

The transporter

Dr Marjana Novič describes the work of her laboratory in chemometric analysis and modelling of the properties of uncharted proteins, and reveals how European collaboration has moved the research forward



Could you outline the membrane transport project?

Our aim was to elucidate the transport mechanism and structural details of bilitranslocase, a membrane protein with a major role in transporting bilirubin from blood to liver cells. We approached this *in silico*, taking advantage of 3D structure information in the Protein Data Bank.

Working with experimentally-tested chemicals, including flavonoids and several purine and pyrimidine derivatives, we developed a computational model for predicting affinity to bilitranslocase. We obtained some interesting results: for example, no other known transporter is able to transport nucleic bases, nucleosides and nucleotides through membranes. The model we developed, with additional information from our recent molecular dynamics simulations and Nuclear Magnetic Resonance (NMR) spectroscopy, will help us to better understand the driving forces of the transport mechanism.

What inspired you to embark on the project?

The first impetus was contact with Professor Sabina Passamonti and her group from the University of Trieste. They had been studying membrane proteins experimentally for several

years and due to the challenging biological functions and structure of these proteins, were looking to collaborate with computational chemists to additionally explore a particular trans-membrane protein, bilitranslocase.

The second trigger to apply for funding was an inspiring collaboration with Professor Milan Randić, who visits our Institute of Chemistry in Ljubljana every year for a couple of months during summer. His broad knowledge of discrete mathematics and his continuous interest in the development of new molecular structure representations for applications in the advanced -omics research area gave me the idea of using discrete mathematics in proteomics.

What is meant by membrane transport, and why is study of this area particularly important from a pharmaceutical perspective?

The cell membrane presents a barrier that has to be passed whenever a molecule from outside 'wants' to reach its target inside the cell or vice-versa. Membranes are lipophilic, so for hydrophilic molecules, which drugs most often are, they are usually not easily permeable. Either a drug needs the help of an active transporter, or it should be 'packed' in an inert or hydrophobic environment to be able to interact with the lipid membrane. How the drug will reach the target if it is inside the cell or in the membrane is important to the pharmaceutical industry. For natural compounds with beneficial effects for human health, such as antioxidants, the results from our studies might help to find a chemical modification that would enable them to better penetrate through cellular membranes.

Could you highlight some of the research with which you have been most recently involved at the L03 Laboratory of Chemometrics?

We have been involved in several European projects, most recently CAESAR, TRACE, IBAAC and now BioChemLig projects, to which we offered informatics support for organic reaction studies, from optimisation of enzyme-

enhanced stereospecific catalysis to theoretical investigation of cyclo-addition reactions. In the CAESAR project, we have been involved in the development of quantitative structure-activity relationship models for different toxicity endpoints. In TRACE we were involved in statistical/chemometrics evaluation of analytical data on food commodities.

Another interesting application of neural network modelling, that we completed last year, was associated with the antioxidative activity of pulvinic acid derivatives. On the basis of carefully chosen influential structural descriptors, the model was able to assess the antioxidative potency of a diverse set of compounds. A new lead compound, coumarine acid derivative, was identified and confirmed with *in vitro* testing. That research was carried out in collaboration with the University of Strasbourg.

Which conclusions have you drawn from your *in silico* modelling of toxic compounds and food traceability?

From modelling toxic compounds, we learned that the available databases were very diverse, with toxicity data obtained by different protocols and much erroneous information regarding molecular structure.

Regarding food traceability, we effected data exploration on water analyses as well as honey and olive oil samples. We found some region- and geology-dependent parameters in the data analysed, so we were able to propose some specifications for the origin of food.

How is TRANS2CARE linked to the membrane project?

TRANS2CARE is a new strategic project for innovation and technology transfer in healthcare. It is financed by the governments of Italy and Slovenia and connects several research institutions, universities and hospitals in the regions close to the Italian-Slovenian border. The project is connected to our membrane research because one of the potential drug targets in TRANS2CARE is also bilitranslocase.

Modelling complexity

Using advanced mathematics, statistics, neural networks and quality methodologies, the Laboratory of Chemometrics at the **National Institute of Chemistry** in Slovenia solves problems in chemistry – with an emphasis on proteomics and genomics – with high dependability

MEMBRANE PROTEINS MAKE up nearly a third of all proteins in human cells. Though their structures are largely uncharted because of the limitations of experimental techniques, they are widely used as drug transporters.

Bilirubin is the substance in bile that gives rise to the yellow pigmentation of bruising, jaundice and urine. A by-product of the breakdown of old haemoglobin, it is moved from the blood to the liver, for eventual excretion, by bilitranslocase, a membrane protein. Until recently, the structure and properties of bilirubin, like many membrane proteins, were unknown, but now a collaborative international study of its properties has ascertained that it has exceptional promise as a delivery mechanism for drugs or diagnostic aids, with unprecedented flexibility and range of applicability, by applying chemometrical techniques alongside *in vitro* experimentation.

DATA DRIVEN MODELLING OF CHEMICAL STRUCTURES

Dr Marjana Novič, Head of the Laboratory of Chemometrics at the Slovenian National Institute of Chemistry in Ljubljana, broadly defines chemometrics as using mathematical modelling to resolve chemical issues. Her involvement with bilirubin arose from a requirement for chemometric analysis from Dr Sabina Passamonti's group at the University of Trieste: "Proteins are challenging because of their multiple roles in the life cycle, their various functions, their structure, synthesis and biological coding," Novič reflects.

In practice, chemometrics is a branch of chemistry that applies both mathematical principles and computational procedures to problems that usually involve high quantities of data, high numbers of variables and the prospect of limited correlation points that will therefore reduce the complexity and number of possible results, so that clear probabilities can be obtained. In Novič's laboratory, the input data is obtained from sources such as imaging and chemical analysis and are then processed

according to the Organisation for Economic Cooperation and Development (OECD)'s five principles for prediction and validation of quantitative structure-activity relationship (QSAR) models to obtain mathematical relationships. The OECD principles for such models state there must be a defined endpoint, an unambiguous algorithm, a defined domain of applicability, appropriate measures of fitness, robustness and predictivity and should support mechanistic interpretation. Having determined a relationship between a chemical structure and a certain biological activity, the model can be used to predict the responses and activities of a particular chemical, along with a rate of error or uncertainty for the prediction.

On another project, Novič's team assessed the carcinogenicity of various toxins and she describes the complex processes involved: "It is very important to perform very carefully the initial step, data compilation and cross-checking for potential errors. The validation step for the models must be carried out properly, bearing in mind the needs of the end users and considering the OECD principles," she notes. The precision of their QSAR model towards hypothesising carcinogenicity was not very high, at around 70 per cent, which is apparently not unusual for such a difficult endpoint as carcinogenicity. "Nevertheless, we could assess how, statistically, selected descriptors which encode information about

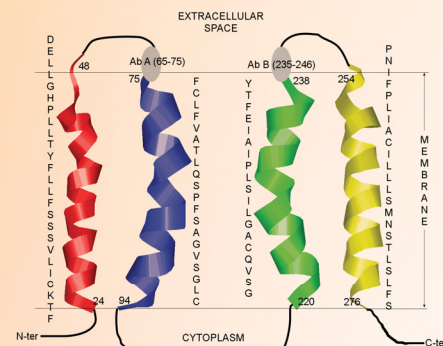


FIGURE 1. BTL-ALPHA HELICES TM

INTELLIGENCE

TRANSPORTATION IN MEMBRANE PROTEINS

OBJECTIVES

Membrane transport is the common step determining the effect of most drugs. The project investigates the mechanism of transport and structural details of bilitranslocase, the transporter of organic molecules from blood to liver cells. It has been found out that bilitranslocase plays a role in the absorption and hepatic metabolism of antioxidants, which is more potent for anthocyanins than for flavonoles. A detailed structural study of the protein indicated four alpha helical transmembrane domains that most likely formed a channel in the cell membrane. A combination of *in silico* modelling along with experimental data (K_a, NMR) supported the hypothesis: bilitranslocase might be a drug transporter.

KEY COLLABORATORS

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DR MARJANA NOVIČ received her degree in Chemistry in 1979, an MSc in Computer Chemistry in 1983 and was awarded PhD status in Chemistry in 1985 from the University of Ljubljana. She is the author or co-author of over 100 scientific papers and 10 book chapters.

Often the experimentalists speak a different language to the chemometricians

general structural features, such as molecular size and shape, as well as specific information on skeletal variation and complexity, correlate with particular groups of chemicals containing structural alerts for carcinogenicity with determined mechanisms and, consequently, with carcinogenic potency.”

IN SILICO MODELLING OF BILITRANSLUCASE

A structure for bilitranslocase was approached by first testing *in vitro* with various endogenous compounds, drugs, purine and pyrimidine derivatives, and then constructing a model according to OECD QSAR principles. Trans-membrane domains were assessed through a second model developed using the limited number of membrane protein 3D structures available in the Protein Data Bank as a basis.

For Novič, defining the variables, adjustable parameters and the properties of the given system are all important parts of solving the problem. Once the system has been defined, then the team can ‘look at it’ using visualisation – a smart projection of multidimensional data. The project participants then choose the modelling method – linear or nonlinear – and after obtaining the first results, go back to the first step. Arriving back at the initial steps allows the team to better define parts of the system or to optimise certain parts of the procedure, improving their results.

Incorporating selected structure variables according to weightings, Novič’s team then interpreted and optimised them in terms of their probable role in transport mechanisms. They classified the model according to active or inactive compounds and used the active classification to predict inhibition factors: “Combining a large amount of information on a particular and well defined object like a biochemical system with limited information on a large number of objects is like merging different perspectives, which greatly improves the clarity of the picture,” says Novič.

The main challenge for the team was obtaining NMR experimental data and expertise on the 3D structure of short protein segments corresponding

to trans-membrane domains. Although they have an 800 MHz NMR spectrometer, it took great effort to engage an NMR expert to help with preparation and to measure and evaluate the results. When the team eventually achieved this, the results confirmed predictions of alpha helices in the trans-membrane domains of bilitranslocase: “Bilitranslocase is a good candidate for transport of polar molecules with therapeutic applications and it should be further evaluated,” Novič adds.

COLLABORATION

A close working relationship between Novič and Professor Milan Randić, the computational chemist responsible for the index for characterisation of molecular branching, has allowed for a strong and effective team to be formed. His enthusiasm for producing new ideas warms Novič and inspires her to continue performing research in this area. Their collaboration in proteomics and genomics studies has blossomed because of this and 20 years on, the partnership between them remains very strong.

Novič regards interdisciplinary research as indispensable when studying proteins because of their complexity and the need for computational and experimental practitioners to constantly feed results of their findings back to one another: “Often the experimentalists speak a different language to the chemometricians. An interdisciplinary approach can explore multiple aspects, from elementary atomic structure to fragile equilibria of molecules within the cells, and most importantly, biological functions including those that depend on protein trafficking,” she elaborates. A combination of different expertise and an interdisciplinary approach within the group also enabled effective distribution of scarce funding.

Novič’s team is currently involved, along with partners from Slovenian industry, in a project to optimise asphalt mixes by recycling waste materials and are also participating in the Italian-Slovenian TRANS2CARE project, which is establishing collaboration in designing novel healthcare products and nurturing young researchers.

If the opportunity arises, Novič would like to continue working with proteins. By concentrating on mathematical means for encoding protein sequences, as well as accompanying information in an innovative and reversible way, the team would be closer to identifying and facilitating experimental data on 3D structures that are not yet available. “But our work does not depend solely on our explorative curiosity,” Novič concludes.

