Cation-π interactions between methylated ammonium groups and tryptophan in the CHARMM36 additive force field

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ABSTRACT: Cation-π interactions between tryptophan and choline or trimethylated lysines are vital for many biological processes. The performance of the additive CHARMM36 force field against target quantum mechanical data is shown to reproduces QM equilibrium geometries but required modified Lennard-Jones potentials to accurately reproduce the QM interaction energies. The modified parameter set allows accurate modeling, include free energies, of cation-π indole-choline and indole-trimethylated lysines interactions relevant for protein-ligand, protein-membrane and protein-protein interfaces.

Cation-π interactions play an important role in biomolecular recognition processes,[1-3] including protein-ligand recognition,[4-6] and in protein structure stability.[7-9] In particular the role of cation-π interactions involving methylated ammonium groups is crucial in epigenetics where aromatic pockets in chromodomains or PHD finger domains recognize methylated lysines of histone tails.[10-12] Recognition of histones by "reader proteins" is important and improper regulation of epigenetic recognition events has implications in several diseases including different types of cancers.[13-17] Choline [-$N(CH_3)_3$] has also been shown to engage in cation-π interactions either in receptor-ligand interactions[5,6] or at protein-membrane interfaces involving choline-containing lipid headgroups.[6,18-20]

The structural and energetic aspects of the formation of cation-π adducts can be captured using molecular dynamics (MD) simulations provided the use of suitable force field parameters.[21] The reliability of the commonly used molecular mechanics (MM) force fields to quantitatively model cation-π interactions is often questioned due to their pairwise additive nature and lack of explicit polarization effects.[7,22] For example Zheng et al. claimed that methylated lysine-tryptophan cation-π interactions in model β-hairpin peptides were not correctly captured with the CHARMM22[23] protein force field and argued in favor of the use of a polarizable force field to correctly describe such interactions.[8] Recently, based on quantum mechanical (QM) calculations, we demonstrated that the CHARMM36[24-26] force field reproduced well the structures of choline-tyrosine cation-π interactions and that simple modifications of its Lennard-Jones potentials were sufficient to reach a quantitative agreement with the QM reference data for both energy and structure.[21] Our QM calculations indeed revealed a dominant dispersion contribution in the calculated interaction energies. In that work, we also demonstrated that the improved parameter set, hereafter referred to as CHARMM36-YF, is readily transferable for phenylalanine-choline cation-π interactions.

We here extend our earlier work to the study of interactions between tryptophan and tetramethylammonium, and use it as a basis to extend the parameter set to include tryptophan-choline and tryptophan-trimethylated lysines cation-π interactions. The new set is coined CHARMM36-WYF. In the following we first show that the performance of CHARMM36 can be improved by comparing it against reference QM calculations. We propose extending the Lennard-Jones potentials to include atom-pair specific terms to reduce the differences between Molecular Mechanical (MM) and QM data. The advantage of this strategy is that the other interactions involving tryptophan or choline will not be affected. We subsequently validate the extended parameter set on the structure of a buried cation-π interactions in a choline-binding protein[5] and on a remarkably stable β-hairpin with a semi-solvent exposed cation-π motif.[9] Also considered is the effect of the proposed force field improvements on free energies of dimerization of the three aromatic amino acids with choline analogues.

Benchmarking QM target data and Energy decomposition. Monomers of indole and tetramethylammonium (TMA) are used to construct the potential energy surfaces (PES). Their relative positions and orientations are defined by the values of R, θ and φ (Figure 1). We consider the approach of TMA perpendicularly to the indole ring (θ, φ= 0°, 0°), and diagonally to the ring (θ, φ= 45°, [0°, 45°, 90°, 270°]). The in-plane approach (θ= 90°) is neglected as in solution these approach angles often do not lead to energetically favorable complexes.[21] SAPT2+/aug-cc-pVDZ is used as the reference QM model chemistry as we demonstrated earlier for phenol-choline complexes that it provides profiles comparable to those obtained with CCSD(T)/cc-pV5Z.[21] Additional calculations show

that neither SAPT0/aug-cc-pVDZ*, MP2/cc-pVQZ, or BLYP-D3/cc-pVTZ perform better for Indole-TMA (Supp. Inf., Figure S2).

The interaction energy between indole and TMA following the (0°, 0°) approach angles is more favorable (-13.20 kcal mol^{-1}) than between benzene and TMA (-9.0 kcal mol^{-1})[21] or phenol and TMA (-9.23 kcal mol^{-1}).[21] This is valid for all tested approach angles except (45°, 0°). The energy decomposition analysis for all the considered approach angles (Table S2) reveals that the interaction energy components greatly depend on the approach angles. However, the contribution of dispersion is always larger than the induction contribution. This observation is also true for phenol-TMA and benzene-TMA systems.[21] As these three systems yield comparable relative contributions from the different energy components in gas phase, solvent screening should affect the PES similarly. Assuming comparable entropic contributions to the free energies of interactions, we expect the cation-π interactions between Trp and choline to yield more favorable free energies in solution than Tyr-choline or Phe-choline.

Performance of the additive CHARMM36 force field and the CHARMM36-YF extension. For all the approach angles, CHARMM36 underestimates the SAPT2+/aug-cc-pVDZ interaction energy at the corresponding identified minima (Table 1). The differences between QM and MM values for approach angles (θ, φ= 0°, 0°), (θ, φ= 45°, 0°) and (θ, φ= 45°, 90°) remain low and around 1.5 kcal mol^{-1} (Figure S2 (A-C)). However for (θ, φ= 45°, 180°) the difference is the highest with the interaction energy underestimated by 3.15 kcal mol^{-1}, and by 2.9 kcal mol^{-1} for (θ, φ= 45°, 270°) (Figure S2 (E)). Yet the distance between the monomers at the minimum of the PES for these two approach angles is similar to the QM reference, and very close to the QM reference for the other approach angles (Table 1 and Figure S2 (A-C)). We performed error analysis on the identified MM equilibrium distances and energies (Table S4). In terms of interaction energy the RMSD between CHARMM36 and QM reference is 2.26 kcal mol^{-1} with a mean absolute percentage error (MAPE) of 28.05%. In terms of equilibrium geometries the corresponding RMSD is 0.11 Å with an MAPE of 1.74% showing that QM equilibrium geometries are correctly modelled by CHARMM36, unlike the interaction energies.

We previously proposed an extension of the Lennard-Jones potential of CHARMM36 for atom pairs involved in cation-π interactions between choline and Tyrosine (Y) or Phenylalanine (F). This parameter set, referred to as CHARMM36-YF, was developed based on QM PES for benzene-TMA and phenol-TMA which were chosen as model systems.[21] Presently we evaluate the applicability of the Lennard-Jones potential of CHARMM36-YF to cation-π interactions between choline and tryptophan (TMA-indole). For the perpendicular approach of TMA to the indole ring, CHARMM36-YF very closely reproduces the energy minima along with the equilibrium distance (Table S1 and Figure S3 (A)); the difference in interaction energy is -0.4 kcal mol^{-1}. The performance for the diagonal approach directions is not as accurate; CHARMM36-YF performs very well for (45°, 0°) and (45°, 90°), but poorly for (45°, 180°) and (45°, 270°) in terms of interaction energy, though the equilibrium distances are similar to the QM reference (Table S1). To sum up, our CHARMM36-YF parameters perform well also for choline-Trp cation-π, and compared to QM reference PES, but the energetics of the diagonal approaches need further improvement, as required to obtain reliable free energy estimates in macromolecular systems.

Atom-pair specific LJ parameters for choline-tryptophan cation-π interactions: CHARMM36-WYF. Following the strategy applied earlier,[21] we modified the Lennard-Jones potentials for six additional atom pairs between indole and TMA. Initially only the epsilon values were changed while maintaining the minimum equilibrium distance term from the original parameter set. We aimed at reproducing the energy minimum for diagonal approach angles (θ, φ= 45°, [0°, 45°, 90°, 270°]) while maintaining the agreement between MM and QM reference for the other approach directions. The best epsilon values among those tested are provided in Table 2. This set is coined CHARMM36-WYF. All PES are provided as Supporting Information (Fig. S3), as well as an error analysis of minimum geometries and energies (Table S4). Although the interaction energy is slightly overestimated for some approach angles CHARMM36-WYF performs well; the RMSD values are 1.19 kcal mol^{-1} and 0.08 Å in terms of interaction energy and equilibrium geometries, respectively. Note that altering the equilibrium distance of the LJ potential (denoted as R_{min} in Table 2 and Table S3) along with the epsilon values did not lead to a better agreement (see CHARMM36-WYF(2) in SI; Table S3, S4, and Figure S3). Of all sets of parameters we have tested, CHARMM36-WYF performs best at reproducing QM PES of cation-π interactions between choline and Trp, without affecting the modeling of interactions obtained with CHARMM36-YF for choline and Tyr or Phe. The final parameter set to use in modeling and MD simulations is provided in Table S5 and in readily usable CHARMM and GROMACS file formats as a separate zipped file in the Supporting information.

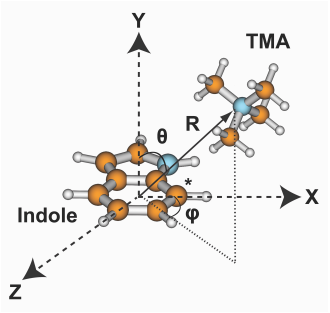

Figure 1. Indole and tetramethylammonium (TMA) model system. The potential energy surface (PES) is constructed as a function of R, θ, and φ.

Table 1. Minimum Interaction Energy Distances, $R_{E_{min}}$ (Å), and Energies, E_{min} (kcal mol^{-1}).

Approach angles (θ,φ)	SAPT2+/aug-cc-pVDZ		CHARMM36		CHARMM36-WYF	
	$R_{E_{min}}$	E_{min}	$R_{E_{min}}$	E_{min}	$R_{E_{min}}$	E_{min}
0°,0°	4.2	-13.20	4.0	-11.62	4.1	-14.21
45°,0°	5.0	-5.44	4.9	-4.06	5.0	-6.35
45°,90°	5.1	-7.52	5.0	-5.87	5.1	-7.30
45°,180°	4.9	-9.28	4.9	-6.13	5.0	-7.16
45°,270°	5.8	-6.16	5.8	-3.26	5.9	-5.38

Solvent-shielded cation-π interactions: choline binding protein. We performed MD simulations of the choline-binding protein ChoX from *Sinorhizobium meliloti* (Figure S4) using CHARMM36, CHARMM36-YF, and CHARMM36-WYF. The ChoX choline-

binding site consists of three tryptophans (W43, W90, W205) and one tyrosine (Y119) all buried in the ligand-bound state (Figure 2 and Figure S4). The protein structure does not undergo any significant conformational change during the simulations, irrespective of the force field variant used. The maximum average RMSD between MD and X-ray structure is 1.27±0.18 Å (Table S6). In order to evaluate the stability of the cation-π interactions we monitor the interactions of each tryptophan with choline using the same reaction coordinates as shown in Figure 1 over the 10 ns simulations (Table S7). The average values for R, θ, and φ from the simulations are compared to their values in the crystal structure. Whichever force field is used, neither the distance (R) nor the angles (θ, φ) deviate significantly from the X-ray values. Their distributions also overlap largely (data not shown). This shows that the unmodified CHARMM36 accurately models the structure of the aromatic cage around choline. Note that the interactions in this complex are shielded from the bulk solvent.

Table 2. Pair-specific Lennard-Jones Parameters (NBFIX terms) used in the modified CHARMM36-WYF force field. R_{min} is the equilibrium distance. Atom types in CGenFF nomenclature. CHARMM36 protein force field nomenclature parameters are provided in Table S5.

NBFIX	epsilon (kcal mol^{-1})		Unaltered R_{min} (Å)
	CHARMM36	CHARMM36-WYF	
CG2R61-CG334	-0.0734	-0.2081	4.2074
CG2R61-NG3P0	-0.1183	-0.2400	3.8424
CG2R51-CG334	-0.0621	-0.0421	4.3150
CG2R51-NG3P0	-0.1000	-0.1000	3.9500
NG2R51-CG334	-0.1241	-0.9241	4.0650
NG2R51-NG3P0	-0.2000	-0.8000	3.7000
CG2RC0-CG334	-0.0873	-0.0173	4.0750
CG2RC0-NG3P0	-0.1407	-0.0107	3.7100

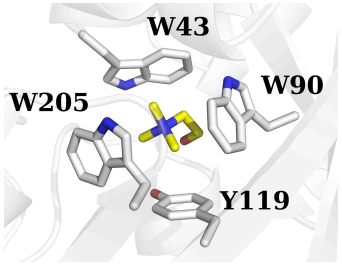

Figure 2. Ligand binding pocket of ChoX protein (PDB id: 2REG). The binding pocket consists of three tryptophans and one tyrosine.

Solvent-exposed cation-π interactions: beta hairpin epigenetic recognition motif. We performed 100 ns MD simulations of a synthetic beta hairpin peptide containing a tri-methylated lysine (K9) interacting with two tryptophans (W2 and W4) through an intramolecular cation-π motif semi-exposed to bulk solvent.[9] The peptide sequence is Ac-R-W-V-W-V-N-G-Orn-K(Me)$_3$-I-L-Q-NH$_2$ as designed and synthesized by Riemen and Waters.[9] The backbone RMSD is lowest with CHARMM36-WYF (Figure S5 and Table S8). We evaluate the structural stability by monitoring the distributions of distances between N and C termini (NTCT), between lysine K9 and each of the tryptophans (W2K9 and W4K9) and between W2 and W4. The main peak of the NTCT distribution is centered around 5.3 Å for all force field variants but shows "shoulders" at higher distances for CHARMM36 (up to 20 Å) and CHARMM36-YF but to a lesser extent (up to 12 Å)(Figure S6 (A)). The distributions of the pairwise cation-π distances (W2K9 and W4K9) are mostly similar irrespective of the force field variants though the distributions obtained with CHARMM36-WYF are somewhat narrower (Figure S6 (B and C)). The distribution of the W2W4 distance obtained with CHARMM36-WYF presents only one peak centered around 8.3 Å and is significantly narrower (Figure S6 (D)). The two other FF variants yield two peaks indicating two distinct arrangements of the pocket residues. Altogether our results indicate that the CHARMM36-WYF parameter set yields the most stable cation-π interactions involving the trimethylated lysines and the tryptophans. Taking the results on the ChoX protein into account, we suggest that the performance of the CHARMM36 depends on the solvent accessibility of the cation-π adducts.

Dimerization free energy of the analogues in explicit solvent. We next evaluate the performance of the force field variants in terms of the energetics of complex formation for TMA with Indole, Benzene or Phenol in water. Dimerization potential of mean force (PMF) calculations were performed using Umbrella Sampling in explicit CHARMM-modified TIP3P water. The results are summarized in Table 3 and Figure 3 (also see Table S9). As neither direct experimental data nor theoretical predictions are available, the force field variants will be qualitatively evaluated using the following criteria. Based on experimental data we earlier estimated cation-π choline-tyrosine interactions at a membrane-protein interface to contribute about 2-3 kcal mol^{-1} to the overall affinity.[18] QM interaction energy for benzene-TMA is comparable to that of phenol-TMA with -9.0 and -9.2 kcal mol^{-1}, respectively (Table S10). Further, the QM energy decompositions of benzene-TMA and phenol-TMA show that the interactions have similar relative contributions from dispersion, electrostatics and induction and should therefore be similarly affected by solvation. We thus expect free energy values in the range of 2-3 kcal mol^{-1} for benzene-TMA and phenol-TMA, within the range of the experimental values for tyrosine-choline interactions. The values obtained with CHARMM36 are out of that range (-0.59 and -0.63 kcal mol^{-1}, respectively) while the CHARMM36-WYF values are within that range, with -2.22 and -2.26 kcal mol^{-1}, respectively.

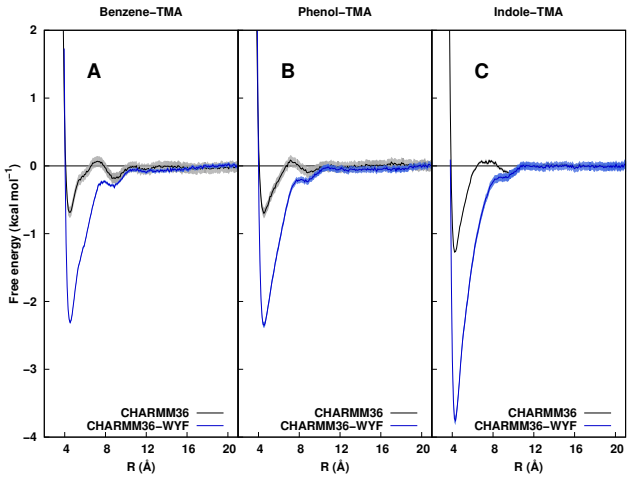

Figure 3. Dimerization PMF for Benzene-TMA (A), Phenol-TMA (B), and Indole-TMA (C). PMF calculations are carried out in explicit water (TIP3P) and using Umbrella sampling. Errors are shown in shaded area along the curves.

We expect the solvent attenuation for Indole-TMA to be similar or more favorable compared to the two other aromatic groups. This

is a reasonable expectation given the magnitude of the dispersion contribution in the three systems, and the similarity in relative importance of the different energy components. Given the QM interaction energy of -13.20 kcal mol^{-1} we would expect a dimerization free energy for indole-TMA more favorable than the -2.2 kcal mol^{-1} of benzene-TMA and phenol-TMA. The PMFs from CHARMM36 and CHARMM36-YF yield free energies of only -1.21 and -2.21 kcal mol^{-1}, respectively. The CHARMM36-WYF value (-3.69 kcal mol^{-1}) seems to be within a more reasonable range. QM interaction energies calculated for these complexes with the COSMO solvation model[27] also reflect a similar trend (Table S10).

Table 3. Obtained energy minima from the dimerization free energies (kcal mol^{-1}) for different complexes.

Force field variants	Benzene-TMA	Phenol-TMA	Indole-TMA
CHARMM36	-0.59	-0.63	-1.21
CHARMM36-WYF	-2.22	-2.26	-3.69

Final remarks. Taken altogether the results presented here show that the performance of CHARMM36 needs to be improved to correctly reproduce the energetics of choline-Phe/Tyr/Trp cation-π adducts but the structures are nicely reproduced, unlike previous reports[8]. Our results also suggest that structures of solvent-exposed cation-π adducts might be more challenging to model accurately. Finally, and while CHARMM36 may perform well for equilibrium simulations, free energy calculations might not lead to accurate energy values. This is also valid for computational mutagenesis using e.g. alchemical transformations which would lead to underestimation of energy contributions. Accordingly, we propose that the CHARMM36-WYF modification be used, as it will capture both accurate structures for cation-π adducts along with reliable free energy contributions. This applies for interactions between any of the three aromatic amino acids and choline or similar groups including trimethylated lysines and is applicable to CGenFF as well as the recent CHARMM36m version of the protein force field.[28]

Calculations of relative binding affinity for rational drug design is one example where subtle difference in energy contribution from individual amino acids is relevant and important. Li et al. revealed the structure of an aromatic cage in a BPTF PHD finger and its role in recognition of the Histone H3K4me3 site.[11] The binding pocket consists of three tyrosines and one tryptophan. Using fluorescence polarization the authors measured the change in binding affinity upon mutations in that pocket. Of particular interest is the decrease from 1.6 ± 0.1 μM for the wild-type to 34.4 ± 1.1 μM for a tryptophan to phenylalanine mutant (W32F). The corresponding change in free energy (ΔΔG) is ≈ 1.82 kcal mol^{-1}. We calculate a difference between our dimerization PMFs for Benzene-TMA and Indole-TMA of 0.62 kcal mol^{-1} for CHARMM36 and 1.47 kcal mol^{-1} for CHARMM36-WYF. Admittedly we cannot account for the other interactions present in the binding pocket of BPTF PHD finger but we predict that CHARMM36-WYF would be able to more accurately calculate the energy loss upon Trp to Phe mutation versus the unmodified CHARMM36 force field. Future investigations are focused on further application of these parameters in a wider range of protein-ligand and protein-lipid interactions.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed Materials and Methods, QM benchmark, Energy minima (Table S1), Energy decompositions (Table S2), Modified force field parameters for comparison (Table S3), Error analysis (Table S4), Final parameter set (Tables S5), Analysis on test cases (Table S6-S10), PES (Figure S1), QM benchmarks (Figure S2), MM force fields comparison with target QM data (Figure S3), Results on test cases (Figures S4-S6) (PDF), Readily usable parameters for MD simulations using CHARMM and GROMACS compatible file formats (as a separate zipped file).

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ABBREVIATIONS

MD, molecular dynamics; PC, phosphatidylcholine; PES, potential energy surface; QM, quantum mechanics; SAPT, symmetry adapted perturbation theory; TMA, tetramethylammonium

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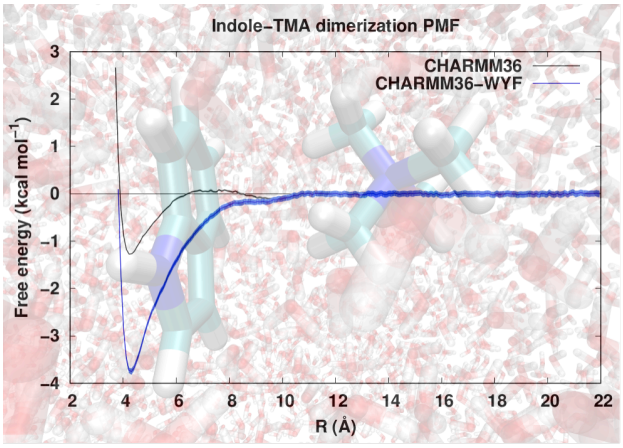
SYNOPSIS TOC.



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