

## Protein modeling in Split

# A Kind of Love Affair

The larger, the better. However, this is not the only reason why Anita Krisko is besotted with the object of her research, apolipoprotein B (apoB), the large carrier protein for cholesterol.

Anita Krisko fell in love with apolipoprotein B100 (apoB) at the laboratories of Rudjer Boskovic Institute while she was still an undergraduate student of molecular biology in Zagreb, Croatia. "As soon as I started learning more about proteins, I was immediately drawn to them", she says. "Up until then, proteins meant meat, something that you need in your daily diet to stay healthy. But then the whole world beyond that opened up for me as I learned about proteins' complexity and variety. They were not simply molecules; to me proteins were more like a whole new species."

## Not just "meat"

Among proteins, apoB was especially attractive for Krisko. It is part of the low-density lipoprotein (LDL) that is the main cholesterol carrier in human plasma. A LDL particle is quite complex; one assembly contains approximately 3,000 lipids stabilised by only one apoB molecule, which has 4,536 amino acid residues and is one of the largest monomeric proteins known.

People are very often drawn to extremes; adrenaline addicts want to conquer the highest mountain or dive into the deepest gorge, while laboratory adventurers want to study the largest protein. So size matters, after all. Thus, Anita Krisko started working on this large protein under the supervision of Greta Pifat-Mrzljak, who was mainly interested in LDL's role in atherosclerosis.

Apolipoproteins are a jolly group of various proteins that combine with a lipid to form different lipoproteins in mammalian blood circulation. ApoB is a distinguished member of this group, as it is the primary constituent of LDL, a usual suspect for causing atherosclerosis and coronary heart disease (CHD). Since CHD is the most common cause of morbidity and mortality in the developed world, with at least one third of the affected individuals younger than 55

years of age, causing vast costs in medical treatment and lost income, everything associated with this disease is worthy of scientific investigation.

LDL oxidation is considered crucial for the initiation and progression of atherogenic processes in the arterial wall, which subsequently lead to CHD. It is not clear how LDL is oxidised *in vivo* but regardless of the mechanism, subsequent processes following oxidation initiation are thought to be the same. They involve loss of antioxidants followed by lipid peroxidation.

When Krisko started out with apoB, she initially studied structural and biochemical modifications of the LDL particle upon its oxidative modification. What she found is that conformational changes of apoB occur even in the earliest stages of oxidation process when the chain reaction of LDL peroxidation has not yet been initiated. This may lead to new insights into the early preven-

relievers, diuretics, cold and allergy medications. She found that physiologically acceptable amounts of caffeine have an anti-oxidative effect on the protein moiety of the LDL particle in the early stages of oxidation (*Clinica Chimica Acta*, 2005, 355, p. 47). The study clearly showed that caffeine plays a protective role, regardless of how oxidation was initiated. This is certainly an encouraging piece of news for all the coffee drinkers and coffee businesses of this world.

## Good news for coffee drinkers

"After several lines of research dealing with LDL's role in biomedical processes, I was ready to tackle the structure of the apoB itself", grins Krisko. After all, she had been in this relationship with apoB for a while but didn't know all that much about it. This was certainly no mean feat. ApoB is highly insoluble in aqueous solutions and it has been, as Krisko says, "highly resistant to both practical and theoretical studies of its structure". Namely, apoB is very large and, except for the N-terminal domain, it shows no significant homology to any known protein in the database.

There have been several attempts to address apoB's secondary structure by different methods with various results, so a consensus on apoB secondary structure has not yet been established. Regarding apoB tertiary structure and the structure of the LDL particle, the resolution of 27 Å has been described but revealed only the overall shape of the particle.

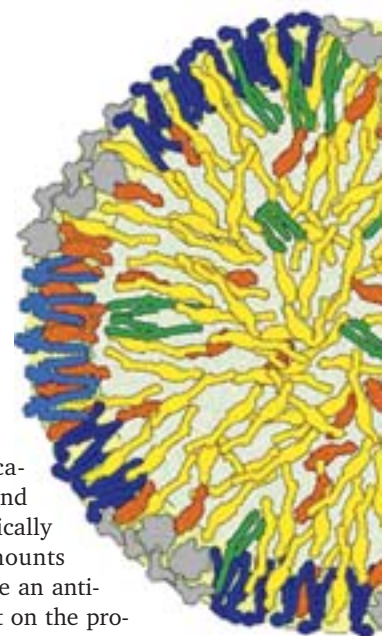
Krisko already had some experience in studying apoB's domain organisation (*Biochimica et Biophysica Acta*, 2003, 1631, p. 239), which gave new clues to understanding the functional role of apoB. But now she was ready for more. At the time, any

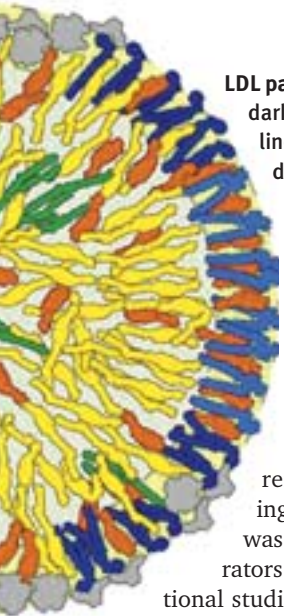


Anita Krisko with one of her most important research tools

tion of atherosclerosis (*Biochimica et Biophysica Acta*, 2007, 1768, p. 2923).

In the context of LDL and atherosclerosis, Krisko also studied the influence of caffeine on LDL oxidation. Many endogenous and exogenous compounds have been tested for their influence on LDL oxidation, of which caffeine is particularly interesting because it is abundant and comprehensively studied. Caffeine is also regularly ingested in food, beverages and drugs such as pain



**LDL particle:**

dark blue – phosphatidylcholine; blue – sphingomyelin; dark yellow – cholesterol ester; orange – cholesterol; green – triglyceride; and grey – apolipoprotein B-100

available knowledge on apoB relied on computational approaches. Krisko remembers, “As interesting as that approach was, I was not able to find collaborators in Croatia for computational studies of the apoB’s tertiary structure. Bioinformatics was not even taught in my undergraduate molecular biology curriculum between 1997 and 2001, so to gain some knowledge I had to rely on personal communication with colleagues that were self-taught in that research field.”

**Threading methods...**

For this risky project she answered the French government’s call for grant applications. She was successful and, finally, at the beginning of 2004, was able to get more closely acquainted with apoB with a little help from a computer and a mentor, Catherine Etchebest from the Université Denis Diderot in Paris. The resulting manuscript was difficult to publish as it was a theoretical model. And in order to produce this model, Krisko had used her own combination of pre-existing computational approaches. Eventually, the results were published (*Proteins*, 2007, 66, p. 342) and Krisko continues to receive numerous requests for reprints, many of them coming from the pharmaceutical industry.

To obtain templates for the modelling of apoB Krisko used threading methods. Parts of the protein, which had no obvious similarities in sequence but had a similar folding to a protein from the database, were yet to be recognised. The average sequence identity between all pairs of similar structures is around 8-10%, which defines the so-called ‘midnight zone’ of protein sequence alignments, mostly populated by protein structure pairs that may have become similar by convergent or divergent evolution. Krisko used threading algorithms to reveal homologous pairs of apoB sequences from the midnight zone of multiple sequence alignments. Based on this she proposed that only the N-terminal domain of the apoB is globular in shape, while all the other domains are

characterised by an extended overall conformation. Thus, she suggested that apoB domains are attached to each other like a string and wrap around the LDL particle in a belt-like manner. She also proposed sites of apoB interaction with the lipid matrix of LDL particles.

**... and machine learning**

Anita Krisko’s multiple publications on apoB’s structure, research methodology and its role in atherosclerosis resulted in numerous invitations to speak at scientific events. Alas, since she is not so fond of flying, she has not yet set foot outside of Europe. Also, owing to her successful relationship with apoB, in early 2007 she became a group leader at the Mediterranean Institute for Life Sciences (MedILS) in Split, Croatia, which is a fairly remarkable accomplishment considering she is under thirty.

Since starting at the MedILS, Krisko opened up a new field of research. Now she is focused on the analysis of proteomes, in search of patterns that reveal different adaptations to extreme environments in microorganisms. The assumption is that proteins that may look alike are designed differently for various extreme environments. With a team of graduate students Krisko is using machine learning to deal with a data set of 192 proteomes, each described with 720 properties and each consisting of about 1,000 proteins.

**Why are big proteins big?**

Although this may seem like a drift away from apoB, it is not necessarily so. In many organisms fond of extreme environments (extremophiles) enormous proteins have been found, with up to 9,000 amino acids. They are huge, just like apoB, and Krisko would like to know whether they have anything? Why are they so big? Why did evolution keep them in one piece? Obviously, there was a big selective pressure to keep them this way. One possible explanation is that this conglomerate is enabling a whole ecosystem on a microscopic level to carry out a certain process at the active site.

Although she has been at MedILS for less than a year, Krisko has already obtained data ready for publication. Right now she is ready to expand her highly productive team. So, graduate students and post-docs interested in extreme science and extreme fun in a campus by the sea should contact her at [anita.krisko@medils.hr](mailto:anita.krisko@medils.hr). Being madly in love with apoB certainly won’t impair the hiring process.

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