

Profile

Life under Extreme Conditions: *Aeropyrum pernix* and Pernisine

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1. BIOGRAPHY



Nataša Poklar Ulrih is a full Professor of Biochemistry and Vice Dean for Scientific Research in the Biotechnical Faculty, University of Ljubljana.

In 2010, Nataša Poklar Ulrih became a full Professor of Biochemistry. Her research work is mostly dedicated to the interactions of proteins and small molecules with biopolymers. Over the last 10 years, the focus of her research has been on the secondary metabolite phytochemicals in plants. Her group is trying to understand how these small molecules interact with membranes, proteins and polysaccharides at the molecular level, and to understand how

these interactions influence the structural and functional relationship in complex matrices, such as foods.

In Autumn 2000, Ulrih moved from one part of the University of Ljubljana to another; from the Faculty of Chemistry and Chemical Technology, to the Biotechnical Faculty. In doing so, she moved from a Teaching Assistant position to Assistant Professor. She then became impressed by the extremophiles (organisms that thrive in extreme environments), and their molecular adaptations to their different environmental conditions. Ulrih and her group started to work on *Aeropyrum pernix*, a hyperthermophilic Archaeon that shows optimal growth at 92°C, pH 7.0, with a salinity of 3.5% and in the presence of oxygen. Ulrih and her young researchers started to grow *A. pernix* in the laboratory, to study its stability, membrane organization and cell components, and to investigate these at the molecular level. She was very excited about the molecular adaptation of cell membranes through the incorporation of special archaeal lipids, which consist of isoprenoid chains attached via ether bonds to the glycerol carbons at the lipid *sn*-2,3 positions. Her group has extensively studied archaeal lipids and archaeosomes as potential delivery systems.

In 2009, Ulrih and her co-workers patented their method for the degradation of prions using the thermostable protease pernisine isolated from *A. pernix*. They demonstrated *in vitro* enzymatic degradation of protein aggregates by pernisine, which included infective prions (PrP^{Sc}) from different origins (i.e., mouse, bovine, deer, human). Despite the decline in classical bovine spongiform encephalopathy cases, prions still represent a serious problem for human health. Even today, there are only a few alkaline and enzymatic detergents that can reduce prion infectivity. Pernisine is a thermostable serine protease from *A. pernix* that has the potential for use in various industries, from the cleaning industry to medical fields, where medium to high temperatures or harsh conditions are encountered (e.g., with denaturants, reductants, detergents). With all of these potential uses of pernisine, they are still trying to define protocols to increase pernisine production. The recent results obtained by Ulrih and her group on the overexpression of pernisine in a heterologous bacterial host

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are promising, which was promoted through their collaboration with the group of Prof. Hrvoje Petković.

- Vice Dean for Scientific Research in the Biotechnical Faculty of the University of Ljubljana;
- Full Professor of Biochemistry in the Biotechnical Faculty of the University of Ljubljana (since 2010);
- Gold Medal for Research and Educational Achievements from the University of Ljubljana;
- Fulbright Visiting Professor at UC Santa Cruz (Prof. Anthony Fink) (in 2006–2007);
- Professorship in the Biotechnical Faculty of the University of Ljubljana (in 2000);
- Postdoctoral position at Rutgers, the State University of New Jersey, USA (Prof. Kenneth J. Breslauer) (in 1995–1996);
- PhD in Chemistry in the Faculty of Chemistry and Chemical Technology, University of Ljubljana (in 1994).

2. PHYTOCHEMICALS IN FOOD

Ulrih explains that being a Professor in the Department of Food Science and Technology means that she is involved in problems that are more ‘realistic’ and come with social responsibilities. She and her research group are involved in many aspects of sustainability, the bioeconomy and nutrition. In recent years, there has been increasing concern for the reduction of the ecological impact of industrial waste related to fruit and vegetables. Additionally, the agronomic, cosmetic and pharmaceutical industries have increased interest in natural extracts from plants, which represent by-products that can be rich in bioactive compounds. This is due to the need to meet the growing demand for natural preservatives and to produce novel ‘functional’ foods that come with significant health benefits. In this respect, olive leaves, onion skin, grape marc, or pomace, are promising plant matrices from which extracts can be obtained that are rich in natural phenolic compounds. Ulrih and her group are working on the optimisation of the extraction of such bioactive compounds from these matrices, to obtain oleuropein, quercetin and resveratrol, respectively. Oleuropein is the most representative polyphenol of olive leaves, and it is responsible for the bitterness of both table olives and extra-virgin olive oil. Several studies have shown a wide variety of *in vitro* and *in vivo* properties for oleuropein, including antioxidant, antiviral, antibacterial and anti-inflammatory activities. Then, to contribute to cost reduction of onion waste disposal while also obtaining value-added products, onion skin can be used to extract quercetin, a natural flavonoid with antioxidant, anti-inflammatory and anti-cancer effects. Finally here, resveratrol has been the focus of scientific research for some time because it is known to have beneficial effects on human health. It has been ascribed anticancer, antidiabetes, and antiaging activities. *Trans*-resveratrol, or 3,5,4'-trihydroxy-*trans*-stilbene, is a phenolic that has two structural isomers, as *trans* and *cis*. Grapes and red wines are among the richest known sources of resveratrol. The main problem with *trans*-resveratrol, however, is its low solubility in water and its poor bioavailability.

As Ulrih explains, an understanding of the molecular basis of interactions among molecules helps to determine the mode(s) of action of

phytochemicals such as the polyphenols with proteins, lipids, nucleic acids and polysaccharides. She has thus continued her basic science research on polyphenol interactions with membranes and proteins. Over the last 5 years, her laboratory has carried out comprehensive molecular analyses of the different polyphenols and their interactions with cell membranes, to understand the mechanisms of their adsorption—how they cross the cell membrane—and their modes of action in the cell. The potential benefits of phenolic compounds are limited by their stability during food processing and storage, and also their bioavailability. This was the reason that Ulrih established a new laboratory in the Biotechnical Faculty, for the stabilisation of bioactive compounds and plant extracts using encapsulation techniques. Encapsulation of such bioactive compounds in different carrier materials (e.g., polysaccharides, proteins and/or lipids matrices) can protect the core compounds and enhance their application for incorporation into diverse food matrices. In addition, encapsulation can provide new approaches for food packaging, through the use of functional coatings that can protect against microbial growth and contamination, and that can enhance the sensorial properties (e.g., flavour, colour, taste), and provide alternative routes of ingestion (e.g., through the skin, mucus membranes). They have successfully encapsulated many compounds and extracts (e.g., folic acid, pantothenic acids, propolis). Indeed, they are very proud of their encapsulation of epigallocatechin gallate, the main catechin from green tea, in liposomes (lipid bilayer vesicles) and in alginate and chitosan microparticles that are reinforced with liposomes. These encapsulation systems provide protection for epigallocatechin gallate in solution at acidic and alkaline pHs, and in fruit juice. They continue to work on many further compounds too, each of which requires a different approach.

3. SCIENTISTS WHO HAVE INSPIRED NATAŠA POKLAR ULRIH

Ulrih was born in Postojna in Slovenia. Her father, Emil, was a teacher of physics and technical instruction, and her mother, Danica, was a shop assistant in a small grocery shop in Podgraje, the village where they lived. Ulrih did not go to kindergarten; she spent a lot of time outdoors, playing with her two younger brothers, or with her mother in the grocery shop, or with her grandmother, who had a huge impact on Ulrih's love of nature. When Ulrih was in primary school, she showed great talent for Mathematics and Natural Sciences. In her secondary school, the Gymnasium of Postojna, she decided to focus on Chemistry. Afterward, she was initially undecided between Medicine and Chemistry; then in 1984, she entered the Faculty of Chemistry and Chemical Technology at the University of Ljubljana.

During her studies at the University of Ljubljana, Ulrih met Prof. Savo Lapanje, who inspired her by his work in the world of proteins. Prof. Lapanje was at that time one of the most famous scientists on the field of proteins. With Prof. Charles Tandford (Duke University, NC, USA), he reported that the intrinsic viscosity $[\eta]$ of proteins in a solution of guanidinium chloride is a function of the polypeptide chain length (n), with this relationship expressed by the equation

$$[\eta] = 0.68n^{0.67},$$

which is universal for all proteins with molecular mass from 3000 to 200,000, regardless of the function of the protein. They were also the

first demonstrate the existence of the random coil conformation. This remains a classical study in the field of Protein Science. In 1978, Prof. Lapanje published a monography entitled, “Physicochemical Aspects of Protein Denaturation” (published by John Wiley & Sons).

Ulrih graduated in 1989 with a Bachelors thesis entitled, “The heat denaturation of β -lactoglobulin”. The work behind this BSc Thesis was published together with Prof. Lapane in Biophysical Chemistry 34, 155–162 (1989), as “Calorimetric and circular dichroic studies of the thermal denaturation of β -lactoglobulin”. She is very proud of this, as her first publication. After obtaining her BSc, she stayed in Prof. Lapanje’s laboratory as a young Researcher for another 5 years. During that time, she was working on all aspects of protein denaturation and preferential solvation. In 1992, she obtained her MSc with her thesis on, “Solvation of β -lactoglobulin in aqueous solutions of alkylureas”. Then in 1994, she obtained her PhD, with a thesis on, “Physicochemical investigation of chymotrypsinogen A denaturation by solvents and heat.”

After obtaining her PhD, Ulrih started to work on the pore-forming protein equinatoxin II with Prof. Peter Maček and his younger colleagues Dr. Gregor Anderluh and Dr. Kristina Sepčič. At that time, she was inspired by the work of O. Ptizin and R.H. Pain on molten globule states, and she was involved in research that showed that equinatoxin forms a molten globule state on the surface of lipid membranes at low pH.

In 1995, she moved to the USA to Rutgers, the State University of New Jersey, as a Postdoctorate in Prof. Kenneth J. Breslauer’s laboratory, which was the main driver in the field of thermodynamics of nucleic acids and DNA. She was very happy that this was the time of the triple helix and she was discovering the world of nucleic acids. She studied cisplatin [*cis*-diammine dichloroplatinum(II)], a widely used anticancer drug with a mechanism of action that binds to and crosslinks DNA. They reported that this crosslinking alters the structure of the host DNA duplex, consistent with a shift from a B-like to an A-like conformation. This lowers its thermal stability and thermodynamic stability, which is enthalpic in origin. However, it does not alter the two-state melting behaviour of the parent, unmodified DNA duplex, despite these significant crosslink-induced changes.

As Ulrih notes, “I was lucky to get the chance to work with the greatest minds of our time in the field of nucleic acids and proteins”. During her stay in the Breslauer laboratory, she published two articles in PNAS of which she is very proud. She stayed in the USA as a Postdoctorate for 15 months, but then decided to return to Slovenia. She continued working in the field of the thermodynamics of protein denaturation and ligand binding to DNA, and she has stayed connected with her colleagues from the Breslauer laboratory. She also used to spend each summer working at the University of Toronto, Canada, in Prof. Tigran V. Chalikian’s laboratory.

For Ulrih, Slovenia is the most beautiful country in the world, and she can now never even think of living somewhere else. However,

being a university Professor and Scientist in Slovenia is not so easy, where there can be a lack of funds, without balanced funding, and often with little recognition of excellent ideas. The system is stagnating, where the main problem is that even well-established Professors are overloaded with teaching hours, from a minimum of 180 h up to 240 h teaching per year. “This system is literally killing you slowly, and sometimes you can see the shadows of once-great minds; it is so sad”.

In the 2006–2007 academic year, Ulrih was awarded a Fulbright Scholarship, and she spend half a year in Prof. Anthony Fink’s laboratory in the Department of Chemistry and Biochemistry at the University of California in Santa Cruz (UC SC), USA. During this time, she moved to California with her husband, Vasja Ulrih, an artist, and their daughter, Gaja, who was 4 years old at the time. It was a very fruitful time for them all. Her husband had two successful exhibitions, one in Santa Cruz and one in San Francisco. She is very emotional when she tells of the opening of Vasja’s exhibition in the University Gallery in Santa Cruz, on 8 February 2007: Cultural Day, and a national holiday in Slovenia. Vasja recited the poem “Zdravljica” in Slovenian, which was written by the greatest Slovenian poet, Dr. France Prešeren. The people in the audience were particularly impressed.

Ulrih was inspired by the work of Prof. Tony Fink in the field of protein aggregation and the molecular basis of diseases. When at UC SC, she started to work on the aggregation of α -synuclein, a protein involved in Parkinson’s disease. Prof. Fink was a world authority on protein folding. Mistakes in this molecular process can lead to degenerative diseases, such as Parkinson’s and Alzheimer’s diseases, and bovine spongiform encephalopathy, or “mad-cow” disease. Prof. Fink’s research was focused on the understanding of what goes wrong, so as to design treatments to repair the damage, or to prevent it from happening in the first place. He worked tirelessly towards the goal of the design of potential drugs and therapeutic methods to combat Parkinson’s and Alzheimer’s diseases.

It is interesting, that Ulrih has always returned to her initial work, to the world of proteins. Her group continues to work on polyphenol interactions with proteins, how polyphenols prevent aggregation of α -synuclein, and how they bind to bovine serum albumin, to understand their transport in the blood.

Greatest hits

- Molten globule state;
- Cisplatin interactions with DNA;
- Molecular basis of diseases of α -synuclein;
- Degradation of prions by pernisine;
- Phytochemical interactions with proteins and lipids;
- Encapsulation of phytochemicals and delivery systems.