# Tracing the Origins of Giant Viruses

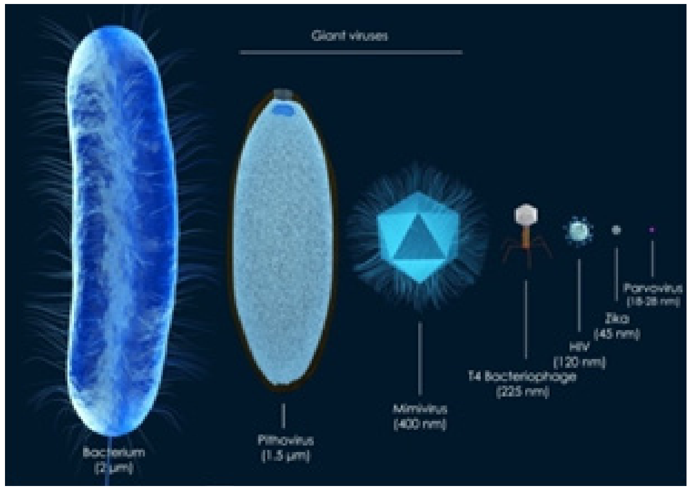


*Photo courtesy: Shutterstock*

**WORKING** at the Indian Institute of Technology Bombay, me and my co-researcher Dr Supriya Patil, have shed light on the origins of Mimivirus and other giant viruses. In a study published in *Molecular Biology and Evolution,* we show that giant viruses may have come from a complex single-cell ancestor, keeping DNA replication machinery but shedding genes that code for other vital processes like metabolism.

Scientists have long been interested in how viruses evolved, especially when it comes to giant viruses that can produce new viruses with very little help from the host — in contrast to most small viruses, which utilise the host's machinery to replicate.

Giant viruses are very common in oceans and other water bodies. They infect single-celled aquatic organisms and have major effects on the latter's population. Because these single-celled organisms greatly influence the carbon turnover in the ocean, viruses have an important role in our world's ecology. So, it is just as important to study them and their evolution, as it is to study the disease-causing viruses.

In the study, me and my co-researcher Dr Supriya Patil performed a series of analyses on major genes and proteins involved in the DNA replication machinery of Mimivirus, the first group of giant viruses to be identified. Our aim was to determine which of the two major suggestions regarding Mimivirus evolution — the reduction and the virus-first hypotheses — were more supported by results. The reduction hypothesis suggests that the giant viruses emerged from unicellular organisms and shed genes over time; the virus-first hypothesis suggests that they were around before single-celled organisms and gained genes, instead.

We created phylogenetic trees with replication proteins and found that those from Mimivirus were more closely related to eukaryotes than to bacteria or small viruses. We also used a technique called multidimensional scaling to determine how similar the Mimiviral proteins are. A greater similarity would indicate that the proteins co-evolved, which means that they are linked together in a larger protein complex with coordinated function. And indeed, our findings showed greater similarity.

We also found that genes related to DNA replication are similar to and fall under purifying selection, which is natural selection that removes harmful gene variants, constraining the genes and preventing their sequences from varying. Such a phenomenon typically occurs when the genes are involved in essential functions (like DNA replication) in an organism.

Taken together, these results imply that Mimiviral DNA replication machinery is ancient and evolved over a long period. This narrows down to the reduction hypothesis, which suggests that the DNA replication machinery already existed in a unicellular ancestor, and the giant viruses were formed after getting rid of other structures in the ancestor, leaving only replication-related parts of the genome.

Because these giant viruses probably predate the diversification of the unicellular ancestor into bacteria, archaea, and eukaryotes, they should have had a major influence on the subsequent evolutionary trajectory of eukaryotes, which are their hosts.

Our work could lay the groundwork for translational research into technology like genetic engineering and nanotechnology. An increased understanding of the mechanisms by which viruses copy themselves and self-assemble means we could potentially modify these viruses to replicate genes we want or create nanobots based on how the viruses function. The possibilities are far-reaching!

*Reference*

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# Rapid COVID-19 variant detection: Anywhere, anytime

*Team MRU at work*

A concerning feature of the second wave was the emergence of several variants of the COVID-19 virus. Some of these variants carry mutations in their genes that enable the virus to avoid the immune system's response to infections and escape detection in antigen-based tests.

Mutations causing changes in the spike protein, which interacts with the host allowing the virus to enter the body, are of special interest. Changes in the viral non-structural proteins which enhance the replication of the virus were also seen during the second wave. These variations increase the transmissibility (increased rate of infection) and virulence (greater damage to health) of the virus. Such variants have been designated "variants of concern" by health authorities worldwide.

It appears that these variants of concern may be able to infect even people who have been vaccinated. One such variant is the Delta, or B.1.617.2, form of the virus. This variant was first identified in India and carries multiple mutations in the spike protein.

Scientists and policymakers now understand the emergent need of sequencing the genome of the virus, to look for variants. The importance of whole-genome sequencing of COVID-19 samples quickly cannot be stressed enough, because of multiple reasons. First, detecting the mutations present in a viral sample is necessary, so that the nature of the changes caused in viral activity may be understood. Second, if a variant of concern is detected, rapid treatment and isolation strategies for a community may be put into action immediately, to prevent its spread. Finally, knowing the changes caused in the amino acid sequences may enable designing better vaccination, detection, and treatment policies.

Our lab (Multidisciplinary Research Unit, DHR-ICMR, Institute of Medical Sciences, Banaras Hindu University) decided to attempt nanopore sequencing – a cheap, quick, and reliable method of detecting changes in the COVID-19 genome. In fact, a quarter of the COVID-19 samples sequenced worldwide were processed using this technique.

Nanopore sequencing uses a protein nanometer-scale pore embedded in a lipid bilayer or solid-state silica membrane, across which a voltage gradient is applied. The process has sufficiently improved over the years and is now a rapid and accurate technique, while still being extremely cost-effective.

Our lab has a MinIon, a small machine that can sequence 96 samples at a time, and a GridIon MK1, which can handle 96x5 samples at one go. It is possible to generate sequencing results within 24 hours, which is fast enough to allow rapid response while remaining cost-effective. Since the hardware involved is small and portable, this means that sequencing efforts may move closer to the point-of-testing or point-of-care.

The majority of the genomes we have sequenced so far turned out to be of the delta variant. This agrees with information from other laboratories, suggesting that the second wave of infection was driven by the delta variant. The second wave posed a staggering challenge to the health care network, and the need for genomics-based surveillance became obvious.

Close collaboration and movement of personnel between COVID-19 testing and sequencing laboratories is now the need of the hour, to enable more and more scientists to receive training in surveillance-level sequencing, so that India may be ready for this menace, anytime, anywhere.

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# Promise of Tackling Genetic Diseases

IN the last 30 years, CRISPR gene editing has evolved as a revolutionary gene-editing tool. Although used in archaea and bacteria as an anti-viral defence system, CRISPR uses a key protein called Cas9 which identifies a target DNA sequence using a guide RNA (gRNA) and a short but important PAM sequence on the DNA. After identification, Cas9 causes a double-stranded break in the DNA. This can be used to precisely target the genome or develop high-yielding crops or even cure genetic disorders.

However, the protein Cas9 faces 2 major challenges. Mismatched bases between DNA and RNA can still lead to cleavage although mutants such as eCas9 and HF1Cas9 reduce this. Secondly, Cas9 has low efficiency as it takes 6 hours to search the target in *E. coli*. For a longer genome such as that of humans, it is expected to take even more time.

DNA-binding proteins often use 3D diffusion or 1D sliding along DNA to locate their target. However, Cas9 shows almost no sliding along DNA. Target search by only 3D diffusion takes a longer time and the stationary mode of Cas9 after binding to DNA can lead to off-target effects. The addition of the sliding function to Cas9 can make a larger area of DNA accessible for target search in the same amount of time. This can also further reduce off-target effects.

Therefore, our purpose was to engineer Cas9 such that it can slide along DNA for faster target search and reduced off-target editing. The results of this approach have been summarised in detail by 'Banerjee, T., Takahashi, H., Subekti, D.R.G. *et al*. Engineering of the genome-editing protein Cas9 to slide along DNA. *Sci Rep* 11, 14165 (2021)'.

To achieve this, two design strategies were used. In the first strategy, I mutated key DNA binding residues which could then facilitate sliding. In the second strategy, I decided to add the sliding-promoting peptide (tail) of another DNA-binding protein Nhp6A to the ends of Cas9 hoping that it may then cause sliding. The tail itself is 16-residue long with 8 positively charged residues.

Both in the absence and presence of gRNA, the addition of tail leads to enhancement of sliding population along with DNA. In fact, one of the mutants showed an 8-fold enhancement in sliding value. This is similar to the sliding coefficient of p53 protein which is another DNA-binding protein of similar size.

So, mutating PAM interacting residues enhances average sliding by 15 times in the absence of gRNA. The addition of the tail causes a larger fraction of molecules to slide in both presence and absence of gRNA. The addition of the tail also enhances the average sliding in the presence of gRNA by 8 times to almost the same as p53.

The added functionality of sliding opens doors to test it *in vivo*. Faster sliding would mean that Cas9 would now be able to locate its target faster and also possibly reduce its off-target effects. This would make it an even more efficient gene editing protein that can be applied for human therapeutic use. The use of Cas9 efficiently can tackle a wide range of genetic diseases such as Albinism, Down's syndrome, etc. and even for cancer therapy. It would revolutionise medicine and pave the way for a future where we may be able to eradicate many genetic disorders.

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# Fermi Lab's Exciting Results

SOME time back, Fermi Lab announced their Muon G-2 results which do not fit with one of the most successful theories, the Standard Model of Particle Physics.

Our search for a theory of everything is perhaps the next great question in physics. And to find the path forward, we need to find loose threads in these theories which might lead us to deeper layers of Physics. And there is this one glitch begging to be tugged and that's the anomalous magnetic dipole moment of the muon – the scientists at Fermilab have just woven it with the muon G-2 experiment.

The standard model of particle physics is a theory describing three of the four known fundamental forces (the electromagnetic, weak, and strong interactions, not including gravity) in the universe, as well as classifying all known elementary particles. Out of all its successes, the prediction of anomalous dipole might be the most miraculous one. It describes how particles with an electric charge interact *via* electromagnetic force. G-2 experiment at the Fermi lab has been precisely doing that. It aims to measure how muons wobble in the magnetic field and have found some astonishing results which disagree with the predictions made by the standard model of particle physics.

But what exactly are muons? Muons are like the cousins of electrons, but heavier. They are about two hundred times heavier than electrons and because of their weight are unstable and decay into lighter particles. This is the reason why muons don't play the roles electrons play in atoms even though they have the same charge and spin as electrons. According to the Standard Model, the magnetic field should make the muons wobble, somewhat like little compass needles, at a predictable rate. But in a new set of studies reported by more than 200 physicists, the muons wobbled at a slightly higher rate.

So how does this process work? Imagine each muon as a tiny analogue clock. As the particle circles the magnet, its hour hand goes round and round at a rate predicted by theory. When the muon's time is up, it decays into a positron that is emitted in the direction of the hour hand. But if that hand turns at a rate different from theory — say, a tick too fast — the positron decay will end up pointing in a slightly different direction. (In this analogy, the hour hand corresponds to the muon's spin, a quantum property that determines the direction of the muon decay.) Detect enough deviating positrons, and you have an anomaly.

# "Liposome" a Nanotechnological Benefactor to the Drug Delivery

**LIPOSOMES,** globular lipid bilayers of 50 to 1000 nm in diameter, have long been looked upon as delivery vehicles for biologically active compounds. Liposomes are used for drug delivery because of their exclusive properties. They contain a wide variety of hydrophilic and hydrophobic diagnostic and therapeutic agents, provide greater drug load with particles and protect encapsulated agents from metabolic processes.

Another property of liposomes is their natural ability to target, which is classified into two categories. One is 'passive targeting' with a bulk recognition mechanism, in which the targeting is initiated by altering the bulk structural characteristics of the carrier such as its hydrophobicity, hydrophilicity, charge density, fluidity, softness, and size of the carrier.

The second is 'active targeting' which is acquired by a molecular recognition mechanism. When liposomes are introduced into the body, primary tissue distribution builds upon a passive targeting mechanism. However, when the liposome and target cell approach each other an active targeting mechanism starts governing the process. Therefore, active targeting is more promising than passive targeting.

Liposomes show less toxicity with the encapsulated drug, many anticancer drugs encapsulated with liposomes target the specific cancer cell or tissue. Since liposomes are digested by phagocytic cells, after intravenous administration several parasitic bacteria which normally reside in the cells can be targeted and diagnosed. Liposomes are used for the treatment of neonatal jaundice. Liposomal antibiotics, steroids, and infusions are nowadays available in terms of vaccine application. Antifungal drug Amphotericin B shows better results in liposomal drug delivery.

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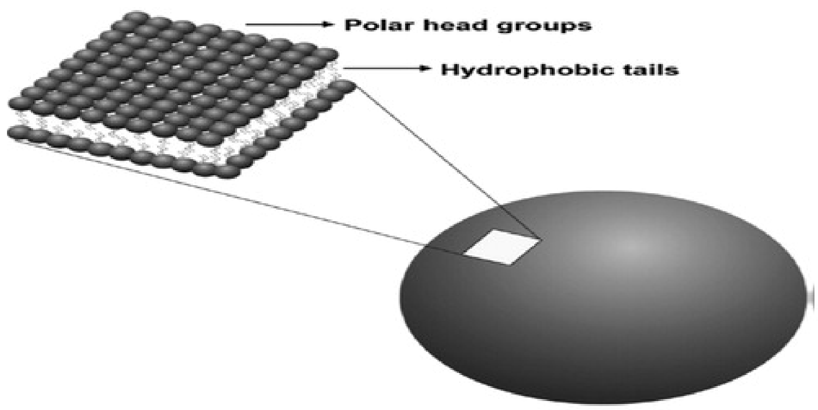


Nanoliposome

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Physicists at the Fermilab detected such an anomaly which was reported in *Physical Review Letters*. The newly updated experimental value for muons deviates from theory by only a minuscule value (0.00000000251). But even this tiny amount can shake particle physics.

The anomaly for the Muon g-2 experiment is 4.1 sigma, meaning it is more than 4 standard deviations from the mean. To put it another way, there is a one-in 40,000 chance that the results of the experiments are due to some statistical fluke. However, the golden standard in particle physics is 5 sigma, which corresponds to 1 in 3.5 million odds of random fluctuation. So there is a 1/40000 chance that it might be just a statistical error and the standard model might rise again triumphantly. But this is not stopping Physicists from being excited as it might open new doors in the world of physics.

If these results are confirmed then it might be the biggest discovery of the decade. But what are the current explanations behind it? The findings suggest that there are other fundamental particles interacting with the field. These particles pop in and out of the vacuum incrementing the change in the magnetic field. But what are these particles and what forces are associated with them? Currently, where our knowledge lies, we have no idea. There seems to be a huge void in the Standard Model which is waiting to be filled.

But what if there is actually new physics behind it? What will be our course of action to learn more about it? Well, we will be needing new tools to explore the true essence of this new physics. For this, we might be needing new colliders, like the Linear collider or the High-luminosity collider which are proposed colliders with higher energy acceleration. Additionally, in the past few months, interest has surged around a muon collider, which multiple papers predict would guarantee physicists the ability to determine the properties of the unknown particle or force affecting the muon.

Whether or not Fermilab is standing on a holy grail of physics remains to be seen in the future as more data and improved theoretical calculations surface but this discovery surely has got us excited as it might pave the path to a new physics. It might help us understand the universe more fundamentally pushing us closer to the grand unified theory.

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