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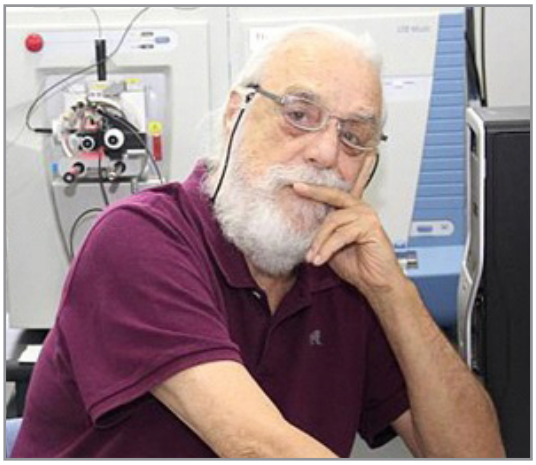
INTERVIEW



Back to the Future with Proteins and Proteomics

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Gilberto B. Domont has a BSc in Chemistry from the University of Brazil and a Ph.D. in Biochemistry from the Universidade Federal do Rio de Janeiro. He did graduate studies at the University of Southern California and Ohio State University and had internships at Technicon Corporation, Applied Biosystems, Institut Pasteur, Lyon, and the W.M. Keck Foundation Biotechnology Research Laboratory, Department of Biochemistry, Yale University. He holds the title of Professor Emeritus of UFRJ. As a member of the Brazilian Academy of Sciences, Dr. Domont is a founding father and ex-President of the Brazilian Society of Biochemistry and Molecular Biology and the Brazilian Society on Toxinology. He was Associate Editor of the Journal of Proteome Research and is currently on the Editorial Board of the Journal of Proteomics. Dr. Domont founded the Brazilian Proteomics Society. He is PI of the Chromosome 15 – Centric Human Proteome Project – Biology/ Diseases and a member of its Executive Committee. Heads the Proteomics Unit of the Institute of Chemistry, UFRJ. Is associated with the European Cancer Moonshot Project at the Dept. of Biomedical Engineering, Div. Clinical Protein Science & Imaging at the Biomedical Center, Lund University, dedicated to the study of the human proteome in cancer. His research interests are also centered on human viral infections, as well as plant, venom, and microorganism proteomics. He is devoted to the application of proteomics techniques to biological systems. His two favorite biological systems are Sonia and Solange, his daughter and wife.

How was your scientific initiation?

In 1956, I was a junior undergraduate enrolled in the Chemistry program at Faculdade Nacional de Filosofia, Universidade do Brasil. Of course, as often happens with so many, I was hooked by Dr. João C. Perrone's lectures on Biochemistry. In the middle of the year, he invited me to join his Protein Chemistry Laboratory at the Instituto Nacional de Tecnologia, granted with a Scientific Initiation scholarship from CNPq. There I lived for 13 years.

Which were your scientific influences to become a scientist? Did you have some teacher that gave you the necessary input to your scientific career?

The scientific atmosphere in Perrone's lab was creative, enthusiastic, critical, and highly scientific. Very limited money was available for research, so one had to have strong commitments to pursue basic science

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and confront difficulties. Perrone's expertise helped us to survive building electrophoresis apparatuses and a gas chromatograph, potentiometer, colorimeter, fraction collector, and amino acid analyzer. Dr. Abrahão Iachan, an industrial chemist, a chemical engineer, and a staff lab senior researcher, contributed with the synthesis of enzyme substrates and protein chemistry reagents. From both of them I learned how to do science and chemistry as well as how to practice their most cherished ethical values.

Why did you decide to be a chemist? How Chemistry motivated you to become a proteomist?

At the age of 15, I was already doing simple chemistry experiments. I liked to use *picpoc* magic to scare my friends. *Picpoc* was made of two kinds of powders: all you had to do was drop a small amount of each powder, one on the thumb and the other on the indicator, and then pop both fingers to hear the sound of an explosion. Fantastic! Of course, as a teenager scientist, I wanted to increase the noise of the explosion to scare my friends even more. The obvious thing to do was to mix both powders; in my brain, having bigger amounts on each finger would provoke a louder explosion! However, what I did not figure out was that when mixing the contents of the two *picpoc* boxes that I had poured over a newspaper page the same explosion would result in friction during mixing. And so, it happened! The consequences were a burned face, eyes, and hair, two months of blindness, and eye surgery. We know a scientist never gives up. I had to understand what had gone on.

"By memory, I recall Fred and Bill chatting on Fenn's MS electron spray experiments. The discussion was about the consequences of Fenn's electrospray technique to scientific research. No one had any idea where it would lead, but everyone was sure that it would revolutionize research in the health sciences."

I was trained and did classical protein chemistry until 1989/1990 when I spent the winter months working at the Yale School of Medicine with Kathy Stone and Kenneth Williams, founder of the W.M. Keck Foundation Biotechnology Research Laboratory, Department of Biochemistry, now The Keck Biotechnology Resource Laboratory. I was there to work on modern protein sequencing techniques. In the department, Fred Richards and William Konigsberg, as well as John Fenn at the Dept of Chemical Engineering, Yale University, were also there, who would drop by sometimes. By memory, I recall Fred and Bill chatting in the large school corridor on Fenn's mass spectrometry electron spray experiments. The discussion was about the consequences of Fenn's electrospray technique to scientific research. No one had any idea where it would lead or what could happen with the science that was done at the time, but everyone was sure that it would revolutionize research in the health sciences. I found the electrospray technique to be my calling. Years after, dinning a hot soup in the relaxing hotel restaurant after the promenade of the II ESPRIT and I EuPA Congress, Valencia, Spain, 2007, I told John how I was hooked by his ESI at Yale.

Did you realize in the early stages of your career that you would be involved with protein science the way you currently are?

Absolutely. As soon as I began working with proteins, I was trapped. Proteins became my partner; love at first sight. No divorce on sight.

What are the recent analytical contributions you consider fundamental for the success of proteomics abroad? Can you comment on your contributions to this field?

It is a long list. Mass spectrometers, analytical techniques, robots, and MS applications are booming. A few examples chosen at random are the following:

- In analytical instruments, contributions were made in robotics and mass spectrometers (MSs), especially for sample preparation and to increase speed, resolution, and sensitivity. Hybrid instruments play an advanced role, especially those that use different analyzers and in ion storage separation devices, giving back a higher resolution and mass accuracy, as well as increased identification of the number of peptides and, hence, proteins. Ion mobility (IMS) has added value for the ion separation capability of MS, especially when coupled to time-of-flight analyzers. TIMS (trapped IMS), PASEF (parallel-accumulation

serial fragmentation), FAIMS (field asymmetric IMS), and SLIM (structures for lossless ion manipulations) have provided other IMS methods for the fractionation of complex mixtures of ions, increasing the number of mass spectra of peptides and more protein identifications. Improvements were seen in the characterization of intact proteins and complexes to verify their structures, proteoforms, and modifications. Two methods to break the protein amide bond which is more robust than the peptide, are electron transfer dissociation (ETD) and ultraviolet photodissociation (UVPD) and they have been used for more efficient fragmentations that can also be achieved using surface-induced dissociation in which a greater amount of kinetic energy of the collision is directed to the ion complex.

- In analytical methodology, immunoprecipitation has powered great advances in the measurements of protein interactions by pull-down, as in the spatial proteomics approach. Techniques for the identification of more proteins by match-between runs or protein quantitation such as parallel reaction monitoring (PRM) or data-independent analysis (DIA) have combined shotgun and targeted proteomics. Other areas of intense research and method development are labeled techniques for protein quantitation, post-translational modifications, especially glyco- and phospho-peptides/proteins, glycomics, and lipidomics, as well as new software for protein searches and statistics. Clinical proteomics is another fast-developing field for testing metabolites, peptides, and proteins for diagnostics, prognostics, and therapeutics.

A scientist has different ways of contributing to society. In my early years in Perrone's lab doing classical protein chemistry, my contributions were centered on the development of automatic analytical methodologies, protein purity and purification, primary structure determination, toxin isolation and sequencing, and plant proteins for feeding. In 1962, jointly with Perrone and Panek, I founded the first graduate program in biological sciences in Brazil, named the Graduate Program in Biochemistry at the Institute of Chemistry, UFRJ. In the program, my protein lab was the embryo for the Proteomics Unit of the last almost 20 years During the proteomics era, the contribution of the group ranged from bioinformatics tools and method development to contributions in understanding the physiological molecular mechanisms of cancer, neurodegenerative diseases, and virus infection diseases. I have also dedicated much of my time to spread the proteomics gospel in talks, conferences, seminars, and international and national courses. As a founding member of SBBqBM, I knew the importance of disseminating proteomics to the Brazilian scientific community, and in many of its annual Congresses I had the chance, with the help of my colleagues, to bring the best possible foreign proteomics scientists and research subjects to a gathering of undergraduates and graduate students, post-docs, and junior and senior scientists.

You are one of the pioneers of proteomics in Latin America. Could you point out the difficulties you faced to increase this area in Brazil and about the creation of the Brazilian Society of Proteomics (BrProt)?

The first big drawback, as always happens in science, is money for research. The second is common to all groups: purchasing reagents and small replacements pieces, maintenance of equipment, administration problems and personnel, accounting, scholarships, grant time validation, etc. Proteomics is expensive, is big money research. MS high-accuracy, high-resolution and high-sensitivity instruments worth US 1million make impossible to renew science labs and parks around the country. Depending on the instrument capability, some problems cannot be even approached. Worse, the consequences are dramatic in terms of data collection and physiological analysis because we cannot do a deeper analysis of any biological sample. In other words, we cannot obtain all the data that more sophisticated instruments provide. The proteomics potential of rare samples will not be explored, and the non-collected data are lost forever together with important discoveries that could have been made. Finally, I want to leave words of hope. As scientists, we deal with the real world and must be realistic in life as well. I am pretty much optimistic when evaluating the course of science in Brazil. My many years of experience mentoring students and dealing with Brazilian science assure me that we have the best people studying, who are highly committed to doing science. By providing more scholarships, research money, and the freedom to pursue ideas, the science

done in Brazil will quickly expand in quality and number!

I did not find it difficulty at all to set up the Brazilian Proteomics Society because the scientific proteomics community fully responded immediately to the call. In a meeting that lasted three days we were able to show great science, discuss relevant themes, create by-laws, and vote and inaugurate elected officials. This is the Brazilian Proteomics Society at it best, our BrProt, and these are the commitments of the members.

Which are your current interests in protein research? You have published interesting scientific papers during your career in both proteomics and in toxinology (and other biochemistry subjects). Could you comment on which ones you consider the most significant papers? Why?

"The most important paper in my career was my first, which dealt with the Electrophoretic Heterogeneity of Trypsin [Perrone JC, Disitzer LV, and Domont GB., Nature 183: 605 (1959)]."

I am now dedicated to advance the Human Proteome Project (HPP) and to study neurodegenerative diseases, cancer, and COVID-19. I am a founding father of the HPP, that involves two projects: the Chromosome-Centric Human Proteome Project (C-HPP) to demonstrate the existence of at least one protein per one of the 19.773 human genes, as accepted by HUPO, and the Biology Disease Human Proteome Project (B/D-HPP), which provides a framework for the study of biology and diseases. Scientifically, our group is responsible to giving life to chromosome 15 proteins, that is for finding its missing proteins, those that have never been identified by mass spectrometry. Administratively, I am a member of the C-HPP Executive Committee and attend Council Meetings.

The most important paper in my career was my first, which dealt with the ***Electrophoretic Heterogeneity of Trypsin [Perrone JC, Disitzer LV, and Domont GB., Nature 183: 605 (1959)]***. Crystallization was considered a criterion of protein purity, and in this work we demonstrated that the widely known and used crystallized trypsin was not a pure enzyme. It is unforgettable that at 25 years old I was the senior author of a Nature manuscript, which was followed by my third one five years later ***(Iachan, A, et al., Fractionations of trypsin by paper electrophoresis. Nature 203: 43, 1964)***.

I should mention that another important work was not a published paper but a printed abstract ***(Silva, MH, et al., Studies on the amino acid sequence of crotamin, in IX International Congress of Biochemistry, Stockholm, in the Abstract Book, IUB, 1973, v.IX)***. We had worked out more than 95% of the amino acid sequence of crotamin, a neurotoxin isolated from *Crotalus durissus terrificus* venom, the first protein to be ***almost*** totally sequenced in Brazil. This work was a fantastic research experience because of the intellectual loneliness I lived in. I was alone; no one did protein sequencing at the time in Brazil, and I had no one to talk with and no one to exchange experiences with in terms of protein sequence techniques and rationales. My interlocutors were the journals; I talked to them every Wednesday at the National Institute of Technology Library, where I perused the recently arrived journal issues. I still remember inserting a hidden tiny pencil dot on the upper left corner to mark the ones I had read. We lost the publication race despite having chosen a niche; a small protein known only by Brazilians.

Natural inhibitors of snake venoms have always been a traditional field of research. Two groups pioneered these studies, setting the standards for understanding the mechanism of action of these inhibitors: Drs Haity Moussatché, Jonas Perales, Ana GC Neves-Ferreira, and Richard H Valente in the Laboratory of Toxinology, Fiocruz, RJ, and us in the Laboratory of Protein Chemistry, UFRJ. Some of these publications were summarized in three book chapters ***[Perales, J, et al., Are inhibitors of metalloproteinases, phospholipases A2 and myotoxins members of the innate immune system? In Andrè Menèz (Org) Perspectives on Toxinology, Wiley & Sons, Ltd, 2001; Neves-Ferreira AGC, et al., Natural Inhibitors: innate immunity to snake venoms. In Stephen P Mackessy. (Org.). Handbook of Venoms and Toxins of Reptiles, CRC Press, 2009; Neves-Ferreira, AGC, et al., Natural Inhibitors of Snake Venom Metallopeptidases, Springer Science, 2015]***.

Interesting results on the molecular mechanisms of neurodegenerative diseases were disclosed in recent years, such as Alzheimer's ***(Mendonça, CF, et al., Proteomic signatures of brain regions affected by tau pathology in early and late stages of Alzheimer's disease Neurobio. Dis. 130: 104509, 2019)*** and schizophrenia ***(Velásquez, E, et al., Synaptosomal proteome of the orbitofrontal cortex from schizophrenia patients using quantitative label-free and iTRAQ-based shotgun proteomics. J. Proteome Res. 16: 4481 2017*** **and** ***Velásquez, E, et al., Quantitative subcellular proteomics of the orbitofrontal cortex of schizophrenia patients. J. Proteome Res. 18: 4240, 2019)***. Through a memorandum of understanding with the University of Lund, the Proteomics Unit became part of the European Branch of the Cancer Moonshot Program, US. The first published manuscript was in melanoma ***[Sanchez, A, et al., Novel functional proteins coded by the human genome discovered in metastases of melanoma patients. Cell Biol Toxicol. 36:261–272 (2020)]***, an upgrade to reduce the number of missing proteins in the HPP.

A recently published multi-omic approach used by five groups to study congenital Zika syndrome ***(Aguiar, RS, et al., Molecular alterations in the extracellular matrix in the brain of newborns with congenital Zika syndrome, Sci. Signal. 13, eaay6736, 2020)*** reports proteomic data from postmortem brain samples of microcephalic stillborn, disclosing the depletion of collagen molecules and the molecular basis of ZIKV infection after vertical transmission.

Of course, we are beginning to work on proteomics and metabolomics of plasma of SARS-CoV-2 human COVID-19-infected subjects.

You are an active member of the Human Proteome Organization (HUPO), and you are very involved with some programs of this institution. Because of this, you know quite well the Brazilian community of chemists and biochemists involved with protein chemistry. Thus, how do you compare the level of qualification of Brazilian proteomists when compared to those from Europe and the USA?

Our students and researchers are the best; they are very well trained in proteomics techniques, including mass spectrometry. I have witnessed praises for the excellent scientific training of our MScs, PhDs, and post-docs who went to work abroad for short terms of for good as well as compliments from many top leading proteomics scientists. Our students and junior scientists have gained international respect and won national and international prizes and highly disputed international scholarships. Frequently, I am asked to recommend graduate students and post-docs for scholarships or staff positions in leading labs in the USA and Europe.

How do you compare the proteomics developed in Brazil with that developed in the USA, Europe, and Asia?

The difference resides in the research topics, which are more frontier-like outside the country, where modern techniques and instruments are used to deeper evaluate the molecular explanations of physiological phenomena as well as scientific structure and policy. This is the result of the social and governmental acceptance of the importance of science for health and social welfare. Brazilian officials and agencies continue to use decades-old science funding policies. The dichotomy of separately funding scholarships and underfunding research projects is untenable anymore. Projects should be the funding unit of grants. The budget of these grants must include all expenses needed to achieve the proposed aims, such as travel, other direct costs like lab supplies and services, equipment, scholarships, administration, etc.

"My entire scientific life was dedicated to having fun doing research with my students, implementing and developing protein chemistry and proteomics, science, teaching, mentoring, and helping students to begin and develop their national and international careers."

You paved the way for many young Brazilian proteomists to launch their careers. Could you comment about your relationship with young scientists?

This is a very kind question. My entire scientific life was dedicated to having fun doing research with my students, implementing and developing protein chemistry and proteomics, science, teaching, mentoring, and helping students to begin and develop their national and international careers. I am very much attached to the intellectual growth of the students. It is an extraordinary and gratifying experience to watch their dedication and to follow their struggle to accomplish and finish their work and become mature scientists. These are highlighted, for instance, in the creation of the Rio de Janeiro Proteomics Network in 2002, whose main operational ideas were to introduce and implement proteomics techniques as well as to congregate protein chemistry students and researchers around the new field.

I always keep my eyes level with my young colleagues; I consider undergraduate or graduate students my professional colleagues. All of us are on the same page, always. The friends I deal with daily in the Proteomics Unit, Department, and Institute are mostly under 40 years of age. It is a relationship based on respect, merit, acceptance of differences, and intellectual leadership. Strictly speaking, everyone is equally treated as a scientist; all are scientific fellows. My students teach and give me too much; I learn a lot from them and owe them a lot, including the privilege of intense collaborations, discussions, warm debates, and the exchange of ideas. Socially, we hang out for beers, barbecues, beaches, social gatherings, and, of course, for science, philosophy of science, and theory of knowledge discussions.

Please, comment on the relationship between BrProt and BrMass.

Initially, in a council meeting with Marcos Eberlin, BrMass leader, I proposed that we should combine the two societies, transforming them into a Mass Spectrometry and Proteomics Society. The idea was not accepted. Years later, in 2018, we met and agreed on organizing a joint meeting – the 4th BrProt / 7th BrMass Annual Congress. Because of the excellent experience and fantastic results, we joined ideals again to organize inside the IMSC2020, a new joint congress adventure.

The relationship between proteomists and mass spectrometrists is excellent because we all speak the same language and have a common basis, mutual human and scientific respect, and friendship. Ideas are freely discussed and adopted or rejected; lectures and symposia are suggested and accepted; and invited speakers are freely chosen. I hope this partnership will improve and will continue for a long time.

"I love science; I love my work; and I enjoy and love my colleagues and students, past and present. I am happy doing science; I cannot stop doing it. It is like breathing. It is a passion I cannot abandon and let expire."

You retired in 1995 and are probably more active today than at the time of your retirement; what is the secret to keeping yourself so motivated?

Absolutely, right! The secret is love. I love science; I love my work; and I enjoy and love my colleagues and students, past and present. I am happy doing science; I cannot stop doing it. It is like breathing. It is a passion I cannot abandon and let expire. Mentally, I never retired. It is easy to understand because I owe everything I have and have done in my life to the Universidade Federal do Rio de Janeiro, UFRJ, including my accomplishments, career, friends, happiness, kindness, patrimony, travels, intellectual activities, etc. During Cardoso's second presidential term, a social security reform was proposed and approved. Then, before its approval, I decided to close my official link as a public servant and changed my employment status. My colleagues proposed, and the Federal University of Rio de Janeiro was very kind in granting me, the title of Emeritus Professor, which, supposedly entitles me to stay and have access to labs and students. I have no plans to quit doing science and the only reason I can imagine doing so is if I was stealing someone else's position at UFRJ.

Could you comment on your impressions about the current crisis (COVID-19 pandemic, economic, political) in Brazilian science? What is your view for the future of Brazilian science? Is there also a window of opportunities created by the new challenges?

All themes – the COVID-19 pandemic chaos and death toll, the economic crisis, and political affairs – have a common explanation, at least for the debacle of the last two years: we are not a scientifically educated society. This, but not only this, explains chloroquine, vermifuges, and others. There is no better demonstration of the absence of reason, criticism, intelligence, or moral behavior than to have an army general such as the Ministry of Health replacing a physician, a Minister of Environment that praises the destruction of forests and rivers, or to have nominated the former incredible Minister of Education. This explains the leader. His ideas and acts tell us the whole story.

The demarcation criterium between science/knowledge (ἐπιστήμη *episteme*) and opinion (*δόξα, doxa*) is verification. To be scientific, it needs to be verified. If it is impossible to verify, then it is just an opinion. Never in my entire life have I witnessed a strange rational moment like this. Before, against facts, there were no arguments; now, against arguments, there are no facts. Shortly, this explains the COVID-19 chaos in the country, the sinking of the economy, and the political pandemonium in a pandemic.

Brazilian science is extraordinary and responded immediately to the Zika epidemics. The same is happening with COVID-19. Some examples are the improvement in health treatment adopted worldwide (heparin), the production of SARS-CoV-2 spike protein for serological tests, new methods for virus detection, and the development of vaccines. One actual issue in Brazil is the slow response of the government to problems. Scientists respond immediately, whereas the government response is pachydermic.

Brazilian science is at a crossroads. A plan for the sciences in the upcoming years is not on the agenda. We need a modern, technological science park. Challenges provide thousands of opportunities. Research opportunities in the application of mass spectrometry to problems in the health and environmental sciences are numerous and diverse, including viral diseases, mental health, environmental quality, immunization, health care, obesity, cardiovascular diseases, and so on. These challenges provide more and better positions for young scientists. We may face less funding for science, scholarships, and positions. These are the prospects for an economy that predates and lives in contingency with little or no money for science. The reaction of the scientific community to these processes is intense. The Brazilian Academy of Sciences, the Sociedade Brasileira para o Progresso da Ciência, other scientific societies, and individual scientists are fighting against and strongly objecting to the disassembly of Brazilian science and the discredit of leading scientists. History teaches us that science and scientists win the race.

Do you want to leave some scientific legacy? What is it?

I want to be acknowledged as a scientist who lived a professional life praising science and scientists with whom I exchanged ideas and ideals and discussed and shared scientific data and ethical values. Those were fantastic moments of joy. I was lucky to spend an entire life of happiness dedicated to science and scientists. It is worth it. I do recommend love and enthusiasm for science and stimuli to and from the students.

"To close, I recommend reflections on Bertold Brecht's words in his play Galileo Galilei: ***'I maintain that the only purpose of science is to ease the hardship of human existence'****. I fully and deeply agree!!!"*

Front row: Hugo Junqueira (Magno´s older son), Renata dos Santos (selphie). From left to right: Magno Junqueira, Solange Guimarães, Gilberto Domont, Rafael Melani, Gustavo Monnerat, Raquel de Farias, Isis Botelho, Vinicius Parracho, Domingos Melo, Mohab Andrade, Fabio Nogueira, Natalia Almeida. Center: Erika Velasquez, Yara Silva, Ana Jacob.
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