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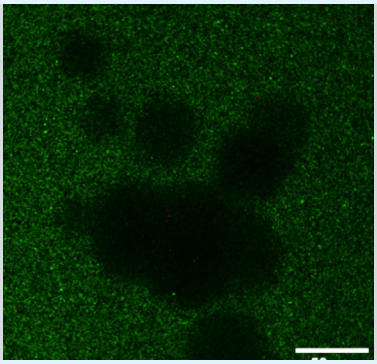
KERNEL

Editorial

Antibiotic resistance has emerged as one of the biggest threats to healthcare in recent decades. In this issue of *Kernel*, read more about how scientists at IISc are exploring alternative approaches to tackle this burgeoning problem.

Our lab feature throws the spotlight on a scientist whose research focuses on the study of systems at the intersection of biology and physics. Also in this issue are stories on how genes influence leaf architecture and how fatty acid uptake is regulated in heart muscles, plus other recent research.

RESISTING RESISTANCE



Clearance of fluorescent bacteria on phage addition (Image: Pallavi Raj Sharma)

FACED WITH THE GROWING THREAT OF ANTIBIOTIC RESISTANCE, RESEARCHERS AT IISc AND ELSEWHERE ARE IN PURSUIT OF ALTERNATIVE STRATEGIES

In the early 1940s, as World War II ravaged several parts of the globe, great uncertainty and a sense of doom loomed large over the soldiers and their families. Even a tiny splinter on the battlefield could prove fatal due to the resulting infection and gangrene, for which effective treatment was scarce. But then, a beacon of hope emerged – a 'wonder drug' called penicillin, Alexander Fleming's accidental discovery. The subsequent mass production of penicillin was instrumental in ushering in the golden era of antibiotics, a class of drugs that has collectively saved millions of lives since then. However, nearly a century later, rising antibiotic resistance threatens to take these efforts back to square one.

Antibiotic resistance is when bacteria develop the ability to block the action of antibiotics – molecules designed to kill them or slow down their growth. This makes bacterial infections harder to treat, and routine procedures like surgeries and transplants risky. The World Health Organisation (WHO) estimates that every year, an alarming 2,30,000 people die worldwide as a result of multidrug-

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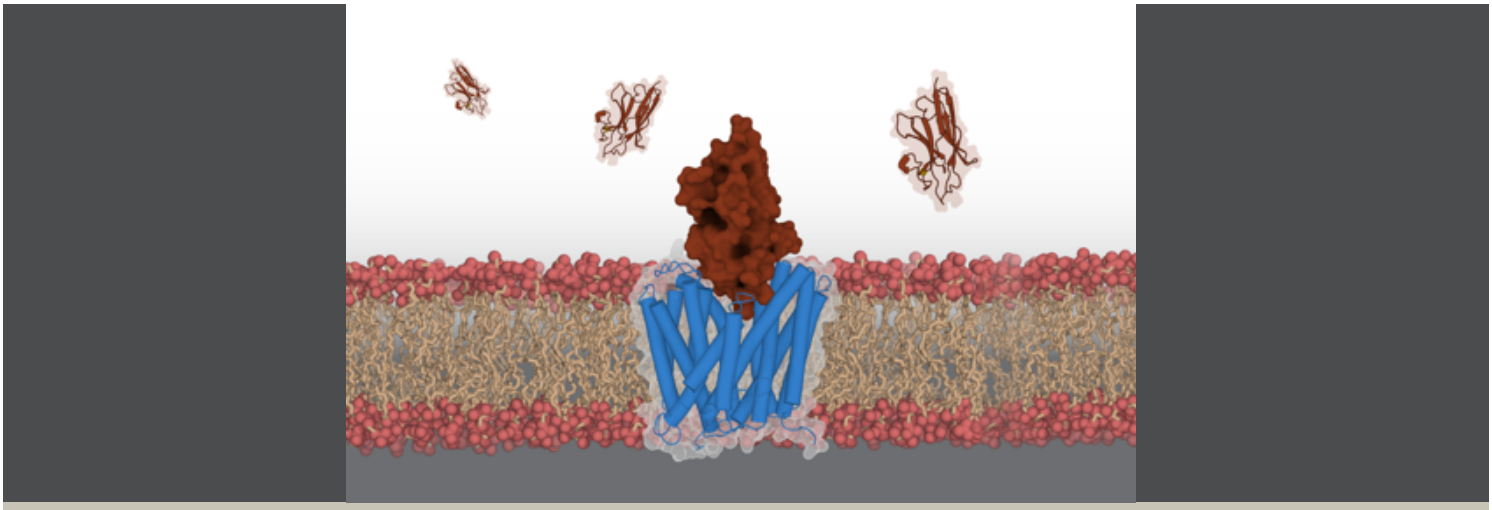


Structure of a camelid antibody in complex with a superbug transporter (Image: Arunabh Athreya)

resistant tuberculosis alone – this figure is much higher if we consider deaths due to other resistant species of bacteria. Even simple skin infections are becoming increasingly difficult to treat, owing to the emergence of strains that are resistant to most known antibiotics.

Given the gravity of the situation, scientists around the world are racing against time to develop more effective antibiotics as well as alternative approaches. Bacteria are adept at developing resistance to drugs that block specific pathways or processes by making small changes to the targeted pathways or molecules. To counter this, scientists have been developing molecules that directly bore holes in the bacterial cell membrane. Rupturing the membrane would disable all essential processes almost immediately, making it difficult for the bacterium to develop resistance. Researchers both at IISc and elsewhere have been developing novel antimicrobial peptides (AMPs) and nanoparticle-based artificial enzymes to accomplish just this.

Another exciting development is the possibility of exploiting the existing prey-predator relationship between bacteria and bacteriophages – viruses that can kill bacteria – to develop new antimicrobials. “Phages were used quite a bit in the early 1900s on the research scale and also on humans. But with the discovery of penicillin and other antibiotics, [they] fell off the way,” says Rachit Agarwal, Assistant Professor at the Centre for BioSystems Science and Engineering, IISc. These bacteriophages – or phages – enter bacterial cells, multiply, and then burst the bacterial cell open to release the new phages, thereby killing the bacterium.

Rachit points out that while there are only a few classes of antibiotics, the repertoire of phages is much larger, and these phages target many different receptors on the bacterial cell membrane – giving us a wider range of options to choose from for therapy. His lab recently tested the use of a mixture of five different phages to treat mycobacterial infections.

Mycobacteria are notorious for their ability to survive in extreme conditions of low oxygen or pH inside our body, and they can stay dormant without dividing for long periods of time, according to Rachit. “We were actually quite surprised to see, at least from whatever lab testing we did, that these phages were quite effective against mycobacteria in all of these pathophysiological environments that we looked at.” When his team combined phage therapy with antibiotics, they found that the ability of the phages to kill the bacteria improved significantly.

Since phages are large molecules, delivering them directly into the niches that bacteria occupy inside our body could make therapy more effective, according to Rachit. Mycobacteria hide within our cells and therefore delivering phages inside these is an additional challenge – this is something that his lab is actively pursuing. “Right now, we’re mostly focused on using some polymeric carriers to load the phages, and modifying these carriers either in size, charge or surface properties to be able to load phages, get internalised by mammalian cells, and be transported to the right intracellular locations,” he says.

Aravind Penmatsa, Assistant Professor at the Molecular Biophysics Unit, IISc, has a different approach to this problem of antibiotic resistance. His lab explores the possibility of blocking certain types of transporter proteins on the bacterial membrane that help it become resistant. These proteins help transport nutrients and other essential molecules.

“Some of these transporters are used by the bacteria … to pump out antibiotics or antibacterial compounds that we use on a day-to-day basis into the external environment. So, this becomes a major mechanism for gaining antimicrobial resistance,” says Aravind. “If you can somehow control this efflux process, and block it, the potential for this strategy is that you can enhance the efficacy of existing antibiotics.”

Aravind’s lab has exploited this principle by developing antibodies derived from mammals like camels which can block a specific efflux pump in *Staphylococcus aureus,* a notoriously resistant species of bacteria. These antibodies are different from conventional Y-shaped antibodies found in humans (and several other species) in that they have a single domain for recognition of the target protein, and are shaped like “a rugby ball,” Aravind explains. This property allows them to interact with crevices of target proteins more effectively, making them an interesting candidate for developing efflux-pump blockers.

However, harnessing this technology to treat bacterial infections will take time, and will require one to choose the right targets, he says. “These single-domain camelid antibodies are phenomenal in their ability to specifically detect the presence of efflux pumps. So even if the whole idea of a therapeutic blocker will take a long time, the idea of using them as diagnostic tools is a much more real and practical application for these antibodies,” he adds.

Although several such efforts have gone into tackling existing antibiotic-resistant strains, preventing the rise of newer antibiotic-resistant species also rests heavily on human behaviour. “A lot of this antibiotic resistance is also due to patients not completing their [therapy] regimen,” says Rachit. “So, if somehow patient compliance can be increased, then the expectation is that the evolution of antibiotic resistance would slow down.”

- Karthika Kaveri Maiappan

Image: Krishna Reddy Challa and Monalisha Rath

STUDY REVEALS HOW GENES INFLUENCE LEAF ARCHITECTURE

A recent study published in *Nature Plants* by researchers at IISc has shed light on how simple leaves – one of the two basic forms of leaves – develop in a plant. The team included researchers from the Department of Microbiology and Cell Biology (MCB) and their collaborators from Shodhaka Life Sciences, Bengaluru.

Plants have either simple or compound leaves. A mango tree, for instance, is said to possess simple leaves because they have a single, intact leaf blade. On the other hand, a gulmohar tree has compound leaves where the leaf blade is dissected into multiple leaflets. However, both simple and compound leaves start out as rod-like structures budding out from the meristem, the tip of the stem where stem cells are present. How these rod-like structures, referred to as primordia, give rise to simple or compound leaves has been a subject of much investigation in the past years.

In this study, the authors identified two gene families that regulate the development of simple leaves through the proteins they code for, in a plant called *Arabidopsis thaliana* – a popular model organism in plant biology. These gene families – *CIN-TCP* and *KNOX-II* – encode proteins called transcription factors that suppress the formation of new leaflets at the margin, thereby giving rise to simple leaves.

The researchers simultaneously suppressed multiple members of the two gene families; this caused the simple leaves to become super-compound leaves that gave rise to leaflets indefinitely. However, when the authors independently suppressed either of the two gene families, the leaves did not turn into compound leaves, suggesting that these genes work in concert.

In addition, these mutant leaves continued to stay young and grow for as long as the necessary growing conditions were available. While *Arabidopsis* leaves typically mature in around 30 days and wither by 60 days, the leaves of these mutant plants with suppressed *CIN-TCP* and *KNOX-II* gene families grew for as long as the researchers observed them (175 days) – and could potentially go on for months or years if given the necessary conditions.

“While scientists have been able to convert compound leaves to simple leaves by manipulating the expression of certain genes, our report is the first one to go the other way around,” Utpal Nath, Associate Professor at MCB and senior author of the paper, says.

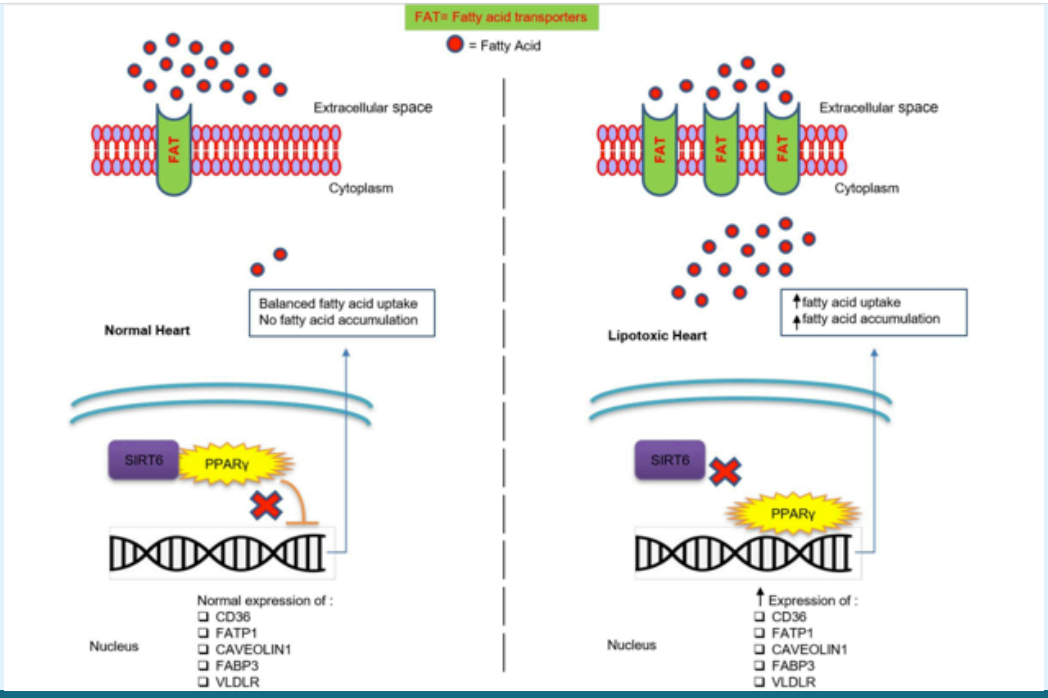
The researchers also found that the leaves of the plants in which the two gene families were suppressed, in contrast to normal *Arabidopsis* leaves, displayed RNA signatures of young immature leaves and actively dividing cells even beyond their typical maturation period. RNA is a chemical messenger which carries instructions from the genes required to synthesise proteins.

Besides providing insights into plant development, in the long run, the findings could initiate and nurture innovations in the food industry. As Krishna Reddy Challa, a former PhD student at MCB and a lead author of the study, says, “one could use this technique to alter the shape of the salad leaves as one chooses, or increase their biomass. For instance, you could change the shape of a spinach leaf to look like lettuce.”

“Since the leaves don’t mature once the *CIN-TCP* and *KNOX-II* genes are suppressed, you can control the longevity of the plant and thereby extend its shelf-life,” adds Monalisha Rath, a PhD student at MCB and another lead author of the study.

- Joel P Joseph

Mechanism by which SIRT6 regulates fatty acid uptake in the heart (Image: Khan et al., 2021/Cell Reports)



REGULATING FATTY ACID UPTAKE IN CARDIAC MUSCLE CELLS: A NEW INSIGHT

Fatty acids are formed when the fat in our diet breaks down during digestion. While many of the body's organs use glucose as their primary energy source, the heart derives most of its required energy (over 70%) from the oxidation of fatty acids. These are crucial for sustaining cardiomyocytes – cardiac muscle cells that control the rhythmic beating of the heart. However, accumulation of excess fatty acids in cardiomyocytes triggers harmful responses, often leading to severe cardiac diseases.

A recent study published in *Cell Reports* by a team of researchers from India and the US, led by scientists at IISc, provides important insights into how fatty acid uptake is regulated in cardiomyocytes. "We identified a mechanism by which fatty acid transport (to cardiomyocytes) is critically regulated by a protein called SIRT6," says team lead Ravi Sundaresan, Associate Professor in the Department of Microbiology and Cell Biology. The study shows that SIRT6 could be a potential therapeutic target for treating several metabolic diseases affecting the heart.

Cardiomyocytes have several fatty acid transporters – specific proteins enhancing the uptake of fatty acids from the blood into the cells – to ensure sufficient supply. The authors say that this is the first study to show that SIRT6 regulates the genes responsible for the formation of these transporter proteins in cardiomyocytes.

The team observed that cardiomyocytes devoid of the SIRT6 protein had higher levels of fatty acid transporters, resulting in higher uptake and accumulation of fatty acids. They also showed that increasing the level of SIRT6 in cardiomyocytes lowered the levels of these transporters, thereby reducing fatty acid uptake and accumulation. The researchers carried out most of the studies in experimental mice models.

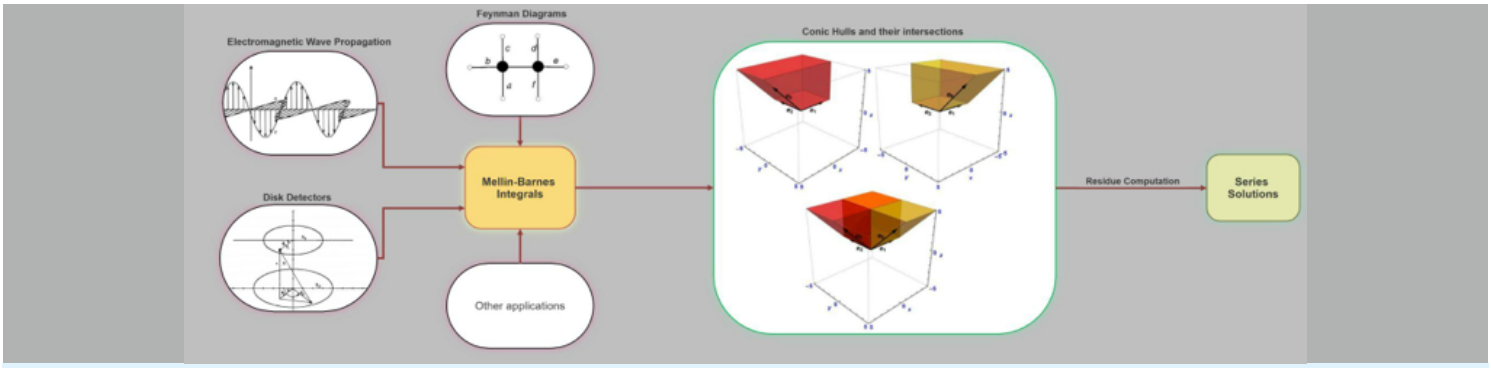
SIRT6 belongs to a family of proteins called sirtuins, which are important biological enzymes that require specific molecules, called cofactors, to function. Surprisingly, the researchers found that SIRT6 neither functions as an enzyme nor does it require any co-factor to regulate fatty acid uptake in cardiomyocytes. Rather, it does so by binding with a specific protein involved in the production of fatty acid transporters. Sundaresan previously led another study which showed that SIRT6 can regulate protein synthesis without functioning as an enzyme.

"Fatty acids are very useful for the heart, if properly utilised," Sundaresan explains. However, accumulation of more fatty acids than the heart can utilise in cardiomyocytes causes lipotoxicity – a condition in which accumulation of lipids can eventually have grave consequences such as inflammation and cell death. Notably, diabetes and obesity are among certain pathological conditions in which levels of fatty acid transporters are reportedly higher. Under such conditions, activating SIRT6 and increasing its expression might be a beneficial therapeutic intervention, the researchers suggest.

The team also included Narayanaswamy Srinivasan, Professor in the Molecular Biophysics Unit, IISc, and researchers from the Tata Institute of Fundamental Research and Sri Jayadeva Institute of Cardiovascular Sciences and Research in India, and Rutgers University and Harvard Medical School in the US.

- Sritama Bose

Photo: Sumit Banik



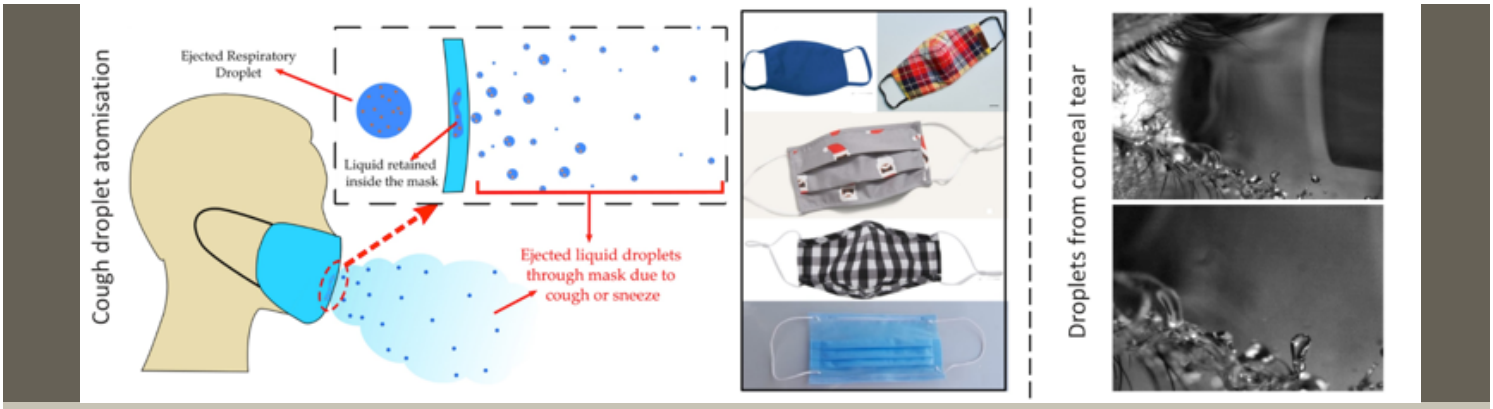
CRACKING A CENTURY-OLD MATHEMATICAL PROBLEM

An important advance has been made towards the full solution of the old mathematical problem of constructing series representations of Mellin-Barnes (MB) integrals of arbitrary complexity. These are a special type of integral whose integrand consists primarily of ratios of products of Euler Gamma functions with complex number arguments.

MB integrals are ubiquitous in physics, engineering, and a variety of quantitative disciplines, and their evaluation – particularly important for quantum field theory and hypergeometric functions theory – has been an unsolved problem for over a century. In the present work, geometrical objects (conic hulls) and multivariate complex analysis have been combined in a new approach to make a breakthrough, allowing the researchers to evaluate MB integrals when the number of integrations is any fixed positive integer. The technique is simple enough to be appreciated and applied by anyone with a firm grasp of high-school geometry. A computer package automating this method has also been produced by the authors, adding a powerful tool to the armoury of physicists and mathematicians. The study was carried out by B Ananthanarayan and Sumit Banik from the Centre for High Energy Physics, along with collaborators in Europe.

- Sumit Banik and B Ananthanarayan

Image: Bal Krishan & Durbar Roy



FLUID DYNAMICS STUDIES ON HOMEMADE FACE MASKS AND NON-INVASIVE EYE PROCEDURES

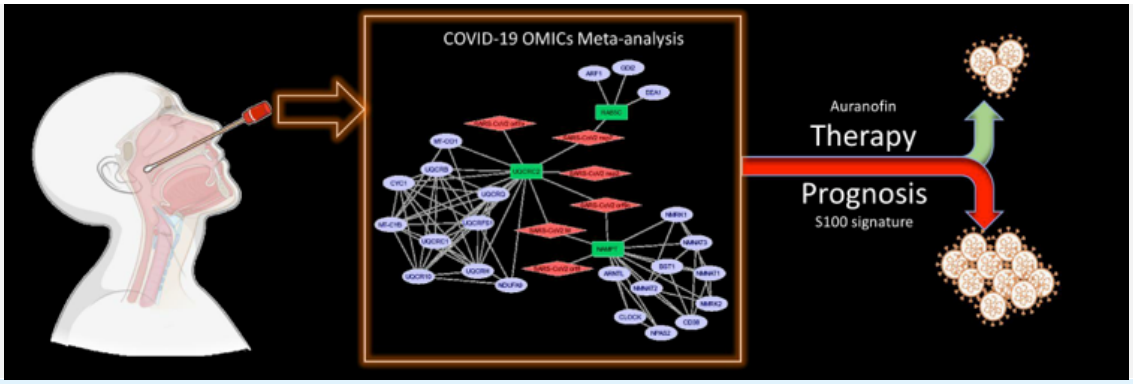
Researchers at IISc led by Saptarshi Basu and Dipshikha Chakravortty have carried out a detailed study on the fate of a large-sized surrogate cough droplet impinging at different velocities on various locally procured cloth fabrics (stole, handkerchief, cotton towel, and surgical masks). A single quantity "ε" was formed by combining the individual effects of pore size and porosity, giving a better insight into the correlation between liquid penetration and fabric properties.

The researchers recommend using a cotton towel (with at least three layers) as a face covering if the person cannot use an N95 or a surgical mask. The team also analysed the effect of washing on mask effectiveness, and results show a negligible influence of washing on mask efficacy up to 70 wash cycles.

In another study, Saptarshi Basu's group collaborated with Narayana Nethralaya Foundation to provide a complete fluid dynamics interpretation of the unexplained droplet generation mechanism from corneal tear film during an eye procedure called non-contact tonometry used routinely for glaucoma detection. The researchers also describe the size scales and velocity ranges of droplets ejected during such procedures, which may help ophthalmologists and medical practitioners conduct these procedures more safely.

- Saptarshi Basu & Dipshikha Chakravortty

Image: Shashank Tripathi



LEADS FOR COVID-19 PROGNOSIS AND THERAPY FROM NASAL SWABS

A recent study, led by Shashank Tripathi from the Centre for Infectious Diseases Research, reports two novel findings: a specific gene signature in nasal swabs which can predict COVID-19 severity, and the potential offered by an FDA-approved drug (Auranofin) for COVID-19 therapy.

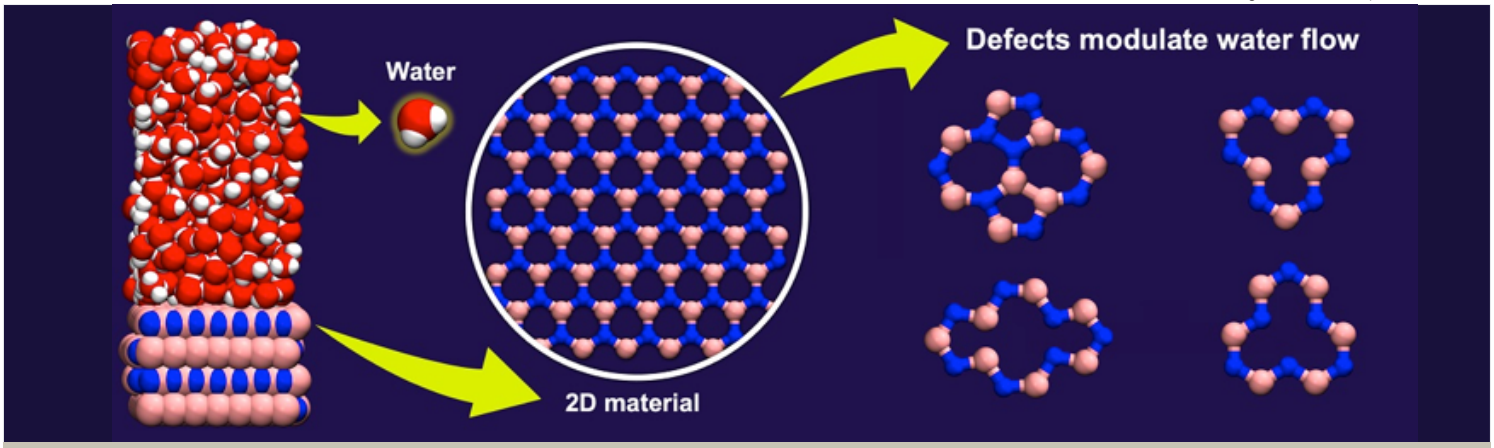
In the study, the researchers analysed COVID-19 data from nasopharyngeal samples and were able to identify specific genes belonging to the S100 family which could serve as prognostic markers of severe COVID-19. This gene signature can be detected by RT-PCR in the nasal swabs which are collected for COVID-19 diagnosis.

The researchers also identified multiple host processes which may be involved in virus replication and disease progression, and may serve as targets for host-directed therapy. Crucially, a redox regulatory protein called Thioredoxin (TXN) was found to be consistently overexpressed in COVID-19 patients.

Auranofin, an FDA-approved drug that targets the enzyme thioredoxin reductase and blocks the thioredoxin pathway, was found to reduce SARS-CoV-2 replication in cell culture as well as in the hamster model. Auranofin is a safe and economical drug used for arthritis treatment. The study, therefore, suggests that it could serve as a promising COVID-19 antiviral.

- Abhijit Biji & Shashank Tripathi

Image: Ananth Govind Rajan & Aniruddha Seal



DEFECTS IN 2D MATERIALS PREDICTED TO MODULATE WATER FLOW

2D materials, which consist of a few layers of atoms, are currently being explored for various applications, including seawater desalination, oil-water separation, and osmotic power harvesting. They often contain defects, which can alter the properties of the material. Although defects are usually thought to increase frictional forces, computer simulations by Ananth Govind Rajan in the Department of Chemical Engineering and Aniruddha Seal from the National Institute of Science Education and Research reveal a notably different picture.

When they analysed a 2D material called hexagonal boron nitride (hBN) interacting with water, they found that some defects, such as a nitrogen (N) vacancy and the Stone-Wales defect (where a boron-nitrogen bond is rotated by 90 degrees in the hBN lattice), surprisingly led to a reduction in water-hBN friction. A boron vacancy increased water-hBN friction almost threefold.

The authors quantified these predictions in terms of “slip length”, a quantity that determines to what extent water molecules “slip” on the hBN surface. They found that, at high defect concentrations, the slip length of hBN with an N vacancy could be comparable to that of graphene, the most slippery 2D material known to date. These findings have implications for the design of devices made using hBN.

- Ananth Govind Rajan & Aniruddha Seal

Prerna Sharma (Photo: Debasmita Mondal)

UNDULATING MEMBRANES MEET LIGHT-GUIDED ALGAE

PRERNA SHARMA'S RESEARCH FOCUSES ON UNDERSTANDING THE NATURE OF SOFT MATTER AT THE INTERFACE OF BIOLOGY AND PHYSICS

Membranes and colloids aren't exactly the first things that come to mind when one thinks of physics. But it took listening to just one talk during her BSc at St Stephen's College for Prerna Sharma to know that is where her destiny lay. Her eyes light up as she recalls that moment. "I knew this is what I wanted to do!" She didn't dip her toes into biophysical waters immediately, though. Her PhD work was broadly oriented around the fluid dynamics of colloids, mixtures in which one component is finely dispersed within another. These mixtures can be biological, such as milk, which is a colloid of fat and water, or non-biological, like fog, a colloid of water crystals and air.

By the time she started her post-doctoral research at Brandeis University in the USA, she had developed a series of tools for studying simple colloidal systems which she wanted to apply to more complicated (read: biological) cases. But unlike conventional biophysicists who study things at the molecular level, like protein folding and protein-protein interactions, her interest was in larger structures.

Now an Associate Professor in the Department of Physics, Prerna focuses on soft-matter physics, the study of systems that are 'squishy' or easily deformed. One of the major systems they study are colloidal membranes. Specifically, they focus on rod-shaped viruses and self-assembly. "Imagine putting a bunch of parts in a box and shaking it, resulting in a fully-assembled computer," says Prerna. That is essentially what her lab does with these viruses. When added to a liquid, under the right conditions, the viruses assemble into a single-layered sheet and orient themselves in the same direction, forming what is called a colloidal membrane. Their lab looks at how these viruses assemble spontaneously under different physical and chemical conditions at the micron scale.

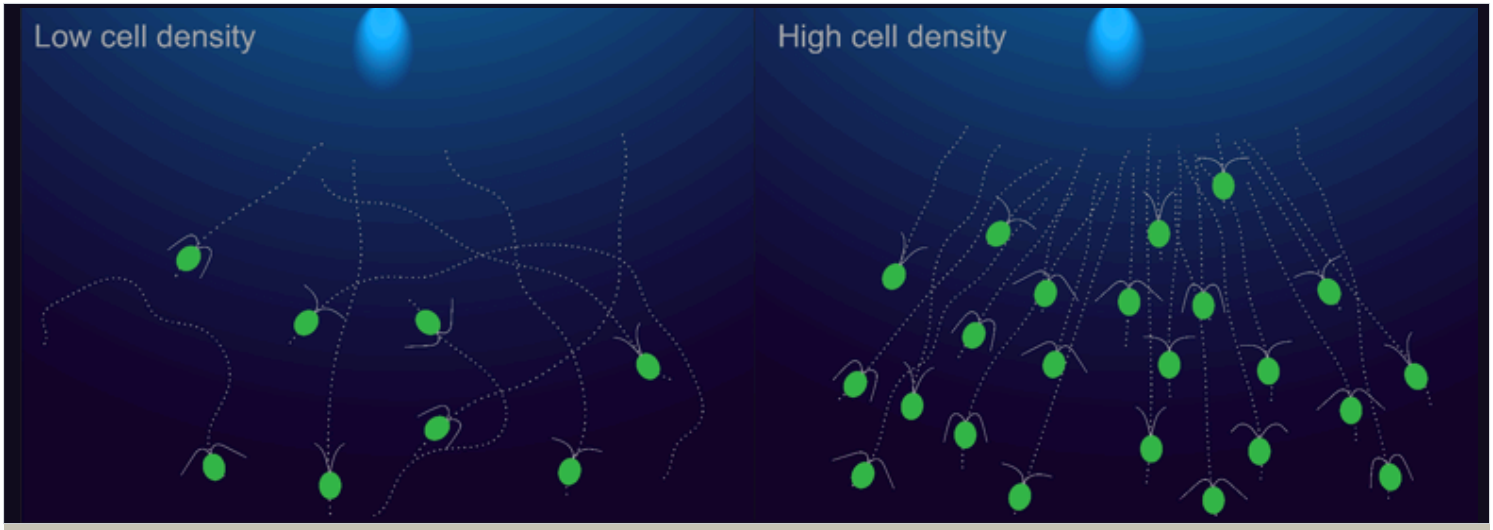
The shapes of colloidal membranes can change depending on how they interact with the components of their environment. One of the ways in which Prerna's lab examines these membranes is by crystallising them, which causes the positions of individual viruses to become fixed and the membrane to adopt a certain shape. In one study, they found that after crystallisation, these membranes became unexpectedly wrinkled and buckled, due to a property called "chirality".

A typical example of chirality is the human hand. The mirror image of a left hand is a right hand, not another left hand. Similarly, the viruses exist as 'left' and 'right' versions. Prerna and her team found that while most of the membrane crystallised, the edges remained fluid-like. The viruses in the fluid regions were able to move and shift positions in response to the forces exerted due to chirality. However, they were unable to do so in the solid, crystallised part, leading to wrinkling and buckling. "It was an unexpected discovery," recalls Prerna, smiling. It was the first major finding from her lab, published in *Nature Communications.*

Since then, the lab has also made significant progress in understanding the nature of the movement of cilia, which are tiny hair-like structures on cells (singular: cilium). Cilia are "active" filaments that perform important functions within the cell, and across tissues. Prerna and her lab purify cilia from an alga called *Chlamydomonas* and study their 'beating' movement in water. The reason they are interested in cilia is because these rod-shaped structures can propel themselves forward using an internal mechanism, without needing an external force.

As the cilium moves by 'beating' or oscillating in a fluid, the stresses generated inside its body need to be balanced out, ideally by external friction – the drag exerted by the fluid on the cilium. However, Prerna's team realised that the effect of this external friction was

Collective motion of algae towards a light source (Image: Sujeet Kumar Choudhary)



too small. Instead, rearrangements within its structure itself were creating enough 'internal friction' to help the cilium move, the team found. These experiments were the first to unambiguously show how internal friction drives cilia movement.

A third area in which Prerna's lab works is collective phototaxis, the spontaneous movement of certain single-celled organisms towards a source of light. Large groups of *Chlamydomonas* are known to be faster than a single cell at detecting light and orienting themselves towards it. Her lab showed that the reason behind this is the crowding from being in a large group, which causes their normal rotating and tumbling motions to slow down, leading to greater efficiency in detecting light. This finding received a lot of attention on Twitter, and one person even stated, "How to do better? Slow down!" while citing her research.

Prerna's work has not only the potential to advance our knowledge of self-assembly in biological systems but also industrial and medical applications. Understanding how thin sheets behave, for example, and pushing them into well-defined shapes can lead to better manufacturing processes. Since the beating of cilia is also responsible for cleaning our lungs of any debris that finds its way there, the lab's work could also one day lead to the creation of a personalised medicine platform for human lungs, according to Prerna.

"I'm not planning the personalised medicine platform immediately," she laughs. In the short term, she wants to create and work with colloidal matchsticks, which are long viral rods with a small bit of protein at one end. These matchsticks naturally assemble into membranous sheets just like the viral rods. Such objects fascinate both biologists – who seek to study how they interact with other biological molecules – and physicists – who might gain insights into symmetry and self-assembly from studying them – alike. For example, they could serve as a platform to study how drugs interact with the protein carried by the matchstick.

In the long term, Prerna wishes to explore deeper questions about emergent behaviour – new behaviours that arise when similar objects come together to form a complex system. "Now that I know how a single cilium behaves, I would like to put two of them together and see if I can predict their behaviour," she states. "And once I know that I would like to look at the behaviour of a larger number. Maybe a whole carpet of cilia."

- Savyasachee Jha

Prerna Sharma and her lab members. From left: Sujeet Kumar Choudhary, Debasmita Mondal, Lachit Saikia, Prerna Sharma, Ayantika Khanra (Photo: Sudhir Kumar Sahu)

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