Modelling the interaction between silver(I) ion and proteins with the 12-6 Lennard-Jones potential: a bottom-up parameterization approach

Luca Manciocchi,[a] Alexandre Bianchi,[b] Valérie Mazan,[c] Mark Potapov,[a] Katharina Fromm,[b] and Martin Spichty,[a]*

[a] Laboratoire d'Innovation Moléculaire et Applications (LIMA), University of Strasbourg|University of Haute-Alsace|CNRS (UMR 7042) – IRJBD, 3 bis rue Alfred Werner, 68057 Mulhouse Cedex, France.
[b] Department of Chemistry, University of Fribourg, Chemin du Musée 9, 1700 Fribourg, Switzerland.
[c] Laboratoire d'Innovation Moléculaire et Applications (LIMA), University of Strasbourg|University of Haute-Alsace|CNRS (UMR 7042) – ECPM, 25 Rue Becquerel, 67087 Strasbourg, France.
* Correspondence: martin.spichty@uha.fr

Abstract: Silver(I) ions and organometallic complexes thereof are well established antimicrobic agents. They have been used in medical applications for many centuries. It is also known that some bacteria can resist to silver(I) treatments by means of an efflux mechanism. The exact mechanism of action, however, is not fully clear yet. All-atom force-field simulations can provide valuable structural and thermodynamic insights on the molecular processes of the underlying mechanism. Lennard-Jones parameters of silver(I) have been available for quite some time; their applicability to describe properly the binding properties (affinity, binding distance) between silver(I) and peptide-based binding motifs remains, however, an open question. We show here that the standard 12-6 Lennard-Jones parameters (previously developed to describe the hydration free energy with the TIP3P water model) drastically underestimate the strength of interaction between silver(I) and methionine as well as histidine. These are two key residues of the silver(I)-binding motifs in the transport proteins SilE. Using free-energy calculations, we calibrated NBFIX parameters for the CHARMM36m force field to reproduce the experimental binding constant between amino acid sidechain fragments and silver(I) ions. We then successfully validated the new parameters on a set of small silver-binding peptides with experimentally known binding constants. In addition, we could monitor how silver(I) ions increase the α-helical content of the LP1 oligopeptide, in agreement with previously reported Circular Dichroism (CD) experiments. Future improvements are outlined. The integration of these new parameters is straightforward for most simulation packages. It sets the stage for the modeling community to study more complex silver(I)-binding processes such as the interaction with silver(I)-binding-transporter proteins.

Keywords: force field, CHARMM, metal ions, peptides, sidechain fragments.

1. Introduction

The antimicrobial properties of silver(I) ions are well known and applied in various medical solutions.[1–3] However, some bacteria are known to resist to silver(I) cytotoxicity through an efficient efflux pump that expels the metal ion out of the cell.[4] The efflux mechanism is based on the interplay between the small periplasmic silver(I)-transporter protein SilF and the tripartite efflux complex SilCBA; the latter functions as a chemiosmotic metal ion/proton antiporter. In addition, the disordered protein SilE acts as molecular sponge[5] to absorb silver(I) ions in the periplasm; probably to regulate the metal ion concentration.[6] The actual role of SilE is, however, still an open question.

Particularly important for the binding of silver(I) ions to SilE are the amino acids histidine (H) and methionine (M); the motifs HXXM and MXXH are privileged binding motifs for silver(I) ions.[7] The influence of the bridging residues XX on the affinity has been studied in great detail,[8] and some tendencies have been identified. Their impact, however, on the dynamic structure of the peptide and the binding affinity is not fully understood yet. An interesting observation is also that silver(I) binding to non-structured proteins and peptides induces the formation of structured, alpha-helical motifs. The actual molecular mechanism remains unclear.[4,7,9]

All-atom molecular dynamics simulations on the nano- to microsecond timescale could provide valuable insights into these open questions. Force-field parameters for silver(I) ions have been developed previously: Won parametrized mono-,di and tri-valent ions including silver(I) to reproduce the hydration free energy (HFE) for Jorgensen's TIP3P water model.[10] To our understanding, these parameters were unfortunately not correctly integrated into the official distribution of the CHARMM force field. The epsilon values lack the proper conversion from kilojoules to kilocalories. This issue is currently checked by the developers of the CHARMM force field (Alex MacKerell; private communication). Merz and coworkers determined alternative 12-6 LJ silver(I) parameters. They aimed to reproduce either the hydration free energy (HFE) or the ion-oxygen distance (IOD) for multiple water models.[11] A 12-6-4 LJ model was also proposed to account for polarizability. The Merz group later proposed specific 12-6-4 LJ parameters for the interaction of silver(I) (among other metal ions) with imidazole and acetate.[12,13] While the 12-6-4 LJ potential is certainly a valuable extension it is not available in most simulation packages by default. We therefore focus only on the standard 12-6 LJ potential in this work.

We first test if the above 12-6 LJ literature parameters can be directly applied to study the interaction of silver(I) ions with a HXXM binding motif described by the CHARMM36m[14] force field. We then present the development of improved 12-6 LJ parameters for the interaction of silver(I) with histidine, methionine and phenylalanine. We thereby employed a bottom up strategy: we first developed parameters for the silver(I) interaction with sidechain fragments for which affinity data was available from literature or that we measured by potentiometric or

spectrophotometric titrations. We then validated the obtained parameters against binding constants data for HXXM and MXXH binding motifs measured from NMR titrations.[7,8] Finally, as an application of the new parameters, we tested them against structural data for the oligopeptide LP1 (also called B1b[9]), in particular the transition from an intrinsically disordered peptide to an alpha-helical structure in the presence of silver(I) ions.

2. Methodology

This section provides a general outline of the chosen methodology. Details of the applied computational procedures are given in the Supplementary Information (ESI).

2.1 Non-bonded interaction potential

In the family of CHARMM force fields the non-bonded interaction between two pairs of atoms is given by the following potential:

$$E_{NB} = E_{elec} + E_{LJ} = \frac{Q}{4\pi\varepsilon_0} + \varepsilon\left[\left(\frac{R^{min}}{r}\right)^{12} - 2\left(\frac{R^{min}}{r}\right)^{6}\right] \quad (1)$$

where r is the distance between the two atoms, Q is the product of their partial charges, ε_0 is the permittivity of vacuum. The parameters ε and R^{min} are in general derived from the Lorentz-Berthelot rule:

$$R^{min} = \begin{cases} R_i^{min}/2 + R_j^{min}/2 & \text{(Lorentz)} \\ & \text{or} \\ R^{NBFIX} & \text{(if specified)} \end{cases} \quad (2)$$

and

$$\varepsilon = \begin{cases} \sqrt{\varepsilon_i \varepsilon_j} & \text{(Berthelot)} \\ & \text{or} \\ \varepsilon^{NBFIX} & \text{(if specified)} \end{cases} \quad (3)$$

where the subscript indicates an atom-type specific parameter of the i-th and j-th atom. Beside this general combination rule, it is also possible with the NBFIX feature of CHARMM to directly specify ε^{NBFIX} and R^{NBFIX} for an interaction between two specific atom types (instead of using the Lorentz-Berthelot rules). It allows to selectively modify the strength and distance for a particular interaction without affecting the other interactions. In what follows we refer to the pair ε^{NBFIX} and R^{NBFIX} as NBFIX parameters.

2.2 Preliminary evaluations

As an initial test, Won's parameter[10] set and Merz' HFE and IOD parameter sets[11] for silver(I) ions were tested in combination with the CHARMM36m force field[14] using the general Lorentz-Berthelot rule. The strong silver-binding tetrapeptide HEFM was chosen as test case because it includes four amino acid side chains that are supposed to bind silver. As shown in the section "Results & Discussion", all three sets drastically underestimated the binding affinity of HEFM and silver(I).

An intuitive strategy to analyze and eventually correct the underestimated affinity is to adopt a bottom-up approach by studying the interaction of each amino acid sidechain with silver(I) separately.[15] We therefore represented each amino acid as a sidechain fragment by removing the backbone and converting the C^{β} and H^{β} atoms into a methyl group (Figure 1); for example, ethylmethylsulfide was chosen as sidechain analogue for methionine. For histidine we converted the C^{α} and H^{α} atoms due to technical reasons (see ESI) and therefore used 4-ethylimidazole as sidechain fragment. By applying the HFE parameter set of Merz,[11] the binding constant was then determined for each fragment with the following procedure:

1) We performed MD based Umbrella Sampling (US) simulations in explicit water to sample along the reaction coordinate of binding, i.e. the distance between silver(I) ion and the coordinated atom;[16]
2) The Potential of Mean Force (PMF) was determined along the binding reaction coordinate through WHAM;[17]
3) From the PMF, the binding constant $\log_{10}(K_{bind,calc})$ was calculated (and, consequently, the binding free energy $\Delta G_{bind,calc}$).[18]
4) We then compared the calculated binding constant with the experimental one from NIST database[19] or from measured values by potentiometric & spectrophotometric titrations (see ESI), and performed a calibration if necessary.

As a side note, our initial test calculations for HEFM were carried out with CHARMM's modified TIP3P (cTIP3P) model in contrast to the parametrizations of Merz or Won that were carried out with Jorgensen's original TIP3P model (sTIP3P). To probe the influence of the two TIP3P solvation models we calculated the PMFs and binding constants of the fragments for both water models.

2.3 Calibration of NBFIX parameters

The main goal of this work is to derive NBFIX parameters for the CHARMM36m force field to better model the silver(I)-methionine and silver(I)-histidine interactions

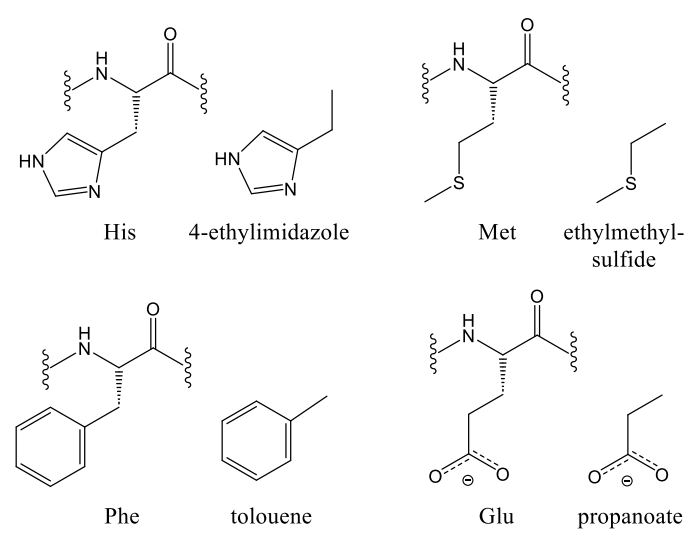

Figure 1. Sidechain fragments to represent histidine, methionine, phenylalanine and glutamic acid.

in particular. The modeling of histidine sidechain is a special case. Indeed, depending on pH, four tautomeric forms are theoretically possible (see Figure 2): one positively charged with both N atoms of the imidazole ring protonated (HSP in CHARMM or HIP in AMBER), two neutral forms with either the proton on the N^{δ} (HSD or HID) or on the N^{ε} (HSE or HIE), and one negatively charged form with no protons on the N atoms (that is, imidazolate-like form). The latter is very rare in biological contexts (see PDB entry 7WAA for an example) as it requires very basic conditions (pK_a =12-14).[20,21] The HSE tautomer was observed by NMR experiments and in many crystal structures of silver(I)-binding small peptides.[9,22] In addition, quantum mechanical calculations showed that 4-methylimidazole (HSE tautomer) features a higher binding affinity than 5-methylimidazole (HSD tautomer).[23] Thus in this work, we only considered only the HSE tautomer (and thus 4-ethylimidazole as sidechain representative molecule), where silver(I) binds to the nitrogen N^{δ}. Note, however that under certain conditions the silver(I) ion can bind to the N^{ε} nitrogen, for example in SilF, where the histidine is buried inside the protein and the HSD tautomer is stabilized through specific interactions.[6] The possibility to include the biologically relevant tautomers through constant-pH computer simulations is discussed in the section "Conclusion & Outlook".

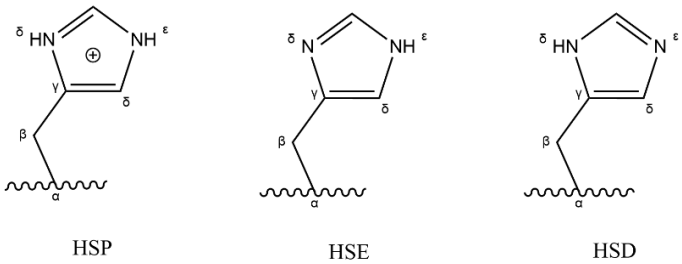

Figure 2. Tautomers of the histidine sidechains as defined in CHARMM force field family: HSP (left, positive), HSE (center, neutral) and HSD (right, neutral).

As a starting point for our optimization we selected the 12-6 LJ HFE parameters of Merz[11] and determined therefrom initial NBFIX parameters for the interaction of silver(I) ion with three specific atom types of the CHARMM36m force field: NR2 (for the N^{δ} atom of histidine), S (for the sulfur atom of methionine) and CA (for the six aromatic atoms of phenylalanine). No NBFIX correction was necessary for the glutamic acid sidechain fragment. Then a calibration procedure was performed to determine the optimal NBFIX parameters that are able to correctly reproduce the experimental binding constant and binding distance of the corresponding side chain fragments. The procedure was the following:

1) Target equilibrium distances ($d_{eq,target}$, Table 2) between silver(I) and coordinated atoms of each fragment were taken either from the literature or determined by DFT calculations; target binding constants ($\log_{10}(K_{bind,exp})$, Table 2) correspond to those of the experiments (see ESI and cited references in Table 2);
2) Using the procedure given in the section 2.2, the PMF and binding constant are calculated;
3) If the minimum of the PMF matches the target distance, we move to the next step; otherwise R^{NBFIX} is adjusted and step 2 is repeated;
4) If the calculated binding free energy is within a given error threshold of 0.5 kcal/mol, the NBFIX parameters are accepted, otherwise ε^{NBFIX} is adjusted and steps 2-3 are repeated.

2.4 Validation on HXXM and MXXH silver-binding motifs and LP1 oligopeptide

The goal of this step was to check the efficacy of the NBFIX parameters when transferred to a larger system. The validation involved calculating two different properties:

- The binding constant of four silver-binding tetrapeptides (HEFM, MNEH, HAAM and MAAH);
- The α-helical content in a longer peptide, LP1 (sequence: AHQKMVESHQRMMG), which was observed to form an α-helical structure upon addition of silver(I) ions.[9]

In both cases, potential-scaled molecular dynamics was employed by scaling down ε to speed up the kinetics of the dissociation process (and adjusting R^{NBFIX} so as to have the same equilibrium distance). Hamiltonian reweighting was then used to retrieve the unbiased binding constant (ESI).

To evaluate the ability of silver(I) to favor the formation of α-helix in LP1, two simulations were performed in parallel. In one the LP1 peptide was simulated alone in solution, whereas in the other it was simulated in the presence of one silver(I) ion. After that, the α-helical content was monitored (see ESI) and compared for the two cases.

3. Results & Discussion

3.1 Testing of existing silver(I) parameters on HEFM tetrapeptide

The silver(I) binding constants obtained for the HEFM tetrapeptide prior the calibration procedure are reported in Table 1. The values are given for different cases, combining box size (30 and 50 Å, to verify potential size effects) and parameters sets (Won, HFE and IOD). In all cases we can notice that the binding constants are lower or equal to 10 ($\log_{10}(K_{bind,calc}) \leq 1$), indicating a drastic underestimation of the interaction strength between silver(I) ion and the HEFM

Table 1. Results for the initial scaled-potential MD simulations of the HEFM tetrapeptide. Three blocks of simulations of 100 ns each were performed for two box sizes and for each parameter set. Values are given with standard error of the mean from block analysis.

Box size (Å)	Number of peptides	Number of Ag^+ ions	Parameter set	Affinity $\log_{10}(K_{bind,calc})$
30	1	1	Won[a]	0.8 ± 0.1
			HFE[b]	1.0 ± 0.1
			IOD[c]	0.5 ±0.1
50	1	1	HFE[b]	0.8 ± 0.2
			IOD[c]	0.6 ± 0.1

[a] Obtained from interpolation of hydration free energy maps.[10]
[b] Optimized to reproduce the hydration free energy.[11]
[c] Optimized to reproduce the metal ion-water oxygen distance.[11]

peptide (experiment: $\log_{10}(K_{bind,exp})$=6.6).[7] This is not really surprising because those parameters were developed to model silver(I)-water interaction only, without any intended correction for interactions with organic solutes.

3.2 Testing of existing parameters on sidechain fragments and calibration of NBFIX parameters

When the HFE parameters of Merz[11] were tested on the interaction of silver(I) with the sidechain fragments (Table 2, column "HFE") we note that the interaction strength is underestimated for all sidechain fragments except for propanoate. For the latter the calculated binding constants were reasonably close to the experimental value (0.89 and 1.25 versus 0.73, respectively for cTIP3P and sTIP3P); the same holds for the equilibrium distance (see also ESI for the PMF profile).

The NBFIX parameters optimized to reproduce the experimental binding constant of the sidechains fragments are reported in Table 2, together with the calculated and experimental binding constants. The fragments 4-ethylimidazole and ethylmethylsulfide possess the highest experimental binding constants of 3.85 and 3.6, respectively (in logarithmic scale). This can explain the high binding constants of the peptides containing such residues ($\log_{10}(K_{bind,exp})$=5-6.7). In the CHARMM36m force field the sulfur of methionine is basically charge neutral (-0.09 *e*) which means that there is no electrostatic stabilization for the interaction with silver(I). Thus, a very strong NBFIX correction (high ε^{NBFIX} in absolute terms) needs to be applied on the sulfur atom of ethylmethylsulfide in order to correct its affinity. This result can be better appreciated observing Figure 3, which contains the PMF profiles of three sidechain fragments before and after the calibration (in black and red lines, respectively). As a general observation, the HFE parameter set yields less attractive PMFs. In particular, in the case of ethylmethylsulfide and toluene, the interaction passes from purely repulsive to attractive after the calibration procedure, which explains why the binding constant of the HEFM peptide was drastically underestimated during the preliminary evaluations.

Table 3. Calculated binding constants for the studied tetrapeptides (HEFM, MNEH, HAAM and MAAH) with the optimized NBFIX paramters. For each peptide eight scaled-potential MD simulations of about 800 ns each were performed (see ESI for more details). The results are shown for both cTIP3P and sTIP3P. Calculated association constants are reported together with the standard error of the mean from block analysis

Peptide sequence	Water model	HFE with NBFIX $\log_{10}(K_{bind,calc})$	Experiment $\log_{10}(K_{bind,exp})$
HEFM	cTIP3P	6.0 ± 0.1	6.6 ± 0.1
	sTIP3P	6.0 ± 0.2	
MNEH	cTIP3P	5.8 ± 0.1	5.4 ± 0.1
	sTIP3P	5.8 ± 0.1	
HAAM	cTIP3P	5.9 ± 0.1	5.7 ± 0.1
	sTIP3P	6.1 ± 0.1	
MAAH	cTIP3P	5.9 ± 0.1	5.4 ± 0.1
	sTIP3P	5.9 ± 0.1	

3.3 Validation on four silver-binding tetrapeptides

To validate the fragment-optimized NBFIX parameters, we calculated the binding constants for the silver-binding tetrapeptides HEFM, MNEH, HAAM and MAAH (Table 3).

Table 2. Optimized ε^{NBFIX} (kcal/mol) and R^{NBFIX} (Å) parameters for the interaction between silver (I) ion and specific atom types of the sidechains fragments. Results are reported for the two water models cTIP3P and sTIP3P. Calculated association constants for the corresponding sidechain fragments are reported together with the standard error of the mean from block analysis. Calculated and experimental target equilibrium distances $d_{eq,calc}$ (corresponding to the minimum of the PMF profile) and $d_{eq,target}$ are also reported.

CHARMM atom type (sidechain fragment)	Water model	ε^{NBFIX}	R^{NBFIX}	$d_{eq,calc}$ [a]	$d_{eq,target}$ [b]	HFE parameters $\log_{10}(K_{bind,calc})$	with NBFIX $\log_{10}(K_{bind,calc})$	Experiment $\log_{10}(K_{bind,exp})$
NR2 (4-ethylimidazole)	cTIP3P	-4.7	2.25	2.08	2.05-2.2[c]	-1.50 ± 0.02	3.82 ± 0.02	3.75[f]-3.85[g]
	sTIP3P	-4.4	2.25	2.11		-0.15 ± 0.01	3.86 ± 0.03	
S (ethylmethylsulfide)	cTIP3P	-11.9	2.50	2.55	2.3-2.5[h]	-1.89 ± 0.02	3.70 ± 0.02	3.6[i] -3.7[j]
	sTIP3P	-11.85	2.50	2.54		-0.29 ± 0.01	3.71 ± 0.03	
CA (tolouene)	cTIP3P	-1.77	2.65	2.29[k]	2.3[k,l]	-2.43 ± 0.03	0.43 ± 0.06	0.44[m]
	sTIP3P	-1.715	2.65	2.29[k]		-2.42 ± 0.01	0.43± 0.03	
N/A.[m] (propanoate)	cTIP3P	N/A[n]	N/A[n]	2.05 (2.43)[o]	2.1-2.4[p]	0.89 ± 0.03	0.89 ± 0.03	0.73[m,q]
	sTIP3P	N/A[n]	N/A[n]	2.05 (2.43)[o]		1.25 ± 0.02	1.25 ± 0.02	

[a] From the minimum of the PMF profile.
[b] Target equilibrium distance (see text and references below).
[c] Literature values.[22,24]
[f] Measured for 4(5)-methylimidazole; this work (see ESI).
[g] Measured for 4(5)-ethylimidazole; this work (see ESI).
[h] Literature values.[24,25]
[i] Measured for ethylmethylsulfide; this work (see ESI).
[j] Measured for dimethylsulfide.[26]
[k] Refers to the distance from the center of the aromatic ring.
[l] From DFT calculations (see ESI).
[m] NIST database.[19]
[n] Not applicable because no NBFIX corrections are necessary (i.e. Lorentz-Berthelot rule applies)
[o] The value in parenthesis refers to the distance between the silver(I) ion and the carboxyl C atom for which the PMF was actually calculated (see ESI).
[p] Literature value.[27]
[q] Measured for acetate.

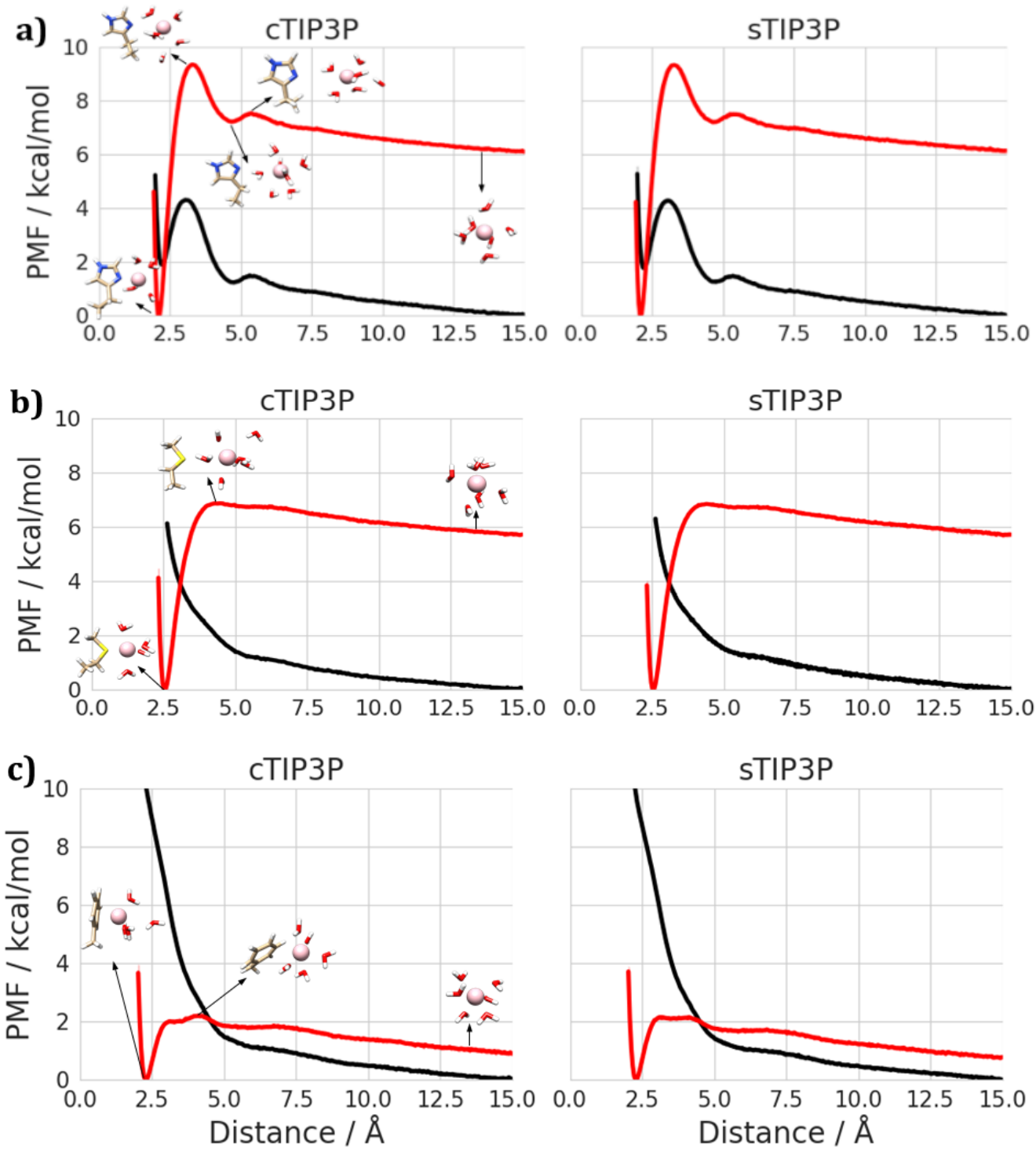

Figure 3. PMF profiles and structures of (a) 4-ethylimidazole, (b) ethylmethylsulfide and (c) toluene with HFE only parameters (black line) and with NBFIX parameters (red line) and in cTIP3P (left) and sTIP3P (right) water models. PMF curves are plotted together with the standard error of the mean obtained from an average over four blocks (lighter colors, barely visible due to the small magnitude). For propanoate, the distance refers to the one from the carboxylic C atom. For toluene, the distance refers to the one from the center of the aromatic ring. Structures are reported for the cTIP3P water model.

Overall, the calculated binding constants are on the same order of magnitude as the experimental values. In terms of Gibb's free energy, they are within 1 kcal/mol of the experimental reference values. Moreover, no significant difference is found when comparing cTIP3P and sTIP3P.

Whereas the experiments measure subtle differences between these peptides in terms of affinity, the calculations predict basically the same binding constant for all peptides. A potential reason for this levelling/flattening is the neglecting of the histidine tautomerism in the calculations above. Here we would like to remind the reader that calculated binding

constants of Table 2 correspond basically to microscopic binding constants for specific peptides where histidine can only exist in its tautomer HSE; HSE is the preferred silver(I)-binding tautomer for small peptides (see above). According to constant-pH simulations of the apo form, the MAAH peptide displays a significantly decreased population of the preferred HSE tautomer (36%) with respect to HAAM (60%). Thus, there is a larger tautomeric penalty to overcome for MAAH than for HAAM; see the companion article for more details on this tautomeric correction.[8] As a result, the calculated binding constant with tautomeric correction, $\log_{10}(K_{bind,corr})$, is 0.22 lower for MAAH (5.47) than for HAAM (5.69) in excellent agreement with the experiment (5.4 ± 0.1 and 5.7 ± 0.1 for MAAH and HAAM, respectively).

3.4 Application to oligopeptide LP1

As a further validation of the parameters, the ability to reproduce structural features such as the α-helical content of the longer silver-binding peptide LP1 was tested. Figure 4 reports the percentage of average α-helical content in the LP1 peptide. Indeed, the presence of the silver(I) ion increases the α-helical content to about 17-18% in the case of the sTIP3P water model (Figure 4b). By contrast, in absence of the ion the α-helical content remains around 5-6%. It should be noted that folding of the peptide into a helix is a slow process and our simulation time of 7 μs could not capture the full dynamics. A part of the problem is that the chosen simulation approach with a scaled potential slows down the folding dynamics since the scaling favors the silver(I) dissociation.

Furthermore, the simulations were carried out only with a single silver(I) ion in the simulation box to simplify the Hamiltonian reweighting scheme. NMR studies indicated, however, that multiple silver(I) ions can bind to LP1. Simulations with more ions are planned.

For the cTIP3P water model no induction of α-helical content can be observed.

4. Conclusion & Outlook

Our new force field parameters for silver(I) ions are capable to reproduce binding constants for tetrapeptides on the same order of magnitude as experimental values. Also, the increased α-helical content of LP1 in the presence of silver(I) was correctly detected with these new parameters. These results validate the applicability of the simple yet accurate LJ model, which can be tightly tuned by means of the NBFIX feature of the CHARMM force field family to selectively correct atom-atom non-bonded interactions, without affecting the rest of the force field. We anticipate that the methodology proposed in this work can be in principle applied to any metal ion provided the existence of reference data for binding constants and distances and the validity of the LJ potential to represent that ion.

We note, however, further improvements for the future simulations of silver(I) interactions with peptides and proteins:

- Histidine tautomerism. The explicit inclusion of the various protonation states of histidine (and in general on all titrable amino acids, especially for cysteine). In this work we assumed that a single static protonation state (HSE) with a microscopic binding constant that coincides with the experimental macroscopic binding constant of 4-ethylimidazole. A more realistic description would allow a dynamic change of histidine protonation states by constant-pH simulations[28–30] where each state binds silver(I) with a specific microscopic binding constant.
- Polarization. This effect is particularly pertinent to deal with the coordination of two or more amino acids to the same silver(I) center as it is observed in more complex systems such as silver-binding oligopeptides (LP1) or

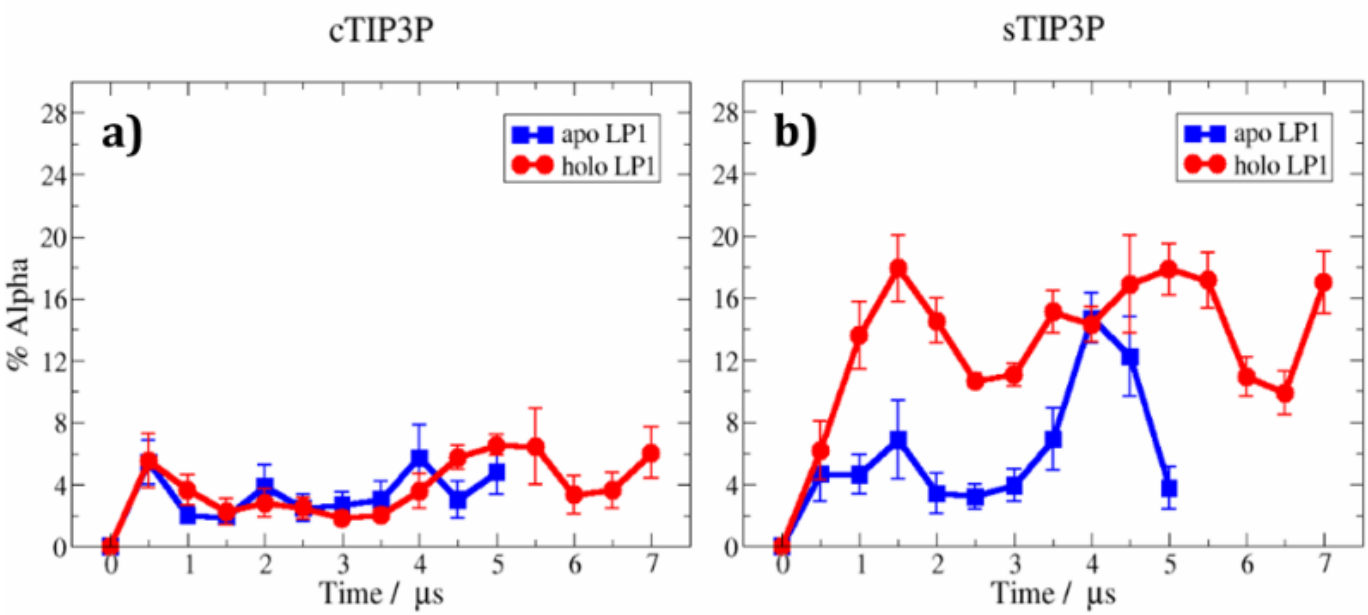

Figure 4. Percentage of α-helical content of LP1 over time (μs) for simulations in cTIP3P (a) and in sTIP3P (b) in the absence (apo, blue) and presence (holo, red) of silver(I).

proteins (SilE). The NBFIX LJ potentials are fully additive and therefore may lead to an overestimation of the affinity. Inclusion of polarization could be done in the context of a polarizable force field such as AMOEBA or CHARMM's Drude force field.[31,32]

- Cationic Dummy Metal Ion Model. To account for particular coordination geometries, the extension to a model with cationic dummy charges could be beneficial.[33]
- Extension of the parametrization to other sidechain fragments and the peptide backbone. The availability of experimental data is the limited factor here. In this light, we would like to emphasize that a careful determination of the reference parameters (equilibrium distance and binding constant) is crucial for the parametrization procedure and can highly influence the sampling.
- Sampling. Finally, improvements may result through the use of enhanced sampling techniques such as Replica Exchange.[34] This could be crucial for an integrative approach that combines the affinity simulation of silver(I) with the constant-pH feature.

Electronic Supporting Information: ESI is available online at https://doi.org/10.1101/2024.11.28.625676. It contains experimental methods and computational details.

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