Crystal clear

Using crystallographic and single-particle techniques, **Professor Dimitrios Fotiadis** analyses membrane proteins to obtain multi-dimensional high-resolution images of their structures and workings

PROFESSOR DIMITRIOS FOTIADIS

Can you begin by outlining the main focus of your research?

Our focus is on membrane proteins, particularly on their structure and supramolecular organisation. Our ultimate goal is to understand the working mechanisms of selected membrane proteins based on their molecular architecture.

What makes membrane proteins such an interesting topic of investigation?

Membrane proteins are embedded in the lipid membranes of the cell, providing a platform for communication with the outside world; import and export of nutrients and metabolic products; energy production; and other vital functions. The extreme importance of membrane proteins is reflected in the wide range of human diseases associated with their malfunction. In our laboratory, and within the National Centre of Competence in Research (NCCR) TransCure, we mainly focus on those membrane transport proteins that are involved in cancer.

Can you elucidate some of the structure determination techniques used in the Fotiadis laboratory?

Our techniques include transmission electron and atomic force microscopy, and electron and X-ray crystallography. Our large repertoire of techniques is quite unique and allows us to study the structure of membrane proteins from different perspectives.

It is fascinating to think of two-dimensional (2D) and 3D crystals composed of highly-ordered membrane proteins. Is it difficult to obtain such crystals?

One of the biggest challenges in structural biology of membrane proteins is obtaining highly ordered crystals. This endeavour faces two major impediments: obtaining milligram amounts of functional and stable protein and growing protein crystals that diffract to high resolution. This is the reason why only about 3 per cent of the crystal structures deposited in the Protein Data Bank are from membrane proteins.

Atomic force microscopy (AFM) is not as common in biology as other techniques such as electron microscopy. Can you comment on the principle and advantages of AFM?

AFM is a relatively young technique. The principle of AFM can be compared to a blind man sensing his environment with a stick. In AFM, the stick is a molecularly sharp tip. For imaging, the tip is raster scanned over a sample surface, while the topography of the sample is recorded simultaneously.

Some of the unique features of AFM are its ability to operate under near-physiological conditions, ie. at temperatures of 4-37 °C; in a buffer solution; and under normal pressure. This is in contrast to electron microscopy and X-ray crystallography, where samples are fixed or frozen. Another exceptional feature of AFM is the strikingly high signal-to-noise ratio and sub-nanometre resolution of the images, which make it possible to visualise and monitor single proteins at work.

Can you describe the concept of the 'TransCure Trias'?

TransCure brings chemistry, structural biology and medicine together to expedite the discovery and validation of new drugs against specific human diseases. We believe that such a multidisciplinary approach will assure success.

What is the main mission of NANOCELL?

NANOCELL is a synthetic biology project to engineer and assemble containers with controllable functions that are not found in Nature. To assemble such containers from synthetic lipids or block copolymers, we produce and engineer molecular machineries of the cell as building blocks, also called modules. These can be placed in the membrane or inside the container to perform particular orchestrated biochemical processes.

The NANOCELL consortium consists of eight European investigators with complementary expertise. The fabrication of such nanocells is an ambitious goal and we are only at the beginning of this endeavour, although we have made progress in recent years.

What would you cite as your proudest research achievement to date?

I am most proud of the revelation of the oligomeric state of rhodopsin in native membranes. This was a fantastic collaboration with Professors Kris Palczewski (Case Western Reserve University, USA) and Andreas Engel (Biozentrum, University of Basel, Switzerland) – two outstanding and inspiring scientists. It reversed current dogma and had an immense impact in the fields of vision and G protein-coupled receptor (GPCR) research. New aspects of the onset and causes of the hereditary disease retinitis pigmentosa then emerged.

Furthermore, GPCR oligomers, not only monomers, will have to be considered conceptually in drug discovery; for example, GPCR oligomers offer interfaces between single proteins where drugs could bind.

And finally, what can be done to speed up progress in research on membrane proteins?

We have to actively advertise the importance of membrane proteins to the public, politicians and funding agencies – and call for greater investment!

Membrane transporters: structure to drug

Professor Dimitrios Fotiadis from the **University of Bern** in Switzerland, and his collaborators investigate the structures of membrane transport proteins in order to understand their molecular working mechanisms and for structure-based design of new drugs to treat specific human diseases

WHILE THE GENE content of the human genome has been fundamentally established, the content of the human proteome is still in the discovery phase. This is particularly apparent with respect to the membrane proteins – whose structures are yet to be determined in most cases – despite the fact that about two thirds of drugs commonly used in treating human diseases target this important class of proteins.

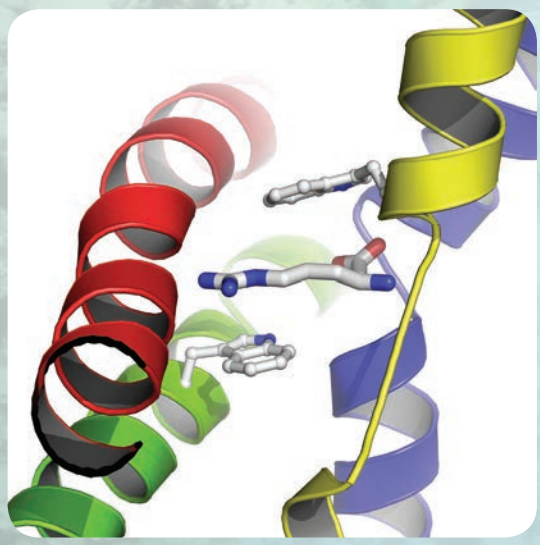
A significant subset of membrane proteins – the transport proteins – transfer signals and materials between cells and between the internal cellular compartments. Membrane transport protein dysfunction is known to be associated with such conditions as cancer, hypertension, osteoporosis and diabetes, as well as with neurodegenerative diseases and psychiatric disorders. Therefore, for public health reasons, understanding the functions of these membrane proteins is a priority.

Professor Dimitrios Fotiadis' laboratory at the Institute of Biochemistry and Molecular Medicine at the University of Bern in Switzerland is currently engaged in three research programmes that are exploring membrane transport proteins using state-of-the-art technology. The main goals are to resolve the question of their structures and molecular mechanisms of transport, and to find inhibitors and positive allosteric activators as new pharmaceutical drugs: "Knowledge of the molecular architecture of the target protein, including its substrate binding site or sites, is crucial for structure-based drug design," Fotiadis asserts. "Membrane proteins mediate numerous vital functions in all living cells – they are at the core of life."

Fotiadis' work seeks not only to obtain structural information, but also to understand the interactions between membrane proteins and how these affect their functions. Often, membrane proteins only become active once they assemble into higher-order complexes: "The structures are simply snapshots of the proteins in a specific conformation that show how they look and how they are built, but often membrane proteins are accompanied by multiple copies of the same or different proteins, thus establishing supramolecular organisations," he explains.

The research being conducted by the Bern scientists on the higher-order structures of membrane proteins builds partly upon Fotiadis' seminal research into rhodopsin, a membrane protein in the retina of the eye. Using atomic force microscopy in an analysis of mouse retinas, he discovered that rhodopsin was not, as previously thought, monomeric: "Rhodopsin is the prototypical G protein-coupled receptor and the primary photoreceptor molecule in the visual signal transduction," he notes. "The dogma of more than 30 years was overturned by our direct visualisation of rhodopsin dimers and higher oligomers in native disk membranes."

FIGURE 1. Substrate binding pocket of the bacterial L-arginine/agmatine transporter AdiC. Bound L-arginine as well as interacting amino acid residues are discerned



OBTAINING MEMBRANE PROTEIN STRUCTURES

With support from the Swiss National Science Foundation (SNSF), Fotiadis is investigating prokaryotic and human proteins of the amino acid, glucose and peptide transporter classes. The properties that he is seeking to illuminate include their structures and conformations, their supramolecular organisations and the molecular bases of substrate binding and transport.

Membrane proteins are naturally embedded in a lipid bilayer. For most analysis purposes, they need first to be extracted, purified and crystallised with the aid of detergents. However, according to Fotiadis, membrane proteins are best studied in their native environment, not in a detergent micelle: "We use transmission electron and atomic force microscopy and electron crystallography to analyse native membranes, proteoliposomes and two-dimensional crystals in which membrane proteins are embedded in lipid bilayers," he explains. "This is one of my major areas of expertise, which I acquired during my time in the laboratory of Professor Andreas Engel at the Biozentrum of the University of Basel."

Isolating tiny amounts of these membrane proteins from natural sources for examination is itself complicated or even impossible. The need to grow crystals so that they are suitable for high-resolution structure determination by crystallographic approaches adds extra complexity to the task. Therefore, heterologous overexpression of recombinant target proteins is required and is currently a limiting factor, particularly for human and eukaryotic membrane proteins. "As for crystals, people in the field say that it is more of an art than a science to grow them," Fotiadis reflects. Because of these limitations, the Bern investigators also analyse equivalent prokaryotic membrane proteins, where success rates for obtaining milligram amounts of target protein for crystallisation and therefore structure determination are significantly higher.

TRANSCURE

Fotiadis is part of TransCure – a translational research programme within the portfolio of the National Centre of Competence in Research (NCCR), funded by the SNSF. The NCCR TransCure, which is hosted by the University of Bern and directed by Professor Matthias Hediger, brings together an interdisciplinary network of Swiss laboratories with expertise in

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membrane transporters in the search for treatments 'from gene to drug'. The concept of TransCure is to foster high-quality basic science research, and to develop therapeutic measures to correct dysfunction of transport proteins or use these for drug delivery. Molecular design, compound screening and analysis can feed the development of prototype drugs, which then can be tested in the laboratory *in vitro* and *in vivo*: "For example, an inhibitor is found by structure-based drug design and scintillation proximity assay (SPA)," Fotiadis continues. "Derivatives can then be synthesised by chemists and tested by SPA iteratively until a strong inhibitor is obtained, from which its inhibitory effect can then be explored by our cell biology and medical colleagues."

Fotiadis' laboratory is contributing their expertise in membrane protein biochemistry and structure determination to the programme and recently, in collaboration with Professor Jean-Louis Reymond at the University of Bern, established a new ligand screening approach using ligand-based virtual screening and SPA: "The innovative aspect of this approach is that purified target protein is used instead of living cells expressing the target protein, thus avoiding interference from endogenous proteins and ligand degradation," Fotiadis elucidates.

NANOCELL

Another programme in which Fotiadis' laboratory is involved is the biomimetic European Science Foundation-funded NANOCELL programme. This synthetic biology programme is led by Professor Daniel Müller (ETH Zürich) and consists of several collaborating laboratories across Europe. The goal of NANOCELL is to engineer and assemble biomimetic molecular machineries of the cell into nanocells with controllable functionalities not found in Nature: "Our contribution is to produce and engineer robust building blocks – also called modules – for nanocells and assemble nanocells containing translocating modules and energising modules in synthetic lipids," enthuses Fotiadis. For example, translocating and energising modules such as proton-driven transporters and light-driven proton pumps are assembled together by Fotiadis' group into small proteoliposomes corresponding to a binary nanocell. If correctly conceived, such 'molecular hoovers' can deplete a solution from toxic molecules by light-driven uptake into the interior of the container. The functionality of nanocells can be increased by adding more modules or extra features that can be placed into membranes or, indeed, into the interior of the nanocells themselves.


THE FOTIADIS GROUP

LOOKING TO THE FUTURE

"Knowledge has exploded during the last decades and will continue to grow exponentially in the future," muses Fotiadis. He believes that one of the biggest advances in structural biology over the next decade will come from the application of free-electron X-ray lasers (FELs). Such instruments will enable the visualisation of extremely fast processes, and thus support the acquisition of new insights into biological structures and dynamics at the atomic level. It is fortunate then that the SwissFEL will go online in 2016 at the Paul Scherrer Institute, which is only about 100 km from his laboratory.

Fotiadis hopes that such advances in technology will help to overcome the problem of having to crystallise membrane proteins, in particular human and eukaryotic membrane proteins, for structural biology in the future, so opening up immense possibilities for structure-based new drug design.

INTELLIGENCE

WHAT DO MEMBRANE PROTEINS LOOK LIKE?

OBJECTIVES

- To understand the molecular working mechanisms of membrane transport proteins
- To unveil the supramolecular organisation of membrane proteins and their complexes
- To identify potent inhibitors for membrane transport proteins as molecular tools and therapeutic agents

To achieve these objectives, structural data from X-ray crystallography, electron microscopy and atomic force microscopy is combined with information from biochemical and biophysical studies.

KEY COLLABORATORS

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PROFESSOR DIMITRIOS FOTIADIS gained his PhD and Habilitation at the Biozentrum of the University of Basel. After a stay in the US, he moved to the University of Bern in 2008 as Professor for Structural Biology at the Institute of Biochemistry and Molecular Medicine.