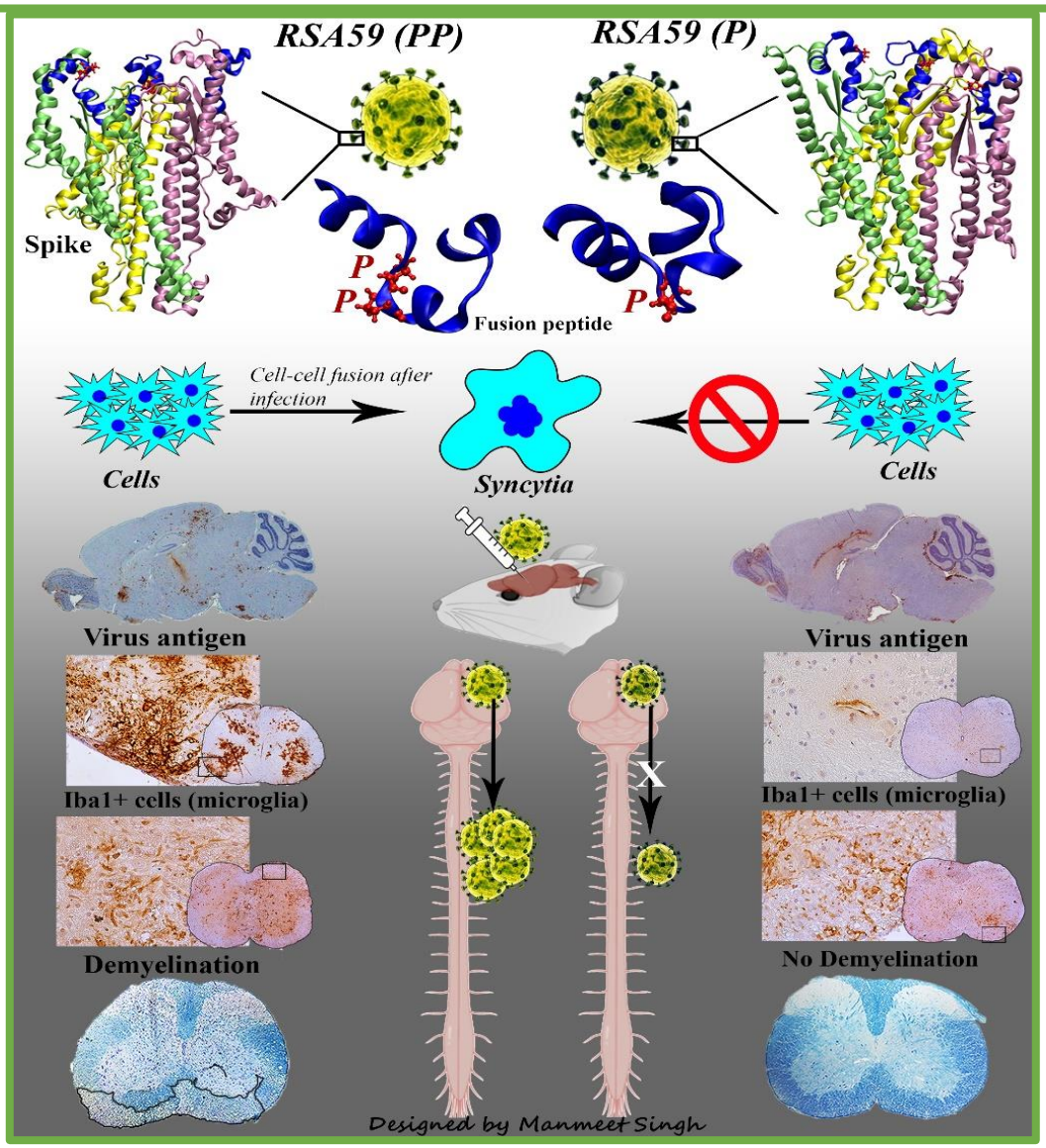
Looking for the mechanism to design an anti-viral for murine β-coronavirus (m-CoV) in an experimental mouse model

Jayasri Das Sarma and her research team at the Department of Biological Sciences work on Murine β-Coronavirus (m-CoV) (Mouse hepatitis virus; MHV) infection in mice as an experimental model system to understand the viral genomic determinants of pathogenic properties to design therapeutic targets. A series of studies including their own demonstrated that spike protein (virus-host attachment protein) mediates many biological properties of MHV, including receptor attachment, virus-cell fusion, its spread, its ability to infect neuroglial cells in the central nervous system and its associated pathogenesis. To dissect the minimal essential motif required for such function, *in silico* molecular modelling with NMR approaches targeted a hydrophobic fusion peptide with two consecutive central prolines in the S2 domain of the Spike protein.

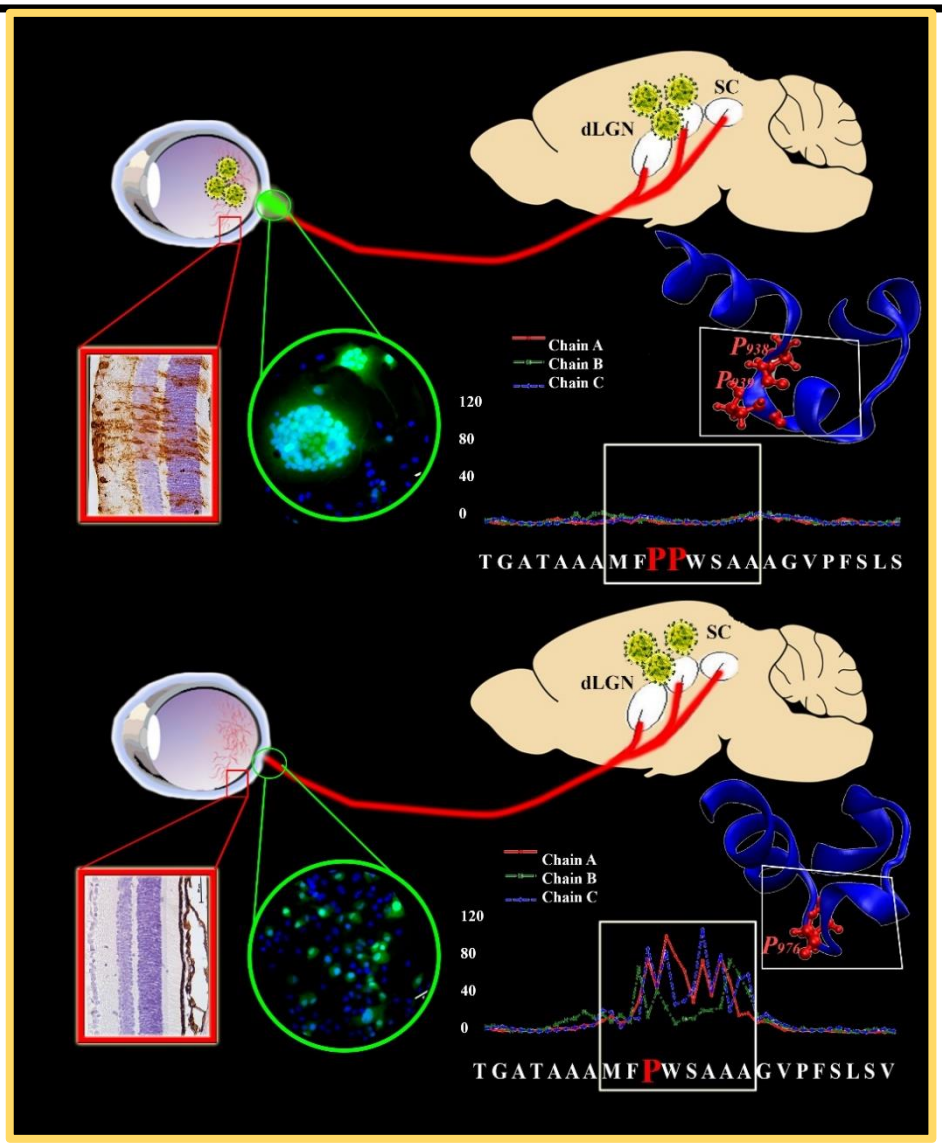
A proline deletion in the spike glycoprotein fusion peptide of mouse hepatitis virus significantly reduces cell- to-cell fusion, its spread and associated neuropathogenesis



Two consecutive central prolines in the fusion peptide of m-CoV play a major role in determining the kinetics of the fusion process and consequent neuropathogenesis. Two consecutive central proline residues also provide rigidity due to its absence of dihedral fluctuation and regulate its cell-to-cell fusion. By deleting one proline by using reverse genetics and targeted recombination system they have shown that deletion of one of such two consecutive proline in the recombinant strain of m-CoV significantly reduces cell-to-cell fusion, and consecutive viral antigen spread form site of the inoculation (brain) to the spinal cord and its associated neuroinflammatory demyelination (***Manmeet Singh#, Abhinoy Kishore#****, Dibyajyoti Maity, Punnepalli Sunanda, Bankala Krishnarjuna, SreeparnaVappala, Srinivasarao Raghothama, Lawrence C. Kenyon, Debnath Pal*, and* ***Jayasri Das Sarma***** "Journal of Biological Chemistry, May 2019, doi: 10.1074/jbc.RA118.004418*).

Moreover, deletion of one proline significantly reduces retrograde axonal transport from brain to the retina of the eye via optic nerve and retinal ganglionic cell degeneration *(**Saurav Saswat Rout, Manmeet Singh**, Kenneth S Shindler, **Jayasri Das Sarma,** Journal of Biological Chemistry, April 2020, doi: 10.1074/jbc.RA119.011918).*

One proline deletion in the fusion peptide of neurotropic mouse hepatitis virus (MHV) restricts retrograde axonal transport and neurodegeneration

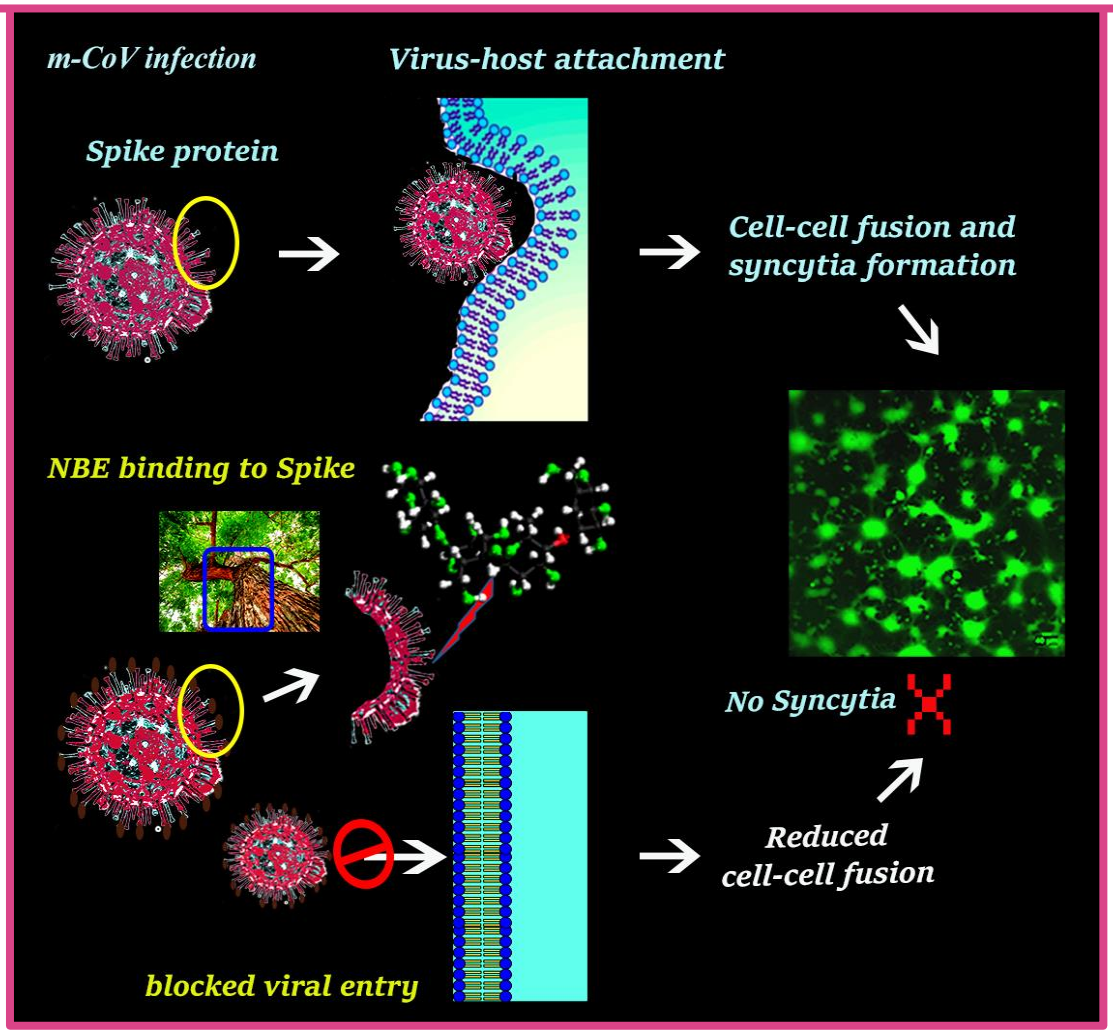


In collaboration with Dr. Debnath Pal, Professor, Department of Computational and Data Sciences, Indian Institute of Science proline-induced rigidity in the m-CoV FP has been reported. The dihedral fluctuation of the FP segment was suppressed whenever two consecutive prolines (PP) were present, in contrast to the presence of a single proline (P) in the chain. Dihedral fluctuation is defined as the standard deviation of the distribution of absolute difference from mean for a dihedral angle in the trajectory.

Together, by combining *in silico* molecular modelling, biophysical NMR data and *in vitro* and *in vivo* comparative studies between the two-proline containing RSA59 (PP) strain and a one proline deleted mutant RSA59, it is evident that proline, a single amino acid, serves as an important entity for in the FP of m-CoV. Two consecutive proline residues are required for neuronal transport from brain to the spinal cord from grey matter to the white matter to induce demyelination, efficient retrograde axonal transport. Altering the rigidity of the FP may play an important role in cell-to cell fusion and viral spread.

As m-CoV fusogenicity is one of the efficient mechanisms of virus spread form cell-to-cell and to evade immune system and establishing persistent infection causing tissue damage, so searches are going on to find out a potential target to control the cell-to-cell fusion.

Cell-to-cell fusion of m-CoV can be contained by bark extract of Azadirachta indica, a traditional Indian medicinal plant



Towards this goal, one of their recent study demonstrated the bark extract of an ethnomedicinal plant *Azadirachta indica (neem)*, effectively restricts viral entry, viral replication, dissemination in the liver and central nervous system, and thus significantly reduces hepatitis, and neuropathogenesis. From the mechanistic stand point in a virus free cell-based reporter assay, it has been shown that NBE can inhibit only spike protein mediated cell-cell fusion *in vitro*, which indicates that NBE might interact with Spike protein and reduces the fusogenicity (*Azadirachta indica A. Juss ameliorates Mouse Hepatitis virus-induced neuroinflammatory demyelination by modulating cell-to-cell fusion in an experimental animal model of Multiple Sclerosis, **Lucky Sarkar**, Ravi Kiran Putchala, Abass Alao Safiriyu, **Jayasri Das Sarma**, Frontiers in Cellular Neuroscience, 2020, doi: 10.3389/fncel.2020.00116).*

Experiments are going on to identify the potential compounds form the NBE by virtual high throughput screening in the form of docking studies in collaboration with Dr. Debnath Pal, IISc Bangalore and Dr. Ujjwal Neogi, Karolinska Institute, Stockholm, Sweden.