CASE STUDY

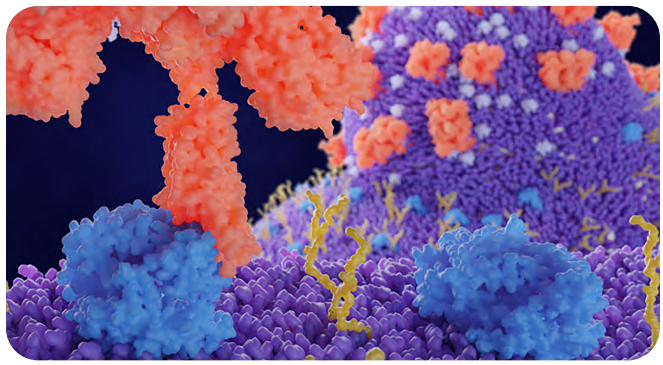
NIH/NCATS helps to unlock the role of SARS-CoV-2 spike protein (S1)

Using accelerated screening research for drug repurposing candidates

COVID-19 has upended the world and proven to be a difficult disease to understand and contain. When a research team at the National Center for Advancing Translational Sciences (NCATS), a part of the NIH, was tasked to help find a possible therapeutic, they turned to Revvity's AlphaLISA™ assay technology and embarked on a drug repurposing strategy as the quickest route to generating data that would help the pharma industry drive towards an effective treatment. The result? In a matter of just a few months, the NCATS team was able to screen 3,384 molecular entities and narrow them down to a field of 25 quality "hits" capable of disrupting SARS-Cov-2 S1 protein:ACE2 receptor binding.

Drug development in the age of COVID-19

Drug development can often take years, but the team's mandate was to move as quickly as possible, while in the midst of a global pandemic, to find potentially relevant drug candidates by screening small molecule compounds that are already approved by regulators while ensuring expected accuracy, safety and efficacy. Compounding this challenge were the restrictions placed upon the team relating to social distancing and other protocols of the pandemic quarantine. Other obstacles included securing resources like the recombinant viral and host proteins needed to run the assay, as well as reduced access to the lab itself.



Tackling the COVID-19 "crown of thorns" protein spikes to unlock new insights

The COVID-19 virus, SARS-CoV 2, is a member of coronavirus family, so named for the molecular crown or 'corona' that surrounds each virus particle. A 'crown of thorns' of spike proteins that interact with molecules on the surface of the cell enables the virus to invade its host. The spike proteins on the surface serve to pick the lock of the receptor to enter the cell. Once inside, the virus mass produces copies of itself, unloading its own genetic instructions into the host cell. And thus, the havoc begins. Every virus chooses a specific receptor to suit its purposes. Identifying this receptor is critical to understanding the virus's spread and developing a therapeutic. COVID-19, like SARS before it, uses its spike proteins to invade cells through the ACE2 (Angiotensin-Converting Enzyme 2) receptor. ACE2 is a common, vital receptor in the human body. It influences blood flow and is involved in many organs. It is particularly prevalent on cells that line the air sacs in lungs, hence the rampaging respiratory effects we've come to associate with the virus. It is, therefore, a tricky target for drugs.

revvity

Putting Revvity's AlphaLISA assay technology to work

Led by Quinlin Hanson and Matthew Hall, the NCATS team's immediate goal was to develop a sensitive and robust assay platform that scientists could use as a template for screening small molecule compounds that might lead to further drug development. First, as a proof of concept, they created a biochemical drug repurposing assay to screen current drugs that block the ACE2 receptor from binding the virus' S1 protein. As a result of their findings, the NCATS team published that data on NCATS' Open Data Portal so other scientists could view the results in order to follow up, collaborate, develop candidate therapeutics on their own or for others to test. Publishing the data on the Open Data Portal proved critical as it allows scientists from all over the world access for the critical opportunity to comment, contribute, and, ultimately, to move their own experimental efforts forward leveraging the NCATS' data. The team's philosophy, in line with NCATS' global mission, was to release knowledge to as broad an audience as possible so that they would no longer be working in an individual silo to address this immense public health problem.

The development process for finding appropriate drugs as therapeutics can take months or even years. Given the nature of the pandemic, the team was aiming to find something in a matter of several weeks or months. First, the team designed a fundamental, biochemical assay as a model that replicates the S1:ACE-2 interaction understood to be the critical entry mechanism for SARS-CoV 2 to infect human lung cells. The S-1 region of the SARS-Cov-2 Spike protein is the region that is believed to be responsible for the docking of the virus to the associated host cells that leads to downstream entry and infection. Based on the importance of this interaction, the ACE2:S1 interaction is frequently the chosen therapeutic target to be disrupted. A highly sensitive proximity assay needed to be developed to differentiate affinities of the compounds in the library that specifically target the ACE2:S1 protein-protein interaction, which led the team to select the Revvity AlphaLISA assay technology. AlphaLISA is a bead-based technology that generates a light signal in response to proteins or any biomolecular interaction, bringing the bead-binding partner complex into close proximity. AlphaLISA beads provide large, molecular, spatial capacity and unique chemical properties that allow for robust assay development with reduced time to results when paired with companion assays that control for assay interference that might falsely "present" as therapeutically relevant hits.

The AlphaLISA beads serve as tiny, spherical, anchors that offer numerous options for recognizing peptide sequences genetically grafted onto target proteins of interest, called tags. Tags offer vital flexibility to use any target protein and they can be removed or manipulated. The team used AlphaLISA donor, acceptor and control TruHit® beads in the central and control assay, respectively, that screened 3,384 molecular entities and found 25 quality "hits" capable of disrupting the S1:ACE2 binding interaction.

The whole process was achieved in a matter of a few months. Their aim was to disrupt the interface between two proteins, the host ACE2 receptor and the SARS-COV-2 spike S-1 protein.

The Revvity AlphaLISA Technology is a homogenous and robust platform that allows for the successful evaluation of protein:protein interactions across a wide dynamic range. This approach can be used in small molecule studies but is also an assay platform that can be applied to virtually any therapeutic. Potential protein therapeutics, polysaccharides, nucleic acid fragments can all be tested. Concerns about a molecular therapeutic being too large are eliminated. Fears about losing the luminescent signal due to distance constraints are alleviated. The sheer number of bead options available allow you to design your assay to minimize interactions with potential contaminants. It results in a highly clean assay.

Throughout the project and due to the restrictions of COVID-19 protocol quarantine, NCATS scientists also found they had to work together in new ways as an interdisciplinary team, taking on responsibilities they each didn't normally have in order to get the job done. For example, scientists who didn't work in compound management helped in this area to coordinate plating of small molecules. The automation team helped establish protocols enabling people to conduct part of their science remotely. Communications became critical even about the smallest logistical challenges such as moving packages from one room to another.

Improving potency, validating findings, peer reviewed study

The next focus for the NCATS/NIH team is to take the 25 hits, find similar small molecules and/or analogs to those hits and improve the potency of each to enhance and further validate their findings.

While the immediate goal of this project was to test for potential small molecule compounds against COVID-19 in a biochemical format, the team also plans to publish a peer-reviewed study which will include rigorous testing on selected molecules, including counter screens where compounds that might interfere with the primary screen are identified.

Conclusion

The NCATS team found this COVID-19 investigation both challenging and exciting. New workarounds had to be developed in the context of significant time pressure that defined a project where multiple skills were brought to bear. The team's open approach to sharing and receiving data was not only helpful but at points inspirational and helped a small band of researchers scale their efforts to take on the most serious global health pandemic in a century.

Hopefully their data and processes will provide helpful ideas to also support the work of fellow scientists and the global science community as it works tirelessly to beat COVID-19.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com