# DEPARTMENT OF MOLECULAR AND BIOMEDICAL SCIENCES

***The research program of the Department of Molecular and Biomedical Sciences is focused mainly on basic research in protein biochemistry, molecular and cellular biology, and genetics. The primary goal of our investigations is the acquisition of new understanding of mammalian pathophysiology with the aim of improving human and animal health.***

**Toxinology**

One of our traditional research topics in the field of toxinology is the study of molecular mechanisms of toxic action of secreted phospholipases $A_2$ ($sPLA_2$s) from animal venoms. In particular, we are focused on those endowed with presynaptic neurotoxicity (β-neurotoxins). The knowledge that we are gaining by studying toxic $sPLA_2$s is helping us to discover the pathophysiological roles of orthologous mammalian $sPLA_2$s, for example, their role in the development of neurodegenerative diseases such as Alzheimer's disease (AD).

In this year we continued the characterization of the interaction of ammodytoxin (Atx), a neurotoxic snake venom $sPLA_2$, with its mitochondrial receptor, cytochrome c oxidase (CCOX), to deepen our understanding of the motoneuron poisoning by Atx and to shed light on the pathophysiological role of a mammalian group IIA $sPLA_2$ (GIIA), an orthologue of Atx, in this organelle. To this end, we prepared recombinant AtxA and rat GIIA, and their enzymatically inactive D49S mutants to synthesize different molecular tools, nanogold- and Alexa-derivatives. Using these tools, we studied the cellular trafficking of $sPLA_2$s by electron- and fluorescent confocal microscopy, and confirmed their internalization in PC12 cells. By heterologous competition experiment, we demonstrated that rat GIIA only weakly competed with $^{125}$I labelled Atx ($^{125}$I-Atx)-binding to CCOX subunit II. Consistently, upon labelling of porcine mitochondrial membranes with $^{125}$I-GIIA, CCOX subunit II was not labelled but another protein of 20 kDa was. These two results suggest that the GIIA binding site on CCOX is different from that of Atx but both binding sites overlap to some extent. According to the apparent molecular mass of the GIIA-binding protein, this protein might represent subunit IV of CCOX that is in contact with subunit II in the CCOX complex. We demonstrated that GIIA inhibits the activity of CCOX both *in vitro* and *ex vivo* in rat brain tissue sections. This led us to hypothesize that the regulation of ATP production is another physiological function of GIIA in mitochondria (Figure 1).

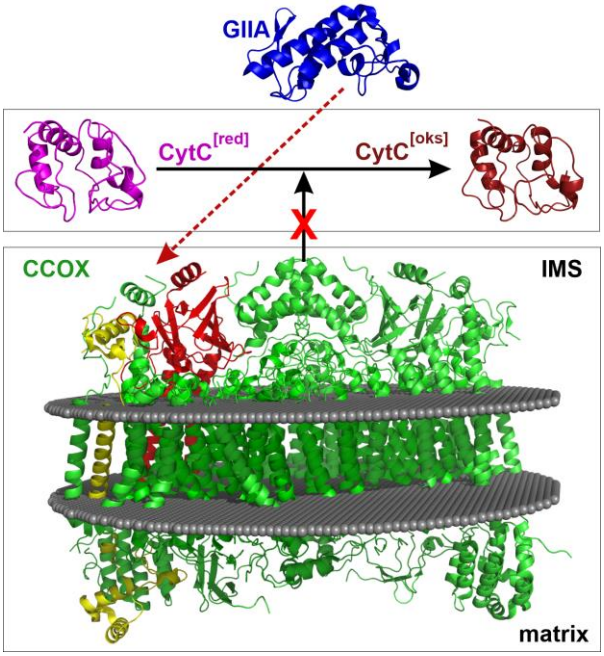


*Figure 1.* ***We proposed the regulation of ATP production as another physiological function of GIIA in mitochondria****. Snake venom or mammalian group IIA phospholipase $A_2$ (GIIA) enters the intermembrane space (IMS) of mitochondria and binds to cytochrome c oxidase (CCOX), the former to its subunit II (red), while the latter most likely to its subunit IV (yellow). By binding to CCOX, either GIIA inhibits enzymatic activity of CCOX—the oxidation of cytochrome c (CytC)—downregulating in this way the production of cellular energy (ATP) by the respiratory chain.*

In pathological conditions, for example in AD, the expression of GIIA is significantly increased and damage inflicted to neuronal mitochondria is very similar to that observed in the Atx-poisoned nerve endings. We have been trying to establish the link between the interaction of GIIA with CCOX and the degeneration of mitochondria, which is potentially very important for early diagnosis of AD and its subsequent treatment.

We prepared a set of recombinant human $sPLA_2$ molecules, GV and GX, as well as their enzymatically inactive mutants, GV(H48Q) and GX(H48Q). These molecules, together with rat and snake venom $sPLA_2$s that have been synthesized before, will be used in electrophysiological studies to probe their effects on nicotinic acetylcholine receptor (nAChR). Unfortunately, due to the Covid-19 pandemics, these experiments could not be performed as planned, in the scope of the bilateral project between Slovenia and Russian Federation (BI-RU/19-20-029). Instead, they will be accomplished by a new partner, the Laboratory of Toxicology and Pharmacology from the Catholic University of Leuven in Belgium. In collaboration with the group from the University of Padova, our recombinant AtxA has been used to study the mechanism of regeneration of AtxA-degenerated motor axon terminals.

We wrote the first draft of a review paper that focuses on the antiviral action of both endogenous mammalian and exogenous (animal venom) $sPLA_2$s.

In 2020, we continued an intensive study of the snake venom proteins that affect the blood coagulation process—haemostasis. We finally succeeded to obtain a 3-year research project J1-2475 from the Slovenian Research Agency (SRA) to boost our studies of a unique serine protease-like anticoagulant protein from the nose-horned viper venom, VaaSPH-1, with ambition to create novel safe anticoagulant drugs.

The results on VaaSP-VX, a serine protease with procoagulant, blood coagulation factor VIIa-like activity from the venom of the nose-horned viper (*Vipera a. ammodytes*, *Vaa*), were published in *Toxins* (Z. Latinović et al., *Toxins*, 12 (2020), 358). The very interesting discovery of the first procoagulant snake venom serine protease with dual, blood coagulation factor V- and X-activating activity was highlighted on the cover page (Figure 2).

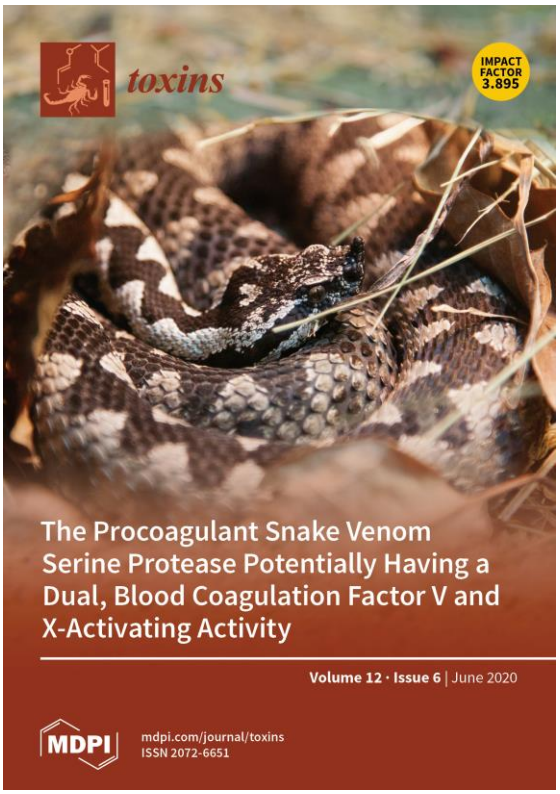


*Figure 2.* ***Cover page of the June issue of Toxins highlighted our paper inside****. Venoms of the Viperidae snakes are rich in proteins that strongly affect the haemostatic system. Our study describes isolation, purification and characterisation of an interesting procoagulant serine protease from the venom of the most venomous viper in Europe, the nose-horned viper (Vipera a. ammodytes). The blood coagulation-promoting effect of VaaSP-VX, as the glycoprotein was named, is most likely based on a simultaneous activation of blood coagulation factors V and X, precursors of the prothrombinase complex. A unique proteolytic specificity along with the resistance to serpin inhibition endow VaaSP-VX with a promising medical potential, for example, for treating patients with haemophilia.*

We performed an extensive characterization of the first member of a novel P-IIIe subclass of snake venom metalloproteinases, lacking the entire catalytic (metalloproteinase) domain, VaaMPIII-3. Our partners from the Institute of Molecular Genetics and Genetic Engineering in Belgrade, Serbia, provided a vector to produce it in bacterial cells. Beside from biochemical point of view, we analysed this unique venom protein also from the pathophysiological point of view by describing its effects on blood coagulation (K. Požek et al., in preparation). In addition, within the scope of our international project on the whole *Vaa* genome sequencing we obtained a contig of about 23 kb presumably harbouring the *VaaMPIII-3* gene. Its nucleotide sequence and overall structure, composed of 10 exons and 9 introns, are importantly different from those of the *Eoc89-like* gene encoding a similar but catalytically active MPIII metalloproteinase from the viperid snake *Echis ocellatus*. It has also been confirmed that lack of the metalloproteinase domain in VaaMPIII-3 is not a result of alternative mRNA splicing but rather due to the corresponding genomic sequence, with a deletion of both the catalytic and subsequent part of the disintegrin domain-coding region, being present already at the gene level.

Zorica Latinović, our doctoral student in this area of research, was awarded the Krka Special Commendation Prize for her PhD thesis entitled "Components of the nose-horned viper venom that affect cardiovascular system".

In collaboration with the colleagues from the University of Zagreb (UZ), we studied toxic activities of the venom of a very rare Croatian karst viper (*Vipera ursinii macrops, Vum*) and comprehensively described its proteomic profile. This snake is not medically important, however, its ecology is very special and it is threatened with extinction. Our data opened the way to unravel a unique insecticidal activity of the venom, potentially leading to new pesticides. Comparing pathological properties of the *Vum* venom with those of the *Vaa* venom, and the proteomes of both venoms, we indicated the existence of neurotoxins in viperid venoms structurally unrelated to $sPLA_2$s (M. Lang Balija et al., *Toxins*, 12 (2020), 187).

Together with researchers from the Veterinary Faculty of the University of Ljubljana (UL), we finalised the study of the first Kunitz-type proteins from viperid venoms that potentiate neuromuscular transmission, and published our results (S. Drofenik et al., *Toxicon*, 187 (2020), 262–270). In this work, we characterized Kunitz-type proteins in *Vaa* venom. These proteins, VaaChi, potently inhibit serine proteases, particularly chymotrypsin. Most interestingly, we found that they also facilitate neurotransmission in a manner similar to that of α-dendrotoxin. They also significantly increased the amplitude of the indirectly evoked simple muscle contraction of the mouse hemidiaphragm, and the amplitudes of the end-plate potential (EPPs) and miniature end-plate potential (MEPPs). VaaChi are thus Kunitz-type proteins with dual functionality, representing the first examples of Kunitz-type proteins from viperid venoms affecting neurotransmission. What is the mechanism behind the facilitation of neuromuscular transmission by VaaChi has not been established, however, blocking of $K^+$ channels, as in the case of α-dendrotoxin, does not seem to be the most probable option. For this work, the student Sabina Drofenik received the Student Prešeren Award at the UL.

In collaboration with colleagues from the Centre for Clinical Toxicology and Pharmacology, University Medical Centre Ljubljana (UMCL), we investigated an interesting clinical effect, a profound, transient and reversible thrombocytopenia of functional platelets in patients envenomed by the nose-horned viper venom. In thromboembolic diseases, such as myocardial infarction and ischemic stroke, platelets play a pivotal role. Currently used antiplatelet drugs have one common side effect—a decreased count of platelets with inhibited function. Such condition represents a high risk of life-threatening haemorrhage especially in interventional cardiology and angiology employing antithrombotic approach. Our findings may pave the way to the development of a new group of antiplatelet agents that will minimise the risk of life-threatening bleeding in antithrombotic approach in interventional cardiology and angiology, and increase the effectiveness of vessel dilatation and emboli aspiration. As we demonstrated, reversible thrombocytopenia in patients

poisoned by the *Vaa* venom is caused by snake C-type lectin-like proteins (snaclecs). To deepen these studies, we succeeded to obtain a 3-year research project J3-2534 from the SRA in 2020.

In the scope of a network including experts from UMCL, the University Hospital and the University of Split, the UZ and our group, we analysed samples collected from patients, envenomed by the nose-horned viper venom and treated with different antivenoms. We submitted a paper for publication in *Clinical Toxicology* suggesting new directives for efficient immunotherapy of the nose-horned viper envenomation (T. Kurtović et al., submitted).

In 2020 we also joined the COST Action "European Venom Network" (CA19144 - EUVEN), an excellent opportunity for establishing new international research collaborations.

**Lipid metabolism and signalling**

Our work in this area is focused on the identification of metabolic and signalling pathways that control lipid acquisition, trafficking and utilization in cancer cells. The resilience of cancer cells to stress depends on the availability of extracellular lipids and on their capacity to synthesize, mobilise or recycle their own intracellular lipids. By studying the ways in which cancer cells use lipids, we aim to reduce their remarkable ability to adapt to the inhospitable tumour microenvironment and thus reduce tumour growth, metastasis and resistance to therapy.

In 2020, our experimental work was focused on the final studies on the involvement of lipid droplets in the production of lipid mediators, such as prostaglandins and leukotrienes that are known promoters of inflammation and tumourigenesis (Figure 3).

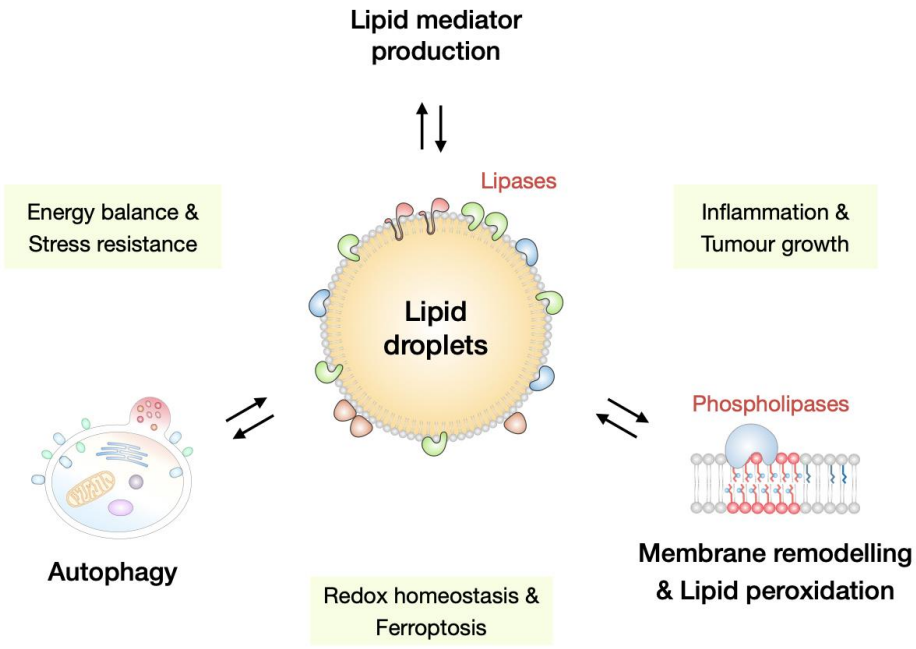


*Figure 3.* ***Lipid droplets are organelles that connect metabolic pathways with inflammation and the resistance of cancer cells to stress****. Lipid droplets are in the core of our research work. These organelles are not only fat-storage repositories but also active modulators of lipid peroxidation, autophagy and the production of inflammatory lipid mediators. By controlling these important aspects of cell biology, they are involved in the maintenance of the energy and redox balance as well as the regulation of inflammation in the tumour microenvironment that fosters tumour growth.*

We have found that lipid droplets transiently store and regulate the release of polyunsaturated fatty acids, which are required for the production of inflammatory lipid mediators. This work is important because it reveals that targeting lipid metabolism in cancer may represent a novel strategy for reducing inflammation and inflammation-related tumorigenesis. The validity and relevance of acquired preliminary results have been confirmed by awarding a post-doctoral research grant to Eva Jarc Jovičić (Z3-2650) by SRA in 2020.

We have continued our work on the SRA research project J7-1818 ("Targeting lipid droplets to reduce cancer cell resistance to stress"), where we are tackling the question of the cooperation between autophagy and lipid droplets in cancer cells. We opened a new promising research field by focusing some of our efforts to investigate the role of lipid droplets in the protection of cancer cells from ferroptosis, a lipid peroxidation-induced form of cell death. Our networking efforts in this field have gained an important impetus by joining the COST Action – "Pan-European Network in Lipidomics and EpiLipidomics" (CA19105 - EpiLipidNET). We were also awarded a grant for a comprehensive proteomic work within the INSTRUCT-ERIC research network hub (PID13338 – "Identification of lipid droplet-associated ferroptotic modulators in cancer"). Our recent work in the field of lipid droplets has been accepted very well by the scientific community as judged by the rapid increase of citation of our work and echo of invited lectures by Toni Petan at the 15$^{th}$ CFGBC Scientific Symposium and at the 1$^{st}$ COST EpiLipidNet meeting. Eva Jarc Jovičič received also the Krka Special Commendation Prize for her PhD thesis entitled "The role of lipid droplets in cancer cell stress resistance".

As part of a special volume of the journal *Reviews of Physiology, Biochemistry and Pharmacology* on "Organelles in Disease", we published an invited review paper entitled "Lipid droplets in cancer" (T. Petan, *Rev. Physiol. Biochem. Pharm.*, (2020), PMID 33074407). In this comprehensive review, we discuss emerging evidence showing that lipid droplets are important parts of cancer metabolic reprogramming. We explore how these fat-laden but highly dynamic organelles consolidate lipid uptake, synthesis, recycling, distribution and breakdown in order to match these entangled lipid fluxes with the requirements for cancer cell survival, growth and metastasis. We focus on the mechanisms that govern lipid droplet function during metabolic stress and reveal their connections with autophagy and ferroptotic cell death. Finally, we discuss how dysregulated lipid droplet turnover may be detrimental to cancer cells, thereby providing exciting therapeutic opportunities in the future. In 2020, also our second invited review paper on lipid droplets appeared, this one in the special issue of *Biochimie* (E. Jarc & T. Petan, *Biochimie*, 169 (2020), 69–87), in which we discussed the principal ways of regulation of the availability of fatty acids by these organelles for the production of lipid mediators and activation of inflammatory signalling pathways.

We contributed to a study led by our colleagues from the National Institute of Chemistry. In 2020 we published the paper in a prestigious journal (V.T. Ha et al., *Proc. Natl. Acad. Sci. USA*, 117 (2020), 25679–25689), in which we revealed a new mechanism of activation of inflammatory pathways by stress-induced extracellular vesicles containing oxidized lysophospholipids. The latter are produced by the synergistic activities of 15-lipoxygenase and GIIA $sPLA_2$, which are upregulated during inflammation and may be therapeutically targeted in inflammatory diseases such as rheumatoid arthritis. Our expertise in $sPLA_2$ enzymology, cell biology and production of recombinant proteins was an important contribution to this important work.

**High-throughput genetics and functional genomics in yeast *Saccharomyces cerevisiae***

Most of the biotechnologically important traits of microorganisms are polygenic, which is one of the reasons for the focus of our research on polygenic traits in yeast *S. cerevisiae*. We performed a comprehensive study demonstrating how backcrossing and high-throughput phenotyping can be used to identify quantitative trait loci (QTLs) for sodium chloride tolerance (Figure 4). We expanded our own method of iterative crossing of genetically diverse yeast strains to identify causative genetic elements for extremely high level of acidotolerance. We continued to

develop our CRISPR-Cas multiplex method, ensuring that we now possess all the necessary technologies to start developing multi-trait industrial yeast strains.

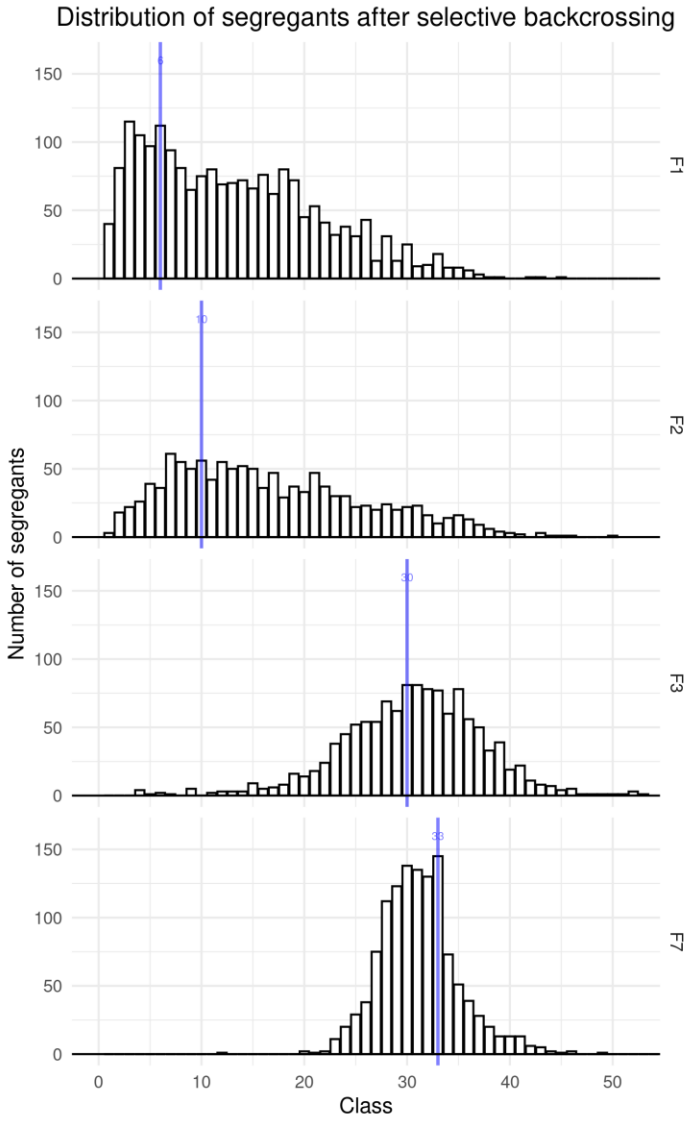


*Figure 4.* ***Identification of halotolerance quantitative trait loci (QTL)****. Two yeast strains with different halotolerance were crossed and sporulated. One thousand individual segregants after each round of backcrossing were assessed for their ability to grow in presence of NaCl in a high-throughput manner. In each generation, the winner was chosen and back-crossed with its parental strain. Distribution shows gradual improvement in mean halotolerance of each generation. Based on persistent genomic markers through generations, quantitative trait loci were predicted.*

We finished our work on a project on environmental condition- and variant-dependent binary physical interactions between selected yeast proteins, which was part of a large consortium's project on proteome-scale maps of binary protein interactions. We have been using the same basic method – yeast two-hybrid assay – to develop an approach that can discriminate between pathogenic and non-pathogenic variants of the human protein MLH1, thus offering an accurate and relatively quick method to clinical geneticists with Lynch syndrome patients.

**Evolutionary genomics**

The diversity and evolution of RNA viruses has been well studied in arthropods and especially in insects. However, the diversity of RNA viruses in the basal hexapods has not been analysed yet. To understand their diversity better, evolutionary histories and genome organizations, we searched for RNA viruses in transcriptome and genome databases of basal hexapods. We discovered ~40 novel RNA viruses, some of which are also present as endogenous viral elements derived from RNA viruses. We demonstrated that basal hexapods host 14 RNA viral clades that have been recently identified in invertebrates. The following RNA viral clades are associated with basal hexapods: Reo, Partiti-Picobirna, Toti-Chryso, Mono-Chu, Bunya-Arena, Orthomyxo, Qinvirus, Picorna-Calici, Hepe-Virga, Narna-Levi, Tombus-Noda, Luteo-Sobemo, Permutotetra and Flavi. We have found representatives of the 9 RNA viral clades that are present as endogenous genomic copies in the genomes of Machilis (Monocondylia) and Catajapyx (Diplura). Our study provided a first insight into the diversity of RNA viruses in basal hexapods and demonstrated that the basal hexapods possess quite high diversity of RNA viral clades (S. Ott Rutar and D. Kordiš, *Peer J.*, 8 (2020), e8336).

Honeybees play a crucial role in global food production as pollinators of numerous crops. Several stressors cause declines in populations of managed and wild bee species, such as habitat degradation, pesticide exposure and pathogens. Viruses act as key stressors and can infect a wide range of bee species. The majority of honeybee-infecting viruses are RNA viruses of the Picornavirales order. Although some ssDNA viruses are common in insects, such as densoviruses, they have not yet been found in honeybees. Densoviruses were, however discovered in bumblebees and ants. In this paper, we demonstrated that densoviruses are indeed present in the transcriptome of the eastern honeybee (*Apis cerana*) from southern China (S. Ott Rutar and D. Kordiš, *Acta Agric. Slov.,* 116 (2020), 383–393). Based on non-structural and structural transcripts, we inferred the genome structure of the *Apis* densovirus. Phylogenetic analysis has shown that this novel Apis densovirus belongs to the *Scindoambidensovirus* genus in the Densovirinae subfamily. Apis densovirus possesses ambisense genome organisation and encodes three non-structural proteins and a split VP (capsid) protein. The availability of complete Apis densovirus genome will enable the analysis of its potential pathogenic impact on honeybees. Our findings will certainly foster research of densoviruses in honeybees and bumblebees.

Satellite DNAs are major constituents of centromeric and pericentromeric regions in many eukaryotes and their role in centromere and kinetochore assembly and heterochromatin formation has been extensively investigated. However, the role of satellite repeats found dispersed in euchromatin, outside of centromere/pericentromere regions, remains largely unexplored. We analysed the dynamics of dispersion of human α-satellite repeats throughout euchromatin during the evolutionary history of primates and the mechanism of their proliferation (I. Feliciello et al., *Genome Biol. Evol.*, 12 (2020), 2125–2138). These results contribute to the understanding of evolutionary and functional significance of satellite DNA repeats spread throughout euchromatin.

In the scope of multi-institutional collaboration, we participated in the study of evolution of molecular resistance of vertebrates to snake venom α-neurotoxins (M.A. Khan et al., *Toxins*, 12 (2020), 638). These toxins bind to nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction, causing muscle paralysis that leads to suffocation. Several venomous snakes and their predators have however evolved resistance to such toxins. The resistance is due to a steric hindrance between glycosylated Asn at positions 187 or 189 in the nAChR ligand-binding domain and α-neurotoxins, by electrostatic repulsion or steric hindrance between positively charged α-neurotoxins and Arg187 of the nAChR. The inhibition of the α-neurotoxins binding to nAChR can also be due to structural changes of the receptor induced by Pro194 or Pro197 replacement. We analyzed the nAChR ligand-binding domain of 148 vertebrate species, and assessed their amino acid sequences for the resistance-associated mutations. We found a widespread convergent evolution of the *N*-glycosylation form of resistance in several taxa including venomous snakes and their lizard preys, but not in the snake-feeding birds. We also documented new lineages with the

Arg form of inhibition. Using an *in vivo* assay in 4 species, we provided further evidence that *N*-glycosylation mutations of nAChR reduce the toxicity of cobra venom. Our research shows that the evolution of α-neurotoxins in snakes may well have prompted arms races and mutations to this ancient receptor across a wide range of vertebrates (Figure 5).

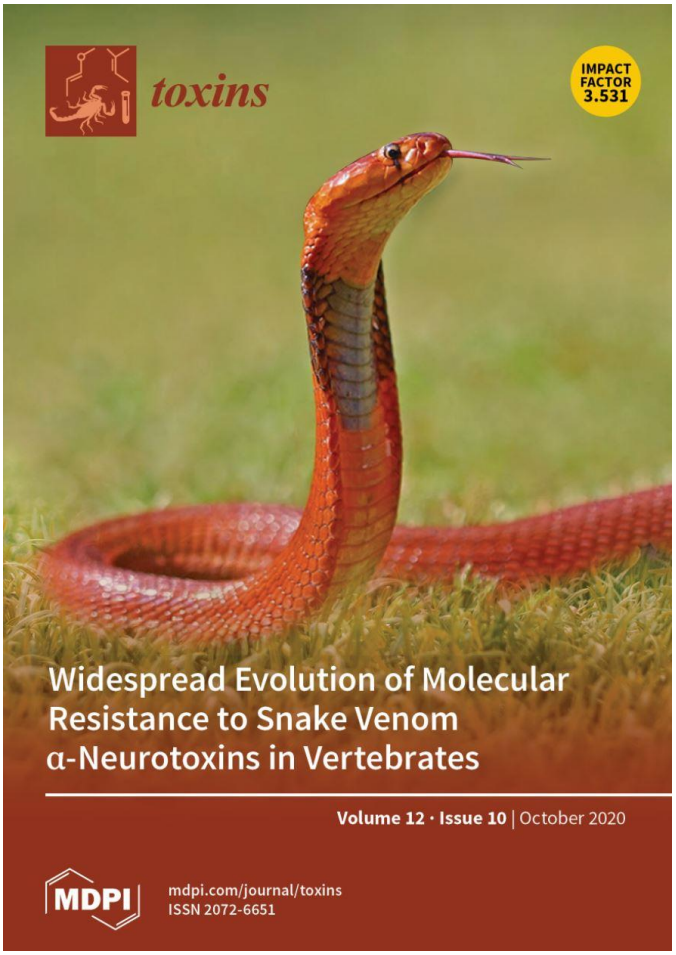


*Figure 5.* ***Another our work deserved highlighting on the cover page, this time of the October issue of Toxins****. In our paper inside, we showed that the resistance to snake venom α-neurotoxins occurred by impairing the binding of these toxins to the nicotinic acetylcholine receptor (nAChR) attained in the process of its convergent evolution. The resistance is due to mutation that results in the attachment of a branched glycan chain to a specific Asn in the ligand-binding region of nAChR. This N-glycosylation then, sterically or electrostatically, prevents binding of α-neurotoxins, large snake venom peptides, to nAChR whereas the binding of a small endogenous neurotransmitter ACh remains normal. We established that the convergent evolution of the resistance to α-neurotoxins by N-glycosylation of nAChR is widely spread in vertebrates.*

**Other subjects**

We also participated in several research projects out of the thematic scope of our department. Two such collaborations resulted in publications in 2020.

Participating with fluorescence microscopy analysis, we co-authored a paper in which we studied glioblastoma, a particularly common and very aggressive primary brain tumour (A. Zottel et al., *Ther. Adv. Med. Oncol.*, 12 (2020), 1758835920915302). One of the main causes of therapy failure is the presence of glioblastoma stem cells that are resistant to chemotherapy and radiotherapy, and that have the potential to form new tumours. Our study was focused on validation of eight novel antigens, TRIM28, nucleolin, vimentin, NAP1L1, TUFM, DPYSL2, CRMP1 and ALYREF, as putative glioblastoma targets, using nanobodies. Indicated for further examination, cells have been exposed to anti-vimentin, anti-NAP1L1, anti-TUFM or anti-DPYSL2 nanobodies. Therapeutically most interesting effects were demonstrated using anti-TUFM and anti-vimentin nanobodies. The former induced a potent inhibition of glioblastoma cell growth after long-term exposure, having only minor effects on astrocytes. The latter efficiently inhibited cell migration.

In the scope of the SRA applicative project L4-1839, led by the colleagues from the Biotechnical Faculty of the UL (BF/UL), we participated at preparing of a review paper on the most distinct

properties of chestnut honey important for its medical application (J. Božič et al., *Acta Biol. Slov.*, 62 (2020), 31–44).

As partners in the SRA project J7-7424, led by colleagues from the Faculty of Electrical Engineering at the UL, we participated with the analysis of nanoparticles' protein corona composition to explain their cytotoxicity and induction of cytokine secretion in THP-1 macrophages. At the end of 2020, the paper was submitted for publication in the prestigious journal *Small* (K. Strojan et al., submitted).

Also in the field of nanoparticles research, this time in collaboration with our partners from the Ruđer Bošković Institute in Zagreb, we participated in establishing the mechanism of formation and morphogenesis of *Arca noae* shell's nanoscale biomineral structures. We accomplished the mass-spectrometric identification of protein components of the shell, potentially involved in the process of biomineralization, i.e. initiation of the extracellular nucleation of aragonite nanocrystals. The paper was prepared and is currently under peer review in *J. Colloid Interface Sci.* (V. Čadež et al., submitted).

In 2020, we started the collaboration on three novel SRA research projects. As experts on proteomics, we are involved in the study J3-2521 of inflammatory process in interstitial cystitis and evaluation of the influence of cannabinoid receptor agonists in urinary bladder (leading institution: the Medical Faculty at the UL). As partners in the project J1-2482 (leading institution: BF/UL), we are participating at assessment of impact of environmentally relevant nano- and micro-plastics on soil invertebrates, while as partners in the project J1-2469 (leading institution: BF/UL), we are contributing at analysis of genomic and transcriptomic data to deepen insight into the exceptional biology of proteus (*Proteus anguinus*).

Following the covid-19 pandemic outbreak, we initiated research on SARS-CoV-2. The first aspect of these studies is genomic analysis and molecular evolution of SARS-CoV-2 and other coronaviruses, with the aims to characterize the evolutionarily constrained regions of the pathogen genome, which should be preferentially targeted to avoid rapid drug resistance mutants, and to identify viral genes interacting with those of its host. Stemming from the latter, we have been working on integrative genomics of SARS-CoV-2 in human cells, specifically by analysing binary protein-protein interactions of viral and human proteins. Based on the results of this research, physiological effects of selected SARS-CoV-2 proteins on cultured human cells will be investigated to validate the functional relevance of the interactions between viral proteins or their variants and their corresponding human protein targets.

**Most important publications in the past year**

1) Latinović, Z., Leonardi, A., Koh, C.Y., Kini, R.M., Trampuš Bakija, A., Pungerčar, J. and Križaj, I.: The procoagulant snake venom serine protease potentially having a dual, blood coagulation factor V and X-activating activity. Toxins, 12 (2020), 358

2) Lang Balija M., Leonardi, A., Brgles, M., Sviben, D., Kurtović, T., Halassy, B. and Križaj, I.: Biological activities and proteomic profile of the venom of *Vipera ursinii* ssp., a very rare karst viper from Croatia. Toxins, 12 (2020), 187

3) Ha, V.T., Lainšček, D., Gesslbauer, B., Jarc-Jovičić, E., Hyötyläinen, T., Ilc, N., Lakota, K., Tomšič, M., van de Loo, F.A.J., Bochkov, V., Petan, T., Jerala, R. and Manček-Keber, M.: Synergy between 15-lipoxygenase and secreted $PLA_2$ promotes inflammation by formation of TLR4 agonists from extracellular vesicles. Proc. Natl. Acad. Sci. USA, 117 (2020), 25679–25689

4) Ott Rutar, S. and Kordiš, D.: Analysis of the RNA virome of basal hexapods. Peer J., 8 (2020), e8336

5) Jarc, E. and Petan, T.: A twist of FATe: lipid droplets and inflammatory lipid mediators. Biochimie, 169 (2020), 69–87