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The Biochemistry Global Summit

25th IUBMB Congress, 46th FEBS Congress, 15th PABMB Congress

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Abstracts submitted to The Biochemistry Global Summit (25th IUBMB Congress, 46th FEBS Congress and 15th PABMB Congress) from 9th to 14th July 2022 and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this supplement. The abstracts are available as two PDF files: Talks (Plenary Lectures, Symposia and FEBS Special Sessions) and Posters.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented (see p.68 for key).

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Atomic and molecular imaging

P-07.1-001
Total synthesis of *Odontosyllis undecimdonta* luciferin and its structural elucidation

Y. Bolt[1], A. Kotlobay[1], M. Dubinnyi[1], R. Zagitova[1], **Z. Kaskova**[1,2], I. Yampolsky[1,2], A. Tsarkova[1,2]
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia,* [2]*Pirogov Russian National Research Medical University, Moscow, Russia*

Odontosyllis undecimdonta is a marine polychaete that has an ability to emit visible green light. During the previous studies we isolated several low-molecular weight compounds, which were then proved to be substrates in the light-emitting transformation process. NMR, mass-spectrometry and X-ray analysis data allowed us to propose the structures of the luciferin oxidation products. We then used this information to suggest a possible structure of *Odontosyllis* luciferin, belonging to a class of 2,3-dicarboxythiochromenes. The main goal of this work was to confirm it by total synthesis. We had to develop new synthetic methods, as literature search showed that there were no reliable approaches to the synthesis of the target compound. The approach employing the use of the thia-Michael/Horner-Wadsworth-Emmons cascade transformations using quinone and diester derivative of a dicarboxylic acid as starting compounds allowed us to obtain *Odontosyllis* luciferin via convergent synthetic scheme from 4-bromo derivative of benzothiophene and dimethylacetylenedicarboxylate. The work was supported by Russian Science Foundation grant № 21-74-10152, https://rscf.ru/en/project/21-74-10152/.

P-07.1-002
Highly-fluorescent benzothiphene-based dye exhibiting a large Stokes shift

Y. Bolt[1], N. Baleeva[1], Y. Nelyubina[2], **A. Andrianova**[1,3], Z. Kaskova[1,4], A. Tsarkova[1,4]
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia,* [2]*A. N. Nesmeyanov Institute of Organoelement Compounds RAS, Moscow, Russia,* [3]*Lomonosov Moscow State University, Faculty of Medicine, Moscow, Russia,* [4]*Pirogov Russian National Research Medical University, Moscow, Russia*

Fluorescent dyes play a significant role in modern science and technology, where they are used as tools in a variety of fields, including nanoscience, biomedicine, and solar energy conversion. The creation of new fluorescent dyes emitting in red and near-infrared regions of the spectrum is a crucial task of modern organic synthesis. Conjugated aromatic compounds with a benzothiophene-based core have fluorescent properties, but mostly possess small Stokes shift, and require conjugation with an additional chromophore group to increase it[1]. A number of conjugated dyes that absorb visible light and emit in the near-infrared spectral region were obtained for different applications[2,3]. Meanwhile, a dimerization of the original dye may become an alternative strategy for the increase of the Stokes shift. Here we report a novel highly fluorescent benzothiophene-based dye comprising five fused rings and exhibiting a large Stokes shift (from $\Delta\lambda$ = 100 nm or $\Delta\nu$ = 3633 cm^{-1} in water to $\Delta\lambda$ = 152 nm or $\Delta\nu$ = 5482 cm^{-1} in ethanol). The compound is obtained via a simple two-step procedure from commonly available aromatic benzenethiol. Due to the presence of a protected carboxyl group, the obtained compound could be easily modified to allow a series of new fluorescent dyes. This work was supported by the Russian Science Foundation grant № 21-74-10152, https://rscf.ru/en/project/21-74-10152/. (1) Jiao C et al. (2011) Org Lett 13, 632. (2) Ni Y et al. (2014) Org Biomol Chem 12, 3774. (3) Lim S. H. et al. (2010) J Med Chem 53, 2865.

P-07.1-003
Synthesis of plant and fungal secondary metabolites – substrates for the biosynthesis of fungal luciferin analogues *in vivo*

A. Silvestrova*[1,2], K. Palkina *[1,3], A. Andrianova[1,4], I. Yampolsky[1,3,5], Z. Kaskova[1,5]
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia,* [2]*Faculty of Chemistry, National Research University Higher School of Economics, Moscow, Russia,* [3]*Planta LLC, Moscow, Russia,* [4]*Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia,* [5]*Pirogov Russian National Research Medical University, Moscow, Russia*

Bioluminescence-based bioimaging systems are widely used in biomedical research. The bioluminescent system of higher fungi was deciphered and successfully reconstructed in heterologous hosts in our laboratory. New fungal luciferin analogs will expand the area of application of fungal bioluminescent system. Wide substrate tolerance of the enzymes of fungal bioluminescent system allows the use of caffeic acid and its synthetic analogs for the biosynthesis of fungal luciferin analogs *in vivo*. We have developed approaches to the biosynthesis of plant and fungal secondary metabolites from caffeic acid analogs in the yeast cell model. This work is an important step in understanding of the process of fungal bioluminescence towards new artificial bioimaging systems. The work was supported by Russian Science Foundation grant № 22-44-02024, https://rscf.ru/en/project/22-44-02024/. *The authors marked with an asterisk equally contributed to the work.

P-07.1-004
A crystallographic snapshot of SARS-CoV-2 main protease maturation process

G. Oliva[1], G. Noske*[1], A. Nakamura*[1], V. Gawriljuk*[1], R. Fernandes*[1], G. Lima[2], H. Rosa*[1], H. Pereira*[1], A. Zeri*[3], A. Nascimento*[3], M. Freire*[1], A. Godoy*[1]
[1]*Institute of Physics of São Carlos, University of São Paulo, São Carlos, Brazil,* [2]*BioMAX, MAX IV Laboratory, Lund, Sweden,* [3]*Brazilian Synchrotron Light Laboratory (LNLS), Campinas, Brazil*

SARS-CoV-2 is the causative agent of COVID-19. The dimeric form of the viral M^{pro} is responsible for the cleavage of the viral polyprotein in 11 sites, including its own N- and C-terminus. The lack of structural information for intermediary forms of M^{pro} is a setback for the understanding its self-maturation process. Herein, we used X-ray crystallography combined with biochemical data to characterize multiple forms of SARS-CoV-2 Mpro. For the immature form, we show that extra N-terminal residues caused conformational changes in the positioning of domain-three over the active site, hampering the dimerization and diminishing its activity. We propose that this form preludes the cis and trans-cleavage of N-terminal residues. Using fragment screening,

we probe new cavities in this form which can be used to guide therapeutic development. Furthermore, we characterized a serine site-directed mutant of the Mpro bound to its endogenous N- and C-terminal residues during dimeric association stage of the maturation process. We suggest this form is a transitional state during the C-terminal trans-cleavage. This data sheds light in the structural modifications of the SARS-CoV-2 main protease during its self-maturation process. *The authors marked with an asterisk equally contributed to the work.

Cellular imaging

P-07.2-001
NIR-emitting pentamethine cyanine dyes for mitochondria visualization with high cytotoxicity to cancer cells

D. Aristova[1], Y. Kot[2], S. Chernii[1], K. Kot[2], D. Babets[2], Y. Slominskii[3], V. Kovalska[1], S. Yarmoluk[1]
[1]Institute of Molecular Biology and Genetics NASU, Kyiv, Ukraine, [2]V. N. Karazin Kharkiv National University, Kharkiv, Ukraine, [3]Institute of organic chemistry NASU, Kyiv, Ukraine

Mitochondria play crucial roles in cellular metabolism. Therefore, compounds that stain mitochondria selectively are essential for tracking their different functional states. Moreover, dyes with high selectivity for mitochondria in cancer cells that also exhibit toxicity can be used for cancer treatment. In this regard, we have studied the series of pentamethine cyanine dyes based on benzothiazole heterocycles with different substituents emitted in the near-infrared region (NIR) as fluorescent probes for mitochondria visualization. Spectral-luminescent properties of the dyes in a free state and in the presence of different biomolecules such as nucleic acids and serum albumins were characterized. Dyes possess low to moderate fluorescence intensity in a free state, but it increases while binding to serum albumins up to 27 times with quantum yield up to 100 times higher than in a free state. The fluorescence emission maxima of dyes in a bound state lie between 680 and 711 nm at the NIR region. We have studied the ability of the dyes to stain live mesenchymal stem cells from human bone marrow (MSC) and the human breast cancer cells MCF-7. The dyes penetrate the cell and stain the cytoplasm components in a shorter incubation time compared to the commercially available dye MitoTracker Green (less than 5 min). Performing co-localization of the studied dyes with MitoTracker Green shows that cyanine dyes colocalize with standard dye, and therefore stain mitochondria in cancer MCF-7 cells. We have also performed SYTOX/Propidium Iodide-based staining assay to investigate the cytotoxicity effect of the dyes. The higher cytotoxicity of studied dyes on MCF-7 cells was shown compared to MSC. Taken together, the combined features of good imaging, NIR emission, and the higher anticancer activity make pentamethine benzothiazole cyanine dyes powerful fluorescent probes applicable for mitochondria visualization in cancer cells and suitable candidates for further theranostic studies.

P-07.2-002
Live imaging with subcellular detail in optical coherence microscopy: is it applicable for quality assessment of mammalian zygotes?

M. Fluks[1], S. Tamborski[2], M. Szkulmowski[2], A. Ajduk[1]
[1]University of Warsaw, Warsaw, Poland, [2]Nicolaus Copernicus University in Torun, Torun, Poland

Confocal and fluorescence microscopy allow for detailed structural studies on intracellular level, but require fluorescent markers to visualize cellular architecture. Bright-field microscopy, although non-destructive, does not provide such data. Optical coherence microscopy (OCM) is a promising alternative, as it does not require sample preprocessing and provides 3D images of intracellular structures, including pronuclei in zygotes, which can be analyzed quantitatively. There were numerous reports indicating that the pronuclear size provides an effective indicator of the embryo's quality, with embryos containing evenly sized pronuclei exhibiting higher developmental potential. We applied OCM to correlate the observed morphological parameters of zygotes, such as number, volume, and distance between pronuclei, with their developmental capabilities, such as the ability to reach the blastocyst stage, morphokinetic parameters, and numbers of cells in the first embryonic cell lineages. We showed that OCM allows for quantitative measurement of the pronucleus volume and distance between the pronuclei in a mouse zygote. As we expected, the size and the distance between the pronuclei correlates with the time of NEBD and the interval between the NEBD and the first mitotic division, but surprisingly, pronuclei volume also correlates with the time required for the 3rd embryonic cell cycle. None of the examined parameters, however, correlated with the total number of cells, or the number of cells in the first embryonic cell lineages in 5-day old embryos. We verified that both scanned and control zygotes have the same ability to complete preimplantation development. In summary, although our results indicate that OCM indeed allows for non-invasive 3D imaging of mammalian zygotes, providing data on pronuclei architecture, it seems the analysis of the volume and distance between the pronuclei, contrary to the earlier reports, might be insufficient for embryo quality assessment.

P-07.2-003
Altered ultrastructure of synaptic mitochondria in Fragile X syndrome linked with metabolic changes

A. Stawikowska, M. Magnowska, M. Dziembowska
Centre of New Technologies, University of Warsaw, S. Banacha 2c, 02-097, Warsaw, Poland

In the synapse, an important pool of mitochondrial proteins is translated locally on the bases of mRNAs transported from cell soma. Moreover, the local synthesis of proteins constituting mitochondrial respiratory chain complexes is increased by synaptic stimulation. In Fmr1 KO mice, a mouse model of fragile X syndrome, proteomic analysis shows dysregulated levels of mitochondrial proteins. Mitochondrial functions are fundamentally linked to their morphology and inner membrane ultrastructure. We used Serial Block Face-Scanning Electron Microscopy (SBF-SEM) to analyze mitochondrial ultrastructure in the hippocampi of Fmr1 KO and WT mice. Mitochondria shapes and volumes were reconstructed with the use of RECONSTRUCT software. We compared the morphology and the number of synaptic