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Force Field Development of β -lactam Class Antibiotics

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ABSTRACT

Molecular dynamics (MD) simulation is a computational chemistry technique used to observe how a molecular system behaves as time passes. MD is based on solving Newton's equations of motion. This requires the use of force fields to describe the potential energy function of each different molecule type in molecular system. In order to develop a force field, charges, bonds, angles, and dihedrals must be parameterized to fit quantum mechanics (QM) data. By basing the force field on QM data, MD simulations have higher accuracy while still using the low computational cost of molecular mechanics. This project focuses on developing well-fit force fields for β -lactam class antibiotics for future MD simulations. Full antibiotics are too large of a molecule to parameterize from scratch, so instead we broke them down into fragments. Smaller molecule fragments allow less terms to be optimized which greatly simplifies force field development. By the transferable nature of parameters in CHARMM force fields, the fragment parameters can be transferred to connecting molecules. Due to this, we can build up larger organic molecule force fields piece by piece. In this work, we developed CHARMM force fields for cephalothin, cefotaxime, ceftazidime, and aztreonam.

1. INTRODUCTION

Molecular dynamics (MD) simulations study the interactions and movement of molecules over a set time window. By solving for Newton's equations of motion, the movement of all molecules in a system are simulated. Molecular mechanics (MM) methods assume that all interactions between atoms and molecules are classical, and it ignores the orbitals of electrons. In general, MM methods are valid approximations at a macroscopic scale. The advantage of molecular mechanics is that it is relatively computationally inexpensive, but at the cost of accuracy. In contrast, quantum mechanics (QM) methods describe molecular systems with wavefunctions of electrons. QM calculations are very computationally expensive which makes computing large-scale systems impractical. Combining the two methods yields a hybrid QM/MM method. QM/MM provides the accuracy of QM and the computational efficiency of MM methods.

The field of computational chemistry has grown rapidly in the past few decades. As computational power has increased, the ability to shed light on complex systems through a computational lens has improved. When experimental and computational chemistry are combined, scientific understanding can be achieved much faster than by either method alone. To combat the development of antibacterial resistance in bacteria, the Tao group is applying machine learning methods to MD simulations to better understand the hydrolysis reaction pathway of β -lactam antibiotics by a class of proteins called β -lactamases. By understanding the reaction pathway, efforts can be made to disrupt or alter the reaction by using different molecules. β -lactamases break apart the structure of the β -lactam ring, thus disabling the antibiotic. In order to conduct accurate MD simulations, force fields for the antibiotics must be developed. Force fields describe the

potential energy function of a molecule. MD simulations use force fields to create a trajectory for a molecule and predict how the system will change each timestep. In other words, the force field is used to predict the way the molecule will behave in a system. The trajectory follows the gradient of the potential energy function, thus requiring a force field.

Currently, the Tao group is focusing on four different β -lactam antibiotics. These antibiotics are cephalothin, cefotaxime, ceftazidime, and aztreonam (Figure 1).

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Figure 1. Structures of the four antibiotics that are being studied.

The first three antibiotics belong to the cephalosporin group. Cephalothin is a 1st generation cephalosporin introduced in 1964 and is still used today. Certain bacteria have been found to be resistant to cephalothin. Due to this, we have chosen it as an antibiotic of study. Cefotaxime and ceftazidime are both 3rd generation cephalosporins which also have experienced

bacterial resistance to a lesser degree than cephalothin. Additionally, cefotaxime and ceftazidime are very structurally similar which will make it easier to pinpoint the structural differences that affect their behavior. By comparing these three antibiotics, we hope to understand which factors and functional groups are responsible for the differences in resistance. The remaining antibiotic, aztreonam, is a single ring β -lactam, which will be used to study the difference between single and double ring β -lactam antibiotics and how their hydrolysis by β -lactamase is affected.

$$\begin{aligned}
 V = & \sum_{\text{stretching}} k_b (b - b_0)^2 + \sum_{\text{bending}} k_\theta (\theta - \theta_0)^2 \\
 & + \sum_{\text{torsion}} k_\phi [1 + \cos(n\phi - \delta)] + \sum_{\text{improper}} k_\omega (\omega - \omega_0)^2 \\
 & + \sum_{\text{Urey-Bradley}} k_u (u - u_0)^2 \\
 & + \sum_{\text{non-bonded}} \left\{ \epsilon \left[\left(\frac{R_{\text{min},ij}}{r_{ij}} \right)^{12} - \left(\frac{R_{\text{min},ij}}{r_{ij}} \right)^6 \right] + \frac{q_i q_j}{\epsilon r_{ij}} \right\}
 \end{aligned}$$

Equation 1. The potential energy function that a force field describes. All the k terms describe force constants. The terms b_0 , θ_0 , ω_0 , and u_0 all describe the equilibrium geometry. Urey-Bradley terms describe a pseudo-bond between an angle, helping to describing scissoring vibrational modes of angles. CHARMM force fields extract the Lennard-Jones potential from QM calculations and parameterize for the Coulomb interactions.

The terms shown in Equation 1 describe the molecule's trajectory during an MD simulation. A more optimized force field will result in more realistic interactions in a system. Chemistry at Harvard Macromolecular Mechanics (CHARMM) is a software developed by Martin Karplus at Harvard. CHARMM contains sets of optimized energy functions that can be used to develop force fields for new molecules. The software was primarily designed to model biological systems.¹ Parametrizing a CHARMM force field to QM data effectively describes the molecule for a system.

CHARMM General Force Field (CGenFF) is a general force field designed through the ParamChem project to be used as a framework to base other force fields. CGenFF contains many common biological groups with a heavy emphasis on heterocyclic rings.² CGenFF assigns atom types based off its library of included molecules and from there, assigns initial parameters. We used both CHARMM and CGenFF to develop the force fields for these antibiotics.

2. METHODOLOGY

A molecular force field describes a mathematical equation for the potential energy function of a molecule. This equation has many parameters that describe its behavior, and these are what are parameterized or altered. In order to parameterize, we followed MacKerell's scheme (Figure 2).⁷

Figure 2. General scheme of parameterization for our force field development.

Parameterizing all the terms simultaneously for large molecule such as a full antibiotic can be very difficult and inefficient. Breaking down the antibiotic into smaller, more manageable fragments can make parametrization much more practical as there are far fewer terms to consider at one time. By looking at the four antibiotics, nine fragments were identified such that any of the four antibiotics could be assembled from these nine fragments (Figure 3). Each fragment had to be parametrized before they could be connected. CGenFF included fragments 1, 2, and 4, shown below, which allowed these fragments to be connected without any further optimization.

Fragment 1*

Fragment 2*

Fragment 3

Fragment 4*

Fragment 5

Fragment 6

Fragment 7

Fragment 8

Fragment 9

Figure 3. The structures of all nine fragments that compose the four antibiotics. The fragments featuring an asterisk (*) were already included in CHARMM General Force Field and did not require any additional optimization.

In the initial step, each fragment was built using Gaussian16³ and GaussView 6.0. An energy optimization was calculated using MP2 level of theory and the 6-31g(d) basis set. This calculation setup offers sufficient accuracy for the generation of the QM data for each fragment. Using Southern Methodist University's Maneframe II, quantum chemistry calculations were carried out for each fragment. Once each calculation was completed, the optimized structure was submitted to CGenFF to generate a stream file. This stream file contains all the parameters for the force field and is the target of all parametrization that is done during development. To fit the force field, three sections of the stream file must be parametrized to yield a well-fitted force field, the charges, geometry, and force constants⁷. CGenFF assigns a penalty to each parameter. A higher penalty signifies that this parameter requires more optimization. Based on the penalties, parameters were

selected to be optimized to the QM data from the Gaussian calculations. The following section will cover the complete parameterization of fragment 8 (Figure 4).

Figure 4. The labeling scheme of the atoms in fragment 8. The atoms were numbered in this manner for the force field of fragment 8. The numbering was determined by following a molecular backbone.

To optimize the charges on the heavy atoms for each fragment, the energies of water interactions were considered. Hydrogens bonded to carbons are already highly optimized from CGenFF, so their charges were all left as constants to avoid having to parametrize too many atoms. Water molecules were placed in line with all atoms capable of hydrogen bonding and the distance between the hydrogen bonding atoms was changed until a minimum in energy was found (Figure 5). The Z-matrix option for optimization in Gaussian16 was used to move the distance between atoms without changing any angles or dihedrals. Z-matrix Gaussian input files cannot specify atoms at 180 degrees because that angle is undefined, therefore a dummy atom was applied to the system only for geometry specification. Using Python scripts created in our research group, the energies from the water interactions were extracted and used as QM data for comparison with the stream file results. Next, a depth first search Python script was used to determine a set of charges such that the difference between the QM and force field energies were at a minimum while keeping the total charge unchanged.

Figure 5. The water interactions considered on fragment 8.

Water Interaction	1	2	3	4	5
Initial Charge	-0.290	-0.230	-0.500	-0.500	-0.500
ΔE (kCal/mol)	-0.4450	-2.4434	0.9430	-0.1264	0.3687
Fitted Charge	-0.430	-0.090	-0.550	-0.550	-0.470
ΔE (kCal/mol)	-0.0560	-0.6196	0.1417	-0.068	0.028

Table 1. Results of parameterizing the charges on the heavy atoms in fragment 8. The ΔE represents the difference in energy between the QM and MM interaction energies.

Using water interactions to parameterize the charges on atoms proved to be an effective method to optimize the Coulombic (charge) interactions. Interaction 2, the nitrogen atom, was more positive than expected. This is likely due to some π bonding between the carbonyl group and the lone pair on the nitrogen. The differences in energy between QM and MM calculations were significantly lowered by parameterizing the charges with high penalties. The next step was optimizing the equilibrium geometry of fragment 8.

Finding the minimum difference in equilibrium geometry only required a comparison between the bonds and angles of the QM data and the calculated MM values from the stream file. Parameters with high penalties selected and their bonds and angles were fit to the original QM structure. This process used a similar depth first search script to generate a set of parameters and then iterate through this set to find the parameters with the smallest difference in bond length and angle. This script was run several times modifying the test parameters until the difference in bond length was less than 0.01 Å and the difference in each angle was less than 3 degrees.

Bond or Angle	C6-C5	N2-S1	C6-C5-C4	C6-C5-N2	C6-C5-H13	C3-N2-S1	C5-N2-S1	N2-S1-O7	N2-S1-O8	N2-S1-O9
Unfitted	0.003	-0.012	1.317	-3.925	1.674	4.155	1.319	-2.207	2.167	-0.844
Fitted	0.003	-0.012	0.633	-2.353	0.801	1.741	1.484	-2.114	2.492	-0.453

Table 2. Parameterization results of the equilibrium geometry of fragment 8. The reported numbers are differences in bond length (Å) and angle (°) between the QM and MM calculated equilibrium geometries.

a)

b)

Figure 6. The structure of fragment 8: a) before optimization b) after bond length and angle parameterization. Orange structure represents QM data and the colored structure represents MM calculated structure.

For fragment 8, CGenFF's initial guess for the equilibrium was good and did not require much optimization. The difference in geometry for angles C6-C5-N2 and C3-N2-S1 was greater than the 3° threshold. As illustrated in Figure 6, not much change occurred from optimizing the equilibrium geometry for fragment 8.

We used two methods to fit the force constants. The first method involved using the natural internal coordinate (NIC) system developed by Fogarasi and Pulay.³ Their NIC system provides a method to better fit ring-shaped structures to their experimental vibrational spectra. With the NIC system, we tried to fit the MM vibrational spectra with the QM spectra. Adjusting the force constant terms in the stream file changed the MM vibrational spectrum, but this method proved very complicated and inefficient even for smaller molecules.

Rather than fit the vibrational frequencies, the other method calculated the potential energy surface (PES) of each bond, angle, improper, and dihedral and fit the stream file to their energy profiles. The force constants to be optimized were determined by the penalty assigned by CGenFF. Through series of Gaussian calculations, the bonds and angles were each stretched slightly both forward

and backward to see how their energies changed. Similarly, the dihedrals to be parametrized were rotated and had their energies at each step extracted. The force constants on bonds were parameterized first, then the angles, and finally the dihedrals. If the molecule had any improper angles, those were parameterized before dihedrals. Improper angles are described as the angle bent out of a planar (flat) portion of the molecule. The force constants on bonds had the highest impact on the differences in energy. Therefore, the bonds were parameterized first. The order of parameterization was determined by impact on energy differences. Fitting the PES scans was a much more efficient way to optimize the force constant terms compared to vibrational frequencies.

Figure 7. The unfitted and fitted energy profiles of rotating the C6 methyl group dihedral on fragment 8.

Plotted in Figure 7, parameterizing the force constants caused the MM energy profile for fragment 8 around the methyl dihedral to be much more consistent with the QM energy profile. The initial force constant terms that described this dihedral were not very good at predicting the next step after the energy minima. This is shown at the points located at around 80, 220, and 330 degrees on the unfitted plot. Increasing the force constants on the terms that describe this dihedral produced much more satisfactory results.

After optimizing the force constants, the charges and equilibrium geometry terms were reparameterized using the same methods to ensure that previous fitting was still accurate.

Figure 8. Example of how the fragments were connected together.

Each of the fragments followed the same procedure. After they were all completed, the same procedure was used to connect them to form the target molecules. An additional step is needed for connecting fragments was to transfer parameters from the fragment force fields. Every time a new fragment was connected, the new molecule had to be parameterized. The optimization for connecting fragments focused on the new bonds, angles, and dihedrals created by connecting the fragments. To balance the charges on the two connecting atoms, the charge of the atom that was removed from the fragment was summed onto the atom from the connecting fragment that replaced it. These steps were repeated every time a fragment was connected until the full antibiotic was completed.

3. RESULTS

At the start of the summer, Zilin Song from our research group had already optimized fragments 3 and 5 as well as the force field for cephalothin. Fragments 1, 2, and 4 were already included in CGenFF so they did not require any fitting. This left the force fields for fragments 6, 7, 8, and 9 to be developed. While developing force fields, I learned that using PES's to fit the force constants was a much quicker and more reliable method compared to fitting the vibrational frequencies. The quality of the optimization on a force field was determined by the variance from the QM target data. For the four molecule fragments I fit this summer, all the parameters fit within the thresholds to be considered well-fit. After repeating the same optimizations for each fragment and connection, it became much easier to determine parameters that required altering.

After finishing the fragments, I began building the aztreonam molecule fragment by fragment. This took a while because each connection required the same optimization as the initial fragments. By the end of the summer, the force field for aztreonam was completed. Using the same methodology, cefotaxime was completed during the fall and the force field for ceftazidime has been started. One of the benefits of using this force field development method is that the completed force fields can be used on similar structures. Using parameters from the aztreonam and cefotaxime, the force field for ceftazidime only requires small adjustment.

Figure 9. The final fitting of aztreonam. Orange structure is the QM structure and MM calculated structure is colored by elements.

Figure 9 shows the overlay of the QM and MM aztreonam structures. This structure is constructed by connecting fragments 5, 7, and 8. The most important fitting for aztreonam is the β -lactam ring, as it is the site of hydrolysis by β -lactamase. The β -lactam ring for the aztreonam force field is well-parameterized which can be seen by the overlap. The biggest difference between the QM and MM structures is the five membered ring. In the QM structure, an amine hydrogen is hydrogen bonding to the SO_3^- group. But due to the backbone of aztreonam, the molecule should be very flexible. As a result, it is expected that there is some variance between the QM and MM structures for the functional groups. Based off of the first antibiotic force field, this method appears to work best for the ring structures.

Figure 10. The final fitted structure force field for cefotaxime. Orange structure is the QM structure and MM calculated structure is colored by elements.

The force field for cefotaxime was constructed using fragments 3, 4, 5, and 6. Cefotaxime's fitted force field, Figure 10, shows good fitting around the bicyclic structure. Like aztreonam's force field, cefotaxime's force

field structure differed from the QM structure in its flexible portions of the molecule. The chain like structures on both sides of the bicyclic ring are slightly rotated out of the QM positions. It is likely that this is due to the flexibility of these chains, and this will not affect the accuracy of the MD simulations. In the MD simulations the antibiotic molecules will be allowed to freely move around. The flexible portions of the antibiotics will likely behave normally.

This methodology of developing force fields for drug-like molecules is efficient. By breaking larger molecules down into smaller pieces makes any larger drug molecule rather simple to develop its force field. When the force fields are being used in the MD simulations, it will become apparent whether further refinement is necessary. Having a well parameterized force field is very important groundwork for MD simulations. The transferability of parameters between connecting fragments has proven to be important for force field development. Transfer of parameters is what allows these molecules to be built piece by piece.

Ceftazidime is the only remaining antibiotic which requires force field development. Due to all the fragments having been finished, it is a simple matter to connect them and develop the final force field. These four antibiotics share similar overall structures which simplifies the force field development, but the fragmentation methodology is very important. Once the last antibiotic is finished, I will transition into conducting the MD simulations of the beta-lactamase hydrolysis pathway. During this stage of research, the performance of these four force fields will be tested. If they perform poorly, adjustments to the parameters will be made to remedy this.

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