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Research Article

Molecular modeling, docking and dynamics analysis of antimicrobial peptides with the ADP ribosylation toxins

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Abstract:
Antimicrobial peptides are cationic, amphiphilic peptides of 12-50 residues with microbicidal activity against bacteria and fungi. The regulartory protein linked with ADP- ribosylation is an important pathological mechanism by which various bacterial toxins affect the eukaryotic cell functions and it is strongly responsible for the actions of several bacterial toxins. The hman-defensins are multifunctional arginine-rich peptides characterized by three intra-molecular disulfide bridges for structure stability. The antimicrobial peptides that are of interest to us are the human alpha and beta-defensins. The ADP-ribosylating 'A subunit' in known structures with PDB ID: 1XTC, PDB ID: 1WGE and PDB ID: 1TI1 are used in this study. Molecular docking analysis of the ADP-ribosylating toxin and antimicrobial peptides using the PATCH DOCK and FIRE DOCK servers were completed. Molecular dynamics dimulation (MDS) was performed for 0.5ns to study the interaction of ADP-ribosylating toxins and antimicrobial peptides. The RMSD values were plotted in a graph format and hydrogen bonds at the interface are calculated and documented. These data show that the toxin is bound to ADP-ribosylating toxin through human alpha defensin 3. Information thus documented find application in combating ADP-ribosylating linked bacterial diseases.

Keywords: Antimicrobial, amphiphilic peptides, ADP-ribosylation, cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* heat-labile toxin

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Background:

Antimicrobial peptides (AMPs) transpire in all kingdoms of life and are integral to host defense. Antimicrobial confrontation has been recognized as a foremost threat to public health and hazardous post-antibiotic era **[1]**. There are so many AMPs identified until now, these peptides can be classified to different groups based on the diverse amino acid components, structures and biological functions of these peptides **[2]**. AMPs link up particularly with the membrane of bacteria and kill the cell by causing seepage of its contents **[3]**. Even though the additional exploration of innate products for their antibiotic activities is costly and the success chances are partial, the recognition of not previously appreciated deliverance methods or products from natural molecules, will open up innovative research perspectives about antimicrobials **[4]**. AMPs are generally endogenous, cationic, amphipathic polypeptides, produced by numerous natural sources. Recently, many biological functions beyond antimicrobial activity have been attributed to AMPs, and some of these have attracted the attention of the cosmetics industry **[5]**. ADP-ribosylation is the addition of one or more ADP-ribose moieties to a protein. These reactions are involved in signaling and the control of many cell processes, including DNA repair and apoptosis. ADP-ribosylation is a ubiquitous regulatory post-translational modification involved in many key processes such as DNA repair, transcription, cell differentiation, apoptosis, and the pathogenic mechanism of certain bacterial toxins. The ADP-ribosylation regulatory cycle has significant interest because of its role in regulating metabolic processes and because it is central to the pathogenic mechanism of a number of toxins. The structure and function of ADP-ribose transferases have been studied widely but very little is known about the mechanism of the hydrolases that remove ADP-ribosylations, providing the regulation's reversibility. In particular, no substrate, product or reaction intermediate complex structures are available for any enzyme removing ADP-ribosylations to serve as foundations for mechanistic suggestions and inhibitor design **[6, 7]**. Human defensins are small cysteine-rich cationic proteins found in both vertibrates and invertibrates. Defensins have also been reported in plants and function as host defense peptides against bacteria, fungi and viruses. They consist of 18-45 amino acids including six to eight conserved cysteine residues. Human neutrophil peptides are found in human atherosclerotic arteries. Human neutrophil-derived alpha-defensins (HNPs) are capable of enhancing phagocytosis. HNP1-3 has been reported to increase the production of tumor necrosis factor (TNF). The capacity of defensins to enhance phagocytosis, promote neutrophil recruitment, enhance the production of proinflammatory cytokines, suppress anti-inflammatory mediators and regulate complement activation argues that defensins upregulate innate host inflammatory defenses against microbial invasion **[8]**. Beta defensins are a family of mammalian defensins. The beta defensins are antimicrobial peptides implicated in the resistance of epithelial surfaces to microbial colonization. β-defensins are coding for genes, which impact the function of the innate immune system.

These genes are responsible for production of antimicrobial polypeptides found in white blood cells such as macrophages, granulocytes and NK-cells, β-defensins are also found in epithelial cells. Single-nucleotide polymorphisms (SNPs) are found in genes coding for β-defensins. β-defensins are cationic and can therefore interact with the membrane of invading microbes, which are negative due to lipo polysaccharides (LPS) and lipoteichoic acid (LTA) found in the cell membrane. The peptides have higher affinity to the binding site compared to Ca^{2+} and Mg^{2+} ions. The peptides will therefore exchange place with those ions, thus affecting the stability of the membrane **[9]**. Many bacterial toxins are proteins, encoded by the bacterial chromosomal genes, plasmids or phages. Lysogenic phages form part of the chromosome. The toxins are usually liberated from the organism by lysis, but some are shed with outer membrane proteins in outer membrane vesicles. An important non-protein toxin is lipopolysaccharide or endotoxin, which is a constituent of the cell wall of gram-negative bacteria. Toxins may damage the eukaryotic cell membrane by combining with some structural component, or otherwise alter its function. Many toxins combine with specific receptors on the surface membrane, frequently glycoproteins or gangliosides, and penetrate the cell to reach their intracellular target. A common mechanism of entry is absorptive endocytosis. Many protein toxins have an A-B structure, B being a polypeptide, which binds to the receptor and A being an enzyme **[10]**. The bacterial exotoxins, cholera toxin (CT), pertussis toxin (PT), and diphtheria toxin (DT), interfere with specific host proteins to cause tissue damage for their respective infections. The common toxic mechanism for these agents is mono-ADP-ribosylation of specific amino acids in eEF-2 proteins, by the catalytic A chains of the toxins. In the absence of acceptor proteins, these toxins also act as NAD^+-N-ribosyl hydrolases. The transition-state structures for NAD^+ hydrolysis and ADP-ribosylation reactions have oxacarbenium ion character in the ribose **[11]** designed and synthesized analogues of NAD^+ to resemble their oxacarbenium ion transition states.

Vibrio cholerae growing in the intestine secretes an exotoxin composed of 5 B subunits, an A subunit and an A2 subunit. On exposure to small bowel epithelial cells, each B subunit binds to a receptor on the gut epithelium. Following binding the A and A2 moieties migrate through the epithelial cell membrane. The A subunit is an ADP-ribosyl transferase that catalyzes the transfer of ADPR from NAD to a guanosine triphosphate (GTP) binding protein that regulates adenylate cyclase activity. The ADP-

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ribosylation of GTP binding protein inhibits the GTP turnoff reaction and causes a sustained increase in adenylate cyclase activity, which results in excess secretion of isotonic fluid into the intestine with resulting diarrhea. *Corynebacterium diphtheriae* cells lysogenized with the b-phage produce a diphtheria toxin which is a bipartite molecule, composed of a B subunit which mediates binding to a specific human cell surface receptor and an A subunit which possesses enzymatic (ADP-ribosyl transferase) activity. The substrate of the reaction is human elongation factor 2 (EF2), an essential part of the protein synthetic machinery. During whooping cough, the *B. pertussis* cell produces an exotoxin composed of an A portion and B portions. The A subunit is an ADP-ribosyl transferase which elevates cAMP but in a way different from cholera toxin. It ribosylates the membrane protein that is specifically binds to guanine nucleotide. Molecular mechanics is to predict the detailed structure and physical properties of molecules. Examples of physical properties that can be calculated include enthalpies of formation, entropies, dipole moments, and strain energies. Molecular mechanics calculates the energy of a molecule and then adjusts the energy through changes in bond lengths and angles to obtain the minimum energy structure. Molecular mechanics can be used to supply the potential energy for molecular dynamics computations on large molecules **[12]**. Molecular Mechanics use classical type models to predict the energy of a molecule as a function of its conformation. This allows predictions of Equilibrium geometries and transition states, relative energies between conformers or between different molecules.

ADP-ribosylation factors (ARFs) belong to the superfamily of Ras-related small GTPases and are believed to participate in vesicular transport and signal transduction events in the cell. Six ARF members have been identified in mammalian cells, but ARF2 is not expressed in humans. Similar to other Ras-related GTPases, the function of ARFs is regulated by their recycling between active GTP-bound and inactive GDP-bound conformations. ARF is activated by various growth factors, such as hepatocyte growth factor, colony stimulating factor-1, and epidermal growth factor (EGF) **[13]**. Arginine adenosine-5′-diphosphoribosylation (ADP-ribosylation) is an enzyme-catalyzed, potentially reversible posttranslational modification, in which the ADP-ribose moiety is transferred from NAD^+ to the guanidino moiety of arginine. At 540 Da, ADP-ribose has the size of approximately five amino acid residues. In contrast to arginine, which, at neutral pH, is positively charged, ADP-ribose carries two negatively charged phosphate moieties. Arginine ADP-ribosylation, thus, causes a notable change in size and chemical property at the ADP-ribosylation site of the target protein **[14]**.

Synthesis of poly (ADP-ribose) from nicotinamide adenine dinucleotide (NAD+) is catalysed by poly (ADP-ribose) polymerase-binding domain containing two zinc-fingers, which is linked to the C-terminal NAD^+-binding domain by a short region containing several glutamic acid residues that are sites of auto-poly (ADP-ribosyl)ation. The intracellular productions of poly (ADP-ribose) are induced by DNA strand-interruption. Unmodified polymerase molecules bind tightly to DNA strand breaks; auto-poly ADP-ribosylation of the protein then affects its release and allows access to lesions for DNA repair enzymes **[15]**. Human defensins are cationic multifunctional arginine-rich peptides (molecular masses ranging from 3.5 to 6 kDa) characterized by three intra molecular disulfide bridges, which stabilize their structure. Defensins display microbicidal activity against a wide spectrum of Gram-negative and Gram-positive bacteria, fungi and viruses. They are also cytotoxic for epithelial cells and chemotactic for T-cells. Based on the presence of six conserved cysteine residues and sequence homology, human defensins are grouped into α and β defensins. The first group (α- defensins) includes human neutrophil peptides (HNP)-1 to 4, major components of the azurophilic granules of neutrophils, and two enteric human defensins, HD-5 and HD-6, isolated from the granules of Paneth cells in the small intestine. The second group (β- defensins) is expressed in epithelial cells of various organs. It is shown that ADP-ribosylation of HNP-1 on arginine 14 reduces its antimicrobial and cytotoxic activities **[16]**. Mono ADP-ribosylation consists in the enzymatic transfer of the single ADP-ribose moiety of NAD to specific amino-acid residues of acceptor proteins coupled to release the nicotinamide (nam). The ADP-ribosylation of host proteins catalyzed by bacterial toxins leads to the interruption of cellular metabolic and regulatory pathways causing severe diseases. Vibrio cholerae toxin (CT) Escherichia coli heat labile enterotoxin, Pseudomonas aeruginosa exoenzyme S and the recently discovered NarE, a toxin-like protein from Neisseria meningitidis recognized arginine as an ADP-ribose acceptor in a similar fashion to ART1 and ART5.

The neutralization of toxins with selected amino-acid specificity is used to hypothesize mono ADP-ribosylation of specific amino acids which blocks defensins ability to inhibit the activities of toxins. The ADP-ribosyltransferase activity is inhibited by HNP-1, the NAD- glycohydrolase (NADase) activity remained unaltered. HNP-1 strongly enhanced the auto-ADP-ribosylation of NarE, a recently discovered catalytic activity of this toxin **[17]**. Antimicrobial peptides are small, cationic, amphiphilic peptides of 12-50 amino acids with microbicidal activity against both bacteria and fungi. The eukaryotic antimicrobial peptides may be divided into four distinct groups according to their structural features: cysteine-free alpha- helices, extended cysteine-free alpha-helices with a predominance of one or two amino acids, loop structures with one intramolecular disulfide bond, and beta-sheet structures

which are stabilized by two or three intramolecular disulfide bonds **[18]**. Defensins are large families of small antimicrobial peptides that contribute to host defense against a broad spectrum of pathogens **[19]**. As components of the innate immune system, antimicrobial peptides in the form of human defensins play an important role in host defense by serving as the epithelial layer's biochemical barrier against local infections. Defensins play a role in cell division, attraction and maturation of immune cells, differentiation and reorganization of epithelial tissues, wound healing and tumor suppression. These antimicrobial peptides may be used directly as a remedy against bacterial and viral infection **[20]**. The mammalian defensins can be subdivided into three main classes n the basis of the position of the cysteines and the disulfide bonding pattern, defensins are grouped into three subfamilies: a-defensins, b- defensins, and h-defensins: the alpha-defensins, beta-defensins and the recently described theta-defensins **[21]**. As components of the innate immune system, antimicrobial peptides in the form of human defensins play an important role in host defense by serving as the epithelial layer's biochemical barrier against local infections. Recent studies have shown these Human α-defensins were first isolated from neutrophils. They are also designated as human neutrophil peptides (HNP). Human polymorph nuclear leukocytes contain four members (HNP1-4) of this antimicrobial peptide subfamily. Two more α-defensins, HD-5 and HD-6, have been detected in Paneth cells of the intestine. HNP1-4 and HD-5 exhibit antimicrobial activities against Gram-positive bacteria, *i.e.*, *Staphylococcus aureus*, and Gram-negative bacteria as *Enterobacter aerogenes* and *Escherichia coli*. No antibacterial activity could be detected for HD-6. HNP1 was highly effective at inactivating *Mycobacterium tuberculosis*. HNP1-3 was able to bind to the lethal factor of the anthrax pathogen, *Bacillus anthracis*, thus causing the inhibition of its enzymatic activity. Human α-defensins, for example, inhibit infections of human immunodeficiency virus (HIV) and herpes simplex virus (HSV) *in vitro* by binding to gp120 of HIV and CD4 receptors **[22]**.

Mammalian alpha-defensins are predominantly found in neutrophils and in small intestinal Paneth cells, whereas mammalian beta-defensins have been isolated from both leukocytes and epithelial cells **[23]**. Beta-defensins are small (3 to 5 kDa in size) secreted antimicrobial and antiviral proteins that are components of innate immunity. Epithelial cells secrete beta-defensins, and they are expressed at high levels in several mucosae, including the mouth, where the concentration of these proteins can reach 100 microg/ml. Because of these properties, we wondered whether they could be part of the defenses that lower oral transmission of human immunodeficiency virus (HIV) compared to other mucosal sites **[24]**. Two novel human beta-defensins, human beta defensin 3 (HBD-3), and human beta defensin 4 (HBD-4) have been discovered. Similar to HBD-1 and HBD-2, HBD-3 has microbicidal activity towards the Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli) and the yeasts Candida albicans and Malassezia furfur. HBD-3 kills Gram-positive bacteria such as Streptococcus pyogenes or Staphylococcus aureus, including multi- resistant S. aureus strains, and even vancomycin-resistant Enterococcus faecium. In contrast to HBD-1 and HBD-2, significant expression of HBD-3 has been demonstrated in non-epithelial tissues, such as leukocytes, heart and skeletal muscle. HBD-4 is expressed in certain epithelia and in neutrophils. Its bactericidal activity against *P. aeruginosa* is stronger than that of the other known beta-defensins **[25]**.

Human alpha and beta defensins contribute substantially to innate immune defenses against microbial and viral infections. Certain nonhuman primates also produce theta-defensins-18 residue cyclic peptides that act as HIV-1 entry inhibitors. The theta defensins (retrocyclins) encoded within the human genome is not expressed as peptides. Theta-defensins in rhesus macaques involves the post-translational ligation of two non-peptides. Human theta-defensin genes can be transcribed and uses aminoglycosides to read-through the premature termination codon found in the mRNA transcripts. This treatment induced the production of intact, bioactive retrocyclin-1 peptide by human epithelial cells and cervicovaginal tissues. The ability to reawaken retrocyclin genes from their 7 million years of slumber using aminoglycosides could provide a novel way to secure enhanced resistance to HIV-1 infection **[26]**. In the human airway, epithelial cells, in concert with intraluminal cells (e.g., macrophages), contribute to the innate immune response. Activated neutrophils, in turn, release azurophilic granules that contain human neutrophil peptides (HNP), small cationic peptides ranging in size from 29-33 aa. HNPs are characterized by high arginine content and contain three pairs of disulfide-linked cysteines. They have a broad spectrum of antimicrobial and cytotoxic activities and play an important role in the innate immune response against both Gram-positive and negative bacteria, fungi, and viruses. A family of eukaryotic and prokaryotic mono-ADP ribosyl transferases (ARTs) catalyzes the transfer of ADP ribose from Nicotinamide adenine dinucleotide (NAD) to arginine residues in proteins**.** Based on high arginine content of defensins and the substrate specificity of ARTs, HNP-1 could be a substrate for ART-1, whether ADP-ribosylation of HNP altered its activities, and ADP-ribosylated HNP-1 exists *in vivo.* Based on this a potentially important function for ADP ribosylation in regulating the activity of basic proteins such as HNP and demonstrate that ADP-ribosylated defensin has unique biological properties **[27]**. ADP-ribosylation of regulatory proteins is an important pathological mechanism by which various bacterial toxins affect eukaryotic cell functions **[6]**. ADP-ribosylation is also responsible for the actions of some bacterial toxins, such as cholera toxin, diphtheria toxin, and pertussis toxin. These toxin

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proteins are ADP-ribosyl transferases that modify target proteins in human cells. Reactions are involved in cell signaling and the control of many cell processes, including DNA repair and apoptosis **[6]**. Cholera toxin (CT) mutants lacking the A subunit alone or both the A and B subunits were deficient for transcytosis **[28]**. When cholera toxin is released from the bacteria in the infected intestine, it binds to the intestinal cells known as enterocytes through the interaction of the pentameric B subunit of the toxin with the ganglioside receptor on the intestinal cell, triggering endocytosis of the toxin. Next, the A/B cholera toxin must undergo cleavage of the A1 domain from the A2 domain in order for A1 to become an active enzyme. Once inside the enterocyte, the enzymatic A1 fragment of the toxin A subunit enters the cytosol, where it activates the G protein G_sa through an ADP-ribosylation reaction that acts to lock the G protein in its GTP-bound form, thereby continually stimulating adenylate cyclase to produce cAMP. The high cAMP levels activate the cystic fibrosis transmembrane conductance regulator (CFTR), causing a dramatic efflux of ions and water from infected enterocytes, leading to watery diarrhea **[29]**. Hence, there is a need to document the molecular modeling, docking and dynamics analysis of antimicrobial peptides with the ADP ribosylation toxins.

Materials and Methods:

Selection of synthetic antimicrobial peptides:

The synthetic antimicrobial peptides were collected from the literature **[19]**. The peptides are human alpha, beta defensins. There are totally 10 defensins included in the present study. These peptides are investigated as inhibitors for ADP ribosylation of cholera toxin, diphtheria toxin, pertussis toxin, *E. coli* labile enterotoxin and NarE toxin using molecular modelling, molecular mechanics and molecular dynamics approach.

Selection of the protein:

The 3D structural atomic coordinates of cholera toxin, diphtheria toxin, pertussis toxin; *E.coli* labile enterotoxin and NarE toxin are referred from Protein Data Bank (PDB) **[30]**. All the proteins are retrieved and only the ADP-ribosylating 'A' subunit is considered for all the present protein-peptide interaction analysis.

Molecular modelling and mechanics of synthetic anti microbial peptides:

The selected synthetic peptides are modelled using maestro Schrodinger software. They are built based on N to C terminus and then the cleanup process is carried out. Open maestro -> edit fragments ->click on aminoacids ->select aminoacid-> forward N-C terminus-> place it in the workspace. Then select grow option and select the rest of the amino acids. Open and edit then cleanup geometry.

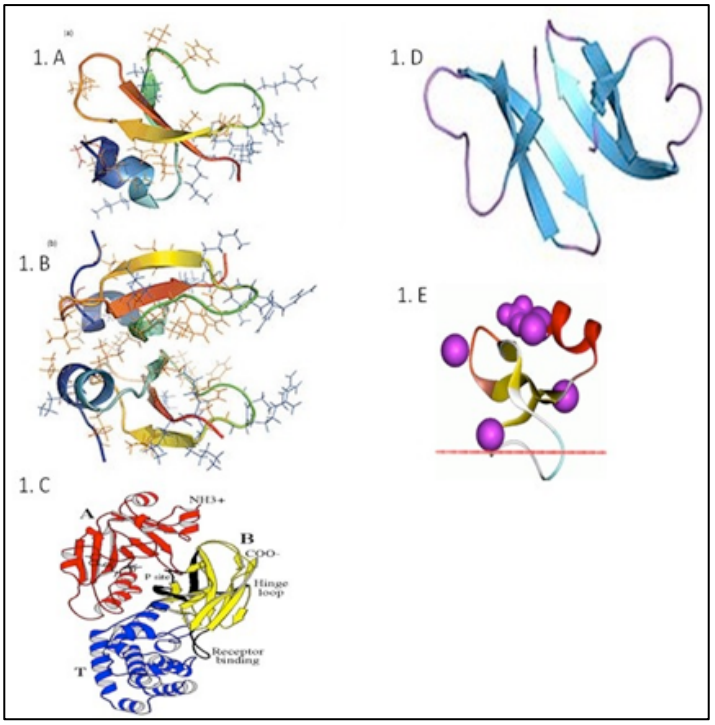

Figure 1: (A) and (B) are the predicted 3D model of Human defensins by using Schrodinger software, (C) the predicted 3D model of Human alpha defensins by using Schrodinger software, (D) Human beta defensin the predicted 3D model of Human beta defensins by using Schrodinger software, (E) Diphtheria Toxin.

Energy minimization of peptides:

The MacroModel Minimization panel is used to set up and submit minimization calculations from within Maestro. The Minimization panel consists of five parts. The first part, the upper portion of the panel, contains controls for general aspects of job set up, such as job name and job source. This portion of the panel also appears on the other MacroModel energy panels. The Minimization panel contains four tabs, the first three of which also appear in the other Macro-Model panels. This is a Steepest Descent (SD) minimization method. The "SD" should not be used to find saddle points, and convergence is poor towards the end of minimization. This is a good method for starting geometries that are far from the minimum, but switching to another method is recommended when derivatives fall below10 KJ/mol Å. Truncated Newton Conjugate Gradient (TNCG) uses second derivatives and line searching and is highly efficient for producing very low gradient structures. It generally converges in one-tenth the number of iterations, but each iteration takes more time.

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Table 1: Human alpha and beta defensins with their sequence

Subunit	Human defensins	Peptide sequence	Length
α	DEF1_HUMAN Neutrophil defensin 1	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	30
α	DEF2_HUMAN Neutrophil defensin 2	CYCRIPACIAGERRYGTCIYQGRLWAFCC	29
α	DEF3_HUMAN Neutrophil defensin 3	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	30
α	DEF4_HUMAN Neutrophil defensin 4	VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRVD	34
α	DEF5_HUMAN Neutrophil defensin 5	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	32
α	DEF6_HUMAN Neutrophil defensin 6	DHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK	36
β	DEFB1_HUMAN b-defensin 1	DHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK	36
β	DEFB3_HUMAN b-defensin 2	GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP	40
β	DEFB3_HUMAN b-defensin 3	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	45
β	DEFB3_HUMAN b-defensin 4	ELDRICGYGTARCRKKCRSQEYRIGRCPNTYACCLRK	37

Table 2: List of ADP ribosylating toxins

Adp ribosylating toxin	PDB ID	Subunit	Number of aminoacids
Cholera toxin	1XTC	A	198
Diptheria toxin	1WGE	A	83
E. coli labile entero toxin	1TI1	A	189

Table 3: Energy minimization of ADP ribosylating toxin-defensin peptides using steepest descent method

Protein	Peptide	Total Energy (KJ/mol)	Strech (KJ /mol)	Bend (KJ/ mol)	Torsion (KJ/mol)	Improper Tortion (KJ/mol)	vdW (KJ/mol)	Electro static (KJ /mol)
Cholera toxin	Defensin3	-31370.5	513.84	2351.4	3064.4	192.8891	-1346.51	-36146.6
Cholera toxin	Defensin5	-31510.8	504.59	2328.9	3068.7	179.7500	-1376.92	-36215.9
Diphtheria toxin	Defensin8	-12086.1	349.58	1291.9	1353.8	99.6912	82.8678	-15264.0
E. coli labile toxin	Defensin8	-33093.3	462.68	1870.6	2293.3	126.2124	-1627.24	-36219.0

Table 4: Energy minimization of ADP ribosylating toxin-defensin peptides using conjugate gradient method

Protein	Peptide	Total Energy (KJ/ mol)	Strech (KJ/ mol)	Bend (KJ /mol)	Torsion (KJ/ mol)	Improper torsion (KJ/ mol)	Vdw (KJ/ mol)	Electrostatic (KJ/ mol)
Cholera toxin	Defensin3	-40804.9	606.59	2316.3	2688.3	174.9725	-1538.3	-45052.8
Cholera toxin	Defensin5	-41956.3	591.38	2363.9	2692.0	164.9962	-1576.8	-46191.8
Diphtheria toxin	Defensin8	-22406.4	305.44	1386.0	1263.2	90.9186	-303.05	-25149.3
E. coli labile toxin	Defensin8	-41948.9	585.80	2091.3	2514.2	158.3773	-1419.6	-45879.0

Docking:

Protein-protein docking was completed using PATCHDOCK (http://bioinfo3d.cs.tau.ac.il/PatchDock/) and Firedock: (http://bioinfo3d.cs.tau.ac.il/FireDock/). The two proteins are uploaded in pdb format and the resultant file which is a complex of ADP ribosylating toxin-defensin is generated. Then the output of the pachdock is refined using FIREDOCK where it refines and gives top ten docking compounds. Based on the global energy values i.e. lower the global energy higher the stability the complexes are prioritized. The hydrogen bonds are identified for the selected complexes defensins-ADP ribosylating toxins using maestro.

Trajectory analysis of the protein-peptide complex:

After the impact dynamics is performed, a file in the auxiliary column in the project table appears as T. click on the T option then a new tab called trajectory tab will open. In that trajectory tab select smoothing factor as 1. Structure: Export to Project table, Selected frames (124 frames) Image: Save image, Format: JPEG, Save to, Movie: Export movie, MPEG, Selected 124 frames, other options: Show simulation box-Show the edges of the simulation box (in purple). Show axes-Show the coordinate axes in green. Replicate system-Enter the number of replicas of the system to display in each of the three directions. This enables you to visualize the movement across the simulation box, Boundaries-These text boxes are unavailable if there are no periodic boundary conditions. Trajectory smoothing is done by averaging the coordinates over the specified number of frames Superimpose on frame-Align the structure in each frame by superimposing a selection of atoms on the corresponding atoms in the frame number given in the text box. Use the Pick atoms to superimpose picking controls to make the atom selection. You should consider picking atoms that do not change their position much during the simulation.

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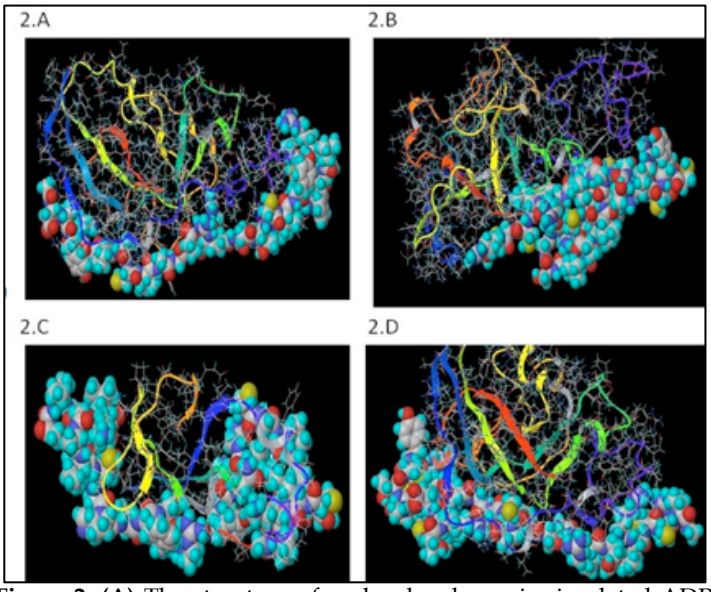

Figure 2: (A) The structure of molecular dynamic simulated ADP ribosylating *cholera* toxin- defensin3 complex along with their distances are showed in the Figures given in the tables below using Schrodinger Software; **(B)** The structure of molecular dynamic simulated ADP ribosylating cholera toxin- defensin 5 complex along with their distances are showed in the Figures given in the tables below using Schrodinger Software; **(C)** The structure of molecular dynamic simulated ADP ribosylating diphtheria toxin-defensin8 complex along with their distances are showed in the Figures given in the tables below using Schrodinger Software; **(D)** The structure of molecular dynamic simulated ADP ribosylating *E. coli* labile enterotoxin-defensin 8 complex along with their distances are showed in the Figures given in the tables below using Schrodinger Software.

RMSD calculation of the protein-peptide complexes:
The root-mean-square deviation (RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. Form the project table select the entries which rmsd should be calculated and totally 12 entries are selected out of 124 frames. For every 10^{th} frame it gets superimposed on 1^{st} frame and rmsd is given. Then the rmsd values are obtained and a graph is plotted for all selected frames on X-axis and RMSD on Y-axis.

Results and Discussion:
Synthetic antimicrobial peptides modelling:
The synthetic antimicrobial peptide sequences are collected from literature **[19]**, which is called as defensins **(Figure 1).** There are totally 10 defensins. The peptides are both alpha defensins **(Figure 1C)** and beta defensins (**Figure 1D**). There are 6 alpha defensins and 4 beta defensins. They are nearly 29 to 45 amino acids lengthen sequences showed in **Table 1**.

Selection of protein:
The proteins for the ADP ribosylating toxins such as cholera toxin, diphtheria toxin (Figure 1E) and E. coli enterolabile toxin showed in Table 2, are retrieved from the protein data bank (PDB) [30]. The ADP-ribosylating "A"- subunit sequence is taken from all the proteins.

Molecular mechanics:
Schrodinger is used for the molecular mechanics. The peptides are built from N to C terminus and the structures are cleaned up by cleanup geometry option. The energy minimization is done individually for all the ten peptides. Both Steepest descent and conjugate gradient two methods are used to minimize the peptide structures. After running the SD for 500 iterations it gives the total energy, stretch energy, bend, torsion, improper torsion, VDW, electrostatic etc in KJ/mol. And finally it displays how many structures are processed. Then Conjugate gradient is run for all the defensins for 1000 iterations. It gives the total energy, stretch energy, bend, torsion, improper torsion, VDW, electrostatic etc in KJ/mol. And finally it displays how many structures are processed.

Docking using PATCHDOCK and FIREDOCK:
PATCHDOCK is the docking server, which can be used for protein- protein docking. Here all the defensins are docked against the ADP ribosylating toxins and the resultant file is a complexed PDB file which is an ADP ribosylating toxin-defensin complex. Patchdock gives the results such as the number of solutions, score represents geometrical complementary score, and area represents the approximate interface area of complex. ACE represents the Atomic Contact Energy; transformation file represents the 3D transformation: 3 rotational angles and 3 translational parameters and finally the predicted complex structure in PDB format. Then the patchdock gives more than 1000 solutions in which the top ten are refined using Firedock, which is a Fast Interactive Refinement molecular DOCKing. The output is a ranked list of all the input solutions. The refined complex structure is generated for up to 100 low-energy candidates. The user can view the complexes in the Jmol applet window and/or download the structures. The results are given in the **Table 3**.

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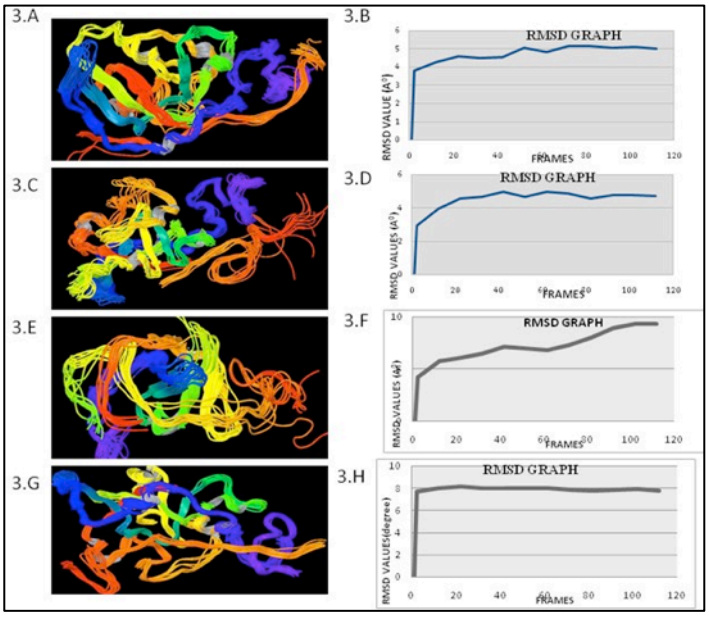

Figure 3: (A) Superimposed structures of ADP ribosylating cholera toxin-defensin3 complex shows the RMSD value of 2.9548 using Schrodinger software for 1000ns; **(B)** Graph plotted against frames on X-axis and RMSD values on Y-axis for ADP ribosylating cholera toxin-defensin3 complex; **(C)** Superimposed structures of ADP ribosylating cholera toxin-defensin5 complex shows the RMSD value of 3.8055 using Schrodinger software for 1000ns; **(D)** Graph plotted against frames on X-axis and RMSD values on Y-axis for ADP ribosylating cholera toxin- defensin5 complex, **(E)** superimposed structures of ADP ribosylating diphtheria toxin-defensin8 complex shows the RMSD value of 9.336 Schrodinger software for 1000ns, **(F)** Graph plotted against frames on X-axis and RMSD values on Y-axis for ADP ribosylating diphtheria toxin-defensin8 complex, (G) superimposed structures of ADP ribosylating labile enterotoxin-defensin8 complex shows the RMSD value of 8.174 using Schrodinger software for 1000ns, (H) Graph plotted against frames on X-axis and RMSD values on Y-axis for ADP ribosylating *E. coli* labile enterotoxin- defensin8 complex, Light orange represents Defensin3 peptide.

Hydrogen bonding analysis:
The results of PATCHDOCK and FIREDOCK are retrieved and they are subjected to maestro. The ADP ribosylating toxin complex with defensin protein is loaded into maestro and the hydrogen bonds are analysed for all the docked complexes. The results are given in the table 4 in which it show very poor interactions in the resultant complexes. Very few numbers of hydrogen bonds are showed.

Molecular modelling of ADP ribosylating toxin-defensin docked complex
For the molecular mechanics, Schrodinger is used. The peptides are built from N to C terminus and the structures are cleaned up by cleanup geometry option. The energy minimization is done individually for all the ten peptides. Both Steepest descent and conjugate gradient two methods are used to minimize the peptide structures. After running the SD for 500 iterations it gives the total energy, stretch energy, bend, torsion, improper torsion, VDW, electrostatic etc in KJ/mol. And finally it displays how many structures are processed. Then Conjugate gradient is run for all the defensins for 1000 iterations. it gives the total energy, stretch cholera toxin-defensin3 complexed protein is given below in which the defensin is represented in van der Waals (CPK) form and the protein in wire and stick frame and their backbone is represented in the ribbon format.

Hydrogen bonds analysis:
Hydrogen bonds are analysed and the distance is measured. Each energy, bend, torsion, improper torsion, VDW, electrostatic etc in KJ/mol. And finally it displays how many structures are processed.

Molecular dynamics simulation of ADP ribosylating toxin and dfensin complexes:
Structure analysis:
Molecular Dynamics simulations examine stable, ground state molecules by applying Newton's equations of motion. The constant volume and temperature (NVT) ensemble is the default ensemble for MD simulations. The constant volume and energy (NVE) and constant pressure and temperature (NPT) ensembles are also supported, as is temperature replica exchange. The widely used velocity Verlet integration algorithm is the default for integrating the equations of motion in standard Cartesian-space molecular dynamics. Stop overall motion option is selected (the default), overall rotational and translational motion (drift) of the system is subtracted from the calculation. Record trajectory option is selected trajectory information is written to the file *jobname*.trj in the Maestro working directory. This information is written in binary format, but can be analyzed using the ANALYSIS task of Impact. A trajectory file contains a sequence of snapshots of the coordinates of the system and velocities. The structure of the ADP ribosylating docked complex has different number of hydrogen bonds in which they shows the strength of the complex. The hydrogen bonds are given below for all the four docked complexes: ADP ribosylating cholera toxin-defensin3 (**Figure 2A**), ADP ribosylating cholera toxin-defensin 5 (**Figure 2B**), ADP ribosylating diphtheria toxin-defensin8 (**Figure 2 C**), ADP ribosylating *E. coli* labile enterotoxin-defensin8 (**Figure 2D**).

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Trajectory analysis of the protein-peptide complexes:
Trajectory file examine individual frames, and export trajectory data in a variety of forms, in the Trajectory panel. The toolbar in the Trajectory panel contains a standard set of controls for playing through the trajectory frames, The Start and End text boxes define the frames at which play starts and ends. Frames are numbered from 0. The Frame slider and frame text box can be used to select the frame to view. The current frame number is displayed in the text box below the slider. The total number of frames is also displayed in a noneditable text box. The Step text box sets the number of frames to step when playing through frames. This value does not affect the Frame slider. The frames that are selected for play can be exported as a selection of frames, using the output buttons. The Time text boxes display the time for the current frame and the total time for the trajectory.

RMSD calculation:
RMSD is calculated for all models. ADP ribosylating cholera toxin-defensin 3 and ADP ribosylating cholera toxin - defensin5 shows the least RMSD value of 2.9548 and 3.8055 respectively. The highest RMSD values are given by ADP ribosylating diphtheria toxin-defensin8 and ADP ribosylating labile enterotoxin-defensin8 as 9.336 and 8.174 respectively. The superposed structures of all the four docked complexes are shown in the [**Figure 3A, C and E**]. And the graph is plotted against selected frames on X-axis and RMSD on Y-axis shown below in the [**Figure 3B, D and F**]. The light orange colour is the defensin peptide and the rest of the molecule is ADP ribosylating toxin. The potential energy graph is plotted number of steps on X-axis and potential energy (KJ/mol) on Y-axis. The plotted potential energy graphs are shown in the **Figure 4**.

Conclusion:
Antimicrobial peptides for innate and adaptive immune responses are relevant **[31].** The use of AMPs in prevention and treatment of infection is critical **[32]**. AMPs can be categorized as unconventional therapeutic molecules with a promising potential to be developed as an alternative treatment for the increasing infections caused by resistant microbes **[33]**. Human defensins are small cysteine-rich cationic proteins found in both vertebrates and invertebrates. The bacterial toxins such as cholera toxin (CT), pertussis toxin (PT), and diphtheria toxin (DT) interfere with specific host proteins to cause tissue damage for infections. The common toxic mechanism for these agents is mono-ADP-ribosylation of specific amino acids in eEF-2 proteins, by the catalytic A chains of the toxins. Hence, we report the molecular modeling, docking and dynamics analysis of antimicrobial peptides with the ADP ribosylation toxins. This AMP data will enable to bridge the gap between the laboratory research and the clinical settings **[34]**. The effect of peptide modifications on selectivity, peptide behaviour in the aqueous and membrane phases are considered **[35]**. The development of AMPs as therapeutics is of their simple design, many AMPs have been synthesized differing in sequence, length, and amino acid composition. Molecules that exhibit amphiphilic properties and antimicrobial activity are known with limited efficiency **[36]**. A cost-efficient strategy to develop new antimicrobials designed to control pathogens that cause harmful pathogenic disease is relevant **[37]**. The designing and improving of AMPs with demonstrated advantages over conventional antibiotics and biocides are required **[38]**. Antimicrobial peptides with anti-*H. pylori* effects have the potential to replace the antibiotics **[39]**. AMPs not only have a broad-spectrum on bacteria and fungi, but also on viruses, protozoa and cancer cells. Compared with traditional antibiotics, their mechanism of action is unique, and it is not easy for AMPs to cause microbial resistance **[40]**. Antimicrobial peptides due to their antimicrobial activity not only kill skin pathogens but also initiate a potent host response to skin infection **[41, 42]**. Thus, the data presented here is helpful for the design of novel drugs to treat the ADP rybosylating bacterial diseases using effective human alpha defensins as inhibitors.

Conflict of Interest:
The authors declare no conflict of interest.

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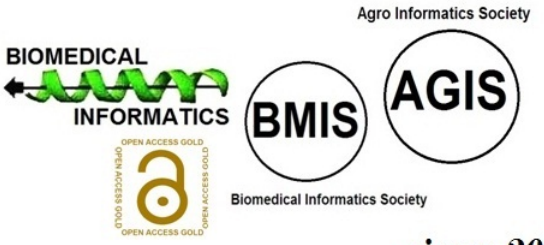

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