Section I

Introduction
Antimicrobial peptides (AMPs) are small molecular weight proteins which act against pathogens like bacteria, viruses and fungi. These peptides have evolved as the components of innate immune system, and thus, they are even able of killing cancerous cells. AMPs (Ganz, 1998) are found in both prokaryotic and eukaryotic organisms, and, also in vertebrates and invertebrates. Nearly whole of the body system of the organism shows the presence of AMPs to confront with the non-self particles (Reddy, 2004), and, as such, they are found in lymph, mucosal surface, body surface, phagocytic cells and reproductive tracts of vertebrates (Matsuzaki, 1999). The antimicrobial activities of secretions, blood leukocytes and lymphatic tissue were recognized during the last fifteen years of the nineteenth century, and, between 1920 and 1950, many of the AMPs were isolated from these secretions and shown to be selective against both Gram positive and Gram negative bacteria. The presence of these antimicrobial substances in tissues and fluids re-establishes their natural resistance to pathogenic micro-organisms. Their inducible nature on exposure of the tissues to micro-organisms led to the confirmation of their relationship with the immune system of the organism beyond doubt. The field of antimicrobial peptide research was born with this observation.

As discussed above, the peptides, labeled as anti-microbial peptides, play a primary role in host defense. Hence, most researchers prefer to call them protein fragments acting as antigens or signal molecules between the cellular components of innate or adaptive immunity. Undoubtedly, these antimicrobial peptides have an ancient lineage. The antibacterial activities of these peptides observed during the last century (in 1960’s and 70’s) were re-confirmed with the indication that all plants, insects and vertebrates have cell-free defense mechanisms in-built in their system. This understanding initiated a thorough search for peptides from diverse sources such as plant tissues, insect
hemolymph, amphibian skin, mammalian neutrophils, etc. In 1980’s, enormous effort was put into the structural and functional characterization of these peptides with a view to develop new and potent antibiotics. This could help to solve the increasing problem of bacterial resistance to antibiotics in human. In the mid 1990’s, several evidences supported the assumption that these AMP’s could also act as signaling molecules for cellular effectors of both innate and adaptive immunity.

Mammalian peptides express a diverse range of immuno-modulatory functions like chemokines, regulation of host gene expression, suppression of sepsis and promotion of wound healing. On the basis of their multifunctional role, these were called host defense peptides. Some of the evidences also indicated the protective role of these peptides in vivo, in reducing the susceptibility to infection. As these peptides play a major role in immunity, their evolution and phylogenetic relationship have become the most focused area of research now.

AMPs from different species share common structural scaffolds which can tolerate a high degree of variation in the amino acid sequence to make them persistence during evolutionary divergence from the remote common ancestors. During the long period of evolution, organisms tended to adapt themselves to the exposed environment which led to their adaptive immunity. It is unique to vertebrates whereas innate immune mechanisms are shared also by diverse range of species across the animal and plant kingdom.

Tomassing and Zanetti (2005) provide an updated review of these peptides. They also suggest that repeated gene duplication gives rise to diverse array of distinct family members of these peptides with specific functions in mammals. Understandably, these peptides are unique and divergent groups of molecules. They are generally between 12 and 50 (< 100) aminoacid-long with net positive charge between +2 to +9. These are characterized by multiple number of the
amino acids like proline, arginine, lysine, tryptophan or phenylalanine and a high proportion (> 30%) of hydrophobic amino acids. A common feature shared by these cationic AMPs is their ability to fold into amphipathic conformation on interaction with membrane.

On the basis of their secondary structure, AMPs can be categorized into four groups (Epand and Vogel 1999, von’t Hoff et al., 2001).

I. \textbf{α-helical}: These are linear amphipathic peptides. The peptides are in disordered structure in aqueous solution but adopt an α-helical structure in hydrophobic solvents or on lipid surface e.g. magainin, cecropinA, temporin. Magainin is highly studied in this group. This is 23 amino acid-long cationic peptide secreted on the skin of African clawed frog \textit{Xenopus laevis} (Zasloff et al., 1987). Some peptides of this group are hydrophobic α-helix with slight negative charge. Anionic peptides are less effective in microbes compared to mammalian cells. One of the negatively charged and hydrophobic peptide is alamethicin (Duclohier and Wroblewski, 2001; Kikukawa and Araiso, 2002). This peptide surrounds ion transport channel after traversing through the lipid bilayer. Plate 1.1 image (a) presents the 3D helical structure of magainin.

II. \textbf{β-sheet}: The β-sheet peptides are cyclic with conserved motifs of six cysteine residues forming three disulfide bonds. The positions of the bonds are between C1-C4, C2-C5, C3-C6. X-ray crystallography suggests that peptides exist as dimers (Hill et al., 1991). The peptides of this group are human β-defensin-2 (Hancock, 2001), tachypleins (Matsuzaki, 1999), protegrins (Harwig et al., 1995), and lactoferricin (Jones et al., 1994), gramicidin S (Prenner et al., 1999), polymyxin B (Zaltash et al., 2000), and tyrocidines (Bu et al., 2002). The peptides exist as β-sheet in solution and further stabilize on interaction with lipid surface. Defensin is well the characterized peptide of this group.
Plate 1.1
Plate 1.1
UCSF Chimera v 1.4.1 rendered images of
(a) α-helical structure of magainin2 (source- *Xenopus laevis*, pdb code- 2MAG)
(b) β-sheet structure of human β defensin-1 (source- *Homo sapiens*, pdb code- 1KJ5)
(c) Extended structure of bovine indolicidin (source- *Bos taurus*, pdb code- 1G89)
(d) Loop structure of nisin (source- *Lactobacillus lactis*, pdb code- 1WCO)

X-ray crystallography (Hill et al., 1991) and NMR (Zhang et al., 1992) studies revealed that defensins either perturb the lipid bilayer or form channels in membrane. This study emphasizes the importance of disulfide linkage for the antimicrobial activity and replacement of cysteine residue by certain other amino acid like Ala, Asp and Leu leads to inactivation of the peptide. Another study on tachyplesin analogs suggested that cyclic structure is essential for antibacterial activity while it may have no role to play in membrane permeabilization (Matsuzaki et al., 1997; Tamamura et al., 1998; Rao, 1999). Image (b) of plate 1.1 is the general 3D structure of defensins group.

III. **AMPs rich in specific amino acids**: The peptides belonging to this group are rich in certain specific amino acids. Histatin, a peptide found in saliva is rich in histidine residues (Brewer et al., 1998; Tsai and Bobek, 1998; Helmerhorst et al., 1999). This peptide targets mitochondria of yeast cell, expressing its unusual antifungal property (Helmerhorst et al., 1999). The peptides produced by porcine neutrophil are rich in proline and arginine or proline and phenylalanine. Examples of these peptides are PR-39 and prophenin which belong to cathelicidin family (Zhao et al., 1995; Linde et al., 2001). Trp rich peptides include tritripticin (Lawyer et al., 1996) and indolicidin (Selsted et al., 1992). Indolicidin permeabilizes the outer membrane of *E. coli* (Falla et al., 1996;
Subbalakshmi et al., 1998) to form channels. Several analogs of these peptides have been synthesized to understand the charge requirement and role of Trp and Pro residues in antibacterial activity (Falla et al., 1996; Subbalakshmi et al., 1996). Image (c) of plate 1.1 presents the structure of the extended form of indolicidin.

IV. Loop structures: Proline and arginine rich peptides can not form amphipathic structure rather they form polyproline type II structure (Boman et al., 1993; Cabiaux et al., 1994). Lantibiotics having a ring structure with thioether bond belong to this group. Nisin is a lantibiotic used as antibacterial agent in food preservation due to its high activity against Gram positive bacteria. This peptide has affinity for Lipid II, which is precursor in the bacterial cell wall synthesis. Image (d) of plate 1.1 is structure of the loop form of nisin.

The activity of AMPs depends on several parameters such as conformation, charge, hydrophobicity, hydrophobic moment, amphipathicity and polar angles. These features are interdependent and influence each other.

1. Conformation (χ): Despite being from diverse sources and having variations in sequence arrangement, AMPs take up two main conformations - α-helix and β-sheet (Hancock, 1997). The α-helical antimicrobial peptides are generally found in extracellular fluids in unstructured form but become helical structure on interaction with amphipathic phospholipid membrane. The β-sheet peptides are highly diverse at the primary structure level but possess distinct hydrophilic and hydrophobic surfaces in solutions.

2. Charge (Q): AMPs have net positive charge and contain cationic domains. The positive charge is due to high proportion of basic amino acid, lysine and arginine, in their sequences. Cationicity of antimicrobial peptides are important for the initial electrostatic attraction to negatively charged phospholipids of the microbial membrane. On increasing positive charge, the membrane activity of
peptides decreases. This is due to strong electrostatic interaction, which anchors the peptide into the negatively charged head group of lipid region (Dathe and Wieprecht, 1999) or the repulsion between the positively charged side chains of intra and intermolecular sequences obstructs the formation of pores (Matsuzaki, 1999). The membrane disruption ability of peptides also depends on the charge and size of the lipid head groups of membrane (Wieprecht et al., 1997; Vogt and Bechinger, 1999).

3. Hydrophobicity: It is the percentage of hydrophobic residues in a peptide and is approximately 50% for most of the antimicrobial peptides (Eisenberg et al., 1984). This parameter measures the peptide affinity for the lipid acyl chain in the core of biological membrane. Histatin and magainin derived peptides confirmed that hemolytic activity is highly related to hydrophobicity but independent of amphipathicity and net peptide charge (Helmerhorst et al., 1999).

4. Amphipathicity and hydrophobic moment: Amphipathicity measures the spatial separation between hydrophilic and hydrophobic side chains in a protein. The amphipathicity of a protein is determined by calculating the hydrophobic moment. Three to four hydrophobic and hydrophilic residues are optimal to interact with amphipathic membrane. The permeabilising and hemolytic activity of a peptide can be increased by increasing the hydrophobic moment. β-sheet also exhibits amphipathicity by several β-strands with very few or no helical domains to create both polar and non-polar surfaces. The β-sheet structure possessed by HNP-3 and by other defensins interacts with target membrane involving amphipathicity and hydrophobic moment.

By the mechanism of biosynthesis, AMPs fall into two categories: non-ribosomally and ribosomally synthesized peptides. Ribosomally produced peptides are synthesized by the process of gene transcription and ribosomal
translation followed by final proteolytic processes, as in case of all functional proteins. They are of utmost significance for the synthesis of active AMP. Magainins are synthesized as long pre-proproteins releasing mature magainin by proteolytic cleavage (Ketchem et al., 1993). In temporins, negatively charged carboxyl terminal is removed by amidation process (Simmaco et al., 1996). Recently, it has been found that some intact proteins have no any antibacterial activity, but after proteolytic cleavage, the released segment exhibits antimicrobial activity. Lactoferrin, a milk protein after digestion with pepsin releases a 25 residue-long peptide lactoferricinB, which expresses a bacteriostatic potency as compared to intact protein (Bellamy et al., 1992).

The remarkable feature of AMPs is their cell specificity by which they kill microbes but being non-toxic to mammalian cells. This property of AMPs is due to the difference in lipid composition between prokaryotic and eukaryotic cell membrane (Dathe and wieprecht, 1999; Matsuzaki, 1999). The outer membrane surface of Gram negative bacteria contains lipopolysaccharide (LPS), while in Gram positive organisms, acidic polysaccharide (teichoic acids), is responsible for the negative charge on its surface. On the contrary, the outer leaflet of mammalian cell is composed predominately of phosphatidylcholine (PC) and sphingomylin (SM) phospholipids (Devaux, 1991; Dolis et al., 1997). The role of the outer membrane and LPS on antimicrobial activity was studied with magainin (Rana et al., 1991). Magainin 2 alters the peptidoglycan complex of outer membrane from wild-type Salmonella typhimurium and a series of LPS mutants, which were susceptible to bactericidal activity of cationic antibiotics. LPS mutants also exhibited the loss of resistance towards the magainin 2 as the length of lipopolysaccharide decreases. Although, there is not always a direct correlation between the antimicrobial activity of a peptide and lipopolysachharide components of the membrane.
The essential component of all the biological membrane is phospholipid bilayer. It is only the phospholipid which displays large diversity in membrane architecture (Kinnunen, 1991; De Kruijff, 1997; Dowhan, 1997) and plays an important role during interaction with antimicrobial peptides (Lohner and Prenner, 1999; Lohner, 2001). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) have no net charge while sphingomyelin (SM), a close analog of PC containing palmitoyl residue, is also neutrally charged. Hydroxylated phospholipids - phosphatidylglycerol (PG), phosphatidylserine (PS) and cardiolipin (CL) - possess a net negative charge. Sterols such as cholesterol and ergosterol found in eukaryotes but rarely in prokaryotes are generally neutral. Cell membrane composed predominately of PG, CL or PS tends to be highly electronegative and is found in many bacterial pathogens. On the contrary, bilayers enriched in zwitterionic phospholipids PE, PC or SM, commonly found in mammalian cytoplasmic membrane, are neutral in charge. Thus, the net charge of a biomembrane is largely based on its phospholipid stoichiometry and architecture. Sterols found in eukaryotic membrane further differentiate mammalian and fungal cells from prokaryotes (Tytler et al., 1995). Antimicrobial peptides with antifungal activity (most of them isolated from plants) are rich in polar neutral amino acids. Cationic antimicrobial peptides bind to membrane containing acidic phospholipids such as PG or PS due to strong electrostatic interaction (Matsuzaki et al., 1991, 1995, 1998. The fluidity and curvature of lipid bilayers also influence the lipid-peptide interaction. Fluid bilayers are more susceptible to the peptides. Magainin permeabilizes lipid bilayer more effectively in liquid-crystalline than gel-phase (Matsuzaki et al., 1991). Cholesterol found in eukaryotic cell membrane is also responsible for fluidity and modulates the activity of the inserted peptide (Matsuzaki et al., 1991). It has been suggested that the formation of hydrogen bonds between
glutamates and cholesterol reduces the antibiotic activity (Tytler et al., 1995). Peptide inserts more slowly into cholesterol containing membranes (Silvestro et al., 1997).

The composition and architecture of prokaryotic and mammalian membranes are neither static nor symmetrical. The distribution of phospholipids within the cytoplasmic membranes is highly asymmetric, for example, only 2% of total PE content in bovine erythrocyte is oriented towards the outer membrane leaflet (Florin-christensen et al., 2001). These differences among the asymmetric distribution, compositional stoichiometry and saturation of phospholipids bilayer highly influence the membrane phase transition and fluidity (Bayer et al., 2000, Verkleij et al., 2000, McIntosh et al., 2001). Interaction of cationic antimicrobial peptide with phospholipids also disturbs the membrane symmetry. Lash et al (1998) demonstrated that a polylysine peptide induces bacterial 1,2-dimyristoyl-PE to separate from lipopolysachharide into a distinct domain. This suggests that interaction with peptide promotes abnormal asymmetry within or between phospholipid leaflets of bilayer membrane.

Many antimicrobial peptides exist in unstructured conformation prior to interaction with the target cells. On binding with the pathogen membrane, peptides undergo conformational dynamics to helical or other structures that effect antimicrobial activity. Tam et al (2000) examined the influence of conformation on membranolytic selectivity of antimicrobial peptides. Unger et al (2001) also provide additional insight into the structural basis for selective toxicity of AMPs. As compared with linear structure, the cyclized peptides are less efficient in initial binding to phospholipid membrane, and, after binding to lipid bilayer, these cyclized peptides revert to 75% of helical structure of their linear analogs. Oren and colleagues (1999) also shed light on the relationship between quaternary structure and selective toxicity among antimicrobial
peptides. Human cathelicidin LL-37 is an antimicrobial peptide cytotoxic to both bacterial and mammalian cells. This peptide exits in equilibrium as monomer and oligomer in solution at low concentration but undergoes self-association within zwitterionic and electronegative phospholipid membranes in vitro. The mechanism of action of LL-37 is detergent-like referred as carpet mechanism, forming a structure induced membrane perturbation. Kol et al (2001) demonstrated that the ability of peptides to induce phospholipid translocation was greater for the peptides containing more lysine or histidine residues, compared with those containing tryptophan.

Antimicrobial peptide mediated cell killing may be rapid. Some linear α-helical peptides kill bacteria so quickly that it is difficult to characterize the steps preceding the cell death (Boman, 1995). There is no any specific mechanism for antimicrobial activity but some specific steps do occur. One such mechanism is electrostatic bonding between anionic or cationic peptide and the structure on the bacterial cell surface. Studies show that magainin 2 and Cecropin A readily insert into monolayers, large unilamellar vesicles and liposomes that contain phospholipids (Zhao et al., 2001; Silvestro et al., 1997). The mechanism by which these peptides permeabilize and traverse microbial membrane is not entirely clear, and, even vary for different peptides. Conventional CD is the tool to determine the peptide secondary structure such as α-helices but it needs optically clear solution (Blondelle et al., 1999, Sitaram and Nagaraj, 1999). Similarly, infrared spectroscopy (e.g. FTIR) is an important tool to study β-sheet peptide conformation. Nuclear magnetic resonance (NMR) study is a powerful technology to obtain structural information for a single residue or at the domain level. Recently developed tools include reverse-phase pressure liquid chromatography and surface plasmon resonance. One of the main events occurring after membrane binding is the process of peptide’s structural or
conformational phase transition. It is most well studied for α helical AMPs. Numerous studies by using various biophysical techniques elucidate that many α-helical antimicrobial peptides are disordered in aqueous environment and exhibit random coil conformation (Bello et al., 1982, Dathe and Wieprecht, 1999). Many peptides rapidly undergo highly structured amphipathic α-helical conformation on interaction with phospholipid bilayer. The frog skin peptide PGLa (the peptide which starts with glycine and ends with leucine amide) is in disordered state but after being exposed to membrane, adopts a helical structure in presence of membrane composed of PG and PE (Latal et al., 1997). Magainins only undergo a helical transition when interacting with anionic membranes (Matsuzaki et al., 1989, 1991). Studies on cecropin analogs also revealed that the extent of α-helical conformation is dependent on the amount of negatively charged phospholipids within the model membrane (Wang et al., 1998). In comparison, β-sheet antimicrobial peptides are much more in ordered state in aqueous solution and membrane environment due to constraint imposed by disulfide bonds. The secondary structure of tachyplesin, a cyclic β-sheet peptide, remains relatively stable on interaction with the target cell membrane.

Antimicrobial peptides may self-associate following the initial interaction with the target membranes. These peptide-peptide interaction and peptide-lipid interaction within membrane create a complex structure, associated with specific antimicrobial mechanism of action. Peptides with well defined hydrophobic and hydrophilic domains efficiently orient themselves towards their respective membrane constituents or corresponding domains in adjacent peptides. This orientation facilitates amphipathic peptides deep into the hydrophobic membrane core.

Various models have been proposed for membrane permeabilization by AMPs. These peptides may act via different mechanisms in distinct membrane
environment. Some models have been proposed in accordance with the prevailing concepts of antimicrobial mechanism of action.

1. The Barrel-Stave mechanism:
This mechanism describes the channel formation during membrane permeabilization. In this model, a number of channel forming peptides are arranged in barrel like ring around an aqueous pore. The initial step in barrel stave pore formation mechanism involves the binding of peptides at the membrane surface most likely as monomers. During binding, the hydrophobic residue/surface of α-helical or β-sheet peptides faces outward whereas the hydrophilic surface forms pore lining (Ehrenstein and Lecar 1997; Breukink and Kruijiff, 1999). After binding, these peptides undergo conformational phase transition, forcing the polar phospholipid head groups to aside, to induce membrane thinning.

When these bound peptides reach a threshold concentration, peptide monomers self aggregate and insert deeper into hydrophobic membrane core. Continued accretion of peptide monomers near the membrane leads to translocation/relaxation of pore. Now, these peptides are transported to the inner membrane leaflet due to concentration gradient of surface-bound peptides. This mechanism of action has been proposed for Alamethicin (Sansom, 1991, Beven et al., 1999, Yang et al., 2001). It has been suggested that four or more trans-membrane spanning peptides are responsible for the channel formation. (Fig:1 on page 16)

2. The Carpet mechanism:
In the carpet model, a high density of peptides is understood to accumulate on the target membrane leading to change in membrane fluidity due to phospholipid displacement, which subsequently leads to membrane disruption. These peptides initially bind to membrane via electrostatic interaction and when
threshold peptide density or concentration is reached, the membrane undergoes favourable energetics resulting in loss of its integrity. This orientation destabilizes the phospholipid packing and causes membrane disruption due to concentration of peptide monomers on the surface (Sitaram and Nagraj, 1999). Peptides follow carpet mechanism of membrane disruption, when the membrane models are rich in PS (Matsuzaki, 1998, 1999). (Fig: 2 on page 16)

3. The Torroid Pore or Wormhole mechanism:

One of the most well characterized peptide membrane interaction is also called supra-molecular complex. It represents a membrane-spanning pore lined with polar peptide surface and phospholipid head groups. The α-helical peptides like magainins and PGLa are known to act by this mechanism. AMPs in the extracellular environment take an α-helical conformation as they interact with charged and hydrophobic bacterial membrane.

These helices are initially oriented parallel to the membrane surface as confirmed by NMR, fluorescence and CD method (Hara et al., 2001). The hydrophobic residues of the bound peptides displace the polar head groups, creating a breach in the hydrophobic region of the membrane. This induces a positive curvature strain in the membrane (Hara et al., 2001). When the peptide reaches a threshold peptide to lipid ratio (estimated to be 1:30 for maganin), they orient themselves perpendicular to the membrane. At this point, helices begin to self associate so that their polar residues are away from the membrane hydrocarbon chains. This transient structure forms the dynamic peptide-lipid supra-molecular or torroidal pore complex. On disintegration of the pore, some peptides become translocated to the cytoplasm surface (Uematsu and Matsuzaki, 2000). (Fig-3 on page 17)
Fig: 1 Barrel stave model for transmembrane pore formation. (a) The peptide monomers bind to cell membrane in an α-helical confirmation, (b) Localization of more peptides on cell membrane, (c) Insertion of peptide helices into hydrophobic core of membrane (d) Additional monomers increase the pore size causing leakage of cytoplasmic material leads to death of cells.

Fig: 2 Carpet model for membrane disruption. (a) Binding of peptides monomers to the phospholipids head groups (b) Alignment of peptides on the membrane surface so that hydrophilic residue face the phospholipids head group (c) Reorientation of peptides to traverse the hydrophobic core of membrane (d) Disintegration of membrane due to disruption of bilayer curvature.
The cytoplasmic membrane is responsible for mediation of many essential functions in microbial pathogens. The functions include selective permeability and maintenance of gradients, cellular energetics driven by electron transport and oxidative phosphorylation in bacteria, and mitochondria in eukaryotic pathogens, synthesis and cross-linking of peptidoglycans, chitin or other biopolymers, motility and processing or display of adhesion or other virulent determinants. Antimicrobial mediated cell death indicates that some peptides kill cell within 2-3 minutes after exposure (Lehrer et al., 1989; Tossi et al., 1997). This rapid cell death is due to consequences of membrane depolarization, loss of ions and metabolite gradients and cessation of other essential functions such as respiration (Blondelle et al., 1999; Hancock and Chapple, 1999). In Gram-negative organisms, antimicrobial peptide interacts independently with
outer and inner membranes. This has been demonstrated for human defensins (Lehrer et al., 1989) in which permeabilization of the outer membrane is followed by that of the inner. For Gram-positive cells, exposure to antimicrobial peptides results in the increase in water and ion flow, efflux of $K^+$ ions, swelling and osmotic dysregulations (Juretic et al., 1989; Ohta et al., 1992; Matsuzaki et al., 1997).

Antimicrobial peptides also inhibit the synthesis of extracellular molecules like peptidoglycan, chitin or other macromolecules. Peptidoglycan synthesis precursor molecules are transported to the cytoplasmic membrane. Cationic or anionic peptides perturb the membrane and peptidoglycan synthesis integrity. Gram-positive organisms are more susceptible due to their high content of peptidoglycan. Antimicrobial agents selectively inhibit protein synthesis (30S or 50S subunit inhibitors) or DNA metabolism. Cationic antimicrobial peptides bind to and inhibit negatively charged nucleic acids. In one experiment, Kragol et al (2001) found that insect peptides pyrrhocoricin, drosocin and apidaecin inhibit the bacterial heat shock protein DnaK leading to cell death. Buforin II also interferes with the intracellular functions (Park et al., 1998). Microcin B17 inhibits DNA replication by binding with DNA gyrase enzyme of *E.coli* (del Castillo et al., 2001).

**Plan of work in the present project:**

The project was conceived with a view to design sequences of desirable amino acids with the required parameters for the antimicrobial properties known till yet on the basis of the structural integrity of some selected AMPs purified from diverse sources. The designed sequences were to be modeled for their possible 3D structure through the application of some well-written algorithm and software. Prediction and structural modeling would have to be based on the templates of the peptides (AMPs) with as much sequence similarity as possible,
and whose 3D structure have already been characterized by X-ray crystallography or NMR technique. The next step was to test the efficacy of the designed sequences by subjecting them to dock with the possible bacterial targets e.g. bacterial membrane components, bacterial cell wall forming enzymes, nucleic acids and the protein synthesizing machinery. These studies were conceived to lead to the selection of potent antibiotic sequences that could commercially be obtained by cloning the corresponding gene in some suitable host and getting it expressed with the help of suitable promoters.

The present project aimed to –
1. To develop a functional algorithm for pattern analysis of the known antimicrobial peptide structures, analyze significant parameters inducing antibiotic capability to suggest some new structures and writing a suitable software (in C++ or Perl) for estimating those properties of the designed peptides,
2. Analyze and model the major antimicrobial peptides with reported 3D structure with the help of the standard visualization/modeling softwares,
4. To design the suggested new sequences and develop predicted models of their 3D structure,
5. To test the de novo designed peptides for requisite parameters and carry out sequence similarity/identity with the help of blast program and related tools at the AMP Database, and
6. To study the interaction of the designed peptides to predict the mechanism of their antibacterial action with the target bacterial components through molecular docking and related approaches, and
7. To plan further for clinical assessment of the peptides.