I, Samrish Variyath, hereby submit this original work as part of the requirements for the degree of Master of Science in Chemical Engineering.

It is entitled:
Molecular Dynamics Simulations of Pseudomonas cepacia lipase in aqueous solutions

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Molecular Dynamics Simulations of *Pseudomonas cepacia* lipase in Aqueous Solutions

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Abstract:

We have performed molecular dynamics simulation of *Pseudomonas cepacia* lipase (PcL) in aqueous solutions under different solution environments. We have also performed simulations of PcL with and without its structurally important Ca$^{++}$ ion. Our simulations have demonstrated the movements of the helical oligopeptide ‘lid’ region of PcL so as to decrease or increase access to its active site cavity. We have discussed the structural integrity of the active site geometry based on root mean squared displacement of the active site residues. The structural interdependence of the lid region and the active site residues has also been established. The effect of point charges on oppositely charged residues of PcL and their effect on secondary and tertiary structure have been demonstrated. Finally, we have made predictions on the immobilization of PcL on positively charged membranes like SBA-15-NH$_2$ based on electrostatic charge density calculations.
Nomenclature:

\( U \) – potential energy, kcal/mol

\( k_i \) – spring constant for bond, angle or dihedral, kcal/mol

\( r_i \) – bond length or distance, Å

\( \Theta_i \) – bond angle, degrees

\( \Phi_i, \gamma_i \) – dihedral angles, degrees

\( q \) – charge, coulomb

\( \sigma \) – finite distance at which inter-particle potential is zero, Å

\( \varepsilon \) – well depth, kcal/mol

\( m \) – mass, kg

\( n \) – multiplicity

\( C_\alpha \) – protein backbone carbon atom
CHAPTER 1 – Introduction:

Part 1

The hydrolysis of esters produces acids and alcohols. In biological systems this process is catalyzed by esterases. Due to the inherent diversity of biological systems, there are different uses of the hydrolysis of esters. As a result, a variety of esterases that significantly differ in their substrate specificity and their biological function exists. [1]

Lipases are one such class of esterases. They are water soluble and catalyze esterification, transesterification, alcoholysis and acidolysis reactions, among others. [1] They are robust in their catalytic action and diverse in their substrate specificity. [2] These properties make them industrially important enzymes. [3] Their characteristic functionality arises from the fact that lipases are activated at the hydrophobic/hydrophilic interface by the preferential movement of a helical oligopeptide unit, often called the ‘lid’, away from its active site, which then participates in the hydrolysis reaction. [4][5]

Lipases do not require cofactors for activation and other aspects of their catalytic functions. However, their activity depends on the presence of a lipid-water interface that exposes their active site. *Pseudomonas cepacia* lipase (PcL) is used as the model lipase in this MS thesis research, in which case, the active site consists of the amino acid residues HIS-286, SER-87 and ASP-264. [5][6]

The helical oligopeptide unit of PcL forming the so-called ‘lid’ [6] that moves preferentially so as to expose the active site in the presence of the hydrophobic-hydrophilic interface is comprised of residues 118 to 166 of the enzyme amino acid sequence. In lipases, the movement of this lid region is facilitated by the presence of a flexible ‘hinge’ region. [7] This stretch of residues is generally referred to as the U1 domain. The structure of lipase is such, that the active site is shielded by the ‘lid’ region in its inactive, i.e., so-called closed conformation. [4][5][8]

As a class, lipases have a long history of being employed as biocatalysts. They catalyze the hydrolysis and synthesis of acyl alcohols, with high regio and stereo selectivity, and have been utilized to: [9][10]

Desymmetrize prochiral molecules by hydrolysis or acylation and
Resolve (kinetic resolution) racemic mixtures \textit{via} hydrolysis or acylation.

Lipases exhibit different structural configurations under different solution conditions, which have a significant impact on their biocatalytic behavior \cite{11,12}. The in-silico modeling of the behavior of the enzyme at different pH, ionic strength and temperature environments in order to understand its biocatalytic activity has not been investigated to date and is the main objective of this MS thesis research.

As lipases are often used as model enzymes for interfacial activation, their crystal structures, as coordinates, are widely available in standard databases. The protein data bank has a range of free and inhibitor complexed enzyme coordinates for lipases. These structures have been used to demonstrate essential motions and active site orientations of lipases in previous studies.

\textbf{Part 2: Essential Dynamics of} \textit{Pseudomonas cepacia} lipase:

The essential motions of PcL have been mapped previously by Lee et al \cite{13}. They conducted molecular dynamics (MD) simulations of PcL in two solvent environments of vacuum and water. The vacuum simulations were considered to model lipase in a hydrophobic environment.

The authors used the essential dynamics approach \cite{14}. This approach first removes all translations and rotations from the trajectory of the MD simulation. A covariance matrix is then built from the atomic fluctuations of this modified trajectory. The matrix is then diagonalized using the QL algorithm \cite{15}. This provides a set of eigenvectors with corresponding eigenvalues.

Using this approach, the authors successfully identified the essential motions of PcL to two domains, namely the U1 (residues 118-166) domain and the U2 (residues 214-261) domain by plotting the absolute value components of their eigenvectors obtained from the protein backbone carbon atom coordinate covariant matrix of the trajectories exported from their simulations.
Lee et al found that the essential motions of lipase were concentrated in the U1 (residues 118-166) and U2 (residues 214-261) domains. They particularly noted the large deviations for the loop regions between the 4th and 5th α helices (residues 128-133) and between the 3rd and 4th β strands (residues 221-222) and suggest that these regions play a major role in the flexibility of their respective domains. They attributed the accessibility of the active site region to the rigidity of the 4th and 5th α helices and the relative flexibility of the loops that connect them.

The catalytic triad of PcL and its lid domains has been identified by previous studies. However, the exact activation mechanism that governs the enzyme’s catalytic action still remains poorly understood. Our objective is the complete characterization of the conformational changes of PcL observed at the atomic level. This research is different from previous molecular dynamics studies of PcL at equilibrium conditions, when its lid regions are either in the closed or open conformation.

In order to understand the dynamics of PcL better, we have chosen different solvation conditions of ionic strength and temperature. The effect of the Ca++ cation in the structural rigidity and on
the active site cavity of the enzyme is explored by MD simulations of PcL with and without this ion. To our knowledge, this is the first such effort for PcL.

The standard protein databases have provided only the open conformation of PcL, either in the free, or in the bound inhibitor form. The coordinates of the closed conformation are not available, but this conformation is predicted both by sequence similarity with other lipase species and by the structural properties of PcL. One of our objectives is to confirm the presence of the closed conformation of PcL wherein the lid domains completely block access of potential substrate to its active site cavity.

CHAPTER 2 – Background:

Part 1: Biocatalytic Membrane Nanosystems

Membrane surfaces for immobilization of enzymes find applications in catalysis, medicine, bioadsorption. The design of highly selective membranes that capture only target proteins can greatly enhance protein separation technology, as well as control desired reaction rate.\textsuperscript{[16][17][18][19]}

Enzyme immobilization is employed to facilitate the separation of reaction products from the biocatalyst while preserving or even improving catalytic activity as compared to a free enzyme in solution. It is desirable to develop methods to control protein surface interactions for applications like biocatalysis, protein recognition and assays for biomedical application.\textsuperscript{[20][21]}

Enzyme encapsulation involves the restriction of an enzyme within an adequately designed retaining device so that the enzyme cannot escape, while reactants and products can freely move into and out of the device. The enzyme may be immobilized on the walls of the encapsulation system, or may be suspended in free solution.

A typical encapsulation and immobilization system, i.e. a Biocatalytic Membrane Nanosystems (BMN), consists of a nano-engineered three layer membrane reactor; a silica nanoreactor layer containing 20-100 nm voids sandwiched between 5-10 nm thick silica layers interconnected with \~2.5 nm mesopores. The silica nanoreactor voids are interconnected through 10-30 nm windows. The middle silica layer will hold the encapsulated enzymes while the outer mesoporous layers
will serve as a selective barrier preventing loss of enzymes while permitting the entry of reactants and exit of products. This BMN concept is part of a National Science Foundation (NSF) funded Nanoscale Interdisciplinary Research Team (NIRT) research collaboration between the University of Cincinnati and Arizona State University.

![Typical design of a Biocatalytic Membrane Nanosystems (BMN) reactor.](image)

**Fig. 2**: Typical design of a Biocatalytic Membrane Nanosystems (BMN) reactor.

In order to understand the process of encapsulation it is necessary to understand the surface chemistry and the biocatalytic functions of encapsulated enzymes. BMN reactors can be designed for protein immobilization or free solution inside membrane nanopores. In both cases, the protein will be entrapped as the size of the nanopores is typically smaller than the enzyme dimensions. Surface chemistry of the BMNs can be designed to control the extent of immobilization. Protein surface electrostatics can be used to design complementary membrane surfaces for better immobilization, higher protein selectivity and enhanced catalytic activity.\(^{[22]}\)\(^{[23]}\) Furthermore, surface chemistry of a protein can lead to the design of membranes with functional groups that uniquely interact with a target protein, which can find applications in protein separation technology.\(^{[24]}\) Finally, there are also some noteworthy protein shape based detection techniques.\(^{[25]}\)

The encapsulation and immobilization of enzymes provides a number of other advantages over free enzymes. Some of these are:\(^{[26]}\)
1. Elimination of the possibility of releasing any genetically altered organisms to the environment.
2. Significant enhancement of biocatalytic activity as compared to free enzyme due to close proximity of enzymes to substrates in nanoscale voids;
3. Fine-tuning of surface chemistry in nanoscale voids via grafting of functional groups (and cofactors, if required) to control enzyme-surface interactions, reactivity and stereo-/regio selectivity;
4. Significantly enhanced efficiency due to high enzyme loading and high mass-transfer rate through large nanopores, flow-through regime and thin-membrane Biocatalytic Membrane Nanosystems (BMN) design;
5. Fine-tuning of the nanoscale size of spherical voids for a particular enzyme and substrate;
6. Ability to use non-aqueous reaction medium to keep reactants and products in solution.

This MS thesis represents the first stage in the process of the development of BMNs where *Pseudomonas cepacia* lipase (PcL) is simulated in free solution in different buffer environments in order to model its conformational changes in free solution and relate observed molecular dynamics (MD) effects to available kinetic data.

**Part 2: Molecular Dynamics**

Molecular dynamics (MD) is a form of computer simulation in which atoms and molecules are allowed to interact for a period of time by approximations of classical physics, resulting in the motion of the atoms and molecules. Because molecular systems generally consist of a vast number of particles, it is impossible to find the properties of such complex systems analytically; MD simulation circumvents this problem by using numerical methods. [27] Protein molecular geometries can be drawn from their published crystal structures which represent the average values of bond lengths and angles. Such models can be created for extremely large molecules in relatively short periods of time. Moreover, the user can visualize molecular scale phenomena with a molecular dynamics (MD) representation far better than in an experiment. Interacting molecules, intermediate states of reaction and active sites of enzymes can be represented *In*
Silico in MD, rather than their existence being speculated by a variety of proposed reaction mechanisms. MD represents an interface between laboratory experiments and theory, and can be understood as a "virtual experiment."

In molecular dynamics, the atoms in the system follow the Newtonian equation of motion given by:

$$m\ddot{r}_\alpha = -\frac{\partial}{\partial r_\alpha} U_{total}(\vec{r}_1, \vec{r}_2, \vec{r}_3, \ldots, \vec{r}_N); \alpha = 1, 2, 3, \ldots, N$$

(1)

m is the mass; r is its position, N is the number of atoms and

$U_{total}$ is the potential energy that depends on all atomic positions and thereby couples the motion of atoms.

In molecular dynamics, the potential energy function represented below is most crucial for the accuracy of the results as it is the input for the simulations that perform calculations on the system in question. The function therefore has to be accurate enough to ensure the credibility of the results that are obtained from the calculations for which it is an input.

The potential energy function generally has the following contributions:

$$U_{total} = U_{bond} + U_{angle} + U_{dihedral} + U_{vdW} + U_{Coulomb}$$

(2)

Where,

$$U_{bond} = \sum_{bonds \ i} k_i^{bond} (r_i - r_{0i})^2$$

(3)

$$U_{angle} = \sum_{angles \ i} k_i^{angle} (\theta_i - \theta_{0i})^2$$

(4)

$$U_{dihedral} = \sum_{dihedral \ i} k_i^{dihedral} [1 + \cos (n_i \phi_i - \gamma_i)] \quad \text{when } n_i \neq 0$$

$$= \sum_{dihedral \ i} k_i^{dihedral} (\theta_i - \gamma_i)^2 \quad \text{when } n_i = 0$$

(5)

(6)

$$U_{vdW} = \sum_i \sum_{j>1} 4C_{ij} \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^6$$

(7)

$$U_{Coulomb} = \sum_i \sum_{j>1} \frac{q_i q_j}{4\pi \epsilon r_{ij}}$$

(8)
In the above equations,

- $U_{\text{bond}}$ is the potential energy of stretching of bonded interactions between atoms.
- $U_{\text{angle}}$ is the potential energy associated with the bending of bonded interactions between atoms.
- $U_{\text{dihedral}}$ is the potential energy associated with the torsion of bonded interactions between atoms.
- $U_{\text{vdW}}$ is the potential energy associated with the van der Waal’s forces approximated by the Lennard-Jones 6-12 potential.
- $U_{\text{Coulomb}}$ is the potential energy associated with the electrostatic interactions.

Also, $\theta$ is the angle term, $\phi$ is the dihedral angle. The parameter $k_i$ represent the spring constants for bonds, angles or dihedrals. The parameter $r_i$ is the bond length in Å, while $r_{0i}$ represents the equilibrium length. The term $r_{ij}$ signifies the distance between the atoms $i$ and $j$. $\gamma$ represents the equilibrium dihedral angle. The charge on the atom is represented by $q$ in coulomb. $\epsilon$ represents the well depth in kcal/mol.\[29\]

Chemistry at Harvard Molecular Mechanics (CHARMM)\[30\] and Assisted Model Building with Energy Refinement (AMBER)\[31\] are examples of force fields that are used in MD.

Nanoscale Molecular Dynamics (NAMD)\[29\] is molecular dynamics (MD) software developed by the Theoretical Nanoscale Biophysics Group (TBG) at Illinois’ Beckman Institute, and is available free of charge for non-commercial use by individuals and academic and research institutions. It is developed using the CHARMM\[30\] parallel programming model, which is noted for its parallel efficiency (using gigabit ethernet) and is therefore used to model large systems (like solvated proteins), typically comprising of millions of atoms.

NAMD uses Visual Molecular Dynamics (VMD)\[32\] for simulation setup and trajectory analysis. NAMD is a valuable tool for MD of large systems like proteins in solution because of the recent advancements in both molecular biology and parallel supercomputing, and is further advocated by the affordability of modern supercomputing power.
Part 3: Periodicity in Molecular dynamics

In molecular dynamics, it is advantageous to represent the system under study in the form of a periodic cell. By using cells, the user ensures that the conditions under which a simulation is performed needs to be represented only once, and that the particles that are enclosed in that cell are replicated to infinity by periodic translations. Thus, there is no need for the user to replicate a whole system, but just a representative part of it with the requisite properties so that the properties of the entire system can be extrapolated from those of the representative cell. MD software ensures that the potential of the cell is conserved by elimination of surface effects. This is achieved by exposing every particle to potential from all particles within the cell and also those in the surrounding ones.\[^{[29]}\]

Periodicity is also useful in determining the full system electrostatics. Since periodicity ensures that every cell is identical to the other, this is done in MD using the particle-mesh Ewald summation method.\[^{[29]}\] The Ewald sum has four terms, namely direct sum, reciprocal sum, self-energy sum and the surface energy sum, as they appear respectively in the equation below.

\[
E_{\text{Ewald}} = E_{\text{dir}} + E_{\text{rec}} + E_{\text{self}} + E_{\text{surface}}
\] (9)

\[
E_{\text{dir}} = \frac{1}{2} \sum_{i,j=1}^{N} q_i q_j \sum_{\vec{n}_r} \text{erfc}(\beta |\vec{r}_i - \vec{r}_j + \vec{n}_r|) - \sum_{(i,j) \in \text{Excluded}} \frac{q_i q_j}{|\vec{r}_i - \vec{r}_j + \vec{v}_{ij}|}
\] (10)

\[
E_{\text{rec}} = \frac{1}{2\pi V} \sum_{\vec{m} \neq \vec{0}} \exp\left(-\frac{\pi^2 |\vec{m}|^2}{\beta^2}\right) |\sum_{i=1}^{N} q_i \exp(2\pi i \vec{m} \cdot \vec{r}_i)|^2
\] (11)

\[
E_{\text{self}} = -\frac{\beta}{\sqrt{\pi}} \sum_{i=1}^{N} q_i^2
\] (12)
\[ E_{surface} = \frac{2\pi}{(2\varepsilon_s + 1)V} \sum_{i=1}^{N} |q_i \vec{r}_i|^2 \]  \hspace{1cm} (13)

In the above equations, \( q_i \) and \( r_i \) are the charge and position of the atom \( i \) respectively and \( n \) is the lattice vector, \( \beta \) is a parameter adjusting the workload distribution for direct and reciprocal sums, \( \varepsilon_s \) is the dielectric constant of the surrounding medium.

The van der Waals potential, describing pairwise interatomic interactions in the system, is truncated at a user-defined cutoff distance in MD simulations beyond which it may be neglected.[29]

In addition to the above potentials, certain forces may be applied by the user to guide the system to a desired configuration of interest. These are generally referred to as steered or interactive molecular dynamics.[27]

**CHAPTER 3 – Objectives:**

The objectives of this research are listed below:

1. To demonstrate the ‘lid’ movements of PcL via MD so as to limit or increase access to its active site cavity. Confirm the presence of the ‘closed’ structure of PcL
2. To determine the influence of counter ions on the structure of PcL
3. To determine the factors affecting the active site geometry and the coordination among active site residues
4. To establish the significance of the Ca\(^{++}\) ion in the structural rigidity of PcL and on its active site geometry
5. To identify possible sites for the effective immobilization of PcL on a surface
CHAPTER 4 – Method:

We used the Nanoscale Molecular Dynamics (NAMD) package in our simulations. Initial coordinates of *Pseudomonas cepacia* lipase were obtained from the Protein Data Bank (PDB) website [10.2210/pdb3LIP/PDB]. The open structure of PcL, represented by the PDB id 3LIP [33] was selected. The CHARMM force field was chosen as it accurately represents large systems, such as solvated proteins [30][34]. CHARMM is an all-atom force field. [35] VMD served as the primary visual interface and its TK console was used to process input data, build initial systems for the purpose of simulation and analyze results. XMGRACE [36] is the primary data presentation software.

All simulations are characterized by PcL surrounded by a periodic water box, which has dimensions of approximately 65 Å x 65 Å x 65 Å. The box size is such that there is at least a 10 Å layer of water surrounding PcL in all directions. Counter ions are added to this box as needed. Two different counter ions were used, which included KCl and NaCl. Biological systems, especially proteins, are known to discriminate between these two ions and sodium tends to interact stronger than potassium with the surfaces of proteins. [37] Also, as point charges, monoatomic ions were shown previously [38] to interact with charged side chains of proteins. Similar effects of the chosen counter ion on charged PcL side chains will be determined. Four additional positive ions are added so as to compensate for the negative charge of PcL [33] and maintain the system in a charge-neutral state. HIS-286 residues were maintained in a neutral state. [39] Two different temperatures were used for each counter ion concentration in order to determine temperature dependence of enzyme flexibility. [40] The Particle Mesh Ewald Sum (PME) [29] method was used for full system periodic electrostatics. The short range van der Waals forces were truncated at a cutoff distance of 12 Å [29].

TIP3 [41] water molecules were used to represent water for the solvation. The periodic box was subject to constant number of particles, temperature and pressure (NPT) constraints.

All simulations were run on the Ohio Supercomputing Center’s (OSC) Glenn cluster. Every simulation was carried out on 10 nodes with 4 processors each. A timestep of 2 fs was used with an equilibration for 50,000 steps. A simulation production runtime of 5,000,000 steps gave us the system dynamics over 10 ns [42].
A snapshot of the system was taken every 4 ps of the simulation for simulations without any counter ions and 10 ps for simulations with counter ions. This was to enable visualization of the system trajectory with the progress of the simulation as well as to calculate Root Mean Squared Deviations (RMSD) of the protein backbone and individual residues. The difference between initial and final positions of the atoms in a structure can be calculated by first superimposing the two structures. This eliminates any differences that arise due to the rotation and translation of a structure during simulation. The positional difference between the atoms in the two structures is called the RMSD and is typically calculated using the formula:

$$\text{RMSD}_\alpha = \sqrt{\frac{\sum_{j=1}^{N_t} \sum_{\alpha=1}^{N_{\alpha}} (\vec{r}_\alpha(t_j) - \overline{\vec{r}}_\alpha)^2}{N_{\alpha}}}$$

Where: $N_{\alpha}$ is the number of atoms whose positions are being compared, $N_t$ is the number of steps over which atomic positions are being compared, $\vec{r}_\alpha(t_j)$ is the position of atom $\alpha$ at time $(t_j)$ and $\overline{\vec{r}}_\alpha$ is the average value of the position of atom $\alpha$ to which the positions of $\vec{r}_\alpha(t_j)$ are being compared.

The essential motions of PcL, i.e. the movements of the U1 (residues 118-166) and U2 (residues 214-261) domains that lead to enzyme activation-deactivation, were compared to those simulated by others [13], in order to validate our methodology. A typical simulation runtime was 70 hours.

NAMD assigns initial velocities to atoms based on the Maxwell – Boltzmann energy distribution and the system temperature. Once assigned, the simulation should maintain the velocities of the atoms so as to follow this distribution. [29] In order to validate our work, we have calculated the correlation coefficient between the final system velocities for our simulations and that predicted...
by the Maxwell–Boltzmann distribution at that temperature for every simulation. We have found that in all of our simulations, this correlation coefficient is greater than 0.93, indicating a greater than 93% agreement with the velocities predicted by the distribution.
CHAPTER 5 – Results:

Results: Part 1 – Simulations with Ca\(^{++}\) ion and no counter ions at 298 K

Graph Set 1: The RMSD of the protein backbone indicates that the system has equilibrated. The RMSD of the individual residues indicates that most residues have large deviations (RMSD > 4 Å). We believe that this is because of the absence of counter ions that balance the charge on PcL. The RMSD of the active site residues are 3.1 Å for SER-87, 3.1 Å for ASP-264 and 4.5 Å for HIS-286. The average RMSD per residue is 3.9 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is approximately 0.95.

Results: Part 2 – Simulations without the Ca\(^{++}\) ion and no counter ions at 298 K

Graph Set 2: The RMSD of the protein backbone indicates that the system has equilibrated. During the simulation, we observed a large movement of the U2 (residues 214-261) domain. This movement is attributed to the absence of the calcium ion and the lack of counter ions balancing the charge on PcL. The RMSD of the individual residues indicates that most residues have large deviations (RMSD > 4 Å). We believe this is because of the absence of the structurally important Ca\(^{++}\) ion and the absence of counter ions that balance the charge on PcL. The RMSD of the active site residues are 2.0 Å for SER-87, 3.9 Å for ASP-264 and 2.4 Å for HIS-286. The average RMSD per residue is 3.2 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is approximately 0.95.
Results: Part 3 – Simulations with Ca$^{++}$ ion

1. P$c$L in water box with 0.1 M KCl counter ions at 310 K

**Graph set 3:** The RMSD of the protein backbone indicates that the system has not equilibrated as towards the end the RMSD is still rising. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 3.4 Å for SER-87, 4.2 Å for ASP-264 and 5.1 Å for HIS-286. The average RMSD per residue is 4.0 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is approximately 0.95.

2. P$c$L in water box with 0.1 M KCl counter ions at 298 K.

**Graph set 4:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are other spikes that are significant at residue number 41, 70 and 286 The RMSD of the active site residues are 3.1 Å for SER-87, 3.6 Å for ASP-264 and 4.0 Å for HIS-286. The average RMSD per residue is 4.5 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is approximately 0.95.
3. **PcL in water box with 0.1 M NaCl counter ions at 310 K.**

   **Graph set 5:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant RMSD peaks for residues 25 – 40 and residues 285 – 290. The RMSD of the active site residues are 2.7 Å for SER-87, 3.8 Å for ASP-264 and 5.1 Å for HIS-286. The average RMSD per residue is 3.9 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is approximately 0.94.

4. **PcL in water box with 0.1 M NaCl counter ions at 298 K.**

   **Graph set 6:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 1.7 Å for SER-87, 1.9 Å for ASP-264 and 3.4 Å for HIS-286. The average RMSD per residue is 2.9 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
5. *PcL* in water box with 0.2 M KCl counter ions at 310 K.

**Graph set 7:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U2 (RESIDUES 214-261) domain. There are significant RMSDs for residues 20-45, 75 and residues 285-290. The RMSD of the active site residues are 2.6 Å for SER-87, 3.0 Å for ASP-264 and 4.0 Å for HIS-286. The average RMSD per residue is 3.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94.

6. *PcL* in water box with 0.2 M KCl counter ions at 298 K.

**Graph set 8:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 1.7 Å for SER-87, 1.8 Å for ASP-264 and 2.5 Å for HIS-286. The average RMSD per residue is 2.8 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
7. *PcL* in water box with 0.2 M NaCl counter ions at 310 K.

**Graph set 9:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant peaks at residues 25, 47 and 285. The RMSD of the active site residues are 2.1 Å for SER-87, 2.2 Å for ASP-264 and 2.8 Å for HIS-286. The average RMSD per residue is 3.8 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

8. *PcL* in water box with 0.2 M NaCl counter ions at 298 K.

**Graph set 10:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U2 (RESIDUES 214-261) domains. There are significant peaks at residues 25, 75 and 290. The RMSD of the active site residues are 1.9 Å for SER-87, 2.9 Å for ASP-264 and 3.6 Å for HIS-286. The average RMSD per residue is 3.6 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.93.
9. *PcL in water box with 0.3 M KCl counter ions at 310 K.*

**Graph set 11:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 2.5 Å for SER-87, 2.9 Å for ASP-264 and 3.7 Å for HIS-286. The average RMSD per residue is 2.8 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

10. *PcL in water box with 0.3 M KCl counter ions at 298 K.*

**Graph set 12:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There is a significant spike at resid 41. The RMSD of the active site residues are 3.0 Å for SER-87, 2.9 Å for ASP-264 and 3.4 Å for HIS-286. The average RMSD per residue is 3.4 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
11. PcL in water box with 0.3 M NaCl counter ions at 310 K.

**Graph set 13:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant spikes for resids 285-300. The RMSD of the active site residues are 2.8 Å for SER-87, 3.1 Å for ASP-264 and 4.2 Å for HIS-286. The average RMSD per residue is 3.1 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

12. PcL in water box with 0.3 M NaCl counter ions at 298 K.

**Graph set 14:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant spikes for residues 285-300. The RMSD of the active site residues are 1.8 Å for SER-87, 3.1 Å for ASP-264 and 4.5 Å for HIS-286. The average RMSD per residue is 2.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
13. \textit{PcL in water box with 0.4 M KCl counter ions at 310 K.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{graph15}
\caption{Graph set 15: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 4.2 Å for SER-87, 4.0 Å for ASP-264 and 4.7 Å for HIS-286. The average RMSD per residue is 4.2 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.}
\end{figure}

14. \textit{PcL in water box with 0.4 M KCl counter ions at 298 K.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{graph16}
\caption{Graph set 16: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 3.2 Å for SER-87, 3.4 Å for ASP-264 and 3.6 Å for HIS-286. The average RMSD per residue is 3.9 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.}
\end{figure}
15. PcL in water box with 0.4 M NaCl counter ions at 310 K.

Graph set 17: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There is a significant spike at resids 41, 71 and 290. The RMSD of the active site residues are 2.3 Å for SER-87, 4.3 Å for ASP-264 and 4.5 Å for HIS-286. The average RMSD per residue is 4.0 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

16. PcL in water box with 0.4 M NaCl counter ions at 298 K.

Graph set 18: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 2.9 Å for SER-87, 2.6 Å for ASP-264 and 3.6 Å for HIS-286. The average RMSD per residue is 3.2 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.93.
17. PcL in water box with 0.5 M KCl counter ions at 310 K.

Graph set 19: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There is a significant increase in RMSDs at residues 286 – 300. The RMSD of the active site residues are 3.7 Å for SER-87, 3.6 Å for ASP-264 and 4.6 Å for HIS-286. The average RMSD per residue is 3.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

18. PcL in water box with 0.5 M KCl counter ions at 298 K.

Graph set 20: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 3.1 Å for SER-87, 3.1 Å for ASP-264 and 3.5 Å for HIS-286. The average RMSD per residue is 3.4 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
19. PcL in water box with 0.5 M NaCl counter ions at 310 K.

Graph set 21: The RMSD of the protein backbone indicates that the system has not equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant spikes at resids 21, 41, 75 and 300. The RMSD of the active site residues are 2.2 Å for SER-87, 2.7 Å for ASP-264 and 3.4 Å for HIS-286. The average RMSD per residue is 3.8 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

20. PcL in water box with 0.5 M NaCl counter ions at 298 K.

Graph set 22: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 1.9 Å for SER-87, 2.7 Å for ASP-264 and 3.2 Å for HIS-286. The average RMSD per residue is 2.9 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.93.
21. *PcL* in water box with 0.6 M KCl counter ions at 310 K.

**Graph set 23:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant spikes at resid 21 and 85 – 300. The RMSD of the active site residues are 1.8 Å for SER-87, 3.6 Å for ASP-264 and 3.7 Å for HIS-286. The average RMSD per residue is 4.1 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.93.

22. *PcL* in water box with 0.6 M KCl counter ions at 298 K.

**Graph set 24:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There are significant spikes at residues 25-50 and residues 285-300. The RMSD of the active site residues are 1.5 Å for SER-87, 2.8 Å for ASP-264 and 4.4 Å for HIS-286. The average RMSD per residue is 3.6 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
23. *PcL* in water box with 0.6 M NaCl counter ions at 310 K.

**Graph set 25:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD $> 4$ Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. There is a significant spike at resid 55. The RMSD of the active site residues are 2.5 Å for SER-87, 2.5 Å for ASP-264 and 3.1 Å for HIS-286. The average RMSD per residue is 3.0 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

24. *PcL* in water box with 0.6 M NaCl counter ions at 298 K.

**Graph set 26:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD $> 4$ Å) residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 2.3 Å for SER-87, 2.7 Å for ASP-264 and 3.0 Å for HIS-286. The average RMSD per residue is 3.1 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
Results: Part 4 – Simulations without Ca\(^{++}\) ion

1. PcL in water box with 0.1 M KCl counter ions at 310 K.

Graph set 27: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 0.8 Å for SER-87, 1.4 Å for ASP-264 and 5.5 Å for HIS-286. The average RMSD per residue is 1.7. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94605.

2. PcL in water box with 0.1 M KCl counter ions at 298 K.

Graph set 28: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. Residues from 285-310 also show high RMSDs. The RMSD of the active site residues are 0.8 Å for SER-87, 1.5 Å for ASP-264 and 5.1 Å for HIS-286. The average RMSD per residue is 1.5 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
3. **PcL in a water box with 0.1 M NaCl counter ions at 310 K.**

   ![Graph set 29](image)

**Graph set 29:** Though the RMSD of the protein backbone is climbing till about 6 ns, it dips thereafter and reaches around 2 Å towards the end. We can conclude that the system has equilibrated. The RMSD of the individual residues indicates that the most mobile (RMSD > 4 Å) residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. Residues from 285-310 also show high RMSDs. The RMSD of the active site residues are 0.7 Å for SER-87, 1.3 Å for ASP-264 and 5.0 Å for HIS-286. The average RMSD per residue is 1.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

4. **PcL in a water box with 0.1 M NaCl counter ions at 298 K.**

   ![Graph set 30](image)

**Graph set 30:** Although the RMSD of the protein backbone indicates is lower than 3 Å throughout the simulation, it is rising and never reaches the desired flat profile. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. However, this simulation indicates very high RMSDs for most residues. The RMSD of the active site residues are 2.9 Å for SER-87, 2.6 Å for ASP-264 and 5.2 Å for HIS-286. The average RMSD per residue is 3.9 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
5. **PcL in a water box with 0.2 M KCl counter ions at 310 K.**

Graph set 31: The RMSD of the protein backbone is fairly flat from 6 ns into the simulation onwards. This indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 3.6 Å for SER-87, 2.7 Å for ASP-264 and 7.0 Å for HIS-286. The average RMSD per residue is 4.8 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94.

6. **PcL in a water box with 0.2 M KCl counter ions at 298 K.**

Graph set 32: The RMSD of the protein backbone shows a fairly flat profile till about 5.3 ns where we observe a spike. The profile then settles down for another half of a nanosecond before rising from about 1.5 Å to 2.5 Å at 10 ns. The RMSD of the individual residues indicates that almost all the residues have a very high deviation. The RMSD of the active site residues are 3.7 Å for SER-87, 4.1 Å for ASP-264 and 7.5 Å for HIS-286. The average RMSD per residue is 5.2 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
7. *PcL* in a water box with 0.2 M NaCl counter ions at 310 K.

**Graph set 33:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime, although we observe a spike at about 8.3 ns. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domain. Residues from 285-310 also show high RMSDs. The RMSD of the active site residues are 0.7 Å for SER-87, 0.8 Å for ASP-264 and 5.9 Å for HIS-286. The average RMSD per residue is 1.5 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94.

8. *PcL* in a water box with 0.2 M NaCl counter ions at 298 K.

**Graph set 34:** Although the RMSD of the backbone keeps rising from about 4 ns to 8.2 ns, it dips thereafter, indicating that the system is stable at the end of the run. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 3.2 Å for SER-87, 3.5 Å for ASP-264 and 5.5 Å for HIS-286. The average RMSD per residue is 3.8 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
9. *PcL* in a water box with 0.3 M KCl counter ions at 310 K.

Graph set 35: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domain. Residues from 285-310 also show high RMSDs. The RMSD of the active site residues are 0.7 Å for SER-87, 1.7 Å for ASP-264 and 5.8 Å for HIS-286. The average RMSD per residue is 1.6 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94.

10. *PcL* in a water box with 0.3 M KCl counter ions at 298 K.

Graph set 36: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime though we observe a spike at 8 ns. The RMSD of the individual residues indicates that all the residues have very high RMSDs. The RMSD of the active site residues are 3.1 Å for SER-87, 3.8 Å for ASP-264 and 6.0 Å for HIS-286. The average RMSD per residue is 4.0 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.93.
11. PcL in a water box with 0.3 M NaCl counter ions at 310 K.

Graph set 37: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domain and the residues from 285-310. The RMSD of the active site residues are 0.68 Å for SER-87, 1.2 Å for ASP-264 and 5.7 Å for HIS-286. The average RMSD per residue is 1.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

12. PcL in a water box with 0.3 M NaCl counter ions at 298 K.

Graph set 38: The RMSD of the protein backbone increases until 5 ns, goes through a bump from 6 ns to 7 ns and then stays below 3 Å for another 2 ns and finally peaks above and dips below 3 ns again. The RMSD of the individual residues shows us that most residues have very high RMSDs. The RMSD of the active site residues are 2.7 Å for SER-87, 3.0 Å for ASP-264 and 7.2 Å for HIS-286. The average RMSD per residue is 4.3 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
13. *PcL in a water box with 0.4 M KCl counter ions at 310 K.*

**Graph set 39:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains and the residues 285-310. The RMSD of the active site residues are 0.67 Å for SER-87, 1.0 Å for ASP-264 and 4.6 Å for HIS-286. The average RMSD per residue is 1.5 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

14. *PcL in a water box with 0.4 M KCl counter ions at 298 K.*

**Graph set 40:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 2.1 Å for SER-87, 1.9 Å for ASP-264 and 4.5 Å for HIS-286. The average RMSD per residue is 3.0 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
15. *PcL* in a water box with 0.4 M NaCl counter ions at 310 K.

**Graph set 41:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains and residues 285-310. The RMSD of the active site residues are 0.75 Å for SER-87, 2.0 Å for ASP-264 and 6.1 Å for HIS-286. The average RMSD per residue is 2.0 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

16. *PcL* in a water box with 0.4 M NaCl counter ions at 298 K.

**Graph set 42:** The RMSD of the protein backbone indicates that the system is quite stable for about 8 ns but has a few sharp peaks towards the end. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. Overall, the individual residues have high RMSDs. The RMSD of the active site residues are 3.6 Å for SER-87, 3.8 Å for ASP-264 and 6.0 Å for HIS-286. The average RMSD per residue is 4.4 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
17. PcL in a water box with 0.5 M KCl counter ions at 310 K.

Graph set 43: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domain. There is also a significant peak between residues 285-310. The RMSD of the active site residues are 0.7 Å for SER-87, 1.5 Å for ASP-264 and 6.9 Å for HIS-286. The average RMSD per residue is 1.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

18. PcL in a water box with 0.5 M KCl counter ions at 298 K.

Graph set 44: The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 2.1 Å for SER-87, 2.2 Å for ASP-264 and 4.0 Å for HIS-286. The average RMSD per residue is 3.4 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
19. *PcL* in a water box with 0.5 M NaCl counter ions at 310 K.

**Graph set 45:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 0.6 Å for SER-87, 2.0 Å for ASP-264 and 4.1 Å for HIS-286. The average RMSD per residue is 1.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.95.

20. *PcL* in a water box with 0.5 M NaCl counter ions at 298 K.

**Graph set 46:** The RMSD of the protein backbone indicates that the system has not equilibrated as the curve is still rising. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domains. The RMSD of the active site residues are 2.1 Å for SER-87, 2.9 Å for ASP-264 and 4.7 Å for HIS-286. The average RMSD per residue is 3.3 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
21. *PcL* in a water box with 0.6 M KCl counter ions at 310 K.

**Graph set 47:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U1 (RESIDUES 118-166) and U2 (RESIDUES 214-261) domain. Residues 285-310 also show significant RMSDs. The RMSD of the active site residues are 0.6 Å for SER-87, 1.6 Å for ASP-264 and 4.8 Å for HIS-286. The average RMSD per residue is 1.5 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94.

22. *PcL* in a water box with 0.6 M KCl counter ions at 298 K.

**Graph set 48:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most residues have high RMSDs. The RMSD of the active site residues are 3.0 Å for SER-87, 4.4 Å for ASP-264 and 3.8 Å for HIS-286. The average RMSD per residue is 3.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.94.
23. *PcL* in a water box with 0.6 M NaCl counter ions at 310 K.

**Graph set 49:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain. The RMSD of the active site residues are 2.1 Å for SER-87, 4.0 Å for ASP-264 and 7.4 Å for HIS-286. The average RMSD per residue is 4.3 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 310 K is 0.94.

24. *PcL* in a water box with 0.6 M NaCl counter ions at 298 K.

**Graph set 50:** The RMSD of the protein backbone indicates that the system has equilibrated over the simulation runtime even though we see a sharp spike towards the end. The RMSD of the individual residues indicates that the most mobile residues are those from the U2 (RESIDUES 214-261) domain, however, RMSDs are very high for all residues. The RMSD of the active site residues are 4.4 Å for SER-87, 4.0 Å for ASP-264 and 5.8 Å for HIS-286. The average RMSD per residue is 4.7 Å. The correlation coefficient of the kinetic energy distribution with the Maxwell Boltzmann energy distribution at 298 K is 0.95.
I. RMSDs of the active site residues

In all our simulations, we began with PDB coordinates of the open conformation of PcL in which the U1 (residues 118-166) and U2 (residues 214-261) domains of PcL have conformations such that the active site cavity of PcL is exposed to solvent. Therefore, any substrate present in solution has access to the active site cavity. The active site residues of PcL are SER-87, ASP-264 and HIS-286. These residues play specific roles in PcL catalyzed reactions, which include proton transfer and binding of intermediates. The sequential progress of reaction depends on the position of the active site residues with respect to one another and with respect to the substrate and any intermediates. If the position of any of these residues is altered significantly, the desired catalytic reaction may not progress at desired rates. It is, therefore, highly important to monitor the geometry and orientation of the active site residues throughout the progress of our simulations. Apart from visualizing the changes by following the system trajectory to the end of the simulation, we have used RMSD values of the Ca atoms of the active site residues to determine if active site geometry and configuration was retained or lost. The following tables show the active site residue RMSDs for each simulation.

<table>
<thead>
<tr>
<th>Table 1: RMSDs of the active site residues of PcL for 0.1 M to 0.3 M counter ions concentration with Ca^{++} ion.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>SER-87</td>
</tr>
<tr>
<td>ASP-264</td>
</tr>
<tr>
<td>HIS-286</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: RMSDs of the active site residues of PcL for 0.4 M to 0.6 M counter ions concentration with Ca^{++} ion.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue</td>
</tr>
<tr>
<td>SER-87</td>
</tr>
<tr>
<td>ASP-264</td>
</tr>
<tr>
<td>HIS-286</td>
</tr>
</tbody>
</table>
Table 3: RMSDs of the active site residues of PcL for 0.1 M to 0.3 M counter ions concentration without Ca$^{++}$ ion.

<table>
<thead>
<tr>
<th>Residue</th>
<th>0.1 M KCl 310 K</th>
<th>0.1 M NaCl 310 K</th>
<th>0.2 M KCl 310 K</th>
<th>0.2 M NaCl 310 K</th>
<th>0.3 M KCl 310 K</th>
<th>0.3 M NaCl 310 K</th>
<th>0.2 M KCl 298 K</th>
<th>0.2 M NaCl 298 K</th>
<th>0.3 M KCl 298 K</th>
<th>0.3 M NaCl 298 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>SER-87</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
<td>2.9</td>
<td>3.6</td>
<td>3.7</td>
<td>0.7</td>
<td>3.2</td>
<td>0.7</td>
<td>3.1</td>
</tr>
<tr>
<td>ASP-264</td>
<td>1.4</td>
<td>1.5</td>
<td>1.3</td>
<td>2.6</td>
<td>2.7</td>
<td>4.1</td>
<td>0.8</td>
<td>3.5</td>
<td>1.7</td>
<td>3.8</td>
</tr>
<tr>
<td>HIS-286</td>
<td>5.5</td>
<td>5.1</td>
<td>5.0</td>
<td>5.2</td>
<td>7.0</td>
<td>5.9</td>
<td>5.5</td>
<td>5.8</td>
<td>6.0</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Table 4: RMSDs of the active site residues of PcL for 0.4 M to 0.6 M counter ions concentration without Ca$^{++}$ ion.

<table>
<thead>
<tr>
<th>Residue</th>
<th>0.4 M KCl 310 K</th>
<th>0.4 M NaCl 310 K</th>
<th>0.5 M KCl 310 K</th>
<th>0.5 M NaCl 310 K</th>
<th>0.6 M KCl 310 K</th>
<th>0.6 M NaCl 310 K</th>
<th>0.5 M KCl 298 K</th>
<th>0.5 M NaCl 298 K</th>
<th>0.6 M KCl 298 K</th>
<th>0.6 M NaCl 298 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>SER-87</td>
<td>0.7</td>
<td>2.1</td>
<td>0.7</td>
<td>3.6</td>
<td>0.7</td>
<td>2.1</td>
<td>0.6</td>
<td>2.1</td>
<td>0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>ASP-264</td>
<td>1.0</td>
<td>1.9</td>
<td>2.0</td>
<td>3.8</td>
<td>1.5</td>
<td>2.2</td>
<td>2.0</td>
<td>2.9</td>
<td>1.6</td>
<td>4.4</td>
</tr>
<tr>
<td>HIS-286</td>
<td>4.6</td>
<td>4.5</td>
<td>6.1</td>
<td>6.0</td>
<td>6.9</td>
<td>4.0</td>
<td>4.1</td>
<td>4.7</td>
<td>4.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Since RMSD values of 4 Å or greater are considered high [29], we can see that HIS-286 residues show very high RMSDs for all the simulations. The lowest RMSD values are for SER-87. ASP-264 also has low RMSD values for most simulations, but crosses the 4 Å limit in 3 out of 24 cases modeled.

Protein conformation in a solvent depends on the hydropathy indices [44] of its constituent amino acid residues. Hydropathy indices are a type of hydrophobicity scale that ranks amino acids according to their relative hydrophobicities. Kyte et al [44] have noted that the hydropathy index is calculated using the transfer free energy, which is derived from the partition coefficient of an amino acid between water and a non interacting isotropic phase, like ethanol. Typically, in aqueous environments, the most hydrophobic (non–polar, positive hydropathy index) amino acids are found hidden inside the folds of the tertiary structure of the protein, while the most hydrophilic (polar, negative hydropathy index) amino acids are exposed to solvent. [45]

The following table shows some important characteristics of amino acids. [44][45]
Table 5: Three letter codes of amino acids with their hydrophobicities, charges and hydropathy indices.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>3 Letter Code</th>
<th>1 Letter Code</th>
<th>Side chain polarity</th>
<th>Side chain charge (pH 7.4)</th>
<th>Hydropathy index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>ALA</td>
<td>A</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>1.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>ARG</td>
<td>R</td>
<td>Polar</td>
<td>positive</td>
<td>-4.5</td>
</tr>
<tr>
<td>Asparagine</td>
<td>ASN</td>
<td>N</td>
<td>Polar</td>
<td>neutral</td>
<td>-3.5</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>ASP</td>
<td>D</td>
<td>Polar</td>
<td>negative</td>
<td>-3.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>CYS</td>
<td>C</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>2.5</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>GLU</td>
<td>E</td>
<td>Polar</td>
<td>negative</td>
<td>-3.5</td>
</tr>
<tr>
<td>Glutamine</td>
<td>GLN</td>
<td>Q</td>
<td>Polar</td>
<td>neutral</td>
<td>-3.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>GLY</td>
<td>G</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>-0.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>HIS</td>
<td>H</td>
<td>Polar</td>
<td>90% neutral 10% positive</td>
<td>-3.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>ILE</td>
<td>I</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>4.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>LEU</td>
<td>L</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>3.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>LYS</td>
<td>K</td>
<td>Polar</td>
<td>positive</td>
<td>-3.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>MET</td>
<td>M</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>1.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>PHE</td>
<td>F</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>2.8</td>
</tr>
<tr>
<td>Proline</td>
<td>PRO</td>
<td>P</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>-1.6</td>
</tr>
<tr>
<td>Serine</td>
<td>SER</td>
<td>S</td>
<td>Polar</td>
<td>neutral</td>
<td>-0.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>THR</td>
<td>T</td>
<td>Polar</td>
<td>neutral</td>
<td>-0.7</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>TRP</td>
<td>W</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>-0.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>TYR</td>
<td>Y</td>
<td>Polar</td>
<td>neutral</td>
<td>-1.3</td>
</tr>
<tr>
<td>Valine</td>
<td>VAL</td>
<td>V</td>
<td>Nonpolar</td>
<td>neutral</td>
<td>4.2</td>
</tr>
</tbody>
</table>

If we consider the amino acid residues surrounding the active site residues, the RMSDs observed during the above simulations can be explained.

a. SER-87 is the only residue between a β-sheet and α-helix and forms a ‘turn’ between them. Although SER is a polar amino acid, the residues surrounding it are primarily non–polar. The residues 81-86 are VAL, ASN, LEU, VAL, GLY and HIS. The β-sheet formed
from these residues consists of 66.67% non–polar residues and has a total hydropathy index of 5.1. The residues 88-99 are GLN, GLY, GLY, LEU, THR, SER, ARG, TYR, VAL, ALA, ALA and VAL. The α-helix formed from these residues consists of 58.33% non–polar residues and has a total hydropathy index of 4.2. The influence of these strong non–polar groups keeps SER-87 in the hydrophobic pocket of the protein and therefore, reflects its low RMSDs in the simulations.

b. ASP-264 is the first residue on a ‘turn’ that consists of residues 264-273. Although ASP is a polar residue, the turn itself is 60% non-polar. The residues that make up this turn are GLY, VAL, VAL, SER, LYS, CYS, SER, ALA and LEU. This region has a total hydropathy index of 7.1. These residues hence play a major role in keeping ASP-264 in the active site cavity, which is reflected in the low RMSDs observed for it in most simulations. Its higher mobility in comparison to that of SER-87 can be explained by the fact that SER-87 is located directly between two secondary structures, which helps retain its position. On the other hand, ASP-264 is located between a coil and a turn, giving its position more flexibility.

c. HIS-286 is part of a α helix formed by residues 285-290. The residues from 285-290 are 66.67% polar and consists of amino acids ASN, HIS, LEU, ASP, GLU and ILE. This region has a total hydropathy index of -5.4. Therefore, this set of residues has a potential for moving away from the hydrophobic center of the protein and have more contact with the solvent. Also, ASN, HIS, ASP, and GLU have very high negative hydropathy indices among amino acids. In addition, aspartic acid and glutamic acid are both negatively charged. As we have used 6 more potassium ions than chloride ions in solution, the solvent is positively charged with respect to the protein. Consequently the region comprising residues 285-290 may further move away from the hydrophobic protein center, where the active site is located. This explains the high RMSDs observed for HIS-286 in all simulations. The RMSD peaks observed for residues 285-305 in graph sets 3-26 above corroborate this observation.
II. Difference in RMSDs due to presence or absence of the Ca$^{++}$ ion.

We have performed simulations with PcL solvated under different counter ions environments and two different temperatures, with and without the presence of the structurally important calcium ion. Similar studies on lipases have been conducted by others $^{[46]}$ who have observed the low energies of unfolding of the Staphylococcus aureus and Staphylococcus hyicus lipases in the absence of the structurally important calcium ion. It has been speculated by others $^{[33]}$ that the calcium ion in PcL could play an important stabilizing role in its active site orientation. We have therefore run simulations with and without this ion in order to determine its influence on the catalytic site geometry.

Lipases typically exhibit a calcium binding pocket. The calcium ion in PcL is four coordinated with PcL. $^{[33],[47]}$ It forms bonds with the carbonyl oxygens of GLN-292, VAL-296 and the delta oxygen atoms of ASP-242 and ASP-288. This bonding confers a cis conformation to the bond between GLN-292 and LEU-293, which in turn allows a hydrogen bond between the amide hydrogen of LEU-293 and the side chain of ASP-242. This complex structure is lost in the absence of the calcium ion from its site.

The average RMSDs per residue for all the simulations performed are tabulated below. From Tables 6-9 it can be observed that the average RMSD per residue increases significantly for the simulations of PcL without the calcium ion. This indicates an increased plasticity for the region surrounding the calcium ion, which contributes to the overall increase in the total RMSD for the protein backbone. The Tables 6-9 therefore demonstrate the importance of the calcium ion in the structural rigidity of PcL. Other subtle influences of this ion on the active site geometry are discussed below.

| Table 6: Average RMSDs of the individual residues of PcL with Ca$^{++}$ at counter ions conc. 0.1 M – 0.3 M |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 0.1 M            | 0.2 M            | 0.3 M            |
| K                | Na               | K                | Na               | K                | Na               |
| 310 K            | 298 K            | 310 K            | 298 K            | 310 K            | 298 K            |
| 4                | 1.2*             | 4.5              | 1.3*             | 3.9              | 1.9*             | 2.9              | 0.9*             | 2.8              | 0.9*             | 2.8              | 0.5*             | 8.0*             | 3.5*             | 3.1*             | 0.8*             | 2.7*             | 0.8*             |

*indicates standard deviation values for that temperature and counter ion concentration
Table 7: Average RMSDs of the individual residues of PcL with Ca\(^{++}\) at counter ions conc. 0.4 M – 0.6 M

<table>
<thead>
<tr>
<th></th>
<th>0.4 M</th>
<th>0.5 M</th>
<th>0.6 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>310 K</td>
<td>298 K</td>
<td>310 K</td>
</tr>
<tr>
<td>Na</td>
<td>310 K</td>
<td>298 K</td>
<td>310 K</td>
</tr>
</tbody>
</table>

*indicates standard deviation values for that temperature and counter ion concentration.

Table 8: Average RMSDs of the individual residues of PcL without Ca\(^{++}\) ion at counter ions conc. 0.1 M – 0.3 M

<table>
<thead>
<tr>
<th></th>
<th>0.1 M</th>
<th>0.2 M</th>
<th>0.3 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>310 K</td>
<td>298 K</td>
<td>310 K</td>
</tr>
<tr>
<td>Na</td>
<td>310 K</td>
<td>298 K</td>
<td>310 K</td>
</tr>
</tbody>
</table>

*indicates standard deviation values for that temperature and counter ion concentration.

Table 9: Average RMSDs of the individual residues of PcL without Ca\(^{++}\) ion at counter ions conc. 0.4 M – 0.6 M

<table>
<thead>
<tr>
<th></th>
<th>0.4 M</th>
<th>0.5 M</th>
<th>0.6 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>310 K</td>
<td>298 K</td>
<td>310 K</td>
</tr>
<tr>
<td>Na</td>
<td>310 K</td>
<td>298 K</td>
<td>310 K</td>
</tr>
</tbody>
</table>

*indicates standard deviation values for that temperature and counter ion concentration.

The importance of the calcium ion for the catalytic functions of lipases has been demonstrated by others.\(^ {46}\) These studies have also demonstrated that the free energy of unfolding of Staphylococcal lipases is significantly lower in the absence of the calcium ion, indicating the lack of concerted movements of vital parts of the enzyme that is often associated with catalytic activation.

Here, we have explained the importance of the calcium ion in the structural rigidity of PcL and gauged its impact on the active site geometry.

As indicated by the higher average RMSDs for PcL without the calcium ion, our results are in agreement with the results obtained by urea unfolding experiments of Simons \textit{et al.}\(^ {44}\) who observed low energy of unfolding of the Staphylococcal lipase. The calcium ion in PcL forms bonds with residues on three different secondary structures. The residues GLN-292, VAL-296 are on the same coil. The residue ASP-242 is on the coil between helices α8 and α9. ASP-288 is the first residue on the α helix formed by residues 288 -290. As a result, our simulations without this ion resulted in poor alignment and coordination between these secondary structural elements.
of PcL. The location of the calcium ion is also very close to the surface of PcL, and the residues it binds are mostly hydrophilic. In the absence of the calcium ion, these polar residues find more freedom in the aqueous solvent, and are displaced further from their initial positions, contributing to the overall higher RMSD of the enzyme.

**III. Effect of the Ca++ ion on the active site geometry:**

The Ca++ ion located near the active site cavity of PcL, \(^{[32, 47]}\) is particularly influential on the position of HIS-286. As reported by others, the calcium ion is six-coordinated to four oxygen atoms of different amino acid residues of PcL and two water molecules. \(^{[48]}\) In the PDB coordinates of PcL, the calcium ion makes contacts with the residues ASP-242, ASP-288, GLN-292 and VAL-296. These bonds with the oxygen atoms of the above residues are very strong and maintained during all of our simulations runs. The bond that the calcium ion makes with ASP-288 is, however, the most critical for the active site geometry.

The calcium ion is coordinated to ASP-242, which is the only residue between the \(\alpha8\) and \(\alpha9\) helices. Moreover, as discussed earlier, the \(\alpha9\) helix is the main helix of the U2 domain (residues 214-261) that moves preferentially to either expose or limit access to the PcL active site. Therefore, when the \(\alpha9\) helix moves, it drags the residue ASP-242, and consequently the calcium ion with it. As the calcium ion is strongly coordinated to ASP-288, this residue experiences the drag imposed by the movement of the U2 domain (residues 214-261), and consequently the active site residue HIS-286 which is just one residue apart in the enzyme sequence. Therefore the calcium ion indirectly influences the stability and configuration of the active site.

The location of the calcium ion is different in different lipases and may be as much as 30 Å off in pancreatic lipase. \(^{[33]}\) This indicates a unique stabilizing role for the ion. In order to understand the role of the calcium ion in greater detail, we have run simulations of PcL without the calcium ion, all other simulation conditions being the same. We observed that without the direct bonding of the residues ASP-242, ASP-288, GLN-292 and VAL-296 with the calcium ion, the region around these residues became structurally very weak, the polar characteristics of the two ASP residues dominated and the region around HIS-286 had much greater RMSDs than those that would retain active site configuration. Histidine RMSDs without the calcium ion ranged from
4.009 Å to 7.532 Å, with an average of 5.590. In contrast, the HIS-286 RMSDs with the calcium ion ranged from 2.495 Å to 5.180 Å, with an average of 3.816 Å, about 1.774 Å lesser. This demonstrates the stabilizing effect of the calcium ion on the active site geometry.

In addition, we have observed that the α9 helix may partially unravel and adopt a coil structure near its C terminus, involving 2-3 residues (PRO-243, SER-244 and THR-245), so as to reduce the impact of its movement on the active site. The increased flexibility of the coil structure as compared to the helix structure allows increased movement of the α9 helix, without tugging on the calcium ion complex. This influence is also attributable to the calcium ion and its strong bond with ASP-242.

The strong influence of calcium ion over the residues ASP-242, ASP-288, GLN-292 and VAL-296 may also unravel the helix formed by residues 286-290 and confer to it a more flexible coil structure. This is observed in simulations with 0.2 M KCl at 310 K, 0.3 M KCl at 310 K, 0.3 M NaCl at 310 K and 0.3 M NaCl at 298 K.

**IV. Decreased access to the active site as a result of the lid movement:**

![Fig 4: The decreased access to the PcL active site cavity due to lid movement. The left and right snapshots are the last frames of the simulation for 0.1 M KCl counter ions at 310 K. The left picture shows PcL active site, U1 domain (residues 118-166) and U2 domain (residues 214-261). The right snapshot shows the entire PcL in cartoon representation. The center picture shows PcL at the beginning of the simulation. Notice that the α-8 helix comprising residues 246-258 of the U2 domain (residues 214-261) moves towards the U1 domain (residues 118-166) as the simulation progresses. The distance between these helices decreases from 20.78 Å to 13.03 Å.

As discussed earlier, PcL has two lid domains, i.e., U1 (residues 118-166) and U2 (residues 214-261). Others have pointed out that the lid domains move preferentially to expose the active site in the presence of a hydrophobic interface. Our simulations reveal that the movement of the lid domains may also significantly influence the active site geometry. This is primarily because the U2 domain (residues 214-261) is located very close to the active site residue ASP-264, i.e.,
separated by only 2 residues (GLN-262 and ASN-263), which are both polar and neutral. Our simulations have shown that the large α-helix formed by residues 246-258 is connected to ASP-264 by a coil formed by residues 258-263. When this helix moves inward, so as to close access to the active site, it tugs on this coil causing ASP-264 to move away from the active site cavity and from the other active site residues. We also observed that the G-10 helix formed by residues 269-271 loses its secondary structure, and becomes a coil when the U2 domain (residues 214-261) moves inward. The G-10 helix has a lysine residue at position 269 in the sequence and the strong interaction with negatively charged chloride ions may justify the loss of the secondary structure. Two chloride ions stay in close proximity to the negatively charged LYS-269 for one nanosecond of the simulation time for PcL at 310 K in 0.1 M KCl counter ions. The following snapshot of the simulation illustrates this point. The same effect is observed for the simulation in 0.2 M KCl at 310 K.

**Fig. 5:** Chloride ion interacting with LYS-269. Chloride ions are shown in yellow, LYS-269 is shown in white, ASP-264 is shown in red. PcL is in ‘cartoon’ representation. The van der Waals spheres of the ion and LYS-269 are observed to overlap. Notice the loss of secondary structure around LYS-269, which is possibly due to the strong interaction with the chloride ion, movement of the U2 domain (residues 214-261), or a combination of both.

### V. Effect of ions on the stability of PcL:

In most of our simulations, we have observed that the RMSDs of the active site of PcL in the NaCl counter ions were lower than the RMSDs observed for KCl counter ions, all other simulation conditions being the same. This trend is also true for the average RMSDs per residue.

Previous studies [49, 50] have shown that sodium interacts more strongly with negatively charged ASP and GLU residues than potassium. The lower RMSDs of PcL in the NaCl counter ions as compared to those in KCl observed in our simulations are in agreement with these findings.

Studies conducted by others [51] have suggested that charged protein unfolding may be triggered by columbic repulsions, whereas neutral protein unfolding may be triggered by centrifugal forces. The centrifugal unfolding of the neutral protein was induced by the authors by using *in vacuo* boundary conditions (free rotations and translations) together with coupling with a
Berendsen thermostat at 293 K. The authors contend that the coupling of the dihedral torsions and the bending modes in the potential energy function they used allowed them to simulate the behavior of a non–rigid molecular rotor.

The authors [51] have gone on to suggest that denatured proteins have typically been found to exist in highly charged states, while proteins that are low in charge are compact, and have a native structure. Other studies [52] have also suggested that the repulsion of charged groups on protein surfaces may decrease the stability of the protein.

As in our simulations, the positively charged sodium ions show strong interaction with the negatively charged amino acid residues of PcL, and since the PcL structure 3LIP we have chosen from the protein data bank has 4 negative charges, we can infer that the ability of PcL to undergo unfolding due to columbic repulsions is diminished in the case of the NaCl counter ions simulations. This is not so in the case of KCl counter ions as the potassium ions tend not to interact with the negatively charged amino acids of PcL as strongly. We therefore observe higher RMSDs for the simulations with the KCl counter ions than the corresponding simulations with equal concentration of NaCl counter ions.

The immobilization of enzymes has been shown in numerous studies to be pH and ionic strength dependent. [53-60] The ionic effects on protein surfaces have been demonstrated to follow a Hofmeister series with the ions on the left side of the series increasing the hydrophobic effects of proteins, leading to them salt out. [61-64] This effect can be used in protein separation, as the more hydrophobic proteins salt out of the solution first. Salis et al. [53] have reported that the amount of adsorbed lysozyme on SBA-15-NH2 silica host follows the Hofmeister series up to concentrations (sodium salts of SCN–, ClO4–, Br–, NO3–, Cl–, SO42–, chloride salts of Na+, Li+, K+, Cs+) of 0.5 M. and that sodium is the only cation for which the amount of adsorbed enzyme does not decrease at 0.2 M counter ion concentration. We have low active site RMSDs for PcL in 0.2 M NaCl counter ion at 298 K, and if PcL shows the same adsorption trends as lysozyme on SBA-15-NH2, it would be interesting to measure its catalytic activity experimentally at the conditions used for our simulations.

If such experiment provides us with high catalytic activity, we can conclude high enzyme loading with high catalytic activity, one of our objectives in the BMN project.
Modified Force Field for MD simulations with ion specific effects:

It is been shown in previous studies that solvated ions modify the structure of water. These effects are believed to follow the Hofmeister series as well. Ions can be classified as kosmotropic or chaotropic depending on their tendency to make or break the structure of water respectively. [34-37][65-67]

Kosmotropic ions in water aggregate hydrophobic solutes, while the chaotropic ions destabilize hydrophobic aggregates, thereby enhancing their solubility. The kosmotropic effect of ions is achieved by the formation of reinforced hydrogen bonds between water molecules in the solvation shell of the hydrophobic particle, as compared to those in bulk water. The Muller – Lee – Graziano simulation model for water includes the kosmotropic and chaotropic effects of small ions on water. [68]

Typically, in experiment, if hydration entropy of solutes is negative, they are considered kosmotropic, as the lower entropies can be explained as an effect of increased ordering of solvent molecules in the hydration shells. [69] Ion effects on solvents have also been studied using static and dynamic light scattering. [70] The authors determined individual influences of strongly chaotropic, nearly neutral and strongly kosmotropic ions on the hydration shells of lysozyme under conditions supportive of protein crystallization.

Paschek [69] has validated five different models of water by comparing the densities of simulation boxes over a range of five different temperatures and constant pressure with a finite number of water ions, with those observed by experiment.

Hribar et al [71] have performed simulations by modifying the force field of a ‘Mercedes Benz’ two dimensional structure of water. They chose this structure as they believe that the model accurately represented certain properties of water as solvent and certain other anomalous properties of interest. The authors successfully modified the force field so as to provide qualitatively correct results for water-water liberation free energies. Their modified force field included the potential for ion – water pair interactions, and explicitly excluded dipole interactions between waters. Finally, the authors compared their simulation results with experimental data for the liberation free energies of the ion – water pairing to validate their method.
Protein solvation results in the formation of hydration shells and solvated ions are thought to influence these solvation shells. Although the actual mechanism of ionic influence on solvation shells is based in quantum theory, we propose that its macro effects can be represented in classical MD quite easily, by modification of the structure of water near the ion. This effect, if represented correctly, can greatly influence the MD results for the effect of specific ions on solvation shells and on protein structure. Moreover, simulation studies have shown that the effects of specific ions on protein solvation shells are not significant beyond the first solvation shell. This makes their representation in MD even easier.

Currently, the kosmotropic or chaotropic phenomena are not represented in classical MD software.

Experimental data can provide information that can help include these effects in the force field used in our molecular dynamics simulations. The inclusion of these effects will help generate more accurate MD results for the effect of specific ions on protein structure.

**VII. The conformation of HIS-286:**

Although histidine can be charged or neutral \[^{30,33}\] in PcL. It has a pKa of 6.3 \[^{77}\] and according to table 5, is mostly neutral at physiological pH 7.4. It was therefore maintained neutral in our simulations. Its structure in the active site cavity for residue 286 is shown in Figure 6. In the PDB coordinates 3LIP for PcL, the active site configuration for the enzyme is the same as that expected for the enzyme in a catalytically active state. This arrangement involves a number of hydrogen bonds, a significant few of which are listed below. \[^{33}\]

a. HIS-286 ring protonated nitrogen hydrogen-bonds with the γ oxygen of SER-87.

b. HIS-286 ring deprotonated nitrogen h-bonds with the Δ1 oxygen of ASP-264.

c. The amide nitrogen of HIS-286 forms a hydrogen bond with the carbonyl oxygen of ASP-264.

d. HIS-286 forms a hydrogen bond with GLU-289.

e. Although both amino acids are positively charged, ASP-264 forms a strong hydrogen bond with GLU-289.

**Fig 6:** Histidine in its neutral form, used in all simulations in this work.
In spite of these H-bonds, in all our simulations, we observed that the HIS-286 residue showed high RMSDs. The high RMSDs of HIS-286 were characterized by the early stage breaking of the hydrogen bonds between SER-87 and ASP-264. The residue then continued to distort its geometry and orientation with respect to the other active site residues. This was achieved by the torsion around its β-carbon atom. This carbon atom provides a very high range of movement for the ring of HIS-286, with the equilibrium torsion angle CA – CB – CG – ND1 in the standard CHARMM topology files being 90°. In the CHARMM standard parameter files, the spring constant for this dihedral angle is 0.91 kcal/mol, with a multiplicity (the number of unpaired electrons in the system) of 3 and angle between planes 0°. This allows considerable movement for the ring segment of HIS-286 which leads to its breaking the hydrogen bonds with its neighboring residues early in the simulation. The residue is rendered free to move about due to the lack of hydrogen bonds and for the rest of the simulation time, it moves further away from its starting position, leading to high RMSDs observed for it in all the simulations.

VIII. Lipase immobilization:

Earlier studies have also shown that protein adsorption was dependent on the concentration of ions in solution around the protein. [53] Our goal was to investigate similar relationships in our studies of PcL immobilization on a solid support, and select specific functional groups that can be used for to enhance the immobilization selectivity and loading.

Enzyme immobilization on surfaces is achieved by virtue of van der Waals, hydrophobic and charged interactions. [53,58,60] The effect of pH in the surrounding media on protein-surface interactions has been widely reported. [57,59]

As mentioned earlier, the PcL active site cavity is surrounded by two main domains, namely U1 (residues 118-166) and U2 (residues 214-261). The calcium ion cleft is also near the active site cavity and is strongly bonded to residues near the active site, i.e., HIS-286 and the α8 helix, which is part of the U2 domain. This region of lipase consists of structurally interdependent parts, as shown previously. Therefore, a direct contact with immobilizing surface in vicinity of these sites is not desirable.

It would however, be advantageous to use the region of PcL located opposite to its active site cavity. This region is structurally rigid and contains a number of negatively charged residues, the
movement of which does not affect the active site geometry or orientation. Also, a direct contact of these residues with a surface would mean that the PcL active site cavity, which is on the opposite side, is exposed to solvent.

These negatively charged residues are GLU-63, ASP-56, GLU-197 and ASP-102. These residues form a cluster of good binding sites near the ‘bottom’ of PcL for a positively charged adsorbent, such as SBA-15-NH₂ (point of zero charge (pzc) ~ 10.) \(^{53}\)

We calculated the charge density of the above negatively charged residues to determine if the interaction with SBA-15-NH₂ at this site would be more favorable than other sites on PcL. For the sake of simplicity, we assumed that the 4 amino acids fall inside a 15.5 Å x 10.5 Å rectangular area. This makes the surface area of the negatively charged region 162.75 Å². Since there are 4 negative charges that fall in this region and the unit negative charge is 1.602 x 10⁻¹⁹ C, \(^{78}\)

\[
\text{Charge density } \sigma = \left[ \frac{(4) (1.602 \times 10^{-19})}{162.75} \right] \text{ C/ Å}^2
\]

Therefore \[
\sigma = \left[ \frac{(4) (1.602 \times 10^{-19})}{(162.75) (10^{-10})^2} \right] \text{ C/ m}^2
\]

Which gives us \(\sigma = -0.39373 \text{ C/ m}^2\) for the above negatively charged amino acid cluster.

The surface charge density of SBA-15-NH₂ at pH 7 was found to be approximately 0.375 C/m². \(^{53}\) This charge density can target the cluster of negatively charged residues shown in Fig 7. The charge densities on PcL’s negatively charged cluster and SBA-15-NH₂ are sufficiently complimentary to each other to expect a favorable interaction, and therefore favor adsorption and immobilization of PcL at this site.
**Fig 7:** A: The electrostatic potential density map of PcL. The positively charged regions are shown in blue while the negatively charged regions are shown in red. Notice the large negatively charged region at the center of Fig. 7A. It consists of the amino acid residues GLU-63, ASP-56, GLU-197 and ASP-102. These negatively charged residues of PcL on the opposite side of the active site region. In Fig. 7B, aspartic acid residues are shown in grey, while glutamic acid residues are shown in silver. PcL is in ‘lines’ representation. Notice the high concentration of negatively charged residues in this region, ideal for interaction with positively charged SBA-15-NH₂ at pH 7, when SBA-15-NH₂ is protonated and thereby positively charged with net positive charge about 0.375 C/m². [53]

The above electrostatic potential energy map for PcL was calculated using the Adaptive Poisson–Boltzmann Solver plugin [79] in VMD which calculates the electrostatic potential energy map using water as the probe molecule for generating the Connolly surface. [80] The Connolly surface is defined as the van der Waals surface of a protein that is accessible to a probe molecule with a non-zero radius. [81]

**IX. Comparison of simulation results with experimental data:**

We have observed the decreased access to the active site of PcL in our simulations with low counter ion concentrations. We have also observed distortion of the active site geometry for our simulations at high concentration of counter ion. We therefore do not expect PcL to show activity at any concentration of NaCl or KCl counter ion environments. This is corroborated by experiment. We performed activity measurements for PcL in the same counter ion environments as performed our simulations (in 0.1 – 0.5 M NaCl or KCl counter ions) and found that PcL is inactive in all cases. Our simulation results are therefore in agreement with experimental results.
CHAPTER 7 – Conclusions:

The results of our simulations indicated that the active site of PcL is susceptible to a number of solution parameters that can influence its conformation. The position of the active site residue HIS-286 is such that it is in a 66.67% polar non-secondary structure region and, therefore, would be comfortable having more contact with aqueous solvent. This characteristic has potential to distort the active site geometry of PcL. This region also contains two negatively charged amino acid residues. In the presence of solvent with excess positive charge, these residues push HIS-286 further away from the other active site residues SER-87 and ASP-264. The lack of rigid secondary structure near HIS-286 makes it easier for its movement. The calcium ion near the active site cavity provides a highly stabilizing role by forming bonds with four amino acids from different secondary structures. In our simulations without the calcium ion, we observed an increased movement of HIS-286 away from the active site cavity.

Chloride ions do not interact strongly with the oppositely charged amino acid residues of PcL. This is true for both PcL with or without the stabilizing calcium ion. This conclusion is in good agreement with results obtained by others for other enzyme systems.

The movements of the U1 (residues 118-166) and U2 domains (residues 214-261), in the absence of a hydrophobic – hydrophilic interface and at low concentration of stabilizing counter ions, decrease the active site accessibility. It is characterized by the movement of the α8 helix of the U2 domain towards the α5 helix of the U1 domain.

As previously observed in other studies, sodium ions show strong affinity for the negatively charged aspartic and glutamic acid residues of PcL. This may explain the low RMSDs observed for most simulations in the NaCl counter ion as compared to those in the KCl counter ion under similar simulation conditions.

CHAPTER 8 – Recommendations:

Current parameters for molecular dynamics need to be tested for the kosmotropic/chaotropic characteristics of ions, in order to have better predictive power.
References:


36. Paul J. Turner Center for Coastal and Land-Margin Research Oregon Graduate Institute of Science and Technology Beaverton, Oregon.


Appendix 1: Part 1 – PcL with Ca\textsuperscript{2+} cation

1. PcL in water box with 0.1 M KCl counter ion at 310 K.

Figure 1: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues towards each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

Figure 2: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.\textsuperscript{[47]}
2. **PcL in water box with 0.1 M KCl counter ions at 298 K.**

**Figure 3:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and 'cartoon' representation. Active site residues are in van der Waals representation. Lid domains are in 'bonds' representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues towards each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 4:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL. [47]
3. **PcL in water box with 0.1 M NaCl counter ions at 310 K.**

![Figure 5](image1.jpg)

**Figure 5:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

![Figure 6](image2.jpg)

**Figure 6:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL. [47]
4. *PcL* in water box with 0.1 M NaCl counter ions at 298 K.

**Figure 7:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues towards each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 8:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on *PcL*. 
5. *PcL* in water box with 0.2 M KCl counter ions at 310 K.

**Figure 9:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues towards each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 10:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on *PcL*. 
6. **PcL in water box with 0.2 M KCl counter ions at 298 K.**

**Figure 11:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues towards each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 12:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
7. PcL in water box with 0.2 M NaCl counter ions at 310 K.

**Figure 13:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues towards each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 14:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
PcL in water box with 0.2 M NaCl counter ions at 298 K.

Figure 15: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (resides 118-166) is in pink and U2 (resides 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (resides 118-166) and U2 (resides 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

Figure 16: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
9. *PcL in water box with 0.3 M KCl counter ions at 310 K.*

**Figure 17:** *PcL active site and lid domains.* Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 18:** *PcL with charged residues and counter ions.* Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
10. PcL in water box with 0.3 M KCl counter ions at 298 K.

**Figure 19:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 20:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
11. PcL in water box with 0.3 M NaCl counter ions at 310 K.

Figure 21: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

Figure 22: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
12. PcL in water box with 0.3 M NaCl counter ions at 298 K.

**Figure 23:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 24:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
13. PcL in water box with 0.4 M KCl counter ions at 310 K.

**Figure 25:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 26:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
14. PcL in water box with 0.4 M KCl counter ions at 298 K.

Figure 27: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

Figure 28: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
15. PcL in water box with 0.4 M NaCl counter ions at 310 K.

**Figure 29:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 30:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
16. *PcL* in water box with 0.4 M NaCl counter ions at 298 K.

**Figure 31:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 32:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on *PcL*.
17. PcL in water box with 0.5 M KCl counter ions at 310 K.

**Figure 33:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 34:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
18. PcL in water box with 0.5 M KCl counter ions at 298 K.

**Figure 35:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 36:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
19. PcL in water box with 0.5 M NaCl counter ions at 310 K.

**Figure 37:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 38:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
Figure 39: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and 'cartoon' representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

Figure 40: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
21. **PcL in water box with 0.6 M KCl counter ions at 310 K.**

**Figure 41:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 42:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
22. *PcL* in water box with 0.6 M KCl counter ions at 298 K.

**Figure 43:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 44:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on *PcL*.
23. PcL in water box with 0.6 M NaCl counter ions at 310 K.

Figure 45: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

Figure 46: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on PcL.
24. *PcL* in water box with 0.6 M NaCl counter ions at 298 K.

**Figure 47:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity. The helix formed by residues 288-290 is shown in magenta and the calcium ion is shown in white.

**Figure 48:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact strongly with positively charged residues possibly due to the overall negative charge on *PcL*. 
Appendix 1: Part 2 – PcL without the Ca^{++} cation

1. PcL in water box with 0.1 M KCl counter ions at 310 K.

**Figure 49:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) is in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 50:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
2. 
PcL in water box with 0.1 M KCl counter ions at 298 K.

**Figure 51:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 52:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
3. PcL in a water box with 0.1 M NaCl counter ions at 310 K.

**Figure 53:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 54:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
4. PcL in a water box with 0.1 M NaCl counter ions at 298 K.

**Figure 55:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 56:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
5. **PcL in a water box with 0.2 M KCl counter ions at 310 K.**

**Figure 57:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to decrease access to the active site cavity.

**Figure 58:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
6. *PcL* in a water box with 0.2 M KCl counter ions at 298 K.

**Figure 59:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 60:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on *PcL*. 
7. PcL in a water box with 0.2 M NaCl counter ions at 310 K.

Figure 61: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 62: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
8. PcL in a water box with 0.2 M NaCl counter ions at 298 K.

**Figure 63:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 64:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
9. PcL in a water box with 0.3 M KCl counter ions at 310 K.

Figure 65: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 66: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
10. PcL in a water box with 0.3 M KCl counter ions at 298 K.

Figure 67: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 68: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
11. PcL in a water box with 0.3 M NaCl counter ions at 310 K.

Figure 69: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 70: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
12. PcL in a water box with 0.3 M NaCl counter ions at 298 K.

**Figure 71:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 1. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 72:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
13. PcL in a water box with 0.4 M KCl counter ions at 310 K.

Figure 73: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 74: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
14. PcL in a water box with 0.4 M KCl counter ions at 298 K.

**Figure 75:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 76:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
15. **PcL in a water box with 0.4 M NaCl counter ions at 310 K.**

**Figure 77:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 78:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
PcL in a water box with 0.4 M NaCl counter ions at 298 K.

Figure 79: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 80: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
17. PcL in a water box with 0.5 M KCl counter ions at 310 K.

**Figure 81:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 82:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
18. *PcL* in a water box with 0.5 M KCl counter ions at 298 K.

**Figure 83:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 84:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on *PcL*.
19. PcL in a water box with 0.5 M NaCl counter ions at 310 K.

Figure 85: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 86: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
20. PcL in a water box with 0.5 M NaCl counter ions at 298 K.

**Figure 87:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 88:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
21. *PcL* in a water box with 0.6 M KCl counter ions at 310 K.

Figure 89: *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 90: *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on *PcL*. 
22. PcL in a water box with 0.6 M KCl counter ions at 298 K.

**Figure 91:** PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 92:** PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (K) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
23. PcL in a water box with 0.6 M NaCl counter ions at 310 K.

Figure 93: PcL active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

Figure 94: PcL with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on PcL.
24. *PcL* in a water box with 0.6 M NaCl counter ions at 298 K.

**Figure 95:** *PcL* active site and lid domains. Orientation before simulation is on the left and after is on the right. Protein is in pale blue color and ‘cartoon’ representation. Active site residues are in van der Waals representation. Lid domains are in ‘bonds’ representation. Active site residue SER-87 is in green, ASP-264 is in red and HIS-286 is in blue. U1 (residues 118-166) are in pink and U2 (residues 214-261) in orange. Notice the movements of active site residues away from each other as the simulation progresses, corresponding to the RMSDs observed in Table 2. U1 (residues 118-166) and U2 (residues 214-261) domains also show movements so as to increase access to the active site cavity.

**Figure 96:** *PcL* with charged residues and counter ions. Negative residues and positive counter ions are shown in the snapshot on the left. Negatively charged residues are Aspartic acid (ASP) shown in grey and Glutamic acid (GLU) shown in silver. Positive ions (Na) are shown in purple. Positively charged residues and negative counter ions (Cl) are shown in the snapshot on the right. Positively charged residues are Lysine (LYS) shown in white and Arginine (ARG) shown in ice blue. Negative ions (Cl) are shown in yellow. Positive ions interact strongly with negatively charged residues. Negative ions do not interact with positively charged residues possibly due to the overall negative charge on *PcL*.
Appendix 2:

Setting up NAMD simulations using VMD, TCL (Tools Command Language) and Material Studio.

This section addresses the integration of all systems and files necessary to run a successful simulation with PcL in a 65 Å x 65 Å x 65 Å water box, with sodium phosphate buffer added. For the sake of simplicity, let’s add 10 molecules of sodium phosphate, which will all be ionized in solution. Therefore, we shall have 10 sodium cations and 10 monovalent phosphate anions inside the water box.

The initial challenge that needs to be solved is that phosphate anion coordinates are not available in the standard PDB located at www.rcsb.pdb.org and also that the topologies and parameters of the monovalent phosphate anion are unknown. All PDB coordinates need to be integrated into one coordinate file in order to generate the protein structure file for a successful NAMD simulation.

Part 1: Generating coordinates of small molecules in the PDB file format

If the coordinates of a particular structure are not available in the standard PDB database, its coordinates have to be generated employing an interactive molecule building software, such as Materials Studio. Here, we discuss the generation of coordinates for the monovalent phosphate anion as an example.

The monovalent phosphate anion has 7 atoms. One phosphorus atom at the center bonded to 4 oxygen atoms. Three of these bonds are single, while one is double. Two of the three singly bonded oxygens are bonded to hydrogens while the third is left with one unsatisfied valency. This unsatisfied valency gives the monovalent phosphate anion its single negative charge.

1. Launch Materials Studio and create a new project.
2. Select New → 3 D Atomistic.
3. Select the Sketch Atom tool and select Carbon from the drop down menu. Click on the 3 D Atomistic document once and press the ESC key.
4. Click on the 3 D Viewer Selection Mode button once.
5. Click again on the carbon atom and click on the Adjust Hydrogen key once. You should have a methane molecule.

6. Click on carbon atom to select it. Click on the drop down menu of the Modify Element key and select Phosphorous. Your carbon atom should change to a phosphorus atom.

7. Similarly, select each of the four hydrogen atoms individually and change them to oxygen atoms. You will have one phosphorus atom surrounded by four single bonded oxygen atoms.

8. Select any one of the bonds between phosphorus and oxygen by clicking on it, midway between the phosphorous atom and the oxygen atom. Click on the Modify Bond button and select a double bond.

9. You should now have phosphorus bonded to four oxygens, one of which is a double bond. The remaining three oxygens each have a single bond with the phosphorus atom and a hydrogen atom.

10. Now select the entire molecule once again by dragging the mouse or double clicking on any atom, and press the Clean button. This will adjust the geometry of the molecule to a tetrahedral structure. Press the Clean button a few more times.

11. Do not add hydrogen atoms to the singly bonded oxygen atoms at this point.

12. Save this file by clicking File → Save.

13. Next, click File → Export. In the pop up window, choose your destination folder, enter your file name, and save as type Protein Data Bank Files (*.pdb, *.ent). Click on the Save button.

You have created a PDB coordinate file for the monovalent phosphate anion. Open this file by right clicking on it and opening with Notepad. If the data is scrambled or not formatted conveniently, it can be opened with Wordpad or even Microsoft Word, but this is not recommended. Make sure that this structure has 5 atoms, one phosphorous and four oxygens. Load this file into VMD and see if all the required bonds are present. The molecule should have a tetrahedral structure, which can be inspected visually this by rotating the molecule in all directions. Click on Graphics → Representations. A new Graphical representations window will pop up. Click on Drawing Method and select CPK. The molecule should look like the snapshot in Figure 1A below.
**Part 2: Modifying the coordinate file using a text editor**

Open the PDB coordinate file just created using Notepad or in a UNIX terminal, and examine it carefully. It should look like the snapshot in Fig. 2A. It need not have the **CONNECT** statements shown in Fig. 2A. In a second window, open the PDB coordinate file of PcL. Scroll down to the last residue of PcL, i.e. VAL 320. The screen should look like the snapshot in Fig. 3A.

![Fig. 2A](image-url) **Fig. 2A:** The coordinate file of the monovalent phosphate anion generated in Part 1 above.
Fig. 3A: The last residue of PcL. The amino acid sequence ends with the TER statement. Note the first HETATM record for the calcium ion which is structurally very important for PcL and plays an active role in the active site geometry. The HETATM records from atom 2341 record the water molecules surrounding PcL.

Refer to the NAMD tutorial appendix for the PDB files. Note that in Figs. 2A and 3A, from left to right, every column has the following entries: Record type, atom id, atom name, residue name, chain id, residue id, coordinates (x, y and z), occupancy, temperature factor, segment name and finally line number. The line number may be absent, which is not important.

In Fig. 2A, notice that the residue name of the phosphate anion atoms is set to MOL by default. It should be changed to something else, so that one can identify a molecule from its residue name. This will be important when one sets up the final system for simulation. The residue name that is assigned can be any combination of capital letters and numbers. In order to help one identify the molecule with its residue name, let’s change all the MOL entries in the phosphate anion coordinate files to PO4. One also has to change the chain ID field from A to B or any capital alphabet letter. This is important as the chain ID field for the PcL atoms is set to A and one needs to distinguish phosphate buffer ions from the PcL atoms. The coordinate file now looks like the snapshot shown in Fig. 4A.
Part 3: Arranging phosphate buffer around PcL

One needs to add 10 phosphate buffer ions around PcL in a random manner. While doing this, one wants to ensure that none of the buffer ions are too close to PcL atoms or overlapping them. This will result in a constraint error when running MD, and the job will fail. Phosphate ions need to be placed at least 2 Å away from PcL atoms and from each other. One can run dynamics for a longer time to note the interactions of the ions with PcL, but should avoid placing the ions too close to PcL at the beginning of the simulation.

Now create 10 buffer ions from the one coordinate file generated using Materials Studio in Part 1. Needless to say, when one generates these 10 different buffer ions, one should have different coordinates for every atom, i.e., there should be 10 different coordinates for phosphorus and 40 different coordinates for oxygens. Moreover, each phosphate must be surrounded by 4 oxygens to form a tetrahedral structure. This can be done using VMD. The following procedure demonstrates how to accomplish this.

1. Launch VMD.
2. Load the PDB coordinates of PcL.
3. Click File \rightarrow New Molecule
4. Load coordinates for the phosphate anion. Main VMD window will look like the following snapshot.
5. Notice that since the phosphate anion coordinates (PO4_1.pdb) were loaded last, it is the Top molecule, indicated by the T in its row, between its id 2 and A for Active Molecule.
6. Click **Graphics ➔ Representations** and change the drawing method to CPK. Observe that the buffer anion is now shown in CPK representation while PcL is still represented by lines.
7. Make PcL the top molecule by double clicking in its row, under T on the VMD Main window.
8. Click **Display ➔ Reset View**. The display will be reset with PcL as the top molecule.
9. Ensure that the mouse pointer indicates that it is in “rotate” mode, and rotate the display until the buffer ion loaded is clearly visible in the CPK representation.
10. Click on **Mouse ➔ Move ➔ Molecule**, or press 8 on the keyboard with the VMD display as the active window.
11. Double click on any of the buffer atoms and drag it to a position desired to be as a buffer around PcL in a water box. Ensure that it is at least 2 Å away from any region of PcL.
12. Click **File ➔ New Molecule** in the VMD main window. Load the original buffer coordinate file again. VMD Main window should now look like the following snapshot.
13. Repeat steps 5 – 11 for this molecule, which has ID 3.
14. Follow the above procedure until all 10 buffer ions surround PcL, such that none of them is too close to the protein, or to one another.
15. Save your work at this point if so desired by clicking File → Save State.
16. Click on the row of the first buffer ion loaded, in order to select it.
17. Click File → Save Coordinates. A new window, called Save Trajectory will appear.

![VMD Interface](image)

18. In the Save Trajectory window, select the first buffer ion coordinate file in the Save Data From field.
19. In the Selected Atoms dropdown menu, select all.
20. In the File Type dropdown menu, select PDB.
21. Click Save. Choose a location and give the file an appropriate name, perhaps po41.pdb.
22. Do this for the other 9 buffer molecule using filenames po42.pdb to po410.pdb in order to get them in some sort of order and sequence.
23. After finishing preceding step, close VMD.

**Part 4: Modifying the newly generated PDB coordinate files**

In step 3, 10 new coordinate files were created for the phosphate buffer ions. Hydrogens have not yet been added to these ions. This can be done later, using the topology files and the psfgen package of VMD. One now need to modify the newly generated files in order for them to be truly unique, so as to represent separate buffer ions around PcL in the simulation box.
Open the file po41.pdb using a text editor, such as Notepad. The chain ID field should be B for this file. Change it to C for all atoms. Also, if we refer to the PcL coordinate file, the residue id field varies from 1 to 320, ranging from the first amino acid in the PcL sequence to the last. We have, therefore, to choose residue id fields for buffer ions to be different from these values. In order to be safe, we can start from the number 601. Therefore, change the residue id fields for the file po41.pdb to 601, po42.pdb to 602 and so on, so that the residue id for po410.pdb is set to 610 for each atom.

Now one has truly separate and distinct buffer ions with unique coordinates with respect to PcL.

Next, follow the NAMD tutorial to generate the file lipp.pdb, i.e., follow the tutorial until completing step 4 of section 1.2 Generating a protein structure file.

Part 5: Generating the topology of the monovalent phosphate anion

We now explore the CHARMM topology files for the phosphate structure. The phosphate anion may not be present in the form required for the system, but its topology can be generated from other molecules that may be present in the available CHARMM files.

1. Explore the folder toppar/stream/
2. Open the file toppar_all27_lipid_model.str for reading only. Do not modify this file.
3. Scroll down till reaching the residue DMPA which represents dimethylphosphate.
4. Carefully browse the file in conjunction with the appendix for the topology files in the NAMD tutorial. Note the charges on the individual atoms and the IC statements. Scroll down to the next residue coded MP_1 which represents methylphosphate. Examine this residue also.
5. Copy the residue DMPA and paste it into the standard topology file top_all27_prot_lipid.inp. We can paste it so that it is the first residue in this topology file, i.e., it is the first to appear after the MASS statement in the file.
6. Examine the MASS statement of the file top_all27_prot_lipid.inp. We will probably not find the mass statements for phosphate atom found in the stream file. We need to copy the mass statement for phosphate from the stream file and paste it in the .inp file.
7. The mass statements for the atom type O2L and OSL, which are the types of oxygen atoms in the phosphate residue DMPA and MP_1 are also not found in the standard
topology inp file. These also need to be copied from the stream file and pasted into the mass statement of the inp file.

8. In the newly pasted entry for DMPA in the topology input file, look at the atom name and atom type for each atom. We may also want to change the name of the residue from DMPA to PHO1 or any other name of our choice. Make sure that the name given to a new residue does not match the residue name for any other residue.

9. We now have the topology for DMPA. We need to get to the topology of the monovalent phosphate anion from this topology. For this, refer to Fig 5 below.

![Fig. 5A: (A) The original dimethyl phosphate anion from the stream file top_all27_lipid_model.str. (B) The modified topology file for monovalent phosphate anion H2PO4⁻.](image)

10. The bonds, angles and dihedrals for the topology file can be measured using VMD. After loading a molecule click Mouse \( \rightarrow \) Labels \( \rightarrow \) Atoms, Bonds, Angles, Dihedrals or use the numeric keys 1, 2, 3 or 4 respectively when the VMD display is the active window.

11. Notice that the topology file must have all hydrogens explicitly mentioned in it. The psf generator script will automatically assign hydrogen coordinates from this topology file to the missing hydrogen atoms in the PDB file. This is why we did not create hydrogen atoms when we were building our anion buffer in Material Studio.

12. After we finish making all changes in our new modified topology input file, we save all changes and close it. It is worth mentioning here that it is best working with topology files in UNIX and not Windows.
Part 6: Generating combined PDB files with all atoms needed for simulation and constructing PSF file

We have generated the PDB coordinates of molecules whose structures are not available in the standard databases. We have done so because these molecules are of interest to us and are to be used in our simulations for our specific research requirements. We now need to integrate these individual PDB files of our protein and 10 buffer molecules into one single PDB coordinate file in order to run our simulations. This is done using the topology file mentioned in part 5 and the psfgen package of VMD. We will also use VMD’s powerful TK console to generate the combined PDB file and PSF file for all atoms. The following is the procedure to do so.

1. Examine the short pgn file provided in the NAMD tutorial. This file is in subsection 5 of section 1.2 Generating a Protein Structure File and has merely 9 lines. Observe the commands invoked by each of these 9 lines.

2. Line 2 invokes the program to read residue topologies from the file name provided. Ensure that the topology file provided here contains the topology of the molecule we have created the coordinates for.

3. Line 3 aliases a residue name found in the PDB file to the corresponding name found in the topology file.

4. Line 4 aliases an atom name that is found in the PDB file to the corresponding name found in the topology file.

We have to ensure that the residue name for our newly created coordinate file matches the residue name of its representative in the topology file. We had chosen to change the resid of our coordinate file from MOL to PO4 in part 4 of this addendum. We also chose to call our residue for the monovalent phosphate anion in the topology file PHO1. We need to direct VMD to understand that we mean the same molecule when we use these two different residue names.

5. After line 4, add the following line to the pgn file: alias residue PO4 PHO1

6. Next, add the line alias atom PHO1 P P1, which tells VMD that the atom P in the coordinate file is actually represented by atom P1 in the topology file. Do this for the other atoms if needed to.
7. After the segment for PcL coordinates is created, add the line `segment AA {PDB po41.pdb}`
8. Next add `coordpdb po41.pdb AA`
9. In 7 and 8 above, we have instructed VMD to create and additional segment called AA, and that it has to read the coordinates for the atoms in this segment from the file po41.pdb.
10. Similarly add the segments for the other 9 phosphate anion molecules.
11. Include the final three lines `guesscoord, writepdb and writepsf` in the pgn file.
12. Save the file and exit the text editor.
13. In a UNIX terminal window, make sure that we have all the coordinate files, PDB coordinate files and topology files in the same folder. In this folder, run the non-graphical version of VMD to execute the pgn script just created. We should have the combined PDB file and a PSF file as output.

**Part 7: Creating the parameter file**

We now have the complete PSF and PDB files for your system. All we need now is the parameter file for running the NAMD jobs. The standard parameters for all the protein atoms, bonds, angels and torsions are available in the standard parameter file `par_all27_prot_lipid.prm`. However this file will not have parameters for atoms PL, O2L, OSL and HOL. These parameters have to be found in other parameter files provided in the CHARMM package.

1. Read thoroughly the section on parameter files in the appendix of the NAMD tutorial
2. Go to the directory `toppar`. Open file `par_all27_lipid.prm`. Do not edit this file.
3. Copy bond parameters for atom pairs HOL OSL, OSL PL and O2L PL and paste these into the ‘BONDS’ section of the standard parameter file.
4. Similarly copy the parameters for ANGLES from this file and past these into the angles section of the standard parameter file.
5. Repeat this procedure for DIHEDRALS and NONBONDED potentials.