



Organoids
Microbiome
Modeling
Precision medicine
Networks
Imaging
Multi-omics



3rd
INTERDISCIPLINARY
SIGNALING
WORKSHOP

VISEGRAD, HUNGARY, 2023

Program & Abstract Book

<https://2023.signalingworkshop.org/>

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Inflammation, infection and cancer in the gut

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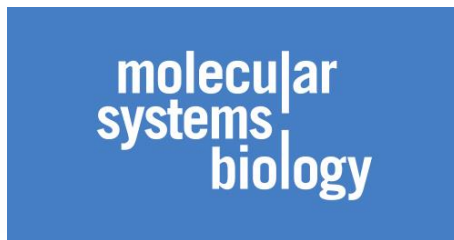
PROGRAM & ABSTRACT BOOK

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Timetable

3rd Interdisciplinary Signaling Workshop - Focus on Inflammation, infection and cancer in the gut - 2023					
	Monday 2023.07.17	Tuesday 2023.07.18	Wednesday 2023.07.19	Thursday 2023.07.20	Friday 2023.07.21
	Arrival & Opening	Disease models and organoids	Microbiome and host-microbe analyses	Modeling and Precision Medicine	Departure
9:00		Keynote lecture: Eduardo Villablanca (Karolinska Institutet, SE) Spatial cell profiling of the whole gastrointestinal tract during intestinal inflammation	Keynote lecture: Jost Entrings (Institut Pasteur, FR) Imaging the modulation of host trafficking and signaling by bacterial effector proteins	Keynote lecture: Chris Tape (University College London, UK) Single-cell Signalling Analysis of Tumour Microenvironment Organoids	
9:45		Expert talk 1: Joana P. Neves (Kings College London, UK) Mucosal organoids reveal 'inside lymphoid cells' functions	Expert talk 1: Paul Wilmes (University of Luxembourg, LU) Systems ecology of human-microbiome interactions	Expert talk 1: Fabian Frohlich (Crick Institute, UK) Anti-Cancer Drug Sensitivity Prediction using Mathematical Models of Signalling Pathways	
10:15		Expert talk 2: Kristine Schauer (Gustave Roussy Institit, FR) Towards a high resolution map of the cancer cell: focus on lysosome dysfunction	Expert talk 2: Cynthia Whitchurch (Quadram Institute, UK) Challenges in imaging microbiome and biofilms on host tissues	Expert talk 2: Laurence Calvete (Institut Curie, FR) Simulating dynamic populations of cell types in cancer: from data to predictive models	
10:45		Tea/Coffee	Tea/Coffee	Tea/Coffee	
11:15		Expert talk 3: Julio Saez-Rodriguez (University of Hordsborg, DE) Knowledge-based machine learning to extract disease mechanisms from multi-omics data	Expert talk 3: Falk Hildebrand (Quadram Institute, UK) Gut Metagenomics: finding good descriptions of bacterial communities and their members	Livia Perletto (University of Rome La Sapienza, IT) A computational strategy to produce actionable and patient-specific networks of intracellular signaling in PDAC	
11:45		Amisha Modasa (Quadram Institute, UK) Assessing the effects of dietary fibre fermentation in an in vitro model of the intestinal epithelium	Federica Ungaro (Università Vita-Salute San Raffaele, IT) Not just a bystander: may the microbiota be the leading actor in gut diseases?	Orsolya Kágyú (Semmelweis University, HU) A systems biological study of ATP4-controlled autophagy induction in inflammatory bowel disease	
12:00		Andrea Checchi (INSERM, FR) The immunospecific cell death (ICD) is a type of cell death that elicits an anticancer immune response	Douglas Brubaker (Curtin Western Institute of Technology, UK) Pharmacobiome: Realizing the Therapeutic Potential of Microbes and Microbial Products	Closing Talk: Tamas Korcsmáros (Imperial College London, UK) Patient- and cell-type specific networks to explain heterogeneity in IBD	
12:30		Nadezhda Doncheva (University of Copenhagen, DK) Cytoscape stringApp 2.0: Analysis and Visualization of Heterogeneous Biological Networks	Bence Szalai (Tübingen AI, HU) Can we overcome bias in machine learning models predicting drug sensitivity and gene essentiality?		
13:00		Lunch	Lunch	Lunch	
14:00	Arrival and registration	Teamwork – part 1	Teamwork – part 2		Departure
14:30				Presentations of teams (15 min / team + discussion)	
15:00					
15:30				Tea/Coffee	
16:00	Opening ceremony	Medieval team building competition, informal discussions in the mesentime	Catanan race on the Danube, informal discussion in the mesentime	Moderated panel discussion on: team generalizations, novel ideas, and concrete actions (e.g. collaborations)	
16:30	Opening Keynote lecture: Jasmin Fisher (University College London, UK) Executable signalling networks: From mechanisms to therapeutic applications			Moderated panel discussion on: Women / Family in Science	
17:00	Opening Keynote lecture: Nick Powell (Imperial College London, UK) Harnessing cytokine-responsive transcriptomics to predict patient trajectories in inflammatory bowel disease			Award presentation for teams and poster presenters and Closing remarks	
17:30					
18:00	Welcome reception and dinner	Special Renaissance Dinner	BBQ Picnic: Dinner in the Garden of the Hotel Visegrád	Workshop Closing Feast in the Court of Foods (some location as Court of Crafts)	
18:30					
19:00					
19:30					
20:00		Double Sunset Cruise			
20:30					
21:00					
21:30	Informal evening program (bowling, table soccer, swimming, etc)	Poster session 1	Poster session 2		
22:00					
22:30		Informal evening program	Informal evening program	Informal evening program with music	

Scientific program Monday - 17 July 2023

14:00	Arrival and registration
16:00	Opening ceremony Chair: Tamás Korcsmáros
16:15	Opening Keynote lecture: Jasmin Fisher (University College London, UK): Executable signalling networks: From mechanisms to therapeutic applications
17:00	Opening Keynote lecture: Nick Powell (Imperial College London, UK): Harnessing cytokine-responsive transcriptomics to predict patient trajectories in inflammatory bowel disease
18:00	Welcome cocktail and dinner
20:00	Informal evening program (bowling, table soccer, swimming, etc)

Tuesday – 18 July 2023

	Disease models and organoids Chair: Diána Papp
9:00	Keynote lecture: Eduardo Villablanca (Karolinska Institutet, SE): Spatial cell profiling of the whole gastrointestinal tract during intestinal inflammation
9:45	Expert talk 1: Joana F. Neves (King's College London, UK): Mucosal organoids reveal innate lymphoid cells' functions
10:15	Expert talk 2: Kristine Schauer (Gustave Roussy Institut, FR): Towards a high resolution map of the cancer cell: focus on lysosome dysfunction
10:45	Tea/Coffee
	Disease models and organoids Chair: Jasmin Fisher
11:15	Expert talk 3: Julio Saez-Rodriguez (University of Heidelberg, DE): Knowledge-based machine learning to extract disease mechanisms from multi-omics data
11:45	Amisha Modasia (Quadram Institute, UK): Assessing the effects of dietary fibre fermentation in an <i>in vitro</i> model of the intestinal epithelium
12:00	Andrea Checcoli (INSERM, FR): The immunogenic cell death (ICD) is a type of cell death that elicits an anticancer immune response
12:15	Nadezhda Doncheva (University of Copenhagen, DK): Cytoscape stringApp 2.0: Analysis and visualization of heterogeneous biological networks
12:30	Lunch
14:00	Teamwork – part 1 Getting know each other
14:30	Teamwork – part 1 Presenting the problem to be solved
16:00	Teamwork – part 1 Medieval team building competition, informal discussions in the meantime
17:30	Special renaissance dinner
19:15	Double Sunset Cruise
21:30	Poster session 1
22:30	Informal evening program

Wednesday – 19 July 2023

	Microbiome and host-microbe analyses Chair: Isabelle Hautefort
9:00	Keynote lecture: Jost Enninga (Institut Pasteur, FR): Imaging the modulation of host trafficking and signaling by bacterial effector proteins
9:45	Expert talk 1: Paul Wilmes (University of Luxembourg, LU): Systems ecology of human-microbiome interactions
10:15	Expert talk 2: Cynthia Whitchurch (Quadram Institute, UK): Challenges in imaging microbiomes and biofilms on host tissues
10:45	Tea/Coffee
	Microbiome and host-microbe analyses Chair: Laura Nolan
11:15	Expert talk 3: Falk Hildebrand (Quadram Institute, UK): Gut metagenomics: finding good descriptors of bacterial communities and their members
11:45	Federica Ungaro (Università Vita-Salute San Raffaele, IT): Not just a bystander: may the microbiota be the leading actor in gut diseases?
12:00	Douglas Brubaker (Case Western Reserve University School of Medicine, US): Pharmacobiome: realizing the therapeutic potential of microbes and microbial products
12:15	László Mérő (Turbine.AI, HU): Can we overcome bias in machine learning models predicting drug sensitivity and gene essentiality?
12:30	Lunch
14:00	Teamwork – part 2 Discussing the problem to be solved
16:00	Tea/Coffee
16:30	Catamaran race on the Danube, informal discussion in the meantime
19:00	BBQ Picnic Dinner in the Garden of the Hotel Visegrád
21:30	Poster session 2
22:30	Informal evening program

Thursday - 20 July 2023

	Modeling and Precision Medicine Chair: Dezső Módos
9:00	Keynote lecture: Chris Tape (University College London, UK): Single-cell signalling analysis of tumour microenvironment organoidsproteins
9:45	Expert talk 1: Fabian Fröhlich (Crick Institute, UK): Anti-cancer drug sensitivity prediction using mathematical models of signalling
10:15	Expert talk 2: Laurence Calzone (Institute Curie, FR): Simulating dynamic populations of cell types in cancer: from data to predictive
10:45	Tea/Coffee
	Modeling and Precision Medicine Chair: Fabian Fröhlich
11:15	Livia Perfetto (University of Rome La Sapienza, IT): A computational strategy to produce actionable and patient-specific networks of intracellular signaling in Pancreatic Ductal Adenocarcinoma
11:30	Avlant Nilsson (Massachusetts Institute of Technology, US): A deep learning model of intracellular networks
11:45	Orsolya Kapuy (Semmelweis University, HU): A systems biological study of ATF4-controlled autophagy induction in inflammatory bowel disease
12:00	Closing Talk: Tamás Korcsmáros (Imperial College London, UK): Patient- and cell-type specific networks to explain heterogeneity in IBD
12:30	Lunch
14:00	Presentations of teams (15 min / team + discussion)
16:00	Tee/Coffee
16:30	Moderated panel discussion on: team presentations, novel ideas, and concrete actions (e.g. collaborations)
17:00	Moderated panel discussion on: Women / Family in Science
17:45	Award presentation for teams and poster presenters and Closing remarks
18:15	Walk and free time in the Court of Crafts
19:00	Workshop Closing Feast in the Court of Foods
22:00	Informal evening program with music

Poster session 1 – 18 July 2023

IBD - Chairs: Federica Ungaro, Julio Saez-Rodriguez

Balázs Bohár	P-01	Systems genomics analysis on gene regulatory networks in inflammatory bowel disease
Margita Ágnes Márton	P-02	A systems biological study of the role of endoplasmic stress upon inflammatory bowel disease
Dezső Módos	P-03	Systems Genomics and Network Approaches Uncover How Single Nucleotide Polymorphisms in Crohn's Disease and Ulcerative Colitis Affect Common Processes Through Different Mechanisms
Francisca Castillo	P-04	Characterization of a novel molecular stratification of Ulcerative Colitis patients
Polina Kornilova	P-05	Paralog proteins play a crucial role in rewiring cell-type specific signalling processes in Ulcerative Colitis and Crohn's disease
Viera Kovacova	P-06	Unveiling the Hidden Potential of Unmapped Reads in Transcriptomic Analyses: Implications for IBD
John Thomas	P-07	Conserved sex differences in intestinal adaptive immunity are present in the healthy and Crohn's disease ileum which are underpinned by sexually dimorphic transcriptional regulation

Disease modelling and networks - Chairs: Nadezhda Doncheva, Joana F. Neves

Veronica Venafrá	P-08	Signaling profiler, a computational strategy to produce actionable and context-specific networks of intracellular signaling
Matthieu Najm	P-09	A systems biology approach for Cystic Fibrosis
Isabelle Hautefort	P-10	Why do we need <i>in vitro</i> co-culture systems?
Gustavo Monasterio	P-11	Spatially resolved proteogenomic atlas of the upper gastrointestinal tract
Marton Olbei	P-12	Cytokine Networks in Immune Mediated Inflammatory Diseases
Dénes Türei	P-13	OmniPath: database knowledge for mechanistic modeling, cell-cell communication and more

Poster session 2 – 19 July 2023

Microbiome - Chairs: Paul Wilmes, Jost Enninga

Carmela Errico P-14	The Caudovirales Class of Viruses is Associated with Dendritic Cell Impairment in CD Patients
Joachim Fritscher P-15	(in) no time to strain: strain-resolved metagenomic profiling with protal
Laura Nolan P-16	Towards a comprehensive understanding of polymicrobial biofilm development and community interactions
Lejla Potari-Gul P-17	Development of a host-microbe interaction pipeline to reveal the cell- and condition-specific effects of bacteria using single-cell transcriptomics data

Cancer - Chairs: Kristine Schauer, Chris Tape

Stefania Cagliani P-18	The role of mfsd2a in the resolution of colorectal cancer-promoting inflammation: implications for innovative therapies
Norbert Deutsch P-19	DisCanVis: Visualizing integrated structural and functional annotations to better understand the effect of cancer mutations located within disordered proteins
Dávid Keresztes P-20	Investigation of the role of COP9 signalosome in the epithelial-mesenchymal transition
Marco Ruscone P-21	Multiscale Model of the Different Modes of Cancer Cell Invasion
Amanda P-22 Facoetti	Investigating the role of gut eukaryotic virome in contributing to colorectal cancer carcinogenesis

Autophagy and modelling - Chairs: Livia Perfetto, Bence Szalai

Valentina Madár P-23	How yeast go multicellular
Yuseok Moon P-24	Integrated stress response facilitates mucoprotective reprogramming against acute renointestinal distress
Luca Csabai P-25	AutophagyNet: High-resolution data source for the analysis of autophagy and its regulation
Bence Hajdú P-26	A systems biological study of oscillatory characteristic of autophagy induction under cellular stress
Márk Kerestély P-27	Reversal of epithelial-mesenchymal transition in a compartmentalized in silico boolean model

ABSTRACTS OF SELECTED ORAL PRESENTATIONS

Assessing the effects of dietary fibre fermentation in an *in vitro* model of the intestinal epithelium

Amisha A Modasia^{1,†}, Fred J Warren¹, Hannah C Harris¹, Alaa Alhasani^{2,3}, Luca Marciani^{2,3}, Gleb Yakubov^{2,3}, Robin C Spiller^{2,3}

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Background

Irritable bowel syndrome (IBS) is a chronic and debilitating functional gastrointestinal disorder. Inulin-type fructans are associated with increased severity of symptoms, however, despite the clinical short-term benefit of exclusion diets, are associated with negative alterations in microbial composition. Microbial fermentation products, short-chain fatty acids (SCFAs), possess multiple beneficial physiological effects on host intestinal barrier function and is therefore unsurprising that altered epithelial barrier integrity and permeability are also reported in IBS patients.

Alternative approaches to regulating colonic fermentation of dietary fibres include supplementation with the viscous fibre psyllium which has been shown to reduce IBS symptoms without inhibiting overall fermentation.

Objectives

To determine the effects on intestinal barrier permeability and integrity from fermentation of inulin and psyllium and identify which products of microbial fermentation may be responsible.

Methods

A single-vessel batch fermentation model with human stool donors was used to assess the fermentation of inulin and psyllium, with samples collected over a 5-day period. SCFAs, that serve as a surrogate marker for intraluminal intestinal fermentation, were assessed by nuclear magnetic resonance (NMR) to determine the magnitude of intestinal fermentation to the different substrates. To determine the impact of fibre fermentation on intestinal barrier function, an *in vitro* cell culture-based approach with Caco-2 cells modelling the intestinal epithelium was used to assess barrier permeability and integrity.

Impact

Supplementation with the resistant fibre psyllium, provides a promising method of alleviating IBS symptoms without restricting prebiotic intake, therefore promoting proper epithelial barrier function.

The immunogenic cell death (ICD) is a type of cell death that elicits an anticancer immune response

Andrea Checcoli

Centre de Recherche des Cordeliers - INSERM UMRS 1138, Paris, France

ICD-inducing agents (Oxaliplatin, Mitoxantrone) induce cancer cells to release some stress/danger signals that attract and activate dendritic cells (DCs) and initiate the cancer-immunity cycle.

Tumor antigens are engulfed and cross-presented by DCs to T lymphocytes, creating a positive feedback loop in which the antitumor immunity further improves the therapeutic efficacy against cancer cells spared by the cytotoxic agent.

Importantly, not all chemotherapeutic drugs currently approved for cancer treatments are able to induce ICD; some commonly used agents (e.g Cisplatin) fail to activate some processes of ICD.

Yet, beside a robust characterization of the ICD hallmarks, we still lack of knowledge of the underlying mechanisms of ICD.

Our work approach is a combination of literature-based and experimental data, including longitudinal transcriptomic and proteomic datasets.

In order to assess the dynamics of both the cancer cell and the immune microenvironment under the regimen of ICD, we combined *in vitro* and *in vivo* approaches to build a mathematical model of ICD.

The model is based on discrete Boolean formalism and simulated with a software based on MaBoSS framework.

We simulate the model behavior by running stochastic simulations with continuous time, considering the dynamics of a multicellular system with appropriate timing of events and population dynamics for each cell type.

We will apply this model to predict the effect of perturbations on the simulated biological system.

These predictions will be experimentally validated.

Altogether, our *in silico* model should facilitate the identification of novel druggable targets and the development of optimized combination regimens including ICD inducers for the care of cancers.

Cytoscape stringApp 2.0: analysis and visualization of heterogeneous biological networks

Nadezhda T. Doncheva¹, John “Scooter” Morris², Henrietta Holze¹, Rebecca Kirsch¹, Katerina Nastou¹, Yesid Cuesta-Astro³, Thomas Rattei⁴, Damian Szklarczyk^{5,6}, Christian von Mering^{5,6}, Lars Juhl Jensen¹

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2 Resource on Biocomputing, Visualization, and Informatics, University of California, San Francisco, USA

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5 Department of Molecular Life Sciences, University of Zurich, Switzerland

6 SIB Swiss Institute of Bioinformatics, Switzerland

Complex biological systems are often represented as biological networks containing several types of molecular entities. Analyzing and visualizing such networks can advance our knowledge of the underlying cellular mechanisms. This can be accomplished with online databases and software tools like STRING, Cytoscape, and its many apps. Specifically, Cytoscape stringApp has focused on providing intra-species protein–protein interactions from STRING to facilitate the interpretation of data from omics studies, such as proteomics and transcriptomics.

Results: Here, we highlight the latest stringApp and STRING functionality that opens new avenues for exploring results from high-throughput experiments. Version 2.0 of stringApp greatly improves the support for heterogeneous networks, thus making it possible to create networks that contain both proteins and interactions from STRING as well as other molecular entities or associations from external sources. We exemplify this on a published SARS-CoV-2 interactome in Cytoscape using stringApp. Thereby, we also showcase the new group-wise enrichment analysis, which can be performed automatically on several subnetworks, as well as the retrieval of both functional associations and physical interactions from STRING. In another example, we demonstrate how the new cross-species query can be used to retrieve protein–protein interactions between the eukaryotic parasite malaria, its hosts human, and its mosquito vector.

Not just a bystander: may the microbiota be the leading actor in gut diseases?

Ungaro Federica¹, Amanda Facchetti¹, Luca Massimino², Carmela Errico¹, Omar Almolla¹, Stefania Cagliani¹, Tommaso Lorenzo Parigi¹, Silvio Danese²

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² *IRCCS Ospedale San Raffaele, Milan, Italy*

The human microbiota is a densely populated community colonizing all mucosal surfaces of our body, especially those lining the gastrointestinal tract, namely the gut microbiota(1). The gut microbiota is a key component of our wellness and a refined equilibrium between the different components of the gut microbiota and between the gut microbiota and the host has to be preserved, to ensure the correct immune and physiological responses and mucosal homeostasis(2).

It is now well-accepted that any unbalance that can discontinue this delicate equilibrium shifts the healthy mucosa to an altered state, activating a series of mucosal responses that may lead to uncontrolled immune and non-immune reactions at the basis of the pathogenesis of chronic inflammatory disorders, such as ulcerative colitis (UC)(1).

UC is a chronic inflammatory disorder of the colonic tract characterized by mucosal ulcerations and complex biological and immune pathways orchestrating its pathogenesis. Despite the availability of several lines of intervention, many patients still do not respond to anti-inflammatory treatment or experience disease relapse. In this multifaceted scenario, microbiota dysbiosis has been already described as a participant in the UC pathogenesis, but if specific microbial entities may be direct triggers of UC is far from being fully defined(3,4).

Our group has been showing during the last years great interest in elucidating further the roles of the microbiota in directing intestinal inflammation and how it can directly impact mucosal physiology and functions. We recently demonstrated the virome-derived protein hepatitis B protein X (HBx) to induce epithelial barrier damage independently of the other components of the microbiota, altering immune functions and provoking mucosal ulcerations that in turn alter the gut bacteriome composition in mice. This viral factor was also associated with UC pathogenesis in a specific cohort of patients, indicating that it could become a targetable molecule for some individuals carrying this protein in their gut microbiota(5).

Therefore, starting from these pieces of evidence, we believe we are on the correct path toward the full understanding of how the microbiota is involved in chronic intestinal disorders, although much more studies are needed and mechanistic insights should be provided to implement the current lines of intervention for the management of chronic inflammatory disorders and ameliorate the overall patients' quality of life.

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The pharmacobiome: Realizing the therapeutic potential of microbes and microbial products

The Brubaker Lab^{1,2,3}, **Douglas K. Brubaker**^{1,2,3}

1 *Weldon School of Biomedical Engineering, Purdue University, West Lafayette, USA*

2 *Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, USA*

3 *Blood Heart Lung Immunology Research Center, University Hospitals, Cleveland, USA*

Several studies have shown interactions between the human microbiome and health, but precise understanding of the functions of microbiomes and their products are not fully elucidated. This lack of knowledge comes in part because systematic methods to functionally characterize microbiomes are lacking. Here, we propose a systems biology concept to characterize microbes and their products termed the Pharmacobiome. The Pharmacobiome is the characterization of a microbe and its products in terms of known drugs and pathways by studying associations between the microbiome and disease states, phenotypes, pathways, networks, known drug functions and how the human clinical demographic characteristics impact the above associations. The Pharmacobiome perspective emphasizes deriving insights from the application of novel computational frameworks to primarily human-context multi-omics data spanning disease states and microbiome sites.

Here, I will share our lab's efforts to (1) understand the regulatory relationships of microbiomes on host signaling pathways, (2) characterize the anti-cancer potential of microbiomes, and (3) identify microbes and microbial products with the potential to mimic the effects of known pharmacologic compounds. The techniques we employ combine existing correlational and data-driven modeling techniques into novel workflows, linking latent variable approaches to interpretable statistical models more powerful than the component methods individually. Examples of our work span both the gut and vaginal microbiomes to include microbiome regulation of mTOR, estrogen, and progesterone signaling and the identification of microbiome mimics of drugs including paclitaxel, cisplatin, and mifepristone.

Can we overcome bias in machine learning models predicting drug sensitivity and gene essentiality?

Bence Szalai, László Mériő

Turbine.Ai, Budapest, Hungary

Machine learning models help cancer research by predicting how cancer cells respond to different drugs and gene knock-outs with the aim to help identify new therapies and personalised treatment options for cancer patients. However, we realised that most currently used performance metrics to evaluate ML models are very sensitive to biases in the data. Even without adversarial construction, most of the performance of sensitivity predictions come from fitting hidden biases in the data. We developed a statistical framework to highlight how much model predictions surpass bias, capturing the biologically interesting, bias independent component of drug sensitivity and gene essentiality. We show how standard machine learning models perform predicting drug sensitivity and gene essentiality in this new framework. We used multimodal models with different complexity (linear regression, Random Forest, Multi Layer Perceptron) with different cell line (gene expression, mutation) and perturbation (chemical structure, target network topology etc.) specific features to predict drug sensitivity from the GDSC and gene essentiality from the DepMap databases. We found that there is indeed interesting additional information above bias in most models, but it's hidden from standard global metrics. We suggest that focusing on the bias independent performance of ML models can boost their usability in drug discovery and precision medicine applications.

A computational strategy to produce actionable and patient-specific networks of intracellular signaling in Pancreatic Ductal Adenocarcinoma

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² Department of Biology and Biotechnology "Charles Darwin", University of La Sapienza, Roma, Italy

³ Computational Biology Research Centre, Human Technopole, Milan, Italy

these authors contributed equally to the project

With a 11% 5-yr survival rate, PDAC is one of the deadliest cancer types. No effective treatment can be offered to patients diagnosed with PDAC as most patients remain refractory to current regimens. A major factor that contributes to PDAC resistance to treatment is its dense fibrotic stroma intertwined with the extracellular matrix, which, together, provide a physical barrier that protects the tumor and promotes its growth and invasiveness. Uncovering these mechanisms at the patient- resolution level will be crucial to underpin mechanisms of PDAC tumorigenesis and progression and to develop efficient therapies.

We recently implemented SignalingProfiler, a generally applicable modelling strategy that combines transcriptomics and phosphoproteomics datasets with prior knowledge annotated in our in-house resource, SIGNOR, to produce context-specific 'mechanistic models' representing the remodeling of the signal transduction cascade at the PTM-resolution level. We recently applied SignalingProfiler to genomics, transcriptomics, proteomics and phosphoproteomics data from 105 treatment-naïve Pancreatic Ductal Adenocarcinoma (PDAC) biopsies, extracted from the CPTAC portal. By this approach we were able to generate patient-specific mechanistic models of PDAC tumorigenesis and progression. These 'mechanistic models' can recapitulate, in a genotype-specific context, the activation status of key signal transduction proteins basing on the regulatory interactions that affect their activities. Integration of these models with clinical data made it possible to stratify patients according to the key activity of signal transduction effectors, revealing the translational impact of the approach.

A deep learning model of intracellular networks

Avlant Nilsson¹, Joshua M. Peters², Nikolaos Meimetis², Daniel Schafer², Bryan Bryson², and Douglas A. Lauffenburger²

¹ Massachusetts Institute of Technology, Cambridge, USA

² Department of Cell and Molecular Biology, Karolinska Institutet, Sweden

Human cells are signal-processors; ligands bind receptors on the cell-surface and the signals are integrated through a molecular network to activate transcription factors (TFs) that govern cell fate. Disruptions to this network are common in disease, e.g. in cancer it can cause unrestricted growth. Simulations of these processes could help predict disease mechanisms and identify suitable drug targets. However, the human signaling network consists of thousands of molecules with tens of thousands of interactions, and it has been challenging to parametrize systems-wide models using traditional approaches.

Aims: To address this, we developed LEMBAS (Large-scale knowledge EMBedded Artificial Signaling networks). It represents the processes as a recurrent neural network with signaling molecules as hidden nodes with interactions constrained to prior knowledge of their connections (Figure 1A). We aimed to evaluate LEMBAS' ability to predict unseen test-data from living cells after fitting it to training data. To this end we measured gene expression in macrophages in response to combinations of 19 different ligands (Figure 1B), including growth factors, interferons, and both inflammatory and anti-inflammatory interleukins.

Results: LEMBAS generalizes to unseen test data (Figure 1C). Through a combination of sensitivity analysis and perturbations on the trained models we inferred causative signaling cascades that could explain signaling under the tested conditions. We are currently expanding the framework to integrate signaling, metabolism, and gene regulation for a more complete mechanistic description of cellular activities.

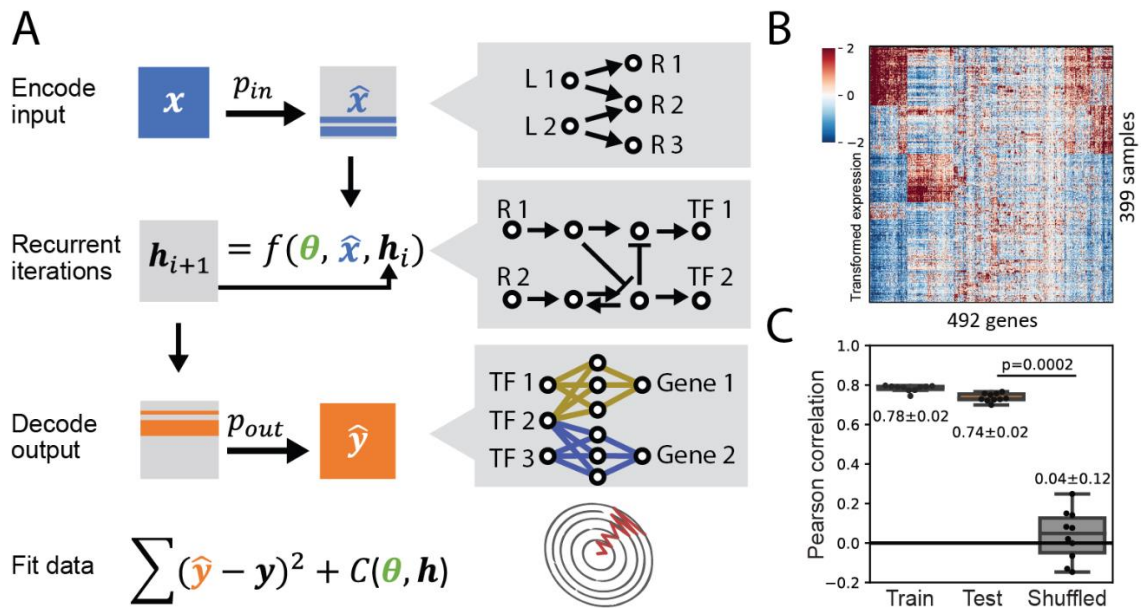


Figure 1. – Deep learning model of macrophage signaling. **A)** model structure. **B)** transcriptomic response to ligand combinations. **C)** 10-fold fold cross validation.

A systems biological study of ATF4-controlled autophagy induction in inflammatory bowel disease

Orsolya Kapuy, Bence Hajdú, Marianna Holczer, Margita Márton

*Semmelweis University, Department of Molecular Biology at the Institute of Biochemistry and Molecular Biology,
Budapest, Hungary*

Inflammatory bowel disease (IBD) is a chronic disease in the gastrointestinal tract. It has shown that disturbance in autophagy-dependent survival might cause IBD, however, the proper dynamical characteristic of IBD regulatory network remains largely unknown. It is well-known that autophagy is essential for the cell to respond various stress events, where the main element of autophagy activator complex, called ULK1, is tightly regulated by the two sensor molecules of nutrient conditions and energy status, called mTOR and AMPK kinases, respectively.

By using various systems biological method, we introduce AMPK-induced activating transcription factor 4, ATF4, as an essential regulatory element of autophagy induction by upregulating ULK1 and down-regulating mTOR in IBD. We propose that ATF4 plays a key role in fine-tuning the induction of autophagy-dependent survival in IBD.

Since ATF4 deficiency can cause severe ulcerative colitis, this study will bring us closer to understand the dynamical features of fatal IBD. Corresponding to experimental data our theoretical analysis has also demonstrated that inhibition of mTOR (e.g. with rapamycin) or activation of AMPK (e.g. with AICAR) can restore dysfunctional autophagy in ATF4-deficiency induced ulcerative colitis, thereby the symptoms of IBD might be postponed.

POSTER ABSTRACTS

P-01 - Systems genomics analysis reveals different upstream biological processes impacting gene regulatory networks in Crohn's disease and ulcerative colitis patients

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Inflammatory Bowel Diseases, including Crohn's disease (CD) and ulcerative colitis (UC), are thought to arise due to complex interactions between multiple single nucleotide polymorphisms (SNPs) and environmental risk factors, which result in pathological immune activation in the gut. Currently, there is still no cure for inflammatory bowel disease (IBD) and the majority of patients fail to achieve long-lasting remission. These shortcomings are reflective of our lack of understanding of IBD pathogenesis. In particular, we are yet to appreciate how these pathomechanisms are likely to vary between individual patients due to the complex gene-environment interactions underpinning IBD. To further understand the genetic risk underpinning IBD, we previously modelled the impact of non-coding IBD-associated SNPs on downstream cell signalling networks in IBD ([Brooks-Warburton et al. 2022](#)). Specifically, we studied how IBD-associated non-coding SNPs can affect the binding of transcription factors (TFs) to transcription factor binding sites (TFBS) in promoter and enhancer regions of the genome, resulting in perturbed downstream cell signalling networks. However, little is known about the upstream signals (including extrinsic and environmental signals) which may influence TFs that bind to IBD-associated non-coding SNPs.

Aim: In this project, we aimed to develop a novel *in-silico* pipeline to infer the likely upstream signals impacting gene regulatory networks using genotype data from a large cohort of UC and CD patients. In other words, we sought to predict potential biological processes that may impact TFs which bind to IBD-associated non-coding SNPs and in turn, their downstream signalling pathways.

Results: We constructed a novel *in-silico* pipeline to map the likely upstream signals impacting TFs that bind to IBD non-coding SNPs by building on the foundations of the iSNP pipeline and also utilising other resources including the gene ontology resource and the IBD Transcriptome and Metatranscriptome Meta-Analysis (IBD TaMMA) framework. Our novel *in-silico* workflow identified different upstream processes impacting gene regulatory mechanisms in UC and CD. In UC patients, we found that the most common upstream signals influencing gene

regulatory networks included B cell differentiation, epithelial cell proliferation, positive regulation of epithelial-to-mesenchymal transition, cellular response to LPS, and cell matrix adhesion. In CD patients, the most frequent upstream signals included positive regulation of VEGF, response to oestradiol, response to virus, regulation of epithelial cell proliferation, and cellular response to IL1. These findings suggest different environmental/extrinsic factors may interact with gene regulatory mechanisms to drive UC (e.g. gram-negative bacteria) and CD (e.g. viruses, oestradiol signalling) pathogenesis. This work could serve as a starting point for future experimental approaches that aim to mechanistically characterise these upstream biological pathways.

P-02 - A systems biological study of the role of endoplasmic stress upon inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic disease in the gastrointestinal tract. IBD is characterised already onset at a young age, and the number of affected patients has risen sharply. Therefore, there is a pressing need to understand the pathologies of IBD and create effective treatments. Recent studies have shown that any disturbance in endoplasmic reticulum (ER) is critical factor, therefore reestablishing intestinal homeostasis by correcting ER-stress induced control mechanism is emerging as a potential therapeutic target for IBD.

Our goal is to explore the essential regulatory motifs of ER-stress induced life-and-death decision in IBD directly focusing on unfolded protein response (UPR). Our scientific questions will be approached from a systems biological perspective by using various theoretical biological tools (such as dynamical modelling and bioinformatic analysis) and molecular biology techniques.

We develop a simple mathematical model which describes the decision making process between autophagy-dependent survival and cell death upon ER stress. We explore the systems-level feedback loops of the control network and confirm the key intra-, and intermolecular-crosstalks of ER-stress induced unfolded protein response. By exploring the key feedback loops we try to understand how autophagy-dependent cellular survival can be extended and therefore the fatal effects of IBD might be postponed. We plan to identify novel agents which might have therapeutical role in the future to expand lifespan of people suffering from IBD.

P-03 - Systems genomics and network approaches uncover how single nucleotide polymorphisms in Crohn's disease and ulcerative colitis affect common processes through different mechanisms

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Inflammatory bowel disease (IBD) has two main types Crohn's disease (CD) and ulcerative colitis (UC). The IBD related single nucleotide polymorphisms are predominantly in non-coding regions of the genome making them challenging to understand. We developed the iSNP pipeline to predict how SNPs in transcription factor (TF) binding sites and miRNA target sites affect signaling networks and contribute to pathogenesis. Here, we further developed iSNP to investigate the pathomechanism of UC and CD in downstream regulatory levels, and to compare these mechanisms.

We analyzed IBD-associated SNP affected genes from 1695 CD and 941 UC patients. To uncover downstream signaling pathways and regulatory processes affected by patient-specific SNPs, we used a heat propagation model to connect SNP-affected proteins to other proteins via a signaling network (using OmniPath), ultimately reaching affected TFs, and their target genes through TF-target gene networks (using DoRothEA).

The iSNP pipeline predicted 83 SNP affected proteins in CD and 121 in UC. Network propagation analysis further identified 518 and 330 proteins affected significantly in CD and UC, respectively; with only 99 proteins overlapping. The functional analysis of the shared proteins indicated the importance of T-cell mediated immunity and the WNT pathway, but also identified disease specific pathways. The affected regulatory networks contain 2067 and 3352 genes in CD and UC, respectively. The patient clusters based on their regulatory networks corresponded to cell specific differentially expressed genes in CD. Furthermore, the regulatory network in UC corresponded to the inflammatory response based on other transcriptomic studies.

Single cell analysis with regulatory network analysis uncovered cell and patient specific manifestation of CD. We showed how different CD and UC associated SNPs use the same biological mechanisms to influence similar immune and developmental pathways with distinguishable outcomes.

P-04 - Characterization of a novel molecular stratification of Ulcerative Colitis patients

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Ulcerative colitis (UC) is a highly heterogeneous chronic inflammatory bowel disease (IBD) that primarily affects the colon. Although there are some available classifications, mostly based on clinical parameters, the lack of a molecular stratification prevents full comprehension of the diagnostic and prognostic heterogeneity of patients, and thus from correctly predicting their response to available treatments. We have recently developed an unbiased stratification of UC patients (UC1/UC2) based on their transcriptomic profiles (Czarnewski P; et al. *Nature Communications*, 2019), which holds great potential for the design of personalized medicine in IBD. However, further characterization is necessary to validate it and consolidate its clinical application. To achieve this goal, we used bulk and single-cell RNA sequencing (sc-RNAseq) datasets from a prospective cohort of Swedish IBD patients to understand whether UC1 and UC2 are different diseases or states of the same disease, and to deeply characterize the cellular composition of UC1 and UC2 patients. In addition, we took advantage of murine spatial transcriptomics datasets of colonic tissues at steady state or during mucosal healing, to get insights into the spatiotemporal dynamics of UC1/UC2 transcriptional profiles.

A longitudinal analysis of UC1/UC2 transcriptomic profiles as well as gene module analysis revealed that UC1 and UC2 profiles are fluctuant during the course of disease, eventually representing different stages of the same disease. scRNAseq analysis allowed us to identify specific cell types characterizing UC2 patients. Finally, while genes defining UC2 patients distributed homogeneously within the murine mouse colon at steady state and during mucosal healing, UC1 genes primarily mapped within damage/regeneration regions.

This study details the differences in immune cell composition and molecular pathways in UC1 and UC2 patients, contributing to a more comprehensive understanding of the new molecular stratification system we have developed for ulcerative colitis patients.

P-05 - Paralog proteins play a crucial role in rewiring cell-type specific signalling processes in Ulcerative Colitis and Crohn's disease

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Cells communicate through signalling pathways which help to coordinate biological processes within multicellular organisms. These processes are complex networks of cross-talks of signalling pathways. Paralog proteins, which derive from homologue genes originating from gene duplication, could be key signalling members and have different functions, leading to a different signalling outcome. The aim of this study is to explore the diverse expression and role of these paralog proteins in signalling pathways in inflammatory bowel disease, including the two main subtype, ulcerative colitis (UC) and Crohn's disease (CD) from biopsies consisting of the three cell lineages (epithelial, immune and stromal cells). Using network biology approaches combined with single-cell RNA-seq data, we identified paralog groups that show cell and/or condition specificity. We were focusing on the JAK/STAT signalling in different cell types. Based on the results, paralog genes show diverse expression patterns in cells depending on the condition. Highlighting the role of JAK and STAT proteins we found that their dimers show IBD condition specific expression. We built up regulatory networks including the target genes of STAT dimers and showed that STAT3 and STAT1 mediate the activation of genes in a condition-specific manner in several immune and epithelial cells. In conclusion, we have developed a pipeline that facilitates the identification of potential key differences among- paralog proteins in cell signalling not only cell-specifically but also in a condition-specific way.

P-06 - Unveiling the hidden potential of unmapped reads in transcriptomic analyses: implications for IBD

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Transcriptomic studies routinely encounter a substantial fraction of unmapped reads, yet these data hold significant potential for refining downstream analysis outcomes. The impact of analytical variations, including alignment methods, mismatch tolerances, and indel handling, on mapping results is considerable and influences the nature of mapped reads, variant calls, and gene expression measurements.

Our work underscores the need to optimize mapping criteria and to perform de novo assembly of unmapped reads. Notably, strict mapping criteria can increase the proportion of unmapped reads. For instance, the genetic divergence at the bacterial sub-species level (5%), equivalent to fifty point mutations in a 1000 bp locus, can reduce the proportion of mapped reads by 10% under the default settings of the STAR aligner.

Further, our exploration of the 'NGS sequencing dark matter' (unmapped reads) from the Inflammatory Bowel Disease Transcriptome and Metatranscriptome Meta-Analysis (IBD TaMMA) illuminates an array of rare human transcripts and transcripts derived from the microbiome. These rare human transcripts are disease-correlated and display significant functional and protein-protein interaction enrichment.

The emerging potential from the often-overlooked fraction of transcriptomic data suggests that revisiting the methodological strategies could be advantageous. Doing so can further enhance our understanding and management of complex diseases like IBD.

P-07 - Conserved sex differences in intestinal adaptive immunity are present in the healthy and Crohn's disease ileum which are underpinned by sexually dimorphic transcriptional regulation

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), exhibits important sex differences in epidemiology, disease course, severity of disease, and therapeutic response. However, the underlying pathomechanisms contributing to sex differences in IBD have been largely understudied thus far. Recent landmark studies in model organisms have revealed that intestinal tissues are highly sexually dimorphic with significant sex differences in intestinal homeostasis including epithelial cell growth, nutrition, and metabolism. Given these findings, we performed explorative *in silico* analyses of bulk and single-cell transcriptomics data from murine and human intestinal mucosal samples to evaluate whether sex differences are present in intestinal immunity in both health and IBD.

We identified that there are consistent sex-biased immune signatures primarily relating to adaptive immunity in the healthy and CD ileum which are conserved across species – specifically, a female sex-bias for B cell signalling, and lymphocyte proliferation and differentiation. Granulocyte chemotaxis also appears to be sexually dimorphic although the directionality of the sex bias was not consistent across species or disease states (healthy vs CD). Using gene regulatory network analysis, we identified that differences in transcriptional regulation may underlie these sex biases - in particular EGR1, which appears to be driving the female sex bias in B cell signalling and lymphocyte proliferation & differentiation in both healthy humans and mice. In the healthy murine ileum, other transcription factors also regulate lymphocyte pathways in females including ESR1, HNF4A, and TFAP2C. In the CD human ileum, these pathways appear to be regulated in females by FOXP3.

The findings that B cell signalling, lymphocyte proliferation, and lymphocyte differentiation are female sex-biased pathways, suggest that there could be sex differences in gut associated lymphoid tissues (GALT) in both the healthy and CD ileum. This may contribute to some of the sex differences observed in CD. Future work will aim to further characterise these sex differences in intestinal humoral immunity *in vivo* using immunophenotyping and single-cell sequencing approaches in both mouse models of ileitis and CD patients.

P-08 - Signaling profiler, a computational strategy to produce actionable and context-specific networks of intracellular signaling

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Cell signaling is composed of an intricate architecture that is finely tuned to maintain homeostasis. Signaling rewiring is the primary strategy for cells to evolve toward a malignant phenotype or to acquire a drug-resistant phenotype^{1,2}. Computational modeling strategies have been exploited in the last 15 years to unravel the complex mechanisms regulating biological systems or to identify promising intervention targets³.

We have recently implemented a generally applicable modeling strategy, dubbed 'Signaling Profiler' (SP)⁴ (<https://github.com/SaccoPerfettoLab/SignalingProfiler>), which builds up mechanistic models recapitulating the key axes driving a disease or a resistant phenotype.

SP is a flexible and modular pipeline composed of three customizable steps:

- **Inference of protein activities:** the context-specific activity modulation of key signaling proteins (transcription factors, kinases, phosphatases, and phosphorylated proteins) is computed from transcriptomic and (phosphor)proteomic data by integrating widely used methods, such as VIPER⁵, with ad hoc developed methodologies, such as PhosphoScore and ProteoScore.
- **Naïve network generation and optimization:** the signal propagation is reconstructed in a two-steps process: (i) generation of a naïve network that connects a user-defined set of receptors to the previously inferred proteins through directed and signed interactions annotated in our in-house resource, SIGNOR⁶; (ii) optimization of causal edges over the experimentally derived activities of proteins through the CARNIVAL algorithm⁷.
- **Model validation through hallmark phenotype activity inference:** SP provides an in-silico validation framework, called PhenoScore, to integrate the signal over system-specific hallmark phenotypes, which are connected to the model exploiting our novel resource, ProxPath.

We successfully applied SP on FLT3-ITD driven AML multi-omics datasets to detect resistance axes to both Tyrosine Kinase Inhibitors⁴ and cytarabine (in press). The SP-identified axes were experimentally validated, and their inhibition could restore sensitivity to drugs in both AML cellular models and ex vivo AML patients' blasts.

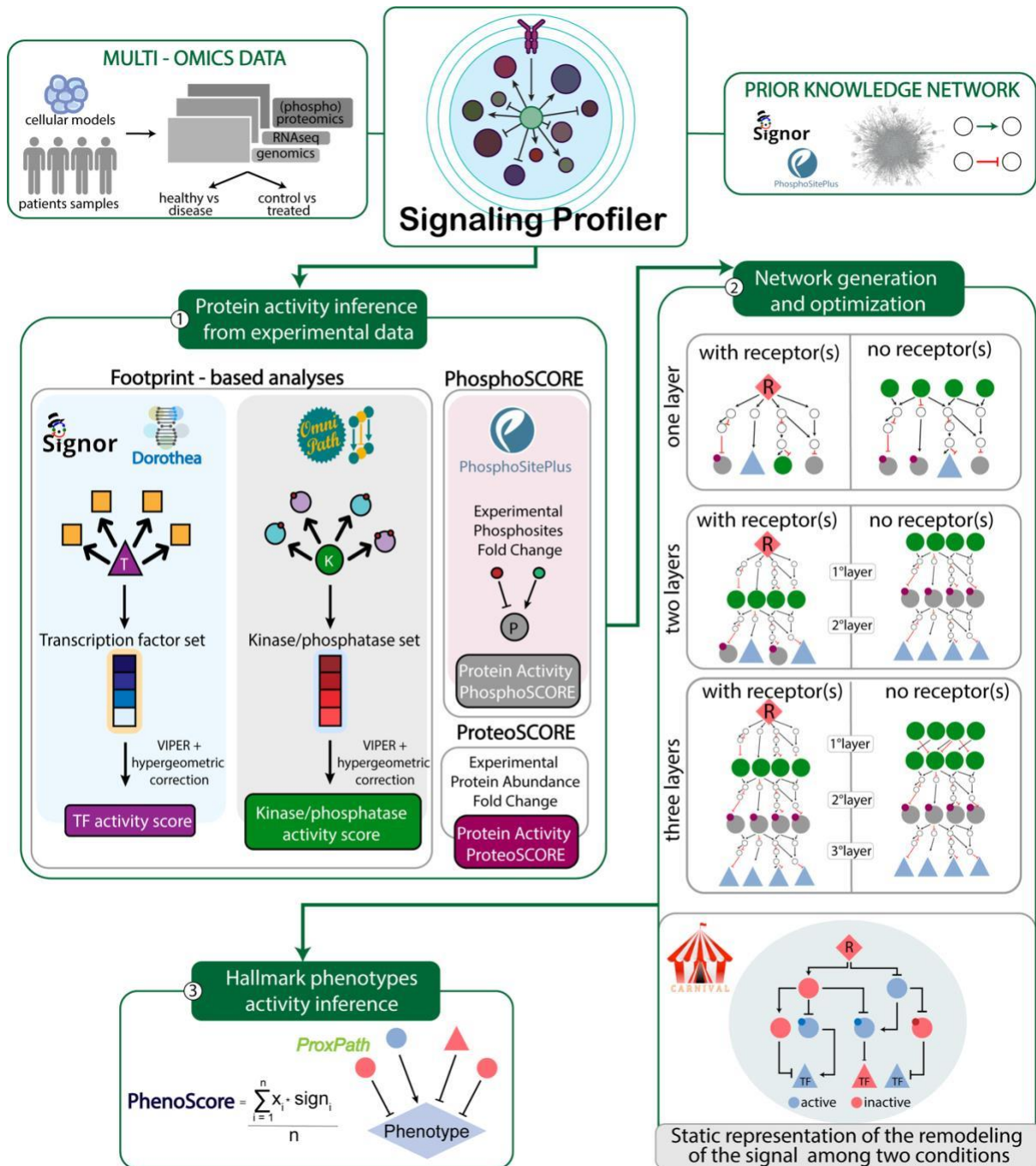


Figure 1. – Signaling Profiler workflow. Signaling Profiler requires as input: (i) multiomics data tables containing the fold-change between two conditions and (ii) a prior knowledge network derived from literature. SP is composed of three steps: 1) Inference of protein activities with three different techniques. The footprint-based analysis (left panel) infers the activity of transcription factors (kinases/phosphatases) from the modulation in the experimental data of their target genes (phosphosites); the PhosphoScore technique (right top panel) assigns to phosphorylated proteins an activity score combining the regulatory role of its phosphosites with their fold-change in the experimental data; the ProteoScore (right down panel) transforms the protein abundance modulation in an activity score. 2) Network construction and optimization, in which a user-defined set of receptors is connected to the previously inferred proteins in a highly customizable way. The user can create three types of naïve networks: a one-layer network in which the receptor(s) is linked to all the inferred proteins; a two-layer network where the receptor is connected to kinases and phosphoproteins and then kinases and phosphoproteins are linked to transcription factors; a three-layer network where the receptor is connected to kinases, then kinases are connected to phosphoproteins (second layer), and finally, the second layer is connected to the transcription factors. Then, CARNIVAL reconstructs the signal propagation combining the naïve network with the protein activities inferred in step one. The network represents the remodeling of the signal due to treatment (if the user is comparing treated vs control) or driving a specific disease (if the user is comparing disease vs healthy). 3) The hallmark phenotypes activity inference provides a model in-silico

validation framework. It allows the user to check if the model recapitulates expected phenotypes. The computation of phenotypes score (PhenoScore) is obtained by integrating the signal over system-specific hallmarks phenotypes, which are connected to the model exploiting our novel resource, ProxPath.

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P-09 – A systems biology approach to Cystic Fibrosis

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Cystic Fibrosis (CF) is caused by mutations in the gene coding the Cystic Fibrosis Transmembrane Regulator (CFTR) protein. However, the overall pathophysiology cannot be solely linked to the loss of the CFTR function. Indeed, although CF is a monogenic disease, CFTR belongs to a yet not fully deciphered network of proteins involved in various biological pathways. Absence of a functional CFTR leads to perturbations of these pathways, leading to deleterious CF phenotypes.

We used publicly available transcriptomic CF datasets to identify the most frequently deregulated biological pathways. We adopted a systems biology approach to connect these pathways and thereby define the CF biological network. CFTR was connected to this network based on its known direct protein interactors (i.e. CFTR interactome) that were present in the network or connected to it. The biological pathways present in the network and their resulting phenotypes were found consistent with today's CF knowledge. This indicates that the approach employed in this study did capture relevant information about the disease.

The topological analysis of the network highlighted a few proteins that may initiate or propagate deregulations from CFTR into the network, and explain the observed CF phenotypes. Importantly, modulators or drugs are known against these proteins, which allows experimental validation, and further offers potential drug repositioning opportunities.

This initial network model can be refined with other types of omics data such as proteomic. Although our research is focused on CF, we believe that our approach could be used to study other monogenic diseases as well.

P-10 – Why do we need *in vitro* co-culture systems?

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Mucosal surfaces, such as the ones found in the intestine or lungs of all mammals, are the stage of many interactions between the different epithelial cell types lining the gut, the underlying tissue on one side including diverse immune cells, enteric neurons and glial cells, and the dynamic and highly complex microbial community residing on the other side. Unfortunately, until recently, no simplified *in vitro* systems were reliably reproducing the *in vivo* cell diversity to decipher cell-cell and cell-microbe interactions. In the last fifteen years, the emergence of self-assembling stem cell-derived 3D structures, called organoids, has revolutionised a wide range of research fields.

Organoids can recapitulate, in a dish, the diverse epithelial cell populations normally found *in vivo*. Many organs can now be modelled using these organoid models. For the first time, tissue development, regeneration and maintenance can be studied in patient-derived organoids, showing great promise for the future of precision and regenerative medicine. However, it is becoming apparent that we now need to bring back elements of the whole tissue complexity in our investigations. Cell-cell interactions and the impact of gut-resident or transiently passing microbes need recapitulating *in vitro* too. For that, access to reliable co-culture systems is imperatively needed.

Many microfluidics systems are being developed to address this need, each one complementing the others. Some are based on single chips that reproduce many of the shear forces and luminal flux observed *in vivo*, improving the degree of differentiation of the epithelial cells, but they do not allow live imaging of the dynamic responses cells may have upon exposure to other cellular protagonists. They are also incompatible with high throughput format assays. Others, more recently developed, have adopted a multiple-chip high throughput format and provide thin observation windows permitting full-thickness imaging of live or fixed samples. They can be applied to multiomic data outputs and enable drug or health-promoting compounds to be screened on patient-derived organoids.

We are developing protocols allowing the culture of human colon organoid-derived cells in the Organoplate microfluidic system from Mimetas (<https://www.mimetas.com/en/our-technology/>). First, we validated the functionality of the system with the Caco-2 immortalised human intestinal cell line. In that system, we succeeded in growing epithelial cell tubes offering access to both the luminal and basal sides of the intestinal epithelial layer. We then attempted to expose Caco-2 cell tubules to biofilm-forming microbes, such as the opportunist pathogen *Pseudomonas aeruginosa*. In this model, we monitored the alteration of tight junctions in the Caco-2 cell tubules by fluorescence immunolabelling and high-content imaging. Currently, we are optimising numerous parameters for growing organoid-derived human epithelial cell tubules. Next, we will infect these tubules with *P. aeruginosa*, and possibly members of the gut microbiota or their metabolites. Successful optimisation will open the door to many projects and lead to future translational applications.

P-11 – Spatially resolved proteogenomic atlas of the upper gastrointestinal tract

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INTRODUCTION: The oral mucosa has long been regarded as a particular barrier with privileged healing properties. However, there is still a poor understanding of the site-specific cellular and molecular circuitry that characterizes this barrier. Here we present the first spatial proteogenomic atlas of the entire murine upper gastrointestinal tract (UGIT) combining spatial transcriptomic (ST, 10x VISIUM) and spatial proteomic (CANOPY, ChipCytometry), together with an innovative dissection/mounting technique (oro-esophagealstomach rolls – ORES rolls). By spatially resolving gene expression and protein detection, we provide a reference atlas for exploring cellular and genetic programs in the upper GI tract, either under steady-state conditions or after challenges, with a special focus on the oral barrier (OB).

METHODS: Male wild type C57BL/6J mice between 8 and 10 weeks of age were used for tissue obtention. Oral, oesophageal and stomach tissues from untreated mice were harvested, rolled, and processed for ST or proteomic studies. To spatially resolve the transcriptomic data, we used non-negative matrix factorization (NNMF) to deconvolved it into 55 factors. Then, each factor was manually annotated based on colocalization with histological features.

RESULTS: As previously shown for colon, using ST and NNMF we were able to identify a previously unappreciated level of molecular regionalization of murine UGIT at steady state, both at proximal-distal and basal-luminal axes. At steady state, we found that Krt76, Krt16, Krt14, Krt6a, and Krt24 were highly expressed in the OB. While Crnn (Cornulin) and Hnrn (Hornerin, involved in cornification of cornified epithelium) were enriched in proximal OB, Iffo2, Gdpd2, and Krt16b were highly expressed in the distal OB. Our data aligns with previous reports showing high Krt76 expression in the OB, and further reveal potential markers defining proximal-to-distal OB molecular regionalization. Finally, using 18-plex SP, we identified a highly compartmentalized regionalization of immune cells along the UGIT.

DISCUSSION & CONCLUSIONS: The current study uncovered spatial transcriptomic patterns that are present at steady state conditions in the UGIT, characterized by unique transcriptional signatures that coincided with different histological processes and immune cells' location. This reference atlas laid the foundation for further exploring the distinctive proteogenomic signatures of the oral barrier, compared to the oesophageal and stomach barriers, at steady state or upon local or distal challenges that can perturb the healing process of the oral mucosa.

P-12 – Cytokine networks in immune mediated inflammatory diseases

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Cytokines, small peptides that facilitate communication between various cell types, are crucial components of the immune system. They form intricate networks of interactions where different cytokines positively and negatively influence each other's production and activity. These networks play a vital role in maintaining the balance and specificity of immune responses during illness and infection. In drug development, targeting cytokine communication is often crucial for managing overactive immune responses in immune-mediated inflammatory diseases (IMIDs), a group of ostensibly unrelated disorders characterised by chronic inflammation and tissue damage resulting from an overactive immune system. Common IMIDs include inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE).

In this study, we created and analysed cytokine-cytokine interaction (CCI) networks for prevalent IMIDs using publicly accessible single-cell RNA-Seq datasets, aiming to uncover shared and distinct immune signalling pathways impacting IMID patients. The generated networks reveal potential intervention points where the exacerbated immune response could be mediated. We established statistically significant CCIs by comparing our results with experimental cytokine response data deposited to the CytoSig database, and determined the likely intracellular pathways connecting upstream and target cytokines using the OmniPath interaction resource and the NicheNet software.

Our research provides new insights into the role of cytokines in IMIDs, and has the potential to aid development of novel treatments for these conditions that could be utilised when patients do not respond to direct intervention of central cytokines.

P-13 – OmniPath: database knowledge for mechanistic modeling, cell-cell communication and more

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OmniPath is a molecular biology database combining more than 170 original resources. It covers a broad variety of data, including signalling, metabolic, gene regulatory and miRNA networks, along with rich annotations of protein and gene function, structure, localization. OmniPath is publicly available by a web API (omnipathdb.org), with convenient access from R and Python by dedicated packages. Thanks to its integration with the DecoupleR and AnnData packages, it seamlessly supports transcription factor and pathway activity inference from bulk or single cell transcriptomics. Its literature curated network of activatory and inhibitory molecular interactions is routinely applied for causal reasoning by the software CARNIVAL and COSMOS, deriving mechanistic insights from transcriptomics or multi-omics data. OmniPath also features comprehensive knowledge about intercellular signalling which, connected to the LIANA module, enables easy inference of cell-cell communication from single cell transcriptomics. These are only a few examples for the applications of OmniPath's diverse data. The integration of OmniPath into the above mentioned workflows is realised with the support of the HPC/Exascale Centre of Excellence for Personalised Medicine in Europe (PerMedCoE) programme.

P-14 – The caudovirales class of viruses is associated with dendritic cell impairment in CD patients

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The gut virome has been established to hold a key role in the pathogenesis of Crohn's disease (CD), belonging to the Inflammatory Bowel Disease (IBD) class. Indeed, some years ago Norman and colleagues described the expansion of Caudovirales as associated with CD pathogenesis and coupled with decreased bacterial diversity¹. To further explore this aspect, we exploited the IBD TaMMA framework², and we found the Autographiviridae, belonging to the class of Caudovirales was highly abundant in the CD colon by comparison with the healthy control (Fig. 1a), confirming the previous study. Therefore, to further investigate the role of this family in the different mucosal cell compartments, CD- and healthy-derived intestinal biopsies were digested to obtain a cell suspension undergoing flow cytometry cell sorting (FACS) to isolate immune and non-immune cells according to cell population-specific markers. Specifically, CD8 and CD4 T cells, B cells, macrophages, dendritic cells, epithelium, endothelium, and fibroblasts were obtained and underwent transcriptomics and metatranscriptomics. Interestingly, the gene ontology (GO) revealed that the sole CD cell populations showing impaired biological response to viral insults by comparison with the healthy were the dendritic cells, fibroblasts, and epithelial cells (Fig. 1b-d) suggesting that in CD mucosa only specific cell compartments were impacted by viral entities. Furthermore, by metatranscriptomics analysis we identified the CD dendritic cells to retain a higher abundance of the Autographiviridae family by comparison with the healthy (Fig. 1e), indicating that the virome dysbiosis in these cells may underlie CD pathogenesis by altering their biological functions. Consistently, the heatmap of differentially regulated genes in CD versus healthy dendritic cells displayed downregulation of the transcripts key for dendritic cell-directed immune response (Fig. 1f).

Similar results have been recently proposed by our group to explain ulcerative colitis (UC) pathogenesis, where we identified a virome-derived protein, belonging to the Hepadnaviridae family, to directly disrupt the epithelial barrier and guide the development of intestinal inflammation³. Therefore, given the growing evidence of the viral entities as triggers for IBD, these data are promising and may pave the way to the discovery of viruses directly involved in CD pathogenesis that can represent a target for the development of novel strategies of intervention, ultimately ameliorating the CD management soon.

¹ Norman, J.M. et al. "Disease-specific alterations in the enteric virome in inflammatory bowel disease." *Cell* vol. 160,3 (2015): 447-60.

² Massimino, L. et al. The Inflammatory Bowel Disease Transcriptome and Metatranscriptome Meta-Analysis (IBD TaMMA) framework. *Nat Comput Sci* 1, (2021):511–515.

³ Massimino, L. et al. Gut virome-colonising Orthohepadnavirus genus is associated with ulcerative colitis pathogenesis and induces intestinal inflammation in vivo. *Gut*. 2023 Feb 14;gutjnl-2022-328375.

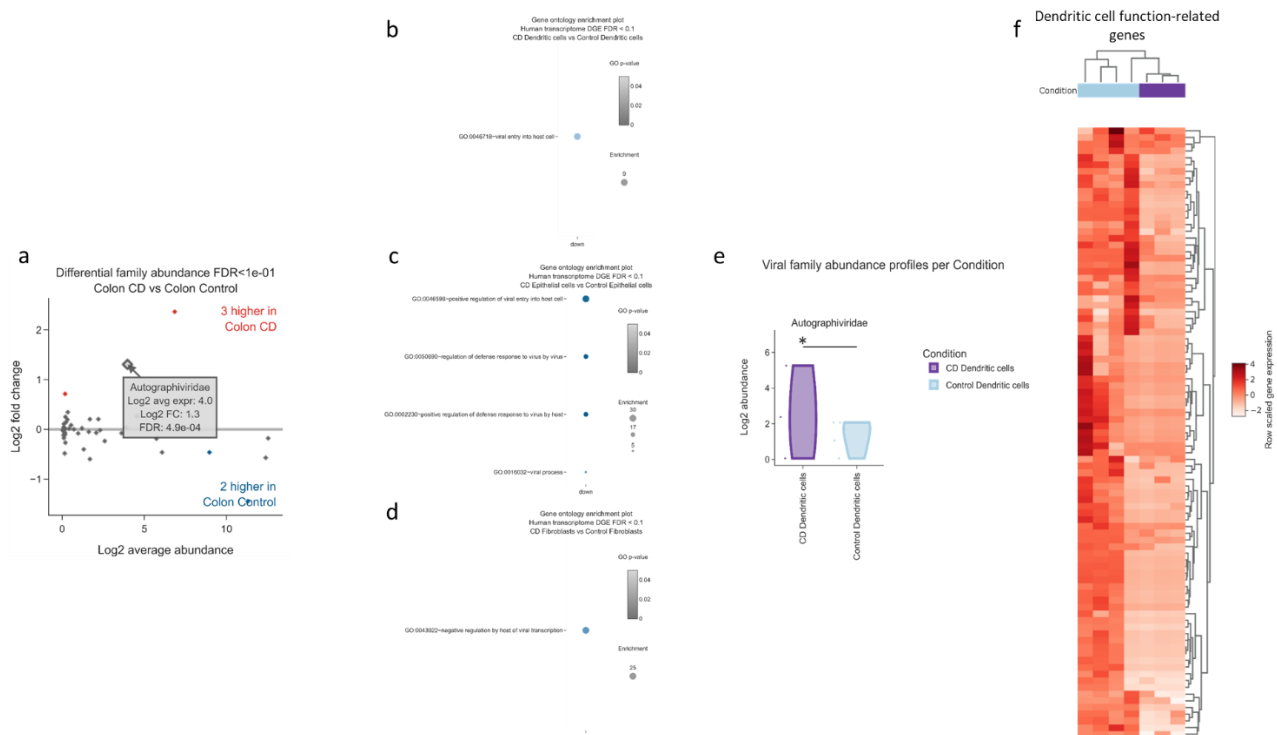


Figure 1 - Autographiviridae viral family abundance in CD colon and the modulation of biological processes to viral insult in CD-derived dendritic cell. a) MA-plot showing Autographiviridae viral family abundance in CD colon compared to control colon. b-d) GO plots displaying gene set enrichments of the specific viral biological processes in CD dendritic cells (b), CD fibroblasts (c) and CD epithelial cells (d) versus healthy counterparts. e) Violin plot showing the differential abundances of Autographiviridae in dendritic cells compared to healthy-derived cell population (*p= 0.031). f) Gene expression heatmap related to dendritic cell functions.

P-15 - (In) no time to strain: strain-resolved metagenomic profiling with protal

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Disclaimer: This data is preliminary and not intended for wider sharing.

Metagenomic profilers process metagenomic sequencing data to reveal the microbial composition and functional potential of microbial communities in a multitude of different environments. Current metagenomic profilers often lack in speed, accuracy and user-friendliness, especially for strain-level. To keep up with the increasing amount of meta-analysis studies encompassing many thousand metagenomic samples and facilitate novel discoveries at reasonable computational cost, profilers need to provide accurate results and fast speeds on species and on strain-level. Here, we present protal (profiling through alignment), a metagenomic profiler operating on strain-level, offering accuracy on-par or better and speed outperforming other state-of-the-art tools.

Aims: Strain-level analysis is still not a standard in metagenomics and tools are either slow or focus on target taxa in a community to reduce computational demands. Protal offers fast strain-resolved profiling for all species of a set of samples and thus allows more researchers to reach strain-level.

Results: We tested protal against mOTUs3, MetaPhlAn3 and MetaPhlAn4 to assess the ability to accurately detect taxa and reconstruct their abundances. Protal has a sensitivity and precision similar or better than state-of-the-art tools for taxonomic classification (Figure 1A) at speeds up to 8x faster than the other tools (Figure 1B). Early development strain-level results on synthetic data with *akkermansia muciniphila* indicate that reads from the same genome consistently cluster together in phylogenetic trees created from the multiple sequencing alignments produced by protal (Figure 2). For protal, most of the strain-level computations are already included in the speed benchmark on species level and it is thus to be expected that the speed difference between protal and other strain-level tools will be even greater.

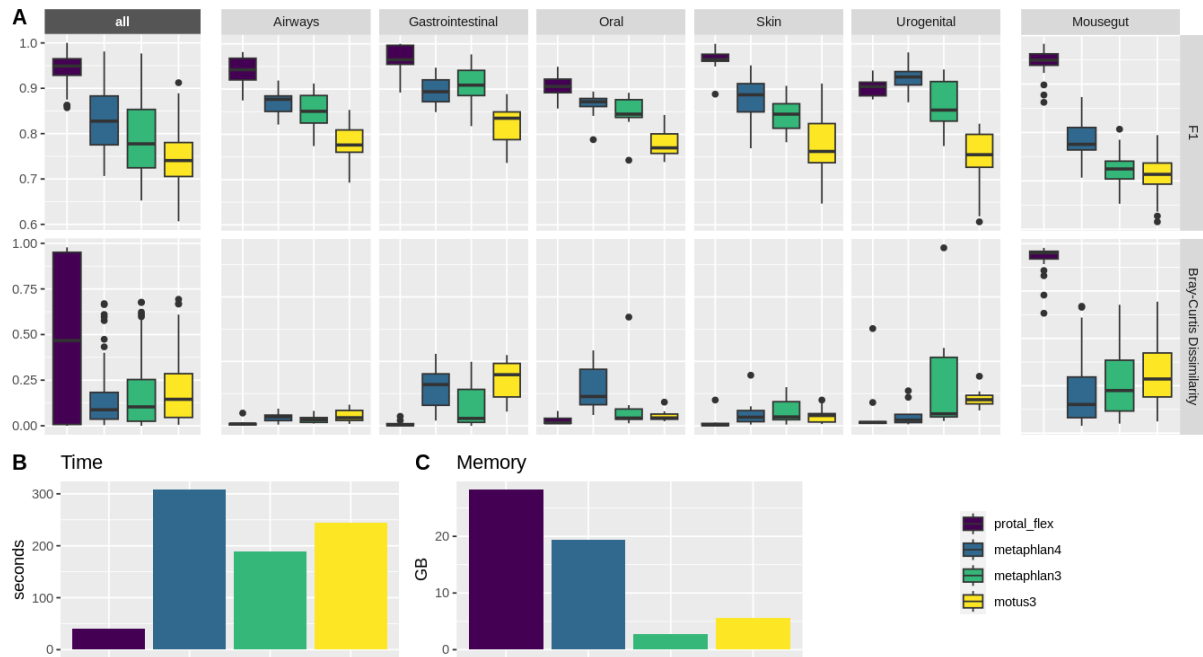


Figure 1. – Protal was tested against mOTUs3, MetaPhlAn3 and MetaPhlAn4 on the synthetic dataset from the CAMI-Challenge (Human data and Mouse gut). **A** Each boxplot with each datapoint within being a sample is the comparison between the tools for a certain simulated environment while the leftmost column is the summary of all environments. The top row contains F1 scores and the bottom row displays the Bray-Curtis dissimilarity. **B, C** The tools were compared regarding the runtime and memory requirement, respectively, on a subset of the data used in A.

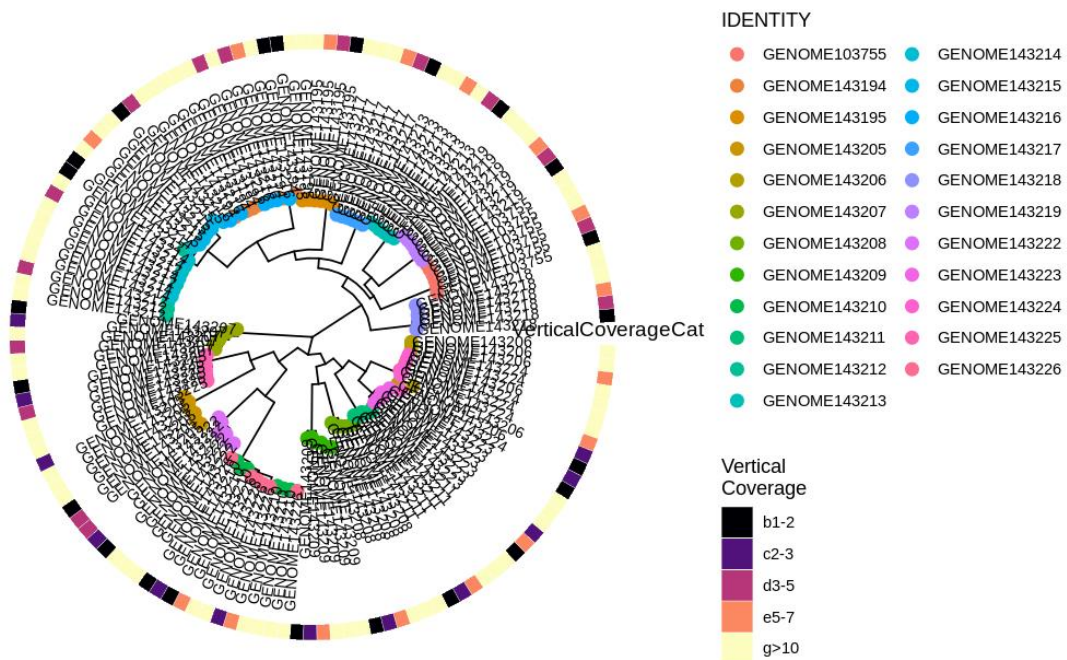


Figure 2. – Reads were simulated from random *Akkermansia muciniphila* strains to assess protals ability to cluster together samples containing the same strain. Tip color indicates the genome whereas the outer ring color encodes for simulated vertical coverage.

P-16 – Towards a comprehensive understanding of polymicrobial biofilm development and community interactions

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Biofilms are the normal mode of growth for microbes. A biofilm is a complex 3D structure where microbes are encased in an extracellular matrix. The matrix protects inhabitants from exogenous stresses like antibiotics, disinfectants, and the immune system, which means that biofilms are often challenging to remove. This recalcitrance can be either beneficial or problematic for the host or local environment. Examples of beneficial biofilms include those in the mammalian gut and oral cavity, in some fermented foods, and in wastewater treatment plants. Conversely, examples of detrimental biofilms include those in the cystic fibrosis (CF) lung, in chronic wounds, on ship hulls, and on spoiled food.

Our overall research aim is to understand microbial biofilm development in relevant settings and how microbe-microbe interactions shape population profile dynamics in these communities. We are working on two main biofilm areas: (1) those associated with the CF lung and (2) with food spoilage. The goal is to use our knowledge to develop interventions that (1) alter the CF lung microbial population profile to obtain the most beneficial outcome for the patient, and (2) develop interventions that reduce food spoilage and extend food shelf-life.

Biofilms are heterogenous at the physical and chemical level, and at the level of community composition, gene expression, and protein and metabolite production, which makes them challenging to study. We are currently using several global 'omics and single-cell approaches to study biofilms however, we are seeking collaborators to develop additional global (metabolomics and proteomics) and single-cell (CLASIFISH and single-cell RNAseq) approaches for our research. Given that all these approaches generate large amounts of data in various formats we are also seeking collaborators to integrate and effectively analyse the multi-omics and single-cell data we are obtaining to get the maximum value out of these datasets and increase our understanding of these complex systems.

P-17 – Development of a host-microbe interaction pipeline to reveal the cell- and condition-specific effects of bacteria using single-cell transcriptomics data

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The human microbiome regulates physiological processes through interactions with the host. Disruptions to the microbiome composition can result in inflammation and the emergence of diseases, such as inflammatory bowel disease (IBD) in the gut. Short linear motifs are short sequences of amino acids that facilitate specific protein-protein interactions through physical connections, usually via domains. These interactions are vital in host-microbe interactions as they have the ability to regulate host immune responses and modify cellular signalling pathways.

We have updated our MicrobioLink pipeline, an algorithm predicting microbe-host interactions through domain-motif and domain-domain interactions using the ELM database. It focuses on cell type-specific effects of bacteria in health and disease through utilising functional analysis and network propagation tools to uncover direct microbe-affected processes and downstream pathways affected by bacteria.

To demonstrate the usefulness of the pipeline, we analysed the impact of *Bacteroides thetaiotaomicron* (Bt) on human immune cells focusing on Toll-like receptor signalling, and found that proteins in extracellular vesicles secreted by Bt may modulate the pathway intracellularly in a cell-type and condition-specific way.

The pipeline offers detailed mechanistic insight into the relationship between bacteria and host cells that will improve our understanding of how microbial proteins may be of therapeutic value in inflammatory diseases, such as IBD.

P-18 – The role of MFSD2A in the resolution of colorectal cancer-promoting inflammation: implications for innovative therapies

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Inflammation is a recognized hallmark of colorectal cancer (CRC), contributing to its development and progression. CRC-associated inflammation may be contrasted by specialized pro-resolving lipid mediators (SPMs) derived from omega-3 polyunsaturated fatty acids as recently proposed. We previously demonstrated that the endothelial Major Facilitator Superfamily Domain-containing 2A (MFSD2A) promotes the resolution of chronic intestinal inflammation by overseeing the release of SPMs. Therefore, we hypothesized that the endothelial *MFSD2A* might have also a role in hampering CRC-associated inflammation.

To test this hypothesis, we performed a transcriptomic analysis on Human Intestinal Microvascular Endothelial Cells (HIMEC). Besides the increased levels of *MFSD2A* transcript in CRC by comparison with the healthy, this analysis pointed out the activation of the resolution phase-related biological processes in CRC as compared to the healthy, proposing the endothelium as a player also in CRC pathogenesis. Furthermore, by lipidomics, CRC HIMEC displayed increased release of SPMs and reduced levels of the pro-inflammatory lipids by comparison with the healthy control cells. Interestingly, upon *MFSD2A* silencing, CRC HIMEC switched to a proinflammatory phenotype, characterized by the activation of pro-inflammatory biological processes and the increased release of pro-inflammatory lipids. Notably, the enhancement of *MFSD2A* expression both *in vitro* and *in vivo* reduces cancer cell growth and shapes tumor microenvironment. These data suggested that *MFSD2A* might contrast the tumor-associated inflammation and ensure the correct balance between pro-inflammatory and pro-resolving milieu, offering novel insights into the development of innovative therapies suitable for CRC patient management.

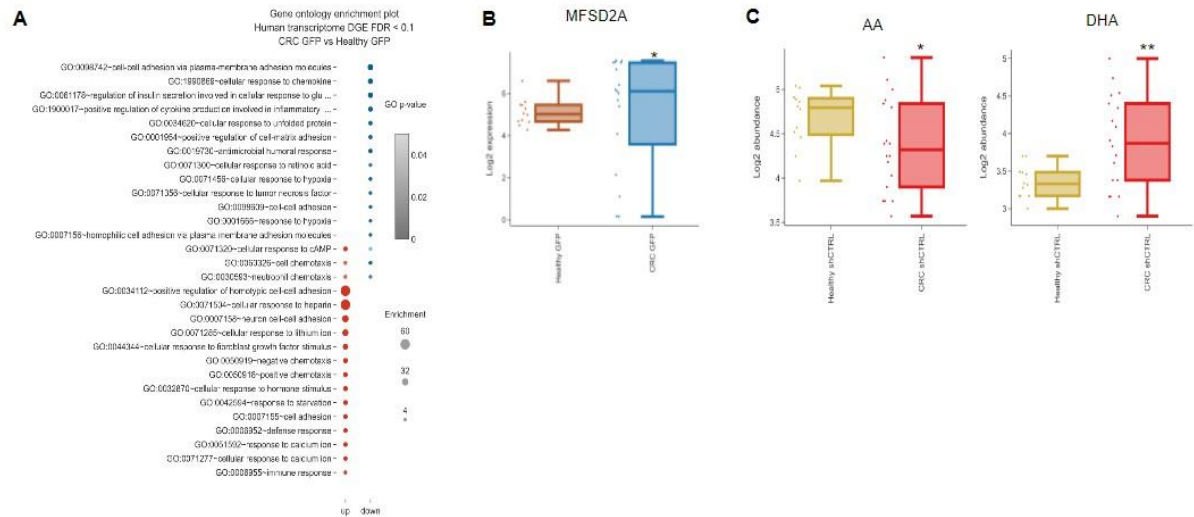


Figure 1. CRC displays an enrichment of inflammation-related pathways, including MFSD2A.

- A.** Gene ontology analysis performed by Gene Set Enrichment Analysis framework for comparison between CRC GFP and healthy GFP cells.
- B.** MFSD2A abundance per conditions (CRC GFP Vs healthy GFP cells). Data are represented as mean $\pm$ s.e.m. * $P < 0.05$.
- C.** AA and DHA lipid abundance per conditions (CRC shCTRL Vs healthy shCTRL cells). Data are represented as mean $\pm$ s.e.m. * $P < 0.05$; ** $P < 0.01$.

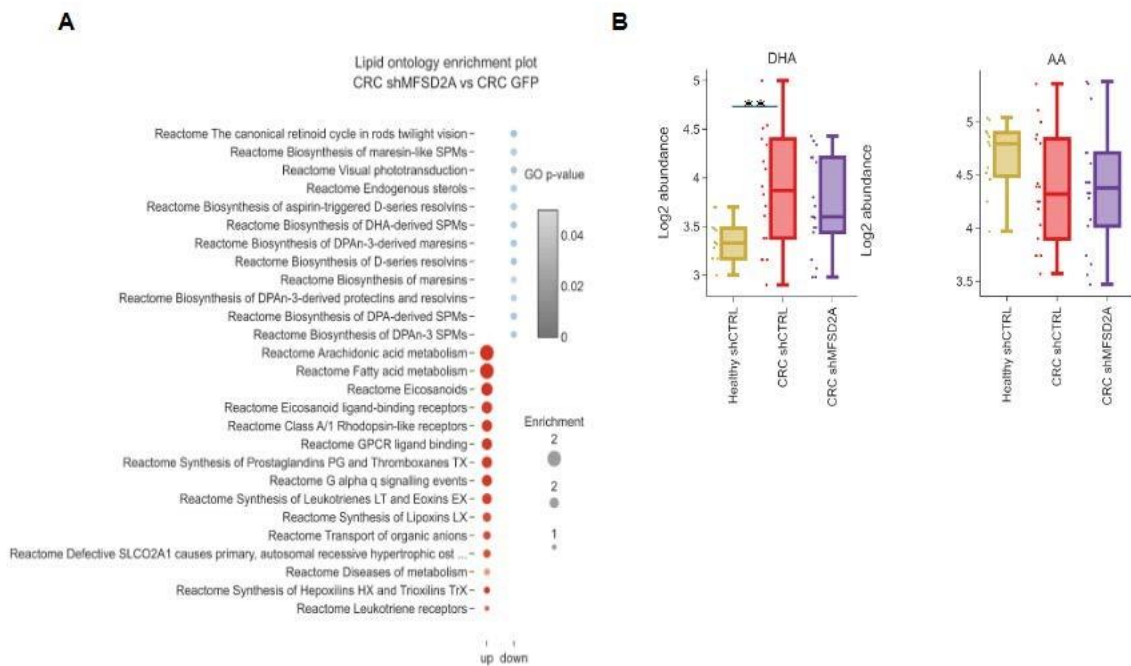


Figure 2. MFSD2A silencing in CRC increases inflammatory processes promoting AA metabolism.

- A.** Lipid ontology analysis performed by Lipid Set Enrichment analysis framework for comparison between CRC shMFSD2A and CRC GFP.
- B.** DHA and AA lipid abundance per conditions (healthy shCTRL, CRC shCTRL and CRC shMFSD2A). Data are represented as mean $\pm$ s.e.m. ** $P < 0.01$.

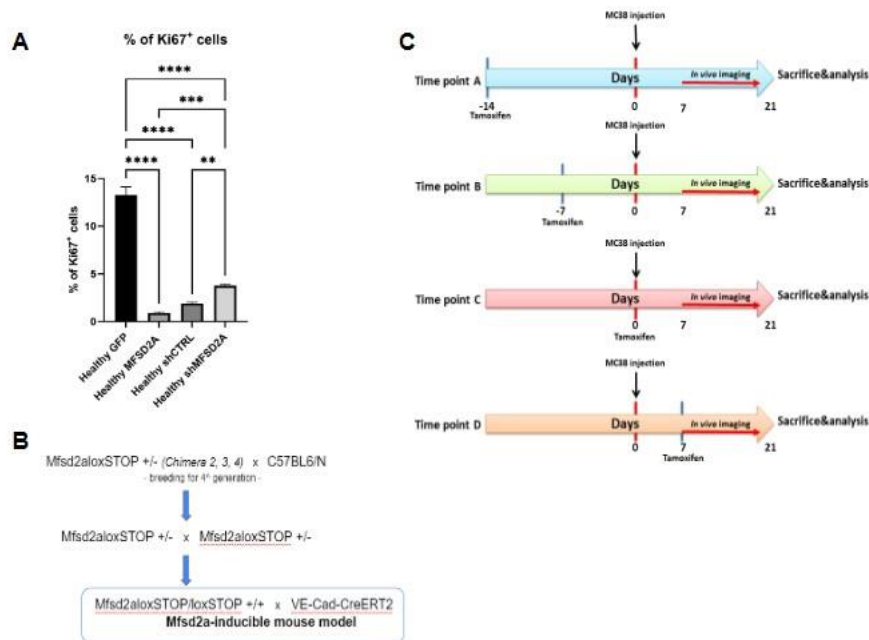


Figure 3. Enhancement of MFSD2A expression reduces cancer cell growth.

A. Percentage of Ki67⁺ CACO cells co-cultured 24h with different HIMEC conditions (healthy GFP, healthy MFSD2A, healthy shCTRL, healthy shMFSD2A). Data are represented as mean $\pm$ s.e.m. ** $P < 0.01$; *** $0.0001 < P < 0.01$; **** $P < 0.0001$.

B. Generation of Mfsd2a-inducible mouse model: by crossing Mfsd2aloxSTOP mouse with the VE-Cad-CreERT2 mouse, the STOP cassette will be removed by Cre upon tamoxifen injections and gene expression will be conditionally reactivated only in the intestinal endothelium, finally restoring Mfsd2a driven by its own regulative region.

C. Exploitation of transgenic mouse model to induce experimental CRC: Mfsd2a expression will be maintained OFF and reactivated at different time-points before and after CRC induction to evaluate the timely Mfsd2a activation.

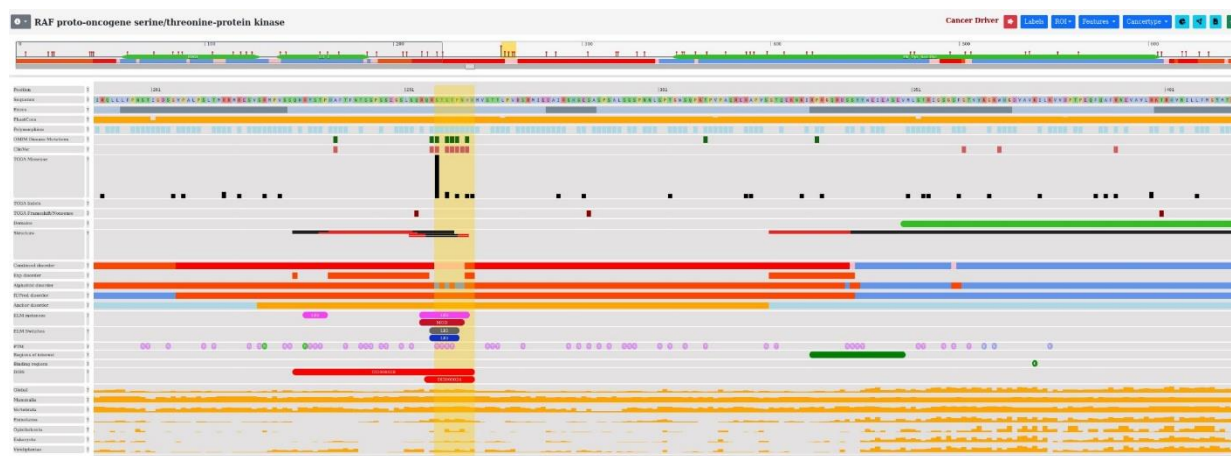
P-19 - DisCanVis: Visualizing integrated structural and functional annotations to better understand the effect of cancer mutations located within disordered proteins

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Keywords: Intrinsically disordered proteins (IDP) , cancer, visualization, functional and structural annotation, web server, tool

Intrinsically disordered proteins (IDPs) play important roles in a wide range of biological processes and have been associated with various diseases, including cancer. In the last few years, cancer genome projects have systematically collected genetic variations underlying multiple cancer types. In parallel, the number and different types of disordered proteins characterized by experimental methods have also significantly increased. Nevertheless, the role of IDPs in various types of cancer is still not well understood. In the presented work I introduce DisCanVis, a novel visualization tool for cancer mutations with a special focus on IDPs. In order to aid the interpretation of observed mutations, genome level information is combined with information about the structural and functional properties of proteins. To ease the access to the data presented in DisCanVis, we introduced multiple tables that group the data based on certain properties, such as known driver genes, significantly mutated regions



or experimentally verified disordered proteins

Figure 1: Example of visualization with RAF proto-oncogene serine/threonine-protein kinase

The web server enables users to inspect individual proteins, collect examples with existing annotations of protein disorder and associated function or to discover currently uncharacterized examples with likely disease relevance. For large scale analysis we offer processed data collection in the web server and programmatic access via REST API.

P-20 - Investigation of the role of COP9 signalosome in the *epithelial-mesenchymal* transition

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Epithelial-mesenchymal transition (EMT) promotes metastasis formation of primary tumors which can develop in parallel with drug resistance. Since EMT is a gradually evolving process, intermediate phenotypic states emerge (hybrid-E/M), showing both epithelial (E) and mesenchymal (M) properties. Hybrid-E/M and M state cancer cells possess invasiveness and chemotherapy resistance. Therefore, reversing EMT - known as mesenchymal-epithelial transition (MET) - has a promising therapeutic potential. In our *in silico* intercellular EMT network model, knock-out of COP9 signalosome complex (CSN) was able to induce MET of the hybrid-E/M and M cells' networks.

Aims: The aim of this project is the *in vitro* validation and further exploration of the CSN complex in the process of EMT/MET, for which appropriate cell lines had to be chosen.

Results: Docetaxel (DOC)-sensitive (PC3, DU145) and -resistant (DU145-DR, PC3DR) prostate cancer cell lines were selected for the experiments. The DR sublines went through EMT during the development of DOC-resistance, which was confirmed by measuring the gene expression of EMT markers (CDH, VIM, ZEB1, ZEB2) using qRT-PCR. The expression of the functional subunits of the CSN (CSN5, CSN6) was determined (Western blot), and their silencing was performed by RNA interference. Based on our measurements, DOC-sensitive cells proved to have E, the PC3-DR cells hybrid-E/M, while the DU145-DR cells proved to have M phenotype. The expression of both CSN5 and CSN6 was detectable in both pairs of cell lines, and their RNA-silencing was also successful.

These results indicate that our cell lines can be considered as suitable *in vitro* systems for the validation of our *in silico* results. In parallel with gene silencing, we will perform functional experiments such as migration, invasion and viability assays, which may prove the role of CSN in EMT, and will also possibly influence DOC resistance.

P-21 – Multiscale model of the different modes of cancer cell invasion

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Motivation: Mathematical models of biological processes altered in cancer are built using the knowledge of complex networks of signaling pathways, detailing the molecular regulations inside different cell types, such as tumor cells, immune and other stromal cells. If these models mainly focus on intracellular information, they often omit a description of the spatial organization among cells and their interactions, and with the tumoral microenvironment.

Methods: We present here a model of tumor cell invasion simulated with PhysiBoSS, a multiscale framework which combines agent-based modeling and continuous time Markov processes applied on Boolean network models. With this model, we aim to study the different modes of cell migration, by considering both spatial information obtained from the agent-based simulation and intracellular regulation obtained from the Boolean model.

Results: Our multiscale model integrates the impact of gene mutations with the perturbation of the environmental conditions and allows the visualization of the results with 2D and 3D representations (Fig. 1). The model successfully reproduces single and collective migration processes and is validated on published experiments on cell invasion (Fig.2). In silico experiments are suggested to search for possible targets that can block the more invasive tumoral phenotypes.

Optional figures:

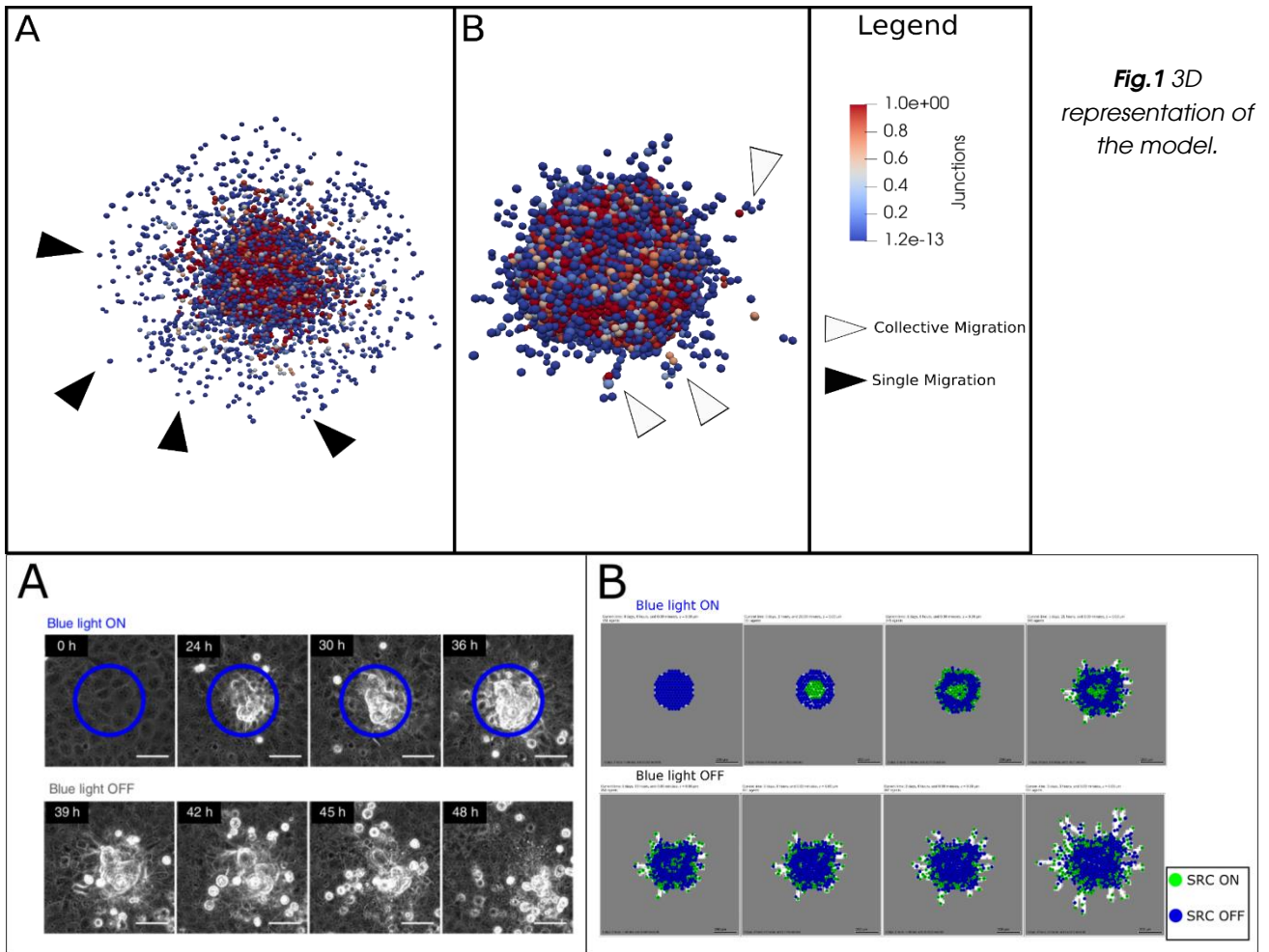


Fig.2 Comparison with In-vitro experiment (Moitrier et al. CommunPhys2, 98, 2019).

P-22 - Investigating the role of gut eukaryotic virome in contributing to colorectal cancer carcinogenesis

Amanda Facchetti¹, Luca Massimino^{1,2}, Salvatore Spanò^{1,2}, Stefania Cagliani¹, Carmela Errico¹, Tommaso Lorenzo Parigi^{1,2}, Silvio Danese^{1,2} and Federica Ungaro^{1,2}

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Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer death in the world. Besides the genetic aberrations, considered the pillars of CRC etiopathogenesis, intestinal dysbiosis and inflammation are widely documented to characterize its pathogenesis. Some of our recent findings pointed out a gut virome-associated *Orthohepadnaviridae* protein, namely Hepatitis B protein X (HBx), to correlate with Ulcerative Colitis pathogenesis and promote intestinal inflammation in mice. Moreover, we showed that HBx is able to induce DNA damage-related biological processes and active biological processes related to the Wnt pathway, known to be involved in colorectal carcinogenesis.

Based on this evidence we hypothesize that HBx may fill the gap existing between intestinal inflammation and CRC onset by stimulating pro-inflammatory and/or pro-carcinogenic effects. Metatranscriptomic analysis revealed that HBx is abundantly present in CRC-derived mucosal samples (**Figure 1A**) compared with healthy individuals, strengthening the link between a virome protein-induced inflammation and tumor development in patients.

Moreover, HBx overexpression in intestinal epithelial cells directly provokes DNA damage (**Figure 1B**), suggesting the direct HBx impact on intestinal epithelium genomic stability.

Interestingly, HBx administration in mice led to the development of polypoid structures in the colonic mucosa (**Figure 1C**) characterized by decreased abundance of T cells, including effector T cells and NK T cells (**Figure 1G**). Of note, *in vivo* HBx modulated the expression of genes involved in the regulation of transcription and activation of the proto-oncogene PIM3 (**Figures 1D-F**) further supporting the role of a viral protein on CRC onset.

This study introduces unconventional and innovative insights for giving CRC studies a new direction for the scientific and clinical investigations, looking at the gut virome as an active component and not as a mere bystander entity of the microbiota, ultimately promoting a new standpoint in the CRC studies and management in the short-term future.

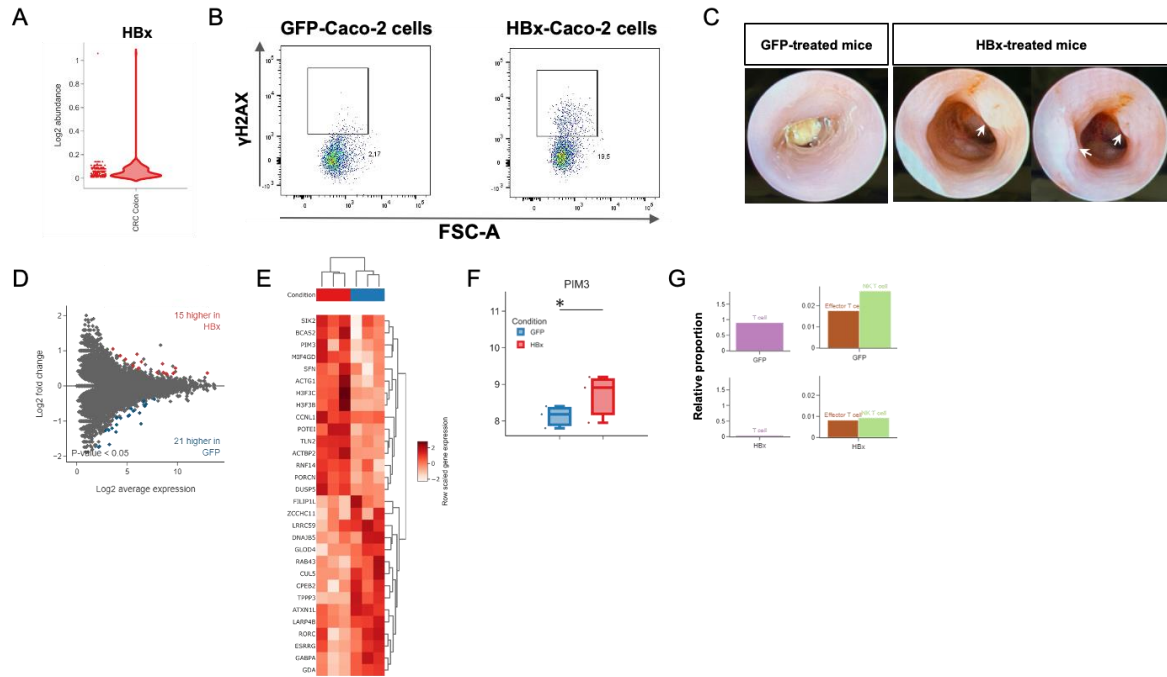


Figure 1. HBx induces carcinogenic properties

(A) Violin plot showing the Log2 abundance of HBx transcript in CRC-derived mucosal samples.

(B) FACS analysis panels showing γH2AX positivity in HBx-overexpressing Caco-2 by comparison with the control.

(C) Representative images of endoscopies performed on HBx- and GFP-treated mice. White arrows indicate polypoid structure in HBx-treated mice.

(D-F) MA-plot (D) and heatmap (E) show differentially expressed genes in HBxtreated mouse colons by comparison with the GFP. Red and blue dots indicate upregulated and downregulated genes, respectively, with statistical significance. In F, box plot shows the differential expression of a cancer marker PIM3 in HBx- versus GFP-treated mice. *P<.05.

(G) Bar plots showing the relative proportion of the indicated cell populations in HBx- and GFP-treated mice.

P-23 - How yeast go multicellular

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Plethora of evolutionary innovation created the world we know today. Several lifestyle strategies have given advantage to organisms to adapt to continuously changing environment. One of these is the emergence of multicellularity, which provided an opportunity for more complex organisms to evolve. The way from unicellularity to multicellularity was a long journey. In this study we want to explore this process with the widely used unicellular model organism: baker's yeast (*Saccharomyces cerevisiae*).

Due to starvation or in insufficient environmental conditions yeasts can form facultative multicellular secondary structures (e.g. pseudohyphae, flocs), that helps the colony to gain nutrients and reduce the deficient environmental impact. Our goal is to mutate this facultative multicellularity to obligate multicellularity and evolve a stay-together strategy in yeast.

ACE2 is a DNA-binding transcription factor involved in positive regulation of septum digestion after cytokinesis. Deletion of this gene results a multicellular „snowflake” phenotype in yeast (Ratcliff et al. 2015). Combined with other modifications (e.g. Flo genes, Septin genes, Wnt signaling pathway) can result in a newer structure and phenotype of a permanent multicellular colony. Through these mutations, the evolutionary development of multicellularity can be better understood.

1 Ratcliff, W., Fankhauser, J., Rogers, D. et al. Origins of multicellular evolvability in snowflake yeast. *Nat Commun* 6, 6102 (2015). <https://doi.org/10.1038/ncomms7102>

P-24 - Integrated stress response facilitates mucoprotective reprogramming against acute renointestinal distress

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The integrated stress response (ISR) plays a pivotal role in the cellular response to stress, primarily through global translational arrest and the upregulation of cellular adaptation-linked molecules. Herein, we assessed whether ISR-driven cellular stress contributes to pathophysiological outcomes in the renointestinal distress. Clinical transcriptome analysis demonstrated that several ISR signals were positively associated with the renal injury in patients. In particular, genetic ablation of NAG as a target of ISR aggravated chemical-induced lesions and muco-foragers in renal tissues and the gut barrier. Moreover, stress-responsive NAG facilitated mucus production and cellular survival via reorganization of the autophagy regulatory network. Collectively, ISR-activated NAG counteracted pathological processes via the protective reprogramming of the autophagic network, thereby providing robust predictive biomarkers and interventions against acute renointestinal distress

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P-25 - AutophagyNet: High-resolution data source for the analysis of autophagy and its regulation

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Autophagy is a highly-conserved catabolic process eliminating unnecessary, dysfunctional cellular components and invading pathogens. Autophagy malfunction contributes to disorders such as cancer, neurodegenerative and inflammatory diseases. Understanding autophagy regulation in health and disease has been the focus of the last decades. The recent advent of omics technologies made precise analysis of autophagy a reality on a systems-level. We previously provided an integrated database for autophagy research, the Autophagy Regulatory Network (ARN). For the last seven years, this resource has been used by thousands of users. Here, we present a new and upgraded resource, AutophagyNet. It builds on the previous database but contains major improvements to address user feedback and novel needs due to the advancement in omics data availability. AutophagyNet contains updated interaction curation and integration of over 280,000 experimentally verified interactions between core autophagy proteins and their protein, transcriptional and post-transcriptional regulators as well as their potential upstream pathway connections. AutophagyNet provides annotations for each core protein about their role: 1) in different types of autophagy (mitophagy, xenophagy, etc.); 2) in distinct stages of autophagy (initiation, elongation, termination, etc); 3) with subcellular and tissue-specific localization. These annotations can be used to filter the dataset, providing customizable download options tailored to the user's needs. The resource is available in various file formats (e.g., CSV, BioPAX and PSI-MI), and data can be analyzed and visualized directly in Cytoscape. The multi-layered regulation of autophagy can be analyzed by combining AutophagyNet with tissue- or cell type-specific using (multi-)omics datasets (e.g. transcriptomic or proteomic data). The resource is publicly accessible at <http://autophagynet.org>.

P-26 – A systems biological study of oscillatory characteristic of autophagy induction under cellular stress

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Autophagy-dependent survival is an essential cellular process to respond various stress events. The main element of autophagy activator complex, called ULK1 is regulated by AMPK and mTORC1 kinases. mTORC1 is a key controller of cellular homeostasis in nutrient rich environment and it inhibits autophagy through the phosphorylation of ULK1. On the other hand, under cellular stress (e.g. starvation), AMPK activates autophagy via ULK1. mTORC1 and AMPK are mutual antagonists, while ULK1 also effects their activity, namely, it inhibits both AMPK and mTOR generating a negative and a double negative feedback loops in the control network. Recently, a freely available mathematical model we developed to confirm that feedback loops of AMPK- mTORC1-ULK1 regulatory triangle and investigate their roles in guaranteeing the switch-like behaviour of autophagy induction. Meanwhile, an oscillatory characteristic was also demonstrated in the presence of rapamycin treatment.

In this study we introduce a theoretical analysis to explore the dynamical characteristic of both negative and double negative feedback loops of ULK1-controlled autophagy induction paying particular attention to understanding how the periodic activation of autophagy with the help of AMPK and mTORC1 kinases upon cellular stress. Parameter estimation and sensitivity analysis are also carried out to validate our model. We suggest an extra regulatory molecule in the control network acting like the key sensor of autophagy induction. Our theoretical analysis explores that the inclusion of this extra protein induced by AMPK ensures the proper dynamics of the control network by up-regulating ULK1 and down-regulating mTORC1 upon cellular stress. The Autophagy Regulatory Network has been used to identify a good number of proteins with similar connections, which could later be important therapeutic targets in various diseases such as inflammatory bowel diseases.

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P-27 – Reversal of epithelial-mesenchymal transition in a compartmentalized *in silico* Boolean model

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It is a major challenge to reverse the epithelial-mesenchymal transition in cancer cells and achieve mesenchymal-epithelial transition (MET) for therapeutic benefit. EMT is involved in the metastasis of a wide range of cancerous diseases including colorectal carcinoma. *In silico* Boolean modelling of EMT signalling is a cost-effective way to gain therapeutically relevant information about its regulation. Subcellular compartmentalization of these Boolean models improves their accuracy by simulating the regulatory role of distinct compartments (e.g., cytoplasm, nucleus) in signalling. Signalling pathways involved in the regulation of both EMT and MET, like the LIF/KLF4 pathway, can be incorporated into these models to model the two-way dynamic of the epithelial-mesenchymal spectrum.

Aims: A limiting factor of current Boolean EMT models is that the stability of their attractors is skewed towards the mesenchymal attractor. We have aimed to assess the effect of compartmentalization (that we had applied to an established Boolean TGFB mediated EMT model) on the stability of the epithelial attractor and its effect on the facilitation of MET by comparing the compartmentalized and original versions of the same model.

Results: In our compartmentalized model, the epithelial attractor was found to be 4 times more stable compared to the original model. (The mesenchymal attractor remained a lot more stable than the epithelial in our model, as expected.) Compared to the 7 perturbations required to restore the epithelial state in the original model, the number of perturbations required to restore the epithelial state was reduced to 5 in the compartmentalized model. By incorporating elements of the LIF/KLF4 signalling pathway into our model, a partial MET was achieved following the activating perturbation of the nuclear GSK3B node. Our compartmentalized approach to modelling EMT increased the stability of the epithelial attractor and shifted the signal transduction towards MET.