

Oral abstracts

Session 1:

Single-molecule FRET Analysis of Mucin PTS Length-Span and Dynamics

Presenting author: Rotem Azulay

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Co-authors: Rotem Azulay¹, Hagen Hofmann¹, Deborah Fass¹ ¹Weizmann Institute of Science, IL

Mucins are characterized by segments rich in proline, threonine, and serine (PTS regions). Some PTS regions have specific roles in mucin self-assembly, whereas others likely contribute to the general properties of mucin hydrogels. Despite the importance of PTS regions in mucin function, the structures and dynamics of these regions are poorly understood, and the relationship between PTS sequence and physical properties is underexplored. To address these issues, we developed a single-molecule fluorescence resonance energy transfer (smFRET) approach allowing the experimental determination of how PTS sequence and glycosylation dictate end-to-end distance and polymer dynamics. By directly comparing isolated unglycosylated PTS peptides to their fully glycosylated counterparts, we provide quantitative evidence that O-glycosylation acts as a primary driver of PTS extension. Furthermore, by tracking a library of engineered variants with varying length, we established how the structural constraints imposed by dense O-glycans stiffen the backbone. Our observations revealed that the end-to-end distance strictly scales with sequence length, adopting an ultra-extended rod-like conformation. This dramatic shift from a flexible coil to a rigid extended state highlights the profound physical impact of glycosylation. Ultimately, these quantitative, single-molecule measurements provide crucial experimental benchmarks for molecular dynamics simulations, helping to elucidate the fundamental biophysical rules governing mucin function.

Session 1:

Tunable, scalable, synthetic mucins for engineering mucus and the glycocalyx

Presenting author: Jessica Kramer

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Mucins are fascinating molecules with unique conformations and glycosylation patterns. Their complexity and heterogeneity has been a barrier to systematically probing their functions in health and disease. To contribute to solving this challenge, we developed chemical and enzymatic methodology to generate synthetic mucins (synMUCs) based entirely on native molecular features. We generated glycosylated polypeptides through tunable, scalable polymerization of amino acid N-carboxyanhydrides with pendent sugars. We also used natural glycosyl transferases to install terminal Sia, Fuc, and sulfated glycans.

Our synthetic materials capture native mucin biophysics as shown through persistence length and spectral data, and they function as biological surrogates for bona fide mucins in microbiology and immunology settings. The synMUCs are chemically defined, reproducible, and fully tunable for many desirable properties including glycosylation (20+ glycans), MW (10-1000 residues), multiple domains, and end groups ready for probe installation.

We have used these synMUCs to engineer signalling-competent MUC1-glycocalyx models to probe infection and cancer, and in mucosal immunity models to probe glycan-dependent host-microbe interactions in the context of viral, bacterial, and fungal infections. We are pleased to share our synMUC tools with the community of researchers studying mucins in health and disease.

Session 2:

Going beyond mucus factories: a new role for goblet cells

Presenting author: Maria Naama

Presenting author institute: Azrieli Faculty of Medicine, Bar-Ilan University, safed, IL

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Colonic goblet cells actively secrete mucus to form a protective mucus barrier that maintains host homeostasis. This mucus barrier effectively separates the host from the luminal microbiota by encapsulating the fecal pellet, preventing bacterial invasion and inflammation. Mucus barrier formation is microbiota-dependent, as germ-free mice lack a mucus barrier. Yet the mechanisms by which gut microbiota regulate mucus barrier formation are not fully understood.

We found that loss of gut microbiota resulted in defective mucus encapsulation despite preserved mucus production and secretion. Using a novel approach to examine goblet cell responses, we uncovered an unexpected mechanism in which microbial regulation of goblet cells promotes mucus encapsulation by regulating epithelial water absorption through aquaporin-8 (Aqp8).

Water absorption in the gut is controlled by the renin-angiotensin-aldosterone system (RAAS), which maintains fluid homeostasis. Consistent with this, microbiota depletion led to increased systemic aldosterone levels and activation of RAAS signaling in goblet cells, suggesting a compensatory host response to altered intestinal water transport.

Together, these findings indicate that goblet cells function not only as mucus-secreting cells but also as an integrative hub where microbial and hormonal signals converge to regulate intestinal barrier function and fluid homeostasis.

Session 2:

Mucus secretion is a novel form of viral egress

Presenting author: Valerie Cortez

Presenting author institute: University of California, Santa Cruz, US

Co-authors: Natalie Pedicino¹, Melanie Kirkpatrick¹, Michelle Ann Pablo¹, Jacob Gulman¹, Nicole Heredia-Osuna¹, Valerie Cortez¹ ¹University of California, Santa Cruz, US

Viruses rely on coordinated host pathways to exit the cell and propagate infection. We have come to appreciate that non-enveloped enteric viruses can use both lytic and non-lytic strategies to exit host cells. However, it remains unclear how astroviruses, a major causative agent of pediatric diarrhea, facilitate this final step in its replication cycle. Because astroviruses infect small intestinal goblet cells, we investigated whether mucus secretion could enable a pathway of non-lytic viral egress. Using the murine astrovirus model, we found that virus release co-occurred almost exclusively with mucus secretion from goblet cells. At the tissue level, mucus secretion also correlated with infection kinetics, characterized by a return to baseline secretion during viral clearance. Further, disruption of mucin biosynthesis reduced viral shedding, and stimulating mucus secretion with carbachol increased viral release. To examine this pathway with human astrovirus, we established a new infection model in LS174T goblet cells. We observed that human astrovirus colocalized with MUC2 within the cell and with isolated mucin granules. Stimulating cells with carbachol enhanced mucus and virus release, which could be blocked by the addition of a muscarinic receptor antagonist. Further, we found that blocking intracellular calcium, which drives mucin granule exocytosis, also reduced virus release. Together, these findings demonstrate that mucus secretion is a novel form of non-lytic enteric viral egress, which deepens our understanding of the astrovirus replication cycle and may hold clues to understanding its pathogenesis.

Session 3:

Quantifying Glycocalyx Crowding Forces from Molecular Exclusion Effects

Presenting author: Matthew Paszek

Presenting author institute: University of California, Davis, US

Co-authors: Lingting Huang¹, Matthew Paszek¹ ¹University of California, Davis, US

The glycocalyx is a dense layer of glycoproteins, proteoglycans, and glycolipids that extends from the plasma membrane and forms a critical barrier against foreign molecules. The high packing density of these macromolecules generates steric crowding, creating an energetic barrier that restricts the penetration of large molecules into the pericellular space. This crowding energy represents a fundamental physical force governing cell-molecule and cell-cell interactions; however, its magnitude in living cells remains poorly quantified.

Here, we quantify glycocalyx crowding by designing size-tunable probes that target endogenous membrane proteins. Using these probes, we developed a competitive binding assay that links glycocalyx crowding energy to differences in the apparent binding affinities of probes with different sizes. Under crowded conditions, larger probes experience stronger steric exclusion than smaller probes, resulting in reduced receptor accessibility and lower apparent binding affinities. By comparing size-dependent binding constants, we extract the free energy associated with glycocalyx-induced crowding using a theoretical framework we developed.

Applying this approach, we quantify, for the first time, how cell-surface mucin expression alters glycocalyx crowding energy, demonstrating that even moderate mucin expression can generate pronounced steric exclusion at the cell surface. Together, these results establish a quantitative framework for measuring glycocalyx crowding energy and reveal how glycocalyx architecture regulates molecular accessibility at the cell surface, providing a physical basis for understanding molecular interactions such as ligand-receptor and cell-cell interactions within the glycocalyx.

Session 3:

Sensing the Gut in Action: An Ingestible Platform for Spatiotemporal Microbiome Activity Mapping toward Digital Gut Twins

Presenting author: Arjan Tibbe

Presenting author institute: IMEC, NL

Co-authors: Arjan Tibbe¹, Neha Awasthi², Annelies Goris¹, Christian Büll² ¹IMEC, NL, ²Radboud University, NL

The growing recognition of the gut microbiome as a central regulator of human health, together with its interactions across organ-associated microbiomes, has driven rapid advances in microbiome research. In parallel, efforts are emerging to enable personalized prediction of microbiome composition and function through the development of a “digital gut twin”.

This digital gut twin is an artificial intelligence (AI)-driven, continuously updated simulation that integrates diverse data streams, such as nutrient intake, host-specific factors, and multi-omic microbiome profiles, to predict individualized responses to dietary interventions, highlighting the need for high-resolution spatiotemporal data on microbiome activity.

Here, we present a novel sensing approach to address this gap. The sensor employs genetically engineered mucins functionalized with predefined glycan structures that can be selectively cleaved by microbial glycosidases, such as sialidases and galactosidases. Glycan removal exposes the mucin backbone to proteolytic degradation, resulting in detachment of mucins from the sensor surface and generating a measurable signal reflecting localized microbial activity.

Integration of this sensor into our ingestible platform, capable of spatially resolved measurements of temperature, pH, and oxidation–reduction potential (ORP), enables coupling of microbiome activity with the local physicochemical environment. This multimodal dataset provides a key input for refining digital gut twins and advancing personalized nutrition and therapy.

Session 4:

A role for CFTR in goblet cell biology

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The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) plays a key role in the regulation of ion and water transport at mucosal surfaces throughout the body. Recent studies have identified that CFTR is expressed in specific subpopulations of mucus-producing goblet cells in both the airways and the gastrointestinal tract, however the role of CFTR in goblet cell biology remains unclear.

Using high-resolution imaging of human and mouse tissue and live cell imaging of mucus secretory responses in small intestinal organoids generated from wildtype and *Cftr* Δ F508 mice we have investigated the role of CFTR in induced secretory responses.

CFTR was found to be localised to the goblet cell theca in close proximity to the mucin granules of small intestinal crypt goblet cells and surface airway goblet cells suggesting a possible role in mucin granule biology. Live cell imaging of *Cftr* Δ F508 organoids revealed that goblet cells from this genetic background were unable to secrete normally when compared to wildtypes following cholinergic stimulation. Whereas short-term treatment (24 hrs) of organoids with Elexacaftor and Tezacaftor rapidly restored liquid secretion as indicated by organoid swelling, mucus secretory responses were not normalised. Long-term treatments (5 days) were necessary for a partial restoration of goblet cell secretory responses.

This study highlights the requirement of a functional CFTR to enable induced goblet cell secretion in the small intestine. The localisation of CFTR to the goblet cell theca at other anatomical sites suggests that CFTR may play a more widespread role in the secretion of mucus, a factor which may have implications for the pathogenesis of cystic fibrosis.

Session 4:

Distinct roles of C-terminal dimerization and N-terminal polymerization in Muc5ac-mediated airway mucus dysfunction

Presenting author: Gilda Opoku

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Co-authors: Gilda Opoku¹, Ana Maria Jaramillo², Christopher Evans¹ ¹University of Colorado, Anschutz Medical Campus, US, ²University of Kansas Medical Center, US

MUC5AC/Muc5ac is overproduced and hypersecreted in asthma and is required for asthma-like obstruction in murine models. To identify potential therapeutic strategies for MUC5AC, we seek to better understand its biosynthesis and polymerization. Widespread mucin disulfide disruption prevents obstruction in allergic mice, so we tested how specific cysteine mutations affect mucin assembly and function.

Polymeric mucins assemble via disulfide bonds within conserved von Willebrand factor (vWF)-like C-terminal cystine knot and N-terminal D3 domains. Prior work implicated 3 cysteines in C-terminal dimerization and 2 in N-terminal polymerization, though residues governing Muc5ac assembly remain undetermined.

In Muc5ac C-terminal fragments, paired (C3378/3380) and triple (C3378/3380/3418) Cys>Ser mutations prevented disulfide assembly. In allergic mice with CRISPR-edited full-length Muc5ac, triple mutants secreted dimers, while paired mutants failed to secrete detectable Muc5ac. Functional studies showed improved airflow in vivo and mucociliary transport in vitro.

N-terminal D3 paired Cys>Ser mutations also prevented assembly in vitro, with similar dimerization occurring in C1135/1177 and C1137/1177 mutants that were replicated in CRISPR-edited mice. However, despite similar polymer disruption as seen in C-terminal mutants, N-terminal mutants showed no improvement in airway hyperreactivity.

These results show disulfide-mediated assembly is required for Muc5ac polymerization, with distinct roles for C-terminal dimerization and N-terminal polymerization in regulating mucus properties. Ongoing studies will dissect how assembly affects glycosylation and trafficking, and how targeted depolymerization may improve mucus function.

Session 5:

Mucin 5AC promotes chemoresistance through OGT/IGF-1R axis in colorectal cancer

Presenting author: Ramesh Pothuraju

Presenting author institute: Rajiv Gandhi Centre for Biotechnology, IN; University of Nebraska Medical Centre, US

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Colorectal cancer (CRC) exhibits significant heterogeneity, often leading to chemoresistance, therapeutic failure, and tumor relapse. Mucin 5AC (MUC5AC), a gel-forming glycoprotein, is aberrantly overexpressed during CRC progression; however, its role in mediating chemoresistance remains unclear. In this study, we investigated the functional and molecular mechanisms underlying MUC5AC-driven therapy resistance in CRC. Immunohistochemical analysis of pre- and post-therapy patient samples revealed elevated MUC5AC expression following chemotherapy. Using azoxymethane/dextran sodium sulfate-induced CRC models, we observed that Muc5ac-deficient mice exhibited a significant reduction in tumor burden upon 5-fluorouracil (5-FU) treatment compared to wild-type controls. In vitro, 5-FU-resistant CRC cell lines showed increased MUC5AC expression, and its knockdown sensitized resistant cells to chemotherapy, as evidenced by reduced tumor growth in xenograft models. Mechanistically, mass spectrometry and biochemical analyses demonstrated that MUC5AC interacts with O-GlcNAc transferase (OGT), facilitating activation of the IGF-1R/AKT signaling pathway, thereby promoting cell proliferation, survival, and resistance to apoptosis. Collectively, our findings identify a novel MUC5AC–OGT–IGF-1R signaling axis that drives chemoresistance in CRC and highlight MUC5AC as a potential therapeutic target to overcome treatment resistance.

Session 5:

Discovery of a MUC4EGF domain-targeting peptide that inhibits the MUC4-ErbB2 complex and resensitizes pancreatic cancer cells to ErbB2 targeted therapies

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers with an increasing incidence. It is mainly due to absence of diagnosis and no effective treatments, and a strong resistance to therapies. It is thus crucial for this devastating cancer to find new therapeutic alternatives. In this aim, we work on the interaction of the mucin MUC4 with receptor tyrosine kinases (RTK), and the targeting of MUC4 using small inhibitory molecules. MUC4, a large glycoprotein overexpressed at the surface of pancreatic cancer cells, is associated with poor prognosis of the tumor, increase of proliferation and migration as well as apoptosis and resistance to therapies. Moreover, we have shown that MUC4EGF domains interact with and upregulates the activity of ErbB2/HER2, this complex being a strong promoter of tumorigenesis. Use of small inhibitory molecules would circumvent the steric hindrance played by MUC4 glycosidic chains. We have now, using molecular dynamics and peptide library screening, identified a specific MUC4EGF domain peptide ligand (i) capable of inhibiting MUC4-ErbB2 complex formation, activity and downstream signaling pathways, (ii) having anti-proliferative activity and (iii) resensitizing pancreatic cancer cells to Trastuzumab and Pertuzumab ErbB2-targeted therapies by restoring their access to their target in vitro, ex vivo and in vivo. Altogether, these results highlight MUC4 as a compelling therapeutic target in PDAC and show that the development of small molecule inhibitors represent a promising strategy to overcome therapeutic resistance in cancers overexpressing MUC4 mucin

Session 6:

Immunological consequences of goblet cell loss: the gut-kidney axis

Presenting author: Sophie Vanhercke

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Goblet cells (GCs) are essential for intestinal homeostasis by secreting a protective mucus layer that segregates the microbiota from the epithelium. Disruption of this barrier triggers chronic inflammation and systemic immune dysregulation. To investigate the immunological consequences of goblet cell loss, we developed goblet cell-specific transgenic mouse models using a Muc2Cre line enabling inducible (Muc2DTR) and constitutive (Muc2DTA) diphtheria-toxin-mediated GC deletion. Constitutive goblet cell loss in Muc2DTA mice resulted in mucus depletion, increased bacterial-epithelial interactions and elevated circulating IgA levels. Chronic GC depletion led to spontaneous mesangial IgA deposition and increased circulating IgA-IgG immune complexes, hallmarks of human IgA nephropathy (IgAN), the most common form of glomerulonephritis. These systemic and renal IgA abnormalities were significantly reduced in germ-free Muc2DTA mice, implicating the microbiota as a key driver of hyper IgA responses and IgAN-like pathology in the absence of a mucus barrier. Loss of the mucus layer also altered the colonic mucosal microbiota, with pronounced enrichment of *Akkermansia muciniphila*, which accounted for approximately 50% of the IgA-coated bacterial fraction in stool. Ongoing work in gnotobiotic Muc2DTA mice examines how specific taxa, including *A. muciniphila*, promote IgA overproduction and IgAN-like pathology. Together, our data show that goblet cell loss alters host-microbiota interactions, driving microbiota-dependent hyper-IgA responses and IgA-mediated kidney disease. These findings uncover a key role for the mucus layer in regulating the gut-IgA-kidney axis and provide new insights into the pathogenesis of IgA-driven disease.

Session 6:

Identification of non canonical goblet cells by scRNAseq is confounded by isolation of doublets

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Single-cell RNA sequencing has expanded our understanding of cellular heterogeneity, subdividing many cell populations into distinct subsets defined by functional specialization and maturation state. Recent studies propose the existence of ‘canonical’ and ‘non-canonical’ GC subsets within the intestinal epithelium. Canonical GCs are characterized by expression of typical GC marker genes (Clca1, Fcgbp) and comprise crypt and intercrypt GCs. Non-canonical GCs are characterized by a mixed gene expression profile of both enterocytes and GCs. However, a major caveat of scRNA-seq is the occurrence of doublets which can potentially generate artificial hybrid populations. We thoroughly investigated this non-canonical GC population to assess potential GC-enterocyte doublet contamination. One feature that may support the doublet hypothesis is the lack of unique non-canonical GC marker genes. Using an imaging flow cytometer on intestinal epithelial cells derived from the R26-tdRFP Muc2iCre line, we identified doublet cells within the intestinal epithelial single-cell suspension. Moreover, most RFP+ GCs expressing enterocyte markers TGM3 and MUC3 are GC-enterocyte doublets (70%), where the RFP+ GC stained negative for enterocyte markers, and the associated RFP- cell was positive for TGM3/MUC3. Only a minority of GC cells were truly double positive (7%). In conclusion, non-canonical GCs in our scRNA-seq dataset of intestinal epithelial cells were largely identified as a doublet artifact. These findings highlight the difficulties in the interpretation of single cell data, especially hybrid cell populations, and the need for careful validation by complementary approaches to avoid mis- or overinterpretation of artifactual populations.

Session 7:

Developmental remodeling of the colonic mucus barrier during weaning coordinates immune homeostasis.

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Weaning is characterized by an expansion of the intestinal microbiota that triggers an immune response known as the weaning reaction. The colonic mucus barrier consists of two distinct layers: a proximal-derived outer layer and a distal-derived inner layer. Here, we investigated the changes in mucus barrier properties during weaning and how a loss of core 1-derived O-glycans affects mucus development as well as immune and epithelial responses in wild-type (WT) and Core 1 β 1,3-galactosyltransferase knockout (KO) mice at 2, 3, and 4 weeks of age. Mucus analyses showed a transient reduction in thickness and increased penetrability in the distal colon at 3 weeks of age in WT mice that was accompanied by an infiltration of innate immune cells into the colonic lamina propria and induction of host defense response to microorganisms by the epithelium. This phenotype was exacerbated in KO mice, which had a thinner more penetrable distal mucus barrier at all time points compared to WT. Impaired mucus barrier integrity in the KO mice resulted in a premature influx of innate immune cells to distal colon at 2 weeks, peaking at 3 weeks and normalizing by 4 weeks with bacterial translocation to colon draining lymph nodes, elevated host defense responses and signs of epithelial invasion of microorganisms at 3 weeks of age. Together, these findings identify a transient weakening of the distal mucus barrier during weaning as part of normal colonic development that promotes immune and epithelial activation. During this period, further impairments to mucus barrier integrity result in a situation where the colonic epithelium is severely exposed to microbial invasion.

Session 7:

Glycans drive composition and function of infant gut synthetic microbial communities

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Dietary and mucin glycans are essential for infant gut microbiota development. Human milk contains oligosaccharides (HMOs) that are digested by the infant gut microbiota. The prebiotic effect of breastmilk is partially mimicked by enriching formula milk with short-chain galacto- and long-chain fructooligosaccharides (scGOS/lcFOS, 9:1) or HMOs. To study infant microbiota-glycan interactions and microbiome dynamics, we created synthetic communities and exposed them to combinations of host- and diet-derived glycans in batch cultivations, and tracked relative and absolute abundance, metabolite production and substrate degradation. Furthermore, we used metaproteomics to study community function. In all conditions with mucin glycans, the specialist mucin glycan degrader *Akkermansia muciniphila* dominated, while *Bifidobacterium* species strongly associated with HMOs and GOS/FOS in the absence of mucin. With complex HMO mixes, *Bifidobacterium bifidum* was dominant, but with GOS/FOS, or GOS/FOS combined with one HMO, *Bifidobacterium infantis* and *Bifidobacterium breve* dominated over *B. bifidum*. Our community was stable, with primary glycan degraders sustaining cross-feeders such as *Escherichia coli* and *Enterococcus faecalis*. Metaproteomics revealed that most of glycoside hydrolases (GHs) detected within the communities originated from primary glycan degraders, and there were differences in metabolite concentrations and substrate degradation between conditions. Feeding distinct glycan mixes to synthetic infant gut microbial communities impacted the community composition and function, providing a rationale for further research into supplementing formula milk with more diverse mixes of oligosaccharides.

Session 8:

Expansion of MUC1 repeats shapes human mucus barrier evolution

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Presenting author institute: Yale University, US

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The human epithelial mucus barrier contains mucin-1 (MUC1), a transmembrane glycoprotein central to pathogen defense. The MUC1 gene harbors exonic variable number tandem repeats (exVNTRs) that differ in copy number among individuals and modulate the length and glycosylation potential of the encoded protein. Despite their functional importance, these repeats have remained inaccessible to evolutionary genetic analysis due to limitations of short-read sequencing. Here, using long-read genome assemblies representing 770 human haplotypes, we comprehensively characterized variation in the MUC1 exVNTR across global populations. We found that repeat copy numbers range from 25 to 116 repeats, with shorter alleles (

Session 8:

Terminal fucosylation influences susceptibility to pneumococcal pneumonia

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Background: The pulmonary glycocalyx regulates lung barrier permeability and harbors terminally fucosylated glycan structures. Around 23% of healthy Europeans, however, lack terminal fucose structures due to Fucosyltransferase (FUT)2 mutations, resulting in altered susceptibility to infection.

Aim: To elucidate whether terminal fucose facilitates *Streptococcus pneumoniae* (S.pn.) infection and susceptibility to pneumococcal disease in patients with bacterial pneumonia and experimental models.

Methods: FUT2 genotypes of patients with bacterial pneumonia were determined by real-time PCR. A murine model of pneumococcal pneumonia was employed including treatment with 2-deoxy-D-galactose (2d-Gal), a fucosylation inhibitor. Glycans shed into bronchoalveolar lavage (BAL) during infection were characterized by mass spectrometry. Lung barrier permeability and immune cell influx were assessed by ELISA and flow cytometry. Dual RNA sequencing was employed to resolve murine and bacterial transcriptomics.

Results: The functional FUT2 allele was significantly enriched in patients with pneumonia compared to healthy individuals, while prevalence of the mutated FUT2 allele was significantly lower compared to the reference allele among the patient cohort. In a murine model of pneumonia, treatment with 2d-Gal led to reduced shedding of terminally fucosylated glycans in addition to significant reduction of bacterial burden. Treatment with 2d-Gal accelerated resolution of neutrophilic inflammation and improved lung barrier function during S.pn. infection.

Conclusions: Limiting fucosylated glycan availability restricts S.pn. infectivity and pulmonary inflammation, suggesting a potential therapeutic strategy targeting host glycosylation.

Session 9:

NOVEL REGULATORS OF SECRETORY APPARATUS STRUCTURE AND MUCIN SECRETION

Presenting author: Kelly Ten Hagen

Presenting author institute: National Institutes of Health , US

Co-authors: Kelly Ten Hagen¹ National Institutes of Health , US

Regulated secretion is an essential process by which proteins, such as mucins, are secreted into the extracellular environment in response to a hormonal or physiological stimulus. Defects in this conserved process are associated with various diseases, such as cystic fibrosis, colon cancer, inflammatory bowel disease and Sjögren's Syndrome. Here, we construct a cell atlas from salivary glands, a major mucin-secreting organ, to identify genes that control various stages of regulated secretion. We identify unique populations of secretory cells that represent distinct temporal stages of secretory granule formation and secretion. Within each population, we identify specific genes that are essential for various aspects of secretory apparatus structure, secretory granule formation and secretion. We show that the loss of one conserved protein associated with human secretory dysfunction, disrupts the formation of secretory granules by influencing the formation of the Tango1 rings to mediate ER-Golgi docking sites. We further show that 2 conserved SNAREs are required for the formation of patent Tango1 pores through which cargo passes from the ER to the Golgi. Finally, we identify other distinct secretory cell populations within the salivary gland that have characteristics of innate immune-like cells, primarily producing diverse antimicrobial peptides and host defense proteins. This study sheds light on essential factors involved in distinct aspects cargo (mucin) movement through the secretory apparatus and identifies unique salivary gland cell populations involved in host defense.

Session 9:

Declining Unfolded Protein Response with Aging Drives Mucin Dysregulation in the Colon

Presenting author: Thishan Illankoon

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Accumulation of misfolded protein induces endoplasmic reticulum (ER) stress, which is resolved by activation of the unfolded protein response (UPR). While increased protein misfolding is recognised as a feature of aging, how age-associated decline in UPR function contributes to intestinal epithelial dysfunction and inflammation remains unclear. We hypothesised that aging compromises epithelial proteostasis, thereby increasing susceptibility to intestinal inflammation.

To investigate this, we analysed human colon biopsies from individuals aged 18-80 years old, and utilised mouse models aged 4-96 weeks old. C57BL/6 mice were used to assess age-related changes in intestinal homeostasis, while Winnie mice (Muc2 mutation) served as a model of chronic ER stress-driven intestinal inflammation.

Aging was associated with a significant decline in UPR gene expression in C57BL/6 mice, which was further exacerbated in older Winnie mice when compared to age-matched controls. Primary colon organoids from aged mice showed reduced capacity to resolve ER stress, indicating impaired epithelial stress responses.

Consistently, human colon biopsies exhibited age-dependent increases in ER stress markers, alongside trends towards reduced UPR activity. Histological analyses revealed that secreted Muc2 production declined with age in C57BL/6 mice and was further reduced in Winnie mice. Additionally, cell surface mucin, Muc13, linked to proinflammatory phenotypes increased with aging.

Together, these findings identify aging as a driver of impaired epithelial proteostasis in the intestine, linking declining UPR capacity with disrupted mucin homeostasis and heightened susceptibility to intestinal inflammation.

Session 10:

The food exposome impacts mucus production: IL-33-driven alterations of MUC2 induced by zinc deficiency in intestinal goblet cells

Presenting author: Christoph Schüßler

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Zinc (Zn) is essential for key physiological functions, such as the immune system [1]. Yet approx. 17% of the global population suffers from Zn deficiency, resulting in impaired immune function, increased susceptibility to infections, and higher incidence of diarrhea [2]. Zn deficiency has also been shown to cause degeneration of the intestinal epithelium and its mucus barrier [3-4]. The latter protects the underlying epithelium, provides a habitat for the microbiota, and contributes to trace element absorption [5]. Prior studies using the human goblet cell model HT29-MTX showed that Zn deficiency alters mucus production, the intestinal O-glycome, and dysregulates mucin gene expression, particularly of MUC2 [4]. Thus, the aim of this study was to identify additional genes and pathways influenced by Zn deficiency, employing transcriptomic analysis to assess gene expression of mucins, ER-stress markers, and cytokines. Zn deficient (ZD) cells had profoundly increased MUC2 expression and secretion, with transcriptomic screening revealing significant interleukin (IL)-33 upregulation, confirmed at the protein level. Stimulation of control cells with IL-33 resulted in significantly increased MUC2 expression. Further steps, downstream of the IL-33 signaling pathway, were studied elaborating the complex signaling cascade that initiates MUC2 expression during ZD conditions. These findings highlight the critical role of Zn in maintaining intestinal mucus homeostasis.

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Session 10:

Oxidation Increases Mucus Viscoelasticity Without Inducing Mucin Crosslinking

Presenting author: Daniel Lauster

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Co-authors: Nico Boback¹, Cosmin Butnarusu¹, Hanna Rulff³, Cihan Baydaroglu³, Kerstin Fentker⁴, Alicia Hartmann¹, Julia Gräber-Dürr², Marcus Mall², Michael Gradzielski³, Philipp Mertins⁴, Daniel Lauster¹ ¹Freie Universität Berlin, DE, ²Charité - Universitätsmedizin Berlin, DE, ³Technische Universität Berlin, DE, ⁴Max-Delbrück-Center for Molecular Medicine, DE

Oxidative stress is a hallmark of airway inflammation and is commonly assumed to increase mucus viscoelasticity through covalent mucin crosslinking via disulfide bond formation. However, direct molecular evidence supporting mucin-mucin crosslinking under oxidative conditions remains limited. Here, we systematically investigate the effects of oxidation on mucus structure and mechanics using bovine submaxillary mucins (BSM) from different sources and human salivary MUC5B.

Oxidative treatment led to increased viscoelasticity in selected samples. However, mass photometry, gel electrophoresis, and western blot analyses did not reveal the formation of higher-order mucin assemblies, indicating the absence of detectable mucin-mucin covalent crosslinking. Proteomic analyses further demonstrated substantial compositional differences between samples, highlighting the potential contribution of non-mucin components.

These findings suggest that oxidation-induced mucus stiffening cannot be explained by mucin-mucin crosslinking alone. Alternative mechanisms may include oxidation-driven non-covalent interactions and potential crosslinking of non-mucin proteins within the mucus matrix.

Together, this work challenges a central assumption in mucus biology and points toward a more complex, composition-dependent mechanism underlying oxidative changes in mucus viscoelasticity.

Poster abstracts

Abstract: 1

A single component mucin base photo-cross linked mucus mimetic self healing hydrogel for barrier properties against microbial pathogens

Presenting author: Ali Abbas

Presenting author institute: Academia Sinica Research Institute , TW; National Tsing Hua University (NTHU), Taiwan , TW

Co-authors: Ali Abbas^{1,2}, Jui Hong Wne¹, Ayesha Iftikhar Cheema¹, Chou Chia Fu¹ ¹Academia Sinica Research Institute , TW, ²National Tsing Hua University (NTHU), Taiwan , TW

Mucus is a viscoelastic biological hydrogel that protects epithelial surfaces from pathogens and environmental stress. Its barrier properties arise mainly from mucins, highly glycosylated proteins that assemble into dynamic disulfide-linked networks. However, the limited availability of physiologically relevant mucus-like materials has restricted in vitro studies of mucosal barrier function, pathogen transport, and drug diffusion. In this study, we developed a mucus-mimetic hydrogel directly from commercially available porcine gastric mucin (PGM) using a chemical modification strategy that introduces redox-active thiol groups onto the mucin backbone. Upon UV (365nm) irradiation, the modified mucin rapidly forms a disulfide-crosslinked hydrogel within minutes, resembling the crosslinking chemistry of native mucus. Rheological measurements showed tunable mechanical properties, with storage moduli (G') ranging from ~ 10 to 100 Pa depending on mucin concentration, overlapping with the viscoelastic range reported for physiological mucus. The material exhibits autonomous self-healing under ambient oxidation and degrades in the presence of physiological glutathione (GSH) concentrations, indicating redox responsiveness. Nanoparticle tracking using 200 nm fluorescent probes revealed restricted diffusion within the gel network, suggesting nanoscale mesh sizes of ~ 200 – 500 nm capable of trapping virus-sized particles. Unlike synthetic mucus mimetics that rely on external polymers or crosslinkers, this platform is constructed directly from native mucin, providing improved biochemical relevance and offering a useful model for studying mucin network mechanics, pathogen interactions, and mucosal barrier function in vitro.

Abstract: 2

Chemotherapies alter mucus to protect tumours from cytotoxicity through multiple mechanisms

Presenting author: Oladimeji Abudu

Presenting author institute: University of Manchester, GB

Co-authors: Oladimeji Abudu¹, Xiangxu Kong¹, David Thornton¹, John Knight¹ ¹University of Manchester, GB

Mucinous colorectal adenocarcinomas are a subset of colorectal tumours which increase mucus production. They are characterised by resistance to frontline chemotherapeutic drugs such as 5-FU and oxaliplatin. Mucus is a protective viscoelastic gel, its functions are hijacked in these tumours to increase chemoresistance. Within mucus, mucin glycoproteins are a key determinant of mucus function and physical properties. Mucins are implicated in chemotherapeutic resistance in multiple cancers including, colorectal cancer, ovarian cancer, and pancreatic cancer. Despite the apparent importance of mucus, the physical properties of mucus from tumours are not well-characterised. Furthermore, the direct interaction and effect of chemotherapeutic drugs on mucus remain poorly characterised.

Our aim is to address two related questions about tumour-derived mucus; do chemotherapies alter the physical properties of mucus and do chemotherapies alter the biological synthesis of mucins? First, we use rate zonal centrifugation to demonstrate that chemotherapeutic drugs such as 5-FU and oxaliplatin alter the density/size of tumour derived mucins. We observed secreted mucin density/size is increased following culture with these drugs at therapeutic concentrations. We theorise that these drugs bind to mucins, leading to an increase in mucin aggregation. Further we demonstrate using immunodot blot, that chemotherapy treatment changes the secretion of proteins involved in chemotherapeutic resistance, which is specific to mucinous tumour cell lines. Altogether, our data show that chemotherapies have multiple effects on mucus.

Abstract: 3

Sensitive and selective molecular tools to study sialidases involved in mucin degradation and microbial pathogenicity

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The cleavage of terminal sialic acids from mucins by neuraminidases (NAs) is a key virulence strategy employed by Gram-negative bacteria, viruses, fungi, and parasites. This process alters mucin barrier properties and exposes underlying receptors for adhesion, further degradation, and ultimately infection. Despite being relevant virulence factors, the study of neuraminidases in complex biological systems remains challenging, mainly due to the lack of selective and sensitive molecular tools. Current tools rely on measuring neuraminidase activity without molecular resolution or further insight into enzyme identity. To address this, we have developed selective molecular probes for neuraminidase detection, tagging, inhibition, and isolation. Our probes enable real-time visualization and trapping of active enzymes in virus preparations, bacterial pellets, and lysates, with the added advantage of inhibiting neuraminidase activity 10-100 times more potently than the commercially available inhibitor DANA. Using these tools, we have characterized neuraminidases from *Hoylella* (formerly *Prevotella*) *timonensis*, *Mannheimia haemolytica*, *Streptococcus pneumoniae*, and influenza A virus. Our approach is straightforward, robust, and adaptable across diverse microbial and host systems. Thus, this strategy can be extended to mammalian cells and applied to study other bacterial neuraminidases, offering a versatile platform to dissect mucin degradation, detect pathogen-associated NAs, and identify specific neuraminidases as potential therapeutic targets.

Abstract: 4

Avoiding Sticky Handshakes: Towards a Mechanistic Model for Regulation of MUC2 Polymerization

Presenting author: Mor Bendek

Presenting author institute: Weizmann Institute of Science, IL

Co-authors: Mor Bendek¹, Deborah Fass¹ ¹Weizmann Institute of Science, IL

The proper function of secreted mucins relies on the formation of head-to-head-tail-to-tail disulfide-bonded polymers. Formation of functional polymers of these large and complex glycoproteins, while avoiding futile bonding patterns, requires a well-regulated stepwise disulfide bonding mechanism, first via the C-terminal cystine knot domain and then via the N-terminal D3 assembly.^[DF1] Although the structures of C-terminal and N-terminal complexes of mucins have been determined and analyzed, a coherent and corroborated mechanism to explain how these complexes form in an orderly and efficient manner remains to be developed. This knowledge gap is intriguing, not only from the biochemical perspective of protein polymerization but also for the critical role these mechanisms play in maintaining healthy mucus barriers.

Here, we studied non-covalent domain-domain interactions in the intestinal mucus MUC2 to shed light on the mechanisms that regulate mucin polymer assembly. First, we aimed to examine the role of the homotypic interaction between the three N-terminal assemblies, originally discovered in MUC2 filaments, in preventing dimerization of the two N-terminal heads of the same C-terminal dimer (closed dimers). Using biochemical and structural techniques, we found that this interaction alone is insufficient to explain N-terminal dimerization regulation. To complete the model, we turned to examine the role of intrinsic elements of the D3-D3 interaction. Considering a recently proposed model of a conformational switch driven by disulfide rearrangement shared by mucins and von Willebrand factor, we studied the effect of different D3 disulfide configurations on non-covalent D3-D3 interactions. We experimentally demonstrated

Abstract: 5

Diurnal dynamics in Muc2 glycosylation

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Mucin glycans vary in monosaccharide composition and linkage, which modulates the biological and biophysical properties of the mucus gel. The host epithelium and microbiome both exhibit robust temporal dynamics with respect to time of day, yet temporal regulation of mucin glycosylation remains unexplored. We have used glycomics techniques to examine temporal patterns of mucin glycosylation in vivo, and found diurnal rhythms in mucin glycosylation. To our knowledge, this is the first demonstration of protein glycan sequences exhibiting a biological rhythm. We then explored the regulation of these diurnal patterns, and the role of the mucus gel as a dynamic medium through which bi-directional interactions occur between the host and the microbiota.

Using germ-free models and antibiotic experiments we have explored how the microbiome shapes host-glycome rhythms. We have also assessed how diurnal variation within the colonic glycome influences susceptibility of mice to pathogens encountered at different times of day. Additionally, genetically manipulated mouse models with altered protein glycosylation have allowed us to determine the role of the mucus-glycome in shaping the composition of the colonic microbiome.

This project evidences that the mucus gel is a diurnal substrate, with mucin glycans in particular exhibiting strong rhythms at the sequence level. We show these dynamics have tangible effects on host-microbiome interactions and pathogen susceptibility. Our work is linking the largely distinct fields of mucus biology and chronobiology, employing novel glycomics techniques to elucidate the fascinating and crucial mechanisms by which host and microbiota coordinate dynamic processes.

Abstract: 6

Using mucus biomimetics to understand mucus-microbiota interaction in the colon

Presenting author: Serena Berberolli

Presenting author institute: University of Leeds, GB

Co-authors: Serena Berberolli¹, Emily Dewhurst¹, Celia Ferreira¹, Daniel Read¹, Mar Collado-González ², Jin Seo³, Francisco Goycoolea ^{1,2}, Anthony Buckley¹ ¹University of Leeds, GB, ²University of Murcia, ES, ³Reckitt, US

The colonic mucus layer plays a critical role in shaping microbial spatial organisation, behaviour, and functionality. However, current in-vitro models fail to accurately reproduce the mucus layer as a mucin network hydrogel, fundamentally limiting our understanding of microbial dynamics in a physiologically representative context. To overcome this limitation, two mucus mimetics were synthesised by direct crosslink of pig gastric mucin with genipin (Collado-González et al., 2025) and 4-arm-PEG-SH (Joyner et al., 2019). This mucus biomimetics were coated onto sampling support structures and compared with traditional mucin/agar or uncoated controls. Using a previously described triple-staged colonic microbiome model (MiGut)(Davis Birch et al., 2023), spatial-temporal analysis was conducted on the biofilms formed in the proximal and distal vessel up to 14 days after inoculation. Alpha diversity was consistently low across all coatings, indicative of high species specificity for bacterial adhesion. Beta diversity revealed biofilm maturation over time in genipin-based structures, while PEG-based mimetic exhibited greater compositional divergence between coatings, particularly between mucin/agar and mucus coating. qPCR confirmed significant enrichment of mucosa associated species known to colonise the colonic mucosa in-vivo, including Akkermansia, Bacteroides, and Bifidobacterium. SEM imaging revealed biofilm structural stability overtime, highlighting the capacity of mucus mimetics to promote bacterial adhesion and colonisation. These findings establish crosslinked mucin hydrogels as promising platforms for next-generation in-vitro gut models for the investigation of mucus-microbiome interaction in health and disease.

Abstract: 7

Disruption of the mucus barrier in a preclinical mouse model of intestinal radiotherapy

Presenting author: Isobelle L Blair

Presenting author institute: Lydia Becker Institute of Immunology and Inflammation, GB; Manchester Cell-Matrix Centre, Faculty of Biology, Medicine and Health, University of Manchester, GB

Co-authors: Isobelle L Blair^{1,2}, David J Thornton^{1,2}, Mark A Travis^{1,2} ¹Lydia Becker Institute of Immunology and Inflammation, GB, ²Manchester Cell-Matrix Centre, Faculty of Biology, Medicine and Health, University of Manchester, GB

Radiotherapy (RT) is widely used to treat abdominal and pelvic cancers, but 90% of patients develop acute intestinal toxicity, representing a major unmet clinical need. Increasing evidence suggests that mucus barrier disruption contributes to RT-induced injury, with the microbiome implicated as a key driver. The intestinal mucus barrier is formed by heavily glycosylated Muc2, where glycan structures regulate mucus stability and microbial interactions. However, whether RT alters Muc2 structure or mucus-microbiome interactions remains unclear.

C57BL/6 mice received 13Gy CT-targeted intestinal RT using a small animal radiation research platform (SARRP). Mucus was collected by sequential extraction with PBS followed by 2M urea and analysed by mass spectrometry, immunoblotting and lectin blotting. To deplete the microbiome, mice were treated orally with broad-spectrum antibiotics.

At 7 days post-RT, during peak inflammation and weight loss, PBS-soluble colonic mucus showed increased abundance of Muc2 and other mucus-associated proteins compared to sham, suggesting increased mucus solubility. Notably, RT induced significant loss of Muc2 glycosylation in PBS-soluble colonic mucus. This loss of glycosylation was not apparent following antibiotic treatment, demonstrating that RT-induced Muc2 glycan loss is microbiome-dependent.

Although Muc2 abundance increased following intestinal RT, its glycosylation was significantly reduced in PBS-soluble mucus. Our data indicates that the microbiome is required for RT-induced glycan degradation. Future studies will determine whether microbial glycosidase activity or host inflammatory responses drive glycan loss, and whether this can be targeted to mitigate RT-induced intestinal toxicity.

Abstract: 8

Secreted Mucins Regulate Neutrophil Extracellular Trap Formation in the Airways

Presenting author: Allison Boboltz

Presenting author institute: University of Maryland, US

Co-authors: Allison Boboltz¹, Vaidehi Rath¹, Gregg Duncan¹ ¹University of Maryland, US

Neutrophil extracellular traps (NETs) are found in the sputum samples of individuals with chronic lung diseases and acute infections. Uncontrolled NETosis in respiratory disease states causes significant damage to host lung tissue and impairs mucociliary clearance. Previous studies have shown that mucins in human saliva induce NETosis via interactions with mucin-associated glycans to maintain oral homeostasis. However, mucin glycosylation in the airways often differs from the oral cavity. The airway mucus barrier is primarily comprised of two secreted mucin subtypes, MUC5B and MUC5AC. Therefore, we investigated the effects of human airway mucus and mucin subtypes on NETosis. We isolated mucus from air-liquid interface cultures of wild type (WT) human airway epithelial (HAE) cells and from HAE cells engineered using CRISPR/Cas9 to knock out (KO) expression of either MUC5B or MUC5AC. We exposed neutrophils to mucus from each HAE culture, finding that WT airway mucus strongly inhibits NETosis, while treatment with MUC5B KO or MUC5AC KO mucus resulted in increased NETosis. These findings were confirmed using a co-culture model in which NETosis was assessed directly on the mucosal surface of WT, MUC5B KO, or MUC5AC KO HAE cultures. In addition, we found that glycosylation plays a critical role in modulating NETosis, as enzymatic removal of sialic acid reduced the inhibitory effects of mucus on NET release. Together these data demonstrate a novel role of airway mucus in modulating innate lung immunity and show that mucins affect NETosis oppositely in the airways compared to the oral cavity. Ongoing studies are exploring how disease-associated alterations to mucus, particularly increased viscoelasticity, upregulate NETosis.

Abstract: 9

Defining a new role for Charcot-Leyden crystals in Type 2 immunity and airway obstruction

Presenting author: Jasmijn Burgelman

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Co-authors: Jasmijn Burgelman^{1,2}, Helena Aegerter^{1,2}, Bart Lambrecht^{1,2} ¹VIB Center for inflammation Research, Ghent, BE, ²Department of Internal Medicine and Pediatrics, Ghent University, BE

Protein crystallization is an important feature of Type 2 inflammation, as evidenced by the convergent evolution of Galectin-10 (Gal10) crystals in humans, and Ym1/2 crystals in mice. Despite the prevalence of crystals in pathogenic mucus in human diseases such as asthma, chronic rhinosinusitis and allergic bronchopulmonary aspergillosis (ABPA), their biological importance has not been explored, due to a substantial lack of tools. We have undertaken several novel approaches to investigate Gal10 (normally absent in mice) crystals in an in vivo and in vitro setting. Bone marrow from IL13 transgenic mice was transferred to mice that endogenously express Gal10 specifically in the lungs showing increased inflammation during house dust mite treatment as well as a striking phenotype of mucus production and airway remodeling; phenotypes which resemble clinical symptoms of severe asthmatics. Recombinant Gal10 crystals were administered to ENaC overexpressing mice (model of spontaneous mucus plugging) showing increased inflammation. In these novel models, we also demonstrate extensive interactions of neutrophils with Gal10 crystals of different sizes leading to neutrophil extracellular trap (NET) formation which contributes to persistent inflammation. This new understanding provides a unique platform to investigate Gal10 crystals as a therapeutic target for an impenetrable aspect of airway disease.

Abstract: 10

B3GNT7 is a Mucus-Modifying Enzyme in the Intestine that Protects Against Colitis

Presenting author: Mary Burns

Presenting author institute: UT Southwestern Medical Center, US

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β 1-3-N-acetylglucosaminyltransferase 7 (B3GNT7) is a Golgi-resident glycosyltransferase that adds GlcNAc to Gal acceptors and thereby participates in polyLacNAc biosynthesis. These polyLacNAc chains can be further modified by fucose, sialic acid, and sulfate. B3GNT7 is highly expressed in the colon and regulated by IL-22, a cytokine that maintains intestinal epithelia barrier integrity. We find that B3GNT7 is necessary for IL-22-regulated polyLacNAcylation in a human goblet cell model and glycosylates components of secreted mucus, including MUC2. To further understand the function of B3GNT7 in intestinal physiology, we generated an intestinal-epithelial specific B3gnt7^{-/-} mouse that shows a defect in secreted mucus, susceptibility to DSS-induced colitis, and susceptibility to *C. rodentium* infection. B3gnt7^{-/-} mice show changes in Muc2 and Lumican, potentially explaining their susceptibility to DSS and infection. These data position B3GNT7 as an important modulator of intestinal mucus glycosylation, and we are currently investigating the biochemical basis of B3GNT7 acceptor substrate specificity, which may explain its non-redundant function in the colon. Future directions include glycomic and glycoproteomic analysis of colonic mucus from B3gnt7^{-/-} mice, immune characterization, and investigating how Lumican glycosylation impacts its function.

Abstract: 11

Bioinspired peptides from respiratory mucins as next generation mucolytic therapy for muco-obstructive lung diseases

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Mucus-obstructive lung diseases, including cystic fibrosis (CF), bronchial asthma, and COPD, feature overproduction of mucins with abnormal amounts of disulfide crosslinks that accumulate in the airways and impair clearance. Current mucolytics (e.g., N-acetylcysteine, NAC) provide limited clinical benefit, creating the need for more potent yet safe drugs. Under these constraints, peptides that can tune disulfide exchange under physiological conditions represent an attractive strategy for developing novel mucolytics.

Inspired by the cysteine-rich respiratory mucins MUC5B and MUC5AC, we mined cysteine-containing motifs to design a panel of peptides. Candidates were screened for solubility, solution stability at different temperatures, thiol reactivity, and cytotoxicity in human bronchial epithelial (HBE) cells up to 5 mM. We found a sequence-dependent toxicity of the compounds, with several lead candidates showing no toxicity up to the maximum tested concentration. Mucolytic efficacy of these lead peptides was quantified ex vivo in airway mucus from CF and asthma patients.

Lead cysteine-peptides showed high reactivity at airway pH 6.8-7.0, remained stable (

Abstract: 12

Uncovering tumour-associated mucin O-glycans recognition and degradation by a mucinase from *Bacteroides caccae*

Presenting author: Alcía Candeias

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Altered mucin O-glycosylation is a hallmark of cancer, yielding tumour-associated glycodomains that modulate host–microbiome interactions in the gastrointestinal tract[1]. These aberrant glycoepitopes, including truncated structures, such as Tn and T antigens, provide key contact points for glycan-recognition systems expressed by gut bacteria, which may promote dysbiosis contributing to early colorectal cancer (CRC) progression[2]. Growth of *B. caccae*, a CRC-enriched bacterium, on mucin O-glycans is associated with increased expression of mucin-degrading enzymes, which includes M60-like peptidases[3]. In this work, we aim to understand the molecular mechanisms underlying mucin recognition and peptide cleavage by a putative mucinase from *B.caccae* (BC16100). To this end, we first addressed the major challenge of preparing well-defined mucin glycosylated domains, obtained chemoenzymatically, that reproduce tumour-associated glycoepitopes. We then employed a multidisciplinary approach combining mass spectrometry, glycopeptide microarrays, affinity measurements, and structural techniques (NMR spectroscopy and X-ray crystallography) to gain mechanistic insights into substrate recognition and cleavage specificity. Together, these studies provide the first molecular-level understanding of how *B. caccae* recognizes tumour-associated antigens and utilizes mucin substrates.

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Abstract: 13

Free Cysteine precipitates Muc5b and reveals potential mucin binding proteins

Presenting author: Guy Carpenter

Presenting author institute: King's College, London, GB

Co-authors: Guy Carpenter¹ King's College, London, GB

Mucins form 10 % of all salivary proteins, are the largest (100-1000 kDa) most highly glycosylated proteins and consequently extremely difficult to purify. As human mucins are not commercially available and the in vitro methods of bacterial/ mammalian cell synthesis are difficult, purification of mucins from saliva is essential. In the current study different amino acids were added to whole mouth saliva containing mucins and other proteins such as amylase. Only cysteine caused the precipitation of mucins from saliva as evidenced by their loss from the supernatants after separation by SDS-PAGE and staining by Coomassie Brilliant Blue and Periodic Acid Schiff's stain. To examine the mechanisms of precipitation other agents were added at the same time as cysteine. Reduction agents such as DTT had no effect but anti-oxidants such as sodium metabisulfite did inhibit the precipitation. Co-incubation of cysteine with other amino acids revealed that arginine, lysine and histidine (all positively charged) inhibited the precipitation, whereas other amino acids had no effect.

When the precipitates were resolubilised and separated by SDS-PAGE other proteins apart from Muc5b and Muc7 were present (based on apparent molecular weight and staining) including amylase, proline-rich proteins and statherin. The presence of these proteins could be inhibited by co-incubation of cysteine with hydrophobic amino acids such as valine or methionine.

In conclusion, the solubility of mucins depends on the cysteine residues in the protein backbone binding with water molecules (presumably through hydrogen bonds). Other proteins (not containing cysteine) appear to bind mucins through hydrophobic interactions.

Abstract: 14

Effects of intermittent fasting on the colonic mucus layer revealed through human to mouse fecal microbiota transplantation.

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Presenting author institute: Departement of Molecular Biology, Umeå University, SE

Co-authors: Héloïse Chat¹, Beate Brandl², Thomas Skurk², Björn Schröder¹ ¹Departement of Molecular Biology, Umeå University, SE, ²Technical University of Munich, DE

Intermittent Fasting (IF) is gaining interest from the population as a strategy to enhance overall health. While several benefits have been reported, prolonged time-restricted eating periods also influence intestinal tissue integrity and gut microbiota dynamics. Most existing studies have focused on bacterial composition, with limited investigation into microbiota function and its interaction with the protective intestinal mucus layer. Using a human-to-mouse microbiota transplantation model, we examined how IF shapes both the structure and function of the mucus barrier. A nutritional intervention was conducted in overweight individuals following a 16:8 intermittent fasting protocol for 12 weeks. Stool samples from the best metabolic responders were transplanted into microbiota-depleted WT C57BL/6J mice. Using a unique ex vivo explant approach, we tested the capacity of the human microbiota to affect mucus function. We assessed mucus growth rate and penetrability in colonic tissue from mice colonized with human microbiota obtained from the participants before and after the fasting intervention. To further characterize the impact of IF-associated microbiota on mucus barrier function, additional analyses will include mucus-related gene expression, short-chain fatty acid production, and microbial carbohydrate-metabolizing capacity. Together, these findings will provide new insights into how IF-driven microbial changes influence the intestinal mucus barrier and may help refine nutritional recommendations surrounding this increasingly popular dietary practice.

Abstract: 15

Evolutionary Dynamics of Mammalian Mucins

Presenting author: Ryan Chen

Presenting author institute: Yale University, US

Co-authors: Ryan Chen¹, Petar Pajic¹, Parker Verran¹, Stacy Malaker¹ ¹Yale University, US

Mucins serve a multitude of biological roles across the tree of life, including barrier formation, lubrication, cell signaling, and microbial interaction. Despite their importance, the evolution of mucins across mammalian species is understudied, largely due to the limitations of short-read sequencing methods in resolving mucin loci. Long-read sequencing technologies are now capable of resolving these regions, enabling downstream evolutionary investigation across species. Using a combination of gene synteny, sequence homology, and annotation-based searches, we documented the presence and absence of 14 canonical mucins across 83 long-read sequenced mammalian genome assemblies. We reaffirmed previous findings that gel-forming mucins are conserved across species. Interesting in resolving transmembrane mucins, we found that MUC22 evolved distinctly in the primate lineage. At a finer scale, we further characterized exonic variable number tandem repeats (exVNTRs) within MUC1, 7, 20, 21, 22, finding instances of repeat expansion and contraction that can directly influence protein length and glycosylation potential. Repeat copy number analysis found that some mucins were more variable across species relative to others. Specifically, we observed that MUC1 and MUC7 exVNTRs differ drastically in copy number between species, in contrast to MUC15, which lacks repeats entirely. Beyond the canonical family, we screened for novel mucin-like proteins and found hotspots of recurrent lineage-specific mucin evolution. Future work will aim to uncover the mechanisms underlying these lineage-specific events and to investigate how these gene products relate to distinct physiological and ecological adaptations.

From Porcine Gastric Mucin to a Mucus-Mimicking Biomaterial (MucinGel®)

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Commercially available purified pig gastric mucin (PGM) type III does not fully reproduce the viscoelastic properties of native mucus, limiting its use in robust in vitro mucosal models. Here, we developed a biomimetic mucus gel by covalently crosslinking commercial PGM type III with genipin and characterized its spectroscopic, physicochemical, rheological and biological properties [1]. Crosslinking was confirmed by UV/Vis, dialysis and FTIR, while dynamic light scattering showed increasing particle size over time. Oscillatory rheology demonstrated viscoelastic gel formation at genipin:mucin ratios higher than 0.08; at G/M 0.11, the material reached G' and G'' values of approximately 10 and 1 Pa and displayed plastic/thixotropic behaviour. SEM showed that the gel could be deposited as homogeneous micron-scale layers onto porous membranes without blocking pores. In vitro, genipin-crosslinked PGM remained cytocompatible for up to 11 weeks. These findings support genipin-crosslinked PGM type III as a tuneable biomimetic mucus material for microbiology, permeability and mucosal interface studies, and enabled downstream technology transfer and commercialization through IDYLLE as MucinGel®.

References

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Abstract: 17

Synthesis & characterization of mucin O-glycans that attenuate virulence in diverse pathogens

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Antibiotic-resistant infections are responsible for an estimated 1.3 million deaths annually, a figure projected to rise to 10 million by 2050. Antibiotic resistance leads to treatment failure, prolonged hospitalization, and substantial economic burden on healthcare systems and society. Innovative strategies for combating infections are therefore urgently required.

Several recent reports have indicated that mucin O-glycans can reduce pathogen load in infections caused by diverse cross-kingdom species (fungal, Gram-positive & Gram-negative bacterial). Rather than directly killing microbes, these glycans reduce the expression of virulence-associated genes and thereby enhance pathogen susceptibility to host immune defenses, indirectly reducing infection load. By exerting activity through virulence attenuation and not broad-spectrum elimination, these compounds are anticipated to exert lower selective pressure for developing resistance.

Despite their broad activity, the molecular mechanisms underlying mucin glycan-mediated virulence attenuation remain poorly understood. Mucins display several hundred distinct O-glycan structures, making it challenging to identify the specific glycans and associated pathways responsible for these effects. To address this, we have developed an in-house platform to generate a library of defined mucin O-glycan structures in quantities sufficient for functional studies (>30 mg). Using this library, we are identifying specific glycan structures that modulate pathogen virulence and investigating their underlying mechanisms of action. This work provides insights into how mucin glycans regulate virulence and supports their further development as novel anti-virulence therapeutics.

Abstract: 18

Establishment and Application of an Inverse Transwell Assay to Assess Mucus Barrier Function Towards Respiratory Virus Infection

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Secreted airway mucus – a front-line defense system against respiratory virus infection – is largely composed of glycosylated mucins that promote virus trapping via steric and adhesive interactions. Still, viruses differ in their size and glycan affinities, and the degree to which mucus can trap specific viruses is unclear. Further, mucin expression and glycosylation are altered in chronic lung disease, with undefined impacts on virus susceptibility. Thus, we devised an inverse Transwell assay (ITA) to probe mucus barrier function towards infection without the confounding effects of ongoing mucus secretion and transport on viral dynamics. The ITA includes BCI-NS1.1 cells, cultured on the basolateral Transwell surface, and a mucus sample in the apical chamber. The cells act as detectors for viral penetrance through the mucus, with quantification of infected cells as a readout. Using the ITA, we assessed the barrier function of human airway culture (HAC)-derived mucus towards influenza (IAV), rhinovirus (RV), adenovirus, and parainfluenza virus. Data showed differences in mucus penetration efficiency between viruses; IAV being the most inhibited relative to no mucus. Notably, IAV and RV penetration through normal mucus was similar to their penetration of mucus sourced from asthmatic HACs. To define the contribution of specific mucins to barrier function, we performed an ITA with mucus harvested from MUC5B- or MUC5AC-knockout HACs. While RV penetration was similar across conditions, IAV was more efficient in breaching MUC5AC-deficient gels. In sum, our data establish the ITA as a tunable platform enabling investigation into mucus penetration by diverse viruses and the effects of altered mucus composition on barrier function.

Abstract: 19

Targeting Cancer-Associated Mucin O-Glycans: From Microbiome Recognition to Engineered GlyCanTargeters

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The heavily O-glycosylated mucins serve as a crucial interface between the host and microbiota. Bacteria of the human microbiome evolved to utilise mucin O-glycans, yet the molecular basis of these interactions and their role in disease remain poorly defined [1,2]. In cancer, mucin O-glycosylation is altered, with truncated glycans marking progression [3]. In this work, we identified glycan recognition by mucin-targeting proteins from gut commensals, including *Bacteroides thetaiotaomicron* and *Bacteroides caccae*. Using bioinformatics, X-ray crystallography, and glycan microarrays, we defined the molecular determinants of glycan recognition and specificity towards defined mucin O-glycans, including truncated and cancer-associated O-glycan epitopes. Building on this, we are currently developing “GlyCanTargeters”: engineered carbohydrate-binding modules (CBMs) with enhanced specificity for mucin O-glycans and enhanced affinity and selectivity for cancer-associated epitopes. Preliminary data in adhesion assays between CBMs and cancer cell models support their application as molecular probes. This work connects microbiome glycan recognition with mucin alterations in cancer and underscores applications in cancer diagnostics.

Abstract: 20

Defining the Cellular and Molecular Basis of Cervical Mucus Plug Formation, Regulation, and Barrier Properties.

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The cervical mucus plug (CMP) is a dynamic structure occupying the cervix during pregnancy. With a dense network of gel-forming mucins (Muc5AC/Muc5B) and antimicrobial peptides, the CMP serves as a physical barrier ensuring uterine sterility during fetal development. CMP dysfunction is linked to preterm birth, which accounts for an annual infant mortality of over 600,000 globally.

Gel-forming mucins and their source - goblet cells (GC) are well described in the pulmonary and gastrointestinal systems but remain understudied in the female reproductive tract (FRT). It is therefore essential to define cellular and molecular mechanisms in the cervical mucosa that governs CMP establishment, maintenance and function.

Histological analysis of pregnant mice indicates the presence of two GC subpopulations with distinct mucin expression patterns. On characterizing CMP in knockout mice, Muc5AC^{-/-} presents altered CMP properties with loosened mucus. Muc5B^{-/-} fails to develop mucus altogether, proving that both Muc5AC and Muc5B are critical for optimal barrier function. Proteomic analysis of CMP indicates high cross-linking between Muc5AC and Muc5B, alongside abundance of mucus-processing associated proteins, such as Fcgbp, Clca1 and Tgm2. This trend mirrors COPD-associated mucus plugging, suggesting that pathways leading to pathological mucus accumulation in lungs potentially drives proper CMP function.

Impaired CMP increases susceptibility to ascending uterine infections that can cause preterm birth. Hence, this work adds to our understanding of how it facilitates favorable obstetric outcomes, building the foundation for future prevention of preterm birth.

Dissecting host-glycan degradation in urbanized and traditional gut microbiomes

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Urban lifestyle reshapes gut microbiota composition and function, including interactions with the host mucus layer. To investigate lifestyle effect on host-glycan foraging, we compared shotgun metagenomes from a traditional Yanomami (n=78) and an urbanized Belgian cohort (n=39). After quality control and assembly, sequences were taxonomically and functionally annotated using KEGG and dbCAN. We then applied a stepwise comparison for host-glycan breakdown genes (terminal uncapping and backbone cleavage), and assimilation of released mucin-related sugars (fucose, galactose, GalNAc, GlcNAc, and sialic acid). The urbanized microbiome exhibited higher potential for key steps of this cascade, including backbone cleavage and desulfation. It also showed increased capacity to assimilate mucin-derived sugars, particularly GalNAc and sialic acid. KEGG pathway analyses reinforced this pattern, revealing higher abundance of galactose metabolism and glycosaminoglycan degradation within this cohort. Taxonomic contributors also differed between lifestyles. These functions were mainly associated with recognized mucin-degrading bacteria, including *Bacteroides*, *Phocaeicola*, and *Blautia* in urban cohort. *Prevotella* is the main contributor in Yanomami; however, this genus is primarily associated with plant fiber breakdown, and several detected CAZyme families have broad substrate specificity. Together, our results suggest that urbanization shifts host-glycan degradation from fiber-adapted taxa toward a more mucin-oriented microbiome with greater downstream capacity to exploit host-derived sugars. In the future, these findings might contribute to unveiling the health effects of mucin-microbiome interactions.

Abstract: 22

Microbiota composition differentially regulates intercrypt goblet cells and mucus glycosylation in the proximal colon

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Intercrypt goblet cells (icGCs) are a specialized goblet cell population located at the luminal surface between colonic crypt openings that produce mucus with distinct properties from those of crypt-resident goblet cells. We previously demonstrated that microbiota depletion increases icGC abundance in the proximal colon through microbiota-derived metabolites. However, whether specific shifts in microbiota composition differentially regulate these cells remains unclear, particularly in the proximal colon where mucus biology remains poorly characterized.

Here, we investigated how distinct microbiota perturbations influence icGCs and the proximal colonic mucus layer. While broad-spectrum antibiotic treatment increased icGC abundance, selective depletion of Gram-positive bacteria using vancomycin led to a marked reduction of icGCs. Loss of icGCs was associated with thinning of the proximal mucus layer and altered mucus glycosylation, characterized by reduced fucosylation. Mechanistically, the vancomycin-induced loss of icGCs required epithelial MyD88 signalling, as demonstrated using epithelial-specific MyD88-deficient mice, indicating that LPS-mediated microbial sensing contributes to icGC regulation.

Consistent with a role for Proteobacteria-derived signals, both *Escherichia coli* mono-colonization of germ-free mice and *Salmonella* infection in SPF mice reduced icGC abundance and altered mucus architecture.

Together, our results identify icGCs as microbiota-responsive regulators of the proximal colon mucus barrier and reveal that distinct microbial configurations can differentially remodel this epithelial niche.

Abstract: 23

Deciphering Muc4 molecular mechanisms in the early onset of PanIN carcinogenetic sequence

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Pancreatic cancer is the 4th cause of death by cancer in the world with a median survival lower than 6 months and an extremely low 5-year survival rate (10%). To study the role of the membrane bound mucin Muc4 in pancreatic carcinogenesis *in vivo*, the Muc4KO mice were crossed with the Pdx1-Cre; LstopL-KrasG12D (KC) mouse model that recapitulate neoplastic progression sequence observed in PDAC patients. We aimed at investigating the molecular mechanisms associated with Muc4 in the early onset of PanIN carcinogenetic sequence.

We confirmed the Muc4 neoexpression in all the PanIN lesions formed in KC (Muc4WT) mice. In KCM4 (Muc4KO) mice, the PanIN lesions were lacking Muc4 expression as expected. We observed increases of the pancreatic mass in KC mice at 3, 6, 9 and 12 months whereas the pancreas weight at 3 months was similar to corresponding controls. KCM4 pancreatic weights were also significantly reduced compared to KC controls. Morphometric analysis on HE stained section showed that KCM4 pancreata displayed a 64% reduction of neoplasia area compared to KC controls.

We performed a spatially resolved proteomics analysis in 3 months old KC and KCM4 pancreata. We selected areas corresponding to normal pancreas and PanIN lesions from IHC slide of FFPE tissue. We observed the detection of 4 proteins (Rab15, Ca3, Hp and Igihg1) in KCM4 normal and PanIN tissues. 12 proteins were also differentially detected in KC and KCM4 PanIN lesions. Eif3i, Eif1 and Eif3c translation factors were notably significantly increased in KCM4 PanINs. Gene ontology analysis showed an enrichment of the KEGG term Protein processing in endoplasmic reticulum and molecular functions terms such as Translation initiation factor activity.

Abstract: 24

Small intestinal mucus barrier dysfunction as a potential driver of dysbiosis influencing colorectal tumorigenesis

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Colorectal cancer (CRC) accounts for ~10% of global cancer cases and is a leading cause of cancer mortality. Epidemiological studies link CRC to environmental factors shaping the intestinal microbiota, including Western-style diets (WSD; high fat, low fiber) and antibiotic exposure, both of which disrupt microbial communities and host–microbe interactions.

The intestinal mucus barrier is a key interface between microbiota and epithelium, regulating microbial access while supporting commensals. Barrier dysfunction is linked to inflammatory bowel disease and can be induced by WSD, but its direct role in colorectal tumorigenesis remains unclear.

While most studies focus on the colon, upstream regions may influence microbial populations in distal sites. The small intestine has emerged as an important site for mucus–microbiome interactions shaping microbial composition. We hypothesize that small intestinal barrier dysfunction alters microbial ecology and contributes to dysbiosis associated with CRC.

To address this, we utilize the $Muc2^{+/-} \times Apc^{Min}$ mouse model, combining impaired mucus barrier function with genetic susceptibility to intestinal tumor formation. Gastrointestinal sampling is performed across experimental conditions including baseline ($Muc2^{+/-}$ vs wild-type), Western-style diet exposure, and broad-spectrum antibiotic treatment. Formalin-fixed Swiss-roll preparations of the small intestine, along with colonic tissues, enable histological assessment of epithelial organization and barrier integrity along the intestinal axis. Preliminary findings suggest small intestinal barrier changes may promote downstream dysbiosis, supporting upstream dysfunction in colorectal tumorigenesis.

Abstract: 25

Elucidating a potential key role of CLCA1 in mucus-mediated defense against *Helicobacter pylori* infection

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The bacterium *Helicobacter pylori* (*H. pylori*) is the main risk-factor for gastric cancer. *H. pylori* easily develops antibiotic resistance. The first layer of defense is the gastric mucus which is primarily made by the MUC5AC-producing surface mucus cells (SMCs). A deeper understanding of *H. pylori* pathogenesis could enable improved treatment and prevention.

H. pylori infection inhibits mucin production. The histamine receptor 3 agonist (R)-alpha-methylhistamine (RaMH) restores mucin production and protects against *H. pylori* infection in mice, but the mechanism is unclear. We have used laser microdissection to analyze the gene expression of SMCs in mice with and without *H. pylori* infection, as well as infected mice with and without RaMH treatment. Differentially expressed genes in common between these two comparisons are potentially more likely to be relevant to mucin-based defence against *H. pylori* than other differentially expressed genes that only show up in either gene expression analysis. There were four significantly down-regulated genes in common between the comparisons infected vs non-infected and vehicle-treated vs RaMH-treated mice. One of these, *Clca1*, has previously been shown to be a key contributor to mucus expansion in the colon.

A pilot *Clca1* immunofluorescence staining indicated no difference between infected and non-infected SMCs, but suggested a trend toward a difference between RaMH and Vehicle-treated mice. To validate this finding, the staining is being repeated in a larger sample set. We are also implementing an organoid-on-a-chip model called transgels, derived from *Clca1*^{-/-} and wt mice, to further investigate the role of *Clca1* in mucus-mediated defense against *H. pylori* infection.

Toward a Systems-Level View of the Salivary Mucinome

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Secreted mucins are primary contributors to the rheological and tribological properties of saliva and play key roles in oral host-microbe interactions through their dense, heterogeneous O-glycosylated domains. However, the mechanisms by which salivary mucins bind to microbes and other salivary proteins remain incompletely understood. This is because previous analyses of salivary mucins lacked tools for O-glycoproteomic mapping of mucin domains, preventing an understanding of glycoprotein structure at the molecular scale. This warranted a thorough characterization of the site-specific O-glycosylation of salivary mucins. Here, with our method GlycoFASP, we prepared and analyzed human whole saliva (WS) samples to categorize the human salivary mucinome. In doing so, we obtained unprecedented sequence coverage of the salivary mucins MUC7 and MUC5B and enabled site-specific mapping of their mucin domains. Furthermore, we used our optimized GlycoFASP protocol to map the mucinome of submandibular-sublingual (SM-SL) saliva, allowing us to determine the O-glycosylation patterns of MUC7 and MUC5B from different sources. Previous studies have observed multiple MUC7 glycoforms in saliva, possibly due to differential glycosyltransferase expression between the SM and SL glands. To explore this, we plan to separate the MUC7 glycoforms from WS by weak anion exchange for downstream glycoproteomic analysis. Through this endeavor, we hope to provide insight into how gland-specific biosynthesis contributes to mucin structural heterogeneity. Overall, our findings have major implications for understanding mucin structure at the molecular level and are essential for parsing the seemingly pleiotropic role of salivary mucins in the oral cavity.

Abstract: 27

Mucosal N-glycosylation as a regulator of the intestinal milieu: impact in the health-to-disease transition in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a chronic debilitating disorder from the gastrointestinal tract comprising Crohn's disease (CD) and ulcerative colitis (UC). Glycans are essential players in IBD immunopathogenesis, even years before disease onset. However, how changes in mucosal glycosylation shape the gut microbiome and impact gut immune responses and inflammation remains unclear. We have shown that alterations in the expression of complex branched N-glycans at gut mucosa surface, modelled in glycoengineered mice, leads to intestinal dysbiosis. This is concomitant with a downregulation of type 3 innate lymphoid cells (ILC3)-mediated immune response, leading to the transition of ILC3 toward an ILC1 proinflammatory phenotype. Indeed, immunofluorescence analysis suggest that mice deficient for branched N-glycans in the gut mucosa seem to display altered expression of AGR2 in goblet cells, which is paramount for the folding and maturation of mucins. Additionally, we demonstrated that the mucosa glycosylation remodelling through prophylactic supplementation with glycans at steady state was able to restore microbial-derived short-chain fatty acids and microbial sensing alongside the rescue of the expression of ILC3 module, suppressing intestinal inflammation and controlling disease onset. Overall, these results unlock the effects of alterations in mucosa glycome composition in the regulation of the intestinal environment, providing new insights on the mechanisms underlying health to intestinal inflammation transition.

Abstract: 28

LC3-independent autophagy as a novel anti-inflammatory mechanism to prevent intestinal inflammation

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The intestinal epithelium maintains a physical barrier formed by the tight contacts between intestinal epithelial cells (IECs), which prevents bacterial infiltration. Preserving this epithelial barrier is crucial for preventing intestinal inflammation, and defects in barrier integrity are commonly observed in inflammatory bowel disease (IBD). IECs are highly sensitive to TNF-mediated cell death, which can trigger tissue damage and disruption of the intestinal barrier under conditions of infection or damage. We recently identified an LC3-independent, ATG9A-mediated form of autophagy as an essential mechanism that protects against TNF-induced cytotoxicity. In this study, we aim to determine the role of LC3-independent autophagy in intestinal homeostasis and inflammation. To this end, we generated IEC-specific Atg9a knockout (Atg9a^{FL/FL} Villin-Cre, Atg9a^{IEC-KO}) mice, which developed normally but were significantly smaller than wild-type controls. Atg9a^{IEC-KO} mice exhibited a notable reduction in the numbers of goblet cells and Paneth cells. In addition, increased immune cell infiltration was observed in the small intestine of Atg9a^{IEC-KO} mice, indicative of inflammation.

A significant number of IECs stained positive for cleaved caspase-3, suggesting epithelial cell death driven by excessive TNF signaling in the small intestine. Consistent with this, Atg9a^{IEC-KO} mice were hypersensitive to TNF, which, upon injection, triggered massive epithelial apoptosis and rapid breakdown of the intestinal barrier, resulting in lethality. Furthermore, RNA-seq data from a cohort of IBD patients revealed reduced ATG9A expression, supporting a role for LC3-independent autophagy in maintaining epithelial homeostasis in the human intestine.

Abstract: 29

Decoding Intestinal Mucins: Single-Cell Insights into Epithelial Dynamics across Inflammatory Bowel Diseases

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Mucins form the structural basis of the intestinal mucus barrier and regulate epithelial barrier integrity and host-microbiome interactions. However, the distribution of mucin genes across intestinal lineages and their modulation during inflammation and therapy remain incompletely defined. To address this, we generated a single-cell mucin expression atlas of the human intestine by analysing three publicly available single-cell RNA sequencing datasets from healthy individuals and patients with ulcerative colitis (UC) or Crohn's disease (CD). Expression of 19 mucin genes was analysed across harmonised cell annotations.

Mucin mRNA expression was restricted to epithelial cells, and subclustering revealed cell-type-specific mucin signatures. Tuft and enteroendocrine cells showed uniformly low mucin expression, while LGR5⁺ stem cells expressed transmembrane and secreted mucins, indicating that the foundation of the mucin landscape is established at the crypt base. Goblet cells showed high MUC2 expression while also expressing transmembrane mucins, suggesting contributions to both mucus secretion and the glycocalyx. Enterocyte subtypes shared a core mucin repertoire with subtle differences along the crypt-surface axis.

Inflamed UC and CD tissues showed highly similar mucin dysregulation patterns, indicating inflammation-driven rather than disease-specific changes. Non-inflamed UC tissue partially resembled healthy profiles but remained altered. Longitudinal analysis of adalimumab-treated patients identified baseline MUC2 expression as a positive predictor of remission. Together, these findings refine the intestinal mucin atlas and highlight mucins as potential biomarkers for therapeutic response in inflammatory bowel diseases.

Abstract: 30

In vitro models of colon epithelium to investigate host responses to a toxin-producing pathogen

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Clostridioides difficile (Cd) infection is exacerbated by antibiotic-induced dysbiosis and disruption of gut epithelial homeostasis. Dissecting the microbial-interaction effects of this disruption from the resulting changes in epithelial immune status is challenging in vivo. To overcome this limitation, models are required wherein we can define, control, and uncouple contributing factors and assess their individual influence on virulence traits and host responses. For this, we use a bottom-up approach involving in vitro models of colon epithelium colonized with Cd and individual commensal bacteria.

We have established Transwell models of a mucus-producing HT-29-E12 colonocyte monolayer, pre-colonized it for 24h with *Bacteroides thetaiotaomicron* (Bt), then infected with *C. difficile*. We integrate metabolomics and triple RNA-seq analyses of the (co-)colonized models with growth, sporulation, and toxin assays to profile host-pathogen-commensal metabolomes and gene expression during their mutual interaction. These analyses reveal multiple cross-feeding mechanisms between Bt and Cd, as well as dampened host inflammatory responses in co-colonized models.

We have also established Transwell models of colon-organoid-derived epithelium to represent a more human colon epithelium. Three days of air-liquid-interface during cultivation promotes differentiation of goblet cells, which remain abundant through 22 days post-seeding. This model also supports (co-)colonization with Bt and Cd, enabling analyses of host-pathogen-commensal dynamics for at least 24h post-infection.

Our results identify these colonocyte models as a valid approach to dissect microbial interactions in vitro and their resulting impact on bacterial and host responses.

Targeting the MUC4 oncogenic interaction platform improves Met-targeted antibody-drug

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers worldwide, with a 5-year survival rate under 11%, an increasing incidence. Patients are often diagnosed at an advanced stage, at which resection is impossible, the only remaining options are harsh and ineffective chemotherapies with high tumor resistance and systematic relapse. It is thus crucial for this devastating cancer to find new therapeutic alternatives. In this aim, we work on the mucin MUC4, a known actor of pancreatic tumorigenesis and therapy resistance. Previously, we showed that MUC4 EGF domains directly interact with ErbB2/HER2 to activate oncogenic pathways. We discovered a specific peptide ligand that disrupts this interaction and resensitizes PDAC tumors to Trastuzumab and Pertuzumab to inhibit tumor growth. In this work, using 2D cell lines, 3D patient-derived tumoroids and mouse xenograft models, we show that MUC4 is an interaction platform at PDAC cell membrane, interacting with tyrosine kinase receptors (RTKs), to generate oncogenic signaling pathways. After screening several targeted therapies toward these RTKs, we identified a potent combination integrating MUC4 peptide ligand (I3) and an Antibody-Drug-Conjugate (Telisotuzumab-Vedotin) targeting Met. We show that the ADC cannot reach Met when the receptor interacts with MUC4, but when we disrupt MUC4-Met complex with the I3 peptide, the ADC reaches Met, gets internalized, and releases its drug to kill PDAC cells in vitro, ex vivo and in vivo. Altogether, these results place MUC4 as a central interaction platform at PDAC cell membrane, and, more importantly, places our ADC-I3 therapeutic combination as a great hope for future applications in PDAC treatment.

Abstract: 32

Hypersecretory mutant MUC2 mucus enhances virulence and cytotoxicity of *Entamoeba histolytica* in colonic epithelial cells

Presenting author: Hayley Gorman

Presenting author institute: University of Calgary, CA

Co-authors: Hayley Gorman¹, France Moreau¹, Maura Ruyechan¹, Gagan Deep Jhingan², Kris Chadee¹
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The MUC2 mucus bilayer is the first line of innate host defence against colonic pathogens, including the parasite *Entamoeba histolytica* (Eh). Alterations in MUC2 production and glycosylation adversely affects epithelial barrier function and protection against disease. Here, we interrogated if mutant MUC2 can alter Eh virulence and pathogenicity. Two cell lines were used: WT and a hypersecretory Mut LS174T goblet-like clone that produce sialylated and fucosylated mucus. Mucus secretion in WT and Mut cells was quantified by ³H-metabolically labeled mucus secretion in response to Eh. Mucus layer integrity was enumerated by Eh adherence to cells and cytotoxicity measured by LDH release. Mucus degradation by Eh was quantified by immunoblotting using MUC2 specific antibodies against the protein and O-glycans. Eh ingestion of WT and Mut mucus (mucophagy) was visualized by confocal microscopy and imaging flow cytometry. Following mucophagy, modulation of Eh virulence genes was measured by RT-PCR, proteomics, and enzymatic activity quantified by enzyme specific substrates. Mut cells secreted more mucus constitutively, and in response to Eh hyper-secreted mucus enhanced Eh attachment, mucophagy and cytotoxicity of goblet cells. Mut mucus was readily degraded and ingested by Eh as compared to WT mucus. Increased ingestion of Mut mucus upregulated Eh virulence with increased enzymatic activity that rapidly degraded Mut mucus in a time- and dose-dependent manner. This study highlights the importance of proper mucus formation in conferring structural and functional integrity of the mucus gel that provides protective innate host defense in the gut against pathogens.

Abstract: 33

FCGBP maintains MUC2 mucus barrier integrity during infection with *Entamoeba histolytica*

Presenting author: Hayley Gorman

Presenting author institute: University of Calgary, CA

Co-authors: Hayley Gorman¹, France Moreau¹, Sarah Wilson¹, Kris Chadee¹ ¹University of Calgary, CA

The first line of innate host defence in the gastrointestinal tract is the mucus layer. MUC2 mucin and FCGBP are major goblet cell proteins that form the mucus barrier to prevent pathogens from invading the underlying epithelial cells. However, the functional/cooperative role of FCGBP together with MUC2 is not well characterized. Here, we interrogated the function of FCGBP in response to the colonic parasite *Entamoeba histolytica* (Eh). Native mucus was collected from LS174T goblet-like cells and organoid monolayers from healthy control patients grown in air-liquid interface (ALI), to investigate the effect of Eh secreted proteases (EhSP) on MUC2 and FCGBP degradation quantified by immunoblotting. Villin Cre *Fcgbpfl/fl* and *Fcgbpfl* ^{-/-} littermates were used to interrogate innate host defences in response to Eh in the colon by quantifying Eh-induced pro-inflammatory cytokines by RT-PCR and a 32-plex cytokine panel. Time- and dose-dependent degradation of mucus derived from ALI showed rapid degradation of FCGBP with low concentrations of EhSP prior to MUC2 degradation. *Fcgbpfl/fl* showed reduced pro-inflammatory cytokines in Eh colonic loops whereas *Fcgbpfl* ^{-/-} littermates elicited an increase in pro-inflammatory cytokines. This study demonstrates that the cleavage of FCGBP is a prerequisite for degrading MUC2 in the mucus layer. Loss of *Fcgbp* dampened a proper innate immune response, suggesting a role for FCGBP in regulating inflammation in the gut to colonic pathogens. These data highlight the need for further research in understanding the cooperative role of MUC2-FCGBP interactions in conferring innate host defense in the gut.

Abstract: 34

Defining the role of mucus barrier dysfunction in microbiota-dependent initiation of colorectal cancer

First author: Karl Hansson **First author institute:** Gothenburg Univ; Dept of Medical biochemistry and Cell biology, SE

Presenting author: Karl Hansson **Presenting author institute:** Gothenburg Univ; Dept of Medical biochemistry and Cell biology, SE

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I prefer to give: A poster presentation

Topic first preference: Mucins in Cancer and Tumour Biology **Topic second preference:** Microbiome-Mucin Interactions

Colorectal cancer (CRC) accounts for approximately 10% of cancer diagnoses worldwide and is strongly associated with environmental factors, particularly dietary patterns high in energy and low in complex carbohydrates, commonly referred to as the Western-style diet (WSD). WSD seems to profoundly influence the intestinal microbiota and may disrupt host–microbe interactions within the colon. The colonic mucus barrier forms the primary interface between epithelial cells and the commensal microbiota, yet its role in CRC initiation remains poorly understood.

Mouse studies suggest that WSD exposure disrupts mucus barrier function and that deletion of the key mucus gel-forming mucin Muc2 results in spontaneous CRC. While the relevance of mucus barrier dysfunction in human CRC is unclear, our state-of-the-art ex vivo analysis of biopsies from healthy individuals and cancer patients reveals compromised mucus barrier function in CRC patients, suggesting its role in oncogenesis. To model microbiota-dependent CRC initiation in vivo, we generated Muc2^{+/-}Apcmin^{+/+} mice, combining reduced Muc2 expression with a predisposition to spontaneous intestinal adenoma formation. Compared to Muc2^{+/+}Apcmin^{+/+} littermates, these mice show increased tumour load in both small intestine and colon. Strikingly, broad-spectrum antibiotic intervention inhibits increased tumour burden in Muc2^{+/-}Apcmin^{+/+} mice, while having little effect on Muc2^{+/+}Apcmin^{+/+} mice.

Together, these findings implicate mucus barrier dysfunction as a key factor in microbiota-driven colorectal tumorigenesis and establish a model system to study barrier–microbiota interactions in CRC.

Abstract: 35

Breaking Barriers: Probing Airway Mucin Glycoproteins in Pneumococcal Infection With StcE.

Presenting author: Niall Hardiman

Presenting author institute: Department of Infectious Diseases, Respiratory Medicine and Critical Care, Charité, DE

Co-authors: Niall Hardiman¹, Cengiz Gökeri¹, Ulrike Behrendt¹, Karen Hoffmann^{1,2}, Sandra Kunder³, Achim D. Gruber³, Stacy A. Malaker⁴, Martin Witzernath¹, Kevin Pagel⁵, Geraldine Nouailles-Kursar¹ ¹Department of Infectious Diseases, Respiratory Medicine and Critical Care, Charité, DE, ²Bundeswehrkrankenhaus Berlin, Department of Internal Medicine and Department of Microbiology and Hospital Hygiene, DE, ³Institute of Veterinary Pathology, Freie University, DE, ⁴Department of Chemistry, Yale University, US, ⁵Department of Biology, Chemistry, and Pharmacy, Freie University, DE

Decoding how the airway epithelial glycocalyx shapes infection outcomes may reveal new strategies to strengthen respiratory defence. The airway glycocalyx, rich in mucin-type O-glycoproteins, is part of the epithelial barrier which forms the first microbial interface, yet its role in regulating infection severity and host tolerance remains poorly defined. We used the mucin-selective protease StcE to cleave pulmonary mucin-type O-glycoproteins during intranasal infection with *Streptococcus pneumoniae*.

Mice were monitored for 48 hours, where infection with active StcE treatment resulted in significantly greater weight loss and clinical deterioration at 24–48 hours post-infection, with significance between the two infection groups. There was a trend toward hypothermia in StcE infected mice. Importantly, bacterial burdens in lungs, blood, spleen, and bronchoalveolar lavage were comparable between groups, indicating worsened disease independent of pathogen load.

Immunohistochemistry demonstrated minimal pulmonary parenchymal infection, restricted to rare intravacuolar bacteria within perivascular neutrophils and macrophages, while airway and alveolar compartments remained largely clear. In contrast, marked extrapulmonary burdens were observed in the pleural cavity, lymph nodes, adipose tissue, and skeletal muscle, predominantly within phagocytes. Bacterial distribution did not differ between the treatments.

Collectively, these findings indicate that disruption of mucin-type O-glycoproteins exacerbates clinical disease without altering bacterial burden or localisation, implicating the airway glycocalyx as a key player during pneumococcal infection.

How mucin concentration and innate mucosal proteins control filament extensibility

Presenting author: Bianca Hazt

Presenting author institute: School of Food Science and Nutrition, University of Leeds, GB

Co-authors: Bianca Hazt¹, George Degen², Lucas Warwaruk², Adam O'Connell³, Daniel Read⁴, Oliver Harlen⁴, Gareth McKinley², Anwesha Sarkar¹ ¹School of Food Science and Nutrition, University of Leeds, GB, ²Department of Mechanical Engineering, Massachusetts Institute of Technology, US, ³Polymer Science Platform, Reckitt Benckiser Healthcare (UK) Ltd, GB, ⁴School of Mathematics, University of Leeds, GB

As the primary gel-forming glycoproteins of the mucus barrier, mucins govern the flowability and extensibility of mucus, properties that are essential to many of its biological functions. While the flow behaviour of mucins under shear has been characterised in considerable detail, their response to extensional deformation remains largely unexplored. To address this gap, we investigated the extensional rheology of bovine submaxillary mucin dispersions using a dripping-onto-a-substrate setup and identified a concentration-dependent transition in their extensional response. We further examined how lactoferrin, an innate protein commonly present in mucosal secretions, modifies mucus flowability and extensibility. At low concentrations, mucin dispersions form highly extensible filaments whose diameters decay exponentially with time. This behaviour reinforced in the presence of native lactoferrin. As mucin concentration increases, viscous stresses become dominant over most of the filament lifetime, and elastic effects emerge only at later stages of thinning. If lactoferrin is incorporated as cross-linked microgels instead, mucin exhibits a similarly reduced elastic extensible response to that observed in concentrated mucin alone. Taken together, these findings demonstrate how mucin concentration redistributes the balance between viscous and elastic stresses during capillary thinning, revealing a concentration-driven reduction in filament extensibility that is reproduced in the presence of bioinspired microgels. This work provides a foundation for new mucosal diagnostic strategies and offers design principles for bioinspired materials with tuneable resistance to filament thinning mediated by macromolecular interactions.

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Barrier Properties of Reconstituted Mucus under Pathophysiological Conditions

Presenting author: Fabio Henkel

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Mucus serves as a highly selective barrier that regulates the entry of small molecules and nanoscopic objects into submucosal tissues. Under healthy conditions, these transport processes are modulated by size-exclusion of larger particles and interaction-based filtration of small molecules. Under pathophysiological conditions, however, these selective transport processes can be disturbed due to endogenous factors such as altered mucus composition or exogenous factors such as mucosal contaminations. Changes in the mucus composition can arise from alterations in the tissue-specific mucin expression such as the de novo expression of regionally atypical mucin subspecies, which is often associated with bacterial infections and gastric cancer. Our research elucidates the influence of MUC5B/MUC2 contaminations on physiologically relevant properties of acidic MUC5AC gels as found in the stomach. Our findings indicate that these properties may be critically altered by the presence of atypical mucin species. A weak integration of a contaminating mucin subtype into the host network weakens the viscoelastic gel but increases its barrier capabilities. Additionally, exogenous contaminations of the mucosal interface with nanoplastic particles can alter the selective permeability of the mucin network, e.g., when cationic nanoplastics adsorb to the mucosal interface. This changes the surface potential of the mucin hydrogel and introduces new, hydrophobic binding sites for small molecules. Understanding these complex properties of mucosal barriers under pathophysiological conditions is crucial for the understanding of mucosal disease progression and for developing drug-carriers to traverse this biological barrier.

Circulating Factors in Multiple Sclerosis and Systemic Lupus Erythematosus Impair Intestinal Epithelial Barrier Function in a Disease-Specific Manner

Presenting author: Inga Viktoria Hensel

Presenting author institute: Department of Medicine 1, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, DE; Deutsches Zentrum Immuntherapie, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, DE; BioMed X Institute, Heidelberg,, DE

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The intestinal epithelium forms a dynamic frontier between the gut lumen and the immune system, maintaining tissue integrity through a single, mucus-producing cell layer. Disturbance of this barrier increases intestinal permeability, a feature commonly seen in individuals with Multiple Sclerosis (MS) and Systemic Lupus Erythematosus (SLE). Yet, the molecular mechanisms driving this phenomenon remain insufficiently understood. In this work, we developed an in vitro platform to examine how MS and SLE influence intestinal epithelial cell function. Human colonic organoids were treated with serum derived from MS or SLE patients and analyzed through combined transcriptomic and functional approaches. This exposure produced a distinctive molecular signature marked by changes in barrier function and antigen sensing (MS) as well as suppression of goblet cell-associated genes and implicated changes in mucus composition (SLE). The organoids displayed disease-specific transcriptomic signature with imbalanced cell populations, impaired mitochondrial activity and enhanced permeability. Analysis of colon biopsy transcriptomes from MS and SLE patients supported these observations. Altogether, our results reveal that circulating factors in MS and SLE can disturb intestinal epithelial homeostasis and may play an active role in promoting barrier compromise in autoimmune disease.

Abstract: 40

Glycosylation profiles of MUC16 expressed in ovarian clear cell carcinoma

Presenting author: Hitomi Hoshino

Presenting author institute: Faculty of Medical Sciences, University of Fukui, JP

Co-authors: Hitomi Hoshino¹, Motohiro Kobayashi¹ ¹Faculty of Medical Sciences, University of Fukui, JP

There are very few cell lines derived from ovarian clear cell carcinoma. This lack of cell lines makes it difficult to understand the molecular mechanism of development and biological behavior of ovarian clear cell carcinoma arising from endometriosis. Recently, we established the MTC-22 cell line from ovarian clear cell carcinoma patient's tissue, and reported that this cell line showed the same expression profiles of ovarian carcinoma-related molecules as seen in the patient's tumor tissue. In addition to MTC-22, we have succeeded in establishing a new ovarian clear cell carcinoma cell line tentatively named TKG-10. Immunofluorescence staining of TTC-25 revealed that the expression patterns of ovarian carcinoma markers, including MUC16 (CA125), were consistent with those of patient's tumor tissue. These results suggest that TKG-10 may also be a useful tool for study on ovarian clear cell carcinoma biology. To clarify the glycosylation patterns of MUC16 in ovarian clear cell carcinoma, we subcloned cDNA encoding the tandem repeat regions of MUC16 and expressed it in MTC-22 and TKG-10 cells. Here, we report the results of glycosylation analysis of MUC16 expressed by these cells.

Abstract: 41

Mechanism-Guided Engineering of Sodium-Mediated Hydration in MUC5B Mucin Films Using Designed Peptides

Presenting author: Zoe HUANG

Presenting author institute: King's College London, GB

Co-authors: Zoe HUANG¹ ¹King's College London, GB

I prefer to give: A poster presentation

Mucin-based lubrication is a fundamental biophysical function of epithelial surfaces, yet the mechanisms governing hydration and friction regulation within mucin films remain incompletely understood. In the oral cavity, lubrication is primarily mediated by MUC5B-rich salivary films, where both macromolecular structure and ionic composition shape interfacial behaviour.

Emerging evidence suggests that sodium ions (Na^+) play a key role in modulating mucin hydration and lubrication. Enrichment of Na^+ within mucin films, together with ion-dependent reductions in friction, supports a model in which Na^+ stabilises hydration layers and maintains low shear resistance. However, the mechanistic basis of ion retention and its coupling to mucin structure and function remains poorly defined.

Here, we adopt a mechanism-guided approach to probe and reconstruct sodium-mediated hydration in mucin systems. Short peptides are rationally designed to integrate three functions: interfacial adsorption for surface retention, selective association with MUC5B for incorporation into mucin films, and sodium-binding capacity to stabilise hydration layers under shear. Design is informed by mucin-interactive motifs and film-stabilising architectures within a computational framework.

Candidate sequences are generated using AI-driven design and evaluated by molecular simulations for MUC5B interaction and Na^+ retention. Functional performance will be assessed using tribological measurements in reconstituted mucin systems and human saliva, linking molecular design to macroscopic friction.

Colibactin-driven colon cancer requires adhesin-mediated epithelial binding

Presenting author: Maude Jans

Presenting author institute: VIB Center for Inflammation Research, BE; Ghent University, BE

Co-authors: Maude Jans^{1,2}, Magdalena Kolata^{3,4}, Gillian Blancke^{1,2}, Aline D'Hondt^{3,4}, Claudia Gräf^{3,4}, Maarten Ciers^{1,2}, Mozes Sze^{1,2}, Alexandra Thiran^{1,2}, Ioanna Petta^{1,2}, Vanessa Andries², Sara Verbandt⁵, Engy Shokry⁶, David Sumpton^{6,7}, Johan Vande Voorde⁷, Geert 1VIB Center for Inflammation Research, BE, 2Ghent University, BE, 3Structural Biology Brussels, Vrije Universiteit Brussel, BE, 4Structural & Molecular Microbiology, VIB-VUB Centre for Structural Biology, BE, 5Department of Oncology, Catholic University Leuven, BE, 6Cancer Research UK Scotland Institute, Glasgow, GB, 7School of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, GB, 8Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, Cornell University, US, 9The Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medicine, AF

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. Using the microbiota-dependent ZEB2-transgenic mouse model of invasive CRC, 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. We also show that allelic switching of FimH strongly influences the genotoxic potential of pks+ *E. coli* and can induce a genotoxic gain-of-function in the probiotic strain Nissle 1917. Together, we show that the oncogenic potential of pks+ *E. coli* critically depends on bacterial adhesion to host epithelial cells 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.

The coordinated role of gastrointestinal mucins during health and inflammation.

Presenting author: Carol Aherne

Presenting author institute: School of Medicine, University College Dublin, IE; Conway Institute, University College Dublin, IE

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Inflammatory bowel disease (IBD), consisting of Crohn's disease (UC) and ulcerative colitis (UC) are debilitating gastrointestinal diseases that are growing in incidence and prevalence worldwide. Disruption of the mucus gel layer (MGL) and the underlying epithelium triggers an inappropriate immune response leading to IBD pathogenesis. Current treatments targeting the immune response fail in 50% of patients. Therefore, understanding how MGL integrity is maintained during health and disease may be key in the development of novel IBD treatments.

Mucins are the major constituents of the MGL. Deletion of Muc2, results in spontaneous murine colitis. Previous studies by our group demonstrate that Muc5ac, the major mucin in the stomach and lungs, is protective in murine colitis. This surprising role for Muc5ac despite the presence of Muc2 suggests a coordinated role for mucins in intestinal barrier function in IBD.

To investigate the independent and possible co-ordinated role of mucins in IBD we deleted Muc2 in C57BL6 mice and in Muc5ac deficient mice using CRISPR, generating two single mucin deficient strains (Muc2^{-/-} and, Muc5ac^{-/-}) and a double deficient strain, Muc2/Muc5ac^{-/-}. Preliminary findings suggest Muc2/Muc5ac^{-/-} mice have reduced viability compared to Muc2^{-/-} or Muc5ac^{-/-}.

To explore the role of mucins in CD, we crossed Muc5ac^{-/-} with the Crohn's-like ileitis model TNFΔARE (Muc5ac^{-/-}/TNFΔARE^{+/-}). Preliminary data demonstrates that, despite the presence of Muc2, Muc5ac^{-/-}/TNFΔARE^{+/-} mice have earlier-onset ileitis, and increased bacterial translocation to the mesenteric lymph nodes.

Overall our findings suggest that Muc5ac expression in the ileum and colon co-ordinates with Muc2 to maintain intestinal health.

Abstract: 44

Recognition of single and double fucosylated H and Lewis antigens by an *Akkermansia muciniphila* α -1,2-fucosidase

Presenting author: Maher Abou Hachem

Presenting author institute: Department of Biotechnology and Biomedicine, Technical University of Denmark,, DK

Co-authors: Mathias Jensen¹, Marina Corbella Morato^{1,2,3}, Jens Preben Morth¹, Carme Rovira³, Maher Abou Hachem ¹ ¹Department of Biotechnology and Biomedicine, Technical University of Denmark,, DK, ²Departament de Farmàcia i Tecnologia Farmacèutica, i Físicoquímica, Universitat de Barcelona (UB), ES, ³Departament de Química Inorgànica i Orgànica and Institut de Química Teòrica i Computacional, Universitat de Barcelona, ES

Several gut microbiome species are known to grow on mucin, but the specialist *Akkermansia muciniphila* is a uniquely dedicated mucin degrader, which is associated with fortified gut barrier function and positive metabolic markers¹. Recently we described the sialidases and fucosidases that *A. muciniphila* deploys to initiate mucin degradation². The *A. muciniphila* α -1,2-fucosidase AmGH95B displays remarkable catalytic efficiency towards H and Lewis antigens on type 1, 2 and 3 chains on mucin O-glycans, which has not been previously reported for any fucosidase. Here we combined X-ray crystallography, molecular dynamics (MD) simulations and mutagenesis to elucidate the structural basis underpinning this substrate multiplicity. We showed that catalytic site occupancy pivots an open-to-closed transition to an active enzyme state. Surprisingly, instead of remodeling its active site, AmGH95B has evolved to recognize multiple substrate-dependent glycan conformations by the same subsites, providing a rationale for the observed broad substrate range. For the first time, we report a dedicated subsite that recognizes secondary fucosyl units in double fucosylated Lewis B/Y and H type 3 antigens. This study unravels an unprecedented recognition of up to 4-5 saccharide units in mucin O-glycans² and human glycolipids³. Our work highlights structural features underlying the enzymatic adaptation of a GM specialist to diverse human glycoconjugates.

1. de Vos, W. M. et al. Gut 2022, 71 (5), 1020-1032. DOI: 10.1136/gutjnl-2021-326789.

2. Shuoker, B. et al. Nat. Commun. 2023, 14 (1), 1833. DOI: 10.1038/s41467-023-37533-6.

3. Jensen, M. et al. Nat. Microbiol. 2024, 9 (5), 1176-1188. DOI: 10.1038/s41564-024-01663-4.

Abstract: 45

Strain-level functional diversity in *Ruminococcus gnavus* drives variation in carbohydrate utilization and host immune responses

Presenting author: Mihovil Joja

Presenting author institute: Luxembourg Institute of Health, LU; University of Luxembourg, LU

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Ruminococcus gnavus is a commensal bacterium present in the majority of human gut microbiomes. Metagenomic sequencing of human stool samples has revealed substantial inter-strain genetic variation, and predicted gene functions involved in carbohydrate metabolism and immune stimulation appear to be overrepresented in disease states such as food allergy and Crohn's disease. To test whether *R. gnavus* strains exhibit extensive functional diversity, we assembled a biobank of diverse *R. gnavus* isolates (N = 67) obtained from healthy adult donors and patients with inflammatory bowel disease. Isolates were assessed for their nutritional capacity on a panel of carbohydrates, revealing substantial differences in substrate utilization. Additionally, mucin foraging was conserved among most strains; however, distinct transcriptomic signatures suggest different strategies for utilizing mucin O-glycans. Subsequently, we tested how this functional variability translates into immune modulation and observed marked changes in both the magnitude of immune stimulation and the T cell polarization. These differences enabled the investigation of specific strain-level genetic characteristics associated with disease-related immune responses. Selected strains will be introduced into gnotobiotic mouse models of colitis and food allergy to assess their impact on disease development. Ultimately, these findings suggest that strain-level functional variation within *R. gnavus*, rather than species presence alone, could shape mucus barrier integrity and downstream host immune responses.

O-Glycan targeting metallopeptidases from *Bacteroides caccae*: tools in cancer research

Presenting author: Calebe Juchem

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Aberrant mucin-type O-glycosylation is a characteristic feature of cancer, contributing to tumour initiation, progression, and resistance to therapy [1]. O-glycopeptidases from commensal bacteria, which specifically recognise and hydrolyse mucin-type O-glycans, offer promising tools to interrogate cancer-associated glycosylation patterns [2,3].

Here, we investigate O-glycopeptidases from *Bacteroides caccae* to develop novel tools for targeting tumour-associated glycans. Modular enzyme constructs comprising catalytic and carbohydrate-binding domains (CBMs) were recombinantly expressed and assessed for glycan recognition and substrate specificity. Glycoprotein and glycopeptide microarrays revealed distinct binding profiles, and enzymatic activity was confirmed towards mucin and mucin-glycopeptide substrates, with enhanced activity following desialylation.

Notably, the CBM of mucinase BC16100 shows high specificity for the Tn antigen (GalNAc α -Ser/Thr) and binds selectively to Tn-enriched pancreatic cancer cells, while the catalytic domain mediates cell surface glycan remodelling. These findings demonstrate the potential of bacterial O-glycopeptidases as selective tools to detect and manipulate tumour-associated O-glycans.

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Abstract: 47

Distinct effects of IL-13 and IL-1 β on mucus properties and epithelial surface hydrophobicity in human airway epithelium

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BACKGROUND. Airway diseases are characterized by heterogeneous inflammatory pathways, but how type 2 and non-type 2 inflammatory signals alter mucus composition, mucus biophysical properties, and epithelial surface features remains incompletely understood.

METHODS. We cultured human airway epithelial cells (AECs) at an air-liquid interface and treated with IL-13 (10 ng/mL) or IL-1 β (10 ng/mL) during the final week of a 21-day differentiation protocol. Mucin gene expression was quantified, mucus biophysical properties were assessed using live-cell rheology and epithelial cell surface hydrophobicity was measured by tensiometry.

RESULTS. IL-13-activated epithelial cells increased MUC5AC expression, whereas IL-1 β -treated cells preferentially increased MUC5B expression compared with untreated controls. Mucus from IL-13-activated cells exhibited increased viscosity and reduced compliance, consistent with previous reports. In contrast, mucus from IL-1 β -treated cells showed rheological properties similar to control mucus. Both IL-13 and IL-1 β treated epithelial cell surfaces exhibited higher hydrophobicity compared to controls; however, IL-1 β resulted in significantly greater surface hydrophobicity than either control or IL-13 conditions.

CONCLUSION. These findings demonstrate that IL-13 and IL-1 β drive distinct mucus and epithelial surface phenotypes, with IL-13 promoting viscoelastic, pathologic mucus and IL-1 β altering epithelial surface properties.

Abstract: 48

Mucus-associated *Bacteroides thetaiotaomicron* modulates the epithelial responses to pathogens

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Bacteroides thetaiotaomicron (Bt) is a prominent gut commensal that contributes to the digestion of diverse carbohydrates and maintenance of intestinal homeostasis, whereas *Clostridium difficile* (Cd), a low-abundance member of the microbiota, can thrive and produce toxins under disrupted gut conditions. Our initial study of Bt and Cd co-colonization in a hypoxic epithelial transwell model revealed elevated pathogen burden, toxin production, and spore formation as compared to Cd mono-infection. Additionally, combined transcriptomics and metabolomics indicated that Bt altered its metabolism to consume host mucus-derived glycans instead of simple sugars in co-colonized models. Bt mucus grazing may facilitate toxin diffusion toward the epithelial layer, indicating a shift in the role of Bt from protective to pro-invasive. Our study aims to characterize how Bt activities shape the mucosal niche and render the epithelium more susceptible to Cd infection. To address this question, we screened Bt transposon mutants of individual polysaccharide utilization loci (PULs) and identified a set of genes involved in mucus utilization. Moreover, we established an imaging framework for the Bt-colonized epithelium model and a newly developed primary colonoid model by combining hybridization chain reaction-fluorescence in situ hybridization and immunohistochemistry, which allowed us to simultaneously visualize Bt and the host mucus layer. Altogether, our work lays the foundation for exploring the Bt metabolism involved in host mucus digestion and for characterizing the spatial organization of Bt in a human gut-like environment, thereby advancing our understanding of microbiota-pathogen-host interactions.

Abstract: 49

Protein synthesis and MUC5AC expression are required for chemoprotection in mucinous colorectal adenocarcinomas

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Presenting author institute: University of Manchester, GB

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Mucinous colorectal adenocarcinomas, account for 15% of colorectal cancer cases, and are characterised by containing over 50% extracellular mucus. This mucus is a result of excessive mucin secretion by tumour cells. The mucus layer acts as a physical and biochemical barrier to chemotherapy, potentially contributing to the poorer prognosis for patients with mucinous tumours. Despite visible differences compared to non-mucinous disease, mucinous colorectal adenocarcinomas are treated identically; no specific therapies exist due to limited understanding of the underlying biology of mucinous disease. To address this, we aim to define the molecular content of secreted mucus, to understand how this is synthesised and whether mucus generates a chemoprotective tumour microenvironment.

Targeting protein synthesis in mucinous cancer cell lines led to a marked reduction in the secretion of multiple mucins. Protein synthesis inhibitors synergised with 5-FU in cell viability assays, indicating that active protein synthesis is essential for mucin production and contributes to chemoresistance. Tumour-derived mucus isolated from patients forms a chemoprotective barrier that limits the efficacy of 5-FU, with optimal protection depending on both mucus concentration and mucin composition. Mechanistically, mucus restricts 5-FU penetration by altering osmotic and diffusion properties, thereby reducing intracellular drug uptake. Reduction of MUC5AC enhanced 5-FU cytotoxicity, increased DNA damage, and promoted 5FU-dependent RNA damage. To further validate and extend these findings, we will employ patient-derived organoid models from the UK Living Biobank and integrate mass spectrometry-based proteomics at both the cellular and organoid levels.

Abstract: 50

Mucus for dinner: Chasing commensal gut degraders

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Deep inside the hidden corridors of our intestines, an invisible army of good bacteria and a powerful mucus barrier work tirelessly to keep us healthy. The mucus, a sticky shield lining the gut, is constantly built and rebuilt to protect the delicate intestinal walls. Beyond its protective function, mucus supplies substrates that sustain beneficial microbes involved in digestion, immunity, and homeostasis. Maintaining this balance is key: when dietary fibers are lacking, microbial metabolism shifts toward mucus glycoproteins, compromising barrier integrity and exposing the epithelium. Most research to date on mucus breakdown and metabolism has focused on Carbohydrate-Active Enzymes (CAZymes) which primarily cleave terminal monosaccharides from mucin O-glycans, while the role of O-glycopeptidases (i.e. mucinases) remains largely unexplored. This project aims to uncover the hidden enzymatic arsenal behind mucin glycan degradation and its link to inflammation. We focus on mapping the repertoire of commensal bacterial enzymes involved in mucus breakdown and understanding their relevance to the disease.

Abstract: 51

Expanding imaging of mucins and glycans

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Nanoscale molecular organization is critical for glycan assembly in the Golgi and for glycan function at the cell surface. However, imaging glycans and key glycoconjugates such as mucins at nanometer-scale resolution remains technically challenging. Here, we advance this goal by developing new chemical tools and protocols for imaging glycans and mucins with expansion microscopy (ExM), a method that improves the effective resolution of light microscopy through physical expansion of samples embedded in a swellable polymer matrix. We designed peptide-based multifunctional linkers that enable anchoring of protein-based probes within the expansion matrix, a key requirement for ExM workflows. Using solid-phase peptide synthesis, these probes can be modularly assembled with diverse chemical handles and fluorophores. We validated probe performance by conjugation to single-chain antibodies against Muc-1 Tn antigen (5E5) and polysialic acid (735). Notably, analysis of Tn-antigen distribution at the cell surface suggested that mucins bearing Tn antigen may be enriched in specific membrane domains, including regions with dense microvilli. We are now evaluating peptide-based probes with an expanded set of mucin-specific binders, including de novo proteins generated through AI-enabled design pipelines. Together, these tools establish a versatile and extensible platform for nanoscale imaging and mapping of cell surface mucins and glycans across complex membrane topology, with broad potential to illuminate the spatial organization of the glycocalyx in health and disease.

Abstract: 52

Exploratory analyses of cervicovaginal mucus O-glycan composition and microbiota profiles in unexplained infertility

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Background: Unexplained infertility accounts for 15–30% of couples seeking fertility treatment, yet the molecular features of the cervicovaginal environment in this condition remain poorly understood. Cervicovaginal mucus (CVM) and the vaginal microbiota are both key components of the reproductive ecosystem and may influence sperm transport, mucosal barrier properties, and treatment success. **Methods:** CVM and vaginal swabs were collected at confirmed ovulation from 19 women undergoing intrauterine insemination for unexplained infertility. Mucin O-glycans were analysed by MALDI-TOF MS/MS. Vaginal microbiota profiles were generated via 16S rRNA sequencing and species-level assignment. Multivariate analyses were used to explore covariance between glycan features and microbial composition. **Results:** We identified diverse, highly fucosylated CVM glycomes comprising 57–68 unique O-glycan compositions, dominated by core 1 and core 2 structures. The vaginal microbiota was highly Lactobacillus-dominant, with Lactobacillus crispatus present in 79% of participants, comprising the highest mean relative abundance. PERMANOVA indicated an association between glycan profiles [NJ1] and dominant taxa ($q = 0.0011$, $R^2 = 0.37$), although the small cohort limited further statistical inference. Unsupervised clustering suggested distinct glycan patterns [NJ2] in samples dominated by less abundant taxa such as Lactobacillus iners or other low-abundance taxa. **Conclusions:** This exploratory study provides a combined characterisation of CVM O-glycosylation and vaginal microbiota in unexplained infertility. The findings suggest non-random co-variation between mucin glycan features and microbial communities.

Goblet cell subpopulation dynamics during inflammation-driven colorectal cancer

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The colonic mucus layer, secreted by goblet cells (GC), constitutes the primary innate defense mechanism of the epithelium, maintaining intestinal homeostasis. However, damage to this barrier leads to tissue injury that activates inflammation. Chronic cycles of inflammation and tissue repair promote genomic instability and increase the risk of colorectal cancer (CRC).

Recent studies show that GCs comprise distinct subpopulations with unique secretory profiles along the crypt–surface axis. However, whether specific GC subpopulations are reprogrammed during chronic inflammation, and how these alterations contribute to the neoplastic transformation, remain unknown. This study aims to define how chronic inflammation reshapes GC subpopulation dynamics and whether these alterations contribute to a tumor-supportive niche.

Using murine models of recurrent inflammation and inflammation-driven carcinogenesis (DSS and AOM-DSS), we mapped GC dynamics across the inflammation–dysplasia–carcinoma. Spatial and temporal analyses revealed progressive alterations in GC differentiation markers and mucin-associated proteins (MUC2, FCGBP, CLCA1) after repeated cycles, most pronounced in regions transitioning to dysplasia. These changes coincided with dysregulation of secretory lineage transcription factors, increased epithelial proliferation, and stress in the biosynthesis pathway of mucus protein, indicating impaired GC maturation during tumorigenesis.

These findings underscore that specific GC subpopulations are dynamically altered during chronic inflammation and neoplastic progression. Further characterization of these changes could be essential for the development of early CRC biomarkers.

Abstract: 54

Colonization of the bovine respiratory mucosal surface by pathogenic bacterium *Mannheimia haemolytica*

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Presenting author institute: Utrecht University, NL

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Bovine respiratory disease (BRD) is a major cause of mortality in calves that is characterized by coinfections of viral and bacterial pathogens, including *Mannheimia haemolytica*. In healthy cows, *M. haemolytica* is a commensal of the upper respiratory tract, but migration of the bacteria into the lower respiratory tract contributes to BRD development. In this study, we investigated the virulence properties of *M. haemolytica* that contribute to colonization and infection of the bovine bronchial mucosal surface. Genomic analysis of *M. haemolytica* clinical isolates demonstrated the presence of genes encoding mucus O-glycan-degrading enzymes, including fucosidases and sialidases. Sialidase activity could be measured for all strains, while fucosidase activity was restricted to the serotype 2 isolate. Proliferation of *M. haemolytica* was increased in mucin-rich environments, seemingly independent of sialidase or fucosidase activity. We grew primary bovine bronchial cultures under air-liquid conditions and observed that *M. haemolytica* serotype 2 strains could efficiently colonize these cultures and removed α 2,3- and α 2,6-linked sialic acids from the mucosal surface. Using glycoengineering experiments, we found that *M. haemolytica* incorporated sialic acid into its lipopolysaccharide (LPS). We are currently investigating if this LPS modulation leads to decreased immune activation. Our findings demonstrate that *M. haemolytica* thrives at the mucosal surface and is capable of exploiting host glycoproteins for proliferation and LPS modification. The main contributions of *M. haemolytica* to BRD might be efficient colonization of the lower respiratory tract, impairment of epithelial barrier integrity and glycan-mediated immune evasion

Abstract: 55

Spontaneous protein crystallization is associated with mucus production and development of progressive pulmonary fibrosis in conditional Nedd4-2 deficient mice

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Spontaneous protein crystallization has recently been described as an amplifier of type 2 inflammatory responses in eosinophilic airway diseases. However, its relevance in other chronic lung diseases remain largely unexplored.

Therefore, we investigated the role of spontaneous protein crystallization in a model of progressive pulmonary fibrosis caused by conditional deletion of Nedd4-2 in the pulmonary epithelium. Lung tissue was analyzed using histology, immunohistochemistry, and electron microscopy to characterize structural alterations and crystal formation. In addition, protein crystals were quantified in bronchoalveolar lavage (BAL) fluid and their abundance was correlated with inflammatory cell counts in BAL and pulmonary compliance. To further explore underlying molecular mechanisms, we performed whole lung transcriptomics.

Chil3- and Chil4-positive crystals were detected in distal airways of conditional Nedd4-2 deficient mice. Crystals were found both within alveolar macrophages and within mucus plugs in terminal airways. The number of crystals recovered from BAL fluid correlated with the degree of pulmonary inflammation and with decline of pulmonary compliance. Transcriptomic analyses revealed gene expression patterns consistent with type 2 inflammation and extracellular matrix remodeling.

Together, these findings demonstrate that conditional deletion of Nedd4-2 promotes spontaneous Chil3 and Chil4 crystallization, accompanied by type 2 inflammation, airway mucus plugs and progressive fibrotic tissue remodeling in the lung. This may represent a previously underappreciated contributor to fibrotic lung disease and could provide new opportunities for therapeutic intervention

Abstract: 56

Quantitative Profiling of Mucinases across Airway Mucins: Identifying Pulmonary Lead Candidates

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Airway mucus forms a protective barrier that limits access to the underlying epithelium. In the lung, this barrier is largely defined by secreted and membrane-tethered mucins, which build the mucus and periciliary layers. Conventional mucolytics show limited ability to disrupt these structures, resulting in modest effects on disease-associated mucus and pulmonary drug delivery, whereas mucinases – enzymes that specifically cleave mucin-domain glycoproteins – may offer a more targeted strategy to overcome airway mucin barriers.

Here, we identified pulmonary lead mucinases by evaluating activity and specificity toward airway-relevant mucins. Selected mucinases (SmE, HC4, StcE, BT4244, OgpA, AM0627, AM0908) were recombinantly expressed in *E. coli*, purified, and screened in a cell-display assay against secreted mucins (MUC5AC, MUC5B) and membrane-tethered mucins (MUC4, MUC16). Engineered HeLa cells presenting fluorophore-linked mucin fragments were incubated with enzyme for 90 min at 37 °C; cleavage released the fluorophore into the supernatant, and fluorescence was quantified. SmE and StcE showed broad activity across airway mucins, with SmE displaying the highest overall activity, whereas HC4 exhibited a more selective profile, with strong activity toward MUC5AC and limited effect on membrane-tethered mucins. Our findings identify SmE and HC4 as complementary lead candidates for pulmonary mucin remodeling and demonstrate that quantitative profiling across airway mucins can distinguish broad-acting from more selective mucinases. Together, these insights support the rational selection of mucinases for targeted modulation of airway mucin barriers. This work was supported by the Scholarship Fund of the Swiss Chemical Industry.

The overlooked glycan barrier on mucus shaping Influenza A virus infection

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Sialic acids (Sia) at O-glycosylated mucin domains of respiratory mucins function as decoy receptors trapping Influenza A virus (IAV) in the mucus by binding to the viral hemagglutinin (HA). Sia binding needs to be compensated by sialidase activity of the viral neuraminidase (NA) to promote traversal of the mucus and binding to epithelial cell surface-displayed Sia receptors that support infection. The glycomes of mucus and cell surface differ from each other and between species. A longstanding hypothesis states that mucus forms a host species-specific restriction barrier to IAV infection that, e.g., prohibits cross-species transmission of human and swine IAVs. Unraveling this mechanism is important in view of the 2009 pandemic that was caused by a swine IAV breaching the species barrier.

Here, infection of primary swine tracheal cell cultures was shown to be absolutely dependent on NA activity. By comparing contemporary swine IAV isolates of distinct clades, we show that parameters like binding affinity and specificity of HA and sialidase activity of NA largely differ. We hypothesize that the balance between these parameters is more relevant than the individual values in relation to infection efficiency. We designed functional HA/NA balance assays, using sialoglycoprotein probes produced in different genetically modified HEK cells to mimic either the cell surface or mucins and correlated this to infection efficiency of these cells. Then, the inhibitory effects of swine and human mucus were quantified in these assays and shown to be different in showing stronger inhibition by human mucus. A comparative mucus analysis of the two species hints at specific glycosylation differences that convey this barrier function.

Abstract: 58

Enhanced mucinase activity by affinity engineering

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Mucus is the first physical barrier to systemic delivery for oral and pulmonary therapeutics. Current approaches do not target the long protein filaments of mucins, the major structural component of mucus. We have recently applied enzymes directly able to cleave mucins (mucinases), to oral drug delivery. One such mucinase, StcE, is highly active, however, many other mucinases with potentially useful specificities, such as HC4, exhibit much lower activity. Here, we investigated enhancing mucinase activity by appending carbohydrate- or mucin-binding modules. We coupled two mucinases (HC4 and OgpA) to the lectins 2Y9G and Hsa and to the mucin-binding module of StcE X409. Their activity was assessed against recombinant model mucin substrates representing gel-forming and secreted mucins (MUC5AC and MUC4) and on mucus retrieved from Calu-3 cells. The mucinases demonstrated enhanced activity with minimal changes to specificity. X409 and the broad-acting lectin 2Y9G showed the strongest effect for both OgpA and HC4, reaching wild-type-equivalent activity at 100-fold lower enzyme concentrations. Multiple particle tracking in model mucus produced by Calu-3 cells revealed a mucinase dependent decrease in micoviscosity, however, no differences between mucinases alone and mucinases coupled to either X409 or 2Y9G were observed. This may reflect differences in glycan composition, or non-productive sequestration by abundant O-glycosylated sites. This work demonstrates the potential of affinity directed mucinase engineering and supports the further tailoring of mucinases to tissue-specific mucus environments. Funding from the Swedish Research Council [grant number 2023–00439] is gratefully acknowledged.

Abstract: 59

Age and Gestational Maturity, Inflammation, and Lipids Shape the Neonatal Intestinal Mucus Barrier

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The intestinal mucus barrier plays a vital role in human health, defending the body against pathogenic invasion. Yet, how the intestinal mucus barrier develops after birth, and how factors such as age and gestational maturity shape its capacity to respond to external challenges in neonatal life remain poorly understood. In this study, we characterize a distinct postnatal developmental trajectory of the mucus barrier in preterm delivered piglets, marked by a more refined mucus microstructure and decreased mucus diffusivity compared with term born piglets. Importantly, we show that necrotizing enterocolitis (NEC) is associated with increased diffusivity of a large pathogen-model compound, establishing a direct link between NEC pathology and impaired mucus barrier function. We further demonstrate that the lipids, sodium decanoate and salcaprozate sodium, modulate mucus diffusivity in ways that depend on both the age and gestational maturity as well as health status of the animals. Collectively, these findings reveal that regulation of the neonatal mucosal barrier is highly dynamic and shaped by age, gestational maturity and disease conditions, underscoring the need for maturity- and disease-specific therapeutic approaches to support optimal gut health in early life.

Abstract: 60

The Role of Mucus-Associated Bacteria in Colorectal Cancer Susceptibility: Lessons from a cohort of patients with common variable immunodeficiency

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Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide, arising from the interplay between genetic susceptibility and environmental factors. Central to CRC pathogenesis is the intestinal mucus barrier: mucosal immunoglobulins maintain its two-layered architecture by confining microbiota to the outer layer, keeping the inner layer sterile. In common variable immunodeficiency (CVID), defective antibody production compromises this barrier, enabling bacteria to breach the inner mucus layer, reach the epithelial surface, and drive chronic inflammation and tumorigenesis. Yet, how these invaders contribute to CRC in immunodeficient hosts remains poorly characterized.

To address this, we isolated, characterized and transplanted bacterial consortia from colonic biopsies of CVID patients into germ-free mice subjected to a colitis-associated cancer model. Mice colonized with CVID-derived consortia developed significantly increased tumor burdens, enhanced inflammation, epithelial damage, and genomic instability compared to control-colonized mice. Sequencing revealed distinct microbial community structures directly correlating with tumor susceptibility.

These findings establish mucus-associated bacteria as active drivers, rather than passive correlates, of CRC in the context of immunodeficiency, identifying the mucus barrier as a critical therapeutic interface for microbiota-targeted cancer prevention.

Abstract: 61

Microbial metabolites regulate epithelial renewal in the intestine depending on microbiota colonization status

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Presenting author institute: University of Gothenburg, SE

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Proliferation of intestinal stem cells (ISCs) maintains epithelial renewal and regeneration in response to signals from the environment. While microbial metabolites are known to influence epithelial renewal, it remains to be elucidated how they regulate ISCs proliferation and if initial exposure to microbial metabolites programs future proliferative responses. The main aim of this work is to determine how microbial metabolites shape epithelial renewal and imprint epithelial memory of proliferative responses in ISCs. Using a microbial metabolite screen in mouse intestinal organoid cultures, we quantified proliferation and production of ROS. We identified that organoids from adult conventionalized mice remained less responsive to certain microbial metabolites than organoids from adult germ-free mice, suggesting that ISCs develop a type of proliferative tolerance upon commensal bacteria colonization. To determine if microbial metabolites imprint epithelial memory of proliferative responses in ISCs, we utilized organoids from neonatal and young mice at weaning with different stages of microbiota maturation. The ISCs from neonatal mice responded to selected microbial metabolites similar to germ-free organoids, while stem cells from weaned mice remained unresponsive like organoids from adult conventionally raised mice. We further investigated the mechanism and evaluated if mouse ISCs responses to microbial metabolites translate to humans, using ISCs from neonatal and adult donors. Our studies indicate that ISCs develop a type of proliferative tolerance to selected microbial metabolites produced upon microbiota colonization and maturation, indicating imprinting of proliferative memory in stem cell compartment during early life.

Abstract: 62

Engineered mucins as a versatile platform

Presenting author: Thomas Crouzier

Presenting author institute: June Bio, DK

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Mucus engineering aims to modulate the properties of mucus via topical treatments and explores the potential of mucins as functional building blocks for diverse biomedical applications. This concept has inspired approaches such as non-hormonal contraceptives that leverage mucus remodeling to control fertility, illustrating how intervening at the mucus level can yield novel therapeutic strategies.

The multifunctionality of mucins offers a remarkable range of opportunities. Their high-density, multivalent glycan display enables high-affinity interactions with cell surface receptors, positioning mucins as agents for controlling inflammation at mucosal surfaces and modulating immune responses. Mucins serve as decoy receptors that bind and inhibit viruses and modulate bacterial virulence. They act as selective substrates for commensal microbiota, functioning as prebiotics and microbial anchor sites that shape the microbiome. Cross-linked into hydrogels, mucins yield biomaterials with potent immunomodulatory and antiviral properties, opening paths toward prophylactic gels against sexually transmitted infections.

In this poster, we survey these diverse application areas, grounding each in current literature and our own work. We also present progress from June Bio, which is building a recombinant mucin platform using engineered *E. coli* to produce mucin glycoproteins with defined amino acid and glycan compositions at scale and low cost. By leveraging glycoengineering to control the identity and density of displayed glycans, this platform generates mucin variants tailored to specific applications, overcoming source heterogeneity, poor scalability, and high cost to make mucins broadly accessible for research and translation.

Characterization of a Novel GH101 Member from *Ruminococcus torques*

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The human gut microbiota has a critical role in health and disease. In the colon these bacteria colonize the mucus layer, a protective barrier where MUC2 is a major component. Despite the complexity of O-glycans some of the microbiota can degrade and utilize mucins and excessive foraging on O-glycans has been associated with disruption of the mucus layer and diseases such as inflammatory bowel diseases (IBD). *Ruminococcus torques* is able to grow on intact mucin domains and it has been shown to be implicated in IBD. Although, the mechanism of utilization of mucins by this bacterium remains unexplored. During growth on mucins, *R. torques* overexpresses two glycoside hydrolases from family 101 (GH101, RT1159 and RT2621), which activity remains unknown. RT1159 and RT2621 have 38.13% sequence identity. Both enzymes release core 1, a typical activity for GH101 enzymes. Although, RT2621 also cleaved sialylated core 1 glycans, a new activity inside this family. To gain insight into this activity, the structure was solved by cryo-electron microscopy at 2.53 Å. The catalytic domain showed the typical GH101 (β/α)₈-barrel fold, flanked at both terminus by multiple β -sandwich domains of unknown function that are dynamic and movable towards the enzyme active site. In the RT2621 active site, GH101 conserved residues were found differently oriented which can explain the novel activity observed. Increasing our understanding of mucin active enzymes extends the knowledge of how the microbiota can utilize complex host glycans and its role in diseases like IBD.

Abstract: 64

Impact of High-Fiber Diet on Translocation and Host Mucin Transcription in Chronic HIV Infection

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In people with HIV (PWH), chronic gut inflammation and "leaky gut" facilitate bacterial translocation, driving systemic immune activation and metabolic syndrome. While high-fiber diet (HFDs) improve outcomes in HIV(+) men who have sex with men (MSM), mechanisms remain unclear. Based on murine models, fiber may prevent host mucin degradation and restore the gastrointestinal barrier, reducing translocation and downstream complications. Using samples from a 4-week randomized intervention, this study assessed the impact of an HFD on translocation, microbial mucolytic enzyme expression, host mucin trafficking/glycosylation genes, and predicted metabolic flux. Plasma, fecal, and colonic biopsy samples were utilized from 36 HIV(+)MSM, 21 HIV(-)MSM, and 9 HIV-negative men who have sex with women. Translocation markers were quantified, and dual RNA-sequencing was performed on biopsy subsets to profile host and microbial transcriptomes. 16S rRNA-based community modeling of fecal microbiomes was applied to predict the flux of mucin and carbohydrate substrates. Flagellin and sCD14 were elevated in HIV(+)MSM but significantly reduced following the HFD. No changes were observed in microbial mucolytic expression or mucin flux; however, the HFD altered the predicted flux of other carbohydrate substrates. The HFD resulted in reduced expression of several host mucins, alongside increased fucosyltransferase and decreased sialyltransferase expression, suggesting diet-induced remodeling of mucin architecture. These findings demonstrate high-fiber interventions effectively reduce systemic translocation in PWH along with host-mediated structural remodeling of the mucin barrier and shifted microbial carbohydrate utilization.

Abstract: 65

Building a mucus barrier: A non-cancerous adult porcine intestinal model producing Muc2

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There are several in vitro models of the intestinal barrier that are used in both academic and industry research. These cellular models, along with microphysiological systems, were developed to mimic human physiology with the aim of reducing the use of animals in research. Despite recent advances, there are currently no low-complexity, non-cancerous adult intestinal models that allow easy luminal access while producing MUC2. This study aimed to establish a new small intestinal heterogeneous cell line using porcine biopsies that could serve as an alternative 2D cellular model. When cultured as a monolayer, the cells formed an integral epithelial layer with expressed tight junction proteins. The presence of goblet cells was confirmed by measuring Tff3 and Muc2 gene expression, Muc2 secretion, and immunocytochemistry staining. The composition of the porcine monolayer was further confirmed using flow cytometry, which allowed for goblet cell quantification. This demonstrated that the presented in vitro model could replicate the adult small intestinal layer with a mucus barrier, facilitating studies into mucus and epithelial barrier function.

Abstract: 66

Investigation of mucosal-related defects and their implication on the development of Environmental Enteric Dysfunction

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Environmental enteric dysfunction (EED) is a subclinical condition characterized by intestinal inflammation, villus blunting and Goblet cells depletion. As the name suggests, EED is caused by constant exposure to poor sanitation and hygiene and thus associated with developing countries. Prolonged exposure can have a significant impact especially on child health, with poor linear growth, delayed neurocognitive development and impaired oral vaccine response. As EED is linked to intergenerational risk of transmission from mother to child, there is a current urgency in understanding the pathophysiology of EED and developing new therapeutic approaches.

Despite present advancements, there is still a lack of specific biomarkers for the diagnosis of EED, and the only available method of detection involves the collection of intestinal biopsies through endoscopic procedures. This invasive diagnostic approach creates a challenge for the detection of the disease in children with this disorder. In addition, it has been shown that nutritional interventions had no effect on the development of EED, suggesting that this problem must be addressed on a cellular level.

In this study, we will examine duodenal biopsy samples, brushings and aspirates collected from patients with mild and moderate EED from Zambia and control samples collected from Sweden. We will focus on the characterization of mucus and mucin-related defects in EED, providing novel insights into how the disease alters the mucinous barrier of the small intestine. This project aims to discover important biomarkers that could provide non-invasive diagnostic tools as well as potential therapeutic markers improving the outcome and progression of EED.

Abstract: 67

Impact of Enteropathogenic Escherichia coli strains on goblet cell secretory function

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Dysregulated mucin secretion compromises the protective mucus barrier, thereby weakening its role in disease prevention. Infections caused by EPEC strains are frequently reported in low- and middle-income countries and may lead to persistent infections during early childhood, contributing to environmental enteric dysfunction. Unlike other diarrheagenic *E. coli* pathotypes, EPEC does not rely on toxin-mediated pathogenicity but instead induces pedestal-like structures on the epithelial surface, a phenomenon known as attaching and effacing (AE) lesions. As part of this process, proteins translocated into host cells promote low-grade inflammatory signalling that suppresses the innate immune response during infection. Despite extensive study of these mechanisms, their impact on goblet cell function and mucus secretory response remains incompletely understood. To address these questions, we evaluated gel-forming and transmembrane mucins in the HT29-18N2 cell line following infection with atypical EPEC strains isolated from children's faecal samples in Brazil and examined inflammatory markers associated with the secretory process. Our bacteria collection was carefully selected based on genetic variability, serotyping, and intimin typing. Remarkably, one strain triggered a massive mucin-5AC release, resulting in goblet cell degranulation and transient mucin hypersecretion. This was accompanied by activation of inflammation-associated signalling, as indicated by increased p-ERK levels followed by a significant rise in p-CREB levels. These findings underscore the mucus release triggered by the EPEC pathogroup and its effects on goblet cell function, potentially contributing to the development of chronic inflammatory disease.

Abstract: 68

Human milk oligosaccharides utilization by *Bacteroides thetaiotaomicron*

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The priming for the development of a beneficial microbial community begins in the infants through their first nutrient source, breast milk. Human breast milk contains a mixture of over a 100 different oligosaccharides, known as human milk oligosaccharides (HMOs). These HMOs pass undigested to the infant gut where they become a freely available nutrient source for the gut bacteria.

Bacteroides are among the most prevalent genera in the human gut microbiota and possess a vast number and variety of carbohydrate-active enzymes. This large number of enzymes allows species such as *Bacteroides thetaiotaomicron* (*B. theta*) to utilize HMOs and complex host and dietary glycans by differentially regulating their gene expression depending on the available carbon source. This makes them an interesting species during weaning when the baby switches from feeding on breast milk to solid foods, as this bacterium can successfully colonize the gut under both conditions.

Here, we have studied the growth of wild-type *B. theta* on a mixture of purified HMOs and compared it to the growths of a library of different mutants that lack specific enzymatic activities. Our results suggest that a complex mixture of glycans is important to upregulate all the proteins required for HMO utilization. Combining glycomics and proteomics analyses has revealed the pivotal role of fucosidases in the breakdown of these complex glycans by *B. theta*. Moreover, we have seen a growth-phase-dependent expression of enzymes, illustrating the bacterium's ability to fine-tune their metabolism to substrate availability. Overall, by understanding the mechanisms of HMO utilization by *B. theta* this work will reveal new insights into the early life gut colonization.

Mucin increases the virulence of enterotoxigenic *Escherichia coli*

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Enterotoxigenic *Escherichia coli* (ETEC) is an important pathogen of humans and other mammals (such as piglets and calves). Mucin, a glycoprotein that forms the major fraction of the intestinal mucus layer, is a key host substance that ETEC encounters in the host environment. This study investigated whether physiologically relevant concentrations of mucin modulate virulence-related phenotypes in ETEC. Growth of ETEC remained unaffected across mucin concentrations from 50 mg/L to 1000 mg/L. Mucin strongly increased swimming motility; at higher concentrations, the bacteria completely covered the motility plates, indicating an increase of motility beyond measurable limits. Further, mucin also increased biofilm formation in a dose-dependent manner. Reverse transcriptase quantitative PCR analysis revealed that exposure to 1000 mg/L mucin resulted in a 3.5-fold upregulation of the heat-stable enterotoxin gene *estP* 2 hours post-treatment, whereas other virulence genes were unaffected. Finally, mucin increased the virulence of ETEC in the invertebrate infection model *Galleria mellonella*, resulting in a 2-fold increase in larval death caused by mucin-treated ETEC when compared to the untreated group. These findings indicate that ETEC senses and responds to mucin and this enhances its virulence-related traits to prime infection. Understanding this host–pathogen interaction may enable the development of novel control strategies against ETEC, an urgent need given rising antibiotic resistance. One approach could involve use of mucin sugars or glycans that block bacterial responses to mucin. As this strategy does not affect bacterial viability, there will be lower selective pressure for resistance compared to conventional antibiotics.

Abstract: 70

Characterization of the Penta-CysD domain cluster of the MUC5AC mucin

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The MUC5AC mucin is the major constituent of the gastric mucus layer protecting the epithelium from acidic gastric juice. In the lungs, MUC5AC synthesis is upregulated during infection and asthma. All gel-forming mucins, with the exception of MUC6, show the same domain organization with three N-terminal von Willebrand D domains followed by variable numbers of repetitive or non-repetitive PTS-rich sequences that after glycosylation form their mucin domains. These mucin domains are separated by several approximately 90 amino acid long cysteine-rich sequences, called CysD domains.

Despite a similar domain organization MUC5B forms linear polymers whereas the 5AC mucin forms net-like structures. It has been shown that the von Willebrand D3 domain of gel-forming mucins is responsible for their polymerization through disulfide-mediated dimerization. One obvious alteration of the domain organization between MUC5AC and 5B is a cluster of five neighbouring CysD domains in the 5AC mucin. These are separated by approximately 70-90 amino acid long non-repeating PTS sequences.

In this study, we have analysed this Penta-CysD domain cluster by chemical cross-linking, mass photometry and electron microscopy. Chemical cross-linking and mass photometry data suggest that this domain cluster forms homodimers. MS data suggest interactions between the 3rd and/or the 5th domain of the respective monomers. Since this cluster of CysD domains is located C-terminal of the D3 domain the dimerization of this cluster might support the tetramerization of MUC5AC providing an explanation for the different structural arrangement of MUC5B and 5AC polymers.

Abstract: 71

Synovial Fluid Glycomics Reveals O-Glycan Remodeling and High-Mannose Signatures in Osteoarthritis

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Presenting author institute: University of California, Davis, US; Cornell University, US

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Osteoarthritis (OA) is a leading cause of chronic joint disease in humans and companion animals, yet early, sensitive biomarkers are lacking. Synovial fluid (SF) provides a window into joint biology, where glycosylation plays key roles in cartilage extracellular matrix organization, cell signaling, and lubrication. Mucins and other heavily O-glycosylated proteins are central to joint homeostasis, and disruption of their glycan structures may reflect early disease processes.

We applied lectin microarray glycomic profiling and explainable machine learning to SF from humans, dogs, and horses to identify conserved glycosylation signatures of OA. Across species, we observed broad glycan remodeling, including changes in sialylation, core fucosylation, and mucin-associated O-glycans. Increases in Core 1 and Core 3 O-glycans in canine and equine OA SF suggest altered mucin biology, potentially linked to proteoglycan 4 and boundary lubrication. In parallel, high-mannose glycans emerged as a conserved cross-species OA signature.

Machine learning accurately distinguished OA from healthy joints and identified key glycan features driving classification. This cross-species approach highlights shared disease mechanisms while revealing species-specific glycan changes.

These findings position mucins and their O-glycans as central to OA pathogenesis and promising biomarker targets. SF glycomic profiling offers a minimally invasive strategy for early detection and improved understanding of joint disease across species.

Abstract: 72

Recombinant Lubricin for Osteoarthritis Therapy: Cross-Species Safety and Efficacy

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Osteoarthritis (OA) is a leading cause of joint dysfunction, motivating the development of biologic therapies that protect cartilage and restore lubrication. Lubricin (proteoglycan 4) is a mucin-like glycoprotein critical for boundary lubrication, cartilage homeostasis, and immune signaling, yet its therapeutic translation has been limited by challenges in scalable production and delivery.

Here, we engineered recombinant lubricin analogs using a modular design that combines species-specific N- and C-terminal domains with an optimized mucin domain to preserve lubrication, tissue binding, and biocompatibility. Recombinant lubricin analogs designed to recapitulate key structural and biological features of native mucin-like glycoproteins were evaluated in preclinical models ranging from rodents to horses. In a rat anterior cruciate ligament transection model, intra-articular recombinant lubricin was well tolerated and mitigated cartilage degeneration, reducing structural damage and preserving joint integrity. In a complementary equine study, repeated intra-articular administration of species-specific recombinant lubricin demonstrated no adverse clinical, biochemical, or histologic effects, supporting safety and biocompatibility in a translational large animal model.

Together, these studies demonstrate that engineered recombinant lubricin can be produced at scale and delivered safely to the joint, where it provides chondroprotective benefits in preclinical models. This cross-species framework supports continued development of recombinant lubricin as a disease-modifying therapy for OA and highlights the translational potential of mucin-based biolubricants.

Abstract: 73

Design of lubricin-inspired glycoproteins for therapeutic use as lubricating, antifouling, and antifibrotic agents

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Lubricin is a mucin-like glycoprotein that assembles on tissue interfaces to form lubricious, antifouling surface layers. This functionality has driven interest in lubricin as a therapeutic for fibrosis, surgical adhesions, dry eye disease, and osteoarthritis. However, lubricin's complex macromolecular structure poses serious challenges to scalable manufacturing. Here, we addressed these challenges by designing lubricin-inspired proteins (LIPs) that preserve core lubricin functionality while enabling scalable production. Each LIP incorporates the mucin-like tandem repeat region of lubricin flanked by collagen-binding domains for selective assembly on tissue surfaces, plus a cleavable Fc domain for one-step purification via protein A affinity chromatography. Recombinant production in glycoengineered HEK293 cells enables tuning of LIP glycosylation profiles and functionality. LIPs applied to collagen-coated substrates and collagen-rich tissues (tendon, cartilage, fascia) showed strong surface binding, as demonstrated by immunofluorescence. LIP-coated surfaces reduced fibroblast spreading by 50% compared to uncoated controls, demonstrating potent antiadhesive activity. Together, these results establish LIPs as rationally designed mucin-like glycoproteins that selectively coat collagen-rich tissue surfaces, with potential applications in protecting and modulating the biological activity of connective tissue interfaces.

Abstract: 74

Modeling Spatial Organization of Gut Microbes in the Intestinal Mucus Layer

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Spatial organization is fundamental to intestinal function. While macroscopic arrangements of gut regions are well understood, microscopic organization between the luminal content (microbes, nutrients) and the host epithelial layer remains less explored. The mucus layer, separating luminal microbes from host cells, consists of a sterile inner layer and an outer layer harbouring microbial communities. Recent research indicated that micron-scale spatial organization plays a key role in host-microbe interactions and gut homeostasis. With the current methodology, 1) intestinal organoid-derived monolayers do not produce a reproducible intact mucus layer on top and while simultaneously maintaining a stem cell niche, and 2) it is challenging to include microbiota on top of the monolayers with an imposed spatial structure. To address these two major limitations in the field, I first optimized the stemcell media composition and added stimulants which induce increased mucus secretion. The mucus produced by the monolayers was then harvested and included in a mucus-agarose bioink in which a thin layer of *E.coli* was incorporated in varying z-axis distance from the monolayer. Confocal microscopy showed that there was a distinct layer of *E.coli* which was spatially separated from the mucus hydrogel below. These moulded hydrogels were placed on the monolayers and showed limited cytotoxicity of the host in response to the moulded *E.coli*. In the future, I will employ a more refined bioprinting approach to arrange several strains in varying microscale patterns in the x and y direction and combine these arrays with the host. These results allow me to investigate the effect of more complex microbial arrangements on the host, and vice versa.

Abstract: 75

Stability of Betacoronaviruses in Physiological Respiratory Fluids

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Respiratory viruses are expelled within mucus-derived droplets composed largely of highly glycosylated mucins, which shape the physicochemical environment, influencing viral persistence in air. Viral stability within these droplets is an important factor in airborne transmission, yet most studies rely on cell culture media that poorly reflect physiological respiratory fluids. Here, we investigated the stability of betacoronaviruses (SARS-CoV-2, HCoV-OC43, and SARS-CoV) in physiologically relevant respiratory fluid mimetics. Model fluids included a baseline airway fluid, an artificial sputum medium mimicking inflamed secretions, and a saliva-like fluid. Viral stability was assessed in 5 μ L droplets at room temperature over 24 hours. Decay kinetics and half-lives were estimated using a Bayesian regression model. Preliminary data show that viral stability varies with both fluid composition and virus type. Stability was highest in artificial sputum, whereas saliva-like fluids showed a rapid loss of infectivity. Despite its high mucin content, saliva did not enhance viral stability, suggesting mucins do not exert a purely protective effect, as seen in the literature. SARS-CoV showed greater stability in lower respiratory tract-like fluids, while SARS-CoV-2 appeared more stable in upper respiratory tract-like conditions. Additional data with increasing mucin concentrations did not increase viral persistence, supporting a non-linear role of mucins. However, in the presence of particulate matter (PM_{2.5}), mucin-rich conditions showed a moderate protective effect. Future work will examine how mucin composition influences viral stability using mucus from human airway epithelial air liquid interface (ALI) cultures.

Abstract: 76

Producing and Characterizing MUC1 Glycoforms for Understanding MUC5B Binding and Oral Mucosal Pellicle Formation

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The oral mucosal pellicle is a dynamic film formed through interactions between salivary components and membrane-associated mucins on the epithelial surface. Despite its importance for lubrication and barrier function, experimental systems that isolate the molecular determinants of pellicle assembly remain limited, often relying on animal mucins or simplified mucin compositions.

Here, a platform is developed to investigate mucosal pellicle formation using defined human mucin components. The model focuses on interactions between the membrane-associated mucin MUC1 and the major salivary gel-forming mucin MUC5B. MUC5B was purified from saliva, while recombinant MUC1 was produced in bacterial and mammalian expression systems to generate non-glycosylated and glycosylated variants, enabling controlled comparison of mucin glycoforms. Glycosylation profiles were characterised using lectins targeting distinct glycan motifs. Functional consequences were assessed by covalently immobilising mucins on tribological substrates to quantify lubrication. Complementary 96-well binding assays were developed in which immobilised MUC1 anchored pellicle formation from saliva or purified MUC5B. Both expression systems yielded purified MUC1 with distinct glycosylation profiles, demonstrating controllable glycoform generation. Surface-immobilised mucins enhanced lubrication, showing that MUC1 glycosylation influences mucin-mucin interactions and ultimately lubrication. Together, these results establish a tuneable and physiologically relevant platform for mechanistic investigation of mucin-mucin interactions and quantitative evaluation of how glycosylation influences pellicle structure and function.

Abstract: 77

Unravelling the role of gut mucosa glycome in the health-to-intestinal inflammation transition

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Inflammatory bowel disease (IBD) is a chronic gastrointestinal disorder with rising global incidence (de Souza & Fiocchi Nat Rev Gastroenterol Hepatol 2016). Crohn's disease (CD), a main IBD form, is an immune-mediated disorder affecting any part of the gut, mainly the distal ileum (Richard et al. World J Gastroenterol 2023). Clinically, CD is extremely challenging, alternating between flares and remission, with post-operative recurrence rates nearing 80% (Petagna et al. Biology Direct 2020), highlighting the urgent need to better understand health-to-intestinal inflammation transition.

Protein N-glycosylation changes play a key role in several diseases, often preceding clinical onset (Gaifem et al. Nature Immunology 2024). In IBD, altered N-glycosylation in the gut microenvironment plays a master role in IBD immunopathogenesis (Dias et al. Hum Mol Genet 2014). However, the role of the mucosal glycome in triggering immune responses and initiating inflammation remains unclear.

This study aims to unravel how gut mucosa glycome dynamics shift from health to inflammation and how it modulates immune responses in disease onset and progression.

Using mass spectrometry imaging (Boyaval et al. Mol Cell Proteomics 2021) we characterized gut mucosa glycome dynamics during the transition to inflammation. Our data strongly suggests a shift in the gut mucosa N-glycome, potentially contributing to disease onset and progression. Concomitantly, we used imaging mass cytometry (Ijsselsteijn et al. Front Immunol 2019) to assess spatial interactions between gut mucosa N-glycome and immune cells. Together, these findings improve understanding of glycome-driven inflammation and highlight new diagnostic and prognostic markers in IBD.

Abstract: 78

Syntaxins 1a, 1b and 3 Together Mediate Airway Mucin Secretion

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Airway mucins are secreted at two rates: a low baseline rate that supports mucociliary clearance and a high stimulated rate that can obstruct airways. Mucin secretion occurs by SNARE-mediated exocytosis, but the key Syntaxin isoform is not known. We evaluated candidate exocytic Stx (1a, 1b, 2, 3, 4, and 11) using IF microscopy and genetic loss-of-function approaches. Stx 1a, 1b, 4, and 11 were deleted in airway epithelium using a Scgb1a1-driven constitutive Cre. Stx3 deletion required a tamoxifen-inducible Scgb1a1-Cre to avoid embryonic lethality, and Stx2 was a whole-animal knockout. Mucin secretion was assessed by PAFS staining with image analysis. Defects in baseline secretion were identified as intracellular mucin accumulation in uninflamed mice, while stimulated secretion defects were assessed following IL-13-induced mucin production and ATP-stimulated secretion.

By IF microscopy, Stx 1a, 1b, and 3 localized to the apical plasma membrane and to mucin granules of secretory cells, Stx4 localized to the basolateral membrane of both secretory and ciliated cells, and Stx11 was not detected in airway epithelium. Deletion of Stx4 disrupted airway viability, while Stx11 deletion had no effect on mucin secretion. Loss of Stx3 resulted in marked baseline mucin accumulation and a severe defect in stimulated secretion. Stx2 knockout mice had minimal baseline accumulation and no defect in stimulated secretion. Deletion of Stx1a had minimal effects, deletion of Stx1b caused modest baseline mucin accumulation, and combined deletion of Stx1a and Stx1b caused substantial baseline mucin accumulation. Thus, Stx3 is the principal mediator of baseline and stimulated secretion, while Stx1a and Stx1b cooperate in baseline secretion.

Abstract: 79

Bacteroides carbohydrate binding modules 32 specifically recognizes O-glycan epitopes in colonic mucins

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The glycosylation of mucins has an impact on the gut microbiota. We hypothesized that the interactions between microbiota and mucin O-glycans play a key role on gut colonization. However, it remains unclear how commensal bacteria recognize and bind to mucin O-glycans.

Bacteroides encode all the required proteins to recognize and utilize a specific glycan in Polysaccharide Utilization Loci (PULs). Previous studies have shown that *Bacteroides fragilis* PUL bf3579-83 encodes the commensal colonization factor (ccf) required during gut colonization and horizontal transmission. This protein (BF3579) contains a carbohydrate-binding domain family 32 (CBM32). However, despite its key role in colonization, the binding specificity of this protein remains unclear. *Bacteroides thetaiotaomicron* (*B. theta*) BT0865 is a homolog of *B. fragilis* ccf. This protein is encoded in a PUL upregulated during growth on O-glycans and in vivo in presence of mucins. Here we show that *B. theta* CBM32 protein binds to human mucins in vitro and in human colonic tissue sections. Further structural and biochemical studies reveal that this protein is a dimer and the binding is dependent on the increased avidity. Indeed, the dimerization of BF3579 was required to promote the binding of this protein. Overall, these results suggest that gut bacteria encode binding proteins that specifically recognize O-glycan epitopes. The over-expression of these binding proteins at the bacteria cell surface increases the avidity and the binding to the target epitope. Therefore, we suggest that the control of the expression of binding proteins has a key role in driving microbiota-glycan interactions that determine the gut colonization with a healthy microbiota.

Abstract: 80

The Structure-Function relationship of the placental CD24 mucin-like molecule in preeclampsia

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Cancer and pregnancy share several biological features, including rapid proliferation, invasion, and immune modulation. At the fetal–maternal interface, a finely regulated immune tolerance is established between the mother and the fetus. CD24, a mucin-like glycoprotein expressed in a broad range of tumor cells, functions as an immune checkpoint molecule. Through interaction with Siglec-10, CD24 delivers a “don’t eat me” signal that promotes immune tolerance.

Placental CD24 is a highly glycosylated protein co-expressed with Siglec-10 on trophoblasts and uterine glands. Altered CD24 expression is associated with preeclampsia. Reduced CD24 levels have been reported in early-onset preeclampsia compared with normal term placentas, whereas CD24 protein expression is elevated in term preeclampsia.

We used BeWo and JEG3 trophoblast cell lines in a preeclampsia model characterized by overexpression of the transcription factor STOX1. We observed a significant reduction in CD24 levels in STOX1–transfected cells compared with mock controls.

Additionally, we found that CD24 and its ligand Siglec-10 were differentially expressed in placentas from patients who had recovered from COVID19 infection, with expression patterns influenced by fetal sex. In maternal plasma, CD24 levels were higher in preeclampsia. In amniotic fluid, elevated CD24 levels were detected exclusively within extracellular vesicles.

In summary, consistent suppression of CD24 expression was observed in preeclampsia placental samples, in STOX-1 cell model, and in a preeclampsia-like syndrome associated with COVID19. Given the established immunosuppressive role of the CD24–Siglec-10 axis, reduced CD24 expression may reflect impaired immune tolerance in preeclampsia.

GALNT12 and intestinal mucus barrier function

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The intestinal mucus layer forms a critical barrier that protects the epithelium from microorganisms and luminal contents. This barrier is primarily composed of mucins, heavily O-glycosylated proteins whose structure and function depend on proper glycosylation. Disruption of mucin glycosylation can compromise mucus barrier integrity and contribute to intestinal inflammation and disease.

GALNT12 is a polypeptide N-acetylgalactosaminyltransferase that catalyzes the initial step of mucin-type O-glycosylation and contributes to the extension of O-glycan structures. Reduced expression and mutations in GALNT12 have been reported in colorectal cancer (CRC), suggesting a role in disease development.

This project aims to determine how loss of GALNT12 affects mucus barrier function. Colon tissue and mucus samples from Galnt12-deficient (Galnt12^{-/-}) and wild-type (Galnt12^{+/+}) mice will be analyzed to assess mucus barrier function, susceptibility of mucus to bacterial proteases, and alterations in mucus-associated proteins. Preliminary results suggest that deletion of Galnt12 leads to altered mucus properties that compromise the intestinal barrier. This impaired barrier function may promote epithelial inflammation and increase susceptibility to CRC.

Abstract: 82

Regulation of intestinal mucosal barrier functions by transmembrane mucins

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The intestinal mucus layer consists of secreted gel-forming mucins and cell-bound transmembrane (TM) mucins that form the epithelial glycocalyx. The different members of the TM mucin family have specific barrier and signaling functions. MUC13 is the most highly expressed TM mucin in the intestinal tract. We discovered that MUC13 negatively regulates tight junction (TJ) complexes (Segui-Perez et al., 2024) and that commensal bacteria interact with MUC13 and enhance intestinal barrier functions (Segui-Perez et al., 2025). MUC13 localized to both the apical surface and the TJ regions on the lateral membranes in in vitro intestinal models including our novel Caco-2 ALI/VIP mucus model (Floor et al., 2025). MUC13 deletion leads to increased transepithelial resistance (TEER) and reduced translocation of small solutes. Removal of the MUC13 cytoplasmic tail (CT) also alters TJ composition but does not affect TEER. To identify bacteria that interact with MUC13 and might enhance intestinal barrier integrity, we performed a screening with a collection of probiotic bacterial strains. A *Bifidobacterium bifidum* strain uniquely degraded the MUC13 O-glycosylated extracellular domain. Further characterization of two probiotic *B. bifidum* strains and the *B. bifidum* wildtype strain demonstrated that the probiotic strains adhered strongly to the apical surface, had high sialidase activity, penetrated the mucus layer, and enhanced epithelial barrier integrity. These unique properties most likely contribute to the probiotic effects of these specific *B. bifidum* strains. We conclude that microbial interactions with TM mucins at the intestinal mucosal surface impact intestinal health and regulation of epithelial barrier functions.

Contribution of disulfide bonds to native bronchial mucus elasticity in stable COPD patients

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Airway mucus is critical to airway protection, but impaired mucus function due to increased elasticity is central to many chronic bronchial diseases, including COPD. Understanding what determines the viscoelastic behavior of mucus in the respiratory tract is prerequisite for any therapeutic action. The aim of this study was to quantify the contribution of mucin cross-linking via disulfide bonds (S-S) to the viscoelastic properties of bronchial mucus of COPD patients and controls.

Passive microrheology was used on mucus samples with 20 μ L volume. Increasing concentrations of the reducing agent TCEP were used to quantify the contribution of S-S density to the mucus viscoelastic properties. A synthetic mucus model with controlled S-S density was used for validation. Measurements were then made in human native mucus collected during bronchoscopy in stable COPD patients and controls (current/former smokers without chronic bronchial disease).

Elasticity of bronchial mucus from controls was of order 10 Pa and the addition of TCEP at 2.5mM was sufficient to get a liquid-like behavior (with S-S bonds density of 10^{-3} nm⁻³). This indicates that their viscoelastic properties were mainly driven by S-S bonds. By comparison, mucus from COPD patients showed higher elasticity (up to hundred of Pa). The addition of TCEP revealed a very different behavior: samples exhibit increasing rheological heterogeneities with some regions becoming liquid-like while others remaining elastic, even when TCEP was in large excess (150mM).

In native bronchial mucus of COPD patients, increased elasticity is driven in part by a increase in S-S bonds density. Our results also suggest that in COPD, components other than S-S bonds contribute to mucus elasticity.

Abstract: 84

Golgi entrapment of transmembrane mucin MUC12 defines early neoplastic progression in colon cancer

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Colon cancer is the third most prevalent type of cancer in the world. Growing evidence links its etiology to gut microbiota dysregulation and abnormal interactions of the microbiota with the colonic epithelium. The colonic epithelium is lined by a monolayer of epithelial cells coated apically by transmembrane mucins.

Transmembrane mucins provide critical protection of the interface between the luminal environment and the epithelium, including the microbiota. Yet the expression profile of transmembrane mucins in colon cancer and whether they are compromised during disease progression remains largely unknown. In this study, we found that transmembrane mucin MUC12 transcripts are enriched in colon adenocarcinomas and the MUC12 protein is enriched in colonocytes. Despite this, MUC12 has not been studied in the context of clinical outcomes of colon cancer. Therefore, we performed histological analysis of patient-matched tumors and adjacent non-affected mucosa from stage I and II colon cancer cohorts. The analysis revealed that MUC12 is predominantly expressed apically in the mucosal samples but abnormally restricted to the Golgi apparatus in the tumor samples, a unique expression pattern that was specific to MUC12 and not observed for other transmembrane mucins. The subcellular MUC12 localization distinguished the mucosa from the neoplastic regions in patient biopsies and correlated with disease progression, outperforming conventional markers for colon cancer. Our findings underscore the importance of monitoring MUC12 protein localization in colon cancer, providing new perspectives on its diagnostic and prognostic utility in the disease.

Genetic Deletion of Muc5b Reduces Interstitial Lung Disease in Neonatal Nedd4-2 Deficient Mice

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MUC5B has been implicated as a major risk factor for the development of pulmonary fibrosis. However, insights into its role in the in vivo pathogenesis of interstitial lung disease (ILD) remain incomplete. We previously developed a mouse model (Nedd4-2^{-/-}) exhibiting severe spontaneous ILD driven by congenital Nedd4-2 deletion. To functionally assess the contribution of MUC5B to ILD pathogenesis, we generated mice with a lung-specific Muc5b knockout on the Nedd4-2^{-/-} background (Nedd4-2^{-/-}/Muc5b^{-/-}).

We conducted a comparative analysis of neonatal Nedd4-2^{-/-}/Muc5b^{-/-} mice against Nedd4-2^{-/-}, Muc5b^{-/-}, and control littermates during the first month of life. Evaluations included survival, oxygen saturation, lung mechanics, BALF analysis, and histology. Muc5b levels were quantified via Western blot, with cellular phenotypes spatially analyzed using Imaging Mass Cytometry (IMC).

Genetic deletion of Muc5b rescued the ILD phenotype of Nedd4-2^{-/-} mice, reducing mortality ($p < 0.05$) and improving pulmonary function. Mechanistically, this was driven by the prevention of airway mucus obstruction and a reduction in key inflammatory markers KC, IL-1 β , IL-13, TNF- α and MIP-2 in BAL. IMC analysis further confirmed that Muc5b deletion attenuated inflammatory cell accumulation and reduced the expression of mesenchymal markers within the lung parenchyma.

Taken together, deletion of Muc5b ameliorates the severity of spontaneous ILD in the neonatal Nedd4-2^{-/-} model by preventing mucus obstruction and subsequent pulmonary inflammation. These data highlight a central pathogenic role for Muc5b in this ILD model and support its potential as a therapeutic target.

Abstract: 86

Highly scalable arrayed CRISPR mediated gene-editing in primary human bronchial epithelial cells for understanding mucus production

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Primary human bronchial epithelial cells (HBEC) cultured at air-liquid interface (ALI) have been established as an integral model for understanding lung diseases, such as asthma. Recent studies utilizing CRISPR/Cas9 gene-editing have highlighted the potential of harnessing HBECs for drug discovery purposes. However, large scale CRISPR screens in HBECs remain challenging. Here we established a robust, scalable, and automated genome editing workflow to investigate MUC5AC production in HBECs. We delivered guide RNA and recombinant Cas9 complexes to primary HBECs using high-throughput liquid handling automation and electroporated using a 384-well nucleofector. To optimize editing efficiency, we tested multiple guide RNA sources and electroporation pulses. Following electroporation, HBECs were cultured at ALI in 96-well Transwells in the absence or presence of IL-13 to model asthma. To demonstrate efficient editing, we targeted the basal cell marker, NGFR, and showed NGFR levels were depleted 5 days post-electroporation and maintained after establishing ALI. To evaluate MUC5AC production, we targeted SPDEF, a transcription factor that regulates MUC5AC, and MUC5AC itself and assessed MUC5AC secretion using a semi-automated MUC5AC dot blot. We confirmed high efficiency editing using flow cytometry and RT-qPCR at mRNA level. This work provides an automated arrayed CRISPR nuclease platform for HBECs which can be combined with various disease specific endpoint assays to prosecute genes for target drug discovery beyond MUC5AC production.

Abstract: 87

Mucin-binding activity in MUB-family adhesins resides in the N-terminal domain, not the MucBP repeats

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The mucus layer is a dynamic microbial niche structured by heavily O-glycosylated mucin glycoproteins. Bacteria colonizing this environment deploy modular surface proteins to engage mucins and other host glycans. Mucus-binding proteins (MUBs), first characterized in *Lactobacillus reuteri*, are widely described as multivalent adhesins in which tandem MucBP repeats constitute the primary mucin-binding units. Prior studies examined full-length MUB or repeat-containing constructs; however, the contribution of the N-terminal region has not been independently evaluated. Using domain-resolved, tag-free constructs, we show this model is incomplete. Mucin-binding activity localizes almost exclusively to a discrete N-terminal domain, while MucBP repeats lack detectable binding when tested in isolation, consistent with a structural scaffolding role. The N-terminal domain exhibits hallmarks of a lectin-like fold, with aromatic residue enrichment and structural similarity to known sialic acid-binding proteins. We further analyzed over 3,000 MUB-repeat-containing proteins spanning human-associated and environmental bacteria. MucBP repeats are comparatively conserved in sequence and structure, whereas N-terminal domains are highly heterogeneous, encompassing lectin-like, collagen-binding, and other ligand-recognition folds. This asymmetry identifies the N-terminus as the primary axis of functional diversification. Together, these findings support a revised model in which a conserved repeat scaffold forms a fibrillar stalk projecting a variable N-terminal effector that determines ligand specificity. This framework reframes how MUB diversity maps onto host-niche specificity and highlights limitations of repeat-based functional annotation.

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The role of intestinal Fcgbp in colitis development: implication of the gut microbiota

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The intestinal mucus layer, secreted by goblet cells, covers the epithelial surface and spatially separates the host from the trillions of microorganisms residing in the gastrointestinal tract, thereby maintaining microbial balance and intestinal homeostasis. However, mucus defects and microbiota alterations are common features of experimental and ulcerative colitis, highlighting the need to understand how mucus-associated proteins regulate barrier function and microbial homeostasis. The mucus layer is primarily built around the highly O-glycosylated polymeric mucin MUC-2, while the roles of other mucus-associated proteins, including Fc fragment of IgG binding protein (FCGBP), remain poorly understood. Here, we investigated the role of FCGBP in intestinal homeostasis using mice lacking FCGBP expression in the intestine. Under basal conditions, FCGBP deficiency resulted only in mild alterations in colonic mucus. In contrast, following dextran sulfate sodium-induced colitis, FCGBP-deficient mice showed increased disease susceptibility, including greater body weight loss, higher disease activity index, increased histopathological scores, enhanced bacterial penetration, and reduced colon length compared with controls. Additionally, changes in housing conditions, and bedding-transfer experiments to standardize horizontal microbial transmission revealed a dysbiotic inflammatory microbial signature associated with FCGBP deficiency that partially drives the disease phenotype. Collectively, these findings identify FCGBP as an important regulator of mucus structure and microbial communities involved in maintaining intestinal homeostasis.

Exploring the role of RetS as a mucin glycan-regulated mediator of virulence in *Pseudomonas aeruginosa*

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The global rise of antimicrobial resistance demands new therapeutic strategies that move beyond the direct killing of pathogens. At mucosal surfaces, mucin O-glycans have been shown to modulate microbial behavior through diverse mechanisms. In *Pseudomonas aeruginosa*, mucin O-glycans can attenuate virulence and thereby increase susceptibility to host immune clearance [1]. However, the specific glycan motifs involved and underlying molecular mechanism(s) remain unclear.

In *P. aeruginosa*, this response may be mediated by the receptor RetS. RetS is known to regulate transitions between acute and chronic phenotypes, and its periplasmic domain, DISMED2, has high homology to carbohydrate-binding proteins. Therefore, we set out to confirm whether mucin glycans interact directly with RetS, and to identify any specific mucin O-glycan motifs involved in this interaction.

Due to their high structural similarity and chemical complexity, individual mucin O-glycan structures cannot be isolated from natural sources nor produced via automated synthesis. Therefore, we have developed an in-house platform to produce individual mucin O-glycans at sufficient scale for biological analysis [2-4]. By recombinantly producing the RetS DISMED2 domain, we have been able to develop glycan-binding assays to identify specific mucin O-glycans that interact with this receptor.

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Abstract: 90

Functional characterisation of the Brazilian Yanomami microbiome along an urbanisation gradient

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The Yanomami represent the largest Indigenous people in the Amazon rainforest, living traditionally through hunting, foraging, and small-scale horticulture. Their microbiota is exceptionally diverse, which is thought to confer resilience against infections and environmental challenges. Conversely, Western populations display reduced microbial diversity, linked to so called 'Western diseases' such as obesity, diabetes, and autoimmune disorders. At the Host-Microbiota-Interaction (HMI) lab, we explore the health-promoting potential of Yanomami microbiota. In collaboration with the Yanomami community, we collected skin, oral, vaginal, and fecal microbiota samples across three villages along an urbanization gradient, alongside a Belgian reference cohort. Fecal samples were transplanted into germ-free mice to generate Human-Microbiota-Associated (HMA) models, which were immunophenotyped and challenged with high-fat diet. Belgian-HMA mice showed accelerated weight gain, and a trend towards elevated fasting glucose and liver pathology compared to Yanomami-HMA mice. Additionally, both innate and adaptive immune compartments differ in the epididymal white adipose tissue (eWAT) of Western vs. Yanomami mice, with Yanomami mice showing reduced frequencies of neutrophils, and elevated frequencies of DCs and GATA3+ CD4 T cells. These findings suggest Yanomami microbiota partially protect against diet-induced obesity and metabolic disease, highlighting the importance of indigenous microbial communities in immunometabolic health.

Abstract: 91

Antibiotics Disrupt Colonic Mucus by Altering Mitochondrial Function and Microbial Metabolites in Cystic Fibrosis

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Cystic fibrosis (CF) is characterized by thickened mucus, chronic respiratory infections, and frequent antibiotic use. Although antibiotics affect mucus production in healthy individuals, their impact on colonic mucus in CF remains unclear. To address this gap, we stained colonic biopsies from non-CF controls and individuals with CF, with or without antibiotic exposure in the last 30 days. We found that both non-CF and CF individuals exposed to metronidazole and amoxicillin had significantly reduced MUC2 compared to those without antibiotics. This reduction was confirmed in mice following antibiotic treatment. To assess epithelial effects, human colonic cells were treated with antibiotics and analyzed by RNAseq. MUC2 transcripts were unchanged, but mitochondrial pathways were altered. Resipher revealed decreased oxygen consumption, impaired respiration, and mucus secretion assays demonstrated reduced goblet cell responsiveness. Microbiome analysis via 16S rRNA sequencing revealed that antibiotic-exposed CF individuals had decreased *Bifidobacterium*, *Anaerostipes*, and *Blautia*. LC-MS/MS metabolomics showed reductions in amino acids and other metabolites. To test microbial contributions to mucus production, we grew stool-derived bioreactor communities from CF and non-CF donors with or without antibiotics. Application of microbial metabolites increased MUC2 expression in epithelial cells, but this effect was lost after antibiotic treatment. An amino acid cocktail depleted in metabolomics restored MUC2 expression. These findings demonstrate that the antibiotics impact the colonic epithelial cells and disrupt gut microbial communities, deplete key metabolites necessary for mucus production, and overall reduce colonic mucus.

Clearing the Path: The role of mucus proteolysis in enteric pathogen colonization

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A common prerequisite for enteric pathogenesis is overcoming the intestinal mucus layer to access the intestinal epithelium. Continuously secreted mucus, which lines the intestinal tract, forms an ever-renewing physical barrier and a first line of defense by preventing direct bacterial–epithelial interactions. The main protein component of the mucus layer is the MUC2 mucin that forms large oligomeric structures. We have demonstrated that Enterotoxigenic *Escherichia coli* (ETEC) uses the specialized protease EatA to degrade MUC2, facilitating access to epithelial cells. Using a novel transgenic mouse expressing human MUC2, we further showed that EatA is adapted to human mucus and lacks activity against mouse Muc2, underscoring the species specificity of the protease and pathogen. Crucially, we discovered that EatA's protease activity is strictly dependent upon the interaction between two distinct domains that are uniquely spaced in human MUC2. EatA belongs to the serine protease autotransporters of Enterobacteriaceae (SPATE) family, which are encoded by multiple enteric pathogens, many of which share structural similarity to EatA. Our results confirm that SPATEs from *Shigella* spp and enteroaggregative *E. coli* (EAEC) known as SepA and Pic rapidly degrade the human mucus layer. Plasmablast derived antibodies from ETEC infected volunteers recognize the essential MUC2-binding domain and inhibit EatA as well as the related SPATEs SepA and Pic.

Together, these findings support mucolytic SPATE proteases as a shared colonization mechanism across enteric pathogens and motivate the development of strategies that could protect the mucus barrier to limit infections.

The role of transmembrane mucin PTS domains in host-bacteria interactions

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The luminal side of enterocytes is decorated with a dense glycocalyx made up of transmembrane mucins, which are characterized by large, repetitive domains rich in proline, serine, and threonine, termed PTS domains. The literature on the role of transmembrane mucins in host-bacteria interactions is highly contradictory, describing transmembrane mucins as either as receptors for bacterial adhesion and invasion, or diffusion barriers that prevent from insulting enterocytes. The configuration of the PTS domain, defined by number of amino acid residues, their sequence, and mucin-type O-glycosylation, varies greatly between different transmembrane mucins. However, the role of the PTS domain in host-bacteria interactions remains unknown. To address this knowledge gap, we established a combinatorial library of MUC1, MUC13, and MUC17 expressed in Caco-2 BBe and HEK293 cells and used the library as a high-throughput platform to define how different PTS domain configurations impact host-microbe interactions. Benchmarking the library to the PTS-specific glycopeptidase StcE revealed varying susceptibility to cleavage, suggesting some PTS domain configurations are protective against bacterial glycopeptidases, while others might be more permissive. To further define the role of PTS configuration in the regulation of bacterial encroachment on enterocytes, we aim to knock-out or knock-in essential host enzymes involved in glycosylation, and further challenge the combinatorial library with live commensal and pathogenic bacteria and their host-engaging proteins. The combinatorial library presents a powerful approach to understanding the role of transmembrane mucins in host-microbe interactions.

Abstract: 94

Biosynthetic sugar barcodes to instruct intestinal immune cells

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Mucins are heavily decorated with sialylated O-glycans. Variation in glycan composition and density across mucins generates a wide range of mucin glycan patterns (MGPs). We propose that these patterns encode biological information that can be recognized by immune receptors. Recent studies show that specific MGPs serve as ligands for immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing sialic-acid-binding immunoglobulin-like lectins (Siglecs), which are abundant on immune cells.

We hypothesize that these self-associated molecular glycan patterns (SAMGPs) deliver inhibitory signals to intestinal immune cells, promoting tolerance. These signals are sensed by innate immune populations, including innate lymphoid cells (ILCs), via Siglecs and related inhibitory receptors. Altered SAMGP presentation is proposed to contribute to IBD.

To test this hypothesis, we will use a synthetic mucin platform that enables controlled biosynthesis of recombinant human mucins with defined glycan structures. These mucin reporters will be used to stimulate primary intestinal immune cells, including ILCs, isolated from human tissue. With functional immunological readouts, such as cytokine production, we will assess how defined glycan patterns modulate immune cell responses. Preliminary data show that IL-22 secretion by ILC3s is reduced when stimulated with a MUC2 reporter glycosylated with the STn/Tn antigen, compared to glycosylation with monosialyl T and disialyl T O-glycans.

Our research aims to uncover the immunological information encoded by mucin glycans and how these 'sugar barcodes' are interpreted by the intestinal immune system, advancing our understanding of the role of SAMGPs in immune homeostasis and intestinal inflammation.

Proteolytic control of mucus dynamics limits bacterial overgrowth in colitis

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An intact colonic mucus barrier separates the microbiota from the epithelium, and barrier defects are strongly associated with inflammation in ulcerative colitis. In experimental colitis, mucus defects precede inflammation, suggesting a causal role, but the underlying mechanisms remain unclear. Here we applied quantitative N-terminomics (TAILS) to colonic mucus to assess regulated proteolysis at steady state and during dextran sodium sulfate (DSS)-induced colitis.

At baseline, extensive processing of structural mucus proteins (Muc2, Fcgbp), proteases (Ctss, Clca1) and antimicrobial proteins (Lgals3, Lgals4) was detected. Structural integration indicated that many cleavage sites lie in constrained regions, consistent with regulated processing rather than indiscriminate degradation.

Mucus barrier dysfunction was evident after 4 days of DSS, preceding overt inflammation. TAILS revealed selective increases in mucus proteolysis, including enhanced Muc2 processing and altered Clca1 processing. This correlated with accelerated proteolysis-driven mucus growth *ex vivo*. Deletion of Clca1 abolished DSS-induced acceleration of mucus growth but did not restore barrier function or alter disease severity. However, Clca1-deficient mice showed increased mucus- and tissue-associated bacterial burden during DSS treatment. Together with an infection model demonstrating that Clca1 limits early bacterial overgrowth, these findings support a role for Clca1-mediated proteolysis in controlling microbial expansion at the mucosal surface. Our data reveal dynamic, regulated proteolytic remodeling of colonic mucus during early colitis and identify Clca1-driven mucus processing as a potential host defense mechanism against bacterial overgrowth.

Abstract: 96

Maturation of human intestinal epithelial cell layers fortifies the apical surface against Salmonella attack

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Intestinal epithelial cells (IECs) play a pivotal role in nutrient uptake and also function as a vital defense barrier against the external environment, including enteric pathogens such as *Salmonella enterica* serovar Typhimurium (*Salmonella*). This duality is achieved by the cells' specialized apical surface displaying dense microvilli, a thick glycocalyx, and a secreted gel-forming mucus layer. However, the interplay between invading enterobacteria and the apical IEC barrier, as well as the factors governing IEC susceptibility, are not fully understood. By performing live-cell infection imaging in human enteroid-/ colonoid-derived IEC layers across maturation states, we were able to show how human IEC maturation and a build-up of the glycocalyx fortify the apical surface against *Salmonella* attack. Therefore, the susceptibility of mature enterocytes/colonocytes is reduced by up to 10-fold compared to immature cell layers. This shift is nullifiable by StcE-enzyme treatment, which cleaves the protein backbone of glycocalyx components like MUC13 in a glycosylation-dependent manner. Moreover, we investigated the physicochemical barrier properties of the secreted mucus layer using air-liquid interface (ALI) cultures. With this culturing approach, we are able to push differentiation of multiple intestinal epithelial cell types, as well as allow the formation of a protective mucus layer on top, showing a significantly delayed *Salmonella* arrival at the apical cell surface. Our findings underscore how the biochemical and structural composition of the apical epithelial surface affects micro-barrier properties and susceptibility to bacterial invasion using organoid technology.

Abstract: 97

Mucus barrier function in patients with ulcerative colitis

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In the healthy intestine, the epithelial surface is protected from direct contact with the gut microbiota by a protective mucus layer that helps to prevent inflammation. Diet is a major determinant of gut microbiota composition and, through this interaction, it can influence the mucus barrier integrity. In ulcerative colitis (UC) the mucosal barrier has been described as defective. Although alterations in the gut microbiota have been observed in UC, their mechanistic role remains poorly understood. This study investigates how gut microbiota and diet contribute to the defective mucus layer in patients with UC. Patients with UC and unaffected controls undergoing routine colon cancer screening are recruited at Norrlands University Hospital, Sweden. Colonic biopsies and faecal samples are collected, and participants complete a food frequency questionnaire to further link diet, microbiota, and mucus function. Mucus function in colon biopsies is assessed by using ex vivo analysis of mucus thickness and growth rate. Mucus penetrability is further evaluated by using bacteria sized fluorescent beads visualized by confocal z-stack imaging to quantify bead distance from the epithelial layer. This study will include samples from 45 participants, including 15 UC patients with acute inflammation, 15 patients in remission, and 15 controls. Ongoing analyses will integrate dietary profiles and microbiota composition with observed mucus phenotypes to identify factors associated with impaired mucus barrier function in UC.

Abstract: 98

Citrobacter rodentium infection disrupts colonic mucus barrier function via ablation of intercrypt goblet cells

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The colonic mucus barrier consists of distinct layers of mucus maintained by specialized goblet cell (GC) subtypes and serves as the first line of defense against enteric pathogens. However, the specific contributions of the inner mucus layer (IML) and the recently described fecal mucus layer (FML) to barrier integrity, as well as the ways in which these layers and GC responses are modulated during infection remain poorly defined. Therefore, by using a combination of *Citrobacter rodentium* infection in mice and ex vivo live mucus imaging, we demonstrate that early epithelial colonization disrupts the IML and increases mucus penetrability. Further, we used a novel ex vivo imaging technique to visualize dynamic alterations in stool associated FML thickness and luminal pathogen distribution. Our data revealed infection induced alterations in FML thickness with progressive accumulation of pathogen clusters within the fecal mucus. Transcriptomic and functional analyses identified Spdef-dependent intercrypt goblet cells (icGCs) as vital for sustaining IML integrity and limiting epithelial access. Spdef-deficient mice exhibited delayed clearance and enhanced crypt invasion, highlighting the protective role of icGCs. Infection while selectively depleting icGCs, collapsed the intercrypt mucus and created permissive niches within the IML. Mechanistically, *C. rodentium* leveraged its type III secretion system, specifically the EspF effector to reprogram GC transcriptional responses, inducing metabolic stress that may promote GC loss. These findings reveal a previously unrecognized virulence strategy in which EspF-driven GC remodeling compromises intercrypt mucus defenses, likely facilitating pathogen persistence and transmission.

Abstract: 99

Developing A High-Throughput Model Mucosal Pellicle to Optimise Interactions & Retention of Oral Actives with the Mouth.

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Mucins play an essential role in oral health, for both oral lubrication and antimicrobial activity. The oral mucosal pellicle (MP), consisting of the mucins MUC1, MUC7 and MUC5B lining the oral epithelium, may act as a key reservoir for retention of oral actives (OAs) in dental care products. These OAs such as commonly used sodium fluoride, stannous fluoride, and chlorhexidine both remineralise damaged enamel and exert antimicrobial effects within the mouth. A large proportion, however, are washed off due to salivary flow and oral rinsing. To optimise the effectiveness of these actives, it is essential for them to be retained for longer within the oral cavity. This project aims to create a physiologically relevant, high-throughput model MP, to optimise interactions with OAs and their effects on dental enamel.

Bovine submaxillary mucin (BSM) was used as a model mucin and dried onto 96-well plates. The model MP was characterised through confocal imaging after fluorescent staining. Nanoparticle retention on the model MP was tested via fluorometric analysis. Up to 80% (+/- 4.28) mucin was retained on the plate surface forming ~60µm-thick mucus-like layers. Fluorescent nanoparticles were successfully retained on the model MP and were released bi-phasically ($t_{1/2}=1.5\text{min}$ in the first 15 mins and $t_{1/2}=4\text{min}$ for the last 45 mins) after initial application.

In conclusion a novel model MP was formed and characterised. Its properties allowed for both size- and charge-dependent nanoparticle retention mechanisms, and was compatible for high-throughput testing. Future work will look to optimise retention of OAs encapsulated within nanoparticles on the model MP and test the effects of optimised OA retention on biofilms and enamel.

Abstract: 100

FFAR2 Regulates Goblet Cell Function and Mucus Dynamics in the Colon

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Goblet cells (GCs) are mucosal epithelial specialised cells in producing gel forming mucins. GCs are essential in maintaining intestinal barrier function, primarily through Muc2, which is essential for maintaining the intestinal mucus barrier. GCs and mucus are regulated by the microbiome, but the mechanism in underlying this regulation remains unclear. In the distal colon, GC intrinsic defence systems regulate host-microbiota interactions, which help prevent mucosal inflammation and colorectal cancer.

Short-chain fatty acids (SCFAs) produced by microbial fermentation of dietary fibre, act as both energy sources and signalling molecules. They bind to free fatty acid receptors (FFARs), which regulate immune activation, proliferation, inflammation, and barrier integrity. FFAR2 is implicated in gut hormone secretion, adipogenesis, and anti-inflammatory responses. Our data suggest FFAR2 is highly expressed in GCs, indicating a potential pathway for their regulation.

In this study, we aimed to define the role of FFAR2 signalling in intestinal GCs using a conditional knockout mouse model (muc2-iCre x FFAR2) to selectively delete FFAR2 in GCs. We combined *Citrobacter rodentium* infection models, antibiotic intervention, metabolic labelling with GalNaz, and motility assays to assess GC function and mucus dynamics.

Our findings show that FFAR2 is highly expressed in colonic GCs and may mediate responses to bacterial metabolites. GC-specific FFAR2 knockout mice exhibited increased resistance to infection, accelerated intestinal motility and slower mucus turnover. Together, these results identify FFAR2 as a key regulator of GC function and intestinal homeostasis.

Abstract: 101

MucinGel® Biomimetic Platforms Reveal Mucus-Dependent Bacterial Responses

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Experimental setups that recapitulate the environment of the living gut are needed to glean understanding of mucus-bacteria interactions. To this end, we developed a surface-based system (2D, coated glass coverslips) and a 3D system (hydrogel matrix microbeads) within a microfluidic device. A mucus-mimetic material from purified porcine gastric mucin crosslinked with genipin (MucinGel®)[1] was used to spin-coat glass coverslips and demonstrated an approximate three-fold increase in biofilm biomass of the mucin-degrading bacterium *Bacteroides thetaiotaomicron* compared with mucin non-degrading *Chlostridium difficile* and *Lactobacillus paracasei*. MucinGel® was also blended with agarose to generate co-gel matrix microbeads for immobilizing bacterial cells at low density in a prototype microfluidic platform for single-cell phenotypic profiling (CellCity®). Two *Escherichia coli* quorum sensing (QS) reporter strains[2] were immobilised on agarose- MucinGel® microbeads and microimaged in CellCity® during 14 h revealing an early onset of the QS response. These platforms provide versatile tools to investigate mucin-dependent microbial behaviors and may contribute to uncover strategies on how to modulate them.

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Abstract: 102

Structural and functional aspects of SIgA–mucin interactions in saliva

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Salivary mucins are key organisers of the oral environment, contributing to lubrication, surface protection and microbial patterning at mucosal interfaces. Among them, the gel-forming mucin MUC5B provides a major structural scaffold within saliva, yet the extent to which it interacts with other host defence factors to shape microbial organisation remains poorly understood. Secretory IgA (SIgA), the predominant immunoglobulin in saliva, is a major mediator of mucosal immunity and has been proposed to influence microbial aggregation and retention. However, the structural and functional basis of SIgA–mucin interactions in saliva remains unclear. Here, we investigate potential interactions between SIgA and MUC5B as a candidate host-derived organising network in the oral cavity. We use protein structure prediction and molecular modelling to explore plausible modes of association and candidate interaction interfaces. These analyses are combined with biochemical fractionation and biophysical characterisation to assess whether SIgA and mucin-associated fractions co-exist in saliva under native conditions. In parallel, fluorescence imaging is used to examine the spatial relationship between SIgA, Muc5B and oral bacteria within salivary samples. Future work will extend this framework using IgA-based microbial profiling to assess patterns of selective bacterial coating in saliva. This work aims to define whether mucin–immunoglobulin interactions contribute to the supramolecular organisation of saliva and thereby influence early bacterial aggregation and colonisation. A better understanding of these processes may provide new insight into how mucin-centred host defence networks support oral mucosal homeostasis in health and disease.

Abstract: 103

Mucosomes as mucin-inspired nanocoatings for translating mucus protection onto artificial surfaces

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Mucus is far more than a passive barrier. It actively restricts microbial adhesion, modulates interactions with bacteria and viruses, and sustains a chemical microenvironment hostile to colonization. These properties stem largely from mucins, the high-molecular-weight glycoproteins that give mucus its distinctive architecture and biological functionality. Translating mucin-derived properties to engineered surfaces could therefore offer a biologically grounded strategy for generating protective interfaces on substrates where microbial colonization leads to infection, functional degradation, or material damage. Mucosomes are glycosylated, mucin-derived nanoparticles designed to retain key mucin bioactivities, including intrinsic antimicrobial properties, while also functioning as carriers for bioactive cargo [1,2]. Their particulate form makes them compatible with surface deposition, raising the possibility of converting diverse substrates into mucus-inspired protective interfaces without requiring chemical modification of the underlying material.

Here, we tested this concept across substrates spanning distinct material classes and application contexts, including polymeric surfaces, organic fibrous materials, and gel-based matrices. Mucosome deposition was achieved across all tested substrate classes, producing detectable nanoparticle coatings that did not meaningfully alter key substrate properties, whether optical or structural. For all the materials tested, mucosome-functionalized surfaces displayed measurable antimicrobial activity, and payload-loaded formulations retained localized release capacity at the surface, functioning as payload-eluting depots. These results provide a proof of principle for mucosomes as a transferable surface-engineering tool that carries the “biological intelligence” of mucus into material systems well beyond native tissue, across a range of fields where controlling the surface-microbe interface is critical.

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Differential Responses to Mucus-Targeted Treatments in Lung Disease Support a Personalized Therapeutic Approach

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Excessive and pathologically altered mucus is a hallmark of chronic respiratory diseases, where increased viscoelasticity impairs mucociliary clearance and promotes infection. This study investigates the rheological effects of mucolytic agents using a reproducible synthetic mucus model based on snail slime mucins cross-linked via disulfide bonds (Milian et al. 2023). Rheological characterization was performed using strain sweep measurements to evaluate key parameters governing mucus behaviour, including viscoelastic modulus (G^*), damping factor ($\tan \delta$), critical strain (γ_c), and critical stress (σ_c), which collectively describe mucus consistency and flow initiation. Four mucolytic agents with distinct mechanisms of action were assessed: isotonic saline (ISS), hypertonic saline (HSS), N-acetylcysteine (NAC), and recombinant human DNase (RhDNase). Results demonstrate mechanism-dependent effects on mucus structure. NAC exhibited the most pronounced activity, reducing G^* by up to 90% and decreasing σ_c by over 80%, indicating substantial disruption of the mucin network and enhanced flow initiation. HSS showed moderate effects consistent with hydration-driven softening, while ISS induced only minor dilution effects. RhDNase displayed negligible impact in this model, likely due to the absence of extracellular DNA in the synthetic system. These results should be interpreted within the scope of the model system. The present study primarily evaluates the response of a controlled synthetic mucus to different mucolytic mechanisms, rather than directly predicting clinical efficacy. While the observed trends are consistent with known modes of action, extrapolation to patient outcomes remains limited. Indeed, clinical evidence highlights heterogeneous responses to mucolytic therapies depending on disease context, for example, RhDNase is effective in cystic fibrosis but shows limited benefit in non-CF bronchiectasis. In the randomized trial by O'Donnell et al. (1998), approximately 300 patients with non-CF bronchiectasis were divided into placebo and RhDNase-treated groups, with overall negative outcomes observed in the treatment arm. However, the mean sputum DNA concentration was similar across groups at time of enrolment, and no stratification based on mucus composition was performed. This raises the possibility that subgrouping patients according to relevant biochemical features, could reveal differential treatment responses that remain obscured in conventional analyses. More broadly, current patient stratification is largely disease-based rather than phenotype-driven, potentially masking subsets of patients who could benefit from targeted mucolytic therapies. These observations underscore that mucus composition and pathology-specific factors critically influence treatment response. As such, the current work should be viewed as a validation of a rheological platform for probing mucus-treatment interactions, providing a foundation for future studies incorporating more complex, biologically relevant samples and supporting the broader goal of phenotype-driven, personalized therapeutic strategies.

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