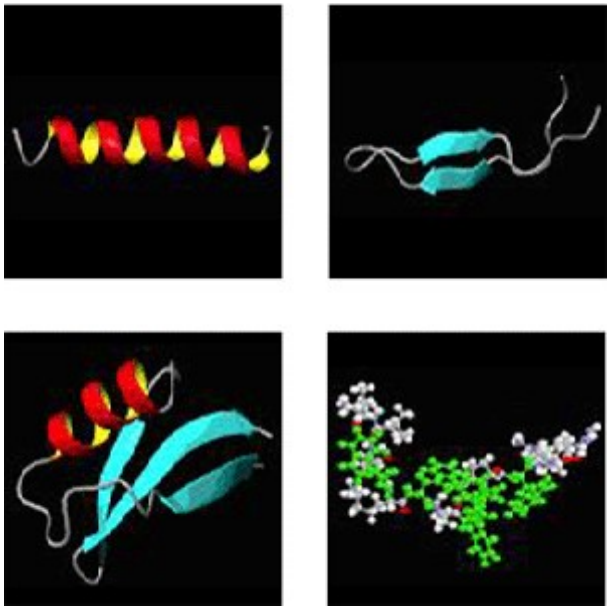
Antimicrobial Peptides

Yesterday, Today, and Tomorrow

A symposium dedicated to the 20th anniversary of the APD



https://aps.unmc.edu/

Omaha, Nebraska, USA

October 6, 2023

University of Nebraska Medical Center

ELSEVIER

pharmaceuticals
an Open Access Journal by MDPI

Introduction

Antimicrobial peptide (AMP) research remains vibrant and is now led by investigations to decipher the functional roles of AMPs in a variety of organisms, studies prompted by an appreciation of the colonizing microbiota shaped by AMPs, and attempts to address the pressing need to develop AMPs into novel antibiotics. AMP research started with the discovery of lysozyme by Sir Alexander Fleming in 1922. It entered a second phase with the exciting discovery of cecropins, defensins, magainins, daptomycin, bactenecin, and histatins in the 1980s. With the completion of the human genome sequence in 2003, we entered the "omics" era. The development and applications of genomic and proteomic approaches in the AMP field initiated a third phase toward a more complete understanding of AMPs. A growing interest in microbiota and its relationship with a variety of human diseases adds a new dimension to antimicrobial peptides, since these molecules can shape the microbiota. To facilitate the research on and education about AMPs, the Wang lab constructed and opened to the public the Antimicrobial Peptide Database (APD) in 2003. This manually curated database (*https://aps.unmc.edu*) is widely utilized due to its high-quality information, user friendliness, powerful search engine, open access, flexibility, continued refinement and expansion, and timely updates.

To celebrate the success and utility of this database for 20 years, a hybrid symposium will be held on October 6, 2023 in Omaha, Nebraska, USA. This pamphlet contains the entire program for the symposium entitled *"Antimicrobial Peptides: Yesterday, Today and Tomorrow"*, including abstracts and a list of registered attendees. In total, over 180 students, postdoctoral fellows, and faculty from around the world have registered for and will attend this symposium. Fifty-two attendees will present posters online (powered by SciForum, *Pharmaceuticals*). We thank Elsevier for sponsoring two "best poster awards" and one "young investigator lectureship award".

We invite colleagues in the field, including those who were unable to attend the symposium, to submit your unpublished work to the special issue on antimicrobial peptides to be published in *Peptides*. Thank you all for your participation and contributions.

Thank you to Dr. Kenneth Bayles, Vice Chancellor of Research and Dr. Joseph Khoury, Chair, Department of Pathology and Microbiology, University of Nebraska Medical Center (UNMC) and Dr. Robert Hancock, Distinguished Professor, University of British Columbia, Canada for their opening remarks. We also wish to give a special thank you to Lyssa White, Liz Van Roekel, Keith Young, Kirsten Stites and the Information Technology Services at UNMC for contributing to the success of this symposium.

This symposium is sponsored by the Department of Pathology and Microbiology, UNMC.

Symposium Chairs:

Charles L. Bevins, MD, PhD, Professor
Monique L. van Hoek, PhD, Professor
Guangshun Wang, PhD, Professor

Special Issue in *Peptides* (Elsevier)

Thank you all for your participation in the symposium *"Antimicrobial Peptides: Yesterday, Today and Tomorrow"* and making valuable contributions. To provide an avenue to unpublished work presented in abstracts and posters at the symposium, a special issue will be edited by the conference chairs and published in *Peptides*. Both original articles and reviews will be considered. This special issue will be more inclusive and can include any topic of interest to the general audience of antimicrobial peptides. There is no publication cost unless you choose “Open Access”.

Submission to the special issue will open on October 1, 2023. The submission website for this journal is located at:

https://www.editorialmanager.com/peptides/default2.aspx

The deadline for manuscript submission will be May 1, 2024.

We look forward to your contributions and continued support.

Guest editors:

Guangshun Wang, PhD, Professor
Monique L. van Hoek, PhD, Professor
Charles L. Bevins, MD, PhD, Professor

ELSEVIER

APD Symposium Schedule

OPENING REMARKS
8:00 a.m. - 8:30 a.m.

Kenneth W. Bayles PhD, Professor, Vice Chancellor for Research
(University of Nebraska Medical Center, USA)

Joseph D. Khoury MD, Stokes-Shackleford Professor, Chair
(Department of Pathology and Microbiology, University of Nebraska Medical Center, USA)

Robert E. W. Hancock PhD, Canada Research Chair, UBC Killam Professor
(University of British Columbia, Canada)

SESSION I: **Database, design and prediction of antimicrobial peptides**
Moderators: Dr. Monique L. van Hoek and Dr. Weiwei Zhang

KEYNOTE SPEAKER 1
8:30 a.m - 8:50 a.m.

Guangshun Wang, PhD, Professor (University of Nebraska Medical Center, USA)
The antimicrobial peptide database as a founding resource for antimicrobial development

9:00 a.m - 9:20 a.m.

Gajendra P. S. Raghava, PhD, Professor (Indraprastha Institute of Information Technology, India)
Machine learning based models for designing therapeutic peptides from the antimicrobial peptide database

9:30 a.m - 9:50 a.m.

Jun Wang PhD, Professor (Chinese Academy of Sciences, China)
Deep learning prediction of antimicrobial peptides from human gut microbiome

9:50 a.m. - 10.00 a.m. **SYMPOSIUM BREAK**

SESSION II: **Promising antimicrobial leads and mechanisms of action**
Moderators: Dr. Guangshun Wang and Dr. Charles L. Bevins

KEYNOTE SPEAKER 2
10:00 a.m - 10:20 a.m.

Kim Lewis, PhD, University Distinguished Professor (Antimicrobial Discovery Center, Northeastern University, USA)
Novel peptide antibiotics with unusual modes of action

10:30 a.m - 10:45 a.m.

Berthony Deslouches, PhD, Assistant Professor (University of Pittsburgh, USA)
Precision design of cationic peptides to overcome bacterial resistance to conventional antibiotics

10:50 a.m - 11:05 a.m.

Evan Haney, PhD, Chief Scientific Officer (Asep Medical Inc., Canada)
Development of AMPs into antibiofilm agents: An industrial view

11:10 a.m - 11:25 a.m.

Michaela Wenzel, PhD, Associate Professor (Chalmers University of Technology, Sweden)
Membrane active antimicrobial peptides: Beyond pore-forming

11:30 a.m - 11:45 a.m.

Renee Fleeman, PhD, Assistant Professor (University of South Florida, USA)
Breaking the Barrier: Polyproline Peptides Disrupt the Matrix of Hypervirulent Klebsiella pneumoniae Biofilms to Release Bacteria from their Protective Barrier

11:50 a.m - 12:40 p.m. **LUNCH BREAK**

APD Symposium Schedule

SPECIAL EVENT 12:40 p.m. - 12:55 p.m.	Sandip Bhattacharyya, PhD., Scientific Review Officer (NIH/NIAID, USA) **NIAID grantsmanship**
SESSION III:	**Microbiota, antimicrobial peptides and human diseases** **Moderators: Dr. Charles L. Bevins, and Dr. Nita Salzman**
KEYNOTE SPEAKER 3 1:00 p.m - 1:20 p.m.	Nita Salzman, MD, PhD, Professor, Program Director (Medical College of Wisconsin, USA) **The microbiota-antimicrobial peptide axis and novel antimicrobial strategies**
1:30 p.m - 1:45 p.m.	Bruno Lemaitre, PhD, Professor (École Polytechnique Fédérale de Lausanne, Switzerland) **A humoral stress response protects Drosophila self-tissues from antimicrobial peptides**
1:50 p.m - 2:05 p.m.	Delphine Destoumieux-Garzón, PhD, Research Director (University Perpignan Via Domitia, France) **Salt-resistant big defensins regulate oyster microbiota**
2:10 p.m - 2:25 p.m.	Bart Thomma, PhD, Professor (University of Cologne, Germany) **Antimicrobial peptides shape plant microbiota**
2:30 p.m - 2:45 p.m.	Eugene B. Chang MD, Martin Boyer Professor, Director (University of Chicago, USA) **A new class of Paneth cell antimicrobial peptides that maintain Candida gut commensalism and innate immunity**
2:50 p.m. - 3:00 p.m.	**SYMPOSIUM BREAK**
SESSION IV:	**Sunlight, vitamin D, human cathelicidin and antimicrobial role** **Moderators: Dr. Monique L. van Hoek and Dr. Guangshun Wang**
KEYNOTE SPEAKER 4 3:05 p.m - 3:25 p.m.	John H. White, PhD, Professor, Chair (Department of Physiology, McGill University, Canada) **Vitamin D, human LL-37 and the antiviral connection**
3:35 p.m - 3:50 p.m.	Jingwei Xie, PhD, Professor (University of Nebraska Medical Center, USA) **Topical delivery of immunomodulating compounds and LL-37 derived peptides for wound management**
3:55 p.m - 4:10 p.m.	Nuch Tanphaichitr, PhD, Professor Emeritus (U Ottawa, Canada) **Development of human LL-37 and related peptides into a spermicide/microbicide**
4:15 p.m.	**CONFERENCE AWARDS AND CLOSING REMARKS**

Molecular characterization of dehydrin PpDHNC from Physcomitrium patens: Potential as an antimicrobial protein

Tanushree Agarwal, Sudipta Ray
Department of Botany, University of Calcutta,
35, Ballygunge Circular Road, Kolkata- 700019, West Bengal, India

Dehydrins, PpDHNA and PpDHNB from Physcomitrium patens provide drought and cold tolerance while PpDHNC shows antimicrobial property suggesting different dehydrins perform separate functions in P. patens. The moss Physcomitrium patens can withstand extremes of environmental condition including abiotic stress such as dehydration, salinity, low temperature and biotic stress such as pathogen attack. Osmotic stress is inflicted under both cold and drought stress conditions where dehydrins have been found to play a significant protective role. In this study, a comparative analysis was drawn for the three dehydrins PpDHNA, PpDHNB and PpDHNC from P. patens. Our data shows that PpDHNA and PpDHNB play a major role in cellular protection during osmotic stress. PpDHNB showed several fold upregulation of the gene when P. patens was subjected to cold and osmotic stress in combination. PpDHNA and PpDHNB provide protection to enzyme lactate dehydrogenase under osmotic as well as freezing conditions. PpDHNC possesses antibacterial activity and thus may have a role in biotic stress response. PpDHNC shows antimicrobial activity against Rhodococcus fascians and Bacillus subtilis. The K segments of PpDHNC are probably associated with the antimicrobial activity. Further investigations involve the use of K segments of PpDHNC alongwith PpDHNA and PpDHNB to form a supra molecule of dehydrin that may show protective properties under multi-stress conditions.

Analysis of the skin secretion of Leptodactylus labyrinthicus, the frog's biohazard protective clothing

Falico, D.A[1*] ; López, J.A[3,4]; Prinsen, E.[1,2]; Van Damme, R[1]

[1]Department of Biology, University of Antwerp (BE),
[2]Department of Chemistry, University of Antwerp (BE),
[3]Instituto Nacional de Limnología (INALI: CONICET-UNL). Ciudad Universitaria. Santa Fe, Argentina.
[4]Departamento de Ciencias Naturales, Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral (UNL). Santa Fe, Argentina.

The secretion of amphibians has been used for many years for different purposes such as religion, culture, agro-economics or even as therapeutic agents. Although these secretions are a mixture of different molecules, especially the active peptides therein have been studied intensively during the past 30 years. Peptides have shown to be able to inhibit the growth of different human microorganisms (making from them good candidates for potential therapeutic agents), and soil microorganisms. The objectives of this work was to analyze the secretion of Leptodactylus labyrinthicus in order to identify bioactive peptides, with emphasis on their biological activity in an ecological context. The results reported here shown that, when fractioning the skin secretion of L. labyrinthicus, the growth of Staphylococcus aureus and Burkholderia cepacia were slowly delayed by fractions 8 and 9, but not any delaying effect was observed on Escherichia coli. Interestingly, the whole secretion (not any individual fractioning done) have shown a growth promotion for Burkholderia cepacia, but not any effect on Staphylococcus aureus and Escherichia coli. On the other hand, two peptides already described in the literature were found in this frog skin secretion. In view of these results we hypothesize that, the secretion of Leptodactylus labyrinthicus is a mix of molecules that act in synergy as a bio-regulator mechanism selecting or benefiting microorganisms on the frog's skin population for ecological, immunological or other purposes not well understood to the moment.

Antimicrobial peptides from Macaronesian fish: in silico and in vitro approach

Patricia Asensio-Calavia[a,b]; Andrea Otazo-Pérez[a,b]; Sergio González-Acosta[a,b]; Beatriz González-Almécija[a]; Antonio Morales-delaNuez[a†]; Manuel R. López[a]; José M. Pérez de la Lastra[a]
[a]Biotechnology of Macromolecules Research Group, Institute of Natural Products and Agrobiology (IPNA-CSIC), San Cristóbal de La Laguna, Spain.
[b]Escuela de Doctorado y Estudios de Posgrado, Universidad de La Laguna, San Cristóbal de La Laguna, Spain.
[†]Current address: Animal Production and Biotechnology Group, Institute of Animal Health and Food Safety, University of Las Palmas de Gran Canaria, Spain.

Fish constantly interact with microorganisms in their aquatic habitat. As a defense mechanism, they possess innate immune system-derived antimicrobial peptides that combat bacteria, viruses, and fungi. The piscidin family's characterization has been limited to Teleostei fish. This study

aimed to select a subset of peptides annotated as piscidins in NCBI and evaluate their bioactivity and structure through both in silico and in vitro methodologies.
Beginning with 51 piscidin sequences, bioinformatic tools were utilized to screen for potential active peptides. These peptides exhibited attributes like α-helical structure, cationic charge, and potent activity against microorganisms and tumors. To explore promising candidates, five novel piscidin peptides were synthesized alongside a well-established active peptide, Epinecidin-1 from Epinephelus coioides. Our initial focus was assessing antibiotic and antifungal activities of each peptide against human pathogens (Escherichia coli, Staphylococcus aureus, Salmonella enterica, Enterococcus faecalis, Pseudomonas aeruginosa, Campylobacter jejuni, and Candida albicans). Outcomes demonstrated noteworthy activity at low concentrations, e.g., 1.56 μM against S. aureus or 3.125-6.25 μM against P. aeruginosa. Peptide toxicity was evaluated using rat erythrocytes and the Vero cell line, revealing toxicity at higher concentrations (25-50 μM) with milder effects at lower concentrations.
In conclusion, these peptides exhibited substantial in vitro antimicrobial activity, particularly against bacteria, aligning with in silico forecasts. These findings strongly underscore the potential of these peptides as agents against microorganisms, potentially aiding in the fight against antibiotic resistance. However, further research is imperative to delve into their mode of action and other potential immunomodulatory attributes.

Sequence diversity and antimicrobial activity of natural homologs of the translation terminator inhibitor Apidaecin.

Authors: **Chetana Baliga[1],[2]*,** Weiping Huang[1]*, Gemma C. Atkinson[3], Nora Vazquez-Laslop[1], Alexander Mankin[1].

Affiliation:
1. Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, Illinois, USA.
2. Department of Biotechnology, Faculty of Life and Allied Health Sciences, Ramaiah University of Applied Sciences, Bangalore, India.
3. Department of Experimental Medicine, Lund University, Lund, Sweden
*Both authors contributed equally to the work.

Apiadecin 1b (Api1b), produced by the honeybee Apis mellifera, is a Proline-rich Anti-microbial Peptide (PrAMP), that enters Gram-negative bacteria via membrane transporters and arrests ribosomes at translation termination, causing cell death. Homologs of Api are found in genomes of several members of bee and wasp families, often, with multiple isoforms found within the same insect. These homologs differ both in their lengths and amino acid composition, especially in the N-terminal part of the peptide. Apidaecins are synthesized by the insect as prepropeptides, which are proteolytically cleaved to yield biologically active peptides, making it challenging to identify the sequence of the active PrAMP from the gene sequence. Our bioinformatics analyses identified several potential homologs of Api1b from genomes of multiple species of bees and wasps. Peptide sequences that differed significantly from Api1b were chemically synthesized and tested against several Gram negative bacteria. Strikingly, a few of the selected peptides showed increased antimicrobial activity compared to Api1b as well as the more potent synthetic variant, Api137. Our in vitro experiments demonstrated that, despite differences in sequence, these active peptides maintain the ability to arrest ribosomes at stop codons, as we described for Api1b and Api137. Interestingly, some of the identified peptides also arrest the terminating ribosomes during in vitro translation but were unable to kill the tested bacteria, suggesting they are unable to penetrate these cells. Future studies may help uncover the sequence requirements for transporter recognition and elucidate whether these peptides have evolved to target specific bacterial species.

Antimicrobial Peptides (AMPs) as future therapeutics

Mugdha Belwalkar[1], Linda Oyama[2], Anushree Lokur[1]*, Mayuri Rege[1]*

1- Department of Microbiology, Ramnarain Ruia Autonomous College, Matunga (E), Mumbai 400019
2- Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, United Kingdom
*- Corresponding authors

Antimicrobial resistance (AMR) is the future pandemic. By 2050, AMR will claim 10 million deaths per year. India is referred to as the AMR capital of the world (Chaudhary & Tomar; 2017). Tackling AMR is a complex problem. Targeted efforts at social, political, policy-making, and research stages are needed. Finding alternatives to existing antibiotics is the need of the hour. Antimicrobial peptides (AMPs) are promising alternatives to antibiotics. AMPs are present in all living organisms. They have novel modes of action

and show less predisposition to antimicrobial resistance. Myriad antimicrobial peptides are being predicted using different peptide databases. AMPs showing inhibition of drug-resistant organisms are being improved to have minimum side effects. Despite the plethora of options available, the number of AMPs undergoing clinical trials is minimal. This is likely because, as compared to traditional antibiotics, high development and production cost limits the number of AMPs under clinical trials. Solid phase synthesis, (a chemical method) is the preferred mode of AMP production. One gram of aminoglycoside costs around $0.80 whereas a similar amount of AMP costs around $50-400. Biological synthesis of AMPs can help to overcome this bottleneck but there have been only a handful of studies that have successfully produced AMPs using biological hosts. We screened many peptides for their suitability for cloning in bacteria. We cloned the selected peptide in plasmid vectors and tested its expression to assess optimal conditions for AMP production. Our approach can serve as a potential platform for cost-effective AMP production.

Novel peptide compounds development using recurrent neural networks

Albert Bolatchiev[1,2,3], Vladimir Baturin[3], Elizaveta Bolatchieva[1,2,3], Evgeny Shchetinin[3]

[1] Peptigen Limited, London, United Kingdom
[2] Albogene LLC, Stavropol, Russian Federation
[3] Stavropol State Medical University, Stavropol, Russian Federation

The search and development of new pharmacologically active peptide compounds is an important and promising task. One of the approaches is so-called de novo drug-design of novel peptides using artificial intelligence and machine learning methods. Previously, Alex Müller et al. (2018) proposed the use of a recurrent neural network (RNN) with generative long-term-short-term memory (LSTM) for de novo design of novel peptide amino acid sequences. However, this method has not been experimentally validated. We de novo designed hundreds of novel peptide sequences by LSTM RNN with our own modifications. Then, using our proprietary bioinformatic platform “PeptiGen” we’ve selected some peptides for synthesis and investigated their antibacterial activity in vitro and in vivo. 8 peptides were active in vitro against carbapenem-resistant isolates of Escherichia coli (n=22), Pseudomonas aeruginosa (n=12), Acinetobacter baumannii (n=8), Klebsiella pneumoniae (n=18) and K. aerogenes (n=12) with minimum inhibitory concentrations range from 0.25 to 8 μg/ml. In addition, we screened the effect of 24 amino-acid peptide PEP-36Emdf in vivo: a single intraperitoneal administration of a 100 μg of PEP-36Emdf 30 min after infection on animal survival in an experimental murine model of K. pneumoniae-induced lethal sepsis. Control group was administrated with sterile saline and showed 0% survival rate. The PEP-36Emdf peptide was shown to provide the survival rate 58.3% (with p=0.0105940). LSTM RNN with our own modifications and “PeptiGen” proprietary bioinformatic “filters” can be used to develop new pharmacologically active and patentable peptide molecules.

Using Molecular Dynamics to Understand the Mechanism of action of AMPs.

Steven R Bowers
George Mason University

In our lab we have been studying the antimicrobial peptide PGLa, and its interaction with the anionic membranes using molecular dynamic simulations. All atom molecular dynamics can gives details of molecular interaction which are not available using other techniques.

In our studies of the antimicrobial peptide PGLa in the anionic DMPC/DMPG bilayer, we measured the secondary structure, the peptide tilt and the orientation of residues within alpha helices. These values were similar to experimental value. We have been able to measure the influx of DMPG and efflux of DMPC as a function of distance from the peptide. We also measured the lipid disordering at different distances from the peptide and found significant disordering near the peptide. Our studies were also able to show significant water insertion into the bilayer caused by the peptide insertion. We also saw that that the orientation of water within the membrane was a significant factor in offsetting the charge of the peptide in the membrane. Our studies revealed 2 major states for PGLa monomers where in one state the peptide was inserted below the lipid Phosphate groups with the Lysine residues pointing up toward the Phosphate groups, while in the other state the peptide was above the Phosphate groups with the Lysine residues pointing down. We also saw rare trans-membrane structures with the peptide interacting with the headgroups from both leaflets. In our study of PGLa diners,we were able to identify the most common inter-peptide interactions as well as the dimer location and orientation within the membrane.

We believe that an understanding the detailed molecular

mechanism of peptide binding to the bilayer, is a necessary factor in designing new better AMPs.

Study of somuncurin-1 behavior in two membrane models using Molecular Dynamics Simulations

N.L. Cancelarich[1], C. Domene[2], M.M. Marani[1], M. Pickholz[3]
[1]IPEEC–CONICET, Puerto Madryn, Argentina;[2] Dept. of Chemistry, Univ. of Bath, Bath, UK; [3]IFIBA-UBA-CONICET, Bs. As, Argentina.

The antibacterial effects of antimicrobial peptides (AMPs) are related to their ability to disrupt bacterial membranes. Understanding the interaction between AMPs and membranes helps the active peptide design. Molecular dynamics simulations are a powerful tool to access the atomic-level scale of the peptide interaction with membranes. Somuncurin-1 is a small AMP isolated from the Patagonian frog Pleurodema somuncurense that showed moderate antimicrobial activity against E. coli and S. aureus, along with low cytotoxicity. To propose modifications in the somuncurin-1 sequence that could increase its performance, we studied its behavior against two membrane models using molecular dynamics simulations. Two types of lipid bilayers were considered: mixtures of palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and lipid mix of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) and 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), which are simple models to mimic mammalian and bacterial membranes, respectively. The results show a differential behavior on these membranes. We found cooperative effects in the peptide-lipid bilayer interaction. Somuncurin-1 was distributed at the hydrophobic core-water interface, and simulations were conducted at temperatures of 303 K, 310 K, and 320 K. Somuncurin-1 exhibited distinct behavior in bilayer-peptide interaction: they display a strong affinity for the lipid interface of the bacterial model. Lys residue is found to anchor at the lipid water interface due to electrostatic interactions with the lipid heads. These simulations provide valuable insights into the behavior somuncurin-1 as a function of its concentration, temperature, and membrane composition in its interactions with lipid bilayers, offering a foundation for optimizing its therapeutic potential.

Antibiofilm and Quorum Sensing Inhibition (QSI) of Antimicrobial Peptide Octopromycin

S. L. Edirisinghe[1], D. C. Rajapaksha[1], Ilson Wang[2] and **Mahanama De Zoysa[1],***

[1]College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Republic of Korea.
[2]National Marine Biodiversity Institute of Korea (MABIK), Seochun-gun 33662,Republic of Korea.
*E-mail: mahanama@cnu.ac.kr

Acinetobacter baumannii is an opportunistic bacterial pathogen that causes severe infections in immunocompromised individuals. A. baumannii forms biofilm and produces extracellular matrix, which supports bacteria to survive under harsh conditions and be resistant to antibacterial treatments. In the present study, we investigated the biofilm and quorum sensing inhibitory effects of antimicrobial peptide, octopromycin in A. baumannii. Field emission scanning electron microscopy results clearly showed significantly reduced biofilm mass and caused a collapse in biofilm architecture at the minimum inhibitory concentration (50 µg/mL) and minimum bactericidal concentration (200 µg/mL) of octopromycin. Antibiotic resistant persister cells of A. baumannii were successfully killed by octopromycin treatment, and it inhibited violacein production in Chromobacterium violaceum in a concentration dependent manner. Octopromycin also inhibited alginate production, surface movements (swarming and swimming), and twitching motility of A. baumannnii, confirming its antiquorum sensing activity. Multiple metabolic pathways, two component regulation systems, quorum sensing, and antibiotic synthesis related pathways in A. baumannii biofilms were strongly affected by octopromycin treatment. The collective findings indicate that the antibacterial peptide octopromycin may control A. baumannii biofilms through multi target interactions. Octopromycin could be a desirable therapeutic option for the prevention and control of A. baumannii infections.

Insights on the Potential Mechanisms of Action of Antimicrobial Peptide, Octominin II Against Candida albicans

J.N.C. Jayasinghe[1], Ilson Whang[2] and **Mahanama De Zoysa[1],***

[1]College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Republic of Korea.
[2]National Marine Biodiversity Institute of Korea (MABIK), Seochun-gun 33662, Republic of Korea.
E-mail: mahanama@cnu.ac.kr

This study introduced a modified version of Octominin,

termed Octominin II, with an 18-amino acid sequence (1GWLIRGAIHAGKAIHGLI18). Octominin II exhibited a random coil secondary structure with a positive charge (+2.46) and hydrophobic ratio of 0.46. It effectively inhibited C. albicans, C. auris, and C. glabrata, with minimum inhibitory and fungicidal concentrations against C. albicans at 80 and 120 µg/mL, respectively. Field emission scanning electron microscopy confirmed Octominin II-induced structural changes in C. albicans cells. Membrane permeability studies using propidium iodide indicated altered cell wall integrity due to Octominin II treatment, while increased production of reactive oxygen species (ROS) was observed. Octominin II downregulated virulence genes (CDR1, TUP1, AGE3, GSC1, SAP2, SAP9) in C. albicans, and concentration-dependent degradation of genomic DNA and total RNA was observed. Octominin II effectively inhibited and eradicated C. albicans biofilms and displayed low cytotoxicity on raw 264.7 cells (0-200 µg/mL) and minimal hemolytic activity on murine erythrocytes (6.25-100 µg/mL). In vivo studies demonstrated Octominin II's ability to reduce C. albicans pathogenicity. Collectively, Octominin II exhibited multi-faceted mechanisms against C. albicans, making it a promising candidate for addressing multi-drug resistant Candida infections.

Precision design of cationic peptides to overcome bacterial resistance to traditional antibiotics

Berthony Deslouches
University of Pittsburgh School of Public Health, Department of Environmental and Occupational Health, Pittsburgh, PA, USA

Antimicrobial peptides (AMPs) have been intensively investigated as an untapped resource for antimicrobial agents to combat bacterial resistance to traditional antibiotics. Failure of AMPs with classical amphipathic structures to reach the clinic, despite numerous structure-function studies, has led to the belief that AMPs are probably not “good therapeutics”. There are several challenges to overcome. Evidently, every successful infection is a indicative of recalcitrance to endogenous AMPs; therefore, AMPs must be engineered to overcome limitations of host defense peptides. This can be achieved through structure-function studies to elucidate the determinants of antimicrobial functions. One problem is that many structure-function studies are often small pilot studies that do not provide definitive answers, which may falsely suggest that the challenges have already been addressed. We have developed a framework for AMP engineering using libraries of cationic peptides differing only by one or two residues at a time, which allows us to effectively titrate the structure-function properties. We found that given a particular amino acid composition, it is possible to generate AMPs with an optimal number of residues to achieve a mean minimum inhibitory concentration (MIC) at low micromolar range while toxicity to primary mammalian cells remains negligible. In addition, the lead candidate AMPs display systemic efficacy in mouse models of bacterial septicemia. Ultimately, our goal is to define the contribution of the cationic and hydrophobic motifs to bacterial target recognition and elimination in order to inform on-demand optimization of AMPs in response to ongoing clinical challenges.

Big defensins: A Diversified Family of Antimicrobial Peptides Confronting Versatile Microbial Environments

Noémie de San Nicolas[1], Aromal Asokan[2], Rafael Da Rosa[3], Vincent Aucagne[2], Karine Loth[2], Jean-Michel Escoubas[1], Viviane Boulo[1], Guillaume M. Charrière[1], Sébastien N. Voisin[4], Philippe Bulet[4], Caroline Montagnani[1], Agnès Delmas[2], **Delphine Destoumieux-Garzón[1]**

[1] IHPE, University Montpellier, CNRS, Ifremer, UPVD, Montpellier, France
[2] Centre de Biophysique Moléculaire, CNRS, Orléans, France
[3] Laboratory of Immunology Applied to Aquaculture, Department of Cell Biology, Embryology and Genetics, Federal University of Santa Catarina, Florianópolis 88040-900, SC, Brazil
[4] Plateforme BioPark d’Archamps, Archparc, Archamps, France ; CR UGA, IAB, INSERM U1209, CNRS UMR5309, La Tronche-Archamps, France*

Big defensins are a family of antimicrobial peptides (AMPs) that has expanded and diversified in mollusks. They are characterized by a unique structure comprising a β-defensin-like C-terminal domain linked to a hydrophobic N-terminal domain with no homology to known proteins. In the oyster Crassostrea gigas, seven genes encoding big defensins have been identified. Cg-BigDef1 was shown to entrap and kill Staphylococcus aureus in peptide nanonets. It has been hypothesized that inhibition of big defensin expression in oysters susceptible to the Pacific Oyster Mortality Syndrome (POMS), may contribute to the fatal dysbiosis leading to oyster death. To test the role of big defensins in the control of oyster microbiota, two Cg-BigDefs, namely Cg-BigDef1 and Cg-BigDef5, were synthesized by

solid-phase peptide synthesis and native chemical ligation. Synthetic Cg-BigDefs showed a broad spectrum of complementary antibacterial activities against a collection of culturable bacteria belonging to the oyster microbiota, at salt concentrations as high as 400 mM NaCl. This revealed that the sequence diversification of Cg-BigDefs has broadened their spectrum of activity. Moreover, synergistic activities were observed between variants of the Cg-BigDefs family. A fine modulation of oyster microbiota by Cg-BigDefs was also observed in vivo by 16S metabarcoding. While much remains to be discovered in terms of Cg-BigDefs mechanisms of action and molecular target specificities, the study of Cg-BigDefs has revealed that AMP diversification is the support of an unexpected specificity in the interaction with oyster microbiota members. Our results further support the hypothesis that the highly diversified immune gene repertoire of oysters has evolved to adapt to rapidly changing microbial environments.

Engineering of a novel skin secretion peptide of an endemic amphibian of Ecuador (Callimedusa ecuatoriana) into promising antimicrobial molecules.

Stefanny M. Bonilla-Jiméneza,[1], Nina Espinosa de los Monteros-Silvaa,[1], Giovanna Morán-Marcillo[a], Sebastián Bermúdez-Pugaa,[b], Andrea Terán [d], José R. Almeidaa,[c], Carolina Proaño-Bolañosa,*

[a] Biomolecules Discovery Group, Laboratory of Molecular Biology and Biochemistry, Universidad Regional Amazónica Ikiam, km 7 ½ vía Muyuna, Tena 150150, Ecuador.
[b] Laboratory of Microbial Biomolecules, Department of Biochemical and Pharmaceutical Technology, University of São Paulo, São Paulo, Brazil
[c] School of Pharmacy, University of Reading, Reading RG6 6UB, UK.
[d] Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Otonga, San Rafael, Quito, Ecuador.
[1] These authors contributed equally to this work.

Studies of amphibian skin secretion have been an important source of broad-spectrum and membrane-targeting antimicrobial peptides, which promise to tackle the antibiotic resistance crisis. It is extremely valuable to assess the diversity of bioactive components in endemic species from biodiverse countries. Callimedusa ecuatoriana from Ecuador is an example of unexplored species, which can hold a library of novel chemical scaffolds with antibiotic action. Despite the potential, all long-term efforts may result in the identification of sequences that are toxic to cells or with low antimicrobial properties. However, the chemical versatility of short-cationic peptides enables engineering of sequence modifications assisted by in silico tools. In this study, we reports a novel skin peptide (PTR-CE1) identified by molecular cloning of mRNA precursor. It demonstrated that it lacks of antimicrobial activity, likely due to its alpha-helix kink structure. So, using the natural sequence of PTR-CE1 as template, we designed and synthesized two analogs (PTR-CE1a and PTR-CE1b). Both engineered peptides displayed high antibacterial activity, even against the ampicillin-resistant bacterial strains. While PTR-CE1b showed MIC values of 106.5-212.99 mM and less than 10% of damage to red blood cells at 3.02 mM, PTR-CE1a displayed a more potent broad-spectrum effect against all the microorganisms, with MIC values of 3.02-12.06 mM, and low hemolytic properties at 6.66 mM. This study highlight the role of the secondary structure for antimicrobial activity, and shows how inactive peptides can be useful as a template for generation of new molecules with high activity and low toxicity.

SAMP: An Accurate Ensemble Model Based on Proportionalized Split Amino Acid Composition for Identifying Antimicrobial Peptides

Junxi Feng[1], Mengtao Sun[2], Weiwei Zhang[3], Guangshun Wang[3], Shibiao Wan[2]*

1. Department of Biostatistics, Harvard School of Public Health, Boston, MA, United States 02115
2. Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, NE, United States 68198
3. Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, United States 68198

*Corresponding contact: Dr. Shibiao Wan, swan@unmc.edu

Antimicrobial peptides (AMPs), a class of innate immune molecules, have received significant attention for their capacity to combat a broad spectrum of pathogens, including viruses, bacteria, and fungi. Predicting AMPs has made it easy and efficient to find AMPs from large datasets with high accuracy. Recent years have witnessed wide applications of computational methods especially machine learning and deep learning for discovering and engineer-

ing AMPs. However, existing methods only use features including compositional, physiochemical, and structural properties of peptide sequences, which cannot fully capture information from AMPs. Here, we present SAMP, an ensemble random projection (RP) based computational model that leverages a new type of features called proportionalized split amino acid composition (PSAAC) in addition to conventional sequence-based features for AMP prediction. With this new feature set, SAMP captures the residue patterns like sorting signals at around both the N terminus and C terminus, while also retaining the sequence order information from the middle peptide fragments. Benchmarking tests on balanced and imbalanced datasets from different species demonstrate that SAMP consistently outperforms existing state-of-the-art methods, such as iAMPpred, in terms of accuracy, sensitivity, specificity and AUC. We further incorporate the ensemble RP architecture in our model, so that our model SAMP is scalable to processing large scale AMP screening with further performance improvement, compared to those without RP. To enhance the impact of SAMP, we have developed a Python package for it, which is freely and publicly available at https://github.com/wan-mlab/SAMP.

Breaking the Barrier: Polyproline Peptides Disrupt the Matrix of Hypervirulent Klebsiella pneumoniae Biofilms to Release Bacteria from their Protective Barrier.

Renee M Fleeman
[1]University of Central Florida College of Medicine
Burnet School of Biomedical Sciences, Division of Immunity and Pathogenesis Orlando, FL 32827 USA

Klebsiella pneumoniae is a dangerous pathogen that has gained much notoriety due to its extreme rate of resistance development. K. pneumoniae acute and chronic wound treatment is complicated by the presence of biofilm formation, which contributes to drug resistance, persistence, tolerance, and slow wound healing. In addition to wound infections, biofilm formation abilities of K. pneumoniae lead to persistent, drug resistant catheter infections. Antimicrobial peptides have shown promise in recent years as a topical treatment and as a device coating to eradicate drug resistant K. pneumoniae. Previous work from our lab revealed a mechanism used by antimicrobial peptides to disrupt the capsule of K. pneumoniae. Recently, we have found that a polyproline peptide bac7 (1-35) can disrupt pre-formed biofilms of hypervirulent K. pneumoniae. Using confocal microscopy of K. pneumoniae NTUH K2044 biofilms with bacteria constitutively expressing GFP and matrix polysaccharides labeled with Texas red tagged Concanavalin A, we show bac7 (1-35) decreases the biofilm matrix material and releases the cells from their protective encasing. Live/dead staining images then revealed the dispersed cells were eradicated by the antimicrobial properties of the peptide. Interestingly, bac7 (1-35) treatment was found to decrease the mucoid phenotype of this species that is provided by capsular polysaccharides. Our results show that in addition to capsular polysaccharides, biofilm matrix polysaccharides are targeted by peptides to potentially sensitize hypervirulent K. pneumoniae to the host immune system and antibiotic therapies.

Bat Cathelicidins as Natural Antimicrobial Agents: A Computational and In Vitro Investigation

Farrais-Solana, Francisco,a Pérez de la Lastra, José Manuela, Asensio-Calavia, Patricia[a,b,*]; Otazo-Pérez, Andrea[a,b]; González-Acosta, Sergioa,[b]; González-Almécija, Beatriz[a]; Morales-delaNuez, Antonio[a†]; López R., Manuel [a]

Affiliation: [a]Macromolecules Biotechnology Research Group, Institute of Natural Products and Agrobiology (IPNA-CSIC), San Cristóbal de La Laguna, Spain.
[b]Escuela de Doctorado y Estudios de Posgrado, Universidad de La Laguna, San Cristóbal de La Laguna, Spain.
[†]Current address: Animal Production and Biotechnology Group, Institute of Animal Health and Food Safety, University of Las Palmas de Gran Canaria, Spain.

*Presenting author: jm.perezdelalastra@csic.es

Antimicrobial peptides (AMPs) are small proteins that play an important role in the innate immune system of various organisms, including plants, animals, and humans. These natural defence molecules have attracted considerable interest due to their potential as alternative antimicrobial agents to combat infectious diseases. In this study, we used computational and in vitro methods to investigate the antimicrobial activity of cathelicidin family peptides from bat species with different ecological niches.
The study of the physicochemical parameters of the peptides (hydrophobicity and net charge), together with the study of the helical regions, allowed us to deduce the antimicrobial character of peptides. To analyse the antimicrobial activity in vitro, we first determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against four different bacteria: E. coli, Salmonella, S. aureus, and E. faecalis. We then se-

lected the most effective peptide and assessed whether it acts as a bacteriostatic or bactericidal agent. Additionally, we investigated the duration of its activity and its ability to lyse bacterial cells. We accomplished this by plotting killing curves during both the exponential and stationary phases of bacterial growth. Finally, we evaluated the peptide's potential to cause hemolysis in rat erythrocytes.
One of our peptides revealed a high antimicrobial activity, with MIC and MBC values ranging from 3.12 to 1.56 µM. It also demonstrated bactericidal properties during the stationary phase but acted as a bacteriostatic agent during the exponential phase. Notably, it exhibited lytic activity against the tested bacteria but no hemolytic activity against rat erythrocytes.
Acknowledgements: This work was funded by projects APOGEO (INTERREGMAC Call-European Funds for Regional Development- FEDER). Patricia Asensio-Calavia and Andrea Otazo-Pérez are recipients of pre-doctoral fellowships from the “Agencia Canaria de Investigación, Innovación y Sociedad de la Información (ACIISI) del Gobierno de Canarias”. We are grateful to CEAMED S.A for providing support in carrying out this research under the agreement with IPNA-CSIC.

Parrot cathelicidins as a new source of antimicrobial agents

Sergio González-Acosta[a,b], Patricia Asensio-Calavia[a,b], Andrea Otazo-Pérez[a,b] Manuel R. López[a], Antonio Morales-delaNueza,[§], José Manuel Pérez de la Lastra[a*]

[a]Biotechnology of Macromolecules Research Group. Instituto de Productos Naturales y Agrobiología (IPNA-CSIC). San Cristóbal de La Laguna, Spain. [b]Escuela de Doctorado y Estudio de Posgrado. Universidad de La Laguna. San Cristóbal de La Laguna, Spain. [§]Current adress: Departamento de Patología Animal, Producción Animal, Bromatología y Tecnología de los Alimentos. Universidad de Las Palmas de Gran Canaria. Las Palmas de Gran Canaria, Spain.

Presenting author: sergi_glez@hotmail.com

The innate immune system of most vertebrates includes a family of host defense peptides named cathelicidins. Avian cathelicidins are excellent candidates for antimicrobial drug development due to their broad spectrum of activity against various microorganisms and low toxicity. Because of their extraordinarily long lifespan, parrots are exposed to a variety of infectious diseases during their lifetime, making them an excellent subject for the study of their immune system. The aim of this study was to evaluate the antimicrobial, hemolytic and cytotoxic activity of parrot cathelicidins and to determine their mode of action. To conduct in vitro tests, we synthesized six peptides: two from Amazona guildingii, two from Eolophus roseicapilla, and two from Gallus gallus (as reference peptides). Our data demonstrated that even at the greatest concentration examined, the peptides had no effect on VERO cells and human erythrocytes. At lower concentrations, the peptides showed strong antimicrobial activity with MIC and MBC values ranging from 12.5 µM to 1.56 µM. However, none of the peptides showed activity against Candida albicans. We used Sytox Green and performed a 4-hours time-kill assay to investigate the mechanism of action. The kinetic assay showed that the peptides required between 20 and 60 min to kill Escherichia coli and that they operated by disturbing the bacterial membrane. In general, parrot cathelicidin-derived peptides showed potent antimicrobial activity and could be used as novel templates for antimicrobial drug development.

Ecology-relevant bacteria drive the evolution of host antimicrobial peptides in Drosophila

M. A. Hanson, L. Grollmus, and B. Lemaitre (Science ; 2023)

Antimicrobial peptides are host-encoded immune effectors that combat pathogens and shape the microbiome in plants and animals. However, little is known about how the host antimicrobial peptide repertoire is adapted to its microbiome. Here, we characterized the function and evolution of the Diptericin antimicrobial peptide family of Diptera. Using mutations affecting the two Diptericins (Dpt) of Drosophila melanogaster, we reveal the specific role of DptA for the pathogen Providencia rettgeri and DptB for the gut mutualist Acetobacter. The presence of DptA- or DptB-like genes across Diptera correlates with the presence of Providencia and Acetobacter in their environment. Moreover, DptA- and DptB-like sequences predict host resistance against infection by these bacteria across the genus Drosophila. Our study explains the evolutionary logic behind the bursts of rapid evolution of an antimicrobial peptide family and reveals how the host immune repertoire adapts to changing microbial environments.

Activity increase and its structural basis after Manduca sexta moricin-6 processing

Chunxiang Hou[1], Om Prakash[2], Haobo Jiang[1]

[1] Dept. of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA
[2] Dept. of Biochem. & Mol. Biophysics, Kansas State University, Manhattan, KS 66506, USA

Insects produce a variety of antimicrobial peptides (AMPs) to kill invading microbes during immune responses. Among them, moricins have a pre-pro-structure, that is, a signal peptide for secretion, a 2 or 4-residue pro-region, and a mature peptide predicted to form an amphipathic α-helix that disrupts bacterial cell membrane. In the tobacco hornworm Manduca sexta, six genes encode moricin-1 through -6. We hypothesize that the pro-moricins become fully active after their (Xaa-Pro)1−2 is removed by a proline-specific dipeptidyl peptidase-4 (DPP4). Two peptides were chemically synthesized: 1) pro-moricin-6, APEPGRLSAIKKGGKIIKKGLGVISAAGTAHEVY SHVKNRRN (42-mer); 2) moricin-6, GRLSAIKKGGKII KKGLGVISAAGTAHEVYSHVKNRRN (38-mer). We produced M. sexta DPP4 in insect cells, treated the precursor with purified DPP4, and detected mature moricin-6 in the reaction mixture. There was a substantial increase in bactericidal activity after the N-terminal tetrapeptide APEP was removed. We have initiated structural and dynamics studies on pro-moricin-6 and moricin-6 using NMR spectroscopy to understand structural differences in the two structures, which may account for the increase in the AMP activity.

Drosocin-like peptides exhibit highly diverse antimicrobial activity and inhibit translation at two different stages.

Weiping Huang[1], Chetana Baliga[1,2], Ariya Chang[1], Gemma C. Atkinson[3], Nora Vazquez-Laslop[1], Alexander Mankin[1].

1. Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, Illinois, USA.
2. Department of Biotechnology, Faculty of Life and Allied Health Sciences, Ramaiah University of Applied Sciences, Bangalore, India.
3. Department of Experimental Medicine, Lund University, Lund, Sweden

Proline-rich antimicrobial peptides (PrAMPs) are short peptides naturally synthesized by arthropods and mammals. According to their ability to inhibit translation, PrAMPs are classified into two subgroups. While type I PrAMPs arrest ribosomes at start codon of open reading frames, type II PrAMPs apidaecin (Api) and drosocin (Dro) stall ribosomes at stop codons. Notably, Api and Dro share sequence similarities specially at their C-termini, which include amino acids critical for target interaction. In this work, we bioinformatically identified Dro-like peptides encoded in the genomes of diverse fruit fly species. By testing the antimicrobial activity of the synthetic version of ten of these peptides, we found that only two of them (besides the already characterized Dro from D. melanogaster) were able to kill the tested GGram-negativebacteria. Further, the identified active peptides had the ability to cause stop codon readthrough in E. coli cells and cause ribosome arrest at stop codons during in vitro translation. Intriguingly, the peptides unable to penetrate and kill the tested strains arrested ribosomes at start codons, resembling the behavior of type I PrAMPs. The outcomes of our study underscore the complexity of predicting MOA solely based on similarity of peptide sequences.

Effects of a chionodracine-derived antimicrobial peptide against bacteria virulence factors

Esther Imperlini, Federica Massaro, Angelica Grifoni, Fernando Porcelli, Stefano Borocci, Francesco Buonocore

Department for Innovation in Biological, AgroFood and Forest Systems, University of Tuscia, Viterbo, Italy

Antarctic fishes, living in an extreme environment and normally exposed to pathogens, are a promising source for antimicrobial peptides (AMPs), fundamental for the innate immune responses of these vertebrates. These natural peptides are emerging as next-generation therapeutics due to their action against bacteria, viruses, yeasts and protozoa. As they show a broad spectrum of activity against multidrug resistant (MDR) bacteria, strong efforts are in progress to bring AMPs into clinical use, in order to counteract the increasing resistance to classical antibiotics. Beyond intrinsic/acquired resistance, MDR species also uses virulence factors (like biofilm formation and protease secretion) to infect hosts. Hence, there is a need for innovative approaches targeting these virulence factors especially in the case of bacteria involved in chronic pathogenesis.
In our research, we used a mutant peptide, named KHS-Cnd, that was obtained from the scaffold of the chionodracine (Cnd), a natural peptide identified in the icefish Chionodraco hamatus.
Among virulence factors, we investigated the effect of KHS-Cnd on protease production of two model Gram-negative/positive bacteria, Escherichia coli and Bacillus ce-

reus. The peptide was tested both at minimum inhibitory concentrations (MICs) and 2x MICs previously determined for the two bacterial strains. A significant reduction in protease activity was observed for both bacteria at the tested concentrations within 1-3 h from the treatment. Moreover, we determined that KHS-Cnd has low cytotoxicity on human primary cells and no hemolytic activity on mammalian erythrocytes at concentrations displaying anti-virulence activity, thus confirming the interesting potential of the peptide as a new drug.

Antimicrobial effector genes and their transcription patterns in an insect, Manduca sexta

Yan He, Xiaolong Cao, **Haobo Jiang**

Dept. of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA

Antimicrobial proteins/peptides (AMPs) are effectors of innate immune systems against pathogen infection in multicellular organisms, which act in concert to suppress or kill bacteria, fungi, viruses, and parasites. We have identified 86 putative AMP genes in the genome of a lepidopteran insect. They encode 15 cecropins, 6 moricins, 6 defensins, 3 gallerimycins, 4 X-tox's, 14 diapausins, 15 whey acidic protein homologs, 11 attacins, 1 gloverin, 4 lebocins, 6 lysozyme-related proteins, and 4 transferrins. Some of the genes (e.g., attacins, cecropins) form large clusters, likely arising after rounds of gene duplication. We compared the amino acid sequences of M. sexta AMPs with homologs from other insects to reveal conserved structural features and phylogenetic relationships. Expression data have shown that many of them are synthesized in fat body and midgut during the larval-pupal molt. Certain genes contain one or more predicted κB binding sites and other regulatory elements in their promoter regions, which may account for the dramatic mRNA level increases in fat body and hemocytes after an immune challenge. Consistent with the mRNA increases, many AMPs become highly abundant in larval plasma at 24 h after challenge, as demonstrated in our proteomic study. Taken together, these data suggest the existence of a large repertoire of AMPs in M. sexta, whose expression is up regulated via immune signaling pathways to fight off invading pathogens in a coordinated manner.

KR-12, the minimal antibacterial peptide of human cathelicidin LL-37: Discovery, engineering and applications

Jayaram Lakshmaiah Narayana[1,2], **D. Zarena** [1,3], and Guangshun Wang*

1. Department of Pathology and Microbiology, College of Medicine, University of Nebraska Medical Center, 985900 Nebraska Medical Center, Omaha, NE 68198-5900, USA.
2. Department of Biotechnology, Dayananda Sagar College of Engineering, Bangalore, India
3. College of Engineering, Jawaharlal Nehru Technological University, Anantapur, India

This poster will provide a summary on the recent research results on KR-12, a 12-residuecationic antimicrobial peptide derived from human cathelicidin LL-37. KR-12 has been shown to have a selective toxic effect on bacteria but not on human cells. The positive charges of KR-12 allow it to interact with negatively charged bacterial membranes. Moreover, KR-12 has been found to possess anti-inflammatory properties useful for development of novel wound dressings. KR-12 has been shown to promote the osteogenic differentiation of human bone marrow stem cells by stimulating BMP/SMAD signaling. In addition, different forms of KR-12 have been designed, including conjugated hybrids, lipidated analogs, and cyclic peptides. Finally, KR-12 has been immobilized on various surfaces to prevent biofilm formation. In conclusion, KR-12 has shown promise for various applications in medicine, food, animal husbandry, agriculture, and aquaculture.

Novel peptide antibiotics with unusual modes of action

Kim Lewis

Antimicrobial Discovery Center, Northeastern University, Boston, MA USA k.lewis@neu.edu

We are experiencing an Antimicrobial Resistance Crisis, a slow-moving pandemic, according to the WHO. This calls for antibiotics that avoid resistance development, and are capable of acting against dormant persister cells responsible for recalcitrance of chronic infections, a source of resistant mutants and an unsolved problem in its own right. We are developing several peptide antibiotics with unusual modes of action that go well beyond a simple inhibition of the target, to address these challenges. Acyldepsipeptide kills persister cells by activating proteolysis. Teixobactin binds to precursors of peptidoglycan and wall teichoic acid,

and forms a supramolecular structure that damages the membrane. Clovibactin binds to a minimal, immutable PiPi moiety of Lipid II, and forms a supramolecular structure that causes cell lysis. There is no detectable resistance to teixobactin and clovibactin. Darobactin and dynobactin bind the “undruggable” β-barrel BamA protein on the surface of Gram-negative bacteria. The binding is based on peptide backbone interactions, precluding resistance development.

Rational design, synthesis, antifungal evaluation and docking studies of antifungal peptide CGA-N12 analogues based on the target CtKRE9

Ruifang Li, Jiasha Wu, Shang Li, Weitong Li, Xin-Hui Zhang

Key Laboratory of Functional Molecules for Biomedical Research, College of Biological Engineering, Henan University of Technology, Zhengzhou, Zhengzhou Henan, 450001, China

Candida tropicalis is a major non-albicans species that causes invasive candidiasis. CGA-N12, an anti-Candida peptide found by our group, disrupted cell wall architecture by inhibiting the activity of the protein killer-resistant 9 (KRE9), a β-1,6-glucan synthase specific to Candida spp. and plants. Herein, a set of CGA-N12 analogues were rationally designed based on the interaction networks between CGA-N12 and C. tropicalis KRE9 (CtKRE9). Seven CGA-N12 analogues with significantly improved antifungal activity against C. tropicalis were screened by reducing the docking energy of CGA-N12 and CtKRE9 and increasing the number of positive charges on CGA-N12 based on a stable three-dimensional model of CtKRE9. CGA-N12 and its analogues exhibited antifungal activity against C. tropicalis and its persist cells; they also inhibited biofilm formation and eradicated preformed biofilms. Compared with fluconazole, they displayed higher activities against the growth of persister cells and more effective preformed biofilm eradication. Among them, CGA-N12-0801, CGA-N12-0902 and CGA-N12-1002 displayed much higher activity and anti-proteinase digestion stability than CGA-N12. Specifically, CGA-N12-0801 was the optimal analogue, with a minimum inhibitory concentration of 3.46 μg/mL and a therapeutic index of 158.07. The results of electronic microscopy observations and KRE9 activity inhibition assays showed that CGA-N12 and its analogues killed C. tropicalis by disrupting the architecture of the cell wall and the integrity of the cell membrane. In conclusion, for the first time, we provide a simple and reliable method for the rational design of antimicrobial peptides and ideal candidates for treating Candida infections that not effectively eliminated by azole drugs.

Keywords: Antimicrobial peptide, Candida tropicalis KRE9, Molecular docking, Molecular design, Antifungal peptide analogues

A novel temporin SSTP1 for killing triple negative breast cancer cells

Shyla, G.[1,2*], Gayathri Mohan[1,3], Soumya K U.[1], Amrutha Mohan[1, 4], Geetha Shanmugam[1], Vineethkumar, T. V.[2], Sreekumar, J. [5], Sanil George[2], and **Tessy T Maliekal[1*]**

[1]Cancer Research, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India, 695014
[2]Interdisciplinary Biology, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India, 695014
[3]Regional Centre for Biotechnology, Faridabad, Haryana, India, 121 001
[4]Manipal Academy of Higher Education, Manipal, Karnataka, India, 576104
[5] 5Statistics, Section of Extension and Social Science, ICAR- Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, 695017

Despite the identification of several antitumor antimicrobial peptides, their clinical application is limited due to various reasons. Since majority of them depend on the membranolytic activity, the high nonspecific effects limit their therapeutic potential. Though induction of apoptosis independent of innate immune regulatory mechanisms are reported, the membrane targets of majority of the peptides are unknown. Here, we show that SSTP1, a novel temporin identified from frog skin secretion binds to IL6/IL6Rα/gp130 complex to induce apoptosis in IL6Rα overexpressing cancer cells, like triple negative breast cancer cells. SSTP1 shifts the proliferative IL6/JAK/STAT signaling to apoptotic IL6/JNK/AP1 pathway. In contrast to IL6 blockers that inhibit JAK/STAT activity, SSTP1 activates JNK/AP1 pathway with concomitant inhibition of STATs. In IL-6Rα-overexpressing cancer cells, SSTP1 induces apoptosis at low concentration through JNK pathway, without causing significant membrane disruption as evidenced by negligible haemolytic activity. Since JNK activation can lead to proliferation, apoptosis or autophagy, we explored its relevance in our study, and found that SSTP1 exclusively induces apoptosis. Our results show that immunomodu-

latory pathways can modulate apoptosis besides their conventional role in immune cell regulation and proliferation in cancer context. Thus we propose that identification of membrane targets of likely anticancer host defense peptides will pave way to the development of therapeutics for targeted therapy.

Cathelicidins in vertebrates: A potential new tool in the fight against Botrytis cinerea

José Manuel Pérez de la Lastra[a], Patricia Asensio-Calavia[a,b]; Andrea Otazo-Pérez[a,b], Sergio González-Acosta[a,b] Beatriz González-Almécija[a], Antonio Morales-delaNuez[a†], Victoria Baca González, Manuel R. López[a]

[a]Macromolecules Biotechnology Research Group, Institute of Natural Products and Agrobiology (IPNA-CSIC), San Cristóbal de La Laguna, Spain.
[b]Escuela de Doctorado y Estudios de Posgrado, Universidad de La Laguna, San Cristóbal de La Laguna, Spain.
†Current address: Animal Production and Biotechnology Group, Institute of Animal Health and Food Safety, University of Las Palmas de Gran Canaria, Spain.

Botrytis cinerea is a fungal phytopathogen with the second largest worldwide impact on the agricultural industry. We evaluated the ability of four peptides from vertebrate cathelicidins to reduce B. cinerea infection in tomato leaves: two proline-rich peptides (LV-RR32 and TT-FR28) and two α-helix (AM-RV28 and TO-KL37). For this purpose, we inoculated four-week-old tomatoes third leaf with a 5 µL droplet of B. cinerea (2X105 conidia/mL) mixed with each peptide a different concentration (200, 100, 50 and 25 µM). After three days of incubation at 24ºC in dark conditions, the average lesion diameter was determined. From the proline-rich peptides examined, the results showed that LV-RR32 was the most effective, fully inhibiting the infection at 100 µM and greatly reducing it at the lowest concentration. TT-FR28, on the other hand, displayed less activity. The larger size and higher proline and arginine content could be responsible of the higher activity of LV-RR32. In the case of α-helices peptides, AM-RV28 inhibited completely the infection at 100 µM and showed good activity at lower concentrations. Meanwhile, TO-KL37 only reduced the infection at the higher concentration tested (200 µM). In this case, AM-RV28 presented the higher hydrophobicity in its α-helix, which could be related with its higher activity against this fungus.
This study is an important step toward harnessing the power of natural defense molecules to address agricultural challenges, and it lays the groundwork for further exploration of the potential of these peptides for crop protection and pathogen management.

Identification of novel antimicrobial peptides from the skin of Leptodactylus chaquensis (Anura Leptodactylidae) frog in northern Argentina

Mariela M. Marani[1], Ana P. Cuzziol Boccioni[1,2], Natalia L. Cancelarich[1], Silvana Aguilar[1], Néstor G. Basso[3]

[1]IPEEC–CONICET, Puerto Madryn, Argentina; [2]FBCB-UNL, Santa Fe, Argentina; [3]IDEAus–CONICET, Puerto Madryn, Argentina.

The search for innovative therapeutic solutions to address the escalating resistance of pathogens to antibiotics is a pressing global challenge. Bioprospecting for novel molecules serves as a cornerstone, providing essential data to fuel databases used in developing AI-driven tools. These tools are crucial for drug design, ushering in a new era of drug discovery and development. Antimicrobial peptides (AMPs) have demonstrated their effectiveness against a broad spectrum of pathogen. These peptides are sourced from various natural origins, including amphibian skin, which is particularly abundant. The northern region of Argentina boasts an unexplored and diverse amphibian biodiversity, promising potential for the discovery of new AMPs. This study aimed to identify AMPs from Leptodactylus chaquensis frog skin collected in Corrientes, Argentina. Total mRNA was extracted from the skin, cDNA was synthesized, and cloned into E. coli DH5α cells. Plasmids of selected colonies were purified, PCR amplified, insert size assessed, and sequences obtained. Three prepro-peptides coding novel mature peptides were identified. The peptides, provisionally named Lch-1, Lch-2, and Lch-3, exhibited lengths of 25, 21, and 9 residues, with net charges of +1, 0, and -1, respectively. Hydrophobic percentages were 38%, 54%, and 44%, with theoretical 3D α-helical content in their structures. Lch-1 and -2 shared 43% and 41% sequence similarity with ocellatin-4 and -6 from Leptodactyus ocellatus. Predicted interactions with membranes suggest antimicrobial potential using the APD tool. Peptides will be synthesized for bioactivity assays. These findings underscore the significance of bioprospecting in uncovering distinct and unique bioactive compounds within each species.

Enhanced Antimicrobial Screening Sensitivity Enabled the Identification of an Ultrashort Peptide KR-8 for Engineering of LL-37mini to Combat Drug-resistant Pathogens

Abraham Fikru Mechesso[1], Yajuan Su[2], Jingwei Xie[2], and Guangshun Wang[1*]

[1]Department of Pathology and Microbiology, College of Medicine, University of Nebraska Medical Center, 985900 Nebraska Medical Center, Omaha, NE 68198-5900, USA
[2]Department of Surgery-Transplant and Mary & Dick Holland Regenerative Medicine Program, College of Medicine, University of Nebraska Medical Center, Omaha, NE, 68198, USA

Identification of novel antibiotics is of top importance because of the threat of antibiotic-resistant pathogens. Antimicrobial screening in Mueller Hinton broth is frequently the first step in antimicrobial discovery. Although widely utilized, this medium is not ideal as it could mask activity of candidates such as human cathelicidin LL-37 against methicillin-resistant Staphylococcus aureus (MRSA). This study identified a sensitive medium where LL-37 displayed excellent activity against numerous pathogens, including MRSA. Our screen of ultrashort overlapping LL-37 peptides led to the identification of KR-8, four-residue shorter than KR-12. KR-8 enabled the design of LL-37mini, which was potent against MRSA, Escherichia coli, and Pseudomonas aeruginosa but was not hemolytic. It also disrupted 24-h preformed biofilms in vitro and killed MRSA in murine wound biofilms. Because LL-37mini can be made cost effectively, it can be developed into new antibiofilm and antimicrobial agents. Our screen condition can increase positive compound hits during antimicrobial screening.

Keywords: Antibacterial susceptibility; antibiofilm; antimicrobial peptides; antimicrobial screen; LL-37mini.

Reptile cathelicidins and their potential as new antimicrobial compounds

Otazo-Pérez, Andrea[a,b]; González-Acosta, Sergio[a,b]; Asensio-Calavia, Patricia[a,b]; González-Almécija, Beatriz[a]; Morales-delaNuez, Antonio[a,†]; López, Manuel R.[a]; Pérez de la Lastra, José Manuel[a].

[a]Macromolecule Biotechnology Research Group, Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez, 3, 38206, San Cristóbal de La Laguna, S/C de Tenerife, Spain.
[b] Escuela de Doctorado y Estudios de Posgrado, Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez, SN. Edificio Calabaza-AN.2D Apdo. 456 38200, San Cristóbal de La Laguna, S/C de Tenerife, Spain.
[†]Current address: Animal Production and Biotechnology Group, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, 35413, Arucas, Las Palmas, Spain.

Cathelicidins are a family of host defense peptides (HDP) with different functions, highlighting their antimicrobial activity, expressed in most vertebrates. These peptides present a high variability, differentiating their sequence, structure, and function among different animal species. In this research, mining the available reptile genomes on NCBI database has been carried out to identify new cathelicidins. An analysis of the structure and physicochemical properties of the peptides was made and 6 of them were selected to synthesize and study their potential as antimicrobial compounds in vitro. For this, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were characterized against 5 human pathogenic bacteria. For peptides that showed the highest activity, their bacteriostatic or bactericidal character as well as their lytic activity and their period of action was studied. For this, kill curves were carried out with the bacteria in the latency and exponential phase. To study the possible toxicity of peptides, a hemolytic activity assay with rat erythrocytes and a MTT cytotoxicity assay with Vero cells were carried out. Novel reptile peptides showed good antimicrobial activity between 0.39 and 25 micromolar against the bacteria which they were tested, exhibiting bactericidal activity when the microorganism is in the latency phase and bacteriostatic activity when it's in the exponential phase. Peptides had an active period of approximately 6 hours and showed lytic activity against bacteria. On the other hand, there was no hemolytic or cytotoxic activity at concentrations that they were effective against microorganisms.

Prediction of the penetration efficiency of proapoptotic peptides by cell-penetrating peptides (CPP) and anticancer peptides (ACP) using bioinformatics frameworks

Hajar Pourabtahi[1] and Shahriyar Abdoli[2*]

[1]Department of New Technologies Biotechnology, Goles-

tan University of Medical Sciences,Gorgan, Iran; hajar.pourabtahi@gmail.com
²Assistant professor in Pharmaceutical Biotechnology, Golestan University of Medical Science,Gorgan, Iran

* Correspondence: drabdoli goums.ac.ir

Cancer is a major cause of death worldwide, and despite advancements in treatment, no effective cure exists. Pro-apoptotic peptides have emerged as a promising alternative to conventional anticancer drugs. However, their clinical application faces challenges due to the cell membrane's hydrophobic barrier, limiting peptide access to intracellular targets. To overcome this obstacle, research suggests that conjugating with cell-penetrating peptides (CPPs) can improve intracellular transport. We propose a bioinformatics approach to evaluate the efficiency of four CPPs (TAT, R8, ATP 128, and Penetramax) for delivering pro-apoptotic sequences BIM, NOXA, BID, and BMF. We also used four anti-cancer peptides (LL37, Pexig, CLS001, and Magainin II) to enhance CPP potency. Our method resulted in the selection of 60 CppProAcp sequences with a high and average probability of absorption efficiency. We evaluated these peptides (CppProAcp) for transfer effect, stability, thermodynamic properties, aggregation potential, folding speed, backbone flexibility, and in vivo administration sensitivity, resulting in the identification of 20 promising structures. Based on our study, this bioinformatic workflow can be universally applied to any CPP-peptide conjugation scheme.
Key Word: pro-apoptotic peptides, cell-penetrating peptides, anti-cancer peptides, Delivery, CPP-peptide

Machine Learning based models for desiging therapeutic peptides from antimicobial peptide database

Gajendra P. S. Raghava, Professor & Head
Department Computational Biology, IIIT, Delhi, India

Therapeutic peptides are a class of molecules that are increasingly gaining attention in the field of medicine due to their potential as highly specific and effective therapeutic agents. Peptides-based therapeutics have become increasingly important in the field of medicine over the last few decades. According to a recent survey, peptide drugs currently account for approximately 10% of the entire pharmaceutical market, and this proportion is expected to increase in the future. Since the early 1980s, a total of 239 therapeutic proteins and peptides have been approved for clinical use by the US Food and Drug Administration (FDA). Thus there is a need to develop peptide based therapeutics aginst emerging strains of pathogens particularly against drug-resistant strains. In order to address this challege, there is a need to develop repositories to maintin experimentally validated therapeutic peptides. In the past number of repositories have been developed to maintain large number of peptides responsible for killing wide range pathogenic strains (e.g., APD3, ParaPep, CAMPR, dbAMP, AntiTbPdb). Antimicobial peptide database (APD) is one of first repository developed in 2003, which is freely avilable for scientific community. These antimicrobial peptides in APD have been heavily used by researchers for developing machine learning based models for predicting and desiging therapeutic peptide. Our group developed many machine-learning based methods (e.g., AntiBP, AntiBP2, AntiCP) using data in APD for desiging wide-range of therapeutic peptides (See https://webs.iiitd.edu.in/raghava/). In this talk, I will cover number of machine learning based models developed for desiging therapeutic peptides particularly antimicrobial peptides.

Progress Toward the Total Synthesis of Transvalencin A

Xavier S. Streety and Steven D. Townsend

Department of Chemistry, Vanderbilt University
Nashville, Tennessee 37235, United States

Initially isolated from a clinical strain of Nocardia transvalensis, transvalencin A is a non-ribosomally encoded peptide (NRP) and zinc chelator with antifungal and antibacterial activity. Despite its initial promise, no work has been published on the molecule since the elucidation of its structure in 2004. Transvalencin A features the common oxazoline, thiazoline, and thiazolidine moieties of NRPs as well as two stereospecific oxidations. Both sites of oxidation result in unusual hemiacetal-like structures. Our ongoing total synthesis seeks to establish conditions for the stereospecific oxidation of modification of the constituent peptides composing tranvalencin A. In addition, our modular approach in the construction of the molecule allows for access to analogues. Once in hand, we seek to further evaluate the antimicrobial properties of transvalencin A beyond that of the initial report and to investigate its effect on metal processing in communities of bacteria.

Functional difference and its structural basis of Manduca sexta pro-moricin-6 and moricin-6

Andy Su[1], Aprajita Jha[1], Nitin Mishra[1], Tomohiro Kimura[1,2], Chunxiang Hou[3], Haobo Jiang[3] and Om Prakash[1]

[1]Department of Biochemistry and Mol. Biophysics, Kansas State University, Manhattan, KS 66506
[2]Department of Chemistry, Kansas State University, Manhattan, KS 66506
[3]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078

Insects produce a variety of antimicrobial peptides (AMPs) to kill invading microbes during immune responses. Among them, moricins have a pre-pro-structure, that is, a signal peptide for secretion, a 2 or 4-residue pro-region, and a mature peptide predicted to form an amphipathic α-helix that disrupts bacterial cell membrane. In the tobacco hornworm Manduca sexta, six genes encode moricin-1 through -6. We hypothesize that the pro-moricin becomes fully active after their (Xaa-Pro)1−2 is removed by a proline-specific dipeptidyl peptidase-4 (DPP4). Two peptides were chemically synthesized: 1) pro-moricin-6, APEPGRLSAIKKGGKIIKKGLGVISAAGTAHEVYSHVKNRRN (42-mer); 2) moricin-6, GRLSAIKKGGKII KKGLGVISAAGTAHEVYSHVKNRRN (38-mer). We produced M. sexta DPP4 in insect cells, treated the precursor with purified DPP4, and detected mature moricin-6 in the reaction mixture. There was a substantial increase in bactericidal activity after the N-terminal tetrapeptide APEP was cleaved. We have initiated structural and dynamics studies on pro-moricin-6 and moricin-6 using NMR spectroscopy to understand structural differences in the two structures, which may account for the increase in the AMP activity.

Development of human LL-37 and related peptides into a spermicide/microbicide

Nongnuj Tanphaichitr[1,2], Seung Gee Lee[1], Wongsakorn Kiattiburut[1], Nopparat Srakaew[1], Jonathan Angel[1,3,4], Guangshun Wang[5]

[1]Chronic Disease Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[2]Department of Obstetrics/Gynecology, University of Ottawa, Ottawa, ON, Canada
[3]Department of Biochemistry, Microbiology, Immunology, Faculty of Medicine, University of Ottawa, ON, Canada
[4]Division of Infectious Diseases, Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada
[5]Department of Pathology and Microbiology, College of Medicine, University of Nebraska Medical Center, Omaha, NE, USA

The concurrent increases in global population and sexually transmitted infections (STIs) have prompted the World Health Organization to promote development of multipurpose prevention technology (MPT) for administration in the female reproductive tract (FRT). We have demonstrated that the antimicrobial peptide LL-37 and its truncated forms, GI-20, GF-17, with known microbicidal effects, can act as spermicides on human and mouse sperm. In mice, these peptides inhibit fertilization in vivo as well as pregnancy when co-injected transcervically with sperm. We have further demonstrated the microbicidal activity of these peptides on Neisseria gonorrhoeae, for which antibiotic resistance is rising. While LL-37/GI-20/GF-17 may warrant the development into MPT agents, they are highly susceptible to degradation by proteases in human cervicovaginal fluid (CVF). Therefore, we have opted to evaluate spermicidal/microbicidal activity of 17BIPHE2, a peptide engineered based on the GF-17 sequence, with 3 L-amino acids (2 Ile's+1 Leu) substituted with D-Leu's and 2 Phe's replaced by biphenylalanines. As expected, 17BIPHE2 is highly resistant to degradation in CVF, and it is a most effective spermicide in CVF, compared with LL-37/GI-20/GF-17. In addition, 17BIPHE2 is active against viruses and numerous antibiotic-resistant bacteria including N gonorrhoeae. Therefore, 17BIPHE2 deserves to be developed into a vaginal MPT agent. However, since >90% of 17BIPHE2 (like LL-37/GI-20/GF-17) remains in the FRT one hour after its administration, hydroxyethylcellulose hydrogel is being tried as an excipient in the delivery system in the mouse FRT. The use of 17BIPHE2 as an MPT agent will empower women to protect themselves against unplanned pregnancies and STIs.

Peptidomimetic oligomers targeting membrane phosphatidylserine exhibit broad antiviral activity

Patrick M. Tate[1], Vincent Mastrodomenico[2], Christina Cunha[2], Joshua McClure[3], Annelise E. Barron[3,4], Gill Diamond[5], Bryan C. Mounce[2], Kent Kirshenbaum[1,3*]

1. Department of Chemistry, New York University, New York, NY 10003, USA

2. Department of Microbiology and Immunology, Loyola University Chicago Medical Center, Maywood, IL 60130, USA
3. Maxwell Biosciences, Austin, TX 78738, USA
4. Department of Bioengineering, Stanford University, Stanford, CA 94305, USA
5. Department of Oral Immunology and Infectious Diseases, University of Louisville School of Dentistry, Louisville, KY 40292, USA
*Corresponding Author: kent@nyu.edu

The development of durable new antiviral therapies is challenging, as viruses can evolve rapidly to establish resistance and attenuate therapeutic efficacy. New compounds that selectively target conserved viral features are attractive therapeutic candidates, particularly for combatting newly emergent viral threats. The innate immune system features a sustained capability to combat pathogens through production of antimicrobial peptides (AMPs); however, these AMPs have shortcomings that can preclude clinical use. The essential functional features of AMPs have been recapitulated by peptidomimetic oligomers, yielding effective antibacterial and antifungal agents. Here, we show that a family of AMP mimetics, called peptoids, exhibit direct antiviral activity against an array of enveloped viruses, including the key human pathogens Zika, Rift Valley fever, and chikungunya viruses. These data suggest that the activities of peptoids include engagement and disruption of viral membrane constituents. To investigate how these peptoids target lipid membranes we used liposome leakage assays to measure membrane disruption. We found that liposomes containing phosphatidylserine (PS) were markedly sensitive to peptoid treatment; in contrast, liposomes formed exclusively with phosphatidylcholine (PC) showed no sensitivity. In addition, chikungunya virus containing elevated envelope PS was more susceptible to peptoid-mediated inactivation. These results indicate that peptoids mimicking the physicochemical characteristics of AMPs act through a membrane-specific mechanism, most likely through preferential interactions with PS. We provide the first evidence for engagement of distinct viral envelope lipid constituents, establishing an avenue for specificity that may enable development of a new family of therapeutics capable of averting the rapid development of resistance.

Antimicrobial peptides shape plant microbiota

Bart Thomma, Cluster of Excellence on Plant Sciences (CEPLAS), Institute for Plant Sciences, University of Cologne, Cologne, Germany

Beneficial plant-associated microbes are found in and on all organs of the plant and help to mitigate (a)biotic stresses. Moreover, plants are able to shape their microbiota and specifically attract beneficial microbes to suppress pathogen attack. Hence, the plant's microbiome can be considered an inherent, exogenous layer that complements its endogenous innate immune system. Microbes typically secrete a plethora of molecules into their environment to promote niche colonization. Especially soil-dwelling microbes are well-known producers of antimicrobials that are exploited to outcompete microbial co-inhabitants in the soil. Plant pathogenic microbes similarly secrete a diversity of molecules into their environment for niche establishment.
Upon plant colonization, microbial pathogens secrete so-called effector proteins that promote disease development. While such effectors are typically considered to exclusively act through direct host manipulation, for instance through the suppression of host immune responses, increasing evidence demonstrates that pathogenic fungi exploit effector proteins with selective antimicrobial properties to promote host colonization through the manipulation of beneficial host microbiota. Given that effector-mediated microbiota manipulation may have evolved in fungal ancestors that encountered microbial competition before symbiosis with land plants evolved, we propose that effector-mediated microbiota manipulation is fundamental to fungal biology.

Vitamin D, human LL-37 and the antiviral connection

John White

The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25D), signals through the vitamin D receptor (VDR), a member of the nuclear receptor family of ligand-regulated transcription factors. The VDR activates gene transcription by binding to cognate vitamin D response elements (VDREs), located in the regulatory regions of target genes. Several year ago, we identified consensus VDREs in promoter-proximal regions of two genes encoding antimicrobial peptides CAMP (LL-37) and HBD2/DEFB4. Subsequent work revealed that 1,25D was a primary and robust inducer of CAMP expression in multiple cell types, whereas it boosted expression of HBD2/DEFB4 when stimulated by inducers of nuclear factor kappa B (NF-κB),

such as interleukin-1β or the pattern recognition receptor NOD2. Notably, treatment of epithelial or myeloid cells in culture led to secretion of antimicrobial activity into conditioned media. These in vitro results were recapitulated in vivo in human lung airway surface liquid (ASL), where it was shown that antimicrobial activity in ASL varied seasonally with circulating vitamin D metabolite levels, that seasonality could be abrogated by vitamin D supplementation, and that antimicrobial activity could be inhibited with a blocking antibody again LL-37. Recent studies have turned towards investigations of the antiviral activity of 1,25D-regulated AMP expression, spurred on by the observations that infections of a variety of viruses or viral mimetics induce local production of 1,25D and induced CAMP gene expression. After providing an overview of vitamin D metabolism and signaling, I will review the latest findings focused on the role of 1,25D-induced AMPs, particularly LL-37, in attenuation of viral infections.

Synthetic molecular evolution to identify host cell compatible antimicrobial peptides effective against drug-resistant, biofilm-forming bacteria in wounds.

Charles G. Starr, Jenisha Ghimire, **William C. Wimley**
Biochemistry and Molecular Biology, Tulane University School of Medicine
New Orleans, LA 70112

Membrane permeabilizing antimicrobial peptides have not succeeded in the clinic, in part due to impediments that limit their applications in vivo. These include low solubility, residual toxicity, susceptibility to proteolysis, development of resistance, and loss of activity due to binding to host cell, tissue and serum proteins. Further, the almost complete lack of quantitative sequence-structure-function rules prevents rational optimization.We have used synthetic molecular evolution to evolve and optimize peptides that lack these impediments. The lead peptides we have discovered have broad-spectrum sterilizing activity against all ESKAPE pathogens, including biofilm-forming pathogens, both in vitro and in vivo. They are not inhibited by host cells, tissue, or serum proteins and retain activity in the protein- and cell-rich environment of a purulent, infected wound. The lead peptides block biofilm formation in vivo and are highly active against existing biofilms in vitro. The lead peptides are highly active against several independent collections of clinical isolates of drug resistant bacteria. Perhaps most importantly, our lead peptides do not induce the development of resistance in Gram negative bacteria under conditions that drive rapid evolution of resistance to conventional antibiotics. These studies demonstrate the power of synthetic molecular evolution in the continued development of antimicrobial peptides.

GK-11: A Novel Synthetic Cationic Peptide that Targets Biofilm Formation and Disruption

Ayşenur Yazıcı[1,2*], Simay Aldağ[1,2], Serkan Örtücü[1,2], Mesut Taşkın[3]

[1]Erzurum Technical University, Faculty of Science, Molecular Biology and Genetics Department, Erzurum, Turkey.
[2]Erzurum Technical University, High Technology Research and Application Centre (YUTAM), Molecular Microbiology Laboratory, Erzurum, Turkey
[3]Atatürk University, Faculty of Science, Molecular Biology and Genetics Department, Erzurum, Turkey

Biofilms are microorganism communities found in 80% of infections. Bacterial biofilms are 10 to 1000-fold more resistant to antibiotics than planktonic cells. This situation causes a significant health problem as it causes antibiotics to be insufficient during the treatment. Therefore, it is crucial to combat biofilms and to discover antibiofilm agents. This study evaluated the antibiofilm properties of GK-11, which we designed with database filtering technology using antibiofilm peptides registered in the APD3 database. The minimum inhibition value (MIC) of GK-11 was demonstrated in vitro against Staphylococcus aureus (ATCC 43300) and Pseudomonas aeruginosa (PAO1) strains. Then, the antibiofilm activity of GK-11 was demonstrated by crystal violet assay and microscopic approaches. Characterization of synthetic GK-11 was also demonstrated by bioinformatics approaches, fouirer transform infrared (FTIR) and circular dichroism (CD) spectroscopy. While GK-11 inhibited the growth of S. aureus in 64 µg/mL, it inhibited P. aeruginosa in 256 µg/mL. However, it achieved 50% inhibition of biofilm formation at 8 µg/mL and 2 µg/mL, respectively. In addition, 50% biofilm disruption was performed at 64 µg/mL and 32 µg/mL, respectively. We suggest that GK-11, which we have shown to have a helix structure, is a potential antibiofilm peptide and can be improved its structure and effects with different modifications.

Key words: Antibiofilm peptide, GK-11, Staphylococcus aureus, Pseudomonas aeruginosa.

Novel Fejervarin peptides promote acute and chronic skin wound healing

Yanmei Zha, Shangjun Jiang, Ting Zhao, Xiao Jin, **Yingxia Zhang***

Key Laboratory of Tropical Biological Resources of Ministry of Education, School of Pharmaceutical Sciences, Collaborative Innovation Center of One Health, Hainan University, Haikou, China
*Corresponding author, e-mail: zhangyingxia@hainanu.edu.cn

The skin wound healing process is complicated and vulnerable to factors such as accidental injury, microbial infection, skin diseases and metabolic dysfunction. Amphibian skin peptides have great potential in the treatment of acute and chronic skin wounds. We firstly cloned a novel group of bioactive peptides from frog Fejervarya moodiei and F. multistriata, showing no amino acid sequence similarities with known bioactive peptides, which is named as Fejervarin (Fej). Mature Fej-1a, Fej-1b, Fej-1c and Fej-1d are composed of 8 amino acid residues, including 2 or 3 negative amino acid residues, with -1 or -2 net negative charges under physiological conditions. In vitro, the Fej peptides significantly promoted proliferation and migration of RAW264.7, HaCaT and HSF cells assessed by CCK-8, EdU proliferation and scratch assays. In vivo, they exhibited the efficacy on promoting acute and chronic skin wound healing on mouse models of full-thickness skin injury and second-degree deep scald, after the mice were treated for 12 and 21 days, respectively. MASSON and H&E stains showed that in the peptide treated group, fibroblasts were formed; inflammation was relieved and the wounds were completely covered by the newly generated epidermis. IHC, WB and RT-PCR assays revealed that the Fej peptides up-regulated EGF and TGF-β expressions to promote the proliferation and migration of epithelial keratinocytes and skin fibroblasts. Collectively, the Fej peptides are promising multifunctional wound healing peptides to reshape the damaged tissue environment, providing effective alternative candidates for the repair and regeneration of acute and chronic cutaneous wounds.

List of Participants

Raja Aadil Hussain Bhat
Karadeniz Technical University

Alaa Abd Elslam
Suez Canal University

Mohamad Aboelenin
National Research Centre (NRC)

Rajan Adhikari
Biooasis lab

Tanushree Agarwal
University of Calcutta

Naseer Ahmed
Gadjah mada university

Diego Alejandro Falico
University of Antwerp

Fahad Alsaab
George Mason University

Lulu Alsalih
George Mason University

Monica A. Arostica
NBC

Patricia Asensio-Calavia
IPNA-CSIC

Javier Badilla
Pontificia Universidad Catolica de Valparaiso

Piyush Baindara
University of Missouri

Chetana Baliga
Ramaiah University of Applied Sciences

Cássio Barcellos Hervé C Hervé
UFSC

Caire Barreto
Johns Hopkins University

Shawn Bearson
USDA/ARS/National Animal Disease Center

Mugdha Belwalkar
Ramnarain Ruia Autonomous College

Francesca Berini
University of Insubria

Charles L. Bevins
UC Davis, School of Medicine

Madhvi Bhakta
Queen's University of Belfast

Sandip Bhattacharyya
National Institute of Allergy and Infectious Diseases (NIAID), NIH

Ahmer Bin Hafeez
Gdansk University of Technology

Albert Bolatchiev
Stavropol State Medical University

Steven R. Bowers
George Mason University

Guilherme Brand
University of Brasília

Francesco Buonocore
University of Tuscia

Thomas C. Caffrey
University of Nebraska Medical Center

Victor Caliva
Universidad de Buenos Aires

Jorge Canas
Indiana University School of Medicine

Natalia L. Cancelarich
National Council of Scientific and Technical Research - CONICET

Constanza Cardenas
Pontificia Universidad Católica de Valparaíso

Eugene Chang
Knapp Center for Biomedical Discovery

Zhan Chen
University of Michigan

Christina Ching
Nationwide Children's Hospital

Martin Conda
University of Nebraska Medical Center

Lauren Coombe
BC Cancer

Lorissa Corrie
University of Victoria

Matthew Crawford
University of Virginia

Xiangli Dang
Anhui Agricultural University

Mahanama De Zoysa
Chungnam National University

Scott Dean
US Naval Research Laboratory

Berthony Deslouches
University of Pittsburgh School of Public Health

Delphine Destoumieux-Garzon
CNRS

Hymonti Dey
UiT the Arctic University of Norway

List of Participants

Rex A. Dunham
Auburn University

Zizy Z. Elbialy
Aquatic and Fisheries Sciences, Kafrelsheikh University

Nina Espinosa de los Monteros-Silva
Universidad Regional Amazónica IKIAM

José M. Fechine
UFPE

Junxi Feng
Harvard School of Public Health

Renee M. Fleeman
The University of Central Florida

Alessio Fragasso
Stanford University

Seung Gee Lee
OHRI

Yitian Go
Wenzhou University

Adrian Gombart
Linus Pauling Institute, Oregon State University

Beatriz Gonzalez
The Spanish National Research Council

Sergio González Acosta
Intituto de Productos Naturales y Agrobiología

Sudipti Gupta
Nationwide Childrens Hospital

Marlies V. Hager
Zoetis

Bob Hancock
University of British Columbia

Evan Haney
Asep Medical Inc

Mark A. Hanson
University of Exeter

Islah Hayati
Jambi University

Caren C Helbing
University of Victoria

Pedro Henrique de Oliveira Cardoso
Universidade Católica Dom Bosco

Kristján Hermannsson
University of Iceland

Leslie Hicks
UNC Chapel Hill

Chunxiang Hou
Oklahoma State University

Weiping Huang
University of Illinois at Chicago

En Huang
UAMS

Esther Imperlini
University of Tuscia

Arup Indra
Oregon State University/ Oregon Health & Science University

Gitali Indra
Oregon State University/ Oregon Health & Science University

Alex Irizarry
George Mason University

Christine Jacobs-Wagner
Stanford University

Godwin James
Donald Danforth Plant Science Center

Håvard Jenssen
Roskilde University

Yifan Jia
Henan University of Technology

Haobo Jiang
Oklahoma State University

Dequan Jiang
University of Nebraska Medical Center

Wenqiang Jiao
Henan Academy of Agricultural Science

Arnoldas Kaunietis
Vilnius University

Prakash Kishore Hazam
Academia Sinica

Suresh Korpole
CSIR-Institute of Microbial Technology

Beena Kumari
University of Victoria

Jayaram Lakshmaiah Narayana
DeDayananda Sagar College of Engineering

Bruno Lemaitre
EPFL

Kim Lewis
Northeastern University

Ruifang Li
Henan University of Technology

List of Participants

Lusheng Li
University of Nebraska Medical Center

Bingyun Li
West Virginia University

Bo-Jyun Liao
National Central University

Chia-Wei Lin
National Central University

Chung-Yen Lin
Academia Sinica, Taiwan

Christopher B. Lockhart
George Mason Universit

Arianna B. Lovati
IRCCS Istituto Ortopedico Galeazzi

Kelli Lynch
Stanford University

Tessy T. Maliekal
Rajiv Gandhi Centre for Biotechnology

Santi M. Mandal
Indian Institute of Technology Kharagpur

José Manuel Pérez de la Lastra
Instituto de Productos Naturales y Agrobiología

Mariela M. Marani
IPEEC-CONICET

Florencia María Aimaretti
Universidad Nacional del Litoral

Flavia Marinelli
University of Insubria

Camila Martins
Federal University of Santa Catarina - UFSC

Elena Mascheroni
University of Insubria

Federica Massaro
Università degli Studi della Tuscia

Abraham Mechesso
University of Nebraska Medical Center

Kai Meng
Henan University of Technology

Fantin Mesny
University of Cologne

Biswajit Mishra
The Miriam Hospital

Ryan E. Moreira
University of Illinois Urbana-Champaign

Sundar S. Nagasundarapandian
Konkuk University

Javier Ortiz
Fundacion MEDINA

Andrea Otazo
Instituto de Productos Naturales y Agrobiología (IPNA-CSIC)

Linda Oyama
Queens University Belfast

Vincenzo Pennone
Gruppo San Donato

Karina Pikalyova
University of Strasbourg

Ambika Pokhrel
The Donald Danforth Plant Science Center

Teodor Postolache
University of Maryland School of Medicine

Hajar Pourabtahi
Golestan University of Medical Sciences

Om Prakash
Kansas State University

Sanjay Prasad Selvaraj
Academia sinica, Taiwan

Carolina Proano
Universidad Regional Amazonica Ikiam

Gajendra Raghava
Indraprastha Institute of information technology (IIIT)

Rakesh Rahangdale
Manipal College of Pharmaceutical Sceinces Manipal

Lakshminarayanan Rajamani
Singapore Eye Research Institute

Monika Rangole
Research Center Borstel

Sudipta Ray
Calcutta University

Ben Raymond
University of Exeter

Seyed Reza Pourhosseini
Kermanshah University of Medical Sciences

List of Participants

Álvaro Rietmann
Universidad Nacional del Litoral

Elena Rosini
University of Insubria

Britta E. Rued
University of Illinois Chicago

Ali Salehi
BC Cancer/UBC/Birol Bioinformatics Technology Lab

Nita Salzman
Medical College of Wisconsin

Dilip Shah
Donald Danforth Plant Science Center

Alvaro S. Siano
Universidad Nacional del Litoral

Matt Sobansky
Zoetis

Alexa Sowers
West Virginia University

Klara Stensvåg
Faculty of Biosciences, Fisheries and Economics

Xavier Streety
Vanderbilt University

Gillian Struble
Oklahoma State University

Andy Su
Kansas State University

Yajuan Su
University of Nebraska Medical Center

Mengtao Sun
University of Nebraska Medical Center

Rebecca R. Sweany
U.S. Department of Agriculture

Ethel Tackie-Yarboi
University of California, San Francisco

Nongnuj (Nuch) Tanphaichit
Ottawa Hospital Research Institute

Thomas Tao
Skidmore College

Patrick Tate
New York University

Bart Thomma
University of Cologne

Ruby Tiwari
Donald Danforth Plant Science Center

Svetoslav Todorov
University of Sao Paulo

Hui-Hsu Tsai
National Central University

Monique L. van Hoek
George Mason University

Nora Vazquez-Laslop
University of Illinois at Chicago

Shibiao Wan
University of Nebraska Medical Center

Jun Wang
Institute of Microbiology, Chinese Academy of Science

Xiuqing Wang
Ningxia Medical University

Guangshun Wang
University of Nebraska Medical Center

Jinhai Wang
Auburn University

Michaela Wenzel
Chalmers University of Technology

John H. White
McGill University

William Wimley
Tulane University School of Medicine

Shao-Chi Wu
National Central University

Di Wu
Wenzhou Medical University

Hanyu Xiao
University of Nebraska Medical Center

Jingwei Xie
University of Nebraska Medical Center

Chao Xiong
Oklahoma State University

Changmou Xu
University of Nebraska-Lincoln

Anat Yanai
BC Cancer

Ayşenur Yazıcı
Erzurum Technical University

Haodong Yin
Oklahoma State University

Ahmed E. Yousef
Ohio State University

D Zarena
JNTUA CE

Yingxia Zhang
Hainan University

List of Participants

Weiwei Zhang
University of Nebraska Medical Center

Shuping Zhang
University of Missouri

Shaojie Zhang
Henan University of Technology

Mei Zhou
Queen's University Belfast

Jingyong Zhu
Zhejiang Sci-Tech University