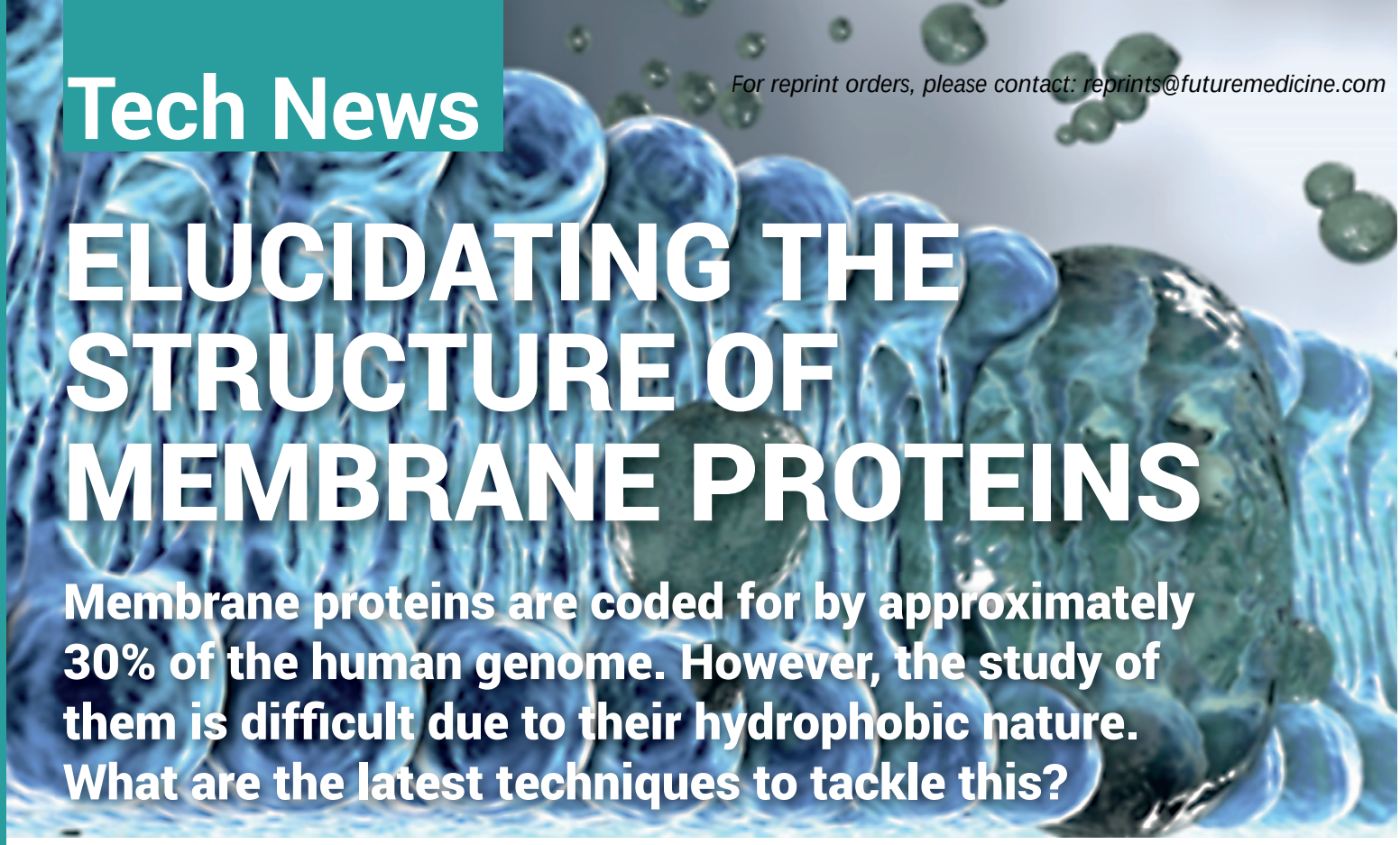

ELUCIDATING THE STRUCTURE OF MEMBRANE PROTEINS

Membrane proteins are coded for by approximately 30% of the human genome. However, the study of them is difficult due to their hydrophobic nature. What are the latest techniques to tackle this?

You may be surprised to learn that membrane proteins are coded for by approximately 30% of the human genome [1]. That's nearly a third of all genetic information in our cells dedicated to the production of membrane proteins. Although elusive, these proteins are critical for cellular function, especially in cell communication and transport pathways.

The cause of their elusiveness can be attributed to their hydrophobic nature, leading to difficulty in structural studies because they can't be dissolved in water and are prevented from crystallizing – a necessary step in techniques such as x-ray crystallography.

Once extracted from cell membranes, the proteins are made water-soluble only when suspended in detergents that mimic the hydrophobicity of a cell membrane. However, these are expensive and there is no 'one size fits all'. Detergents can also disrupt the structure and function of membrane proteins, as they interfere with inter- and intramolecular protein–protein interactions.

> *"If there is no rule to follow, it's difficult for people to understand how to do it..."*

Of ~8000 known membrane proteins found in human cells, only ~50 have a determined structure [2]. With membrane proteins being implicated in many different diseases [3], including heart disease, Alzheimer's and cystic fibrosis, it is of crucial importance that structures are characterized in order for novel ideas for therapies and treatments to come to light.

So, just what new approaches and techniques are being developed to tackle this issue?

GETTING TO THE CRUX OF THE PROBLEM

Developing novel methods and adjusting well-established techniques seem like the most straight-forward and logical ways to solve the issue. But what if the answer was not in adjusting the techniques, but in altering the subject of analysis? After all, the hydrophobicity of membrane proteins is where the crux of the problem lies.

Researchers from MIT (MA, USA) have done just that, developing a method to make these proteins water-soluble. They take a membrane protein and apply personalized computer algorithms that result in the production of a code, which is much simpler than previous attempts to make the proteins soluble. This enables the swapping of some hydrophobic amino acids for hydrophilic ones [4].

"If there is no rule to follow, it's difficult for people to understand how to do it," explained principal research scientist Shuguang Zhang. *"The tool has to be simple, something that anyone can use, not a sophisticated computer simulation that only a few people know how to use."*

Inspired by the late MIT biology professor, Alexander Rich, who asked whether it might be possible to take hydrophobic alpha helices and make them hydrophilic, Zhang began working to find an answer to this question in 2010.

The aforementioned code that allows hydrophobic amino acids to be swapped for hydrophilic ones is based on the understanding that some hydrophobic amino acids are very similar in structure to particular hydrophilic amino acids. Therefore, leucine can be switched to glutamine, isoleucine and valine can both be switched to threonine, and phenylalanine can be switched to tyrosine.

Another important consideration is in the charge of the amino acids. If they were swapped out for ones of opposite charge, it could have a great impact on the structure of the protein. However, all of these amino acids have a neutral charge, so there is no real impact on overall structure.

The code is named QTY after the letter representations of the three amino acids – glutamine, threonine and tyrosine, respectively. The team discovered that all the hydrophobic amino acids needed to be replaced in order for the protein to be completely water-soluble without the need for any detergent.

"It's only when we replace all the hydrophobic residues in the transmembrane regions that we're able to get proteins that are stable and completely free of detergent in an aqueous system," commented first author Rui Qing.

They demonstrated the technique on four different types of G protein-coupled receptors. While they have shown that the modified proteins denature at similar temperatures to their original counterparts, and the modified proteins can also bind the same target molecules although not as strongly, they are yet to obtain precise structures utilizing x-ray crystallography or NMR techniques.

As well as the benefits of this method in terms of elucidating protein structures, it is also hoped that there could be functions for these water-soluble versions of membrane proteins, including in cancer diagnostics, as sensors for environmental pollutants and to capture viruses.

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STEALTHY ARTIFICIAL MEMBRANES

The difficulties in elucidating membrane protein structures lie not only in their hydrophobicity, but in removing them from their native lipid environment. Therefore, a team of researchers from the Institut Laue-Langevin (Grenoble, France), in collaboration with Copenhagen University (Denmark), successfully pioneered a novel technique that allows the visualization of the structure of membrane proteins while embedded in a lookalike lipid membrane.

Artificial nanoscale bilayer discs that carry the membrane proteins in solution and mimic the native lipidic bilayer environment allow for small-angle scattering techniques to be carried out. However, the nanodiscs contribute to measured scattering intensity and make analysis difficult. The team discovered that deuterium labeling the carriers ensures the membrane is invisible to neutron diffraction in 100% D2O, while the structure of the membrane proteins can be highlighted.

These altered nanodiscs have been rightly termed 'stealth' carriers and can be utilized as a platform for low-resolution structural studies of membrane proteins using data analysis tools that were originally developed for water-soluble proteins. The technique was first published in 2014 [5]; however, the first structural study of a membrane protein utilizing this method has only been described in 2018 [6].

Researchers led by Henning Tidow (University of Hamburg, Germany) applied the stealth carrier method strategy to study the structure of an ATP-binding cassette (ABC) transporter protein, MsbA, which is involved in lipid transport functions in bacteria. They utilized the Deuteration Laboratory platform, in combination with small-angle neutron scattering and X-ray scattering, resulting in neutron scattering data that successfully observed the signal from the membrane protein without any input from the surrounding lipid.

The technique also enabled the study of conformational changes in MsbA, demonstrating the sensitivity. This approach is likely to be increasingly important in future membrane protein studies, especially in the development of drugs to target membrane proteins implicated in disease pathways.

OPENING NEW DOORS FOR MASS SPECTROMETRY

Extraction of membrane proteins from their native environment, which is complex and heterogeneous, can alter their integrity. Now, a multi-institutional research team led by the University of Oxford (UK) has developed a new technique that can analyze the structure of intact protein machines within membranes, without any alteration to their structure and functional properties [7].

The new technique uses vibration at ultrasonic frequencies to encourage the cell to fall apart, while electrical currents then apply an electric field to eject the protein machines out of the membrane, directly into a mass spectrometer. Not only did these protein machines then emerge intact, but the analysis also enabled the discovery of their methods of communication, how they find their final location in the membrane and how they transport their molecular luggage into a cell [8].

Lead author Carol Robinson (University of Oxford) commented: *"I wasn't sure this would ever work; I thought the membrane environment would be just too complicated and we wouldn't be able to understand the results. I am delighted that it has because it has given us a whole new view of an important class of drug targets."*

The development of this new technique opens new doors for the application of mass spectrometry: *"With the development of this method, the application of mass spectrometry in biology will be taken to a new level, using it to make discoveries that would not have been possible before,"* explained Steve Matthews (Imperial College London, UK). ▶

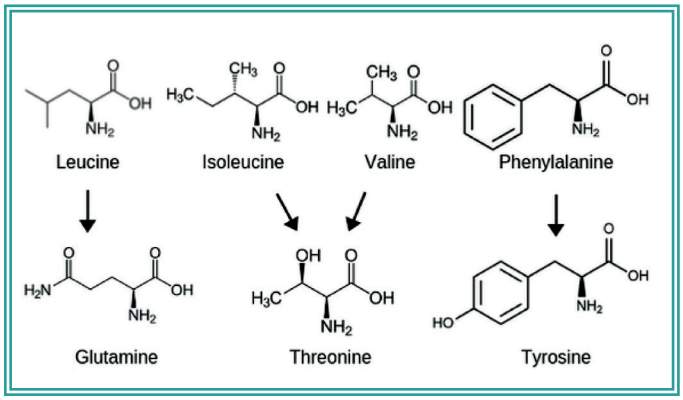

Figure 1. Amino acid structures displaying the switches that can be made from the original hydrophobic amino acids to the similar hydrophilic ones.

Figure 2. Taka-aki Tsunoyama sits at the station from which he controls the microscopes and views membrane protein movements. Credit: Sophie Protheroe, OIST. Reproduced with permission from [11].

Co-author Sarah Rouse (Imperial College London) explained how the development of this new technique to study cell membranes has the potential to transform the way we study disease. *"A longstanding question on the structure of one membrane machine from mitochondria has now been solved using this technique. Mitochondria are particularly interesting because there are several diseases that target them specifically, that we may now be able to design new therapies for."*

This new technique not only sheds light on the interactions between uncovered proteins, lipids and chaperones at unmatched mass resolution but also provides a new perspective on the effects of drugs and disease-associated mutations on target complexes within the context of their native membrane environments.

TWO IS BETTER THAN ONE

In another attempt to study membrane proteins in their native environments, Baker *et al.* developed a hybrid method combining high-resolution solid-state NMR spectroscopy (ssNMR) and electron cryotomography (cryoET) of the same sample [9].

As mentioned previously, most techniques used to study membrane structural biology can significantly alter the structure and function of membrane proteins, through the use of detergents in the purification process. The purified membrane proteins can also be transferred into synthetic lipid bilayers, like the stealth carrier nanodiscs, which have limitations on whether they can completely mimic the complexity of the proteins' natural environment.

Both cryoET and ssNMR provide highly complementary information – conformational and dynamical changes observed by ssNMR help interpret functional results from cryoET. To take advantage of this, Baker *et al.* designed an experimental system with a single sample, suitable for both techniques. This allowed the structure and function of bacterial membrane proteins to be studied in their native environments, across different spatial and temporal resolutions. Moreover, this combination is more powerful than each technique individually [10].

> *"...the development of this new technique to study cell membranes has the potential to transform the way we study disease."*

Whilst the research mentioned here is generally applicable to *E. coli* membrane proteins, this technique has the potential to be expanded to other bacterial and eukaryotic cell lines. Additionally, this approach provides an appealing framework through which the structure, dynamics and function of membrane proteins can be effectively characterized.

WITNESSING PROTEINS DANCING

In order to properly visualize the structure, function and interactions of membrane proteins in relation to the lipid bilayer membrane as a whole, a team of researchers from the Okinawa Institute of Science and Technology Graduate University (Japan) developed an imaging method for live cells named live-cell single fluorescent-molecule imaging (SFMI).

In this, they tag membrane proteins with fluorescent markers to watch the proteins move within the membrane, which they compare to a ballet dancer moving around a stage. *"The proteins in the cell membrane undergo elegantly coordinated dances to relay messages between the cell and its environment,"* commented senior author Akihiro Kusumi [11].

However, the fluorescent markers undergo photobleaching and, over time, lose their fluorescence. Therefore, it was impossible for cell biologists to observe the movements of individual molecules for longer than 10 s. *"It was like randomly taking many 10-second long clips and trying to connect them in the correct order to produce a movie lasting for 5 minutes,"* explained first author Taka-aki Tsunoyama.

Now, a paper published in *Nature Chemical Biology* details an update to SFMI in which photobleaching is suppressed [12]. Previous attempts to suppress photobleaching included the removal of molecular oxygen, which is toxic to living cells, and therefore not very effective. The improved SFMI method involves cells being placed in a low oxygen environment, with the addition of two mild chemicals named trolox and trolox quinone.

This approach successfully reduces the effects of photobleaching while having minimal impact on cell viability, allowing individual molecules to be observed for up to 400 s. *"Our method improves the observation time of fluorescent molecules by fortyfold,"* commented Kusumi.

The increased length of time they were able to observe the molecules for meant they had a much clearer picture of how the molecules move and interact within the cell membrane, without any interruption. *"With our method, we can now follow the movement of each individual molecular dancer for sufficiently long periods of time*

to understand the cellular context," concluded Kusumi.

It is hoped this research could lead to the development of drugs that prevent the migration of cancer cells through the body through improved study of focal adhesion proteins, which link the internal skeleton of the cell to the extracellular matrix.

FUTURE PERSPECTIVE

The abovementioned techniques allow for visualization of the structure of membrane proteins, which has the potential to shed light upon many different diseases, from heart disease to cystic fibrosis. The hope is to use the knowledge gained from these new techniques to develop drugs to target membrane proteins implicated in disease pathways.

Written by Joseph Martin & Abigail Sawyer
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