



Dedicated to the memory of the late Prof. Dr. Igor Kregar (1937–2017)

Igor Kregar, Slovenian and an internationally renowned biochemist in the field of proteolysis and plant molecular biology, unexpectedly passed away on April 11, 2017 in Ljubljana at the age of 79. After almost 40 years of conducting excellent research, teaching and mentoring of undergraduate and graduate students at the Jožef Stefan Institute and Biotechnical faculty at Ljubljana University, he had retired in year 1997 to enjoy his family, friends, travel and some hobbies.

Igor was born on September 11, 1937 in Ljubljana, the son of architect Rado and mother Antonija. In his family reigned an intellectual atmosphere, which resulted in his broad interests for reading books, enjoying traveling, classical music, art and culture in general. Interestingly, as a boy he was highly motivated to read adventure novels set in the American Wild West written by German writer Karl May. Many of May's novels were translated into the Slovenian language, but unfortunately those editions were not accessible in our libraries after World War II for unknown reasons, most probably political. However, Igor did have access to the German editions, which stimulated him to learn the German language. At the same time Igor enjoyed competitive swimming at a local swimming pool. After completing high school with an excellent degree, he decided to study chemistry at the University of Ljubljana. At that time we first met. Soon we established a friendship

that included my classmate Franček Gubenšek. Igor was one of the best students in his class. He chose organic chemistry for his diploma at the Faculty of Chemistry at Ljubljana University. Then Igor joined the Department of Radiobiology at the Nuclear Institute Jožef Stefan (now Jožef Stefan Institute) by enrolling in a master's degree program and completed his PhD dissertation in December 1965 under the mentorship of Prof. Drago Lebez, working on the characterization of intracellular proteinases from the small intestinal mucosa of the rat. At that time intracellular proteases were poorly understood. The main enzyme studied was an acidic hemoglobin-splitting enzyme, called Anson's enzyme in laboratory jargon after the pioneering protein biochemist M. L. Anson. After the lysosome organelle was discovered by C. de Duve, it became clear that this acid protease is a lysosomal enzyme, and in 1960 it was characterized and named cathepsin D. Igor and the author started to collaborate primarily on cathepsin D and also on cathepsin E soon after its discovery. This was at that time an almost unexplored field of research and we were one of the pioneering groups to work in this field.

In 1968 Igor received a Fulbright scholarship to study in Professor John A. Rupley's laboratory in the Department of Chemistry at the University of Arizona, Tucson, USA. Coming from Yugoslavia at that time was for Igor (and later for the author) a completely new world. The Rupley laboratory was modern and very well equipped with adequate funding to buy chemicals. Igor studied the pH dependence of lysozyme-catalyzed hydrolysis of the N-acetylglucosamine hexasaccharide. These kinetic experiments were time consuming and required precise measurements, but later resulted in a high quality publication (1973). Rupley was an excellent biochemist with a great and warm but serious personality. Not to forget, Rupley and his lovely wife, Ila, organized nice parties in their

home, thus keeping and promoting friendly relations in his international laboratory. I can say as one of Rupley's postdocs that those working with him were very fortunate and learned to enjoy hard work and at the same time to be happy. Igor established many friendships in the USA, especially with Rupley's graduate student, Karl Kramer and his wife Virginia, which lasted until Igor's death. In 1968 Igor joined the Kramers on a unique train trip to Mexico where they visited Guadalajara, Mexico City and Taxco. On that trip the train passed through many small Mexican communities, some with bullet-riddled buildings, which were perhaps a reflection of past political conflicts. Igor learned to love Mexican cuisine in part through his many visits to the Kramer's apartment, where Virginia made mouth-watering dishes such as enchiladas, refried beans, flautas, green chiles and spicy meats. Upon his return to Yugoslavia, these experiences motivated Igor to learn how to replicate Mexican meals and to share them with his family and good friends. There was a problem, however, in that many of the ingredients required for Mexican cooking were not readily available in Yugoslavia in the 1970s. Nevertheless, the Kramers were able to supply Igor with Mexican food items such as masa harina, a special red chile powder and various types of chiles in care packages until he was later able to obtain them locally.

Returning to our Department at the Institute after his Fulbright-sponsored study in the USA, Igor continued his research on cathepsins. Development of the first purification of cathepsin D by affinity chromatography allowed him to obtain large quantities of the pure enzyme for numerous biochemical studies including structural and denaturation studies. Almost at the same time an acid sulphhydryl protease was discovered (1973) and later named cathepsin S. This discovery was not immediately recognized and many believed that cathepsin S and L were identical enzymes. Ultimately, this problem was solved by isolating both proteins from the same species and determining their amino acid sequences. Cathepsin S is involved in antigen processing and presentation, degradation of the extracellular matrix, tumor progression, cardiovascular disease and obesity among other roles. A new method for cathepsin B purification from bovine lymph nodes yielded sufficient enzyme for biochemical characterization. All advances in the field resulted in the Second International Symposium on Intracellular Protein Catabolism organized in Ljubljana in 1975. A brief document was prepared by Alan Barrett, Fred Woessner, Igor Kregar and Vito Turk, which highlighted all known 23 intracellular proteases. This modest beginning resulted finally with the establishment of a new classification system and the MEROPS database, which now includes well over 1000 peptidases (Handbook of Proteolytic Enzymes, N.D. Rawlings and G. Salvesen, Preface, Acad. Press, 2013). Igor then extended his research on proteases to those present in the ergot fungus *Claviceps purpurea* and bacterium *Streptomyces rimosus* as well as proteases and their inhibitors from plants

such as the potato. Igor and his coworkers discovered the potato cysteine proteinase inhibitor gene family and characterized some of the expressed recombinant proteins.

Igor was a highly respected scientist and personality. His research resulted in approximately 80 original research papers and reviews published primarily in international journals. He attended many domestic and international symposia and congresses as an invited lecturer. In parallel to his contributions to science, he was an excellent teacher and popular among many students. Most of his students who obtained their PhD degree under his guidance went on to become successful researchers and professors at different universities and institutes. Igor served on various committees at the Institute. He was also the first Secretary General of the Slovenian Biochemical Society (1972–1987) and then Secretary General of the Union of Biochemical Societies of Yugoslavia (1987–1992) until the collapse of Yugoslavia. In 1987 the 18th meeting of the Federation of European Biochemical Societies (FEBS) was held for the first time in Ljubljana, which was a great success from the organizational and scientific points of view. Igor contributed greatly to this success, serving as secretary of this prestigious meeting with more than 1800 scientists attending from all over the world. For his achievements in science, Igor received several distinguished recognitions including the Yugoslav Order of Labour with Silver Wreath Award (1978), Slovenian Boris Kidrič Fund Award (1979) and Lapanje Plaque from the Slovenian Biochemical Society (2009).

Igor's remarkable wife, Anka, died several months after he did. He is survived by two sons, Zlato and Robert, three grandchildren and their families. For us and for those who knew him, Igor always will be remembered. His scientific legacy will live on.

Vito Turk
Jožef Stefan Institute