



INSTITUTE OF MOLECULAR AND  
TRANSLATIONAL MEDICINE

# IMTM 10<sup>TH</sup> ANNUAL IMTM RETREAT REACTOR

JULY 1-3, 2026

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Hotel Hluboký Dvůr, Hrubá Voda

ABSTRACT BOOK

# IMTM REACTOR

## **SCIENTIFIC COMITEE**

Marián Hajdúch, Petr Džubák, Tomáš Oždian

## **ORGANIZING COMITEE**

Tomáš Oždian, Denisa Pavelková

## **GRAPHIC DESIGN & TYPESETTING**

Denisa Pavelková

# PROGRAM

## Wednesday, June 1

10:35	Departure from Olomouc Train station (train M5/Os3543)
11:06	Arrival to Hrubá Voda

### CHAIR: JANA STRÁNSKÁ

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11:10	11:30	<b>Rastislav Slavkovský</b>	Age related clonal haematopoiesis and germline risk factors in healthy donors
11:30	11:50	<b>Lucie Kotková</b>	Bayesian and machine learning approaches in epigenetic age prediction and their correlations with clinical data
11:50	12:10	<b>Jiří Řehulka</b>	Discovery of novel microtubule targeting agents
12:00	13:00	LUNCH	

### CHAIR: SOŇA GURSKÁ

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13:00	13:20	<b>Alžběta Srovnalová</b>	Cell Painting-Based Phenotypic Profiling Reveals a Lysosome-Centered Mechanism of Piritramide Action
13:20	13:40	<b>Anna Šišková</b>	An Interactive R/Shiny Application for Morphological Profiling: From Cell Painting Quality Control to Compound Similarity Analysis
13:40	14:00	<b>Tatiana Mečiarová</b>	Identification of Senolytic Candidates and Assessment of a 5-Azacytidine Polymer as a DNA Demethylating Agent
14:00	14:20	<b>Lukáš Lenart</b>	Development of HiBiT-Based Cystic Fibrosis Cell Model Harboring Premature Termination Codon Mutation
14:20	14:40	<b>Matěj Šamaj</b>	A 53BP1-Based Live-Cell Reporter Platform for DNA Damage Response Profiling and Targeted Screening Applications
14:40	15:00	<b>Kateřina Burgetová Ječmeňová</b>	CELT-228: A high-affinity fluorescent tool for A3 adenosine receptor binding assays
15:00	15:30	COFFEE BREAK	

### CHAIR: ZDENĚK ŠKROTT

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15:30	15:50	<b>Martin Löffelmann</b>	Mitochondria-independent cuproptosis in cancer cells is associated with proteotoxic stress and the p97/NPL4 pathway
15:50	16:10	<b>Adam Kiška</b>	Subcellular and subsecond dynamic study of protein aggregates ubiquitination and heat shock response in live cells using plasmonic TiN nanolayer technology
16:10	16:30	<b>Matthew Lacey</b>	Preliminary exploration of DYRK1B as a senolytic target
16:30	16:50	<b>Martina Omachelová</b>	PARP trapping constrains BRCA1-selective therapeutic window across catalytically active PARP inhibitors
16:50	17:10	<b>Jiří Hodoň</b>	Diazirine conjugates with triterpenes to uncover its mechanism of action
17:10	17:20	COFFEE BREAK	

**CHAIR: TOMÁŠ OŽDIAN**

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17:20	17:40	<b>Julia Osińska</b>	Analysis of the localization and expression of aquaporin 11 (aqp11) in a model of chronic pancreatitis
17:40	18:00	<b>Martina Kintlová</b>	Mass spectrometry as a decision-making tool in small molecule screening
18:00	18:20	<b>Denisa Kroupová</b>	SureQuant™ applied in tear proteomics
18:00	18:15	<b>Jana Václavková</b>	Proteomic signatures in exhaled breath condensate predict paediatric asthma diagnosis and treatment response
18:40	19:00	<b>Miroslav Hruška</b>	Claire: Integrated Developments for Detection and Statistical Evaluation of Rare Peptides in Clinical Proteomics
19:00		<b>DINNER</b>	

**Thursday, June 2****CHAIR: MARTINA MEDVEDÍKOVÁ**

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9:00	9:20	<b>Jan Bouchal</b>	Introduction of prostate cancer group
9:20	9:40	<b>Juan Bautista de Sanctis</b>	CuEt Modulates Activation-Associated Signaling Pathways in CD8+ T Cells and NK Cells
9:40	10:00	<b>Zbyněk Nový</b>	The pros and cons of 161Tb-labelled monoclonal antibodies for cancer imaging and therapy
10:00	10:20	<b>Ihor Kozlov</b>	Identification of the Mutation Dependency of TAU Protein Aggregation Inhibition with Small Molecules Containing a Catechol Moiety
10:20	10:40	<b>Alexander Varečka</b>	Evaluation of NAC-Targeting Peptide Inhibitors Against Pathological $\alpha$ -Synuclein Seeding
10:40	11:00	<b>COFFEE BREAK</b>	

**CHAIR: JOSEF SROVNAL**

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11:10	11:30	<b>Jan Macháň</b>	Presenting PySPRESSO: A Python framework for LC-MS data processing and analysis
11:30	11:50	<b>Stanislav Tricolici</b>	De novo design of DYRK1b inhibitors
11:50	12:10	<b>Michaela Bendová</b>	Harmonizing National Cancer Screening Data in the Czech Republic Using the OMOP Common Data Model
12:10	12:30	<b>Riccardo Fusco</b>	Using acoustic droplet ejection and automated chemistry, we explored a kinase-oriented Groebke-Blackburn-Bienayme chemical space
12:00	12:15	<b>Imma Capriello</b>	The tumor suppressor p53 is mutated in more than half of human cancers
12:30	12:50	<b>Guzel Minibaeva</b>	Open-Source Docking-Guided Hit Discovery
12:45	13:00	<b>Pavel Stejskal</b>	Liquid Biopsy-Based Approaches for Cancer Profiling and Early Detection
13:00	14:00	<b>LUNCH</b>	

**CHAIR: JOSEF SROVNAL**

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14:00	14:20	<b>Barbora Neužilová</b>	Preclinical Comparison of Gallium-68- and Zirconium-89-Labeled Desferrioxamine B Analogues for Bacterial Infection Imaging
14:20	14:40	<b>Katarína Hajdúová</b>	Pretargeted SPECT/CT Imaging of PSMA-Positive LNCaP Tumours Using a DBCO-Based Click Chemistry Approach
14:40	15:00	<b>Dominik Vitek</b>	Eutardigrade <i>H. exemplaris</i> as a new model organism for high-throughput stress and ecotoxicology studies
15:00	15:20	<b>Jiří Voller</b>	NOVEL SELENOLES FOR TREATMENT OF RETINAL AND NEURODEGENERATIVE DISEASES
15:20	15:40	<b>Zeinab Saedi</b>	Therapeutic Targeting of Mutant NPM1 c+ in Acute Myeloid Leukemia
15:40	16:00	<b>Ermin Schadich</b>	High-throughput screening identifies novel antimycobacterial scaffolds
16:00	16:20	<b>Guzel Minibaeva</b>	Open-Source Docking-Guided Hit Discovery
19:00		<b>DINNER</b>	

**Friday, June 3****CHAIR: PETR PAVLIŠ**

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9:00	9:40	<b>Karel Macek</b>	Cybersecurity training
9:40	9:50	<b>Martin Szotkowski</b>	IMTM Helpdesk for dummies
9:50	10:00	<b>Petr Pavliš</b>	What you can do for IMTM and what IMTM offers
10:00	10:20	<b>Anna Sekyrová</b>	Quantification of Extracellular RNA from Plasma EVs
10:20	11:00	<b>COFFEE BREAK</b>	

**CHAIR: PRAVIN PATIL**

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11:00	11:20	<b>Anna Bartáková</b>	LC-MS/MS Profiling of Protein Corona Associated with Melamine Microplastics
11:20	11:40	<b>Elisabetta La Scola</b>	High-throughput experimentation to explore a novel reaction toward triazolopiperazines
11:40	12:00	<b>Kaoud Salama</b>	Multicomponent Access to Oxa- $\beta$ -Lactam Chemical Space and Its Emergence as a Covalent Modifier Scaffold
12:00	12:20	<b>Mayur Mukim</b>	A Split-Ugi-Oxo-Carboxylic Acid Manifold for Topology-Divergent Ring Synthesis
12:20	12:40	<b>Samatha Masineni</b>	Benzodiazepines (BZDs) represent a cornerstone in the pharmacological management
12:40	13:30	<b>LUNCH</b>	
13:51		<b>Train departure from Hrubá voda (M5/Os 3552)</b>	

# Age related clonal haematopoiesis and germline risk factors in healthy donors

*Rastislav Slavkovský, Barbora Kalousová, Pavla Kouřilová, Ondřej Blaták, Zuzana Rožánková, Jiří Drábek, Marián Hajdúch*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, the Czech Republic*

## Abstract

**Background:** Age related clonal haematopoiesis (CH) represents an early pre malignant state characterized by the expansion of hematopoietic stem cell clones carrying somatic mutations, most commonly in DNMT3A, TET2, and ASXL1. Although age is the strongest known risk factor, inter individual variability suggests a contribution of germline genetic factors. Healthy blood donors provide a unique population for studying early CH in the absence of clinical comorbidities.

**Methods:** We analysed peripheral blood samples from 1118 malignancy-free individuals aged 18–65 years. Somatic mutations were detected using a targeted sequencing panel covering recurrently mutated CH genes with a sensitivity of VAF  $\geq 0.5\%$ . Germline variants were obtained from parallel whole genome sequencing and imputed genotypes. Associations between CH status, age, and germline variation were evaluated using logistic regression adjusted for age and sex, with a specific focus on the TERT locus.

**Results:** CH was detected in 18,8 % of donors, with prevalence rising sharply after age 45. The most frequently mutated genes were DNMT3A (9,7%), TET2 (2,5%), Phip (1,1%) and ASXL1 (0,7%). Increasing age correlated with higher VAFs ( $p < 0.001$ ). We identified multiple germline variants within intronic regions of the TERT gene that were significantly associated with CH risk. Several of these intronic genotypes most notably [rs2736099: A allele, rs2853672: C allele, rs2735940: A allele]—showed strong effect sizes (OR  $> 1.6$ ,  $p < 0.05$ ). Carriers of these intronic TERT alleles exhibited higher CH prevalence independent of age. Cluster of intronic TERT variants represented prominent germline signal that supported a role for inherited telomere maintenance pathways in modulating early clonal expansion.

**Conclusions:** CH is detectable in a substantial proportion of healthy malignancy-free donors and increases markedly with age. Our findings highlight a cluster of intronic germline variants in the TERT gene as key determinants of CH susceptibility, suggesting that subtle regulatory variation within the TERT loci influences hematopoietic stem cell dynamics long before clinical disease emerges. Integrating germline information—particularly intronic TERT variation—may improve risk stratification and guide future mechanistic studies into telomere biology and clonal evolution.

## Acknowledgment

This work was funded by The Ministry of Education, Youth and Sports of the Czech Republic (LM2023067 NCLG) and Palacky University Olomouc (IGA LF UP 2026\_005).

# Bayesian and machine learning approaches in epigenetic age prediction and their correlations with clinical data

*Lucie Kotková, Jiří Drábek*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, the Czech Republic*

## Abstract

Using 1040 samples from the ENIGMA cohort, a new MethAge model was trained using a machine learning approach. The study aimed to compare the original Bayesian and new machine learning models' performance and to assess the influence of various clinical and lifestyle features (BMI, smoking status, reproductive health, etc.) on the prediction performance.

## Acknowledgment

This work was supported by EATRIS, the European infrastructure for translational medicine. This work was funded by The Ministry of Education, Youth and Sports of the Czech Republic (LX22NPO5102 EXCELES-NÚVR, LM2023033 BBMRI.cz – Síť českých biobank, CZ.02.1.01/0.0/0.0/16\_026/0008448 ACGT – Analýza českých genomů pro teranostiku) and Palacky University Olomouc (IGA LF UP 2026\_005).

# Cell Painting-Based Phenotypic Profiling Reveals a Lysosome-Centered Mechanism of Piritramide Action

*Srovnalova A., Polishchuk P., Siskova A., Dzubak P., Srovnal J., Jecmenova K., Stejskal P., Vahalikova M., Berta E. and Hajduch M.*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic*

## Abstract

### Background:

Piritramide is a synthetic opioid analgesic widely used for the management of postoperative pain. While piritramide exerts its analgesic effects predominantly through opioid receptor signalling, its potential off-target cellular activities remain poorly understood. We employed Cell Painting-based phenotypic profiling to investigate the cellular mechanism of action of piritramide beyond its canonical receptor activity.

### Methods:

Cell Painting data were analyzed to identify compounds with phenotypic profiles similar to piritramide. Lysosomal and autophagy-related phenotypes were subsequently investigated using LysoTracker staining, LAMP2A immunofluorescence, autophagy marker analysis (LC3 and p62), autophagic flux assays with bafilomycin A1, cathepsin processing studies, opioid receptor antagonist co-treatments, and patient-derived blood samples.

### Results:

Cell Painting analysis identified a cluster of compounds associated with lysosomal function and autophagy modulation that showed strong phenotypic similarity to piritramide. Consistent with this observation, piritramide induced a marked increase in LysoTracker-positive signal and lysosomal membrane marker LAMP2A. Accumulation of LC3-II and p62 indicated disruption of autophagy-lysosome homeostasis, while autophagic flux analysis suggested impaired lysosomal degradation. Furthermore, piritramide altered cathepsin processing, supporting lysosomal dysfunction. The lysosomal phenotype was not reproduced by morphine and was only minimally affected by opioid receptor antagonists, indicating that the observed effects are largely independent of classical opioid receptor signalling. Importantly, increased LAMP2A intensity was also observed in patient-derived blood samples following piritramide treatment, confirming the translational relevance of the findings.

### Conclusions:

Cell Painting-guided phenotypic profiling identified lysosomal perturbation as a previously unrecognized effect of piritramide. Our data support a lysosome-centered mechanism characterized by lysosomal stress, altered cathepsin processing, and impaired autophagy-lysosome function. These findings demonstrate the utility of unbiased phenotypic profiling for uncovering off-target drug activities and reveal a novel cellular response associated with piritramide exposure.

## Acknowledgment

This work was supported by the Technology agency of the Czech Republic project PERMED: T2BA (TN02000109). We also acknowledge the contributions from infrastructural projects CZ-OPENSURE (LM2023052), EATRIS-CZ (LM2023053) and IGA\_LF\_2026\_014.

# An Interactive R/Shiny Application for Morphological Profiling: From Cell Painting Quality Control to Compound Similarity Analysis

*Anna Šišková, Pavel Polishchuk, Alžběta Srovnalová, Petr Džubák*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University and University Hospital Olomouc, Czech Republic*

## Abstract

The Cell Painting assay generates rich morphological profiles, and careful quality control is essential before any biological interpretation. An R/Shiny application guides researchers through the full analysis workflow, from raw profiles to mechanism-of-action exploration.

Quality control includes well-level cell count filtering, plate-level inspection via per-channel intensity maps, Moran's I across all extracted features to flag spatial artifacts (with follow-up visualization on plate maps), and pairwise cosine similarity of positive control wells.

Once data passes QC, an interactive UMAP projection reveals batch effects across plates; batch correction is currently under development. Replicate reproducibility is then quantified per compound by the median pairwise cosine similarity of its replicates, with an adjustable threshold determining which compounds carry forward. Only compounds meeting that threshold proceed to the final stage: comparison of median morphological profiles within each dataset independently and between user-defined test and reference sets.

## Acknowledgment

This work was supported by the National Infrastructure for Chemical Biology, Ministry of Education, Youth and Sports of the Czech Republic (CZ-OPENSURE, LM2023052); the Technology Agency of the Czech Republic (Personalised Medicine: From Translational Research into Biomedical Applications, PerMed, TN02000109); and the Internal Grant Agency of Palacký University Olomouc (IGA LF UP 2026-14).

# Identification of Senolytic Candidates and Assessment of a 5-Azacytidine Polymer as a DNA Demethylating Agent

*Tatiana Mečiarová<sup>1</sup>, Martina Medvedíková<sup>1</sup>, Marián Hajdúch<sup>1,2</sup>*

<sup>1</sup>*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University hospital Olomouc, Czech Republic*

<sup>2</sup>*Institute of Molecular and Translational Medicine, Czech Advanced Technology and Research Institute, Palacky University Olomouc, Czech Republic*

## Abstract

Cellular senescence is a state of permanent cell cycle arrest in which cells lose their proliferative capacity while remaining metabolically active. Senescence is a natural hallmark of aging and serves as a protective mechanism of the organism against the propagation of damaged cells. However, the accumulation of senescent cells can lead to the development of chronic diseases. As a result, the identification of senolytic compounds that selectively target senescent cells has become a promising strategy for prevention and treatment of age-associated disorders. In this work we tested the cytotoxic effect of nine candidate senolytic compounds on multiple senescent cell lines to compare it with their non-senescent counterparts. We identified two compounds with senolytic effect in HT-29 cell line and one in HCT116 cell line.

In parallel, we focused on DNA methyltransferase inhibitory effects of a 5-azacytidine polymer. Aberrant DNA methylations are associated with multiple diseases, including cancer, making DNA methyltransferases (DNMTs) important therapeutic targets. 5-Azacytidine, a cytidine analog, inhibits DNMT activity by forming covalent complexes with these enzymes during DNA replication, resulting in DNA demethylation. We tested the activity of the 5-azacytidine polymer on a cell-based DNA demethylation detection system to compare its effects with those of the monomeric compound.

## Acknowledgment

This work was supported by the internal grant of Palacky University Olomouc IGA LF 2026\_005 and the National Institute for Cancer Research - EXCELES programme, project ID No. LX22NPO5102, funded by the European Union - Next Generation EU.

# Development of HiBiT-Based Cystic Fibrosis Cell Model Harboring Premature Termination Codon Mutation

*Lukáš Lenart*<sup>1</sup>; *Martin Ondra*<sup>1,2</sup>; *Danuta Radzioch*<sup>1,2,3</sup>; *Marián Hajdúch*<sup>1,4</sup>

<sup>1</sup> *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic*

<sup>2</sup> *Research Institute of the McGill University Health Centre, Montreal, Canada*

<sup>3</sup> *Faculty of Medicine and Health Sciences, McGill University, Montreal, Canada*

<sup>4</sup> *Czech Advanced Technology and Research Institute, Palacky University Olomouc, Czech Republic*

## Abstract

Cystic fibrosis (CF) is a progressive hereditary monogenic disease that severely affects lungs, pancreas and other organs. More than 2000 mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR) have been described to alter CFTR structure and function, ultimately resulting in CF. Due to a high prevalence of single mutation, a deletion of phenylalanine in position 508 of the CFTR gene ( $\Delta F508$ ), up to 85% of CF patients are eligible for treatment with CFTR modulators. However, individuals carrying rare CFTR mutations such as premature termination codons (PTCs) remain without effective therapeutic options. To address this unmet need, we optimized our CRISPR/Cas9-based genome-editing strategy to generate a monoclonal cell line, with endogenous expression of a HiBiT-tagged CFTR harbouring the clinically relevant G542X PTC mutation. Together with other HiBiT-based CF models developed by our group, this cell line will be utilized for high-throughput screening of compounds with potential CFTR-rescuing activity.

## Acknowledgment

This work was supported by the internal grant of Palacky University Olomouc IGA\_LF\_2026\_014; the Czech Ministry of Education, Youth, and Sports (EATRIS-CZ, LM2023053) and the Technology Agency of the Czech Republic (Personalised Medicine: From Translational Research into Biomedical Applications, TN02000109).

# A 53BP1-Based Live-Cell Reporter Platform for DNA Damage Response Profiling and Targeted Screening Applications

*Matej Šamaj<sup>1</sup>, Lukáš Lenart<sup>1</sup> and Marián Hajdúch<sup>1,2</sup>*

*<sup>1</sup>Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University and University hospital Olomouc, Czech Republic*

*<sup>2</sup>Institute of Molecular and Translational Medicine, Czech Advanced Technology and Research Institute, Palacký University Olomouc, Czech Republic*

## Abstract

DNA double-strand breaks trigger the rapid recruitment of 53BP1 to sites of damage, resulting in the formation of distinct nuclear foci. Utilizing a U2OS-53BP1-EYFP reporter cell line, this system enables real-time visualization of these foci dynamics over time. In this work, DNA damage was induced by X-ray irradiation and combined with reference DNA damage response (DDR) inhibitors to characterize changes in 53BP1 foci formation and resolution. The results demonstrate the utility of this system for investigating DNA repair mechanisms and support its sensitivity in detecting pathway-specific compound effects.

To expand the translational potential of this platform toward targeted radionuclide therapies, specific tumor targets (PSMA, CAIX, and FAP) were introduced into the 53BP1-EYFP reporter background. Following single-cell cloning, the resulting monoclonal derivative lines were successfully validated for target expression and verified to retain the baseline 53BP1 foci kinetics of the parental line. Current efforts are focused on the methodological optimization of functional radioligand uptake assays. Ultimately, these characterized cell models aim to serve as a robust tool for evaluating novel radiopharmaceuticals and radiosensitizers.

## Acknowledgment

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (European Infrastructure for Translational Medicine EATRIS-CZ-LM2023053); the Technology Agency of the Czech Republic (Personalised Medicine: From Translational Research into Biomedical Applications, TN02000109) and the internal grant of Palacký University Olomouc IGA\_LF\_2026\_014.

# CELT-228: A high-affinity fluorescent tool for A3 adenosine receptor binding assays

*Kateřina Burgetová Ječmeňová<sup>1</sup>, Alžběta Srovnalová<sup>1</sup>, Marián Hajdúch<sup>1</sup>, Petr Džubák<sup>1</sup>*

*<sup>1</sup>Institute of Molecular and Translational Medicine (IMTM), Faculty of Medicine and Dentistry, Palacký University and University Hospital in Olomouc, Hněvotínská 1333/5, CZ-779 00 Olomouc, Czech Republic*

## Abstract

The A3 adenosine receptor (A3AR) represents an important target in pharmacology, yet studying its behavior in living cells has long been constrained by the limitations of radioligand binding assays and the poor signal-to-background ratios of early fluorescent ligands. In this study, we evaluate CELT-228, a subtype-specific fluorescent probe conjugated to a Cy3B fluorophore, as a non-radioactive tool for live-cell imaging and competitive binding assays. Initial radioligand displacement studies confirmed that the fluorescent modification did not critically impair receptor affinity, yielding a  $K_i$  value of 52.7 nM. In confocal microscopy assays, CELT-228 demonstrated selective localization to the plasma membrane of A3AR expressing cells, showing no significant cross-reactivity with the A1AR subtype or wild-type CHO-K1 cells. Furthermore, the probe lacked the extensive intracellular accumulation observed with traditional xanthine amine congener (XAC) scaffolds, providing a highly defined membrane profile at a concentration of 5 nM. Utilizing this high-contrast signal, we established a quantitative high-content screening assay using SIMA software to evaluate a library of novel modulators from the ISVY and SY1-JA series. The assay accurately quantified ligand potencies across a broad dynamic range, identifying highly active compounds such as ISVY130 ( $IC_{50} = 0.376$  nM) and resolving picomolar displacement by the reference antagonist MRS1220 ( $IC_{50} =$

## Acknowledgment

This work was supported by the Czech Ministry of Education, Youth, and Sports (EATRIS-CZ, LM2023053), the European Union – Program EXCELES, ID Project No. LX22NPO5102 and the Technology Agency of the Czech Republic (Personalised Medicine: From Translational Research into Biomedical Applications, TN02000109).

# Mitochondria-independent cuproptosis in cancer cells is associated with proteotoxic stress and the p97/NPL4 pathway

*Martin Loffelmann<sup>1</sup>, Zdenek Skrott<sup>1</sup>, Tomas Pluhacek<sup>2</sup>, Petr Tarkowski<sup>3</sup>, Martin Mistrik<sup>1</sup>*

<sup>1</sup>Laboratory of Genome Integrity, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czechia

<sup>2</sup>Department of Analytical Chemistry, Faculty of Science, Palacky University Olomouc, Olomouc, Czechia

<sup>3</sup>Czech Advanced Technology and Research Institute (CATRIN), Palacky University Olomouc, Olomouc, Czechia

## Abstract

Copper is an essential inorganic element and serves as a cofactor for enzymes involved in metabolism or detoxification. However, excess copper has a detrimental effect on cancer cells and can trigger a recently discovered type of cell death - cuproptosis. Cuproptotic markers are strongly connected with mitochondria. The typical ones were reported as the oligomerization of lipoylated DLAT (dihydrolipoyl transacetylase) proteins, the loss of iron-sulphur cluster proteins, and mitochondrial-metabolism-dependent cells were reported to be more sensitive to this regulated type of cell death. We tested several copper ionophores in the form of their copper complexes, including bis(diethylthiocarbamate) (CuET), pyriithione, NSC319726, and 8-hydroxyquinoline, to assess their ability to induce cuproptosis. The cytotoxic effects of the ionophores were comparable in four cancer cell lines in an oxidative-phosphorylation-dependent state and in glycolysis-driven counterparts. The same comparable effects were observed after using inhibitors of mitochondrial complexes and in a model cell line, Rho0, which lacks mitochondrial DNA. However, we observed that the ionophores induce aggregation and immobilisation of the NPL4 protein (Nuclear protein localisation protein 4), which is a crucial cofactor of p97. NPL4 aggregation disrupts the p97/proteasome pathway, and its aggregation is correlated with cytotoxicity. The proteotoxic stress response was accompanied by the unfolded protein response, the heat shock response, and the accumulation of polyubiquitinated proteins. Furthermore, additional treatment with another non-toxic copper chelator, dibenzylthiocarbamate, or NSC319726 chelator alone, reversed both NPL4 aggregation and ionophore-induced cytotoxicity. These findings provide new information on the copper-dependent cell death mechanism, revealing a prominent proteotoxic component and NPL4 as the main target.

## Acknowledgment

This work was financially supported by the National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) – Funded by the European Union – Next Generation EU, EATRIS-CZ, BBMRI-CZ, the Palacky University Internal Grant Agency in Olomouc (IGA\_LF\_2026\_023), and the Czech Health Research Council (NW26-10-00426).

# Subcellular and subsecond dynamic study of protein aggregates ubiquitination and heat shock response in live cells using plasmonic TiN nanolayer technology

*Adam Kiska<sup>1,2</sup>, Thanh-Lam Bui<sup>3</sup>, Lucie Beresova<sup>1</sup>, Petr Müller<sup>4</sup>, Stepan Kment<sup>3</sup>, Martin Mistrik<sup>1</sup> & Zdenek Skrott<sup>1,2</sup>*

*<sup>1</sup>Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic*

*<sup>2</sup>Institute of Molecular and Translational Medicine Czech Advanced Technologies and Research Institute, Palacky University, Olomouc, Czech Republic.*

*<sup>3</sup>Regional Centre of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Olomouc, Czech Republic.*

*<sup>3</sup>Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*

*<sup>4</sup>Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic.*

## Abstract

Proteostasis, the balance between protein synthesis, folding, and degradation, is essential for cellular functions, as its dysregulation, leading to the accumulation of misfolded aggregated proteins, is a hallmark of cancer and neurodegenerative disorders. Despite its importance, how cells sense, manage, and recover from proteotoxic stress at the molecular and subcellular levels remains poorly understood due to lack of suitable methodological approaches. To overcome these challenges, we present a novel technology based on plasmonic titanium nitride nanolayer. Combined with confocal live-cell microscopy, this technology enables dose-defined microthermal stress within defined subcellular regions, allowing real-time observation of protein unfolding, aggregation, and recovery dynamics with sub-second and micron accuracy, providing unprecedented insights into the orchestration of the heat shock proteins and ubiquitin-proteasome system. Furthermore, molecular characterization using specific inhibitors and disease-relevant mutations demonstrates the versatility of our new technology for drug discovery and for elucidating the mechanistic impact of pathological protein variants.

## Acknowledgment

The study was supported by Palacky University Olomouc Young Researcher Grant (JG\_2023\_33) and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NP05102) - Funded by the European Union – Next Generation EU., and Large RI Project LM2018129 - Czech-Biolmaging).

# Preliminary exploration of DYRK1B as a senolytic target

*Matthew Lacey*<sup>(1,2)</sup>, *Martin Mistrík*<sup>(1)</sup>

<sup>(1)</sup> *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic*

<sup>(2)</sup> *Institute of Molecular and Translational Medicine Czech Advanced Technologies and Research Institute, Palacky University, 77147 Olomouc, Czech Republic.*

## Abstract

Cell Painting analysis revealed a cluster of senolytic compounds with autophagy inhibitory activity, AZ191 co-clustered within this group and was confirmed to demonstrate both autophagy inhibitory activity and preferential toxicity in senescent cells thus qualifying AZ191 as a senolytic. AZ191 is a known inhibitor of DYRK1B, which raises the possibility that DYRK1B may be a suitable molecular target for further senolytic discovery. To explore this possibility, AZ191 and additional DYRK1B inhibitors (AZ-Dyrk1B-33 and INDY) are being tested for senolytic activity across multiple cell lines where DYRK1B expression is also examined.

## Acknowledgment

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# PARP trapping constrains BRCA1-selective therapeutic window across catalytically active PARP inhibitors

*Martina Omachelová<sup>1</sup>, Iva Protivánková<sup>1</sup>, Adam Kiška<sup>1</sup>, Zuzana Machačová<sup>1</sup>, Anoop Kumar Yadav<sup>2</sup>, Hana Polasek-Sedlackova<sup>2</sup>, Zdeněk Škroť<sup>1</sup>, Martin Mistrík<sup>1</sup>, Pavel Moudrý<sup>1</sup>*

<sup>1</sup>*Laboratory of Genome Integrity, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University and University Hospital Olomouc, Olomouc, Czech Republic*

<sup>2</sup>*Department of Cell Biology and Epigenetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic*

## Abstract

Poly(ADP-ribose) polymerase inhibitors (PARPi) have transformed the treatment of homologous recombination-deficient cancers, yet their clinical utility remains constrained by dose-limiting toxicities and limited therapeutic window. PARP1 trapping is widely considered a key contributor to PARPi cytotoxicity, but whether trapping promotes therapeutically useful BRCA1-selective efficacy or instead contributes primarily to non-selective toxicity remains unresolved. Here, we systematically profiled a panel of clinically relevant and next-generation PARPi in isogenic BRCA1-proficient and BRCA1-deficient cellular models, integrating long-term viability, inhibition of cellular ADP-ribosylation and PARP1 trapping. Across inhibitors, catalytic inhibition was observed as a shared pharmacologic activity, but catalytic potency occupied a relatively compressed range and did not independently explain the BRCA1-selective therapeutic window. In contrast, PARP1 trapping varied widely across compounds and inversely associated with selectivity for BRCA1-deficient cells. Multivariable analysis identified trapping as a negative determinant of selectivity, consistent with a model in which trapping increases BRCA1-independent cytotoxicity and thereby narrows the therapeutic window. These findings separate PARPi cytotoxicity from synthetic-lethal selectivity and suggest that the pharmacologic feature most linked to cellular toxicity is not necessarily the feature that best preserves selective anticancer efficacy. Our data support a framework in which productive PARP inhibition is required for BRCA1-selective responses, whereas excessive PARP1 trapping represents a variable liability that constrains therapeutic window.

## Acknowledgment

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# Diazirine conjugates with triterpenes to uncover its mechanism of action

*Jiří Hodoň, Anna Ligasová, Karel Koberna, Petr Džubák, Marián Hajdúch, Milan Urban*

*Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*

## Abstract

Triterpenoids constitute a large class of natural compounds exhibiting diverse biological activities. Our research group focuses primarily on their cytotoxic effects. [1] Triterpenoid derivatives bearing pyridine and pyrazine moieties demonstrated significant cytotoxicity, with IC<sub>50</sub> values ranging from 0.5 to 1.5  $\mu$ M in leukemic cell lines (CCRF-CEM, K-562). Medoxomil-type prodrugs showed unexpectedly high and selective cytotoxicity toward K-562 cells, with IC<sub>50</sub> values of 26–43 nM. [2] However, the mechanism of action of these active derivatives remains unclear. To better understand their mode of action, identification of their molecular protein targets is essential. For this purpose, conjugates of active triterpenes equipped with a bifunctional linker containing a diazirine group and an alkyne moiety were synthesized, enabling photocrosslinking with interacting proteins. Biological experiments were subsequently performed, revealing specific interactions between the triterpenes and their protein targets. The synthesis and future research directions will also be discussed.

## Acknowledgment

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# Analysis of the localization and expression of aquaporin 11 (AQP11) in a model of chronic pancreatitis

*Julia Osińska*

*West Pomeranian University of Technology in Szczecin*

## Abstract

Chronic pancreatitis (CP) is a progressive inflammatory process leading to permanent histopathological changes, which impair the exocrine and endocrine functions of this organ. The aim of the present study was to identify and precisely localize aquaporin 11 (AQP11) in the pancreases of domestic pigs of the Polish Landrace breed (Polska Biała Zwistoucha, PBZ), as well as to analyze changes in the expression of this protein in animals from the control group and after intramuscular administration of cerulein. The study was conducted on domestic pigs of the PBZ breed (n = 10), which were divided into a control group (n = 5) and an experimental group (n = 5). Immunohistochemistry (IHC) was used to identify and analyze the expression of AQP11. In all examined animals, the presence of AQP11 was found in the cells of the islets of Langerhans and in the epithelium lining the pancreatic ducts. These are the first data of this kind in this area. To date, the occurrence of AQP11 has not been analyzed in the pancreas of other animal species or in humans. In animals from the experimental group, AQP11 expression within the pancreatic islets was lower. The obtained results allow the conclusion that lower expression of this protein may primarily contribute to disturbances in insulin secretion in the course of chronic pancreatitis. The presented data may contribute in the future to a better understanding of the course of CP and support the search for new therapeutic strategies.

Keywords: pancreas; water channels; immunohistochemistry; inflammation

# Mass spectrometry as a decision-making tool in small molecule screening

*Martina Kintlová<sup>1)</sup>, Zeinab Saedi<sup>1)</sup>, Tomáš Oždian<sup>1)</sup>, Guzel Minibaeva<sup>1)</sup>, Kaoud Salama<sup>2)</sup>, Petr Džubák<sup>1)</sup>, Marián Hajdúch<sup>1)</sup>, Alexander Dömling<sup>1), 2)</sup>*

<sup>1)</sup> *Laboratory of Experimental Medicine, Institute of molecular a translational medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic.*

<sup>2)</sup> *Innovative Chemistry Group, Regional Centre of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Czech Republic.*

## Abstract

Kinases remain essential therapeutic targets due to their central roles in cell signaling, proliferation, and disease-related regulatory pathways. In our current work, we use the SCIEX Echo® MS system for high-throughput, label-free screening of small-molecule inhibitors across an expanding kinase panel. The workflow has now been established for 26 enzymes, including newly implemented DYRK kinases, enabling rapid assessment of inhibitory activity and compound selectivity in a chromatography-free MS/MS format based on acoustic droplet ejection coupled to electrospray ionization.

Using this platform, recent screening efforts identified positive candidates for the CDK16/CycY complex. These compounds showed a degree of selectivity against other CDK kinases and will be further investigated to better understand their inhibitory profile and biological relevance.

Beyond kinase activity screening, mass spectrometry has also been implemented for routine evaluation of covalent ligand binding to peptide and protein targets. This workflow was applied to screening of covalent ligands against an NPM1-derived peptide, leading to the identification of several ligands from the tested library. The approach was further extended by MS-based localization of ligand attachment sites using electron activated dissociation fragmentation on the ZenoTOF 8600 system.

Together, these results demonstrate the value of mass spectrometry as a decision-making tool supporting compound prioritization, selectivity assessment, and deeper mechanistic characterization in early-stage small-molecule discovery workflows.

## Acknowledgment

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# SureQuant™ applied in tear proteomics

*Denisa Kroupová<sup>1</sup>, Tomáš Oždian<sup>1</sup>, Jana Václavková<sup>1</sup>, Pavla Kouřilová<sup>1</sup>, Petr Džubák<sup>1</sup> and Marian Hajdúch<sup>1</sup>*

*<sup>1</sup> Department of Proteomics, IMTM, Faculty of Medicine and Dentistry, Palacky University, Olomouc, the Czech Republic*

## Abstract

Tear fluid represents a promising, minimally invasive source of biomarkers that may reflect pathological processes associated with the central nervous system. This targeted proteomic study was conducted within the KardioVize cohort to investigate whether selected tear proteins could serve as potential biomarkers.

Tear samples from 180 individuals were collected separately from both eyes, resulting in 360 samples. The samples were randomized into 12 processing and measurement batches, with samples from the left and right eye of each participant included in the same batch. Each sample was analyzed in three technical replicates, resulting in a total of 1,080 LC–MS measurements. Selected peptides were quantified using the SureQuant™ targeted mass spectrometry method with stable isotope-labelled peptides as internal standards. Peptide abundance was evaluated using normalized light-to-heavy peptide ratios.

Several peptides showed significant age-associated differences, predominantly with higher normalized ratios in participants aged  $\geq 70$  years. Significant differences were also observed in the analysis combining age and cognitive status.

These preliminary findings demonstrate that age represents an important confounding factor in targeted tear proteomic studies. No definitive candidate biomarker of cognitive impairment was identified at this stage.

## Acknowledgment

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# Proteomic signatures in exhaled breath condensate predict paediatric asthma diagnosis and treatment response.

*Jana Václavková<sup>1)</sup>, František Kopřiva<sup>3)</sup>, Vendula Látalová<sup>3)</sup>, Pavla Kouřilová<sup>1)</sup>, Jana Vrbková<sup>1)</sup>, Tatiana Gvozdiaková<sup>2)</sup>, Marián Hajdúch<sup>1,2)</sup> and Petr Džubák<sup>1,2)</sup>*

*<sup>1)</sup>Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic*

*<sup>2)</sup>Laboratory of Experimental Medicine, University Hospital Olomouc, Czech Republic*

*<sup>3)</sup>Department of Paediatrics, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic*

## Abstract

Paediatric asthma is a complex disease with variable clinical presentations and treatment responses. This study analysed the proteomic profile of exhaled breath condensate (EBC) samples in children with asthma and healthy controls to identify potential biomarkers and investigate the associated molecular mechanisms. We discovered several differentially expressed proteins in asthmatic children compared to controls.

We performed high-resolution, gel-free shotgun proteomics of EBC collected at the time of diagnosis (prior to treatment initiation) from 124 children (62 patients with asthma, 62 healthy controls). Across whole cohort, we have identified 2421 proteins and of them, 1492 were quantified. We used univariate and multivariate statistical analysis to predict asthma and healthy condition and as well to stratify children for antileukotriene (LTRA) or inhaled corticosteroid (ICS) treatment.

This study underscores the potential of EBC proteomic analysis as a non-invasive diagnostic approach for paediatric asthma, shedding light on its molecular pathogenesis and treatment mechanisms. Future validation of these findings and exploration of the functional roles of these biomarkers must be done using targeted and sensitive analytical methods.

## Acknowledgment

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# Claire: Integrated Developments for Detection and Statistical Evaluation of Rare Peptides in Clinical Proteomics

*Miroslav Hruska, Marian Hajduch*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc*

## Abstract

Reliable detection of rare peptide species remains a major challenge in bottom-up proteomics, especially in large clinical datasets where candidate search spaces are extensive and spectra provide limited evidence. Here, we summarize recent developments in Claire, our cloud-enabled software framework for sensitive detection and evaluation of rare peptides. Claire was applied to large-scale patient proteomics data to analyze variant peptides, ORF-derived peptides, six-frame translation peptides, and alternative splicing peptides, often revealing significant differences in abundance among patient groups.

To support these analyses, Claire was extended with fast and flexible data loading and storage, enabling multi-database searches and integration of heterogeneous peptide classes. Several complementary detection strategies were developed, including target-decoy analysis, hierarchical search with class-level FDR control, and decoy-free prior-aware statistical evaluation of peptide-spectrum matches based on Extreme Value Theory. Additional advances include fragment-ion and immonium-ion analysis, database-free de novo reading of single amino acids, and second-layer statistical evaluation of rare peptide groups against reference peptide populations. Improvements in monoisotopic precursor selection and fragment-spectrum deconvolution further reduce search space size while improving fragment-level signal-to-noise ratio.

Together, these developments provide the basis for a more comprehensive Claire workflow for detecting rare peptides in complex clinical proteomics data. The remaining challenge is to integrate these components into a unified system capable of overcoming the statistical and computational barriers that currently limit robust rare peptide detection.

# CuEt Modulates Activation-Associated Signaling Pathways in CD8+ T Cells and NK Cells

*Juan Bautista De Sanctis, Viktor Valentini, Jenny Garmendia, Helena Besta-Smrčková, Hana Duchová, Marián Hajdúch*

<sup>1</sup> *Institute of Molecular and Translational Medicine; Palacký University Olomouc, Olomouc Czech Republic*

## Abstract

CuEt is a compound with potential anti-cancer properties that activates immune cells at nanomolar concentrations. However, the signaling pathways behind this effect are not fully understood. Notably, highly activated immune cells exhibit an exhaustion-like state (PD-1 expression), reducing their responsiveness to further stimulation. This study examined the impact of nanomolar CuEt and kinase and phosphatase inhibitors: (TPI-1, PTP-1B, PHS1) on naïve and memory CD8+ T cells and NK cells, analyzing key signaling components by Luminex (Milliplex®MAP 7-Plex Human T-Cell Receptor Magnetic Bead Kit (CREB, CD3ε, SYK, ERK/MAP-1/2, LCK, LAT, ZAP70)). The number of assays per condition was 3. Under unstimulated conditions, memory CD8+ T cells exhibited greater ERK1/2 activation than naïve cells (p:0,04), however naïve cells showed higher CREB (p:0,0001), LCK(p:0,0001) and LAT(p:0,025). After PHA stimulation, naïve cells showed higher signaling associated with CREB(p:0,0010) and SYK(p:0,0119), whereas memory cells exhibited greater involvement of ERK1/2(p:0,0045), LCK and LAT were higher as well although not statistically significant at p-value 0,1579 and 0,2841 respectively. Unstimulated NK cells showed higher SYK(p:0,0271) while lower ERK1/2(p:0,005), LCK(p:0,009) and ZAP(p:0,00005) when treated with CuEt compared to control. However when stimulated NK cells exhibited vastly greater activation in all measured targets: CREB(p:0,0018), CD3ε(p:0,0009), SYK(p:0,00000006), ERK1/2(p:0,00048), LCK(p:0,0017), LAT(p:0,000000023) and ZAP70(p:0,05). Overall, these findings indicate that CuEt triggers different immune signaling responses based on cell subtype and activation state.

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# The pros and cons of <sup>161</sup>Tb-labelled monoclonal antibodies for cancer imaging and therapy

Zbyněk Nový

*Institute of Molecular and Translational Medicine; Palacký University Olomouc, Olomouc Czech Republic*

## Abstract

The pros and cons of <sup>161</sup>Tb-labelled monoclonal antibodies for cancer imaging and therapy

Introduction: The monoclonal antibodies – „magic bullets“ – are explored as radiotracer for at least last 40 years. The idea of combing very specific targeting molecule with suitable radionuclide was and still is very attractive especially in the field of cancer imaging and therapy. But several drawbacks have appeared on the way to great new radiotracers.

Methods: We have performed preclinical biological tests mainly focused to *in vivo* methods. The *ex vivo* biodistribution studies in healthy and tumor-bearing mice were the first stage of the testing followed by SPECT/CT imaging with tumor-bearing animals. The last stage of the tracer evaluation was therapy efficacy study with tumor mice. The tested compounds included several different antibodies – pertuzumab, ramucirumab, bevacizumab and amivantamab, all of them labelled with terbium-161 and/or lutetium-177.

Results: All tested monoclonal antibodies shared certain biodistribution patterns – high liver and spleen uptake, surprisingly high kidney and lung uptake. The tumor uptake differed among the tested antibodies with pertuzumab being the most promising one. SPECT/CT imaging results were in good accordance with *ex vivo* biodistribution data, nevertheless they showed the promising potential just for <sup>161</sup>Tb-pertuzumab. The therapy efficacy study was still under investigation at the time of abstract submission.

Conclusions: Despite theoretical high expectations, the most of tested <sup>161</sup>Tb-antibodies failed to show promising properties for tumor imaging or therapy. The only exception was the case of <sup>161</sup>Tb-pertuzumab, which revealed very high tumor uptake and low accumulation in non-targeted organs like liver, spleen, kidneys. But further studies are needed to confirm its clinical potential.

## Acknowledgment

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# Identification of the Mutation Dependency of TAU Protein Aggregation Inhibition with Small Molecules Containing a Catechol Moiety

*Ihor Kozlov, Viswanath Das*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc*

## Abstract

Tauopathies are characterised by a different etiologies, including mutations in the MAPT gene, which contribute to abnormal tau aggregation and alter microtubule-binding ability. The hypothesis was that mutations associated with tauopathies cause structural rearrangements that expose the highly amyloidogenic VQIVYK motif (PHF6), thereby altering aggregation kinetics relative to wild-type (WT) tau protein. Consequently, it is hypothesised that anti-PHF6 compounds, acting as aggregation inhibitors, will demonstrate greater efficacy in preventing fibril elongation and seed spreading in mutant tau variants than in WT tau. The aim of the study was to test this hypothesis and assess the therapeutic potential of repurposed small molecules using representative cellular models.

To mimic and study the pathological factors that determine aggregate formation and seeding, a two-step differentiation protocol was developed for SH-SY5Y cells expressing inducible TauP301L-EGFP. Siding experiments were also conducted using these cells alongside Tau RD P301S biosensor cells. Catechol-O-methyltransferase inhibitors, such as tolcapone and entacapone, demonstrated significantly higher anti-aggregation activity against mutant tau P301S than against WT tau. These results support the hypothesis that mutation-induced exposure of the VQIVYK motif increases susceptibility to specific inhibitors.

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# Evaluation of NAC-Targeting Peptide Inhibitors Against Pathological $\alpha$ -Synuclein Seeding

*Alexander Varečka, Viswanath Das*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic*

## Abstract

Parkinson's disease and related synucleinopathies are characterized by pathological accumulation and prion-like propagation of misfolded alpha-synuclein ( $\alpha$ Syn). Seed-competent  $\alpha$ Syn species promote the conversion of native  $\alpha$ Syn into  $\beta$ -sheet-rich aggregates, contributing to progressive neuronal dysfunction. A central region involved in this process is the non-amyloid component (NAC) domain of  $\alpha$ Syn, which contains hydrophobic residues that support fibril nucleation, stabilization, and seeding competency. Therefore, targeting the NAC domain represents a rational strategy to interfere with pathological  $\alpha$ Syn aggregate on and seed propagation. This PhD project focuses on three previously developed SYNuclein PEptide inhibitors (SYNPEPi) that target hydrophobic residues within the NAC domain of  $\alpha$ Syn. The overall aim is to evaluate whether these NAC-targeting peptide inhibitors can suppress  $\alpha$ Syn aggregation in cellular models, reduce the seeding activity of patient-derived  $\alpha$ Syn seeds, and support further evaluation in synucleinopathy models.

At the current stage, the work focuses on establishing the experimental systems required for inhibitor evaluation. This includes optimizing differentiated SH-SY5Y neuron-like models of  $\alpha$ Syn pathology and establishing seeding conditions using preformed fibrils (PFFs). Cellular readouts include  $\alpha$ Syn aggregation, phosphorylated  $\alpha$ Syn accumulation, cell viability, and neuronal morphology following genetic  $\alpha$ Syn expression, exogenous  $\alpha$ Syn seed exposure, or rotenone-induced stress. In parallel, real-time quaking-induced conversion (RT-QuIC) is being adapted as a functional assay to measure  $\alpha$ Syn seeding activity using defined PFF-based controls. Once the cellular and RT-QuIC assay conditions are established, these systems will be used to evaluate whether SYNPEPi candidates reduce  $\alpha$ Syn aggregation and seeding activity. Subsequent work will extend this approach to  $\alpha$ Syn species enriched from patient-derived samples using immunoprecipitation (IP)-coupled RT-QuIC.

Together, this ongoing work will provide the experimental basis for testing whether NAC-targeting peptide inhibitors can reduce  $\alpha$ Syn aggregation and pathological seeding and will guide the next stage of SYNPEPi evaluation in synucleinopathy models.

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# Presenting PySPRESSO: A Python framework for LC–MS data processing and analysis

*Jan Macháň<sup>1</sup>, Vojtěch Sedláček<sup>2</sup>, Richard Masaří, Martin Trnečka<sup>2</sup>, Lukáš Najdekr<sup>1</sup>*

*<sup>1</sup> Institute of Molecular and Translational Medicine; Palacký University Olomouc; Hněvotínská 1333/5; Olomouc 779 00; Czech Republic*

*<sup>2</sup> Department of Computer Science; Faculty of Science; Palacký University Olomouc; 17. listopadu 12; Olomouc 779 00; Czech Republic*

## Abstract

PySPRESSO is an open-source Python framework for reproducible and automated processing of large-scale multi-batch LC–MS datasets. It brings together key steps of the analysis workflow, including data filtering, normalization, batch correction, statistical analysis, visualization, and report generation, in a modular and transparent environment.

The tool is designed to support standardized LC–MS data analysis while remaining flexible enough to adapt to different experimental designs. Because PySPRESSO runs offline, it is suitable for sensitive or large datasets that cannot be processed through web-based platforms.

In this presentation, I will introduce the main ideas behind PySPRESSO and demonstrate how its developing graphical user interface (GUI) can make LC–MS data processing more accessible. The goal of the GUI is to allow researchers to build and run analysis workflows in an intuitive way, without requiring programming experience, while preserving the flexibility and extensibility of the underlying Python framework.

# De novo design of DYRK1b inhibitors

*Stanislav Tricolici*

*Institute of Molecular and Translational Medicine, Czech Republic*

## Abstract

Dual-specificity tyrosine-phosphorylation–regulated kinase 1B (DYRK1b), a member of the DYRK family, is a key regulator of cellular quiescence and cell-cycle re-entry. By phosphorylating cell-cycle and pro-survival effectors, DYRK1b maintains cancer cells in a reversible, drug-tolerant G0 state and protects them from apoptosis, in part by suppressing reactive oxygen species. Its overexpression and dysregulation are linked to cancer-cell survival and resistance to chemotherapy and radiotherapy across rhabdomyosarcoma, pancreatic, ovarian, and osteosarcoma tumors. Preclinical studies show that DYRK1b inhibition forces quiescent tumor cells out of their protective G0 state and sensitizes tumors to chemotherapy and radiotherapy, highlighting DYRK1b as a promising therapeutic target.

To identify DYRK1b inhibitors, de novo structures were generated with the CReM-dock from a fragment library derived from the Enamine REAL collection of compounds, complemented by CReM-pharm (pharmacophore-guided seeding) and CReM-opt (genetic-algorithm optimization). Compounds were prioritized based on docking score (AutoDock Vina and gnina) and predicted hinge-region interactions. Gnina recovered more hinge-binding compounds than Vina and Morgan (ECFP4) fingerprints were used to assess the chemical novelty and diversity of the prioritized set. The most promising candidates will be evaluated using an absolute binding free-energy protocol to make a final selection of compounds for synthesis and experimental evaluation.

## Acknowledgment

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# Harmonizing National Cancer Screening Data in the Czech Republic Using the OMOP Common Data Model

*Michaela Bendová<sup>1</sup>, Petr Pavliš<sup>1</sup>, Branislav Siska<sup>1</sup>, Marián Hajdúch<sup>1</sup>, Zuzana Hlaváčková<sup>1</sup>*

*Institute of Molecular and Translational Medicine, Czech Republic*

## Abstract

Cancer remains a major public health burden in Europe, with over one million deaths annually, many of which are preventable through effective screening programs. However, the heterogeneity of data structures across national screening initiatives limits large-scale analysis and international collaboration.

This study presents the harmonization of data models from three Czech cancer screening programs—colorectal, cervical, and pilot lung cancer screening—into the OMOP Common Data Model. Source data models obtained from the national screening infrastructure were translated, standardized, and mapped using a combination of automated tools and expert validation.

A high level of harmonization was achieved, with 98% of parameters successfully mapped to standardized vocabularies. The remaining parameters were assigned to the closest higher-level concepts. The resulting harmonized datasets enable consistent, large-scale analyses and support interoperability across institutions and countries.

These findings demonstrate the feasibility of transforming heterogeneous national screening data into a unified framework, facilitating real-world evidence generation and strengthening international research collaboration in cancer prevention."

# Using acoustic droplet ejection and automated chemistry, we explored a kinase-oriented Groebke-Blackburn-Bienayme chemical space

*Riccardo Fusco*

<sup>1</sup> *Institute of Molecular and Translational Medicine, Olomouc, Czech Republic*

<sup>2</sup> *CATRIN, Czech Advanced Technology Research Institute Olomouc, Czech Republic*

## Abstract

Using acoustic droplet ejection and automated chemistry, we explored a kinase-oriented Groebke-Blackburn-Bienayme chemical space built around a fixed 2-aminopyrazine amidine scaffold. Since this scaffold strongly influences GBB feasibility, we used Design of Experiments to test whether the selected chemical space was synthetically reachable and to identify conditions giving analyzable products. The DoE varied catalyst identity and loading, reaction volume, dilution, and imine pre-formation across 245 nanoliter reactions, identifying suitable reaction conditions and a biology-compatible catalyst system.

After defining workable conditions, we executed the chemical-space search in two complementary plates. The first plate used Echo\_CherryPick as a purely random exploration of the enumerated product space; the second followed a more classical row/column logic to map building-block success more directly.

A future perspective is to extend this logic from chemical feasibility to biological relevance. By incorporating biological readouts into the same design framework, the experiment could begin to distinguish molecules that are merely produced efficiently from genuinely active hidden gems. The broader aim is a biology-aware chemical-space search, where synthetic accessibility and kinase activity guide the next experiment together.

# The tumor suppressor p53 is mutated in more than half of human cancers

*Imma Capriello*

*Institute of Molecular and Translational Medicine, Czech Republic*

## Abstract

The tumor suppressor p53 is mutated in more than half of human cancers, leading to loss of its protective functions and, in many cases, acquisition of oncogenic activities. Among the most prevalent structural mutants, p53-Y220C creates a surface cavity that destabilizes the protein while simultaneously providing a unique druggable pocket. This mutation has recently gained considerable attention following the clinical development of mutant-specific stabilizers such as Rezatapopt, which restore p53 function through selective binding to the Y220C cavity. Inspired by the therapeutic tractability of this mutant pocket, we investigated an alternative strategy based on targeted protein degradation. Rather than stabilizing mutant p53, we designed carbazole-based PROTACs (Proteolysis Targeting Chimeras) capable of engaging the Y220C cavity while recruiting E3 ubiquitin ligases to promote ubiquitination and proteasomal degradation of the mutant protein. This approach aims to eliminate both the dominant-negative and gain-of-function activities associated with mutant p53, providing a complementary route to restoring p53 pathway activity.

To accelerate degrader discovery, we synthesized a focused library of 48 carbazole-derived PROTACs using a direct-to-biology (D2B) workflow. This strategy enables rapid progression from synthesis to biological evaluation by testing crude reaction mixtures prior to purification, significantly shortening design–make–test cycles. Ongoing studies compare the activity of crude and purified compounds to evaluate the predictive value of early biological screening and to assess the robustness of the D2B platform for targeted protein degradation campaigns. Together, this work expands the therapeutic landscape of p53-Y220C from functional restoration to selective protein removal, highlighting carbazole-based PROTACs as a promising strategy for precision oncology and demonstrating the utility of accelerated discovery workflows for degrader development.

# Liquid Biopsy–Based Approaches for Cancer Profiling and Early Detection

*Pavel Stejskal<sup>1,2</sup>, Josef Srovnal<sup>1,2</sup>, Monika Vidlařová<sup>1</sup>, Alona Řehulková<sup>1,2</sup>, Marián Hajdúch<sup>1</sup>*

*<sup>1</sup>Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Czech Advanced Technology and Research Institute, Palacky University and University Hospital in Olomouc, Czech Republic*

*<sup>2</sup>University Hospital in Olomouc, Olomouc, Czech Republic*

## Abstract

Liquid biopsy (LB) has emerged as a powerful tool for non-invasive tumor characterization, enabled by advances in molecular biology, next-generation sequencing (NGS), and computational approaches. Unlike conventional tissue biopsy, LB allows repeated assessment of tumor-derived biomarkers circulating in body fluids, particularly peripheral blood. Key analytes include circulating tumor cells (CTCs), circulating tumor nucleic acids (ctDNA and ctRNA), and extracellular vesicles, which provide complementary information on tumor biology and disease progression.

The aim of this study is to contribute to the methodological standardization and broader applicability of LB through the integration of multiple biomarker classes. We demonstrate direct CTC detection using the CytoTrack CT11™ platform, enabling semi-automated immunofluorescent identification of CTCs from whole blood without prior enrichment. In parallel, we continue the optimization of workflows for circulating tumor nucleic acid analysis and molecular profiling.

Furthermore, we are introducing pilot NGS-based profiling of isolated CTCs in patients with glioblastoma multiforme to explore tumor heterogeneity and the feasibility of CTC genomic characterization. We are also implementing novel LB approaches for early cancer detection based on highly sensitive molecular assays and integrative biomarker analyses. Together, these activities aim to advance the clinical utility of liquid biopsy in precision oncology, treatment monitoring, and cancer early detection.

## Acknowledgment

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# Preclinical Comparison of Gallium-68- and Zirconium-89-Labeled Desferrioxamine B Analogues for Bacterial Infection Imaging

*Barbora Neužilová*

*Institute of Molecular and Translational Medicine, Olomouc, Czech Republic*

## Abstract

Desferrioxamine B (DFO-B) is widely used in nuclear medicine as a chelator for radiometals. In addition to its chelating properties, DFO-B acts as a siderophore that can be recognized by bacterial iron transport systems. This unique feature enables the transport of radiolabelled DFO-B into bacterial cells, making it a promising tracer for infection imaging. We have previously demonstrated the feasibility of PET/CT imaging of bacterial myositis using [68Ga]Ga-DFO-B and its analogues.[1] Furthermore, several DFO-B analogues have been developed with improved coordination properties for zirconium, providing stable complexes suitable for radiolabelling with zirconium-89. [2] In the present study, we investigated the biological behavior and infection-imaging potential of DFO-B analogues labelled with 89Zr and compared the results with those previously obtained using 68Ga-labelled compounds.

**Methods:** All DFO-B analogues were radiolabelled with zirconium-89 using HEPES buffer. For comparison, the corresponding compounds had previously been radiolabelled with gallium-68 and evaluated under analogous experimental conditions. Radiochemical purity was determined by RP-HPLC and iTLC.

The *in vitro* uptake of [89Zr]Zr-DFO-B analogues was evaluated in selected microbial cultures under various growth conditions. PET/CT imaging studies were performed in a mouse model of bacterial myositis using selected bacterial strains. Images were acquired at 5 h and 24 h post-injection.

**Results and Discussion:** All DFO-B analogues were successfully radiolabelled with 89Zr, achieving high radiochemical purity. High *in vitro* uptake of the tested [89Zr]Zr-DFO-B analogues was observed in selected strains of *Staphylococcus aureus* and *Streptococcus agalactiae*. These findings were confirmed by PET/CT imaging, which demonstrated pronounced tracer accumulation in the infected left hind limb.

Although the *in vitro* uptake in *Pseudomonas aeruginosa* was lower, a clear PET/CT signal was still detected at the site of infection. In contrast, infection induced by *Escherichia coli*, which showed negligible *in vitro* uptake, produced no detectable PET/CT signal in the corresponding hind limb.

Quantitative analysis of PET/CT images correlated well with the *in vitro* uptake data, demonstrating a relationship between bacterial tracer uptake and *in vivo* signal intensity. Furthermore, the [89Zr]Zr-DFO-B analogues exhibited excellent *in vivo* stability and favorable pharmacokinetic properties, characterized by rapid renal clearance and low background activity. PET/CT imaging revealed high and specific accumulation of the radiotracers in infections caused by *S. aureus*, *S. agalactiae*, and *P. aeruginosa*, resulting in high image contrast. Compared with the previously studied <sup>68</sup>Ga-labelled analogues, the longer half-life of <sup>89</sup>Zr enables imaging at later time points, including 24 h post-injection, while maintaining excellent visualization of the infection site.

**Conclusion:** This study demonstrated that all investigated [89Zr]Zr-DFO-B analogues possess promising *in vitro* and *in vivo* characteristics for bacterial infection imaging. Their high and specific uptake by selected bacterial strains was confirmed both *in vitro* and in a mouse model of infection. The results are consistent with previous findings obtained using [68Ga]Ga-DFO-B analogues, indicating that the DFO-B scaffold remains suitable for infection-targeted PET imaging regardless of the radionuclide used.

Moreover, the longer physical half-life of 89Zr allows high-quality imaging up to 24 hours after tracer administration, providing greater flexibility in imaging protocols and potentially enabling lower administered activities while maintaining diagnostic image quality.

## Acknowledgment

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# Pretargeted SPECT/CT Imaging of PSMA-Positive LNCaP Tumours Using a DBCO-Based Click Chemistry Approach

*Katarína Hajduová, Zbyněk Nový, Miloš Petřík, Martina Benešová-Schäfer, Martin Schäfer, Marián Hajdúch*

*Institute of Molecular and Translational Medicine; Palacký University Olomouc, Olomouc Czech Republic University and University Hospital*

## Abstract

**Background:** Pretargeting strategies based on biorthogonal click chemistry have emerged as a promising approach to improve tumour-to-background contrast while reducing radiation exposure to healthy tissues. Prostate-specific membrane antigen (PSMA) is highly expressed in prostate cancer and represents an attractive target for molecular imaging and/or therapy.

**Aim:** This study aims to evaluate the feasibility of a pretargeted imaging approach for PSMA-positive LNCaP tumours using a DBCO-functionalized PSMA ligand (Z1) and a non-binding negative control compound (Z2) labelled with lutetium-177.

**Methods:** Male mice bearing PSMA-positive LNCaP xenografts receive an intravenous pretreatment with a tetraacetylated aza-mannose (Ac4ManNAz) once a day for three days prior to radioligand administration to enable *in vivo* biorthogonal click chemistry. Control groups receive phosphate-buffered saline (PBS) instead of the tetraacetylated aza-mannose pretreatment. Subsequently, <sup>177</sup>Lu-labelled PSMA-DBCO (Z1) or the corresponding negative control compound lacking the PSMA binding motif (Z2) is administered intravenously. SPECT/CT imaging is performed at 1, 4, 24, and 48 hours post injection to assess tumour targeting, biodistribution, and clearance kinetics. Following the final imaging time point, selected organs, including tumour, kidneys, and liver, are collected and cryopreserved for subsequent histological analyses and assessment of radiation-induced DNA damage.

**Expected Outcomes:** We hypothesize that the PSMA-targeting ligand Z1 will demonstrate selective accumulation in LNCaP tumours, whereas the negative control compound Z2 will exhibit minimal tumour uptake. Furthermore, the tetraacetylated aza-mannose pretargeting strategy is expected to facilitate efficient *in vivo* click chemistry and improve tumour visualization while limiting off-target radiation exposure.

**Conclusion:** This study investigates a novel pretargeted PSMA imaging strategy combining biorthogonal click chemistry with <sup>177</sup>Lu-labelled ligands and multimodal analysis. The findings may contribute to the development of improved targeted imaging and therapy approaches for prostate cancer and provide a foundation for future theragnostic applications.

## Acknowledgment

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# Eutardigrade *H. exemplaris* as a new model organism for high-throughput stress and ecotoxicology studies

*Dominik Vítek*<sup>1</sup>, *Barbora Machová*<sup>2</sup>, *Patricie Neumanová*<sup>3</sup>, *Jitka Nováková*<sup>4</sup>, *Tadeáš Fryčák*<sup>5</sup>, *Marián Hajdúch*<sup>1</sup>, *Jiří Voller*<sup>1,4</sup>

<sup>1</sup> *Institute of Molecular and Translational Medicine, Czech Republic*

<sup>2</sup> *František Palacký Grammar School, Valašské Meziříčí, Czech Republic*

<sup>3</sup> *Jiří Wolker Grammar School, Prostějov, Czech Republic*

<sup>4</sup> *Department of Experimental Biology, Faculty of Science, Palacký University, Czech Republic*

<sup>5</sup> *Faculty of Electrical Engineering, Mathematics and Computer Science, Delft University of Technology, Mekelweg 4, 2628 CD Delft, The Netherlands*

## Abstract

Tardigrades are established model organisms in stress biology and are increasingly being explored in ecotoxicology. Their small size, stress tolerance, and suitability for laboratory culture make them attractive for scalable toxicity testing, but previous experiments have been limited by laborious manual scoring and thus by the number of conditions that can be compared.

Here, we used the laboratory-cultured eutardigrade *Hypsibius exemplaris* to develop a robust workflow combining automated low-magnification brightfield imaging in 384-well plates with deep-learning-based phenotype classification. The pipeline detects and classifies live and dead animals even in images with debris, algal remnants, illumination gradients, overlapping animals, well-edge effects, and toxicant precipitates, suggesting possible extension to poorly soluble or particulate materials such as nanoparticles, filaments, and microplastics. Applying this workflow to 5-day exposures to cadmium(II), mercury(II), and zinc(II) salts, we show that *H. exemplaris* is highly tolerant to metal-induced stress compared with other aquatic invertebrates. We also report toxicity data for a wider set of metals and metalloids, examine temperature dependence of toxic effects, and explore cross-modal stress responses. Finally, we share practical insights from our experience with tardigrade isolation, culture establishment and DNA barcoding.

## Acknowledgment

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# NOVEL SELENOLES FOR TREATMENT OF RETINAL AND NEURODEGENERATIVE DISEASES

JIRÍ VOLLER<sup>a,b</sup>, DOMINIKA TROFIMENKOVÁ<sup>a</sup>, JAKUB BIELCZYŃSKI<sup>c</sup>, DOMINIK VÍTEK<sup>v</sup>, BARTŁOMIEJ PIGULSKIC, MICHAELA MÚDRÁ<sup>a</sup>, IHOR KOZLOV<sup>B</sup>, VISWANATH DAS<sup>B</sup>, VLADIMÍR KRYŠTOF<sup>a</sup>, SŁAWOMIR SZAFERT<sup>C</sup>

<sup>a</sup>Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic

<sup>b</sup>Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 5, 77515 Olomouc, Czech Republic

<sup>c</sup>Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland

## Abstract

Organoselenium compounds are being explored as redox-active drugs for retinal and neurodegenerative diseases, including conditions associated with ferroptosis, oxidative stress, and pathological protein aggregation. However, most biological studies have focused on inorganic selenium compounds, selenium-containing amino acids or compounds with exocyclic selenium. Exploration of organoselenium heterocyclic scaffold is heavily focused on benzisoselenazoles such as clinical candidate ebselen. In contrast, unfused diselenoles but also related compounds (fused diselenoles and selenolanes) remain markedly underexplored, partly because facile synthetic routes to drug-like analogues have been limited. Here, we report the biological evaluation of novel disubstituted 3-methylene-3H-1,2-diselenoles, a scaffold for which only two examples had previously been prepared, serendipitously.

Ferroptosis is a regulated form of cell death driven by iron-dependent peroxidation of membrane lipids when glutathione-based antioxidant defences fail. In the retina, this process is especially relevant because retinal pigment epithelial cells and photoreceptors are exposed to high oxygen tension, light-induced oxidative stress, intense mitochondrial activity, and lipid-rich membrane turnover. Ferroptotic injury has been implicated in age-related macular degeneration, diabetic retinopathy and ischemia-reperfusion injury making ferroptosis an attractive target for retinal cytoprotection. We investigated a new series of organoselenium compounds for their ability to protect retinal pigment epithelial cells ARPE-19 exposed to diverse ferroptosis inducers. Several novel diselenoles ARPE-19 with EC<sub>50</sub> at tens-of-nanomolar concentrations, exceeding about 100x the activity of ebselen, a clinically studied organoselenium compound and GPx4-mimetic reference. Antioxidant and redox-modulating activities were assessed by ORAC, FRAP, DPPH, ABTS, and glutathione peroxidase-like activity assays. Although the compounds displayed stronger direct antioxidant capacity than ebselen, their activity did not exceed that of Trolox, suggesting that radical scavenging alone cannot fully explain their cellular protection.

Because organoselenium compounds may also modulate pathological protein aggregation, selected compounds were tested in tau aggregation assays. The compounds inhibited aggregation of tau R2R3 peptides, including the C291R mutant variant in micromolar concentrations, as shown by thioflavin T fluorescence kinetics. Atomic force microscopy further supported these findings by showing reduced fibril formation and altered aggregate morphology after compound treatment.

These findings identify 3-methylene-3H-1,2-diselenoles as potent modulators of ferroptotic stress in retinal pigment epithelial cells with ability to prevent aggregation of pathological proteins.

## Acknowledgment

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# Therapeutic Targeting of Mutant NPM1<sup>c+</sup> in Acute Myeloid Leukemia

*Zeinab Saedi<sup>1</sup>, Imma Capriello<sup>1</sup>, Riccardo Fusco<sup>1</sup>, Martina Kintlova<sup>1</sup>, Dora Ng<sup>2</sup>, Jan-Henrik Gerhartz<sup>3</sup>, Radoslaw Nowak<sup>3</sup>, Petr Dzubak<sup>1</sup>, Jan Kroenke<sup>2</sup>, Alexander Dömling\*<sup>1</sup>*

<sup>1</sup> *Institute of Molecular and Translational Medicine, Faculty of Medicine and dentistry, and Czech Advanced Technology and Research Institute, Palacky University, Olomouc, Czech Republic,*

<sup>2</sup> *Charité – Berlin University Medicine, Berlin, Germany,*

<sup>3</sup> *Institute of Structural Biology, University Clinics Bonn, Bonn, Germany.*

## Abstract

Mutations in nucleophosmin 1 (NPM1) are among the most frequent genetic alterations in acute myeloid leukemia (AML). The common W288C mutation disrupts the native  $\alpha$ -helical fold of the C-terminal domain (CTD), leading to loss of nucleolar localization signal (NoLS) function. Consequently, NPM1 undergoes aberrant cytoplasmic translocation, which is the characteristic molecular hallmark of NPM1-mutated AML. We hypothesized that covalent targeting of the mutant cysteine with electrophilic compounds could stabilize the disrupted CTD, restore NoLS function, and promote relocalization of mutant NPM1 to the nucleus. To test this hypothesis, a library of 1,234 acrylamide-based electrophiles was evaluated using a cell-based phenotypic assay that monitored the relocalization of mutant NPM1 from the cytoplasm to the nucleus. Phenotypic screening identified a single compound capable of inducing nuclear relocalization of mutant NPM1. However, subsequent biophysical analyses using circular dichroism spectroscopy showed no restoration of  $\alpha$ -helical structure within the NPM1 CTD. Furthermore, mass spectrometry (MS) and nuclear magnetic resonance (NMR) studies revealed neither covalent binding nor detectable non-covalent interaction with the target protein, indicating that the observed cellular activity was unlikely to result from direct target engagement or structural rescue. To determine whether active covalent binders had been missed by the phenotypic screen, the entire library was subsequently subjected to EchoMS-based screening. While the phenotypic assay yielded only a single apparent hit that lacked evidence of direct target engagement, the orthogonal covalent screening identified 22 covalent binders, including five compounds that exhibited dual-cysteine engagement, providing valuable starting points for further mechanistic and cellular investigations. These findings highlight the importance of integrating phenotypic and target-engagement approaches in covalent ligand discovery and establish a foundation for the development of chemical probes targeting mutant NPM1 in AML.

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# High-throughput screening identifies novel antimycobacterial scaffolds

*Ermin Schadich*<sup>(1)</sup>, *Soňa Gurská*<sup>(1,3)</sup>, *Kristina Krupova*<sup>(1,2)</sup>, *Pavlo Polishchuk*<sup>(1)</sup>, *Miroslav Soural*<sup>(1,2)</sup>, *Petr Džubák*<sup>(1,3)</sup>, *Marián Hajdúch*<sup>(1,3)</sup>

<sup>1</sup>*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic*

<sup>2</sup>*Department of Biotechnology, Palacký University, Olomouc, Czech Republic*

<sup>3</sup>*Czech Advanced Technology and Research Institute, Palacký University, Olomouc, Czech Republic*

## Abstract

Tuberculosis, a disease caused by the bacterium *Mycobacterium tuberculosis*, is a serious global concern and a leading cause of death from infectious diseases in the Post-COVID-19 era. According to the WHO, the global burden of *M. tuberculosis* infection is estimated at about one-quarter of the world's population, representing a reservoir of approximately 2 billion people (WHO, 2025). Our study was focused on assessing the *in vitro* antibacterial properties of the 8484 novel compounds from proprietary library against three different mycobacterial strains, *Mycobacterium bovis* substrain Russia and two different *Mycobacterium tuberculosis* strains, standard reference (H37Rv) and multidrug resistant (8666/2010) strain. The antimycobacterial activity of these compounds was identified in the high-throughput screening for growth inhibition of *M. bovis* substrain Russia and validated in dose response assays. The primary high-throughput screen showed that at the 50.0  $\mu\text{M}$  concentration, 271 compounds inhibited bacterial growth at the rate of  $\geq 50\%$ . These primary hits belong to the different chemical classes of compounds. The dose-response analysis showed that the 129 secondary hits had IC<sub>50</sub> values at concentrations smaller than 10.0  $\mu\text{M}$  concentration. Analyses of the cytotoxicity of these secondary hits showed that the 24 compounds were toxic to human BJ fibroblast or mouse J774 cells, and the 105 hits were considered for further analyses. Twenty eight compounds had the IC<sub>50</sub> values against intracellular *M. bovis* at a concentration smaller than the 10.0  $\mu\text{M}$  concentration. They were tested for bacterial growth inhibition of two of *M. tuberculosis* strains, a standard reference (H37Rv) and a multidrug resistant (8666/2010) strain. While 25 compounds had activity against the *M. tuberculosis* standard reference (H37Rv) strain, the 9 compounds were considered as the lead scaffolds with MIC value  $< 10.0 \mu\text{M}$  concentration. These scaffolds belong to modified three classes of compounds, modified nucleosides, thiazolidines and thiazoles. Eight of these leads were active against multidrug resistant (8666/2010) strain. Furthermore, the primary high-throughput screen of 1600 compounds from proprietary library for activity against parasite *Leishmania major* identified the 400 hits. However, in addition to modified nucleosides, thiazolidines and thiazoles, among these hits there are the significant number compounds from the other classes of compounds including triterpenoids. These analyses show the significant pharmacological profiles of compounds from proprietary library.

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# Open-Source Docking-Guided Hit Discovery

*Minibaeva Guzel, Polishchuk Pavel*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc*

## Abstract

There are two main *in silico* approaches to primary hit discovery: virtual screening and *de novo* design. Virtual screening is used to screen existing compounds or enumerated chemical structures. Modern combinatorial libraries contain millions to billions of compounds, and their large-scale screening has led to the discovery of novel and highly active molecules. In practice, however, there has been a lack of fully open-source and automated end-to-end docking pipelines capable of performing all ligand preparation and docking steps without manual intervention. Another limitation is that available computational resources restrict virtual screening to libraries of up to a few billion compounds, and even such libraries cover only a small fraction of the synthesizable chemical space. To explore broader regions of chemical space, *de novo* design methods can be used. These methods iteratively generate new molecules, often guided by docking, to identify promising structures.

To address the first challenge, we developed EasyDock, a docking tool that automates ligand preparation, integrates multiple docking engines, and supports distributed computing, enabling the screening of millions of structures. More recently, we replaced the commercial protonation module with open-source alternatives, making the entire docking pipeline accessible to a wider research community. The latest version of EasyDock was further redesigned to be modular and flexible, with a client-server architecture that facilitates the integration of additional docking engines.

To address the second challenge, we combined EasyDock with the previously developed structure generator CReM to create CReM-dock. This software iteratively grows fragments inside the receptor binding site to generate promising ligand structures. CReM-dock was evaluated in a CDK2 case study, where the potency of designed ligands was assessed using Absolute Binding Free Energy calculations. The computed binding energies of some designed compounds were in good agreement with those of known high-affinity ligands.

However, structures designed by CReM-dock are not necessarily synthetically accessible. To address this limitation, we proposed a pipeline in which *de novo* designed structures are used as templates for similarity searching in ultra-large chemical libraries. We validated this approach in a study aimed at identifying CDK16 inhibitors. Experimental evaluation of selected compounds showed that the proposed pipeline identified five hits, whereas virtual screening of a random subset of the same library retrieved only one.

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# Quantification of Extracellular RNA from Plasma EVs

<sup>1</sup>ANNA SEKYROVA, <sup>1</sup>PAVEL STEJSKAL, <sup>1</sup>JOSEF SROVNAL

<sup>1</sup>Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Czech Republic

## Abstract

**Introduction:** Extracellular vesicles (EVs) are important carriers of biological information, and the RNA they contain (EV-RNA) is considered a promising source of biomarkers for various pathological conditions. However, quantification of EV-RNA is methodologically challenging because RNA isolated from plasma is present at very low concentrations, and commonly used fluorometric and electrophoretic methods often do not provide reliable results. Another issue is the choice of an appropriate reference strategy for RT-qPCR, since classic housekeeping genes are not sufficiently stable or abundant in EV-RNA. The aim of this study was to establish a protocol for EV-RNA quantification using real-time quantitative PCR (RT-qPCR) with suitable reference genes.

**Methods:** EVs were isolated from the plasma of healthy donors using the commercial Norgen ExtraClean Plasma/Serum Exosome Purification and RNA Isolation Kit. The isolated EV-RNA was converted to cDNA by reverse transcription using the SuperScript IV First-Strand Synthesis System kit. Quantification was performed by RT-qPCR using PrimePCR SYBR Green Assay. EV-associated SNRPG, TOMM7, NOP10, and OST4 were tested as reference genes. DNA templates of these genes were used to construct standard curves. RT-qPCR and data analysis were performed on the CFX96 Real-Time PCR Detection System platform.

**Results:** RT-qPCR enabled reliable detection and quantification of EV-RNA even at very low input concentrations. For all tested reference genes, the standard curves were sufficiently linear and amplification efficiency was within the optimal range, confirming the suitability of the experimental design for absolute quantification. The tested EV-RNA samples isolated from plasma, as well as the reference cDNA, fell within the concentration range of the standard curves for all four genes, making it possible to calculate the copy number of individual transcripts per microliter of sample.

**Conclusion:** RT-qPCR using an absolute standard curve represents a sensitive and reliable approach for quantifying plasma EV-RNA even at very low RNA yield. The use of EV-specific reference genes also allows more accurate normalization and contributes to better reproducibility of EV-RNA biomarker analyses.

## Acknowledgment

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# LC-MS/MS Profiling of Protein Corona Associated with Melamine Microplastics

*Anna Bartáková<sup>1</sup>, Dušan Holub<sup>1</sup>, Petr Džubák<sup>1</sup>, Václav Ranc<sup>1</sup> and Marian Hajdúch<sup>1</sup>*

*<sup>1</sup> Department of Proteomics, IMTM, Faculty of Medicine and Dentistry, Palacký University, Olomouc, the Czech Republic*

## Abstract

Microplastics, pollutants found in the environment and in human tissues, may have implications for human health. Therefore, the study aims to investigate how the microplastic surfaces interact with human plasma and cellular proteins.

There are many types of microplastics according to their chemical composition. An example is melamine microplastics, which have been chosen for initial experiments. Melamine is widely used for the manufacture of kitchenware, furniture, etc. These products can release microplastic particles into the environment and enter the human body, where it may affect biological processes.

The initial experiments were focused on examining the plasma protein corona associated with melamine microplastics. For this purpose, the formation of the protein corona on the surface of the melamine microplastics was allowed via incubation of melamine microplastics in diluted plasma. The proteins were subsequently eluted from the melamine surface and processed for LC-MS/MS analysis. The results were compared with control samples.

On average, 383 proteins were identified in protein corona samples. After comparison with control samples, it was shown that on the melamine surface were selectively accumulated proteins, which were mainly involved in cell adhesion, extracellular matrix organization, coagulation, and immune-related processes, and prominently enriched proteins were those associated with complement activation, hemostasis, and the regulation of proteolytic cascades.

Plans of the study are to investigate protein coronas formed on the melamine surface for various time periods, protein coronas formed on the surface of other types of microplastics, and protein coronas formed on the microplastics surface in the

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# High-throughput experimentation to explore a novel reaction toward triazolopiperazines

*Elisabetta La Scola*

*Institute of Molecular and Translational Medicine*

## Abstract

HIGH-THROUGHPUT EXPERIMENTATION TO EXPLORE A NOVEL REACTION TOWARD TRIAZOLOPIPERAZINES  
Triazolopiperazines are privileged scaffolds in medicinal chemistry, combining the heteroaromatic features of triazoles with the sp<sup>3</sup>-rich piperazine ring. Their unique physicochemical properties, including a low calculated LogP and favorable hydrogen-bonding potential, make them ideal for the design of drug-like molecules. Despite their clinical relevance, exemplified by Sitagliptin and Fezolinetant, current synthetic routes rely on lengthy, metal-catalyzed procedures with limited scope and poor sustainability. We report a metal-free, one-pot multicomponent reaction (MCR) that provides modular access to highly substituted triazolopiperazines from readily available building blocks. The method introduces three orthogonal diversification points in a single operation, enabling rapid access to unprecedented substitution patterns, including spirocyclic and ring-expanded analogues, inaccessible by classical routes. The process avoids transition metals, operates under simple conditions, and it is compatible with downstream derivatizations, enabling fast library expansion. The route was also applied to a concise two-step synthesis of Fezolinetant, demonstrating direct industrial relevance. To accelerate optimization and explore the full potential of the reaction, the chemistry was translated into a high-throughput experimentation (HTE) framework. Parallel synthesis in 96-well plates enabled rapid exploration of substrate scope and reaction robustness, while preparative-scale resynthesis of representative hits provided full structural characterization. Reaction optimization was then driven by a three-stage Design of Experiments (DoE) strategy executed via acoustic droplet ejection (ECHO technology), enabling nanoscale parallel synthesis across 384-well plates. This integrated platform provided systematic, data-driven identification of key reaction parameters across hundreds of conditions simultaneously, with significant gains in speed, sustainability, and reaction space coverage. The optimized conditions were then applied to nanoscale synthesis in 1536-well plates, enabling the rapid exploration of an extensive chemical space and the generation of large, structurally diverse triazolopiperazine libraries.

# Multicomponent Access to Oxa- $\beta$ -Lactam Chemical Space and Its Emergence as a Covalent Modifier Scaffold

*Kaoud Salama*<sup>1,2</sup>, *Shabnam Zeinab Saedi*<sup>1,2</sup>, *Martina Kintlova*<sup>2</sup>, *Fiorella Meneghetti*<sup>3</sup>, *Matteo Mori*<sup>3</sup>, *Marian Hajduch*<sup>2</sup>, *Peter Džubák*<sup>2</sup>, *Alexander Dömling*<sup>1,2\*</sup>

<sup>1</sup>Regional Centre of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute (CATRIN), Palacký University Olomouc, Šlechtitelů 27, Olomouc, 783 71, Czech Republic

<sup>2</sup>Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University and University Hospital Olomouc, Olomouc, Czech Republic.

<sup>3</sup>Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche, XtaLab, Via L. Mangiagalli 25, 20133 Milano, Italy

## Abstract

Oxa- $\beta$ -lactams (1,2-oxazetidin-3-ones) constitute the formal oxygen analogues of  $\beta$ -lactams, yet their exploration has been hindered by the lack of general synthetic methods, with previous approaches largely limited to specialized cycloaddition and ring-contraction strategies. Here, we report a multicomponent approach in which the Ugi reaction is diverted from its classical pathway to selectively furnish oxa- $\beta$ -lactams under mild, room-temperature conditions from readily available carbonyl compounds and isocyanides.

To evaluate the scope of the transformation and identify productive substrate classes, automated high-throughput experimentation was employed to rapidly explore a broad and structurally diverse reaction space. The resulting structure–reactivity data enabled efficient identification of successful reaction combinations and guided the translation of selected hits to preparative-scale synthesis. A collection of structurally diverse oxa- $\beta$ -lactam derivatives was prepared and fully characterized, including by single-crystal X-ray diffraction.

Mechanistic investigations, including reaction monitoring and kinetic analysis, provided insight into the sequence of events leading to four-membered ring formation and support a multistep pathway distinct from conventional Ugi product formation. Structural analysis revealed significant distortion of the amide framework within the oxa- $\beta$ -lactam core, consistent with a strained and electronically perturbed heterocyclic scaffold. Preliminary covalent-fragment screening further suggests that this scaffold may provide access to a previously unexplored class of electrophilic heterocycles. Collectively, this work establishes a general entry into oxa- $\beta$ -lactam chemical space and highlights the potential of these underexplored motifs as valuable scaffolds for chemical biology and covalent ligand discovery.

## References

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# A Split-Ugi–Oxo-Carboxylic Acid Manifold for Topology-Divergent Ring Synthesis

*Mayur Mukim*

*Institute of Molecular and Translational Medicine, Czech Republic*

## Abstract

Here we report a new split-Ugi–oxo-carboxylic-acid reaction design in which bis-secondary amines and bifunctional oxo-carboxylic acids generate a common  $\alpha$ -adduct intermediate that gives topology-divergent access to conformationally rich N,O-heterocycles, including medium rings, macrocycles, and bicyclic products.

# Benzodiazepines (BZDs) represent a cornerstone in the pharmacological management

*Samatha Masineni*

*Institute of Molecular and Translational Medicine, Czech Republic*

## Abstract

Benzodiazepines (BZDs) represent a cornerstone in the pharmacological management of central nervous system (CNS) disorders, including anxiety, insomnia, and epilepsy. Acting as positive allosteric modulators of the GABAA receptor, BZDs enhance inhibitory neurotransmission by increasing chloride channel opening frequency<sup>1</sup>. Despite their high therapeutic efficacy and favorable safety profile compared to historical sedatives, their clinical utility is frequently limited by the development of tolerance, physical dependence, and cognitive impairment<sup>2,3</sup>. Despite their established clinical utility, the exploration of rigidified, bio isosteric derivatives in oncology remains a frontier for therapeutic development. In this study, we report a novel, one-step multicomponent reaction (MCR) for the efficient synthesis of benzodiazepine derivatives incorporating a strategic benzo[f]tetrazolo[1,5-a][1,4] diazepine modification. This high-throughput methodology was developed to be fully compatible with Echo acoustic liquid handling systems, enabling rapid, scalable compound generation with nanolitre precision and reduced reagent consumption.

The synthesized library exhibited sub micromolar potency, demonstrating superior anti-proliferative activity compared to the gold-standard BET inhibitor JQ1. The enhanced potency is attributed to the synergistic effects of the benzodiazepine scaffold and the tetrazole bio isostere, which likely optimize molecular interactions and improve metabolic stability. Overall, this work introduces a robust, automated synthetic platform and identifies a promising new class of anticancer candidates, establishing the tetrazolo-fused benzodiazepine framework as a high-value scaffold for future drug discovery.



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