27th Polish Peptide SYMPOSIUM

Book of Abstracts

27th Polish Peptide SYMPOSIUM

Programme and Abstracts

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Filip Maciejowski

Agnieszka Łupicka-Słowik

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The conference, first held in 1967 in Gdańsk and headed by Professor Kupryszewski, has a long and distinguished tradition of bringing together chemists from Poland and around the world. Over the decades, it has evolved into a prestigious event that attracts leading researchers and industry representatives, fostering collaboration and innovation in the field of chemistry. Focusing on peptide chemistry, the event also encompasses a broader range of topics, including biochemistry, structural chemistry, and related disciplines. The conference features keynote speeches from some of the world's top experts, offering attendees the chance to learn about cutting-edge research and advancements directly from the pioneers in the field.

Beyond the scientific program, the conference provides an excellent platform for building professional networks, establishing international collaborations, and exchanging ideas with both academic researchers and industry professionals.

This enduring tradition of excellence and opportunity makes the conference an unmissable event for anyone passionate about advancing the frontiers of chemistry.

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II	1973	Wdzydze near Gdańsk	Andrzej Chimiak
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XIV	1997	Polanica Zdrój	Barbara Lejczak
XV	1999	Waplewo near Olsztyn	Elżbieta Kostyra
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XIX	2007	Pułtusk near Warsaw	Aleksandra Misicka
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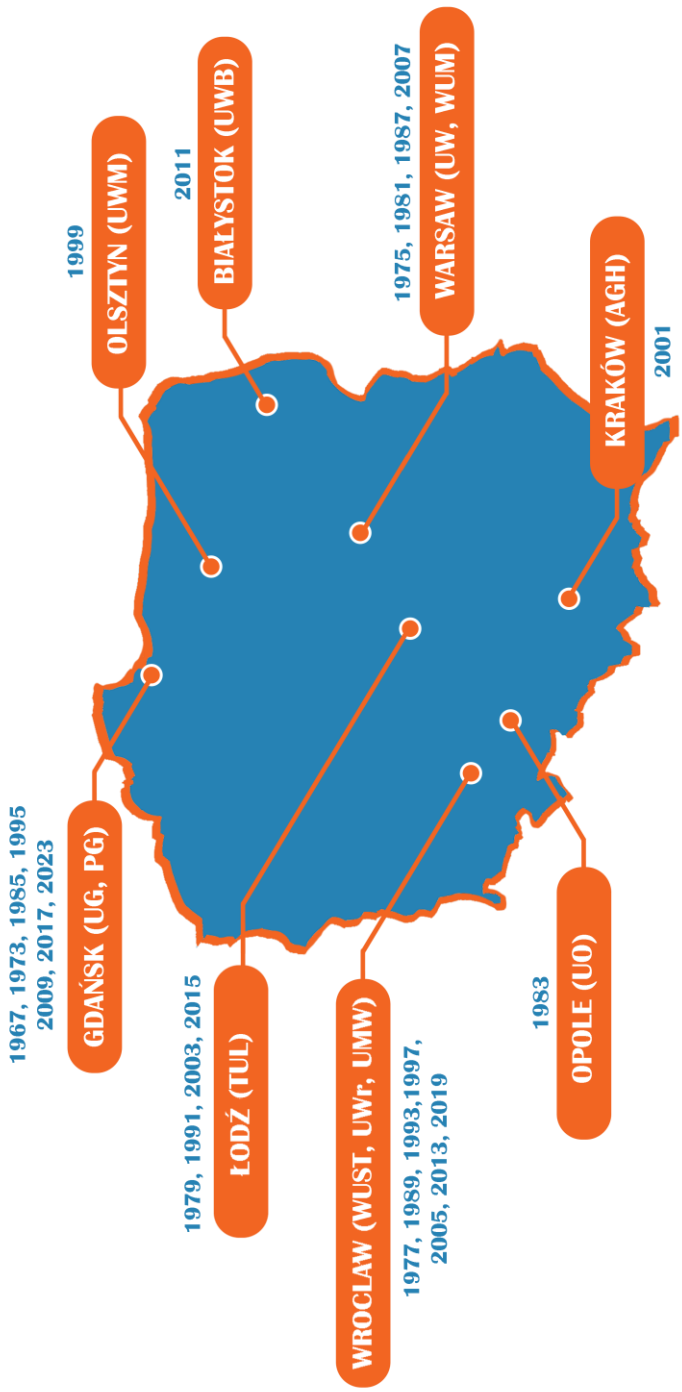
XXI	2011	Supraśl near Białystok	Krystyna Midura-Nowaczek
XXII	2013	Kudowa Zdrój	Rafał Latajka
XXIII	2015	Spała near Łódź	Beata Kolesińska
XXIV	2017	Jastrzębia Góra near Gdańsk	Sylwia Rodziewicz-Motowidło
XXV	2019	Wojanów near Wrocław	Justyna Brasuń
XXVI	2023	Stare Jabłonki near Gdańsk	Agata Gitlin-Domagalska & Natalia Ptaszyńska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1977, 1989, 1993,1997,
2005, 2013, 2019
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1983
OPOLE (UO)

PROGRAMME

Sunday, 7th September	
16^{00}-20^{00}	Registration
19^{00}-20^{00}	Dinner
Monday, 8th September	
9^{00}-9^{15}	Opening ceremony
Session 1 ***9^{15}-11^{10}***	*Chairpersons: Sylwia Rodziewicz-Motowidło, Marcin Sieńczyk*
9^{15}-10^{00}	**Brice Korkmaz** University of Tours, France *First-in-Class Medicines Called Cathepsin C Inhibitors for the Treatment of Neutrophil-Mediated Diseases*
10^{00}-10^{30}	**Adam Lesner** University of Gdańsk, Poland *Peptides as Diagnostic Tools*
10^{30}-10^{50}	**Anna Wardowska** Medical University of Gdansk, Poland *Peptides Targeting the BTLA-HVEM Complex as Potential Regulators of Immune Response – Immunologists' Perspective*
10^{50}-11^{10}	**Aneta Szymańska** University of Gdańsk, Poland *Amyloidogenic Hot Spots in Selected Members of Human Cystatins. Theoretical and Experimental Studies*
11^{10}-12^{00}	Coffee break
Session 2 ***12^{00}-13^{40}***	*Chairpersons: Rafał Latajka, Michael Gütschow*
12^{00}-12^{40}	**Marcin Poręba** Wrocław University of Science and Technology, Poland *Protease-Activated Antibody-Drug Conjugates for Targeted Cancer Therapy*
12^{40}-13^{00}	**Ewa Wieczerzak** University of Gdańsk, Poland *Affibody Molecules in HER2-Positive Breast Cancer Therapy*
13^{00}-13^{20}	**Jacek Plewka** Jagiellonian University, Poland *Peptide-Based Inhibitors Targeting LAG-3/MHCII Interaction for Cancer Immunotherapy*
13^{20}-13^{40}	**Kamil Rolski** Bio-Rad, Poland *Customizable SpyTag® Click-Biochemistry in Design and Construction of Therapeutic Antibodies and Peptides; Synthesis, Purification and Biological Activity Assays*
13^{40}-15^{00}	Lunch break

Session 3	
15^{00}-16^{20}	*Chairpersons: Łukasz Berlicki, Karolina Pułka-Ziach*
15^{00}-15^{40}	**Steven Verhelst** KU Leuven, Belgium *Development and Application of Peptide-Derived Activity- and Affinity-Based Probes Targeting Proteases*
15^{40}-16^{00}	**Rafał Latajka** Wrocław University of Science and Technology, Poland *Phosphonic Analogs of Thiosemicarbazone as Inhibitors of Tyrosinase*
16^{00}-16^{20}	**Marcin Skoreński** KU Leuven, Belgium *New Chemiluminescent Probes for TMPRSS2 and Related S1 Family Proteases with Arginine Selectivity: A Sensitive Detection Strategy*
16^{20}-17^{00}	Coffee break
17^{00}-18^{00}	Poster session 1
19^{00}-20^{00}	Dinner
Tuesday, 9th September	
Session 4	
9^{00}-11^{15}	*Chairpersons: Artur Krężel, Aneta Szymańska*
9^{00}-9^{45}	**Michael Gütschow** University of Bonn, Germany *Modified VHL-binding Peptides for HIF-1α Stabilization and PROTAC Design*
9^{45}-10^{25}	**Beata Kolesińska** Łódź University of Technology, Poland *Materials Mimicking the Natural Surroundings of Cells Based on a Set of Protein Fragments Involved in the Regeneration Process and Polysaccharides*
10^{25}-10^{45}	**Paweł Pięta** Poznań University of Medical Sciences *Synthesis of D-Amino Acid-Modified dNTPs with Potential Application in Developing Oligonucleotide Libraries*
10^{45}-11^{15}	**Giorgio Marini** CEM Corporation, USA *Innovations in Sustainable Peptide Production*
11^{15}-12^{00}	Coffee break

Session 5 ***12^{00}-14^{00}***	*Chairpersons: Beata Kolesińska, Piotr Stefanowicz*
12^{00}-12^{40}	**Artur Krężel** University of Wrocław, Poland *Peptide Models of Protein Structural and Regulatory Zinc Sites: Tools for Elucidating Structure-Stability-Function Relationships*
12^{40}-13^{00}	**Alicja Kluczyk** University of Wrocław, Poland *Fungal Amino Acids and Peptides: Friends, Foes, Tricksters*
13^{00}-13^{20}	**Justyna Brasuń** Wrocław Medical University, Poland *Peptides of the Nervous System and their Derivatives as Target for Metal Ions*
13^{20}-13^{40}	**Ema Kosovic** Tosoh Bioscience GmbH, Germany *Comprehensive Peptide Characterization Using HPLC and MALS: A Multimodal Chromatography Approach*
13^{40}-14^{00}	**Krystyna Niedzielska** Polygen Sp. z o.o, Poland *Integrated Solutions for the Analysis and Purification of Peptides Using LC and MS Techniques*
14^{00}-15^{00}	Lunch
15^{00}-19^{00}	Free time and organized activities
19^{00}-21^{00}	Barbeque
Wednesday, 10th September	
Session 6 ***9^{00}-11^{20}***	*Chairpersons: Justyna Brasuń, Irena Maliszewska*
9^{00}-9^{40}	**Moez Rhimi** INRAE, France *The Gut Microbiome & Disease: from Description to Personalized Medicine*
9^{40}-10^{00}	**Natalia Ptaszyńska** University of Gdańsk, Poland *Dual-Activity Peptide–Drug Conjugates with Selective Antileukemic and Antimicrobial Properties*
10^{00}-10^{20}	**Monika Wojciechowska** University of Warsaw, Poland *Conformational Stabilization of Membrane-Active Peptides via Hydrocarbon Stapling as a Strategy to Improve Antibacterial Activity*
10^{20}-10^{40}	**Puneeth Kumar Drgkoppalu Ramanna** University of Bielefeld, Germany *Anti-Proteolytic AMPs for Targeting ESKAPE Pathogens: A Step Towards Therapeutic Peptides*
10^{40}-11^{00}	**Jannik Paulus** University of Göttingen, Germany *Redox-Responsive Peptide Dimer for the Stabilization and Release of Therapeutic dsRNA*
11^{00}-11^{20}	**Paulina Pohl** Labo24, Poland *Impact of Glassware Quality on Test Results and Safety in the Modern Laboratory*
11^{20}-12^{00}	Coffee break

Session 7	
12^{00}-13^{40}	*Chairpersons: Marcin Poręba, Moez Rhimi*
12^{00}-12^{40}	**Sylwia Rodziewicz-Motowidło** University of Gdańsk, Poland *AI-Assisted Design of a HER2-Targeted, Pro-Apoptotic Amyloidogenic Peptide: From Concept to Structure and Function*
12^{40}-13^{00}	**Piotr Stefanowicz** University of Wroclaw, Poland *Visible-Light-Mediated Transformations of Sulfur- and Selenium-Containing Peptides for Post-Synthetic Modification*
13^{00}-13^{20}	**Dawid Dębowski** University of Gdańsk, Poland *Nature-Inspired Cyclic Peptides as Highly Selective Matriptase Inhibitors: Insights from SAR and Molecular Dynamics*
13^{20}-13^{40}	**Monika Kijewska** University of Wroclaw, Poland *Bridging Food Chemistry and Peptide Science: New Tools to Study Lactosylation*
13^{40}-15^{00}	Lunch
Session 8	
15^{00}-16^{40}	*Chairpersons: Steven Verhelst, Maciej Dawidowski*
15^{00}-15^{40}	**Łukasz Berlicki** Wrocław University of Science and Technology, Poland *Design, Synthesis, Structures, and Functions of Miniproteins*
15^{40}-16^{00}	**Karolina Pułka-Ziach** University of Warsaw, Poland *Folded or Unfolded – Influence of Salt Additives on the Helicity of Oligourea Foldamers*
16^{00}-16^{20}	**Monika Szefczyk** Wrocław University of Science and Technology, Poland *Foldameric Nanostructures and Functional Amyloids as an Example of the Bright Side of Peptide Aggregation Phenomenon*
16^{20}-16^{40}	**Juan Lizandra Perez** Vrije Universiteit, The Netherlands *Design and Characterization of β-catenin Site-Specific Cell-Permeable β-Hairpin-Based Inhibitors*
16^{40}-17^{00}	Coffee break
17^{00}-18^{00}	Poster session 2
20^{00}- 2^{00}	Banquet

Thursday, 11th September	
Session 9 ***9^{00}-11^{00}***	*Young Investigators Day* *Chairpersons: Natalia Ptaszyńska, Jacek Plewka*
9^{00}-9^{15}	**Wiktoria Mallek** University of Gdańsk, Poland *Application of Combinatorial Chemistry Methods in the Characterization of Murine Serine Proteases*
9^{15}-9^{30}	**Jonathan Coene** KU Leuven, Belgium *Novel Legumain Activity-Based Probes*
9^{30}-9^{45}	**Abdulla Al Mamun** Wrocław University of Science and Technology, Poland *Activatable and Membrane-Retained Substrate Probes Reveal Functional Activity of MMPs, ADAMs, and GzmB in Breast Cancer*
9^{45}-10^{00}	**Aleksandra Helbik-Maciejewska** University of Gdańsk, Poland *Cyclic Furin Inhibitors with Disulfide Bridge Mimetics with Anticancer Activity Against Glioblastoma and Lung Cells*
10^{00}-10^{15}	**Izabela Siekierska** University of Warsaw, Poland *Application of Hydrocarbon-Stapled Peptides and Aminoglycosides to Peptide Nucleic Acid Conjugates for Enhanced Uptake and Improved Antibacterial Activity*
10^{15}-10^{30}	**Maksymilian Zabijak** Wrocław University of Science and Technology, Poland *Substrate-Based Probes with DNA-Intercalating Fluorophores for Caspase Activity Analysis in CAR-T Cells*
10^{30}-10^{45}	**Jona Voss** University of Bielefeld, Germany *On Demand Activation of Cryptophycin Drug Conjugates by Click Chemistry*
10^{45}-11^{00}	**Mikołaj Śleziak** University of Wroclaw, Poland *Exploring the Effect of Peptide Stapling on the Antimicrobial Activity and Structural Rigidity of Gramicidin S*
11^{00}-11^{40}	Coffee break
11^{40}-12^{00}	Closing ceremony and announcement of competition winners

LECTURES

L1

First-in-Class Medicines Called Cathepsin C Inhibitors for the Treatment of Neutrophil-Mediated Diseases

Brice Korkmaz[1]

[1] *INSERM UMR-1100, « Research Center for Respiratory Diseases »*
Faculty of Medicine, Université de Tours 37032, Tours, France
brice.korkmaz@inserm.fr

Neutrophils have a critical role in the innate immune response to infection and the control of inflammation. A key component of this process is the release of pro-inflammatory neutrophil serine proteases (NSPs; neutrophil elastase, proteinase 3 and cathepsin G). Normally, NSP activity is controlled and modulated by endogenous antiproteases. However, disruption of this homeostatic relationship can cause diseases in which neutrophilic inflammation is central to the pathology, such as chronic obstructive pulmonary disease (COPD), alpha-1 antitrypsin deficiency, bronchiectasis, and cystic fibrosis, as well as many non-pulmonary pathologies [1-2]. Cathepsin C (CatC), also known as dipeptidyl peptidase 1 (DPP-1), attracts more and more attention from both scientists and clinicians because of its role in the activation of NSPs. Promising preclinical and clinical data suggest that pharmacological inhibition of NSPs might ameliorate these conditions.

Patients with Papillon-Lefèvre syndrome, an autosomal recessive condition with a prevalence of 1-4 per million, have a genetically determined deficiency in CatC but, reassuringly, do not exhibit marked immunodeficiency despite the absence of NSPs in immune defense cells. Hence, the pharmacological control of CatC activity in bone marrow precursor cells represents an attractive therapeutic strategy for NSP-mediated disorders. Recently, positive results are announced from a Phase 3 study with a reversible CatC inhibitor (brensocatib), in patients with non-cystic fibrosis bronchiectasis [3-4], a relatively rare condition in which potential respiratory pathogens frequently colonize the lungs, often leading to exacerbations. Due to overlapping phenotypes and similar underpinning molecular mechanisms for a number of diseases associated with inflammation, a positive effect of brensocatib in bronchiectasis patients could be translated directly to the potential treatment of other NSPs-mediated inflammatory diseases. Ongoing research continues to explore the inhibition of NSP activation by CatC inhibitors in different disease states and the potential clinical benefits of CatC inhibition.

References:

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[2] Korkmaz B.; Lamort A.S.; Domain R. et al. *Biochemical Pharmacology*, **2021**, *194*, 114803.
[3] Chalmers J.D.; Burgel P.R.; Daley C.L. et al. *The New England Journal of Medicine*, **2025**, *392*, 1569-1581.
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L2

Peptide as Diagnostic Tools

Natalia Gruba[1], Honorata Sikora[1], Magdalena Wysocka[1], Adam Lesner[1]

[1] *Faculty of Chemistry University of Gdansk, Gdansk, Poland*
adam.lesner@ug.edu.pl

In this short lecture, an overview of our recent research will be provided. The research focused on the utilization of fluorescently labeled peptides as diagnostic tools for widespread civilization diseases.

For the past decade, the majority of our group's efforts have been dedicated to investigating the relationship between proteolytic activity and the presence of early-stage diabetes sequelae, viral infections, and cancer. The utilization of fluorescent peptide substrates facilitated the establishment of a correlation between the presence of disease and specific proteolytic activity in body fluids, including saliva and urine.

L3

Peptides Targeting the BTLA-HVEM Complex as Potential Regulators of Immune Response – Immunologists' Perspective

Anna Wardowska[1], Marta Spodzieja[2]

[1] *Medical University of Gdansk, Faculty of Medicine, Dębinki 7, 80-211 Gdańsk, Poland*
[2] *University of Gdańsk, Faculty of Chemistry, Wita Stwosza 63, 80-308 Gdańsk, Poland*
anna.wardowska@gumed.edu.pl

Inhibitory immune checkpoints (ICPs) play a crucial role in regulating the immune response. Their fundamental role is to prevent excessive activation of the immune system and to contract natural immune response. These receptors are well-known and exploited as targets in cancer immunotherapy, where tumor cells utilize them to evade immunosurveillance. However, recent studies have highlighted the role of ICP in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus (SLE). It is believed that cancer and autoimmunity are two sides of the same coin; therefore, modifying the activity of inhibitory ICP can be beneficial in both types of medical conditions.

The aim of our current research is to determine whether peptides targeting the BTLA-HVEM complex can affect the activity of immune cells in cancer patients and those with SLE.

To obtain our major goal, we evaluated the influence of several peptides binding to selected members of the BTLA-HVEM complex on the activity of peripheral blood mononuclear cells (PBMC) obtained from healthy donors, melanoma patients, and SLE patients. Following the 72-hour and 120-hour cell cultures with the peptides, the following parameters of T cells were measured using flow cytometry: activation status, proliferation, apoptosis, and the T-cell memory compartment.

The acquired data confirmed the potential usefulness of peptides targeting BTLA and HVEM as an element of cancer immunotherapy, as these compounds were able to rejuvenate the activity of quiescent T cells. Regarding SLE patients, initial studies have been conducted to date; thus, no meaningful conclusions can be drawn at this point. Nevertheless, we assume that modifying the activity of the BTLA-HVEM complex in SLE patients may significantly improve the resolution of ongoing inflammation.

Funding: This study was supported by the National Science Center (Poland), OPUS 22 grant (No. UMO-2021/43/B/NZ7/01022), and SONATA 13 grant (No. UMO-2017/ 26/D/ST5/00919).

L4

Amyloidogenic Hot Spots in Selected Members of Human Cystatins. Theoretical and Experimental Studies

Aneta Szymańska[1], David Pawłowski[1], Rafał Tutkowski[1], Emilia Iłowska[1]

[1] *Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland*
aneta.szymanska@ug.edu.pl

Protein oligomerization and aggregation represents a pivotal pathological process at the core of numerous neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases [1]. In the face of an aging population, these ailments acquire particular significance, posing a substantial challenge to healthcare systems worldwide. Therefore, understanding the molecular mechanisms responsible for the initiation and progression of protein aggregation is essential for the development of effective diagnostic methods, therapies, and preventive strategies.

One of the hypotheses concerning the mechanisms and triggers of the aggregation of peptides and proteins posits that certain regions, referred to as "hot spots", play a crucial role in these processes [2]. These sequences are short fragments of amino acid sequences, distinguished in the primary sequence of a given peptide of protein, capable of initiating and regulating the formation of amyloid structures. Currently, *in silico* methods allow for the identification of such aggregation-prone sites based solely on the amino acid sequence or spatial structure of an analyzed molecule, proving to be invaluable tools in the design of experimental studies on aggregating proteins [3].

The model utilized in the presented research comprised proteins from the cystatin superfamily, such as stefins A and B, cystatin C, and chicken cystatin, which exhibit varying abilities to form amyloidogenic structures [4]. The amyloidogenic hot spots were ascribed using several theoretical approaches, the peptides were synthesized and their amyloidogenic properties checked.

The results obtained from the conducted studies indicate that the synthesized peptides corresponding to the "hot spot" regions of the selected proteins demonstrate diverse amyloidogenic properties, with the course of the fibrilization process and the morphology of the aggregates depending, among other factors, on the ionic strength of the solution. The relation between the amyloidogenicity of the intact protein and its “hot spots” was also established.

Funding: Work supported by grant DS 531-T070-D496-25.

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L5

Protease-Activated Antibody-Drug Conjugates for Targeted Cancer Therapy

Martyna Majchrzak[1], Maria Łęcka[1], Oliwia Gorzeń[1], Marcin Poręba[1,2]

[1] Faculty of Chemistry, and [2] Faculty of Medicine, Wroclaw University of Science and Technology, Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland
marcin.poreba@pwr.edu.pl

Breast cancer remains the most prevalent malignancy among women worldwide. Its diverse subtypes pose unique therapeutic challenges, often necessitating personalized treatment strategies. Standard treatments such as chemotherapy and surgery are frequently associated with significant side effects and limited selectivity, reducing their efficacy in long-term disease management. Antibody-drug conjugates (ADCs) have emerged as a transformative therapeutic modality, combining the tumor-targeting specificity of monoclonal antibodies with the cytotoxic potency of chemotherapeutic agents. A key determinant of ADC efficacy lies in the design of peptide linkers, which enable controlled drug release inside cancer cells while minimizing off-target effects. Conventional linkers such as Val-Cit and Gly-Gly-Phe-Gly are widely used but suffer from poor specificity due to cleavage by multiple proteases (e.g., cathepsins, elastase), potentially limiting their therapeutic precision. Our research explores alternative protease targets for linker activation, focusing on S9 family prolyl oligopeptidases, cysteine cathepsins (specifically cathepsin B and L) and the membrane-bound serine protease matriptase-1. To improve the specificity of linker cleavage and therapeutic efficacy, we utilize the Hybrid Combinatorial Substrate Library (HyCoSuL) technology. This platform enables high-resolution profiling of protease substrate preferences by incorporating unnatural amino acids in the peptide structure. Using HyCoSuL, we have developed novel selective peptide substrates for each of these protease targets and incorporated them into ADC linkers. This approach has yielded conjugates with enhanced protease selectivity and metabolic stability. Preliminary in vitro studies in breast cancer cell lines revealed dose-dependent cytotoxic effects, validating the functional relevance of protease-activated linkers tailored using HyCoSuL profiling. This work underscores the critical importance of precise protease-targeted linker design in ADC development and demonstrates the potential of HyCoSuL-guided strategies to engineer next-generation ADCs with superior specificity and reduced systemic toxicity.

Funding: This project was supported by the OPUS-LAP grant from the National Science Centre in Poland, UMO 2020/39/I/NZ5/03104.

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L6

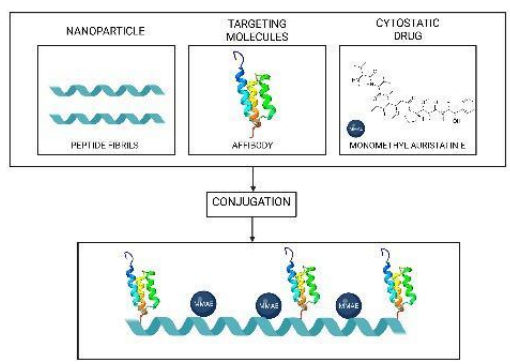
Affibody Molecules in HER2-positive Breast Cancer Therapy

Ewa Wieczerzak[1], Justyna Sawicka[1], Milena Czeszejko[1], Maria Nevarez Martinez[2], Kinga Marszałek[1], Michał Wisiński[1], Sylwia Rodziewicz-Motowidło[1]

[1] *Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdańsk, Poland*
[2] *Center for Integrated Nanotechnologies, Los Alamos National Laboratory, Los Alamos, USA*
ewa.wieczerzak@ug.edu.pl

Breast cancer (BCa) is the most common cancer in women worldwide. In 2020 it caused the death of 8805 women in Poland. One of the four molecular subtypes of BCa constitutes human epidermal growth factor receptor 2 – positive (HER2+). The standard treatment for patients with HER2+ has involved a combination of chemotherapy and HER2-targeted monoclonal antibodies (mAbs) such as Trastuzumab and Pertuzumab. However, BCa cells usually develop resistance to these drugs and to improve patients outcome antibody drug-conjugates (ADC) have been recently approved for treatment of BCa [1]. As an alternative to mAbs, affibodies, engineered affinity proteins, are promising molecules as carriers of cytotoxic drugs for cancer therapy [2]. Affibodies present several advantages over mAbs, e.g. their lower molecular weight provides renal clearance, chemical robustness and thermostability, and a small size enables rapid penetration into tumors [3]. Moreover, they can be produced chemically or in simple procaryotic host cells at lower costs than the production of mAbs.

In our study, we have chemically synthesized the Cys-modified $Z_{HER2:2891}$ affibody and confirmed its strong affinity to HER2. The binding specificity of the affibody to HER2-positive cells was further proved. In the current project, $Z_{HER2:2891}$ is being used as a targeting vector for HER2+ breast cancer. It will be combined with peptide fibrils as a new class of the drug delivery system, and a cytotoxic drug, monomethyl auristatin E (MMAE), thus creating a novel type of nanodrug system with a potential to fight breast cancer. The results of the research conducted to date will be presented.



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L7

Peptide-Based Inhibitors Targeting LAG-3/MHCII Interaction for Cancer Immunotherapy

Jacek Plewka[1], Konrad Barnowski[1], Łukasz Skalniak[1], Katarzyna Nakielska[1], Sylwia Rodziewicz-Motowidło[2], Paulina Stencel[2], Gabriela Całka-Kuc[2], Adam Kulesza[2]

[1] *Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, Krakow, 30-387, Poland*
[2] *Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdansk, Poland*
jacek.plewka@uj.edu.pl

Immune checkpoint blockade has revolutionized cancer therapy, with the inhibition of negative immune regulators earning the Nobel Prize in 2018 [1]. Among these checkpoints, LAG-3 (Lymphocyte-activation gene 3) has emerged as a critical inhibitory receptor that modulates T cell activation and function through its interaction with MHC class II molecules. While monoclonal antibodies targeting LAG-3 have shown clinical promise, their high cost, intravenous delivery, and potential adverse effects limit widespread accessibility.

To address these challenges, our project explores the development of peptide-based inhibitors that disrupt the LAG-3/MHCII interaction. Using a structure-guided modeling approach, we designed peptides mimicking the LAG-3/MHCII interface. These peptides demonstrated low micromolar IC_{50} values in biochemical assays, indicating promising inhibitory potential. Direct binding of the peptides to LAG-3 was confirmed using microscale thermophoresis (MST), validating their mechanism of action.. In cell-based assays, the peptides elicited moderate immune activation responses, comparable to those reported in existing patents. They will also be tested in co-administration with anti-PD-L1 antibody to mimic the FDA-approved nivolumab/relatimab combination [2]

Additionally, we employed phage display to identify novel peptide binders, which are currently undergoing evaluation. Looking ahead, we aim to enhance the therapeutic potential of these peptides by integrating them into a LYTAC (lysosome-targeting chimera) platform to induce targeted degradation of LAG-3.

Our findings support the feasibility of peptide-based immune checkpoint inhibitors as a cost-effective and versatile alternative to antibodies, with potential applications in solid tumor immunotherapy.

Funding: This work has been supported by the National Science Center of Poland as part of the OPUS grant "Innovative small molecule inhibitors targeting LAG-3 and the MHCII complex for cancer immunotherapy" (grant number UMO-2024/53/B/NZ7/02801).

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L8

Customizable SpyTag® Click-Biochemistry in Design and Construction of Therapeutic Antibodies and Peptides; Synthesis, Purification and Biological Activity Assays

Kamil Rolski[1], Proteomic Field Application Scientist II[1]

[1] *Bio-Rad Poland*
kamil_rolski@bio-rad.com

Since the introduction of insulin in 1922, a growing interest in the use of peptide drugs in treatment of various human disease has been observed, gaining increased traction in the last decade. With the best-known examples like Ozempic (treatment of type 2 diabetes/ obesity) and Fuzeon (treatment of AIDS/HIV), there are now 80 therapeutic peptides on the market, 200 in clinical phases, and 600 in advanced pre-clinical stages- the largest proportion targets rare diseases, cancers and diabetes mellitus along with its complications. Taken together, this highlights the need for a reliable, streamlined workflow in peptide/ protein design, purification and characterization [1].

SpyTag/SpyCatcher is a protein coupling approach created by splitting the CnaB2 domain from the fibronectin binding protein FbaB from *Streptococcus pyogenes*. Upon mixing, SpyTag and SpyCatcher associate and spontaneously carry out an amidation reaction promoted by the SpyCatcher residue Glu77, to form an intermolecular isopeptide bond, with a 99% efficiency and at a wide range of conditions, including intracellular assembly [2]. Practical examples include bispecific antibody generation for cancer treatment (anti-CD3/ anti-Her2) [3] and generation of dimeric/ trimeric GLP-1 (showing a 3 to 4-fold increase in activity over the monomeric form) [4].

For the purification and characterization of the peptides, an approach combining liquid chromatography coupled with MALS (multi-angle light scattering) is discussed; this allows for simultaneous fractioning and molecular weight determination. For further characterization and determination of biological activity of the purified fractions, a strategy combining SDS-PAGE and in-cell fluorescent western blotting is proposed, with high specificity and throughput achievable.

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L9

Development and Application of Peptide-Derived Activity- and Affinity-Based Probes Targeting Proteases

Steven H. L. Verhelst[1]

[1] *KU Leuven, Laboratory of Chemical Biology, Department of Cellular and Molecular Medicine*
steven.verhelst@kuleuven.be

Proteases are enzymes that cleave proteins. They are involved in many cellular pathways. Their activity is tightly regulated and dysregulation can lead to a wide variety of human diseases. My lab has set up a research program for the detection of disease-associated proteases by using covalent chemical probes.

In this talk, I will give an overview of my lab's efforts on the development and application of peptide-based covalent chemical probes for serine, cysteine and aspartic proteases with detection ranging from fluorescent microscopy detection of serine proteases involved in inflammation to mass spectrometry-based detection of photoaffinity labeling sites.

L10

Phosphonic Analogs of Thiosemicarbazone as Inhibitors of Tyrosinase

Msanif Msanif[1], Michał Jewgiński[1], Rafał Latajka[1]

[1] *Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, Wrocław*
rafal.latajka@pwr.edu.pl

Tyrosinase (EC 1.14.18.1) is a bifunctional oxidoreductase able to catalyze two reactions: the oxidation of monophenols to o-quinones and the oxidation of o-diphenols to corresponding o-quinones. Both activities are related to the binding of dioxygen to the two copper atoms located in the active site of the enzyme. When L-tyrosine or L-dopa are the substrates, the product of the reaction catalyzed by tyrosinase is dopaquinone, the intermediate in the melanin biosynthesis pathway.

In mammals, including humans, melanin is liable for skin and hair color. Abnormal melanin accumulation in melanocytes in mammals leads to various disorders, which manifest as hyperpigmentation, including melasma, freckles, dark spots, senile lentigo, pigmented acne scars, and melanoma. Tyrosinase also participates in neuromelanin production, and it has been demonstrated that it is involved in the process of neuronal damage, contributing to the development of Parkinson's and other neurodegenerative diseases. The primary strategy to mitigate these undesirable effects is to control melanin biosynthesis by inhibiting tyrosinase. Over the past few decades, numerous inhibitors of the enzyme have been characterized and described in the literature, including kojic acid, azelaic acid, arbutin, aloesin, tropolone, and captopril. As shown in our previous studies, potent inhibitors of tyrosine could be constructed using a thiosemicarbazone scaffold [1-3]. Furthermore, we also demonstrated that derivatives of phosphonic and phosphinic acids can play an important role in inhibiting tyrosinase activity [4]. Therefore, we attempted to combine the inhibitory properties of thiosemicarbazones and the phosphonic group to construct new, effective tyrosinase inhibitors.

Funding: Internal grant of the Faculty of Chemistry, Wrocław University of Science and Technology

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L11

New Chemiluminescent Probes for TMPRSS2 and Related S1 Family Proteases with Arginine Selectivity: A Sensitive Detection Strategy

Marcin Skoreński[1], Emile Verhulst[2], Ingrid De Meester[2], Steven Verhelst[1]

[1] *Laboratory of Chemical Biology, Department of Cellular and Molecular Medicine KU Leuven, Belgium*

[2] *Laboratory of Medical Biochemistry, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Belgium*

marcin.skorenski@kuleuven.be

Serine proteases constitute the predominant mechanistic class of proteolytic enzymes, with type II transmembrane serine proteases (TTSPs) forming a key subgroup of membrane-anchored serine proteases [1]. Although certain TTSPs display autocatalytic activation in vitro, the cellular mechanisms underlying their activation remain poorly understood. A comprehensive characterization of TTSPs is essential for unraveling the complex physiological and pathophysiological signaling pathways regulated by these enzymes. Of particular interest is TMPRSS2, a TTSP that exhibits significantly elevated expression levels in cancerous cells compared to their benign counterparts [2]. This distinctive expression profile positions TMPRSS2 as a promising biomarker for the diagnosis and prognosis of prostate and colorectal cancers, among other potential malignancies. To advance the study, detection, and visualization of TMPRSS2 activity, we have designed and synthesized a focused library of chemiluminescent substrates featuring arginine at the P1 position. These innovative probes enable the sensitive detection and imaging of TMPRSS2 in relevant experimental models, providing valuable tools for exploring its biological roles (**Fig.1**) [3]. Furthermore, we systematically evaluated the activity of these substrates against a diverse panel of serine proteases with specificity profiles similar to TMPRSS2, offering critical insights into substrate selectivity and utility.

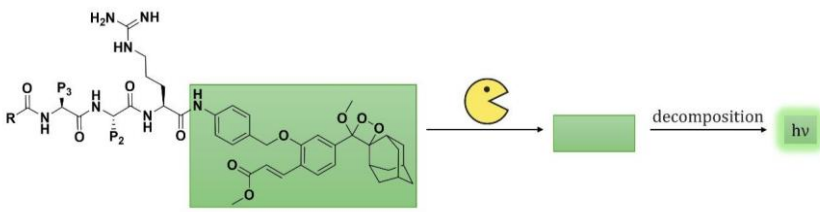

Figure 1. *Activation pathway of developed chemiluminescent substrates.*

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Funded by the European Union

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L12

Modified VHL-binding Peptides for HIF-1α Stabilization and PROTAC Design

Michael Gütschow[1]

[1] *Pharmaceutical Institute, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany*
guetschow@uni-bonn.de

The transcription factor hypoxia-inducible factor 1α (HIF-1α) plays a crucial role in regulating cellular responses to oxygen. When faced with hypoxia, HIF heterodimers activate the expression of genes containing hypoxia-responsive elements which governs e.g. angiogenesis and erythropoiesis. Under normoxic conditions, HIF-1α undergoes proline hydroxylation by prolyl-hydroxylases, facilitating recognition by the von Hippel-Lindau (VHL) tumor suppressor protein, as demonstrated for example by the crystal structure of a complex of VHL with a portion of human HIF-1α (residues 549-582), leading to ubiquitination and subsequent proteasomal degradation [1]. Low molecular weight, peptidomimetic VHL ligands have been utilized for the assembly of proteolysis targeting chimera (PROTAC) degraders.

In the course of our attempts towards PROTACs, intensive efforts have been devoted to the utilization [2], and structural optimization of VHL ligands. A structure-guided and bioactivity-driven design was performed encompassing an iterative and combinatorial strategy to provide VHL inhibitors with dissociation constant lower than 40 nM, independently determined by fluorescence polarization and surface plasmon resonance. Bioactive ligand conformation were explored based on three high-resolution structures of VHL-ligand complexes. The strong HIF-1α-stabilizing properties of these VHL ligands have been demonstrated as well [3].

We assembled PROTACs from a cereblon (CRBN) and a VHL ligase ligand and demonstrated a PROTAC-induced heterodimerization of the two E3 ligases leading to unidirectional and efficient VHL-dependent degradation of CRBN [4].

A recent study explores a PROTAC for NLRP3, the key player in innate immunity. We utilized a thiophene analogue of the NLRP3 inhibitor MCC950 and employed click chemistry for the connection with VHL ligands. PROTAC candidates were evaluated in bidirectional thermal stability studies with NLRP3 and E3 ligases. IL-1β release and protein abundance of NLRP3 were assessed in cellular assays. The first-in-class NLRP3 degrader, PROTAC V2, induces a significant VHL-dependent degradation of NLRP3 and constitutes a valuable tool to decipher the intricate details of the NLRP3 inflammasome [5].

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L13

Materials Mimicking the Natural Surroundings of Cells Based on a Set of Protein Fragments Involved in the Regeneration Process and Polysaccharides

Joanna Lewandowska[1], Justyna Frączyk[1], Angelika Becht[1], Beata Kolesińska[1]

[1] *Institute of Organic Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz*
beata.kolesinska@p.lodz.pl

Dressing materials are used in regenerative medicine to treat wounds, as well as materials supporting the regeneration of damaged tissues [1]. As a part of the research work carried out at the Institute of Organic Chemistry of the TUL, we conduct research on new, biocompatible, multi-component hybrid materials useful in the treatment of non-healing wounds, including diabetic foot syndrome [2,3]. The final hybrid materials stimulate all stages of the wound healing process, thereby providing the basis for a holistic wound treatment tool. The obtained hybrid materials meet all the criteria of a uniform concept for treating chronic wounds - TIME strategy. In the group of diseases occurring with the formation of difficult-to-heal wounds are vascular wounds, diabetic foot syndrome, trophic ulcers, haematological wounds, cancer ulcers and wounds accompanying congenital vascular malformations. The problem of diabetes could affect up to 3 million people in Poland. The diabetic foot syndrome is responsible for about 5% of chronic wounds; in Poland this problem affects 10% of people with diabetes. The risk of ulceration in a diabetic ranges from 12 to 25%. And the risk of amputation in diabetic patients is 30-40 times higher than in the general population. As a result of the synergy between the selected set of biologically active peptides and polysaccharides, it has been found that it is possible to obtain a hybrid material useful in the treatment of difficult-to-heal wounds that affect (i) hemostasis (fragments: α- and γ-chain of fibrinogen, lactoferrin, fibronectin, factor XIII); (ii) inflammation (fragments of pro and anti-inflammatory cytokines); (iii) proliferation (fragments of ECM components: collagens, elastin, fragments of growth factors); and (iv) remodeling of tissue and protection against bacterial infection. Our method was used to select protein fragments involved in the regeneration process, including the synthesis of peptide libraries covering whole proteins (SPOT synthesis) and a dot blot test using specific polyclonal antibodies [4]. Additionally, in the biodegradation process, these materials are decomposed only into natural compounds.

Funding: Financial support, project UMO-2018/31/B/ST8/02760.

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L14

Synthesis of D-Amino Acid-Modified dNTPs with Potential Application in Developing Oligonucleotide Libraries

Paweł Pięta[1], Ewa Burchacka[2], Kacper Pawlak[1,3], Jędrzej Kot[1,3], Amira Tim[1,3], Dominik Mistal[1,3]

[1] *Department of Cell Biology, Poznan University of Medical Sciences, Rokietnicka 5d St., 61-806 Poznan*
[2] *Faculty of Chemistry, Department of Organic and Medicinal Chemistry, Wrocław University of Science and Technology, 27 Wybrzeże Wyspiańskiego St., 50-370 Wrocław*
[3] *Students Scientific Society, Poznan University of Medical Sciences, Fredry 10 St., 61-701 Poznan*
pawel.pieta@ump.edu.pl

Oligonucleotide libraries are used to select functional nucleic acids like aptamers and ribozymes. Their low immunogenicity and toxicity make them ideal candidates for in vivo applications. Introducing chemical modifications into these nucleic acid libraries enhances the DNA's interaction capabilities, thereby expanding its target spectrum [1,2].

The aim of this study was to obtain oligonucleotide libraries modified with D-amino acids. We achieved this objective by:

- synthesizing D-amino acid derivatives of dNTPs.
- developing an enzymatic method for the synthesis of amino acid-modified oligonucleotide libraries.
- biochemically testing the resulting nucleotide libraries.

We synthesized D-amino acid derivatives of deoxynucleotide triphosphates (dNTPs) using a regioselective copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction between ethynyl-dNTPs and azides (D-amino acids) [3]. The resulting products were then used for the enzymatic synthesis of amino acid-modified oligonucleotide libraries via primer extension reactions, employing polymerases from the N9 family. We evaluated the D-amino acid-modified libraries for their suitability as templates in PCR for DNA amplification. Their biological stability was assessed in the presence of S1 nuclease and human serum.

Our research successfully yielded D-amino acid-modified nucleotide triphosphates, as confirmed by mass spectrometry. Incorporation tests of these modified nucleotide phosphates demonstrated their compatibility with enzymatic synthesis of modified oligonucleotides using N9 family polymerases and their amplifiability in PCR using Taq family polymerases. Stability tests further revealed increased stability of the obtained libraries in the presence of nucleases and human serum.

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L15

Innovations in Sustainable Peptide Production

Giorgio Marini[1]

[1] *CEM Corporation, 3100 Smith Farm RoadMatthews, NC 28104, USA*
giorgio.marini@cem.com

Increased global demand for peptide therapeutics has placed a renewed emphasis on improving the efficiency and sustainability of peptide production. This presentation will highlight workflow and methodological improvements for high-throughput peptide synthesis (96-well plates), enabling more efficient access to longer sequences at higher purities. In addition, recent improvements in microwave peptide synthesis related to the total elimination of all washing steps after each cycle (resulting in a massive waste reduction) will be highlighted, with application to both R&D and production scale synthesis. Finally, a new HPLC process for peptide purification that completely eliminates the use of acetonitrile in place of ethanol will be demonstrated. This new process is based on a novel integrated heating system that not only improved peptide recoveries by 50% on average, but also increased the final isolated purity.

L16

Peptide Models of Protein Structural and Regulatory Zinc Sites: Tools for Elucidating Structure-Stability-Function Relationships

Artur Krężel[1]

[1] *Department of Biological Chemistry, Faculty of Biotechnology, University of Wroclaw, Joliot-Curie 14a, 50-383 Wrocław, Poland*
artur.krezel@uwr.edu.pl

Zinc proteins are essential components of the metalloproteomes across all domains of life. As the most prevalent d-block element, zinc (formally Zn(II)) serves various roles as a catalytic co-factor, structural element, or regulatory component. However, questions regarding the (un)folding mechanisms of Zn(II)-dependent proteins, the factors influencing Zn(II) affinity and overall stability, and the impact of toxic metals on native structures and functions remain inadequately addressed. Given the high conservation of structural zinc sites, we can explore the relationships between sequence, structure, and stability by either truncating native folds or mutating specific amino acid residues to gain critical insights. To investigate these relationships, peptide models of zinc-binding sites have proven to be invaluable. By utilizing native or modified sequences of classical zinc fingers, we identified structural elements that modulate Zn(II) affinity, which is linked to the dynamic saturation of these folds in response to cellular Zn(II) fluctuations [1]. Approximately 10% of classical zinc fingers exhibit natural mutations that impact the structure and stability of Zn(II) complexes [2]. Additionally, we studied the central region of the Rad50 protein, which contains a unique interprotein zinc hook motif, demonstrating that this fold possesses exceptional stability and revealing the underlying hidden structural elements that contribute to its unique thermodynamics [3]. Fluorescent models of the zinc clasp motif, crucial for T-cell activation, illustrate how heterodimerization is driven within this small motif [4]. By employing OaAEP1 ligase to ligate peptide fragments, we synthesized a fully functional SmtA metallothionein [5]. We also examined the effects of thiophilic toxic metal ions on the structure and function of structural zinc sites. For example, we found that Ag(I) ions readily damage Cys-containing zinc sites and form complexes dependent on the number of these residues [6]. Both Cd(II) and Hg(II) ions can substitute for Zn(II) in various zinc sites, resulting in newly formed structures that, while structurally similar to their native counterparts, become nonfunctional [7]. These findings were made possible through a series of Cys and His-containing peptide models synthesized via solid-phase peptide synthesis (SPPS).

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L17

Fungal Amino Acids and Peptides: Friends, Foes, Tricksters

Alicja Kluczyk[1], Klaudia Kuźniarska[1], Aleksandra Mitoraj[1]

[1] *Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wrocław, Poland*
alicja.kluczyk@uwr.edu.pl

The medicinal and culinary applications of fruiting bodies of various mushrooms, as well as their poisonous effects attracted a lot of attention throughout the history. The growing interest in fungal metabolites could be attributed to the discovery of penicillin, although macrofungal natural products have been studied even earlier [1]. Nutritional value of mushrooms is related to high protein content. Several bioactive peptides and amino acids were identified (**Fig. 1**), with recent reports on neuroprotective effects of psilocybin and ergothioneine [2].

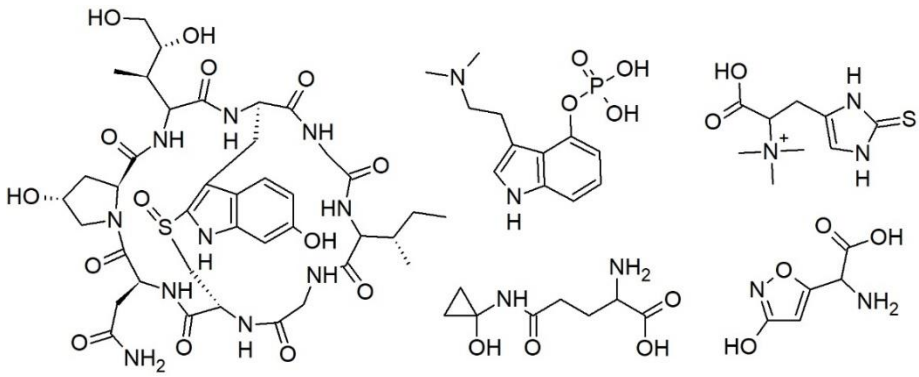

Figure 1. *Selected bioactive mushroom metabolites: α-amanitin, psilocybin, ergothioneine, coprine, ibotenic acid.*

The commercial cultivation of several mushroom species, availability of food supplements, and broad range of research interests require reliable analytical procedures to study the fungal metabolites [3]. In the case of ergothioneine, the limited information on its presence in mushrooms available in Poland, discrepancies in the reported content in various mushrooms and the physicochemical properties, especially the presence of quaternary ammonium motif, prompted us to investigate ergothioneine using liquid chromatography (reversed phase and HILIC) combined with UV and mass spectrometry. Chemical modification of thiol group was also performed. The results indicate that *Boletus edulis* and easily available oyster mushrooms (*Pleurotus ostreatus et alia)* are promising sources of ergothioneine.

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L18

Peptides of the Nervous System and their Derivatives as Target for Metal Ions

Justyna Brasuń[1]

[1] *Wroclaw Medical University, Department of Basic Chemical Sciences*
justyna.brasun@umw.edu.pl

Neuropeptides of the Nervous System represent a broad group of compounds that influence the functioning of the nervous system. This group includes neurotransmitters and neuromodulators involved in various stress-induced responses as: neurobiological, physiological, and psychosomatic which can, in turn, trigger adverse psychological effects such as anxiety or depression. Among these compounds are neuropeptides such as neuropeptide Y (NPY), corticotropin-releasing hormone (CRH), substance P or galanin. Studies have shown that neuropeptide Y acts as a protective factor in response to stress [1]. Another example of a peptide neuromodulator closely linked to stress is galanin. Animal model studies have demonstrated that galanin plays a key role in modulating the stress response; under conditions of chronic stress, elevated levels of this peptide can reduce anxiety-like behaviors and protect neuronal structures from the effects of stress [2].

Stress, especially chronic stress, is an increasingly pressing issue in modern society. For instance, in the United Kingdom in 2018, the group reporting the highest levels of stress consisted of individuals aged 18–24 [3]. A preliminary study on a group of 86 students revealed that over half of the participants experienced high levels of stress [4]. Chronic stress during both early life and adulthood is associated with an increased risk of developing depression [5], partly due to its impact on the homeostasis of metal ions, particularly copper ions Cu^{2+} [6]. The homeostasis of metal ions in the brain may be affected by so-called "stress neuropeptides," which can modulate the availability and distribution of metals in neural tissues. This suggests their potential role in either protecting or damaging neural structures under stress conditions.

Therefore, further research is essential to understand these mechanisms. Such studies will not only deepen our knowledge of the biological underpinnings of stress and depression but may also lead to the development of new, targeted therapeutic strategies.

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L19

Comprehensive Peptide Characterization Using HPLC and MALS: A Multimodal Chromatography Approach

Ema Kosović[1], Andrea Krumm[1]

[1] *Tosoh Bioscience GmbH*
Im Leuschnerpark 4, 64347 Griesheim, Germany
ema.kosovic@tosoh.com

Peptide analysis presents unique analytical challenges due to their structural diversity, charge variability, and susceptibility to degradation. Chromatographic techniques offer robust and versatile solutions for comprehensive peptide characterization and purification. This presentation will drive you through highlighted applications that Tosoh Bioscience provides including Size Exclusion Chromatography (SEC), Hydrophilic Chromatography (HILIC), Hydrophobic Interaction Chromatography (HIC), and Reversed Phase Chromatography (RP).

SEC is employed for assessing molecular size and aggregation state, particularly useful for peptide oligomerization studies. IEX separates peptides based on their charge under specific pH and ionic strength conditions, enabling charge variant profiling and purity assessment. HIC leverages peptide hydrophobicity for selective separation of closely related species, especially in intermediate purification steps. RP-HPLC remains the standard for final peptide purification and impurity profiling due to its high resolution and reproducibility. Complementing these separation techniques, Multi-Angle Light Scattering (MALS) detection provides direct measurement of molar mass and size, enhancing the structural understanding of peptides without reliance on molecular weight standards. The integration of these chromatographic modes with MALS detection enables a comprehensive, orthogonal approach to peptide characterization, supporting both development and quality control in research and biopharmaceutical applications.

L20

Integrated Solutions for the Analysis and Purification of Peptides Using LC and MS Techniques

Krystyna Niedzielska[1]

[1] *Polygen Sp. z o.o., Gliwice, Poland*
krystynan@polygen.com.pl

Polygen, present on the Polish market for over 30 years, offers comprehensive solutions for the analysis and purification of bioactive compounds such as peptides, peptidomimetics, bioconjugates, enzyme inhibitors, and compounds used in biomedical and material sciences. Our portfolio includes advanced HPLC/UHPLC systems with the possibility of integrating fractionation modules and detectors capable of sensitive and universal detection of compounds difficult to determine by classical methods (e.g., lack of chromophore, ionization issues).

The Vanquish Analytical Purification UHPLC system from Thermo Scientific enables precise fractionation, ensuring effective purification of even the most demanding peptide samples.

The CAD (Corona Charged Aerosol Detector) allows for sensitive and universal detection, especially for compounds lacking chromophores or that are difficult to ionize.

A particularly innovative solution is the Plate Express™ system from Advion Interchim Scientific, which works with a mass spectrometer and enables rapid (under 30 seconds) analysis directly from TLC plates. This solution is effective in monitoring reactions, identifying products and impurities, without the need for sample preparation. It significantly reduces the time required for structure confirmation and reaction tracking.

The technologies offered by Polygen comprehensively support peptide synthesis, purification, and quality control processes, increasing their efficiency and reliability of results.

L21

The Gut Microbiome & Disease: from Description to Personalized Medicine

Moez Rhimi[1]

[1] *INRAE-Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, AgroParisTech, Micalis Institute, Université Paris-Saclay, Jouy-en-Josas, France*
moez.rhimi@inrae.fr

The human gut microbiota is involved in wide variety of crucial functions and its association to the human well-being has long been described. Actually, deciphering the microbiota-human crosstalk and how it can be modulated constitutes a real challenge to set out a preventive medicine and to target some diseases including inflammatory disorders. Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are chronically relapsing diseases with an increasing incidence during the last decade. It is demonstrated that IBD patients are characterized by alterations in immune response, disruption of intestinal barrier and alteration of microbiota composition and functions. Fecal transplantation and analysis of the differences between healthy and diseased subjects, in terms of microbiota structure and gene content, were used to establish the involvement of the gut microbiome on the inflammatory bowel diseases. Through functional metagenomics approach our MIHA team demonstrated for the first time that proteolytic homeostasis plays a key role in the gut inflammation. Moreover, we establish the molecular basis of the mode of action of microbial serine proteases and highlighted the protective role of serine proteases inhibitors to protect against inflammation in preclinical models. These pioneer results help us to i) better understand the limited clinical success of dietary intervention in IBD and ii) establish personalized novel therapeutic microbiome-based approaches.

L22

Dual-Activity Peptide–Drug Conjugates with Selective Antileukemic and Antimicrobial Properties

Natalia Ptaszyńska[1], Jan Lica[1,2], Natalia Maciejewska[3], Anna Łęgowska[1], Krzysztof Rolka[1]

[1] *Department of Molecular Biochemistry, Faculty of Chemistry, University of Gdańsk, Poland*
[2] *Department of Medical Biology and Genetics, Faculty of Biology, University of Gdańsk, Poland*
[3] *Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, Poland*
natalia.ptaszynska@ug.edu.pl

Hematopoietic cell transplantation (HCT) is a significant treatment option for leukemia, but its success is often challenged by opportunistic microbial infections, which contribute significantly to mortality in leukemia patients. To address this critical issue, our research group has pursued a novel strategy involving the synthesis of dual-activity compounds combining anticancer and antimicrobial properties. This work represents a continuation and expansion of our earlier studies in this area [1].

To implement this strategy, we synthesized conjugates of fluoroquinolone chemotherapeutics ciprofloxacin (CIP) and levofloxacin (LVX) with cell-penetrating peptides (CPPs) and antimicrobial peptides (AMPs), specifically Transportan 10 (TP10) and lactoferricin B (LfcinB). All discussed conjugates were obtained applying solid-phase method synthesis using various linkers, including redox-sensitive disulfide bridges.

Our studies demonstrate that conjugates contained both LfcinB and TP10 exhibit potent dual activity: selective cytotoxicity towards leukemic cells and broad-spectrum antimicrobial efficacy. LfcinB conjugates, particularly those linked *via* disulfide bridges to ciprofloxacin or fluconazole, showed nanomolar EC_{50} values in HL60 leukemia cells and favorable selectivity indices (BSI, FSI, MSI), with minimal ROS generation and mitochondrial toxicity. Similarly, TP10 conjugates displayed rapid membrane-disruptive effects, nuclear accumulation, and inhibition of both bacterial and human topoisomerases. Computational modeling revealed that TP10 conjugates form membrane-disruptive oligomers and that their metabolites can intercalate into DNA-bound topoisomerase complexes, enhancing cytotoxicity while minimizing mutagenicity.

Importantly, both conjugate classes outperformed equimolar mixtures of their components, underscoring the pharmacological advantage of covalent linkage. These findings support the development of multifunctional peptide–drug conjugates as a novel and promising therapeutic strategy for leukemia treatment, offering to combat both malignancy and associated infections.

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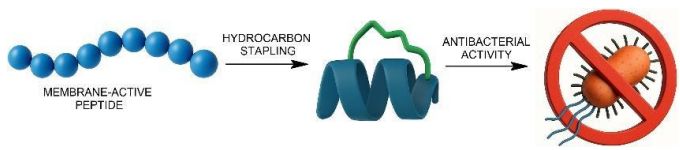
L23

Conformational Stabilization of Membrane-Active Peptides via Hydrocarbon Stapling as a Strategy to Improve Antibacterial Activity

Monika Wojciechowska[1], Małgorzata Lobka[1], Izabela Siekierska[1], Joanna Trylska[1]

[1]*University of Warsaw, Centre of New Technologies, Biomolecular Machines Laboratory, Poland*
m.wojciechowska@cent.uw.edu.pl



The growing crisis of antimicrobial resistance demands innovative therapeutic strategies. Membrane-active peptides (MAPs), including antimicrobial (AMP) and cell-penetrating peptides (CPP), offer promising solutions due to their rapid action and low tendency to induce resistance. However, their clinical translation remains challenging due to poor stability, proteolytic degradation, and cytotoxicity.

We address these limitations by applying hydrocarbon stapling, a chemical strategy that locks peptides into α-helical conformations, enhancing their structural integrity and function. We designed a series of stapled peptides, including anoplin (a naturally occurring AMP with moderate activity) [1], $(KFF)_3K$ (a CPP) [2], and *de novo* amphipathic sequences rich in lysine and leucine [3]. Stapled analogs displayed potent antibacterial activity (MICs 2–4 μM), improved resistance to proteolysis, and lacked hemolytic activity and cytotoxicity.

In a subsequent step, we synthesized peptide–antibiotic conjugates by linking stapled peptides to aminoglycosides (neomycin or amikacin) using cleavable and non-cleavable linkers [4]. These conjugates retained high activity against resistant Gram-negative strains, suggesting a dual mechanism of action: membrane permeabilization combined with antibiotic delivery.

In summary, our results demonstrate that combining stapled peptides with classical antibiotics creates multifunctional hybrids with enhanced antibacterial performance against resistant bacteria. This strategy holds promise for the development of next-generation therapeutics targeting drug-resistant bacteria.

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L24

Anti-Proteolytic AMPs for Targeting ESKAPE Pathogens: A Step Towards Therapeutic Peptides

D. R. Puneeth Kumar[1], Deepanshi Saxena[2,3], Carmela Michalek[1], Rahul Maitra[2,3], Swechcha Singh[2,3], Aparna Sahoo[2,3], Sidharth Chopra[*2,3] and Norbert Sewald[*1]

[1] *Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany*

[2] *Division of Molecular Microbiology and Immunology, CSIR-Central Drug Research Institute, Sector 10, Janakipuram Extension, Sitapur Road, Lucknow-226031, Uttar Pradesh, India*

[3] *Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*

puneeth.dr@uni-bielefeld.de

The two major recently raised concerns about antibiotics are their effects on the environment and the emergence of antibiotic resistance, which is becoming a greater threat to world health. Antimicrobial peptides (AMPs) possess a unique, nonspecific membrane-disruptive action, providing an intriguing approach to addressing the concern of antibiotic resistance. However, in the therapeutic use of AMPs, inadequate proteolytic resistance is a pressing issue that needs to be addressed. Traditional techniques are also being investigated, including stapling, the use of D-amino acids, and complex chemical changes.[1-3] In this work we synthesized a set of novel AMPs with high proteolytic resistance based on a repeating peptide structure unit $(XYZ)_n$ (where X stands for Arg and Lys derivatives, Y for Trp, Z for Leu or γ^4Leu, and n=4) which was developed by sequentially incorporating non-natural amino acids to the α- peptides.[4] With four repeating units, **P3** and **P9** are the most potent of these peptides and have broad-spectrum antimicrobial activity against ESKAPE pathogens. Furthermore, the selectivity index of peptides **P3** and **P9** against strains of *S. aureus* was quite high. With an emphasis on their antibacterial activity and selectivity index, we are going to investigate the structural characteristics, modes of action, and therapeutic potential of Trp- and cation-rich AMPs in this work after considering these initial results. The early results of the newly designed peptides' broad-spectrum antibacterial activity with low toxicity are crucial for developing next-generation antimicrobials in the battle against antibiotic resistance.

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L25

Redox-Responsive Peptide Dimer for the Stabilization and Release of Therapeutic dsRNA

Jannik Paulus[1,2]

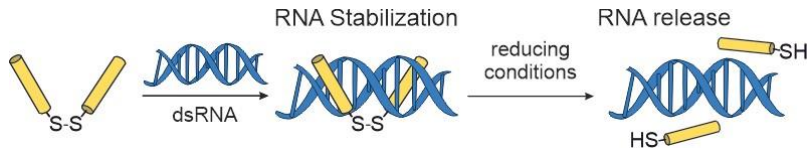
[1] *Department of Chemistry and Pharmaceutical Sciences, VU University Amsterdam, De Boelelaan 1108, 1081 HZ, Amsterdam (The Netherlands)*
[2] *Amsterdam Institute of Molecular and Life Sciences, VU University Amsterdam, De Boelelaan 1108, 1081 HZ, Amsterdam (The Netherlands)*
j.paulus@vu.nl

Double-stranded RNAs (dsRNA) hold significant therapeutic potential but suffer from poor stability and limited cellular uptake.[1] Inspired by the plant viral protein TAV2b—a dsRNA-binding protein that suppresses RNA silencing—RNA-binding peptides have been developed to mimic its structure. Previous stapled peptide designs reproduced helices 1 and 2 of a TAV2b monomer but did not dimerize.[2]

In our approach, we focused on helix 2 of TAV2b and designed a minimal dimeric peptide, covalently linked via a disulfide bridge that replaces the native helical zipper motif. This covalent dimer mimics the functional assembly of the native protein, binds dsRNA with high affinity, and stabilizes it in serum, while also promoting cellular uptake.[3]

Crucially, the disulfide linker enables redox-sensitive control: under intracellular reducing conditions—such as those found in tumour cells with elevated glutathione levels—the dimer dissociates into inactive monomers, triggering RNA release. This mechanism offers a selective and environmentally responsive delivery strategy.[3]

We systematically investigated the impact of the disulfide linkage on RNA binding, stabilization, and release. Our findings highlight this redox-responsive peptide dimer as a promising tool for the targeted delivery of dsRNA therapeutics, including siRNA or miRNA.



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L26

Impact of Glassware Quality on Test Results and Safety in the Modern Laboratory

Paulina Pohl[1], Alexander Gronner[1]

[1] *Labo24, Gliwice, Poland*
paulina.pohl@labo24.pl

This presentation explores how the quality of laboratory glassware affects both the reliability of analytical results and safety in modern labs, especially in biochemical and pharmaceutical research. The introduction includes an overview of the history of glass, its definition, a classification of its main types, and the standards it should meet in laboratory use. Attention is drawn to the growing issue of replacing high-quality glass with cheaper alternatives that differ in physicochemical properties — a practice that may compromise experiment accuracy and increase safety risks.

L27

AI-Assisted Design of a HER2-Targeted, Pro-Apoptotic Amyloidogenic Peptide: From Concept to Structure and Function

Milena Czeszejko[1], Justyna Sawicka[1], Aneta Szymańska[1], Kamila Kitowska[2], Rafał Sądej[2], Sylwia Rodziewicz-Motowidło[1]

[1] *Department of Biomedicinal Chemistry, Faculty of Chemistry, University of Gdańsk, Poland*
[2] *Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, Poland*
s.rodziewicz-motowidlo@ug.edu.pl

The design and analysis of peptides have relied heavily on empirical methods, but recent advancements in artificial intelligence (AI) have begun to transform this landscape. AI, particularly machine learning, enables rapid optimization of peptide sequences by predicting their bioactivity using large datasets [1]. Deep learning tools uncover sequence–activity relationships and guide the rational design of peptides with desirable pharmacological features [2]. AI models also support the prediction of solubility, receptor binding, and stability [3], as well as structural conformation, as seen in breakthroughs such as AlphaFold [4,5]. These tools accelerate the development of therapeutic peptides with improved properties [6].

In this study, we present the AI-assisted design of a multifunctional peptide that combines three features: HER2 receptor targeting (via YCDGFYACY), amyloidogenicity (via [Nle]LVFFAE), and pro-apoptotic activity (via GVYY[β-Ala]TL). The design incorporated glycine-serine linkers (e.g., GSG, GSSGSG) to preserve functional domain separation and reduce undesired proteolytic cleavage. A matrix metalloproteinase (MMP)-sensitive cleavage site (PLGLAG) was introduced before the apoptotic motif to ensure environment-specific activation.

The designed peptide was synthesized and its structure confirmed using circular dichroism (CD) spectroscopy, which revealed characteristic β-sheet content. Its fibrillization ability was verified via transmission electron microscopy (TEM) and Thioflavin T (ThT) fluorescence assay. Results confirmed the formation of amyloid-like fibrils and the peptide's structural integrity under physiological conditions.

Overall, this work illustrates the integration of AI-driven sequence optimization with experimental validation for the development of therapeutic amyloidogenic peptides, opening new avenues for precision nanomedicine and targeted cancer therapy.

Funding: This work was supported by a grant NCN 2022/45/B/NZ7/03889.

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L28

Visible-Light-Mediated Transformations of Sulfur- and Selenium-Containing Peptides for Post-Synthetic Modification

Mateusz Waliczek[1], Piotr Stefanowicz[1]

[1] *Faculty of Chemistry, University of Wroclaw, F. Joliot Curie 14*
piotr.stefanowicz@uwr.edu.pl

We present a series of novel, visible-light-driven methods for the selective modification of peptides containing sulfur or selenium, offering mild and bioorthogonal strategies for post-synthetic peptide editing. Central to these transformations is the use of Rose Bengal as a catalytic photosensitiser in an aqueous environment under LED-type visible light irradiation.

First, we demonstrate the efficient desulfurisation of cysteine residues in peptides and proteins at room temperature with a high yield. This method has been validated using synthetic model peptides, lysozyme C and α-crystallin, and has been successfully incorporated into a one-pot native chemical ligation (NCL)-desulfurisation protocol. This photocatalytic procedure is currently the most efficient and convenient method of converting the cysteine moiety to an alanine residue [1].

Secondly, we report the site-selective conversion of diselenide-containing peptides into dehydroalanine (Dha) via visible light-induced oxidative Se–Se bond cleavage, followed by β-elimination. This transformation proceeds with high chemoselectivity and enables the functionalisation of Dha residues downstream. Additionally, selenocysteine-containing peptides undergo photoactivated selenofunctionalisation reactions with alkenes in the presence of oxygen, providing a practical tool for peptide cyclisation and conjugation. We also describe a photoinduced partial deselenization protocol that leads to selenoethers [2]. These can be combined with diselenide metathesis to create more complex peptide modifications.

These light-mediated, Rose Bengal-catalysed processes collectively establish a versatile platform for biocompatible peptide editing and expand the synthetic toolbox for site-selective functionalisation in chemical biology.

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L29

Nature-Inspired Cyclic Peptides as Highly Selective Matriptase Inhibitors: Insights from SAR and Molecular Dynamics

Dawid Dębowski[1], Aleksandra Helbik-Maciejewska[1], Agata Gitlin-Domagalska[1], Malihe Hassanzadeh[2], Matthieu Lepage[2], Alexandre Joushomme[2], Antoine Désilets[2], Pierre-Luc Boudreault[2], Richard Leduc[2], Krzysztof Rolka[1]

[1]*Department of Molecular Biochemistry, University of Gdansk, Gdansk, Poland*
[2]*Department of Pharmacology-Physiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada*
dawid.debowski@ug.edu.pl

Matriptase (MT1) and matriptase-2 (MT2) are members of type II transmembrane serine proteases family. Despite sharing substantial structural homology and overlapping substrate preferences, these proteases fulfil distinct physiological functions. MT1 is broadly expressed across epithelial tissues, where it plays a crucial role in maintaining epithelial integrity [1]. Notably, an increased expression of MT1 has been documented in a range of epithelial cancers, such as prostate, breast, ovarian, cervical, and stomach cancers. In contrast, MT2 exhibits a tissue-restricted expression profile, being predominantly found in hepatocytes under physiological conditions. Here we present a truncated variant of frog-skin-derived peptide *Huia Versabilis* Bowman-Birk Inhibitor (HV-BBI), that demonstrates high inhibitory activity against MT1 with nanomolar K_i and remarkable - nearly 1000-fold - selectivity over its closest homolog, MT2 [2]. To our knowledge, such a significant preference for MT1 over MT2 has not been previously reported, making our cyclic, non-covalent inhibitor unique among known MT1 inhibitors. Structure–activity relationship (SAR) studies and molecular dynamics (MD) simulations revealed key interactions governing inhibitor binding. Notably, the inhibitor's *C*-terminal region exhibits dynamic behaviour and appears to distance itself from the hydrophobic region of MT1. This observation suggests that hydrophobic and π-π interactions between MT1 and the *C*-terminal Phe13 are less critical than previously thought. Ala-scanning showed that residues located in the middle part of the binding loop Lys5, Ser6, Ile7, and Arg10, are indispensable for binding to MT1. In contrast, substitutions of Gly1, Trp3, Pro11, or Phe13 were well tolerated and did not significantly impair inhibitory activity. Consequently, some of these non-essential amino acids were removed to reduce the overall size of the inhibitor. Moreover, the native disulfide bridge was replaced with more stable surrogates, such as triazole and diselenide linkages. Importantly, this inhibitor retains activity in a cellular context, supporting the continued development of HV-BBI-based scaffolds for targeted protease inhibition.

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L30

Bridging Food Chemistry and Peptide Science: New Tools to Study Lactosylation

Monika Kijewska[1], Michalina Zawadzka[1], Piotr Stefanowicz[1]

[1]*University of Wrocław, Faculty of Chemistry, F. Joliot-Curie Street 14, 50-383 Wrocław, Poland*
monika.kijewska@uwr.edu.pl

Milk is a fundamental food source, rich in proteins, carbohydrates, fats, vitamins, and minerals. However, its susceptibility to bacterial contamination necessitates thermal treatment such as pasteurization or UHT processing.[1] Due to its high lactose and protein content, milk is particularly prone to both early and advanced glycation, especially under high-temperature processing. In some dairy products, such as skim milk powder, up to 50% of lysine residues can be converted to lactulosyllysine.[2] Our studies show that heat treatment of peptide-lactose mixtures leads to lactosylation at the peptide's N-terminus, often accompanied by oxidative deamination of the N-terminal amino acid, forming an α-ketoacyl residue.[3] This reaction limits the utility of direct lactosylation but suggests the potential formation of novel carbonylated peptides—especially in dairy products that are subject to frequent proteolysis, such as hypoallergenic infant formulas. To overcome the challenges of direct thermal lactosylation, we developed a new method for the synthesis of defined non-enzymatic peptide lactosylation products.[4] This approach is based on solid support microwave-assisted synthesis combined with boronate affinity chromatography [5], enabling the synthesis of isotopically labeled lactosylated peptides derived from trypsin digestion. Following the discontinuation of ChemMatrix resin production (used in our procedure both in synthesis and affinity purification), we explored commercially available TentaGel resins and propose an integrated "Affinity on Demand" strategy: a one-pot synthesis and enrichment method utilizing TentaGel-functionalized.[6]

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L31

Design, Synthesis, Structures, and Functions of Miniproteins

Łukasz Berlicki[1]

[1] *Department of Bioorganic Chemistry, Wrocław University of Science and Technology, Wrocław, Poland*
lukasz.berlicki@pwr.edu.pl

Miniproteins, which are defined as polypeptides with a molecular weight not exceeding 10 kDa and stable tertiary structures, show great potential for the development of functional molecules.[1] They combine the advantages of peptides and proteins, namely, synthetic availability and expanded, stable three-dimensional structures.[2] The possibility of synthesis using a solid-phase approach enables the incorporation of any chosen building block, thereby further extending its potential. Miniproteins have already been widely used for the creation of biologically active compounds. In particular, several protein-protein interaction inhibitors have been constructed.[3]

Here, our recent progress in the development of functional miniproteins will be discussed. Miniproteins have been designed *de novo* using the methodology elaborated and validated by us.[4] Subsequently, a highly efficient method for synthesizing miniproteins using flow chemistry has been applied. Obtained miniproteins have been characterized using circular dichroism, differential scanning fluorimetry, and NMR spectroscopy.[5] Molecules exhibiting biological or catalytic activity have been obtained. In particular, compounds that inhibit the PD1/PD-L1 interaction for cancer immunotherapy have been developed.[6] Moreover, enzyme-like catalysts for various reactions have been obtained.[7]

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L32

Folded or Unfolded – Influence of Salt Additives on the Helicity of Oligourea Foldamers

Naveen Gupta[1], Kinga Chaniewicz[1], Rafał Chojnacki[1], Karolina Pułka-Ziach[1]

[1] *University of Warsaw, Faculty of Chemistry, Pasteura 1, 02-093 Warsaw*
karola@chem.uw.edu.pl

Oligourea foldamers [1,2] are unnatural oligomers composed of units connected via urea linkages. These compounds fold into stable 2.5-helices, meaning that one helical turn consists of 2.5 urea residues.[3] These oligomers, referred as helicomimetics, serve as excellent structural mimics of peptides, as the parameters of α-helices and oligourea helices are comparable despite the chemically distinct nature of their backbones. In recent years, it has been demonstrated that, in addition to mimicking the structure of natural peptides, appropriately designed oligoureas can also replicate their function and biological activity.[4]

In this project we have focused on the supramolecular aspects of oligoureas and their isostructural analogs, containing thiourea or guanidine/guanidinium groups. Moreover, the impact of salt additives on the stability of the secondary structures was examined. It was found out that it was possible to disrupt intramolecular hydrogen bonds in helicomimetics with thiourea groups at the C-terminus and replace them with hydrogen bonds formed with external acceptors. In the case of oligomers with guanidine groups, it was shown that for guanidinium salts, anions exhibited a stabilizing effect on the helices. For neutral guanidine oligomers, the way in which the guanidine group was substituted influenced the binding strength of simple anions. The effect of anions (salts) on the helicity of oligourea-based foldamers may be significant and depends on the primary structure of the oligomers.

Foldamer chemistry—including oligoureas and their derivatives—is currently a rapidly developing field at the intersection of organic chemistry, peptide chemistry, and supramolecular chemistry. Research into folding processes and interactions between foldamers and other molecules contributes to the development of new compounds with well-defined properties. Such compounds have potential applications in various areas—from peptidomimetics to nanomaterials.

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L33

Foldameric Nanostructures and Functional Amyloids as an Example of the Bright Side of Peptide Aggregation Phenomenon

Monika Szefczyk[1*], Natalia Szulc[2], Aleksandra Kalitnik[3], Oliwia Polańska[3], Małgorzata Kotulska[3]

[1] Department of Bioorganic Chemistry, Wroclaw University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
[2] Department of Physics and Biophysics, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375, Wrocław, Poland
[3] Department of Biomedical Engineering, Wroclaw University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland
*monika.szefczyk@pwr.edu.pl

Many proteins and peptides have a tendency to aggregate, a characteristic generally considered undesirable as it reduces solubility and hinders their therapeutic use. Aggregation can result in the formation of insoluble protein fibers, known as amyloids, which are linked to different diseases. However, there also exist functional amyloids that fulfill specific physiological roles. Peptides self-association also holds promise in nanotechnology, where peptides can be engineered into nanostructures for a wide range of applications. Here, of particular interest are foldamers, synthetic peptides that adopt well-defined, stable conformations in solution. Moreover, a potential application of bacterial functional amyloids could be exploited with this regard. Our research explores different aspects of peptide aggregation, and how this process can be harnessed beneficially.

Our research explores the amyloidogenic properties and functional potential of amyloids, focusing on cross-interactions. For instance, we identified novel amyloidogenic motifs in fungal NOD-like receptors whose aggregation is modulated by subtle environmental changes, suggesting a role in antifungal defense.[1] Additionally, we showed that the bacterial peptide PSMα3 inhibits insulin and hIAPP fibrillation *in vitro* by disrupting early aggregation through hydrophobic interactions and protects endothelial cells from hIAPP toxicity without being cytotoxic itself.[2]

In parallel, we investigated hierarchical method based on self-assembly for nanostructures formation using foldamers containing cyclic β-amino acids residues as building blocks. These coiled-coil-based foldamers self-organize into nanofibers *via* a zipper-like mechanism, with aggregation behavior determined by residue position.[3]

Altogether, these studies highlight the dual utility of peptide aggregation both for functional biological mechanisms and as a tool for designing novel biomaterials.

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L34

Design and Characterization of β-Catenin Cite-Specific Cell-Permeable β-Hairpin-Based Inhibitors

Juan Lizandra Perez[1,2], Tom N. Grossmann[1,2,3]

[1]*Department of Chemistry and Pharmaceutical Sciences, VU Amsterdam, Amsterdam, Netherlands*
[2]*Amsterdam Institute of Molecular and Life Sciences, VU Amsterdam, Amsterdam, Netherlands*
[3]*Rudolf Faculty of Chemistry, Institute of Organic and Biomolecular, Georg-August-University, Göttingen, Germany*
j.lizandraperez@vu.nl

β-hairpins three dimensional structures and adaptability makes them valuable scaffolds for the engineering of bioactive molecules [1], providing a means to bridge the gap between small molecule drugs (poor specificity) and antibodies (poor cellular uptake) while remaining cost-effective and synthetically accessible. This versatility has increased interest in using β-hairpin motifs to target complex PPIs. Despite significant progress in the design of monomeric soluble hairpins and successful guidelines for their design [2], β-hairpins are still underutilized as the basis for peptide mimetic inhibitors, particularly for intracellular targets. Their potential as therapeutic scaffolds is often limited by several factors. First, they tend to lack interstrand interactions, which leads to decreased uptake and affinity due to lower structural stability in solution. Additionally, these scaffolds have a propensity to aggregate because of their "flat" nature and higher hydrophobicity compared to α-helices [3]. To address these challenges, various strategies have been implemented to improve the stability and affinity of β-hairpins, such as backbone cyclization, interstrand-crosslinking, incorporation of turn mimetics, and backbone modifications [4]. These modifications can positively impact solubility, affinity, and permeability but require detailed structural knowledge and a time-consuming and costly optimisation process. Consequently, there is a growing need for an approach that enables the systematic and straightforward design and characterisation of novel highly selective Permeable β-hairpin-based Inhibitors (PβhI) that can selectively bind specific regions of intracellular targets. The primary goal of this project is to integrate advanced techniques and unlock their synergies in the design of cell permeable binders of so far undruggable intracellular targets. Specifically, the approach will combine site recognition and epitope discovery from de novo design with enhanced affinity achieved through phage-display maturation. Furthermore, it will improve stability and permeability of the β-hairpin designs via interstrand crosslinking (e.g., using bis-electrophiles or disulphides), which will also facilitate library diversification.

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L35

Application of Combinatorial Chemistry Methods in the Characterization of Murine Serine Proteases

Wiktoria Mallek[1], Aleksandra Wróblewska[2], Gabriela Mirecka[1], Agnieszka Piwkowska[2], Adam Lesner[1], Magdalena Wysocka[1]

[1] *University of Gdansk, Faculty of Chemistry, Wita Stwosza 63, 80-308 Gdansk, Poland*
[2] *Mossakowski Medical Research Institute Polish Academy of Sciences, Adolfa Pawińskiego 5, 02-106 Warsaw, Poland*
wiktoria.mallek@phdstud.ug.edu.pl

Diabetes refers to metabolic illnesses that cause hyperglycemia due to impaired insulin synthesis or activity by pancreatic β-cells. Diabetes causes most blindness, renal failure, myocardial infarctions, cerebrovascular accidents, and lower limb amputations, according to WHO data. Around 2 million people died from diabetes and renal disease in 2021. One of the most common and severe consequences of diabetes, diabetic kidney disease (DKD) is characterized by chronic hyperglycemia, cellular insulin resistance, and renal structural damage. Unfortunately, our knowledge of origin and mechanisms of this form of nephropathy is still poor. Recent research suggests that DKD is caused by glomerular podocyte cell structural damage and dysfunction. Increased proteolytic enzyme activity may impair the renal filtration barrier. This damage results in podocyte dysfunction, which is crucial in the onset of proteinuria in diabetic nephropathy. Recent reports indicate that several proteases modulate the glomerular response to diverse metabolic conditions, suggesting that renal damage in diabetes may be linked to altered proteolytic enzyme activity [1-3].

The work aimed to develop novel compounds that can monitor proteolytic activity in cell lysates, podocyte extracellular medium, and urine samples, being the foundation for new diagnostic tools to detect diabetic nephropathy early. Consequently, peptide substrates libraries were synthesized comprising four and six modified positions respectively. Mix and split technique was used for synthesis of library and deconvolution was performed using iterative approach against recombinant mouse neutrophil elastase (rMNE). After synthesizing and determining kinetic parameters, an appropriate rMNE substrate was developed to study neutrophil proteases in podocytes and better understand diabetes-related glomerular barrier dysfunction. These studies will allow us to investigate the impact of proteases in podocyte damage and death, which can lead to renal failure in diabetic nephropathy.

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L36

Novel Legumain Activity-Based Probes

Jonathan Coene[1], Simon Wilms[1], Steven Verhelst[1]

[1] *Laboratory of Chemical Biology, Department of Cellular and Molecular Medicine*
KU Leuven, Belgium
jonathan.coene@kuleuven.be

Legumain, also known as asparaginyl endopeptidase (AEP) or δ-secretase, is a cysteine protease of the CD clan of proteolytic enzymes. It is best known for its high P_1 specificity toward asparagine, and to a lesser extent, aspartic acid. In addition to its endopeptidase activity, legumain also exhibits carboxypeptidase and ligase activity [1-3]. Legumain plays important roles in protein processing and antigen presentation making it pharmacologically relevant in a variety of disease contexts such as cancer [4-6]. While mammalian legumain is primarily localized in the endolysosomal system, recent studies have identified its presence in the extracellular matrix, as well as in the cytosol and nucleus. In these compartments, legumain may retain activity either under pathological acidic conditions or at near-neutral pH through stabilization by interacting proteins or intramolecular complexes [2,3].

Previously, most activity-based probes (ABPs) for legumain incorporated aspartic acid in the P_1 position [7]. However, these probes suffer from limitations such as (1) possible cross-reactivity with caspases, (2) lower affinity for legumain due to its preference for P_1 asparagine, and (3) inability to label legumain under neutral pH conditions. To address these limitations, we have developed a novel series of ABPs containing a P_1 asparagine residue and an alkyne warhead (**Fig. 1**). Moreover, we are currently working to convert these probes into photoactivatable tools by P_1 photocaging, enabling precise spatiotemporal control of legumain inhibition in live-cell or *in vivo* contexts.

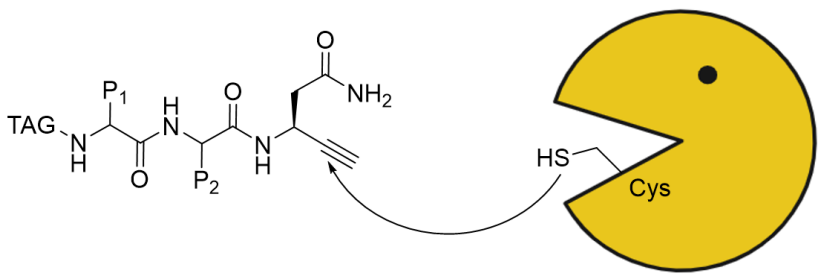

Figure 1. *P_1 asparagine probe covalently labeling the active site cysteine of legumain.*

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L37

Activatable and Membrane-Retained Substrate Probes Reveal Functional Activity of MMPs, ADAMs, and GzmB in Breast Cancer

Abdulla Al Mamun[1], Martyna Majchrzak[1], Julia Nguyen[1], Marcin Poręba[1]

[1] *Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wroclaw University of Science and Technology, 50-370 Wrocław, Poland*

marcin.poreba@pwr.edu.pl

Proteolytic enzymes play central roles in regulating cancer progression and the tumor microenvironment. Among them, Matrix Metalloproteinase (MMPs) and A Disintegrin and Metalloproteinase (ADAMs) are key drivers of tumor invasion, extracellular matrix (ECM) remodeling, and immune evasion. In contrast, granzyme B (GzmB), a serine protease secreted by activated cytotoxic T lymphocytes and natural killer (NK) cells, acts as a major effector of immune-mediated tumor cell killing [1-2]. Together, these protease families define a dynamic proteolytic landscape shaped by both tumor and immune cells. Despite their significance, current tools for studying proteases in cancer focus primarily on gene or protein expression, which often poorly correlates with enzymatic activity. As proteases are tightly regulated at the post-translational level, their biological function depends on their active form, not just their presence. Moreover, there is a critical need for multiplexed, single-cell technologies that can resolve spatial and functional heterogeneity in protease activity across diverse cell populations. We hypothesize that simultaneous, functional detection of active MMPs, ADAMs, and GzmB at single-cell resolution will provide novel insight into tumor-immune protease crosstalk in breast cancer.

To address this, we developed a dual-platform system comprising a suite of fluorescently labelled ACPP probes targeting ADAM10, ADAM17, MMP-2/9, and MMP-14 (**Figure A**). These probes were validated for specificity and activity in MCF-7 and MDA-MB-231 breast cancer cell lines using the IncuCyte system for live-cell imaging. In parallel, we designed an MRS probe that selectively responds to extracellular GzmB activity and is optimized for mass cytometry (CyTOF) (**Figure B**). This integrated approach allows multiplexed detection of functionally active proteases from both tumor and immune compartments in a single CyTOF assay.

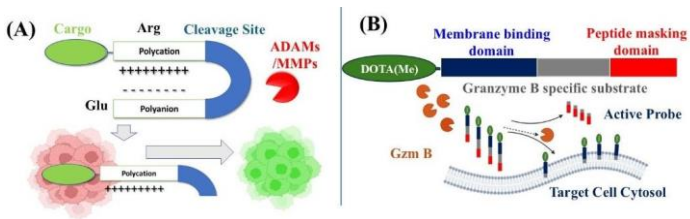

Figure A. *Design and activation mechanism of activatable cell-penetrating Peptide (ACPP), and* ***B.*** *GzmB-responsive membrane-retained substrate (MRS) metal-labelled probe. All probes were synthesized via solid-phase peptide synthesis and validated for stability, specificity, and functional performance.*

Funding: 2022/47/B/NZ5/02405, OPUS, National Science Centre in Poland.

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L38

Cyclic Furin Inhibitors with Disulfide Bridge Mimetics with Anticancer Activity Against Glioblastoma and Lung Cells

Aleksandra Helbik-Maciejewska[1], Agata Gitlin-Domagalska[1], Dawid Dębowski[1], Anna Łęgowska[1], Abdessamad Zerrouqi[2], Beata Pyrzyńska[2], Krzysztof Rolka[1]

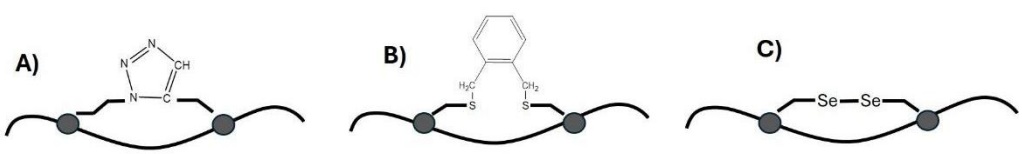
[1] *Department of Molecular Biochemistry, Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland*

[2] *Department of Biochemistry, Faculty of Medicine, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland*

aleksandra.maciejewska@phdstud.ug.edu.pl

Furin is the best-characterized member of the proprotein convertase subtilisin/kexin type (PCSK) family. It is a calcium-dependent transmembrane protease, also known as PCSK3 or paired basic amino acid cleaving enzyme (PACE). The abbreviation PACE refers directly to the canonical cleavage site of this enzyme, which is Arg-X-Lys/Arg-Arg↓ (where X represents any amino acid and ↓ indicates the cleavage site). Furin is widely distributed throughout the organism and is involved in various physiological processes, such as the activation of growth factors, cytokines, metalloproteinases, and adhesion molecules. Moreover, due to its ability to activate a wide range of proteins, furin plays a role in the development of inflammatory diseases, cancers, and infections caused by viruses and bacteria. An imbalance in furin activity may contribute to the formation, progression, and metastasis of various tumors [1]. It is implicated in various cancer types, including lung and brain cancers [2]. Therefore, furin inhibitors are considered potential anticancer therapeutics.

In 2023, we developed a series of potent cyclic furin inhibitors [3]. Recently, we synthesized analogs containing disulfide bridge mimetics to enhance their stability in the reducing, intracellular environment. We replaced the disulfide bridge with triazole, xylene and diselenide bridges. Schematic structures of disulfide bridge mimetics are shown in the Figure 1. As a result, we obtained inhibitors with comparable inhibitory potency against furin and improved anticancer properties against lung cancer and glioblastoma cells. Both, chemical syntheses and biological investigations of synthesized inhibitors will be presented.



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L39

Application of Hydrocarbon-Stapled Peptides and Aminoglycosides to Peptide Nucleic Acid Conjugates for Enhanced Uptake and Improved Antibacterial Activity

Izabela Siekierska[1], Michał Burmistrz[1], Joanna Trylska[1]

[1] *University of Warsaw, Centre of New Technologies, Banacha 2C, 02-097 Warsaw, Poland*
i.siekierska@cent.uw.edu.pl

The rise of antibiotic-resistant bacteria is among the most serious global healthcare threats, while identifying effective alternatives to traditional antibiotics remains a significant challenge. However, several innovative strategies involving antimicrobial peptides [1] or antisense oligomers (ASOs) [2] are gaining increasing interest. In antibacterial applications, the most extensively studied ASOs are peptide nucleic acids (PNAs).[3] These nucleic acid analogs consist of *N*-(2-aminoethyl)glycine units connected via amide bonds. Their unique neutral backbone provides enhanced enzymatic stability and improved binding affinity to naturally occurring nucleic acids. The main limitation restricting the application of PNAs is their inability to permeate complex bacterial membranes. Despite various attempts, such as "Trojan horse" strategies involving conjugation to vitamin B12 [4a] or siderophores [4b], cell-penetrating peptides remain the most widely used PNA transporters.[5]

We investigated two complementary methods for delivering PNA targeting the essential bacterial gene *acpP* into bacterial cells. In the first approach, we evaluated the ability of the naturally occurring peptide anoplin and its hydrocarbon-stapled analog to transport PNA into gram-negative bacteria.[6] These results were subsequently compared with those obtained using the well-characterized cell-penetrating peptide $(KFF)_3K$ and its structurally stabilized analog. The second strategy involved covalent conjugation of PNA oligomers to aminoglycoside (AG) antibiotics, neomycin and amikacin. These cationic small-molecule drugs enter gram-negative bacterial cells, and we hypothesized that they may facilitate the internalization of PNA oligomers. Our results demonstrated that the AG-PNA conjugates' activity against AG-resistant strains is attributed to the antisense mode of action of the PNA oligomer, thereby confirming its delivery into the bacterial cytoplasm. These findings demonstrate the potential of both peptide-mediated and antibiotic-facilitated delivery systems for enhancing PNA-based antimicrobial therapy.

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L40

Substrate-Based Probes with DNA-Intercalating Fluorophores for Caspase Activity Analysis in CAR-T Cells

Maksymilian Zabijak[1], Julia Nguyen[1], Małgorzata Firczuk[2], Marcin Poręba[1]

[1] *Department of Chemical Biology and Bioimaging, Wroclaw University of Science and Technology, Wroclaw, Poland*
[2] *Department of Immunology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*
marcin.poreba@pwr.edu.pl

A wide range of methods for analyzing caspase activity has been developed over the years, including substrate-based probes – chemical compounds consisting of a cleavable peptide sequence and a reporter group. Years ago, a substrate containing a DNA-binding dye was developed.[1] In that probe, fluorescence intensity increases significantly upon binding to DNA (**Figure 1a**), allowing for visualization of caspase activity in real time. To the best of our knowledge, the structure of only one such commercially available caspase-3/7 detection reagent has been published.[1]

Preliminary studies have revealed robust activation of caspases following antigen-dependent CAR-T cell stimulation, suggesting that caspases could serve as biomarkers of CAR-T cell efficacy.[2]

Here, we present the design of novel substrate-based probes equipped with DNA-intercalating fluorophores. By replacing the peptide sequence with those derived from HyCoSuL data, selectivity toward different proteases can be tailored.[3] This, in turn, could enable the identification of the most suitable caspases as potential biomarkers of CAR-T cell activity. The synthesized probes will be used to detect an effector subpopulation of CAR-T cells, which may serve as a biomarker of response to CAR-T therapy in patients (**Figure 1b**).

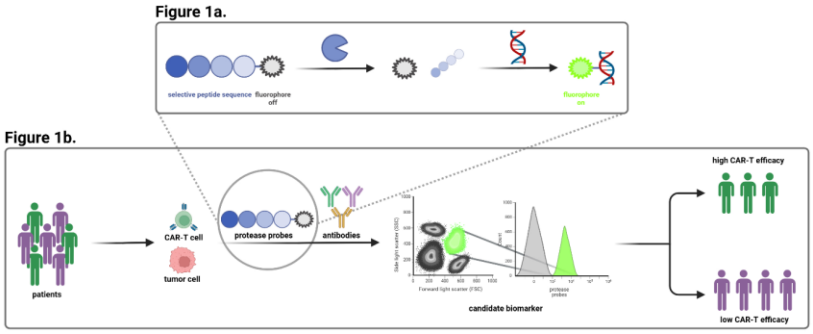

Figure 1. ***a)*** *Intercalation-induced fluorescence.* ***b)*** *Research strategy for dissecting caspase activity in various CAR-T cell populations using a flow cytometry panel.*

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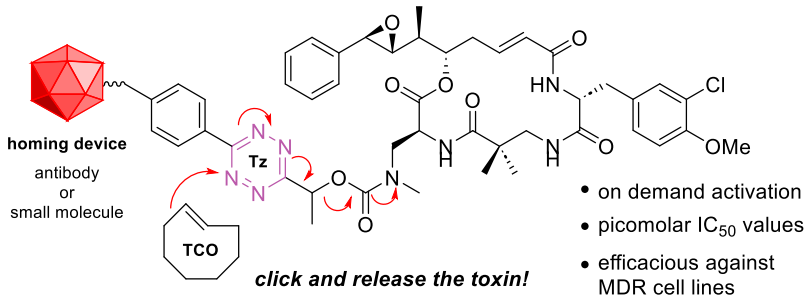
L41

On Demand Activation of Cryptophycin Drug Conjugates by Click Chemistry

Jona Voss[1], Peter Bitsch[2], Harald Kolmar[2], Norbert Sewald[1]

[1]*Organic and Bioorganic Chemistry, Bielefeld University, Bielefeld (Germany)*
[2]*Clemens-Schöpf Institute for Organic Chemistry and Biochemistry, TU Darmstadt, Darmstadt (Germany)*
jona.voss@uni-bielefeld.de

Cryptophycins show great potential as payloads in drug conjugates for targeted cancer therapy by exhibiting single-digit picomolar cytotoxicity combined with a profound efficacy against multidrug resistant (MDR) cell lines. Their outstandingly high cytotoxicity urges the need for high target specificity, making the *click-to-release* strategy a perfect symbiosis. Payloads linked in benzylic position to a 1,2,4,5-tetrazine (Tz) get released quickly and efficiently upon triggering the Diels-Alder reaction with a strained alkene such as *trans*-cyclooctene (TCO).[1]



Combining one of the most toxic cryptophycins developed by our group [2], with a click-cleavable tetrazine linker we synthesised a platform of drug conjugates with an on demand click activation mode. Antibody-Drug Conjugates (ADC) based on internalising and non-internalising antibodies were designed and *in vitro* studies revealed single-digit nanomolar toxicities which were boosted to the picomolar range by activation with TCO, offering a superior activation mode than their enzymatically cleavable analogues.[3] Furthermore, the utilisation of highly target-specific ligands enabled the creation of click-cleavable Small Molecule-Drug Conjugates (SMDC). Tracking internalisation processes and metabolism of these conjugates will be enabled by combination of this SMDC platform with a fluorophore.

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L42

Exploring the Effect of Peptide Stapling on the Antimicrobial Activity and Structural Rigidity of Gramicidin S

Mikołaj Śleziak[1], Jarosław J. Panek[1], Tomasz Janek[2],
Aneta Jezierska[1], Monika Kijewska[1]

[1] *University of Wrocław, Faculty of Chemistry, F. Joliot-Curie St. 14, 50-383 Wrocław, Poland*
[2] *Wrocław University of Environmental and Life Sciences, Faculty of Biotechnology and Food Science, Chełmońskiego St. 37, 51-630 Wrocław, Poland*
mikolaj.sleziak@uwr.edu.pl

The secondary structure of antimicrobial peptides (AMPs) is a critical factor directly correlated with their biological activity, as it influences amphipathicity – a key property underlying their mechanism of action.[1] Consequently, post-synthetic modifications that stabilize secondary structure can enhance both bioactivity and resistance to proteolytic degradation. Peptide stapling could effectively fulfill this role. It is a group of macrocyclization techniques utilized to covalently link two side chains within a peptide's sequence.[2] Stapling is primarily used to modify α-helices; however, there is a significant group of AMPs, such as gramicidin S (GS), that adopt a β-sheet structure. This reveals an underexplored area within peptide stapling that warrants investigation.[3] In our study we synthesized two novel stapled analogs of GS featuring different staple lengths, resulting in varying degrees of structural rigidity. Our methodology involved the substitution of Leu residues with Cys residues. The first stapling strategy employed the introduction of a perfluoroaryl bridge via a thiolate moieties while the second strategy involved the formation of a disulfide bridge between the Cys residues. The bioactivity and structural alterations of the synthesized peptides were systematically evaluated in comparison to unmodified GS and a linear analog characterized by increased flexibility. This assessment was conducted to enhance the understanding of the structure-activity relationship of β-sheet peptides, particularly in relation to the effects of varying rigidity on their antimicrobial efficacy. This evaluation was further supported by molecular dynamics simulations conducted in both aqueous and membrane environments, providing complementary insight into the experimental results (see **Fig. 1.**). Our results suggest that conformational flexibility may play a critical role in the activity of peptides, comparable to the significance of hydrophobicity [4].

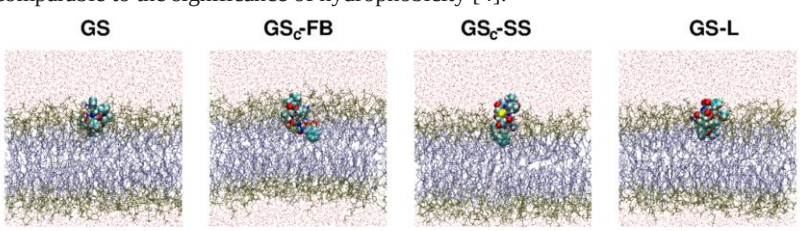

Figure 1. *Differences in the membrane embedding efficiency between analyzed peptides.*

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POSTERS

P1

Bicyclic Peptides as Potential Inhibitors of *H. pylori* HtrA Protease

Maja Aniukiewicz[1], Anna Puszko[1]

[1] *Faculty of Chemistry, University of Warsaw*
m.aniukiewic@student.uw.edu.pl

Helicobacter pylori is a gram-negative bacterium that colonizes the gastric mucosa. Infection usually occurs during childhood, and it is estimated that 50% of the world's population is infected with *H. pylori*.[1] Common treatment for *H. pylori* is based on a combination of antibiotics, but due to increase in resistance against conventional drugs the effectiveness of such therapy is declining. Therefore, there is a constant need to develop innovative active compounds aimed on interaction with new targets. High temperature requirement A (HtrA) protein is a serine protease secreted by *Helicobacter pylori* and other bacteria, which is crucial for infection and survival of bacteria under stressful conditions. In the human stomach it cleaves E-cadherin (transmembrane protein located within adhesive junctions) disrupting epithelial cell-to-cell adhesion.[2] This mechanism (shown in **Figure 1**) is one of the factors causing gastritis. Therefore, HtrA inhibition might be an effective way to suppress *H. pylori* infection.

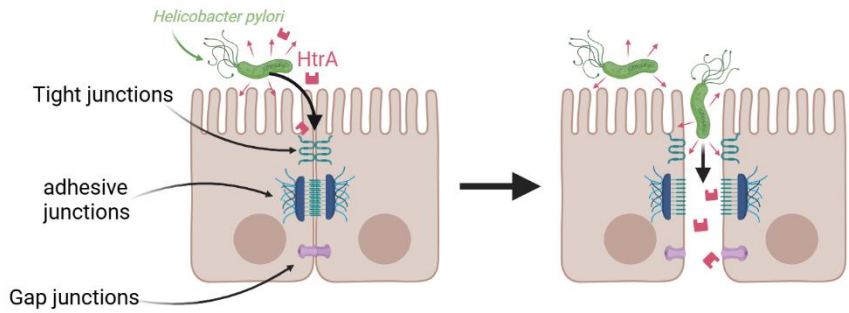

Figure 1. *Mechanism of disrupting epithelial cell-to-cell adhesion by H. pylori. In effect the bacterium can penetrate deeper layers of the epithelium and migrate to other tissues and organs.*

One of the recently discovered molecules effective against another serine protease trypsin, is Sunflower Trypsin Inhibitor-1 (SFTI-1), described as one of the smallest disulfide-bridged cyclic peptides found in nature. Its high affinity for the target enzyme is at a comparable level to the inhibition activity of much larger in size molecules.[3] In our work we used SFTI-1 as a template for engineering peptides that may show inhibition activity against the HtrA enzyme of *Helicobacter pylori*.

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P2

PEG-Conjugated Oligourea Foldamers

Paulina Bachurska-Szpala[1], Karolina Pułka-Ziach[1]

[1] *Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*
p.bachurska@uw.edu.pl

PEG conjugation is known to stabilize α- and β-peptide helices, though the effect depends on the peptide type and PEG size [1-3]. However, aliphatic oligourea foldamers modified with PEG units have not been studied to date.

Foldamers are unnatural oligomers that adopt specific, compact conformations with well-defined secondary structures [4]. Oligoureas even as short as four residues, are capable of forming stable 2.5-helical structures [5], stabilized by three-centered intramolecular hydrogen bonds. In contrast, a stable α-helical conformation typically requires at least ten amino acid residues in peptides.

In the presented project, I investigated the effect of PEG conjugations on the solubility and on the stabilization or destabilization of the helical conformation of foldamer hexamers. To this end, I synthesized two compounds with identical urea residue sequences. The oligomers **6^U–PEG** and **PEG–6^U** differ in the position of the short PEG-3 chain: in the former, the PEG unit is located at the C-terminus, while in the latter, it is attached to the N-terminus. The helices of both oligoureas are stabilized by five three-centered intramolecular hydrogen bonds.

Considering that PEG chains can act as cation receptors, I decided to investigate the ion-binding properties of the synthesized compounds. For this purpose, I performed titrations with $LiClO_4$ and $NaClO_4$ salts, monitored by circular dichroism (CD) spectroscopy and NMR.

Physicochemical studies demonstrated that the PEG-containing foldamers exhibit significantly improved solubility compared to the reference compound. Spectroscopic analyses confirmed that all synthesized foldamers retain a well-defined secondary structure, indicating that PEGylation does not interfere with the ability of oligoureas to adopt the 2.5-helical conformation. Ion-binding studies revealed that the foldamers selectively interact with Li^+ ions. The strongest binding affinity for this cation was observed for **6^U-PEG**. The data indicate that metal cation coordination occurs through the carbonyl oxygen atoms at the C-terminus and is further facilitated by the PEG chain attached at that position.

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P3

Development of a Strategy for Selective Upregulation of Rab GTPases Using Stapled Peptides

Dominika Rubiak[1], Dominika Nielipińska[2], Agnieszka Pietrzyk-Brzezińska[2], Edyta Gendaszewska-Darmach[2], Katarzyna Błażewska[1]

[1] *Institute of Organic Chemistry, Faculty of Chemistry, Lodz University of Technology, Poland*
[2] *Institute of Molecular and Industrial Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Poland*
dominika.rubiak@dokt.p.lodz.pl
katarzyna.blazewska@p.lodz.pl

Eukaryotic cells evolved abundance of specialized membrane-bound organelles. Rab GTPases (Rab proteins) have a prominent role in membrane trafficking and cell homeostasis. Their aberrant functioning leads to such disorders as cancer, diabetes, neurodegenerative diseases, making them very attractive therapeutic target.

The selective targeting of Rab proteins is very challenging, and Rabs remain „undruggable" targets. Most of the existing strategies affect all Rabs by inhibiting the prenylating enzyme, Rab geranylgeranyl transferase (RGGT) [1]. However, such an approach leads to toxicity due to the reduction of activity of above 60 Rab proteins.

Based on crystallographic analysis, we framed a novel approach for the selective up-regulation of Rab protein activity, using stapled peptides. Based on Rab-GAP complex, a series of peptidomimetics derived from a Rab protein were designed and synthesized by SPPS. In order to obtain conformationally rigid and more proteolytically resistant peptides, we used a ring-closing metathesis reaction to staple i, i+4 or i, i+7 positions in a helix [2]. Their purity and structure were confirmed by HPLC, LCMS and CD analysis. Biological evaluation and crystallization of a therapeutically relevant Rab-GAP pair is in progress.

The use of therapeutic peptides gained significant importance over the last three decades in oncology, cardiovascular, and metabolic diseases. However, the idea that the activity of Rab GTPase can be upregulated by targeting their interactions with GAPs has not been studied yet. The successful outcome of these studies will enable extending the methodology to Rab therapeutic targets in type 2 diabetes, allowing better understanding of signalling pathways associated with this condition.

Funding: PRELUDIUM BIS 3 grant (2021/43/O/ST4/01628).

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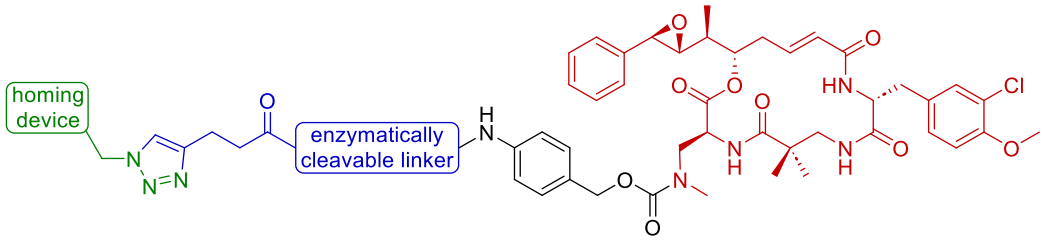
P4

Linkerology of Cryptophycin Drug Conjugates

Jan-Niklas Bollnow[1], Cedric Dessin[1], Norbert Sewald[1]

[1] *Bielefeld University, Bielefeld, Germany*
jan-niklas.bollnow@uni-bielefeld.de

Targeted cancer therapy employs conjugation of cytotoxic drugs (payloads) to targeting ligands (homing devices), which address antigens or receptors that are overexpressed by cancer cells to ensure selectivity, thus widening the therapeutic window. Since the initial discovery of highly potent cryptophycins in 1990 and their first total synthesis in 1994, numerous studies on the structure-activity relationship between the cryptophycin framework and cytotoxicity were conducted, with our group developing a cryptophycin with a methylamino group in unit D that shows single digit picomolar cytotoxicity while also allowing conjugation via said amino group to incorporate into drug conjugates.[1]



The cryptophycin payload (red) is connected to the homing device (green) by an enzymatically cleavable linker (blue) and self-immolative spacer (black) to profit from rapid and traceless release of the drug. Cathepsin B is an enzyme often overexpressed by cancer cells which can be utilized for proteolysis of oligopeptide linkers. Commonly used recognition sequences for cathepsin B cleavable drug conjugates include Val-Ala and Val-Cit. Modifications and extensions of these peptide sequences can be made to increase hydrophilicity, plasma stability, or selectivity regarding other lysosomal proteases.[2] However, the cleavage rate of the linker must not be penalized by these changes to retain high efficacy and selectivity of the drug conjugate.

Herein we scrutinize a variety of cathepsin B cleavable linkers in drug conjugates for their cleavability to establish a relationship between linker systems and cleavage kinetics.

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P5

Synthesis and FRET-Based Cleavage Study of an $\alpha_V\beta_6$-Targeting PDC

Caroline Brinkmann[1], Norbert Sewald[1]

[1] *Organic and Bioorganic Chemistry, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany*
caroline.brinkmann@uni-bielefeld.de

Peptide-drug conjugates (PDCs) are designed to minimize side effects in combating cancer by exploiting tumor-specific surface receptors, such as integrins, for selective drug delivery.[1] Among these, the integrin $\alpha_V\beta_6$ is overexpressed in various cancers, including basal cell carcinoma and breast cancer, and overexpression correlates with aggressive tumor progression.[2]

To study the drug release, an $\alpha_V\beta_6$-targeting PDC was synthesized with a modified cyclic nonapeptide, based on a previously reported structure.[3] The peptide was conjugated via a triazole to a sulfatase-cleavable linker, which has shown high stability in both human and mouse plasma.[4] Instead of a cytotoxic payload, a fluorophore (sulfo-Cy5) was attached to the linker, enabling real-time monitoring of the cleavage by Förster Resonance Energy Transfer (FRET) in conjunction with a second fluorophore (sulfo-Cy3) at the lysine side chain (**Figure 1B**).

Because FRET efficiency is highly distance-dependent, cleavage of the conjugate can be observed by an increase in donor fluorescence (**Figure 1A**).[5] This was confirmed in assays using the Arylsulfatase A (ASA) and the *Helix pomatia* sulfatase (**Figure 1B**). The results demonstrate the utility of a FRET pair for real-time monitoring of $\alpha_V\beta_6$-targeting PDC cleavage *in vitro*. Further studies must be conducted regarding cellular uptake and, prospectively, *in vivo* cleavage.

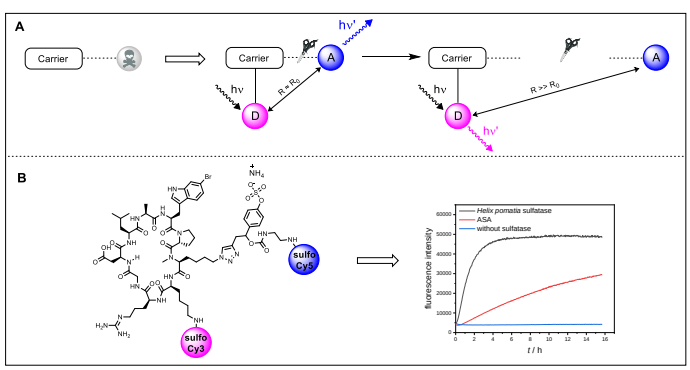

Figure 1. A: *Schematic representation of a PDC and the modified conjugate with the FRET pair. If the distance between the two fluorophores R is less than or equal to the Förster radius R_0, FRET occurs.* ***B:*** *Synthesized conjugate, with which in vitro cleavage assays with the Aryl sulfatase A (ASA) and the sulfatase from Helix pomatia were conducted. The donor fluorescence increases due to cleavage.*

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P6

Synthesis of Peptide-Oligourea Hybrids with Antibacterial Potential

Kinga Chaniewicz[1], Karolina Pułka-Ziach[1]

[1] *University of Warsaw, Faculty of Chemistry, Pasteura 1, 02-093, Warsaw, Poland*
k.chaniewicz@uw.edu.pl

In recent years, we have been dealing with increasing infections caused by bacteria resistant to many of the currently used antibiotics. This phenomenon poses a serious challenge to modern medicine and prompts scientists to search for alternative strategies to treat bacterial infections.

One of the current directions of research focuses on antimicrobial peptides (AMPs), which are naturally occurring in living organisms and exhibit a broad spectrum of activity against various pathogens. A particularly interesting group of antimicrobial peptides are those derived from human breast milk. Studies have shown that these peptides exhibit *in vitro* antimicrobial activity against bacteria, viruses, and fungi, as well as synergistic effects when combined with conventional antibiotics. [1,2] Despite their significant therapeutic potential, natural peptides are highly susceptible to enzymatic degradation. To overcome these limitations, researchers have been designing peptidomimetics—compounds that mimic the structure and function of natural peptides. Among them are urea-based foldamers, which adopt well defined, stable and tunable secondary structures.

The presented project aimed to obtain three peptide–oligourea hybrids that combine elements of natural antimicrobial peptides with oligourea fragments. These compounds feature sequences similar to a peptide isolated from human breast milk and are listed in **Table 1**. Each hybrid is characterized by a different arrangement of peptide and oligourea segments. First, I synthesized the necessary building blocks, which are not commercially available. Then, I performed solid-phase synthesis under microwave-assisted conditions and successfully obtained both the hybrids and the reference peptide. These compounds will be further investigated for their antibacterial properties.

Table 1. *Peptide and hybrids sequences*

peptide	E	L	L	L	N	P	T	K	Q	I	Y	P	V
1	E^u	L^u	L^u		N	P	T	K	Q	I	Y	P	V
2	E	L	L	L	N		T^u	K^u	Q^u	I	Y	P	V
3	E	L	L	L	N	P	T	K	Q	I^u	Y^u		V^u

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P7

Miniprotein-Based Catalysts of Iminium-Based Knoevenagel Condensation in Aqueous Media

Kent Harry Cumpio[1], Łukasz Berlicki[1]

[1] *Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland*
kentharryc@gmail.com

The Knoevenagel condensation of aldehydes or ketones with an active methylene compound is an important tool for building carbon–carbon (C-C) bonds. Several studies have demonstrated the possibility of replacing toxic organic solvents with aqueous media to facilitate the reaction.[1] However, there is a need for alternative catalysts that require milder conditions since heterogeneous catalysts in aqueous media require heating.[2] This study focuses on miniproteins as an alternative class of catalysts capable of catalyzing the Knoevenagel reaction in aqueous media at room temperature. The C-terminal domain of the MvaT miniprotein was repurposed by introducing a catalytic amine residue. This study also introduces a novel approach for the iminium mode of catalysis. Although many amine-catalyzed reactions, including the Knoevenagel reaction, proceed through an iminium intermediate using small molecule catalysts, no known naturally-occurring enzyme has been shown to adopt this activation mode.[3] A three-step procedure was used to design and optimize the miniprotein catalyst: optimization of the active site residues, optimization of residues interacting with the substrate, and optimization of the charge. Initial results after the first two steps showed that the MvaT miniprotein could catalyze the Knoevenagel reaction between cinnamaldehyde and ethyl cyanoacetate, yielding ethyl 2-cyano-5-phenyl-2,4-pentadienoate with a 200-fold increase in $k_{cat,}$ according to the Michaelis-Menten kinetics. Comparison of the kinetic parameters obtained from using lysine, proline, and piperidine shows that the miniproteins have higher catalytic activity in the same reaction conditions. CD characterization showed that the miniproteins retained their structure and thermal stability after a series of modifications to the active site and its surrounding residues.

Funding: De novo design of cavity-incorporating mini-proteins and their application in catalysis, OPUS, NCN, 2021/43/B/ST4/01837.

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P8

Peptide Fibrils as a Nanocarrier for Targeted HER2-positive Therapy

Milena Czeszejko[1], Justyna Sawicka[1], Ewa Wieczerzak[1], Paweł Niedziałkowski[1], Tomasz Kulesza[2], Kamila Kitowska[2], Rafał Sądej[2], Sylwia Rodziewicz-Motowidło[1]

[1] *Department of Biomedicinal Chemistry, Faculty of Chemistry, University of Gdańsk, Poland*
[2.] *Intercollegiate Faculty of Biotechnology of University of Gdańsk and the Medical University of Gdańsk, Poland*
milena.czeszejko@phdstud.ug.edu.pl

Peptide fibrils, classified as one-dimensional nanostructures, represent a compelling example of self-assembling biomolecular systems. Their unique physicochemical properties, including high thermal stability, proteolytic resistance, mechanical rigidity, and chemical modifiability, make them attractive candidates for applications in nanomedicine. Peptide fibrils consist of ordered cross-β sheet structures, in which polypeptide chains are orientated perpendicularly to the fibril axis. Typically, they measure several to tens of nanometres in width and can extend to hundreds of nanometers in length [1]. Most fibrils, used in our project, consist of 2–4 protofilaments that associate laterally or form helically twisted higher-order structures. Their high surface-to-volume ratio facilitates effective drug loading and attachment of target moieties. In this study, we use the peptide GQAGIVV (GFC), a sequence derived by shuffling residues 55–60 of the human cystatin C protein. This peptide retains fibrilogenic properties and forms stable fibrils.

To develop a targeted drug delivery system for HER2-positive breast cancer cells (HER2 + BCa), the GFC peptide was conjugated to the affibody $ZHER_{2:2891}$ [2] through a disulphide bridge. For this purpose, a cysteine residue was added to the *N*-terminus of the peptide (yielding CGQAGIVV) to introduce a reactive thiol group. In parallel, the affibody was selectively modified with a 2-pyridylsulfenyl (SPyr) group, enabling thiol-specific activation. The conjugation strategy relied on the formation of a disulphide bond between the free thiol on the peptide and the SPyr-activated thiol of the affibody. A titration-based protocol was applied to favour heterodimer formation and minimize the occurrence of undesired homodimerization [3]. The resulting conjugate combines the structural stability of peptide fibrils with the specific targeting capabilities of the affibody, offering a promising platform for the development of effective, selective, and biocompatible nanotherapeutics based on self-assembly of fibrillar systems.

Funding: This work was supported by a grant NCN 2022/45/B/NZ7/03889.

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P9

Exploring the Unknown Chemical Space: New Classes of Drug-Like Sulfoximines of Potential Use in Medicinal Chemistry and Peptide Synthesis

Maciej Dawidowski[1], Michał Nowacki[1], Marta Splandesci[1], Martyna Wróbel[1], Anna Puszko[2]

[1] *Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw*
[2] *Faculty of Chemistry, University of Warsaw*
maciej.dawidowski@wum.edu.pl

Sulfoximines, the monoaza-analogs of sulfones, constitute a class of compounds of growing interest in drug discovery. They are chemically stable and offer an additional exit vector to project chemical moieties towards target protein residues as compared to sulfones. Sulfoximines possess the unique H-bond donor/acceptor capabilities and have been shown to mimic the substrate-enzyme transition states, thus allowing for design of enzyme inhibitors. They are considered bioisosteric mainly to sulfones and sulfonamides, but examples of successful replacements have been shown also for secondary amines, hydroxyls and ketones. As a result of the above features, new methods are investigated for introducing drug-like sulfoximine derivatives to the screening libraries [1].

Multicomponent reactions (MCRs) constitute a powerful tool to create molecular diversity of drug-like compounds. They are condensation-type reactions where more than two starting materials react to form a single product essentially containing all the atoms of used reagents. Importantly, they proceed with high atom economy and use simple, one-pot procedures, starting from a wide variety of readily available building blocks. These example features make the MCRs particularly attractive for the rapid synthesis of libraries of compounds for drug-discovery purposes and for manufacturing of active pharmaceutical ingredients (APIs) [2].

Our research focuses on the MCR technologies that give access to the new arrays of drug-like molecules and on sulfoximide functional group. We synthesized a library of sulfoximino-acids, -amides, -phosphonates and -nitriles. These compounds are structurally similar to amino acids and can be used in screening libraries as well as they may constitute useful building blocks for synthesis of therapeutic peptides and peptidomimetics.

Funding: This project is funded by Polish National Science Center (NCN), OPUS24 grant, Reg. No. UMO-2022/47/B/NZ7/01411.

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P10

Cyclative Release Cleavage Integrated into CelluSPOT for High-Purity Peptide Synthesis

Mirko Delpero[1], Bekas Alo[1], Christina Lamers[1]

[1] *Institute of Drug Discovery, Brüderstr. 34, 04103 Leipzig, Germany*
mirko.delpero@medizin.uni-leipzig.de

Peptide synthesis on solid supports remains a cornerstone of modern peptide research, enabling the production of high-purity sequences for therapeutic and screening purposes. The CelluSPOT method [1], which uses functionalized cellulose membranes instead of resin as solid support, was introduced to enable the generation of peptide arrays. However, it can also offer a cost-effective and scalable platform for parallel peptide synthesis. Therefore, we have successfully adapted a peptide synthesis strategy known as *cyclative release* [2]—previously reported on resin—to the CelluSPOT format.

In the cyclative release method the linker towards the solid support contains a thiol group, which is reacted with a thiol-containing amino acid as C-terminus, to immobilize the peptide via a disulfide bond. The final N-terminal amino acid contains a thiol group, which forms a disulfide bridge with the C-terminal thiol in a thiol shuffling mechanism. This results the release of a clean, fully sidechain-protected cyclic peptide from the support.

In this study, we used a model sequence to establish the protocol, which was conducted as manual synthesis on the cellulose-based support using standard Fmoc chemistry. The cyclative release was successfully established and in further steps, we explored the influence of alternative solvents before adapting the protocol for automated synthesis on a MultiPep Synth 1.

Our results demonstrate the compatibility of the cyclative release method with the CelluSPOT approach. This opens the door to a rapid, parallel synthesis of diverse peptide libraries at low cost and with minimal waste. The method holds promise for high-throughput peptide screening and green chemistry applications in early-stage drug discovery and peptide-based research.

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P11

Enzymatic Peptide and Protein Bromination

Annika Deppe[1], Nicolai Montua[1], Norbert Sewald[1]

[1]*Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University*
annika.deppe@uni-bielefeld.de

The bromination of peptides and proteins is particularly valuable for the development of a variety of new drugs as it offers a handle for late-stage functionalization by cross-coupling reactions and increases bioactivity. Flavin dependent Trp-halogenases like Thal are potential enzymes for environmentally friendly peptide and protein halogenation.[1-3] However, Thal showed poor conversions of *C*-terminal tryptophan residues in bromination assays.[1] *In vitro* peptide bromination was improved by structure-based mutagenesis of Thal and screening of different reaction conditions and peptide sequences. This substrate engineering approach led to the identification of a tetrapeptide sequence, termed the "BromoTrp tag", which showed the highest conversion in bromination assays. The BromoTrp tag was subsequently appended to model proteins to investigate its performance in protein modification processes. Using the **bro**minating *Escherichia **coli*** coexpression system ***Brocoli***, efficient *in vivo* protein bromination was achieved in a single cultivation and purification step. Additionally, *in vitro* protein bromination was successfully demonstrated emphasizing the flexible use of Thal for biocatalytic halogenation.

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P12

Peptide Foldamers as Inhibitors of the Immune Checkpoint CTLA-4/B7

Magda Drewniak-Świtalska[1], Łukasz Berlicki[2], Paulina Fortuna[3], Małgorzata Krzystek-Korpacka[1]

[1] *Department of Biochemistry and Immunochemistry, Wroclaw Medical University, Poland*
[2] *Department of Bioorganic Chemistry, Wroclaw University of Science and Technology, Poland*
[3] *Omics Research Center, Wroclaw Medical University, Poland*
magda.drewniak-switalska@umw.edu.pl

Immune checkpoint inhibitors (ICIs) represent one of the most rapidly advancing therapeutic strategies in the treatment of cancer and selected autoimmune diseases [1,2]. Currently, the most commonly used agents are monoclonal antibodies targeting PD-1/PD-L1 and CTLA-4. Despite their high efficacy, these antibodies present significant limitations, such as high production costs, low bioavailability, and the risk of immunogenicity [3]. This has driven the search for alternative solutions, including peptides and their stabilized analogs—foldamers—which offer improved pharmacokinetic profiles and enable precise molecular design [4].

The proposed project aims to develop peptide foldamers that can modulate the interaction between CTLA-4 and B7 proteins, a key immune checkpoint involved in regulating T cell activity [5]. This target was selected due to the availability of structural data for the CTLA-4/B7-2 complex, which enables rational inhibitor design, and due to the lack of well-defined binding pockets in both proteins, which limits the applicability of classical small-molecule inhibitors. The project involves the use of molecular modeling tools (FastDesign algorithm from the Rosetta package) and the incorporation of non-canonical amino acid residues such as trans-ACPC, allowing the design of peptide sequences with enhanced conformational stability and proteolytic resistance [6]. In the subsequent stages, selected peptides are planned to be synthesized using solid-phase Fmoc chemistry with a microwave-assisted peptide synthesizer. The resulting foldamers will be characterized through conformational studies using circular dichroism (CD) and NMR spectroscopy to assess their stability and three-dimensional structure. If promising compounds are obtained, their binding affinity to CTLA-4 and inhibitory potential toward the CTLA-4/B7 interaction will be evaluated using bio-layer interferometry (BLI).

This project addresses unmet clinical needs, especially in cancers like colorectal cancer, where current therapies remain inadequate. The anticipated results may offer new tools for selective immune modulation and advance the development of targeted therapies. Peptide foldamers thus represent a promising alternative to antibodies and a step forward in the design of next-generation biologics.

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P13

Anti-Proteolytic AMPs for Targeting ESKAPE Pathogens: A Step Towards Therapeutic Peptides

D. R. Puneeth Kumar[1], Deepanshi Saxena[2,3], Carmela Michalek[1], Rahul Maitra[2,3], Swechcha Singh[2,3], Aparna Sahoo[2,3], Sidharth Chopra[*2,3] and Norbert Sewald[*1]

[1] Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany

[2] Division of Molecular Microbiology and Immunology, CSIR-Central Drug Research Institute, Sector 10, Janakipuram Extension, Sitapur Road, Lucknow-226031, Uttar Pradesh, India

[3] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

puneeth.dr@uni-bielefeld.de

The two major recently raised concerns about antibiotics are their effects on the environment and the emergence of antibiotic resistance, which is becoming a greater threat to world health. Antimicrobial peptides (AMPs) possess a unique, nonspecific membrane-disruptive action, providing an intriguing approach to addressing the concern of antibiotic resistance. However, in the therapeutic use of AMPs, inadequate proteolytic resistance is a pressing issue that needs to be addressed. Traditional techniques are also being investigated, including stapling, the use of D-amino acids, and complex chemical changes.[1-3] In this work we synthesized a set of novel AMPs with high proteolytic resistance based on a repeating peptide structure unit $(XYZ)_n$ (where X stands for Arg and Lys derivatives, Y for Trp, Z for Leu or γ^4Leu, and n=4) which was developed by sequentially incorporating non-natural amino acids to the α- peptides.[4] With four repeating units, **P3** and **P9** are the most potent of these peptides and have broad-spectrum antimicrobial activity against ESKAPE pathogens. Furthermore, the selectivity index of peptides **P3** and **P9** against strains of *S. aureus* was quite high. With an emphasis on their antibacterial activity and selectivity index, we are going to investigate the structural characteristics, modes of action, and therapeutic potential of Trp- and cation-rich AMPs in this work after considering these initial results. The early results of the newly designed peptides' broad-spectrum antibacterial activity with low toxicity are crucial for developing next-generation antimicrobials in the battle against antibiotic resistance.

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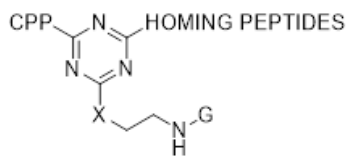
P14

Conjugates Homing Peptide-Cell Penetrating Peptide-Cytostatic/Biomarker Potentially Useful in the Diagnosis or Treatment of Lung Cancer

Piotr Jedrzejczak[1], Justyna Frączyk[1], Beata Kolesińska[1]

[1] *Institute of Organic Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz*
justyna.fraczyk@p.lodz.pl

Lung cancer is responsible for approximately 20% of cancer-related deaths worldwide [1]. Lung cancer is the most common cause of cancer deaths both in Poland (over 23 thousand per year) and worldwide (1.6 million deaths per year). Lung cancer is the malignant tumors with the worst prognosis. The 5-year survival rate in Poland is only about 14%. This is a much lower rate compared to other common cancers. The long-term aim of the project is the development of synthesis methods of conjugates based on s-triazine ring, peptide fragments, and fragment responsible for antitumor activity, or a dye such as carboxyfluorescein, enabling the diagnosis of cancerous lesions. The resulting compounds will be tested on non-small-cell lung cancer cells. Using an s-triazine core allows for connecting three different substituents in one molecule [2]. As peptide substituents, we have chosen epidermal growth factor (EGF) fragments, because EGFR is overexpressed in cancer cells, and cell-penetrating peptides (CPPs), which enhance permeability across cell membranes.



X= NH or O
G= drug or marker

The synthesis of tri-substituted 1,3,5-triazine derivatives was made possible by varying the reactivity of the chlorine atoms of the triazine derivatives. Oligoproline and oligolysine derivatives were used as CPPs. The homing peptides were EGF fragments [3]: YQISEDGEQS, YCLHDGV, YIELDAK, LDKYACNCV, YRITTTPTNG, RYHQRTNTNV, YTIQVLRDGQ, QYRDL, HDGYCLHDGVCMY and QYNVGPSVSK. The structure of the conjugates was confirmed by MS. CD studies were performed to investigate the spatial structure of the conjugates, which is important from the point of view of the ability to interact with EGFR.

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P15

Preliminary Assessment of the Usefulness of Commercial Viral Protease Inhibitors in Inhibition of the HRV B14 3C Protease Activity

Paweł Gierszewski[1]

[1] *Department of Chemistry, Wroclaw University of Science and Technology*
pawelgiersz2004@gmail.com

HRV B14 belongs to the rhinoviruses, which are the cause of nearly 40% of colds. The picornavirus family, which it represents, is characterised by the icosahedral symmetry of the capsid and the encoding on the ssRNA of a polyprotein cleaved by proteases contained in its sequence. Serotype 14 has two cysteine proteases (2A and 3C) responsible for maturation of virions. One way to combat viral infection is to prevent the clevage of the polyprotein by inhibiting these proteases (Wang et al., 1998). In addition, protease 3C by cutting the NLRP1 inflamosome causes an inflammatory response (Tsu et al., 2021) The subject of this study is protease 3C, whose structure with a covalently attached inhibitor (acetyl-LEALFQ-ethylpropionate) was determined by NMR in solution (Bjorndahl et al., 2007). Removal of the inhibitor was followed by docking of five commercially available viral protease targeting drugs (Amprenavir, Darunavir, Indinavir, Nelfinavir, Nirmatrelvir). Amprenavir, Nelfinavir and Nirmatrelvir showed low affinities for the substrate-binding pocket >5.4 kcal/mol. In contrast, Indinavir and Darunavir showed affinities for the substrate-binding pocket in three different conformations (-6.3, -6.2 and -6.1 kcal/mol) for Indinavir and in one (-5.6 kcal/mol) for Darunavir. Despite their rather low affinities, the Darunavir and Indinavir molecules (-6.1 kcal/mol conformation) cover a significant part of the substrate binding pocket preventing substrate access to the catalytic site. Conclusion is that the simulated protease inhibitors did not show significant affinity for the HRV 14 3C protease and, given their non-covalent mechanism of action, would probably not slow down viral maturation. A much more effective way would be to use covalent warheads attached to a preferred peptide or peptidomimetic molecule.

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P16

Halogenated Amino Acids as Substrates for Photoredox-Catalysed Cross Coupling Reactions

Cornelia Göcke[1], Norbert Sewald[1]

[1] *Organic and Bioorganic Chemistry, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany*
cornelia.goecke@uni-bielefeld.de

As cyclic peptides are very important bioactive molecules, we seek to establish a new mode of peptide cyclisation through photoredox-catalysed cross-coupling reactions to enable cyclisation between the C-terminal amino acid and the halogenated side chains of aromatic amino acids.

The reaction proceeds through photoredox-catalysed decarboxylation giving an α-amino radical followed by transition metal catalysed cross-coupling between the radical and the aryl halide.[1] This would potentially allow a peptide cyclisation requiring little functionalisation while still introducing a non-peptidic bond into the molecule, which is usually attributed with higher stability in regard to pharmacokinetics.[2]

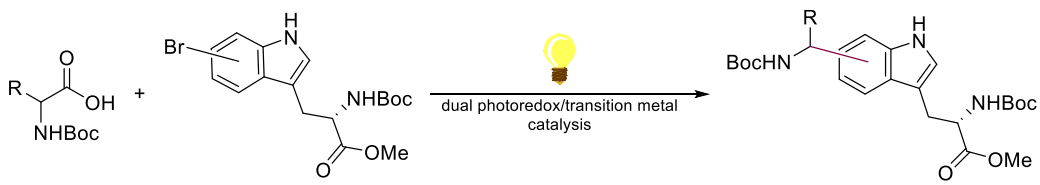

Figure 1. *Schematic representation of the investigated reactions between bromotryptophan and various natural amino acids. Iodophenylalanine was also investigated as the halogenated reaction partner.*

Before attempting the peptide cyclisation, we investigated the reactions between two amino acids, focusing on using bromotryptophan and iodophenylalanine as the halogenated reaction partners and the avoidance of stereoisomer formation regarding the decarboxylating partner (see **Fig. 1**). The main focus for the decarboxylating partner was the use of glycine, as it would not introduce a new stereocentre, but modification of both reaction partners required the adaption of the reaction conditions compared to literature-described procedures.[1] To further broaden the applicability of the system, stereoselective procedures allowing for the use of more diverse decarboxylating partners, were also investigated.[3]

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P17

Hydrophobic Ion Pairs of Omiganan – Synthesis and Antimicrobial Activity

Karol Sikora[1], Adrian Górski[1], Palina Han[1], Małgorzata Paduszyńska[1], Wojciech Kamysz[1]

[1] *Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416, Gdańsk, Poland*
karol.sikora@gumed.edu.pl

Peptides, due to the presence of basic amino acid residues such as arginine, lysine, histidine, and the N-terminal amino group, can interact with acids used in peptide cleavage mixtures and chromatographic purification. However, the role of counter-ions and their influence on physicochemical and biological properties is often overlooked [1]. Hydrophobic ion pairing (HIP) is one method that allows modulation of the solubility and physicochemical properties of charged, hydrophilic molecules, including drugs and peptides. HIP involves replacing existing counter-ions with hydrophobic ones. The formation of such complexes depends on non-covalent interactions between oppositely charged residues and the hydrophobicity of the counter-ion. HIP is commonly employed in formulation and drug delivery systems, facilitating the encapsulation of hydrophilic molecules within emulsions, lipid nanoparticles, or self-emulsifying drug delivery systems [2].

In this study, we investigated the effect of HIP on the antimicrobial activity of the Omiganan peptide. Omiganan was synthesized as a TFA salt, and its HIP complexes with dodecyl sulfate (SDS) and docusate (DOSS) counter-ions were prepared using a precipitation method. All salts, along with the surfactants SDS and DOSS, were subsequently tested for antimicrobial activity. Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method against Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538 and *Enterococcus faecium* ATCC 700221, and Gram-negative bacteria: *Acinetobacter baumannii* BAA 1605 and *Escherichia coli* ATCC 25922.

Omiganan-TFA and its HIP complexes with dodecyl sulfate and docusate exhibited strong activity against Gram-positive bacteria, with MICs ranging from 8 to 16 µg/mL. In contrast, low or no activity was observed against Gram-negative bacteria for most tested compounds, with two exceptions: Omiganan-TFA, which showed a MIC of 32 µg/mL against *E. coli*, and Omiganan-SDS, which significantly reduced the growth of both *A. baumannii* and *E. coli*.

These findings suggest that HIP can influence the antimicrobial properties of peptides and may enhance their activity compared to conventional TFA salts.

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P18

Can Insulin Aggregation Inhibitors be Used to Inhibit the Aggregation of Proteins Responsible for the Development of Neurodegenerative Diseases?

Patrycja Grabarczyk[1], Beata Kolesińska[1]

[1] *Institute of Organic Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz*
250902@edu.p.lodz.pl

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) are associated with pathological protein aggregation and amyloid deposition in the central nervous system [1]. A key feature of the phenomenon of aggregation of protein is formation of β-sheet structure amyloid that arise independently of the proteins' primary amino acid sequence [2].

This project aims to evaluate whether N-methylated peptide inhibitors of insulin aggregation – previously shown to exhibit anti-aggregative activity – can also inhibit the aggregation of amyloidogenic "hot spots" within β-amyloid, α-synuclein, and superoxide dismutase (SOD1), which are implicated in the pathogenesis of these diseases [3,4].

The first stage of the project was the synthesis of N-methylated analogues of hot spot insulins [5], for which the ability to inhibit insulin aggregation was demonstrated [6]. Using solid-phase synthesis and triazine condensing reagents, a pool of analogues of the amyloidogenic hot spot of insulin VEALYL containing N-methylated amino acid residues was obtained. The next stage of the research was to verify the ability to inhibit the aggregation of hot spots of β-amyloid assays by using Congo red, Thioflavin T, and microscopy examination.

It is also planned to test the inhibitor stability against proteolytic degradation in human serum, and cytotoxicity against neuronal cell models. A key aspect of the project is evaluating the blood–brain barrier (BBB) permeability of active compounds [7].

The expected results may pave the way for developing new peptide – based therapeutics targeting pathological protein aggregation in neurodegenerative disorders, which is a key problem in the context of an aging population [1].

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P19

Time-Efficient Peptide N-methylation

Aleksandra Helbik-Maciejewska[1], Agata Gitlin-Domagalska[1], Mladena Glavaš[2], Natalia Ptaszyńska[1], Dawid Dębowski[1], Anna Łęgowska[1], Krzysztof Rolka[1]

[1] *Department of Molecular Biochemistry, Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland*
[2] *Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, 10 000 Zagreb, Croatia*
aleksandra.maciejewska@phdstud.ug.edu.pl

N-methylation of peptides is an essential modification that increases lipophilicity, metabolic stability, and binding affinity or specificity, thereby improving the bioavailability of bioactive peptides [1]. Numerous methods for peptide N-methylation have been developed, which can generally be categorized into two approaches: methylation of amino acid derivatives in solution before coupling them to the peptide, or direct methylation on a solid support during peptide chain elongation.

Methodology described by Naoum *et al.* [2] has been successfully utilized in our laboratory for many years. This three-step procedure, comprising sulfonylation, methylation, and desulfonylation, is fully compatible with solid-phase peptide synthesis (SPPS). However, the process takes 4 hours and requires a laboratory shaker.

We successfully reduced the total reaction time from 4 hours to just 40 minutes. Furthermore, we demonstrated that N-methylation can be performed using a standard laboratory shaker, a common ultrasonic bath, or a microwave synthesizer. Notably, we also proved that this method can be effectively applied to the N-methylation of Cys and His [3], that were reported as particularly problematic by Biron *et al.* [4].

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P20

The Importance of Peptides in a 3D Bioprinted Urinary Bladder Model for Tissue Regeneration Research

Emilia Iłowska[1], Daria Sowik[1], Szymon Mania[2], Adrianna Banach-Kopeć[2], Karol Staszczyk[2], Paweł Dąbrowski[3], Marta Pokrywczyńska[3], Robert Tylingo[2], Sylwia Rodziewicz-Motowidło[1]

[1] *Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdansk, Poland*
[2] *Faculty of Chemistry, Gdansk University of Technology, Gabriela Narutowicz 11/12, 80-223 Gdansk, Poland*
[3] *Faculty of Medicine, Nicolaus Copernicus University, Jurija Gagarina 11, 87-100 Torun, Poland*
emilia.ilowska@ug.edu.pl

Tissue engineering, in combination with modern bioprinting techniques, is making significant strides in the reconstruction of organs, including the urinary bladder. The development of bioprinting inks, such as alginates or methacrylated derivatives of collagen, has become crucial for advancing in this field. However, there remains a need for more specialised approaches, particularly in the context of bladder wall regeneration [1]. The application of 3D bioprinting technology in regenerative medicine has the potential to revolutionise the treatment of bladder injuries, offering solutions that surpass traditional methods. These include a reduced risk of rejection and the elimination of the need for donor tissues. Personalised bladder models could offer better compatibility and functionality, significantly improving the quality of life for patients with bladder disorders [2].

Our project aims to develop an innovative model for the urinary bladder using tissue engineering. The objective is to create a dynamic, multilayered model capable of supporting nutrient transport for cells, based on a chitosan-agarose composite enriched with collagen proteins and/or peptides. In our research, we plan to incorporate peptides that not only enhance angiogenesis but also promote the migration of urothelial cells and exhibit antimicrobial properties. This approach is unprecedented. The use of single peptides for regeneration is known in the literature [3], but the cumulative approach we propose is non-standard. The designed peptides will be incorporated into the corresponding layers of the scaffold. In the outer layer of the 3D model, we will place peptides responsible for angiogenesis – proangiogenic peptides – and those with antimicrobial properties (effective against Gram-negative bacteria). These peptides will form fibrils, enhancing their stability in contact with urine while simultaneously combating G(-). In the next, inner layer, we will introduce peptides that stimulate the migration of urothelial cells. Promigratory peptides will be responsible for restoring bladder integrity and barrier function. For all peptides, we will conduct relevant biological tests to determine their promigratory, antimicrobial, and pro-angiogenic potential using various techniques. Additionally, we will perform structural studies to investigate the structure-activity relationships.

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P21

Zn(II)-Induced Homodimerization of C-terminal Cysteine Cluster of MTF-1 Transcription Factor

Zuzanna Janiec[1], Katarzyna Kluska[1], Anna Kocyła[1], Artur Krężel[1]

[1] *Department of Chemical Biology, Faculty of Biotechnology, University of Wroclaw, Joliot-Curie 14a, 50-383 Wroclaw, Poland*
zuzanna.janiec@uwr.edu.pl
artur.krezel@uwr.edu.pl

Metal-responsive transcription factor-1 (MTF-1) plays a pivotal role in maintaining metal homeostasis by regulating the expression of metallothioneins genes, which are involved in metal detoxification and transport. Metallothioneins are essential for protecting cells against heavy metal toxicity and oxidative stress, a detailed understanding of the regulatory pathways governing their expression is of great importance. MTF-1 activates transcription in response to elevated levels of heavy metals such as Zn(II), Cd(II), and Cu(I). However, the mechanisms underlying this activation remain unclear [1,2]. In this study, we focused on the highly conserved and functionally enigmatic C-terminal CQCQCAC cysteine cluster of MTF-1, which has been proposed to mediate homodimerization. Previous research indicates that mutations in these cysteine residues reduce transcriptional activity, although it remains unclear whether this effect is due to impaired metal binding or cysteine oxidation [3]. To address this, we employed a synthetic peptide model containing the CQCQCAC motif to investigate metal-binding stoichiometry, geometry, and overall affinity. We employed methods such as UV-vis absorption spectroscopy and mass spectrometry to investigate the stoichiometry and metal-binding geometry. To determine overall affinity, we applied fluorescence and UV-vis spectroscopy. Peptide samples were analyzed by SEC under various free Zn(II) conditions to monitor Zn(II)-induced changes in homodimerization. We also introduced alanine substitution variants of the peptides to assess the influence of each cysteine on the affinity of metal binding. Our findings show that the cysteine cluster promotes Zn(II)-centered homodimerization. This discovery provides new insight into the molecular mechanisms of MTF-1 activation and highlights a Zn(II) as a regulatory element essential for cellular metal homeostasis.

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P22

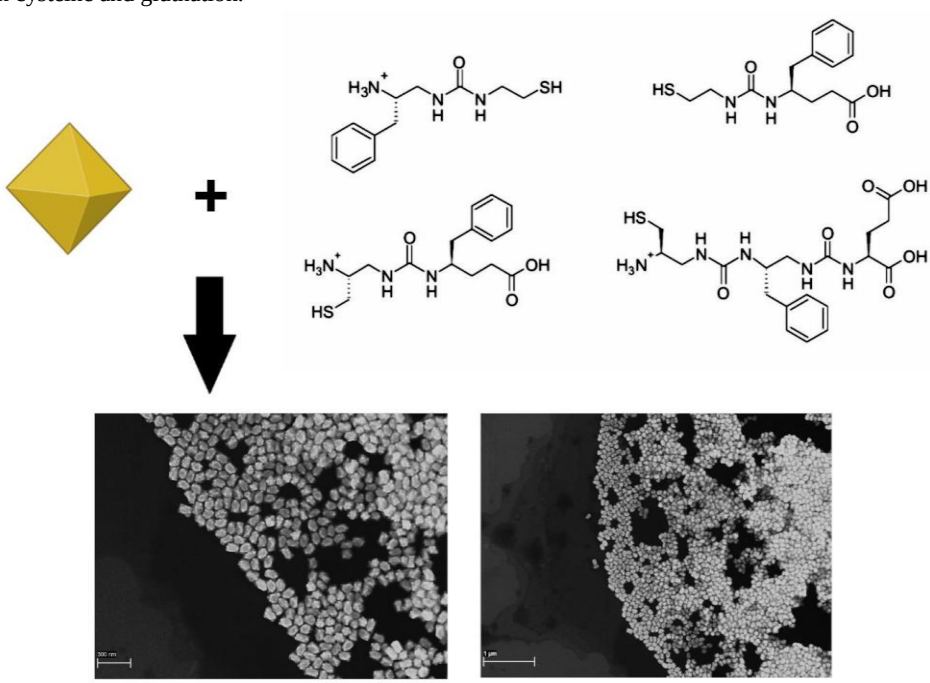
N,N'-Substituted Ureas as Chirality Inductors in Gold Nanoparticles

Bartłomiej Jedliński[1,*], Natalia Kowalska[1], Wiktor Lewandowski[1], Karolina Pułka-Ziach[1]

[1] *Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*
[*] b.jedlinski@student.uw.edu.pl

N,N'-linked oligoureas belong to a group of foldamers – artificial compounds adopting stable and conformationally ordered states, inspired by natural biomacromolecules. Search for various applications of urea-based foldamers relies on their stable helical secondary structure and other α-peptide-like features. Their use range from medicinal chemistry (e.g. antibacterial activity or encapsulation of small molecules) to catalysis and electron transfer.

We propose another application of urea derivatives which is an urea-mediated encoding chirality in gold nanoparticles (AuNPs). Developing chiral nanostructures is crucial to create advanced biosensors based on optical activity. Designed compounds contained a thiol group as an anchor group, as well as amine and carboxyl groups to improve water solubility of ureas and an aromatic ring, the presence of which supports interactions with the metallic surface. Obtained compounds exhibit a moderate level of induction of chirality in gold nanoparticles, yet this study opens up the potential of designing chirality inductors other than well-known cysteine and gluthation.



Includes elements created with *BioRender.com*

P23

Structural Evaluation of Silk Fibroin Sourced from Regionally Bred Bombyx mori

Miłosz Ignacyk[1], Anna Gościniak[1], Przemysław Zalewski[1], Mateusz Szymańczyk[2], Judyta Cielecka-Piontek[1]

[1] *Department of Pharmacognosy and Biomaterials, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland*
[2] *Institute of Natural Fibers and Medicinal Plants, Wojska Polskiego 71B, 60-630 Poznan, Poland*
jpiontek@ump.edu.pl

Silk fibroin is a natural biopolymer traditionally used in the textile industry, but it is gaining increasing attention as a highly biocompatible material for biomedical applications. Due to its favorable physical and chemical properties, it can be processed into various functional forms, including hydrogels, nanofibers, nanoparticles, cast films, and implantable scaffolds. The primary source of fibroin is the silk produced by Bombyx mori silkworms. To make it suitable for medical use, silk must be converted into regenerated silk fibroin (RSF), which involves disrupting and reforming its crystalline structure [1].

This study aimed to characterize the physical properties of silk obtained from a local breeding program (Department of Bioeconomy, Institute of Natural Fibres and Medicinal Plants - National Research Institute, Poznan, Poland) that is aimed at preserving and developing *Bombyx mori* silkworm populations. Regenerated silk fibroin was obtained through a multi-step procedure involving the removal of sericin (degumming), dissolution in lithium bromide (LiBr), dialysis against distilled water to remove residual salts, and subsequent lyophilization. The resulting material was subjected to analytical evaluation. Fourier Transform Infrared Spectroscopy (FTIR-ATR) and X-ray Powder Diffraction (XRPD) were used to assess the structural changes in the silk material. The final product contained 54% fibroin and 18% sericin by weight, relative to the initial mass of raw silk before any degumming steps. FTIR analysis revealed a change in the intensity of several peaks in the raw silk compared to the regenerated fibroin; however, analysis by XRPD presents a halo effect, which indicates the lack of a typical crystal structure for all samples.

These findings indicate that the regeneration process, including degumming, dissolution, and subsequent freeze-drying, induces structural alterations in silk fibroin. Changes in the secondary structure of the protein suggest a partial growth of ordered crystalline domains. This study provides valuable insight into the physicochemical characteristics of fibroin derived from silkworms raised in a locally managed conservation and selective breeding program. It underscores the influence of processing steps on its structural properties.

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P24

The Use of a Heating Clamp Can Accelerate the Attachment of the First Amino Acid on Wang Resin

Adrian Górski[1], Jakub Czekajło[1], Damian Neubauer[1], Wojciech Kamysz[1]

[1] *Department of Inorganic Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416, Gdańsk, Poland*
wojciech.kamysz@gumed.edu.pl

Solid-phase peptide synthesis (SPPS) is a commonly used technique for obtaining peptides for research, industrial, and healthcare applications. Wang resin is the solid support of choice for synthesizing peptides containing a C-terminal carboxyl group. However, this resin require specific treatment when first amino acid is attached. This reaction is typically carried out for 2 to 24 hours at room temperature. Despite this relatively long reaction time, resin loadings are not always satisfactory.

This study evaluated the use of a heating clamp for the temperature-assisted attachment of Fmoc-L-Lys(Boc)-OH to Wang resin. Reactive sites per gram of resin was 0.81 mmol. Fmoc-L-Lys(Boc)-OH, OxymaPure, DIC and DMAP (1:1:1:0.1 eq) were used in threefold molar excess based on the resin. Reagents were dissolved in dichloromethane (DCM) or *N,N*-dimethylformamide (DMF). Coupling was carried out at different temperatures – 25, 35, 50, 60°C. Boiling point of DCM is approx. 40°C therefore mixture was incubated only at 25 and 35°C. All reactions were performed using 1 g of the resin and reagents were dissolved in 15 mL of solvent. After 15, 30, 60, 90, and 120 minutes, a small portion of the resin was placed onto the funnel, thoroughly washed with DMF, DCM, and diethyl ether, and completely dried. Resin samples were tested for loading using standard spectrophotometric method. To 10 mg of resin 15 mL of 20% piperidine in DMF were added and incubated at room temperature with agitation for 15 min. After incubation absorbance at 290 nm was measured and loading was calculated.

In conclusion, elevated temperatures noticeably accelerate the reaction. The highest resin loadings were obtained in DMF at 60°C, while the lowest were observed at 25°C. Therefore, for optimal performance, heating the reaction mixture in DMF above 40°C is recommended. It can be stated that using a heating clamp to the attachment of the first amino acid on Wang resin can significantly reduce time and increase loading. Another advantage is that the heating clamp offers an affordable solution to accelerate solid-phase synthesis due to its compatibility with standard reaction vessels and its high contact surface area.

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P25

Short Binding Loop Fragments of PDGF Family Proteins as Potential Pro-regenerative Compounds

Przemysław Karpowicz[1], Krzysztof Polaczek[1], Małgorzata Zawrzykraj[2], Milena Deptuła[2], Emilia Iłowska[3], Rafał Ślusarz[3], Michał Pikuła[2], Emilia Sikorska[3]

[1] *Department of Biomedical Chemistry, Faculty of Chemistry University of Gdansk, Poland*
[2] *Laboratory of Tissue Engineering and Regenerative Medicine, Medical University of Gdansk, Poland*
[3] *Department of Organic Chemistry, Faculty of Chemistry University of Gdansk, Poland*
przemyslaw.karpowicz@ug.edu.pl

It is estimated that 1–2% of Europeans suffer from chronic wounds each year, consuming up to 2–3% of national healthcare budgets. This problem is particularly severe among individuals with diabetes, where 15–30% of patients experience hard-to-heal wounds, often leading to amputations in up to 20% of cases. The underlying pathophysiology of chronic wound healing remains incompletely understood but may be associated with reduced levels of growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). Current treatments are often insufficient, prompting the search for new therapies, including the use of small-molecule compounds, novel delivery systems, and targeted application of active substances at the wound site. Previous studies, including those from our group, have shown that short fragments of PDGF-BB, known as "loops", which interact within the receptor binding site, exhibit stimulatory effects on cultured skin cell lines and, importantly, accelerate wound epithelialization in a mouse model [1,2].

Encouraged by our previous findings, we extended our approach to synthesize, characterize, and evaluate the biological activity of peptides based on L1 and L3 loop sequences but derived from additional PDGF isoforms - specifically PDGF-A, -C, and -D. The peptides were synthesized using microwave-assisted solid-phase peptide synthesis (MW-SPPS) and purified by chromatographic techniques. Their cytotoxicity and proliferative potential were tested in HaCaT keratinocytes and 46BR.1N fibroblasts. Binding affinity to PDGFRα/β and VEGFR1/2, due to structural similarities, and assessed using a modified ELISA assay. Peptide conformations and receptor interactions were further analyzed by circular dichroism spectroscopy and molecular modeling.

Our results revealed that L1-derived peptides strongly stimulated keratinocytes, but had a weaker effect on fibroblasts. In contrast, L3-based peptides demonstrated higher and more variable receptor-binding affinity, depending on receptor subtype. Interestingly, the most notable biological activity was observed for the peptide derived from the PDGF-A isoform. These findings support further development and refinement of pro-regenerative peptides for therapeutic use and incorporation into wound-healing biomaterials.

Funding: This work was supported by the National Science Centre in Poland (UMO- 2023/51/D/NZ7/02791).

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P26

Identification of Key Structural Elements of Immunomodulatory Proteins - Inhibitors of Antigenic Peptide Transporter TAP Encoded by Selected Alphaherpesviruses

Natalia Karska[1], Małgorzata Graul[2], Krupa Paweł[3], Igor Zhokov[4], Andrea D. Lipińska[2], Sylwia Rodziewicz-Motowidło[1]

[1] Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdańsk, 80-308 Gdańsk, Poland. [2] Laboratory of Virus Molecular Biology, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, 80-307 Gdańsk, Poland. [3] Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668, Warsaw, Poland. [4] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland
natalia.karska@ug.edu.pl

Herpesviruses are among the most widespread pathogens, in addition to the eight species that infect humans, this family of viruses (Herpesviridae) also includes numerous species that cause disease in livestock and wildlife. Often these diseases are epidemic in nature, posing a serious economic problem. The best way to prevent the spread of herpesviruses is through prophylactic vaccines. However, due to the developing resistance of herpesviruses to currently used drugs, another solution is to develop new antiviral therapeutics. The development of effective drugs is possible due to a thorough understanding of the molecular mechanisms of action of herpesviruses, which in the course of evolution have developed a variety of mechanisms to inhibit or evade the innate and acquired immune response of the affected organism. Among the most characteristic features of herpesviruses affecting the course of disease are the ability to reside in the host in a latent state, known as latency, and the ability to modulate the immune response. Cellular immunity, mediated by CD8+ T lymphocytes, is the primary defense mechanism against many viral infections. This immunity depends on the efficient recognition of viral peptides presented by MHC class I molecules on the cell surface. Herpesviruses have developed various strategies to interfere with MHC I processing and antigen presentation, resulting in reduced expression of MHC I molecules on the cell surface. One mechanism for evading the immune response is inhibition of the transporter associated with antigen processing TAP. The physiological role of TAP is to transport antigenic peptides from the cytoplasm to the endoplasmic reticulum for binding to MHC I proteins. Products of the UL49.5 protein orthologs of members of the Varicellovirus genus, including BHV-1, PRV, EHV-1 and EHV4 have been identified as a new class of inhibitors. TAP inhibitors also include proteins of human herpesviruses i.e. EBV and HCMV.

The main objective of the presented project is to determine the 3D structure of immunomodulatory viral proteins (TAP inhibitors) of selected herpesviruses and to study their interaction with lipid bilayer models. To achieve the adopted research goal, the spatial structures of TAP protein inhibitors will be determined using NMR and molecular modeling techniques. In addition, the affinity of these proteins and their strength of interaction with the lipid membrane will be determined using MST, SPR and QCM-D technique. As part of the project, we plan to conduct studies for the following proteins: protein UL49.5 of bovine herpesvirus type 5 (BHV-5), protein UL49.5 of equine herpesvirus types 1 and 4 (EHV-1, EHV-4), protein UL49. 5 of pseudorabies virus (PRV), US6 protein of human cytomegalovirus (HCMV), BNLF2a protein of Epstein-Barr virus (EBV), and CPXV012 protein of cowpox virus (CPX). The proposed research is part of the modern trend of viral protein research. This project may contribute to the development of herpesvirus research, and the determination of the 3D structures of these proteins may help in understanding the pathogenesis and course of the immune response. The results obtained will provide a better understanding of what happens in the human body during disease, but also may allow the development of new therapeutics in the future.

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P27

Studies of the Anti-amyloid Activity of C-peptide Fragments in Human Insulin Solutions

Joanna Lewandowska[1], Beata Kolesińska[1]

[1] *Institute of Organic Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz*
beata.kolesinska@p.lodz.pl

Diabetes, both type I and II, as a disease of civilization of the 21st century, constantly attracts the interest of scientists from various fields of science. Data from the World Health Organization indicate that in 2023, over 830 million patients will fall ill with this disorder [1]. Unfortunately, the number of people diagnosed with diabetes is constantly growing. Despite the dynamically developing market of antidiabetic medications, efforts are still being made to develop formulations that stabilize the native structure of insulin both at the stage of introduction as well as its subsequent biodistribution in the body.

C-peptide is a component of proinsulin, namely it acts as a linker between its A and B chains. Moreover, it supports the correct organization of secondary and tertiary structure by the metabolic hormone, thus allowing the formation of disulfide bridges within it [2]. The performed studies aimed to verify whether the selected fragments of C-peptide **a)** are stable structures in a phosphate buffer at pH 7.2 **b)** show anti-amyloid properties against insulin fibrils. The proposed strategy is a continuation of research on the development of transdermal therapeutic systems supporting long-term anti-diabetic therapy by increasing the stability of the hormone at the stage of its transdermal application and subsequent biodistribution in the body.

The all selected C-peptide fragments (H_2N-^{57}EAEDLQV^{63}-OH; H_2N-^{64}GQVELG^{69}-OH; H_2N-^{76}SLQPLA^{81}-OH; H_2N-^{82}LEGSLQ^{87}-OH) were synthesized on 2-chlorotrityl chloride resin according standard SPPS procedure. The purity of the potential inhibitors was confirmed using high performance liquid chromatography coupled with mass spectrometry. Nextly, both their stability as well as anti-amyloid activity towards insulin, were checked for the incubated samples (37,4°C, phosphate buffer pH 7,2) using following techniques: circular dichroism, absorbance measurements with congo red dye and microscopic analysis.

Based on the results obtained so far, it can be concluded that the fragment showing the most desirable properties as an aggregation inhibitor is the peptide H_2N-^{57}EAEDLQV^{63}-OH (S1). For this sequence, the most unambiguously and repeatable results were obtained in the experimental work. The remaining fragments of the C-peptide demonstrated lower stability in the tested medium, although the growth of insulin fibrils in their presence was also limited.

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P28

Design, Synthesis, and Computational Evaluation of CF_3-Olefinic Amino Acid Analogues as Potential Cathepsin C Inhibitors

Karolina Paszek[1], Tomasz Siodła[1], Maciej Janicki[2], Henryk Koroniak[1], Katarzyna Koroniak-Szejn[1]

[1] *Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland*
[2] *Faculty of Biology, Adam Mickiewicz University, Poznań, Poland*
karolina.paszek@amu.edu.pl
katarzyna.koroniak@amu.edu.pl
tomasz.siodla@amu.edu.pl

In this paper we are reporting the synthesis and theoretical evaluation of novel fluorinated amino acid analogues featuring a CF_3-olefinic moiety as potential inhibitors of Cathepsin C [1], a key protease implicated in inflammatory diseases.[2] Our synthetic approach leverages nucleophilic trifluoromethylation (Ruppert-Prakash reagent) [3] followed by Horner-Wadsworth-Emmons olefination to access diverse CF_3-alkenyl derivatives.[4] Despite challenges in diastereoselectivity and purification, the target compounds were obtained in moderate to good yields.

Molecular docking and dynamics simulations identified promising candidates exhibiting stable binding to Cathepsin C's active site, mimicking native substrate interactions. These compounds formed critical hydrogen bonds with residues Gly277 and Asn380, supported by favourable MM-GBSA binding energies. MD simulations further validated their stability, highlighting their potential as non-covalent inhibitors.

This work underscores the strategic role of fluorine in peptidomimetic design, combining synthetic innovation with computational insights to advance targeted protease inhibition. Our findings open the way for further experimental validation of these analogues as therapeutic leads for conditions like COPD and rheumatoid arthritis.

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P29

Synthesis of Cell-Penetrating Peptides with Peroxisomal Targeting Signal 1 for Targeted Peroxisomal Transport

C. Krofta[1], A. S. Hammann[2], S. Thoms[2], N. Sewald[1]

[1] *Bielefeld University, Department of Chemistry, Organic and Bioorganic Chemistry, Bielefeld, Germany*

[2] *Bielefeld University, Medical School OWL, Biochemistry and Molecular Medicine, Bielefeld, Germany*

christine.krofta@uni-bielefeld.de

Peroxisomes play an unique role in metabolism and signaling pathways in all tissues.[1] Serious diseases such as Zellweger spectrum disorders, X-linked adrenoleukodystrophy, and Refsum's disease show the importance of peroxisomes and thus the urgency of research in this area.[2] A cell penetrating peptide (CPP) and a peroxisomal targeting signal 1 (PTS1) were combined to develop potential *in vitro* carrier with the goal of being able to penetrate the cell membrane and enter the peroxisomes.

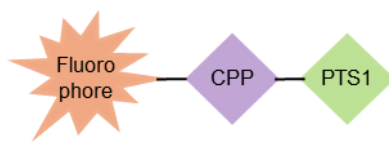

Figure 1. *General structure of possible peroxisomal transporters consisting of fluorophore, CPP and PTS1.*

A library of four peptides was synthesized with the same PTS1 [3] sequence and different CPP sequences, which had previously been proven to be effective on their own.[4-7] A fluorophore was linked to the carrier to visualize its intracellular accumulation using fluorescence microscopy.

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P30

The Application of Automatic Flow Solid Phase Synthesis for Miniproteins

Manish Kumar[1] and Łukasz Berlicki[1]*

[1] *Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland*
*lukasz.berlicki@pwr.edu.pl

Solid phase peptide synthesis (SPPS), since its inception in 1963, has been a major enabling tool for peptide synthesis.[1] Over the past 60 years, several SPPS technologies have been developed, including flow-based SPPS.[2] Recently, it has been demonstrated that automatic fast flow peptide synthesis (AFPS) technology is highly effective.[3] Flow-based SPPS is gaining momentum due to its advantageous features—for example, control over physical parameters and greatly reduced formation of side products. However, the combination of the high purity of crude peptide with a low amount of waste remains a challenge. Nowadays, miniproteins have been of great interest due to their well-defined stable three-dimensional structures, moderate size, and broad applications from basic research to the manufacture of clinically approved therapies.[4–6]

In this contribution, we describe the development of a custom-based fully automatic flow SPPS and optimizations for the synthesis of miniprotein with high purity of crude product. The real-time progress of miniprotein synthesis is monitored using UV. The system is composed of HPLC-based modules, which makes it reliable, cost-effective, and safe. The optimization of synthesis parameters such as flow, pressure, temperature, and duration of individual steps leads to a methodology that allows obtaining peptides and miniproteins with sequences of up to 70 residues in a routine manner. The low usage of solvents and reagents is another advantage of this approach.

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P31

Zn(II) and Cu(I) Binding Properties of Human Metallothionein-4 and Peptide Models of its Domains

Weronika Leszczyńska[1], Artur Krężel[1]

[1] *Department of Chemical Biology, Faculty of Biotechnology, University of Wroclaw, Joliot-Curie 14a, 50-383 Wrocław, Poland*
weronika.leszczynska@uwr.edu.pl
artur.krezel@uwr.edu.pl

Copper and zinc ions are crucible elements and play critical roles in biological processes. These metal ions differ in the body not only in their cellular concentration but also in the functions they perform and the types of complexes they form with metalloproteins. The primary function of metallothioneins (MTs) is to buffer the concentration of these metal ions. Mammalian MTs are small proteins with high cysteine content (~30%) and are characterized by the absence of aromatic residues. This protein family comprises four main isoforms: MT1, MT2, MT3, and MT4. Metallothioneins consist of two domains, α and β, which bind up to seven divalent metal ions, up to three in β and up to four in the α domain. Recently, MT3 and MT4 were isolated, containing bound copper in mixed copper/zinc complexes, suggesting that these isoforms play a significant role in binding, distribution, and metabolism of these ions.[1,2] MT3 has recently been intensively studied for interactions with Cu(I) and Cu(II) ions, but research on MT4 remains unknown.[3] The MT4 domains were synthesized using an SPPS, purified using HPLC, and frozen in the apo form (without bound metal ions). Using spectroscopic methods, it was possible to characterize the formed complexes with Cu(I) and Zn(II) ions. αMT4 and βMT4 domains with Cu(I) interactions were studied under anaerobic conditions to prevent oxidation of both Cu(I) and the peptides. The affinity of Cu(I) ions for MT4 domains was assessed using copper chelator (BCA). Spectroscopic analysis revealed the binding of Zn(II), Cu(I), and Cu(II) ions to MT4 and domains of MT4 and the formation of mixed reduced and oxidized Cu(I)/Zn(II)-MT4 complexes. These complexes suggest the displacement of Zn(II) and binding of Cu(I) ions with varying affinities. Cu(I) binding to MT4 led to the formation of two clusters, Cu_6Cys_9 and Cu_7Cys_{11}, in the β and α domains, respectively. Understanding Cu(I) binding and complex formation is crucial for determining the role of MT4 in copper and zinc metabolism in the cells.

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P32

Overview of Stabilized β-Hairpin Peptidomimetics as Inhibitors of Protein-Protein Interactions

Yun Li[1,2], Felix M. Paulussen[1,2], Tom N. Grossmann[1,2]

[1] *Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit Amsterdam, The Netherlands*
[2] *Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, The Netherlands*
t.n.grossmann@vu.nl

Protein–protein interactions (PPIs) play critical roles in numerous biological processes and are implicated in the pathogenesis of many diseases. However, particularly when involving intracellular targets, PPIs are often deemed "undruggable" due to their large, shallow, and featureless binding interfaces, which are poorly suited for conventional small-molecule therapeutics.[1]

In recent years, peptide-based modalities have emerged as promising alternatives. These molecules combine the surface recognition capabilities of antibodies with a higher potential for membrane permeability and intracellular delivery.[2] Among peptide scaffolds, β-sheet-derived β-hairpin structures—comprising two antiparallel β-strands linked by a tight turn—represent a compelling yet underexplored class of PPI inhibitors.[3] These motifs are inherently stabilized by interstrand hydrogen bonding and turn-inducing residues, but often exhibit insufficient structural stability and limited cellular uptake, ultimately leading to reduced functional efficacy.

To address these limitations, several chemical strategies have been developed to stabilize β-hairpins, such as β-turn optimization, macrocyclization, and covalent crosslinking.[4] These modifications aim to enhance stability, improve binding affinity to PPI targets, and promote favorable pharmacokinetic properties including cellular uptake and proteolytic stability.

This overview reviews recent advances in the design and stabilization of β-hairpin peptidomimetics as inhibitors of intracellular PPIs. Particular focus is placed on the structure–function relationship between hairpin stabilization techniques and biological performance, offering insight into their translational potential as therapeutic agents.

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P33

CD160-derived Peptide as a Bidirectional Inhibitor of the Immune Checkpoints BTLA/HVEM and LIGHT/HVEM

Magdalena Lipińska[1], Piotr Ciura[1], Simon Gumpelmair[2], Peter Steinberger[2], Katarzyna Kuncewicz[1], Emilia Sikorska[1], Anna Wardowska[3], Marta Spodzieja[1]

[1] *Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland*
[2] *Medical University of Vienna, Institute of Immunology, Lazarettgasse 19, 1090 Vienna, Austria*
[3] *Medical University of Gdansk, Faculty of Medicine, Dębinki 7, 80-211 Gdańsk, Poland*
magdalena.lipinska@phdstud.ug.edu.pl

Immune checkpoints (ICPs) are receptors present on the surface of T cells that form complexes with their ligands, present on antigen presenting cells (APCs) [1]. Such complexes can send signals that activate as well as inhibit T cell activation, proliferation, and cytokine production. Co-stimulatory and co-inhibitory immune checkpoints are extremely important for the human body in maintaining immune homeostasis.

Tumour cells are also capable of expressing ligands for inhibitory immune checkpoint receptors on their surface. In cancer patients, signalling pathways that inhibit lymphocyte proliferation are used to suppress the body's immune response. Blocking immune checkpoints involves administering inhibitors – usually monoclonal antibodies – that inhibit complex formation and stimulate the immune system to fight cancer cells [1].

Herpes Virus Entry Mediator (HVEM), which forms complex with B and T Lymphocyte Attenuator (BTLA) is an example of ICP that is still under investigation in a matter of mechanism of action [2]. So far, this complex has been confirmed to play a negative role in the tumour microenvironment of melanoma, colorectal cancer and gastric cancer, and in a number of autoimmune diseases [3].

Besides BTLA, HVEM also interacts with Cluster of Differentiation 160 (CD160), with both receptors sharing overlapping binding sites on the HVEM molecule. Therefore, the aim of this study was to design, synthesize, and evaluate the inhibitory properties of peptides derived from the CD160 protein, targeting the formation of the BTLA/HVEM complex. Our results proved that one designed peptides, referred as A5, has high binding affinity to HVEM and inhibits BTLA/HVEM complex formation. Further molecular dynamic simulation showed that A5 binds not only to cysteine-rich domain 1 (CRD1) of HVEM, which is involved in binding to BTLA, but also to CRD2 and CRD3, which are responsible for the interaction with LIGHT protein. Additional analyses confirmed that A5 inhibits also LIGHT/HVEM complex formation. These results indicate that A5 functions as a dual inhibitor, with potential therapeutic relevance in immune-related disorders.

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P34

NS2B/NS3 Protease of Tick-Borne Encephalitis Virus – a Small Protein of Great Importance

Filip Maciejowski[1], Joanna Białek[1], Renata Grzywa[1], Marcin Sieńczyk[1]

[1] *Department of Organic and Medicinal Chemistry, Wrocław University of Science and Technology, Wybrzeże Stanisława Wyspiańskiego 27, 50-370 Wrocław*
filip.maciejowski@pwr.edu.pl

Tick-borne encephalitis virus (TBEV), an arbovirus belonging to genus *Flavivirus*, is currently one of the major public health concerns across Europe and Asia. TBEV infects the central nervous system, causing encephalitis, meningitis and myelitis, which often lead to permanent neurological complications (up to 50% of acute infection patients) or even death [1,2]. Despite the availability of four vaccines, the number of confirmed TBEV infections has increased over the past years, mainly due to the expanding tick population across Eurasia [3]. Currently, there is no specific treatment for TBEV. Most symptomatic patients receive a supportive care, including analgesics, antipyretics, antiemetics and anticonvulsive agents [4]. The sharp increase in TBEV infection rate, together with the lack of specific treatment, motivates the development of effective, targeted antiviral therapies against TBEV.

The NS2B/NS3 serine protease of TEBV is a key target for the development of novel antivirals. This enzymatic complex plays a crucial role in the post-translational maturation of the viral polyprotein by cleaving it in seven specific spots, enabling the release of both structural and non-structural viral proteins [5]. A promising group of TBEV NS2B/NS3 inhibitors includes guanidine-containing diphenyl esters of 1-aminoalkylphosphonic acids, whose activity has been confirmed against the NS2B/NS3 protease of the West Nile virus - other *Flavivirus* genus member [6].

In this study, we present the design and expression of catalytic variants of the TBEV NS2B/NS3 protease, as well as the synthesis of its inhibitors from the class of 1-aminoalkylphosphonic acids diphenyl esters.

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P35

Modified DAPEG Polymers as Safe Gene Delivery Vectors

Gabriela Mirecka[1], Wiktoria Mallek[1], Agnieszka Piwkowska[2], Adam Lesner[1], Magdalena Wysocka[1]

[1] University of Gdansk, Faculty of Chemistry, Wita Stwosza 63, 80-308 Gdansk, Poland
[2] Mossakowski Medical Research Institute, Polish Academy of Sciences, Adolfa Pawinskiego 5, 02-106 Warsaw, Poland
gabriela.mirecka@phdstud.ug.edu.pl

Transfection is the method of introducing genetic material (DNA/RNA) into eukaryotic cells and it is crucial in gene therapy for genetic abnormalities or cancer. The application of viral vectors has raised concerns over their associated hazards, prompting a trend towards safer, non-viral alternatives including chemical transfection reagents. Efficient transfection agents must exhibit efficient interactions with nucleic acids, guarantee elevated transfection efficacy, and demonstrate minimal cytotoxicity [1,2].

Dendrimers, a promising category of non-viral vectors, are highly branching polymers characterized by a well-defined, three-dimensional, frequently spherical structure. Their positively charged surface groups enable interaction with negatively charged nucleic acids [3].

This study posits that dendrimers consisted of DAPEG building blocks (L-2,3-diaminopropionic acid (DAP) modified with polyethylene glycol (PEG) chains) and modified at the N-terminus with fatty acid residues could serve as efficient and non-toxic DNA carriers for eukaryotic cells [4].

This study aimed to devise strategies for the chemical synthesis of dendrimers incorporating DAPEG fragments and modification of N-terminus with fatty acids. The synthesized compounds were tested for their capacity to bind DNA, transfection efficiency and cytotoxicity against the HEK-293T cell line.

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P36

In Silico Screening of Peptidomimetics Targeting the MβLs

Msanif Msanif[1], Michał Jewgiński[1], Rafał Latajka[1]

[1] Wroclaw University of Science and Technology,
Wyb. Wyspianskiego 27, 50-370 Wroclaw, Poland
msanif.msanif@pwr.edu.pl

Metallo-β-lactamases (MβLs) are a critical group of zinc-dependent enzymes that confer resistance to nearly all β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, the latter being last-resort treatments for multidrug-resistant infections [1]. Variants such as New Delhi Metallo-β-lactamase-1 (NDM-1) and Verona Integron-encoded Metallo-β-lactamase-1 (VIM-1) have emerged as prominent threats due to their broad substrate profiles and rapid global dissemination [2]

Peptidomimetics synthetic compounds that mimic peptide structures, are promising candidates for NDM-1 inhibition due to their enhanced metabolic stability and high target specificity [3]. The urgent need for effective MβL inhibitors has turned attention to peptidomimetics, engineered molecules that replicate the structural features of peptides while offering superior pharmacokinetic properties and resistance to enzymatic degradation [4].

In this study, we demonstrate the pivotal role of computational approaches, including molecular docking and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) free-energy calculations, in facilitating the structure-based design of these inhibitors. These in silico techniques enable high-fidelity modelling of ligand-enzyme binding interactions, particularly at the conserved zinc-active site, thereby streamlining inhibitor optimization while minimizing experimental screening efforts [5].

Collectively, the strategic convergence of peptidomimetic chemistry and computational in silico screening approaches offers a viable therapeutic avenue to counteract metallo-β-lactamase mediated antibiotic resistance and restore the clinical utility of β-lactam antibiotics

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P37

Towards Structural Insights into HVEM/BTLA Disruption: Crystallization of HVEM with Peptide Inhibitor

Marta Orlikowska[1], Marta Piotrowska[1], Katarzyna Kuncewicz[1], Marta Spodzieja[1], Sylwia Rodziewicz-Motowidło[1]

[1] *University of Gdansk, Wita Stwosza 63, 80-308 Gdansk, Poland*
marta.orlikowska@ug,edu.pl

Cancer cells pose a unique challenge to the immune system due to their ability to avoid detection by the immune system, as they have developed advanced strategies to evade it. One such mechanism is the creation of an immunosuppressive environment. A key element of this process is the expression of ligands that can inhibit the immune responses and favor tumor progression. Together with their receptor on lymphocyte T, these ligands are defined as immune checkpoints, and their potential as targets for therapy could inhibit immune responses dependent on T cells, providing hope for effective cancer treatment. Blocking inhibitory immune checkpoints seems to be a valuable approach to activate the immune system.

Tumor cells often exploit the HVEM/BTLA pathway to evade immune detection. By expressing HVEM, cancer cells contribute to immunosuppression and tumor progression across various cancer types [1,2]. Targeting the HVEM/BTLA pathway can reverse this immune suppression, enabling the immune system to recognize and attack cancer cells. Blocking the HVEM/BTLA interaction can block the inhibitory signal, and immune activity can be suppressed. This approach could enhance the function of cytotoxic T cells and other immune cells, promoting anti-tumor immunity.

It is crucial to gather extensive knowledge about the binding site structure and properties to design a sequence of a peptide inhibitor targeting a specific immune checkpoint, minimizing at the same time the negative influence of the inhibitor on the whole organism. Therefore, the main objective of this project is to provide the structural characterization of HVEM in complexes with its known peptide inhibitor [3-5] using X-ray crystallography.

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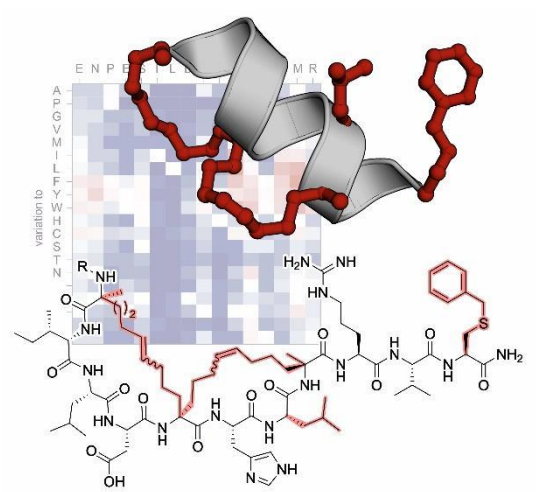
P38

Structure-Based Design of Bicyclic Helical Peptides That Target the Oncogene β-Catenin

Alejandro Yeste-Vázquez[1,2], Felix M. Paulussen[1,2], Mathias Wendt[1,2], Rasmus Klintrot[1,2], Clemens Schulte[3], Kerstin Wallraven[1,2], Lieke van Gijzel[1,2], Boris Simeonov[4,5], Maurice van der Gaag[4,5], Alan Gerber[4,5], Hans M. Maric[3], Sven Hennig[1,2], and Tom N. Grossmann[1,2]

[1] *Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit Amsterdam, The Netherlands*
[2] *Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, The Netherlands.*
[3] *Rudolf Virchow Center, Center for Integrative and Translational Bioimaging, University of Wuerzburg, Germany*
[4] *Department of Neurosurgery, Amsterdam UMC, Amsterdam, The Netherlands*
[5] *Cancer Center Amsterdam, Cancer Biology, Amsterdam UMC, Amsterdam, The Netherlands*
t.n.grossmann@vu.nl

Inhibiting intracellular protein-protein interactions is particularly challenging when the involved interfaces lack pronounced cavities. The transcriptional co-activator protein and oncogene β-catenin exemplifies such a difficult target. Despite extensive efforts, current high-affinity binders are limited to large molecular weight inhibitors, hindering the development of therapeutically useful compounds. In this study, we present the design of a significantly smaller peptidomimetic scaffold derived from the α-helical β-catenin-binding motif of Axin. Through sequence maturation and bicyclization, we created a stitched peptide with a novel crosslink architecture. The binding mode and site were confirmed via crystal structure analysis. Further derivatization produced a β-catenin inhibitor with single-digit micromolar activity in a cell-based assay. This research highlights strategies for designing helix mimetics with reduced molecular weight, thereby enhancing their biological activity.[1]



Graphical abstract showing the discovered peptidic inhibitor of the oncogene β-catenin. The bicyclic inhibitor combines two hydrocarbon staples with i,i+4 and i,i+3 spacing, respectively. After affinity maturation, we obtain a high-affinity binder of the Wnt signaling pathway in a cell-based assay.

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P39

Synthesis and Characterization of Peptides Based on VEGF-binding Loops as Potential Pro-regenerative Compounds

Krzysztof Polaczek[1], Przemysław Karpowicz[1], Małgorzata Zawrzykraj[2], Milena Deptuła[2], Artur Giełdoń[3], Michał Pikuła[2], Emilia Sikorska[4]

[1] *Department of Biomedical Chemistry, Faculty of Chemistry University of Gdansk, Poland*
[2] *Laboratory of Tissue Engineering and Regenerative Medicine, Medical University of Gdansk, Poland*
[3] *Department of Theoretical Chemistry, Faculty of Chemistry University of Gdansk, Poland*
[4] *Department of Organic Chemistry, Faculty of Chemistry University of Gdansk, Poland*
krzysztof.polaczek@phdstud.ug.edu.pl

Impaired wound healing leads to chronic wounds, a major clinical and social challenge in diseases like diabetes and cancer, reducing quality of life and increasing healthcare costs. Protein growth factors, which regulate regenerative processes such as cell proliferation, migration, and angiogenesis, are increasingly used in chronic wound management. Among them, vascular endothelial growth factor (VEGF) plays a key role by activating VEGF receptors (VEGFR) to stimulate angiogenesis and tissue repair. Our group has shown that short "loop" fragments derived from the PDGF-BB protein (a VEGF-related growth factor) can bind receptors, stimulate skin cells, and accelerate wound healing in mice [1,2]. Encouraged by this, we explored similar peptides based on VEGF sequences. While full-length growth factors have therapeutic limitations, peptide mimetics offer targeted, cost-effective alternatives; receptor-binding loops in VEGFs are especially promising for pro-angiogenic therapies.

The aim of this study was to synthesize, characterize, and evaluate the biological activity of peptides based on L1 and L3 loop sequences from human VEGF isoforms (A–D), as well as two phylogenetically distinct isoforms: VEGF-E (viral) and VEGF-F (animal). Peptides were synthesized via microwave-assisted solid-phase peptide synthesis (MW-SPPS) and purified chromatographically. Their cytotoxicity and proliferative effects were assessed in HaCaT keratinocytes and 46BR.1N fibroblasts. Binding to VEGFR1/2 and, due to structural similarity, PDGFRα/β was measured by modified ELISA. Circular dichroism spectroscopy and molecular modeling were used to analyze peptide structure and receptor interactions. The results showed that peptides derived from the L1 loop strongly stimulated keratinocytes, while those based on the L3 loop exhibited higher receptor-binding affinity. Interestingly, the most pronounced cellular effects were observed for peptides derived from VEGF-E and VEGF-F. These findings provide a foundation for further optimization of pro-regenerative peptides for therapeutic applications and their integration into wound-healing biomaterials.

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P40

New Conjugates of Vancomycin with Cell-Penetrating Peptides – Antimicrobial Activity, Cytotoxicity, and BBB Permeability Studies

Katarzyna Prochera[1], Oliwia Bujak[1], Jarosław Ruczyński[1], Natalia Kaźmierczak[2], Katarzyna Kosznik-Kwaśnicka[2], Lidia Piechowicz[2], Piotr Mucha[1], Piotr Rekowski[1]

[1] *Laboratory of Chemistry of Biologically Active Compounds, Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland*

[2] *Department of Medical Microbiology, Faculty of Medicine, Medical University of Gdańsk, Dębowa 25, 80-204 Gdańsk, Poland*

katarzyna.prochera@phdstud.ug.edu.pl

Vancomycin (Van) is a glycopeptide antibiotic commonly used as a last resort for treating life-threatening infections caused by multidrug-resistant bacterial strains, such as *Staphylococcus aureus* and *Enterococcus* spp. However, its effectiveness is currently limited due to the rapidly increasing number of drug-resistant clinical strains and its inherent cytotoxicity and poor penetration into cells and specific regions of the body, such as the brain.

One of the most promising strategies to enhance its efficacy appears to be the covalent attachment of cell-penetrating peptides (CPPs) to the Van structure. A series of synthesized vancomycin conjugates with CPPs – such as TP10, Tat(47–57), PTD4, and Arg_9 – were tested for antimicrobial activity against four reference strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and two clinical drug-resistant strains: methicillin-resistant *S. aureus* and vancomycin-resistant *E. faecium*. Cytotoxicity tests (using a human fibroblast cell line) and blood–brain barrier (BBB) permeability tests (using a parallel artificial membrane permeability assay – PAMPA-BBB assay) were conducted for selected compounds.

Our research demonstrated that some of the Van-CPP conjugates, particularly with Tat(47–57), Arg_9 or TP10, represent significantly higher antimicrobial activity against Gram-positive bacteria such as *S. aureus* and *Enterococcus* spp., lower cytotoxicity and probably a greater ability to penetrate the BBB than unmodified vancomycin.

We conclude that these findings provide a solid basis for the design of novel antimicrobial agents effective in treating infections caused by drug-resistant staphylococcal and enterococcal strains, while also being capable of crossing the BBB.

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P41

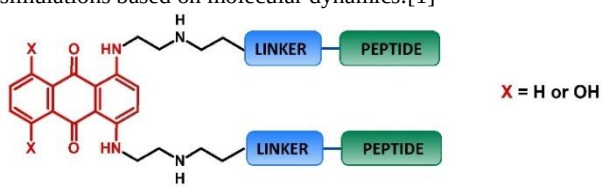
Design of Sequence-Selective Oligopeptide Derivatives of Anti-tumour Intercalators

Anna K. Puszko[1], Alberto Ongaro[2], Luc Demange[3], Giovanni Ribaudo[4], Nohad Gresh[5]

[1] *University of Warsaw, Faculty of Chemistry, Warsaw, Poland*
[2] *Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova 35131, Italy*
[3] *CiTCoM, UMR 8038 CNRS, Faculty of Pharmacy, Paris-Cité University, Paris, France*
[4] *DMMT, University of Brescia, Brescia, Italy*
[5] *Theoretical Chemistry Laboratory, UMR 7616 CNRS, Sorbonne University, Paris, France*
apuszko@chem.uw.edu.pl

Cancer remains a major public health issue due to the rapid development of resistance to chemotherapeutic drugs and the occurrence of hard-to-treat spreading. The existing chemotherapeutics are toxic to all cells, both tumour and normal. This may lead to some serious side effects and sometimes even cause the death of patients.

The aim of our project is the design of novel compounds targeting the major groove of B-DNA unique sequences of six, eight, or ten base pairs. The target sequences are encountered in oncogenes or are involved in essential metabolic processes, overexpressed when cells are deregulated. All target DNA sequences have a central $d(CpG)_2$ site, which is a preferred site for intercalation of conjugated rings of anticancer drugs, including daunomycin or mitoxantrone. The designed compounds are peptide or peptide-like derivatives of intercalating molecules. Two arms are located on the intercalator ring and the structure of each is designed to enable hydrogen bonding interactions of each arm with two consecutive bases on a distinct DNA strand upstream from the anchoring intercalation site. The main sequence used in this project is a self-complementary sequence, a core palindrome of six base pairs – $d(GGCGCC)_2$. The molecules are initially designed on the basis of computer simulations based on molecular dynamics.[1]



The expected outcome of this project is to obtain novel, highly sequence-selective intercalator-oligopeptide derivatives targeting unique oncogenic or retroviral sequences. Mono-, bis- and tris-intercalators targeting six, eight, and ten base-pairs, respectively, would represent a major advance compared to currently used drugs, which do not target sequences longer than three base-pairs.

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P42

Biocatalytic Synthesis of Dihalogenated Tryptophan Derivatives

Bjarne Scharkowski[1], Nicolai Montua[1] and Norbert Sewald[1]

[1] *Organic and Bioorganic Chemistry, Faculty of Chemistry, Bielefeld University, Germany*
bjarne.scharkowski@uni-bielefeld.de

Halogenated aryl-compounds are an abundant motif both in bioactive natural compounds and synthetic pharmaceuticals. In addition to their halo substituents often playing a significant role in bioactivity and metabolic stability, they can serve as activated intermediates for late-stage functionalization via cross-coupling or substitution reactions. Therefore, the regioselective incorporation of two different halo substituents in an aryl moiety could enable the synthesis of halogenated biaryl-compounds provided that both positions show a difference in reactivity sufficient for orthogonal functionalization. Flavin-dependent halogenases (FDHs) are powerful tools for aryl halogenation. They exhibit exceptional regioselectivity towards their native substrates and obviate the need for harsh reaction conditions, replacing the typically employed elemental halogens, high temperatures and Lewis acids with halide salts, ambient temperature, and physiological pH. Flavin-dependent tryptophan halogenases (Trp-FDHs) have been of particular interest in recent years, owing to the diverse applications of tryptophan derivatives in the pharmaceutical, food, agriculture, and dye industries. Protein engineering efforts towards increased stability, substrate scope and catalytic efficiency greatly improved the viability of this enzyme class as biocatalysts for white biotechnology.

Recently, both biocatalytic approaches and metabolic pathways have been described in which different Trp-FDHs work in tandem to produce dihalogenated tryptophan derivatives.[1,2] These were, however, limited to the incorporation of the same halide in both positions. In this work, combinations of different Trp-FDHs were utilized to obtain dihalogenated tryptophan derivatives featuring two different halo substituents, enabling the orthogonal functionalization of the halogenated positions to create diverse building blocks for the use in peptide synthesis.

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P43

Novel SMDCs with Unit D Modified Cryptophycin as Payload

Dominic Seißenschmidt[1], Cedric Dessin[1], Thomas Schachtsiek[1], Norbert Sewald[1]

[1] Department of Chemistry, Bielefeld University,
Universitätsstraße 25, 33615 Bielefeld, Germany
dominic.seissenschmidt@uni-bielefeld.de

Cancer is still one of the most challenging diseases and second leading cause of death in Germany.[1] However classical, non-targeted chemotherapy commonly used for cancer treatment suffers from serious side effects because all rapidly proliferating cells, both malignant and healthy, are affected. An emerging alternative to that approach are SMDCs (small molecule drug conjugates) and ADCs (antibody drug conjugates) which connect the cytotoxic compound with a homing device via an enzymatically cleavable linker, therefore giving the conjugate the ability to accumulate only in tumour tissue.

A promising candidate as a payload is the macrocyclic depsipeptide cryptophycin, which shows excellent activity even against *multidrug resistant* (MDR) tumor cell lines, by inhibition of the microtubuli dynamics.[2] However, these do not contain an addressable functional group for conjugation. Therefore, new synthetic variants with modifications in unit D were developed in our workgroup.[3]

RGD peptides and peptidomimetics are known as homing devices for addressing integrins (e.g. $\alpha_v\beta_3$) which are overexpressed on the surface of several cancer cells. In particular, excellent targeting indices were described of conjugates that contain RGD peptidomimetics.[4] Therefore, cryptophycin-based conjugates were synthesized containing RGD homing device connected via a neutrophil elastase cleavable linker and tested in vitro.

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P44

Small-Molecule Compounds as Potential Inhibitors of the TNF/TNFR2 Complex Formation

Karolina Sławińska[1], Gabriela Całka-Kuc[1], Marta Karpiel[2,3], Justyna Kalinowska-Tłuścik[2], Marta Spodzieja[1], Sylwia Rodziewicz-Motowidło[1]

[1] Department of Biomedicinal Chemistry, Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308, Gdańsk, Poland
[2] Department of Crystallochemistry and Crystallophysics, Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Kraków, Poland
[3] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Lojasiewicza 11 , 30-348 Krakow, Poland
karolina.slawinska@phdstud.ug.edu.pl

The TNF/TNFR2 signaling complex plays a crucial role in tumor progression by directly promoting cancer cell proliferation and indirectly shaping the tumor microenvironment through regulatory T cells (Tregs). High expression of TNFR2 in tumor-infiltrating Tregs improves their immunosuppressive capacity, enabling immune evasion by malignant cells. Blocking the formation of the TNF/TNFR2 complex is therefore considered a promising therapeutic strategy, which can potentially restore antitumor immunity while simultaneously inhibiting cancer growth [1,2].

This project aims to experimentally assess four small-molecule organic compounds, preselected through molecular docking studies, as potential inhibitors of the TNFR2 receptor. This research involves compounds selected from the Enamine PPI library of 40640 compounds [3]. The selection of these compounds was based on the results of virtual docking within the TNFR2 protein binding sites. Based on the theoretical assay, four organic molecules characterised by strong binding to the TNFR2 receptor were selected. Molecules are quinazoline derivatives containing aromatic and amide groups that differ by substituents in the aromatic rings. Given their favourable pharmacokinetics, better tissue penetration, and lower production costs, small molecules may offer a viable alternative to antibody-based therapies targeting immune checkpoints.

The research included competitive ELISA assays to assess whether the molecules can inhibit the formation of the TNF/TNFR2 complex. Then, microscale thermophoresis was measured to determine the binding affinity constants (K_D) of the compounds. The molecules were also tested for cytotoxicity against the OVCAR3 cell line, which is characterised by high overexpression of the TNFR2 receptor.

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P45

Peptide-Based Inhibitors of Immune Checkpoint Interactions as a Strategy for Autoimmune Disease Treatment

Bartosz Bigielmajer[1], Piotr Ciura[1], Adam Sieradzan[1], Peter Steinberger[2], Anna Wardowska[3], Marta Spodzieja[1]

[1] *University of Gdańsk, Faculty of Chemistry, Wita Stwosza 63, 80-308 Gdańsk, Poland*
[2] *Medical University of Vienna, Institute of Immunology, Lazarettgasse 19, 1090 Vienna, Austria*
[3] *Medical University of Gdańsk, Faculty of Medicine, Dębinki 7, 80-211 Gdańsk, Poland*
marta.spodzieja@ug.edu.pl

Immunotherapy harnesses the body's natural defense mechanisms to combat disease. One of its key strategies involves targeting immune checkpoint proteins (ICPs), which regulate immune system activity by maintaining a balance between immune stimulation and suppression across various disease states. Among the stimulatory ICPs are the herpesvirus entry mediator (HVEM) and tumor necrosis factor superfamily member 14 (LIGHT), both of which are expressed on numerous immune cells. The interaction between these proteins modulates T cell activation, and the formation of the HVEM/LIGHT complex has been implicated in several inflammatory and autoimmune diseases, including inflammatory bowel disease, rheumatoid arthritis, and systemic lupus erythematosus [1,2]. A potential therapeutic approach for these conditions involves inhibiting the binding of these proteins. Accordingly, our research focuses on the design of peptide inhibitors to prevent HVEM/LIGHT complex formation.

One of the proteins interacting with LIGHT is decoy receptor 3 (DcR3). DcR3 binds to LIGHT in a similar region to the HVEM protein and inhibits HVEM/LIGHT complex formation [3]. In the presented studies, we used the amino acid sequence of the DcR3 protein, covering the binding fragment of DcR3 to LIGHT, to design peptide inhibitors disrupting HVEM and LIGHT interaction. All peptides were synthesized using solid-phase peptide synthesis and purified using reversed-phase high-performance liquid chromatography. The peptides were then tested for their affinity for the LIGHT protein and their ability to inhibit HVEM and LIGHT binding.

Our results indicate that DcR3-derived peptides bind to LIGHT and inhibit HVEM/LIGHT complex formation. However, further studies are needed to determine their immunological potential, especially in the context of autoimmune disease treatment.

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Rational Design of Novel Miniproteins with Aldolase Activity

Anna Szczepańska[1], Manish Kumar[1], Łukasz Berlicki[1]

[1] *Department of Bioorganic Chemistry, Wroclaw University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370, Wrocław, Poland*
anna.szczepanska@pwr.edu.pl

Although native enzymes are highly efficient and specific, their activity is limited to the narrow range of reactions they have evolved to catalyze. [1] The high conformational stability and compact size of miniproteins make them attractive scaffolds for the *de novo* design of functional peptides, which can be readily synthesized using solid-phase peptide synthesis (SPPS).[2] These characteristics make them particularly well suited for rational enzyme design, providing basis for the development of novel, highly stable catalysts and deepening our understanding of structure - activity relationships.

In this study, we present the *de novo* design and characterization of novel miniproteins that catalyze the aldol reaction between phenylacetaldehyde and 4-nitrobenzaldehyde under mild aqueous conditions.

Candidate sequences were designed using the Rosetta FastDesign protocol and ProteinMPNN, then fine-tuned using Alphafold3.[3-5] To promote folding and enhance rigidity, we incorporated the β-amino acid *trans*-ACPC into the helical secondary structure elements.[6] The resulting miniproteins were synthesized via SPPS and characterized by circular dichroism and nanoDSF, confirming high thermal stability and well-folded structures. Catalytic activity was assessed by HPLC following 1 hour of reaction in phosphate buffer (pH 7.4).

As a result, we identified miniprotein variants capable of catalyzing the aldol reaction with rate enhancements up to 1051 ± 116. Ongoing studies aim to further optimize the activity and specificity. Moreover, the obtained miniproteins demonstrate high thermal stability, making them reusable in the future as scaffolds for different applications.

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P47

Biophysical Characteristics of the Interprotein Zn(II)-binding Motif of DNA Repair Factor CtIP

Martyna Uściła[1], Anna Kocyła[1], Józef Tran[1], Artur Krężel[1]

[1] *Department of Biological Chemistry, Faculty of Biotechnology, University of Wroclaw, Joliot-Curie 14a, 50-383 Wrocław, Poland*
martyna.uscila@uwr.edu.pl, artur.krezel@uwr.edu.pl

DNA damage poses a continuous threat to cellular genetic stability and the accurate transmission of genetic information. Among the most critical types of damage are double-strand breaks, which can initiate carcinogenesis or lead to cell death. CtIP, a key protein in the MRN complex, plays a pivotal role in promoting homologous recombination and participating in DNA end resection. The N-terminal domain of human CtIP exhibits high helical content, containing double-coiled helices, and features a CxxC sequence adjacent to the tetramerization motif, which is capable of binding Zn(II) in a homodimeric configuration [1]. This study investigates the sequential and structural characteristics of CtIP fragments ranging from 4 to 46 amino acids in length, including a protein that encompasses the N-terminal domain. The primary objectives are to determine the Zn(II) binding affinity of CtIP subunits, identify sequence fragments that influence the stability of the resulting complexes, and explore the thermodynamic factors governing these interactions. Spectroscopic techniques were utilized to directly observe Zn(II) binding in the dimeric system, while CD-monitored conformational changes in peptides and proteins upon Zn(II) binding. Spectrophotometric pH-metric titrations revealed that the pK_a values of cysteine thiols in the CxxC motif decrease with increasing peptide length. Thermodynamic analysis indicated that longer peptide fragments exhibit a higher affinity for Zn(II), suggesting that additional structural elements enhance the stability of Zn(II) complexes. This stabilization is likely facilitated by long-range interactions and secondary structures, such as α-helices, which reinforce the Zn(II)-binding domain, increase rigidity, and improve Zn(II) coordination accuracy. Furthermore, a competitive assay with metallothionein confirmed its role as a Zn(II) donor, promoting the formation of the $Zn(CtIP)_2$ complex. This study provides valuable insights into the biophysical properties of Zn(II)-mediated complexes, representing one of the few examples of their structural and functional characterization [2]. Understanding the factors that determine the stability of the $Zn(CtIP)_2$ complex may provide critical perspectives on the role of intracellular Zn(II) concentrations in DNA repair pathways.

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P48

Identification of New BromoTrp Tag for Enzymatic Halogenation and SUZUKI-MIYAURA Cross-Coupling

Maria Walger[1], Norbert Sewald[1]

[1] *Department of Chemistry, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany*
mwalger@uni-bielefeld.de

The post-translational modification of proteins is a great tool for synthesizing bioactive molecules, but it is still challenging in chemical biology. Montua *et al*. combined enzymatic halogenation and transition metal-catalyzed cross-coupling for the introduction of functional groups in proteins. In this approach an engineered variant of the tryptophan 6-halogenase Thal is used for bromination of *C*-terminal tryptophan. A short peptide sequence, called BromoTrp Tag, was identified as substrate of the Thal-variant and can be tagged to the *C*-term of proteins for site selective bromination. In a bio-orthogonal reaction the brominated protein can be further functionalized by cross-coupling.[1] The SUZUKI-MIYAURA coupling can be performed under ambient conditions by using Pd-nanoparticles. Additionally, Dachwitz *et al*. showed that the introduction of methionine in specific positions regarding tryptophane yields in a much faster reaction rate.[2] The results of this research are used to identify an optimized tag for the bio-orthogonal modification of proteins. Different model peptides with a linker are synthesized by solid-phase peptide synthesis and tested on efficient bromination by the Thal-variant. Furthermore, the influence of a possible oxidation of the methionine residue during the enzymatic bromination is investigated regarding the SUZUKi-MIYAURA reaction catalyzed by Pd-nanoparticles.

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