Chapter 5.

The Alchemy Simulation

5.1 Objectives.

Molecular dynamics (MD) simulations can be used advantageously in conjunction with experimental data to investigate proposed mechanisms of calcium selectivity. MD allows a simulated visualization of the chain of events leading up to the experimental observation, and this approach often provides insights that cannot be immediately derived from experimental results. Moreover, MD simulations approximate the energy fluctuations that correlate to specific biochemical events and thereby provide us with a useful quantitative analysis, although it is important to understand the limitations of the methodology when interpreting these results. The accuracy of MD systems is limited in that the electronic configurations are represented by classical electrostatics, instead of quantum mechanical principles. Additionally, even in the treatment of the electrostatics, approximations are employed to make the calculations computationally affordable. Although much progress has been made regarding solvent modeling, the ability to comprehensively represent the many amazing properties of ordinary water still eludes us. However, for systems where the protein structure and nonbonded van der Waals and electrostatic interactions are the prevailing influences on the inherent functional properties, MD simulations often provide reasonable representations. While there is much room for improvement in the degree
of accuracy with which MD calculations represent biological systems, it is nonetheless true that these methods, in spite of the approximations and truncations employed, are frequently able to correctly predict and/or correlate the behavior of proteins.

The first non-equilibrium simulation performed in the pike parvalbumin model system was entitled “Alchemy”. The purpose of the Alchemy simulation was to investigate whether a classical MD model could correlate the sevenfold coordination of Ca\(^{2+}\) and the transition to the sixfold coordination of Mg\(^{2+}\). Depiction of this transition was accomplished through beginning the simulation with parameters that represent Ca\(^{2+}\) in the binding sites, then reducing the van der Waals radius of the bound cations during the simulation, until the radius became representative of Mg\(^{2+}\). We were also interested in observing whether this process was reversible as described by our model, since we know it is, in fact, a reversible transition in parvalbumin. Finally, it was of interest to see if the coordination sphere delimited by the structure of the protein would require that the glutamate at position 12, the last coordinating residue of the loop, be the residue to provide bidentate coordination of Ca\(^{2+}\). There are other carboxylate sidechains coordinating Ca\(^{2+}\) in both binding sites, and the corresponding carboxylate oxygens were all described with equivalent electrostatic charge distributions in the parvalbumin model. Therefore, within the representation of our system, none of the carboxylate sidechains should be electrostatically preferable over another for bidentate ligation. If in our simulations the glutamate at position 12 was accurately, consistently, and reversibly predicted to be the source of bidentate ligation of Ca\(^{2+}\), this would
provide strong evidence that properties of the structural framework of EF-hand binding sites impose this role on the residue at position 12.

5.2 Introduction.

The NAMD (Nelson et al., 1996) Molecular Dynamics Simulations software has been employed to create a computational model of pike parvalbumin using classical mechanics. In these simulations, the energy of the molecule is calculated as a function of the atomic coordinates through the use of empirical force fields (Brunger, 1992; Brooks et al., 1983) of the following form:

\[ E_{\text{total}} = E_{\text{bond}} + E_{\text{angl}} + E_{\text{dihe}} + E_{\text{impr}} + E_{\text{vdw}} + E_{\text{elec}} \]

The total energy of the force field is a summation of empirical conformational energy terms and nonbonded energy terms. The first four energy terms in the above expression are bond and angle conformational energy terms. The last two nonbonded energy terms in the above energy expression represent the van der Waals energy and the electrostatic energy. Atomic trajectories are computed through the numerical solution of Newton’s equations of motion:

\[ m_i \frac{d^2 x_i}{dt^2} (t) = -\nabla x_i E_{\text{TOTAL}} \]
Finally, these methods of solving Newton’s equations of motion cannot be used with dissipative, nonequilibrium systems, or in equilibrium simulations where truncations are employed, without incurring thermal drift effects. Corrections for these effects can be applied in NAMD through implementation of the Berendsen temperature and pressure coupling algorithm (Berendsen et al., 1984). In this method, a coupling constant is parameterized by the user to a value that stabilizes the model during a given simulation while minimizing the disturbance of the system.

5.3 Alchemy protocol.

The protocol for the Alchemy simulation began with 25,000 steps, representing 50 picoseconds (ps) of MD and a van der Waals radius for the bound metal ions that was representative of Ca\(^{2+}\). The ShakeH algorithm was employed to fix the bond between each hydrogen and its mother atom to the ideal bond length specified in the parameter file. This allows a relatively large timestep of 2 fs because the ShakeH algorithm negates the need for timesteps small enough to include hydrogens in the numerical integration. The radius was reduced by 0.1 Å increments in 5 stages. The first four stages were allowed a short, 2,500-step equilibration, representing 4 ps of MD. The final stage of the radius reduction, where the bound cation radius became representative of Mg\(^{2+}\), was simulated for 25,000 steps. To analyze the reversibility of the simulation, the ionic van der Waals radius was increased back to the Ca\(^{2+}\) value, in
an equivalent, but reciprocal manner. The Ca$^{2+}$-bound parvalbumin was modeled at the last stage for 25,000 steps, corresponding to an additional 50 ps of simulation.

The Alchemy simulation was stable, and the root mean square (RMS) deviation from the starting structure never increased above 1.8 Å. The mean RMS deviation value for the simulation was 1.49 Å, with value fluctuations ranging from 1.06 to 1.78 Å (Figure 5-1). The RMS deviation from the starting coordinates exhibited by the Alchemy simulation is well within the acceptable range for MD simulations, particularly simulations where the system is being perturbed, as it is here through the variations in the radii of the bound metal ions.

5.4 Alchemy in the CD site.

First, the performances of the aspartate residues at key loop positions were analyzed. There are coordinating aspartate sidechains in the CD site at positions 1 and 3 of the binding loop, corresponding to residue numbers 51 and 53. These aspartates exhibited the same metal ion-binding behavior in the simulation that they exhibit in crystal structures (Figure 5-2). In both of these residues, one of the oxygens of the aspartate carboxylate sidechain coordinated the Ca$^{2+}$ ion, then moved in to an even shorter Mg$^{2+}$-oxygen bond distance during the Mg$^{2+}$ phase of the simulation. Then, finally, that same oxygen moved back out again to an appropriate Ca$^{2+}$-oxygen distance in the final stage. Also, in both of the aspartates, the other oxygen remained too far away to coordinate the metal ion during all phases of the simulation.
Figure 5-1. Alchemy simulation RMSD from x-ray coordinates.

Graph of the root mean square deviation (RMSD) from the initial x-ray structure in the Alchemy simulation. The duration of the simulation was 190 picoseconds, and a frame was recorded every 0.1 picoseconds, for a total of 1900 frames. The RMSD was never greater than 1.8 angstroms, and the mean RMSD value over the length of the simulation was 1.49 angstroms.
Figure 5-2. The coordinating aspartates in the CD loop of the Alchemy simulation.

(a) ASP 51 OD1 distance to calcium.

(b) ASP 51 OD2 distance to calcium.

(c) ASP 53 OD1 distance to calcium.

(d) ASP 53 OD2 distance to calcium.

The CD loop aspartates exhibited the same metal ion-binding behavior in the simulation that they exhibit in crystal structures. In both residues, one of the sidechain oxygens coordinates the metal ion (a and c), while the other does not (b and d). It is also evident in plots a and c that the coordinating distance decreases when the metal ion's radius decreases.
The model parameterization, as described in section 2.12, required that the charge distribution be modified for the serine at position 5 of the CD site and the backbone carbonyl group that coordinates Ca\(^{2+}\) at position 7 of both loops. This modification was to more accurately represent the polarization of the coordinating oxygens in the presence of a +2 cation, such as Mg\(^{2+}\) or Ca\(^{2+}\). Once the modified charge distribution was applied at these two positions, classical sevenfold coordination of Ca\(^{2+}\) was observed in the parvalbumin MD simulations. The alchemy simulation has been repeated twice with these parameters applied to the CD site. In both of these simulations, the serine at loop position 5 stayed within coordination distance of the metal ion when it was represented as a Ca\(^{2+}\) ion. However, during the phase of the simulation where the ionic radius of the bound cation decreased to represent Mg\(^{2+}\), the serine drifted away from the Mg\(^{2+}\) in one of the simulations (Figure 5-3). Interestingly, in the simulation where the serine moves away from the Mg\(^{2+}\) ion, when the radius increased again, the serine moved back in to coordinate the Ca\(^{2+}\) ion. This movement away from the Mg\(^{2+}\) ion by the serine at position 5 might be an artifact of the imprecision of the charge distribution representation for serine in the MD simulation. On the other hand, it might be suggestive of another way EF-hands discriminate between Mg\(^{2+}\)- and Ca\(^{2+}\)-binding.

There are a variety of EF-hand proteins that contain serine in one of the binding loops (Nelson and Chazin, 1992). The structural information available about EF-hands that include serine at one of the coordinating loop positions implies that serine may not be a good ligand for Mg\(^{2+}\). First of all, in the Mg\(^{2+}\)-loaded pike parvalbumin structure
Figure 5-3. The coordinating serine in the CD loop of the Alchemy simulation.

(a) SER 55 OG distance to calcium.  
Alchemy simulation 12/99.

(b) SER 55 OG distance to calcium.  
Alchemy simulation 03/00.

The serine at loop position 5 coordinates Ca\(^{2+}\) well, but does not coordinate Mg\(^{2+}\). (a) In this Alchemy simulation, the beginning radius is representative of Mg\(^{2+}\), the transition to Ca\(^{2+}\) is complete by frame 700 (70 picoseconds, simulation time) and at frame 1400 the metal ion becomes Mg\(^{2+}\) again. The serine is coordinating the Ca\(^{2+}\) ion in a very stable manner, but tends to disassociate from Mg\(^{2+}\). (b) This simulation begins with the Ca\(^{2+}\) ion bound, transitions to Mg\(^{2+}\) at frame 700, and reverts back to Ca\(^{2+}\) by frame 1400. Here, serine is clearly not coordinating the metal ion when its identity is Mg\(^{2+}\).
published by Declercq et al. (1991), the CD site with the serine at position 5 still retains Ca\(^{2+}\). Thus, in the crystal structure, it is only the parvalbumin EF site that coordinates Mg\(^{2+}\). This circumstance is in spite of the fact that the protein solution used for crystallization was dialyzed successively against 1.5 M MgSO\(_4\), until a Ca\(^{2+}\) concentration corresponding to 0.53 Ca atom per protein molecule was determined by atomic absorption spectrometry. It should be mentioned, though, that a theoretical study has been performed by Allouche et al. (1999) wherein the Ca\(^{2+}\) in the CD site of this pike parvalbumin crystal structure has been transformed computationally into Mg\(^{2+}\). In the Allouche theoretical model, the serine at position 5 coordinates the Mg\(^{2+}\) ion adequately. Moreover, these calculations were performed using the free energy perturbation method, which computes the differences in the binding free energies of the parvalbumin/Mg\(^{2+}\) complex versus the parvalbumin/Ca\(^{2+}\) complex. The obtained value of roughly \(10^3\) for the ratio of Ca\(^{2+}\) and Mg\(^{2+}\) affinity constants in the CD site was in good agreement with experimental observations. However, Allouche and associates cite NMR evidence (Blancuzzi et al., 1993) that titration of apo-parvalbumin with Mg\(^{2+}\) results in only one singly loaded Mg\(^{2+}\) intermediate before formation of the doubly loaded parvalbumin/Mg\(^{2+}\) complex. This singly loaded intermediate has Mg\(^{2+}\) in the EF site and nothing bound in the CD site. The intermediate with Mg\(^{2+}\) in the CD site and nothing bound in the EF site is never seen in these NMR Mg\(^{2+}\) titrations. Furthermore, the titration of fully loaded parvalbumin/Mg\(^{2+}\) with Ca\(^{2+}\) only exhibits a single intermediate species, where Ca\(^{2+}\) is bound in the CD site and Mg\(^{2+}\) is bound in
the EF site. The opposite intermediate, Mg$^{2+}$ in the CD site and Ca$^{2+}$ in the EF site, is never observed.

Also, many constraints were applied in their model that would make it difficult for any of the ligands to move very far away from the bound metal ion. First, the metal ion itself was held fixed. Additionally, all heavy atoms lying more than 9 Å away from the metal were held fixed, and all atoms of any type lying more than 11 Å from the metal ion were also fixed. This makes it impossible for the bulk of the protein just outside of the binding site to be flexible and therefore makes it unlikely that the loop could accommodate much movement away from the metal ion, even if one of the coordinating residues were inclined to do so.

Mg$^{2+}$-loaded structures of both calbindin D9k (Andersson et al., 1997) and the myosin regulatory light chain (Houdusse and Cohen, 1996) contain a serine at loop position 9 in the binding sites containing Mg$^{2+}$. Nevertheless, this serine does not directly coordinate the Mg$^{2+}$ ion in either structure; instead, a water molecule is recruited as a ligand (Figure 5-4). Many other EF-hand proteins have serine residues at key loop positions, but their structures are either unknown or they are only available for the Ca$^{2+}$-bound species. It would require further investigation to state definitively that the trend seen in our simulations for serine to coordinate Ca$^{2+}$ well, but Mg$^{2+}$ only reluctantly, was predictive of true behavior. It does seem worth exploring the possibility, though, that EF-hand proteins might exploit such a property to confer a distinction between Mg$^{2+}$- and Ca$^{2+}$-binding parameters in certain binding sites.
(a) Serine 62 in calbindin D9k is at position 9 of the binding loop, but in this EF-hand site, loop position 9 does not coordinate \( \text{Mg}^{2+} \) or \( \text{Ca}^{2+} \). Instead, a water molecule is recruited to substitute for the coordinating sidechain at position 9 in both \( \text{Mg}^{2+} \) and \( \text{Ca}^{2+} \) coordination. The last half of the loop moves away from the metal ion when \( \text{Mg}^{2+} \) is in the loop. This prevents the glutamate at position 12 from directly ligating the \( \text{Mg}^{2+} \) ion. Instead, it coordinates another water molecule, which in turn coordinates the \( \text{Mg}^{2+} \). When \( \text{Ca}^{2+} \) is bound in this site, the last half of the loop moves in, and the glutamate at position 12 directly coordinates the metal ion with bidentate coordination. (b) Serine 36 at position 9 in the \( \text{Mg}^{2+} \)-site of myosin RLC also does not directly coordinate the \( \text{Mg}^{2+} \) ion. It does, however, coordinate the water molecule that substitutes for a sidechain ligand at position 9. This site is believed to contain \( \text{Mg}^{2+} \) physiologically.
The backbone carbonyl oxygen donated by phenylalanine 57 at loop position 7 also required a more polarized charge distribution representation. The modified charge distribution was calculated in the same manner as for serine 55 (section 2.12); and once applied allowed the carbonyl oxygen in our simulation to correctly emulate wild-type metal ion coordination (Figure 5-5).

The transition from sevenfold Ca\textsuperscript{2+} coordination to sixfold Mg\textsuperscript{2+} coordination was observed in the CD site (Figure 5-6). Furthermore, this transition was reversible in the simulations. In the simulation where the serine at position 5 stayed coordinated to the Mg\textsuperscript{2+} ion, the transition from sevenfold coordination to sixfold coordination was clearly a function of the glutamate at position 12. This glutamate exhibited bidentate coordination of Ca\textsuperscript{2+} and monodentate coordination of Mg\textsuperscript{2+} in the simulation, just as it has been observed to do in crystal structures (Figure 5-7 a, b). The effect was not as clear in the simulation where the serine did not coordinate the Mg\textsuperscript{2+} ion. Although, even in this simulation, one of the glutamate’s carboxylate oxygens moved in to within less than 2 Å from the Mg\textsuperscript{2+} ion, while the other stayed at approximately the same distance it displayed during Ca\textsuperscript{2+}-binding, about 2.3 Å (Figure 5-7 c, d). What was even more noteworthy was that glutamate 59, the coordinating residue at position 9 of the CD loop, did not bind either metal ion in a bidentate fashion (Figure 5-8). The charge distribution on all of the carboxylate sidechains in the CD loop were equivalent; therefore, there was no inherent electrostatic preference for one of the carboxylate sidechains over another. The aspartates in the binding site might be less likely to attain bidentate coordination because they are shorter and less flexible. However, the only
Figure 5-5. The backbone carbonyl oxygen of phenylalanine 57.

In this Alchemy simulation, the initial metal ion radius was representative of Mg$^{2+}$, the transition to Ca$^{2+}$ radius was complete by frame 700, and the transition back to Mg$^{2+}$ occurs during frames 1200 to 1400. The carbonyl oxygen coordinates Ca$^{2+}$ in the simulation at a Ca-O bond distance of about 2.3 angstroms. The Mg-O bond distance in the Alchemy simulation is typically about 2.1 angstroms.
Figure 5-6. Metal ion-binding geometry in the CD site.

(a) Sixfold Mg$^{2+}$ coordination.

(b) Sevenfold Ca$^{2+}$ coordination.

(a) This CD site frame is taken from the Mg$^{2+}$ phase in the Alchemy simulation. The coordinating sidechain oxygen of glutamate 62 at position 12 of the loop is 1.99 angstroms from the Mg$^{2+}$ ion. The noncoordinating oxygen is 2.97 angstroms from the Mg$^{2+}$. (b) In this CD site snapshot taken of a frame during the Ca$^{2+}$ phase of the Alchemy simulation, the uppermost oxygen is 2.24 angstroms from the Ca$^{2+}$, and the other oxygen is 2.58 angstroms from the Ca$^{2+}$ ion. In this simulation the glutamate successfully mimics wild-type metal ion-binding geometry.
The glutamate that is the last coordinating residue of the binding loop exhibited bidentate coordination of Ca\(^{2+}\) and monodentate coordination of Mg\(^{2+}\) in the Alchemy simulation. This is seen quite clearly in graphs (a) and (b) from the simulation where the serine at position 5 stayed at least partially coordinated during the Mg\(^{2+}\) phase. Part (a) represents the sidechain oxygen that stayed coordinated to the metal ion throughout the simulation. In this simulation, Mg\(^{2+}\) was bound at the beginning, the radius increased to represent Ca\(^{2+}\) in the second phase, and then transitioned back to Mg\(^{2+}\) at the end. The sidechain oxygen in part (b) did not coordinate the metal ion when the radius corresponds to Mg\(^{2+}\), but did bind Ca\(^{2+}\). Graphs (c) and (d) are from the simulation where the serine at position 5 does not coordinate the Mg\(^{2+}\) ion. In this simulation the first stage represents Ca\(^{2+}\) in the loop, the second stage Mg\(^{2+}\), and then in the final stage the ion reverts back to Ca\(^{2+}\). It is clear that the oxygen in part (c) is bound to the metal ion throughout the simulation. However, the sidechain oxygen represented in part (d) should move away from the ion during frame number 700 to frame number 1200. It does not, because there are fewer ligating oxygens in this site due to the movement of serine 55, away from the Mg\(^{2+}\). Nevertheless, this sidechain oxygen does not move in as close to the Mg\(^{2+}\) as the other one does.
Figure 5-8. Metal ion coordination by the glutamate at position 9 of the CD loop.

(a) GLU 59 OE1 distance to calcium.

(b) GLU 59 OE2 distance to calcium.

(a) One of the sidechain carboxylate oxygens of glutamate 59 stays too far away from the metal ion to coordinate during the entire Alchemy simulation. (b) The other oxygen coordinates Mg$^{2+}$ at a distance of about 2.0 angstroms in the first and last stages of the simulation, and coordinates Ca$^{2+}$ at a distance of about 2.2 angstroms during the middle simulation stage. This glutamate exhibits monodentate coordination of both species of metal ions in the simulation, as it is known to do in crystal structures.
apparent reason for the system to select the glutamate at position 12 for bidentate coordination of Ca$^{2+}$ over the glutamate at position 9 would be because the geometry of the binding loop prescribes that choice. This observation is a strong indication that the crucial role the residue at position 12 plays in transforming the coordinating sphere through reducing the number of ligands by one when Mg$^{2+}$ is bound, and decreasing the radius of the coordination sphere by extending one oxygen toward the Mg$^{2+}$ ion, is not solely attributable to the length and flexibility of the glutamate sidechain. Instead, it seems this role is at least partly assigned to the residue at position 12 as a function of the structural configuration of the loop.

5.5 Alchemy in the EF site.

The ability to reproduce known parvalbumin metal ion-binding behavior was particularly important in the EF site because the E101D mutation is located in this site. To be able to proceed beyond the Alchemy simulation to simulations mimicking the effects of the E101D mutation, it had to be shown first that this model could suitably represent the wild-type binding site.

Residue numbers 90, 92 and 94, corresponding to loop positions 1, 3, and 5 of the EF site, are all aspartates. As in the CD site, the aspartates in the EF loop displayed wild-type Ca$^{2+}$- and Mg$^{2+}$-binding behavior (Figure 5-9). Similarly, the carbonyl oxygen of methionine 96 behaved well in the Alchemy simulation (Figure 5-10 a). The greatest challenge to the satisfactory depiction of metal ion-binding behavior in the EF
Figure 5-9. The aspartates in the EF loop of the Alchemy simulation.

(a) ASP 90 OD1 distance to calcium. 

(b) ASP 90 OD2 distance to calcium.

(c) ASP 92 OD1 distance to calcium. 

(d) ASP 92 OD2 distance to calcium.

(e) ASP 94 OD1 distance to calcium. 

(f) ASP 94 OD2 distance to calcium.

The coordinating aspartate residues in the EF loop all mimic wild-type metal ion-binding behavior, as shown in graph (a) - (f). In each case, one of the carboxylate sidechain oxygens is bound to the metal ion, and one is not, throughout the entire Alchemy simulation.
Figure 5-10. The carbonyl oxygen and water molecule in the EF site.

(a) MET 96 O distance to calcium.

(b) H$_2$O distance to calcium.

(a) The carbonyl oxygen behaved well in the Alchemy simulation. (b) The water molecule is constrained to stay within 2.37 angstroms of Ca$^{2+}$, and within 2.15 angstroms of Mg$^{2+}$. In the simulation illustrated in both parts (a) and (b), the starting and ending radius is indicative of Ca$^{2+}$ and the middle phase of the simulation contains Mg$^{2+}$. 
loop was properly representing the water molecule that coordinates the cation at position 9 of the loop. Glycine 98 is the residue at position 9 of the EF site. It has no sidechain, thus a water molecule is recruited into the loop for metal ion-binding. Unfortunately, in our model, this water molecule migrated out of the binding pocket.

It is to be expected that there might be exchange between the EF site water molecule and solvent molecules, but in our simulations the water in the binding pocket was not ever replaced by another solvent molecule. It is known from the many crystal structures of EF-hands that have a water molecule at position 9 that this water exhibits a high degree of occupancy. Water therefore cannot wander out of the pocket never to return, as it does in our model. The CHARMm TIP3 water parameters are employed in NAMD, and although these parameters strike a good balance between a reasonable representation for solvent without the generation of excessive computational expense, they are not sophisticated enough to impart appropriate metal ion-binding behavior in the EF loop. It should be noted, though, that it was not our goal in these simulations to predict the behavior of water. On the contrary, the primary goal was to test the behavior of the carboxylate sidechains in the Ca$^{2+}$-binding sites. Hence, the expedient solution to this difficulty with the water at EF loop position 9 was to simply tether the water molecule to the metal ion. Therefore, a Ca$^{2+}$-water oxygen bond distance constraint was enforced on the water molecule in the binding site through changes in the parameter files (see section 2.12), and this bond distance was decreased during the simulation when Mg$^{2+}$ was represented in the loop (Figure 5-10 b).
Figure 5-11. Metal ion-binding geometry in the EF site.

(a) Sixfold Mg$^{2+}$ coordination.

(b) Sevenfold Ca$^{2+}$ coordination.

(a) This snapshot represents a frame of the Alchemy simulation during the Mg$^{2+}$ phase. The coordinating oxygen of the glutamate at EF loop position 12 is 2.07 angstroms from the Mg$^{2+}$ ion. The noncoordinating oxygen is 3.42 angstroms from the Mg$^{2+}$. (b) Both oxygens coordinate Ca$^{2+}$ in this snapshot taken from the Ca$^{2+}$ phase of the Alchemy simulation. The uppermost sidechain oxygen of glutamate 101 is 2.15 angstroms from the Ca$^{2+}$ ion, while the other is 2.48 angstroms from the ion.
As in the CD site, the glutamate at the last coordinating position of the loop showed bidentate coordination of Ca\(^{2+}\), with a reversible transition to monodentate coordination of Mg\(^{2+}\) (Figure 5-11, 5-12). This glutamate was correctly predicted to assume bidentate coordination over three other aspartate carboxylate sidechains that also coordinate metal ions in this loop. This result is probably at least partially dictated by the position of the glutamate oxygens with respect to the other coordinating oxygens in the binding loop. In other words, there are no nearby steric hindrances that would prevent both position 12 sidechain oxygens from binding Ca\(^{2+}\). Also, the other coordinating sidechains are cushioned within the binding loop, where nearby residues can easily make intramolecular hydrogen bonds with the noncoordinating carboxylate oxygens. In contrast, the position 12 glutamate is positioned at the outermost edge of the loop and thus, is very solvent-exposed (Figure 5-13). This leaves both of the carboxylate oxygens very available to the bound metal ion. Hence, it is likely that these circumstances conferred on the residue at position 12 by the structural composition of the loop determine its role as the transitional factor in Ca\(^{2+}\) coordination versus Mg\(^{2+}\).

5.6 Summary

The Alchemy simulation established that the NAMD pike parvalbumin model was able to satisfactorily represent the transition from sevenfold Ca\(^{2+}\) coordination to sixfold Mg\(^{2+}\) coordination in the EF-hand binding sites. This process was shown to be reversible in the simulation by proceeding through three stages. First, the Ca\(^{2+}\)-bound
Figure 5-12. The glutamate at EF site position 12 in the Alchemy simulation.

(a) GLU 101 OE1 distance to calcium.

(b) GLU 101 OE2 distance to calcium.

(a) This glutamate sidechain oxygen coordinates the Alchemy simulation metal ion during all three phases (Ca^{2+}/Mg^{2+}/Ca^{2+}). (b) The second sidechain oxygen stays bound to Ca^{2+} in the first and last stages of the simulation, but moves out of coordination distance in the Mg^{2+} stage of the simulation. This result accurately mimics the bidentate coordination of Ca^{2+}, and the monodentate coordination of Mg^{2+}, that is observed in parvalbumin crystal structures.
Figure 5-13. The glutamate at position 12 is solvent-exposed.

The glutamates that are the last coordinating residues of both Ca$^{2+}$-binding sites in wild-type parvalbumin are highlighted in yellow in this stereo diagram. The stereo view shows that the glutamates are isolated at the edge of the EF-hand loop, and therefore less able to make intramolecular hydrogen bonds than the other coordinating sidechains in the binding site.
stage exhibited sevenfold pentagonal bipyramid coordination geometry, then the Mg$^{2+}$-bound stage transitioned to sixfold octahedral coordination geometry, and lastly, the simulation returned to the Ca$^{2+}$-bound stage and sevenfold coordination.

The Alchemy simulation correctly predicted that the glutamate at position 12 would be the carboxylate sidechain to switch between bidentate ligation of Ca$^{2+}$ and monodentate Mg$^{2+}$ coordination. Because the aspartate and glutamate carboxylate sidechain oxygens were all represented with a uniform charge distribution, the fact that the model was still able to select the glutamate at position 12 as the intermediary factor between sevenfold and sixfold coordination shows that structural effects govern this choice, as opposed to electrostatic effects. These structural effects likely include the position of the glutamate oxygens with respect to the other coordinating oxygens in the binding loop, and the isolation of the position 12 residue from the rest of the protein, making it less likely to form intramolecular hydrogen bonds, and more available for Ca$^{2+}$ coordination.

Finally, the Alchemy simulation exposed a trend for the serine at position 5 to be reluctant to coordinate Mg$^{2+}$. It is possible that this result is because of the rudimentary representation for serine in the MD simulation, and further investigation would be required to infer definitively that serine is a poor Mg$^{2+}$ coordinator. On the other hand, there are no published structures that contain serine in an EF-hand binding site wherein that serine directly coordinates Mg$^{2+}$. Therefore, it is possible that the Alchemy simulation may be predicting an authentic property of serine, one that EF-hand proteins might use to discriminate between Mg$^{2+}$ and Ca$^{2+}$.