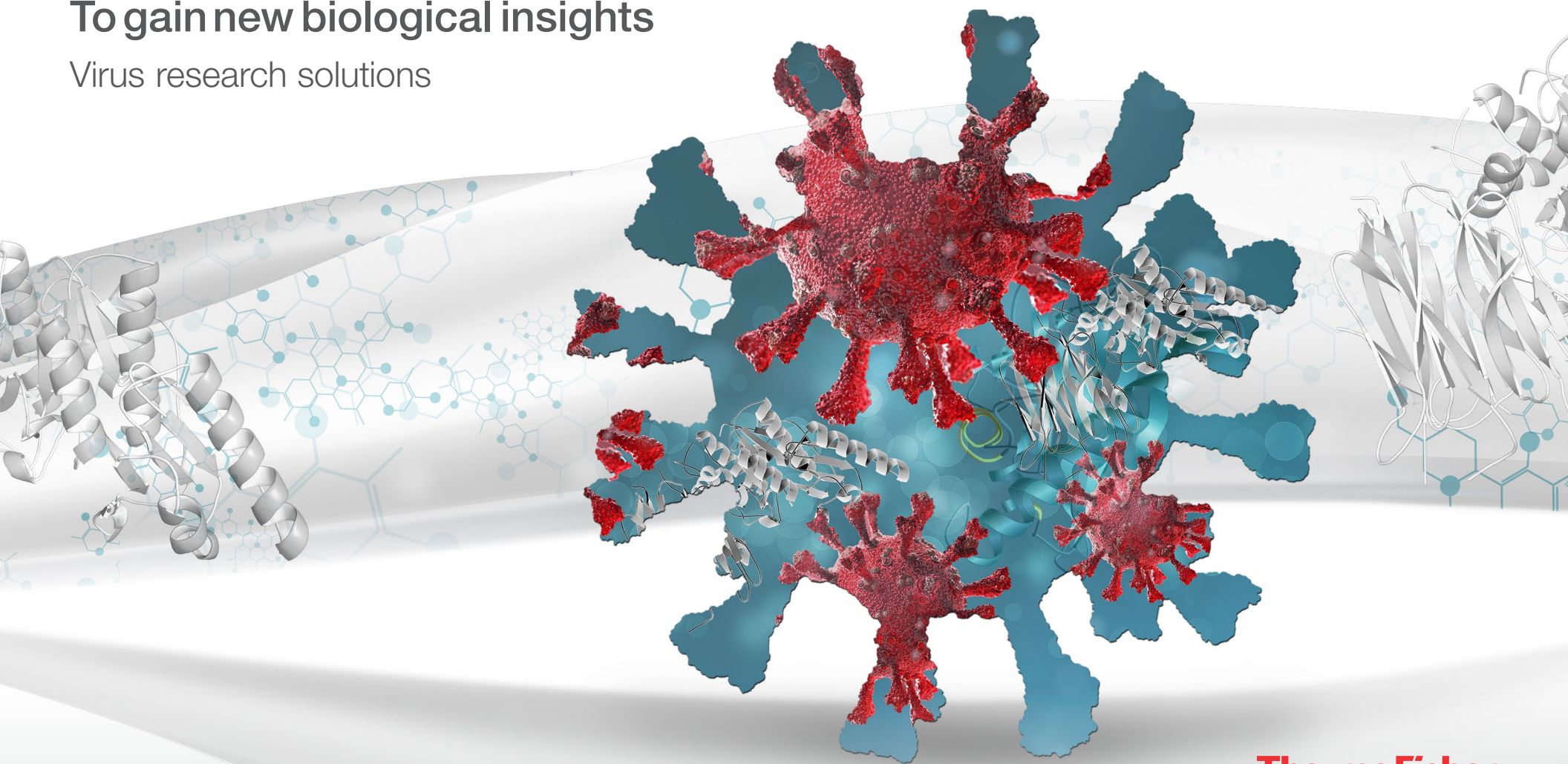


thermo scientific

# Go beyond

To gain new biological insights

Virus research solutions



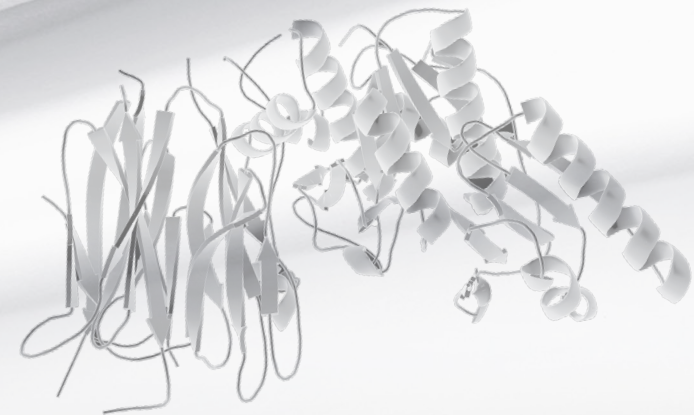
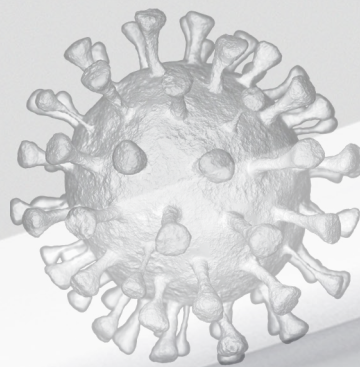
**ThermoFisher**  
SCIENTIFIC



# An urgent need for expanded virus research

Harness the power of omics to accelerate virus research. Thermo Fisher Scientific's proteomics, glycomics, lipidomics and metabolomics mass spectrometry workflows provide virus researchers with tools for studying intact viral particles providing detailed insights into virus structure, function, binding characteristics, and their impacts on the biochemical pathways of host cells upon infection. These tools can contribute to understanding novel viruses and their resulting diseases, and drive actions to mitigate the impact on human health.

Thermo Fisher Scientific offers a complete portfolio of tools and technologies to enable virus research, supporting collaboration and the acceleration of lifesaving discoveries. And we continue to pursue advancements that deliver confidence and rigor in the results.

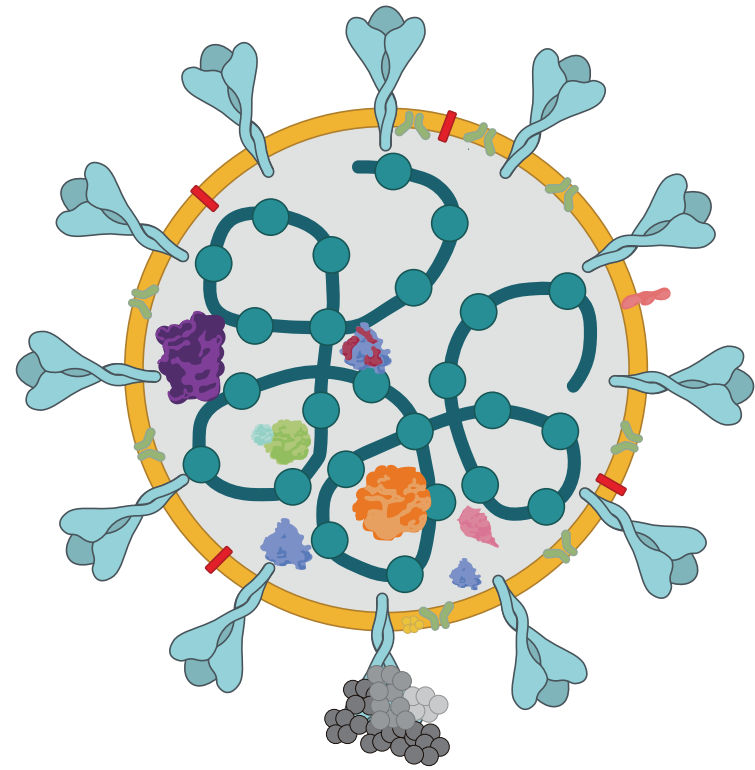




# The role of mass spectrometry in virus research

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has provided a global case study for the need to maintain a responsive and effective ability to rapidly understand emerging viruses. Mass spectrometry (MS) offers many approaches to gain insights into viral behavior by studying intact viral particles, their surfaces and binding characteristics, their protein compositions, and their impacts on the biochemical pathways of host cells upon infection. This information can provide detailed insights into virus structure and function, which may enable virus detection and inform drug and vaccine programs.

For more than 20 years, Thermo Scientific™ mass spectrometers have defined the benchmark in omics research capturing complete profiles of biological systems. That's why we at Thermo Fisher Scientific have invested in developing fit-for-application reagents, software and workflows that live up to the high standards set by our leading instrumentation. This full suite of complementary tools allows you to explore the functions of individual proteins, protein complexes, metabolites, lipids and glycans to determine their places in complex biological systems. Harness their power to help you turn observations into valuable discoveries.

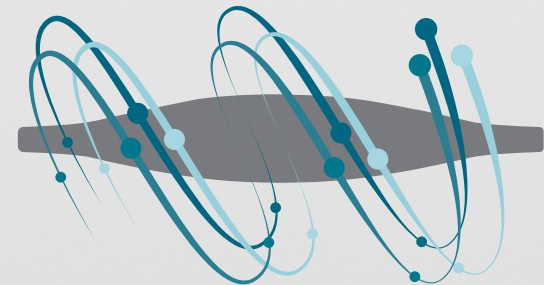


## The core of successful virus research: Orbitrap mass spectrometry

Thermo Scientific™ Orbitrap™ mass spectrometers offers leading mass spectrometry technology for analyzing complex proteomics, metabolomics, lipidomics and glycomics samples, quantifying and characterizing both the sheer number of molecules present and the wide dynamic range they span.

With established sample preparation, chromatographic separation and integrated data analysis tools, the Orbitrap mass spectrometers enables researchers to successfully analyze diverse sample matrices with industry-leading sensitivity.

Featured in more than 75,000 peer-reviewed publications in the last decade, Orbitrap mass analyzer technology has a proven track record as a powerful LC-MS platform for omics.

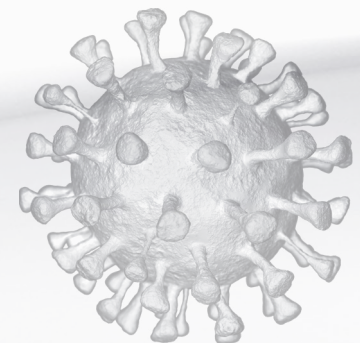
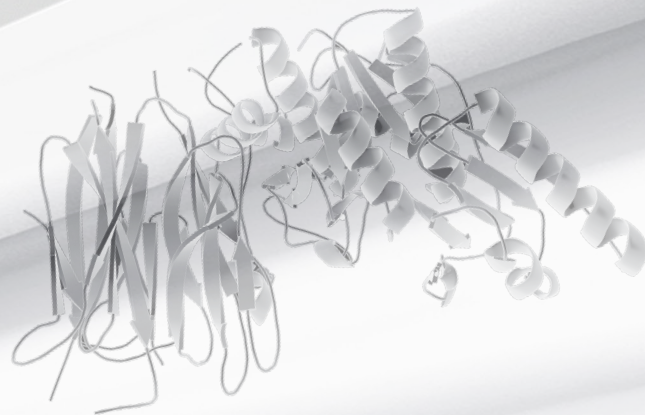




# Quantitative proteomics: Novel insights into virus behavior

Proteomics requires the precise quantitation of all identified proteins, setting the foundation for bolder hypotheses and deeper understanding of biology. In terms of virus research, quantitative proteomics enables the identification and quantitation of viral epitopes and greater understanding of immunogenicity. The characterization and quantitative assessment of the antigenic peptides from viral host proteins are crucial in understanding the specific and effective defenses against a particular viral infection. Additionally, quantitative proteomics can help identify distinct immune response profiles induced by viral infection.

Translational proteomics most frequently involves the discovery of potentially clinically relevant protein biomarkers and transition to verification and validation. Quantitative proteomics workflows such as label-free quantitation (LFQ) and tandem mass tags (TMT) can be used to identify biomarkers for early detection of viral diseases, distinguish distinct profiles induced by disease progression and monitor therapeutic response from patients.

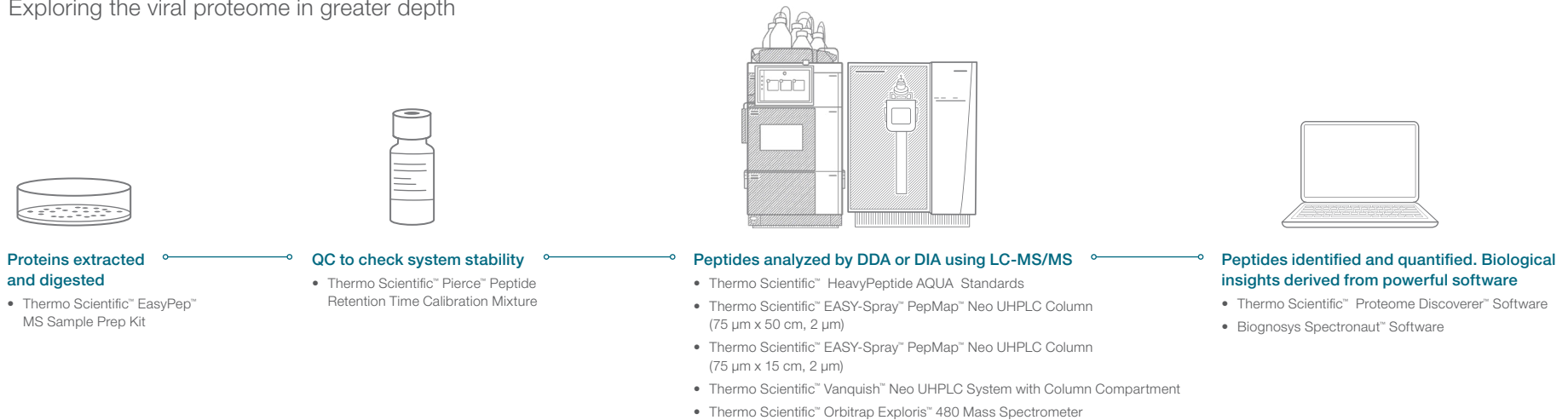




In discovery-based quantitative analyses, the goal is to identify proteins and measure their relative abundance changes across multiple sample sets, usually on a proteome-wide level. The multiple sample sets could potentially represent, for example, different time points in viral infection, a biological pathway, responses to different stimuli, or different cellular locations. High-resolution, accurate-mass (HRAM) Orbitrap MS technology provides the necessary versatility, selectivity, sensitivity and speed for several discovery-based quantitative workflows that include both LFQ and TMT-based quantitation approaches.

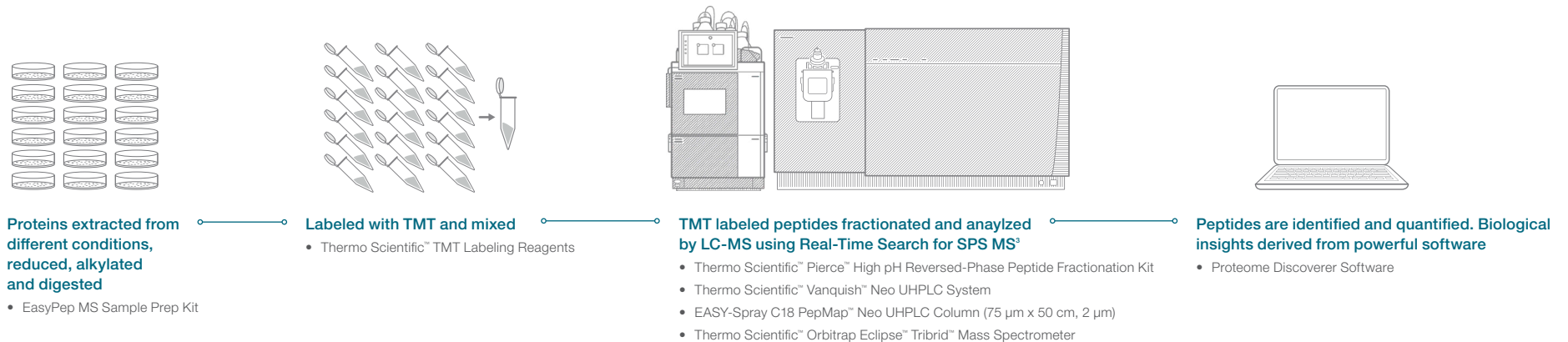
## Label-Free Quantitation

Exploring the viral proteome in greater depth



## Multiplexed Quantitation with TMT Reagents

Multiplying the power of proteomics to explore any objective





## The importance of PTMs on virus life cycle

The surface of the virus envelope is covered with glycoproteins, which are involved in the process of binding to the host cells for viral entry. Glycan diversity on the protein is used by the virus to evade immune system response. Glycans also have significant conformational flexibility contributing to overall conformational dynamics of the molecule that can both generate potentially novel drug binding sites, or shield binding sites predicted from polypeptide-only models. Glycoproteomics can provide information on the glycosylation sites and glycan compositions. Glycomics can expand on glycoproteomics to provide structural details on the glycans.

Post-translational modifications (PTMs) including phosphorylation and polyubiquitination have been shown to potently regulate innate inflammatory responses through the activation, cellular translocation, and interaction of innate receptors, adaptors, and downstream signaling molecules in response to infectious and dangerous signals. Methylation, acetylation, SUMOylation and succinylation are increasingly implicated in the regulation of innate immunity and inflammation. MS can be used to characterize these PTMs, their locations on a protein and the ratios present.



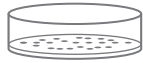
“Mass spectrometry analyses go hand-in-hand with infectious disease experiments, and it is an iterative process. One of the benefits of an Orbitrap Tribrid mass spectrometer is its ability to study complex glycosylation patterns on microbial proteins. Those glycans often modulate the binding of proteins and understanding the composition and structure of those protein modifications in native material is crucial in infectious disease biology.”

—Dr. Lance Wells, University of Georgia



## Glycoproteomics

A sweet revolution in glycopeptide analysis



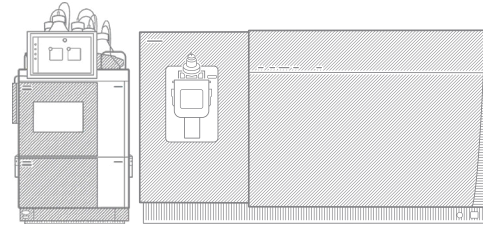
### Proteins extracted, reduced, alkylated and digested

- EasyPep MS Sample Prep Kit



### Glycopeptides enriched

- TMT Labeling Reagents
- Thermo Scientific™ HyperSep™ Retain AX Cartridges



### Intact glycopeptides analyzed by LC-MS/MS on a Thermo Scientific™ Orbitrap™-based MS

- Vanquish Neo UHPLC System
- EASY-Spray C18 PepMap™ Neo UHPLC Column (75 µm x 50 cm, 2 µm)
- Orbitrap Eclipse Tribrid Mass Spectrometer

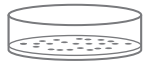


### Glycopeptides identified and biological insights derived from powerful software

- Proteome Discoverer Software with Byonic™ Node

## Phosphoproteomics

Large-scale phosphoproteomics workflow



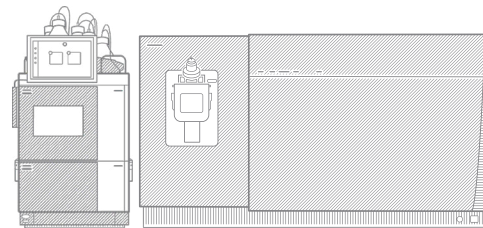
### Proteins extracted, reduced, alkylated and digested

- EasyPep MS Sample Prep Kit



### Phosphopeptides enriched

- TMT Labeling Reagents
- Thermo Scientific™ High-Select™ Fe-NTA Phosphopeptide Enrichment Kit



### Phosphopeptides analyzed by LC-MS/MS on an Orbitrap based MS

- Pierce High pH Reversed-Phase Peptide Fractionation Kit
- Vanquish Neo UHPLC System
- EASY-Spray C18 PepMap™ Neo UHPLC Column (75 µm x 50 cm, 2 µm)
- Orbitrap Eclipse Tribrid Mass Spectrometer

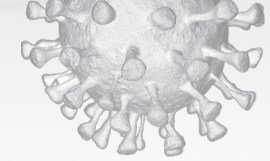


### Phosphopeptides identified and biological insights derived from powerful software

- Proteome Discoverer Software with ptm RS



# Integrative structural biology for viral research



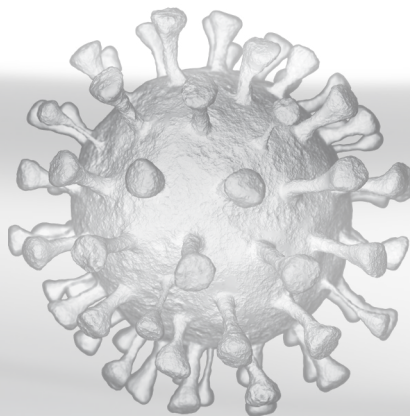
The characterization of viral protein structures, the complexes and interactions they form are essential for understanding virus function and mechanisms of action. MS-based techniques provide the ability to perform experiments at proteome scale, analyze proteins in their native biological state, and do all of this at reduced sample size. MS offers many approaches to gain insights into viral structure and behavior.

Native MS is one such tool allowing for the study of virus structure, virus capsids, quantification of cargo encapsulation, monitoring of capsid assembly, profiling different glycoforms on virus surface proteins and virus-host cell interactions.

A major focus of structural virology is to obtain an understanding of the construction and structural basis for stability of the protein capsids that encapsulate the viral genomic information. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) can be used to measure local conformational dynamics and to gain insight into the mechanisms of assembly and capsid maturation for viruses.

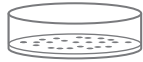
Crosslinking MS (XL-MS) is ideal for examining virus-host cell protein interactions. XL-MS can help identify host cell surface proteins involved in the interaction, their structure or the structure of the complexes, interaction sites, the precise residues involved in binding and the protein interaction topologies between a virus and host cell proteins. Incorporating quantitative mass spectrometry (LFQ, TMT) with XL-MS enables examination of protein complex structure and interaction dynamics, such as measuring changes in levels of interactions or binding affinities.

Affinity purification-MS (AP-MS) is another virus structure tool. It can be used to examine specific protein-protein interactions within protein complexes, or look at protein complexes more globally at the interactome level. Incorporating quantitative MS with AP-MS enables examination of protein-protein interactions under different conditions (across different stages of viral infection), thereby providing a much more dynamic view. AP-MS can also be used to examine PTMs and the role they play in facilitating protein-protein interactions.



## Affinity Purification Mass Spectrometry

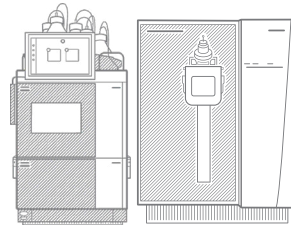
Global insights into viral protein complexes at the interactome level



Proteins extracted



Affinity purified, reduced, alkylated and digested



Peptides analyzed by LC-MS/MS

- Pierce Peptide Retention Time Calibration Mixture
- EASY-Spray C18 PepMap™ Neo UHPLC Column (75 µm x 50 cm, 2 µm)
- Vanquish Neo UHPLC System
- Orbitrap Exploris 480 Mass Spectrometer

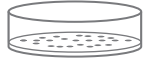


Peptides identified and biological insights derived from powerful software

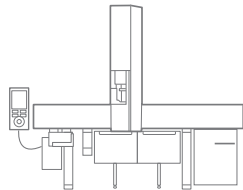
- Proteome Discoverer Software

## Hydrogen-Deuterium Exchange Mass Spectrometry

Elucidating viral protein conformation, structure and dynamics

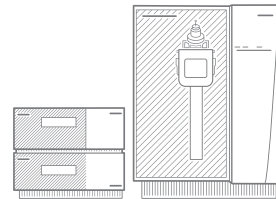


Proteins extracted



Deuterium labeled and in-line digested

- Trajan's LEAP HDX Extended Parallel System
- Thermo Scientific™ Poroszyme™ Immobilized Pepsin Cartridge (2.1 mm x 30 mm)



Analyzed by Orbitrap based MS

- Thermo Scientific™ Acclaim™ PepMap™ 300 µ-Precolumns™, 5 µm Cartridge Columns
- Thermo Scientific™ Acclaim™ PepMap™ 100 C18 HPLC Column (1.0 mm x 5 cm, 3 µm, 100A)
- Thermo Scientific™ UltiMate™ NCS-3500RS Binary Rapid Separation Nano/Capillary Pumps
- Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer

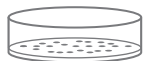


Data analyzed by powerful software

- Thermo Scientific™ BioPharma Finder™ Integrated Software
- HDExaminer Software (Sierra Analytics)

## Crosslinking Mass Spectrometry

Understanding viral protein interactions

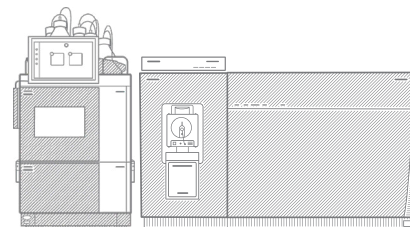


Proteins extracted and labeled with crosslinking reagent

- Thermo Scientific™ DSSO (disuccinimidyl sulfoxide)
- Thermo Scientific™ DSBU (disuccinimidyl dibutyric urea)



Reduced, alkylated and digested



Crosslinked peptides analyzed by LC-MS²-MS²

- Vanquish Neo UHPLC System
- EASY-Spray C18 PepMap™ Neo UHPLC Column (75 µm x 50 cm, 2 µm)
- Thermo Scientific™ FAIMS Pro™ Interface
- Orbitrap Eclipse Tribrid Mass Spectrometer



Crosslinked peptides identified and biological insights derived from powerful suite of software

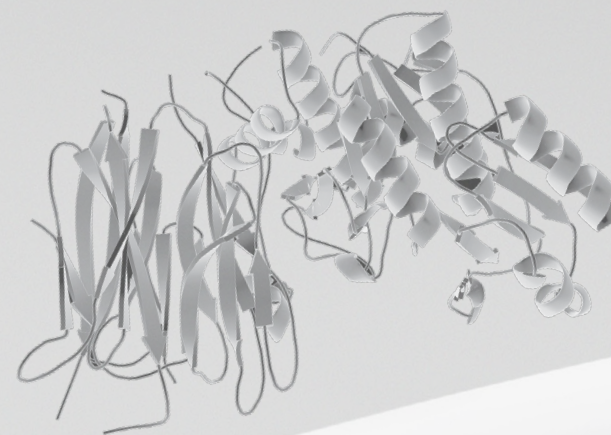
- Proteome Discoverer Software with XlinkX Node



## Integrative structural biology for viral research

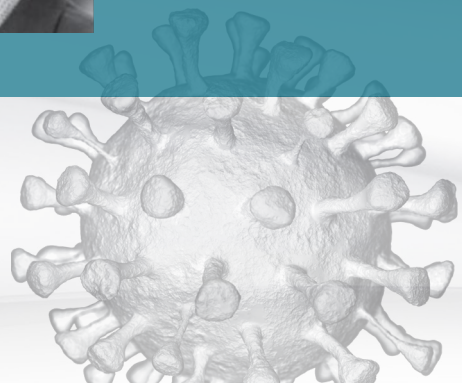
Viral proteomics research endeavors can also benefit from intact/top-down MS analysis or identification at the protein level. The main advantages of the top-down approach include detection of sequence variants, truncation, PTMs localization and the identification at the proteoform level. Such information can be critical for drug design. Top-down analysis can be performed alone or part of native MS and HDX-MS.

Intact protein analysis is performed for a number of reasons, including sequence confirmation, determination of molecular mass and PTMs.



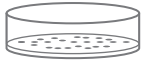
“ In the analysis of large protein assemblies like viruses, ribosomes, and proteasomes, the Q Exactive UHMR mass spectrometer has made things possible that we couldn't do before. The major benefits of the system are the substantial increase in sensitivity and resolution that we get for very large protein assemblies, together with the ability to do MS/MS experiments.”

—Professor Albert Heck, Utrecht University

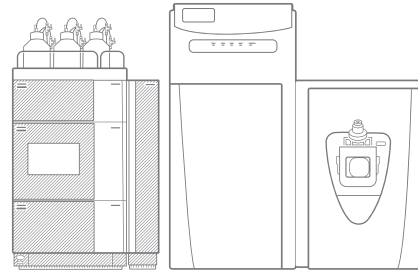


## Native Mass Spectrometry

Insights into viral protein complexes



Proteins extracted



Proteins analyzed by MS

- Thermo Scientific™ Nanospray Flex™ Ion Source and Offline Nano ES Kit
- Thermo Scientific™ Acclaim SEC-1000 Size Exclusion Chromatography HPLC Column (4.6 x 300mm) or Thermo Scientific™ MAbPac™ SEC-1 Size Exclusion Chromatography HPLC Column (2.1 x 300mm)
- Thermo Scientific™ Vanquish™ Horizon UHPLC System or Thermo Scientific™ Vanquish™ Flex UHPLC System
- Thermo Scientific™ Q Exactive™ UHMR (Ultra-High Mass Range) Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

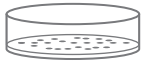


Data analyzed by powerful software

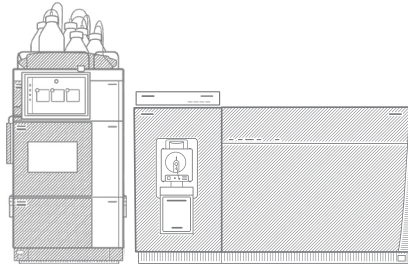
- BioPharma Finder Integrated Software

## Top-Down/Intact Mass Spectrometry

Insights into viral proteoforms



Proteins extracted



Proteins analyzed by LC-MS

- Thermo Scientific™ Pierce™ Intact Protein Standard Mix
- Thermo Scientific™ MAbPac™ RP HPLC Column (2.1 x 100mm) or Thermo Scientific™ EASY-Spray™ Accucore™ 150-C4 HPLC Column (75 µm x 15 cm, 2.6 µm)
- Vanquish Horizon UHPLC System or Vanquish Neo UHPLC System with Column Compartment
- Orbitrap Eclipse Tribrid Mass Spectrometer



Proteins identified and biological insights derived from powerful suite of software

- Thermo Scientific™ ProSightPC™ Software
- Proteome Discoverer Software with ProSightPD Node
- BioPharma Finder Integrated Software



# Targeted assays for virus detection

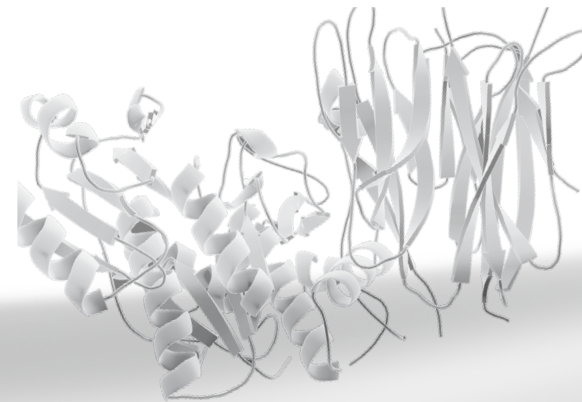
MS provides alternative or complementary virus testing assays to the conventional RT-PCR-based methods to help address issues with testing capacity and shortages in testing supplies. MS-based testing can be used to determine relative and absolute abundance of peptides unique to viral proteins of interest which are then selected for targeted quantitative analysis. This allows for profiling of hundreds of targets in a single experiment. MS-based approaches can be used for the detection of SARS-CoV-2 virus proteins in swabs, mucus and other body fluids.

There are three different approaches to using MS for virus testing:

- Selected reaction monitoring (SRM) using a triple quadrupole mass spectrometer.
- Parallel reaction monitoring (PRM)
- Thermo Scientific™ SureQuant™ Internal Standard (IS) targeted quantitation workflow on an Orbitrap based mass spectrometer.

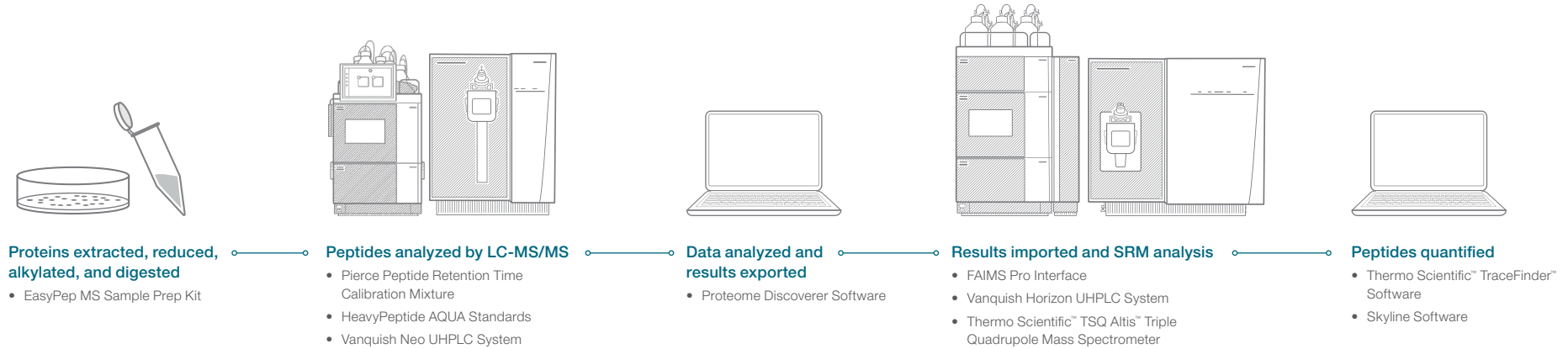
SRM is extremely sensitive, reliable and proficient at detecting large numbers of well-characterized viral proteins. PRM (equivalent to SRM on an Orbitrap mass spectrometer) offers several advantages for targeted detection and quantitation. It eliminates most biological interferences, providing high accuracy and attomole-level limits of detection and quantification.

SureQuant targeted quantitation workflow builds upon the PRM approach by using spiked-in internal standards to dynamically control MS acquisition parameters and optimize instrument duty cycle. This maximizes the number of productive MS scans and improves sensitivity of targeted protein detection.



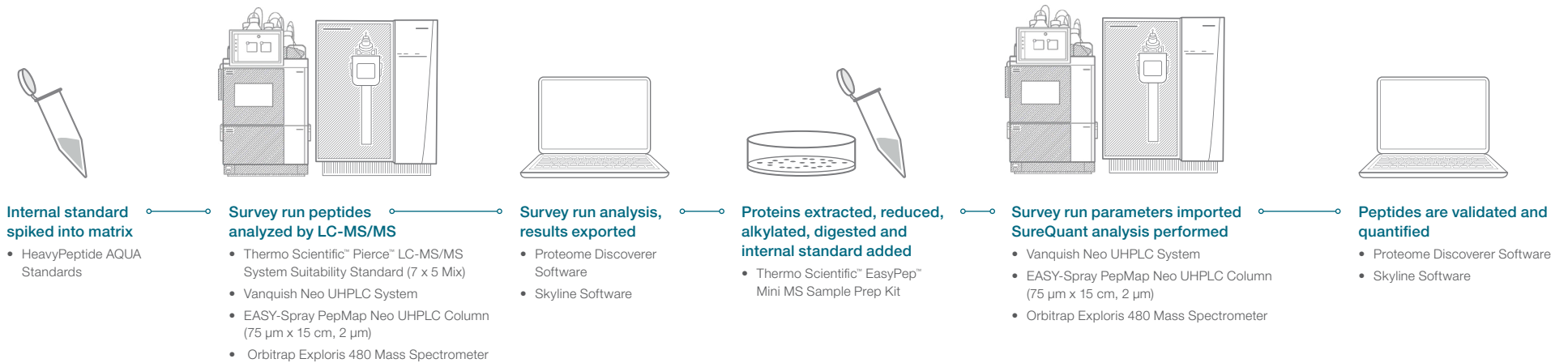
## Selected reaction monitoring targeted workflow

Cost effective platform for virus detection



## SureQuant IS targeted protein quantitation workflow

Quantifying more viral targets in complex matrices with confidence and precision

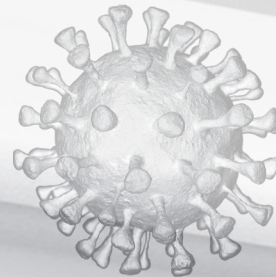
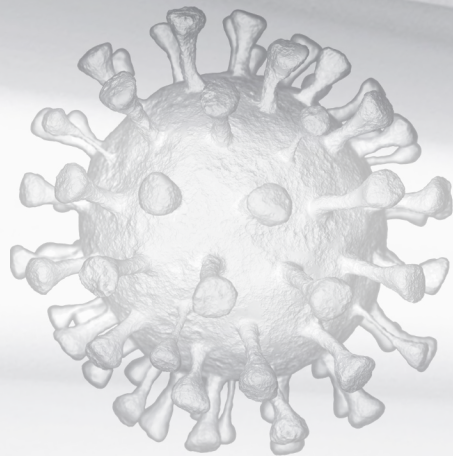




# Metabolomics in virus infection and pathogenesis

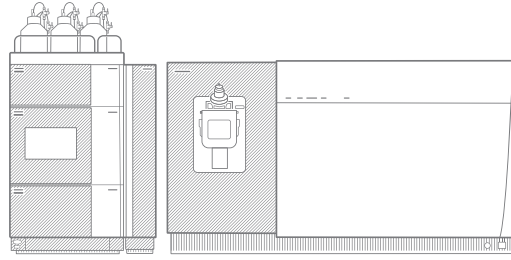
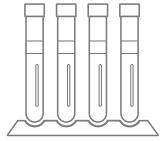
Metabolomics studies reveal metabolic signatures of diseases caused by viral infection leading to insights for translational research and targeted diagnostics that advance precision medicine. MS-based metabolomics workflows can be used to identify metabolic signatures to predict disease severity, progression as well as therapeutic efficacy.

The study of metabolites is also important to understanding the response of the host immune system during viral infection. MS characterization and quantitation of metabolites can yield important insights into how the immune system functions. Since metabolites are diverse in their physicochemical properties and abundance, a truly comprehensive metabolomics study will require sophisticated methods of separation, detection and data processing. The integration of each of the chromatographic separation methods (liquid, gas, and ion) with a high-resolution detection system (Orbitrap mass spectrometer) and powerful differential software, enables broader, deeper, and faster analysis of complex and diverse metabolites—offering a robust system that is more effective than the sum of its parts.



## Untargeted Metabolomics

Deeper and broader analysis of metabolites



Biofluid cell, tissue

Metabolite extraction

Untargeted analyzed by LC-HRAM MSn

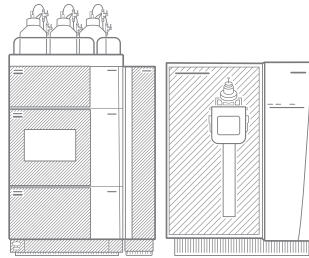
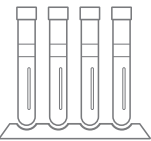
- Thermo Scientific™ Vanquish™ UHPLC System
- Thermo Scientific™ Orbitrap ID-X Tribrid Mass Spectrometer

Data alignment, unbiased peak detection, annotation & identification

- Thermo Scientific™ AcquireX Intelligent Acquisition
- Thermo Scientific™ Compound Discoverer™ Software
- Thermo Scientific™ mzCloud™ HRAM MS<sup>n</sup> Spectral Library
- Thermo Scientific™ Mass Frontier™ Software

## Semi-targeted Metabolomics

Better insights into viral disease outcomes



Biofluid, cell tissue

Metabolite extraction

Semi-targeted analysis by LC-HRAM MS and MS/MS

- Vanquish UHPLC System
- Orbitrap Exploris 240 Mass Spectrometer

Analyze targeted knowns by reference standards and in-house spectral libraries

- AcquireX Intelligent Acquisition
- TraceFinder Software, Thermo Scientific™ mzVault™ Library, Compound Discoverer Software



Discover unknowns with unbiased peak detection, statistics, and annotation tools

- mzCloud HRAM MS<sup>n</sup> Spectral Library Software



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## Integrative structural biology for viral research

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2. The SARS-CoV-2 RNA-protein interactome in infected human cells - *Nature Microbiology* **2021**, 6, 339-353.
3. The SARS-CoV-2 nucleocapsid phosphoprotein forms mutually exclusive condensates with RNA and the membrane-associated M protein - *Nature Communications* **2021**, 12, Article number: 502.

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