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Antimicrobial peptides based on natural marine antimicrobials:
Design and study of their mode of action

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FOREWORD

We are pleased to welcome you to the third Peptide Chemistry Day symposium, organized at the University of Zagreb. Following the great scientific and professional success of the first two meetings, the Organizing Committee aims to establish Peptide Chemistry Day as a biennial symposium—an event that brings together everyone who already loves peptides, or who will soon fall in love with them!

Peptide science, including peptide-based therapeutics, continues to be an innovative strategy for expanding biopharmaceutical portfolios. This symposium aims to bring together prominent researchers from across Croatia, foster discussions on various aspects of peptide chemistry, and highlight the ongoing scientific contributions of Croatian researchers to this dynamic field. Special attention will be given to young scientists by providing them with a platform to share their work, connect with experts, and contribute to the growing peptide research community.

The symposium is jointly organized by the University of Zagreb, the Croatian Chemical Society, and the Ruđer Bošković Institute. We are grateful for the generous support of our sponsors: the European Peptide Society, BICRO BioCentre, and Labtim d.o.o.

We are delighted to have Professor Anna Maria Papini and Professor Paolo Rovero from the University of Florence delivering plenary keynote lectures. We are also honored to welcome Professor Žiga Jakopin, Professor Larisa Zoranić, Professor Gordan Horvat, Dr. Andrea Kišić Rašeta, and Dr. Mihaela Matovina—who will deliver invited lectures and share their expertise and innovative research in peptide chemistry.

We would like to extend our heartfelt thanks to all speakers and participants for sharing their scientific achievements and contributing to the success of this meeting.

Special thanks go to our colleagues from the University of Zagreb—Arijana, Sandra, Maja, Bojana, Marcela, and Danijel—for their invaluable help in preparing promotional materials, managing registration, and spreading positive energy among organizers and attendees.

We sincerely hope you enjoy the friendly atmosphere and take full advantage of the opportunities for meaningful social and scientific exchange at this meeting.

We are already looking forward to the engaging presentations and discussions at the 2027 symposium.

Ruža Frkanec

President of the Organising Committee

PROGRAMME

8:30 – 9:00	**Registration**
09:00 – 9:15	**OPENING ADDRESSES**
09:15 – 10:00	**Prof. Anna Maria Papini**, University of Florence, Department of Chemistry "Ugo Schiff", Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology *From chemical immunology to medicinal chemistry: the power of peptides to elucidate the mystery of molecular mimicry*
10:00 – 10:25	**Prof. Žiga Jakopin**, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Ljubljana *Distinctive Immune Signatures Driven by Structural Alterations in Desmuramylpeptide NOD2 Agonists*
10:25 – 11:00	**COFFEE BREAK / POSTER SESSION**
11:00 – 11:40	**SHORT TALKS** **Marcela Šišić**, mag. chem., University of Zagreb, Center for Research and Knowledge Transfer in Biotechnology *Biomimetic Nanomaterials Functionalized with Peptidoglycan Monomer: Studying Carbohydrate-Mediated Interactions for Biomedical Use* **Lea Pašalić**, mag. chem., Ruđer Bošković Institute, Department of Organic Chemistry and Biochemistry, Laboratory for Engineering of Biomembranes, Zagreb *Cationic-Hydrophobic Peptides and Their Impact on Model Eukaryotic and Prokaryotic Lipid Membranes* **Nurul Azmiera Zamri**, M Sc Med, Department of Molecular Biosciences, Faculty of Natural Sciences, University of Graz *The Antimicrobial Activity and Cytotoxicity Effects of Antimicrobial Peptide Isolated from the Haemolymph of Blow Fly, Chrysomya megacephala* **SPONSOR: Denisa Krevh**, sales representative, Labtim d.o.o. *From Sequence to Sample: Biotage Tools for Efficient Peptide Synthesis and Purification*
11:40 – 12:05	**Prof. Larisa Zoranić**, Department of Physics, Faculty of Science, University of Split *Antimicrobial Peptides in the Fight Against Bacterial Resistance: Progress and Challenges Ahead*
12:05 – 12:30	**Dr. Dubravka Gembarovski, Andrea Kišić Rašeta**, PLIVA, Croatia *Impurity profile characterization of therapeutic peptide using 2D-LC/HRMS*
12:30 – 13:45	**LUNCH BREAK / POSTER SESSION**
13:45 – 14:30	**Prof. Paolo Rovero**, University of Florence, Department of NeuroFarBa, Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology *Bioactive peptides as valuable cosmeceutical ingredients*
14:30 – 14:55	**Prof. Gordan Horvat**, Department of Chemistry, Faculty of Science, University of Zagreb *Anion-binding properties of small cyclopeptides in solution*
14:55 – 15:20	**Dr. Mihaela Matovina**, Ruđer Bošković Institute, Department of Organic Chemistry and Biochemistry, Laboratory for Protein Biochemistry and Molecular Modeling *Unraveling the Role of Dipeptidyl Peptidase 3 in Physiology and Pathology*
15:20 – 15:45	**COFFEE BREAK / POSTER SESSION**
15:45 – 16:00	**Dr. Mladena Glavaš**, Ruđer Bošković Institute, Department of Organic Chemistry and Biochemistry, Laboratory for synthetic Chemsitry, Zagreb *Synthesis and characterisation of peptides with BODIPY fluorophore*
16:00 – 16:15	**Ena Dražić**, mag. med. chem., Faculty of Biotechnology and Drug Development, University of Rijeka *Fmoc-Amino Acid to Supramolecular Hydrogels*
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LECTURES

FROM CHEMICAL IMMUNOLOGY TO MEDICINAL CHEMISTRY: THE POWER OF PEPTIDES TO ELUCIDATE THE MYSTERY OF MOLECULAR MIMICRY

Anna Maria Papini

Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology and Molecular Diagnostics & Life Sciences Centre of Competences
Department of Chemistry "Ugo Schiff", University of Florence
50019 Sesto Fiorentino, Italy

annamaria.papini@unifi.it

Peptides are natural and sustainable products with important and favorable biological properties. They gained increasing attention as innovative and highly useful products in different domains such as pharma, diagnostics, personal care, but also materials. In particular, peptide therapeutics have become one of the hottest topics in pharmaceutical research and indeed the global peptide therapeutic market is expected to reach over 50 billion USD in 2026 (http://www.mordorintelligence.com). This rising interest in peptides is based on many advantages they share compared to protein therapeutics or small molecule drugs, e.g. they present higher potency and specificity than small molecules and lower immunogenic potential and lower manufacturing costs compared to proteins. In addition, robust synthetic strategies not only at the laboratory but also at the large manufacturing scale have been developed as required at the industrial level.

In the pharma arena, the experience of PeptLab at the University of Florence (www.peptlab.unifi.it) ranges from the development of a technology platform for the design and synthesis of side chain-to-side chain stapled peptide analogues as antiviral and/or antibacterial drug candidates [1-10], to supporting API producers in the efficient and GMP-complaint large scale preparation of peptide therapeutics [11,12]. In the field of in vitro diagnostics and biomarkers discovery, fundamental for personalized medicine, we developed peptide probes for characterization of: antibodies as biomarkers of autoimmune diseases [13-15], cross-reactivity with pre-COVID-19 samples from malaria-endemic areas [16], anti-drug antibodies in patients treated with biologics [17,18].

The design of our peptide-based bioactive compounds of pharmaceutical and diagnostic interest has been always accompanied on their production using sustainable synthetic methodologies [19,20], aiming to transfer technology to relevant stakeholders to move the field beyond the state of the art and to launch new start-up companies.

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DISTINCTIVE IMMUNE SIGNATURES DRIVEN BY STRUCTURAL ALTERATIONS IN DESMURAMYLPEPTIDE NOD2 AGONISTS

Žiga Jakopin

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

ziga.jakopin@ffa.uni-lj.si

We report on the design, synthesis and biological evaluation of a series of desmuramylpeptide NOD2 agonists. Structural prerequisites that shape both physico-chemical and immunomodulatory profiles of desmuramylpeptide NOD2 agonists have been delineated. We identified **3**, a butyrylated desmuramylpeptide, as a potent *in vitro* NOD2 agonist (EC50 = 4.6 nM), exhibiting a 17-fold enhancement in potency compared to unsubstituted counterpart **1** (EC50 = 77.0 nM). Novel set of desmuramylpeptides demonstrate unique *in vitro* immunomodulatory activities. They elicited cytokine production in peripheral blood mononuclear cells (PBMCs) in conjunction with lipopolysaccharide (LPS). Spermine-decorated **32** also stimulated LPS-induced cytotoxic activity (2.95-fold) of PBMCs against K562 cancer cells. Cholesterol-conjugate **26** displayed anti-inflammatory actions, highlighted by its capacity to convert the inflammatory monocytes into anti-inflammatory phenotype, and eicosapentaenoylated **23** augmented antigen presentation by mouse bone marrow-derived dendritic cells (BMDCs), thus highlighting its potential as a vaccine adjuvant.

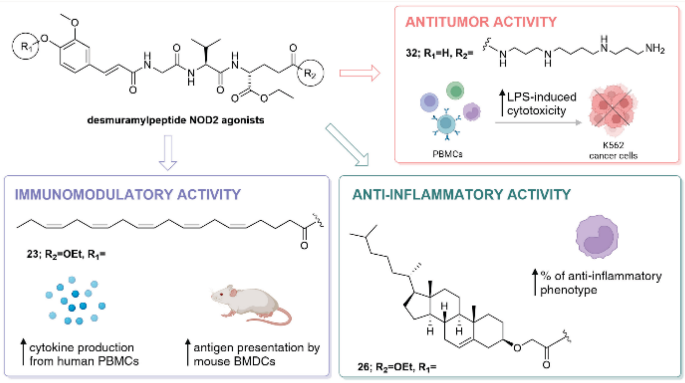

Figure 1. Structural prerequisites that shape immunomodulatory profiles of desmuramyl-peptide NOD2 agonists

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BIOMIMETIC NANOMATERIALS FUNCTIONALIZED WITH PEPTIDOGLYCAN MONOMER: STUDYING CARBOHYDRATE-MEDIATED INTERACTIONS FOR BIOMEDICAL USE

Marcela Šišić,[a,*] Leo Frkanec,[b] Ruža Frkanec[a]

[a] Centre for Research and Knowledge Transfer in Biochemistry, University of Zagreb, Rockefellerova 10, Zagreb, Croatia

[b] Ruđer Bošković Institute, Bijenička c. 52, Zagreb, Croatia

* msisic@unizg.hr

Biomimetic nanomaterials, which mimic the structure and function of natural biological systems, represent a versatile platform for a broad range of biomedical applications.[1] Peptidoglycan (PG), a major component of bacterial cell walls, plays a pivotal role in bacterial viability and in activating the host innate immune response. Due to its absence in higher organisms, PG represents a prototypical pathogen-associated molecular pattern (PAMP) recognized by pattern recognition receptors (PRRs). The peptidoglycan monomer (PGM), GlcNAc-MurNAc-L-Ala-D-isoGln-mesoDAP(ε-NH_2)-D-Ala-D-Ala, isolated from *B. divaricatum*, has demonstrated significant biological activity and serves as a promising molecular scaffold for the development of novel immunomodulatory and antimicrobial agents.[2] This study focuses on the design and synthesis of biomimetic nanomaterials functionalized with PGM and the investigation of their interactions with lectins. Liposomes and gold nanoparticles functionalized with PGM were prepared to enable multivalent glycan presentation for targeted interaction with specific lectins, aiming to elucidate structure–activity relationships. To explore these interactions, complementary label-free biophysical techniques, including isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR), were employed to determine binding kinetics and thermodynamic parameters. The results will deepen our understanding of how nanoscale glycan organization influences specific lectin interactions, thereby enhancing our insight into host–pathogen dynamics and directly supporting the development of novel antimicrobial and immunomodulatory strategies based on biomimetic nanomaterials.

ACKNOWLEDGMENTS

This work is supported by Croatian Science Foundation (IP-2018-01-6910).

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CATIONIC-HYDROPHOBIC PEPTIDES AND THEIR IMPACT ON MODEL EUKARYOTIC AND PROKARYOTIC LIPID MEMBRANES

Lea Pašalić,* Andreja Jakas, Danijela Bakarić

Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia

* lea.pasalic@irb.hr

The plasma membrane, one of the most fundamental parts of the cell, acts as an efficient barrier between the cell's interior and its environment, regulating the movement of substances in and out[1]. This selective permeability ensures the protection of the cell from harmful external factors, but it also poses significant challenges in drug delivery, as therapeutic agents often struggle to penetrate this protective barrier[2]. In this context, the design of cationic hydrophobic peptides offers a promising strategy to enhance membrane interaction and facilitate the delivery of therapeutic compounds. This study is focused on the automatic solid-phase synthesis of peptides (R5F2, K5F2, R5W2, K5W2) and their impact on both eukaryotic and prokaryotic model lipid membranes. The effects of these peptides were examined using differential scanning calorimetry (DSC), temperature-dependent UV-Vis spectroscopy and FTIR-ATR spectroscopy[3]. The DSC and UV-Vis data reveal changes in the phase transition temperatures of the model membranes, while FTIR-ATR spectroscopy offers complementary insights into the peptide-membrane interactions at the molecular level.

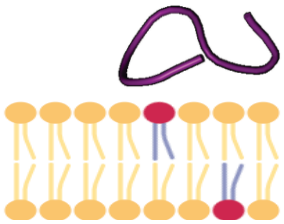

Figure 1. Schematic representation of a peptide- model membrane interaction

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THE ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF A NOVEL PEPTIDE ISOLATED FROM THE HAEMOLYMPH OF THE BLOW FLY, *CHRYSOMYA MEGACEPHALA* (DIPTERA: CALLIPHORIDAE)

Nurul Azmiera[a], Nermina Malanovic[b], Manfred Kriechbaum[c],Hassanain Al-Talib[a], Anna Krasilnikova[d,e], Shariza Sahudin[f,g], Noraziah Sahlan[a], Chong Chin Heo[a]

[a] Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia
[b] Institute of Molecular Biosciences, University of Graz, 8010 Graz, Austria
[c] Institute for Inorganic Chemistry, Graz University of Technology, Stremayrgasse 9, 8010, Graz, Austria
[d] Department of Pharmacology and Therapeutics, School of Medicine, International Medical University, 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000, Kuala Lumpur, Malaysia
[e] Department of Clinical Pharmacology and Intensive Care, Volgograd State Medical University, 1, Pl. Pavshikh Bortsov Square, Volgograd, 400131, Russia
[f] Department of Pharmaceutics, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Selangor, Malaysia
[g] Atta Ur Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Selangor, Malaysia

With the growing threat of antimicrobial resistance, there is an increasing demand for alternative therapeutic approaches. Insects that thrive in microbe-dense habitats are promising sources of novel antimicrobial peptides (AMPs). This study explores the haemolymph of *Chrysomya megacephala* larvae, which naturally inhabit decomposing environments, to identify potential AMPs. Around 200 larvae were infected with a clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) strain. The haemolymph was extracted and purified via Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). Active fractions were analysed using Quadrupole Time-of-Flight Liquid Chromatography Mass Spectrometry (QTOF-LCMS), and peptide sequences were determined through *de novo* analysis. A key peptide (HGCGRLSKWFRQPGLLLSVKR) was identified, showing 100% sequence similarity with *C. megacephala* heat shock protein 70 and *Lucilia cuprina* glycerophosphocholine phosphodiesterase, and 39.13% similarity with the synthetic peptide GNU17. This peptide was synthesised and tested against Gram-positive pathogens including MRSA, *S. aureus*, *M. luteus*, *S. epidermidis*, and *B. subtilis*, with inhibition zones of 8–11 mm at 1 mg/mL. MIC values ranged from 2.5 to 10 mg/L. Differential scanning calorimetry (DSC) revealed lipid-type-dependent membrane interactions, indicating a targeted antimicrobial mechanism. These findings suggest that *C. megacephala*-derived peptides hold promise as novel agents for managing Gram-positive bacterial infections.

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ANTIMICROBIAL PEPTIDES IN THE FIGHT AGAINST BACTERIAL RESISTANCE: PROGRESS AND CHALLENGES AHEAD

Tomislav Rončević[a], Sabrina Pacor[b], Andrea Caporale[c], Anamarija Budimir[d], Matko Maleš[e], Iva Stojan[d], Alessandro Tossi[b], Larisa Zoranić[d,*]

[a] Department of Biology, Faculty of Science, University of Split, Split, Croatia

[b] Department of Life Sciences, University of Trieste, Trieste, Italy

[c] Institute of Crystallography, CNR, Area Science, Park, Basovizza, Trieste, Italy

[d] Department of Physics, Faculty of Science, University of Split, Split, Croatia

[e] Faculty of Maritime Studies, University of Split, Split, Croatia

* larisaz@pmfst.hr

We are in an era of rapid technological progress, yet major global challenges persist such as climate change, energy crises, and rising antibiotic resistance. Antimicrobial peptides (AMPs), natural components of innate immunity with broad-spectrum activity, offer a promising alternative to traditional antibiotics[1]. Despite their potential, AMPs have seen limited clinical adoption due to issues such as stability, toxicity, delivery, and cost[2]. The integration of machine learning is now accelerating AMP discovery and optimization. Still, computational models face challenges like limited data, poor generalizability, and the disconnect between predictions and real-world efficacy[3]. Our recent work on helminth-derived peptides, which are adapted to complex host environments, revealed candidates with strong antimicrobial activity and low toxicity[4]. Mechanistic studies and molecular dynamics simulations suggest they are membrane-active, possibly acting through a novel lipid extraction mechanism. Therefore, a growing body of atomistic and biophysical research on AMPs is emerging, but integrating findings across different scales, such as spatial, temporal, and environmental, remains challenging. Furthermore, a coherent synthesis linking structural, functional, and clinical aspects is still largely lacking. Alternatively, the diversity among AMPs may be too great to support a unified framework, making peptide-specific models tailored to particular clinical or other applications the most promising strategy.

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IMPURITY PROFILE CHARACTERIZATION OF THERAPEUTIC PEPTIDE USING 2D-LC/HRMS

Dubravka Gembarovski,[a,*] Andrea Kišić Rašeta[a]

[a] Analytical Characterization, Analytics, PLIVA R&D Zagreb, Prilaz baruna Filipovića 29, Zagreb, Croatia

* Dubravka.Gembarovski@pliva.com

Peptide drugs, composed of short chains of amino acids, have emerged as a promising class of therapeutics due to their high specificity, efficacy, and lower toxicity compared to traditional small molecule drugs. These peptides can mimic natural biological functions, making them suitable for treating a variety of conditions, including diabetes, cancer, and infectious diseases. However, the synthesis of peptide drugs often results in impurities that can affect their safety and efficacy. Identifying and quantifying these impurities is crucial for ensuring the quality of peptide therapeutics.[1]

Two-dimensional liquid chromatography (2D-LC) coupled with mass spectrometry (MS) is a powerful analytical technique used to identify impurities in peptide drugs. 2D-LC enhances separation efficiency by utilizing two distinct chromatographic columns, each with different separation mechanisms. This approach significantly increases peak capacity and resolution, allowing for the detailed analysis of complex peptide mixtures. When coupled with MS, 2D-LC provides precise mass-to-charge ratio measurements, enabling the identification of impurities based on their molecular weight and fragmentation patterns. This combination of techniques offers a robust method for impurity profiling, ensuring the production of high-quality peptide drugs.

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COLLAGEN MODULATOR PEPTIDES AS VALUABLE COSMECEUTICAL ACTIVE INGREDIENTS

Paolo Rovero

[a] PeptLab, Department of NeuroFarBa, University of Florence, Sesto Fiorentino, Italy

* paolo.rovero@unifi.it

The rising demand for novel cosmeceutical active ingredients has recently driven a surge of interest toward peptides. Most of the peptides used in cosmeceutical preparations have anti-aging claims, as they are designed to interfere with extracellular matrix components, particularly with collagen. Type I collagen is the most abundant protein in the human body and plays a fundamental role in skin firmness, elasticity, and appearance. In fact, healthy skin is characterized by a high density of collagen, resulting from a correct turnover and a well-balanced equilibrium between synthesis and degradation. Thus, an appropriate modulation of collagen turnover is extremely important to prevent pathological conditions and skin changes, including skin aging; in fact, the most visible effects of a reduction in collagen content are the structural collapse of the skin and wrinkles.

Building on the collagen turnover modulation properties of peptides derived from Serpin A1, a physiologic serin protease inhibitor, we initially reported the decapeptide SA1-III. Extensive *in vitro* data underscored the ability of this peptide to enhance collagen concentration in cultured human dermal fibroblasts and suggested a mode of action based on inhibition of collagen degradation [1,2]. Further *in vivo* data supported the use of this peptide as active ingredient of a skincare products range, termed KP1, which optimizes the appearance and texture of the skin, preserving and increasing the level of collagen [3].

More recently we developed and patented AAT11RI, a shorter, second-generation peptide endowed with improved properties. We used the *retro-inverso* approach, based on the use of D-amino acids, to significantly enhance peptide stability against human dermal proteases, while fully maintaining the collagen protection activity [4].

The rational approach we embraced in these studies underscore the added value of substantiated claims in the design of new cosmeceutical ingredients, representing a rarity in the cosmetic field.

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ANION-BINDING PROPERTIES OF SMALL CYCLOPEPTIDES IN SOLUTION

Gordan Horvat

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia

* ghorvat@chem.pmf.hr

Anion binding by various natural and synthetic receptors has been a focus of rapidly developing field of supramolecular chemistry. Cyclopeptides represent a promising class of versatile macrocyclic anion receptors. The anion complexation ability of these compounds can be attributed to the hydrogen-bond donating property of peptide backbone amide groups, flexibility of macrocyclic ring, and subunits variability. They exhibit superior metabolic stability and affinity for charged species compared to their linear analogues.

In this talk our recent contributions to this field will be presented.[1-2] The characterization of receptor-anion complexes was investigated by various experimental methods, as well as molecular dynamics simulations. The solvent effect on anion-complexation equilibria has been also taken into account.

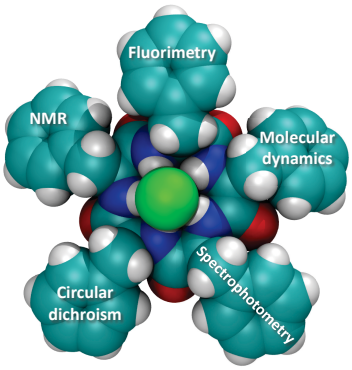

Figure 1. Cyclopentaphenylalanine in complex with chloride anion

ACKNOWLEDGMENTS

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UNRAVELING THE ROLE OF DIPEPTIDYL PEPTIDASE 3 IN PHYSIOLOGY AND PATHOLOGY

Mihaela Matovina

[a] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

mmatovina@irb.hr

Dipeptidyl peptidase 3 (DPP3) is a zinc-dependent peptidase that sequentially cleaves dipeptides from the unsubstituted N-termini of peptides 3–10 amino acids in length, *in vitro*. The physiological roles of human DPP3 are not yet fully elucidated; however, it is presumed to participate in the final stages of intracellular protein turnover and may also play a role in the regulation of blood pressure and pain[1]. Additionally, DPP3 is involved in the regulation of oxidative and electrophilic stress responses via the KEAP1-NRF2 signaling pathway. It interacts with KEAP1, thereby releasing the transcription factor NRF2 from the KEAP1 complex and activating the pathway[2]. NRF2 controls the expression of more than 250 genes and is involved in a wide range of physiological processes. Consequently, dysregulation of NRF2 activity is a hallmark of many diseases[3].

Increased expression or activity of DPP3 has been observed in various cancers. It has also been identified as a biomarker and potential therapeutic target in cardiogenic and septic shock, although the exact mechanisms underlying its involvement in these pathologies remain unclear[1]. Among other types of molecules, peptides and peptidomimetics that inhibit either the peptidase activity of DPP3 or its interaction with KEAP1 are being considered as potential therapeutics for conditions associated with elevated DPP3 expression and/or activity.

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SYNTHESIS AND CHARACTERISATION OF PEPTIDES WITH BODIPY FLUOROPHORE

Mladena Glavaš, Nikola Basarić

Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia

Fluorescence microscopy is a powerful technique used in biology, which relies on the use of fluorescent dyes and allows for the observation of process in living cells[1]. One class of the dyes which are widely used are BODIPY derivatives (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene)[2]. They have a great impact in molecular biology and medicine since they are often used as fluorescence markers[3] or therapeutics[4].
Vasopressin is a nonapeptide comprising a tripeptide tail and a cyclic structure formed by six residues with disulfide bridge[5], that binds to three G protein-coupled receptors. Despite its significant role in the treatment of various diseases, such as diabetes insipidus, vasodilatory shock, hypertension, *etc*., the use of vasopressin has some limitations, such as short biological half-life or lack of specificity for receptors. Therefore, the efforts to solve some of these problems through the discovery of new analogues is very important and can be achieved by incorporation of non-natural amino acids.
The lecture will feature the synthesis of BODIPY dyes, and efforts to incorporate them in different peptides **1**-**3** (Figure 1).

Cys(&)-Tyr-Phe-Gln-Asn-Cys(&)-Pro-AA(^{BODIPY})-Gly-NH_2 **1**

Cys(^{BODIPY})-Phe-Phe-Cys(^{BODIPY})-Gly **2**

Cys(^{BODIPY})-Phe-Phe-Phe-Phe-Phe-Cys(^{BODIPY})-Gly **3**

Scheme 1. Synthesis of BODIPY-labeled peptides.

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FMOC-AMINO ACID TO SUPRAMOLECULAR HYDROGELS

Ena Dražić,[a,c] Patrizia Janković Bevandić,[a,c] Daniela Kalafatović[b,c,*]

[a] University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka 51000, Croatia

[b] University of Rijeka, Faculty of Engineering, Rijeka 51000, Croatia

[c] University of Rijeka, Center for Artificial Intelligence and Cybersecurity, Rijeka, 51000, Croatia

* daniela.kalafatovic@riteh.uniri.hr

Low molecular weight gelators based on Fmoc-amino acids are promising for functional material design due to their ease of hydrogel preparation, tunable properties and biocompatibility[1]. The research presented here focuses on the design and characterization of hydrogels using Fmoc-Histidine and Fmoc-Cysteine, small molecular building blocks capable of forming structures through non-covalent interactions, such as hydrogen bonding and π-π stacking (Figure 1). Histidine, a polar amino acid, is frequently exploited for catalysis in combination with cysteine, a thiol-containing amino acid known for its propensity to create disulfide bridges through oxidation. In this study, hydrogels of Fmoc-Cys and Fmoc-Cys:Fmoc-His (1:1) were obtained, therefore mechanical and catalytic properties of resulting hydrogels were explored. In addition, the crystal structure of self-assembled Fmoc-His was analyzed. Fmoc-Cys:Fmoc-His co-assembled hydrogels exhibit superior catalytic activity compared to self-assembled Fmoc-Cys, likely due to disulfide bridge formation in Fmoc-Cys that hinders its nucleophilic reactivity. In conclusion, this study contributes to the understanding of how low molecular weight gelators work and give insights in their potential applications as minimalistic catalysts.

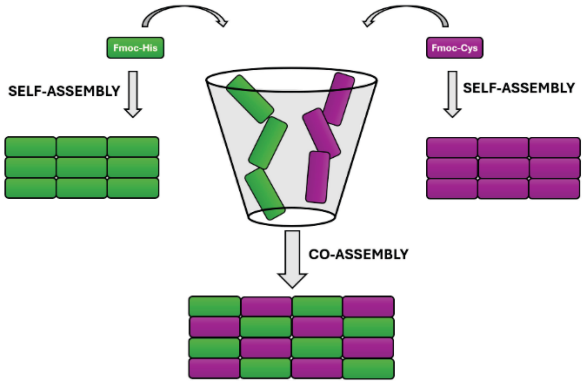

Figure 1. Shematic representation of the assembly pathways for Fmoc-Histidine and Fmoc-Cysteine.

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POSTERS

INFLUENCE OF MUTATION AND PEPTIDE INHIBITOR BINDING ON THE INTERACTION BETWEEN DPP3 AND KEAP1

Antonija Matić,[a,*] Filip Šupljika,[b] Luka Petohleb,[c] Antonija Tomić,[a,*]

[a] Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

[b] Department of Chemistry and Biochemistry, Faculty of Food and Technology, University of Zagreb, Pierottijeva ulica 6, Zagreb, Croatia

[c] Faculty of Science, Horvatovac 102A, Zagreb, Croatia

* amatic@irb.hr, atomic@irb.hr

Dipeptidyl peptidase 3 (DPP3) is a zinc-dependent metalloprotease implicated in various physiological and pathological processes, including the oxidative stress response and cancer progression.[1] DPP3 has been shown to interfere with the Keap1–Nrf2 signaling pathway by competing with Nrf2 for binding to Keap1, thereby promoting the expression of a broad range of antioxidant and cytoprotective genes.[2] Elevated DPP3 expression has been linked to tumorigenesis, particularly in breast and colorectal cancers,[3] where its ability to enhance antioxidant responses supports cancer cell survival by shielding cancer cells from oxidative damage. Our research aims to explore whether modulating DPP3's catalytic activity could provide a strategy for regulating its involvement in the Keap1–Nrf2 pathway, with potential therapeutic implications for cancer and other oxidative stress-related diseases. Specifically, we examined how DPP3 inactivation, achieved through mutation and binding of the IVYPW peptide inhibitor, affects the structural and thermodynamic properties of its interaction with the Kelch domain of Keap1, using a combination of experimental and computational methods.

ACKNOWLEDGMENTS

This work is funded by the European Union – NextGenerationEU, under the project titled "Preliminary study of inhibiting NRF2-dependent transcription by preventing the DPP III – KEAP1 interaction for more effective cancer treatment".

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TOWARDS PHOTOACTIVE PEPTIDES-BOC-HYDRAZINO ACID BASED ON DIARYLETHENE MOIETY

Marija Matković

Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

* mmatkovic@irb.hr

The diarylethene moiety (DAE) has established itself as a molecular photoswitch unit applicable in diverse photoactive molecules.[1] It is shown that molecules with incorporated DAE can have a feature of light controlled bioactivity.[2] This was distinctly demonstrated also on a peptidomimetic structure by incorporating the DAE moiety into gramicidin S analogue *via* Fmoc-hydrazino-DAE acid.[3]

Herein, a preparation of Boc-hydrazino-DAE acid (**Figure 1.**) is presented in a somewhat modified procedure contributing to scale up and overall enhanced simplicity in the isolation of pure target compound.[4]

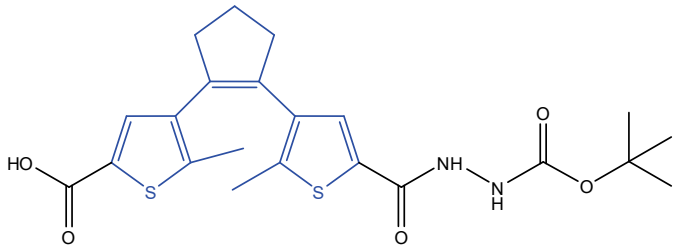

Figure 1. Boc-protected hydrazino-DAE acid

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SOLID PHASE PEPTIDE SYNTHESIS OF ANTIMICROBIAL PEPTIDE ANOPLIN AND ITS DERIVATIVE

Vedrana Krajnović,* Danijela Bakarić, Andreja Jakas

Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

* vedrana.krajnovic@irb.hr

The rapid increase of antibiotic resistant bacteria generated the need for the development of new compounds with antimicrobial properties. Antimicrobial peptides (AMP) with less than 100 amino acids (AA), which are often composed of positively charged and hydrophobic residues, emerged as an alternative to the antibiotics . As they act on the bacterial membrane directly, they displayed profound clinical potential [1]. One of the natural AMPs is anoplin, a decapeptide extracted from the venom of the solitary wasp which is the smallest of the naturally occurring linear α-helical AMPs [2]. Anoplin has a broad-spectrum antibacterial activity that is lower than that of the conventional antibiotics. Moreover, it is also not stable, so many modifications have been made to its structure to improve its activity and stability [3]. We have synthesized anoplin (GLLKRIKTLL) and its derivative K5W5 (KWWKKWKKWW) using solid phase peptide synthesis (SPPS) and characterized them using HPLC and MS techiques. We are working on improving the synthesis in order to achieve a higher yield of the reaction. Further investigation of the peptides will focus on the biological activity and their interaction with membranes.

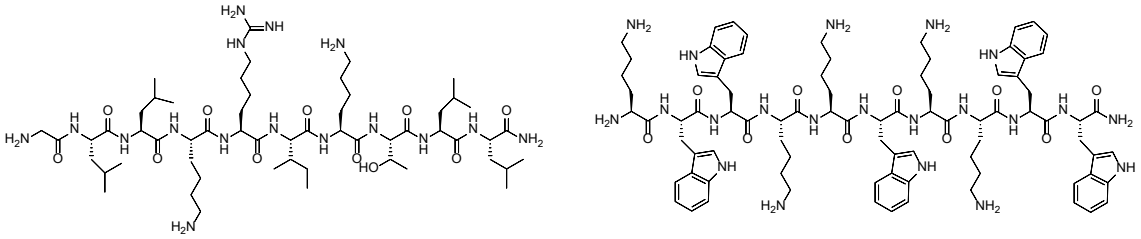

Figure 1. Structures of anoplin and K5W5

ACKNOWLEDGMENTS

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THERMODYNAMICS OF ALKALINE EARTH METAL CATION BINDING BY SMALL LINEAR HOMOPEPTIDES

Matija Modrušan,[a,*] Nikola Cindro,[a] Nikolina Vidović,[b] Giovanna Speranza,[c] Vladislav Tomišić,[a] Gordan Horvat[a]

[a] Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia

[b] Faculty of Biotechnology and Drug Development, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia

[c] Department of Chemistry, University of Milan, Via Golgi 19, 20122 Milan, Italy

* mmodrusan@chem.pmf.hr

In the past few decades cyclic peptides have attracted interest as versatile receptors of cations and anions. These compounds exhibit enhanced binding affinity towards anions compared to their more flexible linear analogues.[1,2] The most challenging step in their synthesis is macrocyclization, i.e. the ability of a linear precursor to bring its reactive termini in close spatial proximity. This reaction step requires templating agents amongst which the most commonly used are simple inorganic ions. Our group recently published the study of the affinity of small linear peptides towards anionic species that could promote cyclization[2,3] but to the best of our knowledge no similar research was done for alkaline earth metal cations. In this work we studied the complexation of alkaline earth metal cations by linear homopeptide methyl esters comprised of various number of phenylalanine subunits (4–6) in acetonitrile and DMF by means of isothermal titration microcalorimetry and spectrofluorimetry. Further insight into the structural characteristics of peptide–cation complexes was obtained by MD simulations.

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DISINFECTING WITH WASTE: NOVEL BIOBASED SURFACTANTS MIMICKING KEY FEATURES OF MEMBRANE-ACTIVE PEPTIDE

Theresa Schwaiger[a], Stefan Schwaiger[b], Markus Hochegger-Krawanja[b], Katalin Barta[b], Nermina Malanovic[a]

[a] University of Graz, Institute of Molecular Biosciences, Humboldstr. 50, 8010 Graz, Austria

[b] University of Graz, Institute of Chemistry, Heinrichstr. 28, 8010 Graz, Austria

* theresa.schwaiger@edu.uni-graz.at

The global rise of multidrug-resistant (MDR) pathogens—driven by antibiotic misuse—threatens a post-antibiotic era, where common infections may become untreatable. As there are no novel drugs available for many resistant pathogens, antimicrobial peptides (AMPs) are promising candidates due to their **non-specific, membrane-disruptive mode of action** and **low resistance development risk**, but clinical development remains difficult. [1]

The PureSurf project aligns with the European Green Deal and climate goals by developing **novel sustainable, bio-based surface-active compounds**. Using a modular design inspired by AMPs, amphiphilic molecules with tunable physicochemical properties were synthesized from lignin-derived monomers and fatty acids from waste cooking oil. Structure–function analysis revealed that cationic headgroups and fatty chain length enhance bacterial targeting and membrane disruption. —characteristics reminiscent of AMP activity.

Lead compounds demonstrated **broad-spectrum antimicrobial efficacy** against both Gram-positive and Gram-negative bacteria and **against MDR strains** from the **ESKAPE panel** through a non-specific, membrane-disruptive mode of action. Their performance was comparable to commercial quats BAC and CTAB. Importantly, the compounds maintained high activity under application-relevant stress conditions, including high protein loads (BSA), in accordance with ISO standards.

The lead candidates exhibited both **bacteriostatic and bactericidal activity**, and their non-specific, AMP-like membrane interaction suggests a **reduced likelihood of resistance** development.

ACKNOWLEDGMENTS

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MARINE-BASED VESICLES: A PRELIMINARY STUDY

Maja Levak Zorinc[a], Ruža Frkanec[b], Blaženka Gašparović[a], Maja Dutour Sikirić, Nadica Ivošević DeNardis[a,*]

[a] Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

[b] Centre for Research and Knowledge Transfer in Biotechnology, Rockefellerova ulica 10, Zagreb, Croatia

[c] Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička c. 54, Zagreb, Croatia

* ivosevic@irb.hr

During the lifespan and after the death of marine organisms, various biochemical substances are released into the seawater. Between 3 major biochememical groups; proteins, carbohydrates, and lipids, lipids are known to be last in order to be consumed by microorganisms in seawater[1]. After the cell death, the cell material can reassemble into reconstructed membrane vesicles[2], most probably, driven by hydrophobic interactions. To study similar processes, we collected marine lipid material from northern Adriatic and aimed to prepare marine-based lipid vesicles.

The vesicles were formed efficiently by thin-film method with diameters of up to 5 µm. The zeta potential of the suspension was – 43 ± 1.2 mV. The detailed structure and morphology of the marine lipid vesicles were imaged for the first time using atomic force microscopy (AFM). The marine-based vesicles imaged in air had a very defined, spherical shape. The average thickness of the dried membrane was 60 ± 30 nm, while the height was 2.5 ± 0.5 nm. When the vesicles were imaged in liquid and adhered to a substrate, they acquired a dome-like to spherical shape with diameters of 2.0 ± 0.9 µm, and heights of 1.1 ± 0.5 µm. We also determined the elasticity of the vesicles with Force Spectroscopy, a method included with the AFM system. Marine-based vesicles appear to be very firm, with values of Young's moduli 2.4 ± 2.5 MPa. This study contributes to the understanding of the spontaneous transformation of marine lipid matter through self-organization processes at the various interfaces in aquatic systems.

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AI-DRIVEN DESIGN AND EVALUATION OF PEPTIDE BINDERS TARGETING ALPHA-BUNGAROTOXIN

Tino Šeba,[a] Mario Gabričević,[a] Tin Weitner[a,*]

[a] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia

* tin.weitner@pharma.unizg.hr

Alpha-bungarotoxin (α-BTX) is a well-characterized neurotoxin that irreversibly binds to nicotinic acetylcholine receptors, making it a valuable target for the development of peptide binders with potential applications in neuropharmacology and toxin neutralization. While earlier binders were identified through experimental screening or structural mimicry, recent advances in deep learning-based protein design now enable the *de novo* computational generation of synthetic binders with enhanced specificity and stability.

In this pilot study, we evaluated two state-of-the-art design pipelines to create novel peptide binders for α-BTX: EvoBind2 utilizes diffusion-based backbone generation conditioned on the target epitope,[1] while BindCraft applies a generative deep learning model trained on nanomolar-affinity binder sequences to generate candidates directly from sequence information.[2] All designed sequences were structurally validated using the Chai-1 model based on AlphaFold3,[3] and their stability was assessed by OpenMM-based energy minimization.[4] Binding free energies of the optimized 3D structures were estimated using the PRODIGY tool.[5]

Both platforms successfully generated binders with favorable structural and binding characteristics. BindCraft provided the best balance between structural integrity, predicted binding affinity, and solubility. EvoBind2 offered more precise epitope targeting but may require further tuning to optimize solubility. These approaches offer complementary advantages, and future research will focus on experimental validation and adaptation to other molecular targets.

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MEMBRANE-TARGETING AMPS: BIOPHYSICAL AND COMPUTATIONAL INSIGHTS INTO ADEPANTINS AND HELMINTH-DERIVED PEPTIDES

Iva Stojan[a], Tomislav Rončević[b], Sabrina Pacor[c], Andrea Caporale[d], Anamarija Budimir[a], Matko Maleš[e], Alessandro Tossi[c], Larisa Zoranić[a]*

[a] Department of Physics, Faculty of Science, University of Split, Split, Croatia
[b] Department of Biology, Faculty of Science, University of Split, Split, Croatia
[c] Department of Life Sciences, University of Trieste, Trieste, Italy
[d] Institute of Crystallography, CNR, Area Science, Park, Basovizza, Trieste, Italy
[e] Faculty of Maritime Studies, University of Split, Split, Croatia
* larisaz@pmfst.hr

The rapid rise of multidrug-resistant microbial strains has spurred significant interest in antimicrobial peptides (AMPs) as a promising alternative to conventional treatments [1]. These naturally occurring and structurally diverse peptides demonstrate broad-spectrum antimicrobial activity alongside immunomodulatory effects [2]. Unlike traditional antibiotics, AMPs exhibit a lower likelihood of inducing resistance due to their rapid and multiple modes of action [3]. Our current project aims to enhance knowledge of the AMPs' structure-function relationship (adepantins [4] and peptides originating from helminths [5]) by integrating molecular dynamics simulations using model membranes with biophysical and biological characterization. By measuring minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) against Gram-positive and Gram-negative pathogens, cytotoxicity assays in human cell lines, structural profiling via circular dichroism spectroscopy, and molecular dynamics simulations—the specificity of their mode of action and their clinical potential has been revealed. Furthermore, a fluorescence-based technique, microscale thermophoresis (MST), will be employed to quantify the strength of molecular interactions in solution between peptides and intact bacterial membranes [6]. Preliminary results indicate that investigated peptides bind to bacterial membranes with micromolar dissociation constants, indicating a membrane-related mechanism of action.

ACKNOWLEDGMENTS

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EQUININS: NEW BROAD-SPECTRUM ANTIMICROBIAL PEPTIDES ISOLATED FROM THE CNIDARIAN *Actinia equina* (LINNAEUS, 1758)

Mariele Staropoli[a,b], Claudia La Corte[a,b], Valentina Catania[b,c], Maria Rosa Trapani[a], Matteo Cammarata[a,b,*], Mariano Dara[a,b], Daniela Parrinello[a,b], Maria Giovanna Parisi[a,b]

[a] Marine Immunobiology Laboratory, Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Viale delle Scienze, Ed. 16, 90128 Palermo, Italy
[b] NBFC—NaQonal Biodiversity Future Center, Piazza Marina 61, 90133 Palermo, Italy
[c] Department of Earth and Marine Sciences (DiSTeM), University of Palermo, Viale delle Scienze, Ed. 16, 90128, Palermo, Italy
* maTeo.cammarata@unipa.it

Among marine invertebrates, the Phylum Cnidaria is considered a promising source of bioactive molecules. The defense and predation mechanisms employed by these organisms involve toxins and antimicrobial peptides (AMPs), which are potentially valuable for therapeutic research.
The antimicrobial activity of acid extracts obtained from the tentacles and body of the Mediterranean Sea anemone *Actinia equina* was tested against Gram-positive and Gram-negative bacteria and fungi. In particular, the acid extracts from the tentacles showed significant minimum inhibitory concentration (MIC) values, up to 0.125 µg/mL, against the tested pathogens.
Subsequent investigations allowed the identification, through solid phase extraction and reversedphase HPLC purification, of the 40% acetonitrile fractions as responsible for the broad-spectrum antimicrobial activity. Peptides 6.2 and 7.3, named Equinin A and Equinin B, respectively, showed MIC values ranging from 0.06 to 0.20 mg/mL.
Sequencing revealed similarities to AMPs found in amphibians, fish, and other cnidarians, with activity against Gram-positive, Gram-negative bacteria, and fungi.
The peptides were then synthesized and tested against the aforementioned bacterial pathogens. In particular, Equinin B exhibited promising antibacterial activity, with MIC and bactericidal concentration values of 1 mg/mL and 0.25 mg/mL, respectively. Furthermore, its genetic organization supports its potential in applied research.
These results highlight the potential of *A. equina* AMPs for therapeutic and biotechnological applications. In particular, Equinin B warrants further studies to optimize its possible clinical use and better understand its mechanism of action.

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EFFECT OF C-TERMINAL AMIDATION ON SELF-ASSEMBLY AND GELATION OF SHORT PEPTIDES

Gaia Hinić[a], Mia Jozić[a], Sophia Mattitsch[d], Ena Dražić[a,c], Patrizia Janković Bevandić[a,c], Daniela Kalafatović[b,c,*]

[a] University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka 51000, Croatia

[b] University of Rijeka, Faculty of Engineering, Rijeka 51000, Croatia

[c] University of Rijeka, Center for Artificial Intelligence and Cybersecurity, Rijeka 51000, Croatia

[d] University of Vienna, Faculty of Life Sciences, Vienna 1030, Austria

* daniela.kalafatovic@uniri.hr

Peptide-based hydrogels show great potential in biomedicine due to their biocompatibility and tunable properties[1]. Self-assembly is a spontaneous process by which peptides form organized structures through intermolecular interactions including hydrogen bonds, π-π stacking, hydrophobic and electrostatic interactions. C-terminal amidation of peptides removes a terminal charge and can thereby modulate peptide interactions. In this work, we examined how this modification influences the self-assembling ability of the IMGIIA peptide. The sequence was generated using a hybrid deep-learning model and shown to aggregate using coarse grained simulations[2]. IMGIIA and IMGIIA-Am were synthesized using Fmoc-based solid phase peptide synthesis and purified. Both peptides showed the capability to form stable hydrogels at the concentration of 5 mM. The hydrogels were further characterized by rheology, circular dichroism (CD) and attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy. These results provide a strong foundation for the future exploration of peptide-based materials.

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LIST OF PARTICIPANTS

Azmiera Zamri, Nurul
Bakarić, Danijela
Barbarić, Lea
Barišić, Lidija
Belovari, Mateja
Besednik, Lucija
Biba, Renata
Brezovečki-Biđin, Vesna
Čipor, Ivona
Čolak, Ante
Dapić, Irena
Dražić, Ena
Frkanec, Leo
Frkanec, Ruža
Gabričević, Mario
Gembarovski, Dubravka
Glavaš, Mladena
Grgičević, Ivan
Habazin, Siniša
Hinić, Gaia
Horvat, Gordan
Jakas, Andreja
Jakopin, Žiga
Janković, Patrizia
Jozić, Mia
Kalafatović, Daniela
Karakaš, Antonija
Katalinić, Maja
Katalinić, Petra
Kišić Rašeta, Andrea
Kovačević, Monika
Krajnović, Vedrana
Krevh, Denisa
Lakošeljac, Ivana
Levak Zorinc, Maja
Malanović, Nermina
Marcelić, Lucija
Maria Papini, Anna
Marković, Marija
Matanović Škugor, Maja
Mathieu, Geoffroy
Matić, Antonija
Matković, Marija
Matovina, Mihaela
Mattitsch, Sophia
Mihalac, Josip
Milatić, Dina
Modrušan, Matija
Mršić, Nataša
Nekola, Irena
Nikolić, Andrej
Pašalić, Lea
Periš, Mihaela
Perković-Hadl, Vedrana
Petrovic-Hunjadi, Martina
Petrović Peroković, Vesna
Ribić, Rosana
Roca, Sunčica
Roje, Marin
Rovero, Paolo
Schwaiger, Theresa
Staropoli, Mariele
Stojan, Iva
Šeba, Tino
Šišić, Marcela
Šporec, Anita
Todorovski, Toni
Tomić, Antonija
Vuković, Martina
Vukovinski Babojelić, Ana
Weitner, Tin
Zandona, Antonio
Zoranić, Larisa