Use of High-field NMR in Covid-19 Drug Development

The Covid-19 NMR Consortium, an international collaboration of NMR experts, is conducting research to determine the ribonucleic acid (RNA) structure of SARS-CoV-2 and its proteins using NMR spectroscopy. One of the consortium's first discoveries, early on in the pandemic, was that a protein within SARS-CoV-2 forms microdroplets with the RNA of the virus. In subsequent months, this discovery enabled scientists to set up experiments to study the interplay between the RNA and the protein, known as the nucleocapsid protein or N protein.

At the invitation of Prof. Schwalbe, Prof. Markus Zweckstetter joined the consortium in late 2020 after the publication of his research on SARS-CoV-2. As part of this 50-strong international collaboration of NMR experts, Prof. Zweckstetter and his team at the Max Planck Institute for Multidisciplinary Sciences in Göttingen, Germany, have been uncovering insights into the hidden workings of Covid-19. Using one of only a few 1.2 GHz NMR instruments in the world, these researchers are helping to elucidate the structure and interactions of the nucleocapsid protein within the SARS-CoV-2 virus and, in doing so, identify promising options for drug targets.

Mechanisms of Covid-19

The etiologic agent of the Covid-19 pandemic is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Many early studies of SARS-CoV-2 focused on the so-called 'spike protein' because this is involved in communication with the host cell. But as understanding of SARS-CoV-2 has evolved, it has become apparent that the nucleocapsid protein, or N protein, also plays a key role because it not only protects the RNA from degradation, but enables the transcription machinery to cluster, and so enhances its ability to replicate.

The viral membrane of SARS-CoV-2 surrounds a helical nucleocapsid in which the viral genome is encapsulated by the nucleocapsid protein. The nucleocapsid protein of SARS-CoV-2 is produced at high levels within infected cells, enhances the efficiency of viral RNA transcription, and is essential for viral replication. Scientists at the German Center for Neurodegenerative Diseases (DZNE) and the Max Planck Institute have now shown that this protein and the RNA can jointly condense into tiny droplets when the virus releases its insides into a host cell.

Continued research determined that RNA induces cooperative liquid–liquid phase separation of the SARS-CoV-2 nucleocapsid protein, where the viral droplets float inside the fluid medium inside the cell. However, this droplet formation is not a specialty of the coronavirus. Such dynamic compartments composed of proteins and other molecules occur naturally inside cells, and they are used as storage sites and reaction chambers. Research by Prof. Zweckstetter and his team shows that the coronavirus also exploits these possibilities, and this is suspected to happen with other pathogens as well.

In agreement with its ability to phase separate *in vitro*, protein associates in cells with stress granules – cytoplasmic RNA/protein granules that form through liquid-liquid phase separation and are modulated by viruses to maximize replication efficiency. This process generates high-density protein/RNA condensates that recruit the RNA-dependent RNA polymerase complex of SARS-CoV-2, providing a mechanism for efficient transcription of viral RNA. Inhibition of RNA-induced phase separation of the nucleocapsid protein by small molecules or biologics thus can interfere with a key step in the SARS-CoV-2 replication cycle.[1]

Identifying Potential Drug Targets

Prof. Zweckstetter and his team believe these findings may offer starting points for drug development. Thanks to collaboration efforts, many groups around the world are now studying the N protein, with a view to assessing its potential as a target for treatment of Covid-19. For example, by interfering with the formation of N protein microdroplets, the viral RNA might become more vulnerable to external damage and less able to replicate reliably.

About the COVID-19 NMR Consortium

The Covid-19 NMR Consortium was initiated at the Goethe University in Frankfurt, Germany, in March 2020 by Prof. Dr. Harald Schwalbe and it has since grown rapidly into an international consortium. Today, scientists from all over the world are collaborating based on open science principles in a unique effort to investigate SARS-CoV-2 using NMR spectroscopy. The goals and the shared scientific targets of the project are coordinated by Prof. Schwalbe and his team at Goethe University. The core team includes five professors and junior group leaders from Darmstadt and Frankfurt and nine senior scientists from the Biological Magnetic Resonance Center (BMRZ) in Frankfurt. The overall goal of this consortium is to join forces to achieve meaningful scientific results in Covid-19 research as quickly as possible.

Another aspect of the work on N protein is to investigate the involvement of kinases in phosphorylating certain residues of N protein, as these enzymes could be promising targets for small-molecule inhibitors. Prof. Zweckstetter's team also discovered that the enzyme SRPK1 kinase, which occurs naturally in the human body, chemically modifies the nucleocapsid protein and influences the formation of the viral droplets.

Insights from NMR Spectroscopy

Consortium scientists investigated these events using NMR spectroscopy and other methods to examine the structure and dynamics of molecules. The Zweckstetter group's research has involved a number of high-field NMR spectrometers from 600 MHz to 950 MHz, which have been central to carrying out the highly sensitive investigations into proteins. Now the group has access to the 1.2 GHz NMR instrument at Göttingen, which they believe will enhance the resolution of three- and higher-dimensional NMR experiments by at least a factor of two compared to existing 950 MHz instrument. That will allow further studies of the structural dynamics of biomolecular

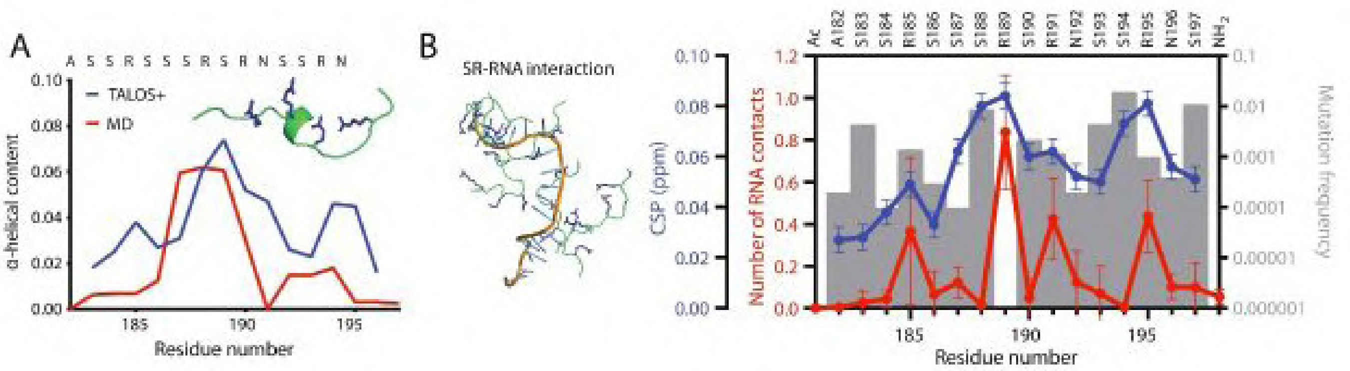

Figure 1: Using NMR to investigate the properties of the A182–S197 region of the nucleocapsid (NCP) protein within SARS-CoV-2, which has a high proportion of serine and arginine resides (known to bind both RNA and proteins). (A) Chemical shift analysis (blue) agrees with molecular dynamics simulations (red) that the residues in this region are very flexible, with a small propensity for α-helical structure next to R189. (B) Re-running the simulations in the presence of polyuridylic acid (a simplified RNA) showed a large number of intermolecular contacts between the arginine residues and the RNA phosphate groups, with a maximum for R189. This agrees with the observation that R189 is the only residue in the region A182–S197 that is not mutated in most of the currently known strains of SARS-CoV-2 (gray bars). Reprinted from ref. [1] under a Creative Commons license (CC BY 4.0, http://creativecommons.org/licenses/by/4.0/).

markers on huge range of time and length scales. The 1.2 GHz NMR instrument uses high-temperature superconductors for the inner coil and regular, low-temperature superconductors for the outer coil. That enables the uniform magnetic field of 28.2 Tesla.

With this boost to NMR science at Göttingen, Prof. Zweckstetter is integrating NMR with other structural biology techniques, thereby obtaining a more complete view of the inner workings of the SARS-CoV-2 virus. For example, further characterisation using high-resolution NMR, molecular dynamics simulations and phase separation experiments could help pinpoint how potential drugs interact with N protein, and whether RNA replication could be influenced.

NMR enables the study of how the biomolecule moves around in solution, and the different shapes it takes on to perform different activities. It also helps with the visualisation of molecules in real-time, gaining crucial insights into how they perform their function and are modified by enzymes. The capabilities of high-field NMR stand in contrast to techniques such as cryoelectron microscopy, which requires frozen samples, and so can only provide snapshots of the molecular action.

For the SARS-CoV-2 research, the team combined molecular dynamics simulations with NMR spectroscopy of 1H, 13C and 15N nuclei, using NOESY, HSQC and total correlation spectroscopy (TOCSY). An example of the insights obtained is shown in Figure 1.

Future Steps

This work has the goal of improving treatment for those with Covid-19. Because the virus is constantly adapting and evolving, it brings a risk of re-infection. To counter the threat it poses, the consortium plans to continue to provide the data to understand the inner workings of the virus and work towards new and better drugs to treat Covid-19.

The Covid-19 NMR project holds further implications that extend beyond the virus. While collaboration is valued in science, the inherent competition between research groups has limited the scope of that collaboration. Whereas normally scientists might work with one or two other teams, the Covid-19 consortium shows the potential when dozens of research teams can collaborate globally. The ability to pool expertise, equipment, and reagents enables much faster progress. Setting up large scientific collaborations to tackle major challenges could be a complementary and maybe even more powerful way of making rapid progress.

REFERENCES

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Dr. Markus Zweckstetter

Prof. Dr. Markus Zweckstetter is head of the research group on Structure determination of proteins using NMR at the Max Planck Institute for Multidisciplinary Sciences in Göttingen, Germany. Since 2012, he has also led the Translational Structural Biology group at the German Center for Neurodegenerative Diseases (DZNE), and is a professor at the University Medical Center in Göttingen. Prof. Zweckstetter received three European Research Council (ERC) grants, which have helped his team uncover protein structure and function using the power of NMR spectroscopy.