Functional Amyloid Meeting

24-26 September 2025
DESY, Hamburg

Book of abstracts

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Dear Participants of the Functional Amyloid Meeting

It is our great pleasure to welcome you to the Functional Amyloid Meeting. This gathering brings together researchers from across disciplines who are united by a common interest in the diverse roles of amyloids—not only as pathological agents but also as fascinating examples of functional biomolecular assemblies.

The abstracts collected in this booklet reflect the breadth and depth of current research in the field. Covering topics ranging from structural biology and biophysics to synthetic biology and biomedical applications. We hope they serve both as a useful reference during the meeting and as inspiration for future collaborations.

We look forward to stimulating discussions new ideas and the continued growth of our vibrant scientific community.

Kind regards

The Organizing Committee

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General information

Conference venue

CSSB, DESY building 15, Hamburg

Main entrance - Notkestr. 85, 22607 Hamburg, Germany

Side entrance - Luruper Chaussee 149, 22761 Hamburg, Germany

The Side entrance is opened for motorists from Monday to Friday 06:00 to 19:00, and closed on weekends. It is opened for pedestrians and cyclists constantly. (It is recommended to ring the bell next to the gate after 19:00 and indicate your destination to the guard-duty).

DESY WLAN: We will provide the information at your arrival eduroam is available on campus, we recommend you to use that if possible.

Registration: Attendees can register onsite in the foyer of building 15. (CSSB)

Meals: Breakfast are available at the DESY cafeteria (opens at 07:00, building 9) at your own expenses.

Coffee breaks and lunch will be in the foyer of building 15 (CCSB).

Conference Dinner: Alsterlagune, Ballindamm 14b, 20095 Hamburg

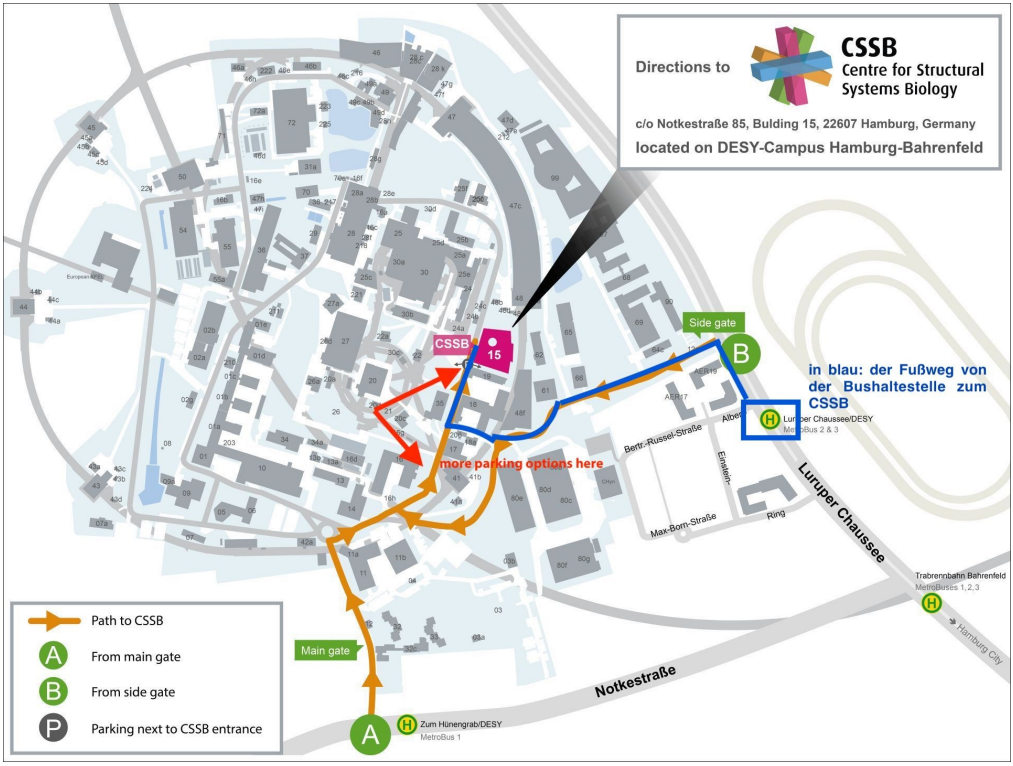
Supermarket: LIDL: From the main gate at Notkestrasse turn right and follow the street (700 – 800m). LIDL will be clearly visible on the left side of the street at the next junction.

BUDNIKOWSKY (Drug store) - Osdorfer Weg 106, 22607 Hamburg

Pharmacy: APOTHEKE an der Osdorfer Landstrasse Osdorfer Landstr.131, 22607 Hamburg

Cash machine/ATM: You will find a cash machine in the foyer of the DESY canteen (Building 9).

How to find the CSSB:



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ThT	✓		✓	✓		
SEC/SEC-MALS		✓				
DLS		✓				
CD					✓	
CryoEM					✓	
AFM					✓	
NMR		✓	✓			
FIDA	✓	✓	✓	✓		✓

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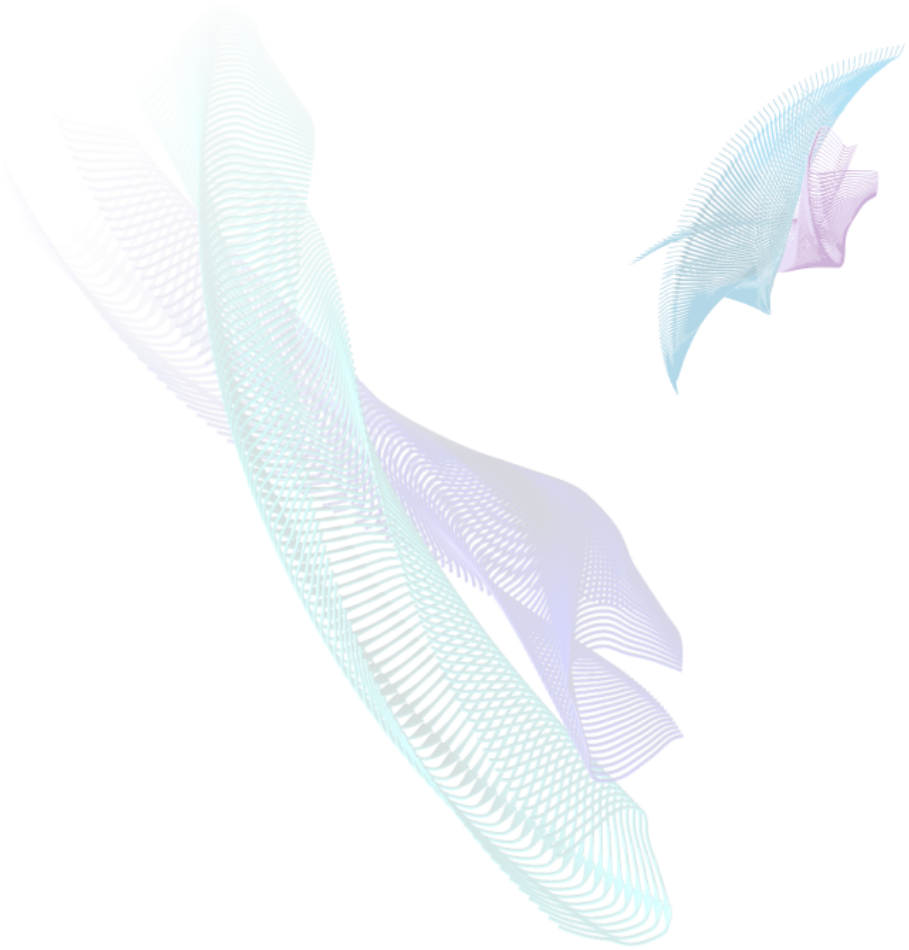
Session 1: Amyloids: From Their Origins to Their Roles Across Kingdoms of Life



Roland Riek, ETH Zurich

Roland Riek is a Full Professor of Physical Chemistry at ETH Zurich. His research bridges structural biology and the origins of life, with a focus on amyloids—protein aggregates that he explores as potential precursors of early life molecules. By studying how amyloids interact with RNA and DNA, his work provides key insights into molecular cooperation in the evolution of life. A pioneer in biological NMR, Roland has significantly advanced our understanding of protein dynamics, misfolded aggregates linked to diseases, and the molecular basis of cellular functions. With a career spanning top institutions, including the Salk Institute in La Jolla, California, he brings a wealth of expertise to the study of functional amyloids.

Abstract: On the potential role of peptide amyloids in the origin of life

It will be discussed whether peptide amyloids could have played an important role in the origin of life and that they may be the LUCA (last universal common ancestor) of protein folds. The following observations will be presented: Functional amyloids exist. An entire genome analysis demonstrates the presence of peptide amyloid segments above random. Peptide amyloids are very stable under harsh conditions. Peptide amyloids can be catalytic active including ATP synthesis from ADP with activated phosphate molecules. Peptide amyloids can replicate themselves in presence of amino acids and COS gas. Peptide amyloids interact with RNA stabilizing each other through avidity. Interactions between RNA and peptide amyloids indicate that the genetic code may be due to an RNA amyloid interplay.

Ina Maja Vorberg
German Center for Neurodegenerative Diseases, Bonn

Dr. Ina Maja Vorberg leads the Prion Cell Biology group at DZNE, where her research explores the molecular mechanisms underlying protein misfolding, aggregation, and intercellular spreading—a hallmark of prion and other neurodegenerative diseases. Her work has significantly advanced our understanding of how prion-like mechanisms contribute to cellular dysfunction, including the role of prion-like domains in protein aggregation and their propagation in neurodegenerative disorders. A key focus is on viral mechanisms that accelerate protein misfolding and dissemination, shedding light on the intersection of infection and neurodegeneration.
Dr. Vorberg is committed to uncovering how these processes can be modulated, offering insights into therapeutic strategies for conditions such as prion diseases and related disorders. Her research, often featured in leading journals, reflects her dedication to solving the challenges posed by protein aggregation and its pathological consequences.

Abstract: Gene products of exogenous and endogenous viruses accelerate the spread of protein misfolding between cells

Neurodegenerative diseases are characterised by progressive protein misfolding and prion-like propagation on pathological protein aggregates throughout the central nervous system. The aetiology of neurodegenerative diseases and the mechanisms of disease progression are incompletely understood. Evidence is accumulating that viruses may be important drivers of pathology due to their ability to induce neuroinflammation, disrupt protein quality control and cause neurotoxicity. Here we show that gene products of endogenous and exogenous viruses can also directly contribute to the intercellular spread of protein misfolding. Derepression of endogenous retroviruses, often observed in neurodegenerative diseases, enhances the intercellular spread of proteopathic seeds. Similarly, envelope glycoproteins encoded by common neurotropic viruses mediate receptor-ligand interactions and membrane fusion, thereby accelerating the transfer of protein cargo from one cell to another. Manipulation of viral gene expression, protein maturation or receptor interactions impairs virus-induced proteopathic seed transmission. Thus, both endogenous and exogenous viruses may be critical modulators of neurodegenerative disease and may represent novel therapeutic targets for disease intervention.

Einav Tayeb-Fligelman
Institute for Genomics and Proteomics, UCLA, Los Angeles

Dr. Einav Tayeb-Fligelman is a project scientist at the University of California, Los Angeles (UCLA), specializing in molecular biochemistry. Her research over the past decade has extensively focused on functional and pathological amyloids, with a current emphasis on developing therapeutic strategies for Alzheimer's disease. At UCLA, she is affiliated with the David Geffen School of Medicine and has been a member of the Eisenberg Lab since 2018. Her work focuses on the molecular architecture of both functional and pathological amyloids, with significant contributions to understanding their structure and how they relate to both pathogenic and functional roles in organisms.
Dr. Tayeb-Fligelman's interdisciplinary approach combines X-ray crystallography, cryo-electron microscopy, and computational biology to elucidate amyloid formation and its impact on cellular function. Her recent publications include studies on the low complexity domains of the nucleocapsid protein of SARS-CoV-2, providing insights into viral protein aggregation.

She holds a Ph.D. in structural biology from the Technion-Israel Institute of Technology and has just accepted a professorship at the Hebrew University of Jerusalem .

Abstract: Structural Investigation of Tau Amyloid from Alzheimer's Brains Treated with Drug Repurposing Candidates

Alzheimer's disease (AD) is the leading cause of dementia and the seventh leading cause of death in the United States. Despite extensive research, current AD therapeutics provide only limited benefits, underscoring the urgent need for novel treatment strategies. A central hallmark of AD is the aggregation of amyloid-beta (Aβ) and tau proteins into amyloids, with tau fibril accumulation closely correlating with disease progression and cognitive decline. Targeting these fibrils represents a promising therapeutic approach for AD.
Toward this end, we employed high-resolution cryogenic electron microscopy (cryo-EM) combined with biochemical assays to identify promising drug repurposing candidates capable of disrupting tau fibrils isolated from the brains of AD patients. Structural analysis provided key insights into the drug's mechanism of action. Our findings suggest that leveraging FDA-approved, blood-brain barrier-permeable compounds in a structure-guided approach could accelerate the development of tau-targeting AD therapeutics.

Jan Münch
Universitätsklinikum Ulm

Dr. Jan Münch is a leading researcher in molecular virology, with a focus on the intersection of amyloid biology and infectious diseases. As director of the Institute of Molecular Virology at Ulm University Medical Center, his groundbreaking studies have illuminated the role of amyloids in enhancing viral infections, particularly HIV-1. His team has identified amyloid fibrils in human semen that facilitate viral transmission, shedding light on their dual roles in infection enhancement and sperm quality control.
Jan's innovative contributions to virology and amyloid research have broad implications for understanding host-pathogen interactions and developing new antiviral strategies, making him a valuable speaker at this conference dedicated to functional amyloids.

Abstract: Functional Amyloids in Viral Infection and Gene Therapy: From SEVI to Synthetic Peptide Nanofibrils

While traditionally associated with neurodegenerative diseases, amyloids also exist as functional structures with key roles in immunity and reproduction. Our research explores this underappreciated class, focusing on semen-derived enhancer of viral infection (SEVI) and related amyloid-forming peptides. SEVI, formed from fragments of prostatic acid phosphatase, was initially discovered for its potent ability to enhance HIV-1 infection by promoting virion attachment to target cells. Follow-up studies have since expanded our understanding of SEVI's physiological relevance. SEVI fibrils bind to bacteria and enhance phagocytic uptake by immune cells and may contribute to sperm selection in the female reproductive tract. Our recent data now show that SEVI fibrils are induced by bacterial surface molecules and exert direct antimicrobial activity against clinically relevant ESKAPE pathogens, uncovering a novel antimicrobial function.

In parallel, we have identified fragments of hemoglobin that form amyloid-like fibrils with inherent antimicrobial activity, suggesting that fibril formation may be a generalizable mechanism of host defense. These insights have informed the rational design of synthetic peptide nanofibrils (PNFs) that mimic SEVI's viral-enhancing properties. PNFs significantly boost lentiviral and retroviral vector transduction efficiency, offering a defined, customizable and scalable alternative to commercial enhancers like Retronectin or Vectofusin. This has direct relevance for improving gene delivery in CAR-T and NK cell engineering.

Altogether, our work highlights the multifaceted role of functional amyloids in host-pathogen interactions and gene therapy, bridging innate immunity and biomedical innovation.

Short Talks of Session 1:

Key role of membrane fluidity in PSMα3-induced membrane disruption, towards its cytotoxicity

Marion Mathelié-Guinlet
Univ. Bordeaux, France

Phenol-soluble modulins α3 (PSMα3) are amphipathic peptides secreted by *Staphylococcus aureus* that self-assemble into cross-α fibrils and disrupt host membranes, contributing to virulence[1,2.] While their cytotoxicity is well-established, the role of membrane physical properties in modulating this activity remains unclear.
We previously showed that both electrostatic and hydrophobic interactions drive lipid binding and membrane insertion of PSMα3[3]. Here, we investigate how membrane fluidity influences PSMα3-induced disruption. Using infrared spectroscopy on model membranes of defined compositions, we found that PSMα3 induces greater perturbation when membranes are more ordered, due to cholesterol enrichment or lipid phase separation. This highlights the importance of hydrophobic core accessibility and membrane rigidity in promoting peptide insertion and membrane destabilization. To validate this mechanism, we have modulated PSMα3 hydrophobicity by changing its native N-formyl group to an acetyl group. This reduced membrane disruption, except for membranes with high-cholesterol content and/or phase separations, confirming that both peptide and membrane properties jointly govern the outcome.

AFM imaging further revealed that both peptides fibrillate at the membrane interface, with membrane thinning coinciding with fibril growth. Despite similar morphology, those fibrils may differ in their secondary structure, potentially affecting subsequent membrane disruption via carpet-like mechanisms.
Altogether, our results demonstrate that membrane properties - particularly fluidity and lipid segregation - are key determinants for PSMα3 activity[4]. These findings likely contribute to PSMα3 cytotoxicity in vivo and, ultimately, *S. aureus* pathogenesis.

1 Tayeb-Fligelman, E. et al., Science, 355 (2017) 831–833
2 Peschel, A. & Otto, M., Nature Reviews Microbiology 11 (2013) 667–673
3 Bonnecaze, L. et al., Nanoscale Horiz., 9 (2024) 1175-1189
4 Bonnecaze, L. et al., In preparation, 2025

Session 1

Atomic Insight into the Amyloid Architecture of the Silkmoth Eggshell: A β-solenoid to Cross-β transition?

Katerina Konstantoulea[1,2,3#], Peter Kunach[1,4#], Harichandra Tagad[1,2], Marc Diamond[1,2], Nikolaos Louros[1,2,3]

1 Center for Alzheimer's and Neurodegenerative Diseases, University of Texas Southwestern Medical Center, Dallas, TX 75390
2 Peter O'Donnell Jr. Brain Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390
3 Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX 75390
4 Department of Neurology, McGill University, Montreal, QC, Canada

Functional amyloids form the outer chorion layer of insect and fish eggs, shielding the embryo from dehydration, oxidative stress and microbial attack while imparting exceptional mechanical strength. In silkmoths, over 200 homologous chorion proteins fall into class A and class B superfamilies; both share a highly conserved central domain composed of imperfect hexapeptide repeats that readily assemble into amyloid-like fibrils[1]. Advance modelling studies predict this core to adopt a compact β-solenoid fold[2], but the fibrillar architecture of the chorion fibrils has remained experimentally unverified. Here, we purified a synthetic fragment representing the class A conserved domain and determined the structure of its self-assembled fibrils by cryo-electron microscopy. Contrary to the prevailing view that β-solenoid proteins form functional amyloids by stacking intact monomeric folds—exemplified by bacterial curli—we show that the silkmoth chorion domain assembles into fibrils whose core exhibits the successive layered cross-β-sheet stacking characteristics of pathological amyloids. These findings explain why short repeat fragments, acting as local aggregation-prone regions, can self-assemble in isolation; it also supports earlier liquid-crystalline nucleation models for chorion assembly[3] and opens avenues for designing biomimetic nanofibres with tunable stiffness.

References

1 Iconomidou, V. A., Vriend, G. & Hamodrakas, S. J. Amyloids protect the silkmoth oocyte and embryo. FEBS Lett 479, 141-145 (2000).
2 Tsiolaki, P. L., Louros, N. N. & Iconomidou, V. A. Hexapeptide Tandem Repeats Dictate the Formation of Silkmoth Chorion, a Natural Protective Amyloid. Journal of Molecular Biology 430, 3774-3783 (2018).
3 Hamodrakas, S. J., Hoenger, A. & Iconomidou, V. A. Amyloid fibrillogenesis of silkmoth chorion protein peptide-analogues via a liquid-crystalline intermediate phase. J Struct Biol 145, 226-235 (2004).

Session 1

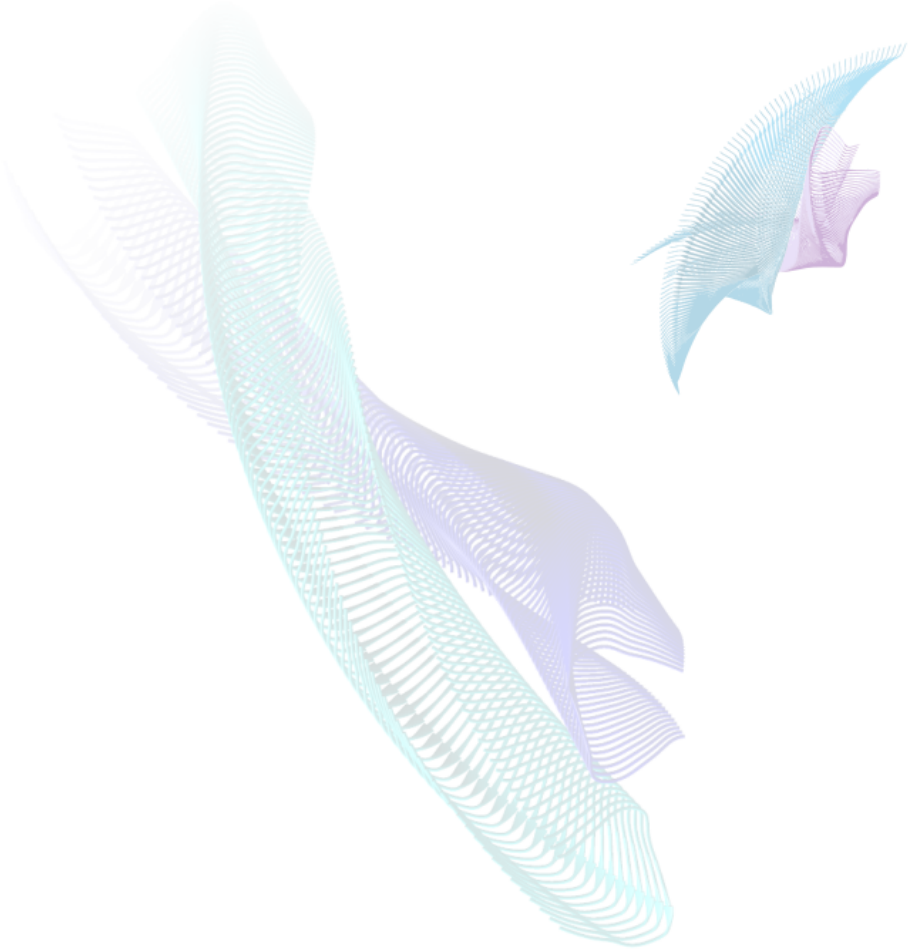
Bacterial Chaperone CsgC Exhibits Multi-Stage Amyloid Inhibition

Divya Kolli[1], Anthony Balistreri[1], Sanduni Wasana Jayaweera[3], Daniel Lundahl[3], Yilin Han[2], Lily Kalcec[1], Emily Goetzler[1], Rachel Alessio[1], Brandon Ruotolo[2], Anders Olofsson[3], Matthew R. Chapman[1]

1 Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA
2 Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA
3 Department of Medical Biochemistry and Biophysics, Umeå University, 901 87 Umeå, Sweden.

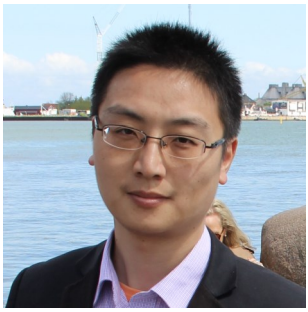
E. coli form biofilms which utilize a matrix composed of DNA, protein, and polysaccharides to protect the bacterial community from stressors. *E. coli* utilizes a functional amyloid called curli as the major proteinaceous component of the bacterial matrix. The major curlin subunit, CsgA, adopts a beta-sheet rich fold upon fibrillization. The chaperone protein CsgC inhibits CsgA amyloid formation. It is currently not known if CsgC inhibits amyloid formation by blocking formation of a pre-fibril nucleus, or if CsgC inhibits monomer addition to the growing fiber. Here, we found that *E. coli* CsgC interacted transiently and weakly with a monomeric, pre-nucleus species of CsgA which delayed amyloid formation. A transient CsgC-CsgA heterodimer was observed using ion mobility-mass spectrometry. When CsgC was added to actively polymerizing CsgA, exponential growth commonly associated with nucleation-dependent amyloid formation was lost. Adding preformed CsgA seeds did not rescue exponential growth, indicating that CsgC also has inhibitory activity during fibril elongation. Indeed, CsgC interacted strongly with CsgA fibers, suggesting that the interaction between CsgC and CsgA fibers can slow new fiber growth. CsgC displays unique inhibitory activity at multiple stages of amyloid formation and acts as an energy-independent chaperone that transiently interacts with prefibrillar CsgA and an amyloid fiber.

Session 2:
From Biological Roles to Practical Applications



Daniel Otzen
Aarhus University

Professor Daniel Otzen is a leading figure in protein biophysics and nanobiotechnology, serving as a Professor at the Interdisciplinary Nanoscience Center (iNANO) at Aarhus University. His pioneering work explores protein aggregation, including the mechanisms behind amyloid fibril formation, which has implications for diseases like Alzheimer's and Parkinson's, as well as functional amyloids in bacterial biofilms. His lab integrates cutting-edge techniques such as spectroscopy, electron microscopy, and calorimetry to unravel the biophysical principles of protein folding and misfolding.
Daniel holds a joint PhD from Aarhus University and Cambridge University, where he worked with Sir Alan Fersht, and has since built an illustrious academic and research career spanning fundamental studies on membrane proteins, detergent-protein interactions, and recently, the activity of cold-active and plastic-degrading enzymes. In addition to over 360 publications, he is known for translating his findings to both health-related and industrial applications.



Huabing Wang
Guanxi Medical University

Professor Wang obtained his PhD degree in protein biophysics with the supervision from Professor Daniel Otzen at Aarhus University in 2011, then he continued one year postdoc at Copenhagen University in Denmark. After that, he worked with Professor Mikael Oliveberg from 2013 to 2021 and obtained a permanent position as researcher at Stockholm University in Sweden. He moved back to China to work at Guanxi Medical University as full professor in 2021. He has made significant contributions to the field of functional amyloids. His recent work explores the potential of functional amyloids as biomaterials of the future, and the interactions between pathological and functional amyloids, contributing to our understanding of how these structures behave in biological systems. His interdisciplinary approach, combining protein folding and structure expertise with materials science, positions him at the forefront of developing novel biomaterials with potential applications in medicine and biotechnology.

Session 2

Abstract: Molecular structure and self-assembly in functional amyloid

Daniel E. Otzen[1], Samuel Peña Díaz[1], Zhefei Zhang[1,2], Jeremias Widmann[1], Yanting Jiang[2], Huabing Wang[2]

1 Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus C, Denmark

2 Department of Clinical Laboratory, the First Affiliated Hospital of Guangxi Medical University, Key Laboratory of Clinical Laboratory Medicine of Guangxi Department of Education, Guangxi Key Laboratory of Enhanced Recovery after Surgery for Gastrointestinal Cancer, Shuangyong Road 6, Guangxi Zhuang Autonomous Region, Nanning, 530021, China

Functional amyloid represents a benign way to use the cross-β fold, otherwise associated with neurodegenerative diseases such as Parkinson's and Alzheimer's. The most well-known examples of FuA are the bacterial proteins CsgA and FapC from *E. coli* and *Pseudomonas*, respectively. The structure of CsgA, solved by a combination of computational and experimental techniques, reveals a simple repetitive β-solenoid. We have recently solved the cryo-EM structure of FapC which reveals a more complex structure with a Greek Key motif and several layers of inner and outer cores, and will present our latest results underpinning this structure by mutational analysis and other material properties related to the amyloid structure. We also present evidence for both intrinsic and engineered catalytic activity within the FapC amyloid fold.

Both CsgA and FapC show a remarkable ability to cross-seed pathological amyloid such as Aβ and α-synuclein; Aβ in turn can inhibit inhibition of functional amyloid, hinting at a protective mechanism. Chaperones which target pathological amyloid also inhibit functional amyloid formation, though by different mechanisms; functional amyloid is typically blocked at the monomer/nucleation level and pathological amyloid at later stages. We will also present evidence that the bacterial microbiome has a number of potent amyloid-blockers whose effects extend to include both functional and pathological amyloid. All this suggests a number of common features linking different amyloid classes, both at the molecular and physiological level.

Maria Andreasen, Aarhus University

Dr. Maria Andreasen is an Associate Professor at Aarhus University in the Department of Biomedicine, where her research delves into the biochemistry and structural biology of functional amyloids, particularly those associated with bacterial biofilms. Her work focuses on understanding how amyloids, such as those formed by the protein FapC in *Pseudomonas* bacteria, contribute to biofilm architecture, antimicrobial resistance, and chronic infections. By exploring the distinct structural and interaction mechanisms of functional amyloids her research sheds light on their role in bacterial survival. Maria's studies have also uncovered the unique properties of amyloid nucleation and aggregation, including surface-catalyzed mechanisms, and their potential implications for developing therapeutic strategies against biofilm-related infections. She has contributed to several impactful publications, advancing our understanding of functional amyloid biology and its broader applications in health and disease.

Abstract: Structure and functionality in functional amyloids

Functional amyloids are found in various organisms ranging from bacteria to humans. They provide different functionalities ranging from virulence during infection to structural scaffolds in biofilms, to storage of hormones, to memory storage and others. The pahogenic *Staphylococcus aureus* produces biofilm that are staiblized by functional amyloids composed of phenol-soluble modulins (PSMs)[1]. PSMs are small (20-45 amino acids) amiphipatic peptides. As monomers, all PSM peptides display an α-helical structure, but they assemble into amyloids with a cross-β structure, except for PSMα3 that forms a unique cross-α fibrillar structure2. PSMα1 is essential in *S. aureus* biofilm assembly as it interacts with all other PSM peptides through cross-seeding and accelerates their assembly[3].

We have recently presented 6 different polymorphs of PSMα1 functional amyloids. The most redominant polymorph revealed a left-handed cross-β fibril composed of two U-shaped protofilaments with subunits tilted out-of-plane. We observe further polymorphism when altering the physio-chemical environment during aggregation giving us unique opportunity to fine tune the structural outcome of the self-assembly process of PSMα1 which also translate into differences in cytotoxicity and catalytic activity. These results are in stark contrast to functional amyloids from *Pseudomonas* formed form the protein FapC. FapC is a 250 aa protein from the Fap operon that upon secretion forms functional amyloids. Interestingly FapC functional amyloids displays a rare monomorphic structure despite changes in the physio-chemical environment.

References: 1. Schwart et al., PLoS Pathog. 2012, 8, e1002744; 2. Tayeb-Fligelman et al., Science. 2017, 355, 831-833; 3. Zaman et al., eLife, 2020, 9, e59776

Margaret Sunde
University of Sydney

Professor Margaret Sunde is a leading researcher in the field of amyloid science, based at the University of Sydney. She completed her PhD at the University of Cambridge and pursued postdoctoral research at both Cambridge and Oxford before moving to Australia in 2001. Her work focuses on unraveling the molecular mechanisms underlying amyloid fibril formation, with an emphasis on their structural and functional diversity. Her pioneering research has illuminated the shared beta-sheet structures of disease-associated and functional amyloids, providing insights into the distinct roles these fibrils play in biology. Margaret's recent studies have explored functional amyloids in microbial infections and viral evasion of host defenses. Notably, her research demonstrated how viral amyloid-forming proteins disrupt programmed cell death mechanisms, offering new perspectives on amyloid's role in host-pathogen interactions. Her multidisciplinary approach integrates structural biology with biophysical and biochemical tools, contributing to potential therapeutic innovations against diseases involving amyloids.

Abstract: The functional amyloid basis for herpes virus inhibition of mammalian necroptosis

Nikhil R. Varghese[1], Chi L.L. Pham[1], Brayden C. Williams[1], Crystal Semaan[1], Chengming He[2], Ann E. McDermott[2], Megan Steain[1] and Margaret Sunde[1]

1 School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney

2 Department of Chemistry, Columbia University, New York

The critical mammalian necroptosis complex is stabilized by a functional amyloid that provides a platform for the recruitment and activation of the kinase RIPK3 and its downstream substrate MLKL. The M45 protein from murine cytomegalovirus can inhibit mammalian necroptosis in human and murine cells; this activity of M45 and related proteins in other herpesviruses allows evasion of the host immune response and viral replication.

We have used cryo-EM and solid-state NMR to determine the core structure of the viral M45 protein in its homomeric amyloid fibril form, as well as the structure of the heteromeric viral:host M45:RIPK3 complex. This is a decoy, competing amyloid structure that prevents the phosphorylation of MLKL by RIPK3, and hence prevents cell lysis. M45 and RIPK3 alternately stack in an S-shaped amyloid structure, with an extended interface in the heteromeric fibrils. The structural integrity of the double turn structure is maintained through several features: two key sets of hydrogen bonds, colocalized polar residue ladders, and colocalized hydroxy group-containing side chains. The details of the interactions between viral and host amyloid-forming proteins shed light on the mechanism of necroptosis inhibition and the potential for the M45 protein to impact murine and human necroptosis. The M45:RIPK3 amyloid reveals the possibility for hybrid functional amyloids to form from imperfectly matched sequences and will inform the future development of necroptosis modulators based on this viral protein.

Raz Jelinek
Ben-Gurion University

Raz Jelinek is a distinguished researcher whose lab's focus spans energy storage, nanotechnology, advanced sensors, biological chemistry – with one of the focuses being functional amyloids. Raz Jelinek obtained his BSc in chemistry from the Hebrew University of Jerusalem, Israel, and his PhD from the University of California, Berkeley. Raz is a Professor at the department of Chemistry at Ben Gurion University, Israel, where he leads a research team focused on exploring the mechanisms that underlie amyloid formation and stability. He is passionate about uncovering how these protein assemblies can be harnessed for novel technologies and therapies. His work bridges the gap between fundamental science and practical applications, highlighting the innovative potential of amyloids in fields such as biotechnology and medicine.

Abstract: Catalytic functional amyloids: the good, the bad, and the beautiful

Protein amyloids have been observed in diverse protein systems and disease pathologies. While functional amyloids have been linked with varied physiological and bacterial systems, their precise "functions" are still debated. A recent groundbreaking study in our laboratory has shown that physiological amyloids – both pathological amyloids such as beta-amyloid as well as functional bacterial amyloids – exhibit remarkable catalytic properties. In this presentation, I will describe intriguing results, demonstrating catalysis by functional amyloids towards a range of biological and chemical reactions, some of which are intimately linked to disease pathologies, yet others play important physiological roles. Further studies will be presented depicting catalytic activity by synthetic amyloids, inspired by our observations.

Short Talks of Session 2:

Amyloid-Inspired Functional Nanomaterials: From Molecular Self-Assembly to their Biomedical Application

Javier Garcia-Pardo, Molood Behbahanipour and Salvador Ventura

Universitat Autònoma de Barcelona

Amyloids are protein assemblies characterized by a highly ordered fibrillar architecture. While many amyloids are associated with human diseases, their intrinsic properties, such as stability, robustness, and tunability, make them attractive building blocks for designing functional nanomaterials (Otzen D et al. Cold Spring Harb Perspect Biol. 2019). Among them, functional prions represent a unique subclass with a modular architecture: a disordered prion domain responsible for self-assembly, and one or more globular domains that confer activity. Notably, the globular region can be replaced by virtually any protein of interest, enabling the creation of fibrils that retain both structure and function (Wang W et al. Nanoscale. 2019). In some cases, steric hindrance may limit activity; however, this can be mitigated by dissecting prion domains into shorter self-assembling segments that preserve accessibility to the functional component.

We recently exploited a short prion-forming sequence from the yeast Sup35 protein to create amyloid-based nanofibrils with antiviral properties. By fusing this sequence to SARS-CoV-2 receptor-binding domain (RBD)-capturing proteins, LCB1 and LCB3, we engineered protein-only fibrillar materials capable of spontaneous self-assembly under controlled conditions (Behbahanipour M et al. J Colloid Interface Sci. 2024). These nanofibrils exhibited high affinity for the SARS-CoV-2 RBD and efficiently inhibited its interaction with the ACE2 receptor. Furthermore, they entrapped and neutralized SARS-CoV-2 virus-like particles with a potency comparable to that of therapeutic antibodies. We also demonstrated their application in creating patterned surfaces that selectively capture SARS-CoV-2 proteins in wet environments. Ultimately, these results highlight the potential of amyloid-inspired nanomaterials as versatile, programmable platforms for the development of next-generation bioengineered functional scaffolds with broad applications in biomedicine.

Session 2

Massive mutagenesis reveals a sweet-spot of amyloid formation which is ideal for RIPKs activity

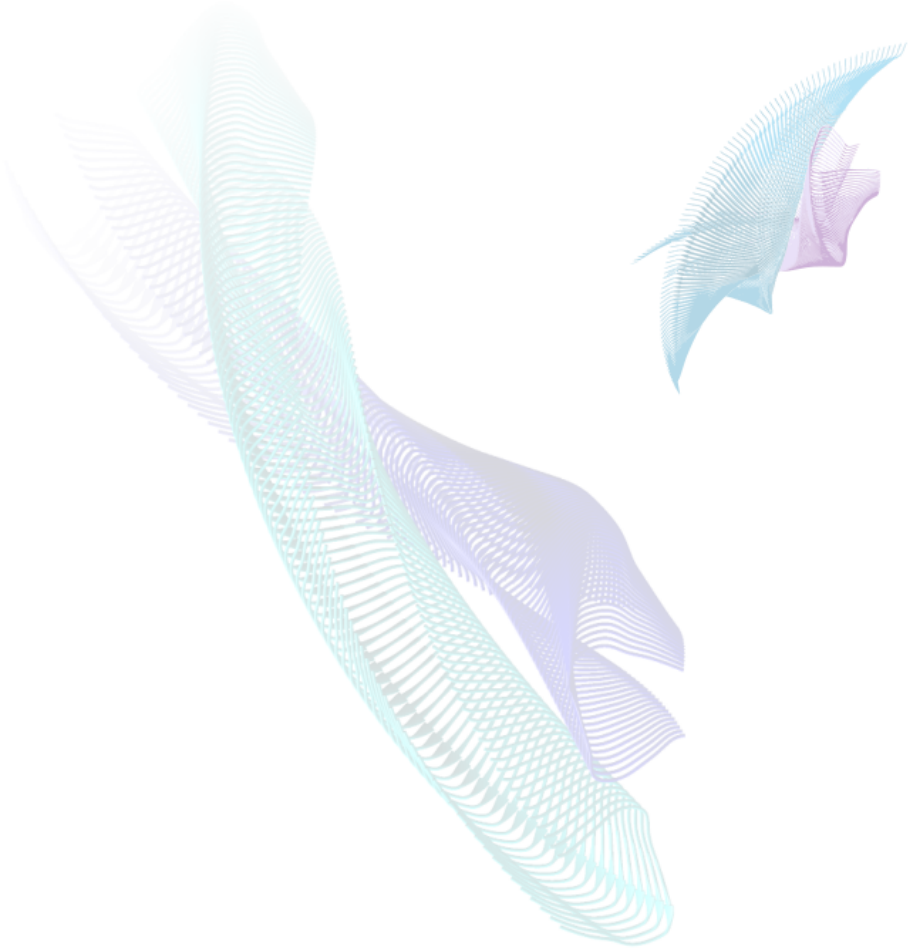
Mariano Martín and Benedetta Bolognesi

Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain.

RIPK1 and RIPK3, two key kinases in necroptosis, form functional amyloid fibrils via their RHIM domains to drive assembly of the necrosome, a macromolecular complex that amplify and propagates the signal leading to necroptotic cell-death. Systematically establishing the relationship between amyloid formation and function has been challenging, partially due to the lack of methods able to report on these two processes at scale.
Here, by combining deep mutagenesis to two massively parallel assays reporting on amyloid nucleation and necroptosis, we quantify the effects of ~3000 amino acid mutations in the two RIPKs. We identify a conserved aliphatic tetrad that forms a key interface at the core of both RIPK1 and RIPK3 fibrils and that is essential for nucleation. Intriguingly, RIPK3 nucleation relies solely on this single interface, whereas RIPK1 requires an additional, second aliphatic patch. Disruption of amyloid nucleation through mutation directly impairs necroptotic signaling, underscoring the functional coupling between fibril formation and kinase activity. Supporting the physiological relevance of amyloid nucleation, we also find that variants altering nucleation are rare in the human population.

Taken together, these results suggest that the RHIM domain evolved to reach an ideal level of amyloid nucleation that is optimal for kinase activity and thus for necroptosis to proceed. Systematically understanding the relationship between amyloid formation and function for these kinases is instrumental for guiding the development of therapeutic strategies that can modulate cell death and more generally impacting synthetic biology approaches towards engineering active amyloids.

Session 3: Functional or Pathological: Which One Are You?



Marcus Fändrich
Universität Ulm

Professor Marcus Fändrich is a leading expert in the structural and biochemical mechanisms of amyloid formation and its implications for human health and biotechnology. As Director of the Institute of Protein Biochemistry at Ulm University, his research spans the molecular understanding of amyloid fibrils in disease contexts, such as systemic amyloidosis, and their potential technological applications. He has contributed extensively to elucidating how protein misfolding and aggregation lead to pathological deposits, with a focus on antibody light chains in amyloidosis and other amyloid-related conditions.
Professor Fändrich leads interdisciplinary efforts utilizing advanced techniques like cryo-electron microscopy to unravel the variability and organ-specific effects of amyloid deposits. His work not only informs fundamental science but also seeks to translate findings into innovative diagnostics and treatments. An accomplished scientist, Professor Fändrich has published widely and spearheaded collaborations addressing the biochemical basis of protein misfolding diseases.

Abstract: Cryo-EM structures of amyloid fibrils in diseases and biotechnology

The advent of cryo-EM boosted our understanding of amyloid fibril structures. During the past 10 years we determined the structures of amyloid fibrils from various human diseases as well as from artificially designed fibrils that were developed for biotechnological purposes. In the presentation I will give an overview over the determined structures, highlighting specific properties of pathological fibrils and their differences from in vitro formed filaments.

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Salvador Ventura
Institute of Biotechnology and Biomedicine

Dr. Salvador Ventura is the director of I3PT-CERCA and leads the Protein Folding and Conformational Diseases group at the Institute of Biotechnology and Biomedicine (IBB), Autonomous University of Barcelona (UAB), where his research focuses on fundamental aspects of protein folding, misfolding, and aggregation. His group works on understanding neurodegenerative diseases related to protein misfolding, combining computational and experimental methods to study the mechanisms underlying these disorders and to design novel self-assembled materials. Dr. Ventura's contributions, including over 300 scientific articles and 20 patents, reflect his dedication to unraveling the complexities of protein aggregation and its pathological consequences. His recent projects focus on uncovering novel proteins that self-aggregate to uncover their functional pathways and their involvement in diseases. Dr. Ventura's work not only advances fundamental science but also holds promise for the development of new therapeutic strategies for protein misfolding diseases and materials for nanotechnology applications.

Abstract: Amyloid-Mediated Biofilm Remodeling by a Bacterial Prion-Like Protein

Prion-like domains (PrLDs) are low-complexity sequences that enable proteins to form amyloid structures with functional roles. Here, we identify *Staphylococcus aureus* SsaA2 as one of the firsts extracellular bacterial prion-like proteins. SsaA2 contains a PrLD adjacent to a CHAP catalytic domain and forms amyloid fibrils in vitro and shows infectivity in yeast. Uniquely, its PrLD acts as a binding module, replacing canonical globular cell wall-anchoring domains and enabling non-covalent association with biofilm matrices. SsaA2 function is essential for biofilm remodeling and complements cell viability in strains lacking the WalKR regulatory system. Mutational studies highlight the critical role of tyrosine and asparagine residues in amyloid formation and biofilm binding. This work reveals a novel mechanism by which a prion-like protein modulates bacterial virulence and suggests that amyloid-based interactions may be widespread in microbial pathogenesis.

Sofie Nyström
Linköping University

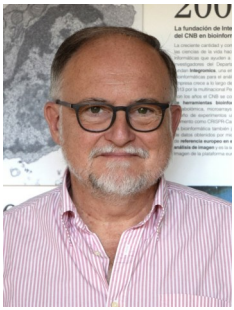
Dr. Sofie Nyström is an Associate Professor at Linköping University, where her research explores the intricate world of amyloids, focusing on their roles in neurodegenerative diseases and functional biological processes. Her groundbreaking work includes elucidating the amyloidogenic properties of the SARS-CoV-2 spike protein and its potential implications in COVID-19-related symptoms, including blood coagulation disturbances.
With expertise spanning protein folding, biomolecular interactions, and the pathological mechanisms of amyloids, Dr. Nyström has contributed significantly to understanding how these fibrillar structures form and their roles in health and disease. Her studies, often leveraging experimental biophysics and molecular biology, provide critical insights into amyloidogenesis and its broader biological relevance.

Abstract: Viral amyloids and their impact on human proteins

Virus encoded proteins comprise many amyloidogenic amino acid stretches. During virus infection, the human body is hence potentially subjected to an abundance of exogenous amyloid fibrils. Epidemiologic evidence of the connection between infection of influenza and herpes viruses as well as the newly emerged SARS-CoV-2 demonstrate that virus infection can provoke amyloid associated disease in humans both acutely and with decade-long latency.

Using a reductionistic approach we use synthetic peptides from virus proteins to investigate the interaction between virus amyloids and human proteins. Based on WALTZ predictions we have generated amyloids from SARS-CoV-2, Herpes simplex 1, Varicella zoster and Influenza A proteins. We then subject proteins purified from human plasma or from recombinant sources to the Virus Protein Amyloid Seeds (VPAS) and monitor their impact on disease relevant processes. Additionally, we use animal models and patient samples to determine the biological relevance of our in vitro findings.

Seven VPAS from SARS-CoV-2 spike were utilized to determine if virus amyloids can be causative agents in the coagulopathic disturbances seen in COVID patients and as adverse events of Spike-based vaccinations. We found the out of the seven tested amyloids, one has detrimental effect on clot lysis when present during a fibrinogen -> fibrin -> plasmin cycle. Additionally, one other Spike amyloid hampers the efficacy of clot formation by depleting the reaction of fibrinogen during the first stage of the reaction cycle. In conclusion, these findings warrant continued research to understand the connection between coagulation mechanisms and VPAS in a broader perspective.



Rafael Giraldo
Centro Nacional de Biotecnología, Madrid

Professor Rafael Giraldo is a leading researcher at the National Center for Biotechnology (CNB-CSIC) in Madrid. He got a PhD in Biology by the Complutense University in Madrid and did a postdoc at the MRC-LMB in Cambridge, UK, before becoming a CSIC permanent staff scientist in 2000. Rafael's work focuses on understanding the molecular mechanisms that regulate the functional and toxic states of intracellular amyloids in bacteria.

One of his landmark contributions is how a functional amyloid structure in the bacterial protein RepA regulates DNA replication through plasmid clustering. Rafael's laboratory bridges structural biology, synthetic biology, and molecular microbiology to shed light on the roles of amyloids in cellular processes and their potential as innovative bioresources and devices for applications in biotechnology and biomedicine.

Abstract: Bacterial Rep-WH1 Prions: From functional amyloids to biotools for One Health

Found in organisms across the whole Tree of Life, prions are proteins with at least two alternative conformations, one of them soluble while the other is a self-templated amyloid aggregate. The latter is vertically (epigenetically) propagated with cell division and, in some cases, also horizontally transmissible between cells (infectious). In prion proteins, the native and the amyloid conformations either have distinctive functions or the amyloid loses function gaining cytotoxicity, as in neurodegenerative and systemic diseases.

Starting from the seminal finding that the WH1 domain in a bacterial protein (RepA) builds functional amyloids to negatively control plasmid DNA replication (1), we have implemented their use in various Synthetic Biology applications (reviewed in 2), and developed a biosafe minimal model of an intracellular generic amyloid disease, which provided insight on the principles of prion propagation (3,4).

In this communication, new research will be presented: A) Progress in the 3D structure of the RepA-WH1 prion filaments, a novel left-handed super-helical coil of helical nanotubes. B) Characterization of the prion-like, bacteriotoxic WH1 domains in Rep proteins from plasmids of the prevalent phytopathogen *Xylella fastidiosa*, paving the way to their use in its containment. C) Engineering an outer membrane porin (OmpF) that enables bacteria to capture and degrade disease-relevant extracellular amyloids (5), either present in gut microbiota, which are potential triggers for neurodegeneration, or infectious prions persistent in natural environments such as soils.

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Short Talks of Session 3:

Fibril formation by the Henipavirus W proteins: a new hallmark of viral pathogenesis?

Frank Gondelaud[1*†], Alexandre Lalande[2†], Giulia Pesce[1], Christophe Bignon[1], Denis Ptchelkine[1], Yu Gu[3], Pierre-Yves Lozach[3], Denis Gerlier[2], Cyrille Mathieu[2*] and Sonia Longhi[1]

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2 CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, CNRS, UMR5308, Université Claude Bernard Lyon 1, Ecole Normale Supérieure de Lyon, 69007, Lyon, France
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The Hendra (HeV) and Nipah (NiV) viruses are zoonotic BSL-4 paramyxoviruses responsible for severe respiratory and neurological disease. In both viruses, the gene encoding the Phosphoprotein (P protein) also encodes the V and W proteins. The P, V and W proteins share a common, intrinsically disordered N-terminal domain (NTD) and have unique and distinct C-terminal domains (CTD). V and W are two key players in the evasion of the host innate immune that act by either counteracting or inhibiting Interferon (IFN) signaling.

We previously showed that the HeV and NiV W proteins form highly flexible, curved fibrils in vitro. Here, we show that the cysteine oxidation state acts as a molecular switch controlling the formation of either amorphous aggregates or flexible fibrils, and that residues 1 to 29 are essential for fibrillation. We also uncover that HeV W can also self-assemble in cellula. HeV W forms distinct types of nuclear condensates that exhibit different dependencies on the cysteine redox-state. While deletion of residues 1-29 prevents formation of nuclear filaments, cysteine-to-serine substitution mainly impairs the formation of non-filamentous condensates. Both infection and HeV W ectopic expression trigger oxidative stress favorable to HeV W condensation. Finally, we show that impaired ability to form redox-sensitive, non-filamentous condensates is associated with a reduced W ability to inhibit the NF-κB pathway, while it conversely enhances W ability to repress the interferon response.

Collectively, these studies provide the first clues on the functional impact of HeV W nuclear condensation. These findings hold promise for the rational design of new therapeutic approaches based on the inhibition of Henipavirus V/W fibrillation.

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Myelin Basic Protein is a new functional amyloid of the mammalian brain

Evgeniy I. Sysoev[1,2] , Aleksandr A. Shenfeld[1] , Tatiana A. Belashova[1,3] , Sergey P. Zadorsky[1,2] , Anna A. Valina[1,2] , Alexey P. Galkin[1,2]

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Myelin is a lipid-rich insulating sheath that surrounds the axons of numerous neurons, facilitating the rapid and efficient transmission of nerve impulses. In the brain, this sheath is produced by the extended, flattened processes of oligodendrocytes. Myelin Basic Protein (MBP) is essential for myelin compaction, as it facilitates adhesion between the opposing cytoplasmic membranes of these processes. Some experimental data suggest that myelin compaction is accompanied by the formation of amyloid-like MBP fibrils [1]. We performed a detailed analysis of the amyloid properties of MBP in vivo, ex vivo, and in vitro. MBP forms SDS-resistant oligomers and aggregates in vivo in the brain of *Rattus norvegicus*, as well as in *Rana temporaria, Trachemys scripta*, and *Gallus gallus domesticus*. Immunohistochemistry revealed colocalization of MBP with amyloid-specific dyes Congo Red (CR) and Thioflavin S in these species as well. Moreover, using a novel approach [2], we demonstrated that native MBP fibrils immunoprecipitated from rat brain bound CR and exhibited apple-green birefringence under polarized light – a characteristic feature of amyloid fibrils. The central region of MBP, comprising amino acid residues 60 to 119, is essential for amyloid fibril formation both in a heterologous yeast model and in vitro. Based on these findings, we propose a model in which MBP not only facilitates adhesion between opposing oligodendrocyte membranes, but also contributes to the longitudinal stabilization of myelin sheaths through amyloid fibril formation. These fibrils may represent an optimal natural structure for myelin compaction and axonal insulation.

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Bovine Meat and Milk Factors (BMMFs) as Zoonotic-Linked Drivers of Cancer and Chronic Diseases – New Variants of Disease-Associated Amyloids?

Gunjan Shukla[1], Grant Hansman[1], Alexander N. Popov[2], Amelie Burk-Körner[1], Anna Koromyslova[1], Barbara Leuchs[1], Veronika Frehtman[1], Nives Cecere[1], Ethel-Michele de Villiers[1], Harald zur Hausen[†1], Timo Bund[1*]

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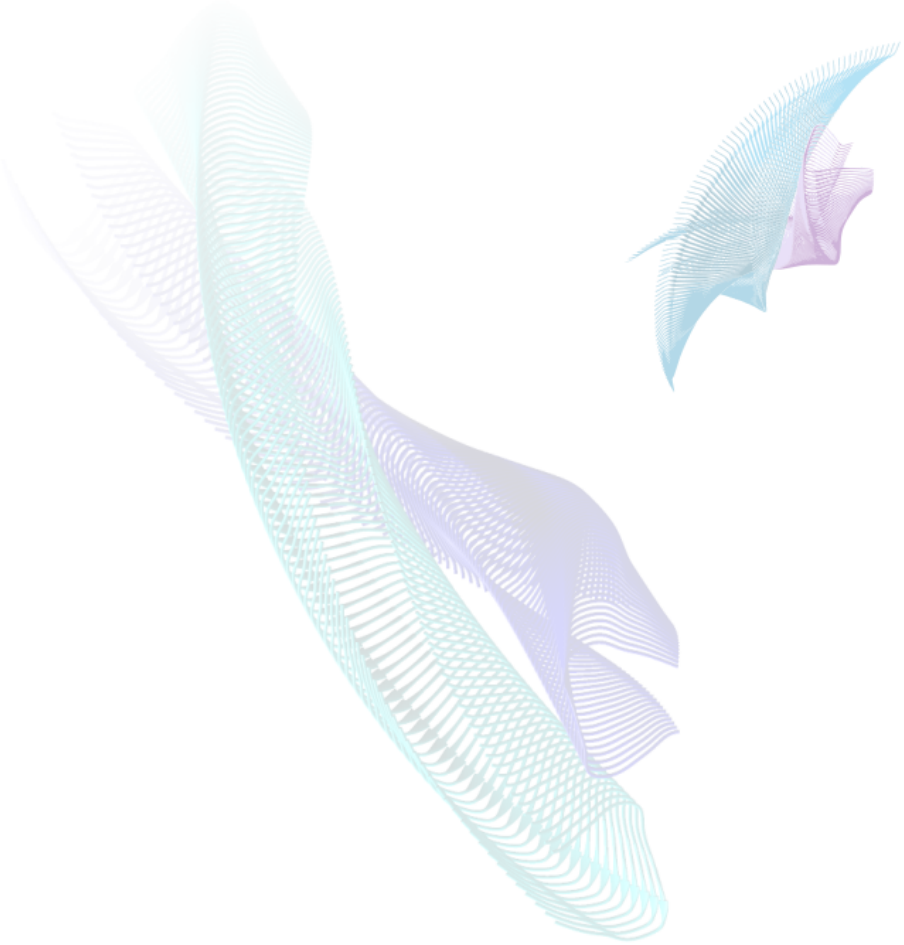
Bovine meat and milk factors (BMMFs) are plasmid-like DNAs that have been frequently detected in bovine meat and milk products and other sources and were proposed to contribute to the development of cancer and neurodegenerative diseases. BMMFs encode a conserved replication protein (Rep) that is actively transcribed and translated in human cells. BMMF Rep expression in tissues of colorectal (CRC), lung, and pancreatic cancer patients histologically aligned with detection of specific populations of macrophages and was increased in cancer versus healthy individuals and therefore described as possible biomarker for cancer. Rep expression coincided with markers of chronic inflammation and DNA damage also in pre-cancerous stages suggesting a causal role of BMMF in chronic inflammation-driven indirect carcinogenesis.

In this study, a Rep WH1 domain encoded on BMMF MSBI1.176 – initially found in multiple sclerosis human brain and CRC tissues - was determined to 1.53 Å resolution by X-ray crystallography. The overall structure of the MSBI1.176 WH1 domain was remarkably similar to other Rep structures e.g. to RepA from *Pseudomonas syringae*, despite having a low (28%) amino-acid sequence identity. The MSBI1.176 WH1 contained elements common to other Reps including an interface for dimerization. Analyses based on electron microscopy, size exclusion chromatography, dynamic light scattering, thioflavin assay as well as in silico predictions suggest BMMF Rep oligomerization likely involving amyloidogenic features. These findings suggest that the MSBI1.176 Rep might have comparable roles and functions to other known and partially amyloidogenic Reps of different origins.

Preliminary analyses support cytotoxicity of Rep in cell culture and support a disease-promoting, pathogenic function of BMMF, which therefore might be of use for (early) detection and monitoring of cancer and other chronic (neuronal) diseases to gain a better understanding of underlying disease mechanisms.

Session 4: Advancing Analytical and Imaging Techniques



Oxana Klementieva, Lund University

Oxana Klementieva is an Associate Professor in Molecular Imaging and head of the Medical Microspectroscopy Laboratory at Lund University, Sweden. Her research focuses on the molecular mechanisms of amyloid formation in neurodegenerative diseases such as Alzheimer's and Parkinson's. She has pioneered advanced imaging techniques, including label-free infrared and nanoscale correlative spectroscopy, to directly study amyloid aggregation and its pathological effects in neurons and tissues. Oxana's contributions include groundbreaking work on amyloid structures and their role in disease progression. Her group also develops innovative methodologies leveraging state-of-the-art facilities such as MAX IV in Sweden. Beyond research, she is dedicated to fostering interdisciplinary collaborations, exemplified through her leadership roles at LINXS, where she advanced the development of multimodal imaging networks to support the research community.

Abstract: Individual amyloids resolved directly in their native environment resolved by optical photothermal infrared microscopy

Spatiotemporal alterations in the chemical and structural makeup of biomolecules play an essential role in the onset and progression of various diseases, including Alzheimer's Disease. Early structural changes at the submicron level often occur well before disease symptoms can be recognized and before morphological changes can be detected using conventional tissue-level methodologies such as spatial proteomics, histology, or immunohistochemical staining. Consequently, there is a critical need for structure-sensitive techniques. Here, I present an approach capable of spatiotemporal chemical imaging of amyloid structures at submicron resolution within their native environment.

Using a recently established technique, the Medical Microspectroscopy Group from Lund University conducted groundbreaking experiments that enabled the monitoring of amyloids in the process of formation, proliferation, and cellular damage directly within living tissues.[1,2] To assess structural changes with sub-micron precision, we employed optical photothermal infrared (O-PTIR) microspectroscopy, a technique sensitive to amyloid structures. By applying O-PTIR to freshly extracted brain tissue from AD mouse model, we documented structural changes in functioning brain tissue, observing the appearance of newly formed amyloids spatially and temporally colocalized with lipid damage.

While time-resolved, submicron in situ imaging of amyloid structures marks a major breakthrough, further progress in understanding amyloid formation, accumulation, and tissue damage requires the integration of highly multiplexed imaging, deep learning–based classification, and multiscale chemical imaging into a unified platform for studying human tissues. In my talk, I will discuss recent advances in combining these technologies, which are essential for linking tissue-level responses with macromolecular composition—pushing the boundaries of what we can visualize and measure in both healthy and diseased states.

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Joost Schymkowitz & Frederic Rousseau
Switch Laboratory, VIB-KU Leuven

Professors Frederic Rousseau and Joost Schymkowitz lead the Switch Laboratory at VIB-KU Leuven, focusing on the intricate mechanisms of protein folding, misfolding, and aggregation. Their groundbreaking work explores how these processes influence diseases and how they can be harnessed for therapeutic innovation. Renowned for their contributions to understanding aggregation-prone regions (APRs) in proteins, they have advanced the field by uncovering how these regions drive amyloid formation while playing roles in both disease and functional biology.
Key innovations include the development of Pept-in™ technology, which leverages protein aggregation for therapeutic purposes, transforming a traditionally pathological phenomenon into a novel drug-design approach. This patented technology has shown potential applications in antimicrobial and oncological therapies. Their research extends beyond fundamental protein science to practical applications in biotechnology and medicine, as evidenced by their spin-off company, Aelin Therapeutics, which is pioneering new therapeutic modalities based on protein aggregation mechanisms.
Both researchers have published extensively in high-impact journals, contributing significantly to our understanding of amyloid structures and their roles in health and disease. Their collaborative efforts bridge theoretical insights with translational applications, making them leaders in the amyloid and protein aggregation research community.

Abstract: Mapping the Structural Landscape and Interactome of Amyloids

The field of amyloid research is undergoing a transformative shift driven by the rapid increase in available high-resolution amyloid structures. With hundreds of fibril structures now accessible, we are at a turning point similar to the structural biology revolution of the 1980s, which led to foundational insights into the principles of protein folding, stability, and interactions, shaping our understanding of how native proteins function and interact with their environment. A comparable opportunity is now emerging for amyloids. By analyzing this expanding structural database, we can start defining how amyloid fibrils are stabilized, what determines their structural polymorphism, and how they interact with other molecules and cellular components.
This talk will present how integrating structural, thermodynamic, and interaction studies can reveal the rules governing amyloid stability and their interactome. Mapping this landscape will help us predict amyloid behavior and develop strategies for modulating both pathological and functional amyloid assemblies.

Susanne Wegmann, DZNE Berlin

Dr. Susanne Wegmann leads the „Protein Actions in Neurodegeneration“ research group at the DZNE in Berlin, where her work focuses on understanding the neuronal tau protein, a key player in Alzheimer’s disease and other dementias. Her research explores the activation of Tau aggregation within neurons, Tau’s functional roles, currently focusing on its interactions with the nucleus and postsynapses. Her group further looks into the condensed phases of Tau and their effects on Tau aggregation and biology. By integrating biochemistry, biophysics, and studies on human brain samples and animal models, Dr. Wegmann adopts a highly interdisciplinary approach to uncover the molecular mechanisms driving neurodegeneration. Her efforts aim to identify novel therapeutic strategies to counteract tau-induced neurotoxicity. A recipient of the prestigious Rainwater Prize for Innovative Early-Career Scientists, Dr. Wegmann is also committed to fostering global scientific talent and promoting diversity in research through initiatives like the Humboldt Foundation’s Henriette Herz Scouting Programme. Her dedication to advancing both science and equity underscores her influential role in the field of neurodegenerative disease research.

Abstract: Tau protein phase transitions towards pathological aggregation

Tau Protein aggregation is a pathological hallmark in Alzheimer's disease and more than 20 other neurological conditions, whereby amyloid-like Tau fibrils form inside neuronal cell bodies. The accumulation of Tau aggregates is thought to rely on templates misfolding and can be initiated through seeded aggregation. The origin of the first seeds, however, is rather unclear. In our research we investigate whether liquid-condensed Tau can mediate Tau seed formation in vitro and in cells, and how this process can be modulated by cellular factors to prevent a priori Tau aggregation in healthy neurons. To achieve these aims, we are using in vitro reconstitution assays with recombinant proteins and cellular and neuronal cell culture models. We employ fluorescence microscopy, optical diffraction tomography, viscoelasticity measurements, crosslinking mass spectrometry to decipher the maturation process of Tau condensates towards amyloid aggregates.

Our data show that Tau phase separation into liquid-like condensates leads to molecular arrangements that favor Tau “seed” formation inside condensates. The maturation is accompanied by a re-organization of molecules inside Tau condensates and a rapid (within minutes to hours) change in viscoelasticity.

Further, different protein interactors of Tau seem to counteract these processes at different stages of condensation and condensate maturation. Based these findings, we propose that – in neurons - multiple Tau interaction partners interfere with Tau condensation and thereby prevent the formation of seeding competent Tau species inside condensates. Disease-associated mutations and PTMs may alter these “protective” interactions thereby allowing for Tau. seed formation and aggregation in tauopathies.

Short Talks of Session 4:

Curli inhibition by host protein, β2-microglobulin leads to impaired *E. coli* biofilm formation

Harshita Agarwal, Neha Jain

Department of Bioscience & Bioengineering, Indian Institute of Technology Jodhpur, Karwar 342030, India.

Functional amyloids are critical structural and functional components of biofilms, forming a key part of the self-secreted 3D extracellular matrix that encases and protects bacterial communities. These amyloids, alongside polysaccharides and eDNA, contribute to the mechanical stability and resilience of the biofilm. By reinforcing biofilm integrity, functional amyloids play a significant role in reducing the efficacy of immune responses, antimicrobial agents, and clinical interventions. This contributes not only to antimicrobial resistance but also to the persistence of chronic infections, delayed wound healing, and exacerbation of diseases. Given their central role in maintaining biofilm architecture, targeting functional amyloid formation offers a promising alternative to conventional antibacterial strategies.
Inhibiting their formation may effectively destabilize the biofilm matrix, thereby enhancing bacterial vulnerability and mitigating resistance without directly promoting selective pressure on bacterial populations. In our study we deciphered that the host protein β2-microglobulin (β2m) inhibits the formation of curli amyloids, key structural components of the extracellular matrix in *uropathogenic Escherichia coli* biofilms. By blocking the polymerization of CsgA, the primary subunit of curli, β2m disrupts amyloid assembly during early biofilm development. This inhibition of curli biogenesis compromises the structural integrity and maturation of the biofilm. Our confocal laser scanning microscopy images revealed that β2m significantly reduced thickness and disrupted matrix distribution, highlighting the essential role of curli in maintaining biofilm architecture. Through an integrated set of biophysical, biochemical, imaging, and in vivo approaches, we demonstrated that β2m substantially attenuates biofilm formation. In a rat skin wound infection model, β2m also accelerated wound healing, demonstrating its potential effectiveness against biofilm-associated infections in vivo. These findings position β2m as a promising endogenous anti-biofilm agent, and reinforce the therapeutic strategy of targeting biofilm matrix components to combat persistent and multidrug-resistant bacterial infections.

Session 4

Making amyloid functional – opportunities and risks with protein nanofibrils in materials design

Christofer Lendel

KTH Royal Institute of Technology, Department of Chemistry, Stockholm, Sweden.

The field of amyloid reaseach has developed from a strong focus on pathological conditions to include also biological function, nanotechnology and material science[1]. The wide variety of proteins that can be transformed into amyloid-like protein nanofibrils (PNFs) opens for many new applications of protein-based materials but also raises critical questions regarding the differences and similarities in structure, formation mechanisms and biological activity for "artificial fibrils" compared to disease-associated amyloid. We have demonstrated the formation of PNFs from a range of agricultural protein resources[2] and how these fibrils can be assembled into ordered, hierarchical structures[3-7]. A key step in the amyloid formation of large and complex proteins is the hydrolytic cleavage of the polypeptide chain into smaller peptides, which occur spontaneously at low pH and high temperature[8]. Our work suggests that this process also dictates the morphology of the PNFs that are formed and thereby an opportunity to control the material properties. Access to morphological distinct fibrils allows us to explore the relationships between nanoscale- and macroscale structures in PNF-based materials. We are also investigating potential cross-seeding between artificial protein fibrils and proteins related to amyloid pathologies[9].

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Session 4

Validating the Presence of Potential Amyloidogenic Proteins with Known Fertilization Functions in the Acrosomal Matrix of Human Spermatozoa

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Amyloids are aggregated proteins with highly organized cross-beta-sheet structures and typically associated with neurodegenerative diseases, prionopathies, and systemic amyloidosis. Pioneering studies in mice revealed the presence of functional amyloids in the sperm acrosomal matrix (AM) and zona pellucida (ZP) of mature oocytes, suggesting a role in fertilization. Studies in our lab showed that amyloid structures are also present in the AM and detergent-resistant AM core isolated from ejaculated spermatozoa of three additional species: *Bos taurus, Macaca mulatta* and *Homo sapiens*. Moreover, mass spectrometry (MS) analysis of their AM and AM core contents identified several potential amyloidogenic proteins described earlier to function in reproductive processes, including sperm-egg binding to the ZP and acrosome reaction. The main objective of this study was to validate the presence of some of these potential amyloidogenic proteins involved in fertilization, and test the hypothesis that human spermatozoa contain such proteins as previously identified in mice. To test this hypothesis, the interactome of fertilization proteins that were identified by MS was built using the protein interaction program STRING, then data mining and Waltz analysis were performed to identify known and potential amyloidogenic proteins, respectively. The presence of known fertilization proteins in the AM and/or AM core, including proteins with amyloidogenic motifs, was validated by indirect immunofluorescence and dot blot experiments. Results from this study support our hypothesis that amyloidogenic proteins are conserved in mammalian spermatozoa, and strongly suggest a functional role for amyloids during fertilization.

Session 4

Virulent and antimicrobial functional fibrils in infections and Neurodegeneration

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Amyloids, traditionally associated with neurodegenerative diseases, are now recognized as structurally stable protein fibrils with diverse physiological functions across all domains of life. In microbes, amyloids can serve as virulence factors that promote infection and biofilm resilience, making them promising targets for antivirulence therapies that may circumvent resistance development linked to bactericidal drugs. In parallel, certain antimicrobial peptides (AMPs) from diverse organisms have been discovered to self-assemble into amyloid fibrils, revealing a direct link between amyloid formation and innate immune defense. Using X-ray crystallography and cryo-electron microscopy, we revealed unexpected structural diversity among both virulent and antimicrobial amyloids, including the discovery of a novel class of cross-α fibrils, alongside canonical cross-β architectures. Many of these peptides exhibit structural polymorphism and switching, adopting different conformations in response to environmental conditions such as pH, lipid membranes, or RNA. This dynamic behavior suggests that amyloid formation can function as a regulated mechanism, modulating bioactivity, specificity, and toxicity. Together, our findings redefine the amyloid fold as a functional and tunable structural state rather than a static endpoint.

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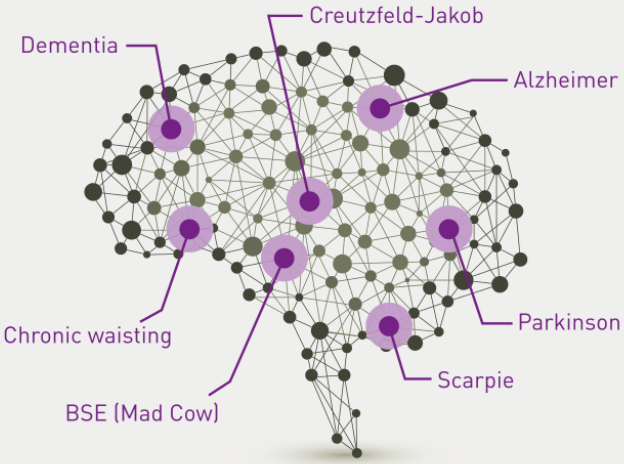

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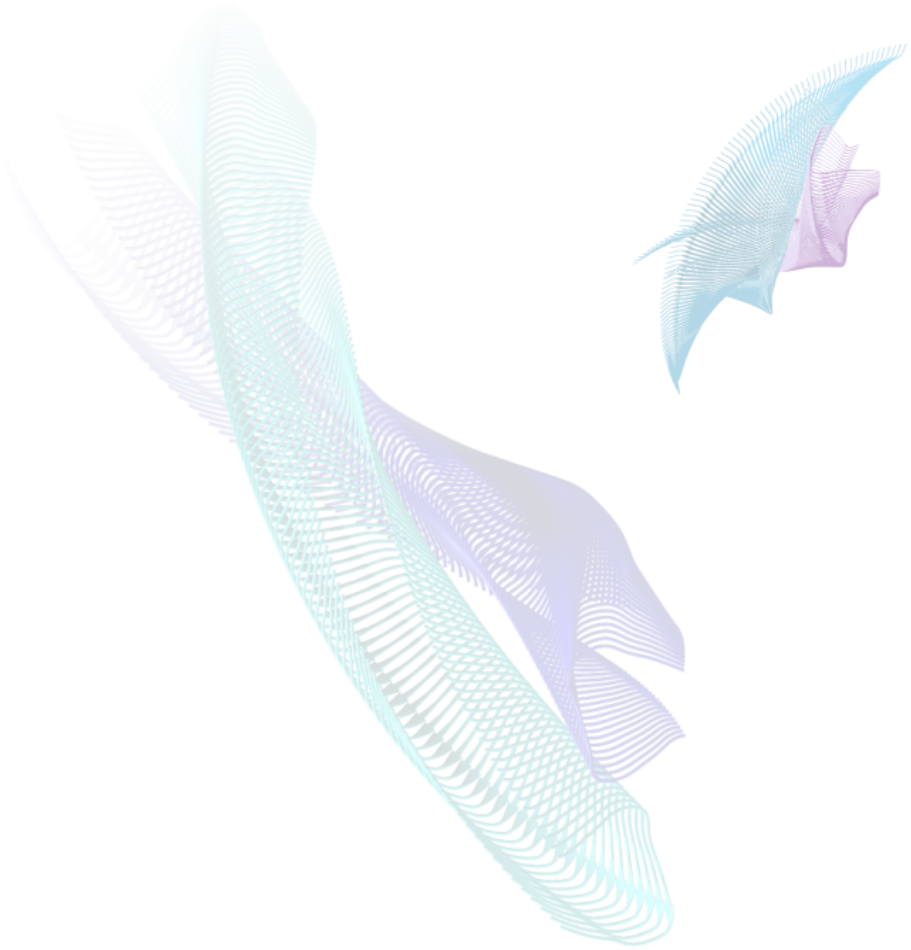
Poster session 1



Flash Talks of Postersession 1:

#1 Dissecting the crosstalk between transthyretin amyloids and the extracellular matrix
Jan-Hannes Schaefer

#7 Amyloidogenicity of virus-derived peptides and their effect on amyloid formation of proteins associated with neurodegenerative disease
Debdeep Chatterjee

#13 Coiled Double Amyloid-Like Fibrils Allosterically Catalyze Hydrolysis of β-Lactam Antibiotics
Sisira Mambram Kunnath

#18 StriFi: Structural and Functional Annotation of Amyloid Fibrils and Their Polymorphic Diversity
Oriol Bárcenas

#1 Dissecting the crosstalk between transthyretin amyloids and the extracellular matrix

Jan-Hannes Schaefer, Robert O'Neill, Hamid Rahmani, Danielle Grotjahn, Evan T. Powers, Jeffery W. Kelly, Gabriel C. Lander

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Neurodegenerative diseases rob the memory, mobility, and independence of millions globally. A hallmark of neurodegeneration is the aggregation of misfolded proteins, known as amyloids. Following Alzheimer's and Parkinson's disease, transthyretin amyloidosis (ATTR) is the third most prominent amyloid disease, originating from transthyretin (TTR), a 55 kDa tetrameric thyroxine carrier protein. Amyloid formation in ATTR begins with the dissociation of TTR-tetramers, followed by partial monomer denaturation, non-native TTR oligomerization, and ultimately formation of amyloid aggregates.

While physiological TTR and isolated amyloids have been studied extensively, the interaction of amyloids with the extracellular matrix (ECM) remains poorly defined.

Our cryo-EM data of ex-vivo amyloid extracts from ATTR patients are selectively enriched in Collagen VI (COLVI), a major component of the ECM. Using a combination of single-particle analysis and sub-tomogram averaging revealed the formation of co-filaments between TTR amyloids and COLVI. Amyloid decoration by ECM components may provide a structural basis for the existing hypothesis for why the clearance of amyloids through phagocytosis is inhibited. Not only does the amyloidogenic state of TTR interact with COLVI, but our in-vitro data shows a strong delay of amyloid formation in the presence of COLVI when using ATTR risk-variants of soluble TTR. This protective effect of ECM components to delay amyloidogenesis could explain why previously reported ECM dysregulation leads to increased amyloid deposition. Overall, our findings might accelerate the development of anti-amyloid drugs targeting their interaction with ECM components

#2 Functional Aggregation or Receptor Affinity? Evolutionary Insights into Peptide Amyloids of the PACAP–Glucagon Superfamily

Dániel Horváth[1,2], Zsolt Dürvanger[1,2], Szebasztián Szaniszlóa[2], Anna Kiss-Szemán[2], Duong Kim Hoang Yen[2], András Perczel[1,2]

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The amyloid aggregation propensity of numerous hormone peptides has been reported in the literature. While some, such as insulin[1], aggregate under non-physiological conditions, others can form functional amyloids under physiological conditions[2,3]—for example, when stored in a condensed form within acidic secretory vesicles. In our previous work[4], we proposed a pH-dependent, reversible self-assembly model for the glucagon-like subfamily of class B G protein-coupled receptor (GPCR) ligands (PACAP–glucagon superfamily), based on the crystal structures of their aggregation-prone regions (APRs). In our current research, we investigate the amyloid aggregation propensity of the receptor-binding regions of PACAP subfamily members to determine whether they retain aggregation-associated functionality despite their divergent amino acid sequences from their evolutionarily related glucagon-like counterparts. Given their broad biological activity, class B GPCR agonist hormones (e.g., GLP-1) and their synthetic derivatives (e.g., semaglutide) are of particular interest. A structure-based understanding of the intrinsic aggregation propensity within the PACAP–glucagon superfamily may provide valuable insights for pharmaceutical development of peptide-based drugs.

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#3 The Impact of Extracellular DNA on αPSM Functional Amyloids from *Staphylococcus Aureus*

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Phenol-soluble modulins (PSMs) from *Staphylococcus aureus* assemble into functional amyloids that play a critical role in biofilm stability and virulence. As these peptides aggregate in the extracellular space, they can interact with various biomolecules, which may influence their aggregation behavior. Heparin, a mimic of heparan sulphate and a polyanion, has previously been shown to both accelerate, decelerate and alter the aggregation mechanism of αPSMs, in a peptide specific manner. This effect is mediated by electrostatic interactions with lysine residues, altering aggregation kinetics without changing fibril structure. In this study, we investigated how extracellular DNA (eDNA) from both *Staphylococcus epidermidis* (a Gram-positive bacteria), and *Escherichia coli* (a Gram-negative bacteria) affects the aggregation kinetics, morphology, secondary structure and cytotoxicity of αPSM amyloids. Our findings reveal that the presence of eDNA significantly alters the aggregation kinetics of PSMα1-4 and the δ-toxin, with both acceleration and deceleration observed depending on the eDNA source. Despite these kinetic changes, fibril morphology remained largely unchanged and eDNA promoted an increased fibril formation across all αPSMs. Additionally, changes in secondary structure were observed via circular dichroism and ANS fluorescence assays, indicating that eDNA influences the structural organization of the aggregates.

Together, these findings suggest that eDNA can modulate PSM α 1-4 and δ-toxin aggregation behavior, primarily by modulating kinetics and secondary structure, while leaving fibril morphology largely intact. This highlights a potential role for eDNA in shaping amyloid assembly with bacterial biofilms.

#4 Elucidating Molecular Determinants of Fibrillation in the V and W Proteins from Paramyxo-virus: Implications for Targeted Therapeutic Strategies

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Nipah and Hendra viruses (NiV, HeV) are biosafety level 4 zoonotic paramyxoviruses with high mortality rates (1). Their V and W proteins, two key players in the host innate immune response evasion, share a common intrinsically disordered N-terminal domain (NTD). We showed that the HeV V protein undergoes a liquid-to-hydrogel phase transition driven by the PNT3 region (amino acids 200–310) that was also shown to be able to form amyloid-like fibrils (2-4). We discovered that the ability to form fibrils is also shared by the intrinsically disordered W proteins (5-7). The W protein fibrillates not only in vitro but also in the nuclei of transfected cells, suggesting a probable functional relevance (6). We hypothesized that fibrillogenicity of W is instrumental for the ability of the virus to evade the host antiviral response (8).

Next, we identified a cryptic amyloidogenic region (CAR) within the PNT1 domain (amino acids 1–110) of the W protein as the main driver of W fibrillation, and showed that it is conserved across Henipaviruses (9). Using advanced techniques such as Taylor dispersion analysis (TDA), Raman spectroscopy, and single-molecule Förster resonance energy transfer (sm-FRET), we are currently investigating the kinetics and structural transitions of PNT1 fibrillation. These studies, aimed at elucidating the molecular basis of fibrillation and at identifying critical residues, will guide the design of small-molecule inhibitors targeting the fibril core. Collectively, these studies are expected to provide new insights into Henipavirus pathogenesis and to pave the way for innovative antiviral therapies.

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#5 Structural Basis of *Pseudomonas* Biofilm-Forming Functional Amyloid FapC Formation

Kasper Holst Hansen[1,2], Mert Golcuk[3], Chang Hyeock Byeon[1], Abdulkadir Tunc[1], Emilie Buhl Plechinger[2], Morten K. D. Dueholm[4], James F. Conway[1], Maria Andreasen[2], Mert Gur[3], Ümit Akbey[1]

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Biofilm-protected *Pseudomonas aeruginosa* causes chronic infections that are difficult to treat. FapC, the major biofilm forming functional-amyloid in *Pseudomonas*, is essential for biofilm integrity, yet its structural details remain unresolved. Using a combination of solution NMR-based structural ensemble of unfolded monomeric FapC, a ~3.3 Å resolution CryoEM density map of FapC fibril, and all-atom MD simulations to capture transition from unfolded to folded monomer to fibrillar fold, we provide a complete structural view of FapC biogenesis. CryoEM reveals a unique triple-layer β-solenoid cross-β fibril composed of a single protofilament. MD simulations initiated from monomeric and fibrillar FapC mapped structural transitions, offering mechanistic insights into amyloid assembly and disassembly. Understanding FapC reveals how *Pseudomonas* exploits functional amyloids for biofilm formation and establishes a structural and mechanistic foundation for developing therapeutics targeting biofilm-related infections.

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#6 N-terminal capping modulates fibrillation and cytotoxicity of PSMα3 under physiological conditions

Laura Bonnecaze[1], Alysson Duchalet[1], Estelle Morvan[2], Birgit Habenstein[3], Lucie Khemtemourian[1], Michael Molinari[1], Sophie Lecomte[1], Sophie Kopp-Marsaudon[1], Marion Mathelié-Guinlet[1]

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The virulence of *Staphylococcus aureus*, a multi-drug resistant pathogen, is tightly linked to the expression of phenol-soluble modulins, notably PSMα3, which self-assembles into cross-α amyloid-like fibrils[1]. While fibrils were initially thought to drive PSMα3's cytotoxic activity, recent findings have shifted attention toward soluble intermediates as key players in membrane disruption[2]. Here, we investigate how N-terminal capping influences PSMα3's aggregation behavior and cytotoxic potential under near-physiological conditions.

Using Thioflavin T (ThT) fluorescence spectroscopy, NMR, and TEM, we compared formylated (f-) and acetylated (ac-) PSMα3 forms. The more hydrophobic ac-PSMα3 exhibits reduced ThT binding and preserves a greater proportion of soluble species, with fibrils observed at late aggregation stages that morphologically differ from f-PSMα3. Importantly, this functionally correlates with significantly reduced cytotoxicity towards HEK293 cells, both in a time- and concentration-dependent manner. Furthermore, we showed that lipoproteins inhibit toxicity, as previously reported, by preventing PSMα3 fibrillation. As neither monomeric nor fibrillar species were toxic, this reinforces the pivotal role of soluble intermediates in PSMα3. Using Laurdan fluorescence, we also demonstrated that only these intermediates led to membrane fluidification, a disruptive mechanism that was further validated in vitro using model membranes of controlled lipid compositions[3].

Taken together, our results highlight the key role of N-terminal capping in modulating the aggregation and subsequent cytotoxicity of PSMα3 *via* membrane permeation[4] , and thus potentially contribute to *S. aureus* pathogenicity in vivo.

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#7 Amyloidogenicity of virus-derived peptides and their effect on amyloid formation of proteins associated with neurodegenerative disease

Debdeep Chatterjee[1], Johan Larsson[1], Vilma Odland[1], Agnes Geite[1], Ranjeet Kumar[2], Pernilla Wittung Stafshede[2], Per Hammarström[1], Sofie Nyström[1] (Corresponding Author)

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Amyloid co-aggregation and cross-seeding are known mechanisms in many pathologic conditions. Herpes simplex virus infection (HSV) has been suggested to play a role in the development of Alzheimer´s disease (Itzhaki et. al., Vaccines, 2021). Recent epidemiologic evidence suggest that neurodegenerative diseases (NDs) can be aggravated by several other viral infections such as Influenza A (Levine et. al., Neuron, 2023) and SARS-CoV-2 (Bonhenry et. al., Lancet Neurology, 2024). Using in vitro protein preparations, we have demonstrated that amyloids of spike protein of SARS-CoV-2 can affect blood clotting (Nyström et. al., JACS, 2022) and cross-seed amyloid fibril formation of ND-proteins such as Aβ and human prion protein (Larsson et. al., bioRxiv, 2023).
In this work we have identified amyloidogenic peptides in several virus proteins using the WALTZ algorithm and tested, in vitro, how SARS-CoV-2 spike as well as Influenza A and Herpes simplex 1 protein peptides form amyloid fibrils. Furthermore, we established the ability of the resulting amyloids to cross-seed and modulate the aggregation of proteins like α-synuclein, Aβ1-40, Aβ1-42 and Tau, which are established as causative agents of NDs. Our in vitro data suggest that the interaction between viral peptide amyloids and the ND-proteins are highly selective and sequence specific. In conclusion, it can be proposed that viral amyloids may be a significant promoter in the initiation of sporadic NDs.

Acknowledgements: Funding of this work was by Olle Engkvists Stiftelse, Swedish Research Council, Konung Gustav V och Drottning Victorias stiftelse.

#8 Functional Amyloid Formation in Staphylococcal Hemolytic Peptides

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While amyloids are classically associated with neurodegenerative diseases, in bacteria, they can serve adaptive roles, such as providing structural support and stabilizing biofilms, leading to enhanced virulence. Here, we investigate whether hemolytic peptides from diverse *Staphylococcus* species form amyloid-like fibrils and how this conformational change might contribute to bacterial pathogenicity.

Building on previous findings that *Staphylococcus aureus* δ-toxin forms amyloid fibrils in vitro, we hypothesized that amyloidogenesis could be a conserved feature among staphylococcal hemolytic peptides. We screened 20 naturally occurring peptide variants from six *Staphylococcus* species using ransmission electron microscopy and amyloid-binding dyes, and found that a subset of seven peptides formed fibrillar aggregates, suggesting that this structural property is both widespread and potentially regulated.

We will also compare the cytotoxic effects and hemolytic activity of the fibrillar aggregates versus their monomeric counterparts to assess how amyloid formation influences peptide function. This comparative study aims to explore the functional consequences of amyloid formation during infection. Previous research on bacterial amyloids indicates that fibrillar assembly may increase peptide stability against proteolytic cleavage or slows it down and contribute to biofilm resilience.

Pathogen-derived amyloid fibrils have also been shown to modulate host immune responses. In addition, bacterial amyloids have the potential to influence host processes by interacting with human amyloidogenic proteins, raising the possibility of cross-seeding events that could link infection to chronic inflammatory or neurodegenerative conditions.

This work contributes to a broader understanding of bacterial pathogenesis and highlights the possible intersection between microbial infection, immune activation, and long-term host pathology.

#9 The structure/function relationship of the Small Basic Protein in *Staphylococcus epidermidis* biofilm formation

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Bacterial resistance to antibiotics poses a significant global threat, causing an estimated 4.95 million deaths in 2019 and is due, in part, to the formation of stable biofilms, clusters of bacterial cells embedded in an extracellular matrix. The biofilm shields bacterial cells from antibiotics and improve their virulence. Biofilm formation is of significant clinical relevance since biofilms are common in hospital-acquired infections. In the case of nosocomial infections caused *by Staphylococcus epidermidis*, biofilm formation involves a 18 kDa small basic protein (Sbp). Sbp is expressed in the early stages of biofilm formation when the bacteria attach to the substrate surface and trigger surface colonization by bacteria1. In parallel, it has been found that Sbp can form amyloid fibrils in vitro and S. *epidermidis* biofilm lacking Sbp expression are ThioflavinS-negative, unlike the WT strain, suggesting that Sbp forms amyloid fibrils within bacterial biofilm.
However, detailed insights into Sbp structures in vitro and within a bacterial biofilm as well as its binding partners within biofilms are still elusive.

To address this knowledge gap, this project is dedicated to elucidating the structures of recombinant Sbp using cryo-electron microscopy. Preliminary results confirm that Sbp can form amyloid fibrils in vitro, with a regular spacing of 4.75 Å observed in the diffraction pattern. Furthermore, to uncover the molecular functions of Sbp within biofilms in its native context, we combine advanced techniques such as correlative light and electron microscopy (cryo-CLEM), focused-ion-beam (FIB) milling and cryo-electron tomography (cryo-ET) to gain unprecedented insights into the structural and functional dynamics of Sbp within lab-grown biofilms. Filamentous assemblies are visible in the extracellular matrix of biofilms whereas such filaments are absent in the knockout mutant. These results can pave the way for novel antimicrobial strategies.

#10 Light-fueled transient catalytic amyloid-like fibrils for antibiotic degradation

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Short peptides that self-assemble into highly ordered fibrillar structures like amyloid fibrils have gained enormous interest in recent years. Amyloid fibrils are linear polypeptide aggregates that are linked to a variety of neurodegenerative diseases. Despite their toxicity, amyloid and amyloid-like fibrils have attracted considerable attention in the fields of catalytic hydrolysis of esters and phosphor-esters, lipid degradation, and catecholamine oxidation. Herein, we present light-fueled transient amyloid-like fibrils from a short peptide with a highly organized structure in water. We employed PFK (PKFKFKFKFKFKP), a short amphiphilic and cationic peptide, and (E)-3-(2-(2-hydroxystyryl)-3,3-dimethyl-3H-indol-1-ium-1-yl) propane-1-sulfonate (MCH), a short-lived photoacid in water for this purpose. Co-mixing of PFK and MCH (1:2 molar ratio) in water under dark conditions results in small random aggregates. Quite surprisingly, within just a minute, highly organized parallel fibrils are generated upon exposure to light, as evidenced by cryo-TEM. The photoacid MCH dissociates under light to release H+ and an aromatic moiety with a pendant sulfonate group, which might interact with the peptides non-covalently to form fibrils under light. Such fibril formation is transient, and the formed fibrils dissociate once the light source is removed. Such spatiotemporal control on the transient supramolecular assembly of peptides into fibrils is a growing area of research in recent years. The light-fueled peptide fibrils are further tested towards catalytic degradation of a ß-lactam antibiotic, an expanding health and environmental risk in wastewater that helps propagate antibiotic-resistant bacterial strains. These catalytic fibrils formed under light can hydrolyze nitrocefin, a ß-lactam antibiotic, demonstrating a novel approach for the degradation of antibiotics in water, providing solutions for tackling this significant environmental and health issue.

#11 Amyloid aggregation of PACAP-glucagon superfamily hormones: dual role of the receptor activating segments?

Zsolt Dürvanger[1,2], Dániel Horváth[1,2], Szebasztián Szaniszló[1,2], Anna Kiss-Szemán[2], Duong Kim Hoang Yen[2], András Perczel[1,2]

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A large number of peptide hormones were suggested to be stored as amyloids in secretory granules[1]. Among these hormones several members of the PACAP-glucagon family can be found, suggesting that amyloid formation might be a common storage mechanism in this family of peptides. As their synthetic derivatives gain more and more attention in drug development, identification of the parameters that influence their amyloid propensity is of utmost importance. Although their tendency to form amyloid aggregates can pose significant challenges in their synthesis, storage and quality control, their natural form of storage might also serve as an inspiration for novel methods of drug formulation in the future.
Recently we have shown that a conserved hexapeptide motif in the glucagon family peptides that is responsible for receptor binding also promotes amyloid formation[2]. Here we extended our study to the whole PACAP-glucagon superfamily to find out whether amyloid formation might be a common storage mechanism for all of its members. As the sequence motif responsible for receptor activation is more conserved throughout the family than the receptor binding motifs, we also aimed to find out how these segments contribute to amyloid formation.

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#12 Germ Granules in Mammalian Spermatozoa: Proteinaceous Carriers of Cytosolic Inheritance?

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Environmental exposures throughout a father's life can cause epigenetic changes in the germline which can be propagated to his children, changing their phenotype and potentially causing disease across multiple generations. Although changes in classical epigenetic marks such as DNA methylation have been observed, the epigenetic carrier in sperm remains unknown. Cytosolic epigenetic inheritance has been documented in *C. elegans*' sperm where phase-transitioning germ granules (GG) function as paternal epigenetic carriers that transmit phenotype to embryos during fertilization. Other studies found that *C. elegans* contain cytosolic functional amyloid that created a heritable phenotype as a result of environmental temperature changes, marking the first instance of amyloid-based inheritance in an animal. In the light of these studies and our preliminary data, we hypothesize that environmentally-responsive GG are present in mammalian spermatozoa and are cytosolic carriers of protein-based epigenetic inheritance. Germgranules, present in many animal species, are dynamic cytosolic biomolecular condensates made up of phase transitioning/amyloidogenic proteins. Since GG contain epigenetic modifying and gene expression regulating proteins bound to their cognate small RNAs (miRNA, piRNA, etc), they are ideal candidates for altering phenotype. However, although vital for spermatogenesis, GG are thought to be absent from mammalian sperm. We began by looking for amyloid in the cytosol of cauda rat sperm and found distinct puncta in the sperm perforatorium, a juxtanuclear cytosolicstructure on the sperm head. Multiple GG markers were detected in the perforatorium puncta, including androgen receptor and DDX5, amyloidogenic proteins involved in gene expression regulation, and BTBD18 and GMCL1, mammalian proteins that closely resemble a key *C. elegans* GG component that is required for paternal epigenetic inheritance. SncRNAsequencing determined the presence of piRNA and miRNA in the perforatorium, and the RNA specific stain RNASelect suggests most of the perforatorium RNA is in the puncta. Our studies suggest GG are present in mammalian spermatozoa and may be a viable mode by which cytosolic amyloid-based inheritance is possible in mammals.

#13 Coiled Double Amyloid-Like Fibrils Allosterically Catalyze Hydrolysis of β-Lactam Antibiotics

Sisira Mambram Kunnath[1,2], Elad Arad[1,2,3] , Ran Zalk[1], Itamar Kass[1], Albert Batushansky[1], Hanna Rapaport[1,4] and Raz Jelinek[1,2]

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The release of antibiotic compounds into wastewater constitutes a significant and growing health and environmental hazard, particularly contributing to the spread of antibiotic resistant bacterial strains. Here, we demonstrate that amyloid fibrils, consisting of an alternating lysine/phenylalanine -sheet forming short peptide, catalyze hydrolysis of β-lactam antibiotics, the most prominent family of antibiotic compounds, which is further widespread in wastewater. Peptide variant analysis, molecular dynamics (MD) simulations, and cryogenic electron microscopy (cryo-EM) reveal that the β-lactam molecules dock onto the fibrils' surface via electrostatic interactions with the lysine sidechains. Importantly, catalytic hydrolysis occurs via an allosteric mechanism mediated by a unique coiled double fibril structure in which the anchored β-lactam molecules are embedded within twisted fiber strands, facilitating nucleophilic attacks by the lysine sidechains. Utilization of the catalytic lysine-displaying amyloid fibrils for hydrolytic degradation and removal of β-lactam antibiotics from water was accomplished through display of the fibrils on silica beads placed in a conventional column filtration setup. Amyloid fibrils displaying lysine arrays may furnish a versatile platform for hydrolysis and removal of β-lactam antibiotics in water, underscoring new avenues for addressing the considerable threat of antibiotics water contamination[1].

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#14 Effects of the ionic strength on functional amyloid fibril formation

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hnRNPDL (heterogeneous ribonucleoprotein D-like) is a human ribonucleoprotein (RNP) involved in transcription and RNA processing. Alternative splicing renders three natural forms, hnRNPDL-2 being the predominant and the only isoform capable of forming amyloid fibrils. It exhibits a modular structure comprising two globular RNA-recognition motifs (RRMs) and a C-terminal low complexity domain (LCD) that hosts missense mutations causing limb-girdle muscular dystrophy D3 (LGMD D3) (Li et al., 2019; Vicente et al., 2020).

Recently, we reported the cryo-electron microscopy (cryo-EM) structure of hnRNPDL-2 amyloid fibrils (Garcia-Pardo et al., 2023), revealing a highly hydrophilic amyloid core that is surrounded by the globular domains in their functional conformation, rendering fibrils that maintain their ability to bind nucleic acids. The architecture and activity of hnRNPDL-2 amyloid fibrils are reminiscent of functional amyloids, suggesting that LGMD D3 might be a loss-of-function disease associated with impaired fibrillation.

Protein aggregation depends on many factors, including environmental conditions such as changes in pH, temperature or ionic strength (Meisl et al., 2017; Owen et al., 2019). Thus, the environmental effects on amyloid aggregation and the molecular mechanisms underlying amyloid transitions are highly relevant and may explain differences in disease progression as well as protein functionality.

In this regard, we investigated the impact of solvent ions on hnRNPDL-2 amyloid aggregation. By using SAXS and molecular dynamic simulations, we have analysed the changes in hnRNPDL-2 conformation induced by different salt concentrations, revealing that the environmental ionic strength affects hnRNPDL-2 conformation and self-assembly. Ultimately, we believe our findings can help understanding disease cause and progression associated to this protein and other functional amyloids involved in human diseases.

#15 Universal methodology for screening and identification of amyloids

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The list of functional amyloids is expanding every year, which indicates the diversity of their localization and molecular role. Screening and identification of unknown amyloids in the proteome of various living organisms is a rather complex task. We have developed two methods, the combination of which allows to solve this problem [1]. The first approach is proteomic screening for amyloids based on the collection of protein aggregates resistant to SDS treatment by ultracentrifugation followed by identification by mass spectrometry. Such approach allows to obtain a list of SDS-resistant candidates for the role of amyloids. The next step is individual analysis of the amyloid properties of selected protein candidates. Protein fibrils are extracted by immunoprecipitation from tissues or cells and then subjected to Congo red staining and birefringence analysis. Additional verification can be applied by staining with Thioflavin S or T, as well as conformation-dependent antibodies.Using proteomic screening and immunoprecipitation of fibrillar proteins, we identified new functional amyloids in vertebrate cortical neurons (the FXR1 protein) and in *Drosophila melanogaster* eggshell (the s36 protein). FXR1 normally forms amyloid RNP particles that protect RNA from degradation. Under stress, FXR1-containing amyloid RNP particles are incorporated into stress granules. The s36 protein forms a network of amyloid fibrils in the fruit fly eggshell. The absence of s36 amyloid fibrils in the eggshell disrupts the endochorion morphology and blocks the development of the micropyle, which leads to sterility. This is the first example of a functional amyloid regulating development and morphogenesis. The methodology we developed has been successfully applied to identify other amyloids in bacteria, yeast, plants and vertebrates.

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#16 Amyloid Fibrils Catalyzing Cationic Ring-Opening Polymerization of Cyclic Esters: Toward Bioinspired Routes to Functional Polyesters

Elinor Slavsky

Catalytic amyloids emerge as robust, self-assembled platforms that mimic enzyme-like activity through ordered β-sheet architectures. Their repetitive display of functional groups enables unique reactivity under mild, aqueous, and often metal-free conditions. These features position amyloids as promising candidates for sustainable and tunable catalysis.
In this work, we present a proof-of-concept study in which amyloid fibrils, formed from a short peptide sequence containing alternating lysine and phenylalanine residues, are harnessed to catalyze the ring-opening polymerization (ROP) of cyclic esters. Specifically, we target the synthesis of poly(ε-caprolactone) (pCL), a biodegradable and biocompatible polyester extensively used in biomedical applications such as tissue engineering, drug delivery systems, and resorbable sutures.
Our design leverages the self-assembled β-sheet structure of the peptide fibrils, which creates a periodic, spatially organized array of lysine side chains embedded within a hydrophobic, π-stacked environment. This arrangement facilitates an activated monomer mechanism, enabling cationic ROP through initiation by lysine residues and stabilization of the growing chain end via non-covalentinteractions within the amyloid matrix. The phenylalanine residues contribute to fibril stability and potentially promote monomer alignment through π–π interactions, further enhancing catalytic efficiency.
Preliminary results suggest that these functional amyloids can initiate and propagate polymerization under mild, metal-free conditions, offering a sustainable, bioinspired strategy for precision polyester synthesis. This study highlights the untapped potential of amyloid materials as catalytically active platforms and suggests a new direction for integrating peptide-based nanostructures into the field of polymer chemistry.

#17 Reversible, Functional Amyloids in Metabolic Regulation and Immune Responses

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Protein aggregation, and in particular amyloid formation, has historically been considered an aberrant, irreversible process, commonly associated with several devastating pathologies, such as Alzheimer's disease, Parkinson's disease, or amyotrophic lateral sclerosis. However, recent insights challenge this view, providing evidence that protein aggregation can also be a regulated, reversible process, fulfilling essential physiological functions. Indeed, reversible amyloids have been implicated in various cellular processes, from peptide hormone storage to metabolic regulation, underscoring their central role in cell physiology.
Our research combines genetic, biochemical, cell biological, and microfluidic approaches to investigate the regulatory mechanisms underlying reversible, functional amyloid formation and disassembly, as well as their physiological implications. Focusing on metabolic enzymes and immune factors, we have identified evolutionarily conserved molecular mechanisms that govern the formation and disassembly of functional amyloids in response to stress [1-3]. Specifically, we uncovered pH- and oxidation-sensitive amyloid cores and elucidated their multi-layered regulation, which involves phosphorylation, protonation, and chaperone binding events [2,3]. Furthermore, we demonstrated that these mechanisms are essential for regulating cellular metabolism and ensuring cell survival under stress conditions [1,2].
These findings highlight the fundamental roles of reversible amyloids in cellular regulation and suggest novel connections to the pathogenesis of amyloid-linked diseases, opening exciting opportunities for the development of innovative therapeutic strategies.

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#18 StriFi: Structural and Functional Annotation of Amyloid Fibrils and Their Polymorphic Diversity

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Amyloid formation is a widespread phenomenon that can either be controlled and involved in cellular processes or be spontaneous and significantly affect pathology and the industrial production of proteins. Although structural biologists have made significant progress in obtaining novel amyloid structures, mainly through advances in cryo-electron microscopy, there is still a lack of comprehensive resources to compile and compare available amyloid structures. These may be non-exhaustive, missing structures from the Protein Data Bank (PDB), lack standardized annotations, or do not focus on fibrils or their structures altogether.

Recognizing the structural diversity of amyloids is essential for studying polymorphism and gaining deeper insights into their biophysical characteristics. To address this gap, we are currently developing StriFi, which features an automated annotation pipeline and a novel algorithm for detecting amyloid cores. It integrates all relevant PDB metadata and precalculated properties, including sequence alignments, in-core residue detection, per-residue flexibility assessments, and subfibril detection. Special emphasis is also placed on advanced structural polymorphism comparison and clustering, highlighting the influence of changes in the sequence or the conditions under which the structures were obtained. StriFi will also continually update itself with new PDB entries, allowing it to grow alongside the influx of new amyloid structures.

Thanks to integrating and enhancing the structural information on amyloids, StriFi facilitates the identification of shared structural motifs and quantitative comparisons between individual amyloids or broader classes, such as pathological and functional amyloids. It also has the potential to allow the development of new methods to predict the structure of amyloids, accelerate the development of therapeutic inhibitors, or design novel nanomaterials inspired by functional amyloids.

#19 Amyloids and RNA: Molecular Cooperation at the Beginning of Life

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Peptide amyloids, due to their repetitive structure, may have played a crucial role in the origin of life by forming interfaces with RNA. We found that RNA molecules as short as trinucleotides can bind to peptide amyloids in a sequence-selective manner, with both the canonical RNA backbone and nucleobases contributing to this interaction. Notably, amyloids and RNA mutually stabilize and protect each other under extreme environmental conditions. The interaction between amyloids and RNA reveals key specificity and affinity determinants, offering potential insights into the emergence of the primitive genetic code.

Furthermore, peptide amyloids show selective binding to nucleotide triphosphates over diphosphates. This selectivity suggests that amyloids could act as selective filters for ATP or, in the presence of Mg^{2+}, serve as primitive catalytic scaffolds that facilitate ATP synthesis despite the thermodynamic challenges involved. In addition, amyloids can catalyze RNA polymerization from 2',3'-cyclic nucleotides under dry, alkaline conditions. The findings support the idea that functional interactions between amyloids and RNA (or nucleotides) may have emerged earlier in evolution than previously assumed, potentially predating the genetic code.

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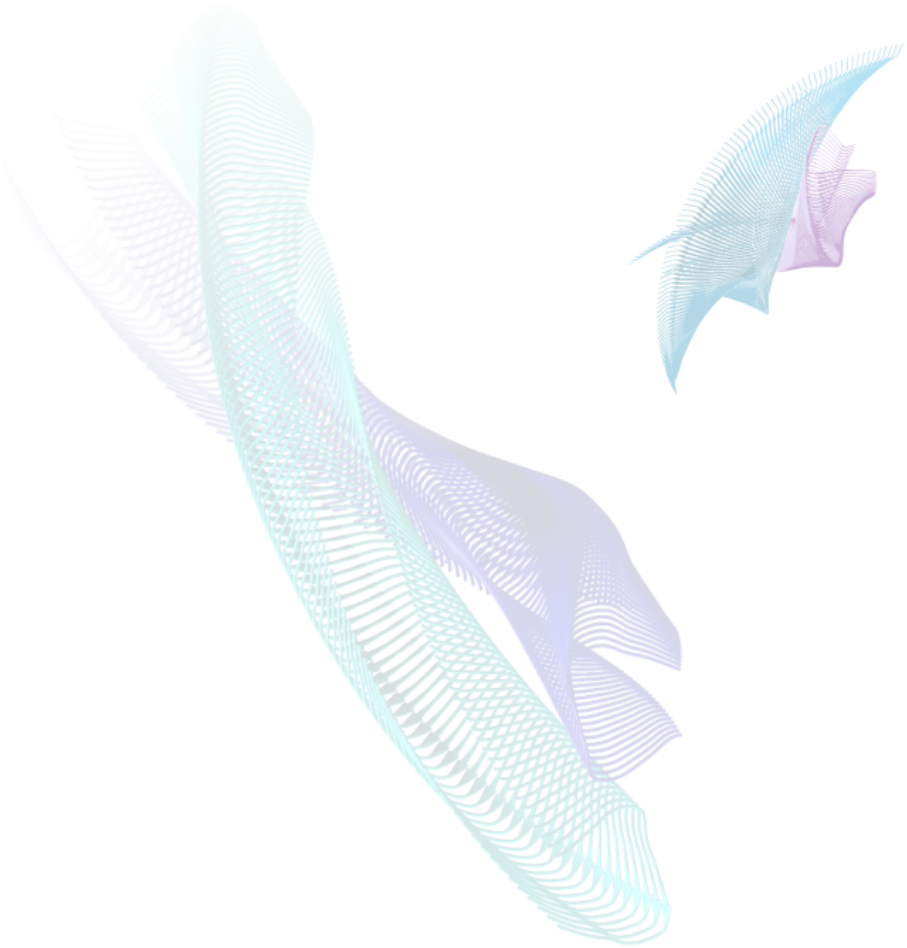
Poster session 2



Flash Talks of Postersession 2:

#21 Impact of Copper Oxide Nanoparticles and Microfluidic Flow on Amyloidogenic Properties of Human Cystatin C
Karolina Rucińska

#27 Bacterial amyloids in the context of neurodegeneration: cross-interactions between Aβ(1–42) and Hpn from Helicobacter pylori, and its fragment
Oliwia Polańska

#33 Intrinsic disordered domains of functional and disease-related amyloids: A combined NMR, EPR, and MD approach
Ansgar B. Siemer

#38 Changing the Game of Amyloid Fibril & Protein Condensate Research with FIDA
Einar Halldórsson; Emil G. P. V. Stender, Ph.D.

#21 Impact of Copper Oxide Nanoparticles and Microfluidic Flow on Amyloidogenic Properties of Human Cystatin C

Karolina Rucińska[1,2], Joanna Maksim[1,2], Łucja Przysiecka[3], Ahmet Kertmen[1], Maciej Kozak[1,4]

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Airborne particulate matter containing metal-based nanoparticles, including copper compounds, is an environmental concern due to its potential role in neurodegenerative processes [1]. Ultrafine particles in PM2.5 dust from industrial and traffic emissions can access the central nervous system via the olfactory route, bypassing the blood-brain barrier [2]. Their high surface reactivity and ability to release metal ions have been linked to oxidative stress, apoptosis, and amyloid fibril formation in neuronal models [3]. Human cystatin C (HCC) is an amyloidogenic protein associated with hereditary cystatin C amyloid angiopathy (HCCAA). Reduced levels of HCC are observed in Alzheimer's disease, where it co-deposits with β-amyloid in senile plaques. Studies show that metal ions, especially copper, modulate the aggregation behavior of amyloidogenic proteins, including HCC, with microfluidic flow conditions further influencing this process. In this study, we used a microfluidic chip to apply physiological shear stress and investigate the combined effects of copper oxide nanoparticles (CuO NPs) and dynamic flow conditions on the aggregation behavior of human cystatin C. Additionally, to characterize protein aggregation, we employed complementary techniques including atomic force microscopy (AFM), thioflavin T fluorescence assay, and small-angle X-ray scattering (SAXS), conducted at the P12 BioSAXS beamline at DESY (Hamburg, Germany). To further assess the potential neurotoxic consequences of these interactions, we performed cell viability assays on SH-SY5Y neuronal cells exposed to the selected aggregates.

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#22 Bacterial protein CsgA modulates β2microglobulin amyloid assembly: Implications of biofilms in kidney-related disorders

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Most urinary tract infections (UTIs), such as cystitis and pyelonephritis, are caused by bacteria such as *Uropathogenic Escherichia coli* (*UPEC*). Bacterial cells can adhere to kidney stones, medical implants, or biological sites via the formation of cell aggregates called biofilms. The bacterial biofilms possess a protective covering called a matrix that provides access and stability to bacterial cells at these sites. The biofilm matrix comprises protein polymers known as amyloids, polysaccharides, extracellular DNA, and water. Bacterial amyloids in the matrix mediate cell-cell adherence, mechanical strength, and biofilm stability. Curli, a functional amyloid in *E. coli* activates host defense mechanisms and inflammatory response. Under pathological conditions, activated immune cells release antimicrobial peptides such as β2 microglobulin (β2m). Studies reported 5-10 times elevated β2m levels due to improper filtration and overexpression under disease conditions, leading to the formation of β2m amyloids that are connected with renal failure. However, the potential contribution of bacterial functional amyloids during β2m amyloidogenesis is poorly understood. In our study, we hypothesize that *UPEC*-derived curli amyloids, particularly the major subunit CsgA, may cross-seed during β2m amyloidogenesis and may contribute to disease aggravation. We aim to gain mechanistic insights and the consequences of cross-talk between CsgA and β2m using various biophysical and biochemical approaches. Further we will monitor the modulation in the release of pro-inflammatory markers (IL6 and TNFα) in THP-1 cells without and with co-aggregation. We believe that the insights gain from this study will help us to understand the inter species amyloid cross-talk that may contribute to disease progression. Understanding of bacterial interaction with the elevated β2m levels will help to novel therapeutic targets to inhibit pathological protein aggregation.

Postersession 2

#23 Cryo-EM structure of a viral transduction enhancing amyloid fibril

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Background:
Amyloid fibrils have emerged as innovative tools to enhance the transduction efficiency of retroviral vectors in gene therapy strategies, such as chimeric antigen receptor T-cell (CAR-T) therapy. The virus-enhancing activity is thought to arise from the ability of these fibrils to bind viral particles and to promote the interactions of the bound virus with the surface of human cells. These features depend in turn on the overall charge of the fibrils, the length of the fibril forming peptide, the presence of amphiphilic residue patterns or the involvement of different charged amino acids. In the absence of an experimentally determined structure of a virus-binding fibril however, it is difficult to estimate how these peptide properties manifest themselves at the quaternary structural level of the assembled amyloid fibril.

Objective:
In this study cryo-electron microscopy was used to determine the molecular structure of an amyloid fibril formed by PNF-18 peptides. This 7-mer peptide was artificially designed as retroviral transduction enhancer and increases HIV-1 infection rates of TZM-bl cells by a factor of more than hundred-fold.

Result & Conclusion:
The results show that PNF-18 peptides undergo a time dependent morphological maturation into polymorphic amyloid fibril structures. These fibrils consist of mated cross-β sheets that interact by hydrophobic residues of amphipathic fibril-forming peptide. The polar residues become exposed to the solvent and create a strongly cationic fibril surface. The structural characteristics revealed by our analysis help to explain the mechanism of retroviral enhancement and provide insight into the molecular plasticity and morphological maturation of amyloid fibril structures.

#24 Structural analysis of Pmel17 using Cryo-EM, a functional amyloid involved in melanin synthesis

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Background: One of the earliest identified human physiological amyloids is Pmel17, which aggregates within melanosomes to form a scaffold crucial for melanin synthesis (McGlinchey et al., 2009). This process involves the proteolytic cleavage of the repeat domain (RPT) of Pmel17, which then assembles into amyloids fibrils at acidic pH levels, facilitating melanin synthesis and deposition. Whole exome sequencing of families with heritable pigment dispersion syndrome and pigmentary glaucoma identified six non-synonymous variants in the RPT (Lahola-Chomiak et al., 2019). These variants exhibit structural defects in Pmel17 fibrillogenesis, which may alter fibril morphology or impair amyloid formation, thereby impacting in its function.

Objectives:

- Determine the atomic structures of wild-type (WT) and RPT mutant fibrils using cryogenic electron microscopy (cryo-EM) to define possible molecular alterations caused by pathogenic variants.
- Comparative functional analysis of WT human Pmel17 RPT, disease-associated mutants, and evolutionarily conserved mouse Pmel17 RPT on melanin synthesis and structural correlation.

Methods: Human Pmel17 RPT, both WT and disease-associated mutant variants were generated via site-directed mutagenesis, in addition to mouse Pmel17 RPT. Proteins were expressed, purified, and assembled into amyloids in mild acidic buffer to simulate native melanosome conditions. Samples were then plunge-frozen in liquid ethane for cryo-EM imaging using a Titan Krios microscope to obtain high-resolution datasets. These datasets were analysed by helical reconstruction in RELION. For melanin synthesis assay, purified RPT were supplemented with DL-DOPA and tyrosinase to catalyse melanin formation. Melanin synthesis activity was quantified by the amount of melanin formed by measuring absorbance at 400 nm.

Results: We successfully generated the WT and mutant RPTs and assembled amyloid fibrils. We are currently working towards the structural determination by cryo-EM. Preliminary helical reconstructions were conducted on the human RPT using reference-free 2D class averages show a cross section with 4 protofilaments arranged in a C2 symmetry.

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#25 Curli-primed immune cells exacerbate pro-inflammatory response to α-synuclein

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The role of the gut-brain axis in neurodegenerative diseases is increasingly recognized. Parkinson's disease (PD) is a fatal neurodegenerative disease that has been associated with alterations in the gut microbiota. Bacterial amyloids are altered protein aggregates produced by certain gut bacteria, such as *Escherichia coli*, as a major component of biofilm. The presence of bacterial amyloids in the gut could potentially trigger or exacerbate Parkinson's disease. However, the exact mechanism remains unknown. This study aims to decipher the immune activation in response to alpha-synuclein when cells (bone marrow-derived macrophages; BMDMs) are primed with bacterial amyloid curli. We observed an enhanced immune response to α-synuclein when macrophages were primed with bacterial amyloid curli. Since the same immune receptors recognize bacterial and human amyloids, we speculate that priming with curli may exacerbate immune activation during the initiation or progression of neurodegenerative diseases. Our data provide evidence that α-synuclein aggravates the inflammatory response in the presence of curli, which may precede neuroinflammation during PD. We believe that deciphering the immune response to amyloid fibrils in the presence of curli will help us understand the contribution of bacterial amyloids in neuroinflammation during PD pathology.

#26 FapC fibril as a scaffold for developing amyloid-based materials: from structure to catalysis

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Functional amyloids, such as CsgA or FapC, are essential structural elements of the bacterial biofilm[1]. Despite they have been widely studied, we still lack high-resolution structures that provide information at residue level. Here, we present a high-resolution cryo-EM structure of a FapC fibril. In resemblance with computational predictions, this fibril shows a Greek key -like conformation with high degree of conservation of the residues constituting and stabilising the core. This degree of sequential and, therefore, structural conservation translates into the possibility of inducing FapC aggregation with other variants upon cross-β stacking. Our data also demonstrates that the extensive H-bond network established by these conserved residues provides an extraordinary resistance to harsh conditions, including extreme pH and high temperature, a property of great interest in the development of new materials[2]. In addition, FapC fibrils showed intrinsic hydrolytic properties performing esterase and lipase reactions in a similar extent than that of its *Escherichia coli* counterpart, CsgA, and better than other reported amyloids. Despite this hydrolytic capacity was first observed in presence of soluble and simple substrates as pNytrophenyl acetate or butyrate, it was later confirmed using more complex reporters (pNytrophenyl palmitate) embedded in lipidic liposomes. Remarkably, this catalytic capacity is retained after fibril deposition in different types of membranes and after multiple washing or substrate addition rounds, proving their recyclable properties. The fibrils also showed significant esterase activity after being exposed to extreme conditions, correlating with ist structural resistance. Altogether, our structural and catalytic characterization lays the foundation for the rational design of bacterial amyloid-based materials[2,3].

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#27 Bacterial amyloids in the context of neurodegeneration: cross-interactions between Aβ(1–42) and Hpn from *Helicobacter pylori*, and its fragment

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Amyloids are associated with their role in neurodegenerative disorders (NDDs), such as Alzheimer's disease (AD), which mainly involves aggregation of beta-amyloid (Aβ). Growing evidence suggests a link between chronic bacterial infections and NDDs. *Helicobacter pylori*, a gastric pathogen, has been implicated in this context [1]. For instance, infected individuals often exhibit elevated levels of Aβ in cerebrospinal fluid and blood [2].

One of the proteins produced by *H. pylori* is Hpn, a small protein composed of 60 amino acids. It is exceptionally rich in histidine residues (47%) and is known for its strong affinity for metal ions, particularly Ni^{2+} [3]. Hpn has been shown to form amyloid-like aggregates [4], though the mechanisms underlying its aggregation and precise biological role of this protein remain unclear.

The aim of this study was to investigate interactions between Aβ(1–42) and full-length Hpn, as well as its highly aggregation-prone fragment Hpn(4–38). Structural and kinetic analyses were performed using Thioflavin T (ThT) fluorescence assays and Fourier-transform infrared (FTIR) spectroscopy. According to our results, in contrast to the previous findings reported by R. Ge et al. for recombinant full-length Hpn [4], the same protein in its synthetic form did not form fibrils under the experimental conditions used. Instead, it assembled into spherical aggregates. Moreover, the presence of Aβ(1-42) significantly altered the aggregation behavior of both Hpn and Hpn(4–38), influencing their structural characteristics and aggregation dynamics. These results suggest potential molecular interactions between bacterial proteins and Aβ(1–42), which may be relevant for understanding mechanisms linking the infection with *H. pylori* to AD pathogenesis.

This work was supported by the National Science Centre, Poland [grant number 2019/35/B/NZ2/03997].

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#28 Bovine Meat and Milk Factors (BMMFs) as Zoonotic-Linked Drivers of Cancer and Chronic Diseases – New Variants of Disease-Associated Amyloids?

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Bovine meat and milk factors (BMMFs) are plasmid-like DNAs that have been frequently detected in bovine meat and milk products and other sources and were proposed to contribute to the development of cancer and neurodegenerative diseases. BMMFs encode a conserved replication protein (Rep) that is actively transcribed and translated in human cells. BMMF Rep expression in tissues of colorectal (CRC), lung, and pancreatic cancer patients histologically aligned with detection of specific populations of macrophages and was increased in cancer versus healthy individuals and therefore described as possible biomarker for cancer. Rep expression coincided with markers of chronic inflammation and DNA damage also in pre-cancerous stages suggesting a causal role of BMMF in chronic inflammation-driven indirect carcinogenesis.

In this study, a Rep WH1 domain encoded on BMMF MSBI1.176 – initially found in multiple sclerosis human brain and CRC tissues - was determined to 1.53 Å resolution by X-ray crystallography. The overall structure of the MSBI1.176 WH1 domain was remarkably similar to other Rep structures e.g. to RepA from *Pseudomonas syringae*, despite having a low (28%) amino-acid sequence identity. The MSBI1.176 WH1 contained elements common to other Reps including an interface for dimerization. Analyses based on electron microscopy, size exclusion chromatography, dynamic light scattering, thioflavin assay as well as in silico predictions suggest BMMF Rep oligomerization likely involving amyloidogenic features. These findings suggest that the MSBI1.176 Rep might have comparable roles and functions to other known and partially amyloidogenic Reps of different origins.

Preliminary analyses support cytotoxicity of Rep in cell culture and support a disease-promoting, pathogenic function of BMMF, which therefore might be of use for (early) detection and monitoring of cancer and other chronic (neuronal) diseases to gain a better understanding of underlying disease mechanisms.

#29 Aggregation determinants of *Staphylococcus aureus* functional amyloids and their tendency to cross-interact with human pathogenic amyloids

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Studying heterogeneous amyloid interactions is crucial for understanding the mechanisms underlying their self-assembly, particularly in the context of pathogenic amyloids, where aggregation can drive disease progression or inhibition. Clarification what triggers or prevents amyloid aggregation is essential, as this may result in the development of novel treatment approaches for amyloid-related diseases. Phenol Soluble Modulins (PSMs) from *Staphylococcus aureus* are short bacterial functional amyloidogenic peptides [1, 2] that can be used as scaffolds to investigate interactions with other amyloids and find sequential determinants that contribute to the aggregation process. Indeed, we showed that the sequences and terminal alterations of PSMs have a significant impact on their aggregation capabilities. Particularly, we demonstrated that synthetic PSMα3 devoid of N-formylation, which is characteristic of wild-type (WT) peptides, does not form amyloid fibrils and, in addition, is not cytotoxic to human cells, in contrast to WT, which is the most toxic among the PSM family [3, 4]. In contrast to WT peptides, synthetic PSMβ1 and PSMβ2 assemble far more slowly and have distinctly different aggregate morphologies. Furthermore, the cross-interaction propensity of synthetic deformylated PSMs varies significantly from that of wild peptides, showing occasionally opposing patterns. As we found out, synthetic PSMα3 [4] and PSMβ1 exhibit a considerable anti-fibrillogenic effect towards a number of human pathogenic amyloids, whereas WT peptides either have no effect at all or, conversely, accelerate the aggregation of both other PSMs [2] and several human amyloids. In conclusion, the aggregation propensity of PSMs varies widely, and the results of cross-interactions are very sensitive to terminal alterations and significantly influenced by both their sequences and the sequences of target human amyloids.

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#30 Structural characterization of Atrial Natriuretic Peptide amyloid fibrils from Isolated Atrial Amyloidosis patients

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Isolated atrial amyloidosis (IAA) is an age-related condition primarily affecting the atria of the hearth, where atrial natriuretic peptide (ANP) is the main constituent of amyloid fibrils. ANP is a 28-amino-acid peptide hormone secreted by atrial cardiomyocytes, involved in regulating blood volume and pressure. The total circulating ANP exists in three molecular forms: mature ANP (28 amino acids) with an intramolecular disulfide bond, dimeric ANP (56 amino acids), and pro-ANP (126 amino acids), which contains the sequence of mature ANP. Under pathological conditions, increased production and secretion of both monomeric and dimeric ANP contribute to amyloid fibril formation, leading to disrupted atrial conduction and an elevated risk of atrial fibrillation.

To date, the lack of structural information on pathological ANP fibrils has hindered the development of hypotheses regarding the aggregation mechanism and the impact of amyloid deposits on atrial fibrillation severity. To address this gap, we collected atrial appendages from three patients during open-heart surgery. Immunohistochemical investigation confirmed the presence of ANP amyloid deposits in these tissues. Cryo-electron microscopy (cryo-EM) analysis on extracted fibrils revealed the presence of two distinct polymorphs of ANP fibrils in all three patients, which were independently solved at resolutions of 2.9 Å and 3.3 Å, respectively. Remarkably, de novo model building identified the presence of covalent dimeric ANP in both polymorphs. While polymorph A exhibited an anti-parallel orientation of the two chains, polymorph B displayed a parallel orientation. Overall, our findings ascribe to dimeric ANP a critical role in amyloid formation, offering promising directions for earlier detection and treatment of IAA.

#31 Aggregating amyloid resources

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Protein aggregation is responsible for several degenerative conditions in humans and also hampers industrial protein production. Bioinformatics tools have been developed to predict and redesign protein solubility more efficiently by understanding the underlying principles behind aggregation. As more experimental data become available, dedicated resources for storing, indexing, classifying and consolidating experimental results have emerged. These resources vary in focus, including aggregation-prone regions, 3D patches, or protein stretches capable of forming amyloid fibrils. Some of these resources also consider the experimental conditions that induce protein aggregation and their impact on the process. We examine the evolution of protein aggregation databases and survey the current state-of-the-art resources[1]. We highlight their applications, complementarity and existing limitations, as well as the propagation of errors between different sources. Moreover, we showcase the existing symbiosis between amyloid-related databases and predictive tools. The list of present and past amyloid databases is available online: https://biogenies.info/amyloid-database-list/.

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#32 Discovery of Amyloid Composites in Seeds and Their Role in Germination

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We hypothesized that the electron-dense features of plant seed storage protein bodies (SSPBs) may share similar molecular signatures with amyloids present in inclusion bodies of other protein aggregates, for example in Huntington's disease. To test this, we used amyloid-specific probes, counterstaining approaches, protoplasts, and biophysical methods, and discovered that SSPBs contain a composite structure of amyloids, amyloid-like aggregates, and native protein structures. Through sequence to structure analysis and peptide fingerprinting, we identified globulin proteins as the main constituents of SSPBs undergoing amyloid transformation. Amyloid composites gradually degrade to supply amino acids during seed germination. This process is responsive to dormancy and germination. Gibberellins promoted amyloid degradation and germination, while abscisic acid had the opposite effect. This research presents the first comprehensive identification of endogenous amyloid composites in plants and highlights their role in seed germination, with potential implications for stress adaptation, plant amyloid biology, life strategies of seeds, and crop productivity.

#33 Intrinsic disordered domains of functional and disease-related amyloids: A combined NMR, EPR, and MD approach

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Structural biology of amyloid fibrils has mostly focused on their cross-β core. However, many amyloid fibrils contain large intrinsically disordered regions (IDRs) in addition to their core. These IDRs often form most of the fibril surface and are binding sites for chaperons, antibodies, and other biomolecules. Consequently, the dynamics and conformational ensemble of this IDRs is important for the function of functional amyloids and the toxicity of amyloids found in disease. Over the years, we have observed such IDRs in the HET-s,[1] RIP1-RIP3,[2] ApCPEB,[3] and Orb2[4,5] functional amyloids. In addition, we characterized IDRs in huntingtin exon-1[6–9] and α-synuclein.[10] From an NMR point of view, these IDRs are neither strictly solid, nor are they truly in solution. These restricted dynamics create unique challenges for obtaining good NMR data especially for regions of intermediate dynamics. Therefore, we are exploring different NMR techniques to spectroscopically access region of intermediate dynamics and combine our NMR data with CW and DEER EPR spectra that do not suffer from the same problem. We are using our NMR and EPR data to benchmark MD simulation with the goal of creating a fibril model that includes both the static amyloid core and ist IDR.
In a next step, we are studying the interaction of proteins with amyloid IDRs. Specifically, we will present data showing that the nature of the interaction between the co-chaperone DNAJB1 and α- synuclein fibrils depends on an important post-translational modification.[10]

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#34 The role of phenol-soluble modulins on *Staphylococcus aureus* biofilm formation

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Staphylococcus aureus is a pathogenic bacterium prone to forming biofilms, dense communities of cells in a self-produced matrix, on surfaces[1] . Biofilms provide high resistance to antibiotics and immune responses, leading to persistent infections like endocarditis and device-related infections.[1] A key factor of their stability is bacterial amyloids, particularly fibrils formed by phenol-soluble modulin (PSM) peptides. They enhance biofilm cohesion, surface adhesion, and resistance to physical and chemical attacks, thus protecting the bacteria. Amyloids also make treatment more challenging by reinforcing biofilm architecture.[2] Targeting amyloid formation could weaken the biofilm scaffold, increasing its susceptibility to antibiotics and immune clearance. This approach offers a promising strategy to combat chronic, biofilm-associated *S. aureus* infections. In this work we show that PSMs are an integral part of *S. aureus* biofilm formation through biophysical assays, confocal scanning laser microscopy and electron microscopy using WT and ΔPSM mutant strains. Our results show that the mutant forms weaker biofilms than WT, further confirming the importance of the PSM to biofilm formation. Additionally, we have repurposed amyloid inhibitors that can also inhibit PSM amyloid formation and applied them to biofilms. The presence of amyloid inhibitors leads to the formation of much weaker biofilms comparable to ΔPSM mutant biofilm, leading us to believe that PSM form amyloids within biofilm and are structurally important to overall biofilm stability. By disrupting a key structural component of the biofilm matrix, these compounds offer a strategic approach to enhancing treatment efficacy against persistent bacterial infections.

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#35 A structural chameleon in cryo-EM: cross-α/cross-β amyloids & nanotubes

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Amyloid fibrils are self-assembled peptide or protein aggregates with cross-β steric zipper backbones that have the notorious reputation of cytotoxic effects and involvement in numerous neurodegenerative diseases. On the other hand, the diverse group of antimicrobial peptides (AMPs) may mediate innate immune responses in conjunction with fibril formation. Our research group has bioinformatically predicted potential fibril-forming AMPs (ffAMPs) (1) that feature sequence traits of α-helices but still possess amphipathic propensities for fibrillation into yet unexplored canonical cross-β or unusual cross-α architectures. Most excitingly, the ambiguities in predicted peptide secondary structures could permit hidden cross-α/-β chameleon switching mechanisms that might drive biological fibril functions in response to environmental changes. Against this background, we screened an amphibian ffAMP candidate of the Temporin family using cell biological, biochemical, biophysical and (cryogenic-) electron microscopy (EM) tools. First, we established that this Temporin is active against pro- and eukaryotic cells, and that it forms fibrils upon incubation. In contrast to other amyloidogenic peptides, EM and circular dichroism (CD) spectroscopy showed that fibril contact with model membranes readily disassembles the fibrils as redissolved peptides adopt α-helical conformations. This membrane-triggered structural switch is most likely also a key-component for observed bactericidal and cytotoxic functions. Further investigation of the peptide's fibrillation behaviour was done using combinations of Thioflavin-T binding, CD spectroscopy, negative stain and cryo-EM. We could reveal a condition-dependent folding and fibril assembly pathway in which an interplay of ionic stabilisation and peptide concentration direct the peptide through a multi-funnelled folding landscape. Thereby, we could obtain high resolution cryo-EM models of both cross-β amyloid and cross-α amyloid fibrils. In addition, the formation of nanotubes could be induced. This highlights the complexity of amyloidogenic peptides, emphasising the need to understand their biological behaviour and context before ultimately developing sequence- and structure-based medical applications.

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#36 The effect of exogenous prion-like proteins in the evolution of neurodegenerative diseases

Jofre Seira Curto, Adan Dominguez Martinez, Genis Perez Collell, Estrella Barniol Simon, Marina Romero Ruiz, Maria Rosario Fernandez, Natalia Sanchez de Groot

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The gut is exposed to a wide range of proteins from ingested substances and resident microbiota. Among them, prion-like proteins can enter and propagate throughout the organism influencing disease development, however, the exact mechanism of this outcome remains unknown. In this study, we employed a multidisciplinary approach to investigate the biophysical properties of exogenous prion-like proteins and their interference in the aggregation of the amyloid beta peptides.
Our research focused on characterizing the amyloidogenic potential of various sequences derived from the gut microbiota. We observed their ability to form amyloid fibrils capable of interfering with amyloid beta peptides, triggering propagation in yeast models and impacting neuroblastoma-derived cell viability (SH-SY5Y). We have also studied how the presence of exogenous prion-like proteins affects homeostasis, interaction, and cross-aggregation of amyloid beta isoforms. Finally, we are characterizing the structure of the resultant amyloid fibers with cryoelectron microscopy. Overall, our findings support the gut as a potential entrance and reservoir for exogenous prion-like proteins which are able to interfere with the amyloid beta peptides indicating a possible role on the development of Alzheimer's disease. This study highlights the importance of considering the impact of exogenous prion-like proteins on host health.

#37 Exogenous Amyloid Sequences: Their Role in Amyloid-Beta Heterotypic Aggregation

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Protein aggregation is a complex process heavily dependent on environmental conditions and molecular interactions. Usually overlooked exogenous interactions can also play an important role in those processes. Cross-seeding in a repeated manner is usually overlooked despite the potential for such events to occur as proteins circulate through the body. In this work we study the impact of exogenous amyloid sequences derived from the gut microbiota on the heterotypic aggregation of Aβ peptides. We utilized ten 21-amino acid peptides derived from bacterial genomes, previously shown to interfere with Aβ40 aggregation and induce memory loss in *Caenorhabditis elegans*. Using cross-seeding assays with Aβ40 and Aβ42, we analyzed the impact of these microbial sequences on aggregation kinetics and seed propagation. Our results show that exogenous peptides can significantly alter Aβ aggregation behavior, leading to variations in fibril morphology and propagation potential. These findings led us to propose the concept of “Interaction History,” which suggests that prior molecular interactions influence the trajectory and properties of Aβ aggregation. This perspective offers insight into the structural diversity of amyloids observed in the disease. Overall, our study highlights the influence of microbial amyloids on Aβ behavior and underscores the need to consider environmental factors in Alzheimer’s pathology and therapy development.

#38 Changing the Game of Amyloid Fibril & Protein Condensate Research with FIDA

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This poster introduces FIDA as a novel solution-based tool for the quantitative study of amyloid fibrils and biomolecular condensates, based on first-principle measurements of hydrodynamic radius. This method used only nanoliters of patient-derived samples such as CSF. Unlike other methods, FIDA enables direct, size-resolved detection of monomers, oligomers, and fibrils without relying on immobilization, labelling, or buffer-specific conditions. Newly developed workflows (MTIS, Capflex, TDIPS) expand application to measuring fibril growth kinetics, binding interactions with oligomeric and full-length fibrils, and thermodynamic stability across polymorphic forms. In condensate research, FIDA allows label-free mapping of phase diagrams and quantification of both dilute and dense phase properties. Together, these capabilities provide a highly accessible and quantitative platform for probing the structural and thermodynamic complexity of amyloid and phase-separated systems.

#39 The Order and Division of Labor in Uropathogenic *E. coli* Biofilms

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Background: Bacteria primarily exist in biofilms—communities of cells living together that are protected from environmental stressors like antibiotics, predation, and extreme temperatures. The extracellular matrix (ECM) is a hallmark of biofilms that confers protection to the bacterial community; for *Escherichia coli* (*E. coli*) biofilms, the ECM consists primarily of protein and carbohydrate polymers known as curli and cellulose, respectively. The production of curli and cellulose are regulated by curli specific gene D (CsgD). *E. coli* stratify into two physically distinct and separable subpopulations within biofilms: the ECM-producing cells, and the **non**-ECM producing cells.

Methods: To understand the difference in gene expression between the two biofilm subpopulations, we performed RNA sequencing on each individual subpopulation and performed differential gene expression analysis comparing the two subpopulation transcriptomes. To determine if there is a more niche division of labor throughout the biofilm beyond the separable subpopulations, we employed a single-cell RNA sequencing (scRNA-seq) approach.

Results: The two subpopulations showed distinct transcriptional profiles. We identified over 200 transcripts as having at least a 2-fold difference between the two subpopulations. The differentially expressed transcripts are involved in motility, curli production, environment sensing, anaerobic respiration, and stress response. Preliminary scRNA-seq experiments resulted in the identification of roughly 150,000 unique cells from *E. coli* biofilms with greater than 100 transcripts per cell.

Conclusions: The two biofilm subpopulations have distinct transcriptional profiles that provide insight on potential mechanisms important for biofilm formation and maturation. To understand the drivers of population differentiation, we will explore genes that are significantly differentially regulated between the major subpopulations and the minor subpopulations identified by scRNA-seq to identify pathways involved in biofilm differentiation and maturation. To our knowledge, this is the first application of scRNA-seq on bacterial biofilms.

Postersession 1

	Title	Presenter
#1	Dissecting the crosstalk between transthyretin amyloids and the extracellular matrix	Jan-Hannes Schaefer
#2	Functional Aggregation or Receptor Affinity? Evolutionary Insights into Peptide Amyloids of the PACAP–Glucagon Superfamily	Dániel Horváth
#3	The Impact of Extracellular DNA on αPSM Functional Amyloids from Staphylococcus Aureus	Emilie Buhl Plechinger
#4	Elucidating Molecular Determinants of Fibrillation in the V and W Proteins from Paramyxo-virus: Implications for Targeted Therapeutic Strategies	Harshita Sawdekar
#5	Structural Basis of Pseudomonas Biofilm-Forming Functional Amyloid FapC Formation	Kasper Holst Hansen
#6	N-terminal capping modulates fibrillation and cytotoxicity of PSMα3 under physiological conditions	Laura Bonnecaze
#7	Amyloidogenicity of virus-derived peptides and their effect on amyloid formation of proteins associated with neurodegenerative disease	Debdeep Chatterjee
#8	Functional Amyloid Formation in Staphylococcal Hemolytic Peptides	Janina Schiller
#9	The structure/function relationship of the Small Basic Protein in Staphylococcus epidermidis biofilm formation	Jim Monistrol
#10	Light-fueled transient catalytic amyloid-like fibrils for antibiotic degradation	Shubhra Kanti Bhaumik
#11	Amyloid aggregation of PACAP-glucagon superfamily hormones: dual role of the receptor activating segments?	Zsolt Dürvanger
#12	Germ Granules in Mammalian Spermatozoa: Proteinaceous Carriers of Cytosolic Inheritance?	Gail A. Cornwall
#13	Coiled Double Amyloid-Like Fibrils Allosterically Catalyze Hydrolysis of β-Lactam Antibiotics	Sisira Mambram Kunnath
#14	Effects of the ionic strength on functional amyloid fibril formation	Andrea Bartolomé-Nafría
#15	Universal methodology for screening and identification of amyloids	Anna A. Valina
#16	Amyloid Fibrils Catalyzing Cationic Ring-Opening Polymerization of Cyclic Esters: Toward Bioinspired Routes to Functional Polyesters	Elinor Slavsky
#17	Reversible, Functional Amyloids in Metabolic Regulation and Immune Responses	Gea Cereghetti
#18	StriFi: Structural and Functional Annotation of Amyloid Fibrils and Their Polymorphic Diversity	Oriol Bárcenas
#19	Amyloids and RNA: Molecular Cooperation at the Beginning of Life	Saroj Rout

Postersession 2

	Title	Presenter
#21	Impact of Copper Oxide Nanoparticles and Microfluidic Flow on Amyloidogenic Properties of Human Cystatin C	Karolina Rucińska
#22	Bacterial protein CsgA modulates β2microglobulin amyloid assembly: Implications of biofilms in kidney-related disorders	Harita Ben
#23	Cryo-EM structure of a viral transduction enhancing amyloid fibril	Matthias Schmidt
#24	Structural analysis of Pmel17 using Cryo-EM, a functional amyloid involved in melanin synthesis	Pablo Adrian Guillen-Poza
#25	Curli-primed immune cells exacerbate pro-inflammatory response to α-synuclein	Neha Jain
#26	FapC fibril as a scaffold for developing amyloid-based materials: from structure to catalysis	Samuel Peña-Díaz
#27	Bacterial amyloids in the context of neurodegeneration: cross-interactions between Aβ(1–42) and Hpn from Helicobacter pylori, and its fragment	Oliwia Polańska
#28	Bovine Meat and Milk Factors (BMMFs) as Zoonotic-Linked Drivers of Cancer and Chronic Diseases – New Variants of Disease-Associated Amyloids?	Timo Bund
#29	Aggregation determinants of Staphylococcus aureus functional amyloids and their tendency to cross-interact with human pathogenic amyloids	Kalitnik Aleksandra
#30	Structural characterization of Atrial Natriuretic Peptide amyloid fibrils from Isolated Atrial Amyloidosis patients	Luca Broggini
#31	Aggregating amyloid resources	Michał Burdukiewicz
#32	Discovery of Amyloid Composites in Seeds and Their Role in Germination	Ashwani Kumar Thakur
#33	Intrinsic disordered domains of functional and disease-related amyloids: A combined NMR, EPR, and MD approach	Ansgar B. Siemer
#34	The role of phenol-soluble modulins on Staphylococcus aureus biofilm formation	Mariana P. Cali
#35	A structural chameleon in cryo-EM: cross-α/cross-β amyloids & nanotubes	Jesse Gayk
#36	The effect of exogenous prion-like proteins in the evolution of neurodegenerative diseases	Jofre Seira Curto
#37	Exogenous Amyloid Sequences: Their Role in Amyloid-Beta Heterotypic Aggregation	Genis Perez Collell
#38	Changing the Game of Amyloid Fibril & Protein Condensate Research with FIDA	Einar Halldórsson; Emil G. P. V. Stender
#39	The Order and Division of Labor in Uropathogenic *E. coli* Biofilms	Kailyn Jessel

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