## KEYNOTE SPEAKER

**Titel**

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**INVITED SPEAKER**

## Secreted mucins – The frontline of host defence

David J. Thornton[1]

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Mucus coats our mucosal surfaces and acts a gatekeeper denying access to harmful agents trying to enter the body. Mucus has bespoke properties at each mucosal site that are tailored to protect against diverse external challenges in terms of its physical and bioactive properties – for example in the colon there is a thick, bi-layered, adherent mucus. Whereas in the lung there much thinner, flowing mucus layer. Dysregulation of the mucus phenotype is associated with disease. For example, a change to a thin, patchy mucus layer in the gut is a feature of inflammatory bowel disease, while a change to a thick, adherent mucus in the lung is a feature of obstructive lung disease.

The structure and protective functions of mucus are provided by a family of closely related, network-forming, multi-domain glycoprotein polymers, the gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6 and MUC19). Animals deficient in gel-forming mucins have been shown to be compromised in host defence. A dominant feature of these mucins is their extensive modification with a diverse array of O-glycan chains, and the different mucins display distinct patterns of O-glycans. These glycans are key drivers of mucin function, including their regulation of microbes and immune cells.

While great progress has been made on many aspects of gel-forming mucin biology, for example, high-resolution structures are now available for the different protein domains within the mucins, the structural basis of mucus barrier formation by mucins, how mucins are synthesised and stored within secretory cells, the molecular components/pathways involved in their secretion, and that changes in mucins can result from external challenges, in particular by pathogens. However, we still lack a complete understanding of the regulation of mucus organisation, and knowledge of how mucins changes with age, infection, and inflammation is under-developed. Expanding our knowledge of these key areas is needed to understand how changes in mucins impact the protective function of mucus in health and disease.

**INVITED SPEAKER**

## A Mucin Odyssey – Tales of MUC1 and other Transmembrane Mucins

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The early years of studying mucins, in particular MUC1, were confusing. One of my earliest introductions to mucins was at the mucus club which was held at various locations in the UK in the mid1980s. Dr. John Sheehan was a pioneer in mucus studies, and he started one meeting with a slide showing a mucin strand extending through three slides in length. The size was a difficult concept, and many of us wondered what we were getting into.
We began the molecular search for mucin proteins with antibodies developed by Dr. Joyce Taylor-Papadimitriou in 1981. The antibodies, now known as HMFG-1 and HMFG-2, were made by immunizing mice with milk fat globule membrane from early lactation milk from women. The repetitive epitopes of the antibodies were confusing, but ultimately led to the discovery that mucins were made up of tandem repeats containing high levels of serine, threonine, and O-linked glycosylation. Similar stories can be told about several of the other large transmembrane mucins. The well-known CA125 antibody developed by Dr. Robert Bast in 1981 recognized the surface of ovarian tumors. The carboxy and amino termini of CA125 were identified 20 years later, in 2001, and described the exceedingly large mucin, MUC16, with a core protein weight of about 2.5 million Daltons and a potential glycosylated molecular mass of twice that. In 2024, long-read sequencing would change the number of repeats from 63 to 19. It seems that the exact molecular sequence and structure of MUC16 are not yet defined. Many of the mucins undergo alternate splicing, resulting in a functionally diverse repertoire of mucin proteins which makes them difficult to characterize. Understanding the gene structure of the transmembrane mucins will aid functional studies and expose therapeutic targets as many of these mucin proteins function in health and disease.

**INVITED SPEAKER**

## Structural and genetic diversity in the secreted mucins, MUC5AC and MUC5B

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Few loci in the human genome have been linked to severe respiratory disease outcomes; the mucin genes, however, are strong candidates due to their critical roles in pathogen entrapment. MUC5AC and MUC5B encode two secreted proteins with variable number tandem repeat sequences (VNTRs) that enable binding to pathogens. These loci have been difficult to assess due to the large and highly polymorphic protein-coding VNTRs. Here, we characterize the genetic structural diversity of MUC5AC and MUC5B by long-read sequencing and assembly of 206 human and 12 non-human primate haplotypes. We find that MUC5B is largely invariant in length (5761-5762aa) in humans; however, 7 haplotypes encode a much larger protein (6291-7019aa) due to a VNTR expansion. In contrast, we discovered 30 allelic variants of MUC5AC that encode 16 distinct proteins (5249-6325aa) with considerable variation in cys domain copy number and VNTR copy/domain number. We show that MUC5AC alleles can be grouped into three phylogenetic clades: H1 (46%, ~ 5654aa), H2 (33%, ~5742aa), and H3 (7%,~ 6325aa). Comparison to non-human ape gene models relative to the two most common human MUC5AC variants (5654aa and 5742aa) suggests an overall reduction in MUC5AC protein length during recent human evolution. Linkage disequilibrium (LD) and Tajima's D analysis reveal that East Asians carry exceptionally large MUC5AC LD blocks and an excess of rare variation ($p<0.05$ autosome-wide). We identified tagging SNPs (tSNPs) in high LD with haplogroups of MUC5AC ($r2 >0.85$; 37 for H1, 7 for H2, 67 for H3) to use for VNTR length imputation. We applied a structural variant genotyping tool called "locityper" to accurately genotype the haplotypes in 2,596 population samples from the 1000 Genomes Project. These findings confirm that the likely ancestral haplotype (H3) has been significantly reduced among Asians at the expense of H1/H2, which show signatures of positive selection. We predict population differences to airway associated disease and provide a more broadly applicable strategy to characterize the genetic impact of the hundreds of complex protein-encoding VNTRs that can now be resolved with long-read sequencing.

**INVITED SPEAKER**

## A mechanism of gene evolution generating mucin function

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How novel gene functions evolve is a fundamental question in biology. Mucin proteins, a functionally but not evolutionarily defined group of proteins, allow the study of convergent evolution of gene function. By analyzing the genomic variation of mucins across a wide range of mammalian genomes, we propose that exonic repeats and their copy number variation contribute substantially to the de novo evolution of new gene functions. By integrating bioinformatic, phylogenetic, proteomic, and immunohistochemical approaches, we identified 15 undescribed instances of evolutionary convergence, where novel mucins originated by gaining densely O-glycosylated exonic repeat domains. Our results suggest that secreted proteins rich in proline are natural precursors for acquiring mucin function. Our findings have broad implications for understanding the role of exonic repeats in the parallel evolution of new gene functions, especially those involving protein glycosylation.

## SELECTED SPEAKER

### AGR2 as a novel regulator of IRE1b in goblet cell quality control

Phaedra Guilbert[1,2], Eva Cloots[1,2], Mathias Provost[2,3], Farzaneh Fayazpour[1,2], Evelien Van de Velde[1,2], Delphine De Sutter[4,5], Savvas Savvides[2,3], Sven Eyckerman[4,5,*], Sophie Janssens[1,2,*]

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The gastrointestinal tract is protected by a mucus layer, mainly consisting of the large glycoprotein MUC2. MUC2 misfolding leads to ER stress, which is mitigated by the Unfolded Protein Response (UPR). Aberrations in the UPR or mucin folding are associated with inflammatory bowel disease (IBD). Anterior Gradient 2 Homolog (AGR2) is a protein disulfide isomerase involved in maturation of mucins, and loss of function mutations have been identified in IBD. The goblet cell (GC)-specific UPR sensor Inositol-requiring Enzyme (IRE)1β protects ER integrity through degradation of excess Muc2 mRNA, but how IRE1β activity is regulated has remained unknown. We identified AGR2 as a regulator of IRE1β. AGR2 interacts specifically with IRE1β in both the human LS174T GC-like cell line and murine colon tissue and inhibits IRE1β through disruption of the catalytically active IRE1β dimer (Cloots et al, EMBO J, 2024; Neidhardt et al., EMBO J 2024; Bertolotti et al., 2024). We found that AGR2 mutants lacking their catalytic cysteine or displaying the disease-associated mutation H117Y were no longer able to dampen IRE1β activity. In vivo, Agr2 deficient mice exhibit depleted Muc2 mRNA levels, that are restored upon compound deficiency with IRE1β. This supports a model where AGR2 functions as a rheostat coupling IRE1β activity to the mucus folding load experienced by GC chaperones such as AGR2.

1. Cloots E, Guilbert P, Provost M, Neidhardt L, Van De Velde E, Fayazpour F, De Sutter D, Savvides S, Eyckerman S, Janssens S (2023). Activation of goblet cell stress sensor IRE1β is controlled by the mucin chaperone AGR2. The EMBO Journal Feb 2 PMID: 38177501 DOI: 10.1038/s44318-023-00015-y
2. Neidhardt L, Cloots E, Friemel N, Weiss CAM, Harding HP, McLaughlin SH, Janssens S, Ron D. The IRE1b-mediated unfolded protein response is repressed by the chaperone AGR2 in mucin producing cells (2023) The EMBO Journal Feb 2 PMID: 38177498 DOI: 10.1038/s44318-023-00014-z
3. Bertolotti A (2024) Keeping goblet cells unstressed. EMBO J Feb 2 PMID: 38308018 https://doi.org/10.1038/s44318-024-00041-4

## SELECTED SPEAKER

### MIA3 is a specialized trafficking protein that is required for MUC5AC production

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Mucus is required for airway defense, but in muco-obstructive diseases such as asthma, mucus is abundant, thick, and poorly transported. Mucus dysfunction is not adequately treated with existing treatments, making it essential to improve our mechanistic understanding of mucin biosynthesis and secretion.
Typically, secretory proteins are produced in two membrane bound intracellular compartments. First, in the endoplasmic reticulum (ER), proteins are folded and stabilized by disulfide bond formation. Late stages of biosynthesis occur in the Golgi apparatus, where sugars are added and secretory cargo is packaged into vesicles fated for secretion. For most secretory proteins, transport between these compartments is also mediated by vesicle transport. These folded proteins exit the ER via a conventional secretory pathway in vesicles whose sizes are restricted by the geometry of coat proteins (COPII coatamers) that limit vesicle diameters to size ranges of 60-90 nm diameter. Because of their massive sizes (500-1000 nm lengths), mucins are not able to fit into COPII vesicles. This task is made even more difficult when mucins are produced in excess, as they are in asthma.
We have identified a non-conventional pathway used by cells adapted to producing other large and polymeric proteins, such as collagen and fibrinogen. This pathway is led by MIA3, a transmembrane protein that localizes to the ER exit sites and binds to the COPII coatamer. Using immunohistochemistry in human lung asthmatic tissue, we found that MIA3 is expressed in goblet cells and in submucosal glands. We then used siRNAs to knockdown MIA3 in A549 cells, and we found that MIA3 is required for MUC5AC secretion. Furthermore, we used shRNA to knock down MIA3 in primary human bronchial epithelial cells (HBECs) grown at air-liquid interface conditions. We observed a 60% reduction in MUC5AC secretion. To fully knock out MIA3, we used a CRISPR/Cas9 approach and observed a severe phenotype characterized by suppressed mucous cell differentiation. Collectively, these findings identify MIA3 as a critical component in the mucin assembly pathway, with implications for MIA3 as a novel target for blocking mucus dysfunction in muco-obstructive diseases.
Currently, we are further dissecting roles of MIA3 in HBECs to test mechanistic functions of MIA3, and its two isoforms (long - 1,907 amino acids, and short - 785 amino acids). Both have been shown to assist in ER to Golgi transport during collagen synthesis. We are also generating conditional knockout mice to better understand the expression and function of MIA3 in vivo. These will be applied to studies of assembly and secretion of mucin and collagen.

## SELECTED SPEAKER

### Autophagy regulates lysosome-mediated degradation of mucin granules

Shailendra Mauyra[1], Katrina Kudrna[1], Elizabeth B. Staab[1], Paul Thomes[2], Bruton F. Dickey[3], John D. Dickinson[1]

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Introduction: Airway diseases such as COPD and asthma are characterized by epithelial mucous metaplasia leading to hypersecretion of mucin granules and subsequent airway obstruction. The factors regulating resolution from mucous metaplasia are poorly understood. We hypothesize that the autophagy pathway is required to eliminate non-secreted mucin granules and present the concept of the mucin cycle to comprehensively explain the factors that regulate mucin granule content of secretory cells.

Methods: To understand the mechanism of mucin granule elimination across the spectrum of degradation pathway from the autophagosome to the lysosome, we first explored the role of autophagy regulatory genes. A secretory cell specific, tamoxifen (TAM) inducible mouse model was generated by crossing Scgb1a1-creERTM+/- with Atg5flox/flox mice (Atg5-/-). Atg5flox/flox mice without Cre (Atg5 +/+) were utilized as controls. Secondly, to assess the lysosomal end of the degradation pathway, we utilized Calu-3 cell lines and primary human airway epithelial cells (hAECs) treated with pharmacologic inhibitors of lysosomal function.

Results: To determine the role of autophagy on mucin granule degradation under homeostatic conditions, total lung Muc5b levels was measured in Atg5+/+ and Atg5-/- mice after Tam induction. We found small, non-significant increase in Muc5b levels in Atg5-/- mice. In response to T2 inflammatory stimuli, OVA-induced mucous metaplasia, we observed Atg5-/- mouse lungs had significantly increased Muc5ac levels. We found a similar increase in Muc5b in Atg5-/- mouse lungs using a IL-1 beta intra-nasal administration model. We next sought to address the back end of the degradation pathway by directly inhibiting lysosomal activity. Inhibition of lysosomal acidification (bafilomycin A1) or lysosomal cathepsin enzyme activity (PepstatinA1-E64D) led to an accumulation of mucin granules in both Calu-3 cells and hAECs. Inhibition of lysosome activity by PepstatinA1-E64D, led to a detection of increased fusion events between mucin granules and Lamp1 labeled lysosomes as detected by super-resolution microscopy and by transmission electron microscopy. This impact of lysosomal inhibition on mucin accumulation in hAECs was greatest during resolution of mucous metaplasia, after withdrawal of IL-13

Conclusion: We propose a new model, the mucin cycle for regulation of intracellular mucin granules in airway secretory cells. Loss of function studies both at the front and back of end of the autophagy-lysosome degradation pathway suggest that mucin granules are degraded. Minimal degradation occurs under homeostatic conditions. Degradation primarily occurs during resolution.

**INVITED SPEAKER**

## Fiber-reinforced slime in hagfishes

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Hagfishes are an ancient group of eel-shaped marine animals that are notorious for defending themselves from predators using large volumes of fibrous slime that they produce within numerous slime glands. The exudate that is ejected from the slime glands consists of two main secretory products, mucous vesicles and slime threads, which are produced from distinct and specialized cell types. When a hagfish is attacked, ejected vesicles and coiled threads interact with seawater and expand by a factor of 10,000 in under 400 milliseconds. Slime deployment involves swelling of the mucous vesicles and unravelling of the coiled threads, resulting in a material that is exceptionally good at conforming to and clogging the gills of predators such as sharks. Hagfishes produce a second kind of fibrous slime from cells in their epidermis, which is produced when the skin is damaged. Epidermal slime also contains mucus and fibers, which are likely the evolutionary precursors of the same components in the glandular slime. In this talk, I will unpack the biophysical mechanisms underlying fibrous slime production and deployment in hagfishes and highlight some of our ongoing research in this area.

**INVITED SPEAKER**

## A novel Cysteine-rich adaptor protein is required for mucin packaging and secretory granule stability in vivo

Liping Zhang[1], Kayla J. Muirhead[1,2], Zulfeqhar A. Syed[1,3], Emilios K. Dimitriadis[4] and Kelly G. Ten Hagen[1*]

Mucins are large, highly glycosylated extracellular matrix proteins that line and protect epithelia of the respiratory, digestive and urogenital tracts. Previous work has shown that mucins form large, interconnected polymeric networks that mediate their biological functions once secreted. However, how these large matrix molecules are compacted and packaged into much smaller secretory granules within cells prior to secretion is largely unknown. Here, we demonstrate that a small cysteine-rich adaptor protein is essential for proper packaging of a secretory mucin in vivo. This adaptor acts via cysteine bonding between itself and the cysteine-rich domain of the mucin. Loss of this adaptor protein disrupts mucin packaging in secretory granules, alters the mobile fraction within granules and results in granules that are larger, more circular and more fragile. Understanding the factors and mechanisms by which mucins and other highly glycosylated matrix proteins are properly packaged and secreted may provide insight into diseases characterized by aberrant mucin secretion.

## SELECTED SPEAKER

### MUC17 is an essential small intestinal glycocalyx component that is disrupted in Crohn's disease

Elena Layunta[1], Sofia Jäverfelt[1], Fleur C. van de Koolwijk[1], Molly Sivertsson[1], Brendan Dolan[1], Liisa Arike[1], Sara Thulin[1], Bruce A. Vallance2, and Thaher Pelaseyed[1*]

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Crohn's disease (CD) is the chronic inflammation of the human ileum and colon triggered by unrestrained immune responses to bacteria, but insights into molecular perturbations at the bacteria-epithelium interface are limited. We report here that membrane mucin MUC17 protects small intestinal enterocytes against commensal and pathogenic bacteria. In the non-inflamed ileum of CD patients, reduced MUC17 levels correlated with a compromised apical glycocalyx barrier, allowing direct bacterial contact with enterocytes. Intestinal-specific Muc17 deletion in a new genetic mouse model, Muc17ΔIEC, rendered the small intestine prone to atypical infection by Citrobacter rodentium while maintaining resistance to experimental colitis. Additionally, the loss of Muc17 resulted in the spontaneous deterioration of epithelial homeostasis and extraintestinal translocation of viable bacteria. Finally, Muc17-deficient mice harbored specific small intestinal bacterial taxa observed in CD patients. In conclusion, our work uncovers a role for the MUC17-based glycocalyx as an essential defense system of the small intestine. The disruption of this system in the non-inflamed ileum of CD patients suggests that defective MUC17 biosynthesis or trafficking is an early epithelial defect that precedes inflammation. Given the limited number of mouse models for small intestinal inflammation, the Muc17ΔIEC model provides opportunities for understanding the molecular mechanisms underlying epithelial cell dysfunction in Crohn's disease.

## SELECTED SPEAKER

### The role of mucus and secreted mucins in the clearance of the normal airways

Brendan Dolan[1], Anna Ermund[1] and Gunnar C. Hansson[1]

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The airways are continuously exposed to external challenges, including dust, bacteria, fungi, and viruses. As such there is a requirement for an efficient system of clearance to maintain the essentially sterile nature of the distal airways. In the proximal airway mucus, liquid secretion and the beating of cilia are the key components of this defensive barrier. However, how these different elements of the airway mucus barrier work together remains to be fully elucidated. Through the use of high-resolution live imaging of explanted tracheal tissue from pigs and human donors we have studied the organisation and dynamic function of the proximal airway mucus barrier. In the normal airway the mucus barrier is organised in a hierarchical manner with the tissue surface protected by the beating of cilia and mucus threads, composed of MUC5AC. The airway surface fluid (ASF) contains large amounts of secreted mucus which continuously moves over the airway surface and finally the MUC5B-rich mucus bundles produced by the submucosal glands. Together these structures work to trap foreign material and efficiently remove it from the airways.
This study highlights the complex organisation and dynamic nature of the airway mucus systems. This barrier is composed of both liquid and mucus secretions and together they work in tandem to efficiently remove foreign material from the airways.

**INVITED SPEAKER**

## New mechanisms in type 2 inflammation

Bart N. Lambrecht[1]

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Spontaneous protein crystallization is a rare event in vivo, yet Charcot-Leyden crystals (CLC) consisting of the protein galectin-10 (Gal10) are frequently observed in asthma mucus plugs in humans. Pseudo-CLCs made up from other proteins are frequently seen in murine asthma. It is unclear if they exacerbate type 2 inflammation or are just markers for disease activity. Release of Gal10 and extracellular crystallization was associated with EEtosis of eosinophils in human primary eosinophils and patients with allergic mucin. We found that recombinant crystalline Gal10 was completely biosimilar to in vivo obtained CLCs and induced innate airway inflammation, whereas a soluble Gal10 engineered to resist crystallization was inert in the airways. When co-administered with harmless antigens, only crystalline Gal10 stimulated adaptive immunity, Th2 sensitization, goblet cell metaplasia and airway eosinophilia. In lung organoids of severe asthmatics, CLCs triggered mucus production. Transgenic mice engineered to overexpress human Gal10 in eosinophils (Galileo mice), ubiquitously (Galactic mice) or in the lungs only (GalacC) had enhanced features of asthma including mucus plugging and bronchial hyperreactivity. CLCs recruited neutrophils, which subsequently underwent NETosis, which contributed to mucus plug formation. To probe for the drugability of this pathway, we generated a panel of antibodies. Antibodies directed against key epitopes of the crystallization interface of Gal10 dissolved pre-existing CLC in patient-derived mucus within hours, and reversed crystal driven inflammation, goblet cell metaplasia, IgE synthesis and bronchial hyperreactivity in a humanized asthma model. Thus, Gal10 and CLC promote mucus formation and inflammation in asthma and can be targeted by crystal dissolving antibodies. In mice, other protein crystals made up of the chitinase like proteins Ym1 and Ym2 (encoded by Chil3 and Chil4) can form in the airways of asthmatic mice. These crystals are induced under the influence of IL-13 and these also enforce type 2 immunity and are drugable by antibodies. Thus protein crystallisation seems to be a unique feature of type 2 immune responses across species, acting to enforce type 2 immune responses, mucus plugging and neutrophil NETosis.

**INVITED SPEAKER**

## Evolutionary Mechanisms for Generating Functional Diversity in Gel-Forming Mucins

Meital Haberman[1], Lev Khmelnitsky[1], Gabriel Javitt[1], Deborah Fass[1]

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The human secreted mucins are huge glycoproteins composed of characteristic amino- and carboxy-terminal folded regions and an extensive, intrinsically disordered, O-glycosylated central segment in which multiple CysD adhesion domains are, in some cases, embedded. During post-translational processing, individual mucin glycoproteins become linked by disulfide bonds to form covalent linear polymers. The mechanism for generating disulfide-bonded mucin polymers stepwise in the secretory pathway is shared by multiple mucins across different organ systems. However, mucus hydrogels comprising different mucins have distinct appearances and biophysical properties, with each mucin adapted to its bodily niche and physiological function. By studying large amino-terminal segments of the mucins MUC2, MUC5B, and, most recently, MUC5AC, we have gained insight into regions of these proteins that may tune the qualities of mucus by modulating covalent and non-covalent supramolecular assembly. Though MUC2, MUC5B, and MUC5AC all self-assemble into “beads-on-a-string” filaments when subjected to acidic pH conditions mimicking those of the late secretory pathway, we found that the filaments can further self-associate in different manners. Whereas the MUC2 amino-terminal segment forms isolated filaments, MUC5B forms bundles of filaments, and MUC5AC forms pairs of filaments arranged in a helix. Major determinants of higher-order mucin filament associations include the angles of bead-bead interactions in the filament and the docking sites of the CysD adhesion domains. CysD domain diversity is coupled to variation in the intrinsically disordered, O-glycosylated segments that separate CysD domains from other globular mucin regions and from one another. In particular, the PTS1 region, to which the key first CysD domain (CysD1) is tethered, has diverged in length and degree of conservation between MUC2, MUC5B, and MUC5AC. A new high-resolution structure of the MUC5AC beaded-filament helix enables a detailed comparison with MUC5B and analysis of the features that may produce functional diversity between these two respiratory mucins.

**INVITED SPEAKER**

## The Unique Exocytosis Mechanism of Large Vesicles in Exocrine Tissues

Tom Biton[1,2], Nadav Scher[1], Shari Carmon[2], Yael Elbaz-Alon[1], Eyal D. Schejter[2], Ben-Zion Shilo[2*], Ori Avinoam[1*]

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Exocrine cells that secrete mucinous cargos utilize large secretory vesicles (LSVs), reaching up to 10 μm in diameter. When LSVs fuse with the apical surface they often recruit an actomyosin meshwork that extrudes their content through dynamic fusion pores that initially dilate to large diameters and subsequently constrict without resealing. In this scenario, the vesicular and apical membranes remain distinct, and membrane homeostasis is maintained. However, the molecular mechanism regulating fusion pore dynamics remains largely uncharacterized. Using live-super-resolution and focused ion beam-scanning electron microscopy (FIB-SEM), we observe that the fusion pore of LSVs in the Drosophila larval salivary glands expand, stabilize, and constrict. Branched actin polymerization is essential for pore expansion and stabilization, while myosin II activity is essential for pore constriction. Furthermore, we identify several conserved Bin-Amphiphysin-Rvs homology (BAR) domain proteins that regulate fusion pore expansion and stabilization. Most notably, we show that the I-BAR protein Missing-in-Metastasis (MIM) localizes to the fusion site and is essential for pore expansion and stabilization in a dose-dependent manner. The MIM I-BAR domain is essential but not sufficient for localization and function. Taken together, our findings show that MIM is part of a dedicated machinery that acts in-concert with actin, myosin II and additional BAR-domain proteins to control fusion pore dynamics, mediating a distinct mode of exocytosis that facilitates content release without perturbing apical membrane homeostasis.

## SELECTED SPEAKER

### The generation of immunogenic gluten peptides occurs during their Goblet cell-mediated transport - Implications for Celiac Disease

Christian V. Recktenwald[1], Jenny K. Gustafsson[1], Brendan Dolan[1], Sjoerd van der Post[1], Sara Thulin[1], Gunnar C. Hansson[1]

1. Department of Medical Biochemistry, University of Gothenburg

Celiac Disease (CeD) is an immune-mediated enteropathy against dietary gluten derived from wheat, rye or barley and one of the most common lifelong food-related disorders worldwide. Morphologically, CeD is characterized by villous atrophy and crypt hyperplasia in the small intestine leading to the malabsorption of nutrients. The prevalence of the disease based on serological diagnosis can be as high as 1.4 % in the general population. The disease is triggered in genetically predisposed individuals harbouring the MHC class II phenotype HLA-DQ2 when tissue transglutaminase (TGM2) has deamidated specific glutamine residues in undigestable gluten peptides. This post-translational modification of the food antigen leads to a higher affinity and stabilization of the HLA-DQ2:Gliadin-complex on the cell surface of Antigen-presenting cells (APCs) and the subsequent activation of CD4+ T cells. When the disease has been established antibodies against deamidated gluten and auto-antibodies against the disease-triggering enzyme TGM2 are produced.
However, it is not understood how these gluten peptides are transferred from the gut lumen to the APCs in the lamina propria. Furthermore, it is not clear if the TGM2-catalyzed deamidation of these peptides takes place extra- or intracellularly. In this study, we have deciphered, by administering luminal injections of gluten peptides in the proximal small intestinal lumen of mice, that the molecular pathway of gluten uptake occurs via Goblet-cell associated antigen passaging (GAP). Furthermore, this pathway was also transferable to humans since the same phenomenon was observed when duodenal biopsies were exposed to labelled gluten peptides ex vivo. In addition, we could show that the TGM2-catalyzed deamidation of gluten peptides occurs both intra- and extracellularly. Surprisingly, the number of deamidated glutamines is increased extracellularly but these peptides do not seem to be taken up by the epithelium thereby suggesting that the GAP-mediated uptake of food antigens is restricted to non-charged molecules.

**INVITED SPEAKER**

## Mucin Glycans and Microbial Virulence: Exploring Therapeutic Potential in Host-Derived Molecules

Katharina Ribbeck[1]

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Mucus, a biological gel lining all wet epithelia including the mouth, lungs, and digestive tracts, is evolved to protect against pathogenic invasion. However, studies on microbial pathogenesis in these systems often neglect the natural, three-dimensional mucus gels, focusing instead on mucus-free environments that lack essential geometric constraints and microbial interactions. To address this gap, our laboratory has developed model test systems utilizing purified mucin polymers, the primary gel-forming constituents of the mucus barrier, and their associated glycans.

Through these models, we investigate the influence of the mucus barrier on microbial virulence and the mechanisms microbes use to breach this protection. Our findings reveal that mucin polymers, particularly their glycans, play a critical role in regulating microbial behaviors such as surface attachment, toxin secretion, quorum sensing, and biofilm formation. Furthermore, we are beginning to identify microbial sensors of mucins and intracellular pathways responsive to mucin regulation. These discoveries position mucin glycans as key regulators of microbial virulence, opening new therapeutic avenues leveraging these host-derived molecules.

**INVITED SPEAKER**

## Mucus gels and innate lung defense

Daniel Song[1], Logan Kaler[1], Elizabeth Engle[1], Ethan Iverson[1], Maria Rife[1], Allison Boboltz[1], Margaret Scull[1], Gregg Duncan[1]

1. University of Maryland

Mucus is a biological gel within the lung designed to behave like an “escalator” with the ability to capture potentially harmful inhaled materials (e.g. pathogens, particulates) and carry these materials via mucociliary clearance up to the throat to be swallowed and sterilized. MUC5B and MUC5AC are large, gel-forming mucins that assemble to form airway mucus gels. MUC5B and MUC5AC are large, gel-forming mucins that assemble to form airway mucus gels. However due to the lack of appropriate models, it is not yet fully understood how MUC5B and MUC5AC individually or synergistically contribute to the biological function of mucus. To understand their unique roles in respiratory health, I will discuss our studies on the rheological properties and transport function of mucus in human airway tissue cultures genetically engineered to secrete either MUC5B or MUC5AC. A breakdown in lung mucus barrier function can lead to increased infections by respiratory viruses, such as influenza, rhinovirus, and coronaviruses, as they are not effectively removed from the airway. For these viral pathogens, it is important to understand the mechanisms through which viral particles avoid adhesion to the mucus barrier and transport to the underlying epithelium to cause infection. To address this, I will discuss our work to quantitatively measure respiratory virus diffusion through and trapping within the mucus barrier using high-speed fluorescent video microscopy and multiple particle tracking.

## SELECTED SPEAKER

### Peroxidase-mediated mucin cross-linking drives pathologic mucus gel formation in IL-13-stimulated airway epithelial cells

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Airway pathological mucus is a feature of airway type 2 inflammation and eosinophil peroxidase promote formation of oxidant acids that cross-link mucins to increase elastic behavior of mucus. Studies in cultured human bronchial epithelial cells (HBECs) show that IL-13 induces formation of pathologic mucus gels but the precise mechanisms underlying these changes remain incompletely elucidated. Here, we optimized a magnetic microwire rheometer (MMWR) to allow in situ characterization of mucus hydrogels secreted by HBECs grown at air liquid interface under control and IL-13-stimulated conditions. The MMWR device applies a force to a magnetic microwire that is placed on mucus secreted by HBECs. The resulting displacement of the microwire in response to the applied force is used to characterize mucus material properties and determine the steady-state compliance and zero-shear viscosity. Using this MMWR technology, we investigated if IL-13 upregulates epithelial cell peroxidases, lactoperoxidase (LPO) or thyroid peroxidase (TPO), to mediate mucin cross- linking and mucus gel elasticity. By applying creep compliance measurements with the MMWR device, we found that mucus secreted by HBECs under control conditions exhibits viscoelastic liquid behavior whereas mucus secreted by HBECs stimulated by IL-13 exhibits solid-like behavior, as revealed by decreased compliance and increased viscosity ($p<0.001$). When mucus from HBECs stimulated by IL-13 is treated with dithiothreitol (DTT) to cleave disulfide bridges between mucin polymers, the compliance and viscosity of the mucus normalize ($p<0.05$). HBECs express two peroxidases, LPO and TPO and we showed that both peroxidases are able to produce oxidant acids oxidizing free thiols into disulfide bridges. We investigated the peroxidase activity in HBECs and found that this activity is higher in IL-13 stimulated cells. To confirm that peroxidases were the mediator of cross-linking, a thiolated hydrogel was placed on IL-13-stimulated HBECs. The hydrogel shows increased stiffening that is prevented by a chemical inhibitor of LPO and TPO ($p<0.01$), confirming peroxidase activity at the apical side of the cells. Finally, gene expression for TPO increased in IL-13 stimulated HBECs as was higher than normal in epithelial brushings from patients with asthma. We conclude that IL-13 causes mucus cross-linking and mucus gel stiffening in HBECs via peroxidase-driven oxidant acid production and that TPO is a candidate mediator of airway mucus plugs in patients with asthma.

## SELECTED SPEAKER

### Topical reinforcement of the cervical mucus barrier to sperm

Ulrike Schimpf[1], Erika Caldas-Silveira[2], Ljudmila Katchan[3], Cécile Vigier-Carriere[3], Isabelle Lantier[2], Gilai Nachmann[1], Sebastian Gidlöf[4], Aino Fianu Jonasson[4], Lars Björndahl[4], Stéphane Trombotto[5], Xavier Druart[2], Thomas Crouzier[1,3]

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3. Cirqle Biomedical Contraception ApS
4. Karolinska University Hospital
5. Polymer Materials Engineering, Lyon University 1

INTRODUCTION: Close to half of the world's pregnancies are still unplanned, reflecting a clear unmet need in contraception. Ideally, a contraceptive would provide the high efficacy of hormonal treatments, without systemic side effects. Our research group has been pioneering several mucus engineering, aims at using mucus and modifying the mucus in situ via topical treatments. Here, we studied topical reinforcement of the cervical mucus by chitosan mucoadhesive polymers as a form of female contraceptive.

METHODS: Using human ovulatory cervical mucus and human sperm, we studied the penetration of sperm into cervical mucus exposed to various chitosan solutions. Capillary tubes were filled with mucus and exposed to formulation and then to sperm. Sperm penetration was measured by microscopy. In vivo efficacy was assessed using the sheep model. Sheep were synchronized, and treated with vaginal gels containing chitosan, followed by artificial insemination with fluorescently labelled ram sperm. The distribution of sperm through the reproductive tract of the ewe was assessed by fluorescence confocal endomicroscopy. Safety of the treatments was assessed using three-dimensional vaginal epithelial cell cultures, by measuring kinematic parameters of sperm exposed to formulations, and by histological analysis of vaginal tissues of the ewes exposed to the formulation.

RESULTS: We found that chitosans larger than 7 kDa effectively cross-linked human ovulatory cervical mucus to prevent sperm penetration in vitro. We then demonstrated in vivo that vaginal gels containing chitosan could stop ram sperm at the entrance of the cervical canal and prevent them from reaching the uterus, whereas the same gels without chitosan did not substantially limit sperm migration. Chitosan did not affect sperm motility in vitro or in vivo, suggesting reinforcement of the mucus physical barrier as the primary mechanism of action. The chitosan formulations did not damage or irritate the ewe vaginal epithelium, in contrast to nonoxynol-9 spermicide. The demonstration that cervical mucus can be reinforced topically to create an effective barrier to sperm may therefore form the technological basis for muco-cervical barrier contraceptives with the potential to become an alternative to hormonal contraceptives.

**INVITED SPEAKER**

## Cell-based Mucin Array for Discovery and Characterization of Mucinases and Carbohydrate Binding Modules

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Mucins arguably represent the last frontier in the analytics of glycoproteins. Most mucins are extremely large and heterogeneous glycoproteins that are resistant to conventional glycoproteomics strategies dependent on proteolytic fragmentation and sequencing. Currently, there are no methods for obtaining human mucin molecules in reasonable purity with defined glycans, and this is a fundamental barrier and limitation for studies of mucins and their complex biology, particularly in the microbiome field. We, therefore, sought to capture the molecular information contained in the tandem repeat regions (TRs) of human mucin and mucin-like O-glycodomains for molecular dissection. We have developed a glycoengineered cell-based platform to display and produce representative mucin TRs with defined O-glycans using mucin reporters containing around 200 amino acids1. The cell-based mucin array enables molecular dissection of microbial interactions with mucin TR sequences and the attached O-glycan structures. Display of the mucin reporters on the cell surface provides the first cell-based display of the human mucins and we have used this to probe and dissect the binding specificities of microbial adhesins, influenza virus, and Siglecs2. We discovered that these adhesins show highly distinct binding preferences for O-glycan patterns displayed on distinct mucin TRs, providing a new level of complexity and diversity to interactions with the mucin glycome. A major power of the cell-based array platform is that it also allows the production of secreted homogenous reporters for wider studies of interactions with GBPs. This we have illustrated by producing a homogenous mucin fragment (MUC1 TRs, 150 amino acids with 25-28 trisaccharide O-glycans) that recently enabled the first single-molecule atomic imaging of glycoproteins by low-temperature scanning tunneling microscopy after soft-landing electrospray ion beam deposition3. We have also shown that the mucin display platform is ideal for the discovery and exploration of mucin-degrading enzymes as well as mucin-binding modules. Using the mucin display, we originally identified a small mucin-binding module (X409)1 on the mucinase StcE which does not bind simple oligosaccharides and hence cannot be discovered by traditional glycan arrays. We also discovered new families of mucinases (O-glycoproteases), including the di-glutamate mucinase HC7, which recognizes dense clusters of O-glycans4 and has family members in eukaryotes. The cell-based glycan and mucin arrays are sustainable resources that we hope to make widely available to the community

References

1. Nason R, Büll C, Konstantinidi A, Sun L, Ye Z, Halim A, Du W, Sørensen DM, Durbesson F, Furukawa S, Mandel U, Joshi HJ, Dworkin LA, Hansen L, David L, Iverson TM, Bensing BA, Sullam PM, Varki A, Vries E, de Haan CAM, Vincentelli R, Henrissat B, Vakhrushev SY, Clausen H, Narimatsu Y. Display of the human mucinome with defined O-glycans by gene engineered cells. (2021) Nat Commun 1;12(1):4070.
2. Büll C, Nason R, Sun L, Van Coillie J, Madriz Sørensen D, Moons SJ, Yang Z, Arbitman S, Fernandes SM, Furukawa S, McBride R, Nycholat CM, Adema GJ, Paulson JC, Schnaar RL, Boltje TJ, Clausen H, Narimatsu Y. Probing the binding specificities of human Siglecs by cell-based glycan arrays. (2021) Proc Natl Acad Sci U S A. Apr 27;118(17):e2026102118.
3. Anggara K, Sršan L, Jaroentomeechai T, Wu X, Rauschenbach S, Narimatsu Y, Clausen H, Ziegler T, Miller RL, Kern K. Direct observation of glycans bonded to proteins and lipids at the single-molecule level. (2023) Science 382(6667):219-223. doi: 10.1126/science.adh3856.
4. Narimatsu Y, Christian Büll, Víctor Taleb, Qinghua Liao, Ismael Compañón, David Sánchez-Navarro, Fabien Durbesson, Renaud Vincentelli, Francisco Corzana, Carme Rovira, Bernard Henrissat, Henrik Clausen, Hiren J. Joshi, Ramon Hurtado-Guerrero*. A Family of Di-Glutamate Mucin Degrading Enzymes that Bridges Glycan Hydrolases and Peptidases. (2024) Nat Catalysis in press.

**INVITED SPEAKER**

## Recent developments in the MS-based analysis of mucin-domain glycoproteins

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Mucin-domain glycoproteins are densely O-glycosylated and play key roles in a host of biological functions. However, their dense O-glycosylation remains enigmatic both in glycoproteomic landscape and structural dynamics, primarily due to the challenges associated with studying mucin domains. Here, we present advances in the mass spectrometric analysis of mucins, including the characterization of mucinases, enrichment techniques, and complete mucinomic mapping of translationally relevant mucin proteins.

## SELECTED SPEAKER

### Mucin O-glycan degradation patterns of Akkermansia muciniphila, Ruminoccocus torques, and a synthetic gut-associated microbial community

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Mucin O-glycans are degraded by the diverse human gut microbiota and used as an important nutrient source. However, the various gut bacteria each have different enzymes and approaches to degrade these O-glycans. Furthermore, the presence of specific O-glycan structures may steer the microbiota composition. Certain bacteria are generalists and have a rather broad enzyme repertoire to degrade numerous different types of O-glycan structures whilst others are specialists and only capable of degrading specific types of sugar linkages within the O-glycans. In this study we investigated the O-glycan degradation patterns of two common gut-associated bacteria Akkermansia muciniphila and Ruminococcus torques by analysing the remaining O-glycan structures in time during mucin fermentation. We compared these degradation patterns with those of a synthetic bacteria community consisting of a diverse range of gut-associated bacteria tin order to recognize dominant mucin degraders and possible cross-feeding. To this end, the bacteria were grown on porcine stomach mucin for 24 hours and sampled every 3 hours. O-glycans were released from the bacterial supernatant samples and analysed using PGC-UPLC-MS/MS. Akkermansia muciniphila showed a slower consistent degradation of all O-glycan structures whilst Rumminococcus torques displayed a preference for targeting individual or specifically fucosylated glycans first. The synthetic community exhibited a faster and more complete degradation compared to the individual bacteria stressing the importance and efficiency of cross-feeding. These findings provide an insight in the mechanisms of gut-associated bacteria in their quest for nutrients via glycan degradation.

## SELECTED SPEAKER

### Unraveling the role of α-(1,2)-fucosylation in bacterial pneumonia

Cengiz Goekeri[1,2], Sebastian M. K. Schickinger[1], Alina Nettesheim[1], Kerstin A. K. Linke[1], Leila Bechtella[3], Gaël M. Vos[3], Elena Lopez-Rodriguez[4], Vladimir Gluhovic[4], Anne Voß[5], Sandra Kunder[5], Jade Bath[6], Katharina Ribbeck[6], Kevin Pagel[3], Achim D. Gruber[5], Matthias Ochs[4,7], Peter H. Seeberger[8], Martin Witzenrath[1,7], Geraldine Nouailles[1]

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Community-acquired pneumonia (CAP) remains a leading contributor towards global communicable disease-mediated mortality and Streptococcus pneumoniae (Spn) is the most frequently detected pathogen in CAP. Mucosal epithelia and biointerfaces, including those of the lungs, are rich in cell-bound and secreted N- and O-glycans, which harbor terminally fucosylated glycans. This study investigates how terminal fucosylation and α-(1,2)-fucosylated glycans influence Spn infection. Pneumococci express fucose utilization genes, which are upregulated upon interaction with fucosylated MUC5AC in vitro. Murine lungs express fucosylated glycans, which are shed into the bronchoalveolar lavage (BAL) during Spn infection. Systemic treatment of mice with 2-deoxy-D-galactose (2-DGal) resulted in incorporation of 2-DGal into the murine glycome and inhibition of fucosylation in vivo. Mice treated with 2-DGal displayed markedly improved physiological parameters such as infection-induced body weight and temperature loss. Treatment with 2-DGal also led to reduced bacterial burden in BAL and systemic spread following Spn infection. Flow cytometry revealed that following 2-DGal treatment, neutrophil recruitment was at similar levels 24 hours post-infection (hpi), but neutrophilic inflammation resolved quicker than sham-treated mice. Moreover, 2-DGal treatment markedly improved lung barrier permeability and led to decreased IL-6 and TNF-α production in Spn-infected mice 48 hpi. Taken together, we conclude that 2-DGal treatment prevents the establishment of pneumococcal infection in mice and alleviates murine pneumonia.

**INVITED SPEAKER**

## Mucin glycan foragers in the human gut: the case of Ruminococcus gnavus

Nathalie Juge[1]

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Ruminococcus gnavus is a prevalent human gut symbiont part of the normal gut microbiota and disproportionately represented in intra- and extra-intestinal diseases from inflammatory bowel diseases to neurological disorders. There is therefore great interest in understanding the mechanisms by which R. gnavus adapt to the gut and communicate with the host.
We showed that R. gnavus' ability to utilise mucin glycans is strain dependent and associated with the utilisation of terminal glycan epitopes. Using a combination of molecular microbiology, biochemical and structural approaches, we uncovered a novel sialic acid metabolic pathway in gut bacteria, which is essential to the capacity of R. gnavus strains to colonise the intestinal mucus niche. From an ecological point of view, since R. gnavus is the only strain reported to produce 2,7-anhydro-Neu5Ac in the gut, the strict specificity of its sialic acid transporter confers a nutritional advantage while supporting the microbial community by enabling access to the uncapped mucin glycan chain.
The interaction of R. gnavus strains with the host at the mucosal interface was further investigated using mouse and human gut-on-chip models, underscoring the importance of studying the health impact of bacteria at the strain level.

**INVITED SPEAKER**

## The adaptation hallmarks of Akkermansia muciniphila enzymes to human O-glycans

Mathias Jensen[1], Linn Stenfelt[1,2], Michael J. Pichler[1], Bashar Shuoker[1,3], Chunsheng Jin[4], Jennifer Ricci Hagman[2,5], Annika Hult[5], Hiroka Sakanaka[1], Haiyang Wu[6], Ana Martínez Gascueña[6], Jining Liu[7], Julia Weikum[1], Tine Sofie Nielsen[1], Jan Holgersson[7], Eva Nordberg Karlsson[3], Nathalie Juge[6], Sebastian Meier[8], Niclas G. Karlsson[4], Jens Preben Morth[1], Martin L. Olsson[2,5] and Maher Abou Hachem[1]

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Akkermansia muciniphila, a core human gut microbiota species, which is a dedicated mucin degrader, relies on mucin both as a carbon and nitrogen source. The relative abundance of A. muciniphila is inversely correlated to the proliferation of mucin-degrading pathobionts, whereas it is positively associated with gut barrier and metabolic health. Despite its pivotal role in mucin turnover and physiological importance, our insight into the mucin O-glycan degradation apparatus of A. muciniphila remains underexplored.
We have recently investigated the initial steps of mucin degradation by A. muciniphila, demonstrating that removal of sialic acid and fucose decapping mediates nutrient sharing to other microbiota groups and is crucial for growth. Inspired by the observed A. muciniphila ability to break down ABO, H and Lewis blood group antigens on mucin, we investigated the adaptation to host-derived ABO antigens on O-glycans. We showed that A. muciniphila possesses exceptionally efficient enzymes to degrade not only the A and B antigens, but additional extensions of these epitopes, which have hitherto received no attention in the context of blood transfusion. Interestingly, the removal of canonical and extended ABO antigens on red blood cells by A. muciniphila enzyme blends significantly improved compatibility with group O plasmas, compared to conversion of A or B antigens alone. Our findings revive hopes for enzymatic production of ABO-universal blood via targeting the hitherto overlooked extended A and B antigens and highlight hallmarks of enzymatic efficiency on host O-glycans by A. muciniphila.

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## SELECTED SPEAKER

### Maturation of Human Intestinal Epithelia involves Fortification of the Apical Surface against Salmonella Attack

Jorik M. van Rijn[1,2,#], Ana C. C. Lopes[1,#], Merve Ceylan[3], Jens Eriksson[1], Alexandra Florbrant[1], Angeliki Ntokaki[1], Rebekkah Hammar[3], Dominic-Luc Webb[4], Per M. Hellström[4], Wilhelm Graf[5], Martin Skogar[5], Magnus Sundbom5, Per Artursson[3], Thaher Pelaseyed6, Maria Letizia Di Martino[1], and Mikael E. Sellin[1,7,*]

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The active invasion of intestinal epithelial cells (IECs) constitutes a key event in the infection cycle of many gut bacterial pathogens. Studies of how the prototype enterobacterium Salmonella enterica Typhimurium (Salmonella) invades transformed cell lines has generated a paradigm for the near-instant, efficient, and type-three-secretion-system (TTSS-1)-driven IEC invasion process, fueled by expansive membrane ruffles. However, recent comparative studies suggest that non-transformed IECs in their native gut context comprise a significantly more challenging target for the bacterial attack. The molecular and cellular features that explain these discrepancies are largely undefined. By live-cell imaging of infections in enteroid- and colonoid-derived epithelial cell layers, our work reveals that the maturation state of human gut epithelia dramatically impacts permissiveness to Salmonella invasion. IEC layers kept under stem- and progenitor-cell-promoting conditions remain permissive to Salmonella invasion, whereas maturation towards an enterocyte/colonocyte end fate markedly reduces the frequency of bacterium-induced epithelial entry structures, and lowers the overall invasion efficiency by up to 10-fold. This phenotypic shift appears coupled to an altered expression of actin regulatory proteins implicated in the Salmonella invasion process, and an increased dependence on the TTSS-1 effector SipA for successful bacterial entry. IEC maturation also involves upregulation of cell surface mucins and distinct shifts in the apical surface glyco-composition, as revealed by multiple lectin stainings. Most notably, enzymatic digestion of this apical glycocalyx converts mature IEC layers back to the Salmonella-invasion-permissive phenotype of their immature counterparts. Taken together, these results showcase how the maturation state of human intestinal epithelia dictate the efficiency, as well as the mechanistic and temporal progression, of the Salmonella invasion process.

## SELECTED SPEAKER

### Characterization of the founding member of the first CBM family known to target sulfated glycans

Elena Fekete[1], Grete Raba[1], Alan Cartmell[2], Ana Luis[1]

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The human gut is colonized by a dense and diverse microbial community: the microbiota. In the healthy intestines, mucus creates a habitat for the commensal microbiota, as well as a barrier to separate the microbiota from the intestinal epithelium(1). Mucin glycoproteins are the major component of intestinal mucus. Mucins are decorated with complex and diverse O-glycans that protect the glycoprotein from degradation by most commensal bacteria. However, some microbiota members, including Akkermansia muciniphila (Akk) can degrade and utilize mucin O-glycans as a carbon source(2). Interestingly, our results show that this bacterium is able to utilize human colonic mucins, but is unable to utilize released mucin O-glycans. This indicates that recognition of the native mucin structures has a key role in mucin utilization by Akk. Understanding the mechanisms of interaction between Akk and mucin glycans will contribute to better understanding of how this bacterium degrades mucins and colonizes the human gut. Previously, it has been shown that some microbiota members express sulfatases that play a key role in O-glycan utilization and in vivo intestinal colonization. A bioinformatic analysis of Akk sulfatases revealed a protein (Amuc_0953) closely related to the key 3S-galactose (3S-Gal) sulfatase identified in the mucin-degrading commensal Bacteroides thetaiotaomicron(3). Amuc_0953 displays a N-terminal domain of unknown function. The crystal structure of this domain reveals a right-handed parallel b-helix fold with 16 turns. Structural alignments show a low similarity to pectin lyases, proteases and the previously characterized CBM89. Using a combination of biochemical and immunofluorescent techniques, we disclosed that the N-terminal domain of Amuc_0953 is the founding member of a novel CBM family that specifically binds 3S-Gal epitopes. Site-directed mutagenesis studies revealed that the recognition of negatively charged sulfated substrates is mediated by positively charged residues. Moreover, this novel CBM potentiates the enzymatic activity of the sulfatase enzyme by increasing binding to the target substrate. Additionally, due to the lack of specific binding proteins that specifically recognize sulfated glycans, we explored this novel CBM as a probe to detect glycosylation alterations associated with cancer, and observed that this binding protein specifically binds to ovarian cancer tissues, known to overexpress 3S-Gal epitopes.
The characterization of this novel CBM revealed the first binding protein that specifically recognizes a sulfated epitope. The expression of these binding proteins in Akk increase binding to glycans epitopes found in mucins, a key step to the utilization of this substrate by Akk. Additionally, this CBM shows the potential to be utilized as a novel probe to target specific epitopes in biological samples.

## SELECTED SPEAKER

### Salmonella enterocyte invasion through MUC1: contributions of the extracellular domain and cytoplasmic tail to invasion and signaling

Jinyi Su[1], Xinyue Li[1], Evelien Floor[1], Karin Strijbis[1]

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Salmonella Enteritidis (Salmonella) is a common food-borne enteropathogenic bacterium that can bypass the mucus layer and invade intestinal epithelial cells. Highly glycosylated mucin proteins are expressed on the apical surface of enterocytes, together called the glycocalyx. Previously, we discovered that the Salmonella giant adhesin SiiE interacts with the glycosylated transmembrane mucin MUC1 which induces apical invasion into enterocytes. Here, we investigate if the MUC1 glycosylated extracellular domain (ED) and/or the cytoplasmic tail (CT) with signaling capacity contribute to Salmonella invasion and/or induction of downstream signaling. As our model, we use HT29-MTX (MUC1-WT) cells, CRISPR/Cas9-MUC1 knockout cells (ΔMUC1), CRISPR/Cas9-MUC1-CT (MUC1-ΔCT) cells that lacked the CT and mucinase-cleaved MUC1-ED (cleaved-ED MUC1) cells leaving the transmembrane domain and CT intact. A significant reduction in bacterial infection was observed in ΔMUC1 and cleaved-ED MUC1 cells, suggesting that MUC1-ED has an essential receptor function. No difference in bacterial infection was observed between MUC1-WT and MUC1-ΔCT cells, showing that the CT is not essential for SiiE-MUC1 invasion. The MUC1-CT was also not required for secretion of IL-8 as measured by ELISA. To determine the contribution of the MUC1-CT, we performed a large RNAseq experiment with uninfected and infected cells. The most striking observation was that the regulation of certain genes by the NFκB family was dependent on the presence of the MUC1-CT. Immunoblot analysis demonstrated that the NFκB transcriptional subunits p50, p52, p65, RELB and c-REL are equally expressed and translocated in all cell types. However, the NFκB cytoplasmic inhibitory subunits p100, p105 and IκBα are significantly upregulated in MUC1-ΔCT and ΔMUC1 cells compared to MUC1-WT cells in the absence of Salmonella but are equally upregulated after Salmonella invasion. Based on these results, we conclude that the MUC1 extracellular domain is essential for Salmonella invasion and MUC1-CT has an anti-inflammatory function by suppressing the NFκB pathway.

**INVITED SPEAKER**

## Transcriptional regulation of invasive mucinous adenocarcinoma of the lung

Motohiro Kobayashi[1]

1. Department of Tumor Pathology, Faculty of Medical Sciences, University of Fukui, Japan

It is important to recognize that tumors are not made up of neoplastic tumor cells alone. Tumor tissue consists of neoplastic tumor cells as well as blood vessels, lymphatic vessels, fibroblasts and immune cells such as macrophages and lymphocytes. As a carbohydrate moiety of mucins, sulfated glycans are expressed not only on neoplastic tumor cells but also on these stromal cells. In this talk, the potential role of sulfated glycans expressed on these two cell types constituting cancer tissue will be discussed..

**INVITED SPEAKER**

## Resolving the role of mucins in extracellular vesicle biology

Matthew Paszek[1]

Cancer cells deploy extracellular vesicles (EVs) to reprogram the local microenvironment and prepare distant niches for metastatic spread. We find that expression of cancer-associated mucins is associated with a progressive increase in EV biogenesis. The mucin induced EVs have physical and biochemical characteristics that are distinct from canonical exosomes and microvesicles, the two primary classes of EVs that are implicated in transmission of molecular messengers between tumor cells and recipient cells. Notably, mucin induced EVs are highly enriched in tetraspanins that are classical biomarkers for exosomes but display size distributions that are more typical of microvesicles. We apply various super-resolution imaging strategies and biophysical techniques to resolve the nanoscale biology of the mucin induced EVs and their cargo selection. Our studies implicate mucins in the generation of membrane curvature, which recruits tetraspanins, including CD63, to the plasma membrane and packaging into EVs. The CD63-containing EVs display a conspicuous mucin corona with broad implications for EV stability, extracellular matrix interactions, and biological function. Together, our results suggest that mucins have a critical but underappreciated role in cancer-associated EV biogenesis.

## SELECTED SPEAKER

### Mucus and epithelial barrier defects promote pks+ driven colorectal cancer development through adhesin-mediated binding mechanisms

Maude Jans[1,2], Magdalena Kolata[4,5], Gillian Blancke[1,3], Maarten Ciers[1,2,3], Anders B. Dohlman[6], Takato Kusakabe[7], Mozes Sze[1,2], Alexandra Thiran[1,3], Geert Berx[2], Sabine Tejpar[8], Geert van Loo[1,2], Iliyan D. Iliev[7], Han Remaut[4,5], Lars Vereecke[1,3]

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Various bacteria are suggested to contribute to colorectal cancer (CRC) development, including pks+ E. coli which produce the genotoxin colibactin that induces characteristic mutational signatures in host epithelial cells. It remains unclear how the highly unstable colibactin molecule is able to access host epithelial cells and its DNA to cause harm. We use experimental and transgenic mouse models to study microbial tumor-driving mechanisms, including Zeb2-transgenic mice, which are characterized by mucus and epithelial barrier defects. We found that pks+ E. coli drives CRC exacerbation and tissue invasion in a colibactin-dependent manner. Using isogenic mutant strains, we further demonstrate that CRC exacerbation critically depends on expression of the E. coli type-1 pilus adhesin FimH and the F9-pilus adhesin FmlH. Blocking bacterial adhesion using a pharmacological FimH inhibitor attenuates colibactin-mediated genotoxicity and CRC exacerbation. Using fluorescently labeled recombinant FimH and FmlH, we characterized E.coli docking sites in healthy and CRC tissue, and found distinct binding patterns associated to mucus and goblet cell subsets. Together, we show that the oncogenic potential of pks+ E. coli critically depends on bacterial adhesion to host epithelial cells and mucus, and is critically mediated by specific bacterial adhesins. Adhesin-mediated epithelial binding subsequently allows production of the genotoxin colibactin in close proximity to host epithelial cells, which promotes DNA damage and drives CRC development. These findings present promising therapeutic avenues for the development of anti-adhesive therapies aiming at mitigating colibactin-induced DNA damage and inhibiting the initiation and progression of CRC, particularly in individuals at risk for developing CRC. Furthermore, my lab has generated various tools for goblet cell research based on our Muc2Cre mouse line, including goblet cell reporter and diphtheria-toxin based depletion models, which we study in the context of infection, inflammation and CRC development.

## SELECTED SPEAKER

### Structural Basis for MUC16 Recognition and Associated Anti-Pancreatic Cancer Activity of Humanized Antibody AR9.6

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Mucin-16 (MUC16), is an attractive target for antibody-mediated immunotherapy due to its central role in oncogenic signaling in pancreatic ductal adenocarcinoma (PDAC). The MUC16 specific monoclonal antibody AR9.6 has shown promise for immunotherapy and imaging of PDAC. Here we report the structural and biological characterization of the humanized AR9.6 antibody (huAR9.6). The structure of humanized antibody huAR9.6 was determined in complex with a MUC16 SEA domain. Binding of huAR9.6 to recombinant, shed and cell-surface MUC16 was characterized and the anti-PDAC activity was evaluated in vitro and in vivo. huAR9.6 bound a discontinuous, conserved epitope with an affinity of ~90 nM. Binding affinity depended on the specific SEA domain(s) present and glycosylation enhanced affinity 3-7-fold. The enhanced affinity was driven by favorable changes in entropy and enthalpy, while transition state thermodynamics revealed that binding to the glycosylated domain proceeded though a distinct pathway. Treatment with huAR9.6 reduced viability, migration, invasion and proliferation of MUC16+ PDAC cells in vitro, disrupted ErbB and AKT signaling and induced apoptosis in cells from patient-derived PDAC xenografts. Tumor burden in PDAC xenograft models was reduced. In addition, huAR9.6 elicited antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Dense avid binding of huAR9.6 to homologous SEA domains on MUC16 may facilitate ADCC and CDC. The avid interaction may sterically inhibit activation of ErbB receptors by MUC16, or alternatively, AR9.6 binding may specifically inhibit ErbB-MUC16 interactions. Either mechanism explain disruption of oncogenic signalling. The results of this study validate the translational therapeutic potential of huAR9.6 against MUC16-expressing PDACs.

**INVITED SPEAKER**

## Mucus Plugging in Asthma – More than an Obstruction to Breathing

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Mucus hypersecretion is a major feature of airway diseases such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis. In allergic asthma, goblet cell hyperplasia and type 2 airway inflammation are important pathophysiologic mechanisms that drive mucus production and the formation of plugs in the airways. It has long been recognized that extensive mucus plugging of the airways in asthma may contribute to airway obstruction and when severe may cause respiratory failure and death in acute exacerbations. More recently it has been shown that the presence of mucus plugs in asthmatics is associated with greater type 2 inflammation and more frequent severe exacerbations. There is also evidence that mucus plugs persist in the same airways over time. Recent studies comparing allergic asthmatics to allergic non-asthmatic control subjects suggest that there is a direct link between inflammation and mucus production in asthmatics that is uncoupled in allergic controls. Furthermore, these studies demonstrate that airway goblet cells are dynamic and develop a pro-inflammatory phenotype with allergen challenge. These data suggest that mucus plugs are more than just physical barriers to airflow and may, along with abnormal goblet cells, provide feed-forward signals that propagate an abnormal airway microenvironment that drives a severe asthma phenotype. Thus, the therapeutics that directly target mucus in the airways may have benefit beyond relieving airway obstruction.

**INVITED SPEAKER**

## The role of mucins and their isoforms in barrier dysfunction and patient stratification

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Mucins are indispensable for the maintenance of mucosal barrier integrity but are still often considered as passive mediators, understating their importance in mediating epithelial barrier function. Furthermore, they act as dual-faceted proteins providing on the one hand an essential nutrient source for the gut microbiota, shaping its community composition and function and regulate epithelial permeability. On the other hand, aberrant mucin expression compromises microbial homeostasis and mucosal barrier integrity further promoting chronic inflammation and even tumourigenesis. Given that abnormal mucin expression is frequently observed in gastrointestinal pathologies, including inflammatory bowel diseases (IBD) and cancer, mucins must be considered as active players impacting mucosal barrier function and, thus, in the onset and course of these diseases. Here, we will provide mechanistic insights on how aberrant MUC13 expression, in cooperation with MUC1, impacts intestinal barrier function upon inflammation and demonstrate that aberrant mucin signatures associate with IBD presentation and gastric cancer outcome. In addition, mucins are highly polymorphic and the presence of genetic differences in mucin genes can give rise to a large repertoire of structurally diverse mRNA isoforms via alternative splicing. While most mRNA isoforms produced from the same mucin gene locus encode similar biological functions, others have the potential to alter protein function resulting in progression towards disease. Using a novel targeted mucin mRNA isoform sequencing approach in conjunction with deep learning and external validation, we unveiled the mucin mRNA isoform landscape in the intestinal tract of IBD and control patients encompassing distinct mucin RNA isoform panels that accurately stratified the heterogeneous IBD patient population in multiple subpopulations based on inflammation, IBD subtype and anatomical location in the intestinal tract.

## SELECTED SPEAKER

### Goblet cells and the circadian clock: a new regulatory mechanism

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The intestinal mucus barrier provides frontline protection to the epithelium and controls daily access of particulates, microbes, and toxins to the underlying mucosal tissues. Specialised epithelial cells, goblet cells, instruct and maintain the protective mucus barrier through the highly dynamic production of gel-forming mucin glycoproteins. A potentially important intrinsic regulator of goblet cell function is the control by the circadian clock. This cell-intrinsic timing mechanism controls rhythmic outputs of gene expression and protein activity in a tissue-specific manner, which allow an organism to anticipate and respond to daily rhythmic changes in their environment, such as feeding/fasting cycles. Research has shown essential roles of circadian rhythms in regulating intestinal tissue homeostasis and microbiota, however, little is known about circadian rhythms in goblet cells. Here, we show that intestinal mucus-associated properties change over a diurnal 24-hour cycle in mice, including the number of goblet cells/crypt and mucin glycosylation. We show that intestinal goblet cells express key components of the molecular clock and that their expression changes over a 24-hour period, supporting a major role for a goblet cell intrinsic clock in the control of mucin production/properties. To assess the importance of the goblet cell clock, we have generated a unique goblet cell “clockless” mouse model (specific knockout of the core clock transcription factor Bmal1 in intestinal goblet cells; Muc2-iCre:Bmal1fl/fl). Bmal1 KO mice show an altered mucus barrier, microbial dysbiosis and a susceptibility rather than resistance to a challenge with the intestinal dwelling helminth, Trichuris muris. These data have identified goblet cell circadian rhythm as a temporal regulatory mechanism for mucin production, revealing fundamental daily regulation at this critical site of defence against inflammation and infectious challenge.

## SELECTED SPEAKER

### Using mucinases to enhance oral delivery of macromolecules

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Peptide drugs hold great therapeutic potential, but their oral delivery often results in subtherapeutic concentrations due to the various gastrointestinal barriers such as mucus, epithelial linings, and digestion processes.1 Despite the use of permeation increasing strategies, clinical success in improving oral bioavailability of peptides remains limited (<1%).1,2 Here, we employ mucinases to reduce the barrier properties of gastrointestinal mucus.3 We evaluated three different mucinases (StcE, BT4244, and AM0627), and a broad-acting O-glycoprotease (OgpA) for their efficacy in degrading the mucin network and reducing the viscosity of porcine small intestinal mucus.4, 5 StcE was identified as the most effective mucin-degrading enzymes, significantly reducing both viscous and elastic properties of mucus within 5 min of application. Using fluorescence recovery after photobleaching (FRAP), we demonstrated that StcE treatment notably increases the diffusivity of peptide surrogates (Cy5-labelled poly(ethylene glycol)s (PEGs); 1, 5, and 20 kDa) through mucus, enhancing both mobile and immobile macromolecule fractions. Subsequently, we assessed the efficacy of StcE and peptide co-delivery in vivo. To that end, we lyophilized StcE and incorporated it together with a novel permeation enhancer and a model drug (desmopressin acetate, ~1 kDa) in commercial enteric capsules. Upon oral administration to dogs, plasma concentration of desmopressin was assessed and compared to those achieved with a control capsule only containing desmopressin acetate. Results achieved with the combination of StcE and permeation enhancer indicated a relative increase in desmopressin plasma levels and area under the curve (AUC) compared to the control, showing a trend towards improved oral bioavailability, however with notable inter-individual variability. Overall, StcE can be employed for reducing the gastrointestinal mucus barrier, enhancing the permeability of macromolecules. StcE has the potential, especially when combined with other delivery strategies, to enhance oral delivery of macromolecules, ultimately improving oral bioavailability and therapeutic efficacy. This research was supported by the ETH Zurich Postdoctoral Fellowship program (Grant No. 22-1 FEL-12).

References:
1. Brown, T. D. et al. Materials for oral delivery of proteins and peptides. Nat Rev Mater (2020) 5, 127–148
2. Moroz, E. et al. Oral delivery of macromolecular drugs: Where we are after almost 100 years of attempts. Adv. Drug Deliv. Rev. (2016) 101, 108–121.
3. Murgia, X. et al. The role of mucus on drug transport and its potential to affect therapeutic outcomes. Advanced drug delivery reviews (2018) 124, 82–97.
4. Lai, S. K. et al. Micro- and macrorheology of mucus. Advanced drug delivery reviews (2009) 61, 86–100.
5. Shon, D. J. et al. Classification, structural biology, and applications of mucin domain-targeting proteases. The Biochemical journal (2021) 478, 1585–1603.