Short oral

Site-specific non-enzymatic PTM of human Hsp27 enhances its chaperone activity

Somnath Mukherjee, Dominik Vogl, Maria Matveenko and Christian F. W. Becker
University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria; FB Rice, 23/44 Market St, Sydney NSW 2000, Australia

Non-enzymatic posttranslational modifications (nPTMs) are believed to affect at least 30% of human proteins, many of which are implicated in various pathological conditions e.g. cataract, diabetes, neurodegenerative diseases and cancer.1 A general lack of structural information of many of such nPTM target proteins coupled with the difficulty in pin-pointing the site of such modifications impede further studies. Since a site-specific installation of most of these nPTMs is not yet possible utilizing the available genetic code expansion techniques, semi-synthesis remains to be the only feasible way to access full-length proteins carrying site-specific and homogeneous modifications. One of such modifications, argpyrimidine (Apy) has been detected in human small heat shock protein Hsp27 (HSPB1) in various tissues of patients suffering from pathological conditions, such as hyperglycemia, cataract and cancer.2 Although, there are as many as 16 arginines in this 205-amino acid long protein susceptible to Apy modification, previous studies based on mutating arginines in Hsp27 to glycine residues followed by expression in HEK-293 cells and subsequent immunoblot analyses, revealed that only mutation at Arg188 leads to loss of recognition by an anti-Apy antibody.3 We were interested to understand the impact of such individual point mutations of arginine residues to Apy at five different arginine sites (R4, R5, R12, R20 and R27) within the unstructured yet functionally crucial N-terminal domain.4 Precisely, how it influences the quaternary structure and thus associated activities of Hsp27. Herein, we present a study combining semisyntheses and biophysical as well as biochemical assays on homogeneously modified Hsp27 analogues with single point Apy mutations at five different arginine sites (R4, R5, R12, R20, R27) within the N-terminal domain of Hsp27 revealing how a seemingly minimal modification within a structurally disordered region of a protein results in altered attributes

References

1. T. Oya-Ito, Y. Naito, T. Takagi, O. Handa, H. Matsui, M. Yamada, K. Shima, T. Yoshikawa, Biochim. Biophys. Acta 2011, 1812, 769–781.

2. P. J. Thornalley, S. Battah, N. Ahmed, N. Karachalias, S. Agalou, R. Babaei-Jadidi, A. Dawney, Biochem. J. 2003, 375, 581–592.

3. H. Sakamoto, T. Mashima, K.Yamamoto, T. Tsuruo, J. Biol. Chem. 2002, 277, 45770–45775.

4. R. Freilich, M. Betegon, E. Tse, S. Mok, O. Julien, D. A. Agard, D. R. outhworth, K. Takeuchi, J. E. Gestwicki, Nat Commun, 2018, 9, 4563.

Short oral

Proteomic approaches to characterize novel neurotoxins from Androctonus mauritanicus scorpion venom

Khadija Daoudi1, 2, Philippe Chan3, Marie-Laure Walet-Balieu3, Laurent coquet3, Magalie dupré4, Julia Chamot-Rooke5, Rachida Cadi2, David vaudry3, 4, Reto Stöcklin6, Naoual Oukkache1

1Laboratory of Venoms and Toxins, Pasteur Institute of Morocco, Casablanca, Morocco 2 Laboratory of Molecular Genetics, Physiopathology and Biotechnology, Faculty of Sciences Ain Chock, Hassan II University of Casablanca, Morocco 3Normandie Univ, Rouen Proteomic Platform (PISSARO), Rouen, France 4Normandie Univ, Inserm, Laboratory of Neuronal and Neuroendocrine Communication and Differentiation, Rouen, France, 5 Atheris Laboratories, Geneva, SwSwitzerland 6 Mass spectrometry for Biology Unit,

Androctonus mauritanicus (A.mauritanicus) scorpion venom showed considerable concern due to its direct involvement in the most lethal envenomation cases in Morocco. Despite its medical relevance, few studies were achieved to elucidate its peptidome. Thereupon, this study aims to reach a more detailed picture of the A.mauritanicus venom peptides using high-throughput mass spectrometry. To this end, two workflows were applied, the first one consists of a combination of top-down and bottom-up approaches to decipher the crude venom, while the second analyzes the venom fractions by the bottom-up approach. Data files were processed using Proteomediscover 2.2 for the analysis of crude venom and Peaks 7.5 for the fractions. The results of the first workflow showed that the A.mauritanicus venom encloses a complex mixture of 269 different compounds, the most abundant ones showed masses from 6185.92 to 7899.53 Da (53.89%), while both approaches' results allowed us to identify a total of 98 peptides. The highest percentage was represented by neuropeptides (87%). The results of LC-MS data from fraction analysis showed that the venom of A.mauritanicus is very complex, counting around 503 MWs ranging from 200 to 8695 Da. LC-MS/MS results allowed the identification of several toxins, which were mainly that targeting sodium (NaScTxs), potassium (KScTxs), and chloride (ClScTxs). Interestingly, this study allowed the identification of several neurotoxins such as Neurotoxin Os3 (P15225), Alpha-insect toxin Lqq3 (P01487), NeurotoxinBmK-II (P59360) and Alpha-mammal toxin Lqq5 (P01481). Besides these NaStxs, two ClScTxs, corresponding to chlorotoxin and chlorotoxin-like peptides were also isolated. This study highlighted the complexity and the molecular diversity of the toxic content of the Moroccan A.mauritanicus. This, in turn, will lead to a deeper understanding of the toxins' mechanism of action and will help uncover those with therapeutic potential.

Short oral

The cyclic cystine-knot motif is indispensable for the immunosuppressive activity of cyclotides

Roland Hellinger#, Edin Muratspahić#, Mina Vasileva#, Carsten Gründemann and Christian W. Gruber*, #
#Center for Pharmacology and Physiology, Medical University of Vienna, 1090 Vienna, Austria
$Institute for Infection Prevention and Hospital Epidemiology, Center for Complementary Medicine, Faculty of Medicine, University of Freiburg, Freiburg, 79111 Freiburg, Germany &Translational Complementary Medicine, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland *Corresponding author: christian.w.gruber@meduniwien.ac.at

'T20K' is well known for its anti-proliferative activity towards immune cells. The peptide has achieved therapeutic effects in the experimental encephalomyelitis autoimmune model which assigned T20K drug candidate status and enabled ongoing clinical investigations for multiple sclerosis therapy [1, 2]. T20K bioactivity on encephalomyelitic T-cells is interleukin-2 dependent, however, its mode of action is not fully explored. Thus mechanistic studies are needed that shed light on the biological function of the drug. In this study, we explored the structure-activity relationship of T20K, with respect to the importance of the prototypic cyclic cysteine knot structural motif for their immunosuppressive activity [3]. Partial or full reduction of the cystine-knot or incorrect folding of T20K resulted in a loss of function in proliferation experiments. Similarly, an acyclic analogue of T20K was inactive. The lack of immunosuppressive activity of all non-native T20K peptides appeared associated with the cell penetrating ability of cyclotides, since cellular uptake experiments demonstrated fast fractional transfer into the cytosol of human immune cells only for the native peptide. Therefore, structural analysis of inactive variants and active T20K using NMR spectroscopy were performed to achieve structure-function relationship data. Although the cyclic cystine knot truncated analogue had native-like confirmation, the backbone of the acyclic T20K was less rigid and the intramolecular hydrogen bond network was affected as compared to the native cyclic T20K. Our study highlights the cyclic cystine-knot motif is a unique bioactive scaffold which governs interactions with, and transport across cellular membranes as well as anti-proliferative activity. Cystine-knot peptides are often used for chemical design of stabilized research probes and peptide therapeutics, hence these observations could provide guidance for the design of novel cyclic cysteine-stabilized molecules.

References

1. Thell, K., Hellinger, R., Sahin, E., Michenthaler, P., Gold-Binder, M., Haider, T., Kuttke, M., Liutkeviciute, Z., Goransson, U., Grundemann, C., Schabbauer, G. and Gruber, C.W. Proc Natl Acad Sci U S A. 2016, 113 (15), 3960-5.
2. Thell, K., Hellinger, R., Schabbauer, G. and Gruber, C.W. Drug Discov Today. 2014,19 (5), 645-53.
3. Hellinger, R., Muratspahić, E., Devi, S., Koehbach, J., Vasileva, M., Harvey, PJ., Craik, DJ., Gründemann, C., and Gruber, CW. ACS Chem Biol, 2021, in press.

Short oral

Chemical strategies to target mucosal biofilms in patients with gastrointestinal disorders

Bernhard Jandl*, Markus Muttenthaler*
*Faculty of Chemistry, Institute of Biological Chemistry, University of Vienna, Vienna, Austria

Gastrointestinal disorders (GI), including inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS), affect 10–15% of the Western population and represent a substantial socio-economic burden to our society.[1] IBD and IBS are progressive diseases related to our lifestyle and the improper and overuse of antibiotics. Biofilm-forming bacteria are an adherent prokaryotic community embedded in a protective layer of extracellular substances and prone to antibiotic resistance.[2] Recently, it was reported that GI biofilms occur in ~60% of IBS patients and ~28% of IBD patients, yet little is known about their function and disease relevance.[3] Antimicrobial peptides (AMPs) of natural origin represent valuable alternatives for treating bacterial infections since they have retained and optimized their activity throughout evolution and triggered little or no resistance.[4] However, their full potential against biofilms has not been revealed yet. Our approach includes (i) synthesis and screening of AMPs from various natural sources for biofilm-specific activity, (ii) chemical strategies to optimize lead compounds as molecular probes and therapeutic candidates, and (iii) characterization of bacterial biofilm isolated from IBD and IBS patients. We want to report our key results from the screening for biofilm-specific therapeutic agents to treat mucosal biofilms in patients with GI disorders.

References

[1] M. R. Brown, P. Gilbert, J Appl Bacteriol 1993, 74 Suppl, 87s-97s; bM. Lang, M. Baumgartner, A. Rożalska, A. Frick, A. Riva, M. Jarek, D. Berry, C. Gasche, Int J Cancer 2020, 147, 2316-2326.

[2] H.-C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S. A. Rice, S. Kjelleberg, Nature Reviews Microbiology 2016, 14, 563-575.

[3] M. Baumgartner, M. Lang, H. Holley, D. Crepaz, B. Hausmann, P. Pjevac, D. Moser, F. Haller, F. Hof, A. Beer, E. Orgler, A. Frick, V. Khare, R. Evstatiev, S. Strohmaier, C. Primas, W. Dolak, T. Köcher, K. Klavins, T. Rath, M. F. Neurath, D. Berry, A. Makristathis, M. Muttenthaler, C. Gasche, Gastroenterology 2021, 161, 1245-1256.e1220.

[4] B. Bechinger, S. U. Gorr, J Dent Res 2017, 96, 254-260.

Short oral

Interfacing the cell functions with supramolecular stimuli

Stavroula Melina Sakellakou
Universität Wien

Nature works in well-defined cellular assemblies, like tissues and organs. The survival of organisms is strictly dependent on the perfect synchronization of all their biological processes in their complexity. The study of the interplay between cellular systems reveals complicated networks that structure intricate architectures coupled with balanced interactions. However, the field of cell-cell interactions is probably one of the most challenging in biochemistry. Researchers have tried to isolate each step in order to understand it and reproduce it in the laboratories in an efficient yet simpler way. For the moment stable contacts have been established among cells.[1] Their assembly is controlled with spatiotemporal precision[2] and their predefined biological functions have been isolated and regulated[3] as well. Nonetheless, until now a system that combines these assets and controls them precisely, independently, and simultaneously has not yet been established. In this context peptides have been playing a crucial role initiating or allowing cellular interactions, movements and specific protein expression. All of these are achieved upon interaction of specific peptides with their respective receptors. In this project, we aim to develop a cellular system that upon a peptide trigger can assemble in confined and well defined space and produce biopharmaceuticals of interest.

References

[1] Z. J. Gartner, C. R. Bertozzi, Proc. Natl. Acad. Sci. 2009, 106, 1–5.

[2] P. Shi, E. Ju, Z. Yan, N. Gao, J. Wang, J. Hou, Y. Zhang, J. Ren, X. Qu, Nat. Commun. 2016, 7, 1–9.

[3] B. Wang, J. Song, H. Yuan, C. Nie, F. Lv, L. Liu, S. Wang, Adv. Mater. 2014, 26, 2371–2375.

Short oral

Constraining peptides via cysteine stapling and cyclization

Julia Kriegesmann1, Thomas Schlatzer2, Kateryna Che1, Dennis Kurzbach1, Rolf Breinbauer2, Christian F. W. Becker1

1 University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria, 2 Graz University of Technology, Institute of Organic Chemistry, Stremayrgasse 9, 8010 Graz, Austria

We recently developed a new chemical method based on Pd-mediated Tsuji-Trost allylation to selectively introduce native prenylations into peptides and proteins.[1] Such prenylations are posttranslational modifications occurring on cysteine side chains, eventually leading to the association of modified proteins to specific membranes in cells.[2] Furthermore, we could demonstrate that this allylation reaction also allows the introduction of a variety of non-native cysteine modifications such as a fluorophore or affinity tags via the respective allylic carbonate reagents.[1] These compounds are easily accessible and sufficiently stable for long-term storage. Here, we report the modification of two or three cysteines within one peptide by bi- or trifunctional allylic carbonate reagents that allow peptide stapling, a concept developed to induce and/or stabilize certain peptide conformations, as well as bicyclization of peptides, respectively.[3] After successful introduction of a variety of staples into di-cysteine peptides, we envisioned secondary functionalization of the stapled peptides via the stapling moiety itself. Staples always carry one or more double bond due to the nature of the allyl carbonate starting material and these can be selectively targeted with additional probes such as fluorophores and affinity tags.

References

[1] T. Schlatzer, J. Kriegesmann, H. Schröder, M. Trobe, C. Lembacher-Fadum, S. Santner, A. V. Kravchuk, C. F. W. Becker, R. Breinbauer, J. Am. Chem. Soc. 2019, 141, 37, 14931-14937.

[2] L. Brunsveld, J. Kuhlmann, K. Alexandrov, A. Wittinghofer, R. S. Goody, H. Waldmann, Angew. Chem. Int. Ed. 2006, 45, 6622–6646.

[3] M. Pelay-Gimeno, A. Glas, O. Koch, T. N. Grossmann, Angew. Chemie Int. Ed. 2015, 54, 8896–8927.

Poster flash talk 1

Posttranslational regulation of the Survival Motor Neuron (SMN) complex

Claudia Cobos C., Filippo Savini, Christian F. W. Becker
Institute of Biological Chemistry, University of Vienna, Austria

Splicing is a crucial event in gene expression in which introns are removed and only coding exons remain. The Survival Motor Neuron (SMN) complex plays an essential role in the assembly of the spliceosomal small nuclear ribonucleoproteins (snRNPs) and the depletion of SMN protein is known to produce malfunction within the involved pathways, affecting human wellbeing. Multiple posttranslational modifications (PTMs) within the constituent protein of snRNPs have been described, however due to a lack of suitable methods to install this modification site-specifically, not much is known about their functions. In this context, we are focusing on the role of PTMs and their effect on the SMN complex. In particular, we recently synthesised, by solid phase peptide synthesis (SPPS), four peptide segments of SMN protein with different PTMs and used native chemical ligation (NCL) to join the four peptides together. Total chemical synthesis, using SPPS, provides full flexibility for modifications of SMN variants that will subsequently be incorporated into the SMN complex to perform in vitro studies to analyse the impact of these modifications on its function in UsnRNP assembly. Additionally, we seek to understand more about the factors required for stable complex formation during the splicing process. For this, we recently expressed the N-terminal portion of SmD1 protein, and we are currently using expressed protein ligation (EPL) to ligate different C-terminal peptides carrying variable numbers of symmetrically dimethylated arginines (sDMA). The information that we will obtain will help to shed light on the gene expression process.

Poster flash talk 2

Exploring IgM Assembly via Protein Semisynthesis

Leopold Dürrauer[1], Oliver Gajsek[1], Aleksandr Kravchuk[1], Tuan Nguyen[2], Johannes Buchner[2], Christian F.W. Becker[1]

[1]Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Austria; [2]TUM Department of Chemistry, Technical University of Munich, Germany; *Correspondence: christian.becker@univie.ac.at

Antibodies, also referred to as immune globulins (Ig), are vital components of the human immune system. Different classes of Igs are produced during various stages of infection. Of these classes, the multivalent, polymeric IgM represents the earliest response of the adaptive immune system. Canonically, immune globulins consist of two covalently linked light (L) and heavy (µ) chains, that form µ2-L2 subunits. In IgM, these subunits are further assembled into (µ2-L2) penta- or (µ2-L2)6 hexamers depending on in the presence or absence of an additional polypeptide, the J (joining) chain. This results in a complex of over 1.200 kDa with higher avidity than any other class of antibody. Previous studies have shown that extending the Cµ4 domain with a 18-aa C-terminal tailpiece (µtp) is sufficient and necessary for the formation of IgM hexamers [1], and that disulfide bond formation between C575 of two Cµ4tp fragments is a prerequisite for assembly [2]. We aim to further explore the underlying mechanism of IgM dimerization via the creation and investigation of µtp-extended Cµ4 domains. To achieve this goal, we have developed strategies [3] for the semi-synthetic production of modified Cµ4tp domains. Currently, we are focusing on the search for yet unknown interaction sites during IgM assembly, via the incorporation of photo-cross-linker-carrying, non-proteinogenic amino acids into our constructs. [4].

References

1. Müller, R., et al., PNAS, 2013. 110(25): p. 10183.
2. Pasalic, D., et al., PNAS, 2017. 114(41): p. E8575.
3. Conibear, A.C., et al., Chem. Soc. Rev., 2018. 47(24): p. 9046-9068.
4. Murale, D.P., et al., Proteome Sci., 2017. 15(1): p. 14.

Poster flash talk 3

Using chemical biology approaches to decipher chromatin ubiquitylation involving RNF168

Pauline Franz, Thierry Pignard, Beat Fierz
Laboratory of Biophysical Chemistry of Macromolecules, EPFL

Recognition, integration and propagation of post-translational modifications (PTMs) on histone proteins play a crucial role in the DNA damage response. In particular, the ubiquitylation cascade mediated by the E3 ubiquitin ligase RNF168 is central in promoting homologous recombination (HR) and nonhomologous end-joining (NHEJ) following DNA double-strand breaks. RNF168 is recruited to DNA damage sites by binding to ubiquitylated linker histone H1 [1], where it further ubiquitylates H2AK13-15 [2], thereby acting as reader and writer of chromatin PTMs. However, the exact mechanisms underlying RNF168 regulation remain elusive, as the limited availability of specifically poly-ubiquitylated H1 restricts mechanistic research on a molecular level. Here, we develop a chemical approach, employing both solution-based and resin-bound protein modification strategies, to poly-ubiquitylate H1, with site- and chain-length specificity. Using a combination of expressed proteins, chemical derivatisation, and in vitro reconstitution approaches, we tightly control and systematically vary the chromatin fibre modification state. Using such ubiquitylated 'designer chromatin', we dissect the interaction and downstream activity of RNF168. This will reveal the chromatin-state-dependent activity of RNF168 in DDR repair pathways on a molecular level.

References

[1] T. Thorslund, A. Ripplinger, S. Hoffmann, T. Wild, M. Uckelmann, B. Villumsen, T. Narita, T. K. Sixma, C. Choudhary, S. Bekker-Jensen, N. Mailand, Nature 2015, 527, 389–393.

[2] F. Mattiroli, J. H. A. Vissers, W. J. Van Dijk, P. Ikpa, E. Citterio, W. Vermeulen, J. A. Marteijn, T. K. Sixma, Cell 2012, 150, 1182–1195.

Poster flash talk 4

Deciphering the impact of posttranslational modification on function and structure of Heat shock pro

Oliver Gajsek1, Christian F. W. Becker1 and Anne C. Conibear2
1Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Währinger Straße 38, 1090, Vienna, Austria , 2School of Biomedical Sciences, University of Queensland, St Lucia QLD 4072, Brisbane, Australia

Elucidating the complex influences of posttranslational modifications (PTMs) on the function and properties of proteins is of paramount importance in order to understand biological processes on a molecular basis. Heat shock proteins (HSPs) play a pivotal role in retaining cellular function by assisting in the folding, transport, maintenance and degradation of proteins under stress and normal conditions.1 Specifically the ubiquitous heat shock protein 90 (Hsp90) is highly regulated by PTMs including phosphorylation, acetylation, SUMOylation, ubiquitination and S-nitrosylation.2 To examine the regulatory function of specific N- and C-terminally located PTMs on essential properties of Hsp90 including its ATPase activity, drug affinity and protein-protein interactions, we aim to generate site-selective modified homogenous variants of human Hsp90α by utilizing a versatile protein semi-synthesis strategy.3 Synthetic peptide segments are ligated to recombinantly expressed protein domains utilizing chemoselective ligation techniques, including native chemical ligation (NCL), diselenide-selenoester ligation (DSL) and serine threonine ligation (STL).4,5,6 We report the successful synthesis of several N- and C-terminal Hsp90 peptides bearing site-selective modifications. Using these N-terminal peptides we are able to generate modified Hsp90 N-terminal domain (NTD) variants utilizing a native chemical ligation based semi-synthesis strategy. Currently extend our approach to access modified NTD variants while, at the same time, working on C-terminal modification of full-length Hsp90. The generated site-selective modified Hsp90 variants will provide new insights into the regulatory function of PTMs on this pivotal cellular protein.

References

1. Schopf, F. H., Biebl, M. M. & Buchner, J. The HSP90 chaperone machinery. Nat. Rev. Mol. Cell Biol. 18, 345–360 (2017).
2. Backe, S. J., Sager, R. A., Woodford, M. R., Makedon, A. M. & Mollapour, M. Post-translational modifications of Hsp90 and translating the chaperone code. J. Biol. Chem. jbc.REV120.011833 (2020) doi:10.1074/jbc.REV120.011833.
3. Conibear, A. C., Watson, E. E., Payne, R. J. & Becker, C. F. W. Native chemical ligation in protein synthesis and semi-synthesis. Chem. Soc. Rev. 47, 9046–9068 (2018).
4. Dawson, P. E., Muir, T. W., Clark-Lewis, I. & Kent, S. B. Synthesis of proteins by native chemical ligation. Science 266, 776–779 (1994).
5. Zhang, Y., Xu, C., Lam, H. Y., Lee, C. & Li, X. Protein chemical synthesis by serine and threonine ligation. Proc. Natl. Acad. Sci. U. S. A. 110, (2013).
6. Kulkarni, S. S., Watson, E. E., Premdjee, B., Conde-Frieboes, K. W. & Payne, R. J. Diselenide–selenoester ligation for chemical protein synthesis. Nat. Protoc. 14, 2229–2257 (201

Poster flash talk 5

Cyclotides isolated from violet plants are inhibitors of human prolyl oligopeptidase

Jasmin Gattringer#, Olivier Eteme Ndogo#, Bernhard Retzl, Carina Ebermann, Christian W. Gruber*, and Roland Hellinger

Medical University of Vienna, Center for Physiology and Pharmacology 1090 Vienna Austria, #These authors contributed equally to this study, *Corresponding author: christian.w.gruber@meduniwien.ac.at

Protease inhibitors have well-established clinical application targeting enzymes such as the angiotensin converting enzyme. Yet there are still numerous proteases available as possible drug targets. Prolyl oligopeptidase (POP), a serine protease, has been discussed as a druggable target for inflammatory or neurological disorders including Parkinson's Disease. While small molecules still account for most lead candidates in this field, their lack of selectivity often hinders drug development. Cyclic peptides have appeared as an interesting alternative. To discover novel peptide enzyme inhibitors we resort to the exploration of traditional medicinal plants ethnopharmacological use. Here we demonstrate the identification and chemical characterization of peptides isolated from the Cameroonian plant genus Allexis and their biological activity for POP inhibition. Fluorescence-based POP inhibition assays identified inhibitory activities in samples of four species Allexis batangae, A. cauliflora, A. obanensis and A. zygomorpha in the range of 15 to 22 µg/mL. Peptidomic analysis identified the expression of cyclotides (cyclic cysteine-rich peptides) in these plants. A. cauliflora was selected for bioactivity-guided isolation of peptides combining preparative high performance liquid chromatography, protease inhibition assays and mass spectrometry analysis. Two peptides, denoted as alca 1 and alca 2 were isolated and shown to inhibit POP with IC50 values of 8.5 µM and IC50 4.4 µM, respectively. The peptides were de novo sequenced using chemical derivatization and mass spectrometry. With yet unknown amino acid motifs in alca-type inhibitors, which are the first bracelet-type cyclotides inhibiting POP, our data provide further insight into the diversity of protease inhibitors of the cyclotide family. The present study emphasizes that nature-derived peptides and in particular cyclotides are a valuable starting point to development novel POP targeting peptide therapeutics.

Poster flash talk 6

Semisynthesis of α-Synuclein (αS) with nPTM implicated in Neurodegenerative Disease

Judith Görlich[1], Somnath Mukherjee[1] and Christian F. W. Becker[1]*

[1] University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria; *Corresponding author: christian.becker@univie.ac.at

α-synuclein (αS) aggregation is known to be associated with Parkinson's disease (PD) and other neurodegenerative disorders generally termed as synucleinopathies.[1] The high number of lysine residues (15) in this relatively short 140 amino acid long, intrinsically disordered presynaptic protein makes it an obvious target for electrophilic metabolites, leading to the formation of advanced glycation end (AGE) products. Intriguingly, AGEs were found to be present in the Lewy bodies (LB), one of the prominent pathophysiological features of PD, with enhanced levels in the frontal cortex of PD brains.[2] Especially for a long-lived protein, such as αS in combination with its high lysine content, the propensity of formation of the above-mentioned AGEs leading to aggregation and thus dysfunction seems to be a plausible route towards neurodegenerative etiologies. Since lysine residues are the common attachment point for αS glycation as well as for ubiquitination, which often leads to its proteasomal degradation. Here we try to understand the interplay between these two different modes of posttranslational modifications. Although a most recent publications report that glycation primarily affects the N-terminus of αS in animal models, we will take a closer look at the individual lysine residue level, to unravel the correlation between the glycated lysine residue on the proteasomal degradation of homogeneously modified αS.[3] To this end, we employ expressed protein ligation (EPL)[4] to access site-specifically Nε-(carboxyethyl)lysine (CEL) modified αS, to generate a library of homogeneously glycated αS, where the site(s) of modification spans the N-terminal domain (K6, K10, K12, K21, K23). These modified αS variants will be tested in degradation assays to determine their effect on processing in the ubiquitin proteasomal system (UPS).[5,6]

References

[1] N. Plotegher, L. Bubacco, Ageing Res. Rev., 2016, 26, 62-71.

[2] I. Sirangelo, C. Iannuzzi, Int. J. Mol. Sci. 2021, 22(12), 6609.

[3] T. Abeywardana, Y. H. Lin, R. Rott, S. Engelender, M. R. Pratt, Chem. Biol. 2013, 20(10), 1207.

[4] R. E. Thompson, T. W. Muir, Chem. Rev., 2020, 120, 6, 3051-3126.

[5] A. Oueslati, M. Fournier, HA. Lashuel, Prog Brain Res., 2010, 183, 115-45.

[6] H. A. Lashuel, C. R. Overk, A. Oueslati, E. Masliah, Nat Rev Neurosci, 2013, 14(1), 38-48.

Poster flash talk 7

Evaporation-induced self-assembly of small peptide-conjugated silica nanoparticles

Cornelia von Baeckmann, Guilherme M. D. M. Rubio, Ann-Jacqueline Herbst, Hanspeter Kählig, Dennis Kurzbach, Michael R. Reithofer and Freddy Kleitz
Department of Inorganic Chemistry – Functional Materials, Institute of Inorganic Chemistry, Department of Organic Chemistry, Department of Biological Chemistry - University of Vienna

Self-assembly directs chaotic units or particles into long-range ordered structures or patterns due to particular supramolecular interactions among the building elements. Unique evaporation-induced self-assembly (EISA) of four different silica nanoparticle systems was realized by functionalizing the particle surface with a small peptide. First, covalent peptide-silica coupling was studied in-depth, starting with a single amino acid (L-serine) grafting and progressing to specific small peptides (up to four amino acids). The particle type (MCM-48-type MSNs, solid nanoparticles, newly designed virus-like nanoparticles) and the amount of peptide linked to their surface did not affect the observed self-assembly. This path provides a good prospect for the design of future advanced drug delivery systems, organized hierarchical sorbents, and nanocatalyst assemblies.

References

C. von Baeckmann, G. M. D. M. Rubio, H. Kählig, D. Kurzbach, M. R. Reithofer, F. Kleitz, Angew. Chem. Int. Ed. 2021, 133, 22882

Poster flash talk 8

Tailor-made ubiquitin chains for targeting the UPS or autophagy

Susanne Huhmann, Marina Kujundzic, Gerhard Niederacher, Christian F. W. Becker
University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria

Defined ubiquitin (Ub) chains are unique tools in deciphering complex degradation signals as the exact molecular composition of Ub tags is critical for recognition by parts of the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway. Over the past decades, various methods have been developed to assemble fully defined Ub chains and Ub-modified proteins including enzymatic approaches,[1] use of genetic code expansion,[2] or intein fusion constructs[3] that allow for chemoselective ligation chemistry; latter also in combination with solid-phase peptide synthesis (SPPS) to obtain Ubs with a large set of modifications.[4] Here, we present the synthesis of activated/mutated recombinant Ubs that are assembled to defined Ub chains by thiol-ene chemistry (TEC) providing isosteric linkages to the native lysine-glycine isopeptide bond, (Figure 1). We focused first on preparation of diubiquitin (diUb) linked via position 48 or 63. The basic Ub building blocks were generated in large amounts by expression of Ub-intein fusion constructs in E. coli. Subsequent cleavage yields the building blocks as C-terminal thioester, hydrazide, allylamide or propargylamide. Apart from that, we synthesized Ub as C-terminal hydrazide in one piece by microwave-assisted SPPS using special side-chain protected and pseudo-proline building blocks. For the assembly of Ub chains longer than two Ub units via TEC, we will make use of phenacyl (PAc) protection of the cysteine residue.[5] Selective removal of PAc allows here to form Ub chains in a defined manner.

References

[1] M. A. Michel, D. Komander, P. R. Elliott, Methods Mol. Biol. 2018, 1844, 73-84.

[2] M. Fottner, A.-D. Brunner, V. Bittl, D. Horn-Ghetko, A. Jussupow, V. R. I. Kaila, A. Bremm, K. Lang, Nat. Chem. Biol. 2019, 15, 276-284.

[3] E. M. Valkevich, R. G. Guenette, N. A. Sanchez, Y.-c. Chen, Y. Ge, E. R. Strieter, J. Am. Chem. Soc. 2012, 134, 6916-6919.

[4] a) G. B. A. van Tilburg, A. F. Elhebieshy, H. Ovaa, Curr. Opin. Struct. Biol. 2016, 38, 92-101; b) P. Gopinath, S. Ohayon, M. Nawatha, A. Brik, Chem. Soc. Rev. 2016, 45, 4171-4198.

[5] a) M. Matveenko, S. Hackl, C. F. W. Becker, ChemistryOpen 2018, 7, 106-110; b) S. Mukherjee, M. Matveenko, C. F. W. Becker, in Expressed Protein Ligation: Methods and Protocols (Ed.: M. Vila-Perelló), Springer US, New York, NY, 2020, pp. 343-358.

Poster flash talk 9

INVESTIGATION OF BIOLOGICAL ACTIVITY OF BIOACTIVE PEPTIDE COMPLEX "TESTONORM" IN VITRO

Yu.A. Khochenkova1, D.A. Khochenkov1, E.R.Safarova2, N.A.Bastrikova2*
1. FSBI “N.N. Blokhin National Medical Research Center for Oncology”, Ministry of Health of the Russian Federation, Moscow, Russia 2. PeptidPro LLC, Moscow, Russia

Recent attention to tissue peptidomes reflects importance of polypeptides in pathophysiology and consequently open the possibility to use tissue-derived peptides as drugs for functional loss recovery. In early studies Testonorm® (TN, bovine testis-derived peptides complex) demonstrated an ability to restore male fertility in mice after radiation damage. The objective of this study was to assess biological activity of TN after oxidative and toxic damages in testicular tissue explants and cell cultures. As the main mechanisms underlying male infertility are oxidative stress and DNA damage in-vitro models with induction of reactive oxygen species (ROS): cisplatin (cisPt)-DNA damage and H2O2-induced lipid peroxidation in porcine (PTP) and bovine (TEB) testicular cell cultures were used. TN activity was assessed by MTT-assay and was compared to L-carnitine (LC) activity as a positive control. TN effects were also examined on PTP and TEB cell viability and migration by wound healing assay (WHA) and primary tissue explants. Results. In both TEB and PTP cell lines the application of 100 mM cisPt or 5 µM H2O2 induced an oxidative stress, reducing the number of live cells to 12-25%. Co-cultivation with TN (0,1-2 µg/ml) had a dose-dependent protective effect on viability: percent of live cells increased to 92–97% in each cell line in the presence of one of ROS agents. LC on contrary did not protect cell cultures from oxidative stress. TN significantly (P<0.05) improved migration activity of TEB and PTP cells in all experimental layouts (100 mM cisPt or 5 µM H2O2): 51.9–81.5% (TN) vs. 31.1–37.9% (LC) relative to intact cells in WHA. TN at 0.1 µg/ml also increased the proliferation of cells from tissue organoids 2.2 times, observed as the growth area around the tissue explants with respect to intact control (P<0.05). Conclusions: a new drug Testonorm (a tissue-derived bioactive peptides complex) demonstrates anti-oxidative, cell proliferative and migration activities in-vitro.

Poster flash talk 10

Development of oral gut-specific peptide drug leads

Thomas Kremsmayr and Markus Muttenthaler

University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry

Orally administered gut-stable peptides present a promising new drug class for gut-specific, non-systemic interaction with luminally accessible gut receptors [1-3]. Such compounds must however resist an evolutionarily highly optimized machinery of endo- and exopeptidases that readily hydrolyse peptide bonds under acidic (stomach) to neutral (intestine) pH conditions. Few chemically developed or nature-derived peptides fulfil these criteria and the structural features conferring these exceptional stability properties remain poorly understood. Broadly varying stability assays and conditions used to characterize peptide stabilities limit such studies as well as comparison across studies [4]. Considering the wide implications that oral and gut-specific peptide therapeutics would have on patients with gastrointestinal disorders and peptide drug development, we systematically investigated approaches to study and enhance peptide gut stability. Here, we present guidance and a robust comparative framework for the design and analysis of gut-stable peptides. Covered examples include a broad structural range of disulfide-rich natural scaffolds, neuropeptides, approved peptide drugs and synthetic modification approaches to improve stability such as scaffold grafting, cyclization and backbone alteration strategies. We further exemplify the application of these concepts in the development of a first-in-class, potent series of therapeutic leads for the gut-specific treatment of chronic abdominal pain.

References

[1] Muttenthaler, M.; King, G. F.; Adams, D. J.; Alewood, P. F., Nat. Rev. Drug Discov. 2021.

[2] Furness, J. B.; Rivera, L. R.; Cho, H. J.; Bravo, D. M.; Callaghan, B., Nat. Rev. Gastroenterol. Hepatol. 2013, 10 (12), 729-40.

[3] Busby, R. W.; Bryant, A. P.; Bartolini, W. P.; Cordero, E. A.; Hannig, G.; Kessler, M. M.; Mahajan-Miklos, S.; Pierce, C. M.; Solinga, R. M.; Sun, L. J.; Tobin, J. V.; Kurtz, C. B.; Currie, M. G., Eur. J. Pharmacol. 2010, 649 (1-3), 328-35.

[4] Cavaco, M.; Andreu, D.; Castanho, M., Angew. Chem. Int. Ed. 2021, 60 (4), 1686-1688.

Poster flash talk 11

Triggering Self-assembly and Function of Yeasts by Light Stimuli

Terézia Morávková, André Oliveira Sequeira, Stavroula-Melina Sakellakou, Davide Bonifazi*
Universität Wien

Yeasts are known as a microbial factory that can produce recombinant proteins as pharmaceuticals, so-called biopharmaceuticals. Yeast is a single monocellular microorganism and its mating reaction is very important for cell-cell communication studies.[1] The fission yeast S. pombe possesses two haploid mating cells P and M cells, that can be differentiated under nitrogen starvation. The M type cells express the Mam2 receptor, which recognizes the P-factor released from P cells.[2] Constructing multicellular assemblies to form a tissue or aggregate with controlled cell-cell interaction is remaining a great challenge. To create the irreversible controlled cell-cell assembly there has been some techniques as modifying the surfaces with single-stranded DNA molecules that are complementary to each other, or using highly specific biotin-streptavidin interaction. Lately, the focus has been moved to create reversible cell-cell interaction. For this purpose, the supramolecular interactions has been used as photo-switchable azobenzene with β-cyclodextrin.[3-5] In our project we are focusing on Mam2 receptor and P-factor interaction. We are using only one-type of yeast that are genetically modified to express the Mam2 receptor independently of the external conditions. Our idea is to modify the P-factor, that is 23 amino acid peptide, in order to attach a motive that can trigger self-assembly or function as production of biopharmaceuticals by external stimuli. The modified P-factor-like-peptide has to have similar interaction behaviour to the Mam2 receptor as the natural P-factor, therefore most of the work is currently focused on this topic.

References

[1] N. Ferrer-Miralles, J. Domingo-Espín, J. Corchero, E. Vázquez, A. Villaverde, Microb. Cell Fact. 2009, 8, 17.

[2] K. Kitamura, C. Shimoda, EMBO J. 1991, 10, 3743–3751.

[3] G. ZJ, B. CR, Proc. Natl. Acad. Sci. U. S. A. 2009, 106, 4606–4610.

[4] D. Sarkar, J. A. Spencer, J. A. Phillips, W. Zhao, S. Schafer, D. P. Spelke, L. J. Mortensen, J. P. Ruiz, P. K. Vemula, R. Sridharan, S. Kumar, R. Karnik, C. P. Lin, J. M. Karp, Blood 2011, 118, 184–191.

[5] P. Shi, E. Ju, Z. Yan, N. Gao, J. Wang, J. Hou, Y. Zhang, J. Ren, X. Qu, Nat. Commun. 2016 71 2016, 7, 1–9.

Poster flash talk 12

Exploring a natural peptide-receptor interface to control cell assembly and function in yeast cells

André Oliveira Sequeira
Vienna University

Cellular communication, essential for the basis of life, is a fundamental tool for the understanding of biological processes and applicable in the development of therapeutic techniques. Cells can proliferate if they receive and process information from the extracellular environment, allowing their metabolic regulation. These signals can be achieved with direct extracellular contacts, due to the presence of a complex array of macromolecules on the cellular surface. Those can occur via intermolecular non-covalent interactions between different cells, that can be regulated, leading to their dynamic assembly. [1] Even though artificial reversible cellular assemblies were accomplished in literature[2-3], no advances were obtained in harnessing such process to control a cellular function. With the aim of conjugating a controlled cellular assembly linked to a cellular function, we developed a model system based on fission yeasts. This unicellular organism was chosen because it contains a specific peptide ligand-GPCR receptor interaction [4] , the P-factor Mam2 interface, involved in mating intracellular signaling. The peptide P-factor can be used on one hand as an anchoring point to mimic the intercellular contacts occurring in the surface of mammalian cells, and secondly exploited in the triggering of a specific function, namely a production of a biopharmaceutical of interest. With this we can establish control over the organization and function of cellular aggregates using two independent triggers, without modifying covalently the cell surface, but instead exploring receptors present in the extracellular space.

References

[1] Armingol, E., Officer, A., Harismendy, O., Lewis, N., Nat. Rev. Genet., 2020, 22, 71-88

[2] Gartner, Z. J., Bertozzi, C. R., Proc. Natl. Acad. Sci. USA, 2009, 106, 4606–4610.

[3] Shi, P., Ju, E., Yan, Z., Gao, N., Wang, J., Hou, J., Zhang, Y., Ren, J., Qu, X., Nat. Commun., 2016, 7, 13088.

[4] Imai, Y., Yamamoto, M., Genes Dev., 1994, 8, 328–338.

Poster flash talk 13

Development of oxytocin receptor tracers for imaging applications

M. Perisic*, P. Kalaba*, E. Keimpema**, C.W. Gruber***, M. Muttenthaler*
* University of Vienna, Department of Biological Chemistry, ** Medical University of Vienna, Center for Brain Research, *** Medical University of Vienna, Center for Physiology and Pharmacology

The oxytocin receptor (OTR) is a G protein-coupled receptor that mediates various physiological functions including reproduction and social behaviour.[1, 2] It is an attractive target for the exploration and therapy of a number of high-profile disorders including cancer, pain, and autism.[3, 4] The design of selective ligands to investigate and visualise OTR is however challenging as OTR shares >80% extracellular sequence homology with the closely related vasopressin receptors.[5] There is also a lack of selective probes (e.g., OTR-specific antibodies) to study OTR expression at the protein level. In this work, we describe the rational design, synthesis, and pharmacological evaluation of a series of peptide tracers for the visualization of OTR. By applying solid phase peptide synthesis and liquid phase labelling methods, we produced a series of OTR-targeting peptide tracers equipped with functional labelling groups such as biotin, fluorophores, near-infrared dyes, and positron emission tomography chelators. We determined the binding affinities of these tracers at OTR and the three closely related vasopressin receptors via radioligand displacement assays and identified a promising lead with a 50-fold selectivity for human OTR. Interestingly, we also demonstrated that linker design could be used to tune the selectivity for OTR. The results of the first ligand series were then used for the design and synthesis of a second series, whose pharmacology is to be determined. These tracers hold a powerful application scope for several in vitro and in vivo studies including positron emission tomography, near-infrared, and fluorescence based imaging techniques that will advance our understanding of the role of OTR in health and disease.

References

[1] C. S. Carter, W. M. Kenkel, E. L. MacLean, S. R. Wilson, A. M. Perkeybile, J. R. Yee, C. F. Ferris, H. P. Nazarloo, S. W. Porges, J. M. Davis, J. J. Connelly and M. A. Kingsbury, Pharmacol. Rev., 2020. 72: p. 829-861.

[2] R. Hurlemann and V. Grinevich, Behavioral Pharmacology of Neuropeptides: Oxytocin, 2018.

[3] H. Liu, C. W. Gruber, P. F. Alewood, A. Möller and M. Muttenthaler, Oncogene, 2020. 39: p. 5917-5932.

[4] M. Busnelli and B. Chini, Curr. Top. Behav. Neurosci., 2018. 35: p. 3-29.

[5] M. Muttenthaler, Å. Andersson, I. Vetter, R. Menon, M. Busnelli, L. Ragnarsson, C. Bergmayr, S. Arrowsmith, J. R. Deuis, H. S. Chiu, N. J. Palpant, M. O'Brien, T. J. Smith, S. Wray, I. D. Neumann, C. W. Gruber, R. J. Lewis and P. F. Alewood, Sci. Signal., 2017. 10: p. 3398.

Poster flash talk 14

Photoactivatable probes to study long-term memory formation

Konstantin Raabe, Markus Muttenthaler

Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Währinger Straße 38, 1090, Vienna, Austria

Memory formation is a crucial neurological process that is not only necessary for adaptive survival but also determines an individual's personality, wellbeing and mental health.[1] The mechanisms responsible for memories, however, are not fully understood. A growing body of evidence suggests increased synaptic strength based on experience-dependent activity patterns, termed long-term potentiation (LTP), as fundamental mechanism for long-term memory formation.[2] Different stimuli can trigger a multitude of intracellular signaling cascades that lead to occurrence of LTP and ongoing research implicates the necessity of de novo or altered protein synthesis for memory to develop.[3] To understand the intracellular machinery, the structural elucidation and identification of memory-proteins is critical and could provide invaluable insights into LTP. Considering that memory formation occurs in a specific brain area at a specific time[4] and considering that over 10,000 proteins regulating physiological processes at any given time,[5] it is extremely difficult to distinguish between the production of memory-proteins and other, memory-unrelated, proteins.[3b] We are therefore developing photocontrollable molecular probes[6] for light-activated bioorthogonal non-canonical amino acid tagging (laBONCAT) that provide the required spatiotemporal resolution for the identification of memory proteins.[7] In addition to probe design and synthesis, the project includes photochemical probe characterization as well as in vitro and in vivo evaluation. In collaboration with the research group of A/Prof. Sadegh Nabavi at the Danish Research Institute of Translational Neuroscience at Aarhus University, Denmark, we will apply these probes in memory-specific in vivo experiments.

References

[1] R. Bisaz, A. Travaglia, C. M. Alberini, Psychopathology 2014, 47, 347-356.

[2] E. R. Kandel, Science 2001, 294, 1030-1038.

[3] aA. Asok, F. Leroy, J. B. Rayman, E. R. Kandel, Trends Neurosci. 2019, 42, 14-22; bE. Klann, J. D. Sweatt, Neurobiol. Learn. Mem. 2008, 89, 247-259.

[4] M. Bocchio, S. Nabavi, M. Capogna, Neuron 2017, 94, 731-743.

[5] O. Y. Naumova, M. Lee, S. Y. Rychkov, N. V. Vlasova, E. L. Grigorenko, Child. Dev. 2013, 84, 76-88.

[6] A. Bardhan, A. Deiters, Curr. Opin. Struct. Biol 2019, 57, 164-175.

[7] S. M. Adelmund, E. R. Ruskowitz, P. E. Farahani, J. V. Wolfe, C. A. DeForest, ACS Chem. Biol. 2018, 13, 573-577.

Poster flash talk 15

Biomimetic silica particles as a vaccine delivery platform

Daniela Reichinger (1), Mirjana Drinic (2), Joshua Tobias (2), Manuel Reithofer (3), Meder Kamalov (1), Beatrice Jahn-Schmid (3), Ursula Wiedermann-Schmidt (2), Christian F. W. Becker (1)

(1) University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Straße 38, 1090, Vienna, Austria; (2) Medical University of Vienna, Department of Pathophysiology, Infectiology and Immunology, Institute of Specific Prophylaxis and Tropical Medicine, Kinderspitalgasse 15, 1090 Vienna, Austria; (3) Medical University of Vienna, Department of Pathophysiology and Allergy Research, Währinger Gürtel 18-20, 3Q, 1090 Vienna, Austria

Peptide-based subunit vaccines provide an excellent safety profile and are easy to produce as well-defined modalities. However, they often elicit a weak immune response which is why they require the use of additional stimulants to obtain the desired effects.[1] Over the past years, increasing focus has been put on biomimetic silica formation for biotechnological applications as well as in biomedical research.[2,3] Peptide-based systems based on the RRIL motif derived from silaffin proteins in diatoms are employed for the formation of morphologically diverse, nanostructured silica particles in vitro under mild, biomimetic conditions.[4,5] In this work, three different antigens are covalently linked to four different peptides containing the RRIL motif via disulfide bonds. These antigen-RRIL conjugates effectively precipitate silica and the morphology of the resulting particles can be characterized by electron microscopy. In addition, RRIL-based silica particles induce the formation of neutrophil extracellular traps (NETs) at a similar level as the well-established adjuvant alum as determined by a NETosis assay and via immunofluorescence.[6] Currently, the immunogenicity of two different silica particles loaded with two model antigens (birch pollen and breast cancer) is tested in BALB/c mice to obtain informations about the induction of IgG antibodies and cytokines after re-stimulation of splenocytes and lung cells. The data readout is expected for the beginning of November. Overall, the described conjugation approach is the first step towards a versatile vaccine adjuvant platform that can be efficiently loaded with a large variety of peptide antigens but also other (bio-)molecules that leads to the stimulation of an immune response. Acknowledgements We gratefully acknowledge funding from Austria Wirtschaftsservice (aws, project SILAVAX to CFWB) and University of Vienna as part of a uni:docs fellowship to DR.

References

[1] Purcell, A. W. et al., J. Nat. Rev. Drug Discovery 2007, 6 (5), 404–414.

[2] Lechner, C. C.; Becker, C. F. W., Marine Drugs 2015, 13 (8), 5297-5333.

[3] Bialas, F.; Reichinger, D.; Becker, C. F. W., Enzyme and Microbial Technology 2021, 150, 109864.

[4] Kamalov, M.; Becker, C. F. W. et al., ChemNanoMat 2018, 4 (12), 1209-1213.

[5] Lechner, C. C.; Becker, C. F. W., Bioorg. Med. Chem. 2013, 21 (12), 3533–3541.

[6] Rada, B., Methods Mol Biol. 2019, 1982, 517-528.

Poster flash talk 16

Plant-derived peptides as ligand of the oxytocin receptor for the treatment of inflammatory bowel disease

Bernhard Retzl (1), Carsten Gründemann (2) and Christian W. Gruber (1)

(1) Medical University of Vienna, Center for Physiology and Pharmacology, Schwarzspanierstr. 17, 1090 Vienna, Austria (2) University of Basel, Translational Complementary Medicine, 4056 Basel, Switzerland

Currently, drugs used to treat inflammatory bowel disease (IBD) are small molecules, e.g. immunosuppressive drugs like azathioprine or large biologics, e.g. infliximab, a monoclonal antibody against TNF-α. Both classes of drugs cannot permanently cure IBD and often have severe side effects. Therefore, we explore an emerging class of molecules with drug-like properties, i.e. circular peptides called cyclotides, as an alternative for IBD treatment. Cyclotides are plant-derived ultra-stable peptides that were discovered during an ethnopharmacological exploration based on the traditional application a decoction of Oldenlandia affinis (Rubiaceae) as uterotonic remedy. Their activity is thought to be mediated via modulation of the oxytocin receptor, which is responsible during birth to induce myometrial contractions. The oxytocin receptor is a G protein-coupled receptor activated by its endogenous ligand oxytocin a nonapeptide synthesized in the supraoptic and paraventricular nucleus of the hypothalamus. Recent studies demonstrate that this may be a new target for IBD therapy: activation of the oxytocin receptor ameliorates clinical and histological damage scores in animal models of inflammatory bowel disease [1]. Presumable, the beneficial effects are at least partly the consequence of an oxytocin receptor mediated polarization of macrophages to the anti-inflammatory phenotype [2]. Peptides, like oxytocin, are rapidly degraded in the gastrointestinal tract and can therefore not be administered via the oral route. Consequently, it is necessary to identify oxytocin receptor ligands with improved stability. We hypothesize cyclotides are gut-stable peptides able to modulate oxytocin receptor signaling in the intestine and its associated immune cells. A plant particularly rich in cyclotides is Viola tricolor. This study will explore the diversity, stability and pharmacological properties of cyclotides isolated from Viola tricolor and test their effects on the polarization of

References

1. Iseri SO, Sener G, Saglam B, Gedik N, Ercan F, Yegen BC. Peptides. 2005;26(3):483-91.

2. Tang Y, Shi Y, Gao Y, Xu X, Han T, Li J, et al. Clin Sci (Lond). 2019;133(18):1977-92.

Poster flash talk 17

Synthesis of Homogeneously Glycosylated Soluble Fas Ligand Variants

Alanca Schmid, Maximilian Schrems, Claudia Bello, Christian F.W. Becker1*
Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria.
University of Florence, Department of Chemistry "Ugo Schiff", Laboratory of Peptide and Protein Chemistry and Biology-PeptLab, Fiorentino, Italy

Glycosylation is one of the most abundant and complex posttranslational modifications of proteins. Since its alteration is frequently observed in many pathogenic conditions[1-2], it is crucial to be able to investigate the impact of individual glycan structures at specific positions in proteins and access to homogeneously glycosylated protein variants is urgently needed[3]. To obtain such homogeneously N-glycosylated variants of soluble Fas ligand (sFasL), an important but yet not well understood player in cancer proliferation, a chemoenzymatic approach was chosen. The protein is assembled from three segments, each of them synthesized by solid phase peptide synthesis (SPPS), using native chemical ligation (NCL) and diselenide-selenoester ligation (DSL)[4]. The C-terminal peptide, containing two native N-glycosylation sites, will be equipped with an N-terminal selenocysteine for DSL and subsequent deselenization and a polyethyleneglycol (PEG) chain linked via a photocleavable auxiliary to enhance peptide solubility[5]. An activated N-glycan core structure, isolated from egg yolk[6-7], can be transferred to the peptide using endoglycosidase mutants. Subsequent elongation with suitable glycosyltransferases would increases the number of accessible glycopeptide variants. In this way, several N-glycan variants of soluble Fas ligand will be obtained and subjected to further chemical and biological investigation. Here, the latest results on the synthesis of all three peptide segments, chemoenzymatic glycosylations tests and assembly of the full-length protein will be presented.

References

[1] Ohtsubo, K., Marth, J. D., Cell, 2006, 126, 855-867.

[2] Stowell, S. R., Ju, T., Cummings, R. D., Annual Review of Pathology: Mechanisms of Disease, 2015, 10, 473-510.

[3] Kajihara, Y., Murakami, M., Unverzagt, C., in Glycochemical Synthesis, 2016, pp. 263-292.

[4] Chisholm, T. S., Kulkarni, S. S., Hossain, K. R., et al., Journal of the American Chemical Society, 2020, 142, 1090-1100.

[5] Kerul, L., Schrems, M., Meli, R., et al., unpublished results.

[6] Seko, A., Koketsu, M., Nishizono, M., et al., Biochimica et biophysica acta, 1997, 1335, 23-32.

[7] Sun, B., Bao, W., Tian, X., et al., Carbohydrate Research, 2014, 396, 62-69.

Poster flash talk 18

Synthesis of a Selenium-based Ligation-Auxiliary for Traceless Peptide Ligations

Maximilian Schrems 1, Claudia Bello 2 and Christian F.W. Becker 1
1 University of Vienna, 2 University of Florence

Chemoselective ligations such as native chemical ligation (NCL)[1] and diselenide-selenoester ligation (DSL)[2] are incredibly valuable tools for peptide chemists to join two unprotected peptide chains under mild conditions, leaving no ligation scar. Historically, two requirements must be fulfilled to use NCL: The respective peptides need to be equipped with a C-terminal thioester on one and an N-terminal cysteine on the other peptide segment. [1] For NCL reactions where these requirements cannot be met, alternative strategies have been developed, including the use of radical desulfurization of cysteine or other β-mercaptoamino acids or cleavable ligation auxiliaries.[3-4] Lately, the use of N-terminal selenocysteine residues in combination with C-terminal selenoesters in diselenide-selenoester ligations (DSL) led to improved reactions kinetics, higher yields and more flexibility with respect to C-terminal amino acids carrying the selenoester even at very low peptide concentrations.[2] Here we present the combination of two of these concepts, namely a photocleavable selenium based ligation auxiliary to provide a highly versatile solution for DSL at almost any ligation site. Starting from the three-component Petasis reaction, synthesis of this auxiliary could be realized with satisfactory yields in only 5 steps. Employing standard SPPS coupling and deprotection reagents, the auxiliary could be attached to a peptide, ready for ligation. Preliminary results show the ligation of short test peptides and further photocleavage indicates the successful removal of the auxiliary. In summary, we developed a concise route for the synthesis of a selenium-based, photocleavable ligation auxiliary for native chemical ligation. Initial experiments show promising results with respect to kinetics and yields, especially when compared to the respective sulfur-containing auxiliary.

References

[1] P. E. Dawson, T. W. Muir, I. Clark-Lewis, et al., Science 1994, 266, 776-779.
[2] T. S. Chisholm, S. S. Kulkarni, K. R. Hossain, et al., J. Am. Chem. Soc. 2020, 142, 1090-1100.
[3] A. C. Conibear, E. E. Watson, R. J. Payne, et al., Chem. Soc. Rev. 2018, 47, 9046-9068.
[4] C. Bello, S. Wang, L. Meng, et al., Angew. Chem. Int. Ed. 2015, 54, 7711-7715.

Poster flash talk 19

Discovery of cyclic peptide analgesic to target the κ-opioid receptor

Edin Muratspahić, Nataša Tomašević and Christian W. Gruber*
Medical University of Vienna, Center for Physiology and Pharmacology, 1090 Vienna, Austria;
* corresponding author

The 21st century opioid crisis has emerged, attributable to over-prescription and misuse of opioid analgesics, followed by rising number of overdose deaths and diagnoses of opioid-use disorder [1]. In this regard, the kappa opioid receptor (KOR), a prototypical G protein-coupled receptor, is appreciated as an alternative therapeutic target for the development of safer pain medications devoid of deleterious side effects commonly associated with the μ-opioid receptor [2]. This has prompted efforts to discover novel KOR ligands by screening diverse sets of circular plant peptide libraries for affinity to KOR. Several hits, including the sunflower trypsin inhibitor peptide, were then used to design optimized ligands by grafting different dynorphin A and difelikefalin-like analogues into the cyclic peptide scaffold. This approach yielded helianorphin-19, a selective, full agonist of the KOR with affinity and potency in a nanomolar range. Moreover, helianorphin-19 significantly enhanced the peripheral analgesic activity in a mouse model of visceral pain, with no impairment of motor coordination or induce central analgesia. This study highlights the potential of plant-derived cyclic peptides as valuable starting points for designing potent, stable and safer peptide ligands for therapeutic targeting of KOR in the periphery.

References

[1] M. Spetea, H. Schmidhammer, Molecules 2020, 25, 5658.

[2] E. Darcq, B. L. Kieffer, Nature Reviews Neuroscience 2018, 19, 499-514.

Poster flash talk 20

Using Organ on Chip (OoC) technology for development of oral peptide therapeutics

Verena Stoeger1, Charalampos Pitsalidis1, Douglas C. van Niekerk1, Chrysanthi-Maria Moysidou1, Alexander J. Boys1, Aimee M. Withers1, David Bulmer2 and Róisín M. Owens1
1Department of Chemical Engineering and Biotechnology, University of Cambridge, UK, 2Department of Pharmacology, University of Cambridge, Cambridge, UK

Peptides, like synthetic insulin for treatment of type I diabetes are of great importance in medical therapeutics. Invasive delivery of peptide drugs via injections promotes efficacy by avoiding gastrointestinal degradation, however this route is often accompanied by a low patient's compliance. On contrary, non-invasive oral administration of drug peptides is limited due to a low bioavailability induced by digestive enzymes, gut barrier permeability and microbial transformations in the gut. As the gastrointestinal tract also hosts a high number of therapeutically relevant receptors like for treatment of inflammatory bowel disease, there is an urgent need for optimizing peptide formulations for oral delivery.[1] Translation of outcomes generated in animal models and simplistic 2D models to humans within pre-clinical phases is limited. OoC technologies are aiming for to build improved human in vitro models for getting better predictions on drug efficacy. Recently, we developed a 3D bioelectronic transmembrane device[2], which allows non-invasive monitoring of gut barrier integrity. Combining principles of Tissue Engineering, Material Sciences and Organic Bioelectronic technology enable intimate cell-electrode coupling. For this purpose the electroactive scaffold PEDOT:PSS is integrated in the transmembrane device and promotes cell hosting due to its tissue like structure. Moreover, it could be demonstrated that activity of a human tri-culture cell model (epithelial, goblet, fibroblasts) can be continuously monitored for ~1 month. Electrical characterization is detected as the epithelial barrier blocks ions present in the cell culture media from penetrating into, and thereby changing the doping state of the conductive polymer PEDOT:PSS. Currently, the influence of nutrients and gut peptides is investigated on different physiological outcome measures in a 2D gut model for comparing and validating the transfer to the 3D electronic transmembrane device.

References

[1] M. Muttenthaler, G. F. King, D. J. Adams, P. F. Alewood, Nat. Rev. Drug Discov. 2021, 20, 309–325.

[2] C. Pitsalidis, D.C Van Niekerk, C. M. Moysidou, A. Boys, A.M. Withers, R. M. Owens, Patent pending 2021

Poster flash talk 21

Zooming in on the microtubule binding region (MTBR) of Tau4 by protein semisynthesis

Dominik P. Vogl*, Mario Migotti**, Vanessa Thoeny*, Robert Konrat**, Anne C. Conibear*** & Christian F. W. Becker*#

*University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria; **Max Perutz Laboratories, Vienna Biocenter Campus 5, 1030 Vienna, Austria; ***The University of Queensland, Faculty of Medicine, School of Biomedical Sciences, St Lucia QLD 4027, Australia; #Corresponding author

Dysfunction of the microtubule associated protein Tau plays a central role in the pathogenesis of several neurodegenerative diseases.[1] Among others, posttranslational modifications (PTMs) located in the microtubule binding region (MTBR) of Tau have been linked to the development of Alzheimer's disease and other tauopathies.[1] Whereas the effects of several site-specific PTMs on microtubule binding and aggregation have been described quite recently by us and others[2,3,4], their impact on structure and dynamics at atomic resolution remains elusive. Based on previous studies on the longest Tau variant (Tau4) [2,3], we now focus on conformational changes of the Tau4-microtubule complex and the impact of PTMs on this interaction. This is achieved by assembling different variants of posttranslationally modified Tau in combination with segmental isotope labelling patterns for NMR analysis. Using this strategy, one can greatly reduce spectral crowding and therefore facilitate resonance assignment of all non-proline backbone residues in large proteins such as Tau.[5] Our semisynthesis strategy is based on generating synthetic peptide segments with PTMs that comprise the MTBR flanked by two recombinantly expressed Tau4 segments.[3] Both, the N-terminal as well as the C-terminal Tau4 fragments, can be labelled with the required stable isotopes (15N, 13C) for comprehensive NMR experiments.[5] Taken together, these modifications should enable valuable insights into molecular determinants of Tau aggregation and binding to microtubules as well as providing guidelines for effective segmental isotope labelling strategies.

References

[1] T. Arendt, Brain Res.Bull. 2016

[2] M. Haj-Yahya and H. A. Lashuel, J. Am. Chem. Soc. 2018

[3] D. Ellmer, M. Brehs, M. Haj-Yahya, H. Lashuel, C. F. W. Becker, Angew. Chem. Int. Ed. 2019

[4] O. Reimann, C. Smet-Nocca, C. P. R. Hackenberger, Angew. Chem. Int. Ed. 2015

[5] D. P. Vogl, Anne C. Conibear, C. F. W. Becker, RSC Chemical Biology 2021

Poster flash talk 23

Design and synthesis of an oligopeptidic potential Y4R radioligand with picomolar binding affinity

Jakob Gleixner, Carina Höring, Max Keller

Institute of Pharmacy, University Regensburg, D-93040 Regensburg, Germany

The family of human NPY receptors comprises four subtypes, designated Y1, Y2, Y4 and Y5 receptor, which are members of the superfamily of G-protein coupled receptors. Pancreatic polypeptide (PP) - a linear 36 amino acid peptide - represents the primary endogenous agonist of the Y4 receptor. In this peptide, the amidated C-terminus (···Tyr-Arg-NH2) is crucial for receptor binding. Therefore, this C-terminal motif is present in reported oligopeptidic Y4R ligands, such as the cyclic peptide UR-AK86c exhibiting high affinity (Ki 0.048 nM) and excellent Y4R selectivity [1]. To date, only two highly selective Y4R radioligands, both derived from the dimeric pentapeptide BVD-74D [2] have been described [3]. These radioligands ([3H]UR-KK193, [3H]UR-KK200) were reported to exhibit a Kd value of 0.67 nM in sodium-free buffer, but, as commonly observed for Y4R agonists, lower affinity in buffers containing a physiological sodium concentration (Kd ca. 10 nM) [3]. Using UR-AK86c as a lead compound, we synthesized cyclic peptidic Y4R ligands containing a propionyl residue, which can be replaced by a commercially available tritiated propionyl residue to get access to radioligands. Labeling precursors ware designed as follows: the N-terminal endocyclic succinyl group in UR-AK86c was replaced by L- or D-aspartate yielding heptapeptides, which were cyclized via the aspartyl alpha- or gamma-carboxyl group and the amino-functionalized modified arginine in position 4. The free N-terminus was propionylated resulting in the "cold" analogues of potential radioligands. Y4R affinities were determined by radioligand competition binding and the functional characterization was performed by measuring miniGi recruitment (split luciferase assay) as well as G-protein activation (Ca2+ aequorin assay). With a Ki value of 44 pM (sodium-free buffer), UR-JG67 proved to be the most promising compound.

References

[1] Konieczny et. al. J. Med. Chem. 2021, accepted manuscript

[2] Balasubramaniam et al. J. Med. Chem. 2006, 49, 2661-2665.

[3] Kuhn et al. J. Med. Chem. 2016, 59, 6045-6058.