Science Last Fortnight

In this column we bring you some snippets from selected scientific literature published between 25th of March and 10th of April – between the last issue of *Current Science* and the current one.

Synthesizing the Smallest Genome

Humans have more than 20,000 genes. *Escherichia coli* carry 5000 genes. *Haemophilus influenza*, 1815 genes, and *Mycoplasma genitalium*, 525 genes. What is the minimum number of genes necessary for an organism that can grow and reproduce?

The mycoplasmas, which typically grow in the nutrient-rich environment of animal hosts, have the smallest known genomes in nature. A comparison of the genome sequences of *Haemophilus influenzae* and *M. genitalium* revealed a common core of only 256 genes. So this was initially proposed to be the minimal gene set for life. But using global transposon mutagenesis in *M. genitalium*, scientists identified 375 essential genes. 150 genes seemed to be nonessential.

To create a minimal organism that can grow and reproduce called for other kinds of experiments. Now the group reports such a work undertaken on *M. mycoides*. *M. mycoides* has more genes than *M. genitalium*. By deleting nonessential genes, transplanting the edited genome to genome-free cytoplasm and checking whether these synthetic organisms could grow and divide, scientists distinguished some quasi-essential genes among what was earlier considered nonessential.

Ultimately they have reduced the number of genes that can make viable organisms to just 473. Scientists have successfully synthesized a minimal cell that is simpler than natural ones.

Science, **351** (6280), (25 Mar 2016)

Monitoring Groundwater Drought

With the unpredictability of rainfall, use of groundwater has gone up in recent decades. Removal of water from underground aquifers, without allowing them time to recharge, can lead to groundwater drought. To measure groundwater recharge deficit in an area, a standardized water-level index (SWI) is used. The index takes into account variations in seasonal water levels. The index is especially useful in relatively dry regions where the standardized precipitation index (SPI), vegetation indices, etc. do not help monitor droughts that impact people.

A study by IIT, Mumbai has come up with a methodology to identify spatio-temporal groundwater droughts and drought-prone zones. They used SWI to determine temporal groundwater drought combined with SPLINE interpolation in GIS to locate spatial groundwater drought.

The SPLINE interpolation technique for GIS minimizes the total surface curvature, simultaneously creating a smooth surface passing through all the sampled points. While the SPLINE function can generate reasonably accurate surface curvatures with a few sampled points, it operates best in cases of low varying surfaces.

Combining these two techniques, the team studied drought conditions in the basin of Krishna tributaries in Mahabubnagar as a test case. The variations in groundwater droughts among various stations of observation were established for the period 1998–2011. The study showed that the region has experienced some instances of mild groundwater droughts.

The methodology offers a successful strategy to monitor and respond to water scarcity in other rainfed drought-prone areas in India. Village-level drought vulnerability assessment, budgeting water use and planning the development of groundwater resources could encourage judicious groundwater exploitation in the country.

Geocarto International, **31**(4), 385–407 (2016)

Early Surra Alert
for bovine health

'Surra' is a disease prevalent in domesticated animals, especially cattle, buffaloes, horses and camels. It is marked by fever, weakness and low productivity in the affected animals. The causative organism is a protozoan parasite, *Trypanosoma evansi*, transmitted by blood-sucking flies. The parasite can survive within hosts for several months, before clinical symptoms appear. And during this time, the protozoa may be transmitted to other healthy animals. Therefore, a surveillance system for early detection of the disease, diagnosis and treatment is an imperative for farmers.

Scientists at the National Institute of Veterinary Epidemiology & Disease Informatics, Bengaluru, have now developed a highly sensitive technique to specifically detect *T. evansi*. They used highly specific monoclonal antibodies raised against the surface proteins of *T. evansi* to detect its presence in animal serum samples. Testing samples from cattle, buffaloes, camels, horses and donkeys, collected across India, Sengupta and team were able to demonstrate a sensitivity of 95.8% and 94.4% specificity for the test.

By eliminating the possibilities of false positives that may be caused by other protozoans, the new test is a good diagnostic tool for mass-screening of animals. But more importantly, data from the study has thrown up an intriguing result: higher prevalence in adult cattle compared to calves!

In science, when one solves a problem, more crop up.

Veterinary Parasitology, **219**, 17–23 (30 March 2016)

Connecting the Dots
Sensing acetone, distinguishing life

Since the accidental discovery, carbon dots have found applications in diverse fields owing to their tunable optical properties. Recently, Pal and his team from IIT Kharagpur have reported a simple process of synthesizing highly fluorescent carbon dots (CDs) that yields particles as small as 2 nm.

The researchers generated CDs through a modified hydrothermal treatment of an alkaline solution containing dopamine and cysteine. The use of an alkaline medium enhanced the emissive property of CDs by stabilizing surface defects like zig-zag edges. However, when the technique was extended to water miscible solvents like acetone, non-fluorescent CDs were produced.

The differing optical properties were due to changes in size and charge.

The fluorescence of CDs originates from surface energy traps and the radiative recombination of electron hole pairs. Substances like acetone, which are good electron donors, can hinder this process and cause quenching. The generated CDs could, therefore, be used to detect acetone in aqueous solutions. It can be easily seen by the reduction of fluorescence.

This has relevance in both industry and biology as acetone – a component of industrial wastes – is also produced under certain physiological conditions of the body. Since the ultra-small particles can easily enter cells, they are also good probes for live cell imaging. Generally, dead cells have a pH of 4–5. This causes the CDs to become non-fluorescent. These extremely cyto-compatible CDs can, therefore, be developed as a novel and economical alternative to the classic 'Live-Dead' assay.

Talanta, **150**, 253–264 (1 April 2016)

Small Peptides to Fight Cancer

Scientists in Kharagpur have been interested in *Abrus precatorius* for some time now. The name *Abrus precatorius* implies a graceful plant that provides rosary beads for prayers. But researchers in IIT KGP had a totally different reason for their interest: cancer treatment.

Earlier, they had demonstrated an immunomodulatory effect of a processed fraction of *rathi* seeds. They had identified two bioactive peptides which they called IR15 and SR11 – to indicate information about their first and last amino acid residues along with the total number of residues. In a recent work, they explore the nature of these peptides and their potential use in cancer therapeutics.

First they showed that the peptides are non-toxic to normal human keratinocyte cell lines. The fluorescently tagged peptides were taken up by cells in a time and concentration dependent manner. By inhibiting peptide uptake, the researchers found clues about a possible mechanism for the entry of the peptides into cells.

The study suggested that the peptides can probably aid the uptake of other biomolecules including components of the active fraction that are cytotoxic towards cancerous cells. So the researchers administered imatinib mesylate – a drug used for cancer treatment – along with IR15 and SR11. The drug uptake was significantly increased, decreasing cell viability.

Interestingly, co-administration of imatinib and the peptides did not show any increase in imatinib uptake by normal cells. This specificity towards cancer cells gives these peptides a potential therapeutic value. An increase in cellular uptake of the drug can help reduce drug dosage and, thus, associated harmful effects.

Although this is only an initial study on human cell lines, it encourages further work on these peptides to evaluate their potential in cancer therapeutics using animal models.

Colloids and Surfaces B: Biointerfaces, **140**, 169–175 (1 April 2016)

Mismatch Mutations:
Nanopore sequencing of DNA

Until now, most sequencing studies have focused on single strand DNA. This method is incapable of detecting the most frequent DNA mutations: incorrect base pairing. A new method suggested by Karmakar and Kundu can solve this problem.

The method relies on the use of a graphene nano-pore, created on a single-layer zigzag graphene nano-ribbon. This can detect current signals. Each base pair has a specific conductance and Local Density Of State (LDOS). Differences in electrical conductance and LDOS patterns could help recognize nitrogenous bases trapped in the nano-pore.

Unlike most physical sensors a graphene sheet is thin enough to capture a single base pair at any given point. This in turn increases the efficiency of the process.

Since the sequencer identifies base pairs rather than individual bases, any diversion from the Watson–Crick base pairing could be located. Nanopore DNA sequencing deals with faster elucidation of the genetic code which can help in identifying genetic defects.

While there is much scope for improvement in the technique, authors maintain that they have an initial blue print for a new, low cost, marker free and reliable DNA sequencing device.

Nanotechnology, **27**(13), 1 April 2016

A Clash of Clans:
Death associated protein kinases

The mutated form of a gene called DAPK3, a member of death associated protein kinases, is frequently found in malignant cells. This particular protein regulates cell replication and cell death. As such, it functions as a tumour suppressor; but the mechanism of its function remains unknown. Scientists from IIT Kharagpur in collaboration with University Sains, Malaysia have unraveled the forces at work.

Researchers identified results of three common mutations – aspartic acid replaced by asparagine at position 161, proline replaced by serine at position 216, threonine replaced by methionine at position 112. Replacements of amino acids cause structural changes in the protein. So they studied the implications of these mutations on enzyme activity.

They first looked at the ATP binding site, since kinases utilize ATP as a phosphate donor. There are 5 separate segments in this site: 3 loops, a hinge region and an activation segment. The mutation that changes the amino acid at position 112 opens up the ATP binding site due to the outward movement of loop1 and 2. In changing the amino acid at position 161 and 216, the loops collapse, moving the site deeper into the protein. Thus, even though the binding pocket remains, distorted conformation results in reduced catalytic efficiency.

There are other factors that influence the enzyme activity of the protein: a well structured hydrophobic spine and Lysine–Glutamate catalytic salt bridge. The mutant proteins lose the catalytic loop. The salt bridge that stabilizes active enzyme conformation was also affected.

The study has thus elucidated the underlying mechanisms involved in tumour progression due to mutations in DAPK3.

Gene, **580**, 17–25 (10 April 2016)

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