EXPLORING DISEASE LANDSCAPES

MAX DELBRÜCK CENTER BIMSB
Berlin Institute for Medical Systems Biology

Harnessing single-cell multi-omics, imaging and computational models

15TH BERLIN SUMMER MEETING 8-9 SEPT. 2022

COMPUTATIONAL AND EXPERIMENTAL MOLECULAR BIOLOGY MEET

Speakers:

Simon Haas
BIMSB, Max Delbrück Center for Molecular Medicine
Berlin, Germany

Markus Morkel
University Hospital Berlin
Berlin, Germany

Noam Stern-Ginossar
Weizmann Institute of Science
Rehovot, Israel

Susanne Wegmann
DZNE
Berlin, Germany

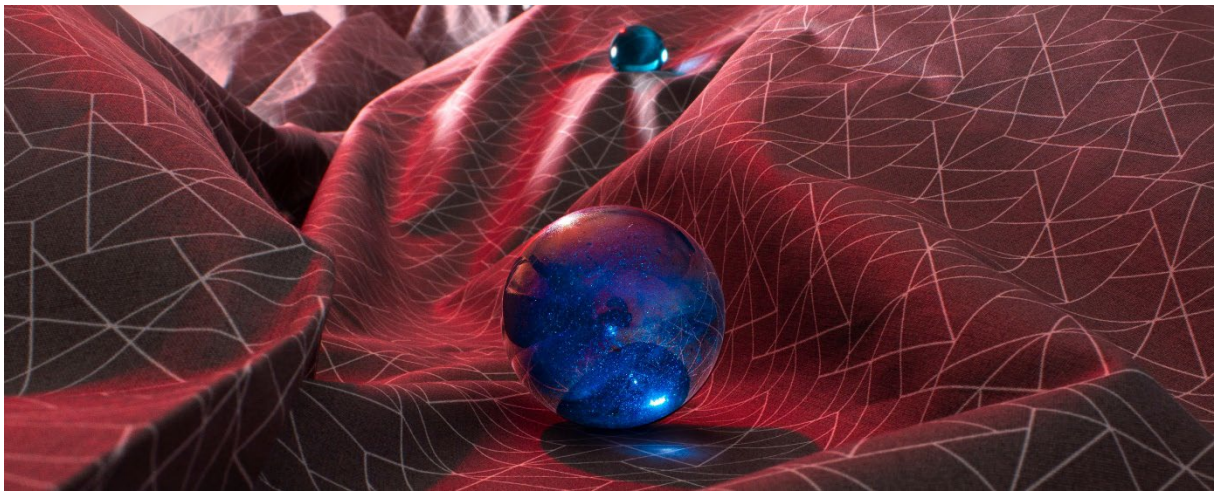
Scientific Committee BIMSB, MDC:

Peran Hayes
Anna Oliveras Martinez
Luiz Gustavo Teixeira Alves
Emanuel Wyler

Contact:
Verena Maier
verena.maier@mdc-berlin.de
+49 30 9406 3076

Mahir Özgören
Mahir.Oezgoeren@mdc-berlin.de
+49 30 9406 3034

www.mdc-berlin.de/15th-berlin-summer-meeting



15th Berlin Summer Meeting 'Exploring Disease Landscapes: Harnessing single-cell multi-omics, imaging and computational models'

8-9 September 2022, Large Conference Room, BIMSB

Dear friends and colleagues,

It is our pleasure to welcome you to the 15th Berlin Summer Meeting 'Exploring Disease Landscapes: Harnessing single-cell multi-omics, imaging and computational models', taking place as a hybrid event, online and on site in the BIMSB Research Building.

The meeting will showcase approaches towards understanding disease from different angles, including investigation of gene regulatory mechanisms, data science, organoid models, imaging, and single cell technologies. We are excited to host both established experts in their respective field and early career scientists as speakers!

To utilize the full potential of finally being able to get together in person again, the program will feature opportunities to network and socialize as well as interactive formats. We are thus looking forward to active and interdisciplinary discussions not only during the general sessions, but also during the breakout session and round tables.

Thank you for joining us – enjoy the meeting!

The BSM2022 Scientific Organizing Committee

Peran Hayes
Anna Oliveras Martinez
Luiz Gustavo Teixeira Alves
Emanuel Wyler

AGENDA

Thursday, September 8, 2022

10:00 am - 10:15 am Welcome address by Nikolaus Rajewsky

Session 1: Gene Regulation
Chair: Emanuel Wyler

10:15 am – 11:00 am **Noam Stern Ginossar**
Weizmann Institute of Science, Rehovot, Israel
mRNA translation – a tug of war during viral infection

11:00 am – 11:20 am **Konrad Chudzik**
Max Planck Institute for Molecular Genetics, Berlin, Germany
Multi-omic single-cell profiling reveals nuclear envelope release precedes gene activation during mouse embryogenesis

11:20 am – 11:40 am **Melyssa Minto**
Duke University, Computational Biology and Bioinformatics, Durham, NC, USA
Multiomic analyses reveal novel co-factors in Zic-mediated gene regulation in the cerebellum

11:40 am – 12 pm **Euphy Wu**
University of North Carolina at Chapel Hill, Department of Biostatistics, NC, USA
Detecting isoform-level allelic imbalance accounting for inferential uncertainty

12:00 pm -1:45 pm *Lunch break*

Session 2: **Single Cell Biology**

Chair: Ivano Legnini

1:45 pm – 2:30 pm	**Simon Haas** Berlin Institute of Health/BIMSB, MDC, Berlin, Germany **Unlocking the mysteries of blood generation by integrated multimodal single-cell analyses**
2:30 pm – 2:50 pm	**Ahmed N. Hegazy** Charité-Universitätsmedizin Berlin, Department of Gastroenterology, Infectious Diseases and Rheumatology/ Berlin Institute of Health, Core Unit Bioinformatics (CUBI) **Multi-omics profiling of the circulating immune cell landscape in inflammatory bowel disease identifies circulating predictors for non-response to anti-integrin treatment**
2:50 pm – 3:10 pm	**Eric Lindberg** MDC, Berlin, Germany **Pathogenic variants damage cell composition and single-cell transcription in cardiomyopathies**
3:10 pm – 3:30 pm	**Maja-Celine Cwikla** BIMSB, MDC/Charité – Universitätsmedizin Berlin, Germany **Intercellular extrachromosomal DNA copy number heterogeneity in neuroblastoma**
3:45 pm – 5:00 pm	Round tables with speakers and PIs (on site only)
5:00 pm – 8:00 pm	*BBQ on the rooftop terrace*

Friday, September 9, 2022

Session 3: Understanding Disease

Chair: Gustavo Teixeira

10:15 am – 11:00 am	**Susanne Wegmann** DZNE, Berlin, Germany **Condensates of Tau – a nexus between function and pathology**
11:00 am – 11:20 am	**Henri Niskanen** Max Planck Institute for Molecular Genetics, Berlin, Germany **Disruption of nucleolar phase separation in human genetic disease**
11:20 am – 11:40 am	**Anna Oliveras** BIMSB, MDC, Berlin, Germany **Tau and ApoE coalescence drives lipid dysregulation in Alzheimer's disease**
11:40 am – 12:00 pm	**Janita Mintcheva** BIMSB, MDC, Berlin, Germany **Cell state transitions and dynamics in zebrafish heart regeneration**
12:00 am – 1:45 pm	Lunch break
12:15 pm – 1:30 pm	Breakout session (on site and by sign-up only)

Session 4: Organoids

Chair: Anna Oliveras Martinez

1:45pm – 2:30 pm	**Markus Morkel** Charité – Universitätsmedizin Berlin, Germany **Oncogenes, signaling networks and cell states in colorectal cancer organoids**
2:30 pm – 2:50 pm	**Kimberly Hartl** Charité – Universitätsmedizin Berlin/BIMSB, MDC, Berlin, Germany **P53 knock-out cells are locked in a regenerative state in the context of colitis**
2:50 pm – 3:10 pm	**Julian Heuberger** Charité – Universitätsmedizin Berlin, Department of Hepatology and Gastroenterology/BIMSB, MDC, Berlin, Germany **IFN-gamma primes colonic epithelia for regeneration**
3:10 pm – 3:30 pm	**Milad Rezvani** Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA/Charité Universitätsmedizin Berlin, Department of Pediatrics, Division of Gastroenterology, Nephrology and Metabolic Medicine/Berlin Institute of Health, Center for Regenerative Therapies, Berlin, Germany **Trajectory Lineage Analyses of Human Hemato-Hepatogenesis Organoids from Human Pluripotent Stem Cells**
3:30 pm	Closing remarks by Peran Hayes

ABSTRACTS

NOAM STERN-GINOSSAR
Weizmann Institute of Science, Rehovot, Israel

mRNA translation – a tug of war during viral infection

To ensure translation of their mRNAs, viruses commonly hijack the host cell's translation machinery to facilitate viral protein production while concomitantly blocking the cell's ability to mount an immune response. On the other hand, many of the host cell-intrinsic immune defenses target translation to incapacitate the infected cell protein synthesis apparatus. Here, I will present our efforts to pertain the molecular principles governing SARS-CoV-2 ability to suppress host protein synthesis. We illustrate the viral protein nsp1 is the main translation shutoff factor of SARS-CoV-2 and we decipher its molecular mechanisms. By generating a viral mutant that lacks nsp1 activity we uncover its functional importance during SARS-CoV-2 infection. We illustrate nsp1 has broad activity and it targets all translated cellular mRNA, but its significance in infection lies explicitly in blocking the type I interferon response. These results illustrate the multipronged approach SARS-CoV-2 is using to interfere with cellular translation and uncovers the Achilles heel of the interferon response - its reliance on de novo protein synthesis.

KONRAD CHUDZIK[*1]
Isabel Guerreiro*[2], Samy Kefalopoulou*[2], Magdalena Schindler[1,3], Robin H. van der Weide[2], Nils Hansmeier[1,3,4], René Hägerling[1,3,4], Lars Wittler[5], Irina Solovei[6], Stefan Mundlos[1,3,4], Jop Kind*[2,7], Michael I. Robson*[1,3,8]

[1]Max Planck Institute for Molecular Genetics, RG Development & Disease, Berlin, Germany,
[2]Hubrecht Institute–KNAW and University Medical Center Utrecht, Oncode Institute, Utrecht, Netherlands,
[3]Charité Universitätsmedizin Berlin, Institute for Medical and Human Genetics, Berlin, Germany,
[4] Berlin Institute of Health at Charité-Universitätsmedizin Berlin, BIH Center for Regenerative Therapies, Berlin, Germany,
[5]Max Planck Institute for Molecular Genetics, Department of Developmental Genetics, Transgenic Unit, Berlin, Germany,
[6]Ludwig Maximilians University Munich, Department of Biology II, Munich, Germany,
[7]Radboud University Nijmegen, Radboud Institute for Molecular Life Sciences, Department of Molecular Biology, Faculty of Science, Nijmegen, Netherlands,
[8]University of Edinburgh, Institute of Genetics and Molecular Medicine, Medical Research Council Human Genetics Unit, Edinburgh, United Kingdom

*These authors contributed equally to this work

Multi-omic single-cell profiling reveals nuclear envelope release precedes gene activation during mouse embryogenesis

Many developmental genes and their enhancer regulatory landscapes dynamically attach to the nuclear envelope in lamina-associated domains (LADs). However, though LADs frequently reconfigure during in vitro differentiation, their precise relationship to gene transcription and regulation remains unknown. Here, we address this with scDam&T in vivo, a new high-resolution method to simultaneously track LAD and transcriptional dynamics in single cells from any tissue during mouse embryogenesis. We observe that LADs are highly dynamic during development and reconfigure both at genes and in their regulatory landscapes. However, altered nuclear envelope attachment can be both coupled and uncoupled from gene expression. Specifically, critical developmental genes and their entire regulatory landscapes, as defined by topologically-associated domains (TADs), can detach from the nuclear envelope prior to later transcriptional activation. What's more, this LAD pre-release appears delimited by TADs themselves and so spreads into neighboring domains when TAD boundaries are removed. Collectively, this suggests LADs have a complex relationship with the regulatory genome but that their release may be required to license critical gene expression changes in development. We are thus attempting to identify the mechanism driving LAD pre-release through single-cell chromatin accessibility profiling.

MELYSSA MINTO[1]
Emiliano Sotelo[2], Vijyendra Ramesh[3], Matthew Vincent[3], Anne E. West[3]

[1] Duke University, Computational Biology and Bioinformatics, Durham, NC, USA
[2] Duke University, University Program of Genetics and Genomics, Durham, NC, USA
[3] Duke University, Neurobiology, Durham, NC, USA

Multiomic analyses reveal novel co-factors in Zic-mediated gene regulation in the cerebellum

The family of Zic transcription factors (TFs) are required for cerebellar development, and their binding is highly enriched at developmentally regulated enhancers active in cerebellar granule neurons at the progenitor stage around postnatal day 7 (P7) and the mature adult stage at P60 in the mouse cerebellum. Interestingly, Zic1/2 ChIP-seq data reveal that the Zics change their binding profile over time. To characterize these differences in Zic targeting between early and late cerebellar maturation, we compared the sequence underlying Zic Peaks, their relationship with active chromatin and gene expression at P7 and P60. From this we found Zic peaks tend to be large and bind open-active chromatin. Strikingly, Zic peaks shift from binding at promoter proximal regions at P7 to distal enhancer regions at P60. We hypothesize that other TFs collaborate with the Zic TFs to change their binding targets and affect their regulatory activity. A multi-tiered approach was used to predict TF binding at those Zic ChIP sites. We assessed motif enrichment (HOMER) and in-vivo TF binding profiles (BART) to determine putative TFs collaborating with Zic to drive this regulation. We then validated the presence of the predicted TFs in granule neurons using gene expression. This workflow identified known and novel distinct collaborators of Zic between early and late development. Early collaborates of Zic includes bHLH factors and chromatin remodelers whereas late collaborators of Zic are activity regulated TFs which are markers of synaptic maturation. To identify Zic's developmental gene targets in cerebellar maturation, we used H3K4me3 PLAC-seq data, which captures promoter-enhancer loops from the adult mouse cerebellum and Hi-C data from the young mouse cerebellum to map genes to Zic bound enhancers. This revealed Zic as a transcriptional activator of late developmental genes in the cerebellum. Using this same approach with in vitro Zic KD, we were able to identify Zic dependent developmental gene targets which have functions in axonogenesis, axon guidance, and ion channel signaling, which are integral in proper maturation of a neuron. These integrated analyses reveal how Zic and other TFs regulate temporal
expression of CGN developmental genes and provides information that will enhance our understanding of the molecular mechanisms that regulate the mode of TF function at enhancers.

EUPHY WU[1]
Noor P. Singh[2], Mohsen Zakeri[2], Matthew Vincent[3], KB Choi[3], Gary A Churchill[3], Cheryl L. Ackert-Bicknell[4], Rob Patro[2], Michael I. Love*[1,5]

[1]Department of Biostatistics, University of North Carolina-Chapel Hill, NC, USA
[2]Department of Computer Science, University of Maryland, College Park, MD, USA
[3]The Jackson Laboratory, Bar Harbor, ME, USA
[4]Department of Orthopedics, School of Medicine, University of Colorado, Anschutz Campus, CO, USA
[5]Department of Genetics, University of North Carolina-Chapel Hill, NC, USA

Detecting isoform-level allelic imbalance accounting for inferential uncertainty

Allelic imbalance (AI) of gene expression in heterozygous individuals is a hallmark of cis-genetic regulation, revealing mechanisms underlying the association of non-coding genetic variation with downstream traits, as in GWAS. Most methods for detecting AI from RNA-sequencing (RNA-seq) data examine allelic expression per exonic SNP, which may obscure imbalance in expression of individual isoforms. Detecting AI at the isoform level requires accounting for inferential uncertainty (IU) of expression estimates, caused by multi-mapping of RNA-seq reads to originating transcripts and alleles. Swish, a previously developed method, can test for differential transcript expression while accounting for IU, and can be applied in a paired setting to detect AI. However, in AI analysis, most transcripts will have high IU across alleles such that even methods like Swish will lose power. Our proposed method, SEESAW, offers AI analysis at various level of resolution, including gene level, isoform level, and optionally aggregating isoforms within a gene based on their transcription start site (TSS), in order to prioritize AI that is a result of cis-genetic regulation at the promoter region. This TSS-based aggregation strategy strengthens the signal for transcripts that may have high IU with respect to allelic quantification, forming transcript groups with reduced IU. SEESAW is primarily designed for experiments with multiple replicates or conditions of organisms with the same genotype, as in an F1 cross or time series experiments of cell lines. We also introduce a new test for detecting dynamic AI from multiple samples profiled across a continuous variable such as a time course experiment that allow us to examine AI changes over time. The SEESAW suite of methods for isoform-level AI is evaluated both on simulated data and applied to an RNA-seq dataset of differentiating F1 mouse osteoblasts.

SIMON HAAS
Berlin Institute of Health/Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany

Unlocking the mysteries of blood generation by integrated multimodal single-cell analyses

AHMED N. HEGAZY
Veronika Horn[1,2], Camila Cancino[1,2,] Konstantin Fritz[1,2], Diana Bösel[1,2], Benedikt Obermeyer-Wasserscheid[3], Marie Burns[2], Axel Schulz[2], Eleni Mantzivi[1], Donata Lissner[1], Elena Sonnenberg[1], Carl Weidinger[1], Britta Siegmund[1], Henrik E. Mei[2], Ahmed N. Hegazy[1,2,4]

[1] Department of Gastroenterology, Infectious Diseases and Rheumatology, Charité-Universitätsmedizin Berlin, Berlin, Germany.
[2] Deutsches Rheuma-Forschungszentrum, ein Institut der Leibniz-Gemeinschaft, Berlin, Germany.
[3] Core Unit Bioinformatics (CUBI), Berlin Institute of Health, Berlin, Germany.
[4] Clinician Scientist Program, Berlin Institute of Health, Berlin, Germany.

Multi-omics profiling of the circulating immune cell landscape in inflammatory bowel disease identifies circulating predictors for non-response to anti-integrin treatment

Inflammatory bowel disease (IBD) is a currently uncurable inflammatory condition of the gut and represents a significant healthcare burden with rising incidence worldwide. Responses to existing therapies are heterogeneous, with only subgroups of patients sustaining clinical responses. Currently, no clinical or paraclinical algorithms or biomarkers accurately predict therapeutic responses in individual IBD patients. A personalized approach would allow clinicians to better deploy the growing number of available IBD therapies, improve outcomes, and reduce side effects. In our project, we established a multi-omics platform for in-depth phenotyping and analysis of blood samples of IBD patients. We included patients receiving Vedolizumab (α4β7-integrin blocker; n = 42) in a prospective, longitudinal study and assessed clinical response after 30 weeks of treatment. To explore possible cellular or transcriptional

biomarkers to predict responses to therapy in individual IBD patients, we performed immune profiling by mass and flow cytometry, single-cell RNA sequencing, T and B receptor profiling, and assessed cytokine response via flow cytometry and serum proteomics. We then developed an algorithm that accurately predicts response to vedolizumab using neural network-based clustering, differential analyses, machine learning, and mathematical modeling. The prediction algorithm was based on cell migration alterations of various immune cell types, transcriptional signature of memory CD4 T cells, and inflammatory serum proteins. Thus, our findings could promote a more personalized medicine approach to predict responses to therapy in individual IBD patients and could significantly improve disease management and reduce healthcare costs.

ERIC L. LINDBERG[1]
Henrike Maatz[1,2], Daniel Reichart[3,4], Nikolay Shvetsov[1], Jonathan G. Seidman[3], Christine E. Seidman[3,5,6], Norbert Hübner[1,4,7*] on behalf of the Cardiomyopathy Single-Cell Consortium

[1]Cardiovascular and Metabolic Sciences, Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany;
[2]DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, Berlin, Germany;
[3]Department of Genetics, Harvard Medical School, Boston, MA, USA;
[4]Department of Medicine I, University Hospital, LMU Munich, Munich, Germany;
[5]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA, USA;
[6]Howard Hughes Medical Institute, Chevy Chase, MD, USA;
[7]Charité-Universitätsmedizin, Berlin, Germany

Pathogenic variants damage cell composition and single-cell transcription in cardiomyopathies

Heart failure is a clinical syndrom and leading cause of death worldwide, caused by functional and structural abnormalities of the heart. Despite previous efforts to characterise molecular changes in the failing heart, little is known on cell-type specific abundance and expression changes, and how individual cell types interact during cardiac remodelling. Using snRNAseq, we characterized the transcriptome of 880,000 nuclei from 18 control and 61 failing, non-ischemic human hearts with pathogenic variants in dilated (DCM; LMNA, TTN, RBM20) and arrythmogenic cardiomyopathy (ACM; PKP2) associated genes or idiopathic disease. DCM is characterized by left ventricular dilatation and impaired contractility. ACM similarly incites ventricular dysfunction, often with more prominent right ventricular involvement and fibrofatty accumulations. Here we hypothesized that individual pathogenic variants in mutated genes evoke distinct single-cell molecular phenotypes. To address this question, we performed genotype-stratified analyses of the ventricular cell lineages and transcriptional states. The resultant DCM and ACM ventricular cell atlas demonstrated distinct right and left ventricular responses, highlighting genotype-associated pathways, intercellular interactions, and differential gene expression at single cell resolution. Genotype-stratified analyses identified multiple transcriptional changes shared only among the hearts harboring pathogenic variants or distinctive for individual and subsets of DCM and ACM genotypes.

MAJA-CELINE CWIKLA[1, 3]
Kerstin Haase[3, 5], Anton Henssen[3], Roland F. Schwarz[1, 2, 4,]

[1] Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany
[2] Institute for Computational Cancer Biology, Center for Integrated Oncology (CIO), Cancer Research Center Cologne Essen (CCCE), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany
[3] Charité - Universitätsmedizin Berlin, Berlin, Germany
[4] BIFOLD - Berlin Institute for the Foundations of Learning and Data, Berlin, Germany
[5] German Cancer Consortium (DKTK), partner site Berlin, and German Cancer Research Center (DKFZ), Heidelberg, Germany

Intercellular extrachromosomal DNA copy number heterogeneity in neuroblastoma

Neuroblastoma is a paediatric cancer characterised by extensive inter- and intra-tumor genetic heterogeneity and varying clinical outcomes. One possible driver for this heterogeneity is extrachromosomal DNA (ecDNA), circular DNA elements which segregate independently to daughter cells during cell division and can lead to rapid amplification of oncogenes. While ecDNA-mediated oncogene amplification has been intensely studied in many tumours including neuroblastoma and has been shown to be associated with poor prognosis, the consequences of intra-tumour variability of ecDNA copy number are still poorly understood. Here we leverage DNA and RNA sequencing data from the same single cells in neuroblastoma cell lines and patients to quantify ecDNA copy number variability and investigate the transcriptional effects of ecDNA-mediated oncogene amplifications including MYCN and its downstream targets. We propose an approach to accurately call ecDNA copy number from single cells by adapting existing copy number calling algorithms to account for the unique ecDNA amplicon structure of individual cell lines and patients. Initial results show substantial heterogeneity of ecDNA across cells and highlight the strong dosis effect of ecDNA copy number on gene expression levels for genes present on ecDNA. We compared high and low MYCN expressing cells within individual tumours and uncovered diverse transcriptomic profiles that affect MYCN target gene expression. Unsupervised gene set enrichment analysis of this intra-tumoural ecDNA heterogeneity uncovers a variety of enriched terms including increased ribosome biogenesis activity in cells with high MYCN expression. Future analyses will focus on further characterisation of ecDNA-mediated phenotypes by investigating distinct ecDNA-driven transcriptional states and state transitions within a tumour cell population.

SUSANNE WEGMANN
DZNE, Berlin, Germany

Condensates of Tau – a nexus between function and pathology

The microtubule associated protein Tau is involved in regulating axonal transport in the brain. Besides its high affinity binding to microtubules, Tau is an intrinsically disordered and highly soluble neuronal protein with a plethora of other interactors. In the human brain, however, Tau can aggregate into intracellular insoluble beta-sheet aggregates, which are a hallmark pathology of Alzheimer's disease and frontotemporal dementia. Intrinsic disorder versus amyloid-like aggregation of Tau are reminiscent of different states of matter, i.e. gas-like and diffuse versus solid and highly ordered. We found that a liquid condensed state of Tau can bridge between soluble and aggregated states of Tau. Furthermore, condensates of Tau enable distinct interactions important for microtubule binding and stability, stress granule association of Tau, and catalyze RNA:Tau interactions. We propose that Tau condensates are an integral part of Tau biology and provide a unique physicochemical interaction mode that combines the physical characteristics of a liquid with biochemical selectivity of the condensed phase.

HENRI NISKANEN[3,*]
Martin A. Mensah[1,2,*], Alexandre P. Magalhaes[3], Shaon Basu[3], Martin Kircher[4], Henrike L. Sczakiel[1,5], Alisa M. V. Reiter[1], Jonas Elsner[1], Peter Meinecke[6], Saskia Biskup[7], Brian H. Y. Chung[8], Gregor Dombrowsky[9], Christel Eckmann-Scholz[10], Marc Phillip Hitz[9], Paul-Martin Holterhus[11], Wiebke Hülsemann[12], Kimia Kahrizi[13], Vera M. Kalscheuer[5], Anita Kan[14], Mandy Krumbiegel[15], Ingo Kurth[16], Jonas Leubner[17], Ann Carolin Longardt[11], Jörg D. Moritz[18], Hossein Najmabadi[13], Karolina Skipalova[1], Andreas Tzschach[19], Eberhard Wiedersberg[20], Martin Zenker[21], Carla Garcia-Cabau[22], René Buschow[3], Xavier Salvatella[22], Matthew L. Kraushar[3], Stefan Mundlos[1,5], Almuth Caliebe[23], Malte Spielmann[5,23,24 $], Denise Horn[1, $], Denes Hnisz[3, $]

[1] Institute of Medical Genetics and Human Genetics, Charité
[2] BIH Biomedical innovation Academy
[3] Department of Genome Regulation, Max Planck Institute for Molecular Genetics
[4] Exploratory Diagnostic Sciences, Berlin Institute of Health at Charité
[5] RG Development & Disease, Max Planck Institute for Molecular Genetics
[6] Institute of Human Genetics, University Medical Center Hamburg-Eppendorf
[7] Center for Genomics and Transcriptomics (CeGaT) GmbH, Tübingen
[8] Department of Pediatrics and Adolescent Medicine, The University of Hong Kong
[9] Department of Congenital Heart Disease and Pediatric Cardiology, University Hospital Schleswig-Holstein
[10] Department of Obstetrics & Gynecology, University Hospital Schleswig-Holstein
[11] Department of Pediatrics, Pediatric Endocrinology and Diabetes, University Hospital Schleswig-Holstein
[12] Handchirurgie, Katholisches Kinderkrankenhaus Wilhelmstift
[13] Genetics Research Center University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
[14] Department of Obstetrics and Gynaecology, Queen Mary Hospital, Hong Kong
[15] Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)
[16] Institute of Human Genetics, Medical Faculty, RWTH Aachen University Hospital
[17] Department of Pediatric Neurology, Charité
[18] Department of Radiology and Neuroradiology, Pediatric Radiology, University Hospital Schleswig-Holstein
[19] Institute of Human Genetics, University of Freiburg
[20] Zentrum für Kinder- und Jugendmedizin, Helios Kliniken Schwerin
[21] Institute of Human Genetics, University Hospital, Otto-von-Guericke University
[22] Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology
[23] Institute of Human Genetics, University Hospitals Schleswig-Holstein, University of Lübeck and Kiel University
[24] DZHK (German Centre for Cardiovascular Research)

* The authors contributed equally
$ These authors jointly supervised this work and are corresponding authors

Disruption of nucleolar phase separation in human genetic disease

Thousands of genetic variants in protein-coding genes have been linked to disease, but the functional impact of most variants is unknown as they occur within intrinsically disordered protein regions that have poorly defined functions. Intrinsically disordered regions were recently found to mediate phase separation, and formation of biomolecular condensates (e.g. the nucleolus), suggesting that

mutations in disordered proteins may alter condensate properties and function. Here we show that a subset of disease-associated variants in disordered regions alter phase separation, cause mispartitioning into the nucleolus, and disrupt nucleolar function. We discovered de novo frameshift variants in HMGB1 that cause brachyphalangy-polydactyly-tibial aplasia syndrome, a rare complex malformation syndrome. The frameshifts replace HMGB1's intrinsically disordered acidic tail with an arginine-rich basic tail. The mutant tail altered HMGB1 phase separation, enhanced its partitioning into the nucleolus, and caused nucleolar dysfunction. We built a catalog of over 200,000 variants in disordered C-terminal tails, and identified over 600 frameshifts that create arginine-rich basic tails in transcription factors and other proteins. For ten out of eleven disease-associated variants tested, the mutation enhanced partitioning into the nucleolus, and several variants altered rRNA biogenesis. These data identify the cause of a rare complex syndrome, and suggest that a large number of genetic variants may dysregulate nucleoli and other biomolecular condensates in humans.

ANNA OLIVERAS[1]
Agnieszka Rybak-Wolf[2], Severine Kunz[3] and Melissa Birol[1]

[1] Single Molecule Biophysics probing Quantitative Neuroscience Lab
[2] Organoid Platform
[3] Electron Microscopy Platform

Applies to all: Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany

Tau and ApoE coalescence drives lipid dysregulation in Alzheimer's disease

Intrinsically Disordered Protein (IDP) aggregation is the late-onset signature of pathology in most neurodegenerative diseases. In Alzheimer's disease (AD), tau protein in neurons accumulates in intracellular deposits that correlate with clinical signs and neurodegeneration. Tau is an intracellular microtube-associated protein which is also released extracellularly upon synaptic activity. Additionally, as an IDP, tau has the ability to bind to lipids and undergo liquid-liquid phase separation (LLPS). Besides, ApoE, an apolipoprotein and the major lipid transporter between neurons and astrocytes, is the strongest genetic factor for AD. Recently, lipid metabolism dysregulation has been placed on focus of AD research and accumulating evidence suggest that ApoE modulates tau pathology. Nevertheless, very little is known about the synergy between tau and ApoE during the onset and progression of AD. Here, we employ a multiscale framework to underpin the structural and molecular complexity
of emerging protein-lipid landscapes in AD harnessing human multicellula2D and 3D models. Our preliminary data strikingly shows that extracellular tau drives ApoE co-internalization in droplet-like structures in brain organoids.
We observe time-dependent and isoform-specific dynamics of tau-ApoE droplets. Interestingly we find that the time-dependent changes in dynamics of tau-ApoE droplets correlate with a build-up of lipid droplets (LDs). We hypothesize that tau and the disease-related isoform of ApoE, ApoE4, coalescence drives early onset homeostatic lipid dysregulation. Here we explore the structural integrity of their coalescence by integrating advanced single-molecule, time-resolved imaging techniques and electron microscopy. We aim to correlate our findings with functional readouts including lipidomics, proteomics and calcium imaging. By combining structural and functional insights, our aim is to unravel the biophysics and molecular mechanism governing ApoE sequestration by tau and elucidate how this results in early-onset lipid dyshomeostasi in AD. Targeting lipid-protein interactions open promising new approaches on AD therapeutic strategies.

JANITA MINTCHEVA
Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany

Cell state transitions and dynamics in zebrafish heart regeneration

Opposed to human and adult mice, zebrafish can regenerate their hearts after injury. A prompt and tightly orchestrated immune response in the acute phase after injury is indispensable for complete regeneration. If delayed, many of the hallmark processes of zebrafish heart regeneration are impaired. However, the diversity and dynamics of immune cell states in the context of heart injury is not well understood. In this project, we are investigating the complex composition and transitions immune cell states in the injured zebrafish heart. Among all the cell types in the heart, *in vivo* scSLAMseq validated macrophage-like cells as the sentinel cells in the injured heart by upregulating the inflammatory signaling pathways of Toll-like receptors, NOD-like receptors and C-type lectin receptors. Moreover, scRNAseq of injured adult zebrafish hearts revealed a diverse and dynamic spectrum of macrophage-like transcriptional cell states across time than the M1/M2 macrophage polarization model suggests. Our analyses validate that macrophages play a key role in the acute response to injury. Additionally, our data will offer the possibility to identify specific macrophage-like cell state(s) that drive the acute response. In future work, we will perform scSLAMseq at later stages to identify the timepoint and characteristics of transition from a broad danger detection response to a more specific, and later pro-regenerative response.

MARKUS MORKEL
Thomas Sell[1,2], Raphael Brandt[1], Mareen Lüthen[1], Stefan Peidli[1,2], Alexandra Trinks[3], Nils Blüthgen[1,2], and Markus Morkel[1,3]

[1] Institute of Pathology, Charité - Universitätsmedizin Berlin
[2] IRI Life Sciences, Humboldt University Berlin
[3] BIH Bioportal Single Cells

Oncogenes, signaling networks and cell states in colorectal cancer organoids

Organoids, stem cell-driven 3D cell cultures relying on a combination of extracellular matrix and growth factors, have become a powerful model to study human disease. For instance, genetically engineered organoids are an excellent tool for examining cancer cell traits in basic research and patient-derived organoids can be employed for studying therapy response in difficult-to-treat patients.
Here, we use organoids as a model system to decipher oncogene activities, cell signaling states and differentiation trajectories of colorectal cancer. We find that oncogenic signals such as these elicited by KRAS are differentiation state-dependent, while, conversely, oncogenic signaling networks inform the cancer cell differentiation space during colorectal cancer progression. In this regard, cancer drivers can have stem cell-promoting or restricting effects. Cell plasticity is thus a hallmark of cancer that is gradually unlocked by sequential mutations during colorectal cancer progression. Furthermore, we define Wnt and MAPK signals as the main drivers of cell development in the normal colon and in colon cancer, respectively. We find that therapeutic targeting of EGFR-MAPK can reverse developmental trajectories, resulting in the formation of therapy-resistant stem-like persister cells. The combination of drug screening with single cell-resolved trajectory analysis could become an important tool in preclinical predictive testing.

KIMBERLY HARTL
Christine Harnack, Hilmar Berger, Hans-Joachim Mollenkopf, Michael Sigal

Applies to all: Charite Universitätsmedizin Berlin/ Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany

P53 knock-out cells are locked in a regenerative state in the context of colitis

Colorectal cancers arise by a defined sequence of mutations that differs between sporadic and inflammation-associated (IA) cancers. In IA tumors, mutations that cause a loss of the tumorsupressor P53 occur earlier. P53 mutant cells were able to outcompete P53 wild type (wt) cells in a mouse model of colitis (Vermeulen et al.) but mechanistic insights are lacking. Therefore, we have developed murine and organoid-based models to study the context-dependent role of Trp53 knock-out. Trp53 KO organoids did not exhibit phenotypic difference compared to wt organoids in full medium. Similarly, loss of Trp53 did not cause severe perturbations of crypt architecture in vivo. In contrast, when we mimic epithelial injury in vitro, we observe a selective advantage of Trp53 KO cells through activation of regenerative pathways. Similarly, in a mouse model of colitis, regenerative Trp53 KO cells expand and persist after the primary epithelial insult. Further in vitro and in vivo experiments will explain how the damaged epithelium selects for P53 mutant cells, which might help to develop screening and preventive measures for patients.

JULIAN HEUBERGER[1,2]
Lichao Liu[1], Manqiang Li[1], Hilmar Berger[1], Steffanie Müllerke[1], Frank Tacke[1] and Michael Sigal[1,2]

[1] Department of Hepatology and Gastroenterology, Charité University Medicine, Berlin, Germany
[2] Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany

IFN-gamma primes colonic epithelia for regeneration

IFN-gamma is a critical cytokine in inflammatory bowel disease and has been shown to impact the severity of colitis. While it controls the recruitment of immune cells, the response of epithelia to IFNg and its consequences are not well studied. Here, we use genetic mouse models and organoids to decipher the role of epithelial responses to IFNg. Dextran-sodium-sulfate (DSS) induced colitis is characterized by a period of bodyweight loss and marked tissue damage followed by a regenerative phase. While wild-type (wt) mice show ulcerative lesions, mice deficient for IFN-gamma receptor 1 (IFNGR1-KO) were less affected. Beside the lesions, we observed distinct IFNg-dependent epithelial changes, such as loss of Wnt activity, proliferation and stemness. This phenotype was recapitulated in live cell imaging of colon organoid cultures that further demonstrated a cell state-dependent IFNg responsiveness. IFNg induces a p21 controlled post-mitotic cell cycle arrest of stem- and proliferative cells. In contrast, differentiated cells die and are extruded from the epithelial layer in response to IFNg. The IFNg post-mitotic cell state is dynamic and cells can be reverted into stem and proliferative cells. Taken together, our data show that IFNg release triggers a directed epithelial reorganization and induce a particular cell state that is primed for regeneration and wound healing.

MILAD REZVANI[1, 2, 3*]
Kyle Lewis[1], Kentaro Iwasawa[1], Susanna Quach[2], Jorge O. Múnera[2], Praneet Chatuvedi[4], Konrad Thorner[4], James M. Wells[4,5,6,7], Brian R. Davis[8], Momoko Yoshimoto[8], Takanori Takebe[1, 4, 6, 7, 9*]

[1] Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.
[2] Charité Universitätsmedizin Berlin, Department of Pediatrics, Division of Gastroenterology, Nephrology and Metabolic Medicine
[3] Berlin Institute of Health, Center for Regenerative Therapies, Berlin, Germany
[4] Division of Developmental Biology
[5] Division of Endocrinology,
[6] Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.
[7] Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA.
[8] Institute of Molecular Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, TX, USA
[9] Institute of Research, Tokyo Medical and Dental University 1-5-45 Yushima, Bunkyo-ku, Tokyo, Japan.

*corresponding authors

Trajectory Lineage Analyses of Human Hemato-Hepatogenesis Organoids from Human Pluripotent Stem Cells

Fetal livers receive early waves of blood progenitors that can demonstrate lifelong multi-lineage contribution in rodents. Although pluripotent stem cell (PSC)-derived human liver organoids generally represent immature hepatic states, the complex interplay between hepatic and hematopoietic ontogeny remains elusive, thereby limiting lineage crosstalk investigations in development and disease. We developed human fetal liver organoids (hFLOs) that harbor syngeneic hepatoblasts-, pericyte-, stellate-, embryonic multipotent blood progenitors (MPP)-like cells and arrays of MPP-derivatives entirely from human PSCs. Longitudinal single-cell analysis and immunophenotyping revealed a pre-hemogenic KDR+/CD235+ yolk sac mesoderm population preceding the emergence of 21 hematopoietic cell states from 24 found in the human fetal liver atlas and symbiotic endoderm. Via CytoTRACE, we identified two hematopoietic populations: hemogenic endothelial cells (HEC) and MPPs. Live-cell imaging demonstrated how HECs undergo an endothelial-to-hematopoietic transition (EHT). MPPs express partial hematopoietic stem cell (HSC) but not liver lymphomyeloid progenitor programs. Single cell ligand-receptor and functional analyses revealed that Notch signaling governs the hematopoietic niche across HECs, MPPs and embryonic stromal cells. To distinguish multipotent from primitive hematopoiesis, we established how hFLOs gave rise to multi-lineage leukopoiesis (Leuko-hFLOs)–including bona fide mature neutrophils–after exposure to a defined

cytokine and small molecule cocktail. In support of functional contribution, bulk RNA-seq and functional characterization–e.g., bacterial phagocytosis–showed that matured Leuko-hFLO-macrophages and neutrophils are indistinguishable from adult

PBMC-derived macrophages and neutrophils. To harness Leuko-hFLOs for poorly modeled lipotoxic stress responses in mice, we added free fatty acids and revealed a positive feedback loop in the signaling of IL-8 to neutrophils. This work supports our hematopoietic liver organoid platform as an effective model for multi-lineage hepatogenesis and complex human hepatitis.