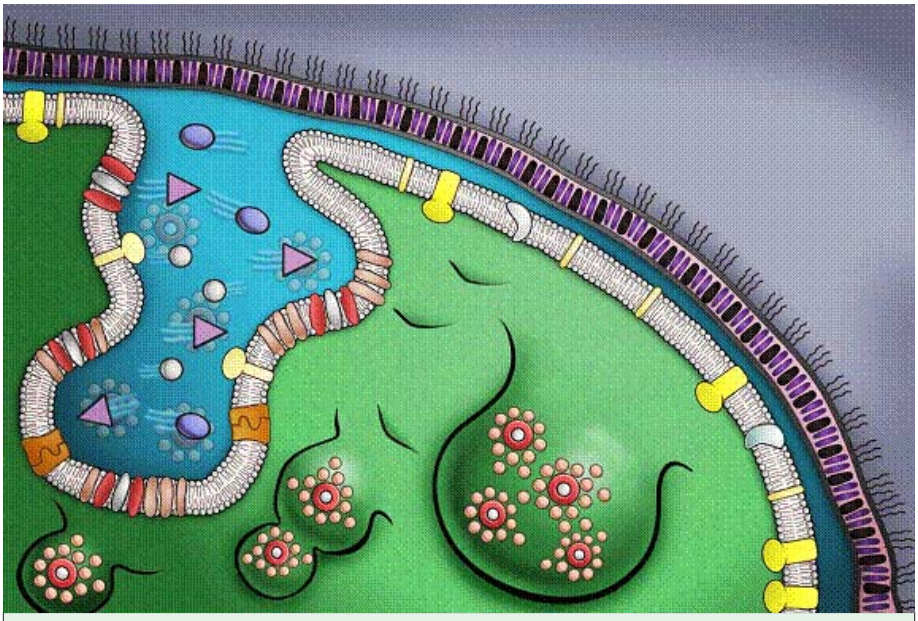
Argonne NATIONAL LABORATORY

Membrane protein 'factory' may lead to new drug treatments

Biologists at Argonne National Laboratory have engineered and patented a bacterial factory that enables the study of membrane proteins. These proteins are challenging to study, but critical to understand because they represent 60 percent of drug targets. Studies of membrane proteins could lead to new and improved pharmaceutical treatments for a broad range of illnesses such as depression, heart disease, addictions and cystic fibrosis.

Membrane proteins perform essential processes in the cell, such as controlling the flow of information and materials between cells and mediating activities like nerve impulses and hormone action. These proteins are located in the rugged, oily two-layered membrane that holds the cell together. One-third of the genome of any organism encodes membrane proteins.



PROTEIN FACTORY – Argonne biologists developed a membrane protein production factory using photosynthetic Rhodobacter*, which can be engineered to express and incorporate the proteins into the cell's cytoplasmic membrane shown in white. Membrane proteins are difficult to study in traditional ways. Since they make up 60 percent of all drug targets, researchers are working to overcome these challenges.*

"When a cell is attacked by a virus or a bacterium, primary entry into the cell is via an association with proteins in the cell membrane," said Argonne biophysicist Phil Laible. "In addition, in many disease states, the essential processes controlled by membrane proteins go awry. That is why so many membrane proteins are drug targets."

Biologists use three-dimensional images of proteins to better understand how proteins work. In drug design, for instance, the 3-D images help researchers develop a drug that specifically blocks binding of a biological attacker that would cause disease.

Researchers in Argonne's Biosciences Division are world leaders in automating the many steps it takes to determine 3-D structures of proteins and have cut time and costs doing it. The structures of thousands of proteins are now known.

"But those are water-soluble proteins," said biochemist Deborah Hanson, who patented the bacterial factory with colleague Laible. "Membrane proteins are harder to study at every step.

"The first step in studying most proteins is to dissolve them in water," Hanson said, "but that does not work with membrane proteins that live in the oily, lipid bi-layer that surrounds the cell."

Researchers studying water-soluble proteins often use commercial *E. coli*-based systems to express, or produce, copies of the protein. When membrane proteins are produced in *E. coli*, they overload the cell's bi-layers and cause the cells to die. The sources that have yielded the majority of the few known membrane-protein structures are organisms in which the target membrane protein is naturally abundant.

Laible and Hanson took advantage of the natural characteristics of the *Rhodobacter* species of photosynthetic bacteria they were working with in another project. Under certain conditions – in response to light or oxygen – *Rhodobacter* naturally produces large quantities of internal membranes.

The biologists developed a system that successfully expresses hundreds of copies of a chosen membrane protein in *Rhodobacter* while simultaneously synthesizing the internal membranes they want to live in.

So far the team has cloned about 500 genes into *Rhodobacter*. "First," Laible said, "we produced a variety of membrane proteins of different sizes, functions and physical properties, and we have had a 60 percent success rate with them. Now we have cloned all of the membrane proteins of *E. coli* and are continuing production."

As they continue to manufacture different membrane proteins, the team is tackling the next step to creating a pathway to protein crystallization for membrane proteins by developing specialized molecules, or reagents.

"We are working," Laible said, "with a multidisciplinary team from the University of Wisconsin-Madison, the University of Illinois-Chicago and deCODE biostructures, Inc. of Bainbridge Island, Washington." They will focus on three types of reagents:

1) Designer detergents that remove the membrane protein from the lipid bi-layer where it resides,

2) Antibodies to stabilize the membrane protein, and

3) Molecules that mimic the lipid bi-layer, or membrane.

Researchers will test the reagents on the membrane proteins produced in the *Rhodobacter* 'factory.'

Funding for this research has been provided by the National Institute of Health's National Institute of General Medical Sciences, which recently granted the biologists a $5 million, five-year research grant to continue their pursuit of a process leading to 3-D structures of membrane proteins. — *Evelyn Brown*

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