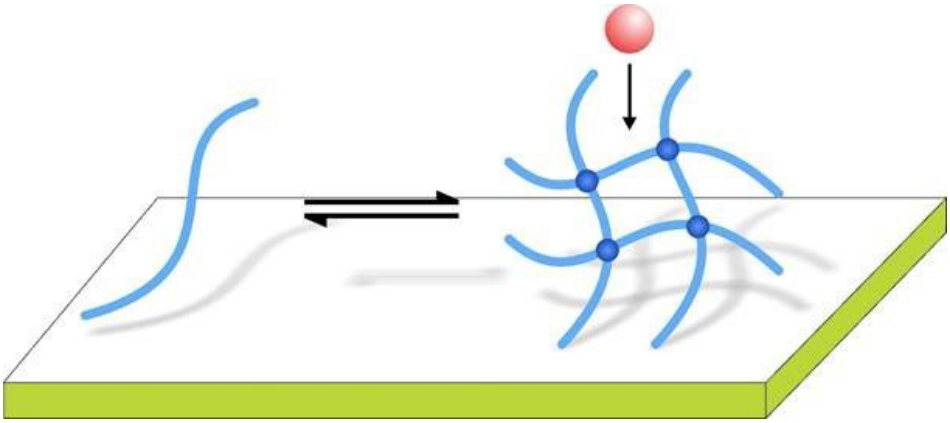
Book of (Poster) Abstracts

3rd International Symposium of the Collaborative Research Center (CRC) 1449 "Dynamic Hydrogels at Biointerfaces" October 5-6, 2026



Venue & Location

Freie Universität Berlin
Institute of Chemistry and Biochemistry
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Program

Monday, OCTOBER 5, 2026

8:30 REGISTRATION

9:00 OPENING
Prof. Rainer Haag | Spokesperson of the CRC 1449, Freie Universität Berlin

SESSION – INTERACTIONS WITH BARRIERS IN THE LUNG

Chair: Marcus Mall

09:15 Clinical features of mucus plug-high asthma and COPD and strategies to treat them
Prof. John Fahy | University of California, San Francisco, USA

10:00 Using glycosaminoglycans to guide precision medicine in the intensive care unit
Prof. Eric Schmidt | Harvard Medical School & Massachusetts General Hospital, USA

10:45 COFFEE BREAK

SESSION – MUCUS HYDROGELS AND GLYCOCALYX

Chair: Beate Koksch

11:15 Modulating the permeability of mucin gels with nanoparticles – nanoplastics vs nanomotors
Prof. Oliver Lieleg | Technische Universität München, D

11:40 Organized surface layers on the apical surface of the alveolar epithelium: Ultrastructure, composition, and component-driven barrier function
Dr. Shailey Twamley (B01) | Charité – Universitätsmedizin Berlin

12:05 Role of *Pseudomonas aeruginosa* in mucus dysfunction in bronchiectasis
Dr. Christine Wong (C04) | Charité – Universitätsmedizin Berlin

12:30 LUNCH BUFFET

SESSION – MUCINS AND GLYCOSYLATION

Chair: Christian Hackenberger

13:30 Mucus Hydrogels – Polymerization and Glycosylation in Models of Lung Disease
Prof. Christopher Evans | University of Colorado Anschutz, USA

14:15 Chemical Precision Tools to Track the Cellular Activity of Glycosyltransferases
Prof. Benjamin Schumann | Technische Universität Dresden, D

14:40 Overcoming barriers - a cell-permeable nanobody to restore CFTR activity in cystic fibrosis
Dr. Luise Franz (C05) | Leibniz-Forschungsinstitut für Molekulare Pharmakologie Berlin, D

15:15 15 SPEED LECTURES from selected posters

1. Luca Rota | MPI KG
2. Friederike Marte | TUD
3. Shirsha Mukherjee | Charité
4. Dr. Andreas Achazi | FUB
5. Annelie Puhlmann | FUB
9. Roman Sprick | TUB
10. Jana Reh | TUM
11. Aditi Löwe | Charité
12. Cody Moose | Stanford
13. Nico Kub | FUB

6. Anton Michel-Busch | TUB
7. Dr. Yannic Kerkhoff | HZB
8. Mostafa Ahmadi | Uni Mainz
14. Mariella Gabler | MPI-KG
15. Oguzhan Aksu | FUB

16:15 COFFEE BREAK & POSTER SESSION

18:30 CONFERENCE DINNER
Alter Krug Dahlem, Königin-Luise-Straße 52, 14195 Berlin

Tuesday, OCTOBER 6, 2026

8:30 REGISTRATION

SESSION – MUCOLYTICS AND RHEOLOGY

Chair: Marina Pigaleva

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Prof. Gerald Fuller | Stanford University, USA

9:45 Mucus plugging in asthma: molecular and cellular insights
Prof. Maude Liégeois | Université de Liège, BE

10:30 Magnetic Microwire Rheology: Theory and Data Analysis
Merten Strehlau (A01) | Technische Universität Berlin

10:50 COFFEE BREAK

SESSION – MUCIN FUNCTION AND HYDROGEL CHARACTERIZATION

Chair: Kevin Pagel

11:20 Regulation of mucus barrier function
Prof. Dave Thornton | The University of Manchester, UK

12:05 Interaction of Mucus and Metals – How Metalloglycobiology Shapes the Mucus Composition
Prof. Maria Maares | Universität Potsdam, D

12:25 PEG-induced collapse of mucin-inspired polymers during gelation revealed by EPR spectroscopy
Dr. Marina Pigaleva (Z01) | Freie Universität Berlin

12:45 LUNCH BUFFET

SESSION – MUCUS BARRIER FUNCTION

Chair: Daniel Lauster

13:45 Mucus and Biofilms as Biological Barriers to Anti-infective Drug Delivery
Prof. Claus-Michael Lehr | Universität des Saarlandes & Helmholtz-Institut für Pharmazeutische Forschung Saarland, D

14:30 Integrative computational analysis to study the interplay between mucus, infection and inflammation in cystic fibrosis
Dr. Laura Schaupp (B05) | Charité – Universitätsmedizin Berlin

14:50 Motion of Salmonella Typhimurium in Bulk, Hydrogels, and Mucus
Prof. Stephan Block (B03) | Freie Universität Berlin

15:15 COFFEE BREAK

SESSION – HYDROGELS FOR CELL CULTURE AND FUNCTION

Chair: Rainer Haag

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Prof. Ronit Satchi-Fainaro | Tel Aviv University, ISR

16:30 The structure of a natural human mucin

Dr. Gaël Vos (C01) | Freie Universität Berlin

16:50 Finding the Sweet Spot: Identifying an Alveolar Biomarker Target

Dr. Karen Hoffmann (C03) | Charité – Universitätsmedizin Berlin

17:10 Decoding protein glycosylation using ion mobility-mass spectrometry

Dr. Leïla Bechtella | Université Evry Paris-Saclay, F

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Prof. Rainer Haag | Freie Universität Berlin

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Effect of Neutrophil Elastase on Mucus Viscoelasticity and Mucociliary Transport in Muco-Obstructive Lung Disease

N. Reuter1,2,3, I. Catanese1,2,3, T. Rubil1,2,3, C. Wong1,2,3, M.A. Mall1,2,3
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Persistent neutrophilic inflammation is a hallmark of muco-obstructive lung diseases such as cystic fibrosis (CF) and bronchiectasis. The imbalance between excessive neutrophil serine proteases (NSP) and insufficient antiproteases drives chronic inflammation, mucus hypersecretion, impaired mucociliary clearance, and airway damage. However, the direct effects of NSP on mucus biophysical properties have not been investigated.
We therefore aimed to evaluate the effects of NSP on mucus rheology and function in CF using ex vivo and in vitro approaches. CF and healthy sputum were treated with either neutrophil elastase (NE) or protease inhibitors (PI), followed by cone-plate rheometry to assess the elastic modulus (G') and viscous modulus (G''). Our results demonstrate that NE exerted a concentration-dependent reduction in viscoelasticity, with the most pronounced decrease in G' and G'' observed at 100 µM NE. PI induced a dose-dependent increase in both G' and G''.
To further investigate the role of NSP on chronic inflammation and impacts on mucus properties, primary airway epithelial cell cultures derived from CF patients were stimulated with IL-1β and NE to mimic chronic inflammatory condition in the airway. Mucus function and rheological properties were assessed via mucociliary transport (MCT) velocity and fluorescence recovery after photobleaching (FRAP). IL-1β stimulation induced mucus secretion and hereby caused an increase in viscosity. NE reduced mucus viscosity within 24 hours and restored MCT within 6 hours.
These data provide initial evidence that NE contributes to the modulation of viscoelastic properties on mucus in muco-obstructive lung diseases, but the regulatory mechanisms remain to be elucidated.

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A cell-permeable nanobody synergizes with CFTR modulators to restore near-normal F508del-CFTR function in airway epithelial cells from patients with cystic fibrosis

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Background

The most common cystic fibrosis (CF)-causing mutation, F508del, resides within nucleotide-binding domain 1 (NBD1) of the CFTR chloride channel. The current gold standard therapy for patients carrying at least one *F508del* allele is the triple combination therapy elexacaftor/tezacaftor/ivacaftor (ETI). Although ETI therapy restores F508del-CFTR function to ~50% of normal in patients, it does not stabilize the thermodynamically disrupted NBD1, and patients on ETI continue to exhibit persistent airway infection and inflammation. Furthermore, natural-history studies indicate that near-normal CFTR function is required to prevent CF-related lung disease. Here, we investigated a novel NBD1-targeting nanobody (NB1) that stabilizes F508del-CFTR and may enhance ETI efficacy, but whose functional impact has remained unclear due to challenges in intracellular delivery.

Methods

We synthesized a cysteine-containing NB1 and conjugated it to a CPP (R10) *via* a disulfide bond. CFBE41o- cells expressing F508del-CFTR were treated with NB1-R10, and uptake of

fluorescently labeled NB1-R10 was assessed by live-cell microscopy and Western blot. Dose-response studies on the effect of NB1-R10 on F508del-CFTR function were conducted using transepithelial short circuit current (I_{sc}) measurements, quantifying CFTR inhibitor-172-sensitive I_{sc} (ΔI_{sc} CFTRinh-172) following cAMP-mediated activation. To assess CFTR maturation, Western blot analysis of whole-cell lysates was performed. Primary airway epithelial cultures were established from nasal brushings of three patients with CF homozygous for *F508del*. Intracellular uptake of NB1-R10 in these cultures was examined by live-cell microscopy. The effect of NB1-R10 monotherapy on F508del-CFTR function, as well as its synergism with ETI treatment, was assessed using I_{sc} measurements.

Results

Conjugation with R10 enabled dose-dependent intracellular delivery of NB1 in CFBE41o- cells, resulting in enhanced maturation and trafficking of F508del-CFTR to the apical cell membrane, and significantly enhanced F508del-CFTR channel function compared to vehicle-treated control. Furthermore, co-treatment with NB1-R10 and ETI led to a ~3.8-fold increase in F508del-CFTR function compared to ETI alone in CFBE41o- cells. In highly differentiated, patient-derived airway epithelial cultures, treatment with NB1-R10 demonstrated efficient intracellular uptake. I_{sc} measurements revealed significantly increased F508del-CFTR function compared to vehicle (ΔI_{sc} CFTRinh-172 = -2.84 ± 0.19 μA/cm² vs. -1.49 ± 0.07 μA/cm²; $p < 0.0001$). ETI therapy alone restored F508del-CFTR function to 55% of normal levels (ΔI_{sc} CFTRinh-172 = -23.01 ± 1.17 μA/cm² vs. -41.52 ± 5.42 μA/cm²). Notably, co-treatment with NB1-R10 and ETI significantly enhanced F508del-CFTR rescue compared to ETI alone (ΔI_{sc} CFTRinh-172 = -36.85 ± 1.54 μA/cm²; $p < 0.0001$), achieving ~88% of normal CFTR function.

Conclusions

Our results show that stabilization of F508del-CFTR via efficient delivery of NB1-R10 enhances the functional rescue of CFTR achieved by ETI in airway epithelial cultures from patients with CF. This study highlights the utility of cell-permeable nanobodies for modulating protein function and underscores their therapeutic potential as next generation biopharmaceuticals for intracellular targeting.

Small Angle Neutron Scattering Investigation of Mucus Mimicking Glycosylated Peptide Hydrogels

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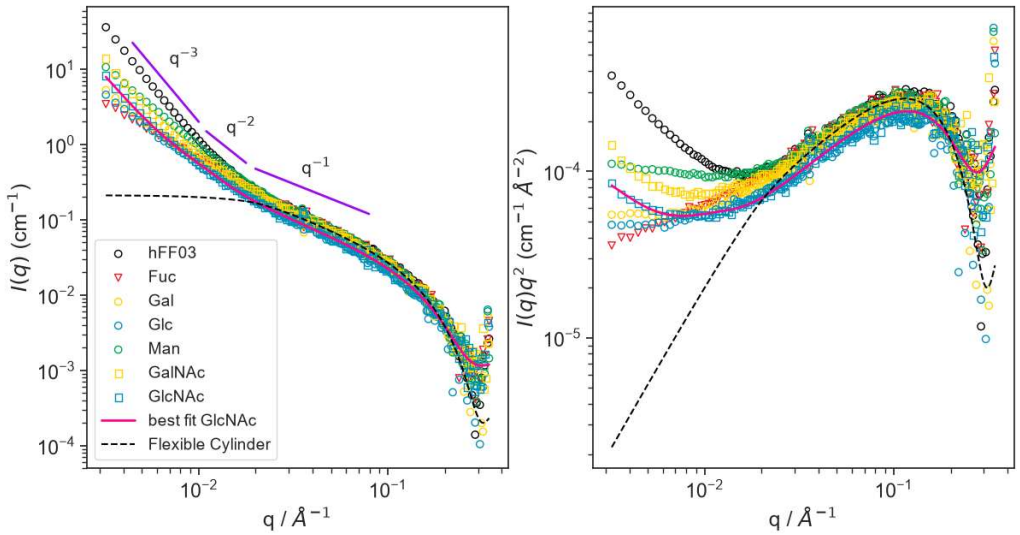

Figure 1: Scattering data of hFF03 peptides with and without glycosilation (0.1 wt% in PBS buffer in D2O). The dashed line represents a simulated flexible cylinder model, the solid red line shows the best fit of the combined flexibly cylinder fractal aggregate model for the *N*-acetylated galactose modified sample.

This study investigates glycosylated peptide hydrogels using small-angle neutron scattering (SANS) to elucidate the influence of glycan modification on the hydrogel structure. The scattering data reveal that the local structure of peptides within self-assembled hydrogels remains consistent across different glycan modifications, exhibiting rod-like particle behaviour with a characteristic q^{-1} intensity scaling at intermediate scattering vector q. Going beyond previous studies, the data are fitted with a combined structure factor-form factor model, based on previous works by Teixera and Pedersen & Schurtenberger.

This analysis, along with model-free fitting, revealed consistent cross-sectional radii of peptide fibrils (1.1–1.3 nm) and persistence lengths (10–30 nm) across modifications, suggesting a stable structural backbone exists in all analysed hydrogel networks. The structure factor model revealed differences in the fractal dimension of the hydrogel network, with denser networks observed for N-acetylated modifications (GlcNAc, GalNAc) and unmodified peptides. At the same time, a weak negative correlation was found between persistence length and fractal dimension, suggesting that more flexible peptide chains facilitate denser network formation. In addition, it was found that glycan modification influenced the swelling of peptide chains, with undecorated peptides exhibiting the highest hydration, highlighting the role of glycosylation in modulating mucus hydration.

These results provide insights into the structural impact of glycan modifications on peptide hydrogel networks, offering a foundation for designing tuneable mucus-mimicking hydrogels for biomedical applications.

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Probing the Viscoelasticity of Mucus-like Hydrogels

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Understanding the viscoelastic properties of mucus is crucial for its function as a barrier against both particles and pathogens. To gain insight into the dependence of viscoelastic properties on microscopic parameters, we study mucus-like model systems through coarse-grained simulations and experiments. On the simulation side, we develop coarse-grained models that yield the structure factor and the viscoelastic spectrum of PEO hydrogels in good agreement with experiments. Experimentally, these systems are characterized using macrorheology and by tracking the diffusion of small tracer particles. In our simulations we also model tracer diffusion, with the aim to disentangle the roles of mesh size and surface adhesion. Further, we experimentally study the properties of hydrogels undergoing cross-linking via hydrophobic domains and hydrogen bonds, which can be done by employing mixtures of PEO and (hydrophobically modified) polyacrylate. Introducing such additional interactions on top of the always present effects of chain entanglement, renders the hydrogels systematically more complex, thereby becoming closer to structure and properties of mucus. In parallel, we are further developing coarse-grained models of transiently cross-linked hydrogels to understand the viscoelastic properties of mucin-like hydrogels. Collectively, our work aims to develop understanding of the structure-dynamics relationship and transport in mucus-mimicking hydrogels, from simple PEO networks to systems with mucin-like transient cross-linking.

Permeability of glycan-presenting, dynamic hydrogels

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Biological hydrogels such as mucus act as selective barriers that regulate the transport of (macro)molecules, thereby protecting the underlying epithelium while enabling controlled molecular exchange across mucosal interfaces [1]. Permeability, however, is not determined by steric obstruction alone: electrostatic and other interactions between the macromolecules and the polymer network critically shape transport, giving rise to selective retardation, trapping or even band-pass filtering [2]. In this project, we aim to quantify how glycan presentation governs macromolecular transport into glycan-decorated hydrogels. To this end, we developed a transwell-based confocal microscopy assay in which the diffusion of fluorescently labelled macromolecules from a bulk compartment into a hydrogel-containing compartment is tracked by recording z-stacks. By analysing the time-dependent concentration profiles, we extract diffusion coefficient and free energy profiles of the macromolecule within the probed hydrogel.

We apply this assay to glycan-decorated hFF03 peptides, which are able to form dynamic hydrogels via coiled-coil interactions and which allow steric and interaction-driven effects to be systematically decoupled [3]. Following the transport of FITC-labeled dextrans, we obtain information of the average hydrogel mesh-size. Furthermore, quantifying the transport of Alexa Fluor 488-labelled wheat germ agglutinin (WGA-AF488) into hFF03 hydrogels presenting varying fractions of the sialylated glycan 3'SiaLac and mannose, we show that lectin–glycan interactions govern WGA transport within the hydrogels. Our results demonstrate that sialylated glycan motifs embedded in the hFF03 hydrogel remain accessible for lectin recognition, and highlight the importance of coupling dynamic transport measurements with interaction-sensitive probe selection when characterizing permeability in soft, glycan-presenting hydrogels.

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Towards Structurally Diverse MUC5AC Glycopeptide Models

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Mucins are highly O-glycosylated proteins that are major components of mucus and play a role in its structural and physical properties. However, the relationship between glycosylation and the conformational properties of long mucin tandem repeat (TR) domains remains poorly understood because structurally defined glycopeptide models are limited.

Previous work in our group established efficient synthetic access to highly GalNAcylated MUC5AC tandem repeat peptides and demonstrated that glycoclustering affects both peptide conformation and backbone rigidity.[1] While progressive α-O-GalNAcylation promoted PPII helical features, pronounced backbone stiffening was observed only at higher degrees of glycosylation.

Native mucins, however, carry a variety of more complex O-glycans. In the present work, we extended the MUC5AC model system beyond α-O-GalNAc towards different and more complex O-glycan structures. Glycoamino acid building blocks representing α-GalNAc, β-GalNAc, β-GlcNAc, Core 1, and Core 3 structures were prepared and incorporated into MUC5AC-derived peptides. A recently developed synthetic protocol from our group was further optimised for these building blocks and extended to longer TR constructs. The resulting series comprises defined glycopeptides that vary in both glycan structure and peptide length.

This series is currently being characterised to investigate how glycan identity and complexity influence the conformational properties of MUC5AC-derived peptides and whether the effects observed for GalNAc clustering extend to more elaborate mucin-type glycans.

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Identifying interactions of Cy3-dyes in nano environments

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Epithelial mucus is the first barrier of our immune-system against external pathogens like bacteria, viruses and other particles. This mucus barrier consists of a fine balance of mucin networks, proteins and water which form a unique structure that enables the capture, encasing and transport of these particles. In respiratory disease, this balance becomes unstable, which can lead to abnormally high viscosity in the hydrogel and an impaired barrier function. Despite an upward trend in respiratory disease worldwide and with chronic obstructive pulmonary disease (COPD) as the third leading cause of death globally in 2023, the mechanics of pathogen penetration, barrier function and disease-related-changes in mucus remains barely understood.

To map spatial viscosity distributions in mucus and mucus-mimetic hydrogels, we utilize fluorescent molecular rotor (FMR) dyes such as Cy3. Each FMR reports the viscosity of its direct vicinity, which can be read out via the FMR's fluorescent lifetime. Combined with fluorescent lifetime imaging microscopy (FLIM), FMR lifetimes, and thus viscosity data, can be quantified with sub-µm spatial resolution. To calibrate FMRs for such applications, we determine their fluorescence decay curves when suspended in simple solutions of known viscosity (*e.g.*, glycerol- and glycan-containing buffer solutions). Applied to the FMRs Cy3-maleimide and Cy3-WGA in glycerol/H_2O solutions at 21°C and 37°C, we observe multiple components in the FMR's decay curves, which differ in their response to the bulk viscosity. We will discuss potential mechanisms for this behavior and the implications for application in FLIM-based viscosity mapping.

Modelling the intestinal mucus barrier in health and disease

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The intestine is site of various pathologies, ranging from infectious diseases to chronic inflammations, as well as cognate malfunctions. One key element affected in these malignancies is the intestinal mucus layer, located between the lumen and epithelium. The use of **patient-derived intestinal organoids** enables access to a model that captures both site- and patient-specific characteristics of the donors. While this model system has already been used to study various mechanisms of epithelial differentiation, a dedicated **characterization of the *in vitro* generated mucus layer** and the mechanisms involved in its generation is still lacking. Here, we present a **transwell filter-based** air liquid interface (ALI) model of the human **colonic and rectal**epithelial mucus layer that shares many characteristics with the *in vivo* barrier. Growth conditions that enable secretory cell generation in the colon produced **viscous secretion**, which was assessed both on a macro- and microscopical level. Rectal mucus generated from donors suffering from **cystic fibrosis** – a disease primarily affecting the lung –revealed that their secretion was more viscous than healthy controls. In the colon, molecular analysis revealed that this secretion indeed contained **acidic and neutral mucins**, as well as various **anti-microbial proteins and peptides**, the expression of which can be modified by exposure to innate immune signals. Lectin staining revealed a **glycosylation pattern** low in fucose but high in *N*-acetylglucosamine and sialic acid. Further, our *in vitro* colonic mucus layer was found impenetrable to pathogenic ***EPEC*** **bacteria**. We believe that these findings have strong implications on **infectious disease research** and beyond. The integration of our model in **organ-on-a-chip** systems to study complex networks such as the gut-lung-axis poses another application opportunity for our model. Our system thus promises to advance our understanding of disease mechanisms both within the gut and beyond.

Molecular Mobility and Diffusion in Mucus-mimicking gels through EPR Spectroscopy

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Mucus hydrogels and their selective barrier properties are defined not only by their macroscopic bulk mechanics but by the molecular-scale environment within their pores — the local chemistry, crowding, and polymer chains dynamics that ultimately governs transport and barrier function. Bulk rheology captures network stiffness and connectivity but cannot access the details of this interior landscape. We use electron paramagnetic resonance (EPR) spectroscopy with site-directed spin labelling to resolve this local environment in mucin-mimetic polymer (MMP) hydrogels, synthetic analogues of the mucus network crosslinked through reversible thiol–pyridyl disulfide chemistry.

We demonstrate that spin labels tethered to the polymer backbone via PEG linkers of varying length serve as reporters at different distances from the network. EPR confirms that local molecular dynamics during network formation are governed by polymer charge rather than bulk mechanics: a sulfated, negatively charged polymer (MMP-SO_3) produces markedly stronger mobility restrictions than its hydroxylated, near-neutral analogue (MMP-OH), despite the two systems being rheologically comparable. Extending the linker length reveals a gradient in mobility across the pore, from a restricted region near the backbone to a more hydrated interior — a structural feature directly relevant to how mucus discriminates between diffusing species by size and surface chemistry. In particular, it was found that local chain collapse occurs within the charged network during gelation and is governed by the increasing PEG concentration in the hydrogel pores.

Extending this framework to molecular transport, we compare probe diffusion in MMP-SO_3 with that in a covalently crosslinked, architecturally distinct extracellular matrix analogue (StemGel®). Results indicate that local probe mobility is sensitive to network type and confinement, consistent with the expectation that crosslink permanence and pore microenvironment together shape diffusive transport.

These findings confirm that molecular-scale dynamics and transport in hydrogels depend on chemistry and microenvironment in ways that bulk characterisation alone cannot resolve.

These results position EPR spectroscopy as a tool for dissecting the molecular basis of mucus barrier function, with implications for understanding pathogen exclusion, drug diffusion, and the failure of this barrier in conditions such as cystic fibrosis.

Mechanical stretch increases sialic acid abundance in the alveolar epithelial glycocalyx

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Abstract

The glycocalyx plays important roles in regulating barrier function, mechanotransduction, host-pathogen interactions, and immune responses. These functions are particularly relevant at the alveolar blood-gas barrier, where the epithelial glycocalyx is directly exposed to mechanical and inflammatory perturbations associated with breathing or mechanical ventilation. However, the composition, regulation and functions of the alveolar epithelial glycocalyx remain poorly defined. Here we identify increased alveolar epithelial glycocalyx sialic acid abundance as a mechanosensitive feature with potential relevance to ventilator-induced lung injury (VILI) and ventilator-associated lung pneumonia (VAP). Primary human alveolar epithelial cells were maintained under static conditions or exposed to 5% or 18% cyclic stretch, mimicking ventilation with physiological tidal volumes or injurious overventilation, respectively. Whereas stretch at 5% produced minimal changes in sialic acid abundance relative to static conditions, high-magnitude stretch markedly enhanced total cell surface sialic acid residues in the glycocalyx via increased *de novo* synthesis of sialylated glycoproteins, RNAs, and lipids. Mechanistically, siRNA-mediated silencing identified the rate-limiting biosynthetic enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (*GNE*) as a principal mediator of this response. Increased glycocalyx sialic acids enhanced binding of influenza A virus hemagglutinin and the immunomodulatory receptors *siglec*-3, -7, and -14 to the alveolar epithelium. Together, these findings reveal mechanical stretch as a regulator of alveolar epithelial sialic acid expression and suggest that glycocalyx remodeling may constitute a previously unrecognized mechanism contributing to viral susceptibility and inflammation in VILI and VAP.

Keywords: sialic acid, glycocalyx, alveoli, mechanical stretch, ventilator-induced lung injury, ventilator-associated pneumonia.

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Comparison of different fluorescence microscopic methods to resolve the structure of the alveolar epithelial glycocalyx

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Abstract: The alveolar epithelial glycocalyx is composed of glycosaminoglycans, proteoglycans and glycoproteins, that is located at the interface between the alveolar epithelium at the lining layer covering the airspaces. This makes the preservation of this structure challenging and the visualization using microscopic methods usually lack enough resolution for a few nanometer structure. With the development of super-resolution microscopy methods, the question about composition and structure can be assessed. We hypothesized that we can combine ultrastructure analysis in electron with fluorescence microscopy. For this purpose we plan to employ epifluorescence, confocal and STED (stimulated emission depletion) microscopy and transmission electron microscopy, together with specific metabolic glycoengineering to label sialic acid. In the future we will be able to correlate fluorescence and electron microscopy to get a first overview of the structure of the glycocalyx and specifically the location of sialic acid.

Surfactant protein A and D regulate sialylation and barrier function of the alveolar epithelial glycocalyx

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Keywords: alveolar epithelial glycocalyx, surfactant protein A, surfactant protein D, neuraminidase, sialic acid

Abstract

Surfactant protein A (SP-A) and D (SP-D), also known as lung collectins, are C-type lectins, calcium-dependent glycan binding proteins with a typical carbohydrate recognition domain. These proteins have been described to bind to sialic acid (SA) and inhibit neuraminidase (NA). Therefore, we hypothesized that they may play a role in the sialylation status of the alveolar epithelial glycocalyx. To test this hypothesis, proteomic and glycomic analyses of bronchoalveolar lavage fluid (BALF) from WT, SP-A$^{-/-}$ and SP-D$^{-/-}$ mice were performed to assess the soluble components of the epithelial glycocalyx. Expression of NA in the lungs of these mice as well as neuraminidase activity were measured. In order to study the physiological relevance of sialylation regulation at the alveolar epithelial glycocalyx, an *in vitro* model of the alveolar epithelium was assessed for epithelial integrity, in the presence or absence of human NA and recombinant (rec) human SP-A and SP-D. Proteomic and glycosaminoglycan analysis of BALF from WT, SP-A$^{-/-}$ and SP-D$^{-/-}$ mice revealed increased differentially expressed proteins and hyaluronan in the absence of SP-D. In addition, glycomic analysis of BALF from WT, SP-A$^{-/-}$ and SP-D$^{-/-}$ mice showed decreased α2-6 sialylation in the absence of SP-D. Lastly, recSP-A and recSP-D decreased SA cleavage and epithelial permeability *in vitro* by interacting with and inactivating NA. In conclusion, SP-A and SP-D regulate sialylation of the glycocalyx via a dual mechanism: 1) by binding to and protecting SA and 2) binding to and inhibiting NA, thus maintaining alveolar epithelial barrier integrity.

2-deoxy-D-galactose modulates the murine glycome and enhances pneumococcal susceptibility to oxidative stress

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Background: The glycome is at the interface of host-pathogen interplay and pathogens significantly alter the glycomic landscape during colonization and infection. Conversely, glycomic alterations may modulate susceptibility or resistance to bacterial infections in mucosal tissues.

Aim: To elucidate the glycome modulating and antibacterial properties of 2-deoxy-D-galactose (2d-Gal) in a murine model of pneumococcal pneumonia.

Methods: A murine model of pneumococcal pneumonia was employed by intranasal infection with *S. pneumoniae* as well as treatment with 2d-Gal. Glycans shed into bronchoalveolar lavage (BAL) during infection were characterized by LC-MS/MS. Lung barrier permeability and immune cell influx were assessed by ELISA and flow cytometry. Dual RNA sequencing was performed to resolve murine and bacterial transcriptomics *in vivo*.

Results: Mice treated with 2d-Gal exhibited milder pneumococcal disease characterized by improved physiological parameters, reduced bacterial burden, reduced acute lung injury and improved lung barrier function. Treatment with 2d-Gal resulted in incorporation of 2d-Gal into the murine pulmonary glycome, the features of which resembled the healthy state in infected mice. Dual RNA sequencing of BAL revealed a reduced pro-inflammatory gene signature in 2d-Gal-treated mice during infection, while bacteria recovered from 2d-Gal-treated mice exhibited significant downregulation in thioredoxin-related genes *in vivo*, which are implicated in resistance to oxidative stress. Growth of *S. pneumoniae* with 2d-Gal *in vitro* resulted in enhanced susceptibility to oxidative stress, which was determined by reduced bacterial survival following exposure to hydrogen peroxide.

Conclusions: Modulation of the pulmonary glycome with 2d-Gal restricts infection by *S. pneumoniae* through enhancing pneumococcal sensitivity to oxidative stress.

Influence of *FUT2* secretor status on pneumococcal infection of the airway epithelium

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Background: Airway epithelial glycoproteins and mucus carry diverse glycans, including terminally fucosylated structures. Approximately 23% of healthy European people are reported to be *FUT2* non-secretors (sese), typically due to single nucleotide polymorphism (SNP) which prevent *FUT2*-dependent α1,2-fucosylation in mucosal secretions. However, the impact of *FUT2* secretor status on pneumonia pathogenesis remains unclear.

Aim: We aim to study the role of α-(1,2)-fucose in S.pn. infection of human preclinical models namely, organoid derived bronchial epithelial cell air liquid interface (ALI) cultures.

Methods: The DNA of patients with community-acquired pneumonia (CAP) was genotyped to identify single nucleotide polymorphisms using real-time PCR-based SNP genotyping assays. Bronchial organoid-derived epithelial cells from homozygous secretor (SeSe) and non-secretor (sese) donors were differentiated at ALI for 3 weeks and infected with S.pn. on the apical surface. Planktonic, cell-adherent, and translocated bacteria were quantified by colony-forming unit (CFU) enumeration. Immunofluorescence microscopy was used to assess extracellular and intracellular bacterial localization.

Results: In the CAP cohort, homozygous secretors were more frequent among hospitalized patients than the non-secretors. In ALI cultures, however, basolateral recovery of S.pn. was higher in sese cultures at 2 and 16 h post-infection. Microscopy detected both extracellular and intracellular bacteria in SeSe and sese ALI cultures.

Conclusion: These findings suggest that *FUT2* secretor status may influence the kinetics of S.pn. interaction with epithelial cells and regulation of epithelial barrier function.

Motility of flagellated bacteria in hydrogels

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How do infectious agents like bacteria interact with and overcome protective biohydrogels such as mucus? To address this question, we recorded and analyzed the motion of flagellated, fluorescently labeled bacteria, such as *E. Coli* and *Salmonella* Typhimurium (*S*. Tm), using fluorescence microscopy and single particle tracking. By determining the bacterial center position and orientation, our tracking framework is able to determine the angle enclosed by the bacterial long axis and the direction of bacterial motion, i.e., the alignment between orientation and motion. For *E. Coli* and *S*. Tm suspended in buffer solutions, we observe, in contrast to previous reports, a very broad distribution of opening angles, ranging from full to no alignment. This observation is further supported by highspeed microscopy measurements, which reveal a precession-type motion of the bacteria, which is more complicated than typically depicted in literature. Preliminary measurements of bacterial motion in mucin-based hydrogels show an increase in bacterial alignment, which is attributed to the confinement generated by the mucins. Complemented with bead-based microrheology, we observe that addition of bacteria changes the spatial fluctuations of the probe beads, which is interpreted as a restructuring of the hydrogel by the bacteria. Simulations of active bacteria in hydrogels demonstrate an intricate dynamic coupling between bacterial motion and hydrogel viscoelasticity.

Abstract for the CRC 1449 3rd international symposium

Synthesis of Mucin Inspired Polymers

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Native mucins are ultra-high molecular weight glycoproteins which form the structural backbone of mucus and protect epithelial surfaces by reducing the virulence of pathogenic viruses, bacteria and fungi through multivalent glycan- mediated interactions. The synthetic replication of native mucins remains a major challenge due to their complex architecture, extreme molecular weights, and dense glycosylation. Overcoming these challenges would provide a powerful platform for studying mucus-pathogen interaction and developing the next generation of anti-virulent biomaterials. Here, we present a scalable synthesis of ultra-high molecular weight mucin inspired polymers with a tailored multivalent glycan presentation for mucin mimetic interactions. The degree of glycosylation can be varied up to a functionalization degree of 60%, yielding polymers with molecular weights up to 5 Mda that closely mimic native mucins in their molecular weight, architecture, morphology, and chain length. This platform establishes a versatile foundation to understand glycan mediated mucus-pathogen interactions and enables a design of the next generation of mucus-inspired anti-virulent biomaterials.

CRC 1449 Symposium Abstract – The Development of Thermoreversible Poly(glycerol)-Poly(oxazoline) Hydrogels for iPSC Culturing and Differentiation

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Matrigel is widely used for induced pluripotent stem cell (iPSC) culture but has a xenogeneic origin and poorly defined composition, limiting reproducibility and clinical translation. Here, we establish a composition-controlled platform of chemically well-defined, thermoresponsive ABA triblock poly(oxazoline)–poly(glycerol)–poly(oxazoline) (POX–PG–POX) copolymers. Telechelic diazido POX–PG–POX copolymers containing 2-ethyl-2-oxazoline (EtOx), 2-n-propyl-2-oxazoline (nPrOx), or 2-cyclopropyl-2-oxazoline (cPrOx) were synthesized via controlled cationic ring-opening polymerization while varying the length of the protected PG-derived block. Oxazoline conversion consistently reached approximately 90% within 24 h under optimized conditions. Systematic composition–property analysis revealed a strong dependence of the lower critical solution temperature (LCST) on oxazoline identity. nPrOx-containing copolymers exhibited tunable LCSTs of 20–28 °C, whereas the EtOx-containing compositions displayed LCSTs near 65 °C or below 4 °C. A heterogeneous, acidified Dowex-mediated deprotection protocol was evaluated for removing acetal protecting groups while preserving the terminal azides required for bio-orthogonal cross-linking. These results identify the nPrOx-containing series as a lead platform for temperature-triggered assembly during warming toward physiological culture temperature. Hydrogel formation with dendritic polyglycerol functionalized with bicyclo[6.1.0]non-4-yn-9-ylmethyl groups (dPG–BCN) is currently being investigated to validate this bio-orthogonally cross-linkable matrix-precursor platform.

CRC1449 Symposium

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Exploring epithelial-driven mechanisms in Crohn's disease using a human terminal ileum model

Abstract

The intestinal epithelial–mucus interface forms a highly dynamic barrier that maintains tissue homeostasis by limiting microbial translocation while preserving selective permeability. Alterations in the composition and biophysical properties of this interface are associated with Crohn's disease (CD), particularly at the terminal ileum. However, how these disease-associated features affect epithelial function remains poorly understood.

Our project aims to translate patient-derived and consortium-generated findings into a human terminal ileum model to investigate epithelial dysfunction in CD. Using terminal ileum organoid-derived epithelial cells, we established polarized 2D monolayers on Transwell inserts to assess epithelial barrier function under inflammatory conditions. Pro-inflammatory stimulation with TNFα and IFNγ induced a dose-dependent reduction in transepithelial electrical resistance (TEER) and increased FITC–dextran permeability. Immunofluorescence further indicated altered and less continuous ZO-1 localization at cell–cell boundaries, consistent with disrupted junctional organization. These findings establish a responsive human ileal epithelial model for investigating barrier dysfunction. To extend this system, organoid-derived epithelial cells are being seeded onto biofabricated hydrogel scaffolds based on methacrylated decellularized small intestinal submucosa (dSIS-MA). Previous work showed that these scaffolds support intestinal lineage-specific gene expression, which was further enhanced under dynamic flow conditions. In parallel, synthetic mucus-like hydrogels are being explored as an apical overlay to mimic key properties of the intestinal mucus layer.

Findings from the different CRC 1449 collaborations will be used to identify relevant disease-associated cues for incorporation into the model, including microbial and metabolite signatures, mucus proteomic and glycosylation features, and altered biophysical properties. These cues will be combined with inflammatory stimulation and biofabricated materials to investigate their effects on epithelial barrier function and phenotype. Together, this approach connects patient-derived disease features with human ileal epithelial responses and biofabricated tissue environments, supporting the development of a more physiologically relevant *in vitro* model for studying intestinal inflammation and barrier dysfunction in Crohn's disease.

CRC1449 Symposium

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Characterising properties of intestinal mucus from *ex vivo* to synthetic sampling

Abstract

Crohn's disease (CD) is a multifactorial inflammatory disorder involving the intestinal mucus layer, epithelium and microbial environment. However, disease-associated changes at the intestinal interface, particularly in the terminal ileum, are still not fully understood. In this project, we bring together complementary approaches to characterize these changes and identify features that can be used to develop disease-relevant *in vitro* models.

For this, B04/AG Siegmund collaborated with several CRC 1449 subgroups to analyse CD and healthy control ileal mucus samples. A01/AG Mall investigates the lung–gut axis by comparing intestinal and airway mucus, helping identify shared disease-associated features across mucosal tissues. B01/AG Ochs uses scanning electron microscopy (SEM) to characterize mucus and tissue architecture, providing structural information on changes in mucus network organization. B05/AG Forslund analyses mucus-associated microbiota to identify microbial signatures that may contribute to mucus–epithelial interactions. C03/AG Pagel investigates mucus glycosylation to identify disease-associated glycan features that may influence mucus properties, while Z01/AG Mertins characterizes the mucus proteome to identify changes in its molecular composition. AG Haag contributes synthetic hydrogels designed to reproduce defined properties of the mucus layer. Together, these approaches provide complementary molecular, microbial, structural and biophysical information that will help define the disease-relevant features to be incorporated into our *in vitro* models. In parallel, a cooperative effort between B04/AG Weinhart and C02/AG Koksch aims at remodelling the synthetic hFF03 peptide for improved cell culture suitability. The hFF03 peptide self-assembles into α-helical coiled-coils that form extended fibrils and gelate rapidly, and has been used as an extracellular matrix mimic. Covalently cross-linkable bonds through functionalization by methacrylic anhydride will open up the possibility for a 3D printable version of the hFF03 peptide with tuneable stiffness.

The results from these collaborations will be brought together to identify relevant disease cues and guide the development of our 2D and 3D intestinal models. By combining patient-derived data from multiple collaborations with human intestinal model systems, we aim to better understand how changes at the intestinal mucus–epithelial interface contribute to Crohn's disease and use these findings to build a more physiologically relevant *in vitro* disease model.

Title:

Boronated Peptide-Rotor Dye Probes Reveal the Heterogeneity of the Glycocalyx and Mucus on Living Cell Surfaces

Authors:

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Abstract:

Glycan-rich extracellular layers, including the glycocalyx and mucus, form heterogeneous molecular environments that regulate transport and mucociliary clearance. Yet their spatial organization on living biointerfaces remains difficult to interrogate because freely diffusible environmentally sensitive dyes lack localization, while conventional bulk measurements erase microscale differences. We developed negatively charged, multivalent boronic-acid peptide probes that bind cell-surface glycans and position Cy3 or CCVJ fluorescent molecular rotors within these extracellular layers. Systematic variation of peptide charge, boronic-acid identity, and valency identified a trisboronated scaffold that stained living A549 lung epithelial cells within 5 min at 1–2 μM, showed minimal internalization, and enabled wash-free imaging with an apparent dissociation constant of 0.9 μM. Fluorescence-lifetime imaging revealed pronounced spatial heterogeneity in the local environments experienced by the surface-bound probes. Perturbation of extracellular disulfide networks with tris(2-carboxyethyl)phosphine (TCEP) produced a clear shift in the fluorescence-lifetime distribution, consistent with altered molecular crowding, confinement, and local viscosity. To enable measurements on conventional microscopes, the Cy3 rotor probe was paired with a weakly environment-responsive Coumarin 343 reference. Ratiometric imaging reproduced the perturbation-induced shift and resolved heterogeneous microenvironmental distributions in human sputum and primary airway epithelial air–liquid-interface cultures. Cystic fibrosis and non-CF samples exhibited distinct probe-response distributions, while time-resolved imaging visualized persistent, mobile, and newly emerging domains during mucociliary transport. These probes provide a rapid strategy for mapping the spatial and temporal heterogeneity of glycan-rich airway biointerfaces.

Engineering Softness: How Molecular Tweaks in Coiled-Coils Shape Hydrogel Behavior

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Hydrogels are three-dimensional polymer networks that retain large amounts of water. They have been used in numerous biomedical and bioengineering applications, for example as drug-delivery systems or as soft contact lenses.[1] The networks themselves are held together by different types of cross-linkages such as hydrogen bonds or ionic interactions that can be fine-tuned by chemical modification and external stimuli, e.g., temperature, ions, or pH.[2]

Herein, we present three strategies for designing and modifying peptide-based hydrogels and investigate their effects on structural and viscoelastic properties. The first two approaches employ the coiled coil-based, hydrogelating fibre-forming peptide hFF03 as a common scaffold.[3] First, histidine was incorporated to introduce potential metal-mediated coordination into the hydrogel network.[4] Inspired by the functional roles of glycans in natural hydrogel systems such as mucins, glycosylation was additionally introduced to investigate its influence on this system.[5] The second approach focuses on terminal modification of hFF03 as a means of modulating hydrogel formation.[6] Here, DOPA was introduced at the C-terminus, N-terminus, or both termini, and the effects on self-assembly and mechanical behavior were compared (Part a). As a third, conceptually distinct approach, we explored an alternative amphiphilic peptide scaffold with a propensity for β-sheet formation. Based on a literature-known peptide hydrogel system, O-linked galactose was incorporated to investigate glycosylation as a strategy for modulating its self-assembly and hydrogel properties (Part b).[7]

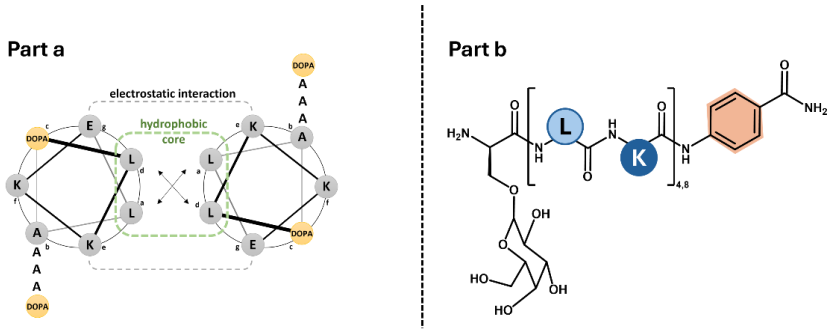

Figure 1: Modifications of hFF03 with C- and N-terminal DOPA (Part a) and O-glycosylated potential peptide system (Part b).

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Rationally designed mucin-like glyco-hydrogels

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Mucus is a complex biological hydrogel that forms an interface between the body and the environment. It hydrates and lubricates tissues, protects the epithelium from mechanical stress and irritation, and provides a first line of defense against pathogens. Its key components are mucins, densely O-glycosylated proteins containing up to 80 wt% carbohydrates. However, their structural diversity makes it difficult to link O-glycan structure to function. Well-defined synthetic mucus models are therefore needed to reveal how composition and structure determine mucus properties.[1,2]

We present a mucin-mimicking glyco-hydrogel that combines controlled glycan presentation with mucus-like physical properties. The system consists of synthetic cellulose oligomers bearing 3,6-dimethylated glucose at the reducing end and various mucin-inspired glycans at the non-reducing end (Figure 1). Upon annealing, the oligomers self-assemble into hydrogels that display mucin-inspired glycans at high density. The methylated units promote transient hydrophobic interactions, resulting in rheological properties within the range of native mucus.[3] The displayed glycans can also be modified enzymatically, for example by sialyltransferases, expanding the range of mucin-like structures accessible with this platform.

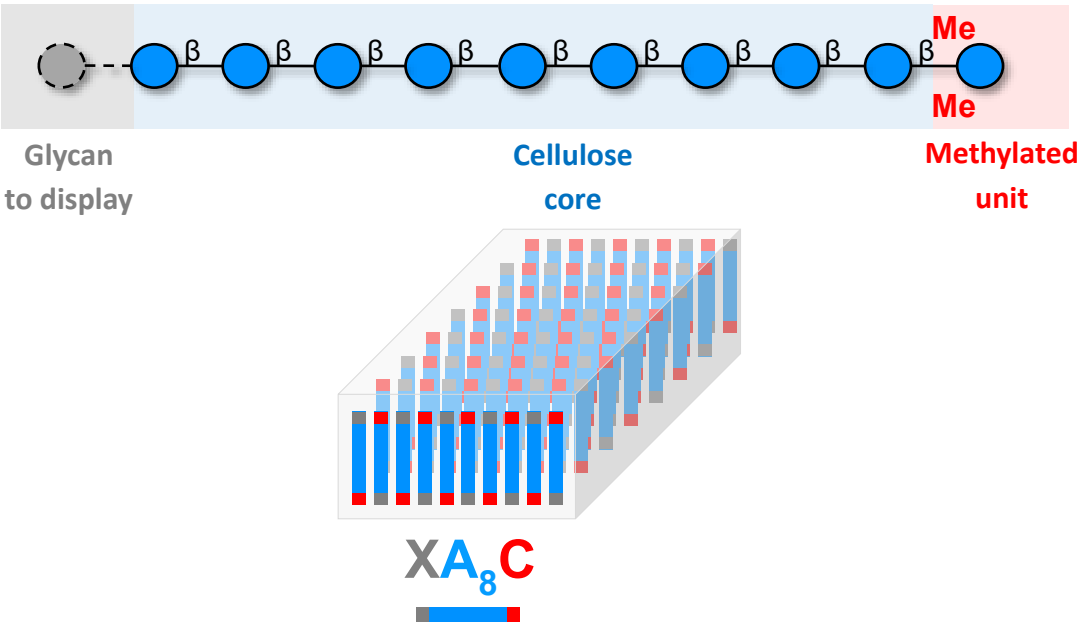

Fig. 1: *Schematic representation of mucin-mimicking glyco-hydrogel.*

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DOI: 10.1002/ange.202515926.

Title:

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

Authors:

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Project: CRC 1449 C03

Abstract:

Decoding how the airway epithelial glycocalyx shapes infection outcomes may reveal new strategies to strengthen respiratory defence. The 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 *O*-mucin-type 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 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.

Title:

Tonsil organoids as a platform for *in vitro* analysis of human adaptive immune responses

Authors:

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Abstract:

Tonsil organoids have emerged as a promising tool for *in vitro* studies on human adaptive immunity. Tonsil cells isolated from the native tissue have been shown to self-aggregate in culture and respond to antigen stimulation by sustaining germinal center reactions and producing high affinity antibodies. The conventional procedure for cell isolation includes a density gradient centrifugation step which might negatively impact the yields of stromal populations like follicular dendritic cells. In order to achieve a more physiologically relevant cell composition within the model, we tested two modified protocol variants. In both of the additional variants density gradient centrifugation was omitted, in one of them an enzymatic digestion step was included.

Tonsil cells isolated through the three protocol variants were successfully cultured into organoids for seven days and stimulated with either a live attenuated influenza vaccine or an inactivated influenza vaccine. Initial signs of B cell responses to live attenuated influenza vaccine stimulation were recorded. These included B cell differentiation and influenza specific antibody production. Conversely, inactivated influenza vaccine stimulation only yielded a marginal response.

Initial results did not reveal significant differences between protocols with regard to their influence on the model's downstream performance. However, due to the small donor number included and the short incubation period, a more extensive comparison needs to be performed in order to ultimately determine which isolation procedure is the most effective for optimal responsiveness of tonsil organoids.

Ileocecal Resection Alters the Ileal Mucus *O*-Glycome in Crohn's Disease Patients

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Intestinal mucus contains *O*-glycans that are key determinants of mucosal barrier function and host–microbe interactions. However, the structural diversity and interindividual variability of *O*-glycosylation in the human terminal ileum remain insufficiently characterized. In this study, the *O*-glycome of terminal ileal mucus was analyzed using porous graphitized carbon liquid chromatography coupled to tandem mass spectrometry. The detected *O*-glycans were predominantly based on core 1 and core 2 structures, with variable chain elongation, sialylation, and fucosylation. FUT2 secretor status was identified as the major source of interindividual variation in the ileal *O*-glycome. In contrast, ABO blood group had only a minor effect on overall glycome composition but strongly affected individual glycan structures through the expression of blood group-specific epitopes. Patients with ileocecal resection exhibited pronounced alterations in the *O*-glycome among FUT2 secretors, characterized by an approximately twofold increase in sialylation accompanied by decreased fucosylation. These results demonstrate that the human ileal *O*-glycome is strongly shaped by FUT2 secretor status and reveal marked alterations in mucosal *O*-glycosylation in patients with ileocecal resection.

Sequential Labeling as a Strategy for Preparative HMO Fractionation

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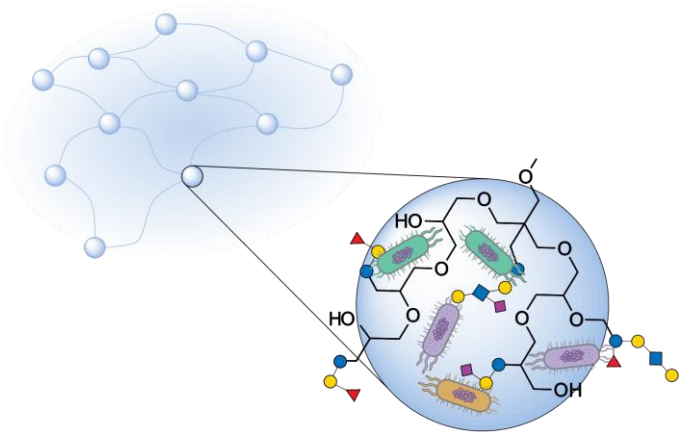
Human milk contains high concentrations of a unique class of unconjugated glycans, known as human milk oligosaccharides (HMOs). These structurally diverse molecules help shape a stable infant microbiome, a critical aspect of early development.[1] Their structural diversity is thought to have evolved in response to extensive exposure to a wide range of microbes. Due to this, HMOs are known to interact with a wide range of bacteria, promoting “healthy” commensals and inhibiting pathogenic strains.[2] However, their structure-function relationship remains under-explored due to challenging synthetic pathway and limited commercial availability.

In our approach, we purify HMOs from the natural source, followed by 2D liquid chromatography employing sequential reversible labeling. The HMO mixture is first labeled with 2,4-dinitrophenylhydrazine (DNPH) and fractionated by preparative HILIC, followed by relabeling of the collected fractions with Girard’s reagent P. The fractions are further separated by PGC chromatography, providing a basis for the preparative separation of HMO isomers.

HMOs generally exhibit weak, monovalent interactions with their targets, which underlies their high abundance. We established a synthetic platform for multivalent presentation of natural HMOs by conjugating them to dendritic polyglycerol (dPG) scaffolds. This system includes crosslinking sites that enable hydrogel formation, mimicking mucus. As a model, we investigated the inhibition of *Pseudomonas aeruginosa* and biofilm suppression by free HMOs, polymer-bound HMOs, and hydrogel format. We found that multivalent presentation retains and enhances the natural antimicrobial activity of HMOs. We observed that 10 kDa HMO-functionalized dPG particles compromise the membrane integrity of *P. aeruginosa*, an effect not observed for unconjugated HMOs. A size-dependent effect was observed, with 10 kDa outperforming 500 kDa particles.

Furthermore, the system enables incorporation of defined HMO structures to probe specific structure-dependent interactions



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Novel Polymeric Reducing Agents as Mucus Modulators in Cystic Fibrosis

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BACKGROUND

Impaired mucociliary clearance leading to airway mucus obstruction is a key feature of cystic fibrosis (CF) lung disease, caused by abnormal viscoelastic properties of CF mucus. The main airway mucus components, secreted mucins MUC5AC and MUC5B form complex polymeric networks via covalent disulfide bonds, which plays an important role for the viscoelastic properties of mucus in the airways. Targeting these crosslinks with reducing agents is a promising mucolytic strategy. The only reducing agent approved for clinical use, N-acetylcysteine, lacks potency and clinical efficacy resulting in an unmet medical need for more effective mucolytic therapies.

OBJECTIVE

The aim of this study was to test the mucolytic activity of a novel thiol-containing polymeric reducing agent (dPGS-SH), by analyzing its effects on viscoelasticity and mucus transport on primary human nasal epithelial cultures from patients with CF and assessing its impact on airway mucus plugging and inflammation in a mouse model of muco-obstructive lung disease.

METHODS

Primary CF nasal epithelial cultures grown at air-liquid interface were treated apically with dPGS-SH for 24 h, to investigate the effect of dPGS-SH-mediated mucolysis in vitro. Magnetic microwire rheology and Fluorescence Recovery After Photobleaching (FRAP) were performed to analyze effects on viscoelastic properties of the native mucus layer. Mucociliary transport was measured by particle tracking of fluorescent beads added to the mucus layer using video microscopy. To test the mucolytic efficacy *in vivo* and determined acute therapeutic effects on airway mucus plugging and airway inflammation, dPGS-SH or vehicle alone was applied to adult βENaC-Tg mice and wild-type littermates by intratracheal instillation in a volume of 1 µl/g body weight three times in one day.

RESULTS

Magnetic microwire rheology showed improvement of compliance (P<0.01) and viscosity (P<0.01) of the native mucus layer in dPGS-SH treated CF nasal epithelial cultures compared to controls. In FRAP measurements, treatment with dPGS-SH increased the fluorescence recovery of the native mucus layer significantly (P<0.01) compared to controls. Analysis of particle tracking of fluorescent beads showed that dPGS-SH treatment increased mucociliary transport velocity (P<0.0001) of CF nasal epithelial cultures compared to PBS treated cultures. Acute treatment with dPGS-SH *in vivo* reduced airway mucus content (P<0.0001) and neutrophil cell counts in bronchoalveolar lavage (P<0.001) in adult βENaC-Tg mice compared to vehicle treated controls.

CONCLUSION

Our results show that dPGS-SH has mucolytic effects on the intact native mucus layer of CF nasal epithelial cell cultures and reduces airway mucus plugging and neutrophilia in βENaC-Tg mice. Together these data provide a rationale for further development of dPGS-SH as a novel mucolytic drug for CF and potentially other muco-obstructive lung diseases.

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Probing charge-dependent protein transport in mucus using supercharged sfGFPs

Fabian Bock, Juliana Serrano, Christian P.R. Hackenberger, Daniel C. Lauster

Mucus represents a selective barrier not only to pathogens, but also to proteins and protein-based therapeutics.[1] Depending on their interactions with the mucin network, proteins may either permeate the mucus layer or become retained in it. Protein transport within this complex matrix is influenced by molecular size and electrostatic interactions at the protein-mucin interface. The high density of negatively charged glycans on mucins makes these interactions an important determinant of protein mobility and retention.[2]

To systematically investigate the role of surface electrostatics at the protein level, variants of superfolder green fluorescent protein (sfGFP) are used as a model system due to their intrinsic fluorescence, high stability, and tolerance towards genetic engineering.[3] A custom designed panel of 16 sfGFPs was generated possessing various net surface charges and is evaluated for binding and diffusion in mucus environments using binding assays and fluorescence microscopy techniques. Beyond global net charge, terminal modifications with lysine- or arginine-rich peptides and PEG are used to probe the influence of localized charge and surface chemistry on protein-mucin interactions.[4] The aim is to determine whether protein transport can be modulated and whether the same principles apply to biologically relevant soluble proteins.

Across the supercharged sfGFP panel, protein mobility decreases with increasing positive net charge in mucin solutions. At higher positive charges, micron-scale fluorescent assemblies emerge together with a depletion of

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freely diffusing protein. The absence of comparable assemblies in mucin-free controls suggests that these structures arise from charge-driven protein-mucin association rather than intrinsic sfGFP aggregation.[5] These observations indicate a charge-dependent transition from freely diffusing proteins towards increasingly retained and potentially multivalent protein-mucin associated states.

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Mucus-Targeted Lectin-Antibody Bioconjugates for Antimicrobial Applications

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Mucosal surfaces represent the first line of defense between the host and invading pathogens and therefore provide an attractive environment for the development of local-acting antimicrobial strategies. Here, we explore two modular protein-engineering approaches for the generation of multifunctional mucus-binding bioconjugates with selective recognition of microbial targets.

Through the use of recombinant lectin-based targeting modes in combination with pathogen-binding antibodies and nanobodies, site-selective protein conjugation strategies were achieved. The resulting constructs are designed to simultaneously interact with components of the mucosal glycocalyx or mucus matrix and molecular structures associated with different pathogens.

Binding, localization, and functional activity of these modular bioconjugates are investigated using biochemical assays as well as physiologically relevant mucus and epithelial model systems. Fluorescent labeling further enables characterization of their interactions with mucosal glycans and assessment of their distribution within these complex biological environments.

By separating mucus targeting from pathogen recognition into independently adaptable modules, this approach provides a versatile platform for generating mucosa-associated antimicrobial drugs, for instance enabling fast response times in the case of pandemic situations and enhancement of antibody-based proteins for more effective treatment.

Mucus Anchoring Protein (MAP)-Antibody Conjugates Enhance Viral Trapping and Antiviral Activity at the Airway Mucosa

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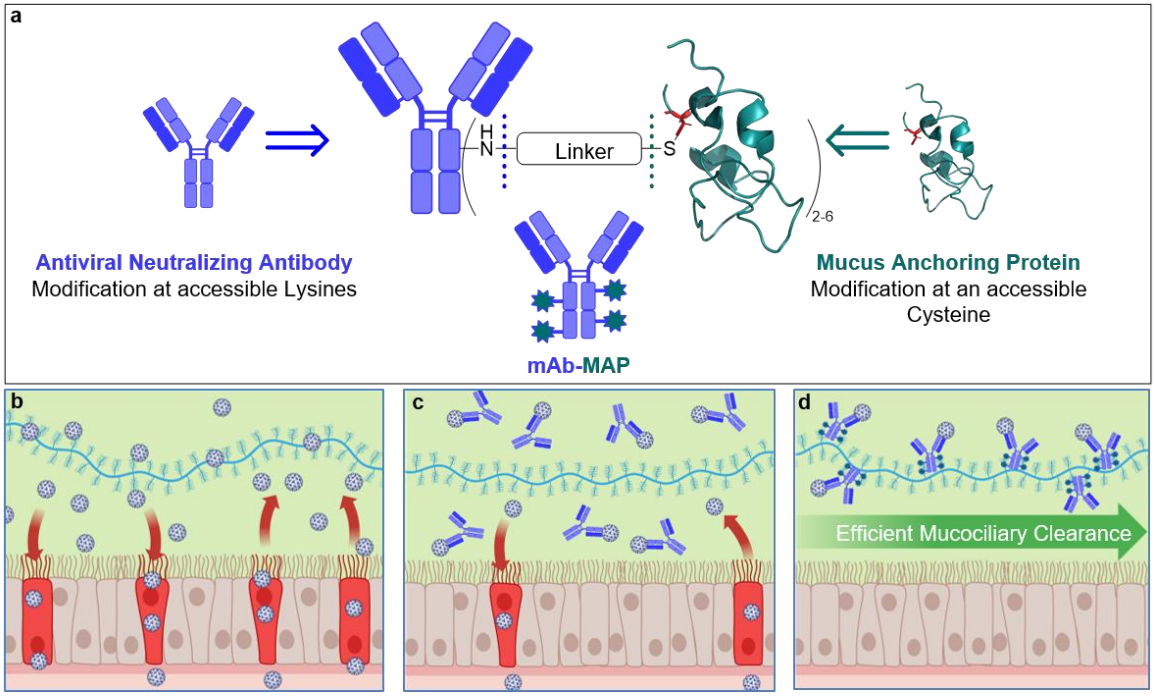

Figure 1. mAb-MAP conjugates (a) general design. (b) Depiction of compromised airways without treatment, in which viral infection and replication occurs in epithelial cell of the airway mucosa (AM). (c) In presence of an antiviral neutralizing antibody infection and replication is decreased, (d) this effect is enhanced by enabling binding to glycotopes in AM proteoglycans and mucins using a Mucus Anchoring Protein.

Airway mucus represents the first biological hydrogel encountered by respiratory viruses and an important interface for locally administered therapeutics.[1] We developed antibody–mucus anchoring protein (mAb–MAP) conjugates to exploit this environment for enhanced antiviral activity. Through late-stage chemical conjugation, neutralizing antibodies are functionalized with the mucus-binding protein Trefoil Factor 3 (TFF3)[2] without requiring genetic engineering. Using Imdevimab as a SARS-CoV-2-targeting antibody, increasing MAP loading enhanced retention within mucin environments and promoted confinement of viral particles, as demonstrated by FRAP and single-virus tracking. These biophysical effects translated into an up to five-order-of-magnitude enhancement in antiviral activity under mucus-containing conditions.[3] Our results demonstrate how engineering interactions at the mucus interface can substantially improve antibody performance against respiratory viruses.

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Upcycling Industrial Lignin into Cationic Hydrogels with Potential for Sustainable Plant Protection

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Poster Abstract

The increasing demand for sustainable agricultural practices has created a need for safer and more efficient alternatives to conventional chemical pesticides. In this work, industrial kraft lignin was upcycled into antimicrobial cationic lignin (CL) and physically embedded within a chitosan–genipin (CH–GPN) hydrogel network to form bioactive CH–GPN–CL hydrogels. These materials were evaluated for the *in vitro* inhibition of the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000.

Kraft lignin was functionalized with glycidyl trimethylammonium chloride (GTMAC) using different lignin:GTMAC mass ratios to obtain CL samples with varying degrees of substitution (DS) and surface charge. The CL library was characterized and screened for antibacterial activity, while plant-tissue compatibility was assessed using *Nicotiana benthamiana* leaf infiltration assays under buffered conditions. Based on strong antibacterial activity and a lower lignin:GTMAC mass ratio, a representative CL sample was selected for incorporation into the CH–GPN hydrogel framework.

The selected CL was physically embedded within the CH–GPN network without preventing gel formation. The resulting CH–GPN–CL hydrogels retained gel-like behavior and porous morphology. Bacterial inhibition assays showed enhanced *in vitro* activity against *P. syringae* pv. *tomato* DC3000 compared with the CH–GPN control, while Live/Dead fluorescence staining indicated increased membrane-compromised bacterial cells after treatment.

Overall, this work demonstrates CH–GPN–CL hydrogels as a promising bio-based material platform that combines lignin valorization, hydrogel formulation, and antibacterial activity for future plant-protection development.

Dynamic in-situ rheometry of micro-volume hydrogel samples

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Characterizing hydrogel properties often requires extracting samples from their formation environment, which can disrupt delicate network structures and alter measured properties. Additionally, many hydrogels undergo dynamic changes during gelation, degradation, or treatment—processes that cannot be captured through single endpoint measurements. We present a micro-volume rheometer (<50μL) designed to measure hydrogels in-situ throughout their complete lifecycle. The instrument performs two complementary measurements: creep tests that intuitively reveal how materials flow or resist deformation under constant stress, and chirp tests that rapidly characterize mechanical properties across frequencies—essential for tracking materials as they evolve. We demonstrate these capabilities on a thiol-crosslinked hydrogel (p(G1MA-co-PDSEMA) + PEG dithiol), which has a backbone similar to other hydrogels previously studied by Bej, *et. al.* (2024). During gelation, we captured the transition from liquid polymer solution to solid gel network, revealing how mechanical properties evolve across different timescales as crosslinks form. Upon adding dithiothreitol (DTT), we tracked the reverse transition as the gel liquefied through crosslink cleavage. The frequency-dependent measurements throughout both transitions provide new insights into network assembly and disassembly dynamics that traditional rheology cannot capture. This approach enables real-time characterization of stimuli-responsive hydrogels, mucus after therapeutic treatment, and other dynamic soft materials in their native state.

Secretion, assembly and structure-function relationship of snail-mucus based materials

Mariella Gabler, Max Renner-Rao, Emilie Duthoo, Peter Fratzl, Franziska Jehle

Snails secrete various types of mucus for an astounding variety of purposes. They use it as a lubricant for locomotion, as an adhesive during diurnal rest, as a sealant during hibernation and as a defensive agent when feeling disturbed or attacked. However, our understanding of how snails are able to finely tune their viscous slimy secretions to different materials is almost non-existent. We investigate the terrestrial snail Cepaea nemoralis, which produces at least five distinct types of mucus-based materials tailored for different biological functions. These materials, though functionally diverse, share common components, including the scaffold protein Collagen VI, calcium in various forms and concentrations, and largely unidentified glyco-components. Our research focuses on the composition, structural organization, mechanical properties, secretion and assembly mechanisms of these mucus types. Using a multidisciplinary approach, we analyze how the snail synthesizes and secretes these materials through specialized gland structures. By characterizing their unique properties, we aim to provide a comprehensive understanding of these biomaterials and their potential for bioinspired applications.

AI-supported quantification of network structures in SEM imaged human mucus

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We present an AI-assisted workflow for automated analysis of pore and filament structures in scanning electron microscopy (SEM) images of human mucus from the respiratory tract and ileum. Our method leverages machine learning within the Fiji platform to classify image regions into pores and filaments, enabling precise and efficient quantification. The workflow includes preprocessing, pixel classification using a trained multi-layer perceptron classifier, feature extraction, and data pooling. This approach significantly reduces analysis time compared to manual measurements while maintaining high precision.

To validate our method, we compared manual and automated measurements on SEM images of airway and ileal mucus samples. High agreement was observed between datasets, with effect sizes indicating that AI-generated data variability is even less than human-to-human inter-observer variation. Additionally, we showed that increasing the counting frame area in automated analysis did not alter resulting distributions notably, confirming the robustness of the method.

This AI-assisted tool supports high-throughput, comparative studies of biological network structures, enhancing accuracy and efficiency. Its application has the potential to accelerate research into structural and functional alterations of mucus in diseases such as cystic fibrosis and Crohn's disease, contributing to a deeper understanding of pathophysiology and the development of targeted therapeutic strategies.

Denaturing and reducing agents improve porcine gastric mucin purification while preserving functional properties

Chiara Gunnella, Lorenzo Sardelli, Federica Giammarino, Prajwal Kargal Gopalakrishna, Alice Di Bernardo, Rafael Cena-Diez, Anders Sonnerborg, Mariangela Cavarelli, Sonja Visentin, Oliver Lieleg

Research on mucus and its key macromolecular component, mucin glycoproteins, has gained considerable attention during the past decade. However, advances in this area are often limited by the fact that commercially available mucins are either not fully functional or come with a low degree of purity. Lab-extracted mucins, in contrast, have been shown to be highly functional, but controlling their level of purity remains critical since mucin-associated proteins are still present after the extraction process. We here modify an existing mucin extraction protocol for stomach mucus, which is based on filtration and size exclusion chromatography; our goal is to enhance the detachment of molecular contaminants from the mucins during the purification routine. In particular, the addition of a denaturing agent (guanidinium chloride, GuHCl) or a reducing agent (dithiothreitol, DTT) at specific steps of the purification cascade is shown to be efficient in removing contaminants attached to the mucin molecule through different molecular interactions: whereas the denaturing agent is more effective in removing physically bound proteins, the reducing agent maximizes the removal of small molecules covalently linked to the mucins. In addition, we find that the lubricating properties of mucins extracted in the presence of the chemical additives are maintained and – in the case of GuHCl addition – the anti-viral efficacy towards HIV is even enhanced, without negatively affecting the chemical architecture of mucin. Importantly, we show how choosing the right dose of the additive as well as the correct position within the sequence of purification steps is crucial to obtain this improved outcome

Kicking Diffusion in the Butt: Nanomotors Stimulate Superdiffusion of Passive Nanoparticles Across Mucosal Barriers

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Abstract

Modern nanomedicine relies on the development of specialized nanocarriers for drug transport. However, despite their diversity, these carrier particles face a common drawback: their transport is limited by Brownian motion. Especially when targeting transmucosal delivery, *e.g.*, in the oral or respiratory tract, the turnover time of mucus defines a narrow time window during which the translocation of such particles needs to be completed to avoid their clearance. To improve the delivery of passive particles across the mucosal barriers, we fabricate Janus-type nanomotors fueled by water. Driven by the active motion

of those nanomotors, the diffusive motion of passive (drug carrier) particles in mucin-based networks is boosted by more than one order of magnitude, and we find stronger enhancement effects for more strongly confined mucin networks. Moreover, to deliver both particle species to the same location on a mucosal surface, we design a muco-responsive microdroplet which is co-loaded with passive particles and nanomotors. Our approach should help leveraging existing (passive) nanoparticle-based drug carriers for applications in oral and pulmonary delivery.

Comparison of gastric, salivary and intestinal mucin coatings

Utku Özdemir and Oliver Lieleg

Even though lab-purified porcine gastric mucins (PGMs) are more easily available than other mucin variants, such other mucins might outperform PGMs due to differences in their molecular architecture, e.g. when used as anti-biofouling layers on medical devices. Here, we investigate the biophysical properties of three secreted mucins: MUC5AC (porcine gastric), MUC5B (bovine salivary) and MUC2 (porcine intestinal), and compare their functional properties when covalently immobilized on polystyrene (PS). In more detail, we determine their size and zeta potential in solution as well as the contact angle and surface potential of coatings assembled from them. Furthermore, we measure the accessibility of peptidic and glycan areas in the different coatings as well as their ability to reduce friction and repel eukaryotic cells, proteins and lipids.

Interestingly, we find that the biochemical differences between the three mucins tested here do not necessarily translate into major differences in the biophysical and anti-biofouling properties of the respective coatings. Our working hypothesis is that the three distinct mucins assume slightly different conformations/molecular orientation on the surface and that this might explain the minor differences in coating properties we observe.

Abstract Berlin - CRC 1449 3rd International Symposium

Composite MUC5AC-based gels with dynamically switchable stiffness on the macro- and micro-scale

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Achieving controlled and reversible stiffening of biopolymer networks remains a significant challenge in materials science. To enable dynamically tunable mechanical properties in mixed mucin/alginate hydrogels on the macro- and micro-scale, the biopolymer networks are cross-linked through a combination of light-induced covalent and ionic interactions. Covalent cross-linking is introduced by functionalization of the biopolymers with methacrylate, whereas ionic cross-links are generated by Ca^{2+} ions and can be removed again by introducing a mix of the chelating agents EDTA and citrate.

To monitor dynamic changes in hydrogel stiffness on the macro-scale, we employ a custom-made rheology setup that enables the manipulation of the cross-linking state of the hydrogels *in situ*, *i.e.*, while quantifying changes in their viscoelastic response at the same time. On the microscale, microgels are generated *via* microfluidics and characterized by microindentation measurements. In both cases, the incorporation of the glycoprotein mucin into the alginate network not only expands the range of available cross-linking strategies but also endows the cytocompatible hydrogel with antibacterial properties.

These well-defined alginate/mucin networks with dynamically switchable mechanical properties provide a versatile platform for applications in cell culture and tissue engineering, where spatially and temporally controlled mechanical cues are of particular interest at both, the macro- and micro-scale.

Mucin-enriched microneedles with bacteria-repellent properties and tunable drug release

Xinke Yu, Leonard R. Gareis, Luca Reichert, Stephan A. Sieber, Jan Torgersen, and Oliver Lieleg

Abstract

Transdermal drug delivery *via* microneedles (MNs) offers a patient-friendly alternative to oral routes by circumventing the first-pass metabolism and overcoming the stratum corneum barrier. However, the clinical efficacy of MNs is often hindered by bacterial biofouling at the tissue interface and the lack of precise spatiotemporal control over multiple therapeutic agents. Here, we develop a multifunctional MN platform by integrating purified mucin glycoproteins into a physically crosslinked polyvinyl alcohol (PVA) matrix *via* three freeze-thaw cycles. We identify that a rather low mucin concentration of 1% (w/v) ($PVA_{14}Muc_1$) provides an optimal balance by triggering a synergistic reinforcement of the matrix stiffness for reliable skin penetration while maintaining potent bacteria-repellent properties against *S. aureus* and *E. coli*. Moreover, to enable sequential delivery of otherwise incompatible drugs, a "particle-in-matrix" strategy is implemented: cationic Vancomycin (Van) is pre-encapsulated into mucin particles to prevent an electrostatic precipitation with anionic Salicylate (Sal) located in the PVA matrix. This architecture facilitates a precise sequential release of both drugs, with a rapid, burst-like liberation of the analgesic Sal, followed by a sustained delivery of the antibiotic Van. This PVA-mucin MN system offers a promising strategy to address the complex requirements of multi-stage dermal drug delivery and wound care management.

Advancing research on Metalloglycobiology – Impact of trace element deficiency in the intestine and endometriosis

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Abstract

Metalloglycobiology focuses on the (modulatory) role of metals for protein and lipid glycosylation. Metal ions, including magnesium and calcium, and trace elements (TE) such as manganese (Mn), selenium (Se) and zinc (Zn), are required for the activity of several key glycosyltransferases (GTs) and their co-factors.[1] Our research focuses on how alterations in metal homeostasis affect glycoprotein synthesis and glycosylation, particularly mucins in the intestine and endometriotic tissue. One major project addresses Zn deficiency, which affects 1 billion people worldwide[2] and has been associated with intestinal epithelium degeneration and disruption of the protective mucus barrier.[3-5] Beyond the intestinal tract, we further explore whether the interplay between TE homeostasis and mucin biology also affects other mucin-rich tissues. The endometrium is of particular interest in this context, as changes in mucin expression and glycosylation patterns have been described in endometriosis (EM), an estrogen-dependent, chronic inflammatory disease affecting approximately 20 % of woman of reproductive age.[6,7] In addition, evidence suggest dysregulated TE homeostasis[7,8] and alterations of genital and intestinal microbiota in EM patients.[9] Given the role of TEs in glycosylation and the role of mucins in epithelial function and microbiota shaping, a potential link between TE status and mucin biology in EM is conceivable but largely unexplored. The aim of this study was to study how alterations in TE status affect mucin expression and glycosylation in mucin-rich tissue, using the intestinal goblet cell (GC) models HT-29-MTX, LS174T and the endometriotic cell line 12Z as complementary *in vitro* models.

A transcriptomic screening of Zn-deprived *in vitro* GCs showed deregulation of multiple mucins and GTs catalyzing *N*- and *O*-glycosylation, in particularly *MUC2* was strongly upregulated, which was confirmed on the protein level analyzing the C-terminal MUC2 fragment and mature protein with WB, slot blot, and immunofluorescence.[10] Additionally, interleukin (IL)-33 was identified as the most pronounced cytokine produced and secreted by ZD cells. To investigate the regulatory role of IL-33 in MUC2 synthesis, *in vitro* GCs were stimulated with human recombinant IL-33, which significantly enhanced MUC2 induction indicating that IL-33 may be a key player in the MUC2 synthesis during Zn deficiency. In patients with EM, inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) revealed altered serum TE levels, particularly decreased Se and Zn and increased Mn, alongside elevated MUC expression and changes in glycosylation within endometriotic lesions assessed by lectin blot and WB. Immunochemical staining of the EM cell line 12Z revealed Se-dependent changes in mucin and T-antigen expression, suggesting an influence of Se on glycosylation. These results suggest a possible link between TE homeostasis and mucin regulation in EM.

Together, our findings highlight Zn and Se as potentially important regulators of mucin-associated processes and the relevance of TE-dependent glycosylation in mucin-rich tissue.

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Title:

Current Hydrogel Research as a Context for Promoting Students' Systems Thinking Skills and Perceived Relevance of Chemistry

Abstract:

Hydrogels at biological interfaces are complex systems in which macroscopic properties and biological functions emerge from interactions between numerous molecular and supramolecular components. Understanding such systems therefore requires the consideration of multiple levels, interactions, and system boundaries – characteristics closely related to Systems Thinking (ST). At the same time, current hydrogel research provides authentic examples of how chemistry contributes to addressing interdisciplinary scientific and societal challenges.

The PhD project *Hydrogel Research in High Schools: Learning Chemistry in Relevant Systems (HyReS)* aims to transfer current research from CRC 1449 into secondary chemistry education. In a research-practice partnership, research contexts are aligned with curricular requirements and transformed into inquiry-based learning modules that combine authentic experiments with a systems-oriented approach. Three learning modules are being developed for different levels of secondary chemistry education.

The accompanying empirical research investigates how learning chemistry through contemporary research contexts affects students' ST skills and their perceived relevance of chemistry. Systems-oriented concept maps are used to investigate the development of students' systems thinking, complemented by qualitative and quantitative approaches to perceived relevance. In addition, the perspectives of students, teachers, and scientists are considered to identify potentials, challenges, and design principles for transferring contemporary research into chemistry education. The poster presents the overall design of HyReS and illustrates how research from CRC 1449 can be transformed into authentic and systems-oriented learning environments for secondary chemistry education.

Collision Induced Dissociation Mass Spectrometry of Imidazolium-tagged Glycopeptides

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O-glycosylation, a complex and highly variable post-translational modification, remains challenging to analyse by mass spectrometry and typically requires a combination of higher-energy collision dissociation (HCD) and electron transfer dissociation (ETD) for comprehensive characterisation.[1]
Previously, to increase the positive charge of the glycopeptide in order to facilitate ETD an imidazolium tag (ITag) was introduced to a synthetic glycopeptide via copper catalysed azide alkyne cycloaddition (CuAAC).[2] As expected the increased charge density lead to more efficient fragmentation by ETD.

We want to further analyse collision induced dissociation (CID) fragmentation patterns of ITag-modified glycopeptides and compare fragmentation patterns at different collision energies. Peptides are first glycosylated in an enzymatic reaction with azide or alkyne containing GalNAc analogues. Different tags can then be introduced to the glycopeptides via CuAAC. First analysis of ITag-modified glycopeptides by CID showed peptide fragments still containing the ITag modifications. These fragments are not observed when subjecting glycopeptides not containing ITag to CID. This suggests that the ITag-modified peptides are more stable towards CID than glycopeptides that are not modified with ITag. This approach could potentially allow for glycosylation site localisation without the need for ETD, facilitating glycoproteomic analysis strategies.

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Structure Diversity Enables Emergent Assembly and Self-Healing in Metallo-Supramolecular PEG–peptide Hydrogels

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Protein aggregation plays a central role in both biological function and the design of advanced functional materials. In our previous work, a poly(ethylene glycol) (PEG)–peptide conjugate with a short spacer and pentapeptide end groups, featuring alternating phenylaniline and histidine residues, formed β-sheet nanofiber junctions and percolated networks below the coil overlap concentration, c^*.[1] However, the strong stretching of short spacers produced a fragile hydrogel with limited modulus level and a poor recovery after network disruption. Here, we address these limitations using a PEG–peptide conjugate with a significantly longer spacer, enabling access to concentrations far above c^* at comparable peptide content. We also enhance the chance of directional metal coordination by employing a heterochiral peptide conjugate. Our results reveal a fundamental tradeoff between the enhanced dynamicity of long polymer spacers and reduced assembly of racemic peptide mixtures. This balance can be uniquely shifted by Zn^{2+} ions, which strengthen β-sheet packing along with individual His complexes, resulting not only in significantly enhanced modulus, but a fast and quantitative network recovery despite prolonged relaxation times. Furthermore, coassembly of a small fraction of long spacer conjugates with the short analogue created a synergistic network architecture, featuring intramolecular assembly of short conjugates connected via long spacers, which overcomes the limitations of each component alone. These findings highlight how molecular diversity can generate emergent assembly and material properties, offering a new principle for designing novel (bio)materials and understanding biological systems.

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Data sharing in CRC 1449: large data transfer between institutional storage and external collaboration partners

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The highly interdisciplinary research conducted at CRC 1449 generates large volumes of diverse raw research data that must be securely shared among researchers at different locations for collaborative processing and analysis. USB drives, email attachments, and Nextcloud are not scalable for larger datasets, nor do they offer uniform access control; furthermore, they create redundant copies of data. Issues such as access and transmission security, authentication, and versioning are addressed using the datasets collected at Freie Universität Berlin – which are stored in the BCP file system (BCPFS) – as examples. We have developed a prototype for data sharing that extends the BCPFS with a comprehensive workflow – from inviting an external project partner to granting them direct access to a shared directory. The data is not duplicated: partners access the original storage via the established Secure File Transfer Protocol (SFTP) and register on the platform to manage their own shared folders. This approach enables CRC members and their external collaboration partners to share data long-term with high security, high performance, and resumable transfers.

Digital Transformation in CRC 1449: ELN Adoption, AI Tools, Computational Chemistry & FAIR Data (INF Project)

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The INF project supports all researchers of CRC 1449 with FAIR-compliant research data management, specifically in the exchange, processing, computer-aided analysis, and archiving of scientific data. The following methods and core services are the main focus:

- **ELN Migration:** Hosting and implementation of Electronic Lab Notebooks (eLabFTW, Chemotion), including institutional IT co-determination processes, while supporting researchers to transition CRC 1449 toward standardized digital documentation under changing conditions.
- **AI Chatbot & Advisors:** Active development and functional expansion of the local, privacy-compliant ZIB Scientific Chatbot (ollama-bcp-zib.de) with subject-specific AI Advisors (e.g., bioimage analysis, biostatistics, FAIR data).
- **Computational Chemistry & ML:** Assisting researchers with quantum chemical calculations to elucidate molecular interactions, as well as providing consultation on ML.
- **NFDI4Chem & Zenodo Community:** Pioneering ELN-to-storage integration as an initiative toward NFDI4Chem standards, alongside curating the CRC 1449 Zenodo Community.
- **Infrastructure & Training:** Provision of the OpenIRIS instrument scheduling platform, and delivery of training on IT-driven workflows for CRC 1449 members.

Through the coordinated integration of these measures, the INF project creates an efficient, AI-supported research data ecosystem for CRC 1449.

Functional DNA materials for data storage and information processing

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DNA offers a unique combination of molecular programmability, specific interactions and self-assembly, making it a powerful building block for functional materials. Here, we combine modelling, coarse-grained simulations and experiments to investigate stimuli-responsive DNA hydrogels as platforms for data storage and information processing. We study how DNA sequence, network architecture and temperature-dependent hybridization govern the structural and mechanical properties of DNA assemblies and hydrogels. By coupling reversible molecular interactions to distinct mechanical states, we explore how information can be encoded, stored and read directly through the physical properties of the material. Our work establishes design principles for programmable DNA-based materials in which information processing emerges from the collective dynamics and mechanics of a responsive polymer network, opening new possibilities for mechanical data storage, sensing and bioinspired computing.