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Interaction of Charged Amino-acid Side Chains with Ions: An Optimization Strategy for Classical Forcefields

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Abstract

Many well-established classical biomolecular forcefields, fitted on the solvation properties of single ions, do not necessarily describe all the details of ion-pairing accurately, especially for complex polyatomic ions. Depending on the target application, it might not be sufficient to reproduce the thermodynamics of ion-pairing, but it may be also necessary to correctly capture structural details, such as the coordination mode. In this work we analyzed how classical force fields can be optimized to yield a realistic description of these different aspects of ion-pairing. Given the prominent role of the interactions of negatively charged aminoacid side chains and divalent cations in many biomolecular systems we chose calcium acetate as a benchmark system to devise a general optimization strategy that we applied to two popular forcefields, namely GROMOS and OPLS-AA. Using experimental association constants and first-principles molecular dynamics simulations as a reference, we found that small modifications of the van der Waals ion-ion interaction parameters allow a systematic improvement of the essential thermodynamic and structural properties of ion-pairing.

keywords: Electrolyte solutions, Molecular Dynamics, Empirical potentials, Biomineralization.

Introduction

Ionic interactions play a key role in a multitude of systems in physics, chemistry and biology. Biomineralisation is a prominent example of processes which delicately depend on the interaction (i.e. binding preferences) of different types of charged species, such as free ions, crystal surfaces and charged (biological) macromolecules. Gaining insight into the pairing of complex ions and its influence on crystal nucleation and growth is the key to understand those phenomena that distinguish biomineralisation from normal crystallisation processes. Details regarding the structure and strength of ion pairs can make a significant difference

and need to be studied for a thorough understanding of the peculiarities occurring during (bio)polymer-controlled crystallisation.

A broad variety of experimental techniques has been applied to study the hydration and association of ions in solution.^{1,2} In view of the limitations that such techniques encounter regarding the determination of structural details and the free energy of pairing, molecular simulations have proven to be a valuable complement to experiments.³⁻¹⁰

Quantum mechanical methods are accurate and transferable but, in spite of advances in computing power, such simulations are limited regarding size and time scales. Simulations based on classical potentials can deliver valuable insight into structural and thermodynamic details of ion-pairing once they have been parametrised thoroughly. Several different approaches to parametrise these potentials exist. In the past years, several studies have shown that a description of ionic interactions based on the homoatomic model parameters of individual ions and the application of a heuristic combination rule often fails.¹⁰⁻¹² Instead, ionic interactions often need to be modeled with pair-specific heteroatomic parameters that have been set up to describe the nonbonded interactions between unlike atoms in a specific surrounding.

Classical simulations have successfully helped to analyze the solvation and ion-pairing of “simple” ions (such as alkali-halides) at a molecular level.¹²⁻¹⁴ However, if more complex (polyatomic) ions are studied, solvation is accompanied by effects which aggravate an accurate description of ion-pairing. Examples of such challenging systems are the charged side chains of aspartate and glutamate peptides, which possess a carboxylate group. Due to strong interactions with cations, these side chains often serve as ligands coordinated to metal cofactors in metalloproteins.¹⁵ They are also well-known for their influence on crystal nucleation and growth of inorganic minerals in living organisms.^{10,16-18}

Metal cations can bind to the negatively charged carboxylate group in several different ways: if the two ions are in direct contact to each other, they are referred to as a contact ion pair (CIP), whereas the so-called solvent-shared ion pair (SIP) is characterised by a stable

layer of solvent in between the two ions (2). Furthermore, the CIP state can be subdivided into one state in which both carboxylate oxygen atoms are coordinated to the cation (from now on referred to as *biCIP*) and one state in which only one of the oxygen atoms is binding to the cation (from now on referred to as *monoCIP*). At first sight, this differentiation between the different types of ions pairs might appear like a minor structural detail. However, the binding-mode (*biCIP* vs. *monoCIP*) of carboxylate groups in metalloproteins is believed to have a major influence on important phenomena in living organisms, such as the signal transduction pathway, and in catalytic processes.^{15,19–23} Furthermore, within the context of biomineralisation it is not unlikely that the binding-mode of the carboxylate group of peptide side chains and metal cations affects the nucleation and crystal growth of the respective mineral.¹⁶ In order to obtain meaningful insights into such crystallisation processes at a molecular scale, it is therefore essential to validate that the applied forcefield yields a realistic description of the association of the ions involved. Such a realistic description of the ion-pairing of complex ions, including structural details such as the binding-mode, delicately depends on a correct balance of ion–ion, ion–water and water–water interactions.

Here we present a procedure to systematically improve standard pairwise forcefields so to match both experimental thermodynamic data and structural information obtained by first-principles simulations. Other groups have made similar analyzes for several forcefields, but mainly focusing on the interaction of amino-acid side chain analogs with monovalent ions (e.g. alkali-acetates).^{14,24} In view of the importance of the interaction of alkaline earth metals (mainly Ca^{2+} and Mg^{2+}) with negatively charged peptide side chains, we focus here on studying the association of acetate (as an analogue for the negatively charged side chain of glutamate) with calcium ions in water. Here we aim at establishing a set of boundaries for the re-parametrization of ion-ion interactions, made of experimental observations and first-principles calculations. Owing to the importance of the relative probabilities of CIPs and SIPs, we do not only focus on the thermodynamics of ion-pairing, but we also consider which forcefield (from now on referred to as FF) gives a realistic description of the structural details.

We will then discuss how a minimal re-parametrization of standard forcefields commonly used for the simulation of biomolecular systems, i.e. GROMOS²⁵ and OPLS-AA,²⁶ performs in the context of the simulation of aqueous salt solutions, compared to state-of-the-art forcefields specifically designed to simulate the formation of biominerals in solution.^{10,27}

Methods

Typical biomolecular forcefields are based on classical pairwise additive non-polarizable potentials. This simplification, which massively reduces computational costs, has recently been further justified by Krekeler and Delle Site, who observed that water molecules in direct contact with monoions are not significantly stronger polarized than their bulk counterparts.²⁸ This may not be the case for divalent ions, which have been observed to induce larger polarization in the water molecules belonging to their first solvation shell.²⁹ To fully take into account such effects one should use polarizable forcefields,³⁰ which yet imply the use of more complicated and computationally expensive numerical algorithms. An alternative approach, followed in,^{10,27} is to keep fixed point charge models, using slightly more sophisticated functional forms for the short-range interactions, i.e. replacing Lennard-Jones potentials with Buckingham potentials. The developers of this scheme have managed to achieve an accurate thermodynamic description of both ions in solution and different mineral phases of calcium carbonate with fixed charge models. Recent successful applications^{8,31} have rendered this type of forcefields very popular for studies connected the (bio)mineralization of calcium carbonate. Since we want to develop a parametrization scheme compatible with standard biomolecular forcefields, we decided to use fixed charge models, and to avoid modifying standard LJ functional form. In the following we will briefly introduce the underlying model structure and the different possible approaches to optimize the chosen models.

Classical forcefield terms and parametrisations

In the framework of classical potentials, intermolecular interactions comprise an electrostatic contribution according to Coulomb’s law (depending on the atomic partial charges q_i) and van der Waals interactions. The latter ones are represented by a Lennard-Jones potential in many popular biomolecular forcefields. The non-bonded interaction between two atoms i and j thus reads:

$$V^{\text{non-bonded}}(r_{ij}) = V_{\text{Coulomb}}(r_{ij}) + V_{\text{LJ}}(r_{ij}) = \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} + \frac{C_{ij}^{(12)}}{r_{ij}^{12}} - \frac{C_{ij}^{(6)}}{r_{ij}^6} = \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} + 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] \quad (1)$$

FF such as GROMOS²⁵ or OPLS-AA²⁶ use heuristic combination rules to define the heteroatomic Lennard-Jones interaction parameters $C_{ij}^{(6)}$ and $C_{ij}^{(12)}$ or σ_{ij} and ϵ_{ij} based on the homoatomic parameters. These two FF apply the geometric-mean combination rule ($\sigma_{ij} = \sqrt{\sigma_{ii}\sigma_{jj}}$ and $\epsilon_{ij} = \sqrt{\epsilon_{ii}\epsilon_{jj}}$), while other FF use the Lorentz-Berthelot combination rule ($\sigma_{ij} = \frac{\sigma_{ii} + \sigma_{jj}}{2}$ and $\epsilon_{ij} = \sqrt{\epsilon_{ii}\epsilon_{jj}}$). Chemical peculiarities of the non-bonded interactions such as the specific mutual polarisation of atoms i and j are therefore not fully taken into account. The validity of an application of such heuristic combination rules depends on the two atom types i and j . Their usage is well tested and confirmed for atom types frequently occurring in biomolecular FF (such as carbon, nitrogen or oxygen) and for the interactions of some alkali-halides in aqueous solution. In the case of ionic species, the self-interaction parameters σ_{ii} and ϵ_{ii} are often fitted to single-ion properties, such as ionic solvation free energies, and do not necessarily yield a realistic description of ion-pair formation and of the resulting thermodynamic properties of electrolyte solutions at higher ion concentrations. Fyta and Netz have shown that for some ion pairs, such as KF, the application of combination rules fails to reproduce experimental thermodynamic properties of ion-pairs, irrespective of the choice of the homonuclear parameters.¹² Only if the heteroatomic interaction parameters σ_{ij} and ϵ_{ij} are optimized independently from the self-interaction parameters (and thereby ignoring the combination rule), both single-ion and ion-pair properties can be modelled in a

satisfying manner.

For a proper description of ion-pairing properties, it is of great importance that the ion-ion, ion-water, and water-water interactions are correctly balanced. If the relative magnitude of these interactions is incorrect (leading to wrong solvation properties of the simulated ion-pair), several changes to the non-bonded interactions can be made. Two major decisions have to be made during such reparametrisation process. The first question concerns which interaction between the different types of species needs to be revised (ion-ion, ion-water, or water-water interactions). As it is often advisable to work with one of the established water models, the water-water interactions are not subject to modifications. If the ion-water interactions have been set-up reliably, based on single-ion solvation properties, it is then sufficient to focus on the modification of the ion-ion interactions.

The second decision that needs to be made applies to the question on how the non-bonded interaction potential (1) between different ions or eventually between ions and water is modified. This is a controversial issue. If the heteroatomic Lennard-Jones parameters σ_{ij} and ε_{ij} are changed, the modification only affects the specific interactions between atom types i and j , while the interaction with any other atom type k remains unaltered. This approach overrules the commonly used heuristic combination rules, but is the one that has the smallest impact on the transferability of the FF. In fact, if the homoatomic Lennard-Jones parameters σ_{ii} and ε_{ii} or the atomic partial charges were modified, this would have an impact on the interactions with any other atom type in the system. The transferability of this parametrisation to other systems would therefore be hampered.

In the past years, several studies have pointed out the importance of a reevaluation of the parametrisation of the interactions of charged amino-acid side chains with water and ions.^{11,32} It was observed for example, that the simulated dynamics of protein-ion interactions show large deviations among three commonly used forcefields GROMOS, OPLS-AA and CHARMM, and that none of the standard parameter sets of these three FF was able to

reproduce experimental thermodynamic data of the respective electrolyte solution.¹¹

Several groups have tried to assess and systematically optimize the description of solvated ions and of ion association properties of different FF, each of them following a different approach.^{10–12,14,32} Reif et al. have recently published a modified version 54A8 of the GROMOS FF that was largely motivated by the need to recalibrate the solvation properties of charged amino-acid side chains and their interactions with ions. During reparametrisation, they did not focus on the interactions of amino acids with specific ions (ion–ion interactions) but rather aimed at a general parametrisation of charged amino-acid side chains. They chose ionic side chain analogues as representatives for the functional groups of the peptide side chains. For the optimization of the solvation properties of these monovalent polyatomic ions (ion–water interactions), absolute intrinsic single-ion hydration free energies served as experimental target data. The changes made to the FF included nearly exclusively the atomic partial charges. Homoeatomic Lennard-Jones parameters were only modified if absolutely necessary. As these changes made to the FF strongly affect the interactions of charged amino-acid side chains with other solvated ions, it is necessary to investigate the consequences with respect to ion-pairing properties. This process has been initiated,²⁴ and it is likely that the parametrisation of other ionic species needs to be revised in order to be compatible with the newly developed description of charged amino-acid side chains.³²

Alternatively one can leave the interactions between amino-acid side chains and water unaltered and focus on the ion–ion interactions.¹¹ The underlying assumption of this approach is that biomolecular FF have been designed to yield a thorough description of solvated proteins. Furthermore, they assume that the monoatomic ions that interact with the amino-acid side chains have also been parametrised carefully, based on single-ion solvation properties. The only remaining degrees of freedom that need to be optimized are then the direct ion–ion interactions. Following this idea, Project et al. used formate as a model for the carboxylate group of an aspartate side chain and reparametrised the calcium–carboxylate interactions based on a comparison between simulated and experimental data on the association constant of

calcium-formate.¹¹ In contrast to Reif et al., they did not change the partial charges in order to modify the non-bonded interactions, but concentrated on the heteroatomic Lennard-Jones parameters instead.

Another interesting approach has been followed by Raiteri et al., who studied the interactions of charged organic species (similar to amino-acid side chains) with different forms of calcium carbonate.¹⁰ Such hetero-interfaces between hard and soft matter pose a great challenge to simulations, as different types of forcefields have evolved for minerals and biomolecules over the past decades. Having been developed by different scientific communities, the forcefields for biological aqueous soft matter systems and for hard matter minerals have been created with different approaches to omit electronic polarisability and to cast its effects into effective classical pairwise additive potentials. These differences affect the transferability of forcefields between hard and soft systems and make them largely incompatible.⁶ With the goal of reproducing experimental thermodynamic properties, Raiteri *et al.*¹⁰ have created a new FF for the nonbonded interactions among the different types of ions and between ions and water. In contrast to all other approaches mentioned so far, Raiteri *et al.* used the Buckingham potential as the basis for some nonbonded van der Waals interactions:

$$V_{\text{Buckingham}}(r_{ij}) = A_{ij} \cdot \exp\left(-\frac{r_{ij}}{\rho_{ij}}\right) - \frac{C_{ij}}{r_{ij}^6} \quad (2)$$

They have optimized the ion–water interactions based on the free energy of solvation of the ions, with experimental data and quantum mechanical calculations serving as references. Regarding the ion–(organic) ion interactions, they rescaled the repulsive part of the Buckingham potential to match experimental free energies of ion pairing.

Forcefields under investigation

We have selected two well-established forcefields from the increasing number of available biomolecular FF. From the family of GROMOS FF,²⁵ we have selected the latest version (54A8),³² which incorporates changes in the description of charged amino-acid side chains. In view of the aim of our work, to analyze the interactions of such charged amino-acid side chains with other solvated ions, a comparison of this version to its predecessors is of special interest. For the system of calcium acetate in water, there is no difference between the former versions 53A6 and 54A7.³³ Besides these “standard” versions, we have also considered the modified version of GROMOS 54A7 as it was proposed by Project et al.¹¹ The other FF of interest is OPLS-AA in its standard version^{34,35} and its modification according to Project.³⁶ Both modified versions of GROMOS and OPLS-AA that were proposed by Project et al. feature revised heteroatomic Lennard Jones parameters $\sigma_{\text{Ca-OM}}$ and $\varepsilon_{\text{Ca-OM}}$ (Table 2), which lead to optimized ion-ion interactions. Taking into account which water models were used during the original FF parametrisation processes and following the results of Hess and van der Vegt,³⁷ we used the SPC/E water model³⁸ in conjunction with the GROMOS FF and the TIP4P-Ew model³⁹ together with the OPLS-AA FF.

Another forcefield which is of great interest here is the one by Raiteri et al.,¹⁰ as it has been constructed with a special focus on the description of the interactions of charged organic species (such as acetate) with calcium ions. In contrast to the other FF, it is largely based on Buckingham potentials and applies a flexible water model. However, in our implementation we used the rigid SPC/E water model instead of the SPC/Fw model⁴⁰ for the ease of use. As these two water models differ only slightly in the partial charges, this deviation from the original FF is not expected to drastically change the solvation properties of the ions. Indeed, our implementation of the forcefield (applying the SPC/E model) does reproduce the Potential of Mean Force of calcium acetate shown in Fig. 3 of Ref. 10 (a comparison of the PMFs is shown in Figure S1 of the Supporting Information).

The models for the calcium ion were also chosen in accordance with the standards of the

respective FF. In conjunction with the OPLS-AA FF the ion model according to Åqvist⁴¹ is usually applied, while the GROMOS FF comes with its own set of parameters for the calcium ion.⁴² The calcium parameters of Raiteri’s FF can be found in the Supporting Information of Ref. 10 and in Ref.³¹

The models for the acetate anion were constructed based on the parameters of the side chain of glutamic acid of the respective FF (GROMOS and OPLS-AA). The acetate model of Raiteri et al. is described in detail in the supporting information of Ref. 10. 1 gives an overview of the atomic partial charges of the different acetate models.

Table 1: Atomic partial charges of acetate. Abbreviations: OM - oxygen atom of the carboxylate group; C_{COO} - carbon atom of the carboxylate group; UA - united atoms; C_{CH₃} - carbon atom of the methyl group

Atom	GROMOS 54A7	GROMOS 54A8	OPLS-AA	Raiteri <i>et al.</i> ¹⁰
	[e]	[e]	[e]	[e]
OM	-0.635	-0.715	-0.80	-0.63
C _{COO}	0.270	0.270	0.70	0.23
CH ₃ (UA)	0.0	0.16	-	-
C _{CH₃}	-	-	-0.28	-0.27
H	-	-	0.06	0.1

Table 2 contains an overview of the nonbonded interaction parameters between the calcium ion and an oxygen atom of acetate for all the different forcefields considered here. Note that table 2 and the following figures already contain values and data of FF modifications (dashed lines) that will be discussed only in the last part of the manuscript.

1 shows a plot of the total nonbonded (Lennard-Jones and Coulomb) interactions between a calcium ion and an oxygen atom of the carboxylate group, according to the functional forms and parameter sets considered in this work.

Table 2: Nonbonded interaction parameters between the calcium ion (Ca) and an oxygen atom of the carboxylate group of the acetate anion (OM). Abbreviations: GROMOS 54A7 EP - GROMOS 54A7 forcefield with modified Ca-OM interactions according to Project et al.;¹¹ GROMOS 54A8 Ca-OM mod. - GROMOS 54A8 forcefield with modified Ca-OM interactions that correspond to 60 % of the modifications as suggested by Project et al.;¹¹ OPLS-AA EP - OPLS-AA forcefield with modified Ca-OM interactions according to Project;³⁶ OPLS-AA Ca-OM mod. - OPLS-AA forcefield with an increase in σ_{Ca-OM} by 5.8 %; Raiteri *et al.* - forcefield as presented in Raiteri et al.¹⁰

<i>LJ-type forcefields</i>	q_O [e]	σ [nm]	ε [kJ/mol]	$C^{(6)}$ [kJ · mol ⁻¹ · nm ⁶]	$C^{(12)}$ [kJ · mol ⁻¹ · nm ¹²]
GROMOS 54A7	-0.635	0.3359	0.2625	$1.508 \cdot 10^{-3}$	$2.165 \cdot 10^{-6}$
GROMOS 54A7 EP ¹¹	-0.635	0.3069	0.5988	$2.0 \cdot 10^{-3}$	$1.67 \cdot 10^{-6}$
GROMOS 54A8	-0.715	0.3359	0.2625	$1.508 \cdot 10^{-3}$	$2.165 \cdot 10^{-6}$
GROMOS 54A8 Ca-OM mod.	-0.715	0.3181	0.4351	$1.803 \cdot 10^{-3}$	$1.868 \cdot 10^{-6}$
OPLS-AA	-0.8	0.2672	1.2857	$1.872 \cdot 10^{-3}$	$0.6811 \cdot 10^{-6}$
OPLS-AA EP ³⁶	-0.8	0.277	1.2857	$2.323 \cdot 10^{-3}$	$1.049 \cdot 10^{-6}$
OPLS-AA Ca-OM mod.	-0.8	0.2827	1.2857	$2.625 \cdot 10^{-3}$	$1.340 \cdot 10^{-6}$

<i>Buckingham-type forcefield</i>	q_O [e]	A [eV]	ρ [Å]	C [eV Å ⁶]
Raiteri <i>et al.</i> ¹⁰	-0.63	2564	0.2715	0.0

Figure 1: Non-bonded (van der Waals and Coulomb) interactions between the calcium cation and an oxygen atom of the carboxylate group of the acetate anion. Abbreviations: cf. caption of table 2.

Computational details of the classical MD simulations

We used the GROMACS simulation package^{43,44} (version 4.5.5) to calculate the Potential of Mean Force (PMF) between one calcium and one acetate ion from classical Molecular Dynamics simulations. The PMF is determined from a series of independent simulations (“blue-moon ensemble”⁴⁵) in which the two ions are constrained at different distances using the linear constraint solver (LINCS) algorithm.⁴⁶ The distance between the calcium ion and the carbon atom of the carboxylate group of the acetate anion was used as reaction coordinate. The PMF is then obtained from integrating the constraint force f_c (including an entropic correction term):^{47,48}

$$V^{\text{PMF}}(r) = \int_{r_m}^r \left[\langle f_c \rangle_s + \frac{2k_B T}{s} \right] ds + C \quad (3)$$

Two system types, both in the shape of a rhombic dodecahedron box with periodic boundary conditions but with different sizes, were used. A “large” simulation box was applied in order to allow large distances r_m between the two ions to probe the unbonded state where the PMF goes asymptotically to zero. This box contained 1031 water molecules besides the ion pair. Furthermore, a “small” simulation box containing 122 water molecules and the ion pair was used to directly compare the results of classical simulations to first-principles MD simulations, in which the electronic problem is solved at the level of density functional theory (DFT). The PMF was determined based on a total number of 91 (large box) or 72 (small box) distances ranging from 0.2 to 1.2 nm (large system) or 0.2 to 0.72 nm (small system). Intervals of 0.005 nm were used between 0.2 and 0.5 nm, while intervals of 0.02 nm were used for distances between 0.5 nm and 1.0 nm and intervals of 0.04 nm for distances larger than 1.0 nm. All simulations were conducted in the NVT ensemble at a temperature of 300 K with the temperature being controlled *via* stochastic velocity rescaling⁴⁹ with a coupling time of 0.1 ps. The equations of motion were integrated with the leap-frog algorithm with a time step of 2 fs. The center of mass translation of the simulation box was removed every 20

fs and the neighbour list was updated every 10 fs. In all simulations, the PME method⁵⁰ was used to treat long-range electrostatic interactions with a grid spacing of 0.116 nm and an interpolation of 4. Owing to the small size of the smaller systems, a real space cut-off of 0.72 nm was used and the Lennard-Jones interactions were truncated at the same distance. In the larger system, the settings for the calculation of the non-bonded interactions differed for the two FF. For the OPLS-AA FF, a switch function was used to switch the Lennard-Jones interactions to zero between 1.05 and 1.1 nm while the real space cut-off for electrostatic interactions was set to 1.4 nm. On the other hand, the Lennard-Jones interactions of the GROMOS FF were truncated at 1.4 nm and real space Coulomb interactions were cut off at 1.0 nm. No long-range dispersion correction was applied to simulations using the GROMOS FF in the large simulation box, while the respective corrections for pressure and energy were applied in all other simulations. Each set-up was equilibrated for 2 ns with additional 8 ns of simulation afterwards for data analysis.

Calculation of the association constant

In order to compare the simulation results to experimental data, the association constant of calcium acetate is computed from the calculated PMF. The association constant of a 1:1 salt is generally defined as the ratio of the activity a_{AC} of the ion complex and the activities a_A and a_C of the free anions and cations in solution:

$$K_a = \frac{a_{AC}}{a_A \cdot a_C} \quad (4)$$

The ion complex AC comprises all sorts of different ion pairs that might occur for the respective ion pair: contact ion pairs (CIP), solvent-shared ion pairs (SIP) and possibly (depending on the ionic species) solvent-separated ion pairs (2SIP). Therefore, this equation holds regardless whether a simple one-step ion pairing model or the so-called “multi-step

Eigen mechanism” of ion-pairing^{1,51} is considered. 4 holds only for dilute conditions where polyionic clusters can be neglected.

According to Chialvo et al.⁵² the association constant (4) can be computed from molecular simulations by integrating over the radial distribution function $g(r)$ of the two ionic species. A prerequisite for the validity of this approach is a low ion concentration as the $g(r)$ must not be affected by multi-body effects and the importance of like-ion pairs must be negligible. In the limit of infinite dilution (the salt concentration ρ_0 goes to zero), the association constant can then be expressed as:⁵²

$$\lim_{\rho_0 \rightarrow 0} K_a = 4\pi \int_0^{R_{\text{cut}}} g_{\text{AC}}^{\text{id}}(r) r^2 dr \quad (5)$$

The radial distribution function of the two ions at infinite dilution $g_{\text{AC}}^{\text{id}}(r)$ can be easily obtained through the PMF that was determined in the NVT ensemble:

$$g^{\text{id}}(r) = \exp\left(-\frac{V^{\text{PMF}}(r)}{k_{\text{B}}T}\right) \quad (6)$$

The restriction to the infinite dilute regime is no hindrance for our analysis as most experimentalists also report the extrapolation of their results to infinite dilution in order to ease comparison.^{53–55} An important quantity in 5 is the upper integration limit R_{cut} , which defines the border between the two states of *paired* (comprising CIPs and SIPs) and *unpaired* ions. The choice of the value of R_{cut} is to a certain extent arbitrary as it marks the distance from which the deviations of $g^{\text{id}}(r)$ from one are negligible. The results obtained for K_a *via* 5 critically depend on the choice of R_{cut} . Therefore, it should be assured that the chosen value fits to the sensitivity, by which the experimental method is able to detect the different types of ion pairs. For most FF considered here, $g^{\text{id}}(r)$ of calcium acetate obtained *via* 6 shows only small deviations from one for ion–ion distances larger than those of SIPs (data not shown). Therefore, the value of R_{cut} was set to the respective (FF-dependent) distance of the minimum which separates the SIP and 2SIP maxima of $g^{\text{id}}(r)$. This choice also considers

that the potentiometric method, used to collect the reference experimental data, identifies both CIP and SIP complexes as associated species.⁵⁶

DFT calculations

First-principles DFT-based Born-Oppenheimer molecular dynamics (BOMD) simulations were performed to calculate the binding free energy of calcium and acetate in solution. We used the generalized gradient approximation exchange-correlation functional by Perdew-Burke-Ernzerhof (PBE)⁵⁷ including Grimme (D3) corrections for dispersion.⁵⁸ The pseudopotential method was adopted so that only valence electrons have been treated explicitly. Pseudopotentials were built according to the scheme by Goedecker, Teter and Hutter (GTH).⁵⁹ The calculations were carried out with the CP2K/Quickstep package,⁶⁰ which employs a mixed basis approach to represent the electronic Kohn-Sham wavefunctions⁶¹ : a double zeta plus polarization function (DZVP) localized basis set was chosen for the real space representation of the valence electrons, while plane waves up to a cutoff energy of 280 Ry were used to expand the density in reciprocal space.

The simulation system consisted of cubic cell of size 1.56 nm containing 123 water molecules and one ion pair. The BOMD equations of motion were integrated using a time step of 0.5 fs and the canonical NVT ensemble was simulated by keeping the temperature at 300 K using a Nose-Hoover thermostat.⁶² The PMF was obtained by thermodynamic integration using the Blue-moon ensemble approach,⁶³ and performing constrained dynamics fixing the distance between the Ca ion and the carbon atom of the carboxylate group of the acetate molecule at 14 distances ranging from 0.286 to 0.565 nm. In the close contact region (0.279-0.325 nm) the distance intervals are separated by 0.007 nm (except 0.01 nm for 0.3 to 0.31 nm and 0.015 nm for 0.31 to 0.325 nm). Then 0.02 nm was used for distances between 0.325 to 0.565 nm. All bonds were constrained using the SHAKE algorithm.⁶⁴ After 2 ps of equilibration in the NVT ensemble, the constraint reaction force was averaged over 8 to 13

ps trajectories. The errors in the PMF are estimated from block data analysis.

In spite of reported shortcomings in reproducing the charge distribution around solvated ions,^{65,66} DFT with semilocal functionals, such as PBE, is nevertheless expected to provide a correct picture of the structure and thermodynamics of monovalent ion pairs in water.²⁸ First-principle molecular dynamics with semilocal density functionals was also shown to render the hydration free energy of a set of monovalent ions within 4% with respect to experimental values,⁶⁷ and even to provide structural and vibrational properties of more complex anions, such as microhydrated sulfate, in good agreement with experiments.⁶⁸

Finally, we have also computed the maximally localized Wannier functions (MLWF) of a significant subset of frames of both CIP, and SIP trajectories, to get the molecular dipoles of water in the first solvation shell of Ca^{2+} and check how significant polarization effects can be for this system.⁶⁹ We obtained an average water dipole of 3.06 (0.28), 3.32 (0.26) and 3.28 (0.28) D, for bidentate CIP, monodentate CIP and SIP, respectively, to compare to an average dipole moment of 3.12 (0.29) D for bulk water. Our calculations show much weaker deviations of the water dipoles in the solvation shell of Ca^{2+} from the average bulk dipole moment with respect to former studies,²⁹ probably due to the choice of different exchange and correlation functionals. These results suggest that the entity of the polarization effects for the system considered here is small enough to justify the use of fixed-charge models on first approximation.

Results and Discussion

In order to improve classical FF in matters of the description of ion-pairing of calcium acetate, the experimental association constant and some first-principles MD (FPMD) simulations of calcium acetate are consulted as reference data. These reference data allow us to consider both the *local* ion-pair structure (comparison to FPMD simulations) and an *integral* (thermodynamic) quantity (comparison to association constant) during the evaluation of classical

FF. The choice of the association constant as a fitting criterion is of course not unique, and can be replaced by other thermodynamic quantities, which can be obtained integrating the PMF of ion pairing, such as, for example, the osmotic pressure.³⁰ In the following, we will show how they can be combined to assess and improve the FF. A further analysis of structural details of the ion-pairs allows to understand the strengths and weaknesses of the different FF types in more detail.

Association constant and pairing free energy

As described in the previous section, the association constant can be determined from the Potential of Mean Force (PMF) of the ions. The PMFs of calcium acetate, as calculated in the “large” cell with the different FF analyzed here, are shown in 2d. Note that 2d already contains data of FF modifications (dashed lines) that will be discussed only in the last part of the manuscript. The minima of the PMF curves correspond to the different types of ion pairs that are observed for calcium acetate: solvent-shared ion pairs (SIP) at C - Ca distances of around 0.5 nm, monodentate contact ion pairs (monoCIP) at distances of approximately 0.34 - 0.36 nm and bidentate contact ion pairs (biCIP) at distances of 0.28 - 0.3 nm. Snapshots of the ion pair at distances which correspond to the different minima of the PMF are shown in 2. 2d shows that there is a marked difference between the standard FF versions of GROMOS and OPLS-AA regarding the relative weight of the two main minima of the PMF. For the standard OPLS-AA forcefield biCIP is the stable configuration, while using GROMOS CIP is only metastable, in favour of SIP pairing. Experimental evidence on the most stable coordination mode of the acetate ion to calcium is sparse. Kondoh and Oi used a combination of ¹³C NMR spectroscopy and IR spectroscopy to analyze the interaction of alkaline earth metal ions with acetate and found that the acetate ion forms a (presumably monodentate) CIP with a calcium ion.⁷⁰

3 shows the common logarithm of the association constant for the different FF of interest calculated based on 5 and on the respective PMF. The standard GROMOS and OPLS-AA

Figure 2: Potential of Mean Force of calcium acetate and snapshots of the different types of ion pairs which correspond to the different minima in the PMF: (a) bidentate CIP (distance between the carbon atom of the carboxylate group of the acetate anion (“C”) and the calcium cation (“Ca”): 0.28 - 0.3 nm), (b) monodentate CIP (C-Ca distance 0.34 - 0.36 nm), (c) SIP (C-Ca distance approx. 0.5 nm), (d) Potential of Mean Force of calcium acetate as a function of the distance C-Ca. By construction, the PMF converges to zero at a distance of 1.20 nm. Abbreviations: cf. caption of Fig. 2; Raiteri *et al.* - PMF taken from Fig. 3 in Raiteri et al.¹⁰

FF and their modifications that have been published so far, clearly fail to reproduce the attraction between the two ions as observed from experiments⁵⁴ (grey-shaded area). For the standard GROMOS 54A7 FF, the attraction between the calcium cation and the acetate anion is by far too weak. The modifications of this FF, that have been published, go into the correct direction by enhancing the ion-ion attraction (leading to an increased association constant), but do not reach the range of experimental data. On the other hand, the standard OPLS-AA FF strongly overestimates the ions attraction. The modification proposed by Project³⁶ improves this shortcoming, yet the association constant of the modified FF does not reach the range of experimental data either. Of all the FF from the literature that we analyzed so far, the one proposed by Raiteri et al.¹⁰ approximates the experimental association constant best, even though it still underestimates the attraction of the ions. Note that 3 already contains data of FF modifications (chequered bars) that will be discussed only in the last part of the manuscript.

Figure 3: Association constant of calcium acetate. The grey-shaded area indicates the range of experimental data according to Daniele et al..⁵⁴ Abbreviations: cf. caption of table 2; Raiteri *et al.* - common logarithm of the association constant calculated from the PMF of calcium acetate as presented in Fig. 3 of Raiteri et al..¹⁰

The analysis of the association constant provides valuable insight into the way in which the interactions between ions are modelled and provides insight on how strongly a FF needs to be modified in order to reproduce the experimental attraction between the two ions. However, the association constant is an integral quantity which measures the total attraction between two species without being able to resolve any information about local details of the interactions. Therefore, even the agreement of calculated association constant with experiments does not guarantee a correct description of the structural details of ion pairing (correct relative probabilities of the different types of ion pairs). As we will show in the following, different FF may exhibit different local structural behaviour while showing very similar association constants.

The difference between the depths of CIP and SIP minima of the PMF is very important (e.g. for nucleation or adsorption processes) as it gives an estimate of the free energy that is necessary to separate a contact ion pair. The OPLS-AA and GROMOS FF versions differ especially in this respect. The great depth of the biCIP minimum of the different OPLS-AA FF (Fig. 2d) causes the strong ion-pair formation which drastically exceeds experimental values (Fig. 3). The PMFs obtained from the different GROMOS FF versions illustrate, how the probabilities of contact ion pairs and solvent-mediated ion pairs are altered depending on the non-bonded interaction parameters that are changed. If only the heteroatomic Ca-OM Lennard-Jones interaction parameters are changed (54A7 modified according to Project et al.: attraction is increased, repulsion is decreased¹¹) compared to the standard 54A7 version, the CIP minima of the PMF become much deeper, while the SIP minimum remains unaltered. On the other hand, if only partial charges are changed (54A8) compared to the standard 54A7 version, not only the CIP minima of the PMF become deeper, but also the SIP minimum is affected, since non-contact ion-ion interactions (mediated by water) are dominated by electrostatic interactions.

It is interesting to see that different FF, which yield similar values for the association constant - as for example the GROMOS 54A8 FF and the one according to Raiteri et al. -

possess different characteristics regarding the balance between CIPs and SIPs. This clearly indicates that further data need to be used to improve forcefields such that they provide the correct molecular structure and balance between CIPs and SIPs. Details on atomic scale structure and dynamics can be indirectly obtained by spectroscopy, however data regarding the relative probabilities of CIPs and SIPs in calcium acetate solutions are not conclusive.⁷⁰ One possibility to study the free energy difference between CIP and SIP states in more detail is to perform first-principles MD (FPMD) simulations based on DFT to calculate the free energy profile of ion-pairing. It is necessary to perform both types of calculations that are to be compared (classical MD simulations and DFT-based calculations) in a system of similar size in order to take size effects into account and thus to ensure comparability. For this reason, we performed the same classical PMF calculations that are shown in 2d in a smaller system. The system contained only 122 water molecules and one ion pair. The classical simulations show that going from the large system that delivers reliable results, to the smaller system, which is more prone to finite size errors, the main characteristics of the PMF curves are preserved. 4 shows for one of the GROMOS and one of the OPLS-AA FF, that the PMF curves of the small and the large system differ by no more than 2 kJ/mol at any ion-ion distance. Given the rather large uncertainties in the reference data (“DFT”-curve in 5) and the overall large free energies of the ion-pairing of calcium acetate, this error can be neglected. Therefore, it is not necessary to correct the outcome of the simulations of the small simulation boxes for their limited size.

5 shows a comparison of the classical PMF calculations (small system) to the results of the DFT-based calculations. In order to ease the comparison of the free energy difference between CIP and SIP states, the PMF curves have been shifted vertically such that the CIP minimum (biCIP or monoCIP) with the lowest energy of each curve (where the error in the DFT-based reference data is smaller than for longer ion-ion distances) is aligned at 0 kJ/mol. Regarding the balance between population of the CIP and SIP states, all FF variations studied here lie between the two extreme cases of the standard OPLS-AA

Figure 4: Potential of Mean Force of calcium acetate as a function of the distance between the calcium cation and the carbon atom of the carboxylate group of the acetate anion for different system sizes. The curves have been shifted vertically such that the minima of biCIP are aligned at 0 kJ/mol. "Small box": 122 water molecules and one ion pair. "Large box": 1031 water molecules and one ion pair.

Figure 5: Potential of Mean Force of calcium acetate as a function of the distance between the calcium cation and the carbon atom of the carboxylate group of the acetate anion. The curves have been shifted vertically such that their lowest CIP-minimum (either bidentate or monodentate) is aligned at 0 kJ/mol. Abbreviations: cf. caption of table 2; Raiteri *et al.* - PMF taken from Fig. 3 in Raiteri *et al.*;¹⁰ DFT - First-Principles Molecular Dynamics simulations based on DFT.

and the standard GROMOS FF. The standard OPLS-AA FF strongly *underestimates* the probability of SIPs (the free energy of the SIP state is approx. 35 kJ/mol higher than the one of the CIP state) while the standard GROMOS FF strongly *overestimates* the probability of SIPs (the free energy of the SIP state is approx. 8 kJ/mol lower than the one of the CIP state). All the proposed modifications of the GROMOS and the OPLS-AA FF improve the balance of CIP and SIP minima with respect to the original FF versions. But only two of the FF from the literature do not only show the correct trend regarding the relative probabilities of CIPs and SIPs, but also agree quantitatively with the DFT-based results (within the error bars of the SIP minimum). The first one is the modification of GROMOS 54A7 as it was proposed by Project et al., which differs from the original version only in an increased value of $\sigma_{\text{Ca-OM}}$.¹¹ The other FF is the one proposed by Raiteri et al. which is parametrised specifically for mineralisation from solution.¹⁰ Note that 5 already contains data of FF modifications (dashed lines) that will be discussed only in the last part of the manuscript.

Comparing the PMF curves of the classical FF to the PMF of the DFT-based simulations draws our attention to another detail of the PMF: the relative probabilities of bidentate and monodentate CIPs. According to the PMF of the DFT-based simulations, the minima of biCIP and monoCIP are comparable in depth (considering the error bars) with a slight tendency towards the monoCIP state. However, considering the limitations in sampling in these FPMD simulations, it is likely that the uncertainty in the PMF is larger than it appears from the error bars in 5. From our simulations, no clear tendency towards either of the two different coordination modes (biCIP and monoCIP) can be inferred. Of the classical FF, only the one suggested by Raiteri et al. shows a similar trend towards the monodentate structure while all other classical FF predict that the bidentate state is more favourable.

Structural analysis

The different GROMOS and OPLS-AA FF underestimate the probability of monoCIPs (with respect to biCIPs) in contrast to the FF by Raiteri et al. and to FPMD. Therefore, it is worth analysing the differences between the classical FF based on the Lennard-Jones interaction potential (GROMOS and OPLS-AA) and based on the Buckingham potential (FF of Raiteri et al.) more closely. The two FF from the literature, where the free energy difference between CIPs and SIPs best matches the DFT results (FF according to Raiteri et al. and the modification of the GROMOS 54A7 FF proposed by Project et al.) are in principle very similar. One difference is in the water models applied in conjunction with the two FF: while the SPC/E water model³⁸ was used for the GROMOS FF, Raiteri et al. utilised the flexible SPC/Fw water model⁴⁰ with their FF. But apart from its flexibility, the SPC/Fw model is quite similar to the SPC/E model. Therefore, it is unlikely that this difference in the water model leads to the observed differences between the two FF. If the water model had such a strong influence on the PMF curves, the OPLS-AA - TIP4P-Ew combination would differ more from the GROMOS - SPC/E results than it actually does. All the other main features of the FF (van der Waals and Coulomb interactions between the ions and between each ion and water) are very similar (cf. Methods section), with two exceptions: (i) The repulsion between oxygen atoms of the acetate anion and of the water molecules is softer for the FF of Raiteri et al. (cf. Supporting Information: figure S2). (ii) Raiteri et al. add a repulsive Buckingham potential to the interaction between hydrogen atoms of water molecules and oxygen atoms of the acetate anion.

Both these differences affect the water structure of the first solvation shell around acetate, which substantially differs for the two FF. 6 shows details of the water structure around the acetate anion for biCIPs. Raiteri’s FF (as it was implemented by us) predicts a structure of the first solvation shell that deviates remarkably from the DFT reference: The OM-OW radial distribution function (Fig. 6a) shows that both oxygen atoms (OM: oxygen atom of the acetate anion; OW: oxygen atom of a water molecule) are able to approach each other

(a) RDF OM-OW

(b) hydrogen-bond angle distribution

Figure 6: Structure of water around the acetate anion for a biCIP configuration. Abbreviations: OM: oxygen atom of the acetate anion; OW: oxygen atom of a water molecule; Raiteri-SPC/E - our implementation of the forcefield by Raiteri et al.¹⁰ combined with the SPC/E water model (cf. Methods section); DFT - First-Principles Molecular Dynamics simulations based on DFT; G54A8 - GROMOS 54A8 forcefield with standard and modified interactions between the calcium ion and the oxygen atoms of the carboxylate group of the acetate anion that correspond to 60 % of the modifications as suggested by Project et al.;¹¹ OPLS-AA - standard OPLS-AA forcefield and modified with an increased $\sigma_{\text{Ca-OM}}$ by 5.8 %.

very closely. A comparison of these results to original data of Raiteri et al. generated with LAMMPS revealed that our implementation of this FF captures these important details correctly (data not shown). This artifact in OM-OW distances influences the hydrogen-bonding angle between acetate and water: The minimum distance between the two oxygen atoms is so small that the hydrogen atom of the hydrogen-bond-donating water molecule is pushed away from the axis between the two oxygen atoms (Fig. 6b: maximum around 45°). This behaviour is not observed as strongly when the two ions are farther apart. In an unpaired situation, both the RDF (OM-OW) and the hydrogen-bond angle distribution of the FF of Raiteri et al. are a lot more similar to the respective DFT-based results (cf. Supporting Information: figure S3). In spite of the success of Raiteri’s FF in describing the single solvated ions and the thermodynamic properties of ion-pairing, it might be worthwhile to consider an alternative, if structural details of ion-pairing are of special interest. From this analysis it appears that modified versions of GROMOS and OPLS-AA provide an accurate structural description of the calcium acetate ion complex in water. A further advantage of

using standard biomolecular FF with suitably modified COO^- -cation interactions is that no further reparametrisation is needed to simulate ion-peptide interactions.

Refinement of GROMOS and OPLS-AA

The modifications of the GROMOS and OPLS-AA FF that were proposed by Project et al.^{11,36} change the short-range interactions between calcium and acetate with the correct tendency. Yet, neither of these two FF reproduces the experimental association constant of calcium acetate (“G 54A7 EP” and “OPLS-AA EP” in 3). This modified version of the OPLS-AA FF furthermore does not reproduce the relative depths of the CIP and SIP minima as predicted by the DFT-based simulations (5). Therefore, it is necessary to optimize the ion-ion interactions even further. As Project et al., we restricted possible modifications of the FF to the van der Waals ion-ion interactions, in order to not corrupt the single ion hydration properties that were optimized before.

The modification of the OPLS-AA FF as proposed by Project consists of an increase in the Lennard-Jones parameter $\sigma_{\text{Ca-OM}}$ by 3.7 % while the other Lennard-Jones parameter $\varepsilon_{\text{Ca-OM}}$ remains unchanged (OM represents the atom type of the carboxylate oxygen of the acetate anion: cf. table 1).³⁶ For the GROMOS FF, Project et al. increased $C_{\text{Ca-OM}}^{(6)}$ by 32.7 % and decreased $C_{\text{Ca-OM}}^{(12)}$ by 22.9 %.¹¹ We further optimized these Lennard-Jones parameters with two major targets in mind: the new parameters should modify the ion-ion interactions such that (i) the association constant matches the experimental results, and (ii) the relative depths of the CIP and SIP minima of the PMF agree with those from the FPMD simulations (within error-bars). For the OPLS-AA FF, these targets are met if $\sigma_{\text{Ca-OM}}$ is increased by 5.8 % with respect to the original FF parameter, while the energy parameter $\varepsilon_{\text{Ca-OM}}$ remains unaltered. In contrast to the work of Project et al., our optimization of the GROMOS FF is based on its latest version (54A8), which comprises an increased partial charge of the OM atom type with respect to its predecessors (table 1). Starting from the standard parameter

values of version 54A8, we increased $C_{\text{Ca-OM}}^{(6)}$ by 19.6 % and decreased $C_{\text{Ca-OM}}^{(12)}$ by 13.7 % in order to meet our two goals. These modifications in $C_{\text{Ca-OM}}^{(6)}$ and $C_{\text{Ca-OM}}^{(12)}$ amount to 60 % of the modifications as proposed by Project et al.. Table 2 contains the new model parameters and 1 shows the resulting optimized ion-ion interactions for both FF (“G 54A8 Ca-OM modified” and “OPLS-AA Ca-OM modified”).

The results in 3 and 5 show that the modified versions of the GROMOS and OPLS-AA FF proposed here both satisfy the two conditions formulated above: The calculated association constants fall into the range of experimental data and the relative depths of the CIP and SIP minima of the PMF curves agree well with the results of the FPMD simulations. From 2d we see that the PMF profiles of these optimized FF differ from those of the respective original FF only at small C-Ca distances (range of contact ion pairs) while the minima of the SIP states remain unchanged. This was to be expected as only the van der Waals interactions were modified and the far-reaching Coulomb interactions - which have the potential to considerably manipulate the minimum of the SIP state - were kept constant.

In spite of the success of these modified FF in modelling important properties of the ion-pairing of calcium acetate, the PMF curves of the two FF possess two features which diverge from the DFT-based reference (5): The free energy of the monoCIP state differs significantly from the one of the biCIP state, which leads to a strong underestimation of the probability of monoCIPs. Furthermore, the free energy barrier between SIPs and CIPs is more pronounced for both FF than for the DFT-based simulations. These two inaccuracies affect the sampling and the dynamics of structural changes of the ion-pairing of calcium acetate. However, a further improvement of the PMF characteristics appears to be not possible, if the FF modifications are limited to the direct ion-ion interactions. This raises the question whether a further optimization of these modified FF is possible, if the search domain is not limited to ion-ion interactions but also includes ion-water interactions. A modification of the ion-water interactions might seem to be imprudent, as these interac-

tions have already been optimized on single ion hydration properties before. However, it is worthwhile to analyze how small changes in ion–water interactions affect the characteristics of the ion–ion PMF in order to assess the effect of potential future FF reparametrisations.

For the FF models, the most important ion–water interactions are those between the calcium ion and the oxygen atoms of water (“Ca-OW”) and those between the oxygen atoms of acetate and water (“OM-OW”). As mentioned above, the only significant differences between the GROMOS FF (based on version 54A7) and the one by Raiteri et al. are the repulsions of the OM-OW and of the OM-HW interactions. Raiteri’s FF performs better with respect to both the depth of the monoCIP minimum and the barrier between CIPs and SIPs. Therefore, it is worth studying whether a softer OM-OW repulsion can be introduced into the Lennard-Jones type FF, leading to an improved depth of the monoCIP minimum and to a decreased barrier between CIPs and SIPs without corrupting the water structure as strongly as Raiteri’s FF does. Furthermore, we studied whether a manipulation of the Ca-OW interactions can lead to similar effects. For this analysis, we have chosen the FF which looks most promising so far: the OPLS-AA FF with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 % (“OPLS-AA Ca-OM modified”). Based on this FF, we have introduced small modifications in the four FF parameters that dominate the (non-electrostatic) ion-water interactions: $\sigma_{\text{Ca-OW}}$, $\varepsilon_{\text{Ca-OW}}$, $\sigma_{\text{OM-OW}}$, and $\varepsilon_{\text{OM-OW}}$.

The Ca-OW radial distribution function (Supporting Information: figure S4) of the optimal OPLS-AA FF shows that this FF nicely reproduces the positions of the RDF-peaks of the DFT-based simulations. From this perspective, no modifications in $\sigma_{\text{Ca-OW}}$ are needed. However, the solvation structure of the calcium ion as it is modelled by the OPLS-AA FF is still far from being optimal. 7 shows that the coordination number of water around the calcium ion is overestimated for ion pair structures (7a) as well as for the unpaired calcium ion (7b). Therefore, it seems worthwhile to modify both $\sigma_{\text{Ca-OW}}$ and $\varepsilon_{\text{Ca-OW}}$ with

the goal to improve the characteristics of the PMF curve and the coordination of water around the calcium ion. figure S5 in the Supporting Information illustrates the scope of the changes in the van der Waals interactions that are introduced by the modifications of $\sigma_{\text{Ca-OW}}$ (decreasing $\sigma_{\text{Ca-OW}}$ by 0.002 nm) and $\varepsilon_{\text{Ca-OW}}$ (decreasing $\varepsilon_{\text{Ca-OW}}$ by 0.132 kJ/mol).

Figure 7: Number of water molecules around the calcium cation as a function of the Ca-OW distance for a biCIP structure and an unpaired ion structure. Abbreviations: cf. caption of 6.

The OM-OW RDF and the respective hydrogen-bond angle distribution (6) of the optimal OPLS-AA FF nicely match FPMD simulations as for the water structure around the acetate anion. The remaining small dissimilarities between the results of classical and FPMD simulations have been observed for the solvation of glutamate before,⁷¹ and can be attributed to the different approaches of the two simulation methods to account for the omitted electronic polarisation. Therefore, the ion–water interactions should only be moderately changed if at all. Figure S2 in the Supporting Information illustrates the scope of the changes in the van der Waals interactions that we introduced into the optimal OPLS-AA FF by the modifications of $\sigma_{\text{OM-OW}}$ (decreasing $\sigma_{\text{OM-OW}}$ by 0.002 nm) and $\varepsilon_{\text{OM-OW}}$ (decreasing $\varepsilon_{\text{OM-OW}}$ by 0.074 kJ/mol).

The modifications of the four ion–water FF parameters $\sigma_{\text{Ca-OW}}$, $\varepsilon_{\text{Ca-OW}}$, $\sigma_{\text{OM-OW}}$, and

$\epsilon_{\text{OM-OW}}$ all have a very similar effect on the PMF of calcium acetate: 8 shows that all of these modifications affect all three minima of the PMF curve. They all improve both the depth of the monoCIP minimum with respect to the biCIP minimum and they improve the barrier between CIP and SIP states. But they also strongly influence the relative depths of the CIP and SIP states (affecting the relative probabilities of CIPs and SIPs) and very likely have a strong effect on the total attraction of the two ions which will become manifest in a significant alteration of the calculated association constant. The optimization of the relative probability of monoCIPs through changes in the ion-water interactions thus has significant side effects on the original goals to meet the experimental association constant and the correct balance between CIPs and SIPs.

A possible alternative approach to improve the details of contact ion pairing and, in general, the accuracy and transferability of forcefields for aqueous solutions would be to explicitly include polarization effects.³⁰ For example a similar approach was successfully exploited to simulate the early stages of calcium carbonate crystallization in solution.^{72,73} However, in the perspective of applying the forcefield refinement scheme devised in this work to complex biopolymer solutions and in the presence of mineral surfaces, the use of polarizable forcefields would not be a practical route, not only due to the higher computational, but also due to the lack of a database of "general purpose" polarizable forcefields for biomolecular systems.

Conclusions

If parametrised with care, classical interaction potentials are in principle capable of describing the peculiarities of ion-pairing in solution. Existing well-established biomolecular forcefields such as GROMOS or OPLS-AA can describe important thermodynamic as well as structural aspects of ion pairing even if complex ions such as charged peptide side chains are involved. However, for the system of calcium acetate dissolved in water, none of the unmod-

(a) modified Ca-OW interactions

(b) modified OM-OW interactions

Figure 8: Influence of modifications in the Ca-OW and OM-OW interaction potentials on the Potential of Mean Force of calcium acetate. The curves have been shifted vertically such that their lowest CIP-minimum (either bidentate or monodentate) is aligned at 0 kJ/mol. Abbreviations: OPLS-AA Ca-OM modified - OPLS-AA forcefield with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 %; OPLS-AA Ca-OM & $\sigma_{\text{Ca-OW}}$ modified - OPLS-AA forcefield with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 % and a decrease in $\sigma_{\text{Ca-OW}}$ by 0.002 nm; OPLS-AA Ca-OM & $\epsilon_{\text{Ca-OW}}$ modified - OPLS-AA forcefield with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 % and a decrease in $\epsilon_{\text{Ca-OW}}$ by 0.132 kJ/mol; OPLS-AA Ca-OM & $\sigma_{\text{OM-OW}}$ modified - OPLS-AA forcefield with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 % and a decrease in $\sigma_{\text{OM-OW}}$ by 0.002 nm; OPLS-AA Ca-OM & $\epsilon_{\text{OM-OW}}$ modified - OPLS-AA forcefield with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 % and a decrease in $\epsilon_{\text{OM-OW}}$ by 0.074 kJ/mol; DFT - First-Principles Molecular Dynamics simulations based on DFT.

ified standard forcefields analyzed here gave a realistic description of thermodynamic *and* structural details of the ion-pair formation. Therefore, small modifications of the forcefield parameters were necessary.

Due to the influence of both the ion–water and the ion–ion interactions on important characteristics of the pairing of ions, it might seem worth *reconsidering* the common practice, to first optimize ion–water interactions based on single ion hydration properties and to adjust the ion–ion interactions to properties of ion-pairs afterwards. However, if one chooses to optimize the ion–ion and ion–water interactions simultaneously, the final set of parameters that results from such a high-dimensional optimization procedure would very likely represent a completely new FF.

Our study shows that such a major effort is actually not necessary. In order to describe the pairing of calcium acetate in solution properly, it is sufficient to modify the van der Waals ion–ion interaction parameters of one of the existing biomolecular forcefields. Thereby, a further modification of the standard ion–water interactions, which were usually optimized on single ion hydration properties before, can be omitted. A prerequisite for the optimization of ion–ion interaction parameters is the availability of thermodynamic as well as structural reference data. For calcium acetate experimental data are sparse. Even though computationally costly, it is sufficient to complement these experimental data with FPMD simulations in order to fine-tune the forcefield, so that it gives a realistic description of the free energy as well as structural details of ion-pairing.

Following this approach, we found that the modified OPLS-AA FF with an increase in $\sigma_{\text{Ca-OM}}$ by 5.8 % describes the ion-pairing of calcium acetate best. The underlying criteria of this choice are the agreement with the experimental association constant (3), and with the most important characteristics of the PMF of the two ions as calculated from FPMD simulations (5: relative probabilities of CIPs and SIPs and height of the free energy barrier between CIPs and SIPs). Due to the uncertainties in the results of the FPMD simulations, a further optimization of the ion–ion Lennard-Jones parameters is currently not possible.

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Supporting Information Available

Supporting information contains a detailed comparison of the structural data obtained with the forcefields discussed in the manuscript. This information is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Marcus, Y.; Hefter, G. Ion Pairing. *Chem. Rev.* **2006**, *106*, 4585–621.
- (2) Collins, K. D. Ions from the Hofmeister Series and Osmolytes: Effects on Proteins in Solution and in the Crystallization Process. *Methods* **2004**, *34*, 300–311.
- (3) De Yoreo, J. J.; Wierzbicki, A.; Dove, P. M. New Insights into Mechanisms of Biomolecular Control on Growth of Inorganic Crystals. *Cryst. Eng. Comm.* **2007**, *9*, 1144–1152.
- (4) Todorova, T.; Hünenberger, P. H.; Hutter, J. Car-Parrinello Molecular Dynamics Simulations of CaCl₂ Aqueous Solutions. *J. Chem. Theo. Comput.* **2008**, *4*, 779–789.
- (5) Aziz, E. F.; Ottosson, N.; Eisebitt, S.; Eberhardt, W.; Jagoda-Cwiklik, B.; Vácha, R.; Jungwirth, P.; Winter, B. Cation-Specific Interactions with Carboxylate in Amino Acid

- and Acetate Aqueous Solutions: X-ray Absorption and Ab Initio Calculations. *J. Phys. Chem. B* **2008**, *112*, 12567–70.
- (6) Harding, J. H.; Duffy, D. M.; Sushko, M. L.; Rodger, P. M.; Quigley, D.; Elliott, J. A. Computational Techniques at the Organic-Inorganic Interface in Biomineralization. *Chem. Rev.* **2008**, *108*, 4823–54.
- (7) Dzubiella, J. Molecular Insights into the Ion-Specific Kinetics of Anionic Peptides. *J. Phys. Chem. B* **2010**, *114*, 7098–103.
- (8) Demichelis, R.; Raiteri, P.; Gale, J. D.; Quigley, D.; Gebauer, D. Stable Prenucleation Mineral Clusters are Liquid-Like Ionic Polymers. *Nature Commun.* **2011**, *2*, 590.
- (9) Anwar, J.; Zahn, D. Uncovering Molecular Processes in Crystal Nucleation and Growth by Using Molecular Simulation. *Angew. Chem.* **2011**, *50*, 1996–2013.
- (10) Raiteri, P.; Demichelis, R.; Gale, J. D.; Kellermeier, M.; Gebauer, D.; Quigley, D.; Wright, L. B.; Walsh, T. R. Exploring the Influence of Organic Species on Pre- and Post-Nucleation Calcium Carbonate. *Farad. Discuss.* **2012**, *159*, 61.
- (11) Project, E.; Nachliel, E.; Gutman, M. Parameterization of Ca^{++} -Protein Interactions for Molecular Dynamics Simulations. *J. Comput. Chem.* **2008**, *29*, 1163–1169.
- (12) Fyta, M.; Netz, R. R. Ionic Force Field Optimization Based on Single-Ion and Ion-Pair Solvation Properties: Going Beyond Standard Mixing Rules. *J. Chem. Phys.* **2012**, *136*, 124103.
- (13) Fennell, C. J.; Bizjak, A.; Vlachy, V.; Dill, K. A. Ion Pairing in Molecular Simulations of Aqueous Alkali Halide Solutions. *J. Phys. Chem. B* **2009**, *113*, 6782–91.
- (14) Hess, B.; van der Vegt, N. F. A. Cation Specific Binding with Protein Surface Charges. *Procs. Natl. Acad. Sci. USA* **2009**, *106*, 13296–300.

- (15) Dudev, T.; Lim, C. Monodentate versus Bidentate Carboxylate Binding in Magnesium and Calcium Proteins: What Are the Basic Principles? . *J. Phys. Chem. B* **2004**, *108*, 4546–4557.
- (16) Hamm, L. M.; Wallace, A. F.; Dove, P. M. Molecular Dynamics of Ion Hydration in the Presence of Small Carboxylated Molecules and Implications for Calcification. *J. Phys. Chem. B* **2010**, *114*, 10488–95.
- (17) Friddle, R. W.; Weaver, M. L.; Qiu, S. R.; Wierzbicki, A.; Casey, W. H.; De Yoreo, J. J. Subnanometer Atomic Force Microscopy of Peptide-Mineral Interactions Links Clustering and Competition to Acceleration and Catastrophe. *Procs. Natl. Acad. Sci. USA* **2010**, *107*, 11–15.
- (18) Fischer, V.; Landfester, K.; Munoz-Espi, R. Stabilization of Calcium Oxalate Metastable Phases by Oligo (L -glutamic acid): Effect of Peptide Chain Length. *Crystal Growth & Design* **2011**, *11*, 1880–1890.
- (19) Rardin, R. L.; Tolman, W. B.; Lippard, S. J. Monodentate Carboxylate Complexes and the Carboxylate Shift: Implications for Polymetalloprotein Structure and Function. *New J. Chem.* **1991**, *15*, 417–430.
- (20) Falke, J. J.; Drake, S. K.; Hazard, A. L.; Peersen, O. B. Molecular Tuning of Ion Binding to Calcium Signaling Proteins. *Quarterly Rev. Biophys.* **1994**, *27*, 219–90.
- (21) Gagné, S. M.; Li, M. X.; Sykes, B. D. Mechanism of Direct Coupling between Binding and Induced Structural Change in Regulatory Calcium Binding Proteins. *Biochem.* **1997**, *36*, 4386–92.
- (22) Ozawa, T.; Fukuda, M.; Nara, M.; Nakamura, A.; Komine, Y.; Kohama, K.; Umezawa, Y. How Can Ca^{2+} Selectively Activate Recoverin in the Presence of Mg^{2+} ? Surface Plasmon Resonance and FT-IR Spectroscopic Studies. *Biochem.* **2000**, *39*, 14495–503.

- (23) Babu, C. S.; Dudev, T.; Casareno, R.; Cowan, J. A.; Lim, C. A Combined Experimental and Theoretical Study of Divalent Metal Ion Selectivity and Function in Proteins: Application to E.-coli Ribonuclease H1. *J. Am. Chem. Soc.* **2003**, *125*, 9318–28.
- (24) 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. Theo. Comput.* **2013**, *9*, 1247–1264.
- (25) Hermans, J.; Berendsen, H. J. C.; Van Gunsteren, W. F.; Postma, J. P. M. A Consistent Empirical Potential for Water-Protein Interactions. *Biopolymers* **1984**, *23*, 1513–1518.
- (26) Kaminski, G.; Duffy, E. M.; Matsui, T.; Jorgensen, W. L. Free Energies of Hydration and Pure Liquid Properties of Hydrocarbons from the OPLS All-Atom Model. *J. Phys. Chem.* **1994**, *98*, 13077–13082.
- (27) Raiteri, P.; Gale, J. D.; Quigley, D.; Rodger, P. M. Derivation of an Accurate Force-Field for Simulating the Growth of Calcium Carbonate from Aqueous Solution: A New Model for the Calcite-Water Interface. *J. Phys. Chem. C* **2010**, *114*, 5997–6010.
- (28) Krekeler, C.; Delle Site, L. Solvation of Positive Ions in Water: The Dominant Role of Water-Water Interaction. *J. Phys.: Condens. Matter* **2007**, *19*, 192101.
- (29) Timko, J.; De Castro, A.; Kuyucak, S. Ab Initio Calculation of the Potential of Mean Force for Dissociation of Aqueous Ca–Cl. *J. Chem. Phys.* **2011**, *134*, 204510.
- (30) Luo, Y.; Jiang, W.; Yu, H.; MacKerell, A. D.; Roux, B. Simulation Study of Ion Pairing in Concentrated Aqueous Salt Solutions with a Polarizable Force Field. *Farad. Discuss.* **2013**, *160*, 135.
- (31) Raiteri, P.; Gale, J. D. Water Is the Key to Nonclassical Nucleation of Amorphous Calcium Carbonate. *J. Am. Chem. Soc.* **2010**, *132*, 17623–17634.

- (32) Reif, M. M.; Hünenberger, P. H.; Oostenbrink, C. New Interaction Parameters for Charged Amino Acid Side Chains in the GROMOS Force Field. *J. Chem. Theo. Comput.* **2012**, *8*, 3705–3723.
- (33) Schmid, N.; Eichenberger, A. P.; Choutko, A.; Riniker, S.; Winger, M.; Mark, A. E.; van Gunsteren, W. F. Definition and Testing of the GROMOS Force-Field Versions 54A7 and 54B7. *Eur. Biophys. J.* **2011**, *40*, 843–56.
- (34) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J. Am. Chem. Soc.* **1996**, *118*, 11225–11236.
- (35) Kaminski, G. A.; Friesner, R. A. Evaluation and Reparametrization of the OPLS-AA Force Field for Proteins via Comparison with Accurate Quantum Chemistry Calculations on Peptides. *J. Phys. Chem. B* **2001**, *105*, 6474–6487.
- (36) Project, E. Elucidating the Ca²⁺ Binding to Calmodulin Family of Proteins by Molecular Dynamics Simulations. Ph.D. thesis, Tel Aviv University, 2010.
- (37) Hess, B.; van der Vegt, N. F. A. Hydration Thermodynamic Properties of Amino Acid Analogues: a Systematic Comparison of Biomolecular Force Fields and Water Models. *J. Phys. Chem. B* **2006**, *110*, 17616–26.
- (38) Berendsen, H.; Grigera, J.; Straatsma, T. The Missing Term in Effective Pair Potentials. *J. Phys. Chem.* **1987**, *91*, 6269–6271.
- (39) Horn, H. W.; Swope, W. C.; Pitner, J. W.; Madura, J. D.; Dick, T. J.; Hura, G. L.; Head-Gordon, T. Development of an Improved Four-Site Water Model for Biomolecular Simulations: TIP4P-Ew. *J. Chem. Phys.* **2004**, *120*, 9665–78.
- (40) Wu, Y.; Tepper, H. L.; Voth, G. A. Flexible Simple Point-Charge Water Model with Improved Liquid-State Properties. *J. Chem. Phys.* **2006**, *124*, 024503.

- (41) Aqvist, J. Ion-Water Interaction Potentials Derived from Free Energy Perturbation Simulations. *J. Phys. Chem.* **1990**, *94*, 8021–8024.
- (42) van Gunsteren, W. F.; Berendsen, H. J. C. *Groningen Molecular Simulation (GROMOS) Library Manual*; 1987.
- (43) Van Der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. GROMACS: Fast, Flexible, and Free. *J. Comput. Chem.* **2005**, *26*, 1701–18.
- (44) Hess, B.; Kutzner, C.; Van Der Spoel, D.; Lindahl, E. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *J. Chem. Theo. Comput.* **2008**, *4*, 435–447.
- (45) Carter, E. A.; Ciccotti, G.; Hynes, J. T.; Kapral, R. Constrained Reaction Coordinate Dynamics for the Simulation of Rare Events. *Chem. Phys. Lett.* **1989**, *156*, 472–477.
- (46) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. LINCS: A Linear Constraint Solver for Molecular Simulations. *J. Comput. Chem.* **1997**, *18*, 1463–1472.
- (47) Sprik, M.; Ciccotti, G. Free Energy from Constrained Molecular Dynamics. *J. Chem. Phys.* **1998**, *109*, 7737.
- (48) Hess, B.; Holm, C.; van der Vegt, N. Osmotic Coefficients of Atomistic NaCl (aq) Force Fields. *J. Chem. Phys.* **2006**, *124*, 164509.
- (49) Bussi, G.; Donadio, D.; Parrinello, M. Canonical Sampling through Velocity Rescaling. *J. Chem. Phys.* **2007**, *126*, 014101.
- (50) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. A Smooth Particle Mesh Ewald Method. *J. Chem. Phys.* **1995**, *103*, 8577.
- (51) Eigen, M.; Tamm, K. Schallabsorption in Elektrolytlösungen als Folge chemischer Relaxation. *Zeitschrift für Elektrochemie* **1962**, *66*, 93–107.

- (52) Chialvo, A. A.; Cummings, P. T.; Cochran, H. D.; Simonson, J. M.; Mesmer, R. E. $\text{Na}^+\text{-Cl}^-$ Ion Pair Association in Supercritical Water. *J. Chem. Phys.* **1995**, *103*, 9379–9387.
- (53) Daniele, P. G.; De Robertis, A.; De Stefano, C.; Sammartano, S.; Rigano, C. On the Possibility of determining the Thermodynamic Parameters for the Formation of Weak Complexes Using a Simple Model for the Dependence on Ionic Strength of Activity Coefficients: Na^+ , K^+ , and Ca^{2+} Complexes of Low Molecular Weight Ligands in Aqueous S. *J. Chem. Soc. Dalton Trans.* **1985**, 2353–2361.
- (54) Daniele, P. G.; Foti, C.; Gianguzza, A.; Prenesti, E.; Sammartano, S. Weak Alkali and Alkaline Earth Metal Complexes of Low Molecular Weight Ligands in Aqueous Solution. *Coord. Chem. Rev.* **2008**, *252*, 1093–1107.
- (55) De Robertis, A.; Di Giacomo, P.; Foti, C. Ion-Selective Electrode Measurements for the Determination of Formation Constants of Alkali and Alkaline Earth Metals with Low-Molecular-Weight Ligands. *Anal. Chim. Acta* **1995**, *300*, 45–51.
- (56) Fournier, P.; Oelkers, E. H.; Gout, R.; Pokrovski, G. Experimental Determination of Aqueous Sodium-Acetate Dissociation Constants at Temperatures from 20 to 240C. *Chem. Geology* **1998**, *151*, 69–84.
- (57) Perdew, J. P.; Burke, K.; Ernzerhof, M. Generalized Gradient Approximation Made Simple. *Phys. Rev. Lett.* **1996**, *77*, 3865.
- (58) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. J. A Consistent and Accurate Ab Initio Parametrization of Density Functional Dispersion Correction (DFT-D) for the 94 Elements H-Pu. *J. Chem. Phys.* **2010**, *132*, 154104.
- (59) Goedecker, S.; Teter, M.; Hutter, J. Separable dual-space Gaussian pseudopotentials. *Phys. Rev. B* **1996**, *54*, 1703.

- (60) The CP2k developers group. <http://cp2k.berlios.de/>.
- (61) Lippert, G.; Hutter, J.; Parrinello, M. A Hybrid Gaussian and Plane Wave Density Functional Scheme. *Mol. Phys.* **1997**, *92*, 477.
- (62) Nosé, S. A Unified Formulation of the Constant Temperature Molecular Dynamics Methods. *J. Chem. Phys.* **1984**, *81*, 511.
- (63) Carter, E. A.; Ciccotti, G.; Hynes, J. T.; Kapral, R. Constrained Reaction Coordinate Dynamics for the Simulation of Rare Events. *Chem. Phys. Lett.* **1989**, *156*, 472–477.
- (64) Ryckaert, J.-P.; Ciccotti, G.; Berendsen, H. J. Numerical Integration of the Cartesian Equations of Motion of a System with Constraints: Molecular Dynamics of n-Alkanes. *J. Comput. Phys.* **1977**, *23*, 327–341.
- (65) Cohen, A. J.; Mori-Sanchez, P.; Yang, W. Insights into Current Limitations of Density Functional Theory. *Science* **2008**, *321*, 792–794.
- (66) Cohen, A. J.; Mori-Sánchez, P.; Yang, W. Challenges for Density Functional Theory. *Chem. Rev.* **2012**, *112*, 289–320.
- (67) Leung, K.; Rempe, S. B.; von Lilienfeld, O. A. Ab Initio Molecular Dynamics Calculations of Ion Hydration Free Energies. *J. Chem. Phys.* **2009**, *130*, 204507.
- (68) Wan, Q.; Spanu, L.; Galli, G. Solvation Properties of Microhydrated Sulfate Anion Clusters: Insights from abInitioCalculations. *J. Phys. Chem. B* **2012**, *116*, 9460–9466.
- (69) Marzari, N.; Vanderbilt, D. Maximally Localized Generalized Wannier Functions for Composite Energy Bands. *Phys. Rev. B* **1997**, *56*, 12847.
- (70) Kondoh, A.; Oi, T. Interaction of Alkaline Earth Metal Ions with Acetic and Lactic Acid in Aqueous Solutions Studied by ¹³C NMR Spectroscopy. *Zeitschrift für Naturforschung A, A J. Phys. Sciences* **1997**, *52a*, 351–357.

- (71) Leenders, E. J. M.; Bolhuis, P. G.; Meijer, E. J. Microscopic Picture of the Aqueous Solvation of Glutamic Acid. *J. Chem. Theo. Comput.* **2008**, *4*, 898–907.
- (72) Bruneval, F.; Donadio, D.; Parrinello, M. Molecular Dynamics Study of the Solvation of Calcium Carbonate in Water. *J. Phys. Chem. B* **2007**, *111*, 12219–12227.
- (73) Tribello, G.; Bruneval, F.; Liew, C.; Parrinello, M. A Molecular Dynamics Study of the Early Stages of Calcium Carbonate Growth. *J. Phys. Chem. B* **2009**, *113*, 11680–11687.
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