Presentations of the Institute's Specialized Laboratories

06.02.2024 r.

Laboratory of Single Cell Analyses

Paulina Jackowiak

ICHB PAN





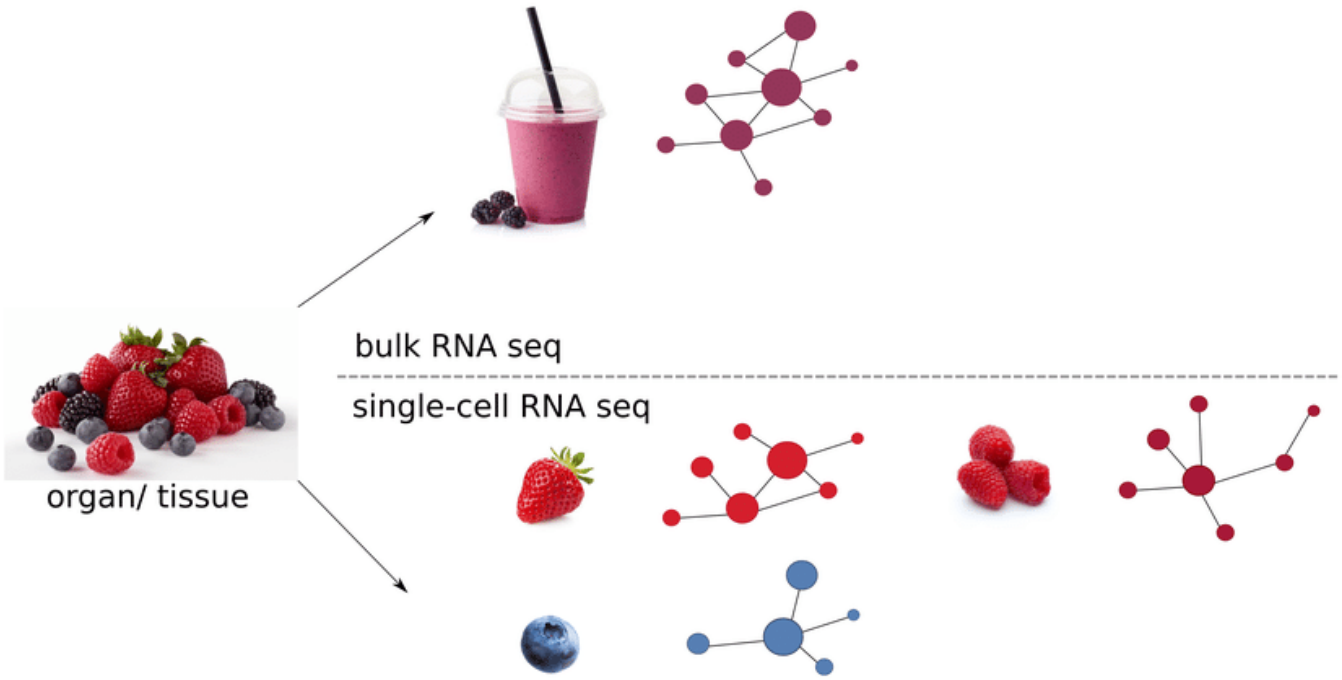

Dr. hab. Paulina Jackowiak Dr. Anna Samelak-Czajka Magdalena Trybus, MSc Dr. Eng. Małgorzata Marszałek-Zeńczak Annasha Dutta, MSc Anastasiia Zaremba, MSc

The infrastructure of the Laboratory is part of a platform for multidimensional imaging of biological processes, established within the framework of NEBI – National Imaging Centre for Biological and Biomedical Sciences.

We integrate experimental and computational approaches to comprehensively characterize diverse biological material at single cell resolution.

Why single cells?

Information obtained based on population average does not provide the whole picture of a sample.

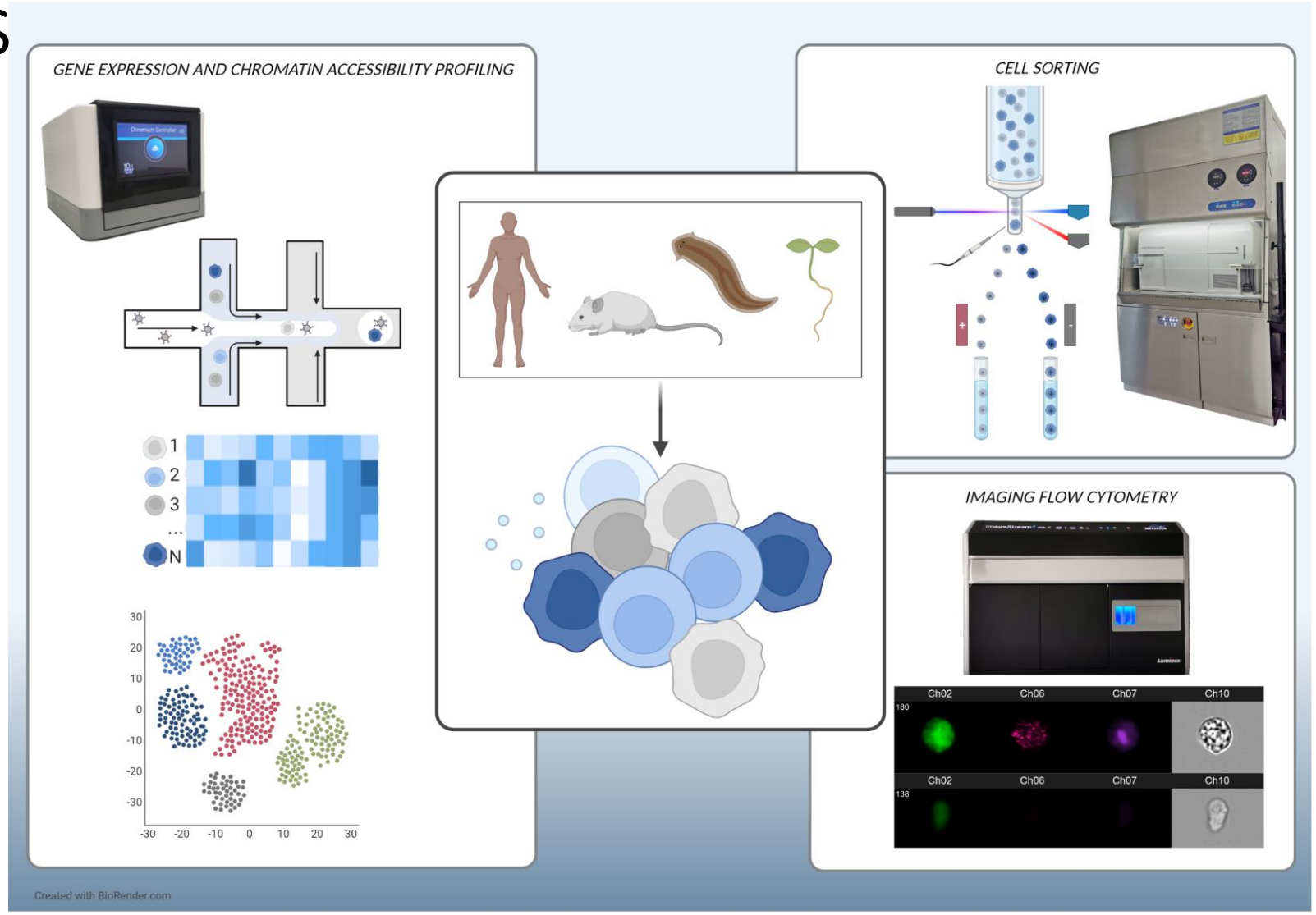


The advantages of taking research one cell at a time:

- identification of cell types and cell states
- identification of distinct subpopulations and differentiation trajectories
- rare cell detection
- exploration of tissue heterogeneity
- more...

Steinheuer et al. (2021). Benchmarking scRNA-seq imputation tools with respect to network inference highlights deficits in performance at high levels of sparsity. biorxiv preprint: 10.1101/2021.04.02.438193.

Our focus



- We offer gene expression and chromatin accessibility profiling, as well as cell sorting and advanced analyses using classical and imaging flow cytometry.
- We provide equipment for quantitative and qualitative analysis of nucleic acids.

Equipment, terms & conditions of use

Equipment available for self-use by trained researchers (the majority of our equipment)

Equipment operated by the Laboratory staff

- LSCA staff performs the experiment, according to the guidelines of the person who submits the samples
- LSCA staff performs the experiment and basic data analysis, according to the guidelines of the person who submits the samples
- LSCA staff performs the experiment, analyzes data and provides substantive intellectual contribution
  (consulting, planning of the experiment, compilation of results, analysis and interpretation of data, modification of the procedure, problem solving)

Terms and conditions for using the resources of the Laboratory of Single Cell Analyses

apply to research groups from IBCH PAS

Which method do you plan to use?

- ○ quantitative analysis of nucleic acids (Qubit 4)
- ○ real-time PCR (CFX96)
- ○ ddPCR (QX200)
- ○ quantitative and qualitative analysis of nucleic acids (TapeStation 4150)
- ◉ classical flow cytometry (Guava easyCyte 12HT; Guava Muse)
- ○ cell sorting (FACSAria Fusion)
- ○ imaging flow cytometry (Amnis ImageStreamX Mk II)
- ○ single cell omics (Chromium 10x Genomics)
- ○ other

How do you plan to use the facility?

- ○ by myself, I only want to use the equipment provided at the Laboratory
- ◉ with the help of the Laboratory staff

Define the scope of tasks outsourced to the Laboratory:

- ○ only performing the experiment, according to my guidelines (collecting flow cytometry data)
- ○ performing the experiment and basic analysis, according to my guidelines
- ○ performing the experiment and requiring substantive intellectual contribution from the Laboratory staff (planning of the experiment, compilation of results, analysis and interpretation of results, modification of the procedure, consulting, problem solving)

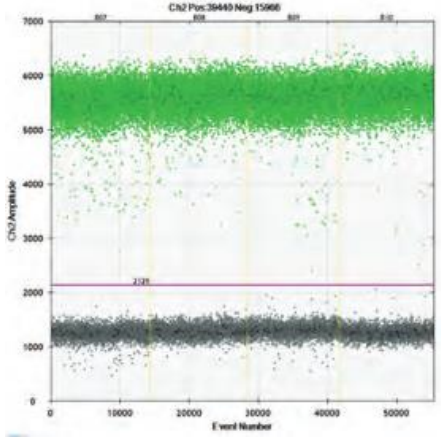
clear form

Quantitative and qualitative analysis of nucleic acids

The QX200 Droplet Digital PCR System provides absolute quantification of target DNA or RNA molecules for EvaGreen or TaqMan hydrolysis probe-based digital PCR applications.

https://www.bio-rad.com

book at: https://reservation.ibch.poznan.pl



Starting sample size, µl	20
QX200 droplet generator capacity	1–8 samples/cartridge
Droplets per 20 µl sample	20,000
QX200 droplet reader capacity	1–96 samples
Sample illumination	Light-emitting diodes
Sample detection	Multi-pixel photon counter
Detection channels	FAM (EvaGreen), HEX (VIC)
Linear dynamic range	5 orders of magnitude

https://www.bio-rad.com

The CFX96 is a precise and flexible, six-channel (five colors and one FRET channel) real-time PCR detection system.

book at: https://reservation.ibch.poznan.pl

https://www.thermofisher.com

The Qubit 4 enables fast and sensitive quantification of DNA, RNA and protein, as well as RNA IQ (integrity & quality) assay.

Quantitative and qualitative analysis of nucleic acids

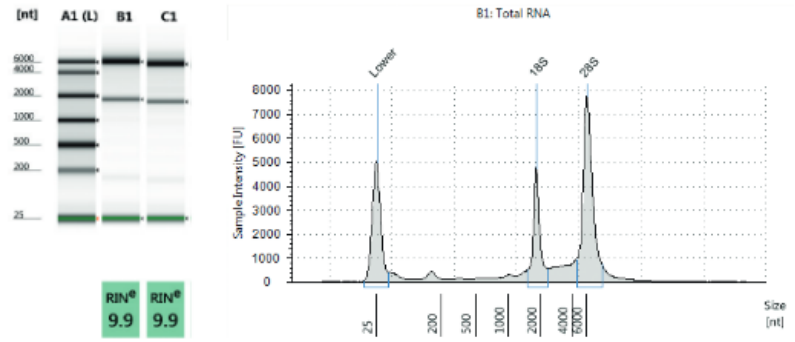
https://www.agilent.com

	RNA QC		DNA QC					
	RNA assay	High Sensitivity RNA assay	Genomic DNA assay	Cell-free DNA assay	D1000 assay	High Sensitivity D1000 assay	D5000 assay	High Sensitivity D5000 assay
Sizing range	100–6000 nt	100–6000 nt	200–60000 bp	50–800 bp	35–1000 bp	35–1000 bp	100–5000 bp	100–5000 bp
Quantitative range	25–500 ng/µL	500–10000 pg/µL	10–100 ng/µL	100–4000 pg/µL	0.1–50 ng/µL	10–1000 pg/µL	0.1–50 ng/µL	10–1000 pg/µL
Sample volume needed	1 µL	2 µL	1 µL	2 µL	1 µL	2 µL	1 µL	2 µL
Limit of detection	5 ng/µL	100 pg/µL	0.5 ng/µL	20 pg/µL	0.1 ng/µL	5 pg/µL	0.1 ng/µL	5 pg/µL
Integrity assessment	RNA integrity number equivalent (RIN^e)	RNA integrity number equivalent (RIN^e)	DNA Integrity Number (DIN)	%cfDNA				

The TapeStation 4150 enables RNA and DNA QC assays, that differ in sizing range and limit of detection. RNA and genomic DNA integrity can be measured.

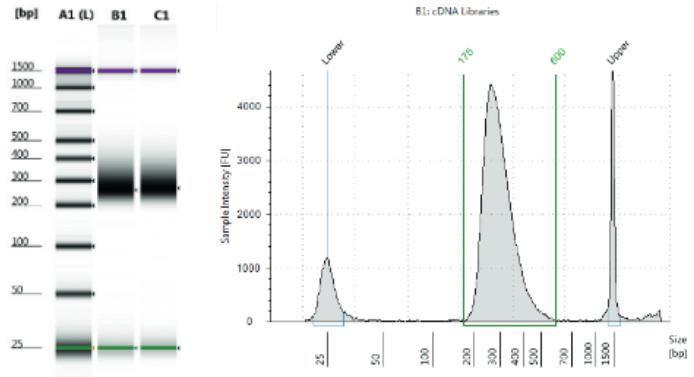
QC: RNA integrity

RNA ScreenTape assay



QC: Sizing (175 – 600 bp)

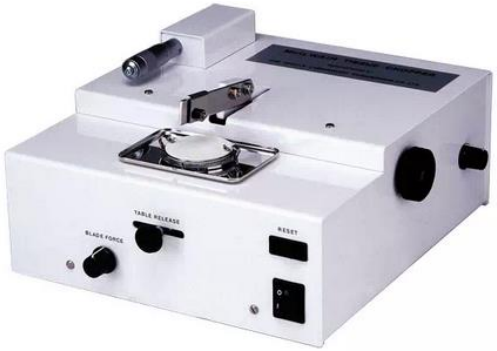
D1000 ScreenTape assay



QC: genomic DNA integrity assay available

Wish to use the equipment? Contact us via email at: lab.single.cell@ibch.poznan.pl

Tissue fragmentation and homogenization



The McIlwain Tissue Chopper enables rapid chopping (slicing) of tissue.

https://campdeninstruments.com

Capacity:

- from 1 to 3 x 2mL, 0.5mL tubes
- 1 x 7mL tube

Speed range:

- 3000, 4000 & 5000 rpm

Locking system:

- Manual (no screw-in system)

Cycles time:

- from 5s to 240s

The Minilys uses bead beating technology for tissue grinding, lysing and homogenization.

https://www.bertin-instruments.com

The gentleMACS Octo Dissociator with Heaters offers a fully automated workflow for tissue dissociation, using optimized built-in or user-defined programs for almost any biological material. All eight positions can be operated independently. Single cell suspensions or homogenates can be obtained.

https://www.pack-icpi.com/

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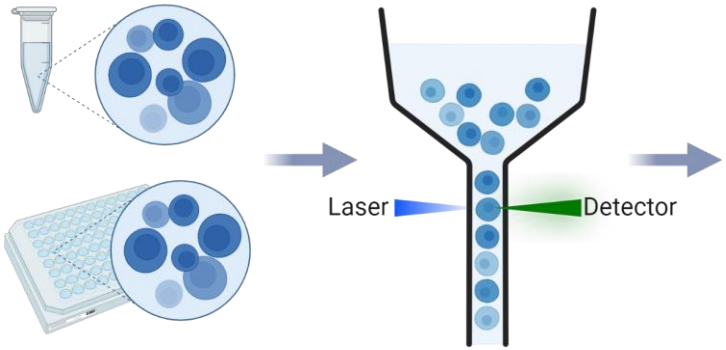
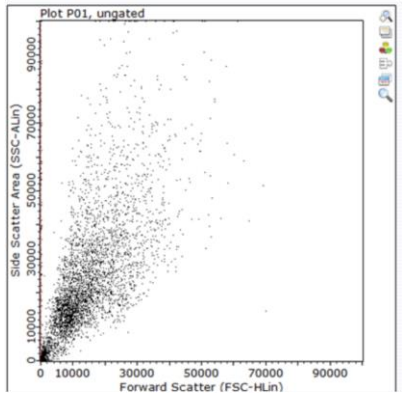
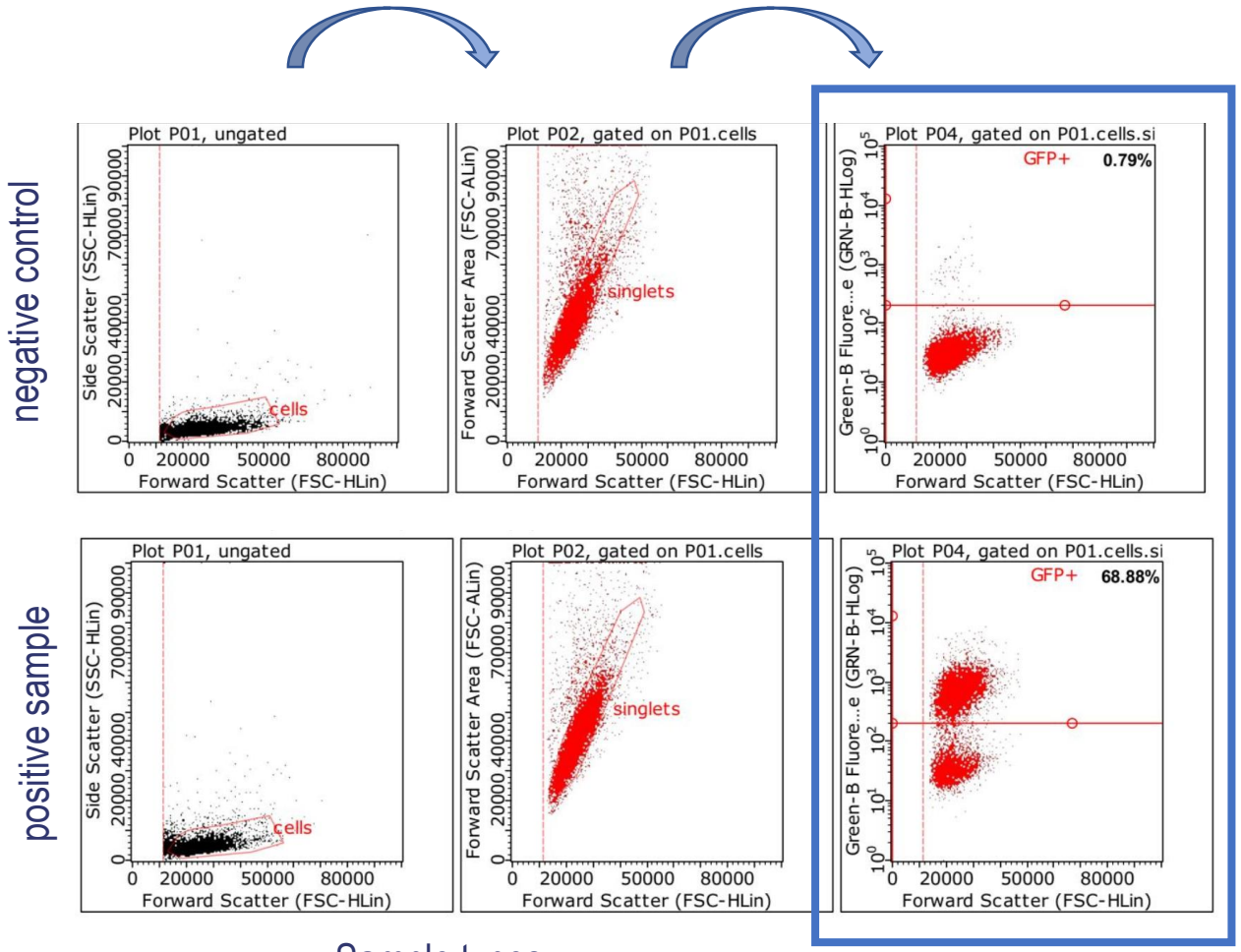
Flow cytometry





- Each dot on a plot represents a single event – usually a single cell.
- All parameters are calculated for each event.
- Statistical significance is achieved from rapid acquisition of thousands of cells.



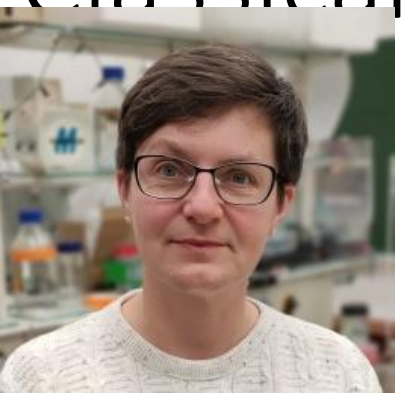
Applications include:

- protein detection, all assays that use fluorescent reporters
- cell viability, cell health (apoptosis, mitochondrial potential, oxidative stress, etc.)
- cell cycle
- cellular pathways (MAPK activation, caspase activation, autophagy)
- multiparametric characterization of diverse biological material
- more...

Sample types:

- eukaryotic cells
- prokaryotic cells
- nuclei

Classical flow cytometry



Magdalena Trybus, MSc

Cytek Guava easyCyte 12HT

- **405**/**488**/**642** nm laser configuration
- 13 detection channels
- sampling system compatible with 1.5 ml tubes and 96-well plates
- pre-defined assays for common applications (dedicated kits and software analysis templates)
- versatile platform for a broad range of cytometric analyses
- user-friendly operation

Cytek Guava Muse

- **532** nm laser
- 2 detection channels
- sampling system compatible with 1.5 ml tubes
- pre-defined assays for a variety of applications (dedicated kits and software analysis templates)
- open module for simple user-defined assays
- easy operation

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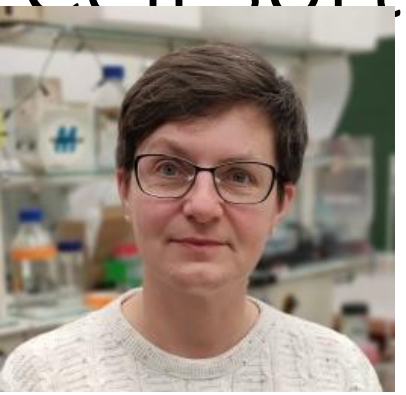
or book at: https://reservation.ibch.poznan.pl

Cell sorting



Magdalena Trybus, MSc

FACSAria Fusion

- 405/488/561/640 nm laser configuration
- integrated into a dedicated BSL-2 laminar flow cabinet
- 4-way sorting
- ACDU unit for directly sorting onto multiwell plates and microscope slides
- sort collection vessels: 1.5/2 ml tube, FACS tube, 15 ml tube, multiwell plates (up to 96 wells), microscope slides
- 70, 85, 100 & 130 µm nozzles – gentle sorting possible
- temperature control for pre-sort and post-sort samples available
- several sort precision modes, including single cell sorting and index sorting

sample types sorted thus far:

- cell lines
- primary cells from dissociated tissue (invertebrates)
- iPSCs
- nuclei (mouse cerebellum, plant root)
- protoplasts (plant root)

- no prokaryotic cells allowed!

Wish to use the equipment? Contact us via email at: lab.single.cell@ibch.poznan.pl

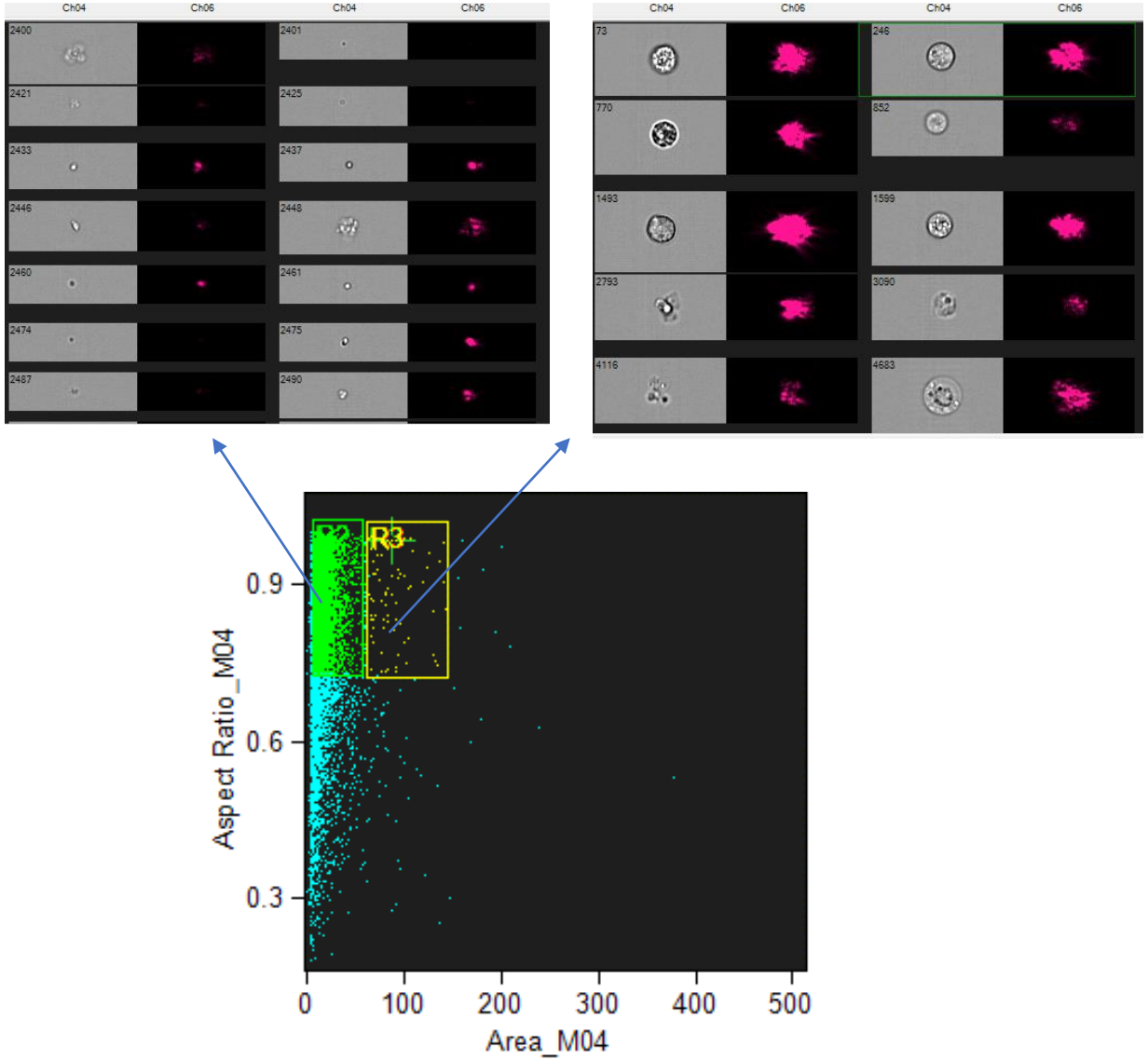
Imaging flow cytometry

Dr. Anna Samelak-Czajka

Cytek Amnis ImageStream^{X} Mk II

- **405**/**488**/**561**/**642** nm laser configuration
- 2 cameras, 12 detection channels
- multimag: 20x/40x/60x
- autosampler, EDF, High Gain mode

Combines high-throughput and multiparameter capabilities of conventional flow cytometry with morphological and spatial information from imaging at single-cell resolution.



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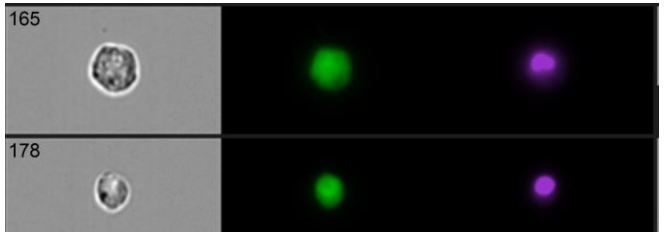
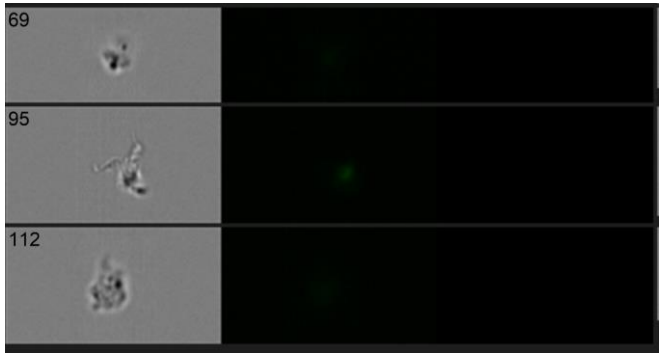
Tissue dissociation QC

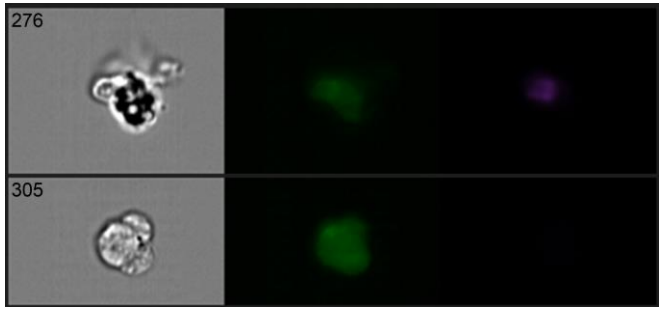
- intact cells



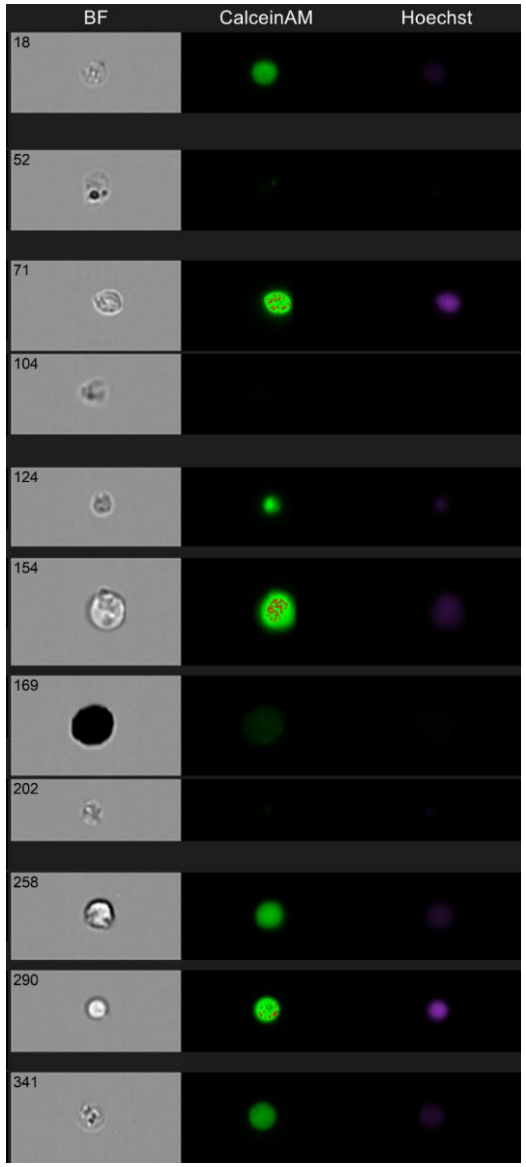
- cell debris, lysed cells



- cells sticking together with debris or small clumps of cells

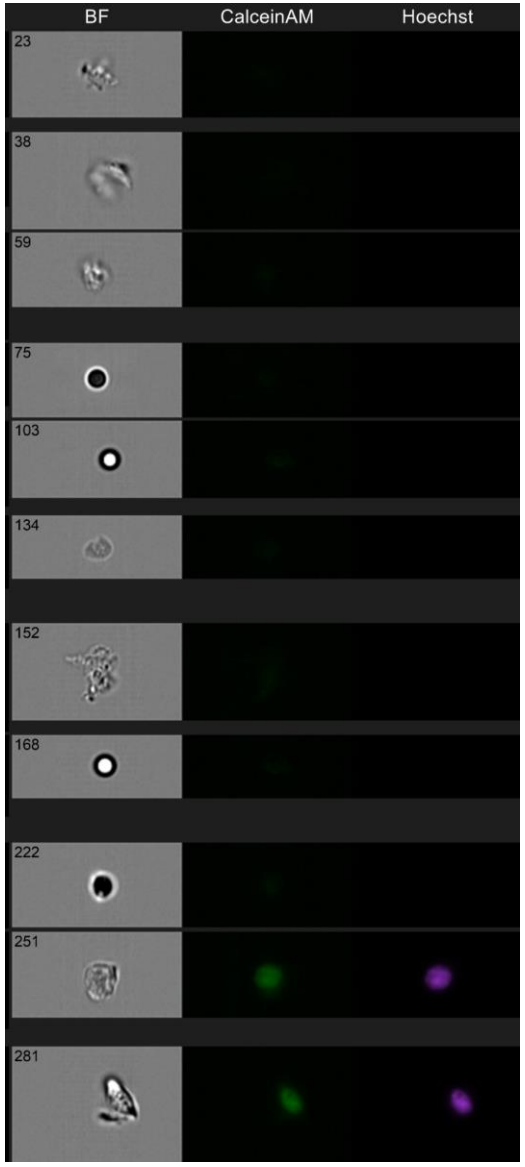


method 1



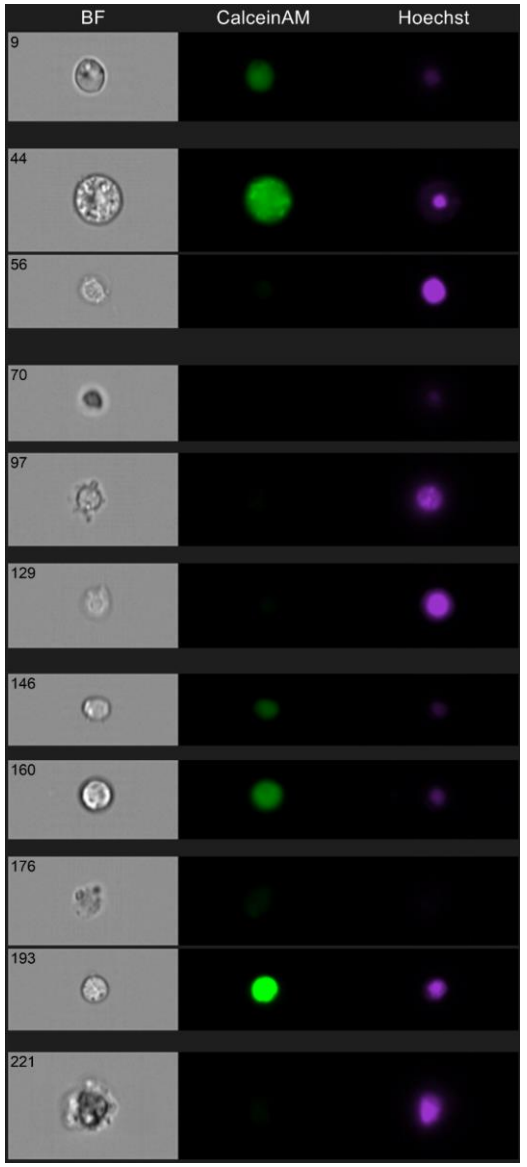
~ 40-50% viability

method 2



~ 40% viability

method 3

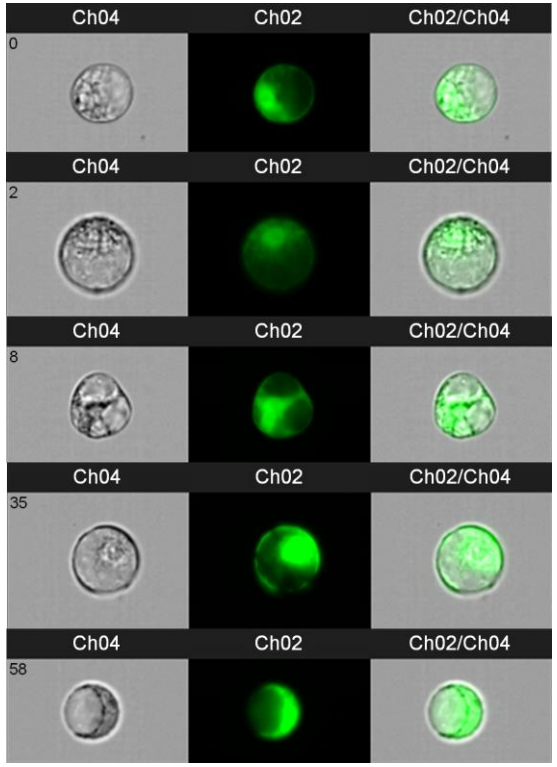


> 70% viability

Tracking intracellular localization of a fluorescent signal

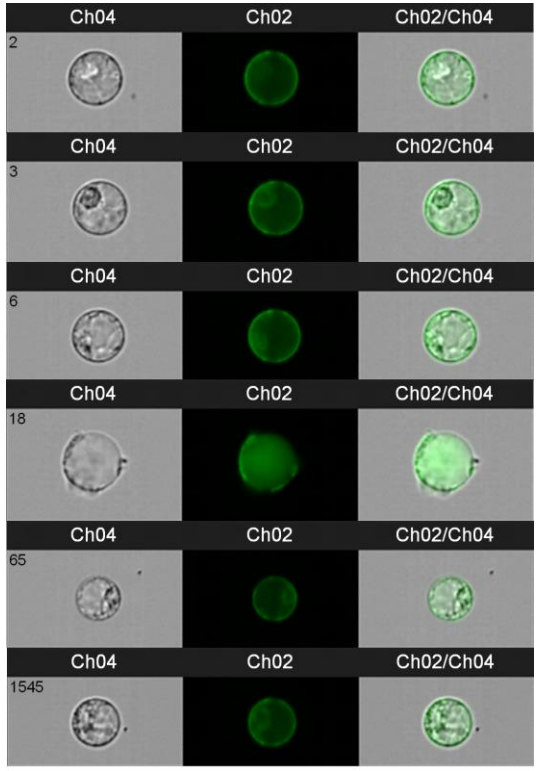
GFP in cell membrane

brightfield *fluorescence* *composite*



GFP in cytoplasm

brightfield *fluorescence* *composite*

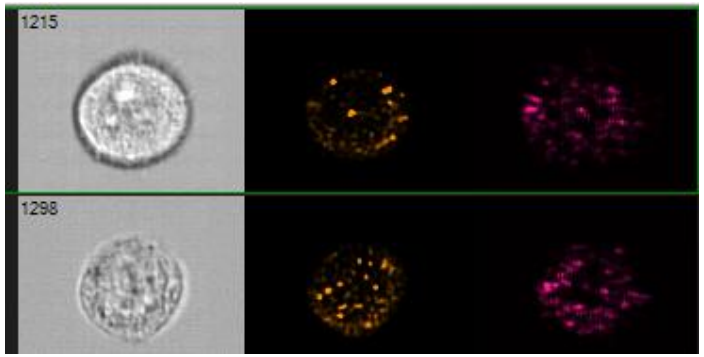


collaboration: Department of Plant Molecular Physiology; Prof. dr hab. M. Jasiński

Identification of intracellular foci, spot counting

mCherry in foci

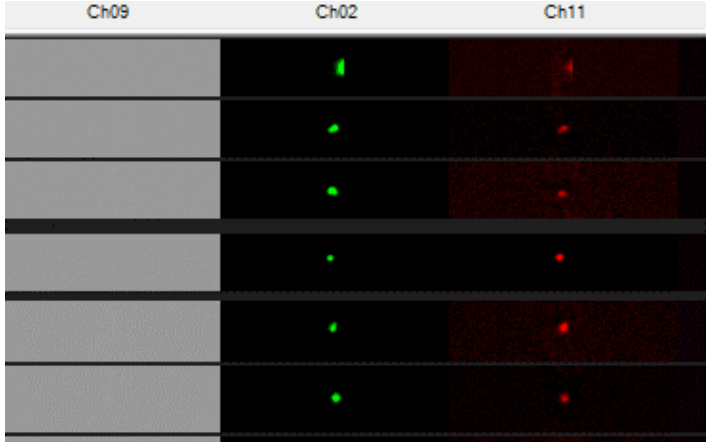
brightfield *fluorescence* *side scatter*



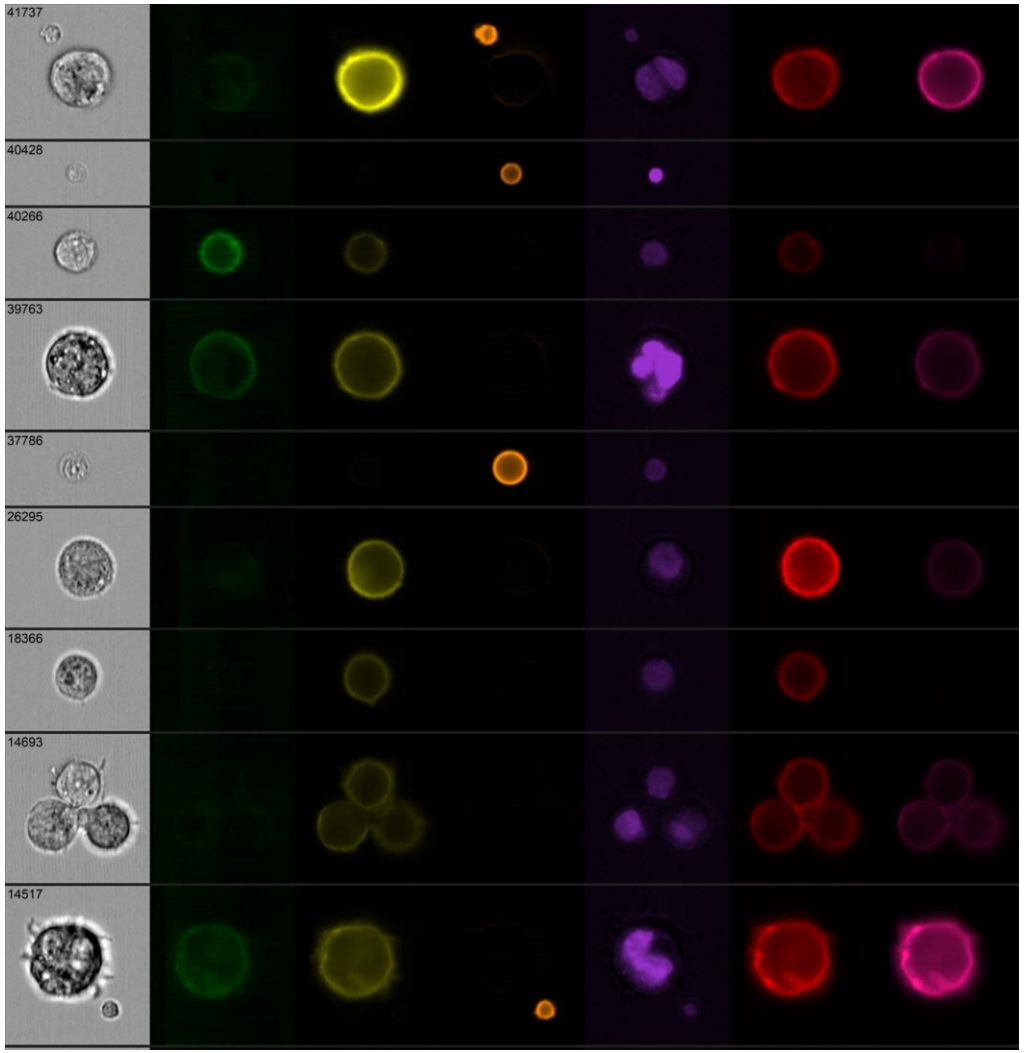
collaboration: Department of RNA Metabolism; Dr. hab. Z. Warkocki

Identification of extracellular vesicles (~30 nm objects)

brightfield *fluorescence 1* *fluorescence 2*



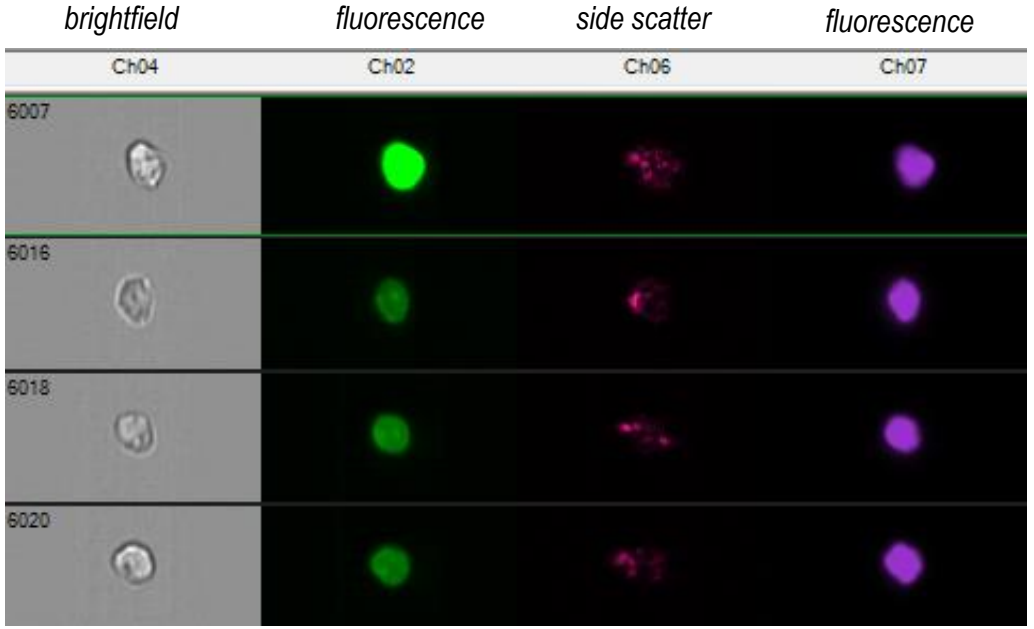
Multiparametric characterization of a clinical sample



collaboration: Dr. M. Zaborowski, Poznań University of Medical Sciences

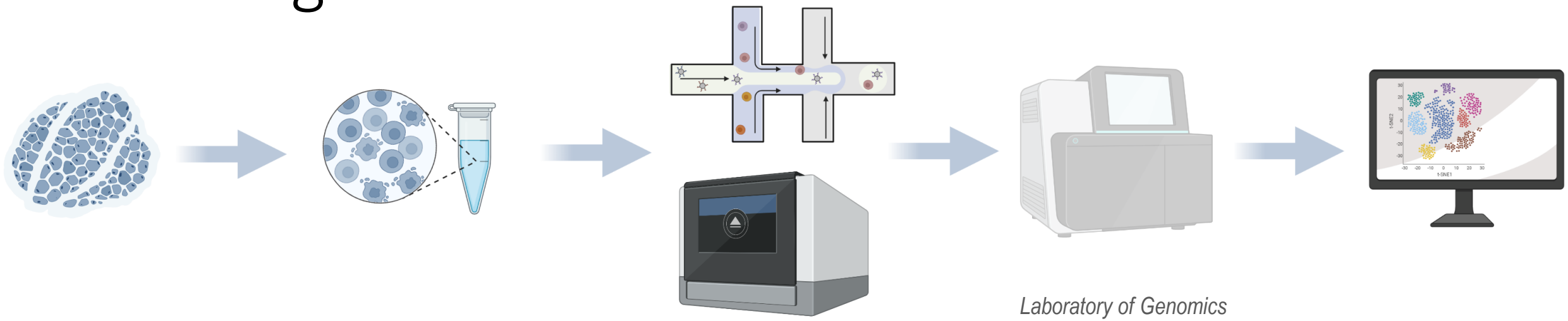
Visualisation of nuclei

GFP in nuclei



collaboration: Department of Neuronal Cell Biology; Dr. P. Świtoński

10x Genomics Chromium Controller platform for single cell multiomics



Laboratory of Genomics

Applications include:

- gene expression profiling
- chromatin accessibility profiling (ATAC-Seq)
- feature barcode technology: integration of gene expression data with cell surface protein detection (antibody-oligonucleotide conjugates required), CRISPR screens

Sample types:

- fixed, fresh, frozen
- whole cells, nuclei

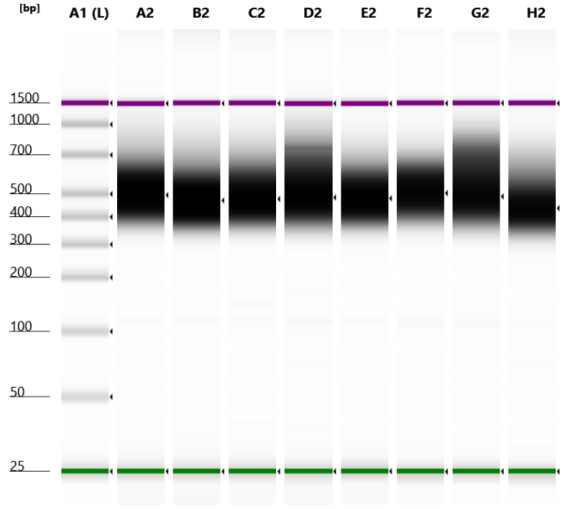
Wish to use the equipment? Contact us via email at: lab.single.cell@ibch.poznan.pl
Available for self-use from June 2024

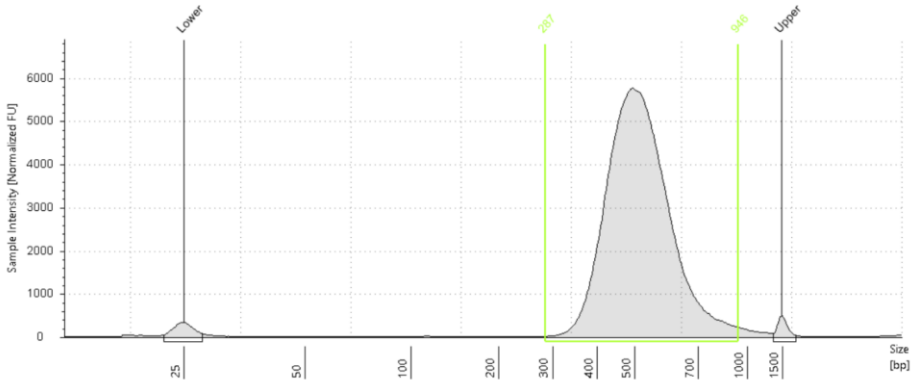
10x Genomics library preparation and data analysis

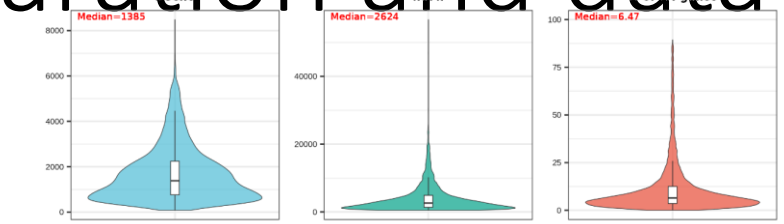
Dr. Anna Samelak-Czajka

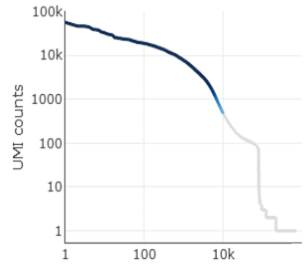
sample types processed thus far:

- PBCM
- primary cultured cells
- dissociated tissue (whole invertebrate)
- plant nuclei

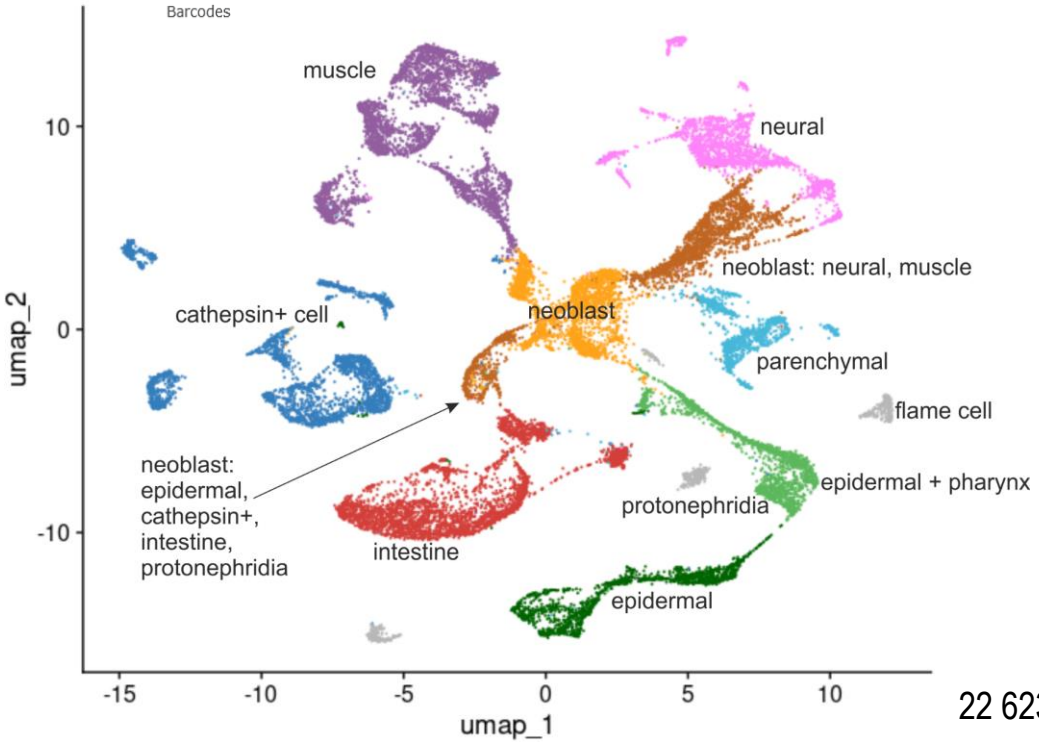








Dr. Eng. Małgorzata Marszałek-Zeńczak



22 623 cells

2nd Guava easyCyte 12HT and Guava Muse Operator Training

- provided by LSCA staff
- small groups, theoretical & hands-on sessions (~4 hours in total)
- September 2024 – save the date!

Droplet Digital PCR Workshop (organized with Bio-Rad)

- provided by Bio-Rad application specialist
- seminar & system demonstration
- date to be announced

Find out more at:
https://portal.ichb.pl/laboratory-of-single-cell-analyses

Contact us at:
lab.single.cell@ibch.poznan.pl

The Laboratory is a part of the infrastructure developed under the project NEBI - National Imaging Centre for Biological and Biomedical Sciences, POIR.04.02.00-00-C004/19, co-financed through the European Regional Development Fund (ERDF) in the frame of Smart Growth Operational Programme 2014-2020 (Measure 4.2 Development of modern research infrastructure of the science sector).

European Funds Smart Growth

Republic of Poland

European Union
European Regional Development Fund


ICHB PAN

INSTITUTE OF BIOORGANIC CHEMISTRY
Polish Academy of Sciences

LABORATORY OF SUBCELLULAR STRUCTURES

ANALYSES

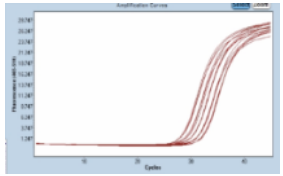
POZNAŃ, 6.02.2024

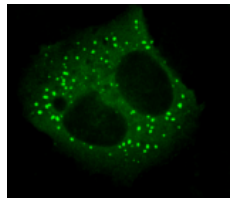
Aim:
investigation of inter- and intracellular processes

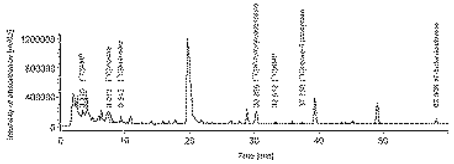
molecular level

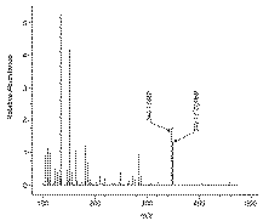
cellular level

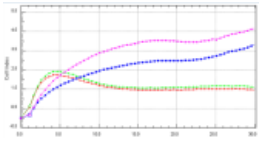
organismal level

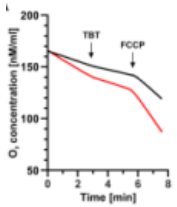


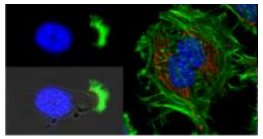


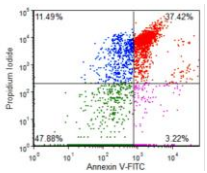


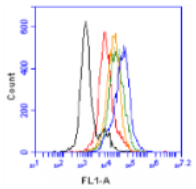


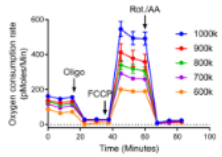


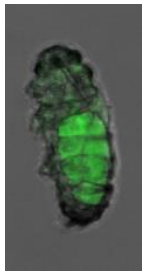




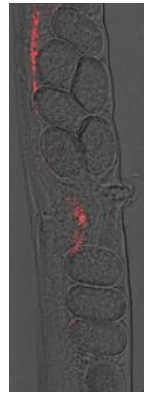




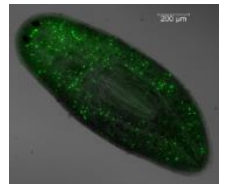




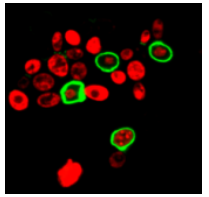
Tardigrada



C. elegans



S. mediterranea



S. cerevisiae

Cell culture room

Cell culture room:

CO_2 incubators

Biohazard BIOBAN hoods

Cell Harvester

Automated cell counter

Fluorescence microscope

Mini centrifuges

Mini incubator

Liquid nitrogen dewar tank

High-speed cooling centrifuges

Additional equipment:

Ultra-low freezers (- 80°C)

Liquid nitrogen dewar tank

Steam Autoclaves

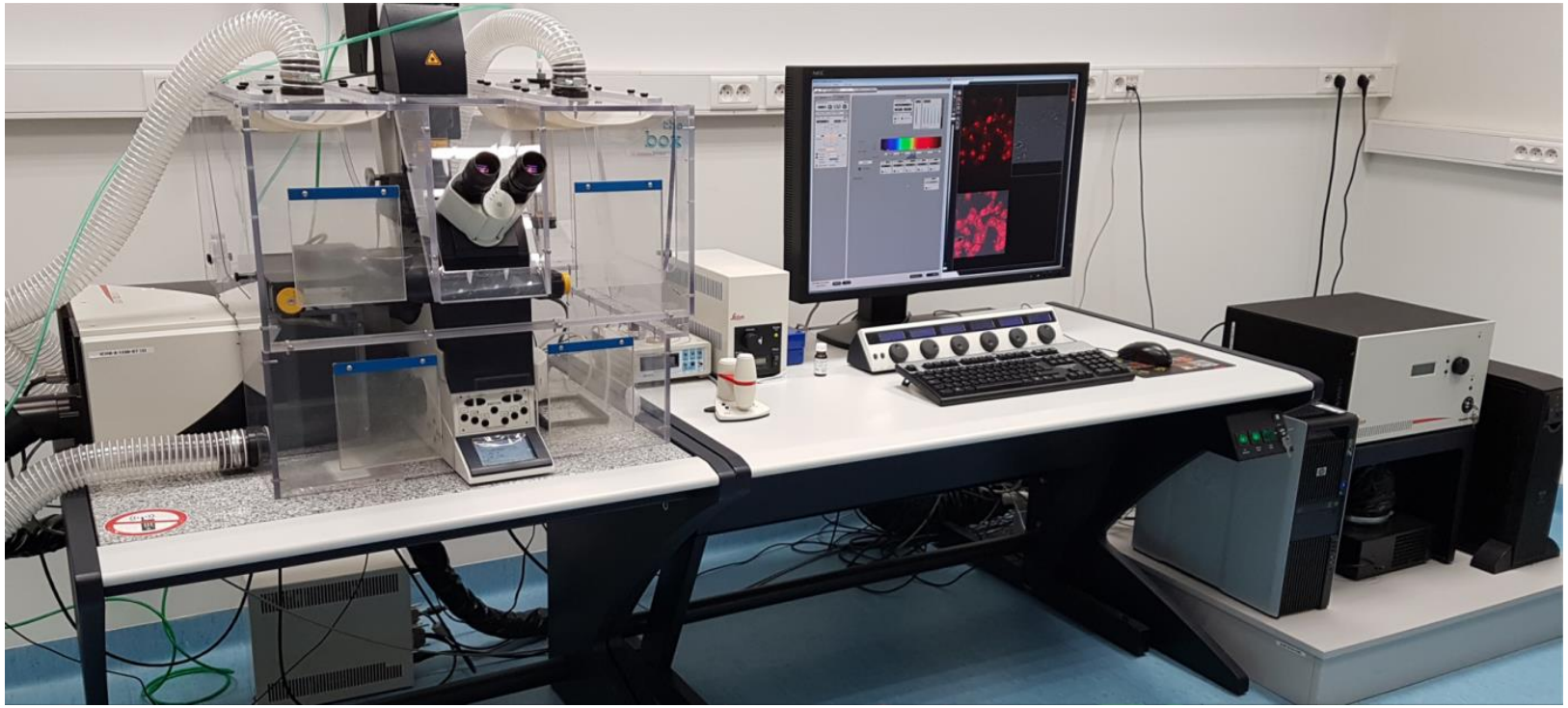
Milli-Q Water Purification System

Ultrasonic homogenizer

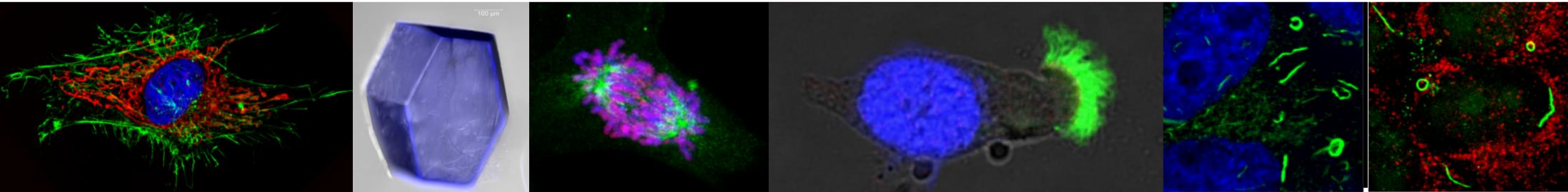
Electroporator

Steam Autoclaves

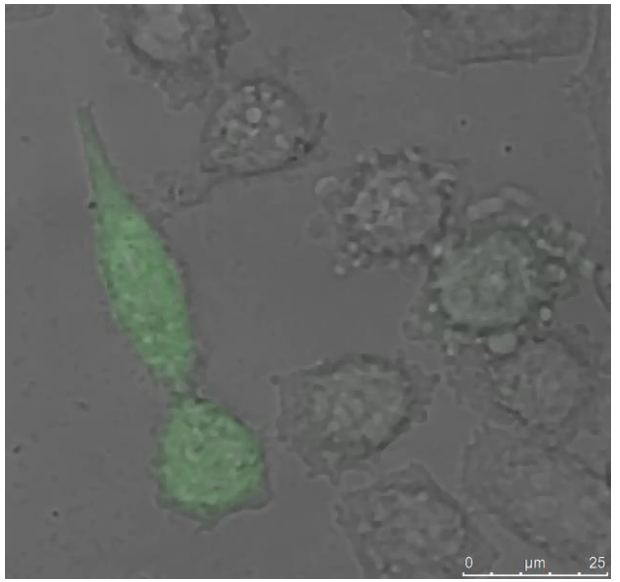
Confocal microscope TCS SP5, Leica



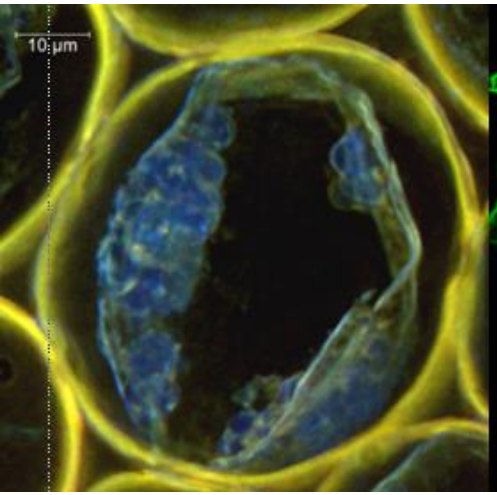
Lasers: White (470-670 nm), 405nm, Argon (458nm, 476nm, 488nm, 496nm, 514nm)
Environmental chamber ensures real time analysis (CO_2, humidity, temperature)



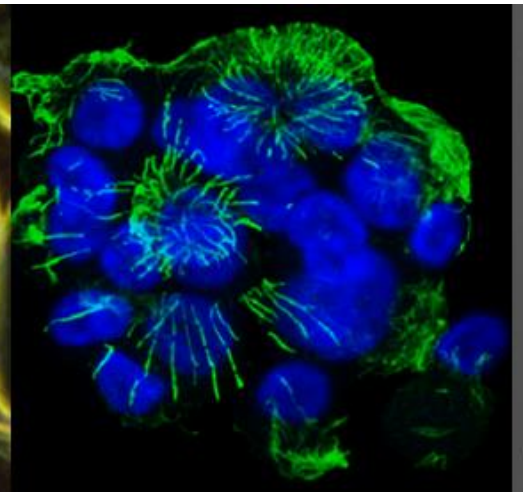
White Light Laser – WLL



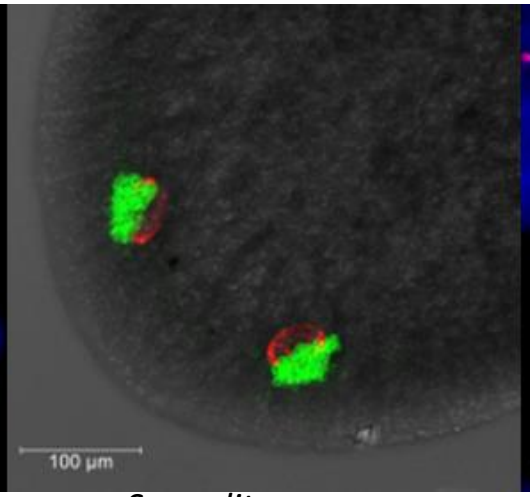
- Selection of any excitation light wavelength in the range of 470-670 nm with 1 nm accuracy for more efficient dye excitation.
- Ability to observe slides with autofluorescence and images with overlapping stain emissions.
- Measurement of co-localization of particles stained with two dyes and their interaction (FRET) and fluorescence intensity .
- Possibility to create absorption spectra of dyes.



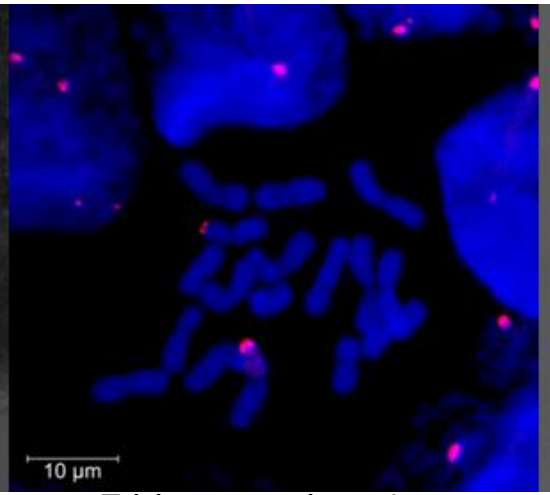
Convallaria majalis L.



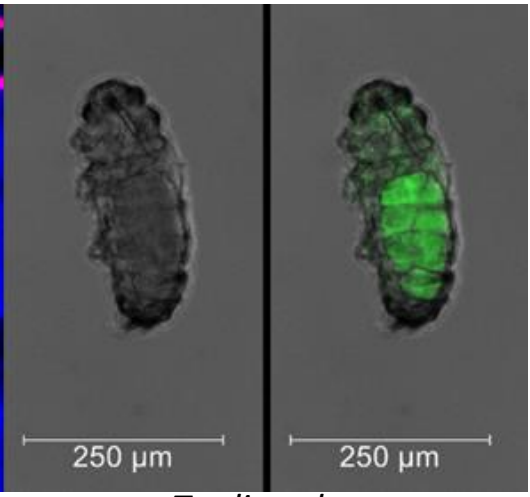
Human epithelial respiratory cells



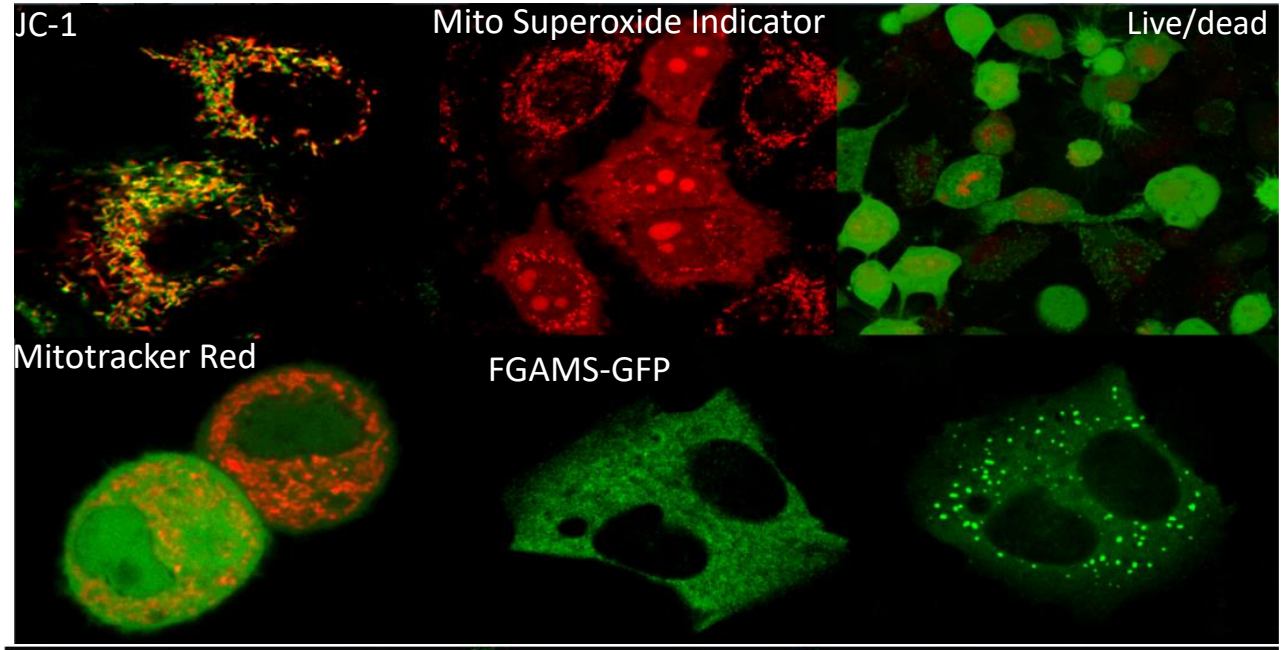
S. mediterranea

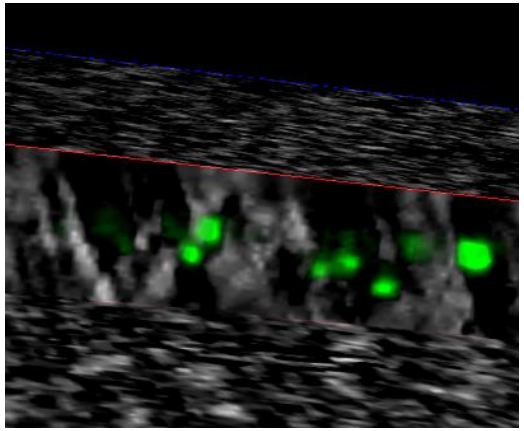


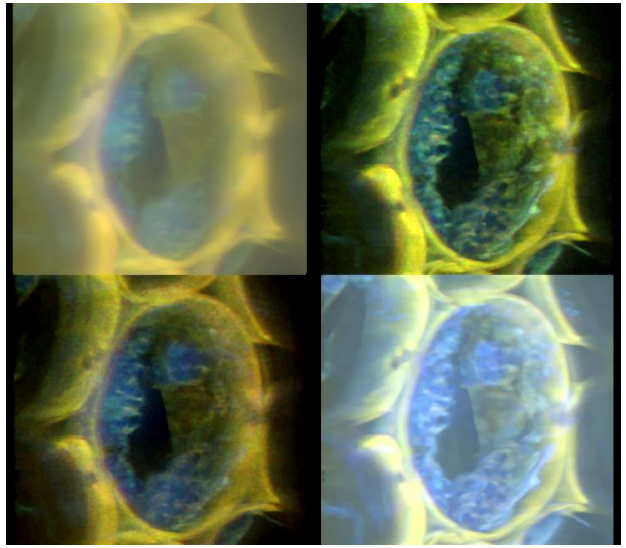
Triticum aestivum L.

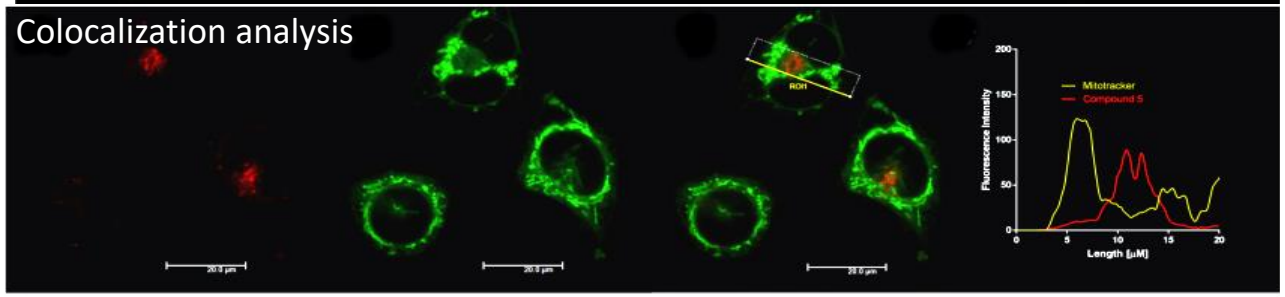


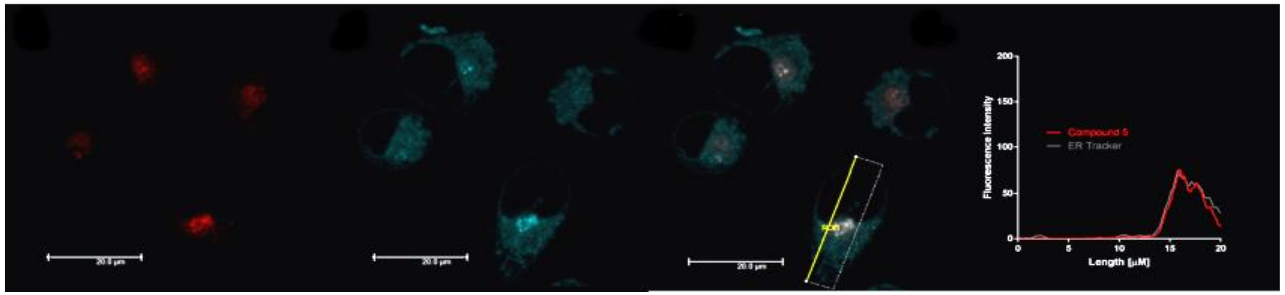
Tardigrada

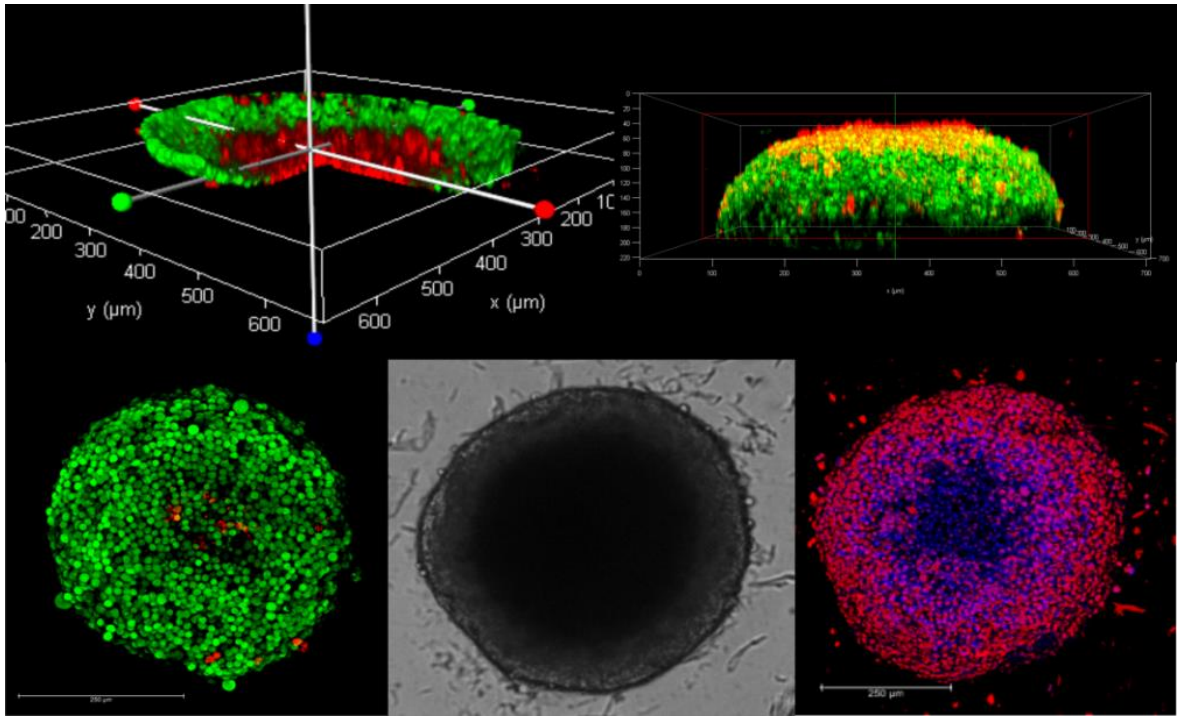

JC-1
Mito Superoxide Indicator
Live/dead
Mitotracker Red
FGAMS-GFP






Colocalization analysis
Mitotracker
Compound 5
Fluorescence Intensity
Length [µM]


Compound 5
ER Tracker
Fluorescence Intensity
Length [µM]


y (µm)
x (µm)

FACSCalibur Flow cytometer, Becton Dickinson

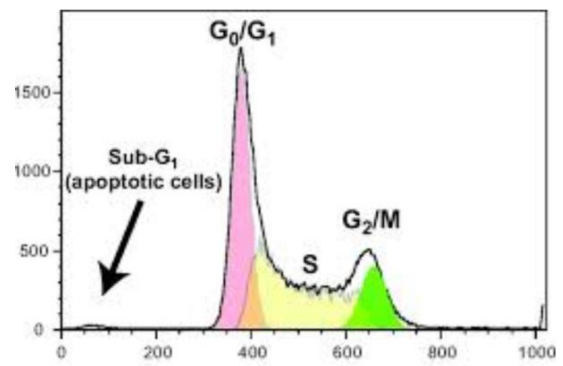
Flow cytometry allows fast analysis of single cells flowing in a stream of liquid. It allows to assess both qualitatively and quantitatively the physical and biological properties of cells and some of their components: nuclei, nucleic acids, mitochondria and chloroplasts.

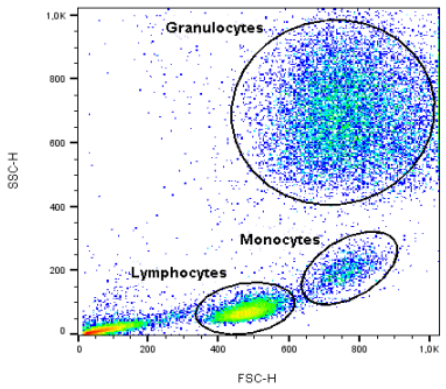
Lasers

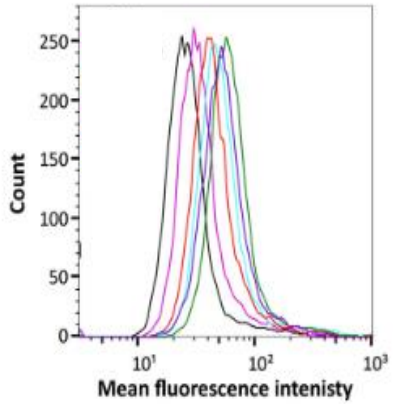
- 488 nm
- 635 nm

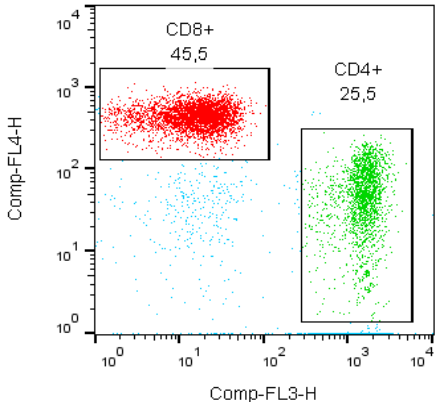
Detectors

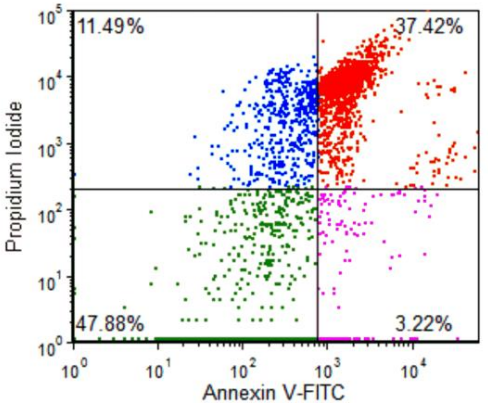
- FL1 (530/30)
- FL2 (585/42)
- FL3 (670 LP)
- FL4 (661/16)







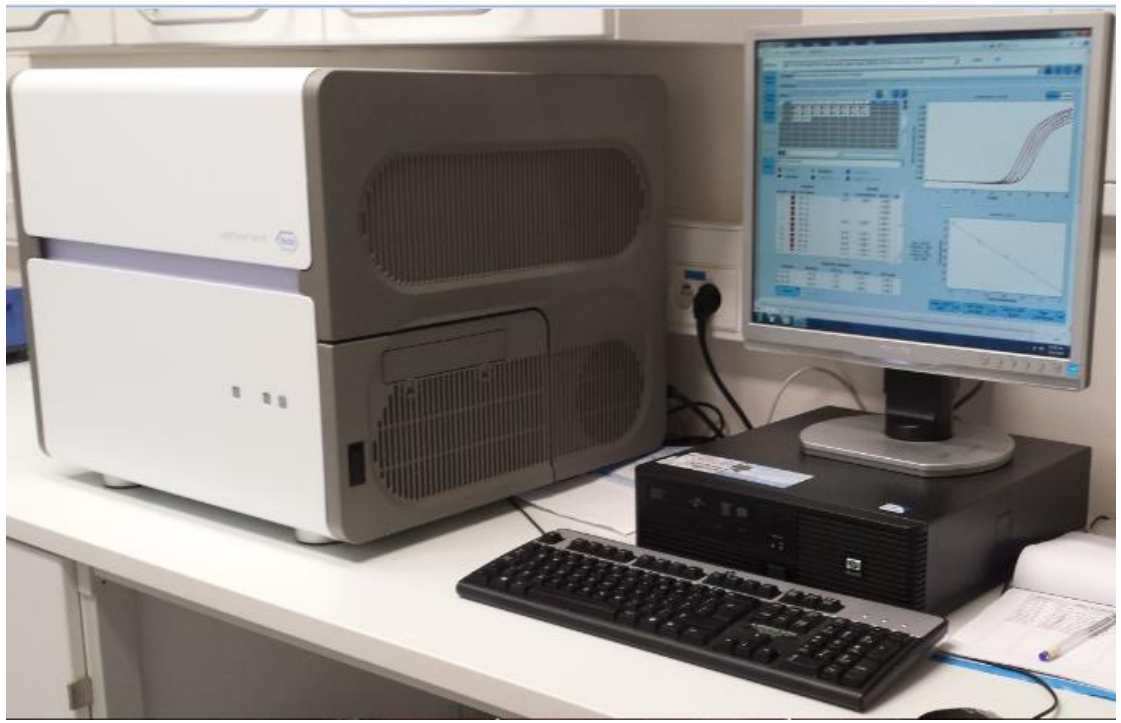




LightCycler 480 II, Roche

It is an integrated system, based on a real-time PCR platform. It allows accurate qualitative and quantitative detection of nucleic acids in a 96-well block.

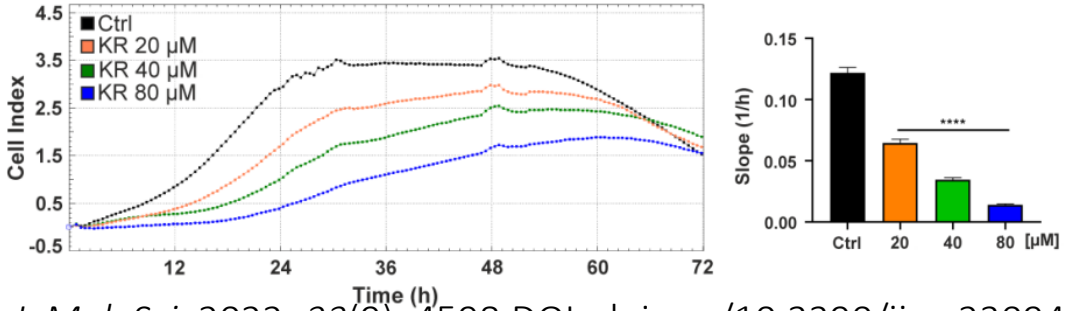
- Improved performance of gene expression analysis and genetic variation analysis.
- HRM analysis of PCR products by melting curve determination enables detection of mutations, polymorphisms and epigenetic differences in double-stranded DNA samples.



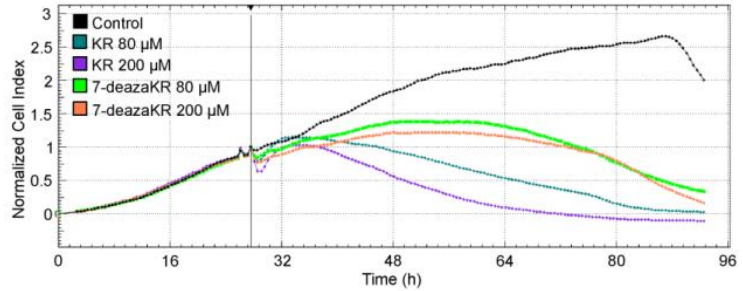
xCELLigence RTCA System, Agilent Technologies

The xCELLigence system allows real-time analysis of eukaryotic cell proliferation, migration and cytotoxicity. The instrument measures impedance-based signals in both cellular proliferation and cell invasion/migration assays – without the use of exogenous labeling.

- The RTCA Analyzer together with the E-Plate is placed into a standard cell culture incubator, what ensures controlled environment throughout the experiment.
- Quantitatively monitors changes in cell number, adhesion, viability, and morphology.
- Analysis of sigle cell line or simultaneous analysis of one or more cell lines in the same experimental conditions.
- Analysis of interactions between two different cell lines.



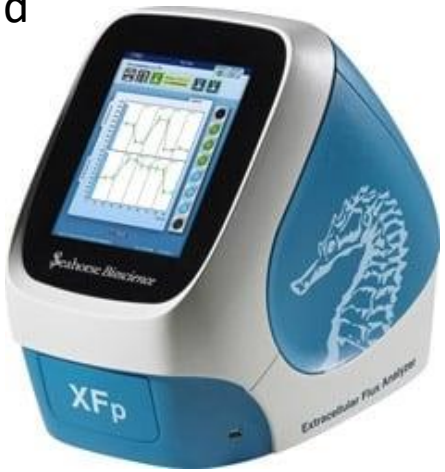
Int. J. Mol. Sci. 2022, *23*(9), 4598 DOI: doi.org/10.3390/ijms23094598



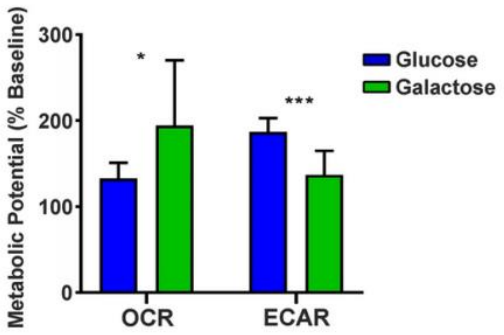
Antioxidants 2021, *10*(6), 950; DOI: doi.org/10.3390/antiox10060950

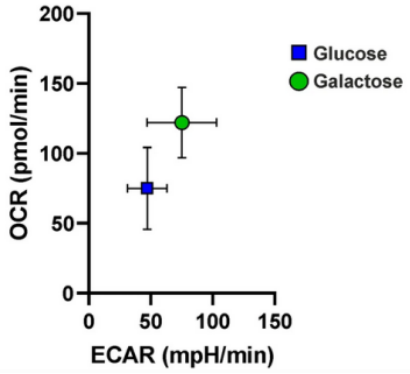
Seahorse XFp Analyzer

- Seahorse XF Analyzers measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of live cells in a multi-well plate, based on key cellular functions such as mitochondrial respiration and glycolysis.



- Analysis of the rate of ATP production from the two key energy pathways (glycolysis and mitochondrial respiration)

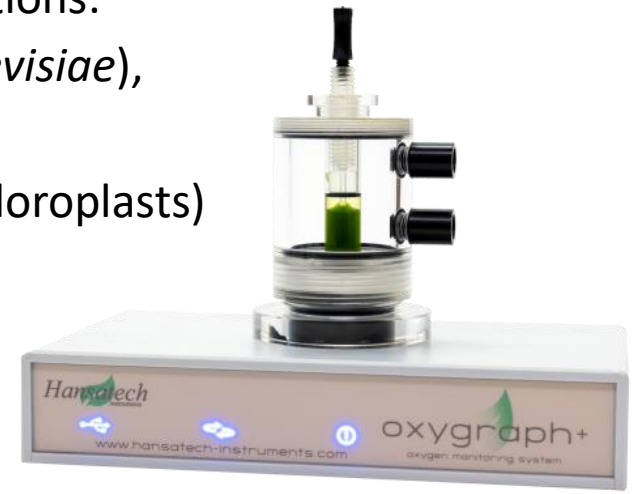


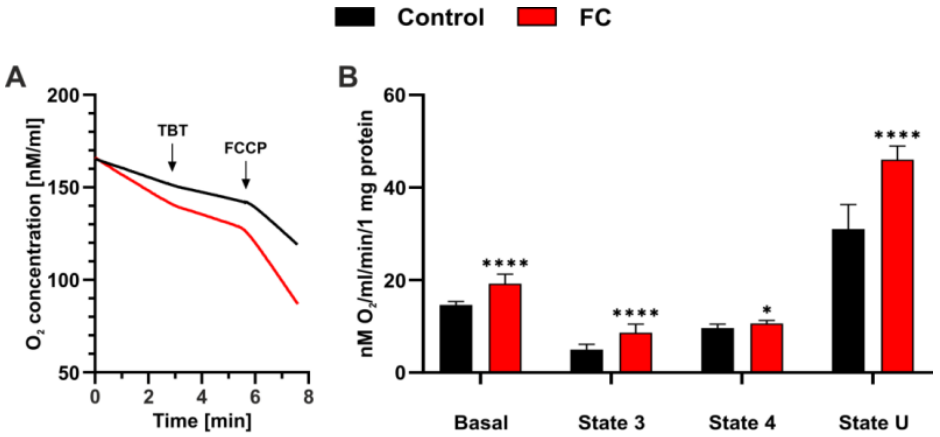


Apoptosis 2020, 25:835–852 DOI: doi.org/10.1007/s10495-020-01637-x

Oxygraph Plus

- Liquid-phase photosyntyhesis and respiration measurement system.
- Provides oxygen uptake measurements across a wide range of applications:
  - organisms (*C. elegans*, *S. cerevisiae*),
  - cells,
  - organelles (mitochondria, chloroplasts)

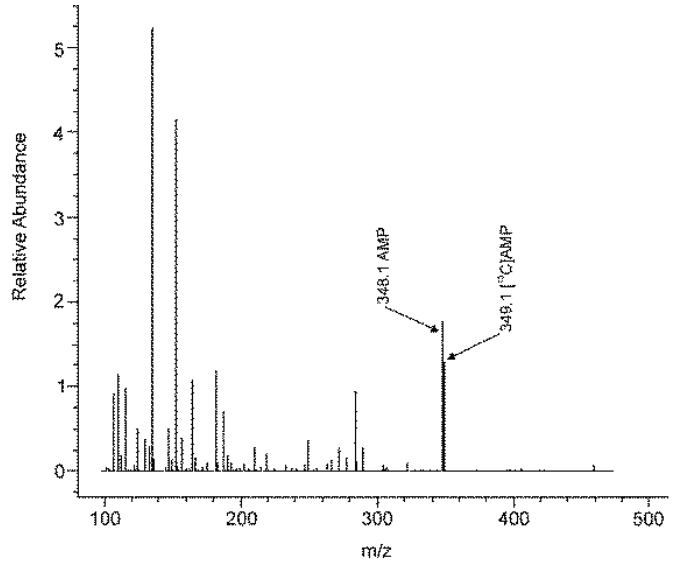


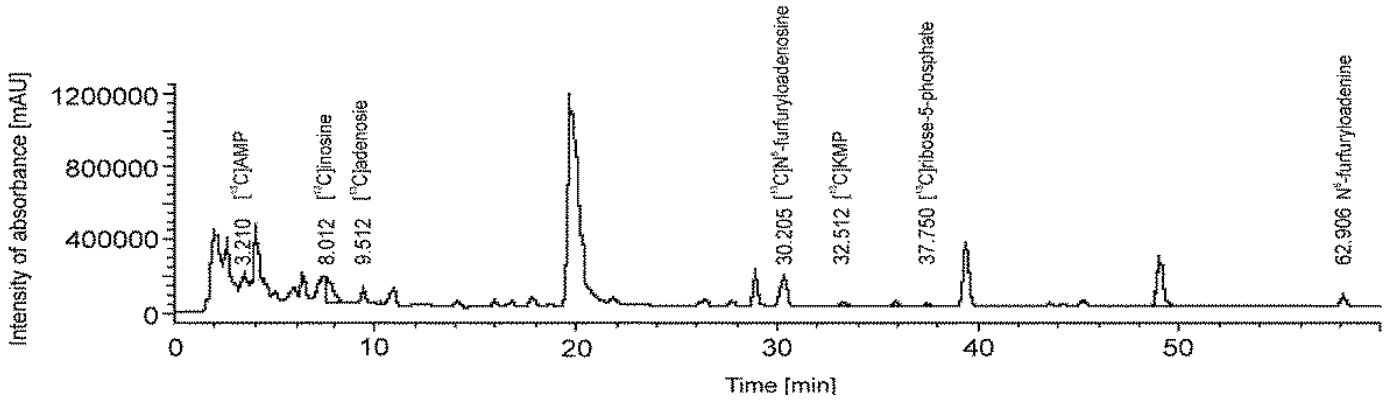


Antioxidants 2022, 11(5), 850 DOI:doi.org/10.3390/antiox11050850

High Performance Liquid Chromatography HPLC LC/MS, ESA Coulochem III electrochemical detector

- Detection of nucleic acids components and their derivatives
- Detection of nucleic acid epigenetic modifications (e. g. m5C)
- Detection of oxidative stress modifications (8-oxo-dG)





Varioskan LUX multimode plate reader, Thermo Scientific

Designed for a variety of applications (6- to 384-well plates):

- Asorbance (UV - Vis)
- Fluorescence intensity (280 - 840 nm)
- Luminescence (360 - 670 nm)
- Time-resolved fluorescence (400-700 nm)

- Integrated gas module for atmospheric control of CO_2 and O_2 for cell-based assays.
- Different measurement modes: endpoint, kinetic, spectra, multipoint and kinetic spectra.
- Two dispensers for easy and accurate reagent addition, enabling follow-up of kinetic reactions, flash-type luminescence reactions, Ca^{2+} studies, and other rapid kinetic applications e.g. ATP, reporter gene assays.
- Control of temperature and shaking.

Colaborations

Colaboration	Type of analysis	Equipment used
Dr hab. Małgorzata Borowiak , prof. AMU, Adam Mickiewicz University ,Poznań	Analysis of pluripotrency markers, proliferation and bioenergetics of pluripotent Hues8-iCas9 cells	confocal microscope, flow cytometer, Seahorse analyzer
Dr hab. Agnieszka B. Olejniczak, prof. IMB PAS, Institute of Medicinal Biology PAS, Łódź	Anaysis of lisosomal membrane permeability and ferroptosis induction in HepG2 cells after 1,8-naphtalamides derivatives treatment.	Comprehensive analysis: experimental workflow, equipment, expertise
Dr hab. Anna Dembska, prof. AMU, Adam Mickiewicz University, Poznań	Analysis of cell membrane permeability to Ag nanoclusters in human cells.	confocal microscope, flow cytometer
Dr hab. Marta Olejniczak, prof. ICHB PAS	Analysis of huntingtin aggregates in brain of YAC128 mice.	Confocal microscope
Prof. Mirosława Z. Naskręt-Barciszewska	Cell cycle and 8-oxo-dG level analysis in T98G, U138 and HaCaT cells using epigenetic modulators (TMZ and metformin).	Cell culture room, HPLC LC/MS with coulochem detector, flow cytometer
Dr hab. Magdalena Łuczak, prof. IBCH PAS	Optimization of endothelial cells cultivation in normal condition and after treatment with serum from CKD and CVD patients.	Cell culture room, confocal microscope, flow cytometer, xCELLigence system
Dr hab. Agata Tyczewska, prof. ICHB PAS	Analysis of gene expression level in aged *C. elegans*	LightCycler 480 II
Prof. Michał Jasiński	Analysis of subcellular localization of plant ABCG transporters, and evaluation of influence of auxins on morphogenetic processes in *M. truncatula* roots.	Confocal microscope
Dr hab. Agnieszka Fiszer, prof. IBCH PAS	Microscope analysis of CAG repeats function in cell lines.	Confocal microscope
Dr hab. Jan Wrzesiński, prof. ICHB PAS	Analysis of real-time proliferation and immunofluorescent visualization of PIWIL1 protein in HEK293 and HK-2 cells	Confocal microscope, FACSCalibur flow cytometer, LightCycler 480 II
Prof. Anna Pasternak	Analysis of influence of G-quadruplexes on cell cycle and viability, and its internalization in HeLa cells	Confocal microscope, flow cytometer
Dr hab. Maciej Figiel, prof. ICHB PAS	Characterictics of brain organoids differantiated form iPSC from patients with juvenile-onset HD and visualisation of neuropathogenesis markers.	Confocal microscope
Dr hab. Zbigniew Warkocki, prof. ICHB PAS	Immunofluorescent analysis of cytoplasmatic bodies LINE-1 in WT cells, analysis of retrotransposone LINE-1 transcript expression in human cells.	Confocal microscope, LightCycler 480 II

List of publications

• Rykowski S, **Gurda-Woźna D, Fedoruk-Wyszomirska A, Orlicka-Płocka M,** Kowalczyk A, Stączek P, Denel-Bobrowska M, Biniek-Antosiak K, Rypniewski W, **Wyszko E**, Olejniczak AB. Carboranyl-1,8-naphthalimide intercalators induce lysosomal membrane permeabilization and ferroptosis in cancer cell lines. J Enzyme Inhib Med Chem. 2023, 38:2171028.

• Barciszewska AM, Belter A, Gawrońska I, **Giel-Pietraszuk M**, Naskręt-Barciszewska MZ. Juglone in combination with temozolomide shows a promising epigenetic therapeutic effect on the glioblastoma cell line. Int J Mol Sci. 2023, 24:6998.

• Rykowski S, **Gurda-Woźna D, Orlicka-Płocka M, Fedoruk-Wyszomirska A, Giel-Pietraszuk M, Wyszko E**, Kowalczyk A, Stączek P, Biniek-Antosiak K, Rypniewski W, Olejniczak AB. Design of DNA intercalators based on 4-Carboranyl-1,8-Naphthalimides: investigation of their DNA-binding ability and anticancer activity. Int J Mol Sci. 2022, 23:4598.

• Barciszewska AM, Belter A, Gawrońska I, **Giel-Pietraszuk M**, Naskręt-Barciszewska MZ. Cross-reactivity between histone demethylase inhibitor valproic acid and DNA methylation in glioblastoma cell lines. Front Oncol. 2022, 12:1033035.

• Rykowski S, **Gurda-Woźna D, Orlicka-Płocka M, Fedoruk-Wyszomirska A, Giel-Pietraszuk M, Wyszko E**, Kowalczyk A, Stączek P, Bak A, Kiliszek A, Rypniewski W, Olejniczak AB. Design, synthesis, and evaluation of novel 3-Carboranyl-1,8-Naphthalimide derivatives as potential anticancer agents. Int J Mol Sci. 2021, 22:2772.

• Wawrzyniak D, Grabowska M, Głodowicz P, Kuczyński K, Kuczyńska B, **Fedoruk-Wyszomirska A**, Rolle K. Down-regulation of tenascin-C inhibits breast cancer cells development by cell growth, migration, and adhesion impairment. PLoS One. 2020 Aug 20;15(8):e0237889.

•Zawirska-Wojtasiak R, **Fedoruk-Wyszomirska A**, Piechowska P, Mildner-Szkudlarz S, Bajerska J, Wojtowicz E, Przygoński K, **Gurda D**, Kubicka W, **Wyszko E**. β-Carbolines in experiments on laboratory animals. Int J Mol Sci. 2020, 21:5245.

• Różycka D, Korycka-Machała M, Żaczek A, Dziadek J, **Gurda D, Orlicka-Płocka M, Wyszko E**, Biniek-Antosiak K, Rypniewski W, Olejniczak AB. Novel Isoniazid-carborane hybrids active in vitro against *Mycobacterium tuberculosis*. Pharmaceuticals (Basel). 2020, 13:465.

•Dembska A, Świtalska A, **Fedoruk-Wyszomirska A**, Juskowiak B. Development of fluorescence oligonucleotide probes based on cytosine- and guanine-rich sequences. Sci Rep. 2020, 10:11006.

Patent application

Zastosowanie 37-merowej sondy oligonukleotydowej, jako indykatora do monitorowania zmian w metabolizmie komórek nowotworowych
P.441574 (2022) A.R. Dembska, N. Lisiak, **A. Fedoruk-Wyszomirska**, B. Rubiś

Projects realized in the Laboratory

Research projects:

- Właściwości przeciwstarzeniowe 4-*N*-furfurylocytozyny w zróżnicowanych wiekowo komórkach eukariotycznych, drożdżach oraz mysim modelu starzenia (NCN OPUS EW)
- Rybozyd kinetyny i jego pochodne – analiza właściwości apoptotycznych i mechanizmu działania w komórkach guzów mózgu (NCN OPUS EW)
- Analiza właściwości pro-regeneracyjnych i przeciwutleniających 4-N-furfurylocytozyny w modelu zwierzęcym *Schmidtea mediterranea* (Planaria) (NCN MINIATURA AFW)
- Optymalizacja warunków biodruku w celu stworzenia komórkowego modelu 3D raka wątroby jako platformy skriningowej do selekcji związków o potencjale terapeutycznym (NCN MINIATURA DGW)

Projects in cooperation:

- Regulacja poziomu neuroaktywnych beta-karbolin w diecie w oparciu o substytuty kawy (NCN OPUS, prof. dr hab. inż. Renata Zawirska-Wojtasiak UP)
- Cząsteczki wiążące DNA – synteza i właściwości interkalatorów DNA zawierających klastry boru (SONATA Bis, dr hab. Agnieszka Olejniczak, prof. IBM PAN)
- pH-wrażliwe oligonukleotydy do monitorowania zmian w metabolizmie komórek nowotworowych wywołanych lekami przeciwnowotworowymi (NCBR TANGO, dr hab. Anna Dembska, prof. UAM)

Head:

Prof. Eliza Wyszko

ext. 1141
eliza.wyszko@ibch.poznan.pl

Staff:

dr. habil. Małgorzata Giel-Pietraszuk, prof. ICHB

dr. Dorota Gurda-Woźna

dr. Agnieszka Fedoruk-Wyszomirska

PhD student:

Paweł Pawelczak, MSc

Contact us:

lab.subcellular.struct@ibch.poznan.pl

https://portal.ichb.pl/pracownia-analiz-struktur-subkomorkowych/


ICHB PAN

LABORATORY OF BIOINFORMATICS

INSTITUTE OF BIOORGANIC CHEMISTRY
Polish Academy of Sciences

STRUCTURE

IBCH PAS

Department of Molecular Genetics

Prof. Piotr Kozłowski

Laboratory of Bioinformatics (2020)

B building, 2nd floor

Room 207 & 208

OUR TEAM

Dr hab. Anna Philips, prof. IBCH PAS - Head of Laboratory

- MSc. in Computer Science (Applied Computer Science)
- Ph.D. in Biology (Bioinformatics)
- In IBCH PAS from 2014

OUR TEAM

Dr Natalia Szóstak

- **MSc. in Biology (Bioinformatics)**
- **Ph.D. in Computer Science (Bioinformatics)**
- **In IBCH PAS from 2020**

OUR TEAM

Dr Arkadiusz Kajdasz

- **MSc. in Biology (Molecular Biology)**
- **Ph.D. in Biology (Molecular Genetics)**
- **In IBCH PAS from 2021 (in Bioinformatics Lab 2023)**

OUR TEAM

Szymon Melewski

- **BS. in Computer Science**

- **In IBCH PAS from 2021 (in Bioinformatics Lab 2024)**

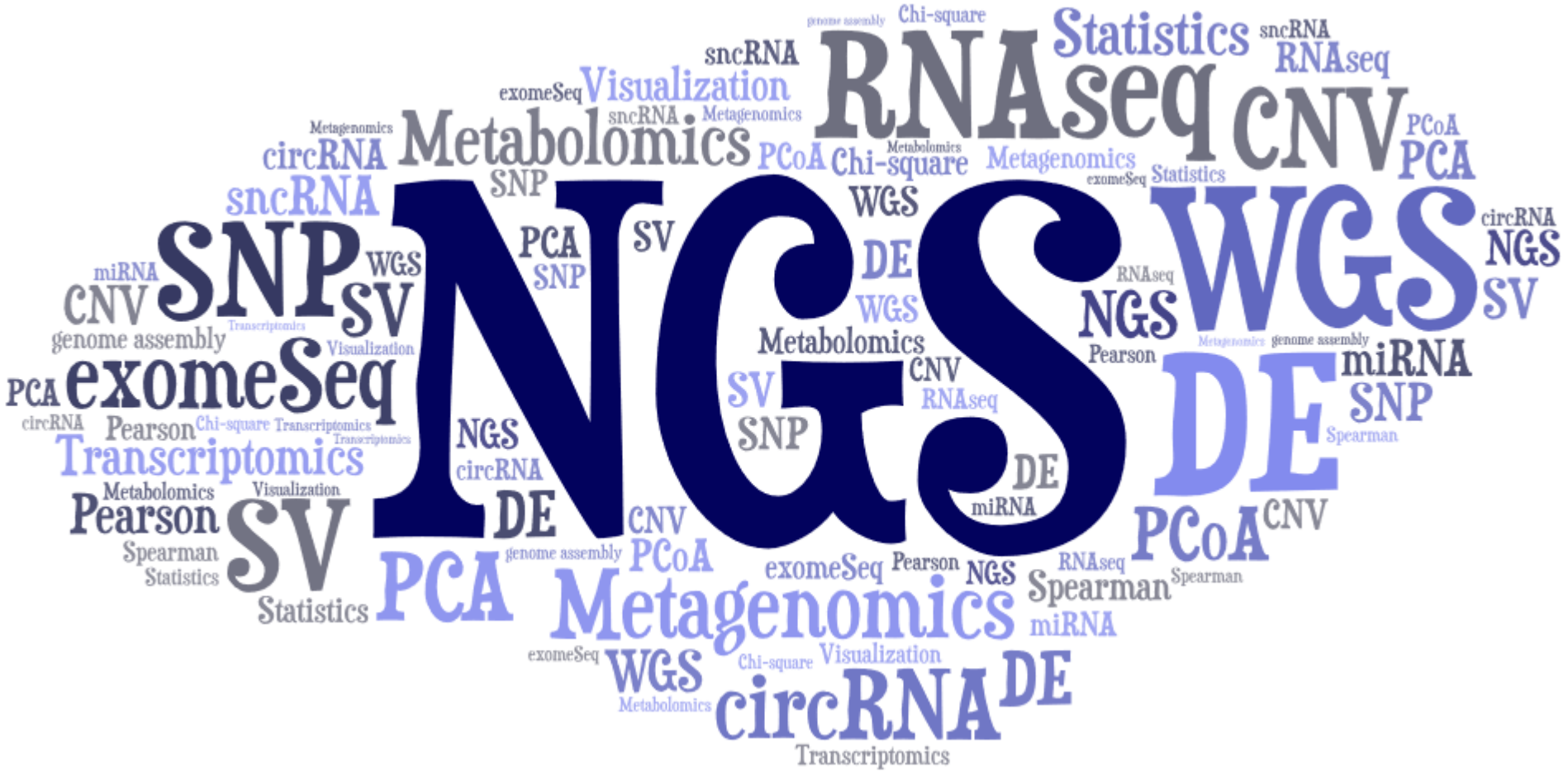
OUR TEAM

Maciej Michalczyk, Ph.D. Student

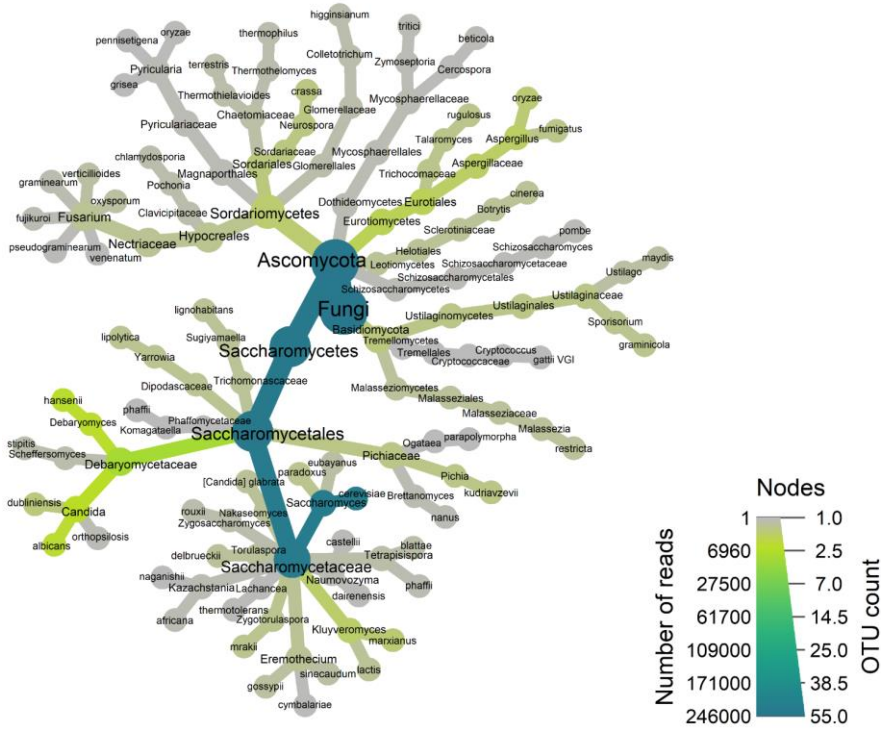
- **MSc. In Biology (Bioinformatics)**

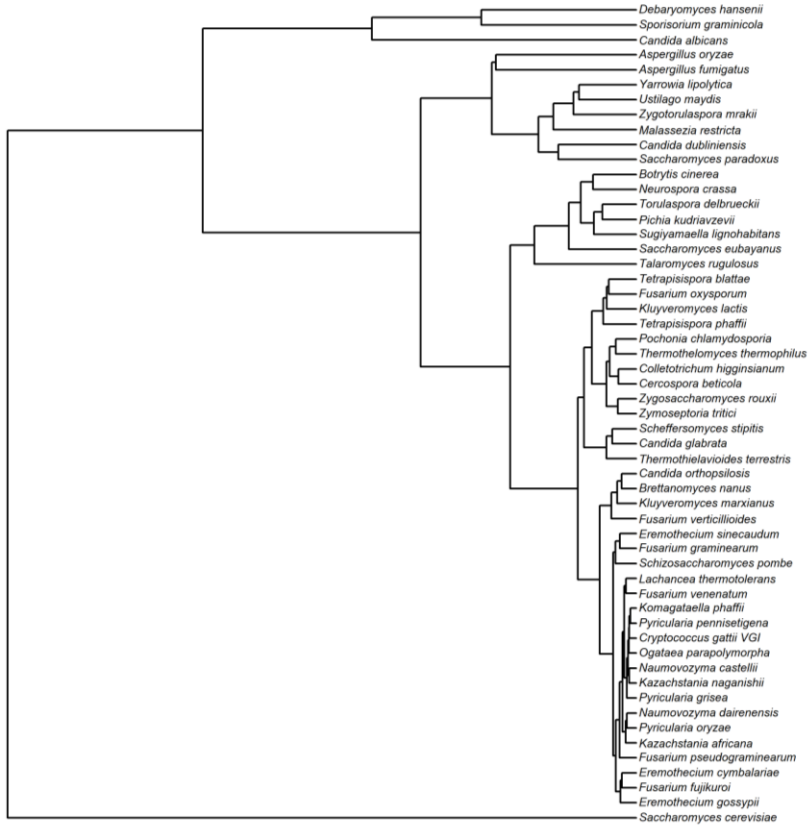
- **In IBCH PAS from 2023**

WHAT WE DO


NGS
RNAseq
WGS
DE
SNP
exomeSeq
Metagenomics
Metabolomics
Transcriptomics
circRNA
sncRNA
CNV
PCA
PCoA
SV
Pearson
Statistics
Spearman
Visualization
miRNA
Chi-square
genome assembly

GENOMICS


Ascomycota
Fungi
Basidiomycota
Saccharomycetes
Saccharomycetales
Saccharomycetaceae
Debaryomycetaceae
Pichiaceae
Sordariomycetes
Eurotiomycetes
Dothideomycetes
Leotiomycetes
Schizosaccharomycetes
Tremellomycetes
Ustilaginomycetes
Malasseziomycetes
Hypocreales
Sordariales
Eurotiales
Helotiales
Schizosaccharomycetales
Ustilaginales
Malasseziales
Trichomonascaceae
Dipodascaceae
Phaffomycetaceae
Malasseziaceae
Ustilaginaceae
Schizosaccharomycetaceae
Sclerotiniaceae
Trichocomaceae
Aspergillaceae
Glomerellaceae
Mycosphaerellaceae
Chaetomiaceae
Pyriculariaceae
Clavicipitaceae
Nectriaceae
Sordariaceae
Magnaporthales
Cryptococcaceae
Tremellales
Talaromyces
Aspergillus
Botrytis
Fusarium
Neurospora
Pochonia
Ustilago
Sporisorium
Schizosaccharomyces
Cryptococcus
Malassezia
Ogataea
parapolymorpha
Pichia
kudriavzevii
Brettanomyces
Saccharomyces
Candida
Debaryomyces
Scheffersomyces
Komagataella
Sugiyamaella
Yarrowia
Lachancea
Naumovozyma
Kazachstania
Nakaseomyces
Zygosaccharomyces
Torulaspora
Tetrapisispora
Zygotorulaspora
Kluyveromyces
Eremothecium
Pyricularia
Thermothelomyces
Thermothielavioides
Colletotrichum
Zymoseptoria
Cercospora
Verticillioides
Graminearum
Oxysporum
Venenatum
Pseudograminearum
Lipolytica
Lignohabitans
Hansenii
Phaffii
Stipitis
Dubliniensis
Albicans
Orthopsilosis
Rouxii
Castellii
Delbrueckii
Naganishii
Africana
Thermotolerans
Mrakii
Sinecaudum
Gossypii
Cymbalariae
Dairenensis
Marxianus
Lactis
Blattae
Phaffii
Nanus
Restricta
Eubayanus
Paradoxus
Cerevisiae
Pennisetigena
Grisea
Terrestris
Thermophilus
Higginsianum
Tritici
Beticola
Crassa
Rugulosus
Oryzae
Fumigatus
Chlamydosporia
Cinerea
Pombe
Maydis
Graminicola
Gattii VGI
Nodes
1
6960
27500
61700
109000
171000
246000
1.0
2.5
7.0
14.5
25.0
38.5
55.0
Number of reads
OTU count


Debaryomyces hansenii
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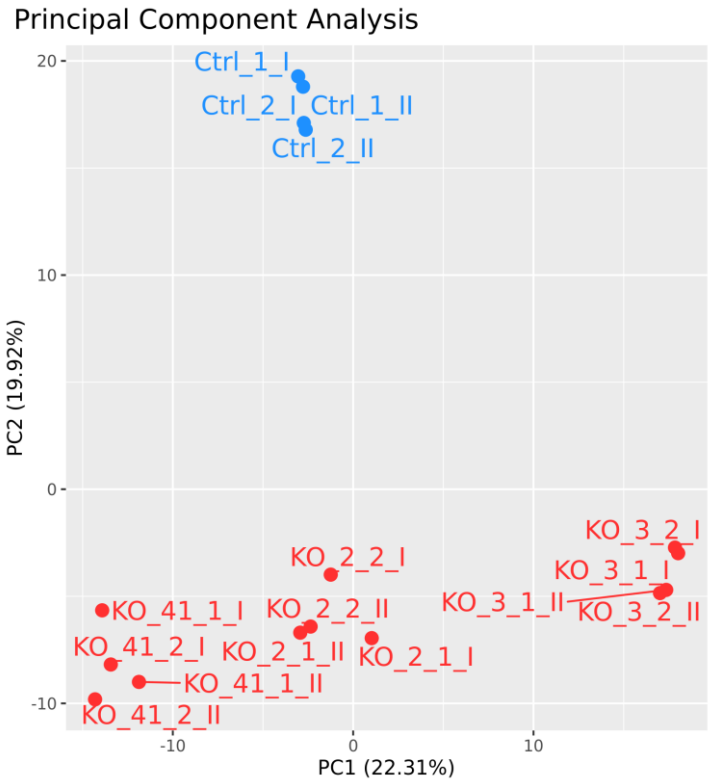
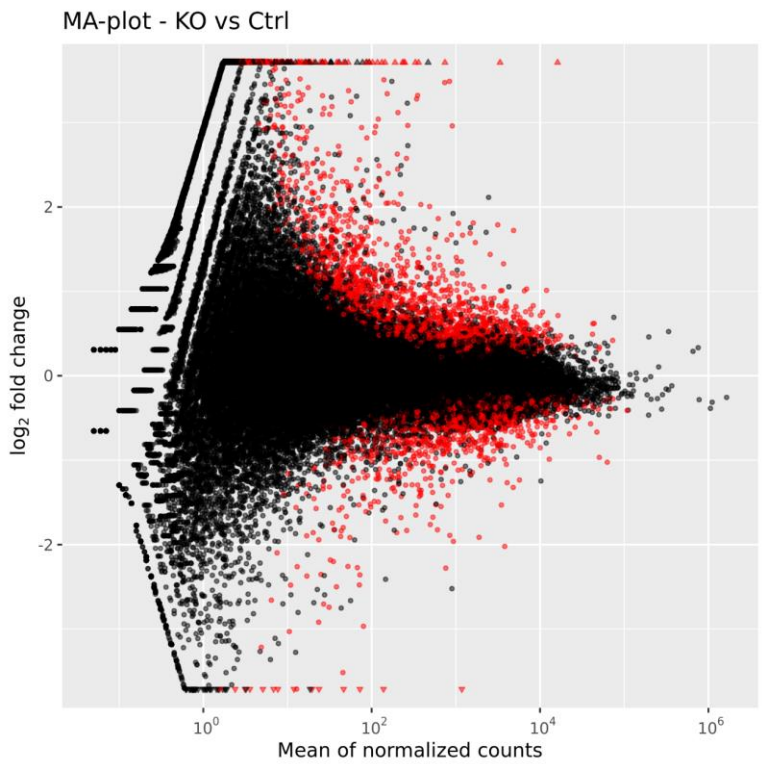
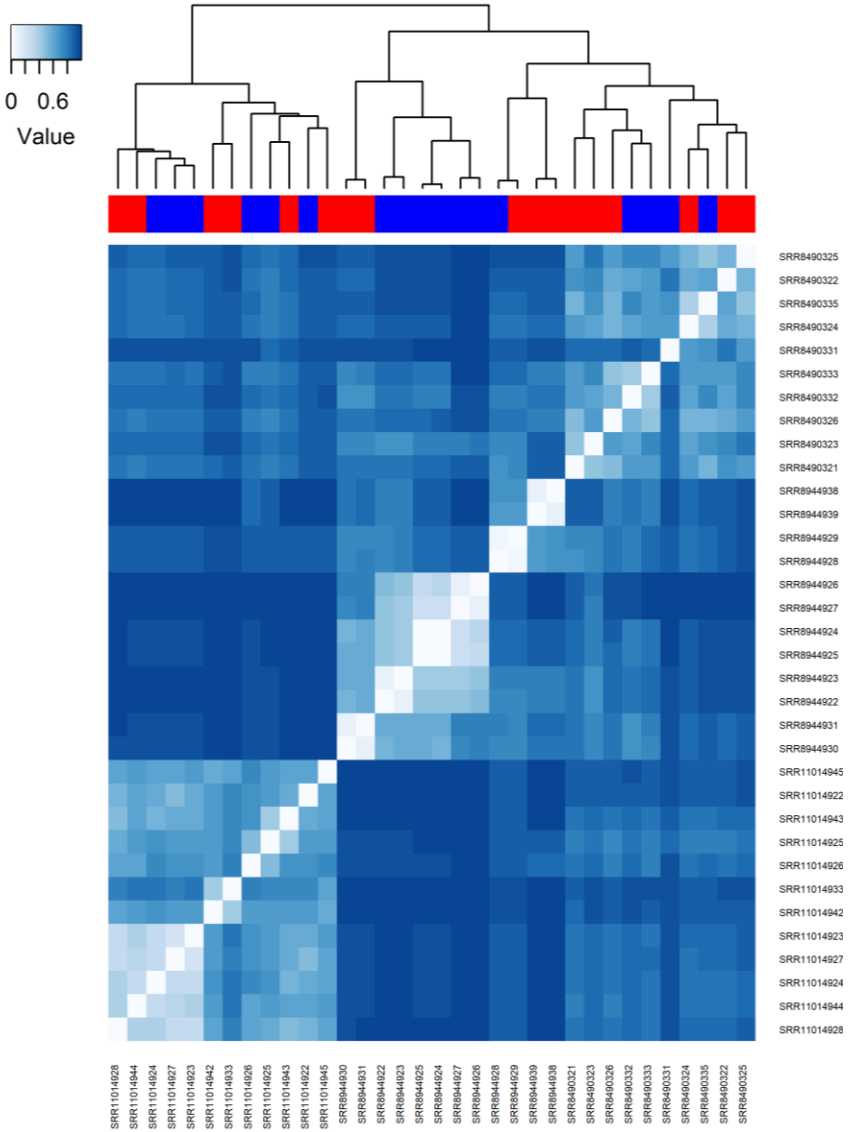
Candida albicans
Aspergillus oryzae
Aspergillus fumigatus
Yarrowia lipolytica
Ustilago maydis
Zygotorulaspora mrakii
Malassezia restricta
Candida dubliniensis
Saccharomyces paradoxus
Botrytis cinerea
Neurospora crassa
Torulaspora delbrueckii
Pichia kudriavzevii
Sugiyamaella lignohabitans
Saccharomyces eubayanus
Talaromyces rugulosus
Tetrapisispora blattae
Fusarium oxysporum
Kluyveromyces lactis
Tetrapisispora phaffii
Pochonia chlamydosporia
Thermothelomyces thermophilus
Colletotrichum higginsianum
Cercospora beticola
Zygosaccharomyces rouxii
Zymoseptoria tritici
Scheffersomyces stipitis
Candida glabrata
Thermothielavioides terrestris
Candida orthopsilosis
Brettanomyces nanus
Kluyveromyces marxianus
Fusarium verticillioides
Eremothecium sinecaudum
Fusarium graminearum
Schizosaccharomyces pombe
Lachancea thermotolerans
Fusarium venenatum
Komagataella phaffii
Pyricularia pennisetigena
Cryptococcus gattii VGI
Ogataea parapolymorpha
Naumovozyma castellii
Kazachstania naganishii
Pyricularia grisea
Naumovozyma dairenensis
Pyricularia oryzae
Kazachstania africana
Fusarium pseudograminearum
Eremothecium cymbalariae
Fusarium fujikuroi
Eremothecium gossypii
Saccharomyces cerevisiae

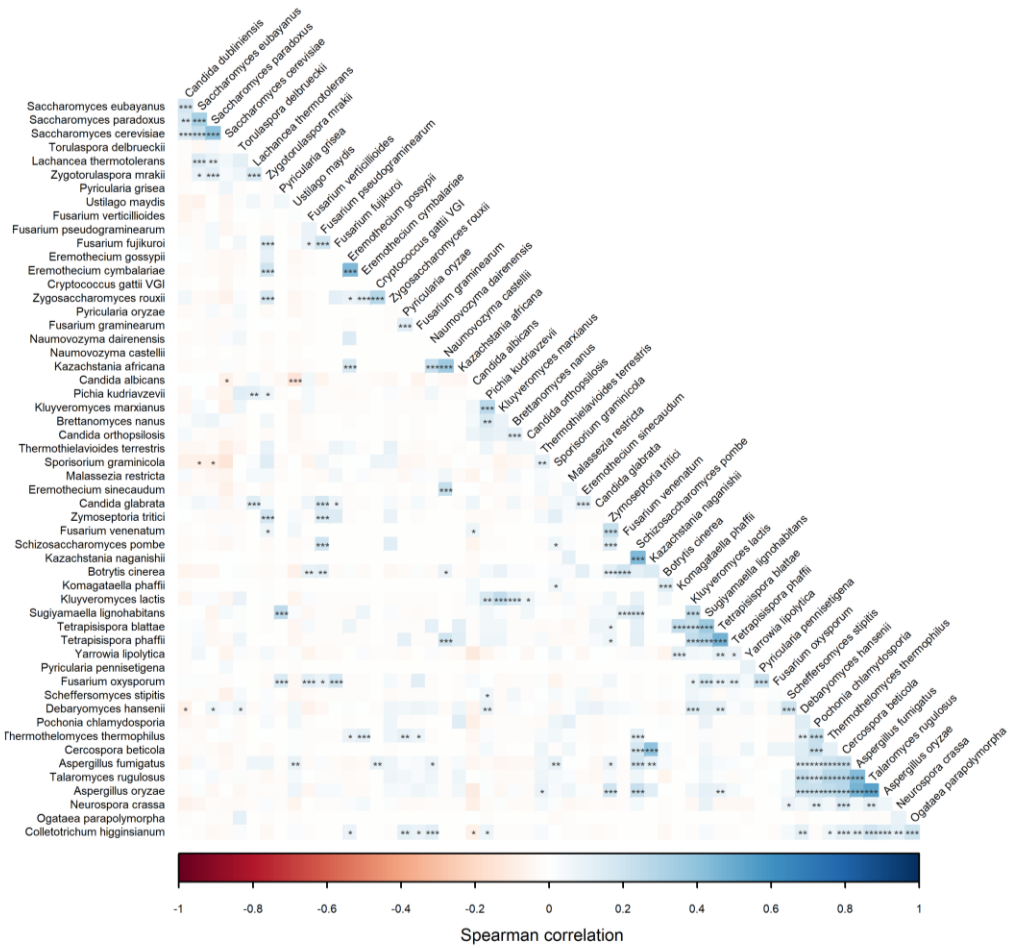
GENE EXPRESSION

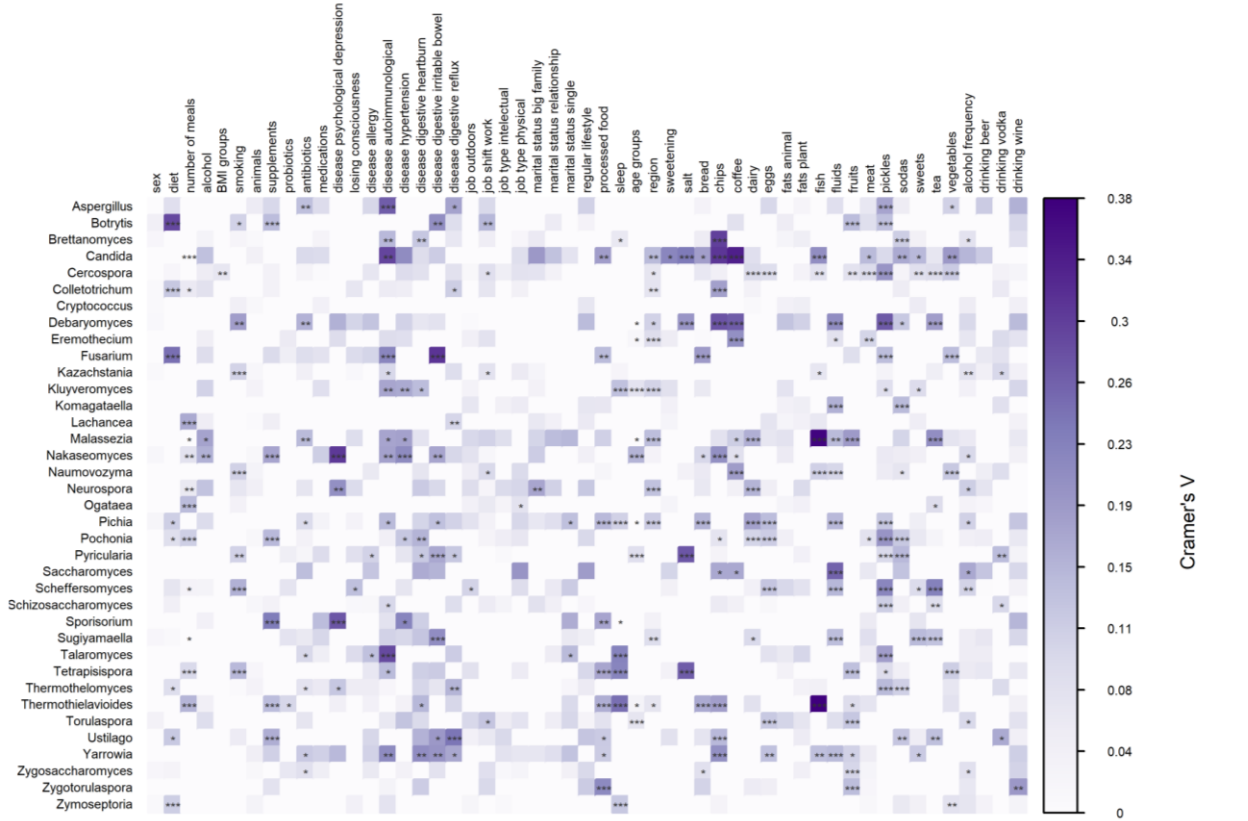




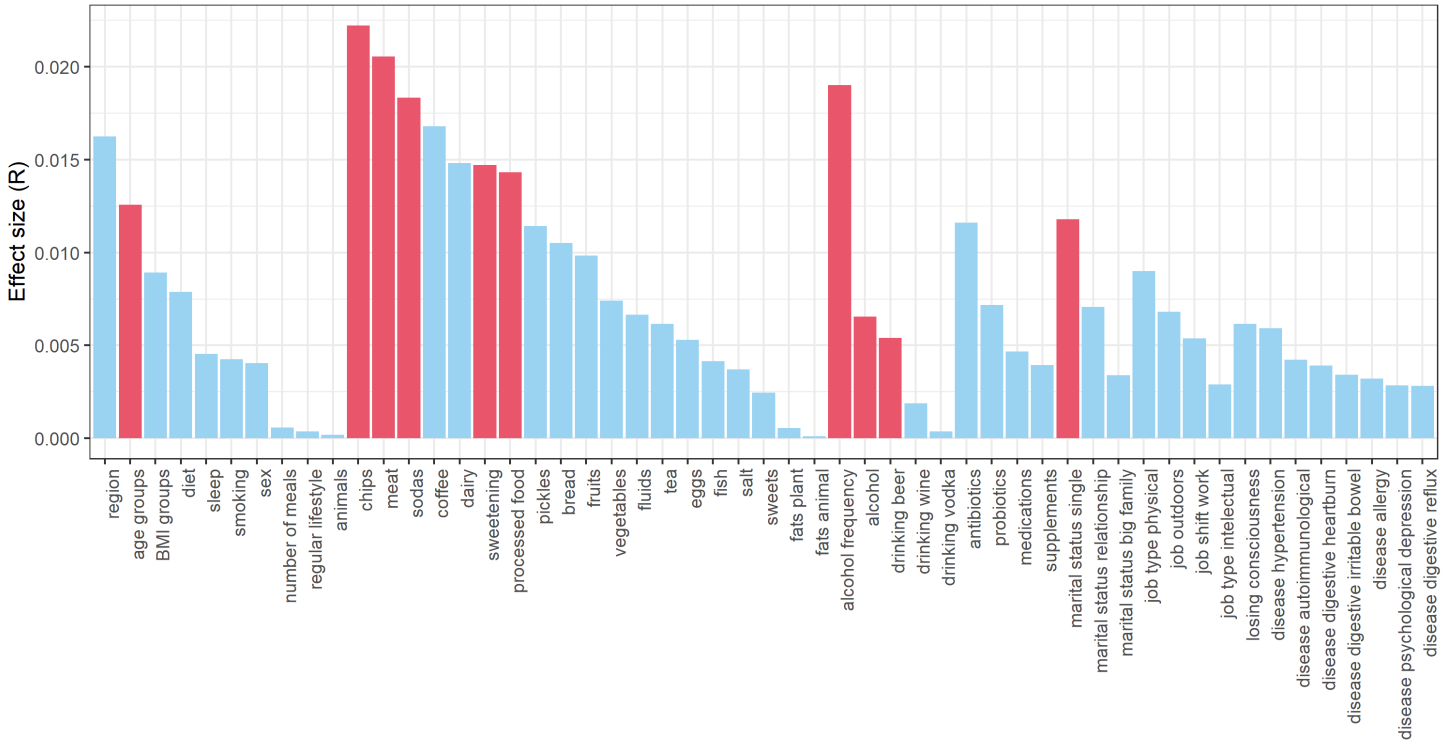


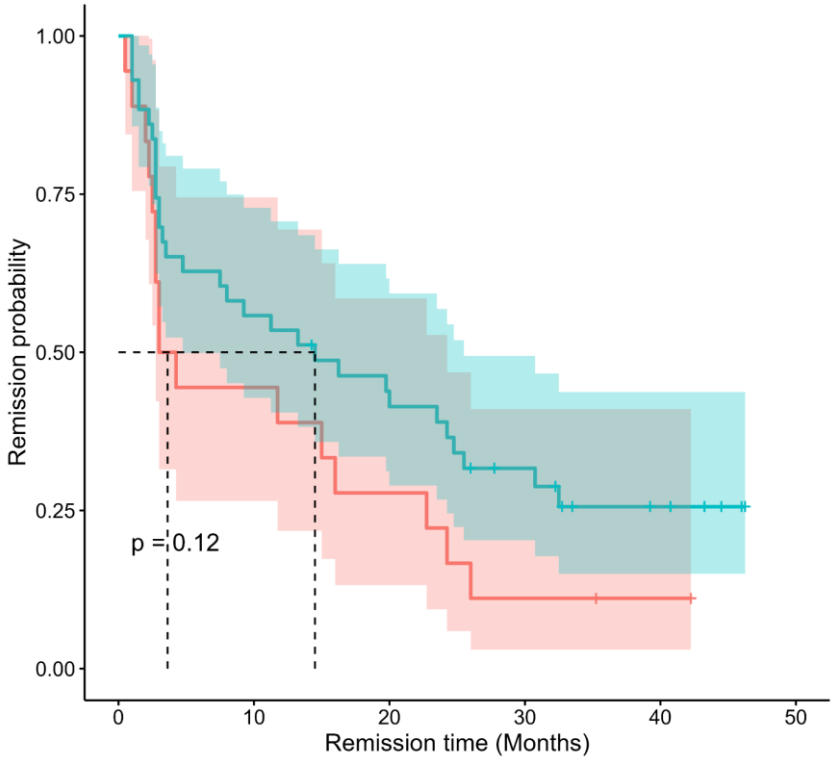
METAGENOMICS

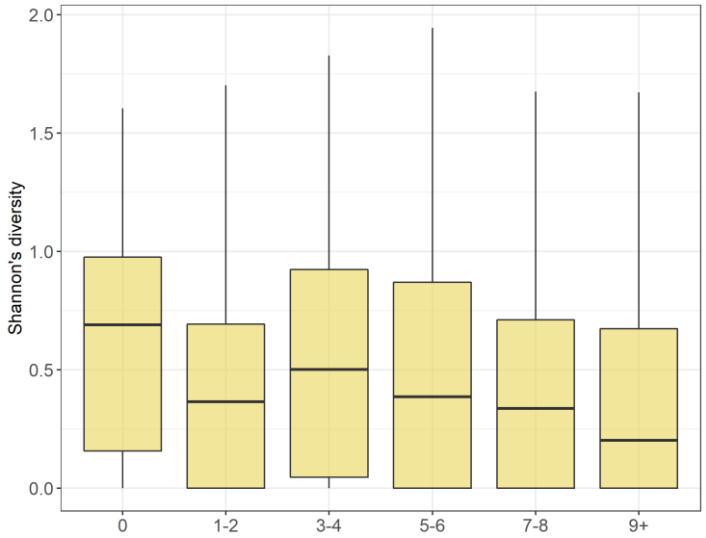




CLINICAL DATA

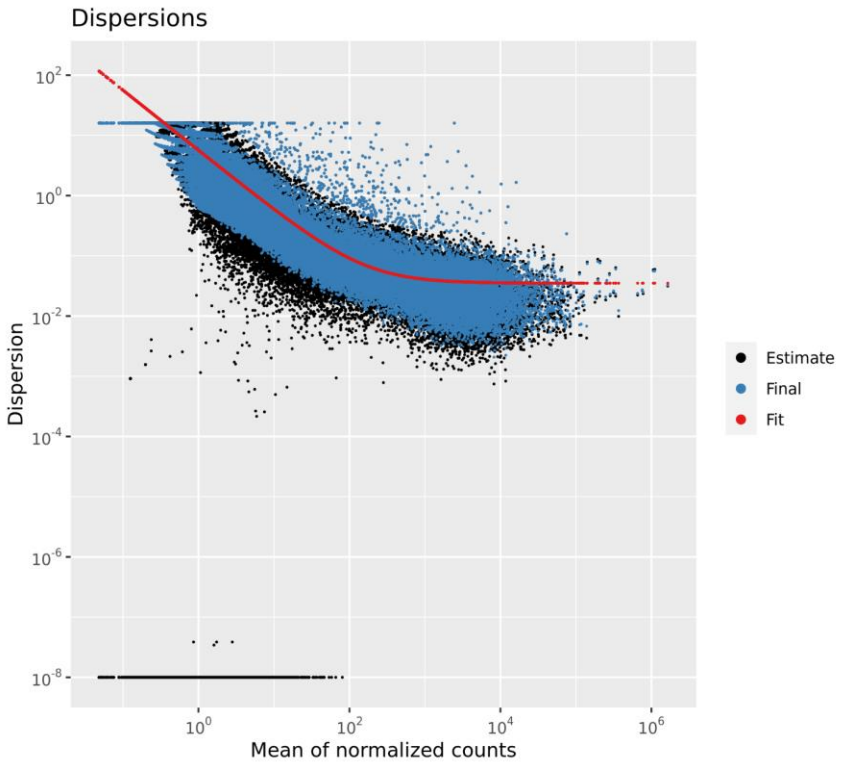


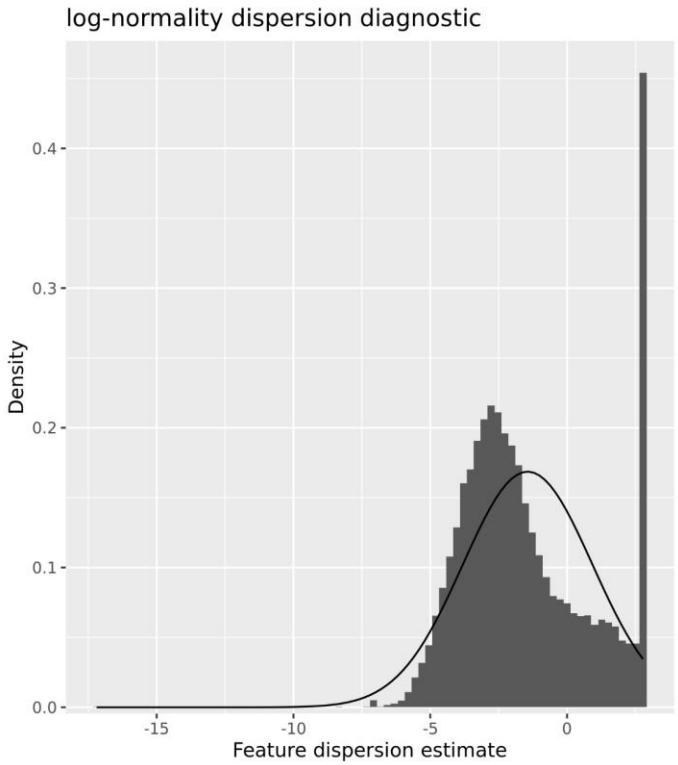


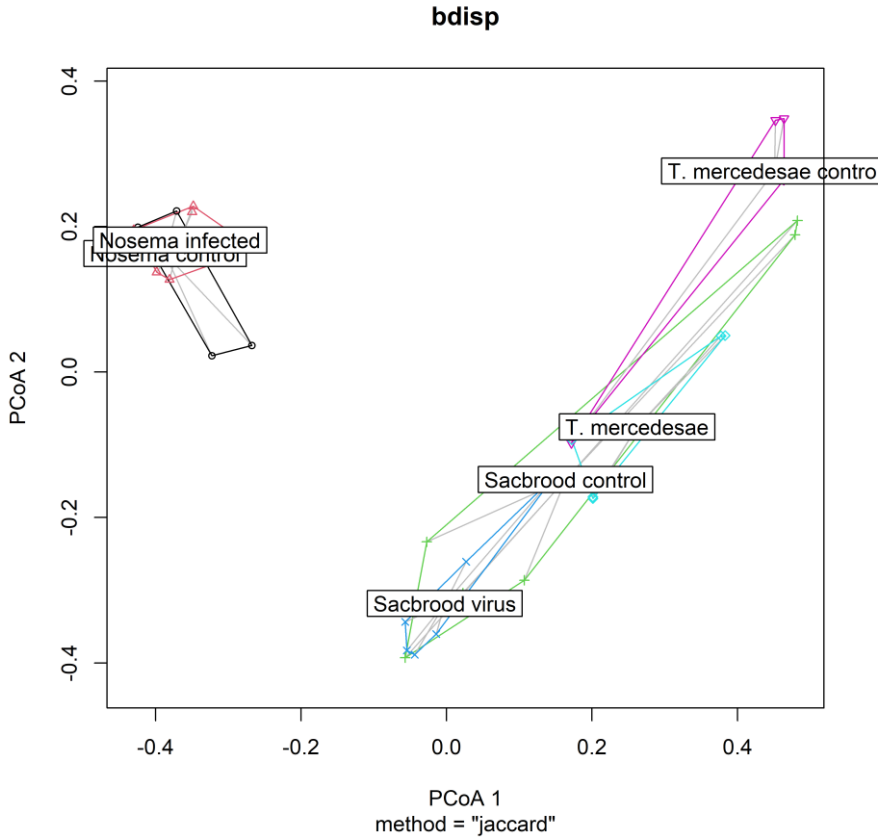


LABORATORY OF BIOINFORMATICS

STATISTICS


Dispersions
Dispersion
Mean of normalized counts
Estimate
Final
Fit


log-normality dispersion diagnostic
Density
Feature dispersion estimate


bdisp
PCoA 2
PCoA 1
method = "jaccard"
Nosema infected
T. mercedesae contro
T. mercedesae
Sacbrood control
Sacbrood virus

OTHERS....

- Data mining
- Big data analyses
- Web services
- Non-standard analyses
- … Or standard, but not popular

HOW WE DO IT

- Published tools
- Python
- R
- Bash
- …
- **PCSS resources**

HOW TO COLABORATE WITH US

1. Talk to us
2. Indicate on the quarterly reports that you use our resources

COLABORATE WITH US

Nucleic Acids Research, 2024, 1–19
https://doi.org/10.1093/nar/gkad1251
RNA and RNA-protein complexes

LINE-1 mRNA 3′ end dynamics shape its biology and retrotransposition potential

Damian M. Janecki[1,†], Raneet Sen[1,†], Natalia Szóstak[2,†], Arkadiusz Kajdasz[1], Martyna Kordyś[1], Kinga Plawgo[1], Dmytro Pandakov[1], Anna Philips[2] and Zbigniew Warkocki[1,*]

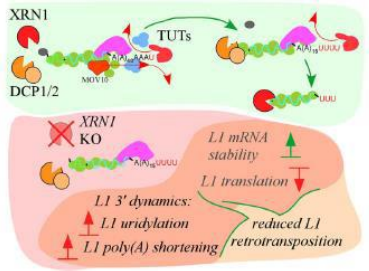
[1]Department of RNA Metabolism, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
[2]Laboratory of Bioinformatics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

*To whom correspondence should be addressed. Tel: +48618528503; Fax: +48618520532; Email: zwarkoc@gmail.com; zwarkocki@ibch.poznan.pl
†The first three authors should be regarded as Joint First Authors.

Abstract

LINE-1 (L1) retrotransposons are mobile genetic elements that create new genomic insertions by a copy-paste mechanism involving *L1* RNA/RNP intermediates. *L1* encodes two ORFs, of which L1-ORF2p nicks genomic DNA and reverse transcribes *L1* mRNA using the nicked DNA as a primer which base-pairs with poly(A) tail of *L1* mRNA. To better understand the importance of non-templated *L1* 3′ ends' dynamics and the interplay between *L1* 3′ and 5′ ends, we investigated the effects of genomic knock-outs and temporal knock-downs of *XRN1*, *DCP2*, and other factors. We hypothesized that in the absence of XRN1, the major 5′→3′ exoribonuclease, there would be more *L1* mRNA and retrotransposition. Conversely, we observed that loss of XRN1 decreased L1 retrotransposition. This occurred despite slight stabilization of *L1* mRNA, but with decreased *L1* RNP formation. Similarly, loss of DCP2, the catalytic subunit of the decapping complex, lowered retrotransposition despite increased steady-state levels of L1 proteins. In both XRN1 and DCP2 depletions we observed shortening of *L1* 3′ poly(A) tails and their increased uridylation by TUT4/7. We explain the observed reduction of L1 retrotransposition by the changed qualities of non-templated *L1* mRNA 3′ ends demonstrating the important role of *L1* 3′ end dynamics in L1 biology.

Graphical abstract



Introduction

Retrotransposons are mobile genetic elements that copy their sequences and insert them in new genomic locations via a mechanism called retrotransposition. Sequencing of the human genome has led to a discovery that nearly half of it derives from repetitive sequences, mostly of retrotransposonal origin (1,2). Retrotransposons in the human genome comprise long-terminal repeat retrotransposons (LTRs), also known as endogenous retroviruses (ERVs), and non-long terminal repeat retrotransposons including long-interspersed elements (*L1*), short-interspersed elements (*Alu*), and a class of *SVA* retrotransposons (3,4). Around 516 000 copies of *L1* occupy nearly

Received: May 11, 2023. **Revised:** December 16, 2023. **Editorial Decision:** December 19, 2023. **Accepted:** December 20, 2023

Nucleic Acids Research, 2024, 1–17
https://doi.org/10.1093/nar/gkae017
Data Resources and Analyses

CMC: Cancer miRNA Census – a list of cancer-related miRNA genes

Malwina Suszynska[1,†], Magdalena Machowska[1,†], Eliza Fraszczyk[1,†], Maciej Michalczyk[2], Anna Philips[2], Paulina Galka-Marciniak[1] and Piotr Kozlowski[1,*]

[1]Department of Molecular Genetics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, 61-704, Poland
[2]Laboratory of Bioinformatics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

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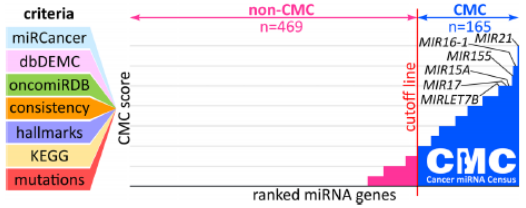
†The first three authors should be regarded as joint First Authors.

Abstract

A growing body of evidence indicates an important role of miRNAs in cancer; however, there is no definitive, convenient-to-use list of cancer-related miRNAs or miRNA genes that may serve as a reference for analyses of miRNAs in cancer. To this end, we created a list of 165 cancer-related miRNA genes called the Cancer miRNA Census (CMC). The list is based on a score, built on various types of functional and genetic evidence for the role of particular miRNAs in cancer, e.g. miRNA–cancer associations reported in databases, associations of miRNAs with cancer hallmarks, or signals of positive selection of genetic alterations in cancer. The presence of well-recognized cancer-related miRNA genes, such as *MIR21*, *MIR155*, *MIR15A*, *MIR17* or *MIRLET7s*, at the top of the CMC ranking directly confirms the accuracy and robustness of the list. Additionally, to verify and indicate the reliability of CMC, we performed a validation of criteria used to build CMC, comparison of CMC with various cancer data (publications and databases), and enrichment analyses of biological pathways and processes such as Gene Ontology or DisGeNET. All validation steps showed a strong association of CMC with cancer/cancer-related processes confirming its usefulness as a reference list of miRNA genes associated with cancer.

Graphical abstract



Introduction

miRNAs are a class of small (18–24nt long), single-stranded noncoding RNA molecules that initiate translation repression and/or mRNA deadenylation, decapping, and degradation by complementary interaction with target sequences usually located in the 3′UTRs of target mRNAs; thus, miRNAs post-transcriptionally regulate (usually downregulate) the expression of most protein-coding genes (1,2). A large body of evidence indicates that miRNAs play an important role in cancer, and many upregulated and downregulated miRNAs are involved in the regulation of different processes in cancer. Among the most well-recognized miRNAs, acting either as oncogenes or tumor suppressors are the let-7 (*MIRLET7*) family, *miR-17-92a-1* cluster (known also as OncomiR-1 or according to HUGO nomenclature *MIR17HG*), miR-21, miR-205, and miR-15a (3,4).

Large cancer-genome projects such as The Cancer Genome Atlas (TCGA) (5,6) or International Cancer Genome Consortium (ICGC) (7) and especially recent whole-genome sequencing efforts such as The Pan-Cancer Analysis of Whole Genomes (PCAWG) (8) increase the potential for identifying cancer-related mutations or cancer-driver genetic elements in noncoding parts of the genome, including genes of miRNAs and other classes of noncoding RNAs. Appropriate reference

Received: June 26, 2023. **Editorial Decision:** December 27, 2023. **Accepted:** January 3, 2024

NAR's 50 years, IBCH PAS in NAR in 2024

LABORATORY OF BIOINFORMATICS

LABORATORY OF BIOINFORMATICS

lab.bioinformatics@ibch.poznan.pl


ICHB PAN

INSTITUTE OF BIOORGANIC CHEMISTRY

Polish Academy of Sciences

Laboratory of Medicinal Chemistry

Centre for Chemical Biology (ERIC)

Dr. Dorota Jakubczyk

Centre for Chemical Biology

Centre for Chemical Biology
Head: Jacek Ł Kolanowski
Research Support Unit, IBCH PAS: dr Michał Gładysz

Laboratory of Molecular Assays and imaging
Head: Dorota Kwiatek

1. High Throughput Screening (HTS)

2. High resolution imaging (MINFLUX)

Laboratory of Medicinal Chemistry
Head: Dorota Jakubczyk

3. Design, synthesis and characterization
- **Bioactive molecules**
- **Targeted chemical libraries**
- **Chemoproteomic probes**

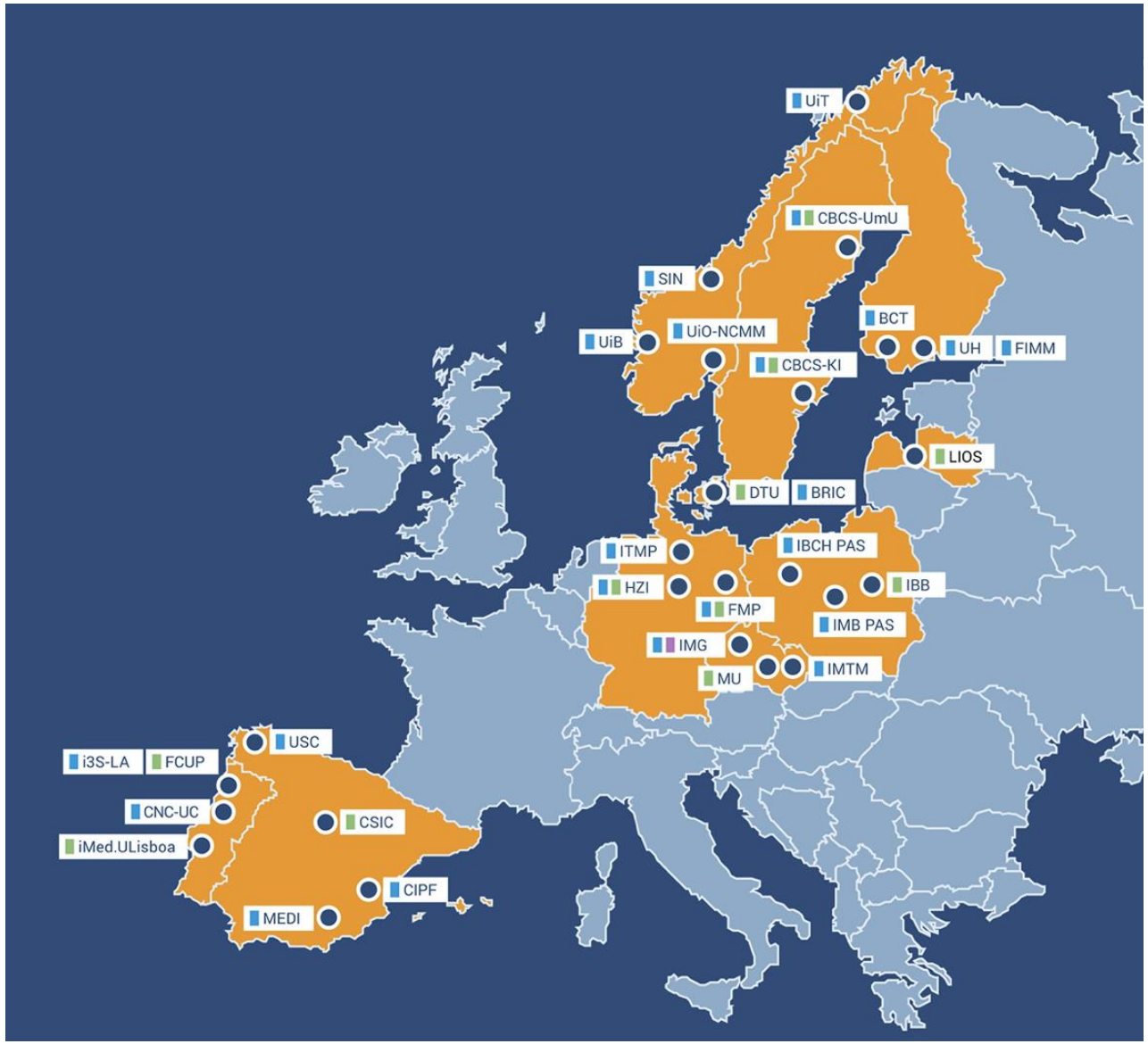
Dept. of Molecular Probes and Prodrugs (DMPP)

Design, synthesis and validation of probes and assays

EU-OPENSCREEN - ERIC

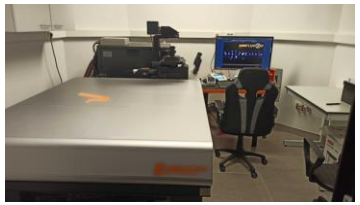
European Research Infrastructure ERIC

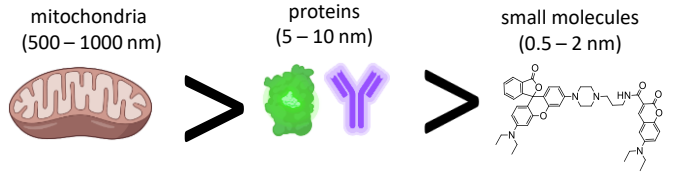
- European Road Map of Research Infrastructure ESFRI
- Dedicated funding in Horizon Europe
- 33 certified partner sites in 10 member countries
- Expertise (partner sites)
  - HTS Screening & Fragment Screening
  - Med chem
  - Chemoproteomics (in formation)
- European Chemical Biology Database
- EU-OS Chemical Compounds Library
  - 100 000 commercial cpds (20kEUR)
  - 40 000 academic cpds
  - Ca. 1000 fragments

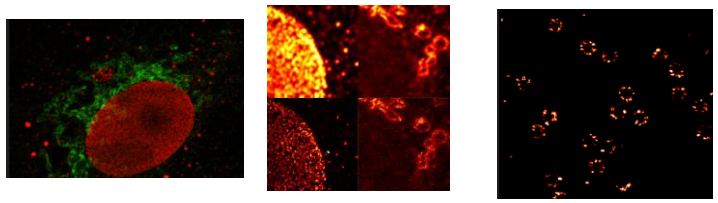


Centre for Chemical Biology

MOLECULAR IMAGING PLATFORM







Imaging technologies available on our platform:

1) Stimulation Depletion Microscopy (STED) - 2D and 3D imaging, multicolor imaging
2) MINFLUX (Minimal Photon Flux) microscopy - 2D and 3D, multicolor imaging, tracking
3) FLIM (Fluorescence Lifetime Imaging) microscopy
4) FCS microscopy (Fluorescence Correlation Spectroscopy microscopy)

VALIDATION/MECHANISM DECONVOLUTION

SCREENING PLATFORM

Assay development
- Single and multi-parametric assays
- Miniaturisation and optimisation
- Automation and assay transfer
- Orthogonal assay

Assay types
- Biochemical assays:
  - Absorbance
  - Fluorescence and derivatives
  - Luminescence
- Cell based assays:
  - Target based (ex. Reporter assay)
  - Phenotypic (ex. Toxicity)
  - High Content Screening
- Format: 384 well plates

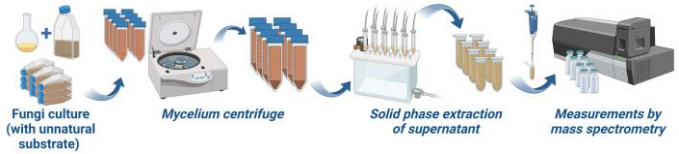
Combinatorial screening (AI – assisted)
- Rapid optimisation of complex mixtures
  - buffers and media
  - drug combinations
  - formulations

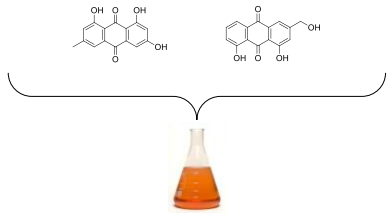
High Throughput Screening
- Primary screening (1k – 100k)
- Hit validation:
  - 3 concentrations
  - IC/EC50 determination
- Counter / orthogonal screening

Examples of screenig assays:
- Cell painting (description below)
- Bioprofiling

SCREENING

MEDICINAL CHEMISTRY PLATFORM





Medicinal Chemistry
- "Hit to lead" - design and synthesis of hit analogues (targeted libraries / HIT molecules optimisation)
- Chemoinformatic design – including selection of already existing libraries for bioactivity tests
- Combination of organic synthesis, semi-synthesis, biocatalysis, biosynthesis and chemical programming of microorganisms
  - E.g. for difficult to make **natural products** analogues
  - Scaling up production of natural compound analogues

- Process automation
- Automated isolation and purification of chemical compounds - from biological material or enzymatic reactions
- Development of chemical probes for pull-down experiments & imaging

HIT OPTIMISATION

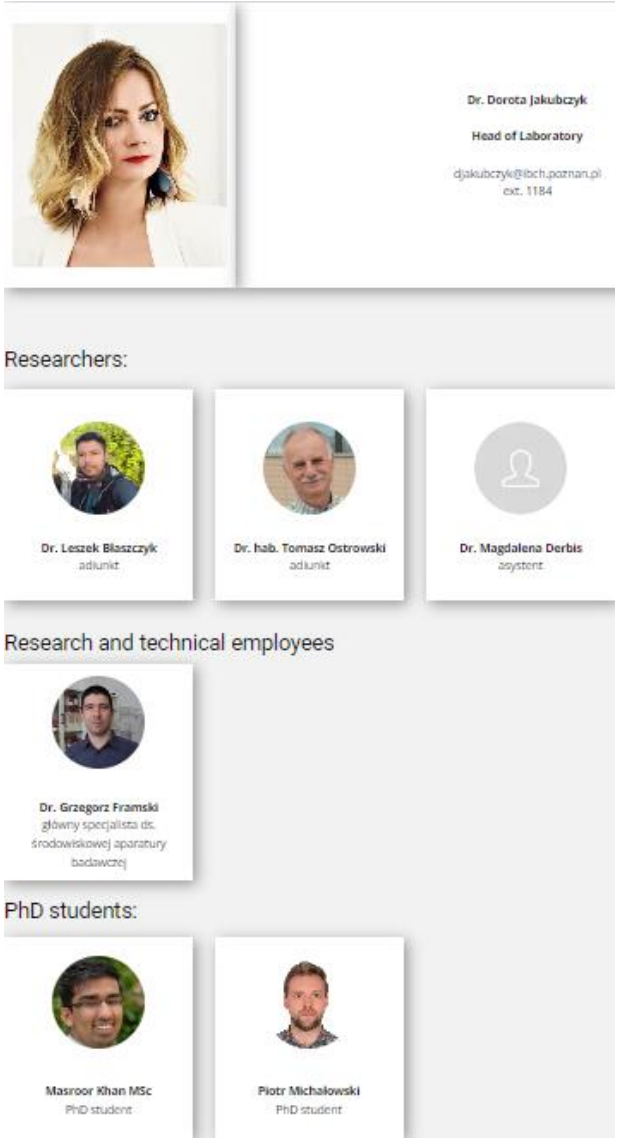
Laboratory of Medicinal Chemistry

Head of Laboratory
Dr. Dorota Jakubczyk

Researchers:
Dr. hab. Tomasz Ostrowski
Dr. Leszek Błaszczyk
Dr. Magdalena Derbis – from DMPP
Dr. Vanrajsinh Thakor - from mid March

Research and technical employees:
Dr. Grzegorz Framski

PhD students:
Masroor Khan M.Sc. – from DMPP
Piotr Michałowski M.Sc. Eng. - Poznan University of Technology



Laboratory of Medicinal Chemistry

Services:

- “Hit to lead” - design and synthesis of hit analogues (targeted libraries / HIT molecules optimisation)
- Chemoinformatic design – including selection of already existing libraries for bioactivity tests
- Combination of organic synthesis, semi-synthesis, biocatalysis and biosynthesis
    - E.g. for difficult to make **natural products** analogues
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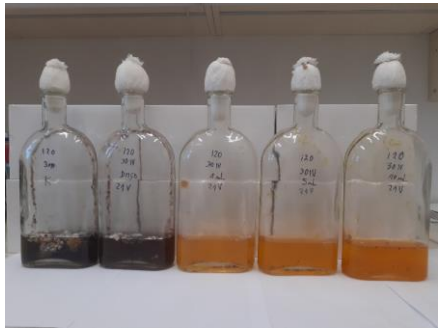


https://www.shutterstock.com/search/flower-flask

Laboratory of Medicinal Chemistry

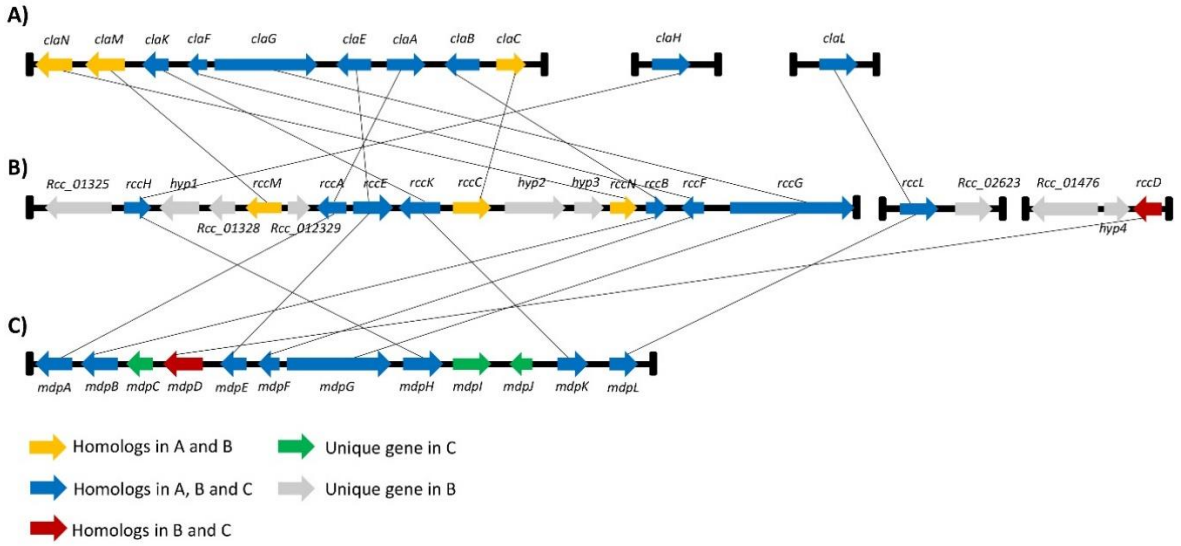
New services

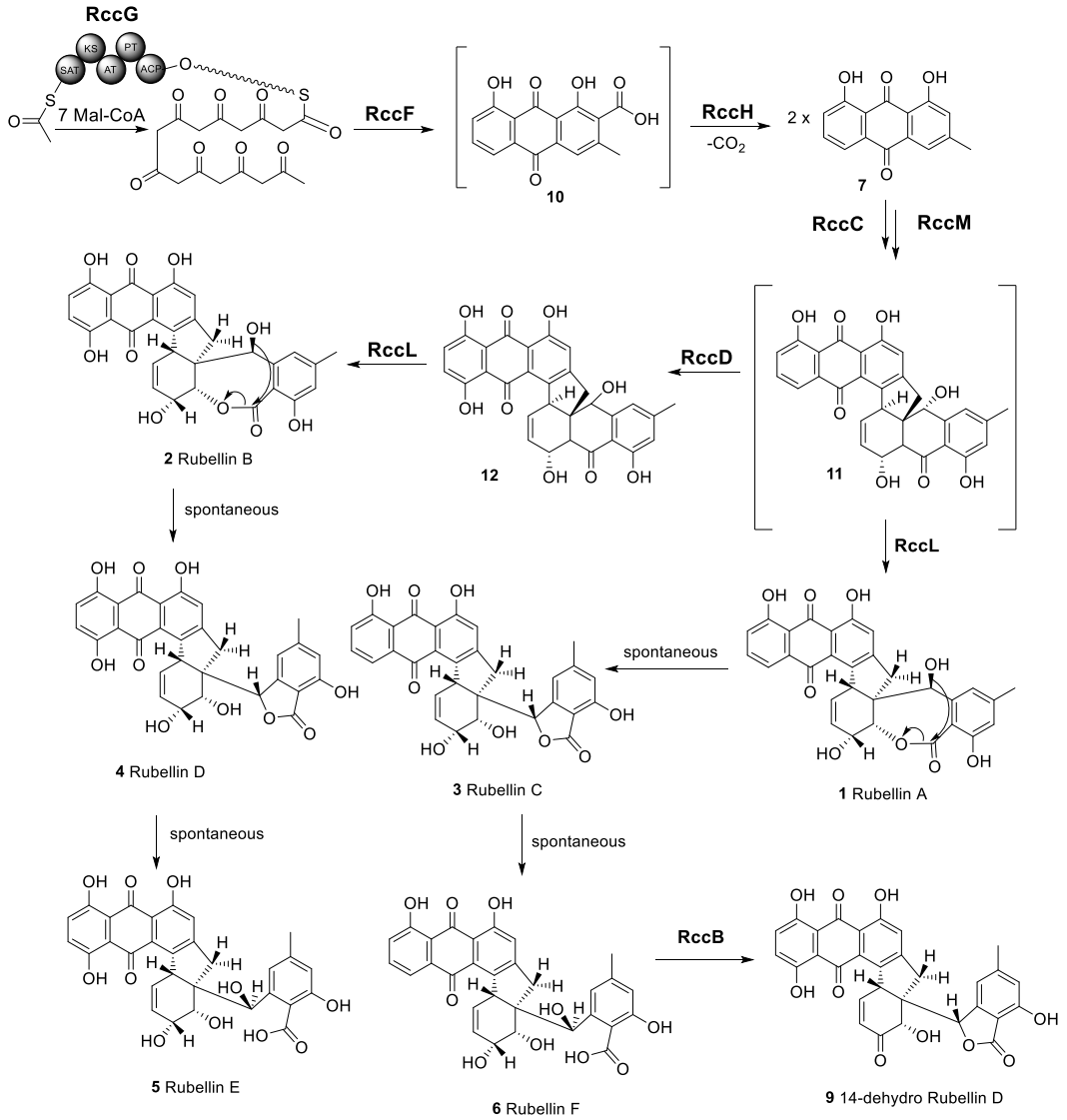
- Design and synthesis of RNA using chemical and biochemical methods along with structural and functional characterization of RNA molecules to understand their role in biological processes (Dr. Leszek Błaszczyk)

- Synthesis of purine and pyrimidine base-modified or/and sugar-modified nucleoside analogues, 1,2,3-triazole nucleosides, as well as quaternary ammonium salts, focused on compounds with required physicochemical properties and potential biological activities (Dr. hab. Tomasz Ostrowski, Dr. Grzegorz Framski)

OUR TECHNOLOGY: chemical programming of microorganisms

Proposed pathway and discovered gene cluster for rubellins biosynthesis





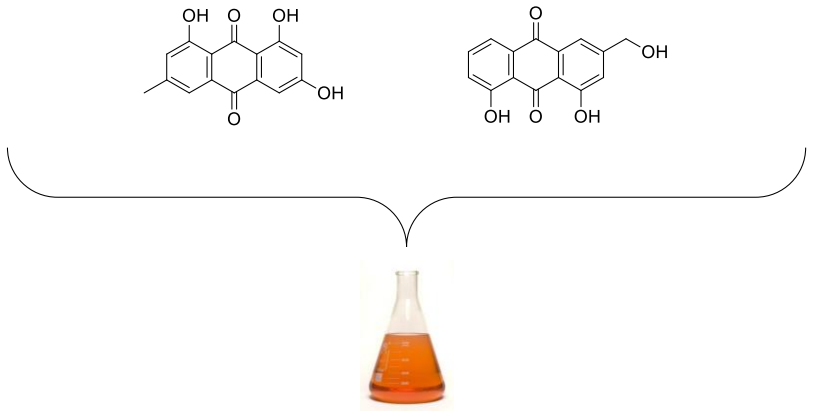
Dorota Jakubczyk
Francois Dussart

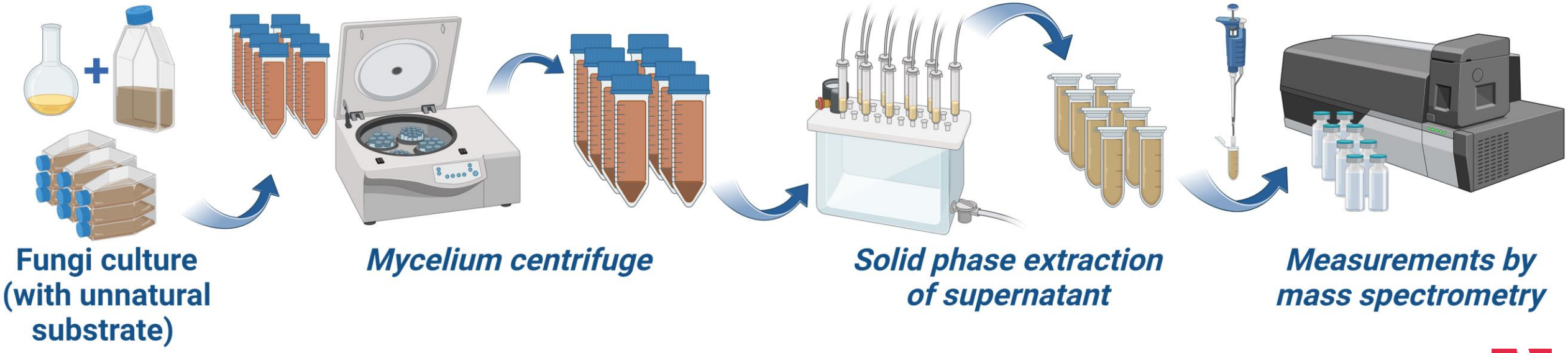
Grant Miniatura 4 by National Science Centre (NCN) No: 2020/04/X/NZ1/01959 – Dr. Dorota Jakubczyk

OUR TECHNOLOGY: chemical programming of microorganisms

Optimisation of bio-production of bioactive natural products

- **Chemical programming** of fungus *Ramularia collo-cygni*





Grant Miniatura 4 by National Science Centre (NCN) No: 2020/04/X/NZ1/01959 – Dr. Dorota Jakubczyk

Users Projects

Dr hab. K. Brzeziński

Identification of the products of the enzymatic reaction of SAHase from Pseudomonas aeruginosa carried out in the presence of transition metal ions

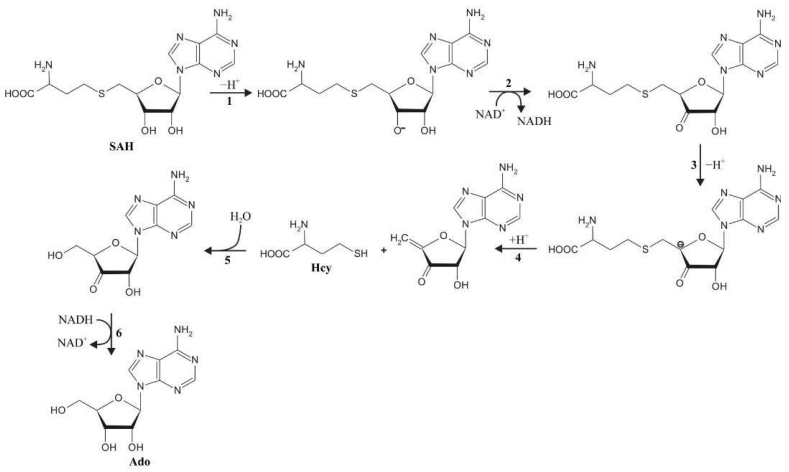


Figure 6
Mechanism of SAH hydrolysis to adenosine (Ado) and L-homocysteine (Hcy) catalyzed by *S*-adenosyl-L-homocysteine hydrolase. The numbers 1–6 indicate the reaction steps described in the mechanism of SAH hydrolysis (see text).

Glu205. As a result, the C3′–H H atom becomes more labile and hydride abstraction by the NAD^+ cofactor is facilitated (2). The products of this reaction step are 3′-keto-AdoHcy and NADH. The acidity of the C4′—H group of the 3′-keto derivative is higher when compared with the Ado molecule, allowing the formation of a $C4'^-$ carboanion through proton transfer to the carboxylic group of Asp139 (3). Proton transfer from the imidazole ring of His62 to the S^δ atom of the 3′-keto-AdoHcy carboanion is followed by β-elimination of Hcy, leading to the formation of 3′-keto-4′,5′-didehydroadenosine (4). As the final step, a water molecule is attached through Michael-type addition (5); consequently, the 3′-keto group is reduced and Ado and NAD^+ are generated (6).

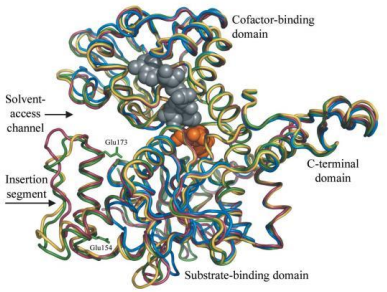


Figure 7
Superposition of the LlSAHase protomer (green) with models of the enzymes from *P. falciparum* (yellow; PDB entry 1v8b), *M. tuberculosis* (raspberry; PDB entry 3ce6) and *H. sapiens* (blue; PDB entry 1li4). The highly conserved Glu154 and Glu173 located in the 40-residue insert of LlSAHase are shown as sticks. Ligands are shown as space-filling models using orange (adenosine) and gray (cofactor) colors.

3.5. The plant-specific insert and the substrate-pocket access channel

An insert of about 40 amino-acid residues is present in all plant SAHases and also in a number of other eukaryotic and bacterial enzymes (Stępkowski *et al.*, 2005). In LlSAHase this segment forms a solvent-exposed region in the catalytic domain (Fig. 7) in an analogous way as in the plasmodial (PDB entry 1v8b) and bacterial (PDB entry 3ce6) enzymes (Tanaka *et al.*, 2004; Reddy *et al.*, 2008). Despite its conserved topology in the protein fold, the insert is in fact the area of the main structural differences between these proteins. The bacterial inserts are several residues shorter, leading to structural divergence. Moreover, differences in the conformation of this surface element could also result from different crystal-packing contacts.

Users Projects

Completed projects:

Prof. dr Paul Schulze-Lefert - Max Planck Institute for Plant Breeding Research - Synthesis and characterization of oxidized fraxetin to investigate the formation of the plant- and microbial-driven coumarin redox cycle (DFG, Germany's Excellence Strategy—EXC 2048/1—Project ID: 390686111)

Dr. hab. K. Brzeziński - Identification of the products of the enzymatic reaction of SAHase from Pseudomonas aeruginosa carried out in the presence of transition metal ions (NCN SONATA BIS nr UMO-2018/30/E/NZ1/00729)

Dr. hab. Miłosz Ruszkowski - Identification of human δ1-pyrroline-5-carboxylate reductase (PYCR1) products (NCN Opus 2021/43/B/NZ7/01611)

Dr. hab. Jacek Kolanowski (POL-OS, Sonata 2017/26/D/NZ1/01234) – Data base creation; Kinetics of bioluminescent probes

Users Projects

Ongoing projects:

Ewa Sobieszczuk-Nowicka, Magdalenia Arasimowicz-Jelonek – Biology Department AMU – synthesis and identyficatin of plant metabolites

Arleta Sierakowska (Miniatura), Chemistry Department AMU – MedChem on new caffeine analogues

Jacek Kolanowski, Dorota Kwiatek – EU-OS (Drive 2 - HORIZON-INFRA-2023-DEV-01-03) - chemoinformatics

Grant proposals (last stage of evaluation):

Jacek Kolanowski, Dorota Kwiatek - Development of the infrastructure POL-OPENSCREEN 2

Dorota Kwiatek, Jacek Kolanowski - Maintenance of the infrastructure: SPUB

Miłosz Ruszkowski – Sonata Bis

Summary of the offer

- **SCREENING (high throughput)**
  - Absorbance, fluorescence, bioluminescence and variants + imaging
  - Biochemical (protein / RNA) and cellular (mammalian and plants), advanced models (3D) Multiparametric tests
- **SUPER-RESOLUTION IMAGING**
  - MINFLUX (2 nm, only one of few in the world, localisation or particle tracking)
  - Multicolour STED (50-30 nm)
  - FRET / Lifetime / FCS
- **MEDICINAL CHEMISTRY**
  - (Bio)synthesis and optimisation of analogues of BIOACTIVE MOLECULES and scale-up
  - Design of dedicated libraries and their synthesis
  - Synthesis of multi-parametric molecular probes

Other services types of the Centre:

Grant proposal preparation

Experiment design

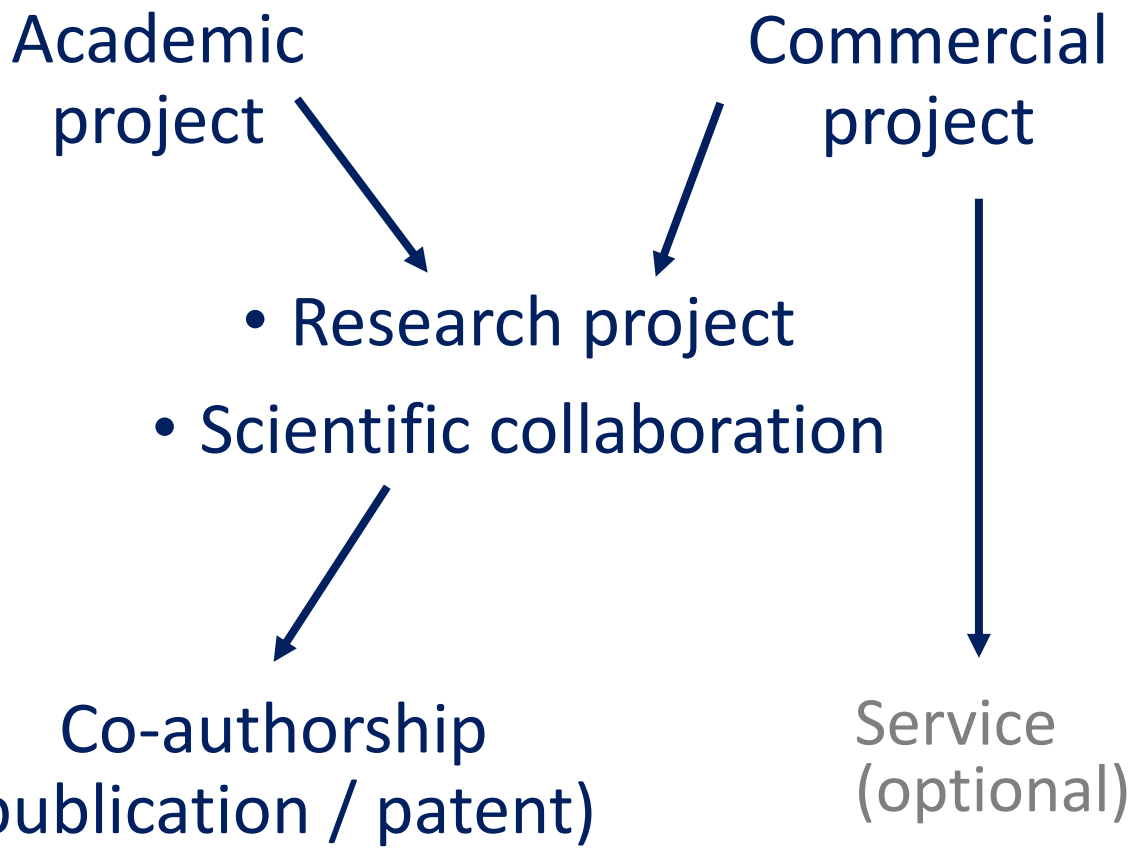
Sample preparation or supervision

Data analysis

Types of activities
- R&D serivces
- Tech development
- Consulting
- Infrastructure collab
- Outreach

Aims / results
- Bioactives
- Tools for imaging and research
- Understanding mech of action

How to work with us



Price depends on:

- Type of collaboration
- Type of experiment
- Number of compounds to screen
- User hands on engagement

Documents:

- Application form
- User agreement

We have EXPERIENCE in and can SCIENTIFICALLY CONSULT on the preparation of joint grant proposals: >35 since 2020 (70% success rate)

Get in touchwith us ☺

Laboratory of Medicinal Chemistry

Dr. Dorota Jakubczyk
djakubczyk@ibch.poznan.pl
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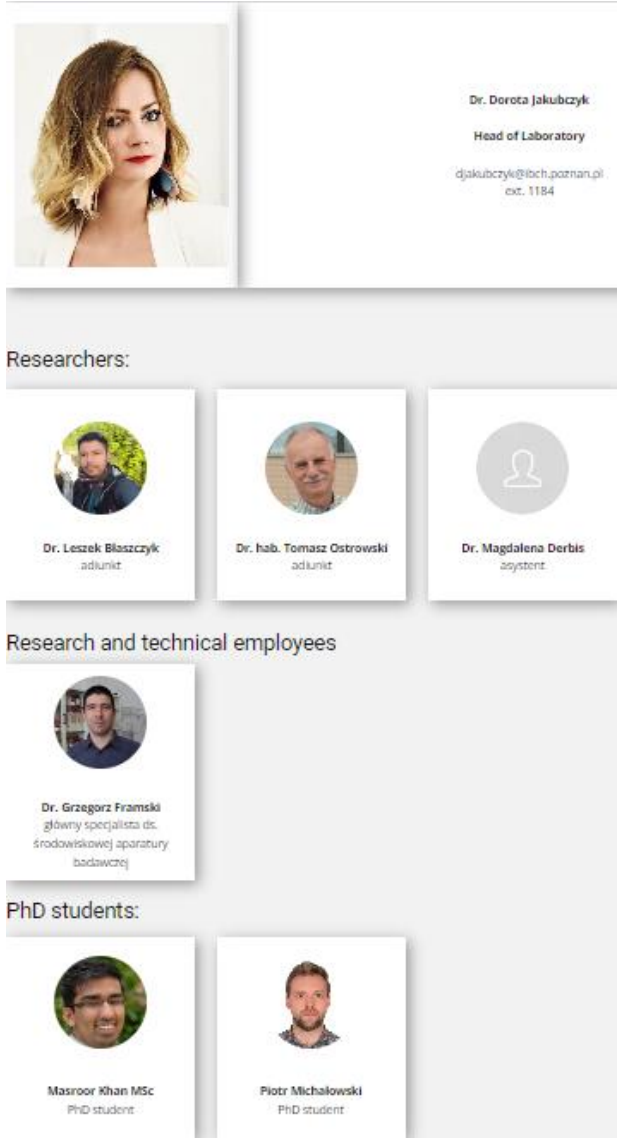
Webpages:
https://chembio.pl/
https://portal.ichb.pl/pracownia-chemii-medycznej/




ICHB PAN

Laboratory of Genomics

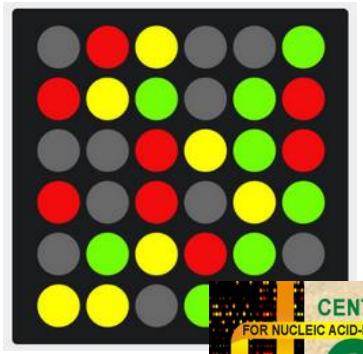
Luiza Handschuh

Presenting: Małgorzata Marcinkowska-Swojak

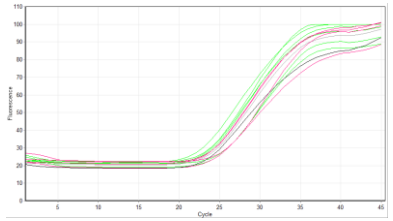
Poznań, 06.02.2024

Brief history

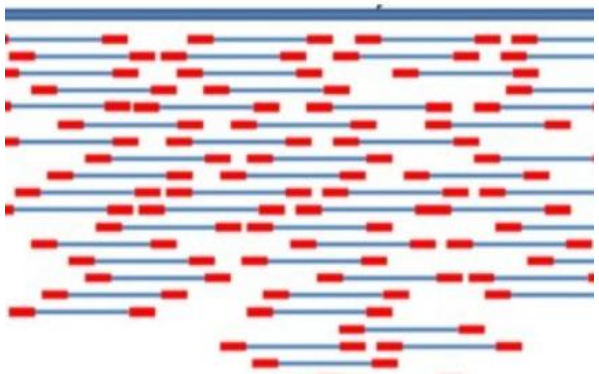
2004
microarrays



2010
quantitative PCR



2010
next generation sequencing

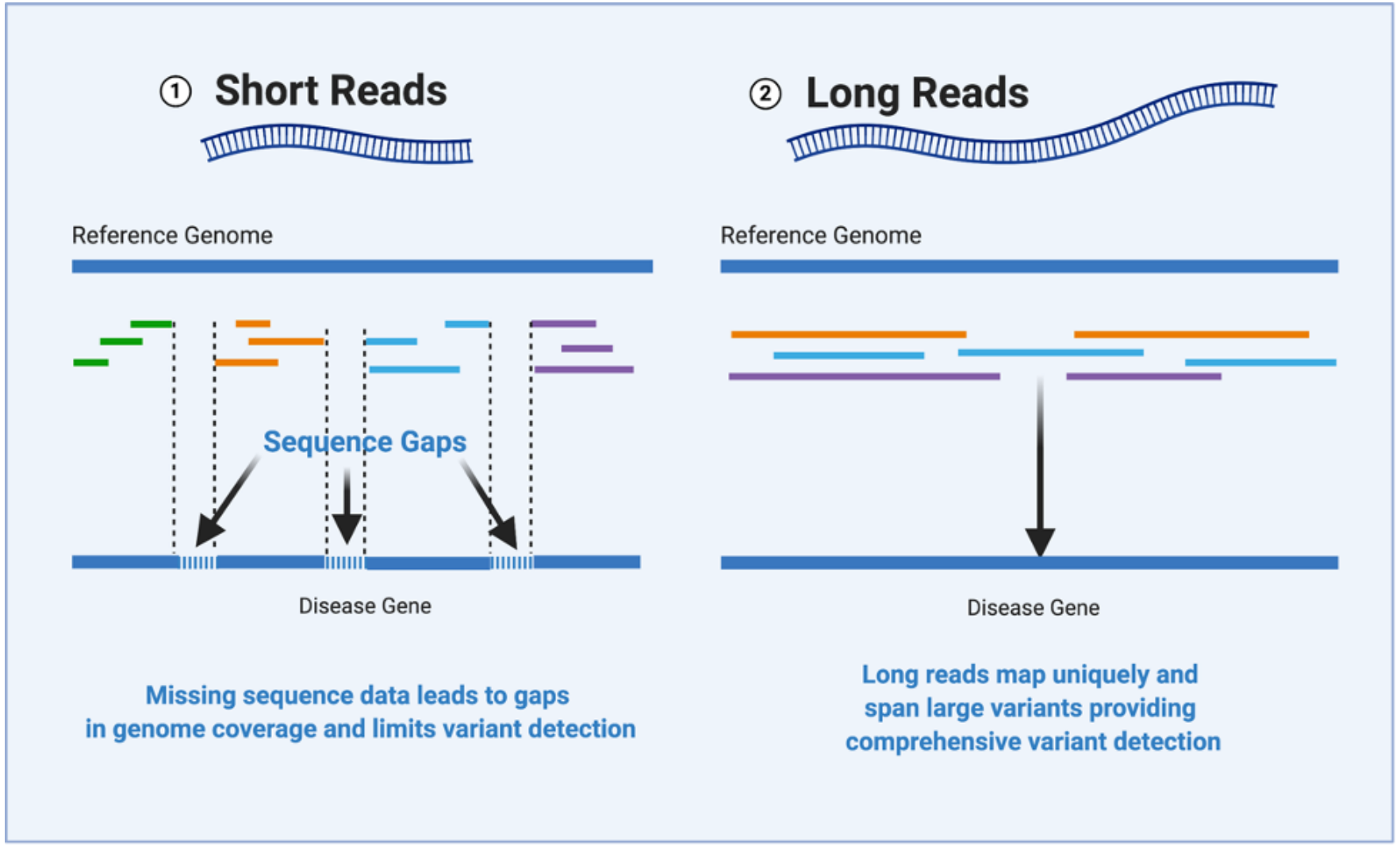


Laboratory of Genomics

2019

Laboratory of Microarrays and Deep Sequencing
established in January 2013

Second and third generation sequencing



2022/2023 - **five** sequencing systems

Illumina sequencing systems

NextSeq 550

MiSeq (dx)

NovaSeq 6000

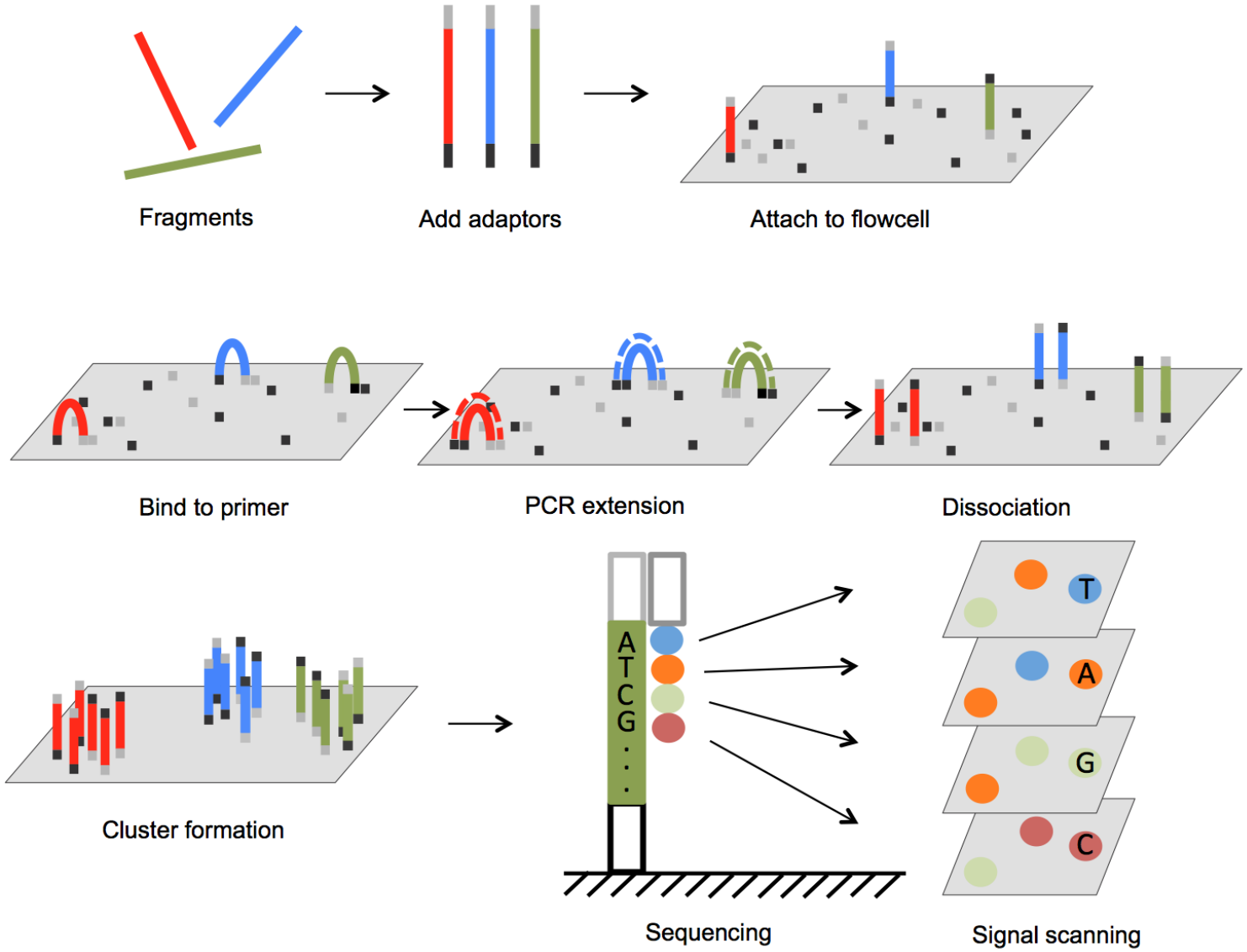
NovaSeq X Plus

2022/2023 - **five** sequencing systems

Pacific Biosciences sequencing systems

Sequel IIe

Illumina sequencing



Illumina sequencers

Benchtop

MiSeq Series

NextSeq 550 Series

Popular Applications & Methods	Key Application	Key Application
Large Whole-Genome Sequencing (human, plant, animal)		
Small Whole-Genome Sequencing (microbe, virus)	●	●
Exome & Large Panel Sequencing (enrichment-based)		●
Targeted Gene Sequencing (amplicon-based, gene panel)	●	●
Single-Cell Profiling (scRNA-Seq, scDNA-Seq, oligo tagging assays)		●
Transcriptome Sequencing (total RNA-Seq, mRNA-Seq, gene expression profiling)		●
Targeted Gene Expression Profiling	●	●
miRNA & Small RNA Analysis	●	●
DNA-Protein Interaction Analysis (ChIP-Seq)	●	●
Methylation Sequencing		●
16S Metagenomic Sequencing	●	●
Metagenomic Profiling (shotgun metagenomics, metatranscriptomics)		●
Cell-Free Sequencing & Liquid Biopsy Analysis		●

Production-scale

NovaSeq 6000 Series

NovaSeq X Series

Popular Applications & Methods	Key Application	Key Application
Large Whole-Genome Sequencing (human, plant, animal)	●	●
Small Whole-Genome Sequencing (microbe, virus)	●	●
Exome & Large Panel Sequencing (enrichment-based)	●	●
Targeted Gene Sequencing (amplicon-based, gene panel)	●	●
Single-Cell Profiling (scRNA-Seq, scDNA-Seq, oligo tagging assays)	●	●
Transcriptome Sequencing (total RNA-Seq, mRNA-Seq, gene expression profiling)	●	●
Chromatin Analysis (ATAC-Seq, ChIP-Seq)	●	●
Methylation Sequencing	●	●
Metagenomic Profiling (shotgun metagenomics, metatranscriptomics)	●	●
Cell-Free Sequencing & Liquid Biopsy Analysis	●	●

Illumina sequencers

MiSeq Series

NextSeq 550 Series

NovaSeq 6000 Series

NovaSeq X Series

1x36 bp - 2x300bp	1x75 bp - 2x150 bp	1x35 bp - 2x250 bp	1x100 bp - 2x150 bp
540 Mb -15 Gb	16,25 - 120 Gb	60 - 3000 Gb	165 Gb - 16 Tb
1 - 50 M reads	130 - 800 M reads	650 M - 20 B reads	1,6 B - 52 B reads
4 - 56 hrs	11 - 29 hrs	13 - 44 hrs	17 - 48 hrs
4 flowcell types	2 flowcell types	4 flowcell types	3 flowcell types
2 100 – 11 100 zł	**7 900 – 33 000 zł**	**13 700 – 91 000 zł**	**11 500 – 96 900 zł**

Illumina sequencers

NextSeq 550 System High-Output Kit	NextSeq 550 System Mid-Output Kit
1 human whole genome	3 exomes
12 exomes	12 enrichment panels
16 transcriptomes	96 amplicon panels

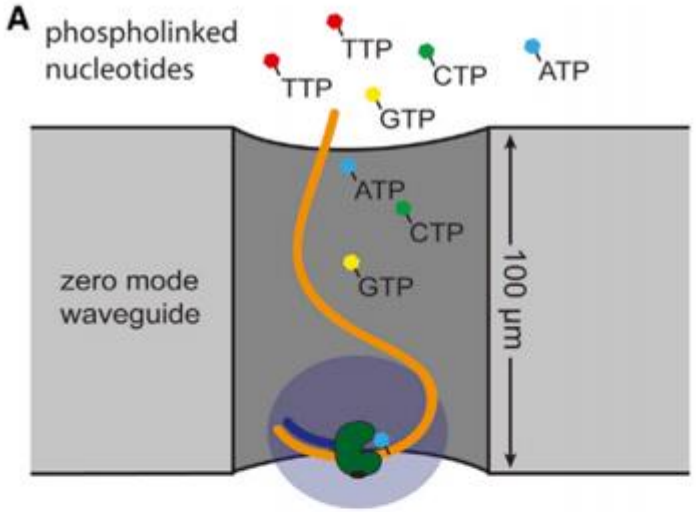
NovaSeq 6000 System

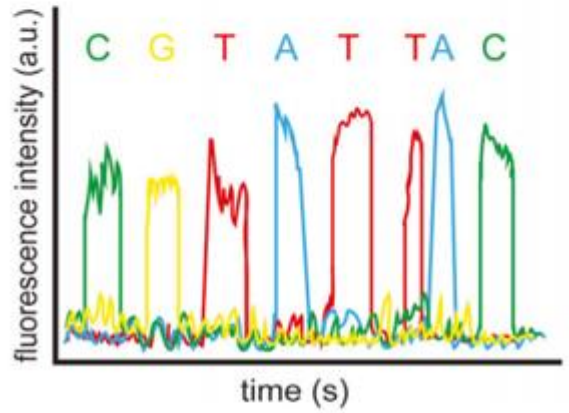
Flow Cell Type	SP	S1	S2	S4
Human Genomes per Run	~4	~8	~20	~48
Exomes per Run	~40	~80	~200	~500
Transcriptomes per Run	~32	~64	~164	~400

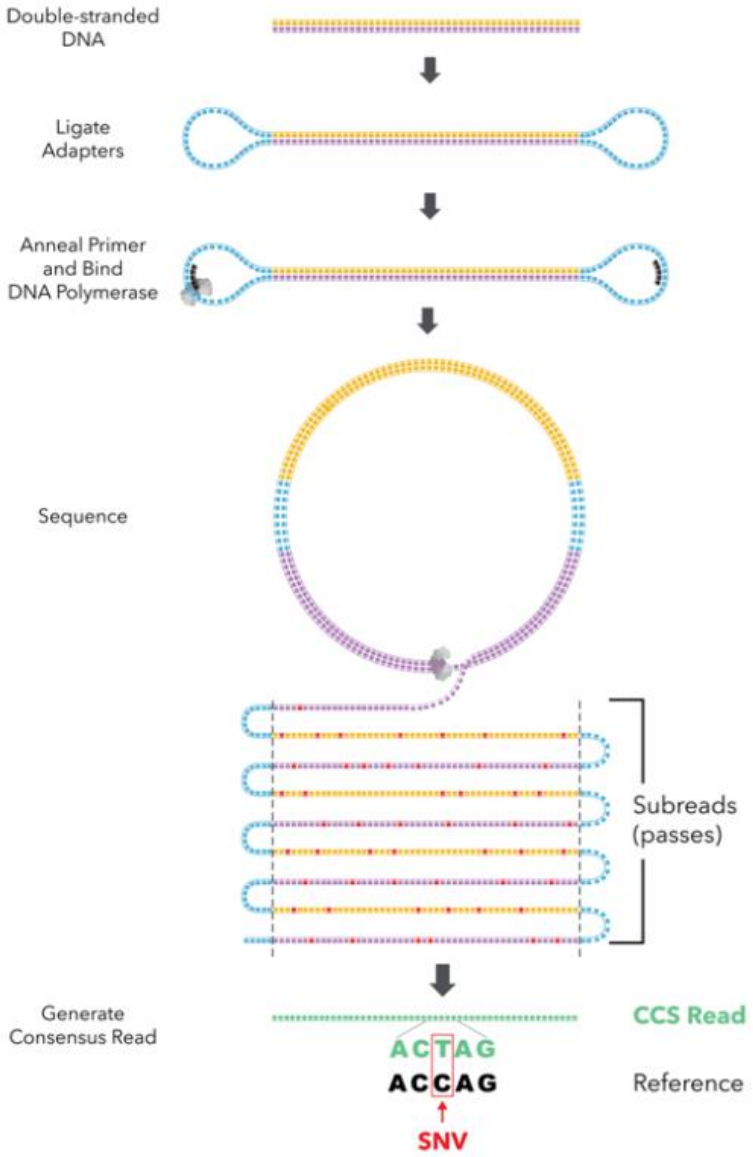
NovaSeq X Series

Flow Cell Type	1.5B	10B	25B
Human Genomes per Flow Cell	~4	~24	~64
Exomes per Flow Cell	~41	~250	~750
Transcriptomes per Flow Cell	~30	~200	~520

Pac-Bio – length and precision







- **Long reads:** 8-15kb, up to 40-70kb
- **High Accuracy:** >99.999% consensus accuracy achieved by sequencing the same molecule multiple times
- **No GC bias**
- **Direct identification of base modification** (epigenetics - 5mC)

Pac-Bio – what can we do with 1 SMRT cell?

SMRT sequencing applications	Samples per SMRT Cell 8M*
Whole genome sequencing	
***De novo* assembly:** Produce reference-quality assemblies for genomes up to 2 Gb	1
Microbial *de novo* assembly: Generate reference-quality assemblies for up to 96 microbial isolates	96
Variant detection: Call single nucleotide, indel, and structural variants and 5mC methylation in a ~3 Gb genome	0.5
RNA sequencing	
Whole transcriptome: Characterize alternative splicing with full-length transcripts	1
Genome annotation: Sequence full-length transcripts and multiplex up to 8 tissues	8
Targeted sequencing	
Amplicon sequencing: Detect variation in specific regions by multiplexing 1,000 samples (1–10 kb)	1,000
HiFiViral sequencing: Multiplex 384 samples and perform full genome sequencing of SARS-CoV-2	384
Metagenome sequencing	
Full-length 16S: Gain species- and strain-level resolution by multiplexing up to 192 samples	192
Metagenome profiling: Profile the composition or functional profile of up to 48 multiplexed samples	48
Metagenome assembly: Generate up to 35 high-quality MAGs from a gut microbiome sample	4

Whole Genome Sequencing

For humans, plants, animals and microbes including *de novo* sequencing and variant detection

RNA Sequencing

In-depth analysis of cDNA sequences across the entire transcriptome or targeted genes

Targeted Sequencing

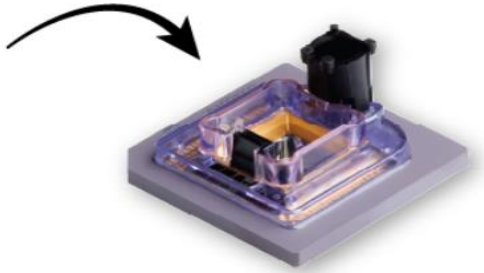
Study relevant genome targets across any regions of interest

Complex Populations

Understand variants among bacterial, viral and cancer cell populations

Epigenetics

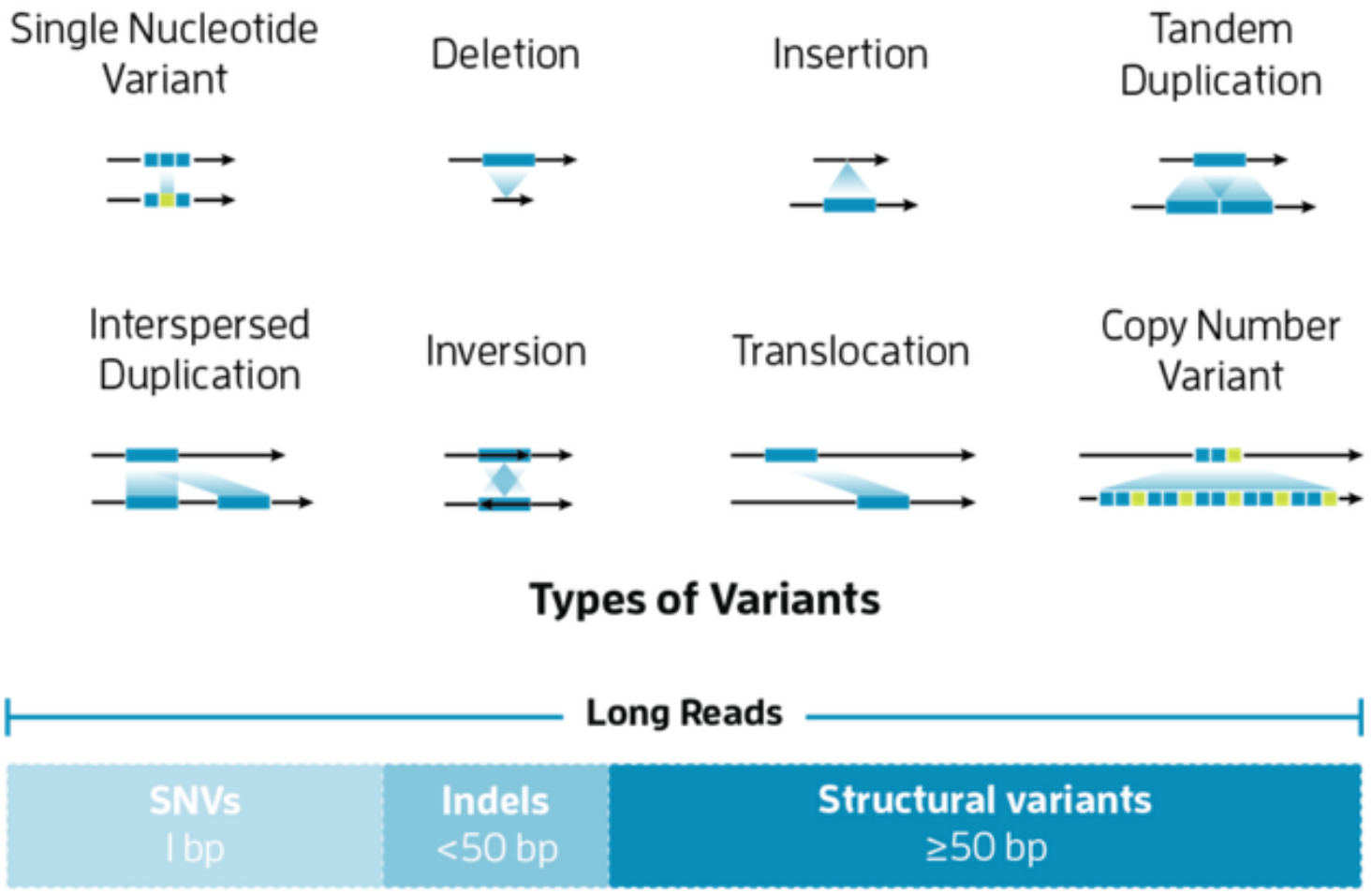
Detect DNA modifications in your samples while you sequence on the PacBio platform



3-4 SMRT cells per 1 **human** genome
1 SMRT cell per 1 **human** transcriptomes

6 500 – 8 500zł / SMRT cell
1 500 – 1 800zł / SMRT cel sequencing

Pac-Bio – genome analysis



Genomic DNA input (higher organisms) - 5-10 µg, high quality (high mol. weight)

Automation (2023)

Automated workstations -Beckman Biomek i5

DNA/RNA isolation

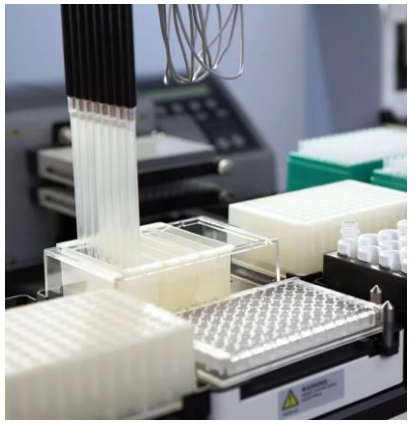
Library preparation

Automation (2023)

Automated workstations -Beckman Biomek i5

DNA/RNA isolation

Library preparation



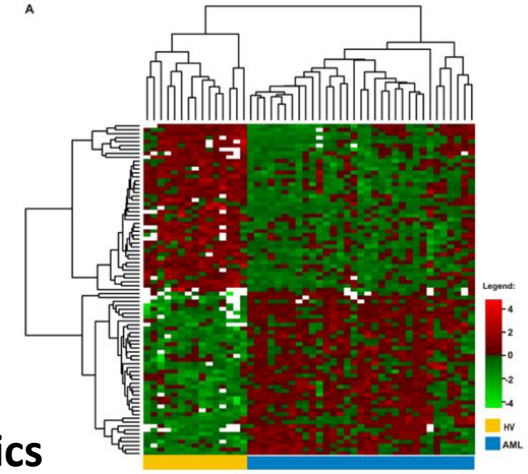
up to 96 samples per run	up to 48 samples per run
4 - 8 hrs	6 - 10 hrs
DNA - Beckman GeneFind	*Genome - Illumina DNA Prep*
RNA - Beckman RNAdvance	*Transcriptome - KAPA RNA HyperPrep Kit (+RiboErase)*

Additional equipment

- **Nanodrop,** ThermoFisher Sci. **(ECBiG)**
- **Qubit,** ThermoFisher Sci. **(ECBiG, ICHB 108A)**
- **Bioanalyzer 2100,** Agilent **(ECBiG)**
- **Bioruptor NextGen Sonicator,** Diagenode **(ICHB)**
- **T100 thermocyclers,** Bio-Rad **(ECBiG, ICHB)**
- **Rotor Gene Q**, Qiagen **(ECBiG)**
- **QX200** droplet digital PCR, Bio-Rad **(ECBiG)**

What we have done

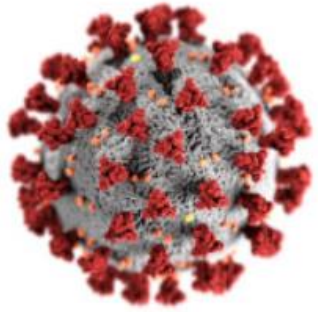
AML transcriptomics



Gene \ Sample	ID014	ID015	ID016	ID019	ID030	ID033	ID034	ID035	ID036	ID041	ID045	ID049	ID050	ID055	ID056	ID058	ID061	ID062	ID070	ID074	ID082	ID087	ID090	ID095	ID101	ID102	ID105	Number of samples with mutation	Percentage of samples with mutation
ASXL1							4.0					4.0				5.0							4.0					4	14.8
BRCA2															9.0					10.								2	7.4
CEBPA			5.0				0.0		0.0															7.0				4	14.8
DNMT3A		4.0										2.0		3.0		4.0	7.0			7.0								6	22.2
FLT3 -ITD			4.0							4.0			4.0															3	11.1
IDH1									-1.5					1.0		-1.5												3	11.1
IDH2												3.0		7.0						7.0								3	11.1
KRAS							9.0																6.0					2	7.4
NPM1		6.0							4.0				4.0	4.0		4.0	4.0			4.0								7	25.9
NRAS								6.0						6.0					6.0					6.0		6.0		5	18.5
RUNX1										3.0					3.0					4.0			3.0			8.0		5	18.5
RUNX1-RUNX1T1																												3	11.1
Number of mutations per sample	0	2	2	0	0	0	3	1	3	1	2	3	2	5	2	4	2	NA	1	3	2	1	3	2	1	2	0	median:	2

AML exome seq

Plant genome and transcriptome seq, circRNA seq



SARS-CoV-2 genome seq

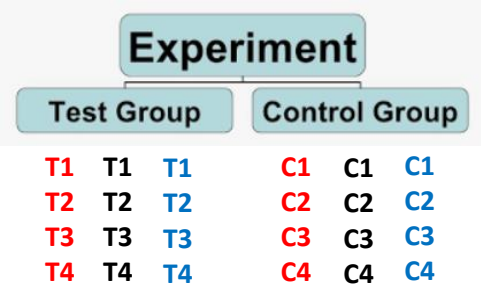
Archaeogenomics

Polish Microbiome Map

MOSAIC

… and other projects

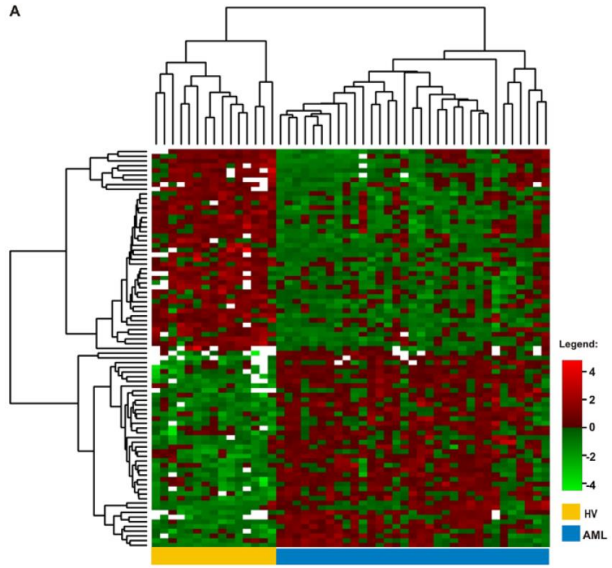
What we can offer



Experiment design

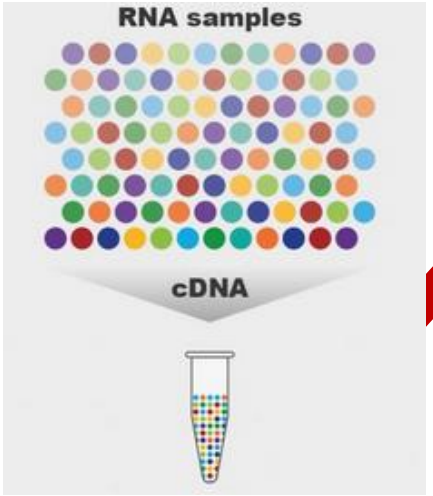
NGS

Sequencing

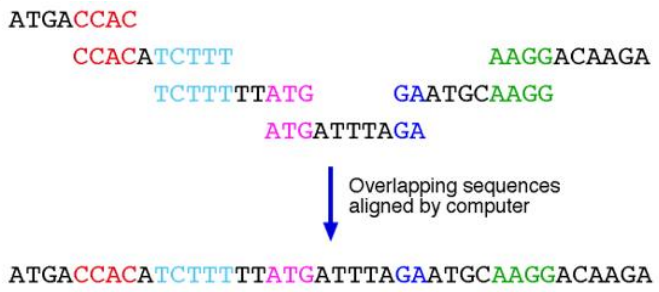


Data analysis (recommended collaboration with the **Laboratory of Bioinformatics**)

DNA/RNA extraction and quality assessment



Library preparation



Team

Head

Luiza Handschuh, prof. ICHBPAN

Wet lab

Magdalena Rakoczy, M.Sc.

Jan Podkowiński, Ph.D.

Małgorzata Marcinkowska-Swojak, Ph.D.

Bioinformatics

Paweł Wojciechowski, Ph.D.

Michał Zeńczak, M.Sc.

Contact

- lab.genomics@ibch.poznan.pl

- **Luiza Handschuh, int. tel. 1249**

luizahan@ibch.poznan.pl

- **Małgorzata Marcinkowska-Swojak, int. tel. 1202**

marcinkm@ibch.poznan.pl


ICHB PAN

Cell and Tissue Culture Laboratory

Natalia Koralewska

Operation areas

Animal cell and tissue cultures

Plant *in vitro* and in soil cultures

Sterilization and autoclaving

Staff

Aleksandra Błaszczak
Jarosław Lewandowski

Mariola Piślewska-Bednarek
Paweł Stróżycki

Hanna Glapiak
Magdalena Puszczyk

Natalia Koralewska

What we offer?

- **Space and equipment** for animal and plant cultures
- Access to a collection of **cell lines**
- **Great ideas, but no biologist? We can do the wet work for you!**
  - ✓ Cell lines culture and maintenance
  - ✓ Analysis of cell morphology, proliferation, adhesion, migration, and viability
  - ✓ Collecting and pre-processing cells for downstream analyses
- **Mycoplasma testing**
- Long-term **liquid nitrogen storage**
- **Training** in best practices for animal and plant cultures
- Technical expertise and hands on support for custom research projects
- Support in preparing research funding proposals involving work with *in vitro* models

IBCH PAS

Animal cell and tissue cultures

BSL2 infrastructure for:

- Cryopreservation and biobanking
- 2D and 3D cultures
- Transfection
- Counting and bioimaging

Localization: 016C

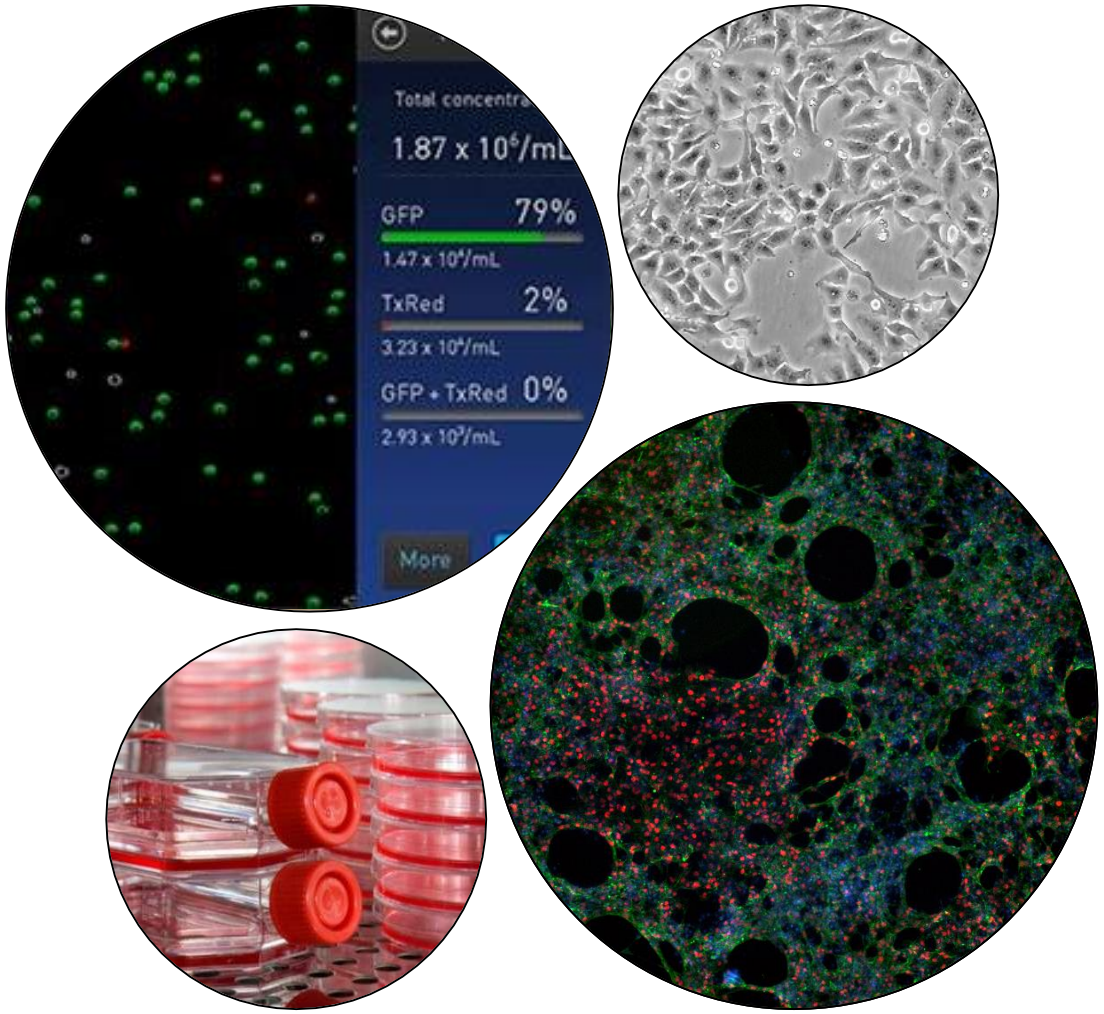
IBCH PAS

Animal cell and tissue cultures

- BSL2 biosafety cabinets
- CO_2 and CO_2/O_2 incubators
- 4°C/-20°C/-80°C/liquid nitrogen storage
- Centrifuges (refrigerated, preparatory)
- Microscopes (inverted, fluorescence, confocal)
- Automated cell counters
- Nucleofector
- Collection of human, mammalian, and insect cell lines

IBCH PAS

Animal cell and tissue cultures



- BSL2 biosafety cabinets
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- Microscopes (inverted, fluorescence, confocal)
- Automated cell counters
- Nucleofector
- Collection of human, mammalian, and insect cell lines

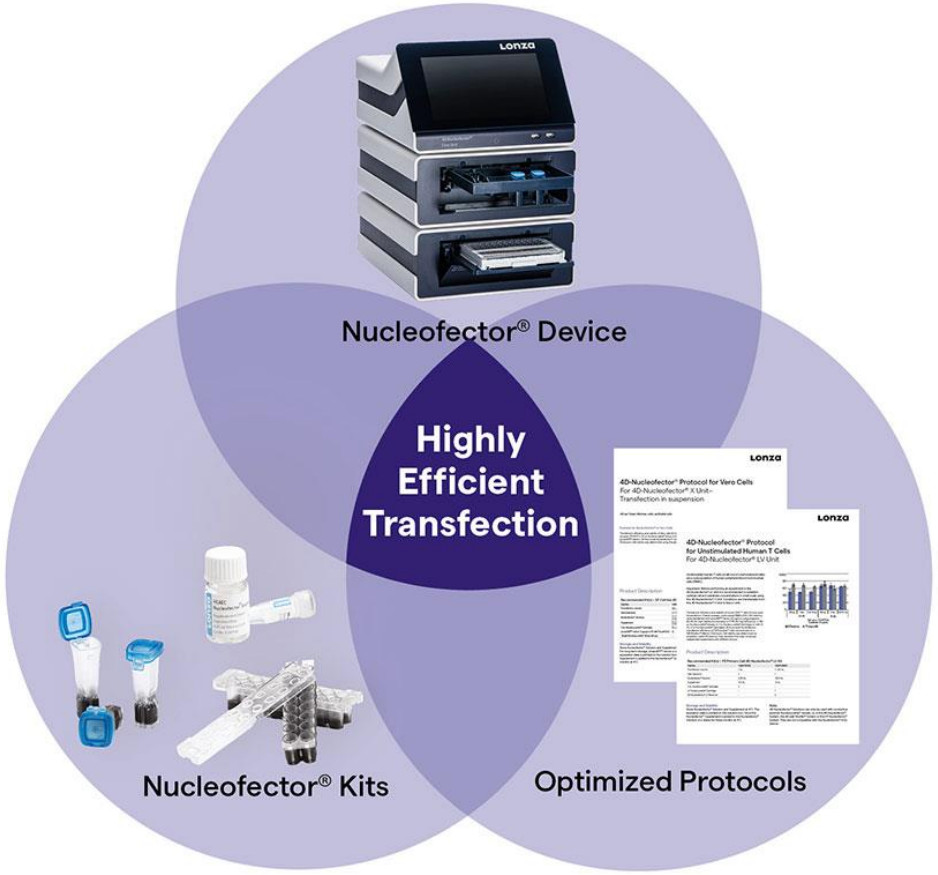
IBCH PAS

Countess® II FL Automated Cell Counter

- Three-channel cell counter
- Bright field and interchangeable LED light cubes: RFP, GFP, Cy5, DAPI
- To assess cell viability, cell cycle phase, apoptotic stage, transfection efficiency, and fluorescent protein expression
- Compatible with a wide variety of eukaryotic cells, incl. clumpy samples

IBCH PAS

4D-Nucleofector®



- Improved electroporation technology
- High transfection efficiency combined with low mortality
- Efficient transfection of hard-to-transfect cells
- A wide range of substrates
- Applicable for overexpression studies, generation of stable clones, RNAi screening, CRISPR/Cas9 genome editing
- 100 µL cuvettes or 20 µL 16-well strips

IBCH PAS

Plant *in vitro* & in soil cultures

- Plant growth chambers and rooms with temperature, humidity, and lighting control
- Greenhouse
- BSL2 biosafety cabinets
- Shaking incubators for bacterial cultures
- Ballistic cell transfection system
- Electroporation system
- 4°C and -20°C storage
- Centrifuges (ultracentrifuge, preparatory)
- Stereoscopic and fluorescence microscope

Localization: 015-016C, greenhouse

IBCH PAS

Sterilization & autoclaving

- Centralized services for dishwashing and autoclaving
- Sterilization of glassware, plastic consumables, media and buffers
- Processing and disposal of hazardous biological waste materials

Localization: 101A

Access

- Trained users are free to operate independently in the facility by reserving time through the reservation system
- Access to the culture laboratories is free of charge
- Users participate in the maintanence costs (CO_2, liquid nitrogen, EtOH, etc.)
- Rates of other services based on project scale – contact us!

IBCH PAS

Contact us

nataliak@ibch.poznan.pl
tel. int. 1505
room 105A


ICHB PAN


ICHB PAN

Dr hab. Anna Urbanowicz

Laboratory of Protein Engineering

affiliated with

Department of Structural Biology of Eukaryotes

Laboratory of Protein Engineering

Team:
Dr hab. Anna Urbanowicz - head
Dr hab. Agata Świątkowska
Dr Joanna Śliwiak
Dr Jakub Barciszewski
Mgr Alina Kasperska
Mgr Martyna Kordyś

Location:
Building A
Basement – level 000A
Building E
Basement – room 009E

- **preparation of bacterial expression vectors, production of recombinant proteins and their purification, evaluation of quality of protein preparations for functional and structural research;**
- **propagation, maintaining and banking of the cell lines, cell tests (cytotoxicity, cell cycle arrest, apoptosis)**
- **physicochemical characteristics of interactions between proteins and other macromolecules: measurement of association and dissociationi constants, determining the number of ligand binding sites and thermodynamic parameters of binding (enthalpy, entropy), measurement of enzymatic kinetics,**
- **high-throughput tests of the initial conditions for crystallization of biomolecules, optimization of the crystallization process to obtain crystals for diffraction experiments, recording diffraction data for protein crystals and nucleic acids using a diffractometer equipped with a rotating anode**

We offer two types of equipment/services:

1. **Available to each IChB employee or PhD student, after prior notification of the Lab Head and initial training**

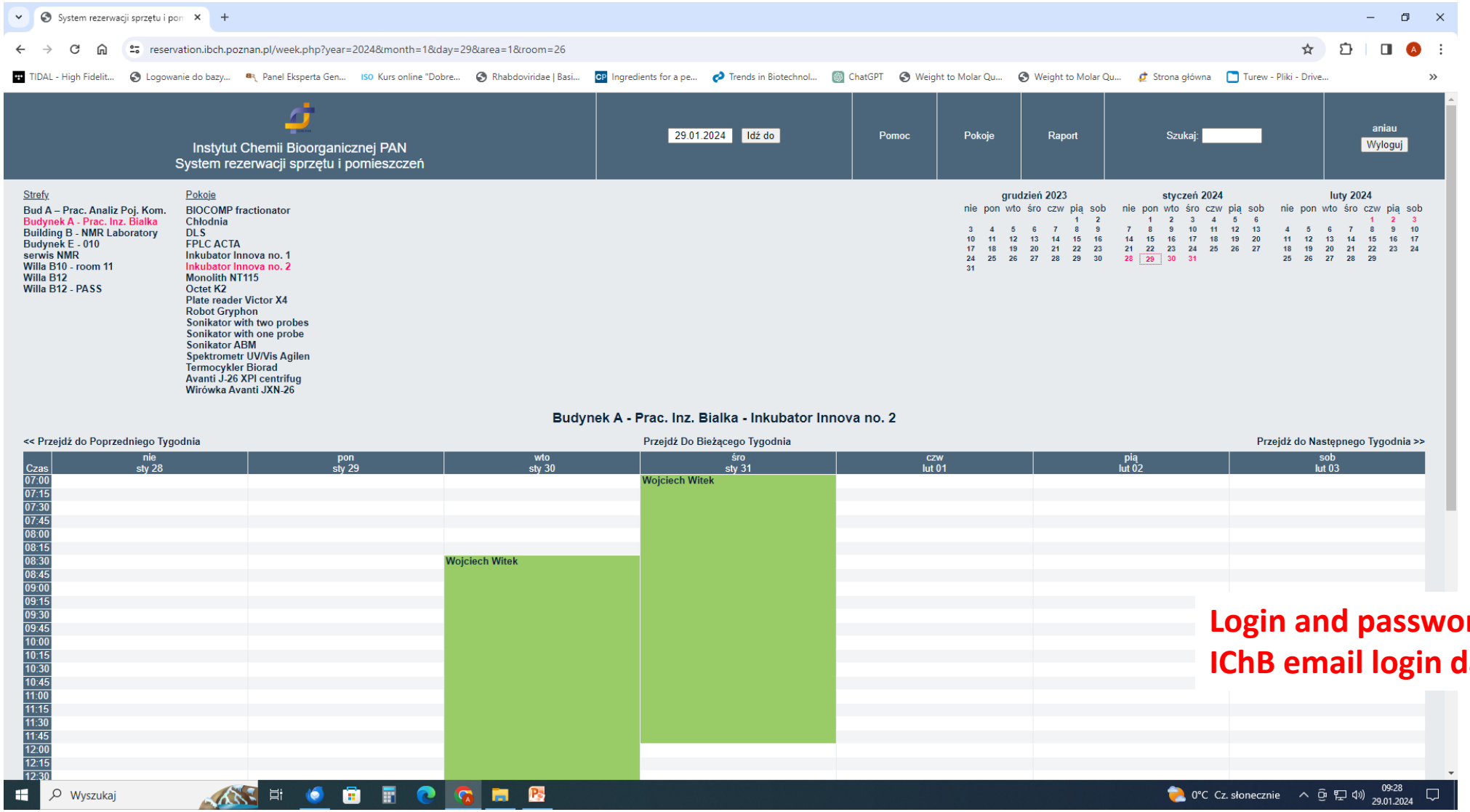
2. **Specialized equipment that may be used only in cooperation with Laboratory Staff**

Equipment available to each IChB employee or PhD student, after prior notification of the Lab Head and initial training:

1. **First usage must be consulted with Lab Head (equipment operation and safety matters)**
2. **Disposable matherials/reagents provided by the user**
3. **Reservation system - obligatory**

- 2 **incubators** with shaking and temperature control in the range of 5 to 50°C,
- 3 **sonicators**,
- 2 **preparative centrifuges** (rotors: 8 x 50 ml and 6 x 500 ml, 6x 1l),
- **Zetasizer** µV analyzer for static and dynamic light scattering measurements (DLS and SLS) in order to assess molecular mass and hydrodynamic diameter of proteins and polymers,
- **nanophotometer** for concentration measurements,
- **UV-visible spectrometer** for precise concentration and kinetics measurements,
- **thermocycler**
- **cold-room** (8 °C) and **crystallization room** (18 °C)

Reservation system - obligatory

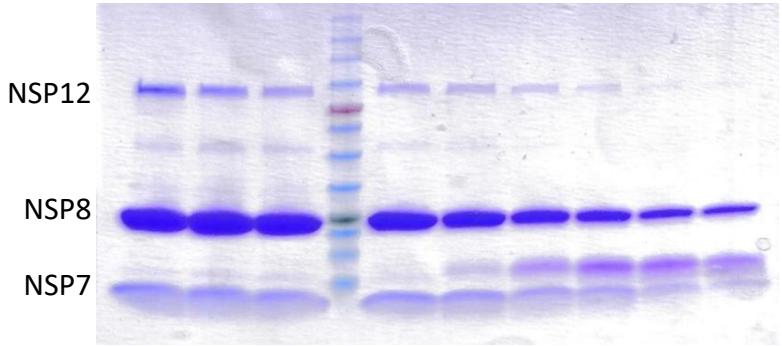


Login and password =
IChB email login data

Specialized equipment that may be used only in cooperation with Laboratory Staff –

AKTA system

- Protein expression and purification, various purification methods (FPLC)
  - **dr hab. Anna Urbanowicz, mgr Martyna Kordyś**



Active recombinant SARS CoV-2 RdRp preparation (NSP7,8,12 complex). The polymerase was produced in *E. coli* and purified by III-step chromatography: Ni-NTA affinity, ion-exchange and, finally SEC (presented fractions after SDS-PAGE).

Specialized equipment that may be used only in cooperation with Laboratory Staff –

Gradient forming and fractionating using Biokomp equipments

- Preparation of the gradient prior to separation of particles through ultracentrifugation or fractionation

  - **mgr Martyna Kordyś**

Applications:

- polysome profiling
- seperation and fractionation of proteins and complexes

Specialized equipment that may be used only in cooperation with Laboratory Staff -

- Fluorescence, luminescence, absorbance, radiometric detection
  - **dr hab. Agata Świątkowska**

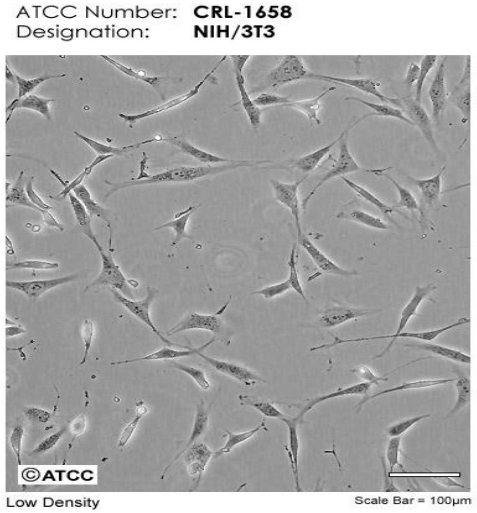
Spectophotometer Victor *X4* Multimode plate reader (Perkin Elmer)
- Fluorescence (fluorescein, GFP, mCherry)
- Luminescence (luciferase system)
- Visible Absorbance

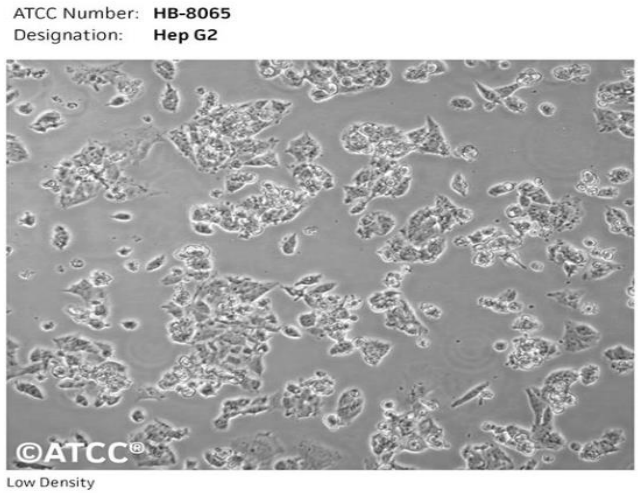
2450 Microplate Counter MicroBeta 2MicroBeta$^{2®}$
Microplate Counter for Radiometric and Luminescence Detection

Specialized equipment that may be used only in cooperation with Laboratory Staff -

- **Propagation, maintaining and banking of the cells, cell tests (cytotoxicity, cell cycle arrest, apoptosis)**

- **dr hab. Agata Świątkowska**





We offer cell lines:

Adherent human cell lines : HepG2, HT-29, HCT116, MCF-7, H1299, HEK-293, BEAS-2B

Adherent mouse cell lines: MEF, NIH3T

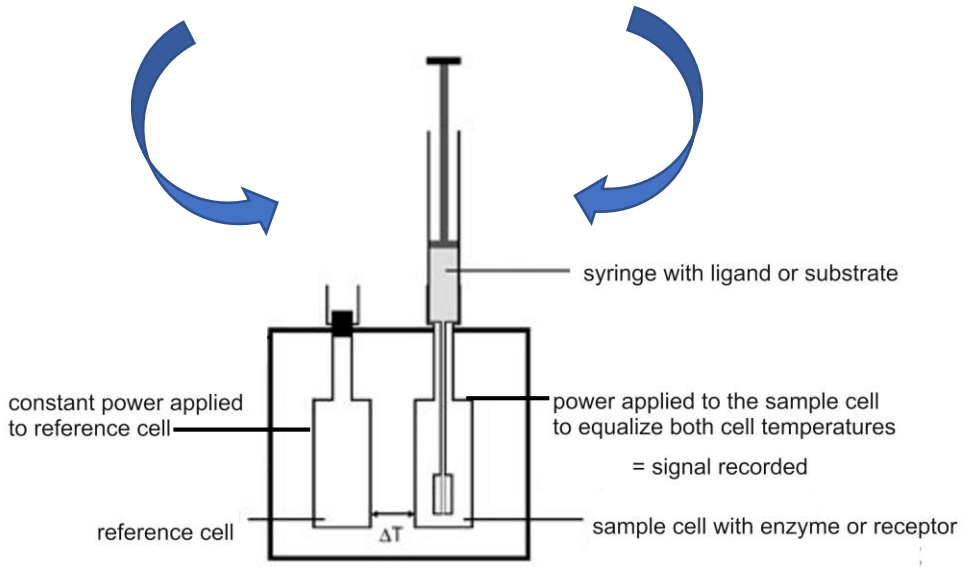
Specialized equipment that may be used only in cooperation with Laboratory Staff -

• Microcal iTC200 and Microcal PEAQ-ITC microcalorimeters

- dr Joanna Śliwiak

iTC-200

PEAQ-ITC



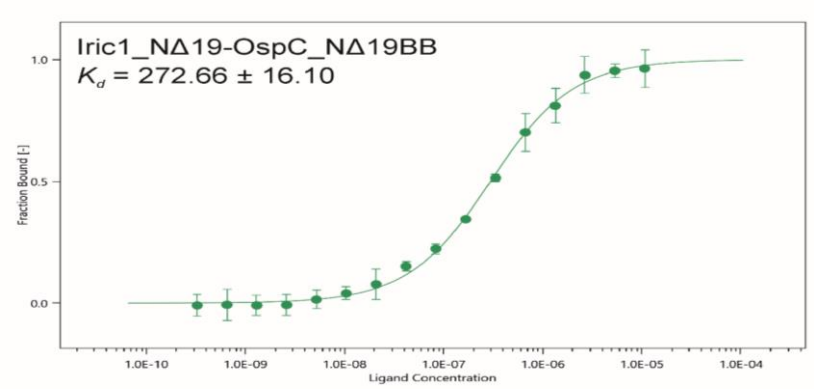
principles of calorimeter operation

- All chemical processes are either exothermic or endothermic – they produce or consume heat.
- Microcalorimetry – ultrasensitive technique, measures very small heat changes coming from these processes
- Dissociation constants, stoichiometry, enthalpy and entropy of bimolecular binding events e.g.:
  - ✓ Parameters of zeatin and giberellin binding by CSBP (Ruszkowski et al. 2014)
  - ✓ The effect of temperaturę of ligand bindng by thermofilic SAHase (Brzezinski et al. 2017)
  - ✓ Influence of monovalent cation on adenosine binding by PaSAHase (Czyrko et al. 2018)
  - ✓ The effect of HISN2 point mutations on AMP binding (Witek et al. 2021)
  - ✓ Binding parameters of different DNA duplexes with transciption factor WRKY (Grzechowiak et al. 2022)
  - ✓ Divalent cations binding by deacetylase (Biniek et al. 2022)
- Enzyme kinetic parameters and total molar enthalpy of enzymatic reaction e.g.:
  - ✓ Temperature and pH effect on work of thermofilic deacetylase (Biniek et al., 2022)
  - ✓ Kinetic parameters of Rhisobium etli asparaginases (Loch et al., 2021, 2023)
  - ✓ Kinetic parameters of AtGDH isoforms (Grzechowiak et al. 2023)
  - ✓ Kinetic parameters of different phyrophosphatase variants (Grzechowiak et al. 2019)

Specialized equipment that may be used only in cooperation with Laboratory Staff –

- **Monolith NT.115 system for measurements of binding affinity using microscale thermophoresis (MST),**

  - **dr hab. Anna Urbanowicz**



K_D range: 1 nM – 500 mM
- molecular size range: 300 Da – 2.5 MDa
- one of the studied molecules has to be fluorescently labelled
- single measurement takes 15 minutes, a small amount of material is needed, a set of 16 ligand dilutions is analyzed

Exemplary MST curves and assessed binding affinities. Binding affinity between Iric1 recombinant protein from *I. ricinus* tick and recombinant OspC protein from *Borrelia*. Bierwagen et al., 2021

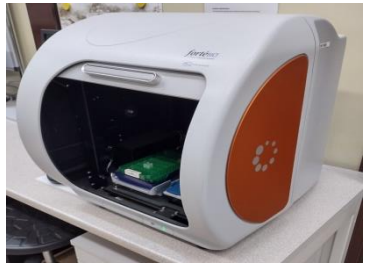
Specialized equipment that may be used only in cooperation with Laboratory Staff –

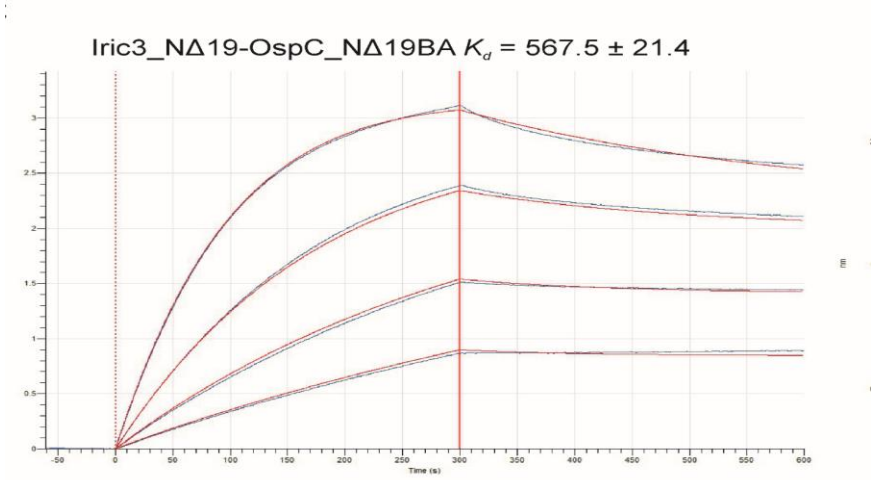
- **Octet K2 system (ForteBio) for measurements of binding affinity using biolayer interferometry (BLI),**

  - **dr hab. Anna Urbanowicz**

real- time measuring association (**ka**) and dissociation (**kd**) kinetics, and affinity constants ($\mathbf{K_D}$)

- K_D range: 0.1 nM – 500 mM
- molecular size range: 150 Da – whole cells
- small amount of material is needed, at least 4 ligand dilutions are analyzed





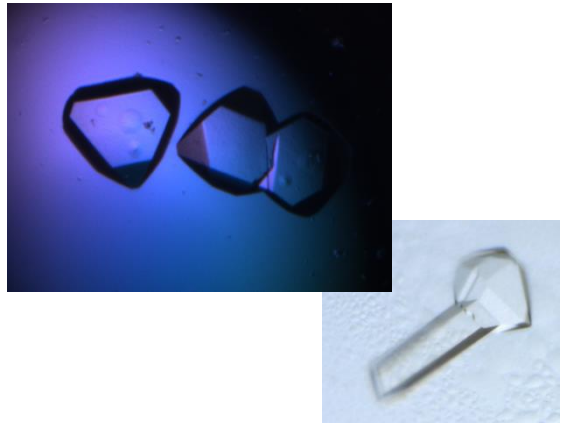
Exemplary BLI curves and assessed binding affinities. Binding affinity between Iric3 recombinant protein from *I. ricinus* tick and recombinant OspC protein from *Borrelia*. Bierwagen et al., 2021

Specialized equipment that may be used only in cooperation with Laboratory Staff –

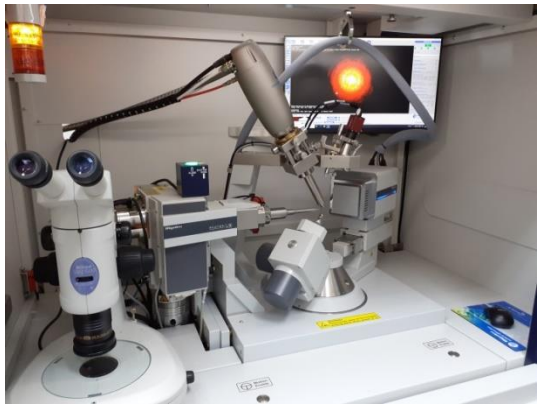
• Crystallization robot ARI Gryphon

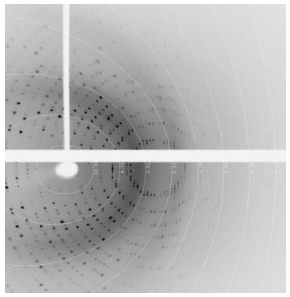
- **dr Jakub Barciszewski**
(Radiological Protection Inspector)

- Crystallization of proteins, nucleic acids and their complexes,
- Determination of initial crystallization condition,
- Optimization of conditions



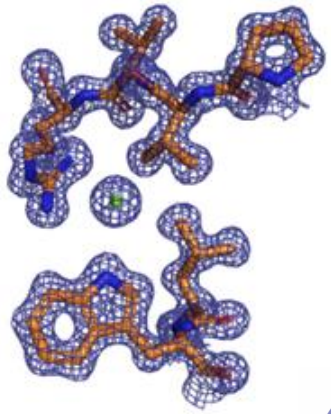
• Diffractometer Rigaku XtaLAB Synergy-R





Assessment of crystal quality

Solving crystal structures of proteins, nucleic acids and their complexes



Dr hab. Anna Urbanowicz

Laboratory of Protein Engineering

affiliated with

Department of Structural Biology of Eukaryotes

We invite you to cooperation!


ICHB PAN

INSTITUTE OF BIOORGANIC CHEMISTRY
Polish Academy of Sciences

Laboratory of Invertebrate Model Organisms

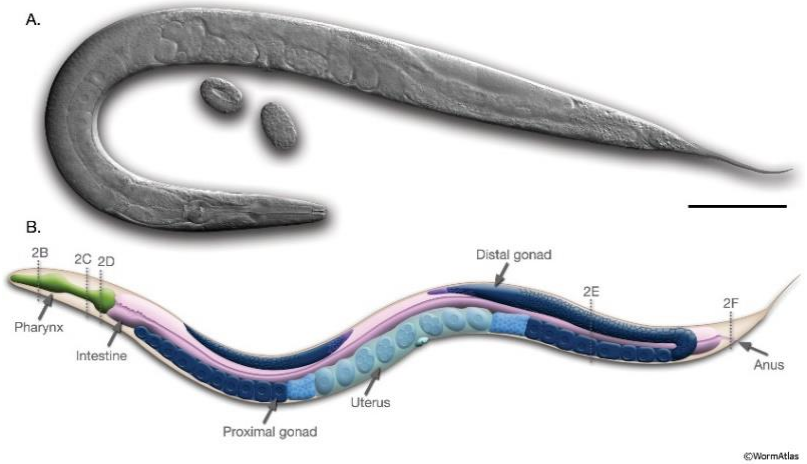
Head: dr. habil. Agata Tyczewska

lab.model.invertebrate@ibch.poznan.pl

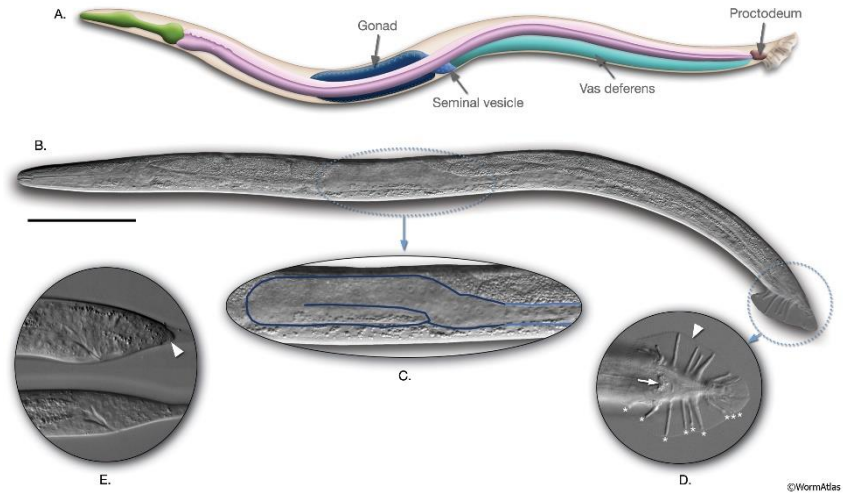
agatat@ibch.poznan.pl

31 January 2023

Caenorhabditis elegans



Anatomy of an adult hermaphrodite (source: Wormatlas).



C. elegans male (source: Wormatlas).

- The life cycle - 2 to 3 weeks, 4 larval stages before reaching maturity.
- Two sexes: male and hermaphrodite. Males arise infrequently (0.1%) at higher frequency (up to 50%) through mating.
- Self-fertilization of the hermaphrodite allows for homozygous worms to generate genetically identical progeny, and male mating facilitates the isolation and maintenance of mutant strains as well as moving mutations between strains.

!!! Ability to self-fertilize is a useful tool because hermaphrodites can reproduce quickly in high numbers.

Caenorhabditis elegans - key benefits of the worm

- Complete genome sequence published in 1998
- *C. elegans* are grown cheaply and in large numbers on plates containing *Escherichia coli*
- Healthy cultures of *C. elegans* can be frozen, defrosted and revived
- *C. elegans* produce approximately 300 eggs/worm within several days
- Short life cycle of only 18-21 days on average
- Very small organism (~ 1mm), convenient to keep in the lab
- The worm is transparent so the behavior of individual cells can be followed through its development
- The anatomy and development of *C. elegans* can be examined easily under a microscope
- The availability of molecular biology tools (i.e., transgenic, gene knockouts, and RNAi knockdowns)

Zhang S et al. 2020, Front Endocrinol (Lausanne), doi: 10.3389/fendo.2020.554994

Caenorhabditis elegans - key benefits of the worm

- *C. elegans* development is very specific, cells divide and specialize in a characteristic way, so each cell can be traced back to the embryo
- Mutant forms of *C. elegans*, can be produced very easily to closely study gene function
- High genetic homology (60–80%) with humans
- Many of the molecular signals controlling *C. elegans* development are also found in more complex organisms, like humans
- Many of the genes in the *C. elegans* genome have functional counterparts in humans, which makes it an extremely useful model for human diseases
- *C. elegans* mutants can be screened with thousands of potential drugs for important diseases
- Studying cell death or 'apoptosis' in the *C. elegans* could hold the key to counteracting the effects of aging in humans as well as providing clues about cancer, diabetes and other diseases

Zhang S et al. 2020, Front Endocrinol (Lausanne), doi: 10.3389/fendo.2020.554994

Caenorhabditis elegans – model organism

- Microbiome Research
- Longevity and Aging
- Parkinson’s disease
- Duchenne Muscular Dystrophy
- Triplet Repeat Diseases
- Neural Development due to the availability of a comprehensive connectivity map and only 302 neurons and ~7000 synapses.
- Mitochondrial Diseases
- Immunology
- Obesity (e.g. nutrient sensing, lipid storage)
- Biomedical and Environmental Toxicology
- Environmental Epigenetics
- Neurotoxic and Behavioral effects of various compounds
- Infection model for a variety of bacterial and fungal pathogens

Caenorhabditis elegans - resources

- *C. elegans* RNAi feeding library – Ahringer

The library has 16,757 clones, made by cloning gene-specific genomic fragments between two inverted T7 promoters. The inserts contain exons and introns and sizes vary from 500 bp to 2.5 kb. (Fraser et al., 2000; Kamath et al., 2003)

- ORFeome-Based RNAi Library – Vidal

The *C. elegans* ORFeome v1.1 library contains 11,511 RNAi clones, each expected to target a single gene. Several of these RNAi clones target the same genes, so the RNAi library in theory can knockdown the expression of 10,953 (~55%) of the 19,920 unique protein-encoding genes predicted in WS112. Of these 10,953 genes, 485 are targeted by two or more RNAi clones. (Rual et al., 2004)

!!! Both libraries target about 94% of *C. elegans* genes.

Caenorhabditis elegans - resources

- **WormBook** <http://www.wormbook.org> is a comprehensive, open-access collection of original, peer-reviewed chapters covering topics related to the biology of *C. elegans* and other nematodes. Wormbook also contains: WormMethods, a collection of protocols for nematode researchers; WormHistory, personal perspectives on *C. elegans* research; and the Worm Breeder's Gazette, an informal, non-refereed, biannual newsletter for the interchange of ideas and information related to *C. elegans* and other nematodes.

- **WormBase** <http://www.wormbase.org> is a major repository for *C. elegans* information, including genomic, genetic, anatomy, people, and literature. WormBase also integrates genetic map information with that of physical map. Genetic Interval Search <http://wormbase.org/db/searches/interval> can return a list of genes that have the potential to map within a specified genetic interval. SNP, Visible Marker, and Strain Search <http://wormbase.org/db/searches/strains> is particularly useful for finding markers for genetic mapping experiments in a small interval.

- **Caenorhabditis Genetics Center** (CGC) <http://biosci.umn.edu/CGC/CGChomepage.htm> is a resource center for *C. elegans* genetics. It is responsible for gene nomenclature, strain collection and distribution, and genetic map construction. CGC homepage is a portal that has links to these and some other related services useful to *C. elegans* geneticists.

- **WormBase Gene Summary** <http://www.wormbase.org/db/gene/gene>. Each gene in WormBase has a summary page which collates together several different aspects of a gene, including identification, genetic and genomic location, function, reagents and bibliography.

Caenorhabditis elegans – in house resources

- **WormBase Genome Browser** <http://www.wormbase.org/db/seq/gbrowse/wormbase> is a physical map browser. Using Genome Browser, one can search and display sequences and sequence-related features; one can also zoom in or out and move along on a chromosome.

- **WormAtlas** <http://www.wormatlas.org/> provides anatomical information of *C. elegans*. The front page lists several useful entry points. One can use the simple text search tool to search the site for information that relates to anatomical terms (e.g. PVT, name of a neuron). Another good way to use this site is to read sections of the “handbook”.

- **The Nematode Expression Pattern Database** (NEXTDB) <http://nematode.lab.nig.ac.jp/db/keysrch.html> provides access to *C. elegans* EST sequences obtained by Yuji Kohara's laboratory and some other experimental results derived from them, such as expression patterns determined by in situ hybridization, which can be searched via a text query tool.

- **BCGSC Expression Patterns** <http://elegans.bcgsc.ca/perl/eprofile/browse> lists GFP expression data which can be browsed directly or searched by gene name, tissue pattern or life stage.

- **Textpresso** <http://www.textpresso.org> allows text searches on primarily *C. elegans* literature, including published papers, personal communications and meeting reports. Two major features distinguish Textpresso from other literature search tools: that it searches full-text contents of publications, and in addition to text strings, that it can search for groups of terms (categories).

Laboratory of Animal Model Organisms - equipment

Mediaclave 10 and MediaJet vario (Integra).

Autoclaving and pouring plates

Mediaclave 10 (Integra) - used for preparing and sterilizing many types of media, min. 1l, max. 10l of broth for sterilization.

MediaJet vario (Integra) - Petri Dish Filler with sets for Petri dishes Ø 35 mm, 60 mm, 90 mm, up to 330 plates/h, equipped with a UV lamp allowing for contamination-free filling of Petri dishes.

Laboratory of Animal Model Organisms - equipment

Stereoscope (Nikon).

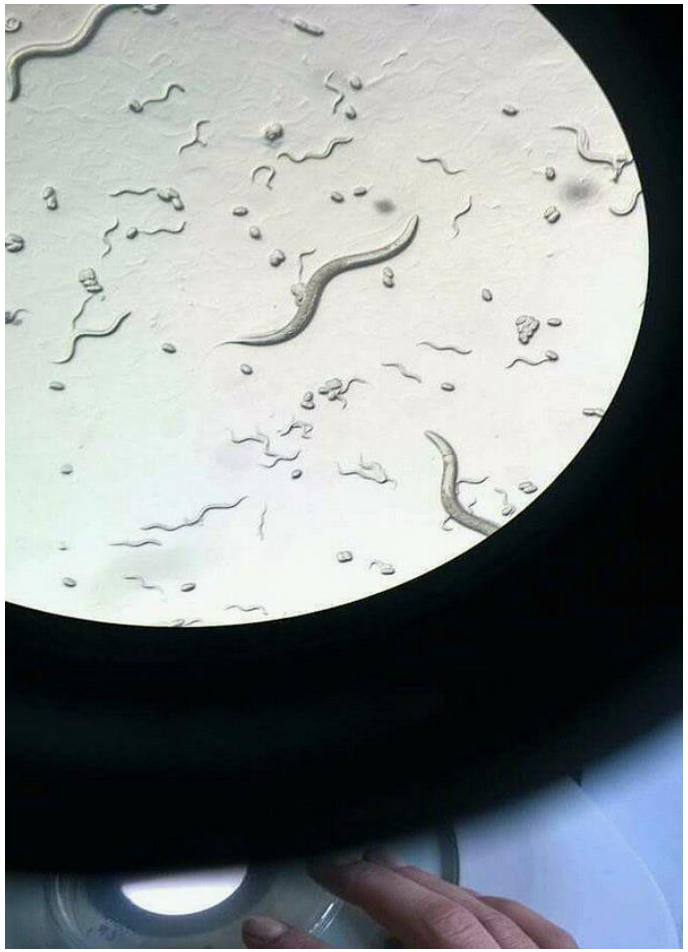


Image viewed under the stereoscope.

Laboratory of Animal Model Organisms - equipment

- **Axio Vert.A1** (Zeiss), with Eppendorf microinjection kit mounted
- **InjectMan®4 micromanipulator** with dynamic motion control and programmable micromembrane
- **FemtoJet®4i** with integrated pressure supply
- **PC-100** (Narishige) – microneedle preparation

A

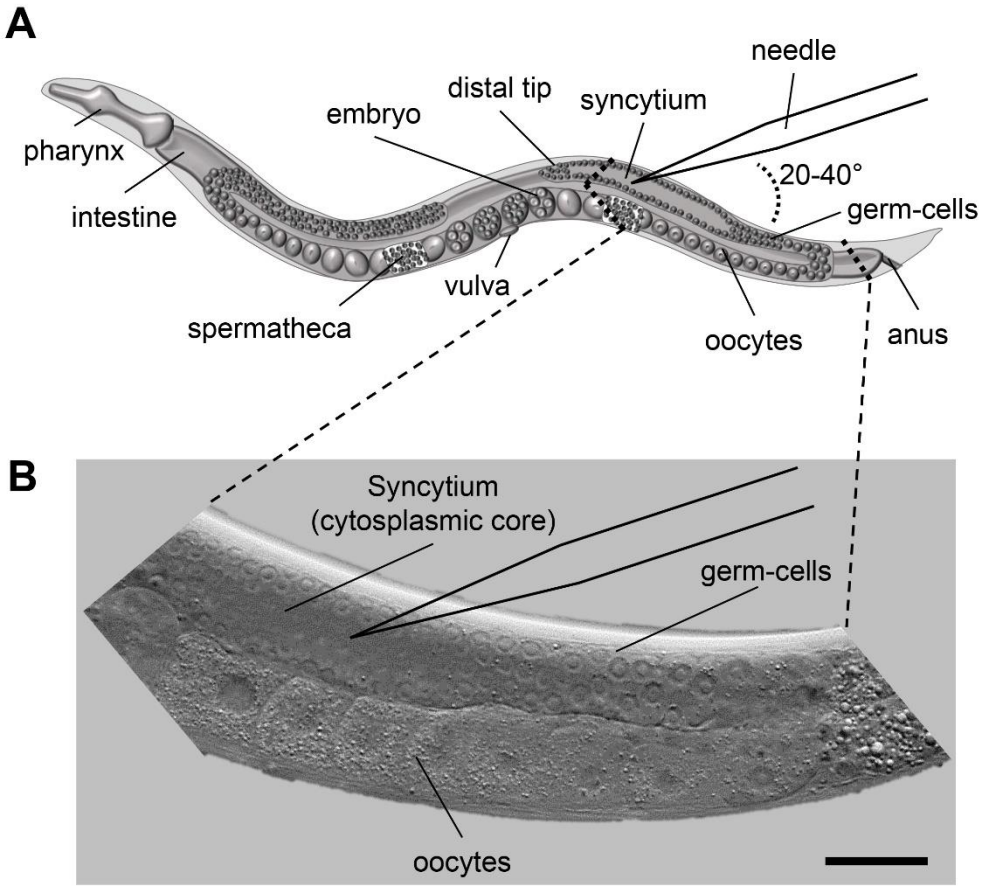
B

C

A. Axio Vert.A1 (*Zeiss*). **B**. FemtoJet 4i (*Eppendorf*). **C**. InjectMan 4 (*Eppendorf*).

PC-100 microneedle puller (*Narishige*).

Laboratory of Animal Model Organisms - microinjection



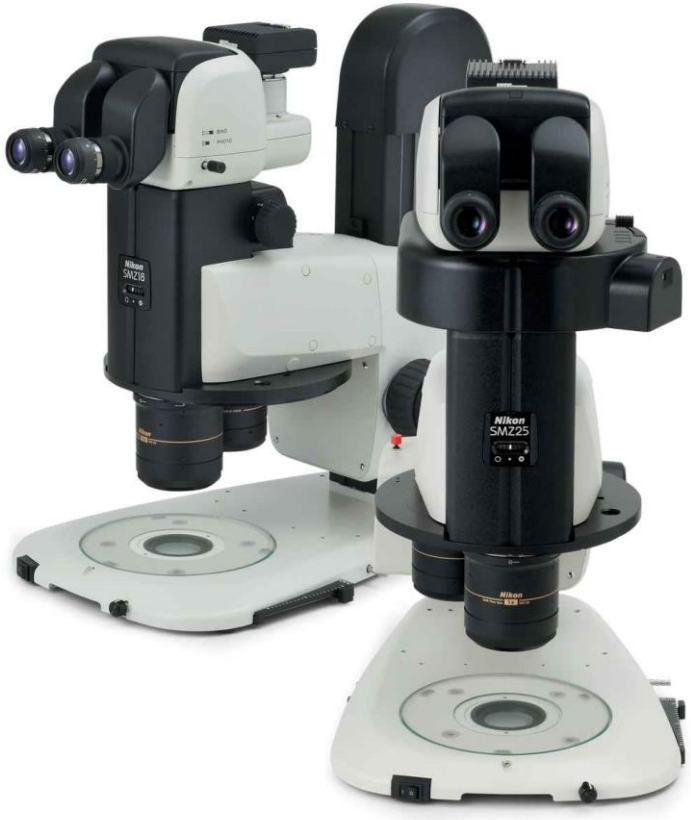
Microinjection is a proven and relatively simple method of introducing DNA into organisms.
Moreover, microinjection is a very effective approach to RNA interference and can be used to deliver synthetic mRNAs or other molecules directly into cells.

Figure 10. Microinjection scheme for *C. elegans*. **A**. Scheme of an adult *C. elegans* displaying the major organs including pharynx, intestine and gonad. When microinjecting *C. elegans* the injection capillary needs to be inserted in the syncytium (cytoplasmic core) of the distal gonad. The inlay indicates the area of interest for injection. **B**. DIC image of the area indicated in 1A. The nuclei of the germ-cells are clearly visible and surround the syncytium. Size bar corresponds to 50 µm. (Rieckher and Tavernarakis, Bio-protocol 2017, 7(19): 578, 10.21769/BioProtoc.2565)

Laboratory of Animal Model Organisms - equipment

Laboratory of Animal Model Organisms - equipment

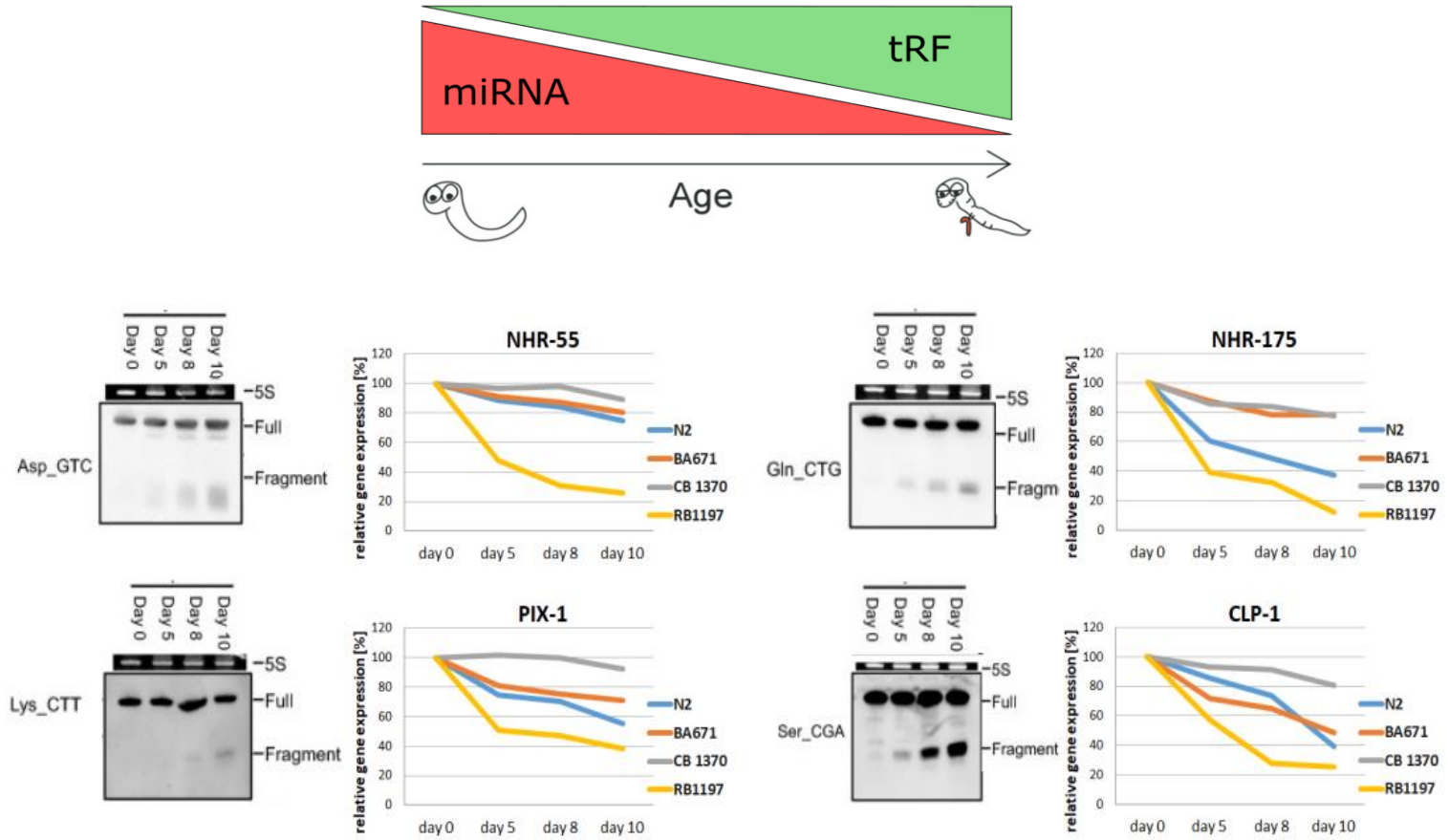
Stereo microscope (Nikon) characterized by a large magnification ratio of 25: 1, high resolution and exceptional fluorescence transmission ability.



Axio Imager 2 (Zeiss) is designed to be a platform for an impressive number of applications in cell biology, neuroscience, molecular genetics and pathology.

Undergoing projects

1. Age-related changes in the expression of small noncoding RNAs

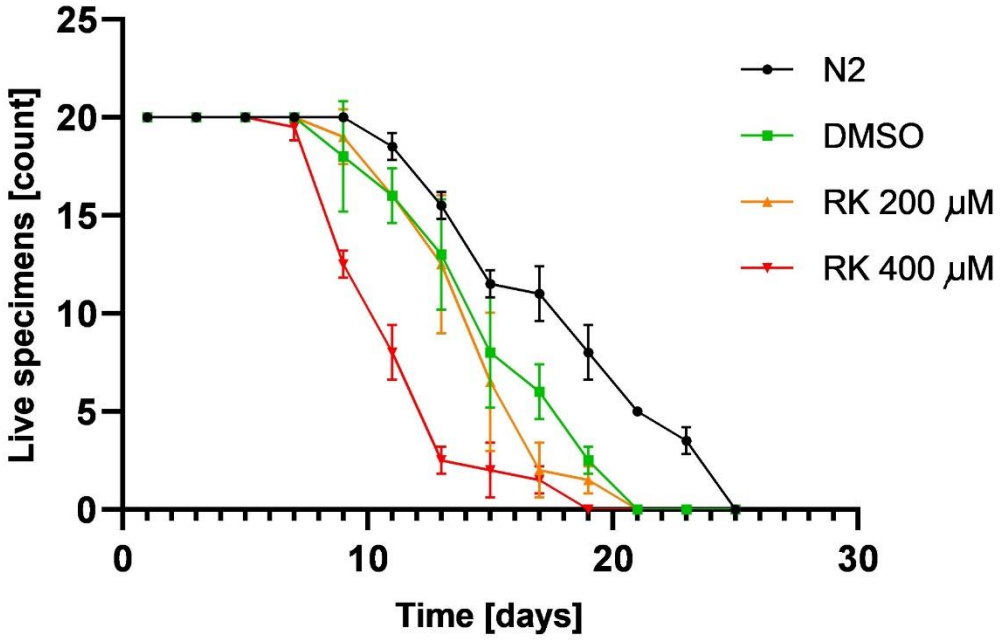


Undergoing projects

1. Analysis of the influence of small molecules on *C. elegans* lifespan (collaboration with **prof. Eliza Wyszko**, Laboratory of Subcellular Structures Analyses, IBCh PAS)

Necessary supplies:

- Petri plates (90 mm),
- *E. coli* OP50,
- NG 2% medium (agar, NaCl, bacto-peptone, cholesterol, $CaCl_2$, $MgSO_4$, KH_2PO_4)
- liquid LB medium
- bleaching solution (sodium hypochlorite, KOH)
- worm strains (10 $ per strain, transport from US)



Undergoing projects

3. Cellular uptake and retention studies of silica nanoparticles in *C. elegans*

Collaboration with **dr. Patrick Perrigue** – NanoBioMedical Centre, Adami Mickiewicz University in Poznań

Article | Open access | Published: 10 January 2023

Cellular uptake and retention studies of silica nanoparticles utilizing senescent fibroblasts

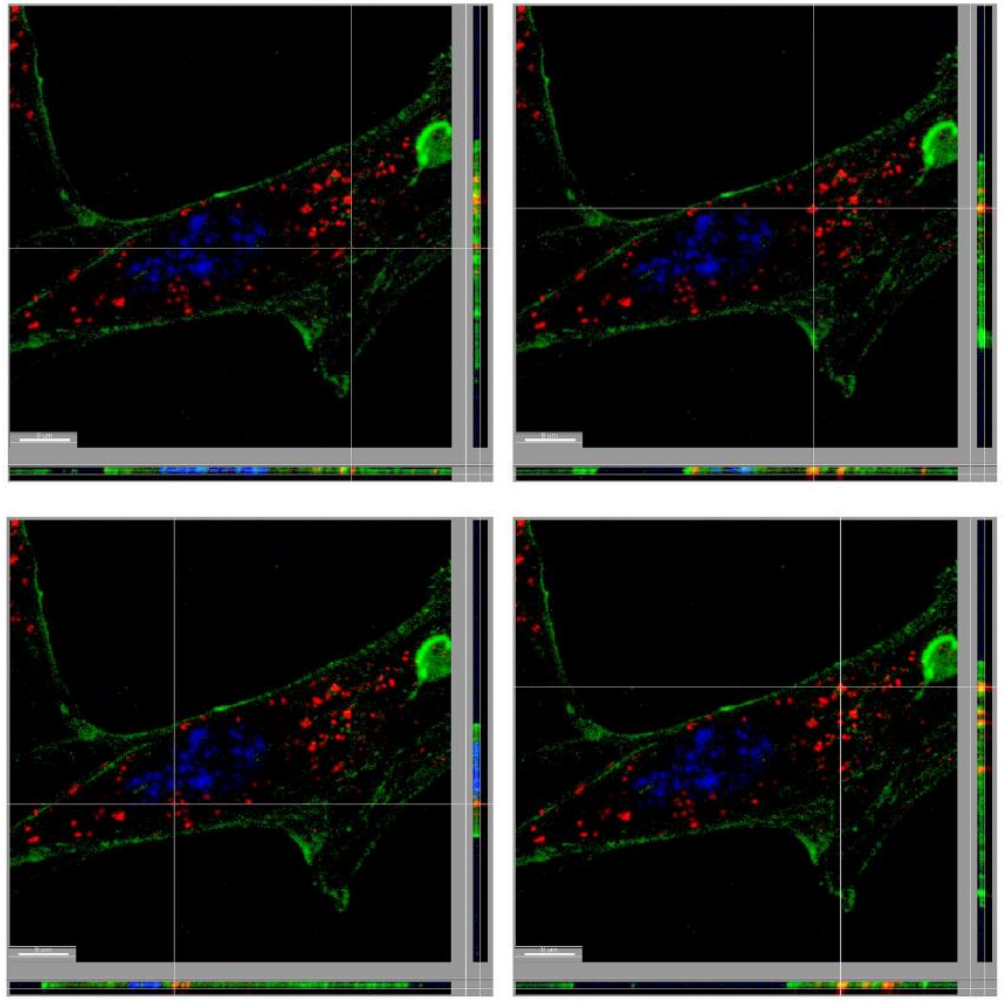
Patrick M. Perrigue, Agata Henschke, Bartosz F. Grześkowiak, Łucja Przysiecka, Kaja Jaskot, Angelika Mielcarek, Emerson Coy & Sergio E. Moya

Scientific Reports **13**, Article number: 475 (2023) | Cite this article

1753 Accesses | Metrics

Abstract

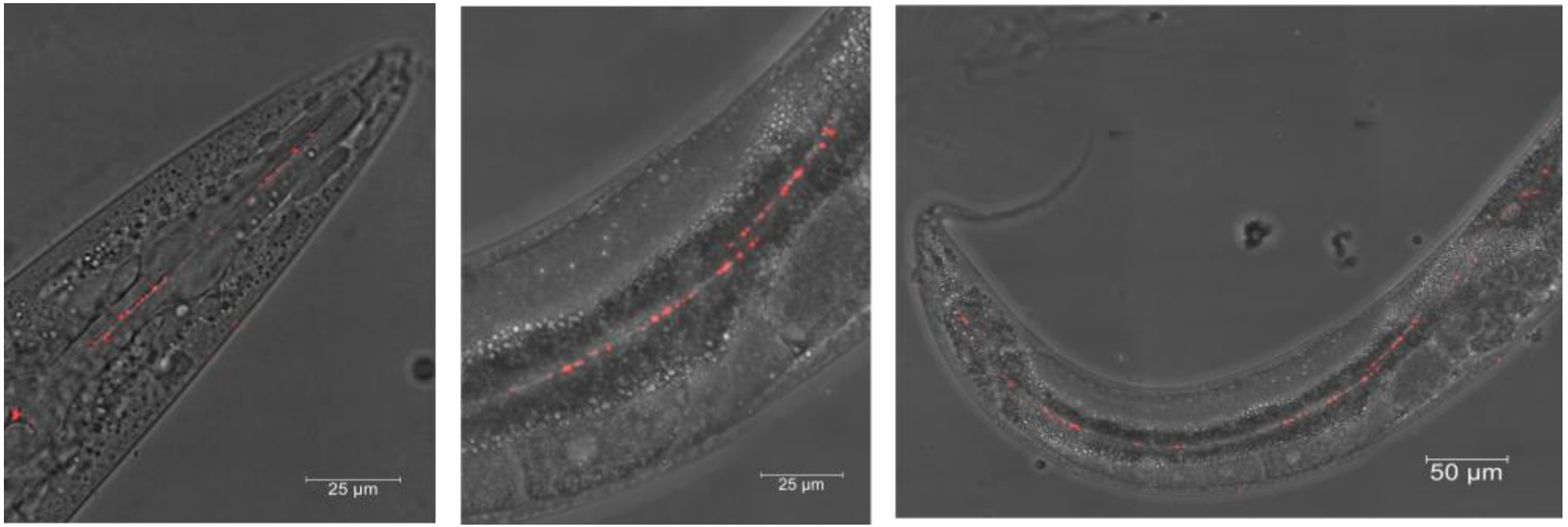
Understanding the interplay between nanoparticles (NPs) and cells is essential to designing more efficient nanomedicines. Previous research has shown the role of the cell cycle having impact on the efficiency of cellular uptake and accumulation of NPs. However, there is a limited investigation into the biological fate of NPs in cells that are permanently withdrawn from the cell cycle. Here we utilize senescent WI-38 fibroblasts, which do not divide and provide a definitive model for tracking the biological fate of silica nanoparticles (SiNPs) independent of cell cycle. We use several methods to measure the cellular uptake kinetics and intracellular retention of SiNPs, including confocal laser scanning microscopy (CLSM), flow cytometry, and transmission electron microscopy (TEM). We demonstrate that SiNPs readily enter into senescent cells. Once internalized, SiNPs do not exit and accumulate in the cytoplasm for long term. Our study provides a basis for future development of NP-based tools that can detect and target senescent cells for therapy.



Cellular uptake and retention studies of silica nanoparticles in *C. elegans*

- *C. elegans* (young adult) fed with *E. coli* OP50 mixed with nanoparticles (500 nm in size, Red fluorescent),
- lifespan analysis
- visualization - Confocal Microscopy, Laboratory of Subcellular Structures Analyses (Head: prof. Eliza Wyszko), IBCh PAS, by **dr. Agnieszka Fedoruk-Wyszomirska**

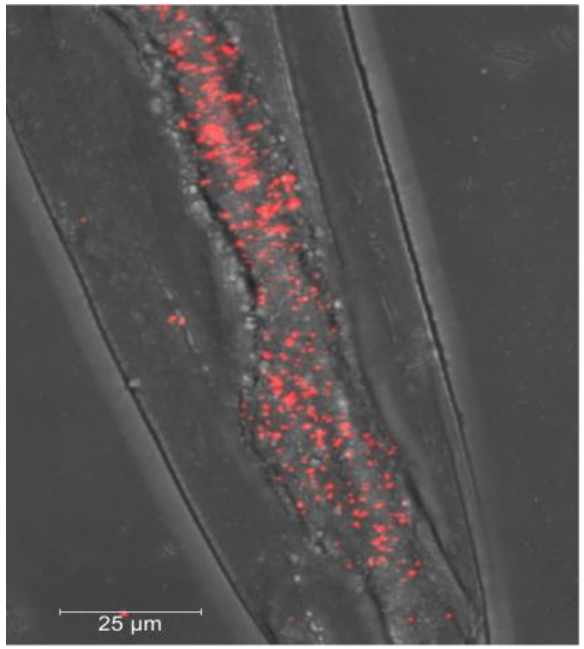
DAY 5

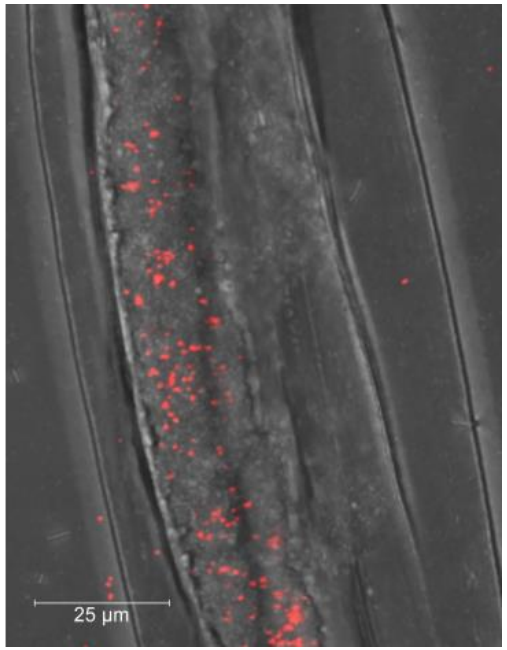


Cellular uptake and retention studies of silica nanoparticles in *C. elegans*

- *C. elegans* (young adult) fed with *E. coli* OP50 mixed with nanoparticles (500 nm in size, Red fluorescent),
- lifespan analysis
- visualization - confocal microscopy, Laboratory of Subcellular Structures Analyses (Head: prof. Eliza Wyszko), IBCh PAS, by **dr. Agnieszka Fedoruk-Wyszomirska**

DAY 18

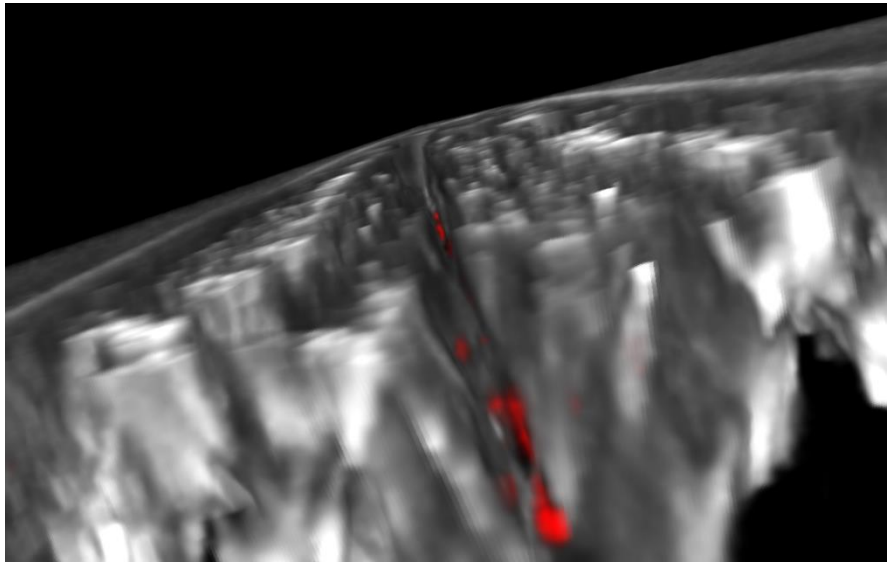




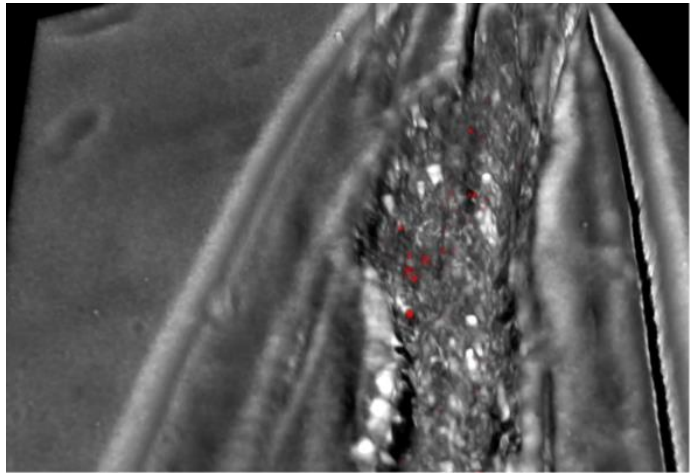
Cellular uptake and retention studies of silica nanoparticles in *C. elegans*

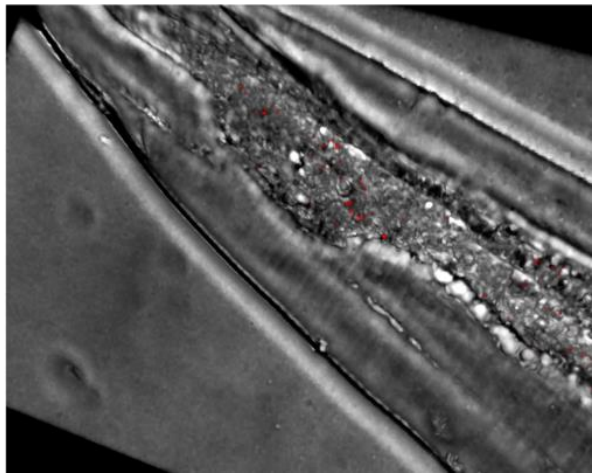
3D imaging

DAY 5



DAY 18

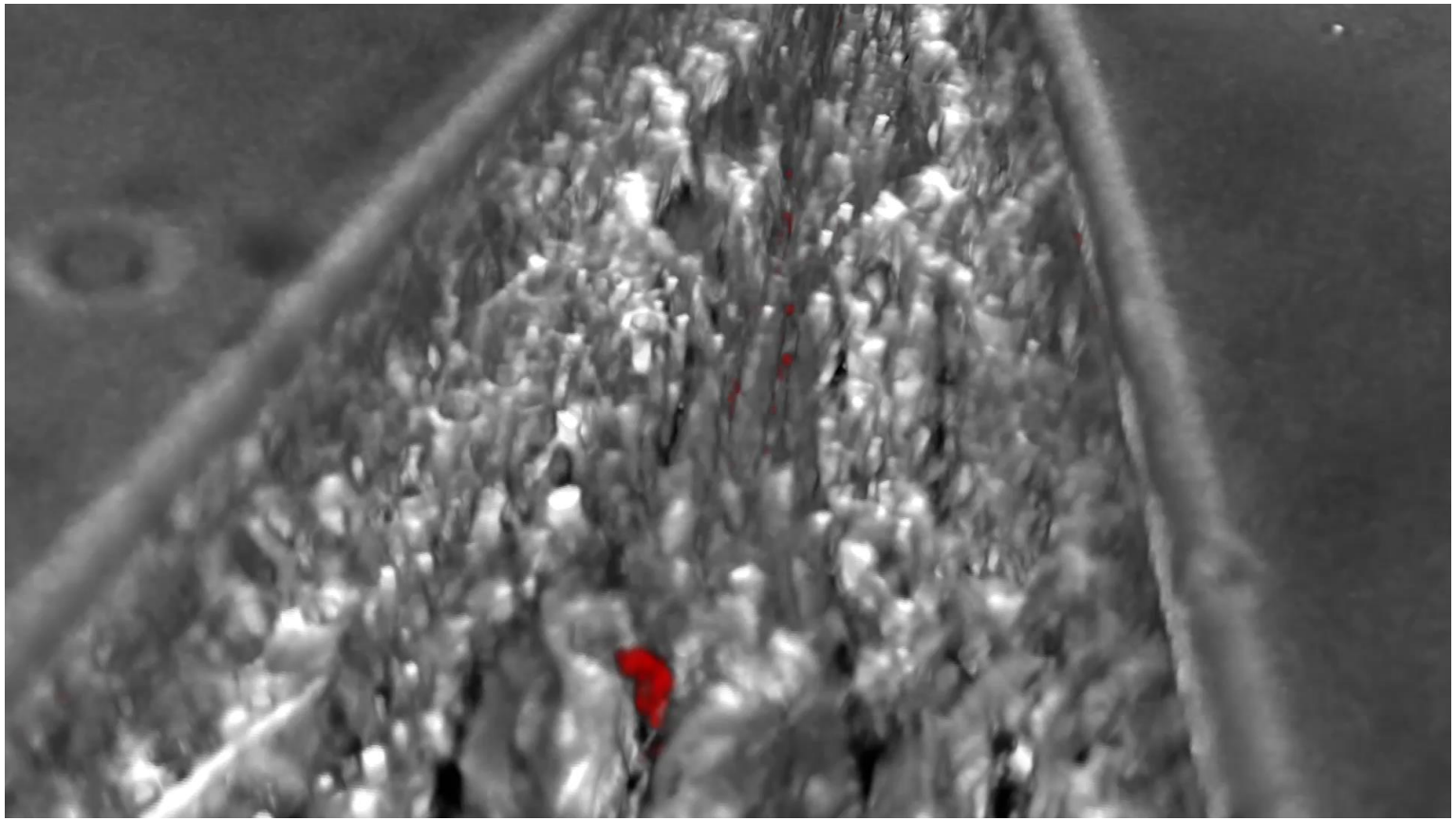




Laboratory of Subcellular Structures Analyses (Head: prof. Eliza Wyszko), IBCh PAS, by **dr. Agnieszka Fedoruk-Wyszomirska**

Cellular uptake and retention studies of silica nanoparticles in *C. elegans*

3D imaging - movies



Laboratory of Subcellular Structures Analyses (Head: prof. Eliza Wyszko), IBCh PAS, by **dr. Agnieszka Fedoruk-Wyszomirska**

Schmidtea mediterranea

1. Freshwater planarian, free-living invertebrate from the phylum Platyhelminthes (flatworms).

2. Three germ layers (endoderm, mesoderm, and ectoderm), bilateral symmetry, and tissues with separate organs.

3. Great ability to regenerate after amputation or injury. In some cases, a full organism can be rebuilt after several days from 1/279 piece of a single worm, although the regenerative abilities of planarians are different across the species.

4. Two distinct strains of *S. mediterranea* exist in nature: a sexual strain (2 cm long) and an asexual strain (slightly shorter).

5. Nervous system of flatworms is comprised of a bilobed 'brain' with different types of neurons and glia.

6. Photo-, chemo- and rheoreceptors located at the front of the planarian's body.

7. Centrally located pharynx is in charge of food intake and removal and is connected to a highly branched intestine, which circulates nutrients within the body

8. Pluripotent stem cells (neoblasts), which are essential for worms' regeneration ability, comprise ~30% of the cells in the adult animal.

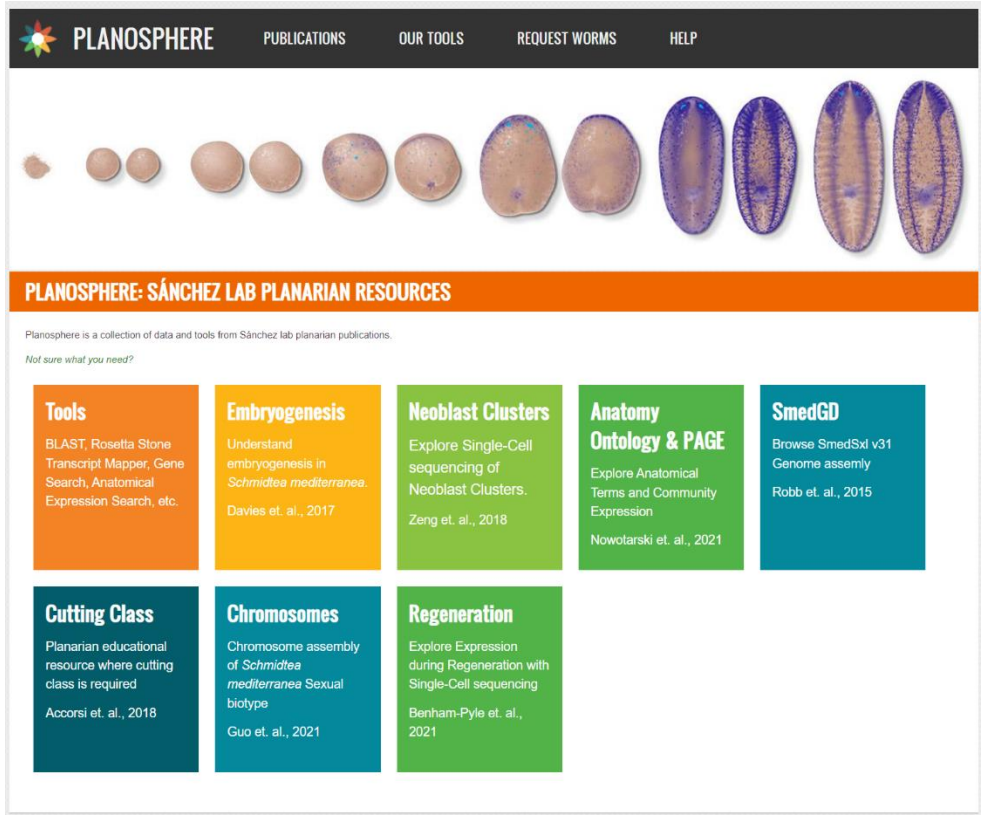
Advantages of the S. mediterranea

1. The maintenance of planarians is relatively easy and cheap, and does not require specialized equipment; only habitat conditions, such as temperature, darkness, feeding, and water culture.

2. The genome of *S. mediterranea* is well annotated - SmedGD: the *Schmidtea mediterranea* genome database.

3. Many of the annotated *S. mediterranea*'s genes have known orthologs (or at least homologs) in the human genome.

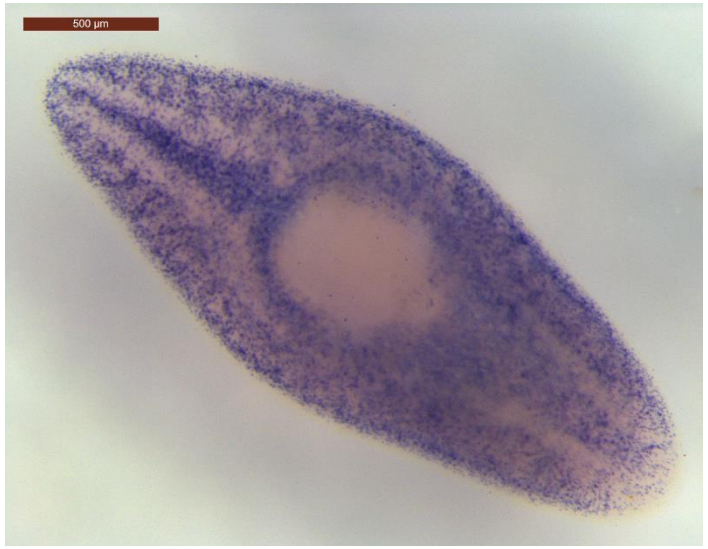
4. Simple modifications of gene expression by knockdown/silencing genes of interest through RNA interference (RNAi) using double-stranded RNA (dsRNA). dsRNA can be administered to the worms by microinjection, by feeding them with dsRNA-containing bacteria, or with food mixed with free dsRNA.

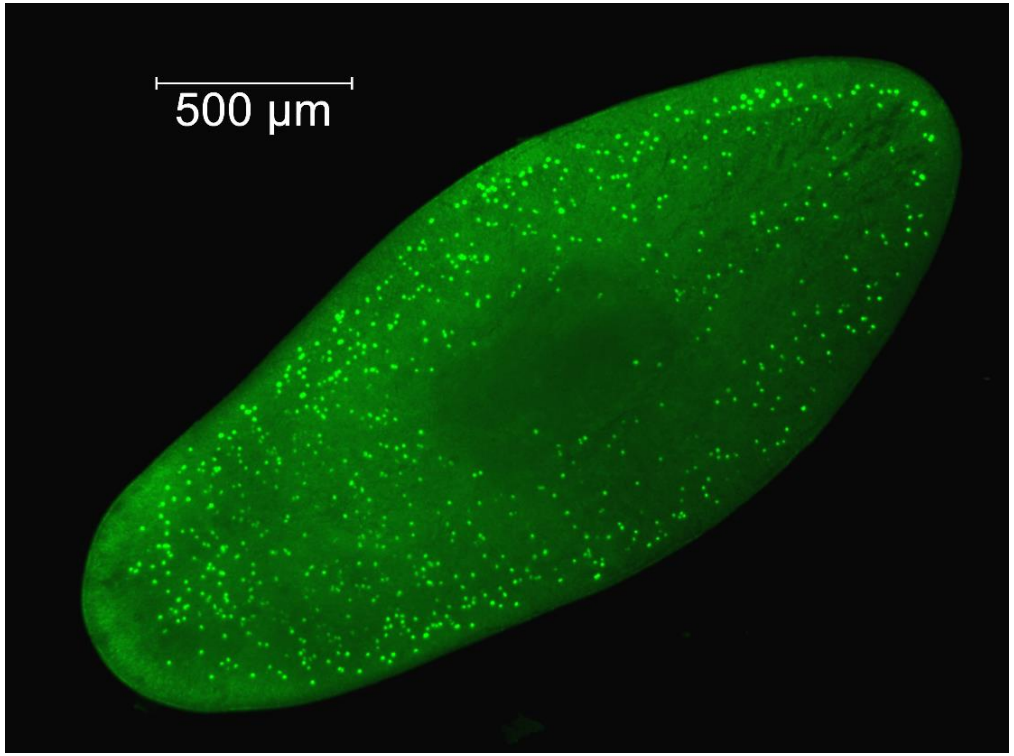


S. mediterranea as a model organism

Model organism for:

- adult stem cel biology research and regeneration
- human motile ciliopathies
- neurotoxicology
- toxicity assessment
- germ cell development
- discovery and characterization of cell-penetrating peptides and bioportides





Laboratory of Subcellular Structures Analyses (Head: prof. Eliza Wyszko), IBCh PAS, by **dr. Agnieszka Fedoruk-Wyszomirska**

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ICHB PAN

Laboratory of Mammalian Model Organisms

Established in January 2020

Poznań, 6.02.2024

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Łukasz Przybył, PhD
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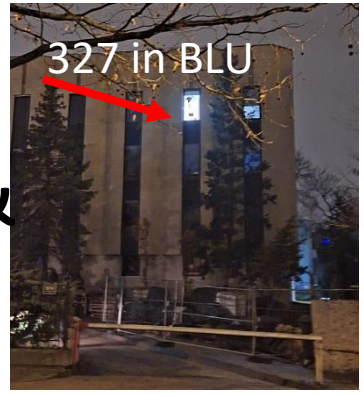
Dorota Wronka MSc, Eng.
biologist

Anna Karlik MSc
product developer

Center for Advanced Technologies; Adam Mickiewicz University

&



DEPARTMENT X

IDEA FOR IN VIVO EXPERIMENT

PLAN | PERMISSIONS | PREPARATION | PERFORMANCE | ANALYSIS

MATERIAL READY FOR ANALYSIS

FIGURES READY FOR PUBLISHING

DEPARTMENT X

WHAT DO WE OFFER?

PLAN

experimental design

planning a budget

PERMISSIONS

Local Ethics Committee or Ministry of Environment or Pharmaceutical Inspectorate

organisation of animal transport

documentation to work with laboratory animals

PREPARATION

training in basic techniques used in animal research

mouse colonies (transgenic or wild-type) and databases

keeping experimental records

PERFORMANCE

In vivo

injections: intramuscular, intraperitoneal, subcutaneous, intracranial
intra-stomach administration (oral gavage)
implantation of osmotic pumps
behavioral testing and phenotyphic observations
transcardial perfusion, organ weighing and sampling, blood withdrawal
en face preparation of whole aorta
brain dissection into structures
acute kidney injury and uninephrctomy
xenograft inoculation
immunization
bronchoalveolar lavage

In vitro

immunophenotyping using flow cytometry
primary cell cultures
hybridoma generation
genotyping
qPCRs

ANALYSIS

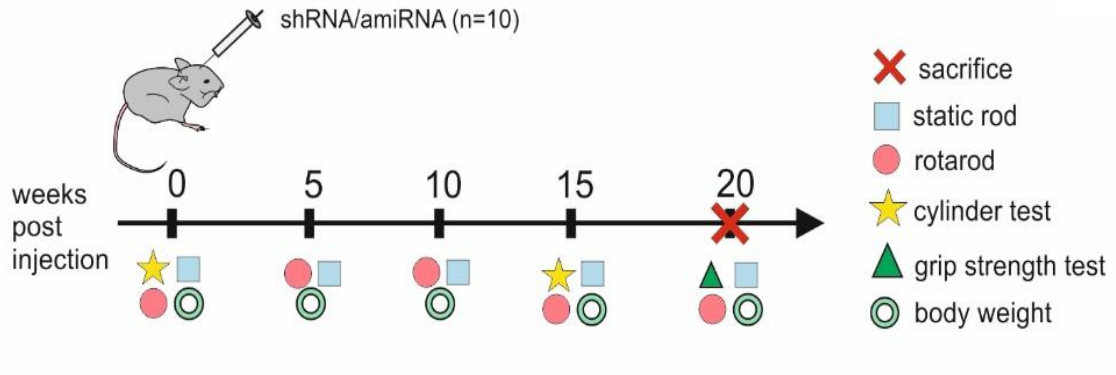
digitalization of data acquired during animal experiment

statistical analysis and figure preparation

PLANNING AND PREPARATION

Experimental design

- Selection of appropriate models and methodology
- Timeline
- Budget



Database and colony management

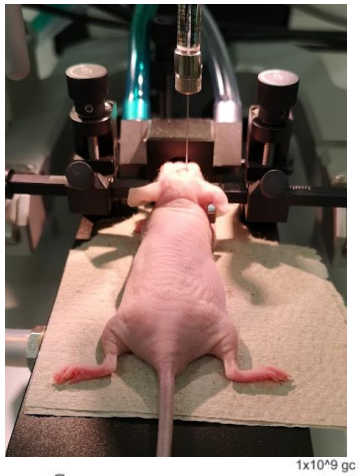
- generate experimental groups
- sustain the mouse lines
- track generations and in-bred factor

Zlecono	Cage number	Animal ID	Genotype	Date of Birth	Sex	Generation	Parent male	Parent female	Experiments planned
Ayca's breeding cages NEW		3539	WT	2021-11-12	F		3365	416	new breeding cage on 16/02 - experimental zone
		2282	WT	2021-12-27	M		413	418	new breeding cage on 16/02 - experimental zone
		3538	WT	2021-11-12	F		3365	416	new breeding cage on 26/01 - experimental zone
		3540	WT	2021-11-12	M		3365	416	new breeding cage on 26/01 - experimental zone
		2217	WT	2021-11-30	F		413	418	new breeding cage on 31/01 - experimental zone
		2218	WT	2021-11-30	F		413	418	new breeding cage on 31/01 - experimental zone
		3541	WT	2021-11-12	M		3365	416	new breeding cage on 31/01 - experimental zone
		3542	WT	2021-11-12	M		3365	416	new breeding cage on 04/02 - experimental zone
		2219	WT	2021-11-30	F		413	418	new breeding cage on 04/02 - experimental zone
		3804	WT	2022-02-16	F		2221	2220	new breeding cage on 04/03 - experimental zone
		3806	WT	2022-02-16	M		2221	2220	new breeding cage on 04/03 - experimental zone
	Breeding zone BC's	416	WT	2021-04-10	F	F:1	1973	1970	*w parze zarodowej*
		418	WT	2021-04-10	F	F:1	1973	1970	*w parze zarodowej*
		2220	WT	2021-11-30	F		413	418	*w parze zarodowej*
		2279	WT	2021-12-22	F		3365	416	*w parze zarodowej*
		2280	WT	2021-12-22	F		3365	416	*w parze zarodowej*
		3365	WT	2021-03-02	M	F:1	1973	1970	*w parze zarodowej*
		413	WT	2021-04-10	M	F:1	1973	1970	*w parze zarodowej*
		2221	WT	2021-11-30	M		413	418	*w parze zarodowej*
		2281	WT	2021-12-22	M		3365	416	*w parze zarodowej*
	1 sentinel								
	2	3901	WT	2022-01-18	F		413	418	
		3902	WT	2022-01-18	F		413	418	
		3802	WT	2022-02-02	F		3365	416	
	3	3807	WT	2022-02-16	M		2221	2220	
		3808	WT	2022-02-16	M		2221	2220	
	4	3818	WT	2022-03-14	F		2281	2280	
		3819	WT	2022-03-14	F		2281	2280	
		3820	WT	2022-03-14	F		2281	2280	
		3821	WT	2022-03-14	F		2281	2280	
		3822	WT	2022-03-16	F		2281	2279	
	5	3832	WT	2022-03-16	M		2281	2279	
		3833	WT	2022-03-16	M		2281	2279	

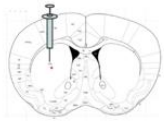
BL6/N BCs | BL6/N F:1 | BL6/N x CDR1as M2 BCs | BL6/N x CDR1 as M2 F:1 | CDR1as M2 BCs | CDR1as M2 F:1/F:2 | OLD BC | Sacrificed

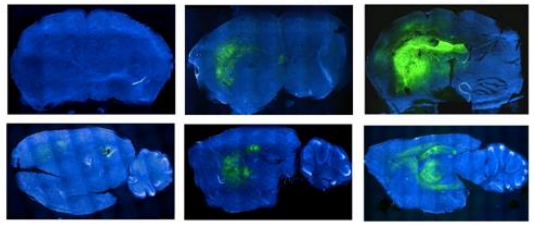
SURGERIES

Intracranial delivery

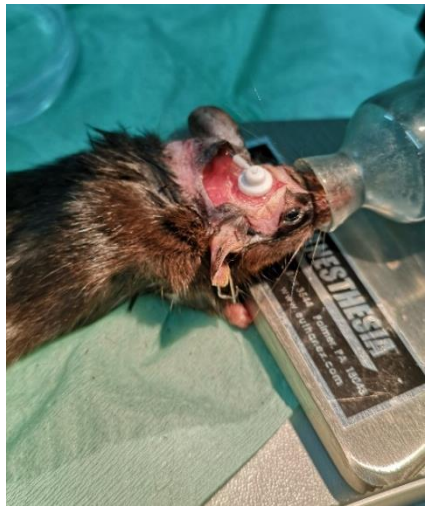


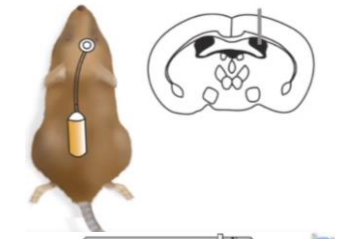
1x10^9 gc 1x10^10 gc 1x10^11 gc

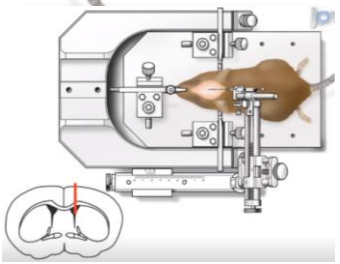




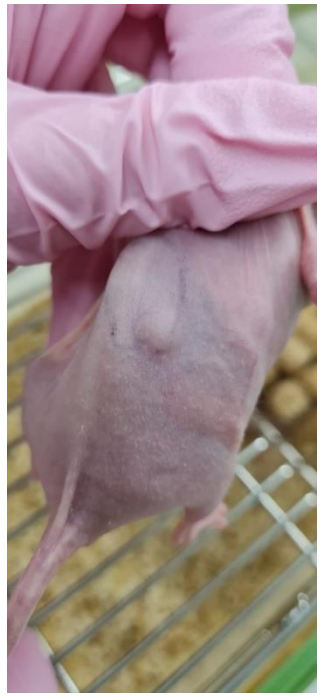
Implantation of osmotic pump



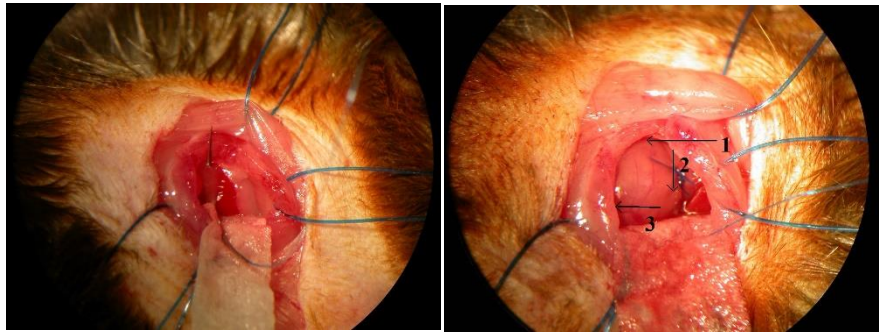




Tumor inoculation

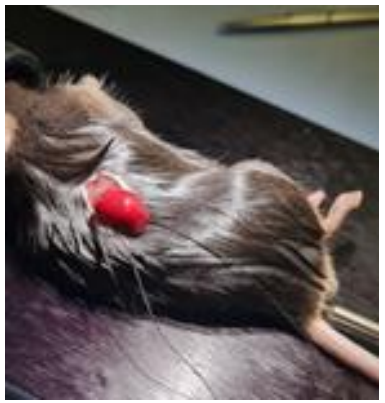


Myocardial infarction and myometrium injections



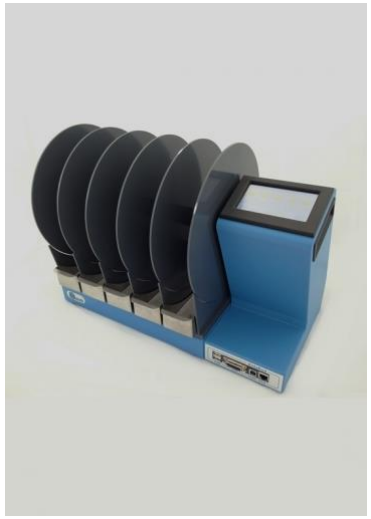
Biopsy and oral gavage

Uninefrectomy / Acute Kidney Injury

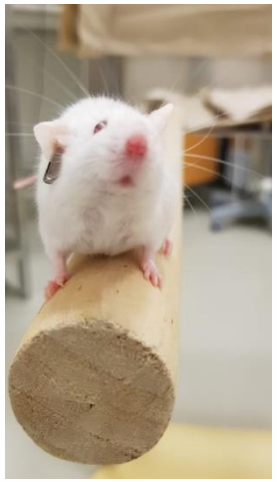


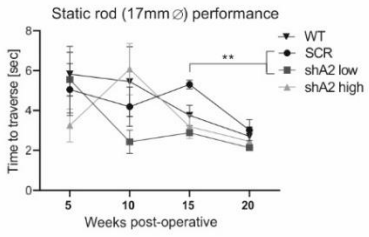
BEHAVIORAL TESTING

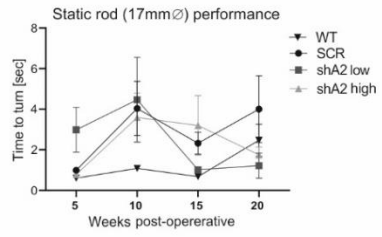
Rotarod Test

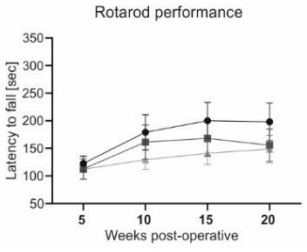


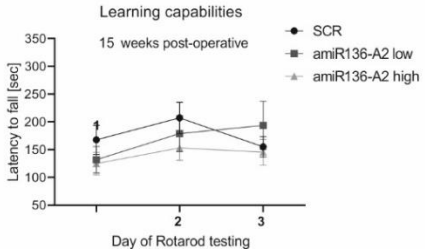
Static rod Test

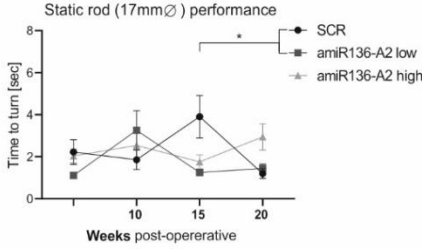




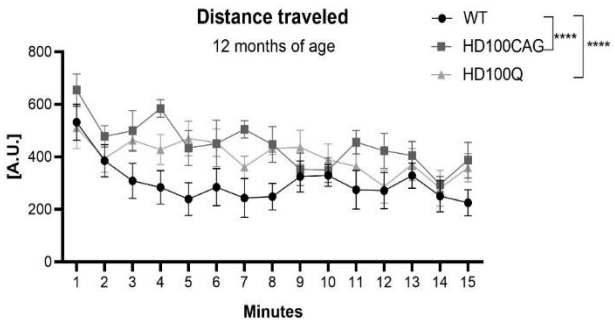




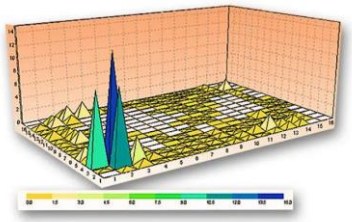


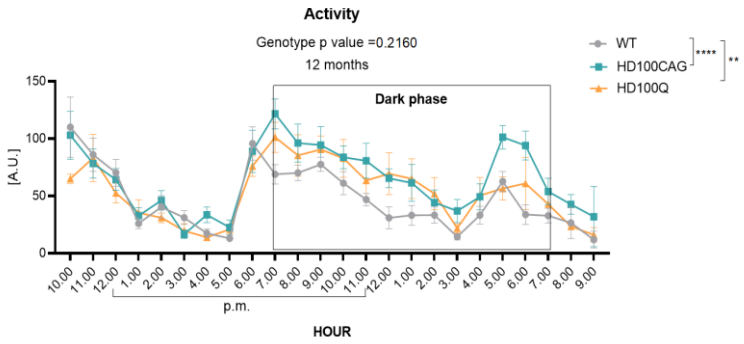


Open-Field Test



Daily Activity Test

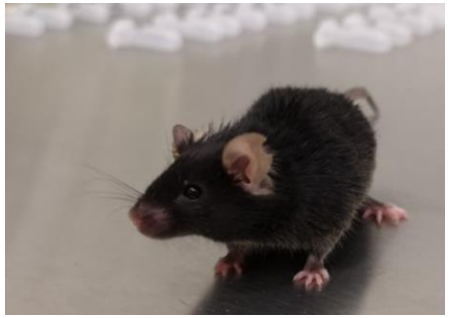




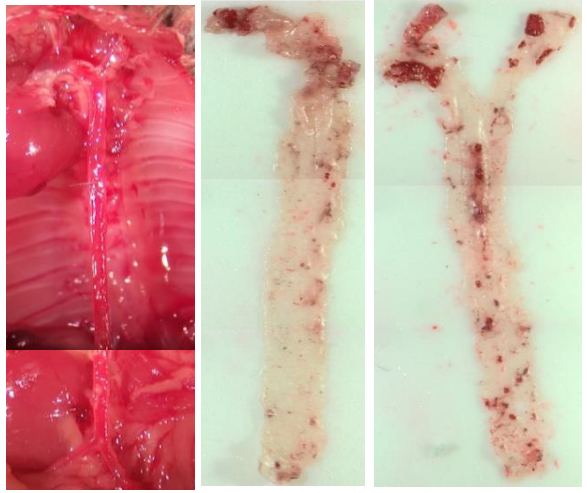
Hindlimb Test

Beaker Test

TISSUE PROCESSING

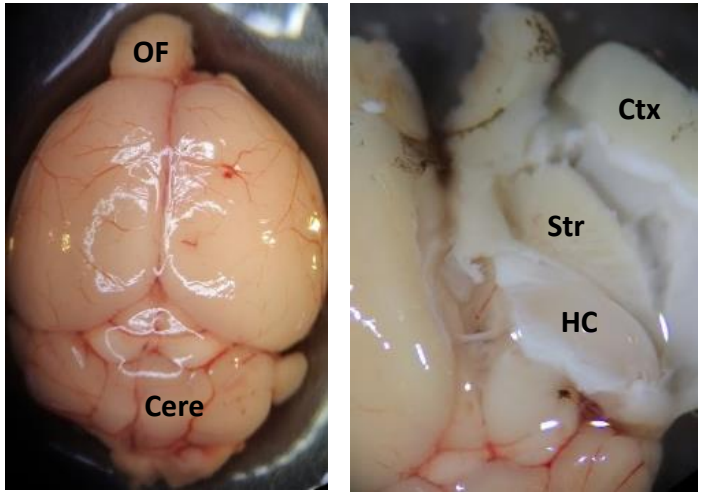


Isolation of aortas *en face*

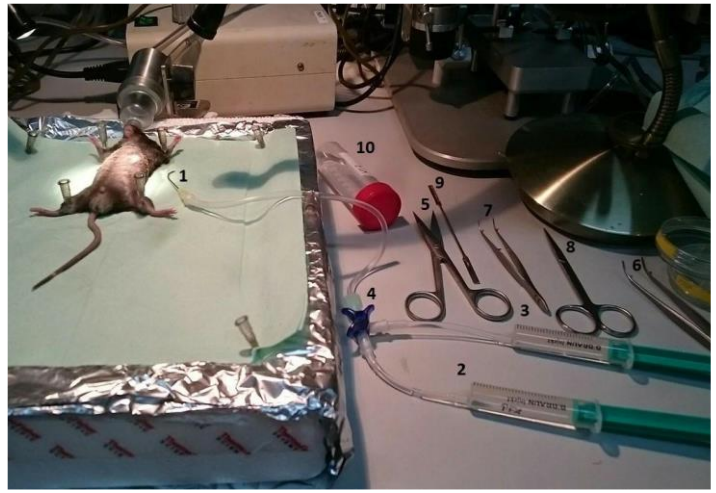


Oil red O

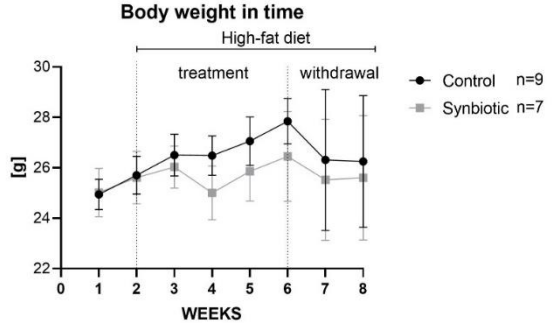
Dissection of brains into structures

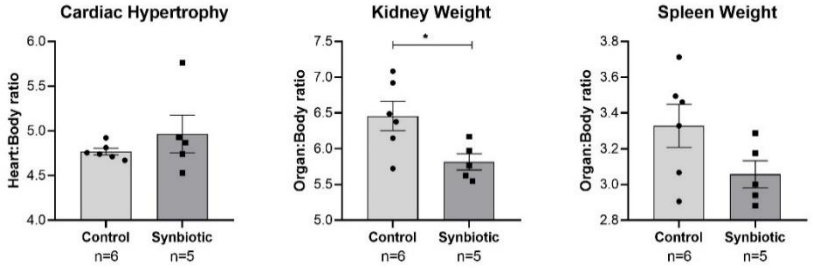


Transcardial perfusion

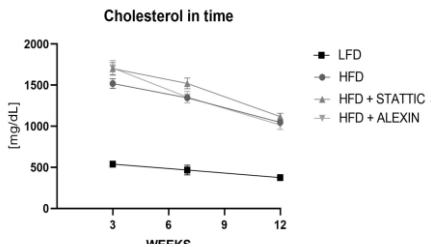


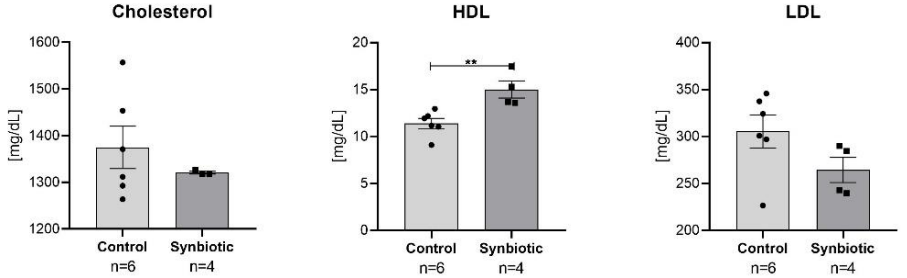
Tissue and animal weighing



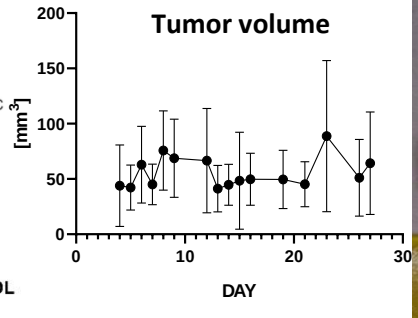


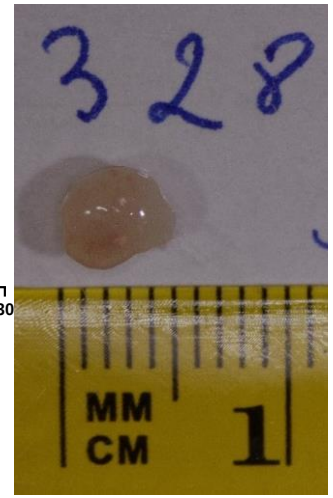
Blood sampling and biochemical analysis in serum/plasma





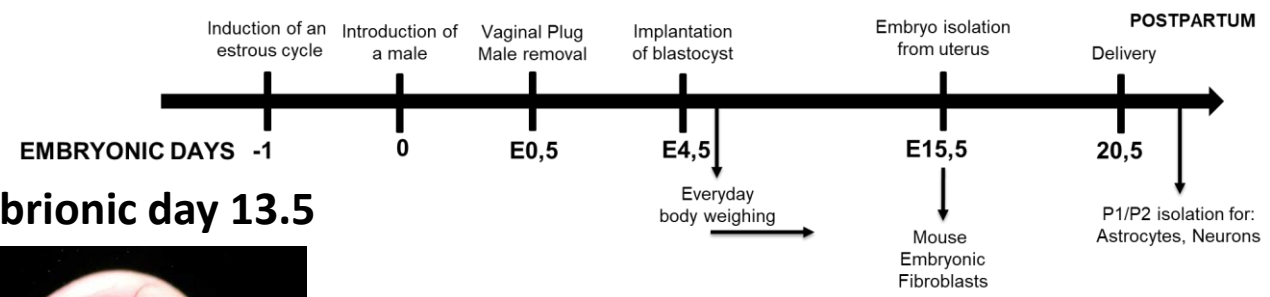
Measuring tumor size



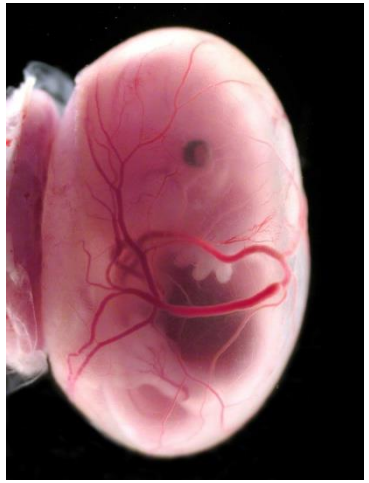


PRIMARY CELL CULTURE

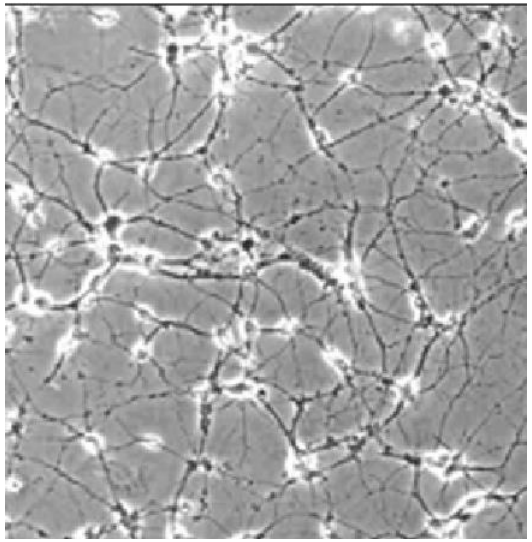
- Mouse embryonic fibroblasts (MEFs)
- Vascular smooth muscle cells (VSMCs)
- Bone marrow-derived macrophages
- Neurons
- Astrocytes



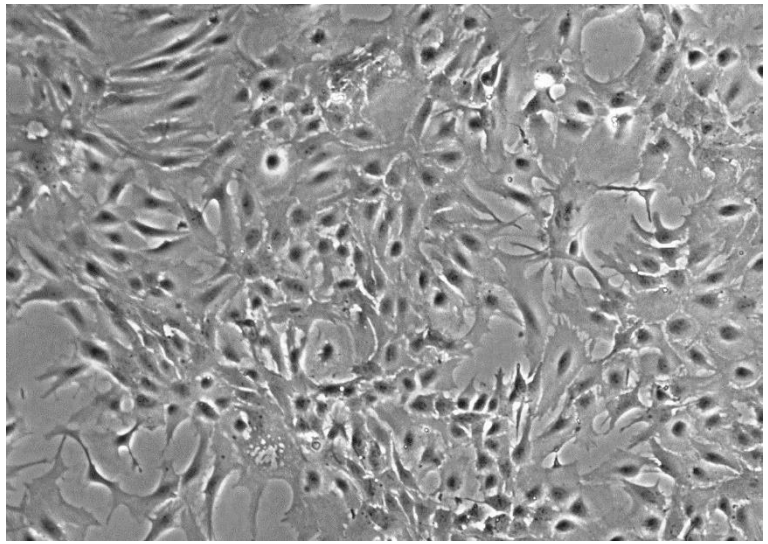
Embrionic day 13.5



Neurons

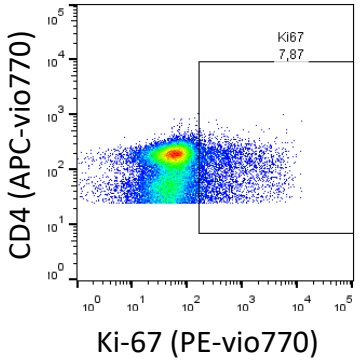


Astrocytes



FLOW CYTOMETRY

- Cell cycle
- Proliferation (Ki67)
- Apoptosis and necrosis (Annexin V, 7AAD)
- Immunophenotyping



Guava 12HT

Mouse:

T cells (effector, helper1, helper17, γδT cells, regulatory, central memory, effector memory, naive)

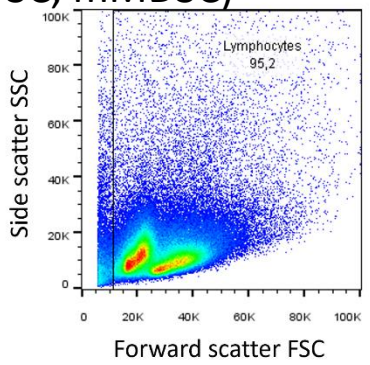
CD3, CD8, CD4, γδTCR, CD44, CD62L, CD69, CD25, FoxP3, T-bet, Helios, RORγt

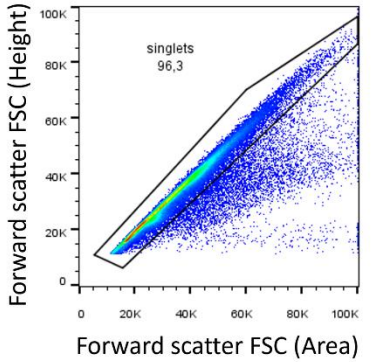
M cells (B cells, monocytes, M1 macrophages, M2 macrophages, gMDSC, mMDSC)

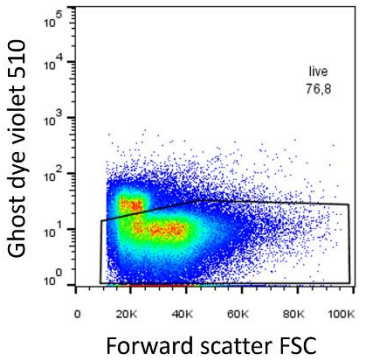
CD11b, CD11c, Ly6C, Ly6G, B220, CD86, CD206

Cytokines

TNF-α, IL-17, IFN-γ, IL-6, IL-1β







Rat:

T cells (helper T cells, effector T cells)

CD3, CD4, CD8

M cells (B cells, NK cells, neutrophils, monocytes, macrophages)

CD43, CD161, B220, His48

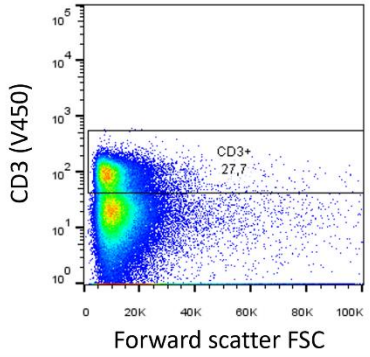
Human:

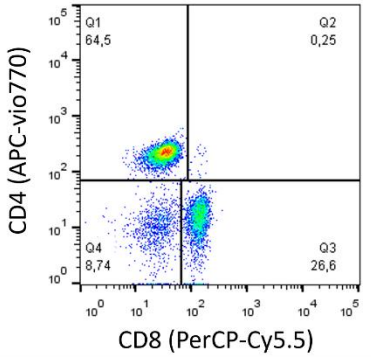
T cells (helper T cells, effector T cells, γδT cells)

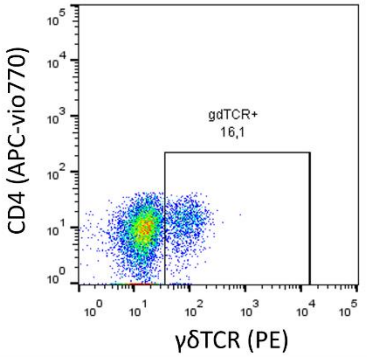
CD3, CD8, CD4, γδTCR, CD44, CD25

B cells

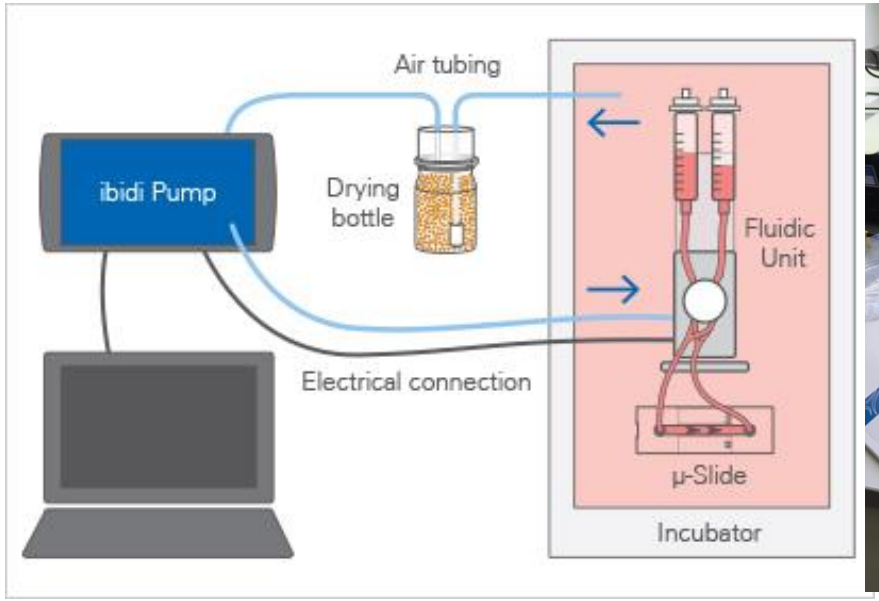
B220

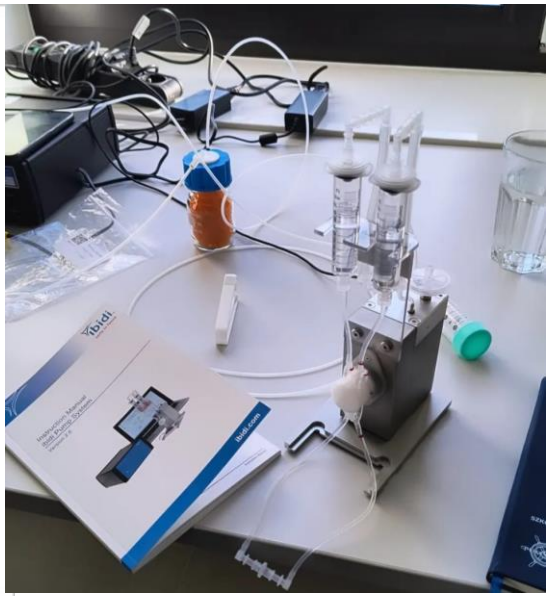






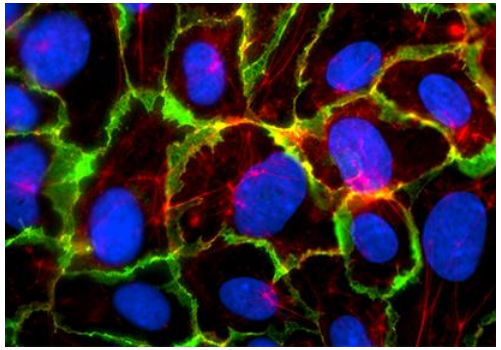
IBIDI PUMP SYSTEM



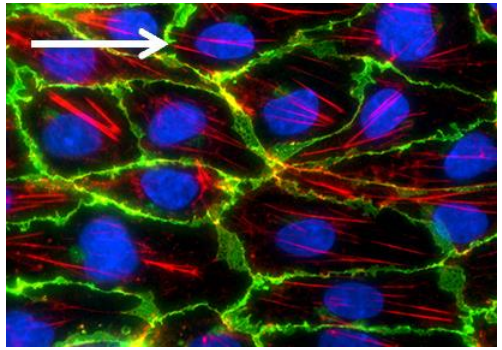


Allows for cell culture and live imaging under flow conditions

Static culture



Flow culture



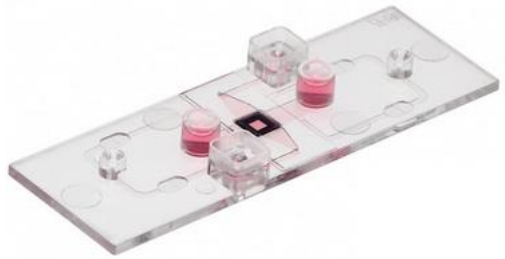
HUVEC (human umbilical vein endothelial cells) structure under static and flow conditions. Flow-conditioned cells are elongated and show distinct F-actin stress fibers (stained with phalloidin, red). VE-cadherins (green), which mark the adherence junctions, are present in both conditions.

Wide variety of slides for different assays

- Adherent cells under flow conditions
- Simulation of blood vessels
- Rolling and Adhesion of Leukocytes assay

- long-term cultivation and perfusion of 3D spheroids or organoids

- 3D Cell and Tissue Assays
- Endothelial barrier model
- Rolling, Adhesion, and Transmigration



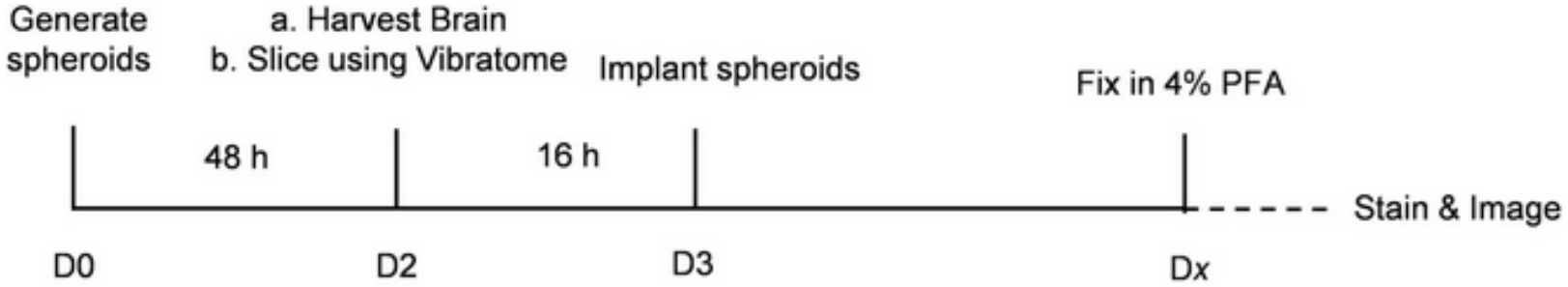
- Blood-brain barrier model
- Trans-Membrane Migration in 2D
- Cell Transport in a 3D Gel Matrix

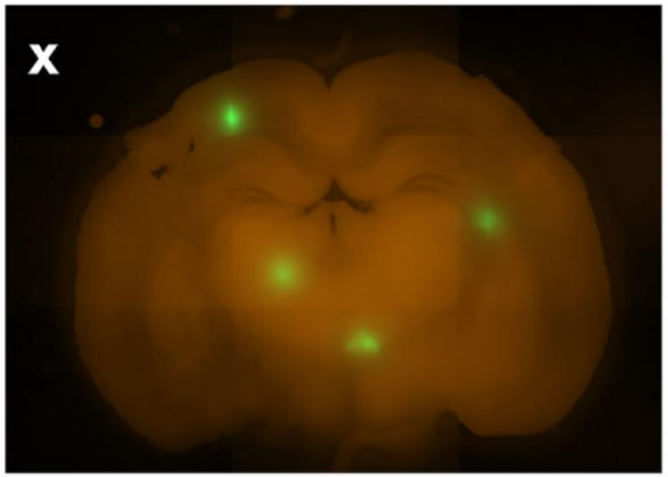
Ibidi.com

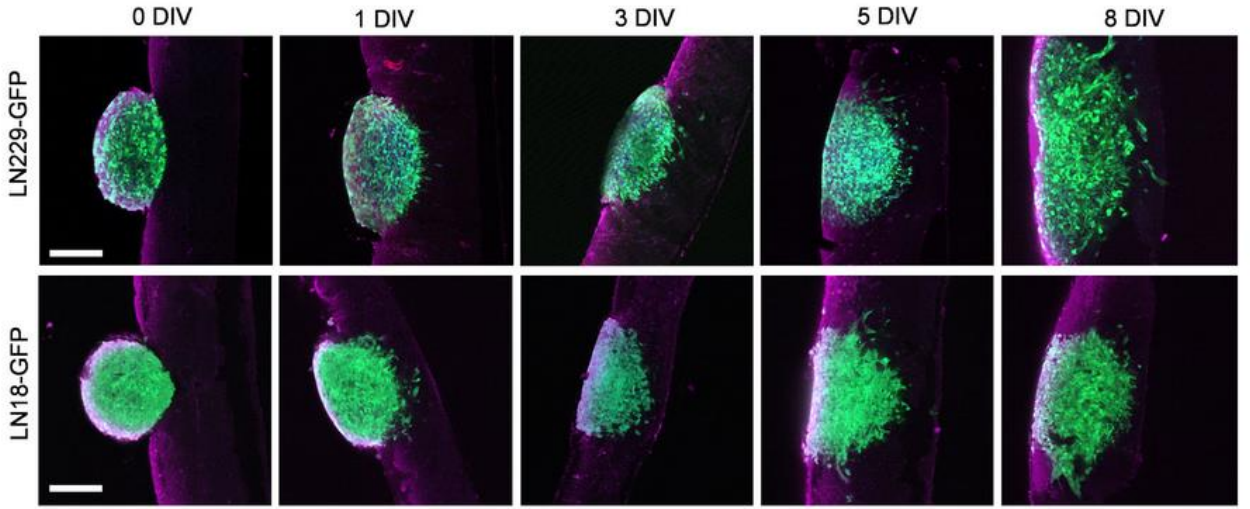
LEICA VIBRATOME VT1200 S @dr hab. Paulina Jackowiak

Fully automated vibrating blade microtome

Development and validation of an advanced ex vivo brain slice invasion assay to model glioblastoma cell invasion into the complex brain microenvironment







By Anna Karlik, Dorota Wronka, Konrad Kuczyński

Decotret LR, Shi R, Thomas KN, Hsu M, Pallen CJ, Bennewith KL. Development and validation of an advanced *ex vivo* brain slice invasion assay to model glioblastoma cell invasion into the complex brain microenvironment. Front Oncol. 2023 Jan 26;13:976945. doi: 10.3389/fonc.2023.976945. PMID: 36793608; PMCID: PMC9923402.

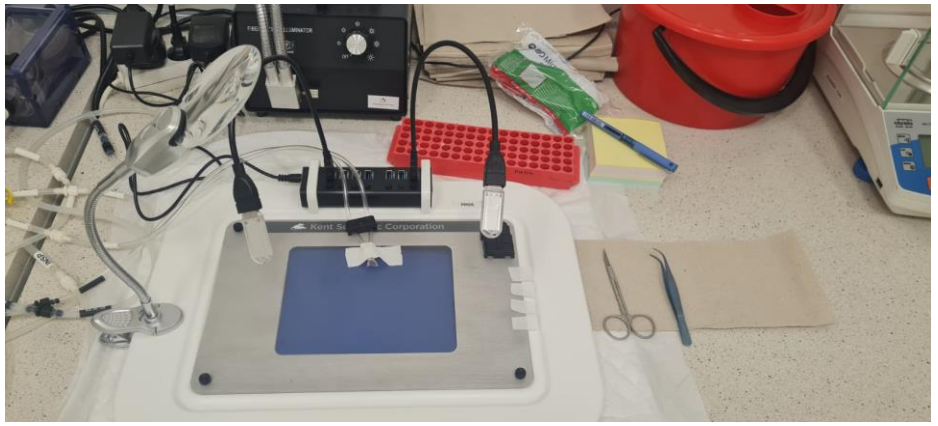
EQUIPMENT

In vivo:

- Digital operating microscope (RWD Life Science)
- Stereo microscope (Nikon)
- Multifunctional surgical platform (Kent Scientific)
- Endotracheal intubation kit for mice and rats (Kent Scientific)
- Low-flow anesthesia system (Kent Scientific)
- System for thermoregulation of mice and rats (RWD Life Science)
- Peristaltic pump (WPI)
- Analytical scales
- Surgical tools (FST/RWD)

In vitro:

- Pump system for long-term flow cell cultures under physiological conditions (Ibidi)
- Cell counter (Bio-Rad)
- Precellys homogenizer (Bertin)
- Bioanalyzer (Agilent)
- QuantStudio3 (Applied Biosystems)
- Thermocycler (Applied Biosystems)
- HPLC with colorimetric detector (Thermo)
- Centrifuge 5424R (Eppendorf)



RESEARCH PROJECTS (past and present)

- *Functional implications of brain-enriched circular RNAs;* PI: dr Monika Piwecka*;* **SONATA BIS 8**
- *Non-coding RNAs at single-cell resolution in the pituitary gland and their role in the regulation of gene expression;* PI: dr Monika Piwecka*;* **OPUS 19**
- *Development of a universal fast-response platform, based on RNA technology, ensuring the national drug and epidemiological safety (*Consortium leader: Polfa S.A., PI: prof. M. Figlerowicz*)* **ABM**
- *Deciphering selective neuronal vulnerability using the direct profiling of degenerating neurons;* PI: dr Paweł Świtoński*;* **SONATA 17**
- *The influence of normal and mutant ataxin 3 on the immune system in the context of the pathogenesis of spinocerebellar ataxia type 3 (SCA3)* – PI dr Łukasz Przybył; **MINIATURA 3**
- *The use of genetic tools in experimental therapy of polyglutamine diseases*; PI prof. Marta Olejniczak; **SONATA BIS 5**
- *The characteristics of the regulatory RNAs landscape in glioblastoma multiforme (GBM). Circular RNAs and micro RNA-like molecules as new players in gliomagenesis and GBM progression and their importance for glioblastoma stem cells*; PI prof. Katarzyna Rolle; **SONATA BIS 7**
- *Anti-aging properties of 4-N-furfyrylocytosine in age-differentiated eukaryotic cells, budding yeast and mouse model of aging*; PI prof. Eliza Wyszko; **OPUS 13**
- *Psychedelics as Therapeutic Candidates in Neurodegenerative Disorders Treatment– a Study in Spinocerebellar Ataxia Type 3 Mouse Model*; PI dr Urszula Kozłowska; **SONATINA 5**
- *Comprehensive analysis of the therapeutic potential of oligonucleotides for the treatment of polyglutamine diseases*; PI prof. Agnieszka Fiszer; **SONATA 9**
- *The novel role of STAT1 in Vascular Smooth Muscle Cell and Macrophage common and –specific transcriptional responses that reflect onset and progression of atherosclerosis*; (cooperation with Adam Mickiewicz University) – PI prof. Johannes Bluyssen; **OPUS 19**

RESEARCH PROJECTS (future)

Granted:

- *Functional or not? Studying positionally conserved vertebrate long noncoding RNA orthologues at subcellular resolution;* PI: dr hab. Barbara Uszczyńska-Ratajczak; **SONATA BIS 11**
- *Antiviral strategies targeting RNA: Peptide nucleic acids (PNA) forming triplexes and their conjugates with low molecular weight ligands specific to conserved structural motifs of influenza A virus RNA and SARS-CoV-2;* PI: prof. Dr hab. Elżbieta Kierzek; **OPUS 21**
- *Vitamin drink enriched with compounds of natural origin with scientifically proven effectiveness supporting the treatment of irratable bowel syndrome;* PI (in consortium): dr hab. Paweł Kołodziejski; **NUTRITECH 1**

Under revision:

- *Modeling and studying tumor invasion in 3D GBM-brain assembloid;* PI: dr. hab. Katarzyna Rolle; **OPUS 26**
- *Design and Development of Serine Hydroxymethyltransferase-2 (SHMT2) Inhibitors Blocking Tumor Growth;* PI: dr hab. Miłosz Ruszkowski*;* **SONATA BIS 13**
- *Organometallic inhibitors of influenza virus and emerging coronaviruses entry;* PI: dr Paweł Zmora*;* **OPUS 26**
- *From third ventricle to pituitary gland: regulatory RNA networks in post-transcriptional modulation of gene expression in neurosecretion;* PI: dr Monika Piwecka; **OPUS-LAP 24**
- *Integrative, Therapeutic Microbiome-empowered Neuroprotective Nutrition;* PI (in consortium): dr Łukasz Przybył; **NUTRIBRAIN**
- *Modeling of human tissues in the study of molecular mechanisms and pathogenesis of human diseases;* PI (in consortium): dr Łukasz Przybył; **STRATEGIC PARTNERSHIPS**

To be submitted soon:

- *Psychobiological stress processing as modulator of neurodegenerative disease severity;* PI (in consortium): dr Łukasz Przybył; **JPND**
- *Development of an innovative targeted therapy for glioma using nucleic acids to modify the tumor microenvironment and the functions of cancer stem cells;* PI (in consortium): dr Łukasz Przybył; **SMART PATH**
- *Development of technology based on parallelized microfluidic systems for cascade filtration of animal cells in continuous perfusion culture on an industrial scale;* PI (in consortium): dr Łukasz Przybył; **SMART PATH**

PUBLICATIONS

Wronka D, Karlik A, Misiorek JO, **Przybyl L.** What the Gut Tells the Brain—Is There a Link between Microbiota and Huntington's Disease? *Int J Mol Sci* 2023

Kotowska-Zimmer A, **Przybyl L,** Pewinska M, Suszynska-Zajczyk J, **Wronka D,** Figiel M and Olejniczak M. A CAG repeat-targeting artificial miRNA lowers the mutant huntingtin level in the YAC128 model of Huntington's disease. *Molecular Therapy - Nucleic Acids* 2022

Majchrzak-Celińska A, Misiorek J, Kruhlenia N, **Przybyl L,** Kleszcz R, Rolle K, Krajka-Kuźniak V. COXIBs and 2,5-dimethylcelecoxib counteract the hyperactivated Wnt/β-catenin pathway and COX-2/PGE2/EP4 signaling in glioblastoma cells *BMC Cancer* 2021

Przybyl L, Wozna-Wysocka M, Kozlowska E, Fiszer A. What, When and How to Measure-Peripheral Biomarkers in Therapy of Huntington's Disease. *Int J Mol Sci* 2021

Wozna-Wysocka M#, Jazurek-Ciesiolka M#, **Przybyl L**#, **Wronka D**, Suszyńska-Zajczyk J, Misiorek JO, Figura G, Ciesiolka A, Sobieszczanska P, Zeller A, Niemira M, Figiel M, Switonski PM*, Fiszer A* Mutant RNA contributes to neuropathology in new mouse models of Huntington's disease**.** *In preparation*

Palani Kumar M, Halami P, Serva Peddha M, **Wronka D, Karlik A,** Bartolomaeus T, Haghikia A, Markó L*, Forslund S*, **Przybyl L*** Synbiotics attenuate hypercholesterolemia in ApoE knockout mouse model by immune-dependent regulation of intestinal cholesterol metabolism *In preparation*

Bębnowska D, Hrynkiewicz R, **Karlik A, Przybyl L,** Niedźwiedzka-Rystwej P Expression of autophagic and apoptotic markers during infection with animal virus causing hemorrhagic fever in rabbits *In preparation*

Zarębska Z, Kuczyński K, Latowska-Łysiak J, Grabowska A, Sajek MP, Piestrzeniewicz R, Barciszewska AM, Kuczyńska B, **Wronka D, Karlik A, Przybyl L**, Rolle K Circular RNA circCLIP2 promotes the invasive properties of glioblastoma by acting as a mediator of EMT pathway and cancer stemness *In preparation*

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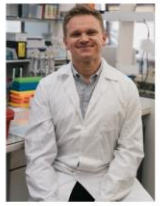
Department of Molecular Virology



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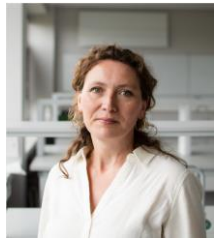
ext. 1306

NATIONAL



Katedra Żywienia Człowieka i Dietetyki
Wydział Nauk o Żywności i Żywieniu Uniwersytetu Przyrodniczego w Poznaniu

prof. dr hab. Joanna Suliburska

KATEDRA BIOCHEMII I BIOTECHNOLOGII 19 56

dr Joanna Suszyńska-Zajczyk

Laboratory of Human Molecular Genetics
Laboratory of High Throughput Technologies

prof. dr hab. Johannes Bluyssen

KATEDRA
FIZJOLOGII, BIOCHEMII I BIOSTRUKTURY ZWIERZĄT

dr hab. Paweł Kołodziejski

UNIWERSYTET SZCZECIŃSKI
INSTYTUT BIOLOGII

dr hab. Paulina Niedźwiedzka-Rystwej

INTERNATIONAL

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

prof. dr Sofia Forslund

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

dr hab. Julian Hellmann-Regen

ECRC Experimental & Clinical Research Center

dr Nicola Wilck

COMPANIES

Medicofarma Biotech
Today's Science is Tomorrow's Medicine

dr Michał Prendecki

bedrocan

dr Mikael Kowal

dystrogene

dr Kris Siemionow

µFraction®

dr Brian Miller

JJP Biologics

prof. dr hab. Tomasz Grabowski

PAST

> 10.000 mice

19 mouse colonies

> 30 people from IBCH administered in animal facility

28 applications to Local Ethics Committee

FUTURE

Myocardial infarction

***In vitro* fertilization/embryo transfer**

Germ-free mice

Dedicated immunophenotyping for rats and human

***In vitro* model of blood-brain barier**

M1/M2 polarisation of macrophages

***Ex vivo* models: Acute brain slices and Precision-cut Lung Slices**

Suppression assay

Good Laboratory Practice

Laboratory of Mammalian Model Organisms

Dr. Łukasz Przybył

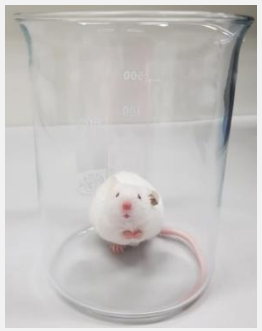
Head of Laboratory

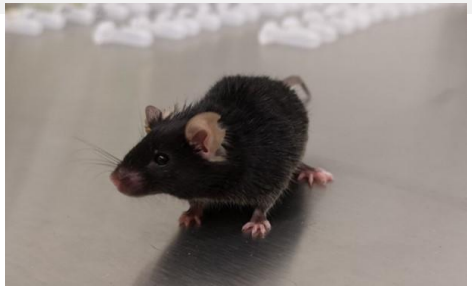
lprzybyl@ibch.poznan.pl
Phone: 61 829 18 58

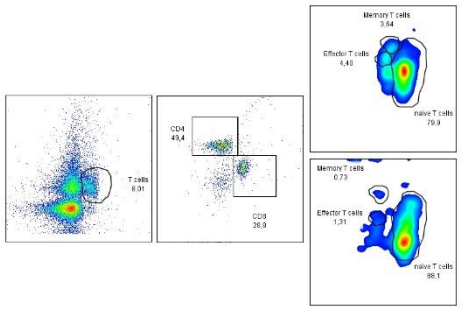
Technical Staff:

Dorota Wronka MSc, Eng.
biologist

Anna Karlik MSc
product developer







Offer

The Laboratory of Model Mammalian Organisms conducts comprehensive experiments on animals as part of **projects implemented at the Institute of Bioorganic Chemistry of the Polish Academy of Sciences.** We offer **assistance** in such aspects of scientific work as:

- planning expenses in grant applications
- designing experiments and appropriate experimental groups in accordance with the 3R and ARRIVE principles
- preparing an application to the Local Ethics Committee, the Ministry of the Environment or the Provincial Pharmaceutical Inspectorate
- organization of transport of experimental animals
- obtaining appropriate consents to work with laboratory animals
- training in basic techniques of working with animals
- maintaining a colony of transgenic or wild-type mice with a database
- obtaining embryonic material at appropriate stages of development
- intramuscular, intraperitoneal, subcutaneous and intracerebral injections
- microsurgery • mouse perfusion, organ weighing, and blood and tissue collection
- keeping experience diaries
- performing behavioral tests
- digitization of data, creation of charts and statistical analysis of data obtained during experiments
- in-depth analysis of the mouse and rat immunophenotype using flow cytometry
- intragastric administration
- implantation of osmotic pumps
- preparation of the entire aorta en face
- dissection of the brain into structures
- Intubations • Uninephrectomy
- Induction of acute kidney injury
- Xenograft inoculations
- Bronchoalveolar lavage
- Culture of cell lines (L929, U87, U251, SP2/ag14) and primary cells (Neurons, Astrocytes, VSMCs, Macrophages)
- Animal immunization and antibody production

Equipment

- Digital operating microscope (RWD Life Science)
- Stereo microscope (Nikon)
- Multifunctional treatment platform, endotracheal intubation kit for mice and rats, low-flow anesthesia system (Kent Scientific)
- Peristaltic pump (WPI)
- System for thermoregulation of mice and rats (RWD Life Science)
- Laminar chamber (Alpina)
- Analytical balances
- Surgical tools
- Precellys Evolution Touch homogenizer (Bertin)
- Ibidi pump system for long-term flow cell cultures under physiological conditions
- CO2 incubators for cell culture
- Capillary electrophoresis apparatus (Agilent)
- HPLC kit with coulometric detector
- UVP laboratory incubator
- QuantStudio3 – Real-time PCR
- Centrifuge 5424R centrifuge with rotor

THANK YOU FOR YOUR ATTENTION!


ICHB PAN

NMR Laboratory

Department of Biomolecular NMR

Presentation of IChB Laboratories, February 6th, 2024

Staff

mgr Anna Teubert

dr Karol Pasternak

Starting 2024!

dr Karolina Zielińska

dr Daniel Baranowski

Equipment

- Spectrometer NMR 400 MHz, AVANCE II, Bruker
- Spectrometer NMR 500 MHz, AVANCE III, Bruker
- Spectrometer NMR 700 MHz, AVANCE III, Bruker

- Circular Dichroism spectrometer (CD), J-815 S, JASCO

- Spectrophotometer UV VIS , V-650, JASCO
- HPLC

INNOWACYJNA GOSPODARKA
NARODOWA STRATEGIA SPÓJNOŚCI

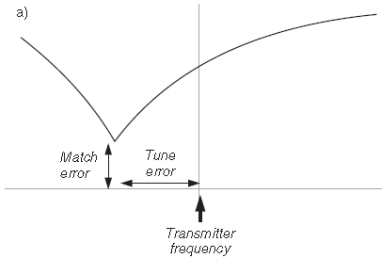
Instytut Chemii Bioorganicznej
Polskiej Akademii Nauk
Poznań

UNIA EUROPEJSKA
EUROPEJSKI FUNDUSZ
ROZWOJU REGIONALNEGO

„Zwiększenie potencjału badawczego Instytutu Chemii Bioorganicznej PAN w zakresie analizy strukturalnej biomolekuł metodami NMR i krystalografii”

Equipment – NMR spectrometers 400 MHz

- Spectrometer equipped with ATMA – *automatic matching i tuning* system



Instrument equipped in broadband probe for wide spectrum of experiments, routine analysis. Available for Institute employees after mandatory training.

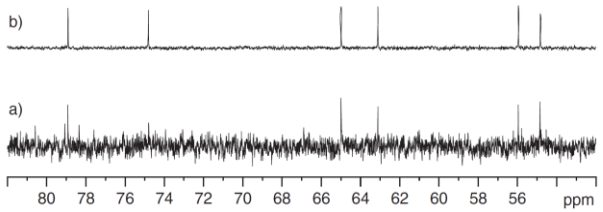
Equipement – NMR spectrometers 500 MHz

- Spectrometer equipped with ATMA – *automatic matching i tuning* system
- TOPSHIM – to achieve best homogenity of sample
- Equipped with autosampler allowing for automated analysis up to 120 samples - controlled by ICON NMR software
- Available for requesting more demanding/complex experiments for every Institute employee

Equipment – NMR spectrometers 700 MHz

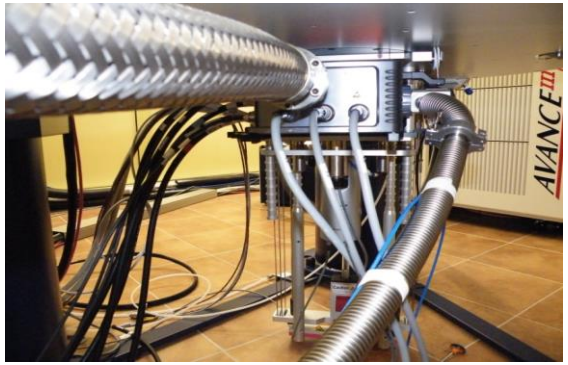
It is ideally suited to the study of **RNA/ DNA and proteins, small molecules and metabolomic samples research.**

400% increase in S/N ratio means that it is possible to run an experiment in 1/16 th of time needed for standard probe (or run an experiment for sample in 4 times lower concentration).



Observation coils are cooled with gas helium ~20K (-253 ºC).

It effects with increasing 400% S/N ratio on ^{1}H and ^{13}C compared to a room temperature standard probe.



INNOWACYJNA GOSPODARKA
NARODOWA STRATEGIA SPÓJNOŚCI
INSTYTUT CHEMII BIOORGANICZNEJ POLSKIEJ AKADEMII NAUK POZNAŃ
UNIA EUROPEJSKA EUROPEJSKI FUNDUSZ ROZWOJU REGIONALNEGO

Equipement

- Circular Dichroism (CD) spectropolarimeter from JASCO.
- Characterization of nucleic acid/protein secondary structure
- Detection of structural changes due to mutagenesis
- Conformational stability in response to changes in: temperature (Tm), pH or buffer composition

- Spectrophotometer UV VIS Jasco V-650

DNA/RNA melting measurements

- HPLC Agilent Tech 1260 Infinity
- Purification of nucleic acids on analytical and semi-preparative scale;
- Characterization

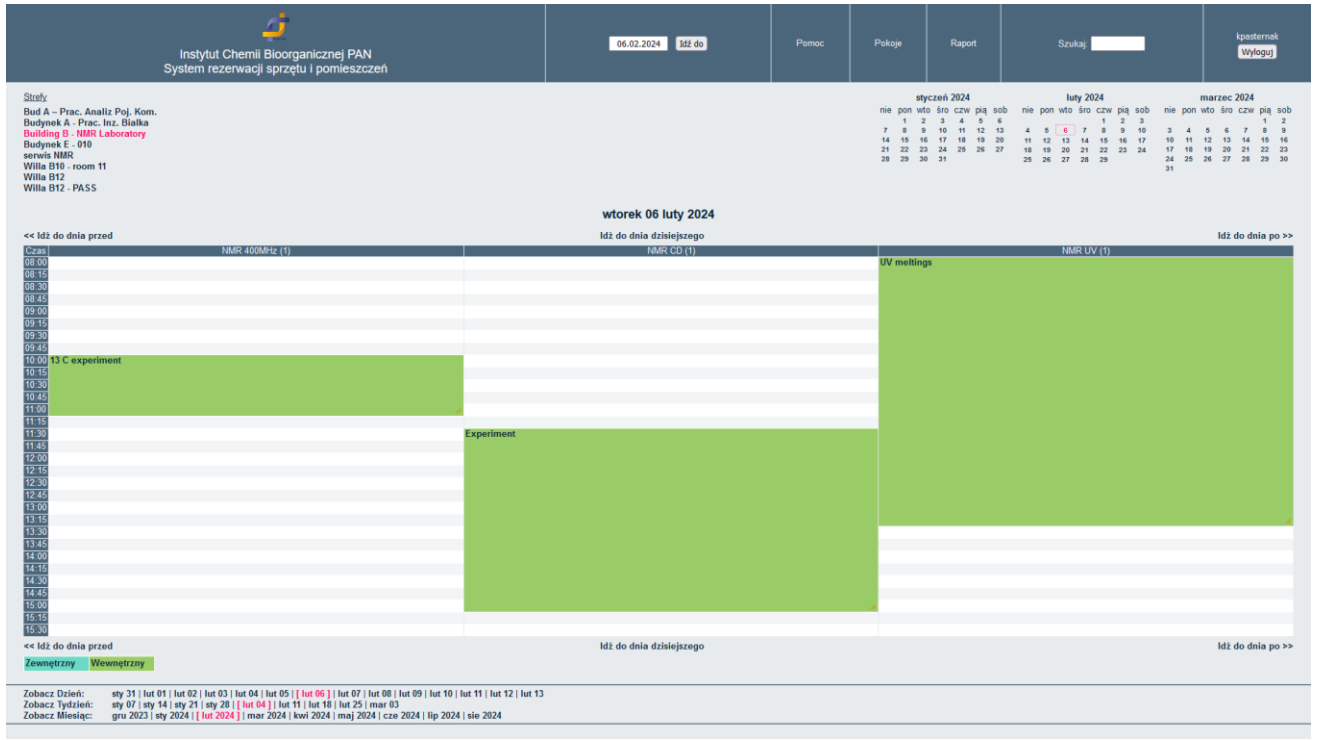
of purity

of nucleic acids

and monomers

e.g. nucleotides

Reservation system



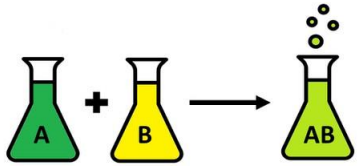
https://reservation.ibch.poznan.pl

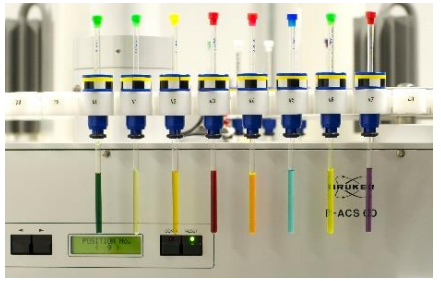
CD – oreder liquid nitrogen for your analysis

400 MHz NMR – mandatory training with Anna Teubert (ext. 1156)

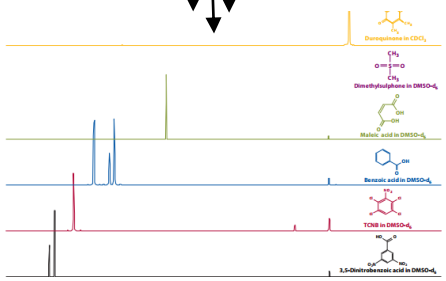
Offer - chemistry

LABORATORY NMR ⟷ DEPARTMENT OF BIOMOLECULAR NMR



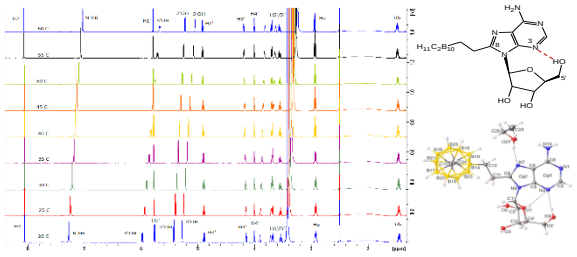


- Identification,
- Confirmation,
- Mechanism analysis
- Temperature influence,
- etc.

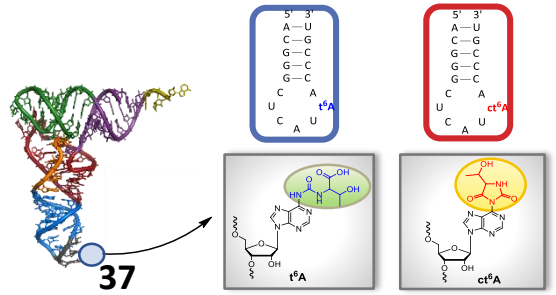


Offer

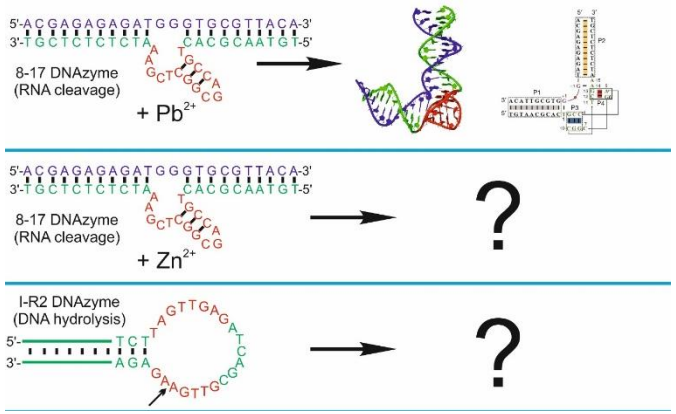
LABORATORY NMR ⟷ DEPARTMENT OF BIOMOLECULAR NMR



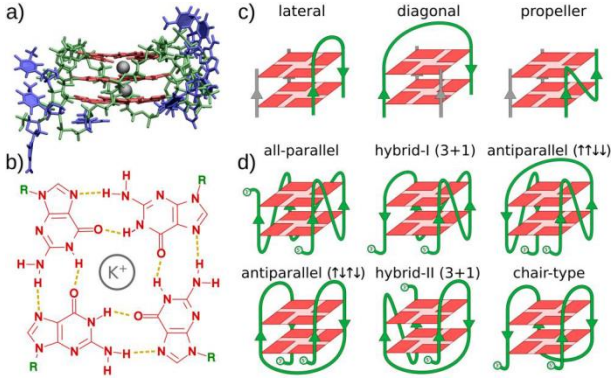
Structure and conformation Adenosie 2-analogues (temperature spectra)



Understanding how the cyclic ct^{6}A modifications influence the structure and function of tRNA.

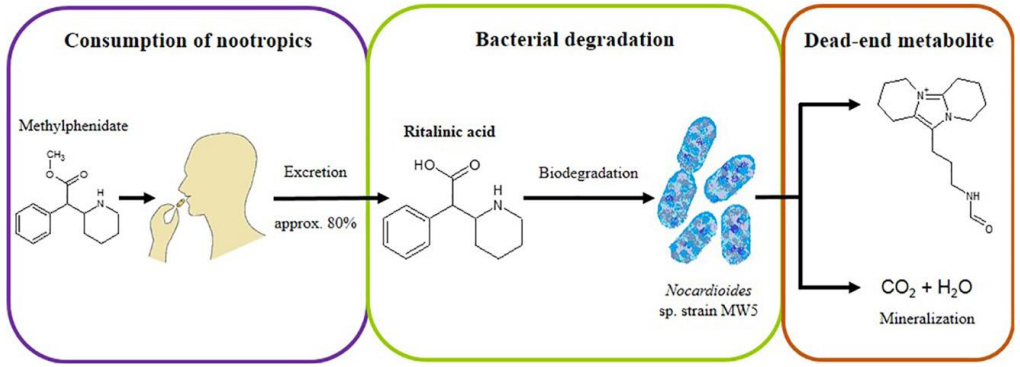


Structural studies and formation of DNA-zymes

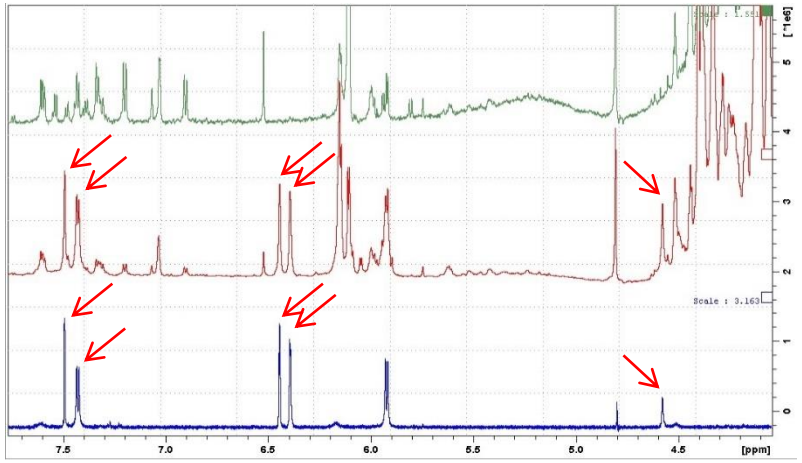


Sequence-structure relationship for the design of DNA G-quadruplexes with a given topology.

Offer



Identification and confirmation of the structure of biodegradation of ritanilic acid.



control

positive

metabolite

Metabolite confirmation form mice tissue (muscles, brain).

LABORATORY NMR ⟷ DEPARTMENT OF BIOMOLECULAR NMR

- There is a broad spectrum of experiments which could be performed using equipement in NMR Laboratory
- NMR is not only for chemists
- Your sample can be recovered
- The experiments could be run in wide range of temperatures and pH values
- Due to the close collaboration with NMR Biomolecular Department we have an expertise in setting up, running and interpretation of variaty of experiments

Feel free to visit us – building B

(entrance next to the first magnet at ICHB PAS)

Thank you for you attention


ICHB PAN

Laboratory of Mass Spectrometry

Łukasz Marczak

Anna Wojakowska

Aleksander Strugała

MS systems:

European Center for Bioinformatics and Genomics:

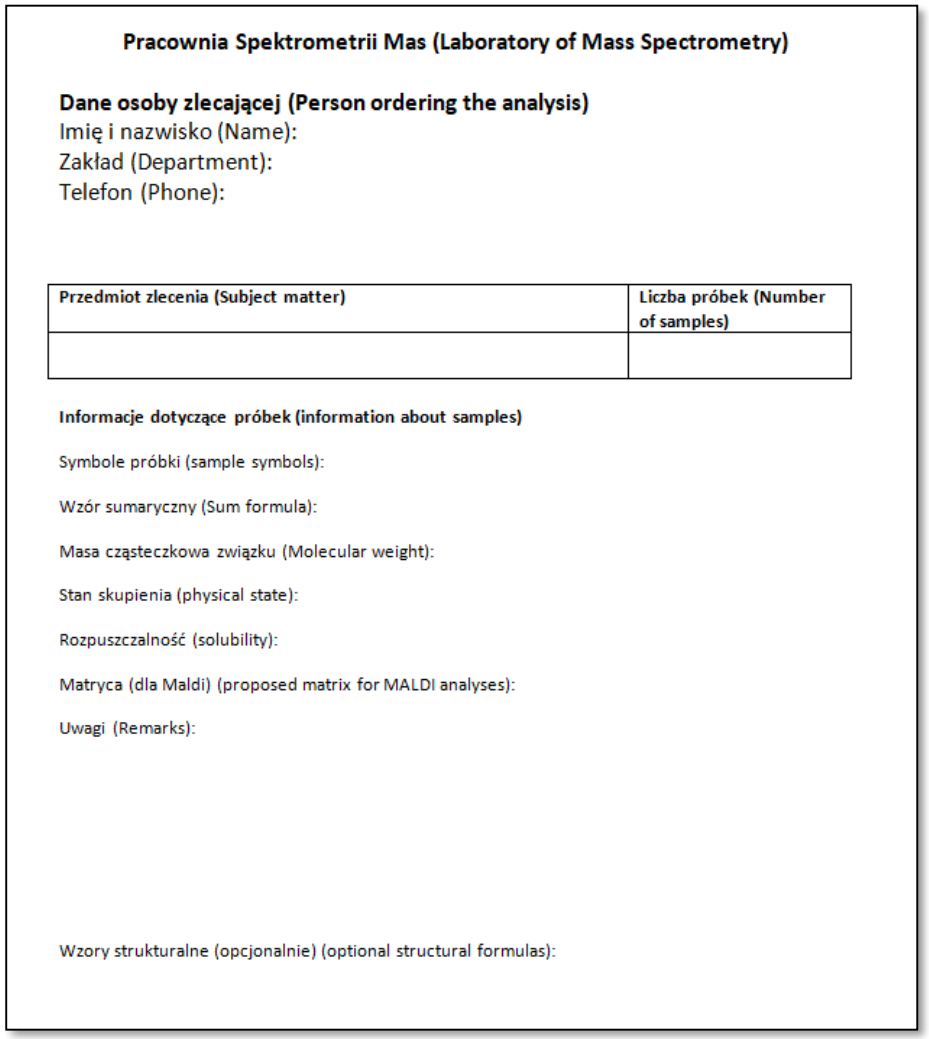
- MALDI-ToF/ToF system
- nano/micro LC-MS (high resolution OrbiTrap)
- GC-MS (TripleQuad)
- GC x GC – MS (ToF)
- nanoLC-MS (ion trap)

Institute of Bioorganic Chemistry (Noskowskiego)

- LC-MS with Ion Mobility separation (NEBI)
- Nano/micro/high flow LC-MS Orbitrap system (MOSAIC)

E-mail: pracownia.ms@ibch.poznan.pl

- Service price list availaible on request
- Prices for Instutute employees are lower than in case of external services
- Payments are settled through the customer's purchase of reagents and small equipment necessary for the day-to-day functioning of the laboratory



Pracownia Spektrometrii Mas (Laboratory of Mass Spectrometry)

Dane osoby zlecającej (Person ordering the analysis)
Imię i nazwisko (Name):
Zakład (Department):
Telefon (Phone):

Przedmiot zlecenia (Subject matter)	Liczba próbek (Number of samples)

Informacje dotyczące próbek (information about samples)

Symbole próbki (sample symbols):

Wzór sumaryczny (Sum formula):

Masa cząsteczkowa związku (Molecular weight):

Stan skupienia (physical state):

Rozpuszczalność (solubility):

Matryca (dla Maldi) (proposed matrix for MALDI analyses):

Uwagi (Remarks):

Wzory strukturalne (opcjonalnie) (optional structural formulas):

What we can measure?

Molecular Weight determination

Low resolution:
- **MALDI – oligonucleotides, peptides, polymers etc.**
- **Structure elucidation (MS/MS)**

High Resolution:
- **Direct infusion – peptides, oligonucleotides, low MW compounds, etc.**
- **LC-MS on Orbitrap**

Proteomic analyses

Identification of single proteins:
- **From polyacrylamide gels**
- **From solution**
- **De novo sequencing**

Analysis of protein modifications

Analysis of proteins in complex mixtures:
- **Identification, PTMs analysis**
- **Identification + quatification (label free, ITRAQs, TMT, etc.)**

Metabolomic analyses

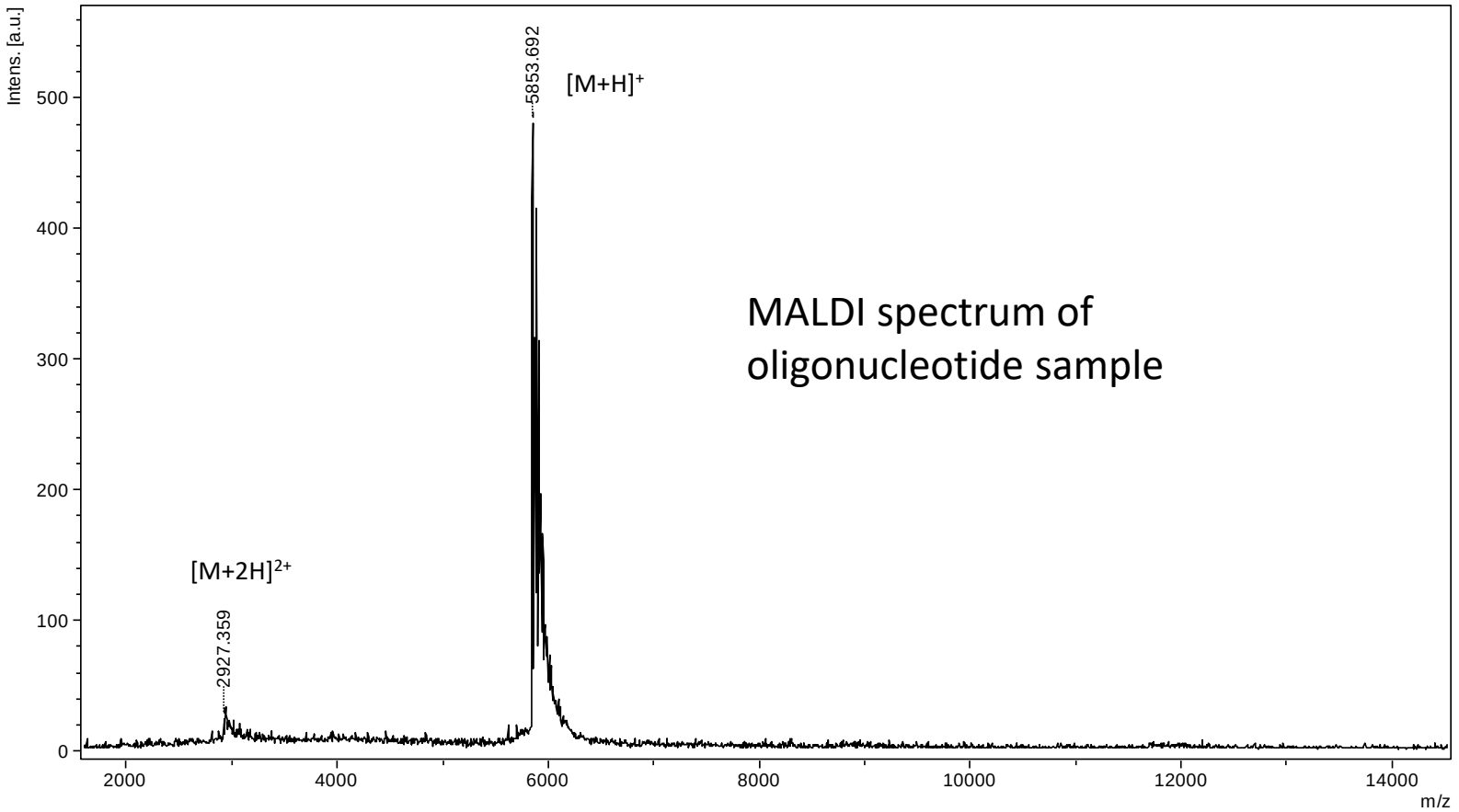
Analysis of compounds in complex mixtures - identification and quantification

- **GC-MS analysis for volatile compounds (+derivatization)**

- **LC-MS analysis for other metabolites**

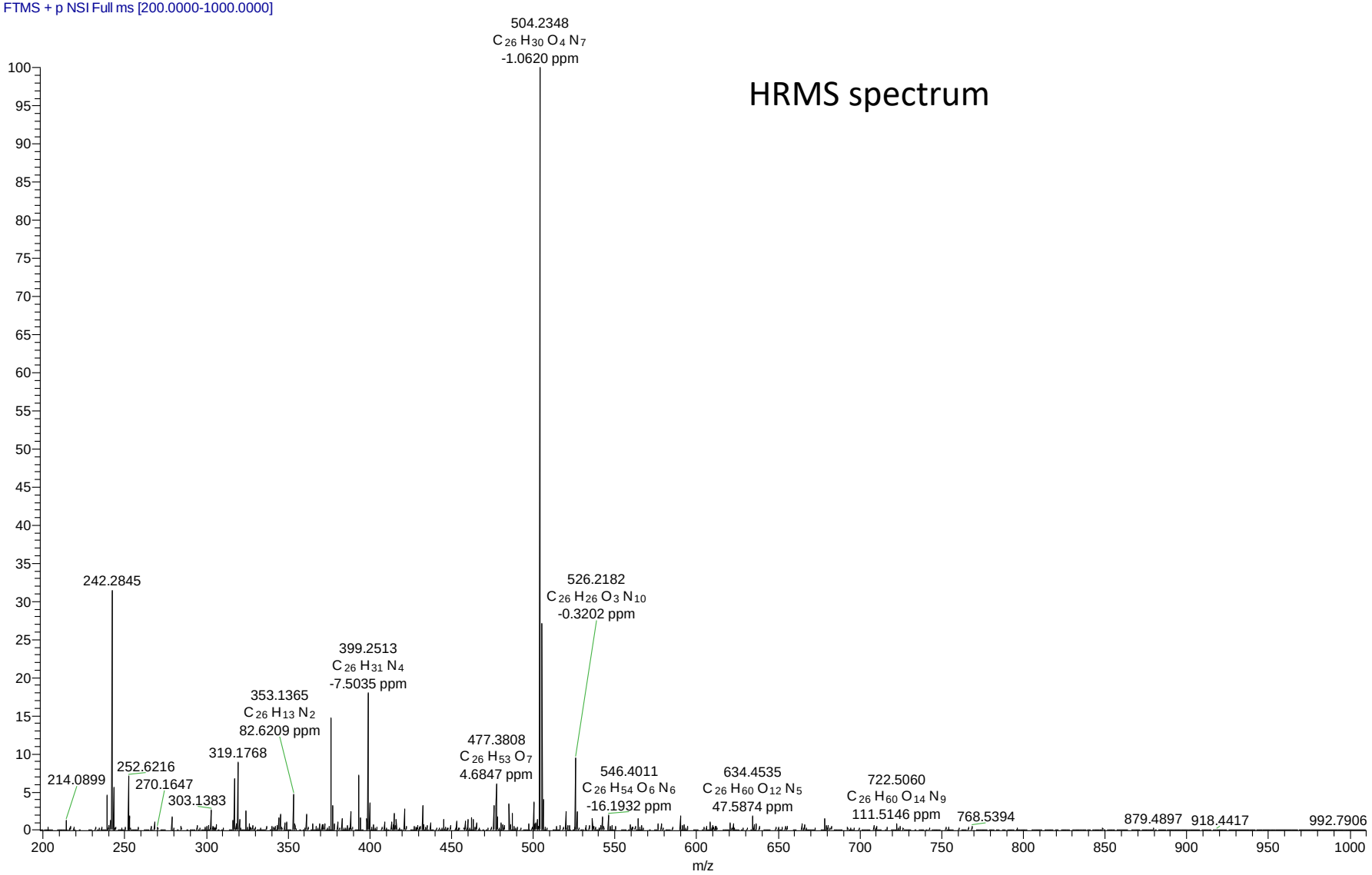
- **Direct Infusion High Resolution analysis with nano source (ie. Lipids)**

Molecular Weight determination

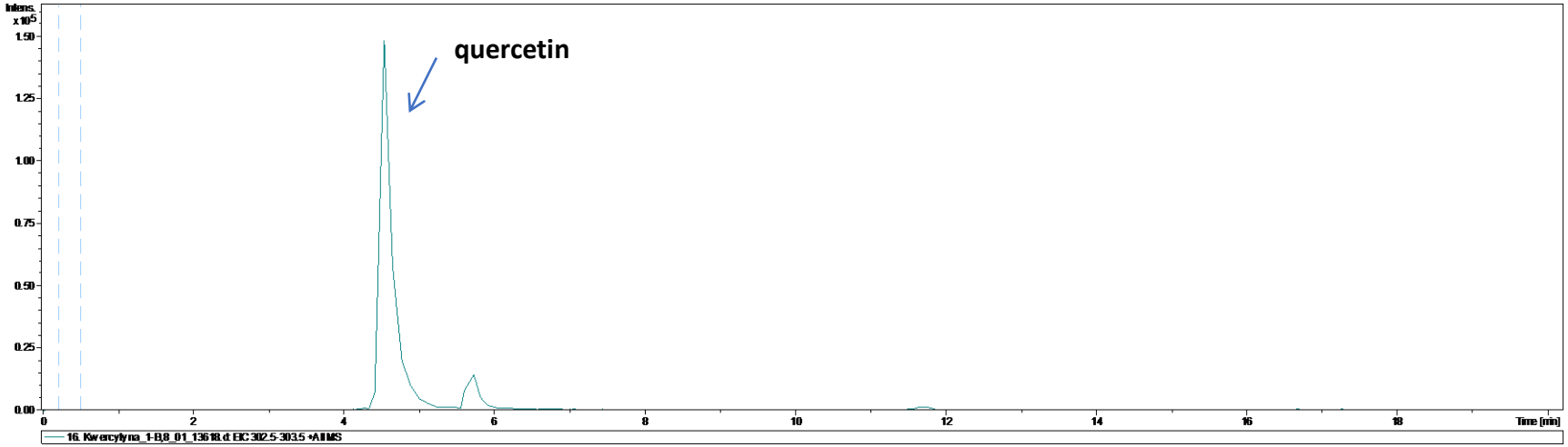


NCD_0.5 #51-52 RT: 0.44-0.45 AV: 2 NL: 1.69E7
T: FTMS + p NSI Full ms [200.0000-1000.0000]

HRMS spectrum

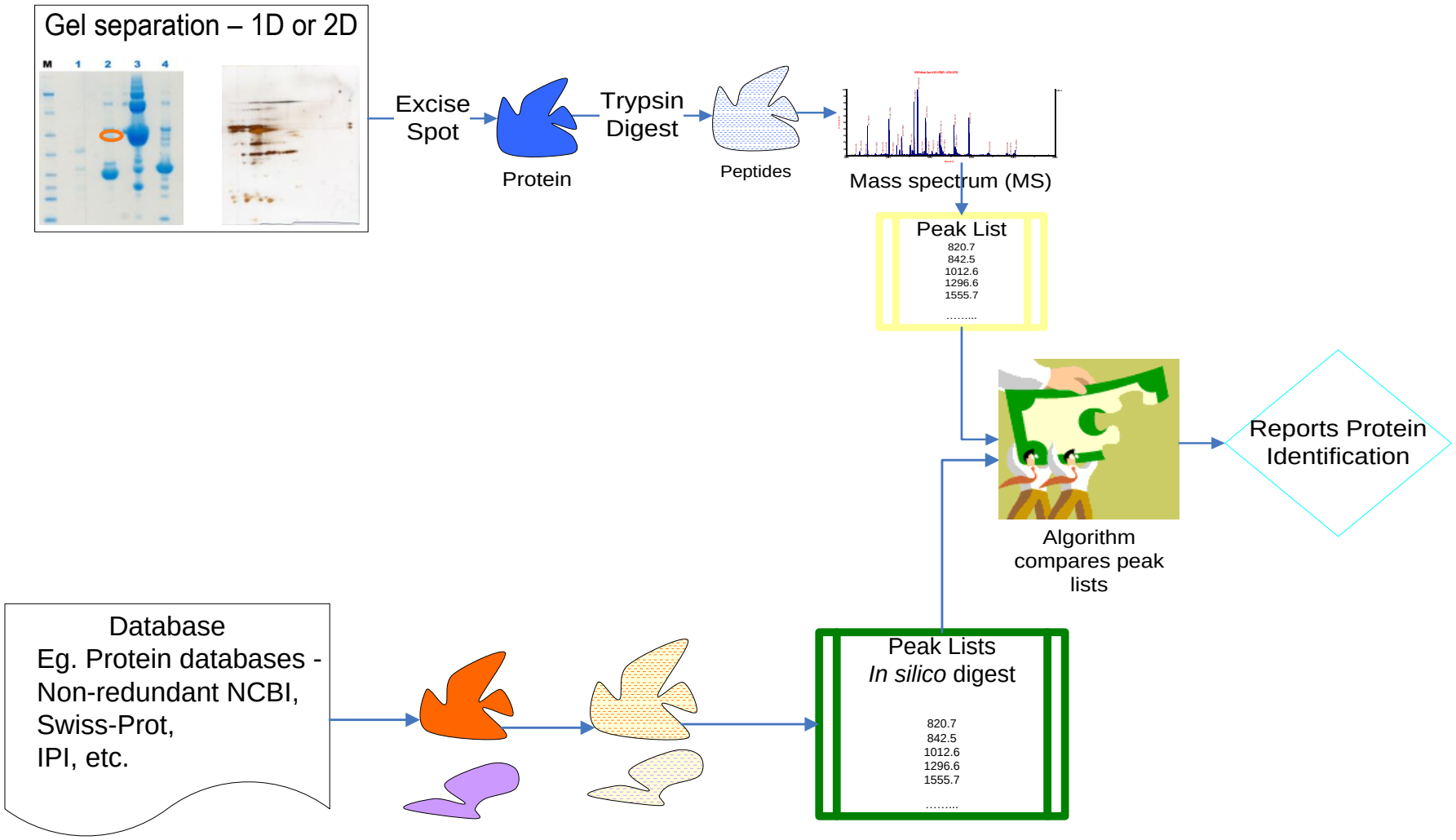


Finding specific compound using LC/MS

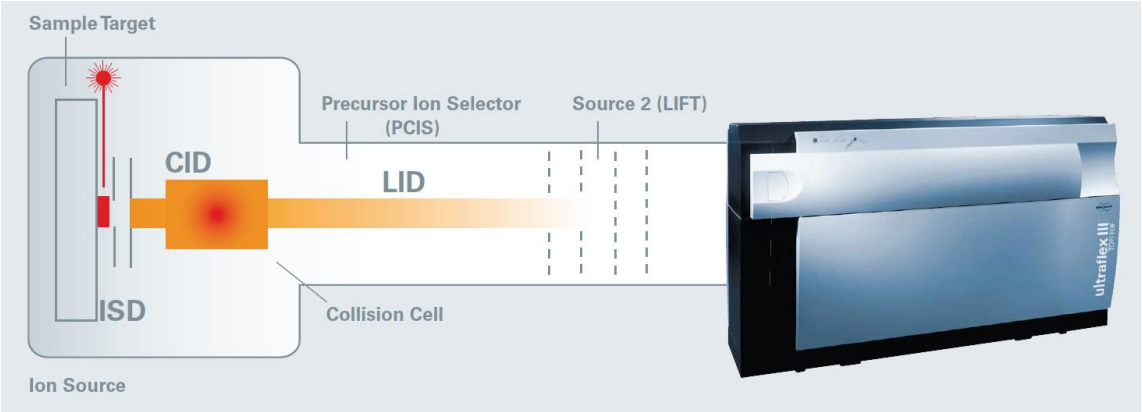


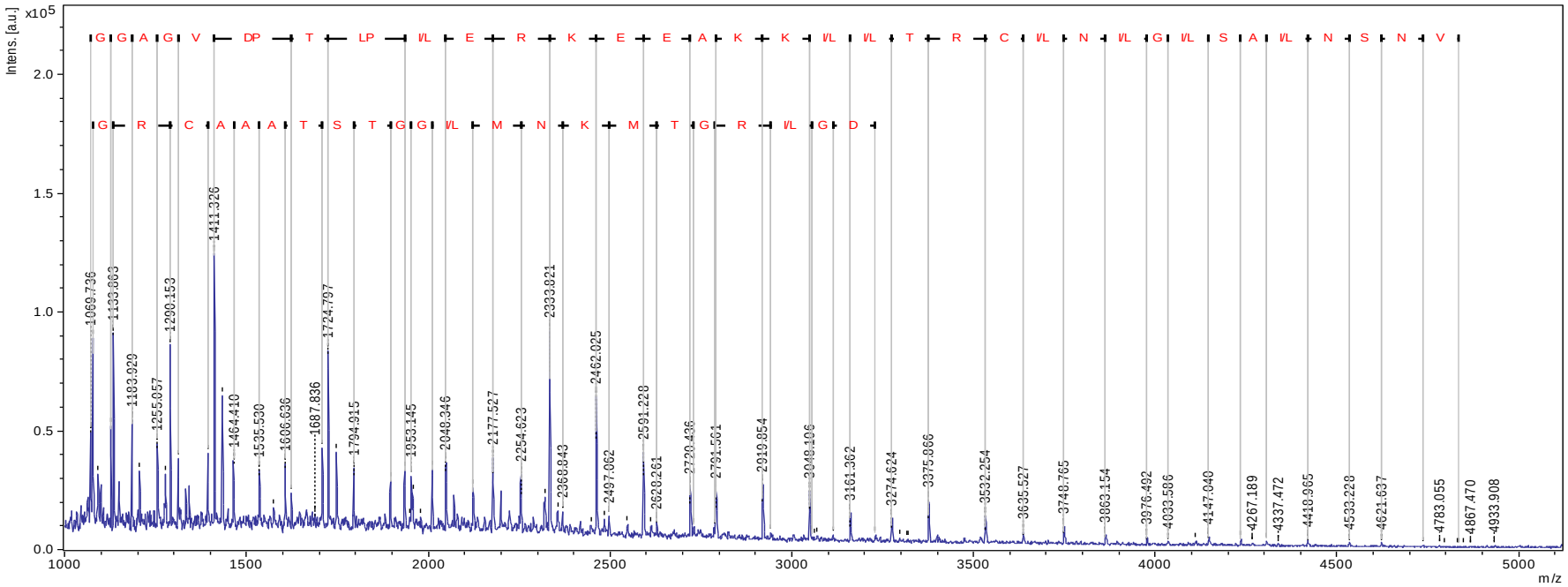
Extracted Ion Chromatogram for 303 m/z (qercetin $[M+H]^+$)

- Identification of proteins from gel bands (PMF and MS/MS)

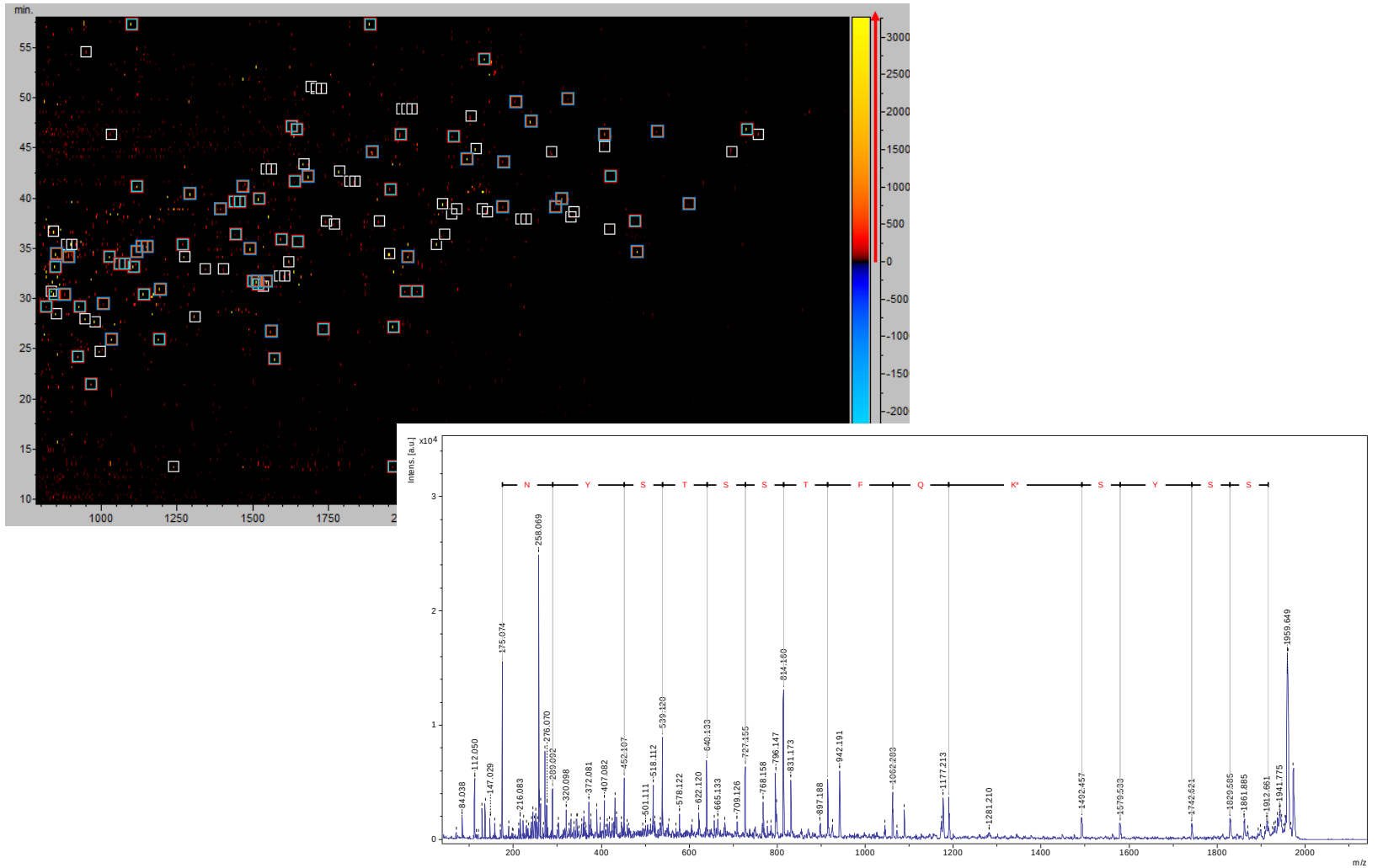


- protein sequencing using the **In Source Decay** (ISD) method





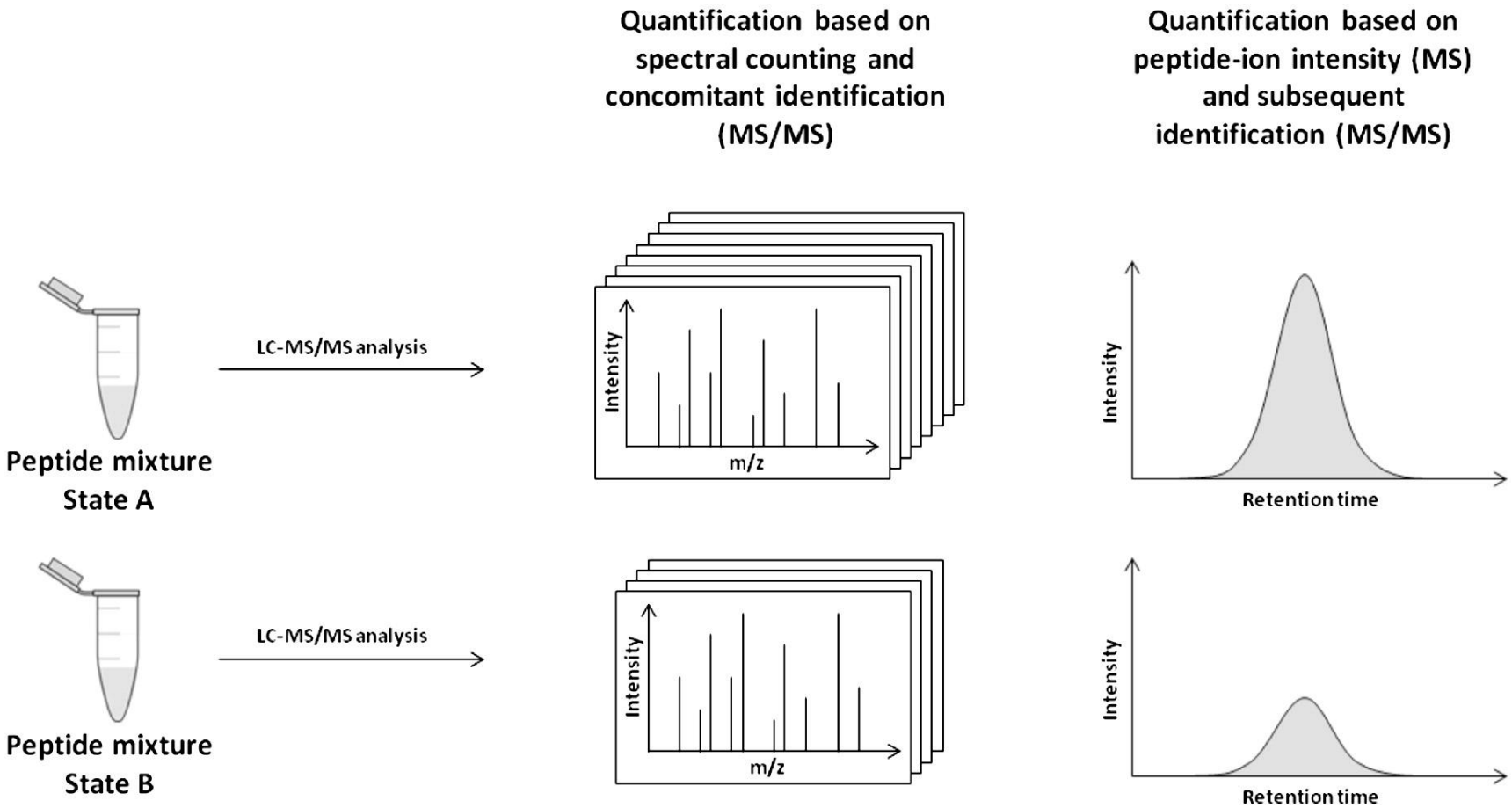
- analysis of complex peptide mixtures with protein identification



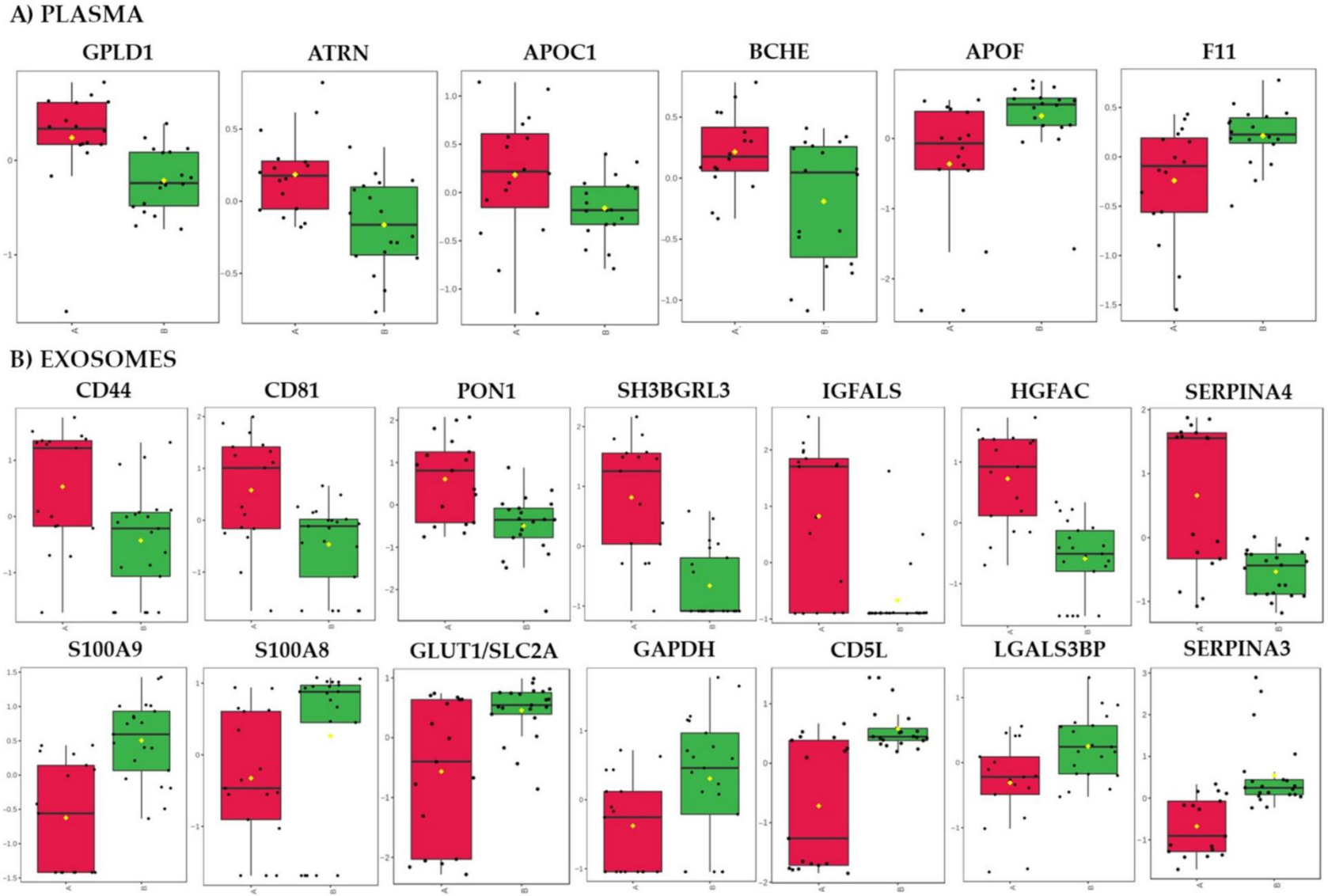
Other applications of the MALDI-TOF/TOF system for protein analysis:

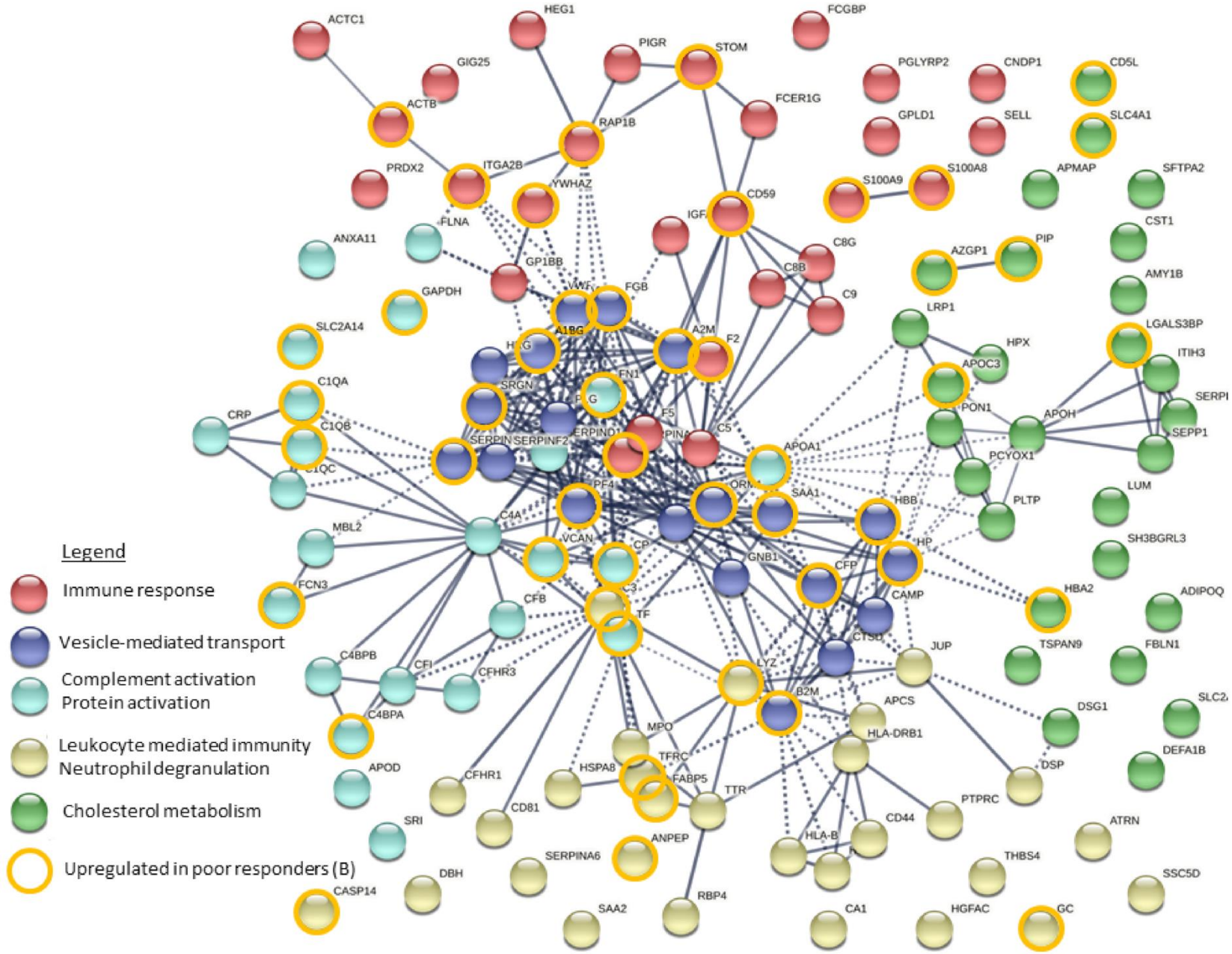
- De novo peptide sequencing (also in mixture – LIFT)
- Analysis of post-translational modifications of proteins (e.g. glycosylation)
- Quantitative analysis of proteins by peptide labeling methods (SILAC, ITRAQ, ICPL, ICAT, etc.)
- Profiling of proteins and peptides

Label free quatitation of proteins using LC-MS/MS



Megger A., et al 2013


A) PLASMA
GPLD1
ATRN
APOC1
BCHE
APOF
F11
B) EXOSOMES
CD44
CD81
PON1
SH3BGRL3
IGFALS
HGFAC
SERPINA4
S100A9
S100A8
GLUT1/SLC2A
GAPDH
CD5L
LGALS3BP
SERPINA3

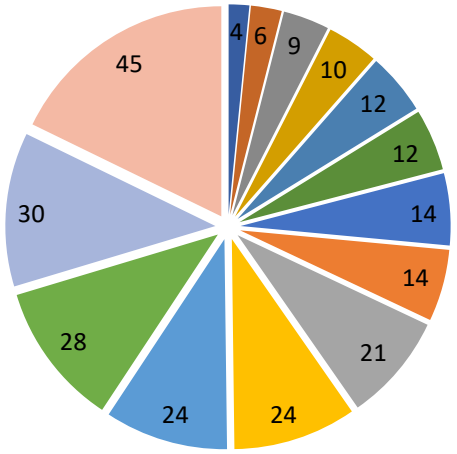

Legend
Immune response
Vesicle-mediated transport
Complement activation
Protein activation
Leukocyte mediated immunity
Neutrophil degranulation
Cholesterol metabolism
Upregulated in poor responders (B)
ACTC1
HEG1
FCGBP
GIG25
PIGR
STOM
PGLYRP2
CNDP1
CD5L
ACTB
RAP1B
FCER1G
GPLD1
SELL
SLC4A1
PRDX2
ITGA2B
YWHAZ
S100A9
S100A8
APMAP
SFTPA2
CD59
FLNA
ANXA11
C8G
AZGP1
PIP
CST1
GP1BB
C8B
AMY1B
GAPDH
C9
LRP1
SLC2A14
LGALS3BP
HPX
ITIH3
C1QA
APOC3
CRP
PON1
APOH
SERPI
C1QB
SEPP1
PCYOX1
C1QC
APOA1
LUM
PLTP
SAA1
HBB
MBL2
SH3BGRL3
HP
FCN3
HBA2
ADIPOQ
CAMP
CFB
TSPAN9
FBLN1
JUP
C4BPB
CFI
CFHR3
LYZ
B2M
APCS
SLC2
DSG1
C4BPA
HLA-DRB1
MPO
DEFA1B
APOD
HSPA8
DSP
CFHR1
FABP5
TTR
PTPRC
CD81
CD44
SRI
ATRN
ANPEP
HLA-B
SERPINA6
THBS4
DBH
SSC5D
CASP14
RBP4
SAA2
CA1
HGFAC
GC

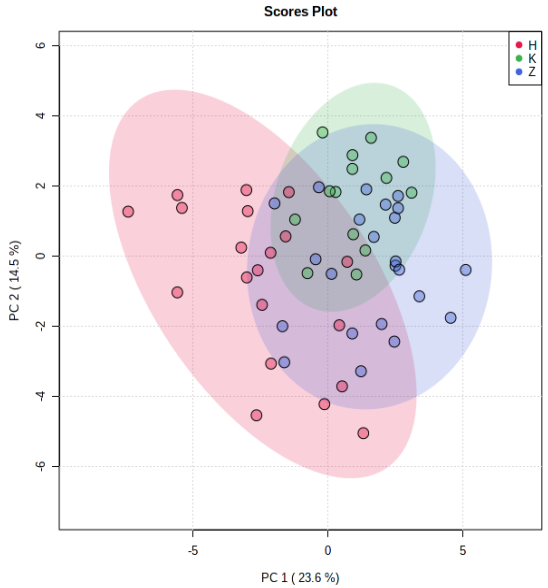
Advion NanoMate TriVersa

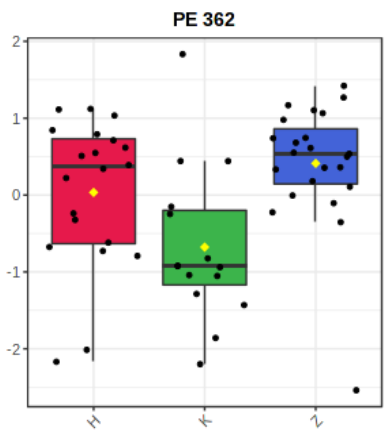
- direct analysis in nanoESI mode (even 1ul of sample)
- HPLC separation with nanoESI ionization
- HPLC separation with nanoESI ionization and collection of fractions for later injection

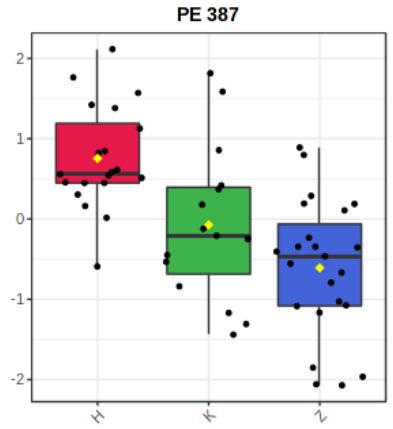
- **lipidomic experiments**

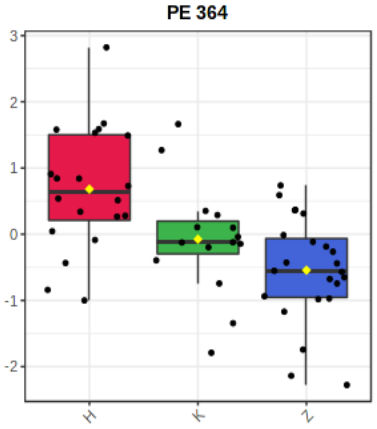
Human plasma lipidomics











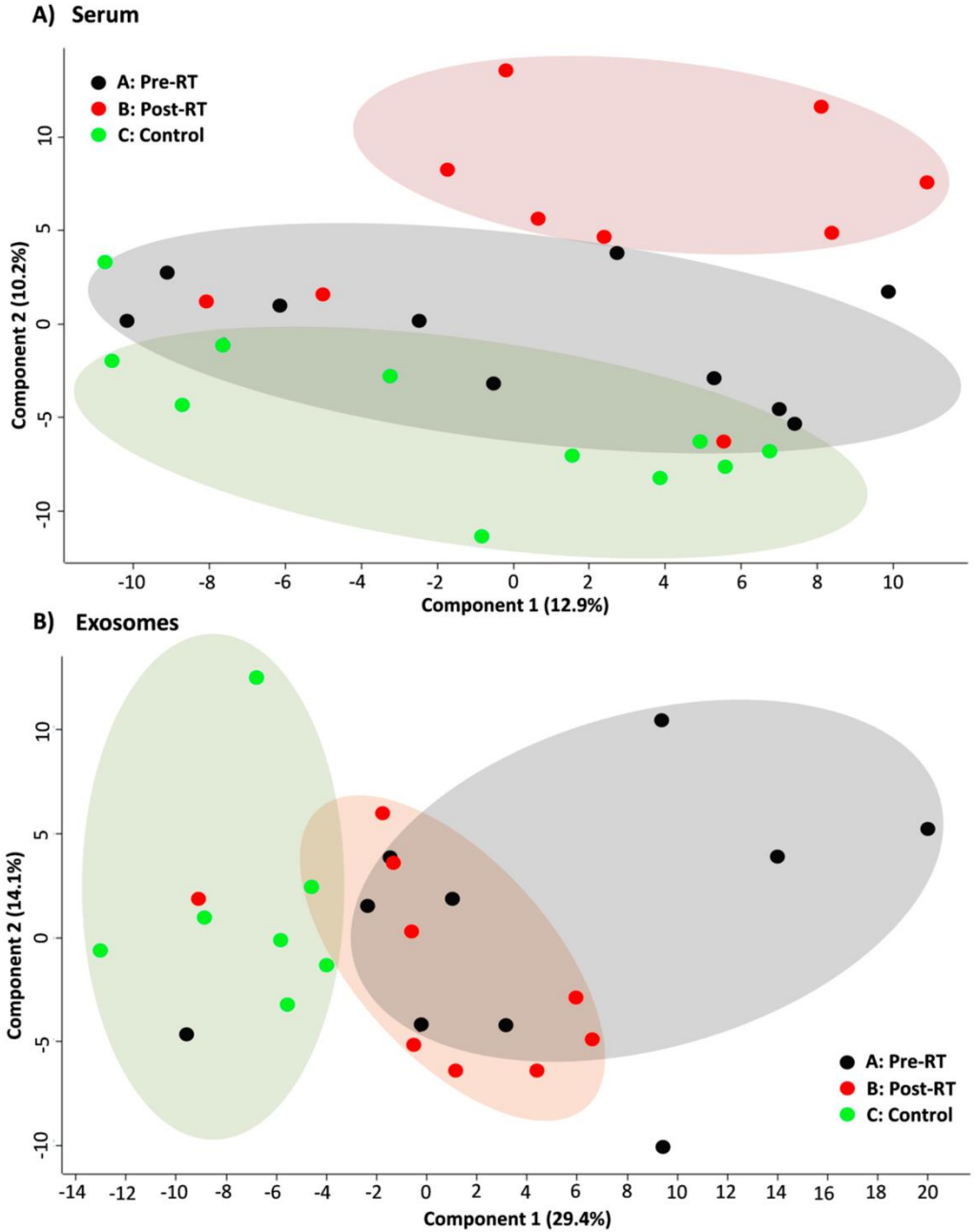
- GC x GC –MS (ToF)

- EI ion
- 2D separation of components of complex mixtures
- high speed (500 spectra / second)
- high sensitivity
- volatile metabolites analysis (qualitative and quantitative)

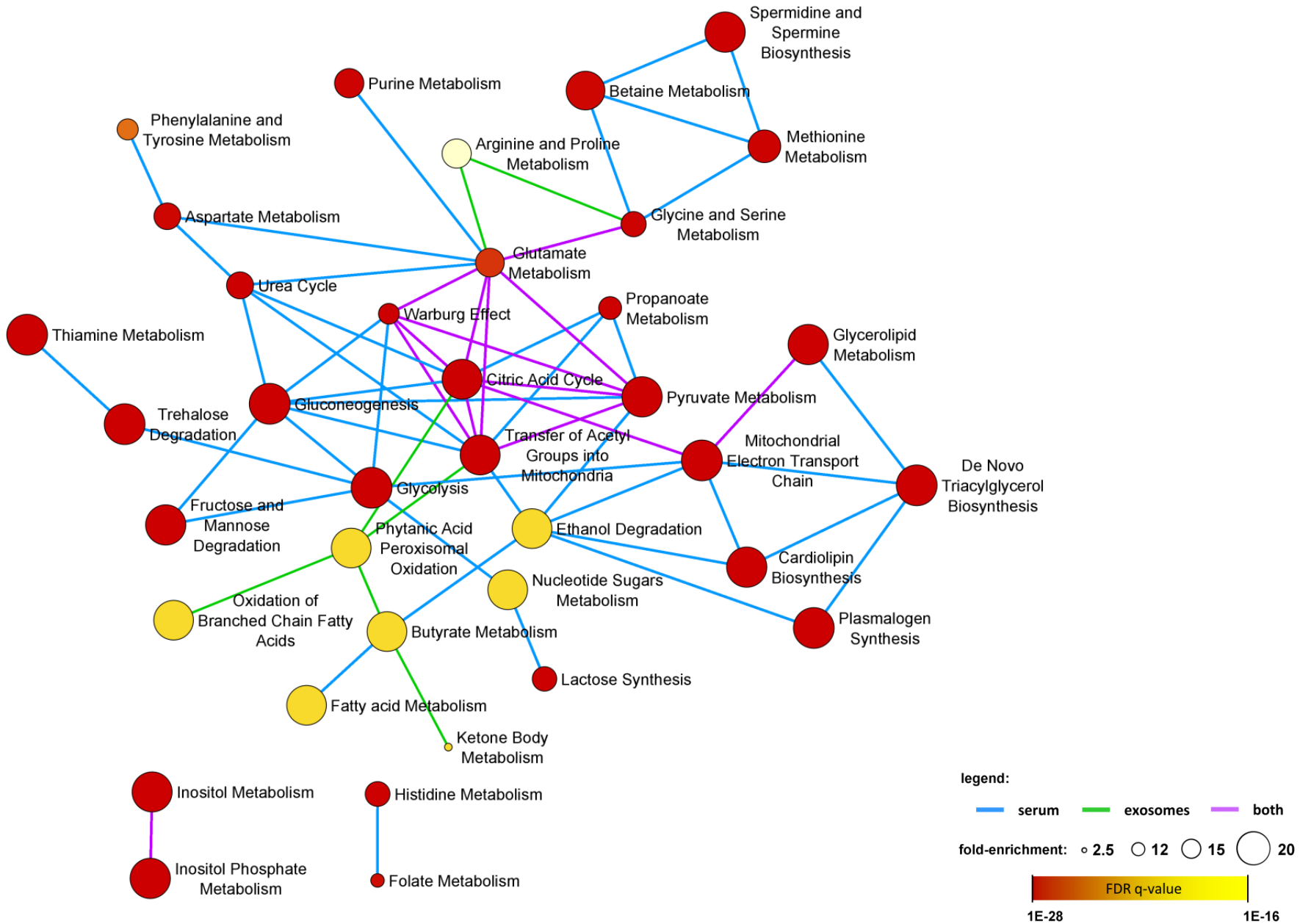
- GC-MS (TripleQuad)

- EI or CI ionization
- additional ion fragmentation in CID mode
- very high sensitivity in MRM mode (up to 500 MRM / second)
- targeted analysis of volatile metabolites (qualitative and quantitative)

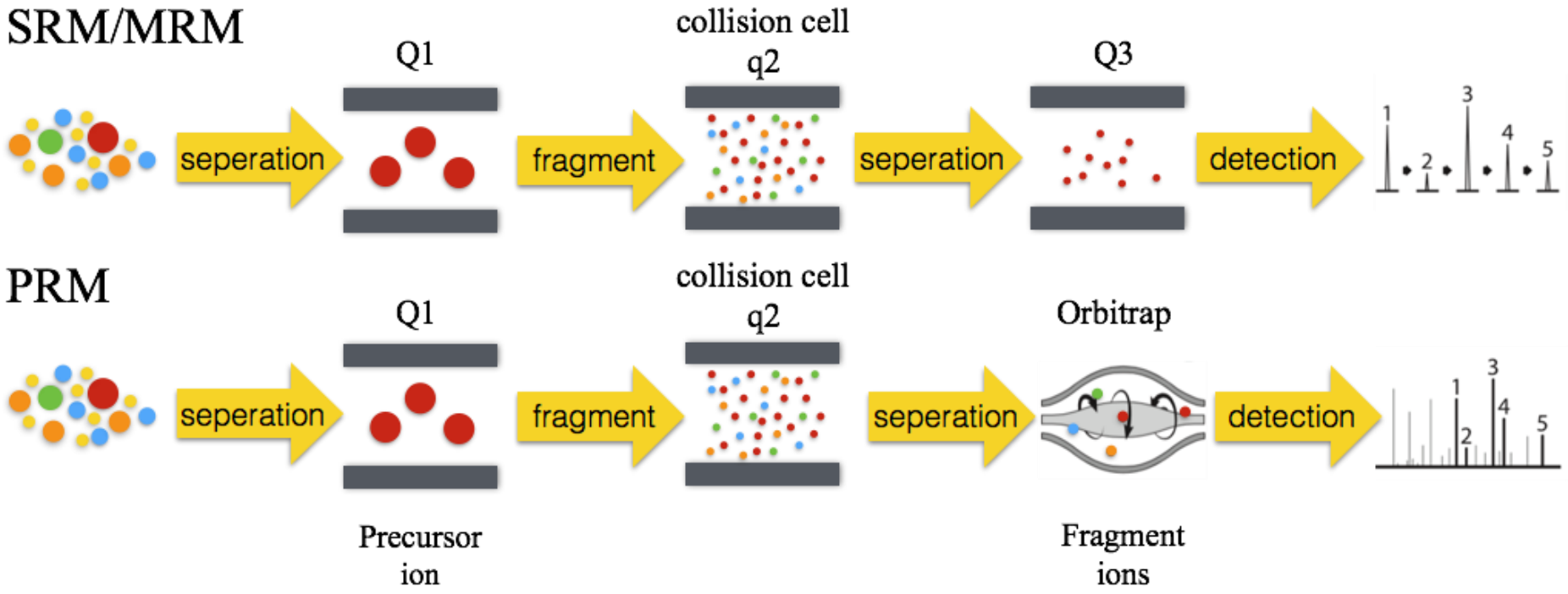
Metabolomic profiling



Metabolic pathways associated with metabolites differentiating head and neck cancer



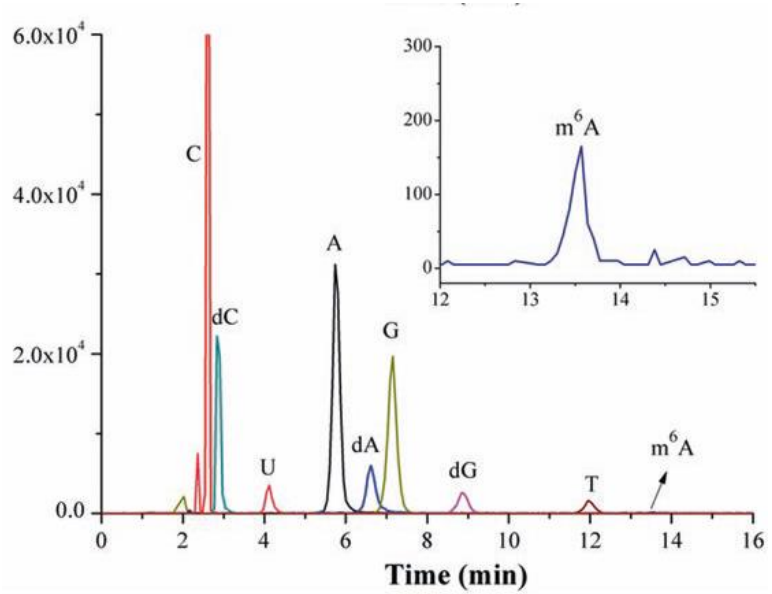
Targeted Analysis

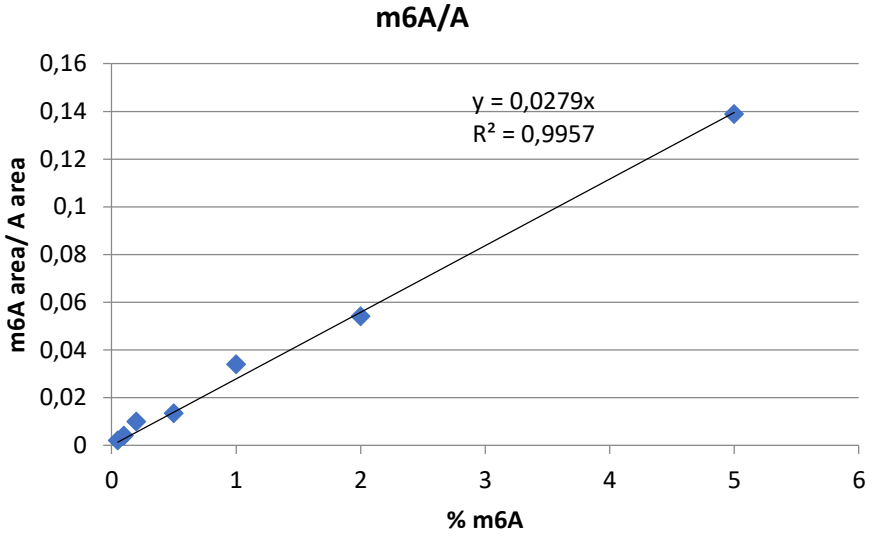


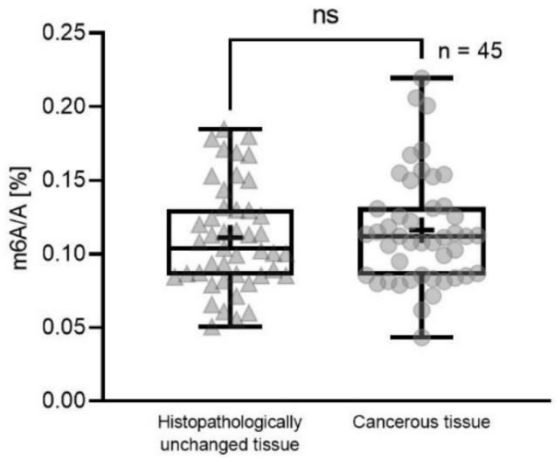
MRM – only using GC-MS

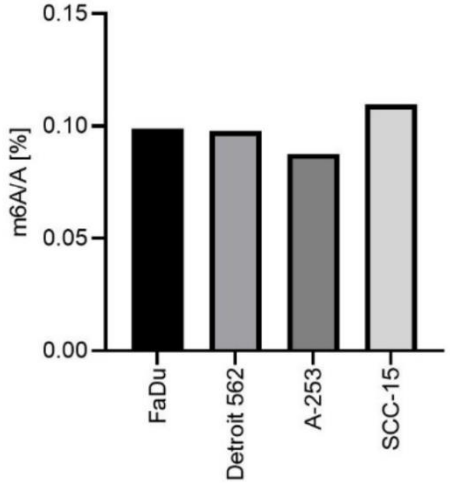
PRM – on LC using Orbitrap

Adenosine methylation analysis (PRM)

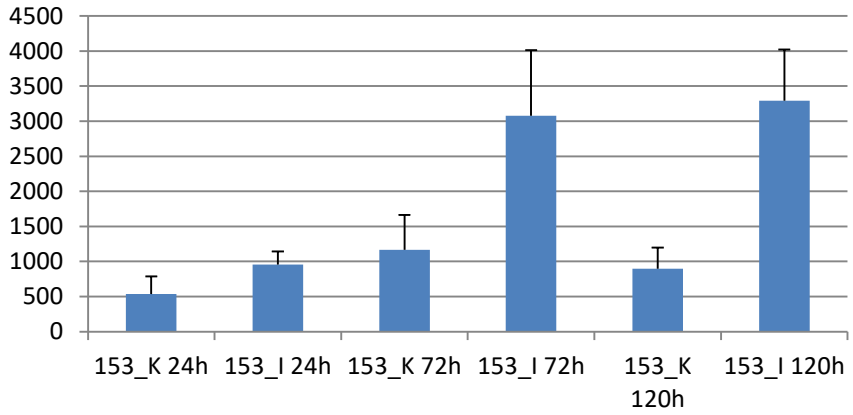




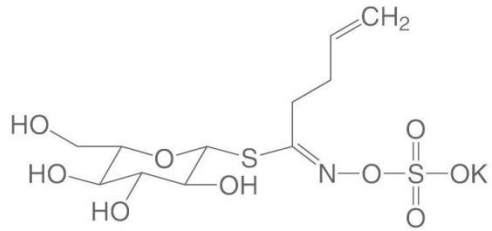


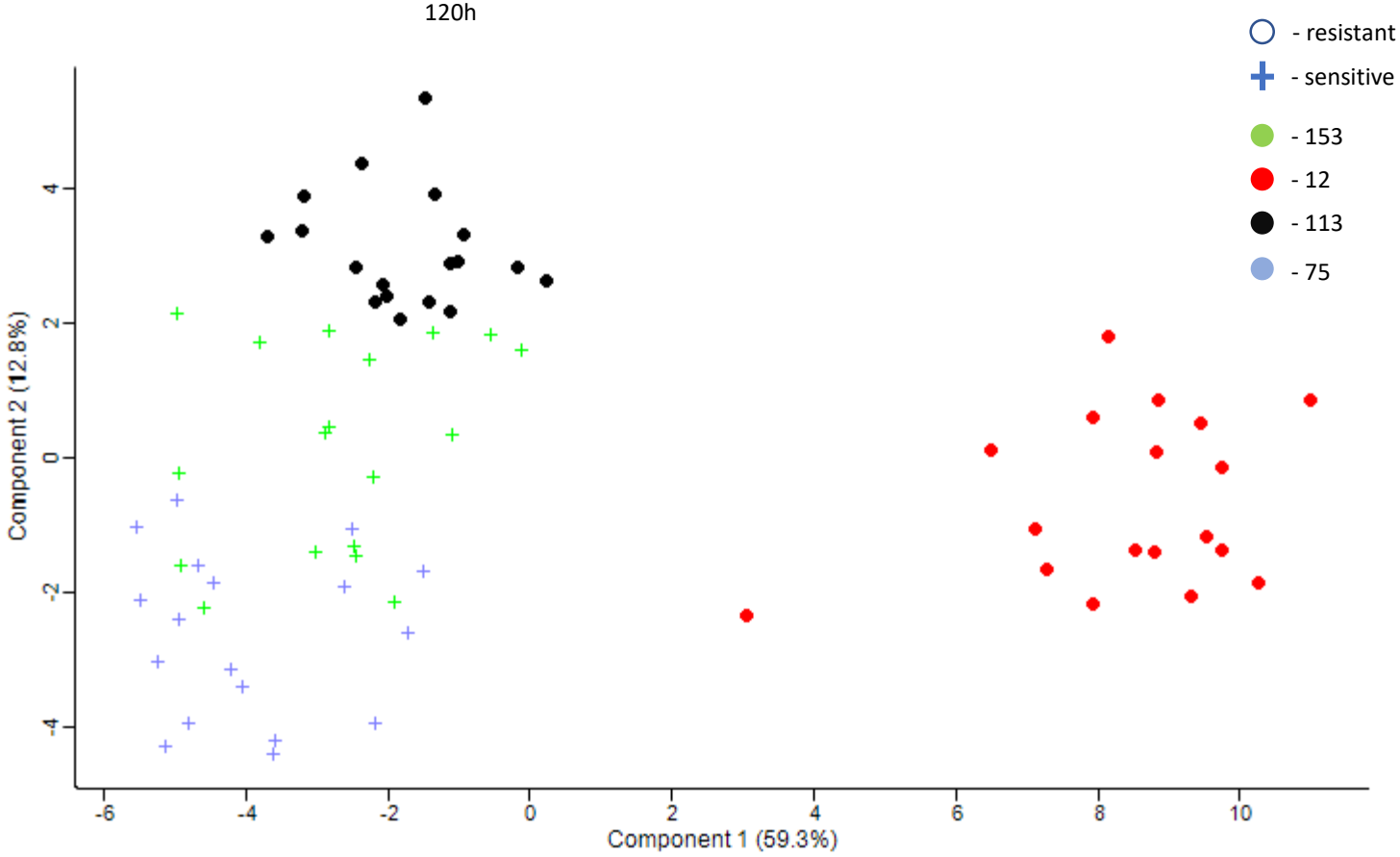


Gluconapin



Glucosinolates MRM





Laboratory offer (summary)

- Accurate determination of molecular (monoisotopic) weight of compounds (HRMS)
- Identification and confirmation of the compound structure on the basis of MS/MS spectra
- Protein identification (from mixtures, gel bands, de novo analysis, etc.)
- Comparative quantitative analysis of proteins in mixtures
- Analysis of post-translational modifications of proteins
- Quantitative analysis of metabolites and other low molecular weight compounds (e.g. amino acids, modified nucleotides, hormones, etc.)


ICHB PAN

INSTITUTE OF BIOORGANIC CHEMISTRY
Polish Academy of Sciences

Laboratory of Molecular Assays and Imaging

head of laboratory: Dorota Kwiatek, PhD
dkwiatek@ibch.poznan.pl

Centre for Chemical Biology ERIC
Institute of Bioorganic Chemistry Polish Academy of Sciences

CENTRE FOR CHEMICAL BIOLOGY ERIC

Head: dr hab. Jacek Ł. Kolanowski
Research Support Unit, IBCH PAS: dr Michał Gładysz

Laboratory of Molecular Assays and Imaging
head: dr Dorota Kwiatek

1. High Throughput Screening (HTS)

dr hab. Joanna Kosman
dr Krzysztof Żukowski
mgr Monika Pyc
mgr Natalia Karczewska

2. Superresolution Imaging (MINFLUX)

dr Volodymyr Cherkas
(Long-term – to support Ukrainian research teams" program
lic. Ihor Panas, mgr Mykyta Bobylyow, mgr Borys Olifirov, dr Yevhenii Sheremet
mgr Adrian Rufli

from Departament of Molecular Probes and Prodrugs

Design, synthesis and validation of molecular probes

dr Michał Jakubczyk
dr Maria Dekaliuk (PACIFIC)
mgr Francesca Canyelles y Font
mgr Anna Wychowaniec

Laboratory of Medicinal Chemistry
head: dr Dorota Jakubczyk

3. Design and synthesis
- Natural product analogs
- Targeted chemical libraries
- Chemoproteomic probes (target IID)

HIGH THROUGHPUT SCREENING

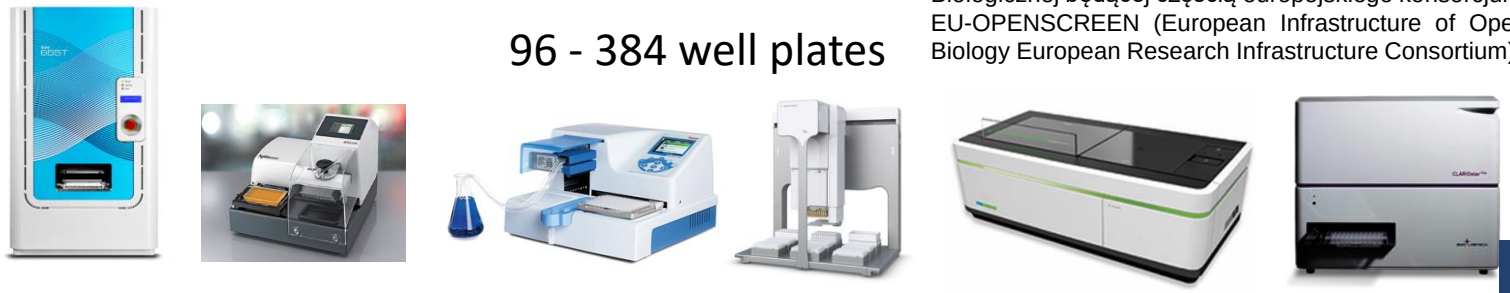
CENTRE FOR CHEMICAL BIOLOGY ERIC

NOT-FOR-PROFIT EUROPEAN RESEARCH INFRASTRUCTURE CONSORTIUM (ERIC)

- FOCUSED ON CHEMICAL BIOLOGY AND DRUG DISCOVERY
- STORES AND DISTRIBUTES COLLECTIONS OF COMPOUNDS
- PROVIDES OPEN ACCESS TO SCREENING AND CHEMISTRY TECHNOLOGIES AND EXPERTISE
- UNITES **33 PARTNER SITES IN 10 EUROPEAN COUNTRIES**

POL-OPENSCREEN - Projekt Polskiej Platformy Infrastruktury Skriningowej dla Chemii Biologicznej będącej częścią europejskiego konsorcjum
EU-OPENSCREEN (European Infrastructure of Open Screening Platforms for Chemical Biology European Research Infrastructure Consortium). Dotacja MNiSW

96 - 384 well plates



Compound from chemical library → **Investigated model / molecular target** → **Adding further reagents** → **Measurements**

Assay types

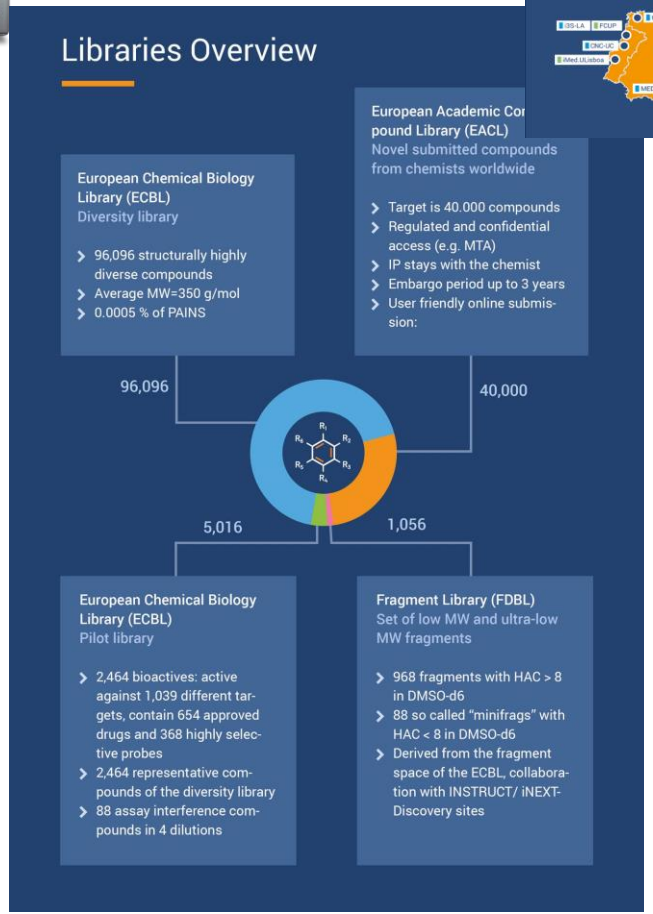
- Biochemical assays:
  - Absorbance
  - Fluorescence and colorimetric (ROS generation assesment – was used for bioprofiling of EU-OS libraries)
  - Luminescence
  - Biouminescence (luciferase based)
- Cell based assays:
  - Target based (ex. Reporter assay)
  - Phenotypic (cell painting)
  - High Content Screening
  - Multiparametic assays (cytotoxicity studies)

Assay development

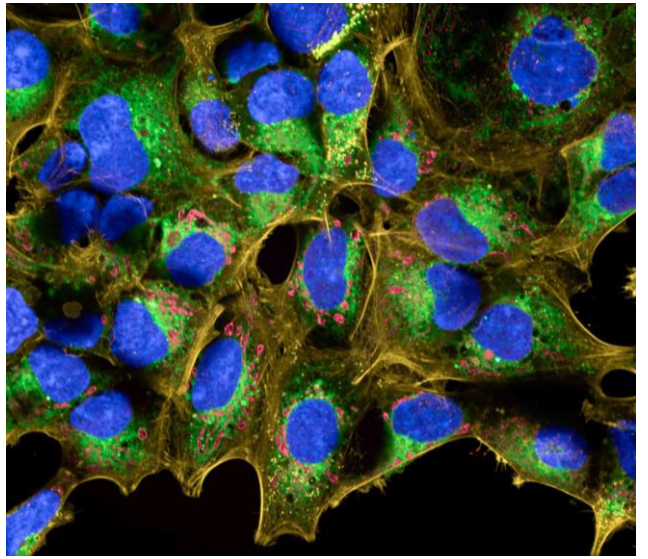
- Single and multi-parametric assays
- Miniaturisation and optimisation
- Automation and assay transfer
- Orthogonal assay

High Throughput Screening

- Primary screening (1k – 100k)
- Hit validation:
  - 3 concentrations
  - IC/EC50 determination
- Counter / orthogonal screening



CELL PAINTING



N.Karczewska, J. Kosman, M. Pyc

High content, image-based, multiparametric assay, used for cytological profiling.

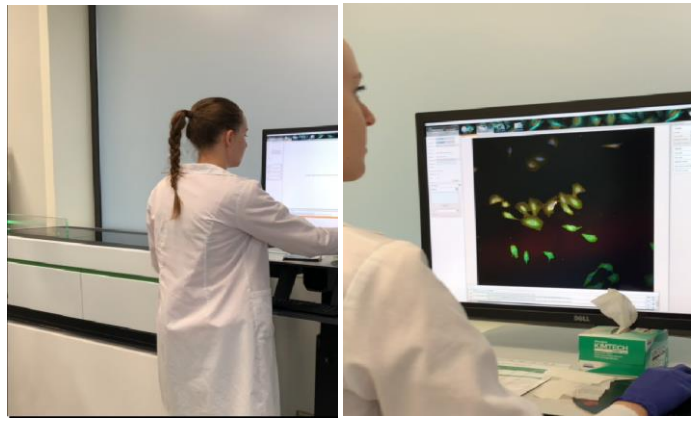
Phenotypic screening method based on staining different subcellular structures with fluorescent dyes.

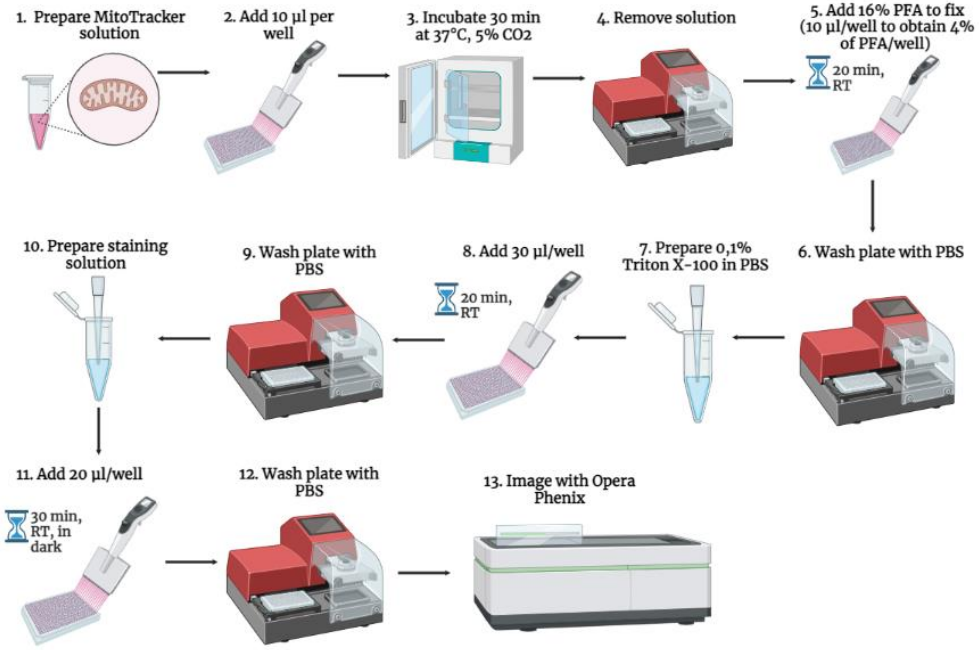
Application of the method:

- Preditcting the mechanism of action (MOA) of compounds
- Grouping compounds based on MOA
- Identifying cell line-specific effects
- Identifying compounds with new MOA
- Evaluation of cytotoxicity of compounds

Examples of studies:

- Dynamic organization of proteins
- Cell viability
- Cell proliferation and toxicity
- DNA damage

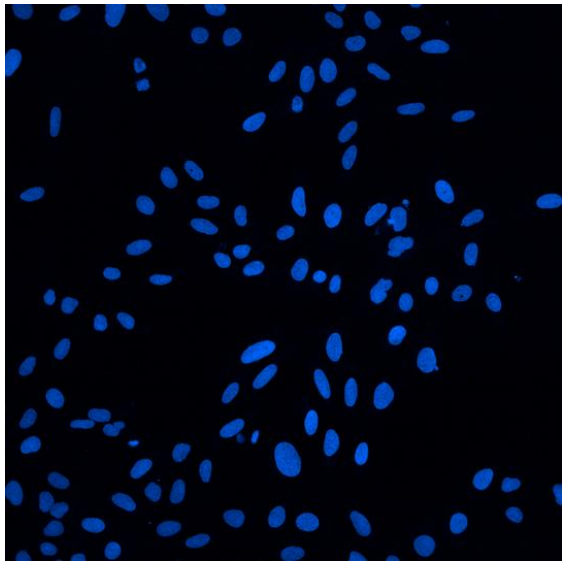




Broad Institute (Bray et al. 2016, Nature Protocols)
https://www.thermofisher.com/pl/

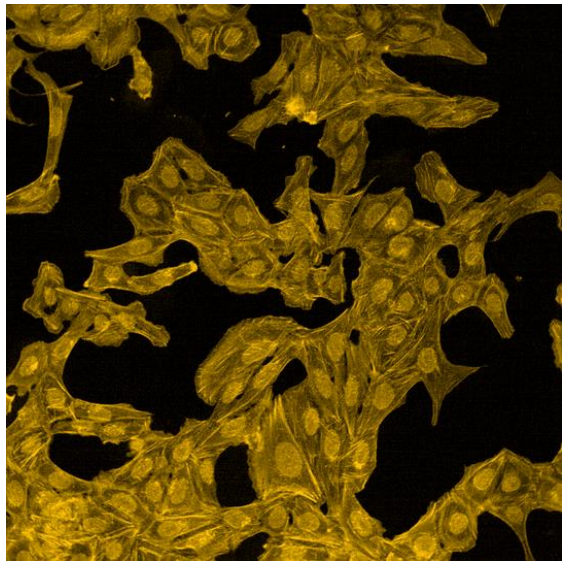
EXAMPLE OF CELL PAINTING IMAGES OBTAINED IN OUR CENTRE

HOECHST33342



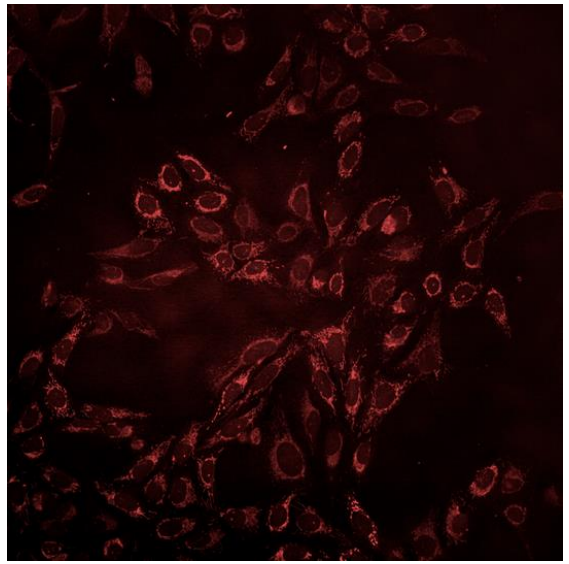
NUCLEI

PHALLOIDIN WGA



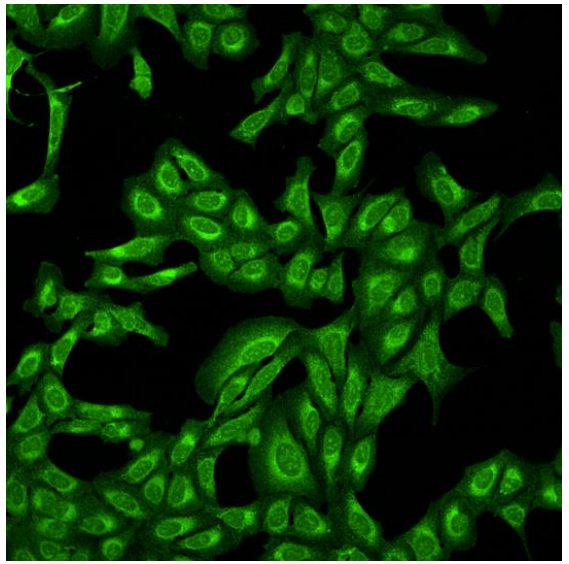
- F-ACTIN (CYTOSKELETON)
- PLASMA MEMBRANE, Ggolgi APPARATUS

MITOTRACKER DEEP RED



MITOCHONDRIA

CONCANAVALIN A SYTO14

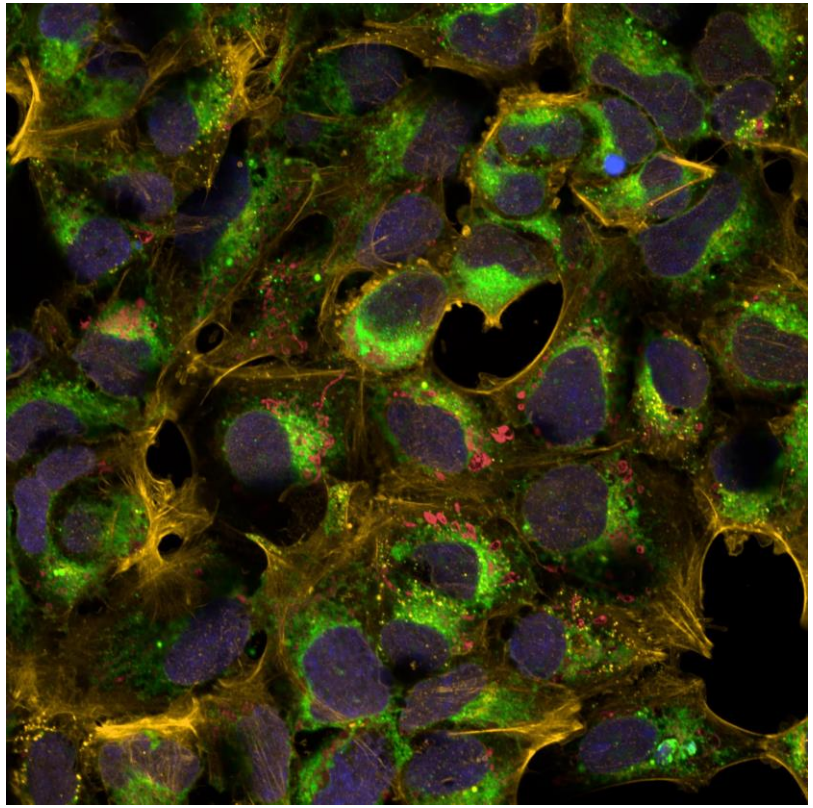


- ENDOPLASMIC RETICULUM
- NUCLEOLI, CYTOPLASMIC RNA

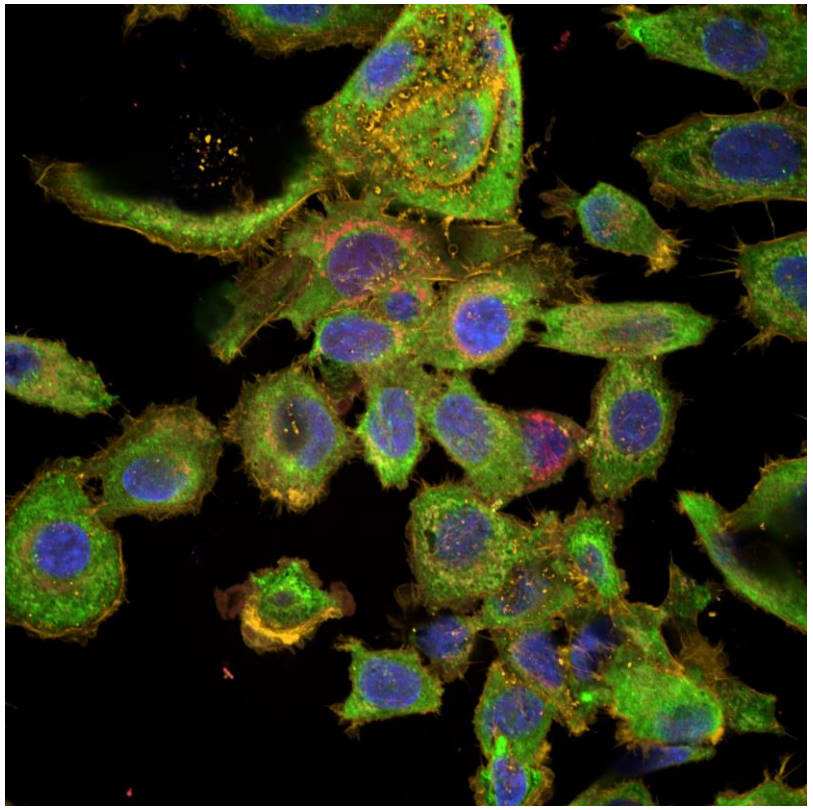
N.Karczewska, J. Kosman, M. Pyc

EXAMPLE OF CELL PAINTING IMAGES OBTAINED IN OUR CENTRE

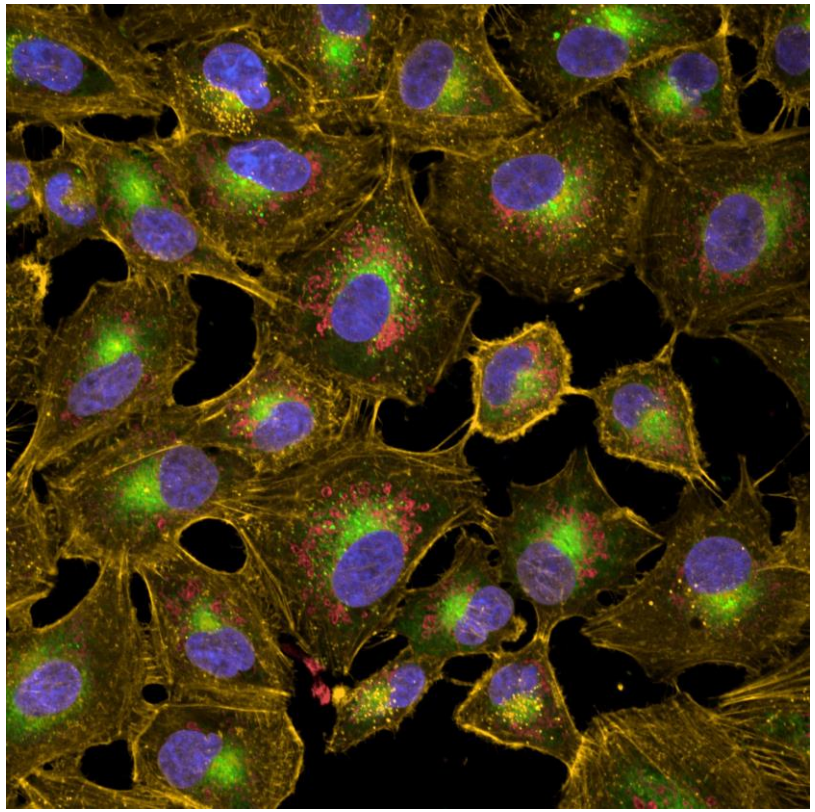
HEK293 cell line



PC3 cell line



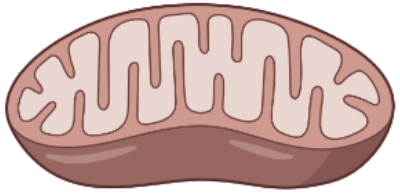
A549 cell line



N.Karczewska, J. Kosman, M. Pyc

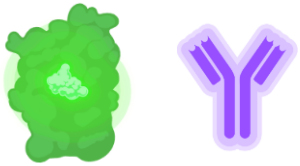
MINFLUX IMAGING SYSTEM – WHAT WE CAN SEE?

mitochondria
(500 – 1000 nm)



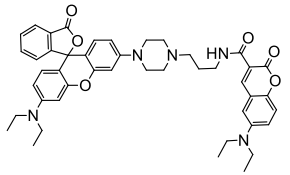
>

proteins
(5 – 10 nm)



>

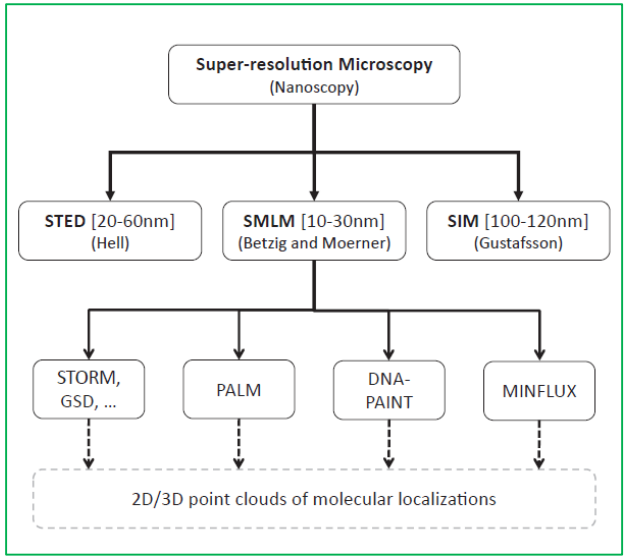
small molecules
(0.5 – 2 nm)

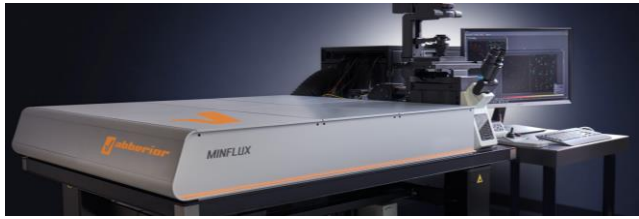


confocal microscopy
(500 nm)

super-resolution microscopy
(20 – 50 nm)

MINFLUX
(1 – 2 nm)





SIZE REFERENCE

Minflux system enables :

- **Localization and measuring interaction with unprecedented resolution**
- **Enables particle tracking**

https://abberior-instruments.com/products/minflux/

Y. Eilers et al., *PNAS*, 2018, vol. 115, p. 6117- 6122
H. Li et al., *Chem. Rev.*, 2018, vol. 118, p.9412 - 9454
G. Vicidomini et al., *Nature Meth.*, 2018, vol. 15, p. 173 – 182

Images were created with BioRender.com

NEBI - Krajowy Ośrodek Badań Obrazowych w Naukach Biologicznych i Biomedycznych (lider konsorcjum: Instytut Biologii Doświadczalnej im. M. Nenckiego PAN
NEBI - National Center for Imaging Research in Biological and Biomedical Sciences (consortium leader: M. Nencki Institute of Experimental Biology of the Polish Academy of Sciences

AVAILABLE TECHNIQUES

STED – STIMULATION DEPLETION MICROSCOPY

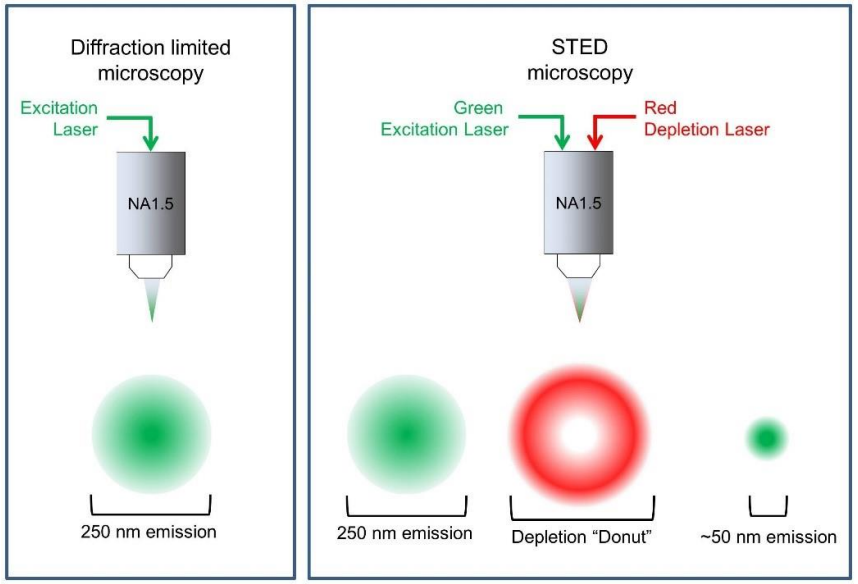
Excitation Pulsed Lasers (STED/Confocal):
488 nm
561 nm
640 nm

Depletion Lasers:
595 nm
775 nm

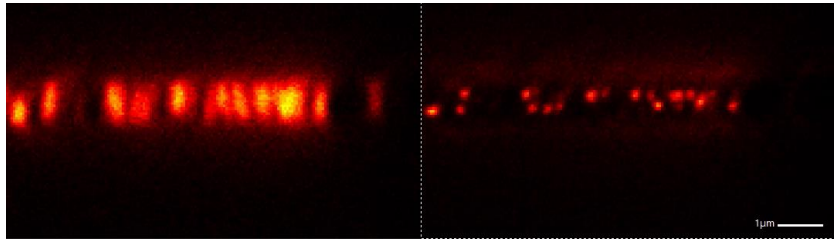
Detection range: 420-750 nm
3 APD detectors with detection filter cubes
long-pass filter
Cy5 far: 685-720 nm
Cy5 near: 650-685 nm
Cy3 cube: 580-630 nm

3 APD detectors with spectral detection modules
short-pass filter

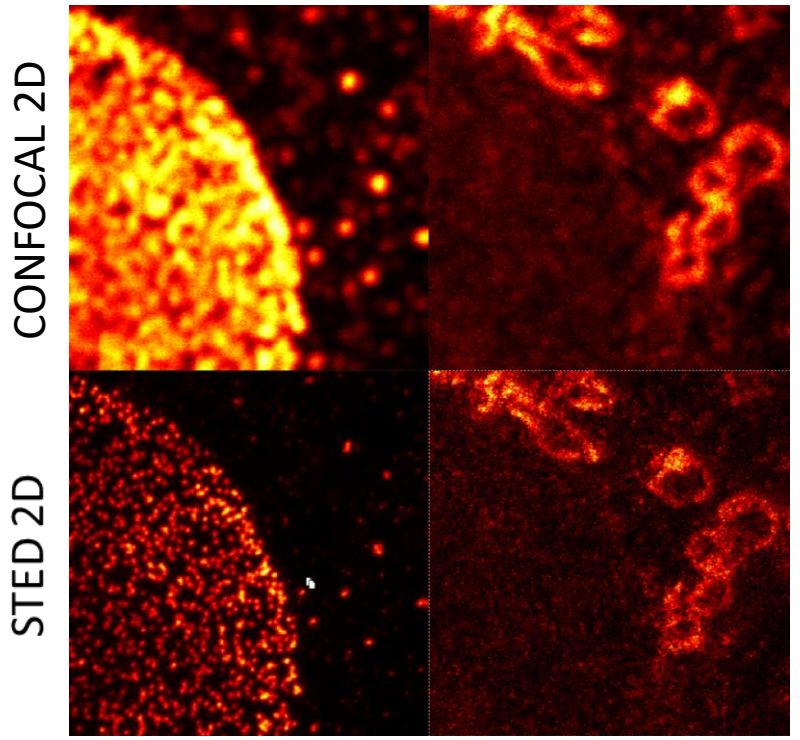
Epifluorescence filter cubes QUAD band

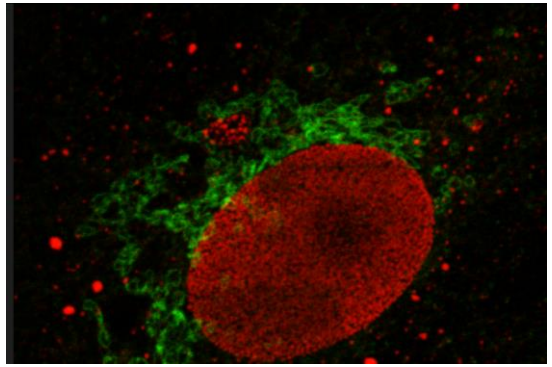


SHORT MOVIE
STED 3D IMAGING OF NPC'S

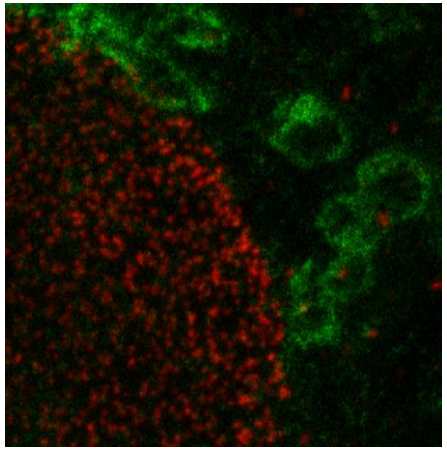


Nup96–SNAP labelled with Alexa Fluor 647



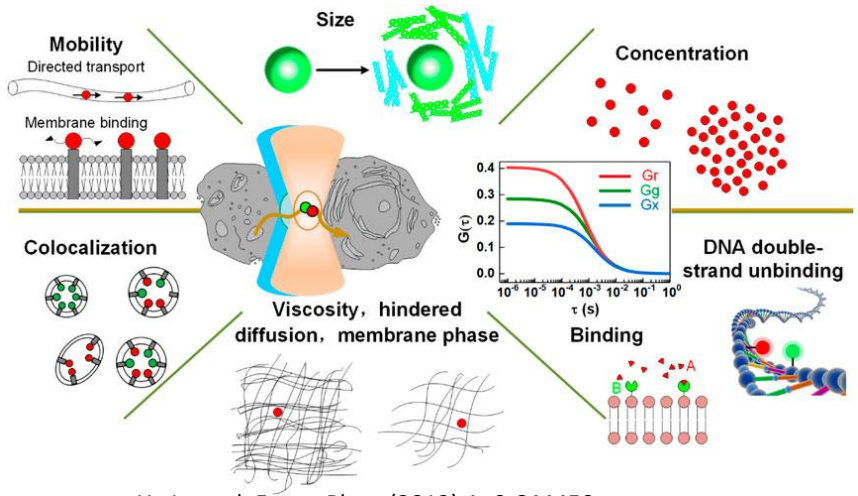


STED 2D, 2 COLOUR



https://doi.org/10.1117/1.JBO.19.8.080901
https://www.technologynetworks.com/neuroscience/articles/what-is-super-resolution-microscopy-sted-sim-and-storm-explained-328572
Vicidomini, G., Bianchini, P. & Diaspro, A. STED super-resolved microscopy. Nat. Methods 15, 173–182 (2018)
Valli, J. *et al.* Seeing beyond the limit: A guide to choosing the right super-resolution microscopy technique. *J. Biol. Chem.* **297**, 100791 (2021)

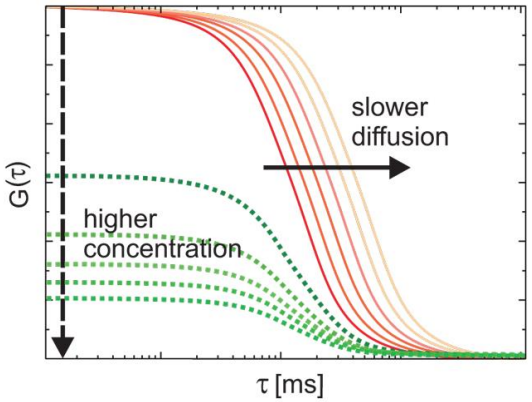
CORRELATION ANALYSIS OF TEMPORAL FLUCTUATIONS IN FLUORESCENCE INTENSITY OF FLUOROPHORES



Yu L, et al. Front. Phys. (2012) A, 9:644450

FCS gives quantitative informations about:

- Average absolute concentrations
- Diffusion coefficients
- Hydrodynamic radii
- Kinetic chemical reaction rates
- Singlet-triplet dynamics
- Binding ratios (FCCS)

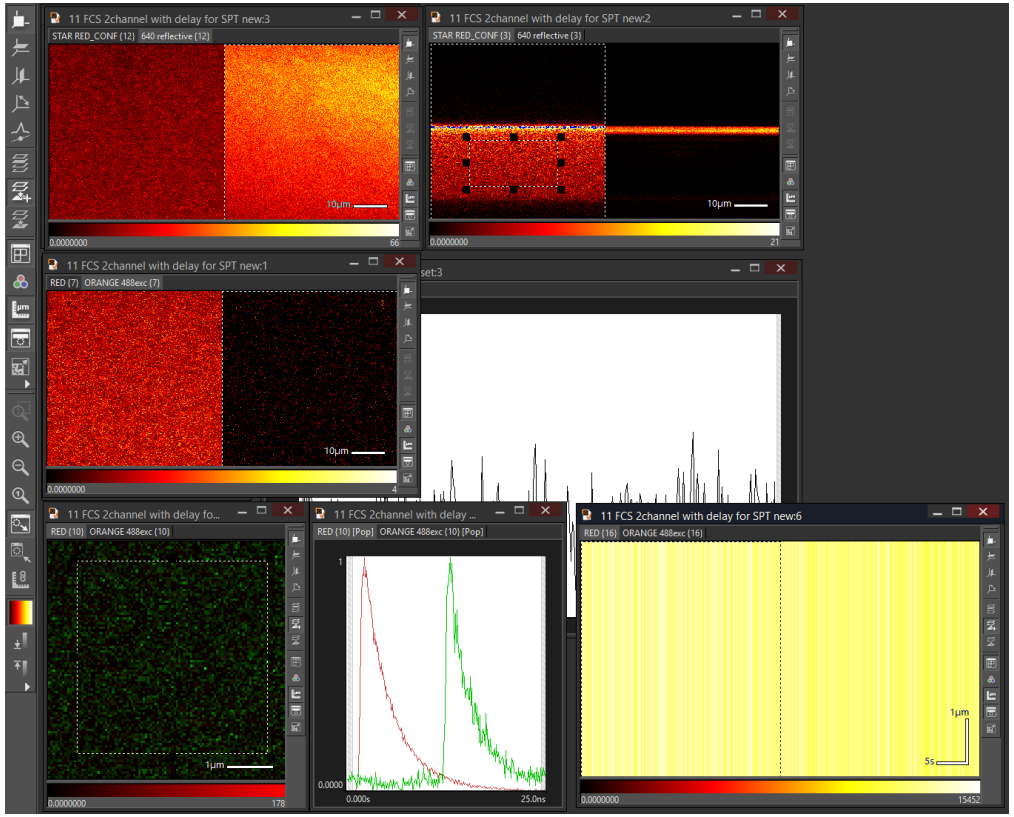


Useful for:

- Detection of molecular association and dissociation
- Determination of the stoichiometry of molecular complexes
- Determination of the kinetic rate constants, i.e. on and off kinetics of complex formation, enzyme dynamics and intramolecular dynamics in cells

(https://www.picoquant.com/applications/category/life-science/fluorescence-lifetime-imaging-flim#tab-4)

STED + FCS – FLUORESCENCE CORRELATION MICROSCOPY



AVAILABLE TECHNIQUES

STED + FLIM – FLUORESCENCE LIFETIME MICROSCOPY

„The fluorescence lifetime - as a sensor parameter for intra- and intermolecular interactions allowing for distance measurements in the nanometer range"

- **Detection of molecular interactions**
- Local environment sensing
- Detection of conformational changes
- Discrimination of labels + background removal

(https://www.picoquant.com/applications/category/life-science/fluorescence-lifetime-imaging-flim#tab-4)

Photochemical & Photobiological Sciences
https://doi.org/10.1007/s43630-023-00401-9

ORIGINAL PAPERS

Check for updates

5-Deazaalloxazine as photosensitizer of singlet oxygen and potential redox-sensitive agent

Małgorzata Insińska-Rak[1] · Anna Golczak[1] · Mateusz Gierszewski[2] · Zubair Anwar[1] · Volodymyr Cherkas[3] · Dorota Kwiatek[3] · Ewa Sikorska[4] · Igor Khmelinskii[5] · Gotard Burdziński[2] · Radek Cibulka[6] · Lucyna Mrówczyńska[7] · Jacek Lukasz Kolanowski[3] · Marek Sikorski[1]

Photochemical & Photobiological Sciences

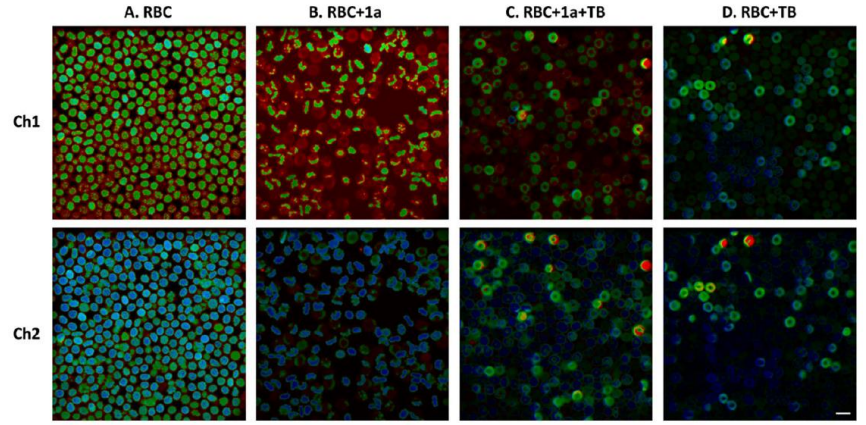


Fig. 5 Images of red blood cells (RBC) as obtained by FLIM (λ_{exc} = 405 nm emission recorded at two channels; channel 1: 420—550 nm and channel 2: 550—780 nm). RBC incubation condition: **A** control, in PBS buffer (24 h, 37 °C), **B** 1.0 mg/mL **1a** in PBS (24 h, 37° C), **C** 1.0 mg/mL **1a** (24 h, 37 °C) followed by incubation with 250 μM TB (1.5 h, at 37 °C), **D** in PBS buffer (24 h, 37 °C) followed by incubation with 250 μM TB (1.5 h, at 37 °C). Representative results are presented. Scale bar 10 μm

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Tetramethylalloxazines as efficient singlet oxygen photosensitizers and potential redox-sensitive agents

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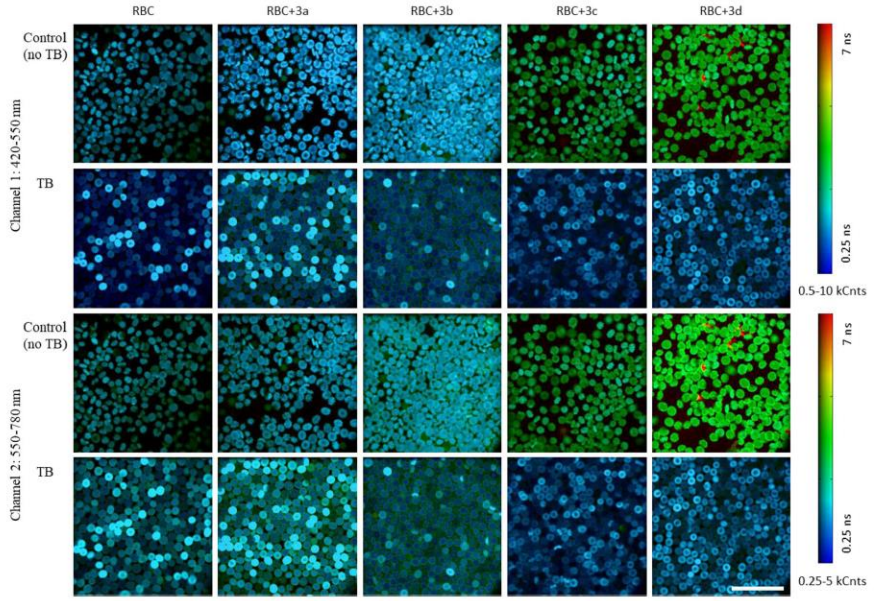


Figure 6. Representative FLIM images (exc 405 nm, 2 channels 420–550 nm and 550–780 nm) of **RBC**, **RBC + X** (where **X** mean preincubation with compounds **3a-3d** at 0.1 mg/1 mL concentration), and similar conditions but followed with TB incubation (oxidative stress, **RBC + TB** and **RBC + X + TB**). Color represents the average lifetime in the pixel (assuming monoexponential decay), while brightness corresponds to the total number of photons per pixel. All images were collected at identical instrument settings. The scalebar size is 50 μm.

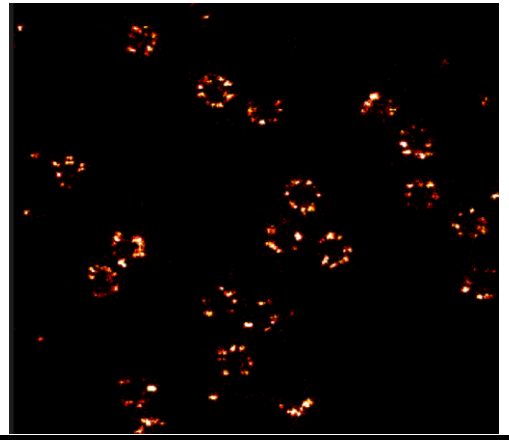
Excitation CW Lasers:
488 nm
561 nm
640 nm

Activation CW/pulsed Laser:
405 nm

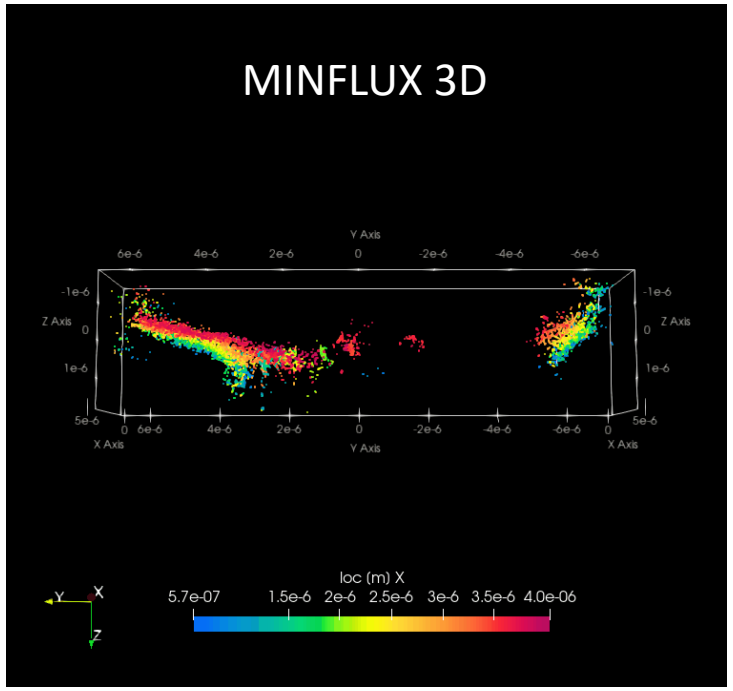
AVAILABLE TECHNIQUES

MINFLUX – MINIMAL PHOTON FLUX

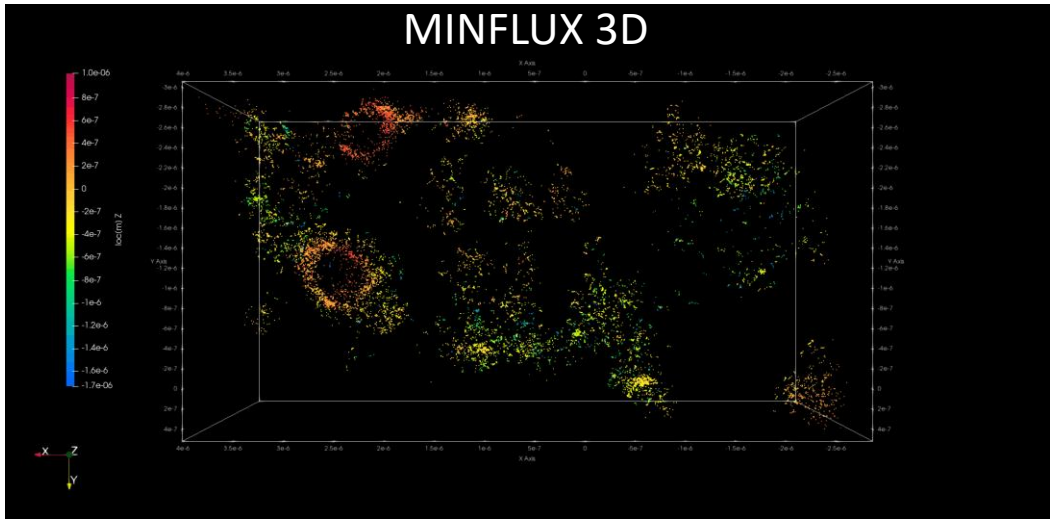
Nup96–SNAP labelled with Alexa Fluor 647

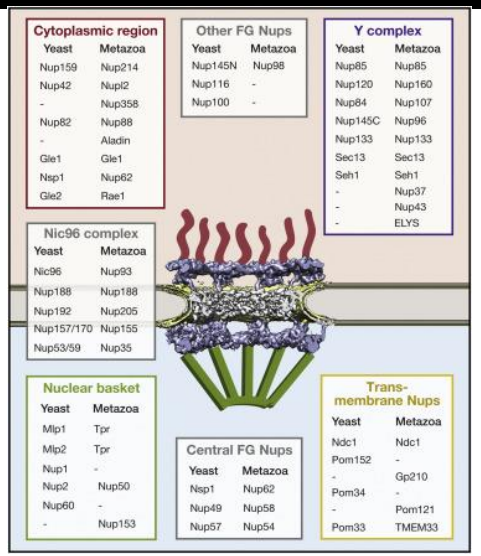


Imaging performed as part of the OPUS project 2021/41/B/NZ2/03781 dr hab. Anna Kurzyńska Kokorniak (Department of Ribonucleoprotein Biochemistry), task: visualization of hDice-RNA/DNA G-quadruplex complexes in human cells using nanoscopy in the Molecular Testing and Imaging Laboratory. Dicer protein (HEK293T line) labeled with FLUX640



Imaging performed as part of the Minflux Test Program for dr Ireneusz Stolarek, Department of Molecular and Systems Biology, ICHB PAN Lamin B1 protein (in human fetal myoblasts) labeled with FLUX640





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Gwosch, K. C. *et al.* MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells. *Nat. Methods* **17**, 217–224 (2020).
Eilers, Y., Ta, H., Gwosch, K. C., Balzarotti, F. & Hell, S. W. MINFLUX monitors rapid molecular jumps with superior spatiotemporal resolution. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 6117–6122 (2018)
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K. E.Knockenhauer, Cell, Volume 164, Issue 6, 10 March 2016, Pages 1162-1171

Tasks realized by users via access to the infrastructure (6 national: one of them from Horizon Europe):
1. Opera Phenix - project ERA-NET-ERARE- 3/III/TreatPolyQ/08/2018 prof. Maciej Figiel ICHB PAN
2. Cytation 3 - project Miniatura-5 (2021/05/X/NZ7/01440) dr Michał Jakubczyk ICHB PAN
3. SONATA 2017/26/D/NZ1/01234: dr hab. Jacek Kolanowski
4. OPUS 15 2018/29/B/ST4/01498501-1332, dr hab. Jacek Kolanowski
5. HOMING Homing/2017-4/33, dr hab. Jacek Kolanowski
6. Pasific: (PAN, MSC cofun,: M. Dekaliuk)

Tasks in realization - international projects within EU-OPENSCREEN (10 tasks in 8 projects)
1. Horizn 2020-INFRADEV-2018-2020 (10 400 EUR
2. EU-OPENSCREEN Academic library screening (2 projects) - for EU-OPENSCREEN
3. ISIDORE – P.I.: Sandeep Ghorpade
4. ISIDORE – P.I. Peter Hart
5. JRA – P.I.Bart Haagmans
6. IMPULSE: IMProving User experience, Long-term sustainability and Services of EU-OPENSCREEN (program HORIZON-INFRA-2023-DEV-01) – 3 tasks
7. AgroServ – Integrated SERvices supporting a sustainable AGROecological transition (HE - HORIZONINFRA- 2021-SERV-01)

Tasks in realization/planned in NCN projects (5 tasks in 4 projects)
1. OPUS 2021/41/B/NZ2/03781 dr hab. Anna Kurzyńska-Kokorniak 1 task
2. OPUS 2021/41/B/NZ1/03819 prof. Elżbieta Kierzek
3. OPUS 2021/43/B/NZ7/0161 dr hab. Miłosz Ruszkowski
4. SONATINA 2021/40/C/NZ3/00323 dr Magdalena Derbis

Tasks in realization/planned within different financing (9 tasks in 6 projects)
1. Natural extracts impact on cancer cells research - service for company – HTS + Minflux tasks
2. 4 tasks in NCBiRPOIR.01.01.01-00-2450/20 NCBR - Szybka Ścieżka 5 (projekt realizowany w konsorcjum, lider projektu: ideas4biology Sp. z o. o.;: dr hab. Anna Kurzyńska-Kokorniak)
3. iCare (Integrated assessment and advanced characterisation neuro-nanotoxicity, HORIZON-CL4-2022-DIGITAL-EMERGING-01 – Centre for Chemical Biology is a WP leader
4. Pasific PAN Marii Skłodowskiej-Curie Action dr Marii Dekaliuk
5. Program wsparcia naukowców z Ukrainy : dr Volodymyr Cherkas
6. Alternative Ends – Alternative gene ends: the crosstalk of RNA cleavage and transcription termination ERC-2021-STG, dr hab. Kinga Kamieniarz-Gdula UAM

Grant proposals:
1. Development of the infrastructure POL-OPENSCREEN 2
2. Maintenance of the infrastructure: SPUB

How to work with us ?

1. Please contact us at least 1 month before your grant application deadline !

2. Meet us live or on-line !

3. What we can do for you?:

a. time and cost estimation

b. help in grant proposal preparation

c. free tests on Minflux (estimation of the microscope application for your purpose)

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room 109E

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dr hab. Joanna Kosman

dr Krzysztof Żukowski

mgr Natalia Karczewska

mgr Monika Pyc

dr Volodymir Cherkas

mgr Adrian Rüfli


ICHB PAN