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UNIVERSITY OF TORONTO

# Advances in Biomedical Research V

**Mediterranean Institute for Life Sciences, Split, Croatia**
**September 15th -19th,2023.**

# Book of Abstracts

Split, September 2023.

Proceedings of the **“Advances in Biomedical Research V”**

Mediterranean Institute for Life Sciences, September 15th - 19th 2023.

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## September 15 (Friday) 2023

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|---|---|
| 17:00 – 18:00 | Arrival & Registration |
| 18:15 – 18:20 | **Opening Lecture INTRODUCTION of the Speaker by Igor Stagljar** |
| 18:20 – 19:00 | "Multispecificity – The Future of Molecular Medicines" **Ray Deshaies** (Senior Vice President, Global Research, Amgen, Thousand Oaks, CA, USA) |
| 19:00 - 21:00 | **Songs, Mediterranean food and wine** |

## September 16 (Saturday) 2023

| | |
|---|---|
| **09:00 - 13:15** | **Session #1** |
| **SESSION CHAIR:** | **Eilika Weber-Ban** (ETH Zurich) |
| 09:00 - 09:30 | "Organoids model human disease" **Hans Clevers** (Head of Roche Pharma Research and Early Development, Basel, Switzerland) |
| 09:30 - 10:00 | "Drugging small GTPases using artificial intelligence and live-cell based technologies" **Igor Stagljar**(Donnelly Centre, University of Toronto, Canada and MedILS, Split, Croatia) |
| 10:00 - 10:30 | "Cystic fibrosis on the African continent: from patients to rare *CFTR* variants" **Hugues Abriel** (University of Berne, Switzerland) |
| 10:30 - 11:00 | *Coffee break* |
| 11:00 - 11:30 | "Chemical Biology for Drug Discovery expands the Druggable Genome" **John Tallarico** (Head, Chemical Biology and Therapeutics at Novartis Institutes for BioMedical Research, Cambridge, MA, USA) |
| 11:30 - 12:00 | "Precision oncology and drug discovery by *ex vivo* image-based drug profiling" **Berend Snijder** (ETH Zurich, Switzerland) |
| 12:00 - 12:30 | "The functional interplay between cell death and inflammation and therapeutic opportunities arising therefrom" **Henning Walczak** (University College London Cancer Institute, London, UK) |
| 12:30 – 13:00 | "Empowering Women in Science: Insights from the Selvita CEO" **Adrijana Vinter** (CEO Selvita, Croatia & Global Head of Drug Discovery) |
| 13:15– 14:00 | *Lunch* |
| 14:00 – 15:30 | ***Poster session I*** */ Coffee break* |

| | |
|---|---|
| **17:30 – 20:00** | **Session #2** |
| **SESSION CHAIR:** | **Ivana Carev** (Medils, Split, Croatia) |
| 17:30 - 18:00 | "Pharmacoproteomics profiling reveals drug effects on the secreted proteome"<br>**Marcus Bantscheff** (Senior Scientific Director at Cellzome, a GSK company, Heidelberg, Germany) |
| 18:00 - 18:30 | "RIP1 is a key mediator of tissue damage and intestinal death in inflammatory diseases"<br>**Domagoj Vučić** (Senior Fellow, Genentech, South San Francisco, CA, USA) |
| 18:30 - 19:00 | "Promoting tissue regeneration through modulation of Wnt signalling"<br>**Stephane Angers** (Donnelly Centre, University of Toronto, Canada) |
| 19:00 - 19:30 | "Computing cancer immunity'<br>**Zlatko Trajanoski** (Medical University of Innsbruck, Austria) |
| 19:30 – 19:50 | "On intrinsic susceptibility of healthy organs to primary cancer or metastasis"<br>**Andrea Gelemanovic** (MedILS, Split, Croatia) |
| 20:00 - 21:00 | *Dinner* |

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| **09:00 – 13:00** | **Session #3** |
| **SESSION CHAIR:** | **Ivana Gašić** (University of Geneva, Switzerland) |
| 09:00 - 09:30 | "Mycobacterial Proteostasis Pathways for Stress Survival"<br>**Eilika Weber-Ban**(ETH Zurich, Switzerland) |
| 09:30 – 10:00 | "Understanding how RNA-based mechanisms control genome stability in cancer"<br>**Lovorka Stojić** (Barts Cancer Institute, Queen Mary University, London, UK) |
| 10:00 - 10:30 | "Enabling affordable and accessible CAR-T cell and other gene-modified cell therapies"<br>**Boro Dropulić** (Executive Director of Caring Cross and CEO of Vector BioMed, Gaithersburg, MD, USA) |
| 10:30 - 10:50 | "The Metabolite Path to Vitality: Detecting, Predicting, and Reversing Gut Inflammation"<br>**Nika Pintar (AniBiome, Zagreb, Croatia)** |
| 11:00 - 11:30 | *Coffee break* |
| 11:30 - 12:00 | "Revealing the Machinery for Production of Proteins in Human Cells"<br>**Nenad Ban** (ETH Zurich, Switzerland) |
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## September 18 (Monday) 2023

| | |
|---|---|
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| **SESSION CHAIR:** | **Lovorka Stojić** (Barts Cancer Institute, Queen Mary University, London, UK) |
| 09:00 - 09:30 | "New drug-like molecules enabling Translational Readthrough: From mechanistic studies to in vivo proof of mechanism in Hurler syndrome models"<br>**Jürgen Reinhardt** (Director, Novartis Pharma, Basel, Switzerland) |
| 09:30 – 10:00 | "Short Term Cultures to Analyse Molecular Response to Therapy in Colorectal Cancer"<br>Mi**chael Bergmann** (Medical University Vienna, Austria) |
| 10:00 – 10:30 | "Intact cells alter the fate of the neighboring cells damaged by UV radiation "<br>**Katarina Trajković** (MedILS, Split, Croatia) |
| 10:30 – 11:00 | "TOR signalling in high resolution"<br>**Robbie Loewith** (University of Geneva, Switzerland) |
| 11:00– 11:30 | "Autophagy Repair of Topoisomerase 1-Cleavage Complexes"<br>**Kristijan Ramadan** (University of Oxford, UK) |
| 11:30 – 12:45 | *WORKSHOP – WOMEN IN SCIENCE*<br>*Moderator:* ***Igor Stagljar***<br>*PARTICIPANTS:* **Eilika Weber-Ban** (ETH Zurich), **Lovorka Stojić** (Barts Cancer Institute, Queen Mary University, London, UK), **Katarina Trajković (**MedILS, Split, Croatia), **Ivana Carev** (Medils, Split, Croatia), **Ivana Gašić** (University of Geneva, Switzerland), **Adrijana Vinter** (Selvita, Croatia) |
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|---|---|
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| 18:00 – 18:30 | "Can we kill resistant microbes with natural products?"<br>**Ivana Carev** (Medils, Split, Croatia) |
| 18:30 – 19:00 | "Epigenome reprogramming during vertebrate embryogenesis"<br>**Ozren Bogdanovic** (Andalusian Centre for Developmental Biology, Seville, Spain) |
| 19:00 – 19:20 | "A structure-guided approach to target the Aurora-A/N-Myc complex in *MYCN*-amplified neuroblastoma<br>**Elisabetta Rubini(** Sapienza University of Rome, Italy) |
| 19:20 – 19:40 | "A Yap-dependent mechanoregulatory program sustains cell migration for embryo axis assembly<br>**María Almuedo-Castillo** (Andalusian Centre for Developmental Biology, Seville, Spain) |
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## September 19 (Tuesday) 2023

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|---|---|
| ***09:00 – 13:00*** | ***Session #5*** |
| ***SESSION CHAIR:*** | ***Katarina Trajković*** *(Medils, Split, Croatia)* |
| *09:00 – 09:30* | *"From protein-protein interactions to therapeutic targets in Huntington's disease"*<br>***Erich Wanker*** *(Max Delbrück Centre for Molecular Medicine, Berlin, Germany)* |
| *09:30 – 10:00* | *"The immune system plays a critical role in the development of Parkinson's disease"*<br>***Michel Desjardins*** *(University of Montreal, Canada)* |
| *10:00 – 10:30* | *"Autoregulation of tubulin mRNA stability"*<br>***Ivana Gašić*** *(University of Geneva, Switzerland)* |
| *10:30 – 11:00* | *Coffee break discussions* |
| *11:00 - 11:40* | ***Closing Lecture***<br>***INTRODUCTION of the Speaker by Igor Stagljar/Miroslav Radman***<br>*"Solving mysteries of the acid-activated chloride channel ASOR"*<br>***Thomas Jentsch*** *(Max Delbrück Centre for Molecular Medicine, Berlin, Germany)* |
| *11:40 - 12:25* | *Coffee break discussions* |
| *13:00 - 14:30* | *Lunch* |

## September 20 (Wednesday) 2023

Departure

# Abstracts

## Multispecificity – The Future of Molecular Medicines

***Ray Deshaies*** *(Senior Vice President, Global Research, Amgen, Thousand Oaks, CA, USA)*

Collectively, the biopharmaceutical industry has had an enormous impact on human health through the discovery and development of numerous safe and effective molecular medicines, including both small molecules and biologics. This success has, inevitably, raised the bar on expectations for future medicines. To thrive going forward, our industry will have to make medicines that are even safer and more effective than what came before. This is a tall order, given the relatively small number of high-conviction, accessible targets in most diseases. Nevertheless, opportunities abound, provided that we can surmount the key barriers that restrain many drug development efforts. These include overcoming biological redundancy, managing on-target toxicity/therapeutic index, and conquering so-called undruggable targets. Multispecific medicines that either attack multiple targets simultaneously, localize the action of a drug, or link targets to natural effectors offer a new approach to vanquish these perennial foes. In my presentation I will review the challenges and opportunities and describe approaches that Amgen is taking to develop the next generation of multispecific small molecule and biologic medicines for the benefit of future patients.

## Organoids model human disease"

**Hans Clevers** (Head of Roche Pharma Research and Early Development, Basel, Switzerland)

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally found that Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. Lgr5 was subsequently found by us to represent an exquisitely specific, yet 'generic' marker for active epithelial stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear, tongue and stomach epithelium.

Single sorted Lgr5+ve stem cells can initiate ever-expanding organoids in the lab. These organoids recapitulate key aspects of the organ from which the stem cells were taken. 3D organoids have been developed for the Lgr5+ve stem cells of human stomach, liver, pancreas, prostate, kidney, breast and many others. Using CRISPR/Cas9 technology, genes can be efficiently modified in these organoids . Organoid technology opens avenues for the study of development, physiology and disease, for drug development and for personalized medicine. In the long run, cultured mini-organs may replace transplant organs from donors and hold promise in gene therapy.

## Drugging small GTPases using artificial intelligence and live-cell based technologies

**Igor Stagljar**(Donnelly Centre, University of Toronto, Canada and MedILS, Split, Croatia)
https://stagljarlab.com

My lab is currently leading major proteomics and chemical genomics projects aimed at deciphering interactions among integral membrane proteins, shedding light on their role in both healthy and diseased cellular contexts. Our objectives include identifying novel therapeutic targets and utilizing artificial intelligence (AI) platforms to screen for new drugs, with a particular focus on lung, colon, and pancreatic cancer.

During my presentation, I will share recent, unpublished data demonstrating the seamless integration of AI-driven computational tools with two high-throughput, live-cell drug discovery technologies recently developed in our lab: Mammalian Membrane Two-Hybrid Drug Screening (MaMTH-DS) and Split Intein Mediated Protein Ligation (SIMPL).

Our work involves the progressive screening of a cohort of traditionally 'undruggable' protein targets, including KRAS and other small GTPases previously considered untargetable. Through this endeavor, we have achieved promising prospective validation of our integrated platform and have rapidly identified actionable compounds. This progress has established a robust pipeline for addressing challenging disease targets associated with currently untreatable cancers.

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## Cystic fibrosis on the African continent: from patients to rare *CFTR* variants

**Hugues Abriel** (University of Berne, Switzerland)

Rare diseases are more common than their name suggests, yet research on their prevalence in African populations is extremely limited. Cystic Fibrosis (CF), a prime example of a genetic disorder caused by mutations in the CFTR anionic channel gene (making it a genetic channelopathy), remains under-researched mainly in Africa, except for a few studies in South Africa, Morocco, and Egypt. My presentation is based on a ten-month academic sabbatical at the University of Kinshasa and CHU of Fès in 2021, where we explored the use of Oxford Nanopore Technologies to sequence genes of interest, notably HBB and CFTR, in patients with hemoglobinopathies and suspected CF. Using these initial findings as a foundation, we recently launched an extended project with Fès, Kinshasa, and Nairobi paediatricians and medical geneticists to investigate CF's incidence and genetic heterogeneity in these regions. A significant advancement in this project is our procurement of three “sweat test” devices, an essential tool for CF diagnosis, soon available for use by our collaborators. One of the focuses of my presentation is our innovative sequencing method that captures the complete CFTR gene using a portable Oxford Nanopore Technology device. This approach is based on ten ~25-kb long amplicons. Once we detect genetic variants, we plan to evaluate their pathogenicity using standard immunoblot expression procedures and patch-clamp electrophysiology. In our ongoing aim to provide personalized treatment solutions for CF patients, we will also assess the potential benefits of emerging CFTR modulator drugs for African patients.

## Chemical Biology for Drug Discovery expands the Druggable Genome

**John Tallarico** (Head, Chemical Biology and Therapeutics at Novartis Institutes for BioMedical Research, Cambridge, MA, USA)

Despite great progress in our ability to creatively modulate the function of proteins for therapeutic benefit, there is still a great number of proteins that have historically been highly challenging to target with small molecules. We call these undraggable targets. First described more than 20 years ago, Targeted Protein Degradation (TPD) has emerged as a solution to some of these challenges. Since the introduction of proteolysis-targeting chimeric small molecules (PROTACs) which utilize the ubiquitin-proteasome system by inducing proximity of a target of interest to an E3 ligase to induce degradation of a specific target, the horizons of TPD have expanded to include molecular glue degraders. This class of molecules is derived from increased understanding over the past decade about the molecular mechanism of action of Thalidomide and related molecules (aka “Imids”). Harnessing this improved understanding to target additional undrugged targets systematically is establishing this approach as a unique solution to some of the thorniest problems in small molecule drug discovery.

## Precision oncology and drug discovery by *ex vivo* image-based drug profiling

**Berend Snijder** (ETH Zurich, Switzerland)

The cellular and molecular systems that determine drug responses in cancer are complex, highly individual, and incompletely understood. As a result, identifying effective treatments for individual patients is still often challenging, particularly in relapsed disease. To tackle this challenge, we are developing Pharmacoscopy, which allows us to measure hundreds of *ex vivo* drug responses in small patient biopsies by immunofluorescence, automated confocal microscopy, single-cell image analysis, and machine learning. In this talk, I will show: 1) Results from interventional clinical trials showing that Pharmacoscopy identifies effective treatments; 2) How we can use deep learning and spatial analyses to discover new cancer and immune cell phenotypes; And 3) how, when combined with patient-centric multi-OMIC measurements and matched patient data, Pharmacoscopy enables the identification of new drugs and associated molecular and cellular systems that govern their treatment response

## The functional interplay between cell death and inflammation and therapeutic opportunities arising therefrom

**Henning Walczak** (University College London Cancer Institute, London, UK)

Henning Walczak, PhD[1,2]

[1]Institute of Biochemistry I & CECAD Research Center, University of Cologne, Germany; [2]Center for Cell Death, Cancer and Inflammation (CCCI), UCL Cancer Institute, University College London, UK

Tumor necrosis factor (TNF) itself and other death ligand members of the TNF cytokine superfamily have been particularly attractive as potential novel cancer therapeutics because of their capacity to directly induce cell death. Soon after TNF was cloned it became clear, however, that recombinant TNF could not be used to treat cancer systemically as it was found to exert lethal inflammation when applied systemically. The same held true for the second death ligand to be identified, Fas ligand (FasL). Yet, for the third death ligand to be discovered, the TNF-related apoptosis-inducing ligand (TRAIL), this was different as TRAIL was capable of selectively inducing apoptosis in cancer cells, yet not in any essential normal cells *in vitro* and *in vivo.* However, in contrast to many cancer cell lines most primary cancer cells turned out to be TRAIL-resistant. We found that combining TRAIL with CDK9 inhibitors overcomes TRAIL apoptosis resistance in a broad range of cancers and that, importantly including cancers resistant to chemo- and/or targeted therapies. With regard to TNF signalling, we discovered linear ubiquitination as a crucial regulator of the balance between cell death and inflammation and that the induction of cell death by TNF, rather than only TNF-induced gene activation, can induce chronic inflammation and autoimmunity. We recently extended this concept of cell death-induced inflammation to further death ligands, including FasL, and our most recent research on this topic will be presented.

## Empowering Women in Science: Insights from the Selvita CEO

**Adrijana Vinter** (CEO Selvita, Croatia & Global Head of Drug Discovery)

Empowering women in science is becoming more and more important topic within communities around the world. Historically, omen were always playing an important role by their scientific contribution. However, despite that, women represent only 33,3% of researchers globally. It is noticeable that their work is not well recognized if we look at the fact that Less than 4 % of Nobel Prizes for science have ever been awarded to women, and only 11 %* of senior research roles are held by women in Europe. Similar situation is happening also in managerial role.

By showing a case of how company Selvita Zagreb was built by women who held more than 80% of managerial positions and 70% of women in scientific disciplines, I will talk about the importance of empowering women in science. I will share a personal and professional experience of mentoring and developing women in different scientific and managerial roles.

## Pharmacoproteomics profiling reveals drug effects on the secreted proteome

**Marcus Bantscheff** (Senior Scientific Director at Cellzome, a GSK company, Heidelberg, Germany)

Adverse drug reactions contribute significantly to late-stage attrition in drug discovery due to their unpredictability and enigmatic underlying mechanisms. In the talk I will discuss how proteomics technologies can contribute to the identification of on- and off-targets of small molecule drugs and to the delineation of dysregulated mechanisms. I will highlight a new study where we applied mass spectrometry-based proteomics to assess the effects of a set of drugs and tool compounds with various levels of concerns for drug induced liver injury on the secretome of a hepatocyte model system. Thermal proteome profiling and other functional studies allowed linking secretion patterns to distinct intracellular mechanisms dysregulated by these compounds. Taken together our data provide insights on a surprisingly large range of mechanisms that are dysregulated by liver damaging drugs and the manifestation of these mechanisms in the secretome of liver cell models suggesting novel marker proteins for monitoring such unwanted effects in preclinical studies and in patients.

## RIP1 is a key mediator of tissue damage and intestinal death in inflammatory diseases

**Domagoj Vučić** (Senior Fellow, Genentech, South San Francisco, CA, USA)

Receptor-interacting protein 1 (RIP1) kinase is a key mediator of TNF induced signaling pathways that regulate inflammatory responses, and caspase-dependent apoptotic and caspase-independent necroptotic cell death. The kinase activity of RIP1 has been implicated in tissue damage and numerous inflammatory and neurodegenerative diseases. Patients suffering from inflammatory bowel diseases (IBD) are particularly sensitive to TNF induced, RIP1 mediated death of intestinal cells when the autophagy regulator ATG16L1 is mutated. We examined how mutations or absence of anti-apoptotic IAP proteins, or critical regulators of NF-kB and MAPK signaling, affect the sensitivity of intestinal tissues to TNF triggered death. Our studies reveal that intestinal and/or whole-body deletion of most signaling and cell death regulators leads to RIP1 mediated cell death. Furthermore, we examined the role of RIP1 activity in Graft-versus-host disease (GVHD), which is the major cause of morbidity and non-relapse mortality (NRM) after hematopoietic cell transplantation (HCT). The RIP1 inhibitor GNE684 prevented GVHD crypt destruction and protected the GI tract from GVHD and improved survival to the same extent as genetic inactivation of RIP1 in allogenic HCT mouse recipients. Importantly, treatment with GNE684 significantly ameliorated GVHD without compromising the graft-versus-leukemia (GVL) effect in two different leukemia models. Finaly, GI biopsies from GVHD patients showed that increased levels of activated phosphorylated RIP1 correlated with tissue damage and predicted non-relapse mortality. Thus, targeting RIP1 represents a novel and promising strategy for treatment of GVHD and many other inflammatory conditions.

## Promoting tissue regeneration through modulation of Wnt signalling

**Stephane Angers** (Donnelly Centre, University of Toronto, Canada)

The Wnt-bcatenin signaling pathway controls stem cell self-renewal and is thereby a master regulator of tissue homeostasis. Through precise control of Wnt signaling it may therefore be possible to promote stem cell activity and boost the regenerative potential of tissues.

We have developed a tetravalent antibody scaffold designed to promote the clustering of the Wnt co-receptors Frizzled and LRP5/6 at the cell surface, which is sufficient to promote their activation and trigger downstream signaling. These antibodies that we call FLAgs (Frizzled and LRP5/6 Agonists), efficiently mimic Wnt proteins and are completely specific for all ten Frizzleds and two LRP5/6 co-receptors.

Our group is studying the efficacy of FLAg antibodies to promote the regeneration of various tissues in vivo. I will present our pre-clinical results describing the ability of a FZD4-LRP5 antibody FLAg to activate bcatenin signaling in retinal endothelial cells, promote angiogesis and reduce permeability in mouse models. Additionally, I will present our data establishing the efficacy of a FZD5-LRP6 FLAg in a DSS-induced colitis model.

In summary, we have developed a biologics pipeline enabling the precise control of Wnt signaling activity in vivo. Leads molecules are now advancing in clinical trials in various unmet medical needs.

## Computing cancer immunity

**Zlatko Trajanoski** (Medical University of Innsbruck, Austria)

Despite major advances in the development of targeted therapies, precision (immune)oncology approaches for patients with colorectal cancer (CRC) continue to lag behind other solid cancers. Using publicly available genomic data we first identified that genotypes are associated with immune phenotypes, suggesting a crosstalk between oncogenic pathways and immune-related signaling. We then showed that kinase inhibitors modulate the immune composition in tumors, implying that pharmacological signaling rewiring could sensitize tumors to immunotherapy. In order to derive mechanistic rationale for precision immune-oncology in individual CRR patients, we developed a functional precision profiling approach based on systematic perturbations of patient-derived organoids (PDOs) followed by quantitative phosphoproteomic measurements. The PDOs were perturbed with kinase inhibitors, and large-scale phosphoproteomic profiling using data-independent acquisition was carried out. Phosphoproteomic profiling revealed crosstalk with immune-related pathways and off-target effects of kinase inhibitors. We then reconstructed topologies of signaling kinase networks and show patient-specific rewiring that is largely unaffected by mutations.
Collectively, we developed a framework that integrates perturbations of PDOs and multimodal data for inferring tumor cell signaling. This work is an important step towards the development of a platform for making therapeutic recommendations for individual CRC patients.

# Mycobacterial Proteostasis Pathways for Stress Survival

**Eilika Weber-Ban**(ETH Zurich, Switzerland)

Charlotte Schilling[1], Andreas U. Müller[1,2], Eva Kummer[1,3], Lena ML Keller[1], Mikhail Kavalchuk[1]

Tatjana von Rosen[1] and Eilika Weber-Ban[1]

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Mycobacteria and other members of Actinobacteria display high adaptability to various stressors, allowing them to thrive under diverse and often harsh conditions (1). Central to their resilience is an intricate network of proteostasis pathways. Part of this network is a gene locus encoding a unique proteasomal complex (2), a post-translational modification system for targeting proteins to this complex (2), and a member of the emerging class of WYL-domain containing transcription factors (3).

I will discuss how this gene locus, referred to as the pupylation-proteasome system (PPS) gene locus, contributes to the survival of mycobacteria under genotoxic stress. I will show how mycobacterial proteasome complexes recruit and process their substrates and which role is played in this process by prokaryotic ubiquitin-like protein Pup (4,5), and I will show how mycobacteria use a unique transcriptional mechanism to orchestrate the DNA damage response (6).

Understanding mycobacterial proteostasis pathways and the role of pupylation and WYL-domain containing transcription factors in stress survival not only sheds light on the remarkable adaptability of these bacteria but also has implications for potential therapeutic strategies against tuberculosis and related diseases.

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(6) Müller AU, Kummer E, Schilling CM, Ban N & Weber-Ban E (2021) *Transcriptional control of mycobacterial DNA damage response by sigma adaptation*. Science Advances 7, eabl4064.

## Understanding how RNA-based mechanisms control genome stability in cancer

**Lovorka Stojić** (Barts Cancer Institute, Queen Mary University, London, UK)

Genome stability is paramount to cellular homeostasis throughout the human lifespan. Cells have developed several surveillance mechanisms to protect the genome from mutations and ensure faithful duplication and transmission of the genetic material. Defects in any of these mechanisms leads to genome instability, which drives cancer evolution and contributes to tumour heterogeneity, drug resistance and poor prognosis. Protein-mediated mechanisms controlling genome stability are well described, however, the biological and regulatory function of RNA-based mechanisms in this context, and in particular the contribution of long noncoding RNAs (lncRNAs), is largely unknown. We have recently identified novel lncRNAs linked to chromosome mis-segregation, a process common to different types of cancer. I will focus on two nuclear localised lncRNAs whose expression is altered in cancer, and highlight mechanisms through which these lncRNAs safeguard genome integrity and their relevance to cancer.

## Enabling affordable and accessible CAR-T cell and other gene-modified cell therapies

**Boro Dropulić** (Executive Director of Caring Cross and CEO of Vector BioMed, Gaithersburg, MD, USA)

Improving access of CAR-T cell and other gene-modified cellular therapies by manufacturing the final product at the place-of-care.

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The current price for a one-time commercial CAR-T cell therapy, not including hospital costs is at least $350,000 per dose. Such pricing is not sustainable in high-income countries, let alone in low- and middle-income countries, leaving many without access to these transformative therapies. A centralized model currently used for the manufacture and distribution of commercial CAR-T cells drives up their cost due to the logistical and infrastructure needs of this model. A more cost-effective way is to manufacture the final CAR-T cell product locally at the point-of-care. In our previous studies, the overall response rate of a CAR-T cell therapy for adult B-cell Lymphoma and Pediatric Leukemia using locally manufactured CAR-T cells produced outstanding clinical outcomes [Nat Med, 2020, https://www.nature.com/articles/s41591-020-1081-3; Nat Com, 2021, https://www.nature.com/articles/s41467-021-27312-6]. Caring Cross is a non-profit that is collaborating with many organizations, including clinical centers around the world to develop and improve the access of CAR-T and other gene-modified cellular therapies that are manufactured locally at, or close to, the hospitals treating the patients needing these therapies. This place-of-care manufacturing significantly reduce the costs of manufacturing CAR-T and other gene-modified cellular products and promises to improve their affordability and access around the world.

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## Revealing the Machinery for Production of Proteins in Human Cells

**Nenad Ban** (ETH Zurich, Switzerland)

Our group is interested in understanding the process of expression of genetic information that leads to the production of functional proteins. This process requires an intricate interplay between the protein synthesis machinery and an ever growing list of cellular components that control protein synthesis and participate in protein biogenesis. Building on our studies that provided some of the first blueprints for understanding the eukaryotic protein synthesis machinery including the cytosolic and the mitochondrial ribosomes, we are now investigating protein synthesis in human cells using a combination of structural, biochemical and biophysical experimental approaches. We are particularly interested in understanding the regulation of protein synthesis and the biogenesis of cytosolic and membrane proteins. I will present examples of recent results that contribute to our understanding of the network and the coordination of cellular factors that interact with translating ribosomes in human cells to control protein synthesis and to ensure accurate protein production.

## Controlling function by conformational trapping

**Tony Kossiakoff** (University of Chicago, IL, USA)

Biological processes are governed by complex sets of molecular recognition events. A major feature of molecular recognition is conformational complementarity between the interacting partners. Many times these partners exist in variable conformational states that are quite different from the one that is required for their complementation except in the particular environment that they function in.
We have developed a high throughput phage display pipeline that generates customized synthetic antibodies using novel phage display libraries and selection strategies that can identify and trap transient conformational intermediates allowing structure-function annotation of the static and dynamic features of a protein system. This allows allosteric control of the protein systems and demonstrates the power of using these reagents to induce conformational states that significantly influence the functional properties of the proteins they bind. As a demonstration of the approach, we have generated synthetic antibodies to CD73/NT5E, a key enzyme in the immunosuppressive adenosinergic pathway, which is highly overexpressed in numerous cancer tumors. These antibodies are customized to bind only in the presence of the tumor microenvironment and leave normal tissue unaffected, thus eliminating deleterious off-target effects.

## Beyond publish and perish: the new culture of preprint peer review

**Thomas Lemberger** (EMBO, Heidelberg, Germany)

Peer review is integral to the scientific process, serving as a crucial step in ensuring the rigor of published research. Despite its importance, the existing system in life sciences faces challenges such as redundant reviews and competition for a limited pool of reviewers, leading to delayed publications. To address these issues, EMBO launched Review Commons, a journal-agnostic peer review system for preprints. Supported by a consortium of publishers, this platform streamlines the publishing process through a single set of high-quality, transparent reviews, which are accepted by affiliated journals. This enables more efficient publishing and alleviates the strain on the reviewer community. Notably, Review Commons posts peer reviews alongside the preprints as soon as a study is submitted to one of the affiliated journals. This is especially beneficial for young scientists, allowing them to showcase their research with expert analyses even before final publication. Moreover, Review Commons fosters editorial collaboration across various journals and accelerates access to peer-reviewed research through publicly reviewed preprints. As preprints gain widespread adoption in life sciences and generative AI-driven tools redefine scientific communication, this talk will discuss the Review Commons initiative in the broader context of the role of open science in a rapidly changing landscape of scientific publishing.

## New drug-like molecules enabling Translational Readthrough: From mechanistic studies to in vivo proof of mechanism in Hurler syndrome models”

**Jürgen Reinhardt** (Director, Novartis Pharma, Basel, Switzerland)

Suppression of premature termination codons (PTCs) by translational readthrough is a promising strategy to treat a wide variety of severe genetic diseases caused by nonsense mutations. In our talk we present two potent readthrough promoters—NVS1.1 and NVS2.1—that restore substantial levels of functional full-length CFTR and IDUA (a-L-Iduronidase) proteins in cellular disease models for cystic fibrosis and Hurler syndrome respectively. In vivo proof of concept studies using an in-house developed Hurler rat model confirmed proof of mechanism and pathway engagement in brain and other tissues. In contrast to other readthrough promoters that affect stop codon decoding, the NVS compounds stimulate PTC suppression by triggering rapid proteasomal degradation of the translation termination factor eRF1. Our presented results show that this occurs by trapping eRF1 in the terminating ribosome, causing ribosome stalls and subsequent ribosome collisions, and activating a branch of the ribosome-associated quality control network, which involves the translational stress sensor GCN1 and the catalytic activity of the E3 ubiquitin ligases RNF14 and RNF25.

## Short Term Cultures to Analyse Molecular Response to Therapy in Colorectal Cancer

Mi**chael Bergmann** (Medical University Vienna, Austria)

We have developed 3 types of short term cultures: i) organoids, ii) slice and tumor fragment cultures and iii) pleural effusion cultures. With respect to organoids we showed that cancer associated fibroblast support organoid growth in the absence of the ENAS medium (Atanasova et al. Cell Mol Gastro Hep 2022). Fibroblasts also change the transcriptome and protein expression of tumor cells and modulate the response to therapy. We have now added myeloid cells to the organoid/fibroblast co-cultures giving raise to triple cultures. Treatment of triple cultures with either oncolytic virus or chemotherapy allow analyses of treatment response using single cell sequencing, FACS analysis or functional assays determining phagocytosis. Organoid culture further reflect interpatient heterogeneity. With respect to slice cultures we analyzed response to T-cell engagers. With respect to pleural effusion cultures we analyzed response to radiotherapy and various T-cell cell stimulating agents. Tumor fragment cultures were used to model effect of radiotherapy in the myeloid system. The observed pro-inflammatory effects of radiotherapy led to a randomized phase II trial investigating the effect of checkpoint inhibitors nivolumab and ipilimumab in combination with radio-chemotherapy in a neoadjuvant setting in locally advanced rectal cancer (NCT 04124601).

## Intact cells alter the fate of the neighboring cells damaged by UV radiation

**Katarina Trajković** (MedILS, Split, Croatia)

Jelena Budimir[1], Nikola Pavlović[1], Andrea Gelemanović[1], Vanda Juranić-Lisnić[2], Swetlana Sperling[3], Milena Ninković[3], Stipan Jonjić[2], Miroslav Radman[1,4], Katarina Trajković[1,4]

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As we age, cells acquire damage in a stochastic manner, which results in phenotypic heterogeneity of cellular populations in the aging tissues[1]. The interplay between cells with different degrees of damage may affect the fate of individual cells. While the detrimental effects of dying cells on healthy cells in their vicinity have been extensively studied[2], little is known about the opposite[3].

In this work we aimed to determine the impact of healthy cells on the neighbouring cells damaged by UV radiation which mimics age-related phenotypic changes. Irradiated cells were grown either in monocultures or in co-cultures with intact cells, and their phenotypes were compared. We found that the intact cells altered the phenotype of highly, but not of moderately damaged cells. In particular, the process of cell death in irradiated cells was accelerated in the presence of intact cells, and this did not depend on direct contact or on extracellular vesicles. Moreover, the transcriptional response to UV light of the damaged cells was almost completely arrested in co-culture with intact cells, suggesting a mechanism of the observed accelerated cell death. These findings emphasize non-cell autonomous factors as a powerful tool for modulating age-related cellular phenotypes.

**References**

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2. Eroglu and Derry, Cell death and differentiation, 2016 (doi:10.1038/cdd.2016.41)
3. Radman, Open biology, 2019 (doi:10.1098/rsob.180250)

## TOR signalling in high resolution

**Robbie Loewith** (University of Geneva, Switzerland)

The Target Of Rapamycin (TOR) is a protein kinase, conserved across eukaryotic species, that plays a fundamental role in the control of cell growth and homeostasis. Mammalian TOR signalling has become well recognized as its dysregulation is implicated in most cancers and many neurodegenerative diseases. TOR operates in two, distinct, multiprotein complexes TORC1 and TORC2, that, like TOR itself, were first discovered in the model eukaryote *Saccharmyces cerevisiae.* In this talk I will aim to show that studies with budding yeast continue to provide exciting new insights into this important signalling network. Specifically, I will present unpublished work from the lab describing our ongoing efforts to discover the molecular mechanisms enabling TORC2 to maintain the biophysical properties of the plasma membrane. This will include new CryoEM structures of TORC2 and unique, endogenous-plasma-membrane-containing structures of the tension-responsive microdomain known as the Eisosome.

## Autophagy Repair of Topoisomerase 1-Cleavage Complexes

**Kristijan Ramadan** (University of Oxford, UK)

Pauline Lascaux[1], Gwendoline Hoslett[1], Annamaria Ruggiano[1], Errin Johnson[2], Iolanda Vendrell[3], Roman Fischer[3] and Kristijan Ramadan[1]*

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Topoisomerase 1 (TOP1) plays a pivotal role in alleviating DNA topological stress preceding DNA replication and transcription. As a part of its enzymatic activity, TOP1 covalently binds to DNA, forming an intermediate known as TOP1 cleavage complexes (TOP1cc) and causing a single-strand DNA break, which facilitates DNA unwinding. If left unrepaired, TOP1cc can impede the progress of DNA replication and transcription, ultimately contributing to genomic instability. Consequently, the stabilisation of TOP1cc with TOP1 poisons, such as Irinotecan and Topotecan, has emerged as a highly effective and widely used approach in cancer therapy. However, the efficient removal of TOP1cc has been shown to lead to resistance in cancer cells.

Until recently, the proteasomal proteolysis of TOP1cc was only observed at excessively high doses of TOP1 poisons (in the micromolar range; µM) that are unattainable in clinical settings, casting doubt on the relevance of proteasome involvement in TOP1 poisons-based cancer therapy and patient response. However, our recent research has uncovered a new facet of TOP1cc repair at clinically relevant doses of TOP1 poisons (in the nanomolar range; nM).

We have discovered that the repair of TOP1cc induced by these clinically relevant doses is dependent on the p97 unfoldase activity, its cofactor TEX264, and the SPRTN protease[1,2]. Moreover, our current findings reveal that the p97-TEX264 complex, operating independently of the SPRTN protease, proteasome, or TDP1 phosphodiesterase activity, is responsible for driving the removal of genotoxic TOP1ccs through autophagy. Specifically, TEX264 functions as the selective autophagy receptor, facilitating the delivery of TOP1cc to the autophagosome in close collaboration with the p97 unfoldase. The p97-TEX264 complex orchestrates the selective autophagic clearance of TOP1cc from stalled DNA replication forks.

Our research not only establishes a direct link between selective autophagy and the processing of nuclear material under conditions of replication stress but also underscores the critical relevance of this novel mechanism for our understanding of genome stability and the response to cancer therapy.

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1 Fielden, J. *et al.* TEX264 coordinates p97- and SPRTN-mediated resolution of topoisomerase 1-DNA adducts. *Nat Commun* **11**, 1274, doi:10.1038/s41467-020-15000-w (2020).

2 Fielden, J., Popovic, M. & Ramadan, K. TEX264 at the intersection of autophagy and DNA repair. *Autopagy* **18**, 40-49, doi:10.1080/15548627.2021.1894059 (2022).

## Metabolomics as a hypothesis generator

**Uwe Sauer "EMBO Member"** (ETH Zurich, Switzerland) **'The EMBO Keynote Lecture'**

The gut microbiome is important for health and disease, and more than a decade of microbiome sequencing has provided important insights in the variation and composition of the gut microbiota. A sobering observation is that although we learned who is there, we gained surprisingly little knowledge on what they are doing and how they interact to establish a community. Exploiting the potential of high-throughput metabolomics by flow-injection mass spectrometry, I will highlight how metabolomics can be used to gain functional insights into what different microbes are doing in the mammalian gut (1). Starting from elucidation of cross-feeding networks in in vitro communities, we will move into mapping the metabolite landscape of the mouse gut (2).

1. Perez Escriva P, Fuhrer T & **U** Sauer (2022). Distinct N and C cross-feeding networks in a synthetic mouse gut consortium. **mSystems** e0148421. doi: 10.1128/msystems.01484-21.

2. Meier KHU, Trouillon J, Li H, Lang M, Fuhrer T, Zamboni N, Sunagawa S, Macpherson AJ & U Sauer (2023). Metabolic landscape of the male mouse gut identifies different niches determined by microbial activities. **Nature Metabolism** 5: 968-980.

## Can we kill resistant microbes with natural products?

**Ivana Carev** (Medils, Split, Croatia)

The pharmacological potential of essential oils derived from the Centaurea genus has long been recognized. Among the myriad constituents within these oils, including ß-caryophyllene, hexadecanoic acid, spathulenol, pentacosane, caryophyllene oxide, and phytol, their antimicrobial properties have garnered particular attention. However, the pivotal role of these dominant components in driving antimicrobial activity remains enigmatic. Here, a comprehensive analysis of existing literature seeks to establish correlations between the chemical compounds found in Centaurea essential oils and their demonstrated antimicrobial efficacy, particularly against *E. coli* and *S. epidermis* microbes. While the analysis of literature data from various Centaurea essential oils supported a positive correlation with antimicrobial activity, our experimental findings, when testing these chemical constituents in isolation, did not substantiate this claim. This intriguing paradox prompted us to employ network pharmacology analysis, suggesting that the antibacterial effects of essential oil constituents may arise from complex synergistic interactions rather than the action of a single component. Furthermore, it presents the inaugural report of antimicrobial activity associated with the representative, pure components such as aromadendrene, germacrene D, spathulenol, longifolene, and a selected mixture of chemical compounds. In summary, our research makes a significant contribution to the understanding of the Centaurea genus and the importance of considering synergistic interactions in the context of antimicrobial activity. It opens the door for further in-depth investigations in this field, offering promising prospects for harnessing natural chemical compounds in the fight against microbial pathogens.

## Epigenome reprogramming during vertebrate embryogenesis

**Ozren Bogdanovic** (Andalusian Centre for Developmental Biology, Seville, Spain)

Evolutionary conservation and divergence of embryonic DNA methylome remodelling

Methylation of cytosines is the most abundant DNA modification in vertebrates that plays crucial roles in cellular differentiation and identity. Therefore, after fertilization, DNA methylation patterns inherited from parental gametes are remodelled into a state compatible with embryogenesis. In mammals, this is achieved through global erasure and re-establishment of DNA methylation patterns. However, in non-mammalian vertebrates like zebrafish, no global erasure has been observed. To investigate the evolutionary conservation of DNA methylation remodelling in anamniotes, we generated base resolution DNA methylome datasets of developing lamprey, medaka and zebrafish-medaka hybrid embryos. In contrast to previous reports, we show that medaka display comparable DNA methylome dynamics to zebrafish with high gametic DNA methylation levels (Sperm: ~90%; Egg: ~75%), and adoption of a paternal-like methylome during early embryogenesis. Similar DNA methylation reprogramming events were also observed during lamprey embryogenesis, however those occurred at a much larger scale (~30% of the genome) and involved transitions in partially methylated DNA (PMD) states. Lastly, we found remarkable evolutionary conservation of DNA methylation remodelling patterns in zebrafish-medaka hybrids, indicative of compatible DNA methylation maintenance machinery in divergent teleost species. Overall, these results suggest strong evolutionary conservation of DNA methylation remodelling pathways in vertebrates, which is distinct from the global DNA methylome erasure seen in mammals.

## From protein-protein interactions to therapeutic targets in Huntington's disease

**Erich Wanker** (Max Delbrück Centre for Molecular Medicine, Berlin, Germany)

*OMICS Approaches and Experimental Models for Target and Drug Discovery in Neurodegenerative Diseases*

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*One of the biggest challenges in disease research is to understand the underlying molecular causes of brain-specific pathologies and to develop effective disease-modifying therapies. We apply quantitative multi-OMICs techniques, disease-relevant experimental model systems and theoretical network modelling approaches to gain insight into the molecular mechanisms of neurodegenerative diseases (NDs) such as Alzheimer's (AD) and Huntington's disease (HD). We have previously generated interactome maps for a large number of ND-associated proteins and recently used MS-based proteomics methods to identify amyloid-b aggregation-associated protein changes in 5xFAD transgenic mice. These investigations revealed an upregulation of the lysosomal protein Arl8b, which was also observed in post-mortem brains of AD patients and CSF, suggesting that it may have potential as biomarker for AD. For HD, we generated multiple transgenic Drosophila lines neuronally expressing aggregation-prone mutant huntingtin exon-1 (mHTTex1) protein variants. We observed a strong correlation between the expression of different pathogenic variants in neurons and the lifespans of transgenic flies, indicating that the model recapitulates key aspects of human HD and enables the investigation of genotype-phenotype relationships. Differential gene expression analysis of RNAseq data from different HD fly strains revealed gene clusters and multiple synaptic proteins that are significantly altered in HD patient brains but not in age-matched controls. Finally, we applied a machine learning approach to identify lifespan-predicting genes using RNAseq data and lifespan measurements as input data. We show that transcriptional downregulation of monoaminergic genes, which control transport and release of neurotransmitters such as dopamine or serotonin, is associated with reduced survival of HD transgenic flies. An improvement of neurotransmitter signaling with drugs may have beneficial effects on disease progression of patients.*

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## The immune system plays a critical role in the development of Parkinson's disease

**Michel Desjardins** (University of Montreal, Canada)

While the contribution of inflammation in the pathological process leading to Parkinson's disease (PD) is well established, a growing body of evidence supports a role for the long-lasting adaptive immune system in the disease. We showed that, in inflammatory conditions, the PD-proteins PINK1 and Parkin negatively regulate the presentation of mitochondrial antigens on MHC I molecules, a process referred to as MitAP (Mitochondrial Antigen Presentation). Over-activation of this pathway, in the absence of PINK1, engages autoimmune mechanisms leading to the establishment of cytotoxic $CD8^+$ T cells that may contribute to dopaminergic neurons (DNs) cell death. We used a pharmacological and genetic approach to characterize the MitAP pathway at the molecular cell level. Our data indicate that this antigen presentation pathway is induced in APCs in response to inflammatory signals through the sequential activation of TLR4, cGAS-STING and the Unfolded Protein Response (UPR). A "UPR motif" present on a STING cytoplasmic domain is shown to specifically activate the UPR sensor IRE1. Remarkably, both the secretion of pro-inflammatory cytokines and antigen presentation on MHC class I molecules are regulated by the PD-related protein LRRK2, in close connection with STING, upstream of the UPR. These data identify LRRK2 as a key regulator of both the innate and adaptive immune response during inflammation.

## Autoregulation of tubulin mRNA stability

**Ivana Gašić** (University of Geneva, Switzerland)

Tubulins are abundant and highly conserved proteins that build the microtubule cytoskeleton. Cells use microtubule cytoskeleton to organize their cytoplasm, move, and divide. Tubulin quantity and quality are critically important for cellular fitness, as loss of either introduces damage to the microtubule network structure and function, and has been associated with ghastly diseases in humans, such as neurodevelopmental disorders and cancer. Cells have therefore evolved multiple mechanisms to ensure tubulin homeostasis. One such mechanism is termed tubulin autoregulation—a pathway through which, when in excess, tubulins trigger cotranslational degradation of their encoding transcripts. Our recent work uncovered the first molecular components of this pathway: the specificity factor required for the recognition of tubulin mRNA (via the nascent tubulin protein; TTC5), the bridging molecule (SCAPER), and the effector mRNA decay machinery (CCR4-NOT complex). In this talk, I will present our ongoing work where we identify tubulin as a sequestration factor and regulator of TTC5 activity: in steady state, TTC5 is inhibited by tubulin. When tubulin levels rise, TTC5 is released to autoregulate tubulin mRNAs. We further identify a TTC5 mutant that fails to bind to tubulin but retains the capacity to autoregulate tubulin mRNA levels. The expression of such a constitutively active mutant in cells causes decreased levels of tubulin encoding mRNAs, ultimately compromising microtubule-dependent division of the genetic material during mitosis.

## Solving mysteries of the acid-activated chloride channel ASOR

**Thomas J. Jentsch** (Max Delbrück Centre for Molecular Medicine, Berlin, Germany)

Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) Berlin

Since its first description (in Sertoli cells, Auzanneau et al, JBC 2003), a mysterious acid-activated chloride current has been found by many groups in all mammalian cells where it had been looked for. This current was mysterious as it needs both strong external acidification and cytoplasm positive voltages for opening, conditions that are almost never reached at the plasma membrane of mammalian cells. Since the molecular identity of the underlying channel (dubbed ASOR for *A*cid-*S*ensitive *O*utwardly *R*ectifying channel) had remained unknown, its biological function was enigmatic.

Using a genome-wide siRNA screen, we recently found that it is composed of (ubiquitously expressed) Tmem206 proteins and identified pore-lining residues by mutagenesis and electrophysiology (1). Cryo-EM studies show that Tmem206 proteins, which contain two transmembrane domains, assemble to trimeric channels whose overall topology resemble ASIC cation channels. In collaboration with Steve Long (Sloan Kettering), we determined the cryo-EM structure of ASOR at different pH values and obtained for the first time structures for the open pore (2). These structures are consistent with our previous results and were further validated by functional assays. In contrast to ASIC and most other channels, opening of ASOR involves a drastic rearrangement of transmembrane domains. Their movement is driven by three pairs of extracellular acidic amino-acids which can approach each other only when protons are interposed (2). pH-dependent gating does not involve a histidine residue that was previously implicated in this process.

The strongly acidic external pH needed to activate ASOR is reached only under exceptional conditions in the extracellular space. Accordingly, ASOR plays a role in acidotoxicity and is deleterious in stroke. However, the main function of ASOR is most likely in intracellular vesicles that are sufficiently acidic to activate ASOR. Emerging evidence shows that it plays distinct roles in endocytic processes. We recently examined its role in macropinocytosis, a ubiquitous from of endocytosis that is of particular importance for immune and cancer cells (3). We found that ASOR, in parallel to TPC cation channels previously implicated by the Grinstein lab in this process, is essential for the shrinkage of macropinosomes. Opening of both channels allows the exit of luminal Na and Cl. This leads to osmotic shrinkage which is followed by vesicle budding. Decreased resolution of macropinosomes results in impaired recycling of membrane receptors and luminal content. Accordingly, cancer cell nutrition by albumin uptake was reduced in *Tmem206*$^{-/-}$ cells (3). ASOR likely modulates several aspects of endocytosis.

Own relevant publications:

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# Short talks

## On intrinsic susceptibility of healthy organs to primary cancer or metastasis

**Andrea Gelemanovic** (MedILS, Split, Croatia)

Andrea Gelemanović[a,e], Tinka Vidović[a,e], Miroslav Radman[a,b,c,d,e], Katarina Trajković[a,b,e]

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Although primary cancer can affect any part of the body, there is a striking variability in cancer prevalence across different organs. Moreover, metastases are non-randomly distributed among organs, with lung, liver, lymph nodes and bone acting as metastatic hotspots. The reasons for such inter-organ variability in the prevalence of primary cancers and metastases are insufficiently understood. In this *in silico* study we show that susceptibility of organs to a primary cancer or a metastasis is linked with their distinctive intrinsic features in the healthy state. In particular, while susceptibility of an organ to primary cancer is associated with the abundance of arterial endothelial cells and atypical gene expression, susceptibility to metastases correlates with high content of immune cells and high expression of immune genes. These data shed light on some fundamental aspects of cancer biology and pave new avenues for mitigation of cancer.

Gelemanovic A, Vidovic T, Radman M, Trajkovic K. On intrinsic susceptibility of healthy organs to primary cancer or metastasis. Research Square; 2023. DOI: 10.21203/rs.3.rs-2868510/v2.

## The Metabolite Path to Vitality: Detecting, Predicting, and Reversing Gut Inflammation

**Nika Pintar** (AniBiome, Zagreb, Croatia)

"The Metabolite Path to Vitality: Detecting, Predicting, and Reversing Gut Inflammation"

Nika Pintar, CEO of Ani Biome, Croatia

https://anibiome.ai/

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Functional gastrointestinal disorders have a profound global impact, indicating the need for exploration of the connection between nutrition, metabolism, and health. Starting from the gut, a Croatian startup Ani Biome initiated the mission to reverse gut inflammation, delivering non-invasive, personalized interventions.

Our groundbreaking approach draws inspiration from ancient biotechnology - fermentation. Through our proprietary multi-level fermentation method, we are developing a new frontier in biomedicine: Metabolites as Medicine. Comprehensive analysis of AgeBiotics' bioactive compounds, powered by artificial intelligence, enables us to predict their influence on human metabolic pathways. AgeBiotics, classified as food, are subjected to a comprehensive scientific approach that elevates their optimization for use as biological interventions. By using an *in vitro* leaky gut model, AgeBiotics show promise in restoring gut barrier integrity and enhancing anti-inflammatory responses. Another integral component of Ani Biome is our mobile app, facilitating non-invasive assessment of gut inflammation and its vitality-related impacts. With a seamless 1-minute daily journey, we longitudinally track psychobiological changes and optimize the product by using proprietary algorithms, in-house machine learning models, and the digital gut model.

Ani Biome mission is clear: to identify, predict, and reverse inflammatory bowel disease, ultimately optimizing vitality and propelling scientific progress. Join us in the Aniverse, an area of innovation and transformation, as we create solutions for a healthier tomorrow.

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## A structure-guided approach to target the Aurora-A/N-Myc complex in *MYCN*-amplified neuroblastoma

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The overexpression of the MYCN oncogene, resulting in an excess of the protein product N-Myc, is a major negative prognostic marker in neuroblastoma (NB) and frequently correlates with poor clinical outcomes. N-Myc is stabilized by its association with the kinase Aurora-A, required for the growth of MYCN-driven NB cells, but largely dispensable for cells lacking MYCN amplification. The physical interaction with Aurora-A sequesters N-Myc from proteolytic degradation, resulting in its accumulation. Since targeting the undruggable N-Myc is a strong, unmet need in pediatric cancer therapy, the destabilization of the Aurora-A/N-Myc complex represents a valid approach to reduce N-Myc cellular levels. Recently, compounds hailed as conformation disrupting (CD) inhibitors stand out as an alternative therapeutic strategy in NB treatment. Among them, PHA-680626 was identified by our research group as the most effective CD inhibitor of the Aurora-A/N-Myc complex in cultured cells [1], suggesting the dependence of its activity on MYCN-amplification status.
Large-scale dose-response experiments have been performed on a panel of cellular models including both non-cancerous and transformed cell lines differentially expressing MYCN, with the aim of ruling-out possible cell specific effects. Results obtained so far demonstrate that PHA-680626 selectively affects the vital parameters of MYCN-amplified cells, whereas it does not seem effective either on non-MYCN amplified and non-transformed cell lines. These outcomes, together with our additional findings on the molecular mechanism of PHA-680626, strongly hint that the marked cytotoxicity displayed in MYCN-amplified cell lines is indeed dependent on the Aurora-A/N-Myc complex disruption. The encouraging biological behavior exhibited by PHA-680626, combined with its favorable drug-likeness, hold the promise of a valuable addition to the repertoire of the pharmacological tools to fight high-risk NB. If successful, the innovative strategy of disrupting the “Unholy Matrimony" between N-Myc and Aurora-A will result in the identification of a leading compound ready to enter more extensive studies, laying the groundwork to rigorously investigate PHA- 680626 in clinical trials.

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https://doi.org/10.3390/ijms222313122

# A Yap-dependent mechanoregulatory program sustains cell migration for embryo axis assembly

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The assembly of the embryo's primary axis is a fundamental landmark for the establishment of the vertebrate body plan. Although the morphogenetic movements directing cell convergence towards the midline have been described extensively, little is known on how gastrulating cells interpret mechanical cues. Yap proteins are well-known transcriptional mechanotransducers, yet their role in gastrulation remains elusive. Here we show that the double knockout of yap and its paralog yap1b in medaka results in an axis assembly failure, due to reduced displacement and migratory persistence in mutant cells. Accordingly, we identified genes involved in cytoskeletal organization and cell-ECM adhesion as potentially direct Yap targets. Dynamic analysis of live sensors and downstream targets reveal that Yap is acting in migratory cells, promoting cortical actin and focal adhesions recruitment. Our results indicate that Yap coordinates a mechanoregulatory program to sustain intracellular tension and maintain the directed cell migration for embryo axis development.

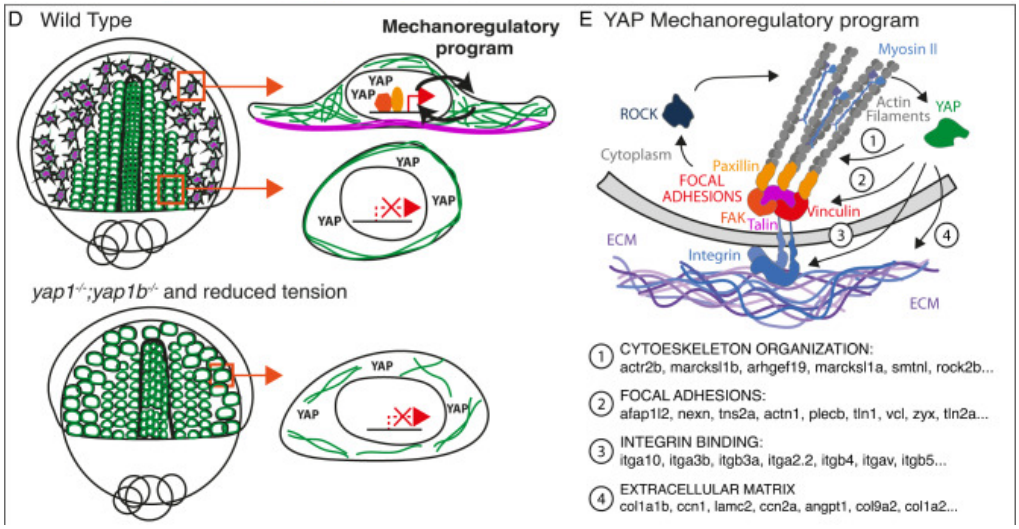


**Figure 1.** Summarizing scheme representing the differences between medial and lateral migrating cells converging to the midline in WT, yap1−/−;yap1b−/− and reduced tension embryos. Main components of the Yap-dependent transcriptional program encode for proteins that provide a link between the ECM and the actin cytoskeleton.

# Posters

## Healthy cells accelerate death of oxidatively damaged cells by blocking their transcriptional response to oxidative damage

**Jelena Budimir** (*Mediterranean Institute for Life Sciences, Split, Croatia)*

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The current research efforts to delay aging and age-related diseases mostly target cell autonomous mechanisms such as oxidative stress and DNA damage. In the recent years, non-cell autonomous mechanisms regulating health and longevity are emerging as promising alternatives[1]. This inspired us to develop a novel concept of cellular parabiosis[2] – phenotypic suppression of aberrant phenotypes in damaged cells or clearing the cell populations from the excessively damaged cells by means of molecular exchange with their healthy neighbours.

Here, we investigate the impact of healthy cells on the phenotype of adjacent damaged cells in a mammalian cell culture model. Specifically, we evaluated the number of oxidatively damaged cells treated with UV light grown in coculture with healthy cells and compared it with the number of the damaged cells in monoculture. Surprisingly, we observed that the number of cells treated with UV significantly decreased when they were cocultured with healthy cells. This “assisted suicide”[3] effect of the healthy cells depended on soluble factors present in the medium conditioned on the healthy cells. To gain deeper understanding of the mechanism, we performed transcriptional analysis of the damaged cells in coculture and monoculture. While the UV treatment induced major transcriptional reprogramming in the cells grown in monoculture, cells in coculture retained the transcriptional signature of the healthy state. In conclusion, we propose a model that healthy cells accelerate death of aberrant cells by a contact-independent mechanism that presumably disables transcriptional response to oxidative damage. Such mechanism could protect cells from the toxic effects of their dying neighbours, ultimately preserving the fitness of the tissue.

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# Oxime blot: a novel method for reliable and sensitive detection of carbonylated proteins in diverse biological systems

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Oxidative stress and the ensuing oxidative protein damage occur in various biological processes and diseases. The carbonyl group on amino acid side chains is the most widely used protein oxidation biomarker. Carbonyl groups are commonly detected indirectly, through their reaction with 2,4-dinitrophenylhydrazine (DNPH) under low pH conditions and further labeling with an anti-DNP antibody. However, the DNPH immunoblotting method lacks protocol standardization, exhibits technical bias, and has low reliability. To overcome these shortcomings, we have developed a new blotting method in which the carbonyl group reacts with the biotin-aminooxy probe to form a chemically stable oxime bond. The reaction speed and the extent of the carbonyl group derivatization are increased by a p-phenylenediamine (pPDA) catalyst under neutral pH conditions. This protocol improvement is crucial because it ensures that the carbonyl derivatization step reaches a reaction plateau and increases the sensitivity and robustness of protein carbonyl detection. Furthermore, derivatization under pH-neutral conditions ensures a good SDS-PAGE protein migration pattern and minimizes protein loss while being compatible with protein immunoprecipitation. This work describes the new Oxime blot method and demonstrates its use in the detection of protein carbonylation in complex matrices from diverse biological samples.

Ladouce R*, Combes, G.F.*, et al. Oxime blot: A novel method for reliable and sensitive detection of carbonylated proteins in diverse biological systems. Redox Biology (2023). https://doi.org/10.1016/j.redox.2023.102743

## Biochemical and functional characterization of proteins relatively resistant to carbonylation

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Protecting proteins from carbonylation is a promising strategy for attenuating aging and delaying the onset of age-related diseases. To get insight into natural protection mechanisms against protein carbonylation, we seek to identify and characterize proteins that are intrinsically more resistant to carbonylation. To that end, bacterial and mammalian cell lysates were exposed to oxidative damage by UV radiation, followed by tagging of the carbonylated proteins by aminooxy-biotin and their subsequent removal by streptavidin beads. The remaining, non-carbonylated proteins were then identified by mass spectrometry (MS) and analyzed using bioinformatics approaches. Gene ontology enrichment analysis of the relatively resistant proteins revealed their involvement in biosynthetic, metabolic, and ribosome-related processes. In addition, they have smaller molecular weight and show an enrichment of proteins that are part of a protein complex. These data shed light on the protein-intrinsic features that protect them from carbonylation and reveal cellular functions that evolved as the most shielded from oxidative damage.

## Intact cells as catalysts for the recovery of dying neighboring cells

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Due to the extended human lifespan in recent decades, age-related diseases have become a significant societal concern. Thus, efforts to postpone or slow down aging and age-related diseases have been intensified in life science research. During degenerative age-related diseases, cells die at variable rates, leading to heterogeneous cell viability status within the cell populations. How the presence of healthy cells affects the phenotype of the dying cells is insufficiently understood.
The concept of cellular parabiosis postulates that aberrant phenotypes in damaged cells can be suppressed by a healthy population of cells by means of intercellular communication. In this study, we investigated how apoptotic cells behave in co-culture with intact cells compared to the apoptotic cells in monoculture. We found that intact cells support the recovery of apoptotic cells. Subsequently, we explored the potential role of tunneling nanotube-mediated intercellular communication in this recovery process. Our findings emphasize the role of non-cell autonomous factors, in particular of the surrounding viable cells, in the control of cellular damage and death.

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# Oxidation modulates aggregation and membrane interaction properties of superoxide dismutase 1

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Amyotrophic lateral sclerosis (ALS) is an age-related neurodegenerative disease characterized by the progressive loss of motor neurons that control voluntary movement. Genetic studies revealed the involvement of superoxide dismutase 1 enzyme (SOD1) in the pathophysiology of the disease. Both wild-type and mutant forms of SOD1 can gain toxic function, which is reflected in their aggregation and cytotoxicity in ALS patients. However, it remains unclear how aging contributes to this toxic gain-of-function in SOD1. Since oxidative damage to proteins accumulates with age, in this study we investigated how oxidation contributes to SOD1 toxicity. Thus, we analyzed the impact of oxidation on SOD1 aggregation properties and cellular behaviour, with the focus on its interactions with the lipid membranes. We found that oxidized SOD1 displayed slower aggregation, yielding smaller aggregates with less □-sheets and more oligomers in comparison with the non-oxidized SOD1. Moreover, externally applied oxidized SOD1 aggregates were more toxic in NSC34 motor neuron-like cell line and more efficient in permeabilizing artificial lipid vesicles. This was accompanied with stronger interactions of oxidized SOD1 aggregates with the lipid membranes *in vitro*. In conclusion, oxidation modulates SOD1 aggregation and thus increases its toxic interactions with the membranes and cellular toxicity. These data shed new light on the role of age-related oxidative damage to proteins at the onset of ALS.

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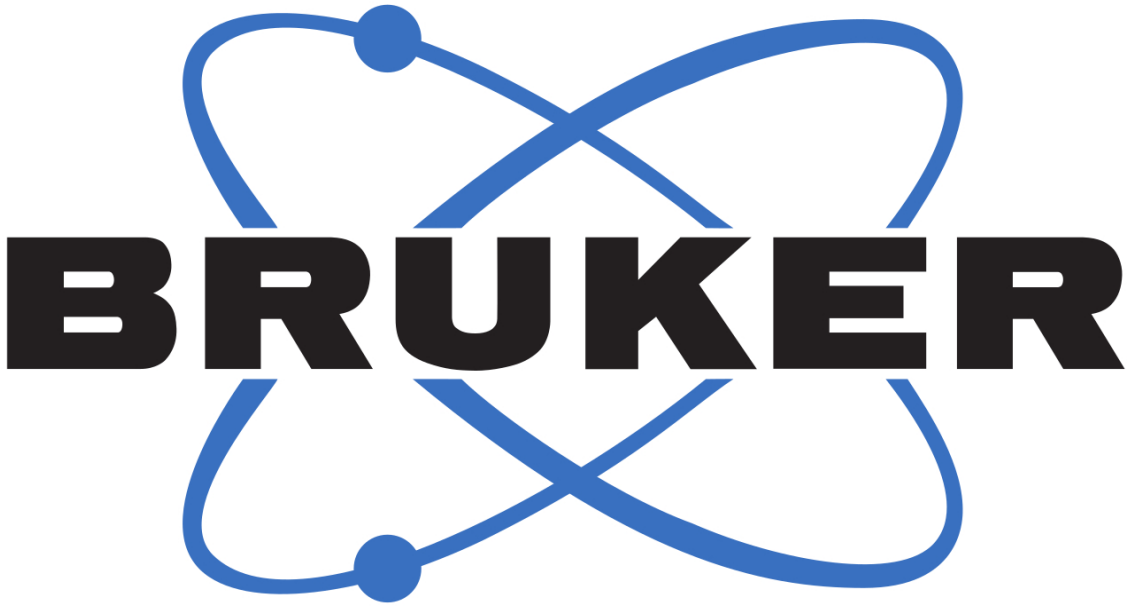


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