INSTITUTE OF BIOORGANIC CHEMISTRY
Polish Academy of Sciences

PSNC

14-15 NOVEMBER 2023
UNDERSTAND

&

DESCRIBE LIFE

Conference

The Institute of Bioorganic Chemistry Polish Academy of Sciences (IBCH PAS) and its affiliated Poznan Supercomputing and Networking Center (PSNC) conduct research at the intersection of three disciplines, biology, chemistry and infromatics. Our common interest lies in understanding and describing life at all levels, from single molecules to entire populations.

The scientific conference "Understand and describe life," organized on the occasion of the 35th anniversary of the IBCH PAS in Poznan and the 30th anniversary of the PSNC, took place on November 14-15 in Poznan. Distinguished scientists from both Poland and abroad, along with doctoral students and representatives from the business sector, gathered to delve into the multifaceted study of life across different levels of organization, fostering valuable discussions and insights.

konferencja@ibch.poznan.pl

life2023.ichb.pl

Sessions 14. 11. 2023

Life at the molecular level – chair JACEK Ł. KOLANOWSKI

JONAS RIES

Superresolution microscopy for structural cell biology

    EMBL, Cell Biology and Biophysics Unit, Heidelberg, Max Perutz Labs.
    University of Vienna, Department of Structural and Computational Biology

MAGDA KONARSKA

Quality control by the spliceosome and its unexpected consequences for the cell

    The International Institute of Molecular Mechanisms and Machines, PAS

SEBASTIAN GLATT

tRNAslational control of eukaryotic gene expression

    Małopolska Centre of Biotechnology, Jagiellonian University

MAKSIM SERDAKOV

Dynamic assrmbly of RNA targeting complexes in bacteria

    International Institute of Molecular and Cell Biology in Warsaw

MARTA SZABAT

RNA G-quadruplexes – noncanonical structures from the influenza A virus genome

    Institute of Bioorganic Chemistry, PAS

Life at the single level – chair PAULINA JACKOWIAK

NIKOLAUS RAJEWSKY

Single cell resolution omics & Artificial Intelligence for personalized medicine

    Berlin Institute for Medical Systems Biology in the Max Delbrück Center, Charite Universitätsmedizin, Berlin

BOŻENA KAMIŃSKA-KACZMAREK

Tumor microenvironment at single-cell resolution – unraveling transcriptional and spatial heterogeneity of immune cells

    Nencki Institute of Experimental Biology, PAS

AGNIESZKA CHACIŃSKA

Protein homeostasis at the crossroads to mitochondria

    The International Institute of Molecular Mechanisms and Machines, PAS

AGNIESZKA CIESIELSKA

Enter the resolution revolution with single cell spatial transcriptomics

    10 × Genomics B.V.

PAWEŁ ŚWITOŃSKI

Isolating Purkinje cell nuclei: a gateway to understanding SCA7 pathology

    Institute of Bioorganic Chemistry, PAS

Life at the organismal level – chair MICHAŁ JASIŃSKI

FRANÇOIS CHAUMONT

Aquaporins: key ubiquitous channels for plant physiology

    Louvain Institute of Biomolecular Science and Technology, UC Louvain

EWELINA KNAPSKA

The central Amygdala as a motivational hub: paving the way for personalized therapies ond behavioral disorders

    The Centre of Excellence for Neural Plasticity and Brain Disorders (BRAINCITY)
    Nencki Institute of Experimental Biology, PAS

WOJCIECH POKRZYWA

Extracellular vesicles in reproductive adaptation: a tale of C. elegans response to internal and external stimuli

    International Institute of Molecular and Cell Biology in Warsaw

SAVANI ANBALAGAN

A ligand-receptor interactome atlas of the zebrafish

    Adam Mickiewicz University in Poznań

ŁUKASZ PRZYBYŁ

New Huntington's disease mouse models prove mutant RNA contribution to phenotype

    Institute of Bioorganic Chemistry, PAS

Sessions 15. 11. 2023

Life at the population level – chair LUIZA HANDSCHUH

TOMASZ GRZYBOWSKI

Prediction of human biogeographic ancestry within Europe

    Department of Forensic Medicine, Ludwik Rydygier Collegium Medicum in Bydgoszcz
    Nicolaus Copernicus University in Toruń

KRZYSZTOF PYRĆ

Advances models for comprehensive understanding of viral infection

    Jagiellonian University in Kraków

PIOTR ZIÓŁKOWSKI

Crossover recombination from the population perspective: interplay between meiotic recombination and polymorphism in plants

    Adam Mickiewicz University in Poznań

KATARZYNA KLONOWSKA

Hidden landscape of tumorigenesis-driving mutations in Tuberous Sclerosis Complex

    Institute of Bioorganic Chemistry, PAS

Synthetic life – chair MARTA OLEJNICZAK

ANDRZEJ DZIEMBOWSKI

Complex metabolic pathways of mRNA therapeutics
International Institute of Molecular and Cell Biology in Warsaw

MAŁGORZATA BOROWIAK

Mechanosignaling in the differentiation and expansion of human pancreatic beta cells
Adam Mickiewicz University in Poznań

MARCIN DRĄG

In search of perfection – the phenomenon of unnatural amono acids
Wrocław University of Science and Technology

KATARZYNA TUTAK

RPS26 a novel RAN translation modifier of RNA harboring expanded CGG repeats in Fragile X-associated syndrome
Adam Mickiewicz University in Poznań

EMILIA IŁOWSKA

Self-assembly peptides – new way in antimicrobial and anticancer research
University of Gdańsk

Digital and virtual life – chair TOMASZ PIONTEK

ROSSEN APOSTOLOV

HPC for life science research: advanced applications for extreme-scale biomolecular simulations
KTH Royal Institute of Technology Stockholm

ALEKSANDRA PĘKOWSKA

Molecular signature of primate astrocytes reveals pathways and regulatory changes contributing to brain evolution
Nencki Institute of Experimental Biology, PAS

CEZARY KĘPKA, JACEK KWIECIŃSKI, MARIUSZ KRUK

AI support in diagnosis and treatment of complicate cardiovascular patients
Advanced post-processing of coronary computed tomography images – benefits for patients and health professionals
The Cardinal Stefan Wyszyński Institute of Cardiology

DEREK GROEN

Simulating conflict-driven population displacement using large-scale computing
Brunel University London

ALEXANDER ZELENKA MARTIN

Hybrid algorithms on a photonic quantum processor
ORCA Ltd.

Isolating Purkinje cell nuclei:
a gateway to understanding SCA7 pathology

Luke C. Bartelt [1,2,3], Mouad Fakhri [4], Grażyna Adamek [4], Grzegorz Bartyzel [5],
Michał Kurkowski [4], Magdalena Trybus [6], Anna Samelak-Czajka [6], Paulina Jackowiak [6],
Agnieszka Fiszer [7], Craig B. Lowe [3], Albert R. La Spada [2,8,9], Paweł M. Świtonski [4]

[1] University Program in Genetics & Genomics, Duke University Medical Center, Durham, USA

[2] Departments of Pathology & Laboratory Medicine, Neurology, Biological Chemistry, and Neurobiology & Behavior, University of California, Irvine, USA

[3] Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, USA

[4] Department of Neuronal Cell Biology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[5] Department of Automatic Control and Robotics, Faculty of Electrical Engineering, Automatics, Computer Science and Biomedical Engineering, AGH University of Science and Technology, Krakow, Poland

[6] Laboratory of Single Cell Analyses, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[7] Department of Medical Biotechnology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[8] Department of Neurology, Duke University School of Medicine, Durham, USA

[9] UCI Center for Neurotherapeutics, University of California, Irvine, USA

Purkinje cells (PCs) are cerebellar neurons crucial for governing voluntary movements, coordination, and motor learning. PC degeneration is a distinctive feature in various neurological disorders, including many spinocerebellar ataxias. However, PCs constitute a small fraction of the total cerebellar cell count, making the exploration of PC-related pathology exceptionally challenging.

In our research, we aimed to establish a protocol for the targeted isolation of PC nuclei, utilizing genetic or immune labeling of nuclear envelopes in conjunction with Fluorescence-Activated Nuclear Sorting.

Using the SUN1-GFP (INTACT) mouse model, we successfully identified and isolated PC nuclei based on elevated green fluorescent protein (GFP) and side scatter signals, distinguishing them from other cerebellar cell types. Validation of PC identity included the robust expression of PC marker genes, increased nuclear size, and a reduced number of nucleoli. To optimize the protocol and avoid the lengthy process of transgenic mouse breeding, we incorporated immunofluorescent labeling of the nuclear membrane protein RanBP2. Through the analysis of PC marker expression, nuclear size, and nucleoli count, we confirmed that a subpopulation with the strongest RanBP2 signal represents a pure fraction of PC nuclei.

Subsequently, we applied this method to purify PCs from both wild-type (WT) and spinocerebellar ataxia type 7 (SCA7) mice, collecting image data of the nuclei through image flow cytometry. Employing the deep spatial autoencoder method, we developed a convolutional neural network that was trained on images of PC nuclei. Afterward, the employed GradCAM method generated heat maps highlighting spatial features that best explained the classification between SCA7 and WT nuclei. Consequently, we identified SCA7 nuclear phenotypes associated with differential DNA heterochromatinization.

Our method of obtaining pure fractions of PC nuclei is valuable for exploring the pathology of PC-related diseases and provides an opportunity to study the long-standing mystery of PC selective vulnerability in spinocerebellar ataxias.

Hidden landscape of tumorigenesis-driving mutations in Tuberous Sclerosis Complex

KATARZYNA KLONOWSKA [1,2]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[2] Brigham and Women's Hospital and Harvard Medical School, Boston, USA

Tuberous sclerosis complex (TSC) is due to inactivating mutations in either *TSC2* or *TSC1* and is characterized by tumor development in multiple tissues, including facial angiofibroma (FAF). Several TSC tumors are known to develop through a two-hit mechanism. In this study, we assessed the frequency of second-hit mutation events in TSC FAF. We developed a multiplex high-sensitivity PCR assay (MHPA) for both *TSC2* and *TP53*, enabling the detection of mutations at extremely low (<0.1%) variant allele frequencies (VAFs) (Klonowska et al., J Clin Invest, 2022). The *TSC2*-MHPA analysis of 81 samples, including 24 FAF biopsies from TSC patients with *TSC2* mosaicism, led to the discovery that UV-induced second-hit mutations causing *TSC2* inactivation was pervasive in TSC facial skin. The analysis identified an average of 4.8 mutations per 2-mm biopsy at a median VAF of 0.08%, generating over 150,000 incipient facial tumors (subclinical "micro-FAFs") in the average TSC subject. Our analysis revealed that TSC facial skin can be viewed as harboring a patchwork of clonal fibroblast proliferations (micro-FAFs) with indolent growth, a small proportion of which develop into clinically observable FAF. The MHPA analysis also led to the identification of a refined UV-related indel signature and a recurrent complex mutation pattern, consisting of both a single-nucleotide or dinucleotide variant and a 1-nucleotide to 9-nucleotide deletion, occuring *in cis*. Additionally, our observations broaden the spectrum of UV-related mutation signatures (Klonowska et al., J Clin Invest, 2022).

New Huntington's disease mouse models prove mutant RNA contribution to phenotype

ŁUKASZ PRZYBYŁ [1#], MAGDALENA JAZUREK-CIESIOŁKA [1#], MAGDALENA WOŹNA-WYSOCKA [1#],
PAWEŁ ŚWITOŃSKI [1], JOANNA SUSZYŃSKA-ZAJCZYK [2], DOROTA WRONKA [1], JULIA MISIOREK [1],
GRZEGORZ FIGURA [1], ADAM CIESIOŁKA [1], PAULA SOBIESZCZAŃSKA [1], MACIEJ FIGIEL [1], ANNA ZELLER [3],
MAGDALENA NIEMIRA [3], AGNIESZKA FISZER [1*]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[2] Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poland

[3] Genomics and Epigenomics Laboratory, Clinical Research Centre, Medical University of Bialystok, Poland

* Corresponding author – e-mail: agnieszka.fiszer@ibch.poznan.pl

Huntington's disease (HD) is classified within the group of neurodegenerative polyglutamine (polyQ) diseases and is caused by the CAG repeat expansion located in the ORF of the *HTT* gene. The extent to which disruptions driven by mutant mRNA influence HD pathogenesis is still a subject of debate, especially when compared to the dominant mechanisms associated with the gain-of-function of the mutant polyQ protein.

This study aimed to assess the involvement of mutant RNA in the pathogenesis of HD. We created two transgenic mouse models of HD using a knock in strategy into the Rosa26 locus. These models express one of the two variants of the human mutant HTT cDNA fragment: either translated – HD/100Q or nontranslated – HD/100CAG. Additional sequences, including an HA tag and MS2 aptamer, were incorporated to facilitate the visualization of the protein and transcript, respectively.

Over 21 months, cohorts of animals were analyzed at 4-month intervals using a comprehensive range of molecular, behavioral, and cognitive methods. Behavioral testing showed a progressive phenotype in both models, with the HD/100Q model displaying a more severe phenotype. Rotarod, static rod, and open-field tests revealed motor deficits during the light phase while ActiMot indicated hyperkinesis during the dark phase. Both models also showed some molecular neuropathological changes in the striatum.

In conclusion, our findings provide *in vivo* evidence supporting a contributory role of mutant RNA in the pathogenesis of HD disease.

Presenting author – e-mail: lukasz.przybyl@ibch.poznan.pl

[#] Equal contribution

Dynamic assembly of RNA targeting complexes in bacteria

MAKSIM SERDAKOV [1], EWELINA MAŁECKA-GRAJEK [1]

[1] Laboratory of Single-Molecule Biophysics, International Institute of Molecular and Cell Biology, Warsaw, Poland

The regulation of mRNA stability and translation by small regulatory RNAs (sRNAs) is crucial for bacterial adaptation to environmental changes, including virulence. The base pairing of two RNAs often involves the chaperone protein Hfq. Downstream regulation includes actions such as inhibiting ribosome assembly on mRNA or RNA degradation through the degradosome. Hfq plays a significant role in these processes, making the stability of ternary Hfq–sRNA–mRNA complexes critical for successful mRNA regulation. This study aims to elucidate the factors governing the dynamics and stability of these ternary complexes. To address this question, we employed single-molecule Total Internal Reflection Microscopy to observe binding and dissociation events on a millisecond scale for various sRNA–mRNA pairs with immobilized Hfq molecules.

Our investigations revealed that the formation of ternary complexes introduces a significant kinetic barrier compared to forming Hfq–RNA complexes. Intriguingly, the efficiency of forming sRNA–mRNA–Hfq complexes was higher when sRNAs were prebound to Hfq, suggesting that sRNAs might require more conformational changes on Hfq than mRNAs. Importantly, our observations indicated that the order in which RNA components join the complex does not significantly affect the lifetime of ternary complexes. RNAs rarely leave Hfq together, but if the complex dissociates, the RNA that joined last is the first to leave. These findings provide valuable insights into the intricacies of sRNA-mediated mRNA regulation.

Noncanonical RNA G-quadruplex structures
in the influenza A virus genome

Marta Szabat *, Maria Nalewaj, Karolina Zielińska, Ryszard Kierzek, Elżbieta Kierzek

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland

* Corresponding author: szabat@ibch.poznan.pl

A comprehensive understanding of RNA structure is essential for gaining valuable insights into its biological functions. Among the secondary structures, G-quadruplexes (G4s) play a significant role in the biological functions of RNA viruses. More recently, it has been revealed that G4s in the genome of SARS-CoV-2, prompting us to inspect the influenza A virus (IAV) genome for the existence of G-rich sequences. Given the severe threat to global public health, influenza is an important research subject for many scientists. Thus, our interest lies in exploring the secondary structure of IAV RNA and its relationship with biological function.

Initially, we investigated the influenza A/California/07/2009 (H1N1) genome for the occurrence of potential G-quadruplex-forming sequences (PQS) and analyzed their conservation across various IAV strains. Subsequently, we examined the ability of PQS to fold into G4s using diverse biophysical methods, including UV-melting, NMR, CD spectroscopy, and the thioflavin T fluorescence assay. Gel electrophoresis was then used to ascertain their folding topology and molecularity.

We identified 12 PQS motifs within the influenza A/California/07/2009 (H1N1) genome, noting that some are highly conserved among different IAV strains. Further confirmation of RNA G4 formation was achieved through NMR spectroscopy, while fluorescence measurements provided additional validation. Additionally, the resulting CD spectra of PQS exhibited a profile typical of parallel G4s. The PAGE experiments revealed differences in G4s folding molecularity, suggesting either bimolecular or tetramolecular structures. In summary, our results revealed that three PQS can form stable G4s. Nonetheless, these G4s may exhibit differences in stability and folding topology depending on the experimental conditions. Interestingly, these PQS are located within segments encoding polymerase complex proteins indicating their possible role in the virus biology. Our current interest revolves around exploring the potential role of G4s in the influenza virus biology.