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New Antimicrobials Project
2nd Workshop
New Compounds & New Strategies for Antimicrobials
University of Trieste, 25-26.5.2012

EUT EDIZIONI UNIVERSITÀ DI TRIESTE

Program

Friday 25th May

Session 1	Chair: *Alessandro Tossi (University of Trieste)*
09.00 – 09.20	*Anna Maria Pirttilä (University of Oulu, NAM project coordinator)* Opening address.
09.20 – 9.50	*Gerald Bills (Fundación MEDINA, Granada)* Endophytic fungal antibiotics: discovery, profiles and mechanisms of action.
9.50 – 10.20	*William M. Shafer (Emory University School of Medicine, Atlanta)* How bacteria modulate their susceptibility to AMPs - implications for anti-infective development and pathogenesis.
10.20 – 10.40	*Mysore V. Tejesvi (University of Oulu, NAM project)* Antimicrobial peptides from endophytes.

Coffee break

Chair: *Anna Maria Pirttilä (University of Oulu)*

11.10 – 11.40	*Delphine Destoumieux (CNRS, Montpellier)* AMPs from marine invertebrates
11.40 – 12.10	*Margherita Sosio (NAICONS scarl, Milan)* From discovery to development of novel antibiotics at NAICONS.
12.10 – 12.30	*Pere Picart (University of Bonn, NAM project)* Piceasin 3 - a new plant defensin isolated from *Picea glauca.*

Lunch

Session 2	Chair: *Maria Luisa Mangoni (University of Rome, La Sapienza)*
14.00 – 14.30	*Ralf Hoffmann (University of Leipzig)* Optimization of insect-derived antimicrobial peptides to treat systemic bacterial infections.
14.30 – 15.00	*Marco Scocchi (University of Trieste, NAM project)* The PRAMP story - towards an understanding of translocation and activities of Proline-rich AMPs.
15.00 – 15.30	*Heike Brötz-Oesterhelt (University of Düsseldorf)* Lipopeptides with novel antibacterial mechanisms or target binding modes.

Coffee break and posters

Session 2 (cont.) Chair: *Margherita Zanetti (University of Udine)*

17.00 – 17.30 *Maria Luisa Mangoni (University of Rome, La Sapienza)* Short membrane-active peptides from amphibian skin to fight microbial pathogens

17.30 – 18.00 *Lidia Feliu (University of Girona)* Antimicrobial peptides for plant protection

Saturday 26th May

Session 3 Chair: *Wiliam M. Shafer (Emory University School of Medicine)*

09.00 – 9.30 *Gregor Anderluh (National Institute of Chemistry Slovenia)* Looking at peptides' and proteins' adventures on membranes using surface plasmon resonance

09.30 – 10.00 *Alessandro Tossi (University of Trieste, NAM project)* Breach or passage - on the multiple modes of antibacterial action of mammalian AMPs

10.00 – 10.30 *Francesca D'Este (University of Udine)* Cathelicidin effects on host cells: on the multiple functions of AMPS

Coffee break

Chair: *Ralf Hoffmann (University of Leipzig)*

11.00 – 11.30 *Francesca Bernardini (Polyphor Ltd, Allschwil CH)* From the natural antimicrobial peptide Protegrin-1 to a novel class of anti-*Pseudomonas* antibiotics

11.30 – 12.00 *Ana Veiga (University of Lisbon)* Correlating molecular biophysics and microbiology using AMPs

12.00 – 12.30 *Tania Schneider (University of Bonn, NAM project)* Cell wall-directed actions of AMPs

Lunch

Session 4 Chair: *Tatiana Ovchinnikova (Shemyakin-Ovchinnikov Inst., Russian Acad. Sci.)*

14.00 – 14.30 *Chiara Falciani (SetLance SRL, Siena)* The branched antimicrobial peptide M33. A new drug against Gram-negative bacteria?

14.30 – 15.00 *Karoline Sidelmann Brinch (Novozyme A/S, NAM project)* Non-medical applications for AMPs

Brief participant talks

15.00 - 15.15 *Karl Lohner (Austrian Academy of Sciences, Graz)* Multiple membrane interaction sites of antimicrobial (lipo)peptides

15.15 - 15.20 *Alberto Pallavicini (University of Trieste)* How many defence peptides in *Mytilus* spp?

Coffee break and posters

Brief participant talks Chair: *Renato Gennaro (University of Trieste)*

16.30 - 16.45 *Davor Juretić (University of Split) In silico* search strategies for finding new AMPs

16.45 - 17.00 *Lorenzo Alibardi (University of Bologna)* Beta-defensins in the soft-shelled turtle *Apalona spinifera*

17.00 - 17.15 *Anton Polyansky (University of Vienna and Russian Academy of Sciences)* Conformational dynamics study of lantibiotics-lipid II interactions.

17.15 - 17.30 *Antonello Romani (Surgery O.U., University Hospital Parma)* A Novel Terapeutic Agent Against Gram-Negative Infections In Cystic Fibrosis

17.30 *Hans-Georg Sahl (University of Bonn, NAM project)* Closing remarks

REGISTERED PARTICIPANTS

Participant	Affiliation	email
Alibardi *Lorenzo*	University of Bologna	lorenzo.alibardi@unibo.it
Anderluh *Gregor*	Nat. Inst. Chem. Slovenia	gregor.anderluh@ki.si
Andersen *Anders Schou*	Novozymes A/S (DK)	ANAS@novozymes.com
Antcheva *Nikolinka*	University of Trieste	nantcheva@units.it
Aquila *Marco*	University of Ferrara	qlamrc@unife.it
Batoni *Giovanna*	University of Pisa	giovanna.batoni@med.unipi.it
Bellanda *Massimo*	University of Padova	massimo.bellanda@unipd.it
Benedusi *Mascia*	University of Ferrara	bndmsc@unife.it
Benincasa *Monica*	University of Trieste	mbenincasa@units.it
Bernardini *Francesca*	Polyphor Ltd Allschwill (CH)	francesca.bernardini@polyphor.com
Bills *Gerald*	Fundació Medina, Granada	gerald.bills@medinaandalucia.es
Binda *Elisa*	University of Insubria	elisa.binda@uninsubria.it
Bobone *Sara*	University of Rome Tor Vergata	sara.bobone@uniroma2.it
Bocchinfuso *Gianfranco*	University of Rome Tor Vergata	gianfranco.bocchinfuso@uniroma2.it
Boniotto *Michele*	University of Paris XI	michele.boniotto@u-psud.fr
Bozzato *Andrea*	University of Padova	andrea.bozzato@bio.unipd.it
Broetz-Oesterhelt *Heike*	University of Düsseldorf	Heike.Broetz-Oesterhelt@uni-duesseldorf.de
Bulfon *Chiara*	University of Udine	chiara.bulfon@uniud.it
Cabassi *Silvia*	University of Parma	cabassis@unipr.it
Carnicelli *Veronica*	University of l'Aquila	veronica.carnicelli@cc.univaq.it
Chiapparini *Fausto*	Biotage AB Milan	fausto.chiapparini@biotage.com
Covaceuszach *Sonia*	CNR Inst. Cristallography	sonia.covaceuszach@ts.ic.cnr.it
Crisma *Marco*	CNR Inst. Biomol. Chem.	marco.crisma@unipd.it
Crovella *Sergio*	Burlo Garofalo Children's Hospital (Trieste)	crovella@burlo.trieste.it
De Gobba *Cristian*	University of Copenhagen	gobba@life.ku.dk
DalBen *Matteo*	University of Trieste	dalben.matteo@yahoo.it
Destoumieux-Garzon *Delphine*	CNRS, Montpellier	ddestoum@ifremer.fr
D' Este *Francesca*	University of Udine	francesca.deste@uniud.it
Di Giulio *Antonio*	University of	antonio.digiulio@cc.univaq.it
Domeneghetti *Stefania*	University of Padova	stefania.domeneghetti@unipd.it
Falciani *Chiara*	Setlance SRL, Siena	falciani@setlance.com
Feliu *Lidia*	University of Girona	lidia.feliu@udg.edu
Freddi *Giuliano*	Innovhub-SSI,	freddi@ssiseta.it
Furlan *Michela*	University of Trieste	mik.furlan@gmail.com
Galletti *Elena*	University of Udine	elena.galletti@uniud.it

Gennaro *Renato*	University of Trieste	rgennaro@units.it
Gobbo *Marina*	University of Padova	marina.gobbo@unipd.it
Guarnaccia *Corrado*	ICGEB Trieste	guarna@icgeb.org
Guida *Filomena*	SISSA Trieste	milenaguida@gmail.com
Henrichfreise *Beate*	University of Bonn	BAEH@novozymes.com
Hodnik *Vesna*	University of Ljubljana	vesna.hodnik@ki.si
Hoffmann *Ralf*	University of Leipzig	hoffmann@chemie.uni-leipzig.de
Ilic *Nada*	University of Split	nada@pmfst.hr
Ivica *Josep*	University of Split	josivi@pmfst.hr
Juretic *Davor*	University of Split	juretic@pmfst.hr
Lamba *Doriano*	CNR Inst. Crystallography	doriano.lamba@ts.ic.cnr.it
Lengvinaite *Simona*	University of Vyatus Magnus	s.lengvinaite@gmf.vdu.lt
Lizzi *Anna Rita*	University of l'Aquila	annarita.lizzi@cc.univaq.it
Lohner *Karl*	Austrian Academy of Science	karl.lohner@oeaw.ac.at
Luca *Vincenzo*	University of Rome La Sapienza	vincenzo.luca@uniroma1.it
Magris *Raffaella*	University of Udine	raffaella.magris@uniud.it
Maisetta *Giuseppantonio*	University of Pisa	gmaisetta@biomed.unipi.it
Manzini *Giorgio*	University of Trieste	manzini@units.it
Mardirossian *Mario*	University of Trieste	mario.mardirossian@phd.units.it
Mangoni *Maria Luisa*	University of Rome La Sapienza	marialuisa.mangoni@uniroma1.it
Meloni *Mauro*	University of Udine	mauro.meloni@uniud.it
Milan *Annalisa*	University of Trieste	annalisafly@aol.it
Miljak *Marija*	University of Split	marija.miljak.1@gmail.com
Mysore V. *Tejesvi*	University of Oulu	mvtejesvi@gmail.com
Ng Choi *Iteng*	University of Girona	iteng.ngchoi@udg.edu
Novkovic *Mario*	University of Split	novkovic.mario@gmail.com
Ovcinnikova *Tatiana*	Russian Academy of Science	ovch@bk.ru
Pacor *Sabrina*	University of Trieste	pacorsab@units.it
Pallavicini *Alberto*	University of Trieste	pallavic@units.it
Pellizzari *Caterina*	University of Trieste	cate_cherry@hotmail.it
Picart Faiget *Pere*	University of Bonn	perepicart@hotmail.com
Pirttilä *Anna Maria*	University of Oulu	am.pirttila@oulu.fi
Planas *Marta*	University of Girona	marta.planas@udg.edu
Polyanski *Anton*	University of Vienna & Russian Academy of Science	newant@gmail.com
Rispoli *Giorgio*	University of Ferrara	rsg@unife.it
Rinaldi *Andrea C*	University of Cagliari	rinaldi@unica.it

Romani *Antonello*	University of Parma	aromani@ao.pr.it
Rosani *Umberto*	University of Padova	umberto.rosani@unipd.it
Rota *Andrea*	CEM Srl Bergamo	Andrea.Rota@cem.com
Runti *Giulia*	University of Trieste	giulia.runti@phd.units.it
Sahl *Hans Georg*	University of Bonn	sahl@microbiology-bonn.de
Sandre *Michele*	CRIBI University of Padova	michele.sandre@gmail.com
Sandrelli *Federica*	University of Padova	federica.sandrelli@unipd.it
Scarsini *Michele*	University of Udine	michele.scarsini@uniud.it
Schneider *Tania*	University of Bonn	tanja@microbiology-bonn.de
Scocchi *Marco*	University of Trieste	mscocchi@units.it
Shafer *William*	Emory University School of Medicine	wshafer@emory.edu
Shamova *Olga*	Russian Academy of Science	oshamova@yandex.ru
Sidelman Brinch *Karoline*	Novozymes S/A (DK)	kbri@novozymes.com
Sillah *Mahmadu*	United Care Roland Clinic	obeng@ieee.org
Simunic *Juraj*	University of Split	jsimunic@pmfst.hr
Skerlavaj *Barbara*	University of Udine	barbara.skerlavaj@uniud.it
Sosio *Marina*	NIACONS scarl, Milan	msosio@naicons.com
Stella *Lorenzo*	Univ. Rome Tor Vergata	stella@stc.uniroma2.it
Stoilova *Tatiana*	Mario Negri Inst. Milan	tatiana.stoilova@marionegri.it
Storici *Paola*	Elettra Sincrotrone Trieste	paola.storici@elettra.trieste.it
Sulek *Franja*	Orthopaedic Hospital Valdoltra (Slovenia)	franja.sulek@ob-valdoltra.si
Suware *Burama*	United Care Roland Clinic	office.ucrc@gmail.com
Tavano *Regina*	University of Padova	regina.tavano@unipd.it
Tettamanti *Gianluca*	University of Insubria	gianluca.tettamanti@uninsubria.it
Tomasinsig *Linda*	University of Udine	linda.tomasinsig@uniud.it
Tossi *Alessandro*	University of Trieste	atossi@units.it
Turcati *Nicole*	University of Trieste	nicole.turcati@alice.it
Vaccari *Lisa*	Elettra Sincrotrone Trieste	lisa.vaccari@elettra.trieste.it
Veiga *Ana Salome*	University of Lisbona	aveiga@fm.ul.pt
Venier *Paola*	University of Padova	paola.venier@unipd.it
Xhindoli *Daniela*	University of Trieste	daniela_xhindoli@yahoo.it
Zachariev *Sotir*	ICGEB Trieste	sotir@icgeb.org
Zanetti *Margherita*	University of Udine	margherita.zanetti@uniud.it
Zlatev *Ventislav*	ICGEB Trieste	zlatev@icgeb.org

PRESENTATION ABSTRACTS

Oral presentations

1) Opening address

Anna Maria Pirttilä (University of Oulu, Finland; NAM Project Scientific Coordinator).

Antibacterial resistance is spreading globally at an alarming rate and continues to increase. New potent antibacterial agents and therapies are therefore continually needed. In recent years, new antibacterial compounds have mainly been additions to the existing classes of drugs and truly new structures have rarely been developed. Pharmaceutical industry has little interest in developing new structures due to high investment and low profit expectancy. The derivatives of existing drugs however raise bacterial resistance much faster than completely new compounds. To overcome the chronic problem of antibiotic resistance, totally new compounds and approaches are required, involving collaboration of researchers from various disciplines, as well as high societal investments.

The NAM (New Antimicrobials) Project is funded by the Marie Curie Actions of the EU Seventh Framework Programme. The aim was to foster collaboration and exchange of personnel between academic groups and companies working on various aspects of antimicrobials: new sources, new approaches and mode-of action. This is the second Workshop organised within the NAM project and its aim is to coalesce the experience of NAM project members with that of participants from other academic and industry groups.

2) Endophytic fungal antibiotics: discovery, profiles and mechanisms of action.

Gerald F. Bills (Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Parque Tecnológico de Ciencias de la Salud, Granada, Spain)

Our lab has developed a suite of integrated tools for high-throughput and species-specific culturing of plant-associated filamentous fungi [1], complementary fermentation methods to manipulate fungal secondary metabolism [2-4], and phylogenetic and mass spectrometry recognition of known antibiotics [5]. Growth of fungi in nutritional arrays, the parallel growth of strains across multiple growth parameters in microplate fermentations, increases the probability of detecting antibiotic signals against Candida albicans and pathogenic bacteria and more thoroughly exploits the metabolic potential of strains. Complementing nutritional arrays with other methods, e.g. fungal growth in chemically inert solid matrices [3] or adsorptive resins [2], likewise enables a more exhaustive search for antibiosis across the filamentous fungi. Known and potentially unknown fungal-produced antibiotics are detected by LC-MS database matching so that chemical isolation work is focused on the most novel chemistry. Results are mapped onto a DNA barcode phylogeny [5]. Phylogenetic mapping has corroborated hypotheses about fungal secondary metabolism gleaned from genomic data and has directly confirmed that antibiosis is a universal and essential trait of the filamentous fungal life cycle. Plant-associated fungi exhibiting significant antibiosis towards model pathogens, *C. albicans* and *Staphylococcus aureus*, have been scaled up to produce enough antibiotic extract to generate

hypothetical mechanism of action profiles in genome-wide fitness tests in *C. albicans* and *S. aureus*, a subsequently often yielding novel metabolites [6, 7].The strategies have revealed new patterns of secondary metabolite distribution among plant-associated fungi while contributing to the discovery of several new antibiotics with new mechanisms of action. The platform will find utility in genomic mining for products of cryptic metabolite pathways.

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2. *de la Cruz, M., J. Martín, V. González-Menéndez, I. Pérez-Victoria, C. Moreno, J.R. Tormo, N.E. Aouad, J. Guarro, F. Vicente, F. Reyes, and G.F. Bills, Chemical and physical modulation of antibiotic activity in Emericella species. Chem Biodivers, 2012. in press.*
3. *Bills, G.F., A.W. Dombrowski, and M.A. Goetz, The "FERMEX" method for metabolite-enriched fungal extracts. Meth Molecul Biol, 2012. In press.*
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5. *Vicente, F., A. Basilio, G. Platas, J. Collado, G.F. Bills, A. González del Val, J. Martín, J.R. Tormo, G.H. Harris, D.L. Zink, M. Justice, J. Nielsen Kahn, and F. Peláez, Distribution of the antifungal agents sordarins across filamentous fungi. Mycol Res, 2009. 113: 754-770.*
6. *Goetz, M.A., C. Zhang, D.L. Zink, M. Arocho, F. Vicente, G.F. Bills, J. Polishook, K. Dorso, R. Onishi, C. Gill, E. Hickey, S. Lee, R. Ball, S. Skwish, R.G.K. Donald, J.W. Phillips, and S.B. Singh, Coelomycin, a highly substituted 2,6-dioxo-pyrazine fungal metabolite antibacterial agent discovered by Staphylococcus aureus fitness test profiling. J Antibiot, 2010. 63: 512-518.*
7. *Roemer, T., D. Xu, S.B. Singh, C.A. Parish, G. Harris, H. Wang, J.E. Davies, and G.F. Bills, Confronting the challenge of natural product-based antifungal discovery. Chem Biol, 2011. 18: 148-164.*

3) How bacteria modulate their susceptibility to AMPs - implications for anti-infective development and pathogenesis.

William M. Shafer (Emory University School of Medicine, Atlanta, GA)

AMPs are important mediators of innate host defense during infection. Given the antibiotic-like properties of AMPs, it is not surprising that microbes have evolved mechanisms to counteract their action. I will discuss systems used by human pathogenic bacteria to thwart the action of AMPs. I will discuss how these mechanisms of AMP resistance impact *in vivo* fitness and survival of pathogens during infection. In a larger context, I will discuss how AMP-resistance systems should be accounted for in the development of new therapeutics.

4) Antimicrobial peptides from endophytes.

Tejesvi MV[a], Picart P[a], Koskimaki, J[a], Ruddock L[a], Tossi A[b], , Kristensen HH[c], Segura DR[c], Andersen B[c], Schnorr KM[c], Olsen PB[c], Neve S[c] Kajula M[a], Hautajärvi H[a], Mattila S[a], Pirttilä AM[a] ([a]Department of Biology, University of Oulu, Finland; [b]Department of Life Sciences, University of Trieste, Italy; [c]Novozymes A/s, Bagsvaerd, Denmark; NAM Project Members)

Endophytes are the microbes which live inside the plants without causing any symptom of disease. Endophytes are well known for the production of novel and biologically active metabolites, which have application in agriculture, medicine and pharmaceutical industry. Staphylococci, Mycobacteria, Streptococci and Enterococci have developed drug resistance and this is a growing threat to world health,

prompting us to look for better antibiotics from new sources such as endophytes. There are many antibiotics already isolated and identified from endophytes, such as Coronamycins, Munumbicins and Leucinostatin A. We have isolated one antifungal and two antibacterial peptides from endophytes using metagenomics, transcriptimcs and genome sequencing tools.

5) AMPs from marine invertebrates (AMPs in the defense of the Pacific oyster - how diversity may compensate for scarcity in the regulation of resident/pathogenic microflora).

Delphine Destoumieux, Paulina Schmitt, Rafael Diego Rosa, Marylise Duperthuy, Julien de Lorgeril, Evelyne Bachère (CNRS, Montpellier, France)

Antimicrobial peptides and proteins (AMPs) participate in the antimicrobial defense of living organisms, protecting hosts from invasion by pathogens and regulating homeostasis. In *Crassostrea gigas* oysters, four families of gene-encoded AMPs have been described to date, namely defensins (CgDefs), proline-rich peptides (Cg-Prps), big defensins (CgBigDefs) and bactericidal permeability/increasing proteins (Cg-BPIs). Unlike in many other marine invertebrates, oyster AMPs are produced at low concentrations. They are expressed by epithelial cells and/or circulating hemocytes. In non-diseased oysters, which are normally populated by an abundant resident microflora, hemocytes express basal levels of Cg-Defs and Cg-Prps that could participate in homeostasis. Upon infection, both AMP families are driven to sites of infection by major hemocyte movements, together with Cg-BPI and given CgBigDef forms, whose expression in hemocytes is induced by infection. Co-localisation of AMPs at sites of infection could be determinant in limiting invasion. Indeed, synergies have been evidenced between peptide families, which are potentiated by the considerable diversity of sequences within the Cg-Def and Cg-Prp families. Besides, diversity takes place at the level of oyster AMP mechanisms of action, which range from membrane lysis for Cg-BPI to inhibition of metabolic pathways for Cg-Defs. We believe that the combination of such different mechanisms of action may account for the synergistic activities observed and compensate for the low peptide concentrations in *C. gigas* cells and tissues. The success of infection by pathogens could then rely on subtle mechanisms of resistance and evasion of the oyster antimicrobial response. Some Vibrio strains pathogenic for oysters are equipped with AMP-sensing systems that trigger resistance. In addition, oyster pathogens have developed diverse mechanisms to resist/evade intracellular killing through phagocytosis and the associated oxidative burst.

6) From discovery to development of novel antibiotics at NAICONS.

Margherita Sosio (NAICONS scarl, Via Fantoli 16/15, 20138 Milan, Italy)

NAICONS (New Anti-Infectives Consortium) is an organization aimed at integrating resources for the discovery and development of new bioactive compounds, particularly antibiotics for serious infections caused by multi-drug resistant pathogens. Naicons' portfolio includes new antibacterial products at the late preclinical/early clinical stage. Our drug discovery programs are based on a

technological platform based on a collection of over 65,000 secondary metabolites producing microorganisms, which includes many rare and hard to isolate strains, and the know-how to exploit this resource. The company explores this potential source of chemical diversity by using rationales that increase the chances of finding new natural products. One approach consists of screening previously (under)explored taxa of Actinobacteria by simple growth-inhibition assays, followed by the rapid assessment of chemical novelty by a combination of analytical techniques and database queries. One particularly promising taxon is represented by the genus Actinoallomurus. A pilot study on 50 strains from this genus has unveiled different classes of secondary metabolites, including new compounds at a high frequency. Naicons is also involved in the development of the antibiotics NAI-107 and NAI-acne. The lantibiotic NAI-107, produced by the actinomycete *Microbispora sp*., has the potential to treat life-threatening infections caused by multidrug-resistant Gram-positive pathogens and is currently undergoing formal toxicology studies. Naicons is coordinating an FP7 project aimed at the optimization of the production process. NAI-Acne is a semisynthetic derivative of the thiazolylpeptide GE2270, produced by the actinomycete *Planobispora rosea*, which exibits a restricted antibacterial spectrum covering mostly *Propionibacterium acnes*. Also in this case, its development can benefit from the optimization of the production process, for which Naicons is coordinating a project supported by Regione Lombardia. During this project, we were able to obtain heterologous production of GE2270 and have started manipulating its biosynthetic pathway.

7) Piceasin 3 - a new plant defensin isolated from *Picea glauca.*

Pere Picart (Institute of Microbiology and Biotechnology, University of Bonn, Germany, NAM Project member).

A novel defensin gene was found by screening the available *Picea glauca* EST database with the BLAST algorithm. A PCR-based strategy was used to isolate the gene *Pgd3* using genomic DNA extracted from *P. glauca*. *Pgd3* encoded for a 77 amino acid peptide with sequence homology to the family of plant defensins and was fully conserved within *Picea* genus. Pgd3 peptide, produced in *Escherichia coli* origami by SUMO technology, had a molecular mass of 5.721 kDa as determined by mass spectrometry. Recombinant Pgd3 was very high heat-stable, moderately inhibited by divalent cations and showed strong antifungal activity against a broad spectrum of plant pathogenic fungi and *Saccharomyces cerevisiae*, with very high levels of activity against the wilting disease causing pathogen *Verticillium dahliae*, and *Botrytis cinerea*, one of the most important pathogens of coniferous trees. The Pgd3 peptide did not induce morphological changes on the treated fungal hyphae, but instead strongly inhibited hyphal elongation. A SYTOX uptake assay suggested that the inhibitory activity of Pgd3 might be associated with altering the membrane permeability of the fungal membranes.

8) Optimization of insect-derived antimicrobial peptides to treat systemic bacterial infections.

Ralf Hoffmann, Uwe Müller, Eszter Ostorhazi, Daniel Knappe, Michael Zahn, Annegret Binas, Oliver Nolte, Norbert Strater, Gottfried Alber (Biotechnology and Biomedicine Centre, University of Leipzig)

The emergence of multi-drug resistant bacterial pathogens in hospitals (nosocomial infections) presents a global threat of growing importance, especially for Gram-negative bacteria in recent years. Our lead compound Api88 was developed in an iterative approach to optimize the native antimicrobial peptide (AMP) apidaecin 1b (produced by honeybee *Apis mellifera*) for increased antibacterial activities against threatening human pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* while maintaining the favorable properties of the original sequence, i.e. no hemolytic activity and no toxicity against mammalian cells. Api88 appears to kill bacteria by the same mode of action as native Pro-rich peptides without lytic effects on cell membranes. It binds with a seven residue long stretch in two different modes to the chaperone DnaK at the substrate binding domain with KD-values around 6 µmol/L.

9) The PRAMP story - towards an understanding of translocation and activities of Proline-rich AMPs.

Marco Scocchi, Monica Benincasa, Giulia Runti, Mario Mardirossian, Renato Gennaro (Department of Life Sciences, University of Trieste, NAM Project member)

Among the galaxy of different AMPs the Proline-rich (PR-AMPs) are a group of cationic peptides with peculiar features. Although not evolutionary related, PR-AMPS of both vertebrates and invertebrates are characterized by short repeated stretches of proline and arginine residues, they selectively kill Gram-negative bacteria and are relatively non toxic towards host cells. Most PR-AMPs have a similar mechanism of action based on the inhibition of largely unknown intracellular targets, while damage to bacterial membranes, unlike many other types of AMPs, is only a secondary effect.

In this study we used Bac7(1-35), an active fragment of a mammalian PR-AMP to further investigate the mechanism of action of this group of peptides. By the screening of *E. coli* mutant libraries with decreased susceptibility to Bac7 we identified SbmA, an inner membrane protein necessary for the transport of Bac7(1-35) and other PR-AMPs to the bacteria, whose deletion caused a marked reduction in peptide susceptibility. Our results indicate that SbmA is a low-specificity active carrier of peptides with unknown function sharing some feature with the ABC transporters and used by host to introduce AMPs into the bacteria. By in vitro affinity binding assays we isolated the heat shock protein DnaK as a first intracellular interactor of Bac7(1-35). Although in vitro interaction with the peptide inhibited the refolding activity of DnaK molecular chaperone system, a DnaK-deficient strain was not significantly affected by the peptide indicating that other vital targets are present in susceptible bacteria. Since DnaK has previously been identified as a target of insect PR-AMPs, our observation indicates an interesting convergence in the mode of action of PR-AMPs both with respect to the transport system and internal target.

Overall these results make PR-AMPs attractive both as anti-infective lead compounds and as a new class of bacteria-penetrating peptides potentially capable of internalizing membrane-impermeant drugs.

10) Lipopeptides with novel antibacterial mechanisms or target binding modes.

Heike Brötz-Oesterhelt (Institute for Pharmaceutical Biology and Biotechnology, Heinrich-Heine University of Düsseldorf, Germany)

Multi-drug-resistant bacteria are spreading within hospitals world-wide and expand into the community. New therapeutic options are continually needed to maintain our therapeutic standard in the treatment of bacterial infections. Our current research focuses on several natural product antibiotics from novel structural classes with resistance breaking properties. We elucidate their molecular mechanisms of action to identify innovative principles for antibiotic intervention and we evaluate their qualification as lead structures for antibiotic development.

Novel acyldepsipeptides designated ADEPs show promising activity against important Gram-positive bacteria. They act by an unprecedented target, the bacterial caseinolytic protease ClpP [1]. Rather than inhibiting their target, the antibiotics deregulate and over-activate it. By abrogating the interaction of ClpP with cooperating and regulatory partner molecules, all normal functions of ClpP in general and regulatory proteolysis are prevented and un-physiological substrates are untimely degraded [2], among these the important cell division protein FtsZ [3]. Structures of ADEP-ClpP complexes unravel the conformational changes that convert ClpP from a strictly controlled precision instrument to a general protein shedder [4].

In contrast, the lipodepsipeptide empedopeptin acts within the classical metabolic pathway of bacterial cell wall synthesis on a well known and highly validated target, the peptidoglycan precursor lipid II. The region of lipid II that is recognised by empedopeptin is distinct from that of the marketed antibiotic vancomycin, thereby omitting cross-resistance [5].

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11) Short membrane-active peptides from amphibian skin to fight microbial pathogens.

Maria Luisa Mangoni (University of Rome, La Sapienza, Italy)

Amphibian skin secretions represent a rich source of antimicrobial peptides (AMPs). In particular, temporins constitute a large family and are among the smallest amphipathic α-helical AMPs (10-16 residues) found in nature to date, and with the lowest number of positively charged amino acids [1]. Some of them possess unique properties including: *(i)* a fast membranolytic effect against a large spectrum of microorganisms; *(ii)* synergism, when combined one with the other, in inhibiting bacterial growth and in endotoxin neutralization [2,3].

Another interesting ,small-sized and membrane-active AMP is esculentin(1-18) corresponding to the N-terminal 1-18 fragment of esculentin-1b, isolated from frog skin. It displays a rapid bactericidal activity against important nosocomial pathogens, such as the Gram-negative *Pseudomonas aeruginosa*. Its *in vivo* antimicrobial activity and mode of action have been studied using the mini-host model of *Caenorhabditis elegans* infected by a multidrug-resistant strain of *P. aeruginosa* [4]. Furthermore, promising results have been obtained from peptide-treatment of cows with clinical mastitis. Overall, these studies suggest frog-skin AMPs as attractive molecules to assist in the future design and manufacturing of new peptide-based anti-infective drugs.

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12) Antimicrobial peptides for plant protection

Lidia Feliu, Esther Badosa, Jordi Cabrefiga, Jesús Francés, Eduard Bardají, Emilio Montesinos, Marta Planas (LIPPSO, University of Girona, Spain)

The main objective of our research is focused on finding peptides as new agents to control economically important plant pathogenic bacteria and fungi for which the available methods are not sufficiently effective. In particular, we are interested in the bacteria *Erwinia amylovora*, *Pseudomonas syringae* and *Xanthomonas vesicatoria*, and the fungi *Fusarium oxysporum* and *Penicillium expansum*. Nowadays, the control of these mincroorganisms is mainly based on copper compounds and antibiotics, which have some limitations and a high environmental impact. On the other hand, antibiotics are not authorized in several countries and resistance has developed in plant pathogens. The identification of peptides able to fight the above bacteria and fungi will represent an alternative to currently used antibiotics or pesticides. The main features expected for these peptides are a high antimicrobial activity, a low eukaryotic cytotoxicity and a high stability to protease digestion.

We have synthesized combinatorial libraries of cyclic decapeptides and linear undecapeptides. These libraries have been screened for antibacterial activity and eukaryotic cytotoxicity, which has led to the identification of lead peptides. The biological profile of these peptides has been further improved by incorporating several modifications into their sequences. These modifications and their influence in the biological activity will be discussed.

13) Looking at peptides' and proteins' adventures on membranes using surface plasmon resonance

Gregor Anderluh[1] and Vesna Hodnik[2] ([1]National Institute of Chemistry and [2]University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia, Ljubljana, Slovenia)

Surface plasmon resonance (SPR) is one of the most important biophysical approaches to study molecular interactions. Most of the published literature describes this approach to study protein-protein interactions. Protein-membrane and

peptide-membrane interactions, although crucial for many cell processes, are less well studied. The most obvious advantages of SPR over other techniques are direct and rapid determination of association and dissociation rates of binding process, no need for labelling of protein or lipids, and low amount of sample that is used in the assay (nM concentrations for proteins).

In a typical experiment, a membrane is prepared on the surface of so-called sensor chip and the protein or peptide is passed over. Different approaches to membrane immobilization were described and will be briefly reviewed in this lecture. It is possible to immobilize intact liposomes on the chip. It is easy to reproducibly prepare stable lipid surface under various experimental conditions, i.e. temperature or pH. SPR also allows studying binding to more complex membrane preparations derived from cells, such as intracellular vesicles preparations, red blood cell ghosts, etc. In the last few years SPR was, therefore, used to study membrane binding of peripheral proteins participating in membrane-mediated cell signalling, membrane binding toxins and peptides, etc.

14) Breach or passage - on the multiple modes of antibacterial action of mammalian AMPs

Alessandro Tossi, Nikolinka Antcheva, Daniela Xhindoli, Filomena Guida, Mario Mardirossian, Marco Scocchi and Renato Gennaro (Department of Life Sciences, University of Trieste, Italy, NAM Project member)

Two of the principal families of mammalian antimicrobial/host defence peptides are the cathelicidins and β-defensins. Cathelicidins are characterized by a conserved pro-region carrying antimicrobial peptides with differentiated sizes and structures, including amphipathic, helical AMPs and proline/arginine rich peptides (PRAMPs) with extended structures. β-defensins have more complex S-S bridged structures consisting of a small β-sheet onto which a helical segment is often packed. These peptides have a multimodal capacity to directly inactivate microbial pathogens, and can also act indirectly by modulating other host immune and healing responses. Their evolutionary patterns range from conservation to positive selection for variation, suggesting that they may have adapted their structures and mechanisms of action to cater for different roles in host defence.

An initial interaction with biological membranes is a common and central theme in modulating the activities of all these types of AMPs, although this subsequently leads to quite different effects (membrane lysis, translocation or binding to a membrane component). To better understand these processes, we are carrying out a systematic analysis of factors affecting the modes-of-action of the human β-defensins hBD2 and 3, the human helical AMP LL-37 and its primate orthologues, and the bovine PR-AMP Bac7. These studies involve the rational design of unnatural analogues to better understand the roles of key structural features in the peptides, the use of membrane models to probe how these mediate membrane interactions, and the use of bacterial strains with specific surface characteristics as tools to probe the mode-of-action.

Our results indicate interesting convergences and divergences in the way that the different types of AMPs interact with membranes, and how this affects subsequent

biological activities. LL-37 and hBD3 are differentially active against both Gram-positive and -negative bacteria and appear to act principally at the microbial surface, eventually leading to membrane breaching. Bac7 is selectively active against Gram-negative bacteria and is internalised by a protein transporter to act on an internal target, without an apparent damage to bacterial membranes. Nonetheless, key residues required for its transport also mediate its capacity to reach the transporter by interacting with the bacterial membranes. For the β-defensins and LL-37, self-aggregation and the presence of an amphipathic helical domain both affect the efficiency of membrane approach and interaction, the selectivity for microbial or host membranes, and ultimately may determine whether the peptides act principally via a direct, membranolytic antimicrobial mechanism, or through other types of mechanisms involving immune stimulation.

15) Cathelicidin effects on host cells: on the multiple functions of AMPs

Francesca D'Este, Linda Tomasinsig, Barbara Skerlavaj, Andrea Sgorbissa, Cristina Bergnach and Margherita Zanetti (Department of Medical and Biological Sciences, University of Udine, Italy)

Accumulating evidence indicates that the role of the antimicrobial peptides (AMPs) in host defence extends far beyond that of simple endogenous antibiotics. This concept is well illustrated by the multiple activities exhibited in vitro and under clinical conditions by the human cathelicidin LL-37. This AMP has been shown to stimulate diverse cellular processes relevant to inflammation and immunity such as chemotaxis, cellular cytokine and chemokine release and angiogenesis. Several protein receptors, also including P2X7, FPRL-1, EGFR, have been implicated in these effects. However the mechanistic basis of their activation by LL-37 is still not fully understood. We have to some extent investigated this issue and have shown that the ability of LL-37 to modulate the function of P2X7 receptor, a cell surface protein involved in the proliferation-inducing activity of LL-37, is strictly dependent on the structural/aggregational properties of LL-37. This finding, together with preliminary data indicating ability of LL-37 to specifically interact with the transmembrane domain of selected membrane proteins, suggests a noncanonical LL-37-receptor interaction mediated by the interaction of the peptide with the cell membrane.

In several instances, the host cell-directed activities of other alpha helical cathelicidins have been shown to differ significantly from those of LL-37. For example, we have shown that the bovine congener BMAP-28 exhibits distinct effects on angiogenesis and on cell death/cell survival processes such as apoptosis, autophagy and proliferation. Also, BMAP-28 displays distinctive anti-endotoxin features by impairing the LPS-induced internalization of the cellular LPS receptor TLR4 without affecting the function of surface TLR4. As a consequence, the activation of the TRAM/TRIF, but not that of the MyD88 TLR4 signalling pathway is inhibited.

16) From the natural antimicrobial peptide Protegrin-1 to a novel class of anti-Pseudomonas antibiotics

F. Bernardini, G. Dale, S. DeMarco, K. Dembowsky, F. O. Gombert, A. Lederer, C. Ludin, P. Misson, A. Wach, J. A. Robinson and D. Obrecht (Polyphor Ltd, Hegenheimermattweg 125, CH-4123 Allschwil, Switzerland)

Antibiotics with new modes of action are urgently needed to combat the growing health threat posed by resistant pathogenic microorganisms. Antibiotic drug discovery in the past two decades has focused efforts on the development of antibiotics against Gram-positive bacteria and more specifically against multidrug-resistant *S. aureus* (MRSA). In contrast, treatment options against some MDR Gram-negative pathogens have become very limited, especially due to the emergence of resistance against the last resort antibiotics, colistin and polymyxin B. Applying the Protein Epitope Mimetics (PEM) Technology, we synthesized a family of peptidomimetic antibiotics based on the antimicrobial peptide Protegrin-1. Several rounds of optimization gave a family of new antibiotics with potent antimicrobial activity specific against *Pseudomonas aeruginosa*. Biochemical and genetic studies showed that these peptidomimetics have a new mode of action and identified the β-barrel protein LptD (Imp/OstA), which is implicated in the outer-membrane biogenesis, as molecular target. Optimized leads show potent antimicrobial activity in murine infection models and POL7080 has been selected as the clinical candidate.

17) Correlating molecular biophysics and microbiology using AMPs

Ana Veiga (University of Lisbon, Portugal)

Antimicrobial peptides (AMPs) are small, amphiphilic peptides with a positive net charge. An understanding of the mechanism adopted by the AMPs is central to their development as a new group of antimicrobial agents. An increasing amount of information on the action of AMPs at the molecular level has not yet been translated into a comprehensive understanding of effects in bacteria. Although some biophysical attributes of AMPs have been correlated with macroscopic features, the physiological relevance of other properties has not yet been addressed. Strong membrane-binding and micromolar therapeutic concentrations of AMPs indicate that membrane-bound concentrations may be reached that are higher than intuitively expected, triggering disruptive effects on bacteria. Experimental evidence obtained with the AMPs Omiganan and BP100 supports this idea and will be discussed in order to bridge the gap between biophysical studies using artificial lipid bilayers and studies using bacteria.

18) Cell wall-directed actions of AMPs

Tania Schneider (Institute of Microbiology and Biotechnology, University of Bonn, Germany, NAM Project member)

Antimicrobial peptides serve a vital role in first-line host defence and can be found throughout the animal and plant kingdom. Although being evolutionary ancient, AMPs have retained antimicrobial efficacy against gram-positive and gram-negative bacteria, fungi and enveloped viruses. Among the antimicrobial peptides, the defensins represent an important peptide family.

The fungal defensin Plectasin is a 40 amino acid peptide produced by the saprophytic ascomycete *Pseudoplectania nigrella*. Plectasin shares primary structural features with defensins from spiders, scorpions, dragonflies and mussels and folds into a cysteine-stabilized alpha-beta-structure.

Unlike conventional antibiotics which act via defined target molecules, antimicrobial defence peptides are assumed to act unspecifically by permeabilising the cell membrane. In contrast to this widely held view, Plectasin specifically targets the bacterial cell wall precursor lipid II, thereby inhibiting cell wall biosynthesis.

A series of cellular approaches and in vitro cell wall biosynthesis assays narrowed down the target pathway and the specific mechanism of action. Structural analysis of the Plectasin:lipid II complex identified, among others, the N-terminal amino group and His18 to contribute to Plectasin binding through interaction with the carboxyl group of *D*-Glu in position 2 of the lipid II stem peptide. In line with this, amidation of *D*-Glu to *D*-iso-Gln, as characteristically found in the peptidoglycan of staphylococci and other gram-positive pathogens, reduced susceptibility towards plectasin.

19) The branched antimicrobial peptide M33. A new drug against Gram-negative bacteria?

Chiara Falciani, Luisa Bracci, Jlenia Brunetti, Stefano Bindi, Barbara Lelli, Silvia Scali, Luisa Lozzi and Alessandro Pini (SetLance SRL, Siena, Italy)

The antimicrobial peptide M33 derives from the random selection of a combinatorial library incubated with E. coli cells and a rational optimization phase that produced a peptide with the typical features of antimicrobial peptides.

M33 peptide is synthesized in tetra-branched form, a structure that makes peptides highly resistant to circulating peptidases, thus becoming particularly suitable for in vivo use. M33 peptide is predominantly active against Gram-negative bacteria with MIC valuess comparable to many traditional antibacterial agents currently used in the clinic. Its mechanism of action has been characterized for membrane interaction, pore formation, biofilm eradication, DNA binding and LPS neutralization. Neutralization of LPS, demonstrated as a reduction in TNF-alpha production by macrophages, is a crucial aspect because it suggests that in vivo the peptide is involved not only in the direct killing of bacteria but possibly also in the reduction of cytokines that generate inflammation. This aspect, along with the low MIC shown by M33 against clinical isolates of P. aeruginosa from patients with Cystic Fibrosis or sepsis, increases the interest of this molecule as a new drug for these diseases where inflammation triggered by bacterial infection is a major element of pathology progression.

The current preclinical development of peptide M33 consists of efficacy experiments in animal models of sepsis, pneumonia and skin infection. Here we present results where strong reduction of bacterial load in-vivo and high animal survival rates are obtained through M33 administration.

20) Non-medical applications for AMPs

Karoline Sidelmann Brinch (Novozymes A/S, denmark, NAM Project member)

In recent times the interest in non-pharma applications for AMP technologies has increased significantly. This talk will review some of the possible areas for such applications of AMPs and discuss some of the emerging challenges which may

present themselves in connection to these uses, including production technologies, cost-in-use, regulatory issues among others.

Brief participant presentations

21) Multiple membrane interaction sites of antimicrobial (lipo)peptides

Dagmar Zweytick[1], Günter Deutsch[1], Yechiel Shai[2], Jörg Andrä[3], Sylvie E. Blondelle[4] and Karl Lohner[1] ([1]Inst. of Biophysics & Nanosystems Research, Austrian Academy of Sciences, Graz, Austria, [2]The Weizmann Institute of Science, Israel, 3Research Center Borstel, Germany, 4Sanford-Burnham Medical Research Institute, San Diego, USA)

Although antibiotic resistance has been an increasingly global health problem, the number of novel antibiotics on the market has declined steadily. In addition, the threat that bacteria being resistant to antibiotics will pass its resistance gene to other pathogens creating multidrug resistance emphasizes the need of novel antibiotics. One promising strategy is based on antimicrobial peptides, which mostly do not target single defined molecular structures, but act on the cell membrane killing bacteria rapidly within minutes, which reduces the likelihood of induced resistance. Understanding the mechanism(s) of membrane damage is thus a critical need to design novel antibiotics.

In general, lipopeptides show an increased activity against bacteria as compared to their non-acylated analogue. Hence, we have studied the effect of N-acylation on synthetic peptides derived from human lactoferricin on bacteria and membrane mimetic systems using various biophysical methods. Linking a hydrophobic moiety resulted in a different mode of bilayer perturbation. Massive membrane damage was only observed in the presence of N-acylated antimicrobial peptides, visualized by incorporation of the cationic dye SYTOX green, which can only enter cells, when its membrane is disrupted. This behaviour was also reflected in a stronger degree of membrane destabilization of *E. coli* and *S. aureus* as well as increased vesicle leakage in the presence of N-acylated antimicrobial peptides. However, these findings did not necessarily correlate quantitatively with the antimicrobial potency, which in several cases was similar for the N-acylated and its parent peptide. This suggests that both types of antimicrobial peptides interact also differently with other targets such as lipopolysaccharides in case of Gram-negative bacteria or lipoteichoic acid in case of Gram-positive bacteria. Electron micrographs support this notion showing formation of extrusions of lipid material, presumably from the outer membrane, in the presence of N-acylated peptides. Both types of peptides though induced membrane ruffling and detachment of the inner and outer membranes clearly indicating a membrane-based mode of action that leads to bacterial killing.

22) How many defence peptides in Mytilus spp?

Venier P, Gerdol M, Rosani U, Domeneghetti S, Manfrin C, De Moro G, Pallavicini A, (Universities of Trieste and Padova, Italy)

Mussels of the genus Mytilus are filter-feeder bivalves constantly surrounded by potential microscopic invaders in the coastal waters. Like other invertebrates, they can account only on rapid and effective innate defences, mainly ascribed to the hemolymph cells. HPLC-based evidence of AntiMicrobial cationic Peptides in *M. galloprovincialis* and

M. edulis dates back to 1996 [1,2]. Since then, both Sanger and high throughput sequencing have greatly speeded the discovery of precursor transcript variants and subsequent functional studies [3].

The availability of new sequence data allowed the definition of various DNA microarray platforms [4]. The analysis of ESTs included in the Mytibase collection drove subsequent amplicon pyrosequencing of the most known *M. galloprovincialis mytilins*, defensins, myticins and mytimycin [5] and revealed in more detail isotype-specific levels of variability of the AMP precursor transcripts, irrespective of the geographical mussel origin and immunostimulation. Both mechanisms underlying such molecular variation and functional meaning remain to be understood.

The high sequence variability of several AMPs is just one feature of an assorted defence repertoire. Illumina and 454 Roche sequencing performed on *M. galloprovincialis* revealed several other short peptides with different putative cysteine motifs. We have initially caracterized two novel AMP families, namely big defensins (about 9 KDa) featured by the motif C-X6-C-X3-C-X13-C-X4-C-C and hydramacin-like/ theromacin-like peptides (7-9 KDa) with four or five disulfide bonds (Gerdol et al. 2011). Their antimicrobial character was confirmed by sequence analysis whereas their tissue-specific patterns of expression definitely persuade us to look better at the AMP expression in tissues other than haemocytes.

Deep DNA/RNA sequencing is currently in progress worldwide on a variety of non-model organisms and it is expected to provide intriguing news and additional details on the defensive strategies of bivalves and other marine species.

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23) *In silico* search strategies for finding new AMPs

Davor Juretić, Damir Vukičević[a], Nada Ilić[a], Mario Novković[a], Juraj Simunić[a], Viktor Bojović[b], Nédia Kamech[c] and Alessandro Tossi[d] ([a]Faculty of Science, University of Split, Split, Croatia; [b]Ruđer Bošković Institute, Zagreb, Croatia; [c]University Pierre and Marie Curie, Paris, France; [d]University of Trieste, Trieste, Italy)

We have developed partially or fully automated knowledge-based methods:

a) For finding natural peptide antibiotics exhibiting promise as lead compounds (by using an indirect search for antimicrobial peptides associated with conserved signal peptides in the EST databases in combination with the therapeutic index (TI) prediction),

b) For suggesting amino acid substitutions expected to decrease toxicity, increase selectivity and maintain antibacterial activity (the Mutator algorithm which uses the TI prediction),

c) For constructing novel classes of peptide antibiotics with very little homology (less than 50%) to any known natural or synthetic antimicrobial peptide (the Designer algorithm).

In-silico designed peptides belong to the class of anuran-like helical peptides for which the TI-predictor http://split4.pmfst.hr/split/dserv1/ has been trained by using the DADP database http://split4.pmfst.hr/dadp/ and confirmed to work well [1-4].
Tests performed at the University of Trieste and at the University Pierre and Marie Curie (Paris) for predicted and measured TI with respect to several identified natural peptides, modified versions of known peptides and ab initio designed peptides have validated the method, with good correlation between predicted and experimental values.

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24) Beta-defensins in the soft-shelled turtle *Apalona spinifera*

L. Dalla Valle, F. Benato, L. Alibardi (Departments of Biology, Universities of Padova and Bologna, Italy)

From a cDNA Library obtained from the skin of the soft-shelled turtle Apalone spinifera we have identified total or partial transcripts for 4 beta-defensins named TuBD1-4 containing 61-66 amino acids and with high homology, in the position of the 6 cysteine-motif and in the N-terminus region. These peptides are expressed in the skin, carapace, but appear to be absent in the liver. Using a specific antibody for TuBD1 the only immunolabeled structures that store this peptide are represented by some non-specific granules localized in heterophyl granulocytes and in sparse granules of the epidermis. It remains unknown whether the content of the epidermal granules may be delivered among corneocytes of the stratum corneous that often contain extracellular or intracellular bacteria.

25) Specific conformational dynamics predefines high selectivity of lantibiotics to lipid II and provides opportunities for design of novel potent antimicrobials

[1,2]Anton Polyansky, [2]Pavel Volynsky, [2]Roman Efremov ([1]Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna and [2]M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow)

Lantibiotics represent a class of highly specific antimicrobial peptides isolated from bacteria. Here we present systematic in silico study of interaction a well-known lantibiotic nisin A with its membrane target Lipid II. We have shown that backbone dynamics of the peptide is strongly constrained by a number of its modified aminoacids and defines selective recognition of the pyrophosphate group of Lipid II. Based on this we have proposed a framework for design of nisin mimics, which can be used in future development of novel antimicrobials.

26) A novel terapeutic agent against gram-negative infections in cystic fibrosis: preliminary results

[1]Antonello Romani, [2]F. Ghidini, [2]S. Cavirani, [3]M.C. Baroni and [2]C.S. Cabassi, ([1]Surgery Operational Unit, University Hospital, Parma; Departments of [2]Animal Health and [3]Internal Medicine and Biomedical Sciences, University of Parma, Italy)

Cystic fibrosis(CF) leads to the production of a hyperviscous secretion within the airway providing a fertile ground for bacteria. The impairment of mucus-ciliary transport and the cleaning function of the upper airway predispose for bacterial colonization and chronic infection by S. aureus, P. aeruginosa and other non-fermenting Gram-negative bacteria. These bacteria are able to acquire antibiotics resistance making them difficult to eradicate thus determining the management of patients troublesome due to inadequate therapeutic options. A library of synthetic AMPs were developed and analysed by using proprietary software developed in house and synthesized. By using an engineered *E.coli* strain ML35pYC, one third of these peptides were able to permeabilise bacterial membrane within the first 30 minutes, almost two third within the first 60 minutes and the remaining peptides within two hours. Four randomly selected peptides were tested against Gram-. AMP72 was the most effective against *P.aeruginosa* ATCC 27853 with an LD_{90} of 1.59 μg/ml, followed by AMP2041 with 4.35 μg/ml and AMP698 with 6.41μg/ml. The less effective was AMP2201 with an LD90 of 16.29 μg/ml. About *Stenotrophomonas maltophilia*, AMP72 was the most interesting with an LD90 of 3.27 μg/ml, followed by AMP2041 with an LD90 of 5.2 μg/ml. The other AMPs have shown an LD_{90} equal or greater than 30 μg/ml. At 125mM salt, three out of 4 peptides had an activity greater than 95% at 12.5 mg/ml, while at 250mM salt only the AMP2041 was able to kill more than 98% of cells at 12.5 μg/ml. The products of this research gave the opportunity to identify novel and effective therapeutic approach to counteract the development, the virulence and the resistance towards conventional antibiotics of common and emergent bacteria involved in pulmonary infection in CF.

Poster presentations

1) Synthesis of beta-defensins

N. Antcheva, F. Guida and A. Tossi - Department of Life Sciences, University of Trieste

In general the defensins are difficult to synthesise considering their length (4-5kDa), the presence of a hydrophobic central core and the presence of six cysteine residues. To overcome those problems, the synthesis of several defensins was performed on a Automated Microwave - Enhanced Solid Phase Peptide Synthesizer (Liberty, CEM, USA) using different resins and conditions. HumanhBD3, bovine LAP and the artificial designed defensin tBD were synthesised using preloaded TGA resins. The coupling

temperature was set at 75°C except for Fmoc-Cys(Trt)-OPfp ester (45°C) to prevent racemisation. For each coupling step a solvent mixture of DMF/DCM 80/20 was used. Double coupling was carried at positions predicted to be difficult. Human hBD2 has a C-terminal Pro, so was synthesised using 2-chlorotrityl chloride resin with low substitution (<0.15 meq/g). The coupling and deprotection temperatures were reduced to 45°C to prevent cleavage of the peptide from the acid labile trityl resin due to release of HCl at higher temperatures. The good quality of the crude peptides permitted us to fold them directly without prior purification, in freshly prepared N2-saturated aqueous buffer pH 7,8 in the presence of the cysteine/cystine pair (20 μM peptide,10×Cys2, 100×Cys). The correct folding was partly confirmed by ESI-MS analysis of fragments obtained by enzymatic digestion (Trypsin/Chymotrypsin), [1]. The antimicrobial activity of the defensin was confirmed by MIC determination, while fluorimetric and flow cytometric assays were used to investigate the effects on prokaryotic cell membranes. Biophysical methods, such as circular dichroism (CD), transmission or reflection IR, SPR and dye release were used to probe structure/activity in the presence of model membranes (anionic DPPC/DPPG, 4/1 and neutral PC/Sm/Ch, 2/2/1 bilayers). These studies indicated that specific structural features modulated the efficiency of membrane insertion and selectivity for microbial or host-cell membranes [2]. The ability of hBD2 to bind to cellular membranes and internalize into host cells such as macrophages or dendritic cells was also studied using flow and confocal microscopy and confirmed its capacity to efficiently internalise into these cells without being accompanied by cytotoxic activity [3].

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2) Biophysical properties of antimicrobial peptides and viroporins studied under strict physiological conditions by means of enhanced patch-clamp technique

Marco Aquila, Anna Fasoli, Giorgio Rispoli and Mascia Benedusi - Institute for Maternal and Child Health IRCCS "Burlo Garofolo"-Trieste, Italy & Department of Biology and Evolution, University of Ferrara, Italy

Many short peptides selectively permeabilize the bacteria plasma membrane, leading to their lyses and death: they are therefore a source of antibacterial molecules, and inspiration for novel and more selective drugs. Another class of short (<100 residues) membrane proteins called viroporins, because they are coded by viral genes, permeabilizes the membrane of susceptible cells during infection of by most animal viruses. The permeabilization leads to host cell lyses and the release of the virus mass, replicated at host cell expense, to propagate the infection. Here, the patch-clamp technique is employed to study the mechanism of membrane permeabilization induced by the pore-forming peptides, under strict physiological conditions. This goal is achieved by recording the ion current through the channels

formed by these peptides, once inserted in the photoreceptor rod outer segment mechanically isolated from the retina of low vertebrates. This is the most suitable cell to carry on the above studies, because it was possible to fully block all its endogenous currents with light, without using any drug that could obstruct the peptide pores or interfere with the pore formation. Peptides, ions, and drugs were applied to (and removed from) the extracellular side in ~50 ms with a computer-controlled microperfusion system, so that the dynamics of pore formation, and the pore selectivity, blockade and gating could be precisely assessed.

When recording large currents, it is necessary to minimize series resistance, to reduce time constant of charging the cell membrane capacitance and error in membrane potential control. A second problem arises from the plasma membrane asymmetry: it is conceivable that peptide permeabilization depends upon the side of the membrane to which it is applied. These two problems could be circumvented by widening the patch pipette shank, through the calibrated combination of heat and air pressure. These pipettes dramatically reduce series resistance, and allow at the same time to insert pulled quartz or plastic tubes very close to the pipette tip, making it possible the delivery of large molecules to the cytosol with a controlled timing. Finally, it is presented here a simple procedure to consistently attain seals with conventional or pressure polished pipettes, made from just one glass type, on a wide variety of cell types, isolated from different amphibian, reptilian, fish, and mammalian tissues, and on artificial membranes made with many different lipid mixtures.

3) Effect of pH on the interaction of the human antimicrobial peptides hepcidin 20 and 25 with *Escherichia coli* ATCC25922 and model membranes

[a]Giuseppantonio Maisetta, [b]Alberto Vitali, [c]Raffaele Petruzzelli, [a]Franca Lisa Brancatisano, [a]Semih Esin, [d]Annarita Stringaro, [d]Marisa Colone, [e]Carla Luzi, [e]Argante Bozzi, [a]Mario Campa, [a]Giovanna Batoni - [a]Dipartimento di Patologia Sperimentale, Biotecnologie Mediche, Infettivologia ed Epidemiologia, University of Pisa, Via S. Zeno 35-39, 56127, Pisa, Italy, [b]Istituto di Biochimica e Biochimica Clinica, Università Cattolica e Istituto per la Chimica del Riconoscimento Molecolare, C.N.R., Largo F. Vito, 1, 00168 Rome, Italy, [c]Dipartimento di Scienze Biomediche, Università degli Studi "G. D'Annunzio", Via dei Vestini 29, 66013 Chieti, Italy [d]Dipartimento di Tecnologie e Salute, Italian National Health Institute, Viale Regina Elena, 299, 00161 Rome, Italy [e]Dipartimento di Scienze e Tecnologie Biomediche, University of L'Aquila, Via Vetoio 2, 67100 L'Aquila, Italy.

The human hepcidin 25 (hep-25) is a peptide mainly produced by the liver and involved in iron homeostasis. Its isoform, hepcidin 20 (hep-20) lacks the first 5 residues at the N-terminus and has an unknown function. The hepcidins are beta-sheet structured peptides endowed with antimicrobial activity against Gram-negative and Gram-positive nosocomial bacterial strains as well as the yeast Candida glabrata. Interestingly, we previously reported that acidic pH enhances the microbicidal effect of both peptides, reducing their active concentrations and shortening their killing times. Aim of the present study was to investigate whether pH influences the mode of hepcidins' interaction with Escherichia coli ATCC 25922 and model membranes. Enzymatic assays on the release of β-galactosidase (a marker of membrane damage)

by hepcidin-treated E. coli cells, revealed a striking release of the enzyme by bacterial cells incubated with both peptides at pH 5.0, while no inner membrane permeabilization capacity was seen at pH 7.4 even at bactericidal concentrations. Similar results were obtained assessing, by flow cytometry, the internalization of the DNA intercalating-dye propidium iodide by hepcidin-treated E. coli cells. Scanning electron microscope imaging revealed that both peptides induced, at acidic pH, a more evident membrane damaging effect (evidenced as significant blebbing on the bacterial surface) than at neutral pH. Furthermore, colorimetric and fluorescence based assays performed on phospholipidic vesicles and liposomes at acidic and/or at neutral pH showed a slight membrane damaging effect at pH 7.4, while at acidic pH a more dramatic effect on vesicle integrity was observed. A DNA retardation migration test, performed at pH 7.4, revealed that both hepcidins bind plasmidic DNA at high affinity suggesting an intracellular targeting mode of action at neutral pH.

On the whole, the above reported data suggest that hepcidins' ability to perturb bacterial membranes is pH-dependent. Since both physiological and pathological conditions are responsible for generating a low pH in vivo, it is tempting to speculate that the enhancing effect of pH on bactericidal activity of human hepcidins may represent a mechanism to promptly control bacterial invasion in cellular compartments or body districts characterized by acidic pH.

4) Biophysical characteristics and pore formation dynamics of cecropin-melittin hybrid peptide (cm15) in a natural membrane

Anna Fasoli, Marco Aquila, Giorgio Rispoli and Mascia Benedusi - Department of Biology and Evolution, University of Ferrara, Italy

The mechanism of membrane permeabilization of the synthetic cecropin-melittin hybrid peptide (Acetyl-KWKLFKKIGAVLKVL-CONH2; CM15) is still unclear. This mechanism was investigated by using a novel technique, consisting in the insertion of CM15 peptide in the plasma membrane of an isolated photoreceptor rod outer segment (OS) of vertebrate retina, which is the solely cell system in nature possessing just one channel type, that can be fully closed by light. Therefore, the ionic current recorded in whole-cell, voltage-clamp configuration from an OS at a given potential (Vh), is flowing entirely through the exogenous pores formed by the peptide channels, if the OS is illuminated. CM15 was rapidly applied (and removed) to (from) the OS in ~50 ms with a computer-controlled microperfusion system, in order to measure the dynamics of pore formation (and disaggregation). Once the main OS endogenous conductance was blocked with light, the OS membrane resistance was ≥1 GΩ, allowing high resolution, low-noise recordings. It was found that CM15 produced voltage-independent membrane permeabilization, repetitive peptide application caused a progressive permeabilization increase, and no single-channel events were detected at low peptide concentrations. Collectively, these results indicate a toroidal mechanism of pore formation by CM15.

Keywords: peptide antibiotics; photoreceptors; ion channels; patch-clamp.

5) Studying glycopeptide antibiotics and their producers in the fight against multi-resistant bacteria

Elisa Binda[1,2], Giorgia Letizia Marcone[1,2], Luciano Gastaldo[1], Flavia Marinelli[1,2] - [1]Università degli Studi dell'Insubria, Varese, Italia, [2]Centro di Ricerca Interuniversitario in Biotecnologie Proteiche "The Protein Factory", Politecnico di Milano, ICRM CNR Milano and Università degli Studi dell'Insubria, Varese, Italia.

Glycopeptide antibiotics are produced by filamentous actinomycetes and inhibit the synthesis of bacterial cell wall by blocking peptidoglycan assembly. They bind to the *D*-alanyl-*D*-alanine (*D*-Ala-*D*-Ala) C terminus of the nascent peptidoglycan and prevent it from being utilized in the following cross-linking reactions catalyzed by transglycosylases and transpeptidases. The structurally related glycopeptides vancomycin and teicoplanin have been introduced in clinical settings since 1958 and 1988, respectively. These drugs are still extensively used against multi-resistant enterococci and methicillin-resistant staphylococci. Concern about vancomycin-resistant enterococci has been increasing during the last decade, and highly vancomycin resistant *Staphylococcus aureus* isolates have recently appeared in clinical specimens. In glycopeptide resistant bacteria, reorganization of the cell wall is directed by the expression of *van* gene clusters. Identification of *van* gene homologous in the genomes of several glycopeptide-producing actinomycetes suggests the involvement of a similar self-resistance mechanism to avoid suicide during production phase. Our research is focalized on two glycopeptide producing actinomycetes: *Nonomuraea* sp. ATCC 39727, which produces A40926 – the precursor of the second generation glycopeptide dalbavancin –, and the teicoplanin producer *Actinoplanes teichomyceticus* ATCC 31121. Part of our work is devoted to the optimization of fermentation process to increase the production of these glycopeptides according to pharmacopeia standards. We are also developing tools for the genetic manipulation of these actinomycetes to improve their antibiotic production. Our studies show that in *A. teichomyceticus* v*anHAX* genes are constitutively expressed to confer a high level of glycopeptide resistance, whereas in *Nonomuraea* sp. a moderate level of self-resistance is due to the activity of a *D,D*-carboxypeptidase encoded by *vanY* gene. The link between glycopeptide resistance and production in these actinomycetes is currently under investigation.

6) Membrane thickness and the mechanism of action of the short peptaibol trichogin GA IV

S. Bobone[1], Y. Gerelli[2], M. De Zotti[3], G. Bocchinfuso[1], B. Orioni[1], A. Palleschi[1], F. Sebastiani[2], E. Latter[4], J. Penfold[4], R. Senesi[5], F. Formaggio[3], C. Toniolo[3], G. Fragneto[2], L. Stella[1], – [1]Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata", Italy; [2]Institut Laue-Langevin, Grenoble, France; [3]ICB, Padova Unit, CNR, Department of Chemistry, University of Padova, Italy; [4]Rutherford-Appleton Laboratory, Harwell Oxford, UK; [5]Department of Physics, University of Rome "Tor Vergata", Italy.*

Trichogin GA IV (Tric) is an antimicrobial peptide belonging to the peptaibol family, the most studied member of which is alamethicin (Alm). It acts by perturbing membrane permeability. Previous data showed that pore formation is linked to Tric aggregation and insertion in the hydrophobic core of the membrane [1-3]. This

behaviour is similar to that of Alm and is in agreement with a barrel-stave mechanism in which transmembrane oriented peptides aggregate to form a channel. However, while the 19-residue Alm has a length comparable to the membrane thickness, Tric is just 10-residue long. Therefore, its helix is about half the bilayer thickness. Molecular dynamics simulations predicted that in its transmembrane orientation Tric interacts strongly with the polar phospholipid headgroups, drawing them towards its N- and C-termini. Thus, using this mechanism Tric might be able to span the entire bilayer. Indeed, neutron reflectivity measurements on a POPC bilayer and a deuterated Tric analog indicated that the peptide inserts in the hydrophobic region of the membrane causing a significant thinning (from about 30 Å to 20 Å). Finally, vesicle leakage experiments demonstrated that Tric activity is significantly higher with thinner membranes, becoming similar to that of Alm when the bilayer thickness is comparable to its size. Overall, these data indicate that a barrel-stave mechanism of pore formation might be possible for Tric despite its relatively small size.

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7) "Minimum bias" molecular dynamics simulations to determine peptide orientation in membranes

G. Bocchinfuso, A. Farrotti, A. Palleschi, B. Bechinger, L. Stella - Department of Chemical Science and Technology, University of Rome Tor Vergata

The development of more effective antimicrobial peptides (AMPs), to fight drug-resistant bacteria, requires a detailed understanding of their mechanism of membrane perturbation, and of their position and orientation inside lipid bilayers. Molecular dynamics simulations can predict such information, but the simulation time-scales are often too short for a peptide to attain its most stable position in a preformed membrane. To overcome this limitation, the so-called "minimum-bias" approach was developed [1,2] in which the simulation starts from a random mixture of lipids, water and a peptide molecule, and a bilayer self-assembles during the trajectory. The high fluidity of the system during this process ensures that the peptide can find its minimum free energy configuration in the final membrane. In this work we provide a very stringent test of the reliability of this approach, by using the artificial AMP LAH4, which contains no cationic amino acids, but comprises four His residues, which change their protonation state with pH. Solid-state NMR experiments demonstrated that at acidic pH values this peptide is always located on the membrane surface, while at pH where the His residues are neutral the peptide inserts in a transmembrane orientation [3]. Several independent simulations were performed for both the charged and neutral peptide state (for a cumulative trajectory length of almost 2 μs). In all cases the charged LAH4 remained in contact with the phospholipid headgroups, while in the neutral state it became embedded in the acyl tails, in agreement with the experimental data. These simulations provide

the possibility to analyze how peptide orientation and charge influence its membrane-perturbing properties.

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8) Development, characterization and antimicrobial activities of new cationic peptides with a wide activity spectrum

[1]Clotilde Silvia Cabassi, [2]Tullia Tedeschi, [1]Sandro Cavirani, [3]Maria Cristina Baroni and [3]Antonello Romani - Departments of [1]Animal Health and [2]Organic and Industrial Chemistry, and [3]Internal Medicine and Biomedical Sciences, University of Parma, [4]Surgery Operational Unit, University Hospital, Parma; Italy)

The therapeutic approach to infection was changed by using antimicrobial drugs. The need for new and even more effective antimicrobial compounds induced pharmaceutical companies to develop novel drugs resulting an armoury of over two hundreds different molecules. However, the developing of drug-resistance to antibiotic compounds is nowadays faster than the discovery of novel classes of antimicrobial drugs. This has forced since 2003 the halting of any efforts to discover novel molecules by pharmaceutical companies.

A potential answer to overcome the antibiotic-resistance is represented by a wide-spectrum novel cyclic antimicrobial peptides (AMPs) recently developed by our research group. These AMPs are characterized by a length ranging from 10 to 30 aminoacid residues and random coil conformation in aqueous solvent. The cyclization was obtained by introducing several cycteines in different positions of sequences. The optimal percentage of hydrophobic aminoacidic residues assures the lacking of eukaryotic membrane perturbation. Moreover, the hydrophobic clustering along three-dimensional structure confer a high salt-tolerance. Finally, they show high solubility, low, if any, hemo-and cyto-toxicity.

The antimicrobial activity against Gram-negative bacteria such as *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* e *Stenotrophomonas maltophilia* indicated E.coli LD90 ranging from 0.83 uM (1.5 ug/ml) e 10.64 uM (19.63 ug/ml) while for P. aeruginosa tra 0.87 uM (1.59 ug/ml) e 7.68 uM (16.29 ug/ml). Then the LD90 obtained with *Stenotrophomonas maltophilia* ranging between 1.88 uM (3.42 ug/ml) and 2.67 uM. Interesting are, moreover, the results obtained against Gram-positive bacteria and in particular against MRSA. Two tested peptides showed a LD90 between 0.85 uM (1.65 ug/ml) and 0.90 uM (1.72 ug/ml). Similarly, using *S.aureus* methicillin-sensitive the LD90 were from 1.42 uM (2.89 ug/ml) and 13.4 uM (28.77 ug/ml). The AMPs developed were also active against fungi and yeast such as Candida albicans. The LD90 ranged from 0.68 uM (1.24 ug/ml) to 39 uM (circa 70 ug/ml).

In conclusion, such peptides can be effectively used as primary agents or adjuvants in the treatment of infectious diseases of human and animals

9) Antimicrobial lipopeptaibiotic trichogin GA IV: role of Aib to L-Leu substitutions on conformation and bioactivity

Barbara Biondi, Marta De Zotti, Yoonkyung Park, Kyung-Soo Hahm, Claudio Toniolo, Fernando Formaggio, and Marco Crisma - Institute of Biomolecular Chemistry, Padova Unit, CNR

The lipopeptaibiotic trichogin GA IV is a natural, non-ribosomally synthesized, antimicrobial peptide remarkably resistant to the action of hydrolytic enzymes. This feature may be connected to the multiple presence in its sequence of the noncoded residue α-aminoisobutyric acid (Aib), which is known to be responsible for the adoption of particularly stable helical structures already at the level of short peptides. To investigate the role of Aib residues on the 3D-structure and bioactivity of trichogin GA IV, we synthesized and fully characterized four analogs where one or two Aib residues are replaced by L-Leu. Our extensive conformational studies (including an X-ray diffraction analysis) and biological assays performed on these analogs showed that the Aib to L-Leu replacements do not significantly affect the resistance to proteolysis, but modulate the bioactivity of trichogin GA IV in a 3D-structure related manner

10) Analysis of Defb1 regulatory SNPs in cystic fibrosis patients from north eastern Italy

Marcello Morgutti[1], Annalisa Fabris[1], Anna Vendramin[1] Furio Poli[2], Chiara Trevisiol[3], Sergio Crovella[1] - [1]Genetic Section, IRCCS Burlo Garofolo, Trieste (Italy), [2]Pediatric Unit, IRCCS Burlo Garofolo, Trieste (Italy), [3]Pediatric Unit, San Polo Hospital, Monfalcone (GO), (Italy)

In our study we analyzed the three 5' UTR regulatory DEFB1 SNPs -52 (G/A), -44 (C/G) and -20 (G/A) in a group of cystic fibrosis patients from North Eastern Italy, and in healthy controls, with the aim of verifying the possible involvement of these functional SNPs in CF pathogenesis.

Genotypes and alleles frequencies of the -44 (C/G) and -20 (G/A) SNPs were similar in CF patients and healthy controls and no significant difference was evidenced between the two analyzed groups.

Conversely, a difference in the distribution of the -52 (G/A) SNP genotypes was evidenced when comparing CF patients and healthy controls (p=0.0081): DEFB1 -52 GG genotype was more frequent in CF patients (0.52) than in healthy controls (0.30).

11) Modulation of cytokine gene expression by cathelicidin BMAP-28 in LPS-stimulated and -unstimulated macrophages

Francesca D'Este, Linda Tomasinsig, Barbara Skerlavaj and Margherita Zanetti - Department of Medical and Biological Sciences, University of Udine.

Apart from direct bacterial killing, antimicrobial host defence peptides (HDPs) exert various other biological activities that also include modulation of immune responses to infection. The bovine cathelicidin BMAP-28 has been extensively studied with regard to its direct antibacterial activity while little is known about its effects on immune cell function. We have investigated its ability to affect inflammatory

pathways and to influence the proinflammatory response induced by LPS in RAW 264.7 macrophages, in terms of modulation of TLR4 activation and cytokine gene induction. BMAP-28 on its own elicited ERK1/2, p38 and NF-κB activation leading to upregulation of IL-1β gene expression in these cells, suggesting it has the capacity to activate selected cellular pathways through direct effects on macrophages. As expected based on its in vitro LPS-binding properties, BMAP-28 blocked LPS-induced cytokine gene expression when added to the cell culture in combination with LPS. However it enhanced the induction of IL-1β and IL-6 genes and suppressed that of IFN-β when added prior to or following LPS stimulation over a 30-60 min time interval, or when co-administered with taxol as another TLR4 stimulant. It did not inhibit the expression of IFN-β induced by the TLR3 ligand poly(I:C). Overall these results, and the fact that BMAP-28 increased the LPS-stimulated activation of NF-κB while diminishing that of IRF-3, suggest that the peptide potentiates the early TLR4–mediated proinflammatory cytokine response while inhibiting the TLR4/TRAM/TRIF signalling pathway leading to IRF-3 activation and IFN-β gene expression. Using a TLR4-specific antibody we also found that BMAP-28 decreased the LPS-induced internalization of surface TLR4 required for initiating the TRAM/TRIF signalling pathway, which provides a mechanism for the inhibitory effect of the peptide on the TLR4/TRAM/TRIF pathway.

12) Antimicrobial Peptides from food sources

C. De Gobba, J. H. Otte - Department of Food Science, University of Copenhagen

Food proteins can be processed into peptides with desirable activities against important diseases, such as cardiovascular diseases, diabetes and cancer. Peptides derived from milk or fish proteins are known to inhibit angiotensin converting enzyme (ACE) involved in blood pressure regulation, affect immune defence, inhibit microbial activity, and have antioxidative as well as antiproliferative activities on human breast cancer cell lines.

The release of high-value health-promoting peptides from larger food proteins is performed by means of proteolytic enzymes. The commonly used proteolytic enzymes such as trypsin and lactic acid bacteria enzymes are most active around 35-40 °C and normal atmospheric pressure, so all processes based on these enzymes may give rise to hygiene or organolepsis problems. Therefore, the aim of the multi-partner collaborative project NOVENIA is to find novel enzymes for industrial use; we will use novel cold-active proteolytic enzymes from psychrophilic microorganisms and pressure-stable proteolytic enzymes from barophilic bacteria in cold processes to produce bioactive peptides from protein sources like milk and fish.

The purpose of our part of the project is to set up screening assays to detect bioactive peptides in hydrolysates made by the action of the novel enzymes on food proteins.

This poster will present the results from the screening for antimicrobial peptides using hydrolysates of bovine milk casein proteins. These hydrolysates are prepared using enzymes taken from the supernatant of a culture of the bacterium *Arsukibacterium ikkense*, the commercial enzyme papain and a novel enzyme from the carnivorous plant *Dionaea muscipulan* (Venus flytrap).

13) Characterization of the salmonid cathelicidins and of their biological activities.

Michela Furlan, Giuseppe Cannone, Monica Benincasa, Alberto Pallavicini, Renato Gennaro, Marco Scocchi - University of Trieste, Dept. of Life Sciences, Trieste, Italy

Cathelicidins are a family of cationic antimicrobial peptides (AMPs) and an important component of the innate immune responses. Members of this family have recently been identified in salmonids and other fishes. The analysis of the Cath-1 and Cath-2 salmonid cathelicidin sequences evidenced an organization with a conserved cathelin domain and a relatively long and varied glycine/serine-rich C-termini domain.

In this study we characterized the antimicrobial activity spectrum, the mode of action and tissue localization of some of the C-termini peptides of salmonid cathelicidins. Different peptide fragments representing specific regions of CATH1 and/or CATH2 of rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), grayling (*Thymallus thymallus*) and brown trout (*Salmo trutta fario*) have been chemically synthesized and their antimicrobial activity evaluated against standard bacterial strains and some fish pathogens. Most peptides showed a medium-dependent antimicrobial activity with MIC values ranging from 4 µM to 64 µM. Killing kinetics and membrane permeabilization assays indicated that these peptides rapidly kill their bacteria targets by permeation of the cell membranes. Hemolytic assay on peripheral human blood evidenced a very low toxicity against eukaryotic cells. To identify CATH-1 in trout tissues and to study its processing, a recombinant CATH-1 derived from *O. mykiss* spleen cDNA has been expressed in *E. coli* and purified as a glutathione S-transferase fused form. Western blot analysis, performed with a polyclonal antibody raised against the whole protein by using the recombinant CATH-1 as an antigen, revealed that the protein is abundantly expressed in spleen and head kidney tissues and it is detected as multiple forms of different MW. Experiments are ongoing to determine eventual CATH-1 posttrasductional modifications as well its tissue distribution. Results will contribute to a comparative understanding of the functions of cathelicidins in the vertebrates.

14) Antimicrobial photodynamic therapy: synthesis, conformational properties and antibacterial activity of peptide-porphyrin conjugates

V. Sella, E. Frezza, R. Dosselli, E. Reddi, A. Ferrarini, M. Gobbo - Department of Chemical Science, Univeristy of Padova

The worldwide rise of antibiotic resistance stimulates the search for new strategies for controlling bacterial infections based on the use of agents different from the common antibiotics. Two promising approaches are the application of photodynamic therapy (PDT) and of cationic antimicrobial peptides (CAMP) in the treatment of localized infection.

PDT involves the use of non-toxic dyes or photosensitizers (PS), that can generate reactive oxygen species upon exposure to light in the presence of oxygen. It is well establish that singlet oxygen is produced as the main species responsible for cell death.

CAMP are components of the innate defense mechanism of many organisms. They are short peptides (10-50 amino acids), with an overall positive charge

(generally +2 to +9) and a substantial proportion (≥30%) of hydrophobic residues. These properties permit the peptide to fold into an amphipathic structure, often upon contact with membranes, and ensures accumulation at the poly-anionic microbial cell surfaces.

In general neutral, anionic or cationic PS molecules can efficiently kill Gram-positive bacteria, whereas Gram-negative bacteria are less susceptible to phodynamic killing and only cationic porphyrins can induce their photo-inactivation. On the contrary CAMP exhibit a broad spectrum of antimicrobial activity and do not easily induced resistance compared to conventional antibiotics. Thus the use of CAMP in combination with PDT is expected to enhance the effectiveness of PDT.

Recently we have shown that the conjugation of apidaecin 1b, a 18-residue peptide, to a 5(4'-carboxyphenyl)-10,15,20-triphenylporphyrin (cTPP) photosensitizer afforded a new antibacterial agent, with a broader spectrum activity with respect to that of the two individual components or a mixture of them [Rosselli R et al ACS Med. Chem. Lett. (2010) 1, 35].

Here, we present the synthesis of a new conjugate between cTPP and the membrane active peptide magainin 2 and a preliminary investigation of its antibacterial activity, in the dark and under light-activation. Moreover, the conformational properties of the porphyrin-peptide conjugates will be compared to those of the parent peptides and an interpretation of the circular dichroism spectra with respect to the assembling of these systems in aqueous environment will be presented.

15) Features of the primary structure of the PR-AMP Bac7 important for its internalization capacity and antimicrobial activity.

[1]Filomena Guida, [1]Monica benincasa, [2]Sotir Zachariev, [1]Nikolinka Antcheva, [1]Marco Scocchi, [1]Renato Gennaro and [1]Alessandro Tossi - [1]Department of Life Sciences, University of Trieste and [2]International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

The proline-rich peptides are a group of linear AMPs, isolated from both mammals and invertebrates, active mainly against Gram-negative bacteria, which act intracellularly being internalized without an apparent damage to bacterial membranes.

The sequence of the bovine cathelicidin Bac7, a reference Pro-rich AMP (PRAMP) has been subjected to several studies identifying the N-terminal region as responsible for the antimicrobial activity. To search for the minimal entry sequence into bacteria, and to investigate whether this overlaps with the minimal antimicrobial fragment, a set of progressively shortened labelled N-terminal fragments of Bac7 were synthesized and tested for their antibacterial activity and internalization capacity into *E. coli* cells by flow cytometry and confocal microscopy. While N-terminal fragments as short as 16 residues were both fully active and efficiently internalized into the cells, further shortening significantly reduced both these functions.

In addition the role of the two key N-terminal Arg residues of Bac7 on penetration of the outer membrane and translocation through a putative inner membrane transporter was probed by systematically substituting with lysine, *D*-Arg,

nitro-Arg [4], dimethyl-Arg [4], peptoid Arg and citrulline, or by omitting them and by using mutants of *E.coli* strains with deleted transport system or altered outer membrane characteristics.

The results indicated that stereochemistry, charge and H-bonding all seem to be important requirements for the activity and internalization of this PR AMP. These are relevant to both outer membrane transit and the translocating role of inner membrane transporter, which was confirmed. These studies have helped evaluate Bac7 as a potential anti-infective agent selective for Gram-negative bacteria, as well as a possible vehicle for internalization of antibiotic cargo into these, and suggested unnatural variants with potentially improved activity.

16) Cell wall biosynthesis in cell wall-less endobacteria - new options for antibiotic treatment?

A. Gaballah[a], A. Kloeckner[a], C. Otten, H. Buehl[a], S. De Benedetti[a], T. Schneider[a], H.G. Sahl[a], B. Henrichfreis,[a,b] - [a]Institute of Microbiology and Biotechnology, University of Bonnand b Novozymes A/S, NAM Project members

Searching for new antibacterial targets in chlamydiae we focus on co-ordination of cell wall biosynthesis and cell division, two essential processes driven by partially overlapping multi-protein machineries whose activity needs to be tightly controlled to maintain cell integrity. Previously, we discussed that maintaining biosynthesis of the completed cell wall building block, lipid II, in cell wall-lacking endobacteria like *chlamydiae* may reflect a vital role of lipid II biosynthesis in prokaryotic cell division. Currently, we investigate orchestration and regulation of chlamydial lipid II biosynthesis and cell division in vitro. We analyze how the chlamydial actin ortholog MreB is functionally organizing lipid II biosynthesis and demonstrate increased transferase activity of the cell wall enzyme MraY in the presence of MreB. In addition, we investigate whether elongation factor EF-Tu serves as a cytoskeletal component in chlamydiae as shown before for the orthologous protein in Bacillus. EF-Tu interacts with MreB and both proteins mutually increase their polymerization. Moreover, we study the regulatory function of eukaryote-like Ser/Thr kinases and phosphatases and prove that biosynthesis of cell wall precursors is affected by kinase PknD and that phosphatase Cpn0397 exhibits dephosphorylation activity. Deeper insight in co-ordination and regulation of lipid II biosynthesis and cell division will provide a basis for the design of new drugs to combat diseases caused by chlamydiae and other bacterial pathogens.

17) The interaction of nep1-like proteins with lipids

Vesna Hodnik[1], Isabell Kuefner[2], Thorsten Nürnberger[2] and Gregor Anderluh[1,3] - [1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia, 2ZMBP; [2]Plant Biochemistry, Auf der Morgenstelle 5, 72076 Tuebingen, Germany, [3]National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

The family of necrosis- and ethylene-inducing peptide 1 (NEP1)-like proteins (NLPs) elicit diverse defense reactions and cell death in dicotyledonous plants. They are cytolytic proteins, structurally similar to actinoporins, pore forming toxins from

marine invertebrates. A membrane disrupting activity was demonstrated for NLPs [1]. The other proteins that have similar structure are fungal lectins. All these proteins interact with specific structures at the membranes surface; actinoporins with sphingomyelin and lectins with carbohydrates. When plasma membranes were pretreated with different proteases and glycosidases the NLP proteins still bound to the membranes, therefore, the target molecule on the host cell is most probably of lipid origin. We have used multilamellar vesicles prepared from lipid extract from tobacco leaves in a binding assay for different NLPs (NLPPp from Phytophthora parasitica, NLPPcc from *Pectobacterium carotovorum* subsp. *carotovorum* and NLPPya from *Pythium aphanidermatum*). The secondary structure of proteins at different pH values was evaluated by using circular dichroism. Crystal structure of NLPPya reveals a single-domain molecule with a fold consisting of a central β-sandwich, with 3 strands in the first sheet and a 5-stranded antiparallel second sheet. Three helices encompass the second sheet at the top of the sandwich. At the base of the protein an uneven surface is established mainly by 3 broad loops [1]. We evaluate stability of the protein in different buffers using circular dichroism (CD) and differential scanning fluorimetry (DSF). According to the far UV circular dichroism spectra the proteins do not undergo structural changes upon pH change from 7.4 to 5.5. When comparing the near UV circular dichroism spectra we observed changes when lowering pH to 5.5 in the range where phenylalanine has a characteristic profile. DSF revealed that NLPPp is most stable at pH 6.0 and is more stable when disulfide bonds are preserved. The binding of proteins to small unilamellar vesicles from lipids extracted from plasma membranes from *Arabidopsis thaliana* and *Commelina communis* was evaluated using surface plasmon resonance.

18) Adepantins, computationally designed, glycine-rich peptide antibiotics, with low toxicity and very high G- selectivity

Nada Ilić[1], Mario Novković[1], Filomena Guida[2], Daniela Xhindoli[2], Monica Benincasa[2], Alessandro Tossi[2], Davor Juretić[1] - [1]Department of Physics, Faculty of Science, University of Split, Croatia; [2]Department of Life Sciences, University of Trieste, Italy

Adepantins (ADPs) are glycine-rich antimicrobial peptides (AMPs), constructed using the "Designer" algorithm, which is based on known characteristics of anuran AMPs [1]. Parameters used to predict the primary structure of ADPs include various physicochemical properties, such as lengthwise asymmetry and predicted therapeutic index (TI). ADP1 was previously tested and has shown high antimicrobial activity (MIC) against E. coli (2-4 μM) and low cytotoxic activity (HC_{50}) against human red blood cells (480 μM). To confirm the high selectivity of peptides from this group, additional tests were performed for monomeric, dimeric and fluorescently labelled versions of ADP2 and ADP3, using different bacterial strains, host cells and model membrane systems. They are selective for Gram-negative bacterial cells (MIC = 0.5 - 4 μM) both with respect to human erythrocytes (HC_{50} > 400 μM for monomers), as with ADP1, and with respect to Gram-positive bacteria (MIC > 128 μM). Dimers have high haemolicity and exceptional antimicrobial activity. All adepantins structure as alpha helices when in contact with PG/dPG liposomes, while maintaining a random coil structure in the presence of PC/SM/Ch membranes. At sub-toxic concentrations,

dimers exhibit much higher permeabilization of both bacterial membranes in various E. coli strains than monomers. The "Designer" algorithm thus identified novel AMPs less than 50% homologous to any other natural or synthetic AMP, highly selective for Gram-negative bacteria and with a high TI (up to 400).

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19) Synthesis and efficiency evaluation of RND type efflux pump inhibitors

S. Lengvinaite, R. Daugelavicius - Department of Biochemistry and Biotechnologies, Vytautas Magnus University, Kaunas, Lithuania

Salmonella enterica serovar *Typhimurium* is the main cause of food poisoning and is the second most common cause of bacterial diarrhea [1-2]. RND-type multidrug resistance efflux pumps are the main reason of *Salmonella enterica* cell resistance to antibiotics. It is important to investigate the mechanism of efflux pump inhibition. The aim of our work was to synthesize small dipeptide inhibitors and to evaluate the efficiency RND-type efflux pump inhibition in S. enterica cells. In this work we present data on the electrochemical assay of accumulation of an indicator compound of efflux pumps in bacteria tetraphenylphosphonium (TPP^+) ions. We studied effects of the synthesized RND-type efflux pump inhibitors on accumulation of TPP^+ ions in S. enterica. Data on the effective concentrations of the inhibitors and the efficiency of inhibition of RND-type efflux pump activity will be presented.

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20) The effect of *D*-amino acids in the target cell selectivity of the frog skin peptide temporin L

Vincenzo Luca, Paolo Grieco, Alfonso Carotenuto, Luigia Auriemma, Maria Rosaria Saviello, Pietro Campiglia, Ludovica Marcellini, Maria Luisa Mangoni - Department of Biochemical Science, University of Rome La Sapienza

Temporin L (TL, 13-residues long) is a frog-skin peptide with a wide and potent spectrum of antimicrobial activity, but with a toxic effect on mammalian cells at its microbicidal concentration. Previous studies indicated [Pro3]TL as an analog with a slightly lower hemolytic activity than the native TL. Here, a systematic replacement of single residues within the alpha-helix domain of Pro3 TL (Lys7 to Leu13) with D aminoacids, known as helix breakers, has been carried out. Structure-activity relationship studies, by means of CD/NMR spectroscopy analysis and antimicrobial/hemolytic assays have been performed leading to a better understanding on the structural elements that are responsible for the cell selectivity of TL. Most importantly, we have found how a single L-to D aminoacid substitution can preserve the strong anti-candida activity of [Pro3]TL, making it completely harmless towards human cells.

21) Reduced phospohorylation of LPS decreases E. coli susceptibility to the human cathelicidin LL-37.

M. Mardirossian, K. Bociek, S. Ferluga, M. Benincasa, M. Scocchi and R. Gennaro - Department of Life Sciences, University of Trieste, Italy

The human cathelicidin LL-37 is an α-helical, cationic antimicrobial peptide (AMP) showing both direct antibacterial activity and multifunctional immunomodulatory capacities. Like many other AMPs, this peptide kills bacteria primarily by altering membrane integrity, even thought its exact mechanism of action is still undefined. To increase our knowledge on the bacterial component involved in the mode of action of LL-37, we screened an E. coli knock-out mutant library in search for mutations conferring altered susceptibility to this peptide. As first, a library was created by random insertion of the Tn5 transposon into the bacterial genome. Secondary, mutants with decreased susceptibility to LL-37 were selected by plating E. coli cells of the bank on peptide-supplemented solid medium. The different susceptibility among the wild-type and mutant cells was assessed by measuring the effect of the peptide on the kinetics of bacterial growth, on the kinetics of bacterial killing, and evaluating, using flow citometry, the LL-37's binding to bacteria and its effect on their integrity.

In 15 out of 20 LL-37-resistant mutants, the Tn5 trasposon was found to be inserted into the waaY gene, which encodes for a specific kinase responsible of the phosphorylation of the Hep II residue in the core region of bacterial lipopolysaccharide (LPS). This modification decreased the peptide's ability to kill bacterial cells, correlating with a reduced binding to bacteria and with a decreased membrane permeabilisation. Susceptibility of these mutants to several other AMPs of different structural classes was however unaffected, suggesting a specific and selective link between the gene waaY and the mechanism of action of LL-37. Summarising, waaY inactivation determines a decreased antibacterial activity. The results reveal a putative LPS-binding site for LL-37 and stress the importance of the electrostatic interactions between bacterial surface and antimicrobial peptides.

22) DADP: the Database of Anuran Defense Peptides

Mario Novković[1], Juraj Simunić[1], Viktor Bojović[2], Alessandro Tossi[3] and Davor Juretić[1]
Department of Physics, Faculty of Science, University of Split and [2]Centre for Informatics and Computing , Ruđer Bošković Institute, Zagreb, Croatia; [3]Department of Life Sciences, University of Trieste, Italy

Anuran tissues, and especially skin, are a rich source of bioactive peptides and their precursors. As potential antibiotics, host defense peptides from amphibian species are a fast growing group in the UniProtKB database. We here present a manually curated database (http://split4.pmfst.hr/dadp/) of antimicrobial and other defense peptides with a total of 2571 entries, 1766 full precursors with demarcated signal peptide (SP), acidic proregion(s) and bioactive moiety(s) and 805 bioactive defense peptides, encompassing a total of 167 anuran species from 12 families [1]. Each DADP entry ID contains detailed information about peptide names and sequences, tripartite structure, taxonomy, biogeography data (ecozone and distribution), SP class, tissue, biological function, links to UniProtKB, references and activity data in the form of minimal inhibitory concentration (MIC, μM) for *E. coli*

and/or S. aureus and concentration required for 50% haemolysis of red blood cells (HC50). Precursor structure is informative about origin, function and evolution of anuran host defense peptides, while more than 900 mature peptides with known MIC values in DADP are a valuable resource for structure-activity studies. The DADP focus on precursors and their conserved SPs has allowed the individuation of six distinct SP classes. The more conserved of these can be used for searching cDNA, UniProtKB or other protein databases for novel bioactive peptides, for creating PROSITE search patterns, and for phylogenetic analysis.

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23) Cyclic lipopeptides: design, synthesis and antimicrobial activity

Sílvia Vilà, Cristina Orozco, Esther Badosa, Emilio Montesinos, Lidia Feliu and Marta Planas - LIPPSO, University of Girona, Spain

Antimicrobial peptides are secondary metabolites isolated from a wide range of organisms and have been shown to be effective against plant pathogenic bacteria and fungi. These peptides are considered a good alternative to traditional antibiotics for plant disease control. Among antimicrobial peptides, cyclolipopeptides, such as surfactins, fengycins or iturins, are produced by several plant-associated and soil-inhibiting bacteria, and display antifungal, antibacterial, and cytotoxic activities. For these compounds, it has been observed that the presence of a hydrophobic tail in their structure favours their insertion into the cytoplasmatic membrane and, therefore, improves their activity.

Recently, we have identified antimicrobial peptides active against the phytopathogenic bacteria and fungi. In particular, de novo designed cyclic peptides, with the general structure cyclo(X3–Phe–X5–Gln) where X = Lys or Leu, have been prepared and screened for their biological activity. The most active peptide was c(KKLKKFKKLQ) (BPC194) which also displayed low hemolysis. With the aim of improving the biological activity of BPC194, in this work, this cyclic peptide was derivatized with different fatty acids. For this purpose, one or two lysine residues present in the sequence were acylated. The resulting cyclolipopeptides were evaluated in vitro against phytopathogenic bacterial and fungal strains, and assayed for their hemolytic activity.

24) Specific conformational dynamics predefines high selectivity of lantibiotics to lipid II and provides opportunities for design of novel potent antimicrobials

Anton Polyansky, Pavel Volynsky, Roman Efremov - Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna & M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

Lantibiotics represent a class of highly specific antimicrobial peptides isolated from bacteria. Here we present systematic *in silico* study of interaction a well-known lantibiotic nisin A with its membrane target Lipid II. We have shown that backbone

dynamics of the peptide is strongly constrained by a number of its modified aminoacids and defines selective recognition of the pyrophosphate group of Lipid II. Based on this we have proposed a framework for design of nisin mimics, which can be used in future development of novel antimicrobials.

25) A novel dendrimeric peptide with antimicrobial properties: structure-function analysis of SB056

Scorciapino MA, Pirri G, Vargiu AV, Ruggerone P, Giuliani A, Casu M, Buerck J, Wadhwani P, Ulrich AS, Rinaldi AC - Department. of Biomedical Sciences, University of Cagliari, Italy

The novel antimicrobial peptide with a dimeric dendrimer scaffold, SB056, was empirically optimized by high-throughput screening. This procedure produced an intriguing primary sequence whose structure-function analysis is described here. The alternating pattern of hydrophilic and hydrophobic amino acids suggests the possibility that SB056 is a membrane-active peptide that forms amphiphilic β-strands in a lipid environment. Circular dichroism confirmed that the cationic SB056 folds as β-sheets in the presence of anionic vesicles. Lipid monolayer surface pressure experiments revealed unusual kinetics of monolayer penetration, which suggest lipid-induced aggregation as a membranolytic mechanism. NMR analyses of the linear monomer and the dendrimeric SB056 in water and in 30% trifluoroethanol, on the other hand, yielded essentially unstructured conformations, supporting the excellent solubility and storage properties of this compound. However, simulated annealing showed that many residues lie in the β-region of the Ramachandran plot, and molecular-dynamics simulations confirmed the propensity of this peptide to fold as a β-type conformation. The excellent solubility in water and the lipid-induced oligomerization characteristics of this peptide thus shed light on its mechanism of antimicrobial action, which may also be relevant for systems that can form toxic β-amyloid fibrils when in contact with cellular membranes. Functionally, SB056 showed high activity against Gram-negative bacteria and some limited activity against Gram-positive bacteria. Its potency against Gram-negative strains was comparable (on a molar basis) to that of colistin and polymyxin B, with an even broader spectrum of activity than numerous other reference compounds.

26) Chitosan-carrageenan based polymeric bioabsorbable systems loaded with antimicrobial peptides in the management of ascendent cholangytes: an overview

[1]A. Romani, [2]C.S. Cabassi - [1]University Hospital Parma, and Department of Animal Health, Animal Infective Diseases section, University of Parma, Italy

Treatment of neoplastic or flogistic diseases that cause biliary duct stenosis involves extrahepatic biliary duct resection and anastomosis between bile duct stump and intestine (Roux-en-Y hepaticojeunostomy). Often this treatment is burden with septic complications (cholangites) and anastomotic stenosis. Although mortality of no-treated cholangites is near 100%, with proper treatment it is close to 20-25%. Similar complications occur in the chronic allograft liver rejection.

Our research group have recently developed a multilayered bioabsorbable chitosan-based prosthesis recently patented (MI2011/3425A) granted by Regione Emilia-Romagna, Programma di Ricerca Regione-Università,2007-2009. During the last three years the research group have obtained significant results about the implantation of this prosthesis in preclinical trials on pigs. The results showed the efficient and complete biological substitution of prosthesis within 6 months.

In an effort to reduce the occurrence of septic complication, the inner layer of prosthesis was modified to obtain a composite film loaded with antimicrobial peptides for delayed drug release. This composite film was based on chitosan and carrageenan solution. Carrageenans are water soluble polymers extracted from red algae. They are used in food and pharmaceutical industries as gelling, stabilizing agents and immobilization of drugs and enzymes. Several isomers of carrageenan are known as k-, l-, and i-carrageenan. The gelling power of K-and L-carrageenans impart excellent film forming properties.

The k-carrageenan/chitosan composite films were prepared by dissolving the carrageenan in distilled water and chitosan solutions was prepared as described in patent WO2008/077949. The composite films were prepared in antimicrobial peptide/k-carrageenan:chitosan optimal proportion. The film was obtained as described in WO2008/077949.

The dried composite films were cutted in 5mm-diameter disks and placed in flat-bottom 96-multiwell plates. 50UL of *E.coli* strain ATCC 25922 suspension was layered onto composite disk. Every 24h, the disk was transferred into another well and 50 uL of fresh bacterial suspension was added. The antimicrobial activity was evaluated by transferring 20 uL of bacterial suspension onto McConkey agar plates for CFU count. After 24h of incubation at 37°C the MIC and LD_{90} values were obtained.

The preliminary results showed a significant reduction of 90-100% CFU load (vs control) after two hours of incubation. The drug releasing was sustained for the following two weeks with a CFU reduction ranging from 50% (vs control) to 85%. This promising results will be further investigated. (granted by Regione Emilia-Romagna, Programma di Ricerca Regione-Università,2007-2009)

27) New insights into the architecture and function of SbmA, an unusual transporter of the inner membrane of *E. coli*

G. Runti, T. Stoilova, M. Benincasa, R. Gennaro, M. Scocchi - Department of Life Sciences, University of Trieste

SbmA/BacA is a protein of the inner membrane of many Gram-negative bacteria, predicted to be the transmembrane domain of an ABC transport system. sbmA-deleted E. coli strain shows an increased resistance to the antimicrobial peptide Bac7 and other proline-rich peptides due to their reduced uptake. In addition, BacA of some Gram-negative species is essential to establish chronic infection in animal and plant hosts.

The aim of this study was to characterize the architecture and function of the SbmA transport system in E. coli. By affinity chromatography and fluorescence anisotropy assays we showed that SbmA is able to bind Bac7 also without the

requirement of a substrate-binding protein. By using a bacterial two-hybrid system and in vitro cross-linking assay we showed that SbmA forms dimers both *in vivo* and *in vitro*. Flow cytometry analysis using different metabolic inhibitors suggests that the proton motive force rather than ATP hydrolysis represents the driving force for the translocation of peptide substrates.

Searching for possible interactors of SbmA, we showed that the *yaiW* gene, located downstream of *sbmA*, is part of the same operon. The two genes are cotranscribed but an interaction between the corresponding proteins has not been detected neither at the cytoplasmic nor at the inner membrane level. However, *ΔyaiW* strain showed an increased resistance to Bac7 suggesting the involvement of this protein in the SbmA-mediated uptake of the peptide.

Overall, these findings suggest that SbmA is an unusual peptide transporter, likely derived from the widespread ABC transporters, which has evolutionarily lost/changed some typical features of this transport systems, such as the requirement of a substrate-binding protein and the energizing source.

28) *In vitro* activity of cathelicidins against azole-susceptible and -resistant human pathogenic Candida species

M. Scarsini, L. Tomasinsig, , B. Skerlavaj, A. Arzese and M. Zanetti - Department of Medical and Biological Sciences, University of Udine

The antimicrobial peptides (AMPs) of innate immunity exert potent and broad-spectrum antimicrobial activity and have low propensity to select resistant mutants. Some (e.g., the human cathelicidin LL-37) may stimulate additional defence responses, making AMPs attractive candidates for development as novel antiinfective agents combining antimicrobial and immunostimulating properties.

Candida spp are the most common cause of vaginal infection, affecting 75% of fertile age women with 5-10% incidence of recurrent infections. *C. albicans* is by far the most common species responsible for clinical infections although, less frequent species may cause recurrent and persistent infections. The decreased susceptibility of *Candida* spp to currently used antifungals is mainly due to the biofilm-forming ability.

In this study we have compared the anti-Candida activities of LL-37 and LL-37 orthologs with those of conventional antifungals, using microdilution susceptibility and XTT reduction assays and optical microscopy. The LL-37 ortholog BMAP-28 was found to be highly effective against 27 vaginal isolates of *Candida* spp. (MIC50, 4 microM) also including azole-resistant strains, and against reference C. albicans strains in planktonic and biofilm states, in standard media and in simulated vaginal fluid. LL-37 was less effective than BMAP-28 as fungicidal (MIC50, 64 microM) although, it prevented Candida biofilm growth by inhibiting cell adhesion to medical grade silicone surface. Ongoing toxicity studies on vaginal epithelial cells will allow the selection of peptides with the highest anti-Candida activity and lowest cytotoxicity.

29) Study of the toxic action of a proline-rich antimicrobial peptide ChBac3.4 on mammalian cells

O.Shamova, D.Orlov, T.Pazina, V.Kokryakov - Institute for Experimental Medicine Russian Accademy of Medical Sciences

ChBac3.4 is a proline-rich peptide from goat leukocytes. This peptide appears to differ from structurally similar ChBac5 and other proline-rich cathelicidins in virtue of its increased ability to damage microbial membranes and to exert toxic effects for mammalian cells. We explored the cytotoxic activity of ChBac3.4 towards selected tumor and normal human cultured cells in comparison with that of membranolytic protegrin 1 (PG1). Unlike PG1, even high concentrations of ChBac3.4 were not significantly hemolytic for human erythrocytes. But ChBac3.4 was selectively cytotoxic, damaging human K562 erythroleukemia cells, U937 hystiocytic lymphoma cells, HL60 promyelocytic leukemia but not normal human skin fibroblasts and some other human target cells. The toxic action of PG1 had been occurred within 15-20 min, while ChBac3.4 demonstrated more delayed effects on cells viability. An increase of caspase 3 activity was observed in U937 cells and to a much lesser degree in K-562 cells treated with varied concentrations of ChBac3.4. After an incubation of U-937 cells with the peptide a significant amount of apoptotic cells was revealed using Annexin V-Cy3 apoptosis detection kit; in the case of K-562 line most of the cells exposed features of necrosis. We have not observed any signs of apoptosis in both types of target cells treated with PG1 in the range of concentration 1.25 – 40 microM. The proline-rich peptide ChBac3.4 warrants further study, especially with respect to its various effects on tumor and normal mammalian cells, and its antineoplastic potential.

30) Membrane-perturbing effects of antimicrobial peptides: a systematic spectroscopic analysis

D. Roversi[1], L. Giordano[1], M. De Zotti[2], G. Bocchinfuso[1], A. Farrotti[1], S. Bobone[1], A. Palleschi[1], Y. Park[3], K.-S. Hahm[4], F. Formaggio[2], C.Toniolo[2], L. Stella[1] - [1]Department of Chemical Sciences and Technologies, University of Rome "Tor Vergata"; [2]ICB, Padova Unit, CNR, Department of Chemistry, University of Padova; [3]Department of Biotechnology, Chosun UniversityGwangju, Korea; [4]BioLeaders Corporation, Daejeon, Korea

Antimicrobial peptides (AMPs) exhibit a strong activity against a wide range of microorganisms, mainly by perturbing the permeability of bacterial membranes through the formation of pores. However, AMPs effects on membrane properties probably extend beyond pore-formation. We performed a systematic spectroscopic analysis of the effects on membrane structure and dynamics of two very different AMPs: the cationic PMAP-23, which creates pores according to the “carpet” model,1 and alamethicin, which forms “barrel-stave” channels.2 By using fluorescence anisotropy measurements on liposomes comprising probes localized at different depths in the bilayer, we measured peptide effects on membrane fluidity and order. Laurdan spectral shifts provided indications about water penetration in the bilayer. In the case of PMAP-23, it was possible to focus specifically on the lipids surrounding the peptide by following the membrane-probe fluorescence due to FRET from the

peptide Trp residues. Finally, peptide-induced perturbation of lateral mobility and domain formation were determined by several methods. All experiments were compared with liposome-leakage measurements: while for PMAP-23 all membrane-perturbing effects are correlated with the vesicle leakage process, alamethicin does not significantly influence membrane dynamics at the concentrations in which it forms pores. Surprisingly, in all cases the most significant peptide-induced effect is a reduction in membrane fluidity.

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31) Short Peptaibiotics as New Antitumor Agents

Marta De Zotti[1], Barbara Biondi[1], Fernando Formaggio[1], Cristina Peggion[1], Emanuele Papini[2] and Regina Tavano[2] - [1]ICB CNR, Padova Unit, Department of Chemistry, University of Padova, 35131 Padova, Italy; [2]Department of Biomedical Experimental Sciences, University of Padova, 35121 Padova, Italy

Trichogin GA IV, first isolated from the fungus *Trichoderma longibrachiatum* [1], is the prototype of lipopeptaibols, a sub-class of short-length peptaibiotics exhibiting membrane-modifying properties. The primary structure of the 10-amino acid peptide trichogin GA IV is as follows:

n-Oct-Aib1-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile10-Lol

where n-Oct is n-octanoyl, Aib is α-aminoisobutyric acid, and Lol is the 1,2-amino alcohol leucinol. In this work, we synthesized, purified, and fully characterized a set of Trichogin GA IV analogues in which the four Gly residues at positions 2, 5, 6, and 9, lying on the poorly hydrophilic face of the helical structure, are substituted by one (position 2 or 9), two (positions 5 and 6), three (positions 2, 5, and 9), and four (positions 2, 5, 6, and 9) strongly hydrophilic Lys residues. Another analogue with the triple Lys replacement, characterized by the incorporation of an additional helix-inducing Aib residue at position 6, was also prepared.

It is well known that Trichogin GA IV and its synthetic analogues are membrane active and that they can induce liposomal permeabilization. In cellular systems red blood cell (rbc) haemolysis is a well characterized phenomenon linked to membrane destabilization. In addition, cell death induction (cytocidal effect) may represent an effective functional assay, since permeabilizing peptides able to form pores or to alter the lipid bilayer of the plasma membrane are supposed to induce cell necrosis.

To study the effects of Trichogin GA IV on eukaryotic cells, several variants of the wt Trichogin GA IV have been tested using three in vitro cell-based assays: i. human rbc lysis ii. cell mortality assays in total human blood leukocytes and in separate sub-populations (PMNs, monocytes, macrophages, lymphocytes); iii. cell mortality of four tumor-derived stable cell lines (HeLa, A541, A431, HL60).

The goal was the understanding of the structural features required for cellular cytocidal effects and the identification of peptides targeting tumor-derived cells, but devoid of haemolytic/leukocydal potentially dangerous side effects, in the

perspective of use of Trichogin analogues as anti-tumor drugs. It appears that tumor cells are affected by almost all of our Trichogin analogues, while some of them leave the leukocytes unaffected.

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32) Short-term interaction of the human peptide beta-Defensin 2 (hBD2) with dendritic cells explored by SR-IRMS

Francesca Morgera, Sabrina Pacor, Luisa Creatti, Nikolinka Antcheva, Alessandro Tossi, Lisa Vaccari - Department of Life Sciences, University of Trieste and SISSI Beamline, Elettra Sincrotrone S C.p.A, Trieste, Italy

Beta-Defensins are antimicrobial peptides that exert their host-defense functions at the interface between the host and microbial biota. They display a direct, salt and medium sensitive cidal activity, in vitro, against a broad spectrum of bacteria and fungi and there is increasing evidence that they also play a role in alerting and enhancing cellular components of innate and adaptive immunity. Their interaction with biological membranes plays a central role in both these types of activities. In this study we have investigated the interaction of fluorescently labelled human beta-defensin 2 (hBD2) with monocytes, macrophages and immature dendritic cells, observing a differential capacity to be rapidly internalised into these cells. Complementary microscopy techniques (TEM, optical and synchrotron radiation infrared microspectroscopy) were used to explore the functional and biological implications of these interactions on immature dendritic cells. Short-term exposure to the peptide resulted in significant alterations in membrane composition and re-organization of the endomembrane system, with induction of degranulation. These events may be associated with the antigen-presenting activities or the chemotaxis of iDC, which appears to occur via both CCR6-dependent and independent mechanisms.

33) The human cathelicidin LL-37: effect of self-aggregation on the mechanism of action

Daniela Xhindoli , Nikolinka Antcheva, Alessandro Tossi - Department of Life Sciences, University of Trieste, Italy

LL-37 is the only member of the cathelicidin family of the antimicrobial peptides, found in humans, and is an important multifunctional component of the innate immune system. Its direct antimicrobial activity is reportedly due to its ability to interact with and to permeabilize bacterial membranes by a detergent-like “carpet” mechanism or to the formation of discreet channels. Studies on the evolution of LL-37 orthologues in primates [1] indicate an important role for oligomerization and aggregation that need to be further investigated to elucidate its mode of action. Therefore, we have synthesized a photoreactive analog by replacing the Phe in the 5th position with the unnatural amino acid, p-Benzoyl-L-Phenylalanine (Bpa). Irradiation with UV light enables covalent cross-linking, so that self-assembly of the LL-37 monomer in PIL buffer could be somehow quantified.

Another strategy was to synthesize three different disulfide-linked LL-37 dimers by adding a Cys residue to either the C- or N-termini, (C- or N-terminal-linked parallel,

and antiparallel dimers) as obligatorily aggregated forms. SAR studies revealed that the dimeric forms have an increased propensity to form stacked helices in bulk-solution and when in contact with model membranes, and act differently on both bacterial and host cells. We found a lower antimicrobial activity for dimers against both Gram-positive and Gram-negative bacteria, so that the generally lower antimicrobial activity of LL-37 with respect to its orthologues in the primate such as RL-37 may derive from its greater tendency to aggregate. The haemolytic effect was different amongst the diverse dimeric forms and particularly high for C-terminal dimer.

Surface Plasmon Resonance was used to study both its self-aggregation and interaction with zwitterionic or anionic LUVs. Membrane interaction was found to be quite different from that of the mmuRL-37, which tends not to aggregate under the conditions used. This comfirmed the importance of conformation and self-aggregation on the mode of action and biological activity of LL-37.

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34) Novel antimicrobial peptides from marine invertebrates

Tatiana V. Ovchinnikova[1], Zakhar O. Shenkarev[1], Sergey V. Balandin[1], Pavel V. Panteleev[1], Ekaterina I. Finkina[1], Alexander S. Paramonov[1], Dmitry A. Altukhov[1], Albina K. Gisatulina[1], Vladimir N. Kokryakov[2], Alexander S. Arseniev[1] - [1]Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya str., 16/10, 117997 Moscow, Russia, [2]Institute of Experimental Medicine, Russian Academy of Medical Sciences, Academica Pavlova str., 12, 197376 Saint-Petersburg, Russia*

Marine invertebrates lack an acquired immunity with a system of antibody diversification and rely solely on innate immune mechanisms. The survival of marine invertebrate animals in microbe-laden environment suggests that their innate immune system is effective and robust. Antimicrobial peptides (AMP) are a major component of the innate immune defense system in marine invertebrates. Novel AMP from marine organisms could afford design of new antibiotics manifesting broad-spectrum antibacterial activity in the presence of physiological or elevated NaCl concentrations.

A new family of small (21-residue) antimicrobial peptides, termed arenicins, was discovered in coelomocytes of marine polychaeta lugworm *Arenicola marina*. These AMP exhibit activity against Gram-positive, Gram-negative bacteria and fungi. Molecular masses (2758.3 Da and 2772.3 Da) and complete amino acid sequences were determined for each isoform. Arenicins have one disulfide bond (Cys3-Cys20). In order to determine a high-resolution three-dimensional structure of arenicin-2, the recombinant peptide was overexpressed as a fused form in *Escherichia coli*. Arenicin-2 in aqueous solution adopts the significantly twisted β-hairpin conformation without pronounced amphipathicity. To assess the mechanism of arenicin action, the spatial structure and backbone dynamics of the peptide in membrane-mimicking media were studied.

A novel 40-residue antimicrobial peptide, aurelin, exhibiting activity against Gram-positive and Gram-negative bacteria, was purified from the mesoglea of a

scyphoid jellyfish *Aurelia aurita*. Molecular mass (4296.95 Da) and complete amino acid sequence of aurelin were determined. Aurelin has six cysteines forming three disulfide bonds. To our knowledge, aurelin is the first AMP isolated from cnidarians (class Scyphozoa). Aurelin reveals partial similarity both with defensins and K+ channel blocking toxins of sea anemones and belongs to ShKT domain family. Overlapping of biological properties of marine animal AMP and toxins along with their sequence homology might be a consequence of divergent evolution from a common ancestor. Recombinant aurelin and its 15N-labeled analogue were overexpressed in *E. coli* and purified. The spatial structure, backbone dynamics and interaction of aurelin with lipid vesicles and detergent micelles were studied.

35) Trans2Care project

Sabina Passamonti, Renato Gennaro - Department of Life Sciences, University of Trieste, Italy

Trans2Care is a joint project of academic, research, healthcare and technology transfer institutions from Italy and Slovenia, with University of Trieste as leading partner. The network works in tight cooperation with industry and end-users, in order to address unmet medical needs.

Trans2Care's mission is to promote knowledge dissemination and technology transfer, favouring innovative practices and products for disease prevention, early diagnosis, personalized therapies.

The objectives are to set up a shared, cross-border network of institutions dedicated to the permanent development of new protocols and biotechnological devices for the prevention, early diagnosis and cure of neurodegenerative, cardiovascular, orthopaedic, infective and oncologic diseases.

Within Trans2care, groups at Trieste and Udine Universities and Valdotra Orthopaedic hospital are working on the development and transfer of novel applications for antimicrobials.

36) Innovaqua project

Michela Furlan, Lucia Corich, Marco Scocchi and Sabina Passamonti - Department of Life Sciences, University of Trieste, Italy.

Innovaqua is a network of 8 partners, including academic institutions (Universities of Trieste - Lead Partner, Udine, Padova and Nova Gorica), research institutions (Istituto Zooprofilattico delle Venezie, Slovenian Chamber of Agriculture and Forestry) and companies related to aquaculture (Vodomec d.o.o., Shoreline soc. coop.).

The project coordinates the sharing knowledge and training young researchers on various aspects of fish farming, including management and prophylaxis of infectious diseases, actively involving them in knowledge transfer to fish farmers.

The human resources involved in the project include team managers, researchers and industry experts, and are divided into working groups that share technologies and research methodologies. Four of these are collaborating in the development of endogenous antimicrobial peptides (AMPs) from trout and sea bass as novel vaccine

adjuvants for use in the control of infectious diseases in economically important fish stocks.

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