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# **PUSHING** **Frontiers** **SINGAPORE**



**NANYANG TECHNOLOGICAL UNIVERSITY**

**RESEARCH & INNOVATION**



## Membrane Protein Sciences in Singapore

By Jaume Torres and Pär Nordlund

# Tools for Rational Discovery of Novel Therapeutics and Diagnostics Targeting Integral Membrane Proteins

Lipids, nucleic acids, sugars and proteins are the main basic components of living organisms. The latter constitute about half of the dry body weight in humans. Proteins are simply strings of amino acids joined by so-called peptide bonds, and when these strings fold in a specific three-dimensional structure they can adopt many different functions, from purely structural, acting as simple building blocks or frames, to catalytic (enzymes), speeding up chemical reactions.

Proteins can be divided into two classes: water-soluble ones, that can be found inside or outside cells, and integral membrane proteins (IMPs), which inhabit only the lipidic (oily) membranes that surround all cells or their internal compartments (Figure 1). This localisation seriously hinders many types of studies that are routine in water-soluble proteins.

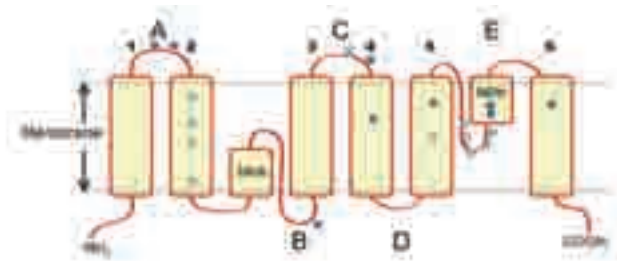


Figure 1. Topology of a typical IMP, an  $\alpha$ -helical membrane protein that spans the membrane several times. The scheme corresponds to the water channel protein aquaporin, which has characteristic hemi-loops inserted partially into the membrane.

IMPs are very abundant – they constitute a third of all the proteins in most organisms, from humans to bacteria – and they are critical for the survival of the organism. In the human cell, receptors for hormones, neurotransmitters, sugar transporters, ion-channels and enzymes play important roles in controlling critical signalling and metabolic events. Also many human pathogens such as HIV or influenza viruses and malaria parasites enter human cells by binding first to IMP receptors.

The understanding of disease mechanisms at the molecular level critically depends on structural and biochemical information. However, only ~170 unique structures of IMPs are known, with only a small fraction being of mammalian origin or of therapeutic relevance. This has to be compared to the tens of thousands of structures available for water-soluble proteins.

Structural and biochemical information is also of great importance for accelerating the discovery of novel lead drugs. Currently ~40% of drugs in the clinic are targeting IMPs. Early provision of high-resolution structures can substantially accelerate drug design efforts and the impact of structural biology in drug discovery is predicted to increase dramatically in the next decade. The availability of purified protein is essential for structure determination, and also to study the interaction of proteins and drugs. However, production of IMPs in the traditional host, i.e., the bacterium *Escherichia coli*, is notably difficult. This problem has been overcome in some cases by the use of other organisms such as yeasts, insect and mammalian cells, and 'cell free' extracts that contain just the essential components for protein production.

*This project is led by Principal Investigator Jaume Torres, Associate Professor at the School of Biological Sciences, College of Science, NTU. NTU Visiting Professor Pär Nordlund, Assoc Prof Julien Lescar, Asst Prof Tobias Cornvik, Assoc Prof Said Eshaghi and Assoc Prof Konstantin Pervushin are co-PIs in the project that received funding of up to S\$10 million (US\$7.9 million) under the National Research Foundation (NRF) Competitive Research Programme (CRP) Funding Scheme.*



One of the objectives of our research programme is to produce IMPs for the scientific community. The groups of Tobias Cornvik and Pär Nordlund have set up a Protein Production Platform at NTU@One North (Biopolis) (Figure 2, right).

When IMPs can be produced, the next problem is purification and handling. By their very nature, IMPs need to be constantly surrounded by lipids or by detergents, otherwise these proteins form unusable aggregates. This requirement poses additional problems, because the presence of artificial environments not only may affect protein stability and function, but makes crystal formation – and subsequent study by X-ray diffraction methods – more difficult.

One aspect of the programme is the establishment of general methods to increase the stability of IMPs to high temperatures (thermostabilisation). The strategy is based on a proprietary Colony-Filtration blot system created by Tobias Cornvik and Pär Nordlund, to screen for thermostable proteins at the colony level, i.e., before any culture is grown.

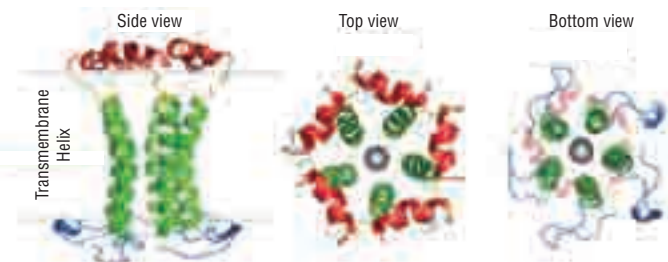
Most IMPs are poorly soluble, unstable, partially unstructured or highly flexible. All these factors make crystallisation efforts futile. A way to solve this problem is to crystallise IMPs in complex with antibodies, used normally by our immune system, which bind tightly and specifically to parts of a protein. Recently the simpler monomeric antibodies from *Camelidae*, e.g., llamas and camels, which are more stable and easier to manipulate and produce than traditional antibodies, are being favoured.

The IMP targets of our programme involve a wide range of size, function and organisms. For example, the group of Pär Nordlund targets enzymes involved in the production of messenger molecules that are potential targets for anti-inflammatory drugs. Konstantin Pervushin aims for structural studies of  $\gamma$ -secretase, an integral membrane enzyme that is involved in Alzheimer's disease. The group of Said Eshaghi attempts to unravel the mechanism of the so-called 'CRAC' channel, which is central to the activation of the immune system.



Figure 2. The Protein Production Platform at NTU@One North (Biopolis) aims to serve the scientific community by producing purified IMPs.

Figure 3. Typical structure of a small ion channel inserted in a lipid bilayer (delimited by the dotted lines), and top and bottom view of the same channel, where the grey central circle represents an ion. This structure was obtained using NMR by the groups of Jaume Torres and Konstantin Pervushin.



Channels of viral origin are the focus of Jaume Torres and Konstantin Pervushin (Figure 3). These proteins are multifunctional, and are critical for virus survival. Such proteins are found in coronaviruses, influenza, hepatitis or HIV. The group also investigates the activity modulation of aquaporins, critical components in the water homeostasis of the cell.

IMPs present in pathogens like Dengue virus causing Dengue haemorrhagic fever are investigated by the group of Julien Lescar. Some of these proteins enhance the cleavage of the viral polypeptide chain during virus replication.

Our combined programme attempts to address some of the fundamental technological hurdles in membrane protein science that are relevant for both the industrial and academic sectors. We aim at providing tools for drug discovery and for the generation of diagnostics that target IMPs.



3D protein visualisation on the multi-touch table. Assoc Prof Wolfgang Müller-Wittig (right), Director of Fraunhofer IDM@NTU, and Prof Gerhard Grüber (left) from the School of Biological Sciences are shown visualising a 3D model of an ATP synthase.

By Nicola Wittekindt

## Virtual and Augmented Reality for Science and Education

Interactive and digital media (IDM) take centre stage at Fraunhofer IDM@NTU, the first such centre in Asia for Fraunhofer-Gesellschaft, Europe's largest institution for applied research. The centre is part of NTU's efforts to advance applied research in New Media, one of five thrusts in NTU's five-year strategic plan to bring the university into the global league of excellence. It looks at solving real-world problems through enabling technologies, such as visualisation as well as virtual and augmented reality.

The S\$14 million (US\$11 million) centre conducts research with applications in education, industry, entertainment and tourism. Among its projects is a multi-touch table with an array of 3D applications. The multi-functional workspace allows multiple users to access various documents concurrently on a single horizontal touch table, while a big vertical screen displays functional information in various formats such as 3D models, videos or documents. Simple hand movements on the touch table allow users to move through visualisations of 3D models; a valuable application for instance for architects and urban developers to "roam" 3D models of buildings and cities while changing the viewing perspectives.

Mobile maintenance

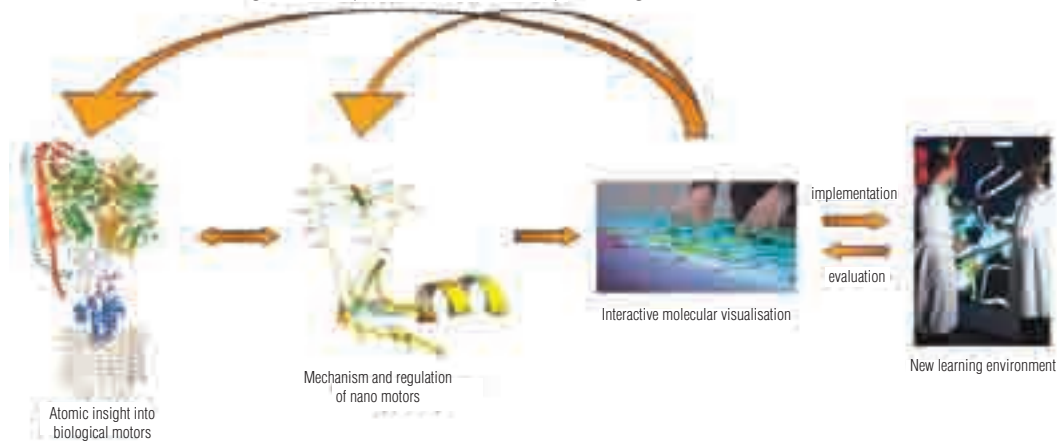


Furthermore, the multi-touch table can be connected to multiple screens that allow for visualisation of images in different scales.

3D protein visualisation is another highly regarded application for the multi-touch table. The technique to visualise 3D models of high-molecular proteins was developed jointly by Assoc Prof Wolfgang Müller-Wittig, Director of Fraunhofer IDM@NTU, and Prof Gerhard Grüber of NTU's School of Biological Sciences. They created a communication platform for the planning, interpretation and presentation of atomic structures of the smallest biological motor ATP synthase, which produces the molecule ATP (adenosine triphosphate), the energy currency of cells. Prof Grüber had previously determined the atomic structures of the energy producer A1AO-ATP synthase, a protein complex comprised of several subunits.

The use of a multi-touch table allows for an intuitive interaction with the dynamic motor protein on an atomic level. The simulation results provide a platform to study the mysteries of protein folding and the interplay of protein structures and to gain insights and inspiration for new biological experiments. Given the availability of their atomic structures, other proteins and multi-protein complexes can be visualised on the multi-touch table, facilitating communication among scientists. In addition, the visualisation, probing, and manipulation of such complex intramolecular processes provide a new teaching and learning tool for complicated biological systems. Virtual manipulations of the protein structures – by changing data points corresponding to individual parts of the molecular structures – allow for the analysis

## Platform To Design New Experiments And Therapeutic Agents



3D protein visualisation facilitates the design of new experiments and therapeutic agents.

of structure-function relationships such as the impact of structural changes on movements of motor protein subunits. Thus, the system enables virtual “experiments” on intra- and inter-molecular processes that can be applied to biomedical research, materials testing or other areas of biological or chemical sciences. Finally, the virtual representation enables fruitful discussions on future projects and investments. This new level of quality in representing protein structures was presented at the world’s largest computer exhibition, CeBIT, in Hannover, Germany, in March 2010 and 2011.

Other research projects at Fraunhofer IDM@NTU exploit Augmented Reality for education. The Augmented Chinese Learning Game allows learners to mix and match Chinese character cards that are read via a web camera to produce the relevant 3D object and audio pronunciation. Real-time experimentation is provided in the Augmented Physics Lab, where students can design virtual electrical circuits to power a real light bulb by placing cards representing components like resistors or transistors.

Mixed reality physics lab



Augmented Reality for mobile devices makes virtual guides possible and facilitates mobile e-learning. For instance, pointing the built-in camera of your phone at a building will display the building’s history – in historical pictures overlaying the actual one – and elaborate on its architecture or important historical or cultural events.

In a further application, the On-Demand Maintenance System enables engineers and technicians to monitor the well-being of technical systems. By pointing the camera of a mobile device at nodes or other equipment parts of the system, the Augmented Reality technology compares the current status to the default status and visualises correct functionality versus discrepancies by highlighting the respective parts in green or red, respectively.

Thus, Virtual Reality, Augmented Reality and Visual Analytics applications developed by Fraunhofer IDM@NTU help to solve problems in science and engineering by improving discovery processes as well as teaching and learning in a wide variety of domains.

Chinese learning game



## Self-healing Anti-rust Coating System



Self-healing coating (left) and control coating (right) after 24-hour immersion in salt water

Asst Prof Yang Jinglei from the School of Mechanical & Aerospace Engineering (MAE) and his team have successfully developed a special anti-rust coating system for metals. The anti-corrosion coat contains microscopic capsules loaded with chemicals that are highly reactive to water and enable scratched surfaces to self-heal. When the coating is scratched, the microcapsules break and release the chemicals that react with water or moisture in the air. Using its 'superglue' properties, the coating will form a protective layer of polymer over the scratch, acting as a sealant and preventing rust and corrosion from setting in. The self-healing coat has also been tested under harsh environmental conditions including humidity and concentrated salt water. In wider applications, this breakthrough reduces maintenance costs due to rust and corrosion on ships, planes, bridges, and infrastructures and buildings.

The article, "*Facile microencapsulation of HDI for self-healing anticorrosion coatings*", was published in *Journal of Materials Chemistry*, 2011, 21, 11123-11130.

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## A Simple Blood Test to Predict Heart Attacks and Strokes

NTU's Asst Prof Newman Sze and his collaborators at the Interuniversity Cardiology Institute of the Netherlands have discovered more than 10 types of unique proteins in the blood of stroke or heart attack patients that can be used as a diagnostic tool to pinpoint the risk of heart disease in a person. The researchers seek to do a similar study locally in Singapore's multi-racial population. The study will be facilitated by new cutting-edge research equipment — the Agilent HPLC-Chip LC/MS Triple Quadrupole system — that allows for parallel and simultaneous measurement of all biomarkers of interest in multiple samples. The local study will last one year and involve some 2,000 samples collected in Singapore and the Netherlands.

