What a Virus **really Needs**

When a virus infects a cell, it interacts in many ways with its host. Prof. Andreas Pichlmair investigates these interactions at the protein level applying modern mass spectrometry technologies. He uses the collected data for network analyses. As a virologist, Pichlmair's goal is to understand which cellular components and signaling pathways are essential for viruses' propagation. His goal is to promote the development of new medications that target these pathways.

Kurzfassung · Langfassung: www.tum.de/faszination-forschung-25

Was ein Virus wirklich braucht

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Viren infizieren Wirtszellen und bedienen sich der molekularen Maschinerie der Zelle für ihre eigene Vervielfältigung. Dafür sind viele Interaktionen des Virus mit den Proteinen der Zelle notwendig. Prof. Andreas Pichlmair, Virologe der TUM, nutzt die Massenspektrometrie, um die beteiligten Proteine zu detektieren. Diese eignen sich potenziell als Ausgangspunkt für die Entwicklung von Therapien, auch gegen das neuartige Coronavirus SARS-CoV-2. Die Massenspektrometrie misst die Masse von Molekülen. Weil jedes Molekül eine einzigartige Masse besitzt, können die Forscher ihrem Messwert anhand einer Datenbank den jeweiligen Namen zuordnen. Zusätzlich lässt sich auch die Menge des jeweiligen Proteins erfassen. Pichlmair und sein Team beobachten, wie sich die Proteinzusammensetzung nach einem Virusinfekt in bestimmten Zeitintervallen verändert. Die Veränderungen, die sie dabei beobachten, verrechnen die Forscher auf Basis der Daten von Netzwerkanalysen. So lassen sich Signalwege der Zellen erkennen, die für Viren überlebensnotwendig sind. Parallel dazu versuchen sie herauszufinden, ob diese Signalwege auch bei anderen Erkrankungen eine Rolle spielen, für die es bereits Medikamente gibt. Denn dies könnte die Entwicklung neuer Wirkstoffe deutlich abkürzen. □

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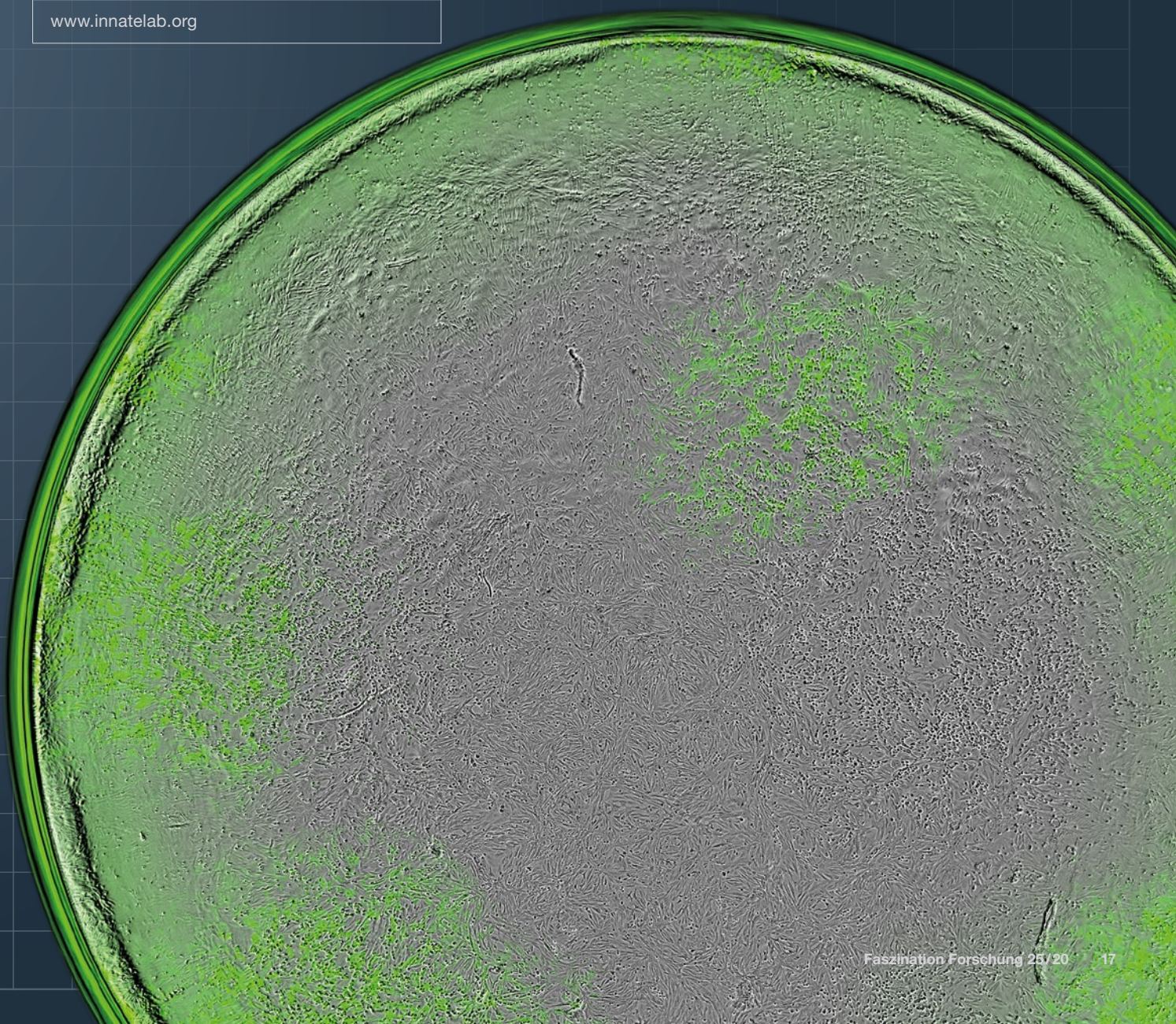
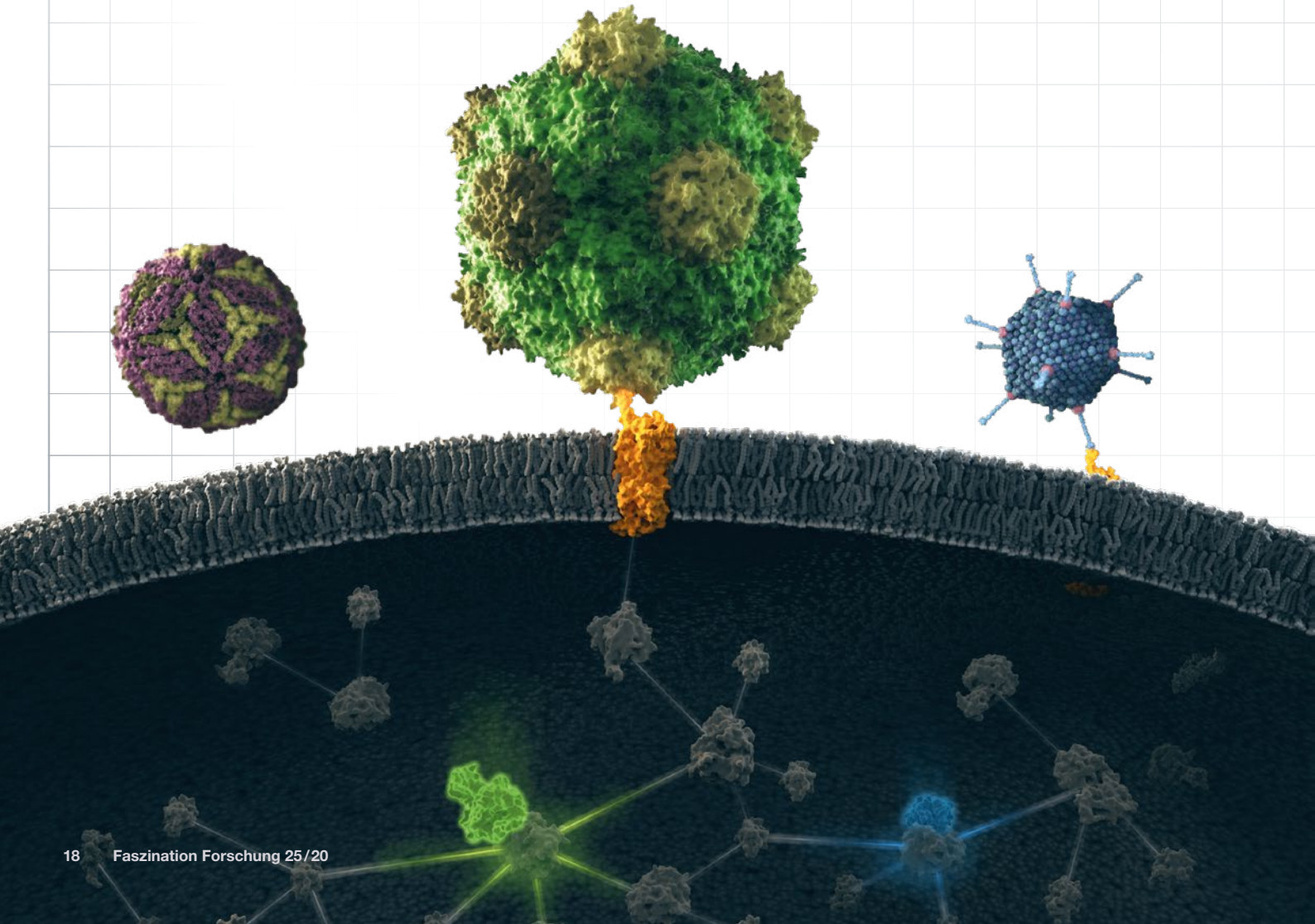


Graphics: ediundsepp; Picture credit: TUM

Viruses depend on foreign cells to multiply. Without a host, they cannot reproduce. After settling in a host cell, viruses take advantage of the cell's molecular machinery for their own reproductive purposes. First, however, they need to escape their host's immune system. They achieve this either by disguising themselves to avoid detection or by manipulating the signaling pathways that the host's immune system would otherwise use to raise the alarm. All of these steps involve interactions between the virus and certain cellular factors. Prof. Andreas Pichlmair is interested in the proteins involved in this process: The ones which the virus often specifically disables in the host cell to stay unrecognized and others which facilitate the virus's reproduction. As the TUM virologist explains, "these proteins could provide a starting point from which to develop therapies", including for the novel coronavirus (SARS-CoV-2).

Viruses control the cell's protein production

If a virus successfully delivers its genetic material into a host cell, it takes control of the cellular production mechanisms. This means, for instance, that the virus is able to restrict the production of proteins that the host cell's immune system requires to defend itself. At the same time, it can ramp up production of the proteins it requires for its own reproduction. Both cells and viruses are predominantly made up of proteins that form structures and, in the form of enzymes, facilitate chemical reactions. Each cell contains around 20,000 different genes carrying the code for specific proteins. While some proteins are not produced (expressed) at all or only with a few copies, others occur in millions of copies. Pichlmair and his team are using mass spectrometry to investigate which proteins are of greatest interest to viruses. This technique measures the mass of molecules such as proteins. Since



Viruses cause cytopathic effects in infected cells. Cells are fixed and stained with a violet dye. Wherever the virus replicates, it destroys the cell layer and thus reduces the violet color in the respective dish.

every molecule has a unique mass, scientists can identify the repertoire and quantity of individual proteins in a given sample. The researchers then compare the proteins in healthy cells with those in infected cells. This allows them to examine how the protein composition of cells changes at set intervals after a viral infection.
After conducting their measurements, the researchers ultimately obtain a list of several thousand proteins and their respective quantities.

The function of the proteins serve as an approach for possible medications

Certain proteins, for instance the ones required for energy metabolism, change in the case of almost all infections. Other proteins, however, only change when a cell is infected by a specific virus. All proteins that occur in greater or lesser quantities following an infection are presumably important for the virus. The precise function of many of these proteins remains unknown – and it is these proteins that interest Pichlmair. He wants to understand why a virus needs them. It would allow new medications to be developed quickly in response to specific viruses. The researchers are applying network analysis, a technique based on the analysis of complex processes within cells. Different stimuli within cells trigger a wide range of interactions between molecules. Some of these molecules activate or deactivate other molecules. Others enable or disable the production of proteins. While some of these interactions within cells have already been identified, our understanding of others remains rudimentary. Protein production often plays a role. These interactions inside cells are also known as signaling cascades or pathways as one reaction acts like a signal and triggers and often amplifies numerous other reactions. The fact that these signaling cascades often interact with one another gives the cell a wide opportunity to respond to pathogens, but makes it more complicated for scientists to understand the processes involved.

The scientists get clues from network analyses

A number of these cascades have been identified and are stored in databases enabling Pichlmair to analyze the list of proteins generated through mass spectrometry. He uses the results of this analysis to help him interpret what happens in a cell once it is infected; his aim is to determine the reactions that take place within cells and pinpoint which signaling pathways are activated when a virus has infected a cell. “The more of this complex data we have, the more precise the picture becomes,” says Pichlmair. By the same token, the more we know about a specific ▷

Picture credits: Andreas Pichlmair, Magdalena Jooss

signaling pathway, the easier it is to identify whether the virus needs it. Pichlmair, however, also issues a warning: "There are many measurement readings we simply cannot interpret because the network data is too sketchy or is missing entirely."

Coronavirus: Searching for analogies with other diseases

The novel coronavirus SARS-CoV-2 is made up of around 27 proteins. While the functions of some of these proteins are known, others remain unclear. "What mass spectrometry shows us is that, as in other viruses, there are certain proteins both in the virus itself and in the host cell that are of particular importance to the virus," says Pichlmair. The researchers' goal is to identify signal cascades that are activated when a cell is infected with the coronavirus. In parallel with this, they are seeking to discover whether these cascades might also play a role in other diseases for which medications are already available. If so, it would significantly accelerate the identification of therapeutic agents that may be active against SARS-CoV-2. This approach, known as drug repurposing, looks at medications that have been approved for use with other diseases – or are at least at an advanced stage of testing. Medications that intervene in signal cascades are primarily known for their use in treating cancers. ▷

≈20,000

proteins are engaged by the virus

29

proteins are expressed by the virus

1,000

proteins are bound by the virus proteins

"What's special about our work is that we are linking mass spectrometry, bioinformatic analyses, virology and cell biology."

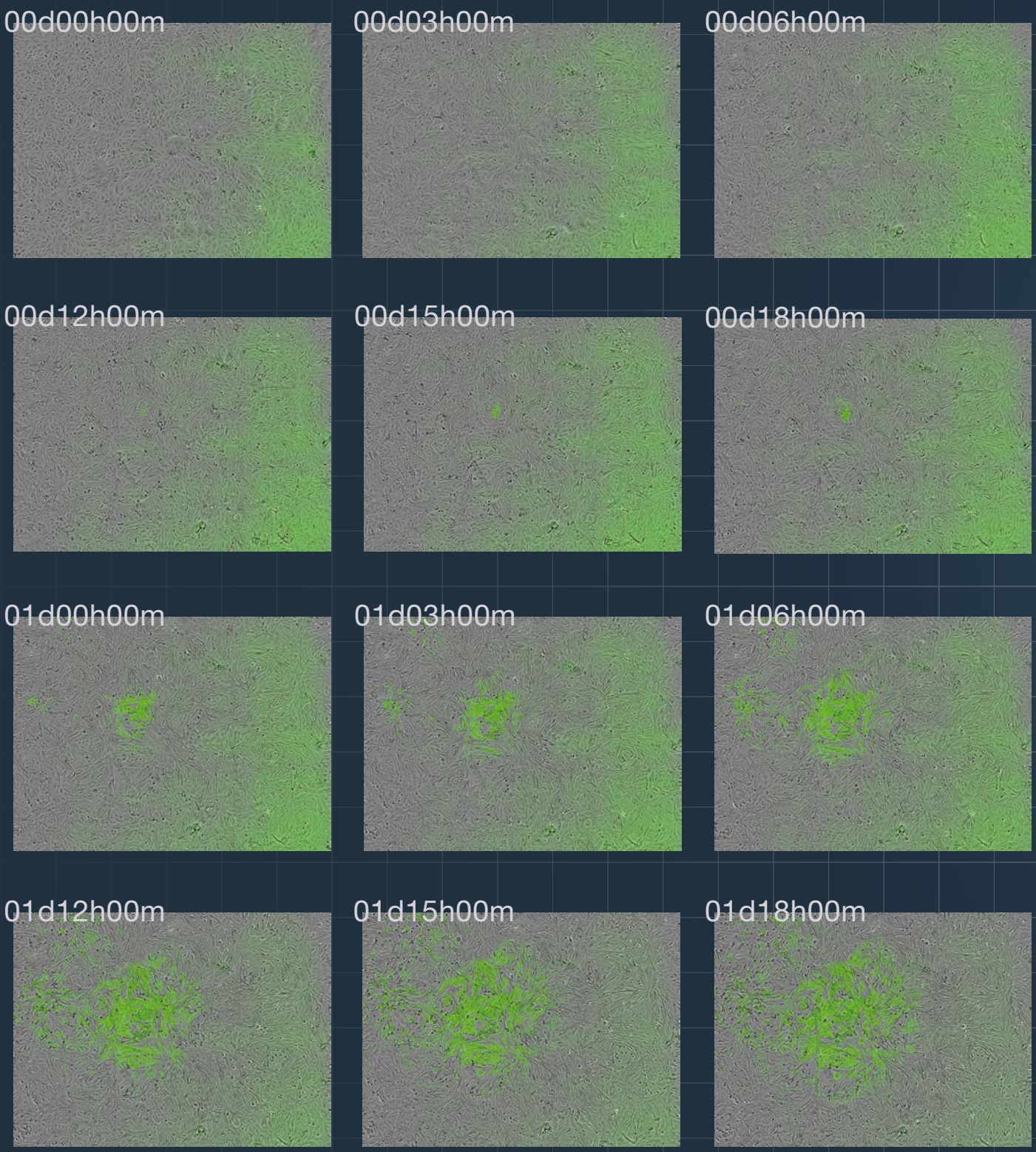
Andreas Pichlmair

The cells are stored in nitrogen.

Picture credits: Magdalena Jooss

Prof. Andreas Pichlmair

Andreas Pichlmair studied at the University of Veterinary Medicine, Vienna, and performed his doctoral studies at the University of Freiburg/Breisgau. Subsequently, he obtained his doctorate from the London Research Institute of Cancer Research UK (now the Francis Crick Institute) and then spent three years as a postdoc in Vienna at the Austrian Academy of Sciences' Center for Molecular Medicine (CeMM). From 2011 to 2017, he established his own laboratory at the Max Planck Institute of Biochemistry in Munich as a Research Group Leader. Pichlmair was appointed Associate Professor at TUM in 2017. His research focuses on the interaction between viral proteins and their host organisms. He primarily uses mass spectrometry and combines this with other techniques, such as network analyses and other systems analyses. As a virologist, Pichlmair aims to understand which cellular components and signaling pathways are essential to viruses' survival – and thereby represent suitable targets for the development of drugs.



A cell culture is exposed to a SARS-CoV-2 which is marked with a green fluorescent protein. Infected cells in which the virus is reproducing show up in green. The screening microscope (right) allows the same measurement to be performed every three hours to follow the replication of the virus. The development and spread of the virus can thus be seen over time.

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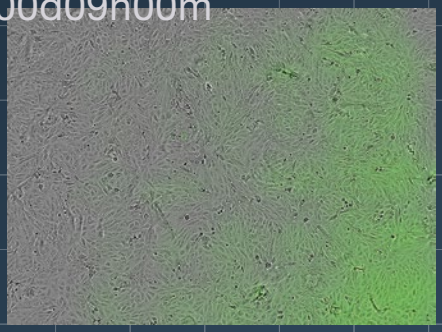

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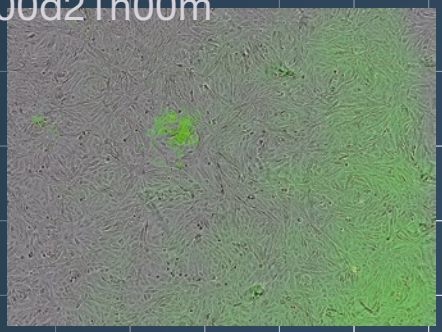

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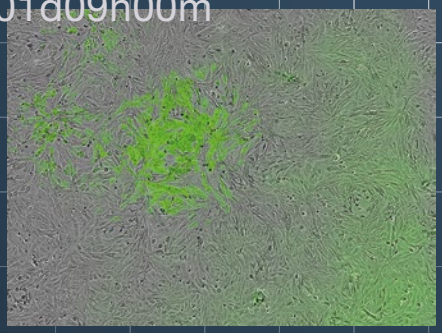

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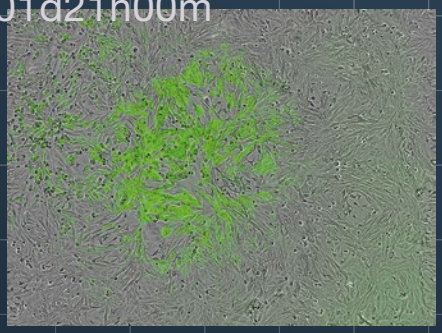
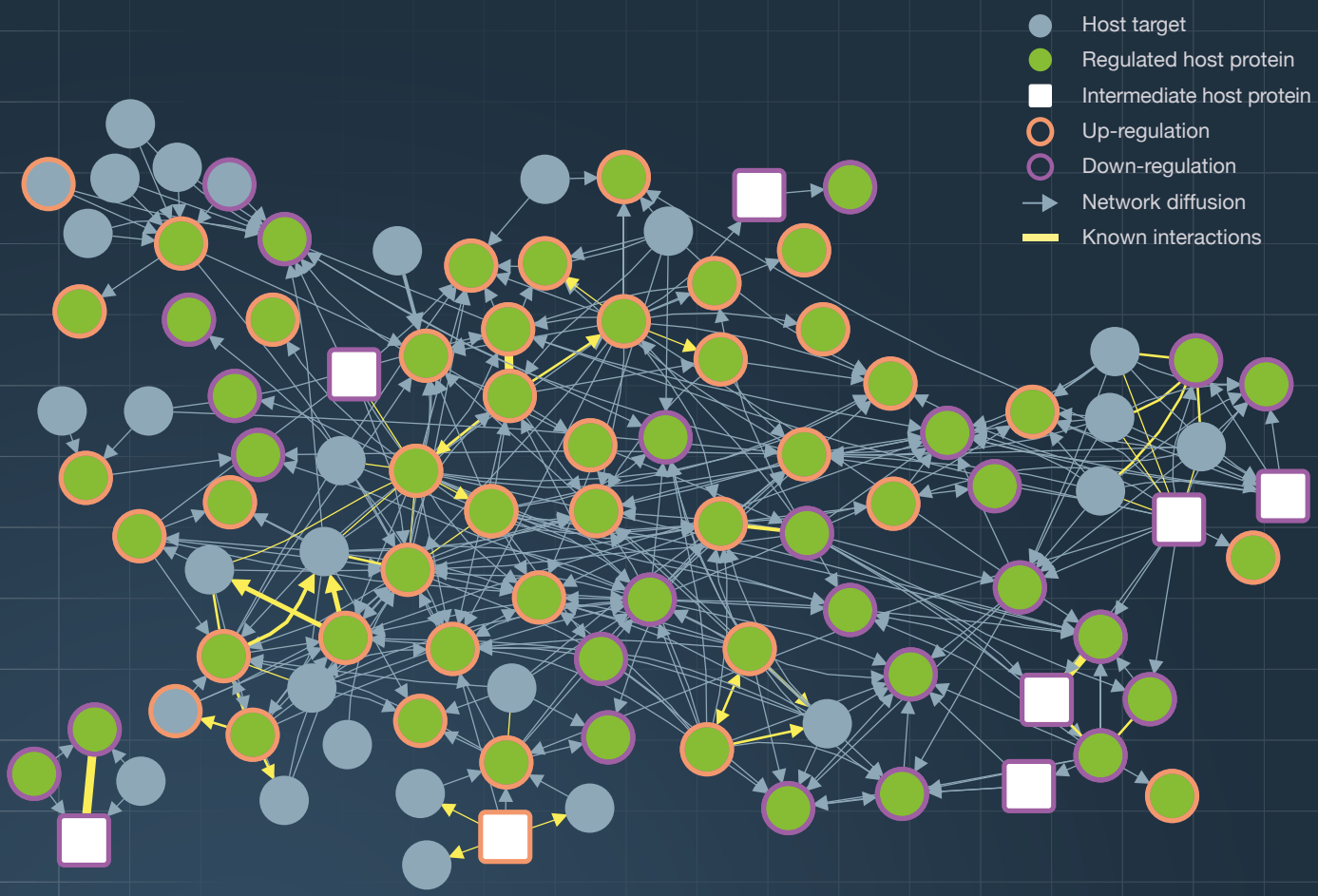
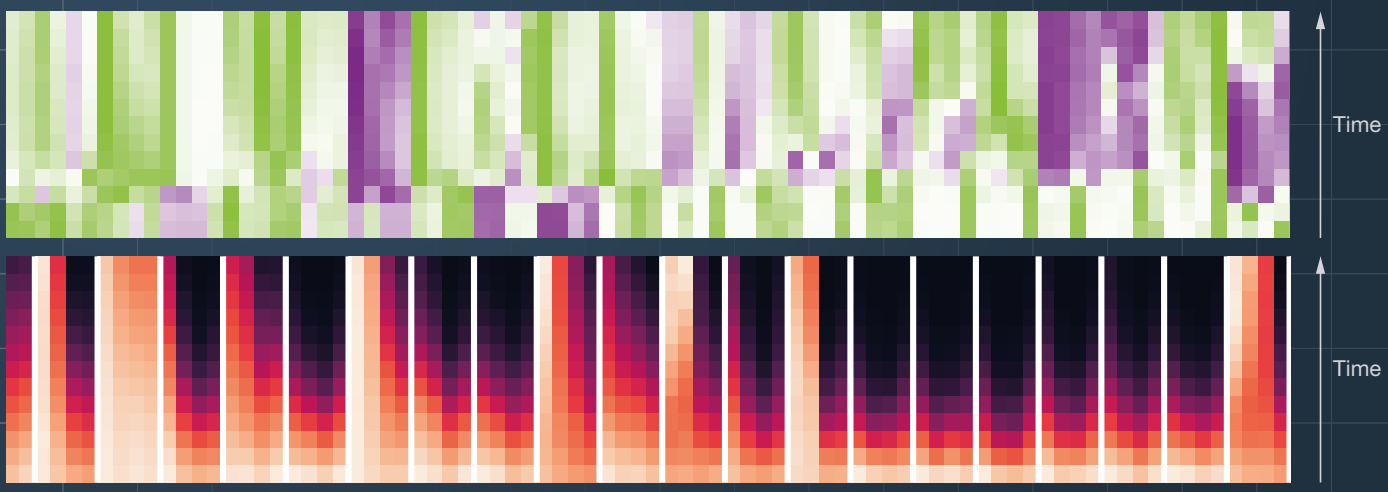


Picture credit: Andreas Pichlmair, Magdalena Jooss



The network in this picture shows what happens after the SARS-CoV-2 ORF7a protein is expressed in a cell. Each network node depicts a specific protein. Grey nodes indicate cellular proteins, to which the virus protein binds. Green nodes illustrate the downstream changes in host protein expression. An orange border stands for an abundance increase, purple for decrease. These proteins are targets for the viral protein, as it changes their abundance rate. The yellow lines denote the current knowledge of molecular interactions in the cell. Thus the network analysis helps to gain a deeper molecular understanding of the virus protein activity in the cell.



Results of a screening as shown on the previous page. Top: Signal of the green fluorescent protein; less green/more purple indicates less virus. Bottom: Cell growth; darker colors indicate higher cell densities. Each column represents the test with a different compound, each square within that column stands for one measurement. Dark red/purple colors indicate compounds which seem effective against the virus.

While some of Pichlmair's team continue to collect and analyze data, others are already testing the efficacy of identified substance classes. Do they still allow viruses to reproduce or do they inhibit this process? To do this, the researchers use cell cultures and coronaviruses marked with a fluorescent green dye. Infected cells in which the virus is reproducing show up in green. In cases where agents prove to be effective, the green coloring either disappears or does not appear at all. The researchers use a screening microscope, which allows them to process high volumes of samples. The device has been co-funded by the Max-von-Bauernfeind Association, a longstanding partner of TUM. Like all experiments which involve direct use of the virus, these tests are conducted in biosafety level 3 (BSL-3) laboratories. BSL-3 laboratories are sealed off and isolated from the outside world and are designed in such a way that pathogens cannot inadvertently escape. Thanks to their modern microscope, Pichlmair and his team can perform 574 independent measurements every three to four days.

A truly interdisciplinary team

"What's special about our work is that we are linking mass spectrometry, bioinformatic analyses, virology and cell biology," says Pichlmair. This approach demands extensive experience in entirely different fields. Pichlmair's multidisciplinary team comprises virologists, cell biologists, engineers and bioinformaticians. His team of eighteen work hand in hand, each of them a specialist in their field and each contributing his or her part to complete the puzzle. At the same time, other team members are performing quality checks to avoid analyzing artifacts. Together, the team are working to understand exactly what viruses need in order to reproduce. "The challenge lies in not simply conducting blind tests where you don't understand what's happening. Instead, it lies in identifying substance classes of interest that, when combined with our knowledge, can help us to develop forward-thinking treatments," says Pichlmair.

Karoline Stürmer

Many of the experiments are conducted in biosafety level 3 (BSL-3) laboratories. BSL-3 laboratories are sealed off and isolated from the outside world and are designed in such a way that pathogens cannot inadvertently escape. A special "dress code" and HEPA-filtered ventilation devices protect scientists from getting infected.

Graphics: ediundsepp (source: TUM); Picture credit: Magdalena Jooss