Optimized Protein–Excipient Interactions in the Martini 3 Force Field

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Abstract

High drug dosages required for biotherapeutics, such as monoclonal antibodies (mAbs), and the small volumes that can be administered to patients via subcutaneous injections pose challenges due to high-concentration formulations. The addition of excipients such as arginine to high-concentration protein formulations can increase solubility and reduce the tendency of protein particle formation. Studying high-concentration mAb systems with molecular dynamics (MD) simulations can provide microscopic insights into the mode of action of excipients but requires large system sizes and long time scales that are currently out of reach at the fully atomistic level. Computationally efficient coarse grained models such as the Martini 3 force field can

tackle this challenge but require careful parametrization, testing, and validation. This study extends the popular Martini 3 force field towards realistic protein-excipient interactions of arginine and glutamate excipients, using the Fab domains of the therapeutic mAbs trastuzumab and omalizumab as model systems. A novel Martini 3 mapping of the amino acid excipients is introduced, which explicitly captures the zwitterionic character of the backbone. The Fab-excipient interactions of arginine and glutamate are characterized at the single-residue level concerning molecular contacts. The Martini 3 simulations are compared with results from all-atom simulations as a reference. Our findings reveal an overestimation of Fab-excipient contacts in the original Martini 3 force field, suggesting a too strong attraction between protein surface residues and excipients. Therefore, we reparametrized the protein-excipient interaction parameters in Martini 3 against all-atom simulations. The excipient interactions obtained with the new Martini 3 mapping and Lennard-Jones (LJ) interaction parameters, coined Martini 3-exc, agree closely with the all-atom reference data. This work presents an improved parameter set for mAb-arginine and mAb-glutamate interactions in the Martini 3 coarse grained force field, enabling large-scale simulations of high-concentration mAb formulations and the stabilizing effect of the excipients.

Introduction

Monoclonal antibodies (mAbs) are successfully used across diverse targets and disease areas, such as asthma, infectious diseases, immunology, and oncology.[1] Subcutaneous injection is the preferred route of administration for many antibody therapeutics for reasons that include self-administration for home medications, ease of use, reduction of hospitalization, and patient convenience. However, the high dosages required (ca. 1 to 10 mg of mAb per kg of body weight) and the small volumes that can be injected subcutaneously or into the muscle imply high-concentration formulations, typically higher than 100 mg/mL.[2] Due to close intermolecular distances between mAbs at high concentrations, intermolecular interactions become non-negligible. Challenges arise due to non-ideal solution behavior at such concentrations, like high viscosity, high opalescence, phase separation, aggregation or gelation, or the increased propensity for protein particle formation, which can negatively affect several steps during manufacturing and likely impair shelf-life of the drug product.[2–6] Moreover, high viscosity adversely impacts the delivery of antibody therapeutics. This is because the injection needles of syringes are typically thin, ranging from 27 to 29 G (diameters of 0.41 mm to 0.33 mm, respectively) for subcutaneous injections, with a range of 30 to 32 G (0.31 to 0.23 mm).[7] This leads to increased tissue back pressure and high injection forces, which can compromise patient comfort.[7–12] In addition, aggregation poses risks to both efficacy and patient safety, including the potential for allergic responses and anaphylaxis.[13]

Current strategies for stabilizing protein drugs primarily rely on time- and resource-intensive trial-and-error methods. These approaches often involve empirically derived heuristics to screen various combinations of buffers and excipients, including salts, amino acids, polyols, antioxidants, and surfactants.[14,15] One of the effective and frequently used amino acid-based excipients to mitigate protein aggregation and reduce viscosity are arginine salts.[16–18] The mechanisms underlying the effect of arginine on solubility and aggregation are challenging to investigate, especially at the microscopic or even molecular level. Molecular Dynamics (MD) simulations provide, in principle, the necessary resolution to obtain the detailed in-

sights desired to complement and interpret experimental results.[19,20] Present MD studies investigating the effect of arginine on mAbs typically focus on the Fab domain.[21–23] Tilegenova et al. found in their MD simulations that aromatic protein residues are main interaction sites with arginine.[21] Golovanov et al. reported suppression of aggregation and improved protein solubility with glutamate as counterions to arginine.[24] In our previous atomistic MD simulation study on excipient interactions of the Fab domains of the therapeutic antibodies trastuzumab (Herceptin®) and omalizumab (Xolair®), we found preferential interactions of arginine with specific residues on the Fab surfaces (in particular charged and aromatic amino acids), and arginine-facilitated recruitment of glutamate towards the Fab surfaces, with predominantly stronger excipient interactions of omalizumab than trastuzumab.[23] These computational findings are in line with experimental long-term storage results[23] and shed light on the intricate intermolecular interactions that govern the macroscopic properties of protein-excipient formulations. Focusing on the Fab domains when studying the impact of excipients on mAbs is partly attributed to the significant influence of the Fab domains on protein-protein interactions, protein particle formation, viscosity, and solubility.[25] All-atom simulations of full-length mAbs in explicit solvent are computationally very expensive and therefore usually limited to single mAbs (monomers)[26–30] or mAb dimers.[31] Previously, we tackled this significant challenge and used multi-microsecond all-atom MD simulations of 4 (identical) mAbs in a simulation box to predict the viscosity of such high-concentration systems.[32] However, ideally simulation systems comprising of more than 4 mAbs and for up to millisecond simulation times would be needed to generate the sampling required for statistically precise viscosity calculations and to enable the computational screening of a large number of systems and different formulation conditions. These requirements are currently out of reach for all-atom simulations.

Coarse-grained (CG) models simplify molecular representations by combining groups of atoms into single beads, thereby saving computation time.[19,33] These approaches range from high-level, where a single CG bead is a complete biopolymer, via single bead per amino

acid residue models to low-level (i.e., detailed) models such as Martini.[34,35] The widely used Martini model assigns fragments of 2-4 non-hydrogen atoms (including all covalently bonded hydrogens) to a single CG-bead. Combined with its computational efficiency, the level of detail and chemical specificity retained in the CG-Martini model is beneficial for studying protein-excipient interactions.[36,37] Especially with the release of Martini 3, which has improved protein-protein interaction parameters compared to its predecessor Martini 2,[38] large-scale multi-protein systems became more accessible.[34,39,40] At the same time, there is still room for improvements. On the one hand, Thomasen et al. observed too compact intrinsically disordered proteins (IDPs) and multidomain protein structures in Martini 3, which could be improved by either increasing the attractive protein-water interactions by 10 % or decreasing the attractive protein-protein interactions by 12 %.[41,42] On the other hand, Pedersen et al. and Lamprakis et al. reported that protein-protein complexes that were found to be stable in all-atom simulations were unstable in Martini 3 simulations, suggesting underestimation of specific protein-protein interactions.[43,44] These findings suggest that the underlying factors for the remaining inaccuracies of the model are more complex than simple global under- or overestimation of protein-protein or protein-water interactions. In Martini, amino acid-based excipients, such as arginine and glutamate, are modeled with the same CG bead types that are used for describing the respective amino acid as part of a polypeptide chain, which we speculate could be a possible source of deviations.

In this work, we investigated the protein-excipient interactions in Martini 3 of arginine (Arg) and glutamate (Glu) excipients with the Fab domains of the mAbs trastuzumab and omalizumab. First, we propose a novel CG mapping in which two charged CG beads are assigned to the amino acid backbone of the excipient molecule (following the building-block principle of Martini 3), thereby explicitly capturing the zwitterionic nature of the individual (free) amino acid excipient molecules. Then we compute the Fab-excipient interactions of arginine and glutamate for different excipient systems, 150 mM Arg/Cl and 150 mM Na/Glu (protein-to-excipient molar ratio of 1:90). We compare the molecular contacts of the excipi-

ents to the trastuzumab Fab domain surface residues with results from all-atom simulations carried out with the Charmm36m-NBF force field that includes the recent refinement of cation–π interactions of Liu et al.[45] The molecular contacts of the excipient molecules with the protein amino acid residues (or residue types) in Martini 3 vs. CHARMM36m-NBF is used to optimize the protein-excipient interaction parameters in the Martini 3 force field. Finally, the improved parameter set is verified with simulations of a different mAb, omalizumab, and of trastuzumab in an equimolar excipient mixture of 75 mM Arg/Cl and 75 mM Na/Glu. Overall, this work presents an improved parameter set for mAb-arginine and mAb-glutamate interactions in Martini 3 and a straightforward method to optimize protein-excipient interactions.

Methods

Setup of Simulation Systems

Atomistic systems

The atomic coordinates of the omalizumab Fab domain were obtained from the protein data bank (PDB: 2XA8).[46] For trastuzumab, the structure of the D185A light chain mutant (PDB: 6BHZ)[47] was used, in which missing residues were added and the alanine in the light chain at position 185 was replaced with aspartate using SWISS-MODEL and Swiss-Pdbviewer.[48] Each Fab domain was placed in a cubic simulation box with ca. 10 nm edge length. Arginine and/or glutamate molecules were introduced at the desired concentrations. To neutralize the net charge of the simulation boxes due to the introduction of the positively charged arginine and negatively charged glutamate excipients, corresponding amounts of chloride and sodium ions were added. Furthermore, trastuzumab Fab was also simulated in an equimolar mixture of 75 mM arginine and 75 mM glutamate (including 75 mM sodium and 75 mM chloride ions to maintain consistent ionic strength across all three systems). The remaining net charge in

each simulation box was neutralized by adapting the number of chloride and sodium ions.

Coarse-grained systems

The coarse-grained models of the Fab domains of omalizumab and trastuzumab were generated with the Martinize 2 script[49] using the atomistic simulation systems described above as input. For the all-atom to coarse-grained conversion, we defined updated mapping instructions for the arginine and glutamate excipients, representing the backbone with two beads instead of one (which is the default in Martini), see Figure 1A. Not only does this approach capture the zwitterionic character of the backbone, but the molecular surfaces also match those from all-atom models much closer than the CG-Martini models of the amino acid residues with single backbone beads (Figure 1B, C). The C_α and ammonium group were assigned to a Martini SQ4p bead type, while the carboxylate group was assigned to an SQ5n bead (Figure 1A), following the recommended Martini 3 mappings for these functional groups.[39]

Molecular Dynamics Simulations

The MD simulations in this work were carried out with GROMACS (version 2021.1).[50] The details of the all-atom simulations are described in our previous work.[23] Briefly, the CHARMM36m-NBF[45,51] force field with improved cation–π interactions[45] was used, which we implemented into GROMACS (force field files in GROMACS format are provided as Supporting Information). For the present study, cation–π interactions are important for contacts of arginine excipients with aromatic amino acid side chains. For water, the CHARMM version of the TIP3P water model was used.[51] After energy minimization, the systems were equilibrated for 2 ns with position restraints on the proteins at a constant temperature of 298 K, implemented with the velocity rescaling thermostat with a stochastic term[52] and constant pressure of 1 bar using the stochastic cell rescaling barostat.[53] Finally, for each simulation system, five independent production MD simulations were performed for 500 ns

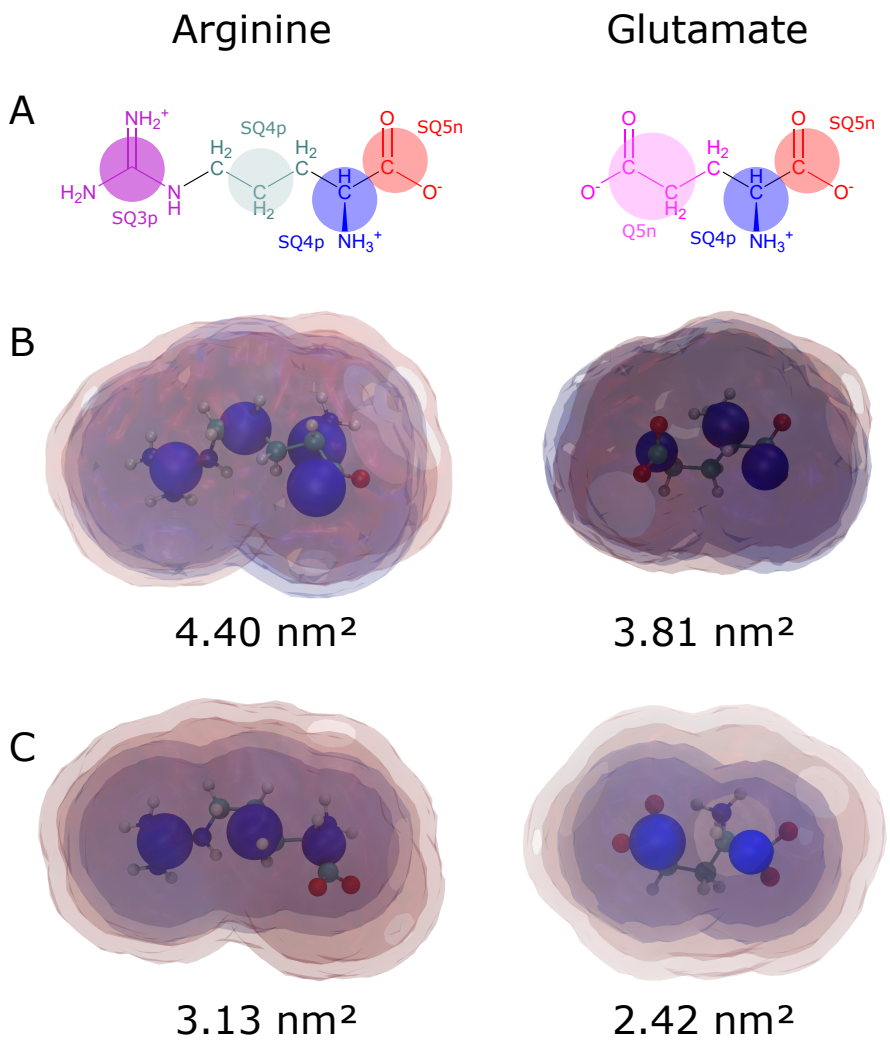

Figure 1: Coarse-grained arginine (left column) and glutamate (right column) models in comparison with the all-atom structures. A) Lewis structures of the excipients and schematics of the mapping of the atoms to the coarse-grained (CG) beads. B) and C) Connolly surfaces (transparent blue surfaces) and Martini 3 CG beads (blue spheres). The red volumetric objects show the Connolly surfaces of the underlying atomistic structures. The coarse-grained models presented in B) are the new CG models with two backbone beads developed in this work, while the models shown in C) are the default Martini 3 representations with a single backbone bead. The numbers below the 3D renderings denote the solvent-accessible surface area (SASA) of the CG structures. The SASAs of the corresponding atomistic arginine and glutamate structures are 4.68 nm^2 and 3.86 nm^2, respectively.

each, initiated with different random seeds for generating the initial velocities.

The corresponding coarse-grained simulations employed the Martini 3 force field[39] with the new excipient models introduced in this work. The same protocol as mentioned before was followed, except for equilibrating for 20 ns and production runs of 1 μs. The recommended "new-rf"[54] simulation settings were used, which included the use of a 1.1 nm nonbonded cutoff with reaction field electrostatics and 20 fs integration time steps. Temperature and pressure were kept constant at 300 K and 1 bar, respectively (with the same thermostat and barostat as in the all-atom simulations).

Parametrization of Martini 3 Arginine and Glutamate Excipient Models

The intramolecular interaction parameters were generated using all-atom simulations (mapped to CG coordinates) as a reference. To that end, three cubic simulation boxes with 8 nm edge length were generated and filled with 1) 150 mM Arg/Cl, 2) 150 mM Na/Glu, and 3) 75 mM Arg/Cl + 75 mM Na/Glu, each solvated with water. MD simulations were performed for 100 ns using the CHARMM36m-NBF force field[45] (at constant temperature of 298 K and pressure of 1 bar). The trajectory was saved to disk with 10 ps time spacing and mapped to the CG representation with the Martinize 2 script.[49] The distributions of distances and angles were used to initially estimate and then adjust the bond and angle parameters of the CG excipient models. Then, Martini 3 simulations analogous to the excipient-only all-atom simulations were performed to first, validate the final bond and angle distributions against the corresponding ones from the all-atom reference simulations, and second to compute the excipient-excipient radial distribution functions (RDFs). The intermolecular Lennard-Jones (LJ) interactions between excipients were scaled in Martini 3 to match the integrals of the RDFs (as a measure of the effective interaction strength) obtained with the CHARMM36m-NBF force field.

Parametrization of Protein-Excipient Interactions in Martini 3

A key analysis performed in this work is based on molecular contacts between excipient molecules and protein surface residues. For each frame of the MD trajectories, whenever at least one atom (or bead, for the CG simulations) of a given excipient molecule was within a cutoff distance of at least one atom (or bead) of a particular protein surface residue, one molecular contact was counted. These contacts were then averaged over all trajectory frames. The cutoff distances used for contact counting were 0.5 nm and 0.75 nm in the atomistic and Martini 3 simulations, respectively. The different cutoff values are required due to the different resolutions of the two models. To verify the direct comparison of contacts obtained with 0.5 nm and 0.75 nm cutoffs used in the analyses of the all-atom and coarse-grained simulations, respectively, the all-atom trajectory was mapped to the Martini 3 representation and the contacts were compared (Figure S1). The Pearson correlation coefficients between the contacts directly computed from the all-atom trajectory (with 0.5 nm cutoff) and the ones from the same trajectory analysed in terms of its CG coordinates (with 0.75 nm cutoff) are $r = 0.98$ for arginine and $r = 0.96$ for glutamate (with slopes of the linear fits of 0.995 and 0.989, respectively), showing that the two cutoff values provide highly similar results.

To adjust the protein-excipient interactions in the Martini 3 force field, we scaled the well depths $\epsilon_{\text{res-exc}}$ of the LJ 12-6 interaction potentials between the protein residues and the excipients. In Martini 3, the pairwise LJ interactions between all bead types are explicitly defined in an interaction matrix. Since the residue-excipient interactions are to be reparametrized for each residue type separately, we expanded the Martini 3 interaction matrix by adding residue type- and excipient type-specific bead types, whose LJ parameters were copied from their respective default values. The new interaction parameters between the amino acid residues and the excipients were then calculated via

$$\epsilon_{i+1,\text{res-exc}} = \epsilon_{i,\text{res-exc}} \cdot \left[1 + \frac{1}{x} \cdot \left(\frac{con_{\text{AA,res-exc}}}{con_{\text{CG,res-exc}}} - 1\right)\right] \tag{1}$$

Here, $\epsilon_{i,\mathrm{res},\mathrm{exc}}$ is the depth of the LJ potential well. The indices "i", "res", and "exc" denote the iteration counter, residue type and excipient, respectively. The number of contacts is indicated by "*con*", where "AA" and "CG" correspond to all-atom and coarse-grained simulations, respectively. The "learning rate" x is introduced to avoid too strong scaling in subsequent iterations, with its value chosen between 10 and 20. A value of $x = 10$ was employed during the early iterations, with adjustments in subsequent iterations depending on the difference of contact counts in the Martini 3 and CHARMM36m-NBF simulations. In the above scaling procedure, we did not differentiate between the different beads within a given protein residue or excipient, that is, the same scaling of LJ interaction strength was applied to all beads of that residue type or excipient. Removing this restriction would enable a more flexible fitting procedure at the expense of increasing the dimensionality of the parameter space. In light of the results obtained with the simpler strategy adopted in this work (see below), we leave this possible extension for future work. We used the protein-excipient contacts from the simulations with trastuzumab as reparametrization target and the simulations with omalizumab for validation.

Results and Discussion

Excipient Parametrization

Here we introduce new CG Martini 3 models of arginine and glutamate excipients with two CG beads describing the backbone of these free amino acids. This approach requires to define a new AA-to-CG mapping scheme and the use of different bead types (Figure 1). It has two main advantages. First, the zwitterionic character of the backbone is explicitly represented by the positively and negatively charged beads for the N- and C-terminus, respectively. Second, as shown in Figure 1, the solvent-accessible surface areas (SASAs) of the excipients of 4.40 nm^2 (arginine) and 3.81 nm^2 (glutamate) match the all-atom SASAs (4.68 nm^2 and 3.86 nm^2, respectively) much closer than the default models with a single backbone bead

(3.13 nm^2 and 2.42 nm^2, respectively). The bonded (intramolecular) force field parameters of the new CG models for arginine and glutamate were generated with atomistic simulations as reference (see Methods). The CG and all-atom distributions are plotted in Figures S2 and S3 and the final intramolecular parameters are listed in Table 1. For the majority of the cases, the bond and angle distributions from the atomistic excipient-only simulations closely agree with the corresponding CG distributions, thus the bonded parameters were only slightly adjusted.

Table 1: Intramolecular interaction parameters for the adapted Martini 3 models of arginine and glutamate. BBO and BBN are the SQ5n and SQ4p beads for the backbone carboxyl and amino-groups, respectively, and SC1 (and SC2, for arginine) refer to the side chain beads (Figure 1A). The parameters r_{eq}, θ_{eq}, k_{bond}, and k_θ are the equilibrium bond length and angle and the force constants of the harmonic bond and angle potentials, respectively.

	Arginine		Glutamate	
		Bonds		
Beads	r_{eq}/nm	$k_{bond}/\frac{kJ}{mol \cdot nm^2}$	r_{eq}/nm	$k_{bond}/\frac{kJ}{mol \cdot nm^2}$
BBO-BBN	0.251	99103	0.252	99103
BBN-SC1	0.293	7647	0.331	991
BBO-SC1	0.377	1810	0.468	2143
SC1-SC2	0.354	5119	–	–
		Angles		
	θ_{eq}/deg	$k_\theta/\frac{kJ}{mol \cdot rad^2}$	θ_{eq}/deg	$k_\theta/\frac{kJ}{mol \cdot rad^2}$
BBO-BBN-SC1	50.722	146.3	42.210	53.690
BBO-SC1-SC2	139.833	33.5	–	–
BBN-SC1-SC2	132.656	15.5	–	–

After having optimized the intramolecular interaction parameters of the excipients, we performed CG-MD simulations with only excipients (no proteins). Figure 2 shows the excipient-excipient RDFs obtained from these simulations. The dashed orange lines show the RDFs with the original Martini 3 parameters (that is, before adjusting the intermolecular interaction strengths), which exhibit reduced attraction compared to the atomistic reference data obtained with the CHARMM36m-NBF force field. The backbones of the excipients consist of SQ4p and SQ5n beads in the CG model, which are among the most polar Mar-

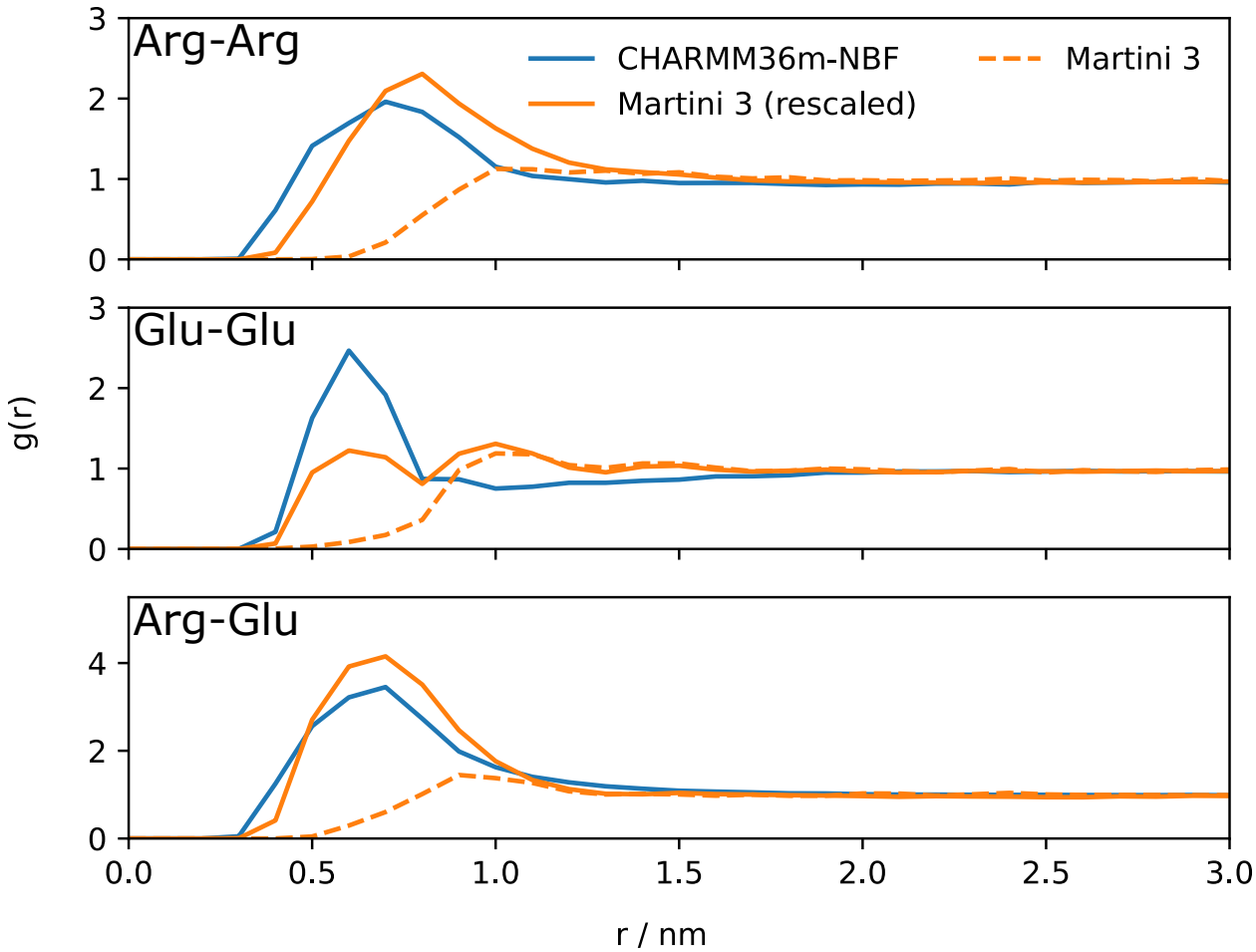

Figure 2: Radial distribution functions of the centers of mass of the excipients. The graphs from top to bottom were obtained from the 150 mM Arg/Cl, 150 mM Na/Glu and 75 mM Arg/Cl + 75 mM Na/Glu simulations, respectively.

tini 3 beads. We decided to specifically adjust the excipient-excipient and protein-excipient interactions. This parametrization was achieved by globally rescaling the Lennard-Jones well depths of all involved molecule-specific bead types, see equation 1. To match the coarse-grained and all-atom results for the excipients, the LJ interaction strengths in the Martini 3 force field between the arginine excipients (arginine-arginine) were increased by 65 %, and those between glutamate excipients (glutamate-glutamate) were increased by 40 %. The area under the curve of the Arg-Arg RDF obtained with this reparametrized model (Figure 2, orange line) agrees with the area under the CHARMM36m RDF, which is plotted in blue in Figure 2.

Notably, in both the original and the reparametrized Martini 3 force fields, the Glu-Glu RDF (Figure 2, middle panel) has two maxima, a direct contact and a solvent-separated one at ca. 0.6 nm and 1.0 nm, respectively. LJ rescaling leads to the maximum at 0.6 nm, which is lower in intensity compared to the CHARMM36m-NBF maximum at this distance. However, after rescaling the areas below the atomistic and coarse-grained RDFs are similar, so we decided not to increase the Glu-Glu interactions further. We speculate that the additional maximum at 1.0 nm might be due to packing effects related to the 3-bead model of glutamate. Notably, after the reparametrization of the Arg-Arg and Glu-Glu interactions described above, Arg-Glu rescaling was not necessary to obtain agreement with the atomistic reference data (bottom panel of Figure 2).

Martini 3 Before Protein-Excipient Reparametrization

After introducing the new zwitterionic backbone CG-Martini 3 models of arginine and glutamate excipients and parametrizing the excipient-excipient interactions in Martini 3, we simulated the trastuzumab Fab domain with two different excipient conditions, 150 mM Arg/Cl and 150 mM Na/Glu. Preferential excipient-residue interactions were analyzed by counting molecular contacts. In the atomistic simulations, a molecular contact was counted if at least one excipient atom was within 0.5 nm of one or more atoms of a protein residue. In the CG simulations, this cutoff value for the bead-bead contacts was increased to 0.75 nm (Figure S1). The results obtained with the new Martini 3 excipient models (but the original, that is, unscaled LJ interactions as defined in Martini 3) are compared to the all-atom simulations with the CHARMM36m-NBF force field in Figure 3.

Figure 3A shows the correlation plot of trastuzumab Fab/arginine molecular contacts in both force fields. With a Pearson correlation coefficient (PCC) of $r = -0.03$, Martini 3 and Charmm36m-NBF do not correlate. On average, the number of molecular contacts observed in Martini 3 is about two to three times higher than in the all-atom simulations, suggesting a systematically too strong attraction between protein surface residues and excipients.

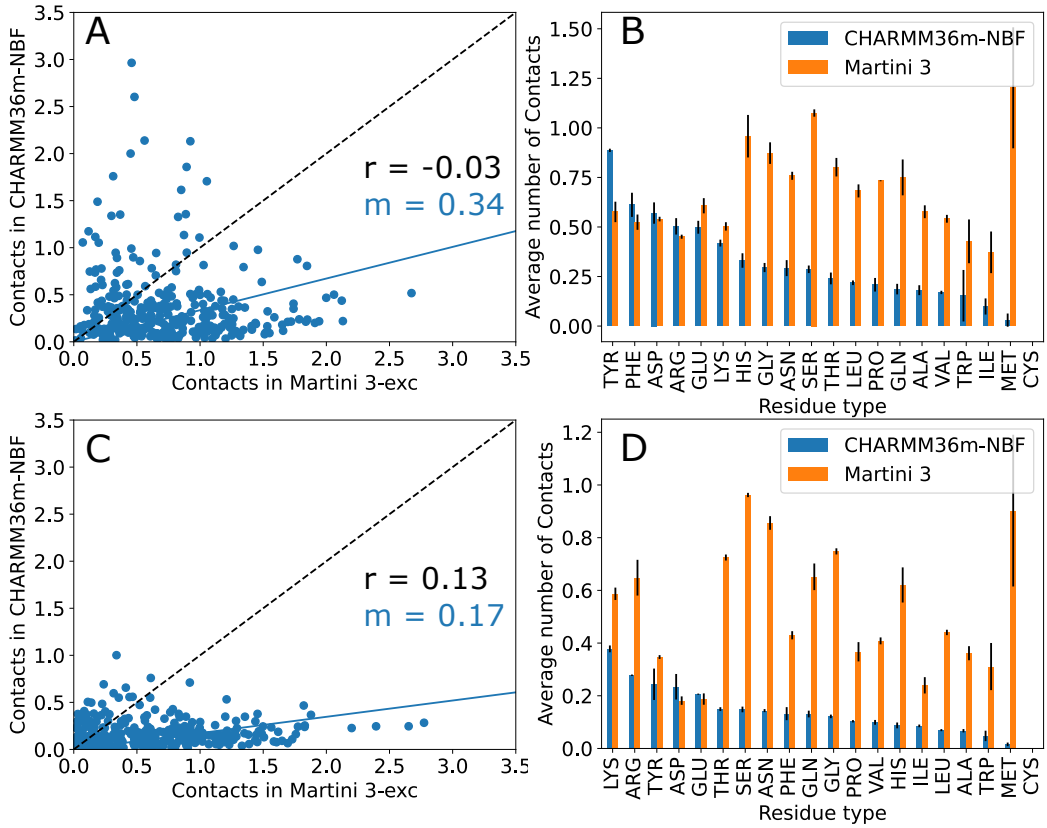

Figure 3: Comparison of residue-excipient contacts for the trastuzumab Fab domain with unscaled Martini 3 and CHARMM36m-NBF. The residue-arginine contacts are shown in A and B, the residue-glutamate contacts in C and D. In the correlation plots (A and C), the residue-excipient contacts with Martini 3 and CHARMM36m-NBF are compared (r denotes the Pearson correlation coefficients, m the slope of the linear fits (blue lines)). The dashed black lines are the diagonals ($y = x$). The average number of excipient-residue contacts with CHARMM36m-NBF and Martini 3 are shown in B and D for the different amino acid residue types. The error bars in panels B and D represent the standard deviations.

Furthermore, the specificity seen in the different high-contact regions differs significantly, especially concerning the distinct pattern of preferential excipient interactions seen in the complementarity-determining regions (CDRs, Figure S4). This is also reflected in the over-estimation of the residue type-averaged contacts with arginine excipients (Figure 3B). The general trend of preferable trastuzumab Fab domain interactions with arginine excipients significantly differs between the all-atom and the coarse-grained force fields. While the average local interactions of arginine excipients with Phe, Asp, Arg, Glu and Lys amino acid residues match between Martini 3 and CHARMM36m-NBF, they are underestimated for Tyr

residues and overestimated for other amino acid residue types. Furthermore, the all-atom simulations revealed on average significantly fewer contacts of glutamate excipients with the trastuzumab Fab domain compared to arginine (compare upper and lower panels in Figure 3). The glutamate contacts in Martini 3 compared to CHARMM36m-NBF are presented in Figure 3C and D, showing a similar overestimation of molecular contacts in the original Martini 3 interaction parameters as seen for arginine excipients. Furthermore, with original interaction strengths, the number of Fab-glutamate contacts is as high as the Fab-arginine contacts, in strong contrast to the all-atom simulations in which a preferential interaction of the Fab domain with arginine excipients (over glutamate) is observed. Therefore, for a more accurate description of specific protein-excipient interactions, we concluded that it is necessary to adjust the protein-excipient interaction parameters in Martini 3.

Reparametrized Martini 3-exc Force Field

The differences in excipient-residue contacts shown in Figures 3B and D serve as a target to adjust the Martini 3 interaction parameters. In Martini 3, the beads used to describe amino acid residues in proteins are assigned to represent the key chemical fragments (or "building blocks") of each residue. Some of these fragments are represented by the same bead type in Martini 3, for example, Phe, Tyr, and Trp have TC3 type beads describing the aromatic ring. To reparametrize the excipient-residue interactions we defined residue-specific and excipient-specific bead types with, at the beginning of the parametrization procedure, the same LJ interaction parameters as in the original Martini 3 force field. Then, the LJ parameters were adjusted iteratively to match the molecular contacts of the CHARMM36m-NBF data (see Methods). The final results (after 15 iterations, equation 1) obtained with the reparametrized Martini 3 force field, coined Martini 3-exc, are presented in Figure 4 and the final scaling factors of the LJ well-depths are listed in Table 2.

Figure 4A depicts the arginine contact correlation plot of CHARMM36m-NBF with the Martini 3-exc simulations. The PCC of the arginine contacts between both models is $r =$

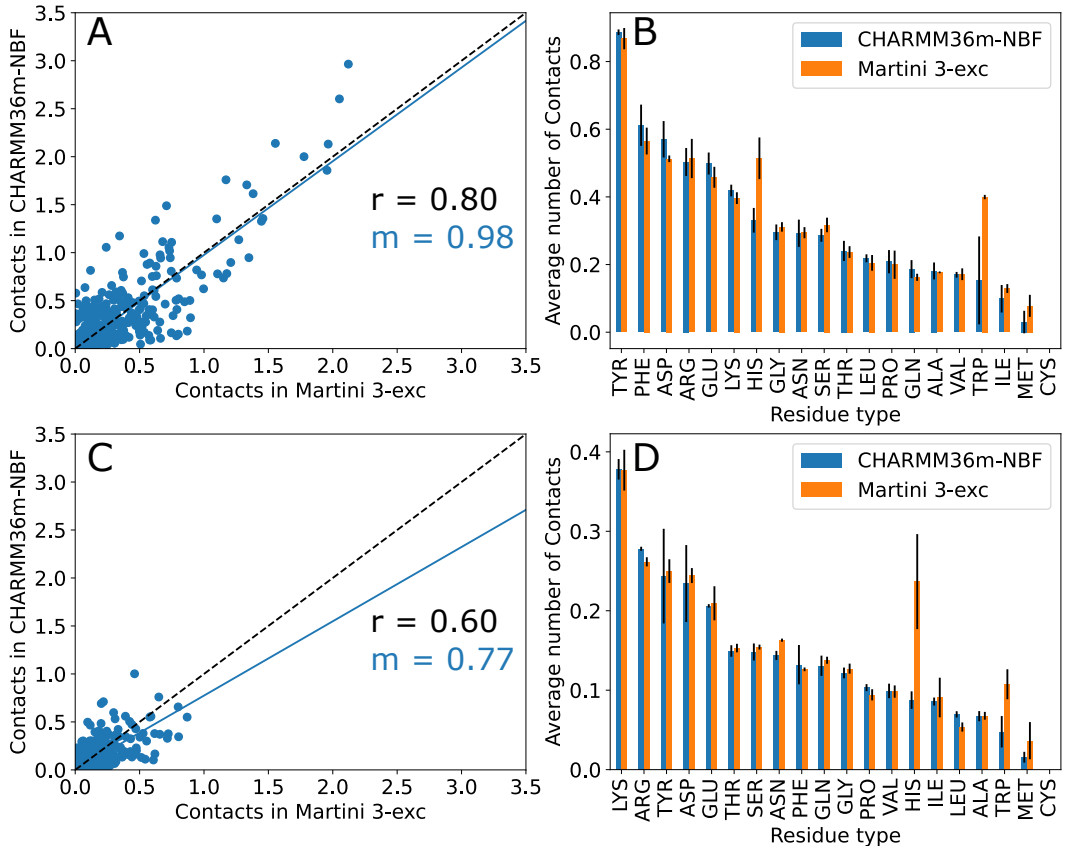

Figure 4: Residue-excipient contacts from the Martini 3-exc simulations of the trastuzumab Fab domain with reparametrized residue-excipient LJ interaction strengths are compared to the CHARMM36m-NBF results. The residue-arginine contacts are shown in A and B, and the residue-glutamate contacts in C and D. In the correlation plots (A and C), the residue-excipient contacts with Martini 3-exc are compared to CHARMM36m-NBF (r denotes the Pearson correlation coefficient, m is the slope of the linear fits (blue lines)). The dashed black lines are the diagonals ($y = x$). The average number of excipient-residue type contacts are shown in B and D with error bars presenting the standard deviations.

0.80, which is significantly higher compared to the original Martini 3 force field (Figure 3). The arginine-residue type contact comparison in Figure 4B also highlights the major improvements achieved with the new interaction parameters. Only the arginine contacts with His and Trp residues still deviate from the all-atom reference values. However, these deviations have to be interpreted with care due to the low number of His and Trp residues at the surface of trastuzumab (4 and 3 residues, respectively), which implies limited statistics.

Similar to arginine, also for glutamate the agreement between the atomistic and coarse-grained simulations is considerably improved (Figure 4C, D), with a PCC of 0.60 compared to

0.13 with the original Martini 3 interactions strengths. The smaller correlation found for the glutamate-residue contacts can be explained by the smaller range of the values. The average number of contacts of trastuzumab residues with arginine is 0.38, with a broader range of values and a significant fraction of data points above 1.5 (Figure 4A), whereas the average number of glutamate contacts is only 0.13. Therefore, small differences between the all-atom and coarse-grained data have a larger effect on the correlation of the glutamate contacts. In Figure 4D the glutamate-residue type contacts are compared between the Martini 3-exc and CHARMM36m-NBF simulations. As discussed above for arginine, the His and Trp contacts in Martini 3-exc are still overestimated, which did not improve with more iterations (equation 1). This is likely related to the low number of His and Trp surface residues in the trastuzumab Fab domain (4 and 3, respectively).

Table 2: Scaling factors of the Lennard-Jones well-depths between protein residues and excipients in Martini 3-exc. Residue types that occur fewer than 5 times at the surface of trastuzumab are marked with an asterisk.

Residue	Arginine Scaling Factor	Glutamate Scaling Factor
Ala	0.85243063	0.78866005
Arg	1.42525544	1.05145992
Asn	0.88332562	0.78235118
Asp	1.17903206	1.43715351
Gln	0.92320811	0.90999592
Glu	1.14044223	1.43277269
Gly	0.82831058	0.80240124
His*	0.65421264	0.52236041
Ile*	0.67424864	1.06553751
Leu	0.91315563	0.88385436
Lys	1.14728312	1.05905944
Met*	0.50578418	0.46673367
Phe	1.32787235	1.24383757
Pro	0.82090591	1.03344047
Ser	0.80059120	0.73218966
Thr	0.82067907	0.89391894
Trp*	0.49368134	0.59695372
Tyr	1.17262296	1.14480006
Val	0.88504739	0.97775697

The majority of arginine-residue type contacts in our reparametrized Martini force field

reached good agreement with the atomistic data after scaling down the arginine-residue type interactions (Table 2), that is, making them less attractive (reduced well-depth of the LJ potential). In contrast, arginine interactions with Arg, Asp, Glu, Lys, Phe, and Tyr were increased to match the CHARMM36m-NBF data. The same trend is observed for the interactions of glutamate excipients. At first glance, the necessity to increase selected excipient-residue interactions seems counterintuitive, because the contacts shown in Figure 3 are overestimated for all residue types (except Tyr and Phe). However, excipient-residue interactions cannot be attributed to individual residues alone, but they are also affected by corecruitment due to excipient interactions with neighboring amino acid residues on a local patch on the protein surface.[23]

The LJ interaction strength reparametrization presented in this work was carried out for the trastuzumab Fab domain, and generalizations about protein-excipient interactions in Martini 3 should be made with caution. However, the results suggest that the effective repulsion between equally charged molecules is overestimated, while the attraction between oppositely charged molecules is underestimated (Table 2).

The residue types in Table 2 marked with asterisks are rare on the trastuzumab Fab surface (less than 5 surface residues of the respective type, none in case of Cys). Therefore, the limited sampling in terms of the local surface patch context of these residues might influence the average number of excipient-residue contacts, implying that the resulting interaction parameters are unlikely to be applicable to significantly different proteins. In particular, the interaction strengths of the excipients with His and Trp were scaled down, but still overestimate the molecular contacts compared to CHARMM36m-NBF (Figure 4B and D).

Notably, the results presented here were generated to optimize the excipient-Fab interactions for trastuzumab. Overall 16 iterations with varying x in Equation 1 were performed, which systematically improved the agreement between the coarse-grained and all-atom force fields step by step. The improvements between consecutive refinement iterations decrease steadily, which means that already after a few (e.g., 4 or 5) iterations markedly improved

results can be obtained. Overall, the new Martini 3-exc force field parameters yield molecular contacts for trastuzumab that closely match the all-atom reference data. We expect that Martini 3-exc should also lead to an improved description of the excipient interactions for other mAbs due to the similarity of antibodies, especially in the consensus constant regions of the same subclass.

Martini 3-exc with Equimolar Excipient Concentrations

The arginine-excipient and glutamate-excipient parameters of Martini 3-exc were optimized with single excipient systems with chloride (for arginine) or sodium (for glutamate) counterions, respectively. To test for transferability, simulations with 75 mM concentrated equimolar mixtures of arginine and glutamate excipients (with 75 mM Na/Cl) were performed and the molecular excipient-residue contacts compared between Martini 3-exc and Charmm36m-NBF.

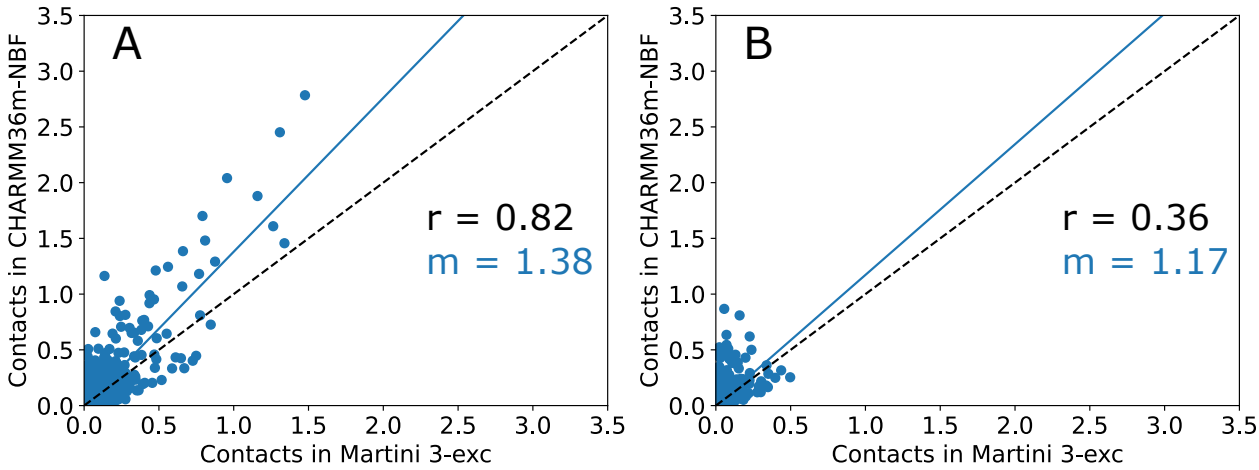

Figure 5: Correlation of protein-excipient contacts between CHARMM36m-NBF and Martini 3-exc simulations of the trastuzumab Fab domain with equimolar concentrations of 75 mM Arg/Cl and Na/Glu (r denotes the Pearson correlation coefficient, m is the slope of the linear fits (blue lines)). The dashed black lines are the diagonals ($y = x$). The arginine-residue and glutamate-residue contacts are depicted in panel A and B, respectively.

Figure 5 presents the respective correlation plots. The molecular contacts of arginine, (Figure 5A) demonstrate a high level of consistency between the two force fields, with a

strong correlation ($r = 0.82$). Consequently, we expect the new arginine-residue interactions in Martini 3 to be robust towards changes in both arginine excipient concentration and (minor) changes in the solvation environment. The molecular contacts of glutamate (Figure 5) have a PCC of $r = 0.36$ and are more strongly affected by the changes of the solvation conditions. Due to the low number of molecular contacts of glutamate to trastuzumab, this result supports the notion that excipients with comparatively weak protein interactions are more influenced by changes in the environment. However, when averaged over all trastuzumab residues, the Martini 3-exc glutamate interactions are reasonably close to those obtained from the all-atom reference simulations with average contact counts of 0.05 and 0.09, respectively.

Crossvalidation of Martini 3-exc for a Different Antibody

To test the transferability of the new Martini 3-exc parameter set to other antibody Fab domains that were not included in the parametrization procedure, we simulated the omalizumab Fab domain with the excipients arginine and glutamate. As described above for trastuzumab, we analyzed the molecular contacts by comparing the results to all-atom simulations carried out with the CHARMM36m-NBF force field. The omalizumab and trastuzumab Fab domains have sequence identities of 82 % and 91 % for the heavy and light chains, respectively. The correlation plots of excipient-residue contacts between CHARMM36m-NBF and Martini 3-exc are presented in Figure 6 for both arginine (Figure 6 A) and glutamate (Figure 6 B). The corresponding correlation plots with the unscaled Martini 3 force field are shown in Figure S5.

There is a high correlation in the number of arginine-residue contacts between reparametrized Martini 3-exc and the CHARMM36m-NBF force field (Pearson correlation coefficient of 0.78). The slope of 0.84 also highlights a close match between overall interaction strengths between both contact data sets. We conclude that the parametrization strategy works well for arginine excipients and that the Martini 3-exc parameters can provide more accurate results also for Fab domains other than trastuzumab. However, for glutamate the correla-

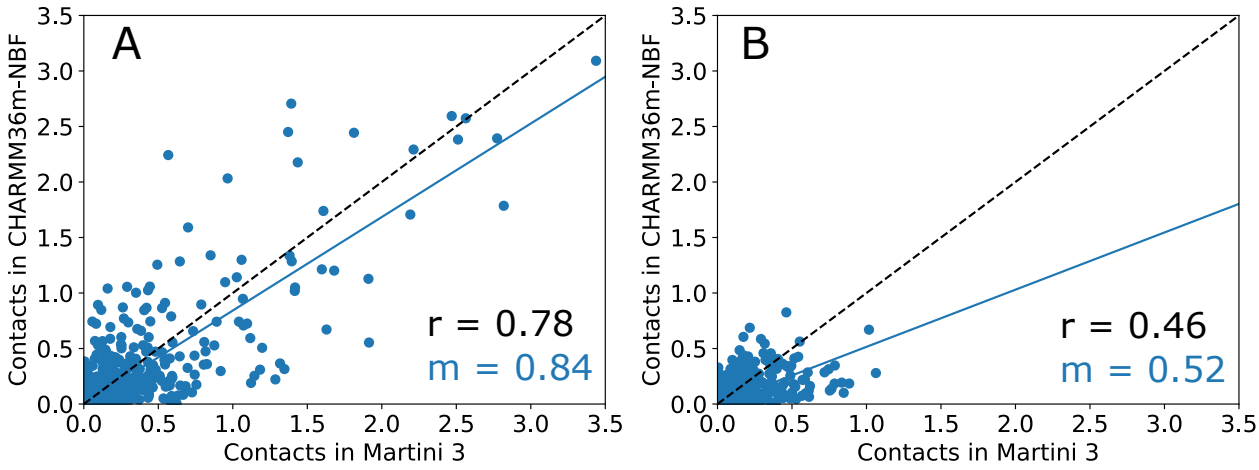

Figure 6: Correlation of protein-excipient contacts between CHARMM36m-NBF and Martini 3-exc simulations of the omalizumab Fab domain (r denotes the Pearson correlation coefficient, m is the slope of the linear fits (blue lines)). The dashed black lines are the diagonals ($y = x$). The arginine-residue and glutamate-residue contacts are depicted in panels A) and B), respectively.

tion of the excipient-residue contacts in omalizumab is lower between the two force fields ($r = 0.46$), which can again partly be attributed to the smaller value range of the glutamate contacts compare to the arginine contacts. Nevertheless, while the specificity of glutamate-residue contacts at the individual residue level is only partially captured by Martini 3-exc, the average number of glutamate contacts (averaged over all omalizumab surface residues) is 0.19, which is in reasonable agreement with the value of 0.12 from the CHARMM36m-NBF simulation (Figure S4). This result suggests that, at the global level, the description of the protein interactions with both excipients, arginine and glutamate, is improved in the Martini 3-exc model.

General Discussion

Taken together, the new Martini 3-exc parameter set for excipient-residue interactions more closely agrees with the reference all-atom simulations. The arginine contacts observed in the validation system (omalizumab Fab domain) match the reference data. Substantial improvements of protein-excipient interactions are expected for Fabs of the IgG1 subclass

in general. For glutamate excipients, the new Martini 3-exc parameters yield improved molecular contacts on a global average level, but contacts of a given specific residue cannot be reliably predicted by the CG force field. However, this result has to be carefully interpreted in light of the overall small number of glutamate-Fab contacts.

In general, we expect the force field parametrization approach presented here to be transferable to protein interactions with excipients or ligands that have attractive interactions with the proteins of interest. Molecules that have less favorable (or even repulsive) interactions with the proteins, such as glutamate, are less robust because they are subject to increased statistical noise and they are more sensitive to perturbations, and thus small changes of the environmental conditions or the parameters can have relatively large consequences. One popular strategy to improve protein-protein interactions in Martini, first suggested by Stark et al. for Martini 2, is to scale the LJ well-depths of all protein-protein bead interactions.[55] In Martini 3, Thomasen et al. obtained improved agreement with experimental SAXS and NMR data by either downscaling the protein-protein interactions (less attractive) or upscaling the protein-water interactions (more attractive).[41,42]

We observed that increasing the favorable interactions of arginine and glutamate with Arg, Asp, Glu, Lys, Phe, and Tyr residues, while decreasing the interaction strength with all other residue types was necessary to obtain results closer to the atomistic CHARMM36m-NBF force field (except of glutamate excipients with Ile). For full-length antibodies, which also include the Fc domain, the parameters derived here should provide more accurate protein-excipient interactions, but additional refinement may be necessary. This is challenging also in light of the glycosylation of the Fc domain, and would also require to further refine Martini 3 carbohydrate parameters[56,57] to glycosylated amino acid side chains. However, once these are available, we expect the parametrization strategy followed in this work to be promising for parametrization of excipient-glycan interactions as well.

Summary and Conclusions

The Martini force field is a computationally efficient coarse-grained model that is promising for studying biomolecular systems on time and length scales that are currently out of reach for all-atom simulations. By construction, the parameters of CG force fields can be expected to be transferable to only within a narrow range (and their applicability within that range needs to be carefully tested), and thus systems or conditions that differ substantially from those used in the original parametrization are usually not as accurately described with the default parameters. However, the modular structure of the Martini force field facilitates adaptations and optimizations for new systems and compounds, as this work demonstrated for protein-excipient interactions as a case example.

In the present work, we introduce an adapted Martini 3-exc coarse grained force field of the amino acid excipients arginine and glutamate, which was parametrized against all-atom simulations as a reference. To study the effects of excipients on aggregation and solubility of antibody Fab domains at a near-atomic level, accurate excipient-residue interactions are crucial. On that account, the Fab domain of the monoclonal antibody trastuzumab was used in excipient-Fab simulations to compare the local excipient-residue interactions, using the new CG Martini 3-exc excipient models described in this work. The excipient-residue interaction parameters were reparametrized to match molecular contacts of reference simulations using the CHARMM36m-NBF all-atom force field. The new Martini 3-exc was validated with the omalizumab Fab domain, which was not used in the parametrization procedure.

The adjusted excipient-residue interaction parameters of Martini 3-exc yield significantly improved results for the interactions of the trastuzumab Fab domain with arginine and glutamate excipients. The validation simulations with the omalizumab Fab domain (which was not used in the parametrization procedure) showed that for arginine excipients the new interaction parameters of Martini 3-exc provide results in agreement with the all-atom reference simulations, but for glutamate the Fab-excipient contacts were only slightly improved compared to the original Martini 3 force field (see Figure S5).

We expect the adopted parametrization strategy to be applicable also to other excipients or ligands that have attractive interactions with proteins. In the context of protein systems unrelated to Fab domains and antibodies, the adjusted excipient-residue parameters of Martini 3-exc are expected to perform better than the original Martini 3 force field, but they should be carefully checked and validated, and further refinements are likely necessary.

In conclusion, the new Martini 3-exc parameter set provides improved descriptions of excipient-Fab interactions, opening the way towards studying the molecular underpinnings of the stabilizing effects of arginine and glutamate excipients on the solution state properties of biopharmaceutical high-concentration monoclonal antibody formulations using CG-Martini simulations.

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Supporting Information Available

Correlation of molecular contact cutoff of 0.5 nm and 0.75 nm in coarse-grained and all-atom resolution. Histograms of bonds and angles of the 4-bead arginine model after reparametrization compared to the all-atom histograms. Histograms of bonds and angle of the 3-bead glutamate model after reparametrization compared to the all-atom histograms. Arginine contacts with trastuzumab residues in Martini 3, Martini 3-exc and CHARMM36m-NBF. Correlation of molecular contacts between arginine and glutamate with omalizumab in Martini 3 compared to CHARMM36m-NBF.

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