



Dedicated to the memory of the late Prof. Dr. Franc Gubenšek

Following several years of illness Franc Gubenšek died on August 17th 2010.

Franc Gubenšek was born in 1937. In 1961 he graduated in physical chemistry at the Technical faculty, University of Ljubljana and was immediately appointed to the Department of Radiobiology in the then Nuclear Institute, today the Jožef Stefan Institute (JSI) in Ljubljana. He received his Ph.D. in 1965 in the field of physical chemistry at the Faculty of Natural Sciences and Technology, University of Ljubljana. Soon after the defence of his doctoral thesis he obtained a Fulbright fellowship and, in 1967, started his post-doctoral study at the University in Arizona, Tucson with John Rupley. There he encountered protein science, then a very promising and rapidly developing field, which attracted him at once and to which he became bound for the rest of his life.

Following his return from the USA, he started his life work on protein toxins in the group of Professor Drago Lebez at the JSI, the founder of toxinology research in Slovenia. When Lebez left the JSI in 1976, Franc Gubenšek – Franček to his colleagues and friends – took his position and assumed part of his toxicological problematics – he focused on the protein components of the venom of the most venomous European snake, the horn-nosed viper, *Vipera ammodytes ammodytes*. He was especially interested in proteins with phospholipase activity as one of the major pharmacologically active components of the venom, showing them to belong to the group of secreted phospholipases A₂ (sPLA₂s). Today, those that possess strong presynaptic neurotoxicity and anticoagulant activity are known as ammodytoxins and those that are myotoxicity or not toxic, ammodytins. At the beginning of the 1980s Franček was attracted by the structure of proteins. Initially, he was studying their secondary structure, using circu-

lar dichroism spectroscopy. Even more important was his role in introducing protein primary structure determination into Slovenia and, under his lead, the first protein primary structures were determined. Based on complementary methodological approaches, he directed his group in the early 1990s into four separate pathways of snake venom research.

First, by developing an immunological approach they mapped the structure of the so-called »toxic site« on the molecule of ammodytoxin. To accomplish this task they introduced the technology of preparing monoclonal and site-directed antibodies into Slovenia.

Secondly, they introduced molecular biology and protein engineering into toxinological studies. They determined cDNA sequences of all the ammodytoxins and ammodytins. The first method for preparing recombinant ammodytoxin in *E. coli* was then successfully developed, followed by highly effective bacterial production of these toxins. This formed the basis for the biosynthesis of a number of ammodytoxin mutants over the following years. Designed replacements of amino acid residues revealed the complexity of the »neurotoxic site« in the molecule of ammodytoxin. In this way, the areas of interaction of the ammodytoxin molecule with various receptors, and the role of particular amino acid residues in the molecule in binding to phospholipid membranes were determined, a step important in determining the specificity and enzyme activity of every sPLA₂.

A big challenge was, and still is, to understand the mechanism of blockade of neuromuscular communication by sPLA₂ toxins at the molecular level. So, thirdly, they started to look for the reason for the specific action of ammodytoxins on the presynaptic membrane of the motoneuron by introducing methods to study membrane proteins and by characterizing membrane receptors. The key to identification of the first binding proteins for ammodytoxin in the presynaptic membranes of bovine cerebral cortex was

the successful development of a method for preparing radioactively labelled ammodytoxin. The discovery and description of additional high-affinity membrane receptors for ammodytoxin in different nervous tissues followed. It was a big surprise when the first intracellular high-affinity binding protein for ammodytoxin was found to be calmodulin. However, soon after, further intracellular ammodytoxin-binding proteins were found – 14-3-3 proteins, like calmodulin also localized in the cytosol, and R25, a protein in the mitochondrial membrane. Doubt concerning the dogma of exclusively extracellular action of sPLA₂s was further strengthened with the first proofs about the entrance of ammodytoxin into nerve cells in culture and its biological activity in the cytosol of a eukaryotic cell. Following the demonstration of the possibility of sPLA₂ action in the cytosol, mitochondria and nucleus, various explanations for the numerous effects of these enzymes on mammalian cells have been advanced.

Fourthly, Frančec and his colleagues entered the exciting area of molecular evolution. They studied the evolution of toxins, DNA transposable elements and the structure of their genes. In this particular activity of his group it is especially important to emphasize the discovery of a novel mobile element of DNA, retrotransposon ART-2 or Bov-B LINE. They clearly demonstrated that this retrotransposon moved, more than 45 million years ago, from the genome of an evolutionarily old snake to the genome of an ancestor of today's ruminants. This first, clear experimental demonstration of the so-called horizontal transfer of genetic material between such distant classes of vertebrates stimulated significant interest in the scientific community and well merited publication in *Nature Genetics*.

One of Frančec's great qualities was that he trusted us, his younger colleagues, and allowed us to take initiatives. He never tried to enforce his opinion but he polished many of our ideas in constructive dialogue, with his extensive knowledge and ingenuity. One consequence of this characteristic is the existence today at the JSI of the only group of experts in Slovenia trained and equipped to perform high-throughput yeast genomics experiments, operating in the department that evolved from his research group. With this facility the picture of the mechanism of action of neurotoxic sPLA₂s has been refined and, in addition, the ability to study the mechanisms of action of drugs, and lipid-associated and lipid-mediated diseases in humans has been facilitated.

His group collaborated extensively with prominent research groups from all over the world. With his collaborators he published about 140 research papers in the best journals for the area of investigation. With them he acquired high international recognition for himself and his group in the field of toxinology. In acknowledgement of his important achievements in toxinology he was elected, from 1989 to 1996, President of the European Section of the International Society on Toxinology (EIST) and, from 1997 to 2000, President of the worldwide IST.

He organized several international scientific conferences and took part in many scientific boards of meetings in Slovenia and abroad. Three times, in 1977, 1989 and 2004, he and his team organized the EIST symposia. For many years he served as a member of the Editorial Boards of *Toxicon* and *Acta Chimica Slovenica*.

His work as a teacher, starting in 1974, was also very rich. In 1986 he became a Full Professor of biochemistry at the University of Ljubljana. He taught various areas of the subject at undergraduate and postgraduate levels in the Faculty of Che-

mistry and Chemical Technology (FCCT), Biotechnical Faculty and Medical Faculty. In 1997 he was fully engaged at the FCCT, in the Chair of Biochemistry which he then led until his retirement in 2004. His leading role in the creation and organization of the new, independent study of biochemistry at the FCCT has to be especially emphasized. Frančec was an experienced mentor and a great motivator of researchers. Under his leadership many students graduated and presented their master's and doctoral theses. Many of them hold important positions today in Slovenian and international institutions and in industry.

Scientific, pedagogical and group-leading activities did not prevent Frančec from dedicating his energy to organization and promotion of the development of biochemistry and molecular biology in the broader community. In this respect, he was a very active member of the Slovenian Biochemical Society from its beginning. From 1989 to 1998, he also headed the Society. From 1997 he was a representative of the Republic of Slovenia in the European Molecular Biology Conference (EMBC). The numerous important duties that he accepted, he always accomplished accurately and comprehensively. Besides his professional work he carried out a number of responsible organisational and executive functions at the JSI, the most important being a member of its Executive Board from 1992 and its President from 1997 to 2005.

His scientific achievements and status were recognized internationally by his membership of the European Molecular Biology Organization (EMBO) to which he was elected in 1998 as the first Slovenian scientist. In 1992 he was nominated as an Honorary Visiting Professor at the Guangxi Medical University in Nanning, China. In 1996 he was honoured by the Croatian Biochemical Society. Frančec also received several domestic awards, including the Zois Award in 1997. For his substantial contribution to the development and organization of biochemistry and molecular biology in Slovenia he was nominated in 2008 as an Honorary Member of the Slovenian Biochemical Society. In 2003, he was elected a member the Slovenian Academy of Sciences and Arts.

Sadly, progressive Alzheimer's disease prevented Frančec from continuing his work and collaboration with the research team that he created and headed until his retirement in 2004. His extensive legacy, however, facilitated the evolution of his research group into a strong and independent department, the Department of Molecular and Biomedical Sciences at the JSI.

We, his colleagues and friends, are grateful to him for all he gave us, as scientist and friend. For this reason, we will always keep him in cherished memory. We all remember Frank's boisterous laughter, the twinkle in his eyes during meetings among friends, and his sharp and witty jokes. These memories will linger on and act as an inspiration to younger scientists.

In this special issue of *Acta Chimica Slovenica*, dedicated to his memory, you will find fourteen articles written by some of the World's prominent toxinologists to whom he was very close.

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