Lipids, Nanoparticles and Beyond!

You've heard of fats – one of the primary nutrients our body needs. Well, fats are just one type of lipid – a group of biomolecules known for their inability to dissolve in water.

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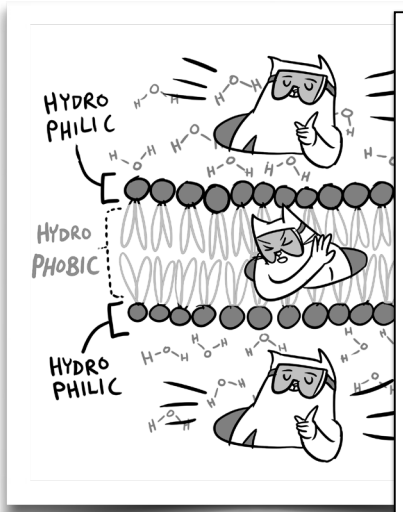

Lipids also make up biological membranes – the sacs that contain and protect cells. Because of their love-hate relationship with water, membrane lipids are typically arranged as a bilayer, with the hydrophobic ("water fearing") tail facing inwards and the hydrophilic ("water loving") head outwards.

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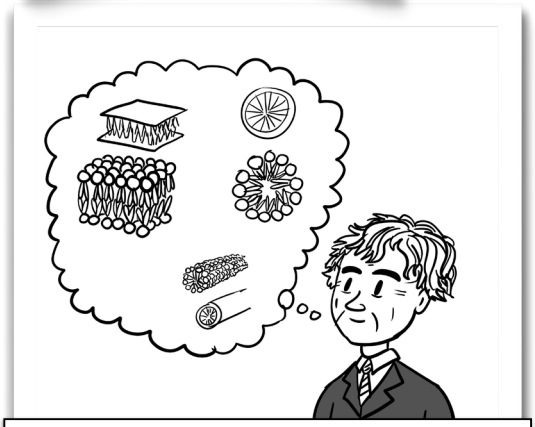

Though lipids are known for their bilayer structure, they can form all sorts of different shapes. But what drove the differences in lipid membrane shape and structure? That was the question of Dr. Pieter Cullis, physicist-turned-biochemist.

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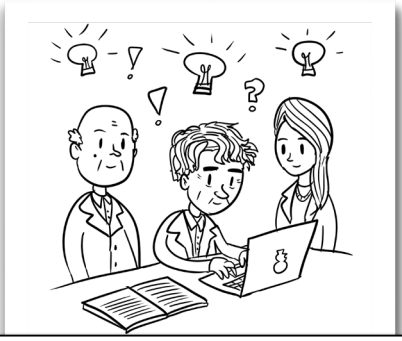

Because biological membranes have many types of lipids, they can be difficult to study. However, Dr. Cullis had a plan. He and his team would use artificial "model membranes" to isolate and characterize individual lipids.

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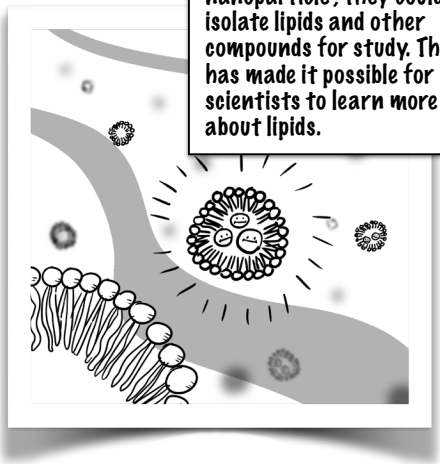

By developing what they called a "lipid nanoparticle", they could isolate lipids and other compounds for study. This has made it possible for scientists to learn more about lipids.

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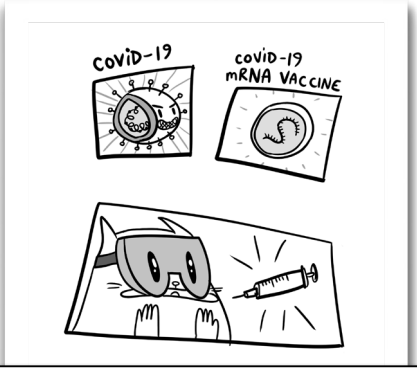


As well, these lipid nanoparticles could also be used to deliver compounds into cells. One important example of this is the revolutionary work that led to the COVID-19 mRNA vaccines, changing the face of medicine forever.

Art by Armin Mortazavi and text by Braydon Black. October 2022

Lipids, Nanoparticles and Beyond!

The biochemistry of your cell membrane leads to insights that saves millions of lives.

Written by Braydon Black
Art by Armin Mortazavi

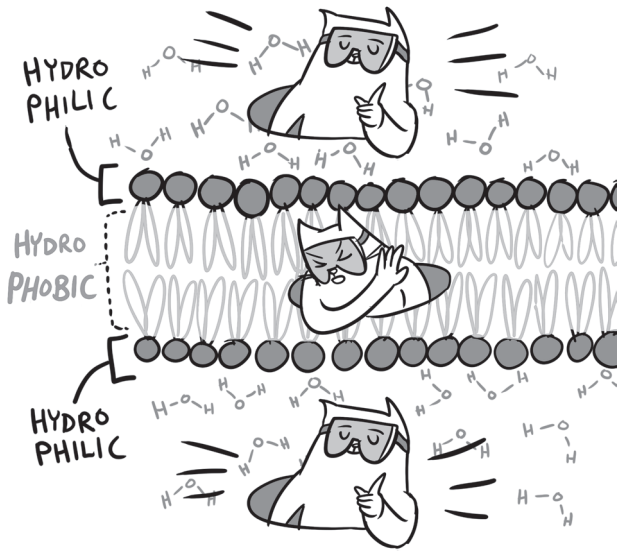
October 2022

The human body is a fascinating and complex thing. Our bodies contain trillions of cells that work together to keep us alive. These **cells**, each with their own function, are what make you *you*! They help keep you nourished and hydrated, and allow you to breathe, see, feel, and even think. Cells can do all of this, partly because they have distinct internal compartments. In other words, the inside of the cell is separated from the outside. Having this compartmentalization means that individual cells can be protected from external threats, but also that individual cells can have specific jobs.

But what are cells, anyways? And how do they function? Well, cells are a basic building block of life – think of them as tiny machines working in massive numbers that allow our bodies to work. Each cell, itself, has many parts, but the three main components for human cells include the **nucleus** (where DNA is stored), the **cytoplasm** (the fluid that fills the cell), and the **cell membrane** (the envelope that surrounds the cell and keeps it together). In particular, the cell membrane is like a good coat for the cell – it protects the good stuff inside and keeps bad stuff out. In order to do this, cell membranes need a few things, but one type of molecule in particular is especially key — **lipids**.

Lipids are a type of chemical that are defined by their inability to dissolve in water. Fats, for instance, are one type of lipid, and fats and water just don't mix (try adding oil to water to see this).

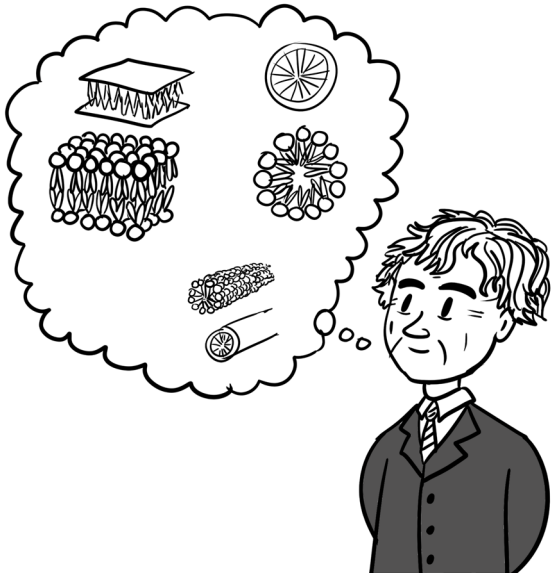
Because of their love-hate relationship with water, cells use lipids to construct cellular membranes. These lipids, many of which are called **phospholipids**, make up the cell membrane bilayer. They have a **hydrophobic** ("water fearing") tail facing inwards and the **hydrophilic** ("water loving") head facing outwards, meaning that they can interact with the aqueous or water environment inside and outside of the cell, but do not allow water or other polar substances to freely cross the membrane.



One lipid, two lipid, red lipid, blue lipid

The peculiar nature of lipid bilayers sparked the interest of Dr. Pieter Cullis, physicist-turned-biochemist. Though lipids were known to form

bilayers in water-based environments, this wasn't always the case. Lipids, he found, could form all sorts of structures, and the distribution of lipids in these membrane structures was asymmetrical – a property that was driven by the differing chemistries inside and outside of the membrane. This meant that the asymmetrical nature of membrane lipids was not necessarily random - that different types of lipids could be distributed throughout the membrane in an organized way – providing areas of a membrane that have their own set of biophysical properties and functions.



However, one major problem stood in the way – how to study the many, many types of lipids that made up the cell membrane? A cell membrane can contain thousands of different types of lipids, making it difficult to isolate and study the function of each type. But Dr. Cullis and his team had a plan. His team devised a system they called **model membranes** – a set of artificial membrane sacs (or vesicles) that could be used to isolate and control these membrane lipids for further study. By manipulating the salt environment or pH around these lipids, they found that they could generate creation of asymmetrical lipid bilayers similar to those seen in living cells.

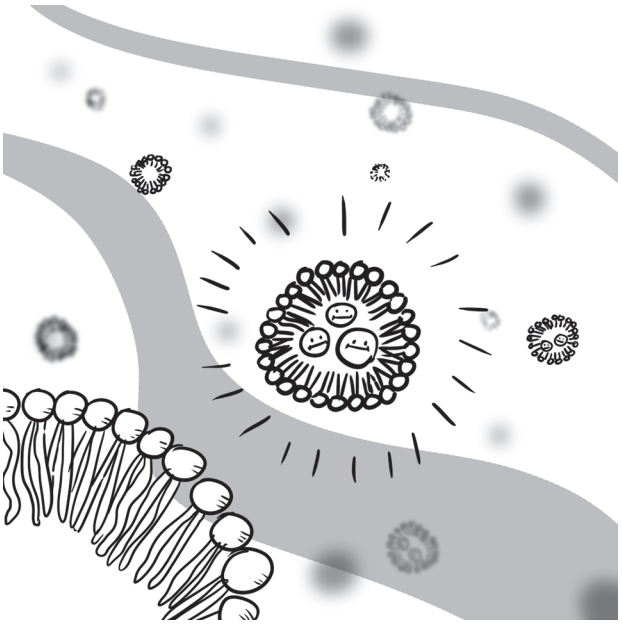
To do this, Dr. Cullis and his team developed model membranes using **ionizable cationic lipids**. These are lipids that can exist in either a neutral or positively charged form, sort of like a switch. Using these ionizable lipids was crucial to the model membrane design – it meant that the electrical charge of the membrane could be manipulated based on the physiological environment.

But why did this matter? Well, at the time it was known that cationic (or positively charged) vesicles could be taken up by cells. This concept intrigued researchers, as it provided a potential avenue to transport precious cargo (such as drugs) directly into cells. However, cationic vesicles themselves were toxic to the body making them poor candidates for drug delivery. For Dr. Cullis, there was another way. His solution was to use these ionizable cationic lipids that could remain neutral (and therefore safe) outside cells but could then become positively charged once taken up by cells. That way, lipid vesicles with targeting molecules could move through the body and bind to their target cells. They could then enter these cells and upon turning positively charged, and could release their cargo without causing harm to other parts of the body.

His idea was ground-breaking and had enormous implications for medicine and biology. This technology could enable direct targeting and delivery of compounds to different parts of the body with unprecedented precision. Cancer drugs, for example, could be packaged up and delivered directly to tumor cells, avoiding the often-harmful side effects that come with untargeted treatments like chemotherapy. Using these vesicles to package and deliver drugs offered many other advantages over traditional drug treatments. Not only could drugs be directed to avoid off-target effects – they would also be more stable during transit, have better absorption by cells, and would be less susceptible to drug resistance.

The potential of this technology – now termed the **lipid nanoparticle** (LNP) – was tremendous! Dr. Cullis saw an opportunity to bring it to medical research, and started a business centered around LNP drug delivery. At first, they focused on the targeted delivery of cancer drugs to cancerous tissue using LNPs, and their results were promising. However, business was tough – there wasn't much money to be had in delivering drugs that were already on the market. They needed something

new to put LNPs on the cutting edge of medical research.



Gene therapy and the future of medicine

It was around this time that the concept of **personalized medicine** was on the upswing. According to personalized medicine, medical treatments should consider genetic differences between individual people. Why? Well, most medicine focuses on how drugs and treatments affect broad populations – for example, the effectiveness of a drug on average for a given population. However, prescription drugs and treatments don't always work well for everyone. The reality is that each person may respond differently to the same dose of medication or may be at higher or lower chance of certain outcomes, good and bad. Because of these individual genetic differences, a "one size fits all" model can't provide everyone with the medical treatments that they need.

After starting their anticancer drug delivery business, Dr. Cullis and his team realized that the popularity of personalized medicine was taking off. And one of the next big areas of research on the horizon was **gene therapy** – a form of personalized medicine focused on treating disease by altering a person's genetic makeup. Many diseases such as cancer, cystic fibrosis, hemophilia, sickle cell anemia, and countless others have a strong genetic component, meaning that they are linked to missing or malfunctioning genes in the genome. Gene therapy offers a potential way to correct these genes by using foreign DNA (or other genetic material, such as RNA) to restore normal gene function.

However, it's very difficult to just deliver DNA or RNA to cells. The problem with introducing DNA and RNA is that they are often considered as "foreign" by the body's immune system and will be broken down in the same way that an infection might be, in order to defend the body. Luckily, the LNPs Dr. Cullis had been using are immunologically safe and could be used to transport large molecules such as DNA and RNA to the cell. This sophisticated delivery system of LNPs began to revolutionize treatment of genetic diseases. For example, the LNP-based drug *Patisiran* was developed using RNA to target mutated genes responsible for amyloidosis.

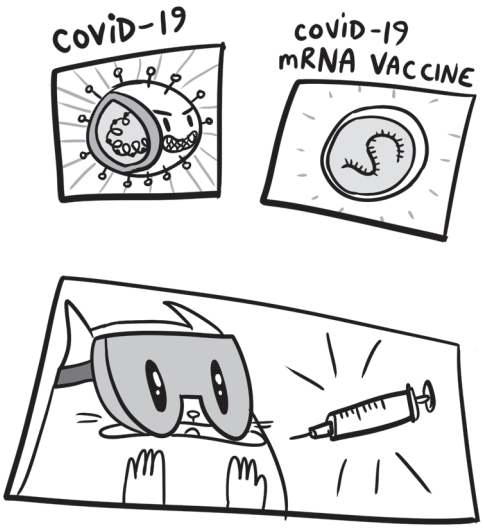
The advent of mRNA vaccines

Around the same time, Drs. Drew Wiesmann and Katalin Karikó saw the potential of LNP therapeutics for vaccine development. Until only recently, viral vaccines (such as the flu vaccine) were developed using modified or inactivated viruses and often had limitations with loading efficiency, safety, and launching an effective immune response. However, they had a novel idea – what if the mRNA blueprints for viral proteins could be packaged up and sent into our cells in the form of a vaccine? Once in our cells, the body would produce that viral protein and launch an immune response against that virus.

Clinical trials with **mRNA vaccines** against Zika virus began shortly thereafter, and the results were good. But in March 2020, the focus shifted – it was the start of the COVID-19 pandemic. Safe and effective vaccines were needed urgently around the world to stop the spread of SARS-CoV2 – the virus that causes COVID-19. Researchers had to act fast, and LNP-based mRNA vaccines were one of the answers. Today, both the Pfizer/BioNTech and Moderna vaccines for COVID-19 use LNPs to deliver viral spike protein mRNA to our cells. In doing so, our bodies can produce and recognize

this spike protein to fend off invading SARS-CoV2 that might otherwise cause serious harm.

Because of the work of Drs. Cullis, LNPs have delivered mRNA vaccines to billions of arms around the world and have kept SARS-CoV2 at bay. The future of medicine has also changed. Now, researchers can develop medical treatments in a whole new way, including gene therapies that can change the lives of millions with genetic disorders.



Despite his outstanding achievements, Dr. Cullis remains humble. His advice? To remain ever curious. “If you get fascinated by something, follow the fascination, “ he says, “and don’t be afraid to change directions. Build your confidence, try new things, and learn from what you have done. Because you never know where it will take you.”