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KERNEL

Editorial

Among the many vaccines being developed for COVID-19, what makes the one being developed at IISc unique?

Read more about it in this issue of *Kernel.*

Other stories in this issue feature magnetic nanomotors that can selectively target cancer cells, a new species of water bear that can survive UV radiation, and the profile of an astrophysicist on a quest to solve the mysteries of the universe.

GRAPPLING WITH DEADLY VIRUSES

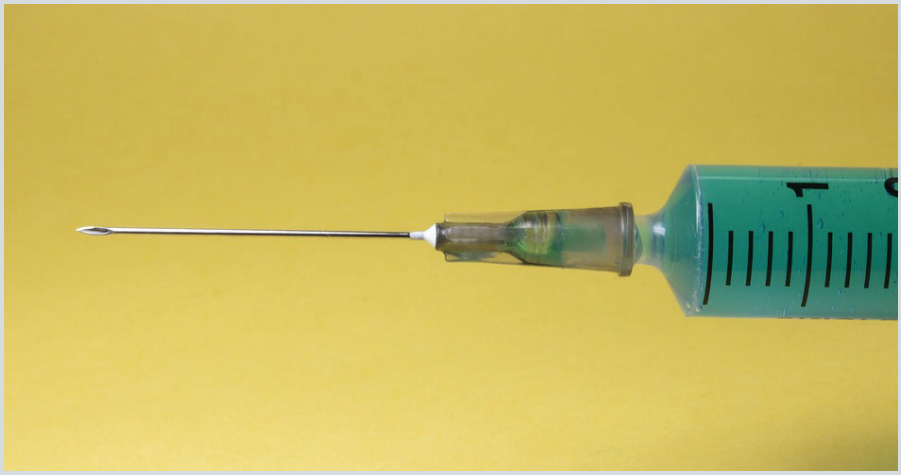

Photo: Pixabay/HeungSoon

RESEARCHERS AT AN IISc LAB AND START-UP ARE WORKING ON EFFECTIVE VACCINE STRATEGIES AGAINST TWO DEADLY VIRUSES – SARS-CoV-2 AND HIV

In the midst of the raging pandemic, hope bloomed this November. Two research groups announced within days of each other that their COVID-19 vaccine candidates were more than 90% effective at preventing infection. The first doses of these vaccines could be available before the end of 2020, at least in the US, if an emergency use authorisation is granted by the country's Food and Drug Administration (FDA).

But here's the kicker: both vaccines need to be stored and shipped at extremely cold temperatures to prevent them from losing their potency. At this point, countries like India simply do not have enough freezer boxes and supply chains to store and transport millions of doses of such vaccines, especially to far-flung corners.

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Mynvax team (Photo courtesy: Mynvax)

A more feasible option for a country like India would be a vaccine that can be stored at room temperatures. One such heat-tolerant vaccine is currently being developed at IISc by researchers in the lab of Raghavan Varadarajan, Professor at the Molecular Biophysics Unit, and Mynvax, an IISc-incubated start-up that Varadarajan co-founded with alumnus Gautham Nadig.
In a study published recently in the *Journal of Biological Chemistry,* the team showed that their vaccine candidate, which triggered a strong immune response in guinea pigs, could remain stable for a month at a balmy 37°C.

Many of the 200-odd COVID-19 vaccines being developed contain the spike protein of the novel coronavirus, a molecule that sticks out of the virus's surface. The hope is that injecting this protein, or a modified form of it, into our body will prompt our immune system to produce antibodies that may help us fight the virus if and when we get infected. But instead of the full spike protein, which needs more stringent purification protocols, Varadarajan's team decided to use just a small portion of it – a short stretch within a region called the receptor binding domain (RBD), the part that actually binds to our cells.

First, they took the genes coding for this RBD portion and inserted it into a carrier molecule called plasmid. The plasmid was then planted into mammalian cells in the lab, which 'read' the genes and churned out copies of the RBD portion. These copies were then injected into guinea pigs in the lab. After a few weeks, the guinea pigs started producing antibodies against the RBD portion, enough to prevent infection. This was the expected part.

What was unexpected was that their RBD portion also turned out to be extremely sturdy – it remained active for a month at 37°C, and freeze-dried versions could handle temperatures up to 100°C. "That was a matter of luck," says Varadarajan. The full spike protein, on the other hand, quickly lost its activity beyond 50°C.

The team will now have to carry out safety and toxicity studies in rats, along with process development and manufacture of a clinical trial batch, before they are tested in humans. "Those studies can cost about Rs 10 crore," he says. "Unless the government funds us, we might not be able to take it forward."

SARS-CoV-2 isn't the only deadly virus that Varadarajan's lab has been taking on; they have also been working on HIV. A second study published by them in the journal *Proceedings of the National Academy of Sciences* describes an effective method to pinpoint which parts of the HIV surface are targeted by antibodies.

Like SARS-CoV-2, HIV has an envelope protein that helps it latch on to cells in our body. Scientists have struggled to pinpoint exactly which parts of this protein – called epitopes – are targeted by neutralising antibodies, a subset of our immune system's antibodies which can block virus entry. Vaccines based on these antibody-binding regions have a better chance of triggering a strong immune response, according to the authors.

Existing methods like X-ray crystallography and cryo-electron microscopy can help map such regions, but they are time-consuming, complicated and expensive.

Cryo-electron microscopy, for example, would allow one to identify the sites where antibodies bind to, but cannot distinguish which of these are neutralising antibodies. Therefore, Varadarajan and his team decided to explore alternative approaches.

First, the team mutated the virus so that a molecule called cysteine would pop up in several places on the envelope protein. They then added a chemical label that would stick to these cysteine molecules, and finally, treated the virus with neutralising antibodies. If the antibodies could not bind to crucial sites on the virus because they were blocked by the cysteine label, the virus could survive and cause infection. These sites were then identified in one shot by sequencing the genes of the surviving mutant viruses.

"This is a rapid way of figuring out where antibodies are binding and is useful for vaccine design," says Varadarajan. It doesn't need expensive equipment, just gene sequencers readily accessible to most labs, he adds.

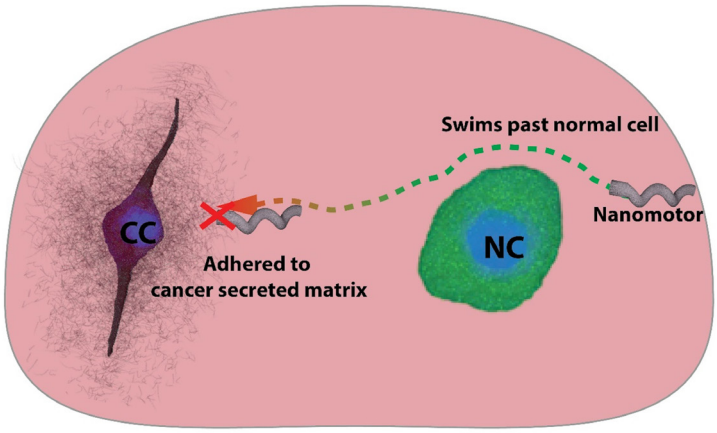
The researchers used this method to analyse the sera – the portion of blood containing mixed antibodies – from a HIV patient. "We used the sera to map unknown epitopes using the same approach," says Rohini Datta, first author and former PhD student. "We found that the patient sera targeted two sites on the envelope protein. The fact that this sera targets these specific sites was not known before."

Another advantage is that it can be used to identify multiple epitopes from multiple sera in parallel, she says. This could be helpful during clinical trials, for example, to see how different people's sera react to the same vaccine.

"In principle, researchers could adapt this methodology to any virus, including SARS-CoV-2," says Varadarajan.

- Ranjini Raghunath

Image: Debayan Dasgupta



NANOMOTORS AS PROBES TO SENSE CANCER ENVIRONMENT

MAGNETICALLY-DRIVEN NANOMOTORS CAN STICK TO THE MATRIX SURROUNDING CANCER CELLS, WHICH CAN HELP PROBE THEIR ENVIRONMENT AND SELECTIVELY TARGET THEM

An interdisciplinary team of researchers from IISc has used a 3D tumour model and magnetically-driven nanomotors to probe the microenvironment of cancer cells. The team consists of researchers from the Centre for Nano Science and Engineering (CeNSE) and Department of Molecular Reproduction, Development and Genetics (MRDG).

In their work, published in *Angewandte Chemie,* the team steered helical nanomotors remotely via an external magnetic field through the tumour model to sense, map and quantify changes in the cellular environment. The model comprises both healthy and cancer cells embedded within a reconstituted basement membrane matrix, and mimics the breast cancer environment.

The study highlights a new way of targeting cancer cells by manoeuvering nanomotors inside a tumour and waiting for them to localise in the vicinity of the cancerous site. "We tried driving the nanomotors toward cancer cells in a tumour model and observed them getting stuck to the matrix near cancer cells, but this was not observed near normal cells," says Debayan Dasgupta, a co-first author and PhD student at CeNSE.

The extracellular matrix (ECM) is a complex 3D network of proteins and carbohydrates secreted by living cells into their neighbourhood. However, when cancer cells secrete fresh material into the ECM, it disrupts the chemical and physical composition of the native ECM surrounding healthy cells, degrading the local environment. Therefore, understanding how the cellular microenvironment is altered due to cancer cells and measuring these changes quantitatively could be vital in understanding the progression of cancer.

In the current study, the researchers discovered that as the nanomotors approached the cancer cell membrane, they stuck to the matrix more strongly than they would to normal cells. To measure how strongly the nanomotors bound to the matrix, the team calculated the magnetic field strength required to overcome the adhesive force, and move forward.

"This means that the cancer cells are doing something. So, we did some measurements and discovered that it [the adhesive force] depended on the type of cells, the strength of interaction and also which side of the cell the nanomotor approached," explains Ambarish Ghosh, Associate Professor at CeNSE and one of the senior authors. "In the end, we really ended up discovering a physical property of an important biological environment."

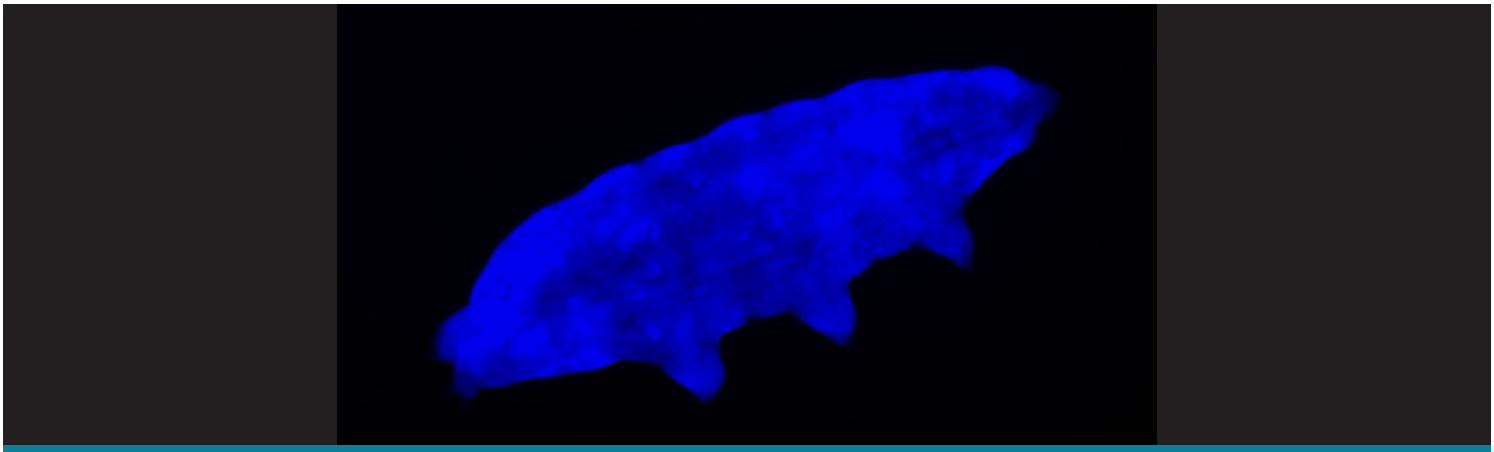
The reason why the nanomotors appear to stick to the cancer cells better is their charged ECM. This may be due to the presence of 2,3-linked sialic acid, a sugar-conjugated molecule which confers a negative charge on the cancer cell environment, the researchers found. They visualised the distribution of these sugars using fluorescent markers and found that sialic acids were distributed up to 40 micrometres from the cancer cell surface – the same distance until which the nanomotors experienced strong adhesion.

To counter this adhesive effect, the team coated the nanomotors with a polymer which shielded them from the charged environment. The coated nanomotors did not stick to the matrix near the cancer cells, whereas the uncoated motors clung to the matrix, confirming the fact that the negatively charged cancer microenvironment interacts with the incoming nanomotors, rendering them immobile.

"What came as a beautiful surprise was that within such a milieu, we found that aggressive cancer cells ended up remodelling their surroundings by making them stickier, and richer in specific charged sugars," says Ramray Bhat, Assistant Professor at MRDG and one of the senior authors. "This charging can potentially be used to target and kill tiny populations of cancer cells hidden among their normal counterparts, for which we are extending these studies to living animals."

- Gouri Patil

A fluorescent tardigrade from IISc campus (Photo: Harikumar R Suma and Sandeep M Eswarappa)



SURVIVAL SECRETS OF TARDIGRADES

STUDY FINDS THAT TARDIGRADES CAN SURVIVE ULTRAVIOLET RADIATION BY GLOWING IN THE DARK, A FEAT THAT ADDS TO THEIR REPUTATION AS AN INDESTRUCTIBLE MICRO-ANIMAL

Tardigrades are tiny, millimeter-sized creatures that have fascinated the scientific community for a while now – understandably so – as not many animals can claim the ability to survive five mass extinctions. Due to their appearance, tardigrades are endearingly called moss piglets or water bears.

Sandeep Eswarappa, Assistant Professor in the Department of Biochemistry, has been studying these water bears for the past five years. "I watched this popular science-based TV series called *Cosmos* presented by Carl Sagan and later by Neil deGrasse Tyson. One of the episodes that stuck talked about the microcosmos present in a single drop of water. It mentioned tardigrades and how they have survived five mass extinctions. This episode got me interested in them," he explains. Incidentally, his postdoc mentor had trained under Carl Sagan. As a biochemist, the nudge to study less explored model systems – *E. coli,* yeast and mice are already well explored – also pushed him closer towards tardigrades.

Although tardigrades were first discovered by German zoologist Johann August Ephraim Goeze in 1773 – he gave them the name water bears – research on them is even now in its nascent stage.

What makes these creatures unique is the fact that they can tolerate harsh conditions such as extreme temperature and pressure, ionising radiations, osmotic stress, and even the vacuum of space. By studying tardigrades, researchers hope to learn the mechanisms by which they can tolerate extreme physical stresses, and hopefully apply these findings to help humans cope with similar conditions.

Eswarappa points out that understanding tardigrades' stress tolerance has significantly improved in the last five years and this could potentially lead to useful applications.

In a recent study, for example, his lab discovered a new species of tardigrades (*Paramacrobiotus* sp.) that can tolerate harmful UV radiation. When exposed to lethal UV radiation, these tardigrades withstood the rays by absorbing them and releasing a fluorescent glow. "They use a fluorescent shield that absorbs harmful UV radiation and emits harmless blue light as fluorescence. Interestingly, we could transfer this UV tolerance property to another tardigrade, *Hypsibius exemplaris,* which is otherwise sensitive to UV radiation," says Eswarappa.

Their study offers direct evidence that fluorescence in organisms provides photoprotection, he points out. "Photoprotection against UV radiation has been suggested as a possible function of fluorescence in some organisms such as amphioxus, comb jellies and corals. However, there was no direct experimental proof of this in any organism, until our study," he says. His team is now focusing on identifying the chemical nature of the fluorescent compound.

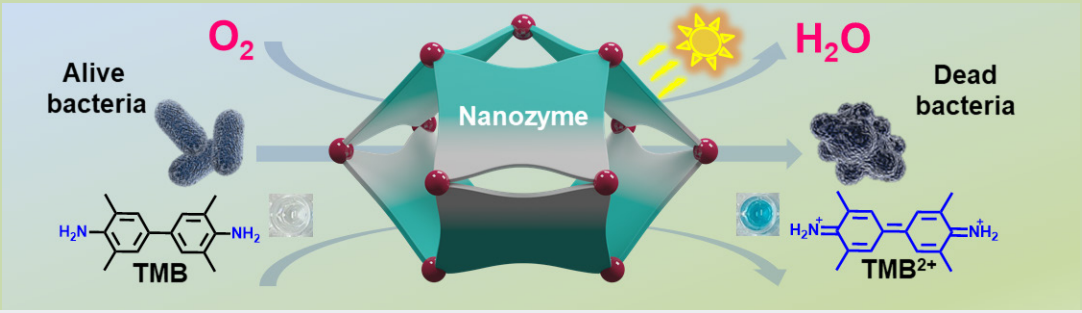
Such insights could even come in handy in our day-to-day lives. "This research can potentially lead us to develop a novel UV-protective (sunscreen) compound," he points out. The same compound could also be used to make UV-protective windows and screens.

Eswarappa's lab is also collaborating with physicists and chemists to learn more about these fascinating creatures. "We plan to investigate their ability to tolerate complete desiccation and thermotolerance. We are also interested in studying their metabolism and translation [one of the steps in protein synthesis] during their hibernation state, called 'tun' state."

There are still many mysteries yet to be solved when it comes to tardigrades. A recent study, for example, showed that some tardigrades cannot survive extreme heat, unusual for an animal pegged to survive almost anything. But that may not be as surprising because there are over 1,000 known species of tardigrades, says Eswarappa. "Not all of them will show thermotolerance. They have evolved tolerance according to their environment."

- Vaishalli Chandra

Image: Soumalya Bhattacharyya



MOLECULAR CAGES THAT MIMIC ENZYMES AND KILL BACTERIA IN WATER

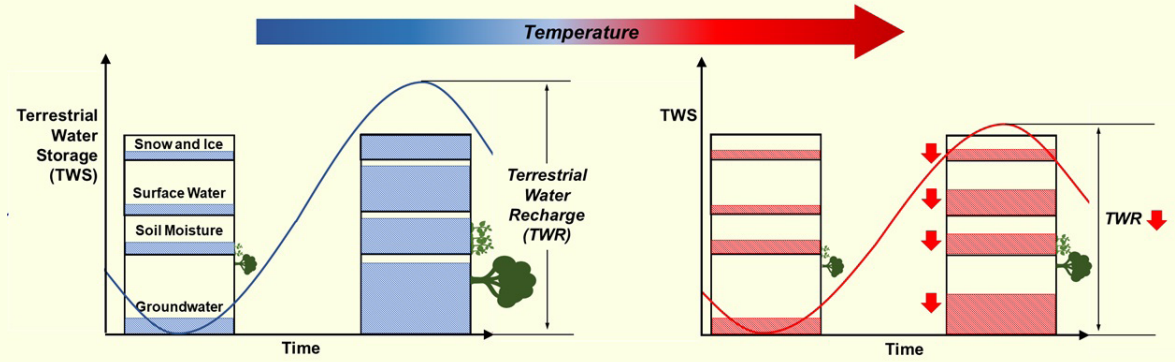
Researchers from the Departments of Inorganic & Physical Chemistry and Organic Chemistry have synthesised molecular architectures that can kill disease-causing bacteria in water, including the infamous methicillin-resistant *Staphylococcus aureus.* They fashioned these molecules to mimic natural enzymes, using the principles of supramolecular chemistry, which deals with how molecules assemble spatially, and the intermolecular forces responsible for their organisation.

In one of their studies, the researchers designed a "molecular cage" called PMB1 via self-assembly of a benzothiadiazole-based ligand and platinum-based units. The benzothiadiazole unit is a photosensitiser, which absorbs light efficiently and produces reactive oxygen species that disrupt the bacterial cell membrane. The positively charged PMB1 cage also enhances the adhesion of the bacteria to the cage and damages the bacterial cell membrane.

In another study, they propose a water-soluble nanozyme cage structure consisting of benzothiadiazole-based and palladium-based units, which imitates the activity of an enzyme called 'oxidase' and generates reactive oxygen species due to the light absorption by the benzothiadiazole unit. These kill the bacteria present in water.

- Gouri Patil

Image: Chandan Banerjee



RISING TEMPERATURES ARE LEADING TO REDUCED WATER RECHARGE

Water availability in a region can be denoted by the total water annually recharging the natural water bodies above and below the ground level such as lakes, rivers, groundwater, and so on.

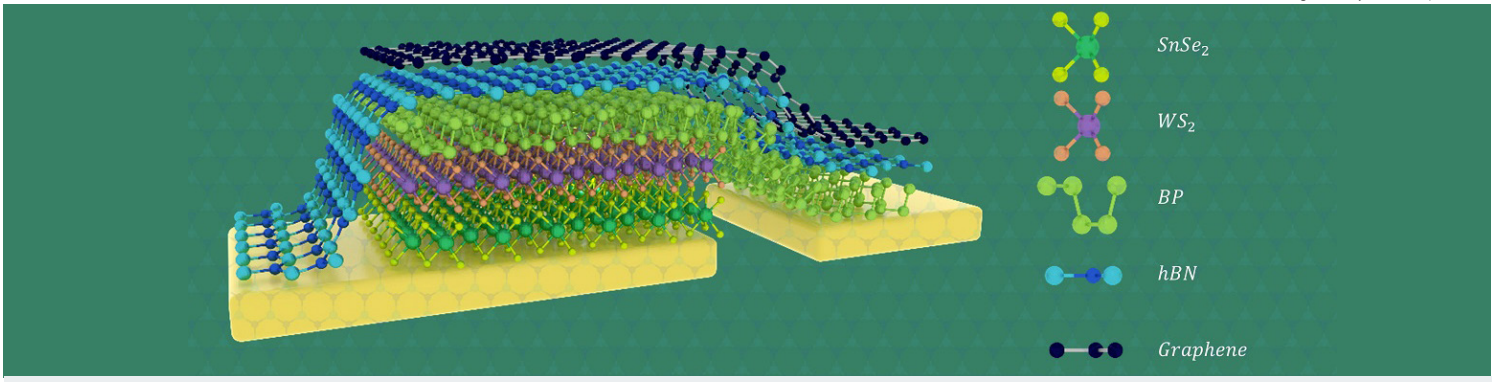
Researchers at IISc and the University of New South Wales used satellite-derived estimates of total annual recharge to investigate the effect of rise in temperature for areas drained by 31 major rivers around the world.

These include the Amazon, Ganges, Brahmaputra, Indus, Nile, Tigris-Euphrates, Mekong and Mississippi, alongside which most of the global population resides.

The researchers find that the areas drained by 23 out of these 31 rivers show reduced recharge with increase in temperature. Vegetation growth was also found to reduce due to the decline in the annual water recharge. Given that this is a result of just 0.9°C rise in global temperature, the impact of the expected 3.5°C rise by the end of this century is a major concern.

The findings of this work, based on Gravity Recovery and Climate Experiment (GRACE) satellite observations, are the first of their kind and in line with future projections from mathematical models.

Image courtesy: Kausik Majumdar lab



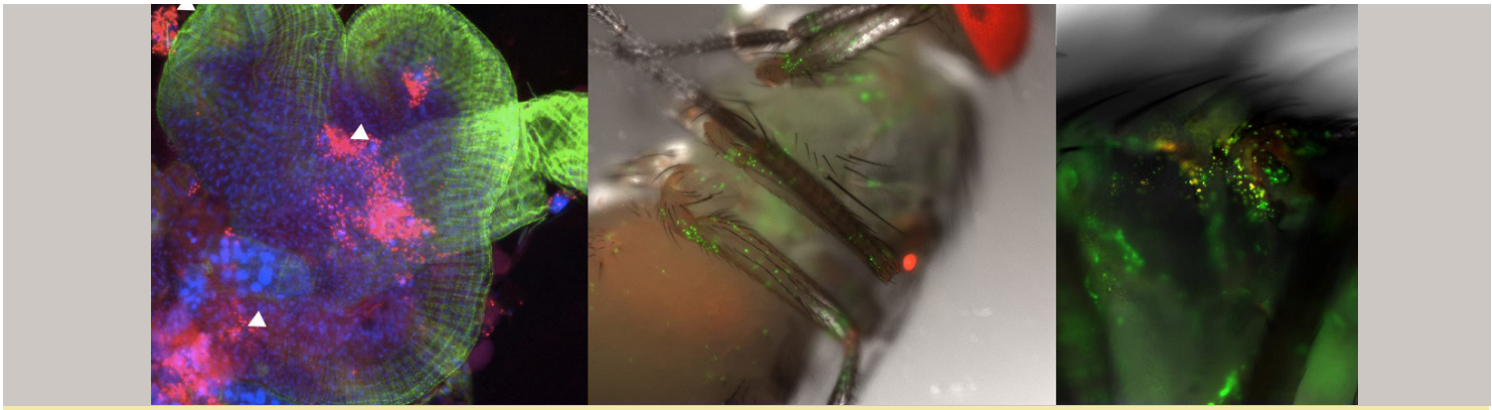
A VERSATILE TUNNEL DIODE FOR LOW-POWER ELECTRONICS

With increasing demand for low-power electronic chips that can perform diverse functions, alternatives to traditional electronic circuit components are actively being explored. One such component is the Esaki tunnel diode, hailed as a landmark discovery in the semiconductor industry, which has now become a key element in electronic circuits.

In a recent study, researchers from the Department of Electrical Communication Engineering have designed and experimentally demonstrated a highly versatile form of this tunnel diode, where the active layers are separated by just a few tenths of a nanometre thick spacer.

The team was able to transform this tunnel diode into both a voltage-controlled oscillator and an ultra-low-power single element memory cell. The oscillator can be easily integrated onto a chip. The memory cell is ideal for low-power electronics as it consumes very less energy, and is also suitable for high density memory applications. The proposed tunnel diode retains its versatile operation at very low temperatures (-269°C), making it suitable for cryogenic electronics as well.

Image: Sveta Chakrabarti



UNRAVELLING SIGNALLING PATHWAYS IN WOUND HEALING

Our body can detect tissue damage and wounds using specific signals from cells called damage-associated molecular patterns (DAMPs). To study the signalling pathways linked to wound healing, researchers have been using the fruit fly as a model.

In a new study, researchers from the Department of Molecular Reproduction, Development and Genetics (MRDG) show that hydrogen peroxide produced from a wound activates specific signalling pathways in the fruit flies' blood cells (also called hemocytes). Hydrogen peroxide acts as a DAMP signal to help home in hemocytes to the site of damage and activate wound-healing pathways. Hemocytes, in turn, help produce more hydrogen peroxide near the wound using an enzyme called DUOX.

The researchers also found that a water channel called aquaporin helps increase intracellular hydrogen peroxide in blood cells following an injury, which is critical for their activation.

Another immune pathway (Toll) was activated upon injury, which is protective for the flies from subsequent bacterial infection. This points to a role that the injury has in training the immune response to fight a potential disease-causing agent.

Nirupam Roy (Photo: Parijat Dey)

ON A COSMIC TRAIL

NIRUPAM ROY'S LAB OBSERVES THE COSMOS THROUGH RADIO TELESCOPES

Nirupam Roy's foray into the field of astrophysics happened by chance. During his BE days, he came across a tender notice from the Tata Institute of Fundamental Research (TIFR) in the local newspaper. He wrote a letter, addressing it to the head of the Department of Physics, hoping to get his physics-related questions answered. This letter ended up reaching Probir Roy, a high energy physicist in TIFR, who replied, and later encouraged Nirupam to apply for a PhD.

Nirupam went on to pursue a doctoral degree in astrophysics at the National Centre for Radio Astrophysics (NCRA-TIFR), where he met the late Govind Swarup, widely regarded as the "father of Indian radio astronomy", from whom he learnt about the early days of astronomy research in the country. He then joined the National Radio Astronomy Observatory in New Mexico as a Jansky Fellow, and later the Max-Planck Institute for Radio Astronomy in Bonn, Germany for his postdoctoral studies.

During his time abroad, Nirupam would get periodic emails from Govind Swarup encouraging him to "come back to India after his fellowship" and "consider teaching". "It was part of the reason I joined IISc," he says. After he returned to India, he worked briefly at IIT Kharagpur, before joining as an assistant professor in the Department of Physics at IISc in 2016.

In IISc, his lab focuses on two fascinating aspects of stars: the interstellar medium and supernova remnants. Interstellar medium, as the name suggests, is the collection of low-density gases and dust that are present in the space between the stars. It plays an important role in the formation of new stars. In a recent study, a student at his lab looked at the temperature distribution of the gases in the interstellar medium. To his surprise, he found definitive evidence that interstellar turbulence is driving a large fraction of the gas to the temperature range that was so far considered unstable and mostly devoid of gases.

Studying the interstellar medium may also shed light on another fascinating aspect of the universe: dark matter. When researchers estimate the mass of galaxies or galactic clusters from their dynamics, that mass is always higher than the directly observed mass, and this discrepancy is attributed to dark matter.

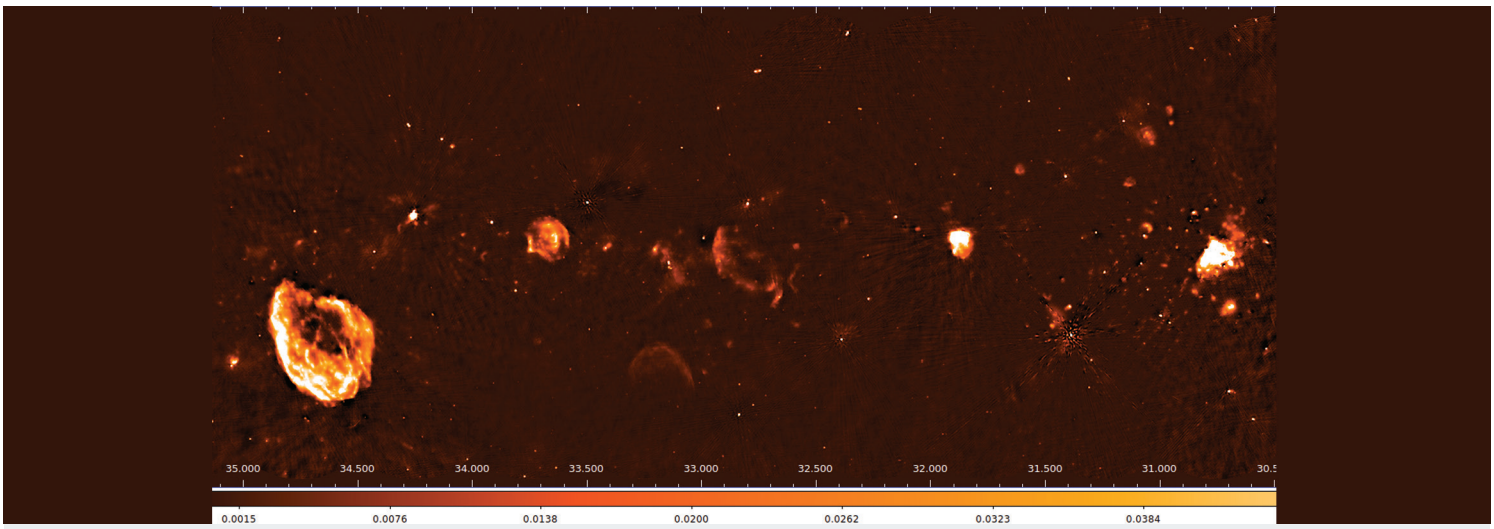
"We don't see any light being emitted or absorbed by it. But we see that there is a gravitational effect from it. This is called dark matter and its exact nature is not known. It is known that it exists. It is known that the overall dynamics of the universe is strongly influenced by the dark matter components. However, we do not know what it consists of," he explains. His lab is trying to observe and model the mass distribution of both stellar and dark matter mass in the galaxy.

The second important aspect of stars that his lab works on is the supernova. Some massive stars, towards the end of their life, undergo violent acts of explosion called supernovae and leave behind some of their mass as a remnant, much like the shell of a bullet. His group has discovered new supernova remnants and found associated neutral hydrogen jet in them for the first time.

Jointly with an international team, they also plan to carry out a survey to search for supernova remnants in the low frequency range (250-900 MHz) using the Giant Metrewave Radio Telescope (GMRT) located in Pune.

Nirupam is also part of a team of Indian scientists participating in an international collaborative project called the Square Kilometer Array (SKA), which

Low frequency sky image from GMRT pilot survey (Image: Rohit Dokara)



aims to build the world's largest radio telescope. "It will have much better sensitivity than the existing telescopes, and it will be able to do many of these things that we are doing today with much better accuracy," he explains.

In astrophysics, the farther one looks across the universe, the farther back in time you go. Shortly after the big bang, the universe began to reionise. Very little is known about this epoch of reionisation. The SKA aims to study this by capturing the low frequency signal of neutral hydrogen from the farthest corners of the universe corresponding to this epoch. "You are trying to construct the overall evolution of the universe. You are trying to answer when exactly the past galaxies and stars and quasars started existing in our universe," Nirupam explains excitedly.

Aside from unravelling the deepest mysteries of our universe, Nirupam enjoys reading fiction, writing for Bengali magazines and keeping himself abreast of the sociopolitical situation of the country.

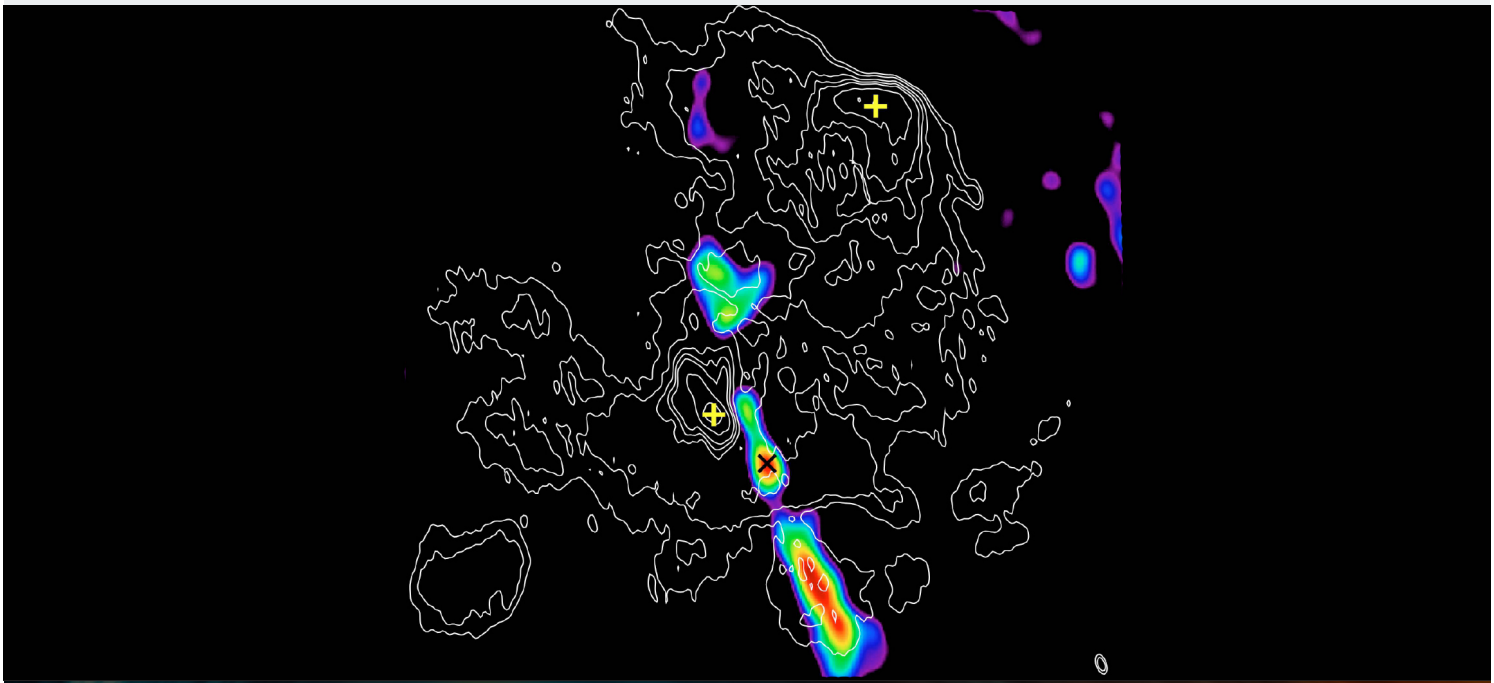
In recent months, the pandemic has slowed down his work, but only to a small extent since his research mostly involves analysing data from observatories, and theoretical modelling.

Though some deadlines have been missed, he feels it is important to not get stressed out. "At the end of about 27 years of education, it does not matter if it is 27.5 or 28 years, so it is important to not worry about the delay, but to keep the bigger picture in mind," he says.

He proposes the same advice to students who are in academia. Running behind that one publication today for something in the future, while completely ignoring the present is not an ideal thing to do, he says. "Look at it from the outside, once in a while. I'm not discouraging anyone from doing hard work, but you have to enjoy [the work] while you are doing it."

- Rohini Murugan

Neutral hydrogen jet associated with supernova remnants (Image: Veena et al.)



Office of Communications (OoC)
Indian Institute of Science (IISc)
Bengaluru - 560012
kernel.ooc@iisc.ac.in | office.ooc@iisc.ac.in

भारतीय विज्ञान संस्थान

EDITORIAL TEAM
Deepika S
Karthik Ramaswamy
Ranjini Raghunath (Coordinator)
Samira Agnihotri
Vaishalli Chandra

DESIGN
TheFool.in