules become oriented, losing orientational freedom.

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Figure 2. The parametrization process used in the development of the CHARMM FFs. The process is reiterated until satisfactory reproduction of target data is achieved. Adapted with permission from [59], copyright © 2011 John Wiley and Sons.
and hydrogen bond contacts. This is associated with a reduced water density on the surface, called de-wetting. In this context, the adsorption of a peptide can be regarded as minimizing the surface contact with water and so returning its favourable entropy and reducing interfacial tension [70,71]. This can be a significant contributor to binding [72], and so must be addressed by any surface model used for peptide binding.

Current methods of experimentally determining the bulk properties at the solid–water interface use some combination of surface tension, interfacial tension, water contact angle, zeta potential or heat of immersion. The water contact angle, an implicit measure of the polarity of the surface, is a technique readily available in most laboratories and has a large number of published data for most surfaces of interest. In simulation, the water contact angle can also be determined at the nanoscale to compare with experimental measurements, with some added considerations. While experimental contact angles represent the average of trillions of molecular interactions, nanoscale droplets can be somewhat influenced by the specific composition of a handful of polar and non-polar groups on the surface; however this effect can be quantified and removed by consideration of the solvent-accessible surface area of those groups [73]. The process differs from macroscale measurements—while on some simulated surfaces it is enough to simply fit a circle to the two-dimensional density profile of a nanodroplet of water [47,74] and measure the angle at the interface, for other surfaces the line tension can affect the size of the droplet [75]. This relationship between the energy at the perimeter of the droplet and the size of the circumference of the droplet at the surface can lead to size dependence of the calculated water contact angles, and so the level of dependence on this value should be determined [76] before calculation, as in [77].

Macroscopic properties like heat of vaporization or the free energy of hydration are also common measures. Clearly, heat of vaporization is not appropriate for a solid-phase surface. With lipids, the free energy of solvation has been determined in simulation using a free energy method that involves transforming the target molecule into and out of a water box [78], but it is unclear how this approach would be implemented for surfaces, and there are no published examples using the biomolecular FFs mentioned here. The enthalpy of aqueous immersion, however, is an experimental quantity that can also be calculated in simulations. Determined using calorimetry, this quantity can be calculated using three separate simulations of the surface–water interface, the surface alone, and the water alone, as demonstrated for metal [46], silica [47] and most recently hydroxyapatite [79].

Using a thorough surface characterization such as through the methods outlined here, highly heterogeneous or amorphous surfaces can be modelled without exact atomic coordinates of the surface. If the functional groups on the surface are present in the correct area density, and the surface is parametrized so as to reproduce bulk thermodynamic quantities, then it is likely that it can also quantitatively measure the adsorption free energy of peptides and thus be used as a predictive tool for understanding peptide–surface interactions. To our knowledge, there are no published examples of this process being followed rigorously for heterogeneous surfaces—in which the exact atomic coordinates of the surface are used for free energy validation and prediction. However, a number of studies have used organic models for surfaces and explored the trends resulting from altering the atomic composition of the surface. Dai et al. [74] developed a model for polypropylene with parameters borrowed from the CHARMM FF, and compared the effect of common hydrophilization procedures on water contact angle in experiment. The model reflected the trends from experiment, but the parameters were not optimized for polypropylene so absolute values could not be compared. Most recently, the dual-FF philosophy for CHARMM originally developed for SAMs was applied to high-density polyethylene [58]. Surface characterization included atomic composition and water contact angle, but these were not used for optimization of the surface parameters. These models represent promising approaches, yet the lack of parametrization in each case precludes any assessment of their potential for predictive simulations.

Surface models that are justified in their atomic composition and bulk thermodynamic properties must then be validated in order to be quantitative for peptide interactions. The quantity of most interest is the free energy of adsorption, because it accounts for both the enthalpic interaction with the surface as well as the entropic change in the system. Experimentally, the free energy of the interaction is more difficult to determine for peptides on surfaces than it is for interactions between functional proteins. In experimentally measuring the binding affinity of small molecules to proteins, there is often some functional effect (such as current–voltage curves for ion channels) that will be altered by some critical concentration of the small molecule, and this concentration can be used to determine the binding free energy. On surfaces, however, the binding free energy of a peptide is more difficult to determine. On electrically conductive surfaces, SPR is a common technique to observe and measure peptide adsorption [65,80,81]. For surfaces with more than a nanometre-thick non-conductive layer such as organic polymers, Thyparambil et al. have measured peptide binding by determining a correlation between desorption via atomic force microscopy and the binding free energy from SPR experiments. Atomic force microscopy is a powerful method of measuring adsorption free energy. Because a pulling force is applied, it does not rely on adsorption and subsequent desorption of the peptide, which can limit SPR measurements to only those peptides with reversible binding.

Importantly, there are several simulation analogues that can be used to compare with experiment for validating model surface parameters. Umbrella sampling is one method, in which peptides are restricted at increasing distances from the material surface. At each distance, the force of attraction between the peptide and the surface can be calculated from the movement away from the restrictive potential towards the surface. These positional data can then be deconvoluted into a continuous free energy profile, and this is used to determine an adsorption free energy, as in [82], to compare with experimental adsorption free energies. There is another popular approach that may have advantages over umbrella sampling in terms of simplicity and parallelization. In this approach, steered molecular dynamics is used to pull the bound peptide away from the surface while measuring resistance. This is a non-equilibrium process, which used to be considered inappropriate for determining equilibrium quantities like free energy. However, using a statistical mechanical relation discovered relatively recently [83], the free energy of adsorption can be calculated. This has already been used for surface adsorption free energies [84]. While this technique is suitable for determining relative binding free energies, and is attractive for its ease of use, it offers no sampling benefits
over umbrella sampling when calculating absolute values for adsorption free energy [85].

Another enhanced simulation technique that has recently been applied to interfacial peptide surface adsorption is metadynamics. Here, one or two degrees of freedom are biased via the potential energy function, forcing the peptide to escape from low free energy wells while also recreating the free energy landscape as it is explored. As the computational effort grows dramatically with each degree of freedom that is biased, generally only one or two are chosen—such as the peptide distance or angle from a surface. To ensure full sampling while only biasing a couple of degrees of freedom, metadynamics has been combined with replica exchange [86] to measure the free energy of adsorption for peptides at interfaces. This approach was demonstrated on the alanine dipeptide on gold [87] and has been used to calculate the free energy of adsorption for oligopeptides on SAM surfaces [88], silica [89] and on gold [44]. These studies also demonstrate how a re-weighting scheme can be applied to undo the metadynamics bias and analyse additional degrees of freedom in an equilibrium Boltzmann distribution to assess their free energy landscape.

4. Polarizable force fields for interfacial systems

While this review focuses on additive FFs, it is important to consider it in the context of the ongoing development of polarizable FFs. Ultimately, if a rigorously parametrized surface fails to reproduce peptide adsorption free energies using an additive FF, this is most likely due to the lack of polarizability, assuming proper sampling of the peptide conformational entropy at the surface. On this basis, a polarizable FF will be needed. The GollP-CHARMM FF [43] mentioned previously incorporates polarization in a gold surface using a rotating rod model, retaining the fixed-charge description in the aqueous phase. Walsh and co-workers have also recently applied this method to silver [34] and graphite [39] surfaces, where they take advantage of the computational efficiency afforded by the fixed-charge treatment of the aqueous phase to calculate amino acid binding free energies.

There are also two fully polarizable FFs of interest for simulating biological interfaces. The AMOEBA FF introduces polarization with multipole–multipole interactions and has recently been extended for use with proteins [90]. While the most recent update to the FF has not been applied to interfaces, previously an FF based on AMOEBA was extended for use with peptides on a graphene surface [91]. The CHARMm Drude-2013 FF is similar to the CHARMM additive FF, with the addition of a charged particle within non-hydrogen atoms that moves in response to the electric field, also known as the charge-on-spring [26]. The use of these FFs in protein simulations has been reviewed recently [92], but current parameterization efforts focus mostly on biomolecules in aqueous environments and there is significant work yet to be done towards simulating an interfacial system.

5. Conclusion

Understanding peptide–surface interactions has enormous potential in biotechnological applications, but their characterization at the atomic level is beyond the resolution of current experimental techniques. For this reason, molecular mechanics is used to model this interaction in atomic detail. However, most FFs currently in use are not tailored for the interaction of proteins with surfaces and do not account for polarization effects. New FFs for the simulation of peptide–surface interactions developed with a rigorous parametrization procedure taking into account these effects are required. In order for the model to accurately reproduce binding behaviour the parametrization must be balanced, reproducing both atomic-scale interactions and bulk thermodynamic properties. This will allow it to account for both the enthalpic and entropic contributions to peptide adsorption. Additionally, it must be followed by validation with experimental quantities that were not used during parametrization. Significant ground has already been covered in understanding the process of peptide adsorption, but strict parametrization and validation promise to advance this field even further in future.

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