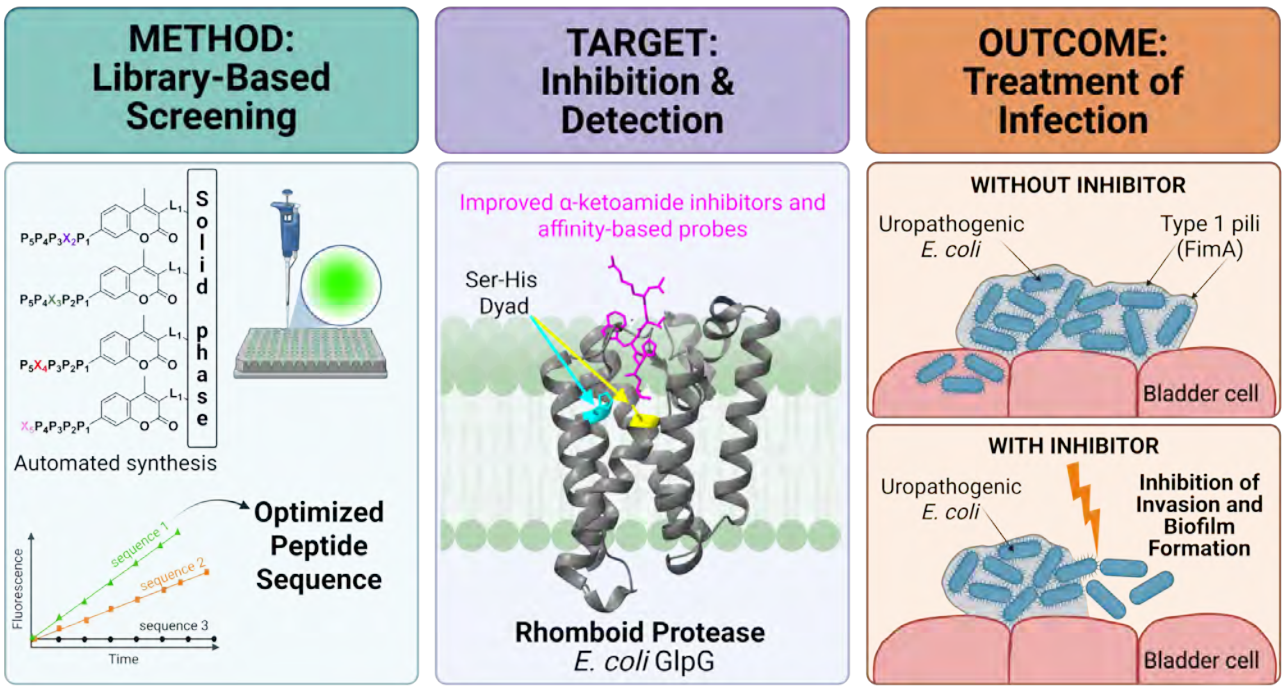
43rd Winter School on Proteinases and Their Inhibitors

Tiers am Rosengarten
March 11 - March 15, 2026

Program & Abstract Book



Library-based Approach for Profiling of Sequence Preferences of Rhomboid Proteases

Courtesy of Yurii Dubanych, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague

The cover shows a view of Tiers am Rosengarten.

43rd Winter School on Proteinases and Their Inhibitors
in Tiers (Italy)
March 11 - March 15, 2026

Organizing Committee

Klaudia Brix (Bremen)
Thomas Reinheckel (Freiburg)
Boris Turk (Ljubljana)
Hans Brandstetter (Salzburg)

Editorial Team

Claudine Wernsperger & Hans Brandstetter
Department of Biosciences and Medical Biology
University of Salzburg

Supported by

HENNER GRAEFF Foundation

GENERAL INSTRUCTIONS

Accommodation

All Winterschool participants are accommodated in the hotels Paradies, Laurin or in nearby appartments, all of which are within a few minutes walking distance. If you are unsure, please ask at the registration of Hotel Paradies. They have a list of all participants and are happy to help you.

While accommodation and meals including mineral water are covered, **any extras are not**:

- Please pay all drinks (except mineral water) directly after the meal.
- Please do not forget to pay your good night-drinks before you leave for the night.
- Please do not forget to pay all your extras (personal orders, …) at your hotel before departure.

If you want to rent ski equipment, please ask at the Hotel Paradies.

Scientific lectures

All lectures will take place at the *Haus der Dorfgemeinde* (City Hall).

Practical trainings

• Wednesday night, 21:00	Drug discovery in pardise (Hotel Paradies)
• Thursday evening, 20:00	Acoustics and kinetics in enzymology, featuring InhibiTIERS (City Hall)
• Friday evening, 20:00	Solutions in proteolytic assays (Hotel Paradies)

Social events

• Saturday afternoon	Hiking tours, excursions
• Saturday evening, Hotel Paradies	Gala Dinner & Farewell

Disclaimer

To enable and foster the unrestrained presentation and discussion of new results and ongoing research, every Winter School attendee must respect that all contents presented and discussed at the Winter School are private communications, not allowed for public use. Consequently, recording of lectures or photographing/ copying of slides or presentations is prohibited.

Wednesday, March 11

18:00 DINNER

20:00 – 21:15 Wednesday evening session: DRUG DISCOVERY IN ALLERGY

20:00 – 20:10 Welcome: Hans BRANDSTETTER, Klaudia BRIX and Thomas REINHECKEL

20:10 MAUN Henry (Genentech, Inc., San Francisco) *Mechanistic studies of therapeutic anti-tryptase antibodies for the treatment of asthma*

21:15 Hands-on session on DRUG DISCOVERY IN PARADISE (Hotel Paradies)

Please note:

Please consider time for discussion for your talk.
Standard talks are scheduled for **15 min**, i.e. **10 min presentation plus 5 min discussion**

Thursday, March 12

09:00 – 10:00 Thursday morning session I: CANCER

09:00 – 09:10 **Chairs:** Julian FRÄDRICH and Christoph PETERS

09:10 KUTSCHHEIT Kira (University of Freiburg) *Cancer-associated neutrophils and their protease ASPRV1 in murine breast cancer*

09:25 GONZALEZ QUEVEDO Cassandra (University of Kiel) *Cleavage of Golgi-Resident Substrates by Meprin β in Colorectal Cancer Cells*

09:40 KELLY Hannah (University of Oxford) *ADAM10 is a key regulator of MT1-MMP-mediated cancer cell invasion*

09:55 BRANDSTETTER Hans (University of Salzburg) The HENNER GRAEFF Foundation and the REMOD-HEALING MSCA Consortium

10:00 – 10:30 COFFEE BREAK, GROUP PHOTO

10:30 – 11:55 Thursday morning session II: INHIBITORS

10:30 – 10:40 **Chairs:** Henry MAUN and Dušan TURK

10:40 HILLER Lukas (Helmholtz Institute for Pharmaceutical Research Saarland (HIPS)) *Dipeptidic Phosphonates: Potent Inhibitors of Pseudomonas aeruginosa Elastase B Showing Efficacy in an in vivo Murine Keratitis Model and an ex vivo Human Cornea Assay*

10:55 HÖLL Anna (University of Salzburg) *Development of selective PC7-inhibitors*

11:10 LYTRA Elpida (Technical University of Denmark) *De novo design and experimental validation of a selective MMP-8 inhibitor*

11:25 ZARIC Miki (Jožef Stefan Institute) *Structural and proteomic analysis of the mouse cathepsin B-DARPin 4m3 complex reveals species-specific binding determinants*

11:40 KSIAZEK Mirosław (Jagiellonian University) *α2-macroglobulin (A2M) from Tannerella forsythia is the first bacterial A2M active as a dimer*

12:00 LUNCH

14:30 – 17:00 Thursday afternoon session I: SUBSTRATES, INTERACTOME

14:30 – 14:40 Chairs: Pitter HUESGEN, Konstantinos KALOGEROPOULOS & Christopher OVERALL

14:40 HUBER Hanna Mereth (University of Freiburg) *Protease Substrate Discovery by Improved C-Terminome Analysis*

14:55 DUBANYCH Yurii (IOCB Prague) *Library-based Approach for Profiling of Sequence Preferences of Rhomboid Proteases*

15:10 DEFANT Pauline (University of Salzburg) *Functional characterization of cytosolic legumain*

15:25 KLAUSHOFER Rupert (University of Salzburg) *New tools to study legumain activities*

15:40 SURIANO Alessia (Swedish University of Agricultural Sciences and Linnean Center for Plant Biology) *Evolutionary conservation of metacaspase type II activation following mechanical damage*

15:55 – 16:15 COFFEE BREAK

16:15 – 17:15 Career session - Open discussion on strategies, complementary methods, hot research areas and more

Chairs: Klaudia BRIX, Anna MOLES, Thomas REINHECKEL

18:00 DINNER

20:15 – 23:45 ACOUSTICS AND KINETICS IN ENZYMOLOGY: InhibiTIERS AllStar Band (CITY HALL)

Friday, March 13

09:00 – 10:25 Friday morning session I: IMMUNITY, INFLAMMATION, METABOLISM

09:00 – 09:10 **Chairs:** Klaus-Peter KNOBELOCH, Anna MOLES FERNÁNDEZ, Paloma RUIZ BLÁZQUEZ, Qun YANG

09:10 YANG Qun (Swedish University of Agricultural Sciences) *Protease-mediated plant immunity in response to damage*

09:25 GONCHARENKO Lina (University of Freiburg) *Dipeptidyl peptidase 8 and its impact on bone marrow derived macrophage differentiation*

09:40 RUIZ-BLAZQUEZ Paloma (Spanish National Research Council) *Cathepsin D-driven lysosomal activity contributes to macrophage mediated collagen remodeling during kidney fibrosis*

09:55 HENDEL Michel (Technical University of Munich) *A TIMP-1-dominated Immunosuppressive Secretory Signature accompanies malignant Progression in Pancreatic Ductal Adenocarcinoma*

10:10 FRÄDRICH Julian (Technical University of Munich) *Targeting TIMP-1-dependent Immunosuppression in Pancreatic Ductal Adenocarcinoma*

10:25 – 11:00 COFFEE BREAK

11:00 – 12:15 Friday morning session II

11:00 SOMMER Alexander (Technical University of Munich) *Gene Co-Expression Network Analysis Reveals Distinct Transcriptional Profiles of TIMP-1-Associated Cancer Cell-Subtypes in Pancreatic Ductal Adenocarcinoma*

11:15 ELSAIFY Menna (Technical University of Munich) *TIMP1 as a central regulator of stromal–immune interactions and ECM pathways in LUAD*

11:30 BRUNNER Vanessa (Technical University of Munich) *Pancreatic Ductal Adenocarcinoma-derived TIMP-1 Reprograms Hepatic Metabolism Towards Lipid Loss*

11:45 MANEVSKI Damjan (Technical University of Munich) *Cachexia-associated Impairment of Lipid Accumulation by TIMP-1-Triggered Suppression of mTOR Signalling in Adipocytes*

11:50 BERGER Heidrun (Technical University of Munich) *ESCRT-III–linked Immediate Early Gene regulation modulates AP-1–dependent TIMP-1 expression in neurons*

12:30 LUNCH

14:30 – 15:55 Friday afternoon sessions: PATHOGENS & INFECTIOUS DISEASES

14:30 – 14:40 Chairs: David JURNECKA, Brice KORKMAZ, Jan POTEMPA and Silja WESSLER

14:40 BRIX Klaudia (Constructor University Bremen) *Epithelial cell interactions with SARS-CoV-2 spike protein variants*

14:55 URSIC Tadej (Jožef Stefan Institute) *Processing of EBOV GP by cysteine Cathepsins*

15:10 MIKRUTA Katarzyna (Jagiellonian University) *Structural basis of activation and multi-megadalton dodecahedron assembly in the metallopeptidase zuzalysin*

15:25 WOCKENFUSS Gina (University of Greifswald) *Jep expression under physiological relevant conditions*

15:40 WADHWA Saruchi (University of Salzburg) *Protease mediated CagA cleavage in Helicobacter pylori infected immune cells*

16:00 – 16:30 COFFEE BREAK

16:30 – 18:00 Friday afternoon sessions: PATHOGENS & INFECTIOUS DISEASES II

16:30 KADEN Chelsea (University of Greifswald) *Functional expansion of the Staphylococcus aureus ClpX interactome using the TIE-UP-SIN workflow*

16:45 JURNECKA David (Czech Academy of Sciences) *Extreme C-terminus of filamentous hemagglutinin precursor is essential for interaction of Bordetella pertussis with ciliated epithelial cells*

17:00 – 17:05 DRUG DISCOVERY IN RESPIRATORY DISEASES
Chair: Brice KORKMAZ

17:05 CHAZEIRAT Thibault (INSERM) *First-in-class dual elastase/proteinase 3 inhibitor for inhaled treatment of neutrophil-driven lung disease*

17:20 PICCHI Elena (Chiesi) *First-in-class dual elastase/proteinase 3 inhibitor for inhaled treatment of neutrophil-driven lung disease*

17:35 REINHECKEL Thomas (University of Freiburg) *Survive or Die: An Intro to Tiers Extreme Hiking*

18:00 DINNER

20:00 – 23:00 Solutions in proteolytic assays (Hotel Paradies)

Saturday, March 14

09:00 – 11:15 Saturday morning session: SHEDDASES

09:00 – 09:10 Chairs: Kira BICKENBACH, Marcin DRAG and Walter STÖCKER

09:10 BICKENBACH Kira (University of Kiel) *Shedding of transferrin receptor I by meprin β regulates cellular iron uptake and iron homeostasis in vivo*

09:25 BÜLCK Cynthia (University of Kiel) *N-terminomic analysis reveals proteolytic processing of Cadherin-17 by ADAM10*

09:40 BECKINGER Silje (University of Kiel) *Targeting genetically inducible systemic inflammation through pharmacological intervention in a meprin α expressing mouse model*

09:55 HEßLER Emily Charlotte (University of Kiel) *Meprin β influences blood brain barrier (BBB) integrity*

10:10 BASTARDO TORIO Mariana Sofia (University of Kiel) *Regulated Ectodomain Shedding of the Polymeric Immunoglobulin Receptor (pIgR) by metalloproteases in the intestinal mucosa*

10:25 HELFRITZ Hannah-Louisa (University of Kiel) *Posttranslational modifications of meprin β and their impact on its intestinal role*

10:40 – 11:00 COFFEE BREAK

11:00 TURK Dušan (Jožef Stefan Institute) *Molecular civilization we call life*

11:25 Hans BRANDSTETTER (University of Salzburg)
Closing remarks & end of scientific sessions

12:00 LUNCH

SATURDAY AFTERNOON: HIKING, EXCURSION, ROUND TABLE DISCUSSIONS

12:00 – 17:00 Locally organized skiing tours (skiing equipment can be rented at the Frommer Alm and lunch package will be provided)

12:09 – 16:51 Public bus to Bozen (Tour of Bozen, Visit Ötzi the Iceman & more)

13:00 – 17:30 'Extreme' hiking tour

19:00 GALA DINNER AT THE HOTEL PARADIES

Sunday, March 15

09:09 Public bus to Bozen main station

10:09 Public bus to Bozen main station

11:09 Public bus to Bozen main station

12:09 Public bus to Bozen main station

ABSTRACTS

Mechanistic studies of therapeutic anti-tryptase antibodies for the treatment of asthma

Henry R. Maun[1], Caleigh M. Azumaya[2], Benjamin T. Walters[2,3], Rajesh Vij[4], Ashley Morando[3,5], Kelly M. Loyet[3], James T. Koerber[4], Alexis Rohou[2], Robert A. Lazarus[1]

Departments of Biological Chemistry[1] and Early Discovery Biochemistry[1], Structural Biology[2], Biochemical and Cellular Pharmacology[3], Antibody Engineering[4], Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA

Human β-tryptase, a tetrameric serine protease, is a major mediator of allergic inflammation. In severe asthma, patients with low Type 2 (T2) inflammation often derive limited benefit from current biologics, representing a significant unmet clinical need. We demonstrate that mast cell tryptase is elevated in severe asthma regardless of T2-biomarker status. Furthermore, active β-tryptase allele count correlates with systemic tryptase levels and predicts a reduced clinical response to anti-IgE therapy.

While active-site small molecule inhibitors often lack specificity, we developed a panel of highly selective inhibitory antibodies that allosterically dissociate active tryptase tetramers into inactive monomers. Using cryo-EM, X-ray crystallography, and HDX-MS, we mapped distinct molecular mechanisms of action, revealing the structural basis for the destabilization of the small and large tetramerization interfaces. We identify both monovalent and bivalent-dependent mechanisms; notably, one IgG acts as "molecular pliers," utilizing its hinge region to physically “pull the tetramer apart”. In functional models, an anti-tryptase antibody potently inhibited enzymatic activity, reducing IgE-mediated systemic anaphylaxis in humanized mice and suppressing airway tryptase in *Ascaris*-sensitized cynomolgus monkeys with a favorable pharmacokinetic profile. These findings establish allosteric tryptase inhibition as a promising therapeutic approach for severe asthma, particularly for the T2-independent patient subset.

Cancer-associated neutrophils and their protease ASPRV1 in murine breast cancer

Kira Kutschheit[1,2,3,4], Nelli Hautz[1], Carolin Suppe[1], Tobias Blessing[1], Thomas Reinheckel[1,3,4], Martina Tholen[1]

1 Institute of Molecular Medicine and Cell Research, University of Freiburg, Germany
2 Faculty of Biology, University of Freiburg, Germany
3 German Cancer Research Centre (DKFZ), Heidelberg, Germany
4 German Cancer Consortium (DKTK), partner site Freiburg, Germany

Measured by incidence, breast cancer is the second most common cancer type worldwide. In that context, recent research suggested that neutrophils potentially exert a more substantial influence on cancer pathogenesis than previously understood. Especially neutrophil proteases play crucial roles in many physiological as well as pathological processes. Thus, this research aims to shed light on a fairly unknown neutrophil protease in breast cancer, with a primary focus on its biochemical attributes and its functional significance.

A proteomic screen could identify adverse levels of a rather understudied protease in CD11b-enriched splenocytes of breast cancer-bearing mice, namely retroviral-like aspartic protease 1 (ASPRV1). In this screen, ASPRV1 levels exceeded those of the common myeloid marker S100A8. To biochemically explore this protease, a reintroduction of ASPRV1 translation start mutants into an ASPRV1 knockout SCF ER-Hoxb8 cell line demonstrated that the translation start might be misannotated leading to an exclusion of a putative transmembrane domain, potentially resulting in cytoplasmic location. Finally, to investigate ASPRV1 functionally in a cancer context, an *in vivo* 4T1 breast cancer model was used. Here, we could reveal that ASPRV1 showed higher levels in $CD11b^{+}Ly6G^{+}$ bone marrow cells of breast cancer-bearing mice. Additionally, lung and tumour tissues of these mice indicated elevated levels of ASPRV1 when compared to their healthy counterparts.

Further studies will explore additional biochemical and functional aspects of ASPRV1 as well as its potential as a novel therapeutic target in breast cancer.

Cleavage of Golgi-Resident Substrates by Meprin β in Colorectal Cancer Cells

Cassandra González Quevedo, Daniel Meisinger, Christoph Becher-Pauly, Kira Bickenbach, Silje Beckinger, Matthias Voss

1 Biochemical Institute, Christian-Albrechts-University Kiel, Otto-Hahn-Platz 9, 24118 Kiel, Germany

Meprin β is a metalloprotease highly expressed in intestinal epithelium and it has also been suggested to contribute to migration and invasiveness of colorectal cancer cells. Until now established substrates of meprin β are largely cell-surface or extracellular matrix proteins. Considering this, our N-terminomics experiments in a colorectal cancer cell line unexpectedly revealed candidate meprin β substrates implicated in glycosylation reactions catalyzed in the Golgi apparatus. Such Golgi membrane proteins are known targets of SPPL3, an intramembrane protease localizing predominantly to the mid-Golgi and known to regulate protein glycosylation by cleaving Golgi glycosylation enzymes. To further extend our initial data, we aim to use meprin β knock-out and active-site mutant knock-in colorectal cancer cells to perform more extensive N-terminome and Golgi proteome analyses. Moreover, we are validating candidate Golgi-resident meprin β substrates by immunoblotting and are investigating a possible interplay of meprin β and SPPL3 in the Golgi apparatus. Our findings suggest a potential role of meprin β in the Golgi apparatus, raising the possibility that this protease is already active early in the secretory pathway and that this unexpected behavior could contribute to tumorigenesis in colorectal cancer.

ADAM10 is a key regulator of MT1-MMP-mediated cancer cell invasion

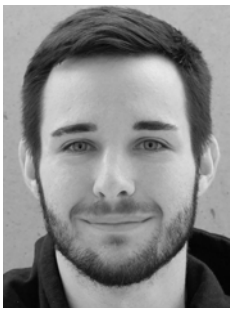
Hannah Kelly[1], Anna Hoyle[1], Iolanda Vendrell[2], Roman Fischer[2], Tonia Vincent[1], Yoshifumi Itoh[1]

1 Kennedy Institute of Rheumatology, University of Oxford, Roosevelt Drive, OX3 7FY, England
2 Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Old Road Campus, OX3 7FZ, England

Cancer invasion and metastasis are some of the greatest challenges in treating cancer. For cancer cells to escape from an initial tumour and invade other tissues, they must break down the extracellular matrix (ECM), as the ECM represents a physical barrier to migrating cancer cells. A crucial enzyme involved in this process is membrane type-I matrix metalloproteinase (MT1-MMP). MT1-MMP localises to the leading-edge of invading cancer cells, enabling these cells to degrade the ECM in a directional manner. This localisation step is a crucial step in MT1-MMP regulation and involves its transport to the leading-edge by kinesin superfamily proteins (KIFs). Our group previously showed that KIF3A and KIF13A transport MT1-MMP-containing vesicles to focal adhesions while KIF9v1 competes to transport these vesicles to other parts of the cell membrane, away from the leading-edge. However, it is still unclear how KIFs specifically recognise MT1-MMP-containing vesicles.

KIFs do not directly recognise MT1-MMP-containing vesicles. An adaptor protein (or multiple adaptors) likely mediates the recognition of vesicles by KIFs. These adaptors, therefore, must play a crucial role in the leading-edge localisation of MT1-MMP. We have identified ADAM10 as a potential adaptor protein for KIF3A to recognise the vesicle. ADAM10 is a metalloproteinase highly expressed in invasive cancer. We have shown that ADAM10 knockdown in HT1080 fibrosarcoma cells impairs MT1-MMP function. ADAM10 knockdown decreases proMMP2 activation, collagen film degradation, and Transwell invasion through a collagen film. We have also shown that ADAM10 colocalises with MT1-MMP in intracellular vesicles. Live-cell imaging has shown dynamic colocalization of MT1-MMP and ADAM10 in vesicles transported to the leading-edge of invasive HT1080 fibrosarcoma cells. Identifying ADAM10 as an adaptor protein involved in MT1-MMP vesicle transport has revealed a new mechanism of action for ADAM10 in invasive cancer and may lead to the identification of novel therapeutic strategies to prevent cancer metastasis.

Dipeptidic Phosphonates: Potent Inhibitors of *Pseudomonas aeruginosa* Elastase B Showing Efficacy in an in vivo Murine Keratitis Model and an ex vivo Human Cornea Assay



Lukas Hiller[1,2,3], Alexander F. Kiefer[1,2], Christian Schütz[1,2], Colya N. Englisch[4], Dominik Kolling[5], Andreas M. Kany[1,2], Roya Shafiei[1,2,3], Ahmad Aljohmani[6], Niklas Wirschem[1,2,3], Daniela Yildiz[6], Katharina Rox[2,7], Jesko Köhnke[5], Tim Berger[8], Markus Bischoff[4], Berthold Seitz[8], Jörg Haupenthal[1,2], Anna K. H. Hirsch*[,1,2,3,9]

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8 Department of Ophthalmology, Saarland University Medical Center (UKS), Kirrberger Straße 100, 66424 Homburg/Saar, Germany
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Pseudomonas aeruginosa (PA) is a widespread ubiquitous and opportunistic pathogen causing severe infections in organs such as the lungs, skin, and eyes. Notorious for its ability to gain antibiotic resistances, it is classified by the WHO as a high-priority target for new treatment strategies.[1,2] Due to its role in microbial keratitis, a major cause of blindness worldwide, targeting PA in the eye is especially important.[3]

A promising approach involves inhibiting LasB, an extracellular zinc-dependent protease crucial to the virulence of PA. LasB degrades structural proteins like elastin and collagen, as well as immune components such as cytokines and antibodies, causing tissue damage and aiding in immune evasion.[4,2] Therefore, inhibiting LasB may reduce pathogenicity and mitigate harm to patients without directly killing the bacteria, thereby lowering selection pressure for resistant forms.[4]

In a structure-based drug design campaign, highly potent dipeptidic phosphonates were synthesized, showing favorable pharmacokinetic profiles, excellent selectivity and good tolerability in vitro as well as in mice. Furthermore, a selected LasB inhibitor proved efficacious in a PA keratitis mouse model when combined with meropenem, underlining the potential for a synergistic treatment approach.[5]

To additionally enhance preclinical evaluation, a human ex vivo cornea assay was developed. Using non-transplantable corneal tissue, LasB-driven collagen degradation was quantified via hydroxyproline release, which after chemical transformation could be measured spectrometrically. Based on this proof of concept, the dose-dependent inhibition by selected compounds was confirmed, supporting the role of this assay as a useful tool for evaluating LasB inhibitors in microbial keratitis treatment and highlighting the potential of disrupting the virulence of PA to minimize irreversible damage to corneal tissue.

References:
[1] WHO - Antimicrobial Resistance Division (ARM) & Impact Initiatives and Research Coordination (IRC) WHO Bacterial Priority Pathogens List, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance; World Health Organization, 2024.
[2] Wretlind, B.; Pavlovskis, O. R. Pseudomonas aeruginosa elastase and its role in pseudomonas infections. Reviews of Infectious Diseases 1983, 5 Suppl 5, S998-1004. DOI: 10.1093/clinids/5.supplement_5.s998
[3] Hilliam, Y.; Kaye, S.; Winstanley, C. Pseudomonas aeruginosa and microbial keratitis. J Med Microbiol 2020, 69 (1), 3-13. DOI: 10.1099/jmm.0.001110.
[4] Everett, M. J.; Davies, D. T. Pseudomonas aeruginosa elastase (LasB) as a therapeutic target. Drug Discovery Today 2021, 26 (9), 2108-2123. DOI: https://doi.org/10.1016/j.drudis.2021.02.026.
[5] Kiefer, A. F.; Schütz, C.; Englisch, C.; et al. Dipeptidic Phosphonates: Potent Inhibitors of Pseudomonas aeruginosa Elastase B Showing Efficacy in a Murine Keratitis Model, Ver. 1. ChemRxiv, September 24, 2024. DOI: 10.26434/chemrxiv-2024-3mtxb.

Development of selective PC7-inhibitors

Anna Höll[1,2], Torsten Steinmetzer[3], Veronika Temml[4], Daniela Schuster[4], Hans Brandstetter[1,2], Sven O. Dahms[1,2]

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4 Department of Pharmaceutical and Medicinal Chemistry, Paracelsus Medical University, Strubergasse 21, 5020 Salzburg, Austria

Proprotein convertase 7 (PC7) is the most ancestral and yet the least investigated member of the furin-like proprotein convertase (PC) family. PC7 mediates the maturation of several disease-associated substrates and hence represents an interesting target to treat certain cancer types, infections, anxiety or cardiovascular diseases. However, the development of selective PC7 inhibitors is challenging due to the highly conserved substrate-binding pockets among PCs.

To overcome this limitation, we aim to target a less conserved cryptic binding pocket that has previously been described for the related PC furin. We started with generating a pharmacophore model with LigandScout4.5 based on a structure of PC7 in complex with a known cryptic-pocket binder predicted with AlphaFold3. With this model, more than 350 000 compounds from the SPECS and DrugBank databases were screened *In Silico*. Approximately 1 500 hits were obtained and subsequently evaluated using AlphaFold3 to identify compounds capable of inducing cryptic pocket opening. Eleven candidates were selected for experimental testing, of which three showed inhibitory activity against PC7. One compound inhibited PC7 with a K_i of 16 µM while showing no inhibition of furin and only minimal activity against PACE4, demonstrating high selectivity. AlphaFold3 predictions suggest that this compound fits well into the PC7 cryptic pocket but is sterically incompatible with the corresponding pocket in furin. Based on our lead compound, next-generation inhibitors were designed and synthesized, showing improved potency. In summary, this study reports the first selective PC7 inhibitors and establishes a foundation for subsequent optimization toward potent and selective PC7-targeted compounds.

De novo design and experimental validation of a selective MMP-8 inhibitor

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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that play central roles in extracellular matrix remodeling and cell signaling. Dysregulated MMP activity plays an important role in a wide range of pathological conditions, including cancer, chronic inflammation, and fibrotic diseases. Despite decades of effort, therapeutic inhibition of MMPs has largely failed in clinical settings, primarily due to poor selectivity across the MMP family and consequent dose-limiting side effects. These challenges highlight the need for fundamentally new strategies to achieve precise and controllable MMP inhibition.

In this project, we explore the use of AI-driven de novo protein design to design selective protein inhibitors targeting human MMPs. We utilize state of the art computational protein design tools and in silico screening to create and test for binders with high predicted affinity and specificity toward individual MMP family members. In our preliminary work, we generate and characterize a specific protein inhibitor against MMP8 with substrate assays and mass spectrometry-based degradomics. Furthermore, I will discuss how inhibitors created with de novo protein design can be evaluated through an integrated experimental pipeline including enzymatic activity measurements, biophysical characterization, and mass spectrometry-based degradomics to assess selectivity and off-target interactions.

Structural and proteomic analysis of the mouse cathepsin B-DARPin 4m3 complex reveals species-specific binding determinants

Miki Zarić [1,2], Livija Tušar [1,3], Lovro Kramer [1,†], Olga Vasiljeva [1,4], Matej Novak [1,2], Francis Impens [5], Aleksandra Usenik [1,3], Kris Gevaert [5], Dušan Turk [1,3], Boris Turk [1,6]

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5 VIB-UGent Center for Medical Biotechnology, UGent Department of Biomolecular Medicine, Ghent 9052, Belgium
6 Faculty of Chemistry and Chemical Engineering, University of Ljubljana, Ljubljana SI-1000, Slovenia
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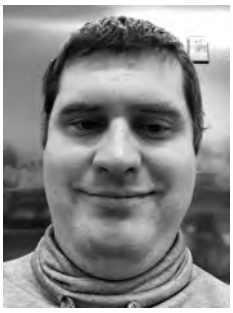
Cathepsin B (CatB) is a lysosomal cysteine protease that plays a major role in various pathologies and is therefore considered a valuable therapeutic target. To address species-specific inhibitor challenges, we characterized the selective binding of designed ankyrin repeat protein (DARPin) 4m3 toward mouse cathepsin B (mCatB) over human CatB (hCatB). The mCatB–DARPin 4m3 complex was validated by size-exclusion chromatography (SEC), nano-differential scanning fluorimetry (nano-DSF), and surface plasmon resonance (SPR), revealing high affinity binding (K_D = 65.7 nM) and potent inhibition (Ki = 26.7 nM; mixed competitive/noncompetitive). DARPin 4m3 showed no binding/inhibition toward hCatB. The 1.67 Å crystal structure of the complex—the first for mCatB—identified key interaction residues (e.g., I65/Q66 in mCatB vs. S65/M66 in hCatB) conferring selectivity. Proteomic analysis of endogenous substrates using a support vector machine (SVM) revealed greater similarity between mCatB and hCatB cleavages (Area Under the Curve (AUC) = 0.733) than between mCatB and other human cathepsins (AUC = 0.939–0.965). Clustering and SVM methods offer broadly applicable tools for protease specificity profiling in drug discovery. This study demonstrates the utility of DARPins for species-selective targeting and highlights the importance of integrated structural and proteomic approaches for dissecting protein–protein interactions.

a2-macroglobulin (A2M) from Tannerella forsythia is the first bacterial A2M active as a dimer



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α2-macroglobulins (A2M) are large, multidomain inhibitors that target a broad spectrum of proteases across all catalytic classes with disparate substrate specificities. It is achieved through unique trapping mechanisms, in which significant structural rearrangements in the A2M molecule are triggered by the prey protease hydrolysing the "bait region". As a result of A2M action, the protease becomes encapsulated, usually accompanied by the formation of a covalent bond between the protease and A2M. Mammalian A2Ms, including human (h) A2M, are active as a dimer and are very effective protease inhibitors. In stark contrast, bacterial A2M weakly inhibits proteases; protease encapsulation is negligible, and the key step in the inhibition mechanism is the formation of a covalent link with the protease. Here, we show that A2M from the human periodontopathogen *Tannerella forsythia* (TfA2M) is more similar to hA2M than to other bacterial A2Ms. The TfA2M monomer was completely inactive and gained inhibitory activity upon dimerisation. TfA2M efficiently inhibited various proteases (association rate constant, $k_{ass}>10^5$ $M^{-1}s^{-1}$) to form stable covalent inhibitor complexes. Interestingly, the lack of covalent linkage to the protease did not affect the inhibition efficiency of TfA2M. Protease inhibition was achieved using an exceptionally short bait region, and efficient inhibition was observed even with peptides of 5 kDa. The resolved three-dimensional structure revealed complete encapsulation of the prey protease, fully explaining the efficient inhibition of proteases by TfA2M. To conclude, TfA2M is much more similar to hA2M in its protease inhibition mechanism than to characterised A2Ms from other human bacteria.

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Protease Substrate Discovery by Improved C Terminome Analysis

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Proteins, fundamental components of life, are involved in almost every biological process within living organisms. Their functions are not only determined by their primary amino acid sequence, but also by their three-dimensional structure, post-translational modifications, and interactions with other molecules. Of particular interest are the terminal regions of proteins, specifically the C-termini, which plays different roles in various biological functions, including signal transduction, complex formation, and protein degradation. C-termini can be modified be many processes such as alternative splicing, post-translational modifications and proteolytic processing which generates new C-termini with every proteolytic cleavage. Therefore, many important fragments can only be distinguished at the C-termini, without a robust method to enrich and analyze these regions our understanding of protease activity and substrate specificity remains incomplete. This comprehensive substrate identification is essential for fully elucidating the roles of proteases in both physiological processes and disease.

In recent years, mass spectrometry-based proteomics has become the preferred approach for investigating protein structure and function on a large scale. This powerful technique enables proteome-wide identification, quantification, and characterization of proteins, including their modifications and interactions. However, standard bottom-up proteomics workflows face significant challenges in identifying and characterizing protein C-termini.

Here we introduce an optimized workflow based on 2-pyridinecarboxaldehyde (2-PCA) and undecanal labeling for selective isolation of protein C-termini. Its application enables comprehensive mapping of proteolytic processing and identification of caspase substrates involved in regulated cell death.

Library-based Approach for Profiling of Sequence Preferences of Rhomboid Proteases

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Rhomboid proteases are conserved across all kingdoms of life and fulfill diverse roles, ranging from quorum sensing in bacteria to the regulation of developmental signaling in *Drosophila*. In humans, they regulate key pathways such as ER-associated degradation, mitophagy, apoptosis, epithelial tissue maintenance, and cell signaling. In a pathological context, rhomboid proteases have been associated with Parkinson's and Alzheimer's diseases (PARL), type II diabetes (PARL), epithelial (RHBDL2) and colorectal (RHBDL4) cancers, malaria, and bacterial virulence, including biofilm formation in uropathogenic *Escherichia coli*. These associations highlight rhomboid proteases as attractive therapeutic targets. However, rhomboid-targeted drug development has been hindered by the lack of efficient methods to systematically probe rhomboid substrate preferences.

The most advanced rhomboid protease inhibitors to date are based on the α-ketoamide scaffold with oligopeptide substrate-derived 'head' and hydrophobic 'tail', whose composition strongly influences potency and specificity. Here we present a library-based fluorescence assay that enables rapid mapping of rhomboid substrate sequence preferences using fluorogenic peptide substrates. We validated this approach on *E. coli* rhomboid protease GlpG and the human protease RHBDL2. The identified sequence preferences were then applied to optimize α-ketoamide inhibitors, resulting in improved potency. In addition, optimized substrate sequences were used to generate activity-based probes. By directly linking substrate profiling to inhibitor and probe design, this approach provides pharmacological tools for studying rhomboid protease biology *in vitro* and *in vivo* and supports the development of selective rhomboid-targeted therapeutics.

Functional characterization of cytosolic legumain

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Legumain is a lysosomal cysteine protease that is primarily localized to the endolysosomal system. However, its overexpression and translocation to the cytosol or extracellular space have been associated with severe pathologies, including cancer and Alzheimer's disease. Notably, the active form of legumain, termed asparaginyl endopeptidase (AEP), has been shown to cleave substrates in the neutral cytosolic environment, despite being conformationally unstable at pH values above 6. Previous work from our group demonstrated that extra-lysosomal legumain activity can be maintained through intra- or intermolecular stabilization, including interactions with the prodomain, integrin αvβ3, or endogenous protein inhibitors.

In this study, we investigated the presence of legumain in the cytosol of cancerous and non-cancerous human cells and aimed to identify potential interaction and stabilization partners. Cytosolic co-immunoprecipitation assays were performed using HEK293T and adenocarcinoma (AGS) cells with antibodies targeting (i) the inactive zymogen prolegumain, (ii) active AEP, or (iii) the legumain stabilization and activity modulation (LSAM) domain. Interacting proteins were identified by data-independent acquisition LC–MS. Using this approach, we provide evidence for the presence of prolegumain in the cytosol of HEK293T cells and identify several candidate interaction partners. Computational modeling using AlphaFold predicted Forkhead box protein P4 (FoxP4) as a potential LSAM interactor. Importantly, initial microscale thermophoresis experiments provide first experimental evidence supporting a direct interaction between FoxP4 and the LSAM domain.

Taken together, this study provides a framework for subsequent analyses of cytosolic legumain and associated proteins in the cytosol of human cells. Ongoing work involves validation of candidates identified in the initial experiments and the establishment of assays incorporating AGS and immune (THP) cells in the context of *Helicobacter pylori* infection.

New tools to study legumain activities

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The cysteine protease legumain typically localizes to the endolysosomal system, where it is an important player for the immune system. Notably, besides its well characterized protease activity, legumain harbors an additional ligase activity. Both activities reside in the same active site and require substrates harboring an asparagine residue at the P1 position. Although both activities overlap, they show distinct pH preferences: legumain's protease activity is favored under acidic conditions, while the ligase activity requires neutral pH environments. Interestingly, translocation of legumain into compartments with neutral pH was observed under pathophysiological conditions, for example in the context of Alzheimer's disease or in different types of cancer. These opposing enzymatic activities raise important questions regarding their respective roles in disease, highlighting the need for novel tools to study legumain function.

To investigate the ligase activity of human legumain, we employed the sunflower trypsin inhibitor precursor peptide I (SFTI-I) as a model substrate. Using a positional scanning approach, we developed an optimized substrate for human legumain with increased cyclic product formation. In a next step, we aimed to convert the established MS-based assay into a FRET-based assay. However, we observed high levels of background FRET signal in the non-cyclized peptide, indicating an insufficient distance between the donor and acceptor fluorophores in the precursor peptide. To address this limitation, we aim to increase the distance between the FRET donor and acceptor by introducing a spacer into the peptide sequence.

To study the protease activity of legumain, we evaluated novel alkyne-based fluorescent probes. Co-crystallization with legumain enabled determination of the probe-enzyme complex structure and elucidation of the binding mode of these new probes.

Evolutionary conservation of metacaspase type II activation following mechanical damage

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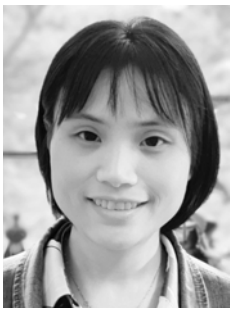
Plants are subjected to various abiotic and biotic stressors, including mechanical damages which can create entry points for pathogens and microorganisms. To counter these threats, plants have evolved mechanisms to recognize and respond to wounding. Metacaspases (MCAs) are a class of cysteine proteases conserved across different plant species, fungi, protists, and bacteria, playing a crucial role in stress-defence responses. In plants, these proteases are categorized into type I and type II. Additionally, type II MCAs can be activated either by calcium-dependent or calcium- independent mechanisms. Previous research has identified a calcium-dependent type II MCA in Arabidopsis, known as MCA4 (AtMC4/AtMCA-IIa), which is activated by injury, leading to the release of Pep1, a pivotal immunogenic peptide in plant wound signaling.

This study aims to explore the evolutionarily conservation of the activation mechanisms of MCAs in three evolutionary distant plant models *Chlamydomonas reinhardtii*, *Marchantia polymorpha*, and *Arabidopsis thaliana*. These models were selected for their ease of laboratory handling and broad evolutionary distribution, making them ideal for investigating MCA activation during damage. MCA-II activation levels were evaluated in *C. reinhardtii* subjected to mechanical damage such freeze-and-thaw and vortexing with glass beads.

Overall, this research aims to deepen our understanding of the evolutionary conservation of MCA-II activation by wounding across different plant species.

Protease-mediated plant immunity in response to damage



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Plant damage can range from death of a few cells to removal of entire organs, resulting from single or repeated events, such as by chewing insects. As a universal response to damage, cells release so-called damage-associated molecular patterns (DAMPs), in parallel to specific plant wound hormones, such as jasmonic acid (JA), and volatiles to alert the surrounding tissues of the impending danger. DAMPs, hormones and volatiles feed into downstream cellular responses through calcium (Ca2+) signalling, reactive oxygen species (ROS) formation, phosphorylation cascades, and defence gene expression in the surrounding tissue to deter herbivores and prevent water loss, pathogen infection, and ultimately plant mortality. While the read-out of wound responses and genetic players in plants have become increasingly better known, the mechanistic insight into the generation and transduction of signals at protein level is largely lacking, especially the involvement of proteases in plant wound responses.

Proteases are evolutionarily conserved enzymes with key regulatory roles and are classified according to their active site amino acid or proteolytic mechanism into aspartic-, cysteine-, glutamic-, metallo-, serine-, and threonine-type. Next to complete degradation, proteolysis can induce highly targeted cleavage in substrate proteins to alter their function. Classically, papaya papain is known to be activated within two minutes of damage, resulting in latex coagulation that physically seals off wound sites and deters herbivore feeding. In maize, the similar papain-like cysteine protease called Maize Insect Resistance 1 (Mir1), was found to confer resistance to fall armyworm caterpillars. Here, cysteine protesase will be used an example to discuss their role in response to damage.

Dipeptidyl peptidase 8 and its impact on bone marrow derived macrophage differentiation

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The amino-dipeptidyl peptidase 8 (DPP8) is an ubiquitously expressed intra-cellular serine peptidase. DPP8 cleavage specificity is at the N-terminus of substrate proteins if the penultimate amino acid is a proline or alanine. DPP8 and its homologue DPP9 were both described to take part in immune processes such as pyroptosis in monocytes and macrophages but also taking part in pathologies of the immune system and inflammation. Due to the high similarity between DPP8 and DPP9, selective inhibitors for these two peptidases are lacking, therefore making a discrimination of their functions difficult. To elucidate the DPP8 influence on the development of bone marrow derived macrophages, we genetically deleted DPP8 using a Cre^{ERT2} loxP- system in freshly obtained murine bone marrow.

The DPP8 deficient bone marrow cells showed decreased viability from the start of M-CSF induced macrophage development. Analysis of the cell death mechanism revealed a none-lytic and rather apoptotic way of cell death upon DPP8 deletion. Priming the DPP8 deficient cells with lipopolysaccharides (LPS) led to an unexpected rescue of cell viability, proposing a DPP8 independent way of myeloid cell survival and differentiation during LPS-activated inflammatory processes.

Taken together, our study reveals a novel role for DPP8 in early stages of none-inflammatory myeloid cell differentiation.

Cathepsin D-driven lysosomal activity contributes to macrophage mediated collagen remodeling during kidney fibrosis

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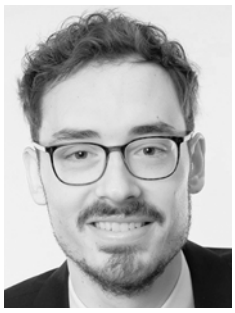
Background and Aims: Renal fibrosis is a common histopathological feature of chronic kidney disease (CKD) characterized by progressive and excessive accumulation of extracellular matrix (ECM) leading to impaired kidney function. Macrophages mediate ECM remodeling via lysosomal recycling. Hence, lysosomal protease inbalance in macrophages might be essential for fibrosis progression and regression in the kidney. Thus, the aim of this study was to investigate the proteolytic and degradative pathways associated to macrophages during renal fibrosis.

Methods: A macrophage-specific cathepsin D knockout ($CtsD^{\Delta Myel}$) mouse was generated by breeding LysMCre mouse with CtsD-floxed mouse. Renal fibrosis was induced by unilateral ureteral obstruction (UUO) for 10 days and folic acid administration for 30 days and assessed histologically. Fibrosis reversion was evaluated in a UUO reversal model by collagen degradation (R-CHP) staining and RT-qPCR of MMPs and macrophage polarization markers. In peritoneal macrophages, the secretome was analyzed using a protease array. Collagen degradation and endocytosis were assessed using DQ-Collagen I, FITC-Dextran and WB. scRNA-seq analysis was performed using GSE140023 dataset. Finally, macrophage subpopulations in fibrotic kidneys were determined by flow cytometry.

Results: scRNA-seq analysis of CtsD revealed high expression in macrophages after UUO-7days. $CtsD^{\Delta Myel}$ mice showed enhanced renal fibrosis in both fibrotic models by SR staining and αSMA levels. Further scRNA-seq analysis revealed that macrophage with highest CtsD expression exhibit a pro-resolutive phenotype. In agreement, a flow cytometry myeloid panel, confirmed an increase in CtsD expression during fibrosis and demonstrated that Arg1+ve and Mrc1+ve macrophages are the subtypes with the highest CtsD expression. $CtsD^{\Delta Myel}$ macrophages showed reduced proteolytic capacity of collagen I without impairment of the Endo180-mediated endocytosis. In addition, $CtsD^{\Delta Myel}$ macrophages also exhibited altered secretome. Finally, in a fibrosis resolution model, $CtsD^{\Delta Myel}$ mice exhibited impaired collagen degradation analyzed by R-CHP staining, accompanied by downregulation of MMP2 and MMP7 and a reduction in pro-resolving macrophage markers.

Conclusion: CtsD is expressed in pro-resolutive macrophages contributing to collagen remodeling and participating in renal fibrosis resolution.

A TIMP-1-dominated Immunosuppressive Secretory Signature accompanies malignant Progression in Pancreatic Ductal Adenocarcinoma



Julian Frädrich[1], Michel Hendel[1], Alexander Sommer[1], Vanessa Brunner[1], Damjan Manevski[1], Stefanie Bärthel[6,7,8], Ihsan Ekin Demir[2,3,4,5], Dieter Saur[3,6,7],Achim Krüger[1]

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Targeting immunosuppressive effects of the tumor microenvironment (TME) remains a critical challenge in treating pancreatic ductal adenocarcinoma (PDAC) patients. Despite recent advances in elucidating the orchestration of a pro-tumorigenic TME during malignant progression of PDAC, key targets for therapy options are still missing. Analysis of single-nucleus RNAseq data of 17 PDAC patients and subsequent trajectory analysis revealed insight in the malignant progression stages of the pancreatic epithelium from acinar into ductal and finally highly aggressive basal-like cancer cells. Integrating bulk transcriptomics of human and murine PDAC and proteomic data identified a cancer-immunoinstructive secretory signature (CISS) of 19 pro-inflammatory factors, associated with worse survival of PDAC patients. Expression levels of CISS concomitantly increased with malignant progression of pancreatic epithelial cells and was highest in basal-like cancer cells. The emerging pro-inflammatory cytokine Tissue Inhibitor of Metalloproteinases (TIMP)-1 was consistently induced throughout the progressing epithelium and the most dominantly expressed CISS factor in basal-like cancer cells. TIMP-1/CISS expression in basal-like cancer cells was associated with substantial suppression of anti-tumor activity of tumor-infiltrating lymphoid cells, most prominently of natural killer cell. Targeting TIMP-1/CISS expression in basal-like cancer cells therefore constitutes a promising therapeutical approach for the treatment of PDAC patients, as it will be shown by Frädrich et al. in this session.

Targeting TIMP-1-dependent Immunosuppression in Pancreatic Ductal Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a highly immunosuppressive tumor microenvironment (TME) and resistance to virtually all targeted therapies including immune checkpoint-blockade (ICB). Immunosuppression in PDAC correlates with upregulation of a cancer-immunoinstructive secretory signature (CISS), an expression-set of cancer cell-derived inflammatory proteins dominated by Tissue Inhibitor of Metalloproteinases (TIMP)-1. This upregulation of TIMP-1 expression peaks in the most aggressive basal-like PDAC cancer cell subtype. Here, we integrated bulk and single-cell transcriptomics, proteomics, functional approaches, and clinical parameters in human and murine PDAC leading to elucidation of cancer cell-derived TIMP-1 as a functional and targetable determinant of immunosuppression in PDAC. TIMP-1-dependent immunosuppression mainly affected cytotoxicity of tumor-infiltrating natural killer (NK) cells. TIMP-1 expression together with impaired NK cell cytotoxicity served as a prognostic predictor allowing stratification of patients at high risk of local cancer recurrence and metastasis. Toward a clinically exploitable approach to target TIMP-1, we identified a kinase activity pattern including MEK/ERK and receptor tyrosine kinases as crucial regulators of TIMP-1 expression in PDAC cells. Employing clinically approved kinase inhibitors trametinib and nintedanib synergistically suppressed TIMP-1 expression in PDAC cells *in vitro* and *in vivo*, restored intratumoral NK cell activity, and re-sensitized basal-like PDAC to ICB. This study is the first example of implementing a therapeutic opportunity of interference with the disease-promoting expression of the emerging cytokine TIMP-1 in PDAC.

Gene Co-Expression Network Analysis Reveals Distinct Transcriptional Profiles of TIMP-1-Associated Cancer Cell-Subtypes in Pancreatic Ductal Adenocarcinoma

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Tumors of Pancreatic ductal adenocarcinoma (PDAC) harbor two major cancer cell subtypes of the less malignant classical and the highly aggressive basal-like subtype. It was shown that basal-like cancer cells are the major source of TIMP-1, an emerging cytokine correlating with poor survival across virtually all cancer entities. However, the gene regulatory networks governing elevated TIMP-1 expression during progression of basal-like cancer cells remain incompletely understood. We here applied high-dimensional Weighted Gene Co-expression Network Analysis (hdWGCNA) on single nucleus RNA sequencing data from tumors of PDAC patients. This led to identification of classical and basal-like cancer cell-specific gene expression modules. *TIMP1* was identified as one central component of the basal-like–specific transcriptional program, and the congruency with the previously found correlation of TIMP-1 expression and cancer cell progression confirmed the suitability of hdWGCNA. hdWGCNA can now be used to dissect complex transcriptional profiles and establish interpretable co-expression modules mapped to distinct cancer cell subtypes.

TIMP1 as a central regulator of stromal–immune interactions and ECM pathways in LUAD

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Lung adenocarcinoma (LUAD) progression is shaped by complex interactions within the tumor microenvironment (TME), where stromal and immune cells modulate tumor evolution. Using single-cell RNA sequencing of 36,281 cells from treatment-naïve LUAD patients, combined with ligand–receptor inference via NicheNet and spatial transcriptomics, we identified coordinated signaling networks among macrophages, fibroblasts, and epithelial cells. Top ligands mediating these interactions included TGFB1, MMP14, CTHRC1, and IL6, each regulating multiple target genes across cell types. To investigate how the multifunctional protease inhibitor TIMP1 integrates into this network, we performed pseudobulk correlation analysis across cell types. TIMP1 expression was strongly correlated with CTHRC1 in fibroblasts ($\rho=0.65$, adj. $p<10^{-6}$) and moderately with TGFB1, while bulk correlations revealed additional associations with MMP14 ($\rho=0.54$) and IL6 ($\rho=0.26$), highlighting its potential as a central node in TME signaling. Extending this analysis to pathway activity using GSVA, TIMP1 expression was most strongly associated with extracellular matrix organization, collagen formation, angiogenesis, and IGF signaling pathways ($\rho=0.64$–0.73, adj. $p<10^{-31}$), linking ligand expression to broader functional programs in the TME. Overall, these findings suggest that TIMP1 acts as a key integrator of stromal–immune signaling and ECM remodeling in LUAD, coordinating multiple ligand–receptor interactions and pathway activities, and providing candidate targets for therapeutic intervention.

Pancreatic Ductal Adenocarcinoma-derived TIMP-1 Reprograms Hepatic Metabolism Towards Lipid Loss

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Pancreatic ductal adenocarcinoma (PDAC) profoundly reprograms systemic metabolism contributing to disease severity and mortality. The liver, the central regulator of systemic metabolism, is functionally dependent on hepatic lipid homeostasis. The emerging cytokine Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) is systemically elevated in virtually all inflammatory diseases including cancers such as PDAC and was previously shown to impact gene expression of various cells in the liver by interacting with its receptor CD63. Here, we investigated whether TIMP-1 impacts lipid metabolism of hepatocytes. Single nucleus RNA sequencing of PDAC-primed livers of TIMP-1-competent and TIMP-1-ablated mice revealed a TIMP-1-dependent induction of lipid catabolism-associated gene expression in hepatocytes. Complementary targeted metabolomics demonstrated a TIMP-1-dependent reduction of triacylglyceride (TAG) stores in the liver and the blood. This role of TIMP-1 was corroborated in the clinical context, as, in the blood of PDAC patients, elevated TIMP-1 levels correlated with decreased liver-associated TAGs. Exposure of hepatocytes to recombinant TIMP-1 was sufficient to induce an AMPK-dependent β-oxidation-associated catabolic phenotype and was abrogated by interference with a CD63 antibody. TIMP-1/CD63 interaction was necessary for AMPK activation and reduced hepatic lipid storage in livers from CD63-ablated PDAC bearing mice. Altogether, we identified the TIMP-1/CD63/AMPK axis as a tumor-induced trigger of hepatic lipid loss in PDAC. This tumor-liver reprograming axis suggests that TIMP-1 is a central mediator of metabolic disruption in inflammatory diseases.

Cachexia-associated Impairment of Lipid Accumulation by TIMP-1-Triggered Suppression of mTOR Signalling in Adipocytes

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Adipose tissue wasting is a direct consequence of impaired lipid storage in adipocytes, contributing to the severity of many chronic inflammatory diseases and leading to cachexia. Tissue Inhibitor of Metalloproteinases (TIMP)-1 is an increasingly recognized cytokine involved in systemic inflammatory signaling and a systemically elevated marker of cachexia in cancer. Here, we aimed to unravel a mechanistic role of TIMP-1 on lipid wasting in adipocytes. A murine preadipocyte cell line (3T3-L1), as well as primary preadipocytes isolated from murine and human adipose tissue were differentiated into mature adipocytes. Exposure to recombinant TIMP-1 increasingly impaired their capacity to accumulate lipids over time as determined by flow-cytometric analyses. TIMP-1 exposure led to reduction of lipid catabolism-associated mTOR/S6 signaling and AMPK activity. These data point at a functional role of TIMP-1 in lipid loss in adipocytes. This may be of broader clinical interest, since elevated TIMP-1 levels are a biomarker of virtually all cancers and other cachexia-associated diseases.

ESCRT-III–linked Immediate Early Gene regulation modulates AP-1–dependent TIMP-1 expression in neurons

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Neuronal plasticity relies on tightly coordinated transcriptional and extracellular remodelling processes. Tissue inhibitor of metalloproteinases-1 (TIMP-1) plays a central role in synaptic plasticity and neuroinflammatory conditions. TIMP-1 transcription is regulated by AP-1 consisting of the immediate early gene (IEG) products cFos and Jun family members. However, upstream mechanisms controlling IEG expression and subsequent AP-1–dependent gene expression in post-mitotic neurons remain incompletely understood. Here, we investigated the impact of CHMP7, a nuclear envelope–associated recruiter of the ESCRT-III membrane-remodelling machinery, on the expression of IEGs including AP-1 components and its impact on TIMP-1 expression in neurons. Using shRNA-mediated knockdown of *Chmp7*, we established a loss-of-function model in primary cortical neurons from neonatal mice. qPCR and mRNA sequencing upon CHMP7 depletion revealed significant downregulation of the AP-1–associated genes *Fos* and *Junb*. This was accompanied by a strong reduction of TIMP-1 expression suggesting that ESCRT-III–linked nuclear processes influence IEG transcription and modulate AP-1–controlled TIMP-1 expression. Hence, we propose a conceptual model in which nuclear envelope dynamics influence the expression of the protease inhibitor and emerging pro-inflammatory cytokine TIMP-1, which is relevant for synaptic remodelling and inflammatory processes in the brain.

Epithelial cell interactions with SARS-CoV-2 spike protein variants

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COVID-19 patients often suffer from gastro-intestinal (GI) tract complications during acute infection with SARS-CoV-2. The virus may even contribute to the development of Long COVID with ongoing symptoms, if it persists in the body. The intestinal mucosa serves as a reservoir, harboring SARS-CoV-2 and its spike (S) protein, which both exert damaging effects. It is therefore important to study the interactions of the S protein with intestine mucosal cells to delineate how it may contribute to the pathophysiology of SARS-CoV-2

We showed that intestine mucosal cells express SARS-CoV-2 relevant proteins in cell type-specific manner, which function as viral entry (co-)receptors (ACE2, Basigin and DPPIV) and as S protein-processing enzymes (Cathepsin L, legumain and TMPRSS2). Infection, replication and spreading of SARS-CoV-2 from intestine mucosal cells *in situ* can be studied *in vitro* using cell culture models. Thus, we used Caco-2 and HT29-MTX cells as surrogates of intestine epithelial and goblet cells, respectively. While ACE2-positive Caco-2 cells are infected by S protein pseudo-typed vesicular stomatitis virus (VSV) particles, ACE2-negative HT29-MTX cells were not. However, cytopathic effects on the actin cytoskeleton were observed in both cell lines, indicating that these were not exclusively initiated through ACE2 but may also depend on other receptors. Upon incubation of Caco-2 cells with increasing concentrations of S protein, ACE2 was down-regulated and DPPIV was up-regulated in a SARS-CoV-2 variant-specific manner. Moreover, the S protein-processing enzymes cathepsin L and legumain were up-regulated, whereas TMPRSS2 was less affected.

We conclude that the S protein of SARS-CoV-2 triggers common mechanisms via its binding to viral receptors and co-receptors, leading to molecular changes, eventually resulting in intestinal cell damage. This hypothesis is further supported by the notion that the cytopathic effects evoked by authentic SARS-CoV-2, S protein pseudo-typed VSV particles, and recombinant S protein all result in a similar outcome, namely, a leaky barrier function of the intestinal mucosa.

Our future investigations will yield a better understanding of mechanisms leading to acute and long-term cytopathic effects, with the long-term goal to identify cellular defense mechanisms for Long COVID prevention or therapy.

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Processing of EBOV GP by cysteine Cathepsins

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Ebola virus (EBOV) is a negative-strand RNA virus that causes an often fatal hemorrhagic fever in humans and primates. A key factor in viral entry into host cells is the interaction between viral glycoproteins and host cellular factors. The Ebola virus glycoprotein (EBOV GP) facilitates viral attachment and entry via the endosomal trafficking pathway, and cleavage of EBOV GP by cysteine cathepsin is believed to be important for EBOV entry into host cells.

EBOV GP ectodomain constructs were designed based on Lee et al. Acta Cryst. 2009, D65, 1162-1180 using the Zaire ebolavirus isolate sequence (NC_002549). The transmembrane domain was removed and C-terminal TEV recognition site followed by His-tag was added. EBOV GP was produced using transient expression in FreeStyle 293-F cells and the baculovirus vector expression system (BEVS). Furin was co-expressed to ensure efficient cleavage of EBOV GP into mature GP1 and GP2 two domain form, linked by disulfide bridge. The protein was purified by immobilized Ni-affinity chromatography and size exclusion chromatography, and maturation was confirmed by SDS-PAGE and western blot analysis.

The goal of this project is to find out whether the available endosomal cysteine cathepsins will process and/or degrade the EBOV GP at near physiological conditions. We hope that this work will contribute to understanding of physiological roles of endosomal proteases in Ebola virus cell entry.

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Structural basis of activation and multi-megadalton dodecahedron assembly in the metallopeptidase zuzalysin

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Porphyromonas gingivalis is a keystone pathogen of periodontitis, a highly prevalent chronic inflammatory disease affecting approximately 20% of the world population. Its pathogenicity relies heavily on proteolytic activities that underpin tissue invasion, immune modulation, and disease progression. Here, we investigate zuzalysin (ZUZ), a ~95-kDa metallopeptidase that defines an unprecedented mode of catalytic assembly in bacteria.

ZUZ is produced as a latent monomer that undergoes calcium-dependent activation coupled with hierarchical oligomerization. Calcium binding induces formation of catalytically active pentamers, which further associate into bipentamers and tripentamers, ultimately assembling into a ~5.6-MDa, ~355-Å virus-like dodecahedron. High-resolution structures spanning this assembly pathway were determined by X-ray crystallography and single-particle cryo-electron microscopy at 1.8-3.6 Å resolution, revealing the molecular basis of activation, inter-subunit interfaces, and proteolytic competence. The fully assembled particle contains 20 main entry pores and a central lumen lined by 60 active sites, forming a compartmentalized proteolytic chamber.

Functional studies using a *zuz* deletion mutant demonstrate that ZUZ contributes significantly to *P. gingivalis* virulence in a murine infection model. Quantitative proteomic analyses further reveal that loss of ZUZ alters protein networks associated with outer membrane homeostasis and the oxidative stress response, indicating a broader physiological role beyond proteolysis. Bioinformatic analyses show that ZUZ homologs are conserved across multiple members of the Bacteroidota phylum, suggesting that this assembly paradigm is broadly relevant beyond *P. gingivalis*.

Collectively, these findings establish ZUZ as the largest catalytic protein assembly resolved at high resolution to date and uncover a structurally and functionally sophisticated protease system central to bacterial pathogenicity and homeostasis.

Jep expression under physiological relevant conditions

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Staphylococcus aureus is a pathogen that causes various diseases. The conventional *in vivo* model for the bacterium is the murine model. Human-adapted *S. aureus* strains are less virulent in murine models, a phenomenon attributable to their elevated host specificity[1]. Consequently, mouse-adapted strains are used increasingly to overcome this limitation. One such strain is JSNZ, which secretes the JSNZ extracellular protease (Jep) at very elevated levels[2]. This investigation examined how Jep is expressed under different infection-relevant conditions and the co-regulation of other proteins to identify potential regulators of Jep.

The strain JSNZ was cultivated at control conditions (37°C), constant high temperature (42 °C), constant low temperature (32 °C), iron-limiting (with Bipyridine) and oxygen-limiting conditions. The expression of *jep* was analyzed at seven time points on transcriptomic level and on proteomic level (cell fraction and supernatant).

Secreted Jep makes up about 75 % of the supernatant fraction when grown at 42 °C, at 37 °C or under iron-limitation. This finding was confirmed at transcript level, where it was shown that *jep* expression increases over time, reaching a plateau in the stationary growth phase. Interestingly, iron limitation caused the highest expression in exponentially growing cells, which then declined, in contrast, the secreted Jep levels accumulated in the supernatant, seemingly without degradation. At 32 °C, both JSNZ growth and Jep secretion showed a delay. Under oxygen limitation, there was barely any Jep detectable. As it is highly similar to the serine-protease-like proteins (*spls*), it was hypothesized that *jep* could have a similar regulation pattern. However, the most positively correlated protein was another protease, ScpA. *In silico* prediction of possible regulators of *jep* indicated a high potential for PurR and SaeR. These findings demonstrate that the regulation of *jep* is complex and possible due to an interaction between various regulators, which requires further investigation.

1. Trübe, P. et al. (2019) Int. J. Med. Microbiol.

2. Wolfgramm, H. et al. (2025) Curr. Res. Microb. Sci.

Protease mediated CagA cleavage in *Helicobacter pylori* infected immune cells

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Helicobacter pylori is a gram-negative bacterial pathogen that is uniquely adapted to colonize the epithelial lining of the human stomach. It is recognized as type-1 carcinogen and is known to colonize nearly half of the world's population. *H. pylori* expresses a large number of virulence factors that influence its pathogenicity. Cytotoxin-associated gene A (CagA) is one of the most important virulence factors expressed by *H. pylori* and found to be associated with gastric cancer and mucosa - associated lymphoid tissue (MALT) lymphoma. CagA is injected into the cytoplasm of host cells via the T4SS system. The disrupted epithelial barrier allows the passage of *H. pylori* to the underlying tissue and permits direct contact with infiltrating immune cells. As *H. pylori* directly infects also infiltrating immune cells, it translocates CagA into these cells as well. Translocation of CagA has been observed in monocytic as well as B- lymphocytic cell lines including U937, Mec1, THP1 and Josk-M. CagA has been intensively studied in gastric epithelial cells but not many studies have been conducted in immune cells. CagA is not only translocated into immune cells but is also cleaved into a 100 kDa N-terminal and a 35-40 kDa C-terminal fragment. It has been observed that cleavage of CagA occurs within an asparagine (N) – rich region (aa 885 – aa 889).

Our findings suggest that CagA cleavage is a regulated process mediated by specific host protease rather than nonspecific degradation. In this study, we investigated the host proteases involved in CagA processing and characterized the cleavage site, highlighting the critical requirement of an intact asparagine stretch for efficient cleavage. Together, this work provides new insights into the host-mediated processing of CagA and identifies regulated proteolytic cleavage as a potential therapeutic target in *H. pylori*–associated disease.

Functional expansion of the *Staphylococcus aureus* ClpX interactome using the TIE-UP-SIN workflow

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Maintaining protein homeostasis is essential for sustaining life. In *Staphylococcus aureus* precise control of protein degradation is critical not only for adaptation to and survival of changing environmental and cell status conditions, but also for virulence regulation. Thus, proteins involved in proteostasis and proteolysis, particularly the Clp protease system, inherit great potential for therapeutic targeting. The Clp degradation machinery comprises the proteolytic core ClpP and associated ATPases, ClpX and ClpC, which also function independently as chaperones facilitating protein folding. Within this network, the ClpXP complex is of particular interest due to its involvement in essential cellular processes and pathogenicity. Although several ClpXP substrates have been identified using trapping mutants of ClpP, its interaction network remains incompletely defined.

In this study we aimed to expand the understanding of Clp-ATPase function by detailed functional characterization of the AAA+ ATPase ClpX.

To identify putative interaction partners, we employed a novel quantitative mass spectrometry approach, **TIE-UP-SIN** (**T**argeted **I**nteractome **E**xperiments for **U**nknown **P**roteins by **S**taple **I**sotope **N**ormalization) [1]. This method combines *in vivo* crosslinking of adjacent proteins *via* formaldehyde followed by affinity purification of Twin-Strep-tagged ClpX complexes. A triple-sample isotopic design using unlabeled and ^{15}N-labeled media enables robust normalization and discrimination of specific versus nonspecific interactions.

Using this workflow, we captured a reproducible and high-confidence set of *S. aureus* ClpX-associated proteins. The resulting interactome complements known ClpXP substrates and provides a pioneering experimental framework for dissecting Clp-mediated proteostasis and its role in bacterial adaptation and virulence.

Our approach opens new avenues for exploring the regulatory complexity of bacterial proteolytic systems and for uncovering novel targets in the fight against antibiotic resistance.

1 Schedlowski, M. et al. TIE-UP-SIN: a novel method for enhanced identification of protein–protein interactions. Front. Microbiol. 16, (2025).

Extreme C-terminus of filamentous hemagglutinin precursor is essential for interaction of Bordetella pertussis with ciliated epithelial cells

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Filamentous hemagglutinin (FhaB) is a large two-partner secretion (TPS) protein that mediates the attachment of Bordetella pertussis, the causative agent of whooping cough, to the motile cilia of respiratory epithelial cells. Although mature FHA, generated through multi-step proteolytic processing, has long been considered the primary functional adhesin, the molecular mechanisms of FhaB-mediated adhesion remain poorly understood. Here, we demonstrate that the extreme C-terminal domain (ECT) of FhaB is a highly conserved, structurally autonomous element essential for B. pertussis colonization and host interaction. While ECT deletion does not impair FhaB secretion, folding, or surface presentation, it results in severe defects in nasal colonization, bacterial shedding, and transmission in mouse infection models. Using primary human nasal epithelial cells differentiated at the air-liquid interface (ALI), we further show that ECT is indispensable for FhaB-mediated adhesion to ciliated cells. Notably, epithelial attachment abrogates FhaB's proteolytic processing, suggesting a functional role for the C-terminal prodomain in host-pathogen interactions. These findings redefine FhaB as a multifunctional virulence factor and identify ECT as a critical determinant of B. pertussis adhesion and colonization.

First-in-class dual elastase/proteinase 3 inhibitor for inhaled treatment of neutrophil-driven lung disease

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Neutrophils have a critical role in the innate immune response to infection, and in the regulation of inflammatory processes. A key component of neutrophilic activity is the secretion of proteolytically active neutrophil elastase-related serine proteinases (neutrophil elastase [NE], proteinase 3 [PR3] and cathepsin G), which are known to play a role in immune modulation and tissue repair following injury. Under physiological conditions, neutrophil serine proteinases activity is controlled by endogenous antiproteinases. However, disruption of the proteinase-antiproteinase balance can cause diseases progression in which neutrophilic inflammation is central to the pathology including non-cystic fibrosis bronchiectasis (NCFB) and cystic fibrosis (CF). We investigated the biochemical and pharmacological properties of CHF6333.04, a novel potent dual NE and PR3 inhibitor designed for the treatment of neutrophilic inflammatory lung diseases by inhalation. CHF6333.04 inhibited NE and PR3, respectively, by competition and by an allosteric mechanism. Displacement assays were performed by Microscale Thermophoresis using MCPR3-7, a conformation-specific antibody recognizing a structural state accessible in both the zymogen-like (Z*) and inactive mature (E*) forms of PR3. This setup is consistent with CHF6333.04 exerting its allosteric mechanism by affecting regions within the activation domain of PR3, as suggested by HDX-MS data. CHF6333.04 had no effect on the antibody-stabilized Z* conformation but displaced MCPR3-7 on the mature enzyme, indicating selective modulation of the pre-existing E/E* conformational equilibrium. The activities of membrane-bound forms of NE and PR3 from purified activated human blood neutrophils were fully controlled by CHF6333.04. Furthermore, CHF6333.04 inhibited NE and PR3 activity in sputum samples from patients with NCFB and CF translating into an anti-elastinolytic effect which was confirmed by elastin degradation in *ex vivo*. Our results show the potential of this first-in class dual NE and PR3 inhibitor to restore the proteinase-antiproteinase balance in chronic inflammatory diseases characterized by neutrophilic inflammation.

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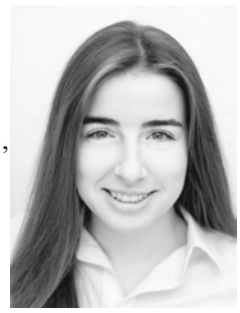
Shedding of transferrin receptor I by meprin β regulates cellular iron uptake and iron homeostasis *in vivo*



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Iron is indispensable for numerous biological processes; however, an excess of iron is cytotoxic. Accordingly, the maintenance of iron homeostasis requires tight regulation. The main protein mediating cellular iron uptake is transferrin receptor I (TfRI). TfRI also exists in a soluble form in human serum, which is employed as a diagnostic biomarker for anemia. Despite its clinical relevance, the proteases responsible for TfRI shedding have remained elusive.

Using mass spectrometry-based degradomic approaches, we identified the metalloproteases meprin β, ADAM10, and ADAM17 as sheddases of TfRI. *In cellulo*, these enzymes decrease cell surface TfRI abundance, associated with a significant impairment of cellular holo-transferrin uptake.

Notably, while ADAM10 and ADAM17 cleave TfRI at multiple sites within the stalk region of TfRI, cleavage by meprin β is limited to R109↓110E, fitting to the strong cleavage preference of meprin β for acidic amino acids at the P1' position. We have generated a neo-epitope specific antibody, specifically detecting human soluble TfRI (sTfRI) starting at E110, as generated by meprin β. This neo-epitope specific antibody could serve as a powerful tool for the specific detection of sTfRI generated by meprin β in human samples.

In vivo relevance for meprin β was observed in knock-out mice (*Mep1b*$^{-/-}$), which show increased serum iron concentrations accompanied by decreased sTfRI and hepcidin serum levels, supporting a regulatory function of this protease in systemic iron homeostasis. Furthermore, TfRI is highly expressed at the blood–brain barrier (BBB) and is intensively investigated as a therapeutic target for receptor-mediated drug delivery. In a disease-associated mouse model, we demonstrate that increased meprin β expression in brain endothelial cells diminishes TfRI-dependent transcytosis of holo-transferrin in primary murine brain endothelial cells.

N-terminomic analysis reveals proteolytic processing of Cadherin-17 by ADAM10

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Proteolytic processing of cell surface proteins is a critical regulatory mechanism in cellular signaling, cell-cell adhesion, tissue homeostasis, and related diseases such as cancer. Members of the A Disintegrin and Metalloprotease (ADAM) family are key mediators of ectodomain shedding, thereby modulating the function and availability of numerous transmembrane proteins. Among them, ADAM10 is a major sheddase with a broad substrate spectrum, including E-, N- and VE-Cadherin, Notch receptor as well as growth factor receptors, and is critically involved in epithelial biology and tumor progression.

Here, we employed HUNTER-based N-terminomics to systematically identify proteolytic cleavage events dependent on ADAM10 expression in human colorectal cancer cells. Using Colo320 WT and Colo320 cells deficient for ADAM10 (Colo320 *ADAM10*$^{-/-}$), we identified the cell adhesion molecule Cadherin-17 as a potential substrate of ADAM10. Cadherin-17 is an intestine-specific member of the cadherin superfamily. Although Cadherin-17 has been implicated in intestinal epithelial homeostasis and cancer progression, the molecular mechanisms governing its proteolytic regulation remain poorly understood. Notably, Cadherin-17 is used clinically as a diagnostic marker for primary tumors and metastases of gastrointestinal origin.

In vitro analyses confirmed that ADAM10 is the primary metalloprotease responsible for proteolytic processing of Cadherin-17 in Colo320, Caco-2, and HEK293T cells. ADAM10-mediated ectodomain shedding of Cadherin-17 resulted in reduced cell-cell adhesion, suggesting a functional role for this shedding event in the dynamic regulation of epithelial integrity.

In summary, this study identifies Cadherin-17 as a novel substrate of ADAM10 and provides new insights into ADAM10-dependent irreversible posttranslational regulation of cadherins. These results expand the current understanding of ADAM10 function in intestinal physiology and cancer biology and highlight ADAM10-mediated Cadherin-17 cleavage as a potential regulatory mechanism with relevance for gastrointestinal pathologies.

Targeting genetically inducible systemic inflammation through pharmacological intervention in a meprin α expressing mouse model

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The metalloprotease meprin α is expressed by specific cells in various organs, such as epithelial cells of the kidney and intestine, as well as keratinocytes of the stratum basale in the epidermis. Due to its unique cleavage specificity, meprin α modulates the processing of extracellular proteins and thereby contributes to tissue homeostasis. However, as the substrate spectrum of meprin α also includes numerous inflammation-associated mediators, altered expression levels and mislocalization of the protease have been linked to several inflammatory diseases, such as psoriasis vulgaris and systemic inflammation.

To mimic pathology-associated meprin α expression in the skin, we generated an inducible cre-based mouse model K5Mα. Induction of meprin α expression in keratinocytes resulted in a severe inflammatory skin phenotype and, within three weeks, a lethal, systemic inflammatory syndrome developed. Disease progression correlated with increased serum levels of IL-6, TNFα, and IL-1, cytokines that are also critically involved in human sepsis.

In order to assess the pathological relevance of these proinflammatory cytokines in the K5Mα model, mice were treated with Etanercept, Anakinra, and Olamkicept to inhibit TNFα, IL-1, and IL-6 trans-signaling, respectively. Pharmacological inhibition of these pathways showed no therapeutic effect on the development of the pathological skin phenotype or the lethal systemic inflammatory syndrome. However, mice that constitutively overexpress the soluble fusion protein gp130Fc (K5Mα sgp130 model), which selectively blocks IL-6 trans-signaling while preserving classic IL-6 signaling, developed inflammation-associated characteristics, previously described in the K5Mα model, albeit with markedly reduced severity and delayed onset.

Collectively, these findings identify pathological meprin α expression as a potent upstream driver of systemic inflammation. Further meprin α expression *in vivo* modulates IL-6 trans-signaling to a relevant extent, and this pathway may represent a critical contributor to the development of the systemic inflammatory failure observed in K5Mα mice.

Meprin β influences blood brain barrier (BBB) integrity

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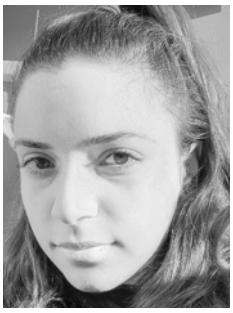
Dysregulation of the metalloprotease meprin β is associated with inflammation, cancer and Alzheimer's disease (AD). In AD patients' brains meprin β levels are elevated compared to control patients, contributing to the generation of neurotoxic amyloid β. Moreover, meprin β influences the BBB permeability, which is found to be increased in AD patients' brains.

We have identified meprin β as a regulator of the tight junction (TJ) proteins claudin-5 and occludin, controlling integrity and the function of the BBB. Meprin β knock out mice (*Mep1b*$^{-/-}$) showed increased protein levels of claudin-5 and occludin, associated with an increased barrier tightness, shown by elevated transendothelial electrical resistance (TEER) of primary brain endothelial cells, lower IgG levels in cerebrospinal fluid and reduced brain water content compared to wildtype mice. In comparison to that, an inducible brain endothelial cell specific meprin β overexpressing model shows decreased TJ protein levels and therefore an increased BBB permeability and lower TEER.

By using cellular overexpressing models, we proved that claudin-5 is a proteolytic substrate of endogenous membrane-bound as well as soluble meprin β. Cleavage takes place in the second extracellular loop of claudin-5. Now we further investigate the regulation of meprin β-mediated cleavage as well as a potential influence on TJ proteins on the transcriptional level.

Regulated Ectodomain Shedding of the Polymeric Immunoglobulin Receptor (pIgR) by metalloproteases in the intestinal mucosa



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Meprin metalloproteases are highly expressed in mucosal epithelia, particularly in the kidney and gut. In the small intestine, meprin β mediates cleavage and detachment of the major mucus component mucin-2, thereby preventing bacterial overgrowth and infection. In the colon, meprin β together with meprin α is involved in the proteolytic processing of galectin-3, a key mechanism for bacterial agglutination and microbiome regulation. Under chronic inflammatory conditions, both meprin α and meprin β are significantly reduced, as demonstrated in intestinal tissue from patients with inflammatory bowel disease. Thus, meprins are essential for maintaining intestinal mucus layer homeostasis, host defense against pathogens, and regulation of the commensal microbiome.

Employing N-terminomics, we identified the polymeric immunoglobulin receptor (pIgR) as a potential mucosal substrate of meprin α/β. pIgR mediates the transcytosis of dimeric IgA from the basolateral to the apical surface of gut enterocytes, where IgA is released into the mucus binding commensal and pathogenic bacteria to prevent epithelial interaction and invasion. For bacterial capture, IgA-bound pIgR must be shed from the apical epithelial surface. However, the protease responsible for this processing remains unknown. The identified cleavage site in pIgR between Glu589 and Glu590 is consistent with the cleavage specificity of meprins, which preferentially target negatively charged amino acids.

Preliminary in vitro data indicate that ectodomain shedding of pIgR can be mediated by meprin α/β but also by their regulatory interactors ADAM10/17, and MT1-MMP. We therefore propose the existence of a proteolytic shedding complex composed of meprin α/β, ADAMs and MT1-MMP that orchestrates the liberation of soluble pIgR and the subsequent release of bound dimeric IgA into the intestinal lumen. Overall, pIgR represents a crucial component of the first line of mucosal immunity, and its regulated proteolytic processing may be a key mechanism in host–microbiome interactions and pathogen defense.

Posttranslational modifications of meprin β and their impact on its intestinal role

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A healthy commensal microbiome and an intact epithelial barrier are central determinants of intestinal mucosal homeostasis. Disruption of these systems is a hallmark of chronic inflammatory bowel diseases (IBD). The metalloproteases meprin α and meprin β are highly expressed in mucosal epithelia, particularly in the kidney and gastrointestinal tract. Notably, expression of meprins is significantly reduced under chronic inflammatory conditions, as observed in intestinal tissue from IBD patients, suggesting a protective physiological role of these proteases in maintaining mucosal integrity.

Meprin β exerts distinct functions along the intestinal tract that depend on its cellular localization. In the small intestine, the soluble shed meprin β cleaves the major mucus component mucin-2 (MUC2), thereby promoting mucus turnover, limiting bacterial overgrowth, and preventing pathogen invasion. In contrast, in the colon, membrane-bound meprin β mediates proteolytic processing of galectin-3, a key molecule involved in bacterial agglutination, microbiome composition, and the regulation of host–microbe interactions. Thus, the intestinal function of meprin β is tightly regulated by its subcellular distribution.

Therefore, ectodomain shedding of meprin β represents a central regulatory switch that controls the balance between soluble and membrane-bound protease pools and is crucial for intestinal homeostasis. However, the molecular mechanisms regulating meprin β ectodomain shedding at the apical surface of epithelial cells remain poorly understood.

Preliminary data indicate that the intestinal microbiome plays a decisive role in controlling meprin β shedding. In germ-free mice, ectodomain shedding is completely absent, whereas in wildling mice with a complex microbiota, both membrane-bound and soluble meprin β are detectable, thus enabling different substrate cleavage. Furthermore, initial *in vitro* experiments suggest that glycosylation patterns within the known shedding region of meprin β critically regulate this process. Understanding this regulatory mechanism may provide insight into protease-driven pathology and barrier dysfunction in IBD.

Molecular civilization we call life

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There is not much to argue that science is about discoveries and inventions. Discoveries require assays - where an assay may be a laboratory experiment or an observed natural phenomenon. In this context, discovery represents the formulation of new knowledge by a discoverer. While such formulation can be preformed in stages, once established, repetitions of the assay do not alter that fundamental act. Similarly, it is with inventions. Inventions are implementation of knowledge made by inventor. They provide means of control over the process of manufacturing a product. Once the control is established, the invention is completed, perhaps improved later. However, these “Eureka!” moments cannot be repeated, therefore, they are not reproducible. As science today is all about reproducibility, this science cannot address either discoveries nor inventions as they are both one-time events.

How can science address inventions of nature? Science must be transformed to encompass one-time, non-repetitive events, such as making of inventions, into its narrative. Thus, the established laws of nature must be expanded to incorporate the laws of science shaped above. Consequently, the study of unique events is the next frontier in science, which is currently blocked by the ceiling of repetition. To harness evolution for our own good, we need to discover the mechanisms of making inventions. The first step towards harnessing of natural tools of evolution is admission of their existence.

It is a common understanding that life invented myriad of tools and processes, including the genetic code. Where the agreement stops is how this happened. This is the point where the modern synthesis (Darwinian) approach gets into trouble. While it touts the accidental origin of genetic code modifications, it clashes with the second law of thermodynamics, which suggests that the appearance of higher order is very unlikely by increasing disorder. Another problem a similarly shaped approach faces is the origin of the spark of life, phrased in questions like “Which mixture of salts makes life going?” Whereas life is a whole lot about the inheritance of information stored in DNA, both ways of thinking hint that life came to be from a state devoid of prior information, which implies a genesis from chaos.

So let me rephrase the question: how did life happen? Considering that the evolution of life is a history of inventions, the question arises: who invented these myriad tools and processes, and how. Natural selection perhaps? Is natural selection the assay which shapes discoveries and inventions without the discoverer and inventor? Do only humans have the right to discover and invent? My pat project to be published as a book addresses these questions and suggests that life did not appear from chaos, but is a result of long evolution of processes that exhibit self-awareness and a desire to sustain and expand control. If the DNA code did not come from nothing but was invented, where is the origin of inheritance? My take: Life speaks quantum. The chemistry of life resides in the quantum domain, which exhibits awareness and memory. The processes within the quantum domain can predate the long lived chemical memory written in DNA. When these processes invented the genetic code and learned how to use it, they became chemically literate and thus civilized. Hence, there was not only one spark of life, but a myriad of them in the forms of discoveries and inventions. To conclude, the book "Molecular civilisation we call life" is the one you will want to read.

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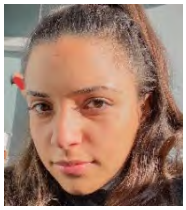

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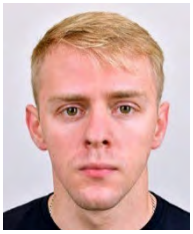

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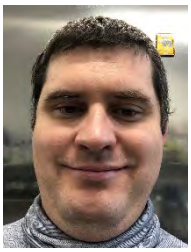

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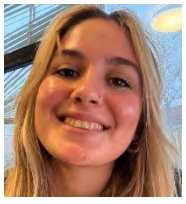

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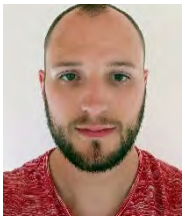

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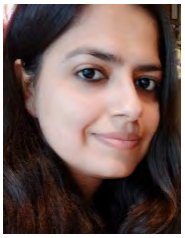

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The foundation is dedicated to fund scientific research on protease systems, related biological factors, and the molecular mechanisms underlying these processes, with a particular emphasis on tumor progression and metastasis.

Support will be primarily given to students, doctoral candidates, and early career researchers.

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Applicants

Doctoral candidates or post-docs (<35 years old) planning a short-term research stay at institutions in Germany or abroad.

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Leaders of research groups from two institutions in Europe, one of which must be from Germany, involved in a cooperation project.

Funding is provided for one visit each of the group leaders plus up to three additional representatives from one institution to visit the cooperation partner. The other participants should ideally be younger members of the applicants' groups (students, doctoral candidates, post-docs).

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Applicants

Medical doctors or scientists (<40 years old) with an active third-party funding application as the principal applicant, who are pursuing an academic career.

One-time grant of max. € 10,000. The financial support can be freely allocated for project-related expenses.

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