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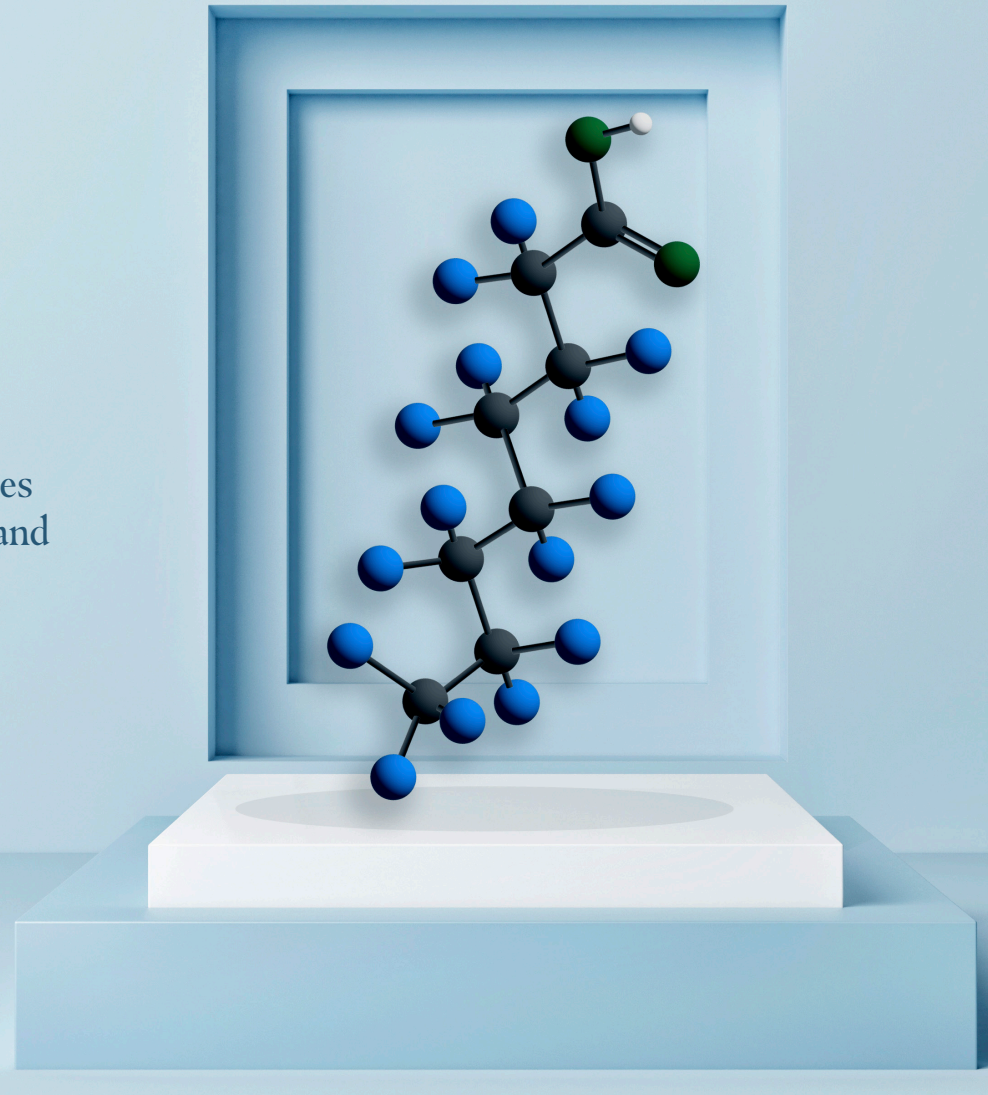
THE EMERGING CRISIS OF "FOREVER" CHEMICALS



Could We Develop Vaccines for Depression and Anxiety?

The "Streetlight Effect" in Proteomics

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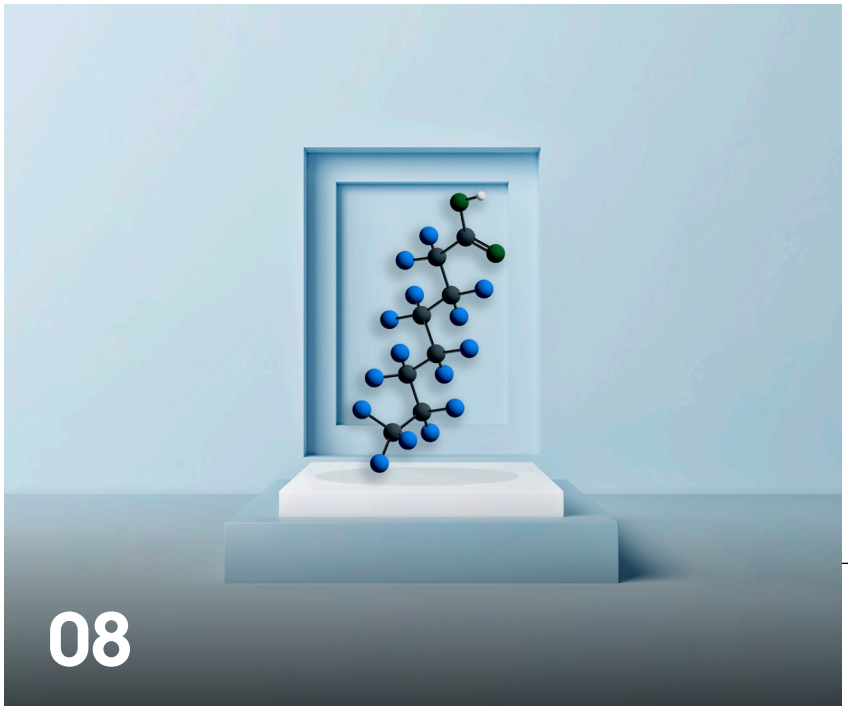

FEATURE

The Emerging Crisis of "Forever" Chemicals

Kerry Taylor-Smith

EDITORS' NOTE

Welcome to issue eighteen of *The Scientific Observer*, the monthly online magazine brought to you by *Technology Networks*.

This month the newsroom explores studies uncovering signaling pathways that may contribute to the astounding longevity of Indian jumping ant queens, the links between influenza vaccines and stroke risk, as well as the mechanisms responsible for our urge to eat fatty foods.

After existing for almost a century largely unnoticed, per- and polyfluoroalkyl substances are garnering increasing attention due to growing evidence of their damaging impact on the environment and human and animal health. But what do we know about these substances, why are they such a problem and do they really last "forever"? Kerry Taylor-Smith addresses these questions and more in this month's feature article, *The Emerging Crisis of Forever Chemicals*.

Focus is also given to the development of pharmaceuticals to treat and prevent mental health conditions. Simon Spichak discusses the potential of vaccines to be used as a preventative for depression and anxiety, while Ruairi J MacKenzie investigates the current state of the non-hallucinogenic psychedelics field.

We hope you enjoy exploring this issue of *The Scientific Observer*. Subscribe to make sure you never miss an issue.

Have an idea for a story?

If you would like to contribute to *The Scientific Observer*, please feel free to email our friendly editorial team.

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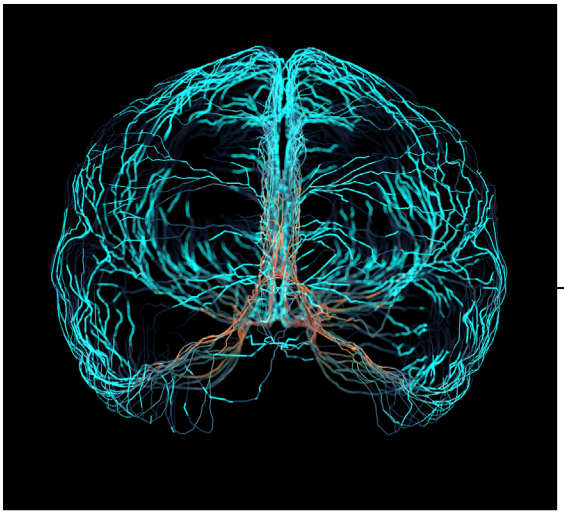
From the Newsroom

OUR URGE TO EAT FATTY FOODS IS CAUSED BY A GUT-BRAIN SIGNAL

RUAIRI J MACKENZIE

The temptation of a burger or pizza has surprisingly little to do with our taste buds, finds a new study. Instead, connections between our gut and brain dictate our obsession for fatty foods.

JOURNAL: *Nature.*



COULD FLU VACCINES PROTECT AGAINST STROKE?

MOLLY CAMPBELL

A new study has identified an association between receiving an influenza vaccine and a reduced risk of stroke.

JOURNAL: *Neurology.*

Harpegnathos saltator worker ant.

ANTI-INSULIN SIGNALS HELP QUEEN ANTS LIVE LONG AND PROSPER

SARAH WHELAN

Researchers have discovered that modified insulin signaling pathways in Indian jumping ant queens may be responsible for their astounding longevity and reproductive ability.

JOURNAL: *Science.*

Louis Hansel on Unsplash, Hua Yan/New York University

To Hallucinate or Not

RUAIRI J MACKENZIE

Psychedelics drugs are, after a near-50-year absence from research, back in vogue. There are multiple milestones by which a neutral outsider might measure the extent of psychedelics' revival. They *do* have their own Netflix show (actually, they now have several) but have not yet been approved by the US Food and Drug Administration. One smaller, nonetheless significant sign that the tide around psychedelic science is changing is the decision by the organizers of the recent Federation of European Neuroscience Societies (FENS) conference, the largest such neuroscience event in Europe, to carve out a slot for this burgeoning area of research.

Previous editions of FENS had featured little mention of psychedelics. While some sessions briefly touched on the antidepressant capabilities of the non-classical psychedelic ketamine, FENS 2022 broke new ground, featuring both a mini-conference, held in conjunction with the European College of Neuropsychopharmacology, and a symposium reviewing the advent of psychedelic compounds without hallucinogenic effects.

Technology Networks attended this latter symposium, which featured talks from Maastricht University's Dr. Kim Kuypers, Stanford University's Dr. Lindsay Cameron, Dr. Scott Thompson of the University of Maryland and Rafael Moliner from the University of Helsinki. The symposium aimed to tackle the idea, growing in popularity among researchers, that the rollout of potential psychedelic medicines could be limited by the hallucinatory trips that these compounds induce.

PSYCHEDELICS WITHOUT THE TRIP

"If psychedelics are able to be dissociated from the therapeutic effects, then it would be useful in reducing the amount of time that a patient has to spend. In preparation, during the drug administration, with follow up – that's a lot of time on the patient's side," explains Cameron in an interview with *Technology Networks*.

Cameron also hopes that making psychedelics hallucination-free could reduce cost for patients and reassure individuals who are unsure about the effects of a trip that could potentially last hours and require close supervision from a nurse or therapist throughout.

Cameron and her mentor, University of California, Davis Professor David

Olson, have made the development of non-hallucinogenic psychedelics the focus of their research. Cameron marked a significant breakthrough with the publication of a paper on a compound called tabernanthalog, an analog of the psychedelic ibogaine.

Ibogaine, a compound produced by plants in the family Apocynaceae, has shown some potential in early-stage trials for the treatment of addiction. However, its effects are long-lasting, and concerns have been raised over the compound's toxicity and cardiac side effects.

Tabernanthalog, in comparison, is non-hallucinogenic and non-toxic. In a small study in rats, Cameron showed that the compound could reduce alcohol- and heroin-seeking behavior, raising the possibility that it might mimic ibogaine's anti-addiction function in humans. "The idea here," Cameron explains, "is that we have a compound that seems to be therapeutically active but would no longer be producing hallucinations. Our lab coined the term 'psychoplastogen' as something that is able to cause this change, to mold brains." The speakers came back to this focus on the brain-structure altering, or neuroplastic, effect of psychedelics throughout the symposium.

A SEROTONIN DEBATE

While the presenters were aligned on their interest in exploring non-hallucinogenic compounds, their data didn't always sync up. Three researchers – Thompson, Cameron and Moliner – each looked at the role of the serotonin 5-HT_{2A} receptor, widely thought to be essential to the hallucinatory action of psychedelics. It's unclear right now whether these compounds' antidepressant effects are *also* derived from signaling through this receptor. Cameron's data suggested that tabernanthalog, at least, required 5-HT_{2A} activation to produce its effects. Thompson, working in rodents with psilocybin, investigated how the drug affects the brain's reward system in the context of chronic stress – a process that his results suggested to be independent of 5-HT_{2A}. A final talk from Moliner highlighted why such conflicting data will be common at this early stage of our understanding of psychedelics' molecular action: these brain processes are incredibly hard to fine-tune.

Moliner showed data suggesting that ketanserin, a compound widely used in pre-clinical research as a pharmacological tool to block 5-HT_{2A} receptors, might not be very selective at all, instead acting to additionally block other subtypes of 5-HT receptor. Teasing out how these different molecular actors mediate the psychedelic experience will be key not just to developing better hallucinogenic and non-hallucinogenic compounds, but to understanding how depression and other mental disorders are realized in the brain.

These differing paradigms within a field are part of what makes scientific conferences so stimulating to better research. But there are debates around psychedelics that were largely bypassed at FENS 2022.

LEAVING BEHIND PSYCHEDELICS' TABOO

Dr. Muad Abd El Hay, a postdoctoral researcher at the Ernst Strüngmann Institute in Frankfurt, went to every psychedelic talk he could at FENS; he tells *Technology Networks* that he has "attended specialist psychedelics conferences since 2014". To Abd El Hay, the presence of a psychedelics slot at FENS represents a huge shift from previous years. In the past, he explains, "[psychedelics] was a taboo subject ... to do it right, you basically needed to sacrifice careers in a way."

Now the field has flipped, Abd El Hay says. As larger labs – known for breakthroughs in non-psychedelic areas of science – began to publish landmark papers, the taboo has faded away. But Abd El Hay says that the decision by FENS to focus on the non-hallucinatory effects of psychedelics was potentially a non-representative introduction to the field: "The narrative at the end was, 'We don't need that psychedelic effect for anything to happen in mice, neuroplasticity is everything and we don't even need the 5-HT_{2A} receptor.' It was really cool data, but a lot of people would disagree with this. They weren't represented here."

"What I knew to be a very collaborative field is suddenly turning to a very competitive one."

– Dr. Muad Abd El Hay

There are many key questions still to be answered around psychedelics, but as they start to be recognized more widely in neuroscience, there is an undeniable sense of momentum. "We're finally starting to make progress to get these as therapeutics," says Cameron.

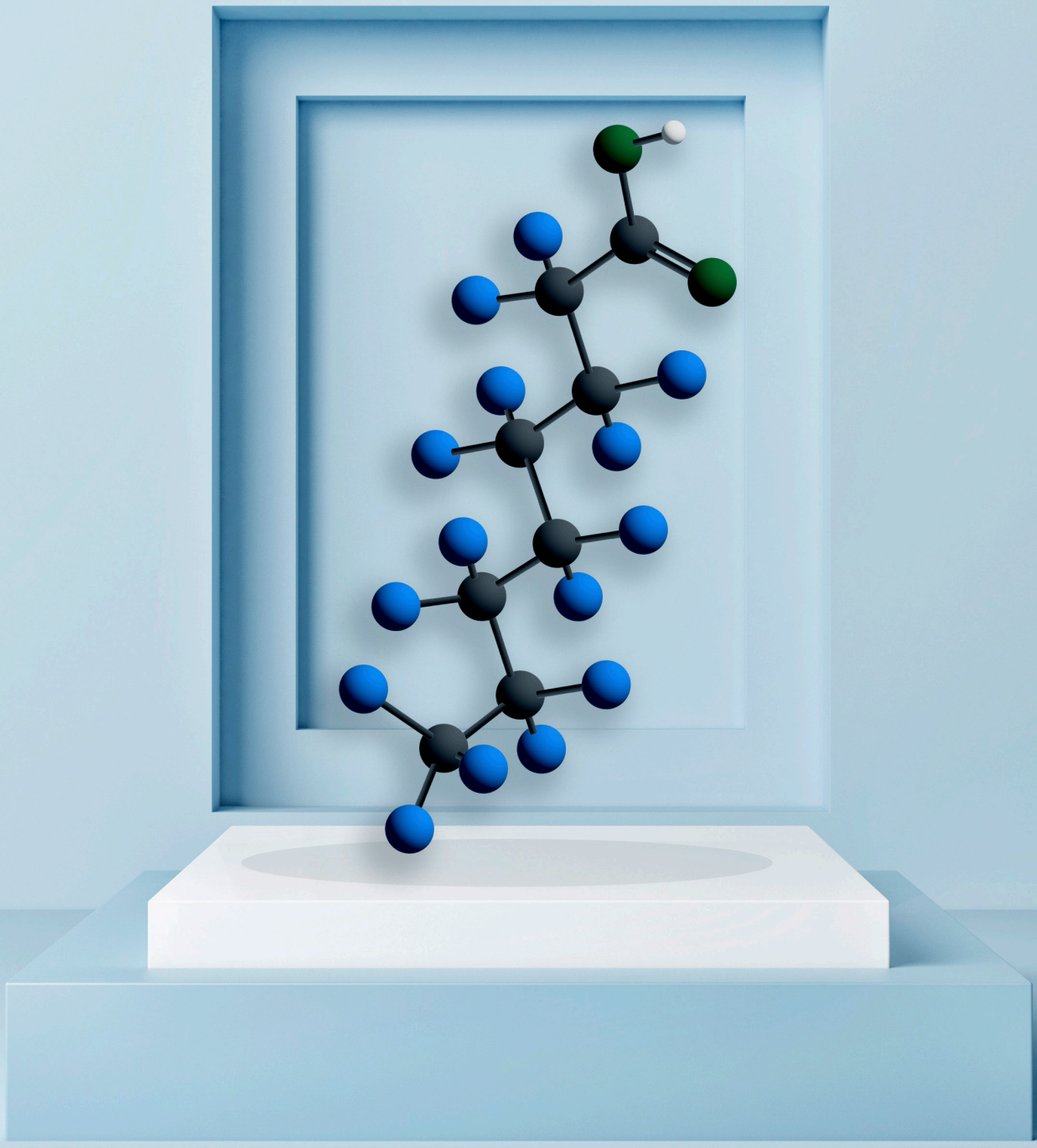
What seems less clear is whether the culture of psychedelic research will survive the field's move to the big time. To Abd El Hay, there was a noticeably different mood among the researchers he spoke to at FENS compared to those from psychedelics-only conferences. "I feel a little bit sad, because what I knew to be a very collaborative field is suddenly turning to a very competitive one," he says.

That change might be inevitable as psychedelic research hits the headlines more and more. The first day's joint symposium featured a speaker from growing and aspirational psychedelic pharma company Compass Pathways, which has been criticized for its aggressing patenting of psychedelic molecules.

A non-hallucinogenic, synthetic and more corporate future for psychedelics might be required to reach more patients and help more people, but it's hard to see how that is compatible with the field's past mindset of sharing and collaboration. Abd El Hay, nevertheless, says he did still manage to meet some future collaborators at FENS and have fruitful discussions, even if they didn't come as easily as at previous psychedelic meetups. "I am not giving up on this [field], but it's a bit sad to see that these problems are coming," he says.

THE EMERGING CRISIS OF "FOREVER" CHEMICALS

KERRY TAYLOR-SMITH



Per- and polyfluoroalkyl substances (PFAS) have penetrated every corner of the globe; they've infiltrated rainwater, contaminate our food and drinking water and are ever-present in the environment – even in the most remote locations. These "forever chemicals" have existed for almost a century but have gone largely unnoticed until relatively recently – perhaps because of their invisible nature. PFAS are everywhere, and they're persistent; evidence is increasingly revealing their damaging impact on the environment and human and animal health.

What do we know about them, why are they such a problem and do they *really* last "forever"?

WHAT ARE PFAS?

PFAS are a family of highly toxic fluorinated chemicals dubbed "forever chemicals" because of their inability to degrade in the environment. "PFAS are manmade chemicals with unique properties – oil and water repellent, temperature resistant and friction reducing – which makes them difficult to treat in the environment," explains Amy Dindal, PFAS program manager at Battelle, the world's largest independent non-profit applied science and technology organization.

These unique physical and chemical properties are the consequence of one of the strongest bonds found in organic chemistry: the carbon–fluorine bond, and that's why PFAS are unable to degrade without intervention.

Invented in the 1930s, they were initially used in nonstick and waterproof coatings, but by the 1950s, PFAS were used on a large-scale to create consumer and industrial products resistant to heat, oil, stains, grease and water. In the late 1960s, the US Navy began working with a major PFAS manufacturer to develop aqueous film-forming foam (AFFF) to rapidly extinguish fires. In the same decade, traces of PFAS began to appear in human blood samples, but the chemicals continued to be used.

Figures vary, but approximately 4,500 PFAS have been identified in hundreds of everyday products, from nonstick cookware to greaseproof paper, fast-food containers, stain-resistant textiles to cleaning products and paint – and even personal care products like shampoos, dental floss and cosmetics have shown traces of PFAS.

"The same properties that make them [PFAS] so useful for multiple applications also make them very difficult to remove from the environment, as well as our bodies," Dindal says. "The sheer number and variety mean uncertainty about their impacts is high, as not all PFAS chemicals have been studied for human health impacts."

Invented in the 1930s, PFAS were initially used in nonstick and waterproof coatings.

Some PFAS have been utilized more extensively, and specific examples, like perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are more widely studied, so it's important to note that what we *know* about PFAS is based on just a handful of chemicals, not their entire catalog.

PFAS are long-lasting and move throughout the environment. It's their persistence, rather than toxicity, that's the problem – it allows them to travel over large distances causing long-term, and even life-long, exposure.

HOW ARE WE EXPOSED TO PFAS?

PFAS are ubiquitous in global environments – in the soil, air, water and even our food. There is no "safe space" on earth to avoid PFAS and it's vitally important they are rapidly restricted, according to researchers from Stockholm University that discovered levels of POFS and PFOA in rainwater "greatly exceeded" US EPA Lifetime Drinking Water Health Advisory levels.

It's estimated there are almost 3,000 sites in the United States alone contaminated with PFAS. How do they get there?

Many are released during production and use or following the disposal of PFAS-containing material. They're usually present at relatively low levels, but higher concentrations are typically found at contaminated sites, like fluorochemical manufacturing plants, metal plating factories or facilities using PFAS to produce goods.

PFAS exposure occurs in many ways, and it's difficult to know which is the most detrimental. A Centers for Disease Control (CDC) survey revealed most people in the US are exposed to some PFAS at relatively low levels, typically through their diet as the chemicals can be present in fish, meat and dairy. Drinking water is another major exposure source, especially in contaminated communities where people and animals may be exposed to higher levels of PFAS through ground and surface water sources. Occupation may also be a factor, with firefighters or those working in chemical or manufacturing plants using PFAS at a higher risk.

Exposure pathways are similar for animals. Animal studies suggest some PFAS

are linked with liver toxicity, tumors in multiple organ systems, disruption of the immune and endocrine systems, adverse neurobehavioral effects, neonatal toxicity and death. However, the bioaccumulation process in animals is different from that of humans, so existing metrics must be adapted, alongside new mechanistic models, before any meaningful conclusions can be made.

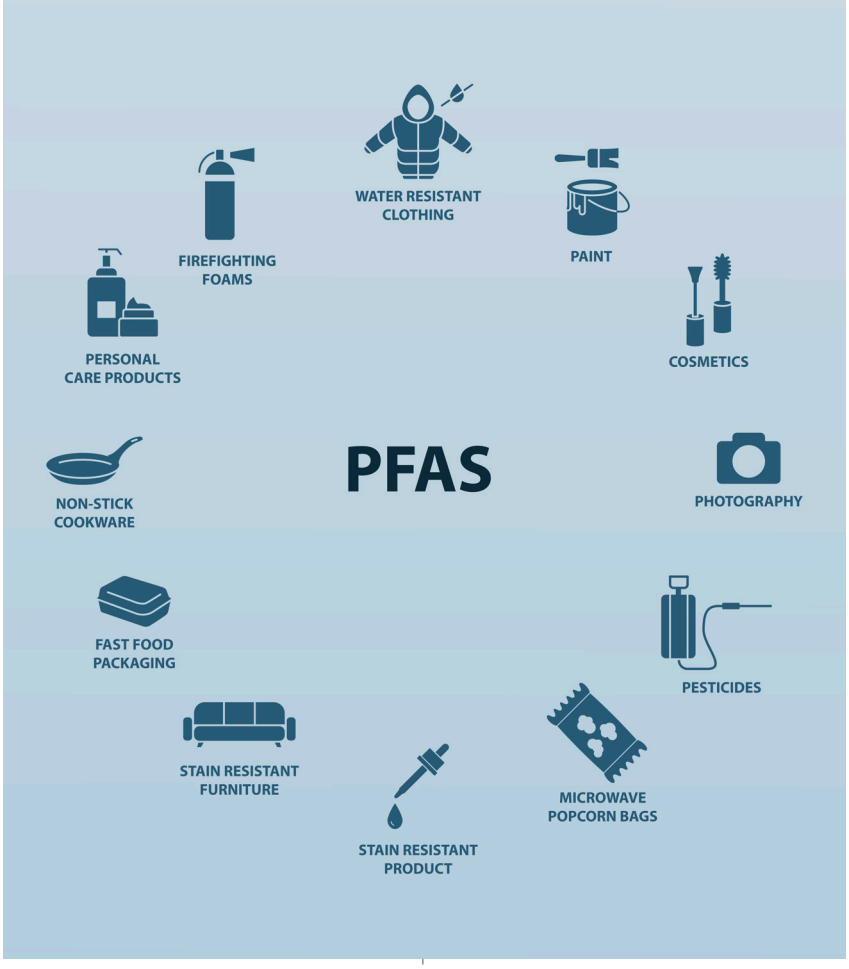
IMPACT ON HUMAN HEALTH

Studies have shown that some PFAS in the environment can bioaccumulate to the point where they are detectable in the blood of humans and animals all over the world – even in the Arctic and Antarctica. Although these remote areas register low levels, they still exceed even the most stringent guidelines designed to protect human health.

What do we know about the impact of PFAS on the human body? Our current understanding of the biological impacts of PFAS is based on just four of the thousands of different chemicals in the group: PFOS, PFOA, PFHxS and PFNA. This is because there is not enough data on the biological risks of the entire catalog of PFAS, especially where the chemicals are found in very small quantities. Consequently, most human exposure assessments focus on these and, likewise, wildlife studies are limited to targeted PFAS – around 30 in total.

PFAS bind to proteins, such as albumin, and circulate throughout the body where they linger after exposure.

A study conducted in pregnant women found that maternal PFAS concentrations were associated with an increased risk of late-onset preeclampsia. There is some indication that exposure to PFOA, PFOS and PFHxS can diminish a child's antibody response to vaccination, increasing their risk of developing infectious diseases. Results from epidemiological studies have indicated associations between PFAS exposure and the risk of developing some cancers, namely prostate, kidney and testicular cancer, in addition to increased risk of high cholesterol levels and adverse developmental outcomes. For many of these associations identified in human subjects, data is corroborated by animal studies.



A 2018 CDC report showed a link between 14 different PFAS chemicals and cancer, birth defects, thyroid disease and liver damage, Dindal says: "Other studies have linked consumption of PFAS-contaminated water to high cholesterol and nerve disorders."

Children may be more sensitive to the harmful effects of PFAS and are thought to be exposed when putting their hands to their mouths after crawling or playing on carpets and upholstery treated with PFAS to be stain and water repellant. The chemicals are associated with low birth weight, developmental delay, accelerated puberty, bone variation, and behavioral changes in children.

IMPROVING OUR UNDERSTANDING

It's vitally important to improve our understanding of PFAS and the risks they pose. Research exploring the substances is ongoing, but it's challenging to study and assess the potential risk of each individual chemical due to the thousands of PFAS used in consumer, commercial and industrial products.

Most studies over the last decade have focused on a limited number of substances and are unable to account for the different ways people are exposed at different stages of life. The types of PFAS and how they are used have

also changed over time, and there isn't enough data on exposure sources for the general population, or the contribution that food processing and packaging makes to PFAS levels in food.

Simply put, we still don't know how harmful PFAS are – finding the answer will require a long-term in-depth evaluation. Improved laboratory methods are required to more effectively identify and measure PFAS in all media. Current analytical methods, such as liquid chromatography-mass spectrometry (LC-MS), collect data on specific substances with high precision and sensitivity, however, new techniques are needed to reveal the presence of unidentified PFAS in the environment and their characteristics.

We need to do more than just test for these dangerous chemicals; we need to better understand the release and environmental degradation pathways of PFAS to determine how the chemicals can be managed and disposed of.

Of particular interest is how to remove PFAS from drinking water sources. Current methods remove contaminants through techniques like filtration. While effective, it transfers these contaminants to other media, generating secondary waste that requires incineration or landfilling.

Researchers from Northwestern University have developed a method to "slot into" the water purification process after PFAS have been removed. The process is inexpensive, uses low temperatures and destroys PFOA using sodium hydroxide, commonly used to make household products like soap. It could be used to treat concentrated PFAS waste in a more energy-efficient manner.

"The degradation uses very simple conditions," study lead Dr. Brittany Trang explains. The process involves "chopping off" the carbon-based head of the PFOA molecule, leaving the rest to fall apart. "However, integrating this into any industrial system would take much more optimization than we currently have done – we aren't ready for that yet!"

Trang says further research is required before this method can be used on other PFAS, like perfluorosulfonic acids, but it's promising and she hopes it will encourage people in the PFAS degradation field to think about designing destruction methods differently.

PFAS destruction using oxidation is a more attractive option, according to Dindal, "because it completely removes the chemicals from the environment," and doesn't generate any secondary waste.

Our current understanding of the biological impacts of PFAS is based on just four of the thousands of different chemicals in the group: PFOS, PFOA, PFHxS and PFNA.

TACKLING THE PFAS PROBLEM

PFOS and PFOA pollution is just the tip of the iceberg; there are thousands more PFAS that we know little about, and we know even less about the risks they pose.

The damage PFAS can have has been increasingly recognized, particularly in the last 20 years, and there are many safety levels in place that have been reduced over the years. However, these are often only advisory and so are not legally enforceable.

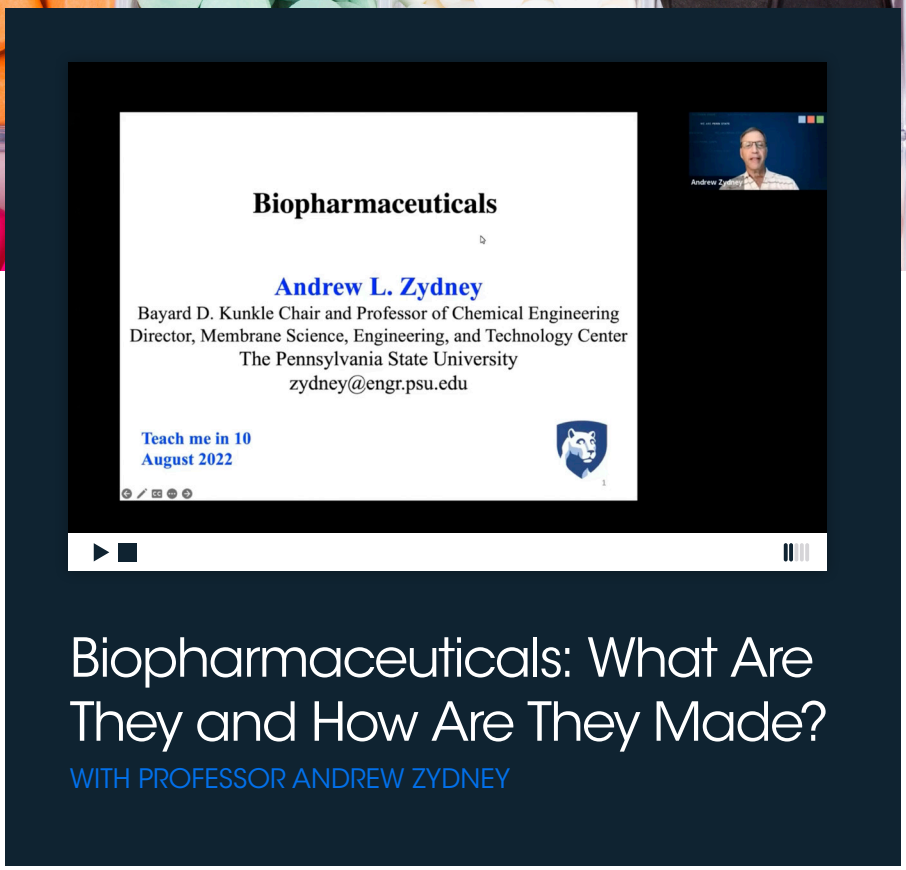
In 2002, one major manufacturer voluntarily phased out the production of PFOA and PFOS globally as a precautionary measure, including materials used to produce certain repellents and surfactant products, AFFF and coatings for food packaging

More recently, carpet manufacturers, who'd recently replaced one type of PFAS with another, eliminated all PFAS after learning they too could be problematic. They were encouraged by the Madrid statement, signed by hundreds of scientists who say no PFAS should be used as they never degrade, and could cause harm to health.

These are just two of the many strategies in place to reduce emissions, production and uses of specific PFAS. While many PFAS have already been phased out or substituted, there are plans to further restrict usage, manufacture and import of certain PFAS, expected on a chemical-by-chemical basis.

In June 2022, the EPA released a national testing strategy that requires PFAS manufacturers to provide the agency with toxicity data and information on categories of PFAS chemicals. The agency has selected PFAS to be tested based on an approach that breaks the large number of PFAS into smaller categories based on similar features and considers what existing data are available for each category. The aim is to address the data gap and investigate all PFAS one at a time, although this will be a challenging and time-consuming task.

It's clear that there is much more to be done. We need more research into the different types of PFAS and the damage they can do, more information on their replacements and alternatives – and any risks they might pose – and we need to discover more ways to destroy and eliminate the PFAS that are already in the environment. They might not have to be "forever" chemicals, as we once thought.



Biopharmaceuticals: What Are They and How Are They Made?

WITH PROFESSOR ANDREW ZYDNEY

Biopharmaceuticals – sometimes referred to as biologics – are drugs that are produced using living cells or organisms. Since their introduction, biopharmaceuticals have dramatically altered the landscape of modern medicine, providing treatment options for a broad range of diseases.

In less than 10 minutes, Professor Andrew Zydney talks us through: what are biopharmaceuticals? How do they differ from traditional small-molecule pharmaceuticals? How are they made? What factors might influence the high cost of biopharmaceuticals?

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Could We Develop Vaccines for Depression and Anxiety?

SIMON SPICHAK

In response to the pandemic, scientists around the world developed COVID-19 vaccines in record-time, saving millions of lives. Vaccines have been hailed "one of the greatest medical advances of modern times". They work by introducing a harmless form of a pathogen to the immune system, which teaches adaptive immune cells how to respond to the real pathogen if they're ever exposed in the future. Could the same technology that trains the immune system against pathogens also be used as a preventative for depression and anxiety?

THE LINK BETWEEN INFLAMMATION AND MENTAL HEALTH

While there isn't a specific microbe that directly causes mental illness, there are some (like SARS-CoV-2) that adversely impact the immune system, which in turn can affect mental health. The effects of COVID-19 infection – and associated stressors such as grief and lockdowns – over the last two years have likely contributed to the 25% increase in the prevalence of the most common mental illnesses in the world: depression and anxiety.

Inflammation is a key component of the body's immune response, often activated in response to tissue damage. However, a growing amount of research is now demonstrating the role of inflammation in the pathogenesis of human diseases, including mental health disorders.

In certain subtypes of depression, scientists have identified elevated levels of the stress hormone cortisol and other inflammatory signaling molecules in the blood and the cerebrospinal fluid. One meta-analysis published in *Psychological Medicine* analyzed 37 studies of depression, looking for

increased levels of C-reactive protein (CRP)– a molecular biomarker of an acute inflammatory response. Depressed patients were 46% more likely to have elevated levels of this protein in their blood.

Another study published in *Molecular Psychiatry* found that depressed patients who responded to antidepressant treatment showed a reduction in the inflammatory cytokine TNF-alpha. While these are interesting links, more research is warranted to understand the directionality of cause-and-effect and whether these cytokines can cause depression.

There are similar findings suggesting systemic inflammation plays a role in anxiety disorders. A meta-analysis published in *BMJ Open* found that patients with generalized anxiety disorder had higher levels of CRP in their blood compared to controls. However, the authors noted that there is only preliminary evidence that the cytokines play a role in the development of the disease.

Could a vaccine be used to help teach the immune system to taper its response to stress?

The psychological stress response is known to contribute to the etiology of mental health illnesses, but the idea that the inflammatory response against microbial organisms can also cause similar outcomes is controversial. Researchers are hypothesizing that, if they can find a type of bacteria that stimulates a similar response to psychological stressors, then it might be possible to inoculate or vaccinate against future stressors and their consequences on mental health.

Dr. Christopher Lowry is an associate professor of integrative physiology at the University of Colorado Boulder. His research is laying the groundwork to bring this idea to fruition. "If we're going to inject the bacterium prior to a stressor, the immunization could alter the host's immune system in a way that would prevent inflammation," he posits. "Then we should be able to prevent any negative outcomes of stress exposures that are dependent on inflammation."

MYCOBACTERIUM VACCAE AS AN IMMUNE REGULATOR

In 1999, Lowry began his research career at the University of Bristol, where he studied how the vagus nerve facilitated communication between the lungs and the brain. His supervisor at the time, Professor Stafford Lightman, and a colleague, Professor Graham Rook, had become interested in a bacterium isolated from soil in Uganda, called *Mycobacterium vaccae*. *M. vaccae* may already be capable of vaccinating humans against tuberculosis, a respiratory infection that often leads to the development of depression.

"They [Rook and his collaborators] showed that if you injected mice with this bacterium, it could prevent allergic airway inflammation in models of allergic asthma," Lowry says. Their findings were published in *Nature Medicine* in 2002.

Lowry wanted to see what neurons would fire if heat-killed *M. vaccae* was injected into the airways of mice. "We looked at what that did to serotonin systems and found that it was highly selective for activating a group of neurons that we believed *should* have antidepressant properties," he says. These neurons belong to the serotonergic system. When the researchers tested the effects in mice, they obsegvved antidepressant-like responses in behavioral tests. *M. vaccae* increased the activation of serotonin neurons as well as serotonin metabolism in the prefrontal cortex, mimicking some of the effects of antidepressants. When Lowry published the findings in 2007, it captured the public's imagination, he recalls: "It genuinely seemed to have these antidepressant-like properties, and people had a hard time getting their head around that," Lowry says. "You're injecting a bacterium and getting antidepressant-like effects, that doesn't make sense."

For Lowry, it was clear that the bacterium exerted anti-inflammatory effects by promoting the balanced expression of regulatory and inflammatory T-cells, which are typically associated with longer-term immune responses. Another study conducted by Rook found that the heat-killed bacterium provided protection against the allergic air-way response for more than 12 weeks after immunization. After being exposed to an airway allergen, the researchers found no change in inflammatory cytokine or antibody levels. Perhaps it could also provide long-term protection against the negative inflammatory impacts of stress?

Lowry teamed up with a researcher from Germany named Stefan Reber next, conducting research in a mouse model of an inflammatory form of post-traumatic stress disorder (PTSD). A state of chronic anxiety is induced in the model using a colony housing (CSC) paradigm. Lowry and Reber tested whether inoculating the mice with a preparation of *M. vaccae* prior to CSC exposure could provide some level of protection against stressor-induced inflammatory responses. "We saw that we could prevent stress-induced colitis, stress-induced exaggeration of pro-inflammatory cytokine release from mesenteric lymph node cells, and we could prevent stress-induced exaggeration of anxiety in that model," Lowry says.

MAKING SENSE OF SERENDIPITOUS FINDINGS

Why does a bacterium found in soil have such profound effects? To figure that out, scientists needed to understand where its immune-modulating activity comes from.

Normally, heating microbes denatures their proteins, rendering them inert, but *M. vaccae* has some unique properties allowing the bacterium and its lipids to remain intact after death. "At a scanning electron microscopy level, after you heat-kill these bacteria, they are morphologically intact," Lowry says.

"When you pre-expose macrophages – a type of innate immune cell – to the lipid, and then challenge them with lipopolysaccharide, one specific lipid isolated from *M. vaccae* suppresses the inflammatory response in the macrophage," Lowry says. "We fo-

cused on a potential receptor for the lipid which turned out to be a host receptor, a transcription factor called PPAR-alpha."

In nature, *M. vaccae* might be inhaled or swallowed up by animals and then eaten by phagocytic immune cells. Then the bacteria could release lipids into the cell to shut off the inflammatory cascade and survive. "I like to point out that mammals and bacteria had 65 million years to solve these problems and co-exist together," Lowry adds. Due to the unique properties of *Mycobacterium*, different species are currently being screened to determine whether they might also have similar properties.

LINKING MICROBES AND MENTAL HEALTH

Many scientists believe that one of the reasons inflammatory disorders – including anxiety, depression and PTSD – are on the rise is due to a lack of exposure to microbes earlier in life. This idea, called the hygiene hypothesis, may explain why a soil bacterium could have the ability to inoculate against future stress exposures.

One of Lowy's collaborators conducted a study comparing the stress responses of young men who grew on farms close to animals to those who were raised in cities. "The people that grew up in cities had an exaggerated inflammatory response to the psychosocial distress," Lowry explains. "This supports the idea that if you grew up in the city, without exposure to animals, you're at higher risk of repeated chronic low-grade inflammation in response to purely psychological stressors." While provocative, the study was limited because many other important factors related to the microbiome (including mode of delivery, formula or breast milk in early life, and diet) were not considered.

Even if *M. vaccae* doesn't directly cause a disease, the hygiene hypothesis could provide one rationale for inoculating individuals against stress-related disorders. It has been revisited by epidemiologists in recent years, questioning the links between early microbial exposure and inflammatory disease. According to the Microbiological Society, there is little evidence that the increase in inflammatory disorders is related to personal or household hygiene and cleanliness, instead positing that exposure to beneficial microbes from the natural environment is necessary for regulating the immune system. Even then, there is substantial debate over what these beneficial microbes are and if it is even possible to define a healthy gut microbiome that would promote healthy immune development. While other studies show links between the gut microbiome and depression, it isn't clear whether they contribute to the development of the disease, act as a confounder or are a consequence of depression itself.

In certain subtypes of depression, scientists have identified elevated levels of the stress hormone cortisol and other inflammatory signaling molecules in the blood and the cerebrospinal fluid.

ARE THERE ANY VACCINES THAT IMPACT MENTAL HEALTH?

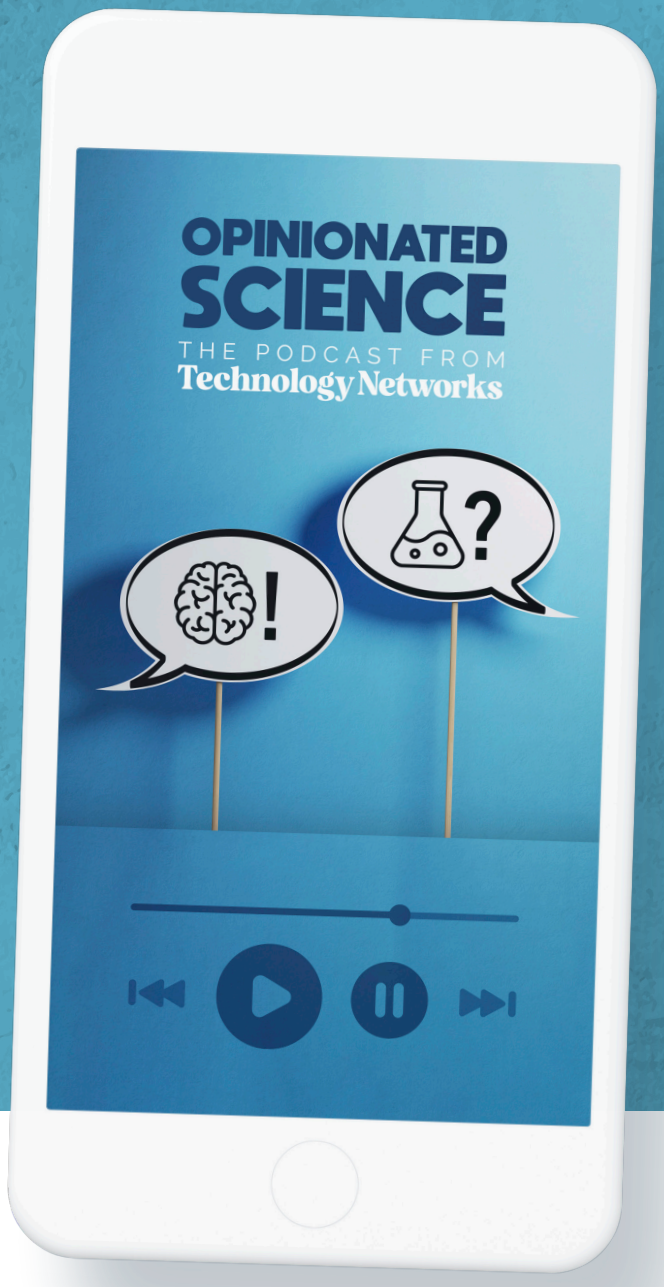
A growing body of evidence suggests that regulating the immune system has an impact on the brain and behavior. This is further demonstrated by the effects that vaccines designed against infectious diseases can have on mood and mental health.

Research proposes that the impact of flu vaccines on mood is more negative in people who have anxiety or depression, suggesting another link between the immune system and our mental state. Intriguingly, the COVID-19 vaccines appear to be associated with a lower prevalence of anxiety or depression, though it is unclear why. A recent large, cross-sectional study found that people who received a vaccination were 13% less likely to develop anxiety and 17% less likely to develop depression. The nature of the study design prevents causal inference, but the data enhances our understanding of how vaccination could offer a preventative route for mental health conditions.

HOW LONG UNTIL CLINICAL TRIALS?

According to Lowry, it may be five years until *M. vaccae* is tested in humans as a potential vaccine against anxiety or depression: "The strain that we're studying has been used in many clinical trials, including phase III trials, but not in the context of psychiatry," Lowry said, adding that the bacterium already has a good safety record, which can make it easier to receive approval for testing the vaccine for other conditions in humans.

It may take more than a decade before there is long-term data to inform us whether *M. vaccae* can reduce the risk or severity of developing depression over a five-year period. But if it *does* work, it could become an incredible vaccine success story and jumpstart the development of more vaccines for mental health conditions in the future.



Pig Skin Corneas and Memory Repair With Electricity

On this episode of *Opinionated Science*, the team investigate two technologies that take the "fi" out of "scifi". Listen now to find out how one research group is repairing blindness with an unexpected source of cellular help, and another is using alternating electrical current to enhance memory performance.

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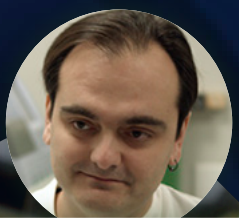

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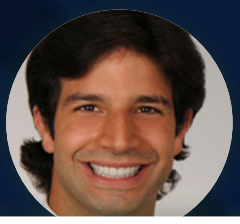

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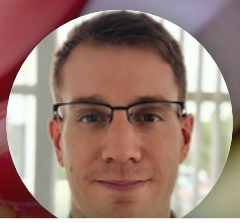

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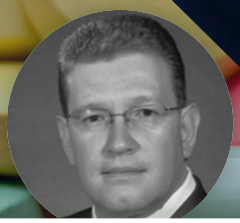

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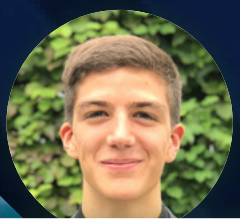

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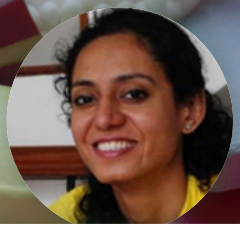

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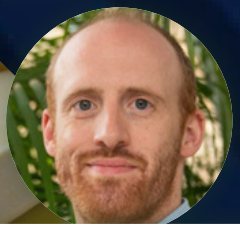

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The "Streetlight Effect" in Proteomics

MOLLY CAMPBELL

Our current understanding of human, animal and plant biology is largely derived from the insights provided by studying the DNA code. However, this code is just one of the integral components of biology's central dogma. DNA must be read and converted to proteins, the "workhorses" of the cell, responsible for coordinating and conducting specific functions.

The introduction of high-throughput technologies, bioinformatic tools and artificial intelligence (AI)-based methods have progressed the field of proteomics over recent years. While not yet "in the clinic" so to speak, the study of proteins expressed in healthy or diseased states is guiding the development of diagnostic biomarkers, the identification of drug targets and the production of novel biopharmaceuticals. Across the broader life sciences, the applications of proteomics are numerous and varied.

The Human Proteome Project (HPP), which aims to generate a map of protein-based molecular architecture of the human body, has discovered 93.2% of the human proteome, identifying 18,407 proteins.

"Proteomics has been transformed from an isolated field into a comprehensive tool for biological research that can be used to explain biological functions" – write Yahui Liu et al.

The future of proteomics is no doubt bright. However, a commentary article published in *Nature Methods* by Kustatscher et al. earlier this year brought attention to an underlying problem in the field: some proteins are getting more research attention than others.

The publication states that an estimated 500 proteins (approximately 25% of the human proteome) account for 95% of all life science publications. Most of these proteins were already known to the scientific community in the pre-human genome project era. Tumor protein 53 (p53), sometimes nicknamed the "guardian of the genome" due to its role in DNA repair and cell division, is one of the most frequently studied proteins. "One of the many chilling statistics revealed is the fact that p53 is the subject of 2 publications per day," says Professor Kathryn Lilley, professor of cellular dynamics at the University of Cambridge, and a co-author of the publication.

WHY DOES THIS ANNOTATION BIAS EXIST?

This inequality in protein annotation occurs due to a variety of different factors, Lilley explains: "Firstly, there are practical reasons why a protein might remain unannotated. This could be down to the fact that it is expressed at low levels and therefore rarely 'measured' in an experiment."

Extremely small proteins, or those that possess certain properties (such as being hydrophobic, can prove challenging for even the most sophisticated analytical technologies. Some proteins can adopt unstable states that are present for a fraction of a second but play key biological roles – known as "fleeting proteins", which are likely not captured in most studies.

"It could be that its corresponding and gene or transcript do not appear as 'interesting/significant' in genomics studies, or it is not associated with any disease states. Moreover, it may be that the protein does not resemble any other protein in terms of likely domain structure, well documented motifs or clear evolutionary trajectory," Lilley says.

She describes the non-practical reasons as being "less palatable" to her mind: "There is security in numbers in scientific research. If a protein is well-studied, there may be more resources available which can be shared amongst different groups. If a protein is perceived to be of great interest by the scientific community, there is more chance of having research outputs published via high impact mechanisms, leading to high citation and subsequently a greater chance of continued funding."

This cycle perhaps isn't unique to the field of proteomics and speaks to wider issues within scientific research. But in this instance, it's fueling what Lilley calls a "self-perpetuating microcosm of the well-studied proteome" at the expense of taking risks.

"When studies unearth sets of proteins that require further investigation, it is frustrating to trawl the literature only to find that historically such proteins have been ignored, many as simply not of significant interest to pursue, not trendy enough to attract funding, or generally considered to be a bit 'dull'," – Lilley.

WHY ARE UNDERSTUDIED PROTEINS PROBLEMATIC?

Bias towards well-studied proteins inhibits our knowledge of cellular function, dysfunction and ultimately hinders progress across life science research. "The understudied proteome contains many examples of protein essential for proliferation, a key cellular process, whose aberrant function underpins many diseases, cancer being the most pertinent in many avenues of research. This bias will extend to most cellular processes, and hence without functional annotation of this subset of proteins, we'll have little to no chance of fully understanding how cells work."

Many of the drugs used to treat human diseases target proteins. Data from the DrugBank database suggests that the entire collection of drugs approved by the US Food and Drug Administration (FDA) target 620 proteins in total, including transporters, enzymes, ion channels and receptors. "The understudied proteome contains a considerable number [of proteins] that are expected to be druggable," says Lilley.

To create a new drug, there are various stages of preclinical and clinical development required. Bench research and preclinical trials are reliant on models that enable scientists to interrogate the drug's function *in vitro* and *in vivo*. However, if our basic knowledge of cellular mechanisms is flawed, our models could be too. "Knowledge of the function and role in disease of this considerable subset of the proteome may result in a step change in drug discovery going forward," Lilley notes.

THE UNDERSTUDIED PROTEIN INITIATIVE

Kustatscher and colleagues have brought the scale of the issue to light – but how do we tackle it? A change is clearly required within proteomics approaches to bring the perpetuating cycle to a halt. The Understudied Protein Initiative, a novel Wellcome Trust-funded initiative developed by Kustatscher et al., outlines a solution: a coordinated effort from the functional proteomics community. The initiative suggests that sufficient data be gathered on an understudied protein – perhaps on its interactions, localization or expression – such that hypotheses on its function can be made. "In an ideal world, researchers could carry out some systems level functional assays, where every protein is tested for a specific function. A good example of this, is testing whether a protein binds RNA or not. There are many routine methods to

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– Lilley

carry out such a functionality screen and that also can be applied across many conditions; some proteins may only bind RNA under a certain set of circumstances," explains Lilley.

Using this functional data, it would be easier to then clarify which field or laboratory is best suited to conduct further, detailed studies of that protein. In essence, the task is divided into two parts: large-scale pre-characterization by omics scientists, followed by focused molecular biology studies. "More systems-wide studies will need agreement of the biological system, sets of conditions tested, sharing of resources and a holistic set of methods to 'prod and poke' the understudied proteome," says Lilley. "What will be particularly essential will be data sharing, curation, integration of databases and creation of dynamic cellular models. Building on resources such as MuSIC 1.0, a hierarchical map of the cell from Ideker lab, being a very good starting point."

She continues, "As a word of caution, however, the task at hand is almost uncalculatable in size. We have yet to adequately compute the size of the proteome. If one takes into consideration the number of proteoforms that may exist, in other words, the number of distinct chemical entities though post transcriptional and post translational processing and the likely combinatorial nature of this processing, the size of the proteome expands by multiple orders of magnitude."

No matter the size of the anticipated challenge, a start must be made somewhere. The Understudied Proteins Initiative published an open invitation to researchers, outlining its "roadmap" for the project. An openly accessible survey has been launched as a first step, which presents a randomly selected human protein and asks the user to assign it to an annotation level. Next, the survey asks the user to describe which tools, resources and considerations they would put forward for that assessment.

"Based on the responses to the survey, we aim to define the challenge for a community effort to tackle protein annotation bias. We will present and discuss the results in a workshop," the initiative leaders state. Core questions to be addressed during the workshop include:

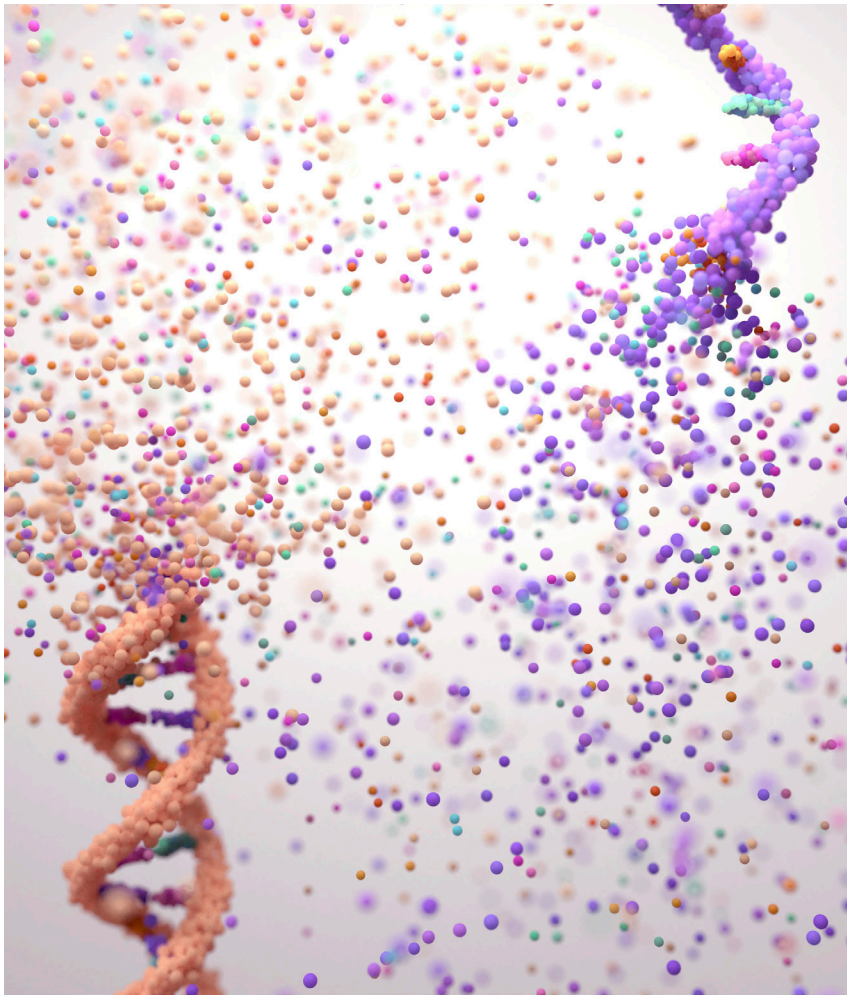
- What new information on an uncharacterized protein would spark detailed mechanistic studies?
- What tool(s) would provide that information?
- How could a consortium be structured?
- How would the information efficiently reach molecular biologists to instigate change?

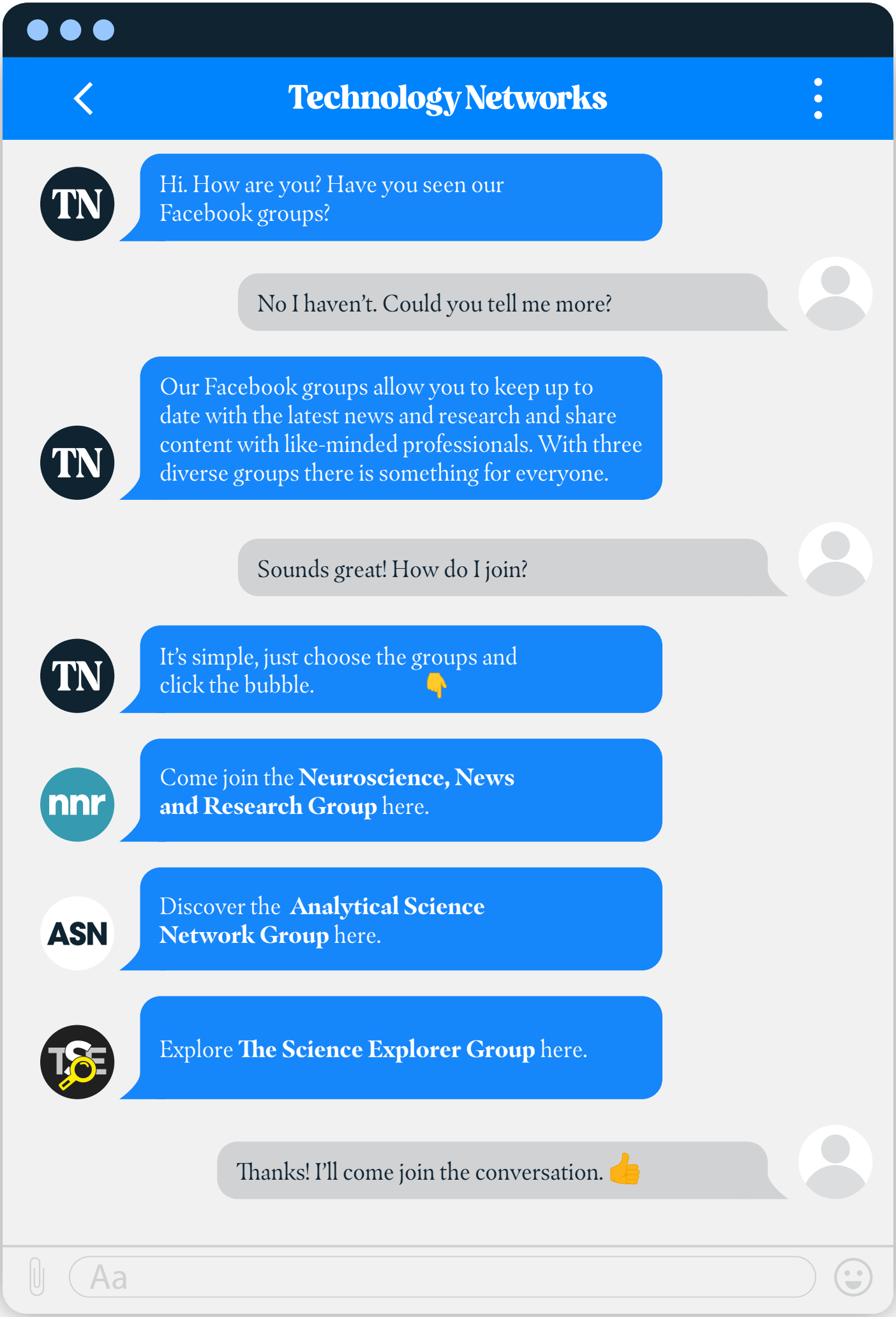
TAKING ACTION

Some of the greatest triumphs in science have been based on taking a potential risk. It seems imperative – arguably now more than ever – that researchers feel confident and comfortable pursuing studies on lesser-known or understood proteins, irrespective of the anticipated analytical challenge or the perception that the protein is "dull". Who knows what we might find – maybe solutions to some of the most challenging scientific conundrums of our time?

The Understudied Protein Initiative is leading the way and encourages the community to get involved by participating in the survey and spreading the word.

"By providing a basic molecular characterization of all proteins, the Understudied Proteins Initiative will catalyze mechanistic investigations of understudied proteins, drive new biomedical research, and boost our understanding of the human proteome and its role in disease," – The Understudied Protein Initiative.

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