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Molecular dynamics simulation of Chlorotoxin

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Molecular dynamics simulation of Chlorotoxin

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DEDICATION

To my parents.

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NOMENCLATURE

CADD	Computer Aided Drug Design
CLTX	Chlorotoxin
DFT	Density Function Theory
BD	Brownian Dynamics
MD	Molecular Dynamics
VMD	Visual Molecular Dynamics
NAMD	NAnoscale Molecular Dynamics program
EM	Energy Minimization
EE	Energy Equilibration
PR	Production Run
N	Number of the particles
V	Volume
T	Absolute Temperature
P	Pressure
NVT	Ensemble with constant number of particles, constant volume and constant temperature
NPT	Ensemble with constant number of particles, constant pressure and constant temperature
MMP-2	Matrix Metalloprotease series (MMP-1~28)
TIMP-2	Tissue Inhibitor of Matrix Metalloproteinase-2
MT2-MMP	Membrane Type 2 Matrix Metalloprotease

ECM	Extracellular Membrane
C	Cysteine
X-Ray	X-ray Crystallography
NMR	Nuclear Magnetic Resanonce
PDB	Protein Data Bank
PSF	Protein Structure File
RMSD	Root Mean Square Deviation
RDF	Radial Distribution Function
Rg	Radius of Gyration

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ABSTRACT

Nature inspires us to address the most contemporary scientific challenges. Advances in the drug delivery system at the nano/micro-scale hold promise to treat some fatal diseases in the next few decades. Scientists have discovered that the biomolecule Chlorotoxin, a naturally occurring biomaterial, can target tumor cells in the human brain with great precision. The application of Chlorotoxin can assist future surgeons to avoid the risk of damaging healthy tissues in the human brain. Chlorotoxin purified from scorpion venom is essentially a peptide containing 36 amino acids and demonstrates high affinity particularly to glioma and neuroectodermal tumor. Knowledge of the molecular structure and stability is immensely useful to understand transport of Chlorotoxin in a blood saturated environment. Equilibrium molecular dynamics simulations are employed to examine the stability of Chlorotoxin at various temperatures and ion concentrations of the surrounding solvent environment. The analyses of the root mean square deviation, radial distribution function, and radius of gyration from the molecule's atomic trajectories facilitate prediction of the structural stability of Chlorotoxin under different thermodynamic environments and the optimal temperature and ion concentration for its diffusion in blood.

CHAPTER I

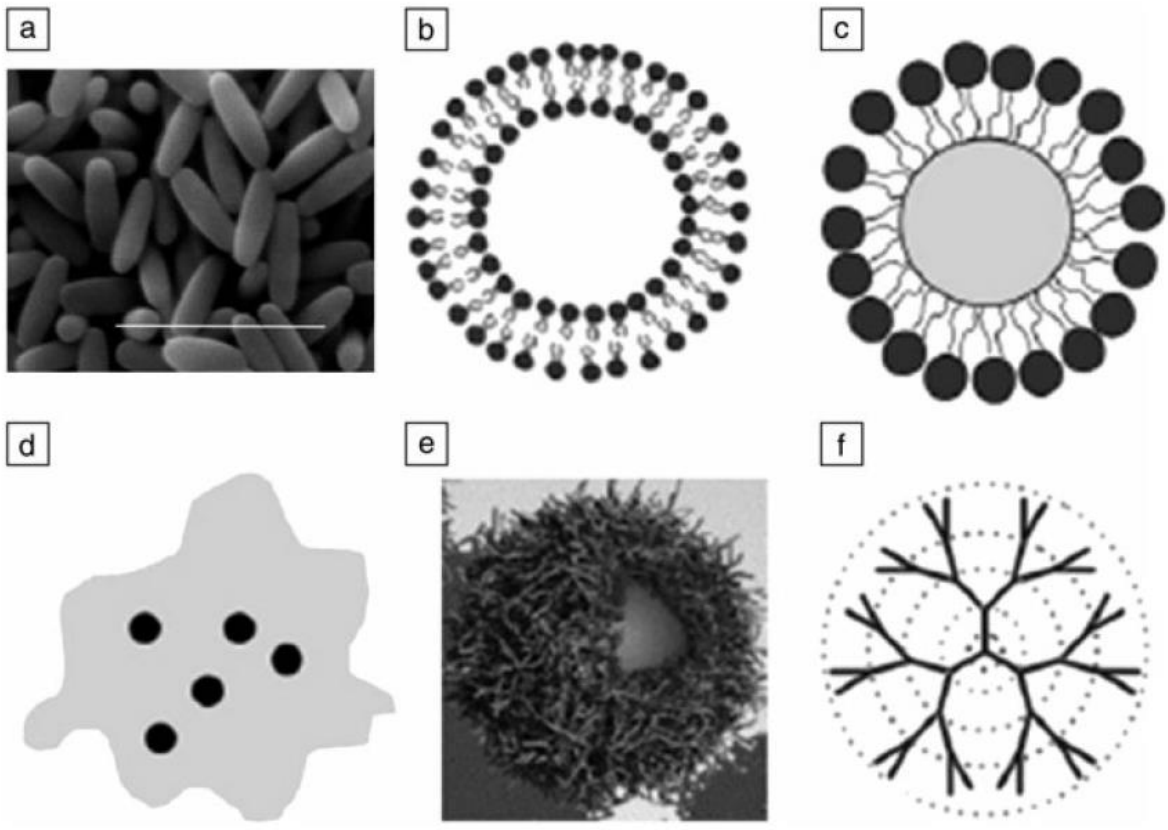
INTRODUCTION

1.1 Motivation

Disease is an obstacle for human civilization, aside from war. Nanoscience and nanotechnology holds promise for a possibility to conquer some of these fatal diseases. In particular, nano-bio-technological material and devices are increasingly attracting scientists to focus on the pharmaceutical industry. The general features of a nano-system typically require that the system itself or its essential components be man-made in the 1–100 nm range in at least one dimension, usually integrating novel material structures for devices and systems with fundamentally new properties and functions [1-2]. George Whitesides [3] and Robert Langer [4] both suggested that less limitations should be put on the dimension of the medicine and instead the interest should be on the function of the system and tools to derive it. The investigation of nanoparticles is presently of interest for medical applications due to its therapeutic and diagnostic potential. The application of nanoparticles to treat diseases encounters two major issues: efficacy and efficiency [5–8]. Specifically, as Theresa pointed out that many of the early problems of conventional drugs can be resolved with several nanotechnology-based drug delivery systems [6]. For example, the conventional drugs have problems such as poor solubility, tissue damage on extravasation, rapid breakdown of the drug in vivo, unfavorable pharmacokinetics, poor bio-distribution, and lack of selectivity for target tissues [6]. One can also read this information from Table 1 below. Moreover, another challenge of treating cancer is the prediction of precancer in an earlier stage [1].

Table 1: Non-ideal properties of drugs and their therapeutic implications.[6]

Problem	Implication	Effect of DDS
Poor solubility	A convenient pharmaceutical format is difficult to achieve, as hydrophobic drugs may precipitate in aqueous media. Toxicities are associated with the use of excipients such as Cremphor (the solubilizer for paclitaxel in Taxol).	DDS such as lipid micelles or liposomes provide both hydrophilic and hydrophobic environments, enhancing drug solubility.
Tissue damage on Extravasation	Inadvertent extravasation of cytotoxic drugs leads to tissue damage, e.g., tissue necrosis with free doxorubicin.	Regulated drug release from the DDS can reduce or eliminate tissue damage on accidental extravasation.
Rapid breakdown of the drug in vivo	Loss of activity of the drug follows administration, e.g., loss of activity of camptothecins at physiological pH.	DDS protects the drug from premature degradation and functions as a sustained release system. Lower doses of drug are required.
Unfavorable Pharmacokinetics	Drug is cleared too rapidly, by the kidney, for example, requiring high doses or continuous infusion.	DDS can substantially alter the PK of the drug and reduce clearance. Rapid renal clearance of small molecules is avoided.
Poor distribution	Drugs that have widespread distribution in the body can affect normal tissues, resulting in dose-limiting side effects, such as the cardiac toxicity of doxorubicin.	The particulate nature of DDS lowers the volume of distribution and helps to reduce side effects in sensitive, nontarget tissues.
Lack of selectivity for target tissues	Distribution of the drug to normal tissues leads to side effects that restrict the amount of drug that can be administered. Low concentrations of drugs in target tissues will result in suboptimal therapeutic effects.	DDS can increase drug concentrations in diseased tissues such as tumors by the EPR effect. Ligand-mediated targeting of the DDS can further improve drug specificity.



Examples of organic material-based carriers for drug delivery: (a) rod-shaped polymer particles (scale bar: 1 μm) (courtesy of Aaron Anselmo), (b) liposomes, (c) solid-lipid nanoparticles, (d) protein-based nanoparticles (drug bound to protein), (e) layer-by-layer capsules,[39] and (f) dendrimers.[40] Reprinted with permission from Reference 38. © 2009 Wiley.

Figure 1: Novel materials applied to drug delivery system.
(Samir Mitragotri, MRS bulletin, volume 39, March 2014)

To address the challenges of drug delivery system, it is essential for us to employ the novel materials and current nanotechnology, such as experimental tool, imaging tool and computational tool to make a breakthrough on the disease treatment. Researchers have discovered a reservoir of nanomaterials applied to the drug delivery system, including inorganic materials, such as gold, silica, gel and PEG, organic materials such as protein,

lipids, oxide nanoparticles and so on.[7-8] Figure 1 lists some of the novel organic biomaterials applied to drug delivery system that are under investigation. Those materials present special properties and functions corresponding to the demands of drug delivery design. Strikingly, one such materials is venom [9–14], a controversial material in nature because of its traditional image of poison, driven by the recent discovery of its potential as a pharmacological material.

The most prominent feature of venom is its targeting ability that has emerged after thousands of years' evolution in the process of attacking prey, defending predator, and intimidating other species. We have always considered venom as poison or chemical weapon. However, derivative venom can be applied to resolve some intriguing medical challenges, such as in drug delivery system, analgesic, and tissue engineering [14–16]. In this research, we mainly focus on the application of Chlorotoxin (CLTX) to diagnose and treat cancer [15–20]. Typically, in the literature on CLTX, most of them focus on the application of imaging tumor cells for surgeons. The literature [18–20] about imaging applications present us a paradigm on how to conjugate CLTX with an imaging agent to target and indicate the tumor sites. CLTX is not only useful in the area of imaging, but it also has therapeutic effect to treat different diseases apart from cancer. More and more researchers are exploiting the therapeutic applications although the advances are still in the early stage. Sontheimer's group [21-22] from the University of Alabama at Birmingham published a few papers about the therapeutic mechanism of CLTX treating cancer.

Except from the emerging materials available for exploring novel medicine, there is an accelerating development on the computational method of drug discovery. The computational method not only provides a physical feeling of the processes at the nanoscale,

but it also can enlighten us with details about the reaction mechanisms [23–26] of medicine and diseased site by the calculation of binding energies. William L. Jorgensen [26] had claimed that proficiency with computations is advantageous for delivering new drug candidates more quickly and at lower cost. Specifically, the application of computational method to design venom-based derivative drug can resolve the challenges of potential side effects due to interactions of toxins with off-target receptors [25]. Gordan [24] has suggested that computations have the potential to provide lower cost alternatives for exploring the effects of new compounds on ion channels.

Figure 2: Venom extraction from a scorpion to make anti-cancer medicine. *(http://i.imgur.com/xzu9b.jpg)*

Nanotechnology is an interdisciplinary field which contains biology, statistical mechanics, physics, chemistry, molecular engineering and biotechnology. Accelerated and high-throughput screening strategies are required to combine materials and transport methodologies for validating the new drugs [27].

The venom-derived drug can not only treat some fatal diseases, it also improves the drug efficiency in our body [10]. Figure 2 presents the venom extraction from a scorpion that is used to make anti-cancer medicine. A complete report on the development of peptide, which contains venom and its derivatives, as therapeutic or diagnostic drugs can be requested from http://www.peptidetherapeutics.org. Memorial Sloan Keltering Cancer Center also demonstrates the application of venom in treating with analgesic, anti-inflammatory, cancer treatment, alleviating chemotherapy side effects, immunostimulation and radiation side effects. Besides, there are six venom-derived drugs approved by FDA and there are several venom drugs in clinical trial.

1.2 Objective

There are three fundamental parts comprising the drug delivery system, which are platform agent, targeting agent and drug agent. The function of platform agent is to carry out other agents in the blood stream and protect other agents from disruption by the protease or ions in the blood system; the targeting agent is to locate the diseased site rather than interact with normal tissues; the drug agent is to release medicinal drug in a certain amount and interact with diseased tissue cells. Table 2 provides a summary of the components of drug delivery system. Furthermore, there are other considerations except from those three parts, such as poor solubility, EPR effect, and other side effects.

Table 2: The three components of drug delivery system

Drug delivery system	Function
Platform agent	Carry out and protect other agents
Targeting agent	Locate the diseased site instead of attaching normal tissues
Drug agent	Interact with bad tissues

Being targeting agent is one of the most prominent features of CLTX. A fundamental understanding of CLTX reaction mechanisms with glioma cell membrane can be extended to delivery processes of CLTX-based conjugates that constitute the drug circulating in human body. Current therapeutic methods of curing cancer always encounter two challenges, the efficacy and cytotoxicity. We apply computer-aided drug design (CADD) method to identify a potential drug candidate and optimize the discovered drug. Such a drug candidate can be useful during surgical applications and chemotherapy, which are two ways to treat glioma cells. From a surgery perspective, biomarker such a CLTX-Cy5.5 nano-probe [21- 22] could potentially be used to image resections of glioma brain tumors. From chemotherapy standpoint, the CLTX reacting with Chloride channel can be a potential chemotherapeutic inhibitor to stop tumor cells from proliferating.

Extensive effort has been put on the imaging application because of its predictability of cancer in an earlier stage. Scientists have discovered that CLTX can target tumor cell in human brain with great precision. In practice, surgeons have to remove the tumor cells with their visual inspection that poses the risk of damaging healthy cells in the normal tissues. Thus, it is important to devise a method of locating and identifying the tumor cells. One of the promising approaches of using CLTX to detect tumor cell is ongoing. Dr. Jim Olsen named this potentially powerful technique as “tumor paint” [28]; because of its special ability of blocking Chloride channel, scientists named it as CLTX. Most importantly, it binds with high particular affinity to glioma and neuroectodermal tumor cells, but not with the healthy tissues [29]. The most challenging problem is to understand the protein-ligand binding mechanism which can help us resolve the off-target issue and improve its efficacy to interpret the interaction mechanism.

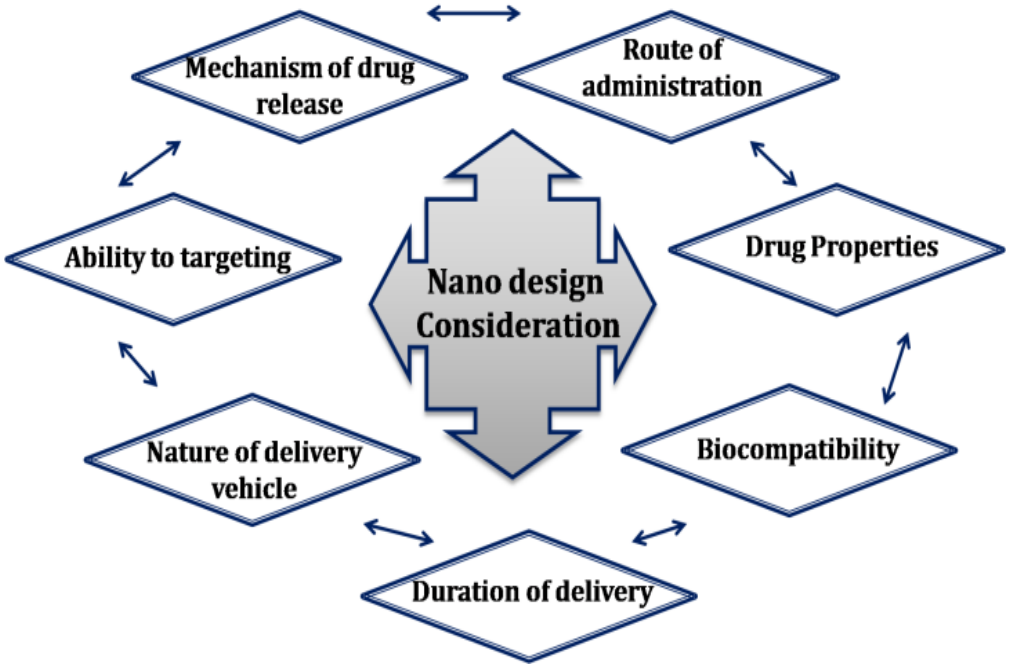


Figure 3: Factors for simultaneous consideration in the design of a nanoparticle for smart drug delivery system
("Application of Nanotechnology in Drug Delivery", book edited by Ali Demir Sezer)

Another objective of this thesis is to understand how CLTX behaves in an aqueous environment that approximately mimics the environment of a typical drug delivery system. Figure 3 presents the factors accountable for the design of nanomaterial-based drug delivery system. Chlorotoxin plays a significant role in all the constraints factors, especially duration of delivery, ability to targeting and biocompatibility. Unlike micelle, liposome or gel material, which has hydrophobic matrix to support its structure, CLTX can resist from protease in the blood cell and hardly could be affected by thermal or ionic disturbances.

Usually, there are two types of platform agents. The common one holds the drug agents inside of the platform geometry which can protect the drug carrier from attack by external force. Based on the predictions by King [12, 30], we can estimate that the four

disulfide bonds in CLTX play a key role in determining the stability of the structure. More and more attention has been paid to the research of disulfide-rich protein in the pharmacological tool and therapeutics [31–33]. This thesis facilitates an understanding of the CLTX structure in an aqueous environment with temperature and ionic variations using atomistic computations.

Computational predictions are increasingly gaining attention from researchers since they provide an economically feasible framework to test new theories with high resolution. Computational techniques can be divided into hierarchical multiscale modeling scheme based on the particle size scale [34], such as quantum mechanical level, molecular mechanical level and mesoscale level. The molecular dynamics (MD) method has emerged as a powerful technique for comprehension of the atomistic features of material systems. In the standard MD method, the Newtonian equations of motion are employed to resolve numerically the trajectory of the particles in a fixed periodical cell of volume *V*. MD simulation has three major types of applications [35], which are refinement of structure, descriptions of the system at equilibrium and examination of actual dynamics. In our work, we apply molecular dynamics to investigate Chlorotoxin structure in different environments, specifically, at different temperatures within an aqueous environment and different ions solvated in the solution. NAnoscale Molecular Dynamics program (NAMD) [36] and Visual Molecular Dynamics (VMD) [37] are utilized to simulate the atoms and to illustrate the molecular motion observed in the simulations. In order to obtain the results, the simulation techniques follow different stages: initializing to energy minimization, to energy equilibration, to production run.

Three structural and transport properties are employed to describe the evolution of CLTX due to interactions with water molecules and ions at different environments. The investigations of these properties educate us about the stability of the molecule as expected in a more complicated neighborhood of human blood flow. This provides us a reference to understand the possibility of transportation of therapeutic protein in blood.

1.3 The Major Proteins on the Surface of Tumor Cells

In order to understand the inhibitor/targeting mechanism of CLTX, we have to learn how tumor cells try to invade the normal tissue. Scientists have substantiated that CLTX has a strong correlation with tumor cells than normal brain cells [38–41]. Now there are numerous researchers vigorously focusing on the interaction mechanism of CLTX and tumor cells. Based on the current knowledge of this interaction, the tumor cell invasion mechanism is considered to be an extremely complex and hence important to understand, as explained in the literature [42]. In essence, the main aspect lies in explaining the invasion mechanism through the roles and functions of MMP-2 and ionic channels (Cl^- and K^+ channel).

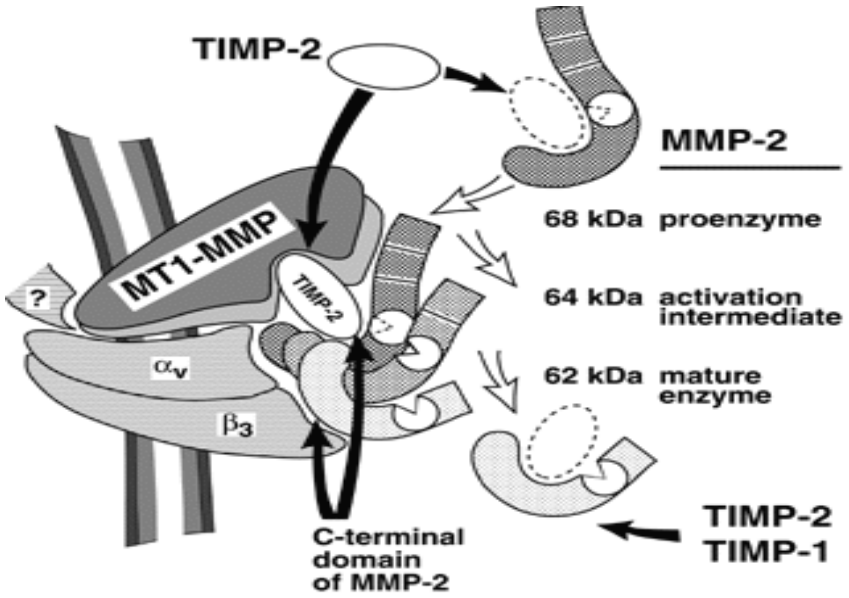


Figure 4: Schematic of the MMP-integrin molecular assembly
(Alex Strongin, Professor, Medical discovery institute)

As mentioned from above, the interaction first occurs at the surface of the tumor cells and normal tissues. Scientists have identified a few major proteins in the surface of tumor cells. They are matrix metalloprotease series (MMP-1~28), $\alpha_v\beta_3$ integrin, membrane type 2-matrix metalloprotease (MT2-MMP), and tissue inhibitor of matrix metalloproteinase-2 (TIMP-2). Figure 4 shows a few major proteins isolated from the surface of tumor cells. Because of the presence of these proteins, it is difficult to identify which major protein is the receptor of CLTX. MMP-2 mainly consists primarily a 72-kda band and with five domains.

The functions of these proteins are summarized in Table 3 below [40].

Table 3: The functions of the four major proteins on the surface of the tumor cells

Major protein on the surface of tumor cells	Function
MMP-2	In degradation and remodeling of the ECM
$\alpha_v\beta_3$ integrin	A membrane-type MMP activates MMP-2
MT2-MMP	Promotes the maturation and release of MMP-2; Forms a hemophilic complex through the hemopexin domain keeping the MT1-MMP molecules together facilitating pro-MMP-2 activation
TIMP-2	Endogenous inhibitor

1.4 Chlorotoxin and Its Receptor

There is no conclusive evidence of the receptor that reacts with CLTX on the surface of tumor cells. Table 4 presents the fours receptors that can bind with CLTX. The first one is the Chloride channel which gives rise to the name CLTX as describe by Debin et al. in 1993. The second receptor is MMP-2 that is a 72-kDa band. Jessy Deshane[40] has identified MMP-2 as a receptor by the method of weight and also examined whether CLTX inhibits MMP-2 catalytic activity at this article [40]. The third receptor is Annexin A2 [43]. Annexin A2 is a calcium-dependent phospholipid binding protein on the extracellular side of the plasma membrane of various tumor cells and endothelial cell types [43]. The fourth receptor is the complex of the few major proteins on the surface of the tumor cells [40]. From the

literature we can conclude that CLTX exerts some effect on the major proteins on the surface of the tumor cells, but we are unsure it is on individual protein MMP-2 or on the complex of the proteins.

More and more evidence has proven that there is a high possibility that CLTX interacts with MMP-2. However, the receptor for CLTX and the mechanism underlying its anti-invasive effect are still unknown. In order to advance the research, it is assumed that MMP-2 is the receptor. Now, since the site on the MMP-2 molecule that interacts with CLTX is unknown, attempts are ongoing to find the active site of MMP-2 by the means of high accuracy equipment, such as X-ray, NMR and SEM and by the computational predictions.

Table 4: The possible receptors that CLTX targets

	CLTX
Receptor 1	Small conductance CL- channels were shown to be potently blocked by CLTX.
Receptor 2	MMP-2 involved in ECM degradation associates with the interaction of CLTX and tumor cells.
Receptor 3	Annexin A2 as CLTX receptor.
Receptor 4	CLTX interacts with a combination of the major few proteins on the surface of tumor cells.

A more fundamental research topic is to explore the interaction of CLTX and glioma cell membrane, characterized by the Chloride channel and matrix metalloproteinase-2 (MMP-2) in its morphology. MMP-2 is a protein anchoring to the glioma cell membrane. Since CLTX binding with glioma cells offers two functions (blockage of Chloride Channel

and attachment to MMP-2) advanced understanding of binding mechanism of CLTX reacting with MMP-2 can be achieved by molecular simulations. The application of such investigation is in the design of a biomarker to treat cancer in preoperative diagnostic and intraoperative pathology at cellular-level resolution.

MMP-2 is a type of Gelatinase and its structure consists of five different domains. CLTX is notable of its two functions: blocking Channel and attaching to MMP-2. The function of MMP-2 is described by previous literature in three aspects: the ability of MMP-2 to digest normal cell membrane, MMP-2 generated by the active agents inducing gene expression, and the activity of MMP-2 in destroying endogenous inhibitors. Even though the mechanism of glioma cells invasion of normal cells is unclear, scientists have made progress on the understanding of MMP relevant activities. Next, we have to obtain the complex coordinates (CLTX and MMP-2 binding) file (PDB), which indicates the binding site of the two molecules. While it is simple to obtain separate coordinates files (PDB) for both the molecules (CLTX and MMP-2), the complex combined coordinate file is absent to extend the simulation. In absence of such information about the binding sites of the two molecules, the computations are limited in the investigation of the interaction mechanism.

Most of the earlier literature presents, based on experiments, a general conclusion that CLTX has ability to stop MMP-2 invasion. Surprisingly, there is no work using X-ray and NMR to investigate the interaction mechanism. It is significant for resolving the structure of the binding molecules by the approach of imaging. Now, it is available for the two molecule structures: CLTX and MMP-2, but they have an extensive surface; hence we do not know where the binding site is.

If we do know the binding site structure coordinates, then it opens large possibilities to investigate interactions which can be divided into two categories: explicit and implicit. First is the method of Single Molecule Force Microscopy to investigate two-protein (or two residues, two atoms) interaction by unbinding. The magnitude of this rupture force of unbinding can help us decide the reaction type, such as enzymatic reaction, covalent reaction, noncovalent reaction or diffusive movement. It can facilitate in resolving the problems in drug delivery: encapsulation in transportation, controlled release and targeting bad tissue.

Also, there is an implicit computational method to interpret the interaction mechanism using a statistical-thermodynamic basis. Considering the complexity of molecule, there are numerous articles on landscape energy, MM/PBSA and so on to calculate free energy.

However, those methods have limitations. While we can employ single molecule force microscopy to measure the force required to unbind the bond of CLTX and MMP-2, the structure information from the X-Ray Crystallography and NMR data is absent. This prevents applying AFM to measure unbinding force or employ molecular dynamics simulation to compute the rupture force explicitly since it is unclear what the loading regime is. Moreover, since there are no experimental work reporting types of the interaction (such as chemical reaction, covalent, noncovalent, enzymatic, diffusely movement), choosing a reasonable implicit method is difficult.

In order to apply molecular dynamics to compute the thermodynamics properties and interpret the interaction mechanism, some assumptions must be established. First, we will have no experimental data to compare with our computational work. Second, we assume the active site as the binding regime for the interaction. The active sites are the functional

residues as presented when the molecules interact with other particles. This will allow us to compute the free energy of the interaction in an implicit manner and can enlighten us of the interaction mechanism.

CHAPTER II

FUNCTION AND APPLICATION OF CHLOROTOXIN

2.1 Structure of Chlorotoxin

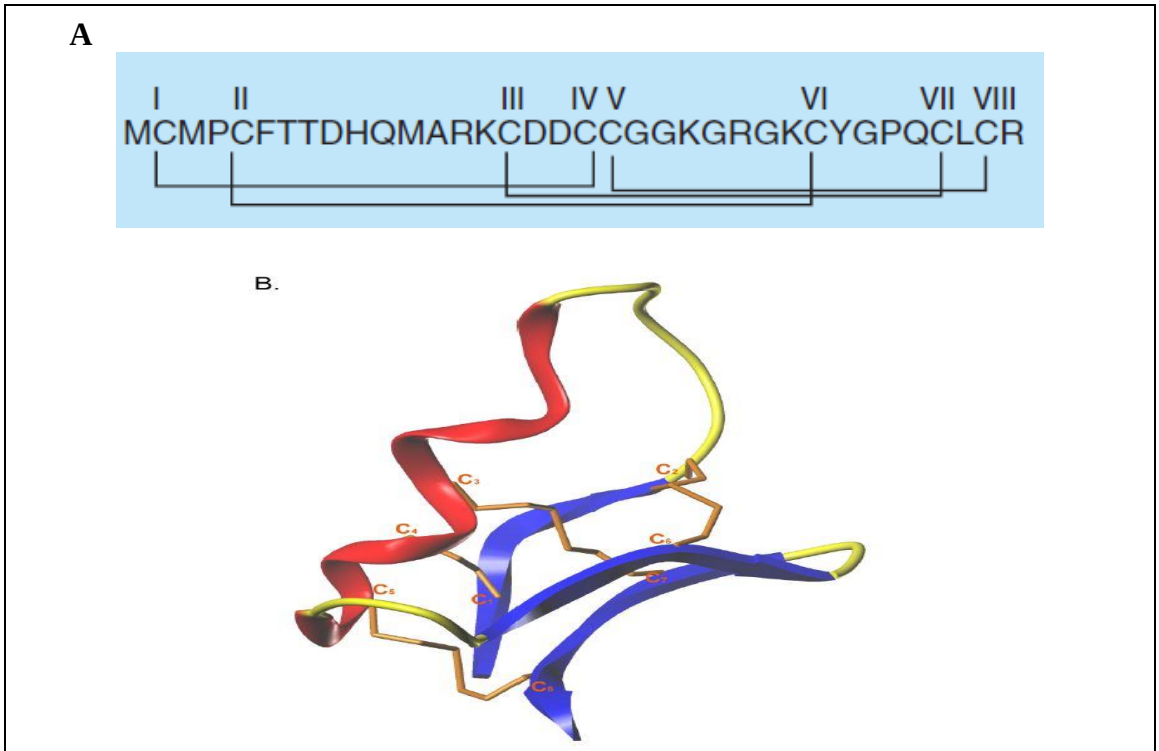


Figure 5: Amino acid sequence and 3 D representation of CLTX
(A) The disulfide connectivity is shown using solid lines and the cysteine residue are labeled using Roman numerals from I to VIII [33]
(B) 3 D structure of CLTX, α- helix in red, β -sheets in blue and disulfide bridge in orange
(Jessy Deshane, The Journal of Biological Chemistry, vol. 278, No. 6)

CLTX purified from scorpion venom is essentially a peptide containing 36 amino acids and demonstrates high affinity particularly to glioma and neuroectodermal tumor [44]. The structure of CLTX was resolved by experimental method with the means of NMR and X-ray crystallography. Since the Hydrogen atom is too small, which cannot be detected by those tools, our computational tools have to guess those missing hydrogens.

While the earlier has focused on primary and secondary structures, the current interest is in the details of the tertiary structure. Next, we summarize the CLTX protein structure in a hierarchical way.

2.1 a Primary Structure

CLTX consisting of 36 amino acids is a protein. The amino acids sequence is shown in Figure 5 A. It compares with the sequence of toxin family of molecules. From the sequence, it is obvious that CLTX is cysteine-rich protein.

2.1 b Secondary Structure

Molecular structure of CLTX, α- helix in red and β-sheets in blue and Disulfide Bridge in orange are presented in Figure 5 B. Three are three β-sheets and one α helix. Scientists predict that the landscape energy of CLTX may determine the reaction mechanism due to such structure.

2.1 c Tertiary Structure

A compact structure is maintained by four disulfide bonds that connect the eight cysteine residues. The cysteine pattern adopted is of the type C1-C4, C2-C6, C3-C7 and C5-C8. The disulfide connectivity is shown using solid lines and the cysteine residues are labeled using Roman numerals from I to VIII in Figure 5 B. We observe that toxin family is a type of protein rich in cysteine. The disulfide bridges may contribute to the stability of CLTX. Figure 6 presents a tattoo from Jim Olson who is a pioneer in imaging cancer by the use of CLTX.

Figure 6: Representation of the knot of disulfide bonds at the center of the Chlorotoxin molecule. Jim Olson shows off his tattoo, which represents the knot of disulfide bonds at the center of the Chlorotoxin molecule. *Courtesy John Clark*

Table 5: The hierarchical structure of CLTX

Protein: CLTX	Structures features
Primary Structure	36 amino acids; the sequence and the α/β motif
Secondary Structure	Three α sheets and one β sheets
Tertiary Structure	Knot structure
Quandary Structure	It's a small protein

The toxin family of molecules has three disulfide bridges in general. The CLTX, however, has four disulfide bridges. Earlier reports conclude that the fourth disulfide bridge contributes to the specific affinity of CLTX to glioma cell, since the other family members of toxin do not bind with glioma cell. A future area of research is to apply molecular dynamics to compute the free energy for the different disulfide residue to interpret CLTX functions.

Table 5 summarizes the four level structure information of CLTX.

2.2 Function and Application of Chlorotoxin

There are two general functions of CLTX that are applicable to the pharmacy industry. The first one is use in therapeutic medicine. In order to penetrate the normal cells, glioma cells need to shrink by using ionic channels (Cl- and K+ channel). CLTX can bind with the ionic channels to stop the ions communications within and outside of the glioma cells. The other function of CLTX is specificity and affinity. As we have analyzed, there are four possible receptors reacting with CLTX and although the binding mechanism is still inconclusive, CLTX has shown a high efficiency to target glioma cells.

Table 6: Application, function and structure of Chlorotoxin

Function	Application	Structure
Interact with ionic channel	Therapeutic Medicine	Essentially primary and secondary structure dominates determine
Interact with four possible receptors and without knowing the active site of the receptor	Diagnostic Medicine	Unsure about how structure determine the function

Based on the function of CLTX, there are also two applications of CLTX used in treating human diseases. Table 6 generalizes how the structure determines the function that in turn determines the application.

CHAPTER III

MOLECULAR SIMULATION

3.1 Methodology

In order to understand the binding mechism of CLTX and receptor, there are three major methods to move ahead. These are experimental method, computatonal method and advanced imaging. This thesis considers only the computational approach. Figure 7 presents examples of length scales in nature. For example, 1 Angstrom to 1 nm, *ab initio* first principles calculations such as density function theory (DFT) is applied. At the range of 10-100 nm, classical MD simulations are employed. Larger length scales call for browinan dynamics (BD) simulations and course grained modeling.

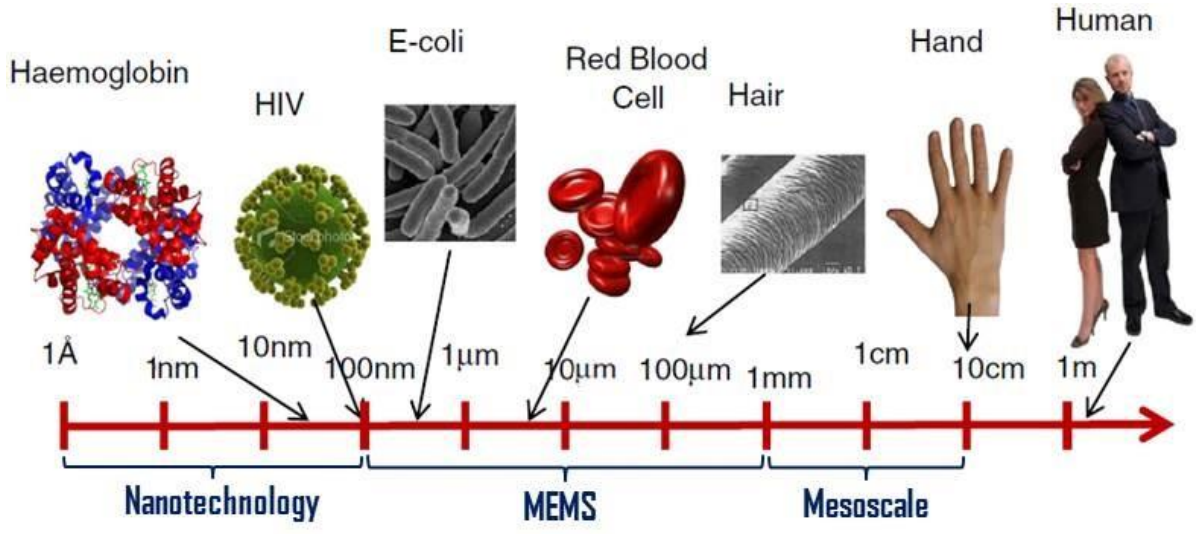


Figure 7: Dimensional scale of MEMS and Nanotechnology.
(Adapted from Nguyen et al.)

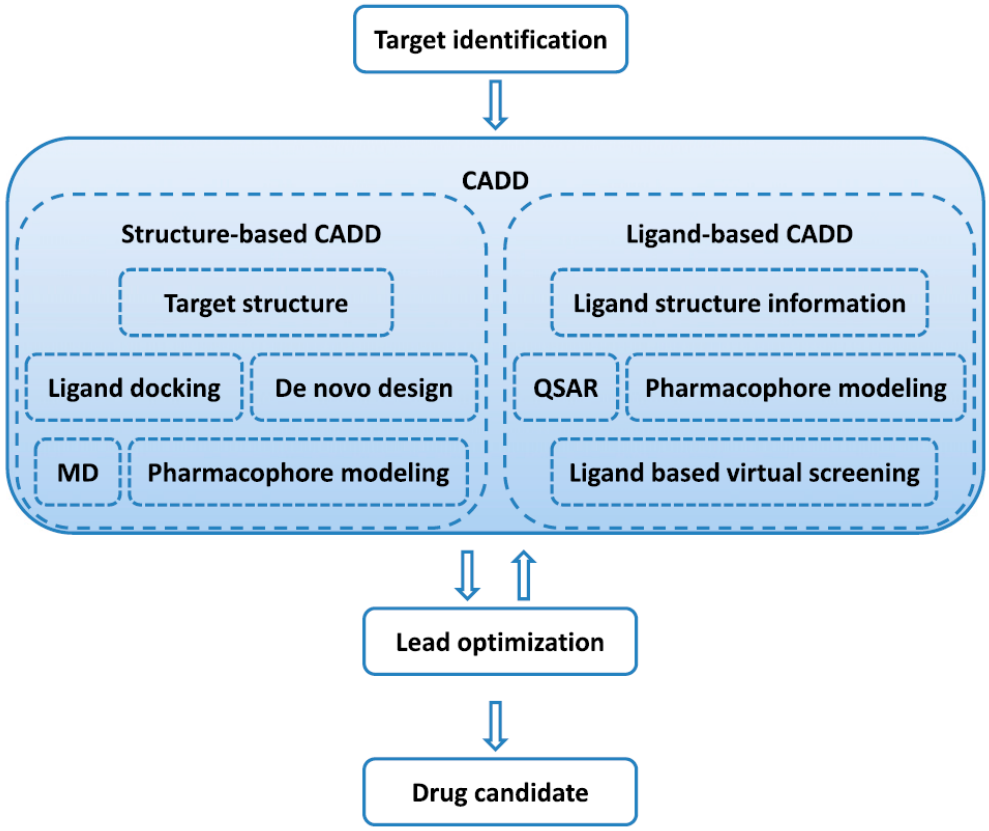


Fig. 8: CADD in drug discovery/design pipeline. A therapeutic target is identified against which a drug has to be developed. Depending on the availability of structure information, a structure-based approach or a ligand-based approach is used. A successful CADD campaign will allow identification of multiple lead compounds. Lead identification is often followed by several cycles of lead optimization and subsequent lead identification using CADD. Lead compounds are tested in vivo to identify drug candidates.[23]

By the means of computation, researchers have applied to protein adsorption, protein and protein recognition, and investigate the dynamics properties of the molecules based on the problem requirements. In general, there are two directions in the process of computational drug design as shown in Figure 8. They are structure-based CADD and ligand-based CADD.

3.2 Simulation details

In order to investigate CLTX stability in water, we intentionally implement two parametric scenarios, temperature and ion concentration. First, the simulation is performed at different temperatures, 280K, 290K, 300K, and 310K, respectively. Second, the simulation at 300 K is performed by adding three different ionic concentrations in the aqueous environment, 10 Na/13Cl, 50 Na/53Cl and 100 Na/103Cl. VMD is used for simulation setup, visualization and analyses while NAMD is employed for the simulations. There are three extra negative ions added for the sake of neutralizing the system. In the following descriptions, we use 10 ions concentration representing for 10 Na/13Cl, 50 ions representing for 50Na/53Cl, and 100 ions representing for 100Na/103Cl. The overall process of the simulation is categorized into several stages executed separately but in sequence of the standard protocol: EM > NVT > NPT [45][46].

The first step is to set up the system and optimize the molecular geometry. A cubic box with dimension of 50 Angstrom x 50 Angstrom x 50 Angstrom contains the peptide CLTX. To mimic the cellular environment, the CLTX was placed in a water solvent by utilizing VMD Solvation Package. We generate the various concentrations of ions with the Autoionize Plugin. The geometry of the freely obtained protein data bank (PDB) structure file from the RCSB database (derived experimentally via NMR or X-ray crystallography) is optimized by Energy Minimization (EM). The total energy of the system evolving with step time is plotted in Figure 9.

The next step is Energy Equilibration (EE). Based on Hamiltonian mechanics, the particles undergo energy conversion inside the system. Employing Newton’s second law, and CHARRM potential force field functions [47], the energy and forces of intermolecular

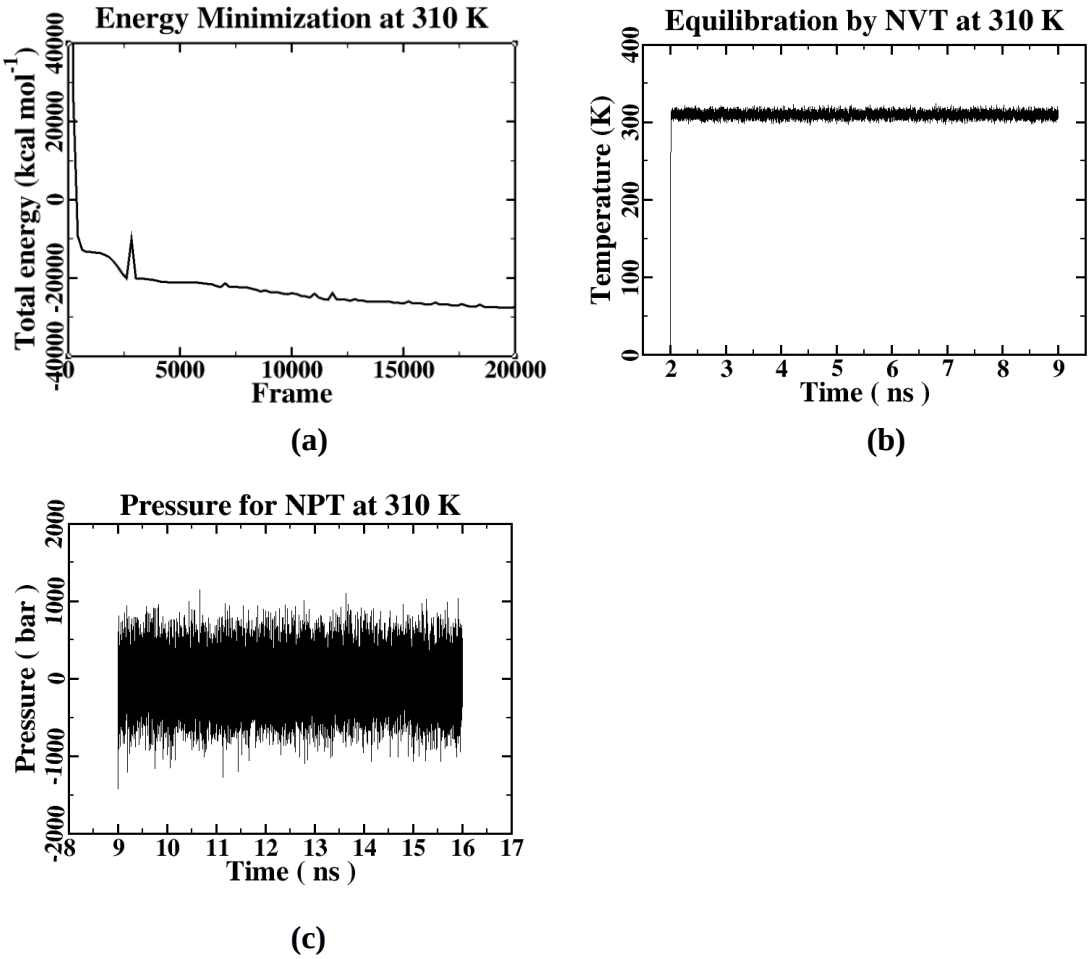


Figure 9: Different stages of simulation for equilibrating Chlorotoxin in water. Figure (a) is the indicator that total energy dropped to the system's minimum value much before 20000 frames. Figure (b) presents temperature evolution with time at the assigned value of 310 K and shows when energy equilibration is achieved under the NVT ensemble. Figure (c) shows the trend of the pressure vs. time fluctuations from 1 bar under the NPT ensemble. Although the average pressure agrees with the assigned value, the large fluctuations are due to the incompressibility effects of water.

interactions are described. The system is initialized with a velocity distribution to atoms corresponding to the temperature using the NVT ensemble. From the plot of the temperature vs. time in Figure 8, the system is found to equilibrate after about 7 ns. Then, using the last configuration from NVT ensemble, we continue to equilibrate the system with NPT ensemble. We plot pressure vs. time to identify the required equilibration time, shown in Figure 9. The final stage is Production Run (PR) that employs the NPT ensemble for 10 ns. We analyze the molecular trajectories obtained from the PR to compute the root mean square deviation (RMSD), radius of gyration (Rg) and radial distribution function (RDF). The observed results with temperature and ion concentration dependencies are presented next with discussions on RMSD, Rg and RDF.

CHAPTER IV

RESULTS AND DISCUSSION

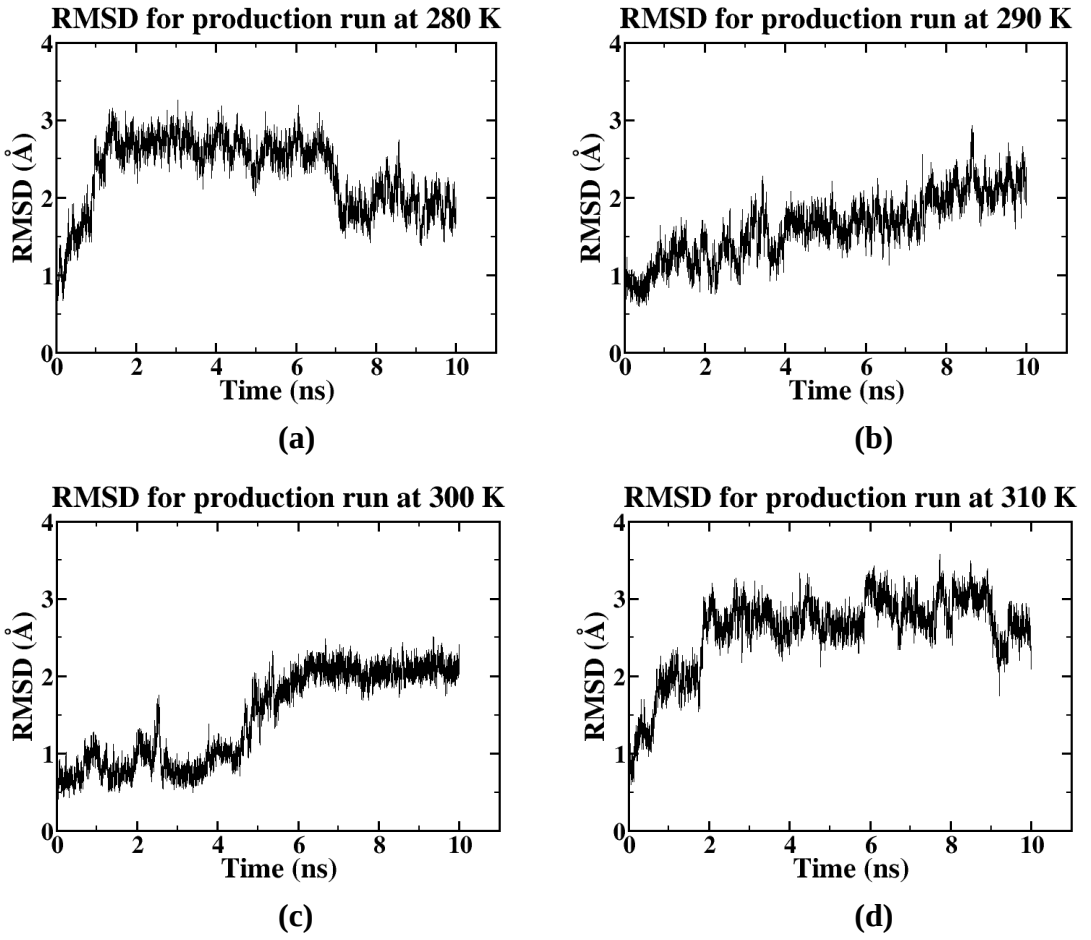


Figure 10: RMSD variation with temperature. The average difference of each Chlorotoxin atom position at beginning and every evolved time are calculated in Angstrom.

4.1 Predictions from simulations

Figures 10 (a), (b), (c), and (d) are the PR results of RMSD at different temperatures. The aggregated differences of each CLTX atom position between the initialized time step and every evolved time step are calculated in Angstrom. The RMSD plots suggest that the peptide CLTX is most stable at 300 K as inferred from the RMSD values that become constant at last few nanoseconds. The reduction of RMSD in Figure 10(a) with temperature 280 K and increment in Figure 10(b) with temperature 290 K indicate either molecule's instability or that the system has not attained EE. Under the different ion concentrations, from Figures 11 (a) and (b), the protein is relatively stable at 10 Na/13Cl and 50 Na/53Cl because of the RMSD value persists around 1 Angstrom. However, Figure 11 (c) shows the RMSD value increasing from 1 to 4 Angstrom that implies the mobility of the protein atoms. Since RMSD only concentrates on the atoms of CLTX, we cannot conjecture whether atomic velocities of CLTX or water molecule or ions impact on the RMSD. Hence, we cannot derive much detail for the interactions among CLTX, water and ions just from the RMSD analyses.

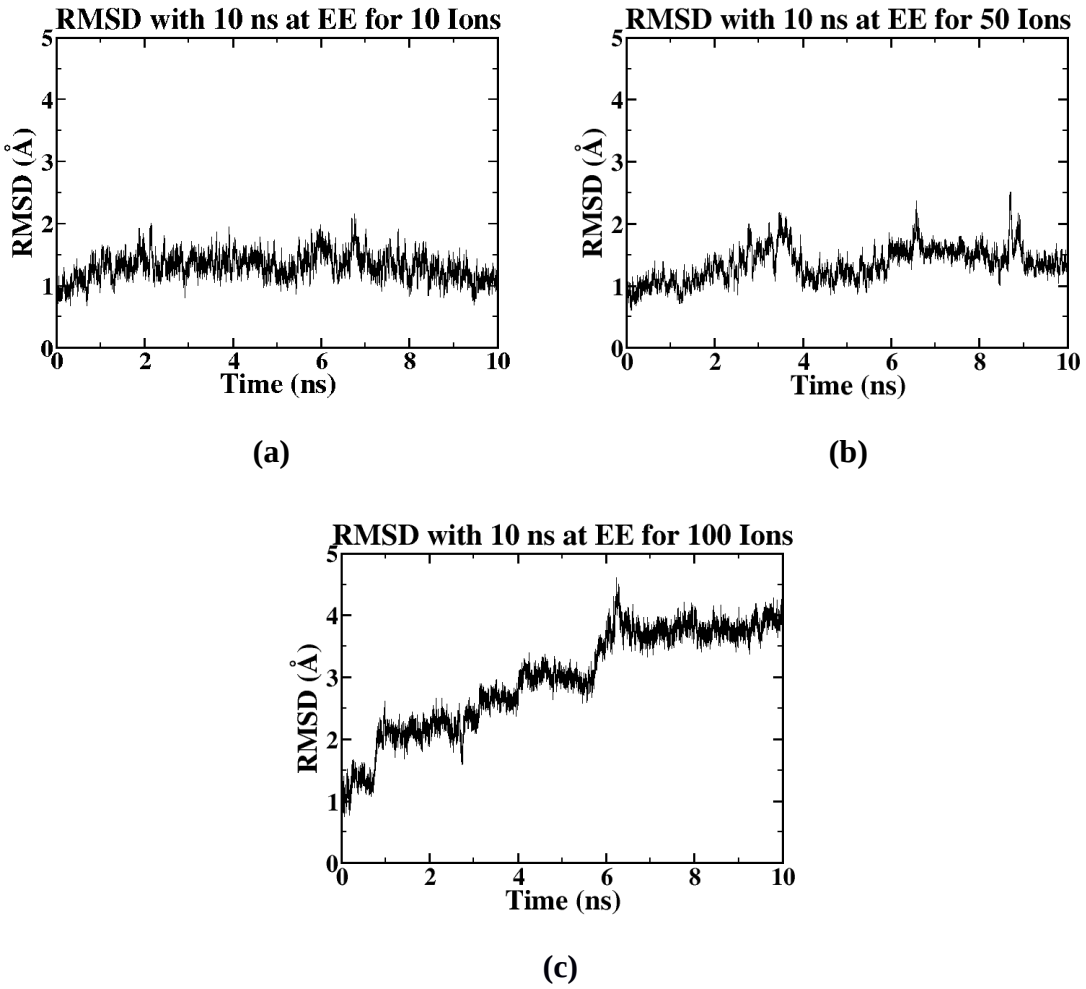


Figure 11: RMSD variation with ion concentration. The average difference of each Chlorotoxin atom position at beginning and every evolved time are calculated in Angstrom.

While RMSD provides the information of the CLTX atoms' displacements with time, Rg informs us about the expansion or compression of the whole CLTX molecule relative to its center of mass. Figures 12 (a), (b), (c), and (d) present the Rg value at different temperatures. By observing the Rg trend in all the four plots --- at 280 K it is varying irregularly, at 290 K it is increasing from 9.25 to 9.75 Angstrom, at 300 K it is approximately 9.25 Angstrom and at 310 K it is fluctuating around 9.25 Angstrom regularly but with a wide range. As a result, temperature 300 K, Figure 12 (c), shows the relatively stable environment.

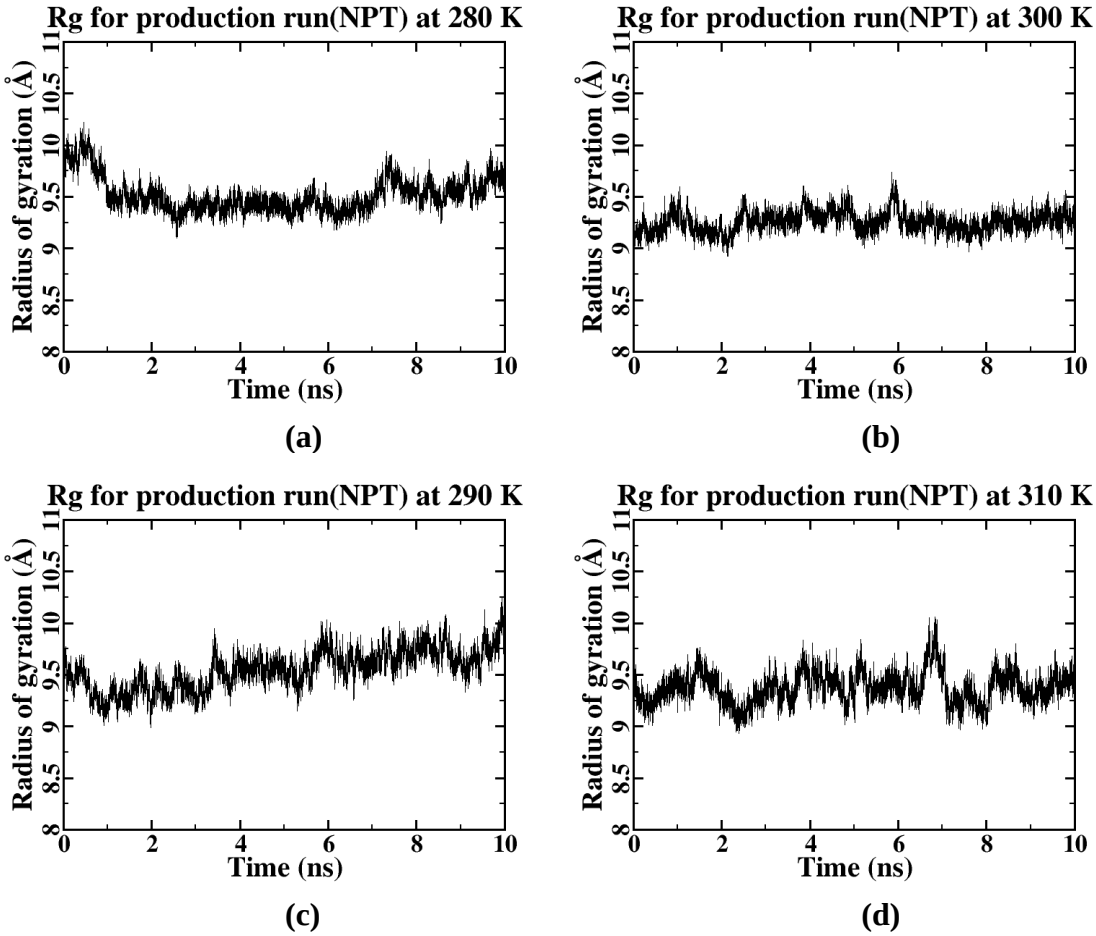


Figure 12: Variation of Rg with time at different temperatures. The average distance of atoms of Chlorotoxin expanding or compressing corresponding to its center at different times is represented in Angstrom.

We next present the Rg results in Figure 13 for 10 Na/13Cl, 50 Na/53Cl and 100 Na/103Cl environments. The Rg variation can be observed from the plots at different ion concentrations. Figure 13 (a), 10 Na/13Cl, indicates the Rg value approximated to 9.75 Angstrom. Figure 13 (b), 50 Na/53Cl, indicates the Rg value close to 9.5 Angstrom. Figure 13 (c), 100 Na/ 103Cl, shows the Rg value fluctuating between 9.0 and 9.75 Angstrom. These results imply that the protein stability decreases as the ion concentrations increases. On the other hand, compared ion concentrations result with temperature results of Rg, we notice that the values of Rg are different. This change suggests that ion concentrations contribute more to the CLTX stability compared to different temperature environments. Since the temperature environments become more complicated by adding ions, the interactions of CLTX atoms with other particles also become more frequent. Similar to RMSD, Rg only concentrates on the interaction of CLTX atoms with itself and provides no input on its relative interaction with water.

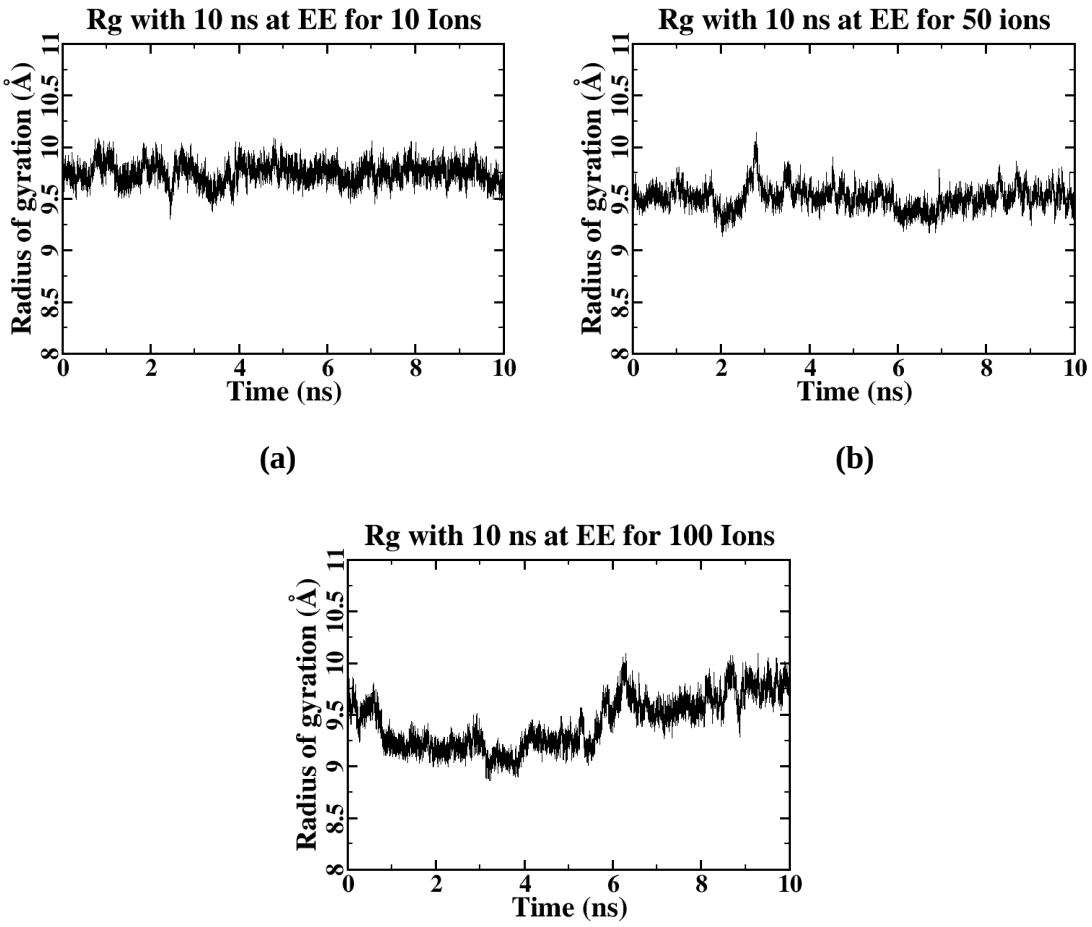


Figure 13: Variation of Rg with time at different ion concentrations. The average distance of atoms of Chlorotoxin expanding or compressing corresponding to its center at different times is represented in Angstrom.

After investigating the conformation of CLTX at varied environments by RMSD and Rg, we select RDF to explore the interaction of CLTX atoms with other particles, such as water molecules and ions. RDF measures the density of water molecule or ions corresponding to a reference CLTX site as a function of radial distance and Coordination number calculates the number of particles around CLTX as a function of radial distance.

Since CLTX is composed of 36 residues, it is meaningful to investigate the interaction of water molecules and ions surrounding each residue. Then we can discover the relatively active site of the protein, the hydrophobic and hydrophilic residues by using RDF. Since there are 36 residues in CLTX, it requires 36 RDF and CN distributions to investigate each residue.

RMSD, Rg and RDF are combined to interpret the stability of CLTX. From investigation of RMSD, we discover that the relative stable environment is at 300 K and low ion concentrations. Theoretically, RMSD reflects the mobility of each of the protein atoms. Rg reflects the whole molecule responsiveness when the molecule is stimulated by external force, such as temperature determining the atom velocity and ionic forces. Thus, it is significant to notice that the most stable temperature is at 300K that is between 280 K and 310 K. The geometrical structure of the protein determines the aggregated response through the atoms interacting with each other.

Figure 14 presents the peaks value of RDF and variation of CN which demonstrate the density of chloride, sodium and water relative to Tyrosine (TYR) at different environments. We can get particle distribution between residue TYR and water and between residue TYR and ions. It is apparent that TYR residue has a preference to interact with positive ion sodium.

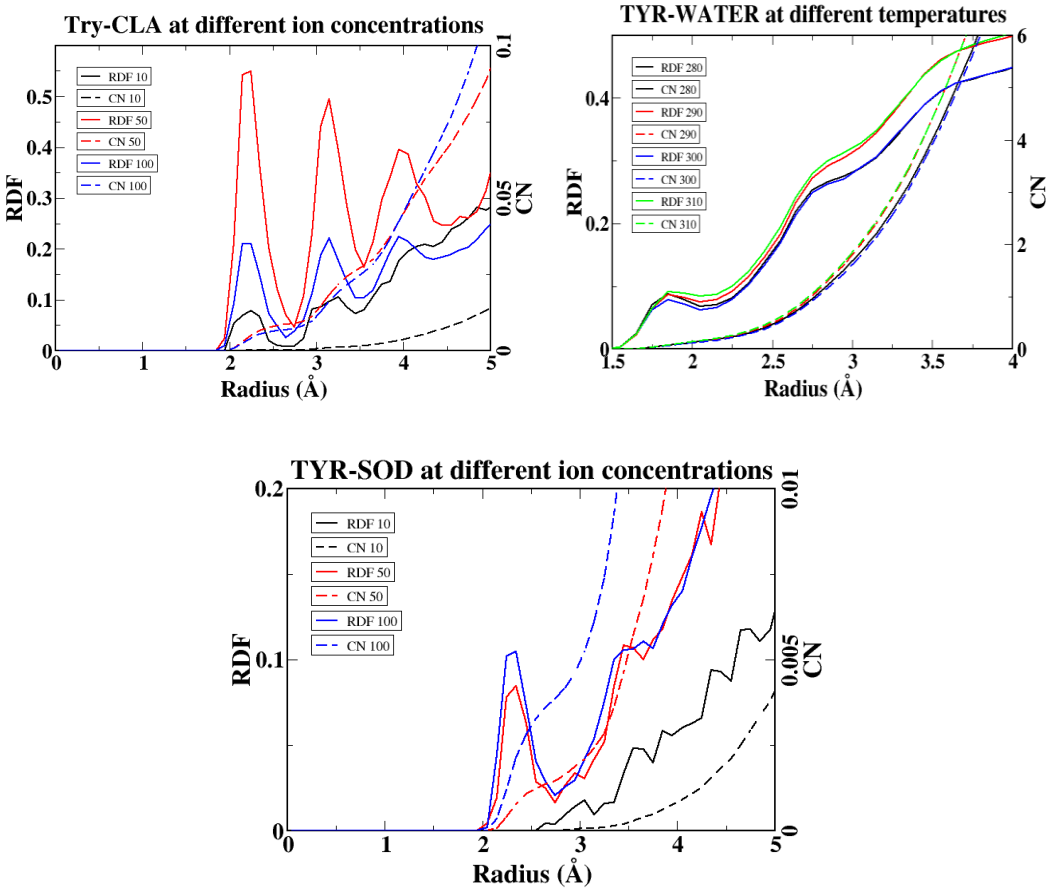


Figure 14: The RDF and variation of CN showing the density distribution of chloride, sodium and water relative to Tyrosine at different environments.

CHAPTER V

CONCLUSION AND FUTURE WORK

Stability can be immensely useful when investigating role of CLTX transport in blood and ionic environment. Equilibrium molecular dynamics simulations have been performed to examine the stability of CLTX described by molecular structure in the solvent environment over a range of temperatures and salt concentrations in the solution. We have investigated three properties, namely, RMSD, Rg and RDF to analyze stability of CLTX at different temperatures and different ion concentrations. The range of varied temperature is from 280 K to 310 K which can provide us with details about the molecule structure. We conclude that the peptide CLTX is most stable at 300 K by investigating RMSD and Rg, and the TYR residue shows affinity towards the positively charged ions. Future efforts will be focused on understanding the binding mechanism between CLTX and MMR-2, and their control with external stimulus.

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