Structure-activity relationship studies of lysine-based -peptide/-peptoid antimicrobial peptidomimetics

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TAKING NATURE’S LEAD IN ANTIMICROBIALS

An international conference held under the auspices of Roskilde University, the University of Copenhagen and the Protein & Peptide Science Group of the Royal Society of Chemistry
Friday August 25

09:15 Welcome & Opening of IMAP 2017
Paul Robert Hansen & Håvard Jessen

Session 1: Structure, Function & Design of AMPs
Co-Chairs: Håvard Jessen, Edwin Veldhuizen
09:25 L1: Engineered Peptide Therapeutics as Next-Generation Medicines
César da la Fuente, MIT
10:00 L2: Small, cysteine-rich and cationic proteins from Penicillium chrysogenum: antimicrobial potential and mode of action
Florentine Marx, Medical University of Innsbruck
10:30 L3: Critical factors that may contribute to the high in vivo efficacy of proline-rich antimicrobial peptides
Ralf Hoffmann, Leipzig University
10:50 Coffee

Session 2: Rapid Communications
Chair: Ralf Hofmann
12:00 R1: Antifungal Activity of NP339: A Novel Antifungal Peptide
Joshy Jacob, Emory University
12:10 L14: Urumin, an amphibian host defense peptide with selective anticancer activity
Neeloffer Mookherjee, University of Manitoba
12:30 R2: In vivo activity of the novel AMP cWFW in Pseudomonas aeruginosa
Zvi Hayouka, Hebrew University of Jerusalem
12:45 Lunch
12:30 L15: The Design and Development of Peptide-Based Anti-Infectives
Steven Cobb, Durham University
14:05 L16: Novel anti-septic polypeptides for the neutralization of pathogen- and damage-associated patterns
Klaus Brandenburg, Brandenburg Antiinfektiva GmbH
14:40 L17: Novexatin® (NP213): A cyclic antifungal peptide in development for the treatment of onychomycosis
Derry Mercer, Novabiotics Ltd.
15:00 Coffee

Session 4: Peptide Biomaterials & Polymers
Co-Chairs: Marc Devocelle, Maren von Köckritz-Blickwede
15:30 L18: Building simple synthetic polymers of membrane-active peptides.
Greg Tew, University of Massachusetts
16:05 L19: Explorations of the self-assembling intestinal peptide human defensin 6
Elizabeth Nolan, MIT
16:40 L20: Synthetic Mimics of Antimicrobial Peptides – New Polymers for the Fight Against Multi-resistant Bacteria and Biofilms
Karen Lienkamp, University of Freiburg
17:15 L21: Self-assembled tripeptide hydrogels: towards minimalist AMPs that can be switched on/off?
Silia Marchesan, University of Trieste
17:35 L22: The Development of Ultrashort Antimicrobial Peptide Nanoparticles with Potent Antimicrobial and Antifibroin Activities against Multidrug Resistant Bacteria.
Ammar Almaayyah, Jordan University of Science & Technology
17:35 Close of Day 2
19:30 Conference Dinner (reserved and pre-paid only)

Session 5: Susceptibility Testing of AMPs
Co-Chairs: César da la Fuente, Deborah O’Neil
09:00 L23: Technical challenges of testing susceptibility of bacterial isolates from human and animals to host defense peptides.
Maren von Köckritz-Blickwede, Stiftung Tierärztliche Hochschule Hannover
09:35 L24: Susceptibility Testing of Proline-rich Antimicrobial Peptides
Daniel Knappe, EnBiotix GmbH
10:10 L25: Testing the susceptibility of planktonic cultures and biofilms towards host defense peptides that potentiate their antimicrobial activity in the presence of metal ions
Alfredo Angeles-Baza, University of Connecticut
10:45 Panel discussion (workshop style)
11:00 Coffee

Session 6: Membrane Interactions, Physical Studies & Immunomodulatory Aspects of AMPs
Co-Chairs: Alfredo Angeles-Baza, Steven Cobb
11:30 L26: Toward the comprehension of the mechanism of action of antimicrobial peptides: studies of the interactions of peptides with bacterial cells.
Alessandra Romanelli, University of Naples
11:50 L27: Stored membrane curvature energy modulates the synergistic activity of magainin peptides.
Karl Lohner, University of Graz
12:10 L28: Interspecies cationic peptide downregulation reveals divergence in antimicrobial activity, TLR modulation, chemokine induction and regulation of phagocytosis
Maaike Scheentra, Utrecht University
12:30 Presentation of Prizes
12:45 Close of Meeting

The IMAP series began in 2008 in Leipzig and continued in 2012, also in Leipzig. In 2013 and 2015 the meetings were held in London and in 2014 took place in Graz. In 2016 the meeting returned to Leipzig.

The Organising Committee would like to welcome you all to the 7th in the series as IMAP moves to Copenhagen. We look forward to many stimulating talks and posters as well as fruitful discussions and future collaborations.

Organising Committee: Paul Robert Hansen, University of Copenhagen (Co-Chair); Kai Hilpert, St. George's, University of London; Ralf Hoffmann, Leipzig University; Håvard Jessen, Roskilde University (Co-Chair); Karl Lohner, University of Graz; Alessandra Romanelli, University of Naples; Edwin Veldhuizen, Utrecht University.
L1: Engineered Peptide Therapeutics as Next-Generation Medicines

César de la Fuente-Borja

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Peptides hold great promise as drugs. Their primary amino acid sequences can be tuned to achieve specific biological functions in living cells. Their vast combinatorial space (20^n, unparalled by other classes of compounds) allows for the rapid screening of large numbers of sequences. Furthermore, biological specificity can be incorporated into peptide sequences. In this talk, I will discuss our work in engineering a family of naturally occurring peptides, called antimicrobial peptides (AMPs). I will introduce a multidisciplinary approach that combines bio-inspired peptide design, synthetic and computational biology, and animal models, to adapt the peptides to the unique environment of bacterial pathogens, which can eradicate biofilms and difficult-to-treat bacterial infections. These synthetic peptides kill bacteria directly, and do not target the innate immune response of the host. In addition, the sequential peptidization that accompanies conventional antibiotics to combat biofilms. I will also discuss recent efforts to design peptides for precision microbial engineering to combat infectious diseases and antibiotic resistance as well as my overarching vision of generating a Peptide Encyclopedia designed to treat every medically relevant microbe. Finally, I will present novel protein based therapies that are derived from synthetic peptides, in the development of novel antibiotics, peptide discovery, and the evolutionary design of peptides. In summary, I will discuss multiple approaches to exploiting the untapped potential of engineered AMPs.

L2: Two small, cysteine-rich and cationic proteins from Penicillium chrysogenum: antimicrobial potential and mode of action

Anna Huber1, Cézard P. Sonnerdégere2, György Váradi3, Gyula Batta3, László Galgócz4, Florentine Marx5

1. Institute of Biotechnology, University of Debrecen, Debrecen, Hungary; 2. Department of Organic Chemistry, Faculty of Science and Technology, Babeș-Bolyai University, Cluj-Napoca, Romania; 3. Institute of Biochemistry, Department of Chemistry and Biotechnology, University of Leipzig, Leipzig, Germany

In recent years, the novel antimicrobial peptides (PrAMPs), which are identified as cyclized small peptides with antimicrobial activity, might find wide application in medicine, agriculture and food preservation. The mould Penicillium chrysogenum (rDNA Q176) is the ancestor of all industrial penicillin-producing strains and is recognized as a “safe organism” by the US Food and Drug Administration. Here, we present new insights into the discovery and the mode of action of two secreted antimicrobial proteins from P. chrysogenum (Q176): the extensively studied natAP1 [1-3] and the novel, currently characterized nPrAMP1.

These bio-molecules are small, cysteine-rich and cationic. PAF and nPrAMP1 inhibit the growth of opportunistic pathogenic human parasites, such as Balamuthia mandrillaris, Trichomonas vaginalis, Trypanosoma brucei, but lack anti-bacterial activity. The toxicity of both antimicrobial proteins is closely linked to their energy-dependent uptake into vacuoles and their subsequent release into the cell cytosol.[1,2] Although they exhibit a very similar and highly stable β-sheet structure their species specificity and killing dynamics differ, which let us assume that the mechanistic function is different. To investigate in much more detail the structure of the proteins, we established a Penicillium chrysogenum-based expression system for the bulk production of cysteine-rich, anti-fungal proteins that vary in their primary structure [4]. The spontaneously occurring dimerisation and the acetylation of the proteins that regulate the interaction of PAF with the fungal cell [4,5]. Furthermore, a short, synthetic peptide that spans one distinct motif is antimicrobial active. Finally, we applied rational design to produce PAF variants and synthesized modified peptides with increased positive net charge and lower hydropathy, which significantly improved their antimicrobial efficacy.[6,7]

L3: Critical factors that may contribute to the high in vivo efficacy of proline-rich antimicrobial peptides

Liliya Horswill, Keira Schnitzler, Ennina A. Hiss, Luzia Albrecht, Jörn L. Reymond

1. Department of Biotechnology and Biomedicine, Swiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Zurich, Switzerland; 2. leipzig, Germany

Antimicrobial peptides (ACPs) provide an opportunity in the fight against cancer. Following the observation of the oxytocic activity of aminoglycoside antibiotics in the early 1990s, hundreds of anticancer peptides have been identified and collected in specialized databases.[1] Using this a priori knowledge and a support vector machine classifier model that allowed to distinguish between anticancer active and inactive peptides, this model was used to analyse three different peptide libraries which were generated in silico:[2] (i) amphipathic peptides with varying hydrophobic arcs, (ii) peptides with a hydrophobic gradient along their sequence, and (iii) peptides with the amino acid composition of known allophycocyanins. Several peptides from each library predicted to be active or inactive, respectively, were synthesized and tested against two different cancer cell lines. 10 out of the 12 predictions turned out to be correct. This model showed a promising potential to improve the selectivity of the most active peptide.[3]

L4: Chemical space guided discovery and optimization of topologically diverse antimicrobial peptides against Pseudomonas aeruginosa and its biofilms

Ivan Dibonaventura, Xin Jin, Daniel Protib, Thissa N. Siriwardena, Ricardo Visni, Bee-Han Gan, Sacha Javari, Tam Darby

Department of Chemistry and Biotechnology, Swiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Zurich, Switzerland.

A chemical space is a multi-dimensional virtual space in which all molecules correspond to a point (molecular fingerprint). Chemical space is a well-known concept in the area of drug discovery. Chemical space dimensions correspond to different numerical descriptors of molecular structures. A chemical space is a multi-dimensional virtual space in which all molecules correspond to a point (molecular fingerprint). This model was used to analyse three different peptide libraries which were generated in silico:[2] (i) amphipathic peptides with varying hydrophobic arcs, (ii) peptides with a hydrophobic gradient along their sequence, and (iii) peptides with the amino acid composition of known allophycocyanins. Several peptides from each library predicted to be active or inactive, respectively, were synthesized and tested against two different cancer cell lines. 10 out of the 12 predictions turned out to be correct. This model showed a promising potential to improve the selectivity of the most active peptide [3].

L5: In silico adaptive design of peptides with selective anticancer activity

Gisela Gabernet, Damian Gautsch, Alex T. Müller, Jan A. Hiss, and Gisbert Schneider

1. Swiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Zurich, Switzerland.

Anticancer peptides (ACPs) provide an opportunity in the fight against cancer. Following the observation of the oxytocic activity of aminoglycoside antibiotics in the early 1990s, hundreds of anticancer peptides have been identified and collected in specialized databases [1]. Using this a priori knowledge and a support vector machine classifier model that allowed to distinguish between anticancer active and inactive peptides, this model was used to analyse three different peptide libraries which were generated in silico [2]: (i) amphipathic peptides with varying hydrophobic arcs, (ii) peptides with a hydrophobic gradient along their sequence, and (iii) peptides with the amino acid composition of known allophycocyanins. Several peptides from each library predicted to be active or inactive, respectively, were synthesized and tested against two different cancer cell lines. 10 out of the 12 predictions turned out to be correct. This model showed a promising potential to improve the selectivity of the most active peptide [3].

L6: Beyond membrane permeabilization: Novel mode of action for a membrane targeting antimicrobial peptide cWFV

Henrik Strahl

1. Department of Cell and Molecular Biosciences, Newcastle University, UK

Due to the easy accessibility for extracellular agents, the bacterial cytoplasmic membrane is a major target for antimicrobials such as membrane-active peptides. These natural or synthetic antimicrobial peptides represent a largely untapped reservoir of potentially promissory candidates for the development of new antibiotics. Initially perceived to primarily trigger segregation of peripheral and integral membrane proteins, more recent work has revealed mechanisms, i.e., transporter-mediated peptide uptake and the underlying PrAMP-specific mode of action, synergistic activities, fast bacterial uptake, and a long lasting post-antibiotic effect [4, 5]. The first step of the main PR-AMPs killing mechanism involves the selective uptake of peptides and this triggers pore formation. Solution NMR studies revealed that nisin binds lipid II in a so-called pyrophosphate cage [1] and this high-resolution structure of complex is used for the prediction of the antibiotic activity. The results of the present study will provide proof-of-concept for the applicability of active membrane targeting AMPs for clinical use [2].

L7: Mammalian proline-rich AMPs inhibiting protein synthesis in the ribosome and preventing the elongation phase

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After decades of antibiotic resistant pathogens, proline-rich antimicrobial peptides (Pr-AMPs) emerged as good candidates for the development of new antibiotics due to their high antimicrobial potency and clinical translatability. Over the last years and new efforts have been put to finely characterize their targets and mode of action. The first step of the main Pr-AMPs killing mechanism involves the selective uptake of peptides and this triggers pore formation. Solution NMR studies revealed that nisin binds lipid II in a so-called pyrophosphate cage [1] and this high-resolution structure of complex is used for the prediction of the antibiotic activity. The results of the present study will provide proof-of-concept for the applicability of active membrane targeting AMPs for clinical use [2].


Physiological relevant state. Here we present a cutting-edge solid-state NMR study in native-like lipid membranes that enables, for the first time, to study the nisin-Lipid II pore at high resolution. We unambiguously demonstrate that the nisin pore structure in native-like membranes media strongly diverges from hitherto published data (Figure 1). We present substantial progress towards the determination of the nisin-Lipid II pore, including the nisin structure and the nisin – Lipid II interface.

Moreover, we present a novel ultra-sensitive solid-state NMR approach that enabled us to investigate the nisin pore structure directly in native cellular bacterial membranes. Our results clearly show that the nisin pore gets modulated by the cellular bacterial environment. Altogether, our approach enables a straightforward structural analysis of AMP-Lipid II interactions in physiological and even truly native conditions, which we expect to be critical for rational design.

For millions of years, the first line of defence against infections in multicellular organisms has relied on Antimicrobial Peptides (AMPs). These cationic and amphiphilic sequences represent promising leads for the development of antibiotics delaying the emergence of resistance in bacteria. However, their clinical applications have often been limited by an inadequate margin of safety [1].

A prodrug approach can overcome a toxicity barrier in drug delivery. AMP prodrugs can be generated by transiently reducing or annealing their net positive charges, through modification with a negative promoiety which can be reversed by an enzyme (bacterial or human) confined to sites of infection. For example, neutrophil elastase (NE), a human protease involved in chronic airway inflammation and infections associated with cystic fibrosis (CF), can restore the cationic property of AMPs modified with oligo-glutamate promoieties. Consequently, their bactericidal activities against the CF pathogen *Pseudomonas aeruginosa* are restored by NE in CF bronchoalveolar lavage fluids. The potential of this prodrug approach in reducing the in vivo toxicity of AMPs was demonstrated in a murine model of lung delivery [2]. Additionally, an in vitro nebulisation study performed with a vibrating mesh nebuliser showed that a high level of dosing in the lung can be achieved for AMP prodrugs.

the cellular infiltration there was no significant increase or decrease in bacterial load in the lungs and spleen or the cytokines IL-6 and KC.

In conclusion Hc-Cath attenuates inflammation in a mouse model of LPS induced acute lung inflammation. Furthermore Hc-Cath reduces cellular infiltration into the lungs in a mouse model of pulmonary infection without increasing susceptibility to bacterial spread.

L12: Immunomodulatory functions and therapeutic potential of cationic host defense peptides in chronic inflammatory disease.
Hadeesa Piyadasa1,2, Anthony Altiere3, Mahadevappa Hemeshkhar1,2, Ka-Yee Choi3,4 and Neeloffer Mohorker1,2
1 Department of Immunology, 2 Manitoba Centre for Proteomics and Systems Biology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada. neeloffer.mohorker@umanitoba.ca
Cationic host defense (antimicrobial) peptides are immunomodulatory molecules that contribute to resolution of infections and immune homeostasis. Synthetic derivatives of these natural peptides are known as innate defense regulator (IDR) peptides. Exogenous administration of IDR peptides can confer protection and control inflammation in a variety of infection models [1]. We are examining the effects of exogenous administration of IDR peptides in chronic inflammatory diseases such as rheumatoid arthritis [2] and asthma. We have demonstrated that administration of specific IDR peptides derived from cathelicidins can control the disease process in murine models of arthritis and asthma. This talk will focus on our recent findings in asthma, and the mechanisms underlying related immunomodulatory functions of IDR peptides. We have shown that a specific IDR peptide significantly improves airway hyper-responsiveness (breathing capacity) and infiltration of inflammatory cells in the lungs, in a murine model of asthma. Transcriptomic analyses revealed that the peptide suppresses the expression of several candidates within the inflammatory network activated in the lungs. Interrogation of mechanisms underlying regulation of inflammation demonstrated that IDR peptides suppress the production of critical chronic inflammatory cytokines such as IL-33 in bronchial epithelial cells (in murine lung tissue and in human bronchial epithelial cells). We have also shown that the anti-inflammatory function of IDR peptides is mediated by the activation of the dual phosphatase MKP-1. Our studies provide the foundation for the development of IDR peptides as immunomodulatory therapy for chronic inflammatory diseases. The advantage of IDR peptide-based therapy is the ability to control inflammation without compromising resolution of infections.


L13: The preventative and therapeutic potential of cathelicidins in respiratory viral infections
Silke M. Currie1, Emily Goyer Findlay2, Amanda J. McFarlane1, Paul M. Fitch1, Bettina Böttcher1, Nick Colegrave1, Allan Paras1, Agnieszka Jozwik3, Donald J. Davidson1
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Respiratory syncytial virus (RSV) is a leading cause of respiratory tract infection in infants, causing significant morbidity and mortality. No vaccine or specific, effective treatment is currently available. A more complete understanding of the key components of effective host response to RSV, and other pulmonary viruses, and novel preventative and therapeutic interventions, are urgently required.

Cathelicidins are host defence peptides, expressed in the inflamed lung, with key microbicidal and modulatory roles in innate host defence against infection. Here we demonstrate that the human cathelicidin LL-37 mediates an antiviral effect on RSV and influenza. LL-37 directly damages the viral envelope of RSV, disrupting particles and decreasing virus binding to, and infection of, epithelial cells. Delivery of exogenous LL-37 is protective in vivo in murine models of pulmonary RSV and influenza infection, demonstrating maximal efficacy when applied concomitantly with virus. Furthermore, endogenous murine cathelicidin, induced by infection, has a fundamental role in protection against disease following infection with RSV. Finally, higher nasal levels of LL-37 are associated with protection in a healthy human RSV infection model.

These data lead us to propose that cathelicidins are a key, non-redundant component of host defence against pulmonary viral infections; functioning as a first point of contact “antiviral shield”, and having additional later phase roles in minimising the severity of disease outcome. Consequently, cathelicidins represent an indiscernible target for preventative strategies against infection and may inform the design of novel therapeutic analogues for use in established infection.

L14: Urumin, an amphibian host defense peptide is virucidal H1 influenza viruses
David Holthausen, Song Hye Lee and Joshy Jacobs
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Influenza is the most common recurring human respiratory virus. Worldwide there are 3.5-5 million cases of severe influenza infection annually, which affects 5-10% of adults and 20-30% of children and results in 250-500 thousand deaths. Current vaccination strategies do offer protection, but pandemic outbreaks occur unexpectedly, limiting our ability to develop vaccines in a timely manner. Additionally, for seasonal influenza viruses, mismatches frequently occur between the vaccine and the circulating strains. In addition, the emergence of drug-resistant influenza viruses is also a major concern; currently, only neuraminidase inhibitors are recommended, as adamantane-resistant influenza strains have become widespread. Thus, there is a pressing need to develop new antivirals against influenza viruses. Antimicrobial host defense peptides are an ancient, evolutionarily conserved arm of the innate immune system that exists in all living organisms and confers protection to the host. These peptides have evolved to protect the host against a wide variety of pathogens. Here we screened peptides derived from the skin of the South Indian frog, *Hydrophylax bahuvistara* for anti-viral activity against influenza A viruses. We present a novel host defense frog peptide that we named Urumin, which is virucidal for H1 hemagglutinin-bearing human influenza A viruses. Furthermore, we show that the peptide can protect naïve mice from lethal influenza virus infection. This peptide specifically targets the conserved stalk region of H1 hemagglutinin and is also effective against even drug-resistant H1 influenza viruses. In conclusion, Urumin peptide represents a novel class of anti-influenza virucide that targets hemagglutinin.

L15: The Design and Development of Peptoid-Based Anti-Infectives
Steven Cobbe
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Protozoan parasites represent a severe threat to global human health as they are responsible for infection in Malaria, and, a range of Neglected Tropical Diseases (NTDs) including Chagas disease, leishmaniasis and African sleeping sickness. Often treatments for NTDs are limited in their efficacy and drug resistance is an emerging problem. The current efforts to develop new treatments for NTDs have met with limited success and as such novel compound classes for development are actively being sought. Peptides are peptidomimetics that have showed promise as antibacterial agents but their application in the field of NTDs is highly underdeveloped. As part of an ongoing project within the group to develop new agents for the treatment of NTDs, we have designed and prepared peptoids containing a wide variety of chemical functionalities and evaluated their activity against a range of protozoan targets. From our studies in this area we have identified several peptides that have potent anti-parasitic activity and good selectivity indices (SI). For example, the cationic linear peptoid [NamyNspeNspe]2 had an IC50 of 0.089 µg/mL against Plasmodium falciparum and a SI > 100. Our related work towards the development...
of novel peptide-based antimicrobials (e.g. targeting mixed species biofilms) will also be discussed.

Novexatin (NP213) is currently the subject of a large, multicentre Phase IIb clinical trial as a topical therapy for treatment of mild to moderate onychomycosis, with results expected to be released in early 2018.

**Session 4: Peptide Biomaterials, Polymers and Membrane Interactions of AMPs**

**L18: Building simple synthetic polymers of membrane-active peptides.**

Gregory N. Tew

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Our primary aim is to create new materials using a combination of principles, many of which are inspired by biology. Using both defined model lipid oligomers and easier to produce, but heterogenous, polymers, it has been possible to generate scaffolds with biological potency superior to the natural analogues. We will illustrate some of these new materials and medical applications, and discuss on conformationally flexible backbonesthat are antifungal. These synthetic polymers mimic the essential physicochemical properties of natural human host defense peptides, like Magainin and Defensin. We will discuss our newest results in which we have successfully mimicked that biological activity of protein transduction domains like HIV-TAT. The versatility of these synthetic mimics provides the opportunity to discover analogues with superior properties compared to their native sequences. In general, these molecules interact directly with the lipid membrane generating new curvature. This has direct analogs to pores in biological membranes, a central feature of biology that has attracted considerable interest for decades. These endeavours represent one specific example of how synthetic systems of biological molecules interact with and manipulate the plasma membrane. Modern biophysical assays interrogate the interplay between the synthetic scaffold and lipid composition leading to negative Gaussian curvature, a requirement for pore formation. The complexity of this interplay between lipids, bilayer components, and the scaffolds remains to be better resolved, but significant progress has been provided. It has become clear that the combination of molecular design, biophysical models, and biological evaluation provide a robust approach to novel protein mimetics.

**L19: Explorations of the self-assembling intestinal peptide human defense 6 Elizabeth M. Bailey

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Onychomycosis (nail fungus) is an infection of the nail unit caused by dermatophytes, yeasts, or non-dermatophyte molds that affects ~10% of the general population and is a serious problem in the diabetic population. Onychomycosis is difficult to treat, with ~20 – 25% of patients not responding to treatment and the recurrence rate after apparently successful treatment is high (20 – 50%). Systemic therapies are generally more effective than topical therapies, but their use can be limited by toxicity, drug interactions and other side effects. Patients often prefer and request topical treatment.

NP213 is a novel antifungal peptide, designed and specified specifically to penetrate the nail, a notoriously difficult tissue for antifungal agents to access. NP213 demonstrated significant ex vivo and in vivo efficacy against dermatophytes. NP213 does not bind to keratin and is minimally impacted by protease activity. Activity of NP213 is retained within the infected nail. TEM demonstrates that NP213 accumulates within the parakeratotic stratum (parakeratotic ex vivo nail infection model). Dermatophytes did not develop in vitro resistance to NP213. The rapid fungicidal activity of NP213 predicts fast clinical response, which is supported by clinical and preclinical data demonstrating resolution of infection following daily administration of NP213 for only 28 days, unlike the 24 weeks treatment normally associated with topical treatments.

In addition, it sustained the delivery of poorly soluble ciprofloxacin by means of co-assembly in the nanostructured hydrogel [3].

**L20: Synthetic Mimics of Antimicrobial Peptides (SMAPs) – New Polymers for the Fight Against Multi-resistant Bacteria and Biofilms**

E. Likens-king and A. Al-Hajjami

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Bacterial contamination of catheters and other medical devices is a serious issue. A new class of US FDA approved biocompatible antibiotics form biofilms on surfaces. These cause life-threatening infections. Additionally, bacterial resistance against traditional antibiotics is continuously increasing, particularly in novel antibiotic resistant biofilms. Indeed, more than hundred thousand people worldwide die from infections related to biofilm-associated infections. In this context, antimicrobials as well as peptides are of crucial importance. Antimicrobial peptides can be used in two ways: at low molecular weights, they mimic the properties of natural antimicrobial peptides (AMPs), and at high concentrations, like in membranes [1]. As polymer coatings, they make medical devices contract against bacteria and slow down biofilm formation [2].

We here present our current research in both fields. We present polypeptide-based Synthetic Mimics of Antimicrobial Peptides (SMAPs) [3,4], their mechanism of action as a drug, [1,4] and how it is possible to turn them into biocompatible-materials [2]. We synthesized SMAPs with precisely tuned antimicrobial activity and cell-surface-activity; and could show that their mode of action is similar to AMPs, which are known to cause only very little bacterial resistance [5]. This promising candidates for drug applications.

Additionally, surface-attached SMAPs were shown to selectively kill bacteria without harming human cells, and are thus interesting coating material for medical applications. We also found concentration limits of these coatings (adhesion of the debris of dead bacteria) and present our most recent solutions for this issue (self-regenerating coatings and bifunctional polymers).


**L21: The Development of Ultrashort Antimicrobial Peptide Nanoparticles with Potent Antimicrobial and Antifouling Activities against Multidrug Resistant Bacteria.**

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Conventional antibiotics are facing strong microbial resistance that has recently reached unprecedented critical levels. This situation is leading to a significant limitation in the use of antimicrobial agents currently available in the clinic. Antimicrobial peptides (AMPs) could provide the medical community with an alternative strategy to traditional antibiotics for combating microbial resistance. AMPs consist of an enormous group of molecules that display efficient and potent antimicrobial activities against wild type and multidrug resistant bacteria. However, the development of AMPs into clinical use is hampered by their relatively low stability, toxicity and high manufacturing costs. Current research efforts are focusing on designing and developing technologies that might reduce the toxicity of AMPs and retaining their potent antimicrobial activity and possibly enhance their delivery. In this study, a novel in house designed potent ultrashort antimicrobial peptide nanoparticles (UCP-NPs) has been synthesised by immobilising antifouling bioactive synthetic micellar nanoparticles (CS-NPs) based on the ionotropic gelation method. The encapsulation efficacy reported for (RBRBR) into CS-NPs was 97.5%. The UCP-NPs was successfully prepared by mixing the RBRBR from the nano-carrier exhibited slow release followed by a progressive linear release for 14 days. The antibacterial kinetics of RCP-NPs against multidrug resistant Staphylococcus aureus for 4 days, and the developed RBRBR-CS-NPs exhibited 3-log decrease in the number of colonies when compared to CS-NP and 5-log decrease when compared to control bacteria. The encapsulation...
peptide nanoparticle formulation managed to limit the toxicity of the free peptide against both mammalian cells and human erythrocytes. Additionally, the peptide nanoparticles showed inhibition of biofilm formation when tested against biofilm forming bacteria. Loaded USAMP into CS-NPs could represent an innovative approach to develop delivery systems based on AMPs, preserving potent antimicrobial effects against multi-resistant biofilm forming bacteria with negligible systemic toxicity and reduced synthetic costs that are obstructing the clinical development of AMPs generally.

Session 5: Susceptibility Testing of AMPs

L23: Technical challenges of testing susceptibility of bacterial isolates from human and animals to host defense peptides.

Melissa N. Langer1, Marita Meurer2, Stefanie Blodkamp3, Martin Beyrach3, Andrea Feller1, Nicole de Buhr3, Thomas Gunzburger1, Lothar Kreienbrock1, Stefan Schwarz2, Marvin von Knoblick-Wedekind2

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Antimicrobial susceptibility testing by determination of minimal inhibitory concentrations (MICs) values is a standard diagnostic tool in clinical microbiology. We report on the information how susceptible or how resistant a bacterium is to a specific antimicrobial agent. To determine the MIC distribution for specific antimicrobial agents among bacteria of a certain species, a number of unrelated clinical field isolates is commonly tested for their MICs. For host defense peptides/antimicrobial peptides (AMPs), susceptibility testing is currently not feasible as critical tests are not performed.

In this study, we have statistically compared AMP microdilution assays for their homogeneity of variances when comparing the MIC values of two bovine cathelicidins in BAPM-27 and BAPM-28 for 55 different E. coli strains and for 111 different S. aureus or E. coli isolates. Importantly, the results revealed a high peptide-specific inhomogeneity of MIC values in case of S. aureus, but not for E. coli. Secondly, polypolyene plates were compared with polystyrene plates regarding their homogenity of variances and Student’s t-test for comparison of the means of both polymers. Interestingly, also by increasing the rate of killing [1]. We have also been interested in a family of AMPs containing a copper binding motif, known as the ATCUM motif. In the tick Ixodes scapularis, the ATCUM motif present in the HDP lusus has been shown to be important to its antimicrobial role [2]. Whether copper binding is a major part of the antimicrobial activity of the more than 60 ATCUM-containing AMPs remains to be determined. To advance this research, our group has modified susceptibility testing to address whether copper inhibition can be described for their immune-mediatory function. Many of these findings have been either described solely in the context of the human LL-37 and/or murine HDPs. Thus, most studies evaluated varying copper concentrations. The current study examines 12 different cathelicidins, selected from 6 different microbial species for their antimicrobial activity and immunomodulatory capacity as well as their in vitro stability. This allows for a better understanding of the conservation of cathelicidin functions.

For example, the ATCUS cathelicidin 3 in C. albicans is strongly inhibited for its activites against a large number of microorganisms, including some emerging pathogens. However, the synthetic antimicrobial activity of NP339 against C. albicans was found to be variable.

NP339 demonstrates the potential as a novel and effective fungicidal therapeutic candidate with activity against Mucorales, including Rhizomucor and Rhytisma. The MIC for NP339 against the latter was 2 µg/ml, indicating its potential for use in the treatment of fungal infections, including phagocytosis.

The results of our study indicate that NP339 is a potential therapeutic agent for the treatment of fungal infections, including phagocytosis.

R2: Inflammatory responses mediated by cytoplasmic LPS are suppressed by anti-endotoxin peptides

Ana Pfälzler, Lena Heinebrock1, Qi Su2, Klaus Brandenburg1, Lothar Kreienbrock1

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 Toll-like receptor (TLR) 4-independent recognition of intracellular lipopolysaccharides (LPS) by inflammatory caspases leads to pyroptotic cell death and cell death. The novel mechanism of caspase-1 activation by LPS is considered as ‘cytoplasmic LPS’.

The results showed that the synthetic anti-endotoxin peptide Pep19-2.5 efficiently suppresses the pro-inflammatory activities of immune cells and reduces the expression of pro-inflammatory cytokines.

A PORTION OF THE TEXT HAS BEEN SPOKEN OUT TO ENSURE THAT THE INFORMATION IS ACCURATE. THE TEXT CONTINUES AS FOLLOWS:

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strongly reduced IL-1β and LDH secretion triggered by intracellular LPS. In contrast, the TLR4 signaling inhibitor TAK-242 abrogated LPS-induced GSK3β and NF-κB activities in THP-1 monocytes, Pep29-2.5 further suppressed LPS-induced HMGB1 production and caspase-1 activation. Consistent with this observation, we found that impaired R7 and IL-1 receptor signaling in primary monocytes stimulated with LPS and reduced LDH release and IL-1β and IL-1α expression in LPS-transfected HaCat keratinocytes in the presence of Pep29-2.5. Additionally, Pep29-2.5 completely abolished IL-1β release induced by LPS/ATP in macrophages via canonical inflammasome activation. Notably, anti-endotoxin peptides reduced IL-1β and LDH secretion induced by LPS in a dose-dependent manner with no apparent effects on LPS access to the cytosol under physiological conditions. In conclusion, we provide evidence that anti-endotoxin peptides inhibit the inflammasome activation by inducing pro-inflammatory LPS sensing in myeloid cells and keratinocytes and activation of the classical inflammasome by LPS-ATP which may contribute to the protection against bacterial cells and keratinocytes and activation of the classical inflammasome by LPS-ATP induced inflammatory effect.

Over 60% of all nosocomial infections are due to the presence of biofilms on medical devices, such as catheters, which cause infections and can lead to sepsis. Cathelicidins are a class of innate immune peptides that are produced by the innate immune system and have a broad range of antimicrobial activities due to their ability to form pores in the bacterial membrane, leading to cell death. Cathelicidins are known for their anti-biofilm properties and have been shown to be effective against a wide range of pathogens, including Pseudomonas aeruginosa, which is a major cause of nosocomial infections, especially in patients with cystic fibrosis.

In this study, we investigated the anti-biofilm activity of a library of novel cathelicidins synthesized on resin. We determined the minimal inhibitory concentration (MIC) of different cathelicidins against Pseudomonas aeruginosa ATCC 27853 and evaluated their ability to disrupt pre-formed biofilms. Our results showed that some cathelicidins were able to inhibit biofilm formation and disrupt pre-formed biofilms, suggesting that cathelicidins have potential for the treatment of infections caused by Pseudomonas aeruginosa.

References:

P4: Characterisation of novel antimicrobial peptides targeting gram negative bacteria

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Over the last century, the discovery of antimicrobial peptides (AMPs) has revolutionized the field of infectious diseases. AMPs are endogenous, small peptides that are produced by the innate immune system and have a broad spectrum of activities, including anti-bacterial, anti-viral, and anti-fungal effects. They are particularly promising for the treatment of infections caused by Gram-negative bacteria, which are resistant to many conventional antibiotics.

In this study, we investigated the anti-bacterial activity of a library of novel synthetic antimicrobial peptides (AMPs) against a panel of Gram-negative bacteria, including Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. Our results showed that some AMPs were able to inhibit the growth of these bacteria and disrupt pre-formed biofilms, suggesting that AMPs have potential for the treatment of infections caused by Gram-negative bacteria.

References:
Antimicrobial peptides (AMPs) are key components of the innate immune repertoire of animals and plants, providing broad and rapid protection against invading pathogens.

Despite their importance, the genetic and functional diversity of avian b-defensins (AvBDs) is poorly understood. In mollusks [Anas platyrhynchos], evolutionary analyses have shown that the majority of AvBD genes are under strong purifying selection, with a single predominant allele per gene, whereas other genes are undergoing balancing selection, resulting in higher diversity. In cephalopods, no antimicrobial peptide (AMP) has been identified to date.

In cephalopods, no antimicrobial peptide (AMP) has been identified to date. With an original approach, we designed and synthesized a library of peptides using computational tools, we identified potential antibacterial sequences. Ten peptides from the library were further confirmed with a high-throughput assay.

In the cuttlefish Sepia officinalis, we identified 15 AMPs in the cuttlefish stomach. The purified, mature PAFB effectively inhibited the growth of human pathogenic fungi and bacteria.

The peptides exhibit a variety of modes of action, including killing bacteria and fungi, as well as interfering with biofilm formation. The peptides show promise for the development of novel AMPs for use in agriculture, medicine, and biotechnology.

Overall, these results suggest the potential utility of antimicrobial peptides and their derivatives in the eradication of multidrug-resistant bacteria and fungal infections.
Pharmacophore modeling led to the screening of 136 different potential scaffolds and the identification of two candidates, Pγvar and Pγvar'.

Pγvar is a well-known neutrophil-defensive peptide, whereas Pγvar' is a newly discovered fragment.

Pγvar' is found to be up to nine times more potent than Pγvar in vitro.

The study shows the potential of Pγvar' as a novel therapeutic agent for the treatment of chronic infections.

Conclusions:

1. The study highlights the importance of pharmacophore modeling in the discovery of new antimicrobial peptides.
2. Pγvar' shows promising antifungal activity and could be a potential candidate for future development.
3. The findings contribute to the growing body of knowledge on the potential of new antimicrobial peptides for the treatment of drug-resistant infections.

References:


P17: LL-37 fragments have antimicrobial activity against Staphylococcus epidermidis biofilms

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Department of Science and Environment, Roskilde University, Roskilde, Denmark

Staphylococcus epidermidis is a common nosocomial pathogen able to form biofilms, which are sessile communities of cells surrounded by extracellular polymeric substances.

The biofilms are highly resistant to antibiotics and represent a significant challenge in the treatment of infections.

Previous studies have shown that LL-37, a human cationic antimicrobial peptide, has the potential to disrupt the formation and stability of biofilms formed by Staphylococcus epidermidis.

In this study, we investigated the effect of LL-37 fragments on the formation and dispersion of Staphylococcus epidermidis biofilms.

Materials and Methods:

- Biofilm formation was induced on polystyrene plates using an 18-hour culture of Staphylococcus epidermidis.
- LL-37 fragments were synthesized and tested for their ability to disrupt the biofilms.
- The biofilm dispersion was quantified using a crystal violet assay.

Results:

- The LL-37 fragments were able to disrupt the biofilms in a dose-dependent manner.
- The fragments with the highest activity were those containing the acidic residues Glu and Asp.

Conclusions:

- The LL-37 fragments have potential for the treatment of Staphylococcus epidermidis biofilm infections.
- Further studies are needed to evaluate the effect of these fragments on the virulence factors of the bacteria.

References:


P18: Chemical space guided optimization of antimicrobial peptide dendrimer GSK3

Theresa N. Sinwarden, Xin Jin and Jean-Louis Reynaud

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The development of novel antimicrobial peptides is crucial to combat antibiotic-resistant infections.

Antimicrobial peptides (AMPs) are a promising class of materials due to their broad-spectrum activity and low toxicity.

However, the development of AMPs is challenging due to the stochastic nature of their generation and the need for high-throughput screening to identify active compounds.

In this study, we used a chemical space-guided optimization approach to design a novel antimicrobial peptide dendrimer.

Materials and Methods:

- The chemical space of LL-37 was constructed based on a nearest-neighbor search.
- The dendrimer was designed to maximize the overlap with the antimicrobial region of the chemical space.
- The performance of the dendrimer was evaluated using a high-throughput screening assay.

Results:

- The dendrimer showed potent activity against a panel of Gram-positive and Gram-negative bacteria.
- The dendrimer exhibited low toxicity in vitro.

Conclusions:

- The chemical space-guided optimization approach is a promising method for the design of novel antimicrobial peptides.
- Further studies are needed to evaluate the long-term stability and pharmacological properties of the dendrimer.

References:

Antimicrobial peptides (AMPs) represent an efficient part of innate immunity and as antibiotic drugs, not just in triggering unchecked immunomodulatory properties. To create druggable AMPs, NovaBiotics has developed recombinant AMPs derived from engineered peptides including Novarfyn (NP432), a novel antibiotic peptide with potent bactericidal activity against both Gram positive and Gram negative multidrug resistant pathogens.

Both microbiodiversity antimicrobial susceptibility testing was used to assess the efficacy of NP432 against clinical MRSA and drug resistant A. baumannii. The in vitro bactericidal activity was determined by enumeration following exposure to NP432 with either pathogen for up to 24 h. Electron microscopy was conducted according to standard procedures following the killing of 0.4 and A. baumannii to NP432 for 15 min, 30 min or 60 min. Data presented shows NP432 is a rapidly bactericidal compound that kills bacterial cells within 1 h in a range of physiological conditions, outperforming a number of clinically relevant AMPs.

Staphylococcus aureus is a significant human pathogen causing a range of infectious diseases; from skin and soft tissue infections, hospital-acquired infections (HAI) to potentially fatal bacteremia and endocarditis. Nasal carriage of S. aureus and S. epidermidis and as a carrier for antibiotics.

We determined the amount of the released peptide in the bacterial media containing pathogenic bacteria (methicillin-resistant S. aureus, S. epidermidis and A. baumannii). NP432 inhibited bacterial adhesion to peptide-functionalized Ti disks was observed in the case of S. epidermidis, approximately 60% with respect to bare titanium. Additional immobilization experiments are currently underway aimed at increasing peptide surface coverage to further improve antimicrobial efficiency of peptide-functionalized surfaces.

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S. aureus, S. epidermidis and A. baumannii). NP432 inhibited bacterial adhesion to peptide-functionalized Ti disks was observed in the case of S. epidermidis, approximately 60% with respect to bare titanium. Additional immobilization experiments are currently underway aimed at increasing peptide surface coverage to further improve antimicrobial efficiency of peptide-functionalized surfaces.

Thus, it is desirable to functionalize the materials surface to confer antibiofilm and immunomodulatory properties. We used poly(methylmethacrylate)-based bone cement (Palacos®r) which is currently being explored for the fixation of joint replacements and as a carrier for antibiotics.

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Infection of orthopaedic implants is difficult to treat and may lead to implant failure[1]. Staphylococcus aureus and Staphylococcus epidermidis are the most common Gram-positive bacteria causing nosocomial infections. NovaBiotics is currently developing Luminaderm® (NP108), a new antibiotic peptide that shows promising in vitro activity against a variety of hospital-acquired infections. NovaBiotics, First Faculty of Medicine, Charles University in Prague and University Hospital in Motol, Prague, Czech Republic. vajekj@ujf.cas.cz

Antimicrobial efficacy was evaluated by measuring adhesion of S. aureus and S. epidermidis reference strains to Ti samples upon 2-h incubation. Viability of Ti-attached bacteria was determined by colony forming units (CFU) counts after detachment from Ti disks by a vortexing step. A stronger inhibition of bacterial adhesion to peptide-functionalized Ti disks was observed in the case of S. epidermidis, approximately 60% with respect to bare titanium. Additional immobilization experiments are currently underway aimed at increasing peptide surface coverage to further improve antimicrobial efficiency of peptide-functionalized surfaces.

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P31: Antimicrobial peptide-based therapy of antibiotic-resistant infections. Our work provides guidelines for the development of efficient CAP-based antimicrobial therapies against multidrug-resistant infections. Additionally, we observed that PGLA restores antibiotic efficacy against antibiotic-resistant bacteria showing widespread resistance to the antibiotic used in the combination. This was true for antibiotic-resistant strains. Our best candidate was a novel anionic antimicrobial peptide first identified in Chinese giant crab Scylla paramamosain, which is proved to exert a major role in innate immunity defence molecules with broad spectrum and effective against antibiotic resistant pathogens. However, there is little known about resistance to conventional antibiotics that leads to cross-resistance between different CAPs. The mechanism of collateral sensitivity described here provides a basis for the development of efficient CAP-based antimicrobial therapies against multidrug-resistant infections.

P32: Antimicrobial Peptide PGLA Restores Antibiotic Susceptibility and Mitigates Resistance Evolution

Károly Palkó, Balázs Papp, Zsuzsanna Lázár, Viktoria Lázár, Réka Spohn, Bálint Csörgő, Csaba Pál
1. Synthetic and Systemic Biology Unit, Institute of Marine Biology, Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; 2. Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary.

Cationic antimicrobial peptides are short peptides with a broad spectrum of antibacterial activities, and are considered as promising novel alternatives to conventional antibacterial agents. In our previous work we identified peptide PGLA (Pseudomonas aeruginosa, a common clinic opportunistic pathogen that causes chronic lung infections). The PGLA treatment with the recombinant protein significantly exerted resistance to conventional antibiotics, has caused various clinical diseases. It is urgent to develop potent antimicrobial agents to deal with this problem. Antimicrobial peptides (AMPs) are known to be capable of innate immunity defence molecules with broad spectrum and effective antimicrobial activity against microbial pathogens, and considered to be a potential new class of anti-infection agents. However, a cationic, cysteine-rich antimicrobial peptide, has been well studied as a significant regulator of iron metabolism while its antimicrobial mechanism is less focused. In our study, we investigated the antimicrobial activity of AS-hepc3 and its antimicrobial mechanisms against Staphylococcus epidermidis and AML12 were not affected, indicating the cancer specificity of the protein. Furthermore, combined application of SCY2 and its interacting protein showed significant synergistic effect in inhibiting the growth of Hela and T24. These findings suggested that SCY2 in combination with AS-hepc3 may be effective in inhibiting cancer growth and merit further study and evaluation.

P33: A new protein interacting with the antimicrobial peptide SCY2 from Scylla paramamosain induces tumor cell apoptosis

Zhao Tran, Ke Jia, Gergő Fekete, Gábor Grzézal, Gábor Olajos, Tamás A. Martinek, Balázs Papp, Csaba Pál
1. Synthetic and Systemic Biology Unit, Institute of Marine Biology, Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; 2. Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary.

Multidrug-resistant bacterial infections are an emerging global health threat. Thus, there is an urgent need for antimicrobial agents with new mechanisms of action in the fight against multidrug-resistance. Cationic antimicrobial peptides (CAPs) are considered promising novel alternatives to conventional antibiotics. However, little is known about resistance to conventional antibiotics that leads to cross-resistance between different CAPs. We systematically addressed this question by studying the susceptibilities of a comprehensive set of 60 antibiotic resistant Escherichia coli strains towards 24 cationic antimicrobial peptides. Strikingly, antibiotic resistant bacteria frequently showed collateral sensitivity to CAPs, whereas cross-resistance was relatively rare. We identified clinically relevant multidrug-resistance mutations that increase sensitivity to certain CAPs. This pattern of collateral sensitivity arises as a by-product of genomic expression changes that modify the lipopolysaccharide composition of the bacterial outer membrane. This observation surfaces the bacterial chemical, and thereby enhances the killing efficiency of the membrane-interacting CAPs. The mechanism of collateral sensitivity described here provides a basis for the development of efficient CAP-based antimicrobial therapies against multidrug-resistant infections.

P34: Antimicrobial peptides AS-hepc3 and its interacting protein SCY2 are novel antimicrobial agents which induces tumor cell apoptosis

Ke Jia, Gergő Fekete, Gábor Grzézal, Gábor Olajos, Tamás A. Martinek, Balázs Papp, Csaba Pál
1. Synthetic and Systemic Biology Unit, Institute of Marine Biology, Research Centre, Hungarian Academy of Sciences, Szeged, Hungary; 2. Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary.

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### Presenting Author | Poster Number & Title
--- | ---
Sonavane, Yogesh | P19: Molecular Dynamic Simulations of PGLa-H Tandem-repeat Peptides
Sonderegger, Christoph | P20: Design and characterisation of improved antifungal peptides
Spohn, Réka | P31: Antibiotic resistant bacteria show widespread collateral sensitivity to antimicrobial peptides
Torres, Marcelo | P21: Novel wasp venom derived antimicrobial peptides with no hemolytic activity
Veldhuizen, Edwin | P2: Activity of Cathelicidins against Pseudomonas aeruginosa biofilms.
Volejníková, Andrea | P26: Antimicrobial peptides loaded into bone cement prevent bacterial biofilm formation on its surface
Weingarth, Markus | P29: Computational studies on the membrane-binding mode of CSαβ defensins
Yang, Ying | P33: A new protein interacting with the antimicrobial peptide SCY2 from Scylla paramamosain induces tumor cell apoptosis
Zhu, Depeng | P34: Antimicrobial mechanisms of cysteine-rich antimicrobial peptide hepcidin derived from Black Porgy (Acanthopagrus schlegelii)

### Poster Competition
The poster competition is sponsored by the Protein & Peptide Science Group of the Royal Society of Chemistry. There will be a first prize of € 100 and two runner-up prizes of € 50 each. The competition is open to all early-stage researchers (graduate students and post-docs). Please inform the registration desk if you do NOT want to enter the poster competition. The judges will review all eligible posters during the poster session on Friday and draw up a short list. The final selection will be made during the breaks on Saturday and Sunday, so posters should be left in place until the end of the meeting.

### Presentation of Awards
The poster competition prizes will be awarded at the end of the final session on Sunday, just before the close of the meeting.

### Acknowledgements
The organisers are grateful to NovaBiotics Ltd. for their generous financial support of the meeting.

NovaBiotics Ltd is a leading clinical-stage biotechnology company focused on the design and development of first-in-class anti-infectives for difficult-to-treat, medically unmet diseases. The Company’s advanced portfolio of antimicrobial therapeutic candidates targets large and important markets with significant unmet clinical needs, including Lynovex (in oral and inhaled form), an orphan drug candidate for cystic fibrosis (CF), Novexatin, a potential step change therapy for onychomycosis (being co-developed with and out-licensed to Taro Pharmaceuticals) and Novamycin, a novel antifungal peptide in development for the treatment of aspergillosis, candidiasis and cryptococcosis. Novexatin and Novamycin are among the first in class peptide anti-infectives which the company continues to derive from its proprietary antimicrobial peptide rational drug design platform.

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