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Title

Harnessing the Chemical Toolbox to Fine-tune β-Catenin Oncogenic Signaling

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Abstract

β-catenin is an intracellular protein and a key regulator in many biological processes throughout many human cell lines. Its transcriptional activity dictates cellular metabolism, proliferation, and differentiation, which requires the strict regulation of β-catenin activation, translocation, and subsequent degradation. These key roles are orchestrated by post-translational modifications, which are primarily located at the intrinsically disordered N-terminus of β-catenin. Herein, four consecutive phosphorylation events (S33, S37, T41, S45) trigger the recruitment of E3 ligase ß-TrCP, causing ubiquitination and subsequent proteasomal degradation, thereby suppressing the oncogenic potential of β-catenin. The exact mechanism by which β-catenin is regulated by phosphorylation is still unknown, and the impact phosphorylation has on the protein structure and function is highly debated since the N-terminus is missing from most structural studies. Obtaining homogenous, site-specifically modified variants from biological systems is impractical and their structural prediction is difficult owing to their dynamic nature. In this study, we show the chemical synthesis, site-specific modification, and structural characterization of the β-catenin N-terminus with its site-specific phosphorylation modifications which prime proteasomal degradation. The unique access to site-specifically phosphorylated variants allows their structural investigation via NMR spectroscopy, with which we can study conformational changes and dynamic transitions on a molecular level induced by phosphorylation. This enables us to determine which phosphorylation sites are critical for β-catenin degradation and whether they alter the structure, thereby potentially regulating binding to partner proteins. Understanding how PTMs regulate the structure-function relationship of the ß-catenin N-terminus have the potential to expand our opportunities to guide the development of new therapeutics that target the degradation of oncogenic β-catenin.

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Title

Fatty acid conjugated EPI-X4 derivatives with increased activity and in vivo stability

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Abstract

Dysregulation of the CXCL12/CXCR4 axis is implicated in autoimmune, inflammatory, and oncogenic diseases, positioning CXCR4 as a pivotal therapeutic target. We evaluated optimized variants of the specific endogenous CXCR4 antagonist, EPI-X4, addressing existing challenges in stability and potency. Our structure-activity relationship study investigates the conjugation of EPI-X4 derivatives with long-chain fatty acids, enhancing serum albumin interaction and receptor affinity. Molecular dynamic simulations revealed that the lipid moieties stabilize the peptide-receptor interaction through hydrophobic contacts at the receptor's N-terminus, anchoring the lipopeptide within the CXCR4 binding pocket and maintaining essential receptor interactions. Accordingly, lipidation resulted in increased receptor affinities and antagonistic activities. Additionally, by interacting with human serum albumin lipidated EPI-X4 derivatives displayed sustained stability in human plasma and extended circulation times in vivo. Selected candidates showed significant therapeutic potential in human retinoblastoma cells in vitro and in ovo, with our lead derivative exhibiting higher efficacies compared to its non-lipidated counterpart. This study not only elucidates the optimization trajectory for EPI-X4 derivatives but also underscores the intricate interplay between stability and efficacy, crucial for delineating their translational potential in clinical applications.

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Title

Pseudoproline Derivatives and By-Product Formation in Flow Peptide Synthesis

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Abstract

Pseudoproline derivatives, like Thr(ΨPro)-OH, are widely used in peptide synthesis to minimize aggregation and prevent aspartimide (Asi) formation. This study investigates unexpected by-products, including aspartimide and an imine derivative of Thr(ΨPro), formed during flow peptide chemistry. We explored the oxazolidine structure of the pseudoproline moiety and proposed mechanisms for its two-way ring opening, leading to these by-products. Our findings suggest that Asi formation is catalyzed by pseudoproline under high temperature and pressure.

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Title

Consequences of Site-Specific Glycation on α-Synuclein Function and Aggregation

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Abstract

Synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) are neurodegenerative disorders characterized by the pathological aggregation of α-synuclein (αSyn). In contrast, monomeric αSyn is a highly abundant protein in the central nervous system, playing a crucial role in neurotransmitter release and synaptic vesicle recycling. A key question in synucleinopathy research is what triggers αSyn aggregation and how to prevent these pathological processes. Only 5 – 10 % of PD cases have a genetic background, while the vast majority occurs sporadically. Risk factors such as aging, environmental toxins, and comorbidities like type 2 diabetes are strongly linked to the accumulation of non-enzymatic posttranslational modifications (nPTMs) through protein oxidation and glycation. These modifications are increasingly recognized as major factors influencing αSyn function and aggregation. To investigate this hypothesis, we developed a semi-synthetic approach to site-specifically incorporate N6-carboxyethyllysine into αSyn. N6-Carboxyethyllysine is the main product of lysine glycation by methyl glyoxal, which is a reactive byproduct of carbohydrate metabolism and a major contributor to diabetic complications. In total, we synthesized 11 αSyn variants with N6-carboxyethyllysine modifications at positions K6, K10, K12, K21, K23, K30, K32, K43, K45, K58, and K60 at a micromolar scale. These glycated αSyn variants were compared to wild-type αSyn in various bioassays to assess the impact of glycation on both pathological processes, such as aggregation and neurotoxicity, as well as physiological functions, including degradation and membrane interactions.

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Title

Photocaged oxytocin and vasopressin probes to decipher neuropeptide signalling pathways

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Abstract

The ancient oxytocin/vasopressin (OT/VP) signalling system is highly conserved across evolution, playing a pivotal role in a myriad of physiological functions within the periphery and the central nervous system, including neuromodulation, emotional responses, and learning. Dysfunctions in this system have been implicated in various pathophysiological and pathopsychological conditions, such as breast cancer, autism spectrum disorder, or major depressive disorder. However, the intricate neuropeptide-mediated pathways underlying many of these functions, especially in the brain, remain elusive. Long diffusion pathways of neuropeptides through brain tissues after injections can lead to receptor activation away from the original injection side, making it complex to pinpoint connections between neuropeptide activity in certain areas and the macroscopic outcome on behavioural levels. To allow a more in-depth exploration of neuropeptide signalling within the brain, we aimed to expand the toolbox for neuroscientists. Therefore, we developed advanced photoprobes for OT and VP, utilizing photopharmacology to achieve spatiotemporal control over neuropeptide activity. We synthesized three classes of photolabile protecting groups (photocages) based on coumarins, nitrophenethyls, and BODIPYs, allowing controlled release of OT and VP upon light exposure. The photocaged OT/VP probes were characterized in cellular assays targeting central receptors (hOTR, hV1aR, hV1bR) and validated *in vitro* in HEK-293 cells and hippocampal neurons using confocal microscopy. Our probes showed no dark activity and were able to activate their receptors after irradiation, while neither compounds nor light exposure showed adverse effects on neurons, thereby illustrating their potential to enable precise receptor activation in defined brain regions and to facilitate the study of neuropeptide signaling in future *ex vivo* and *in vivo* experiments. This approach offers a powerful tool for investigating the complex roles of OT and VP in physiological and pathological conditions, advancing our understanding of neuropeptide signaling and overcoming the limitations of conventional methods.

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Title

Discovery and development of macrocyclic peptide modulators of the cannabinoid 2 receptor

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Abstract

The cannabinoid type 2 receptor (CB_2R), a prototypical G protein-coupled receptor (GPCR), is an important regulator of immune cell function and a promising target to treat chronic inflammation and fibrosis. While CB_2R is typically targeted by small organic molecules, including endo-, phyto- and synthetic cannabinoids, peptides – owing to their size – may offer alternative binding sites to facilitate differential interactions with the receptor. Here we explore plant-derived cyclic cystine-knot peptides as ligands of the CB_2R. Cyclotides are known for their exceptional biochemical stability. Recently they gained attention as modulators of GPCR signaling and as templates for designing peptide ligands with improved pharmacokinetic properties over linear peptides. Cyclotide-based ligands for CB_2R were profiled based on a peptide-enriched extract library comprising nine plant species. Employing pharmacology-guided fractionation and peptidomics analysis we identified novel cyclotide vodo-C1 from sweet violet plant (*Viola odorata*) as a full agonist of CB_2R with an affinity (K_i) of ~1 µM and a potency (EC_{50}) of ~8 µM. Leveraging deep learning networks we verified the structural topology of vodo-C1 and modelled its molecular volume in comparison to the CB_2R ligand binding pocket. Utilizing a fragment-based approach, we designed and characterized vodo-C1-based bicyclic peptides (vBCL1-4), aiming to achieve reduced size and improved potency. Intriguingly, the design of cyclotide-inspired bicyclic peptides led to an unexpected transformation of the molecular mode of action from an agonist to the discovery of modulators with negative allosteric and neutral antagonist properties for CB_2R. This study introduces a first-in-class macrocyclic peptide phyto-cannabinoid, which served as template for the development of synthetic CB_2R peptide modulators, and hence these findings offer opportunities for future peptide-based probe and drug development at cannabinoid receptors.

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Title

Membrane Permeabilizing Coacervates and Use for Intracellular Delivery of IgGs

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Abstract

We have previously reported the facilitated infusion of IgG into cells from coacervates formed with Alexa Fluor 488-labeled IgG (Alexa488-IgG) and FcB(L17E)3, the conjugate of an Fc-binding peptide with the trimer of the intracellular IgG delivery peptide L17E [1]. Contact of the coacervates with the cell membrane resulted in spontaneous influx and distribution of Alexa488-IgG throughout the cells in serum-containing medium. The negative charge of Alexa488-IgG was critical for coacervate formation with positively charged FcB(L17E)3. The use of IgG antibodies with negative charge modifications at predetermined positions is important for a precise evaluation of the interaction modes between Alexa488-IgG and FcB(L17E)3 in coacervate formation and the properties of coacervates as carriers of IgG. Therefore, we designed IgG antibodies with anionic peptide tags consisting of glutamic acid and tyrosine, which were attached to the C-terminus of the heavy and light chains of the IgG antibodies to serve as scaffolds for coacervate formation. Anionic tag-bound antibodies with FcB(L17E)3 formed a coacervate, and easy cytosolic delivery of IgG antibodies was achieved by treating cells. [1] T. Iwata et al. (2021) *ACIE 60*, 19804.

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Title

BLOOD BRAIN BARRIER – PEPTIDE SHUTTLES FOR DRUG DELIVERY INTO THE CNS

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Abstract

The permeability of the blood-brain barrier (BBB) is essential for drugs targeting the central nervous system. However, the majority of molecules are unable to traverse the BBB due to its rigorous transport regulation. The 'Trojan Horse' approach exploits receptor-mediated transcytosis to deliver cargos across the BBB. Despite promising data in peptide-based BBB shuttles, the proteolytic instability of peptides, remains a substantial problem in the development of peptide therapeutics. Utilizing natural peptides as scaffolds in drug design can enhance stability since they frequently have inherent stability. In this project, we used the molecular grafting approach to design a set of peptide probes by modifying proteolysis-stable cyclic sunflower trypsin inhibitor 1 (SFTI-1) to include peptides with reported transport across the BBB, in particular peptide 22 and MiniAp-4. We implemented a brain endothelial cell transport assay for the bioactivity testing, quantifying transport with LC-MS. Tight junction protein expression was stable from day 6 onwards. Lucifer Yellow permeability measurements yielded apparent permeability (Papp) values of ~2x10^-6 from day 5 onwards. Papp values for the negative (atenolol) and positive control (quinidine) were 1x10^-6 and 8x10^-6, respectively, confirming assay functionality. The most successful probe SFTI-1-peptide 22 obtained a considerable permeability (Papp 9x10^-6) compared to peptide 22 (Papp 3.5x10^-6) alone and SFTI-1 alone (Papp 5.3x10^-6). In conclusion, the development of novel BBB shuttle probes yielded peptides with favorable transport in cell-based assays. Next, we will assess BBB permeability through a series of experiments to determine the unbound brain-to-plasma ratio (Kp,uu,brain). Our findings will demonstrate that a nature-derived peptide scaffold can enhance the stability of an incorporated linear peptide while facilitating transcellular transport, offering proof-of-concept for designing stabilized peptide BBB shuttles.

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Title

Pharmacological Characterization of Oxytocin-like Signaling Systems in Tardigrada and Onychophora

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Abstract

Oxytocin (OXT) and arginine-vasopressin (AVP) are critical regulators of physiological processes in mammals, including social behavior, stress response, vasoconstriction and water homeostasis, via their G protein-coupled receptors (GPCRs), i.e. the oxytocin (OXTR) and three vasopressin receptors (V1aR, V1bR, and V2R). Intriguingly oxytocin-like neuropeptides and receptors are one of the most ancient signaling systems present in many invertebrates whereas their molecular structure and function remain largely uncharacterized in these animals. The two phyla Tardigrada('water bears') and Onychophora ('velvet worms') are over 600 million years old, making them ideal model systems for exploring the evolutionary conservation of oxytocin-like signaling in invertebrates. Therefore this project aims to discover and characterize the oxytocin-like signaling system in Hypsibius exemplaris (Tardigrada) and Euperipatoides rowelli (Onychophora). Transcriptome mining allowed identification of one oxytocin-like peptide and three oxytocin-like receptors in H. exemplaris and one peptide along with two oxytocin-like receptors in E. rowelli. Specific primers were designed for validation of transcripts and to quantify precursor/receptor expression via qPCR, followed by cloning of receptor genes into the pEGFP-N1 vector for transient expression in HEK293 cells and chemical synthesis of the peptide. Overall this study is designed to compare signaling profiles of endogenous peptide/receptor pairs from Panarthropoda with the human oxytocin/vasopressin system utilizing bioluminescence resonance energy transfer (BRET)-based biosensor assays. For drug discovery purpose, we aim to study the signaling profiles of the invertebrate neuropeptides at the human receptors. Ultimately, the findings may provide insights into conserved neuropeptide signaling across evolutionary lineages and contribute to the development of novel pharmacological probes or drugs targeting oxytocin-related receptors.

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Title

Novel cyclotide POP modulator as a research tool for synucleinopathy therapeutics

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Abstract

Synucleinopathies, including Parkinson's disease (PD), are a group of neurodegenerative disorders characterized by the accumulation of alpha-synuclein (αSyn) fibrils within Lewy bodies and Lewy neurites. Prolyl oligopeptidase (POP) has been demonstrated to augment αSyn aggregation in Parkinson's disease models, whereas the inhibition of POP has shown promise in diminishing aggregation and facilitating autophagy, indicating it may serve as a compelling therapeutic target for synucleinopathies. Our work focuses on the cyclotide psysol 2, a plant-derived peptide that modulates POP activity as a mixed-type inhibitor with allosteric-like pharmacology. This project aims to unravel the mechanisms by which psysol 2 affects POP and therefore the protein network (e.g., αSyn or protein phosphatase 2A), providing further insights into its role in synucleinopathies.
To confirm the efficacy of POP in a cellular setting, a fluorescence-based assay was used to demonstrate that psysol 2 significantly increases autophagy at 10 μM in a similar level as the autophagy-inducer rapamycin and the specific POP inhibitor KYP-2047. This supports psysol 2's potential to modulate the POP protein network. However, a more detailed interaction model remains elusive to date since the peptide/protein complex has been difficult to crystallize. To address this limitation, we resorted to molecular dynamics (MD) simulations and molecular docking to developed a predict the binding interactions between psysol 2 and POP. To validate the computational model, we will perform amino acid mutation studies and site-specific mutagenesis to identify key inhibitory sites, guiding SAR work toward designing more potent POP inhibitors." In conclusion, this study explored the molecular details of a plant-derived peptide modulator of POP and its role in cell-based context. At a more general level, psysol 2 and its derivaties may serve as probes to study POP and its protein network as drug target in synucleopathies.

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Title

A naturally occurring 22-amino acid fragment of human hemoglobin A inhibits autophagy and HIV-1

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Abstract

Autophagy is an evolutionary ancient catabolic pathway and integral to anti-viral innate immunity. While the core machinery of autophagy is well characterized, many aspects of its physiological regulation, especially via peptides remain unclear. As a central homeostatic pathway, dysregulation of autophagy is associated with various diseases ranging from cancer and neurodegeneration to infectious diseases. Thus, therapeutic modulation of autophagy would help mitigate these disorders. However, currently available agents lack specificity and are accompanied by severe side effects in patients. Therefore, there is an urgent need for specific autophagy modulating compounds. Here, we isolate a C-terminal fragment of human hemoglobin A (HBA1, amino acids 111-132) from human bone marrow as an inhibitor of autophagy initiation. Mechanistically, the rapid acting HBA1(111-132) adopts a flexible conformation in solution, binds to the cell surface and reduces lipidation of the autophagy marker protein LC3B. Structure-activity relationship studies revealed that the C-terminal 13 amino acids of HBA1(120-132) are sufficient to inhibit autophagy, two charged amino acids (D127, K128) mediate solubility, and two serines (S125, S132) are required for function. Successful viruses like human immunodeficiency virus 1 (HIV-1) evolved strategies to subvert autophagy for virion production. Our results show that HBA1(120-132) reduced virus yields of primary HIV-1. In summary, our data shows the identification of a naturally occurring HBA1 fragment (111-132) as a physiological, non-inflammatory antagonist of autophagy. Optimized derivatives of HBA1(111-132) may offer perspectives to restrict autophagy-dependent viruses and for rebalancing in diseases associated with dysregulated autophagy.

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Title

Design of a novel cyclic peptide modulator of T-lymphocyte migration

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Abstract

Tight regulation of the migration of immune cells through our body is vital for maintaining proper function of the immune system. However, in various inflammatory and autoimmune disorders, cells evade these regulatory mechanisms contributing to disease progression. Pharmacological modulation of immune cell migration has already been approved for clinical use (e.g. FTY-720, natalizumab). Pepitem, a recently discovered endogenous peptide, regulates lymphocyte transendothelial migration by targeting sphingosine-1-phosphate receptor signalling. However, the peptide is susceptible to fast metabolic inactivation by endogenous proteases, which limits its therapeutic application. In this study, we aimed to develop stabilized peptides based on the pepitem pharmacophore. After *in silico* analysis of the bioactive epitope we utilized molecular grafting to stabilize the sequence in a cystine-rich, plant-derived peptide scaffold. The designed six novel peptides demonstrated enhanced stability in human serum, with half-lives exceeding 48 hours compared to pepitem with only 3.1 hours. The most promising peptide, named VhTI-pep 2, inhibited CD3+ T-lymphocyte migration* in vitro* with EC50 = 10.6 ± 16.5 nM, comparable to pepitem (EC50 = 6.0 ± 6.4 nM), and effectively reduced migration of different T-lymphocyte populations. Additionally, using multiple sclerosis-patient derived lymphocytes we confirmed inhibition of the migration of lymphocyte subsets with relevance in the disease progression by the lead candidate peptide. These findings highlight the potential of VhTI-pep 2 as a modulator of immune cell migration. Further *in vivo* characterization of the peptide in disease models such as the EAE mouse model of multiple sclerosis will provide further evidence on the efficacy of the peptide to modulate immune cell migration. If successful, the novel peptide could provide the basis for new therapeutic approaches in autoimmune diseases such as multiple sclerosis.

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Title

Site-specific acetylation of HMGN1 within the nucleosome binding domain

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Abstract

Chromatin is a complex of RNA, DNA, and proteins to store the genome of eukaryotes in physiological conditions and to organize DNA metabolic pathways. DNA replication, transcription, recombination, and chromosome segregation are the major biological processes that chromatin is involved in. Nucleosomes are the repeating units in chromatin structure, and can undergo certain modifications. Several regulatory proteins, including non-histone proteins like HMGN1, can participate in chromatin structure modification and may result in functional change. HMGN1 is subjected intensely to posttranslational modifications (PTMs), mainly lysine acetylation and serine phosphorylation. Although various biological functions are attributed to HMGN1 and its PTMs, the precise effects of its PTMs on the biological function remain largely unknown. We aim to investigate the effects of lysine acetylation in the nucleosome binding domain of HMGN1 on interactions of HMGN1 with nucleosomes. We present the synthesis of site-specifically acetylated HMGN1 variants by a semi-synthetic approach. We performed structural analysis of the site-specifically acetylated HMGN1 variants by CD spectroscopy, which shows that both unmodified and acetylated variants of HMGN1 are intrinsically disordered, as reported in the literature. We also conducted preliminary thermal stability shift assays to examine the interactions of HMGN1 variants with nucleosomes. We anticipate the assays will provide more insights on how HMGN1 accomplishes specific functions within the cells due to PTMs at precise positions.

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Title

Probing Oxytocin/Vasopressin receptor pharmacology utilizing evolutionary conserved signalling systems

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Abstract

The two neuropeptides oxytocin (OXT) and arginine-vasopressin (AVP) regulate a plethora of physiological processes via binding and subsequently activating the four G protein-coupled receptors (GPCRs) OXTR, V1aR, V1bR, and V2R. High extracellular sequence homology of these related receptors poses a major challenge in developing selective ligands. Invertebrates comprise over 97% of all known animal species. In this project we utilize this incredible biodiversity to mine unique OXT/AVP-like peptides from invertebrate genomes/transcriptomes for computational and pharmacological characterization. Invertebrate species typically harbour only one OXT/AVP-like peptide and receptor. Therefore, investigation of these cognate peptide/receptor pairs, and comparative studies with the human signalling system may be useful to obtain molecular details of receptor selectivity and ligand-induced pathway bias. In silico mining revealed a total of 52 OXT/AVP-like peptides across different invertebrate phyla, of which 14 ligand-receptor pairs (from the phyla Echinodermata, Insecta, Annelida, Mollusca, Brachiopoda, Cephalochordata, Rotifera, Tardigrada, Onychophora, Platyhelminthes, Crustaceans, Bryozoa, Hemichordata, Alveolata) were selected for synthesis and radioligand binding assays. One peptide (OXTL-19 discovered in the rotifer B. asplanchnoidis) was selected for detailed pharmacological analysis, based on preferential binding to the OXTR over the three vasopressin receptors. Biosensor assays demonstrate a significantly diminished ability of OXTL-19 to induce β-arrestin recruitment to the OXTR (~9% efficacy for β-arrestin 1 and ~30% for β-arrestin 2 as compared to OXT). G protein dissociation assays reveal an inverse signal (-100% efficacy compared to OXT), suggesting efficacy as inverse agonist. At a more general level, the discovery of invertebrate neuropeptide ligands with enhanced subtype selectivity at the human GPCRs, followed by pharmacological characterization and computationally-driven sequence analysis will help to address current limitations in the development of optimized peptide GPCR ligands. As long-term goal this project aims to increase our knowledge of the molecular signatures of ligand-induced GPCR-interaction. This will ultimately help to develop safer peptide therapeutics.

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Title

Effects of site-selective post-translational modifications on tau protein

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Abstract

Few diseases are as notorious as Alzheimer's disease (AD), a neurodegenerative disease (ND) characterized by severe dementia. Therefore, many studies been carried out to elucidate the molecular mechanisms leading to AD, and the insoluble, proteinaceous material deposits closely associated with this disease. These comprise β-amyloid and microtubule-associated protein tau aggregates, respectively. Remarkably, in spite of tau aggregates having been associated with other NDs, termed tauopathies, their exact composition and structure appears to be distinct for each disease, evidenced by protein isoforms and numerous post-translational modifications (PTMs) found in patients. In AD, the noticeable PTMs are hyperphosphorylation, acetylation, and ubiquitylation. Yet, individual contributions of these PTMs to the aggregation process remain largely unexplored, due to the difficulty of attaining homogenous, site-specifically modified protein sample. However, by combining recombinant expression and solid phase peptide synthesis (SPPS) to generate semisynthetic tau variants, PTMs can be introduced site-selectively into peptide segments via SPPS. The novel semisynthetic strategy devised for this project will allow us to introduce PTMs to the R3 and R4 repeat domains of the microtubule binding domain of tau. The resulting tau variants will then be employed to study aggregation, tubulin binding but also the effects of specific PTMs on its liquid-liquid phase separation (LLPS) behavior. Supposing LLPS regulates cellular processes by enrichment of proteins in liquid-like droplets, aberrant LLPS could favor aggregation of modified protein in disease.

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Title

Development of advanced molecular tools to study the oxytocin receptor system

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Abstract

The oxytocin receptor (OTR), a G protein-coupled receptor (GPCR), plays key roles in reproduction and social behaviors and is a promising therapeutic target for conditions like cancer, pain, and autism [1, 2]. Translating these findings into the clinic as proven difficult due to a poor undestanding of OTR expression, its functions in different tissues, and interactions with other receptors such as the dopamine D2 receptor (D2R) [3]. These gaps limit research advancements and targeted therapy development. To deepen our understanding of OTR's role in health and disease, we addressed existing limitations by developing and applying innovative tools to study OTR and OTR-D2R complexes. We created a selective fluorescent tracer for OTR, which we validated as a research tool for *in vitro* OTR detection using confocal microscopy, fluorescence-activated cell sorting, and *ex vivo* detection through intracerebroventricular injection in mice. Additionally, we applied this tracer in live-cell single-molecule microscopy, enabling real-time tracking of OTR at the cell membrane. To explore the effects of dimerization on biological responses, we also designed bivalent ligands specifically targeting OTR-D2R heterodimers, with ongoing evaluation of their pharmacological properties. We investigated OTR and D2R dimerization *in vitro via* bioluminescence resonance energy transfer and plan to confirm and further analyze these findings with confocal microscopy. With our molecular tools, we aim to enhance our understanding of OTR, its interactions with other receptors and its complex roles in health and disease. This knowledge may ultimately lead to new therapeutic approaches targeting OTR, benefiting patients affected by various disorders.

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Title

BrainBike peptidomimetic enables efficient transport of bio- and nano-therapeutics across brain endothelium

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Abstract

Therapeutics cannot reach the brain in sufficient amounts because of their low permeability across the blood-brain barrier (BBB). Although brain shuttle peptides may enhance BBB transport of large therapeutics via receptor-mediated transport, high lability to proteases limits their efficiency. We have previously shown that increasing protease resistance generates more efficient peptide shuttles. However, the capacity of these shuttles to transport proteins and certain nanocarriers was limited.[1] Here we develop BrainBikes, a novel family of bicyclic protease-resistant peptide shuttles capable of increasing transport of various types of cargo. Utilizing a chemical linker, we generated several bicyclic analogs from a linear peptide that target the transferrin receptor (TfR1).[2] All analogs exhibit increased metabolic resistance. In addition, one of the analogs, BrainBike-4 showed a 7-fold higher affinity for cells with high levels of TfR1. We site-specifically conjugated BrainBike-4 to a model protein (GFP), a single chain variable antibody fragment (scFv), and to a non-viral gene delivery vector based on poly(β-aminoester)s (pBAEs). Conjugation of BrainBike-4 resulted in a 4-fold increase in GFP transport and a 5-fold increase in scFv transport across a human cell-based BBB model, significantly outperforming previously reported peptide shuttles.[3] Furthermore, we have recently found that BrainBike-4 is also capable of significantly enhancing the transport of pBAE gene delivery nanovehicles in the same model. Our results highlight the potential of bicyclic peptidomimetics as brain shuttles and offer new possibilities for bio- and nano-therapeutic transport.

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Title

A Modular ABPP Approach to Target Protease AgrB in the Pathogen S. aureus

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Abstract

The human pathogen *Staphylococcus aureus* uses the *agr* quorum sensing system to coordinate the production of a broad spectrum of virulence factors to facilitate infection of its host. Hereby, the enzyme AgrB, a cysteine endopeptidase, possesses a central role in this quorum sensing system since it catalyses the biosynthesis of the autoinducing peptide (AIP) the *agr* QS signal. We hypothesize that an inhibition of the protease AgrB of *S. aureus* would be feasible to block AIP maturation and secretion. In absence of the signal molecule, the agr response system would not be activated and the pathogen will not be able to produce virulence factors. We use activity-based probes to label the target AgrB both recombinantly expressed in *E. coli* and in different *S. aureus* strains and establish a suitable competitive assay to screen for potential inhibitors. The probes are developed based on recent findings of α-haloacetamide warheads and ligand selection strategy for rapid probe scaffold tailoring. With the structural information gained from the ABPP and complimentary docking experiments potential electrophilic inhibitors targeting the catalytic cysteine can be designed. As a future perspective the developed toolbox will subsequently be applied towards other pathogens. *Agr*-like or similar QS signalling systems controlling the virulence have been discovered in various species of* Staphylococcus, Clostridium,* *Bacillus*, *Enterococcus*, and *Streptococcus* and also play a role as a phage encoded infection sensing system.

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Title

Production of trefoil factor family peptide 2 – a long-standing challenge

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Abstract

Production of trefoil factor family peptide 2 – a long-standing challenge Dóra Bogdán1, Markus Muttenthaler1,2

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Human trefoil factor family peptides (hTFF1-3) play an essential role in maintaining and restoring the integrity of the gastrointestinal epithelium, particularly following injury or infection. hTFF2 consists of 106 amino acids and has two trefoil domains formed by 6 disulfide bonds. This leads to a characteristic and compact three-looped structure. Its 3D structure and interaction partners are still not completely identified due to hTFF2 production challenges and aggregation problems. The challenges of producing it recombinantly or by solid-phase peptide synthesis have limited the exploration of its therapeutic potential. Here, we investigated the recombinant expression of hTFF2 using two fusion constructs: His-tagged TEV-fused and His-tagged SUMO-fused peptides, both expressed in *Escherichia coli*. We focused on optimising expression conditions to achieve high yield and solubility while addressing the issue of inclusion body formation. Expressions with His-tagged TEV construct resulted in significant inclusion body formation, necessitating solubilisation strategies. We employed both traditional methods and mild solubilisation techniques to recover biologically active hTFF2 from these aggregates. The His-tagged SUMO-fusion construct significantly improved solubility, with no observable inclusion body formation under similar conditions. The SUMO tag enhanced the solubility of hTFF2, facilitating easier purification and reducing the need for post-expression processing steps. This study provides valuable insights into optimising the expression and purification processes for producing hTFF2 which will facilitate studies into its structural properties and interaction behaviour, with the long-term goal of enabling its therapeutic application.

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Title

Unraveling the functional implications of site-specific phosphorylations on heat shock protein 27: a semisynthetic approach

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Abstract

Posttranslational modifications, particularly phosphorylations, can have profound influence on proteins with severe implications for diverse pathological conditions such as cancer or neurodegenerative diseases.[1] Our focus lies on Hsp27, a member of the small heat shock protein (sHsp) family, known for its role in cellular stress response and apoptosis regulation.[1,2] The N-terminal domain of Hsp27 undergoes dynamic modulation through serine phosphorylations at residues 15, 78 and 82, mediated by cellular stress-induced activation of e.g. the MAPK pathway. These modifications induce a shift in oligomerization dynamics, thereby impacting chaperone activity.[2] Investigating the influence of these phosphorylations will contribute to our understanding of the relationship between site-specific modifications and their effect on functional dynamics of Hsp27. Existing studies, employing cell induction methods or site-specific mutagenesis, fall short in dissecting the nuanced impact of individual phospho-variants.[3] Therefore, our objective is to generate variants of Hsp27 phosphorylated at positions 15, 78, and 82, using a semi-synthetic approach, combining solid-phase peptide synthesis (SPPS), recombinant protein expression and chemoselective ligation approaches. To this end, suitably functionalized peptide segments of Hsp27 have been obtained through both SPPS and expression. For the expressed peptide segments, a thiol protection strategy with a phenacyl group will be strategically applied to preserve the native cysteine at position 137 in Hsp27 during ligation reactions, enabling a traceless semi-synthesis. The overall strategic approach allows for the preparation of full-length, functional Hsp27 with phosphorylations at distinct sites through sequential ligation and subsequent desulfurization. Further, we aim to conduct biochemical assessments, including chaperone activity assays and Hsp90 interaction studies, to unveil the functional properties of the obtained phospho-variants.

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Title

The peptides within R5-templated silica particles resemble a condensed liquid-like phase

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Abstract

Biomimetic silica nanoparticles templated by diatom-derived peptides, notably the R5 peptide, show significant promise for drug delivery and enzyme encapsulation applications due to their inherent biocompatibility, tunable surface properties, and capacity for molecular encapsulation.[1] Optimizing these nanoparticles for biomedical use requires detailed insights into the structure and molecular dynamics of the peptide matrix that directs their formation. However, the primary technique for probing peptide and protein dynamics in aqueous environments, nuclear magnetic resonance (NMR) spectroscopy, typically struggles with high molecular weight systems when relying on conventional proton detection. To overcome this limitation and study larger peptide assemblies involved in biomineralization, we present an integrated approach that combines carbon-detected NMR with electron paramagnetic resonance (EPR) spectroscopy enhanced by site-directed spin labeling (SDSL) to characterize the structural dynamics of R5 peptide assemblies both before and after encapsulation within a silica matrix.[2] Our findings reveal that R5 assemblies exist in a condensed, liquid-like phase prior to mineralization. Upon incorporation into the silica matrix, a portion of the R5 peptides adopts a more restricted dynamic state, while another subset retains liquid-like mobility. This study not only deepens our understanding of the principles underlying biomimetic material design but also underscores the critical role of liquid-liquid phase separation in peptide-guided biomineralization.

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Title

Identification of intermediates in peptide-guided biomineralization through Dissolution Dynamic Nuclear Polarization (D-DNP) with over 100-fold enhancement in multidimensional NMR

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Abstract

The crystallization pathway of calcium phosphate (CaP) in the presence of osteopontin (OPN) follows a non-classical route, characterized by rapid, transient intermediates that are challenging to capture with conventional techniques. Nuclear Magnetic Resonance (NMR) is typically low in sensitivity but unmatched in providing atomistic resolution; however, its effectiveness for fast-evolving processes is limited, as signal-to-noise ratios improve only with the square root of the number of scans thus making 2D NMR spectra unsuitable. While increasing scans can enhance resolution, this approach is impractical for capturing quick dynamics. To address this limitation, dissolution Dynamic Nuclear Polarization (dDNP) is employed to enhance sensitivity, enabling the detection of transient fast evolving states in real time1. By integrating dDNP, a custom-made injection device2 and in combination with artificial intelligence-driven peak picking algorithms3, high-resolution spectra can be recorded within less than a minute. AI processing improves the resolution of the hyperpolarized spectra allows the acquisition of two-dimensional nuclear magnetic resonance experiments with superior sensitivity. Utilizing both techniques allows for a detailed exploration of CaP's behavior in the presence of OPN. This approach provides insights into the dynamic interactions and structural transformations within the system within a timeframe of less than a minute.

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Title

Photocatalytic Diselenide Contraction as a Tool for Site-selective Bioisosteric Ubiquitylation

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Abstract

Ubiquitylation is a highly conserved post-translational modification (PTM) in eukaryotes, which serves as critical regulatory mechanism for protein homeostasis, cellular transport, signal transduction pathways and numerous other functions. The biological function of ubiquitylation is dictated predominantly by the topology of its linkage. Deciphering ubiquitin's complex biochemistry necessitates novel synthetic methods that deliver well defined, biosimilar ubiquitylation. To this end, a semisynthetic strategy relying on the recombinant expression of ubiquitin combined with chemoselective photocatalytic diselenide contraction (PDC) [1] was established to enable site-selective biomimetic selenalysine-linked ubiquitylation. The modification of ubiquitin with a C-terminally selenol was fine-tuned to avoid hydrolysis. The conditions of the PDC reaction, such as solvent composition, phosphine concentration and irradiation were optimized for efficient ubiquitylation of the Tau peptide. Furthermore, it is demonstrated, that the selenalysine linkage undergoes efficient cleavage by deubiquitylating enzymes, comparable to the native isopeptide linkage. The presented method expands the toolbox of site-selective ubiquitylation techniques. It is tolerant to many functional groups and will help to further elucidate the complexities of ubiquitylation.

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Title

Modular access to structurally defined ubiquitin chains

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Abstract

Ubiquitination is a ubiquitous highly diverse PTM strongly related to protein homeostasis via the ubiquitin-proteasome system (UPS). Nature constructs ubiquitin tags with high spatiotemporal precision to execute defined functions, which critically rely on the exact molecular composition of the ubiquitin chain.[1] Deciphering the complex ubiquitin code is therefore of paramount interest in biology resulting in a demand for structurally homogeneous ubiquitin chains as well as ubiquitinated target motifs. As enzymatic approaches suffer from inherent drawbacks like hardly controllable length or type of Ub chains and substrate-specificity,[2] we harness a combination of recombinant expression and high precision chemical tools to tackle this challenge. Our semisynthetic strategy, relies on expression of Ub-intein fusion constructs giving access to high quantities of defined ubiquitin building blocks with versatile C-terminal reactive handles such as thioesters, hydrazides or allyl amides. Linkage between ubiquitin monomers or target peptides and proteins is achieved site selectively via photoinitiated thiol-ene click (TEC) chemistry resulting in nearly bioisosteric connections.[3] Using DUB-assays, we further demonstrate the recognition of this artificial linkage by the Ub processing machinery. The presented work combines minimal synthetic effort by semisynthesis with high fidelity linkage chemistry paving the way towards homogeneously ubiquitinated proteins to help elucidating the biological role of the ubiquitin code on a molecular basis.

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Title

Novel gut-stable peptide ligands targeting gut MC4 receptors for the treatment of metabolic disorders

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Abstract

Gut peptides play a pivotal role in the pathophysiology of metabolic disorders, having key functions in appetite regulation and glycemic control. Of particular interest are enteroendocrine L cells, which secrete the glucoregulatory/ satiety-promoting peptides glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). These cells express the melanocortin 4 receptor (MC4R) and respond to its endogenous agonist α-melanocyte-stimulating hormone (α-MSH) by augmenting the release of GLP-1 and PYY. Targeting gut MC4R, therefore, is an innovative and highly promising therapeutic strategy for tackling metabolic disorders. Our aim is to develop chemically modified variants of α-MSH, which resist the harsh proteolytic environment in the gut, making them suitable for targeting intestinal MC4R after oral administration.

Using solid-phase peptide synthesis (SPPS) and employing different macrocyclization strategies and site-specific modifications at experimentally identified cleavage sites, we synthesized a library of peptides with substantially enhanced gut stability ($t_{1/2} \geq 8$ h) while retaining or even improving potency ($EC_{50} \leq 1$ nM) and selectivity for MC4R. Structure-stability-activity relationship studies further guided the selection of lead structures and informed the design of optimized lead compounds.

Developing oral peptide therapeutics remains a challenging yet highly desirable goal. Beyond improving patient compliance, oral delivery of peptides holds particular promise for regioselectively targeting receptors in the gut. For example, many gut peptides have receptors located near their release sites in the gut, such as on vagal afferent neurons, where they transmit satiety and glucoregulatory signals to the brain. Oral delivery of gut peptide analogs may better replicate the distribution pattern of endogenous gut peptides, achieving high concentrations locally in the gut, and therefore could more effectively engage the gut-brain axis for managing type 2 diabetes mellitus and obesity.

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Title

Structure of peptide assemblies by combined NMR and molecular dynamics simulations.

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Abstract

The natural recognition systems that drive the formation of nanostructures have inspired considerable work on the self-assembly of synthetic peptides, leveraging their inherent propensity to adopt specific secondary structures. These include α-helices, β-strands, and polyproline helices, which have been strategically applied in designing fibrous peptide materials, hydrogels, and other biomimetic materials with tailored structural and functional properties [1].

In this context, we propose a computational workflow that combines molecular dynamics simulations with experimental spectroscopic constraints to investigate the self-mineralization of synthetic peptides. The process begins with an initial simulation step, where multiple peptide monomers are added to a small simulation box to initiate aggregation. Once stable oligomers are observed, they are extracted from the initial box and transferred to a larger simulation environment, where additional copies of the oligomers are added. This step enables further aggregation, ultimately resulting in larger, polymer-like self-assemblies with morphologies that reflect the structural characteristics of the constituent peptides.

The validation of these simulated structures involves quantitative comparison with experimentally derived NMR data, specifically through measurements of diffusion coefficients and fractal dimensions. [2].

This workflow was applied to study the self-assembly behavior of several peptides, each known to form distinct shapes such as rods, helices, and spheres. Our findings demonstrate that the secondary structure and aggregation behavior of the peptides have a direct influence on the morphology of the resulting mineral structures. The peptide-induced morphologies translate to specific silica particle shapes, from tubular to spherical forms. This insight highlights the potential of tailoring particle morphology through precise control over peptide sequence and assembly conditions, offering a powerful route for creating specialized nanomaterials with defined structural features [3].

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Title

Antibiofilm peptides to target gastrointestinal biofilms

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Abstract

Gastrointestinal biofilms are mucus-adherent prokaryotic communities embedded in a protective matrix of extracellular substances, including DNA, proteins, polysaccharides, and lipids. These biofilms are associated with gut disorders such as irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD, Crohn's disease, ulcerative colitis),1, 2 affecting 10–15% of the Western population and imposing a substantial socioeconomic burden. Intestinal biofilms are present in 57% of IBS and 34% of ulcerative colitis patients, compared to only 6% of healthy individuals.3 Despite this prevalence, no biofilm-specific treatments exist. Many natural antimicrobial peptides (AMPs) have evolved to combat bacterial infections and are a valuable resource for drug discovery. However, their full potential against gut mucosal biofilms remains largely unexplored. Our strategy for developing gut-restricted antibiofilm peptides includes (i) synthesising AMPs from diverse natural sources, (ii) screening them for antibiofilm activity against biofilm-forming bacteria from biofilm-positive patients, and (iii) employing chemical modifications to enhance gut stability and therapeutic efficacy. Here, we present our latest findings on developing antibiofilm drug leads for the treatment of mucosal biofilms in patients with GI disorders.

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Development and pharmacological characterisation of bivalent ligands targeting OTR-D2R heterodimers

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Abstract

One of the central stimulators of social behaviour is the neuropeptide Oxytocin (OT). The oxytocinergic system is considered as a potential drug target to treat neuropsychiatric disorders, including schizophrenia and autism spectrum disorder (ASD), which feature deficits in neuronal social-behavioural domains[1,2]. Recent discoveries suggested that G protein-coupled receptor (GPCR) complex formation is involved in these diseases. GPCR heteromers can attain altered expression levels, ligand pharmacology, signalling, and intracellular trafficking, rendering them promising new therapeutic targets[3,4]. In our study, we investigate the pharmacological characteristics of oxytocin receptor (OTR) interacting with other neuropsychiatric-relevant GPCRs, notably the dopamine D2 receptor (D2R), forming OTR-D2R heterodimers. To specifically target the OTR-D2R heterodimer, we develop bivalent ligands with different combinations of agonistic and antagonistic ligand types for the comprehensive pharmacological investigation of the consequences of dimer formation. Through the development and application of bivalent ligands, we aim to facilitate a promising therapeutic toolbox to effectively target OTR-D2R complexes and study the function of heteromerization and its influences on health and disease.

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Surface mapping strategies to understand folds of artificial proteins.

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Abstract

Characterizing solvent-exposed surfaces on biomolecular structures is essential for understanding their functional interactions, stability, and dynamics under physiological conditions. Traditional nuclear magnetic resonance (NMR) spectroscopy faces inherent sensitivity limitations, often necessitating high sample concentrations that hinder the physiological relevance of the data. We address this challenge by leveraging hyperpolarized water via dissolution dynamic nuclear polarization (DDNP) to selectively amplify signals from solvent-exposed regions on peptide. This method enables high-resolution, residue-specific mapping of solvent-exposed surfaces at low micromolar concentrations and physiological conditions. By selectively enhancing proton signals of exposed residues through hyperpolarized water transfer, this approach delivers real-time, residue-resolved insights into the dynamics of protein-solvent interactions. Our results highlight significant structural rearrangements in various biomolecules, providing detailed surface accessibility maps that reveal critical solvent-exposed regions and transient binding sites. This hyperpolarized NMR technique represents a robust, sensitive tool for elucidating biomolecular surfaces. Herein, its application will be expanded to artificial proteins based on single chain nanoparticles.

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Title

Supramolecular peptide lamellae with antimicrobial activity

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Abstract

To combatthe threat of antimicrobial resistance, the constant search for new compounds with unique mechanisms of action is required. In this regard, supramolecular assemblies, inspired by filament forming natural peptides, combined with the improved biostability of non-natural compounds, could offer a promising alternative to current small molecule antibiotics. To this end, we designed supramolecular peptide structures for which the assembly process is triggered by bacterial cell surface lipopolysaccharides (LPS). Inspired by the alternating chirality backbone pattern of some effective peptide antimicrobials, we employed lysine-rich heterochiral beta3-peptides, termed lamellin-2K and lamellin-3K, with optimal residual spacing for enhanced coordination on the phosphate groups of LPS [1]. Combined molecular dynamics simulations (MD), cryo-EM, TEM, and bacterial assays confirmed that the phosphate-induced conformational change of these lamellins led to the formation of thin, striped lamellar layers, where each stripe represents double arrays of H-bonded peptide molecules that are interconnected by phosphate ions. Detailed image analysis demonstrated that the lamellae penetrating deep into the bacterial cell have a rather uniform size distribution and, surprisingly, only a few of these supramolecules are sufficient to cause major cell wall damage making them efficient in destroying target cells. Besides morphological insight, the results also provide a missing mechanistic link for membrane-targeting agents, connecting how the antibiotic mechanism is built up from individual molecules through on-site formation of the active supramolecules that could lead to bactericidal activity.

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Title

Assessing Peptide structure and function through Nuclear Overhauser Effects for Enhanced NMR Sensitivity

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Abstract

Enhancing nuclear magnetic resonance (NMR) signal sensitivity for aliphatic protons in biomolecules holds significant potential for structural biology, particularly in studying large proteins. We investigate a cascade of nuclear Overhauser enhancement (NOE) pathways from hyperpolarized water to aliphatic proton sites. Through dissolution dynamic nuclear polarization (dDNP) of water [1,2,3], hyperpolarized protons are introduced into a target peptide solution, followed by intermolecular NOE transfer. This doubly relayed proton exchange strategy achieves rapid, positive signal enhancement in aliphatic protons, overcoming previous challenges of NOE signal suppression observed in direct water-to-side chain hyperpolarization attempts. In a model alanine-glycine dipeptide, sequential NOE transfer yields a substantial signal boost to alanine methyl protons within milliseconds, revealing a promising mechanism for hyperpolarization-based NMR of methyl groups—a common probe in large protein NMR [4]. These findings pave the way for enhanced, residue-specific detection of aliphatic protons, expanding the utility of hyperpolarization techniques for biomolecular studies. In a proof-of-concept this method can be expanded to applications to DNA and larger proteins.

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Title

Fluorogenic cell surface labelling using fluorescent molecular rotor-labelled peptide-boronic acid conjugates as viscosity sensors

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Abstract

Respiratory illnesses such as Chronic Obstructive Pulmonary Disease (COPD) or Cystic Fibrosis (CF) feature an extraordinarily viscous mucus hydrogel layer. The current knowledge of the viscoelastic properties of mucus is based on rheometry, particle tracking or diffusivity measurements1. These methods do not provide information about spatial viscosity distributions on the in thin layers of mucus formed on living tissue cultures. To this end, we devised fluorescent molecular rotor (FMR) dye-peptide conjugates with functional groups for targeting and binding cell surfaces, i.e. glycans. Cell impermeable peptide conjugates of FMR dyes with C-terminal hydrazides enabled fluorogenic cell surface staining via covalent linkage to sialic acids. Ratio imaging after co-labelling with an environmentally insensitive dye showed that the glycan bound FMRs respond to changes induced by the mucolytic agent TCEP.2 With an aim to characterize mucus hydrogels under physiological conditions we investigated the ability of more than 30 peptide probes to effectively label cell surfaces. From this screening, it emerged that a minimum of three boronic acids and at least five aspartates was essential to label the surfaces of the context relevant A549 cell line. Our most successful probe functionalized with Wulff-like boronic acid receptors3 (KD = 0.9 µM) exceeded similar probes having benzoxaboroles or acylphenylboronic acids. Further, wash-free cell surface labelling was achieved by utilizing FMRs like Cy3 or CCVJ thanks to their fluorogenic properties. In dual labelling experiments, we attached a viscosity-sensitive FMR probe in combination with a non-responsive fluorophore (Coumarin343) to surfaces of living cells. Ratiometric imaging of A549 cells, patient sputum samples as well as air-liquid interface cultures from patient derived lung epithelial cells revealed the spatial heterogeneity of mucus viscosity.

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Title

Addressing sustainability challenges in peptide synthesis with flow chemistry and machine learning

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Abstract

In the era of peptide therapeutics, solid phase peptide synthesis is becoming increasingly important in the pharmaceutical industry and related research. However, the high cost and the large amount of toxic waste generated during production overshadow the current technology, requiring the reduction of excess reagents and the replacement of the solvents used. Advances have been made to replace N,N-dimethylformamide with moderate success. Here, we report a recyclable anisole/dimethyl sulfoxide based and carefully tuned solvent system that is compatible with flow chemistry and outperforms DMF. By exploring the solvent parameter space, we have selected several mixtures, tested their swelling ability, amino acid solubility, coupling efficiency, and Fmoc-cleaving capacity, and found the Anisole/DMSO (17:3) mixture to be ideal for coupling. By adjusting the flow parameters, racemization was reduced to <2% in the case of His, and <1% for Cys. Several mixtures were screened for optimal Fmoc cleavage, selected to cover the solvent parameter space uniformly. To test the selected solvent mixtures for aspartimide formation, and Fmoc-cleavage efficiency, both scorpion toxin II (VKDGYI) and JR10-mer (WFTTLISTIM) challenging sequences were synthesized and new correlations between reaction rates and solvent parameters were found. Further parameter optimizations were performed using a machine learning algorithm (Bayesian optimization) to reduce aspartimide formation and maximize Fmoc-deprotection. With the final parameters obtained, the Aib-ACP (10-mer), the glucagon like peptide 1 (GLP-1, 30-mer) and bovine pancreatic trypsin inhibitor (BPTI, 58-mer) polypeptides were synthesized with high efficiency and synthetic speed (12 min/cycle). The method is ideal for high temperature synthetic approaches. Based on sustainability metrics, the applied synthetic flow chemistry protocol, with the greener solvent mixture (Anisole/DMSO) performs outstandingly well compared to traditional methods and to state-of-the-art synthesizers.

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Title

Chemoselective Peptide Transformations: From Synthetic Tools to Bioorthogonal Concepts

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Abstract

Chemoselective transformations are powerful tools in modern peptide and protein chemistry. In addition to being used for functionalization approaches, chemoselective transformations have also been employed in the field of chemical protein synthesis, for example as conjugation methods or, more recently, for the design of late-stage protecting groups.(1) In particular, reversible click reactions with high levels of chemoselectivity offer a variety of new possibilities. Recently, we reported tetrazine-thiol exchange (TeTEx) as a powerful tool for the chemoselective conjugation of cysteines to a variety of cargos.(2) In contrast to existing approaches, TeTEx offers both the possibility of reversible and irreversible attachment. We applied this technology for the in situ cyclization of peptides without the need for additional activation reagents or protecting group reshuffling.(3) With the help of mechanism-based design, we chemically tailored the reagents to achieve site-selective cysteine labelling with high precision and fidelity.(4) In recent work, we demonstrated that the use of this dynamic chemistry can be extended to the field of chemical biology, allowing for precision synthesis within a biological environment.(5)

(1) Nature Communication 2020, 11, 982
(2) RSC Chemical Biology, 2023, 4, 685-691
(3) Journal of Peptide Science, 2024, e3548.
(4) Manuscript in preparation
(5) Manuscript in preparation

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Title

Exploiting the potential of a SpyTag/SpyCatcher system in protein ubiquitination

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Abstract

The attachment of ubiquitin (Ub) chains to proteins is one of the most important posttranslational modifications, as it influences a broad range of cellular processes, such as protein degradation, DNA damage response and intracellular trafficking.[1] Ub can either be attached as a single moiety or as a polymeric chain consisting of several Ub units, linked by isopeptide bonds, mostly to lysine side chains.[2] Since all the seven lysine residues in Ub can be involved in forming the Ub chain, there are many different linkage possibilities that lead to a wide variety of signals in cells. In order to study the role of ubiquitination in biological studies, the modified protein has to be prepared in a homogeneous form and workable quantities. As this can be challenging by enzymatic methods, several synthetic and semisynthetic methods have been developed to generate homogeneously ubiquitinated proteins.[3] In this project, we aim to devise a new method for preparing ubiquitinated proteins based on the SpyTag/SpyCatcher system. The aspartic acid within the SpyTag peptide spontaneously forms a covalent bond with the lysine in the SpyCatcher protein when both are in close proximity.[4] In our strategy we attach the SpyTag sequence (16 AA) to one of the Ub unit(s) and we recombinantly express the SpyCatcher protein fused to the protein of interest (POI). When the different ubiquitin variants carrying the SpyTag are reacted with the POI-SpyCatcher, this results in POI-SpyCatcher/SpyTag-Ub conjugates. We plan to use this approach to prepare ubiquitinated proteins with K48-linked chain to test it in proteasomal degradation.

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Title

Continuous-Flow Peptide Synthesis: Greening the Process

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Abstract

The synthesis of peptides is crucial due to their roles in peptide-based medicines and drug discovery. Since its introduction by Merrifield, peptide synthesis has primarily been carried out on solid supports, and solid-phase peptide synthesis (SPPS) has undergone significant advancements. However, traditional SPPS methods still require a large excess of amino acids, and the widespread use of green solvents remains a challenge. Recently, continuous-flow (CF) techniques have gained prominence in synthetic methodologies. Here, we demonstrate that by applying CF technology and optimizing reaction parameters, the amino acid equivalents required for SPPS can be drastically reduced to approximately 1.5 equivalents. Additionally, the use of propylene carbonate (PC), regarded as one of the greenest solvents, was implemented in SPPS, leading to successful scale-up. Under optimized conditions, all 20 proteinogenic amino acids were coupled with excellent conversions using just 1.5 equivalents. Difficult peptide sequences were synthesized automatically with purities comparable to those reported in the literature, but with significantly less consumption of amino acids and solvents. Moreover, β-peptide foldamers with alicyclic side chains were synthesized in high yields, as well as N-methylated peptides using Fmoc-protected N-methylated amino acids. The technology was also applied to synthesize protected sequences rapidly, incorporating exotic and expensive amino acids at lower costs with minimal amino acid equivalents. Additionally, we confirmed that PC could replace N,N-dimethylformamide (DMF) in SPPS without any further optimization. This highlights the sustainable nature of PC as one of the greenest available solvents.

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Title

Fine-Tuning the Immune-Stimulatory and Cancer Cell Binding Properties of Immune System Engagers (ISErs)

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Abstract

Immunotherapeutic approaches, such as the well-established monoclonal antibodies (mAbs), are highly successful in treating cancer. Despite their therapeutic successes, this form of therapy faces challenges such as poor tumor penetration, high production costs, and overlong bioavailability.[1] A novel approach for targeting cancer are immune system engagers (ISErs), a class of synthetically accessible, peptide-based immunotherapeutic assemblies that show potential in addressing the limitations of mAbs. The design of ISErs includes at least two cell surface binders that recognize cancer-specific targets and are connected via inert polyethylene glycol chains with an effector peptide. Here, the short N-formylated fMIFL peptide agonist of bacterial origin is applied, that targets formyl peptide receptors on innate immune cells, leading to the recruitment of neutrophils to the cancer site.[2] The synthesis of ISErs is enabled via solid-phase peptide synthesis combined with chemo-selective ligation techniques, e. g., copper-catalyzed azide-alkyne cycloaddition reactions, which provide flexible and facile synthesis opportunities to obtain compounds of medium size (5-7 kDa).[3] The primary goal is to generate ISEr variants with optimized binding affinities and avidities towards different cancer markers and customized immune-stimulating properties. To achieve this, various aspects of the ISEr structure are modified, including the length of the inert PEG linker, the binder sequence, and the attachment of additional immune system agonists.

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Title

Discovery of an anti-inflammatory peptide in human placenta

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Abstract

The innate immune system is a powerful anti-microbial defense system. However, excessive or chronic innate immune activation causes and contributes to inflammatory disease. This is best illustrated in auto-inflammatory diseases such as type I interferonopathies, characterized by the chronic presence of type I interferon (IFN). However, current anti-inflammatory therapies are plagued by adverse effects, lack efficiency or are too costly. The human peptidome provides a unique source of highly potent immunoregulatory agents. Thus our aim was to discover novel human physiological anti-inflammatory peptides and characterize their molecular mechanism. Human peptide libraries were generated by chromatographic separations of extracts from pooled placenta samples. The individual fractions were probed for type I interferon (IFN) reduction using A549 epithelial reporter cells, expressing a Luciferase controlled by an IFN stimulated response element promoter. Hit fractions were iteratively re-chromatographed and re-analyzed for bioactivity and peptide content. Eventually, after four rounds of purification the contained peptides were identified by mass spectrometry. The lead peptide (PL03), a 16-mer, was identified by de novo machine learning aided discovery and is not templated in the human genome. Synthesized PL03 reduces type I IFN signaling at a low micromolar IC50 in A549 and THP-1 cells as well as in primary fibroblasts, in the absence of any cellular toxicity and without affecting related cellular pathways like the NFκB pathway. In summary, we discovered the peptide PL03 in human placenta that efficiently and specifically downregulates IFN-mediated signaling. Clarification of the physiological mechanism of PL03 will give unique insights into the regulation of innate immunity and prevention of auto-inflammatory diseases. Application and optimization of the peptide may pave the way for novel therapeutic approaches against diseases associated with excessive or chronic inflammation, including type I interferonopathies, sepsis and neurodegeneration.

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Title

Sea Anemone β-Defensin-Like α-Amylase Inhibitors as Promising Agents for Postprandial Hyperglycemia Control

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Abstract

Type 1 and 2 diabetes mellitus are widespread diseases, affecting approximately 9% of adults, with Type 2 diabetes often resulting from metabolic disorders linked to overfeeding, physical inactivity, and disrupted circadian rhythms. Diabetic patients often experience abnormally high blood glucose levels, leading to serious body damage. One of the active strategies for glycemic control is the inhibition of α-amylase and α-glucosidase, which reduces the degradation of polysaccharides. In this study, we report two α-amylase inhibitors, Mgf and Mgf-2, isolated from the mucus of the sea anemone Heteractis magnifica, which belong to the β-defensin family. Both peptides effectively inhibited mammalian pancreatic and salivary α-amylases, with inhibition constants (Ki) in the sub-nanomolar range, five orders of magnitude lower than the FDA-approved glucose-lowering agent acarbose. Structural characterization using 15N relaxation and molecular dynamics simulations (MDS) revealed that the YIYH-containing reactive loop of Mgf-2 is highly mobile. Ensemble protein-protein docking combined with MDS, and scanning mutagenesis identified hydrophobic interactions between the YIYH loop of Mgf-2 and the hydrophobic “rim” surrounding the amylase active site as the major determinant of the inhibitor-enzyme interaction. Additionally, the conservative residue M27, located on the second loop of Mgf-2, was found to play a key role in binding affinity. Oral administration of Mgf-2 to mice with induced Type 1 and Type 2 diabetes significantly reduced postprandial glucose levels, and repeated administration resulted in a reduction of fasting glucose levels. Finally, a BLAST search of the NCBI database revealed that homologues of these β-defensin-like α-amylase inhibitors are widespread across various species of sea anemones and corals. These peptides exist both as independent domains and as fusions with trefoil-like factor 2 protein, underscoring their significance for animal survival and suggesting potential directions for future research. This work was supported by the Russian Science Foundation (RSF 21-74-20147).

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Title

Optimizing peptide purification: HPLC scale-up process for high purity and efficiency

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Abstract
Peptides have emerged as promising therapeutic agents in recent years, offering high specificity and selectivity in treating various diseases. Effective peptide purification is essential to ensure drug efficacy and safety. Here, we present a systematic workflow for the linear scale-up of high performance liquid chromatography (HPLC) methods to purify synthetic peptides, using Angiotensin I, a peptide precursor in blood pressure regulation, as a model. The scale-up process from an analytical HPLC method involved gradient optimization, method adjustments to parameters such as particle size, flow rate, and column dimension, as well as overload studies to ensure the consistent purification of the crude peptide. The KNAUER HPLC Method Converter [1] was used to calculate the preparative scale method parameters. The optimized preparative HPLC method resulted in a purity increase of Angiotensin I to over 99% with minimal sample loss. To further enhance throughput and flexibility, a preparative liquid handler could be integrated into the system allowing for injection of large volumes and quantities, as well as fraction collection and re-injection of target fractions using one instrument. Our approach can serve as a guideline for efficient peptide purification workflows, optimizing both purity and productivity while maintaining chromatographic performance at a larger scale.
[1] https://www.knauer.net/lc-method-converter